PSG Institute of Medical Sciences & Research
Post Box No. 1674, Peelamedu, Coimbatore 641 004, INDIA
Phone : 91 422 - 2598822, 2570170 • Fax : 91 422 - 2594400
E-mail : psgmedschool@gmail.com Website : www.psgimsr.in

6th January,2022

SELF-DECLARATION

I, Dr T M SubbaRao, Principal, do hereby declare that PSG Institute of Medical Sciences and Research, Coimbatore has the prescribe number of teaching faculty, infrastructure etc., as per the statutes of the National Medical Commission, New Delhi and the TN Dr MGR Medical University, Chennai, for conducting Undergraduate and Postgraduate Courses.

The above said information is true to the best of my knowledge.

Dr T M SubbaRao
Principal

Dr. T.M. Subba Rao, MD.,
PRINCIPAL
PSG Institute of Medical Sciences
& Research / PSG Hospitals
Coimbatore - 641 004.

	Information of the medical college/institution Year of Inception: Government/private:	PSG Institute of Medical Sciences & Research 1985 PRIVATE
1.	Name	**PSG Institute of Medical Sciences & Research**
2.	Address with pin code	Post Box.No.1674 Peelamedu Coimbatore – 641 004 Tamil Nadu, India
3.	University address with pin code	The TN Dr.MGR Medical University, Anna Salai, Guindy, Chennai-600 032 Tamil Nadu, India
4.	Official website	www.psgimsr.ac.in
5.	Dean/ Principal/ Director	Dr T M SubbaRao
6.	Mobile Number	0422 4345802
7.	Email ID of Dean /Principal	psgmedschool@gmail.com
8.	Hospital	PSG HOSPITALS Post Box No.1674, Peelamedu, Coimbatore – 641 004. Phone 0422 – 4345802, 2598822 E.mail: psgmedschool@gmail.com Web: www.psghospitals.com
9.	Date and Year of Registration of the Hospital (DD/MM/YYYY)	November 15, 1989
10.	Number of Beds	1130
11.	Number of Beds for emergency	30 Beds
12.	Date of the First Letter of Permission(LoP) of MBBS (DD/MM/YYYY) & number of seats	• G.O.Me.No.1196, Health dated. 24thJuly 1985- Permission to start PSGIMS&R. • Lr. No.MCI 34(41)/96-Med./14804, dated.20.9.1996 - MCIRecognition of admission for MBBS 100 seats. • Lr.No.U.12012/864/2008-ME(P-II) dated.19.6.2009 - LOP issued by Central Govt., Ministry of Health & Family welfare for increase of seats in MBBS course from 100 to 150

		students. • Lr.No.MCI-34(41)(UG)/2014-Med/TN/117205 dt.01.07.2014 - MCI Recognition for increase of seats in MBBS course from 100 to 150 students. • MCI letter No:MCI 34(41)(UG)/ 2019-Med/157716,Dated22.10.19 – Continuation of recognition of MBBS degree with 150 admissions. • Lr. No. MCI 37(1)(UG)(I-26)/2019-Med/77. Dated. 24.09.2020 - LOP issued by BOG in supersession of MCI towards increase of seats in MBBS course from 150 to 250. • Lr. No. NMC/UGR/2020/000298/021304. Dated. 22.09.2021–Renewal of Permission (1st Renewal) for MBBS course for 2nd batch– against the increase intake of seats from 150 to 250 seats.
13.	Status of Recognition	The MBBS course with 150 seats is recognized as on 22.10.2019 and valid for 5 years. The Institute has obtained permission to increase MBBS admission from 150 to 250 seats in 2020. The institute offers Post Graduate programs in 24 disciplines covering Broad and Super Specialities. All of them are recognized.
14.	Number of MBBS and PG broad specialty and super specialty students admitted in this session*	MBBS: 250 MD/MS: 19 DM/MCh: 4
15.	Inpatients registered and admitted (01.01.2021-31.12.2021)**	2021 - 96781 2020 - 70908 2019 - 81963
16.	Outpatients registered (01.01.2021-31.12.2021)**	2021 - 1047920 2020- 986320 2019- 878085

17.	Number of Deaths reported to the Municipality/ village register	2021 - 1019 2020 - 825 2019– 657
18.	Address and pin code of the Corporation/village where the Death records are reported	Birth and death Registrar, 56th ward, Chandragandhinagar, Sowripalayam, Coimbatore -641028
19.	Website link/ email ID/ hyperlink of the corporation in case Death Records are reported	https://www.crstn.org/birth_death_tn/
20.	Number of Births reported	2021 - 2741 2020 - 2981 2019 – 3685
21.	Address and pin code of the Corporation/village where the Birth records are reported	Birth and death Registrar, 56th ward, Chandragandhinagar, Sowripalayam, Coimbatore -641028
22.	Website link/ email ID/ hyperlink of the corporation in case Birth Records are reported	https://www.crstn.org/birth_death_tn/
23.	Number of Rooms in Men's Hostel and students accommodated	475 Rooms, Occupied by 389 students.
24.	Total Number of Rooms in Women's Hostel and students accommodated	475 Rooms, Occupied by 415 students.
25.	Name of the Grievance Redressal Officer (PIO & CPIO):	PIO – Dr K Bhuvaneswari. Professor & Head Department of Pharmacology, PSGIMSR, Coimbatore.
26.	Address with Pin code	Post Box No.1674, Peelamedu, Coimbatore – 641 004. Phone 0422 – 4345802, 2598822 E.mail: psgmedschool@gmail.com Web: www.psghospitals.com
27.	Telephone Number Email Id	Phone 0422 – 4345802, 2598822 E.mail: psgmedschool@gmail.com
28.	Grievances reported	2021 - 2020 - 2019 -

29. Details of Post- Graduation Courses offered

Post- Graduate Course	Year of Commencement of the Course	Number of Students Currently pursuing the Course			Number of Students admitted in the current session
		2019	2020	Total	2021
MD- ANAESTHESIOLOGY	01.04.2011	4	4	8	ADMISSION PROCESS NOT YET STARTED
MD- BIOCHEMISTRY	31.03.2009	1	1	2	
MD-DERMATOLOGY	16.12.2002	5	5	10	
MD- MICROBIOLOGY	21.08.2001	3	3	6	
MD-PATHOLOGY	21.06.2001	4	4	8	
MD-TUBERCLOSIS & RESPIRATORY DISEASE	31.03.2011	2	2	4	
MS-OTO-RHINO-LARYNGOLOGY	23.03.2011	2	2	4	
MS - SURGERY	31.03.2009	4	4	8	
MD- GENERAL MEDICINE	21.06.2001	6	6	12	
MD- PAEDIATRICS	31.03.2009	6	6	12	
MD-PHARMACOLOGY	31.03.2009	2	1	3	
MD-PHYSIOLOGY	21.06.2001	0	0	0	
MD-PSYCHIATRY	31.03.2009	4	4	8	
MS-ORTHOPAEDICS	31.03.2009	2	2	4	
MD-ANATOMY	24.03.2011	0	0	0	
MD-RADIO DIAGNOSIS	31.03.2011	3	3	6	
MS -OPHTHALMOLOGY	31.03.2009	3	3	6	
MD-COMMUNITY MEDICINE	10.10.2001	2	1	3	
MS-OBSTETRICS & GYNAECOLOGY	21.08.2001	7	7	14	
D ORTHO	21.06.2001	0	2	2	
DM CARDIOLOGY	15.07.2009	1	1	2	
DM GASTROENTEROLOGY	10.07.2009	1	3	4	
DM NEUROLOGY	10.07.2009	0	0	0	
DM NEPHROLOGY	31.03.2009	1	0	1	

30. Department wise list of Faculty Members

PSG Institute of Medical Sciences & Research

DEPARTMENT OF ANATOMY

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1.	Dr. G. AMUDHA MBBS,MS-ANATOMY 54415	Professor & HOD 16.02.2015	Regular	RBC course: 02.08.2016 to 04.08.2016	2021 2020 2019 2018 2017	Professor	No. = 17 Topics covered: 1. General Anatomy I – Anatomical terminologies and Introduction to Systems of body 2. Mammary gland 3. Brachial plexus 4. Dissection of back 5. Shoulder Joint 6. Hand I 7. Blood supply of Lower Limb 8. Lungs 9. Blood supply of Heart & Cardiac plexus 10. Venous & lymphatic drainage of Thorax 11. Introduction to Histology of GIT, Esophagus & Stomach 12. Liver 13. Face 14. Cranial cavity with meninges and pituitary gland 15. Pharynx 16. Spinal cord with blood supply

Department of Anatomy

2.	Dr. M. JAMUNA MBBS, MS-ANATOMY 54783	Professor	Regular	RBC course:20th,21st and 22nd Sptember2016 Research Methodology – 2nd and 3rd November 2007	2021 2020 2019 2018 2017	Professor	No. = 18 Topics covered: 1. General anatomy, 2. Mammary gland, 3. Brachial plexus, 4. Shoulder joint, elbow, radioulnar, wrist joints, 5. Clinical anatomy charts of upper limb, 6. Front of thigh, 7. Hip joint, 8. Arches of foot, 9. Mediastinum and superior mediastinum, 10. Posterior abdominal wall and thoracolumbar fascia, 11. Histology of liver,gall bladder and pancreas, 12. Urinary bladder, 13. Deep cervical fascia, 14. Facial nerve, 15. Larynx, Sulci and gyri and functional areas of cerebrum, 16. White matter of cerebrum and basal ganglia

3.	Dr. A.K. MANICKA VASUKI MBBS, MD-ANATOMY 63015	Associate Professor 01.07.2019	Regular	RBC course:10th - 12th April 2017 Research Methodology / NPTEL course: Mar- June 2020	2021 2020 2019 2018 2017	Associate professor Associate professor Associate professor from 1st July Assistant professor Assistant professor	No. = 40 Topics covered: 1. Introduction of upper limb and pectoral region, 2. General Embryology I,II, III, IV, V, VI, VII, VIII, IX, 3. Hand II, 4. Front of thigh, 5. Sole of Foot, 6. Ankle Joint & Joints of foot, Pericardium, 7. Development of Heart I & II, 8. Introduction to Abdomen & Anterior Abdominal wall, 9. Scrotum & Testis, 10. Stomach with Coeliac trunk, 11. Development of GIT I & II, 12. Development of GUT I, II & III, 13. Parotid region, 14. Submandibular region, 15. Trigeminal nerve, 16. Development of face, palate and pharyngeal arches, 17. Fourth ventricle, 18. 3rd and lateral ventricles, 19. Development of brain and spinal cord.

4.	Dr. C. NANDHINI AISHWARYA MBBS, MD-ANATOMY 106910	Assistant Professor 12.03.2019	Regular	RBC course: Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	Assistant Professor, PSGIMS&R Assistant Professor, PSGIMS&R Assistant Professor, PSGIMS&R Senior Resident, PSGIMS&R -	No. = 24 Topics covered: 1. Axilla - boundaries and contents, axillary artery 2. Back of arm and structures under cover of deltoid 3. Front of arm 4. Histology of bone 5. Histology of blood vessels 6. Hand II 7. Anterolateral Compartment of leg and Dorsum of Foot 8. Nerves of Lower Limb 9. Ribs & Introduction to vertebral column 10. Heart 11. Genetics I 12. Revision Anterior Abdominal wall, Scrotum & Testis 13. Genetics II 14. Genetics III 15. Genetics IV 16. Introduction to perineum, perineal pouches and ischiorectal fossa 17. Midline structure of neck and thyroid gland 18. Suboccipital region

							19. Eye ball and lacrimal apparatus 20. Medulla I & II 21. Cranial nerve 3, 4 and 6
5.	Dr. R. PRAVEENA MBBS, MD-ANATOMY 91993	Assistant Professor	Regular	RBC course: Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	Assistant Professor, PSGIMS&R Assistant Professor, PSGIMS&R Senior Resident, JIPMER Senior Resident, JIPMER --------	No. = 18 Topics covered: 1. Adductor compartment of thigh 2. Back of thigh& Popliteal fossa 3. Osteology of Tibia, Fibula 4. Sternum & Thoracic vertebrae 5. Histology of Lungs & Trachea 6. Other chambers of heart 7. Pleura & Lungs 8. Rectus sheath & Inguinal canal 9. Anterior Abdominal wall, Scrotum & Testis 10. Histology of Small intestine, Large intestine & Appendix 11. Portal system with development 12. Prostate and Urethra 13. Triangles of Neck 14. Orbit - boundaries and contents 15. Ear with 8th cranial nerve 16. Blood supply of brain
6.	Dr. S. SUGANYA MBBS, MD-ANATOMY 84171	Assistant Professor 19.05.2020	Regular	RBC course: 12th-14th April 2021 Research Methodology / NPTEL course:	2021 2020	Assistant professor in PSGIMSR Assistant professor in PSGIMSR (from 19th May 2020)	No. = 22 Topics covered: 1. General Anatomy-Skin, Superficial fascia and Deep fascia 2. Bones & Joints

				March-June 2021	2019	Assistant professor in KFMSR	3. Muscular System 4. Circulatory system 5. Nervous system 6. Axillary & musculocutaneous nerves 7. Front of forearm 8. Hand I 9. Radial, ulnar & median nerves 10. Introduction to lower limb 11. Knee joint 12. Radiology & clinical charts of lower limb 13. External features of heart & right atrium 14. Peritoneum 15. Large intestine, Caecum, Appendix, Interior mesenteric artery 16. Uterus, tubes and ovary 17. Introduction to head and neck and scalp 18. Temporal and Infratemporal region 19. Nose 20. Pons 21. Thalamus, hypothalamus and limbic system 22. Clinical charts and Radiology of head and neck and neuroanatomy
					2018	Assistant professor in KFMSR	
					2017	-----	

7.	Dr. R. RAJA RAJESHWARI MBBS, MD-ANATOMY 106393	Assistant Professor 11.05.2021	Regular	RBC course: Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	Assistant professor Senior resident till May Assistant professor after May Senior resident ----- -----	No. = 31 Topics covered: 1. General Histology I – Introduction to Histology, Cells & Tissues 2. General Histology II – Epithelium and Glands I 3. C1 - Histology of Connective tissue 4. Histology of cartilage 5. Histology of cartilage 6. Front of arm 7. Cubital fossa 8. Axillary & Musculo cutaneous nerves 9. Histology of muscle 10. Clinical anatomy, charts of upper limb 11. Histology of lymphatic system 12. Histology of Nervous tissue 13. Articulated foot 14. Histology of skin 15. Pleura 16. Mediastinum 17. Duodenum, small intestine with superior mesenteric artery 18. Pancreas & Extrahepatic biliary apparatus 19. Rectum and Anal canal 20. Pre and Para vertebral region

							21. Temporomandibular joint and muscles of mastication 22. Oral cavity, tongue and palate and tonsil 23. Mid brain 24. Optic nerve and pathway
8.	Mrs. K. RADHIKA M.Sc TNMC Registration Number: Not Applicable	Assistant Professor	Regular	RBC course: Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	Assistant professor	No. = 17 <u>Topics covered:</u> Demo Lectures 1. Thoracic vertebra, ribs and sternum 2. Histology of Bone and skin 3. Introduction to skull, norma frontalis and mandible 4. Histology of Blood vessels, lymphatic system 5. Norma verticalis and occipitalis 6. Histology of nervous tissue 7. Norma lateralis, cervical vertebra 8. Norma basalis and cranial fossa 9. Frontal, parietal and occipitalis and hyoid bone 10. Tibia, fibula and articulated foot 11. Histology of female reproductive system 12. Femur, patella 13. Histology of male reproductive system 14. Pelvis and hipbone

							15. Histology of kidney, ureter, urinary bladder and cartilage 16. Sacrum 17. Histology of small intestine, large intestine and appendix
9.	Mrs. DEBORAH JOY HT M.Sc TNMC Registration Number: Not Applicable	Assistant Professor	Regular	RBC course: Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	Assistant professor	No. = 13 <u>Topics covered:</u> 1. Axilla - boundaries and contents, axillary artery, 2. Back of arm and structures under cover of deltoid, 3. Cubital fossa, 4. Back of forearm and Dorsum of hand, 5. Elbow, wrist and radioulnar joints, 6. Radial, ulnar and median nerves, 7. Introduction to lower limb, 8. Adductor compartment of thigh, 9. Introduction to thorax and Intercostal spaces, 10. Posterior mediastinum, 11. Intercostals spaces & Mechanism of Respiration, 12. Lumbar vertebrae, Sacrum & Pelvis.

PSG Institute of Medical Sciences & Research

DEPARTMENT OF **PHYSIOLOGY**

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification & TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr K Deepalakshmi MD (Physiology) 76503	Professor & HOD 01.08.2020	Regular/ Permanent	RBC course: **27th -29th August 2018** Research Methodology / NPTEL course: **March – June 2020**	2021	PSGIMS&R, Physiology, Professor & HOD	No. = **21** **Theory Topics covered:** 1. Transport across cell membrane I 2. Blood coagulation II 3. Skeletal muscle I 4. Properties of cardiac muscle I 5. ECG II 6. Circulation 7. Introduction to RS 8. CO2 Transport 9. Introduction to GIT 10. Gastric circulation 11. Intestine 12. Tubular function 13. Pituitary hormone I 14. Spermatogenesis & Testosterone 15. Pregnancy I 16. Eye IV
					2020	PSGIMS&R, Physiology, Professor	
					2019	PSGIMS&R, Physiology, Associate Professor	
					2018	PSGIMS&R, Physiology, Associate Professor	
					2017	PSGIMS&R, Physiology,	

						Associate Professor	17. Synapse 18. Extrapyramidal tract 19. Vestibular apparatus and maintenance of equilibrium 20. Hypothalamus 21. CSF & BBB **Practical :** **No of sessions : 30** **No of hours : 77** 1. Microscope & Neaubauer chamber 2. Estimation of Total RBC Count 3. Estimation of Total WBC Count 4. Absolute Eosinophil Count 5. Differential Count 6. Blood Indices 7. Blood Grouping & Typing 8. Bleeding Time & Clotting Time 9. Packed Cell Volume Erythrocyte Sedimentation Rate & Hemoglobin Estimation 10. Examination Of RS 11. Respiratory Efficiency Tests 12. Spirometry , Stethograph & Vitalograph

							13. Examination Of Pulse & BP 14. Examination Of CVS 15. Examination Of Sensory System & Cerebellar Function Test 16. Examination Of Motor System & Examination Of Reflexes 17. Examination Of 1-12 Cranial Nerves 18. All Practical Revision
2	Dr. R Nagashree MD (Physiology) 50532	Professor 01.07.2007	Regular/ permanent	RBC course: **20th -22nd September 2016** Research Methodology / NPTEL course: **NA**	2021	PSGIMS&R, Physiology, Professor	No. = **20** **Theory Topics covered:** 1. Introduction 2. RBC I 3. WBC 4. Properties of nerve fiber 5. Cardiac cycle 6. BP I 7. Mechanism of respiration 8. Coronary circulation 9. Regulation of Respiration II 10. Exocrine pancreas 11. Introduction to excretion 12. Acid base balance I
					2020	PSGIMS&R, Physiology, Professor	
					2019	PSGIMS&R, Physiology, Professor	
					2018	PSGIMS&R, Physiology, Professor	

					2017	PSGIMS&R, Physiology, Professor	22. Pituitary hormone II 13. Pancreatic hormone I 14. Female reproductive system 15. Contraception 16. Ear I 17. Receptor 18. Muscle spindle 19. Cerebral cortex 20. EEG & RAS **Practical :** **No of sessions : 30** **No of hours : 77** 1. Microscope & Neaubauer chamber 2. Estimation of Total RBC Count 3. Estimation of Total WBC Count 4. Absolute Eosinophil Count 5. Differential Count 6. Blood Indices 7. Blood Grouping & Typing 8. Bleeding Time & Clotting Time 9. Packed Cell Volume Erythrocyte Sedimentation Rate & Hemoglobin Estimation 10. Examination Of RS

							11. Respiratory Efficiency Tests 12. Spirometry , Stethograph & Vitalograph 13. Examination Of Pulse & BP 14. Examination Of CVS 15. Examination Of Sensory System & Cerebellar Function Test 16. Examination Of Motor System & Examination Of Reflexes 17. Examination Of 1-12 Cranial Nerves 18. All Practical Revision
3	Dr G V Lathadevi MD (Physiology) 45596	Professor 01.12.2012	Regular/ permanent	RBC course: **10th – 12th April 2017** Research Methodology / NPTEL course: **NA**	2021	PSGIMS&R, Physiology, Professor	No. = **20** **Theory Topics covered:** 1. Body fluid compartments 2. Immunity I 3. NMJ 4. Cardiac muscle 5. BP II 6. Pulmonary circulation 7. O2 Transport 8. Pulmonary function tests 9. Liver 10. Digestion and absorption of CO2 11. Acid base balance II 12. Thyroid Hormone I
					2020	PSGIMS&R, Physiology, Professor	
					2019	PSGIMS&R, Physiology, Professor	
					2018	PSGIMS&R, Physiology, Professor	

					2017	PSGIMS&R, Physiology, Professor	13. Pancreatic hormone II 14. Ovarian hormones 15. Eye I 16. Ear II 17. Ascending tracts & sensory cortex 18. Reflexes 19. Cerebellum 20. Sleep **Practical :** **No of sessions : 30** **No of hours : 77** 1. Microscope & Neaubauer chamber 2. Estimation of Total RBC Count 3. Estimation of Total WBC Count 4. Absolute Eosinophil Count 5. Differential Count 6. Blood Indices 7. Blood Grouping & Typing 8. Bleeding Time & Clotting Time 9. Packed Cell Volume Erythrocyte Sedimentation Rate & Hemoglobin Estimation 10. Examination Of RS 11. Respiratory Efficiency Tests

							12. Spirometry , Stethograph & Vitalograph 13. Examination Of Pulse & BP 14. Examination Of CVS 15. Examination Of Sensory System & Cerebellar Function Test 16. Examination Of Motor System & Examination Of Reflexes 17. Examination Of 1-12 Cranial Nerves 18. All Practical Revision
4	Dr V Kannan MD (Physiology) 50576	Associate Professor 01.12.11	Regular/ permanent	RBC course: **12th – 14th December 2017** Research Methodology / NPTEL course: **NA**	2021	PSGIMS&R, Physiology, Associate Professor	No. = **5** **Theory Topics covered:** 1. Transport across cell membrane II 2. Blood Groups 3. Lymph 4. Skeletal muscle III 5. Properties of cardiac muscle II **Practical :** **No of sessions : 30** **No of hours : 77** 1. Microscope & Neaubauer
					2020	PSGIMS&R, Physiology, Associate Professor	
					2019	PSGIMS&R, Physiology, Associate Professor	
					2018	PSGIMS&R, Physiology,	

						Associate Professor	chamber 2. Estimation of Total RBC Count 3. Estimation of Total WBC Count 4. Absolute Eosinophil Count 5. Differential Count 6. Blood Indices 7. Blood Grouping & Typing 8. Bleeding Time & Clotting Time 9. Packed Cell Volume Erythrocyte Sedimentation Rate & Hemoglobin Estimation 10. Examination Of RS 11. Respiratory Efficiency Tests 12. Spirometry , Stethograph & Vitalograph 13. Examination Of Pulse & BP 14. Examination Of CVS 15. Examination Of Sensory System & Cerebellar Function Test 16. Examination Of Motor System & Examination Of Reflexes 17. Examination Of 1-12 Cranial Nerves 18. All Practical Revision
					2017	PSGIMS&R, Physiology, Associate Professor	

5	Dr N Shuba MD (Physiology) 56724	Professor 01.12.2021	Regular/ permanent	RBC course: **12th – 14th December 2017** Research Methodology / NPTEL course: **Mar- June 2021**	2021-Dec	PSGIMS&R, Physiology, Professor	No. = **21** **Theory Topics covered:** 1. Second messengers 2. Introduction to Nerve 3. Skeletal muscle I 4. ECG I 5. Cardiac failure 6. Splanchnic & fetal circulation 7. Gas Exchange 8. High attitude & deep sea physiology 9. LFT 10. Digestion and absorption of fats 11. Mechanism of urine dilution 12. Urinary Bladder 13. Thyroid hormone II 14. Parathyroid hormones 15. Menstrual cycle 16. Eye II 17. Eye IV 18. Pain 19. Posture regulating mechanism 20. Speech and cerebral dominance 21. Thalamus
					2021-Jan - Nov	PSGIMS&R, Physiology, Associate Professor	
					2020	PSGIMS&R, Physiology, Associate Professor	
					2019	PSGIMS&R, Physiology, Associate Professor	
					2018	PSGIMS&R, Physiology, Associate Professor	
					2017	PSGIMS&R, Physiology, Associate	

						Professor	**Practical :** **No of sessions : 30** **No of hours : 77** 1. Microscope & Neaubauer chamber 2. Estimation of Total RBC Count 3. Estimation of Total WBC Count 4. Absolute Eosinophil Count 5. Differential Count 6. Blood Indices 7. Blood Grouping & Typing 8. Bleeding Time & Clotting Time 9. Packed Cell Volume Erythrocyte Sedimentation Rate & Hemoglobin Estimation 10. Examination Of RS 11. Respiratory Efficiency Tests 12. Spirometry , Stethograph & Vitalograph 13. Examination Of Pulse & BP 14. Examination Of CVS 15. Examination Of Sensory System & Cerebellar Function Test 16. Examination Of Motor System & Examination Of Reflexes 17. Examination Of 1-12 Cranial

							Nerves 18. All Practical Revision
6	Dr S Vijayabaskaran MD (Physiology) 54566	Assistant Professor 1.8.2016	Regular/ permanent	RBC course: **27th -29th August 2018** Research Methodology / NPTEL course: **March – June 2020**	2021	PSGIMS&R, Physiology, Assistant Professor	No. = **18** **Theory Topics covered:** 1. Cell Physiology 2. Blood coagulation I 3. RMP 4. Skeletal muscle IV 5. Cardiac output II 6. Shock 7. Cerebral Circulation 8. Cutaneous circulation 9. Hypoxia & artificial respiration 10. GI Hormones 11. GFR 12. Maintenance of ECF volume 13. Skin and temperature regulation 14. Adrenal medulla 15. Introduction to Reproduction 16. Parturition & Lactation 17. Eye III 18. Smell & taste **Practical :** **No of sessions : 30**
					2020	PSGIMS&R, Physiology, Assistant Professor	
					2019	PSGIMS&R, Physiology, Assistant Professor	
					2018	PSGIMS&R, Physiology, Assistant Professor	
					2017	PSGIMS&R, Physiology, Assistant Professor	

							No of hours : 77 1. Microscope & Neaubauer chamber 2. Estimation of Total RBC Count 3. Estimation of Total WBC Count 4. Absolute Eosinophil Count 5. Differential Count 6. Blood Indices 7. Blood Grouping & Typing 8. Bleeding Time & Clotting Time 9. Packed Cell Volume Erythrocyte Sedimentation Rate & Hemoglobin Estimation 10. Examination Of RS 11. Respiratory Efficiency Tests 12. Spirometry , Stethograph & Vitalograph 13. Examination Of Pulse & BP 14. Examination Of CVS 15. Examination Of Sensory System & Cerebellar Function Test 16. Examination Of Motor System & Examination Of Reflexes 17. Examination Of 1-12 Cranial Nerves 18. All Practical Revision

7	Dr R Pavitra Vyshnavi Yogisparan MD (Physiology) 96323	Assistant Professor 22.10.2021	Regular/ permanent	RBC course: **17th – 19th August 2021** Research Methodology / NPTEL course: **March – June 2020**	Oct 2021	PSGIMS&R, Physiology, Assistant Professor	No. = **2** **Theory Topics covered:** 1. Limbic system 2. ANS **Practical :** **No of sessions : 5** **No of hours :13** 1. Examination Of Sensory System & Cerebellar Function Test 2. Examination Of Motor System & Examination Of Reflexes 3. All Practical Revision
					Jan - Oct 2021	KMCH, Physiology, Assistant Professor	
					Dec-2020	KMCH, Physiology, Assistant Professor	
					2020	KFMSR Physiology, Assistant Professor	
					July 2019	KFMSR Physiology, Assistant Professor	
					Jan – May 2018	PSGIMS&R, Physiology, Tutor Cum PG	

					2017	PSGIMS&R, Physiology, Tutor Cum PG	
8	Dr S Vidhya MD (Physiology) 87211	Tutor 02.09.21	Regular/ permanent	RBC course: Research Methodology / NPTEL course: **Mar- June 2021**	2021	PSGIMS&R, Physiology, Tutor	No. = **6** **Theory Topics covered:** 1. Ear I 2. Introduction to CNS 3. Descending tracts and pyramidal tract 4. Applied aspect of spinal cord 5. Basal ganglia 6. Learning & memory **Practical :** **No of sessions : 17** **No of hours : 44** 1. Packed Cell Volume Erythrocyte Sedimentation Rate & Hemoglobin Estimation 2. Examination Of RS 3. Respiratory Efficiency Tests 4. Spirometry , Stethograph & Vitalograph 5. Examination Of Pulse & BP
					2020	PSGIMS&R, Physiology, Tutor Cum PG	
					2019	PSGIMS&R, Physiology, Tutor Cum PG	
					2018	PSGIMS&R, Physiology, Tutor Cum PG	
					2017		

							6. Examination Of CVS 7. Examination Of Sensory System & Cerebellar Function Test 8. Examination Of Motor System & Examination Of Reflexes 9. Examination Of 1-12 Cranial Nerves 10. All Practical Revision

PSG Institute of Medical Sciences & Research

DEPARTMENT OF BIOCHEMISTRY

SERVICE PARTICULARS

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr.G.Jeyachandran MBBS,MD Biochemistry 52723	Professor & HOD 09.12.2013	Regular/Permanent	RBC course:10th to 12th April 2017 Research Methodology / NPTEL course: 26.09.2021	2021	PSGIMSR, Biochemistry, Prof & HOD	No. = 26 Topics covered:1.Classification And Isomerism. 2. Monosaccharides Properties And Functions 3. Sphingolipids, Cholesterol 4. Lipid Storage Disorders 5. PDH 6. Cholesterol Synthesis (2) 7.Lipoprotein Disorders,Lipid Profile 8.Eicosanoid Metabolism (2) 9. Fatty Liver 10.Primary,Secondary, Tertiary Quarternary Structure Of Insulin 11.Regulation Of Blood Glucose 12.Tyrosine 13.Activation Energy, Mechanism Of Action 14. Electrolytes (3) 15.Cancer Biology(2) 16.Peptides 17.Aids 18.Adrenal Function Tests + Peptides 19.Lactic acidosis 20. Estimation Of Cholesterol / Hdl 21.Estimation Of Uric Acid
					2020	PSGIMSR, Biochemistry, Prof & HOD	
					2019	PSGIMSR, Biochemistry, Prof & HOD	
					2018	PSGIMSR, Biochemistry, Prof & HOD	
					2017	PSGIMSR, Biochemistry, Prof &HOD	

2	Dr.B.Gayathri MBBS,MD Biochemistry 73005	Professor 01.05.2015	Regular/ Permanent	RBC course:22nd May 2020 Research Methodology / NPTEL course: Nil	2021	PSGIMSR, Biochemistry, Prof	No. =21 Topics covered: 1. Phospholipids 2. Hemoglobinopathies, Hbs 3. Glycolysis(2) 4. Obesity,Metabolic Syndrome, Atherosclerosis 5. Metabolism Of Adipose Tissue 6. Urea Cycle 7. Nitric Oxide 8. Structure, Synthesis , Porphyrias 9. Metabolic Acidosis 10. Purine And Pyrimidine Chemistry 11. Nucleic Acid Structure (2) 12. Translation (3) 13. Vit- D 14. Iron 15. Metabolism In Fed State 16. Metabolism Of Fasting State 17. Estimation Of Triglyceride
					2020	PSGIMSR, Biochemistry, Prof	
					2019	PSGIMSR, Biochemistry, Prof	
					2018	PSGIMSR, Biochemistry, Prof	
					2017	PSGIMSR, Biochemistry, Prof	
3	Dr.G.Sumithra MBBS,MD Biochemistry 76949	Associate Professor 01.06.2016	Regular/ Permanent	RBC course:14th Nov 2019 Research Methodology / NPTEL course:	2021	PSGIMSR, Biochemistry, Associate Professor	No. = 24 Topics covered: 1. Homopolysaccharides 2. Secondary Structure 3. Tertiary Structure
					2020	PSGIMSR, Biochemistry,	

				18.Dec.2020		Associate Professor	&Quarternery structure of proteins 4. Hemoglobin Structure 5. Fructose, Galactose Metabolism 6. Glycogen Metabolism (3) 7. Fatty Acid Synthesis (2) 8. Breakdown And Jaundice 9. Factors Affectingenzyme Activity 10. Pyrimidine Metabolism And Disorders (2) 11. Mutation 12. Molecular Biology Techniques (4) 13. B1 14. Miscellneous 15. CSF 16. Practical- Color Reactions Of Proteins 17. Estimation Of Plasma Glucose
					2019	PSGIMSR, Biochemistry, Associate Professor	
					2018	PSGIMSR, Biochemistry, Associate Professor	
					2017	PSGIMSR, Biochemistry, Associate Professor	
4	Dr.A.S.Meenakshisundram MBBS,MD Biochemistry 83221	Assistant Professor 13.05.2013	Regular/ Permanent	RBC course:31thMarch, 1nd& 2ndApril 2015 Research Methodology / NPTEL course: 18.12.2020	2021	PSGIMSR, Biochemistry, Assistant Professor	No. = 22 Topics covered: 1. Disaccharides Properties And Functions 2. Aminoacid Structure (2) 3. ETC (4) 4. Types And Classification 5. Hypoglycemia 6. OGTT 7. Acute & Chronic Complications
					2020	PSGIMSR, Biochemistry, Assistant Professor	
					2019	PSGIMSR, Biochemistry, Assistant Professor	

					2018	PSGIMSR, Biochemistry, Assistant Professor	(2) 8. Hyperammonemia 9. Catecholamine, Pheochromocytoma 10. Effects Of [S] On Velocity, Lineweaver Burk Plot 11. Regualtion Of Gene Expression (2) 12. Vit B6 13. Vit B1 14. Precipitation Reactions Of Proteins 15. Colorimetry &Sphrectrophotometry 16. Normal Constituents Of Urine
					2017	PSGIMSR, Biochemistry, Assistant Professor	
5	Dr.R.Sujatha MD(Biochemistry) 89225	Associate Professor 01.12.2021	Regular/Permanent	RBC course:20th to 22nd sep 2016 Research Methodology / NPTEL course: 18.Dec.2020	2021	PSGIMSR, Biochemistry, Assistant Professor	No. = 17 Topics covered: 1. GAG, Lectin,Sialic Acid, Proteoglycans, Glycoproteins (2) 2. TCA cycle (2) 3. Digestion And Absorption 4. Transamination & Deamination 5. Protein Degradation 6. Glycine (2) 7. Introduction And Buffers 8. Replication (3) 9. Targeting 10. Tumor Markers & Anti Cancer Drugs (2) 11. Copper
					2020	PSGIMSR, Biochemistry, Assistant Professor	
					2019	PSGIMSR, Biochemistry, Assistant Professor	
					2018	PSGIMSR, Biochemistry, Assistant Professor	
					2017	PSGIMSR, Biochemistry,	

						Assistant Professor	12. Estimation Of Serum Urea 13. Abnormal Constituents Of Urine
6	Dr.D.Vijaya Ph D (Biochemistry)	Assistant Professor 30.11.2019	permanent	RBC course:Nil Research Methodology / NPTEL course:Nil	2021	PSGIMSR, BiochemistryAssistant Professor	No. = 20 Topics covered: 1. Hb Gene, Thalessemia 2. Glut, SGLT 3. Hmp Shunt (2) 4. Lipoprotein Chemistry 5. Lipoprotein Metabolism (2) 6. Digestion And Absorption 7. Phenylalanine 8. Tryptophan Metabolism 9. Inhibitors 10. Regulation Of Enzyme Activity (2) 11. Vit A 12. Biotin 13. Electrophoresis 14. Alcohol Metabolism 15. TFT 16. Carbohydrate Reactions 17. Estimation Of Ca/P
					2020	PSGIMSR, BiochemistryAssistant Professor	
					2019	PSGIMSR, Biochemistry, Assistant Professor	
					2018	PSGIMSR, Biochemistry, Tutor	
					2017	PSGIMSR, ProfessorBiochemistry Quality Manager in Lab	
7.	Dr.B.Dhanalakshmi MD(Biochemistry) 96937	Assistant Professor 09.06.2021	permanent	RBC course:Nil Research Methodology / NPTEL course: 23.10.21	2021	PSGIMSR, Biochemistry, Assistant Professor	No. = 14 Topics covered: 1. Cysteine (2) 2. Carcinoid Syndrome 3. RFT (2) 4. Purine Metabolism And
					2020	KMCHIHSR Biochemistry, Assistant Professor	

					2019	KMCHIHSR Biochemistry, Assistant Professor	Disorders (4) 5. Pantothenic Acid 6. Nutrition (5) 7. Chromatography (2) 8. Radioactivity 9. Basic Principles Of Lab Equipments (3) 10. Enzyme Estimation
					2018	KMCHIHSR Biochemistry, Assistant Professor	
					2017	PSGIMSR, Biochemistry,Tutor	
8.	Dr.D.Saranya MD(Biochemistry) 109841	Assistant Professor 15.10.2020	permanent	RBC course:Nil Research Methodology / NPTEL course:22.AUG.2021	2021	PSGIMSR, Biochemistry, Assistant Professor	No. = 18 Topics covered: 1. Classification And Functions, Fatty Acids 2. Digestion And Absorption 3. Gluconeogenesis (2) 4. Definition, Classification 5. Metabolic Alkalosis 6. Transcription (3) 7. K 8. Folate 9. Free Radicals & Antioxidants (2) 10. Immunoglobulins (3) 11. Lactic acidosis 12. Precipitation Reactions Of Proteins 13. Estimation Of Creatinine 14. Dna Extraction
					2020	PSGIMSR, Biochemistry, Assistant Professor	
					2019	PSGIMSR, Biochemistry, Tutor	
					2018	PG- VSS Medical College, Orrisa	
					2017	PG- VSS Medical College, Orrisa	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF PATHOLOGY

SERVICE PARTICULARS

S No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	**DR.S.SHANTHAKUMARI** **MD(PATHOLOGY)** **TNMC REG. NO: 47529**	Professor and Head Promoted on 17.4.11 as professor As HOD Pathology 11.8.2021	REGULAR/ PERMANENT	RBC course: 10th-13th March 2010 SRMC-RC, Porur Chennai 22-25, Sept. 2015 – (RBCW AETCOM – CMC Vellore NC) Co-ordinator – Orientation Programme 1st July 2014 CMC Vellore. Research Methodology / NPTEL course: N.A. 20th Aug- 22nd Sept. 2007(ICMR NIH Course)	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	No. = 24 (12 lectures x 2 batches = 24) **Topics covered**: Introduction and cell injury, Platelet and bleeding disorders CVS, Renal diseases
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY – PROFESSOR	

2	**DR. PRASANNA N KUMAR** **MD(PATHOLOGY)** **TNMC REG. NO: 36511**	PROFESOR OF PATHOLOGY 03.05 1995	REGULAR/ PERMANENT	RBC course: 12th to 14th Dec.2017 Research Methodology / NPTEL course: N.A.	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	No. = 10 LECTURES X 2 BATCHES - 20 4 PRACTICALS/BATCH - 8 Topics covered: HAEMATOLOGY – RBC DISORDERS
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY – PROFESSOR	
3	**DR T M SUBBARAO** **MD(PATHOLOGY)** **TNMC REG. NO: 52354**	PROFESSOR & PRINCIPAL	REGULAR/ PERMANENT	RBC course: 10th -12th Dec.2017 ; BCW : 14th to 16th June 2012 Research Methodology / NPTEL course: N.A.	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	No. = 5 Topics covered: Cell injury & adaptation
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY – PROFESSOR	
4	**DR G UMAMAHESWARI** **MD(PATHOLOGY)** **TNMC REG. NO: 68485**	PROFESSOR 01.01.2020	REGULAR/ PERMANENT	RBC course: 12th to 14th Apr 2021 Research Methodology / NPTEL course: Aug-Dec 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	No. 30 Topics covered : Haemodynamicdiseases Female genital tract Breast Central Nervous System & eye for 2 batches
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY – ASSOCIATE PROFESSOR	
					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY - ASSOCIATE PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY – ASSOCIATE PROFESSOR	

5	**DR S VIDHYALAKSHMI** **MD(PATHOLOGY)** **TNMC REG. NO: 64978**	PROFESSOR 01.12.2021	REGULAR/ PERMANENT	RBC course: 12-14 December 2017 Research Methodology – December 2020 NPTEL course: Mar - June 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY –ASSOCIATE PROFESSOR till 30.11.2021 PROFESSOR FROM 01.12.2021	No. 26 Topics covered : Neoplasia, Liver, Endocrine system
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY –ASSOCIATE PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSOCIATE PROFESSOR	
					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSOCIATE PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY – ASSOCIATE PROFESSOR	
6	**DR S SAKTHISANKARI MD** **MD(PATHOLOGY)** **TNMC REG. NO: 90358**	ASSOCIATE PROFESSOR 01.12.2021	REGULAR/ PERMANENT	RBC course: 10-12th April 2017 Research Methodology / NPTEL course March-June 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY –ASSISTANT PROFESSOR till 30.11.2021 ASSOCIATE PROFESSOR FROM 01.12.2021	No. 37 Topics covered : Tissue repair Tuberculosis, Breast Lower urinary tract Male Genital tract Lymph nodes, Spleen Inflammation
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	

					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY – ASSISTANT PROFESSOR	
7	**DR ELIZ THOMAS MD** **MD(PATHOLOGY)** **TNMC REG. NO: 152882**	ASSISTANT PROFESSOR	REGULAR/ PERMANENT	RBC course: 12-14th April 2020 Research Methodology / NPTEL course March-June 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	No. 21 Topics covered : Amyloidosis, Inflammation, Healing and repair, Infectious diseases, Environmental and Nutritional Disorders, WBC Disorders
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY – ASSISTANT PROFESSOR	

8	**DR NIDHYA G MD** **MD(PATHOLOGY)** **TNMC REG. NO: 82654**	ASSISTANT PROFESSOR	REGULAR/ PERMANENT	RBC course: 27TH TO 30TH August 2018 Research Methodology / NPTEL course March-June 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY – ASSISTANT PROFESSOR	No. 15 Topics covered : White blood cell disorders (4) Blood Banking and Transfusion (2) Infectious disease (2) Transfusion Medicine (2) Liver, Gall bladder & Pancreas
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY – ASSISTANT PROFESSOR	
					2018	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2017	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH – PATHOLOGY – ASSISTANT PROFESSOR	
9	**DR G LAWANYA** **MD(PATHOLOGY)** **TNMC REG. NO:80624**	ASSISTANT PROFESSOR	REGULAR/ PERMANENT	RBC course: Research Methodology / NPTEL course March-June 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	No. 13 Topics covered : Head and Neck, Bones&Joints, Skin, GIT
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR	

10	**DR K R PRIYADARSHINI MD** **MD(PATHOLOGY)** **TNMC REG. NO: 99344**	ASSISTANT PROFESSOR 28.07.2020	REGULAR/ PERMANENT	RBC course: Research Methodology / NPTEL course : March-Jun 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY –ASSISTANT PROFESSOR	No. 13 Topics covered : 1. Disease of immune system 2. Head and Neck 3. Gastrointestinal system 4. Haemorrhagic disorders 5. Plasma cell disorders
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY TUTOR - Till27.07.2020 ASSISTANT PROFESSOR (From 28.07.2020))	
					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH - PATHOLOGY –TUTOR (From 15.07.2019)	
11	**DR PREETHI M S MD** **MD(PATHOLOGY)** **TNMC REG. NO: 104238**	ASSISTANT PROFESSOR 01.03.2021	REGULAR/ PERMANENT	RBC course: Research Methodology / NPTEL course Sep-Dec 2019	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH- PATHOLOGY - ASSISTANT PROFESSOR(From 01.03.21)	No. 16 Topics covered : Inflammation, Healing & Repair, Genetics, Diseases of infancy and childhood, Environment and Nutritional disorders, Skin, Lymph node, Spleen and Thymus
					2019	PSG INSTITUTE OF MEDICAL SCIENCES &RESEARCH - PATHOLOGY - TUTOR (Tutor : 09.10.2019 to 28.02.2021)	

12	**DR A ABINAYA SUNDARI MD** **MD(PATHOLOGY)** **TNMC REG. NO: 97430**	ASSISTANT PROFESSOR	REGULAR/ PERMANENT	RBC course: Research Methodology / NPTEL course Mar-Jun 2020	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH PATHOLOGY - ASSISTANT PROFESSOR KMCHIHSR, COIMBATORE - ASSISTANT PROFESSOR (Till 8th April 21)	No. 16 Topics covered : Respiratory system, Heart Blood Vessels , Haemodynamics
					2020	KMCHIHSR, COIMBATORE - ASSISTANT PROFESSOR	
					2019	KMCHIHSR, COIMBATORE - ASSISTANT PROFESSOR	
					2018	KMCHIHSR, COIMBATORE - ASSISTANT PROFESSOR (From July 2018)	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF MICROBIOLOGY

SERVICE PARTICULARS

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr. B. Appalaraju MD, Microbiology TNMC Reg. No - 153464	Professor & Head 08.04.1997	Regular	RBC course: 27.08.2018 to 30.08.2018 Research Methodology / NPTEL course: Not applicable.	2021	PSGIMS&R , Microbiology, Professor & Head	No. of Theory = 34 Topics covered: General bacteriology, Immunology, Virology, Mycology, Parasitology, Applied Microbiology, Infection Control.
					2020	PSGIMS&R , Microbiology, Professor & Head	
					2019	PSGIMS&R , Microbiology, Professor & Head	
					2018	PSGIMS&R , Microbiology, Professor & Head	
					2017	PSGIMS&R , Microbiology, Professor & Head	
2	Dr. S. Parvathi MBBS, DNB, Microbiology TNMC Reg. No - 38417	Professor 01.09.2009	Regular	RBC course: 20.09.2016 to 22.09.2016 Research Methodology / NPTEL course: Not applicable.	2021	PSGIMS&R , Microbiology, Professor	No. of Theory = 31 Topics covered: General bacteriology, Virology, Mycology, Parasitology.
					2020	PSGIMS&R , Microbiology, Professor	
					2019	PSGIMS&R , Microbiology, Professor	
					2018	PSGIMS&R , Microbiology, Professor	
					2017	PSGIMS&R , Microbiology, Professor	
3	Dr. R. Someshwaran	Associate	Regular	RBC course:	2021	PSGIMS&R , Microbiology, Associate Professor	No. of Theory = 38

	MD, Microbiology TNMC Reg. No - 89450	Professor 20.09.2018		25-07-2016 to 28-07-2016 Research Methodology / NPTEL course: March 2021 to June 2021	2020	KMCHIHSR, Microbiology, Associate Professor 01.01.2020 to 07.07.2020 PSGIMS&R, Microbiology, Associate Professor 09.07.2020 to 31.12.2020	Topics covered: General bacteriology, Immunology, Virology, Mycology, Parasitology, Applied Microbiology, Infection Control.
					2019	KFMS&R, Microbiology, Associate Professor 01.01.2019 to 10.06.2019 KMCHIHSR, Microbiology, Associate Professor 14.06.2019 to 31.12.2019	
					2018	KFMS&R, Microbiology, Assistant Professor 01.01.2018 to 19.09.2018 KFMS&R, Microbiology, Associate Professor 20.09.2018 to 31.12.2018	
					2017	KFMS&R, Microbiology, Assistant Professor	
4	Dr. S. Lavanya MD, Microbiology TNMC Reg. No - 140547	Assistant Professor 01.09.2015	Regular	RBC course: 12.12.2017 to 14.12.2017 Research Methodology / NPTEL course:	2021	PSGIMS&R , Microbiology, Assistant Professor	No. of Theory = 11 Topics covered: General bacteriology, Immunology.
					2020	PSGIMS&R , Microbiology, Assistant Professor	
					2019	PSGIMS&R , Microbiology, Assistant Professor	
					2018	PSGIMS&R , Microbiology, Assistant Professor	
					2017	PSGIMS&R , Microbiology, Assistant Professor	

				Nil.			
5	Dr. Ganesh Perumal P MD, Microbiology TNMC Reg. No - 81356	Assistant Professor 01.02.2018	Regular	RBC course: 12.04.2021 to 14.04.2021 Research Methodology / NPTEL course: March 2021 to June 2021	2021	PSGIMS&R, Microbiology, Assistant Professor.	No. = 28 Topics covered: General bacteriology, Immunology, Virology, Mycology, Parasitology, Applied Microbiology, Infection Control.
					2020	PSGIMS&R , Microbiology, Assistant Professor.	
					2019	PSGIMS&R , Microbiology, Assistant Professor, 14.10.2019 to 31.12.2019 PES Institute of Medical Sciences, Microbiology, Assistant Professor, 03.01.2019 to 12.10.2019	
					2018	Govt. Medical College, Palakkad, Microbiology, Assistant Professor.	
					2017	Govt. Medical College, Palakkad, Microbiology, Tutor.	
6	Dr. M. Mohamadiya Rizwana MD, Microbiology TNMC Reg. No - 84785	Assistant Professor 15.10.2020	Regular	RBC course: 12.04.2021 to 14.04.2021 Research Methodology / NPTEL course: Nil.	2021	PSGIMS&R, Microbiology, Assistant Professor.	No. = 28 Topics covered: General bacteriology, Immunology, Virology, Mycology, Parasitology, Applied Microbiology, Infection Control.
					2020	PSGIMS&R, Microbiology, Assistant Professor 15.10.2020 to 31.12.2020 PSGIMS&R, Microbiology, Tutor.	
					2019	PSGIMS&R, Microbiology, Tutor.	
					2018	-	
					2017	-	

7	Dr. S. Shanmugapriya MD, Microbiology TNMC Reg. No - 90458	Assistant Professor 11.05.2021	Regular	RBC course: Nil. Research Methodology / NPTEL course: Nil.	2021	PSGIMS&R, Microbiology, Assistant Professor 11.05.2021 to 15.12.2021 (Till date)	No. = 17 Topics covered: General bacteriology, Virology, Parasitology, Applied Microbiology, Infection Control.
					2020	PSGIMS&R, Microbiology, Assistant Professor	
					2019	-	
					2018	PSGIMS&R, Microbiology, Tutor	
					2017	PSGIMS&R, Microbiology, Tutor	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF PHARMACOLOGY

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr. K. Bhuvaneswari, MBBS, MD., PGDBE., TN MC Reg. No: 43193	Professor & HOD Date of promotion – 01/09/2004	Regular/ Permanent	RBC course:18th to 21st October 2016 Research Methodology – 28 to 30 November 2018- TNMGRUniversity NPTEL course: Mar – June 2021	2021	PSG IMSR, Pharmacology, Professor & HOD	No. = **13** Topics covered: **2019 Batch:** Absorption AETCOM Myocardial Infarction General Anesthetics Bronchial asthma & COPD AETCOM Antiarrhthymics **2018 Batch:** Antiarrhythmics Treatment of shock Treatment of MI **PG Lecture:**
					2020	PSG IMSR, Pharmacology, Professor& HOD	
					2019	PSG IMSR, Pharmacology, Professor& HOD	
					2018	PSG IMSR, Pharmacology, Professor & HOD	
					2017	PSG IMSR, Pharmacology, Professor & HOD	

							Cytokines in therapy Molecular drug delivery Drugs in pregnancy & lactation
2	Dr. S. Mohamed Musthafa, MBBS, MD., TN MC Reg. No: 37828	Associate Professor Date of Joining – 10/10/2019	Regular/ Permanent	RBC course: 12th to 14th April 2021 Research Methodology16th to 18thMarch 2021 TN MGR University NPTEL course: Mar – June 2021	2021	PSG IMSR, Pharmacology, Associate Professor	No. = **21** Topics covered: **2019 Batch:** Excretion Cholinergic drugs Skeletal Muscle relaxants Anti fungaldrugs Immunosupressants NSAIDS Antianginal drugs Anticoagulant drugs Antiepileptic drugs Alcohol Oestrogens& SERM Drugs affecting calcium balance Diuretic & antidiuretic Anti viraldrugs Anthelmintic drugs
					2020	PSG IMSR, Pharmacology, Associate Professor	
					2019	PSG IMSR, Pharmacology, Associate Professor	
					2018		
					2017		

							2018 Batch: Treatment of CHF I& II Treatment of urinary tract infections Antimalarial drugs **PG Lecture:** Therapeutic drug monitoring Paediatric Pharmacology
3	Dr. Ramanujam Narayanan, MBBS, MD (Pharmacology), TN MCI Reg. No. 82834; PhD, PG Diploma in Bioethics (PGDBE),	Associate Professor, Department of Pharmacology, Date of promotion – 01/07/2018	Regular/ Permanent	RBC course: 20th-22nd September 2016 Research Methodology / NPTEL course: Mar – June 2021	2021	PSG IMSR, Pharmacology, Associate Professor	No. = **28** Topics covered: **2019 Batch:** Pharmacodynamics I & II New drug development Adrenergic drugs Antiadrenergic drugs I Corticosteroids DMARD & Antigout Anticancer drugs I & II Betalactams II Quinolones Hematinics Drug abuse and deaddiction Anterior pituitary
					2020	PSG IMSR, Pharmacology, Associate Professor	
					2019	PSG IMSR, Pharmacology, Associate Professor	
					2018	PSG IMSR, Pharmacology, Assistant & Associate Professor	
					2017	PSG IMSR, Pharmacology, Assistant Professor	

							Androgens & anti androgens + drugs for erectile dysfunction Diabetes mellitus I & II Antiparkinson drugs Anti-ulcer drugs Anti malarial drugs Treatment of UTI and gram Negative infections Iron poisoning case discussion **2018 Batch:** Anti hypertensives Systemic Corticosteroids Antiviral drugs Anticancer drugs **PG Lecture:** Chronopharmacology Gene knockout models in drug development
4	Dr. S. Shanmuga Priya, MBBS, MD., TN MCI Reg. No 76725	Associate Professor, Department of Pharmacology, Date of	Regular/ Permanent	RBC course: 10th to 12thApril 2017 Research Methodology:	2021 2020	PSG IMSR, Pharmacology, Associate Professor PSG IMSR, Pharmacology, Associate Professor	No. =**21** Topics covered: **2019 Batch:** Distribution

		promotion – 01/07/2019		March 30-April 1 2021 TN MGR University NPTEL course: March – June 2020	2019	PSG IMSR, Pharmacology, Assistant & Associate Professor	Pharmacovigilance Antiadrenergic drugs I Histamine & antihistamines Aminoglycoside Broad spectrum & Miscellaneous Calcium Channel blockers Hypolipidemic drugs Opioids Antitubercular drugs Antipsychotic drugs Antihypertensives Drugs for Constipation and Diarrhea Cyanide poisoning case discussion **2018 Batch:** Diuretics & Antidiuretics Antitubercular drugs Bronchial asthma Introduction & Sources of drugs **PG Lecture:** PharmacovigilanceMelatoni
					2018	PSG IMSR, Pharmacology, Assistant Professor	
					2017	PSG IMSR, Pharmacology, Assistant Professor	

							n Therapeutics gases
5	Dr. A. Umamaheswari, MBBS, MD., TN MC Reg. No: 92266	Assistant Professor Date of joining 17.10.2016	Regular/ Permanent	RBC course: 10th to 12th April 2017 Research Methodology / NPTEL course: Aug – Dec 2020	2021	PSG IMSR, Pharmacology, Assistant Professor	No. = **23** Topics covered: **2019 Batch:** Metabolism AntiCholinergic drugs Antimicrobial intro Prostaglandins Seratonin and treatment of migraine Betalactams I Sulfonamide & macrolide Anti platelet drugs Drugs affecting RAS Hypnosedatives Posterior pituitary, Drugs acting on uterus Progestin & anti progestin & oral contraceptives Thyroid and anti-thyroid drugs Antidepressant Treatment of cardiac failure I &
					2020	PSG IMSR, Pharmacology, Assistant Professor	
					2019	PSG IMSR, Pharmacology, Assistant Professor	
					2018	PSG IMSR, Pharmacology, Assistant Professor	
					2017	PSG IMSR, Pharmacology, Assistant Professor	

							II Antiretroviral drugs Antiamoebic drugs Pandemic module **2018 Batch:** Thyroid Hormones & Antithyroid drugs) Drugs affecting Calcium Balance Antiretrovirals **PG Lecture:** Geriatric Pharmacology
6	Dr. K. Archana , MBBS , MD., TN MCI Reg. No :100090	Tutor Date of Joining 16.09.2021	Regular/ Permanent	RBC course: Research Methodology: 29th to 31st August 2018 TN MGR University NPTEL course: Aug – Dec 2020	2021	PSGIMS &R , Pharmacology, Tutor	No. = **9** Topics covered: **2019 batch:** Treatment of hepatitis Chelating agents Anti leprotic drugs Salicylate poisoning case discussion **2018 Batch:** Introduction & Routes of drug administration
					2020	PSGIMS &R , Pharmacology, PG Tutor	
					2019	PSGIMS &R , Pharmacology, PG Tutor	
					2018	PSGIMS &R , Pharmacology, PG Tutor	
					2017	-	

							Absorption Mechanism of drug action Anticholinergics Beta blockers

PSG Institute of Medical Sciences & Research

DEPARTMENT OF FORENSIC MEDICINE & TOXICOLOGY

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr. K.Meiyazhagan MD Forensic Medicine TNMC Reg. No 30971	Professor & HOD 20.02.2009	Permanent/Regular	Revised Basic Course in Medical Education - 12th to 14th - April 2021.	2021	Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences &Research Professor & Head of the Dept.	No. = 36 & 46 Practicals. Topics covered: Theory 1. Medical Ethics. 2. Medico legal Aspects of wound. 3. Identification. 4.Legal Procedure 5. Post mortem changes. 6. Medico legal autopsy 7. Regional Injuries 8. Toxicology 9. X-Ray, Dentition 10. Drunkenness 11. Infant Death 12. Dentition <u>Practical</u>
					2020	Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences & Research, Professor & Head of the Dept.	
					2019	Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences & Research, Professor &	

						Head of the Dept.	1. Dentition 2. Examination of Fetus 3. Starvation 4. Anaesthetic operative Deaths 5. Blood & semen examination 6. Forensic Science Lab 7. Skeletal Remains examination. 8. Starvation, Anaeasthetic Operative Death. 9. Blood& semen Examination 10. Forensic Science Laboratory. 11. Postmortem Artefacts. 12. Drug Dependance Sexual Abuse. <u>Practicals</u> 1. Skeletal Remain examination. 2. Estimation of age by radiological examination. 3. Estimation of age by Physical and Dental examination. 4. Examination and Certification of a case of drunkenness.
					2018	Professor, Dept of Forensic Medicine, Government Mohan Kumaramangalam Medical College Salem Professor.	
					2017	Professor, Dept of Forensic Medicine, Government Mohan Kumaramangalam Medical College Salem Professor.	

							5. Collection, Preservation, Packing and Forwarding of Viscera for a Chemical analysis to the Forensic Science laboratory. 6. Wound Certification 7. Examination of a weapon and correlation with the Wound. 8. Writing / Documentation of Medico-Legal cases. 9. Certification of Death / Certification of Leave with Return to Duty. 10. Examination of Foetus. 11. Examination of Bundle of Bones. 12. Examination of a case of a Victim of a Sexual Assault. 13. Examination / Collection of evidence from an individual who is an Accused in a case of a Sexual Assault. 14. Toxicology – discussion.

2	Dr.Jegadheeshwararaj.J MD Forensic Medicine TNMC Reg. No 81596-	Associate Professor 10.06.2011	Permanent/Regular	Revised Basic Course in Medical Education - 12th – 14th December 2017 NPTEL – BCBMR November – 2021.	2021	Associate Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences &Research Associate Professor	No. = 46 & 52 Practicals Topics covered: Theory 1. Introduction of Legal Procedure. 2. Medical Ethics 3. Identification 4. Post mortem Changes. 5. Regional Injuries. 6. Virginity, Infertility, Delivery 7. Criminal Abortion 8. Skeletal Remain examination. 9. Starvation,Anaeasthetic Operative Death. 10. Blood& semen Examination 11. Forensic Science Laboratory. 12. Postmortem Artefacts. 13. Drug Dependance Sexual Abuse <u>Practicals</u> 1. Skeletal Remain examination. 2. Estimation of age by radiological examination. 3. Estimation of age by Physical and Dental examination.
					2020	Associate Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences &Research Associate Professor	
					2019	Associate Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences &Research Associate Professor	
					2018	Associate Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences &Research Associate Professor	
					2017	Associate Professor, Dept of Forensic Medicine, PSG Institute of Medical	

						Sciences &Research Associate Professor	4. Examination and Certification of a case of drunkenness. 5. Collection, Preservation, Packing and Forwarding of Viscera for a Chemical analysis to the Forensic Science laboratory. 6. Wound Certification 7. Examination of a weapon and correlation with the Wound. 8. Writing / Documentation of Medico-Legal cases. 9. Certification of Death / Certification of Leave with Return to Duty. 10. Examination of Foetus. 11. Examination of Bundle of Bones. 12. Examination of a case of a Victim of a Sexual Assault. 13. Examination / Collection of evidence from an individual who is an Accused in a case of a Sexual Assault. 14. Toxicology – discussion.

3	Dr. JEGAN.R.S. MD Forensic Medicine TNMC Reg. No 105926-	Assistant Professor 22.07.2019	Permanent/Regular	Revised Basic Course in Medical Education - 12th to 14th April-2021	2021	Assistant Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences & Research	No. = 53 & 52 Practicals Topics covered: Theory 1. Fire Arm & Proximal Ballistics. 2. Death and its Causes 3. Forensic Psychiatry 4. Thermal Injuries 5. Infant Death 6. Identification 7. Legal Procedure Medico legal Autopsy . Medico legal Aspect of Wound. 8. Mechanical Injuries. 9. Asphyxial Death. 10. Thermal Injuries. 11. Sexual Offence 12. Pregnancy. 13. Forensic Psychiatry 14. Wound Certificate 15. Firearm Injuries. 16. Deliriant Poison <u>Practicals</u> 1. Skeletal Remain examination. 2. Estimation of age by radiological examination. 3. Estimation of age by
					2020	Assistant Professor, Dept of Forensic Medicine, PSG Institute of Medical Sciences & Research	
					2019	Tutor Dept of Forensic Medicine, PSG Institute of Medical Sciences & Research Post Graduate / Tutor, Dept of Forensic Medicine, Mandya Institute of Medical Sciences, Mandya – Karnataka.	
					2018	Post Graduate / Tutor, Dept of Forensic Medicine, Mandya Institute of Medical Sciences, Mandya-Karnataka	

					2017	Post Graduate / Tutor, Dept of Forensic Medicine, Mandya Institute of Medical Sciences, Mandya-Karnataka	Physical and Dental examination. 4. Examination and Certification of a case of drunkenness. 5. Collection, Preservation, Packing and Forwarding of Viscera for a Chemical analysis to the Forensic Science laboratory. 6. Wound Certification 7. Examination of a weapon and correlation with the Wound. 8. Writing / Documentation of Medico-Legal cases. 9. Certification of Death / Certification of Leave with Return to Duty. 10. Examination of Foetus. 11. Examination of Bundle of Bones. 12. Examination of a case of a Victim of a Sexual Assault. 13. Examination / Collection of evidence from an individual who is an Accused in a case of a Sexual Assault. 14. Toxicology – discussion.

PSG Institute of Medical Sciences & Research

DEPARTMENT OF COMMUNITY MEDICINE

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr.Sudha K S MD (Community Medicine) TNMC Reg. No 54168	Professor and Head 24/02/2021 Professor 01/05/2015	Permanent / Regular	RBC course: 23-25 September, 2015 Research Methodology / NPTEL course: Not applicable	2021	Professor and Head Community Medicine PSG institute of Medical Sciences and Research	No. = 10 Topics covered: • Epidemiology, prevention & control of Diphtheria and Pertussis • Epidemiology, prevention & control of Malaria • Social Obstetrics – Introduction and • Antenatal care • Alcoholism & drug dependence • Epidemiology and control of cancer • Protein Energy Malnutrition • Introduction to Clinicosocial case
					2020	Professor Community Medicine PSG institute of Medical Sciences and Research	
					2019	Professor Community Medicine PSG institute of Medical Sciences and Research	
					2018	Professor Community Medicine PSG institute of Medical Sciences and Research	
					2017	Professor Community Medicine PSG institute of Medical Sciences and Research	

2	Dr S L Ravishankar MD (community Medicine) TNMC Reg. No 62480	Professor 21-01-2005	Permanent / Regular	RBC course: 20-22 September, 2016 Research Methodology / NPTEL course: Not Applicable	2021	Professor, Dept of Comminity medicine, PSGIMSR	No. = 9 Topics covered: 1. CSC discussion: Anemia CSC allotment – Batch 1&2: TB; Batch 3&4:Anemia 2. Clinico Social Case (CSC): Explanation to students about the CSC form 3.Introduction to the Department of Community Medicine, objectives of the programme 4. Epidemiology, prevention & control of Acute Respiratory Infections 5. Epidemiology, prevention & control of Filariasis 6. Intranatal and Postnatal care 7. Genetics and health 8. Epidemiology and control of cancer 9. CSC discussion- ARI Case allotment: Dengue fever
					2020	Professor, Dept of Comminity medicine, PSGIMSR	
					2019	Professor, Dept of Comminity medicine, PSGIMSR	
					2018	Professor, Dept of Comminity medicine, PSGIMSR	
					2017	Professor, Dept of Comminity medicine, PSGIMSR	
3	Dr Sivamani M MD (community Medicine) TNMC Reg. No - 48777	Professor 01-07-12	Permanent / Regular	RBC course: 20-22 September, 2016	2021	2021-Profeessor, Dept of Community medicine, PSGIMSR	No. = 13 Topics covered: 1.Introduction to Dept of Community Medicine
					2020	2021-Profeessor ,Dept of Community medicine, PSGIMSR	

				Research Methodology / NPTEL course: Not Applicable	2019	2021-Profeessor ,Dept of Community medicine, PSGIMSR	2.Epidemiology of Tuberculosis 3.Epidemiology of Dengue 4.Health problems of under five children 5.Clinico social case discussion- systemic hyper tension 6. Descriptive epidemiology: Measurements of Morbidity (Prevalence and Incidence) and Mortality 7. Measles: Measurements of Morbidity (Attack rate, Secondary Attack Rate) Standardized Mortality Rate 8. Analytical epidemiology: Case Control study 9. Analytical epidemiology: Cohort study 10. Experimental epidemiology: Clinical trial - Efficacy of a vaccine 11. Demographic exercises: CBR, CDR, Growth Rate, Sex Ratio etc. 12. Demographic exercises: Fertility rates
					2018	2021-Profeessor, Dept of Community medicine, PSGIMSR	
					2017	2021-Profeessor, Dept of Community medicine, PSGIMSR	

							13. MCH indicators
4	Dr G.M. Muhammad M.B.B.S, M.D TNMC Reg. No - 54625	Associate Professor 01.09.2011	Permanent / Regular	RBC course: 27th -30th August 2018 Research Methodology / NPTEL course: 30.12.2021	2021	Associate Professor, Department of Community Medicine, PSG IMSR	No. = 9 Topics covered: 1. Epidemiology – Principles and concepts 2. Descriptive Epidemiology 3. Analytical Epidemiology – Case control study 4. Cohort study 5. Experimental epidemiology – Randomized control trials 6. Non randomized trials- Association and causation 7. No of Clinico social Case discussions – 2 8. Tuberculosis case discussions 9. Practical - Spotters in Public health - 7 classes
					2020	Associate Professor, Department of Community Medicine, PSG IMSR	
					2019	Associate Professor, Department of Community Medicine, PSG IMSR	
					2018	Associate Professor, Department of Community Medicine, PSG IMSR	
					2017	Associate Professor, Department of Community Medicine, PSG IMSR	
5	Dr. K. Suvetha MD (Community Medicine)	Professor 1.12.2021	Permanent / Regular	RBC course: Nov 2017	2021	1.12.21- till date- Professor	No. = 10 Topics covered: <u>Theory:</u>

	TNMC Reg. No - 67065			Research Methodology / NPTEL course: Mar-Jun2020		Jan 2021 to 30.11.21- Associate professor, Department of community medicine, PSGIMSR	1. Social issue affecting children & Adolescents 2. General epidemiology 3. Revision: Epidemiological tools: Ratio; Proportion; Rate; 4. Types of epidemiological studies 5. Descriptive Epidemiology 6. Analytical Epidemiology: Case control study 7. Analytical Epidemiology: Cohort study 8. Experimental Epidemiology: Randomized Control Trial <u>Practicals</u> (3 batches): 1. Introduction to community health program 2. Clinico social case discussion- Diabetes and dengue fever 3.
					2020	Associate professor, Department of community medicine, PSGIMSR	
					2019	Associate professor, Department of community medicine, PSGIMSR	
					2018	Associate professor, Department of community medicine, PSGIMSR	
					2017	Associate professor, Department of community medicine, PSGIMSR	
6.	Dr.S.Karthikeyan MD Community Medicine	Associate Professor	Permanent / Regular	RBC course:	2021	PSG Institute Of Medical Sciences & Resarch Department of Community Medicine	No. = 36 Topics covered:

	TNMC Reg. No - 83717	19.07.2018		12-14 December 2017 Research Methodology / NPTEL course: March-June 2020		Associate Professor	1. Epidemiology, prevention & control of Acute Diarrheal Diseases 2. Epidemiology, prevention & control of Leprosy 3. Tribal health 4. Screening for disease 5. Health education: definition, approaches, models 6. Health education: communication process, types, barriers 7. Determinants of health 8. Describe and discuss the natural history of disease 9.CHP- CSC discussion- PNC(2) 10.CHP- CSC discussion- Anemia (2) 11. CHP-Procedures for students to follow during the visit to PHC, Sub centre and ICDS centre. (3) 12. community orientation programme-COP(14)
					2020	PSG Institute Of Medical Sciences & Resarch Department of Community Medicine Associate Professor	
					2019	Government medical college, Palakkad Department of Community Medicine Associate Professor(upto 17.8.19) 2.PSG Institute Of Medical Sciences & Resarch Department of Community Medicine Associate Professor(from 22.08.21)	
					2018	PSG Institute Of Medical Sciences & Resarch Department of Community Medicine Assistant Professor(upto 18.07.19) 2.Government medical college, Palakkad	

						Department of Community Medicine Associate Professor(From 19.7.19)	13. Entomology & applied aspects: a. Mosquitoes- Anopheles, Culex, Aedes: Adults& Eggsb. House-fly, Sand-fly, Tick c. Rat Flea, Itch mite, Louse, Cyclops 14. Vaccines and cold chain equipments 15. F.P. methods (Condom, Oral pills, Copper T) 16. MCH record card; Growth chart, Mid Arm Circumference tape 17.Cereals, Pulses and Animal foods 18.Vegetables, Fruits, Sugars and Oils 19.OSPE-NHP-Part I
					2017	PSG Institute Of Medical Sciences & Research Department of Community Medicine Assistant Professor	
7	Dr. Jenit Osborn MD (Community Medicine) TNMC Reg. No - 88589	Assistant Professor	Permanent / Regular	RBC course: Research Methodology / NPTEL course: AUG-DEC (2020)	2021	PSG IMSR COMMUNITY MEDICINE ASSISTANT PROFESSOR	No. = 7 Topics covered: 1. Epidemiology, prevention & control of amoebiasis, ascariasis and hook worm 2. Epidemiology, prevention &
					2020	PSG IMSR COMMUNITY MEDICINE ASSISTANT PROFESSOR	
					2019	PSG IMSR COMMUNITY MEDICINE	

						ASSISTANT PROFESSOR	control of STD and HIV/ AIDS 3. Health problems of elderly and components of prevention 4. Epidemiology and control of coronary heart disease 5. Health education: methods 6. Clinico-social case discussion – Leprosy 7. CRRI Training for management of common medical illness
					2018	PSG IMSR COMMUNITY MEDICINE ASSISTANT PROFESSOR	
					2017	PSG IMSR COMMUNITY MEDICINE ASSISTANT PROFESSOR	
8.	Dr S Suganathan MD Community Medicine TNMC Reg. No - 95571	Assistant Professor	Permanent / Regular	RBC course: - Research Methodology / NPTEL course: -	2021	PSG INSTITUTE OF MEDICAL SCIENCES & RESARCH Department of Community Medicine Assistant Professor	No. = 10 Topics covered: 1. Health Care system in INDIA 2.Role Of Family In Health And Disease 3. Social Determinants in Health
					2020	PSG INSTITUTE OF MEDICAL SCIENCES & RESARCH Department of Community Medicine Assistant Professor	

					2019	PSG INSTITUTE OF MEDICAL SCIENCES & RESARCH Department of Community Medicine Assistant Professor	4.Introduction to demography and planning cycle 5. Role of Rate-Ratio-Proportion 6. Epidemiology and control of diabetes type 2 7.Mental health type-causes 8.Medical entomology 9. Balanced diet 10.Sewage disposal
					2018	SAVEETHA MEDICAL COLLEGE Department of Community Medicine Assistant Professor 18.07.2018	
					2017	SAVEETHA MEDICAL COLLEGE Department of Community Medicine Assistant Professor	
9.	Dr.Vijayageetha M MD , DNB TNMC Reg. No - 92572	ASSISTANT PROFESSOR	Permanent / Regular	RBC course: Research Methodology / NPTEL course: December 2020	2021	ICMR- National Institute of Epidemiology, Chennai; Division-Online Courses; Consultant II PSG Institute of Medical Sciences and Research, Coimbatore; Department of Community Medicine; Assistant Professor	No. = 14 Topics covered: Bronchogenic Carcinoma (IT) Health Education methods Practical aspects in Community Medicine (Nutrition, Environment, Medical Entomology)
					2020	ICMR- National Institute of Epidemiology, Chennai; Division-Online Courses; Consultant II	
					2019	JIPMER, Puducherry; Department of Preventive and Social Medicine; Senior Resident	

						ICMR- National Institute of Epidemiology, Chennai; Division- Online Courses; Consultant II	
					2018	JIPMER, Puducherry; Department of Preventive and Social Medicine; Senior Resident	
					2017	JIPMER, Puducherry; Department of Preventive and Social Medicine; Senior Resident	
10.	Dr.Sandhiya V MD Community Medicine TNMC Reg. No - 117679	Tutor 04.10.2021	Permanent / Regular	RBC course: Research Methodology / NPTEL course: Basic course in biomedical research: AUG – DEC 2020	2021	PSG IMSR, Community Medicine, Tutor	No. = 27 Topics covered: COP 1. Primary healthcare 2. Introduction to Community orientation program, Family health study 3. School health services 4. Solid waste management 5. Biomedical waste management 6. Sewage treatment plant 7. Water purification methods 8. Vaccination strategies including vaccine development and
					2020	PSG IMSR, Community Medicine, Tutor / PG	
					2019	PSG IMSR, Community Medicine, Tutor / PG	
					2018	PSG IMSR, Community Medicine, Tutor / PG	
					2017	-	

							implementation, vaccination centre. 9. Visit: Family vist, PSG Urban vaccination centre, PSG Sewage treatment plant, PSG hospital waste managementt, PSG RO Plant, ICDS 10. Emerging and re-emerging infections, early identification and control of new infections. 11. National mental health program CHP 12. Clinico-social case discussion- Protein energy malnutrition 13. Clinico-social case discussion- Hypertension 14. Clinico-social case discussion PNC Spotters: 15. Housefly, Sand-fly, Tick, rat flea, itchmite, louse, Cyclops 16. Universal immunization program Theory

							17. Fertility 18. Introduction to environment and health 19. Water quality 20. Surveillance of drinking water quality, hardness of water, swimming pool sanitation 21. Air: indices of thermal comfort – air pollution 22. Ventilation, lighting, housing 23. Concept of health 24. Concept of wellbeing 25. Concept of disease and its causation 26. Natural history of disease, concepts of control, levels of prevention and mode of intervention 27. Community diagnosis, community treatment, diseases classification.
11.	Dr Anil C Mathew MSc Biostatistics PhD Biostatistics	Biostatistician (Professor) 01-04-2008	Permanent / Regular		2021 2020	Biostatistician Professor, Dept of Community medicine, PSGIMSR Biostatistician Professor, Dept of Community medicine, PSGIMSR	No. = 10 Topics covered: 1.Classification and Tabulation 2.Graphic and diagrammatic presentation 3.HHealth Information system 4.Sampling methods

					2019	Biostatistician Professor, Dept of Community medicine, PSGIMSR	5. Measures of averages 6. Measures of dispersion 7. Tests of significance t test 8.Tests of significance Z test 9. Tests of significance Chi square test 10.Screening tests evaluation
					2018	Biostatistician Professor, Dept of Community medicine, PSGIMSR	
					2017	Biostatistician Professor, Dept of Community medicine, PSGIMSR	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF GENERAL MEDICINE

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)				Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr.T.Saravanan MD TNMC Reg:60877	Professor:1.6.13	Regular	**RBC course:** 027.8.18-29.8.2018 Research Methodology / NPTEL course:Not applicable	2021	PSGIMSR	GENERAL MEDICINE	Professor & HOD	No. = 7 **Topics covered:** 1.Cor pulmonale 2.Pleural effusion 3. Ascites 4. Megaloblastic anemia 5. Pancreatitis 6. CNS Tumours 7.Diabetes Mellitus Management
					2020	PSGIMSR	GENERAL MEDICINE	Professor & HOD	
					2019	PSGIMSR	GENERAL MEDICINE	Professor & HOD	
					2018	PSGIMSR	GENERAL MEDICINE	Professor	
					2017	PSGIMSR	GENERAL MEDICINE	Professor	
2	Dr.K.Jayachandran MD	Professor:1.9.97	Regular	**RBC course:** 23rd-25th June 2014	2021	PSGIMSR	GENERAL MEDICINE	Professor	No. = ___5__ Topics covered: 1.Pulmonary TB 2.INFECTIVE ENDOCARDITIS
					2020	PSGIMSR	GENERAL MEDICINE	Professor	

	TNMC Reg:31478			Research Methodology / NPTEL course: Not applicable	2019	PSGIMSR	GENERAL MEDICINE	Professor &HOD	3.Pulmonary TB 4.PANCREATITS 5.CARDIOMYOPATHIES
					2018	PSGIMSR	GENERAL MEDICINE	Professor & HOD	
					2017	PSGIMSR	GENERAL MEDICINE	Professor HOD	
3	Dr.Sujaya Menon MD TNMC Reg:41246	Professor:1.4.2004	Regular	**RBC course:** 10.7.2013-12.7.2013 Research Methodology / NPTEL course: Not applicable	2021	PSGIMSR	GENERAL MEDICINE	Professor	No. = 5 **Topics covered:** 1.GBS 2. Aplastic Anemia 3. Anemia classification & iron deficiency anemia 4. Portal HT 5.RHD/MS/MR
					2020	PSGIMSR	GENERAL MEDICINE	Professor	
					2019	PSGIMSR	GENERAL MEDICINE	Professor	
					2018	PSGIMSR	GENERAL MEDICINE	Professor	
					2017	PSGIMSR	GENERAL MEDICINE	Professor	
4	Dr.Sujithkumar MD TNMC Reg:58810	Professor:1.8.11	Regular	**RBC course: - 14th -16th June 2012** Research	2021	PSGIMSR	GENERAL MEDICINE	Professor	No. =6 **Topics covered:** 1.Alzheimer's Disease 2. Cranial Nerve Disorders
					2020	PSGIMSR	GENERAL MEDICINE	Professor	

				Methodology / NPTEL course: Not applicable	2019	PSGIMSR	GENERAL MEDICINE	Professor	3. IDA 4. Hemolytic anemia 5. ILD 6. PULMONARY EMBOLISM
					2018	PSGIMSR	GENERAL MEDICINE	Professor	
					2017	PSGIMSR	GENERAL MEDICINE	Professor	
5	Dr.A.Murali MD TNMC Reg:56948	Professor:1.9.13	Regular	**RBC course:** **17th-19th Jan 2013** Research Methodology / NPTEL course: Not applicable	2021	PSGIMSR	GENERAL MEDICINE	Professor	No. =6 **Topics covered:** 1.Cushing's syndrome 2. Meningitis & Encephalitis 3.UTI 4. DM Part 2-Acute & Chronic complications & management 5. Approach to UGI bleed 6. COPD
					2020	PSGIMSR	GENERAL MEDICINE	Professor	
					2019	PSGIMSR	GENERAL MEDICINE	Professor	
					2018	PSGIMSR	GENERAL MEDICINE	Professor	
					2017	PSGIMSR	GENERAL MEDICINE	Professor	
6.	Dr.R.Tolstoy MD TNMC Reg:65231	Professor:1.6.14	Regular	**RBC** course:10.7.12 to 12.7.12 Research	2021	PSGIMSR	GENERAL MEDICINE	Professor	No. = 6 Topics covered: 1.Seizure Disorder 2.PERICARDIAL
					2020	PSGIMSR	GENERAL MEDICINE	Professor	

				Methodology / NPTEL course: Not applicable	2019	PSGIMSR	GENERAL MEDICINE	Professor	DISEASE 3.Occupational lung disease 4.Acute & chronic pancreatitis 5.Acute and chronic Hepatitis other than 5.Hepatitis B 6.Myasthenia Gravis
					2018	PSGIMSR	GENERAL MEDICINE	Professor	
					2017	PSGIMSR	GENERAL MEDICINE	Professor	
7.	Dr.Chandrasekaran.S MD TNMC Reg:35751	Professor :13.11.2010	Regular	RBC course: - Research Methodology / NPTEL course: Not Applicable	From Sep 2020	PSG IMSR	General Medicine	Professor	No. = 15 Topics covered: 1.Irritable bowel syndrome 2.Anemia Megaloblastic Anemia 3.Bleeding and coagulation disorders 4.Acute kidney injury/ 5.Chronic kidney disease 6.CAD MI/IHD/ Athero sclerosis 7.BRANICAL ASHTHMA 8.Pulmonary TB 9.Pneumonia/Br.Asthma
					Till March 2020	Velammal Medical College	General Medicine	Professor	
					2019	Coimbatore Medical College	General Medicine	Professor	
					2018	Coimbatore Medical College	General Medicine	Professor	
					2017	Coimbatore	General	Professor	

						Medical College	Medicine		10.Additions disease 11.DKA 12.Anterior circulation stroke 13.Diabetes Mellitus part 1 14.Parkinson's Disease 15.Diabetes Mellitus pathophysiology
8.	Dr.L.S.Somasundaram MD TNMC Reg:31512	Professor:1.9.2000	Regular	RBC course: - Research Methodology / NPTEL course: Not applicable	2021	PSG IMSR	General Medicine	Professor	No. = 6 Topics covered: 1.Cirrhosis of Liver - Aetio pathogensis 2. Disorders of absorption 3.DM- II complications and management 4.Systemic Hypertension 5.Congenital Heart Disease 6.Enteric fever
					2020	PSG IMSR	General Medicine	Professor	
					2019	PSG IMSR	General Medicine	Professor	
					2018	PSG IMSR	General Medicine	Professor	
					2017	PSG IMSR	General Medicine	Professor	
9 .	Dr.Denesh Narasimhan MD	Professor:1.7.18	Regular	**RBC course:** 1.4.2015-2.4.2015	2021	PSG IMSR	General Medicine	Associate Professor	No. = 6 Topics covered: **1.**PNEUMONIA 2.ARDS
					2020	PSG IMSR	General	Associate	

	TNMC Reg:68619			Research Methodology / NPTEL course: - Not Applicable			Medicine	Professor	3.Viral Hepatitis B infection 4.Hepatitis B 5. Head ache 6.Rheumatic fever
					2019	PSG IMSR	General Medicine	Associate Professor	
					From July 2018	PSG IMSR	General Medicine	Associate Professor	
					2017 Till June 2018	PSG IMSR	General Medicine	Associate Professor	
10.	Dr.Nandhakumar MD TNMC Reg:33335	Associate Professor:1.3.21	Regular	RBC course: - Research Methodology / NPTEL course:	2021	PSG IMSR	General Medicine	Associate Professor	No. = 14 Topics covered: 1.Pulmonary Hypertension 2.Diabetes Mellitus 3.HYPERBILIURBINEMIA AND GI BLEEDING 4.MULTIPLE MYELOMA 5.HIV 6.Headache overview, 7.Seizure and status epileptics 8.Inflammatory bowel disease 9.T.B/ Bronchitectasis
					2020	-	-	-	
					2019	-	-	-	
					2018	-	-	-	
					2017	-	-	-	

									10.DIAGNOSTIC TOOLS IN RESPIRATORY DISORDERS 11.Inflammatory bowel disease 12.Cushings disease 13.DM Part 1- introduction & Pathophysiology 14.ATRIAL FIBRILLATION
11.	Dr.G.Jagadeeswaran MD TNMC Reg:77403	Associate Professor:1.3.2014	Regular	**RBC course:** **10.4.2014-12.4.2014** Research Methodology / NPTEL course: -	2021	PSG IMSR	General Medicine	Associate Professor	No. = 10 Topics covered: 1.Acute diarrhoeal diseases 2.Approach to Anemia 3.Cirrhosis of Liver - complications and management 4.SYSTEMIC HYPERTENSION 5.CVA 6.PERIPHERAL VASCULAR DISEASE 7.Acute diarrhoeal diseases 8.Nephrotic syndrome
					2020	PSG IMSR	General Medicine	Associate Professor	
					2019	PSG IMSR	General Medicine	Associate Professor	
					2018	PSG IMSR	General Medicine	Associate Professor	
					2017	PSG IMSR	General Medicine	Associate Professor	

									9.Myopathies 10.Movement Disorders
12.	Dr.P.Velammal DNB TNMC Reg:72057	Associate Professor:1.5.2019	Regular	**RBC course:** 27.8.18-29.8.18 Research Methodology / NPTEL course: -	2021	PSG IMSR	General Medicine	Associate Professor	No. = 8 Topics covered: 1.CA.Lungs 2.Encephalitis and meningitis 3.BRONCHIECTASIS 4.Acute and Chronic hepatitis(excluding Hepatitis B) 5.PUO 6.APLA and Hypercoagulation 7.Infective Endocarditis 8.Diabetes Mellitus Part 2
					2020 From May 2019	PSG IMSR	General Medicine	Associate Professor	
					2019 Till Apr	PSG IMSR	General Medicine	Assistant Professor	
					2018	PSG IMSR	General Medicine	Senior Resident	
					2017				
13.	Dr.K.Bhargavi MD TNMC Reg:86679	Assistant Professor:14.2.2018	Regular	RBC course:- Research Methodology / NPTEL	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 8 Topics covered: 1.Cardiac failure 2.Bronchial Asthma
					2020	PSG IMSR	General Medicine	Assistant Professor	

				course:mar-june 2020	2019	PSG IMSR	General Medicine	Assistant Professor	3.INFLAMMATORY BOWEL DISEASE 4.SLE 5.Rheumatoid Arthritis 6.Malaria 7.Sleep apnea 8.Addison's Disease	
					2018	PSG IMSR	General Medicine	Assistant Professor		
					2017	PSG IMSR	General Medicine	Senior Resident		
14.	Dr. Srinivasan K MD TNMC Reg:83303	Assistant Professor 1.2.16	Regular	RBC course: - Research Methodology / NPTEL course:-	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 11 Topics covered: 1.Pulmonary Embolism 2.PANCREATITS 3.Bronchial Asthma 4.Bleeding and coagulation disorders 5.Rheumatic Fever 6.ILD 7.Platelet disorders 8.Aplastic anemia 9.Anemia classification & iron deficiency anemia 10.Hemolytic anemia 11.APLA	
					2020	PSG IMSR	General Medicine	Assistant Professor		
					2019	PSG IMSR	General Medicine	Assistant Professor		
					2018	PSG IMSR	General Medicine	Assistant Professor		
					2017	PSG IMSR	General Medicine	Assistant Professor		

15.	Dr. Yoganathan C MD TNMC Reg:68345	Assistant Professor 1.11.16	Regular	RBC course: - Research Methodology / NPTEL course:-	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 14 Topics covered: 1.Pulmonary Embolism 2.Acute Diarrhoeal disease 3.Malabsorption syndromes 4.THYROID DISORDERS 5.Cranial nerve disorders 6.SHT, 7. Rheumatic fever 8.Malaria 9.Hemoglobinopathy 10.Acid peptic disease & GERD 11.DM Part 1- introduction & Pathophysiology 12.Hypothroidism & myxedema coma 13.Multiple sclerosis	
					2020	PSG IMSR	General Medicine	Assistant Professor		
					2019	PSG IMSR	General Medicine	Assistant Professor		
					2018	PSG IMSR	General Medicine	Assistant Professor		
					2017	PSG IMSR	General Medicine	Assistant Professor		

									14. MENINGITIS
16.	Dr. Karthikeyan P S MD TNMC Reg:97190	Assistant Professor 8.12.17	Regular	RBC course: - Research Methodology / NPTEL course:-	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 6 Topics covered: 1.Pleural Effusion 2.CVA 3.Pleural Effusion 4.ARDS 5.INFECTIVE ENDOCARDITIS 6.Megaloblastic anemia
					2020	PSG IMSR	General Medicine	Assistant Professor	
					2019	PSG IMSR	General Medicine	Assistant Professor	
					2018	PSG IMSR	General Medicine	Assistant Professor	
					2017	Vinayaka Mission KVMCH	General Medicine	Assistant Professor	
17.	Dr. Senthil Kumar MD TNMC Reg:53831	Assistant Professor 15.6.2011	Regular	RBC course: - Research Methodology / NPTEL course	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 5 Topics covered: 1.CVA 2.Platelet Disorders 3.DISORDERS OF CARDIAC RHYTHM-ATRIAL ARRTHYTHMIAS 4.Lungs in systemic disease 5.Approach to Jaundice
					2020	PSG IMSR	General Medicine	Assistant Professor	
					2019	PSG IMSR	General Medicine	Assistant Professor	
					2018	PSG IMSR	General Medicine	Assistant Professor	

					2017	PSG IMSR	General Medicine	Assistant Professor	
18.	Dr. Krishna S Nair MD TNMC Reg:50138	Assistant Professor 1.8.20	Regular	RBC course: - Research Methodology / NPTEL course	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 6 Topics covered: 1.Pneumothroax 2.RHEUMATIC FEVER 3.CIRRHOSIS LIVER 4.Rheumatic Heart Disease 5.TB- MANAGEMENT 6.Dengue fever
					2020 From 1.8.20	PSG IMSR	General Medicine	Assistant Professor	
					2019 till 31.7.20	PSG IMSR	General Medicine	Senior Resident	
					2018	-	-	-	
					2017	Amirtha Vishwa Vidha peeth	General Medicine	PG/Tutor	
19.	Dr. Nirmala S MD TNMC Reg:99587	Assistant Professor 1.12.20	Regular	RBC course: - Research Methodology / NPTEL course	2021 From Dec 2020	PSG IMSR	General Medicine	Assistant professor	No. = 5 Topics covered: 1.Diagnostic tools in respiratory disorders 2.Meningitis 3.Systemic Hypertension 4.Pneumothroax
					2020 Till Nov 2020	KMCH	General Medicine	Assistant Professor	
					From	KMCH	General	Assistant	

					Sept 2019		Medicine	Professor	5.PLATELET DISORDERS
					From june 17 to Aug 18	MGMCRI	General Medicine	PG/Tutor in General Medicine	
					2017 Till May	MGMCRI	General Medicine	PG/Tutor in General Medicine	
20.	Dr. NekkantiAbilash MD TNMC Reg:103499	Assistant Professor 1.3.21	Regular	RBC course: - Research Methodology / NPTEL course	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 5 Topics covered: 1.CAD 2CIRRHOSIS LIVER 3.Headache overview, 4.Seizure and status epileptics 5.HIV-MANAGEMENT
					2020	PSG IMSR	General Medicine	Senior Resident	
					2019 from 12.12.19	PSG IMSR	General Medicine	Senior Resident	
					2018	-	-	-	
					2017	-	-	-	
21.	Dr. Jayakumar M MD TNMC Reg:49791	Assistant Professor Nov 21	Regular	` RBC course: - Research Methodology /	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 4 Topics covered: 1.Hypopitutarism 2.Approach to
					2020	-	-	-	

				NPTEL course	2019	-	-	-	Jaundice 3.Hemolytic Anemia 4.Posterior circulation stroke
					2018	-	-	-	
					2017	-	-	-	
22.	Dr. Clement Jenil dhas MD TNMC Reg:111045	Assistant Professor 5.12.2021	Regular	RBC course: - Research Methodology / NPTEL course	2021	PSG IMSR	General Medicine	Assistant Professor	No. = 2 Topics covered: Infective Endocarditis Diabetes Melliltus
					2020 Dec	Karpagam Faculty of Medical science	General Medicine	Assistant Professor	
					2020	Karpagam Faculty of Medical science	General Medicine	Senior Resident	
					2019	Karpagam Faculty of Medical science	General Medicine	Senior Resident	
					2018	PSG IMSR	General	PG/Tutor	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF RESPIRATORY MEDICINE

SERVICE PARTICULARS

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr.R. KARTHIKEYAN MD (CHEST), TNMC 73510	PROFESSOR & HOD	REGULAR/ PERMANENT	RBC course Research Methodology / NPTEL course: Not applicable	2021	PSG- IMSR RESPIRATORY MEDICINE PROFESSOR & HEAD	No. = 18 Topics covered: A. Topics in clinical postings • Introduction to TB • Clinical features • MDR TB • EPTB • TB-HIV B. Bedside teaching in early clinical exposure session. C. Large group teaching on common topics in Respiratory Medicine
					2020	PSG- IMSR RESPIRATORY MEDICINE PROFESSOR	
					2019	PSG- IMSR RESPIRATORY MEDICINE PROFESSOR	
					2018	PSG- IMSR RESPIRATORY MEDICINE	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF **DERMATOLOGY**

SERVICE PARTICULARS

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr. Reena Rai MD DVL TNMC Reg No 44619	Professor 01.01.2005	Regular	RBC course: 10th, 11th& 12th April 2017 Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	PSGIMSR, DEPT. OF DERMATOLOGY, COIMBATORE PROFESSOR & HEAD	No. = 1 Topics covered: Hansens disease
2	Dr. Mahadevan K MD DV TNMC Reg No 37425	Coimbatore Medical College07.11.2004 to 28.02.2015 PSGIMSR Professor 08.05.2015		RBC course: 27,28,29,30 April 2018 Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	PSGIMSR, DEPT. OF DERMATOLOGY, COIMBATORE PROFESSOR	No. = 2 Topics covered: Sexually transmitted diseases HIV

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
3	Dr. Shanmugasekar C MD DVL TNMC Reg No 72292	Professor 17.08.2015	Regular	RBC course: 12-14th Dec. 2017 Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	PSGIMSR, DEPT. OF DERMATOLOGY, COIMBATORE PROFESSOR	No. = 1 Topics covered: Drug reaction
4	Dr. Setu Mittal TNMC Reg No 133499	Senior Resident 01.02.2019	Regular	RBC course: Research Methodology / NPTEL course: 18,19 Oct. 2019	2021 2020 2019 2018 2017	PSGIMSR, DEPT. OF DERMATOLOGY, COIMBATORE SENIOR RESIDENT - -	No. = 1 Topics covered: Cutaneous Bacterial Infections

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
5	Dr. Yuvasri G MD DVL TNMC Reg No 111031	Senior Resident 04.11.2020	Regular	RBC course:Nil Research Methodology / NPTEL course:	2021	PSGIMSR, DEPT. OF DERMATOLOGY, COIMBATORE SENIOR RESIDENT	No. = Nil Topics covered:
					2020		
					2019	-	
					2018	-	
					2017	-	
6	Dr. Lakshmi Prabha P MD DVL TNMC Reg No 121741	Senior Resident 01.12.2021	Regular	RBC course:Nil Research Methodology / NPTEL course: 29.08.2018 to 31.08 2018	2021	PSGIMSR, DEPT. OF DERMATOLOGY, COIMBATORE SENIOR RESIDENT	No. = Nil Topics covered:
					2020	-	
					2019	-	
					2018	-	
					2017	-	

						PROFESSOR	
					2017	PSG- IMSR RESPIRATORY MEDICIN E PROFESSOR	
2	Dr.K.ANUPAMA MURTHY M.D (CHEST), PGDGM , FAIMER FELLOW TNMC REG NO: 107428	PROFESSOR 12/07/2011	REGULAR/ PERMANENT	RBC course: Research Methodology / NPTEL course: Not applicable	2021	PSG-IMSR RESPIRATORY MEDICINE PROFESSOR	No. = 18 Topics covered: A. Topics in clinical postings • TB-HIV • Adverse effect of ATT • Chemotherapy of TB • Clinical features • Introduction to TB B. Bedside teaching in early clinical exposure session. C. Large group teaching on common topics in Respiratory Medicine
					2020	PSG-IMSR RESPIRATORY MEDICINE PROFESSOR	
					2019	PSG-IMSR RESPIRATORY MEDICINE PROFESSOR	
					2018	PSG-IMSR RESPIRATORY	

						MEDICINE PROFESSOR	
					2017	PSG-IMSR RESPIRATORY MEDICINE PROFESSOR	
3	Dr. KANCHERLA ROOPA DNB (Respiratory Medicine) TNMC REG NO:76308	ASSOCIATE PROFESSOR	REGULAR / PERMANENT	RBC course: Research Methodology / NPTEL course:	2021	PSG – IMSR RESPIRATORY MEDICINE ASSOCIATE PROFESSOR	No. = 20 Topics covered: A. Topics in clinical postings • Clinical features of pulmonary • Extra pulmonary • TB Manifestation • MDR TB • Micro-TB • Pneumonia • Diagnostic C X R • TB-HIV
					2020	PSG-IMSR ASSISTANT PROFESSOR	
					2019	PSG-IMSR ASSISTANT PROFESSOR	
					2018	PSG-IMSR ASSISTANT	

						PROFESSOR	B. Bedside teaching in early clinical exposure session. C. Large group teaching on common topics in Respiratory Medicine
					2017	PSG-IMSR ASSISTANT PROFESSOR	
4	DR.VARUN MD MD (Respiratory Medicine), TNMC REG NO: 92589	ASSISTANT PROFESSOR	REGULAR/ PERMANENT	RBC course: 14.05.2021 Research Methodology / NPTEL course: NOV-2021	2021	PSG-IMSR RESPIRATORY MEDICINE ASSISTANT PROFESSOR	No. = 23 Topics covered: A. Topics in clinical postings • Clinical case based discussion • Diagnostic X-ray • Pathogonisis • Astic chest X-ray • RNTCP B. Bedside teaching in early clinical exposure session. C. Large group teaching on common topics in Respiratory Medicine
					2020	PSG-IMSR RESPIRATORY MEDICINE ASSISTANT PROFESSOR	
					2019	PSG-IMSR RESPIRATORY MEDICINE SENIOR RESIDENT/ASSISTANT PROFESSOR	

					2018	PSG-IMSR RESPIRATORY MEDICINE ASSISTANT PROFESSOR	
					2017	ST JOHNS MEDICAL COLLEGE RESPIRATORY MEDICINE SENIOR RESIDENT	
5	Dr. G. THARINI MD(Pulmonary Medicine),DNB TNMC REG NO: 102605	ASSISTANT PROFESSOR	REGULAR/ PERMANENT	RBC course: Research Methodology / NPTEL course: Not applicable	2021	PSG-IMSR RESPIRATORY MEDICINE ASSISTANT PROFESSOR	No. = 25 Topics covered: A. Topics in clinical postings • Introduction & tuberculosis. • Diagnosis of tuberculosis. • MDR TB • TB PL.effession • MDR TB • Asthma • HIV TB • Diagnosis of TB
					2020	PSG-IMSR RESPIRATORY MEDICINE ASSISTANT PROFESSOR	
					2019	PSG-IMSR RESPIRATORY MEDICINE	

						SENIOR RESIDENT/ASSISTANT PROFESSOR	B. Bedside teaching in early clinical exposure session. C. Large group teaching on common topics in Respiratory Medicine
					2018	PSG-IMSR RESPIRATORY MEDICINE SENIOR RESIDENT	
					2017	-	
6	Dr. R. JAYAKUMAR MD (Pulmonary Disease), DNB (Respiratory Disease), MRCP (UK) TNMC REG NO: 75483	ASSISTANT PROFESSOR	REGULAR/ PERMANENT	RBC course: 14.05.2021 Research Methodology / NPTEL course: MAR-JUN 2021	2021	PSG-IMSR RESPIRATORY MEDICINE ASSISTANT PROFESSOR	No. = 25 Topics covered: A. Topics in clinical postings • Pathogonisis of tuberculosis. • Parmandory of anti TB drugs. • TB-HIV • Case based discussion • Microbiology • TB-HIV • Case based discussion B. Bedside teaching in early clinical
					2020	PSG-IMSR RESPIRATORY MEDICINE ASSISTANT PROFESSOR	
					2019	-	
					2018	-	

					2017	-	exposure session. C. Large group teaching on common topics in Respiratory Medicine

DEPARTMENT OF PAEDIATRICS

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification & TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr A Jayavardhana MD (Paed) TNMC Reg No - 68374	Professor 01.05.2017 & HOD 24.02.2021	Regular & Permanent	RBC course: 22.09.2016 Research Methodology / NPTEL course: June 2021	2021	PSG IMSR, Paediatrics , Professor From 24.02.21 HOD	Nos. = 4 Topics covered: **2017 Regular batch** 1. Typhoid fever and leptospirosis 2. Cerebral Palsy 3. Acute respiratory infection in children **2018 Regular Batch:** 4. Acute respiratory infection in children - Upper & Lower
					2020	PSG IMSR, Paediatrics Professor	
					2019	PSG IMSR, Paediatrics Professor	
					2018	PSG IMSR, Paediatrics Professor	
					2017	PSG IMSR, Paediatrics Professor	

2	Dr K Neelakandan MD (Paed) TNMC Reg No - 32705	Professor 06.07.2016	Regular & Permanent	RBC course:22.09.2016 Research Methodology / NPTEL course: Not applicable.	2021 2020 2019 2018 2017	PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor	Nos. = 5 Topics covered: **2017 Regular batch** 1. Introduction to Pediatrics & Child development 2. Acute and chronic diarrhea 3. Congenital cyanotic heart disease 4. Short Stature **2018 Regular batch** 5. Introduction to Pediatrics & Child development, Mortality Rate
3	Dr K Jothilakshmi DNB (Paed) TNMC Reg No - 52532	Professor 01.02.2010	Regular & Permanent	RBC course: 14.04.2021 Research Methodology / NPTEL course: December 2021.	2021 2020 2019 2018 2017	PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor PSG IMSR, Paediatrics, Professor	Nos. = 5 Topics covered: **2017 Regular Batch :** 1. Nutrition in children & Obesity 2. Malaria, Filaria & Chicken Pox 3. Acute and Recurrent urinary tract infection 4. Epilepsy, Status epilepticus, Febrile Seizure **2018 Regular batch** 5. Nutrition in children, Obesity 6.

4	Dr S Ramesh MD (Paed) DM (Neo) TNMC Reg No - 67060	Assoc. Professor & 22.12.2017	Regular & Permanent	RBC course: 12.04.2017 Research Methodology / NPTEL course: June 2021	2021	PSG IMSR, Paediatrics ,Assoc.Professor	Nos. = 4 Topics covered: **2017 Regular Batch:** 1. Neonatal infections 2. Seizures in New Born 3. Metabolic disturbances in Newborn 4. Congenital malformations in neonates and birth injuries
					2020	PSG IMSR, Paediatrics, Assoc.Professor	
					2019	PSG IMSR, Paediatrics, Assoc.Professor	
					2018	PSG IMSR, Paediatrics, Assoc.Professor	
					2017	PSG IMSR, Paediatrics, Asst. Professor	
5	Dr P Venkateshwaran MD (Paed) TNMC Reg No - 66197	Assoc. Professor	Regular & Permanent	RBC course: 29.08.2018 Research Methodology / NPTEL course: June 2021	2021	PSG IMSR, Paediatrics, Assoc. Professor	Nos. = 3 Topics covered: **2017 Regular Batch :** 1. Tuberculosis in children 2. Rheumatic fever including chorea 3. Snakebite Envenomation & Scorpion sting
					2020	PSG IMSR, Paediatrics, Assoc. Professor	
					2019	PSG IMSR, Paediatrics, Assoc. Professor	
					2018	PSG IMSR, Paediatrics, Assoc. Professor	
					2017	-	

6	Dr. N.T. Rajesh MD (Paed) TNMC Reg No - 72214	Assoc. Professor & 01.04.2016	Regular & Permanent	RBC course: 14.12.2017 Research Methodology / NPTEL course: June 2020	2021	PSG IMSR, Paediatrics, Assoc.Professor	Nos. = 5 Topics covered: **2017 Regular Batch** 1. Immunization (National Immunization Program / Pulse Polio Program / Cold Chain) 2. Pyrexia of unknown origin 3. Acute Flaccid Paralysis 4. Hydrocephalus & Microcephaly **2018 Regular Batch** 5. Immunization (National immunisation program, Pulse Polio, Cold Chain
					2020	PSG IMSR, Paediatrics, Assoc.Professor	
					2019	PSG IMSR, Paediatrics, Assoc.Professor	
					2018	PSG IMSR, Paediatrics, Assoc.Professor	
					2017	PSG IMSR, Paediatrics, Assoc.Professor	

7	Dr Bharathi E DNB (Paed) TNMC Reg No - 76115	Asst. Professor & 01.06.2016	Regular & Permanent	RBC course: 14.12.2017 Research Methodology / NPTEL course: June 2021	2021	PSG IMSR, Paediatrics, Asst. Professor	Nos. = 6 Topics covered: **2017 Regular Batch** 1. Vitamins in Children (Requirements / Deficiency States / Hypervitaminosis) 2. Developmental assessment & Behavioural disorders 3. Hypertension in children 4. Breast feeding including BFHI 5. Hemolytic Anemia **2018 Regular Batch :** 6. Vitamins in Children - Requirements, deficiency states, hypervitaminosis
					2020	PSG IMSR, Paediatrics, Asst. Professor	
					2019	PSG IMSR, Paediatrics, Asst..Professor	
					2018	PSG IMSR, Paediatrics, Asst. Professor	
					2017	PSG IMSR, Paediatrics, Asst. Professor	

8	Dr.B. Muruganantham MD (Paed) TNMC Reg No - 92416	Asst. Professor & 22.12.2017	Regular & Permanent	RBC course: 30.08.2018 Research Methodology / NPTEL course: June 2020	2021	PSG IMSR, Paediatrics, Asst. Professor	Nos. = 8 Topics covered: **2017 Regular Batch:** 1. Protein energy malnutrition 2. Helminthic infestations, Giardiasis and amebiasis 3. Gastroesophageal Reflux 4. Prematurity, IUGR, Gestational age assessment 5. Approach to anemia / Iron deficiency anemia 6. Puberty and Adolescence 7. Poisoning in children **2018 Regular Batch** 8. Protein energy malnutrition
					2020	PSG IMSR, Paediatrics, Asst. Professor	
					2019	PSG IMSR, Paediatrics, Asst. Professor	
					2018	PSG IMSR, Paediatrics, Asst. Professor	
					2017	PSG IMSR, Paediatrics, Senior Resident	

9	Dr J Suchithra DNB (Paed) TNMC Reg No - 98135	Asst. Professor & 01.10.2019	Regular & Permanent	RBC course: 14.04.2021 Research Methodology / NPTEL course: June 2021	2021	PSG IMSR, Paediatrics, Asst. Professor	Nos. = 5 Topics covered: **2017 Regular Batch** 1. Vaccines (Principles, Hep A & B Influenza) 2. Acute renal failure (briefly CRF) 3. Respiratory distress in New Born 4. Coagulation disorder **2018 Regular Batch** 5. Vaccines (Principles, specific vaccines, vaccine preparations available)
					2020	PSG IMSR, Paediatrics, Asst. Professor	
					2019	PSG IMSR, Paediatrics, Senior Resident 01.10.2019 onwards Asst. Professor	
					2018	PSG IMSR, Paediatrics, Senior Resident	
					2017	PSG IMSR, Paediatrics, Senior Resident	
					2020	PSG IMSR, Paediatrics, Asst. Professor	
					2019	PSG IMSR, Paediatrics, Senior Resident 01.10.2019 onwards Asst. Professor	
					2018	PSG IMSR, Paediatrics, Senior Resident	
					2017	PG/TUTOR – Manipal	
10	Dr.S.Gayathri MD (Paed) DNB (Paed) TNMC Reg No - 90559	Asst. Professor & 01.10.2019	Regular & Permanent	RBC course: Not attended Research Methodology / NPTEL course: Dec 2020	2021	PSG IMSR, Paediatrics, Asst. Professor	Nos. = 4 Topics covered: **2017 Regular Batch** 1. Acute and chronic liver cell failure 2. Congestive cardiac failure 3. Meningitis, Encephalitis including sequelae
					2020	PSG IMSR, Paediatrics, Asst. Professor	
					2019	PSG IMSR, Paediatrics, Senior Resident 01.10.2019 onwards Asst. Professor	
					2018	PSG IMSR, Paediatrics, Senior Resident	

					2017	-	4. Diabetes Mellitus
11	Dr Nitin Srinivasan DNB (Paed) TNMC Reg No - 64780	Asst. Professor & 01.04.2020	Regular & Permanent	RBC course: Not attended Research Methodology / NPTEL course:	2021	PSG IMSR, Paediatrics, Asst. Professor	No. =3 Topics covered : 1 Congenital diseases 2 Infectious diseases 3 Cardio Pulmonary Resuscitation
					2020	PSG IMSR, Paediatrics, Asst. Professor	
					2019	-	
					2018	-	
					2017	-	
12	Dr. Abraar Sheriff M, MD (Paed) DM (Paed Critical Care) TNMC Reg No - 92531	Asst. Professor & 15.06.2021	Regular & Permanent	RBC course: Not attended Research Methodology / NPTEL course:	2021	PSG IMSR, Paediatrics, Asst. Professor	No. = 3 Topics covered: **2017 Regular Batch** 1. Acute Leukemia & Lymphoma 2. Status epilepticus / Status Asthmaticus / Shock Management **2018 Regular Batch:** 3. Fluid & Electrolyte management in Newborn & Children
					2020	-	
					2019	-	
					2018	-	
					2017	-	

DEPARTMENT OF PSYCHIATRY

SERVICE PARTICULARS

S. No	Name of the faculty,Qualification& TNMCregistrationnumber	CurrentDesignation &Date ofpromotion	Nature ofemployment Regular/permanentor contract/ outsourced	Mentionthedates ofcompletionof:	Details of Service in the Last 5years (Mention name of institute,departmentanddesignation)		Number of lectures taken in2021until15.12.2021. Listthetopicscovered
1	Dr.G. Raghuthaman, MD(Psy), DNB (Psy),TNMCRegNo:50498	Professor 01/08/2008	Regular/ Permanent	RBCcourse: 08/11/2021& 09/11/2021 ResearchMethodology /NPTELcourse:	2021	Professor&Head	No.=11 Topicscovered: • History&MSE • Schizophrenia • ChildPsychiatry • OrganicPsychiatry • SubstanceDependence • GeriatricOnset Psychiatricdisorder • AnxietyDisorder • Non–Pharmacological Treatmentinpsychiatry • Depression • Dissociativedisorder
					2020	Professor&Head	
					2019	Professor&Head	
					2018	Professor&Head	
					2017	Professor&Head	

2	Dr.I. Anand, DPM(Psy),MD(Psy) TNMCRegNo:52512	Professor 01/05/2011	Regular/ Permanent	RBCcourse: 29/10/2021& 30/10/2021 Research Methodology/ NPTELcourse:	2021 2020 2019 2018 2017	Professor Professor Professor Professor Professor	No. =5 Topicscovered: • SubstanceAbuse • AnxietyDisorder • OrganicPsychiatry • Community Psychiatry&Rehabilitation • SubstanceUseDisorder

3	Dr.SyedUmmar,MDPsy TNMCRegNo:72310	Associate Professor 01/06/2013	Regular/ Permanent	RBC course:10/04/2017, 11/04/2017 & 12/04/2017 29/10/2021&\ 30/10/2021 ResearchMethodology /NPTEL course:March–June2020	2021 2020 2019 2018 2017	AssociateProfessor AssociateProfessor AssociateProfessor AssociateProfessor AssociateProfessor	No. =6 Topicscovered: • ChildPsychiatry • PsychiatryEmergency • Depression • BipolarDisorder • Somatoformdisorder • History&MSE

Dr.P.Pradeep,MD(Psy) TNMCRegNo:76343	Associate Professor 11/04/2016	Regular/ Permanent	RBCcourse: Research Methodology/ NPTELcourse: March–June2020	2021 2020 2019 2018 2017	Associate Professor Associate Professor Associate Professor Associate Professor Associate Professor	No. =7 Topicscovered: • AnxietyDisorder • SubstanceDependence • BipolarDisorder • AnxietyDisorder • DoctorPatientrelationship & Mentalhealthnormalcy,stressandMSE. • OrganicMentaldisorder • Dissociative &somatoformdisorder

Dr.R. SureshKumar,MD(Psy) TNMCRegNo:76408	Associate Professor 01/01/2020	Regular/ Permanent	RBCcourse: 12/12/2017, 13/12/2017,14/12/2017 Research Methodology/ NPTELcourse: March–June2020	2021 2020 2019 2018 2017	Associate Professor Associate Professor Assistant Professor Assistant Professor Assistant Professor	No. =5 Topicscovered: • Bipolar Disorder • Depression • Schizophrenia • Mood Disorder • Sexual Disfunction

PSG Institute of Medical Sciences & Research

DEPARTMENT OF GENERAL SURGERY

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ Outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr S Rajesh Kumar MBBS, MS (Gen. Surgery) TNMC Reg No 61657	Professor & HOD 01.07.2013	Regular	RBC course: 10.4.2017 to 12.4.2017 Research Methodology / NPTEL course: NA	2021	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR & HEAD.	No. = 12 Topics covered: 1. Gastric ulcer 2. Gastri c operation for morbid obesity and other conditions 3. Renal and ureteric calculi 4. Rectal prolapsed and proctitis, salitary rectal ulcer 5. Acute peritonitis 6. Carcinoma penis 7. Spleen- Splenomegaly, hypertension and splenectomy 8. Oral malignancy- Etiopathlogenesis/Pre malignant condition 9. Oral malignancy- Clinical
					2020	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	
					2019	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	
					2018	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	
					2017	PSGIMSR- COIMBATORE, GENERAL SURGERY - PROFESSOR	

							presentation/Diagnosis management 10. Oral carcinoma-Occult primary/Neck node management 11. Benign breast disease 12. Carcinoma of the breast
2	Dr S Premkumar MBBS, MS (Gen. Surgery) TNMC Reg No 33296	Professor 14.01.2014	Regular	RBC course: 27.8.2018 to 30.8.2018 Research Methodology / NPTEL course: NA	2021	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	No. = 11 Topics covered: 1. Parathyroid- Hyper and hypothyroidism 2. Multinodular goiter 3. Obstructive jaundice 4. Inguinal hernia 5. Umbilical hernia, epigawtric hernia, rare external hernia 6. Pelvic abscess 7. Special forms of peritonisis/TB Perinotitis 8. Mesentric injury, Omental tortion, mesenteric cyst, Retroperitoneal neoplasms 9. Oesophagus- Anatomy, physiology, symptoms of oesophagial disorders and investigation for oesophageal disease 10. Benign tumours/Carcinoma rectum
					2020	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	
					2019	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	
					2018	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	
					2017	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	

							11. Anal/anal canal- Surgical anatomy/examination of anal, canal, congenital abnormalities
3	Dr Vimal Kumar Govindan MBBS,MS (Gen.Surgery) TNMC Reg No 38609	Professor 01.04.2003	Regular	RBC course: 10.4.2017 to 12.4.2017 Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	No. = 12 Topics covered: 1. Carcinoma oesophagus 2. Benign tumours and carcinoma rectum 3. Testis- Undescended testis, Ectopic testis, Tortion Testis, Varicocele 4. Carinoma of panscres and endocrine tumours of pancreas' 5. Thyroid neoplasms 6. Burst abdomen, incisional hernia, divertication of rectus/neoplasm of anterior abdominal wall 7. Acute peritonitis 8. Congenital abnormalities of oesophagus, foreign bodies, oesophageus, oesophagial performation 9. The Rectum- Surgical anatomy, clinical features, rectal disease/injuries/FB rectum 10. Rectal prolapsed and proctitis 11. Incontinence of urine/Retension of urine

							12. Bladder stones foreign bodies in bladder, ,diverticulum of bladder,, urinary fistula
4	Dr S Karthikeyan MBBS,MS (Gen.Surgery) TNMC Reg No 60622	PROFESSOR 01/06/2013	REGULAR	RBC course: 10th – 12th April, 2017 Research Methodology / NPTEL course: NA	2021 2020 2019 2018 2017	PSGIMSR- COIMBATORE, GENERAL SURGERY - PROFESSOR	No. = 11 Topics covered: 1. GIST/Duodenal Tumours 2. Carcinoma tongue 3. Surgical techniques, skin sutures and sutures materials 4. Bile duct obstruction/strictures of bile duct/primary sclerosing cholangitis 5. Spleen- Splenomegaly/Hyper splenism/splenectomy 6. Vermi form appendix 7. Inguinal hernia 8. Haematemesis and malena 9. Gastric outlet obstruction 10. Gastric cancer 11. Gastric stromal tumours/GIST/Duodenal tumour
5	Dr S Saradha MBBS, MS (Gen. Surgery) TNMC Reg No 39688	Professor	Regular	RBC course: Research	2021 2020 2019	PSGIMSR- COIMBATORE, GENERAL SURGERY – PROFESSOR	No. = 11 Topics covered: 1. Oral malignancy- Etiopathogenesis and premalignant condition 2. TAO 3. Portal hypertension

				Methodology / NPTEL course: NA	2018	Govt. Medical College, Hospital, Pudukottai, Dean	4. Liver trauma 5. Parathyroid-Hyperparathyroidism/Hypotheyroidism 6. Hiatus hernia/GERD 7. Thyroid neoplasms 8. Salivary glands-Minor, sublingual and submandiblar 9. Angiodysplasia, Blind loop syndrome, diverticular disease 10. TB peritonitis/spl form of peritonitis 11. Intussusception volvulus/adynamic obstruction
					2017	Govt. Medical College, Hospital, Pudukottai, Dean	
6	Dr Shaikh Afzal Rubby MBBS, MS (Gen. surgery) TNMC Reg No 10273	Associate Professor	Regular	RBC course: Research Methodology / NPTEL course:	2021	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSOCIATE PROFESSOR	No. = 10 Topics covered: 1. Rectum- Surgical anatomy, clinical features, rectal disease, injuries, FB rectum 2. Benign thyroid disease 3. Urinary Bladder-Anatomy/Incontinence and Retention of urine 4. Sterile precautions and theatre surgery 5. Acute life support and critical care 6. Fluid balance, electrolyte balance and
					2020	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSOCIATE PROFESSOR	
					2019	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSOCIATE PROFESSOR	
					2018	ESI Medical College, Joka, General surgery, Asst. Prof.	
					2017	ESI Medical College, Joka, General surgery, Asst. Prof.	

							acid base balance 7. Retention of urine 8. Anaemia 9. Gastric operations for morbid obesity and other gasric conditions 10. Management of bile duct constructions and strictures of bile duct
7	Dr Arun Prasath S.V MBBS, MS (Gen. surgery) TNMC Reg No 90433	Associate Professor 01.10.2019	Regular	RBC course: 27.8.2018 to 30.8.2018 Research Methodology / NPTEL course	2021 2020 2019 2018 2017	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO.PROFESSOR PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO. PROFESSOR PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO. PROFESSOR PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST. PROFESSOR PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST. PROFESSOR	No. = 09 Topics covered: 1. Hyperthyroidism 2. Benign breast diseases 3. Management of bile duct obstructions and strictures 4. Kidney Neoplasms 5. Varicose veins 6. Intestinal obstruction 7. Shock 8. Accident and emergency surgery 9. Cyst, ulcer, sinuS
8	Dr Chitra R MBBS, MS (Gen. surgery) TNMC Reg No 90922	Associate Professor 27.07.2020	Regular	RBC course: 27.8.2018 to 30.8.2018 Research Methodology / NPTEL	2021 2020 2019 2018	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO. PROFESSOR PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO. PROFESSOR PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST. PROFESSOR PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST. PROFESSOR	No. = 09 Topics covered: 1. Parathyroid gland – Disorders 2. Bronchial cyst/Bronchial fistula/cystic hygroma/Thyroglossal cyst 3. Benign breast disease 4. Cyst, ulcer and sinus

				course	2017	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST. PROFESSOR	5. Breast carcinoma 6. Surgical Jaundice 7. AIDS 8. Carcinoma of pancreas and endocrine tumours of pancreas 9. H.Pylori/gastritis
9	Dr Vishnuvarthan MBBS,MS (Gen.Surgery) TNMC Reg No 84172	Associate Professor	Regular	RBC course: Research Methodology **/ NPTEL** course **June 2021**	2021	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO.PROFESSOR	No. = 10 Topics covered 1. Oral carcinoma- Occult primary/Neck node management 2. Foreign bodies in oesophagus, perforation, Mallory- Weiss syndrome 3. The Rectum- Anatomy/Rectal disease and injuries 4. Inguinal hernia 5. Hydronephrosis 6. Burns 7. Adrenal glands- disorder so adrenal medulla 8. Gangrene/amputation 9. Carcinoma of tonue 10. Bladder diverticulum and urinary fistula
					2020	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO.PROFESSOR	
					2019	Karpagam Faculty of Medical Sciences & Research, Coimabtore,General Surgery-Asso. Prof. till 30.6.2019	
					2018	Karpagam Faculty of Medical Sciences & Research, Coimabtore,General Surgery-Asso. Prof. from 1.10.2018	
					2017	Karpagam Faculty of Medical Sciences & Research,Coimabtore,General Surgery-Asst. Prof.	

10.	Dr Sunay Narasimha Bhat MBBS, MS (Gen.Surgery) TNMC Reg No 78854	Associate Professor 25.07.2020	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO.PROFESSOR	No. = 08 Topics covered 1. Thyroid neoplasm 2. Hiatus hernia/ GERD 3. Rectal prolapsed and proctitis 4. Prostate – Anatomy and BPH 5. Disorders of adrenal cortex 6. Prostate- BPH and Neoplasms 7. Thyroid- Anatomy, Hypothyrodism and hyperthyroidism 8. Foreign bodies in oesophagus, perforation, Mallory-Weiss syndrome
					2020	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO.PROFESSOR	
					2019	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST.PROFESSOR	
					2018	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST.PROFESSOR	
					2017	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASST. PROFESSOR	
11	Dr R Ramasamy MBBS,MS (Gen.Surgery) TNMC Reg No 50530	Associate Professor	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR- COIMBATORE, GENERAL SURGERY – ASSO.PROFESSOR	No. = 2 Topics covered 1. Sinus and fistula 2. RECONSTRUCTIVE SURGERY- Basic principles-Skin/graft
					2020	-	
					2019	-	
					2018	-	
					2017	-	
12	Dr Ajay Sivakumar MBBS, MS (Gen.surgery) TNMC Reg No 95451	Assistant Professor 01.08.2017	Regular	RBC course: 04.10.2017 to 06.10.2017 Research	2021	PSGIMSR ,Coimbatore General Surgery , Asst. Prof.	NO: 5 Topics covered 1. Thyroid- Hypothyroidism, Thyroiditis 2. Incontinence of Urine and Retension of urine 3. Carcinoma breast
					2020	PSGIMSR ,Coimbatore General Surgery , Asst. Prof	
					2019	PSGIMSR ,Coimbatore General Surgery , Asst. Prof	

				Methodology **/ NPTEL** course **June 2021**	2018	PSGIMSR ,Coimbatore General Surgery , Asst. Prof	4. Anal canal- Anatomy and disorders 5. Neck secondaries
					2017	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
13	Dr Kamalraj S MBBS,MS (Gen.Surgery) TNMC Reg No 100677	Assistant Professor 28.04.2018	Regular	RBC course: 27.8.2018 to 30.8.2018 Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof.	No. = 11 Topics covered 1. Inguinal hernia 2. Breast carcinoma 3. Volvulus and intussusceptions 4. Urinary Bladder- Surgical anatomy/innervations/congenital defects/bladder trauma 5. Wound, Tissue repair and scars 6. Principles of Ethics in surgery 7. Nutrition and fluid therapy 8. Cyst, ulcer and sinus 9. Anaesthesia- Principles of general, Regional ad local anaesthesia 10. Blood and blood components- Indications and appropriate use of blood and blood products, complications of blood transfusion 11. Benign breast disease
					2020	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
					2019	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
					2018	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
					2017	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	

14	Dr Abraham Benjamin MBBS,MS (Gen.Surgery) TNMC Reg No 104697	Assistant Professor 01.10.2019	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery, Asst. Prof.	No. = 3 Topics covered 1. Oesophagus- Benign disorders 2. Urinary system- Causes, investigations and management 3. Blood and blood components- Indications and appropriate use of blood and blood products, complications of blood transfusion
					2020	PSGIMSR ,Coimbatore General Surgery, Asst. Prof	
					2019	PSGIMSR ,Coimbatore General Surgery, Asst. Prof	
					2018	PSGIMSR ,Coimbatore General Surgery, Senior Resident	
					2017	PSGIMSR ,Coimbatore General Surgery, Senior Resident	
15	Dr Priya S MBBS,MS (Gen.Surgery) TNMC Reg No 94200	Assistant Professor 01.01.2020	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR, Coimbatore General Surgery, Asst. Prof.	No. = 4 Topics covered 1. Carcinoma prostate 2. Disorders of adrenal medulla 3. Fluid and electrolyte and acid balance 4. Shock
					2020	PSGIMSR, Coimbatore General Surgery, Asst. Prof	
					2019	PSGIMSR, Coimbatore General Surgery, Senior Resident	
					2018	PSGIMSR, Coimbatore General Surgery, Senior Resident	
					2017	PSGIMSR, Coimbatore General Surgery, Senior Resident	
16	Dr Tracy Rosalin	Assistant	Regular	RBC course:	2021	PSGIMSR, Coimbatore	No. = 7

	Poulose MBBS,MS (Gen.Surgery) TNMC Reg No 109221	Professor 01.03.2021		Research Methodology / NPTEL course		General Surgery, Asst. Prof.	Topics covered 1. Benign thyroid disorders 2. Varicose veins 3. Benign breast disease 4. Wound, tissue repair and scar 5. Gangrene/Amputation 6. Congenital anomalies of genitourinary system-Clinical features, investigations and management 7. Vascular disease- Applied anatomy of venous system of lower limb
					2020	PSGIMSR, Coimbatore General Surgery, Senior Resident	
					2019	PSGIMSR, Coimbatore General Surgery, Senior Resident	
						KMCH, Coimbatore General Surgery, Senior Resident	
					2018	PSGIMSR, Coimbatore General Surgery, Junior Resident.	
					2017	PSGIMSR ,Coimbatore General Surgery, Junior Resident.	
17	Dr S Syed Mohamed Ashiq MBBS,MS (Gen.Surgery) TNMC Reg No 98195	Assistant Professor 01.03.2021	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	No. = 4 Topics covered 1. Anal fissure, Anal Warts/Anal neoplasms 2. Thyroid – Hypothyrodism 3. Hyperthyroidism
					2020	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
					2019	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
					2018	Saveetha Medical	

						College,Chennai,General Surgery-Senior Resident	4. Hydrocele/Epidymo orchitis
					2017	Sree Balaji Medical College, Junior Resident	
18	Dr R Sivakumar MBBS,MS (Gen.Surgery) TNMC Reg No 93861	Assistant Professor	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR, Coimbatore General Surgery, Asst. Prof	No. = 9 Topics covered 1. Liver trauma 2. Salivary gland disorders 3. Carcinoma penis 4. Acute peritonitis 5. Cholecystitis 6. Gall bladder disorders 7. Burns 8. Benign disorders of thyroid 9. Shock
					2020	PSGIMSR, Coimbatore General Surgery, Asst. Prof	
					2019	PSGIMSR, Coimbatore General Surgery, Asst. Prof	
					2018	Tamilnadu Govt. Service	
					2017	JIPMER, General Surgery, Senior Resident	
19	Dr S Mahendran MBBS,MS (Gen.Surgery) TNMC Reg No 94936	Assistant Professor	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	No. = 4 Topics covered 1. Gastric out let obstruction 2. Breast- Carcinoma of the breast 3. Inguinal hernia 4. Surgical infections- Etiology/Pathogenesis and management
					2020	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
					2019	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
					2018	Govt. medical College,Kozhigode,General surgery, Senior Resident	
					2017	Govt. medical College,Kozhigode,General surgery, Senior Resident	

20	Dr Yuvaraj Karthick MBBS, MS (Gen. surgery) TNMC Reg No 122519	Assistant Professor	Regular	RBC course: 12.4.21 to 14.4.2021 Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	No. = 6 Topics covered 1. Breast- Anatomy, Investigations, Nippledisorders 2. Acute peritonitis 3. Anal Cana-Surgical anatomy, Examination, congenital abnormalities 4. Wound, tissue repair and scar 5. Inguinal hernia 6. Parathyroid gland disorders
					2020	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
						Amitha School of Medicine, General surgery,Asst. Prof	
					2019	Amitha School of Medicine, General surgery,Asst. Prof	
					2018	Amitha School of Medicine, General surgery,Asst. Prof.	
					2017	Amitha School of Medicine, General surgery,Asst. Prof.	
						PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
21	Dr Balamurugan MBBS, MS (Gen. surgery) TNMC Reg No 91836	Assistant Professor 01.01.2020	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	No. = 2 Topics covered 1. Thyroid- Hypothyroidism, Thyroiditis 2. Breast- Carcinoma of the breast
					2020	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
					2019	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
					2018	-	
					2017	-	

22	Dr P Suganth Sarvesh MBBS, MS (Gen. surgery) TNMC Reg No 107011	Assistant Professor 01.11.2021	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	No. = 4 Topics covered 1. Portal hypertension 2. 2. Portal hypertension 3. Benign breast disorders 4. Perioperative management-Principles of surgical procedures
					2020	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	
					2019	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
					2018	PSGIMSR ,Coimbatore General Surgery ,Junior Resident	
					2017	PSGIMSR ,Coimbatore General Surgery ,Junior Resident	
23	Dr V Naveen Kumar MBBS, MS (Gen. surgery) TNMC Reg No 107013	Assistant Professor 01.11.2021	Regular	RBC course: Research Methodology / NPTEL course	2021	PSGIMSR ,Coimbatore General Surgery ,Asst. Prof	No. = 3 Topics covered 1. Oral malignancy- Clinical presentation and diagnosis management 2. Skin sutures and suture materials 3. Skin and subcutaneous tissue- clinical features and management of various cutaneous and subcutaneous infections
					2020	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
					2019	PSGIMSR ,Coimbatore General Surgery ,Senior Resident	
					2018	PSGIMSR ,Coimbatore General Surgery ,Junior Resident	
					2017	PSGIMSR ,Coimbatore General Surgery ,Jr.Resident	

DEPARTMENT OF ORTHOPAEDICS

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr. Dinakar rai BK D. Ortho, MS Ortho TNMC Reg: 73800	Professor 01.09.2000	Regular	RBC course: 27.8.2018 to 29.8.2018 Research Methodology / NPTEL course:	2021	PROFESSOR & HEAD, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.Garre's osteomyelitis and brodie's abscess, atypical/ fungal osteomyelitis / salmonella osteomyelitis 2.Perthes diseases 3. Lumbosacral plexus and sciatic nerve course
					2020	PROFESSOR& HEAD, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	PROFESSOR& HEAD, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2018	PROFESSOR& HEAD, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2017	PROFESSOR& HEAD, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	

2	Dr. Arvind Kumar S M MS Ortho TNMC Reg: 62553	Professor 01.06.2014	Regular	RBC course: 27.8.2018 to 29.8.2018 Research Methodology / NPTEL course:	2021	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 2 Topics covered: 1.Septic arthritis-adult and neonate / infant and its sequel 2.SCFE Median nerve and ulnar nerve
					2020	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2018	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2017	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
3	Dr. N. Venkatesh Kumar D.Ortho, DNB Ortho TNMC Reg: 57659	Professor 01.06.2017	Regular	RBC course: Research Methodology / NPTEL course:	2021	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.Introduction and overview of skeletal TB
					2020	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG	

						IMSR, COIMBATORE	2.Congenital Coxa Vara 3. Foot drop – etiology and treatment approach
					2018	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2017	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
4	Dr. Major K Kamalanathan D. Ortho, DNB Ortho, MS Gen Sur TNMC Reg : 37633	Professor 31.10.2014	Regular	RBC course: Research Methodology / NPTEL course:	2021	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.TB of hip/ Knee 2.Introduction skeletal dysplasia 3.Poly trauma & concepts of triage system. Note on Damage control Orthopaedics
					2020	PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	-	
					2018	-	
					2017	-	
5	Dr. Prasanna. C MS Ortho	Associate Professor	Regular	RBC course: Research Methodology /	2021	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 2 Topics covered:

	TNMC Reg: 74342	01.06.2014		NPTEL course:	2020	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	1.Spaces of hand and web space infection 2.Approach to Achondroplasia & related topics
					2019	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2018	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2017	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
6	Dr. Sandeep MMR MS Ortho TNMC Reg: 136311	Associate Professor 01.05.2017	Regular	RBC course: 12.4.21 to 14.4.21 Research Methodology / NPTEL course:	2021	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.Complications of fracture elaborate on compartment syndrome
					2020	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	

					2018	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	2.Tuberculosis of spine Part I 3.Outline of metabolic disorders and approach on work up
					2017	ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, MAHATMA GANDHI MEDICAL COLLEGE & RESEARCH INSTITUTE, PUDHUCHERRY	
						ASSOCIATE PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
7.	Dr. Sathyamurthy. P MS Ortho TNMC Reg: 94106	Assistant Professor 22.12.2017	Regular	RBC course: 12.4.21 to 14.4.21 Research Methodology / NPTEL course:	2021	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.Fracture healing and factors influencing fracture healing 2.Osteomyelitis – Pathophysiology,
					2020	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	

							elaborate onAcuteosteomyelitis 3.Diaphysial aclasis, Osteopetrosis
					2018	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2017	SENIOR RESIDENT, DEPT OF ORTHOPAEDICS, VMMC, SAFDARJUNG HOSPITAL, NEW DELHI	
8.	Dr. V. Chandan Noel MS Ortho TNMC Reg: 92352	Assistant Professor 01.06.2019	Regular	RBC course: Research Methodology / NPTEL course:	2021	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.Principles of Internal fixation 2.Tuberculosis of spine Part II 3.Pagets disease and Fibrous dysplasia
					2020	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2018	SENIOR RESIDENT, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2017	ARTHROSCOPY FELLOWSHIP AT ORTHO	

						ONE HOSPITAL	
9.	Dr. C. J Arun Raja D .Ortho, DNB Ortho TNMC Reg: 75397	Assistant Professor 01.11.2019	Regular	RBC course: Research Methodology / NPTEL course:	2021	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.Introduction to septic arthritis 2.CDH Part I 3. Parathyroid disorders
					2020	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	SENIOR RESIDENT, KARPAGAM FACULTY OF MEDICAL SCIENCES, COIMBATORE	
					2018	SENIOR RESIDENT, KARPAGAM FACULTY OF MEDICAL SCIENCES, COIMBATORE	
					2017	SENIOR RESIDENT, KARPAGAM FACULTY OF MEDICAL SCIENCES, COIMBATORE	

10.	Dr. Anupam Gupta D .Ortho, DNB Ortho TNMC Reg : 51753	Assistant Professor 01.08.2007	Regular	RBC course: Research Methodology / NPTEL course:	2021	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.Evolution and classification overview o f GCT, Osteosarcoma and bone tumor 2.Osteoporosis 3. Scurvy, flurosis, hypervitaminosis D
					2020	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, IQ CITY MEDICAL COLLEGE	
					2018	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, TAGORE MEDICAL COLLEGE	
					2017	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, TAGORE MEDICAL COLLEGE	

11.	Dr. Reguvaran N J MS Ortho TNMC Reg: 103355	Assistant Professor 02.07.2018	Regular	RBC course: Research Methodology / NPTEL course:	2021	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 4 Topics covered: 1.Chronic osteomyelitis - difference types sequestrum 2.Osteogenesis imperfecta 3. Osteoporosis 4. Etiology and approach to genu valgus and varum
					2020	ASSISTANT PROFESSOR, VELAMMAL MEDICAL COLLEGE, MADURAI	
					2019	ASSISTANT PROFESSOR, VELAMMAL MEDICAL COLLEGE, MADURAI	
					2018	ASSISTANT PROFESSOR, MAHATMA GANDHI MEDICAL COLLEGE	
					2017	SENIOR RESIDENT, MAHATMA GANDHI MEDICAL COLLEGE	

12.	Dr. K. Vivek MS Ortho TNMC Reg: 113406	Assistant Professor 01.11.2021	Regular	RBC course: Research Methodology / NPTEL course:	2021 NOV	ASSISTANT PROFESSOR, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	No. = 3 Topics covered: 1.CTEV 2.Etiology and approach to genu valgus and varum 3.Osteogenesis imperfecta
					2021	SENIOR RESIDENT, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2020	SENIOR RESIDENT, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2019	SENIOR RESIDENT, DEPT OF ORTHOPAEDICS, PSG IMSR, COIMBATORE	
					2018	-	
					2017	-	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF OTO-RHINO-LARYNGOLOGY

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr.S.Palaninathan, MS(ENT), TNMC Reg. No - 29364	Professor & HOD of ENT 01.04.2011	Permanent	RBC course: December 2017 Research Methodology / NPTEL course: Not Applicable	2021	PSGIMSR, DEPARTMENT OF ENT, PROF. & HOD	No. = 21 Topics covered: **Theory:** 1. Tumors of Pharynx 2. Neck swelling 3. Anatomy of& Physiology of Esophagus 4. CA Larynx 5. Laryngeal infection 6. Voice disorders & vocal cord paralysis **Clinics:** Symptoms in Ear Examination of Ear
					2020	PSGIMSR, DEPARTMENT OF ENT, PROF. & HOD	
					2019	PSGIMSR, DEPARTMENT OF ENT, PROF. & HOD	
					2018	PSGIMSR, DEPARTMENT OF ENT, PROF. & HOD	
					2017	PSGIMSR, DEPARTMENT OF ENT, PROF. & HOD	

							Examination of Nose Examination of Throat Case Discussion - CSOM Bedside Teaching Xrays Common Diseases of ENT Seminar Discussion
2	Dr.A.Dayanand, DLO, DNB TNMC Reg. No - 60476	Associate Professor, 01.01.2013	Permanent	RBC course: 10th, 11th, 12th April'2017 Research Methodology / NPTEL course: NA	2021	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	No. = 22 Topics covered: **THEORY:** 1. Introduction to ENT 2. CSOM 3. Meneire's Disease 4. Nasal Polyps 5. FESS 6. Anatomy of Larynx 7. Neck spaces **CLINICS:**
					2020	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	
					2019	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	
					2018	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	

					2017	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	Introduction History Taking Symptoms in Ear Small group Teaching Examination of Nose Examination of Throat Case Discussion - Adenitonsilitis OSCE
3	Dr.V. Ravisankar, MS(ENT) , DNB, TNMC Reg. No - 69362	Associate Professor 01.10.2012	Permanent	RBC course: Research Methodology / NPTEL course:	2021	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	No. = 20 Topics covered: **THEORY:** 1. Anatomy of the Ear 2. Complications of CSOM 3. Operative Surgical Procedure in Ear 4. Acute & Chronic Sinusitis &
					2020	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	
					2019	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	

					2018	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	Complications 5. Physiology of the Ear 6. Granulomatous disease of Nose **CLINICS:** Introduction History Taking Symptoms in Ear Examination of Ear Examination of Nose Examination of Throat Case Discussion –Nasal Polyps Seminar Discussion
					2017	PSGIMSR, DEPARTMENT OF ENT, ASSOCIATE PROFESSOR	
4	Dr.K. Karthikeyan, DLO,DNB,MNAMS., TNMC Reg. No - 68220	Assistant Professor	Permanent	RBC course: 12th, 13th, 14th April'2021 8th & 9th October '2021	2021	PSGIMSR, DEPARTMENT OF ENT, ASSISTANT PROFESSOR	No. = 21 Topics covered: **THEORY:** 1. Disease of External Ear 2. Otosclerosis 3. Anatomy & Physiology
					2020	PSGIMSR, DEPARTMENT OF ENT, ASSISTANT PROFESSOR	
					2019	-	
					2018	-	

				Research Methodology / NPTEL course: Mar-Jun'2021	2017	-	of Nose 4. Neoplasms of Nasal cavity & PNS 5. Tonsillitis & Adenoid hypertrophy **CLINICS:** 1. Symptoms in Ear 2. Examination of Ear 3. Examination of Nose 4. Examination of Throat 5. Case Discussion – Ca Larynx 6. Seminar Discussion 7. XRays 8. OSCE 9. Osteology of Skull & facial bones 10. Radiology of Ear, PNS & Neck

PSG Institute of Medical Sciences & Research

DEPARTMENT OF OPHTHALMOLOGY

SERVICE PARTICULARS

Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
Dr. K.Divya MS(Ophthalmology), DNB TNMC REG. NO: 72387	Professor& HOD, Promoted on 01.08.20 as Professor As HOD24.02.2021	Permanent	RBC course: 3rd to 5thMarch2020 CMC Vellore 12 -14, Dec 2017 Research Methodology / NPTEL course: Not applicable	2021	Professor & Head of the Department of Ophthalmology PSGIMSR.	No. 7 Topics covered: 2018 Regular batch 1. Anatomy of Uveal tract 2. Uveitis- classification, clinical features and management 3. Congenital anomalies ClinicsNo.12 (4X 3 batches) Topics covered 1. Revision of Anatomy, embryology and Physiology of eye
				2020	Professor Department of Ophthalmology PSGIMSR.	
				2019	Associate Professor Department of Ophthalmology PSGIMSR.	
				2018	Associate Professor Department of Ophthalmology PSGIMSR.	
				2017	Associate Professor Department of Ophthalmology PSGIMSR.	

						2. Clinical evaluation of patient with cataract I 3. Evaluation of patient with red eye II 4. Evaluation and management of patient with corneal opacity
Dr. D. Sundar, MS (Ophthalmology), DO TNMC REG. NO: 37979	Professor, Promoted on 01.07.99 as Professor	Permanent	RBC course: 12 -14, Dec. 2017 Research Methodology / NPTEL course: Not applicable	2021	Professor Department of Ophthalmology PSGIMSR.	No. 8 Topics covered: 2018 Regular batch 1. Anatomy and Embryology 2. Sclera and Vitreous 3.Retina Clinics No.12 (4 X 3 batches)
				2020	Professor & Head of the Department of Ophthalmology PSGIMSR.	
				2019	Professor & Head of the Department of Ophthalmology PSGIMSR.	
				2018	Professor & Head of the Department of Ophthalmology PSGIMSR.	

				2017	Professor & Head of the Department of Ophthalmology PSGIMSR.	Topics covered 1.History taking in ophthalmology 2.Clinical evaluation of patient with cataract II 3.Evaluation of lid swelling 4.Evaluation of patient with squint I
Dr. S. Padmanaban MS(Ophthalmology), DO, TNMC REG. NO: 39587	Associate Professor	Permanent	RBC course: 23rd to 25th Jan 2018 Research Methodology / NPTEL course: Not applicable	2021	Associate Professor Department of Ophthalmology PSGIMSR.	No. 7 Topics covered: 2018 Regular batch 1.Lens Anatomy, 2. Embryology, 3. Physiology, diseases (cataract)and developmental anomalies Clinics No.12 (4 X 3 batches)
				2020	Associate Professor Department of Ophthalmology PSGIMSR.	
				2019	Associate Professor, Department of Ophthalmology PSGIMSR. (12.11.19) Associate Professor, Coimbatore Medical College, Coimbatore, Ophthalmology, (19.01.17 to 30.09.2019)	
				2018	Associate Professor & Head of the	

					Department of Ophthalmology, Coimbatore Medical College, Coimbatore, Ophthalmology, (19.01.17 to 30.09.2019)	Topics covered 1.Clinical examination of Eye I 2.Surgical Management of cataract I 3.Evaluation of patient with watering eye 4.Evaluation of patient with squint II
				2017	Associate Professor & Head of the Department of Ophthalmology, Coimbatore Medical College, Coimbatore, Ophthalmology, (19.01.17 to 30.09.2019)	
Dr.Vidhyadevi.R DO(Ophthalmology), DNB TNMC REG. NO: 70298	Assistant Professor, 18.11.19	Permanent	RBC course: - Research Methodology / NPTEL course: Basic Course in Biomedical Research: 24 Oct 2021	2021	Assistant Professor Department of Ophthalmology PSGIMSR.	No. 9 Topics covered: 2018 Regular batch 1.Lacrimal Apparatus, 2.Lids 3. Conjunctiva 4.Surgical procedures in Ophthalmology 5. Red Eye
				2020	Assistant Professor Department of	

					Ophthalmology PSGIMSR	6.Keratitis, Keratoconus.
				2019	Assistant Professor Department of Ophthalmology PSGIMSR	Clinics No.12 (4 X 3 batches)
				2018	-	Topics covered
				2017	Senior Resident, Department of Ophthalmology PSGIMSR. (07.8.15 to 30.11.17)	1.Clinical examination of Eye II 2.Surgical Management of cataract II 3.Evaluation of case of ptosis 4.Evaluation of patient with anterior uveitis
Dr. Swetha. M.H MS(Ophthalmology), DNB, Fellowship in Cornea and Micro Surgery TNMC REG. NO: 152027	Assistant Professor, 09.03.20	Permanent	RBC course: - Research Methodology / NPTEL course: Not applicable	2021	Assistant Professor Department of Ophthalmology PSGIMSR	No. 6 Topics covered: 2018 Regular batch 1.Cornea 2.Hypopyon 3.Retinitis Pigmentosa
				2020	Assistant Professor (09.03.20 to till date) Department of Ophthalmology PSGIMSR.	
				2019	-	
				2018	-	

				2017	Senior Resident (01.02.17 to 14.11.17) Kasturba Medical College, Mangalore, Ophthalmology, Senior Resident (17.08.15 to 29.01.17) Government Medical College, Kozhikode, Ophthalmology,	4.Glaucoma 5.Optic Atrophy Clinics No.12 (4 X 3 batches) Topics covered 1.Investigations and procedures in ophthalmology I 2.Complications of cataract surgery 3.Evaluation of case of entropion, ectropion Bells palsy 4.phthalmic evaluation in patients with systemic diseases

Dr. Saudhamini. S MS(Ophthalmology), DNB.,Fellowship in Clinical Glaucoma TNMC REG. NO: 153544	Senior Resident, 23.02.21	Permanent	RBC course: - Research Methodology / NPTEL course: Not applicable	2021	Senior Resident, Department of Ophthalmology PSGIMSR. From 23.02.21 to till date	No. 3 Topics covered:3 2018 Regular batch 1. Glaucoma I 2. Glaucoma II 3.Glaucoma III Clinics No.9 (3 X 3 batches) Topics covered 2018 Regular batch 1.Investigations and procedures in ophthalmology II 2.Pre OP evaluation and Post operative case of cataract 3.Evaluation of patient with corneal ulcer I
				2020	-	
				2019	-	
				2018	Senior Resident, Gandhi Medical college, Secunderabad, Ophthalmology, (from 08.08.2017 to 31.03.2018)	
				2017	Senior Resident, Gandhi Medical college, Secunderabad, Ophthalmology, (from 08.08.2017 to 31.03.2018) Junior Resident, Gandhi Medical college, Secunderabad, Ophthalmology, (from 10.07.14 to 09.07.2017)	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

SERVICE PARTICULARS

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr Lathamaheswari. S DGO,DNB TNMC Reg 54416	Professor & Head of the Department 01.02.2010	Regular	RBC course: 12th to 14th Dec, 2017 Research Methodology / NPTEL course:	2021	PSG IMSR, Obstetrics &Gynaecology, Professor & HOD	No. =6 Topics covered: 1. Fetus in Utero 2. Ectopic Pregnancy 3. Ovarian tumours 4. Hypertensive disorders in Pregnancy 5. CIN & cervical cancer 6. Preterm labour
					2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Professor	
2	Dr Seetha Panicker MD, DNB TNMC Reg 38408	Professor 01.09.2000	Regular	RBC course: 20th to 22, Sep, 2016 Research Methodology / NPTEL course:	2021	PSG IMSR, Obstetrics &Gynaecology, Professor Professor	No.= 6 Topics covered: 1. Occipito posterior position 2. Gynaec Ca screening modalities – Pap smear, LBC, colposcopy 3. Heart disease in Pregnancy 4. Rh negative pregnancy 5. Gynaec Ca screening modalities – Pap smear, LBC, colposcopy (2018 Add Batch) 6. Ectopic pregnancy
					2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Professor Professor& HOD	

3	Dr Chitra T V MD, DNB TNMC Reg 38407	Professor 01.09.2002	Regular	RBC course: 31st Mar,1st& 2nd Apr, 2015 Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Professor	No. = 6 Topics covered: 1. Breech presentation 2. Anemia in pregnancy 3. Abortion & MTP 4. AUB 5. UV prolapse 6. GTD
4	Dr Reena Abraham DGO, MD TNMC Reg 108163	Professor 01.04.2006	Regular	RBC course: 10th to 12th Apr, 2017 Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Professor	No. = 5 Topics covered: 1. Pelvis and skull 2. Placenta previa 3. CPD 4. Fibroid uterus 5. Classify, define and discuss the aetiology and management of abortions including threatened, incomplete, inevitable, missed and septic abortion
5	Dr Kanchanamalai	Professor	Regular	RBC course:	2021	PSG IMSR, Obstetrics	No. = 4

	MD TNMC Reg 30488	01.04.2008		23rd to 25th June 2014 Research Methodology / NPTEL course:	2020 2019 2018 2017	&Gynaecology, Professor	Topics covered: 1. Diabetes in pregnancy 2. Infertility 3. Postmenopausal bleeding 4. Describe, discuss and demonstrate the clinical features of pregnancy, derive and discuss its differential diagnosis, elaborate the principles underlying and interpret pregnancy tests.
6	Dr Ramya MD, DNB TNMC Reg 62902	Professor 01.04.2019	Regular	RBC course: 12th to 14th Dec, 2017 Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Professor PSG IMSR, Obstetrics &Gynaecology, Associate Professor	No. = 6 Topics covered: 1. Transverse lie, cord presentation & compound presentation 2. USG in Obstetrics 3. PID 4. GDM 5. USG in Obstetrics (2018 Add Batch) 6. Previous LSCS
7	Dr Poornima C MS TNMC Reg 100091	Assoc. Professor 01.09.2012	Regular	RBC course: 10th to 12th Apr, 2017 Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Assoc. Professor	No. = 5 Topics covered: 1. Preterm labour 2. Fetal Growth Restriction 3. Diabetes in pregnancy 4. Describe and discuss the physiology of puberty, features of abnormal puberty, common problems and their management 5. Enumerate the causes and describe the

							investigations and management of hyperandrogenism
8	Dr Chippy Tess Mathew MD TNMC Reg 107766	Assoc. Professor 01.12.2012	Regular	RBC course: 31st Mar,1st& 2nd Apr, 2015 Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Assoc. Professor	No. = 5 Topics covered: 1. Conduct of labour 2. Abnormal uterine bleeding 3. Placenta Previa 4. Endometriosis 5. Describe and discuss the common causes pathogenesis, clinical features, differential diagnosis, investigations, principles of management of fertility
9	Dr Lalitha N MD, MRCOG TNMC Reg 65300	Assoc. Professor 01.01.2020	Regular	RBC course: 12th to 14th Apr, 2021 Research Methodology / NPTEL course: Mar-June, 2021	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Assoc. Professor PSG IMSR, Obstetrics &Gynaecology, Asst. Professor	No. = 4 Topics covered: 1. Physiology of pregnancy 2. Laparaoscopy and hysterectomy 3. Fertilisation& Implantation 4. Define, classify and discuss abnormal uterine bleeding, its aetiology, clinical features
10	Dr Maheswari S MD TNMC Reg 78424	Assoc. Professor 01.01.2020	Regular	RBC course:27th to 29th Aug, 2018 Research Methodology /	2021 2020 2019	PSG IMSR, Obstetrics &Gynaecology, Assoc. Professor PSG IMSR, Obstetrics	No. = 5 Topics covered: 1. Physiology of labour 2. Heart disease in pregnancy

				NPTEL course:	2018 2017	&Gynaecology, Asst. Professor	3. Hypertensive disorders in pregnancy 4. Postdated pregnancy 5. Normal puerperium
11	Dr Pooranidevi A MD TNMC Reg 77886	Asst. Professor 01.06.2016	Regular	RBC course: Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Asst. Professor	No. = 6 Topics covered: 1. Face & Brow presentation 2. Abruptio Placenta 3. History taking – Antenatal case 4. Placenta 5. Describe and discuss the physiology of lactation 6. Introduction to contraception, counseling for FP, Natural FP and barrier methods
12	Dr Krishnapriya MS,DGO,DNB TNMC Reg 78907	Asst. Professor 01.09.2016	Regular	RBC course: 12th to 14th Apr, 2021 Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics &Gynaecology, Asst.Professor	No. = 5 Topics covered: 1. Physiology of labour 2. Primary amenorrhoea 3. Hormonal contraceptives, IUCD, sterilisation 4. Describe and discuss the etiology, pathology, clinical features, differential diagnosis, investigations, management and long term implications of genital tubeculosis 5. Describe and discuss the etiopathogenesis; clinical features; differential diagnosis; investigations; management, complications of PCOS

13	Dr Sumangali. T MS TNMC Reg 75422	Asst. Professor 01.01.2018	Regular	RBC course: Research Methodology / NPTEL course: 9th& 10th Sep, 2016	2021 2020 2019 2018	PSG IMSR, Obstetrics &Gynaecology, Asst.Professor	No. = 4 Topics covered: 1. Applied anatomy – female genital tract 2. Endometriosis 3. Vesicular mole and other GTD 4. IUCD, Hormonal contraceptives
					2017	PSG IMSR, Obstetrics &Gynaecology, Sr. Resident	
14	Dr Anupriya. K MD TNMC Reg 80894	Asst. Professor 01.09.2018	Regular	RBC course: Research Methodology / NPTEL course:	2021 2020 2019 2018	PSG IMSR, Obstetrics &Gynaecology, Asst.Professor	No. = 7 Topics covered: 1. History taking – Gynaec case 2. GTN 3. Secondary amenorrhoea 4. Applied anatomy – female genital tract 5. Normal mullerian development and sex differentiation 6. Describe and discuss the etiology, pathology, clinical features, differential diagnosis, investigations, management and long term implications of sexually transmitted infections 7. Describe and discuss the etiology, pathology, clinical features, differential diagnosis, investigations, management of long term implications of HIV
					2017	PSG IMSR, Obstetrics &Gynaecology, Senior Resident	
15	Dr Savitha. V MD TNMC Reg 78908	Asst. Professor 01.01.2020	Regular	RBC course: Research Methodology /	2021 2020	PSG IMSR, Obstetrics &Gynaecology, Asst.Professor	No. = 7 Topics covered: 1. Postdated pregnancy
					2019	PSG IMSR, Obstetrics	

				NPTEL course:	2018 2017	&Gynaecology, Senior Resident	2. Sterilisation (Male and female) 3. Conduct of labour 4. Menopause and HRT 5. Abnormal mullerian development 6. Primary and secondary amenorrhoea 7. Premalignant lesions of cervix
16	Dr Aswini. P DGO,DNB TNMC Reg 81194	Assistant Professor 01.11.2021	Regular	RBC course: Research Methodology / NPTEL course:	2021 Dec 2020 – 21 Nov 2019 2018 2017	PSG IMSR, Obstetrics & Obstetrics &Gynaecology, Asst. Professor PSG IMSR, Obstetrics & Obstetrics &Gynaecology, Senior Resident Karpagam Faculty of Medical Sciences & Research, Obstetrics &Gynaecology, Senior Resident -	No. = 6 Topics covered: 1. Introduction to contraception, counseling for FP, natural FP & barrier methods 2. Normal puerperium & Physiology of lactation 3. STD 4. Diabetes in pregnancy 5. Abruptio placenta 6. Describe the clinical characteristics of physiological vaginal discharge
17	Dr Vishranthi MD TNMC Regn.No:71870	Senior Resident 08.10.2019	Regular	RBC course: Research Methodology / NPTEL course:	2021 2020 2019 2018 2017 2017	PSG IMSR, Obstetrics & Gynaecology, Senior Resident - - -	No. = 4 Topics covered: 1. Induction of labour 2. Discuss the aetiology, clinical features, differential diagnosis of acute abdomen in early pregnancy (with a focus on ectopic pregnancy) and enumerate the principles of medical and surgical management 3. Enumerate and discuss the physiology of normal

							labour, mechanism of labour in occipito-anterior presentation; monitoring od labour including partogram 4. Gynaec Ca screening modalities – VIA, VILI, Pap smear, LBC, Colposcopy, Tumour markers
18	Dr Kanmani. M MS TNMC Regn.No:112609	Senior Resident 02.09.2020	Regular	RBC course: Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics & Gynaecology, Senior Resident - - -	No. = 5 Topics covered: 1. Normal mullerian development and sex differentiation 2. Placenta 3. Physiology of pregnancy 4. Normal puerperium & Physiology of lactation 5. Reproductive physiology
19	Dr Bavanisree. P MS TNMC Regn.No:111055	Senior Resident 21.09.2020	Regular	RBC course: Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics & Gynaecology, Senior Resident MAPIMS, OBG, Senior Resident - -	No. = 5 Topics covered: 1. Physiology of pregnancy 2. Describe the physiology of ovulation, menstruation, fertilization, implantation and gametogenesis 3. Describe and discuss the basic embryology of fetus, factors influencing fetal growth and development, anatomy and physiology of placenta, and teratogenesis 4. Describe and discuss the development and anatomy of the female reproductive tract,

							relationship to other pelvic organs, applied anatomy as related to Obstetrics and Gynaecology. 5. Describe and discuss the changes in the genital tract, cardiovascular system, respiratory, haematology, renal and gastrointestinal system in pregnancy
20	Dr Aneri Parikh MS G-60009 G-27945	Senior Resident 11.03.21	Regular	RBC course: Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics & Gynaecology, Senior Resident GMERS Medical College, GOTRI, OBG, Senior Resident - - -	No. = 5 Topics covered: 1. Diagnosis of pregnancy 2. Physiology of labour 3. Pubertal and adolescent problems 4. Enumerate, describe and discuss the objectives of antenatal care, assessment of period of gestation, screening for high-risk factors 5. Describe the etiopathology, impact on maternal and fetal health and principles of management of hyperemesis gravidarum
21	Dr Athilakshmi MS TNMC Regn.No:78067	Senior Resident 12.07.2021	Regular	RBC course: Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Obstetrics & Gynaecology, Senior Resident - - - -	No. = 2 Topics covered: 1. Primary amenoorhoea 2. Describe and discuss the physiology of menopause, symptoms, prevention, management and the role of hormone replacement therapy

22	Dr Vishnupriya MS TNMC Regn.No:106963	Senior Resident 15.11.2021	Regular	RBC course: Research Methodology / NPTEL course:	2021	PSG IMSR, Obstetrics & Gynaecology, Senior Resident	No. = 2 Topics covered: 1. Tuberculosis of genital tract 2. Enumerate and discuss the diameters of maternal pelvis and types
					2020	GMC, MANJERI, OBG, Sernior Resident	
					2019	-	
					2018	-	
					2017	-	

DEPARTMENT OF ANAESTHESIOLOGY

SERVICE PARTICULARS

S. No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	Dr.C.Ganesan Professor & Head TNMC Reg No 49255	Professor & Head 1.1.2009	Regular& Permanent	RBC course: 20 -22 Sep 2016 Research Methodology / NPTEL course: Not Applicable	2021 2020 2019 2018 2017	PSG IMSR, Department of Anaesthesiology, Professor&Head .	No. = 10 Topics covered: 1.Valvular heart diseases & Anesthesia 2.Cardiopulmonary bypass 3.Anesthesia & burns 4.Anemia complicating pregnancy 5.Thoracic anesthesia 6.One lung ventilation 7.IHD & Anesthesia 8.Myocardial protection 9.TOF & Anesthesia 10.PDA & Anesthesia

2	Dr.G.Dhanabagyam Professor TNMC Reg No 48894	Professor 1.7.2011	Regular & Permanent	RBC course: 10 – 12 Apr 2017 Research Methodology / NPTEL course: Not Applicable	2021 2020 2019 2018 2017	PSG IMSR, Department of Anaesthesiology, Professor	No. = 7 Topics covered: 1.Mitral stenosis 2.Case Presentation – Burns 3.PIH 4.Atrial septal defect 5.Monitoring in anaesthesia 6.Basic Life support 7.Preoperative Assessment
3	Dr.Prasanthkumar.M.S Professor TNMC Reg No 70825	Professor 15.3.2021	Regular & Permanent	RBC course: 20 -22 Sep 2016 Research Methodology / NPTEL course:	2021 2020 2019 2018 2017	PSG IMSR, Department of Anaesthesiology, Professor PSG IMSR, Department of Anaesthesiology, Associate Professor	No. = 7 Topics covered: 1.Congenital Heart Disease 2.Obstructive Jaundice 3.Case Presentation – Gestational Diabetes Mellitus 4.Tetralogy of Fallot

							5.Anaesthesia Machine checklist 6.Anaesthesia for liver transplant 7.Thromboelastogram
4	Dr.Arunkumar.R Associate Professor TNMC Reg No 66112	Associate Professor 1.10.2019	Regular & Permanent	RBC course: 12.04.2021 to 14.04.2021 Research Methodology / NPTEL course:	2021 2020 2019	PSG IMSR, Department of Anaesthesiology, Associate Professor	No. = 7 Topics covered: 1.Chronic Obstructive airway disease 2.Basic Life Support 3.Cirrhosis of Liver 4.How to perform well in university examination 5.Ischaemic Heart Disease 6.Obstructive jaundice 7.Advanced Cardiovascular Life Support
					2018 2017	PSG IMSR, Department of Anaesthesiology, Assistant Professor	
5	Dr.Silvy Anna Varughese Assistant professor TNMC Reg No 154526	Assistant professor	Regular & Permanent	RBC course: Research Methodology / NPTEL	2021 2020 2019 2018	PSG IMSR, Department of Anaesthesiology, Assistant Professor	No. = 7 Topics covered: 1.Introduction, preoperative evaluation, premedication in anesthesia

				course: December 2020	2017		2.Pulmonary Function test 3.Pacemaker & Anesthesiologist 4.Anaesthetic considerations in Liver Transplantation 5.Neonatal Resuscitation 6.Tracheal tubes 7.Intercostal Drainage
6	Dr.Dhivya Assistant professor TNMC Reg No 90013	Assistant professor 01.02.2019	Regular & Permanent	RBC course: 12.04.2021 to 14.04.2021 Research Methodology / NPTEL course:	2021	PSG IMSR, Department of Anaesthesiology, Assistant Professor	No. = 9 Topics covered: 1. Epidural Anaesthesia 2. Capnography 3. Diagnosis and treatment of arrhythmias 4. Obesity and Anaesthetic implications 5. Pathophysiology and assessment of blood loss 6. Massive blood transfusion 7. Muscle relaxants 8. Vapourizer
					2020	PSG IMSR, Department of Anaesthesiology, Assistant Professor	
					2019		
					2018	PSG IMSR, Department of Anaesthesiology, Senior Resident	
					2017		

							9. Spinal & Epidural needles
7	Dr.Aswin.A.B Assistant professor TNMC Reg No 112384	Assistant professor 1.10.2019	Regular & Permanent	RBC course: Research Methodology / NPTEL course: Aug –Dec 2020	2021 2020 2019 2018 2017	PSG IMSR, Department of Anaesthesiology, Assistant Professor PSG IMSR, Department of Anaesthesiology, Senior Resident	No. = 8 Topics covered: 1. LASER and Anaesthesia 2. One lung Ventilation and Double lumen tubes 3. Vasopressors nad Inotropes 4.Anaesthetic considerations in Renal Transplantation 5. Obstetric emergencies 6. Neuro Muscular Monitoring 7. Cerebral physiology and CSF circulation 8.Advanced Cardiac Life Support
8	Dr.JayaprakashArunachalam Assistant professor TNMC Reg No 105748	Assistant professor 1.10.2019	Regular &Permanent	RBC course: Research Methodology / NPTEL	2021 2020 2019	PSG IMSR, Department of Anaesthesiology, Assistant Professor	No. = 8 Topics covered: 1. Carbondioxide transport and Regulation of respiration 2. Hyperbaric Physiology and

				course:	2018 2017	PSG IMSR, Department of Anaesthesiology, Senior Resident	Anaesthesia 3. Regional anaesthesia and Anticoagulant 4. Renal function tests and its implications in anaesthesia 5. Blood Conservation strategies 6. Journal club 7. Circle System 8.Spinal Anaesthesia
9	Dr.Arun.P Assistant professor TNMC Reg No 106384	Assistant professor 01.09.2020	Regular & Permanent	RBC course: Research Methodology / NPTEL course:	2021 2020	PSG IMSR, Department of Anaesthesiology, Assistant Professor	No. = 8 Topics covered: 1. Regulation of Acid base balance and Interpretation of ABG 2. Nebulisation in anaesthetic practice 3. Conduct of GA and failed Intubation Drill in pregnancy 4. Neonatal emergencies and Neonatal Resuscitation 5. Neonatal emergencies 6. Factors regulating Cerebral blood flow and influence of anaesthesia on
					2019	PSG IMSR, Department of Anaesthesiology, Senior Resident	
					2018 2017	Kasturba Medical College, Junior Resident / PG	

							cerebral blood flow 7. Various approaches of tracheostomy 8.Basic Life Support
10	Dr.Renjith Viswanath Assistant professor TNMC Reg No 153206	Assistant professor	Regular & Permanent	RBC course: Research Methodology / NPTEL course:	2021	PSG IMSR, Department of Anaesthesiology, Assistant Professor	No. = 6 Topics covered: 1. CVP Monitoring 2. Pain Pathway of Labour& Methods of labour analgesia 3. Caudal Anaesthesia and JR circuit 4. Safety features in anaesthesia machine 5. Double lumen tubes &Bronchial blocker 6.Oxygen Therapy
					2020	All India Institute of Medical Sciences, New Delhi, Senior Resident	
					2019		
					2018	Amala Institute of Medical Sciences, Junior Resident/PG	
					2017		
11	Dr.Santhosh Raja Assistant professor TNMC Reg No 97713	Assistant professor 01.04.2021	Regular & Permanent	RBC course: Research Methodology / NPTEL course:	2021	PSG IMSR, Department of Anaesthesiology, Assistant Professor	No. = 4 Topics covered: 1. Pain Pathway of Labour& Methods of labour analgesia 2. Various approaches of tracheostomy
					2020	PSG IMSR, Department of Anaesthesiology, Senior Resident	

							3. Foreign body in the bronchus 4. Regional anaesthesia and Anticoagulant
12	Dr.Archana.S.K Assistant professor TNMC Reg No 90616	Assistant professor 1.11.2021	Regular & Permanent	RBC course: Research Methodology / NPTEL course:	2021	PSG IMSR, Department of Anaesthesiology, Assistant Professor	No. = 7 Topics covered: 1. Local Anaesthesia 2. Coronary circulation & factors regulating cardiac output 3. Anatomy of Lumbar plexus and lower limb blocks 4. Foreign body in the bronchus 5. Anatomical, Physiological & Pharmacological consideration in Pregnancy 6. Head Injury – Diagnosis & Management 7. ICP Monitoring
					2020 2019	PSG IMSR, Department of Anaesthesiology, Senior Resident	
					2018 2017 2019 2018 2017	PSG IMSR, Department of Anaesthesiology, Junior Resident	

DEPARTMENT OF RADIO DIAGNOSIS

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years(Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021.List the topics covered
1	Dr. Devanand. B MD (RD) TNMC Reg: 50495	Professor & Head 01.03.2006	Regular	RBC course: 12th – 14th Dec 2017 Research Methodology / NPTEL course:	2021	PSG IMSR, Radiology, Professor	No. = 15 Topics covered: 1. MRI MSK - Wrist / ankle 2. Diffusion weighted imaging 3. Chest X-ray Collapse/ Consolidation 4. USG of acute abdomen 5. USG – Elastography 6. CT Abdomen – Pancreas & Spleen
					2020	PSG IMSR, Radiology, Professor	
					2019	PSG IMSR, Radiology, Professor	
					2018	PSG IMSR, Radiology, Professor	
					2017	PSG IMSR, Radiology, Professor	

							7. CT Angiogram - Part 2 8. Introduction to Radiology 9. Onsite (CT/ MR/ USG)
2	Dr. Vikrant. K MD (RD) TNMC Reg: 76720	Professor 01.08.2020	Regular	RBC course: 20th – 22nd Sep 2016 Research Methodology / NPTEL course:	2021	PSG IMSR, Radiology, Professor	No. = 17 Topics covered: 1. MRCP 2. Fetal MRI 3. Imaging evaluation of Neck nodes 4. Radiography of skeletal trauma 5. USG Breast 6. Contrast USG 7. CT Abdomen – Trauma 8. MRI Basics – Physics
					2020	PSG IMSR, Radiology, Professor	
					2019	PSG IMSR, Radiology, Associate Professor	
					2018	PSG IMSR, Radiology, Associate Professor	
					2017	PSG IMSR, Radiology, Associate Professor	

							9. FRS (Chest/ Abdomen / Bones) 10. Contrast media 11. Ultrasonogram 12. Chest X- Ray 13. Bones and joints
3	Dr. Elango. N MD (RD) TNMC Reg: 34024	Associate Professor DOJ: 12.02.2014	Regular	RBC course: 12th – 14th Dec 2017 Research Methodology / NPTEL course:	2021	PSG IMSR, Radiology, Associate Professor	No. = 15 Topics covered: 1. Cardiac MRI – Basics protocol 2. Perfusion Imaging 3. Radiography of acute abdomen 4. USG Abdomen - common pediatric pathology 5. USG guided procedures – Part I 6. CT Abdomen – GIT 7. CT guided procedures – Biopsy / FNAC
					2020	PSG IMSR, Radiology, Associate Professor	
					2019	PSG IMSR, Radiology, Associate Professor	
					2018	PSG IMSR, Radiology, Associate Professor	
					2017	PSG IMSR, Radiology, Associate Professor	

							8. Chest X-Ray 9. Introduction to Radiology 10. X-Ray Imaging 11. Chest X-Ray, Abdomen X-Ray, X-Ray procedures
4	Dr. Rajakumar. R DNB (RD), DMRD TNMC Reg: 57622	Associate Professor DOJ: 06.01.2014	Regular	RBC course: 20th – 22nd Sep 2016 Research Methodology / NPTEL course:	2021	PSG IMSR, Radiology, Associate Professor	No. = 16 Topics covered: 1. MRS / DWI 2. Cardiac MRI – Interpretation 3. Recent Advances in MRI 4. Radiography of inflammatory arthritis 5. USG of small parts 6. USG guided procedures – Part II 7. CT Abdomen – GUT
					2020	PSG IMSR, Radiology, Associate Professor	
					2019	PSG IMSR, Radiology, Associate Professor	
					2018	PSG IMSR, Radiology, Associate Professor	
					2017	PSG IMSR, Radiology, Associate Professor	

							8. CT guided procedures –Special Neurolysis / Drainage / RFA 9. CT Scan 10. Abdomen X- Ray 11. CT and USG Basics
5	Dr. Maheswaran. V DNB (RD), PDCC TNMC Reg: 63405	Associate Professor 01.08.2020	Regular	RBC course: 27th – 30th Aug 2018 Research Methodology / NPTEL course:	2021	PSG IMSR, Radiology, Associate Professor	No. = 16 Topics covered: 1. MRI Abdomen – Bowel enteroclysis 2. MRI of gynecological malignancies 3. Mediastinal masses 4. Contrast Media 5. USG OBS – First trimester bleeding 6. CT Basics –
					2020	PSG IMSR, Radiology, Associate Professor	
					2019	PSG IMSR, Radiology, Assistant Professor	
					2018	PSG IMSR, Radiology, Assistant Professor	
					2017	PSG IMSR, Radiology, Assistant Professor	

							Physics & Equipment 7. Acute abdomen 8. MRI Basics – Equipment 9. Contrast Media 10. Bones and joints 11. MRI
6	Dr. Vijayakumar. C MD (RD), DNB, PDCC TNMC Reg: 85396	Assistant Professor 21.03.2017	Regular	RBC course: 12th – 14th April 2021 Research Methodology / NPTEL course: BCBR: Sep –Dec 2019 NPTEL: 17th June 2020	2021	PSG IMSR, Radiology, Assistant Professor	No. = 16 Topics covered: 1. Urography 2. Fusion Imaging – Technique / Applications 3. Neonadal& pediatric abdominal pathologies 4. Contrast Media – Management of complications. 5. USG OBS – Target scan 6. CT Basics –
					2020	PSG IMSR, Radiology, Assistant Professor	
					2019	PSG IMSR, Radiology, Assistant Professor	
					2018	PSG IMSR, Radiology, Assistant Professor	
					2017	PSG IMSR, Radiology, Assistant Professor	

							Technique 7. CT Thorax – Anatomy 8. MRI Basics - Protocols – Brain / spine 9. Reading Abdominal X-Ray, Abdominal USG and Abdominal CT 10. Contrast Media 11. X- Ray Procedures
7	Dr. Jayaprakash. N MD (RD) TNMC Reg: 82219	Assistant Professor 01.02.2018	Regular	RBC course: 12th – 14th April 2021 Research Methodology / NPTEL course:	2021	PSG IMSR, Radiology, Assistant Professor	No. = 14 Topics covered: 1. MRI – Liver 2. MRI prostate including spectroscopy 3. Imaging of inflammatory bowel disease
					2020	PSG IMSR, Radiology, Assistant Professor	
					2019	PSG IMSR, Radiology, Assistant Professor	
					2018	PSG IMSR, Radiology, Assistant Professor	

					2017	PSG IMSR, Radiology, Senior Resident	4. Basics of barium Procedures – Technique 5. USG TRUS & TVS – Common indications and pathology 6. Common CT protocols 7. CT Thorax – HRCT Lung 8. Reading Chest X-Ray and CT Thorax 9. X- Ray Procedures 10. USG Basics
8	Dr. Thilagarajan. S MD (RD), DNB TNMC Reg: 77073	Assistant Professor 01.10.2018	Regular	RBC course: Research Methodology /	2021	PSG IMSR, Radiology, Assistant Professor	No. 14 Topics covered: 1. MRI Defaecography 2. Glial Neoplasms 3. Acute & chronic
					2020	PSG IMSR, Radiology, Assistant Professor	
					2019	PSG IMSR, Radiology, Assistant Professor	

					2018	PSG IMSR, Radiology, Assistant Professor	cholecystitis 4. Barium Procedures in inflammatory bowel disease 5. USG Doppler studies – Part 1 6. CT Brain – Anatomy 7. CT Thorax – CTPA 8. CT Basics 9. USG Basics 10. Reading Skeletal X-Ray
					2017	PSG IMSR, Radiology, Senior Resident	
9	Dr. Arunkumar. V. B MD (RD) TNMC Reg: 94595	Assistant Professor 01.12.2020	Regular	RBC course: Research Methodology / NPTEL course:	2021	PSG IMSR, Radiology, Assistant Professor	No. = 14 Topics covered: 1. MRI MSK – Shoulder / Knee 2. Imaging of Acute Stroke
					2020	PSG IMSR, Radiology, Assistant Professor	
					2019	PSG IMSR, Radiology, Senior Resident	

					2018	PSG IMSR, Radiology, Senior Resident	3. Diffuse liver parenchymal disease 4. Fluoroscopy contrast studies GUT 5. USG Doppler studies – Part 2 6. CT Brain – Cerebro vascular disease 7. CT Thorax – Pleura & Chest wall 8. Special Procedures and Patient Preparation 9. CT Basics 10. MRI Basics and Images
					2017	PSG IMSR, Radiology, Senior Resident	

PSG Institute of Medical Sciences & Research

DEPARTMENT OF DENTAL SURGERY

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	DR.K.ABHIRAMI- MDS TNMC Reg No. 5529	ASSOCIATE PROFESSOR 01.12.2010	REGULAR/PERMANENT	MET course: 17 /01/2013 to 19/01/2013 15/09/2020 &16/09/2020 12/04/2021 to 14/04/2021 Research Methodology / NPTEL course: -	2021	PSGIMSR, DENTAL OPD, ASSOCIATE PROFESSOR	No. = 5 Topics covered: Clinical posting: 1) Aware of malocclusion and the tissues that cause it. 2) Enumerate the impact of malocclusion on aesthetics, health. 3) Identify malocclusion. 4) Counsel patients with respect to correction of malocclusion and the role it might have on oral health specifically on the TMJ. Theory class: 1) Malocclusion
					2020	PSGIMSR, DENTAL OPD, ASSOCIATE PROFESSOR	
					2019	PSGIMSR, DENTAL OPD, ASSOCIATE PROFESSOR	
					2018	PSGIMSR, DENTAL OPD, ASSOCIATE PROFESSOR	
					2017	PSGIMSR, DENTAL OPD, ASSOCIATE PROFESSOR	
2	DR.P.GOWTHAM SURESH	ASSISTANT PROFESSOR	REGULAR/PERMANENT	MET course:	2021	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	No. = 6

	MDS TNMC Reg No. 10927	01.06.2016		4/10/2017 to 6/10/2017 27/08/2018 to 29/08/2018 30/08/2018 Research Methodology / NPTEL course: -	2020	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	Topics covered: Clinical posting: 1) Enumerate the parts of the tooth and supporting structures. 2) Enumerate the common diseases that affect the periodontium and identify local and systemic causative factors. 3) Identify periodontal disease. 4) Discuss the role of periodontal disease as a focus of sepsis. 5) Counsel patients with respect to oral hygiene, diet and the direct bearing on systemic health and vice versa. Theory class: 1) Periodontal disease
					2019	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	
					2018	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	
					2017	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	
3	DR.B.DINESH BABU MDS TNMC Reg No. 14509	ASSISTANT PROFESSOR 01/09/2018	REGULAR/PERMANENT	MET course: 4/10/2017 to 6/10/2017	2021	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	No. = 6 Topics covered: Clinical posting:
					2020	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	

				12/04/2021 to 14/04/2021 Research Methodology / NPTEL course: -	2019	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	1) Discuss the various causes for partial/complete loss of teeth and associated structures. 2) Discuss the local and systemic sequelae of the above. 3) Identify complete complement of teeth and identify missing teeth. 4) Enumerate common ways of restoring the edentulous state. 5) Counsel patients on the importance of restoring missing teeth/tissues with respect to the benefits on oral and systemic health. Theory class 1) Edentulous disease
					2018	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	
					2017	PSGIMSR, DENTAL OPD, SENIOR RESIDENT	
4	DR.C.VINODHA MDS TNMC Reg No. 14316	ASSISTANT PROFESSOR 01/07/2019	REGULAR/PERMANENT	MET course: 29/10/2021 & 30/10/2021 Research Methodology / NPTEL course: -	2021	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	No. = 6 Topics covered: Clinical posting: 1) Enumerate the parts of the tooth.
					2020	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	
					2019	PSGIMSR, DENTAL OPD, ASSISTANT PROFESSOR	

					2018	PSGIMSR, DENTAL OPD, SENIOR RESIDENT	2) Discuss the role of causative microorganisms in the aetio-pathogenesis of dental caries. 3) Identify dental caries. 4) Discuss the role of dental caries as a focus of sepsis. 5) Counsel patients with respect to oral hygiene, diet and the direct bearing on systemic health. Theory class: 1) Dental caries.
					2017	PSGIMSR, DENTAL OPD, SENIOR RESIDENT	

DEPARTMENT OF PHYSICAL MEDICINE AND REHABILITATION (PMR)

SERVICE PARTICULARS

S.No	Name of the faculty, Qualification& TNMC registration number	Current Designation & Date of promotion	Nature of employment Regular/ permanent or contract/ outsourced	Mention the dates of completion of:	Details of Service in the Last 5 years (Mention name of institute, department and designation)		Number of lectures taken in 2021 until 15.12.2021. List the topics covered
1	**Dr V RAMAMOORTHY** MD., (PMR) DNB (PMR) TNMC Registration number : 45391	PROFESSOR 01-12-2011	REGULAR	RBC course: 27,28,29, & 30 August 2018 Research Methodology / NPTEL course:	2021	PSG IMS&R, PMR, PROFESSOR	No. = 3 <u>Topics covered</u>: 1. Rights of persons with disabilities 2. Disability etiquette 3. Health Insurance & Economics
					2020	PSG IMS&R, PMR, PROFESSOR	
					2019	PSG IMS&R, PMR, PROFESSOR	
					2018	PSG IMS&R, PMR, PROFESSOR	
					2017	PSG IMS&R, PMR, PROFESSOR	
					2017		

DEPARTMENT OF ANATOMY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pub med (Y/N)	Scopus (Y/N)	
1.	G.Amudha	Anila A Mathews,*, Prathyusha K, Thomas V. Chacko, Amudha GEffectiveness of interactive case-based learning in medical microbiology Journal of Education Technology in Health Sciences, September-December,2017;4(3):112-115	N	N	Index Copernicus Google Scholar
		A.K.Manicka vasuki*,G.Amudha,C.Nandhini Aishwarya, Deborah Joy Hepzibah,V.A.Kesavan, Morphometric study of Jugular foramen and Jugular fossa of dried Adult Human skulls and its clinical significance – an original article, National Journal of Clinical Anatomy,Dec'2019:8:160-164	N	N	DOAJ
		G. Amudha, Sandeep Diwan, Anatomy of Lumbar Plexus and Implications to Regional Anaesthesiologist. International Journal of Regional Anaesthesia Vol. 2 (2) July-December, 2021:17-21	N	N	Open access journal
2.	M.Jamuna	A.K.Manicka vasuki*, M.Jamuna, Deborah Joy Hepziba, M.Nirmaladevi, K.Radhika, Anatomical study of Right Coronary artery and its clinical significance – Cadaveric study – an Original article, Indian Journal of Clinical Anatomy and Physiology,June 2017,vol.4,iss.2,pp185 – 89	N	N	Index Copernicus & Google scholar
		A.K.Manicka vasuki*, M.Jamuna, Deborah Joy Hepziba, M.Nirmaladevi, Beulah Priyalatha Swamicken, K.Radhika,K.Kalyana sundaram, Anatomical study of Left Coronary artery and its clinical significance – Cadaveric study – an Original article, Journal of Clinical and Diagnostic Research, Jan 2018,vol.12(1),AC01 – AC05	N	N	DOAJ
		A.K.Manicka vasuki*, M.Jamuna, M.Nirmaladevi, Deborah Joy	N	N	Index Copernicus

		Hepziba, K.Radhika, Kailash Krishnan, An Osteological study of Foramen Transversarium of Cervical vertebrae and its clinical significance – an Original article, International Journal of Anatomy and Research.Feb2018,vol.6(1.2):4906-13			
		A.K.Manicka vasuki*, K.Kailash Krishnan,M.Jamuna, Deborah Joy Hepziba,K.Kalyana sundaram,Anatomical study of Lobes & Fissures of Lungs & its clinical significance – Cadaveric study – an Original article, International Journal of Anatomy, Radiology & Surgery,Jan2019,vol-8(1):A015-A019	N	N	DOAJ
		A.K.Manicka vasuki*, M.Jamuna, M.Mekala,S.Elavarasi, Deborah Joy Hepzibah, An Anatomical study of Profunda femoris artery And its variations –Cadaveric study, an Original article, Journal of Human Anatomy, Sep'2019,3(2):000141	N	N	Google scholar
3.	A.K.Manicka Vasuki	A.K.Manicka vasuki*, M.Jamuna, Deborah Joy Hepziba, M.Nirmaladevi, K.Radhika, Anatomical study of Right Coronary artery and its clinical significance – Cadaveric study – an Original article, Indian Journal of Clinical Anatomy and Physiology,June 2017,vol.4,iss.2,pp185 – 89	N	N	Index Copernicus & Google scholar
		A.K.Manicka vasuki*, M.Jamuna, Deborah Joy Hepziba, M.Nirmaladevi, Beulah Priyalatha Swamicken, K.Radhika,K.Kalyana sundaram, Anatomical study of Left Coronary artery and its clinical significance – Cadaveric study – an Original article, Journal of Clinical and Diagnostic Research, Jan 2018,vol.12(1),AC01 – AC05	N	N	DOAJ
		A.K.Manicka vasuki*, M.Jamuna, M.Nirmaladevi, Deborah Joy Hepziba, K.Radhika, Kailash Krishnan, An Osteological study of Foramen Transversarium of Cervical vertebrae and its clinical significance – an Original article, International Journal of Anatomy and Research.Feb2018,vol.6(1.2):4906-13	N	N	Index Copernicus
		A.K.Manicka vasuki*, K.Kailash Krishnan,M.Jamuna, Deborah Joy	N	N	DOAJ

		Hepziba,K.Kalyana sundaram,Anatomical study of Lobes & Fissures of Lungs & its clinical significance – Cadaveric study – an Original article, International Journal of Anatomy, Radiology & Surgery,Jan2019,vol-8(1):A015-A019			
		A.K.Manicka vasuki*, M.Jamuna, M.Mekala,S.Elavarasi, Deborah Joy Hepzibah, An Anatomical study of Profunda femoris artery And its variations –Cadaveric study, an Original article, Journal of Human Anatomy, Sep'2019,3(2):000141	N	N	Google scholar
		A.K.Manicka vasuki*,G.Amudha,C.Nandhini Aishwarya, Deborah Joy Hepzibah,V.A.Kesavan, Morphometric study of Jugular foramen and Jugular fossa of dried Adult Human skulls and its clinical significance – an original article, National Journal of Clinical Anatomy,Dec'2019:8:160-164	N	N	DOAJ
		A.K.Manicka vasuki*, M.Nirmaladevi, Morphometric study of Atypical Lumbar vertebrae and their clinical significance – an original article, International Journal of Anatomy,Radiology & Surgery,Oct'2021,vol.10(4):A045-A049	N	N	DOAJ
4.	R. Praveena	Prathapamchandra V, Ravichandran P, Shanmugasundaram J. Vascular foramina of navicular bone: a morphometric study. Antomy and Cell Biology 2017; 50(2):93-98.	Y	Y	Google scholar CrossRef Central
		Prasanna LC, Andrade LS, Ravichandra P, Hosapatna M. An insight into the circle of Willis, its variation in the formation and component vessels in the adult human cadavers. Ethiopian Medical Journal 2020; 58(02).	Y	N	DOAJ
5.	Dr. S.Suganya	Suganya Saminathan*, A study on the conversion of laparoscopic cholecystectomy owing to preoperative complications(Aberrant Anatomy)- an original article, Journal of Evolution of Medical and Dental sciences, J. Evolution Med. Dent. Sci./eISSN- 2278-4802, pISSN- 2278-4748/ Vol. 7/ Issue 38/ Sept. 17, 2018	N	N	Google scholar

		Suganya Saminathan*, A cadaveric study of the myocardial bridges - an original article, Journal of Evolution of Medical and Dental sciences, J. Evolution Med. Dent. Sci./eISSN- 2278-4802, pISSN- 2278-4748/ Vol. 8/ Issue 02/ Jan. 14, 2019	N	N	Google scholar
		Suganya Saminathan*, Morphological study of coronary ostia - an original article, International Journal of Advanced Research, *Int. J. of Adv. Res.* 8 (Feb). 102-106] (ISSN 2320-5407). Int J Health Sci Res. 2021; 11(2):8-11	Y	N	Google scholar
		Suganya Saminathan*, A Cadaveric Study on Coronary Dominance in South Indian Population - an original article, International Journal of Health Sciences and Research,	N	N	Google scholar
6.	R. Raja Rajeshwari	Devi RR, Trivedi DJ, Chauda SM. Accessory head of Coracobrachialis and its clinical uses. International journal of Advanced research. 2017 7(1), 864-867	N	N	Index Copernicus
		Devi RR, Trivedi DJ, Chauda SM. Occipitalization of atlas: A case report. Journal of the Anatomical Society of India. 2017 Aug 1;66:S109-10.	N	N	Google scholar Index Copernicus
7.	Mrs.T.Deborah Joy Hepzibah	A.K.Manicka vasuki, M.Jamuna, Deborah Joy Hepzibah*, M.Nirmaladevi, K.Radhika, Anatomical study of Right Coronary artery and its clinical significance – Cadaveric study – an Original article, Indian Journal of Clinical Anatomy and Physiology,June 2017,vol.4,iss.2,pp185 - 89	N	N	Index Copernicus Google scholar
		A.K.Manicka vasuki, M.Jamuna, Deborah Joy Hepzibah*, M.Nirmaladevi, Beulah Priyalatha Swamicken, K.Radhika,K.Kalyana sundaram, Anatomical study of Left Coronary artery and its clinical significance – Cadaveric study – an Original article, Journal of Clinical and Diagnostic Research, Jan 2018,vol.12(1),AC01 – AC05	N	N	DOAJ
		A.K.Manicka vasuki, M.Jamuna, M.Nirmaladevi, Deborah Joy Hepzibah*, K.Radhika, Kailash Krishnan, An Osteological study of	N	N	Index Copernicus

Annexure 1 - Publication in Vancouver Reference Style

		Foramen Transversarium of Cervical vertebrae and its clinical significance - an Original article, International Journal of Anatomy and Research.Feb2018,vol.6(1.2):4906-13			
		A.K.Manicka vasuki, K.Kailash Krishnan,M.Jamuna, Deborah Joy Hepzibah*,K.Kalyana sundaram,Anatomical study of Lobes & Fissures of Lungs & its clinical significance – Cadaveric study – an Original article, International Journal of Anatomy, Radiology & Surgery,Jan2019,vol-8(1):A015-A019	N	N	DOAJ
		A.K.Manicka vasuki, M.Jamuna, M.Mekala,S.Elavarasi, Deborah Joy Hepzibah*, An Anatomical study of Profunda femoris artery And its variations –Cadaveric study, an Original article, Journal of Human Anatomy, Sep'2019,3(2):000141	N	N	Google scholar
		A.K.Manicka vasuki,G.Amudha,C.Nandhini Aishwarya, Deborah Joy Hepzibah*,V.A.Kesavan, Morphometric study of Jugular fossa and Jugular foramen of Adult dried Human skulls and its clinical significance – an original article,National Journal of Clinical Anatomy,Dec'2019:8:160-164	N	N	DOAJ

PSG Institute of Medical Sciences & Research

DEPARTMENT OF PHYSIOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr.No	Faculty Name	Publications in Vancouver Referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr K Deepalakshmi	1.Kaliyaperumal D, Bhargavi K, Ramaraju K, Nair KS, Ramalingam S, Alagesan M. Electrocardiographic Changes in COVID-19 Patients: A Hospital-based Descriptive Study. Indian J Crit Care Med 2022; 26 (1):43-48.	Y	Y	EBSCO, Google Scholar
		2.Kaliyaperumal D, RK K,Alagesan M, Ramalingam S. Characterization of cardiac autonomic function in COVID-19 using heart rate variability a hospital based preliminary observational study. J Basic Clin Physiol Pharmacol. 2021 Mar 12;32(3):247-253.	Y	Y	EBSCO, Embase, Web of Science
		3. Deepalakshmi K,Vijayabaskaran S, Thamaraiselvi R., Nagashree R. Association between Quality of Sleep and Academic Performance in Medical Students: A Preliminary Study. International Journal of Physiology April-June 2020;8(2):0273-76.	N	N	Index Copernicus, Pubmed (for selected citation)
		4.Deepalakshmi K, Vijayabaskaran S, Nagashree R. Comparison of time domain measures of short-term heart rate variability in type 2 diabetic patients with and without microalbuminuria- a preliminary study. Int J Basic Appl Physiol 2020;9(1):105-108.	N	N	Index copernicus

Department of Physiology

		5.Deepalakshmi K,Elango Y,Iswarya S.Fatigue, depression and anxiety among nurses working in shift. Int J Res Med Sci July 2019;7(7):2655-2659	N	N	Medicus & Index copernicus
		6.Kaliyaperumal D, Vijayabaskaran S, Varsha V, Murali A. Effect of age and hypertension on cognition - A cross sectional study. Journal of Clinical and Diagnostic Research September 2018;12(9): OC05-OC08	N	Y	Embase, DOAJ & Index copernicus
		7. Kaliyaperumal D, Ramesh V, Leelakrishnan V, Shanmugam K. Clinical and manometric profile of patients with GERD in a tertiary care hospital. International Journal of Advances in Medicine. December 2017;4(6):1658-1661	N	N	Index Medicus & Index copernicus
		8.Deepalakshmi K, Yaal E, Murali A, Iswarya S. Effects of Sleep Deprivation on the Cognitive Performance of Nurses Working in Shift. Journal of Clinical and Diagnostic Research. August 2017;11(8): CC01-CC03	Y	Y	Embase & Index copernicus
		9.Deepalakshmi K,Vijayabaskaran S, Murali A. Comparison of microalbuminuria and heart rate variability in prediction of cardiovascular complications in diabetic population a pilot study. International journal of Advances in Medicine. June 2017; 4(3):819-823	N	N	Index Medicus & Index copernicus
		10.Deepalakshmi K, Sathyavathi P, Kumar PA ,Anith Kumar M, Denesh N. Students and faculty perspectives towards early clinical experience in learning neurosciences. National Journal of Research in Community Medicine. Jan.-Mar 2017; 6 (1):038-043	N	N	Index copernicus

		11.Silpita K, Murali A, Shanmugasundaram R, Deepalakshmi K, Anith Kumar M. Correlation of Reciprocal Changes and QRS Amplitude in ECG to Left Ventricular Dysfunction, Wall Motion Score and Clinical Outcome in First Time ST Elevation Myocardial Infarction. Journal of Clinical and Diagnostic Research. July 2017; 11(7): OC04-OC08	Y	Y	Embase & Index copernicus
2.	Dr R Nagashree	1.Jeyabanu M, Nagashree R, Pushparaj T. Peripheral nerve conduction tests in patients with hypothyroidism and healthy volunteers: a comparative study National journal of physiology, pharmacy and pharmacology.2018 ;8(9):1265-1268.	N	N	Embase, Index medicus Index copernicus,
		2. R Nagashree, Praveena M Effect of obesity on semen parameters National Journal of physiology. 2019;(7)2: 33-39.	N	N	Association Journal
		3. Deepalakshmi K,Vijayabaskaran S, Thamaraiselvi R., Nagashree R. Association between Quality of Sleep and Academic Performance in Medical Students: A Preliminary Study. International Journal of Physiology. April-June 2020;8(2):0273-76.	N	N	Index Copernicus, Pubmed (for selected citation)
		4.Deepalakshmi K, Vijayabaskaran S, Nagashree R, Comparison of time domain measures of short-term heart rate variability in type 2 diabetic patients with and without microalbuminuria- a preliminary study. Int J Basic Appl Physiol. 2020;9(1):105-108.	N	N	Index copernicus
3.	Dr G V Lathadevi	Hemavardhini CS , Lathadevi GV. Hypolipidemic Effects Of Fenugreek And Atorvastatin-Comparative Study On High Fat Fed Dyslipidemic Rats. The Journal of Clinical Diagnostics and Research Sep,2018;12(9): CC01-CC04.	N	Y	Central, Embase, EBSCO, Index Copernicus

		Raja Priya S, Lathadevi GV, Kumari MM. A Study on the quality of life in children and adolescents with type I Diabetes Mellitus. National Journal of Physiology, 2019; 7(2): 25-32.	N	N	Association Journal
4.	Dr V Kannan	Kannan V. A Commentary on the role of "Female Gender" in receiving the nobelprize for Physiology/ Medicine. Research & Reviews: Journal of Medical and Health Sciences. Sep 2019; 6(3): 41-43.	N	N	Index Copernicus Google Scholar
5.	Dr N Shuba	1.Nirangjhana S, Shuba N. Platelet Indices as a tool in assessing the progression of Type 2 Diabetes Mellitus. International Journal of Scientific Research.2020;9(1):46-48.	N	N	Index medicus, Index Copernicus, DJOF
		2.Nirangjhana S, Shuba N. Comparison of Sensory Nerve Conduction between overweight and obese Diabetic Peripheral Neuropathy patients based on Asian Indian Body Mass Index standard. International Journal of Scientific Research.2019;8(12): 44-46.	N	N	Index medicus, Index Copernicus, DJOF
		3.Shuba N, Vidhya KG. Association of Platelet Indices with severity of Acute Ischemic Stroke. International Journal of Scientific Research.2019;8(8): 57-60.	N	N	Index medicus, Index Copernicus, DJOF
		4.Shuba N, Praba V, Prithiviraaj P. Association of Platelet Indices with Disease Activity in Rheumatoid Arthritis. Journal of Evolution of Medical and Dental Science.2018;7(36):3940-3945.	N	N	Index Copernicus, Index Medicus, EBSCO
		5.Shuba N, Prakash B. Effects of Age, Gender and Education Level on Cognition in Elderly People. Journal of Evolution of Medical and Dental Sciences. 2017;6(95):6952-6956.	N	N	Index Copernicus, Index Medicus, EBSCO
		6.Prakash B, Pai PK, Chaitra V, Ramasamy P, Shuba N. A Case of Acute Paraplegia due to Aortic Dissection in Marfan's Syndrome. Journal of Neurosciences in Rural Practice. 2017 April-June; 8(2): 316-319.	Y	Y	DOAJ, EBSCO

		7.Shuba Narasimhan, Prakash Balasubramaniam. Role of Vitamin D in the Outcome of Ischemic Stroke-A Randomized Controlled Trial. Journal of Clinical and Diagnostic Research. 2017 Feb; 11(2): CC06-CC10.	Y	N	Embase & Index copernicus
6.	Dr S Vijayabaskaran	1. Deepalakshmi K,Vijayabaskaran S, Thamaraiselvi R., Nagashree R. Association between Quality of Sleep and Academic Performance in Medical Students: A Preliminary Study. International Journal of Physiology April-June 2020;8(2):0273-76.	N	N	Index Copernicus, Pubmed (for selected citation)
		2.Deepalakshmi K, Vijayabaskaran S, Nagashree R, Comparison of time domain measures of short-term heart rate variability in type 2 diabetic patients with and without microalbuminuria- a preliminary study. Int J Basic Appl Physiol. 2020;9(1):105-108.	N	N	Index Copernicus
		3.Kaliyaperumal D, Vijayabaskaran S, Varsha V, MuraliA. Effect of age and hypertension on cognition- A cross sectional study. Journal of Clinical and Diagnostic Research. September 2018;12(9): OC05-OC08	N	Y	Embase, DOAJ & Index Copernicus
		4.Deepalakshmi K, Vijayabaskaran S, Murali A. Comparison of microalbuminuria and heart rate variability in prediction of cardiovascular complications in diabetic population a pilot study. International journal of Advances in Medicine. June 2017; 4(3):819-823	N	N	Index Medicus & Index copernicus
7.	Dr R Pavitra Vyshnavi Yogisparan	1. Yogisparan P, Ramanathan R, Jayabal M, Selvaraj V, Shanmugam J. Comparison of high-resolution manometry parameters among type II diabetes patients and normal volunteers. National Journal of Physiology, Pharmacy and Pharmacology. August 2021; 2(2): 1-5.	N	N	EMBASE, Index Copernicus

PSG Institute of Medical Sciences & Research

DEPARTMENT OF BIOCHEMISTRY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	FacultyName	Publications in Vancouver referencingstyle.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr.G.Jeyachandran	Sowndharya J, Jeyachandran G. Occurrence of thyroid dysfunction in patients with psychiatric disturbances - A hospital based retrospective study. Indian Journal of Basic and Applied Medical Research; March 2019: Vol.-8, Issue- 2, P. 491 -500	N	N	Index Copernicus
		Dayakar S, Pillai HR, Kalpathodi S, Chandran GJ, Nair RR. A Guide to Total Quality Management System (TQMS) in Molecular Diagnostics from Experiences in Seeking Accreditation and Implementation. SN Comprehensive Clinical Medicine. 2019 Feb 1;1(2):123-33.	Y	N	
		J Sowndharya, S Aruna, G Jeyachandran. Study on the biochemistry laboratory performance in notifying critical alerts. MedPulse International Journal of Biochemistry. January 2020; 13(1): 01-03.	N	N	Index Copernicus
2	Dr.B.Gayathri	Sujatha R, Gayathri B. Correlation between lipid profile and myeloperoxidase levels in type-2 diabetes mellitus. Int J Med Res Rev 2016;4(9):1538-1542. doi:10.17511/ijmrr.2016.i09.04	N	N	Index Copernicus

3	Sumitra G	Mahto M, Mohapatra S, Sumitra G, Kaushik S, Mishra TK, Saxena A. A case of light chain deposition disease (LCDD) in a young patient. Indian Journal of Clinical Biochemistry. 2011 Oct;26(4):420-2.	Y	Y	
		Patel S, Sumitra G, Koner BC, Saxena A. Role of serum matrix metalloproteinase-2 and-9 to predict breast cancer progression. Clinical biochemistry. 2011 Jul 1;44(10-11):869-72.	Y	Y	
		Govindarajan S, Vellingiri K. Effect of red yeast rice and coconut, rice bran or sunflower oil combination in rats on hypercholesterolemic diet. Journal of clinical and diagnostic research: JCDR. 2016 Apr;10(4):BF05.	Y	N	Index Copernicus
		Govindarajan S, Raghavan VM, Rao AC. Plasma Myeloperoxidase and total sialic acid as prognostic indicators in acute coronary syndrome. Journal of clinical and diagnostic research: JCDR. 2016 Aug;10(8):BC09.	Y	N	Index Copernicus
		Kavitha S, Sumitra G, Vijaya D, Kavitha M, Bhuvaneshwari S. Awareness about Research Ethics among General Practitioners not Attached with Research Institutions. Journal of clinical and diagnostic research: JCDR. 2016 Feb;10(2):BL01.	Y	N	Index Copernicus
		Govindarajan S, Vasanth G, Kumar PA, Priyadarshini C, Radhakrishnan SS, Kanagaraj V, Balasubramanian N, Kumar PN, Divya TJ, Aishwarya CN. Impact of a comprehensive early clinical exposure program for preclinical year medical students. Health Professions Education. 2018 Jun 1;4(2):133-8.	N	N	
		Govindarajan S, Rajaragupathy S, Subramanian K, Karthikeyan J. Effectiveness of team teaching in biochemistry lectures for undergraduate students. Biochemistry and Molecular Biology Education. 2021 Apr 17.	Y	Y	
		Govindarajan S,Rajaragupathy S. Online team based learning inteaching Biochemistry for first year MBBS students during COVID-19 pandemic Biochem Mol BiolEduc. 2021;1–6. https://doi.org/10.1002/bmb.21598	Y	Y	
4	Dr.Meenakshi Sundaram A.S.	Sundaram A, Prabhu K. Plasma Total Thiols and Total Thiol/Albumin Ratio in Patients Suffering from Depression. Journal of clinical and diagnostic research.2021 Apr;Vol-15(4): BC05-BC07	N	N	Directory of open access journals (DOAJ)
		Sundaram Andra Suryanarayana M, Vellingiri K, Agarwal N S, Mohan B. Can Early Thyroid Profiling Help Avert Spontaneous Abortions/Early	Y	N	Pubmed,Pubmedcentral ,DOAJ

		Pregnancy Loss: A Retrospective Study.Cureus.2021 Sep 15; 13(9):e18003.			
5	Dr Sujatha R	Sujatha R, Kavitha S. Atherogenic indices in stroke patients: A retrospective study. Iran J Neurol 2017; 16(2): 78-82.	Y	N	Directory of Open Access Journals (DOAJ), Directory of Research Journal Indexing (DRJI)
		Sujatha R, Jayagowri K. Assessment of Palliative Care Awarenessamong Undergraduate Healthcare Students. Journal of Clinicaland Diagnostic Research 2017; 11(9): JC06-JC10.	Y	N	Emerging Sources Citation Index (Web of Science, Thomson reuters), Index Copernicus ICV 2019: 129.24, Academic Search Complete Database, Directory of Open Access Journals (DOAJ), Embase
		Govindarajan, S, Rajaragupathy, S, Subramanian, K, Karthikeyan,J. Effectiveness of team teaching in biochemistry lectures forundergraduate students. Biochem Mol Biol Educ. 2021; 1– 5.	Y	Y	-
		Sujatha R, Jayagowri K, Lavanya N. Comparison of albumin-bilirubingrade with Child-Pugh and Model for End Stage Liver Disease scores in chronic liver disease. BiochimicaClinica 2021; 45(2) 141-145.	N	Y	-
		Sujatha R, Kailash K. Pentraxin-3 as an emerging reliable noninvasiveserum marker in the detection of alcoholic fibrosis. Malay.J. Biochem. Mol. Biol. (2020) 23 (3): 98-102.	N	Y	-

DEPARTMENT OF PATHOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr.No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr.S.SHANTHAKUMARI	Harikrishnan V, Kumari S, Ramkumar S, Sankaran R, Ramalingam S, Sairam T. Correlation of the Expression of BRAF V600E Mutation With Various Phenotypic Expressions of Thyroid Neoplasms. Cureus. 2021 Jun;13(6).	Y	N	
		Sakthisankari S, Vidhyalakshmi S, Shanthakumari S, Devanand B, Nagul U. The combination of ACR-Thyroid Imaging Reporting and Data system and The Bethesda System for Reporting Thyroid Cytopathology in the evaluation of thyroid nodules—An institutional experience. Cytopathology. 2021 Apr 27.	Y	N	
		Pillai MM, Sathishkumar G, Houshyar S, Senthilkumar R, Quigley A, Shanthakumari S, Padhye R, Bhattacharyya A. Nanocomposite-coated silk-based artificial conduits: The influence of structures on regeneration of the peripheral nerve. ACS Applied Bio Materials. 2020 May 27;3(7):4454-64.	N	Y	
		Pillai MM, Gopinathan J, Senthil Kumar R, Sathish Kumar G, Shanthakumari S, Sahanand KS, Bhattacharyya A, Selvakumar R. Tissue engineering of human knee meniscus using functionalized and reinforced silk-polyvinyl alcohol composite three-dimensional scaffolds: understanding the in vitro and in vivo behavior. Journal of Biomedical Materials Research Part A. 2018 Jun;106(6):1722-31.	Y	N	

		Sakthisankari S, Vidhyalakshmi S, Shanthakumari S, Devanand B, Nagul U. The combination of ACR-Thyroid Imaging Reporting and Data system and The Bethesda System for Reporting Thyroid Cytopathology in the evaluation of thyroid nodules—An institutional experience. Cytopathology. 2021 Apr 27.	Y	N	
2	Dr. PRASANNA N KUMAR	World Health Organization. Guidance for clinical case management of thrombosis with thrombocytopenia syndrome (TTS) following vaccination to prevent coronavirus disease (COVID-19) 2021.	N	N	
		Hailu D, Fufu Hordofa D, Adam Endalew H, Karimi Mutua D, Bekele W, Bonilla M, Çeliker MY, Challinor J, Dotan A, Habashy C, Kumar PN. Training pediatric hematologist/oncologists for capacity building in Ethiopia. Pediatric Blood & Cancer. 2020 Dec;67(12):e28760.	Y	N	
		Sakthisankari S, Kumar PN, Syed T. Anaplastic Multiple Myeloma-a Morphological Challenge--A Case Series. Indian Journal of Medical &Paediatric Oncology. 2020 May 1;41(3).	N	N	Scopus, Web of Science
		SHANMUGASUNDARAM S, GURUSAMY U, KUMAR PN, JOHN BJ. Pancreatic Plasmacytoma Misdiagnosed as Neuroendocrine Tumour: A Pathologist's Dilemma. Journal of Clinical & Diagnostic Research. 2020 Feb 1;14(2).	Y	N	
		V.T.Anjali, Reena Rai G.Umamaheswari, Prasanna N Kumar HaemophagocyticLymphohistiocytosis complicating Methotrexate Toxicity Indian Dermatol Online J [Internet]. 2020 11(2):219–21	Y	N	

		Ganesan N, Shanmugham HB, Kumar PN. Analysis of Bacterial Contamination in Blood Bank: Comparison of Two Different Donor Arm Disinfectant.	Y	N	
		Hailu D, Adamu H, Fufa D, Karimi D, Alexander T, Habashy C, Dotan A, Wali RM, Kumar PN, Bonilla M, Bekele W. Training pediatric hematologists/oncologists for capacity building in Ethiopia.	Y	N	
		Govindarajan S, Vasanth G, Kumar PA, Priyadarshini C, Radhakrishnan SS, Kanagaraj V, Balasubramanian N, Kumar PN, Divya TJ, Aishwarya CN. Impact of a comprehensive early clinical exposure program for preclinical year medical students. Health Professions Education. 2018 Jun 1;4(2):133-8.	Y	N	
		SakthisankariShanmugasundaram, Prasanna N Kumar, S Vidhyalakshmi, V Chaitra Xeroderma Pigmentosum and Bone Marrow Aplasia : A Rare Association Indian Journal of Medical and Paediatric Oncology 2018 ; 39 : 247-9.	N	Y	
		Anjali VT, Rai R, Maheswari GU, Kumar PN. Hemophagocytic lymphohistiocytosis complicating methotrexate toxicity. Indian Dermatology Online Journal. 2020 Mar;11(2):219.	Y	N	
		Ganesan N, Kumar PN. Chediak–Higashi Syndrome in Accelerated Phase. Indian Journal of Hematology and Blood Transfusion. 2018 Jan;34(1):146-7.Published online : 27 March 2017.	Y	N	
3	Dr T M SUBBARAO	Alagesan M, Franklin J, Jayalakshmi J, Rao TS, Chaithra V. Basidiobolomycosis-Miss Me; It Hurts You. The Journal of the Association of Physicians of India. 2019 Jan;67(1):83-4.	Y	N	
		Subashchandrabose P, Madanagopaal LR, Rao TM. Diagnosis and classification of acute leukemia in bone marrow trephine biopsies, utility of a selected panel of minimal immunohistochemical markers. International journal of hematology-oncology and stem cell research. 2016 Jul 1;10(3):138.	Y	N	

		Vijaya R, Rao TM, Kumar PA. Morphological study of thymus in anencephalic and normal fetus. Journal of Morphological Sciences. 2017 Jan 16;30(2):0-.	N	N	
		Murali A, SubbaRao TM, Haridas A, Gopalakrishnapillai N. SERIES OF UNFORTUNATE EVENTS: UNUSUAL MANIFESTATIONS OF IDIOPATHIC HYPEREOSINOPHILIC SYNDROME.	N	N	
		Kurian SS, Rai R, Madhukar ST. Amyloidosis cutis dyschromica. Indian dermatology online journal. 2013 Oct;4(4):344.	Y	N	
4	Dr G UMAMAHESWARI	Shanmugasundaram S, Venkataswamy C, Gurusamy U. Pathologist's role in identifying cardiac defects—a fetal autopsy series. Cardiovascular Pathology. 2021 Mar 1;51:107312.	Y	N	
		SHANMUGASUNDARAM S, GURUSAMY U, KUMAR PN, JOHN BJ. Pancreatic Plasmacytoma Misdiagnosed as Neuroendocrine Tumour: A Pathologist's Dilemma. Journal of Clinical & Diagnostic Research. 2020 Feb 1;14(2).	N	N	Index Copernicus, Cross Ref. Google scholar, DOAJ
		Anjali VT, Rai R, Maheswari GU, Kumar PN. Hemophagocytic lymphohistiocytosis complicating methotrexate toxicity. Indian Dermatology Online Journal. 2020 Mar;11(2):219.	Y	N	
		Arunprasath P, Rai R, Umamaheswari G. Erythrokeratodermaen cocardes–A rare phenotype. Indian Journal of Paediatric Dermatology. 2020 Jul 1;21(3):191.	N	N	DOAJ, Emerging Sources Citation Index, Indian Science Abstracts, Web of Science
		Kumaresan M, Gurusamy U, Rai R, Srinivas CR. Plucked hair as a substrate for indirect immunofluorescence in cases of pemphigus vulgaris. International Journal of Trichology. 2020 May;12(3):114.	Y	N	
		Ganesan N, Gurusamy U. Morphologic Spectrum of Undetermined Causes of	Y	N	

		Hemoptysis-A Pathologist's Role. Turkish Journal of Pathology. 2020 Jul 21;36(3):237-45.			
		Mathew CT, Maheswari U, Shanmugam K. Co-relation of endometrial thickness by transvaginal sonogram with histopathology pattern in abnormal uterine bleeding: a study from South India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2020 Apr 1;9(4):1416-22.	N	N	Index Medicus for South-East Asia Region (WHO) Index Copernicus ScopeMed CrossRef
		Mathew CT, Maheswari U, Ramu A. One year retrospective analysis of PAP smears-from a tertiary hospital in Tamil Nadu.	N	N	Google Scholar Crossref Scilit Index Copernicus
		Natarajan L, UmaMaheswari G. Gestational hyperglycemia on diet and medication: impact on placental pathology and pregnancy outcomes. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Aug 1;8(8):3350-7.	N	N	Index Medicus for South-East Asia Region (WHO) Index Copernicus ScopeMed CrossRef
		Ramya T, Umamaheswari G, Chaitra V. Placental pathology in maternal autoimmune diseases-new insights and clinical implications. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2017 Sep 1;6(9):4090-8.	N	N	Index Medicus for South-East Asia Region (WHO) Index Copernicus ScopeMed CrossRef

		Umamaheswari G, Ramya T, Chaitra V. Nonimmune hydrops foetalis (NIHF): value of fetal autopsy and comparison with ultrasound findings. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018 Aug 1;7(8):3330-8.	N	N	Index Medicus for South-East Asia Region (WHO) Index Copernicus ScopeMed CrossRef
		Gurusamy U, Thangavelu R, Venkataswamy C. Abnormalities of the umbilical cord: correlation with placental histology and perinatal outcome. Int J Res Med Sci. 2017;5:5009-16.	N	N	Index Copernicus Index medicus for south east Asia region (WHO) ScopeMed CrossRef Google Scholar
		Venkataswamy C, Gurusamy U, Lakshmi SV. Second-trimester fetal autopsy: A morphological study with prenatal USG correlations and clinical implications. Journal of laboratory physicians. 2018 Jul;10(03):338-45.	Y	N	
		Gurusamy U, Venkataswamy C, Sivaraman A. Evaluation of alopecia: A new processing technique combining vertical and transverse sections from a single scalp biopsy specimen. International journal of trichology. 2018 Jan;10(1):11.	Y	N	
		GUrUSamy U, Venkataswamy C. Hair Loss in Paediatric and Adolescent Age Group: A Clinico-Pathological Analysis in a Tertiary Health Care Centre. Journal of Clinical & Diagnostic Research. 2017 Dec 1;11(12).	Y	N	
5	Dr. S VIDHYALAKSHMI	Sakthisankari S, Vidhyalakshmi S, Shanthakumari S, Devanand B, Nagul U. The combination of ACR-Thyroid Imaging Reporting and Data system and The Bethesda System for Reporting Thyroid Cytopathology in the evaluation of thyroid nodules—An institutional experience. Cytopathology. 2021 Apr 27.	Y	N	

		Narayanan R, Muthusamy S, Lakshmi SV, Ramaraju K, Balasubramaniam G, Sankaran R. Design and development of whole-body rodent inhalation chamber for exposure to Aroclor 1232 in Swiss albino mice. International Journal of Environmental Science and Technology. 2021 Jul 6:1-2.	Y	N	
		Vishnu N, Kulkarni AV, Vidhyalakshmi S, Sambandam S, Garg P, LeelaKrishnan V, Janarthan K, Singh G, Kaur M, Chitra TV, John BJ. Fibrolamellar variant of hepatocellular carcinoma presenting during pregnancy: management dilemmas. Annals of hepato-biliary-pancreatic surgery. 2017 Feb 1;21(1):48-51.	Y	N	
		Vidhya Lakshmi, Kharishma Nair and Karthikeyan Shanmugam 2017. "The role of body mass index (BMI) as an independent risk factor and prognostic factor in breast cancer – A hospital based epidemiological study ", International Journal of Current Research, 9 (12), 62653-62656	N	Y	
		S. VL, Raj RV, Sahanand S. Histological Evaluation Of Articular Cartilage Damage Following Radiofrequency Chondroplasty. EJMED [Internet]. 2020 Feb. 13 [cited 2021 Dec. 16];2(1).	N	N	Index Copernicus
		Rabia S. THE EXPRESSION OF BASAL CYTOKERATINS IN BREAST CANCERS. Medico Research Chronicles. 2018 Jun 30;5(3):252-9.	N	N	NLM

		Venkataswamy C, Gurusamy U, Lakshmi SV. Second-trimester fetal autopsy: A morphological study with prenatal USG correlations and clinical implications. Journal of laboratory physicians. 2018 Jul;10(03):338-45.	Y	N	
		SUNDARAM SS, VIDHYALAKSHMI S. Histomorphological Spectrum of Mediastinal Masses with Special Emphasis on Rare Lesions. Journal of Clinical & Diagnostic Research. 2020 Aug 1;14(8).	N	N	Index Copernicus
6	Dr S SAKTHISANKARI	Shanmugasundaram S, Venkataswamy C, Gurusamy U. Pathologist's role in identifying cardiac defects—a fetal autopsy series. Cardiovascular Pathology. 2021 Mar 1;51:107312.	Y	N	
		Sakthisankari S, Vidhyalakshmi S, Shanthakumari S, Devanand B, Nagul U. The combination of ACR-Thyroid Imaging Reporting and Data system and The Bethesda System for Reporting Thyroid Cytopathology in the evaluation of thyroid nodules—An institutional experience. Cytopathology. 2021 Apr 27..	Y	N	
		Shanmugasundaram S, Kartikayan RK. The Value of Duodenal Biopsies in the Evaluation of Megaloblastic Anemia. Journal of Laboratory Physicians. 2021 Jul 12.	Y	N	
		Sundar PM, Shanmugasundaram S, Nagappan E. The role of the IAC Yokohama System for Reporting Breast Fine-Needle Aspiration Biopsy and the ACR Breast Imaging-Reporting and Data System in the evaluation of breast lesions. Cytopathology.	Y	N	
		Shanmugasundaram S, Balan K, Arumugam D. Immunohistochemical profile and distribution of Non-Hodgkin and Hodgkin lymphoma-An experience in a medical college hospital in Tamil Nadu. Indian Journal of Medical and Paediatric Oncology. 2020 Sep;41(05):695-701.	N	Y	

		SUNDARAM SS, VIDHYALAKSHMI S. Histomorphological Spectrum of Mediastinal Masses with Special Emphasis on Rare Lesions. Journal of Clinical & Diagnostic Research. 2020 Aug 1;14(8).	N	N	DOAJ Web of Science
		Shanmuga SS, Angaraju A, Ganesan BM, Ramalingam S, Dhanabalan RT. CD5 positive follicular lymphomas: A diagnostic dilemma in a resource restricted laboratory setting. Annals of Pathology and Laboratory Medicine. 2017 Dec 10;4(6):A621-625.	N	N	Index Copernicus
		Rajagopala S, Sankari S, Kancherla R, Ramanathan RP, Balalakshmoji D. Miliary Sarcoidosis: does it exist? A case series and systematic review of literature. Sarcoidosis, Vasculitis, and Diffuse Lung Diseases. 2020;37(1):53.	Y	N	
		Sakthisankari S, Kumar PN, Syed T. Anaplastic Multiple Myeloma-a Morphological Challenge--A Case Series. Indian Journal of Medical &Paediatric Oncology. 2020 May 1;41(3).	N	Y	
		SHANMUGASUNDARAM S, GURUSAMY U, KUMAR PN, JOHN BJ. Pancreatic Plasmacytoma Misdiagnosed as Neuroendocrine Tumour: A Pathologist's Dilemma. Journal of Clinical & Diagnostic Research. 2020 Feb 1;14(2).	N	N	DOAJ Web of Science
		Shanmugasundaram S, Kumar P, Lakshmi S, Chaitra V. Xeroderma Pigmentosum and Bone Marrow Aplasia: A Rare Association. Indian Journal of Medical &Paediatric Oncology. 2018 Apr 1;39(2).	N	Y	
7	Dr ELIZ THOMAS	Gunaseelan L, Thomas E, Kumar PN, Menon S. Osteoblasts to the Rescue–A Rare Case of Secondary Myelofibrosis. Journal of Applied Hematology. 2021 Jan 1;12(1):48.	N	Y	

		Thomas E, Venkatesh V. Primary laryngeal leishmaniasis: an unusual diagnosis at an unusual site. Tropical doctor. 2017 Apr;47(2):181-3.	N	N	Index Copernicus
		Thomas E, Venkatasubramanian A. Profile of uncommon primary adult hepatic malignancies at a tertiary care center. Journal of Pathology of Nepal. 2020 Sep 30;10(2):1718-21.	N	N	DOAJ
8	Dr NIDHYA G	Ganesan N, Gurusamy U. Morphologic Spectrum of Undetermined Causes of Hemoptysis-A Pathologist's Role. Turkish Journal of Pathology. 2020 Jul 21;36(3):237-45..	Y	N	
		Nidhya Ganesan, Vidhyalakshmi S, Kartikayan R K, Muruganand M. A rare case of small bowel penumatosis intestinalis. Int. J. Med.Lab. Resp 2020, 5(1) : 31-34.	N	N	Index Copernicus
		Rajesh H, Kuppusamy B, Venkataswamy C, Ganesan N. Enterobius vermicularis Infection of the Uterine Endometrium in an Infertile Female. The Journal of Obstetrics and Gynecology of India. 2020 Feb;70(1):89-91.	Y	N	
		Ganesan N, Shanmugham HB, Kumar PN. Analysis of Bacterial Contamination in Blood Bank: Comparison of Two Different Donor Arm Disinfectant.	N	N	Index copernicus

		Rajagopala S, Kancherla R, Ganesan N, Balalakshmoji D, Karuppannasamy D, Ramanathan RP, Gopalan R. An unusual cause of breathlessness and profuse micronodules. Lung India: Official Organ of Indian Chest Society. 2018 Sep;35(5):431..	Y	N	
		Ganesan N, Kumar PN. Chediak–Higashi Syndrome in Accelerated Phase. Indian Journal of Hematology and Blood Transfusion. 2018 Jan;34(1):146-7.	Y	N	
		Ganesan N, Phansalkar MD, Ambroise MM, Varghese RG, Validating micronucleus score in Effusion fluids. J cytol. 2017 Oct-Dec, 34(4) : 193-196	Y	N	
9	Dr LAWANYA G	Gunaseelan L, Thomas E, Kumar PN, Menon S. Osteoblasts to the Rescue–A Rare Case of Secondary Myelofibrosis. Journal of Applied Hematology. 2021 Jan 1;12(1):48.	N	Y	
		S Boobalan, G Lawanya. Clinico-cytological and radiological correlation of sputum analysis in a tertiary care hospital.In: MedPulse International Journal of Pathology. June 2019; 10(3): 169-174.	N	N	Index Copernicus
10	Dr PREETHI M S	Sundar PM, Shanmugasundaram S, Nagappan E. The role of the IAC Yokohama System for Reporting Breast Fine-Needle Aspiration Biopsy and the ACR Breast Imaging-Reporting and Data System in the evaluation of breast lesions. Cytopathology.	Y	N	
11	Dr A ABINAYA SUNDARI	Veena Venkatesh, Shiv Priya R, Abinayasundari A Variation of Platelet Indices among patients with type 2 Diabetes Mellitus : Int. Journal of Health and Clinical Research 2021 ; 4 (6) : 167-171	N	N	Google Scholar

DEPARTMENT OF MICROBIOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr. B. Appalaraju	Uma Maheswari, M. and Appalaraju. 2017. Molecular Detection and Subtyping of Newdelhi Metallobetalactamases (NDM-1) in Clinical Isolates of Escherichia coli and Klebsiella pneumoniae in a Tertiary Care Hospital, South India. Int.J.Curr.Microbiol.App.Sci. 6(5): 2264- 2270. doi: https://doi.org/10.20546/ijcmas.2017.605.252	N	N	Index Copernicus
		M. Rizwana, S. Parvathi and B. Appalaraju, Phenotypic and genotypic characterization of Enterococci from clinical isolates in a tertiary care hospital. *Int. J. Curr. Microbiol. App. Sci.*2017; 6(7): 1160-1173.	N	N	Index Copernicus
		Rizwana MM, Parvathi S, Appalaraju B. Prevalence of Vancomycin Resistant Enterococci Carriers from Rectal Swabs among High Risk Patients in a Tertiary Care Hospital. *NJLM*, 2020. 9(3):ML01-02.	N	N	Index Copernicus
		Dinoop Korol Ponnambath, Swaran Kumar M, Appalaraju Boppe, Karthikeyan Shanmugam. Clinico – mycological and antifungal susceptibility profiles of candiduria in A tertiary care hospital from South India. National NJLM. 2017;6(4): MO07-MO12.	N	N	Index Copernicus
		Sai Keerthana, Marina Thomas, Appalaraju. Haemophilus influenza in community acquired infections. 2017. *Int. J. Curr. Microbiol. App. Sci*. 2017; 6(4): 929-937.	N	N	Index Copernicus
		Appalaraju B, Baveja S, Baliga S et al. J Antimicrob Chemother In vitro activity of a novel antibacterial agent, levonadifloxacin, against clinical isolates collected in a prospective, multicentre surveillance study in India. 2020;75(3):600-608. doi: 10.1093/jac/dkz493. PMID: 31840170.	Y	N	-

		Xavier C. Rajan*, Sivamani Manivasagan, Appalaraju Boppe. Prevalence and the factors influencing soil transmitted helminths among school age children of 5 to 14 years in a rural area of Coimbatore district. *Int J Community Med Public Health.* 2019 Nov;6(11):4924-4928.	N	N	Index Copernicus
		Arunaloke Chakrabarti et al. Characteristics, outcome and risk factors for mortality of paediatric patients with ICU-acquired candidemia in India: A multicentre prospective study Mycoses. 2020; 63(11): 1149-1163.	Y	N	-
2	Dr. S. Parvathi	M. Rizwana, S. Parvathi and B. Appalaraju, Phenotypic and genotypic characterization of Enterococci from clinical isolates in a tertiary care hospital. *Int. J. Curr. Microbiol. App. Sci.*2017; 6(7): 1160-1173.	N	N	Index Copernicus
		Rizwana M, Parvathi S, Appalaraju B. Prevalence of Vancomycin Resistant Enterococci Carriers from Rectal Swabs among High Risk Patients in a Tertiary Care Hospital. *NJLM*, 2020. 9(3):ML01-02.	N	N	Index Copernicus
3	Dr. R. Someshwaran	Keerthana, N., R. Someshwaran and Dhanasekaran, A. Comparative Evaluation of Performance of Qualitative and Semiquantitative Slide Widal Test versus Quantitative Tube Widal Test in Diagnosing Enteric Fever. *Int. J. Curr. Microbiol. App. Sci.* 2018. 7(11): 1714-1719.	N	N	Index Copernicus
		Karthiga, S., A. Dhanasekaran and Someshwaran, R. 2018. Bacteriological Profile and Antibiogram of Catheter Related Blood Stream Infections (CR-BSIs) among Chronic Hemodialysis Patients Attending Tertiary Care Medical College Hospital. *Int. J. Curr. Microbiol. App. Sci.* 7(11): 1121-1128.	N	N	Index Copernicus
4	Dr.Ganesh Perumal P	Perumal P.G, Jnaneshwara K.B, Patil A.B, Akshay R. Incidence of infections with extended spectrum beta Lactamase (ESBL)-producing gram-negative bacteria among patients admitted in medical intensive care unit of tertiary care hospital. *Trop. J. Path. Micro.* 2017;3(2):168-173. doi: 10.17511/jopm.2017.i2.17.	N	N	Index Copernicus
5	Dr. M. Mohamadiya Rizwana	Rizwana MM, Parvathi S, Appalaraju B. Prevalence of Vancomycin Resistant Enterococci Carriers from Rectal Swabs among High Risk Patients in a Tertiary Care Hospital. *NJLM*, 2020. 9(3):ML01-02.	N	N	Index Copernicus

		M. Rizwana, S. Parvathi and B. Appalaraju, Phenotypic and genotypic characterization of Enterococci from clinical isolates in a tertiary care hospital. *Int. J. Curr. Microbiol. App. Sci.*2017; 6(7): 1160-1173.	N	N	Index Copernicus

DEPARTMENT OF PHARMACOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr Bhuvaneswari K,	Subhashini R, Bhuvaneswari K, Umamaheswari A. Impact of a sensitization program on prescription order writing skills of second year MBBS students: a prospective study. Int J Basic Clin Pharmacol. **2020** Jun;9(6):906-911.	N	N	Index Copernicus, EBSCO
		Shanmugapriya S, Bhuvaneswari K, Veena RM. A Study to Identify the Effect of Momordica Charantia on Smooth Muscle Activity in Isolated Guinea Pig Ileum. Indian J PhysiolPharmacol. **2019**;63(4):317-23.	N	Y	-
		Karthika P, Bhuvaneswari K, Balachander R. Momordica Charantia Nano Dry Powder Oral Formulation In Controlling Histamine-Induced Bronchospasm. Asian J Pharm Clin Res, Vol 12, Issue 8, **2019**, 137-140.	N	N	Index Copernicus
		Umamaheswari A, BhuvaneswariK ,Muthuraman PL. Effect of EmblicaOfficinalisFruit Dry Power on Behavior Changes and Antioxidant Levels: An in Vitro and in Vivo Study. Asian J Pharm Clin Res, Vol 12, Issue 7, **2019**, 291-294.	N	N	Index Copernicus
		Ramamoorthy S, Bhuvaneswari K. A cross sectional study on the status of Inflammatory markers in polycystic ovary syndrome (PCOS) in Indian Population. Biomed Pharmacol J **2019**;12(4):1975-1983.	N	Y	-
		Subramani B, Krishnamurthy B. Effects of Momordica Charantia (Bitter gourd) on Oxidative Stress and Pro-Inflammatory Marker in Metabolic Syndrome Using a High-Ffructose Diet Induced Rat Model. Biomedical and Pharmacology Journal. **2019** Mar 25;12(1):305-24.	N	Y	-
		Umamaheswari A, Bhuvaneswari K, Senthilkumar R. To Compare the Anti-inflammatory effect of oral hypoglycemic drugs in type 2 diabetes mellitus. Biomed Pharmacol J **2018**;11(3):1667-1674.	N	Y	-

		Krishnamurthy B, Kumar PA, Sathyanarayanan A. Comparison of the effects of zonisamide and flupirtine on paclitaxel induced peripheral neuropathy in rats. J Clin Diagno Res. **2018**;12(1): FC05-FC08	N	Y	-
		Bhuvaneswari K, Deena Sangeetha C. SimMan 3G – High Fidelity Simulation: A Method of Facilitate Pharmacology Learning by MBBS Students. Int.j.med.sci.educ. July-September **2017**; 4(4):293-302.	N	N	Index Copernicus
	Dr. K. Bhuvaneswari	Bhuvaneswari K, Veena RM. Medication Practices of School Teachers in Coimbatore. Biomedical & Pharmacology Journal. **2017**;10(3):1167-74.	N	Y	-
		ShanmugaPriya S, Nivedhithaa S, Bhuvaneswari K. A study on the effects of agomelatine on food intake and body weight in restraint stress model in adult Swiss albino mice. Asian Journal of Pharmaceutical and Clinical Research. **2017;**10(9):141-3.	N	Y	-
		Bhuvaneswari K, Jeyalakshmi D, Umamaheswari A. Knowledge, awareness and attitude towards rational use of medicines by community pharmacists in and around Coimbatore, India. Int J Basic Clin Pharmacol. **2017** Oct;6(10):2422-2427	N	N	Index Copernicus, EBSCO
		Narayanan R, Gilbert B, Ravichandran S, Bhuvaneswari K. Psychopharmacological characterization of effects of Ferulaasafoetida Linn formulation in mouse on a Y-maze, EPM, and open field apparatus. Int J Basic Clin Pharm. **2017**Sep;6:2254-8.	N	N	Index Copernicus, EBSCO
		Bhuvaneswari K., Swarna R. M. P. L., Amudhan Aravind.Anti asthmatic effect of Momordica Charantiaand its comparison with montelukast an in vitro and in vivo model. Int J Basic Clin Pharmacol. **2017** Jul;6(7):1810-1815	N	N	Index Copernicus, EBSCO
		Shanmugapriya S, Bhuvaneswari K, Karthika P. Audit to evaluate the completeness of adverse drug reporting in an ADR monitoring centre in Tamilnadu. Indian Journal of Medical Specialities. **2017** Oct 1;8(4):175-8.	N	N	Web of Science: Emerging Sources Citation Index; EBSCO
		Shanmugapriya S, Bhuvaneswari K, Rashmi K. A study on the effect of low molecular weight on potassium homeostasis in patients administered heparin for thromboprophylaxis in a tertiary care hospital. Int J Pharm Pharm Sci. **2017**;9(6):85-9.	N	N	Index Copernicus

2	Dr. N. Ramanujam	Ramalingam S, Narayanan R, Muthusamy S, Veronika M, Sankaran R, Toscano W. Persistent organic pollutants-environmental risk factors for diabetes mellitus?–A population-based study. Indian Journal of Occupational and Environmental Medicine. **2021** Jul;25(3):157.	Y	Y	Web of Science, EBSCO, EMBASE, Emerging Science Citation Index
		Narayanan R, Muthusamy S, Lakshmi SV, Ramaraju K, Balasubramaniam G, Sankaran R. Design and development of whole-body rodent inhalation chamber for exposure to Aroclor 1232 in Swiss albino mice. International Journal of Environmental Science and Technology. **2021** Jul 6:1-2.	N	Y	EBSCO, Emerging Science Citation Index
		Ramanujam N, Sivaselvakumar M, Ramalingam S. Fast and parallel determination of PCB 77 and PCB 180 in plasma using ultra performance liquid chromatography with diode array detection: A pharmacokinetic study in Swiss albino mouse. Biomedical Chromatography. **2017** Nov;31(11):e4000.	Y	Y	Web of Science, EBSCO, EMBASE, Science Citation Index (Expanded)
		Narayanan R, Gilbert B, Ravichandran S, Bhuvaneswari K. Psychopharmacological characterization of effects of Ferulaasafoetida Linn formulation in mouse on a Y-maze, EPM, and open field apparatus. Int J Basic Clin Pharm. **2017**Sep;6:2254-8.	N	N	Index Copernicus, EBSCO
3	Dr.S Shanmuga Priya	Shanmugapriya S, Saravanan A, Shuruthi S, Dharsini JS, Saravanan T. Association of gastroesophageal reflux disease with anxiety, depression, and sleep disorders. Journal of Medical Sciences. **2021** Jan 1;41(1):9.	N	Y	EBSCO, EMBASE
		Shanmugapriya S, Saravanan T, Saravanan A, Devi Y, Kishore V. Lipid Lowering Effect of Teneligliptin in Comparison to Simvastatin in Diet Induced Hyperlipidemic Rats. Biomedical and Pharmacology Journal. **2020** Mar 28;13(1):33-9.	N	Y	-
		Shanmugapriya S, Bhuvaneswari K, Veena RM. A Study to Identify the Effect of Momordica Charantia on Smooth Muscle Activity in Isolated Guinea Pig Ileum. Indian J PhysiolPharmacol. **2019**;63(4):317-23.	N	Y	EMBASE, Web of Science
		Shanmugapriya S, Dhandapani N, Saravanan T. A study comparing cognitive function assessment in type-2 diabetes mellitus using Rowland Universal Dementia Assessment Scale and Mini Mental State Examination. International Journal of Health & Allied Sciences. **2018** Jul 1;7(3):184-190.	N	N	Index Copernicus, EBSCO
		Shanmugapriya S, Saravanan T, Rajee SS, Venkatrajan R, Thomas PM. Drug prescription pattern of outpatients in a tertiary care teaching hospital in Tamil Nadu. Perspect Clin Res. **2018** Jul-Sep;9(3):133-138.	Y	Y	EBSCO

	Dr. S Shanmuga Priya	Saravanan T, Shanmugapriya S. A study on the effectiveness of teaching the principles and methods of clinical audit to medical postgraduates. Current Medicine Research and Practice. **2018** Mar 1;8(2):59-63.	N	N	EMBASE
		Shanmugapriya S, Sangeetha D, Sankaran R. Knowledge and Attitude Towards the Translation of Medical Research to Clinical Benefit Amongst Researchers in A Tertiary Care Hospital. J Clin Diagno Res.**2017** Dec 1;11(12):FC01-5.	N	Y	-
		ShanmugaPriya S, Nivedhithaa S, Bhuvaneswari K. A study on the effects of agomelatine on food intake and body weight in restraint stress model in adult Swiss albino mice. Asian Journal of Pharmaceutical and Clinical Research. **2017;**10(9):141-3.	N	Y	-
		Shanmugapriya S, Saravanan T, Janani K. Antibiotic sensitivity pattern to urinary tract infections in a tertiary care hospital in South India. Int J Basic Clin Pharmacol. **2017** Jun;6(6):1445-1450.	N	N	Index Copernicus, EBSCO
		Saravanan T, Shanmugapriya S, Sumitra G, Saravanan A, AK MV. Hepato-protective effect of folic acid and vitamin B12 in comparison to n-acetylcysteine in experimentally induced acetaminophen toxicity in rats. Biomedical and Pharmacology Journal. **2017** Jun 20;10(2):549-55.	N	Y	-
		Shanmugapriya S, Bhuvaneswari K, Karthika P. Audit to evaluate the completeness of adverse drug reporting in an ADR monitoring centre in Tamilnadu. Indian Journal of Medical Specialities. **2017** Oct 1;8(4):175-8.	N	N	Web of Science: Emerging Sources Citation Index; EBSCO
		Shanmugapriya S, Bhuvaneswari K, Rashmi K. A study on the effect of low molecular weight on potassium homeostasis in patients administered heparin for thromboprophylaxis in a tertiary care hospital. Int J Pharm Pharm Sci. **2017**;9(6):85-9.	N	N	Index Copernicus

4	Dr A. Umamaheswari	Subhashini R, Bhuvaneswari K, Umamaheswari A. Impact of a sensitization program on prescription order writing skills of second year MBBS students: a prospective study. Int J Basic Clin Pharmacol. **2020** Jun;9(6):906-911.	N	N	Index Copernicus, EBSCO
		Umamaheswari A, BhuvaneswariK ,Muthuraman PL. Effect of EmblicaOfficinalisFruit Dry Power on Behavior Changes and Antioxidant Levels: An in Vitro and in Vivo Study. Asian J Pharm Clin Res, Vol 12, Issue 7, **2019**, 291-294.	N	N	Index Copernicus
		Umamaheswari A, Bhuvaneswari K, Senthilkumar R. To Compare the Anti-inflammatory effect of oral hypoglycemic drugs in type 2 diabetes mellitus. Biomed Pharmacol J **2018**;11(3):1667-1674.	N	Y	-
		Umamaheswari A, AbishekPrasenaa A, Mohamed Akkram Khan.Role of Fresh Juice and Dry Powder Preparations of BenincasaHispida and LagenariaSiceraria Fruits in Inhibiting Cellular Stress By Measuring Dpph Assay. EJPMR, **2018**,5(9), 338-341.	N	N	Index Copernicus
		Bhuvaneswari K, Jeyalakshmi D, Umamaheswari A. Knowledge, awareness and attitude towards rational use of medicines by community pharmacists in and around Coimbatore, India. Int J Basic Clin Pharmacol. **2017** Oct;6(10):2422-2427	N	N	Index Copernicus, EBSCO
		Umamaheswari A, Umamageswari MS, SathiyaVinotha AT. Assessment of medical students' attitude towards didactic lectures taken in pharmacology in a private medical college. Int J Basic Clin Pharmacol. **2017;**Jun;6(6): 1451-1455.	N	N	Index Copernicus, EBSCO

PSG Institute of Medical Sciences & Research

DEPARTMENT OF FORENSIC MEDICINE & TOXICOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pub med (Y/N)	Scopus (Y/N)	
1	Jegadheeshwararaj J	Jegadheeshwararaj J, Thejaswi HT, Murari A. A Study of Bony and Cartilaginous Neck Structures in Deaths Due to Suicidal Hanging at New Delhi. Medico-Legal Update. 2017 Jul 1;17(2).	N	Y	Embase, Index copernicus
		Jegadheeshwararaj J, Thejaswi HT, Murari A. Crucial Factors Frequently Overlooked in Deaths due to Hanging: A Prospective Study. Indian Journal of Forensic Medicine & Toxicology. 2017 Jul 1;11(2).	N	Y	Embase, Index copernicus

PSG Institute of Medical Sciences & Research

DEPARTMENT OF COMMUNITY MEDICINE

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	FacultyName	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr Sudha KS	1. Ramalingam S, Narayanan R, Muthusamy S, Veronika M, Sankaran R, Toscano W. Persistent organic pollutants-environmental risk factors for diabetes mellitus?–A population-based study. Indian Journal of Occupational and Environmental Medicine. 2021 Jul;25(3):157.	Y	Y	
		2. Ramalingam S, Radhakrishnan S, Kaliappan T, Gopalan R, Subrahmanian M, Sankaran R. The genetics of cardiac failure: Role of a G protein-coupled receptor polymorphism in therapeutic response in an Indian population. Journal of Clinical and Translational Research. 2021 Aug 26;7(4):501.	Y	N	
		3. Mani K, Thirumalmuthu K, Kathiresan DS, Ramalingam S, Sankaran R, Jeyaraj S. In-silico analysis of Covid-19 genome sequences of Indian origin: impact of mutations in identification of SARS-Co-V2. Molecular and cellular probes. 2021 Jun 16:101748.	Y	Y	
		4. Harikrishnan V, Kumari S, Ramkumar S, Sankaran R, Ramalingam S, Sairam T. Correlation of the Expression of BRAF V600E Mutation With Various Phenotypic Expressions of Thyroid Neoplasms. Cureus. 2021 Jun;13(6).	Y	N	
		5. Rosario P, Viswash R, Seenivasan T, Ramalingam S, Sellgren KL, Grego S, Trotochaud L. Potential Pitfalls in Wastewater Phosphorus Analysis and How to Avoid Them. Environmental health insights. 2021 May;15:11786302211019218.	Y	Y	
		6. Muthusamy S, Palanisamy S, Ramalingam S. Exposure of bisphenol A in breast cancer patients—quantitatively assessed by sensitivity-enhanced high-performance liquid chromatography coupled with fluorescence detection: A case–control study. Biomedical Chromatography. 2021 Apr 7:e5137.	Y	N	
		7. Sasidaran S, Kachoria P, Raj A, Ramalingam S, Stoner BR, Sellgren KL, Grego S. Physical properties of menstrual hygiene waste as feedstock for onsite disposal technologies. Journal of Water, Sanitation and Hygiene for Development. 2021 May;11(3):474-82.Doi: https://doi.org/10.2166/washdev.2021.262	N	N	

		8. Kaliyaperumal D, Rk K, Alagesan M, Sudha Ramalingam. Characterization of cardiac autonomic function in COVID-19 using heart rate variability: a hospital based preliminary observational study. J Basic Clin Physiol Pharmacol. 2021 Mar 11. PMID: 33705614	Y	N	
	Dr Sudha KS	9. Osborn J, Satheesh T, Ramalingam S. Knowledge, Attitude and Practice of COVID 19 and the factors influencing them among a Rural Population in South India. Indian Journal of Community Health. 2021 Jan 1;33(1).	N	N	
		10. Meenu S, Ramalingam S, Sairam T, Appinabhavi A, Panicker S, Oommen S, Sankaran R. Comparison of Polymerase Chain Reaction (PCR), Microbiological and Histopathological Observations in the Diagnosis of Endometrial Tuberculosis. The Journal of Obstetrics and Gynecology of India. 2020 Dec;70(6):510-5.	Y	N	
		11. Meenu S, Pradeep B, Ramalingam S, Sairam T, Rai R, Sankaran R. Papillon–Lefèvre syndrome (PLS) with novel compound heterozygous mutation in the exclusion and Peptidase C1A domains of Cathepsin C gene. Molecular Biology Reports. 2020 Jul;47(7):5681-7.	Y	N	
		12. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton AZ, Benjamin EJ, Benziger CP, Bonny A. Global burden of cardiovascular diseases and risk factors, 1990–2019: update from the GBD 2019 study. Journal of the American College of Cardiology. 2020 Dec 22;76(25):2982-3021.	Y	N	
		13. Rajan R, Divya KP, Kandadai RM, Yadav R, Satagopam VP, Madhusoodanan UK, Agarwal P, Kumar N, Ferreira T, Kumar H, Sreeram Prasad AV. Genetic architecture of Parkinson's disease in the Indian population: harnessing genetic diversity to address critical gaps in Parkinson's disease research. Frontiers in neurology. 2020 Jun 18;11:524.	Y	N	
		14. Ramalingam S, Ganesan S. End-of-life practices in rural South India: SocioCultural determinants. Indian journal of palliative care. 2019 Apr;25(2):224.	Y	Y	
		15. Ganesan S, Ravishankar SL, Ramalingam S. Are body image issues affecting our adolescents? A cross-sectional study among college going adolescent girls. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2018 Dec;43(Suppl 1):S42.	Y	N	
		16. Arora NK, Swaminathan S, Mohapatra A, Gopalan HS, Katoch VM, Bhan MK, Rasaily R, Shekhar C, Thavaraj V, Roy M, Das MK. Research priorities in maternal, newborn, & child health & nutrition for India: An Indian Council of Medical Research-INCLEN Initiative. The Indian journal of medical research. 2017 May;145(5):611.	Y	N	

		17. Sairam T, Patel AN, Subrahmanian M, Gopalan R, Pogwizd SM, Ramalingam S, Sankaran R, Rajasekaran NS. Evidence for a hyper-reductive redox in a sub-set of heart failure patients. Journal of translational medicine. 2018 Dec;16(1):1-3.	Y	Y	
2.	Dr S.L. Ravishankar	1. Ganesan S, Ravishankar SL, Ramalingam S. Are body image issues affecting our adolescents? A cross-sectional study among college going adolescent girls. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2018 Dec;43(Suppl 1):S42.	Y	N	
		2. Rajan XC, Ravishankar. A community based study to assess the prevalence and sociodemographic factors influencing undernutrition among children aged 6 to 14 years in a rural area of Coimbatore. Int J Community Med Public Health 6:2549-54 (2019)	Y	N	
		3. Gopalakrishnan M, Ravishankar SL, Raghuthaman G, Senthilkumar R. Depression among elderly diabetics at Peripheral health centres attached to a private medical college in Coimbatore: a cross sectional study. National Journal of Research in Community Medicine. 2019 Dec 31;8(4):312-5.	N	N	Index copernicus
		4. Jenit A. Osborn, Sriram Rm, Karthikeyan S, Ravishankar S.L. A study on contraceptive prevalence rate and factors influencing it in a rural area of Coimbatore, South India. Journal of Family Medicine and Primary Care 2021;10:2246-51	Y	N	
3.	Dr M Sivamani	1. Arora NK, Das MK, Poluru R, Kashyap NK, Mathew T, Mathai J, Aggarwal MK, Haldar P, Verstraeten T, Zuber PL, INCLEN Vaccine Safety Study Group. A Prospective Cohort Study on the Safety of Infant Pentavalent (DTwP-HBV-Hib) and Oral Polio Vaccines in Two South Indian Districts. The Pediatric infectious disease journal. 2020 May;39(5):389.	Y	N	
		2. Sam CJ, Arunachalam PA, Sivamani Manivasagan TS. Parental satisfaction with pediatric day-care surgery and its determinants in a tertiary care hospital. Journal of Indian Association of Pediatric Surgeons. 2017 Oct;22(4):226.	Y	N	
		3. A review of research projects done by undergraduate medical students over a period of fifteen years in a medical college in Coimbatore. Senthilkumar SK,Thanigai Nayaki KP,Sivamani M. Int J Preven Curat Comm Med 2017;3(4):19-22.	N	Y	
		4. Pediatric Appropriate Evaluation Protocol for India (PAEP-India): Toolfor Assessing Appropriateness of Pediatric Hospitalization Mk Das, N k Arora, R Poluru, A Seth, A Aggarwal,A P Dubey, PC Goyal, G Gathwala, A malik, AK Goel,A Chakravarty, S Arya, A Upadhyay, M Gupta, T Mathew,RK Pillai, J mathai, S Manivasagan, S Ramesh, MK Aggarwal, CG Maure and Patrick lf zuber. Indian Pediatrics Dec,2018;55(4),1041-45.	Y	N	
		5. Prevalence and the factors influencing soil-transmitted helminths among school age children	Y	N	

		(5–14 years age) in a ruralarea of Coimbatore district.V Xavier Christu Rajan, M Sivamani, B Appalaraju. Trop Parasitol,2020;10(2),74-8			
4	Dr G M Muhammad	1. Osborn AJ, Muhammad GM, Ravishankar SL, Mathew AC. Prevalence and correlates of anemia among women in the reproductive age (15–49 years) in a rural area of Tamil Nadu: An exploratory study. Journal of Education and Health Promotion. 2021;10.	Y	Y	DOAJ, Index Copernicus,Emerging sources Citation Index, Scimago Journal Ranking, Web of Science
		2. Gopalakrishnan M, Ravishankar SL, Raghuthaman G, Senthilkumar R. Depression among elderly diabetics at Peripheral health centres attached to a private medical college in Coimbatore: a cross sectional study. National Journal of Research in Community Medicine. 2019 Dec 31;8(4):312-5.	N	N	Index copernicus
		3. Ganesan S, Chacko TV, Muhammad GM. Are our rural adolescents eating healthy?: Implications for redesigning school health interventions–A cross sectional study in rural Coimbatore. Indian journal of public health. 2019 Oct 1;63(4):293.	Y	Y	DOAJ, Emerging Sources Citation Index, Indian Science Abstracts, IndMed, MEDLINE/Index Medicus, Scimago Journal Ranking, SCOPUS, Web of Science, Social Sciences Citation Index
		4. Muhammad GM, Ganesan S, Chacko TV. Effectiveness of multi-strategic health screening cum educational intervention model in promoting health of school children in rural Coimbatore. Indian journal of public health. 2019 Apr 1;63(2):133	Y	Y	DOAJ, Emerging Sources Citation Index, Indian Science Abstracts,

					IndMed, MEDLINE/Index Medicus, Scimago Journal Ranking, SCOPUS, Web of Science, Social Sciences Citation Index
5	Dr Suvetha K	1. Kannappan S, Gupta M. A toolkit for strengthening health care policies and infrastructure of industries in developing countries.Indian J Occup Environ Med 2021;25:163-8.	Y	Y	
		2. Sakthivel A, Kannappan S, Panicker AS. Prevalence of mental health problems among high school students. Indian J Community Med 2021;46:574-5	Y	Y	
		3. Anisha Rodrigues, Suvetha Kannappan, Bhakti Sadhu. A primary care approach for oral health promotion among HIV patients: An interventional study . J Indian Assoc Public Health Dent. 2021 | January-March | Volume 19 | Issue 1	N	N	DOAJ
		4. Suvetha.K, Chacko TV. Academia-Industry collaboration to provide interdisciplinary experiential learning opportunities in Public Health Professions Education and improve health of female factory workers. Educ Health 2020; 33:55-60.	Y	Y	
		5. Chandrashekar BR, Chacko TV, Anand KM, Suvetha K, Jaishankar HP, Suma S. Enhancing identification and counseling skills of dental undergraduate students using a customized Tobacco Counseling Training Module (TCTM) - A piloting of the process using ADDIE framework. Indian J Cancer. 2020;57(3):296-310.	Y	Y	
		Jothilakshmi K, Vinoth V, Kannappan S. Pattern and prevalence of metabolic syndrome in obese adolescents aged 10-18 years: a community based study. Int J Contemp Pediatr 2020;7:1344-8.	N	N	
		7. Suvetha.K, Karthikeyan.S. Peer Educators as change leaders- Effectiveness of peer education process in creating awareness on reproductive health among women workers in textile industry. Indian Journal of Community medicine. 2019;44 (3):252-255	Y	Y	
		8. Abirami. S, Suvetha.K, Subhashini.G. Psychological well- being of women workers in a garment factory of South India-A cross sectional study. National journal of research in Community Medicine.2019;8(2):189-91	N	N	Copernicus

		9. Suvetha K, Subhashini G, Sivan.Y.S, Dharshan.M. Evaluation of community orientation program for first year medical undergraduate students. Indian Journal of public health research and development. 2019; 10(2): 50-56	N	N	
		10. Srinivasan R, Kannappan S. Oral feeds with or without dextrose half normal saline for treatment of hypernatremia in breastfed late preterm and term neonates: A comparison study. Journal of Clinical Neonatology. 2018; 7: 202-207	N	Y	
	Dr Suvetha K	11. Senthilkumar SK. Thomas V. Chacko, K Suvetha. Nutritional status assessment of children aged 0-5 years and its determinants in a tribal community of Coimbatore district. International Journal of Community Medicine and Public Health. 2018; 5(7): 2835-2845	N	N	Copernicus
		12. Divya K, Suvetha K, Sen A, Sundar D. Needs assessment of ophthalmology education for undergraduate medical students– A study from a medical college in South India. Education for Health 2017; 30: 223-7	Y	Y	
		13. Ramesh S, Suvetha K. Predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates. Indian Journal of Child Health. 2017; 4(4): 561-565	N	N	Copernicus
6	Dr. Karthikeyan S	1. Hamsaa Nandini S, Karthikeyan S An educational intervention on the risk factors of lifestyle diseases among men aged 30-50 years in an urban slum in Coimbatore, Tamil Nadu, India International Journal of Community Medicine and Public Health | January 2017 | Vol 4 | Issue 1:181-185	N	N	INDEX COPERNICUS
		2. Dinoop Korol Ponnambath, Swaran Kumar M, Appalaraju Boppe, Karthikeyan Shanmugam Clinico-mycological and anti fungal susceptibility profiles of candiduria in a tertiary care hospital from south India National Journal of Laboratory Medicine. 2017 Oct, Vol: 6(4): MO07-MO12	N	N	INDEX COPERNICUS
		3. Lekha T, Prasad HN, Sarwate RN, Patel M, Karthikeyan S. Intravitreal bevacizumab for choroidal neovascularization associated with angioid streaks: Long-term results. Middle East African Journal of Ophthalmology. 2017 Jul 1;24(3):136	Y	Y	
		4. Deepalakshmi K, Vasanthi R, Venkatakrishnan L, Karthikeyan S. Clinical and manometric profile of patients with GERD in a tertiary care hospital. International Journal of Advances in Medicine. 2017 Nov 22;4(6):1658-61	N	N	INDEX COPERNICUS
		5. Vidhya Lakshmi, Kharishma Nair Karthikeyan ShanmugamThe role of body mass index (BMI) as an independent risk factor and prognostic factor in breast cancer-A hospital based	N	N	INDEX COPERNICUS

		epidemiological study International Journal of Current Research, Vol. 9, Issue, 12, pp.62653-62656, December, 2017			
		6. Peer educators as change leaders – Effectiveness of peer education process in creating awareness on reproductive health among women workers in textile industry Suvetha Kannappan, Karthikeyan Shanmugam Indian journal of Community Medicine 2019 ,Volume : 44,Issue : 3,Page : 252-255	Y	Y	
	Dr. Karthikeyan S	7. MATHEW, Chippy Tess; MAHESWARI, Uma; SHANMUGAM Karthikeyan. Co-relation of endometrial thickness by transvaginal sonogram with histopathology pattern in abnormal uterine bleeding: a study from South India. International Journal of Reproduction Contraception, Obstetrics and Gynecology, [S.l.], v. 9, n. 4, p. 1416-1421, mar. 2020	N	N	INDEX COPERNICUS
		8. Suja S, Saravanan T, Karthikeyan S. Profile of hematological abnormalities and its correlation with absolute CD4 count and human immunodeficiency virus viral load in human immunodeficiency virus-infected patients in a tertiary care hospital. Indian Journal of Sexually Transmitted Diseases and AIDS. 2020 Jul 1;41(2):156	Y	Y	
		9. Sunny AA, Iyer RS, Kumaran SG, Bunshaw NG, Shanmugham K, Govindaraj U. Affordability, availability and tolerability of anti-seizure medications are better predictors of adherence than beliefs: Changing paradigms from a low resource setting. Seizure. 2020 Nov 12	Y	Y	
		10. Abhirami K, Vallig, Karthikeyan S, Dental Health Status among College Students and the Treatment Needs, JRes Med Dent Sci, 2020, 8 (6): 131-135	N	N	INDEX COPERNICUS
		11. Osborn JA, Sriram R, Karthikeyan S, Ravishankar SL. A study on contraceptive prevalence rate and factors influencing it in a rural area of Coimbatore, South India. Journal of Family Medicine and Primary Care. 2021 Jun;10(6):2246	Y	Y	
		12. Vallippalam J, Krishna S, Suresh N, Sunny AA, Karthikeyan,Iyer RS. Stroke education focusing on recognition and response to decrease pre-hospital delay in India: Need of the hour to save hours. Interdisciplinary Neurosurgery. 2021 Dec 1;26:101309	Y	Y	
7	Dr. Jenit Osborn A	1. Subramaniyan P, Chacko T V, Sriram R M, Osborn A J. Usage of personal protective equipment among workers of a foundry in South India: an interventional study. Nat J Res Community Med2019;8(1): 65-69.	Y	N	
		2. Osborn J, Priya KK, Satheesh T, Ramalingam S. Knowledge, Attitude and Practice of COVID 19 and the factors influencing them among a Rural Population in South India. Indian Journal	Y	N	

		of Community Health. 2021 Mar 31;33(1).			
		3. Osborn JA, Sriram R, Karthikeyan S, Ravishankar SL. A study on contraceptive prevalence rate and factors influencing it in a rural area of Coimbatore, South India. Journal of Family Medicine and Primary Care. 2021 Jun;10(6):2246.	Y	N	
		4. Osborn AJ, Muhammad GM, Ravishankar SL, Mathew AC. Prevalence and correlates of anemia among women in the reproductive age (15–49 years) in a rural area of Tamil Nadu: An exploratory study. Journal of Education and Health Promotion. 2021;10.	Y	N	
		5. Jothinath K, Balakrishnan S, Raju V, Menon S, Osborn J. Clinical efficacy of levosimendanvsmilrinone in preventing low cardiac output syndrome following pediatric cardiac surgery. Annals of Cardiac Anaesthesia. 2021 Apr 1;24(2):217.	Y	N	
8	Dr. S. Suganathan	1. Soundararajan SS. A study on activities of daily living among elderly in a rural population of Tamilnadu. Children. 2017; 172:38-2. Jan- March 2018 Vol 7 issue 1	N	N	Index corponicus
9	Dr.Vijayageetha M	1. Subramanian S, Sarkar S, Vijayageetha M, AdhinarayananS.Experiences of Volunteers and Patient Satisfaction in Home Based Palliative Care, Puducherry, South India—A Descriptive Qualitative Study. Journal of Palliative Care. 2021.	Y	Y	
		2. Jayaram S, Saya GK, RajkumariN, Ulaganeethi R, Roy PK, Vijayageetha Mathavaswami. Prevalence of intestinal parasitic infection and its associated factors among children in Puducherry, South India: a community-based study. Journal of Parasitic Diseases. 2021:45(4):897-902.	Y	Y	
		3. Vinayachandran G, Patel N, Vijayageetha M, Lakshminarayanan S. Food portion size educational programme in a workplace setting in Puducherry – Is it effective? Journal of Family Medicine and Primary Care.2021: 10(9):3368-3373.	Y	N	
		4. Krishnamoorthy Y, Vijayageetha M, Saya GK. Validation and Reliability Assessment of the Mini-Nutritional Assessment-Short Form Questionnaire among Older Adults in South India. Indian J Community Medicine. 2021;46(1):70-74.	Y	Y	
		5. Vemuri MB, Rajaram M, Mohapatra MM, Vijayageetha M, Negi VS, Adithan S et al.Distribution of interstitial lung diseases in a tertiary care centre of South India. International Journal of advances in medicine. 2021;8(3):420-426.	N	N	Index Copernicus, Index Medicus
		6. Khemani MC, Premarajan KC, Menon V, Olickal JJ, Vijayageetha M, Chinnakali P. Pathways to care among patients with severe mental disorders attending a tertiary health-care	Y	Y	

		facility in Puducherry, South India. Indian J Psychiatry. 2020;62:664-9.			
		7. Vijayageetha M, Sen A, Subramanian S, Anuja M, Anushree J, Arya A B et al. Presumptive pulmonary tuberculosis and its associated factors among adult patients availing out-patient services in a tertiary care center, Puducherry, South India. Int J Community Med Public Health. 2020;7(7):2703-2707.	N	N	Index Copernicus, Index Medicus
		8. Rajaram M, Malik A, Mohapatra MM, Vijayageetha M, Babu VM, Vally S et al. Comparison of Clinical, Radiological and Laboratory Parameters Between Elderly and Young Patient With Newly Diagnosed Smear Positive Pulmonary Tuberculosis: A Hospital-Based Cross Sectional Study. Cureus. 2020;12(5):	Y	N	
	Dr.Vijayageetha M	9. Ayiraveetil R, Sarkar S, Chinnakali P, Jeyashree K, Vijayageetha M, Thekkur P et al. Household food insecurity among patients with pulmonary tuberculosis and its associated factors in South India: a cross-sectional analysis. BMJ Open.2020;10:e033798.	Y	Y	
		10. Patel BH, Jeyashree K, Chinnakali P, Vijayageetha M, Mehta KG, Modi B et al. Cash transfer scheme for people with tuberculosis treated by the National TB Programme in Western India: a mixed methods study. BMJ Open. 2019;9:	Y	Y	
		11. Vijayageetha M, Kumar AMV, Ramakrishnan J, Sarkar S, Papa D, Mehta K, Joseph NM, Rajaram M, Rajaa S, Chinnakali P. Tuberculosis screening among pregnant women attending a tertiary care hospital in Puducherry, South India: is it worth the effort?.Glob Health Action. 2019;12(1):1564488.	Y	Y	
		12. Sahu SK, Rajaa S, Vijayageetha M, Selvaraj K, Sambath PM, Roy G. Strengthening growth monitoring among under-5-year children to fight childhood undernutrition in India. J Family Med Prim Care. 2019;8:231-8.	N	N	Index Copernicus, Index Medicus
		13. Krishnamoorthy Y, Chandar D, Jayaseelan V , Vijayakumar K, Sivaranjini K, Vijayageetha M. Household survey on public awareness and attitudes toward dengue infection in rural Tamil Nadu, South India. J Edu Health Promot. 2018;7:171.	Y	Y	
		14. Krishnamoorthy Y, Vijayageetha M, Sakthivel M, Sarveswaran G. Emerging public health threat of e-waste management: global and Indian perspective. Reviews on Environmental Health. 2018;33(4):321-29.	Y	Y	
		15. Krishnamoorthy Y, Vijayageetha M, Kumar SG, Rajaa S, Rehman T. Prevalence of malnutrition and its associated factors among elderly population in rural Puducherry	N	N	Index Copernicus, Index Medicus

		using mini-nutritional assessment questionnaire. J Family Med Prim Care. 2018;7(6):1429-33.			
		16. Mahalakshmy T, Premarajan KC, Soundappan K, Rajarethinam K, Krishnamoorthy Y, Rajalatchumi A, Vijayageetha M et al. A Mixed Methods Evaluation of Adolescent Friendly Health Clinic Under National Adolescent Health Program, Puducherry, India. Indian J pediatr. 2018;86:132-139.	N	Y	
		17. Krishnamoorthy Y, Vijayageetha M, Sarkar S. Awareness about rabies among general population and treatment seeking behaviour following dog-bite in rural Puducherry: a community based cross-sectional study. International Journal of Community Medicine and Public Health. 2018;5(6):2557-63.	N	N	Index Copernicus, Index Medicus
10	Dr Anil C Mathew	1. ANIL C MATHEW, ELBIN SIBY, AMAL TOM, SENTHIL KUMAR R Applications of Proportional od logistic regression models and continuation ratio models in examining the association of physi inactivity with erectile dysfunction among type 2 diabetes patients. Physical Activity and Nutrition, Vol.25(1),Page 30-34, 2021	Y	N	
		2. OSBORN JENIT, MUHAMMAD GM, RAVISHANKAR SL, ANIL C MATHEW Prevalence and correla of anemia among women in the reproductive age-An exploratory study Journal of Education a Health Promotion ,Vol.10, 355,2021	Y	N	
		3. NOEL GEORGE,ALAN JOSE,SIDHARTH DHARAMSI, LEELAKRISHNAN VENKATAKRISHNAN, ANIL C MATHEW Prognostic survival factors in acute liver failure patients in India Advanced Biomedical Research, Vol.9 (37), 2020	Y	N	
		4. NARENDRA KUMAR ARORA, MANOJA KUMAR DAS, RAMESH POLURU , NEERAJ KUMAR KASHYAP, THOMAS MATHEW, JOHN MATHAI, ANIL C. MATHEW (INCLEN VACCINE SAFETY STUDY GROUP). A Prospective cohort study of the safety of Infant Pentavalent(DTwP-HBV-H and oral polio vaccines in two South Indian districts. The Pediatric Infectious Disease Journal. Vol. 39(5), Page 389-396,2020	Y	N	
		5. ANIL C MATHEW, RAMAMOORTHY V, SUBASH P, XAVIER CHRISTU RAJAN. Morbidity profile of truck drivers; A South Indian Cross sectional Study. International Journal of Community Medicine and Public Health, Vol.6 (10),Page 4450-4455, 2019	N	N	
		6. ANIL C MATHEW, SANOOP THOMAS, RICHU RAVIKUMAR, JITHIN THOMAS, ARUN PADMANADAN Socio demographic and life style factors associated with internet addiction among medical and health sciences students. National Journal of Research in Community	N	N	

		Medicine,Vol.8(4),Page 240-244,2019			
		7. VISWAKUMAR P, THAMIIZHAMATHI T,ANIL C MATHEW, VIMAL KUMAR GONINDAN, VIGNESH KANNAN, TRACY ROSALIN POULOSE CR-Possum-Can it be used to predict morbidity? A single-centre retrospective study Indian Journal of Surgical Oncology, Volume 10, Issue 1, Page 174-179,2019	Y	N	
		8. ANIL C MATHEW, DHANYA C, JIBIN K BENNY, DONA MARIA PHILIP, GHURUPRIYAN M, RAMESH S, NEELAKANDAN K The effect of Mode of Delivery on Breastfeeding Outcomes: A Hospital Based Study. Asian Pacific Journal of Health Sciences ,Vol.5(3), Page 241-45,2018	N	N	
		9. ANIL C MATHEW, DONA MARIA PHILIP, JIBIN K BENNY, DHANYA C, MANJU T M, RAMESH S, NEELAKANDAN KFactors Associated with Timely Initiation of Breastfeeding: a Hospital Based Study.Asian Pacific Journal of Health Sciences, Vol.5(3), Page 318-24,2018	N	N	
	Dr Anil C Mathew	10. ANIL C MATHEW, SOMY KRISHNAN , MELVIN JOY, DARSHAN MS, AMITHVARSHAN A,SENTHIL KUMAR R Chocolate consumption and its relation to type 2 diabetes mellitus International Journal of Advances in Medicine, Vol. 4(5),Page 1473-1476, 2017	N	N	
		11. ANITH KM, MURALI A,SIVARAMAKRISHNAN G, ANIL C MATHEW, VENU G, JAYACHANDRAN K, MADHAV CM Evaluation of iron status in patients with end stage of renal disease. International Journal of Advances in Medicine,Vol.4(5),Page 1415-1421,2017	N	N	
		12. ANIL C MATHEW,MELVIN JOY,SOMY KRISHNAN,DARSAN MANOJ S, KAVITHA V,SENTHIL KUMA R, YUNSHNENG Ma Consumption of tea and its relation to risk of type 2 diabetes. Asian Pacific Journal of Health Sciences, Vol. 4(3),Page 83-87 (2017)	N	N	
		13. ANIL C MATHEW, JIBIN K BENNY, DONA MARIA PHILIP, DHANYA C, SANDHIYA V, RAMESH S, NEELAKANDAN K Socio-demographic and clinical correlates of exclusive breastfeeding up to si months of age. Nepal Journal of Epidemiology, Vol.9(3),Page 727-734,2019	Y	N	

DEPARTMENT OF RESPIRATORY MEDICINE

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

S.NO	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			PUBMED Y/N	SCOPUS Y/N	
1	Dr. R KARTHIKEYAN	Chhabra S K, Sahay S, Ramaraju K. Allergic Bronchopulmonary Aspergillosis complicating childhood asthma. Indian J Pediatr. 2009 mar; 76 (3):331 – 2.	Y	N	
		Ramaraju. K, Aggarwal B, Kulsrestha R, Chhabra S. Unusual Presentation of an uncommon lung malignancy. Lung India. 2008 Jul;25 (3): 132 – 4.	Y	N	
		Karthikeyan Ramaraju, Srikanth Krishnamurthy, Smrithi Maamidi, Anupama Murthy Kaza, and Nithilavalli Balasubramaniam. Is serum cholesterol a risk factor for asthma? Lung India. 2013; 30(4): 295–301.	Y	N	

		Ramaraju Karthikeyan, Srikanth Krishnamoorthy, Smrithi Maamidi, Anupama Murthy Kaza, Nithilavalli Balasubramanian. Effect of inhaled corticosteroids on systemic inflammation in asthma. Perspectives in clinical research 2014; Vol 5 (2): 75-79.	Y	N	
		Anupama Murthy Kaza, Ramaraju Karthikeyan, Nithilavalli Balasubramaniam. Simulation based approach to improve competencies in respiratory emergencies among interns. International journal of innovative research and development 2015; Vol 4 (11): pp213 - 217.	N	N	Google Scholar
		Prashanth Ashok Kumar, R.Karthikeyan. A survey on the pattern of referral and management of patients with Tuberculosis by medical practitioners in an urban area. Journal of Patient Safety & Infection Control 2015; 3(2):49.	No	Yes	

		Ramaraju Karthikeyan, Anupama Murthy Kaza, Nithilavalli Balasubramaniam, Suddhuraj Chandrasekaran, Predicting healthcare utilization by patients admitted for COPD exacerbation. Journal of clinical and diagnostic research 2016; Vol 10(2):pp OC13 – OC17.	Y	N	
		Prasanth Ashok Kumar, Ramaraju Karthikeyan and Anupama Murthy Kaza. Public private partnership for tuberculosis control in India: Are the private practitioner anywhere near to this RNTCP sponsored mission? IOSR Journal of dental and medical sciences 2016; Vol 15(4): PP 107-112.	N	N	Google Scholar, Indian Citation Index
		Ramaraju Karthikeyan, Anupama K Murthy, Punitha Murugesan, Nithilavalli Balasubramaniam, Siddhuraj Chandrasekar. Is Serum Cholesterol A Risk Factor For Ischemic Heart Disease In Patients With Chronic Obstructive Pulmonary Disease? IOSR Journal of Dental and Medical Sciences 2016; Vol 15(3): PP 88-9.	N	N	Google Scholar, Indian Citation Index

		Shweta Paulraj, Prasanth AK, Kavitha S Karthikeyan R. "Role of Oxidative Stress in COPD. Can we Use A Novel Biomarker To Measure It?" IOSR Journal of Dental and Medical Sciences 2017;16(9): 52-56.			Google Scholar, Indian Citation Index
		Lekha T, Karthikeyan R. Multimodal imaging of choroidal tubercles. Indian journal of ophthalmology. 2018 Jul;66(7):995..	Y	N	
		Kaliyaperumal D, Karthikeyan RK, Alagesan M, Ramalingam S. Characterization of cardiac autonomic function in COVID-19 using heart rate variability: a hospital based preliminary observational study. Journal of basic and clinical physiology and pharmacology. 2021 May 1;32(3):247-53.	Y	N	
		Gopikrishnan N, Narasimhan D, Karthikeyan R, Nair KS, Murali A. Post COVID19 infections: A case series of 5	Y	N	

		different pathogens. Open J Clin Med Case Rep. 2021; 1789.			
		Deepalakshmi Kaliyaperumal , Kumar Bhargavi , Karthikeyan Ramaraju , Krishna S Nair , Sudha Ramalingam , Murali Alagesan. Electrocardiographic Changes in COVID-19 Patients: A Hospital-based Descriptive Stud.Ind J Crit Care Med (2021): 10.5005/jp-journals-10071-24045	Y	N	
		Viyannan M, Kumar PA, Eswarswamy, S, Murugesan G, Ramaraju K. 3D Printed Model of Airway for Clinical Simulation. Asian J. Med. Radiol. Res. 2020;8(2):50-56.	N	N	Index Copernicus
		Narayanan R, Muthusamy S, Lakshmi SV, Ramaraju K, Balasubramaniam G, Sankaran R. Design and development of whole-body rodent inhalation chamber for exposure to Aroclor 1232 in Swiss albino mice.	Y	Y	

		International Journal of Environmental Science and Technology. 2021 Jul 6:1-2.			
		Kaliyaperumal D, Bhargavi K, Ramaraju K, Nair KS, Ramalingam S, Alagesan M. Electrocardiographic Changes in COVID-19 Patients:A Hospital-based Descriptive Study. Indian J Crit Care Med 2022; 26(1):43-48.	Y	Y	EBSCO, Google Scholar
2	Dr.ANUPAMA MURTHY	Karthikeyan R, Anupama K, Murthy AK. Is Serum Cholesterol a Risk Factor for Ischemic Heart Disease in Patients with Chronic Obstructive Pulmonary Disease. IOSR Journal of Dental and Medical Sciences. 2016;15(3):88-91.	N	N	Google Scholar, Indian Citation Index
		Murthy KA, Ramaraju K, Balasubrramanian BN, Kumar PA. Simulation Based Approach to Improve Competencies in Respiratory Emergencies amongst Interns. International Journal of Innovative Research and Development. 2015 Oct 31;4(11).	N	N	Google Scholar

		Karthikeyan Ramaraju, Srikanth Krishnamurthy, Smrithi Maamidi, Anupama Murthy Kaza, and Nithilavalli Balasubramaniam. Is serum cholesterol a risk factor for asthma? Lung India. 2013; 30(4): 295–301.	Y	N	
		Ramaraju K, Kaza AM, Balasubramanian N, Chandrasekaran S. Predicting healthcare utilization by patients admitted for COPD exacerbation. Journal of clinical and diagnostic research: JCDR. 2016 Feb;10(2):OC13.	Y	N	
		Karthikeyan R, Anupama K, Murthy AK. Is Serum Cholesterol a Risk Factor for Ischemic Heart Disease in Patients with Chronic Obstructive Pulmonary Disease. IOSR Journal of Dental and Medical Sciences. 2016;15(3):88-91	N	N	Google Scholar, Indian Citation Index
		Prasanth Ashok Kumar, Ramaraju Karthikeyan and Anupama Murthy Kaza. Public private partnership for tuberculosis control in India: Are the private practitioner	N	N	Google Scholar, Indian Citation Index

		anywhere near to this RNDCP sponsored mission? IOSR Journal of dental and medical sciences 2016; Vol 15(4): PP 107-112.			
3	Dr. KANCHERLA ROOPA	Rajagopala S, Kancherla R, Ramanathan RP. Tuberculosis-associated chylothorax: case report and systematic review of the literature. Respiration. 2018;95(4):260-8.	Y	N	
		2Kancherla R, Ramanathan RP, Appalaraju B, Rajagopala S. Pulmonary Nocardiosis Presenting as Exacerbation of Chronic Pulmonary Disease. Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine. 2019 Oct;23(10):467.	Y	N	
		Arumugam M, Dhanasekaran S, Roopa K, Govindharaj P. Effectiveness of pulmonary rehabilitation in lymphangioleiomyomatosis. Indian Journal of Respiratory Care. 2021 May 1;10(2):252.	Y	N	

		Chennaiyan B, Bhatt AN, Kancherla R, Kuriakose CK, Dev AV, Philip GA. Validity of tuberculous pleuritis diagnosed in a resource-constrained setting in Dindigul district of Tamil Nadu. Journal of family medicine and primary care. 2016 Jul;5(3):615.	Y	N	
		. Chennaiyan B, Bhatt AN, Kancherla R, Kuriakose CK, Dev AV, Philip GA. Validity of tuberculous pleuritis diagnosed in a resource-constrained setting in Dindigul district of Tamil Nadu. Journal of family medicine and primary care. 2016 Jul;5(3):615.	Y	N	
		Rajagopala S, Sankari S, Kancherla R, Ramanathan RP, Balalakshmoji D. Miliary Sarcoidosis: does it exist? A case series and systematic review of literature. Sarcoidosis, Vasculitis, and Diffuse Lung Diseases. 2020;37(1):53.	Y	N	
		Preditors of post discharge home oxygen therapy and bilevel positive airway preesure therapy in stable			Index Copernicus

		hypercapnoea: An experience from tertiaty care health center in south India :Pulmon journal Vol 23, Issue 2,May-Aug 2021			
		Unfamiliar territory of family drug: INH induced erythroderma :Euracian journal of pulmonary medicine	N	N	DOAJ

DEPARTMENT OF GENERAL MEDICINE

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr.T.Saravanan	Shanmugapriya S, Saravanan T, Janani K. Antibiotic sensitivity pattern to urinary tract infections in a tertiary care hospital in South India. Int J Basic ClinPharmacol 2017; 6(6):1445-50.	N	N	Copernicus
		Saravanan T, Shanmugapriya S, Sumitra G, Dhayananth RS, Saravanan A, Manicka Vasuki AK. Hepato-protective effect of folic acid and vitamin B12 in comparison to N-acetylcysteine in experimentally induced acetaminophen toxicity in rats.Biomed. &Pharmacol. J. 2017; 10(2): 549-555.	N	Y	
		Salim B, Athira MV, Kandaswamy A, Vijayakumar M, Saravanan T, Sairam T. Microfluidic device for novel breast cancer screening by blood test using miRNA beacon probe. Biomedical microdevices. 2017 Dec;19(4):1-1.)	Y	N	
		Krithika.E , Venkatrajan.R , Saravanan.T , BrittoDuraisingh.L*. A comprehensive analysis on uses and side effects of steroids with respect to different routes International Journal of Pharma Sciences and Research (IJPSR) ISSN :0975-9492 Vol 8 No 08 Aug 2017	N	N	EBSCO
		Shanmugapriya S, Saravanan T, Rajee SS, Venkatrajan R, Thomas PM. Drug prescription pattern of outpatients in a tertiary care teaching hospital in Tamil Nadu. Perspectives in clinical research. 2018 Jul;9(3):133.	Y	N	
		Saravanan T, Shanmugapriya S,*. Study on the effectiveness of teaching the principles and methods of clinical audit to medical postgraduates. Current Medicine Research and Practice 8 (2018) 59–63.	N	N	Embase
		Shanmugapriya S, Dhandapani N, Saravanan T. A study comparing cognitive function assessment in type-2 diabetes mellitus using Rowland	N	N	EBSCO

		Universal Dementia Assessment Scale and Mini Mental State Examination. International Journal of Health & Allied Sciences. 2018 Jul 1;7(3):184-.			
	Dr.T.Saravanan	Petchiappan V, Hussain TM, Thangavelu S. Can serum ferritin levels predict the severity of dengue early?: an observational study.	N	N	Coper nicus
		Aashiqahamed shukkoor1, Nimmyelizabeth george1, SaravananThangavelu2*. N-acetylcysteine, a boon for yellow phosphorus-induced acute liver failure? A case report. Asian j pharm clin res, vol 12, issue 8, 2019, 1-3	N	N	EMBASE & EBSCO
		Kumar B, Thangavelu S. Looking beyond lupus in lupus; Journal of Clinical and Diagnostic Research. 2019 Dec, Vol- 13(12): OD01-OD03. DOI: 10.7860/JCDR/2019/42862.13331	N	N	Web of science
		Kumar B, Thangavelu S, Prabhakar R. Euglycemic diabetic ketoacidosis: another masquerader!.	N	N	Copernicus
		Thangavelu S, Jenil Dhas C, Zeya Ansari M. Dengue Fever Complicated By Hemophagocytosis–A Difficult To Manage Case. The Open Hematology Journal. 2019 Feb 28;7(1).	N	N	EMBASE & EBSCO
		Shukkoor AA, Thangavelu S, George NE, Priya S. Dapsone hypersensitivity syndrome (DHS): a detrimental effect of dapsone? A case report. Current drug safety. 2019 Mar 1;14(1):37-9.	Y	N	
		Kumar B, Thangavelu S, Shukkoor AA, Ramakrishnan N. To err is… Drug? Patient? Doctor? Healthcare System?. Indian Journal of Pharmacy Practice. 2020 Oct;13(4):363.	N	Y	
		Kumar B, Prabhakar R, Thangavelu S. Tenofovir-induced delayed nephro-osteo toxicity. The Journal of the Royal College of Physicians of Edinburgh. 2020 Sep 1;50(3):291-4.	N	N	coper nicus
		Shanmugapriya S, Saravanan A, Shuruthi S, Dharsini JS, Saravanan T. Association of gastroesophageal reflux disease with anxiety, depression, and sleep disorders. Journal of Medical Sciences. 2021 Jan	N	Y	

		1;41(1):9.			
	Dr.T.Saravanan	Vishnupriya S, Andhuvan G, Saravanan T, Velammal P. A comparative study on the effect of sodium-glucose cotransporter-2 inhibitors and dipeptidyl peptidase-4 inhibitors as an add on therapy in patients with type 2 DM. Int. J. Res. Pharm. Sci., 2020, 11(1), 630-634	N	N	
2.	Dr.K.Bhargavi	Kumar B, Thangavelu S. Looking beyond lupus in lupus; Journal of Clinical and Diagnostic Research. 2019 Dec, Vol- 13(12): OD01-OD03. DOI: 10.7860/JCDR/2019/42862.13331	N	N	-
		Kumar B, Thangavelu S, Prabhakar R. Euglycemic diabetic ketoacidosis: another masquerader!.	N	N	Web of science
		Kumar B, Prabhakar R, Thangavelu S. Tenofovir-induced delayed nephro-osteo toxicity. The Journal of the Royal College of Physicians of Edinburgh. 2020 Sep 1;50(3):291-4.	N	N	Copernicus
		Kumar B, Thangavelu S, Shukkoor AA, Ramakrishnan N. To err is… Drug? Patient? Doctor? Healthcare System?. Indian Journal of Pharmacy Practice. 2020 Oct;13(4):363.	N	Y	N
		Kumar, B., Anees, M., Manickam, S. and Thangavelu, S., 2021. Complications and Outcome of H1N1 Influenza in a Tertiary Care Hospital in South India –A Retrospective Study. *Current Respiratory Medicine Reviews*, 17.	N	N	
3.	Dr.Denesh Narasimhan	Narasimhan D, Katragadda S. Retrospective Analysis of Demographic and Clinical Profile of Dengue Infection in a Tertiary Care Hospital. December 2017 National Journal of Research in Community Medicine 6(4):271	N	N	Coper nicus
		Denesh Narasimhan, Silpita Katragadda, M. Sathish Retrospective analysis of ultrasound features of dengue infection. International Journal of Research in Medical ScienceInt J Res Sci. 2017/Dec:5 (12); 5260-5262	N	N	Coper nicus
		Narasimhan D, Ponnusamy P, Sathish M. Analysis of liver function tests in dengue fever. International Journal of Advances in Medicine. 2018;5(1):47.	N	N	Coper nicus

		Narasimhan D, Katragadda S, Sathish M. Retrospective study of white blood cell count and hematocrit in dengue fever. International Journal of Advances in Medicine. 2018;5(1):24.	N	N	Coper nicus
4.	Dr. Jayachandran	R V D, Rajendiran D, Jayachandran D, Aruna D. Insulin resistance and significance of postprandial lipid profile in normoglycemic coronary artery disease. International Journal of Medical Research and Review. 2017;5(1):15-19.	N	N	Coper nicus
		Manickam S, Sethuraman G, Kuppusamy J. A study of the symptoms of gastro oesophageal reflux disease and the associated risk factors in a tertiary care centre.	N	N	Index coper nicus
		Petchiappan V, Pedireddy D, Manickam S, Kuppusamy J. A prospective study on the clinical profile and outcome of meningoencephalitis in adults in a South Indian tertiary care centre. J R Coll Physicians Edinb. 2019; 49: 282–6	N	N	copernicus
		Mambatta A, Alagesan M, Meeran M, Rein J, Ganesan S, Mathew A et al. Evaluation of iron status in patients with end stage renal disease. International Journal of Advances in Medicine. 2017;4(5):1415.	N	N	Coper nicus
		Manickam S, Chidambaram Y, Kuppusamy J, Dhas CJ, Kumar S. Association of N-terminal-Pro-B-type Natriuretic Peptide with Localization of Thrombus and Clinical Outcome in Acute Pulmonary Embolism. International Journal of Contemporary Medical Research Vol.7/issue.4/April 2020.	N	N	Google scholar
5.	Dr.A.Murali	Mambatta A, Alagesan M, Meeran M, Rein J, Ganesan S, Mathew A et al. Evaluation of iron status in patients with end stage renal disease. International	N	N	Coper nicus

		Journal of Advances in Medicine. 2017;4(5):1415.			
		Katragadda S. Correlation of Reciprocal Changes and QRS Amplitude in ECG to Left Ventricular Dysfunction, Wall Motion Score and Clinical Outcome in First Time ST Elevation Myocardial Infarction. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH. 2017;.	N	N	Web of science
		Kaliyaperumal D, Elango Y, Alagesan M, Santhanakrishanan I. Effects of Sleep Deprivation on the Cognitive Performance of Nurses Working in Shift. J Clin Diagn Res. 2017 Aug;11(8):CC01-CC03. doi: 10.7860/JCDR/2017/26029.10324. Epub 2017 Aug 1. PMID: 28969117; PMCID: PMC5620757.	N	N	Web of science
		Deepalakshmi K, Vijayabaskaran S, Murali A. Comparison of microalbuminuria and heart rate variability in prediction of cardiovascular complications in diabetic population: a pilot study. International Journal of Advances in Medicine. 2017;4(3):819.	N	N	Coper nicus
		Dhas C, A. M, C. Y. Eosinophilic gastroenteritis in an immunosuppressed patient: a case report. International Journal of Advances in Medicine. 2018;5(4):1078.	N	N	Coper nicus
		Kaliyaperumal D, Varadharajan VK, Viswanathan V, Alagesan M. Effect of Age and Hypertension on Cognition: A Cross-sectional Study. Journal of Clinical & Diagnostic Research. 2018 Sep 1;12(9).	N	N	Web of science
		Yoganathan Chidambaram, Murali Alagesan, Clement Jenil Dhas. Human brucellosis in a non-susceptible host: a case report. International Journal of Advances in Medicine. 2018. 5(4).	N	N	Coper nicus

		Alagesan Murali, Gauri Shankar J, Senthur Nambi P, Shanthakumari S, Aswatha Rabindranath Nambi, Ram Gopalakrishnan. Non Hodgkin's lymphoma of B cell type with hemophagocytosis in a patient with HIV infection – Open J of Clinical and Medical Case reports. Vol 3. Issue 17 (2017). 1306.	N	N	Google scholar
		Alagesan Murali, Vivek Koushik, Ram Gopalakrishnan, Senthur Nambi P. IRIS due to cryptococcal meningitis ten years after the initial episode in a HIV positive patient with a high CD4 count and suppressed viral load – A case report. Open J Clin Med Case Rep: Volume 3 (2017). 1316	N	N	Google scholar
		Murali Alagesan, Joel Franklin, J Jayalakshmi, TM Subba Rao, V Chaithra. Basidiobolomycosis- Miss me; it hurts you. Journal of the Association of Physicians of India 2019. 67; 99-101	N	N	Coper nicus
		Rhoshini Rajasekaran, Murali Alagesan, Yoganathan, Azhar, Venkatakrishnan Leelakrishnan. Kikuchi Fujimoto Disease: A case report. Open J Clin Med Case Rep: Volume 4 (2018)	N	N	Google scholar
		Deepalakshmi Kaliyaperumal, Karthikeyan RK, Murali Alagesan, Sudha Ramalingam. Characterization of cardiac autonomic function in COVID-19 using heart rate variability: a hospital based preliminary observational study. J Basic Clin Physiol Pharmacol 2021. DOI: https://doi.org/10.1515/jbcpp-2020-0378	N	Y	
		Nilaa Gopikrishnan; Denesh Narasimhan; Karthikeyan R; Krishna S Nair; Murali	N	N	Coper nicus

		A. Post COVID 19 infections: A case series of 5 different pathogens. Open Journal of Clinical & Medical Case Reports Volume 7 (2021) Issue 12. 1789.			
6.	Dr.C.Yoganathan	Sundaramoorthy V, Mambatta AK, Chidambaram Y, Gopalan R, Menon S. Prevalence of metabolic syndrome in patients with premature coronary artery disease proven by coronary angiogram. Int J Res Med Sci. 2017 Nov;5(11):5021-5.	N	N	Coper nicus
		Yoganathan Chidambaram, Murali Alagesan, Clement Jenil Dhas. Human brucellosis in a non-susceptible host: a case report. International Journal of Advances in Medicine. 2018. 5(4).	N	N	Coper nicus
		Dhas C, A. M, C. Y. Eosinophilic gastroenteritis in an immunosuppressed patient: a case report. International Journal of Advances in Medicine. 2018;5(4):1078.	N	N	Coper nicus
		Manickam S, Chidambaram Y, Kuppusamy J, Dhas CJ, Kumar S. Association of N-terminal-Pro-B-type Natriuretic Peptide with Localization of Thrombus and Clinical Outcome in Acute Pulmonary Embolism. International Journal of Contemporary Medical Research Vol.7/issue.4/April 2020.	N	N	Google scholar
		Chidambaram Y, Mambatta A, Sivaraj S. Tropical sprue in megaloblastic anemia. International Journal of Research in Medical Sciences. 2017;5(9):4133.	N	N	Google scholar

7.	Dr.Velammal P	Petchiappan V, Selvam S, Dhanush M, Prabu N. Assessment of Handgrip Strength in patients with Rheumatoid arthritis and Fibromyalgia- Do They differ -An observational Study. Archives of Medicine 2018 Vol No:10 Iss No:4:3 ISSN 1989-5216	N	N	EBSCO
		Vishnupriya S, Andhuvan G, Saravanan T, Velammal P. A comparative study on the effect of sodium-glucose cotransporter-2 inhibitors and dipeptidyl peptidase-4 inhibitors as an add on therapy in patients with type 2 DM. Int. J. Res. Pharm. Sci., 2020, 11(1), 630-634	N	N	EMBAS
		Petchiappan V, Pedireddy D, Manickam S, Kuppusamy J. A prospective study on the clinical profile and outcome of meningoencephalitis in adults in a South Indian tertiary care centre. J R Coll Physicians Edinb. 2019; 49: 282–6	N	N	copernicus
		Manickam S , Arun P , Petchiappan V, Menon S. Is serum uric acid an added risk factor for micro-vascular complications of diabetes mellitus? – a prospective study. International Journal of Contemporary Medical Research 2019; 6(7):G30-G34.	N	N	Index coper nicus
		Manickam S, Srinivasan T V, Petchiappan V, Sujith Kumar S. A prospective study of correlation of magnetic resonance imaging of brain including MR angiogram and carotid vertebral arterial doppler study with the clinical features in ischemic stroke. International Journal of Contemporary Medical	N	N	Coper nicus

		Research 2019; 6(6):F37-F40.			
		Petchiappan V, Sivakrishna N, Manickam S, Menon S. Glycaemic control and C - reactive protein levels in type 2 diabetes mellitus -how well they co-relate? : A prospective study. Int J Res Med Sci 2019; 7: 1818-21.	N	N	Index coper nicus
	Dr.Velammal P	Manickam S, Franklin J , Petchiappan V , Menon S. A study of serum albumin levels in acute ischemic stroke and its correlation with clinical outcome. International Journal of Contemporary Medical Research IJCMR 2019;6(3):C17-C20.	N	N	Index coper nicus
		Petchiappan V, Hussain TM, Thangavelu S. Can serum ferritin levels predict the severity of dengue early? : an observational study. Int J Res Med Sci 2019; 7:876-81.	N	N	Coper nicus
		Petchiappan V, Priyadarshini P, Prabu N. Comment on Long-Term Dietary Changes after Diagnosis of Rheumatoid Arthritis in Swedish Women: Data from a Population-Based Cohort. International Journal of Rheumatology Volume 2019, Article ID 1939686, https://doi.org/10.1155/2019/1939686	N	Y	
		Petchiappan V, Priyadarshini P, Prabu N. Dietary restrictions in rheumatoid arthritis-what the patient's feel: A questionnaire based study. Archives of General Internal Medicine. 2018; 2(3):27-31. DOI: 10.4066/ 2591-7951.1000054	N	N	Googl scholar
		Petchiappan V, Guhan A, Selvam S, Nagaprabu VN. ANA immunofluorescence versus profile-how well they perform in autoimmune diseases: an analysis of their clinical utility in a tertiary care centre. International Journal of Research in Medical Sciences .2018 Sep;6(9):3140-3146 DOI:	N	N	Coper nicus

		http://dx.doi.org/10.18203/2320- 6012.ijrms20183659			
8.	Dr..R.Tolstoy	Jagadeeswaran G, Ansari MZ, Rajangam T. Urinary tract infection in diabetics-a five year retrospective study on the prevalence of bacterial isolates and its antibiotic susceptibility patterns in a tertiary care hospital in South India. Int J Contemp Med Res. 2018;5:33-8.	N	N	Coper nicus
		Rajangam T, Priyashini RM, Manickam S. A study of prevalence of depression in type 2 diabetic patients in a tertiary care centre. IJCMR. 2018;5:113-5.	N	N	Coper nicus
		Prabu S, Rajangam T, Paul TV, Thomas N, Mandalam SS. Effect of Dietary Calcium Intake on Bone Mineral Density in Elderly South Indian Men. Annals of Health and Health Sciences. 2018;5(1):26-30.	Y	N	
		Ansari MZ, Tolstoy R, Jagadeeswaran G. A Rare Etiology of Severe Thrombocytopenia in Patient with Chronic Liver Disease. The Journal of the Association of Physicians of India. 2018 Mar 1;66(3):86-7.	Y	N	
		Rajangam T, Govindarajan J, Ansari MZ. A Tertiary Care Hospital based Study on the Prevalence of Pulmonary Hypertention in Patients with Glomerular Filtration Rate less than 30 Ml/Min Per 1.73 Meter Square on Dialysis	N	N	Coper nicus
		Kumar PA, Kaza AM, Rajangam T, Kumar MA, Mohanasundram P, Reddy A. An Organised Programme of Simulation-based Clinical Training for the Medical Interns. Journal of Research in Medical Education & Ethics. 2018;8(1):30-5.	N	N	Coper nicus

9.	Dr.G.Jagadeeswaran	Jagadeeswaran G, Ansari MZ, Rajangam T. Urinary tract infection in diabetics-a five year retrospective study on the prevalence of bacterial isolates and its antibiotic susceptibility patterns in a tertiary care hospital in South India. Int J Contemp Med Res. 2018;5:33-8.	N	N	Coper nicus
		Ansari MZ, Tolstoy R, Jagadeeswaran G. A Rare Etiology of Severe Thrombocytopenia in Patient with Chronic Liver Disease. The Journal of the Association of Physicians of India. 2018 Mar 1;66(3):86-7.	Y	N	
		Rajangam T, Govindarajan J, Ansari MZ. A Tertiary Care Hospital based Study on the Prevalence of Pulmonary Hypertention in Patients with Glomerular Filtration Rate less than 30 Ml/Min Per 1.73 Meter Square on Dialysis	N	N	Coper nicus
10.	Dr.Sujaya Menon	Manickam S, Prashanth A, Velammal P, Menon S. Is Serum Uric Acid an Added Risk Factor for Micro-Vascular Complications of Diabetes Mellitus?–A Prospective Study.	N	N	Index coper nicus
		Petchiappan V, Sivakrishna N, Manickam S, Menon S. Glycaemic control and C - reactive protein levels in type 2 diabetes mellitus -how well they co-relate? : A prospective study. Int J Res Med Sci 2019; 7: 1818-21.	N	N	Index coper nicus

		Manickam S, Franklin J , Petchiappan V , Menon S. A study of serum albumin levels in acute ischemic stroke and its correlation with clinical outcome. International Journal of Contemporary Medical Research IJCMR 2019;6(3):C17-C20.	N	N	Index coper nicus
		Sundaramoorthy V, Mambatta AK, Chidambaram Y, Gopalan R, Menon S. Prevalence of metabolic syndrome in patients with premature coronary artery disease proven by coronary angiogram. Int J ResMed Sci.2017Nov;5(11):5021-5.	N	N	Coper nicus
11.	Dr.S.Sujithkumar	Chidambaram Y, Mambatta A, Sivaraj S. Tropical sprue in megaloblastic anemia. International Journal of Research in Medical Sciences. 2017;5(9):4133.	N	N	Google scholar
		Manickam S, Srinivasan T V,Petchiappan V, Sujith Kumar S. Aprospective study of correlation of magnetic resonance imaging of brain including MR angiogram and carotid vertebral arterial doppler study with the clinical features in ischemic stroke.International JournalofContemporaryMedica Research 2019; 6(6):F37-F40.	N	N	Coper nicus
		Manickam S, Chidambaram Y, Kuppusamy J, Dhas CJ, Kumar S. Association of N-terminal-Pro-B-type Natriuretic Peptide with Localization of Thrombus and Clinical Outcome in Acute Pulmonary Embolism. International Journal of Contemporary Medical Research Vol.7/issue.4/April 2020.	N	N	Google scholar

DEPARTMENT OF DERMATOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sl. No	FacultyName	Publications in Vancouver referencingstyle.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr. Reena Rai	**PUBLICATION** Yuvasri G, Rai R. Comparison of efficacy of autologous platelet-rich fibrin versus Unna's paste dressing in chronic venous leg ulcers: A comparative study. Indian dermatology online journal. 2020 Jan;11(1):58.	Y	N	
		Rai R, Harikumar MV. Comparison of direct immunofluorescence of plucked hair and skin for evaluation of immunological remission in pemphigus. Indian dermatology online journal. 2017 Sep;8(5):319.	Y	N	
		Rai R, Shenoy MM, Viswanath V, Sarma N, Majid I, Dogra S. Contact sensitivity in patients with venous leg ulcer: A multi-centric Indian study. International wound journal. 2018 Aug;15(4):618-22.	Y	N	
		Rai R, Adityan J. Thrombocytopenia induced by intermittent therapy with Rifampicin--A case report. Leprosy Review. 2018 Jun 1;89(2):170-3.	Y	N	
		Periyasamy MK, Sekar SC, Rai R. Analysis of hypersensitivity in fragrance series by patch testing. Indian dermatology online journal. 2019 Nov;10(6):657.	Y	N	
		Periyasamy MK, Sekar CS, Rai R. Effectiveness of dual sequential wavelength laser in the treatment of portwine stains–A retrospective study. Indian dermatology online journal. 2019 Jul;10(4):418.	Y	N	
		Yuvasri G, Rai R. Comparison of efficacy of autologous platelet-rich fibrin versus Unna's paste dressing in chronic venous leg ulcers: A comparative study. Indian dermatology online journal. 2020 Jan;11(1):58.	Y	N	
		Rai AK, Janani K, Rai R. Efficacy of topical timolol versus saline in chronic venous	Y	N	

		ulcers: a randomized controlled trial. Journal of Cutaneous and Aesthetic Surgery. 2020 Jan;13(1):18.			
	Dr. Reena Rai	Anjali VT, Rai R, Maheswari GU, Kumar PN. Hemophagocytic lymphohistiocytosis complicating methotrexate toxicity. Indian Dermatology Online Journal. 2020 Mar;11(2):219.	Y	N	
		Meenu S, Pradeep B, Ramalingam S, Sairam T, Rai R, Sankaran R. Papillon–Lefèvre syndrome (PLS) with novel compound heterozygous mutation in the exclusion and Peptidase C1A domains of Cathepsin C gene. Molecular Biology Reports. 2020 Jul;47(7):5681-7.	Y	N	
		Arunprasath P, Rai R, Umamaheswari G. Erythrokeratodermaen cocardes–A rare phenotype. Indian Journal of Paediatric Dermatology. 2020 Jul 1;21(3):191.	Y	N	
		Kumaresan M, Gurusamy U, Rai R, Srinivas CR. Plucked hair as a substrate for indirect immunofluorescence in cases of pemphigus vulgaris. International Journal of Trichology. 2020 May;12(3):114.	Y	N	
		Nagarajan H, Rai R. Contact dermatitis to minoxidil. Contact Dermatitis. 2021 Jan;84(1):57-.	Y	N	
		Arunprasath P, Rai R, Venkataswamy C. Comparative analysis of BIOCHIP mosaic-based indirect immunofluorescence with direct immunofluorescence in diagnosis of autoimmune bullous diseases: A cross-sectional study. Indian dermatology online journal. 2020 Nov;11(6):915.	Y	N	
		Arunprasath P, Rai R, Venkataswamy C. Pirfenidone-induced dose-dependent phototoxicity–a distinct drug reaction. Indian Dermatology Online Journal. 2020 Nov;11(6):1007.	Y	N	
		Nagarajan H, Rai R. Contact dermatitis to minoxidil. Contact Dermatitis. 2021 Jan;84(1):57.	Y	N	
		Rai R, Nagarajan H. COVID-19 Presenting as Enanthem and Itchy Exanthem. The Journal of the Association of Physicians of India. 2021 Apr;69(4):11-2.	Y	N	
		Rai R, Anand JB, Shanmugasekar C, Arunprasath P, Chaitra V, Zillikens D, Schimdt	Y	N	

		E. Anti-P 200 pemphigoid–The most common floor binding subepidermal autoimmune bullous disease in a tertiary care center in south India. Indian Journal of Dermatology, Venereology and Leprology.:1-5.			
	Dr, Reena Rai	**BOOKS** Chapter in text book Leg ulcers Reena Rai Acs(I) Textbook Of Cutaneous & Aesthetic Surgery (2 Vols) 2017 Chapter in text book Disease of vascular system, vasculitis and leg ulcersReena Rai IADVL Concise textbook of dermatology2019 Chapter in text book Peripheral Vascular disease Reena Rai SkinClinical Dermatology2019 Chapter in text book Role of PRP and PRF in managing leg ulcers Reena Rai Leg Ulcers: Diagnosis & Management2019 Text book of post graduate dermatology 2021 chapter onErythema multiforme, steven Johnson, Toxic epidermal necrolysis. Jaypee Brothers 2021	N	N	

Sr. No	FacultyName	Publications in Vancouver referencingstyle.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
2	Dr. Shanmugasekar C	Annabathula A, Sekar CS, Srinivas CR. Fractional carbon dioxide, long pulse Nd: YAG and pulsed dye laser in the management of keloids. Journal of cutaneous and aesthetic surgery. 2017 Apr;10(2):76.	Y	N	
		Srinivas CR, Sekar CS. SOAP PUVA. Indian dermatology online journal. 2018 May;9(3):175.	Y	N	
		Periyasamy MK, Sekar SC, Rai R. Analysis of hypersensitivity in fragrance series by patch testing. Indian dermatology online journal. 2019 Nov;10(6):657.	Y	N	
		Periyasamy MK, Sekar CS, Rai R. Effectiveness of dual sequential wavelength laser in the treatment of portwine stains–A retrospective study. Indian dermatology online journal. 2019 Jul;10(4):418.	Y	N	
		Rai R, Anand JB, Shanmugasekar C, Arunprasath P, Chaitra V, Zillikens D, Schimdt E. Anti-P 200 pemphigoid–The most common floor binding subepidermal autoimmune bullous disease in a tertiary care center in south India. Indian Journal of Dermatology, Venereology and Leprology.:1-5.	Y	N	
		Ponmariappan LP, Sekar CS, Rai R, Chaithra V. Unusual presentation of trichoblastoma. Journal of Cutaneous and Aesthetic Surgery. 2021 Jul 1;14(3):374.	Y	N	
		BOOKS Chapter in text book Wound healing Shanmugasekar C ACSI Text Book of Dermatosurgery 2017	N	N	

Sr. No	FacultyName	Publications in Vancouver referencingstyle.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
3	**Dr.Yuvasri G**	**Publication** Yuvasri G, Rai R. Comparison of efficacy of autologous platelet-rich fibrin versus Unna's paste dressing in chronic venous leg ulcers: A comparative study. Indian dermatology online journal. 2020 Jan;11(1):58.	Y	N	
4	**Dr. Lakshmi Prabha P**	Ponmariappan LP, Sekar CS, Rai R, Chaithra V. Unusual presentation of trichoblastoma. Journal of Cutaneous and Aesthetic Surgery. 2021 Jul 1;14(3):374.	Y	N	

Department of Psychiatry

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

S.NO	Faculty Name	PublicationsinVancouver referencing style.	Indexed in		If indexed in any other,please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr.G.Raghuthaman	1. Gopalakrishnan M, Ravishankar SL, Raghuthaman G,Senthilkumar R. Depression among elderly diabetics atPeripheral health centres attached to a private medicalcollegeinCoimbatore:across sectionalstudy. NationalJournalofResearchinCommunityMedicine.2019Dec31;8(4):312-5.	Y	N	-
		2. Gopal R, Chennatte SS, Shilpa S. Comparing 24-hoursymptomtriggeredtherapyandfixedscheduletreatmentfor alcohol withdrawal symptoms-A randomized controlstudy.Asianjournalofpsychiatry.2020Feb;48:101888.	Y	N	-
		3. DoraiBL,RaghuthamanG.Phenomenologyof delirium in decompensated liver disease patients-a prospective observational study. Kindly Journal of Psychiatry. 2021 Oct 28.	Y	N	-
2.	Dr.SyedUmmar	1. Ibrahim SU, Kalyanasundaram VB, Ramanathan SA,Ramasamy S. Trajectory of insight on various dimensions among bipolar disorder in-patients. Industrial PsychiatryJournal.2020Jul1;29(2):285.B.	Y	N	`-

		2. Priyadharshini BS, Ummar IS. Prevalence and sociodemographic profile of lithium-induced cutaneous side effects in bipolar affective disorder patients: a 1-year prospective observational study in South India. Indian journal of psychological medicine. 2017 Sep;39(5):648- 52.	Y	N	`-
		3. Afreen S. Effectiveness of mood stabilizer in euthymic BPAD patients: An one year prospective observational study,comparing lithiumv/s divalproate sodium(Doctoral dissertation, PSG Institute of Medical Sciences and Research,Coimbatore)..	N	N	PIC
		4. Syed Ummar Ibrahim, Fathima nasreen jaffar hussain, Jayachandran G, Sureshkumar Ramasamy. A comparative study of effects on working and logical memory in psychiatry patients receiving combined electroconvulsive therapy with pharmaco therapy and pharmacotherapy only. International Journal of Psychology. June 2019; 10(3): 19-23.	N	N	PIC
		5. Ramasamy S, Thaj RR, Ibrahim SU, 2nd to 4th Digit Ratio (2D:4D) – Is It a maker for Dyslexia? Indian Journal of Mental Health 2019;6(4).	N	N	PIC
		6. M. Baskaran1*, i. Syed ummar2, a. Jayasudha1 andbrundha gandhi1 , pre experimental study to assess the effectiveness of structured teaching program onknowledge regarding management ofviolent behavior amongprimary care givers of mentally illintertiarycare hospital in Coimbatore india,	N	N	PIC/DOAJ
	Dr.SyedUmmar	7. Dr. Syed Ummer. I1*, Dr. SushithSugathan. C2, Study onCorrelation between Psychotic Symptoms and Suicide in aCohortof PatientswithSeveredepression,the international journal of Indian psychology 2019,450-456.	N	N	PIC

		8. Dr.Syedummar.I1*,dr.Sushithsuganthan.C2,clinicalandsocio-demographic correlates in depressive patients withsuicidal ideas & suicidal attempts, the international journalofindianpsychology,2017,vol4, issue2,87-99.	N	N	PIC
3.	Dr. Pradeep	1. Iyer A, Palaniappan P. Biological dysrhythm in remitted bipolar I disorder. Asian journal of psychiatry. 2017 Dec1;30:218-24.	N	N	Index Copernicus
		2. Easwarana K, Palaniappan P. Social cue recognition andattributional bias in remitted bipolar disorder. Are thesefactorsassociatedwithsocisalfunctioninginbipolardisorder?. Psychiatryresearch.2021Nov22:114300.	Y	N	-
		3. Palaniappan P, Easwaran K. Theory of Mind Deficits andTheir Influence on Functional Impairment in RemittedPhase of Bipolar Disorder. Indian Journal PsychologicalMedicine.2021 May1:0253717620930315	Y	N	-
4.	Dr. Suresh Kumar	1. Ramasamy S, Thaj RR, Ibrahim SU, 2nd to 4th Digit Ratio (2D:4D) – Is It a maker for Dyslexia? Indian Journal of Mental Health 2019;6(4).	N	N	Index Copernicus
		2. Ibrahim SU, Kalyanasundaram VB, Ramanathan SA,RamasamyS.Trajectoryofinsightonvariousdimensionsamong bipolar disorder in-patients. Industrial PsychiatryJournal.2020Jul1;29(2):285.	N	N	Index Copernicus
		3. Ramasamy S, Bharath S. Clinical characteristics ofpatients with non-affective, non-organic, late onsetpsychosis. Asian	Y	N	-

		journal of psychiatry. 2017 Feb 1;25:74-8.			
		4. Syed Ummar Ibrahim, Fathima nasreen jaffar hussain, Jayachandran G, Sureshkumar Ramasamy. A comparative study of effects on working and logical memory in psychiatry patients receiving combined electroconvulsive therapy with pharmaco therapy and pharmacotherapy only. International Journal of Psychology. June 2019; 10(3): 19-23.	Y	N	-
		5. Suresh Kumar Ramasamy1, Syed Ummar Ibrahim2, SarahAfreen3,SendhilRaj4 ,Effectivenessofmoodstabilizerin euthymic BPAD patients: An one year prospectiveobservational study, comparing Lithium v/s Divalproatesodium , International Journal of Psychology , 2019 , Vol10, Issue2, 648-652.			Index Copernicus
	Dr. Suresh Kumar	6. Meera Saravanan, Dr. A Jayasudha, Dr. R Sureshkumar, Chinchu S Giju, A Srinidhi, Sri Gayathri and M Tamilselvi , Managing levels of insomnia in psychiatric patients , international journal of intellectual disability , 2021, 1-4	N	N	Index Copernicus
		7. Dr. Sureshkumar Ramasamy1*, Dr. Shilpa Srinivasan2, Dr. Shree Aarthi Ramanathan3, Standards of Lithium Treatment Monitoring In a Teaching Hospital in South India among Bipolar Affective Disorder , The International Journal of Indian Psychology ,2017 , 139- 145.	N	N	Index Copernicus

DEPARTMENT OF PAEDIATRICS

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr.No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr A Jayavardhana	Arumugam J, A. Arthi Ponnusamy. A study on community-acquired methicillin-resistant Staphylococcal aureus infections in Coimbatore children. Indian journal of applied research. Feb 2014 ;Volume : 4 (2): ISSN - 2249-555X.	N	N	CITATION INDEX
		Arumugam J, Malini. Parental Perception on Pediatric Obesity– Implications on Early Recognition of Obesogenic Factors and Management. Original article. Indian Journal of applied research. Volume : 4 (3) Mar 2014 ISSN - 2249-555X.	N	N	CITATION INDEX
		Arumugam J, Devaprasath. Sleep pattern and problems in healthy toddlers. S. Indian Journal of Basic and Applied Medical Research; December 2015 ; Vol.-5, (1) : P. 217-220	N	N	INDEX COPERNICUS
		Riyaz chungathu, Arumugam J. Current pattern of Salmonella Typhi antimicrobial susceptibility in the era of antibiotic abuse. Indian Journal of Basic and Applied Medical Research; December 2015; Vol.5 (1): P. 400-404.	N	N	INDEX COPERNICUS
		Rajamani K, Arumugam J. PREVALENCE OF SEROPOSITIVITY AGAINST HEPATITIS B VIRUS IN PREVIOUSLY IMMUNIZED ADOLESCENT CHILDREN. Journal of evidence based medicine and Health care ; July 2016 : Volume 3(55), PP 2850-2853.	N	N	INDEX COPERNICUS
		Aghil RB, Arumugam J. THE IMPACT OF PREMATURITY ON INFANT BRAINSTEM AUDITORY EVOKED RESPONSES. Journal of evidence based medicine and Health care ; January 2016 ; Vol.3 :PP 4950-4953 (4).	N	N	INDEX COPERNICUS

02.	Dr K Jothilakshmi	Rajesh NT, Alagarasu K, Patil JA, Bharathi E, More A, Kakade MB, Veronika M, Jothilakshmi K, Shah PS. Serotype-specific differences in the laboratory parameters among hospitalized children with dengue and genetic diversity of dengue viruses circulating in Tamil Nadu, India during 2017. Journal of medical virology. 2020 Aug;92(8):1013-22.	N	N	SCIMAGO
		Jothilakshmi K, Vinoth V, Kannappan S. Pattern and prevalence of metabolic syndrome in obese adolescents aged 10-18 years: a community based study.	Y	N	SCIMAGO INDEX COPERNICUS
		INCLEN Intussusception Surveillance Network Study Group. Risk of intussusception after monovalent rotavirus vaccine (Rotavac) in Indian infants: A Self-controlled case series analysis. Vaccine. Jan 2021 3; 39(1): 78-84. Multicentric study in 18 centers in India. By INCLEN Investigator for PSG Institutions.	Y	N	MANY OTHERS
		Manoja K, Narendra K, Bini G, Apporva S, John M, Cenita J, Jothilakshmi K, Pavai A. Intussusception in children aged under two years in India: Retrospective surveillance at nineteen tertiary care hospitals. Vaccine. October 2020; 38(43): 6849-6857	Y	N	MANY OTHERS
		Pavai Arunachalam CJ, Lakshmi KJ, Shanmugham HB. A rare case of pediatric osteochondroma presenting as hemothorax. Journal of Indian Association of Pediatric Surgeons. 2020 Sep;25(5):316.	Y	N	
		"Prospective surveillance for intussusception in Indian children aged under two years at nineteen tertiary care hospitals." *BMC pediatrics* 20 (2020): 1-13.	Y	Y	CAB ABSTRACT CNKI EBSCO EMBASE

		Das MK. Experience of establishing and coordinating a nationwide network for bidirectional intussusception surveillance in India: lessons for multisite research studies. BMJ open. 2021 May 1;11(5):e046827.	Y	N	
		Manoja Kumar D, The INCLEN Intussusception Surveillance Network Study Group. Association of meteorological parameters with intussusception in children aged under 2 years: results from a multisite bidirectional surveillance over 7 years in India. BMJ Open May 2021; 11(5): e043687.	Y	N	
		Das MK, Arora NK, Mathai J, Sam CJ, Rajamani G, Krishnaswamy R, Jothilakshmi K, Arunachalam P, Gupta B. Profile and Epidemiology of Intussusception in Children Under-Two Years of Age: A Prospective Surveillance. Indian Journal of Pediatrics. 2021 May 31:1-8.	Y	N	
		Balagurunathan M, Natarajan T, Karthikeyan J, Palanisamy V. Clinical spectrum and short-term outcomes of multisystem inflammatory syndrome in children in a south Indian hospital. Clinical and experimental pediatrics. 2021 Oct;64(10):531.	Y	N	
03.	Dr.Ramesh.S	Ramesh S, Suvetha K. Predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates. Indian Journal of Child Health. 2017 Dec 25;4(4):561-5.	N	N	Index copernicus
		Ramesh S. Predisposing factors and outcome of acute kidney injury in neonates. Indian Journal of Child Health. 2018 Jan 25;5(1):46-9.	N	N	Index copernicus
		Ramesh S, Kumutha J. Role of perfusion index in pulse oximetry screening for critical congenital heart disease in neonates. Indian Journal of Child Health. 2018 Mar 26;5(3):200-3.	N	N	Index copernicus
		Srinivasan R, Kannappan S. Oral feeds with or without dextrose half normal saline for treatment of hypernatremia in breastfed late preterm and term neonates: A comparison study. Journal of Clinical Neonatology. 2018 Oct	Y	N	

		1;7(4):202.			
		Mathew AC, Dhanya C, Benny JK, Philip DM, Ghurupriyan M, Ramesh S, Neelakandan K. The effect of mode of delivery on breastfeeding outcomes: A Hospital based study. Asian Pac. J. Health Sci., 2018; 5(3); 241- 245.	N	N	Index copernicus
		Mathew AC, Philip DM, Benny JK, Dhanya C, Manju TM, Ramesh S. Factors associated with timely initiation of breast feeding: a hospital based study. Asian Pacific J Heal Sci. 2018;5(3):318-24.	N	N	Index copernicus
		Das MK, Arora NK, Poluru R, Seth A, Aggarwal A, Dubey AP, Goyal PC, Gathwala G, Malik A, Goel AK, Chakravarty A. Pediatric Appropriate Evaluation Protocol for India (PAEP-India): tool for assessing appropriateness of pediatric hospitalization. Indian pediatrics. 2018 Dec;55(12):1041-5. (Dr Ramesh S is one of the co-author)	Y	N	
		Lekha T, Ramesh S, Sharma A, Abinaya G. MII RetCam assisted smartphone based fundus imaging for retinopathy of prematurity. Indian journal of ophthalmology. 2019 Jun;67(6):834.	Y	N	
		Mathew AC, Benny JK, Philip DM, Dhanya C, Joy M, Sandhiya V, Ramesh S, Neelakandan K. Socio-Demographic and Clinical Correlates of Exclusive Breastfeeding practices up to Six Months of Age. Nepal journal of epidemiology. 2019 Sep;9(3):772.	Y	N	
		Arora NK, Das MK, Poluru R, Kashyap NK, Mathew T, Mathai J, Aggarwal MK, Haldar P, Verstraeten T, Zuber PL, INCLEN Vaccine Safety Study Group. A Prospective Cohort Study on the Safety of Infant Pentavalent (DTwP-HBV-Hib) and Oral Polio Vaccines in Two South Indian Districts. The Pediatric infectious disease journal. 2020 May;39(5):389. (Dr Ramesh S is one of the co-author, as a study group member)	Y	N	
		Palanisamy S, Srinivasan R Clinical profile of congenital hypothyroidism identified through new-born screening: a retrospective observational study. Int J Contemp Pediatr. 2020 Oct;7(10):1968-1972.	N	N	Index copernicus

		Palanisamy S, Srinivasan R, Kannappan S, Ganesan J. Serum Thyroid Stimulating Hormone Screening and Influence of Perinatal Factors. Indian Journal of Pediatrics. 2021 May;88(5):516-.	Y	N	
		Velumani SR, Srinivasan R, Kumar PN. Late-onset hyporegenerative anemia in an infant with rhesus isoimmunization. Journal of Clinical Neonatology. 2021 Apr 1;10(2):117.	Y	N	
		Palanisamy S, Srinivasan R, Perumal T. Unusual presentation of cow's milk protein allergy in a neonate. Journal of Clinical Neonatology. 2021 Oct 1;10(4):239.	Y	N	
04.	Dr Rajesh NT	Elangovan B. Clinical and laboratory predictors to differentiate severe dengue from scrub typhus in children.	N	N	National Library of Medicine Index copernicus
		Kanagaraju V, Elangovan B. Gallbladder motility in children with chronic functional abdominal pain.	N	N	National Library of Medicine Index copernicus
		Rajesh NT, Alagarasu K, Patil JA, Bharathi E, More A, Kakade MB, Veronika M, Jothilakshmi K, Shah PS. Serotype-specific differences in the laboratory parameters among hospitalized children with dengue and genetic diversity of dengue viruses circulating in Tamil Nadu, India during 2017. Journal of medical virology. 2020 Aug;92(8):1013-22.	Y	N	
		Elangovan B, Rajesh NT, Subrahmanian M. Apolipoprotein-E Gene Polymorphism and Lipid Composition among IUGR and AGA Neonates. Journal of Pediatric Genetics. 2021 Feb 11.	Y	N	
05.	Dr P Venkateshwaran	CHELLAPPAN, Indumathi; PALANISAMY, Venkateshwaran; ELANGOVAN, Bharathi. Clinical and laboratory profile of COVID-19 in children below 15 years. International Journal of Contemporary Pediatrics, [S.l.], v. 8, n. 9, p. 1489-1495, aug. 2021. ISSN 2349-3291. doi:http://dx.doi.org/10.18203/2349-3291.ijcp20213308.	N	N	National Library of Medicine Index copernicus
		Balagurunathan M, Natarajan T, Karthikeyan J, Palanisamy V. Clinical spectrum and short-term outcomes of multisystem inflammatory syndrome in children in a south Indian hospital. Clinical and experimental pediatrics. 2021	Y	N	

		Oct;64(10):531.			
		P Venkateshwaran, Dr.N.T.Rajesh, Dr.Pavai Arunachalam. Sublingual Ulceration Due to Natal Tooth – Riga – Fedes Disease. Indian journal of applied research. Volume : 4/ Issue: 7 /July 2014 / ISSN – 2249-555X.	-		
06.	Dr Bharathi E	Elangovan B. Clinical and laboratory predictors to differentiate severe dengue from scrub typhus in children.	N	N	National Library of Medicine Index copernicus
		Kanagaraju V, Elangovan B. Gallbladder motility in children with chronic functional abdominal pain.	N	N	National Library of Medicine Index copernicus
		Rajesh NT, Alagarasu K, Patil JA, Bharathi E, More A, Kakade MB, Veronika M, Jothilakshmi K, Shah PS. Serotype-specific differences in the laboratory parameters among hospitalized children with dengue and genetic diversity of dengue viruses circulating in Tamil Nadu, India during 2017. Journal of medical virology. 2020 Aug;92(8):1013-22.	Y	N	
		Indumathi Chellappan, Venkateshwaran Palanisamy*, Bharathi Elangovan. Clinical and laboratory profile of COVID-19 in children below 15 years. International Journal of Contemporary Pediatrics Chellappan I et al. Int J Contemp Pediatr. 2021 Sep;8(9):1489-1495 . pISSN 2349-3283 | eISSN 2349-3291	N	N	
		Elangovan B, Rajesh NT, Subrahmanian M. Apolipoprotein-E Gene Polymorphism and Lipid Composition among IUGR and AGA Neonates. Journal of Pediatric Genetics. 2021 Feb 11.	Y	N	

07.	Dr B Muruganantham	Balagurunathan M, Natarajan T, Karthikeyan J, Palanisamy V. Clinical spectrum and short-term outcomes of multisystem inflammatory syndrome in children in a south Indian hospital. Clinical and experimental pediatrics. 2021 Oct;64(10):531.	Y	N	NIL
08.	Dr.S.Gayathri	Gayathri S, Mohan BV. Analysis of incidence and risk factors for retinopathy of prematurity among preterm neonates in a tertiary care neonatal unit. RESEARCH JOURNAL OF PHARMACEUTICAL BIOLOGICAL AND CHEMICAL SCIENCES. 2015 Nov 1;6(6):1565-70.	N	Y	
09.	Dr.Nitin Srinivasan	Srinivasan N, Chandramathi J, Prabhu AS, Ponthenkandath S. Vitamin D deficiency and morbidity among preterm infants in a developing country. Int J Contemp Pediatr. 2017 Mar;4(2):499-502.	N	N	Index Copernicus
		Maya P, Rajam L, Srinivasan N. Fulminant Hepatic Failure with White Phosphorus: A Case Report. J Indian Soc Toxicol. 2005;1(1):28-9.	N	N	Index Medicus Medline
10.	Dr Abraar Sheriff M	Sheriff A, Rameshkumar R, Chidambaram M, Maulik K, Kumar RS, Jamal A, Bhowmick R, Biswal N, Mahadevan S. Epinephrine Plus Vasopressin vs Epinephrine Plus Placebo in Pediatric Intensive Care Unit Cardiopulmonary Resuscitation: A Randomized Double Blind Controlled Clinical Trial. Indian Pediatrics. 2021 Feb 19.	Y	Y	-

Department of General Surgery

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

S:No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr S Rajesh Kumar	Vinoth D, Rajeshkumar S, Sali SA. A case series of adult intussusception diagnosed by ultrasound in South India: an uncommon notion. International Surgery Journal. 2020 Jun 25;7(7):2363-70.	N	N	Google scholar, Index copernicus
2	Dr .S. Premkumar	Periyanarkunan MR, Elavarasan S, Sivaraman P, Chinnasamy G. Aorto-oesophageal fistula: rarest cause of haematemesis-is it salvageable?. BMJ Case Reports CP. 2021 Nov 1;14(11):e245396.	Y	N	N
3	Dr Vimal Kumar Govindan	Prabakaran V, Thangaraju T, Mathew AC, Govindan V, Kannan V, Poulose TR. CR-Possum—Can It Be Used to Predict Morbidity? A Single-Centre Retrospective Study. Indian journal of surgical oncology. 2019 Mar;10(1):174-9.	Yes	Yes	Google scholar, Index Copernicus
		Kathirvel M, Jayarajan J, Sivakumar A, Govindan V. Risk factors for the diabetic foot infection with multidrug-resistant microorganisms in South India. International Surgery Journal. 2018 Jan 25;5(2):675-82.	N	N	Google scholar, Index Copernicus

4	Dr S Karthikeyan	S Karthikeyan, DG Manoj Prabhakar, Guhan Ramasamy et al. Vacuum Assisted Closure Versus Conventional Wound Therapy in the Management of Diabetic Wounds. New Indian J Surg. 2019;10(5):501-506. *DOI: https://dx.doi.org/10.21088/nijs.0976.4747.10519.7* DOI: https://dx.doi.org/10.21088/nijs.0976.4747.10519.7	N	N	Index Copernicus, Poland: 2018: 91.08, 2016: 78.33, 2015: 70.33; MedLine/ Locatore plus, USA; Genamics JournalSeek; WorldCat; Gaudeamus Academia; Science Library Index; The International Committee of Medical Journal Editors (ICMJE).
		S. Karthikeyan, Subbaiah S., Vimal Raj et al. Analysis of Antibiotic Therapy in Acute Severe Pancreatitis. New Indian J Surg. 2018;9(3):323-27. DOI: https://dx.doi.org/10.21088/nijs.0976.4747.9318.14 DOI: https://dx.doi.org/10.21088/nijs.0976.4747.9318.14	N	N	Index Copernicus, Poland: 2018: 91.08, 2016: 78.33, 2015: 70.33; MedLine/ Locatore plus, USA; Genamics JournalSeek; WorldCat; Gaudeamus Academia; Science Library Index; The International Committee of Medical Journal Editors (ICMJE).
		The Characteristics of routine Histopathological Examination of Appendicectomy Specimen: A Study from a Tertiary Care Centre S. Karthikeyan a, Raja S.R. b,Vimal Raj b, Priya b, Sivaprasanna K. c, Vijay Muthukumaran ca Professor b Assistant Professor c Resident, Dept. of General Surgery, PSG Institute of Medical Sciences & Research & PSG Hospitals, Coimbatore 641004, Tamil Nadu, India. New Indian Journal of Surgery , Volume 8 Number 3, July September 2017 DOI: https://dx.doi.org/10.21088/nijs.0976.4747.8317.14	N	N	Index Copernicus, Poland: 2018: 91.08, 2016: 78.33, 2015: 70.33; MedLine/ Locatore plus, USA; Genamics JournalSeek; WorldCat; Gaudeamus Academia; Science Library Index; The International

		DOI: https://dx.doi.org/10.21088/nijs.0976.4747.8317.14			Committee of Medical Journal Editors (ICMJE).
		Antibiotic Prophylaxis and Surgical Site Infection S. Karthikeyan*, Priya**, Vimal Raj**, Sivaprasanna***, Akash*** *Professor **Assistant Professor ***Resident, Department of General Surgery, PSG Hospitals, Coimbatore, Tamil Nadu New Indian Journal of Surgery , Volume 8 Number 1, January - March 2017 DOI: https://dx.doi.org/10.21088/nijs.0976.4747.8117.2 DOI: https://dx.doi.org/10.21088/nijs.0976.4747.8117.2	N	N	Index Copernicus, Poland: 2018: 91.08, 2016: 78.33, 2015: 70.33; MedLine/ Locatore plus, USA; Genamics JournalSeek; WorldCat; Gaudeamus Academia; Science Library Index; The International Committee of Medical Journal Editors (ICMJE).
		Predictors of Major Lower Limb Amputation among Type II Diabetic Patients S. Karthikeyan*, Priya**, Vimal Raj**, Akash*** *Professor **Assistant Professor ***Resident, Department of General Surgery, PSG Hospitals, , Coimbatore, Tamil Nadu New Indian Journal of Surgery , Volume 7 Number 3, September December 2016 DOI: https://dx.doi.org/10.21088/nijs.0976.4747.7316.4 DOI: https://dx.doi.org/10.21088/nijs.0976.4747.7316.4	N	N	Index Copernicus, Poland: 2018: 91.08, 2016: 78.33, 2015: 70.33; MedLine/ Locatore plus, USA; Genamics JournalSeek; WorldCat; Gaudeamus Academia; Science Library Index; The International Committee of Medical Journal Editors (ICMJE).
4	Dr S Karthikeyan	To operate or not to operate, that is the question: review of management of splenic trauma in our institution over the past 15 years S. Karthikeyan*, Hemachandran**, Adharsh Kumar M.***, Bichu Joseph Maliakal*** *Professor, **Assistant Professor, ***Resident, Department of General Surgery, PSG Hospitals, Coimbatore, Tamil Nadu	N	N	Index Copernicus, Poland: 2018: 91.08, 2016: 78.33, 2015: 70.33; MedLine/ Locatore plus, USA; Genamics JournalSeek; WorldCat; Gaudeamus

		New Indian Journal of Surgery Volume 7 Number 2, May - August 2016. DOI: https://dx.doi.org/10.21088/nijs.0976.4747.7216.11			Academia; Science Library Index; The International Committee of Medical Journal Editors (ICMJE).
		Follow Up of Surgical Treatment for Varicose Veins in the Last Three Years in our Institution *S. Karthikeyan , S. Hemachandran , Bichu Joseph Maliakal , Adharshkumar M. Department of General and GI Surgery, PSG Institute of Medical Sciences and Research Peelamedu, Coimbatore, Tamil Nadu *New Indian Journal of Surgery , Volume 7 Number 1, January April 2016* *DOI: https://dx.doi.org/10.21088/nijs.0976.4747.7116.3*	N	N	Index Copernicus, Poland: 2018: 91.08, 2016: 78.33, 2015: 70.33; MedLine/ Locatore plus, USA; Genamics JournalSeek; WorldCat; Gaudeamus Academia; Science Library Index; The International Committee of Medical Journal Editors (ICMJE).
4	Dr Shaikha Afzal Rubby	Venkatachala S, Krishnakumar R, Rubby SA. Soft palate schwannoma. Indian Journal of Surgery. 2013 Jun;75(1):319-21.	Y	N	N
		Rubby SA, Rangaswamy P, Sundar P. A prospective study comparing laparoscopic and open ventral hernia repair. International Surgery Journal. 2016 Dec 13;4(1):170-6.	N	N	Google scholar
5	Dr Vishnuvarthan	Kumar RA, Vishnuvarthan S. Lateral partial internal sphincterotomy-a complete relief in chronic fissure in ANO. International Surgery Journal. 2017 Sep 27;4(10):3347-9.	N	N	ICV, Google scholar
		Vishnuvarthan S. *Study on the conversion of laparoscopic cholecystectomy owing to per*	N	N	Index Copernicus

		operative complications (Doctoral dissertation, Stanley Medical College, Chennai).			
		Vishnuvarthan S. *Study on the conversion of laparoscopic cholecystectomy owing to per operative complications* (Doctoral dissertation, Stanley Medical College, Chennai).	N	N	ICV, Google scholar
		Saminathan S, Selvaraj V. A CADAVERIC STUDY OF THE MYOCARDIAL BRIDGES. Journal of Evolution of Medical and Dental Sciences. 2019 Jan 14;8(2):133	N	N	ICV, Google scholar
6	Dr Ajay Sivakumar	Kathirvel M, Jayarajan J, Sivakumar A, Govindan V. Risk factors for the diabetic foot infection with multidrug-resistant microorganisms in South India. International Surgery Journal. 2018 Jan 25;5(2):675-82.	N	N	Google scholar, Index Copernicus
7	Dr Mahendran S	Ragesh KV, Mahendran S, Mathad S. Outcome of skin incision by cautery versus steel scalpel in hernia surgery: a prospective cohort study at a tertiary medical college hospital in South India. International Surgery Journal. 2017 Apr 22;4(5):1521-4.	N	N	Google scholar, Index Copernicus
8	Dr Arun Prasath S.V	SV Arun Prasath[1], Vinoth D[2], Rajesh Kumar [3], [1]Assistant Professor, [2] Senior Resident [3]Professor, Department of General and GI surgery, PSG Institute of Medical Sciences & Research, Coimbatore, India. Monitoring Postoperative Pain Management in General Surgical Wards: A Clinical Audit of Feedback pain protocol in our Institution. New Indian Journal of Surgery, July- August 2019,Volume 10, Number 4.	N	N	N
		Vijay Muthukumaran G. *Acetic Acid based dressings on Pseudomonas Aeruginosa infected Diabetic Ulcers: A Randomized Controlled study* (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Google scholar, Index Copernicus

9	Dr Abraham Benjamin	Abraham Benjamin Muthunayagam[1], Chitra R[2], Muruganand Myilsamy[3], Vinoth [4]. [1,2,3,4]Assistant Professor, Department of General and GI surgery, PSG Institute of Medical Sciences & Research, Coimbatore, India. Comparison of CTSI and BISAP Scoring in the Assessment of Severity and Mortality in Case of Acute Pancreatitis. New Indian Journal of Surgery, November – December 2019, Volume 10: Number 6.	N	N	N
10	Dr R Chitra	Vaishnavi J. *Retrospective study on patients undergoing laparotomy to assess the risk factors of re-laparotomy* (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Google scholar,
11	Dr Sunay N Bhat	Bhat SN, Vinoth D. Retracted: Studying the impact of the cost-effective Suvidha off-loading dressing in healing neuropathic ulcers in diabetic foot: a case series of 83 cases from South India. International Surgery Journal. 2020 Jun 25;7(7):2371-7.	N	N	Google scholar, Index Copernicus
		Vinoth D, Bhat SN, Samuel J. Routine baseline serum carcinoembryonic antigen as a negative predictor of peritoneal metastasis in colorectal malignancies: a cost-effective tool in South India. International Surgery Journal. 2020 Jun 25;7(7):2159-64.	N	N	Google scholar, Index Copernicus
12	Dr Priya.S	Vupputuri H, Kumar S, Subramani P, Venugopal K. A single-blind, randomized controlled study to compare Desarda technique with Lichtenstein technique by evaluating short-and long-term outcomes after 3 years of follow-up in primary inguinal hernias. International Journal of Abdominal Wall and Hernia Surgery. 2019 Jan 1;2(1):16.	N	N	Google scholar, Index Copernicus
		Dr Sudharshan Babu K.G[1], Dr Priya.S[2], Dr Madhusudhan H.R[3] Clinical Study and Management of Calculus in Upper 1/3rd of the Ureter. Journal of Medical Sciences and Clinical Research, December 2017, Volume 05: Issue 12.	N	N	Index Copernicus

13	Dr Tracy Rosaline Poulose	Prabakaran V, Thangaraju T, Mathew AC, Govindan V, Kannan V, Poulose TR. CR-Possum—Can It Be Used to Predict Morbidity? A Single-Centre Retrospective Study. Indian journal of surgical oncology. 2019 Mar;10(1):174-9.	Yes	Yes	Google scholar, Index Copernicus

DEPARTMENT OF ORTHOPAEDICS

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr. Dinakar rai	• Sandeep MM, Rai D. A modified dynamic hip screw in unstable intertrochanteric fracture: A retro-prospective study. International Journal of Orthopaedics. 2020;6(3):841-4.	N	N	Index Copernicus
2.	Dr. Arvind Kumar SM	• Arvind Kumar SM, Kumar NV, Udayamoorthy S. Radiological assessment of inter-prosthetic joint movement in bipolar hip hemiarthroplasty for fracture neck of femur. International Journal of Orthopaedics. 2017;3(2):583-9.	N	N	Index Copernicus
		• Chandan Noel V. Assessment of proximal femur anthropometry in south indian population through cadaveric bones and radiologically. Correlating difference if any between other ethnic groups, assessment of best fit implant for indian population (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Index Copernicus
		• Arvind Kumar SM, Krishna YR, Kumar BA. Mechanical comparison of reconstruction nail and dynamic condylar screw in the treatment of unstable Subtrochanteric fracture in vitro study. International Journal of Orthopaedics. 2019;5(4):646-51.	N	N	Index Copernicus
		• Arvind Kumar S. M., Arun Kumar B Proximal femoral nail antirotation II in fixation of intertrochanteric fractures:	N	N	Index Copernicus

		retrospective and prospective observational study			
		• Kumar NV, Arvind Kumar SM, Udayamoorthy S, Kumar K. Platelet rich plasma in treatment of plantar fascitis. Int J Res Orthop. 2017 May;3(3):350-3.	N	N	Index Copernicus
		• Venkatesh Kumar N, Arvind Kumar SM, Vetri Ganapathi and Sairamakrishnan S Issues in management of fractures complicated by compartment syndrome DOI: https://doi.org/10.22271/ortho.2017.v3.i4e.44	N	N	Index Copernicus
		• Venkatesh Kumar N, SM AK, Vinayagamoorthy SS. Acetabular erosion after hemiarthroplasty. International Journal of Research in Orthopaedics. 2018 Jan;4(1):53.	N	N	Index Copernicus
3.	Dr. Venkatesh Kumar. N	• Kumar NV, Arvind Kumar SM, Udayamoorthy S, Sanjeev S. Functional outcome of Tibial condyle fractures treated by minimally invasive plate osteosynthesis. International Journal of Orthopaedics. 2017;3(1):792-6.	N	N	Index Copernicus
		• Kumar NV, Arvind Kumar SM, Udayamoorthy S, Kumar K. Platelet rich plasma in treatment of plantar fascitis. Int J Res Orthop. 2017 May;3(3):350-3.	N	N	Index Copernicus
		• Chandan Noel V. Assessment of proximal femur anthropometry in south indian population through cadaveric bones and radiologically. Correlating difference if any between other ethnic groups, assessment of best fit implant for indian population (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Index Copernicus
		• Arvind Kumar SM, Kumar NV, Udayamoorthy S. Radiological	N	N	

		assessment of inter-prosthetic joint movement in bipolar hip hemiarthroplasty for fracture neck of femur. International Journal of Orthopaedics. 2017;3(2):583-9.			Index Copernicus
		• Venkatesh Kumar N, Arvind Kumar SM, Vetri Ganapathi and Sairamakrishnan S Issues in management of fractures complicated by compartment syndrome DOI: https://doi.org/10.22271/ortho.2017.v3.i4e.44	N	N	Index Copernicus
		• Venkatesh Kumar N, SM AK, Vinayagamoorthy SS. Acetabular erosion after hemiarthroplasty. International Journal of Research in Orthopaedics. 2018 Jan;4(1):53.	N	N	Index Copernicus
		• L. Priya, N.Venkatesh Kumar, M.SanjayBalaji, C.V.RevanthA mechanical device for the reduction of distal radius fracture, International Journal of Innovative Technology and Exploring Engineering	N	Y	
4.	Dr. Major K Kamalanathan	• Primary total hip arthroplasty in fracture neck of femur	N	N	
		• Padmanaban K, Kamalanathan M, Palaniswamy B, Ramasamy V, Cyriac AP. Prospective study of distal tibia fracture with anterolateral plating by MIPPO. Indian Journal of Orthopaedics. 2017;3(2):153-9.	N	N	

5.	Dr. Prasanna. C	• Chandiralingam P, Baba V. The Charcot foot: Is it a commonly overlooked entity in diabetic population. International Journal of Orthopaedics. 2020;6(1):1267-9.	N	N	Index Copernicus
		• Chandiralingam P. Low back pain in pregnancy: Prevalence among south Indian population. International Journal of Orthopaedics. 2020;6(1):1027-9.	N	N	Index Copernicus
		• Gupta A, Prasanna C, Rai D. Functional outcome of adhesive capsulitis treated by manipulation. International Journal of Orthopaedics. 2020;6(3):10-4.	N	N	Index Copernicus
		• Analysis of surgical management of proximal humeral fractures	N	N	Index Copernicus
6.	Dr. Sandeep. MMR	• Gopal K, Sandeep MR, Prakash A, Jose A. Correlation between magnetic resonance imaging and arthroscopy in internal derangement of knee. International Journal of Research in Orthopaedics. 2017 May;3(3):476-81.	N	N	Index Copernicus
		• Sandeep MMR1, Surendher Kumar R.2, Prabhu T.3, Ashish Jose1, Krishnagopal RA study of clinical and radiological outcome of tibial plateau fractures treated with minimally invasive percutaneous plate osteosynthesis	N	N	Index Copernicus
		• Sandeep MM, Rai D. A modified dynamic hip screw in unstable intertrochanteric fracture: A retro-prospective study. International Journal of Orthopaedics. 2020;6(3):841-4.	N	N	Index Copernicus

		• Sandeep MM. A prospective study to access the Co-relation between osteoporosis and serum homocysteine level. International Journal of Orthopaedics. 2020;6(4):93-6.	N	N	Index Copernicus
7.	Dr. Anupam Gupta	• Gupta A, Prasanna C, Rai D. Functional outcome of adhesive capsulitis treated by manipulation. International Journal of Orthopaedics. 2020;6(3):10-4.	N	N	Copernicus
8.	Dr. Reguvaran. NJ	• Dhiyanesh K. Functional and radiological outcome of lateral approach for hemiarthroplasty for hip. International Journal of Orthopaedics. 2019;5(1):200-4.	N	N	Index Copernicus

DEPARTMENT OF OTO-RHINO-LARYNGOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr.S.Palaninathan	1. Dayanand A, Madeswaran M, Palaninathan S. A clinical study on etiopathogenesis of hoarseness of voice.	Y	Y	
		2. Dayanand A, Harshavardhan B, Dheebika J, Palaninathan S. Study on temporalis fascia graft versus temporalis fascia with cartilage graft.	Y	Y	
		3. Dayanand A, Dheebika J, Prathula S, Palaninathan S. A Study on Prevalence of Hearing Loss as a Complication of Diabetes. European Journal of Medical and Health Sciences. 2020 Aug 29;2(4).	Y	Y	
2.	Dr.A.Dayanand	1. Dayanand A, Madeswaran M, Palaninathan S. A clinical study on etiopathogenesis of hoarseness of voice.	Y	Y	
		1. Dayanand A, Harshavardhan B, Dheebika J, Palaninathan S. Study on temporalis fascia graft versus temporalis fascia with cartilage graft.	Y	Y	
		2. Dayanand A, Dheebika J, Prathula S, Palaninathan S. A Study on Prevalence of Hearing Loss as a Complication of Diabetes. European Journal of Medical and Health Sciences. 2020 Aug 29;2(4).	Y	Y	

DEPARTMENT OF OPHTHALMOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr.No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr. K. Divya	Karuppannasamy D, Venkatesan R, Thankappan L, Andavar R, Devisundaram S. Inverse association between serum bilirubin levels and retinopathy in patients with type 2 diabetes mellitus. Journal of clinical and diagnostic research: JCDR. 2017 Feb;11(2):NC09.	Y	Y	
		Divya K, Suvetha K, Sen A, Sundar D. Needs assessment of ophthalmology education for undergraduate medical students–A study from a medical college in South India. Education for Health. 2017 Sep 1;30(3):223.	Y	Y	
		Lekha T, Venkatakrishnan L, Divya K, Lavanya P. Periorbital and mediastinal emphysema after upper gastrointestinal endoscopy: case report of a rare complication. Journal of ophthalmic & vision research. 2017 Jul;12(3):345.	Y	Y	
		Rajagopala S, Kancherla R, Ganesan N, Balalakshmoji D, Karuppannasamy D, Ramanathan RP, Gopalan R. An unusual cause of breathlessness and profuse micronodules. Lung India: Official Organ of Indian Chest Society. 2018 Sep;35(5):431.	Y	Y	
		Karuppannasamy D, Andavar R, Arumugam J, Muthuvel K. DRESS syndrome secondary to carbamazepine therapy	Y	Y	

		presenting with bilateral acute anterior uveitis and angle closure glaucoma. Journal of ophthalmic & vision research. 2019 Jul;14(3):382.			
		Raghuram A, Divya K, Narendran V, Ganesh MR, Saravanan VR. Needle subretinal fluid drainage assisted pneumatic retinopexy for primary rhegmatogenous retinal detachment. Egyptian Retina Journal. 2019 Jan 1;6(1):16.	N	N	Index Copernicus
		Divya K, Dhivya N, Ganesh MR, Sundar D. Dry eye disease and tear dysfunction in patients with type 2 diabetes: A hospital-based study from South India. Sudanese Journal of Ophthalmology. 2019 Jan 1;11(1):14.	N	N	Index Copernicus
		Divya K, Kanagaraju V, Devanand B, Jeevamala C, Raghuram A, Sundar D. Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging. Indian journal of ophthalmology. 2020 Jun;68(6):1108.	Y	Y	
		Divya K, Ganesh MR, Sundar D. Relationship between myopia and central corneal thickness–A hospital based study from South India. Kerala Journal of Ophthalmology. 2020 Jan 1;32(1):45.	N	N	DOAJ, Index Copernicus
		Kanagaraju V, Divya K, Raajaganesh M, Devanand B. Evaluation of resistive index of orbital vessels using color doppler imaging in patients with type 2 diabetes mellitus. Journal of Medical Ultrasound. 2021 Apr 1;29(2):111.	Y	Y	
		Divya K, Indumathi C, Vikrant K, Padmanaban S. Pseudotumor cerebri complicating multisystem inflammatory syndrome in a child. Journal of Current Ophthalmology. 2021 Jul;33(3):358.	Y	Y	
2	Dr. D. Sundar	Divya K, Raghuram V, Lekha T, Raghuram A, Sundar D. Inverse Association between serum Bilirubin Levels and Retinopathy in	Y	Y	

		Patients with type 2 Diabetes Mellitus. Journal of Clinical and Diagnostic research. 2017 Feb;11(2):NC09-NC12.			
		Divya K, Suvetha K, Sen A, Sundar D. Needs assessment of ophthalmology education for undergraduate medical students–A study from a medical college in South India. Education for Health. 2017 Sep 1;30(3):223.	Y	Y	
		Divya K, Dhivya N, Ganesh MR, Sundar D. Dry eye disease and tear dysfunction in patients with type 2 diabetes: A hospital-based study from South India. Sudanese Journal of Ophthalmology. 2019 Jan 1;11(1):14.	N	N	Index Copernicus
		Divya K, Kanagaraju V, Devanand B, Jeevamala C, Raghuram A, Sundar D. Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging. Indian journal of ophthalmology. 2020 Jun;68(6):1108.	Y	Y	
		Divya K, Ganesh MR, Sundar D. Relationship between myopia and central corneal thickness–A hospital based study from South India. Kerala Journal of Ophthalmology. 2020 Jan 1;32(1):45.	N	N	DOAJ, Index Copernicus
		Brayan J, Sundar D, Singh SB, Singh NB, Thomas M, Chandrakanth P. Relationship between levels of vascular endothelial growth factor in blood and severity of diabetic retinopathy. TNOA Journal of Ophthalmic Science and Research. 2021 Jan 1;59(1):5.	N	N	DOAJ (Directory of Open Access Journals)
3	Dr.S.Padmanaban	Karthikeyan V, Padmanaban S, Vinupal A, Natarajan D. Astudy of Prognostic Factors Influencing Visual Outcome inPatients ofIndirect Traumatic Optic Neuropathy.	N	N	Cross Ref
		Swetha K. *Analytical study on epidemiology of pterygium in a*	N	N	Cross ref

		tertiary care centre (Doctoral dissertation, Coimbatore Medical College, Coimbatore).			Index Copernicus
		Padmanaban S, Jeevakala C, Saravanan J, Shalini G. Sight threatening ocular complications in dengue fever-A prospective study. Indian J Basic Appl Med Res. 2018;7:635-40.	N	N	Cross Ref Index Copernicus
		Mohana Priya P. *Study the prevalence of diabetic retinopathy and dry eye in diabetes mellitus patients and comparing with duration and urea, creatinine level* (Doctoral dissertation, Coimbatore Medical College, Coimbatore).	N	N	Cross Ref Index Copernicus
		Padmanaban S, Sumathi P, Prayagi Kandoth RP. Congenital capillary hemangioma arising from palpebral conjunctiva of a neonate. Indian journal of ophthalmology. 2017 Nov;65(11):1221.	Y	Y	
		Divya K, Indumathi C, Vikrant K, Padmanaban S. Pseudotumor cerebri complicating multisystem inflammatory syndrome in a child. Journal of Current Ophthalmology. 2021 Jul;33(3):358.	Y	Y	

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr Latha Maheswari. S	1. Suganya K, Subbarayan LM. Maternal and perinatal outcomes in women with first trimester vaginal bleeding. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Nov 1;8(11):4320-4.	N	N	Indexed in Copernicus
		2. Abraham R, Arunachalam P. Adnexal torsion-five-year retrospective study. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018 Feb 1;7(2):508-13.	N	N	Indexed in copernicus
		3. Reshma Shri R. *Evaluation of first trimester fasting blood glucose as a predictor of gestational diabetes mellitus* (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Indexed in copernicus
2	Dr Seetha Panicker	1. Bhat C, Panicker S. Labor Pattern in South Indian Population: A Retrospective Study. Recent Developments in Medicine and Medical Research Vol. 12. 2021 Nov 2:120-7.	N	Y	
		2. Chitra TV, Panicker S, Wesley G. Efficacy of intermittent iron versus daily iron in antenatal women.	N	N	Indexed in copernicus
		3. Maheswari S, Poornima C, Panicker S. Review of 113 cases of autoimmune disorders in pregnancy. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Dec 1;8(12):4786-93.	N	N	Indexed in copernicus
		4. Meena Priyadharshini V, Panicker S. Universal screening for hypothyroidism in pregnancy: is it necessary?. International Journal of Reproduction, Contraception, Obstetrics and Gynecology.;8(7):2684.	N	N	Indexed in copernicus
		5. Ramya T, Sabnis SS, Chitra TV, Panicker S. Cesarean myomectomy: an experience from a tertiary care teaching hospital. The Journal of Obstetrics and Gynecology of India. 2019 Oct;69(5):426-30.	Y	N	

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

		6. Meenapriyadharshini, Seetha panicker. First Trimester Cervical Length And Pre Term Birth. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) Volume 17, Issue 10 Ver. 12 (October. 2018), PP 31-34	N	N	Indian citation Index
		7. Panicker S, Chitra TV. Surveillance of surgical site infections to determine incidence, risk factors and microbiologic spectrum following obstetric and gynaecological surgeries. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018 Oct 1;7(10):4029-35.	N	N	Indexed in copernicus
		8. S., M., C., P., N., L. and Panicker, S., 2018. Analysis of various types of ectopic pregnancies: a five-year review. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, 7(6), p.2410.	N	N	Indexed in copernicus
		9. Aravind Chander V. *To find the association between intrapartum amniotic fluid lactate level and labour outcome* (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Indexed in copernicus
		10. Chitra, T.V, Seetha Panicker. MODERATE TO SEVERE THROMBOCYTOPENIA IN THE PARTURIENT. International Journal of Development Research Vol. 07, Issue, 07, pp.13959-13962, July, 2017	N	N	Google scholar
3	Dr Chitra T V	1. Dr Chitra Bhat. A usual parasite in an unusual location –incidental detection of strongyloids stercoralis in liquid based cytology. Journal of postgraduate Medicine. Accepted for publication	N	N	
		2. Bhat C, Panicker S. Labor Pattern in South Indian Population: A Retrospective Study. Recent Developments in Medicine and Medical Research Vol. 12. 2021 Nov 2:120-7.	N	Y	
		3. Chitra TV, Panicker S, Wesley G. Efficacy of intermittent iron versus daily iron in antenatal women	N	N	Indexed in copernicus
		4. Ramya T, Sabnis SS, Chitra TV, Panicker S. Cesarean myomectomy: an	Y	N	

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

		experience from a tertiary care teaching hospital. The Journal of Obstetrics and Gynecology of India. 2019 Oct;69(5):426-30			
		5. Panicker S, Chitra TV. Surveillance of surgical site infections to determine incidence, risk factors and microbiologic spectrum following obstetric and gynaecological surgeries. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018 Oct 1;7(10):4029-35	N	N	Indexed in Copernicus
		6. Chitra, T.V, Seetha Panicker. MODERATE TO SEVERE THROMBOCYTOPENIA IN THE PARTURIENT. International Journal of Development Research Vol. 07, Issue, 07, pp.13959-13962, July, 2017	N	N	Google scholar
4	Dr Reena Abraham	1. Dr Reena Abraham. Role of third trimester OGTT in the detection of late onset gestational diabetes in the Indian population. International Journal of Diabetes in developing countries. Accepted for publication	N	Y	Google scholar
		2. Saira R. *Effect of antenatal yoga on labour outcome* (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Google scholar
		3. Abraham R, Arunachalam P. Adnexal torsion-five-year retrospective study. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018 Feb 1;7(2):508-13.	N	N	Indexed in Copernicus
5	Dr Kanchanamalai	1. Malathi TM, Kanchanamalai K. A comparative study of safety and efficacy of vaginal isosorbide mononitrate (40mg) with dinoprostone gel (0.5 mg). International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018 Oct 1;7(10):4197-202.	N	N	Indexed in Copernicus
6	Dr Ramya T	1. Thangavelu R, Natarajan L. Towards safe motherhood to--are we moving in the right direction?: experience from an urban health facility in South India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Jul 1;8(7):2851-6.	N	N	Indexed in copernicus
		2. Ramya T, Sabnis SS, Chitra TV, Panicker S. Cesarean myomectomy: an experience from a tertiary care teaching hospital. The Journal of Obstetrics	Y	N	

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

		and Gynecology of India. 2019 Oct;69(5):426-30			
		3. Umamaheswari G. Nonimmune hydrops foetalis: value of perinatal autopsy and placental examination in determining aetiology. International Journal of Research in Medical Sciences. 2018 Oct;6(10):3327.	N	N	Indexed in copernicus
		4. Umamaheswari G, Ramya T, Chaitra V. Nonimmune hydrops foetalis (NIHF): value of fetal autopsy and comparison with ultrasound findings. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2018 Aug 1;7(8):3330-8.	N	N	Indexed in copernicus
		5. Gurusamy U, Thangavelu R, Venkataswamy C. Abnormalities of the umbilical cord: correlation with placental histology and perinatal outcome. Int J Res Med Sci. 2017;5:5009-16.	N	N	Indexed in copernicus
		6. Ramya T, Umamaheswari G, Chaitra V. Placental pathology in maternal autoimmune diseases-new insights and clinical implications. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2017 Sep 1;6(9):4090-8.	N	N	Indexed in copernicus
7	Dr Poornima. C	1. Maheswari S, Poornima C, Panicker S. Review of 113 cases of autoimmune disorders in pregnancy. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Dec 1;8(12):4786-93.	N	N	Indexed in copernicus
		2. S., M., C., P., N., L. and Panicker, S., 2018. Analysis of various types of ectopic pregnancies: a five-year review. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, 7(6), p.2410.	N	N	Indexed in copernicus
		3. 2021. [online] Available at: <https://www.researchgate.net/publication/312273271_Comparative_study_on_gynaecological_issues_in_adolescence_among_private_and_government_school_girls> [Accessed 17 December 2021].	N	N	Indexed in copernicus
8	Dr Chippy Tess Mathew	1. Mathew CT, Maheswari U, Shanmugam K. Co-relation of endometrial thickness by transvaginal sonogram with histopathology pattern in abnormal	N	N	Indexed in Copernicus

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

		uterine bleeding: a study from South India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2020 Apr 1;9(4):1416-22.			
		2. Dr Sairajan, Dr Reena Abraham, Dr Chippy Tess Mathew . Effect of Antenatal Yoga on Labour Outcome- A Non-Randomized Control Study from a Tertiary Hospital in South India". International Journal of Gynaecology and Obstetric Research. Vol. 5(1), pp. 038-046, February, 2020.	N	N	Indexed in Copernicus
		3. Mathew CT, Maheswari U, Ramu A. One year retrospective analysis of PAP smears-from a tertiary hospital in Tamil Nadu.	N	N	Google scholar
		4. Dhivya M. *Association of Serum Neutrophil Gelatinase Associated Lipocalin (NGAL) Levels with Preeclampsia* (Doctoral dissertation, PSG Institute of Medical Sciences and Research, Coimbatore).	N	N	Indexed in Copernicus
9	Dr Lalitha N	1. Natarajan L, Srinivasan D, Sekar D, Krishnamoorthi P, Kumar P. Fetal medicine as a part of early clinical exposure. International Journal of Health & Allied Sciences. 2021 Jul 1;10(3):231-.	N	N	Google scholar
		2. Rajesh H, Natarajan L, Panicker S, Mithraprabhu S. Hyperreactio luteinalis following frozen embryo transfer managed by fetal reduction. BMJ Case Reports CP. 2021 Mar 1;14(3):e236455.	Y	N	
		3. Doraiswamy V, Natarajan L, Venkatesh CT. Supraventricular tachycardia in one of the twins: The ethical dilemmas involved in treatment. Annals of Pediatric Cardiology. 2020 Apr;13(2):150..	N	N	Indexed in Copernicus
		4. Natarajan L, Gosavi AT, Wataganara T, Su LL, Amin Z, Leung TY, Choolani M, Biswas A, Mattar CN. Mentoring a surgical team towards procedural competence in the early learning curve for selective fetoscopic laser photocoagulation. Singapore Medical Journal. 2020 Sep 21.	Y	N	

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

		5. Natarajan L, UmaMaheswari G. Gestational hyperglycemia on diet and medication: impact on placental pathology and pregnancy outcomes. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Aug 1;8(8):3350-7.N	N	N	Indexed in Copernicus
		6. Thangavelu R, Natarajan L. Towards safe motherhood to--are we moving in the right direction?: experience from an urban health facility in South India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Jul 1;8(7):2851-6.	N	N	Indexed in Copernicus
		7. S., M., C., P., N., L. and Panicker, S., 2018. Analysis of various types of ectopic pregnancies: a five-year review. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, 7(6), p.2410.	N	N	Indexed in Copernicus
10	Dr Maheswari. S	1. Maheswari S, Poornima C, Panicker S. Review of 113 cases of autoimmune disorders in pregnancy. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2019 Dec 1;8(12):4786-93.	N	N	Indexed in Copernicus
		2. S., M., C., P., N., L. and Panicker, S., 2018. Analysis of various types of ectopic pregnancies: a five-year review. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, 7(6), p.2410.	N	N	Indexed in Copernicus
11	Dr Pooranidevi. A	1. Pooranidevi.A,Tamilarasu kaliappn, Nimmy Elizabeth George et al. Antenatal echocardiographic screening:An overrated routine practice in Asymptomatic mothers.Journal of card.dis.res:Vol.12,issue:03,2021	N	Y	
12	Dr Sumangali. T	1. Panicker S, Thirunavukkarasu S, Bhat C. Analysis of Classification Systems and Outcome of Labor in Women Undergoing Induction of Labor in South Indian Population. J South Asian Feder Obs Gynae 2021; 13 (5):306-310.	N	Y	

DEPARTMENT OF ANAESTHESIOLOGY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr.No	FacultyName	Publications in Vancouver referencingstyle.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1	Dr.Ganesan	Ganesan C, Biradar PA, Sampathkumar SV, Sunny A. A Comparative Study of Right and Left IJV Cannulation Using Surface Anatomy or USG Guidance: A Prospective Randomized Study Indian Journal of Anaesthesia and Analgesia.Vol 4 No 2 Apr – Jun 2017 Page 203-208	N	N	Index Copernicus
		Kumar RA, Ganesan C. A clinical audit on cancellation of elective surgery on a scheduled day in a teaching.institution . Medpulse International Journal of Anesthesiology. Nov 2019;12(2):161-165 .	N	N	Index Copernicus
		Periyanarkunan MR, Elavarasan S, Sivaraman P, Chinnasamy G. Aorto-oesophageal fistula: rarest cause of haematemesis-is it salvageable?. BMJ Case Reports CP. 2021 Nov 1;14(11):e245396.	Y	N	
2	Dr.Dhanabagyam	Rohini S, Kumar RA, Dhanabagyam G. Comparison of C-MAC blade and McCoy blade for laryngoscopy in adult patients undergoing tracheal intubation for elective surgeries with simulated cervical spine injury using manual in line stabilization.MedPulse International Journal of Anesthesiology. August	N	N	Index Copernicus

		2019;11(2):129-135..			
3	Dr.Prasanthkumar	MS Prasanthkumar, S Shaik Mushahida. Comparison of Intrathecal bupivacaine with fentanyl to intra thecal bupivacaine for elective lower abdominal and lower Limb surgeries. MedPulse International Journal of Anesthesiology. April 2020; 14(1): 09-13 Sulekha, M S Prasanth Kumar. Efficacy of paracetamol when added as an adjunct to lignocaine in intravenous regional anaesthesia- A prospective randomised double blinded study. MedPulse International Journal of Anesthesiology. October 2019; 12(1): 43-47 R Arun Shankar, M S Prasanth Kumar, G Dhanabagyam. Comparison of two doses of intravenous Dexmedetomidine 0.6mcg/kg and 1mcg/kg given prior to induction in attenuation of hemodynamic stress response of laryngoscopy and endotracheal intubation. MedPulse International Journal of Anesthesiology. October 2018; 8(1): 24-28. J Keerthana, M S Prasanth Kumar, S Shaik Mushahida. Comparison of perineural and intravenous dexamethasone on duration of analgesia in supraclavicular brachial plexus block. MedPulse International Journal of Anesthesiology. March 2022; 21(3):99-104	N N N N	N N N N	Index Copernicus Index Copernicus Index Copernicus Index Copernicus

4	Dr R Arun Kumar	Arun Kumar R (2019 Incidence of Post Dural Puncture Headache With Different Size Quincke Spinal Needles In Parturients Undergoing Caeserean Section, International Journal of Current Advanced Research.08(07),pp.19428-19432	N	N	1.Google Scholar
		Rohini S, Kumar RA, Dhanabagyam G. Comparison of C-MAC blade and McCoy blade for laryngoscopy in adult patients undergoing tracheal intubation for elective surgeries with simulated cervical spine injury using manual in line stabilization.MedPulse International Journal of Anesthesiology. August 2019;11(2):129-135..	N	N	1.Index Copernicus 2.Scilit
		Mohan KC, Kumar RA, Vinotha AS, Dhatchinamoorthi D. Efficacy of dexamethasone in reducing the severity of postoperative sorethroat after endotracheal intubation. MedPulse International Journal of Anesthesiology.November 2019; 12(2): 155-160	N	N	1.Index Copernicus
		Kumar RA, Ganesan C. A clinical audit on cancellation of elective surgery on a scheduled day in a teaching.institution . Medpulse International Journal of Anesthesiology. Nov 2019;12(2):161-165	N	N	1.Index Copernicus
		Kumar RA, Preetha I. Comparing the efficacy and haemodynamic response between intrathecal hyperbaric 0.5% bupivacaine with isobaric 0.75% ropivacaine using fentanyl as adjuvant in patients undergoing infra umbilical surgeries. Indian J Clin Anaesth 2020;7(3):444-449	N	N	1.Index Copernicus 2.Google Scholar 1.Index Copernicus

		Kumar RA, Ammu S. Comparing the efficacy of tramadol, ketamine and dexmedetomidine in the prevention of intraoperative shivering in patients undergoing surgery under subarachnoid blockade. Indian J Clin Anaesth 2021;8(3):446-451			2.Google Scholar
5	Dr.Silvy anna varughese	Varughese SA, Saxena KN, Taneja B, Sarin YK. Comparative evaluation of single level paravertebral block versus caudal block for postoperative analgesia in pediatric inguinal surgery. Northern Journal of ISA. 2017;2: 47-51. Pillai VS, Ramesh B, Varughese SA, Resident S. Safety and efficacy of multiple site thoracic paravertebral nerve block vs. modified pectoral nerve block for postoperative analgesia after modified radical mastectomies-a randomised controlled trial. InIJCA 2018 Jul (Vol. 5, pp. 445-50).	N N	N N	Non Index Copernicus Index Copernicus
6	Dr.Renjith Viswanath	Viswanath R, Pulickal R, Chirayath JJ, Mullapilly K. Comparison of vital capacity rapid inhalation and tidal ventilation induction with sevoflurane in adults: a prospective cohort study. Medical Gas Research. 2021 Jul;11(3):100. Aggarwal R, Bhatia R, Kulshrestha K, Soni KD, Viswanath R, Singh AK, Iyer KV, Khanna P, Bhattacharjee S, Patel N, Aravindan A. Clinicoepidemiological Features and Mortality Analysis of Deceased Patients with COVID-19 in a Tertiary Care Center. Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication	Y Y	N N	

		of Indian Society of Critical Care Medicine. 2021 Jun;25(6):622. Sryma PB, Mittal S, Mohan A, Madan K, Tiwari P, Bhatnagar S, Trikha A, Dosi R, Bhopale S, Viswanath R, Hadda V. Effect of proning in patients with COVID-19 acute hypoxemic respiratory failure receiving noninvasive oxygen therapy. Lung India: Official Organ of Indian Chest Society. 2021 Mar;38(Suppl 1):S6..	Y	N	
7	Dr.P.Arun	Arun P, Krishna HM. Comparative study between insulin bolus regimen and glucose insulin infusion regimen on effectiveness of intraoperative blood glucose control in patients with type 2 diabetes mellitus undergoing non-cardiac surgery. Sri Lankan Journal of Anaesthesiology. 2019 Jul 16;27(2).	N	Y	

DEPARTMENT OF RADIO DIAGNOSIS

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	**Dr.Devanand. B**	Saravanakumar A, Vaideki K, Govindarajan KN, Jayakumar S, Devanand B. Assessment of regional pediatric computed tomography dose indices in Tamil Nadu. Journal of medical physics. 2017 Jan;42(1):48.	Y	N	
		Garg P, Pandey S, MAHESHWARAN V, DEVANAND B, RAJAKUMAR R. Qualitative and Quantitative Assessment of Enhancement and Washout Characteristics of HCC and non HCC Hypervascular Liver Tumours. Journal of Clinical & Diagnostic Research. 2018 Feb 1;12(2).	Y	N	
		Rajagopala S, Kancherla R, Ganesan N, Balalakshmoji D, Karuppannasamy D, Ramanathan RP, Gopalan R. An unusual cause of breathlessness and profuse micronodules. Lung India: Official Organ of Indian Chest Society. 2018 Sep;35(5):431.	Y	N	
		Dr. Maheswaran, Dr. Suganya, Dr. Devanand. B. Congenital biotinidase deficiency – Clinching the Diagnosis with Classic imaging features. IOSR Journal of Dental and Medical Science. November 2019; Volume 18, issue 11: P– 17 -20.	N	N	Google scholar
		Maheswaran, Muthusubramanian, Rajakumar, Devenand, L. Venkatakrishnan. Correlation of splenic ARFI with endoscopic finding of esophageal varices in chronic liver parenchymal disease. Indian Journal of Basic and Applied Medical Research. December 2019; Vol 9, Issue – 1: 27 – 34.	N	N	CrossRef, National Science Library, Index Copernicus, International Committee of Medical Journal Editors (ICMJE), EBSCO, Hinari, Sherpa Romeo , Genamics &

					Publons
		Maheswaran, Sandhya, Elango, Devanand, Balakrishnan. Correlation of diffusion tensor imaging with clinical and neurological findings in patients with cervical spondylotic myelopathy. Indian Journal of Basic and Applied Medical Research. Dec 2019; vol - 9, Issue – 1: 40- 50.	N	N	Index Copernicus
		Divya K, Kanagaraju V, Devanand B, Jeevamala C, Raghuram A, Sundar D. Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging. Indian journal of ophthalmology. 2020 Jun;68(6):1108.	Y	Y	
		Kanagaraju V, Sukithra S, Jayaprakash N, Devan B. Utility of ultrasound elastography in evaluation of thyroid nodules and correlation with cytology. West African Journal of Radiology. 2020 Jan 1;27(1):52.	N	N	Index Copernicus
		Kanagaraju V, Ashlyin PV, Elango N, Devanand B. Role of transrectal ultrasound elastography in the diagnosis of prostate carcinoma. Journal of Medical Ultrasound. 2020 Jul;28(3):173.	Y	Y	
		Kanagaraju V, Rakshith AV, Devanand B, Rajakumar R. Utility of ultrasound elastography to differentiate benign from malignant cervical lymph nodes. Journal of Medical Ultrasound. 2020 Apr;28(2):92.	Y	Y	
		Kanagaraju V, Dhivya B, Devanand B, Maheswaran V. Utility of ultrasound strain elastography to differentiate benign from malignant lesions of the breast. Journal of Medical Ultrasound. 2021 Apr 1;29(2):89.	Y	Y	
		Maheswaran Viyannan, Sucharitha Periyasamy, Balalakshmoji Devanand. Analysis of different pathologies presenting as acute abdomen and performance of MDCT in making the diagnosis with clinical, intra-operative and histopathology correlation. Int J	N	N	Index Copernicus

		Radiol Diagn Imaging 2020;3(1):36-43			
		Viyannan Maheswaran, Vinu Adithi Vaishnavi, Balalakshmoji Devanand. Evaluation of acoustic radiation force impulse (ARFI) elastography as a tool for non invasive detection and grading of liver fibrosis with histopathology correlation. Int J Radiol Diagn Imaging 2020;3(1):22-26.	N	N	Index Copernicus
		Viyannan M, Balalakshmoji D, Leelakrishnan V. Hereditary hemorrhagic telangiectasia of liver: Pathophysiology with role of radiology in diagnosis and treatment. The Indian Journal of Radiology & Imaging. 2020 Jan;30(1):98.	Y	N	
		Kanagaraju V, Divya K, Raajaganesh M, Devanand B. Evaluation of resistive index of orbital vessels using color doppler imaging in patients with type 2 diabetes mellitus. Journal of Medical Ultrasound. 2021 Apr 1;29(2):111.	Y	Y	
		Viyannan M, Mohamed SK, Nagappan E, Balalakshmoji D. Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction. Journal of Ultrasound. 2020 Nov 18:1-8.	N	N	Springer Link
2.	**Dr. Vikrant. K**	Govindarajan S, Vasanth G, Kumar PA, Priyadarshini C, Radhakrishnan SS, Kanagaraj V, Balasubramanian N, Kumar PN, Divya TJ, Aishwarya CN. Impact of a comprehensive early clinical exposure program for preclinical year medical students. Health Professions Education. 2018 Jun 1;4(2):133-8.	N	N	Plumx metrics
		Kanagaraju V, Elangovan B. Gallbladder motility in children with chronic functional abdominal pain.	N	N	Index Medicus, Index Copernicus
		Divya K, Kanagaraju V, Devanand B, Jeevamala C, Raghuram A, Sundar D. Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging. Indian journal of ophthalmology. 2020 Jun;68(6):1108.	Y	Y	
		Kanagaraju V, Sukithra S, Jayaprakash N, Devan B. Utility of ultrasound elastography in evaluation of thyroid nodules and correlation with cytology. West African Journal of Radiology. 2020 Jan 1;27(1):52.	N	N	Index Copernicus

		Kanagaraju V, Ashlyin PV, Elango N, Devanand B. Role of transrectal ultrasound elastography in the diagnosis of prostate carcinoma. Journal of Medical Ultrasound. 2020 Jul;28(3):173.	Y	Y	
		Kanagaraju V, Rakshith AV, Devanand B, Rajakumar R. Utility of ultrasound elastography to differentiate benign from malignant cervical lymph nodes. Journal of Medical Ultrasound. 2020 Apr;28(2):92.	Y	Y	
		Kanagaraju V, Dhivya B, Devanand B, Maheswaran V. Utility of ultrasound strain elastography to differentiate benign from malignant lesions of the breast. Journal of Medical Ultrasound. 2021 Apr 1;29(2):89.	Y	Y	
		Kanagaraju V, Divya K, Raajaganesh M, Devanand B. Evaluation of resistive index of orbital vessels using color doppler imaging in patients with type 2 diabetes mellitus. Journal of Medical Ultrasound. 2021 Apr 1;29(2):111.	Y	Y	
		Divya K, Indumathi C, Vikrant K, Padmanaban S. Pseudotumor cerebri complicating multisystem inflammatory syndrome in a child. Journal of Current Ophthalmology. 2021 Jul;33(3):358.	Y	Y	
3.	**Dr. Elango. N**	Maheswaran, Sandhya, Elango, Devanand, Balakrishnan. Correlation of diffusion tensor imaging with clinical and neurological findings in patients with cervical spondylotic myelopathy. Indian Journal of Basic and Applied Medical Research. Dec 2019; vol - 9, Issue – 1: 40- 50.	N	N	Index Copernicus
		Jose A, Kumar AS, Govindarajan KN, Devanand B, Elango N. Assessment of adult diagnostic reference levels for panoramic radiography in Tamil Nadu region. Journal of medical physics. 2019 Oct;44(4):292.	Y	N	
		Kanagaraju V, Ashlyin PV, Elango N, Devanand B. Role of transrectal ultrasound elastography in the diagnosis of prostate carcinoma. Journal of Medical Ultrasound.	Y	Y	

		2020 Jul;28(3):173.			
		Viyannan M, Mohamed SK, Nagappan E, Balalakshmoji D. Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction. Journal of Ultrasound. 2020 Nov 18:1-8.	N	N	Springer Link
		Ravitha Rajalakshmi N, Vidhyapriya R, Elango N, Ramesh N. Deeply supervised u-net for mass segmentation in digital mammograms. International Journal of Imaging Systems and Technology. 2021 Mar 1;31(1):59-71.	Y	Y	
4.	**Dr. Rajakumar. R**	Garg P, Pandey S, MAHESHWARAN V, DEVANAND B, RAJAKUMAR R. Qualitative and Quantitative Assessment of Enhancement and Washout Characteristics of HCC and non HCC Hypervascular Liver Tumours. Journal of Clinical & Diagnostic Research. 2018 Feb 1;12(2).	Y	N	
		Maheswaran, Muthusubramanian, Rajakumar, Devenand, L. Venkatakrishnan. Correlation of splenic ARFI with endoscopic finding of esophageal varices in chronic liver parenchymal disease. Indian Journal of Basic and Applied Medical Research. December 2019; Vol 9, Issue – 1: 27 – 34.	N	N	CrossRef, National Science Library, Index Copernicus, International Committee of Medical Journal Editors (ICMJE), EBSCO, Hinari, Sherpa Romeo , Genamics & Publons
		Kanagaraju V, Rakshith AV, Devanand B, Rajakumar R. Utility of ultrasound elastography to differentiate benign from malignant cervical lymph nodes. Journal of Medical Ultrasound. 2020 Apr;28(2):92.	Y	Y	
		Garg P, R Rajakumar, Bandla A & Devanand. (2021) Is CT Cisternography in CSF Rhinorrhea Really Antiquated? Clinical Review of 14 Cases with Pictorial Depiction. J Neurosurg Imaging Techniques, 6(1): 328-336.	N	N	SciTech Central
5.	**Dr. Maheswaran. V**	Garg P, Pandey S, MAHESHWARAN V, DEVANAND B, RAJAKUMAR R. Qualitative and Quantitative Assessment of Enhancement and Washout Characteristics of HCC and non HCC Hypervascular Liver Tumours. Journal of Clinical & Diagnostic	Y	N	

		Research. 2018 Feb 1;12(2).			
		Dr. Maheswaran, Dr. Suganya, Dr. Devanand. B. Congenital biotinidase deficiency – Clinching the Diagnosis with Classic imaging features. IOSR Journal of Dental and Medical Science. November 2019; Volume 18, issue 11: P– 17 -20.	N	N	Google scholar
		Maheswaran, Muthusubramanian, Rajakumar, Devenand, L. Venkatakrishnan. Correlation of splenic ARFI with endoscopic finding of esophageal varices in chronic liver parenchymal disease. Indian Journal of Basic and Applied Medical Research. December 2019; Vol 9, Issue – 1: 27 – 34.	N	N	CrossRef, National Science Library, Index Copernicus, International Committee of Medical Journal Editors (ICMJE), EBSCO, Hinari, Sherpa Romeo , Genamics & Publons
		Maheswaran, Sandhya, Elango, Devanand, Balakrishnan. Correlation of diffusion tensor imaging with clinical and neurological findings in patients with cervical spondylotic myelopathy. Indian Journal of Basic and Applied Medical Research. Dec 2019; vol - 9, Issue – 1: 40- 50.	N	N	Index Copernicus
		Maheswaran Viyannan, Sucharitha Periyasamy, Balalakshmoji Devanand. Analysis of different pathologies presenting as acute abdomen and performance of MDCT in making the diagnosis with clinical, intra-operative and histopathology correlation. Int J Radiol Diagn Imaging 2020;3(1):36-43	N	N	Index Copernicus
		Viyannan Maheswaran, Vinu Adithi Vaishnavi, Balalakshmoji Devanand. Evaluation of acoustic radiation force impulse (ARFI) elastography as a tool for non invasive detection and grading of liver fibrosis with histopathology correlation. Int J Radiol Diagn Imaging 2020;3(1):22-26.	N	N	Index Copernicus
		Viyannan M, Balalakshmoji D, Leelakrishnan V. Hereditary hemorrhagic telangiectasia of liver: Pathophysiology with role of radiology in diagnosis and treatment. The Indian Journal of Radiology & Imaging. 2020 Jan;30(1):98.	Y	N	
		Viyannan M, Mohamed SK, Nagappan E, Balalakshmoji D. Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction. Journal of Ultrasound. 2020 Nov 18:1-8.	N	N	Springer Link

6.	**Dr. Jayaprakash. N**	Jayaprakash N, Kumar A, PS SD. Determination Of gestational age in third trimester using foetal transcerebellar diameter and assessment of foetal growth using Tcd/Ac ratio. IOSR J Dental Med Sci. 2018;17(8):54-60.	N	N	Google scholar
		Arun Kumar S, Jayaprakash N, & Prasad Hegde. (2020). New Generation Contrast Media and Renal Saftey. *Asian Journal of Medical Radiological Research*, *7*(2), 175-177.	N	N	Index Copernicus
		Kanagaraju V, Sukithra S, Jayaprakash N, Devan B. Utility of ultrasound elastography in evaluation of thyroid nodules and correlation with cytology. West African Journal of Radiology. 2020 Jan 1;27(1):52.	N	N	Index Copernicus

PSG Institute of Medical Sciences & Research

DEPARTMENT OF DENTAL SURGERY

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

Sr. No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pubmed (Y/N)	Scopus (Y/N)	
1.	Dr.K.Abhirami	1. Kannan A, Govindarajan V. The ill effect in the oral healthamong the adults due to the consumption of aerated drinks. J Evid Based Med Healthc. 2017;4(43):2656-8..	N	N	DOAJ,Index copernicus
		2. Kannan A, Govindarajan V. PREVALENCE OF DENTAL CARIES AMONG SCHOOL CHILDREN IN SULUR-COIMBATORE.	N	N	DOAJ,Index copernicus
		3. Dr.K.Abhirami, comparison of TMA,stainless and TIMOLIUM for friction,load deflection and surface characteristic , journal of Indian dental association tamilnadu. 2018;8(2):56-64.	N	N	DOAJ
		4. Abhirami K, Valli G. Evaluation for prevalence of malocclusion among adults and need for orthodontic treatment: A cross sectional study.	N	N	DOAJ,Index Copernicus international
		5. Abhirami K,Vallig, Karthikeyan MS, Dental health status among college students and the treatment needs, J Res Med Dent Sci,2020, 8(6):131-135	N	N	DOAJ
2.	Dr.P.Gowthamsuresh	1. Arthanari KK, Annamalai S, Thangavelu A, Palanivelu C, Suresh G, Anbuselvan S. Mucormycosis during Coronavirus Disease pandemic: A diagnosis we cannot afford to miss. Journal of Pharmacy and Bioallied Sciences. 2021 Nov 1;13(6):1769.	N	Y	-
3.	Dr.B.Dineshbabu	1. Kumar Y, Bharate V, Babu D, Verma C, A simple and innovative technique for prosthetic rehabilitation of continuous maxillary and orbital	N	N	Index Copernicus international

		defect,international journal of prosthodontics and restorative dentistry. 2017; 7(2):1-4. 2. Babu BD, Jain V, Pruthi G, Mangtani N, Pillai RS. Effect of denture soft liner on mandibular ridge resorption in complete denture wearers after 6 and 12 months of denture insertion: A prospective randomized clinical study. The Journal of the Indian Prosthodontic Society. 2017 Jul;17(3):233.	Y	Y	-
4.	Dr.C.Vinodha	1. Vinodha C, Simon AK. AWARENESS OF ORAL HYGIENE AMONG PATIENTS ATTENDING DENTAL OUT PATIENT DEPARTMENT IN A TERTIARY HOSPITAL IN COIMBATORE A CROSS SECTIONAL STUDY.	Y	N	-
			Y	N	-
		2. Ramachandran VS, Shankari V, Shanmugasundaram MR, Chandrasegaran V, Kumaraguru K. Management of Three Rooted Mandibular First Molar with Six Canals: A Case Report. Cureus. 2019 Dec;11(12).	Y	N	-
		3. EVALUATION OF SURFACE TENSION OF THREE ROOT CANAL SEALERS USING PENDANT DROP METHOD, Dr.Vinodha.C, Dr.Indira.R. INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH: 2019; 8(5):38-39. 4. INVITRO COMPARATIVE EVALUATION OF CONTACT ANGLE OF THREE ROOTCANAL SEALERS, Dr. Vinodhachandrasegaran, INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH,2020; 9(3):22-25.	Y	N	-
		5. COMPARATIVE EVALUATION OF LATERAL PENETRATION DEPTH OF ROOT CANAL SEALERS UNDER CONFOCAL LASER SCANNING MICROSCOPE, Dr.Vinodha.C, Dr.Indira.R, INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH: 2019,8(6),36-37.	Y	N	-

DEPARTMENT OF PHYSICAL MEDICINE AND REHABILITATION

DETAILS OF PUBLICATION FOR THE PAST FIVE YEARS (FROM 2017 TILL DATE)

S.No	Faculty Name	Publications in Vancouver referencing style.	Indexed in		If indexed in any other, please mention in this column
			Pub med (Y/N)	Scopus (Y/N)	
1	DR V RAMAMOORTHY	Veyilmuthu R, Govindan S, Venugopalan M, Panicker S. Effect of transcutaneous electrical nerve stimulation on labour pain relief among primigravida and multigravida mothers. Int J Reprod Contracept Obstet Gynecol. 2017;6:980-5.	N	N	Index Copernicus ScopeMed; crossRef; Google Scholar; J-Gate.
		Chankaramangalam MA, Ramamoorthy V, Muthuraja D, Saravanan PA, Rajan V. Factors associated with hypertension among truck drivers: a cross sectional study at a check post on a national highway in South India. International Journal of Medical Research & Health Sciences. 2017;6(5):126-9.	N	N	Index Copernicus ScopeMed; crossRef; Google Scholar;
		Arumugam M, Chandrasekaran B, Ramaiya MP, Veyilmuthu R. Comparison of functional ramp walk test and 6-min walk test in healthy volunteers: A new approach in functional capacity evaluation. International Journal of Advanced Medical and Health Research. 2017 Jul 1;4(2):75.	N	N	DOAJ; GoogleScholar; National scienece Library; ProQuest
		Ramamoorthy V. Physiatrist's Assessment and Management of Diabetic Foot.	N	N	Index Copernicus Google Scholar; Journal Index; J-Gate.

		Mathew AC, Veyilmuthu R, Palanisamy S, Xavier CR. Morbidity profile of truck drivers: a South Indian cross-sectional study.	N	N	Index Copernicus Google Scholar; Journal Index; Index Medicus for South-East Region (WHO)
		Dr. V Ramamoorthy . Effect of therapeutic exercise vs retro walking in individuals with knee . International journal of health sciences and research June 2019, Volume 9, Issue 6, Page130 - 136	N	N	Index Copernicus Google Scholar; Journal Index; J-Gate.
		Dr. V Ramamoorthy – Co- author **Efficacy of swiss ball therapy in balance rehabilitation of hemiplegic stroke participants.** International journal of research and review June 2019, Volume 6, Issue 6, Page 303 – 307	N	N	Index Copernicus Google Scholar; Journal Index; J-Gate.
		Dr. V Ramamoorthy – Co- author **Micro current therapy as a potential measure for treatment of pressure ulcer. A case report.** International journal of current research February 2020, Volume 12, Issue 2, Page 10077 - 10079	N	N	Index Copernicus Google Scholar; Open Academic Journal Index; DAJI.
		Ramamoorthy V. Physiatrist's Management of Diabetic Foot Ulcer. International Journal of Health Sciences and Research. 2020 Jan;10(1).	N	N	Index Copernicus Google Scholar; Journal Index; J-Gate.
		Dr. V Ramamoorthy - Primary author **Effectiveness of intense sensory motor stimulation on improving functional recovery in post stroke individuals** International journal of health sciences and research July 2020, Volume 10, Issue 7, Page 152 -159	N	N	Index Copernicus Google Scholar; Journal Index; J-Gate.

		Jayasanthi M, Ramamoorthy V, Parthiban A. Improved ICA algorithm for ECG feature extraction and R-peak detection. International Journal of Adaptive Control and Signal Processing. 2021 Jan;35(1):38-50.	N	N	Google Scholar

Annexure 2 - Proof of Publication by Faculties

Original Research Article DOI: 10.18231/2393-8005.2017.0028

Effectiveness of interactive case-based learning in medical microbiology

Anila A Mathews[1,*], Prathyusha K[2], Thomas V. Chacko[3], Amudha G[4]

[1,3,4]Professor, [2]Assistant Professor, [1,2]Dept of Microbiology [3]Dept of Community Medicine and Medical Education, [4]Dept. of Anatomy, [1,2]Government Medical College, Palakkad Kerala, [3]Believers Church Medical College, Thiruvalla, Kerala, [4]PSG, Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India

***Corresponding Author:**
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Abstract

Introduction: In Indian medical schools, most of the undergraduate teaching is mainly theoretical and mostly teacher centric, though now it is slowly changing to learner centric. In Microbiology, the second year medical students are taught "organism based" instead of "infection based". Interactive Case-based learning (CBL) helps in better understanding of the basic concepts and helps in clinical correlation.

Objective of the Study: To evaluate the effectiveness of an interactive case based learning methodology in teaching microbiology to undergraduate medical students.

Methodology: The crossover interventional study was undertaken at government medical college, Palakkad. The second year MBBS students were briefed regarding the study and were divided into two groups (test and control group). The case-based learning modules were prepared after discussion with the department- faculty and clinician and then validated by renowned faculties from different medical colleges and implemented. Pre and post tests were conducted and the results analyzed using unpaired T test. Feedback from students and faculty too were obtained and analyzed.

Results: The study showed that there was significant difference in the post test scores of students who were part of Case based learning methodology. The feedback analysis using the Likert scale also gave a positive feedback for the CBL sessions.

Conclusion: The interactive case based learning helped the students to understand the basic microbiological concepts better. It also motivated the students and helped them in clinical correlation.

Keywords: Case based learning, Learning modules, Microbiology.

Introduction

Present day Educational training programs in microbiology are mainly theoretical and concentrate more on micro-organisms rather than on common infections in the medical schools in India. In this process, students become disinterested, as they don't realize its clinical importance. Current attitudes of medical students towards basic science courses points to a need to change the training methodology. The method of teaching clinical microbiology based on clinical concepts will facilitate higher ordered learning, critical thinking and self-directed learning.[1] The goal of case-based learning is to prepare the students for clinical practice through the use of authentic clinical cases.[1] It prepares the students to become better clinicians.

Goal: To help the MBBS students understand and apply the Microbiological concepts in the diagnosis of common infectious diseases

Objectives

1. To design learning modules for the case based learning sessions
2. To apply the designed CBL modules as a routine to teach clinical microbiology to MBBS students
3. To evaluate the effectiveness of case based learning methodology

Methodology

Institutional human ethical clearance was obtained. Informed consent was taken from the students for the interventional study. The learning modules were prepared based on KEMPs instructional design model.[2,3] The clinical cases were decided after discussion with five department faculty and a clinician and 20 learning modules were prepared for the selected clinical cases. The learning modules comprised of the clinical case scenario, few important learning objectives, few critical thinking questions and reading material on the same.[1] The learning modules were validated for construct and content validity with the help of external subject experts (belonging to the same specialty but from different colleges in India) and FAIMER faculty based on Waltz and Bausell's criteria.[4]

After validation, the case- based modular learning (CBL) was implemented. The second MBBS students (82 students) were randomly divided into study group and control group. The students were divided into small groups of 8-10 students. Each CBL sessions were held for two hours each. Informed consent was obtained from the students. All the students had the regular lecture class, which was followed by interactive case-based learning for the study group. Pre and post test questions were prepared and they were validated by subject experts and then they were administered to both the groups. A pre-test before the CBL session followed by the post test was administered. The results of the pre

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Morphometric Study of Jugular Foramen and Jugular Fossa of Dried Adult Human Skulls and Its Clinical Significance

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Abstract

Introduction Jugular foramen is one of the most fascinating foramina of the human skull. It is a complex, irregular bony canal located between the occipital bone and petrous part of the temporal bone. Many important structures, like 9th, 10th, 11th cranial nerves, meningeal branch of occipital and ascending pharyngeal arteries, internal jugular vein, and inferior petrosal sinus, are passing through it. The jugular fossa has a septum and a dome. The septum divides the foramen into two compartments: anteromedial compartment (pars nervosa) and posterolateral compartment (pars vascularis). The dome contains superior bulb of internal jugular vein. The architecture of the foramen varies in size, shape, and laterality besides differences related to sex and race. The morphometric measurements of jugular foramen are very important for neurosurgeries and head and neck surgeries.

Objectives The aim of the present study is to study the morphology of jugular foramen along with its dimensions, compartments, presence of partial or complete septa and dome.

Materials and Methods A total number of 60 jugular foramina were examined from 30 adult dry human skulls of unknown age and sex from the Department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore. Measurements were taken using Digital Vernier calipers. Results were analyzed statistically.

Results The length, width, and surface area of jugular foramen of right side were measured and compared with the left side. Length and width of the jugular foramen was significantly higher on the right side. The presence of partial septum was found in 27 skulls (90%) on the right side and 29 skulls (99.7%) on the left side, respectively. Dome was present in 100% of the jugular foramina on the right side and 90% of the jugular foramina on the left side. Separate opening for inferior petrosal sinus was found in eight skulls (27%) on the right side and four skulls (13%) on the left side.

Conclusion This study provides a clear understanding of anatomy of jugular foramen and supports the reported morphometric variations. The morphometric variations of jugular foramen in the parameters of the skull are probably due to ethnic and racial factors. Knowledge of these variations is important for neurosurgeons and radiologists who deal with space occupying lesions of the structures surrounding jugular foramen. This study may be helpful for ENT surgeons while performing middle ear surgeries.

Keywords
- jugular foramen
- internal jugular vein
- dome
- septum
- cranial nerves

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Basic Science

Anatomy of Lumbar Plexus and Implications to Regional Anaesthesiologist

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Abstract

Lumbar plexus is one of the two nerve plexuses which supply the lower limb. It is formed in the posterior abdominal wall within the psoas major muscle. The branches of the plexus exit via the medial and lateral borders as well as its ventral surface. It is a complex plexus which gives a branch to complete the formation of lumbo sacral plexus. The branches mainly supply the groin, anterior and medial compartments of thigh. They also supply the hip and knee joints. The cutaneous innervation by the branches of lumbar plexus is limited to the anterior, lateral and medial parts of the thigh, medial side of the leg and foot and also the lower part of anterior abdominal wall and perineum. Regional anaesthesia is a highly skilled and precise technique used widely in the patients to reduce the drug usage and decrease the intra and post operative complications. Lumbar plexus block can be used in surgeries related to hip joint and anterior part of thigh and groin. To execute the procedure successfully, sound knowledge in anatomy of lumbar plexus is required.

Keywords: Lumbar plexus, Branches, Regional anaesthesia.

Introduction:

Lumbar plexus is one of the nerve plexuses which supply the lower limb. It is a plexus which is formed inside the substance of the psoas major muscle and its branches emerge out of the muscle through its medial and lateral borders and also through its anterior surface. It is predominantly formed by the ventral rami of spinal nerves L 1, 2, 3 and 4. There is a small contribution from the T12 spinal nerve too. [1] The branches of lumbar plexus contribute to the innervation of lower limb predominantly the hip joint, knee joint, anterior and medial compartments of thigh. They also give cutaneous innervation to the anterior, lateral and medial surfaces of thigh, medial surface of the leg and foot. A small area parallel to inguinal ligament in the anterolateral part of lower abdomen and groin also is supplied by its branches. It gives motor innervation to the lower part of muscles of anterior abdominal wall and cremaster. Communication between the sensory branches of lumbar plexus will result in sensory overlap. [2]

Formation:

The roots of spinal nerves from L1 to L5 emerge in front of the transverse processes of lumbar vertebra. These roots enter into the part of psoas major muscle which is in between the muscle fibres taking origin from the bodies of lumbar vertebra and intervertebral discs and muscle fibres arising from the transverse processes of lumbar vertebra. [4]

When traced from body of L1 to lower lumbar vertebra, the plexus shares the same coronary plane at L1 but when it comes down, it is more anterior in position. The first four lumbar ventral rami with a contribution from subcostal nerve form the lumbar plexus. The plexus is complex and prone to have variations. The incidence of variations in lumbar plexus is around 20%.

The L1 ventral rami with a branch from ventral rami of subcostal nerve form a common trunk which divides and give rise to iliohypogastric and ilioinguinal nerves. L1 ventral ramus gives a branch which joins with a branch from L2 ventral rami to form genitofemoral nerve.

The ventral rami of L2 L3 and L4 spinal nerves divide into ventral and dorsal divisions. The ventral divisions of L2, L3

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Anatomical study of right coronary artery and its variations – Cadaveric study

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Abstract

Introduction: Knowledge of the normal and variant anatomy and anomalies of coronary circulation are vital component in the management of coronary artery disease. Any coronary artery disorder may reduce the blood supply to the heart and may lead to Myocardial infarction and death.

The aim of the study was to identify right coronary artery branching pattern and its variations. This may indirectly affect the patient's prognosis. This study describes the presence of normal arterial pattern and variations of right coronary artery in fifty heart specimens.

Materials and Method: This study was carried out on fifty formalin fixed adult human cadaveric heart specimens of both sexes. Visceral pericardium and subepicardial fats were removed. The right coronary artery and its branches were carefully dissected and followed till their termination.

Results: The right coronary artery was found to be arising from pulmonary trunk in three heart specimens in our study. Third coronary artery was found in one specimen. Sinu atrial nodal artery was arising from right coronary from right coronary artery in 94% and from left coronary artery in 6% Right dominance was observed in 35(70%) specimens. Fistula between right coronary artery and pulmonary artery was found in one specimen.

Conclusion: Branching pattern of right coronary artery is important for interpretation of coronary angiography and surgical revascularization of myocardium.

Keywords: Coronary artery, Sinu – atrial nodal artery, Fistula, pulmonary artery

Introduction

Coronary arteries represent the only supply to the myocardium. The two main coronary arteries, right and left coronary arteries arise from ascending aorta. Right coronary artery arises from right anterior aortic sinus and left coronary artery arises from left posterior aortic sinus. Patency of left coronary artery is vital for sufficient perfusion of the heart. The left coronary artery is responsible for irrigation, not only of most of the left ventricle, but also considerable portion of right ventricle.[1]

Right coronary artery runs between pulmonary trunk and right auricle and then runs downwards and is divided into two segments. First segment runs along the right border of the heart up to inferior border of heart. It gives right conus artery and supplies infundibulam of the right ventricle. If the right conus artery arises separately from the aortic sinus, then it is called as Third coronary artery. The second segment runs up to the apex as right marginal artery and supplies right ventricle. Sometimes it may runs in the posterior interventricular groove and may give rise to Posterior interventricular branch. Hence according to the origin of posterior interventricular artery, the coronary artery dominance has been considered. Right coronary artery ends in the crux by anastomosing with circumflex artery. It supplies the right atrium, right ventricle, atrioventricular septum and part of left ventricle.

Sinu atrial nodal artery may arise as atrial rami from initial segment of right coronary artery in 65% of the cases and from circumflex artery in 35% of the cases. Atrioventricular nodal artery arises as first septal ramus of posterior interventricular artery in 90% of the cases and 10% from the left coronary artery.[2]

Numerous studies on the variations of the arteries have been reported, but still it is better to study them further with respect to their clinical significance.

Coronary anomalies are a poorly understood topic in modern cardiology. Clinicians should be aware of such anomalies because some of them may produce sudden death.

The incidence of congenital coronary artery anomalies is 5–6%. By definition, the term anomalous or abnormal is used to define any variant form observed in less than 1% of the general population.[3]

The aim of the study is to identify the normal pattern of the coronary arteries and its variations. Though the modern techniques of revascularisation and coronary bypass surgeries are available, the knowledge about the anatomy of coronary arterial pattern and its variations are important for the cardiologists and cardio – thoracic surgeons.

Materials and Method

Fifty formalin fixed heart specimens of both sexes with intact coronary arterial pattern were studied in Anatomy department, PSG IMS & R, Coimbatore. The right coronary artery and its branches were carefully dissected out till their termination and were analysed for normal and abnormal patterns of coronary arteries.

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Original Article

Anatomy Section

Anatomical Study of Left Coronary Artery and its Variations–Cadaveric Study

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ABSTRACT

Introduction: Variations of the anatomy of the Coronary arteries are quiet common and have been reported previously in the literature. Right and Left coronary arteries which supply blood to the heart originates from the ascending aorta. Any coronary artery disorder may have serious implications by reducing the blood supply to heart leading to myocardial infarction and death.

Aim: To identify left coronary artery branching pattern and its variations.

Materials and Methods: Fifty formalin fixed wet specimens of heart of both sexes with intact coronary arterial pattern were collected and studied. The left coronary artery and its branches were carefully dissected out and were analysed for variable pattern in each specimen.

Results: Left coronary artery (LCA) was found to be hypoplastic in six (12%) specimens. In one specimen (2%), hypoplastic LCA was crossed by a vein and had communication with the vein. Trifurcation of LCA was seen in 17 (34%) specimens, Circumflex artery was short in one (2%) specimen. Kugel's artery was observed in three (6%) specimens. Median artery was found in seven specimens (14%). The left coronary artery and circumflex artery were absent in one (2%) specimen. In case where left coronary artery was absent, left anterior descending artery and circumflex artery were taking origin directly from left anterior aortic sinus. Left anterior descending artery showed variation in the form of short course (2 cm), discontinuous and embedded within myocardial tissue in 2% of cases each. However the circumflex artery also showed short course in one (2%) case.

Conclusion: The variations of the left coronary artery observed in the study are important for interpretation of coronary angiography, surgical revascularization of myocardium and embryological interpretations.

Keywords: Angiography, Circumflex artery, Kugel's artery, Left Anterior descending artery

INTRODUCTION

Left coronary artery (LCA) originates from left posterior aortic sinus. Patency of left coronary artery is vital for sufficient perfusion of the heart. The left coronary artery supplies most of the left ventricle and considerable portion of right ventricle [1].

LCA bifurcates into left anterior descending and circumflex artery and passes between pulmonary trunk and left auricle. The left anterior descending artery (LAD) runs in the left anterior interventricular groove and ends at the apex or may run up to crux of the heart [2]. Circumflex artery (CX) then runs in the posterior atrioventricular groove and ends at the crux of heart by anastomosing with right coronary artery (RCA) or may end before the crux. Sometimes posterior interventricular artery may arise from circumflex artery and may run in the posterior interventricular groove as left coronary dominance [2].

Numerous studies on the variations of the arteries have been reported [3,4]. Coronary artery (CA) variations is a topic of interest in modern cardiology. Cardiologists, cardiothoracic surgeons and radiologists should be aware of such anomalies because some of them may produce sudden death. Such rare coronary anomalies are poorly understood thus a detailed variation in them will add onto the existing clinical knowledge. The incidence of congenital coronary artery anomalies is 5–6%. By definition, the term anomalous or abnormal is used to define any variant form observed in less than 1% of the general population [3]. The incidence of all coronary anomalies is 0.23% in autopsy series and ranges between 0.3 to 5.6% in angiographic series [4]. The recent introduction of selective coronary angiography provides information about the normal coronary arterial pattern and also its variations [3].

The frequency of various anomalies of left coronary artery and the arterial dominance of the heart were observed in current study.

Numerous studies [3,4] on the variations of the arteries have been reported, but still it is better to study them further with respect to their clinical significance. The aim of the study was to identify the normal arterial branching pattern of left coronary artery and its variations in fifty heart specimens.

MATERIALS AND METHODS

The Cross sectional observational study was conducted from September 2016 to November 2016. The study was carried out after obtaining ethical clearance from Institutional ethical committee. Fifty formalin fixed heart specimens of both sexes with intact coronary arterial pattern were studied. Specimens with damaged arterial pattern were excluded. The left coronary artery and their branches were carefully dissected out till their termination and were analysed for variable patterns.

Length of the arteries were measured with the help of thread and Vernier caliper from the point of origin of the arteries to the point of termination. The course of artery was delineated and thread was passed around the vessel. Then thread with mark was measured using the Vernier caliper. Percentage incidence of various patterns was recorded and tabulated.

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Original Research Article

AN OSTEOLOGICAL STUDY OF FORAMEN TRANSVERSARIUM OF CERVICAL VERTEBRAE AND ITS CLINICAL SIGNIFICANCE

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ABSTRACT

Introduction: Seven cervical vertebrae constitute the skeletal framework of the neck. Along with the other identification points, the cardinal feature of a cervical vertebra whether typical or atypical is the presence of Foramen transversarium on the transverse process. This foramen transmits the Vertebral artery, Vertebral vein and Sympathetic nerves. These foramina are known to have variations in size, shape and numbers and may be absent, incomplete or duplicate, which may lead to various symptoms. Their etiology is related with the variations in the course of the Vertebral artery due to developmental reasons.

Materials and methods: Three hundred dry human cervical vertebrae were collected and studied in the department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore. We included all the Cervical vertebrae including Atlas, Axis and all other cervical vertebrae. We observed the presence, size, shape and variations of Foramen transversarium of cervical vertebrae during our study.

Observations and results: Out of three hundred Cervical vertebrae, One hundred and twenty five cervical vertebrae (42%) had variations of Foramen transversarium like Complete / incomplete Accessory foramen, triple foramen on one side, absence of foramen on one side and unequal size of foramen on both the sides. The different types of shapes of foramen transversarium were also analysed and tabulated. The areas of foramen transversarium were measured after measuring all the diameters.

Conclusion: Variations in the Foramen transversarium may indicate, the variation in the course of the Vertebral artery. The present study was important clinical implications for Head and neck and vascular surgeries. These variations should be kept in our mind during surgeries on cervical spine.

KEY WORDS: Foramen transversarium, shape, cervical vertebrae, accessory, absent and variations.

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Original Article

Anatomy Section

Anatomical Study of Lobes and Fissures of Lungs and its Clinical Significance-A Cadaveric Study

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ABSTRACT

Introduction: The human lungs are divided by fissures into lobes which permit movements of lobes to one another. The fissures act as barriers to avoid the spread of the diseases. Knowledge of variations in the lobes and fissures of the lungs is important for the Radiologists to interpret the radiological images and for the cardiothoracic surgeons to plan for segmental resection or lobectomy.

Aim: To study the patterns of fissures and lobes of the lungs and their variations and to find their clinical implications and to compare with the previous studies.

Materials and Methods: Forty pairs of lungs were used for the study. They were obtained from formalin fixed cadavers. The patterns of lobes, fissures and hilar anatomy of lungs and its variations were noted and specimens were photographed.

Results: In the present study, incomplete horizontal fissure was found to be in 23(57.5%) right lung specimens and incomplete oblique fissure was observed in 20(50%) left lung specimens. Absence of horizontal fissure was about 10(25%) and oblique fissure was about six (15%) in right lung specimens. Absence of oblique fissure was about four (10%) in left lung specimens. According to Craig and Walker classification, Grade 1 and Grade 3 fissures were found to be more in both the lung specimens. Accessory fissure was observed in 5 (12.5%) specimens in the right lung and 10 (25%) specimens in the left lung. Single lobe was observed in both the lungs, 7.5% in the right lung and 5% in the left lung.

Conclusion: Variations of fissures and lobes and of hilar anatomy is of great significance. Knowledge of these variations is important for Radiologists and Cardiothoracic surgeons for interpreting the data and to plan for the surgeries.

Keywords: Accessory fissure, Hilar anatomy, Lobectomy

INTRODUCTION

The lungs are a pair of essential organs of respiration located within the thoracic cavity, on either side of heart and other mediastinal contents. Each lung is half – conical in shape, divided into lobes by fissures. Anatomically, left lung is divided into upper and lower lobes by oblique fissure whereas right lung is divided into upper, middle, lower lobes by oblique and horizontal fissures. In each lung, the oblique fissure begins from mediastinal surface above and behind the hilum and cuts the posterior border of the lung about 2.5 cm lateral to the junction of the T3 &T4 spine. Then, it runs along the costal surface, cuts the inferior border of the lung and will reappear on the mediastinal surface and ends at the lower end of the hilum. The horizontal fissure begins at the oblique fissure, courses along the costal surface, cuts the anterior border and appears to end at the hilum [1].

Finding of an accessory fissure in lung specimens is not uncommon but appreciating them on radiographs and Computed Tomography (CT) scans is difficult and hence, they are not appreciated as distinct entities or are completely misinterpreted. They usually occur at the boundaries between bronchopulmonary segments [2]. The commonly found accessory fissures are superior accessory fissure, inferior accessory fissure and left minor accessory fissure. The superior accessory fissure separates superior segment from the rest of the segments of lower lobe of lung, the inferior accessory fissure separates small infracardiac lobe from other segments of lower lobe of lung on the diaphragmatic surface and the left minor fissure separates the lingula from the other segments of upper lobe of left lung [2].

The fissures may vary in the degree of completeness and tend to divide the lobe into smaller divisions. Complete fissures have continuity of lobes at their bottom. The adjacent lobes are connected by a small portion of pulmonary tissues because of clefts it fails to reach the hilum in the incomplete fissures. The fissure may be absent altogether. The fissures are important for uniform expansion of lobes. They allow movements of lobes in relation to each other. This is more involved in the lower lobes at the time of breathing [3].

The appearance of accessory lung fissure may be seen as in relation to its completeness or its depth and that varies with X-ray and CT scan [4].

Knowledge of classical accessory lobes and fissures is necessary for radiological interpretation. This gives proper guidance to Cardio thoracic surgeons in performing segmental lung resection and lobectomies to have an uncomplicated perioperative outcome.

To assess the patterns of lobes and fissures of lung and to find out any variations in the fissures and lobes and hilar structures of the lungs in human cadavers and to find their clinical significance and to compare with them with the previous studies.

MATERIALS AND METHODS

Forty pairs of lung specimens (32 lung specimens from male cadavers and 8 lung specimens from female cadavers) free from pathological lesions, removed at the time of teaching for first year of MBBS from the 10% formalin fixed human cadavers from the Department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore were included in the study. The study was conducted in the month of November 2016 to April 2017 after Ethical clearance (Proposal Number.16/291). The lung specimens were observed and were photographed for:

1. The patterns of lobes and fissures;
2. Variations in fissures, complete or incomplete;
3. Accessory fissure if any;
4. Hilar structures and its variations.

The anatomical classification proposed by Walker WS et al., was followed to determine the presence of completeness of fissures [5].

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Anatomical Study of Profunda Femoris Artery and it's Variations – Cadaveric Study

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Abstract

Background and Aims: Profunda femoris artery is the largest branch of femoral artery. It is the principal supply to the muscles of the thigh as well as head and neck of femur. Its branches form anastomosis around the head of the femur. Profunda femoris artery is also used for arteriography. It is frequently used in vascular reconstructive procedures in the proximal thigh. It forms main route of collateral circulation in occlusion of femoral artery. The study of variation of Profunda femoris artery is of great value for radiologists and surgeons during diagnostic and surgical intervention.

Aim of the study is to observe the origin of Profunda femoris artery, to measure the distance between midinguinal point and site of origin of Profunda femoris artery and to study the anatomy of Profunda femoris artery and its branches and to find out any variations in its course and its branches.

Materials and Methods: The study was conducted as a prospective study from April 2018 to October 2018. All the lower limbs with the intact Femoral artery and Profunda femoris artery and their branches were included. Femoral artery and its branches had got cut during dissection were not included in the study. Thirty two Profunda femoris arteries (20 on the right side and 12 on the left side) were studied in the department of Anatomy. The femoral triangle was exposed by making incisions along the inguinal ligament from pubic symphysis to anterior superior iliac spine. Midpoint was taken as Midinguinal point (MIP). MIP was marked with a coloured pen. The distance between MIP and the site of origin of

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Anatomical study of right coronary artery and its variations – Cadaveric study

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Abstract

Introduction: Knowledge of the normal and variant anatomy and anomalies of coronary circulation are vital component in the management of coronary artery disease. Any coronary artery disorder may reduce the blood supply to the heart and may lead to Myocardial infarction and death.

The aim of the study was to identify right coronary artery branching pattern and its variations. This may indirectly affect the patient's prognosis. This study describes the presence of normal arterial pattern and variations of right coronary artery in fifty heart specimens.

Materials and Method: This study was carried out on fifty formalin fixed adult human cadaveric heart specimens of both sexes. Visceral pericardium and subepicardial fats were removed. The right coronary artery and its branches were carefully dissected and followed till their termination.

Results: The right coronary artery was found to be arising from pulmonary trunk in three heart specimens in our study. Third coronary artery was found in one specimen. Sinu atrial nodal artery was arising from right coronary from right coronary artery in 94% and from left coronary artery in 6% Right dominance was observed in 35(70%) specimens. Fistula between right coronary artery and pulmonary artery was found in one specimen.

Conclusion: Branching pattern of right coronary artery is important for interpretation of coronary angiography and surgical revascularization of myocardium.

Keywords: Coronary artery, Sinu – atrial nodal artery, Fistula, pulmonary artery

Introduction

Coronary arteries represent the only supply to the myocardium. The two main coronary arteries, right and left coronary arteries arise from ascending aorta. Right coronary artery arises from right anterior aortic sinus and left coronary artery arises from left posterior aortic sinus. Patency of left coronary artery is vital for sufficient perfusion of the heart. The left coronary artery is responsible for irrigation, not only of most of the left ventricle, but also considerable portion of right ventricle.[1]

Right coronary artery runs between pulmonary trunk and right auricle and then runs downwards and is divided into two segments. First segment runs along the right border of the heart up to inferior border of heart. It gives right conus artery and supplies infundibulam of the right ventricle. If the right conus artery arises separately from the aortic sinus, then it is called as Third coronary artery. The second segment runs up to the apex as right marginal artery and supplies right ventricle. Sometimes it may runs in the posterior interventricular groove and may give rise to Posterior interventricular branch. Hence according to the origin of posterior interventricular artery, the coronary artery dominance has been considered. Right coronary artery ends in the crux by anastomosing with circumflex artery. It supplies the right atrium, right ventricle, atrioventricular septum and part of left ventricle.

Sinu atrial nodal artery may arise as atrial rami from initial segment of right coronary artery in 65% of the cases and from circumflex artery in 35% of the cases. Atrioventricular nodal artery arises as first septal ramus of posterior interventricular artery in 90% of the cases and 10% from the left coronary artery.[2]

Numerous studies on the variations of the arteries have been reported, but still it is better to study them further with respect to their clinical significance.

Coronary anomalies are a poorly understood topic in modern cardiology. Clinicians should be aware of such anomalies because some of them may produce sudden death.

The incidence of congenital coronary artery anomalies is 5–6%. By definition, the term anomalous or abnormal is used to define any variant form observed in less than 1% of the general population.[3]

The aim of the study is to identify the normal pattern of the coronary arteries and its variations. Though the modern techniques of revascularisation and coronary bypass surgeries are available, the knowledge about the anatomy of coronary arterial pattern and its variations are important for the cardiologists and cardio – thoracic surgeons.

Materials and Method

Fifty formalin fixed heart specimens of both sexes with intact coronary arterial pattern were studied in Anatomy department, PSG IMS & R, Coimbatore. The right coronary artery and its branches were carefully dissected out till their termination and were analysed for normal and abnormal patterns of coronary arteries.

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Original Article

Anatomy Section

Anatomical Study of Left Coronary Artery and its Variations–Cadaveric Study

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ABSTRACT

Introduction: Variations of the anatomy of the Coronary arteries are quiet common and have been reported previously in the literature. Right and Left coronary arteries which supply blood to the heart originates from the ascending aorta. Any coronary artery disorder may have serious implications by reducing the blood supply to heart leading to myocardial infarction and death.

Aim: To identify left coronary artery branching pattern and its variations.

Materials and Methods: Fifty formalin fixed wet specimens of heart of both sexes with intact coronary arterial pattern were collected and studied. The left coronary artery and its branches were carefully dissected out and were analysed for variable pattern in each specimen.

Results: Left coronary artery (LCA) was found to be hypoplastic in six (12%) specimens. In one specimen (2%), hypoplastic LCA was crossed by a vein and had communication with the vein. Trifurcation of LCA was seen in 17 (34%) specimens, Circumflex artery was short in one (2%) specimen. Kugel's artery was observed in three (6%) specimens. Median artery was found in seven specimens (14%). The left coronary artery and circumflex artery were absent in one (2%) specimen. In case where left coronary artery was absent, left anterior descending artery and circumflex artery were taking origin directly from left anterior aortic sinus. Left anterior descending artery showed variation in the form of short course (2 cm), discontinuous and embedded within myocardial tissue in 2% of cases each. However the circumflex artery also showed short course in one (2%) case.

Conclusion: The variations of the left coronary artery observed in the study are important for interpretation of coronary angiography, surgical revascularization of myocardium and embryological interpretations.

Keywords: Angiography, Circumflex artery, Kugel's artery, Left Anterior descending artery

INTRODUCTION

Left coronary artery (LCA) originates from left posterior aortic sinus. Patency of left coronary artery is vital for sufficient perfusion of the heart. The left coronary artery supplies most of the left ventricle and considerable portion of right ventricle [1].

LCA bifurcates into left anterior descending and circumflex artery and passes between pulmonary trunk and left auricle. The left anterior descending artery (LAD) runs in the left anterior interventricular groove and ends at the apex or may run up to crux of the heart [2]. Circumflex artery (CX) then runs in the posterior atrioventricular groove and ends at the crux of heart by anastomosing with right coronary artery (RCA) or may end before the crux. Sometimes posterior interventricular artery may arise from circumflex artery and may run in the posterior interventricular groove as left coronary dominance [2].

Numerous studies on the variations of the arteries have been reported [3,4]. Coronary artery (CA) variations is a topic of interest in modern cardiology. Cardiologists, cardiothoracic surgeons and radiologists should be aware of such anomalies because some of them may produce sudden death. Such rare coronary anomalies are poorly understood thus a detailed variation in them will add onto the existing clinical knowledge. The incidence of congenital coronary artery anomalies is 5–6%. By definition, the term anomalous or abnormal is used to define any variant form observed in less than 1% of the general population [3]. The incidence of all coronary anomalies is 0.23% in autopsy series and ranges between 0.3 to 5.6% in angiographic series [4]. The recent introduction of selective coronary angiography provides information about the normal coronary arterial pattern and also its variations [3].

The frequency of various anomalies of left coronary artery and the arterial dominance of the heart were observed in current study.

Numerous studies [3,4] on the variations of the arteries have been reported, but still it is better to study them further with respect to their clinical significance. The aim of the study was to identify the normal arterial branching pattern of left coronary artery and its variations in fifty heart specimens.

MATERIALS AND METHODS

The Cross sectional observational study was conducted from September 2016 to November 2016. The study was carried out after obtaining ethical clearance from Institutional ethical committee. Fifty formalin fixed heart specimens of both sexes with intact coronary arterial pattern were studied. Specimens with damaged arterial pattern were excluded. The left coronary artery and their branches were carefully dissected out till their termination and were analysed for variable patterns.

Length of the arteries were measured with the help of thread and Vernier caliper from the point of origin of the arteries to the point of termination. The course of artery was delineated and thread was passed around the vessel. Then thread with mark was measured using the Vernier caliper. Percentage incidence of various patterns was recorded and tabulated.

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Original Research Article

AN OSTEOLOGICAL STUDY OF FORAMEN TRANSVERSARIUM OF CERVICAL VERTEBRAE AND ITS CLINICAL SIGNIFICANCE

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ABSTRACT

Introduction: Seven cervical vertebrae constitute the skeletal framework of the neck. Along with the other identification points, the cardinal feature of a cervical vertebra whether typical or atypical is the presence of Foramen transversarium on the transverse process. This foramen transmits the Vertebral artery, Vertebral vein and Sympathetic nerves. These foramina are known to have variations in size, shape and numbers and may be absent, incomplete or duplicate, which may lead to various symptoms. Their etiology is related with the variations in the course of the Vertebral artery due to developmental reasons.

Materials and methods: Three hundred dry human cervical vertebrae were collected and studied in the department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore. We included all the Cervical vertebrae including Atlas, Axis and all other cervical vertebrae. We observed the presence, size, shape and variations of Foramen transversarium of cervical vertebrae during our study.

Observations and results: Out of three hundred Cervical vertebrae, One hundred and twenty five cervical vertebrae (42%) had variations of Foramen transversarium like Complete / incomplete Accessory foramen, triple foramen on one side, absence of foramen on one side and unequal size of foramen on both the sides. The different types of shapes of foramen transversarium were also analysed and tabulated. The areas of foramen transversarium were measured after measuring all the diameters.

Conclusion: Variations in the Foramen transversarium may indicate, the variation in the course of the Vertebral artery. The present study was important clinical implications for Head and neck and vascular surgeries. These variations should be kept in our mind during surgeries on cervical spine.

KEY WORDS: Foramen transversarium, shape, cervical vertebrae, accessory, absent and variations.

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Original Article

Anatomy Section

Anatomical Study of Lobes and Fissures of Lungs and its Clinical Significance-A Cadaveric Study

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ABSTRACT

Introduction: The human lungs are divided by fissures into lobes which permit movements of lobes to one another. The fissures act as barriers to avoid the spread of the diseases. Knowledge of variations in the lobes and fissures of the lungs is important for the Radiologists to interpret the radiological images and for the cardiothoracic surgeons to plan for segmental resection or lobectomy.

Aim: To study the patterns of fissures and lobes of the lungs and their variations and to find their clinical implications and to compare with the previous studies.

Materials and Methods: Forty pairs of lungs were used for the study. They were obtained from formalin fixed cadavers. The patterns of lobes, fissures and hilar anatomy of lungs and its variations were noted and specimens were photographed.

Results: In the present study, incomplete horizontal fissure was found to be in 23(57.5%) right lung specimens and incomplete oblique fissure was observed in 20(50%) left lung specimens. Absence of horizontal fissure was about 10(25%) and oblique fissure was about six (15%) in right lung specimens. Absence of oblique fissure was about four (10%) in left lung specimens. According to Craig and Walker classification, Grade 1 and Grade 3 fissures were found to be more in both the lung specimens. Accessory fissure was observed in 5 (12.5%) specimens in the right lung and 10 (25%) specimens in the left lung. Single lobe was observed in both the lungs, 7.5% in the right lung and 5% in the left lung.

Conclusion: Variations of fissures and lobes and of hilar anatomy is of great significance. Knowledge of these variations is important for Radiologists and Cardiothoracic surgeons for interpreting the data and to plan for the surgeries.

Keywords: Accessory fissure, Hilar anatomy, Lobectomy

INTRODUCTION

The lungs are a pair of essential organs of respiration located within the thoracic cavity, on either side of heart and other mediastinal contents. Each lung is half – conical in shape, divided into lobes by fissures. Anatomically, left lung is divided into upper and lower lobes by oblique fissure whereas right lung is divided into upper, middle, lower lobes by oblique and horizontal fissures. In each lung, the oblique fissure begins from mediastinal surface above and behind the hilum and cuts the posterior border of the lung about 2.5 cm lateral to the junction of the T3 &T4 spine. Then, it runs along the costal surface, cuts the inferior border of the lung and will reappear on the mediastinal surface and ends at the lower end of the hilum. The horizontal fissure begins at the oblique fissure, courses along the costal surface, cuts the anterior border and appears to end at the hilum [1].

Finding of an accessory fissure in lung specimens is not uncommon but appreciating them on radiographs and Computed Tomography (CT) scans is difficult and hence, they are not appreciated as distinct entities or are completely misinterpreted. They usually occur at the boundaries between bronchopulmonary segments [2]. The commonly found accessory fissures are superior accessory fissure, inferior accessory fissure and left minor accessory fissure. The superior accessory fissure separates superior segment from the rest of the segments of lower lobe of lung, the inferior accessory fissure separates small infracardiac lobe from other segments of lower lobe of lung on the diaphragmatic surface and the left minor fissure separates the lingula from the other segments of upper lobe of left lung [2].

The fissures may vary in the degree of completeness and tend to divide the lobe into smaller divisions. Complete fissures have continuity of lobes at their bottom. The adjacent lobes are connected by a small portion of pulmonary tissues because of clefts it fails to reach the hilum in the incomplete fissures. The fissure may be absent altogether. The fissures are important for uniform expansion of lobes. They allow movements of lobes in relation to each other. This is more involved in the lower lobes at the time of breathing [3].

The appearance of accessory lung fissure may be seen as in relation to its completeness or its depth and that varies with X–ray and CT scan [4].

Knowledge of classical accessory lobes and fissures is necessary for radiological interpretation. This gives proper guidance to Cardio thoracic surgeons in performing segmental lung resection and lobectomies to have an uncomplicated perioperative outcome.

To assess the patterns of lobes and fissures of lung and to find out any variations in the fissures and lobes and hilar structures of the lungs in human cadavers and to find their clinical significance and to compare with them with the previous studies.

MATERIALS AND METHODS

Forty pairs of lung specimens (32 lung specimens from male cadavers and 8 lung specimens from female cadavers) free from pathological lesions, removed at the time of teaching for first year of MBBS from the 10% formalin fixed human cadavers from the Department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore were included in the study. The study was conducted in the month of November 2016 to April 2017 after Ethical clearance (Proposal Number.16/291). The lung specimens were observed and were photographed for:

1. The patterns of lobes and fissures;
2. Variations in fissures, complete or incomplete;
3. Accessory fissure if any;
4. Hilar structures and its variations.

The anatomical classification proposed by Walker WS et al., was followed to determine the presence of completeness of fissures [5].

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Anatomical Study of Profunda Femoris Artery and it's Variations – Cadaveric Study

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Research Article
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Abstract

Background and Aims: Profunda femoris artery is the largest branch of femoral artery. It is the principal supply to the muscles of the thigh as well as head and neck of femur. Its branches form anastomosis around the head of the femur. Profunda femoris artery is also used for arteriography. It is frequently used in vascular reconstructive procedures in the proximal thigh. It forms main route of collateral circulation in occlusion of femoral artery. The study of variation of Profunda femoris artery is of great value for radiologists and surgeons during diagnostic and surgical intervention.

Aim of the study is to observe the origin of Profunda femoris artery, to measure the distance between midinguinal point and site of origin of Profunda femoris artery and to study the anatomy of Profunda femoris artery and its branches and to find out any variations in its course and its branches.

Materials and Methods: The study was conducted as a prospective study from April 2018 to October 2018. All the lower limbs with the intact Femoral artery and Profunda femoris artery and their branches were included. Femoral artery and its branches had got cut during dissection were not included in the study. Thirty two Profunda femoris arteries (20 on the right side and 12 on the left side) were studied in the department of Anatomy. The femoral triangle was exposed by making incisions along the inguinal ligament from pubic symphysis to anterior superior iliac spine. Midpoint was taken as Midinguinal point (MIP). MIP was marked with a coloured pen. The distance between MIP and the site of origin of

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THIEME OPEN ACCESS

Morphometric Study of Jugular Foramen and Jugular Fossa of Dried Adult Human Skulls and Its Clinical Significance

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Abstract

Introduction Jugular foramen is one of the most fascinating foramina of the human skull. It is a complex, irregular bony canal located between the occipital bone and petrous part of the temporal bone. Many important structures, like 9th, 10th, 11th cranial nerves, meningeal branch of occipital and ascending pharyngeal arteries, internal jugular vein, and inferior petrosal sinus, are passing through it. The jugular fossa has a septum and a dome. The septum divides the foramen into two compartments: anteromedial compartment (pars nervosa) and posterolateral compartment (pars vascularis). The dome contains superior bulb of internal jugular vein. The architecture of the foramen varies in size, shape, and laterality besides differences related to sex and race. The morphometric measurements of jugular foramen are very important for neurosurgeries and head and neck surgeries.

Objectives The aim of the present study is to study the morphology of jugular foramen along with its dimensions, compartments, presence of partial or complete septa and dome.

Materials and Methods A total number of 60 jugular foramina were examined from 30 adult dry human skulls of unknown age and sex from the Department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore. Measurements were taken using Digital Vernier calipers. Results were analyzed statistically.

Results The length, width, and surface area of jugular foramen of right side were measured and compared with the left side. Length and width of the jugular foramen was significantly higher on the right side. The presence of partial septum was found in 27 skulls (90%) on the right side and 29 skulls (99.7%) on the left side, respectively. Dome was present in 100% of the jugular foramina on the right side and 90% of the jugular foramina on the left side. Separate opening for inferior petrosal sinus was found in eight skulls (27%) on the right side and four skulls (13%) on the left side.

Conclusion This study provides a clear understanding of anatomy of jugular foramen and supports the reported morphometric variations. The morphometric variations of jugular foramen in the parameters of the skull are probably due to ethnic and racial factors. Knowledge of these variations is important for neurosurgeons and radiologists who deal with space occupying lesions of the structures surrounding jugular foramen. This study may be helpful for ENT surgeons while performing middle ear surgeries.

Keywords
- jugular foramen
- internal jugular vein
- dome
- septum
- cranial nerves

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Original Article

Anatomy Section

Morphometric Study of Atypical Lumbar Vertebrae and their Clinical Significance

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ABSTRACT

Introduction: Lumbar pedicles are short and strong. They form an important part of lumbar spine and play role in transmission of weight from neural arch to the anterior part of vertebral column. Fractures, tumours and degenerative diseases of lumbar vertebrae are common. Pedicle screw fixation is needed to stabilise the vertebrae if required. Only few studies are available for atypical lumbar vertebrae.

Aim: To determine the various morphometric measurements of fifth lumbar vertebrae and to discuss the various applied aspects of the study.

Materials and Methods: This cross-sectional study was conducted for a period of one year from March 2016 to February 2017. Eighty-five adult human fifth lumbar vertebrae were identified with its atypical features and were examined. Various morphometric measurements- pedicle length, pedicle height and pedicle width, inter pedicle distance, transverse pedicle angle, sagital pedicle angle, chord length, vertebral body height, vertebral body width and vertebral canal measurements were taken using sliding vernier caliper with a least count of 0.1 mm. The data were entered in Microsoft excel and were analysed.

Results: A total of 85 fifth lumbar vertebrae were examined. Mean Pedicle Height of L5 vertebrae on both the sides were equal (8.4±3.74). Mean pedicle length of L5 vertebrae and mean transverse pedicular angle of L5 vertebrae were more on the right side than on the left side. Mean pedicle width, mean sagital pedicular angle and chord length of L5 vertebrae were more on the left side than on the right side in this study. All the measurements were compared with previous authors and found to have similar results.

Conclusion: Various morphometric measurements of pedicle of fifth lumbar vertebrae were taken in this study. These morphometric knowledge of the pedicles may be helpful for the orthopaedic surgeons and radiologists for proper selection of the pedicle screw and operative repair.

Keywords: Lumbar pedicle, Pedicular angle, Vertebral canal

INTRODUCTION

There are five lumbar vertebrae between the rib cage and the pelvis. The largest segments of the vertebral column are lumbar vertebrae and are characterised by the presence of accessory tubercles in transverse processes and mammillary tubercles in superior articular facets and by the absence of foramen transversarium in the transverse process (as only found in cervical region) and by the absence of facets on the sides of the body (as only found in thoracic region). They are designated L1-L5. The lumbar vertebrae support the weight of the body and permit movement [1].

Lumbar vertebral pedicles are short, thick and flat dorsal projections from superior part of the body at the junction of its lateral and dorsal surface. Pedicles form the concavity by the curved superior and inferior borders. Superior vertebral notch is shallower than inferior. When vertebrae articulate by the intervertebral disc and facet joints, these adjacent vertebral notches contribute to formation of intervertebral foramen. The foramen contains a segmental mixed spinal nerve and its sheaths, 2-4 recurrent meningeal nerves, variable number of spinal arteries and plexiform venous connections between the internal and external vertebral venous plexus. These structures particularly the nerves may be affected by trauma or diseases affecting the adjacent tissue. The complete perimeter of an intervertebral foramen consists of the notches, dorsal aspects of parts of adjacent vertebral bodies, the intervertebral disc and the capsule of the plane synovial facet joints [1].

The fifth lumbar vertebrae is the only atypical lumbar vertebrae differs from others by having massive body, its anterior surface is more extensive than posterior surface, transverse processes are substantial and they encroach on the sides of the body from the junctions of pedicles and laminae and the space between inferior articular processes are wider than that of superior articular processes [2]. Pedicles are thick, strong projections from the junction of lateral and dorsal surface of body of lumbar vertebrae. They are made up of inner cancellous and outer cortical bone. Pedicles have an important role in weight transmission from neural arch to anterior part of vertebral column in lumbar spine. Pedicle of fifth lumbar vertebrae helps in forward slide of L5 over S1. In the lumbar region, where the concavity is posterior, a part of compressive force of anterior column is transmitted to posterior [3].

Low back pain is a major public health problem all over the world. An estimated 75% of all the people may experience back pain at some time in their lives out of which most of them recover without surgery while 3-5% of the patient's present herniated disc and 1-2% have compression of nerve root. Treatment can be conservative or by surgical decompression-laminectomy in persons experiencing severe pain, claudication, neurological deficit or Myelopathy [4].

Fracture of lumbar pedicles is common in older age group due to osteoporosis and requires open surgical or percutaneous pedicle screw fixation to stabilise the vertebrae. Hence morphometric knowledge of lumbar pedicle is important [5]. Transpedicle fixation of the spine is a safe way of gaining effective vertebral stabilisation in the management of different spinal ailments fracture in lumbar spine, removal of tumours in vertebral bodies, gross spondylolesthesis and lumbar unsteadiness [6]. Fracture of the pedicle might result from the usage of relatively oversized screw. Hence, choice of the screw for operation is determined by the minimum diameter of the pedicle [7].

Transpedicle screws enable devices like plates, rods or wires to be applied to spine for fixation. The integrity of the pedicles and vertebral

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Vascular foramina of navicular bone: a morphometric study

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Abstract: The navicular bone is supplied by more than one artery. The knowledge about the vascular foramina is important to understand the pathogenesis and management of navicular fractures. The objective of the present study is to analyze the morphology and morphometry of vascular foramina of dried human navicular bone in Indian population. The study was carried out by using 100 navicular bones (50 right and 50 left) collected from our institute and other medical institutes in and around Puducherry. The bones were macroscopically studied for vascular foramina with respect to its location, number, size, and shape. The data collected were statistically analyzed. The vascular foramina were present on dorsal, plantar, medial, and lateral surfaces of navicular bone. Kruskal-Wallis test followed by series of Mann-Whitney test for *post hoc* analysis showed the number of nutrient foramina observed on dorsal surface were significantly greater than those observed on the plantar (U=2,755, P=0.001), medial (U=43, P=0.001), and lateral (U=626.5, P=0.001) surfaces of the navicle. About 97.6% of foramina were circular and 2.5% were oval in appearance. About 96.7% of vascular foramina were <1 mm in size and 3.3% were ≥1 mm in size. Spearman's rank correlation coefficient done showed a strong, positive correlation between vascular foramina of <1 mm size and circular shape, which was statistically significant (r_s=0.981, P=0.001). We believe the present study has provided additional information on the vascular foramina of navicular bone and useful to surgeons in foot surgeries.

Key words: Navicular, Stress fractures, Vascular foramina

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Introduction

The navicular bone is one of the tarsal bones present on the medial side of the foot. The word navicular in Latin means a little ship [1]. It articulates with talus proximally and with the three cuneiforms distally. It also gives attachment to the tendon of tibialis posterior muscle [2]. The navicular bone derives its dorsal blood supply from a branch of dorsalis pedis artery and plantar blood supply from medial plantar branch of posterior tibial artery [3]. These branches enter the bone through the numerous vascular foramina present on the surface of the bone.

Navicular is the most frequently injured lesser tarsal bone and an important constituent in the transverse tarsal locking mechanism [1]. Acute navicular fractures are broadly classified into three types: avulsion fractures, tuberosity fractures, and body fractures [4]. Tuberosity and displaced body fractures are treated surgically while others are treated conservatively [5]. Navicular stress fracture is common among athletes and may have devastating consequences including avascular necrosis [6, 7].

Thus, the knowledge about the vascular foramina of the navicular bone becomes important for orthopaedic and vascular surgeons. An enhanced knowledge about the vascula-

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ORIGINAL ARTICLE

AN INSIGHT INTO THE CIRCLE OF WILLIS, ITS VARIATION IN THE FORMATION AND COMPONENT VESSELS IN THE ADULT HUMAN CADAVERS

Prasanna LC, MD[1*], Andrade LS, PhD[1], Ravichandra P, MD[1], Hosapatna M, MD[1]

ABSTRACT

Introduction: *Circle of Willis, is an arterial circle, furnishing arterial supply to most of the brain and meninges. It is formed by the branches of anterior cerebral, middle cerebral and posterior cerebral arteries. However, its formation and a complete arterial circle, differs among individuals.*
Objectives: *In the present study, an attempt has been made to identify those variations and to segregate them into anterior and posterior circulation.*
Methods: *Thirty-two cadaveric brains were used for the study. The circle of Willis was identified and painted for better visualisation and then photographed.*
Results: *The results show that the variations of the anterior circulation exceeded those observed in the posterior circulation.*
Conclusions: *These variations would add on to the previous findings and thus help the neurosurgeons while operating this region.*
Key words: *Anterior cerebral artery; basilar artery; brain, cadaver; circle of Willis; communicating artery; internal carotid; posterior cerebral*

INTRODUCTION

The optimum functioning of an individual at any given point of time is highly dependent on constant neural activity going on in the brain, which reflects the high metabolic activity and the amount of energy requirement. This enormous demand is met with through a profuse network of blood vessels called the circle of Willis/circulus arteriosus cerebri which serve to convey an uninterrupted supply of nourishment and oxygen to the brain (1).

The circle is formed by the terminal branches of internal carotid and vertebro-basilar arterial systems in the interpeduncular fossa at the base of the brain. Although a complete circular channel almost always exists, one vessel is usually sufficiently narrowed to reduce its role as a collateral route rendering the circle functionally incomplete (2,3). The cerebral and communicating arteries may be absent, hypoplastic, double or even triple (4,5).

Considering the complexity of the developmental pattern in arterial circle, arterial variations occur mostly because of absence of a vessel normally present or abnormal persistence of an embryonic stage of a vessel (6).

Keeping in view the surgical importance of this region, the present study was undertaken to observe the formation of the Circle of Willis, in terms of origin and branching pattern of the component vessels, to evaluate any variations in the component vessels, and to note the frequency of occurrence of different patterns of variant circles (in terms of anterior and posterior circulations). The study not only adds clinically significant unreported variations to the literature but also finds out the prevalence of these variations in the study population.

MATERIAL AND METHODS

Thirty-two cadaveric specimens were procured from the routine dissection done for the undergraduate students in the Department of Anatomy, Kasturba Medical College, MAHE, Karnataka, India. After cleaning and carefully removing the overlying meninges without damaging the vessels, the pattern of formation and the existing anatomical variations of the constituent vessels of the circulus arteriosus cerebri was noted. Each specimen was photographed using a digital camera with the variations noticed. The observations were carried out by a single person. Ethical clearance was obtained from the institutional ethical committee (IEC). The number of the ethical clearance is IEC 331/2012.

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STUDY ON THE CONVERSION OF LAPAROSCOPIC CHOLECYSTECTOMY OWING TO PER OPERATIVE COMPLICATIONS

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ABSTRACT

BACKGROUND

In the modern medical era, laparoscopic approach to surgical conditions has reached to a height that it is performed even for malignant conditions. The minimal access surgery which has grown up from minimally invasive surgery has given us the faith that nearly all surgeries can be done by laparoscopy. The first laparoscopic cholecystectomy was done by Prof. Dr. Med Erich Muhe of Germany in the year 1985. About ten lakh cholecystectomies are performed ever year, of which 96% are done by laparoscopic method. Laparoscopic cholecystectomy is the most common laparoscopic procedure. By number of studies and research works, it is stated that laparoscopic cholecystectomy is superior to conventional open method because of its advantages like smaller incisions, early recovery, less post-operative pain and hospital stay with better cosmesis. However, laparoscopic cholecystectomy also has got its own disadvantages and complications. In 1992, an NIH consensus conference held in Bethesda approved laparoscopic cholecystectomy as the treatment of choice for symptomatic cholelithiasis. Conversion to open technique is a major morbidity of laparoscopy, as it loses its supremacy over open technique once the conversion takes place. With growing experience of laparoscopic cholecystectomy and completion of the learning curve, the indications for laparoscopic cholecystectomy have been extended approaching that of open cholecystectomy. Complications of laparoscopic cholecystectomy have been minimised to as low as 2 - 6%. However, a substantial proportion of patients had to be converted to open surgery because of technical difficulties or intraoperative complications. Conversion rates of 2.6% to 14% had been described in different studies. The factor to be considered with conversion is that it should never be considered a complication, but rather a correct judgement by the surgeon.

The aims of the present study are:

1. To study the incidence of conversion of laparoscopic cholecystectomy.
2. To analyse the reasons for the conversion of laparoscopic cholecystectomy owing to per operative complications like-
   a) Arterial injury,
   b) Hepatobiliary tract injury,
   c) Dense adhesions,
   d) Aberrant anatomy,
   e) Technical issues.

MATERIALS AND METHODS

This prospective observational study was conducted in patients who underwent laparoscopic cholecystectomy from November 2016 to November 2017 in the Department of General Surgery, Karpagam Faculty of Medical Sciences and Research, Coimbatore. There were totally 98 patients, of which 36 were males and 62 were females. The study has been done after the patient's informed consent. All these patients were evaluated in a proper manner as given in the proforma and had been assessed pre-operatively and operated under perfect anaesthetic fitness. This study mainly focussed on the patients who had been converted to open method. They were analysed further regarding the reasons for conversion to open method due to per-operative complications.

RESULTS

From November 2016 - November 2017, a total of 98 patients had undergone laparoscopic cholecystectomy in the Department of General Surgery, Karpagam Faculty of Medical Sciences and Research, Coimbatore. Out of 98 patients who underwent laparoscopic cholecystectomy, 13 patients (13.26%) were converted to open cholecystectomy owing to per-operative complications. All patients underwent cholecystectomy as an elective case. Though some morbidity was there in terms of post-operative complications, which we are not analysing, no mortality was reported.

CONCLUSION

After analysing the results of our study, we conclude that the incidence of conversion of laparoscopic cholecystectomy is 13.26%. The reasons for conversion in descending order of frequency are: 1. Dense adhesions (5.1%), 2. Difficult anatomy around Calot's triangle (3.06%), 3. Arterial injury (3.06%), 4. Hepatobiliary injury (1.02%) and 5. Technical issues (1.02%).

KEY WORDS

Laparoscopic Cholecystectomy, Cholelithiasis, Per-Operative Complications, Bile Duct, Cystic Artery, Adhesions, Calot's Triangle.

HOW TO CITE THIS ARTICLE: Vishnuvarthan S, Suganya S. Study on the conversion of laparoscopic cholecystectomy owing to per operative complications. J. Evolution Med. Dent. Sci. 2018;7(38):4952-4956, DOI: 10.14260/jemds/2018/929

BACKGROUND

In the modern medical era, laparoscopic approach to surgical conditions have reached to a height that it is performed even for malignant conditions. The minimal access surgery, which has grown up from minimal invasive surgery has given us the faith that nearly all surgeries can be done by laparoscopy. The first laparoscopic cholecystectomy was done by Prof. Dr. Med Erich Muhe of Germany in the year 1985. About ten lakh cholecystectomies are performed ever year, of which 96% are done by laparoscopic method. Laparoscopic cholecystectomy

A CADAVERIC STUDY OF THE MYOCARDIAL BRIDGES

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ABSTRACT

BACKGROUND

Myocardial bridging is a congenital anomaly, where a segment of an epicardial coronary artery is undercover of myocardial fibres for a variable distance and the underlying arterial segment is described as a mural coronary artery or a tunnelled artery. The history of myocardial bridges dates back to 1737 when it was first recognized by Reyman and then by Black in 1805. The first detailed post mortem examination of myocardial bridges was done by Geiringer in 1951 and the first radiological description was given by Portmann and Iwig in 1960. The bridged segment of the coronary artery is susceptible to various pathophysiological mechanisms leading to myocardial ischemia, especially when associated with other cardiovascular diseases. The myocardial bridges were initially thought to be innocent bystanders in the development of acute coronary syndromes. But in the last two decades, the outlook has changed with the increasing incidence of sudden cardiac deaths in young athletes, where myocardial bridges were the only finding at autopsy. The mechanism by which the myocardial bridges induce clinical symptoms is uncertain, but among the proposed are vasospasm, thrombus formation, endothelial dysfunction and impaired coronary flow reserve. Most important of these is the dynamic systolic compression of the tunnelled artery with sustained early diastolic diameter reduction.

The aim of this study is to provide more information on the incidence of myocardial bridges, arterial preponderance for myocardial bridging, the length of the bridged segment and multiple bridging.

MATERIALS AND METHODS

A descriptive study of fifty formalin fixed hearts from the department of anatomy were dissected by conventional dissection methods as guided by Cunningham.

RESULTS

Myocardial bridges were found in 18 (36%) hearts with a total of about 28 bridges. Most common artery involved was the anterior inter ventricular artery (66.6%), which even had multiple bridges over it. The mean length of the bridges was 26.54 mm.

CONCLUSION

From the present study, we infer that the presence of myocardial bridges in an unsuspected population is relatively higher. The definitive role of these bridges in acute coronary syndromes is yet uncertain. But owing to its higher rate of detection in those with myocardial ischemia, it is better to screen for the bridges along with other aetiological factors and treat the patients prophylactically. Of the imaging modalities, CT Coronary Angiography is found to be the investigation of choice. In the near future, holistic studies involving cadaveric, radiologic and clinical data are mandatory in researching the myocardial bridges.

KEY WORDS

Myocardial Bridges, Myocardial Ischemia, Atherosclerosis, Tunnelled Artery, Systolic Compression.

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BACKGROUND

Myocardial bridging is a congenital anomaly, in which an epicardial coronary artery lies in the myocardium for part of its course. The bridged segment of the coronary artery is susceptible to various pathophysiological mechanisms leading to myocardial ischemia, especially when associated with other cardio vascular diseases. Recently, myocardial bridges have become a main topic of interest owing to its probable causation of sudden cardiac death in young athletes.

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The aim of this study is to provide more information on the arteries with myocardial bridges. Of the fifty formalin fixed hearts dissected, myocardial bridges were found in 18 (36%) hearts with a total of about 28 bridges. Most common artery involved was the anterior inter ventricular artery, which even had multiple bridges over a single artery. The mean length of the bridges was 26.54 mm.

Myocardial bridging is a congenital anomaly, where a segment of an epicardial coronary artery is undercover of myocardial fibres for a variable distance and the underlying arterial segment is described as a mural coronary artery or a tunnelled artery.[1] (Fig. 1).

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RESEARCH ARTICLE

MORPHOLOGICAL STUDY OF CORONARY OSTIA

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Manuscript Info

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Abstract

Objective: To study the normal and variant anatomy of the coronary artery ostia in south Indian population.

Introduction: Sudden cardiac deaths, especially in young are on the rise in recent days. Of all causes for sudden cardiac death, anomalous origins of coronary arteries also comprise a significant percentage. A cadaveric study of the origin of coronary arteries in an unsuspected population in south India paves way for understanding the normal variants & to determine the prevalence of anomalies and the need for screening for such anomalies.

Methods: Fifty adult heart specimens of unknown age and sex were dissected. The number of ostia and their positions within the respective sinuses were observed. Vertical and circumferential deviations of the ostia were observed.

Results: Of all hearts,the coronary arteries arose from the aortic sinuses in 99 hearts; no openings were present in the pulmonary artery or the non-coronary sinus. In one heart, the left coronary artery was absent, wherein the anterior inter ventricular artery and the left circumflex artery had independent origins from the aortic sinuses.The number of openings in the aortic sinuses varied from 2-3 in the present study; multiple ostia were seen in the right posterior sinus. The majority of the ostia lay below the sinutubular ridge (89% for RCA & 92% for LCA) and at or above the level of the upper margin of the cusps (84%). Left ostial openings were mainly centrally located (80%), whereas the right coronary ostia were often shifted towards the right posterior aortic sinus (59%).

Discussion: The usual location of the ostia was within the sinus and above the cusps, but below the sinutubular ridge. On occasion, normal variants like multiple ostia, vertical or circumferential shift in the position, and slit-like ostia may create confusion in interpreting the images and pose a difficulty during procedures like angiography, angioplasty, and coronary artery bypass surgery.

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Original Research Article

A Cadaveric Study on Coronary Dominance in South Indian Population

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ABSTRACT

Background: Adequate insight about coronary dominance is important to understand coronary artery diseases, interpret the radiological images and to devise a proper treatment protocol in cardiovascular diseases. It determines the amount and the area of myocardium being perfused by the left or right coronary arteries.
Aim: The aim of the present study is to observe the origin of posterior interventricular artery which determines the coronary dominance.
Materials and Methods: The study was done on 50 formalin fixed adult heart specimens in the Institute of Anatomy, Madras Medical College irrespective of age, sex, socio-economic status, religion and education status. The coronary arteries were examined by gross dissection and analyzed statistically.
Results: Right dominance was seen in 26(52%) hearts, left dominance in 10 (20%) hearts and balanced or codominance was seen in 14(28%) hearts.
Discussion: The coronary artery dominance has an important clinical & surgical significance. Most of the studies have reported a higher percentage of right dominance including the present study.
Conclusion: The present study on coronary dominance would be of assistance to interventional cardiologist and cardiothoracic surgeons to plan ahead during angioplasty & coronary artery bypass surgeries. Coronary dominance is also considered as an indicator in the incidence of myocardial infarction & also used as a prognostic factor during recovery.

Key Words: codominance, coronary artery, coronary diseases, coronary dominance, coronary preponderance

INTRODUCTION

Coronary artery disease is one of the leading causes of mortality and morbidity across the world. Coronary artery disease is one of the major causes of death in developed countries. The incidence of coronary artery disease is increasing today in developing countries too, because of a sedentary life style, binge eating of junk food, reduced physical activity and stress. Recent trends show a rise in coronary artery associated death in young people. It is seen significantly both in males and females irrespective of socio economic status [1]. In the young, the primary cause of death is non atherosclerotic coronary abnormalities [1]. Dominance pattern of the heart has got important clinical significance. Left dominance was found to have significantly higher mortality than right dominance and codominance [2]. Dominance also plays important role in inferior wall infarcts of the heart. Inferior wall infarcts although less extensive than anterior infarcts, are more important as they can cause various degrees of atrioventricular blocks in approximately 30% of cases. The dominanat right coronary artery usually supplies atrioventricular node. Therefore an inferior wall infarcts caused by occlusion of right coronary artery will have higher risk of AV blocks [3].

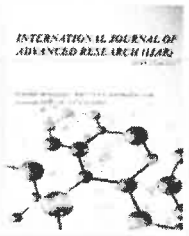
The arterial supply to the heart is by two coronary arteries: Right coronary artery (RCA) and left coronary (LCA). There is a

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RESEARCH ARTICLE

ACCESSORY HEAD OF CORACOBRACHIALIS AND ITS CLINICAL USES.

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Key words:-
Coracobrachialis, Struther's ligaments, median nerve, brachial artery.

Abstract

Coracobrachialis is the most medial of three anterior compartment muscles of arm. In some mammals it is tricipital in origin. The lower head is usually suppressed in man. Sometimes it is represented by a fibrous band, the ligament of Struther's. During our dissection regime of I[st] MBBS students in the department of anatomy, BJMC, we observed an accessory head of coracobrachialis on the right side of a male embalmed donated cadaver .The muscle was described as being functionally unimportant. However, the muscle has recently attracted interest with its potential use for contouring the infraclavicular area or covering the exposed axillary vessels specifically in postmastectomy reconstructive operations, muscle transfer for the treatment of paralysis, as a guide for axillary artery access and anaesthetic blockade of the brachial plexus.

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Introduction:-

Coracobrachialis is the most medial of three anterior compartment muscles of arm.

It arises from the tip of coracoid process in common with the short head of biceps brachii and inserted by fleshy fibres to the medial border of the middle of the shaft of humerus where the nutrient foramen of the bone is usually located. The muscle is supplied by musculocutaneous nerve, a branch from the lateral cord. It is said that the nerve pierces the muscle and supplies it before piercing. It is a weak flexor of shoulder joint. Coracocobrachialis represents the adductor muscle of arm but such action is insignificant in man. In some mammals it is tricipital in origin. Upper 2 heads are fused to take origin from coracoid process and enclose the musculocutaneous nerve between them. The lower head is usually suppressed in man. Sometimes it is represented by a fibrous band, the ligament of struthers , which extends from an occasional bony projection, the supratrochlear spur, from the anteromedial surface of lower part of the humerus to the medial epicondyle. Median nerve or brachial artery may pass beneath the ligament which compress upon them producing vascular spasm or median nerve palsy[2]. The muscle was described as being functionally unimportant [3].

However, the muscle has recently attracted interest with its potential use for contouring the infraclavicular area[4,7] or covering the exposed axillary vessels specifically in postmastectomy reconstructive operations[5], muscle transfer for the treatment of paralysis, as a guide for axillary artery access and anaesthetic blockade of the brachial plexus[4,6].

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Occipitalization of Atlas -

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Abstract:

Occipitalization of atlas is an osseous anomaly of the cranio-vertebral junction which occurs at the base of the skull in the region of the foramen magnum due to failure of segmentation and separation of the most caudal occipital sclerotome and the first cervical sclerotome during the first few weeks of foetal life. Atlas is the first cervical vertebra and the atlanto-occipital joint belongs to ellipsoid variety of synovial joints. It does not have a body like other vertebrae. Rarely, it gets occipitalized, where the lateral masses of the atlas vertebra fuse with the condyles of the occipital bone. One skull showing Occipitalization of Atlas was observed in the Department of Anatomy, B.J. Medical College, Ahmedabad, Gujarat. The knowledge of such a fusion may be of importance for radiologists, anaesthesiologists, orthopaedic and neurosurgeons because skeletal abnormalities at the cranio-cervical junction may result in sudden unexpected death. It can result in dysphagia, dysarthria or torticollis because of compression of cranial nerves. They should be aware that such an anomaly may exist without any typical symptomatic presentation, and thus, serious consequences of upper cervical spinal manipulative therapy may arise when a complete and adequate clinical assessment is missed.

Key words: Atlanto-Occipital joint, Atlas, Occipital bone, Occipitalization.

Introduction:

Atlas is the first cervical vertebra. It is ring like structure, consisting of anterior and posterior arches including two lateral masses along with the transverse processes. Two lateral masses bear a kidney shaped superior articular facets which articulate with occipital condyles to form atlanto-occipital joint of ellipsoid variety. Inferior articular facets of these lateral masses are almost circular and flat. It is oriented more obliquely to the transverse plane than the superior articular facet and faces more medially. Inferior articular facets of atlas form lateral atlanto-axial plane joint[1]. The anterior arch which forms anterior 2/5 of ring is slightly convex anteriorly and bears anterior tubercle. The anterior longitudinal ligament and longus colli muscles are attached to anterior tubercle. Its upper border provides attachment to the anterior atlanto-occipital membrane which often gets ossified. The posterior surface of the anterior arch bears concave circular facet for dens[1]. This condition was initially described by Rokitansky in 1844 and was demonstrated radiologically by Schuller in 1911 [2]. According to Yochum and Rowe[8] occipitalization

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Anatomical study of right coronary artery and its variations – Cadaveric study

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Abstract

Introduction: Knowledge of the normal and variant anatomy and anomalies of coronary circulation are vital component in the management of coronary artery disease. Any coronary artery disorder may reduce the blood supply to the heart and may lead to Myocardial infarction and death.

The aim of the study was to identify right coronary artery branching pattern and its variations. This may indirectly affect the patient's prognosis. This study describes the presence of normal arterial pattern and variations of right coronary artery in fifty heart specimens.

Materials and Method: This study was carried out on fifty formalin fixed adult human cadaveric heart specimens of both sexes. Visceral pericardium and subepicardial fats were removed. The right coronary artery and its branches were carefully dissected and followed till their termination.

Results: The right coronary artery was found to be arising from pulmonary trunk in three heart specimens in our study. Third coronary artery was found in one specimen. Sinu atrial nodal artery was arising from right coronary from right coronary artery in 94% and from left coronary artery in 6% Right dominance was observed in 35(70%) specimens. Fistula between right coronary artery and pulmonary artery was found in one specimen.

Conclusion: Branching pattern of right coronary artery is important for interpretation of coronary angiography and surgical revascularization of myocardium.

Keywords: Coronary artery, Sinu – atrial nodal artery, Fistula, pulmonary artery

Introduction

Coronary arteries represent the only supply to the myocardium. The two main coronary arteries, right and left coronary arteries arise from ascending aorta. Right coronary artery arises from right anterior aortic sinus and left coronary artery arises from left posterior aortic sinus. Patency of left coronary artery is vital for sufficient perfusion of the heart. The left coronary artery is responsible for irrigation, not only of most of the left ventricle, but also considerable portion of right ventricle.[1]

Right coronary artery runs between pulmonary trunk and right auricle and then runs downwards and is divided into two segments. First segment runs along the right border of the heart up to inferior border of heart. It gives right conus artery and supplies infundibulam of the right ventricle. If the right conus artery arises separately from the aortic sinus, then it is called as Third coronary artery. The second segment runs up to the apex as right marginal artery and supplies right ventricle. Sometimes it may runs in the posterior interventricular groove and may give rise to Posterior interventricular branch. Hence according to the origin of posterior interventricular artery, the coronary artery dominance has been considered. Right coronary artery ends in the crux by anastomosing with circumflex artery. It supplies the right atrium, right ventricle, atrioventricular septum and part of left ventricle.

Sinu atrial nodal artery may arise as atrial rami from initial segment of right coronary artery in 65% of the cases and from circumflex artery in 35% of the cases. Atrioventricular nodal artery arises as first septal ramus of posterior interventricular artery in 90% of the cases and 10% from the left coronary artery.[2]

Numerous studies on the variations of the arteries have been reported, but still it is better to study them further with respect to their clinical significance.

Coronary anomalies are a poorly understood topic in modern cardiology. Clinicians should be aware of such anomalies because some of them may produce sudden death.

The incidence of congenital coronary artery anomalies is 5–6%. By definition, the term anomalous or abnormal is used to define any variant form observed in less than 1% of the general population.[3]

The aim of the study is to identify the normal pattern of the coronary arteries and its variations. Though the modern techniques of revascularisation and coronary bypass surgeries are available, the knowledge about the anatomy of coronary arterial pattern and its variations are important for the cardiologists and cardio – thoracic surgeons.

Materials and Method

Fifty formalin fixed heart specimens of both sexes with intact coronary arterial pattern were studied in Anatomy department, PSG IMS & R, Coimbatore. The right coronary artery and its branches were carefully dissected out till their termination and were analysed for normal and abnormal patterns of coronary arteries.

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Original Article

Anatomy Section

Anatomical Study of Left Coronary Artery and its Variations–Cadaveric Study

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ABSTRACT

Introduction: Variations of the anatomy of the Coronary arteries are quiet common and have been reported previously in the literature. Right and Left coronary arteries which supply blood to the heart originates from the ascending aorta. Any coronary artery disorder may have serious implications by reducing the blood supply to heart leading to myocardial infarction and death.

Aim: To identify left coronary artery branching pattern and its variations.

Materials and Methods: Fifty formalin fixed wet specimens of heart of both sexes with intact coronary arterial pattern were collected and studied. The left coronary artery and its branches were carefully dissected out and were analysed for variable pattern in each specimen.

Results: Left coronary artery (LCA) was found to be hypoplastic in six (12%) specimens. In one specimen (2%), hypoplastic LCA was crossed by a vein and had communication with the vein. Trifurcation of LCA was seen in 17 (34%) specimens, Circumflex artery was short in one (2%) specimen. Kugel's artery was observed in three (6%) specimens. Median artery was found in seven specimens (14%). The left coronary artery and circumflex artery were absent in one (2%) specimen. In case where left coronary artery was absent, left anterior descending artery and circumflex artery were taking origin directly from left anterior aortic sinus. Left anterior descending artery showed variation in the form of short course (2 cm), discontinuous and embedded within myocardial tissue in 2% of cases each. However the circumflex artery also showed short course in one (2%) case.

Conclusion: The variations of the left coronary artery observed in the study are important for interpretation of coronary angiography, surgical revascularization of myocardium and embryological interpretations.

Keywords: Angiography, Circumflex artery, Kugel's artery, Left Anterior descending artery

INTRODUCTION

Left coronary artery (LCA) originates from left posterior aortic sinus. Patency of left coronary artery is vital for sufficient perfusion of the heart. The left coronary artery supplies most of the left ventricle and considerable portion of right ventricle [1].

LCA bifurcates into left anterior descending and circumflex artery and passes between pulmonary trunk and left auricle. The left anterior descending artery (LAD) runs in the left anterior interventricular groove and ends at the apex or may run up to crux of the heart [2]. Circumflex artery (CX) then runs in the posterior atrioventricular groove and ends at the crux of heart by anastomosing with right coronary artery (RCA) or may end before the crux. Sometimes posterior interventricular artery may arise from circumflex artery and may run in the posterior interventricular groove as left coronary dominance [2].

Numerous studies on the variations of the arteries have been reported [3,4]. Coronary artery (CA) variations is a topic of interest in modern cardiology. Cardiologists, cardiothoracic surgeons and radiologists should be aware of such anomalies because some of them may produce sudden death. Such rare coronary anomalies are poorly understood thus a detailed variation in them will add onto the existing clinical knowledge. The incidence of congenital coronary artery anomalies is 5–6%. By definition, the term anomalous or abnormal is used to define any variant form observed in less than 1% of the general population [3]. The incidence of all coronary anomalies is 0.23% in autopsy series and ranges between 0.3 to 5.6% in angiographic series [4]. The recent introduction of selective coronary angiography provides information about the normal coronary arterial pattern and also its variations [3].

The frequency of various anomalies of left coronary artery and the arterial dominance of the heart were observed in current study.

Numerous studies [3,4] on the variations of the arteries have been reported, but still it is better to study them further with respect to their clinical significance. The aim of the study was to identify the normal arterial branching pattern of left coronary artery and its variations in fifty heart specimens.

MATERIALS AND METHODS

The Cross sectional observational study was conducted from September 2016 to November 2016. The study was carried out after obtaining ethical clearance from Institutional ethical committee. Fifty formalin fixed heart specimens of both sexes with intact coronary arterial pattern were studied. Specimens with damaged arterial pattern were excluded. The left coronary artery and their branches were carefully dissected out till their termination and were analysed for variable patterns.

Length of the arteries were measured with the help of thread and Vernier caliper from the point of origin of the arteries to the point of termination. The course of artery was delineated and thread was passed around the vessel. Then thread with mark was measured using the Vernier caliper. Percentage incidence of various patterns was recorded and tabulated.

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Original Research Article

AN OSTEOLOGICAL STUDY OF FORAMEN TRANSVERSARIUM OF CERVICAL VERTEBRAE AND ITS CLINICAL SIGNIFICANCE

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ABSTRACT

Introduction: Seven cervical vertebrae constitute the skeletal framework of the neck. Along with the other identification points, the cardinal feature of a cervical vertebra whether typical or atypical is the presence of Foramen transversarium on the transverse process. This foramen transmits the Vertebral artery, Vertebral vein and Sympathetic nerves. These foramina are known to have variations in size, shape and numbers and may be absent, incomplete or duplicate, which may lead to various symptoms. Their etiology is related with the variations in the course of the Vertebral artery due to developmental reasons.

Materials and methods: Three hundred dry human cervical vertebrae were collected and studied in the department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore. We included all the Cervical vertebrae including Atlas, Axis and all other cervical vertebrae. We observed the presence, size, shape and variations of Foramen transversarium of cervical vertebrae during our study.

Observations and results: Out of three hundred Cervical vertebrae, One hundred and twenty five cervical vertebrae (42%) had variations of Foramen transversarium like Complete / incomplete Accessory foramen, triple foramen on one side, absence of foramen on one side and unequal size of foramen on both the sides. The different types of shapes of foramen transversarium were also analysed and tabulated. The areas of foramen transversarium were measured after measuring all the diameters.

Conclusion: Variations in the Foramen transversarium may indicate, the variation in the course of the Vertebral artery. The present study was important clinical implications for Head and neck and vascular surgeries. These variations should be kept in our mind during surgeries on cervical spine.

KEY WORDS: Foramen transversarium, shape, cervical vertebrae, accessory, absent and variations.

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Original Article

Anatomy Section

Anatomical Study of Lobes and Fissures of Lungs and its Clinical Significance-A Cadaveric Study

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ABSTRACT

Introduction: The human lungs are divided by fissures into lobes which permit movements of lobes to one another. The fissures act as barriers to avoid the spread of the diseases. Knowledge of variations in the lobes and fissures of the lungs is important for the Radiologists to interpret the radiological images and for the cardiothoracic surgeons to plan for segmental resection or lobectomy.

Aim: To study the patterns of fissures and lobes of the lungs and their variations and to find their clinical implications and to compare with the previous studies.

Materials and Methods: Forty pairs of lungs were used for the study. They were obtained from formalin fixed cadavers. The patterns of lobes, fissures and hilar anatomy of lungs and its variations were noted and specimens were photographed.

Results: In the present study, incomplete horizontal fissure was found to be in 23(57.5%) right lung specimens and incomplete oblique fissure was observed in 20(50%) left lung specimens. Absence of horizontal fissure was about 10(25%) and oblique fissure was about six (15%) in right lung specimens. Absence of oblique fissure was about four (10%) in left lung specimens. According to Craig and Walker classification, Grade 1 and Grade 3 fissures were found to be more in both the lung specimens. Accessory fissure was observed in 5 (12.5%) specimens in the right lung and 10 (25%) specimens in the left lung. Single lobe was observed in both the lungs, 7.5% in the right lung and 5% in the left lung.

Conclusion: Variations of fissures and lobes and of hilar anatomy is of great significance. Knowledge of these variations is important for Radiologists and Cardiothoracic surgeons for interpreting the data and to plan for the surgeries.

Keywords: Accessory fissure, Hilar anatomy, Lobectomy

INTRODUCTION

The lungs are a pair of essential organs of respiration located within the thoracic cavity, on either side of heart and other mediastinal contents. Each lung is half – conical in shape, divided into lobes by fissures. Anatomically, left lung is divided into upper and lower lobes by oblique fissure whereas right lung is divided into upper, middle, lower lobes by oblique and horizontal fissures. In each lung, the oblique fissure begins from mediastinal surface above and behind the hilum and cuts the posterior border of the lung about 2.5 cm lateral to the junction of the T3 &T4 spine. Then, it runs along the costal surface, cuts the inferior border of the lung and will reappear on the mediastinal surface and ends at the lower end of the hilum. The horizontal fissure begins at the oblique fissure, courses along the costal surface, cuts the anterior border and appears to end at the hilum [1].

Finding of an accessory fissure in lung specimens is not uncommon but appreciating them on radiographs and Computed Tomography (CT) scans is difficult and hence, they are not appreciated as distinct entities or are completely misinterpreted. They usually occur at the boundaries between bronchopulmonary segments [2]. The commonly found accessory fissures are superior accessory fissure, inferior accessory fissure and left minor accessory fissure. The superior accessory fissure separates superior segment from the rest of the segments of lower lobe of lung, the inferior accessory fissure separates small infracardiac lobe from other segments of lower lobe of lung on the diaphragmatic surface and the left minor fissure separates the lingula from the other segments of upper lobe of left lung [2].

The fissures may vary in the degree of completeness and tend to divide the lobe into smaller divisions. Complete fissures have continuity of lobes at their bottom. The adjacent lobes are connected by a small portion of pulmonary tissues because of clefts it fails to reach the hilum in the incomplete fissures. The fissure may be absent altogether. The fissures are important for uniform expansion of lobes. They allow movements of lobes in relation to each other. This is more involved in the lower lobes at the time of breathing [3].

The appearance of accessory lung fissure may be seen as in relation to its completeness or its depth and that varies with X–ray and CT scan [4].

Knowledge of classical accessory lobes and fissures is necessary for radiological interpretation. This gives proper guidance to Cardio thoracic surgeons in performing segmental lung resection and lobectomies to have an uncomplicated perioperative outcome.

To assess the patterns of lobes and fissures of lung and to find out any variations in the fissures and lobes and hilar structures of the lungs in human cadavers and to find their clinical significance and to compare with them with the previous studies.

MATERIALS AND METHODS

Forty pairs of lung specimens (32 lung specimens from male cadavers and 8 lung specimens from female cadavers) free from pathological lesions, removed at the time of teaching for first year of MBBS from the 10% formalin fixed human cadavers from the Department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore were included in the study. The study was conducted in the month of November 2016 to April 2017 after Ethical clearance (Proposal Number.16/291). The lung specimens were observed and were photographed for:

1. The patterns of lobes and fissures;
2. Variations in fissures, complete or incomplete;
3. Accessory fissure if any;
4. Hilar structures and its variations.

The anatomical classification proposed by Walker WS et al., was followed to determine the presence of completeness of fissures [5].

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Anatomical Study of Profunda Femoris Artery and it's Variations – Cadaveric Study

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Research Article
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Abstract

Background and Aims: Profunda femoris artery is the largest branch of femoral artery. It is the principal supply to the muscles of the thigh as well as head and neck of femur. Its branches form anastomosis around the head of the femur. Profunda femoris artery is also used for arteriography. It is frequently used in vascular reconstructive procedures in the proximal thigh. It forms main route of collateral circulation in occlusion of femoral artery. The study of variation of Profunda femoris artery is of great value for radiologists and surgeons during diagnostic and surgical intervention.

Aim of the study is to observe the origin of Profunda femoris artery, to measure the distance between midinguinal point and site of origin of Profunda femoris artery and to study the anatomy of Profunda femoris artery and its branches and to find out any variations in its course and its branches.

Materials and Methods: The study was conducted as a prospective study from April 2018 to October 2018. All the lower limbs with the intact Femoral artery and Profunda femoris artery and their branches were included. Femoral artery and its branches had got cut during dissection were not included in the study. Thirty two Profunda femoris arteries (20 on the right side and 12 on the left side) were studied in the department of Anatomy. The femoral triangle was exposed by making incisions along the inguinal ligament from pubic symphysis to anterior superior iliac spine. Midpoint was taken as Midinguinal point (MIP). MIP was marked with a coloured pen. The distance between MIP and the site of origin of

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Morphometric Study of Jugular Foramen and Jugular Fossa of Dried Adult Human Skulls and Its Clinical Significance

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Abstract

Introduction Jugular foramen is one of the most fascinating foramina of the human skull. It is a complex, irregular bony canal located between the occipital bone and petrous part of the temporal bone. Many important structures, like 9th, 10th, 11th cranial nerves, meningeal branch of occipital and ascending pharyngeal arteries, internal jugular vein, and inferior petrosal sinus, are passing through it. The jugular fossa has a septum and a dome. The septum divides the foramen into two compartments: anteromedial compartment (pars nervosa) and posterolateral compartment (pars vascularis). The dome contains superior bulb of internal jugular vein. The architecture of the foramen varies in size, shape, and laterality besides differences related to sex and race. The morphometric measurements of jugular foramen are very important for neurosurgeries and head and neck surgeries.

Objectives The aim of the present study is to study the morphology of jugular foramen along with its dimensions, compartments, presence of partial or complete septa and dome.

Materials and Methods A total number of 60 jugular foramina were examined from 30 adult dry human skulls of unknown age and sex from the Department of Anatomy, PSG Institute of Medical Sciences and Research, Coimbatore. Measurements were taken using Digital Vernier calipers. Results were analyzed statistically.

Results The length, width, and surface area of jugular foramen of right side were measured and compared with the left side. Length and width of the jugular foramen was significantly higher on the right side. The presence of partial septum was found in 27 skulls (90%) on the right side and 29 skulls (99.7%) on the left side, respectively. Dome was present in 100% of the jugular foramina on the right side and 90% of the jugular foramina on the left side. Separate opening for inferior petrosal sinus was found in eight skulls (27%) on the right side and four skulls (13%) on the left side.

Conclusion This study provides a clear understanding of anatomy of jugular foramen and supports the reported morphometric variations. The morphometric variations of jugular foramen in the parameters of the skull are probably due to ethnic and racial factors. Knowledge of these variations is important for neurosurgeons and radiologists who deal with space occupying lesions of the structures surrounding jugular foramen. This study may be helpful for ENT surgeons while performing middle ear surgeries.

Keywords
- jugular foramen
- internal jugular vein
- dome
- septum
- cranial nerves

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ORIGINAL ARTICLE

Electrocardiographic Changes in COVID-19 Patients: A Hospital-based Descriptive Study

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Abstract

Background: Coronavirus disease-2019 (COVID-19) infection is a multisystem disease not restricted to the lungs. It has a negative impact on the cardiovascular system by causing myocardial damage, vascular inflammation, plaque instability, and myocardial infarction. The presence of myocardial injury is a poor prognostic sign. Electrocardiogram (ECG), a simple bedside diagnostic test with high prognostic value, can be employed to assess early cardiovascular involvement in such patients. Various abnormalities in ECG like ST-T changes, arrhythmia, and conduction defects have been reported in COVID-19. We aimed to find out the ECG abnormalities of COVID-19 patients.

Methods: We performed a cross-sectional, hospital-based descriptive study among 315 COVID-19 in-patients who underwent ECG recording on admission. Patients' clinical profiles were noted from their records, and the ECG abnormalities were studied.

Results: Among the abnormal ECGs 255 (81%), rhythm abnormalities were seen in 9 patients (2.9%), rate abnormalities in 115 patients (36.5%), and prolonged PR interval in 2.9%. Short QRS complex was seen in 8.3%. QT interval was prolonged in 8.3% of the patients. Significant changes in the ST and T segments (42.9%) were observed. In logistic regression analysis, ischemic changes in ECG were associated with systemic hypertension and respiratory failure.

Conclusion: In our study, COVID-19 patients had ischemic changes, rate, rhythm abnormalities, and conduction defects in their ECG. With this ongoing pandemic of COVID-19 and limited health resources, ECG-a simple bedside noninvasive tool is highly beneficial and helps in the early diagnosis and management of cardiac injury.

Keywords: Coronavirus disease-2019, Electrocardiogram change, Rate abnormalities, ST-T changes.

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Introduction

A cluster of pneumonia cases were reported due to "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) at the end of 2019 in the city of Wuhan, in the Hubei Province of China. Soon coronavirus disease-2019 (COVID-19) was declared as a pandemic owing to its rapid spread across the countries.[1] Initially regarded as a respiratory infection, COVID-19 is now known to affect all major systems in the body. Quite a lot is discussed in literature last year about COVID-19 and its effect on lungs and systemic response. However, very little is debated about cardiovascular involvement in COVID. It has been observed that lung involvement is more severe in patients with preexisting cardiac involvement. However, in sharp contrast new-onset cardiac involvement is also noted in a few patients and few patients do present with cardiac symptoms alone without lung involvement.[2] The spectrum of presentation is wide-ranging from patients having no cardiac disease at all, asymptomatic but with elevated cardiac markers, having symptoms of overt cardiac disease such as angina, cardiogenic shock, heart failure, cardiac arrhythmias, and sudden cardiac death.

Arrhythmia and acute cardiac injury were reported in 16.7 and 7.2% of the COVID patients.[3] In addition to the systemic inflammatory response, the physiological mechanisms identified to cause cardiac involvement in COVID-19 patients are hypoxemia-related myocardial cell injury and endothelial cell damage due to upregulated expression of angiotensin-converting enzyme 2 (ACE 2) in the heart and lungs.[4]

The electrocardiogram (ECG) changes reflect cardiac involvement with diverse manifestations. Arrhythmia and conduction defects are found to be more prevalent among SARS-CoV-2-infected individuals.[5] Myocardial ischemia, myocarditis, shock, hypoxia, and electrolyte abnormalities were the factors identified to cause arrhythmias.[6] The presence of cardiac involvement may imply poor prognosis and an adverse outcome.[7] Therefore, it is pertinent to assess and monitor the cardiac abnormalities paving way for a prompt action. ECG, a simple bedside diagnostic test with high prognostic value, can be employed to assess cardiovascular involvement in COVID-19 patients. We aimed to find out the ECG abnormalities of patients with SARS-CoV-2 infection.

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Deepalakshmi Kaliyaperumal*, Karthikeyan RK, Murali Alagesan and Sudha Ramalingam

Characterization of cardiac autonomic function in COVID-19 using heart rate variability: a hospital based preliminary observational study

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Abstract

Objectives: The novel corona virus disease, which was initially reported in China in late 2019, has become a global pandemic affecting 330 million cases. COVID-19 affects predominantly the respiratory system, in addition to other organ systems, mainly the cardiovascular system. One of the hypotheses is that virus entering the target cells by binding to angiotensin converting enzyme 2 affecting hypothalamic pituitary axis could lead to dysautonomia which is measured by heart rate variability (HRV). HRV is a non-invasive measure of autonomic function that facilitates identification of COVID-19 patients at the risk of developing cardiovascular complications. So, we aimed to assess HRV in COVID patients and compare between COVID patients and normal controls.

Methods: In a case control design, we compared 63 COVID-19 infected patients with 43 healthy controls matched for age and gender. Along with clinical characterization, heart rate variability was evaluated using ambulatory 5 min ECG in lead II and expressed in frequency and time domain measures. Statistical analysis was performed using SPSS 17.0.

Results: Mean age of the study population was 49.1 ± 14.2 years and 71 (66.9%) were males. Frequency domain measures high (HF) and low (LF) frequency powers were significantly decreased in COVID-19 patients compared to controls. HF/LF and LF/HF ratios were not different between groups. Time domain measures rMSSD (root mean square of successive RR interval differences) and SDNN (standard deviation of NN intervals) were significantly increased among COVID-19 subjects. COVID-19 infection was associated with increased parasympathetic activity as defined by rMSSD>40 {adjusted odds ratio 7.609 (95% CI 1.61–35.94); p=0.01} and SDNN>60 {adjusted odds ratio 2.620 (95% CI 1.070–6.44); p=0.035} after adjusting for age, gender and comorbidities.

Conclusions: Our study results showed increased parasympathetic tone in COVID patients. Early diagnosis of autonomic imbalance in COVID patients is needed to plan management and limit progression of disease.

Keywords: heart failure; pulmonary hypertension; right heart.

Introduction

In December 2019, a novel coronavirus disease (nCoV) epidemic emerged in Wuhan City, Hubei Province, China. Owing to its high transmissibility and infectivity rate it spread across the world and WHO declared it as a pandemic on March 11, 2020 [1, 2]. As this virus predominantly affects the respiratory system it has been renamed as severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) and the disease caused by this virus has been named as corona virus disease 19 (COVID-19) [3].

COVID-19 shows wide range of clinical presentations ranging from asymptomatic/mild symptoms (fever, cough, dyspnea, myalgia, fatigue, anosmia, dysgeusia and diarrhea) to severe illness like acute respiratory distress syndrome (ARDS), arterial and venous thrombosis, myocarditis and varieties of neurological manifestations [4–7]. A report from WHO says that "80% of infections are mild or asymptomatic, 15% are severe infections and 5% are critical infections" [8]. Co-morbid conditions like obesity, type 2 diabetes mellitus, coronary artery disease, chronic liver disease, chronic kidney disease and chronic respiratory disease are known to be risk factors for developing severe disease [9–11]. Wide spectrum of manifestations is explained by SARS CoV-2 having target cells in multiple organs and systems like respiratory tract including lung parenchyma,

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Association between Quality of Sleep and Academic Performance in Medical Students: A Preliminary Study

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Abstract

Background: Medical students are more prone to daytime sleepiness, sleep deprivation and irregular sleep schedules, as they have less free time, longer courses and working hours compared to other professional students.

Aim: To assess the quality of sleep and to find out the association between sleep quality and academic performance among medical students.

Methodology: This cross sectional study was conducted among 176 medical students. They were subjected to Pittsburgh Sleep Quality Index (PSQI) questionnaire to assess their sleep quality over a one-month period. A PSQI global score of 5 and greater than 5 was considered indicative of poor sleep. Academic performance was assessed from their internal assessment scores conducted during that period. The data was analyzed using the unpaired students 't' test and Pearson's correlation test

Results: Our study included 176 students from all the phases of MBBS curriculum. 24 percentage students had poor quality of sleep. In our study, there was no statistically significant difference in the performance of good sleepers (57.94 ±18.06) and poor sleepers (58.80 ±17.37) with a p value of 0.785. Our study results showed a negative correlation between global PSQI scores and assessment scores of the students ($r = 0.57$ and $P = 0.449$).

Conclusion: In our study association between sleep quality and academic performance was not statistically significant. Assessment of sleep quality helps to indentify the students at risk and plan the specific programs to improve the quality of sleep.

Keywords: *Sleep quality, academic performance, medical students.*

Introduction

Medical students are exposed to multitude of changes when they enter into their professional life after completing their school education. Owing to highly demanding professional and academic requirements medical students are considered to be a vulnerable population of poor quality of life.[1] Medical students have compromised quality of life as they have to undergo long and intensive academic years.

Medical students are more prone to daytime sleepiness, sleep deprivation and irregular sleep schedules as they have less free time, longer courses and working hours compared to other professional students. Over the period of time, poor sleep quality will lead to chronic inability to recover from stressful conditions

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COMPARISON OF TIME DOMAIN MEASURES OF SHORT TERM HEART RATE VARIABILITY IN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT MICROALBUMINURIA- A PRELIMINARY STUDY

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Abstract: Background & objectives: Diabetes is a complex and multi-factorial endocrine disorder with increased prevalence worldwide. Microalbuminuria is an independent marker of endothelial dysfunction and increases the risk of arrhythmia and atherosclerosis that affects cardiac autonomic function. Cardiovascular pathology remains a primary cause of death in patients with diabetes mellitus .So we aimed to compare time domain measures of short term Heart Rate Variability (HRV) in type 2 diabetic patients with and without microalbuminuria **Methods:** This prospective comparative study was conducted among diabetic patients with (n=14) and without (n=15) microalbuminuria. Patients with hypertension, dyslipedemia, coronary artery disease, cerebrovascular accident, family history of young MI, hyper or hypothyroidism and on beta-blocker treatment were excluded. Study participants were subjected to general clinical examination. Presence of microalbuminuria and their HbA_1C values were noted from their clinical records. Heart rate variability was assessed using an ambulatory ECG system in lead II for 5 minutes. The series of RR intervals obtained was subjected to time domain analysis. Time domain measures SDNN, rMSSD and NN50and pNN50 were obtained. **Results:** 29 diabetic patients participated in the study, out of which 14 were diabetics with microalbuminuria, and 15 were without microalbuminuria. On analysis of short-term time domain measures of HRV, the mean SDNN was decreased (51 ± 11.5) in diabetic patients with microalbuminuria, when compared to diabetic patients without microalbuminuria (54.96 ± 10.93) with p value 0.45. pNN50 and rMSSD was decreased in diabetic patients with microalbuminuria (1.09 ± 2.73, 14.32±8.17) when compared to diabetic patients without microalbuminuria (1.82 ± 3.06, 18.99 ± 7.71) with p value 0.51 & 0.13 respectively .

Conclusion: Our findings indicate cardiac autonomic function was altered in diabetic population with microvascular involvement

Key Words: Microalbuminuria, Heart rate variability, Diabetes Mellitus

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Introduction:

Diabetes mellitus is a complex and multi-factorial endocrine disorder affecting all age groups .Its prevalence has increased worldwide and it was estimated to be 2.8% in 2000 and 4.4% in 2030.[1] Microalbuminuria is a independent marker of endothelial dysfunction and known to cause cardiovascular morbidity and mortality among individuals with diabetes.[2] Microalbuminuria increases the risk of arrhythmia and atherosclerosis which affects cardiac autonomic function.[3]

Early recognition of autonomic dysfunction plays a significant role in prediction of cardiovascular mortality in diabetes mellitus. Decreased Heart Rate Variability (HRV) is associated higher risk (32–45%) of cardiovascular morbidity and mortality in diabetic individuals and as well in healthy controls.[4,5,6]

HRV is a reliable non invasive tool used in the assessment of cardiac autonomic function. Evaluation of HRV by time domain measure is the simplest and most popular method.[7] Parameters considered during time a domain analysis are standard deviation of all R-R intervals (SDNN), root mean square of successive RR- interval differences (RMSSD) and number of intervals differing by > 50ms from adjacent interval (NN50) and

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Original Research Article

Fatigue, depression and anxiety among nurses working in shift in a tertiary care hospital in South India

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ABSTRACT

Background: Shift work has become a social and economic necessity, as most sectors like the healthcare require round the clock operation. Extended and variable working hours leads to desynchronization of the body's circadian rhythm. Thus, shift work is commonly associated with various physical and mental disorders like insomnia, fatigue, depression, anxiety, cognitive impairment, etc. The aim of the study was to assess the prevalence of sleep deprivation and hence, the levels of fatigue, depression and anxiety among shift working nurses.
Methods: This cross-sectional study was conducted among shift working nurses. Sleep deprivation among 97 female and 3 male healthy nurses of age 20-50 years was assessed by Epworth Sleepiness Scale (ESS). Fatigue Severity Scale, Patient Health Questionnaire (PHQ-9) and Hamilton Anxiety Rating Scale (HAM-A) were used for assessing fatigue, depression and anxiety respectively. Level of Sleep deprivation, Depression and Anxiety were expressed in Proportion. Difference in fatigue levels during day and night shift was analyzed using Wilcoxon Signed-Ranks Test.
Results: Analysis of ESS, showed, 69% of shift working nurses had poorer sleep quality. The level of fatigue experienced following a night shift work was significantly higher than that following day shift work ($Z = 3.34$, $p = 0.0008$). Moderate to severe depression and anxiety was observed in 15% and 16% of the nurses working in shift.
Conclusions: Thus, shift work is inevitably associated with sleep deprivation. There exists an increased prevalence of fatigue, depression and anxiety among shift working nurses.

Keywords: Anxiety, Depression, Fatigue, Shift work, Sleep deprivation

INTRODUCTION

Sleep plays vital role in promoting good physical, mental and emotional health. Adequate quantity and quality of sleep each night is necessary for the maintenance of homeostasis. Sleep deprivation (SD) is a condition where 'an individual is either partially or completely prevented from obtaining their usual amount of sleep in 24 hours'.[1] This serious health hazard is likely to result from inadequate quantity of sleep, poor quality of sleep, or sleep at the wrong time of the day. SD is common public health problem that affect individuals of all age groups. The need for nonstop work in certain sectors like industrial set up and health care has forced the workers into shift work.[2] Cognitive performance worsens with progressive sleep deprivation, as evidenced by its effects on general intellect, mental speed, response inhibition, reaction time, attention, working memory and other complex cognitive functions like decision making and multitasking.[3] Shift work has resulted in lifestyle modifications that affect the biological clock, i.e. the circadian rhythm. Disruption of this natural rhythm for a

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Original Article

Internal Medicine Section

Effect of Age and Hypertension on Cognition: A Cross-sectional Study

DEEPALAKSHMI KALIYAPERUMAL[1], VIJAYABAS KARAN SHANMUGA VARADHARAJAN[2], VARSHA VISWANATHAN[3], MURALI ALAGESAN[4]

ABSTRACT

Introduction: Cognitive impairment has evolved as major health and social issue. As age advances cognitive tasks involving processing speed, working memory and executive function get affected. Cognitive impairment is also influenced by modifiable risk factors such as hypertension, diabetes mellitus and hypercholesterolemia.

Aim: To find out the effect of age and hypertension on cognition.

Materials and Methods: The total study participants were divided into three groups based on their age as group I (18-29) group II (30-59) and group III (above 60) with 20 participants (n= 20) in each group. Their cognition was assessed using Mini Mental State Examination, Picture word Learning test, Stroop colour word test, Letter digit coding test, Verbal fluency and Logic memory tests. Disease history and known co morbidities (hypertension) were noted. Post-hoc test (Bonferroni) was employed to assess age-wise comparison of cognition and Pearson's chi-square test was used to compare cognition between hypertensive and normotensives.

Results: Group II had impaired cognition when compared with group I (p-value- 0.000). Group III had impaired cognition when compared with group I (p-value- 0.000) and group II (p-value 0.001). The hypertensives in group II and III had statistically significant impairment in cognition when compared with normotensives (p-value -0.001) in the same group.

Conclusion: Cognitive functions are impaired as a result of increase in age. Furthermore, there is also a significant decrease in cognitive functions amongst those suffering from hypertension. Hence, we conclude there is decrease in cognitive function as age advances and the co-existence of hypertension hastens this decline.

Keywords: Cognitive impairment, Mini mental state examination, Old age

INTRODUCTION

The word cognition comes from the Latin verb *cognosco* meaning 'I know, perceive' or 'to recognise or conceptualise. Cognitive functions are those which include cerebral activities that lead to knowledge and encompass reasoning, memory, attention and language. Ageing can also have diverse effects on cognitive function. The spectrum of cognitive decline ranges from normal cognitive ageing (healthy" or "successful" Ageing), mild Cognitive impairment (does not prevent them from performing daily activities) and severe cognitive deficits (impede the ability to live independently [1]. Cognitive impairment has evolved as major health and social issue as the life span of both men and women has increased dramatically. Cognitive impairment accounts for about 40% of hospital admissions among the older individuals [2]. As age advances cognitive tasks involving rapid processing and transformation of information, which includes processing speed, working memory and executive function gets affected [3].

These age-related cognitive declining results from structural and functional changes that affect neuronal structure like loss of synapses and dysfunction of neuronal networks [4]. Cognitive impairment is also influenced by modifiable risk factors such as hypertension, diabetes mellitus and hypercholesterolemia [5]. Among these factors hypertension, which causes vascular changes resulting in cerebral infarction and diffuse ischaemic changes contribute to cognitive impairment [6].

Age related cognitive decline is already stated by several studies [7,8] earlier; but none of them used six tests which examine the different domains of cognition and there are only a few studies [9,10] that have included co-morbid condition such as hypertension to study the decline in cognition as age advances.

Hence, we aimed to conduct a study to find out the effect of age on cognition with the help of series of cognitive assessment tests. In this study we also analysed the effect of more common and prevalent (33.8% in urban parts of India) [11] co-morbid condition, hypertension on cognition especially amongst the vulnerable elderly group of individuals.

MATERIALS AND METHODS

This cross-sectional study was conducted during the month of January 2014 for the period of two months among students, staff and patients of PSG Institute of Medical Sciences and Research, Coimbatore after obtaining the ethical clearance from institutional ethical committee (Ethics registration file No 13/328). A total of 60 participants after getting their written informed consent were included in the study. Participants were grouped based on their age Group-I (18-29), Group II (30-59), Group III (above 60). Participants younger than 18 years of age and those suffering from co-morbidities apart from hypertension such as diabetes mellitus; chronic respiratory diseases, chronic liver diseases, thyroid diseases, renal and neurological disorders were excluded from the study. Hypertensive participants (Systolic BP of more than 140mm Hg, Diastolic BP of more than 90 mm Hg) for more than two years duration and on anti hypertensive treatment were included the study.

Study participants were assessed for their cognitive performance by using a series of tests. An initial interview of subjects was conducted where they were asked about their education, occupation and age at retirement for group III. Disease history and known co-morbidities (hypertension) were noted.

Mini Mental State Examination [12]

It is an 11 question measure that tests five areas of cognitive function: orientation, registration, attention and calculation, recall and language. The maximum score is 30, a score of 23 or lower is indicative of cognitive impairment.

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Original Research Article

Clinical and manometric profile of patients with GERD in a tertiary care hospital

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ABSTRACT

Background: Abnormal esophageal motility and low Lower Esophageal Pressure (LES) play an integral role among various etiologies implicated in pathogenesis and severity of Gastro Esophageal Reflux Disease (GERD). Delayed clearance of refluxate leads to prolonged mucosal exposure to gastro duodenal contents which promotes esophagitis and its complications. To find out the association of ineffective esophageal motility (IEM) and low Lower Esophageal Sphincter (LES) pressure with endoscopic esophagitis in patients presenting with symptoms of GERD. Settings and designs: Prospective cross sectional study done in a tertiary medical care center in south India from October 2016 to March 2017.

Methods: This cross sectional study was carried out among patients presenting with symptoms of GERD (heart burn and regurgitation,) at least twice per week for past three months. Based on their endoscopy findings patients with GERD are grouped into two having erosive and nonerosive reflux disease. After general and systemic clinical examination, High resolution Manometry was performed, and data was analyzed using Trace 1.2.3a V software. Statistical methods: Data was analysed using SPSS version 19. Chi square test was used to analyze categorical variables and independent 't' test was used for continuous variables.

Results: A total of 66 patients with GERD symptoms were subjected to esophageal manometry. Out of these 66 patients 42 were males and 22 were females with their mean age 40.82±12.86 years. Among 66 patients presented with GERD symptoms 26.7% were found to have ineffective esophageal motility and only 11.7% had low LES pressure. Ineffective esophageal motility was also found to be more common among older age group individuals with their mean age being 44.64 ±14.154. Among the patients with erosive reflux disease (70.21%), 66.7% had low LES pressure and 75 % had IEM; the association was not statistically significant.

Conclusions: The incidence of Ineffective esophageal motility in patients with GERD is found to be higher than that of low LES pressure. There was no significant association observed between IEM and low LES pressure with endoscopic esophagitis in patients with GERD.

Keywords: Chicago classification, Hypotensive sphincter, High Resolution Manometry, Ineffective peristalsis

INTRODUCTION

Globally Gastro Esophageal Reflux Disease (GERD) remains a highly prevalent disease with considerable geographic variation; estimated prevalence rate was found to be 18.1-27.8% in US and 8.8-25.9% in Europe, and less than 10% in Asian countries.[1] GERD causes significant pain and discomfort which compromise

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Original Article

Physiology Section

Effects of Sleep Deprivation on the Cognitive Performance of Nurses Working in Shift

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ABSTRACT

Introduction: Sleep deprivation and altered circadian rhythm affects the cognitive performance of an individual. Quality of sleep is compromised in those who are frequently involved in extended working hours and shift work which is found to be more common among nurses. Cognitive impairment leads to fatigability, decline in attention and efficiency in their workplace which puts their health and patients' health at risk.

Aim: To find out the prevalence of sleep deprivation and its impact on cognition among shift working nurses.

Materials and Methods: Sleep deprivation among 97 female and three male healthy nurses of age 20–50 years was assessed by Epworth Sleepiness Scale (ESS). Cognition was assessed by Montreal Cognitive Assessment (MoCA) questionnaire. Mobile applications were used to test their vigilance, reaction time, photographic memory and numerical cognition. The above said parameters were assessed during end of day shift and 3-4 days after start of night shift.

Results: Poor sleep quality was observed among 69% of shift working nurses according to ESS scores. The cognitive performance was analysed using Wilcoxon signed rank test. The MoCA score was found to be lesser among 66% of nurses during night (25.72) than day (26.81). During the night, 32% made more mathematical errors. It was also found that, 71%, 83% and 68% of the nurses scored lesser during night in the Stroop's colour test, vigilance test and memory tests respectively. Thus, impairment in cognitive performance was statistically significant ($p<0.001$) among shift working nurses.

Conclusion: Cognitive performance was found to be impaired among shift working nurses, due to poor sleep quality and decreased alertness during wake state. Thus, shift work poses significant cognitive risks in work performance of nurses.

Keywords: Circadian rhythm, Night shift, Sleep quality

INTRODUCTION

Sleep plays an integral part in the health and well-being of the individual. Sleep is controlled by the Supra Chiasmatic Nucleus (SCN) of the hypothalamus, endogenous clock which regulates the production of melatonin, a hormone that induces sleep [1]. Physiological systems that follow circadian rhythm like sleep-wake cycle, hormone secretions, core body temperature are influenced by signals from the environment especially light [2]. Altered circadian rhythm results in physiological and psychological variations [3].

The recommended sleep duration for a healthy adult is seven to nine hours per day [4]. Shift work results in a conflict between a days oriented circadian physiology and requirement for work and sleep at the wrong biological time of day. Internally driven circadian biological clocks regulate the periods of sleepiness and wakefulness. Disruption of circadian rhythm and inadequate sleep are associated with derangement of physiological functions and leads to obesity, cardiovascular disease, and cognitive impairment. Thus, shift work, night work, and longer, irregular working hours are associated with reduced sleep duration and poor quality of sleep [5,6].

Sleep Deprivation (SD) is defined as, "obtaining inadequate sleep to support adequate daytime alertness" [7]. Long-term SD has a detrimental effect on cognitive function, attention, learning, and working memory and is associated with exaggerated neural reactivity [8]. Moreover, it is also associated with mal adaptations of physiological and behavioral functions. Sleep deprivation affects the ability of health workers who are involved in multitude of life saving tasks which need more attention and concentration. Extended work shifts of twelve hours or longer is common with nurses, whose insufficient sleep puts patients' health and their health at risk [9]. SD among nurses is the major cause for making an error in patient care and significant decrease in vigilance of the job [10]. The aim of this study was to assess the prevalence of SD among nurses working in shift and its impact on their cognitive performance.

MATERIALS AND METHODS

This cross-sectional study was conducted among staff nurses of Peelamedu Samanaidu Govindasamy Hospitals, Coimbatore, Tamil Nadu, India, who were on a rotating work schedule i.e., staff posted to day shift for one month and the same staff posted to night shift for the next one month during August 2016 to September 2016 after obtaining the ethical clearance from the Institution Ethical Committee.

Sample Size

Hundred staff nurses were chosen whose age ranged from 20 years to 50 years. Sample size (96), was calculated considering prevalence of sleep deprivation among staff nurses as 51% with 20 % precision [11].

Inclusion Criteria

Staff nurses of all departments like Medicine, Surgery, OBG, Paediatrics, etc., who were working eight hours shift a day for six days a week, not suffering from any known medical or mental illness and had a previous work experience of at least one year in rotating shift work, were included.

Exclusion Criteria

Staff nurses with a history of medical illness especially neurological diseases, sleep disorders (insomnia, somnolence, etc.,) and smoking, alcohol, any other drug consumption or any medications (within the last 14 days), those who do overtime and other jobs during off duty were excluded from the study.

The participants were informed about the test procedure and a written consent was obtained from them. The staff nurses were assessed for sleep deprivation using the Epworth Sleepiness Scale (ESS) [12]. The ESS was used to measure day time sleepiness and

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Original Research Article

Comparison of microalbuminuria and heart rate variability in prediction of cardiovascular complications in diabetic population: a pilot study

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ABSTRACT

Background: Microalbuminuria is considered as an early marker and strongly associated with risk of cardiovascular complications in diabetic patients. Autonomic imbalance due to micro vascular damage to parasympathetic and sympathetic fibers results in reduced heart rate variability; also predicts increased risk for cardiovascular events in diabetics. Hence it is necessary to identify an early and effective predictor of diabetic micro vascular complications. Objective of the study was to compare heart rate variability of controls with type 2 diabetes with and without microalbuminuria.

Methods: This comparative study was conducted among individuals without diabetes, hypertension and dyslipidemia (controls) and Type II diabetics with and without microalbuminuria (cases). Cases and controls were subjected to general clinical examination; microalbuminuria and HbA1C were noted. heart rate variability was assessed using digital physiograph. Frequency (HF, LF, LF/HF ratio) domain readings were noted.

Results: Mann Whitney U test was employed to analyze nonparametric data. Diabetics with microalbuminuria when compared with controls showed statistically significant (p-value=0.015) reduction in vagal activity. When compared with diabetics without microalbuminuria (180.4±151.7) they showed reduction in HF with no statistical significance. When diabetic group without microalbuminuria (0.56±0.31) was compared with controls they showed alternation in LF/HF ratio which has no statistical significance. Diabetics with microalbuminuria when compared with controls showed a statistically significant (p-value=0.009) alternation in LF /HF ratio implying an autonomic imbalance.

Conclusions: This study shows there is significant vagal inhibition and autonomic imbalance in diabetic patients with microalbuminuria compared to controls. To ascertain the role of HRV as an early predictor of cardiovascular complications we propose to conduct study with a larger sample size in future.

Keywords: Autonomic imbalance, Diabetes mellitus, Heart rate variability, Microalbuminuria

INTRODUCTION

The magnitude of non-communicable diseases (cardiovascular diseases, diabetes, malignancies and chronic respiratory diseases) has brought about a negative socioeconomic impact and sufferings to the mankind.[1] 36 million deaths have been reported every year due to diabetes and its complications. Moreover, 9 million people succumb to death prematurely.[2] Improved and effective medications have increased the lifespan of diabetic population. Hence there is a need to identify and intervene at the earliest in order to prevent complications and provide them a better quality of living. Microalbuminuria is considered as an early marker. It

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Original Research Article

Students and faculty perspectives towards early clinical experience in learning neurosciences

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Date of Submission: 16.12.2016 **Date of Acceptance: 04.03.2017**

Abstract

Background: Medical education has undergone major changes over a period which redefines conventional teaching strategies. It is necessary to reinforce and link basic sciences with clinical aspects in order to enhance better understanding, knowledge retention, application and development of clinical skills. With this view we introduced a method of exposing students to clinical environment early in their curriculum. Our study aimed at determining post exposure academic performance of students and faculty perceptions towards early clinical experience (ECE) in a hospital setting. **Methods**: Validated teaching module comprising of specific learning objectives, lesson plan, students and facilitators guide was developed and implemented to 150 students. After conventional didactic lectures in Anatomy and Physiology on Facial nerve, students were taken to hospital wards in five batches of 30 each. They observed interaction between physician and patients to appreciate clinical signs of facial nerve palsy. Pre and post tests were conducted to assess effectiveness of ECE. Students and faculty were provided with feedback questionnaires to know their perceptions towards the program. **Results**: Out of 150 students 119 students attended all sessions. Descriptive statistics was employed to assess their responses. Significant difference in scores of the students before (2.91±2.35) and after (5.93±3.07) ECE was observed with p value <0.01. 87% of students agreed that module was student centered, facilitated active learning and fostered critical thinking. Faculty involved revealed that this module satisfied current disciplinary (Anatomy and Physiology) and interdisciplinary (clinical) expectations. **Conclusion**: This active teaching learning strategy was well conceived by students and faculty. It helps in integration of basic and clinical sciences and paves way for application of basic science concepts in clinical practice.

Key words: Medical education, Perspective, Early Clinical Exposure, Active learning.

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Introduction:

Medical Science is an ever evolving field which invariably demands a periodic revisit of learning as well as teaching methods. Globally more emphasis is being laid on early clinical exposure of students, which includes both horizontal and vertical integration that eventually is expected to bring contextual learning. The traditional undergraduate medical curriculum in India is designed such that the students are exposed to clinical subjects during the second year of their course after prior exposure to basic sciences in the form of didactic lectures. On observing and screening the pattern of present medical education curriculum, the academicians have strongly recommended that the students need to be introduced to clinical medicine early in their

Original Article

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Internal Medicine Section

Correlation of Reciprocal Changes and QRS Amplitude in ECG to Left Ventricular Dysfunction, Wall Motion Score and Clinical Outcome in First Time ST Elevation Myocardial Infarction

SILPITA KATRAGADDA[1], MURALI ALAGESAN[2], SHANMUGASUNDARAM RATHAKRISHNAN[3], DEEPALAKSHMI KALIYAPERUMAL[4], ANITH KUMAR MAMBATTA[5]

ABSTRACT

Introduction: Electrocardiogram (ECG) is the simplest tool for diagnosing ST Elevation Myocardial Infarction (STEMI). We can use a12 lead ECG for prognostication purposes also.

Aim: The aim of the study was to find out the role of ECG as a prognostic marker in terms of clinical outcome and wall motion abnormality.

Materials and Methods: It was a prospective study done in PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India, from January 2014 to September 2014. Patients aged above 18 years admitted with first episode of ST EMI as per the inclusion and exclusion criteria were recruited for the study. Presence of reciprocal changes and QRS amplitude was measured from ECG. Presence of Left Ventricular Dysfunction (LVD) and wall motion score were calculated from ECG along with clinical outcome during first follow up visit. Statistical analysis was done using SPSS software. Probability was calculated using chi-square test, independent t-test and ANOVA analysis.

Results: A total of 120 patients were recruited for the study of which six were excluded based on the exclusion criteria. Among 114 patients analysed, 55 had reciprocal changes; 38 of them developed LVD which was statistically significant (p=0.002). Of the 78 patients with Anterior Wall Myocardial Infarction (AWMI), 35 had reciprocal changes; 15 (42.9%) of them had NYHA Class 1 symptoms, 14 (40%) had Class II and 4 (11%) had class III symptoms at follow up. The association was statistically significant (p=0.001). Similar statistically significant association was found in patients with Inferior Wall Myocardial Infarction (IWMI) who had reciprocal changes and NYHA symptoms at follow up (p=0.004). The mean wall motion score in patients with AWMI and reciprocal changes was 24.83 ± 4.1; whereas without reciprocal changes was 23.98 ± 3.6; the association was not statistically significant. The mean QRS amplitude of all patients with LVD was 33.25 ±16.34. The association between QRS amplitude and LVD was not statistically significant. The overall mean wall motion score was 24.86 ± 3.91. The association between QRS amplitude and wall motion score was statistically significant (r value = 0.210). The association between QRS amplitude and wall motion score was statistically significant when we analysed AWMI (r= -0.147, p=0.199) and IWMI (r= -0.359, p=0.031) separately.

Conclusion: ECG can be used as a tool for prognostication in acute STEMI. The presence of reciprocal changes in the ECG can signify poorer outcome on follow up. Lower QRS amplitude can be used as a predictor of larger infarct.

Keywords: Coronary artery disease, Heart failure, Prognostic marker

INTRODUCTION

Myocardial infarction is one of the most important causes for hospitalization and is commonly seen in all populations worldwide [1]. The clinical approach to a patient suspected to have an acute coronary syndrome consists of detailed history taking, ECG changes, cardiac biochemistry evidence and imaging. The reliability of each of these modalities as a diagnostic tool is dependent on multiple factors, the most significant being the window period between the time of infarction and the time the patient seeks medical attention [2]. The current approach to patients presenting with typical or atypical features of myocardial ischemia or infarction starts with making the provisional diagnosis of acute coronary syndrome [3,4]. Depending on the changes seen on the 12 lead ECG, acute coronary syndrome is classified into STEMI, Non ST Elevation Myocardial Infarction (NSTEMI) or unstable angina [5].

Inspite of major leaps in the diagnostic tools and treatment of myocardial infarction, STEMI persists as one of the leading cause for mortality and morbidity in both the developed and developing world [6]. The prevalence of coronary disease and myocardial infarction is also on rise in the developing countries, with further worsening of adverse cardiovascular events due to disadvantages like inadequate primary prevention policies and limited availability of medical help [7].

There has been extensive research done in the field of cardiovascular medicine focused on ST elevation myocardial infarction and the dynamics of complications surrounding it. One particular area of interest in recent studies appears to be the study of extent of infarct over imaging modalities and its correlation with various events in the course of infarction [8,9]. Other parameters gaining importance are non traditional ECG changes like reciprocal changes, ST depression, QRS morphology, q waves etc., [10-17].

The simplest and oldest diagnostic tool for myocardial infarction is the 12 lead ECG. In daily practice, even though the ECG remains an important test in diagnosis and detection of progression of disease, there still remains ample potential for its role as a prognostic marker [18,19]. The fundamental advantage that stands to be gained by

Original Research

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Peripheral nerve conduction tests in patients with hypothyroidism and healthy volunteers: A comparative study

Jeyabanu Murugiah, Nagashree Ramasamy, Pushparaj Thangaraj.

PlumX Metrics

ABSTRACT

IJMPS. 2014; 5(3): 09-14

CORRELATION OF LIPID PROFILE AND RR INTERVAL WITH TSH IN HYPERTHYROIDS AND EUTHYROIDS

Rashmi Ramanathan, Mohan Jayapal, Manishankar subramaniyam, Nagashree Ramasamy, Jyothi Sivalingiah, Rajeswari M..

ABSTRACT

Abstract

Aim: Excess thyroid hormones has a profound effect on carbohydrate, protein, lipid metabolism and also on Cardiovascular system. The current study was aimed to determine the association between serum lipid profile, RR interval with TSH in hyperthyroids and euthyroids

Methodology: Study was carried out in PSG Hospitals ,Coimbatore among 30 untreated hyperthyroids and 30 age matched Euthyroids. Thyroid profile including Triiodothyronine (T3), Thyroxine (T4) and Thyroid stimulating hormone (TSH) were measured by using ECLIA (Electro Chemi Luminescence Immuno Assay). Serum Lipid profile including Cholesterol, Triglycerides, LDL, and HDL were measured by Immuno Turbidimetry method. ECG was recorded using in lead II for 5 minute. Heart rate and RR interval were noted for all the subjects.

Results: All lipid parameters were significantly reduced in Hyperthyroids than controls. Among the Euthyroids, Significant ($p<0.05$)

Key words: Lipid Profile, TSH, RRinterval, Hyperthyroids

Original Research Article

Effect of Obesity on semen parameters

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Abstract

Background: Obesity is a non-communicable disease and has grown to pandemic in recent years in western as well as in developing countries. It should be considered as an etiology of male infertility, as there is an increase in incidence of overweight and obesity in men of reproductive age. **Aim: To** study the effect of obesity on semen parameters. **Materials and methods:** This was a cross sectional study, which involved 75 male participants, aged between 20 to 50 years. Paternal height and weight, life style habits and semen analysis data were collected from medical records of fertility department. Men were divided into three groups based on their body mass index (BMI). Group1 (n = 25)- Normal (18.5 -24.99 kg/m^2), Group 2(n = 25) - Overweight (25.0 -29.99 kg/m^2), Group 3 (n = 25)- Obese (BMI>30 kg/m^2). Semen parameters were analysed by applying WHO 2010 criteria as normal cut off values. The data obtained was analysed using logistic regression. **Result: T**his study showed statistically significant association between obesity and abnormal sperm concentration, low sperm motility, low sperm vitality with an adjusted odds ratio of 26.36 (3.05 – 227.74),14.34 (1.18–174.00),8.59 (2.34 – 31.49) respectively after adjusting for age, smoking habits, intake of alcohol and physical activity. **Conclusion:** This study finding strongly indicates that obesity has an association with semen parameters. In future, it is needed to work in that direction to reduce such factors, which can affect the fertility of males.

Key words: Body mass index (BMI), Male infertility, Obesity, Semen parameters.

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Introduction

Obesity is a global health problem not only in Western countries but also in developing countries like India.[1] American Medical Association (2013), recently classified obesity as a disease. It affects not only cardiovascular system but also has a role in many other related health disorders. It accounts for 7.5% of the total burden of disease. In India, the prevalence of obesity is 12.6% in women as compared to 9.3% in men.[2]The rate of obesity in reproductive-age men has nearly tripled. Obesity reaches epidemic proportions with 1.6 billion adults being classified as overweight and an extra 400 million adults classified as obese.[3]Obesity adversely affects male reproduction by endocrine, thermal, genetic and sexual mechanisms.[3] It has

Association between Quality of Sleep and Academic Performance in Medical Students: A Preliminary Study

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Abstract

Background: Medical students are more prone to daytime sleepiness, sleep deprivation and irregular sleep schedules, as they have less free time, longer courses and working hours compared to other professional students.

Aim: To assess the quality of sleep and to find out the association between sleep quality and academic performance among medical students.

Methodology: This cross sectional study was conducted among 176 medical students. They were subjected to Pittsburgh Sleep Quality Index (PSQI) questionnaire to assess their sleep quality over a one-month period. A PSQI global score of 5 and greater than 5 was considered indicative of poor sleep. Academic performance was assessed from their internal assessment scores conducted during that period. The data was analyzed using the unpaired students 't' test and Pearson's correlation test

Results: Our study included 176 students from all the phases of MBBS curriculum. 24 percentage students had poor quality of sleep. In our study, there was no statistically significant difference in the performance of good sleepers (57.94 ±18.06) and poor sleepers (58.80 ±17.37) with a p value of 0.785. Our study results showed a negative correlation between global PSQI scores and assessment scores of the students (r = 0.57 and P =0.449).

Conclusion: In our study association between sleep quality and academic performance was not statistically significant. Assessment of sleep quality helps to indentify the students at risk and plan the specific programs to improve the quality of sleep.

Keywords: *Sleep quality, academic performance, medical students.*

Introduction

Medical students are exposed to multitude of changes when they enter into their professional life after completing their school education. Owing to highly demanding professional and academic requirements medical students are considered to be a vulnerable population of poor quality of life.[1] Medical students have compromised quality of life as they have to undergo long and intensive academic years.

Medical students are more prone to daytime sleepiness, sleep deprivation and irregular sleep schedules as they have less free time, longer courses and working hours compared to other professional students. Over the period of time, poor sleep quality will lead to chronic inability to recover from stressful conditions

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COMPARISON OF TIME DOMAIN MEASURES OF SHORT TERM HEART RATE VARIABILITY IN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT MICROALBUMINURIA- A PRELIMINARY STUDY

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Abstract: Background & objectives: Diabetes is a complex and multi-factorial endocrine disorder with increased prevalence worldwide. Microalbuminuria is an independent marker of endothelial dysfunction and increases the risk of arrhythmia and atherosclerosis that affects cardiac autonomic function. Cardiovascular pathology remains a primary cause of death in patients with diabetes mellitus .So we aimed to compare time domain measures of short term Heart Rate Variability (HRV) in type 2 diabetic patients with and without microalbuminuria **Methods:** This prospective comparative study was conducted among diabetic patients with (n=14) and without (n=15) microalbuminuria. Patients with hypertension, dyslipedemia, coronary artery disease, cerebrovascular accident, family history of young MI, hyper or hypothyroidism and on beta-blocker treatment were excluded. Study participants were subjected to general clinical examination. Presence of microalbuminuria and their HbA_1C values were noted from their clinical records. Heart rate variability was assessed using an ambulatory ECG system in lead II for 5 minutes. The series of RR intervals obtained was subjected to time domain analysis. Time domain measures SDNN, rMSSD and NN50and pNN50 were obtained. **Results:** 29 diabetic patients participated in the study, out of which 14 were diabetics with microalbuminuria, and 15 were without microalbuminuria. On analysis of short-term time domain measures of HRV, the mean SDNN was decreased (51 ± 11.5) in diabetic patients with microalbuminuria, when compared to diabetic patients without microalbuminuria (54.96 ± 10.93) with p value 0.45. pNN50 and rMSSD was decreased in diabetic patients with microalbuminuria (1.09 ± 2.73, 14.32±8.17) when compared to diabetic patients without microalbuminuria (1.82 ± 3.06, 18.99 ± 7.71) with p value 0.51 & 0.13 respectively .

Conclusion: Our findings indicate cardiac autonomic function was altered in diabetic population with microvascular involvement

Key Words: Microalbuminuria, Heart rate variability, Diabetes Mellitus

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Introduction:

Diabetes mellitus is a complex and multi-factorial endocrine disorder affecting all age groups .Its prevalence has increased worldwide and it was estimated to be 2.8% in 2000 and 4.4% in 2030.[1] Microalbuminuria is a independent marker of endothelial dysfunction and known to cause cardiovascular morbidity and mortality among individuals with diabetes.[2] Microalbuminuria increases the risk of arrhythmia and atherosclerosis which affects cardiac autonomic function.[3]

Early recognition of autonomic dysfunction plays a significant role in prediction of cardiovascular mortality in diabetes mellitus. Decreased Heart Rate Variability (HRV) is associated higher risk (32–45%) of cardiovascular morbidity and mortality in diabetic individuals and as well in healthy controls.[4,5,6]

HRV is a reliable non invasive tool used in the assessment of cardiac autonomic function. Evaluation of HRV by time domain measure is the simplest and most popular method.[7] Parameters considered during time a domain analysis are standard deviation of all R-R intervals (SDNN), root mean square of successive RR- interval differences (RMSSD) and number of intervals differing by > 50ms from adjacent interval (NN50) and

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Original Article

Physiology Section

Hypolipidemic Effects of Fenugreek and Atorvastatin-Comparative Study on High Fat Fed Dyslipidemic Rats

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ABSTRACT

Introduction: Dyslipidemia is the current medical problem of utmost concern with an increased prevalence among the males between 31-40 years. Various extracts from fenugreek (*Trigonellafoenum-graecum*), methi (in Hindi), have known to have effect on the metabolic parameters, lipid lowing properties being one of them.

Aim: The objective of this study was to evaluate the efficacy of fenugreek, one of the commonest Indian spice in lowering the serum lipid levels and comparing its efficacy with lipid lowering properties of a pharmacological agent-atorvastatin so that it could be incorporated as a part of life style modifications among the dyslipidemic individuals.

Materials and Methods: Twenty four adult male Wistar rats were divided equally into four groups. Group A was kept as the control and fed on normal diet. Hyperlipidemia was induced in Groups B, C and D with high fat diet containing groundnut oil cake and dried coconut for 12 weeks. The hyperlipidemic rats were then subjected to the various hypolipidemic regimens. Group B- 2mL emulsion of fenugreek (1 gm/kg/day) orally, Group C- 2mL emulsion of atorvastatin (30 mg/kg/day) orally, Group D- 4mL emulsion of fenugreek and atorvastatin combined. The serum samples were analysed for lipid profile at the beginning and end of four weeks. The statistical analysis for the data collected was done using One-way Anova analysis in the software- SPSS Version 20.0.

Results: Administration of fenugreek to hyperlipidemic rats (Group B) showed a significant reduction of serum triglycerides, cholesterol and body weight ($p<0.05$) and a significant increase in the serum High Density Lipoprotein (HDL) levels ($p<0.05$). The serum Low Density Lipoprotein Cholesterol (LDL-C) values showed a statistical reduction in Group C ($p<0.01$) administered with atorvastatin monotherapy. In Group D the combination therapy with fenugreek and atorvastatin showed a statistical significant increase of High Density Lipoprotein Cholesterol (HDL-C) $p<0.01$.

Conclusion: Fenugreek is proved to be a potent hypolipidemic agent. Lifestyle modification with dietary supplement of fenugreek can be taken as an effective hypolipidemic element initially to combat the increasing risks of dyslipidemia. If they do not respond then the individuals can switch over to pharmacological agents like atorvastatin along with fenugreek to control dyslipidemia.

Keywords: Dietary supplement, Lifestyle modification, Metabolic disorder, Statins, Trigonellafoenum-graecum

INTRODUCTION

Dyslipidemia as defined by the abnormal levels of lipid parameters such as LDL-C, triglycerides, HDL-C and atherogenic indices in the blood, is the current medical as well as social problem of utmost concern leading to increasing morbidity and mortality. Studies have shown that regional disparity exists in India with the highest rates of hypercholesterolemia observed in Tamil Nadu (18.3%) among which the urban residents had the highest prevalence of lipid abnormalities compared to rural residents [1,2]. Studies have also revealed that dyslipidaemia is more common among the males aged 30-40 years leading to increased prevalence of young infarcts due to coronary artery disease in this group [3,4]. Current AACE guidelines on the prevention and management of dyslipidemia suggest that it is the major prerequisite for the development of coronary artery disease and may even be the primary risk factor occurring before other major risk factors comes into play [5].

Several methods for combating this life threatening metabolic disorder have been emphasized upon in various researches in the recent times. The armamentarium for managing it now includes "statins" that can decrease the LDL-C levels by up to 55% [6]. Statins are hypolipidemic agents with anti-atherosclerotic properties [7]. A study on comparison of safety and efficacy among statins showed that atorvastatin is more effective when compared to simvastatin and pravastatin in patients with hyperlipidemia [8,9]. Multiple clinical trials conducted across the world show that a significant number of patients on statins continue to have a high residual risk of coronary artery disease [10,11]. The various adverse effects associated with the statin group of drugs provide a clinical challenge in management of dyslipidemia with such pharmacological modalities.

The modern medicine draws its potential from the rich legacy of traditional medicine. Fenugreek (*Trigonellafoenumgrae*-cum) is one of the oldest medicinal plants, dating back to the Hippocrates and ancient Egyptian times and known to have immense therapeutic value as proven by several experiments on animal and human models [12]. It is also said to have a high levels of iron hence it can be an useful supplement in iron-deficient patients [13]. In vivo studies using fenugreek extracts has an inhibitory effect on the growth of cancer cells and reduces inflammation [14]. The other beneficial nutritive effects of fenugreek seeds include hypolipidemic and anti-diabetic effects [15]. Lipid lowering effects of fenugreek have been attributed to the various chemical extracts such as ethyl acetate extract which was found to have-Naringenin, the abundant flavonoid compound found to have significant hypocholesterolemic effects [16].

This study was aimed at determining the hypolipidemic potential of fenugreek in comparison to the pharmacological agents like atorvastatin in order to include fenugreek as part of the dietary modification in the hypolipidemic regimen taken up by the dyslipidemic individuals.

Original Research Article

A study on the quality of life in children and adolescents with type I Diabetes Mellitus.

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Abstract

Background: Diabetes mellitus is expected to increase from 425 million in 2017 to 629 million by 2045 worldwide. Diabetes mellitus diagnosed during childhood presents an increased risk of complications in an early and productive stage of life, leading to a reduction in mean life expectancy of 10 to 20 years which is especially prevalent in developing countries. Children and adolescents with Type 1 diabetes Mellitus are at a heightened risk for mental health issues, including diabetes distress, depression, anxiety, and disordered eating. Health-Related Quality of Life (HRQOL) is an essential health outcome in clinical trials and healthcare. **Aim:** To evaluate the quality of life in children and adolescents with Type 1 Diabetes Mellitus for an effective management using Pediatric Quality of Life Inventory (PedsQL) tool questionnaire. **Materials and Methodology:** This was a prospective study. After ethical approval, 60 subjects who qualify the inclusion criteria were enrolled. The PedsQL tool questionnaire were given separately to child and parent. The child self-report and parent proxy-report were obtained and the PedsQL 4.0 Generic Core Scales were calculated. Statisticalanalysis was done using paired student 't' test. **Results:** The PedsQL score of children was 77.94 ± 15.59 and that of parents was 74.12 ± 17.78. There was a significant difference between the child's self-report and the parents'- proxy report. The p value was statistically significant (p =0.000). **Conclusion:** These findings emphasizes the importance of the psychosocial assessment and regular counselling to the children and adolescents with Type 1 DM which is a chronic disease along with their routine medical treatment which insists on good glycemic control.

Key words: type1 diabetes mellitus, HRQOL, PedsQL

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Introduction

Diabetes mellitus (DM) is one of the most common metabolic diseases worldwide. Its prevalence has increased in the past decade. Almost 90% of cases classified as Type 1 DM, with an autoimmune cause are children aged under 15 years. It represents one of the major chronic disease in pediatric age group. The Diabetes Atlas 2017 estimates that there are 128,500 children and adolescents with diabetes in India.[1]

Type 1 DM, formerly known as juvenile or insulin-dependent diabetes is characterized by deficient insulin production. Symptoms include excessive

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A Commentary on the Role of "Female Gender" in Receiving the Nobel Prize for Physiology/Medicine

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Commentary

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Keywords: Nobel, Genetics, Cell biology, Research

ABSTRACT

In browsing through the list of Nobel Prize Laureates, only about 5.3% of women have won the Alfred Nobel Prize for Physiology and Medicine. In countries with highest number of Nobel laureates like USA & UK, only about 4% of them are Females. This review article analyses the role played by the "female gender" in receiving the nobel prize for Physiology and Medicine.

INTRODUCTION

Nobel Prize is one of the highly prestigious awards to be won by the mankind. Each recipient receives a medal, a diploma and a cash prize of about 8 million Swedish Krono that has varied through the years **(Figures 1-3)**. Now cash prize is equivalent to 6 crore and 25 lakhs. Nobel prize medals are handmade with recycled gold with careful precision. So far only 48 women have received (822 men) the distinction and among them, only 12 women got the honor for Physiology/Medicine whereas 211 individuals have received Nobel Prize for Physiology/Medicine.

The first woman to receive the Nobel Prize for Physiology/Medicine was in the year 1947. It is about 46 years after inception of the prize (1901).



Figure 1. Picture of Albert Nobel medal.

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INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PLATELET INDICES AS A TOOL IN ASSESSING THE PROGRESSION OF TYPE 2 DIABETES MELLITUS

Physiology

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ABSTRACT

Background: Type 2 Diabetes mellitus is considered as a prothrombotic state that increases the risk for microvascular complications. Studies associating platelet indices with severity of diabetes have yielded inconsistent results.

Aim: To compare platelet indices such as platelet count, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) between diabetic and non-diabetic population.

Materials and methods: The cross-sectional study was conducted in the Department of Medicine with 30 diabetic patients and 30 controls. Platelet indices were measured using Beckman Coulter LH 780. SPSS software was used for statistical analysis.

Results: MPV, Platelet count and PDW were significantly increased in diabetic subjects compared to non diabetic subjects. MPV and Platelet count showed significant positive correlation with HbA1c in diabetic patients.

Conclusion: Platelet indices especially MPV, PDW assesses the thrombogenic state and hence can be used to monitor treatment.

KEYWORDS

Diabetes mellitus, Mean platelet volume, Platelet distribution width.

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized chiefly by hyperglycemia resulting from absolute or relative deficiency of insulin [1]. The resultant metabolic dysfunction in diabetes leads to microvascular and macrovascular complications. These vascular complications in turn results in secondary damage to multiple organ systems [2].

The prevalence of diabetes has risen worldwide from 108 million cases in 1980 to 422 million cases in 2014. According to WHO, Diabetes was the seventh leading cause of death in 2016. A recent estimate in 2016 has suggested that diabetes causes almost 1.6 million deaths globally [3]. Type 2 diabetes mellitus is the predominant form of diabetes worldwide accounting for 90% of cases. India has the second-largest prevalence of diabetes accounting for 62.1 million cases in 2013 [2].

Type 2 Diabetes mellitus is considered as a prothrombotic state with enhanced platelet reactivity that increases the risk for microvascular complications like retinopathy [4]. Insulin antagonizes the effect of platelet agonists like collagen, adenosine diphosphate, epinephrine and platelet-activating factor. Hence insulin resistance leads to increased reactivity of platelets [5].

Platelet indices such as platelet count, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) have been used to assess the platelet activity. MPV indicates the average size and activity of platelets. This in turn reflects either a change in platelet stimulation or a change in the rate of platelet production [6].

Increase in MPV has been demonstrated in metabolic syndrome and stroke, which are prothrombotic states [6]. Platelet distribution width (PDW) which indicates heterogeneity in platelet size and shape is a sign of active platelet release [7].

Investigations such as fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT) assesses the prothrombotic state. Many of these tests cannot be employed for screening prothrombotic state in diabetes routinely.

Hence in their place; platelet indices that include MPV, PDW which are done during routine hematological analysis can be used [4].Not many studies have been conducted in South India emphasizing the association of platelet indices with glycemic control in diabetes mellitus. Studies associating platelet indices with progression of diabetes have yielded inconsistent results. Hence this study was chosen to compare platelet indices between diabetic and non-diabetic population and to correlate platelet indices with HbA1c in diabetic patients.

MATERIALS AND METHODS

This cross-sectional comparative study was conducted in the Department of Medicine, from May 2018 to April 2019 after getting Institutional Ethics Clearance. Sample size was calculated with Open Epi software version 3.01 using 80% power, 95% confidence interval and by substituting mean ± SD values of platelet indices obtained from a study conducted by Shilpi K, et al [4]. The calculated sample size was 60 with 30 subjects without diabetes mellitus in Group A and 30 subjects with type 2 diabetes mellitus in Group B.

Inclusion criteria

- Age of the subjects: 35-70 years
- Gender: Both males and females
- Subjects with type 2 diabetes mellitus on treatment. Diagnosis of type 2 diabetes mellitus was made using WHO criteria [11]

Exclusion criteria

- Subjects who were pregnant.
- Subjects with acute infections, trauma or recent myocardial infarction.
- Subjects with hematological disorders like anemia (hemoglobin values <12g/dl in females and <13g/dl in males), polycythemia, lymphoma, multiple myeloma or with bleeding disorders.
- Subjects with autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus.
- Subject with hypertension, thyroid disorders like hypothyroidism
- Subjects with cardiac failure, hepatic failure, renal failure, malignancies or poor general conditions.
- Subjects on antiplatelet medications like aspirin, clopidogrel.
- Subjects who were not willing to participate.

After getting informed and written consent, the data was collected using a data collection tool. Under sterile aseptic precautions, 2ml of venous blood was drawn from the antecubital veins of the subjects. The withdrawn blood was anticoagulated with EDTA. Platelet indices were computed using Beckman Coulter LH 780. Plasma glucose level was measured by the glucose oxidase method and HbA1c was measured by HPLC, if they have not been already done in the study population.

The data collected were analyzed using IBM SPSS software version 24. Normally distributed variables were presented as mean ± SD. Categorical variables were presented as frequencies and proportions. Independent t-test was used to compare the variables between the two groups. Karl Pearson's correlation was used to compare the continuous variables which were normally distributed. p-value < 0.05 was considered to be statistically significant.

RESULTS

The mean age of the subjects in groups A (non-diabetic) and B

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COMPARISON OF SENSORY NERVE CONDUCTION BETWEEN OVERWEIGHT AND OBESE DIABETIC PERIPHERAL NEUROPATHY PATIENTS BASED ON ASIAN INDIAN BODY MASS INDEX STANDARDS

Physiology

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ABSTRACT

Background: Obesity is a major component of metabolic syndrome that accentuates the inflammatory and oxidative stress in diabetic peripheral neuropathy (DPN). Based on the Asian Indian BMI standards, the prevalence of overweight is on the rise in India.

Aim: To compare the sensory nerve conduction parameters such as latency, amplitude and nerve conduction velocity in the median, ulnar and sural nerves between overweight and obese DPN patients.

Materials and methods: This cross-sectional study was conducted in the Department of Neurology with 80 type 2 DPN patients with Body mass index (BMI) > 23 Kg/m2. Sensory nerve conduction studies were performed. SPSS software was used for statistical analysis.

Results: Except for sural nerve, the nerve conduction studies in other nerves did not show a statistical significant difference between overweight and obesity.

Conclusion: Overweight affects sensory nerve conduction study in diabetic peripheral neuropathy similar to obesity.

KEYWORDS

Diabetic peripheral neuropathy, Overweight, BMI

INTRODUCTION

Type 2 diabetes mellitus is the predominant form of diabetes worldwide accounting for more than 90% of diabetic cases [1]. Among the microvascular complications of diabetes; diabetic neuropathy occurs in more than 50% of individuals with type 2 diabetes mellitus [2]. Diabetic neuropathy can manifest in the forms of polyneuropathy, mononeuropathy and/ or autonomic neuropathy. Out of these forms, distal symmetric polyneuropathy (DSPN) accounts for about 75% of the caseload in diabetic neuropathies [3]. A prevailing view for the pathogenesis of diabetic peripheral neuropathy is the inflammatory and oxidative stress that occurs as a result of metabolic syndrome [3]. Obesity is a major component of metabolic syndrome [4].

Based on the BMI classification criteria for Asian Indians, the prevalence of overweight and obesity (BMI $\geq$23 kg/m ^{2}) in India is on the rise [5]. For long it was known that obesity is a major risk factor for amplifying the severity of diabetic neuropathy. Now with the advent of newer guidelines for BMI classification [6] even diabetic patients with overweight (BMI 23-27.4 Kg/m^2) could be under risk for worsening severity of peripheral neuropathy on par with obesity.

Hence using nerve conduction study as a tool, we decided to compare the sensory nerve conduction study parameters such as latency, amplitude, and nerve conduction velocity in the median, ulnar and sural nerves between overweight and obese diabetic peripheral neuropathy patients (DPN) patients. Since the sensory component of the nerves is more affected in diabetic peripheral neuropathy [7], we have chosen sensory nerve conduction study. The null hypothesis employed here is that there is no difference in sensory nerve conduction between overweight and obese DPN patients. Sensory nerve conduction study parameters in DPN patients with both overweight and obesity were also correlated with BMI.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Neurology from December 2017 to December 2018 in 80 DPN outpatients after getting Institutional Ethics Clearance. The sample size was calculated with Open Epi software version 3.0 using the prevalence of overweight and obesity from ICMR-INDIAB study 2015 [8] and 95% confidence interval.

Inclusion criteria

- Age: 35-70 years
- BMI $\geq$ 23 Kg/m^2 (Subjects with BMI 23-27.4 Kg/m^2 were considered overweight and those with BMI > 27.5 Kg/m^2 were considered to be obese)
- Gender: Both males and females
- Subjects with a history of type 2 diabetic peripheral neuropathy for more than 1-year duration on treatment.

Exclusion criteria

- Subjects with hemolytic or aplastic anemias
- Subjects with vitamin B12 deficiency
- Subjects with thyroid disorders
- Subjects with inflammatory conditions such as connective tissue disease, CIDP (Chronic Inflammatory Demyelinating Polyneuropathy) or neuromuscular disease
- Subjects with signs of peripheral vascular disease
- Subjects with a history of cerebral infarction, cervical spondylosis or lumbar spondylosis.
- Subjects diagnosed with malignancies.
- Subjects with cardiac failure, hepatic failure or poor general conditions.

After getting informed and written consent, the data was collected. Recorders Medicare System (RMS) EMG EPM 2K version 1 was used to record the sensory nerve conduction parameters such as latency, amplitude and nerve conduction velocity (NCV). Onset latency (ms) was measured from the onset of stimulus artifact to the initial negative peak in antidromic conduction. Amplitude (μV) was measured from the baseline to the negative peak of the biphasic response obtained after supramaximal nerve stimulation and NCV (m/s) was measured by applying a stimulus at a single stimulus site. The formula utilised for calculating NCV was

$$\text{Conduction velocity} = \frac{\text{Distance between stimulating and recording electrodes (cm)}}{\text{Onset of latency (msec)}} \times 10$$

Fasting (FBS) and postprandial plasma glucose levels (PPBS) were estimated using hexokinase, UV, kinetic methods. Glycosylated hemoglobin (HbA1c) was assessed using high-pressure liquid chromatography (HPLC).

Serum cholesterol and triglycerides (TGL) were measured using enzymatic endpoint methods. Serum high-density lipoproteins (HDL) and low-density lipoproteins (LDL) levels were measured using direct enzymatic assays. Serum urea and creatinine were determined using enzymatic methods. Estimated glomerular filtration (eGFR) was calculated based on chronic kidney disease epidemiology collaboration (CKD – EPI) equation (2009).

The data collected were analyzed using SPSS IBM software version 24. Shapiro-Wilk test was used to test the normality of variables. Normally distributed data are presented as mean $\pm$ standard deviation (SD) and variables with skewed distribution are presented as median (interquartile range).

Independent t-test was used to compare the variables with normal distribution between DPN with overweight and those with obesity. Mann-Whitney U test was used to compare the variables with skewed

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INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ASSOCIATION OF PLATELET INDICES WITH SEVERITY OF ACUTE ISCHEMIC STROKE

Physiology

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ABSTRACT

Background: Stroke serves as the leading cause of death and physical disability. This study was conducted to find the relationship between platelet indices and severity of acute ischemic stroke.

Materials and methods: This was a cross-sectional study conducted among 60 ischemic stroke patients divided into severe and mild stroke based on modified Rankin Score(mRS) with 30 patients in each group and 30 normal individuals. Platelet indices(Platelet count and Mean Platelet Volume) were obtained from CBC report.

Results: There was a significant decrease in platelet count and increase in Mean Platelet Volume(MPV) in stroke patients when compared with normal persons and also in severe stroke patients when compared with mild stroke patients with p values 0.001 and < 0.001 respectively in both comparisons.

Conclusion: Patients with severe stroke had lowest platelet count and the highest MPV in our study. There is a positive association between platelet indices and the severity of acute ischemic stroke. Platelet indices can serve as an important predictor for the prognosis of acute ischemic stroke. It also helps in risk stratification in ischemic stroke patients so that they can be given more vigilant and aggressive care that can lead to decrease in morbidity and mortality.

KEYWORDS

Acute ischemic stroke, Platelet Indices, Platelet count, Mean Platelet volume

INTRODUCTION:

Central nervous system infarction is brain, spinal cord or retinal cell death attributable to ischemia, based on neuropathological, neuroimaging and/or clinical evidence of permanent injury (1). Stroke is the second leading cause of death, after ischemic heart disease accounting for 9% of deaths worldwide (2). Ischemic stroke is an episode of neurological dysfunction caused by cerebral, spinal or retinal infarction (1). As per Global burden of disease data on stroke on 2013, there were about 25.7 million stroke survivors, 6.5 million stroke deaths and 10.3 millions new strokes. Thus stroke is disease of immense public health importance with serious economic and social consequences (3). The two major mechanisms causing stroke are ischemia and haemorrhage. About 85% of all strokes are ischemic in origin (4).About one-fifth of patients with an acute stroke die within one month of the event and at least half of those who survive are left with a physical disability(5).

Cortical strokes which are 60% of ischemic stroke follow in situ cerebral thrombosis or cardiac source in which platelet play an important role. However lacunar stroke comprising 25% of ischemic stroke is due to lipohyalinosis of end arteries, which is unlikely to involve platelets (6). In recent years, there is an increased interest on research documenting the platelet indices (platelet count, mean platelet volume (MPV), platelet distribution width) in association with various ischemic strokes. Increased platelet reactivity is considered to be a relevant pathophysiological factor in approximately 50% of all strokes (7). Large platelets due to greater content in granules are more reactive than ordinary size platelets, produce more prothrombotic factors and show greater aggregation to Adenosine diphosphate (ADP), collagen and secrete more thromboxane A2 (TXA2). Increased platelet size has been described in patients with vascular risk factors such as diabetes, hypercholesterolemia and metabolic syndrome (8).

Platelet indices are calculated during routine blood analysis along with Complete Blood Count but these are generally not considered by clinicians. Mean Platelet Volume and platelet count are inversely associated so that the total platelet mass remains approximately constant (9).Studies have detected increased MPV in different subtypes of stroke both in acute phase and long after disease. Also stroke patients with high mortality have been found to have low platelet count (8). Moreover, studies on association of MPV with severity of ischemic stroke yielded inconsistent results (10).

Our study finds the association between the platelet indices(Platelet count and Mean Platelet Volume) and severity of acute ischemic stroke thus helps to know the diagnostic value of platelet indices in ischemic stroke. It also helps in risk stratification of patients with ischemic stroke, so that such patients can be given more vigilant and aggressive care that can lead to decrease in morbidity and mortality.

MATERIALS AND METHODS:

This was a cross sectional study, conducted among 60 acute ischemic stroke patients admitted in PSG Hospitals, Coimbatore, Tamilnadu and 30 healthy persons. The study was conducted after obtaining clearance from Institutional Human Ethical Committee, of our institution and obtaining written informed consent from the subjects.

Study population: Patients presenting with acute ischemic stroke (without previous history of stroke) in the age group of 40-70 years admitted in Neurology ward are cases. Controls were healthy persons attending Master Health Check up department.

Inclusion criteria:

- Patients with acute ischemic stroke for the first time proven by CT scan
- Patients admitted within 24 hours of onset of symptoms in neurology ward
- Patients of both genders within the age group of 40-70 years
- Known case of diabetes mellitus and hypertension under control

Exclusion criteria:

- Patients with previous history of stroke
- Transient ischemic attack
- Patients with haemorrhagic stroke, cerebral venous sinus thrombosis
- Patients with late admission of stroke (> 24 hours after stroke onset)
- Patients with co-morbidities like chronic renal disease, chronic liver disease, bypass heart surgery.
- Patients who are on drugs which modify platelet morphology like NSAIDS for long time.
- Patients with haematological malignancies and patients showing atypical cells in peripheral smear were excluded.

Sample size: 90 subjects, 60 acute ischemic stroke patients divided into 2 groups (groupI and group II) of 30 patients in each group and 30 healthy subjects(group III)

Calculation of sample size was done by using Open Epi software version 3.01, by using simplified formula for difference in means of

ASSOCIATION OF PLATELET INDICES WITH DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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ABSTRACT

BACKGROUND

Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disease of unknown aetiology that mainly affects peripheral joints in a symmetric pattern. Prevalence of RA is approximately 1% worldwide, being two to three times more common in women than men. Effective management in RA requires close monitoring of disease activity. Platelet activation is an important event in synovial inflammation, but very few studies have used platelet indices to assess inflammation.

Thus, the aim of the study was to correlate the platelet indices (platelet count, mean platelet volume and platelet distribution width) with disease activity in Rheumatoid Arthritis.

MATERIALS AND METHODS

This descriptive comparative study was conducted among 60 RA patients and 30 healthy controls within the age group of 30 to 70 years. Every consecutive RA patient who attended the Rheumatology Outpatient Department was included in the study. The severity of RA was assessed by the Disease Activity Score Calculator for Rheumatoid Arthritis (DAS-28). The platelet indices assessed in the study include platelet count, Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) obtained from their CBC report. The study population was divided into three groups. Group I (Moderate disease activity) with DAS-28 score of 3.2-5.1; Group II (High disease activity) with DAS-28 score above 5.1. Thirty patients in Group I and thirty in Group II were taken for the study. Group III: 30 healthy controls who attended the Master Health Check-Up Department. Data analysis was done by using SPSS software and independent t-test. P value < 0.05 was taken as statistically significant.

RESULTS

There was statistically significant increase in platelet count, decrease in MPV and PDW in high-grade RA patients when compared with normal individuals (p= 0.004, p= 0.001, p= 0.019 respectively). We also found similar statistical significance between high and moderate grade RA patients (p= 0.001 for platelet count, p= 0.007 for MPV and p= 0.012 for PDW).

CONCLUSION

Our study suggests that platelet indices can be considered as inflammatory markers for disease activity in RA. However, further studies with larger number of patients are warranted to cross validate our findings.

KEY WORDS

Rheumatoid Arthritis, Platelet Indices, DAS 28.

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BACKGROUND

Rheumatoid Arthritis is a chronic autoimmune inflammatory disease characterised by tenderness, swelling and stiffness of the joints with progressive destruction of cartilage and bone.[1] RA affects approximately 0.92% of the adult population of India with 20 to 40 new cases per lakh population every year[2] and is two to three times more common in women than men as other autoimmune diseases.[3]

Early diagnosis and aggressive therapy can usually prevent permanent disability and improve the quality of life.[2] Effective management in RA requires close monitoring of disease activity.

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To determine the disease activity, clinicians use clinical signs and symptoms, radiological findings and Rheumatoid Factor (RF) values and C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR). Other blood tests are required for assessment of systemic involvement (Liver and kidney function) and monitoring of adverse drug events. So far, immune cells were considered to play the central role in RA pathophysiology and until recently platelets have not been expected to have a role in the pathogenesis of the disease. Inflammation creates an imbalance of normal physiological anticoagulant mechanism, induces activation of coagulation system with interleukin-6 and tumour necrosis factor alpha (TNFα) thus affecting platelet indices.[4]

Dense platelet bodies contain adenosine nucleotides, calcium and serotonin that exert effects in the initial phase of inflammation. They release pre-stored inflammatory mediators and can also produce eicosanoids such as thromboxane A_2, prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and PGE_2, to regulate inflammatory response and can synthesize peptide mediators such as interleukin (IL)-1β on stimulation.[5,6]

From the 1950s, many attempts were made to design a disease activity measurement tool. Tools available are in the form of patient questionnaires, joint counts or lab tests. Most

EFFECTS OF AGE, GENDER AND EDUCATION LEVEL ON COGNITION IN ELDERLY PEOPLE

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ABSTRACT

BACKGROUND

With rapidly increasing number of older people in our community cognitive impairment is a growing public health concern in India. It affects the quality of life for the person affected and the caregiver. By preserving the function, we can help older adults to remain independent for as long as possible.

The aim of this study is to investigate the cognitive function in elderly people in various age groups and to find correlation of gender and education with cognition.

MATERIALS AND METHODS

This is a cross-sectional study conducted on 100 elderly people above 60 years at PSG Hospitals, Coimbatore. Cognition was assessed by Mini-Mental State Examination (MMSE) and Modified Mini-Mental Status examination (3MS) and was scored for 30 and 100 respectively. Correlation of age, gender and education with cognition scores was found. Statistical analysis was done using SPSS software by independent t test and chi square test and p value <0.05 was considered to be statistically significant.

RESULTS

We found statistically significant decline in cognition scores in elderly people as age advances by both tests ($p < 0.001$). Correlation of gender with cognition scores was not found to be significant ($p = 0.254$ and 0.268) and correlation of education with cognition scores showed decreased cognition in persons with lower level of education.

CONCLUSION

In general, we detected a significant decline in cognitive scores as age advances by both MMSE and 3MS. Higher education and younger age group were associated with better cognitive performance. By identifying those people at risk and by evaluation of any causes for cognitive decline and by proper treatment at an early stage, dementia can be prevented.

KEYWORDS

Cognition, Dementia, Education, Gender, MMSE, 3MS.

HOW TO CITE THIS ARTICLE: Shuba N, Prakash B. Effects of age, gender and education level on cognition in elderly people. J. Evolution Med. Dent. Sci. 2017;6(95):6952-6956, DOI: 10.14260/jemds/2017/1509

BACKGROUND

The elderly population in India above 60 years was 8% in 2010, is expected to increase to 19% in 2050 according to United Nations Population Division (UN 2011). By 2050, India's 60 and older population is expected to encompass 323 million people.[1] Ageing entails many physical, biological, chemical, and psychological changes. Ageing can have diverse effects on cognitive function. Cognitive impairment affects the quality of life for the person affected and the caregiver.

In 2010, 3.7 million Indians over 60 years had dementia and are expected to double by 2030. Prevalence rates of mild cognitive impairment varies from 3% to as high as 59% with a conversion rate to dementia varying from 8% to 15% shows the need for diagnostic tests for dementia.[2]

Mini-Mental state Examination (MMSE), by Folstein et al in 1975 is the most widely used standardised instrument for cognitive impairment in the world.[3] Modified Mini-Mental status Examination (3MS) was developed by Teng and Chui in 1987, it is an expanded version of MMSE.

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The 3MS is significantly a better predictor of functional outcome than the MMSE and is found to have higher sensitivity.[4] Even though various studies show decline in cognition as age advances, there are not much studies involving correlation of various factors with cognition in elderly people. This study finds the correlation of various physiological factors among elderly people with cognitive impairment.

We tested the hypothesis that age, gender and education play an important role in cognitive function in elderly people. By preserving function, we can help older adults remain independent for as long as possible, which can improve their quality of life and delay the need for costly longterm care. By this study elderly people who are at risk of developing dementia and Alzheimer's disease can be identified and by initiating effective treatment and care, the disease can be prevented or progression of the disease can be slowed down.

Objective

The objective of the study is to assess the cognitive function in elderly people above 60 years by MMSE and 3MS, and to find correlation of gender and education level with cognitive impairment.

MATERIALS AND METHODS

This is a cross-sectional study done by collecting data afresh from study participants. Clearance from Human Ethics Committee, PSG IMS&R was obtained and the study was

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LETTER TO THE EDITOR
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A case of acute paraplegia due to aortic dissection in Marfan syndrome

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Full Text

Sir,

A 45-year-old woman went to bed well by 10 pm after finishing strenuous household works. She woke up around 4 am due to chest and back discomfort with sweating. She could not turn over or sit-up. The lower limbs were felt cold, numb, and weak. The emergency department physician had suggested X-ray of the spine to check for compressive pathology. She had cardiac arrest at the radiology department, where she was revived and shifted to intensive care unit, sedated, and intubated. After 6 h of stabilization, she could be extubated. There was no history of fever, diarrhea, trauma, visual impairment, chest pain, palpitation, or dyspnea preceding the event. However, she had episodes of unexplained chest discomfort at rest for the previous 2 weeks. There was no other significant medical history. Clinically, she was conscious and afebrile, but was in discomfort due to back pain, and could not lie down properly. She could not describe her problems and was not able to localize the tender spot in the spine. Her peripheral pulses were well felt in all the four limbs. The pulse rate was 76/min, and blood pressure was 140/50 mm of Hg, with a collapsing pulse in the upper limbs. Early diastolic murmur was made out by auscultation. Respiratory system and abdominal examination were normal. Neurologically, cranial nerve and upper limb examinations were normal. No Horner's syndrome was noted. She had weakness of Medical Research Council grade 0/5 in both lower limbs from hip downward with hypotonia. Deep tendon reflexes in the upper limbs were brisk, which could not be elicited in lower limbs. She was able to describe the absent pinprick below her D-10 dermatome but could not cooperate well for posterior column examination. Plantar reflexes were absent on both the sides. In view of the above findings, the clinical diagnoses of myocardial infarction (MI), aortic regurgitation (AR), with anterior spinal artery (ASA) embolism causing paraplegia were made. Aortic disease was not considered initially as the peripheral pulses were normal. The patient did not have typical pain of dissection. Early diagnosis of Aortic Dissection (AD) at critical setup is often difficult [1] Furthermore, paraplegia as a presenting manifestation of AD is rare.[2] Other differential diagnoses of spinal arteriovenous malformation, dural arteriovenous fistula, epidural abscess, and spinal cord demyelination were also considered. However, a single consolidated diagnosis which explains all the findings could not be arrived at this point. The patient was subjected to investigations. X-ray of the dorsal spine did not reveal vertebral collapse or other abnormalities. Her routine blood investigations, liver function tests, and electrolytes were normal. Electrocardiogram showed anterior wall MI. The troponin-T level was high (80.97 pg/ml). Her echocardiogram showed mild left ventricular dysfunction, global hypokinesia, ejection fraction of 45%, mild AR, mild mitral regurgitation with dissection flap in ascending and arch of aorta extending into descending aorta. Three attempts of doing magnetic resonance imaging (MRI) went in vain as she could not lie down for more than few minutes and not sedated in view of hemodynamic instability. However, we could complete computed tomography (CT) angiogram of the aorta which revealed a Stanford-A, de-Bakey type-1 dissection of the aorta with entry point at the coronary sinus, extending upward to involving root, arch of aorta, descending thoracic and abdominal aorta extending into both common iliac and proximal external iliac arteries. All the three major branches of arch of aorta were involved. The true lumens of left main, right coronary arteries were compressed and small throughout their length. The left renal artery was arising from the true lumen at the site of narrowing [Figure 1]. Patient was advised to undergo emergency surgery under high life risk with poor outcome of paraplegia, which was explained in detail and her attendees agreed after prolonged counseling. The operation was performed through a midline sternotomy. Cardiopulmonary bypass was achieved. The aortic dissection was found to have originated in the right coronary sinus in close relation to the right coronary ostium. The left main coronary ostium looked clear without any obstructions. Under moderate hypothermia, the aortic root was replaced with a St. Jude Medical valved Dacron conduit. A reversed saphenous vein graft was sutured to the right coronary artery and connected proximally to the aortic graft. The distal aorta was anastomosed to the Dacron graft. The patient was weaned off bypass with stiff ionotropic support and shifted to the intensive care unit. She made slow but steady recovery with good hemodynamics, with no fresh focal neurologic deficit and gradual improvement in creatinine. She was transferred to the postoperative ward on day 8 and fit for discharge on the 14th postoperative day. Despite adequate physiotherapy, medial nursing, and nutritional care, she had developed sacral sores and the sensory level had ascended to D-2 dermatome. There was no improvement in lower limb power. She also continued to have double incontinence. A CT scan of the brain done on the 5th postoperative day did not show any abnormality. The marfanoid features were noted later after the diagnosis of AD was made. She had wrist sign, thumb sign, probable malar hypoplasia, and arm span-height ratio being 171.165 (>1.04) [Figure 2]. The patient's only son also had marfanoid features. The dissected aorta was sent for histopathology. The gross sample was 2 cm in length and 3 cm in diameter. Microscopy revealed focal loss of elastic and mucoid material of media. There are areas of disruption of elastic laminae replaced by smooth muscle, mucoid material, and collagen. The above features suggest aortic dissection secondary to cystic medial degeneration of ascending aorta [Figure 3].{Figure 1}{Figure 2}{Figure 3}

Discussion

Acute AD is a cardiothoracic emergency and acute paraplegia is neurological emergency. The diagnostic difficulties encountered in our patient include poor description of t the diagnosis he pain due to her restlessness as she had recent cardiac arrest, missing marfanoid features clinically due to supine position and sickness. Normal peripheral pulses and the inability to perform MRI also had made the diagnosis difficult. Embolic spinal cord infarction was initially suspected due to the occurrence of simultaneous MI with elevated troponin-T. Echocardiogram had given the first clue about aortic dissection. Unfortunately, MRI of the spine could not be done post operatively also due to recent surgery and staples. This case was presented due to its rare combination of paraplegia due to spinal cord infarction, caused by aortic dissection, secondary to Marfan syndrome associated with acute MI and cardiac arrest, without compromising carotid and renal circulations, and to share the difficulties encountered in making a prompt initial diagnosis. The clinical features of Marfan syndrome include ectopia lentis, joint laxity, dolichostenomelia, scoliosis, and pectus deformities. Retinal detachment, early cataract, and glaucoma are other features. The life expectancy is reduced in severe forms who have greater diameter of aortic root and arch (60%). If the difference in aortic root diameter is more than 5 mm in a patient with family history, prophylactic aortic root replacement should be considered. The aortic dissection is one of the major Ghent criteria [3] for the diagnosis of Marfan syndrome.

Original Article

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Physiology Section

Role of Vitamin D in the Outcome of Ischemic Stroke- A Randomized Controlled Trial

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ABSTRACT

Introduction: Vitamin D deficiency has reached epidemic proportions in India with prevalence rates of 70-100% in the general population. Low vitamin D levels are associated with worse outcomes in ischemic strokes. The outcome of stroke after vitamin D replenishment has not been much studied.

Aim: To compare the stroke outcome between patients receiving vitamin D supplementation and patients without vitamin D supplementation by Scandinavian Stroke Scale (SSS) and thus, to assess the long term outcome of ischemic stroke patients with low vitamin D level by supplementation of vitamin D.

Materials and Methods: This is a non blinded randomized controlled trial conducted in ischemic stroke patients. The patients were divided into two groups (A and B) where Group A received a single dose of 6 lac IU of Cholecalciferol Intramascular (IM) injection and Group B were not given vitamin D. Finally 30 patients in each group were analyzed. SSS was applied to evaluate the stroke severity at the onset and at the follow up after three months in both groups. The difference in SSS was analyzed using SPSS software. Independent t-test was applied and p-value < 0.05 was taken as significant.

Results: Mean±SD of vitamin D levels in Group A and Group B were 17.98±3.81ng/ml and 18.44±4.69 ng/ml respectively. Mean±SD of SSS at time of admission and after three months for Group A was 32.50±11.61 and 38.89±8.34; and for Group B 35.82±8.56 and 38.32±7.10. The difference in SSS from time of admission and after three months, in Group A (6.39±...) Group B (2.50±2.20) were analyzed statistically and found to be highly significant (p<0.001).

Conclusion: The results showed that there is a significant improvement in the stroke outcome after three months in those patients who were supplemented with vitamin D. Thus screening for vitamin D deficiency in stroke patients is essential and vitamin D replenishment will improve the stroke outcome.

Keywords: Scandinavian stroke scale, Stroke severity, Vitamin D deficiency, Vitamin D supplementation

INTRODUCTION

Vitamin D deficiency has reached an epidemic in India with prevalence rate being 70-100% in the general population [1]. Vitamin D deficiency is widely prevalent in tropical countries like India despite abundant sunlight [1]. It is the most under diagnosed and untreated nutritional deficiency in the world. Vitamin D deficiency is associated with increased risk of vascular disease and ischemic stroke in healthy individuals [2,3]. Vitamin D deficiency is associated with ischemic stroke risk and contributing factors being hypertension, hyperlipidemia, diabetes mellitus and ischemic heart disease [4]. Stroke is the second leading cause of death worldwide, accounting for 9% of deaths. There are an estimated 5.7 million stroke deaths which are expected to increase to 7.8 million by 2030 [5,6]. Vitamin D deficiency in stroke patients preceded stroke and prevalence of vitamin D deficiency was more in stroke patients than general medical patients [7]. Vitamin D supplementation in post stroke patients is helpful in prevention of recurrent stroke and is suggested to reduce neurological, psychological and musculoskeletal disorders [8]. It also reduces morbidity, mortality and improves functional outcome after stroke [9]. American Stroke Association International Stroke Conference 2015 has found that stroke patients with low vitamin D levels have large ischemic infarcts, suffer severe strokes and have poor health outcomes after stroke than those with normal vitamin D level [10].

There are various methods and scales to assess severity of stroke. SSS is reliable and widely used to assess functional outcome in patients with ischemic cerebrovascular disease and has been successfully used in several clinical trials [11].

Even though studies have shown association of vitamin D deficiency in stroke, studies have not been done by supplementation of vitamin D to ischemic stroke patients, which form the basis of our study. We tested the hypothesis that there is improved functional outcome in ischemic stroke patients supplemented with high dose of vitamin D compared with ischemic stroke patients receiving regular conventional treatment. Thus, we have assessed the long term outcome of ischemic stroke patients with low vitamin D level, by supplementation of vitamin D.

MATERIALS AND METHODS

This is a non blinded, randomized controlled trial conducted at PSG Hospitals, Coimbatore, Tamil Nadu, India. On obtaining approval from Institutional Human Ethics committee, the study was conducted in ischemic stroke patients admitted in Neurology ward from April-2014 to March- 2016. Informed written consent was obtained from the patients.

At the time of planning for the study, there were no trials of vitamin D supplementation in ischemic stroke patients. Therefore, sample size was based on Mean±SD estimates of vitamin D level in ischemic stroke patients and normal persons as per advice by statistician and the study done by Wu C et al., was used for calculation of sample size [12]. Sample size was calculated by Open Epi software version 3.01, by using simplified formula for difference in means of power 80%, confidence interval 95% and α level of 0.05 and was found to be 28 persons in each group.

The study included both males and females of age group from 50 to 80 years with vitamin D insufficiency (Serum 25- hydroxy vitamin D level 21-29 ng/ml) and vitamin D deficiency (Serum 25- hydroxy vitamin D level ≤ 20 ng/ml). Diagnosis of stroke was based on clinical assessment supported by Computed Tomography (CT) scan and Magnetic Resonance Imaging (MRI). Ischemic stroke

Association between Quality of Sleep and Academic Performance in Medical Students: A Preliminary Study

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Abstract

Background: Medical students are more prone to daytime sleepiness, sleep deprivation and irregular sleep schedules, as they have less free time, longer courses and working hours compared to other professional students.

Aim: To assess the quality of sleep and to find out the association between sleep quality and academic performance among medical students.

Methodology: This cross sectional study was conducted among 176 medical students. They were subjected to Pittsburgh Sleep Quality Index (PSQI) questionnaire to assess their sleep quality over a one-month period. A PSQI global score of 5 and greater than 5 was considered indicative of poor sleep. Academic performance was assessed from their internal assessment scores conducted during that period. The data was analyzed using the unpaired students 't' test and Pearson's correlation test

Results: Our study included 176 students from all the phases of MBBS curriculum. 24 percentage students had poor quality of sleep. In our study, there was no statistically significant difference in the performance of good sleepers (57.94 ±18.06) and poor sleepers (58.80 ±17.37) with a p value of 0.785. Our study results showed a negative correlation between global PSQI scores and assessment scores of the students ($r = 0.57$ and $P = 0.449$).

Conclusion: In our study association between sleep quality and academic performance was not statistically significant. Assessment of sleep quality helps to indentify the students at risk and plan the specific programs to improve the quality of sleep.

Keywords: *Sleep quality, academic performance, medical students.*

Introduction

Medical students are exposed to multitude of changes when they enter into their professional life after completing their school education. Owing to highly demanding professional and academic requirements medical students are considered to be a vulnerable population of poor quality of life.[1] Medical students have compromised quality of life as they have to undergo long and intensive academic years.

Medical students are more prone to daytime sleepiness, sleep deprivation and irregular sleep schedules as they have less free time, longer courses and working hours compared to other professional students. Over the period of time, poor sleep quality will lead to chronic inability to recover from stressful conditions

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COMPARISON OF TIME DOMAIN MEASURES OF SHORT TERM HEART RATE VARIABILITY IN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT MICROALBUMINURIA- A PRELIMINARY STUDY

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Abstract: Background & objectives: Diabetes is a complex and multi-factorial endocrine disorder with increased prevalence worldwide. Microalbuminuria is an independent marker of endothelial dysfunction and increases the risk of arrhythmia and atherosclerosis that affects cardiac autonomic function. Cardiovascular pathology remains a primary cause of death in patients with diabetes mellitus .So we aimed to compare time domain measures of short term Heart Rate Variability (HRV) in type 2 diabetic patients with and without microalbuminuria **Methods:** This prospective comparative study was conducted among diabetic patients with (n=14) and without (n=15) microalbuminuria. Patients with hypertension, dyslipedemia, coronary artery disease, cerebrovascular accident, family history of young MI, hyper or hypothyroidism and on beta-blocker treatment were excluded. Study participants were subjected to general clinical examination. Presence of microalbuminuria and their HbA_1C values were noted from their clinical records. Heart rate variability was assessed using an ambulatory ECG system in lead II for 5 minutes. The series of RR intervals obtained was subjected to time domain analysis. Time domain measures SDNN, rMSSD and NN50and pNN50 were obtained. **Results:** 29 diabetic patients participated in the study, out of which 14 were diabetics with microalbuminuria, and 15 were without microalbuminuria. On analysis of short-term time domain measures of HRV, the mean SDNN was decreased (51 ± 11.5) in diabetic patients with microalbuminuria, when compared to diabetic patients without microalbuminuria (54.96 ± 10.93) with p value 0.45. pNN50 and rMSSD was decreased in diabetic patients with microalbuminuria (1.09 ± 2.73, 14.32±8.17) when compared to diabetic patients without microalbuminuria (1.82 ± 3.06, 18.99 ± 7.71) with p value 0.51 & 0.13 respectively .

Conclusion: Our findings indicate cardiac autonomic function was altered in diabetic population with microvascular involvement

Key Words: Microalbuminuria, Heart rate variability, Diabetes Mellitus

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Introduction:

Diabetes mellitus is a complex and multi-factorial endocrine disorder affecting all age groups .Its prevalence has increased worldwide and it was estimated to be 2.8% in 2000 and 4.4% in 2030.[1] Microalbuminuria is a independent marker of endothelial dysfunction and known to cause cardiovascular morbidity and mortality among individuals with diabetes.[2] Microalbuminuria increases the risk of arrhythmia and atherosclerosis which affects cardiac autonomic function.[3]

Early recognition of autonomic dysfunction plays a significant role in prediction of cardiovascular mortality in diabetes mellitus. Decreased Heart Rate Variability (HRV) is associated higher risk (32–45%) of cardiovascular morbidity and mortality in diabetic individuals and as well in healthy controls.[4,5,6]

HRV is a reliable non invasive tool used in the assessment of cardiac autonomic function. Evaluation of HRV by time domain measure is the simplest and most popular method.[7] Parameters considered during time a domain analysis are standard deviation of all R-R intervals (SDNN), root mean square of successive RR- interval differences (RMSSD) and number of intervals differing by > 50ms from adjacent interval (NN50) and

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Original Article

Internal Medicine Section

Effect of Age and Hypertension on Cognition: A Cross-sectional Study

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ABSTRACT

Introduction: Cognitive impairment has evolved as major health and social issue. As age advances cognitive tasks involving processing speed, working memory and executive function get affected. Cognitive impairment is also influenced by modifiable risk factors such as hypertension, diabetes mellitus and hypercholesterolemia.

Aim: To find out the effect of age and hypertension on cognition.

Materials and Methods: The total study participants were divided into three groups based on their age as group I (18-29) group II (30-59) and group III (above 60) with 20 participants (n= 20) in each group. Their cognition was assessed using Mini Mental State Examination, Picture word Learning test, Stroop colour word test, Letter digit coding test, Verbal fluency and Logic memory tests. Disease history and known co morbidities (hypertension) were noted. Post-hoc test (Bonferroni) was employed to assess age-wise comparison of cognition and Pearson's chi-square test was used to compare cognition between hypertensive and normotensives.

Results: Group II had impaired cognition when compared with group I (p-value- 0.000). Group III had impaired cognition when compared with group I (p-value- 0.000) and group II (p-value 0.001). The hypertensives in group II and III had statistically significant impairment in cognition when compared with normotensives (p-value -0.001) in the same group.

Conclusion: Cognitive functions are impaired as a result of increase in age. Furthermore, there is also a significant decrease in cognitive functions amongst those suffering from hypertension. Hence, we conclude there is decrease in cognitive function as age advances and the co-existence of hypertension hastens this decline.

Keywords: Cognitive impairment, Mini mental state examination, Old age

INTRODUCTION

The word cognition comes from the Latin verb *cognosco* meaning 'I know, perceive' or 'to recognise or conceptualise. Cognitive functions are those which include cerebral activities that lead to knowledge and encompass reasoning, memory, attention and language. Ageing can also have diverse effects on cognitive function. The spectrum of cognitive decline ranges from normal cognitive ageing (healthy" or "successful" Ageing), mild Cognitive impairment (does not prevent them from performing daily activities) and severe cognitive deficits (impede the ability to live independently [1]. Cognitive impairment has evolved as major health and social issue as the life span of both men and women has increased dramatically. Cognitive impairment accounts for about 40% of hospital admissions among the older individuals [2]. As age advances cognitive tasks involving rapid processing and transformation of information, which includes processing speed, working memory and executive function gets affected [3].

These age-related cognitive declining results from structural and functional changes that affect neuronal structure like loss of synapses and dysfunction of neuronal networks [4]. Cognitive impairment is also influenced by modifiable risk factors such as hypertension, diabetes mellitus and hypercholesterolemia [5]. Among these factors hypertension, which causes vascular changes resulting in cerebral infarction and diffuse ischaemic changes contribute to cognitive impairment [6].

Age related cognitive decline is already stated by several studies [7,8] earlier; but none of them used six tests which examine the different domains of cognition and there are only a few studies [9,10] that have included co-morbid condition such as hypertension to study the decline in cognition as age advances.

Hence, we aimed to conduct a study to find out the effect of age on cognition with the help of series of cognitive assessment tests. In this study we also analysed the effect of more common and prevalent (33.8% in urban parts of India) [11] co-morbid condition, hypertension on cognition especially amongst the vulnerable elderly group of individuals.

MATERIALS AND METHODS

This cross-sectional study was conducted during the month of January 2014 for the period of two months among students, staff and patients of PSG Institute of Medical Sciences and Research, Coimbatore after obtaining the ethical clearance from institutional ethical committee (Ethics registration file No 13/328). A total of 60 participants after getting their written informed consent were included in the study. Participants were grouped based on their age Group-I (18-29), Group II (30-59), Group III (above 60). Participants younger than 18 years of age and those suffering from co-morbidities apart from hypertension such as diabetes mellitus; chronic respiratory diseases, chronic liver diseases, thyroid diseases, renal and neurological disorders were excluded from the study. Hypertensive participants (Systolic BP of more than 140mm Hg, Diastolic BP of more than 90 mm Hg) for more than two years duration and on anti hypertensive treatment were included the study.

Study participants were assessed for their cognitive performance by using a series of tests. An initial interview of subjects was conducted where they were asked about their education, occupation and age at retirement for group III. Disease history and known co-morbidities (hypertension) were noted.

Mini Mental State Examination [12]

It is an 11 question measure that tests five areas of cognitive function: orientation, registration, attention and calculation, recall and language. The maximum score is 30, a score of 23 or lower is indicative of cognitive impairment.

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Original Research Article

Comparison of microalbuminuria and heart rate variability in prediction of cardiovascular complications in diabetic population: a pilot study

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ABSTRACT

Background: Microalbuminuria is considered as an early marker and strongly associated with risk of cardiovascular complications in diabetic patients. Autonomic imbalance due to micro vascular damage to parasympathetic and sympathetic fibers results in reduced heart rate variability; also predicts increased risk for cardiovascular events in diabetics. Hence it is necessary to identify an early and effective predictor of diabetic micro vascular complications. Objective of the study was to compare heart rate variability of controls with type 2 diabetes with and without microalbuminuria.

Methods: This comparative study was conducted among individuals without diabetes, hypertension and dyslipidemia (controls) and Type II diabetics with and without microalbuminuria (cases). Cases and controls were subjected to general clinical examination; microalbuminuria and HbA1C were noted. heart rate variability was assessed using digital physiograph. Frequency (HF, LF, LF/HF ratio) domain readings were noted.

Results: Mann Whitney U test was employed to analyze nonparametric data. Diabetics with microalbuminuria when compared with controls showed statistically significant (p-value=0.015) reduction in vagal activity. When compared with diabetics without microalbuminuria (180.4±151.7) they showed reduction in HF with no statistical significance. When diabetic group without microalbuminuria (0.56±0.31) was compared with controls they showed alternation in LF/HF ratio which has no statistical significance. Diabetics with microalbuminuria when compared with controls showed a statistically significant (p-value=0.009) alternation in LF /HF ratio implying an autonomic imbalance.

Conclusions: This study shows there is significant vagal inhibition and autonomic imbalance in diabetic patients with microalbuminuria compared to controls. To ascertain the role of HRV as an early predictor of cardiovascular complications we propose to conduct study with a larger sample size in future.

Keywords: Autonomic imbalance, Diabetes mellitus, Heart rate variability, Microalbuminuria

INTRODUCTION

The magnitude of non-communicable diseases (cardiovascular diseases, diabetes, malignancies and chronic respiratory diseases) has brought about a negative socioeconomic impact and sufferings to the mankind.[1] 36 million deaths have been reported every year due to diabetes and its complications. Moreover, 9 million people succumb to death prematurely.[2] Improved and effective medications have increased the lifespan of diabetic population. Hence there is a need to identify and intervene at the earliest in order to prevent complications and provide them a better quality of living. Microalbuminuria is considered as an early marker. It

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RESEARCH ARTICLE

Comparison of high-resolution manometry parameters among type II diabetes patients and normal volunteers

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ABSTRACT

Background: Gastrointestinal (GI) autonomic neuropathy and reflux esophagitis are common in chronic diabetics. To gauge the adequacy of peristalsis, high-resolution esophageal manometry is considered as a reliable test. **Aim and Objective:** To compare the GI symptoms and high-resolution manometry parameters between type II diabetes patients and normal volunteers. Materials and Methods: This comparative study was conducted on 35 known type II diabetes mellitus patients (hemoglobin A1c [HbA1c] levels ≥6.5%) and 35 normal volunteers between the age group of 35 and 70 years. Sociodemographic details, drug history, disease duration, and GI symptoms were elicited among the study participants. HbA1c and High-resolution manometry were measured for all the participants. **Results:** The frequency of epigastric pain (31.48%), heartburn (34.28%), pharyngeal irritation (31.43%), and loose stools (37.14%) were significantly higher in the diabetics. Significant difference ($P < 0.05$) was observed in mean basal expiratory pressure (MBEP) and mean basal inspiratory pressure (MBIP) values between diabetics and controls. There was a positive significant correlation between esophagogastric junction contractile integral and MBIP ($r = 0.557$), MBEP ($r = 0.583$), Median Integrated Relaxation pressure ($r = 0.410$). **Conclusion:** Hyperglycaemia seems to be an independent risk factor for gastroesophageal reflux disease (GERD). Obesity significantly increments the occurrence of GERD in diabetics. Strict adherence to preventive measures such as weight reduction, diet management, glycaemic control will have a great impact on the occurrence of GERD in diabetics.

KEY WORDS: High-resolution Manometry; Hemoglobin A1c; Type 2 Diabetes Mellitus; Gastrointestinal Symptoms

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder with high blood glucose levels, either the pancreas does not produce adequate insulin or due to insulin resistance.[1] India is known as the "diabetes capital" of the world due to its growing prevalence and the causes may be attributed to lifestyle changes, genetic predisposition, and urbanization. Majority of diabetics may encounter episodes of gastrointestinal (GI) symptoms like heartburn and acid regurgitation.[2] The GI symptoms such as diarrhea, epigastric pain, nausea, heartburn, vomiting, dysphagia, constipation heartburn, and fecal incontinence are collectively known as Diabetic enteropathy.[3] Autonomic dysfunction can be the primary cause if a type II Diabetic person presents with upper GI symptoms most often.[4] Gastroesophageal reflux disease (GERD) was predominantly observed in diabetics than the euglycemic population.[5]

His angle and flap valve, Crural diaphragm, lower esophageal sphincter (LES) are the components of Esophagogastric

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Original Article

Biochemistry Section

Plasma Total Thiols and Total Thiol/Albumin Ratio in Patients Suffering from Depression

AS MEENAKSHI SUNDARAM[1], KRISHNANANDA PRABHU[2]

BY-NC-ND

ABSTRACT

Introduction: Increased oxidative stress has been reported in patients who suffer from depressive disorders. Albumin acts as a target for plasma protein oxidation during oxidative stress. The plasma thiols act as significant in-vivo antioxidants. Major SH-groups are found on the surface of albumin molecules. Since depressive disorders are related to oxidative stress. Only a few studies have been done that correlate plasma total thiols with major depressive disorder. The authors intended to draw a relationship between oxidative stress, thiols and major depressive disorder by estimating total plasma thiols and calculating the ratio of thiol/albumin. The study may throw some light in understanding whether the use of anti-oxidant supplements to counter oxidative stress in depressed patients.

Aim: To estimate plasma thiols, albumin and obtain a plasma thiol/albumin ratio in people suffering from depression and compare the levels with the control group.

Materials and Methods: This case control study was conducted in Kasturba Medical College, Manipal. Plasma thiols were estimated using Ellman's method. Plasma albumin levels were estimated using Bromo cresol green dye binding method. Mann-Whitney U test was used for analysing the data for total thiols and thiol/albumin ratio. A p-value <0.05 was considered significant.

Results: The study group was made up of 43 (22 males and 21 females) patients and the control group was made up of 40 (18 males and 22 females) healthy controls. Plasma thiol levels and the plasma total thiol/albumin ratio were significantly elevated (p<0.001) in cases (depression) as compared to that of the controls.

Conclusion: Plasma total thiols can be used as an early marker for understanding the risk for major depressive disorders and also be used as a prognostic indicator in the follow up of patients suffering from major depressive disorder who are under treatment.

Keywords: Major depressive disorder, Malon-di-aldehyde, Reactive oxygen species

INTRODUCTION

Elevated oxidative stress identified by elevated free radicals has been studied in patients with unipolar depressive disorder. As a common understanding, 12% of patients presenting to primary health care set up suffer from depression [1,2]. Antioxidant effects seen in antidepressants that are offered as a treatment of depression supports the concept that free radical stress may have a part in the pathology of depressive disorders. The stress may cause neuro-degeneration and lowered neurogenesis. There are data suggested that elevation in oxidative stress can be related to major depression and is proved by an elevated serum Malondialdehyde (MDA) levels and Super oxide dismutase (SOD) levels [3]. High levels of antioxidant enzymatic activity (SOD and glutathione peroxidase) have been found in patients with depression [4]. Albumin gets to be a target during oxidative stress for oxidation of plasma proteins. Since thiols are found to exhibit in-vivo anti-oxidant properties and the major free sulphydryl groups are present over albumin [4,5] the study was intended to estimate the same and correlate them with the depressive disorders. The study can help us to understand if in future anti-oxidants or the drugs that can increase total thiols, may be helpful in the treatment of depression.

MATERIALS AND METHODS

This was a case-control study conducted between September 2010 and September 2012.

The study was conducted at:

1. Departments of Biochemistry and Psychiatry, Kasturba Medical College and Hospital.
2. Department of Psychiatry, Dr. A. V. Baliga Memorial Hospital, Udupi.

After obtaining the approval from the Institutional Ethics Committee (Approval number: I.E.C./44/2011). of Kasturba Hospital, Manipal and the Director of Dr. A. V. Baliga Hospital, Udupi, study has been done in accordance with Helsinki guidelines for human studies. The study was conducted after getting the proper informed written consent of the cases' legal guardians and healthy controls.

Inclusion criteria: Cases: Patients of both sexes of age group 30 to 70 years, who were freshly diagnosed clinically as suffering from depressive disorders and not on any treatment for depressive disorders.

Controls: Healthy subjects of both sexes of age group 30 to 70 years, who have scored less than four major points (or) fourteen minor points after answering the Goldberg's general health questionnaire-28 [6].

Exclusion criteria: Subjects less than 30 years and more than 70 years of age/those suffering from depression but having symptoms of psychosis/with disorders suggestive of psychosis (schizophrenia)/tuberculosis/diabetes mellitus/Acquired Immunodeficiency Syndrome (AIDS)/thyroid gland based illness/seizure disorders /long standing defects in kidney, liver or brain function and any chronic illnesses and subjects who were on treatment for any of the above conditions. Subjects who were chronic alcohol and nicotine consumers were excluded too.

Serum Separation

Samples were collected by venepuncture into the Lithium heparin containers in the fasting period from each patient before admission.

Blood was allowed to clot in an upright position for atleast 30 minutes but not longer than one hour before centrifugation. Centrifugation was done for atleast 15 minutes at 2200-2500 RPM within one hour of collection.

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Can Early Thyroid Profiling Help Avert Spontaneous Abortions/Early Pregnancy Loss: A Retrospective Study

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Abstract

Presence of thyroid autoantibodies in euthyroid women can result in various complications such as miscarriages and pre-eclampsia. Women who are hypothyroid have an increased risk of anaemia, infertility, and preterm birth. Spontaneous miscarriages have been reported in association with women with antithyroid antibodies. This can be utilised as a supplementary marker for the mother's immune system failure. The goal of this study was to compare the thyroid profiles of pregnant women who had a normal delivery to those who had a high-risk obstetric history, and to see if early thyroid profiling can help prevent poor pregnancy outcomes. In conclusion, our analysis has demonstrated that women with abnormal values of T4, T3, anti-thyroid peroxidase (TPO), and TSH were associated with fetal demise when compared to women with normal values of T4, T3, anti-TPO, and TSH. Anti-TPO levels were shown to be elevated in women with a poor obstetric history, making early thyroid profiling improve outcomes in pregnancy. Hypothyroidism with increased TSH and anti-TPO levels may have a negative impact on obstetric history, resulting in the loss of an early pregnancy.

Categories: Internal Medicine, Obstetrics/Gynecology
Keywords: thyroid profile, pregnancy loss, spontaneous abortions, anti-tpo antibodies, tsh levels

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Introduction

Thyroid peroxidase (TPO) is an enzyme found in the thyroid gland's apical membrane that aids in the conversion of iodine ions (I-) to iodine (I) atoms, a process known as organification. For the formation of thyroxine (T4) and triiodothyronine, these iodine atoms will bind to the tyrosine residues connected to thyroglobulin molecules (T3). TPO plays a supportive role in the formation of the thyroglobulin molecule as well as thyroid hormone coupling events. Anti-TPO antibodies, which belong to the IgG immunoglobulin class, are autoantibodies directed against thyroid peroxidase protein [1]. These antibodies are seen in patients with thyroid dysfunction (hypothyroidism or hyperthyroidism), but there is also evidence of these antibodies in healthy individuals. In Hashimoto's thyroiditis, anti-TPO antibodies can be one of the autoantibodies targeting the gland [2]. Euthyroid women with autoantibodies can cause complications in pregnancy such as repeated miscarriages and pre-eclampsia, according to one study [3]. Euthyroid women are also at a higher risk of anemia, infertility, and preterm deliveries [4]. According to one study, spontaneous miscarriages are linked to a higher incidence of antithyroid antibodies [5]. This can be used as a secondary marker for immune system failure in the mother [6]. The goal of this study was to compare the thyroid profiles of pregnant women who had a normal delivery with those who had a complicated obstetric history, and to see if early thyroid profiling can help prevent bad pregnancy outcomes.

Materials And Methods

From December 2019 to December 2020, investigators at PSG Institute of Medical Sciences and Research in Coimbatore conducted a retrospective analysis. After receiving approval from the institutional ethics committee, we reviewed data from 164 patients, including 81 patients with thyroid problems and 83 healthy pregnant women. Age, BMI, anti-TPO levels, T3, T4 levels, mode of delivery, and thyroid medication were all taken into consideration. The information obtained on chart review of the 164 patients was documented on a password-protected online tool and accessed by the investigators only. None of the patients protected health information (PHI) were documented as per the institutional Health Insurance Portability and Accountability Act (HIPAA) policy. The mean, standard median, and confidence interval were used to express all quantitative data. A student t-test was used to quantify the magnitude of the mean difference between groups, and a p-value of 0.05 was considered statistically significant.

Results

The mean age group in normal individuals was 27.67 years old, while thyroid patients were 27.43 years old.

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Original Article

Biochemistry Section

Plasma Myeloperoxidase and Total Sialic Acid as Prognostic Indicators in Acute Coronary Syndrome

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ABSTRACT

Introduction: Acute Coronary Syndrome (ACS) is the leading cause of death worldwide. Oxidative stress and inflammation play important role in the destabilization of plaques leading to ACS. Markers which reflect this pathophysiologic mechanism may have prognostic value. Myeloperoxidase (MPO) and Sialic acid are markers of inflammation and oxidative stress. Both these markers are increased in patients with ACS. Their prognostic value in ACS is not well established.

Aim: To analyse the prognostic value of plasma myeloperoxidase and total sialic acid levels in patients with suspected acute coronary syndrome.

Materials and Methods: A prospective study was conducted on 93 consecutively admitted patients with chest pain from July 2011 to September 2011. Plasma MPO and total sialic acid levels on admission were estimated spectrophotometrically. These were compared with extent of disease, development of complications during the hospital stay, left ventricular ejection fraction and mean duration of stay in hospital.

Results: Plasma MPO and total sialic acid levels were significantly higher in patients with myocardial infarction than those with unstable and stable angina ($p<0.001$ and $p<0.007$ respectively). The levels of plasma MPO and sialic acid levels were significantly higher in patients who developed complications like heart failure, arrhythmias, renal failure during their stay in hospital ($p<0.011$ and $p<0.006$ respectively). Ejection fraction was significantly low in patients with high MPO levels ($p<0.011$).

Conclusion: In patients with ACS, plasma MPO and total sialic acid levels on admission could predict the development of complications during their hospital stay. MPO levels correlated with ejection fraction in patients with ACS.

Keywords: Left ventricular ejection fraction, Major adverse cardiac event, Myocardial infarction

INTRODUCTION

Ischemic Heart Disease (IHD) is the leading cause of death worldwide, accounting for 12.7% of global mortality [1]. Acute Coronary Syndrome (ACS) covers a wide spectrum of clinical conditions ranging from unstable angina to Non-ST Elevation Myocardial Infarction (N-STEMI) and ST Elevation Myocardial Infarction (STEMI). Insufficient coronary perfusion due to variable degrees of acute plaque change, thrombosis and vasospasm over chronic, progressive atherosclerotic narrowing of the coronary arteries is the underlying pathology. Although they share this pathophysiology, outcomes and effective management strategies differ by the patient's place in the spectrum [2]. As new treatments and management guidelines emerge for care of patients with ACS, risk stratification is the cornerstone in the initial evaluation of these patients [2]. Simple prognostic markers that reflect pathophysiologic mechanism may aid in tailoring the therapy to match the intensity of patient's disease [3].

Oxidative stress and inflammation play vital roles in pathogenesis and progression of Coronary Artery Disease (CAD) leading to Acute Coronary Syndromes (ACS) [4]. Inflammation is implicated in all stages of atherosclerosis, from early lipid deposition to plaque rupture. Inflammation mediates the weakening of fibrous cap and makes the plaque more susceptible to rupture, thus precipitating ACS [5]. A study shows that the inflammatory process persists even after the resolution of clinical symptoms and there is a link between recurrent ischemic episodes and persistent inflammation [6]. Thus, inflammatory biomarkers may provide independent information regarding pathophysiology, prognosis and help in formulating optimal therapeutic strategies.

Myeloperoxidase (MPO) is present in neutrophils and monocytes and is released when they undergo degranulation [7]. It is a marker of inflammation and oxidative stress that has been consistently demonstrated to be elevated in patients with ACS [7]. Several experimental and epidemiologic studies indicate MPO as a candidate marker for clinical application [7]. In addition to being a causative factor for CVD and acute cardio vascular events, MPO can also extend the prognostic information gained from traditional biochemical markers. So, further investigation is needed to expand our knowledge on the prognostic role of MPO in ACS.

Sialic acids are 10 carbon derivatives of neuraminic acid. They comprise the terminal sugar of the oligosaccharide chain in glycolipids and glycoproteins. Studies have shown that serum total sialic acid is elevated in cardiovascular disease; which is attributed to the increase acute phase reactants in these patients [8]. Following acute myocardial infarction, shedding or secretion from damaged cell can lead to elevated sialic acid [9]. Though many studies have evaluated its role in pathogenesis of atherosclerosis its role as a prognostic marker has not been studied.

The purpose of the present study was to analyse the prognostic value of plasma myeloperoxidase and total sialic acid levels in patients with suspected acute coronary syndrome. In samples of patients with suspected ACS, myeloperoxidase and total sialic acid levels were measured and their concentrations were compared with certain prognostic markers of ACS.

MATERIALS AND METHODS

This prospective study was conducted at the Department of Biochemistry, PSG Institute of Medical Sciences and Research, Coimbatore, India. It was approved by the Institutional Human Ethics Committee (ethical clearance letter no:11/206).

Study subjects

Consecutively admitted patients with chest pain in PSG Hospitals from July 2011 to September 2011 were studied. Patients with cardiac chest pain as shown by ECG changes or elevated cardiac Troponin T levels (> 0.03ng/ml) were included in the study. Patients

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COVID-19

Biochemistry and Molecular Biology Education WILEY

Online team based learning in teaching Biochemistry for first year MBBS students during COVID-19 pandemic

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Abstract

The emergence of Corona Virus Disease-2019 (COVID-19) pandemic had unprecedented effects on medical education worldwide. Sustaining student engagement in virtual learning is an arduous task. Team based learning (TBL) is a learner-centered approach that can facilitate better student engagement. Adapting TBL to online platform may address the challenges faced in virtual learning. This study was conducted to implement and evaluate online TBL among first year MBBS students in Biochemistry. After obtaining informed consent, three online TBL sessions were planned. The Individual Readiness Assessment Test, Group Readiness Assessment Test, and team application were assigned in Google classroom. The students used online platforms for their team interactions. After submitting their assignments, they joined online discussion with facilitator. Peer evaluation was done via Google forms. At the end of all the sessions, the students' perceptions on the process were collected using a structured anonymous feedback with open comments. Analysis of their feedback showed that the students found the process useful and it helped in fostering their team skills. Five major themes emerged on content analysis of the open comments; 'Enriched team skills', 'Gain in knowledge', 'Impact on attitude', 'Interaction during pandemic', and 'Emotional responses'. Students perceived that the online TBL is effective in improving their engagement, learning, and team skills.

KEYWORDS

medical students, pre-clinical, small group discussion, team skills, virtual learning

1 | INTRODUCTION

Indian Medical curriculum faced two challenges in the year 2019, first, being the most anticipated shift from traditional curriculum towards Competency Based Medical Education and second, the emergence of COVID-19 pandemic. When the new curriculum was rolled out, colleges had to close mid-academic year due to COVID-19 lockdown restrictions across the country. The break in academics, initially expected to be for a brief period, continued for more than a year.

Medical schools had to witness a sudden transition from conventional face-to-face learning to virtual learning. This was a demanding task and the stakeholders faced many challenges in the process. Lack of technical and time management skills, inadequate infrastructure, and poor communication at various levels were some of the challenges faced by the faculty and institutions.[1] Students faced several problems; anxiety, distractions, isolation, lack of interaction with peers, and difficulty in staying focused in online class.[2]

Team based learning (TBL) is a highly learner-centered, group based instructional approach that can

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Letter to Editor

Biochemistry Section

Awareness about Research Ethics among General Practitioners not Attached with Research Institutions

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Keywords: Bioethics, Workshops, Private doctors

Dear sir,

Research in medical field is important to help and improve our knowledge in medicine. Especially there is increase in research in India in the past few decades. Research ethics is an important factor in any research protecting the welfare of participants. Many International guidelines exist for the same [1,2]. Complaints regarding research show that there is increase of awareness among public as well as the practices of doctors. General practitioners working in a hospital do conduct clinical trials. This study was designed with the aim to evaluate the awareness and knowledge of general practitioners about research ethics.

After obtaining approval from Institutional Ethics committee, a cross sectional study was conducted among 25 general practitioners in the local town. They were explained about the study and asked for willingness to participate. After getting informed written consent, they were recruited. A questionnaire was made by the authors based on literature. First part of questionnaire included demographic data, details about research involving human participants in the past and present and participation in any programs on research ethics. Second part contained a series of questions or statements which tested the knowledge of the doctors in research ethics.

Twenty five general practitioners or working in hospitals participated in the study. All the participants filled up the questionnaire and returned it to us whose identity was blinded. Mean age of the participants was 41.5 years. Of 25, three were MBBS, three were PG Diploma, 15 were MD/MS/ DNB holders and two were super specialty. Currently one was involved in research.

Eight participants had conducted clinical trials before and 76% were interested in future research. 8% participants had undergone formal training but, 52% felt that they are familiar with Research Ethics. All the 25 participants were aware that patient confidentiality has to be maintained. A 96% participants knew that ethical clearance has to be obtained for all the projects and all potential risks has to be explained to patients before enrolling them in the study. A 92% participant knew that patients had the right to withdraw from study without forgoing their right for treatment.

A total of 16% participants responded that stored samples can be used in other research projects without patient permission. A 16% were also not clear about conflict of interest while conducting clinical trials, 16% felt that ethical committee need not be informed about the progress of the study. Considerable number of participants (28%), were unclear about consent issues in vulnerable populations namely – children, students, etc.

Medicine is a growing field and research and it forms the platform for expansion of knowledge. Doctors working in research institutes undergo formal training in research ethics and general practitioners have less access to the same. Most of the participants knew the importance of patient confidentiality which is similar to the study conducted by Gopinath NM et al., to study the knowledge about research ethics among dental faculty [3]. But they were not aware of the ethical issues involving vulnerable population. Participants were knowledgeable about the need for ethics committee approval before starting the research but they felt that reporting study progress only delayed research. This is similar with El-Dessouky HF et al., study among dental faulty [4].

Though half of the participants said they are knowledgeable about research ethics, only 2 out of 25 participants had undergone any formal training which indicates a need for workshops or Seminars to general practitioners.

Knowledge about research ethics involving human subjects among Doctors not associated with research institutions is fairly good even though majority of them have not undergone formal training. Probably this knowledge was acquired during their PG training. However complete knowledge about research ethics involving human subjects is mandatory for all Doctors. This gap can be covered by conducting periodic training on Research ethics for all medical practitioners.

REFERENCES

[1] Declaration of Geneva (1948). Adopted by the General Assembly of World Medical Association at Geneva Switzerland.
[2] The Nuremberg Code (1949) Trials of War Criminals before the Nuremberg Military Tribunals under Control Council Law No. 10, Washington DC: USGovernment Printing Office. 2: 181-182.
[3] Gopinath NM, John J, Senthil kumar E, Nagappan N. Knowledge Awareness and Attitude about Research Ethics among Dental Faculties in India. *J Contemp Dent Pract*. 2014;15(5):608-13.
[4] El-Dessouky HF, Abdel-Aziz AM, Ibrahim C, Moni M, Fadl RA, Silverman H. Knowledge, Awareness, and Attitudes about Research Ethics among Dental Faculty in the Middle East: A Pilot Study. *International Journal of Dentistry*. 2011;2011:694759, 13 pages.

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Role of serum matrix metalloproteinase-2 and -9 to predict breast cancer progression

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ABSTRACT

Objective: Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, have been reported as putative tumor markers because of their involvement in cancer invasion and metastasis. The aim of our study was to elucidate the possible role of MMP-2 and -9 as serum prognostic biomarker for breast cancer classification and correlate it with the clinicopathological variables.

Design and Methods: Our study consisted of 60 females with primary breast cancer, 40 cases of benign breast disease and 60 healthy female volunteers as controls. The serum MMP-2 and -9 levels were quantitatively measured by ELISA technique.

Results: A significantly raised MMP-2 and MMP-9 levels were observed in breast cancer patients. Significant rise in serum MMP-9 concentration was found in patients presenting with metastasis as well as in those cases who presented with a duration of less than 1 year. ROC analyses depicted a serum cutoff value of 315 ng/mL for MMP-9 to discriminate the breast cancer patients from the control group.

Conclusion: Our results suggest that serum MMP-9 level is a better marker than serum MMP-2 in predicting the breast cancer development and progression.

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Introduction

Breast cancer continues to be one of the leading cause of cancer related mortality in women in the Western world [1]. It is well documented that breast cancer patients with the same stage of disease can have markedly different outcome and therapy responses [2]. The current screening methods like mammography, self breast examination and clinical breast examination are not sufficient for an early diagnosis in all these patients. Hence there is a need for biomarkers that can complement existing screening tools and used in clinical decision making to predict the responsiveness to therapies. An ideal biomarker should correlate well with disease and be easily detectable in blood. So far no single soluble biomarker for breast cancer with these attributes has been identified.

The mammary tissue microenvironment, consisting of structural, biochemical and cellular entities, is known to contribute to tumor cell fate [3]. Degradation of the stromal connective tissue and basement membrane components are key elements in tumor invasion and metastasis. Tumor invasion and metastasis are the major causes of treatment failure or death for carcinoma patient, and hence, molecules involved in these processes are obvious candidates to be identified as new prognostic markers in breast cancer. The matrix metalloproteinases (MMPs) in humans are a family of 23 members that have been associated with remodeling of the extracellular matrix (ECM). Because of their involvement in processing of ECM, MMPs were implicated in cancer invasion and metastasis [4,5]. Because tissue MMP may also be secreted into the blood stream and increase the circulating levels, it is believed that MMP profile in the blood could serve as biological markers for disease onset, progression and monitoring [6]. Gelatinase A (MMP-2) and gelatinase B (MMP-9) differ from other MMPs because of their ability to degrade gelatin and type IV collagen, the main component of basement membrane (BM), which is the main barrier separating in situ and invasive carcinoma [7]. Recent evidence suggests that MMP-2 and MMP-9 may also be involved in breast cancer initiation and growth through complex interactions with the main oncogenes and tumor suppressor genes involved in early stages of tumorigenesis [8]. Recent studies in breast cancer patients have shown that an increase activity level of both MMP-2 and MMP-9 in their sera is a strong and independent indicator of biologically aggressive disease with an unfavorable outcome and that their increased level in patients with advanced TNM staging might reflect the severity of invasive breast cancer [9–11]. Especially in breast, colorectal, bladder and ovarian cancers, serum MMP-9 levels have been shown to have prognostic significance [12]. Few studies have associated MMP-2 positivity with unfavorable prognosis in both premenopausal and postmenopausal node positive breast carcinoma patients [13–15], while few others have suggested that MMP-2 negativity may be linked with a favorable prognosis in node negative breast carcinoma [16]. Although available reports suggest that elevated levels of MMP-2 and MMP-9 occur in serum and plasma of

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ARTICLE

Biochemistry and Molecular Biology Education WILEY

Effectiveness of team teaching in biochemistry lectures for undergraduate students

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Abstract

Team teaching is an innovative method to engage the large group and make lectures more interesting. The aim of the study is to evaluate the impact of team teaching in first year biochemistry lecture classes. After obtaining ethical clearance, 150 first year medical undergraduate students participated in the study on a voluntary basis. Topic identified for team teaching was "Protein biosynthesis" in Biochemistry, which was designed to be carried out in 5 h of lecture by three teachers. At the end of the team teaching module and traditional lectures, a structured anonymous feedback was obtained from the students. At the end of each class, students' understanding of the lecture was assessed by an MCQ test comprising 5–6 items, which mainly tested the recollection and understanding levels of Bloom's taxonomy. This was compared with traditional lectures. The mean MCQ test score was significantly higher for team teaching when compared to traditional teaching. The students had increased attention span and were able to answer questions from later part of the class in team lectures. The students had reported that lectures taught by team were more interesting and informative. They also felt that the team was more receptive to the doubts raised, stimulated discussion on the topic and had a better rapport with the class.

KEYWORDS

medical education, team-teaching, undergraduate

1 | INTRODUCTION

Lecture is still the dominant teaching strategy in Medical schools worldwide.[1] Although lecture has the advantage of organized presentation of concepts to a large body of audience with economical use of time, it has always suffered the disrepute of being boring and unattractively long. Attention span for most of the adult learners is said to be around 20 min.[2] Capturing the attention of the large group and maintaining it through the lecture, encouraging active learning are some of the challenges faced by the lecturer. Many strategies have been explored to make the lectures more interactive and interesting. With an easily distracted audience of 150 (annual intake in most medical colleges in India), taking an effective lecture is a Herculean task.[3]

Collaborative teaching or Co-teaching involves a group of instructors working purposefully, regularly, and cooperatively to help a group of students learn. There are many possible models of Co-teaching—supportive, parallel, alternative, complementary, and team teaching. Team teaching is defined as "well planned, team taught lessons,

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CASE REPORT

A Case of Light Chain Deposition Disease (LCDD) in a Young Patient

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Abstract Light chain disease is a variant of multiple myeloma in which the malignant population of marrow cells produces free monoclonal light chains but no heavy chain or complete immunoglobulin. The monoclonal light chains are small enough to be freely filtered by the kidneys and become Bence–Jones protein. Light chain disease comprises about 18% of multiple myeloma patients. Here we present a case report of a 38-year-old man who initially presented with complaints of pain in back and low grade fever off and on. He was found to have collapse of D9 and D12 vertebrae along with ascites and right pleural effusion and massive proteinuria. Multiple myeloma was considered as a differential diagnosis based on the investigations but eventually the patient was lost to follow up. This case is reported here as the light chain variant of multiple myeloma leading to deposition disease is less commonly reported and presents considerable difficulties in diagnosis.

Keywords Multiple myeloma · Light chain disease · Bence–Jones protein

Introduction

Light chain disease (LCD) is a variant of multiple myeloma in which the malignant plasma cells of the bone marrow produce free monoclonal light chains [1]. These light chains can be deposited in many organs leading to light chain deposition disease [2]. Renal involvement is most frequent and it manifests as nephrotic range proteinuria and renal insufficiency. Light chain deposition disease is found in 5% of the myeloma patients at autopsy. The monoclonal light chains are small enough to be freely filtered by the kidneys and become Bence–Jones protein. The clinical features [3] include:

1. A more malignant course as compared to classical myeloma.
2. Extremely rapid doubling time.
3. Tendency for more osteolytic lesions.
4. Increased hypercalcemia.
5. Higher incidence of renal failure (including azotemia at presentation) as compared to other patients with multiple myeloma.
6. Higher incidence of amyloidosis and plasma cell leukaemia in the terminal stages of the disease. Renal failure is the principle cause of death in these patients.

Here we report a variant of multiple myeloma manifesting as excess kappa light chain production leading to extramedullary involvement in the form of hepatosplenomegaly, ascites, pleural effusion and renal failure.

Case Report

A 38-year-old male presented to the hospital with complaints of back ache for 2 months associated with low grade fever off and on. There were no complaints of bowel and bladder. He admitted to having a decreased appetite associated with weight loss. There was no history of any trauma or swelling in any part of the body.

Clinical examination of the abdomen revealed hepatosplenomegaly and presence of free fluid. On CNS examination power was 4/5 in bilateral lower limbs. B/L Plantars

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Impact of a Comprehensive Early Clinical Exposure Program for Preclinical Year Medical Students

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Abstract

Purpose: To study the impact of an early clinical exposure program designed to provide a wide variety (cognitive, affective and psychomotor) of learning experiences for the preclinical year students.
Method: One hundred and fifty preclinical students were posted in small groups to selected departments – Transfusion medicine, Catheterization lab, Simulation lab, Radiology, Neurology, Nephrology, Respiratory medicine and General surgery. Each student had atleast ten hours of clinical exposure under this program. The program was evaluated through a series of pre and post-test questionnaires, which were designed based on the learning objectives of each session. Students who wished to participate in the program evaluation gave informed consent, took up the pre / post test and were also asked to give their written open comments about the program.
Results: There was a significant increase in the post-test scores (ranging from 9.14 ± 2.67 to 36.65 ± 6.62) when compared to the pre-test scores (ranging from 7.94 ± 2.31 to 28.69 ± 6.11) for all the sessions (p value < 0.001, $n = 144$). Analysis of the open feedback showed that the program had significant impact on the cognitive, psychomotor and affective domains. "Application of basic sciences in clinical practice", "motivation to learn", "got familiar with various specialties", "insight about what the patient undergoes" were the themes identified from the open comments.

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Original Article

Biochemistry Section

Effect of Red Yeast Rice and Coconut, Rice Bran or Sunflower Oil Combination in Rats on Hypercholesterolemic Diet

SUMITRA GOVINDARAJAN[1], KISHORE VELLINGIRI[2]

ABSTRACT

Introduction: Dietary supplements provide a novel population based health approach for treating hyperlipidemias. Red yeast rice is known to have lipid lowering effects. Combination of red yeast rice with various oils is taken by different population around the world.

Aim: In this present work, we aimed to compare the effects of red yeast rice with different oil (coconut, rice bran and sunflower oil) supplementations on lipid levels and oxidative stress in rats fed on hypercholesterolemic diet.

Materials and Methods: A Randomized controlled study was conducted on 28 male Sprague Dawley rats. It included 4 arms-Control arm (hypercholesterolemic diet), Test arm A (hypercholesterolemic diet +Red yeast rice + Rice bran oil), arm B (hypercholesterolemic diet +Red yeast rice + Coconut oil) and arm C (hypercholesterolemic diet +Red yeast rice + Sunflower oil). At the end of one month, serum cholesterol, triglycerides, MDA and paroxanase was measured. The mean values of analytes between the different groups were compared using student 't-' test.

Results: The rats fed with red yeast rice and rice bran oil combination showed significantly lower levels of serum cholesterol, triglycerides and MDA when compared to the controls. The serum paroxanase levels were significantly higher in this group when compared to the controls. The rats fed with red yeast rice and coconut oil combination showed significantly lower serum cholesterol and MDA levels when compared to the controls. The mean triglyceride and paroxanase levels did not show any statistically significant difference from the controls. The rats on red yeast rice and sunflower oil combination did not show any statistically significant difference in the lipid levels and oxidative stress parameters.

Conclusion: The food combination which had best outcome in preventing the development of hyperlipidemia and oxidative stress in rats fed with hypercholesterolemic diet was red yeast rice and rice bran oil. Combining red yeast rice with coconut oil and sunflower oil gave suboptimal benefits.

Keywords: Cholesterol, Hyperlipidemia, Lipid peroxidation, MDA, Paroxanase, Triglyceride

INTRODUCTION

Cardiovascular disease is a major cause of mortality and morbidity worldwide. In India, rapid urbanization has led to rising trends in the prevalence of conventional risk factors of cardiovascular diseases [1]. Steadily increasing prevalence of Cardio vascular disease and its risk factors highlight the need to promote primary prevention strategies [1].

Hyperlipidemia is a major risk factor for cardio vascular diseases. A recent study states that prevalence of hypercholesterolemia in India is about 14% and hypertriglyceridemia is about 30% [2]. Though several pharmacological agents are used to lower cholesterol levels such as statins, niacin, resins and fibrates, each has its array of adverse effect [3]. Diet therapy could play a dominant role in the treatment of hyperlipidemia [4]. In addition to being inexpensive, it has the potential to become habitual and augment drug therapy [4]. Most importantly diet therapy can be adopted by the entire family and thus help in primary prevention of acquired hyperlipidemias [4]. Over years many lipid lowering dietary factors have come to light from various cultures across the world [5]. Many of these are being studied for their applicability and effectiveness in different populations [5].

Red yeast rice, a fermented rice product used widely in China and Japan, is known to have lipid lowering effects [6]. Red yeast rice is a promising therapeutic alternative in patients with hypercholesterolemia [5]. Incidentally in regions which traditionally use red yeast rice, rice bran oil is also widely used. Rice bran oil has also been shown to have cholesterol lowering properties [7]. The effect of these two could be additive.

In various regions of India, traditionally people have been using different cooking oils ranging from coconut oil, gingelly oil, mustard oil, to safflower and sunflower oil. The efficacy of the red yeast rice in lowering lipid levels and oxidative stress when combined with these widely used edible oils has not been studied.

AIM

In this present work, we aimed to compare the effects of red yeast rice with different oil (coconut, rice bran and sunflower oil) supplementations on lipid levels and oxidative stress in rats fed on hypercholesterolemic diet.

MATERIALS AND METHODS

A randomized controlled study was carried out in the animal facility of PSG Institute of Medical Sciences and Research (PSG IMSR), Coimbatore, during the period Apr 2014 to Aug 2014. The study was approved by the Institutional Animal Ethics Committee and the experiments were carried out according to the guidelines of Institutional Animal Ethics Committee, PSG IMSR. Twenty eight healthy 2-3-month-old male Sprague Dawley rats, weighing 200-300 gm, were included in the study. Irritable rats and overweight rats were excluded. These were housed in steel cages under controlled temperature (22±2°C) and humidity (55±5%) with a fixed 12 hours light–dark cycle. The rats were randomly divided into 4 groups - control (n=10), Group A (n=6), Group B (n=6) and Group C (n=6).

Hypercholesterolemic diet [8] contained 1% cholesterol, 0.2% bile salts and 5% butter added to the base feed. The base feed used was commercial Laboratory animal feed preparation supplied by Sai Industries, Bangalore. Its composition was Carbohydrates 65%, crude protein 21.5%, crude fat 4.4 %, crude fiber 3.2%, total ash 5.1%, moisture 9.7%, calcium 1.4% and phosphorous 0.4%. In addition to the hypercholesterolemic diet, the test groups were

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Atherogenic indices in stroke patients: A retrospective study

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Keywords
Stroke; Lipid Profile; Atherogenic Indices

Abstract

Background: Stroke makes a significant cause of morbidity and mortality worldwide. Although derangements in the lipid profile have been suggested as a risk factor for the development of stroke, various studies show inconsistent results on the association between lipid profile and stroke. A very few studies have commented on the status of lipid indices in stroke patients.

Methods: After obtaining ethical medical records of the study populations were analyzed, and data collected from patients admitted to the hospital with clinically diagnosed stroke and control group consisted of apparently healthy volunteers selected from the master health checkup department. Baseline characteristics and lipid profile parameters and the number of days of hospital stay for stroke patients were collected. Lipid indices were calculated using following formulae. Atherogenic index of plasma (AIP) = log triglyceride/high-density lipoprotein cholesterol (HDLc), Castelli's Risk Index (CRI-I) = Total cholesterol/HDLc, CRI-II = Low density lipoprotein cholesterol/HDLc, atherogenic coefficient (AC) = (Total cholesterol−HDLc)/HDLc, and non-HDLc (NHC) = Total cholesterol–HDL.

Results: The study included 620 participants of which 290 were stroke patients and 330 healthy volunteers. 61% of stroke patients were hypertensives and 38% were diabetics 28% were both diabetic and hypertensives. In this study, the lipid parameters and the indices were significantly higher in stroke patients than the control group. Three indices, namely, CRI-I, AC, and NHC were found to be contributing to the risk of stroke significantly. There was no statistically significant correlation between the duration of hospital stay and lipid indices or individual parameters of lipid profile.

Conclusion: In this study, the atherogenic lipid indices were significantly higher in stroke patients compared to controls.

Introduction

Stroke is defined as "an acute neurologic dysfunction of vascular origin with sudden (within seconds) or at least rapid (within hours) occurrence of symptoms and signs corresponding to the involvement of focal areas in the brain."[1] It is one of the leading causes of mortality and disability globally.

The prevalence of stroke in India varies in different regions of country, and the estimated prevalence rates increase from 0.3/1000 in < 45 years age group to 12-20/1000 in the 75-84 years age group.[2]

There are several studies in India determining risk factors of stroke. Goldstein et al.[3] described the various non-modifiable and modifiable risk

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Comparison of albumin-bilirubin grade with Child-Pugh and Model for End Stage Liver Disease scores in chronic liver disease

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ABSTRACT

Introduction: chronic liver diseases are characterized by injury to hepatocytes, chronic inflammation and progressive substitution of liver parenchyma by scar tissue or fibrosis. Albumin-bilirubin grade (ALBI) includes serum albumin and bilirubin levels, two most commonly performed and cost-effective parameters measured in the Clinical Laboratory. This study aims to establish an association between ALBI grade and Model for End stage Liver Disease (MELD) and Child-Pugh (CP) scores among chronic liver disease patients.
Methods: after ethical approval, information such as age, gender, provisional diagnosis, serum albumin and serum total bilirubin levels, prognostic scores of patients with liver disease were obtained from patient records. Albumin-bilirubin grading system was calculated as following: [$\log_{10}$ bilirubin (µmol/L) × 0.66] + [albumin (g/L) × −0.0852].
Results: this cross-sectional descriptive study recruited 100 patients with chronic liver disease and 100 healthy controls. Alcoholism was the common aetiology among the cases. ALBI was calculated; the mean (SD) grade among cases and controls were -0.94 (0.15) and -3.17 (0.23) respectively with a p-value of <0.001.There was statistically significant difference in ALBI grades between CP scores B and C (p value = 0.001). Pearson's correlation between MELD score and ALBI grade showed a statistically significant correlation (r = 0.723 with significance 0.001).
Discussion: our study demonstrated that there was a significant association between ALBI grade and the most commonly used prognostic scores such as CP and MELD.

INTRODUCTION

Chronic liver diseases are characterized by injury to hepatocytes, chronic inflammation and progressive substitution of liver parenchyma by scar tissue or fibrosis. Chronic liver diseases arise from various causes; the most common being alcoholic and non-alcoholic fatty liver diseases (NAFLD). However, other relevant causes exist: infections, toxins, metabolic and autoimmune causes, malignancy.

The prognosis of chronic liver diseases is being assessed by scores like Child Pugh (CP) and Model for End stage Liver Disease (MELD). CP score calculation requires albumin, bilirubin, International Normalized Ratio, presence of hepatic encephalopathy and ascites. The presence of encephalopathy and ascites require a subjective evaluation; albumin serum concentration and ascites are related to each other leading to variations in grading. MELD score calculation requires serum creatinine, bilirubin and International Normalized Ratio; it is well known that serum creatinine values are influenced by factors like age, gender, muscle mass causing a large variation among individuals. A number of studies have shown that variability in creatinine and International Normalized Ratio determination in different laboratories have led to inconsistency in MELD scores (1,2). As CP and MELD scores show the mentioned limitations, there is an urgent need for other prognostic markers.

One novel indicator is the Albumin-Bilirubin (ALBI) grade utilized by Johnson et al. in 2015 for hepatocellular carcinoma (3). The ALBI grade includes serum albumin and bilirubin levels, the two most commonly performed and cost-effective parameters that are a part of routine liver function tests. Since the first paper, various studies have been carried on to verify its effectiveness and usefulness in hepatocellular cancer. Wang et al. conducted a study to compare ALBI grade with CP score and concluded that ALBI was better than CP in distinguishing patients into different groups based on prognosis of liver cancer; post-operative survival was also better defined in ALBI-classified patients than in CP-classified groups (4). A massive research had been done

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Original Article

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Education Section

Assessment of Palliative Care Awareness among Undergraduate Healthcare Students

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ABSTRACT

Introduction: Palliative care knowledge is being given meager importance in the curriculum of medical and other allied medical sciences. It is vital that all health care practitioners including medical, pharmacy, physiotherapy and nursing are aware and apply the best principles of palliative care.

Aim: To assess the awareness of palliative care among undergraduate students of medical, nursing, pharmacy and physiotherapy.

Materials and Methods: The study population included total of 200 students. Among 200 students, 50 were from each of the colleges of medicine, nursing, pharmacy and physiotherapy. After obtaining informed consent, questionnaire was given. The questionnaire contained the sociodemographic profile and 35 statements under nine groups, for which the respondents were expected to answer one out of the three options (Yes, No, Don't know). The groups of statements deal with palliative care definition, its philosophy, communication issues, non-pain symptoms, medications use and context of application of palliative care.

Results: It was found that less than 20% of nursing students were unaware of palliative care. Among the undergraduates of college of pharmacy, more than 50% had no knowledge of palliative care. More than 80% of physiotherapy, nursing and medical students agree that death should occur without any pain or symptoms. The need of palliative care was well understood by more than 70% of students of physiotherapy, pharmacy, nursing and medical colleges.

Conclusion: Basic knowledge about palliative care was inadequate among the undergraduate students related to healthcare.

Keywords: Knowledge, Questionnaire-based study, Undergraduate medical education

INTRODUCTION

The number of patients with cancer and other life-limiting diseases continue to increase. The last decades have seen a rapid growth in the concern for the severely ill and dying patients. Palliative care is a methodology that improves the quality of life of patients and solves the problems associated with life-threatening illness, faced by the patients and their families. The prevention and relief of suffering can be achieved through early diagnosis, assessment and treatment not restricting to physical pain and other problems but also in other aspects like psychological, social, etc., [1]. Palliative care is not restricted to cancer or terminally ill patients alone. Palliative care also includes diseases that last for many years and other life-threatening conditions. Health professionals function largely within a culture that focuses on cure, and many avoid the patient who is dying inspite of a significant population suffering from end-of-life illnesses.

The significance of palliative care in the undergraduate syllabus of health professionals has been well recognized in the western world. Following this, it was introduced in the St John's National Academy of Health Sciences [2]. Most countries have led to the establishment of palliative care services in a variety of settings with varied staff composition and different quality standards. Palliative care education at medical schools has also increased within the last years [3]. However, undergraduate education in palliative care shows wide variation and there is no standardized core curriculum. There is considerable gap in the knowledge of palliative care among undergraduate students in our country according to Sadhu S et al., [4].

Palliative care is a multidisciplinary approach. A palliative care team may consist of a general physician, specialist, nurses, local pharmacist, medical practice social worker, physiotherapist and many others. In order to provide effective care, the members of the team need to have a common ideal and understanding of the role and contribution each of them have to make an order to achieve successful outcomes.

This study aims to assess the level of adequacy in various aspects of palliative care among undergraduate students of medicine, nursing, pharmacy and physiotherapy. This study may thus pave a way to include palliative care in the syllabus of undergraduate health care students.

MATERIALS AND METHODS

This cross-sectional questionnaire based study was conducted between November 2016 and March 2017. After obtaining approval from the Institutional Human Ethical Committee, questionnaires were given to final and pre-final year students of medicine, nursing, physiotherapy and pharmacy studying in a tertiary care teaching hospital in Southern India. A written informed consent form was given and those who were willing to participate in the study were included in the study and those not willing to participate were excluded. A total of, 200 students, 50 students each from college of nursing, physiotherapy, pharmacy and medicine gave consent to participate in the study.

Sample size calculation was done according to the formula $4pq/d^2$. (p-prevalence of awareness of palliative care from previous studies; q-100 minus prevalence percentage; d-precision which can range from 10%-20%). The prevalence of awareness of palliative care from previous studies was around 60%. The precision was kept around 15% and the calculation provided a sample size of 145.4. We included a few more participants to improve the strength of the study. The questionnaire contained the sociodemographic profile and 35 statements under nine groups for which the respondents were expected to answer one of three options (Yes, No, Don't know). The groups of statements deal with palliative care definition, its philosophy, communication issues, non-pain

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PENTRAXIN-3 AS AN EMERGING RELIABLE NON-INVASIVE SERUM MARKER IN THE DETECTION OF ALCOHOLIC FIBROSIS

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Keywords:

Pentraxin-3, alcoholic liver disease, fibrosis, Child Pugh score, MELD score

Abstract

Alcoholic cirrhosis is the end spectrum of alcoholic liver disease, which includes fatty liver, alcoholic hepatitis, fibrosis, cirrhosis and superimposed hepatocellular carcinoma. Fibrosis is central to the pathology of all types of chronic liver disease. Pentraxin-3 is involved in numerous roles like regulation of inflammation and innate resistance to pathogens. It is strongly expressed on collagen bundles and fibrotic areas. After ethical clearance, demographic and laboratory details were collected from medical records. Leftover samples were used for serum pentraxin-3 estimation using an ELISA kit. The study included a total of 40 participants who were males 20 known cases of alcoholic liver disease and the control group included 20 healthy controls. The average value of serum pentraxin-3 in alcoholic liver disease cases was significantly ($p=0.006$) elevated as compared with healthy volunteers (0.51 ± 0.13ng/ml vs 0.27 ± 0.1ng/ml).This study concludes that serum pentraxin-3 could emerge as one of the novel non-invasive biomarkers in predicting the progression of fibrosis in alcoholic liver disease patients.

INTRODUCTION

Alcoholic liver disease (ALD) is a major cause of alcohol-related morbidity and mortality. Its presentation ranges from fatty liver to hepatitis, cirrhosis, and hepatocellular carcinoma. In South India, the prevalence of alcohol use varies between 33% - 50%, with an increased prevalence among the illiterate and the poor [1]. Alcoholic cirrhosis is the end spectrum of alcoholic liver disease, which includes steatosis to steatohepatitis, alcoholic hepatitis, fibrosis, cirrhosis and superimposed hepatocellular carcinoma. Fibrosis that occurs in alcoholic cirrhosis is a wound-healing response that occurs in all types of chronic liver disease. It is characterized by excessive accumulation of collagen and other extracellular matrix proteins [2]. Albeit, steatosis is visualized in all heavy drinkers, however only 10-20% of individuals might develop cirrhosis. Meanwhile, in compensated patients, advanced fibrosis or cirrhosis is the main predictor for long-term survival and clinically it is highly important to diagnose the advanced fibrosis before decompensation to enhance abstinence and survival.

Pentraxin-3is classified under the pentraxin family and is involved in a mélange of roles like regulation of inflammation and innate resistance to pathogens. It is strongly expressed on collagen bundles and fibrotic areas. It is produced by macrophages, endothelial cells, epithelial cells and fibroblasts. It is strongly associated with human and murine fibroblast differentiation [3]. Previous studies show that plasma pentraxin-3 levels are significantly increased in patients diagnosed with acute myocardial infarction, non-alcoholic steatohepatitis and sepsis [4,5]. Pentraxin-3 is highly populated in fibrotic areas and associated with collagen deposition and further aids the differentiation of monocytes to fibroblasts [6]. Narcisso-Schavion et al. showed that the median pentraxin-3 level is significantly higher in cirrhotic patients than control subjects [7]. However, ALD is a common pathological condition encountered among the Indian population. The majority of

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ARTICLE

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Effectiveness of team teaching in biochemistry lectures for undergraduate students

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Abstract

Team teaching is an innovative method to engage the large group and make lectures more interesting. The aim of the study is to evaluate the impact of team teaching in first year biochemistry lecture classes. After obtaining ethical clearance, 150 first year medical undergraduate students participated in the study on a voluntary basis. Topic identified for team teaching was "Protein biosynthesis" in Biochemistry, which was designed to be carried out in 5 h of lecture by three teachers. At the end of the team teaching module and traditional lectures, a structured anonymous feedback was obtained from the students. At the end of each class, students' understanding of the lecture was assessed by an MCQ test comprising 5–6 items, which mainly tested the recollection and understanding levels of Bloom's taxonomy. This was compared with traditional lectures. The mean MCQ test score was significantly higher for team teaching when compared to traditional teaching. The students had increased attention span and were able to answer questions from later part of the class in team lectures. The students had reported that lectures taught by team were more interesting and informative. They also felt that the team was more receptive to the doubts raised, stimulated discussion on the topic and had a better rapport with the class.

KEYWORDS

medical education, team-teaching, undergraduate

1 | INTRODUCTION

Lecture is still the dominant teaching strategy in Medical schools worldwide.[1] Although lecture has the advantage of organized presentation of concepts to a large body of audience with economical use of time, it has always suffered the disrepute of being boring and unattractively long. Attention span for most of the adult learners is said to be around 20 min.[2] Capturing the attention of the large group and maintaining it through the lecture, encouraging active learning are some of the challenges faced by the lecturer. Many strategies have been explored to make the lectures more interactive and interesting. With an easily distracted audience of 150 (annual intake in most medical colleges in India), taking an effective lecture is a Herculean task.[3]

Collaborative teaching or Co-teaching involves a group of instructors working purposefully, regularly, and cooperatively to help a group of students learn. There are many possible models of Co-teaching—supportive, parallel, alternative, complementary, and team teaching. Team teaching is defined as "well planned, team taught lessons,

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Original Article

Occurrence of thyroid dysfunction in patients with psychiatric disturbances - A hospital based retrospective study

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Abstract:

Introduction: The TFT results of psychiatric patients are frequently abnormal. Though the inter relation of neural networks & the interference of the drugs are known, our quest is to examine whether any pattern exist in TFT results of psychiatric patients.

Methods: All patients belonging to the Psychiatry department of a tertiary care Hospital in Coimbatore for whom thyroid functions are evaluated during the period for six months period, are included in this study. Ethics clearance was obtained from Institutional Human Ethics Committee. Details of patients including age, gender, ICD10 based diagnosis, TFT results, treatment given and associated co-morbidities were noted from their medical records.

Results:

- Age distribution is mainly between 18 to 70 years.
- Most of the patients (93%) belong to 3 groups – F3 69%, F2 14% & F4 10%.
- Female predominance is seen in F2, F3 & male predominance in F1, F4.
- Dysthyroid status is common in F3(Hypo 14%, Hyper 6%) and F2 (Hypo 15% & Hyper 2%) as shown in Figs: 5,6 & 7.
- Interestingly, only 1 out of 43 patients on Lithium had TSH above 10mIU/Ml.

Conclusion: Form this study , we conclude , in F3 mood disorders both hypothyroidism and hyperthyroidism plays a confounding role. F2 Schizophrenia is associated with hypothyroidism and Lithium doesn't affect thyroid status much.

Keywords: Thyroid hormones, Lithium

Introduction

The TFT results of psychiatric patients are frequently abnormal. Though the inter relation of neural networks & the interference of the drugs are known, our quest is to examine whether any pattern exist in TFT results of psychiatric patients. The psychiatric disturbances which accompany hyperthyroidism and hypothyroidism, the two commonest thyroid disorders, mimic mental illness. People with an overactive thyroid may exhibit marked anxiety and tension, emotional liability, impatience and irritability, distractible over activity, and fluctuating depression with sadness and problems with sleep and the appetite. In extreme cases, they may appear schizophrenic, losing touch with reality and becoming delirious or hallucinating. An underactive thyroid can lead to progressive loss of interest and initiative, slowing of mental processes, poor memory for recent events, fading of the personality's colour and vivacity, general intellectual deterioration, depression with a paranoid flavor, and eventually to dementia and permanent harmful

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MEDICINE

A Guide to Total Quality Management System (TQMS) in Molecular Diagnostics from Experiences in Seeking Accreditation and Implementation

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Abstract

The molecular diagnostic laboratories in developing countries are progressively improving and attaining international accreditation. Hence, understanding and implementing the concept of quality are the needs of the hour. Total quality management system (TQMS) in a diagnostic laboratory is an integrated program involving all laboratory staff aiming for integration, consistency, and increase in efficiency. A comprehensive understanding of such a system is unfortunately only through practice. Our endeavors in establishment of TQMS for laboratory accreditation has helped us gain comprehensive understanding of the process and have compelled us to share the knowledge with the community engaged in such diagnostics. A number of accreditation bodies have put forth guidelines for laboratories seeking accreditation; however, such documents can be overwhelming at times leading to confusion and difficulty in implementation. We aim to present to the reader a lucid script of basic reasoning behind such accreditation guidelines in conjunction with thorough and practical generalized workflows for TQMS implementation. The goal of TQMS is to provide the best possible care for patients; and to achieve top-notch quality, there should be a continuous drive for improvement. The clinical vignettes presented in this paper illustrate the value of applying select ISO 15189:2012 recommendations in a molecular diagnostic laboratory to efficiently ensure quality through TQMS and a basic understanding of nuances for its practical implementation. Implementation of TQMS in a diagnostic setting enables seamless flow of laboratory results and instills confidence in clinicians regarding disease interpretation and treatment.

Keywords Molecular diagnostics · Total quality management system · Next-generation sequencing · Real-time PCR/PCR · ISO15189:2012 · Accreditation

Introduction

At present, globally, healthcare management relies on a battery of molecular diagnostic tests that help clinicians to take highly informed decisions for better prognosis of disease. It is a dynamic area leading to insights in research and treatment that are revolutionizing health care. Molecular diagnostics detect and measure the presence of genetic material or proteins associated with a specific health condition. It helps to uncover the underlying mechanisms of disease and also enables clinicians to individualize therapy, thus facilitating the practice of personalized medicine. These tests cover an array of diseases and contribute to early and precise diagnosis of the disease aiding in preventing disease progression [1••]. Molecular diagnostics gained wide acceptance and attention owing to its quick turnaround time and reliability [2•]. Though there has been a steep increase in the number of laboratories performing such tests on clinical samples for disease diagnosis, the documentation or guidelines for efficient functioning of such laboratories are often tabooed as complicated and incomprehensible for use in regular practice. Nonetheless, it does not diminish the fact that these labs must strictly abide by uniform

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Original Research Article

Study on the biochemistry laboratory performance in notifying critical alerts

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Abstract

Background: Critical alert interval is the interval of examination (test) results that indicates an immediate risk to the patient of injury or death. **Materials and Methods:** All consecutive critical data of the month January 2019 are taken from the critical alert register from a tertiary care hospital/DC/BC/Reg/RR/09-Critical. From LIS, we generated the list of data satisfying the critical alert conditions in the same period January 2019. **Results:** The distribution had two peaks. The first peak is the tallest i.e.171 alerts are given within 25minutes. ABG and electrolyte samples processed immediately, contribute to this. This peak is superimposed on a normal distribution with a peak at 50 to 75 minutes interval and a long tail on the right side. **Conclusion:** Critical alert recording is missed in 26.3% of total alerts triggered. After correction, 50% of beta HCG is not recorded in the register. In 171 instances only we have given the alert within 25minutes.
Key Words: Critical alert, Parameters, Samples, Notification and authorized personnel.

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INTRODUCTION

Critical alert interval is the interval of examination (test) results that indicates an immediate risk to the patient of injury or death. When examination results fall within established "alert" or "critical" intervals:

-A physician (or other authorized health professional) is notified immediately.

-Records are maintained of actions taken that document date, time, responsible laboratory staff member, person notified and examination results conveyed, and any difficulties encountered in notifications.

This study is about how effectively it is practiced in the laboratory

MATERIALS AND METHODS

Institutional human ethics committee approval was obtained. All consecutive critical data of the month January 2019 are taken from the critical alert register from a tertiary care hospital/DC/BC/Reg/RR/09-Critical. From LIS, we generated the list of data satisfying the critical alert conditions in the same period January 2019.

FOLLOW UP

- We have a LIS based system to trigger, initiate action and record critical alert in 100% instances after this study.
- We have been working to reduce the time taken for issuing alert and efficiency of tracking the authorized personnel to issue the alert.

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Original Research Article

Variation of platelet indices among patients with type 2 diabetes mellitus

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Abstract

Diabetes Mellitus (DM) is a global pandemic disease with high prevalence as well as increased morbidity. Platelet function plays an important role in the pathogenesis of atherothrombosis in type 2 Diabetes Mellitus. Altered platelet parameters have been reported in patients with Diabetes Mellitus and have been associated with increased risk of vascular complications in these individuals. A hospital-based prospective study was conducted over a period of 12 months from January 2020 to December 2020. The study population was divided into two groups: Cases (n=200) and Controls (n=200). Detailed history, complete clinical evaluation and relevant investigations were obtained, and the results were compared. It was found that Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) were significantly increased in diabetic patients than in non-diabetic individuals. It was also observed that elevated MPV and PDW were seen in diabetic patients with complications. There was no significant difference in platelet count between the two groups. Hence, MPV and PDW are cost-effective platelet parameters which can be used as markers in predicting the vascular complications in DM.

Keywords: Diabetes Mellitus, Platelet parameters, vascular complications, HbA1C

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Introduction

Diabetes mellitus (DM) is perhaps one of the oldest known diseases with diverse terminologies. It is emerging as one of the dominant public health concerns globally with a prevalence increasing in middle and low-income countries. International Diabetes Federation (IDF) indicate that 425 million had DM in 2018 and is expected to further aggravate to 642 million by the year 2040. It is also recognized as one of four priority non-communicable diseases (NCDs). World Health Organization (WHO, 2013) devised targets with a vision of 25% reduction in mortality from NCDs by 2025. The Sustainable Development Goals (SDG) also comprised a target of one-third reduction in the premature deaths caused due to NCDs including diabetes by 2030. Especially in Asian countries like India, the unprecedented economic growth and rapid urbanization led to the transition in health concerns from communicable to NCDs[1]. Among the NCDs, DM and cardiovascular diseases are the leading health problems[1]. An alarming surge in diabetic subjects from 26 million in 1990 to 65 million in 2016 was recorded in India. The prevalence in Indian adults aged 20 years or older escalated from 5.5% in 1990 to 7.7% in 2016. The prevalence in 2016 was highest in Tamil Nadu and Kerala. Untreated type II DM (T2DM) is ultimately leading to macro vascular and micro vascular complications. It is well known that T2DM is known for its expression after 40 years of age.

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However, it is now becoming more apparent in young individuals even at the age of ten. Young-onset T2DM prevalence has been reported in many countries with different ethnic and cultural backgrounds with increasing prevalence of the sedentary life style and obesity. It has more aggressive phenotype with adverse impact on quality of life augmenting the possibility of future public heath catastrophe[2].

DM is a growing health problem associated with increased risk of micro vascular and macro vascular complications[3]. Diabetic patients have large platelets that are young, more reactive with increased tendency to aggregate. They contain denser granules, secrete more serotonin, betathromboglobulin and thromboxane A_2 than smaller and less active platelets. This suggests an association between the platelet functions and diabetic vascular complications. Thus, indicating changes in mean platelet volume (MPV) and platelet distribution width (PDW) reflect the state of thrombogenesis[4]. Therefore, MPV and PDW can be considered as predictive markers for vascular complications of Diabetes Mellitus. Identification of such patients can be easily made by simple routine haematological analysis which helps in easy detection of prothrombotic state of the patients. It is a cost-effective tool that could possibly benefit the diabetic patients by preventive actions as a large number suffer from preventable vascular complications. Analysing the platelet parameters can act as an alarm for diagnosing and progression of complications of DM. Hence in view of this, we studied and compared the platelet parameters in diabetic patients and healthy controls.

Materials and methods

A total of 200 subjects who were known diabetic patients and 200 control subjects who had normal blood sugar were taken in this study. The study was conducted in the Department of Pathology, KMCH Institute of Health Science and Research over a period of one year from January 2020 to December 2020 ,after obtaining approval in

institutional ethics commitee.

Inclusion criteria

1. 200 diagnosed cases of type II DM, as per guidelines of American Diabetic Association. (Diabetes is diagnosed if HbA1C is >6.5%, Fasting glucose >126mg/dl, Post Prandial glucose>200 mg/dl)
2. 200 controls with normal blood glucose levels.

Exclusion criteria

1. Male patients with Hemoglobin <12gm and female patients with hemoglobin < l0gm
2. Non diabetic subjects with coronary artery disease.
3. Subjects with any diagnosed malignancy, thrombocytopenia or thrombotic disorders.
4. Patients on anti-platelet drugs such as aspirin and clopidogrel.
5. Pregnant women

This hospital-based study was carried out fulfilling the inclusion and exclusion criteria attending either out-patient or in-patient clinic. They were compared with 200 controls who had normal blood glucose levels.

Detailed history was taken from both cases and control subjects and complete clinical evaluation was done regarding any associated macro or micro vascular complications. Relevant investigations were obtained like blood glucose levels and HbA1C levels and complete blood count analysis was done using Beckman coulter.

Values of platelet indices like Platelet Distribution Width (PDW), Mean Platelet Volume (MPV) and platelet count were noted.

Statistical analysis

Statistical analysis was performed using SPSS version 16.0. Comparison between different groups regarding categorical variables was performed using the student's t-test and Pearson's correlation test (r value as the coefficient) and Probability (p-value).

Results

200 samples of diabetic cases and 200 samples of non-diabetic healthy controls were studied. We studied age distribution and platelet parameters.

Platelet parameters such as MPV, PDW and Platelet count were studied in

a) Diabetic cases and non-diabetic healthy controls
b) Diabetics under glycemic control (Group A) and not under glycemic control (Group B)
c) Diabetics with complications (Group C) and without complications (Group D)

In this study, among the diabetics, 3 patients were less than 40 years, 28 patients were between 41 and 50 years, 45 patients were between 51 and 60 years and 24 cases were above 60 years. Among the control population, 12 individuals were below 40 years, 36 individuals were between 41 and 50 years, 45 individuals were 51 - 60 years and 7 individuals were above 60 years. (Figure 1)

It is observed that the median MPV (mean platelet volume) among the diabetic population was 15fl and it is found to be increased than the median MPV among the control population which was 9fl (Figure 2). 76% of diabetic patients in the study had increased median platelet volume and 24% of diabetic patient had median platelet volume within normal range where as 19% of control population had increased mean platelet volume and 81% of control population had mean platelet volume within normal range. There was a statistically significant difference of between diabetic and non-diabetic population. ($p < 0.001$)

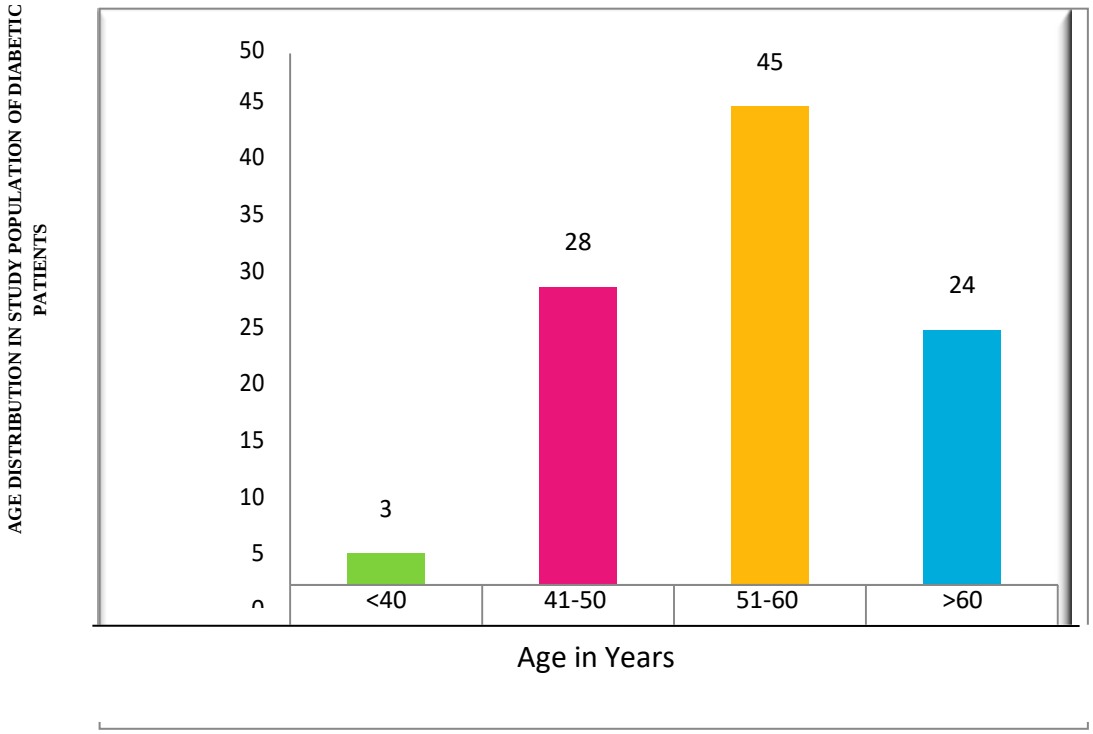


Fig 1:Age distribution in study population of diabetic patients

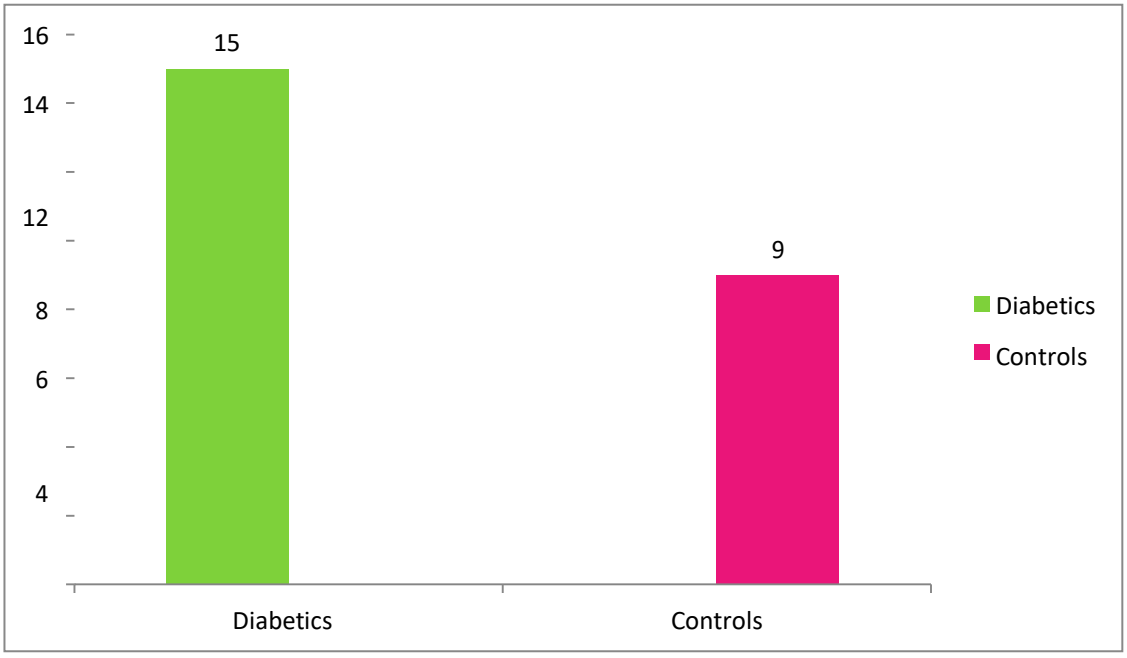


Fig 2:Comparison of median values of MPV between diabetic cases and non-diabetic controls

The median platelet distribution width (PDW) was reported to be higher in diabetic population (21%) when compared to the median platelet distribution width in control population (11%) (Figure 3). The platelet distribution width was found to be increased in diabetic population than non-diabetic and there was a statistically significant difference between them. ($p < 0.001$).

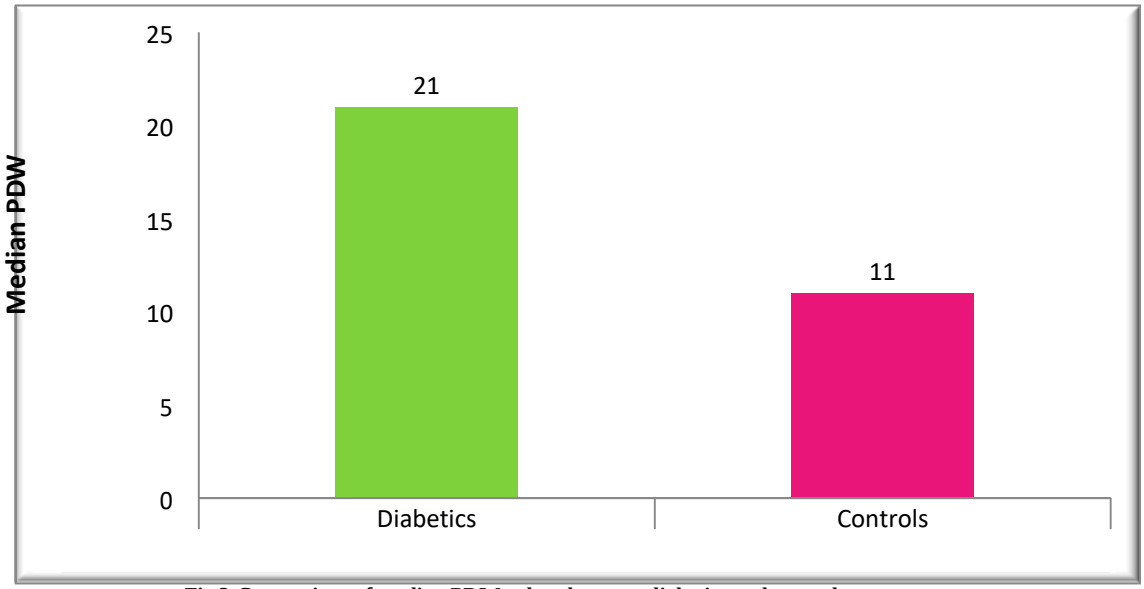


Fig 3:Comparison of median PDW values between diabetics and controls

Median platelet count of diabetic population was $2.66x10^9$/L and that of normal
Among the diabetic population, 7% had decreased platelet count, 90% had normal platelet count and 3% had increased platelet count.
In case of normal healthy individuals, 1% had decreased platelet count, 97% had normal platelet count and 2% had increased platelet count. There was no statistically significant difference of platelet count between diabetics and no diabetic individuals. ($p= 0.207$)
Platelet parameters were compared between diabetics and healthy controls and statistically significant difference of MPV and PDW values were obtained. (Table 1).

Table 1:Comparison of Median values of platelet parameters between diabetic cases and non-diabetic controls

PARAMERTERS	GROUP	NUMBER	MEDIAN	p VALUE
MPV (fl)	Cases	200	15.0	
	Controls	200	9.0	<0.001
PDW (%)	Cases	200	21.0	
	Controls	200	11.0	<0.001
PLATELET COUNT (x10^9/L)	Cases	200	2.77	
	Controls	200	2.60	0.207

Diabetic patients were divided into two groups based on HbA1C levels (glycemic control):
Group A: Diabetics under glycemic control (HbA1C < 6.5) - 33%
Group B: Diabetics not under glycemic control. (HbA1C >= 6.5) - 67%
Patients with complications were categorized as group C and without complications were categorized as group D.
The mean value of mean platelet volume in Group C (14.2) was found to be higher than the mean platelet volume in Group D (9.5fl).
Among group C patients, 96% were found to have increased mean platelet volume and 3% had normal mean platelet volume. Among group D patients, 67% had increased mean platelet volume and 33% had normal mean platelet volume. There was a statistically significant difference between the two-population group C and group D (p< 0.001).
The mean platelet count in Group C patients was 2.45x10^9/L and it was found to be little decreased than the mean platelet counts in Group D (2.66x10^9/L). There was no statistically significant difference of platelet count between Group C and Group D (p= 0.244).
Platelet parameters are compared between Group C and Group D. (Table 2). A statistically significant difference of MPV and PDW values are obtained.

Table 2:Comparison of platelet parameters between diabetics with and without complications

PARAMETERS	COMPLICATIONS	MEDIAN	p VALUE
MPV (fl)	PRESENT (group C)	14.2	<0.0001
	ABSENT (group D)	9.55	
PDW (%)	PRESENT (group C)	18.6	<0.001
	ABSENT (group D)	11.8	
PLATELET COUNT(x10^9/L)	PRESENT (group C)	2.45	0.244
	ABSENT (group D)	2.66	

Discussion

Diabetes Mellitus (DM) refers to a group of common metabolic disorders which share the phenotype of hyperglycemia. The etiology of DM includes reduced insulin secretion and decreased glucose utilization. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems which impose a tremendous burden on the individual with DM and on the health care system[5].

The common feature in all the complications of diabetes is vasculopathy both "micro" and "macro", characterized by progressive narrowing of lumen as well as abnormal permeability to proteins[6]. Macro and microvascular diseases are currently the principal causes of morbidity and mortality in patients with type I and type II DM. Loss of the modulatory role of the endothelium may be an initiating and a critical factor in the development of diabetic vascular disease[7].

In our study, platelet parameters such as mean platelet volume (MPV), platelet distribution width (PDW) and platelet count were compared between diabetic population and normal healthy individuals. Platelet volume is a marker of the platelet function and activation. Platelet distribution width (PDW) is an indicator of the distribution of platelet size and also a marker of platelet activation.

In our study, the median platelet volume values were higher in diabetic patients as compared to non-diabetic population and there was a statistically significant difference between diabetic and non-diabetic population. (p < 0.001).Same observations were found in the study conducted by Buchet al[8], Bhattacharjee et al[9], Yilmaz et al[10], Dermitas et al[11], Gupta AV et al[12] which revealed that mean platelet volume was significantly raised in diabetics than non-diabetic healthy individuals.

In our study, median platelet distribution width levels were higher in diabetic population when compared with non-diabetic subjects. There was a statistically significant difference between diabetic population and non-diabetic healthy controls (p <0.001). Observation in our study correlated with study conducted by Alhadaset al[13], Jabeen et al[14], Buch et all[8].

There was no statistically significant difference of platelet count between diabetic and non-diabetic healthy individuals (p=0.207) in this study. Similar results were found in the study done by Buchet al[8], Yilmaz et al[10], Jabeen et al[14] and Kodiattee et al[15].

In our study, there was a statistically significant differences of median platelet volume between diabetics with and without complications. (p <0.001). Similar higher values of mean platelet volume were seen in the study done by Papanas et al[16] and Buch et al[8].

There was also a significant differences of mean platelet distribution width between diabetics with and without complications in our study (p <0.001). Similar results were observed in the study done by Buchet et a1[8].

In case of platelet count there was no significant difference in our study and similar finding was seen in the study conducted by Dermitas et al[11].

Conclusion

Platelet parameters such as Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet count were compared between 200 diabetic cases and 200 non diabetic healthy controls. It was found that median Platelet Volume and Platelet Distribution Width were significantly increased in diabetic patients than non-diabetic healthy individuals. Similarly, elevated MPV and PDW were seen in diabetic patients with complications compared to diabetic patients without

complications. Mean Platelet Volume and platelet distribution width are indicators of platelet activity. Increased MPV and PDW are associated with increased risk of thrombogenic events leading to vascular complications of diabetes mellitus. Our study indicates that Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) are increased in diabetic patients and they can be used as a cost-effective marker in predicting the vascular complications in diabetes mellitus.

References

1. Rajeev Chawla, Sujoy Ghosh, Banshi Saboo, Sanjay Kalra, On behalf of the RSSDI-ESI Consensus Group RSSDI-ESI. Clinical Practice Recommendations for the Management of Type 2 Diabetes Mellitus 2020. Indian J Endocrinol Metab. 2020;9:9
2. Aleksandra Korniluk, Olga Martyna Koper-Lenkiewicz, Joanna Kamińska, Halina Kemona, Violetta Dymicka-Piekarska. Mean Platelet Volume (MPV): New Perspectives for an Old Marker in the Course and Prognosis of Inflammatory Conditions. Mediators Inflamm. 2019:1
3. Xue-feng xu, Feng-lin jiang, Mei-jun ou, Zhi-hui zhang. The association between mean platelet volume and chronic atrial fibrillation and the presence of thrombotic events. Biomed Rep. 2015; 3(3): 388–394.
4. Endler G, Klimesch A, Sunder-Plassmann H, Schillinger M, Exner M, Mannhalter C, et al. Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. Br J Haematol 2002; 117:399-404.
5. Adnan Bashir Bhatti, Farhan Ali, Siddique Akbar Satti.Relationship of Hypertensive Retinopathy with Mean Platelet Volume Among Hypertensive Patients. Cureus 2015.
6. Yi Lin, Zhongjie. Current views on type 2 diabetes. Sun J Endocrinol 2010:1
7. Tesfaye S, Andrew JM. Dyck PJ, Freeman R, Horowitz M, et al. Diabetic Neuropathies: Update on Definitions, Diagnostic Criteria, Estimation of Severity, and Treatments. Diabetes Care. 2010; 33: 2285-93.
8. Buch A, Kaur S, Nair R, Jain A. Platelet volume indices as predictive biomarkers for diabetic complications in Type 2 diabetic patients. J Lab Physicians 2017;84-88
9. Bhattacharjee, Datta A, Debbarma R.K., Das S.K. Platelet indices in diabetics and influence of glycemic control — a hospital-based study in North-East India. Int J Med Res Rev 2016:78
10. TolgaYilmaz, AhuYilmaz. Relationship between altered Platelet Morphological Parameters and Retinopathy in Patients with Type 2 Diabetes Mellitus. J Ophthalmol. 2016:9
11. Arjun Ved Gupta, AnujVed Gupta, Anannya Mukherji. Platelet Indices and Endothelial Dysfunction in patients of Diabetes Mellitus type 2. Sch. J. App.Med. Sci.,2016:8
12. Levent Dermitas, Husnu Degirmenci. Emin Murat Akbas, AdaletOzcicek et al. Association of hematological indices with diabetes, impaired glucose regulation and microvascular complications of diabetes. Int J ClinExp Med. 2015:09
13. KamillaR.Alhadas; Sandra N.Santos; Marcel Mara S. Freitas et al. Are platelet indices useful in the evaluation of type 2 diabetes mellitus? J Bras Patol Med Lab 2016:12
14. Jabeen F., Fawwad A., Rizvi H. A., Alvi F. Role of platelet indices, glycemic control and CRP in pathogenesis of vascular complications in type-2 diabetic patients. Pakistan Journal of Medical Sciences 2013:57
15. Kodiatte TA, Manikyam UK, Rao SB, Jagadish TM, Reddy M, Lingaiah HK, et al. Mean platelet volume in type 2 diabetes mellitus. J Lab Physicians. 2012:9
16. Papanas N, Symeonidis G, Maltezos E, Mavridis G, Karavageli E, Vosnakidis T, et al. Mean platelet volume in patients with type 2 diabetes mellitus. Platelets. 2004;15:475-8.

Conflict of Interest: Nil
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Case Report

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Osteoblasts to the Rescue – A Rare Case of Secondary Myelofibrosis

Lawanya Gunaseelan, Eliz Thomas, Prasanna N. Kumar, Sujaya Menon[1]

Abstract:

A 36-year-old female patient presented with joint pain, generalized weakness, and bleeding gums. There was no significant history of other illnesses or intake of drugs. Examination revealed pallor and splenomegaly. Peripheral smear examination showed pancytopenia. A large number of osteoblasts and a few scattered osteoclasts were seen in the bone marrow aspirate. The trephine biopsy revealed marrow fibrosis. A suggestion to investigate for hyperparathyroidism was made. Serum calcium, ionized calcium, and PTH were increased. 99m Tc-Sestamibi scan was done and adenoma was detected in the right inferior parathyroid and subsequently excised. Postsurgery, hematological parameters and bone markers improved. Follow-up bone marrow biopsy revealed hypercellular marrow with marked regression of fibrosis. Splenomegaly was reversed. We concluded that pancytopenia was due to bone marrow fibrosis, resulting from primary hyperparathyroidism. It is important to consider secondary causes of myelofibrosis in the appropriate clinical setting.

Keywords:

Osteoblasts in the marrow, secondary myelofibrosis, marrow fibrosis

Background

Primary myelofibrosis is commonly a disease of the elderly. Secondary myelofibrosis occurs due to various pathological processes that involve the bone marrow such as malignancy, infection, metabolic disorders, radiation, and drugs. Hyperparathyroidism as a precipitating factor for myelofibrosis has rarely been reported. [1,2] It is important to consider secondary causes of myelofibrosis in the appropriate clinical setting. Our patient improved, similar to others after surgery. Diagnosis was confirmed when fibrosis in the bone marrow reversed after surgery.

Case Report

We report a 36-year-old female who presented with joint pain, generalized weakness, and bleeding gums. There was no significant history of other illnesses or intake of any drugs. Examination revealed pallor and massive splenomegaly. Peripheral smear examination showed pancytopenia. Bone marrow aspiration showed a large number of osteoblasts and a few scattered osteoclasts [Figure 1]. The trephine biopsy revealed marrow fibrosis of grade 3 [Figure 2]. On the basis of the presence of abundant osteoblasts and fibrosis, a suggestion to investigate for hyperparathyroidism was made. On further investigation, serum calcium, serum ionized calcium, and PTH were found to be increased [Table1]. 99m Tc-Sestamibi scan was done, and adenoma was detected in the right inferior parathyroid and was subsequently excised [Figure 3].

As early as 6 months postsurgery, hematological parameters and bone markers improved without any intervention. During the follow-up, bone marrow biopsy revealed hypercellular

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Table 1: Investigations

Investigations	Initial
Hemoglobin (normal: 12-15 g/dl)	7.4
PCV (normal: 36%-46%)	22.7
WBC (normal: 4-10×10^3 µl)	1.7×10^3
Platelet count (normal: 150-400×10^3)	11×10^3
Bone marrow aspirate	Hypocellular marrow with increase in osteoclasts and osteoblasts
Bone marrow trephine	Grade 3 fibrosis
Ionized PTH (normal: 15-65 pg/ml)	1484
Serum calcium (normal: 8.8-10.2 mg/dl)	15.3
Ionized calcium (normal: 1.1-1.3 mmol/L)	2.224
Serum phosphorus (normal: 2.7-4.5 mg/dl)	2.3

PCV=Packed cell volume; WBC=White blood cell; PTH=Parathyroid hormone

Table 2: Investigations post surgery

Investigations	6 months postsurgery
Hemoglobin (normal: 12-15 g/dl)	11.2
PCV (normal: 36%-46%)	34.2
WBC (normal: 4-10×10^3 µl)	4.9×10^3
Platelet count (normal: 150-400×10^3)	71×10^3
Bone marrow aspirate	Hypercellular marrow with erythroid hyperplasia. Occasional osteoblasts seen
Bone marrow trephine	No evidence of fibrosis
Ionized PTH (normal: 15-65 Pg/Ml)	55
Serum calcium (normal: 8.8-10.2 mg/Dl)	8.0
Ionized calcium (normal: 1.1-1.3 mmol/L)	1.184
Serum phosphorus (normal: 2.7-4.5 mg/dl)	4.0

PCV=Packed cell volume; WBC=White blood cell; PTH=Parathyroid hormone

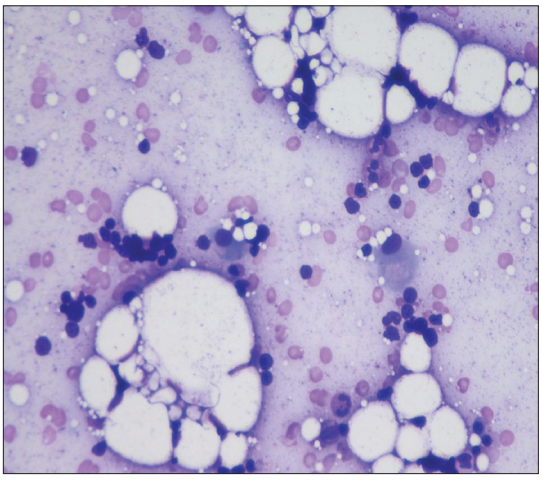


Figure 1: Osteoblasts seen in the bone marrow aspirate

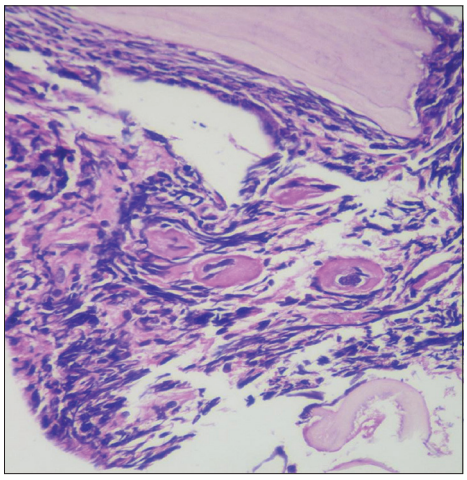


Figure 2: Bone marrow trephine biopsy showing fibrosis

marrow and marked regression of fibrosis. The spleen also gradually decreased in size. We concluded that the pancytopenia was because of bone marrow fibrosis resulting from primary hyperparathyroidism.

Investigations

Differential diagnosis

All the causes for pancytopenia were ruled out one by one. Due to the presence of osteoblasts and osteoclasts in the marrow aspirate, along with fibrosis in the biopsy, a suggestion to investigate for hyperparathyroidism was made.

Treatment

Fluids, diuretics, bisphosphonates, and other supportive measures were administered to treat hypercalcemia. Thalidomide and prednisolone were given in tapering doses.

The parathyroid adenoma was excised and confirmed by histopathology.

Outcome and follow-up

Six months post-surgery, hematological parameters and bone markers improved [Table 2]. Bone marrow biopsy

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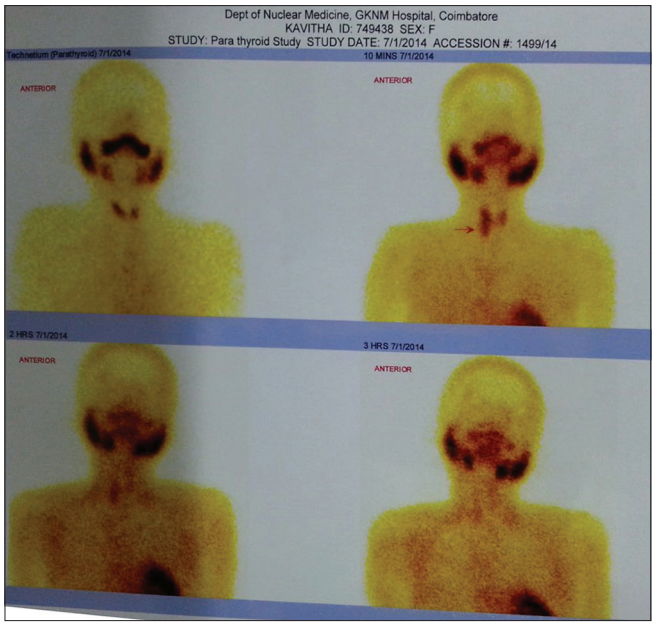


Figure 3: Radioisotope study revealed inferior parathyroid adenoma

revealed hypercellular marrow and marked regression of fibrosis [Figure 4]. The spleen gradually decreased in size.

Discussion and Conclusion

Pancytopenia is a common hematological entity, and the various causes have to be evaluated for. Myelofibrosis is one such cause and primary myelofibrosis is known to occur in the elderly. Secondary myelofibrosis can be due to various etiologies such as drugs, neoplasms, collage diseases, hyperparathyroidism, and renal osteodystrophy. Literature says that asymptomatic primary hyperparathyroidism is rare.[2-5] Our patient had features of advanced hyperparathyroidism such as elevated blood calcium levels, PTH levels, ALP levels and radiological evidence of subperiosteal bone resorption and osteopenia. After the curative parathyroid surgery, improvement in hematological and metabolic parameters is very suggestive of marrow fibrosis due to primary hyperparathyroidism.

Learning points/take home messages

- Evaluation for secondary myelofibrosis in correlation with clinical and biochemical parameters is of paramount significance
- Hyperparathyroidism must be considered with the presence of osteoblasts in the marrow aspirate.

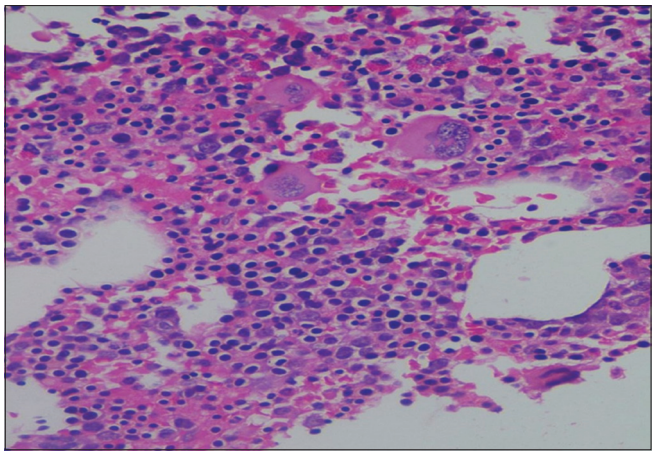


Figure 4: Bone marrow biopsy post surgery shows presence of hematopoietic elements

Ethics approval and consent to participate

Ethics approval was obtained and consent to participate was taken from the patient.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Elaraj DM, Clark OH. Current status and treatment of primary hyperparathyroidism. Perm J 2008;12:32-7.
2. Bhadada SK, Bhansali A, Ahluwalia J, Chanukya GV, Behera A, Dutta P, *et al*. Anaemia and marrow fibrosis in patients with primary hyperparathyroidism before and after curative parathyroidectomy. Clin Endocrinol (Oxf) 2009;70:527-32.
3. Boxer M, Ellman L, Geller R, Wang CA. Anemia in primary hyperparathyroidism. Arch Intern Med 1977;137:588-93.
4. Kumbasar B, Taylan I, Kazancioglu R, Agan M, Yenigun M, Sar F, *et al*. Myelofibrosis secondary to hyperparathyroidism. Exp Clin Endocrinol Diabetes 2004;112:127-30.
5. Lim DJ, Oh EJ, Park CW, Kwon HS, Hong EJ, Yoon KH, *et al*. Pancytopenia and secondary myelofibrosis could be induced by primary hyperparathyroidism. Int J Lab Hematol 2007;29:464-8.

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Profile of Uncommon Primary Adult Hepatic Malignancies at a Tertiary Care Centre

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Abstract

Context: Hepatocellular carcinoma has been the most common primary malignancy of the liver in adults, followed by cholangiocarcinoma. The less common malignancies arise from the vascular endothelial cells, neuroendocrine cells, hematolymphoid tissues, and mesenchymal tissues. Imaging studies alone may pose a diagnostic challenge due to variable appearances. Histological review of the tissue specimen, along with immunohistochemical stains is imperative for diagnosis. However, a multidisciplinary approach is necessary to make an accurate diagnosis and help in management. Out of the 24 liver malignancies encountered, six unusual primary malignancies were seen. Radiologic impression and biochemical parameters helped arrive at a definitive opinion as the lesions were not typical of this location. A high index of suspicion along with the immunohistochemical profile finally facilitated the diagnosis.

Aims: To study unusual adult primary hepatic malignancies in a 2-year study period with clinic-radiological and biochemical correlation and also determine the age and sex distribution.

Methods and Material: This study was performed in 2 years in the Department of Pathology. Ethics approval was obtained. A total of 24 liver malignancies were encountered. Metastatic tumors and tumor-like lesions were excluded. The patient's clinico-radiological findings and laboratory investigations were noted. A total of 6 unusual non-hepatocellular malignancies were seen and identified based on morphology and special stains. Statistical analysis used: SPSS version 23.0.

Results: Out of 6 non-hepatocellular malignancies, 3 were neuroendocrine carcinomas, 2 were leiomyosarcomas and 1 was a hematolymphoid malignancy.

Conclusion: The cases of primary non-hepatocellular malignancies are unexpected. A multidiciplinary approach is mandatory.

Keywords: Hepatopathology; non-hepatocellular liver malignancy; liver cancer; Primary non-hepatocellular malignancies.

Key Messages: Unusual primary non-hepatocellular liver malignancies in adult.

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1. Introduction

Primary liver cancers are rare. Because the liver is made up of several different types of cells, several types of tumors can form there.

Hepatocellular carcinoma has been the most common primary malignancy of the liver in adults, followed by cholangiocarcinoma. The less common are those arising from the vascular endothelial cells, neuroendocrine cells, hematolymphoid tissues, and mesenchymal tissues. [1]

Many times, imaging studies alone may pose a diagnostic challenge due to the variable appearances of the tumors. Histological review of the tissue specimen along with immunohistochemical stains is imperative for definitive diagnosis. A multidisciplinary approach is necessary for accurate diagnosis and management.

2. Materials and Methods

2.1 Ethics statement

Approval from the Institutional Human Ethics Committee (IHEC) was obtained (Approval number – 19/137). Consent from patients was not applicable.

2.2 Study design

A retrospective study for a period of 2 years was conducted in a tertiary care center. The study included the collection of data from the patients' record sheets available in the Medical Records Department. Information pertaining to the cases was recorded and tabulated.

All investigated cases of primary non-hepatocellular hepatic malignancies of 2 years were included in the study. Metastatic tumors and tumor-like lesions were excluded.

2.3 Statistical analysis

All the data were analyzed using SPSS version 23.0 (Armonk, NY: IBM Corp.). It is represented as proportions and percentages.

3. Results

We encountered a total of six unusual cases of primary non-hepatocellular hepatic malignancies, which included three neuroendocrine carcinomas, two leiomyosarcomas, and one hematolymphoid malignancy. All were biopsies.

3.1 Age:

The mean age at presentation for the patients was 56 years, the majority of them being males. No cases were reported in childhood or adolescence.

3.2 Symptoms at presentation

There were varied symptoms at presentation, ranging from abdominal pain to asymptomatic presentation. The most common presenting symptoms were abdominal pain and loss of appetite. Other presentations include yellowish discoloration of eyes, tiredness and nausea. Almost all the cases had palpable mass in the right hypochondrium.

3.3 Liver function test

The liver enzymes were deranged in all the cases.

3.4 Imaging Studies

Imaging studies done were ultrasound, computed tomography (CT) scan or both. Diagnosis of malignancy was established for all the cases with most favouring a hepatocellular carcinoma.

3.5 Histopathology

Of the six non-hepatocellular primary hepatic malignancies, three were neuroendocrine carcinomas, two were leiomyosarcomas and one was a hematolymphoid malignancy. Search for primary origin elsewhere was negative, and metastasis was ruled out.

The diagnosis was established with the help of immunohistochemical markers.

Figure 1: Neuroendocrine tumor. The tumor cells show immunopositivity with synaptophysin (C) and chromogranin (D)

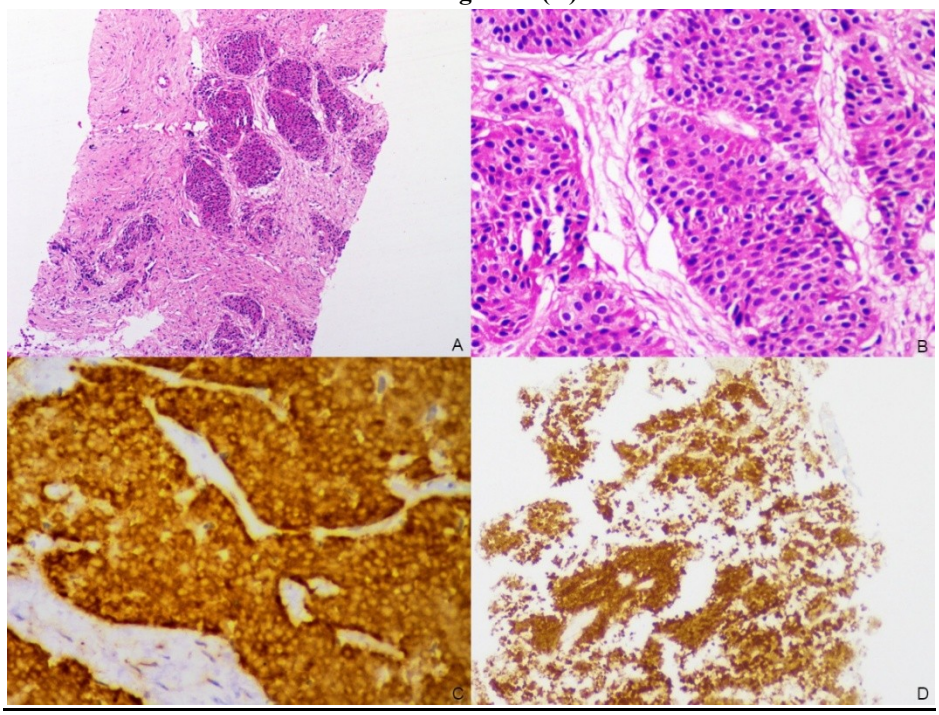


Figure 2: Leiomyosarcoma. The tumor cells show strong immunopositivity with sma (C) and vimentin (D).

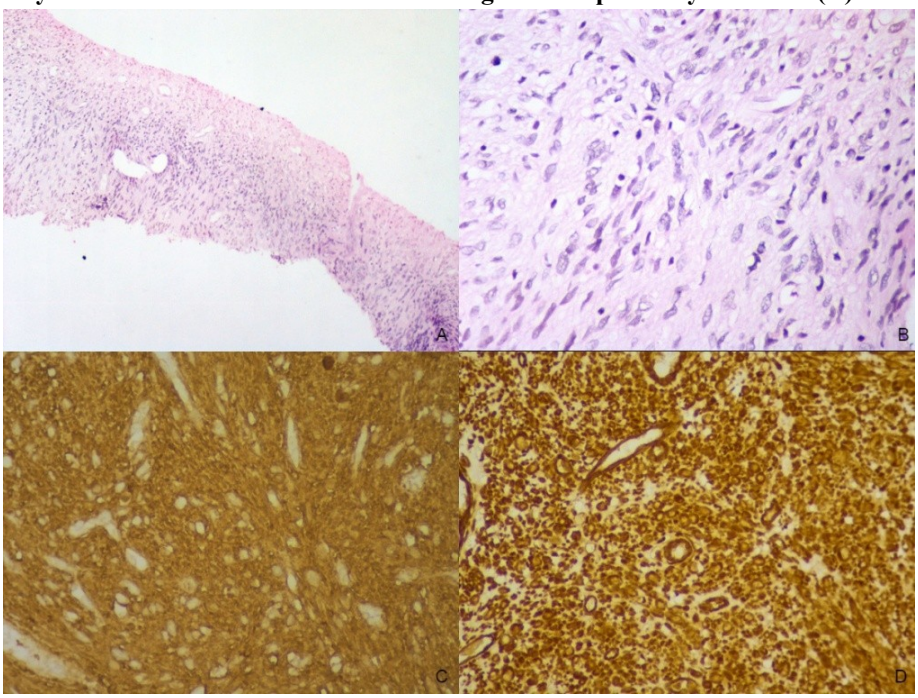
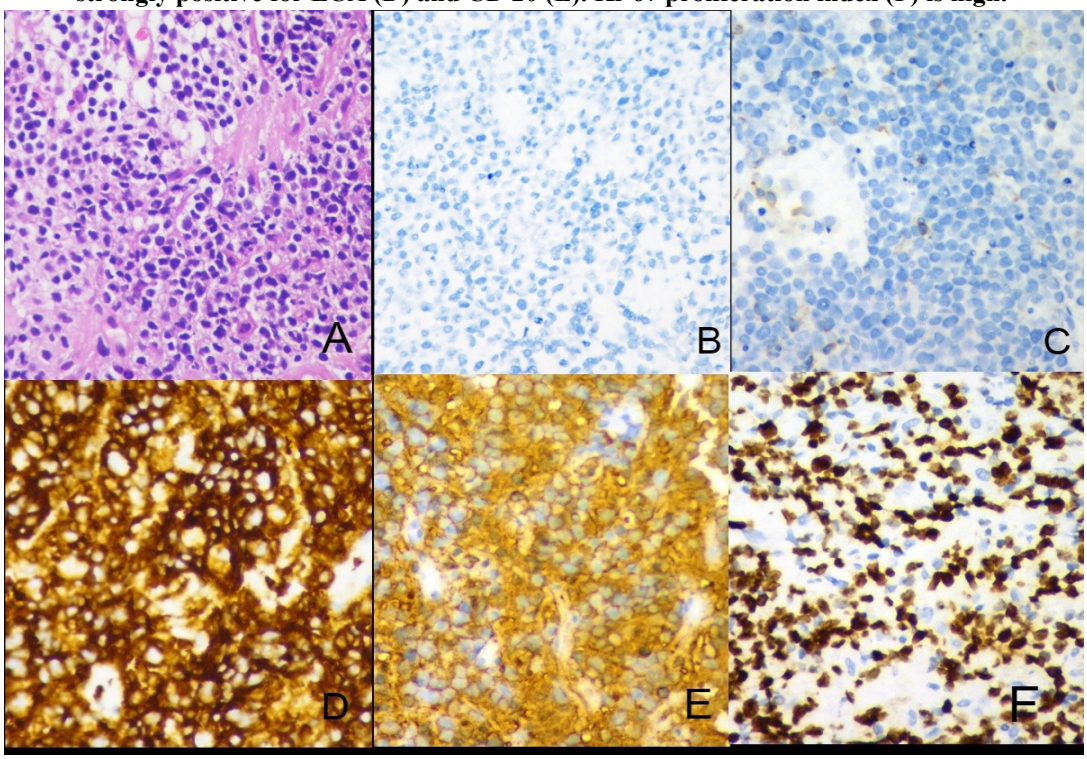


Figure 3: B-cell non-Hodgkin lymphoma. The tumor cells are negative for Heppar-1 (B) and CD 3 (C). They are strongly positive for LCA (D) and CD 20 (E). Ki-67 proliferation index (F) is high.



4. Discussion

A variety of hepatic lesions can present as hepatic masses. Amongst the malignant neoplastic lesions, primary hepatocellular carcinoma and cholangiocarcinoma are the most common. Primary non-hepatocellular hepatic malignancies although rare, are known to occur. [2,3] Prior to pathological examination for primary hepatic malignancies, misdiagnosis is frequent due to similar presentation and imaging findings in both hepatocellular and non-hepatocellular malignancies. [4,5]

Approximately 50- 90% of all neuroendocrine tumors cases are known to arise from the gastrointestinal

tract and the occurrence as primary hepatic location is rare (0.3%) [6].

Definitive diagnosis of primary neuroendocrine carcinoma is possible only after hepatic biopsy and ancillary tools like special stains and immunohistochemistry. Therefore, it can be established that biopsy and pathological examination are the gold standard for diagnosis.[2]

On histopathological examination, neuroendocrine tumors (NET) are composed of uniform round to polygonal cells with pale eosinophilic granulated cytoplasm, monotonous, round, centrally located finely stippled nuclei and small inconspicuous nucleoli. Mitosis and necrosis are infrequent.

The tumor cells show immunoreactivity with synaptophysin and chromogranin and are negative for Heppar-1. This was seen in all our cases reported as NET [Figure 1].

Mesenchymal tumors are also rarely encountered in adults. [7] Amongst the primary malignant mesenchymal tumors, hepatic angiosarcomas are most common. Primary hepatic leiomyosarcoma are again rare tumors and not many cases have been reported in the English literature.[8,9] It is often associated with immunodeficiency or viral infections (HCV, EBV, etc). [10] The cases in our study were not associated with any such underlying condition.

Histomorphology of leiomyosarcoma shows a spindle cell lesion with features of anaplasia which has to be distinguished from other spindle cell lesions of this region.

Our cases were confirmed to be primary, by ruling out metastases from other origin. Immunohistochemical markers helped in confirming the diagnosis [Figure 2].

Hematolymphoid malignancies with hepatic involvement are a relatively common presentation of extranodal disease. Rarely, lymphomas can involve the liver exclusively (primary hepatic lymphomas). As defined by previous studies, primary hepatic lymphoma is to be considered when the patient gives negative history of prior or subsequent lymphoma diagnosis, nil biopsy-proven hematolymphoid neoplasm at a nonhepatic location, and no other reason for clinical concern for hematolymphoid malignancy elsewhere in the body. [11,12] Most common primary hepatic lymphoma reported so far, is diffuse large B-cell lymphoma (DLBCL) which is closely associated with HCV infection and immunocompromised states, including HIV infection. [13,14] Our case was diagnosed as primary non-Hodgkin lymphoma of B-cell type and further typing could not be done as the tissue was insufficient [Figure 3]. The patient was immunocompetent.

5. Conclusion

The correlation of clinical, serological, radiological and histopathological findings is helpful in the early detection, diagnosis, and management of hepatic masses. Imaging studies is a reliable tool to detect hepatic malignancy. However, histological examination with IHC remains gold standard for diagnosis and confirmation of different types of malignancies arising from the liver.

Ethics approval and consent to participate: Ethics approval was obtained and consents to participate was taken from the patient.

Availability of data and materials: The datasets during or analysed during the current study available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests**.**

Funding: Nil

Authors' contributions: VA collected all the patient data from the records. TE analyzed and interpreted the patient data regarding the disease & the histolopathological examination of the biopsies. ET was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

References

[1]. Jiang K, Al-Diffalha S, Centeno B. Primary Liver Cancers-Part 1. *Cancer Control* 2018; 25:107327481774462.

[2]. Chen Z., Xiao H.-E., Ramchandra P., Huang H.-J. Imaging and pathological features of primary hepatic neuroendocrine carcinoma: an analysis of nine cases and review of the literature. *Oncology Letters*. 2014; 7:956–962.

[3]. Baek S. H., Yoon J. H., Kim K. W. Primary hepatic neuroendocrine tumor: gadoxetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging. *Acta Radiologica Short Reports.* 2013; 2:6.

[4]. Scarsbrook A. F., Ganeshan A., Statham J., *et al.* Anatomic and functional imaging of metastatic carcinoid tumors. *Radiographics.* 2007; 27:455-76.

[5]. Kellock T, Tuong B, Harris AC, Yoshida E. Diagnostic Imaging of Primary Hepatic Neuroendocrine Tumors: A Case and Discussion of the Literature. *Case Rep Radiol* 2014; 2014:156491

[6]. Camargo ÉS, Viveiros Mde M, Corrêa Neto IJ, Robles L, Rezende MB. Primary hepatic carcinoid tumor: case report and literature review. *Einstein (Sao Paulo)* 2014; 12:505-8.

[7]. Ah Kim K *et al*. Unusual Mesenchymal Liver Tumors in Adults: Radiologic-Pathologic Correlation. *AJR* 2006; W481-W489.

[8]. Shivathirthan N., Kita J., Iso Y. Primary hepatic leiomyosarcoma: case report and literature review. *World J Gastrointest Oncol.* 2011; 3:148–52.

[9]. Iida T., Maeda T., Amari Y., Yurugi T., Tsukamoto Y., Nakajima F. Primary hepatic leiomyosarcoma in a patient with autosomal dominant polycystic kidney disease. *CEN Case Rep.* 2017; 6:74–8.

[10]. Ke-Lei Zhu and Xiu-Jun Cai. Primary hepatic leiomyosarcoma successfully treated by transcatheter arterial chemoembolization: A case report. *World J Clin Cases.* 2019:525–31.

[11]. Yang XW, Tan WF *et al*. Diagnosis and surgical treatment of primary hepatic lymphoma. *World J Gastroenterol*. 2010; 16:6016-9.

[12]. Agmon-Levin N, Berger I, Shtalrid M *et al*. Primary hepatic lymphoma: a case report and review of the literature. *Age Aging* 2004; 33:637–40.

[13]. El-Sharkawi D, Ramsay A, Cwynarski K *et al*. Clinicopathologic characteristics of patients with hepatic lymphoma diagnosed using image-guided liver biopsy techniques. *Leukemia Lymphoma* 2011; 52:2130-34.

[14]. Noronha V, Shafi NQ, Obando JA *et al*. Primary non-Hodgkin's lymphoma of the liver. *Crit Rev Oncol Hematol* 2005; 53:199-207.

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ORIGINAL ARTICLE

Type II endometrial cancers: A case series

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ABSTRACT

Introduction: Endometrial carcinoma ranks 3rd in India among gynecological malignancies. Endometrial cancer (EC) can be classified into two distinct groups – type I and type II, based on histology, which differs in molecular, clinical and histopathological profiles. Type II is nonestrogen dependent, nonendometrioid, more aggressive and carries poor prognosis. Although type II cancers contribute only about 10% of EC incidence, they present at advanced age and cause approximately 50% recurrence and deaths with a low 5-year, overall survival rate. Type II EC are also characterized by genetic alterations in p53, human epidermal growth factor-2/neu, p16 and E-cadherin.
Materials and Methods: Endometrial carcinomas diagnosed from endometrial biopsies and hysterectomy specimens received in the Department of Pathology, Kasturba Medical College, Mangalore, from January 2007 to June 2012 were included in the study. Clinicopathological analysis of the 84 cases of EC was done with emphasis on morphology. p53 immunostaining was performed in two cases of serous carcinoma.
Results: Out of a total of 84 cases of EC, ten cases were of type II (11.9%). Out of which, eight were serous carcinoma (9.5%) and two clear cell (2.4%). p53 immunostain was strongly positive in the serous papillary carcinomas. The age of the patients ranged from 45 to 75 years. Myometrial invasion was more than half. Treatment was hysterectomy followed by aggressive chemotherapy.
Conclusion: Of the type II EC, serous carcinoma is the most common type. Clinical presentation and prognosis differs in comparison to type I EC, thus the recognition of this type of EC is pivotal.

Key Words: Clear cell carcinoma, serous papillary, type II endometrial cancer

INTRODUCTION

Endometrial carcinoma ranks 3rd in India amongst the gynecological malignancies.[1] Endometrial cancer (EC) can be classified into two distinct groups – type I and type II, based on histology, which differs in molecular as well as in clinical and histopathological profiles.[2] Type II is nonestrogen dependent, nonendometrioid, with higher grade histologies, more aggressive and carries an adverse prognosis.[3,4] Although type II cancers contribute only about 10% of EC incidence, they present at advanced age and cause approximately 50% recurrence and deaths with a low 5-year, overall survival rate of 35%.[5,6] Type II cancers typically arise in an atrophic endometrial background, and often have deep myometrial penetration and demonstrate lymph node spread. Type II EC is also characterized by genetic alterations in p53, human epidermal growth factor (HER)-2/neu, p16 and E-cadherin.[7]

MATERIALS AND METHODS

Endometrial carcinomas diagnosed from endometrial biopsies and hysterectomy specimens received in the Department of Pathology, Kasturba Medical College,

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Mangalore, from January 2007 to June 2012 were included in the study. All specimens were fixed in 10% neutral buffered formalin and paraffin embedded for histological examination with hematoxylin and eosin staining. The clinicopathological analysis of the cases of EC was done with emphasis on morphology. p53 immunostaining was performed in two cases of serous carcinoma; using mouse monoclonal antibody. p53 immunoreactivity was regarded as positive when brown staining was localized to the tumor cell nuclei.

RESULTS

During the study period, a total of 84 cases of EC were reported. Of these, ten cases were classified as type II EC (11.9%) as per histology. Out of which, eight were serous carcinoma (9.5%) and two clear cell (2.4%). The age of the patients ranged from 45 to 75 years, with mean age being 62 years. Grossly, the type II EC frequently appeared bulky on a background of an atrophic endometrium, with tumor filling almost the entire cavity [Figure 1]. Myometrial invasion was more than half in seven of the cases. p53 immunostain was performed in two of the serous papillary carcinomas which showed strongly positive (>75% of tumor cells nuclei) staining in the nuclei. The tumor, node, metastasis staging and FIGO staging (2008) was done for all the cases, with four cases of stage III disease [Table 1].

Treatment was surgical hysterectomy in all cases, with one case being followed with aggressive chemotherapy due to metastases in the liver.

DISCUSSION

Type II endometrial carcinomas are nonestrogen related, nonendometrioid type. These generally occur in women a decade later than type I carcinoma, and in contrast to type I carcinoma they usually arise in the setting



Figure 1: Endometrial serous carcinoma gross specimen: Uterus with fleshy tumor filling the endometrial cavity with papillary excrescences

Table 1: Clinical profile and pathological features of type II endometrial cancers

S.No	Age (years)	Nature of specimen	Diagnosis	Grade/Stage	Gross features	Myometrial invasion
1.	58	Endometrial biopsy	Endometrial serous carcinoma	FIGO Stage Ib. TNM - T1bN0M0	Multiple dark brown tissue bits	> 50%
2.	75	Endometrial curettage	Endometrial serous carcinoma	FIGO Stage Ib. TNM - T1bN0M0	Multiple dark brown tissue bits	>50%
3.	45	TAH+BSO+ lymph node dissection	Clear cell carcinoma with squamous differentiation	FIGO Stage Ib. TNM - T1bN0M0	Grey white sessile growth arising in the posterior wall near right cornua	>50%
4.	69	TAH+BSO+ lymph node dissection	Clear cell carcinoma	FIGO Stage IIIc. TNM - T3bN1M0	Bulky uterus on a background of an atrophic endometrium, with tumour filling almost the entire cavity	>50%
5.	65	TAH+BSO+ lymph node dissection	Endometrial serous carcinoma	FIGO Stage IIIc. TNM - T3bN1M0	Enlarged uterus with tumour in entire cavity	>50%
6.	64	TAH+BSO+ lymph node dissection	Endometrial serous carcinoma	FIGO Stage Ia. TNM - T1aN0M0	Fleshy endometrium with large polyp in the cavity	<50%
7.	67	TAH+BSO+ lymph node dissection	Endometrial serous carcinoma	FIGO Stage IIIc. TNM - T3bN1M0	Fleshy endometrium with papillary excrescences; tumour filling entire cavity	>50%
8.	62	TAH+BSO+ lymph node dissection	Endometrial serous carcinoma	FIGO Stage Ia. TNM - T1aN0M0	Bulky uterus on a background of an atrophic endometrium, with tumour almost filling the entire cavity	<50%
9.	59	TAH+BSO+ lymph node dissection	Endometrial serous carcinoma	FIGO Stage Ia. TNM - T1aN0M0	Fleshy endometrium with polyp in the cavity	<50%
10.	60	TAH+BSO+ lymph node dissection	Endometrial serous carcinoma	FIGO Stage IV TNM - T3bN1M1	Bulky uterus on a background of an atrophic endometrium, with tumour almost filling the entire cavity	>50%

*TAH: Total Abdominal Hysterectomy *BSO: Bilateral salphingo-oophorectomy

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of endometrial atrophy. Of the type II EC, serous carcinoma is the most common type. Endometrial serous carcinomas (ESCs) constitute only 10% of ECs but have a substantially higher case-fatality rate than their more common endometrioid counterparts.[8] This is attributable, at least in part, to the advanced stage at which many patients with ESC present.[8]

The patient's age at the time of diagnosis is often a critical factor in determining the prognosis of a patient with endometrial carcinoma.[9] Generally, endometrial carcinoma in younger women, particularly before the menopause, is associated with a 5-year survival approaching 100%. One study found a 5-year survival of 96% for women aged 40–49 years compared with only 53% for women of 70–79 years.[10] Increasing age is associated with a higher grade and stage of endometrioid tumors and the likelihood of a nonendometrioid type tumor, but this accounts for only part of the difference in survival. An additional factor may be that a relative lack of immunocompetence is more prevalent in older patients.[11] In our series too, the mean age was 62 years, and the histomorphological features were of high grade (poorly differentiated).

Out of the 84 cases of EC reported during the study period, eight were serous carcinoma and two were clear cell carcinoma. This showed that serous carcinomas were the most common type of type II ECs. This was in concordance with studies and case reviews done earlier in literature.[4-8] Uterine bleeding in the postmenopausal woman was the presenting feature in all the cases.

ESCs are morphologically characterized by a complex pattern of papillae with cellular budding, cytologic pleomorphism, frequent lymphovascular and/or myometrial invasion, and not infrequently containing psammoma bodies.[12] Histologically, clear, glycogen-filled cells and hobnail cells that project individually into lumens and papillary spaces, and large, highly pleomorphic nuclei characterize the typical clear cell adenocarcinoma.[12]

In our study, ESC showed complex, branching papillae with fibrovascular core [Figure 2]. The papillae are covered by a stratified epithelium with a prominent and characteristic tufting or budding pattern. One case also showed a glandular pattern, lined by polygonal cells. Marked nuclear pleomorphism and large macronucleoli along with occasional bizarre and hyperchromatic giant nuclei was seen. Lymphovascular invasion and deep myometrial invasion was also present. In the clear cell carcinoma, the cells had clear cytoplasm with regular cell outline. The enlarged and pleomorphic nuclei were appearing to protrude into the lumens, presenting a "hobnail" appearance.

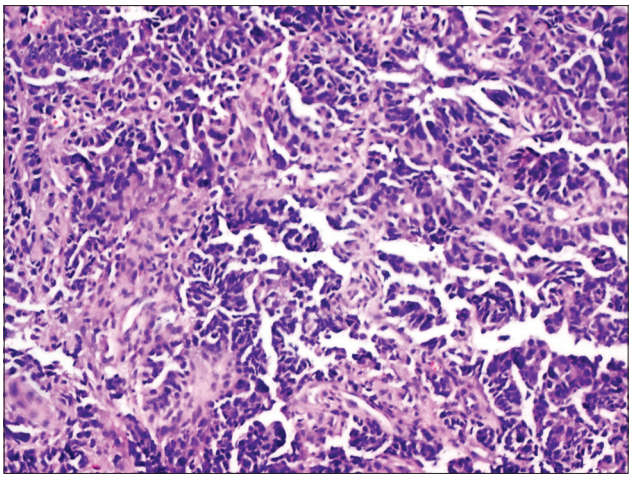


Figure 2: Endometrial serous carcinoma. Complex branching papillae (H and E; ×10)

The precursor of serous carcinoma, endometrial intraepithelial carcinoma, consists of cells identical to those of serous carcinoma but lacks identifiable stromal invasion.[13] Mutations in p53 are found in approximately 75% of these lesions, suggesting that mutation of p53 is an early event in serous endometrial carcinoma.[13] Thus, serous carcinoma presumably begins as a surface epithelial neoplasm that extends into adjacent gland structures and later invades endometrial stroma. Their generally poorer prognosis is thought to be a consequence of a propensity to exfoliate, undergo transtubal spread, and implant on peritoneal surfaces like their ovarian counterparts.

Myoinvasion is described by the presence of a constellation of features, including irregular, jagged contours of the neoplastic glands, single tumor cells or clusters in the myometrium, desmoplastic stroma, haphazard distribution of neoplastic glands in myometrium, etc.[14] Once the presence of myoinvasion is established, the depth of invasion is measured using the deepest undulation of endomyometrial junction. Other authors also advocate this approach.[14] Patients with more than 50% myometrial thickness invasion are at increased risk for extrauterine metastases, including pelvic and para-aortic lymph node metastases. These patients often require more aggressive surgical staging, which may include pelvic and para-aortic lymphadenectomy, as well as postoperative adjunctive therapy.[15] In our findings, seven of the cases (70%) had more than half of myometrial thickness invasion. One of the cases had distant metastases to the liver. For all the cases, Wertheim's hysterectomy was done. Postoperative chemotherapy was given additionally to the case with distant metastases.

The molecular changes of p53 mutation in 80–90% of serous carcinomas is reflected in p53 immunohistochemistry, which typically shows a diffuse and intense nuclear staining

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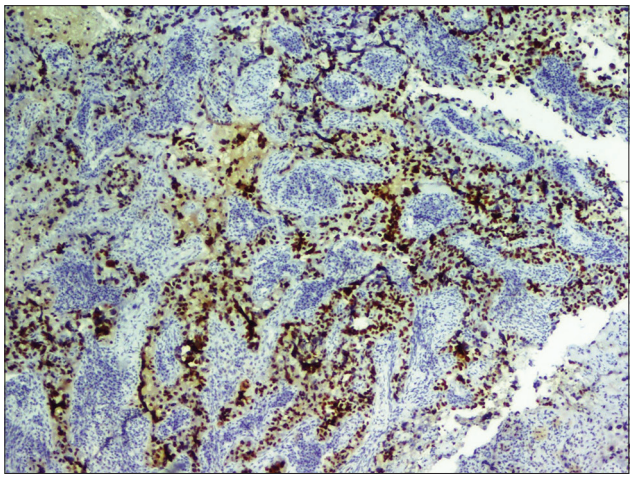


Figure 3: Endometrial serous carcinoma. Immunohistochemistry p53 staining. Diffuse strong positivity seen in the tumor cell nuclei

pattern involving almost all tumor cells.[16] In our study too, intense p53 staining was seen in 2 of the cases diagnosed as ESC and progesterone receptor marker was negative [Figure 3]. Thus, there is a significant association between p53 overexpression and ESC.

CONCLUSION

Our case series shows that the incidence of type II EC is less than that of type I. Among the type II EC, serous carcinomas are higher in number. The aggressiveness and the poorer survival rates of type II EC makes the diagnosis of this type very crucial for the histopathologist and the clinician.

Financial support and sponsorship

Manipal University.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Devi K. Current status of gynecological cancer care in India. J Gynecol Oncol 2009;20:77-80.
2. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983;15:10-7.
3. Amant F, Vandenput I, Van Gorp T, Vergote I, Moerman P. Endometrial carcinosarcoma with osteoclast-like giant cells. Eur J Gynaecol Oncol 2006;27:92-4.
4. Mendivil A, Schuler KM, Gehrig PA. Non-endometrioid adenocarcinoma of the uterine corpus: A review of selected histological subtypes. Cancer Control 2009;16:46-52.
5. Singh P, Smith CL, Cheetham G, Dodd TJ, Davy ML. Serous carcinoma of the uterus-determination of HER-2/neu status using immunohistochemistry, chromogenic *in situ* hybridization, and quantitative polymerase chain reaction techniques: Its significance and clinical correlation. Int J Gynecol Cancer 2008;18:1344-51.
6. Bansal N, Yendluri V, Wenham RM. The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. Cancer Control 2009;16:8-13.
7. Llobet D, Pallares J, Yeramian A, Santacana M, Eritja N, Velasco A, *et al.* Molecular pathology of endometrial carcinoma: Practical aspects from the diagnostic and therapeutic viewpoints. J Clin Pathol 2009;62:777-85.
8. Fadare O, Zheng W. Insights into endometrial serous carcinogenesis and progression. Int J Clin Exp Pathol 2009;2:411-32.
9. Abeler VM, Kjørstad KE. Endometrial adenocarcinoma in Norway. A study of a total population. Cancer 1991;67:3093-103.
10. Connelly PJ, Alberhasky RC, Christopherson WM. Carcinoma of the endometrium. III. Analysis of 865 cases of adenocarcinoma and adenoacanthoma. Obstet Gynecol 1982;59:569-75.
11. Mutter GL, Matias-Guiu, Lax SF. Endometrial adenocarcinoma. In: Robboy SJ, Mutter GL, Prat J, Bentley RC, Russell P, Anderson MC, editors. Robboy's Pathology of the Female Reproductive Tract. 2nd ed. China: Elsevier; 2009. p. 410-21.
12. Tavassoli F, Devilee P. World Health Orgaization Classification of Tumors. Pathology and Genetics of Tumors of the Breast and Female Genital Organs. Lyon: IARC Press; 2003.
13. Crum CP. The Female genital tract. In: Kumar V, Abbas AK, Fausto N. Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia: Elsevier; 2010. p. 1032-3.
14. Ali A, Black D, Soslow RA. Difficulties in assessing the depth of myometrial invasion in endometrial carcinoma. Int J Gynecol Pathol 2007;26:115-23.
15. Larson DM, Connor GP, Broste SK, Krawisz BR, Johnson KK. Prognostic significance of gross myometrial invasion with endometrial cancer. Obstet Gynecol 1996;88:394-8.
16. Lax SF, Kendall B, Tashiro H, Slebos RJ, Hedrick L. The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: Evidence of distinct molecular genetic pathways. Cancer 2000;88:814-24.

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Case Report

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Osteoblasts to the Rescue – A Rare Case of Secondary Myelofibrosis

Lawanya Gunaseelan, Eliz Thomas, Prasanna N. Kumar, Sujaya Menon[1]

Abstract:

A 36-year-old female patient presented with joint pain, generalized weakness, and bleeding gums. There was no significant history of other illnesses or intake of drugs. Examination revealed pallor and splenomegaly. Peripheral smear examination showed pancytopenia. A large number of osteoblasts and a few scattered osteoclasts were seen in the bone marrow aspirate. The trephine biopsy revealed marrow fibrosis. A suggestion to investigate for hyperparathyroidism was made. Serum calcium, ionized calcium, and PTH were increased. 99m Tc-Sestamibi scan was done and adenoma was detected in the right inferior parathyroid and subsequently excised. Postsurgery, hematological parameters and bone markers improved. Follow-up bone marrow biopsy revealed hypercellular marrow with marked regression of fibrosis. Splenomegaly was reversed. We concluded that pancytopenia was due to bone marrow fibrosis, resulting from primary hyperparathyroidism. It is important to consider secondary causes of myelofibrosis in the appropriate clinical setting.

Keywords:

Osteoblasts in the marrow, secondary myelofibrosis, marrow fibrosis

Background

Primary myelofibrosis is commonly a disease of the elderly. Secondary myelofibrosis occurs due to various pathological processes that involve the bone marrow such as malignancy, infection, metabolic disorders, radiation, and drugs. Hyperparathyroidism as a precipitating factor for myelofibrosis has rarely been reported. [1,2] It is important to consider secondary causes of myelofibrosis in the appropriate clinical setting. Our patient improved, similar to others after surgery. Diagnosis was confirmed when fibrosis in the bone marrow reversed after surgery.

Case Report

We report a 36-year-old female who presented with joint pain, generalized weakness, and bleeding gums. There was no significant history of other illnesses or intake of any drugs. Examination revealed pallor and massive splenomegaly. Peripheral smear examination showed pancytopenia. Bone marrow aspiration showed a large number of osteoblasts and a few scattered osteoclasts [Figure 1]. The trephine biopsy revealed marrow fibrosis of grade 3 [Figure 2]. On the basis of the presence of abundant osteoblasts and fibrosis, a suggestion to investigate for hyperparathyroidism was made. On further investigation, serum calcium, serum ionized calcium, and PTH were found to be increased [Table1]. 99m Tc-Sestamibi scan was done, and adenoma was detected in the right inferior parathyroid and was subsequently excised [Figure 3].

As early as 6 months postsurgery, hematological parameters and bone markers improved without any intervention. During the follow-up, bone marrow biopsy revealed hypercellular

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Table 1: Investigations

Investigations	Initial
Hemoglobin (normal: 12-15 g/dl)	7.4
PCV (normal: 36%-46%)	22.7
WBC (normal: 4-10×10^3 µl)	1.7×10^3
Platelet count (normal: 150-400×10^3)	11×10^3
Bone marrow aspirate	Hypocellular marrow with increase in osteoclasts and osteoblasts
Bone marrow trephine	Grade 3 fibrosis
Ionized PTH (normal: 15-65 pg/ml)	1484
Serum calcium (normal: 8.8-10.2 mg/dl)	15.3
Ionized calcium (normal: 1.1-1.3 mmol/L)	2.224
Serum phosphorus (normal: 2.7-4.5 mg/dl)	2.3

PCV=Packed cell volume; WBC=White blood cell; PTH=Parathyroid hormone

Table 2: Investigations post surgery

Investigations	6 months postsurgery
Hemoglobin (normal: 12-15 g/dl)	11.2
PCV (normal: 36%-46%)	34.2
WBC (normal: 4-10×10^3 µl)	4.9×10^3
Platelet count (normal: 150-400×10^3)	71×10^3
Bone marrow aspirate	Hypercellular marrow with erythroid hyperplasia. Occasional osteoblasts seen
Bone marrow trephine	No evidence of fibrosis
Ionized PTH (normal: 15-65 Pg/Ml)	55
Serum calcium (normal: 8.8-10.2 mg/Dl)	8.0
Ionized calcium (normal: 1.1-1.3 mmol/L)	1.184
Serum phosphorus (normal: 2.7-4.5 mg/dl)	4.0

PCV=Packed cell volume; WBC=White blood cell; PTH=Parathyroid hormone

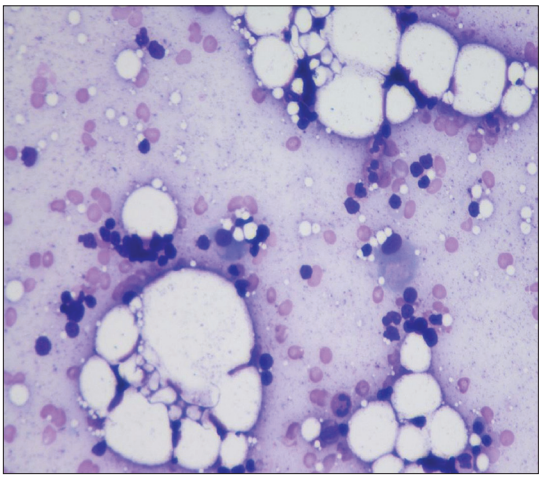


Figure 1: Osteoblasts seen in the bone marrow aspirate

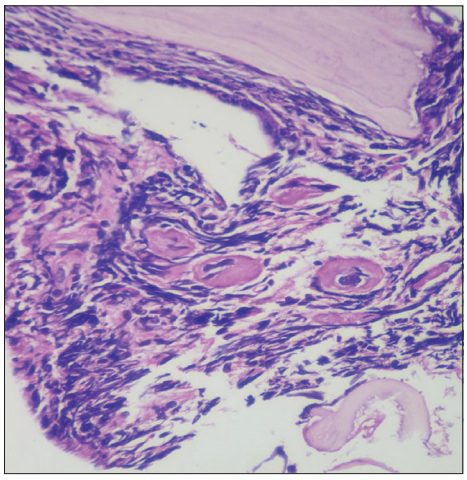


Figure 2: Bone marrow trephine biopsy showing fibrosis

marrow and marked regression of fibrosis. The spleen also gradually decreased in size. We concluded that the pancytopenia was because of bone marrow fibrosis resulting from primary hyperparathyroidism.

Investigations

Differential diagnosis

All the causes for pancytopenia were ruled out one by one. Due to the presence of osteoblasts and osteoclasts in the marrow aspirate, along with fibrosis in the biopsy, a suggestion to investigate for hyperparathyroidism was made.

Treatment

Fluids, diuretics, bisphosphonates, and other supportive measures were administered to treat hypercalcemia. Thalidomide and prednisolone were given in tapering doses.

The parathyroid adenoma was excised and confirmed by histopathology.

Outcome and follow-up

Six months post-surgery, hematological parameters and bone markers improved [Table 2]. Bone marrow biopsy

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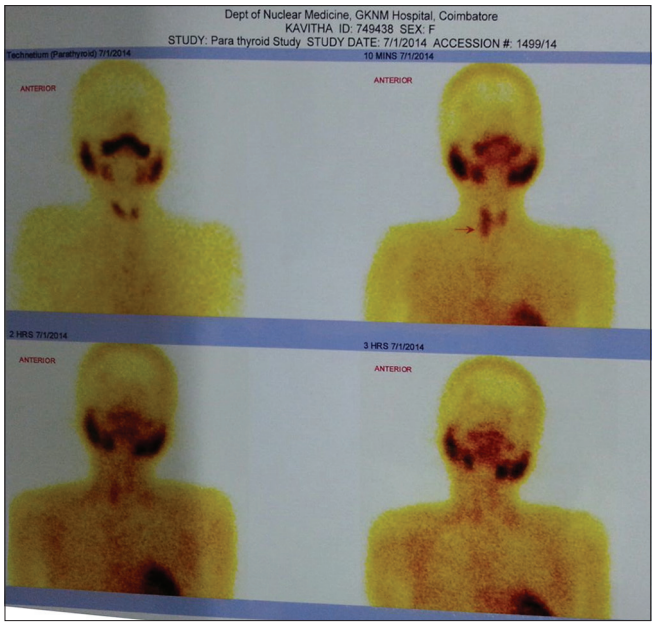


Figure 3: Radioisotope study revealed inferior parathyroid adenoma

revealed hypercellular marrow and marked regression of fibrosis [Figure 4]. The spleen gradually decreased in size.

Discussion and Conclusion

Pancytopenia is a common hematological entity, and the various causes have to be evaluated for. Myelofibrosis is one such cause and primary myelofibrosis is known to occur in the elderly. Secondary myelofibrosis can be due to various etiologies such as drugs, neoplasms, collage diseases, hyperparathyroidism, and renal osteodystrophy. Literature says that asymptomatic primary hyperparathyroidism is rare.[2-5] Our patient had features of advanced hyperparathyroidism such as elevated blood calcium levels, PTH levels, ALP levels and radiological evidence of subperiosteal bone resorption and osteopenia. After the curative parathyroid surgery, improvement in hematological and metabolic parameters is very suggestive of marrow fibrosis due to primary hyperparathyroidism.

Learning points/take home messages

- Evaluation for secondary myelofibrosis in correlation with clinical and biochemical parameters is of paramount significance
- Hyperparathyroidism must be considered with the presence of osteoblasts in the marrow aspirate.

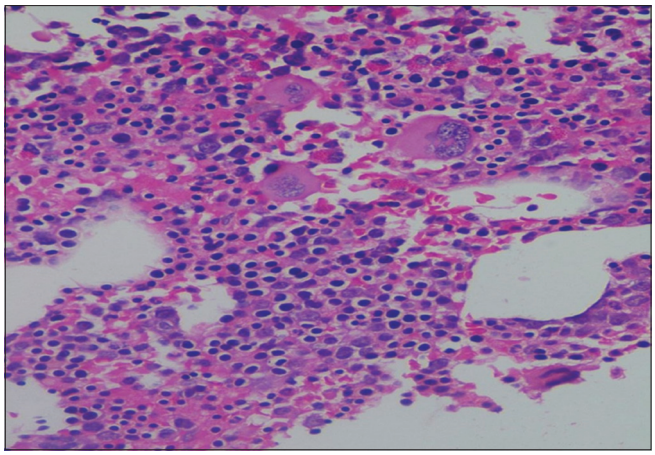


Figure 4: Bone marrow biopsy post surgery shows presence of hematopoietic elements

Ethics approval and consent to participate

Ethics approval was obtained and consent to participate was taken from the patient.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Elaraj DM, Clark OH. Current status and treatment of primary hyperparathyroidism. Perm J 2008;12:32-7.
2. Bhadada SK, Bhansali A, Ahluwalia J, Chanukya GV, Behera A, Dutta P, *et al*. Anaemia and marrow fibrosis in patients with primary hyperparathyroidism before and after curative parathyroidectomy. Clin Endocrinol (Oxf) 2009;70:527-32.
3. Boxer M, Ellman L, Geller R, Wang CA. Anemia in primary hyperparathyroidism. Arch Intern Med 1977;137:588-93.
4. Kumbasar B, Taylan I, Kazancioglu R, Agan M, Yenigun M, Sar F, *et al*. Myelofibrosis secondary to hyperparathyroidism. Exp Clin Endocrinol Diabetes 2004;112:127-30.
5. Lim DJ, Oh EJ, Park CW, Kwon HS, Hong EJ, Yoon KH, *et al*. Pancytopenia and secondary myelofibrosis could be induced by primary hyperparathyroidism. Int J Lab Hematol 2007;29:464-8.

Original Research Article

Clinico-cytological and radiological correlation of sputum analysis in a tertiary care hospital

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Sputum, a specialized product of the respiratory tract, is formed as a result of interaction between mucociliary apparatus, immune system of the host and animate, inanimate invaders from the environment. It is not only limited to the diagnosis of tuberculosis and malignancy, but is also equally important in the diagnosis of other diseases. The aim is to study the cellular morphology in the satisfactory sputum samples and to correlate the cytological features with clinical and radiological findings. **M** This study emphasizes the role of sputum cytology in the diagnosis of non-neoplastic lung diseases in correlation with clinical and radiological features. This is a prospective study, undertaken in the Department of Pathology, from July 2016 to December 2018. During this period, 200 sputum samples were collected. In the study period, 200 sputum samples received account for 1.56% of the total cytological samples. Age group ranged from 6 to 84 years with a mean age of 55.41 years. Majority of the patients belonged to the age group of 61-70 years (32%). Male to female ratio was 3.65:1. Among 200 patients, 98 cases were clinically diagnosed to have pulmonary tuberculosis (49%), of which cytological diagnosis was made in 59 cases (60.2%). Of the 32 clinical Bronchial asthma cases, 31 chronic bronchitis, 15 acute bronchitis and 13 pneumonia cases, cytological diagnosis was made in 25, 28, 11 and 10 cases respectively. Three patients each with clinical diagnosis of lung abscess and candida infection had consistent cytological diagnosis. Similarly, one patient each who were diagnosed with Aspergilloma, Actinomycosis, Pulmonary edema, Bronchiectasis, pulmonary eosinophilia clinically, had consistent cytological diagnosis. Sputum cytology is a simple, cheap, non-invasive and free of complications for the early diagnosis of lung diseases. Although fiber optic bronchoscopy and fine needle aspiration provide better diagnostic value, pulmonary cytology provides the primary laboratory means for diagnosing and treating patients with lung diseases.

Clinical correlation, Non neoplastic lung lesions, Sputum cytology

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Introduction

Diagnostic cytology is an art and science of interpretation of cells from the human body that either exfoliate (desquamate) from the epithelial surface freely or are removed from tissue sources by various clinical procedures[1]. Diagnostic cytology is based on three sampling techniques i.e. Exfoliative cytology, Abrasive cytology and Aspiration cytology. Rapid urbanization, industrial growth, and increasing pollution have led to dramatic increase in respiratory diseases. On the global basis, ranking of cancer sites in the decreasing order showed lung at the top of the list[2]. There are numerous investigations available, namely sputum cytology, radiological studies, Bronchoalveolar lavage (BAL), Bronchial brushings cytology and fine needle aspiration cytology, etc., to study the morphology of lung diseases. "Sputum cytology" is an example of "Exfoliative cytology" which is based on spontaneous shedding of cells derived from the lining of an organ into a cavity from where they can be removed by a non-abrasive means[1,3,4].Sputum is a specialized product of respiratory tract, produced as a result of interaction between mucociliary apparatus, immune system of the host and animate, inanimate invaders from the environment[5]. Sputum cytology affords a simple, accurate, reliable, cost effective and non-invasive procedure for the assessment of respiratory diseases including pre-invasive and invasive pulmonary malignancies. The cellular and non-cellular components of sputum reflect the pathological

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process. Sputum examination plays a vital role in the diagnostic cytopathology. It is not only limited to the diagnosis of tuberculosis and malignancy but also equally important in the diagnosis of other diseases like bacterial, viral, fungal, and parasitic infections of the respiratory tract, necessitating the study of sputum cytology[1].

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This study emphasizes the role of sputum cytology in the diagnosis of non-malignant lung diseases in correlation with clinical and radiological features. This is a prospective study, undertaken in the Department of Pathology from July 2016 to December 2018. All the sputum samples were sent to the Department of Pathology for cytological examination. Inclusion criterion: Non-neoplastic lung diseases. Exclusion criterion: Neoplastic and Unsatisfactory sputum samples. All the patients in this study were examined in detail and routine necessary investigations were done. These patients were given prior instructions about the technique of producing good sputum. They were instructed to brush their teeth, and/or rinse the mouth and gargle with water, upon awakening in the morning. They were then asked to inhale deeply to the full capacity of the lungs and exhale the air with an expulsive cough. This early morning deep sputum samples, were collected in sterile glass bottle (fresh and unfixed sputum) and sent to the laboratory immediately for cytological examination. Radiological investigations were done at the discretion of attending physician. Sterile disposable sticks were used to spread sputum thinly, and the specimen was examined carefully with naked eye and hand lens. The suspicious material, tissue particles and blood stained areas were picked and smeared on the albuminized slides (pick and smear method). Four such smears were prepared for each sputum sample. Among these, two smears were air dried and were stained with Giemsa stain and Ziehl-Neelsen stain (using 20% sulphuric acid) for demonstrating acid fast bacilli. The remaining two were promptly fixed in a fixative containing equal amounts of ether and 95% ethyl alcohol. These two were stained with Papanicolaou's stain and Haematoxylin and Eosin stain.

□□□□□□□

In this study, 200 sputum samples account for 1.56% of the total 12,835 cytological specimens received during the study period. Age group of the patients investigated for sputum cytology ranged from 6 years to 84 years with a mean age of 55.41 years. Majority of the patients belonged to the age group of 61-70 years followed by 51-60 years, 41-50 years, 71-80 years and 31-40 years. (Figure: 1)□

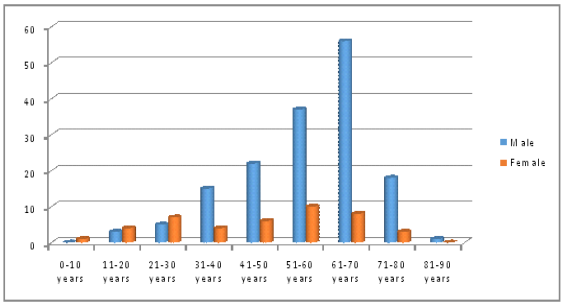


Figure 1: Distribution of Cases According to age And Sex

In this present study, males outnumber females. Male to female ratio was 3.65:1. Among 200 patients, 98 cases were clinically diagnosed to have pulmonary tuberculosis (49%). Rest in the decreasing order, were diagnosed to have bronchial asthma (16%), chronic bronchitis (15.5%), acute bronchitis (7.5%), pneumonia (6.5%), pulmonary candidiasis (1.5%), lung abscess (1.5%), pulmonary edema (0.5%), bronchiectasis (0.5%), aspergilloma (0.5%), actinomycosis (0.5%), and pulmonary eosinophilia (0.5%). (Table No: 1). The cytological diagnosis of pulmonary tuberculosis was made in 59 cases (60.2%), chronic bronchitis in 28 (78.12%), acute bronchitis in 11 (73.3%), pneumonia in 10 (76.92%), lung abscess in 3 (100%), candida infection in 3 (100%), aspergilloma, actinomycosis, pulmonary edema, bronchiectasis and pulmonary eosinophilia were diagnosed in one patient each, which correlated 100% with clinical diagnosis. The above clinico-cytological correlation of various non-neoplastic lung lesions was depicted in Table No: 2. Cytological features of lung lesions are enumerated in Table No: 3.

Table 1: Clinical Diagnoses

Non neoplastic pulmonary diseases	Males	Females	Total	Percentage
Acute bronchitis	15	0	15	7.5
Pneumonia	10	03	13	6.5
Lung abscess	02	01	03	1.5
Pulmonary tuberculosis	**77**	21	98	49
Aspergilloma	01	0	01	0.5
Actinomycosis	01	0	01	0.5
Candida	03	0	03	1.5
Pulmonary edema	0	01	01	0.5
Chronic bronchitis	25	06	31	15.5
Bronchiectasis	01	0	01	0.5

Bronchial asthma	22	10	32	16
Pulmonary eosinophilia	0	01	01	0.5
Total	**157**	**43**	**200**	**100**

Table 2: Clinico-Cytological Correlation Of Non

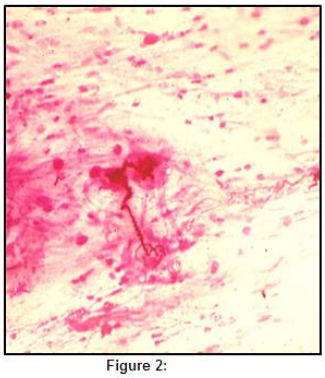
S. No	Diseases	Number of clinically diagnosed cases	Cytological features Consistent with clinical diagnosis	Percentage
1	Acute bronchitis	15	11	73.33
2	Pneumonia	13	10	76.92
3	Lung abscess	3	3	100
4	Aspergilloma	1	1	100
5	Actinomycosis	1	1	100
6	Candida	3	3	100
7	Pulmonary edema	1	1	100
8	Chronic bronchitis	31	28	90.32
9	Bronchiectasis	1	1	100
10	Bronchial asthma	32	25	78.12
11	Pulmonary eosinophilia	1	1	100

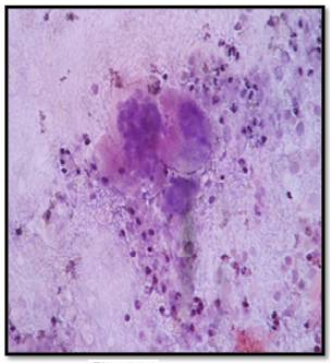
Table 3: Cytological Features Of Lung Diseases

Sr No	Cytological features	Acute Bronchitis (11)	Pneumonia (10)	Lung abscess (3)	Pul. TB (59)	Aspergilloma (1)	Candida (3)	Pul. Edema (1)	Ch. Bronchitis (28)	Bronchiectasis (1)	Bronchial Asthma (25)	Pulmonary Eosinophilia (1)
1	Squamous cells	11	10	3	59	1	3	1	28	1	25	1
2	Neutrophils	11	10	3	59	1	3	1	28	1	25	1
3	Degenerated epithelial cells	11	10	3	59	1	3	1	28	1	25	1
4	Alveolar carbon laden macrophage	11	10	3	59	1	3	1	28	1	25	1
5	Cellular debris	11	10	3	55	1	3	1	28	1	25	1
6	Curschmann spirals	1	2	2	6	0	0	0	9	0	16	0
7	Lymphocytes	7	8	3	57	1	3	1	25	1	22	1
8	Ciliated columnar cells	2	4	1	52	1	2	0	22	1	19	1
9	Epithelioidhistiocytes	0	0	0	55	0	0	0	0	0	0	0
10	Langhans giant cells	0	0	0	51	0	0	0	0	0	0	0
11	Metaplastic squamous cells	2	1	1	26	0	1	0	10	0	12	0
12	Erythrocytes	1	1	0	11	0	0	0	0	0	0	0
13	Acid fast bacilli	0	0	0	13	0	0	0	0	0	0	0
14	Eosinophils	0	0	0	3	1	2	0	11	0	13	1

Table 4: AFB Positivity In Clinically Suspected Pulmonary Tuberculosispatients In Comparison With Other Studies

Serial No	Authors	Clinically suspected pulmonary tuberculosis No of cases	Percentage of cases having AFB positive samples
1	Gokhale *et al*[98] (1990)	2100	14.28
2	Panda *et al*[99] (1995)	100	13.00
3	Khagi A K *et al*[100] (2009)	250	53.60
4	Hepple *et al*[34] (2010)	280	43.20
5	Mindolli *et al*[101] (2013)	84	29.76
6	Goyal *et al*[102] (2013)	388	07.47
7	**Present study**	**98**	**13.26**


Figure 2:


Figure 3

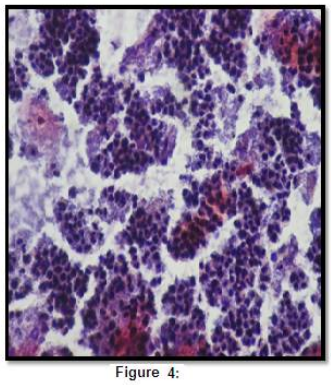

Figure 4:


Figure 5:

Figure 2: A Curschmann spiral with dense central core of inspissated mucus in a sputum of patient with bronchial asthma (Hematoxylin and eosin, x400); **Figure 3:** Sputum showing multinucleated giant cells of Langhans type (Papanicolaou stain, x400); **Figure4:** X ray showing Chroniccavitatory Pulmonary Aspergillosis; **Figure 5:** Numerous neutrophils in sputum of patient with pulmonary lung abscess (Papanicolaou stain, x400)

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In this study, number of sputum samples studied comprises only 1.56% of the total cytological specimens, which indicates that sputum cytology has not gained much popularity among the physicians. However, this procedure has certain advantages over other investigations, in being noninvasive, cheap and repeatability[1,5,6] . Majority of the patients in this study belonged to the age group of 61-70 years (32%). Many of the respiratory diseases are common in this age group, necessitating sputum examination. In this study male patients outnumber female patients with male to female ratio of 3.65:1 and our findings are similar to other studies[7]. Many of the respiratory diseases are common in males[7] and most adult males are habitual smokers in this geographical location, which is in line with Longo *et al* study. In this study clinically diagnosed respiratory diseases in decreasing order are pulmonary tuberculosis (48%), bronchial asthma (16%), chronic bronchitis (15.5%), acute bronchitis (7.5%), pneumonia (6.5%), pulmonary candidiasis (1.5%), lung abscess (1.5%), pulmonary edema (0.5%), bronchiectasis (0.5%), aspergilloma (0.5%), actinomycosis (0.5%)and pulmonary eosinophilia (0.5%). These relative incidences indicate the relative incidence of respiratory diseases in the general population. Pulmonary tuberculosis is the most common respiratory disease in our country[7].Roby *et al* (1988) suggested the adequacy criteria for sputum samples, according to which, sputum samples were regarded as adequate if there were at least 150 macrophages per slide from a nonsmoker or at least 300 macrophages per slide from a smoker. However, quantitative sputum cytology was not done in this study[6]. In the present study, cytological diagnosis was made in 11 cases of acute bronchitis, which revealed predominant neutrophils, a few lymphocytes, ciliated columnar cells, Curschmann spirals and cellular debris. Curschmannspirals□□□□□ **2**□ are considered to be pathognomonic of chronic bronchial disorders such as chronic bronchitis and bronchial asthma. The full significance of Curschmann spirals in the pathology of the respiratory tract has not been fully assessed as yet[1]. Investigations done by Walker and Fullmer (1970) documented that over 90% of asymptomatic cigarette smokers have Curschmann spirals in their sputum. Occurrence of Curschmann spirals in acute bronchitis, seen in this study, is probably related to smoking. Ten cases of pneumonia and three cases of lung abscesses encountered in this study, showed predominant population of neutrophils □□□□□□□ Gram stain and bacterial culture studies were not done in these cases. Gray W *et al* suggested that the overgrowth of the commensals from oropharynx should not be mistaken for an infection. They are unlikely to be significant if mixed with sputum and if found mainly in areas of saliva without accompanying inflammatory cells[6].Cytological diagnosis was made in 59 cases of pulmonary tuberculosis. Epithelioidhistiocytes were observed in 55 cases and Langhans giant cells were observed in 51 cases. The combination of epithelioidhistiocytes and Langhans giant cells (Fig.3) is highly suggestive of tuberculosis but is not pathognomonic since either or both cell types can be seen in other diseases with granulomatous inflammation[6]. Presence of epithelioid cell granuloma and Langhans giant cells in sputum cytology in cases of pulmonary tuberculosis is of very uncommon occurrence. Only a few reports could be found in the English language literature. Presence of epithelioid cell granuloma and Langhans cells, along with demonstration of AFB in routine sputum cytology, is capable of providing a conclusive diagnosis in the appropriate clinical context, obviating the need for additional

investigation[8]. Long, slender acid fast bacilli were demonstrated in eight cases (Grade-II) and five cases (Grade-III). □□□□□□ □□□□□ show AFB positivity in clinically suspected pulmonary tuberculosis patients in comparison with other studies. Dawson *et al* (1981) suggested that the presence of striking pleomorphism and/or cross banding in acid fast bacilli found in smears of sputum is usually indicative of mycobacteria other than mycobacterium tuberculosis[9]. However, atypical strains of mycobacteria causing lung infection can be morphologically similar to mycobacterium tuberculosis and can only be distinguished by culture[6]. In two patients, who were clinically diagnosed with pulmonary tuberculosis, sputum cytology revealed pulmonary candidiasis and Actinomycosis. In these patients, chest x ray showed apical opacities in the lung fields. Therefore concomitant pulmonary tuberculosis could not be ruled out in these cases, though acidfast bacilli were not seen in these sputum samples. Clinical diagnosis of aspergilloma was made in a 70 year old man who was earlier treated for tuberculosis. In this case sputum examination was done, which revealed Aspergillosis. Inhaled air borne spores of aspergillusfumigatus may lodge and germinate in damaged pulmonary tissue, and an aspergilloma (a ball of aspergillus fungus) can form in any area of damaged lung, in which there is a persistent abnormal space. The most common cause of such pulmonary damage is tuberculosis, but an aspergilloma can develop in an abscess cavity, a bronchiectatic space, or even in a cavitatedtumour. Most, but not all are caused by Aspergillusfumigatus[7]. The presence of fungi in sputum specimens raises one of the three possibilities. Firstly, they may be contaminants from the mouth or atmosphere; secondly, there may be saprophytic colonization of an area of pre-existing diseased lung such as a cavity, without any invasion of tissues; or thirdly, there could be an active infection (mycosis), with growth of fungi in the lung parenchyma[6]. Inflammatory debris is usually present when there is invasion of tissues by fungi or if a fungal ball (mycetoma) has formed in cavitatedlung[6] □□□□□□□□In a patient clinically diagnosed with chronic bronchitis, sputum cytology revealed Respiratory Syncytial Virus infection. Patients with viral infection may exfoliate reactive bronchial epithelial cells in tridimensional clusters with prominent nucleoli, mimicking cells derived from a bronchogenic adenocarcinoma. These cells usually disappear within 2 weeks after the recovery of the lung infection[6]. Sputum cytology in a case of clinically diagnosed pulmonary edema revealed superficial squamous epithelial cells, neutrophils, lymphocytes, and carbon laden alveolar macrophages in a mucinous background. Cytological features were non-specific. Cytological diagnosis was made in 28 cases of chronic bronchitis, where sputum samples showed clusters of hyperplastic bronchial epithelial cells in two and Curschmannspirals in nine. Cytological features were non-specific. Gorska *et al* (2008) observed that, neutrophils, macrophages, and lymphocytes are the most significant inflammatory cells found in the airways of patients with COPD[10]. Hyperplastic epithelial cell clusters need to be distinguished from adenocarcinoma and metastatic carcinoma cells. The presence of a few cilia around the border of some of the groups is evident of reactive change. Nuclear features may not be clearly discernible for evaluation of malignancy, but macronucleoli are suspicious of malignancy. Adenocarcinoma cells tend to exfoliate more profusely than benign cells. In this study, clinical diagnosis of bronchiectasis was made in one case, where cytological features were non-specific. Cytological diagnosis was made in 25 out of 32 clinically diagnosed bronchial asthma cases. Curschmann spirals were noted in sixteen patients. Findings such as Charcot–Leyden crystals, Curschmann spirals or Creola bodies have been considered characteristic in patients with asthma[1,5,6]. Pizzichini *et al* (2009) reported that proportion of eosinophils in sputum is a more accurate marker of asthmatic airway inflammation than the proportion of blood eosinophils or serum eosinophilic cationic protein[11]. In one patient, Creola bodies consisting of clusters of ciliated bronchial epithelial cells were seen. They have come to known as Creola bodies after a young girl in whom they were reported, assuming initially that they were adenocarcinoma cells.None of the sputum samples showed Charcot leydencrystals. This is probably related to the absence of clinical evidence that external allergens play a role in the production of late onset asthma (non atopic)[6]. In one case of pulmonary eosinophilia, sputum cytology revealed squamous epithelial cells, a few neutrophils, lymphocytes, erythrocytes and plenty of eosinophils in a mucinous background. Blood peripheral smear of this patient showed increase in eosinophils. Transient pulmonary lesions characterize pulmonary eosinophilia, eosinophilia in the blood, and a benign clinical course. CT scans are often quite striking, with shadows of varying size and shape in any of the lobes suggesting irregular intrapulmonary densities[12].

□□□□□□□□□□

Sputum cytology has been the traditional focus for studying lung diseases for many years. Sputum cytology is a cost effective, non invasive and free of complications for the early diagnosis of lung diseases. However, the emphasis has been altered by the introduction of fiber optic bronchoscopy and fine needle aspiration. Recently,

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automated systems to increase the productivity and accuracy in the cytology laboratory are beginning to appear. As there is no single diagnostic method to exclude the diseases, combinations of various techniques for obtaining material from respiratory tract yield better results. Pulmonary cytology provides the primary laboratory means for diagnosing and treating patients with lung diseases.

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1. Melamed M R. The lower respiratory tract in the absence of cancer. Tumors of the lung conventional cytology and aspiration cytology. In: Koss G L. Diagnostic Cytology and Its Histopathologic Basis. 5th ed. Philadelphia: JB Lippincott Williams and Wilkins Company; 2006. p. 569-711.
2. Park K. Non communicable diseases. In: Park's text book of preventive and social medicine. 21st ed. Jabalpur: BanarsidasBhanot Publishers; 2011. p. 359-61.
3. Ammanangi AS, Dombale VD, Miskin AT, Dandagi GL, Sangolli SS. Sputum cytology in suspected cases of carcinoma of lung (sputum cytology a poor man's bronchoscopy!). Lung India. 2012; 29: 19-23.
4. Gupta K.B, Garg S. Sputum Induction–A Useful tool in diagnosis of respiratory diseases. Lung India. 2006; 23: 82-86.
5. Johnston W W, Elson C E. Respiratory Tract. In: Bibbo M, Wilbur C D. Comprehensive Cytopathology.3rd ed. Philadelphia: WB Saunders Company; 1997. p. 301-360.
6. Gray W, Thomas E, Giles, Julie, McCarthy. In: Diagnostic cytopathology. 5 th ed. Newyork: Churchill Livingstone; 2010. p. 17-114.
7. Longo *et al*. Approach to the Patient with Disease of the Respiratory System. In: Harrisons principles of internal medicine. 18 th edition. The McGraw-Hill Companies. United states of America; 2012.
8. Kumar B. Granuloma in sputum cytology of pulmonary tuberculosis: a case report. ActaCytol. 2009; 53(3): 341-3.
9. Dawson *et al*. Differential identification of mycobacteria in smears of sputum. Tubercle and lung disease. 1981: 62; 257-262.
10. K. Gorska *et al*. Eosinophilic airway inflammation in chronic obstructive pulmonary disease and asthma. Journal of physiology and pharmacology. 2008; 59(Suppl 6): 261–270.
11. Al Obaidi *et al*. The predictive value of eosinophil cationic protein and lactate dehydrogenase in asthma: a comparative study of serum versus sputum. WAO Journal.2009; 2:144–149
12. Aliya N. Husain. The Lung. In□□Robbins and Cotran Pathologic Basis of disease. 8th Ed. Saunders Elsevier, Philadelphia; 2014. P. 669-727.

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Original Article

Validating Micronucleus Score in Effusion Fluids

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Abstract

Background: Identifying malignant cells in effusion fluid is vital in staging and management of cancers. Differentiating reactive mesothelial cells from malignant cells in effusion fluid is a challenging task and there is an ongoing need for simpler and cost effective tool to aid the diagnosis. Micronucleus is an additional smaller nucleus in the cytoplasm, formed by chromosomes or chromosomal fragments formed during cell division. **Aims:** The aim of this study was to assess the significance of micronucleated cell in effusion fluids to distinguish adenocarcinomatous from reactive mesothelial effusions. **Materials and Methods:** Thirty cases of unequivocal malignant effusion fluids and 30 benign cases with reactive mesothelial cells as control were studied. Number of microucleated cells present per1000 well-preserved cells in Leishman-stained smears were counted. **Results:** Mean (±SD) micronucleated score in malignant and benign effusions were 15.77 ± 9.78 and 1.87 ± 1.78, respectively. The median scores were 13 and 2, respectively. Mann–Whitney test showed that this difference was statistically significant ($P < 0.001$). This study revealed that there was a significant difference in micronucleus scoring between benign and malignant effusions. **Conclusions:** Micronucleus score can be used as an additional biomarker in the interpretation of routinely stained cytosmears.

Keywords: Adenocarcinoma, effusion fluid, micronucleus

Introduction

Identifying malignant cells in effusion fluid is of paramount importance in staging and management of cancers. Differentiating reactive from malignant cases in effusion fluid is difficult at many times. We need a simple, inexpensive, and reliable tool to identify them. Micronucleus (MN) test is a simple test which is done on routine staining, thus avoiding the need of expensive methods and the use of immunocytochemistry panels and flow cytometry.[1-5]

MN is an additional smaller nucleus in the cell cytoplasm. Its presence indicates chromosomal damage or breakage and mitotic dysfunction of the cell. MN is round to oval in shape and is separated from the main nucleus. The mean diameter is 1/16th to 1/3rd of the main nucleus and is nonrefractile. Color and texture is the same as that of the main nucleus.[6,7]

Utility of MN scoring have been studied in cervical smears,[8,9] exfoliated buccal and bladder cells,[10] colorectal carcinoma,[11] and lymphocytes[12] to detect chromosomal damage, exposure to carcinogens, and genotoxic agents.

Because limited number of MN studies has been reported on effusion fluids, the present study was undertaken to distinguish malignant cells from reactive mesothelial cells.

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The aim of this study was to assess the significance of micronucleated cells in effusion fluids to distinguish malignant cells from reactive mesothelial cells in unequivocal cases. Herein, we found out the utility of micronucleated cell scoring in cytological smears of effusion fluid to distinguish benign and malignant cases.

Materials and Methods

The present study was carried out in our department after obtaining approval from the ethical research committee. A total of 60 cases sent for fluid analysis were considered for the study, 30 were malignant and 30 were benign (reactive).

Relevant clinical details including the age, sex, type of effusion, and site of malignancy were noted from the departmental data base. Additional information regarding radiological findings/tumor markers was collected from hospital records.

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All malignant cases included in the study were supported either by histopathology or by radiology. Benign cases included were approved by three cytopathologists independently as benign.

Smears of diagnosed cases of metastatic adenocarcinoma and reactive mesothelial cells stained with Leishman stain were studied for MN scoring. The smears were analyzed by light microscopy under oil immersion (1000×) separately and independently by two scorers and an average score was calculated. Only the noninflammatory cells were considered for scoring. Separately lying cells without overlap were studied. The number of micronucleated cells per 1000 well-preserved cells under oil immersion lens (1000×) was counted. MN [Figure 1] were detected according to the following criteria:[13]

1. Should be within the cytoplasm of the cell
2. Round to oval in shape and nonrefractile
3. Mean diameter less than 1/3 of the main nucleus
4. The shape, color, and texture of MN are similar to those of the original nuclei
5. It should be in the same optical plane as the original nucleus
6. Distinctly separate from the main nucleus with a similar staining intensity.

Degenerated cells, apoptotic cells, cytoplasmic fragments, and overlapping and inflammatory cells were exempted from counting and scoring. Cells with single/multiple MN were counted as one.

Descriptive statistics used was mean (SD) for continuous variables and percentage for categorical and dichotomous variables. Receiver operator characteristic (ROC) analysis was used to determine the sensitivity and specificity. Mann–Whitney test (nonparametric test) was used to detect MN score difference between two groups. MN score in both the groups was expressed as mean ± SD and median (min–max) where appropriate. Area under the curve (AUC) is a measure of the overall performance of a diagnostic test. It was assessed using ROC curve analysis to determine the cut-off point between the two groups. The ROC plots the true positive rate (sensitivity) against the false-positive rate (1-specificity). The closer the AUC is to 1 the better is the overall diagnostic performance of the test, and a test with an AUC value of 1 is one that is perfectly accurate. The practical lower limit for the AUC of a diagnostic test is 0.5. Data processing and analysis were performed using SPSS Statistics for Windows (version 20.0. Armonk , New York: IBM corporation).

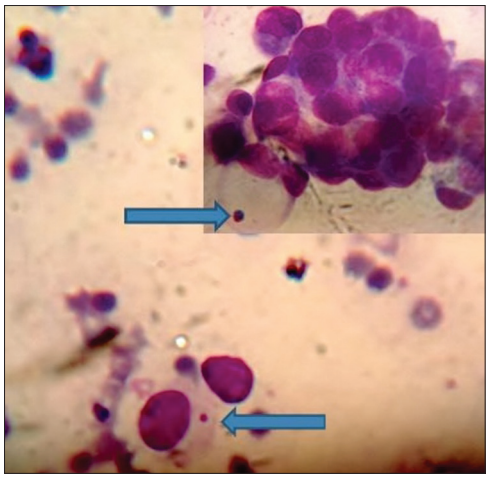


Figure 1: Micronucleus (indicated with an arrow) in a mesothelial cell and (inset) in a signet ring cell, (Leishman stain x1000)

Results

The present retrospective study was done on 60 effusion fluids which included 30 benign (reactive) and 30 malignant cases. Age ranged 33–85 years and females had a higher preponderance than males. The mean ± SD number of MN score in malignant cases was 15 ± 9.78 with a maximum of 50 and a minimum of 6. Median score was 13. Maximum number of MN seen in a cell was 3. The mean ± SD number of MN score in benign cases was 1 ± 1.78 with a maximum of 5 and minimum of 0. Median score was 2 [Table 1]. Mann–Whitney test scoring revealed a statistically significant difference in MN score between the two groups. The results of the ROC analysis showed that MN counts possessed a high degree of sensitivity and specificity. Using the ROC curve method, we found out a cut-off of 5.5 for MN count giving a sensitivity of 100% and a specificity of 100% at this point ($P = 0.000$ and AUC = 1).

Discussion

To the best of our knowledge there are currently only two published studies of MN scoring on effusion fluids, however, these studies have been done on other samples such as exfoliated buccal, vaginal, cervical, and lymphocytes. These studies noticed significant difference in MN scoring between benign and malignant groups. The present study was undertaken to examine the difference in MN score between the two groups (benign and malignant) objectively, and if possible, to evaluate the cut-off point between the two groups by validation of MN score in effusion fluids.

The study conducted by Kaur and Dey on ascitic fluid effusions using MGG stain included 20 and 15 cases of malignant cells and benign mesothelial cells, respectively.[14] In their retrospective study, test group age ranged from 34 to 71 years and females were more than males. They concluded that MN scoring was significantly higher in malignant effusions and their mean MN score in malignant cases was 21 compared to 2.9 in benign cases. Another study done by Tyagi *et al.* on ascitic fluid including 60 benign and 40 malignant fluids showed mean MN score in malignant group to be 13.2 and

Table 1: Result of statistical analysis between two groups

Category	Number of cases	MN score mean ± SD (min-max)	Median
Malignant	30	15.77±9.78 (6-50)	13
Benign	30	1.87±1.78 (0-5)	2

the MN score ranged from 1 to 58. Benign group showed mean a MN score of 0.57 ranging from 0 to 5.[15] Our results correlate with their results [Table 2]. The standard deviation showed wide variation in malignant group and were 9.78 and 1.78 in malignant and benign groups, respectively. A similar finding was noted in many other studies. They suggested that more variation in malignant cases was probably due to type and staging of the primary tumor, carcinogen exposure, and genetic causes. We considered few additional factors to explain this variation in fluid cytology. First factor may be the variable admixture of reactive mesothelial cells with malignant cells in malignant effusions which might lead to extreme differences in MN scoring. It is well-known that malignant effusions also show reactive mesothelial cells. Admixture of more reactive mesothelial cells might have led to low scores. Second, the presence of malignant signet ring cells pose another problem as these cells have peripherally compressed cytoplasm which may obscure the detection of micronucleus, leading to a low score [Figure 1 inset]. These problems were unique to effusions and not noticed in vaginal or oral smears.

Table 2: Comparison of MN scores with previous fluid studies

Study	Sample size	MN Score mean (min-max)	*P**
Kaur *et al*.	T - 20	21 (9-34)	<0.001
	C - 15	2.9 (0-10)	
Tyagi *et al*.	T - 40	13.2 (1-58)	<0.001
	C - 60	0.5 (0-5)	
Present study	T - 20	15.7 (6-50)	<0.001
	C- 20	1.87 (0-5)	

T: Test group, C: Control group

Table 3: Difficulties encountered in detecting MN

Mimickers of MN	Availability of single cells	Other factors
Debris	Cell balls in malignant cases	Signet ring cells
Platelets	Overlapping cells	
Lymphocytes		
Apoptotic cells		
Stain deposits		



Figure 2: Mimickers of micronucleus – nuclear debris (indicated with an arrow), (Leishman stain x1000)

MN scoring can be difficult in situations, as stated in Table 3, where structures mimicking MN such as stain deposits, nuclear debris, overlapping platelets, and lymphocytes were present [Figure 2]. When encountered with such problems, comparing the structures with texture and staining characteristics of original nucleus helps us to isolate the artefacts. Apoptotic cells also mimic MN and can be easily differentiated by noting the absence of original (main) nucleus. The problems of keratohyaline granules mimicking MN were seen only in studies where squamous cells were seen. The present study included only adenocarcinoma and we did not encounter this problem. We did not find any significant difference in MN scoring in relation to age and site of primary cancer. Normal cells during cell division can also rarely give rise to micronucleus, however, this is a less common phenomenon when compared to preneoplastic and neoplastic conditions.

MN scoring is a simple and effective tool to distinguish between benign and malignant effusion in routine cytological analysis. The limitations of our study were smaller sample size and absence of use of specific nuclear stains. Hence, we advocate prospective larger scale studies with adequate sample size and use of specific nuclear stains.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Mohanty S, Dey P. Serous effusions: Diagnosis of malignancy beyond cytomorphology- An analytic review. Postgrad Med J 2003;79:569-74.
2. Bedrossian CW. Diagnostic problems in serous effusions. Diagn Cytopathol 1998;19:131-7.
3. Saha I, Dey P, Vhora H, Nijhawan R. Role of DNA flow cytometry and image cytometry on effusion fluid. Diagn Cytopathol 2000;22:81-5.
4. Saleh HA, El-Fakharany M, Makki H, Kadhim A, Masood S. Differentiating reactive mesothelial cells from metastatic adenocarcinoma in serous effusions: The utility of immunocytochemical panel in the differential diagnosis. Diagn Cytopathol 2009;37:324-32.
5. Pillai V, Cibas ES, Dorfman DM. A simplified flow cytometric immunophenotyping procedure for the diagnosis of effusions caused by epithelial malignancies. Am J Clin Pathol 2013;139:672-81.
6. Leach NT, Jackson-Cook C. Micronuclei with multiple copies of the X chromosome: Do chromosomes replicate in micronuclei? Mutat Res 2004;554:89-94.
7. Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. The Human MicroNucleus Project-An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat Res 1999;428:271-83.
8. Ambroise MM, Balasundaram K, Phansalkar M. Predictive value of micronucleus count in cervical intraepithelial neoplasia and carcinoma. Turk Patoloji Derg 2013;29:171-8.
9. Samantha S, Dey P, Nijhawan R. Micronucleus in cervical intraepithelial lesions and carcinoma. Acta Cytol 2011;55:42-7.
10. Singaraju M, Singaraju S, Parwani R, Wanjari S. Cytogenetic biomonitoring in petrol station attendants: A micronucleus study. J Cytol 2012;29:1-5.

11. Karaman A, Binici DN, Kabalar ME, Calikusu Z. Micronucleus analysisin patients with colorectal adenocarcinoma and colorectal polyps. World J Gastroenterol 2008;14:6835-9.
12. Iarmarcovai G, Ceppi M, Botta A, Orsiere T, Bonassi S. Micronuclei frequency inperipheral blood lymphocytes of cancer patients: A meta - analysis. Mutat Res 2008;659:274-83.
13. Belien JA, Copper MP, Braakhuis BJ, Snow GB, Baak JP. Standardization of counting micronuclei: Definition of a protocol to measure genotoxic damage in human exfoliated cells. Carcinogenesis 1995;16:2395-400.
14. Kaur J, Dey P. Micronucleus to distinguish adenocarcinoma from reactive mesothelial cell in effusion fluid. Diagn Cytopathol 2010;38:811-3.
15. Tyagi R, Dey P, Uppal R, Rajwanshi A. Analysis of morphological markers of chromosomal instability in ascitic fluid. Diagn Cytopathol 2015;43:855-8.

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Clinicopathological Conference

An unusual cause of breathlessness and profuse micronodules

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ABSTRACT

We describe a 21-year-old male with a history of smoking and subacute onset of breathlessness with normal cardiorespiratory examination. The presence of "track marks" and digital infarcts prompted evaluation for infective endocarditis and confrontational history taking revealed anorexia, weight loss over 3 months along with intravenous drug abuse of reconstituted tablets of tapentadol. Echocardiography was normal and blood cultures were sterile; computed tomography showed bilateral, diffuse, small centrilobular nodules with "tree-in-bud" appearance. In this clinicopathologic conference, we discuss the clinical and radiological differential diagnosis of centrilobular nodules, lung biopsy findings, and management options for patients with such a presentation.

KEY WORDS: Excipient lung disease, intravascular talcosis, pulmonary foreign body angiogranulomatosis, talc granulomatosis

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PRESENTATION OF THE CASE

A 21-year-old college student presented with breathlessness and left-sided chest pain for a week. Breathlessness was insidious in onset and persisted on walking at own speed at level. Chest pain was left-sided, intermittent, pricking and non-exertional. There was no history of fever, rhinitis, headache, hemoptysis, cough, or joint pains. He did not raise pets but admitted to smoking 7–8 cigarettes/day and consuming whisky (192 g of alcohol weekly). He denied any recent worsening of educational performance. He did not report any significant prior medical illness or pertinent illness in family or occupational dust exposure. He was initially seen in cardiology and evaluation showed normal cardiac and respiratory examination without any pallor, clubbing, or organomegaly. Body mass index was 18.6 kg/m^2. The examination also showed extensive track marks over both arms with evidence of multiple peripheral digital infarcts [Figure 1], raising suspicion for infective endocarditis (IE). On confronting, he disclosed daily intravenous self-injections of three–four powdered and reconstituted 50 mg tablets of Tapentadol (Tydol, Sun Pharma) in sterile distilled water two–three times a day over the last 2 years. He also admitted to using inhaled cannabis twice weekly over the last 2 years. There was associated anorexia and unquantified weight loss over the last 3 months. Three sets of blood cultures were sterile. Electrocardiography showed T-wave inversions in leads V3–V6 and II, III, and aVF. Transthoracic echocardiography (TTE) showed normal valves without any evidence of vegetations or pulmonary hypertension and normal left ventricular function. Serum creatinine

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Figure 1: Composite image of the clinical photographs (left) of the right forearm showing "track marks," indicating healed superficial septic thrombophlebitis and (right) both hands showing multiple bilateral distal digital pulp infarcts. Similar findings were also seen in both plantar surfaces (not shown)

was 0.6 mg/dL; urine microscopy was normal without any albuminuria. Hemoglobin was 14.2 g/dL with normal leukocyte and platelet counts. He remained afebrile. Transesophageal echocardiography (TEE) was planned; in the meanwhile, review of his chest radiographs showed diffusely scattered fine nodules bilaterally [Figure 2, left]. Spirometry showed mild restriction with reduced diffusion capacity for carbon monoxide (forced expiratory volumes at 1 s 2.25 L [65%]%, forced vital capacity 2.87 L [71%], DLco 62%). There was desaturation to 92% while walking 6 minutes, with preserved walk distance (545 meters, 89% predicted). Human immunodeficiency virus (HIV), anti-nuclear antibodies, hepatitis B, and hepatitis C antibodies were negative by enzyme-linked immunosorbent assay. Immunoglobulin E levels were 9 IU/mL (normal <100 IU/mL). High-resolution computed tomography (HRCT) of the thorax showed bilateral profuse evenly distributed micronodules with "tree-in-bud" [Figure 2, right]. There was no sputum production on induction with 3% saline. A procedure was planned for diagnosis.

DIFFERENTIAL DIAGNOSIS

Important features of this case

This HIV-negative young adult with significant smoking history, cannabis use, and intravenous drug abuse with reconstituted tablets over 2 years has breathlessness, anorexia, and weight loss with radiological findings of micronodules. There is evidence of distal embolic infarcts, but initial evaluation for endocarditis was negative. The reduced DLco, restriction on spirometry and unexpectedly low walk distance in a young individual with desaturation on walking implies extensive parenchymal and/or pulmonary vascular disease. The differential diagnosis of profuse bilateral micronodules is extensive [Table 1] but can be narrowed by careful history and analysis of HRCT findings in conjunction with the expected pulmonary complications of intravenous drug abuse [Table 2].[1] The first step is to characterize the

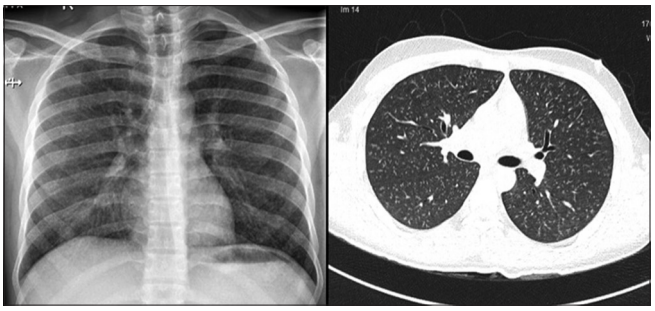


Figure 2: Composite image of the chest radiograph (left) showing small nodular opacities and high-resolution computed tomography of the thorax (right) showed bilateral profuse evenly distributed micronodules with tree-in-budding; pulmonary artery was normal in size, and there were no associated lymphadenopathy. Expiratory computed tomography did not show any air trapping (not shown)

Table 1: Causes of diffuse micronodular disease on computed tomography

With tree-in-budding
Infectious causes
Tuberculosis, nontubercular mycobacteria (typically MAC)
Bronchopneumonia: *Haemophilus influenzae*, *Mycoplasma pneumoniae*, Chlamydia
Viral bronchiolitis
Airway-invasive aspergillosis
Aspiration
Bronchiectasis: Cystic fibrosis, ciliary disorders, immunodeficiency
Immunologic
Allergic bronchopulmonary aspergillosis
Diffuse panbronchiolitis
Hematogenous: intravascular metastases (rare)
Without tree-in-budding
Infective causes
Tuberculosis
Fungi: *Histoplasma capsulatum*, blastomycosis, candidemia
Varicella zoster
Immunologic causes
Tropical pulmonary eosinophilia
Hypersensitivity pneumonitis (subacute)
Follicular bronchiolitis
Respiratory bronchiolitis
Sarcoidosis
Diffuse pulmonary amyloidosis
Allergic bronchopulmonary aspergillosis (rarely)
Occupational lung disease
Berylliosis, silicosis, coal worker's pneumoconiosis
Idiopathic
Pulmonary alveolar microlithiasis
Idiopathic pulmonary hemosiderosis
Malignancy
Metastasizing leiomyoma
Pulmonary Langerhans cell histiocytosis (early)
Metastatic adenocarcinoma, renal cell carcinoma, papillary thyroid carcinoma
Others
Metastatic calcification (renal failure)
Pulmonary arteriolar causes
Pulmonary edema and hemorrhage, excipient lung disease, plexogenic arteriopathy, veno-occlusive disease, pulmonary capillary hemangiomatosis

MAC: *Mycobacterium avium* intracellulare, *Wuchereria bancrofti*

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Table 2: Pulmonary problems in intravenous drug abusers

Mechanism of Injury	Observed side-effects
Pharmacodynamic properties of injected drug	Noncardiogenic pulmonary edema (cocaine, opioids) Cocaine intoxication: pulmonary edema, pulmonary hemorrhage, cardiogenic pulmonary edema (coronary spasm) Methylphenidate intoxication: hemoptysis
Complications of unsafe intravenous injections	Lung abscess (with loss of consciousness, seizures, and aspiration) Infective endocarditis Septic pulmonary embolism Infections acquired from needle sharing (HIV, hepatitis B, hepatitis C)
Effects of contaminants	Excipient lung disease* Emphysema (lower lobe predominant "Ritalin" lung with methylphenidate) and Bullous lung disease Pulmonary vascular disease Group 1: Pulmonary hypertension (cocaine and methamphetamine) Group 3: Pulmonary hypertension (emphysema, foreign body granulomatosis, and interstitial lung disease) Interstitial lung disease Pneumothorax (subclavian injections, cocaine, and bullous lung disease), septic emboli
Related to associated abused substances, poverty, malnutrition	Pneumonia Tuberculosis More frequent exacerbations of asthma (fatal asthma)

*Talc, starch, crospovidone, and microcrystalline cellulose are used as fillers. HIV: Human Immunodeficiency Virus

distribution of the micronodules spatially within the lung and also the secondary pulmonary lobule. These nodules were uniform in size and were distributed evenly in a cephalocaudal direction, without any preferential lobar involvement. None of the nodules abut the pleural surface or the fissures [Figure 3]; there is clear evidence of extensive bilateral branching nodules in centrilobular distribution. There is no evidence of bronchiectasis; while no contrast was administered, in conjunction with echocardiography, there is no dilated pulmonary artery or lymphadenopathy. These findings limit the radiological differentials to causes of micronodules in a centrilobular distribution and extensive uniform "tree-in-bud" appearance. "Tree-in-bud" indicates the presence of dilated centrilobular bronchioles with lumen impacted by mucus, fluid, or pus and associated with peribronchiolar inflammation. "Tree-in-bud" indicates small airway disease and is associated with airway infection in the large majority but can also be rarely caused by arteriolar disease.[2] Its presence makes three common causes of centrilobular nodules unlikely: hypersensitivity pneumonitis, follicular bronchiolitis, and respiratory bronchiolitis.

Endobronchial tuberculosis

Mycobacterium tuberculosis is endemic in India, with more than 2.2 million new cases each year. Delayed recognition

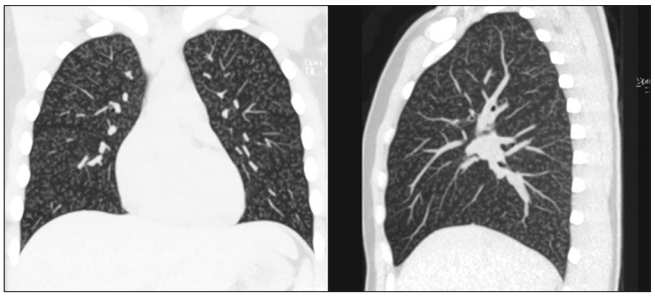


Figure 3: Coronal and sagittal reconstruction of the maximum intensity projection formatting of the high-resolution computed tomography images of the thorax showing diffuse, extensive, bilateral centrilobular nodules with branching and without any lobar predominance

and extensive disease are related to associated substance abuse, poverty, and malnutrition, all of which were present in this patient. Extensive endobronchial spread of tuberculosis can cause extensive tree-in-bud opacities although asymmetry is usually seen.[3] Tuberculosis is also the most common cause of micronodules with a random pattern (miliary). Even though the "pretest probability" of tuberculosis is high in this patient, the marked symmetry and the absence of any cavitation or consolidation is odd; digital infarcts are unusual but could be caused by an associated immune complex phenomenon that can occur with tuberculosis. *Mycobacterium avium*-intracellulare infection can also cause centrilobular nodules with "tree-in-bud," bronchiectasis, and consolidation. While it can be seen in immunocompetent patients, the classic phenotype is a middle-aged woman with slender build and without any constitutional symptoms.

Bronchopneumonia and viral bronchiolitis

Infectious bronchiolitis due to viruses, *Mycoplasma*, and chlamydia is a common cause of profuse and symmetric centrilobular nodules with "tree-in-bud;"[3] however, the prolonged illness and associated embolic phenomenon are unusual.

Allergic bronchopulmonary aspergillosis

Centrilobular nodules with "tree-in-bud" or miliary nodules can be caused by allergic bronchopulmonary aspergillosis but the absence of asthma, central bronchiectasis, and normal IgE levels rule out this diagnosis.[2,4]

Diffuse panbronchiolitis

Diffuse panbronchiolitis (DPB) is a rare disease of unknown cause presenting in males between the second and fifth decade with productive cough, diffuse bilateral centrilobular nodules with "tree-in-bud" and sinusitis. While reported in Indians,[5] it is extremely rare in our population but can explain all the clinical and radiological features.[6] Cold agglutinins are frequent in DPB and pulp infarcts may be due to an associated connective tissue disease. The absence of productive sputum, obstruction on spirometry, sinusitis, and air trapping on expiratory CT are unusual in DPB and necessitate further evaluation with surgical lung biopsy to establish the diagnosis.[6]

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Excipient lung disease

Centrilobular nodules are usually caused by bronchiolar disease but can rarely be caused by arteriolar disease. Arteriolar causes include pulmonary edema and hemorrhage, excipient lung disease (ELD), granulomatosis with polyangiitis (GPA), plexogenic arteriopathy (PH), veno-occlusive disease (VOD), pulmonary capillary hemangiomatosis (PCH), and metastatic calcification.[2] There was no evidence of heart failure, renal failure or anemia and hemoptysis to suggest alveolar hemorrhage. The absence of pulmonary hypertension makes centrilobular nodules due to PH, VOD, and PCH very unlikely. Nodules due to GPA are larger, fewer, and associated with cavitation. Excipients are insoluble inert particulate filler materials that bind and protect the active drug during production as well as shape and lubricate tablets for easy swallowing. Excipients include talc (hydrated magnesium silicate), microcrystalline cellulose, crospovidone, and starch. Typically, excipients constitute 5% of a tablet's mass. On intravenous injection, excipient particles lodge in pulmonary arterioles and trigger an angiogranulomatous reaction; progressive disease is associated with dyspnea and pulmonary hypertension.[7] However, the presence of finger pulp infarcts, "tree-in-bud" appearance and absence of pulmonary hypertension are unusual in ELD.

CLINICAL DIAGNOSIS

In summary, the differential diagnosis of extensive centrilobular nodules with "tree-in-bud" without bronchiectasis or pulmonary hypertension in this young adult with smoking, cannabis use, and intravenous drug abuse of reconstituted tablets can be narrowed down to extensive endobronchial tuberculosis, diffuse panbronchiolitis, and ELD.

Dr. RM. PL. Ramanthan: With these clinicoradiological possibilities in mind, this patient underwent fundus examination and bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial lung biopsy, with a plan for surgical biopsy if evaluation was inconclusive. Can we now have the findings?

Dr. K. Divya: Dilated fundus examination confirmed refractile particles scattered along both the retinal arteries and retinal veins [Figure 4], confirming systemic embolization of small (<5 μm) talc particles, consistent with intravenous talcosis. Inhalational talc exposure does not cause such marked retinal deposition as the inhaled particles are larger and are restricted by bronchioles and pulmonary circulation.[8,9]

Dr. Nidhya Ganesan: BAL was negative for acid-fast bacilli and for *M. tuberculosis* by Xpert MTB/RIF; occasional needle-shaped crystals were seen on cytology. The transbronchial lung biopsy specimen confirmed perivascular granulomatous reaction to talc particles [Figure 5, left] that appear as birefringent needle-shaped crystals within the granulomas under polarized light [Figure 5, right]. Review of the composition of Tydol confirmed the presence of talc as excipient. Microcrystalline cellulose appears rod like while starch has a maltese cross pattern and crospovidone is nonbirefringent on polarization.[7]

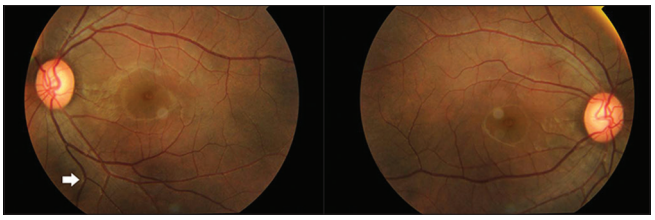


Figure 4: Fundus photograph of both eyes showing refractile particles scattered along both the retinal arteries and retinal veins, confirming the diagnosis of intravenous talcosis

Dr. Srinivas Rajagopala: A diagnosis of ELD (talc granulomatosis) was made. Psychiatry services were involved, and the patient underwent counseling, arrangement for family supervision, and daily-observed methadone replacement. He has successfully discontinued intravenous drug abuse and remains stable at 3 months of follow-up. Serial spirometry, DLco, and radiographs have been advised at 6 monthly intervals. Three unusual findings need an explantation; 1) The presence of "tree-in-bud" in a process that is hematogenous in origin 2)The absence of pulmonary hypertension in ELD and 3)The cause of the bilateral digital infarcts observed.

Dr. B. Devanand: Diseases involving pulmonary arterioles can cause centrilobular nodules as arterioles run in the peribronchovascular interstitium adjacent to bronchioles. Talcosis associated with intravenous abuse is most commonly associated with diffuse fine centrilobular nodules, ground-glass abnormalities or a combination of nodules and lower-lobe emphysema (Ritalin lung with methylphenidate).[9,10] The "tree-in-bud" pattern is unusual for arteriolar disease; it can be caused by intra-arteriolar accumulation of talc with associated adjacent perivascular granulomatous reaction giving the appearance of branching centrilobular nodules and has been described in a few cases of ELD earlier.[11]

Dr. Srinivas Rajagopala: What is the course of ELD? Can the disease progress despite cessation of abuse?

Dr. Kancherla Roopa: Lung fibrosis and pulmonary hypertension are related to the extent of vascular obstruction and granulomatous inflammation and are usually irreversible with talc, cellulose, and crospovidone as these cannot be mobilized; starch can be removed by metabolism and pulmonary hypertension can be reversible to a large degree. Disease progression can occur despite cessation due to ongoing granulomatous foreign-body reaction to the deposited, unmobilizable talc. The initial series of patients described all had progressive symptoms leading to death over 9 months to 5 years.[10,12] The index

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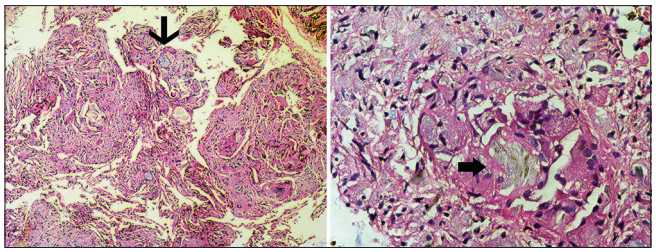


Figure 5: Photomicrographs of the transbronchial lung tissue biopsy specimen (left) demonstrating perivascular foreign body granulomatous reaction to the talc particles (thin arrow, H and E, ×100). Under polarized light, birefringent 20–95 μm needle-shaped crystals were seen in the granuloma, consistent with talc (block arrow, H and E, ×400)

patient has a large load of perivascular disease and is at risk for pulmonary hypertension and disease progression despite cessation of further abuse.

Dr. Srinivas Rajagopala: How common is systemic embolization of talc given that the lung acts as an effective filter?

Dr. K. Divya: The talc that is trapped in the pulmonary arterioles is typically around 10 μm, but smaller particles can escape to the systemic circulation and be detected in the retina, spleen, and virtually every organ on autopsy.[12] Finding excipient crystals in fundoscopy is a rapid noninvasive method to confirm intravenous talcosis.

Dr. Srinivas Rajagopala: Given the presence of digital infarcts, is TEE needed to exclude IE in this patient?

Dr. G. Rajendiran: The first consideration in this patient with possible unsafe needle practices, no murmurs, weight loss, breathlessness, and digital embolic manifestations was subacute native valve IE. TTE has low sensitivity compared to TEE (46% vs. 93%) although both are highly specific (96%).[13] Embolic manifestations are seen in about half of patients with IE.[14] He was not administered any antibiotics, three sets of blood cultures were sterile, and TTE showed all valves clearly without any vegetations. TEE is restricted in our practice to high probability for IE or in whom TTE is rendered less sensitive due to obesity, lung hyperinflation, or prosthetic valves. The index patient met only 2 minor Modified Duke's criteria and was considered low risk for IE after the initial evaluation and TEE deferred.[15] Furthermore, a clear alternative explanation for the observed distal infarcts in the form of talc deposition in the distal microcirculation with thrombosis is available and corroborated by fundoscopy. While this physical finding has not been described in ELD previously, it is consistent with the distal digital embolization and subsequent microthrombosis caused by talc.

Dr. Srinivas Rajagopala: What are the options for those with progressive breathlessness despite cessation of intravenous drug abuse?

Dr. RM. PL. Ramanthan: There are previous small series of lung allografts after a careful selection process in ELD with excellent outcomes.[16] Cadaveric bilateral sequential lung transplantation is a reality in our country today, and he can be listed for the same at our center if he meets criteria and remains free of substance abuse for at least 6 months with adequate psychiatry counseling.

SUMMARY

Clinical examination findings continue to have relevance in this era of investigation-driven medicine. Excipient lung disease can result from intravenous injection of reconstituted tablets and can present with ground glass opacities, small nodules, and emphysema. Pulmonary arteriolar diseases must be considered in the differential diagnosis of centrilobular nodules on CT.

Acknowledgments

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that names, identifying marks, and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Marchiori E, Souza AS Jr., Franquet T, Müller NL. Diffuse high-attenuation pulmonary abnormalities: A pattern-oriented diagnostic approach on high-resolution CT. AJR Am J Roentgenol 2005;184:273-82.
2. Webb R, Muller NL, Naidich DP. HRCT Findings: Multiple nodules and nodular opacities. In: High-Resolution CT of the Lung. 5th ed. Philadelphia: LWW Wolters Kluwer; 2014. p. 125-9.
3. Walker CM, Abbott GF, Greene RE, Shepard JA, Vummidi D, Digumarthy SR, *et al*. Imaging pulmonary infection: Classic signs and patterns. AJR Am J Roentgenol 2014;202:479-92.
4. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, *et al*. Allergic bronchopulmonary aspergillosis: Review of literature and proposal of new diagnostic and classification criteria. Clin Exp Allergy 2013;43:850-73.
5. Nath A, Aggarwal AN, Gupta R. Diffuse panbronchiolitis: Report of a rare disease from India. Indian J Chest Dis Allied Sci 2010;52:43-5.
6. Poletti V, Casoni G, Chilosi M, Zompatori M. Diffuse panbronchiolitis. Eur Respir J 2006;28:862-71.
7. Nguyen VT, Chan ES, Chou SH, Godwin JD, Fligner CL, Schmidt RA, *et al*. Pulmonary effects of i.v. Injection of crushed oral tablets: "Excipient lung disease". AJR Am J Roentgenol 2014;203:W506-15.
8. Akira M, Kozuka T, Yamamoto S, Sakatani M, Morinaga K. Inhalational talc pneumoconiosis: Radiographic and CT findings in 14 patients. AJR

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Am J Roentgenol 2007;188:326-33.
9. Ward S, Heyneman LE, Reittner P, Kazerooni EA, Godwin JD, Müller NL, *et al*. Talcosis associated with IV abuse of oral medications: CT findings. AJR Am J Roentgenol 2000;174:789-93.
10. Feigin DS. Talc: Understanding its manifestations in the chest. AJR Am J Roentgenol 1986;146:295-301.
11. Bendeck SE, Leung AN, Berry GJ, Daniel D, Ruoss SJ. Cellulose granulomatosis presenting as centrilobular nodules: CT and histologic findings. AJR Am J Roentgenol 2001;177:1151-3.
12. Sieniewicz DJ, Nidecker AC. Conglomerate pulmonary disease: A form of talcosis in intravenous methadone abusers. AJR Am J Roentgenol 1980;135:697-702.
13. Bashore TM, Cabell C, Fowler V Jr. Update on infective endocarditis. Curr Probl Cardiol 2006;31:274-352.
14. McDonald JR. Acute infective endocarditis. Infect Dis Clin North Am 2009;23:643-64.
15. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr., Ryan T, *et al*. Proposed modifications to the duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;30:633-8.
16. Weinkauf JG, Puttagunta L, Nador R, Jackson K, LaBranche K, Kapasi A, *et al*. Long-term outcome of lung transplantation in previous intravenous drug users with talc lung granulomatosis. Transplant Proc 2013;45:2375-7.

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RESEARCH ARTICLE

Pulmonary alveolar microlithiasis: an interesting case report with systematic review of Indian literature

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Abstract **Pulmonary alveolar microlithiasis is a rare disease characterized by intra-alveolar presence of microliths. This study reports an interesting case of pulmonary alveolar microlithiasis and provides a systematic review of cases reported from India. A 23-year-old female presented with a history of cough, wheeze, chest pain, and episodic wheeze for five months. Pulmonary function tests demonstrated an obstructive pattern, and chest X-ray showed fine micronodular opacities predominantly involving the middle and lower zones of both lungs. Transbronchial lung biopsy revealed the diagnosis. She responded well to inhaled steroid therapy. A systematic review of literature was performed and identified 73 cases of pulmonary alveolar microlithiasis reported from India. The mean (SD) age of the patients was 28.8 (14.9) years, with an almost equal male:female ratio. Many patients were asymptomatic at presentation. Breathlessness and cough were the most common symptoms, and the disease progressed into respiratory failure associated with cor pulmonale. About one-third of the cases were initially misdiagnosed and treated as pulmonary tuberculosis. Extra-pulmonary manifestations and co-morbidities were also evident in our series. This systematic review helps to determine epidemiological and clinical characteristics of pulmonary alveolar microlithiasis. Further research is needed to elucidate the etiopathogenesis, diagnosis, and therapeutic options, which are beneficial in developing and identifying cost-effective treatment for pulmonary alveolar microlithiasis.**

Keywords pulmonary alveolar microlithiasis; alveolar microlithiasis; microliths; rare diseases

Introduction

Pulmonary alveolar microlithiasis (PAM) is a rare disease characterized by intra-alveolar presence of microliths. It is an autosomal recessive disorder and runs in families. PAM occurs because of mutation in the sodium co-transporter (*SLC34A2*) gene [1]. Affected patients may range from early childhood to elderly and are usually asymptomatic during the early phase of the disease. This disease has a long and progressive course with deterioration of lung function [2].

Malphighi from Italy (1686) was the first to describe the macroscopic features of the disease, and Puhr from Hungary is credited for naming the disease in 1933 [2]. The first PAM case in India was reported by Viswanathan in 1961 [3]. This disease is extremely rare; hence, a systematic review of published cases is helpful in understanding its epidemiology, etiopathogenesis, clinical features, and diagnosis. Two interesting national reviews have already been published from Turkey and Italy [4,5]. In this study, we present an interesting case of PAM and review the literature of 52 case reports (73 cases) from India [3,6–56]. Majority of the case reports have been published in our national journals.

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Our case report

A 23-year-old female, born of second-degree consanguineous marriage, presented with cough with expectoration, chest pain, and episodic wheeze for five months. The chest pain was associated with coughing. Sputum production

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was minimal and not foul smelling or blood tinged. No history of fever, hemoptysis, or loss of weight was present. She did not have any past history of pulmonary tuberculosis, and her personal history was unremarkable. She was not a smoker and had no history of exposure to toxic gases and fumes. Pedal edema, cyanosis, and clubbing were not present. On lung auscultation, vesicular breath sounds were heard bilaterally. Oxygen saturation was 96% on room air. Pulmonary function test revealed an obstructive pattern, with a forced vital capacity of 2.83 L (109% of predicted value), a forced expiratory volume in 1 s of 1.86 L (83% of predicted value), a FEV1/FVC ratio of 65.7 (78% of predicted value), and a PEF of 2.87 L/s (46% of predicted value).

Routine blood investigations were within normal limits, except for a mild eosinophilia. Chest X-ray showed diffuse fine micronodular opacities, predominantly involving the middle and lower zones of both lungs with relative sparing of the apices (Fig. 1). Miliary tuberculosis was initially suspected, but sputum smear and culture were negative for acid-fast bacilli. High resolution computed tomography (HRCT) of chest showed diffuse bilateral micronodules of 1–2 mm size, involving all segments of the lung (Fig. 2).



Fig. 1 Chest X-ray showing fine, diffuse, and symmetric micronodular calcifications.

On bronchoscopy, the respiratory mucosa showed mild congestion without any significant abnormality. BAL was performed, and biopsy specimens were obtained by transbronchial lung biopsy (TBLB) from the right middle lobe. BAL did not reveal respiratory pathogens or any specific pathology. TBLB exhibited dilated alveolar spaces with laminated calcific concretions (Figs. 3 and 4). The calcospherites positively stained with von Kossa stain. Our final diagnosis was pulmonary alveolar microlithiasis with associated bronchial asthma.



Fig. 2 High resolution CT scan showing diffuse bilateral miliary shadows.

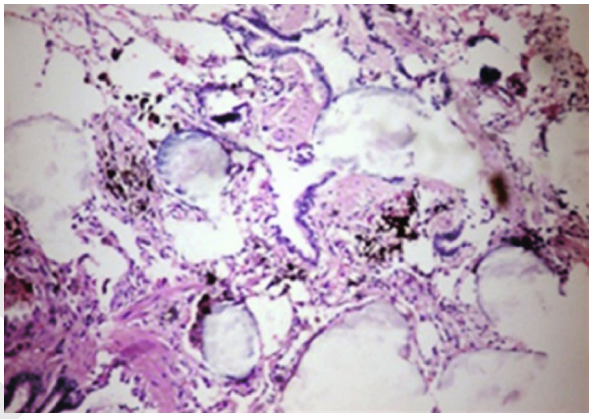


Fig. 3 Calcospherites within the dilated alveolar spaces (H&E stain, 100 ×).

We also assessed the sibling (elder brother) of our patient. He was in good health, and his chest X-ray was unremarkable. The patient was started on inhaled fluticasone propionate/formoterol combination therapy. She improved symptomatically and came regularly for follow-up visits for nine months. A repeat radiograph was performed eight months after her initial presentation, and significant change was not observed (Fig.5). Unfortunately, she was lost to follow-up thereafter.

Methods

Search strategy

A PubMed search was performed with the terms

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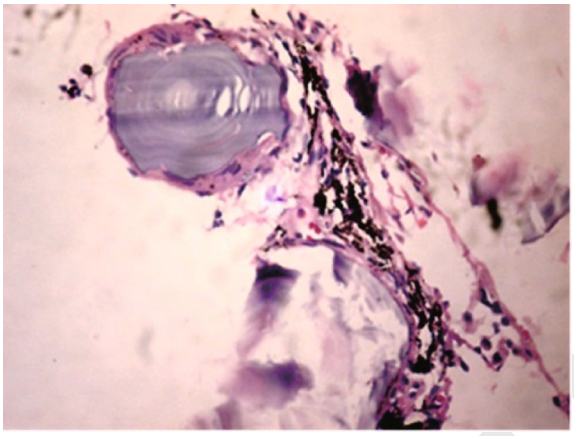


Fig. 4 Concentric calcification (H&E stain, 400 ×).

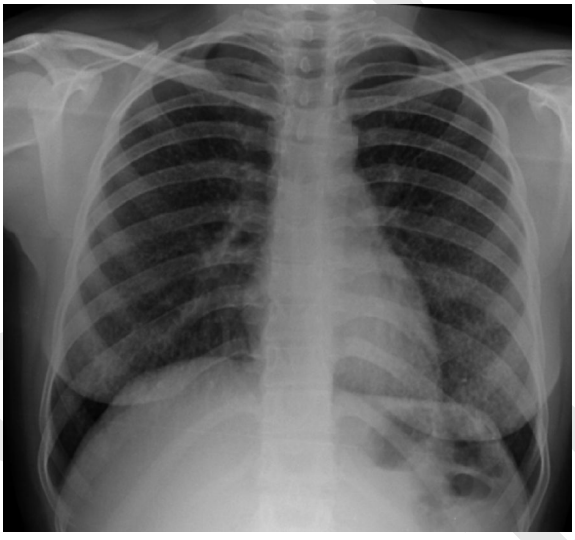


Fig. 5 Repeat chest X-ray also showing diffuse micronodular calcifications.

"pulmonary alveolar microlithiasis" OR "alveolar microlithiasis" OR "microlithiasis alveolaris pulmonale" AND "India." The search was limited to articles published in the English language. Additional publications were also identified from the reference list of the published articles. The websites of national journals of India, such as *Lung India* and *Indian Journal of Pathology and Microbiology* were also searched to identify further citations. Cases published as a case report and subsequently in a case series or as an "interesting image" were not counted separately.

Inclusion criteria

We included only case reports and short case series from India published between 1961 and 2013. All cases diagnosed as PAM from India based on radiological investigations or biopsy were included in our study.

Exclusion criteria

Review studies were excluded. Cases without complete clinical and diagnostic details were also excluded.

Two authors independently screened the title and abstracts of the reports identified in the search. Full text articles were retrieved for relevant citations. The final list of articles was selected by consensus.

Data collection and statistical analysis

Clinical data and basic demographic details of individual cases were extracted and compiled using Microsoft Excel 2010. Categorical data and continuous variables were expressed as percentages and as mean and standard deviation, respectively.

Results

Our analysis included 73 PAM cases (52 case reports/short case series) reported from India between 1961 and 2013 [3,6–56]. The details of familial screening were available in 38 patients. A familial preponderance was evident in 21 cases. All the familial cases were siblings, except for a familial occurrence in a mother and her two daughters. The proband (mother) was married to her maternal uncle who was not affected by the disease.

A total of 37 females and 36 males with a mean ± SD age of 28.8 ± 14.9 years (median age 27.0 years; range 4–60 years) were found. Maximum cases were reported in the age group of 21–30 years (Table 1). The cases were reported from all geographic locations of India. Although occupational history was not mentioned in all the cases, the majority were housewives, students, and farmers. Three cases had history of exposure to respiratory irritants, but the occupational exposure was probably not associated with the development of PAM. A 43-year-old male had worked for four years in a cement retail shop, 15 years before developing PAM [3]. A 25-year-old housewife was employed part time in making biris (local hand-rolled cigarettes) [45]. Both the cases were familial, and their siblings were not exposed to these occupational hazards. Similarly, radiological evidence of the disease was not observed in the coworkers of a 26-year-old machine operator employed in a Calico printing factory [13].

Clinical features

Table 1 shows the frequency of common clinical signs and symptoms in various age groups. Twenty-two cases (30%) were misdiagnosed as pulmonary tuberculosis based on

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Table 1 Clinical manifestations of pulmonary alveolar microlithiasis (PAM) in different age groups (73 cases)

Age group in years	Number of cases in each age group	Non-symptomatic	Breathlessness	Cough	Fever	Sputum expect-oration	Clubbing	Cyanosis	Cor pulmonale
0–10	8	3	0	1*	3	1	1	0	0
		37.5%	0.0%	12.5%	37.5%	12.5%	12.5%	0.0%	0.0%
11–20	16	6	6	6	1	1	4	0	0
		37.5%	37.5%	37.5%	6.3%	6.3%	25.0%	0.0%	0.0%
21–30	17	5	6	6	3	3	3	3	3
		29.4%	35.3%	35.3%	17.6%	17.6%	17.6%	17.6%	17.6%
31–40	15	3	10	9	1	4	6	2	4
		20%	66.7%	60.0%	6.7%	26.7%	40.0%	13.3%	26.7%
41–50	10	0	9	7	1	6	4	2	2
		0.0%	90%	70.0%	10.0%	60.0%	40.0%	20.0%	20.0%
⩾51	7	0	6	5	0	2	2	2	3
		0.0%	85.7%	71.4%	0.0%	28.6%	28.6%	28.6%	42.9%
Total	73	17	37	34	9	17	20	9	12
		23.3%	50.7%	46.6%	12.3%	23.3%	27.4%	12.3%	16.4%

* For example, 1/8 PAM cases in 0–10 age group (12.5% of cases in 0–10 age group) presented with cough.

radiological features and started on ATT, but only one of them was sputum positive. This particular case was treated for pulmonary tuberculosis twice; the first treatment was based on sputum positivity for acid-fast bacilli, and the second treatment was based on radiological abnormalities. The patients did not improve symptomatically on ATT, and further investigations, such as lung biopsy, revealed PAM. Interestingly, only one case had developed superadded sputum positive pulmonary tuberculosis almost after 12 years of diagnosis.

Six patients aged between 24 and 60 years (mean age of 39.6 ± 14.2 years) had died; five of them died within a week of admission, and one patient died within three months of admission. These patients had symptom duration that ranged from 3 months to 10 years. The 24-year-old patient developed pneumothorax following a bout of coughing and died of respiratory failure. Others died because of respiratory insufficiency associated with cor pulmonale. The 60-year-old female had worsening symptoms for five years and was treated at several centers with antibiotics and anti-tubercular drugs. The patient was finally diagnosed as PAM in a tertiary care center; unfortunately, she died within a week of admission. Two other patients, aged 50 years and 30 years, also had symptoms for 7 and 10 years, respectively; nevertheless, the diagnosis was established only before their death.

Long-term follow-up details were not available for majority of the patients in our series.

Imaging

Chest radiograph was performed in all patients except for one case. The characteristic finding in most of the cases were sand-like calcific micronodules (sandstorm pattern) diffusely involving both middle and lower lobes bilaterally with relative sparing of the apices. The calcific densities were confluent and denser at the bases. Other common roentgen findings included a dark radiolucent strip between the ribs, the markedly radiopaque lung parenchyma (black pleural line or negative pleural sign), and obscuration of the cardiac outline, diaphragm, and hilar vessels (the "drowned heart syndrome"). Twenty-five patients (34%) were evaluated using CT scan/high resolution CT scan. However, about 57% of the cases diagnosed after 1990 were evaluated using CT scan. Technetium-99m bone scan performed in nine patients showed diffuse increase in radiotracer uptake in both lungs.

PAM was associated with nephrolithiasis (one case), appendicitis (one case), and cholelithiasis (two cases). PAM was also associated with infertility (three males and two females).

Pulmonary function tests were performed in 40 patients. Restrictive pattern was evident in 34 patients (85.0%), and obstructive pattern and normal study in one patient (2.5%) and five patients (12.5%) were observed, respectively. Bronchoalveolar lavage was performed in nine patients and revealed the characteristic calcospherites in three cases. Lung biopsy proved to be confirmatory in 37 patients. CT-guided FNAC revealed the characteristic laminated microliths in a patient [26]. Necropsy was carried out in only one case [6].

Majority of the cases were treated conservatively, and none of the cases underwent lung transplantation. The cases in our series were predominantly reported from reputed tertiary care centers and probably represent the tip of the iceberg. A number of PAM cases are possibly undetected and even worse misdiagnosed as miliary tuberculosis in India.

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Table 2 Epidemiological characteristics, clinical features, and diagnostic tests in India and other countries

Review	Turkish series [4]	Italian series [5]	Our series
Number of cases	52	48	73
Age (mean, year)	27.3	30.5	28.8
Male	65.4%	39.6%	49.3%
Female	34.6%	60.4%	50.7%
Clinical features			
Asymptomatic	19.2%	35.4%	23.3%
Dyspnea	67.3%	67.7%	50.7%
Fever	NA	9.6%	12.3%
Cough	38.5%	32.2%	46.6%
Cyanosis	13.5%	29%	12.3%
Clubbing	23.1%	NA	27.4%
Pulmonary function tests			
Normal	32%	64.2%	12.5%
Restrictive	68%	28.7%	85%
Obstructive	0	7.1%	2.5%
Diagnostic investigations			
CT scan	9.6%	23.0%	34.3%
Tc-99 scintiscan	13.5%	NA	12.3%
BAL	11.5%	16.7%	12.3%
Biopsy	76.9%	33.3%	50.7%
Necropsy	NA	16.7%	1.4%

NA, not mentioned.

Discussion

PAM has been reported worldwide and has no specific geographic or racial distribution. It is widely prevalent in Turkey, Italy, Japan, India, and USA. Recently, PAM is also being reported frequently from other Asian countries, such as China. PAM has no obvious sex predilection. Although PAM affects all ages, it has been most frequently discovered from birth up to 40 years old [2]. The youngest reported cases were premature twins, who died soon after birth [57]. PAM occurs in families, as well as sporadically [2,4,5,58]. Familial cases account for about half of the reported cases [4]. PAM usually affects siblings and less frequently cousins in a horizontal pattern. Parents and children are rarely affected in a vertical pattern [2]. PAM is a recessively inherited disease with complete penetrance, and no role for non-genetic mechanisms seems to exist [59,60]. Familial PAM is common in our series, and all the familial cases were siblings, except for a rare involvement of a mother and her two daughters [11]. Although the male: female ratio was equal in our series, a female predominance was noted in our familial cases (1:2 ratio). History of significant exposure to specific environmental or occupational risk factors was not observed in almost all our cases.

Recent studies have shown that PAM resulted from the mutation of the *SLC34A2* gene, a type IIb sodium phosphate co-transporter gene involved in phosphate homeostasis in several organs. *SLC34A2* is expressed in the apical membrane of type II alveolar cells in the lungs. These cells synthesize surfactants using phospholipids and are also involved in recycling and degrading the outdated surfactant, thereby releasing phosphates in the process. The clearance of phosphate from the alveolar space is possibly reduced because of SLC34A2 dysfunction, hence leading to the formation of microliths [1,60]. Dogan *et al.* analyzed three siblings with PAM in an inbred family. The genetic analysis revealed homozygous mutation in the *SLC34A2* gene in all three siblings diagnosed with PAM. Both their parents (consanguineous marriage) were carriers, with heterozygous mutation of *SLC34A2* gene. Thus, the impaired activity of the *SLC34A2* gene may be responsible for familial PAM [61].

Autopsy studies revealed that the lungs in PAM are very hard, heavy, and incompressible. The outer surfaces of the lung appear irregular and granular because of microlith protrusion under the visceral pleura. The lungs are gritty on sectioning, and the cut surface has a sandpaper texture. The sandy microliths can be squeezed out [6,62–64]. Bullous emphysematous areas can be found in the anterior margin and at the apex of the lungs [64]. Moreover, the microliths were also found within the bronchial walls in an autopsy case study [65]. Histologically, concentrically laminated calcospherites are observed within dilated alveolar spaces. The pulmonary interstitium shows varying degrees of fibrosis. Recently, morphometric analysis of CD34 immunostained sections in an autopsy case demonstrated a

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significant reduction in the pulmonary capillary beds, which probably leads to pulmonary hypertension [62]. Chemically, the calcospherites are composed of calcium and phosphorus salts. Traces of potassium, iron, copper, and magnesium can also be found [66].

Clinical course

PAM occurs in different age groups, but is frequently diagnosed in the second and third decades. Microliths are probably formed early in childhood, but the manifestations occur much later. The youngest patient in our series was four years old. Many patients were asymptomatic at presentation and were diagnosed incidentally. Breathlessness and cough are the most common symptoms (Tables 1 and 2). Cough probably results from the direct stimulation of the C fibers or other receptors through the microliths [58]. Clubbing is also detected frequently, and wheeze and chest pain are present in less than 10% of our cases. The disease worsens over time and usually progresses into respiratory failure associated with cor pulmonale. Death can also occur because of tension pneumothorax. In our series, the mean age at death is 39.6 years. Mascie-Taylor estimated the mean age at death to be 41.7 years [67]. Mean age at death is not mentioned in the Turkish and Italian series [4,5].

About one-third of the cases from India were misdiagnosed and treated for pulmonary tuberculosis. PAM was also misdiagnosed as miliary tuberculosis in 29% of the Italian cases [5].

Extra pulmonary manifestations

PAM can also be presented with extra-pulmonary manifestations and co-morbidities. Approximately 8% of males in our series were infertile. Semen analysis showed azoospermia in a 40-year-old male [36]. A detailed work up of infertility in a 27-year-old male revealed calcific deposit in the seminal vesicles [28]. Epididymal and periurethral calcifications causing azoospermia and punctate calcification in the seminal vesicles are associated with PAM [68,69]. Calcification of the sympathetic nervous system and possible involvement of the testes in a case of PAM is also reported [70]. Moreover, mutations in SLC34A2 are possibly associated with testicular microlithiasis [60]. These observations make an interesting aspect of PAM, which requires further research.

Co-morbidities, such as mitral stenosis [71], autosomal recessive Waardenburg anophthalmia syndrome [72], lymphocytic interstitial pneumonitis [73], milk-alkali syndrome [74], and diaphyseal aclasia [75], are rarely reported. A case of PAM with concomitant valve calcification, cholelithiasis, and idiopathic medullary nephrocalcinosis is also reported [76]. PAM is rarely associated with pulmonary tuberculosis.

Investigations

Chest X-ray, CT, and high-resolution CT findings are often diagnostic of PAM. Chest radiographs reveal diffuse areas of micronodular calcifications involving both lungs symmetrically. Tomographic evaluation confirms chest X-ray features. Minimal structural changes of the lung parenchyma can also be detected using high resolution CT scans. Most commonly, the CT scan reveals diffuse ground-glass opacities, which are associated with confluent and diffuse calcified nodules [63,77]. Ground-glass attenuation occurs because of small calculi in the alveoli. The calcifications can be observed along the bronchovascular bundles and interlobular septae. The diffuse ground glass attenuation and septal thickening with calcified nodules is referred to as the "crazy-paving" pattern in HRCT scans [77]. Meanwhile, the occurrence of calcification along the interlobular septa is attributed to the accumulation of calcospherites within the peripheral lobular parenchyma adjacent to the septa. HRCT scans can also reveal small sub-pleural cysts and linear subpleural calcifications. The small thin-walled sub-pleural cysts form a peripheral translucent band between the calcified lung parenchyma and the ribs, probably explaining the black pleural sign observed in chest radiographs [77–79]. These small sub-pleural cysts represent dilated alveolar ducts histologically [80]. Apical bullae can also be detected by chest X-ray or CT scans. Multiple calcified pleural plaques [49] and extensive interstitial fibrosis with traction bronchiectasis are observed rarely in our series [45].

The micronodular pattern observed in PAM can mimic diseases, such as miliary tuberculosis. However, PAM involves the middle and lower lobes with relative sparing of the apices, in contrast to pulmonary tuberculosis. The characteristic dissociation between the extensive radiographic alterations and minimal symptoms also helps in diagnosing PAM. Biopsy may be needed to confirm the disease, specifically in regions with a high incidence of pulmonary tuberculosis. In the youngest patient in our series aged four, the lesions were exudative in nature during the earliest phase of the disease [13]. The exudative nodules later got calcified. Discerning these exudative lesions from miliary tuberculosis in young children who presented at an early phase of the disease can be difficult. Small calcifications in children can be difficult to detect on a chest X-ray [73].

Pulmonary function tests usually demonstrate a restrictive pattern, although patients may have a normal lung function initially. An obstructive pattern is rarely observed (Table 2). Our case showed an obstructive pattern probably due to associated bronchial asthma. Brown *et al.* demonstrated that the vital capacity level correctly predicts the development of pulmonary hypertension with exercise in PAM. This relationship suggests the presence of a

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restricted pulmonary vascular bed [81], as demonstrated recently by morphometric analysis [62].

BAL has proved to be a useful tool in the diagnosis of PAM. In our series, BAL revealed the diagnosis in 3/9 cases and in 4/6 cases in the Turkish series. Occasional cases have been diagnosed by sputum examination [36].

Treatment

Many authors emphasize that lung transplantation is the only effective treatment for PAM. Cases from India have generally been treated supportively. However, our case showed a good clinical response to steroid therapy. The role of steroids or other drugs in the management of PAM is not clear. Literature review clearly reveals that steroid cannot cure PAM. Several cases from India were treated with steroids before the diagnosis, but did not show any improvement. By contrast, a few authors have reported clinical improvement with steroids. A 56-year-old male in our series showed minimal improvement in the follow-up chest X-ray following steroid therapy [55]. Another patient initially showed some improvement of dyspnea and cyanosis, but later deteriorated [7].

A 10-year-old girl who presented with associated lymphocytic interstitial pneumonitis showed a rapid improvement in pulmonary function and exercise tolerance in response to steroid therapy [73]. Improvement was also evident in an eight-month-old infant treated with corticosteroid and hydroxychloroquine [82]. A 45-year-old woman who developed PAM after a varicella zoster virus infection with concomitant antiphospholipid syndrome and discoid lupus erythematosus responded to inhaled steroids (200 μg of budesonide per day) [83]. Pulmonary function tests revealed an obstructive pattern in our patient. Bronchial asthma has probably resulted from the chronic irritation caused by the microliths. PAM can also involve the central airways in addition to the alveoli as previously mentioned. Thus, literature review shows that only few patients have benefited from steroid therapy. Steroids cannot cure PAM, but they have provided symptomatic relief in some cases. Moreover, steroid therapy is unlikely to be effective in the late stages. Further research is needed to identify patients who are likely to benefit from steroids.

Few authors have also described the utility of disodiumetidronate, a bisphosphonate inhibiting the precipitation of hydroxyapatite microcrystals. Ozcelik *et al.* [84] reported clinical and radiological improvement of two patients with PAM who were treated with disodium etidronate for 9 and 11 years, respectively. The patients receiving this drug have to be closely monitored for possible side effects, such as rickets, bone fractures, and hyperphosphatemia. The drug was not effective in an earlier case treated for 18 months [85]. The effectiveness of disodium etidronate for patients who present in the late phase of the disease should be evaluated.

Lung transplantation is the only effective treatment for end-stage pulmonary disease and can be performed safely even in elderly patients. Shigemura *et al.* [86] reported a case of successful double lung transplantation in a 63-year-old recipient with end-stage PAM. Recently, a 64-year-old woman underwent a right-lung transplant and is able to lead an active life without any complications over a 5-year follow-up period [87]. Although complications related to transplantation have occurred in some cases, recurrence has not been reported to date [86–89].

Recent advances

Recent studies, specifically from Turkey, China, and Japan, have helped us better elucidate the genetic basis of PAM [1,60,61,90]. The mutation of *SLC34A2* gene is responsible for PAM, as discussed earlier. Eleven different homozygous mutations of *SLC34A2* gene involving exons 1, 2, 3, 4, 6, 7, 8, 11, 12, and 13 have been identified. The predominant result of these mutations was protein truncation [90]. Interestingly, mutations seem to vary among races. Mutations in Chinese and Japanese are more likely to affect exons 7 and 8 [90]. Yin *et al.* [90] suggested that exon 8 may be the screen target for Chinese patients. No disease-causing mutations or single nucleotide polymorphisms in the *SLC34A2* gene have also been reported [90,91]. Genes other than the *SLC34A2* gene could possibly be involved in the pathogenesis of PAM. Advanced molecular research tools, such as genome-wide single nucleotide polymorphism analysis, will certainly help us gain better insights and devise novel therapeutic strategies.

Takahashi *et al.* [92] showed that surfactant protein-A and surfactant protein-D are potential serum markers to monitor the disease activity and progression of PAM. Dysfunction of *SLC34A2* gene reduces the clearance of phosphate, hence leading to the formation of microliths. Thus, drugs targeting phosphate metabolism rather than calcium metabolism could prove to be beneficial [1].

Conclusions

Physicians should have a high degree of suspicion for this rare disorder in regions with a high incidence of pulmonary tuberculosis. Siblings of a patient with PAM should be screened for PAM even if they are asymptomatic. The clinical manifestations vary with the disease stage. PAM has a prolonged course and can be associated with extra pulmonary manifestations. Long-term follow-up and genetic analysis will help us to better understand the etiopathogenesis. Cost-effective treatment should also be established for PAM in developing nations.

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Compliance with ethics guidelines

Nidhya Ganesan, Marie Moses Ambroise, Anita Ramdas, King Herald Kisku, Kulwant Singh, and Renu G' Boy Varghese have no conflicts of interest to declare. This manuscript is a systematic review article and does not involve a research protocol requiring ethics committee approval.

References

1. Huqun, Izumi S, Miyazawa H, Ishii K, Uchiyama B, Ishida T, Tanaka S, Tazawa R, Fukuyama S, Tanaka T, Nagai Y, Yokote A, Takahashi H, Fukushima T, Kobayashi K, Chiba H, Nagata M, Sakamoto S, Nakata K, Takebayashi Y, Shimizu Y, Kaneko K, Shimizu M, Kanazawa M, Abe S, Inoue Y, Takenoshita S, Yoshimura K, Kudo K, Tachibana T, Nukiwa T, Hagiwara K. Mutations in the *SLC34A2* gene are associated with pulmonary alveolar microlithiasis. Am J Respir Crit Care Med 2007; 175(3): 263–268
2. Castellana G, Lamorgese V. Pulmonary alveolar microlithiasis. World cases and review of the literature. Respiration 2003; 70(5): 549–555
3. Viswanathan R. Pulmonary alveolar microlithiasis. Thorax 1962; 17 (3): 251–256
4. Ucan ES, Keyf AI, Aydilek R, Yalcin Z, Sebit S, Kudu M, Ok U. Pulmonary alveolar microlithiasis: review of Turkish reports. Thorax 1993; 48(2): 171–173
5. Mariotta S, Guidi L, Papale M, Ricci A, Bisetti A. Pulmonary alveolar microlithiasis: review of Italian reports. Eur J Epidemiol 1997; 13(5): 587–590
6. Bhattacharjee BK, Barua R, Duta JK. Pulmonary alveolar microlithiasis. Indian J Chest Dis 1965; 7: 51–53
7. Banerjea JC, Mukerji A, Maiti CR. Chronic cor pulmonale due to pulmonary alveolar microlithiasis. Indian Heart J 1967; 19(1): 47–56
8. Rajagopalan CR, Krishna KVS, Rao M, Babu TN. Pulmonary alveolar microlithiasis. Report of a case. Indian J Pediatr 1967; 34 (236): 328–331
9. Rao CS, Rao YM, Reddy DB, Sarada D. Pulmonary alveolar microlithiasis. J Indian Med Assoc 1968; 50(9): 427–428
10. Khattri HN, Sodhi JS, Gujeral JS, Dutta BN. Microlithiasis alveolaris pulmonale. J Assoc Physicians India 1971; 19(4): 335–337
11. Thiruvengadam KV, Arunachalam K, Murali MR, Sarma AV, Varadarajan MG, Shetty BS, Swamidasan G. Pulmonary alveolar microlithiasis in a family. J Assoc Physicians India 1972; 20(9): 707–712
12. Prabhakar BR, Sabharwal BD, Prabhakar H, Sethl RS. Pulmonary alveolar microlithiasis. A case report with review of literature. Indian J Pathol Bacteriol 1974; 17(3): 193–196
13. Bhatia JL, Thind GS. Pulmonary alveolar microlithiasis. Ind J Tub 1976; 23(3): 110–115
14. Mathur AS, Gupta DK, Garg RK. Pulmonary alveolar microlithiasis. Indian JRadiol 1977; 31(3): 207–208
15. Thind GS, Bhatia JL. Pulmonary alveolar microlithiasis. Br J Dis Chest 1978; 72(2): 151–154
16. Taneja VP, Sen D. Pulmonary alveolar microlithiasis (case report). J Assoc Physicians India 1981; 29(5): 411–414
17. Gupta SK, Maini VK, Bedi RS. Pulmonary alveolar microlithiasis. Indian J Chest Dis Allied Sci 1982; 24(1): 29–32
18. Saigal RK, Gupta K, Sandhu SK, Sidhu PK. Pulmonary alveolar microlithiasis. (A case report.) J Postgrad Med 1982; 28(4): 235–237
19. Chetty A, Paul VK, Kumar A, Mitra DK, Chopra P. Alveolar microlithiasis — a case report with review of literature. Indian J Pediatr 1983; 50(405): 453–455
20. Challa S, Dusaj IS, Kumar V, Mehrotra R. Pulmonary alveolar microlithiasis. Indian J Chest Dis Allied Sci 1984; 26(4): 263–266
21. NatrajaIyer K, Sasikumar S, Devi SS. Pulmonary alveolar microlithiasis — a case report. Indian J Radiol 1986; 40(1): 63–65
22. Bhadur P, Seetharaman ML, Krishnamurthy H. Pulmonary alveolar microlithiasis — a case report. Lung India 1987; 5(4): 187–188
23. Pant K, Chawla R, Chhabra SK, Jain SK, Malik GB, Gaur SN. Pulmonary alveolar microlithiasis — a case report and review of literature. Indian J Chest Dis Allied Sci 1988; 30(2): 141–147
24. Swarnkar JS, Wakodkar TD, Kirolikar SK, Diwakar RK. Pulmonary alveolar microlithiasis — a report of two cases. Lung India 1989; 7 (3): 140–142
25. Venkateswarlu J, Waghray P, Subbarao K, Kishore LT, Subbalakshmi M. pleural calcification in pulmonary alveolar microlithiasis — a case report. Lung India 1989; 7(3): 143–145
26. Pant K, Shah A, Mathur RK, Chhabra SK, Jain SK. Pulmonary alveolar microlithiasis with pleural calcification and nephrolithiasis. Chest 1990; 98(1): 245–246
27. Rao SD, Rekha S, Chandrasekhara MK, Shetty N, Srikrishna. Pulmonary alveolar microlithiasis in siblings. Indian Pediatr 1991; 28(10): 1206–1210
28. Sharma SK, Sharma S, Mukhopadhyaya S, Padhy AK, Vijayraghavan M. Pulmonary alveolar microlithiasis — report of three cases with pulmonary function and exercise studies. Indian J Chest Dis Allied Sci 1992; 34(4): 205–215
29. Babu MK, Kumar KR, Thomas JA. Pulmonary alveolar microlithiasis. Indian J Pathol Microbiol 1992; 35(2): 149–152
30. Malhotra B, Kailan BM. Pulmonary alveolarmicrolithiasis — a case report. Ind J Tub 1993; 40(1): 31–33
31. Malur PR, Ajay K, Jayaram N. Alveolar microlithiasis — report of two cases. Indian J Pathol Microbiol 1993; 36(2): 163–167
32. Boghani A, Kumaresan K, Sundaram C, Bhaskar G. Pulmonary alveolar microlithiasis revisited with CT scan, bone scan and broncho-alveolar lavage. Lung India 1993; 11(2): 37–39
33. Gaude GS, Pinto MJW, Bagga AS. Pneumothorax in pulmonary alveolar microlithiasis: a case report. Antiseptic 1994; 91(4): 135–136
34. Singh NK, Gupta A. Pulmonary alveolar microlithiasis. J Assoc Physicians India 1995; 43(9): 647–648
35. Kashyap S, Kumar M, Pal LS. Pulmonary alveolar microlithiasis in a Himachali woman. J Assoc Physicians India 1996; 44(1): 56–57
36. Chatterji R, Gaude GS, Patil PV. Pulmonary alveolar microlithiasis: diagnosed by sputum examination and transbronchial biopsy. Indian J Chest Dis Allied Sci 1997; 39(4): 263–267
37. Vidyasagar B, Ranjana SD, Jayan R, NagarajaSetty KL, Shivalingappa GS. Pulmonary alveolar microlithiasis. Lung India 1998; 16 (2): 80–82

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http://journal.hep.com.cn/

38. Dwivedi MK, Pal RK, Borkar PB. Pulmonary alveolar microlithiasis. Indian J Radiol Imaging 1999; 9(4): 195–196
39. Hira HS, Singh T, Chowdhary V. Pulmonary alveolar microlithiasis: role of transbronchial lung biopsy. J Assoc Physicians India 2000; 48(8): 832–833
40. Gupta D, Aggarwal AN, Jindal SK. Lung static pressure-volume analysis can demonstrate alveolar involvement in pulmonary alveolar microlithiasis. Lung India 2000; 18(2): 64–65
41. Shah MS, Nanavati KJ, Airon A, Shah RR, Joshi BD. Case report—pulmonary alveolar microlithiasis. Indian J Radiol Imaging 2003; 13 (3): 277–279
42. Bhalotra B, Gogia A, Gupta P, Jain N. A pulmonary snowstorm. Med J Aust 2004; 180(9): 454
43. Patro SS, Kar CR. Pulmonary alveolar microlithiasis. Indian J Radiol Imaging 2006; 16(4): 879–881
44. Unnithan J, Nandagopal V. A rare case of pulmonary alveolar microlithiasis. Pulmon 2006; 8(1): 27–29
45. Mitra S, Ghoshal AG. A family of pulmonary alveolar microlithiasis— a case report. Lung India 2000; 18(3): 97–100
46. Hephzibah J, Oommen R, Mugesh B, Pradhip J. Pulmonary alveolar microlithiasis scinitigraphic features. Internet J Pulmonol Med 2006; 9(1). https://ispub.com/IJPM/9/1/12504
47. Thapa R, Ganguly D, Ghosh A. Pulmonary alveolar microlithiasis in siblings. Indian Pediatr 2008; 45(2): 154–156
48. Chandra S, Mohan A, Guleria R, Das P, Sarkar C. Pulmonary alveolar microlithiasis. BMJ Case Rep 2009; 2009: bcr09. 2008.1012
49. Malhotra B, Sabharwal R, Singh M, Singh A. Pulmonary alveolar microlithiasis with calcified pleural plaques. Lung India 2010; 27 (4): 250–252
50. Gayathri Devi HJ, Mohan Rao KN, Prathima KM, Das JK. Pulmonary alveolar microlithiasis. Lung India 2011; 28(2): 139–141
51. Shah M, Joshi JM. Bone scintigraphy in pulmonary alveolar microlithiasis. Indian J Chest Dis Allied Sci 2011; 53(4): 221–223
52. Gayathri B, Keerthivasan S. Pulmonary alveolar microlithiasis. J Med 2011; 12(1): 55–57
53. Sonwalkar P, Karthik M, Vishal NS, Praveen M, Patil MM. Pulmonary alveolar microlithiasis. JCDR 2012; 6(7): 1296–1297
54. Galhotra R, Gupta K. Images—pulmonary alveolar microlithiasis (PAM). J Pulmon Resp Med 2012; 2: 124
55. Flynn A, Agastyaraju AD. Pulmonary alveolar microlithiasis. Int J Case Rep Images 2013; 4(2): 108–110
56. Sahoo MK, Karunanithi S, Bal CS. Pulmonary alveolar microlithiasis: imaging characteristics of planar and SPECT/CT bone scan versus 18F-FDG and 18F-sodium fluoride PET/CT scanning. Jpn J Radiol 2013; 31(11): 766–769
57. Caffrey PR, Altman RS. Pulmonary alveolar microlithiasis occurring in premature twins. J Pediatr 1965; 66(4): 758–763
58. Mariotta S, Ricci A, Papale M, De Clementi F, Sposato B, Guidi L, Mannino F. Pulmonary alveolar microlithiasis: report on 576 cases published in the literature. Sarcoidosis Vasc Diffuse Lung Dis 2004; 21(3): 173–181
59. Castellana G, Gentile M, Castellana R, Fiorente P, Lamorgese V. Pulmonary alveolar microlithiasis: clinical features, evolution of the phenotype, and review of the literature. Am J Med Genet 2002; 111 (2): 220–224
60. Corut A, Senyigit A, Ugur SA, Altin S, Ozcelik U, Calisir H, Yildirim Z, Gocmen A, Tolun A. Mutations in SLC34A2 cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis. Am J Hum Genet 2006; 79(4): 650–656
61. Dogan OT, Ozsahin SL, Gul E, Arslan S, Koksal B, Berk S, Ozdemir O, Akkurt I. A frame-shift mutation in the *SLC34A2* gene in three patients with pulmonary alveolar microlithiasis in an inbred family. Intern Med 2010; 49(1): 45–49
62. Terada T. Pulmonary alveolar microlithiasis with cor pulmonale: an autopsy case demonstrating a marked decrease in pulmonary vascular beds. Respir Med 2009; 103(11): 1768–1771
63. Chalmers AG, Wyatt J, Robinson PJ. Computed tomographic and pathological findings in pulmonary alveolar microlithiasis. Br J Radiol 1986; 59(700): 408–411
64. Lauta VM. Pulmonary alveolar microlithiasis: an overview of clinical and pathological features together with possible therapies. Respir Med 2003; 97(10): 1081–1085
65. Sears MR, Chang AR, Taylor AJ. Pulmonary alveolar microlithiasis. Thorax 1971; 26(6): 704–711
66. Moran CA, Hochholzer L, Hasleton PS, Johnson FB, Koss MN. Pulmonary alveolar microlithiasis. A clinicopathologic and chemical analysis of seven cases. Arch Pathol Lab Med 1997; 121(6): 607–611
67. Mascie-Taylor BH, Wardman AG, Madden CA, Page RL. A case of alveolar microlithiasis: observation over 22 years and recovery of material by lavage. Thorax 1985; 40(12): 952–953
68. Kanat F, Teke T, Imecik O. Pulmonary alveolar microlithiasis with epididymal and periurethral calcifications causing obstructive azospermia. Int J Tuberc Lung Dis 2004; 8(10): 1275
69. Arslan A, Yalin T, Akan H, Belet U. Pulmonary alveolar microlithiasis associated with calcifications in the seminal vesicles. J Belge Radiol 1996; 79(3): 118–119
70. Coetzee T. Pulmonary alveolar microlithiasis with involvement of the sympathetic nervous system and gonads. Thorax 1970; 25(5): 637–642
71. Yesner R, Skorneck AB. Pulmonary alveolar microlithiasis in mitral stenosis. Am J Pathol 1956; 32(3): 611
72. Schmidt H, Lörcher U, Kitz R, Zielen S, Ahrens P, König R. Pulmonary alveolar microlithiasis in children. Pediatr Radiol 1996; 26(1): 33–36
73. Ratjen FA, Schoenfeld B, Wiesemann HG. Pulmonary alveolar microlithiasis and lymphocytic interstitial pneumonitis in a ten year old girl. Eur Respir J 1992; 5(10): 1283–1285
74. Portnoy LM, Amadeo B, Hennigar GR. Pulmonary alveolar microlithiasis: an unusual case (associated with milk alkali syndrome). Am J Clin Pathol 1964; 41(2): 194–201
75. Ritchie DA, O'Connor SA, McGivern D. An unusual presentation of pulmonary alveolar microlithiasis and diaphyseal aclasia. Br J Radiol 1992; 65(770): 178–181
76. Giallauria F, Giallauria G. Pulmonary alveolar microlithiasis. Thorax 2011; 66(9): 840
77. Gasparetto EL, Tazoniero P, Escuissato DL, Marchiori E, Frare E Silva RL, Sakamoto D. Pulmonary alveolar microlithiasis presenting with crazy-paving pattern on high resolution CT. Br J Radiol 2004; 77(923): 974–976
78. Marchiori E, Gonçalves CM, Escuissato DL, Teixeira KI, Rodrigues R, Barreto MM, Esteves M. Pulmonary alveolar microlithiasis: high-resolution computed tomography findings in 10 patients. J

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http://journal.hep.com.cn/

Bras Pneumol 2007; 33(5): 552–557
79. Siddiqui NA, Fuhrman CR. Best cases from the AFIP: pulmonary alveolar microlithiasis. Radiographics 2011; 31(2): 585–590
80. Sumikawa H, Johkoh T, Tomiyama N, Hamada S, Koyama M, Tsubamoto M, Murai S, Inoue A, Nakamura H, Tachibana T, Müller NL. Pulmonary alveolar microlithiasis: CT and pathologic findings in 10 patients. Monaldi Arch Chest Dis 2005; 63(1): 59–64
81. Brown J, Leon W, Felton C. Hemodynamic and pulmonary studies in pulmonary alveolar microlithiasis. Am J Med 1984; 77(1): 176–178
82. Dahabreh M, Najada A. Pulmonary alveolar microlithiasis in an 8-month-old infant. Ann Trop Paediatr 2009; 29(1): 55–59
83. Yilmaz MI, Koc B, Kantarcioglu M, Akinci SB, Ayta H, Bulucu F, Bal S. Pulmonary alveolar microlithiasis after Varicella zoster infection in a patient presenting with antiphospholipid syndrome and discoid lupus. Rheumatol Int 2002; 22(5): 213–215
84. Ozcelik U, Yalcin E, Arıyurek M, Ersoz DD, Cinel G, Gulhan B, Kiper N. Long-term results of disodium etidronate treatment in pulmonary alveolar microlithiasis. Pediatr Pulmonol 2010; 45(5): 514–517
85. Jankovic S, Pavlov N, Ivkosic A, Erceg I, Glavina-Durdov M, Tocilj J, Dragisic-Ivulic S, Primorac D. Pulmonary alveolar microlithiasis in childhood: clinical and radiological follow-up. Pediatr Pulmonol 2002; 34(5): 384–387
86. Shigemura N, Bermudez C, Hattler BG, Johnson B, Crespo M, Pilewski J, Toyoda Y. Lung transplantation for pulmonary alveolar microlithiasis. J Thorac Cardiovasc Surg 2010; 139(3): e50–e52
87. Borrelli R, Fossi A, Volterrani L, Voltolini L. Right single-lung transplantation for pulmonary alveolar microlithiasis. Eur J Cardiothorac Surg 2014; 45(2): e40
88. Edelman JD, Bavaria J, Kaiser LR, Litzky LA, Palevsky HI, Kotloff RM. Bilateral sequential lung transplantation for pulmonary alveolar microlithiasis. Chest 1997; 112(4): 1140–1144
89. Samano MN, Waisberg DR, Canzian M, Campos SV, Pêgo-Fernandes PM, Jatene FB. Lung transplantation for pulmonary alveolar microlithiasis: a case report. Clinics (Sao Paulo) 2010; 65 (2): 233–236
90. Yin X, Wang H, Wu D, Zhao G, Shao J, Dai Y. *SLC34A2* gene mutation of pulmonary alveolar microlithiasis: report of four cases and review of literatures. Respir Med 2013; 107(2): 217–222
91. Olauson H, Brandenburg V, Larsson TE. Mutation analysis and serum FGF23 level in a patient with pulmonary alveolar microlithiasis. Endocrine 2010; 37(2): 244–248
92. Takahashi H, Chiba H, Shiratori M, Tachibana T, Abe S. Elevated serum surfactant protein A and D in pulmonary alveolar microlithiasis. Respirology 2006; 11(3): 330–333

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Original Research Article

Analysis of Bacterial Contamination in Blood Bank: Comparison of Two Different Donor Arm Disinfectant

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Abstract

Bacterial contamination of transfusion products, especially platelets are the most common cause of transfusion associated morbidity and mortality. Minimizing bacterial contamination in blood products is a priority for safe transfusion practices. A prospective study was done comparing the efficiency of two different disinfectant techniques by studying the flora on donor's arm before and after disinfection. This study was undertaken as there are very few reports on this important transfusion protocol from Southern India. Mean number of colonies in post disinfection swab culture with 2% povidone iodine was found to be greater than that of 2.5% Chlorhexidine in 75% isopropyl alcohol. 2.5% chlorhexidine disinfection offers more effective reduction in bacterial contamination than 2% povidone iodine group.

Keywords: Chlorhexidine, Povidone iodine, Bacteremia.

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INTRODUCTION

There are reports to indicate that every year India requires 40 million units of blood but only 4million units are available. Hence the collection and preservation of this precious resource requires utmost care [1]. In India, approximately 57% of transfusion transmitted reactions have been found to be associated with bacterial contamination of donated blood [2, 3].The French Haemovigilance surveillance system cited bacterial transfusion transmission as the most identified cause of transfusion associated deaths [4].In the UK, between 1995 to 2003, bacterial transfusion transmissions accounted for 58% of transfusion reactions [5]. Bacteriological screening tests for platelet units were introduced in the US in 2004 and the study showed that the average prevalence of bacterial contamination in platelets from the whole blood was 33.9/100,000 units, that of platelets from apheresis 51/100,000, while that of red cell concentrates was 2.6/100,000[6].

In India, antisepsis with three-step regimen of spirit-10% povidone iodine-spirit for donor arm is followed currently. The US-FDA has approved use of alcoholic-chlorhexidine as highly effective for donor arm disinfection. However, it is not being used in India. Also, tincture iodine which was used earlier is now discontinued due to its irritant nature [7].

It is observed that Gram positive skin commensals such as coagulase negative Staphylococci and Bacillus cereus are the organisms most often recovered from donated blood which are thought to originate principally through phlebotomy[8]. The introduction of a needle through the skin to collect blood causes the passage of live microbes or the movement of microbes from small skin flaps produced by the needle itself into the collection bags[9].This is found to be effectively reduced by efficient donor arm disinfectants[10].

Approximately 16% of the deaths due to transfusion reactions have been associated with bacterial contamination, which are usually multi-Drug resistant [7]. Hence, it becomes mandatory to analyse the gaps in the process and implement corrective action to prevent them. SHOT(serious hazards of transfusion), BaCON (Bacterial contamination associated with transfusion) and French haemovigilance surveillance system, studies stated that resident skin flora accounted for 90% of platelet concentrates and 70% of red cell concentrates causing transfusion reactions[11,12].

A Blood &Transplant study conducted by NHS revealed reduction in contamination of blood products by 57% using improved disinfection strategies [13].

Review of the literature revealed only a few reports from India on this topic, and since blood safety is a very vital issue, it was thought worthwhile to undertake this study. The aim of this review is to consider the scientific evidence in supporting the efficacy of improved Donor arm disinfection in preventing bacterial contamination of blood.

AIMS & OBJECTIVES

The chief objectives of this study are:

- To assess the rate of bacterial contamination in blood bank setting.
- To compare the results from two different donor arm disinfectant techniques.

METHODOLOGY

This study was a randomised control study using prospective data. 100 subjects were included in the study after calculating the sample size.

The Voluntary donor arrives at the blood bank was tested for eligibility. After obtaining consent form for study, pre-Disinfection sample was collected in sterile swab. Disinfectant was applied for an area of 10cm on cubital fossa in concentric pattern. 2.5% Chlorhexidine in 75% isopropyl alcohol was applied as disinfectant (group 1) and while 70% Alcohol followed by 2% povidone iodine followed by 70%Alcohol was used as disinfectant (group 2). Post-Disinfection sample was collected in sterile swab. Both the swabs were cultured in blood agar and colony count was performed.

Sterile swabs for the project were made on the same day, supplied by the Department of Microbiology. Samples were collected from the cubital fossa of the donor using sterile forceps by gloved hands and immediately plated on 5% Sheep blood agar under bio-safety cabinets with positive pressure and laminar air flow.

All the patients who have received transfusion from the donors of the sample size were followed up for transfusion reactions. Any allergic reactions were to be notified and treated accordingly. To ensure quality periodical controls for swabs and Disinfectant were established. Technical officers were trained on proper method of disinfection techniques. The waste samples were discarded as per hospital's standard waste disposal system.

Data was entered in Microsoft Excel spreadsheet and analysis was done using SPSS software by Microsoft V.22.0. Association between two factors was expressed in form of odds ratio. P<0.005 was considered significant.

OBSERVATION & RESULTS

A total of 98 male and 2 female donors were selected for the study and disinfectant used was randomly assigned to them. Both the pre- and post-disinfection swabs were cultured on blood agar and their efficacy was determined by the number of colonies formed on the blood agar (Table I).

Table-I: Provides the result of swab culture with two disinfectants.

DISINFECTANT USED	n	Colonies-before disinfection	Colonies-after disinfection
Povidone iodine	50	40-210	0-10
Chlorhexidine	50	35-190	0-4

STATISTICAL ANALYSIS

Independent student 't' test was done to compare the colony count of post-disinfection swab culture between 70% Alcohol followed by2% povidone iodine followed by 70% alcohol and 2.5% Chlorhexidine in 75% isopropyl alcohol . Analysis of covariant was done using General linear model on SPSS 24.0 to compare the mean difference in post-disinfection swab culture adjusted to the bacterial load on pre-disinfection swab culture.

Table II presents the mean (SD) of the post-disinfection swab cultures between the two disinfectants and a statistically significant difference was observed. The mean (SD) of the post-disinfection swab culture for 2.5% Chlorhexidine in 75% isopropyl alcohol was 0.2800(0.70102) and 70% Alcohol followed by2% povidone iodine followed by 70% alcohol was 0.9000(1.97174).t=2.095;p<0.05.

Table-II: Comparison of post disinfection swab culture between the two disinfectants

DISINFECTANT USED	MEAN	SD	p
Povidone iodine	0.9000	1.97174	P<0.05
Chlorhexidine	0.2800	0.70102	

Analysis of covariant showed that the mean difference of the post-disinfection swab culture adjusted to the bacterial load on pre-disinfection swab culture was statistically significant F=4.974; p<0.05.

DISCUSSION

The hazards of post transfusion septicaemia became less common when closed plastic bags replaced glass containers for collection of blood. Even though it

has seen a massive decrease, it has not disappeared entirely and there seems to have been a resurgence of interest in it due to modern day increase in genetically improved resistant organisms. It is not always easy to identify the source of bacterial contamination in blood bags. Post transfusion septicaemia can be endogenous which is due to organisms circulating in the blood and in other hand, exogenous post transfusional septicaemia stems from organisms on donors' skin and equipment contamination. Endogenous post transfusional septicaemia is comparatively less than exogenous post transfusional septicaemia due to vigorous donor selection criteria hence extra attention should be given to exogenous post transfusional septicaemia which can be significantly reduced by optimal disinfection of phlebotomy site, since majority of the organisms contaminating the blood products belong to the group of skin commensals[14].

Our skin contains two types of bacterial flora. One is transient flora which can be easily washed away by water and soap. The other is resident flora that requires extensive disinfection with an efficacious disinfectant which we tried to identify in this study.

In our study encompassing 100 donors' disinfection with 2.5% chlorhexidine in 75% isopropyl alcohol was comparatively more efficacious than 70% Alcohol followed by2% povidone iodine followed by 70%alcohol. Mean values of colonies of 70% Alcohol followed by2% povidone iodine followed by 70% alcohol is greater than what was observed in 2.5% Chlorhexidine in 75% isopropyl alcohol in the swab culture of post-disinfection swabs.

We compared our study with similar studies. Lee C K *et al*. reported a relative risk reduction of 3.4% of chlorhexidine over povidone Iodine in disinfection of donors' arm [15].Ramirez Arcos *et al*. also observed that disinfection by70% alcohol followed by chlorhexidine is highly effective having 98.74% of reduction rate in bacterial contamination of blood components than what is seen with isopropyl alcohol and iodine which correlates with the results of our study [16]. Also Benjamin RJ *et al*. proved that the 1.5ml chlorhexidine disinfection method offers more effective reduction in bacterial contamination of apheresis platelets than 70% Alcohol followed by2% povidone iodine followed by 70%alcohol [17]

Chlorhexidine is a broad spectrum agent with cumulative actions, which is recommended in the 'Guidelines on drawing blood' by WHO, Centre For Disease Control (CDC) and Health Care Infection Control Practices Advisory Committee (HIPAC) as an optimal skin disinfectant for variety of uses like phlebotomy, vascular catheterization and pre-op preparation of skin[12].

It is also less irritant and non-staining on skin. We found that both the donors and the staff of the blood bank expressed an increased compliance towards it during the period of this study. On application it disrupts the cell wall and is more effective against gram positive organisms than gram negative organisms. In our study we also detected most of the gram positive organisms are inhibited, but due to the small numbers that were studied, analysis and comparison of data cannot be made at this juncture.

A study done by Lee C K *et al*. detected predominantly Bacillus species and Coagulase-negative staphylococci as the most common pathogens [15]. In this study organisms like Coagulase-negative Staphylococci, Micrococci and Diptheroid were mainly isolated in Pre-disinfection swabs, while only Coagulase-negative staphylococci were identified in post- disinfection swabs. This is explained by the emerging resistance of staphylococci to Chlorhexidine [18]. The use of conventional agar plates and gram-staining to identify the morphology of the organism limits the scope of identifying varied bacterial flora which is more evident on automated culture systems like BACTEC/BacTALERT.

This study prompts us to conclude that there is a decrease in bacterial contamination with 2.5% chlorhexidine in 75% isopropyl alcohol compared to 70% alcohol followed by 2% povidone iodine followed by 70% Alcohol.

Since there is no significant difference in cost between these two methods tested in our study, Chlorhexidine should be affordable in blood banks of resource limited nations as well.

CONCLUSION

- Bacterial contamination of blood components remains a major challenge in the practice of transfusion medicine.
- Optimal skin antisepsis of the phlebotomy site is essential to minimize the risk of contamination of collected blood.
- Every reaction of post transfusional septicaemia must be identified, reported, evaluated and the cause for contamination must be identified in order to prevent future post transfusional septicaemia and maintain safe blood practices.

Compliance with Ethical Standards

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee

Conflict of Interest

The authors declare that they have no conflict of interest

Informed consent

Informed consent was obtained from all individual participants included in the study

REFERENCES

1. India facing a blood shortage of 3 million units of blood. (2016). Retrieved 21 Nov 2016 from http://timesofindia.com.
2. Patel, T. G., Shukla, R. V., & Gupte, S. C. (2013). Impact of donor arm cleaning with different aseptic solutions for prevention of contamination in blood bags. *Indian Journal of Hematology and Blood Transfusion*, *29*(1), 17-20.
3. Hoppe, P. A. (1992). Interim measures for detection of bacterially contaminated red cell components. *Transfusion*, *32*(3), 199-201.
4. Debeir, J., Noel, L., Aullen, J. P., Frette, C., Sari, F., Mai, M. P. V., & Cosson, A. (1999). The French haemovigilance system. *Vox sanguinis*, *77*(2), 77-81.
5. Stainsby, D., Cohen, H., Jones, H., Knowles, S., Milkins, C., Chapman, C., & Revill, J. (2003). Serious hazards of transfusion (SHOT). *SHOT annual report*, 1-88.
6. Blajchman, M. A., Ali, A. M., & Richardson, H. L. (1994). Bacterial contamination of cellular blood components. *Vox sanguinis*, *67*, 25-33.
7. Shah, S., Mehta, N. A., & Jadhav, S. G. (2014). Prospective evaluation of 2%(w/v) alcoholic chlorhexidine gluconate as an antiseptic agent for blood donor arm preparation. *Asian journal of transfusion science*, *8*(2), 92.
8. Leo, A. M., Salvadego, M. M., Piva, M. G., Ruffato, G., Valverde, S., Trabuio, E., ... & Gessoni, G. (2007). From the donor's arm to blood product: a study on bacterial contamination of apheresis platelet concentrates. *Blood Transfusion*, *5*(3), 130.
9. Stramer, S. L. (2007). Current risks of transfusion-transmitted agents: a review. *Archives of pathology & laboratory medicine*, *131*(5), 702-707.
10. McDonald, C., McGuane, S., Thomas, J., Hartley, S., Robbins, S., Roy, A., ... & Barbara, J. (2010). A novel rapid and effective donor arm disinfection method. *Transfusion*, *50*(1), 53-58.
11. Perez, P., Salmi, L. R., Folléa, G., Schmit, J. L., De Barbeyrac, B., Sudre, P., ... & Bacthem Group the French Haemovigilance Network. (2001). Determinants of transfusion-associated bacterial contamination: results of the French BACTHEM Case-Control Study. *Transfusion*, *41*(7), 862-872.
12. McDonald, C. P. (2011). Interventions implemented to reduce the risk of transmission of bacteria by transfusion in the English National Blood Service. *Transfusion Medicine and Hemotherapy*, *38*(4), 255-258.
13. Dubey, A., Sonker, A., & Chaudhary, R. (2017). Snake in the grass: A case report of transfusion reactions due to contaminated donor arm disinfectant. *Asian journal of transfusion science*, *11*(1), 50.
14. Puckett, A., Davison, G., Entwistle, C. C., & Barbara, J. A. (1992). Post transfusion septicaemia 1980-1989: importance of donor arm cleansing. *Journal of clinical pathology*, *45*(2), 155-157.
15. Lee, C. K., Ho, P. L., Chan, N. K., Mak, A., Hong, J., & Lin, C. K. (2002). Impact of donor arm skin disinfection on the bacterial contamination rate of platelet concentrates. *Vox sanguinis*, *83*(3), 204-208.
16. Ramirez-Arcos, S., & Goldman, M. (2010). Skin disinfection methods: prospective evaluation and postimplementation results. *Transfusion*, *50*(1), 59-64.
17. Benjamin, R. J., Dy, B., Warren, R., Lischka, M., & Eder, A. F. (2011). Skin disinfection with a single-step 2% chlorhexidine swab is more effective than a two-step povidone-iodine method in preventing bacterial contamination of apheresis platelets. *Transfusion*, *51*(3), 531-538.
18. Goldman, M., Roy, G., Frechette, N., Decary, F., Massicotte, L., & Delage, G. (1997). Evaluation of donor skin disinfection methods. *Transfusion*, *37*(3), 309-312.

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Morphologic Spectrum of Undetermined Causes of Hemoptysis- A Pathologist's Role

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ABSTRACT

Objective: Hemoptysis is the expectoration of blood or blood-streaked sputum from the tracheobronchial tree. The etiology may derive from nonneoplastic conditions such as infections, chronic pulmonary diseases, and vasculitis or neoplastic causes. Sometimes a definitive cause for hemoptysis cannot be found after ample diagnostic workup. The role of biopsy in such cases is to help the clinician in arriving at the final diagnosis. Diffuse alveolar hemorrhage is the main histopathological finding in hemoptysis and it appears with diffuse chest infiltrates radiologically.

Material and Method: A retrospective study of 2 years duration was conducted to identify the morphological spectrum of diseases presenting with hemoptysis. A total of 243 lung biopsies obtained by various methods were retrieved in this study period and 20 cases with hemoptysis of undetermined etiology were detected.

Results: Based on imaging and histopathology findings, the etiological causes of hemoptysis were divided into hemoptysis with and without capillaritis or due to tumor/tumor-like lesions and due to miscellaneous conditions. The most common etiology was vasculitis followed by infections.

Conclusion: Histopathology helps to detect the etiology, particularly in cases of hemoptysis due to non-immunologic causes. In immunologic cases, histopathological findings may support the diagnosis in correlation with the clinical/imaging features.

Key Words: Hemoptysis, Immunologic, Diffuse alveolar hemorrhage, Capillaritis

INTRODUCTION

Hemoptysis is the expectoration of blood from the pulmonary parenchyma or the tracheobronchial tree below the level of the glottis. The expectoration of blood can vary from a small amount of blood-streaking sputum to massive bleeding with life-threatening consequences. The clinical diagnostic algorithm should aim to differentiate and identify the etiology of hemoptysis, since massive hemoptysis may result in a fatal outcome (1, 2).

The common causes of hemoptysis include pulmonary tuberculosis (TB), malignancy, bronchiectasis, chronic bronchitis, pneumonia, and fungal infections. The other rare causes are vasculitis due to collagen vascular diseases and hereditary hemorrhagic telangiectasia, essential cryoglobulinemia, Behcet's disease, acute lung transplantation rejection, drug-induced (e.g., chemotherapeutic agents, propylthiouracil), idiopathic pulmonary hemosiderosis, and toxin or inhalation injury (3).

About 30% of cases attending respiratory clinics and presenting with hemoptysis pose a problem in clinical diagnosis, and the histopathology plays a vital role in diagnosing these conditions. The condition can be localized or diffuse depending upon the underlying etiology (4). Intra alveolar hemorrhage, fibrin deposition, and hemosiderin-laden macrophages associated with or without capillaritis are the common findings observed in cases of diffuse alveolar hemorrhage (DAH) (5). Radiologically, DAH shows patchy/ diffuse bilateral areas of lung consolidation mainly involving the lower lobes and perihilar regions (6).

A literature search revealed only a few reports on using histopathological interpretation in diagnosing unknown causes of hemoptysis. This is vital to diagnose and treat the condition earlier, as it prevents disease progression, and constituted the reason for conducting the current study. This study aims to identify the various uncommon aetiologies of hemoptysis based on histopathological features and the pathologist's approach in diagnosing this condition.

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MATERIAL and METHODS

All lung biopsies, including lobectomy specimens received in the department of pathology, between Jan 2018 to Jan 2020 (2 years duration) were retrospectively reviewed. Of the received 243 specimens, 20 cases had hemoptysis with undetermined causes. The obtained specimens were derived from various procedures and included 117 transbronchial lung biopsies (TBLB), 62 endo bronchial lung biopsies (EBLB), 28 guided (Ultra sonogram /CT) biopsies, 19 thoracoscopic/Video-assisted (VATS) biopsies, and 17 lobectomies. The cases with a history of hemoptysis associated with common causes such as tuberculosis (TB), carcinoma, bronchitis, bronchiectasis, and interstitial lung diseases (ILD) were excluded from the study. The clinical data obtained were age, sex, presenting complaints, clinical diagnosis/differential diagnosis, and significantly associated co-morbidities. Laboratory investigations such as autoimmune workup (ANCA, ANA, etc.), sputum for culture & acid-fast bacilli (AFB), bronchoalveolar lavage (BAL) for culture and cytology, and other relevant information were collected. The findings of imaging (high-resolution computerized tomography - HRCT) and bronchoscopic findings were recorded if available (not always performed).

Ethical approval for this study was obtained from PSG Institute of Medical Sciences & Research (Approval number: 20/117, Date: 27.05.2020).

RESULTS

Out of 243 lung biopsies received, a total of 20 cases with symptoms of hemoptysis due to undetermined etiology were included in our study.

Histopathological Findings

Based on imaging and histopathological findings, the etiology of hemoptysis was divided into localized and diffuse/bilateral pulmonary involvement (Table I). Depending upon the histological findings, the causes of hemoptysis were further divided into

- Hemoptysis with capillaritis (Table II)
- Hemoptysis without capillaritis (Table III)
- Unusual tumors and tumor-like lesions (Table IV)
- Miscellaneous uncommon causes

Miscellaneous uncommon causes are listed below:

Pulmonary Alveolar Microlithiasis (n=1)

HRCT of a 34-year-old male who presented with hemoptysis showed bilateral ground-glass densities with septal calcification -? Interstitial lung disease. TBLB sections showed distended alveoli containing concentric lamellated, calcific material with few hemosiderin-laden macrophages.

Pulmonary Endometriosis (n=1)

A 43-year-old man with a history of cirrhosis secondary to hepatitis 'C' had recurrent episodes of hemoptysis. Bronchoscopy revealed a mucosal white patch in the left lingular segment. The biopsied lesion showed lung tissue intimately admixed with a focus of endometrial glands and stroma. CD 10 immunostaining of the stroma, as well as estrogen and progesterone receptor staining of the glandular component, were both consistent with endometriosis (Figure 1A-D).

Table I: Etiological classification based on imaging & histopathological findings.

Causes of hemoptysis			
Localized	**Number of cases**	**Diffuse /bilateral**	**Number of cases**
Pulmonary hamartoma	1	GPA	3
Aspergillosis	1	Silicosis	3
Myelolipoma	1	EGPA	2
Mucor	1	Invasive aspergillosis	1
Pulmonary endometriosis	1	Invasive mucor+aspergillosis	1
Mucoepidermoid carcinoma	1	Multiple metastases from a leiomyosarcoma	1
Probably benign mesenchymal neoplasm	1	PAM	1
		Pulmonary intravascular hemangioma	1

GPA: Granulomatosis with Polyangiitis / Wegener's, **EGPA:** Eosinophilic granulomatosis with polyangiitis / Churg-Strauss syndrome, **PAM:** Pulmonary alveolar microlithiasis.

Table II: Causes of hemoptysis with capillaritis.

	Granulomatosis with Polyangiitis (GPA)			**Eosinophilic granulomatosis with polyangiitis (EGPA)**	
	Case - 1	**Case-2**	**Case-3**	**Case 1**	**Case 2**
Age, sex	40, M	46, F	51, F	43, M	42, M
Complaints	Cough, hemoptysis, and dyspnoea				
Serology	Anti-CCP, PR3-ANCA&RF: positive	PR3-ANCA positive	PR3-ANCA positive	RF: positive	PR3-ANCA: weakly positive
Imaging studies	Bilateral nodules	Ground glass changes, calcified nodule	Bilateral consolidation with few nodules	Bilateral nodules	Bilateral nodular and cavitating lesions.
Type of biopsy	EBLB	CT guided biopsy	CT guided biopsy	TBLB	VATS lung biopsy
HPE findings: **Vascular changes:**	• Capillaritis, venulitis • Fibrinoid necrosis (Figure 2A)	Vague outlines of thrombosed blood vessels	Vasculitis and Fibrin thrombi (Figure 2C- D)	Eosinophilic infiltration of the vessel wall	• Necrosis of the vessel walls with inflammatory cells infiltration (Figure 3A-C) • Organizing, recanalizing and fibrin thrombi
Parenchymal changes	• Granulomatous inflammation with MNG cells (Figure 2B) • Interstitial inflammation	• Necrosis, karyorrhexis, hematoxyphilic nuclear dust • Neutrophilic micro abscess	• Necrosis, karyorrhectic debris • Granulomatous response with MNG cells • Neutrophilic micro abscess	• Eosinophilic microabscess • Palisading granuloma	• Intraalveolar hemorrhage (Figure 3D) • Eosinophilic microabscess, parenchymal necrosis • Perl's stain highlights the hemosiderin-laden macrophages (Figure 4)

MNG cells: Multinucleate giant cells, **HPE:** Histopathological examination, **EBLB:** Endobronchial lung biopsy, **CT:** Computed tomography, **VATS:** Video-assisted thoracoscopic surgery, **TBLB:** Transbronchial lung biopsy.

Table III: Causes of hemoptysis without capillaritis.

	Silicosis			**Fungal infection**			
	Case -1	**Case-2**	**Case-3**	**Case-1**	**Case-2**	**Case-3**	**Case-4**
Age, sex	61, M	54, M	58, M	28, M	56, M	45, F	15, F
Complaints	Cough, hemoptysis and dyspnoea.			Cough, hemoptysis, and dyspnoea			
Another relevant history	Exposure to silicon dust +			Post Koch's sequelae	DM, CKD	Post TB, DM.	DM, DKA^{+}, Mild PAH
Imaging studies	Bilateral lung nodules&hilar nodes	Calcified nodes	B/L nodules, calcification	NA	Cavitatory consolidation	B/L subpleural nodules	B/L cavitating consolidations
Type of biopsy	TBLB			Right upper lobe segmentectomy	EBLB	Right lower lobectomy	TBLB
HPE Findings	• Nodules of epithelioid and pigment laden histiocytes, predominantly around the vascular channels (Figure 5A,B)			• Dilated bronchi forming a cavity containing the fungal ball • Tissue, angioinvasion & infarct • Fibrosis, chronic granulomatous inflammation • Oxalate crystals (Figure 6A,B)			
Special study	Polarizer: birefringent crystals (Figure 5B inset)			GMS & PAS stains: septate, acute-angled branching hyphae of aspergillus	PAS & GMS stains: broad, aseptate and wide-angle branching hyphae of mucor	PAS & GMS stains: Tangles of fungal elements, consistent with aspergillus and mucor	

DM: Diabetes Mellitus, **CKD:** Chronic kidney disease, **TB:** Tuberculosis, **NA:** Not available, **DKA:** Diabetic ketoacidosis, **PAH:** Pulmonary artery hypertension, **B/L:** Bilateral, **HPE:** Histopathological examination, **EBLB:** Endobronchial lung biopsy, **TBLB:** Transbronchial lung biopsy, **GMS:** Grocott Methenamine Silver Stain.

Table IV: Unusual tumors and tumor-like lesions of the lung.

Diagnosis	Pulmonary hamartoma (n=1)	High-grade Spindle cell sarcoma - metastatic LMS (n=1)	Benign mesenchymal neoplasm (n=1)	Pulmonary myelolipoma (n=1)	Endobronchial mucoepidermoid carcinoma, high grade (n=1)
Age, sex	46, F	59, M	51, M	65, M	39, F
Complaints	Cough, hemoptysis, and dyspnoea				
Imaging studies	Solitary pulmonary nodule.	Nodular masses in both lungs - suggested metastases.	-	Endobronchial nodules -? TB/ sarcoidosis	-
Bronchoscopy	NA	NA	Irregularisation in left lingula? a blood clot? growth	-	Left main bronchus tumor with a possible endobronchial carcinoid.
Type of biopsy	Lobectomy	EBLB	EBLB	EBLB	Left Pneumonectomy
Gross	Glistening lobulated mass of 3x2.5 cm	-	-	-	Endobronchial ulcerated solid, yellow-white polypoidal mass
HPE findings	Circumscribed lesion of mature cartilage interspersed by adipose tissue, smooth muscle, and fibrovascular tissue. Foci of ossification, chondromyxoid area.	• Pleomorphic spindle cells • Coagulative necrosis. Positive for SMA and desmin.	• Polypoidal lesion • Fascicles of spindle cells • Cellular myxoid stroma. • Express Vimentin and SMA	• Circumscribed lesion of thin trabecular bone enclosing adipocytes and hematopoietic elements • MPO-highlighting the myeloid cells (Figure 7A-C).	• Infiltrating neoplasm, • Solid nests, cribriform pattern, and mucin filled acini. • Squamoid morules (Figure 8A,B). • Positive for Pan CK and P63

LMS: Leiomyosarcoma, **NA:** Not available, **SMA:** Smooth muscle actin, **MPO:** Myeloperoxidase, **HPE:** Histopathological examination, **EBLB:** Endobronchial lung biopsy, **TB:** Tuberculosis.

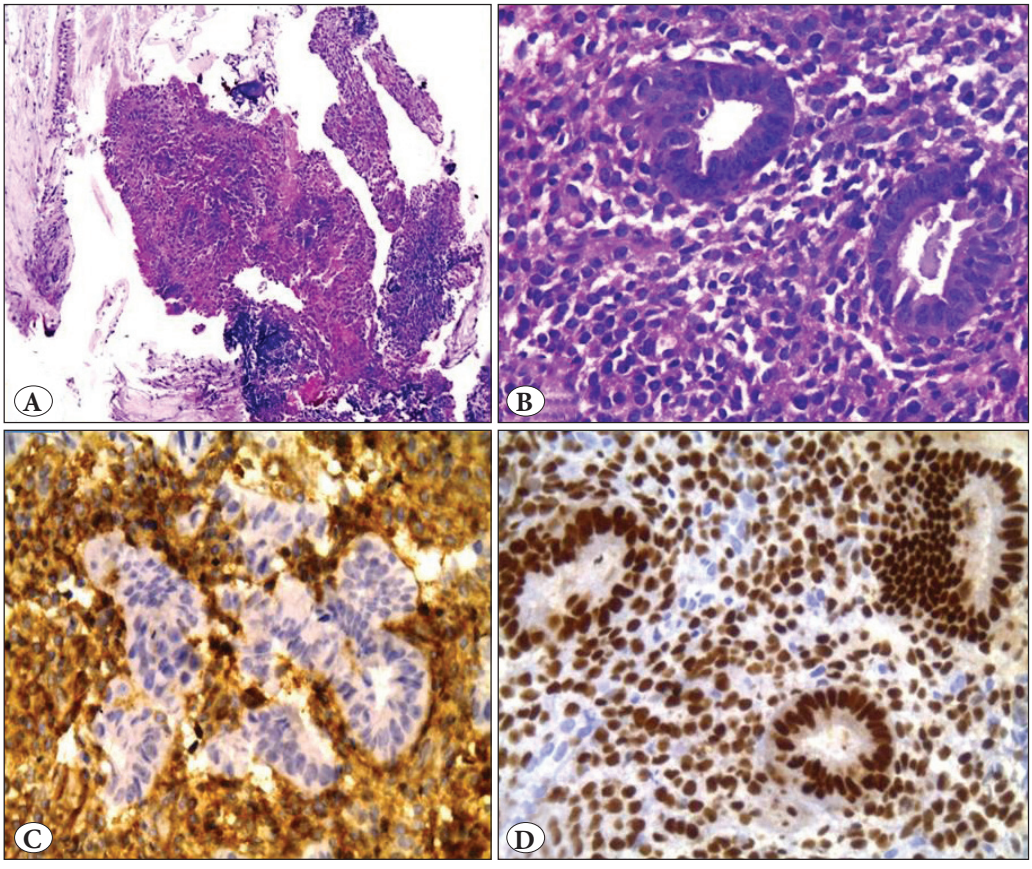


Figure 1: Bronchial endometriosis. **A)** Bronchial mucosa with a focus of endometrial glands and stroma (H&E; x4). **B)** High power view (H&E; x40). **C)** CD10 positive stromal cells (IHC; x40). **D)** ER positive epithelial & stromal cells (IHC; x40).

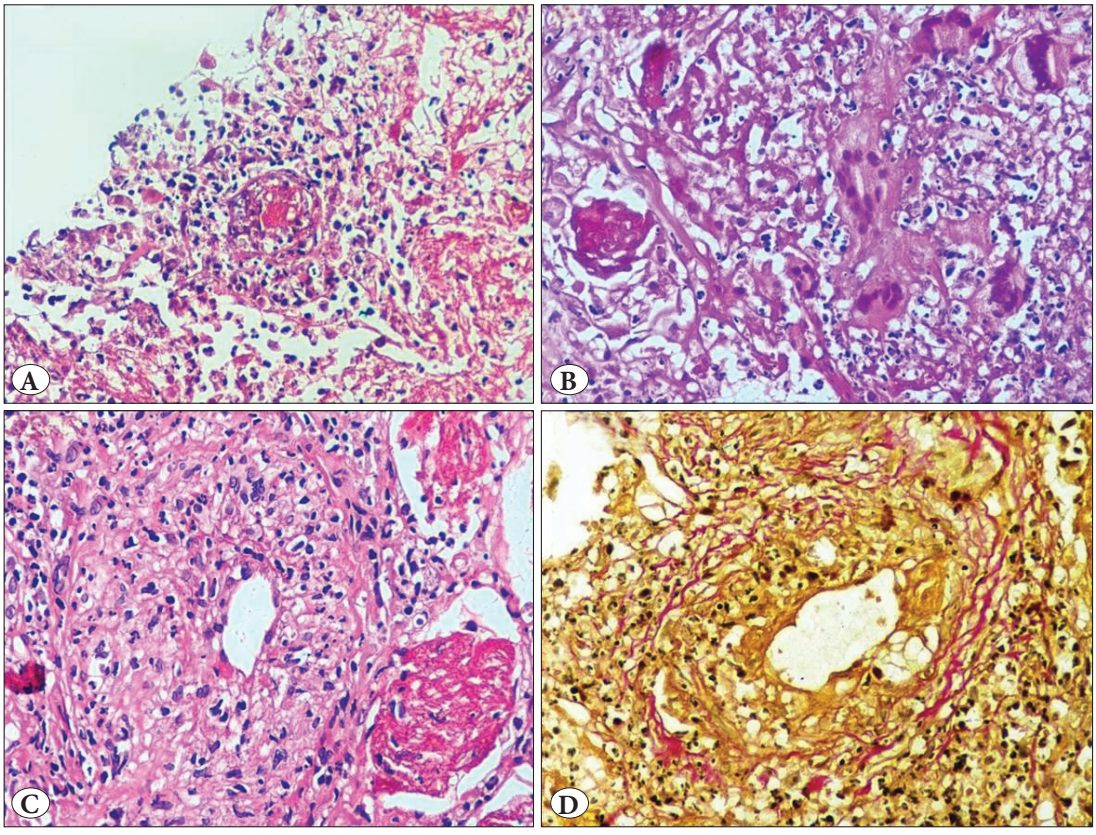


Figure 2: Granulomatosis with polyangiitis. **A)** Capillaritis with fibrin thrombi (H&E; x10). **B)** Scattered multinucleated giant cells, karyorrhectic debris & parenchymal necrosis (H&E; x40). **C)** Medium-sized vessel wall with neutrophilic infiltration and destruction (H&E; x10). **D)** Verhoeff-Van Gieson (VVG) stain highlights the outline of a vessel wall (VVG; x40).

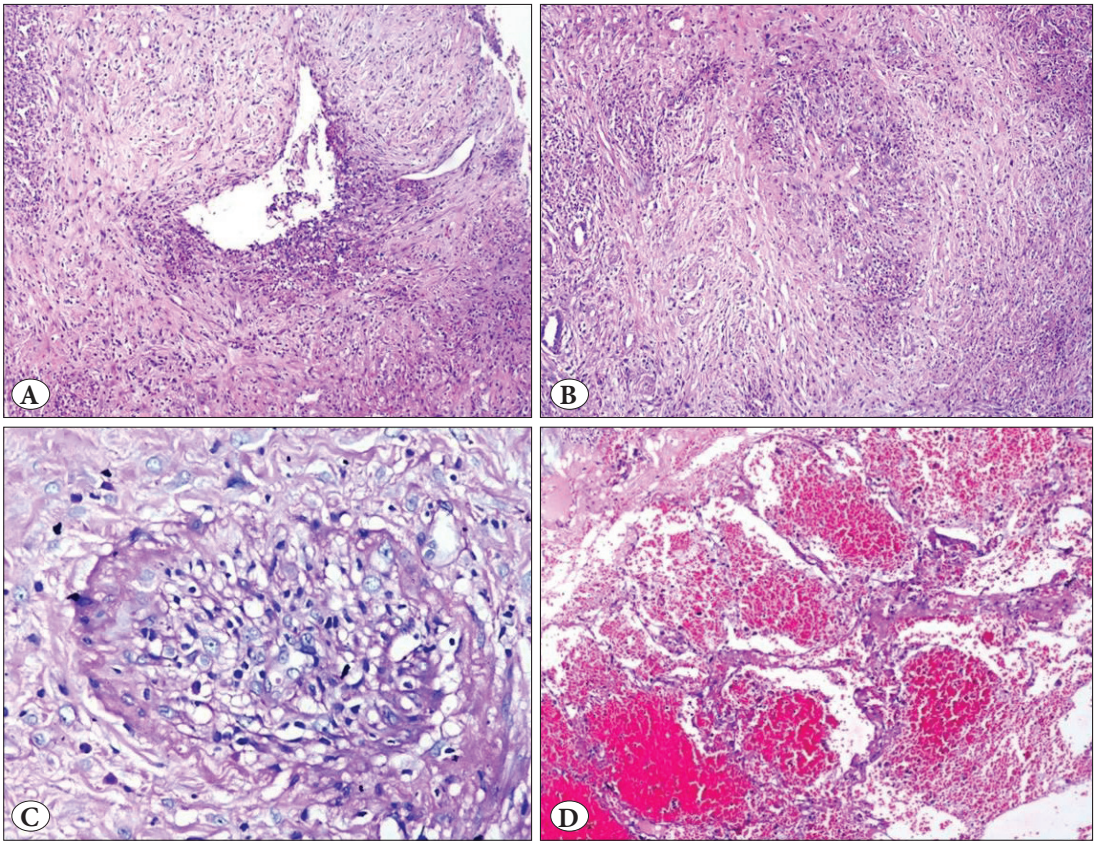


Figure 3: Eosinophilic granulomatosis with polyangiitis. **A-B)** A medium-sized artery infiltrated by eosinophils and histiocytes with partial destruction of a vessel wall (H&E; x10 & x20). **C)** (PAS stain; x40). **D)** Intra-alveolar hemorrhage (H&E; x10).

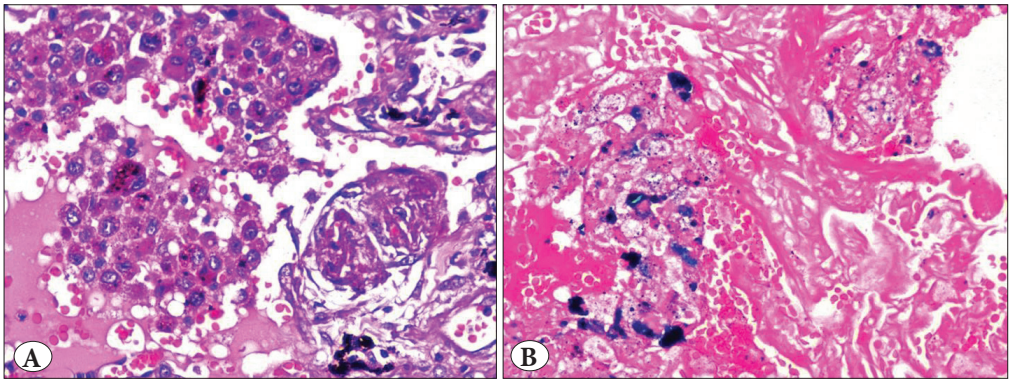


Figure 4: Hemosiderin-laden macrophages **A)** (H&E; x40). **B)** (Perl's stain; x40).

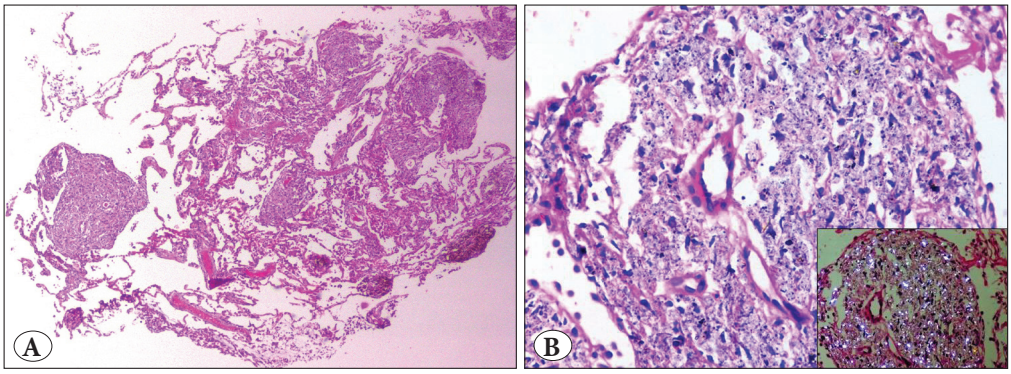


Figure 5: Silicosis. **A)** Multiple, patchy silicotic nodules (H&E; x10). **B)** Perivascular nodular collection of pigmented histiocytes (Inset-birefringent crystals under polarized light) (H&E; 40).

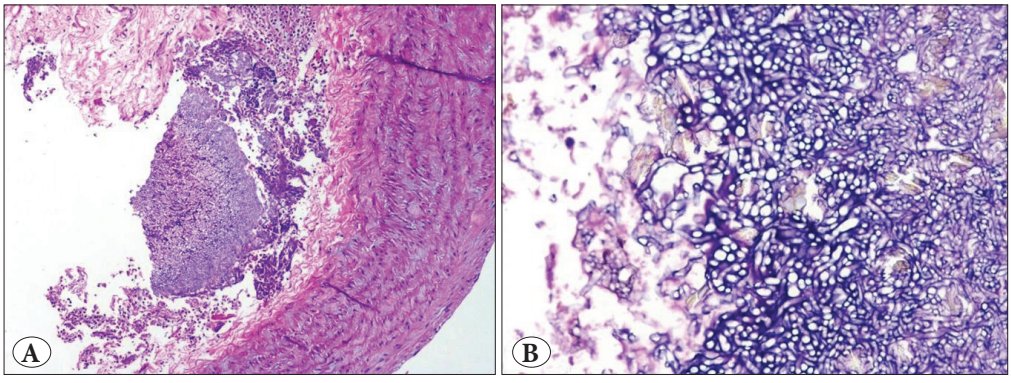


Figure 6: Fungal infection. **A)** Intravascular plugs of fungal organisms (H&E; x4). **B)** Septate, acute angle branching hyphal fungal organisms consistent with aspergillus with oxalate crystals (PAS; x10).

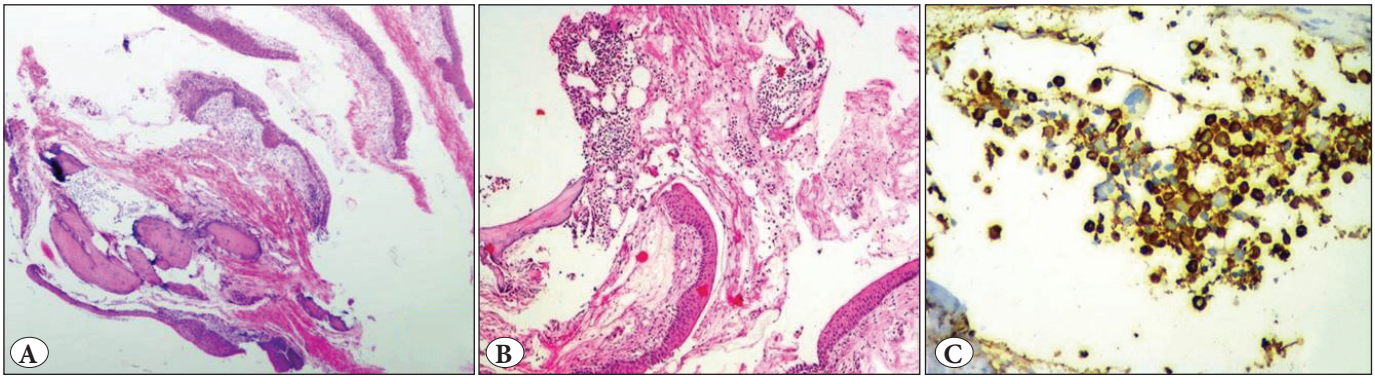


Figure 7: Myelolipoma. **A)** Bronchial mucosa with squamous metaplasia and a fairly circumscribed lesion composed of adipocytes admixed with hematopoietic elements (H&E; x4). **B)** High power view (H&E; x10). **C)** Myeloperoxidase stain (IHC; x10).

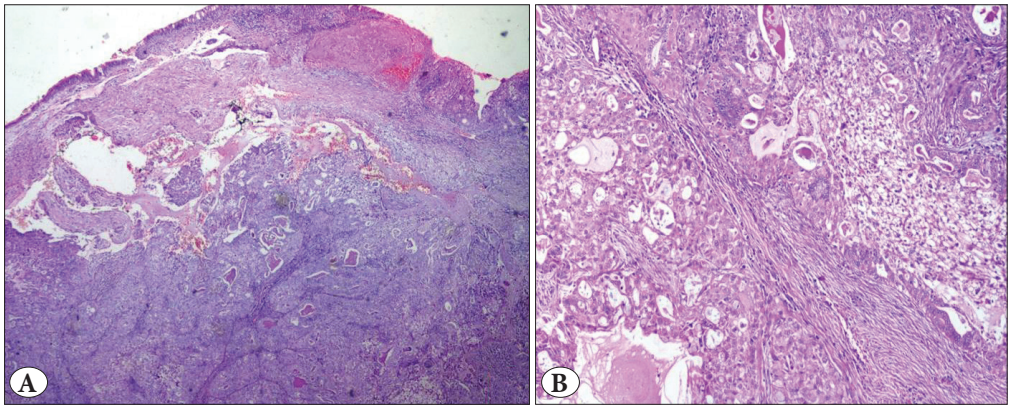


Figure 8: Endobronchial mucoepidermoid carcinoma. **A)** Showing admixture of squamous and glandular elements (H&E; x4). **B)** High power view (H&E; x10).

Pulmonary Intravascular Haemangioma (n=1)

HRCT of an elderly male showed multiple small nodules in both the lungs and a lobulated enhancing soft tissue lesion attached to the anterior wall of the right atrium-? Malignancy. TBLB sections showed lung parenchyma with medium to large-sized vessels containing an intraluminal, adherent lesion of tubular vascular spaces lined by endothelial cells. CD31 & CD34 immunostaining highlighted the endothelial cells. Since the right atrial mass could not be biopsied, the clinical/imaging findings were correlated and the final HPE diagnosis made was intravascular capillary haemangioma/?fragmented from cardiac haemangioma.

DISCUSSION

Haemoptysis is considered to be a serious medical condition and has a broad differential diagnosis. All cases with microscopic evidence of hemorrhage do not present with hemoptysis clinically. Similarly, all patients who presented with hemoptysis will not show alveolar hemorrhage histologically (4).

Haemoptysis with Capillaritis (Granulomatosis with Polyangiitis & Eosinophilic Granulomatosis with Polyangiitis)

Pulmonary vasculitis is characterized by inflammation of the small and medium-sized vessel wall, causing pulmonary hemorrhage. Imaging studies show multiple lung cavitary nodules/masses, which may mimic metastasis. Also in some conditions, the lung infiltrates are transitory making the diagnosis a challenging one (7).

Schnabel et al. stated that histopathological findings may vary depending upon the type of biopsy. They found out that the yield of guided lung biopsy is high when compared to TBLB since gross lesions are easily visible. Similarly, most of our cases had undergone guided biopsies and were adequate (8).

Travis et al. suggested that capillaritis is a morphologic marker of DAH in their study. They concluded that capillaritis and necrotizing granulomatous inflammation with mixed inflammatory cell infiltrate were pathognomonic features of GPA. Parenchymal necrosis, extravasated fibrin, and scattered multinucleate giant cells in a biopsy could suggest the diagnosis of a GPA even in the absence of granuloma. The destruction of the vessel wall can be highlighted by VVG elastic stains. However, correct sampling is essential for the maximum yield (9, 10).

The diagnosis of EGPA can be made if the biopsy shows granulomatous vasculitis with extravasated eosinophils and evidence of eosinophilic pneumonia (11). Correspondingly, three cases in our series showed capillaritis and the remaining two were diagnosed based on relevant clinical and imaging findings.

Haemoptysis without Capillaritis

Fungal Infection: Alveolar hemorrhage resulting from fungal infection is infrequent. The possible pathogenesis of hemoptysis in fungal infection could be 1) due to chronic inflammation as the granulation tissue lining the cavity is rich in capillaries, 2) these new vessels are rather fragile and can rupture easily, and 3) erosion of the blood vessels adjacent to the cavity (12). The presence of calcium oxalate crystals in a patient with a cavitary lesion will raise suspicion of a fungal infection and the pathologist should search for the same in the adjacent area (13). Likewise, our cases also had similar histology which would have led to hemoptysis.

Silicosis: An autopsy case study by Kyeong et al. revealed that unlike silicosis which affects mainly the lung parenchyma, silico - tuberculosis also affects the pulmonary vasculature causing hemoptysis (14). Our cases did not have evidence of tuberculosis. Streak-type hemoptysis seen in our cases could be explained by the destruction of small-sized blood vessels by fibrosis and chronic inflammation (15).

Unusual Tumors and Tumor-Like Lesions of the Lung

Primary Pulmonary Mesenchymal Tumors: Accounts for less than 1% of all lung malignancy. These tumors show lineage differentiation and can be diagnosed in the lung with similar histological and immunological criteria as in other sites (soft tissue) (16). Pulmonary hamartoma is the most common benign mesenchymal tumor of the lung, and is predominantly seen in older males**.** Geramizadeh et al. and Ahmed et al. in their study stated that the occurrence of hemoptysis in hamartoma is very rare and results from giant or endobronchial tumors causing erosion of the vessels (17, 18). In contrast to their findings, our patient was a middle-aged female with a small peripherally located pulmonary hamartoma.

According to Hashimoto et al., it is important to identify and differentiate metastatic mesenchymal tumors correctly, since most mesenchymal tumors presented as occult/unknown primary with pulmonary metastatic foci (19). Identically, one of our cases had metastatic LMS with a known diagnosis of primary LMS of the thigh.

Mucoepidermoid Carcinoma (MEC): MEC is a rare salivary gland type endobronchial tumor with mucous secreting cells, squamous cells, and intermediate cells. A definitive diagnosis requires bronchoscopy and representative biopsy as this tumor exhibits morphological heterogeneity. It can be misdiagnosed as primary non-small cell lung carcinoma if the biopsy is inadequate (20, 21). The diagnosis of this rare neoplasm was possible since ours was a pneumonectomy specimen.

Myelolipoma: Pulmonary myelolipoma is distinctly unusual and rarely presents with hemoptysis. Embolic origin, reticuloendothelial cell metaplasia, and proliferation of hematopoietic stem cells are possible mechanisms of histogenesis. Tumour arising adjacent to the bronchial cartilage may show bony trabeculae and this explains the possible histogenesis of the metaplastic theory (22). Our case also revealed a similar histology.

Miscellaneous Uncommon Causes

Pulmonary Endometriosis: Pulmonary endometriosis is a rare form of extra pelvic endometriosis, occasionally reported in men. Cases of male endometriosis have typically been linked to increased circulating estrogen levels. Peripheral conversion of androstenedione and testosterone to the circulating estrogens was noted in men with cirrhosis of the liver (23). Our patient had a history of cirrhosis, possibly leading to an altered hormonal state that interacted with a reactive/metaplastic process.

Pulmonary Alveolar Microlithiasis (PAM): PAM is a rare autosomal recessive lung disease characterized by the accumulation of concentrically laminated calcospherites within the alveolar spaces. Lauta in his study stated that there is a striking clinico-radiologic disparity in this condition (24). Since our patient did not have other system involvement, the possibility of PAM over secondary calcification was favored by correlating the clinical and imaging features.

Pulmonary Intravascular Haemangioma: The co-existence of cardiac and pulmonary haemangioma is very rare and very few reports of such an association have been described so far. Imaging studies may help in diagnosing cardiac haemangioma while histopathology is essential for confirmation. Complete excision is the treatment of choice in resectable cardiac haemangioma and solitary pulmonary haemangioma (25). Since the general condition of the patient was poor, resection was not possible in our case. Fragmented emboli from a cardiac haemangioma could also be considered as a differential.

In conclusion, in immunologic causes of hemoptysis, histopathological findings may support the diagnosis in correlation with the clinical/imaging features. However, in cases of non-immunologic causes of hemoptysis, a histopathologic examination is mandatory over clinical/imaging studies. The cases presented here are incredibly rare diseases with an uncommon presentation. The diagnosis is often missed or delayed, due to a low index of suspicion. Histopathological examination is imperative in unexplained cases of hemoptysis as it plays a vital role in therapy decisions.

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CONFLICT of INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Haro Estarriol M, Vizcaya Sanchez M, Jimenez Lopez J, Tornero Molina A. Etiology of hemoptysis: Prospective analysis of 752 cases. Rev Clin Esp. 2001;201:696-700

2. Lee BR, Yu JY, Ban HJ, Oh IJ, Kim KS, Kwon YS, Kim YI, Kim YC, Lim SC. Analysis of patients with hemoptysis in a tertiary referral hospital. Tuberc Respir Dis (Seoul). 2012;73:107-14.
3. Quadrelli S, Dubinsky D, Solis M, Yucra D, Hernandez M, Karlen H, Brigante A. Immune diffuse alveolar hemorrhage: Clinical presentation and outcome. Respir Med. 2017;129:59-62.
4. Colby TV, Fukuoka J, Ewaskow SP, Helmers R, Leslie KO. Pathologic approach to pulmonary hemorrhage. Ann Diagn Pathol 2001;5:309- 19.
5. Park MS. Diffuse alveolar hemorrhage. Tuberc Respir Dis (Seoul). 2013;74:151- 62.
6. Castaner E, Alguersuari A, Gallardo X, Andreu M, Pallardo Y, Mata JM, Ramírez J. When to suspect pulmonary vasculitis: Radiologic and clinical clues. Radiographics. 2010;30:33-53.
7. Lynch JP, Leatherman JW. Alveolar hemorrhage syndromes. In: Fishman, editor. Pulmonary Diseases and Disorders. New York: McGraw Hill; 1998. 1193-1210.
8. Schnabel A, Holl-Ulrich K, Dalhoff K, Reuter M, Gross WL. Efficacy of transbronchial biopsy in pulmonary vasculitides. Eur Respir J 1997;10:2738-43.
9. Travis WD, Colby TV, Lombard C, Carpenter HA. A clinicopathologic study of 34 cases of diffuse pulmonary hemorrhage with lung biopsy confirmation. Am J Surg Pathol. 1990;14:1112-25.
10. Travis WD, Hoffman GS, Leavitt RY, Pass HI, Fauci AS. Surgical pathology of the lung in Wegener's granulomatosis. Review of 87 open lung biopsies from 67 patients. Am J Surg Pathol. 1991;15:315- 33.
11. Suster S, Moran CA. Biopsy interpretation series. Biopsy interpretation of lung. 1st ed. Epstein JI, editor. Philadelphia: Lippincott Williams and Wilkins; 2013.
12. Corrin B, Nicholson AG. Pathology of lungs. 3rd ed. Churchill Livingstone: Elsevier; 2011.
13. Mukhopadhyay S. Nonneoplastic pulmonary pathology. An algorithmic approach to histologic findings in the lung. 1st ed. Erika D, editor. United Kingdom: Cambridge University Press; 2016.
14. Kyeong HE, Cheong H, Kim HJ, Choi YS. Pulmonary hemorrhage with progressive massive fibrosis in a silicosis patient: An autopsy case. Korean J Leg Med 2012;36:186-9.
15. Anna-Luise A. Katzenstein MD. Diagnostic Atlas of Non-Neoplastic Lung Disease: A Practical Guide for Surgical Pathologists. 1st ed. David DA, editor. New York: Springer; 2016.
16. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of Tumours of the Lung, Pleura, Thymus, and Heart. 4th ed. International Agency for Research on Cancer: Lyon; 2015.
17. Geramizadeh B, Mottavvas M, Zeyaian B, Amirian A. Giant hamartoma of lung presented with massive hemoptysis: A rare case report and review of the literature. Rare Tumors. 2019; 11:1-4.
18. Ahmed S, Arshad A, Mador MJ. Endobronchial hamartoma; A rare structural cause of chronic cough. Respir Med Case Rep. 2017;22:224-7.
19. Hashimoto H, Tsugeno Y, Sugita K, Inamura K. Mesenchymal tumors of the lung: Diagnostic pathology, molecular pathogenesis, and identified biomarkers. J Thorac Dis. 2019;11:9- 24.
20. du Toit-Prinsloo L, Bunn BK. Massive hemoptysis due to primary mucoepidermoid carcinoma of the lung in a 12-year-old. Forensic Sci Med Pathol. 2016;12:380- 3.
21. El-Sameed YA, Al Marzooqi SH. Primary mucoepidermoid carcinoma of the lung. J Bronchology Interv Pulmonol. 2012;19:203-5.
22. Sato K, Ueda Y, Katsuda S, and Tsuchihara K: Myelolipoma of the lung: A case report and brief review. J Clin Pathol. 2007;60: 728-30.
23. Jabr FI, Mani V. An unusual cause of abdominal pain in a male patient: Endometriosis. Avicenna J Med. 2014;4:99-101.
24. Lauta VM. Pulmonary alveolar microlithiasis: An overview of clinical and pathological features together with possible therapies. Respir Medi. 2003;97:1081-85.
25. Wang C, Chen H, Sun L, Mei Y. Cardiac cavernous haemangioma coexisting with pulmonary cavernous hemangiomas and giant hepatic hemangioma. Ann Thorac Surg. 2017;103:149-52.

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IMAGES

Chediak–Higashi Syndrome in Accelerated Phase

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A two-month-old boy, born to consanguineous parents, presented with high grade fever for 5 days. On examination he was febrile, pale with generalized hypo pigmentation of skin and hair, hepatosplenomegaly and cervical lymphadenopathy. He had a past history of recurrent respiratory tract infections. Splenomegaly of 2.5 cm was noted at birth. Complete blood count—total count 3.1 × 10^3/µl, haemoglobin 7.4 g/dl, and platelet count 24 × 10^3/µl. Primary blood and urine cultures were sterile. Peripheral smear showed pancytopenia with large eosinophilic inclusions in the lymphocytes (Fig. 1). Bone marrow revealed large coarse azurophilic granules in the cytoplasm of many of the myeloid precursors and mature granulocytes including eosinophils (Figs. 2, 3).

Erythrophagocytosis was evident in the marrow histiocytes (Fig. 4). Serum iron was 28 µg/dl, ferritin >2000 ng/ml and triglycerides were 325 mg/dl. With the presence of six of the eight diagnostic criteria for HLH being present i.e. fever, splenomegaly, pancytopenia, hypertriglyceridemia, hyperferritenemia and haemophagocytosis in the bone marrow, a diagnosis of Chediak–Higashi syndrome with haemophagocytic lymphohistiocytosis (accelerated phase) was made. The child was treated with IV ceftriaxone and vancomycin. However his condition deteriorated and he expired.

Chediak–Higashi syndrome (CHS) is a rare, autosomal recessive disorder that affects multiple systems of the body mainly the immune system [1]. The clinical manifestations of

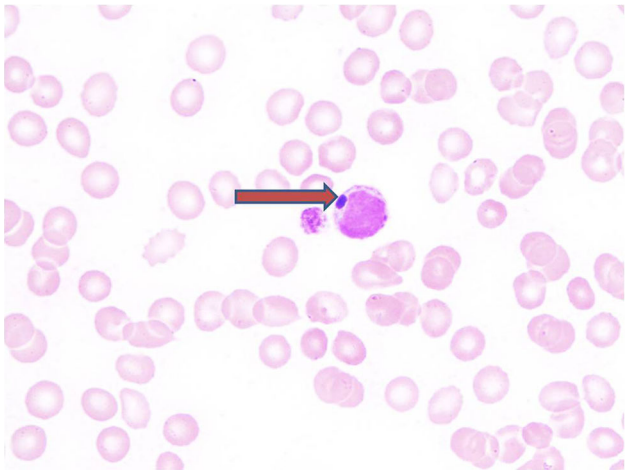


Fig. 1 Lymphocyte inclusion (indicated with *red arrow*)

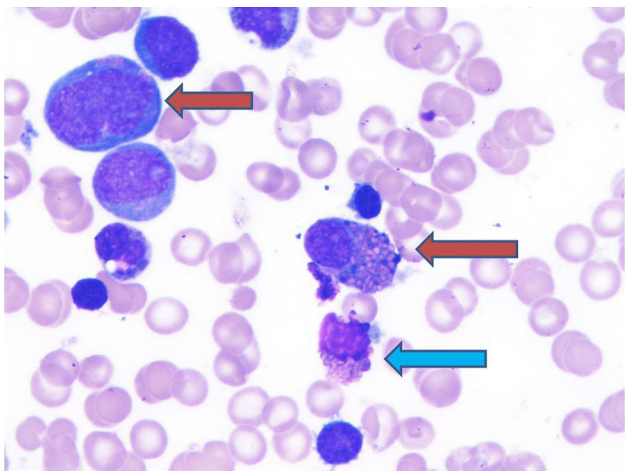


Fig. 2 Large coarse granules are present in the cytoplasm of the myeloid precursor (indicated with *red arrow*) and eosinophils (indicated with *blue arrow*)

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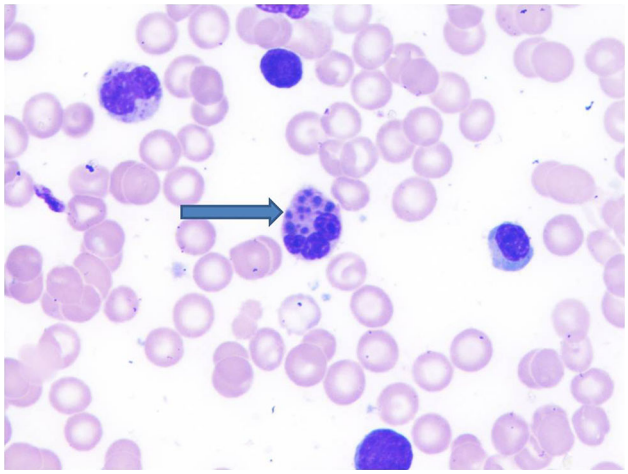


Fig. 3 Giant granules in the cytoplasm of neutrophils (indicated with *blue arrow*)

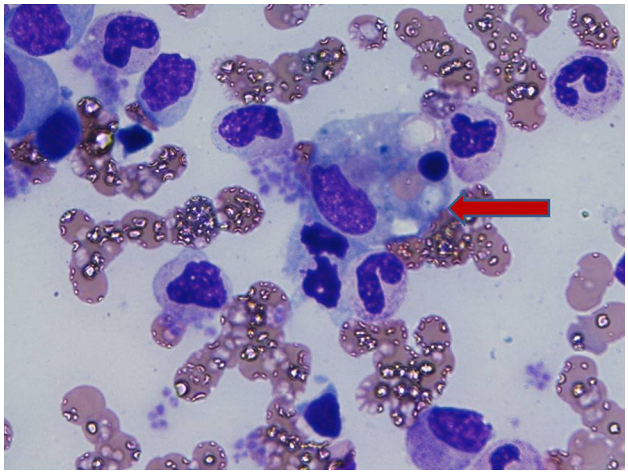


Fig. 4 Erythrophagocytosis (indicated with *red arrow*)

CHS in accelerated phase include high fever, neurological abnormalities, lymphadenopathy, hepatosplenomegaly, pancytopenia, abnormal giant granules in peripheral blood and bone marrow, lymphohistiocytic infiltration in liver, spleen and lymphnodes [2].The only treatment is allogenic haematopoietic stem cell transplantation (HSCT). The prognosis is poor [3].

Compliance with ethical standards

Conflict of interest Authors declared that they have no conflicts of interest.

References

1. Ward DM, Shiflett SL, Kaplan J (2002) Chediak–Higashi syndrome: a clinical and molecular view of a rare lysosomal storage disorder. Curr Mol Med 2(5):469–477
2. Bouatay A, Hizem S, Tej A, Moatamri W, Boughamoura L, Kortas M (2014) Chediak–Higashi syndrome presenting in accelerated phase: a case report and literature review of the literature. Indian J Hematol Blood Transfus 30(1):223–226
3. Kaplan J, De Domenico I, Ward DM (2008) Chediak–Higashi Syndrome. Curr Opin Hematol 15(1):22–29

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SHORT COMMENTARY

Enterobius vermicularis Infection of the Uterine Endometrium in an Infertile Female

Hema Rajesh[1] · Balu Kuppusamy[2] · Chaitra Venkataswamy[3] · Nidhya Ganesan[3]

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Introduction

Enterobius vermicularis is the most common human helminth infecting the intestine. Due to its low pathogenicity, infection is usually asymptomatic. Extraintestinal infections of *E. vermicularis* are extremely rare. The commonest extraintestinal site of infection is the female genital tract [1]. The male worm dies in the bowel during copulation, and the gravid female worm can migrate from the perianal region to the vagina and can ascend and reach peritoneum through the uterine cavity and fallopian tube. Various reports are available in the literature of the worm in the vagina, myometrium, endometrium, fallopian tube, ovary and pelvic peritoneum as well as an interesting incident of invasion of a human embryo [2–4].

Enterobius vermicularis infection is rarely reported to be associated with infertility. We report the detection of uterine endometrial *E. vermicularis* infestation in a woman who presented with primary infertility and subsequently conceived after treatment for the infection.

Case Report

A 38-year-old woman presented to our fertility clinic for evaluation of primary infertility. She was married for 15 years and had regular menstrual cycles. She had twice attempted intrauterine insemination unsuccessfully elsewhere. A hormonal assay showed normal results (FSH: 3.2 mIU/mL, LH: 5.5 mIU/mL, TSH: 1.6 mIU/mL, PRL: 7.03 ng/Ml and AMH: 1.2 ng/mL). An ultrasound evaluation revealed normal-appearing uterus and ovaries and a small right adnexal cyst. A sonohysterogram showed a normal uterine cavity, and a hysterosalpingogram confirmed the patency of both fallopian tubes. A semen analysis of her husband was also within normal limits.

An informed consent was obtained from the patient. She subsequently underwent hysterolaparoscopy. Laparoscopy revealed normal-appearing uterus and ovaries and a small fimbrial cyst which was excised. Histopathology evaluation suggested a paratubal cyst. On hysteroscopy, the uterine cavity appeared adequate. The endometrium, however, was looking irregular and polypoidal. Endometrial sampling was done and sent for histopathological evaluation which demonstrated the larvae of *Enterobius vermicularis* embedded within the endometrium (Figs. 1, 2). She was treated with a single dose of 400 mg of albendazole which was repeated after 2 weeks. In view of prolonged infertility and advanced age of the female, in vitro fertilization (IVF) was offered which resulted in a successful conception. She is into the sixth month of pregnancy at the moment.

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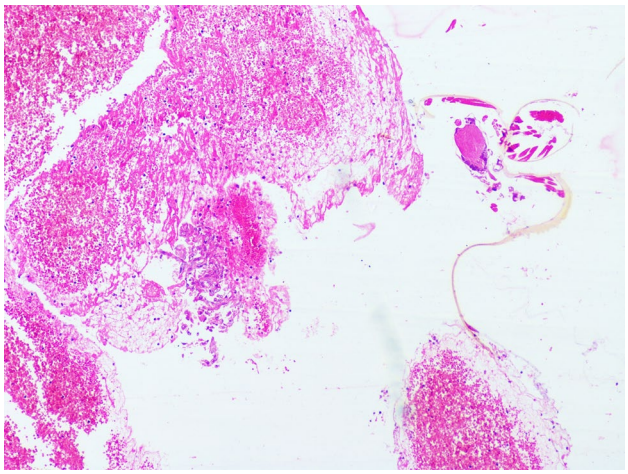


Fig. 1 Hematoxylin & eosin stain of the endometrial sample showing *E. vermicularis* larva

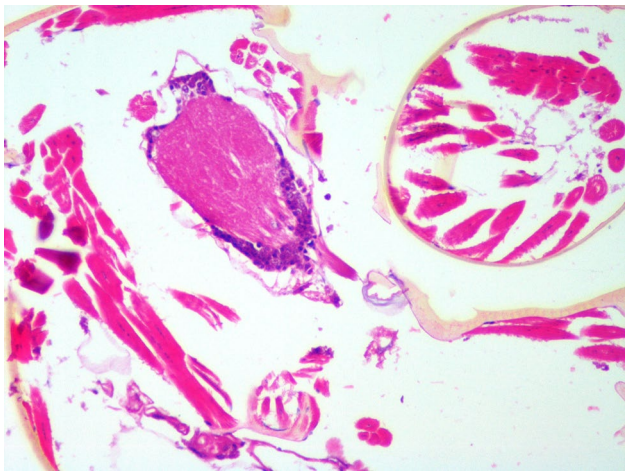


Fig. 2 Hematoxylin & eosin stain of the endometrial sample showing the larva of *E. vermicularis* with its outer chitinous layer and internal organs

Discussion

Enterobiasis caused by *E. vermicularis* is one of the most common helminthic infestations of the cecum and adjacent gut. Ingestion of eggs present in the contaminated hands or food results in infection by this pinworm [2]. Scratching the perianal skin where the female worm has laid eggs results in hand contamination. The gravid female worm can migrate from the perianal area to the vagina and ascend through the fallopian tube to the peritoneum [3]. The worm can also pass through the intestinal wall and cause pelvic peritoneal granuloma. Such navigation by the larval and adult parasite can result in fallopian tube infiltration, tubo-ovarian abscess, salpingooophoritis or granulomata of the vulva, vagina, uterus, fallopian tubes or ovaries and rarely destruction of the human embryo [4].

Enterobius vermicularis granulomas do not usually cause clinical problems and are incidental findings. They have been reported to cause vulvovaginitis and can masquerade as cervical and endometrial carcinoma. Involvement of the pelvic peritoneum and the fallopian tube resulting in infertility has been reported [5, 6]. Ours is the first instance of uterine endometrial infection with the worm resulting in infertility. Since the patient has never undergone IVF before, the causality of this association cannot be established. The presence of unexplained infertility and its reversal after treatment of worm infestation may suggest causality. The diffuse endometrial inflammation caused by the worm could interfere with implantation and thus cause infertility. The subtle uterine irregularity was visible only on hysteroscopy.

Our case highlights the importance of ectopic enterobiasis in female reproductive ill health, especially in the tropical countries. In addition to the previously reported instances of ectopic enterobiasis-related pelvic inflammatory disease and tubal infection resulting in infertility, uterine endometrial involvement by the worm also seems to be another potential cause for infertility. Co-infection with other bacteria from the intestine and skin is another major concern as they seem to move to these sites along with the pinworm and contribute toward infection and inflammation [7]. We stress on the importance of close observation of the uterine cavity through hysteroscopy in all cases of prolonged unexplained infertility. An endometrial sampling can identify the pinworm infection and allow treatment which might restore fertility.

Compliance with Ethical Standards

Conflict of interest None.

Ethical Statement This work has not been published before; it is not under consideration for publication anywhere else; its publication has been approved by all co-authors, as well as by the responsible authorities.

References

1. Sizer AR, Nirmal DM, Shannon J, et al. A pelvic mass due to infestation of the fallopian tube with *Enterobius vermicularis*. J Obstet Gynaecol. 2004;24:462–3.
2. Erhan V, Zekioglu O, Ozdemir N, et al. Unilateral salpingitis due to *Enterobius vermicularis*. Int J Gynecol Pathol. 2000;19:188–9.
3. Smolyakov R, Talalay B, Yanai-Inbar I, et al. *Enterobius vermicularis* infection of female genital tract: a report of three cases and review of the literature. Eur J Obstet Gynecol Reprod Biol. 2003;107:220–2.
4. Mendoza E, Jorda M, Rafel E, et al. Invasion of human embryo by *Enterobius vermicularis*. Arch Pathol Lab Med. 1987;111:761–2.
5. Neri A, Tadir Y, Grausbard G, et al. *Enterobius (Oxyuris) vermicularis* of the pelvic peritoneum—a cause of infertility. Eur J Obstet Gynecol Reprod Biol. 1986;23:239–41.
6. Young C, Tataryn I, Kowalewska-Grochowska KT, et al. *Enterobius vermicularis* infection of the fallopian tube in an infertile female. Pathol Res Pract. 2010;206(6):405–7.
7. Siteti MC, Injete SD, Wanyonyi WA. Helminthiases: a neglected cause for reproductive ill-health and social stigma. Parasitol United J. 2015;8:87–94.

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Case Report

Pathology Section

Disseminated Histoplasmosis in an Immunocompetent Patient Diagnosed on Bone Marrow Aspirate – A Rare Presentation from a Non-Endemic Area

NIDHYA GANESAN[1], REETIKA SHARMA[2], MANJIRI DILIP PHANSALKAR[3], RENUG'BOY VARGHESE[4]

ABSTRACT

Histoplasmosis causing systemic fungal infection is commonly seen in endemic areas. In India, disease prevalence is more in eastern part of the country and there have been very few reports from southern part of India. The occurrence of disseminated histoplasmosis in immunocompetent individual is rare. We report a case of disseminated histoplasmosis in an immunocompetent individual with no underlying risk factors. The disease was not suspected clinically and was diagnosed by bone marrow aspirate incidentally.

Keywords: Bone marrow aspirate, *Histoplasma capsulatum*, Immunocompetent, Incidental finding

CASE REPORT

A 55-year-old female patient hailing from Kumbakonam (Tamilnadu) was admitted to our hospital with complaints of abdominal pain and progressive abdominal distention for the last one month. She also gave a history of fever on and off for the last three months. She had undergone aortic valve replacement for calcific aortic valve stenosis three years ago. There was no history of travel to the endemic areas. Her general physical examination revealed pallor and abdominal distention. There was no hepatosplenomegaly. Rest of the general and systemic examination was within normal limits. Her Laboratory results were as follows, Hemoglobin-10.4 gm/dl, Total leukocyte count - 12,500 cells/cumm; Polymorphs - 75%, Lymphocyte - 22%, Eosinophil -2%, Monocyte - 1%. Erythrocyte sedimentation rate - 22. Liver function test, renal function test and serum electrolytes were within normal limits. She was also tested for Malaria and Dengue antigen and was found negative.

Serological tests for Human Immunodeficiency Virus (HIV), Syphilis and Antinuclear Antibody (VDRL and ANA) were negative. Urine routine and culture reports were also normal. Her chest radiograph was within normal limits. Contrast Enhanced Computed Tomography (CECT) abdomen showed massive ascites and no organomegaly.

Her blood culture, both aerobic and anaerobic, showed no growth. Sputum for Acid Fast Bacilli (AFB) also tested negative. Stool for occult blood was found to be negative.

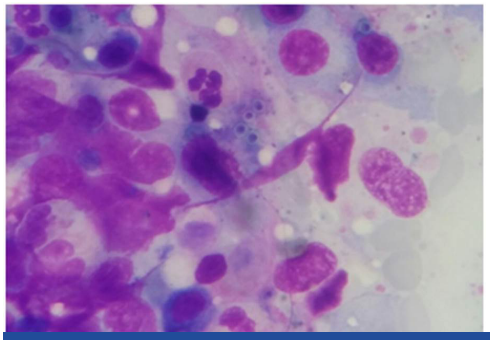
She underwent ascitic fluid aspiration twice and the sample was sent for cytology. It showed reactive mesothelial cells with few macrophages and no malignant cells/organisms. Biochemical evaluation of the fluid revealed exudative effusion.

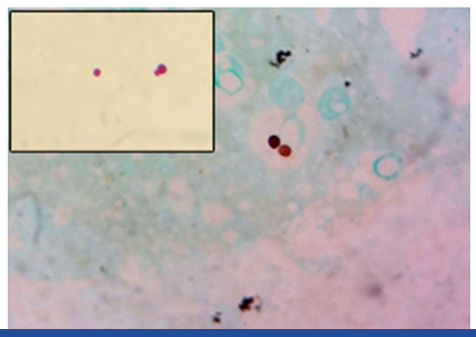
In view of her age and persisting ascites, a high index of suspicion for malignancy was noted and she underwent a bone marrow aspiration (BMA) and biopsy. BMA showed normocellular marrow with myeloid: erythroid ratio being 2:1. Erythroid series showed normoblastic maturation. Myeloid and megakaryocytic series were within normal limits. Plasma cells were increased in number (8%). In addition there were extracellular organisms measuring 3-4 microns in diameter, singly scattered as well as in groups [Table/Fig-1]. Some of them were showing narrow based budding and were surrounded by a clear space resembling *Histoplasma capsulatum*. This was confirmed by special stains (PAS, GMS & Perl's Stain) [Table/Fig-2,3]. Retrospectively we searched for the organisms in buffy coat and it also showed the presence of the organism. Patient was given injection Amphotericin B 0.7 mg for two weeks and tablet Itraconazole 200mg. Patient improved symptomatically and was advised to continue itraconazole for 12 months. Patient did not come for checkup after 6 months possibly due to relief of the symptoms.

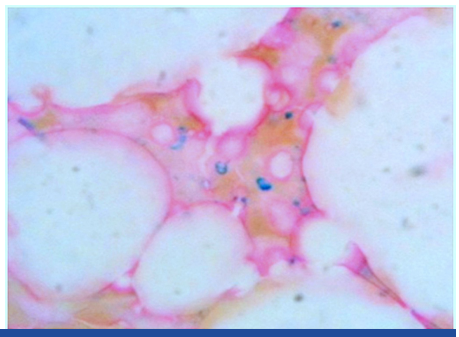
DISCUSSION

Histoplasmosis is endemic in the U.S (Ohio and Mississippi valley). It has also been reported worldwide in countries such as Africa, sub –Saharan Africa, South East Asia and India [1].

It is a dimorphic fungus i.e. it can exist as mycelial form (environment) and yeast form (inside the body). The transmission is through aerosols and not through person to person contact [1]. Our patient







[Table/Fig-1]: Bone marrow aspirate showing extracellular 3-4um sized organisms showing halo around these (MGG, 100X) **[Table/Fig-2]:** Encapsulated brown to black coloured structures (GMS, 100X); Inset shows PAS positive structures one of them showing narrow based budding (PAS, 100X) **[Table/Fig-3]:** These organisms showed positivity to perl's stain (Perls stain, 40X) (MGG-May Grunwald Giemsa, GMS- Gomori Methamine Silver, PAS- Periodic Schiff)

was exposed to bird droppings and this might be the probable cause for her to develop the disease. The clinical manifestations vary from asymptomatic to acute or chronic pulmonary histoplasmosis and disseminated histoplasmosis (spread to other organs and bone marrow). The progressive disseminated histoplasmosis usually affects the immunocompromised patients particularly those affected with HIV. It indicates impaired cell mediated immunity [1,2].

In India the first case of disseminated histoplasmosis (DH) was reported by Panja and Sen in 1954. There have been very few reports of disseminated histoplasmosis in immunocompetent individuals from non-endemic areas (South India) [3]. Histoplasmosis in immunocompetent individual is usually asymptomatic and sometimes manifest as minor illness such as cough, fever and chest pain. Chest radiograph shows hilar/mediastinal lymphadenopathy. These changes may mimic tuberculosis and may mislead the clinician [4,5]. Our patient had no pulmonary symptoms and had a normal chest x-ray which rules out pulmonary histoplasmosis. Acute pulmonary histoplasmosis is caused due to intense exposure to spores and can lead to fulminant acute form with shock and increased mortality.

The spores through the aerosol route are inhaled and deposited in the alveoli. The germinated spores are ingested by the pulmonary macrophages and spread to hilar and mediastinal lymph nodes and can spread to other organs via blood stream resulting in disseminated histoplasmosis [5].

The estimated incidence of disseminated histoplasmosis in healthy adults is approximately 1 per 2000 cases [6]. The study of disseminated histoplasmosis from South India was reported by Subramanian et al., in 2005 which included 19 patients in which only 4 patients were immunocompetent and only one was from southern region of India (Tamil nadu). BMA were done in 11 out of 19 patients and positive reports were seen only in 7 patients. In their study they stated that diagnosis of histoplasmosis by histopathology/ culture from bone marrow, adrenal, lymph node is confirmatory [1]. Deodhar et al., (2013) in their study on DH found that 6 patients out of 37 were immunocompetent. This study indicates the rise of presentation of DH in immunocompetent individual [7]. Histoplasmosis may cause ascites [5,8,9] due to peritonitis [8]. This might be the cause for developing ascites in our patient.

The diagnosis of organisms by means of culture testing takes 2 to 6 weeks and may not be an option in acute fulminant form which needs rapid interpretation and treatment and also in cases of self-limited disease demonstration of organism is difficult in culture [4].

Bone marrow examination has a 75% sensitivity in diagnosing fungal stains in HIV patients [3,10]. The differential diagnosis considered are *Leishmania, Candida, Cryptococcus, Blastomycosis and Pneumocystis carnii*. Amastigote form of leishmania may resemble the yeast form of histoplasmosis but can be differentiated by bar shaped kinetoplast and negative fungal stain. *Cryptococcus* and *pneumocystis carnii* are usually larger (2-20 microns) than histoplasmosis and present extracellularly. Blastomycosis has broad based budding when compared to histoplasmosis. The demonstration of histoplasmosis in Perl's Prussian blue iron stain was reported by Caldwell in 1982. *Candida* and *Cryptococcus* usually don't stain with Prussian blue stain [11]. Our case showed positive staining in Prussian blue stain and confirms the diagnosis.

Other diagnostic modalities include histoplasmin skin test which is due to delayed hyper sensitivity reaction, antigen detection from serum or urine and serum antibody assay by complement fixation and immune diffusion [12].

Amphotericin B is the drug of choice for this disease followed by azole groups. Lifelong treatment is required in HIV patients as relapse rates is more in these groups [1,13]. Our patient was given Inj. Amphotericin B 0.7 mg/kg for 2 weeks and she was continued with oral Iatraconazole 200mg thrice daily for 0ne year.

This case warrants discussion due to the nature of presentation i.e. from a non-endemic area (South India) in an immunocompetent individual who on presentation had no or minimal features of histoplasmosis which on contrary is common in most of the presented case series till date [14].

CONCLUSION

Awareness of histoplasmosis is important as this disease is still rare in non-endemic areas of India, has varying clinical course and its features overlap with those of other systemic illnesses including tuberculosis and malignancy. Presence of DH has to be borne in mind while treating spectrum of patients who are immunocompetent and additional testing methods have to be employed (BMA) to rule out histoplasmosis as early diagnosis and treatment is vital in this otherwise fatal condition.

REFERENCES

[1] Subramanian S, Abraham OC, Rupali P, Zachariah A, Mathew MS, Mathai D. Disseminated Histoplasmosis. *JAPI*. 2005;53:185-89.

[2] Mukherjee A, Tangri R, Verma N, Gautam D. Chronic disseminated histoplasmosis bone marrow involvement in an immunocompetent patient. *Indian J Hematol Blood Transfus*. 2010;26(2):65–67.

[3] Subbalaxmi MVS, Umabala P, Paul R, Chandra N, Raju Y, Rudramurthy SM. A rare presentation of progressive disseminated histoplasmosis in an immunocompetent patient from a non-endemic region. *Med Mycol Case Rep*. 2013;2:103–07.

[4] Kurowski R, Ostapchuk M. Overview of histoplasmosis. *Am Fam Physician*. 2002;66(12):2247-52.

[5] Ubesie AC, Okafo OC, Ibeziako NS, Onukwuli VO, Mbanefo NR, Uzoigwe JC, et al. Disseminated Histoplasmosis in a 13-year-old girl: a case report. *Afr Health Sci* 2013; 13(2):518-21.

[6] Sane SY, Patel MG, Patel BM, Kokal KK. Disseminated histoplasmosis (a case report). *J Postgrad Med*. 1983;29:270.

[7] Deodhar D, Frenzen F, Rupali P, David D, Promila M, Ramya I, et al. Disseminated histoplasmosis: A comparative study of the clinical features and outcome among immunocomprimised and immunocompetent patients. *Natl Med J India*. 2013;26:214-15.

[8] Reddy PA, Brasher CA, Christianson C, Gorelick DF. Peritonitis due to histoplasmosis. *Ann Intern Med*. 1970;72:79-81.

[9] Ahmed S, Shazzad N, Rahaman MFU, Kader A, Azad AK, Haq SA. Disseminated Histoplasmosis Without Pulmonary Involvement in An Immunocompetent Host - A Case Report. *BSMMU J*. 2010;3(1):44-46.

[10] Pamnani R, Rajab JA, Githang'a J, Kasmani R. Disseminated histoplasmosis diagnosed on bone marrow aspirate cytology: report of four cases. *East Afr Med J*. 2009;86(12):102-05.

[11] Caldwell CW, Taylor H. Visualization of Histoplasma capsulatum in bone marrow with Prussian blue iron stain. *J Clin Microbiol*. 1982;15(1):156–58.

[12] Hasliz M , Nur Atiqah NA, Lim CB, Hussain IHMI. Disseminated Histoplasmosis in a non-immunocompromised child. *Med J Malaysia*. 1999;56(1):120-24.

[13] Srikrishna A, Sitalakshmi S, Shantala Devi AM, Damodar P, D'Souza GA. Disseminated histoplasmosis in an AIDS patient diagnosed on bone marrow. *Indian J Pathol Microbiol.* 2002;45(3):333-34.

[14] Loulergue P, Bastides F, Baudouin V, Chandenier J, Mariani-Kurkdjian P, Dupont B, et al. Literature review and case histories of *Histoplasma capsulatum* var. duboisii infections in HIV-infected patients. *Emerg Infect Dis*. 2007;13(11):1647–52.

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Case Report

Platelet phagocytosis in peripheral blood during acute phase of dengue virus infection

Nidhya Ganesan, Indira Gunasekaran, Somanath Padhi, Anita Ramdas, Manjiri Phansalkar

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Abstract We aimed to describe peripheral blood phagocytosis of platelets as a possible underlying mechanism of thrombocytopenia during the acute phase of dengue virus infection in a young male. Furthermore, enhanced platelet phagocytosis through the apoptotic mechanism is also briefly highlighted.

Key Words: Apoptosis, dengue virus, peripheral blood, phagocytosis, thrombocytopenia

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INTRODUCTION

Dengue, a *Flavivirus* and transmitted by *Aedes aegypti* mosquito, is a great public health concern in India. Over the last decade, dengue has reached pan-Indian epidemic proportions leading to significant morbidity and mortality. Four serotypes of dengue virus (DENV) are known to cause the disease such as DENV-1, DENV-2, DENV-3, and DENV-4. A protective immunity against one infecting serotype lasts lifelong, but this is very short lasting (3–4 months) against other serotypes. Therefore, the second infection after the protective period often results in severe disease.[1]

Thrombocytopenia and increased vascular permeability are the two major characteristics of dengue fever (DF).[2] As per 2009 WHO recommendation, dengue cases can be classified into: (i) dengue without warning signs, (ii) dengue with warning signs (abdominal pain/persistent vomiting/mucosal bleed/increase in hematocrit with decrease in platelet count), and (iii) severe dengue (severe plasma leakage, severe bleeding, and severe organ involvement).[3] Thrombocytopenia associated with dengue infection is postulated to have has several underlying mechanisms.[4] In this manuscript, we aimed to describe the occurrence of platelet phagocytosis by peripheral blood monocytes, and highlight apoptosis-phagocytosis as another mechanism of dengue associated thrombocytopenia.

CASE REPORT

A 19-year-old male college student was evaluated for a 3-day history of high-grade fever, generalized myalgia, and the loss of appetite. He had no organomegaly, lymphadenopathy, skin rash, or any bone tenderness. His routine laboratory evaluation, on day 2 postadmission, showed hemoglobin - 130 g/L (reference 120–140 g/L), hematocrit - 43% (37–54%); total leukocyte count - 6 × 10^9/L with differentials of neutrophil - 40%, lymphocytes - 55%, and monocytes - 5%; total platelet

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count - 60 × 10^9/L (150–450 × 10^9/L), mean platelet volume - 11 fL (6–11 fL), and platelet distribution width - 23% (11–18%). Leishmann stained peripheral smear evaluation revealed normocytic normochromic red cells, relative lymphocytosis with reactive lymphoid cells, thrombocytopenia with numerous giant platelets, and no blasts. Besides these, there was an evidence of platelet phagocytosis by monocytes [Figure 1, ×400]. His biochemical parameters showed raised liver transaminases (serum glutamic-oxaloacetic transaminase - 105 IU/L, serum glutamic pyruvic transaminase - 115 IU/L, reference < 50 IU/L), and coagulation parameters were within normal reference range. His microbiological workup for HIV, hepatitis B and C viruses, *Salmonella,* scrub typhus, malaria, and leptospira were negative. DENV serology revealed positive nonstructural antigen-1 by ELISA. He was managed symptomatically and finally discharged on 8th-day postadmission with a platelet count of 100 × 10^9/L, and his liver transaminases at the time of discharge were 65 and 59 IU/L, respectively.

DISCUSSION

The proposed mechanisms of DENV associated thrombocytopenia are: (i) DENV induced direct suppression of bone marrow megakaryopoiesis, and (ii) Immune-mediated increased the peripheral destruction.[5] An increased level of platelet associated IgG is frequently observed in patients with chronic idiopathic thrombocytopenic purpura.[6] The previous studies have also found an inverse correlation between the levels of platelet associated antibodies and platelet count during the acute phase of dengue infection. It is hypothesized that increased macrophage clearance of peripheral platelets (coated with immune complexes) through both F_c receptor and complement receptor mechanisms, as well as direct platelet lyses, are the likely causes of thrombocytopenia in these patients.[7]

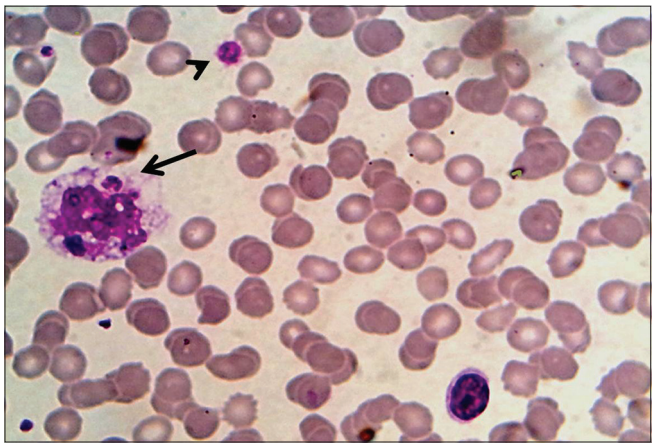


Figure 1: Peripheral blood smear from a young male patient with acute phase dengue virus infection demonstrating platelet phagocytosis by monocytes (black arrow). The platelet count at the time of admission was 60 × 10^9/L. Note the presence of a giant platelet (back arrow head) and a reactive lymphoid cell at the right lower corner of the field (Leishmann, ×400)

Recent *in-vitro* studies on dengue patients have shown that DENV induces increased peripheral platelet clearance by phagocytosis secondary to the apoptotic mechanism, during the acute and early convalescence phases of the infection.[8,9] By using flow cytometry, it was demonstrated that compared to healthy volunteers (n = 38), platelets isolated from dengue patients (57 with DF, 24 with dengue hemorrhagic fever [DHF]) showed significantly higher (P < 0.001) upregulation of proapoptotic markers such as active caspase-3 on their membranes (2.82 ± 1.05% vs. 16.82 ± 11.31%, respectively), increased Annexin V binding (3.27 ± 0.91% vs. 19.67 ± 10.72%, respectively); and thus increased phagocytosis by macrophages (20.29 ± 5.77% vs. 55.46 ± 29.66%, respectively). Furthermore, these findings were significantly (P < 0.05) observed from patients with DHF than those from DF.[8] The outcome of the above study is intriguing because of the very fact that platelet being anucleate particles lacks the machinery to express the apoptotic genes! Hence, the detailed molecular mechanism of DENV induced peripheral phagocytosis of activated platelets remains to be fully understood. As suggested by Honda *et al*., the percentage of phagocytosis should be correlated with platelet associated immunoglobulin titers.[9]

Our observation of peripheral platelet phagocytosis, in the present case, supports the hypothesis that increased peripheral clearance is an important mechanism in DENV associated thrombocytopenia. More prospective studies are required to validate the apoptotic hypothesis as suggested by Alonzo *et al*.[8] However, we do suggest that platelet phagocytosis in peripheral blood should be mentioned, as well as quantified, in clinical pathology reporting in cases with dengue associated thrombocytopenia.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Cecilia D. Current status of dengue and chikungunya in India. WHO South East Asia J Public Health 2014;3:22-7.
2. de Castro RA, de Castro JA, Barez MY, Frias MV, Dixit J, Genereux M. Thrombocytopenia associated with dengue hemorrhagic fever responds to intravenous administration of anti-D (Rh(0)-D) immune globulin. Am J Trop Med Hyg 2007;76:737-42.

[Downloaded free from http://www.jcrsmed.org on Friday, December 20, 2019, IP: 192.168.30.31]

3. Cecilia D, Shah PS, Alagarasu K. Dengue: Achievements in the last decade. In: Arankalle VA, Cecilia D, editors. NIV Golden to Diamond Jubilee: The Glorious Decade. Pune, India: Golden Jubilee Publication; 2012. p. 141-62.
4. Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: An integrated view. Clin Microbiol Rev 2009;22:564-81.
5. La Russa VF, Innis BL. Mechanisms of dengue virus-induced bone marrow suppression. Baillieres Clin Haematol 1995;8:249-70.
6. Rand ML, Wright JF. Virus-associated idiopathic thrombocytopenic purpura. Transfus Sci 1998;19:253-9.
7. Saito M, Oishi K, Inoue S, Dimaano EM, Alera MT, Robles AM, *et al.* Association of increased platelet-associated immunoglobulins with thrombocytopenia and the severity of disease in secondary dengue virus infections. Clin Exp Immunol 2004;138:299-303.
8. Alonzo MT, Lacuesta TL, Dimaano EM, Kurosu T, Suarez LA, Mapua CA, *et al.* Platelet apoptosis and apoptotic platelet clearance by macrophages in secondary dengue virus infections. J Infect Dis 2012;205:1321-9.
9. Honda S, Saito M, Dimaano EM, Morales PA, Alonzo MT, Suarez LA, *et al.* Increased phagocytosis of platelets from patients with secondary dengue virus infection by human macrophages. Am J Trop Med Hyg 2009;80:841-5.

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IMAGES

Chediak–Higashi Syndrome in Accelerated Phase

Nidhya Ganesan[1,2] · **Prasanna N. Kumar**[1]

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A two-month-old boy, born to consanguineous parents, presented with high grade fever for 5 days. On examination he was febrile, pale with generalized hypo pigmentation of skin and hair, hepatosplenomegaly and cervical lymphadenopathy. He had a past history of recurrent respiratory tract infections. Splenomegaly of 2.5 cm was noted at birth. Complete blood count—total count 3.1 × 10^3/µl, haemoglobin 7.4 g/dl, and platelet count 24 × 10^3/µl. Primary blood and urine cultures were sterile. Peripheral smear showed pancytopenia with large eosinophilic inclusions in the lymphocytes (Fig. 1). Bone marrow revealed large coarse azurophilic granules in the cytoplasm of many of the myeloid precursors and mature granulocytes including eosinophils (Figs. 2, 3).

Erythrophagocytosis was evident in the marrow histiocytes (Fig. 4). Serum iron was 28 µg/dl, ferritin >2000 ng/ml and triglycerides were 325 mg/dl. With the presence of six of the eight diagnostic criteria for HLH being present i.e. fever, splenomegaly, pancytopenia, hypertriglyceridemia, hyperferritenemia and haemophagocytosis in the bone marrow, a diagnosis of Chediak–Higashi syndrome with haemophagocytic lymphohistiocytosis (accelerated phase) was made. The child was treated with IV ceftriaxone and vancomycin. However his condition deteriorated and he expired.

Chediak–Higashi syndrome (CHS) is a rare, autosomal recessive disorder that affects multiple systems of the body mainly the immune system [1]. The clinical manifestations of

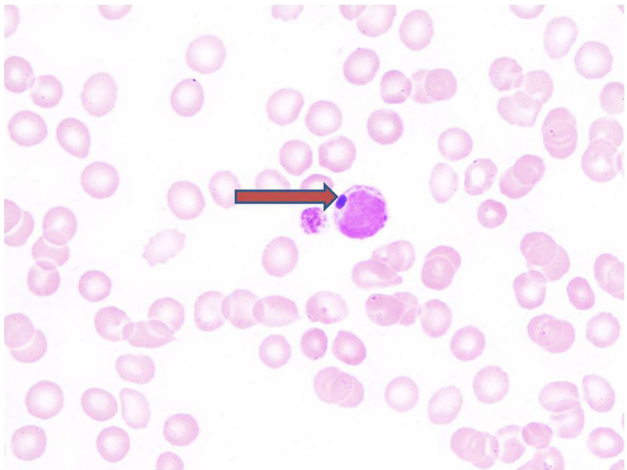


Fig. 1 Lymphocyte inclusion (indicated with *red arrow*)

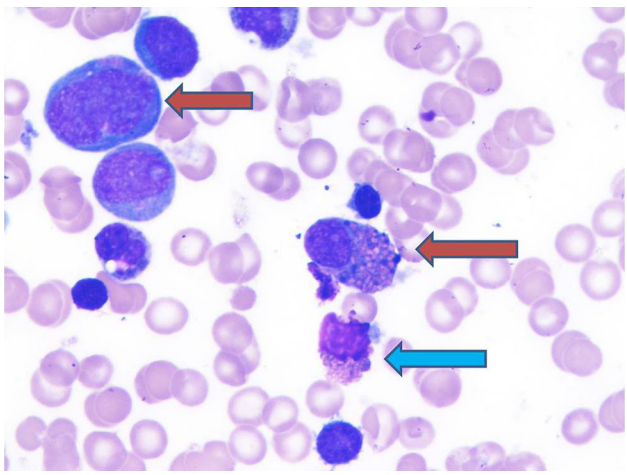


Fig. 2 Large coarse granules are present in the cytoplasm of the myeloid precursor (indicated with *red arrow*) and eosinophils (indicated with *blue arrow*)

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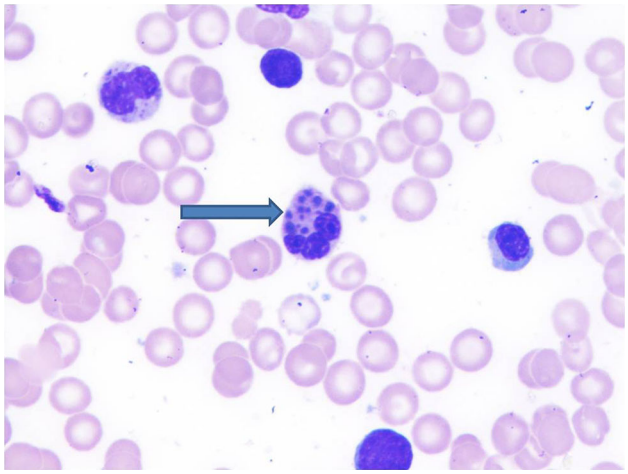


Fig. 3 Giant granules in the cytoplasm of neutrophils (indicated with *blue arrow*)

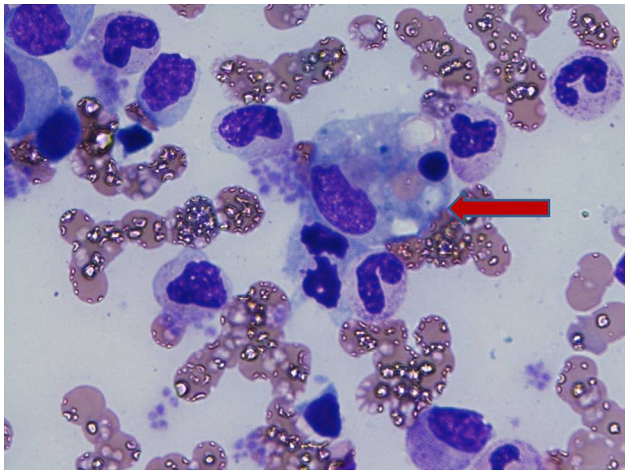


Fig. 4 Erythrophagocytosis (indicated with *red arrow*)

CHS in accelerated phase include high fever, neurological abnormalities, lymphadenopathy, hepatosplenomegaly, pancytopenia, abnormal giant granules in peripheral blood and bone marrow, lymphohistiocytic infiltration in liver, spleen and lymphnodes [2].The only treatment is allogenic haematopoietic stem cell transplantation (HSCT). The prognosis is poor [3].

Compliance with ethical standards

Conflict of interest Authors declared that they have no conflicts of interest.

References

1. Ward DM, Shiflett SL, Kaplan J (2002) Chediak–Higashi syndrome: a clinical and molecular view of a rare lysosomal storage disorder. Curr Mol Med 2(5):469–477
2. Bouatay A, Hizem S, Tej A, Moatamri W, Boughamoura L, Kortas M (2014) Chediak–Higashi syndrome presenting in accelerated phase: a case report and literature review of the literature. Indian J Hematol Blood Transfus 30(1):223–226
3. Kaplan J, De Domenico I, Ward DM (2008) Chediak–Higashi Syndrome. Curr Opin Hematol 15(1):22–29

Original Article

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Laboratory Diagnosis of Renal Disorders: Automated Urine Sediment Analyzer Compared with Manual Methods

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ABSTRACT

Background: Urinalysis is one of the earliest methods used to screen and detect patients with kidney diseases. It also helps to monitor and assess the severity of the disease process in already diagnosed patients. Microscopy plays a vital role in routine urinalysis and gives more information when analyzed together with chemical strip tests. Introduction of automation of the conventional methods of urinalysis has reduced the disadvantages of manual methods in terms of accuracy of results and turnaround time.

Method: Aim of the present study is to evaluate the performance of an automatic urinalysis system – FUS-100 (which in cooperates an automatic urine chemistry analyzer H-800) manufactured by Dirui Industrial Co. Ltd., China in patients with renal diseases and compare the results of microscopy with manual microscopic analysis. In addition, our study aims to find out the possibility of safely reducing the number of manual microscopy analyses by cross-interpretation of the results generated by FUS-100 automated urine particle analyzer with manual methods. The urine sediments of five hundred urine samples were examined by these two methods.

Result: Automation of urine analysis decreases the turnaround time and is less labour intensive leading to better accuracy and precision.

Conclusion: Preanalytical errors related to centrifugation and sediment preparation are prevented in automation. Our study tends to suggest that automation of urine microscopy therefore is a more standardized procedure and makes urine microscopy a more objective investigation.

Keywords: *Automation, Renal Disorders, Manual Methods, Urinalysis, Casts, Crystals, Cells*

Introduction

Urinalysis provides information and clues to many diseases and can also be an indication of the condition of a patient's health. In patients with nonspecific symptoms and severe kidney diseases, urinalysis serves as one of the early laboratory investigations to initiate treatment.[1,2]

Routine urinalysis is mainly done by inspection, chemical testing and microscopic examination of the urinary deposits which can have cells, casts, crystals or microorganisms. Traditionally, microscopy has always been done by manual methods. Manual examination of the urinary sediment, even though considered a time tested and standard method is labour intensive and involves considerable time, leading to delays in reporting. Automation of urinalysis has been introduced to reduce the disadvantages of the manual method.

The present study evaluates the results generated by an automated urine analyzer FUS -100, which incorporates an automatic urine chemistry analyzer H-800(manufactured by M/S. Dirui industrial Co.Ltd., China) in patients with renal diseases and compares the results of microscopy obtained by automation with manual microscopic analysis. Simultaneously, control samples from a population without knowing renal disorders were also studied. It was felt that such a study would be useful, if it is designed to find out the possibility of safely reducing the number of manual microscopy analyses by cross-interpretation of the results of the FUS-100 automated urine particle analyzer with manual methods.[1,2,3]

Materials and Methods

This was a prospective observational study, performed on urine samples received in the Clinical Pathology laboratory, Department of Pathology. Routine urinalysis done during a period of one year was analysed. A total of 500 urine samples collected from both outpatients and inpatients were examined within an hour of receipt. Physical and chemical analysis including tests for detecting glucose, ketones, proteins, blood, urobilinogen, pH, specific gravity, haemoglobinuria, myoglobinuria, hemosiderinuria, bile salts and bile pigments were performed on these samples. All the samples were collected exclusive of preservatives. The study included patients with confirmed renal disorders and those suspected to have renal disorders based on abnormal renal function tests. The clinical details and diagnosis related to all the urine samples were obtained from the test request forms.

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After receiving the samples, a dipstick chemical strip analyses of the urine samples were performed using the automatic urine chemistry analyzer H-800. Results obtained were used to screen further microscopic urine analysis. An abnormal urine was considered as one in which any of the following parameters tested were out of range - blood, proteins, nitrites, ketones, urobilinogen, bilirubin, WBCs and glucose. The rest of the urine samples were considered as normal controls.

For performing automated microscopic analysis (FUS-100), uncentrifuged urine samples were placed on the sample rack provided with the analyzer. The FUS100 aspirates 0.95 mLof urine and at the end of one minute, the artificial intelligence identification software within the analyzer classifies the particles into twelve categories (Red blood cells, white blood cells, white blood cell clumps, hyaline casts, pathological casts, squamous epithelial cells, non-squamous epithelial cells,bacteria,yeast, crystals,sperm, mucus) present in the given sample by capturing each of their images. Using this classification the software calculates the visible component concentration by taking into account the number of images and the scanned urine volume. All visible component images of a given urine sample are shown on the monitor in separated gridsand presented in the form of numbers/low power or high power field. FUS Focus, FUS positive control, and FUS negative control were run according to the manufacturer's instructions were run every day before testing the urine samples.

The samples apportioned for manual microscopy were gently mixed and centrifuged in a centrifuge test tube at 1500rpm for five minutes and the supernatant was discarded. A drop from the remaining sediment was placed onto a microscope glass slide and covered by means of a cover slip. Microscopic examination of the sediment was done initially under low power (10X) to identify casts and then high power (40X) was used for erythrocytes, leukocytes, crystals, epithelial cells, bacteria, yeast and other significant findings. An average of ten fields was taken and the results were calculated semi-quantitatively and expressed as a range (Table-1).

Among the randomly examined samples with renal function abnormalities, the microscopic sediments quantified from the manual method was compared with that of the automated analyzer.

Results

The following are the results for the various constituents of the urine sediment examined through automation and by manual microscopy.

The area under the curve (AUC) and sensitivity for detecting RBCs by the manual method were 0.8648 and 82%, respectively. By automated method using FUS-100 these values were 0.8763 and 86.5% respectively, which are higher than the values obtained by manual method (Table 2). The changes noticed were seen to be statistically significant, suggesting that FUS-100 analyzer is more efficient than the manual method at detecting RBCs when compared to the manual method. However, the specificity and PPV for detecting RBCs through manual method was found to be higher (Table 2). The AUC for WBCs was registered as 0.6568 by FUS-100 while with manual method was found to be 0.6524 (Table 2). This result reflects that the accuracy of both the methods had comparable results in detecting WBCs, though the result of FUS-100 is marginally higher. The Sensitivity for detecting WBCs was once again marginally increased with the investigations performed by FUS-100 (50.5%), as against the manual method (48%) (Table 2). However, we found a higher specificity for WBCs (80.28%) with the manual method. Finally, the PPV for detecting WBCs by manual method is higher (63%) than with FUS-100 analyzer which is 62%.

In the people without any renal abnormality, nearly 92.7% were detected to have epithelial cells (within a range of 0 to 5) by automated microscopy whereas only 84.7% were detected to have them by manual method. The specificity of manual method in recognizing epithelial cells was 98% and positive predictive value was 70%. However FUS-100 had a better negative predictive value which was 59 % (Table 2).

Among the study population, 482(96.4%) cases detected by FUS-100, 283(94.3%) cases detected positive for bacteria were patients who did not have any renal abnormalities, whereas in the manual method such cases constituted only 265 (88.3%) (Table 3). This shows that automated microscopy is more efficient in detecting bacteria when compared to the manual method whereas,yeasts were detected with equal accuracy by both the methods. However, since simultaneous cultures were not done in any of the cases studied, it is not possible to determine if these bacteria are contaminants or clinically significant entities.

Among the 200 patients with renal abnormalities, the sediments contained a variety of crystals like Ammonium Biurate, Amorphous urates, calcium carbonate, uric acid, etc. Among these, some of the crystals were identified by both the methods, while a few others were identified only by one of the methods. The details of distribution of these deposits are tabulated (Table 4). Additionally, the FUS-100 analyzer classified some of the amorphous urates and calcium carbonates as RBCs, which were reclassified while reviewing the analyzed images.

In addition to the entities mentioned above, FUS-100 identified a total of 74 cases to have hyaline casts out of which only 35 cases truly had the casts. Out of the 35 true positive cases, 3 cases were seen in patients with renal abnormalities in the range of 6-10. However, the manual method was able to detect 29 cases of hyaline casts out of which 26 were in the range of 0 to 5 and the other 3 cases in the range of 6 to 10. Waxy casts, noted in a patient by manual microscopy, was not detected by the automated analysis (Table 4). However, FUS-100 categorizes most of the detected casts and crystals as "unclassified" making it essential for the operator to review the images on the screen before finalizing the reports from the analyzer.

Statistical Analysis: All the diagnostic test evaluation analyses sensitivity, specificity, positive predictive values and negative predictive values for FUS-100 automated urine sediment analyzer and manual microscopic method were done using Medcalc statistical software & STATA statistical software package release 11. Simple calculations like percentages, proportions and mean values were derived. A type I error of 0.05 was considered in all analyses.

Table 1: Semi- quantitative range classification of urine particles used for this study.

Parameters	Ranges				
Erythrocyte/ HPF	0 – 5	6 – 10	11 – 20	21 – 30	> 30
Leukocyte/ HPF	0 – 5	6 – 10	11–20	21-30	>30
Epithelial cells/ HPF	0 – 5	6 – 10	11-20	21-30	>30
Casts/LPF	0 -5		5-10		>10
Crystals/ HPF	Positive		Negative		

Abbreviations: HPF, High power field; LPF, Low power field.

Table 2: Diagnostic accuracy of urine sediment analysis between the automated and manual methods.

	RBC		WBC		Epithelial Cells	
	Automated	Manual	Automated	Manual	Automated	Manual
AUC	0.8763	0.8648	0.6568	0.6524	0.5141	0.5124
SE	0.0159	0.0167	0.0218	0.0216	0.0119	0.0076
95% CI	0.8441 to 0.9038	0.8312 to 0.8937	0.6130 to 0.6986	0.6083 to 0.6946	0.4681 to 0.5598	0.4648 to 0.5597
Z	23.7	21.9	7.21	7.04	1.19	1.63
P Value	<0.01	<0.01	<0.01	<0.01	>0.05	>0.05
Sensitivity	86.5	82	50.5	48	8.08	3.66
95% CI	81.0 - 90.9	76.0 - 87.1	43.4 - 57.6	40.9 - 55.2	4.7 - 12.8	1.5 - 7.4
Specificity	83.61	84.48	79.25	80.28	94.6	98.82
95% CI	78.9 - 87.6	79.8 - 88.5	74.2 - 83.7	75.2 - 84.7	91.3 - 96.9	96.6 - 99.8
PPV	78	79	62	63	52	70
95% CI	71.9 - 83.2	72.3 - 83.9	54.4 - 69.8	54.6 - 70.4	33.1 - 69.8	32.8 - 94.1
NPV	90	87	70	69	59	58
95% CI	86.1 - 93.5	82.7 - 90.9	64.9 - 75.1	63.8 - 74.0	54.4 - 63.7	52.9 - 62.4

Abbreviations: AUC, area under the curve; SE, standard error; CI, confidence interval; PPV, Positive Predictive Value; NPV ,Negative Predictive Value.

Table 3: Distribution of cells other than RBCs and WBCs in the study population

Cells Other than RBCs and WBCs	Automated			Manual		
	Normal renal function	Abnormal renal function	Total	Normal renal function	Abnormal renal function	Total
Urine without other particles	16	0	16	34	0	34
Bacteria	283	199	482	265	199	464
Yeast	1	1	2	1	1	2
Total	**300**	**200**	**500**	**300**	**200**	**500**

Table 4: Details of crystals and casts identified in the study population.

Casts/crystals	Automation			Manual		
	Normal renal function	Abnormal renal function	Total	Normal renal function	Abnormal renal function	Total
Nil	267	159	426	273	158	431
Ammonium Biurate	0	6	6	0	6	6
Amorphous Urates	0	1	1	0	1	1
Calcium Carbonate	0	2	2	0	2	2
Cholesterol crystals	0	1	1	0	1	1
Calcium Oxalate	1	18	19	1	18	19
Uric acid crystals	0	4	4	0	4	4
Sulfa crystals	0	1	1	0	1	1
Triple phosphate crystals	0	4	4	0	4	4
Hyaline casts	32	3	35	26	3	29
Granular casts	0	1	1	0	1	1
Waxy casts	0	0	0	0	1	1
Total	**300**	**200**	**500**	**300**	**200**	**500**

Discussion

Urinalysis is one of the earliest methods used to screen and detect patients with kidney diseases. In many laboratories, urinalysis is restricted to chemical strip analysis, which in fact, is a cost-effective and widely accepts screening method. However, microscopy plays a vital role in routine urinalysis and gives more information when analyzed together with chemical strip tests.[4] Better results can be obtained only with the use of standardized techniques for this procedure.

Manual microscopy of the urine may be affected by preanalytical variables such as the speed and time for centrifugation, sediment preparation and interpretation all of which may lead to imprecise and inaccurate results. For a reliable urine microscopy report, the examiner should be skilled to recognize the constituents of the urine sediment correctly and possess knowledge about the clinical importance of detecting substances in urinary sediments. With the introduction of automation in all spheres of clinical laboratory practice, it is no wonder that in the recent past, companies have come out with equipments that automate urine microscopy. [5,6]

A number of instruments offering automated urine analyses are now available. These include the IRIS iQ200, the UF1000i, sediMAX and more recently the DIRUI FUS -100 Series urine sediment analyzer.[7] However, there are only a few studies that have compared the results from automated urine analyzers and microscopic methods in parallel.

This study made use of fresh samples of urine, examined within an hour of collection. However, in reality, when there is a delay in transporting urine samples, they are processed after refrigeration. The reliability of results for such samples derived from the FUS analyzerhas not been studied and is unknown.

Manual examination of urine requires the sample to be centrifuged for five minutes, after which it is examined. It is thus advantageous that the FUS-100 uses urine samples for analysis which are not centrifuged and reduces the turnaround time.

In our study, we found that the sensitivity for RBCs was better in FUS-100 while the specificity for detecting erythrocytes was higher in the manual method. This may be because of the fact that FUS-100 classifies calcium crystals, yeast and sometimes amorphous urates as RBCs. Hence, this will require reclassification. Literature shows similar studies which have made a comparison of FUS-100 with UriSed and manual methods. These studies reveal higher sensitivity for detection and discrimination of RBCs with FUS-100 than with UriSed and manual method.[5,7]

Yuksel et al reported that FUS-100 shows high sensitivity (73%) and high negative predictive value for RBCs (95%) when compared with manual microscopy. Similar to their results, in our study we found that the sensitivity (86.5%) negative predictive value (90%) and also AUC (0.8763) were higher for RBCs with FUS-100 which indicates minimal false negative results even though the possibility of false positive results are more when compared with manual microscopy.[5,7] The automated analyzers iQ200 and UF-100 recognize dysmorphic RBCs in their routine urinalysis and this has been substantiated through studies. [7,8] However this recognition is not possible with the FUS-100.

Leukocytes are routinely noticed in normal urine (upto 5 cells/HPF) and also in interstitial nephritis and proliferative glomerulonephritis.

In our study, we have shown that FUS-100 has a higher sensitivity for RBCsand WBCs in comparison with conventional manual methods. This may be due to the fact that the analyzer recognizes epithelial cells, RBCs and crystals as WBCs. We have been able to detect squamous epithelial cells in the range of 0 to 5 cells/HPF more frequently with FUS-100 (99%) when compared with manual method (95.5%). In the case of the iQ200, David et al in their study state that cell counts for erythrocytes, WBCs and epithelial cells correlate well with manual counts using counting chambers. They also found out that the AUC did not alter even after correcting the captured images for erythrocytes, WBCs and squamous epithelial cells. He concluded that manual review can be greatly reduced when the interpretation of automated analyzer is combined with chemical strip test.

Increased numbers of hyaline casts in urine indicate pathological conditions like acute glomerulonephritis and other renal diseases with proteinuria. We found that the FUS-100 classifies 14.8% (74 cases) of particles as hyaline casts out of which only 7% (35 cases) were true hyaline casts as evidenced by microscopy. The stored images which were captured by the in-built imaging system were also reviewed and the findings were in agreement with what was seen in the manual microscopy.

The falsely elevated hyaline cast count (14.8%) generated by the FUS-100 is due to the inclusion of folded squamous epithelial cells. This was also seen with other analyzers like the sediMAX, iQ200 and UriSed, built imaging system. Granular casts are usually associated with tubulointerstitial disease.[5,7,8] We found that FUS-100 recognized granular casts in patient suspected to have renal abnormalities as did the manual method.

Waxy casts are usually associated with serious renal pathologies like chronic diseases and amyloidosis. [9,10]

Increased uric acid crystals may be associated with hyperuricosuria and acute nephropathy.[7] In our study, we noticed four cases with uric acid crystals in patients with abnormal renal function. All the four cases were recognized by both automated and manual microscopic methods.

This study reveals that even though the FUS-100 was able to recognize and classify RBCs, WBCs, and squamous epithelial cells, all the crystals and casts other than hyaline casts are categorized as “unclassified” by this analyzer. This therefore requires technical assessment of the components of the urine sediment. This observation was also reported in the study done by Yuksel et al. and by Wah DT et al who recommend that a technician review the captured images by the iQ200 analyzer for all casts, WBC clumps and yeasts for confirmation. All the above studies support our observations with the FUS-100. The inability to identify casts in automated urine microscopy is an intrinsic limitation of this technology.

In order to eliminate the false positive results seen in automated urine microscopy a dedicated and well trained technician is required for the urinalysis workstation to visualize and correct the wrongly labeled cells and to classify the images shown as abnormal casts or “unclassified” before finalizing the results. However, the results that were analysed in this study were unedited results from the analyzer. For the correct identification of unknown particles and pathological casts, the manual method is still required for confirmation.

Conclusion

The FUS-100 automated urine particle analyzer performance is better than the manual microscopy for detecting RBCs, WBCs and squamous epithelial cells when compared with the manual method. Though it recognizes some crystals and casts, it fails to classify them. Therefore, manual urine microscopy must supplement the urine examination, especially in patients with abnormal renal function and abnormal urine chemical analysis.

Conventional microscopy is still required for identifying dysmorphic erythrocytes, yeasts, Trichomonas, oval fat bodies, and for the differentiation of various casts and crystals.

Automation of urine analysis decreases the turnaround time and is less labour intensive leading to better accuracy and precision. Automated urine microscopy combined with dipstick results can be used as a screening procedure for large numbers of urine samples in places with a high workload.

Abbreviations

RBC – Red blood cells
WBC –White blood cells
PPV- Positive predictive Value
NPV- Negative predictive Value
AUC-Area under the curve
HPF- High power field

References

1. McPherson RA, Ben-Ezra J. Basic examination of urine. In: McPherson, Pincus MR. Editors. Henry's Clinical diagnosis and Management by Laboratory methods. 22nd Ed. Ch. 28. Philadelphia: Saunders Elsevier; 2011.
2. Clinical and laboratory standards Institute, Urinalysis: Approved guideline- 3rd Ed. 2009.
3. European urinalysis group. European urinalysis guidelines. Scand J Lab Invest 2000; 60:1-96.
4. Darci RB, John CL. Automated urinalysis in the clinical lab. Medical laboratory observer; 2012.
5. Yuksel H, Kilic E.E, Ekinsi A, Evliyaoglu O. Comparison of fully automated urine sediment analyzers H800-FUS100 and LabumatUrised with manual microscopy. J Clin Lab Anal. 2013; 27:312-316.
6. Coppens A. Speeckaert M, Delanghe J. The pre-analytical challenges of routine urinalysis. Acta Clin Belg. 2010; 65:182-9.
7. Foggazi GB, Verdesca S, Garigali G. Urinalysis: Core curriculum 2008. Am J Kidney Dis. 2008; 51:1052-1067.
8. Budak YU, Huysal K. Comparison of three automated systems for urine chemistry and sediment analysis in routine laboratory practice. Clin Lab. 2011; 57:47-52.
9. Winkel P, Statland BE, Jorgensen K. Urine microscopy, an ill defined method, examined by a multifactorial technique. Clin Chem. 1974; 20:436-439.
10. Brody LH, Sallada JR, Ambaster K. Urinalysis and the urinary sediment. Med Clin North Am. 1971; 55:243-266.

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Case Report

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Pathology Section

Pancreatic Plasmacytoma Misdiagnosed as Neuroendocrine Tumour: A Pathologist's Dilemma

SAKTHISANKARI SHANMUGASUNDARAM[1], UMAMAHESWARI GURUSAMY[2], PRASANNA N KUMAR[3], BIKU JOSEPH JOHN[4]

BY-NC-ND

ABSTRACT

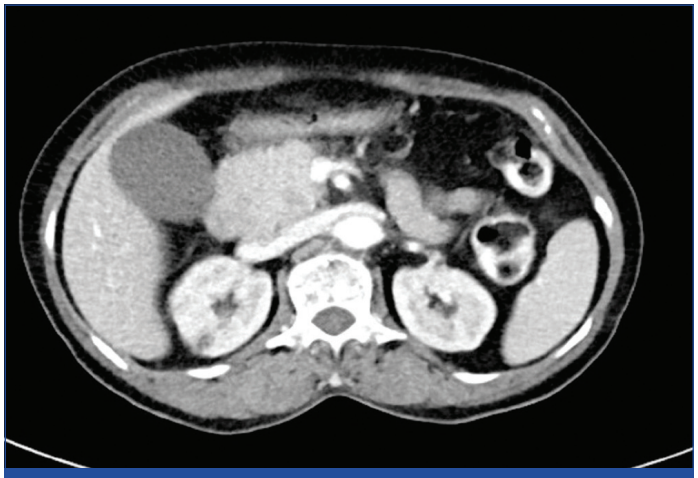
Extramedullary plasmacytomas are rare, constituting to about 5% of plasma cell neoplasms. Pancreatic involvement is uncommon constituting to about 2.3%. Localised solitary plasmacytomas has a good response to radiation. When they are associated with pre-existing multiple myeloma, their prognosis is poor. A case of plasmacytoma of pancreas presenting with obstructive jaundice preceding plasma cell leukaemia is presented here. The lesion was misdiagnosed by fine needle aspiration cytology as neuroendocrine neoplasm. The patient did not present with symptoms of myeloma initially. The present case highlights the significance of considering other causes of pancreatic mass than primary carcinomas.

Keywords: Extramedullary plasmacytoma, Pancreatic neoplasm, Plasma cell leukaemia

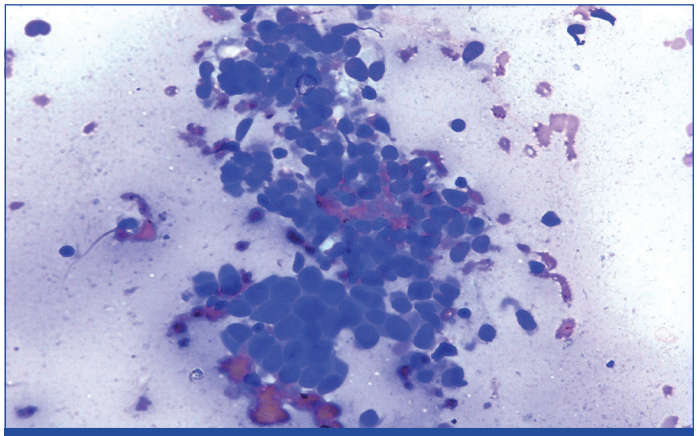
CASE REPORT

A 62-year-old female presented with complaints of fever on and off for 15 days, a vague, non-radiating, intermittent right upper quadrant, paraumbilical pain and vomiting for five days. There was no history of gastrointestinal bleed, loss of weight and loss of appetite. She was a known hypertensive and diabetic for five years and was on treatment with oral hypoglycemics and antihypertensives. Her vitals were within normal limits and there was no organomegaly.

Ultrasound abdomen showed a hypoechoic mass in the pancreatic head dilating the main pancreatic duct. Computed Tomography (CT) revealed an isodense mass lesion with well defined borders in the head of pancreas measuring 4.8×5.5 cm [Table/Fig-1]. Acoustic Radiation Force Impulse (ARFI) elastography showed mean shear velocity of 3.6. The provisional diagnosis included primary pancreatic tumour. Endoscopic Ultrasound Guided Fine Needle Aspiration Cytology (EUS-FNAC) was planned for the evaluation of the pancreatic mass. EUS showed a 3×3.1 cm iso and hypoechoic mass in the head of pancreas with dilated common bile duct measuring 9 mm in the suprapancreatic portion. Dilated main pancreatic duct (6.5 mm) distal to the mass in the pancreas was identified. Visualised tail of pancreas and portal vein appeared normal. Cytological examination of the mass showed sheets and scattered plasmacytoid cells suspicious of a neuroendocrine neoplasm [Table/Fig-2].



[Table/Fig-1]: CT scan showing isodense lesion in head of pancreas.



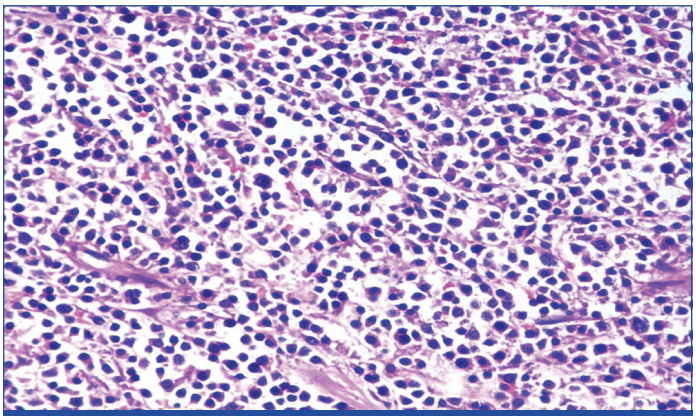
[Table/Fig-2]: Cytology smears showing plasmacytoid cells (40x).

Whipple resection was done with a clinical and cytological diagnosis of a pancreatic neuroendocrine tumour. Pancreas showed an ill-defined mass occupying the head [Table/Fig-3]. Haematoxylin & Eosin (H&E) sections of the mass showed a diffusely infiltrating neoplasm composed of monotonous, plasmacytoid cells infiltrating the acini and ducts [Table/Fig-4]. Few binucleate and multinucleate plasma cells and lymphocytes were present. Morphological differential diagnosis included plasmacytoma and lymphoplasmacytic lymphoma. Immunohistochemical staining was done for confirmation of diagnosis. The tumour cells showed strong membranous positivity for CD138 [Table/Fig-5], CD45 and selective Kappa staining [Table/Fig-6]. They were negative for CD20 and Lambda immunostains. A diagnosis of Plasmacytoma was made and radiological and biochemical workup for multiple myeloma was suggested. Protein electrophoresis was normal. Urine for Bence Jones Protein was negative. Serum calcium and renal parameters were normal. CT spine survey did not reveal any osteolytic lesions. The case was treated as a solitary extramedullary plasmacytoma and suggested follow-up. A month later, the patient developed severe anaemia (Hb-6.8 g/dL). Total count was elevated and peripheral smear showed 40% plasma cells suggestive of plasma cell leukaemia [Table/Fig-7]. Reviewing the patient's peripheral smear and complete haemogram taken a month back, there was normocytic normochromic anaemia and no demonstrable plasma cells in peripheral smear. ESR was elevated (93 mm/hr). Bone marrow aspiration done showed infiltration by sheets of immature

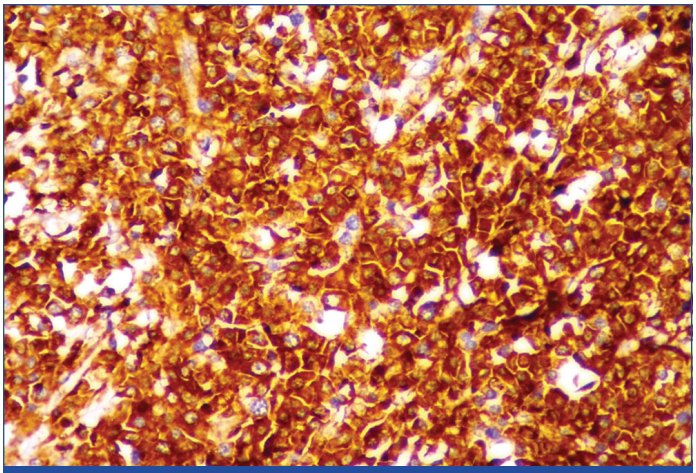
and mature plasma cells. On immunohistochemistry, these cells showed kappa restriction. A diagnosis of plasma cell leukaemia was made. Investigations done at presentation and a month later are tabulated [Table/Fig-8]. The patient was referred to higher centre for further treatment.



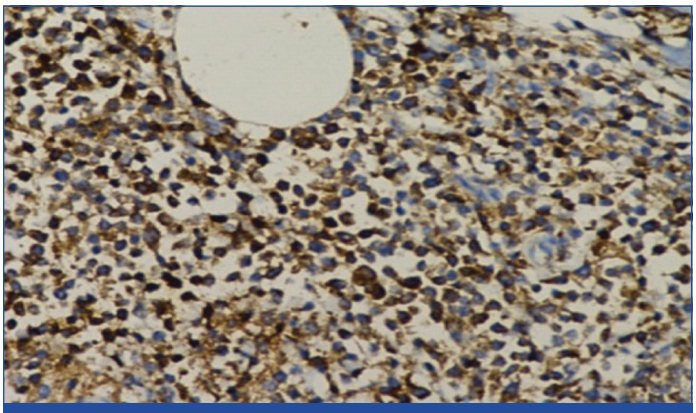
[Table/Fig-3]: Whipple's specimen showing ill-defined lesion in pancreas.



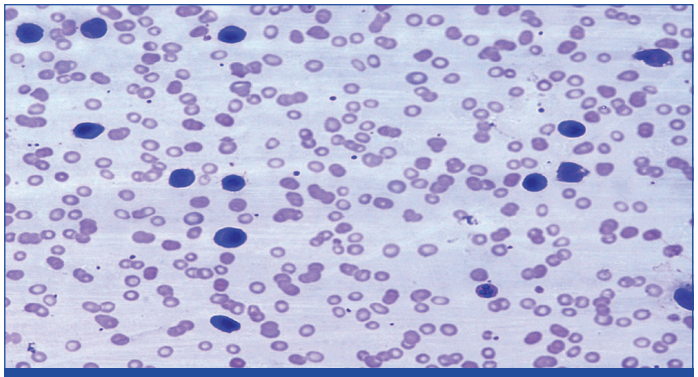
[Table/Fig-4]: Diffuse infiltration by plasma cells (H&E, 40x).



[Table/Fig-5]: Strong membranous expression of CD138 by tumour cells (IHC, 40x).



[Table/Fig-6]: Neoplastic cells with kappa expression (IHC, 40x).



[Table/Fig-7]: Peripheral smear with plasma cell leukaemia (40X).

Parameters	At initial presentation	A month later
Serum urea	42 mg/dL	81 mg/dL
Serum creatinine	1.06 mg/dL	3.82 mg/dL
Direct bilirubin	3.3 mg/dL	1.8 mg/dL
Haemoglobin	9.5 g/dl	6.8 g/dL
Total WBC count	4.3×10^3/µL	30.5×10^3/µL
Peripheral smear	Normocytic anaemia	Plasma cell leukaemia
Serum calcium	1.116 mmol/L	1.178 mmol/L

[Table/Fig-1]: Biochemical and haematological investigations.

DISCUSSION

Plasma cell neoplasms include Plasma Cell Myeloma (PCM) which includes smouldering myeloma, non-secretory myeloma, plasma cell leukaemia and plasmacytoma which includes solitary bone and extraosseous plasmacytoma. In majority of the patients with PCM, features of end organ damage in the form of one or more of the following, hypercalcaemia, Renal insufficiency, Anaemia, and Bone lesions (CRAB) are present. The clonal proliferation usually begins in bone marrow. Renal failure occurs due to tubular damage caused by excessive synthesis of monoclonal immunoglobulins [1]. It constitutes 10 to 15% of haematological malignancies. Median age at diagnosis is 66 years [2]. The clinical spectrum varies from asymptomatic to highly aggressive.

Extramedullary Plasmacytomas (EMPs) are localised proliferation of monoclonal plasma cells forming solitary lesions outside the bone marrow. EMPs can be a manifestation of advanced multiple myeloma or can represent a solitary plasmacytoma without involving the bone marrow [3]. EMPs are uncommon and represent 5% of all plasma cell tumours. The most frequent site of involvement being upper respiratory tract followed by gastrointestinal tract and reticuloendothelial system [2].

Primary pancreatic plasmacytoma is rare constituting 2.3% of EMPs. Majority of the reported pancreatic plasmacytoma are secondary [3]. EMPs have the median age of presentation at 55 years (A decade younger than patients with multiple myeloma) and the majority (three-fourths) are males [4].

Most of these lesions are located in the pancreatic head. Diffuse enlargement and presenting as mass in body (4 cases) [5] and tail of the pancreas have also been reported. The typical presenting symptoms include abdominal pain and obstructive jaundice from bile duct compression when there is involvement of head. Diffuse lesions are picked up rather late as they tend to be asymptomatic [6]. The case presented here also had obstructive jaundice raising a concern for primary pancreatic carcinomas [7].

Radiologically plasmacytoma is characterised by a homogenously enhancing multilobular, solid mass on CT, which is non-specific and resemble that of neuroendocrine tumours or carcinoma [6]. The case described presented as an isodense lesion in the pancreatic head. Differentiating plasmacytomas radiologically from other pancreatic tumours is challenging.

Endoscopic Ultrasound (EUS) is a well recognised technique for confirmation of pancreatic masses by histology. EUS- FNAs are reported to have an accuracy of 78-95% in the diagnosis of pancreatic masses. Neuroendocrine tumour presents as a, homogeneous, isoechoic or hypoechoic mass on EUS while plasmacytoma shows heterogenecity with uneven margins. NETs are hypervascular [5,8].

When FNA was done, the lesion was morphologically misdiagnosed as neuroendocrine neoplasm since both share plasmacytoid morphology. In a rare site, one would be more likely to suspect a primary lesion with plasmacytoid features, particularly endocrine neoplasm. Islet cell tumours have been reported to be a great mimic of plasmacytoma with regards to clinical, cytological and radiological appearance [9]. EUS guided core biopsy from the lesion and immunophenotyping with cytokeratin for carcinoma and CD138, kappa and lambda for identifying monoclonality of plasma cells would have helped in arriving at the diagnosis. EUS guided FNA also serves as a platform for obtaining material for flow cytometry.

The patient described here did not present with any symptoms pertaining to myeloma prior to surgery. The patient progressed to plasma cell leukaemia within a short time (one month) which is evident from the investigations [Table/Fig-8]. Plasma cell leukaemias are primary in 60-70% of cases and secondary to multiple myeloma in 30-40 % cases [10,11].

To our knowledge, this is the first case of plasmacytoma of pancreas being the initial manifestation of a plasma cell leukaemia. Two other cases of plasma cell leukaemia have been reported in association with gastric plasmacytoma. One case reported also had a normal WBC and platelet count with normocytic normochromic anaemia three weeks prior to the diagnosis of gastric plasmacytoma. The authors had hypothesised that secondary plasma cell leukaemia would have occurred as a result of rapid transformation of possibly underdiagnosed light chain multiple myeloma. The present case could also represent a similar rapid transformation from a non-secretory myeloma presenting with an extramedullary plasmacytoma [12].

From the previous reports it is clear that in any mass lesion of a solid organ in a patient with multiple myeloma the possibility of secondary plasmacytoma is to be considered [3,4,7]. Present case in addition highlights the fact that plasmacytoma is to be considered when plasmacytoid cells are identified in EUS-FNAs even when previous diagnosis of myeloma or symptoms pertaining to myeloma is not there at presentation. Immunocytochemistry can be used as an ancillary technique to diagnose with certainty in such cases. Presurgical diagnosis is essential as the management varies between primary pancreatic carcinoma and plasmacytoma.

CONCLUSION(S)

This case highlights the importance of considering other causes of pancreatic head mass than a primary carcinoma in a patient with obstructive jaundice. It also emphasises the fact that pathologists must be aware of the existence of pancreatic plasmacytoma as initial manifestation of myeloma despite its rarity, a high index of suspicion being essential.

REFERENCES

[1] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). IARC: Lyon 2017.
[2] Pallavi R, Ravella PM, Popescu MA. An unusual pancreatic mass: A case report and literature review. Transl Gastrointest Cancer. 2014;3:106-10.
[3] Hue SSS, Azhar K. Plasmacytoma of the pancreas: An unusual manifestation of multiple myeloma. Singapore Med J. 2013;54:105-07.
[4] Padda MS, Milles T, Adeniran AJ, Mahootic S, Aslaniana HR. Pancreatic and gastric plasmacytoma presenting with obstructive jaundice, diagnosed with endoscopic ultrasound-guided fine needle aspiration. Case Rep Gastroenterol. 2010;4:410-15.
[5] Lu T, Pu H, Zhao G. Primary pancreatic plasmacytoma: A rare case report. BMC Gastroenterol. 2017;17:167.
[6] Smith A, Hal H, Frauenhoffer E. Extramedullary plasmacytoma of the pancreas: A rare entity. Case Reports in Radiology. 2012;2012:798264.
[7] Williet N, Dumas O, Rinaldi L. Difficult endoscopic diagnosis of a pancreatic plasmacytoma: Case report and review of literature. World Journal of Clin Oncolog. 2017;8:91-95.
[8] Kim JH, Paik WH, Joo M, Kim JG, Kim JW, Bae WK, et al. Extramedullary plasmacytoma mimicking pancreatic cancer: A case report and literature review. Endosc Ultrasound. 2017;6:269-72.
[9] Dodd LG, Evans DB, Symmans F, Katz RL. Fine-needle aspiration of pancreatic extramedullary plasmacytoma: Possible confusion with islet cell tumour. Diagn Cytopathol. 1994;10:371-75.
[10] Singh S, Rath A, Yadav S. Primary plasma cell leukaemia-Case report and review of the literature. Sultan Qaboos University Med J. 2018;18(3):397-401.
[11] Jain AG, Faisal-Uddin M, Khan AK, Wazir M, Shen Q, Manoucheri M. Plasma cell leukaemia-one in a million: A case report. World J Clin Oncol. 2019;10(3):161-65.
[12] Puria A, Laua M, Zhaoa X, Chana K, Badera K. A rare case of a minimally secretory pasma cell leukaemia with a haemorrhagic gastric plasmacytoma. Leukaemia Research Reports. 2018;9:23-27.

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Original Research Article

Analysis of Bacterial Contamination in Blood Bank: Comparison of Two Different Donor Arm Disinfectant

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Abstract

Bacterial contamination of transfusion products, especially platelets are the most common cause of transfusion associated morbidity and mortality. Minimizing bacterial contamination in blood products is a priority for safe transfusion practices. A prospective study was done comparing the efficiency of two different disinfectant techniques by studying the flora on donor's arm before and after disinfection. This study was undertaken as there are very few reports on this important transfusion protocol from Southern India. Mean number of colonies in post disinfection swab culture with 2% povidone iodine was found to be greater than that of 2.5% Chlorhexidine in 75% isopropyl alcohol. 2.5% chlorhexidine disinfection offers more effective reduction in bacterial contamination than 2% povidone iodine group.

Keywords: Chlorhexidine, Povidone iodine, Bacteremia.

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INTRODUCTION

There are reports to indicate that every year India requires 40 million units of blood but only 4million units are available. Hence the collection and preservation of this precious resource requires utmost care [1]. In India, approximately 57% of transfusion transmitted reactions have been found to be associated with bacterial contamination of donated blood [2, 3].The French Haemovigilance surveillance system cited bacterial transfusion transmission as the most identified cause of transfusion associated deaths [4].In the UK, between 1995 to 2003, bacterial transfusion transmissions accounted for 58% of transfusion reactions [5]. Bacteriological screening tests for platelet units were introduced in the US in 2004 and the study showed that the average prevalence of bacterial contamination in platelets from the whole blood was 33.9/100,000 units, that of platelets from apheresis 51/100,000, while that of red cell concentrates was 2.6/100,000[6].

In India, antisepsis with three-step regimen of spirit-10% povidone iodine-spirit for donor arm is followed currently. The US-FDA has approved use of alcoholic-chlorhexidine as highly effective for donor arm disinfection. However, it is not being used in India. Also, tincture iodine which was used earlier is now discontinued due to its irritant nature [7].

It is observed that Gram positive skin commensals such as coagulase negative Staphylococci and Bacillus cereus are the organisms most often recovered from donated blood which are thought to originate principally through phlebotomy[8]. The introduction of a needle through the skin to collect blood causes the passage of live microbes or the movement of microbes from small skin flaps produced by the needle itself into the collection bags[9].This is found to be effectively reduced by efficient donor arm disinfectants[10].

Approximately 16% of the deaths due to transfusion reactions have been associated with bacterial contamination, which are usually multi-Drug resistant [7]. Hence, it becomes mandatory to analyse the gaps in the process and implement corrective action to prevent them. SHOT(serious hazards of transfusion), BaCON (Bacterial contamination associated with transfusion) and French haemovigilance surveillance system, studies stated that resident skin flora accounted for 90% of platelet concentrates and 70% of red cell concentrates causing transfusion reactions[11,12].

A Blood &Transplant study conducted by NHS revealed reduction in contamination of blood products by 57% using improved disinfection strategies [13].

Review of the literature revealed only a few reports from India on this topic, and since blood safety is a very vital issue, it was thought worthwhile to undertake this study. The aim of this review is to consider the scientific evidence in supporting the efficacy of improved Donor arm disinfection in preventing bacterial contamination of blood.

AIMS & OBJECTIVES

The chief objectives of this study are:

- To assess the rate of bacterial contamination in blood bank setting.
- To compare the results from two different donor arm disinfectant techniques.

METHODOLOGY

This study was a randomised control study using prospective data. 100 subjects were included in the study after calculating the sample size.

The Voluntary donor arrives at the blood bank was tested for eligibility. After obtaining consent form for study, pre-Disinfection sample was collected in sterile swab. Disinfectant was applied for an area of 10cm on cubital fossa in concentric pattern. 2.5% Chlorhexidine in 75% isopropyl alcohol was applied as disinfectant (group 1) and while 70% Alcohol followed by 2% povidone iodine followed by 70%Alcohol was used as disinfectant (group 2). Post-Disinfection sample was collected in sterile swab. Both the swabs were cultured in blood agar and colony count was performed.

Sterile swabs for the project were made on the same day, supplied by the Department of Microbiology. Samples were collected from the cubital fossa of the donor using sterile forceps by gloved hands and immediately plated on 5% Sheep blood agar under bio-safety cabinets with positive pressure and laminar air flow.

All the patients who have received transfusion from the donors of the sample size were followed up for transfusion reactions. Any allergic reactions were to be notified and treated accordingly. To ensure quality periodical controls for swabs and Disinfectant were established. Technical officers were trained on proper method of disinfection techniques. The waste samples were discarded as per hospital's standard waste disposal system.

Data was entered in Microsoft Excel spreadsheet and analysis was done using SPSS software by Microsoft V.22.0. Association between two factors was expressed in form of odds ratio. P<0.005 was considered significant.

OBSERVATION & RESULTS

A total of 98 male and 2 female donors were selected for the study and disinfectant used was randomly assigned to them. Both the pre- and post-disinfection swabs were cultured on blood agar and their efficacy was determined by the number of colonies formed on the blood agar (Table I).

Table-I: Provides the result of swab culture with two disinfectants.

DISINFECTANT USED	n	Colonies-before disinfection	Colonies-after disinfection
Povidone iodine	50	40-210	0-10
Chlorhexidine	50	35-190	0-4

STATISTICAL ANALYSIS

Independent student 't' test was done to compare the colony count of post-disinfection swab culture between 70% Alcohol followed by2% povidone iodine followed by 70% alcohol and 2.5% Chlorhexidine in 75% isopropyl alcohol . Analysis of covariant was done using General linear model on SPSS 24.0 to compare the mean difference in post-disinfection swab culture adjusted to the bacterial load on pre-disinfection swab culture.

Table II presents the mean (SD) of the post-disinfection swab cultures between the two disinfectants and a statistically significant difference was observed. The mean (SD) of the post-disinfection swab culture for 2.5% Chlorhexidine in 75% isopropyl alcohol was 0.2800(0.70102) and 70% Alcohol followed by2% povidone iodine followed by 70% alcohol was 0.9000(1.97174).t=2.095;p<0.05.

Table-II: Comparison of post disinfection swab culture between the two disinfectants

DISINFECTANT USED	MEAN	SD	p
Povidone iodine	0.9000	1.97174	P<0.05
Chlorhexidine	0.2800	0.70102	

Analysis of covariant showed that the mean difference of the post-disinfection swab culture adjusted to the bacterial load on pre-disinfection swab culture was statistically significant F=4.974; p<0.05.

DISCUSSION

The hazards of post transfusion septicaemia became less common when closed plastic bags replaced glass containers for collection of blood. Even though it

has seen a massive decrease, it has not disappeared entirely and there seems to have been a resurgence of interest in it due to modern day increase in genetically improved resistant organisms. It is not always easy to identify the source of bacterial contamination in blood bags. Post transfusion septicaemia can be endogenous which is due to organisms circulating in the blood and in other hand, exogenous post transfusional septicaemia stems from organisms on donors' skin and equipment contamination. Endogenous post transfusional septicaemia is comparatively less than exogenous post transfusional septicaemia due to vigorous donor selection criteria hence extra attention should be given to exogenous post transfusional septicaemia which can be significantly reduced by optimal disinfection of phlebotomy site, since majority of the organisms contaminating the blood products belong to the group of skin commensals[14].

Our skin contains two types of bacterial flora. One is transient flora which can be easily washed away by water and soap. The other is resident flora that requires extensive disinfection with an efficacious disinfectant which we tried to identify in this study.

In our study encompassing 100 donors' disinfection with 2.5% chlorhexidine in 75% isopropyl alcohol was comparatively more efficacious than 70% Alcohol followed by2% povidone iodine followed by 70%alcohol. Mean values of colonies of 70% Alcohol followed by2% povidone iodine followed by 70% alcohol is greater than what was observed in 2.5% Chlorhexidine in 75% isopropyl alcohol in the swab culture of post-disinfection swabs.

We compared our study with similar studies. Lee C K *et al*. reported a relative risk reduction of 3.4% of chlorhexidine over povidone Iodine in disinfection of donors' arm [15].Ramirez Arcos *et al*. also observed that disinfection by70% alcohol followed by chlorhexidine is highly effective having 98.74% of reduction rate in bacterial contamination of blood components than what is seen with isopropyl alcohol and iodine which correlates with the results of our study [16]. Also Benjamin RJ *et al*. proved that the 1.5ml chlorhexidine disinfection method offers more effective reduction in bacterial contamination of apheresis platelets than 70% Alcohol followed by2% povidone iodine followed by 70%alcohol [17]

Chlorhexidine is a broad spectrum agent with cumulative actions, which is recommended in the 'Guidelines on drawing blood' by WHO, Centre For Disease Control (CDC) and Health Care Infection Control Practices Advisory Committee (HIPAC) as an optimal skin disinfectant for variety of uses like phlebotomy, vascular catheterization and pre-op preparation of skin[12].

It is also less irritant and non-staining on skin. We found that both the donors and the staff of the blood bank expressed an increased compliance towards it during the period of this study. On application it disrupts the cell wall and is more effective against gram positive organisms than gram negative organisms. In our study we also detected most of the gram positive organisms are inhibited, but due to the small numbers that were studied, analysis and comparison of data cannot be made at this juncture.

A study done by Lee C K *et al*. detected predominantly Bacillus species and Coagulase-negative staphylococci as the most common pathogens [15]. In this study organisms like Coagulase-negative Staphylococci, Micrococci and Diptheroid were mainly isolated in Pre-disinfection swabs, while only Coagulase-negative staphylococci were identified in post- disinfection swabs. This is explained by the emerging resistance of staphylococci to Chlorhexidine [18]. The use of conventional agar plates and gram-staining to identify the morphology of the organism limits the scope of identifying varied bacterial flora which is more evident on automated culture systems like BACTEC/BacTALERT.

This study prompts us to conclude that there is a decrease in bacterial contamination with 2.5% chlorhexidine in 75% isopropyl alcohol compared to 70% alcohol followed by 2% povidone iodine followed by 70% Alcohol.

Since there is no significant difference in cost between these two methods tested in our study, Chlorhexidine should be affordable in blood banks of resource limited nations as well.

CONCLUSION

- Bacterial contamination of blood components remains a major challenge in the practice of transfusion medicine.
- Optimal skin antisepsis of the phlebotomy site is essential to minimize the risk of contamination of collected blood.
- Every reaction of post transfusional septicaemia must be identified, reported, evaluated and the cause for contamination must be identified in order to prevent future post transfusional septicaemia and maintain safe blood practices.

Compliance with Ethical Standards

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee

Conflict of Interest

The authors declare that they have no conflict of interest

Informed consent

Informed consent was obtained from all individual participants included in the study

REFERENCES

1. India facing a blood shortage of 3 million units of blood. (2016). Retrieved 21 Nov 2016 from http://timesofindia.com.
2. Patel, T. G., Shukla, R. V., & Gupte, S. C. (2013). Impact of donor arm cleaning with different aseptic solutions for prevention of contamination in blood bags. *Indian Journal of Hematology and Blood Transfusion*, *29*(1), 17-20.
3. Hoppe, P. A. (1992). Interim measures for detection of bacterially contaminated red cell components. *Transfusion*, *32*(3), 199-201.
4. Debeir, J., Noel, L., Aullen, J. P., Frette, C., Sari, F., Mai, M. P. V., & Cosson, A. (1999). The French haemovigilance system. *Vox sanguinis*, *77*(2), 77-81.
5. Stainsby, D., Cohen, H., Jones, H., Knowles, S., Milkins, C., Chapman, C., & Revill, J. (2003). Serious hazards of transfusion (SHOT). *SHOT annual report*, 1-88.
6. Blajchman, M. A., Ali, A. M., & Richardson, H. L. (1994). Bacterial contamination of cellular blood components. *Vox sanguinis*, *67*, 25-33.
7. Shah, S., Mehta, N. A., & Jadhav, S. G. (2014). Prospective evaluation of 2%(w/v) alcoholic chlorhexidine gluconate as an antiseptic agent for blood donor arm preparation. *Asian journal of transfusion science*, *8*(2), 92.
8. Leo, A. M., Salvadego, M. M., Piva, M. G., Ruffato, G., Valverde, S., Trabuio, E., ... & Gessoni, G. (2007). From the donor's arm to blood product: a study on bacterial contamination of apheresis platelet concentrates. *Blood Transfusion*, *5*(3), 130.
9. Stramer, S. L. (2007). Current risks of transfusion-transmitted agents: a review. *Archives of pathology & laboratory medicine*, *131*(5), 702-707.
10. McDonald, C., McGuane, S., Thomas, J., Hartley, S., Robbins, S., Roy, A., ... & Barbara, J. (2010). A novel rapid and effective donor arm disinfection method. *Transfusion*, *50*(1), 53-58.
11. Perez, P., Salmi, L. R., Folléa, G., Schmit, J. L., De Barbeyrac, B., Sudre, P., ... & Bacthem Group the French Haemovigilance Network. (2001). Determinants of transfusion-associated bacterial contamination: results of the French BACTHEM Case-Control Study. *Transfusion*, *41*(7), 862-872.
12. McDonald, C. P. (2011). Interventions implemented to reduce the risk of transmission of bacteria by transfusion in the English National Blood Service. *Transfusion Medicine and Hemotherapy*, *38*(4), 255-258.
13. Dubey, A., Sonker, A., & Chaudhary, R. (2017). Snake in the grass: A case report of transfusion reactions due to contaminated donor arm disinfectant. *Asian journal of transfusion science*, *11*(1), 50.
14. Puckett, A., Davison, G., Entwistle, C. C., & Barbara, J. A. (1992). Post transfusion septicaemia 1980-1989: importance of donor arm cleansing. *Journal of clinical pathology*, *45*(2), 155-157.
15. Lee, C. K., Ho, P. L., Chan, N. K., Mak, A., Hong, J., & Lin, C. K. (2002). Impact of donor arm skin disinfection on the bacterial contamination rate of platelet concentrates. *Vox sanguinis*, *83*(3), 204-208.
16. Ramirez-Arcos, S., & Goldman, M. (2010). Skin disinfection methods: prospective evaluation and postimplementation results. *Transfusion*, *50*(1), 59-64.
17. Benjamin, R. J., Dy, B., Warren, R., Lischka, M., & Eder, A. F. (2011). Skin disinfection with a single-step 2% chlorhexidine swab is more effective than a two-step povidone-iodine method in preventing bacterial contamination of apheresis platelets. *Transfusion*, *51*(3), 531-538.
18. Goldman, M., Roy, G., Frechette, N., Decary, F., Massicotte, L., & Delage, G. (1997). Evaluation of donor skin disinfection methods. *Transfusion*, *37*(3), 309-312.

Guidance for clinical case management of thrombosis with thrombocytopenia syndrome (TTS) following vaccination to prevent coronavirus disease (COVID-19)

Interim guidance

19 July 2021

World Health Organization

WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance document will expire 2 years after the date of publication.

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Abbreviations and acronyms

AEFI	Adverse event following immunization
aHIT	Autoimmune heparin-induced thrombocytopenia
Ad26.COV2-S vaccine	Johnson & Johnson (J&J) Janssen COVID-19 Ad26.COV2-S vaccine
APTT	Activated partial thromboplastin time
BC	Brighton Collaboration
ChAdOx-1 vaccine	AstraZeneca COVID-19 ChAdOx-1 vaccine
CT scan	Computerized tomography scan
CTPA	CT pulmonary angiogram
CVST	Cerebral venous sinus thrombosis
DIC	Disseminated intravascular coagulation
DVT	Deep vein thrombosis
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EtD	Evidence-to-decision-making
FEU	Fibrinogen Equivalent Units
GACVS	Global Advisory Committee on Vaccine Safety
GDG	Guideline Development Group
GRADE	Grading of Recommendations Assessment, Development and Evaluation (a method of assessing the certainty of evidence)
HIT	Heparin induced thrombocytopenia
ITP	Idiopathic thrombocytopenic purpura
IVB	Immunizations, Vaccines and Biologicals Department
IVIG	Intravenous immunoglobulin
LMICs	Low-and-middle income countries
MRI	Magnetic resonance imaging
MSD	Mental Health and Substance Use Department
NCD	Noncommunicable Diseases Department
NHAC	Non-heparin-based anticoagulants
PCR	Polymerase chain reaction
PE	Pulmonary embolism
PF4	Platelet factor 4
PICO	Patient/intervention/comparator/outcome)
QNS	Quality Norms and Standards
RPQ	Regulation and Prequalification
SMR	Standardized morbidity ratio
SVT	Splanchnic vein thrombosis
TTP	Thrombotic thrombocytopenic purpura
TTS	Thrombosis thrombocytopenia syndrome
WHE	WHO Health Emergencies Programme

Key points

Background	Thrombosis with thrombocytopenia syndrome (TTS) has been reported in individuals vaccinated with COVID-19 non-replicant adenovirus vector-based vaccines (AstraZeneca COVID-19 ChAdOx-1 vaccine and Johnson & Johnson (J&J) Janssen COVID-19 Ad26.COV2-S vaccine).
Scope	The present document aims to provide interim guidance on the recognition and management of thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination.
Case definition	TTS is defined by the presence of a thrombosis/thromboembolism, generally in uncommon locations, such as cerebral venous sinus or splanchnic veins and marked thrombocytopenia (<50 x 10^9/L) following vaccination with a COVID-19 non-replicant adenovirus vector-based vaccine. Cases of thrombosis/thromboembolisms (i.e., pulmonary, deep vein thrombosis, coronary arteries, cerebral arteries) in common location have also been reported following vaccination with a COVID-19 non-replicant adenovirus vector-based vaccine.
Incidence	The cumulative incidence of TTS following vaccination with a non-replicant adenovirus vector-based vaccine ranges from 0.5 to 6.8 cases per 100 000 vaccinees. Incidence rates differ depending on the vaccine, age, sex, geographical distribution and interpretation of the case definition. The observed-to-expected rate is higher following vaccination with the ChAdOx-1 vaccine, in females and in patients aged <60 years. Most TTS cases have been reported within 3 to 30 days following vaccination with a COVID-19 non-replicant adenovirus vector-based vaccine. Information from low- and middle-income countries will be fundamental for understanding the incidence of TTS better, given that the adenovirus vector-based vaccines have been used more extensively in these countries.
Risk factors	The main risk factors for TTS following vaccination with COVID-19 adenovirus vector-based vaccines are the use of non-replicant adenovirus vector-based vaccines and younger age. There is currently no evidence that traditional risk factors for thrombosis/thromboembolisms increase the risk of TTS in this context.
Pathophysiology	TTS has been associated with the presence of anti-platelet factor 4 (anti-PF4) antibodies. There are similarities with autoimmune heparin-induced thrombocytopenia (aHIT). TTS may be caused by the binding of anti-PF4 to platelets, causing platelet activation and aggregation, thrombosis, platelet consumption, and thrombocytopenia. However, the exact mechanisms are still unclear and should be further investigated.
Clinical presentation	TTS should be suspected in patients presenting with severe and unusual headache, abdominal pain with or without vomiting, sudden onset of breathing difficulty, chest pain or limb pains, particularly in those aged under 60 years, within four weeks following vaccination. Patients with suggestive clinical symptoms should promptly undergo investigations to rule out thrombotic events and presence of thrombocytopenia.
Laboratory diagnosis	Individuals who present with thrombosis within four weeks following vaccination should be evaluated for thrombocytopenia, increased D-dimer and positive anti-PF4 antibodies. An enzyme-linked immunosorbent assay (ELISA) should be used to detect anti-PF4 antibodies, as rapid immunoassays are not as sensitive. The presence of anti-PF4 antibodies in a patient with a thrombotic event and thrombocytopenia following COVID-19 vaccination is highly suggestive of TTS. Other biomarkers can be helpful in the laboratory diagnosis of TTS, including D-dimer, fibrinogen, and blood smear to confirm reduced platelets and to rule out platelet clumping. The case definition implies the absence of a better alternative explanation for the condition.

Imaging	Suitable imaging examinations should be performed in patients with suspected TTS as soon as possible, depending on anatomical location, especially in those who present with thrombocytopenia within 30 days post-vaccination.
Clinical case management	Vaccinated individuals should be advised to seek immediate medical attention if they develop symptoms including severe or persistent headache, blurred vision, shortness of breath, chest pain, leg swelling, persistent abdominal pain or unusual skin bruising and/or petechiae (tiny purple, red, or brown spots on the skin) occurring within four weeks after vaccination, although some cases have been reported later than 30 days post-vaccination. These patients should be investigated for thrombosis and thrombocytopenia. Reporting these symptoms must be made easy for the vaccine recipients, and could include helplines and hospital vaccine centre, online reporting systems.
Treatment	WHO advises against the use of heparin in individuals with TTS in the context of COVID-19 vaccination (*conditional recommendation, very low certainty*). WHO recommends against the use of platelet infusion for individuals with TTS in the context of COVID-19 vaccination in all cases other than emergency situations where surgery is strongly indicated, thrombocytopenia is severe (platelets <50 000/μL), and platelet transfusion is required to be able to proceed with emergency surgery *(strong, very low certainty).* WHO recommends the use of intravenous immunoglobulins (IVIG) and/or non-heparin-based anticoagulants for individuals with TTS following COVID-19 vaccination *(strong, very low certainty).* WHO does not provide any recommendation for steroid treatment, but notes the general use of steroids and the likelihood that steroids will usually be given in combination with other treatments.

Background, scope, and rationale

Since March 2021, cases of thromboses associated with thrombocytopenia have been reported in patients vaccinated with the Oxford-AstraZeneca ChAdOx1-S and Johnson & Johnson (J&J) Janssen Ad26.COV2-S COVID-19 vaccines. Evaluation of the cases by national and international bodies concluded that there was a plausible causal link between these two adenovirus vectored vaccines and the events (1-3).

The association was based on the temporal association with vaccination, an increased incidence when compared with expected baseline rates, for cerebral venous sinus thrombosis (CVST), the presence of simultaneous multiple thromboses in some patients, the presence of thrombocytopenia and anti-platelet factor 4 antibodies (anti-PF4), and a higher mortality rate than that reported in the literature (1-30).

The purpose of this document is to provide interim guidance on the recognition and clinical management of this rare adverse event, known as thrombosis with thrombocytopenia syndrome (TTS), following vaccination. This newly reported syndrome has received different names, including vaccine-induced immune thrombotic thrombocytopenia (VIITT), vaccine-induced prothrombotic immune thrombocytopenia (VIPIT), and vaccine-induced thrombotic thrombocytopenia (VITT). **In the present document, the term TTS will used in the context of COVID-19 adenovirus-vector vaccines unless otherwise specified.**

Knowledge about TTS following vaccination with a COVID-19 adenovirus vector-based vaccine is rapidly evolving. This document aims to increase awareness about TTS in the context of COVID-19 vaccination and thereby help healthcare providers in the assessment and management of potential TTS cases. Individuals and healthcare providers must be aware of the symptoms of possible TTS to enable prompt diagnosis and early treatment. Healthcare providers should be aware of the relevant diagnostic tests and know which treatments should be given and which should be avoided. This document reviews the existing information on the epidemiology, risk factors, aetiology, diagnosis and clinical management protocol for TTS with specific considerations for low-and-middle income countries (LMICs). It will be revised as new evidence emerges. Detailed data regarding the methodology used in the guideline development is available in Annex 5: Methods for guideline development. TTS is a very rare adverse event following immunization (AEFI) and the benefits of COVID-19 vaccination clearly outweigh the potential risks.

Case definition

An interim case definition that could be practical to use in clinical settings has been developed with input from a representative of the Brighton Collaboration (BC) group, to ensure the harmonization with the BC definition of TTS (*version 10.16.3-May-23-2021*). The present interim case definition aims to provide an optimal balance between sensitivity and specificity while ensuring applicability across all resource settings. It is based on the existing evidence and as needed, can be updated with the publication of new cases and data (32).

It is important to note that there is a difference between the clinical management of a potential case, and the case definition. The clinical management algorithms (see below) aim to identify all possible cases with the intent of offering the most adequate treatments while continuing to confirm or rule out TTS, and avoid potentially harmful treatments. Since platelet counts can evolve over time and results for some investigations are not available instantly, the classification of cases should ideally be finalized once all available and existing data have been analysed. The investigations must be repeated if symptoms do not resolve.

The definition of TTS is based on the combined presence of a thrombosis and new onset thrombocytopenia (**Table 1**). Three levels of certainty are proposed, based on the anatomical location of the thrombosis, the severity of thrombocytopenia and the outcome of laboratory investigations (**Table 2**). The most common thromboses in the general population are limb vein thrombosis, pulmonary artery/vein thrombosis, cerebral artery thrombosis or myocardial artery thrombosis. However in the case of TTS, the thromboses have been observed mainly in cerebral and splanchnic veins. Multiple-organ thromboses have also been observed, although less commonly. In view of these observations, the term ‘unusual location’ thrombosis is used to describe the thromboses in TTS.

WHO classification of TTS following vaccination with a COVID-19 vaccine is based on the degree of certainty (**Table 2**). It includes three mandatory criteria (A, B and C) with C defining the degree of certainty based on the combination of major and minor criteria presented in **Table 1**:

A. Vaccination against COVID within last 30 days.
B. No alternative explanation for the condition (i.e., no heparin exposure within the previous 100 days).
C. Combination of thrombosis and thrombocytopenia.

Table 1: Major and minor criteria for thrombocytopenia, thrombotic events and laboratory examinations.

Classification	Major criteria	Minor criteria
Thrombosis	**CONFIRMED** diagnosis of thrombosis by imaging study, surgical, or pathology findings consistent with thrombosis/thromboembolism in an **uncommon location**: • cerebral veins OR • splanchnic veins OR • multiple organ	**CONFIRMED** diagnosis of thrombosis by imaging study, surgical, or pathology consistent with thrombosis/thromboembolism in a **common location:** • pulmonary arteries/veins OR • limb veins OR • coronary arteries OR • cerebral arteries OR • other arteries/veins OR **SUGGESTIVE** thrombosis by supporting imaging or laboratory findings **suggestive but not definitive** of thrombosis/thromboembolism in any location OR **SUGGESTIVE** thrombosis by specific **clinical syndromes** consistent with thrombosis or thromboembolism event in any location
Thrombocytopenia	Platelet count: <50 x 10^9/L AND Confirmatory peripheral smear showing reduced platelets AND No evidence of platelet clumping	Platelet count: > 50 x 10^9/L - <150 x 10^9/L OR >50% decrease from baseline platelet count
Laboratory (other than thrombocytopenia)	Positive anti-platelet factor 4 antibodies (with ELISA) or platelet functional assay (i.e., serotonin release assay)	D-dimer > 4000 µg/L fibrinogen equivalent units (FEU)

Table 2. WHO classification of TTS following vaccination with a COVID-19 vaccine is based on the degree of certainty.

Classification	Level 1 (Confirmed case)		Level 2 (Probable case)		Level 3 (Possible case)
Thrombosis	Major / Minor	Major	Minor	Major	Minor
Thrombocytopenia	Major / Minor	Major	Major	Minor	Minor
Laboratory (Other than thrombocytopenia)	Major	Minor	Minor	Minor	Minor / No laboratory

Cases with incomplete investigation or the presence of other possible explanations should be classified as possible cases of TTS until better evidence is available. Detailed information on these diagnostic criteria including imaging modalities, surgical and pathological findings and clinical symptoms for TTS are available in more detail below.

The clinical diagnosis of TTS is summarized in Figure 1.

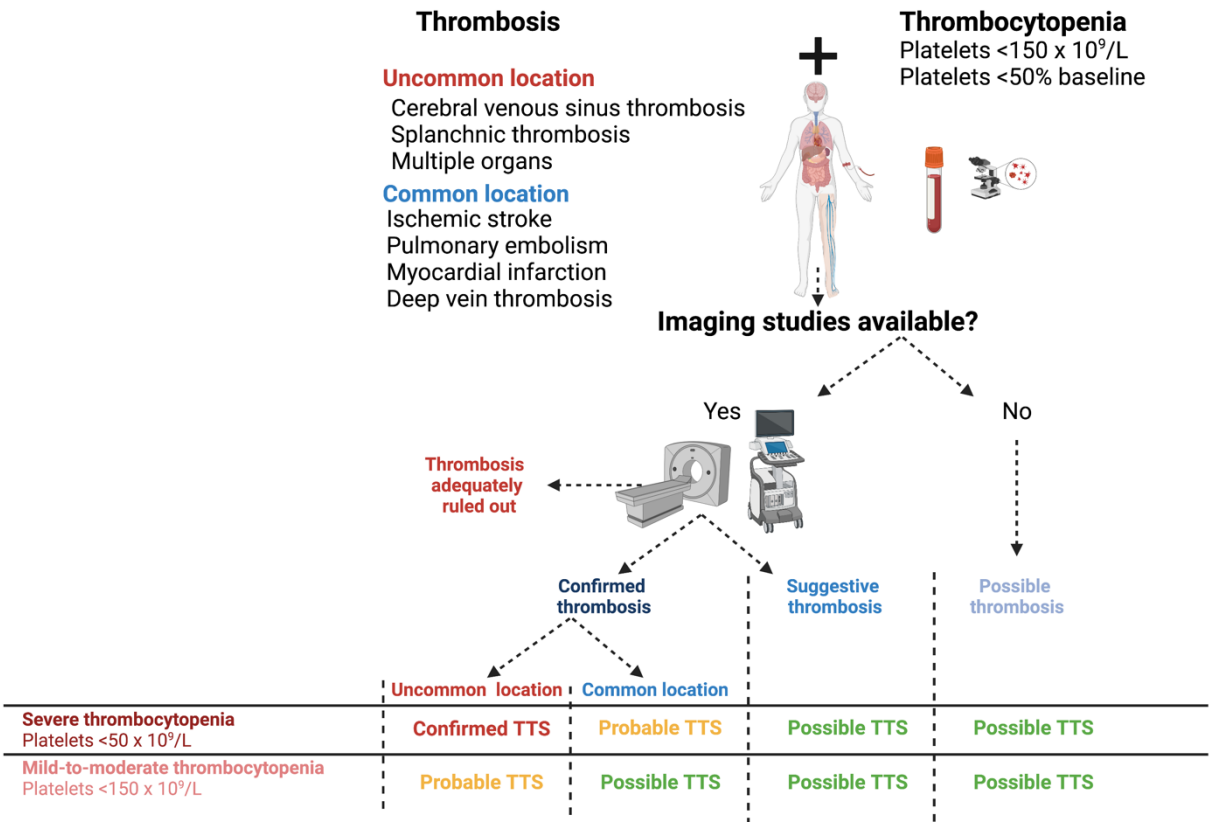


Figure 1: Algorithm for the clinical diagnosis of thrombosis thrombocytopenia syndrome

Figure created with BioRender.com

Incidence

Overview

The epidemiology of TTS is a rapidly evolving field, and its interpretation requires special considerations. The incidence may be influenced by the patient profile in each country and the specific vaccines used. The risk may depend on age, sex or other factors. Public awareness may also influence the clinical outcome as patients who present earlier could receive treatment sooner, resulting in lower morbidity/mortality. In most studies and official reports, the observed case rates are from spontaneous notifications and therefore these are the notification rates rather than with the real case rates, which may be under-estimated due to underreporting. In addition, the epidemiology of uncommon thrombotic events, such as splanchnic thrombosis, is not well established in the general population, so increased risk may be difficult to estimate. In addition, a precise denominator is needed, i.e., the numbers of those vaccinated in different age groups and by gender. A study analysing the background rates of TTS from eight countries reported significant population-level heterogeneity in the rates, suggesting that estimates from one country or region should be interpreted with caution, due to systematic error associated with the databases used to estimate the control rates (33-35).

TTS incidence can be estimated using the crude cumulative incidence per 100 000 persons or the standardized morbidity ratio (SMR), also known as observed-to-expected analysis, that analyses the ratio between the observed number of cases in the population and the number of cases that would be expected according to baseline incidence, with a 95% confidence interval (CI) (1-3).

Incidence of CVST in the general population and in COVID-19 patients

The incidence of CVST in the general population is estimated to be between 1.2 and 2.0 cases/100 000 person/year (36). In a national study done in the United States of America from 2006 to 2016, the incidence varied by sex (more frequent in women), age (more frequent between 18-44 years) and race (more frequent in African American > Caucasian > Asian). The incidence of venous thromboembolism has been reported to be almost 70% less frequent in southeast Asian populations compared to those of European descent (36).

Thrombotic complications are frequent in patients with active or recent severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection (37). Thrombocytopenia (<150 000 platelets/µL) has been reported to occur in about 15% of COVID-19 patients with CVST. The relative risk of having CVST for patients with COVID-19, compared with those without, is estimated to be at least 14 times higher. The incidence of CVST in COVID-19 patients was analysed in a study collecting real-time data from electronic medical records between 24 March 2020, and 1 March 2021. Among the 667 551 COVID-19 patients at risk there were 42 CVST cases and among the 65 796 480 non-COVID patients there were 1022 cases (rate 0.0001 vs. 0.000002, OR: 41.0; 95% CI: 30.1-55.8), with a higher mortality rate in the COVID-19 group (11.9% vs. 2.8%, OR: 4.6; 95% CI: 1.3-13.0) (35). Thrombocytopenia may also be present in patients with COVID-19 infection, including patients with thrombotic complications, however it is generally milder than thrombocytopenia reported in patients with TTS (37). The presence of an active SARS-CoV-2 infection was ruled out in most TTS cases reported to date (1-30).

Incidence of CVST following vaccination with a COVID-19 non-replicant adenovirus-vectored vaccine

Table 1 in Annex 4 summarizes the existing evidence from studies reporting the incidence of TTS after vaccination with a non-replicant adenovirus vector-based COVID 19 vaccine as of 27 May 2021 (18, 39, 41–48). A report from the European Medicines Agency (EMA), published on 8 April 2021, assessed the number of reported cases in the European Economic Area member states and the United Kingdom up to 22 March 2021, using databases from Italy (Agenzia Regionale di Sanità, ARS) and Spain (Foundation for the Promotion of Health and Biomedical Research of Valencia Region, FISABIO) as comparators, reported a global observed-to-expected rate of 7.73 (95% CI: 5.3-10.8) per 100 000 persons over 14 days (7). A population study conducted in Denmark and Norway in patients who received the first dose of ChAdOx-1 vaccine, from 9 February 2021 to 11 March 2021, reported an observed-to-expected incidence of 20.25 (95% CI: 8.14-41.73) cases within 28 days of vaccination, and an excess of 2.5 (95% CI: 0.9-5.2) CVST cases per 100 000 vaccinations over 28 days. The observed-to-expected rate for venous thromboembolic events was 1.97 (95% CI: 1.5-2.54) with 11 (95% CI: 5.6-17.0) excess events per 100 000 doses (38).

The incidence of CVST after vaccination with a non-replicant adenovirus vector-based COVID 19 vaccine is also proportionally higher in women (although this may have been due to vaccination of priority groups that may have included more women) and patients aged <60 years (1, 2). The incidence of cerebrovascular events per 100 000 person-years after ChAdOx1-S vaccination was higher in women (29.4; 95% CI: 19.8-42.0) compared with men (6.2; 95% CI: 1.3-18.0) within one month in a German study (18). The median age was 40 years, with few cases occurring in patients aged >60 years. This study reported a CVST rate of 20.52 (95% CI: 5.59-52.5) per 100 000 person-years in females aged >60 years, but not in males aged >60 yeas (18). The EMA signal assessment report published on 8 April 2021, reported an increased SMR for CVST over 14 days, in patients vaccinated with the ChAdOx1-S COVID-19 vaccine, compared with the background rate, particularly in patients aged 18-60, in patients with and without thrombocytopenia. The association with disseminated intravascular coagulation (DIC) or other embolic and thrombotic events was inconclusive at that time.

Most cases of TTS have been reported after the first dose of ChAdOx-1, but there are still insufficient data to establish whether the risk of TTS differs between the first and second dose because substantially fewer second doses of the ChAdOx-1vaccine have been given. The Ad26.COV2-S vaccine is administered as a single dose. Data about Ad26.COV2-S was initially reported in the United States of America. The Center for Disease Control and Prevention reported 28 confirmed TTS cases among 8 739 657 people vaccinated, as of 12 May 2021, which appears less frequent than for ChAdOx1-S, with the highest rate reported being 12.4 cases per million doses for women aged 30-39 years (39). At the time of publication of this interim guidance, no TTS cases have been reported following vaccination with other adenovirus vector-based COVID-19 vaccines or mRNA COVID-19 vaccines (40). It is also important to acknowledge that although TTS events have been reported following vaccination with the two adenovirus vector-based vaccines, other vaccines using adenovirus as a vector, should also be under careful surveillance since the absence of TTS cases may be due to underreporting, given the absence of well-established pharmacovigilance systems in some countries. Country-level active surveillance could be considered in addition to passive surveillance systems.

Risk factors

The main risk factors for TTS are age (41-48) and the type of non-replicant adenovirus vector-based COVID-19 vaccine, with a higher risk associated with ChAdOx1-s than with Ad26.COV2-S (1-30). To date, there is no evidence that supports an increased risk of vaccine-related TTS or a more severe clinical presentation in patients with pre-existing thrombotic risk factors. The frequency of thrombotic risk factors in TTS (approximately 30%) does not appear to differ from the reported frequency of thrombotic risk factors (37-84%) in patients with thrombotic events unrelated to vaccination (49-58). However, the presence of additional causes for the thromboses must be evaluated according to the local standard of care, to ensure that patients are treated appropriately, whenever necessary, particularly in common location thromboses, such as deep vein thrombosis (DVT) or pulmonary embolism (PE). The list of thrombosis risk factors is available in Annex 1.

Pathophysiology

The aetiology of TTS is thought to be immune-mediated (59). The presence of anti-PF4 antibodies, the temporal association with immunization, the clinical onset of symptoms and the presence of multiple thromboses supports this hypothesis. Since there are several similarities with autoimmune heparin-induced thrombocytopenia (aHIT), it is thought that the pathophysiology of TTS is similar to that of aHIT's. Hence, like for aHIT, TTS may be caused by the binding of PF4 antibodies to an unknown (or yet to be described) polyanion, which then leads to a conformational change in the PF4 antibodies which reveals a new antigen, resulting in IgG antibodies being developed against this neo complex of polyanion-PF4 antibodies (4–6, 10, 59–64). The PF4-polyanion bound to the anti-PF4 antibodies subsequently binds to the Fc-gamma receptors of platelets, thereby crosslinking the platelets which results in platelet activation and aggregation. This leads to platelet consumption and thrombocytopenia as well as microparticle production and thrombin generation that contributes to development of thrombosis (59-69). There are clinical and laboratory similarities between TTS and aHIT, however, the explanation for unusual intracranial or splanchnic vein locations is currently unknown. Some of the cases reported had normal platelet counts but positive anti-PF4 antibodies/abnormal platelet function test or a highly suggestive clinical presentation but were negative for anti-PF4 antibodies (1–30). It is still unclear whether cases without thrombocytopenia are variations of the same syndrome or due to a different cause. The technique and the timing of laboratory tests may partially explain the negative results. (69–74)

Clinical presentation

The most specific elements of TTS are the **delayed onset** after vaccination and **greater severity** (1-30). Most adverse events and symptoms following immunization occur within the first 72 hours and tend to resolve spontaneously, while vaccine-related TTS typically presents after these have resolved. The median time from vaccination to symptom onset is 8 to -9 days with a range of 1 to 37 days. As of 27May 2021 only one of the 21 case series reported cases that occurred within the first 72 hours (18). Onset within the first 72 hours was described for 4 of the 62 cases who had all received the ChAdOx-1 vaccine in that study (18), while the remaining 165 cases reported so far, all occurred within 3 to 25 days, based on the available data, with one case occurring after day 30, reported on day 37 (1-30). Cases could be expected to occur within 30 days, the estimated elimination time of antibodies. Therefore, any case of concurrent thrombosis and thrombocytopenia within 30 days after vaccination with an adenovirus vector-based vaccine must be managed as a potential TTS case, with platelet count monitoring. However, cases occurring between 30-100 days should also be carefully monitored.

TTS is associated with a more severe thrombosis profile, with greater extension of thrombi and a higher frequency of concomitant intracranial haemorrhage in the case of CVST. Mortality rates (20-25%) are two-to-three times higher compared with non-vaccine related thrombotic events, however, there may be a significant reporting bias and the reasons for these findings are still yet to be clarified. In addition, the use of heparin-based anticoagulants could have influenced the clinical outcome of the initial cases that have been reported. Importantly, the clinical symptoms of thromboses in TTS **may be similar** to those of thrombotic events in the general population (75-82).

Table 2 in Annex 4 summarizes the clinical presentation of the most common locations of thrombosis in TTS. Possible symptoms and signs must be recognized, however the clinical evaluation is not sensitive or specific, so a high index of suspicion applies to in patients at risk for TTS. As the clinical symptoms for TTS are non-specific and can be associated with other conditions, physicians should be aware of the possibility of TTS in patients who have received a COVID-19 vaccine.

1. Cerebral venous sinus thrombosis

The most frequently reported vaccine-related TTS location is CVST. The most frequent symptom of CVST is headache (50%), mostly severe, which typically presents with other symptoms or signs (Table 2 in Annex 4, Figure 2). Vaccine-related CVST presents with an increased rate of intracranial haemorrhage (approximately 40% of patients) and a higher mortality rate, compared with non-vaccine related CVST. The mortality rate of CVST in patients with COVID-19 is similar to that in patients with vaccine-related TTS, however, thrombocytopenia is less frequent in COVID-19-related CVST and when present, tends to be less marked (between 100 000 and 150 000 platelets per µL).

2. Splanchnic vein thrombosis

The second most common TTS involves splanchnic vein thrombosis (SVT), including portal, superior mesenteric and/or splenic veins, reported in approximately 30% of cases. The symptoms of SVT are expected to be similar in vaccine-related TTS and TTS related to another aetiology, with the most frequent symptom being abdominal pain.

3. Deep vein thrombosis and pulmonary embolism

DVT and PE have been described in the reported cases of patients with TTS following vaccination with an adenovirus vector-based COVID-19 vaccine. The clinical symptoms of the DVT and PE are non-specific, and these symptoms are as likely to be due to DVT or PE as other alternate diagnoses. Consequently, clinical prediction tools, such as the Wells score for PE and DVT, which takes into consideration the symptoms and signs of DVT/PE, risk factors, and the possibility of an alternate diagnosis, have been derived and validated. Patients who are determined to be 'likely' based on such prediction scores should undergo further investigation with appropriate imaging to confirm or exclude DVT or PE.

4. **Multiple organ thromboses**

Approximately 20-25% of patients with vaccine-related TTS have multiple-organ thromboses, which requires thorough clinical examination and appropriate investigations, as necessary.

Laboratory diagnosis

1. Platelet count

Haemogram (complete blood count) must be done for all patients. Thrombocytopenia is defined as a platelet count <150 000/µL or a 50% decrease from a recent previous platelet count, if available. In most of the reported cases, the platelet nadir was <50 000 platelets/µL, with a median value around 25 000 platelets/µL (83-88).

A few of the reported cases had a normal platelet count but they were positive for anti-PF4 antibodies which was not explained by another cause of thrombosis. If the platelet count is normal, follow-up haemograms must be repeated daily as the patient might be in the early stages of TTS. In inconclusive cases, an increased D-dimer values may be equally suggestive and additional laboratory parameters may be helpful (1-30).

2. Blood smear/film

A peripheral blood smear should be done to rule out a pseudo thrombocytopenia, caused by platelet clumping. The presence of schistocytes (fragmented red blood cells) was reported in one case of TTS (6).

3. D-dimer

A D-dimer value of four times the upper limit of the normal range (i.e., usually >4000 µg/L FEU (fibrinogen equivalent units)) is also highly suggestive, and a value of between 2000 and 4000 µg/L FEU may be suggestive (3-6).

4. Anti-platelet factor 4 antibodies

The presence of anti-PF4 antibodies, in the absence of heparin therapy, is highly specific for TTS (4–6). However, the sensitivity of the assay is influenced by the type of technique used (ideally based on ELISA,

since rapid immunoassays are neither sensitive nor specific and should not be used, if possible) and the timing of the analysis. The anti-PF4 ELISA must be done on a sample taken prior to the administration of intravenous immunoglobulin (IVIG) treatment, as IVIG can interfere with the results.

5. Fibrinogen

Fibrinogen concentrations tend to increase in TTS, but when the blood fibrinogen concentrations decrease or remain low (<1.5 g/L), this should be considered a sign of worsening TTS.

6. Other coagulation parameters: prothrombin time, activated partial thromboplastin time

Prothrombin time (PT) and activated partial thromboplastin time (APTT) should be evaluated whenever possible, as part of a differential diagnosis for other coagulation disorders, such as DIC.

7. SARS-CoV-2 tests

SARS-CoV-2 real-time polymerase chain reaction (PCR) test on a oropharyngeal swab sample should be done for all patients with a suspected TTS and the result provided at the time of the notification. Serum antibody testing should also be considered to evaluate potential past exposure to SARS-CoV-2.

Table 3 in Annex 4 summarizes the laboratory differential diagnosis between TTS, immune thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP) and DIC. Patients with isolated thrombocytopenia and continued absence of thrombosis may have post-vaccination ITP and not TTS. Other entities that should be included in the differential diagnosis of thrombosis/disseminated thrombosis and thrombocytopenia are listed in Table 4 in Annex 4.

Imaging diagnosis

Imaging and clinical workup for thrombosis should be based on the symptoms and location. Table 5 in Annex 4 summarizes the specific diagnostic modalities consistent with a confirmed diagnosis of thrombosis or thromboembolism and the diagnostics modalities that may be supportive but not definitive (89-98).

Table 6 in Annex 4 summarizes the typical and specific radiological signs and the optimal and/or alternative imaging modalities that may be useful for TTS diagnosis. The concept of optimal is based on sensitivity, specificity and availability. In case of suspected CVST, imaging studies should be done promptly, not only for confirming/supporting the diagnosis, but also to rule out the presence of intracranial findings that might increase the risk of complications or may need urgent neurosurgical treatment. This should be considered in patients with altered fundoscopy, decreased level of consciousness, seizures or focal neurological symptoms/signs.

Clinical case management

Patients with suspected TTS within 30 days post-vaccination must be referred urgently to a hospital emergency room/urgent care for evaluation. Management of patients with suspected TTS may benefit from multidisciplinary evaluation, whenever available, including haematology, neurology, neurosurgery, radiology, intensive care, internal medicine and emergency department clinicians. Patients must be hospitalized and whenever possible, transferred to a tertiary-care hospital equipped with all the facilities listed above (83, 84, 99–102).

Clinical evaluation of patients must include the presence of symptoms and signs of thrombosis. CVST may present with headache, visual disturbances, seizures, altered mental status, decreased level of consciousness, focal neurological symptoms and/or vomiting; splanchnic thrombosis may present with abdominal pain, nausea, diarrhoea/constipation, fever, anorexia, back pain or gastrointestinal bleeding; deep vein thrombosis manifesting as limb swelling, pain or tenderness, redness and vein distension; pulmonary embolism may present as dyspnea, chest pain, difficulty to perform physical exercise, haemoptysis, syncope or palpitations; myocardial infarction may present with chest or left arm pain, shortness of breath or cyanosis; and ischemic stroke may present as sudden onset focal neurological symptoms (for further information, please see Table 2 in Annex 4). In settings of high COVID-19 incidence and transmission, patients should be tested for COVID-19, and, whenever possible, fundoscopy carried out to assess for papilledema. Imaging studies should be requested based on the clinical index of suspicion and should not be delayed while waiting for the PCR result or the fundoscopy. The different diagnostic options, including the optimal and alternative tests are described in Table 6 in Annex 4. Patients must be monitored closely, since new-onset thrombosis might occur, and multiple thromboses have been reported in 20-25% of patients.

If a venous or arterial thrombosis is diagnosed or suspected and has a temporal relationship with vaccination with an adenovirus vector-based COVID-19 vaccine, laboratory workup should be requested as outlined above, with at least a **haemogram** (full blood count), and, whenever possible, a D-dimer test. If the index of suspicion for TTS remains high in a patient who presents with a normal platelet count, then **the platelet count must be rechecked at least daily.** Additional laboratory tests that can be done include peripheral blood smear, D-dimer test, fibrinogen concentration, additional coagulation parameters and anti-PF4 antibodies. The differential diagnosis of thrombosis, based on the laboratory results is described in Table 3 in Annex 4.

It is important to consider that patients with high D-dimer and persistently low platelet count, or organ specific laboratory abnormalities (such as increased liver enzymes) may have subtle or paucisymptomatic thrombosis.

Reporting these symptoms must be made easy for the vaccine recipients, and could include helplines and hospital vaccine centre and online reporting systems.

Figures 2 and 3 summarize the clinical workup for patients.

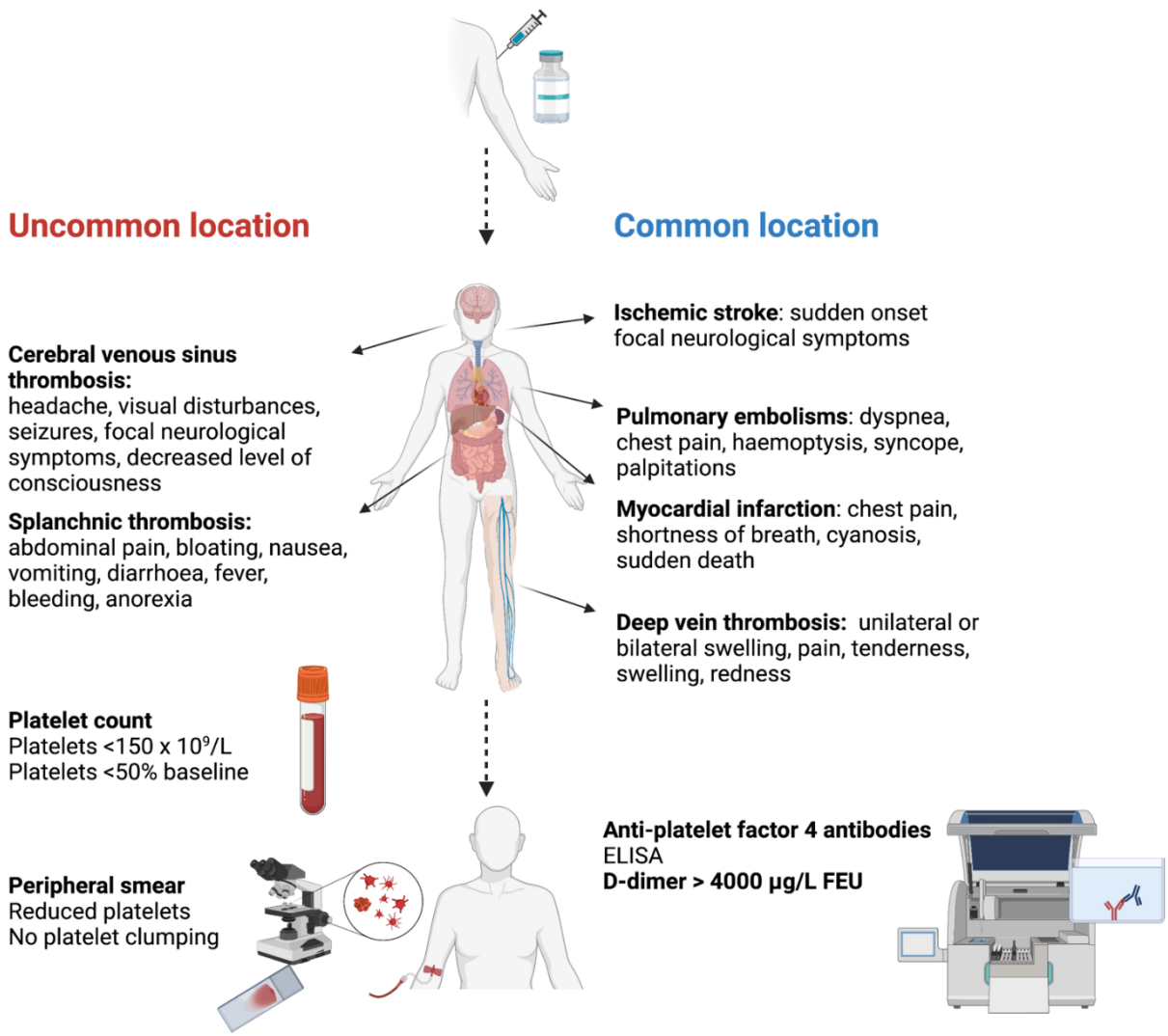


Figure 2: Clinical workup in patients with clinical symptoms and signs suggestive of thrombosis with 30 days of vaccination with a COVID-19 adenovirus vector-based vaccine

Figure created with BioRender.com

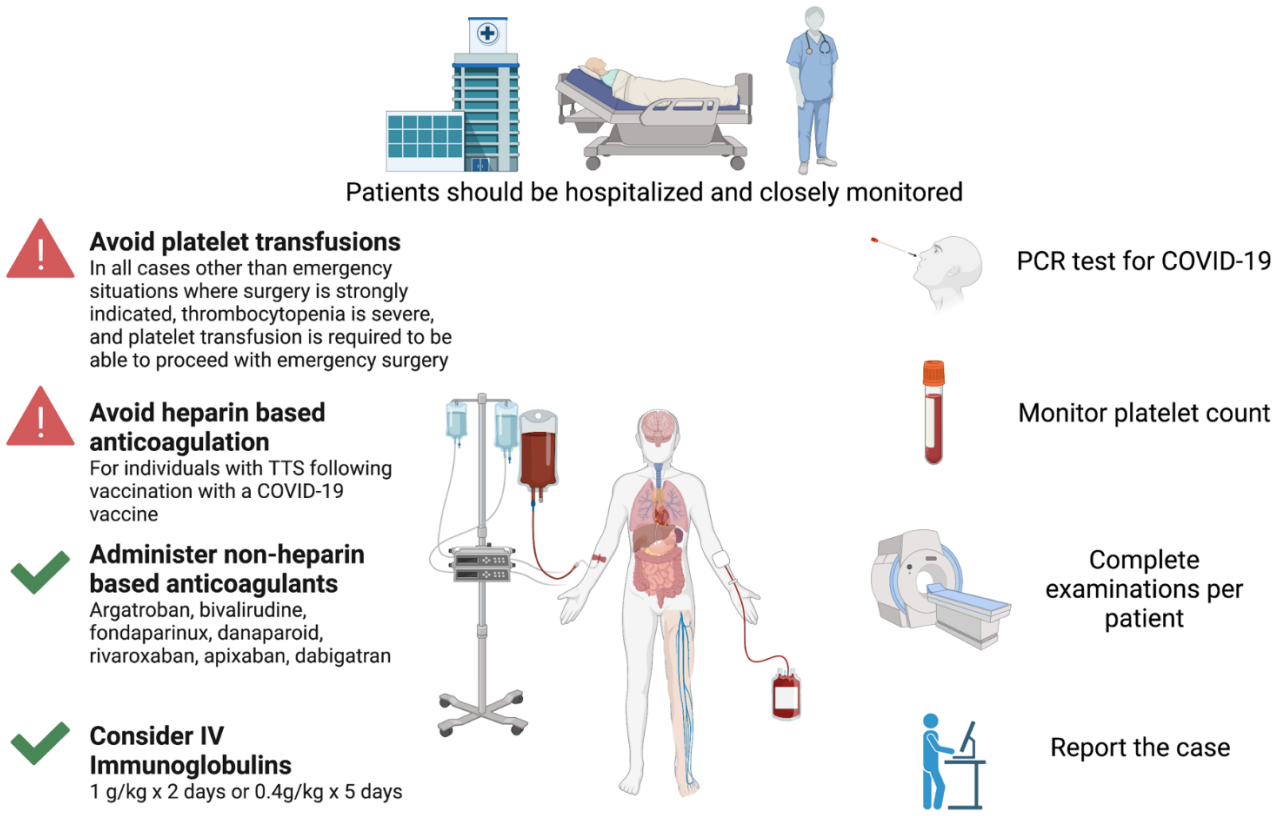


Figure 3: Clinical management of patients with confirmed, probable or possible vaccine related TTS

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Treatment recommendations

More complete details on the methods used for evidence assessment and formulation of these recommendations can be found in Annex 2 and Annex 3. Consult the Summary of findings and the Evidence tables for full details of the data used to formulate these recommendations.

Recommendation 1: WHO advises against the use of heparin for individuals with TTS following vaccination with a COVID-19 vaccine (*conditional recommendation, very low certainty evidence)*

Recommendation 2: WHO recommends against platelet infusion for individuals with TTS following vaccination with a COVID-19 vaccine in all cases other than emergency situations where surgery is strongly indicated, thrombocytopenia is severe, and platelet transfusion is required to be able to proceed with emergency surgery *(strong, very low certainty).*

WHO recommends the use of IVIG and non-heparin-based anticoagulants (NHAC) for individuals with TTS following vaccination with a COVID-19 vaccine *(strong, very low certainty)*

Full details on the evidence-to-decision process are available in Annex 6.

Table 7 in Annex 4 provides examples of non-heparin anticoagulants based on guidelines and national recommendations for TTS management that are available at present (103 -150). A GRADE evaluation of existing cases was carried out based on available information with these options. A GRADE evaluation of individual treatment options could not be carried out at present because of insufficient number of cases for each option. GRADE evaluation of treatment options will be possible in future versions of this guideline as global experience in TTS case management increases.

The GDG did not provide any judgment on steroid treatment, but noted the general use of steroids and the likelihood that steroids would usually be given in combination with other treatments.

Recommendations addressing vaccination, prevention and lifestyle changes in post-recovery TTS patients and the general population

No studies that provide information about post vaccine-related TTS, prophylaxis and lifestyle were identified. Important considerations include:

1. **Future vaccination**
   Patients with TTS following non-replicant adenovirus-vector-based vaccine should not receive the second dose of this vaccine to avoid repeated exposure to the antigen that caused the syndrome.
2. **Possible prophylactic medication to prevent thrombotic/thrombocytopenic events or other prophylactic measures**
   Although no studies have yet assessed the use of prophylactic medication in patients with post vaccine-related TTS, there are some studies on the long-term use of anti-thrombotic drugs to prevent TTS in patients recovering from COVID-19 disease. There is presently no indication or guideline for prophylactic treatment, including prophylactic anticoagulation or anti-aggregation in patients with other risk factors for thrombosis.
3. **Contraindicated drugs**
   The use of heparin-based anticoagulants is not recommended in patients with suspected TTS. There is insufficient evidence to contraindicate other drugs that have been associated with immune thrombocytopenia syndrome.
4. **Contraindication of adenovirus vector-based COVID-19 vaccines**
   Adenovirus vector-based COVID-19 vaccines and other adenovirus vector-based vaccines must be avoided in patients with a prior history of HIT or major venous and arterial thromboses occurring with thrombocytopenia.

References

1. European Medicines Agency. 29 March 2021 update. COVID-19 vaccine safety update VAXZEVRIA AstraZeneca AB. Available at: https://www.ema.europa.eu/en/documents/covid-19-vaccine-safety-update/covid-19-vaccine-safety-update-vaxzevria-previously-covid-19-vaccine-astrazeneca-29-march-2021_en.pdf, accessed 22 April 2021.
2. European Medicines Agency. COVID-19 Vaccine Janssen: EMA finds possible link to very rare cases of unusual blood clots with low blood platelets. 20 April 2021. Available at: https://www.ema.europa.eu/en/news/covid-19-vaccine-janssen-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood, accessed 30 April 2021.
3. European Medicines Agency.14 April 2021 update. COVID-19 vaccine safety update VAXZEVRIA AstraZeneca AB. Available at: https://www.ema.europa.eu/en/documents/covid-19-vaccine-safety-update/covid-19-vaccine-safety-update-vaxzevria-previously-covid-19-vaccine-astrazeneca-14-april-2021_en.pdf, accessed 22 April 2021.
4. Scully M, Singh D, Lown R, Poles A, Solomon T, Levi M, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. N Engl J Med. 2021;384(23):2202-2211.
5. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med. 2021;384(22):2092-2101.
6. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.
7. European Medicines Agency. Signal assessment report on embolic and thrombotic events (SMQ) with COVID-19 Vaccine (ChAdOx1-S [recombinant]) – Vaxzevria (previously COVID-19 Vaccine AstraZeneca) (Other viral vaccines). Available at: https://www.ema.europa.eu/en/documents/prac-recommendation/signal-assessment-report-embolic-thrombotic-events-smq-covid-19-vaccine-chadox1-s-recombinant_en.pdf, accessed 30 May 2021.
8. Franchini M, Testa S, Pezzo M, Glingani C, Caruso B, Terenziani I, et al. Cerebral venous thrombosis and thrombocytopenia post-COVID-19 vaccination. Thromb Res. 2021;202:182-183.
9. D'Agostino V, Caranci F, Negro A, Piscitelli V, Tuccillo B, Fasano F, et al. A rare case of cerebral venous thrombosis and disseminated intravascular coagulation temporally associated to the COVID-19 vaccine administration. J Pers Med. 2021;11(4):285
10. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
11. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after "COVID-19 Vaccine AstraZeneca" exposure. J Clin Med. 2021;10(8):1599.

12. Castelli GP, Pognani C, Sozzi C, Franchini M, Vivona L. Cerebral venous sinus thrombosis associated with thrombocytopenia post-vaccination for COVID-19. Crit Care. 2021;25(1):137.
13. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. N Engl J Med. 2021;384(20):1964-1965.
14. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
15. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
16. Yocum A, Simon EL. Thrombotic thrombocytopenic purpura after Ad26.COV2-S vaccination. Am J Emerg Med. 2021:S0735-6757(21)00376-4. doi: 10.1016/j.ajem.2021.05.001.
17. Jamme M, Mosnino E, Hayon J, Franchineau G. Fatal cerebral venous sinus thrombosis after COVID-19 vaccination. Intensive Care Med. 2021:1–2. doi: 10.1007/s00134-021-06425-y.
18. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
19. Bjørnstad-Tuveng TH, Rudjord A, Anker P. Fatal cerebral haemorrhage after COVID-19 vaccine. Tidsskr Nor Laegeforen. 2021;141. 10.4045/tidsskr.21.0312.
20. Blauenfeldt RA, Kristensen SR, Ernstsen SL, Kristensen CCH, Simonsen CZ, Hvas AM. Thrombocytopenia with acute ischemic stroke and bleeding in a patient newly vaccinated with an adenoviral vector-based COVID-19 vaccine. J Thromb Haemost. 2021;19(7):1771-1775.
21. Porres-Aguilar M, Lazo-Langner A, Panduro A, Uribe M. COVID-19 vaccine-induced immune thrombotic thrombocytopenia: An emerging cause of splanchnic vein thrombosis. Ann Hepatol. 2021;23:100356. doi: 10.1016/j.aohep.2021.100356.
22. Hocking J, Chunilal SD, Chen V, Brighton T, Nguyen J, Tan J, et al. The first known ChAdOx1 nCoV-19 vaccine-induced thrombotic thrombocytopenia in Australia. Preprint. Med J Austral Available at: https://www.mja.com.au/journal/2021/first-known-chadox1-ncov-19-vaccine-induced-thrombotic-thrombocytopenia-australia, accessed 17 May 2021.
23. Chatterjee S, Ojha UK, Vardhan B, Tiwari A. Myocardial infarction after COVID-19 vaccination-casual or causal? Diabetes Metab Syndr. 2021;15(3):1055–1056.
24. McCrae KR. Thrombotic thrombocytopenia due to SARS-CoV-2 vaccination. Cleve Clin J Med. 2021. doi: 10.3949/ccjm.88a.ccc078.
25. Colarossi G, Schnöring H, Trivellas A, Betsch M, Hatam N, Eschweiler J, et al. Prognostic factors for patients with heparin-induced thrombocytopenia: a systematic review. Int J Clin Pharm. 2020;43(3):449-460.
26. Borhani Haghighi A, Edgell RC, Cruz-Flores S, Feen E, Piriyawat P, Vora N, et al. Mortality of cerebral venous-sinus thrombosis in a large national sample. Stroke. 2012 Jan;43(1):262-264.
27. Ferro JM, Canhão P, Stam J, Bousser MG, Barinagarrementeria F; ISCVT Investigators. Prognosis of cerebral vein and dural sinus thrombosis: results of the International Study on Cerebral Vein and Dural Sinus Thrombosis (ISCVT). Stroke. 2004 Mar;35(3):664-670.
28. Zhu FC, Li YH, Guan XH, Hou LH, Wang WJ, Li JX, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet. 2020;395(10240):1845-1854.
29. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al.; Oxford COVID Vaccine Trial Group. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020;396(10249):467-478.
30. Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, Hou LH, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet. 2020;396(10249):479-488.
31. GRADE Handbook Available at: https://gdt.gradepro.org/app/handbook/handbook.html#h.ged5uqebmir9, accessed 30 May 2021.
32. Brighton Collaboration. Interim case definition of thrombosis with thrombocytopenia syndrome. Available at: https://brightoncollaboration.us/thrombosis-with-thrombocytopenia-syndrome-interim-case-definition/, accessed 22 April 2021.
33. Burn E, Li X, Kotska K, Stewart HM, Reich C, Seager S, et al. Background rates of five thrombosis with thrombocytopenia syndromes of special interest for COVID-19 vaccine safety surveillance: incidence between 2017 and 2019 and patient profiles from 20.6 million people in six European countries. medRxiv. doi: https://doi.org/10.1101/2021.05.12.21257083. Version May 14, 2021.

34. Li X, Ostropolets A, Makadia R, Shaoibi A, Rao G, Sena AG, et al. Characterizing the incidence of adverse events of special interest for COVID-19 vaccines across eight countries: a multinational network cohort study medRxiv. doi: https://doi.org/10.1101/2021.03.25.21254315; version posted April 17, 2021
35. Smadja DM, Yue QY, Chocron R, Sanchez O, Lillo-Le Louet A. Vaccination against COVID-19: insight from arterial and venous thrombosis occurrence using data from VigiBase. Eur Respir J. 2021:2100956. doi: 10.1183/13993003.00956-2021.
36. Otite FO, Patel S, Sharma R, Khandwala P, Desai D, Latorre JG, et al. Trends in incidence and epidemiologic characteristics of cerebral venous thrombosis in the United States. Neurology. 2020 Oct 20;95(16):e2200-e2213.
37. Thakur KT, Tamborska A, Wood GK, McNeill E, Roh D, Akpan IJ, et al. Clinical review of cerebral venous thrombosis in the context of COVID-19 vaccinations: Evaluation, management, and scientific questions. J Neurol Sci. 2021;427:117532.
38. Pottegård A, Lund LC, Karlstad Ø, Dahl J, Andersen M, Hallas J, et al. Arterial events, venous thromboembolism, thrombocytopenia, and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: population based cohort study. BMJ. 2021;373:n1114.
39. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
40. Pawlowski C, Rincon-Hekking J, Awasthi S, Pandey V, Lenehan P, Venkatakrishnan AJ, et al. Cerebral venous sinus thrombosis (CVST) is not significantly linked to COVID-19 vaccines or non-COVID vaccines in a large multi-state US health system. medRxiv. doi: https://doi.org/10.1101/2021.04.20.21255806. Version April 23, 2021.
41. Spanish Agency of Medicine and Healthcare products. Pharmacovigilance report, published on 9 April 2021. Available at: https://www.aemps.gob.es/acciones-informativas/boletines-de-la-aemps/boletin-mensual-de-farmacovigilancia/, accessed 22 April 2021.
42. Medicines & Healthcare products Regulatory Agency. Coronavirus vaccine - weekly summary of Yellow Card reporting. Updated 27 May 2021. Available at: https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting#contents, accessed 30 May 2021.
43. Chan BTB, Bobos P, Odutayo A, Pai M. Meta-analysis of risk of vaccine-induced immune thrombotic thrombocytopenia following ChAdOx1-S recombinant vaccine. medRxiv. doi: https://doi.org/10.1101/2021.05.04.21256613. version posted May 8, 2021.
44. Shay DK, Gee J, Su JR, Myers TR, Marquez P, Liu R, et al. Safety Monitoring of the Janssen (Johnson & Johnson) COVID-19 Vaccine - United States, March-April 2021. MMWR Morb Mortal Wkly Rep. 2021;70(18):680-684.
45. MacNeil JR, Su JR, Broder KR, Guh AY, Gargano JW, Wallace M, et al. Updated recommendations from the Advisory Committee on Immunization Practices for use of the Janssen (Johnson & Johnson) COVID-19 vaccine after reports of thrombosis with thrombocytopenia syndrome among vaccine recipients - United States, April 2021. MMWR Morb Mortal Wkly Rep. 2021;70(17):651-656.
46. Food and Drug Administration. Fact sheet for healthcare providers administering vaccine (vaccination providers) emergency use authorization (EUA) of the JANSSEN COVID-19 vaccine to prevent coronavirus disease 2019 (COVID-19). Available at: https://www.fda.gov/media/146304/download, accessed 15 May 2021.
47. Australian Government, Department of Health. ATAGI update following weekly COVID-19 meeting – 23 June 2021. Available at: https://www.health.gov.au/news/atagi-update-following-weekly-covid-19-meeting-23-june-2021, accessed 2 July 2021.
48. Medicines & Healthcare products Regulatory Agency. Coronavirus vaccine – summary of yellow card reporting. First published on 5 February 2021, Updated on 10 June 2021. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/992984/20210610_Coronavirus_vaccine_-_summary_of_Yellow_Card_reporting.pdf, accessed 16 June 2021).
49. Valeriani E, Riva N, Di Nisio M, Ageno W. Splanchnic vein thrombosis: current perspectives. Vasc Health Risk Manag. 2019;15:449-461.
50. Qi X, De Stefano V, Senzolo M, Xu H, Mancuso A. Splanchnic vein thrombosis: etiology, diagnosis, and treatment. Gastroenterol Res Pract. 2015;2015:506136.
51. Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocytopenia. N Engl J Med. 2001;344(17):1286-1292.
52. De Stefano V, Qi X, Betti S, Rossi E. Splanchnic vein thrombosis and myeloproliferative neoplasms: molecular-driven diagnosis and long-term treatment. Thromb Haemost. 2016;115(2):240-249.
53. Ramrakhiani N, Sharma DK, Dubey R, Gupta P, Sharma A, Sharma KK. Clinical profile, risk factors and outcomes in patients with cerebral venous sinus thrombosis: a study from Western India. J Assoc Physicians India. 2019;67(9):49-53.

54. Ferro JM, Canhão P, Stam J, Bousser MG, Barinagarrementeria F; ISCVT Investigators. Prognosis of cerebral vein and dural sinus thrombosis: results of the International Study on Cerebral Vein and Dural Sinus Thrombosis (ISCVT). Stroke. 2004;35(3):664-670.
55. Kalita J, Misra UK, Singh RK. Do the risk factors determine the severity and outcome of cerebral venous sinus thrombosis? Transl Stroke Res. 2018;9(6):575-581.
56. Pan L, Ding J, Ya J, Zhou D, Hu Y, Fan C, et al. Risk factors and predictors of outcomes in 243 Chinese patients with cerebral venous sinus thrombosis: A retrospective analysis. Clin Neurol Neurosurg. 2019;183:105384.
57. McBane RD 2nd, Tafur A, Wysokinski WE. Acquired and congenital risk factors associated with cerebral venous sinus thrombosis. Thromb Res. 2010;126(2):81-87.
58. Saadatnia M, Fatehi F, Basiri K, Mousavi SA, Mehr GK. Cerebral venous sinus thrombosis risk factors. Int J Stroke. 2009;4(2):111-123.
59. Greinacher A, Selleng K, Wesche J, Handtke S, Palankar R, Aurich K, et al. Towards understanding ChAdOx1 nCov-19 vaccine immune thrombotic thrombocytopenia (VITT). Preprint. Research Square. 10.21203/rs.3.rs-440461/v1. Posted 20 April 2021.
60. Greinacher A. Heparin-Induced Thrombocytopenia. N Engl J Med. 2015;373(19):1883-1884.
61. Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. J Thromb Haemost. 2017;15(11):2099-2114.
62. Hofherr SE, Mok H, Gushiken FC, Lopez JA, Barry MA. Polyethylene glycol modification of adenovirus reduces platelet activation, endothelial cell activation, and thrombocytopenia. Hum Gene Ther. 2007;18(9):837-838.
63. Cichon G, Schmidt HH, Benhidjeb T, Löser P, Ziemer S, Haas R, et al. Intravenous administration of recombinant adenoviruses causes thrombocytopenia, anemia and erythroblastosis in rabbits. J Gene Med. 1999;1(5):360-371.
64. Schnell MA, Zhang Y, Tazelaar J, Gao GP, Yu QC, Qian R, et al. Activation of innate immunity in nonhuman primates following intraportal administration of adenoviral vectors. Mol Ther. 2001;3(5 Pt 1):708-722.
65. Wolins N, Lozier J, Eggerman TL, Jones E, Aguilar-Córdova E, Vostal JG. Intravenous administration of replication-incompetent adenovirus to rhesus monkeys induces thrombocytopenia by increasing in vivo platelet clearance. Br J Haematol. 2003;123(5):903-905.
66. Varnavski AN, Calcedo R, Bove M, Gao G, Wilson JM. Evaluation of toxicity from high-dose systemic administration of recombinant adenovirus vector in vector-naive and pre-immunized mice. Gene Ther. 2005;12(5):427-436.
67. AbdelMassih A, Hozaien R, El Shershaby M, Kamel A, Ismail HA, Fouda R. Is the heparin-induced thrombocytopenia-like syndrome associated with ChAdOx vaccine related to the vaccine itself or to an autoimmune reaction to severe acute respiratory syndrome 2 coronavirus: insights and implications from previous reports in infected cases? New Microbes New Infect. 2021;41:100884.
68. Smith CW, Kardeby C, Di Y, Lowe GC, Lester WA, Watson SP. Platelet activation by vaccine-induced immune thrombotic thrombocytopenia (VITT) patient serum is blocked by COX, $P2Y_{12}$ and kinase inhibitors. medRxiv. Doi: https://doi.org/10.1101/2021.04.24.21255655. Version posted April 27, 2021.
69. Kadkhoda K. Post-adenoviral-based COVID-19 vaccines thrombosis: A proposed mechanism. J Thromb Haemost. 2021;19(7):1831-1832.
70. Parums DV. Editorial: SARS-CoV-2 mRNA Vaccines and the possible mechanism of vaccine-induced immune thrombotic thrombocytopenia (VITT). Med Sci Monit. 2021;27:e932899.
71. von Hundelshausen P, Lorenz R, Siess W, Weber C. Vaccine-induced immune thrombotic thrombocytopenia (VITT): targeting pathomechanisms with bruton tyrosine kinase inhibitors. Thromb Haemost. 2021. doi: 10.1055/a-1481-3039.
72. Xie C, Vincent L, Chadwick A, Peschl H. COVID-19 vaccine induced prothrombotic immune thrombocytopenia. Eur Heart J. 2021:ehab237. doi: 10.1093/eurheartj/ehab237.
73. Elalamy I, Gerotziafas G, Alamowitch S, Laroche JP, van Dreden P, Ageno W, et al. SARS-CoV-2 vaccine and thrombosis: an expert consensus on vaccine-induced immune thrombotic thrombocytopenia. Thromb Haemost. 2021. doi: 10.1055/a-1499-0119.
74. Mattioli AV, Bonetti L, Zennaro M, Ambrosio G, Mattioli G. Heparin/PF4 antibodies formation after heparin treatment: temporal aspects and long-term follow-up. Am Heart J. 2009;157(3):589-595.
75. Kiliç T, Akakin A. Anatomy of cerebral veins and sinuses. Front Neurol Neurosci. 2008;23:4-15.
76. Singh RJ, Saini J, Varadharajan S, Kulkarni GB, Veerendrakumar M. Headache in cerebral venous sinus thrombosis revisited: Exploring the role of vascular congestion and cortical vein thrombosis. Cephalalgia. 2018;38(3):503-510.
77. Wasay M, Kojan S, Dai AI, Bobustuc G, Sheikh Z. Headache in cerebral venous thrombosis: incidence, pattern and location in 200 consecutive patients. J Headache Pain 2010;11(2):137-139.
78. García-Azorín D, Monje MHG, González-García N, Guerrero ÁL, Porta-Etessam J. Presence of red flags in patients with cerebral venous sinus thrombosis admitted to the emergency department because of headache: A STROBE compliant cohort-study. Medicine (Baltimore). 2020;99(29):e20900.

79. Mehta A, Danesh J, Kuruvilla D. Cerebral venous thrombosis headache. Curr Pain Headache Rep. 2019;23(7):47.
80. Silvis SM, de Sousa DA, Ferro JM, Coutinho JM. Cerebral venous thrombosis. Nat Rev Neurol. 2017;13(9):555-565.
81. Bayas A, Menacher M, Christ M, Behrens L, Rank A, Naumann M. Bilateral superior ophthalmic vein thrombosis, ischaemic stroke, and immune thrombocytopenia after ChAdOx1 nCoV-19 vaccination. Lancet. 2021;397(10285):e11. doi: 10.1016/S0140-6736(21)00872-2.
82. Greinacher A, Farner B, Kroll H, Kohlmann T, Warkentin TE, Eichler P. Clinical features of heparin-induced thrombocytopenia including risk factors for thrombosis. A retrospective analysis of 408 patients. Thromb Haemost. 2005;94(1):132-5.
83. Avendaño-Solá C, Cámara R, Castellanos M, Ezpeleta D, García-Azorín D, Martínez CI, et al. Diagnostic and treatment recommendations from the FACME ad-hoc expert working group on the management of cerebral venous sinus thrombosis associated with COVID-19 vaccination. Neurologia. 2021 doi: 10.1016/j.nrl.2021.05.001.
84. Thakur KT, Tamborska A, Wood GK, McNeill E, Roh D, Akpan IJ. Clinical review of cerebral venous thrombosis in the context of COVID-19 vaccinations: Evaluation, management, and scientific questions. J Neurol Sci. 2021;427:117532. doi: 10.1016/j.jns.2021.117532.
85. Sørvoll IH, Horvei KD, Ernstsen SL, Laegreid IJ, Lund S, Grønli RH, et al. An observational study to identify the prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in Norwegian health care workers after COVID-19 vaccination. J Thromb Haemost. 2021;19(7):1813-1818.
86. Nazy I, Sachs UJ, Arnold DM, McKenzie SE, Choi P, Althaus K, et al. Recommendations for the clinical and laboratory diagnosis of VITT against COVID-19: Communication from the ISTH SSC Subcommittee on Platelet Immunology. J Thromb Haemost. 2021;19(6):1585-1588.
87. Oldenburg J, Klamroth R, Langer F, Albisetti M, von Auer C, Ay C, et al. Diagnosis and management of vaccine-related thrombosis following AstraZeneca COVID-19 vaccination: guidance statement from the GTH. Hamostaseologie. 2021;41(3):184-189.
88. Platton S, Bartlett A, MacCallum P, Makris M, McDonald V, Singh D, et al. Evaluation of laboratory assays for anti-Platelet Factor 4 antibodies after ChAdOx1 nCOV-19 vaccination. J Thromb Haemost. 2021. doi: 10.1111/jth.15362.
89. Linn J, Pfefferkorn T, Ivanicova K, Müller-Schunk S, Hartz S, Wiesmann M, et al. Noncontrast CT in deep cerebral venous thrombosis and sinus thrombosis: comparison of its diagnostic value for both entities. AJNR Am J Neuroradiol. 2009;30(4):728-735.
90. Buyck PJ, Zuurbier SM, Garcia-Esperon C, Barboza MA, Costa P, Escudero I, et al. Diagnostic accuracy of noncontrast CT imaging markers in cerebral venous thrombosis. Neurology. 2019;92(8):e841-e851.
91. Bonatti M, Valletta R, Lombardo F, Zamboni GA, Turri E, Avesani G, et al. Accuracy of unenhanced CT in the diagnosis of cerebral venous sinus thrombosis. Radiol Med. 2021;126(3):399-404.
92. Tayyebi S, Akhavan R, Shams M, Salehi M, Farrokh D, Yousefi F, et al. Diagnostic value of non-contrast brain computed tomography in the evaluation of acute cerebral venous thrombosis. Sci Rep. 2020;10(1):883.
93. Xu W, Gao L, Li T, Ramdoyal ND, Zhang J, Shao A. The performance of CT versus MRI in the differential diagnosis of cerebral venous thrombosis. Thromb Haemost. 2018;118(6):1067-1077.
94. Gao L, Xu W, Li T, Yu X, Cao S, Xu H, et al. Accuracy of magnetic resonance venography in diagnosing cerebral venous sinus thrombosis. Thromb Res. 2018;167:64-73.
95. Sato T, Terasawa Y, Mitsumura H, Komatsu T, Sakuta K, Sakai K, et al. Venous stasis and cerebrovascular complications in cerebral venous sinus thrombosis. Eur Neurol. 2017;78(3-4):154-160.
96. Weimar C. Diagnosis and treatment of cerebral venous and sinus thrombosis. Curr Neurol Neurosci Rep. 2014;14(1):417.
97. Ferro JM, Aguiar de Sousa D. Cerebral venous thrombosis: an update. Curr Neurol Neurosci Rep. 2019;19(10):74.
98. Ciccone A, Zanotti B; working group on cerebral venous thrombosis after COVID-19 vaccination. The importance of recognizing cerebral venous thrombosis following anti-COVID-19 vaccination. Eur J Intern Med. 2021:89:115-117.
99. British Society of Haemotology. Guidance produced from the Expert Haematology Panel (EHP) focused on syndrome of Thrombosis and Thrombocytopenia occurring after coronavirus vaccination. Updated guidance on management. Version 1.7. Available at: https://b-s-h.org.uk/media/19590/guidance-version-17-on-mngmt-of-vitt-20210420.pdf, accessed 23 April 2021.
100. Brasil. Ministério da Saúde. Coordenação Geral do Programa Nacional de I. Orientações para identificação investigação e manejo da síndrome de trombose e trombocitopenia (TTS) no contexto da vacinação contra o COVID-19 no Brasil. 2021. Available at: http://fi-admin.bvsalud.org/document/view/j33vc, accessed 30 May 2021.
101. Franchini M, Liumbruno GM, Pezzo M. COVID-19 vaccine-associated immune thrombosis and thrombocytopenia (VITT): Diagnostic and therapeutic recommendations for a new syndrome. Eur J Haematol. 2021. doi: 10.1111/ejh.13665.

102. Cuker A, Arepally GM, Chong BH, Cines DB, Greinacher A, Gruel Y, et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: heparin-induced thrombocytopenia. Blood Adv. 2018;2(22):3360-3392.
103. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
104. Thaler J, Ay C, Gleixner KV, Hauswirth AW, Cacioppo F, Grafeneder J, et al. Successful treatment of vaccine-induced prothrombotic immune thrombocytopenia (VIPIT). J Thromb Haemost 2021. doi: 10.1111/jth.15346.
105. Einhäupl K, Stam J, Bousser MG, De Bruijn SF, Ferro JM, Martinelli I, et al. EFNS guideline on the treatment of cerebral venous and sinus thrombosis in adult patients. Eur J Neurol. 2010;17(10):1229-1235.
106. Saposnik G, Barinagarrementeria F, Brown RD Jr, Bushnell CD, Cucchiara B, Cushman M, et al. Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2011;42(4):1158-1192.
107. Ferro JM, Bousser MG, Canhão P, Coutinho JM, Crassard I, Dentali F, et al. European Stroke Organization guideline for the diagnosis and treatment of cerebral venous thrombosis - Endorsed by the European Academy of Neurology. Eur Stroke J. 2017;2(3):195-221.
108. Einhäupl KM, Villringer A, Meister W, Mehraein S, Garner C, Pellkofer M, et al. Heparin treatment in sinus venous thrombosis. Lancet. 1991;338(8767):597-600.
109. Mehraein S, Schmidtke K, Villringer A, Valdueza JM, Masuhr F. Heparin treatment in cerebral sinus and venous thrombosis: patients at risk of fatal outcome. Cerebrovasc Dis. 2003;15(1-2):17-21.
110. Busch MA, Hoffmann O, Einhäupl KM, Masuhr F. Outcome of heparin-treated patients with acute cerebral venous sinus thrombosis: influence of the temporal pattern of intracerebral haemorrhage. Eur J Neurol. 2016;23(9):1387-1392.
111. Coutinho JM, Ferro JM, Canhão P, Barinagarrementeria F, Bousser MG, Stam J, et al. Unfractionated or low-molecular weight heparin for the treatment of cerebral venous thrombosis. Stroke. 2010;41(11):2575-2580.
112. Misra UK, Kalita J, Chandra S, Kumar B, Bansal V. Low molecular weight heparin versus unfractionated heparin in cerebral venous sinus thrombosis: a randomized controlled trial. Eur J Neurol. 2012;19(7):1030-1036.
113. Afshari D, Moradian N, Nasiri F, Razazian N, Bostani A, Sariaslani P. The efficacy and safety of low-molecular-weight heparin and unfractionated heparin in the treatment of cerebral venous sinus thrombosis. Neurosciences (Riyadh). 2015;20(4):357-361.
114. Qureshi A, Perera A. Low molecular weight heparin versus unfractionated heparin in the management of cerebral venous thrombosis: A systematic review and meta-analysis. Ann Med Surg (Lond). 2017;17:22-26.
115. Furie KL, Cushman M, Elkind MSV, Lyden PD, Saposnik G; American Heart Association/American Stroke Association Stroke Council Leadership. Diagnosis and management of cerebral venous sinus thrombosis with vaccine-induced immune thrombotic thrombocytopenia. Stroke. 2021;52(7):2478-2482.
116. Matthai WH Jr, Hursting MJ, Lewis BE, Kelton JG. Argatroban anticoagulation in patients with a history of heparin-induced thrombocytopenia. Thromb Res. 2005;116(2):121-126.
117. Colarossi G, Maffulli N, Trivellas A, Schnöring H, Hatam N, Tingart M, et al. Superior outcomes with Argatroban for heparin-induced thrombocytopenia: a Bayesian network meta-analysis. Int J Clin Pharm. 2021 doi: 10.1007/s11096-021-01260-z.
118. Gleichgerrcht E, Lim MY, Turan TN. Cerebral venous sinus thrombosis due to low-molecular-weight heparin-induced thrombocytopenia. Neurologist. 2017;22(6):241-244.
119. Barreto AD, Alexandrov AV, Lyden P, Lee J, Martin-Schild S, Shen L, et al. The argatroban and tissue-type plasminogen activator stroke study: final results of a pilot safety study. Stroke. 2012;43(3):770-775.
120. Barreto AD, Ford GA, Shen L, Pedroza C, Tyson J, Cai C, et al.; ARTSS-2 Investigators. Randomized, multicenter Trial of ARTSS-2 (Argatroban With Recombinant Tissue Plasminogen Activator for Acute Stroke). Stroke. 2017;48(6):1608-1616.
121. Berekashvili K, Soomro J, Shen L, Misra V, Chen PR, Blackburn S, et al. Safety and feasibility of argatroban, recombinant tissue plasminogen activator, and intra-arterial therapy in stroke (ARTSS-IA Study). J Stroke Cerebrovasc Dis. 2018;27(12):3647-3651.
122. Hwang SR, Wang Y, Weil EL, Padmanabhan A, Warkentin TE, Pruthi RK. Cerebral venous sinus thrombosis associated with spontaneous heparin-induced thrombocytopenia syndrome after total knee arthroplasty. Platelets. 2020:1-5. doi: 10.1080/09537104.2020.1828574.
123. Sun Z, Lan X, Li S, Zhao H, Tang Z, Xi Y. Comparisons of argatroban to lepirudin and bivalirudin in the treatment of heparin-induced thrombocytopenia: a systematic review and meta-analysis. Int J Hematol. 2017;106(4):476-483.
124. Thorsteinsson GS, Magnussson M, Hallberg LM, Wahlgren NG, Lindgren F, Malmborg P, et al. Cerebral venous thrombosis and heparin-induced thrombocytopenia in an 18-year old male with severe ulcerative colitis. World J Gastroenterol. 2008;14(28):4576-4579.
125. Kuo KH, Kovacs MJ. Fondaparinux: a potential new therapy for HIT. Hematology. 2005;10(4):271-275.

126. Shankar Iyer R, Tcr R, Akhtar S, Muthukalathi K, Kumar P, Muthukumar K. Is it safe to treat cerebral venous thrombosis with oral rivaroxaban without heparin? A preliminary study from 20 patients. Clin Neurol Neurosurg. 2018;175:108-111.
127. Fayyaz M, Abbas F, Kashif T. The role of warfarin and rivaroxaban in the treatment of cerebral venous thrombosis. Cureus. 2019;11(5):e4589.
128. Maqsood M, Imran Hasan Khan M, Yameen M, Aziz Ahmed K, Hussain N, Hussain S. Use of oral rivaroxaban in cerebral venous thrombosis. J Drug Assess. 2020;10(1):1-6.
129. Connor P, Sánchez van Kammen M, Lensing AWA, Chalmers E, Kállay K, Hege K, et al. Safety and efficacy of rivaroxaban in pediatric cerebral venous thrombosis (EINSTEIN-Jr CVT). Blood Adv. 2020;4(24):6250-6258.
130. Fatima M, Asghar MS, Abbas S, Iltaf S, Ali A. An observational study to evaluate the effectiveness of rivaroxaban in the management of cerebral venous sinus thrombosis. Cureus 2021;13(3):e13663.
131. Esmaeili S, Abolmaali M, Aarabi S, Motamed MR, Chaibakhsh S, Joghataei MT, et al. Rivaroxaban for the treatment of cerebral venous thrombosis. BMC Neurol. 2021;21(1):73.
132. Covut F, Kewan T, Perez O, Flores M, Haddad A, Daw H. Apixaban and rivaroxaban in patients with cerebral venous thrombosis. Thromb Res. 2019;173:77-78.
133. Rao SK, Ibrahim M, Hanni CM, Suchdev K, Parker D, Rajamani K, et al. Apixaban for the treatment of cerebral venous thrombosis: A case series. J Neurol Sci. 2017;381:318-320.
134. Li H, Yao M, Liao S, Chen J, Yu J. Comparison of novel oral anticoagulants and vitamin K antagonists in patients with cerebral venous sinus thrombosis on efficacy and safety: A systematic review. Front Neurol. 2020;11:597623.
135. Bose G, Graveline J, Yogendrakumar V, Shorr R, Fergusson DA, Le Gal G, et al. Direct oral anticoagulants in treatment of cerebral venous thrombosis: a systematic review. BMJ Open. 2021;11(2):e040212.
136. Riva N, Ageno W. Direct oral anticoagulants for unusual-site venous thromboembolism. Res Pract Thromb Haemost. 2021;5(2):265-277.
137. Riva N, Carrier M, Gatt A, Ageno W. Anticoagulation in splanchnic and cerebral vein thrombosis: An international vignette-based survey. Res Pract Thromb Haemost. 2020;4(7):1192-1202.
138. Stam J, Majoie CB, van Delden OM, van Lienden KP, Reekers JA. Endovascular thrombectomy and thrombolysis for severe cerebral sinus thrombosis: a prospective study. Stroke. 2008;39(5):1487-1490.
139. Salottolo K, Wagner J, Frei DF, Loy D, Bellon RJ, McCarthy K, et al. Epidemiology, endovascular treatment, and prognosis of cerebral venous thrombosis: US center study of 152 patients. J Am Heart Assoc. 2017;6(6):e005480.
140. Liao CH, Liao NC, Chen WH, Chen HC, Shen CC, Yang SF, et al. Endovascular mechanical thrombectomy and on-site chemical thrombolysis for severe cerebral venous sinus thrombosis. Sci Rep. 2020;10(1):4937.
141. Keller E, Pangalu A, Fandino J, Könü D, Yonekawa Y. Decompressive craniectomy in severe cerebral venous and dural sinus thrombosis. Acta Neurochir Suppl. 2005;94:177-183.
142. Riva N, Ageno W. Cerebral and splanchnic vein thrombosis: advances, challenges, and unanswered questions. J Clin Med. 2020;9(3):743.
143. Karnam A, Lacroix-Desmazes S, Kaveri SV, Bayry J. Vaccine-induced prothrombotic immune thrombocytopenia (VIPIT): Consider IVIG batch in the treatment. J Thromb Haemost. 2021;19(7):1838-1839.
144. Aryal MR, Gosain R, Donato A, Katel A, Chakradhar R, Dhital R, et al. Effectiveness of intravenous immunoglobulin use in heparin-induced thrombocytopenia. Blood Coagul Fibrinolysis. 2020;31(5):287-292.
145. Dougherty JA, Yarsley RL. Intravenous immune globulin (IVIG) for treatment of autoimmune heparin-induced thrombocytopenia: a systematic review. Ann Pharmacother. 2021;55(2):198-215.
146. Onuoha C, Barton KD, Wong ECC, Raval JS, Rollins-Raval MA, Ipe TS, et al. Therapeutic plasma exchange and intravenous immune globulin in the treatment of heparin-induced thrombocytopenia: A systematic review. Transfusion. 2020;60(11):2714-2736.
147. Guetl K, Gary T, Raggam RB, Schmid J, Wölfler A, Brodmann M. SARS-CoV-2 vaccine-induced immune thrombotic thrombocytopenia treated with immunoglobulin and argatroban. Lancet. 2021;397(10293):e19.
148. Greinacher A, Eichler P, Lubenow N, Kwasny H, Luz M. Heparin-induced thrombocytopenia with thromboembolic complications: meta-analysis of 2 prospective trials to assess the value of parenteral treatment with lepirudin and its therapeutic aPTT range. Blood. 2000;96(3):846-851.
149. Treschan TA, Schaefer MS, Geib J, Bahlmann A, Brezina T, Werner P, et al. Argatroban versus Lepirudin in critically ill patients (ALicia): a randomized controlled trial. Crit Care. 2014;18(5):588.
150. Bourguignon A, Arnold DM, Warkentin TE, Smith JW, Pannu T, Shrum JM, et al. Adjunct immune globulin for vaccine-induced thrombotic thrombocytopenia. N Engl J Med. 2021. doi: 10.1056/NEJMoa2107051.

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Annexes

Annex 1: Thrombosis risk factors

The main risk factors for venous thromboses in the general population include:

- inherited prothrombotic disorders (Factor V Leiden, prothrombin G2021A mutation, antithrombin deficiency, protein S deficiency, protein C deficiency);
- pregnancy/puerperium;
- hormone therapy or contraceptive treatment;
- obesity;
- cancer;
- myeloproliferative neoplasms;
- immobility;
- dehydration;
- smoking;
- infections;
- inflammatory or immune-mediated conditions (inflammatory bowel disease, Behçet's disease, thyroid disease, systemic lupus erythematosus, antiphospholipid syndrome, nephrotic syndrome, sarcoidosis, paroxysmal nocturnal haemoglobinuria);
- jugular vein catheterization;
- trauma;
- severe anaemia;
- recent neurosurgical procedure;
- post-surgical state.

Chronic liver disease is the main risk factor for splanchnic vein thrombosis, but myeloproliferative neoplasms, cancer, chemotherapy, fatty liver, diabetes and coeliac disease are also known risk factors. Patients with COVID-19 also have a significantly increased risk of thrombotic events, including CVST, however, there were no markers of an active or recent COVID-19 infection in most of the reported cases.

Annex 2: PICO 1: evidence-to-decision formulation

Results of the rapid literature review

The review identified eight studies providing valid data for the outcome of recovery [7 case series and 1 case report] in 127 patients, 45 of who were treated with heparin (5, 6, 10, 11, 14, 15, 18, 39). At the time of publication of the individual studies, 68/127 (53.5%) of patients had recovered. The recovery rate in patients treated with heparin was 7/14 (50%) based on data from 4 studies including 14 patients (5, 6, 10, 11).

Nine studies provided valid data for mortality rates [7 case series and 2 case reports] in 128 patients, 46 of who were treated with heparin (5, 6, 10, 11, 13, 14, 18, 39). The overall mortality rate was 30/128 (23.4%). The mortality rate in patients treated with heparin was 6/32 (18.7%) based on data from 5 studies including 32 patients (5, 10, 11, 14, 15).

Eight studies providing valid data for haemorrhage [6 case series and 2 case reports] including 67 patients, 34 of them treated with heparin (5, 6, 10, 11, 13-15, 39). The overall intracranial haemorrhage rate was 27/67 (40.3%). It was 7/21 (33.3%) in patients treated with heparin, based on data available in 6 studies (5, 6, 10, 11, 13, 14).

Certainty of evidence was very low for recovery, mortality, and haemorrhage.

Certainty of evidence

All studies were observational, either case series or case reports. There were nine valid studies for the mortality outcome and eight for haemorrhage and recovery. There were 127 patients for the recovery outcome, 128 for the mortality outcome and 67 for the haemorrhage outcome. The total number of treated patients was 14 for the recovery outcome, 32 for the mortality outcome and 21 for the haemorrhage outcome. The risk of bias was judged to be very serious, with possible selection bias, as fatal cases were more likely to be quickly published as case reports (within a range of between 5 and 8 days). Not all confounding factors influencing the prognosis were analysed (age, time between symptom onset and treatment, concomitant treatments, presence of multiple organ thrombosis) and follow-up was incomplete in many cases. The degree of inconsistency was judged to be serious, since the dose was not described in many studies, the duration and follow-up protocols varied within the different studies. Imprecision was judged to be very serious, since the number of patients included was less than the number of patients that would be needed for a single adequately powered trial, according to a sample size calculation. There was also a strong suspicion of publication bias.

Balance of benefits versus harms

The evidence was limited and the Guideline Development Group (GDG) agreed with the very low certainty rating. In judging the balance of benefits and harms, the GDG members also discussed that that in heparin induced thrombocytopenia (HIT), which has a similar presentation to TTS, heparin-based anticoagulants could be more harmful than beneficial and that, based on what is known about the pathophysiology of TTS, heparin may also be harmful in TTS. The GDG experts agreed on the need for more and better data, and some stated that heparin could be harmful, and that no benefit had been demonstrated for TTS.

Values and preferences

No data was available to inform how patients judge the value or benefit of heparin. Heparin is the main treatment for most thromboses and thromboembolisms. It is also the most frequently used anticoagulation treatment. Members of the GDG noted that if patients were aware of the uncertainty of the data and aware of the possible harms, some may prefer not to receive it. However, overall, the GDG agreed that there was likely to be major uncertainty and variability in how patients would value heparin for the treatment of TTS.

Resources use

The GDG discussed that not all heparin-based anticoagulants require the same resources. Non-fractioned heparin requires continuous intravenous administration and frequent laboratory monitoring in order to adjust the dose, whereas low-molecular-weight heparin does not need regular monitoring, but its administration requires specific training.

The GDG also noted that not all heparin-based anticoagulation treatments are equally cost-effective, with the cost being associated with not only to the direct cost of the product, but also with laboratory checks and monitoring visits.

Given the lack of specific data available to inform this judgement, the GDG judged that the costs for heparin treatment were likely to be variable and some GDG members reported costs would be large.

Equity and human rights

The GDG discussed that equity was likely to be reduced if other non-heparin treatments were recommended, instead of heparin, as many are not widely available, and choice would therefore be limited. Heparin is available in many LMIC settings, while the other non-heparin treatment options may not be.

Acceptability and feasibility

The GDG members believed that not all non-heparin-based anticoagulants would be equally acceptable to all stakeholders and that not all non-heparin-based therapies are equivalent, and also some of them are not widely available in all settings. The GDG members noted that heparin is the most widely used parenteral anticoagulant, and that many clinicians may not be trained or aware of other non-heparin-based treatments.

Recommendation rationale

The GDG formulated a conditional recommendation *against* heparin use for patients diagnosed with TTS following COVID-19 vaccination because:

1. of the uncertainty of the currently available data on the benefits and harms of heparin in TTS patients;
2. the present recommendations are applicable exclusively for emergency interim use and can be modified or completely changed when evidence becomes available; and
3. heparin may be the only therapeutic drug available in some countries who may not have the resources for treatment with IVIG and non-heparin anticoagulants.

Full details regarding the evidence-to-decision, GRADE tables and the literature review are available in supplementary data: methods for guideline development.

Annex 3: PICO 2: evidence-to-decision formulation

Results of the rapid literature review

Recovery

Six case series, including 110 patients with TTS, provided valid data for recovery rates., 77 who had been treated with NHAC. The global recovery rate was 65/110 (59.1%) (6, 11, 15, 18, 39, 103). Information about recovery in patients treated with non-heparin-based anticoagulants (NHACs) was available in 5 studies including 44 patients. The recovery rate in these patients was 28/44 (63.6%) (11, 6, 15, 39, 103).

Seven studies reported the use of IVIG [2 case reports and 5 case series] in 113 patients, 55 of them had been treated with IVIG (6, 10, 14, 15, 18, 39, 103). The global recovery rate was 64/113 (56.6%). The recovery rate in patients treated with IVIG was 6/9 (66.7%) from 4 studies including 9 patients (6, 10, 14, 103).

Five studies provided valid data about platelet transfusions, [2 case reports and 3 case series], including 48 patients, 16 of them had received platelet transfusion (8, 10, 14, 15, 39). The global recovery rate was 25/48 (52.1%). The recovery rate in patients who had received platelet transfusion was 0/6 (0%) from 3 studies including 6 patients (8, 10, 14).

Four case reports, including nine patients, reported data about steroid treatment seven of them had been treated with steroids (10, 14, 16, 103). The global recovery rate was 4/9 (44.4%). In patients treated with steroids it was 4/7 (57.1%) from four case reports including seven patients (10, 14, 16, 103).

The GDG noted that recovery data indicated some benefit, while noting that the studies were small and heterogenous and many studies were likely to have included combinations of drugs rather than single drugs. The GDG judged benefits as moderate but uncertain. The GDG noted that the data suggested there was no benefit from platelet transfusion although data were only available for 6 patients.

Mortality

Six case series provided data on mortality in 110 patients; 77 had been treated with NHAC (6, 11, 15, 18, 39, 103). The global mortality rate was 17/110 (15.4%). The mortality rate was 1/15 (6.7%) in 4 studies including 15 patients treated with NHAC (6, 11, 15, 103).

Eight studies [3 case reports and 5 case series] reported use of IVIG in 114 patients, 55 had been treated with IVIG (6, 10, 13-15, 18, 39, 103). The global mortality rate was 22/55 (40.0%). The mortality rate in patients treated with IVIG was 4/16 (25.0%) from 5 studies including 16 patients (6, 10, 14, 15, 103).

Five studies provided valid data about platelet transfusions [2 case reports and 3 case series] in 48 patients; 16 had received platelet transfusion (8, 10, 14, 15, 39). The global mortality rate was 12/48 (25.0%). The mortality rate in patients who had received platelet transfusion was 5/6 (83.3%) from three studies including six patients (8, 10, 14).

Six studies provided data about steroids [4 case reports and 2 case series] in 82 patients; 14 of had been treated with steroids (10, 14-16, 18, 103). The global mortality rate was 19/82 (23.2%). The mortality rate in patients treated with steroids was 3/7 (42.9%) from four studies including seven patients (10, 14, 16, 103).

Intracranial haemorrhage

Five case series provided valid data about intracranial haemorrhage in 49 patients; 44 had been treated with NHAC (6, 11, 15, 39, 103). The global intracranial haemorrhage rate was 20/49 (40.8%). The intracranial haemorrhage rate was 7/18 (38.9%) in 18 patients treated with NHAC from 4 studies (6, 11, 15, 103).

Seven studies [3 case reports and 4 case series] described the use of IVIG in 54 patients; 35 had been treated with IVIG (6, 10, 13-15, 39, 103). The global intracranial haemorrhage rate was 25/54 (46.3%). The intracranial haemorrhage rate in patients treated with IVIG was 5/10 (50.0%) from 5 studies including 10 patients (6, 10, 13, 14, 103).

Five studies [2 case reports and 3 case series] provided valid data about platelet transfusions in 48 patients, 16 had received platelet transfusion (8, 10, 14, 15, 39). The global intracranial haemorrhage rate was 23/48 (47.9%). The intracranial haemorrhage rate in patients who had received platelet transfusion was 4/6 (66.7%) from 3 studies including 6 patients (8, 10, 14).

Five studies [4 case reports and 1 case series] providing valid data for steroid treatment in 21 patients; 10 had been treated with steroids (10, 14-16, 103). The global intracranial haemorrhage rate was 12/21 (57.1%). The intracranial haemorrhage rate in patients treated with steroids was 3/7 (42.9%) from four studies including seven patients (10, 14, 16, 103).

The GDG noted that death was reduced in patients treated with NHAC or IVIG compared with the global rates and the intracranial haemorrhage rates were similar. Some GDG members noted that the harms were small and uncertain. The GDG noted a signal that mortality and haemorrhage were markedly increased in patients who had received platelet transfusion. The GDG noted that the events were reported for a small number of patients and acknowledged that platelet transfusion is usually only considered when there are limited alternatives to save the patient or prior to surgery which may introduce bias for the observed events. Uncertainty was also noted in these results., The GDG judged the harms of steroid treatment to be uncertain.

Certainty of evidence was very low for recovery, death and intracranial haemorrhage.

Certainty of evidence

The GDG agreed with the very low certainty rating. All studies were observational, either case series or case reports. The number of valid studies ranged from four to eight, the total number of included patients ranged from 9 to 113 for the recovery outcome, from 48 to -110 patients for the mortality outcome and from 21 to 54 patients for the intracranial haemorrhage outcome. The total number of treated patients ranged from 6 to 44 for the recovery outcome, from 6 to 16 for the mortality outcome and from 6 to 18 for the intracranial haemorrhage outcome. The risk of bias was judged to be very serious, with possible selection bias, as fatal cases were more likely to be quickly published as case reports (within a range of between 5 and 8 days). Not all confounding factors influencing the prognosis were analysed (age, time between symptom onset and treatment, concomitant treatments, presence of multiple organ thrombosis) and follow-up was incomplete in many cases. The degree of inconsistency was judged to be serious, since the dose was not described in many studies, the duration and follow-up protocols varied within the different studies. Imprecision was judged to be very serious, since the total number of patients included was less than the number of patients that would be needed for a single adequately powered trial, according to a sample size calculation. In addition, there was a strong suspicion of publication bias.

Balance of benefits versus harms

The GDG judged the benefits to be probably greater than the harms for NHAC and IVIG treatments. This was informed by the data but also by previous clinical experience and expert opinion, as well as pathophysiology mechanisms. The GDG judged the harms to be greater than the benefits for platelet transfusion. However, the GDG noted that when surgery is considered for thrombosis, there is a role for platelet transfusion. The GDG did not provide any judgment for steroid treatment, but noted the general use of steroids and the likelihood that steroids would usually be given in combination with other treatments.

Values and preferences

The GDG noted that from a patient perspective, given the lack of clear evidence of a single beneficial treatment modality, patients would probably show important variability and uncertainty in their choice of intervention to receive and the value they placed on specific outcomes.

However, some GDG members noted that there was probably no important uncertainty or variability, particularly with respect to not wanting to receive platelet transfusion. Mortality was noted to be the outcome the most highly valued by patients.

Resources use

Most members of the GDG described the current treatments, such as NHAC and IVIG, as expensive and the facilities and human resources required to administer them as costly. However, it was acknowledged that governments may be able to reduce costs of treatments, especially as vaccination is rolled out, in order to ensure appropriate treatment for TTS following COVID-19 vaccination. They noted that this may vary from setting to setting.

Equity and human rights

Equity was likely to be reduced if expensive interventions, such as for NHAC and IVIG that are not widely available, were recommended and this would therefore limit choice. However, it was noted that use of steroids would be unlikely have an impact on equity as these are widely available on the essential drug list.

The GDG noted that if platelet transfusions were not recommended, this would not have an impact on equity.

Acceptability and feasibility

The GDG members said they believed that all treatments were widely accepted by clinicians. The GDG said that despite high cost and requirements for specialist facilities, the treatments are feasible and should be considered as the rollout of adenovirus vector-based vaccines gathers speed in LMICs. Good referral systems and access will be essential (implementation considerations).

Recommendation rationale

The GDG formulated a strong recommendation ***in favour of*** the use of IVIG and NHAC for individuals with TTS following COVID-19 vaccination because:

1. The balance of benefit and harms was deemed to be in favour of their use despite the very low certainty of the evidence; and
2. Both IVIG and NHAC were likely to be widely acceptable to stakeholders and were feasible although costs and availability may continue to be a barrier to use in some settings.

The GDG formulated a strong recommendation ***against*** the use of platelet transfusion in individuals with TTS following COVID-19 vaccination because:

1. The extremely high rate of harms (death and intracranial haemorrhage) reported in the studies using platelet transfusion. Despite the very low certainty of this evidence, the life-threatening nature of the situation warranted a strong recommendation as the potential harms are considered to be catastrophic;
2. There was likely to be minimal variability in how patients value platelet transfusion, and most would not want to receive platelet transfusion, based on current evidence; and
3. The recommendation is strong, with the caveat that it is permissible where surgery is strongly indicated, and thrombocytopenia is severe.

The GDG did not make a specific recommendation on the use of steroids, noting that these were often used in combination with other treatments, and that use was variable and not indicated for all patients.

Full details regarding the evidence-to-decision, GRADE tables and the literature review are available in the Supplementary data: methods for guideline development.

Annex 4: Tables

Table 1. Cumulative incidence of TTS following vaccination with a non-replicant adenovirus vector-based vaccine (27 May 2021)

First author/source	Date of publication	Study period	Country	Vaccine	Dose	Cumulative incidence (95% CI) [cases per 100 000 vaccinees]
Schultz (10)	9/4/21	Unknown-20/03/2021	Norway	ChAdOx-1	First	3.8 (95% CI 1.4-9.3)
Spanish Medicines Agency (41)	11/5/21	01/02/2021-25/04/2021	Spain	ChAdOx-1	First	0.5 [1.3 in patients aged 30-39]
Centers for Disease Control and Prevention (39)	12/5/21	Unknown-07/05/2021	United States of America	BNT162b2, Ad26.COV2S	First	Global: 0.32 [1.2 in female patients aged 30-39]
Schulz (18)	13/5/21	Unknown-14/04/2021	Germany	ChAdOx-1 and BNT162b2	First	6.5 (95% CI 4.4-9.2) overall; 17.9 (95% CI 11.8-26.1) for ChAdOx1
Medicines & Healthcare products Regulatory Agency (48)	27/5/21	09/12/2020-26/05/2021	United Kingdom	ChAdOx-1	First and second	1.4 first dose, 0.13 second dose
Chan (43)	Preprint	Unknown-15/04/2021	Norway, Denmark, The Netherlands, Italy, Canada, United Kingdom, Germany, Australia, France, Spain	ChAdOx-1	First	0.73 (95% CI 0.43-1.23). Age <65 years: 1.60 (95% CI 0.71-3.62), Age 55-64 years: 0.41 (95% CI 0.1-1.65)

Table 2. Clinical syndromes suggestive of thrombosis or thromboembolism

Thrombosis location	Symptoms	Signs

Cerebral venous sinus	• New onset/unexplained headache:
	  • In some cases, may have headache-specific red flags, including resistance to symptomatic treatment and progressive worsening, sudden onset, strict unilaterality in some cases
	  • Headache may present with or without symptoms of increased intracranial pressure, as worsening by decubitus, sudden onset, strict unilaterality, worsening with Valsalva manoeuvres.
	  • Mild headaches immediately after vaccination are common. Headaches associated with TTS typically start or worsen 3-4 days after vaccination and progressively become very severe
	• Visual disturbances: blurred vision, diplopia, ophthalmodynia
	• Seizures
	• Altered mental status/encephalopathy
	• Decreased level of consciousness/coma
	• Focal neurological symptoms: weakness, sensory abnormalities, gait instability, speech disorders, dysarthria
	• Vomiting with or without nausea.
		• Meningeal irritation signs (Kernig sign, Brudzinski sign, Jolt accentuation sign);
		• Papilledema
		• Focal neurological signs (dysphasia, dysarthria, hemiparesis, hemi-hypoesthesia, hemianopia, aphasia, dysarthria, cranial nerve palsies, ophthalmoplegia, ataxia)
		• Cushing triad may suggest increased intracranial pressure: bradycardia, bradypnea (low respiratory rate) and arterial hypertension.

Abdominal veins (portal, superior mesenteric, splenic, hepatic)	• Abdominal pain • Bloating, nausea, vomiting • Diarrhoea/increased frequency of bowel movements • Constipation • Fever, anorexia • Back pain • Gastrointestinal bleeding	• Portal hypertension signs • Abdominal distension/ascites • Hepatomegaly • Jaundice
Deep vein	• Unilateral or bilateral swelling • Painful and tenderness • Limb swelling • Redness, distended veins	• Homan’s sign (discomfort or pain in the calf, or behind the knee, or involuntary flexion of the knee with forced dorsiflexion of the foot) • Asymmetric limb perimeter circumference
Pulmonary veins or arteries	• Dyspnoea, with sudden onset, shortness of breath, cough • Chest pain, with pleuritic characteristics • Difficulty to perform any physical exercises • Haemoptysis • Syncope, palpitations	• Increased respiratory rate • Tachycardia • Arterial hypotension

Thrombosis location	Symptoms	Signs
Myocardial arteries	● Chest pain, often cruising in nature ● Left arm pain ● Shortness of breath, cyanosis ● Sudden death	● Arrythmias, including asystole
Ischemic stroke/cerebral arteries	● Sudden onset focal neurological symptoms ● Weakness, sensory abnormalities, gait instability, speech disorders, dysarthria ● Abnormal gait	● Focal neurological signs (dysphasia, dysarthria, hemiparesis, hemi-hypoesthesia, hemianopia, aphasia, dysarthria, cranial nerve palsies, ophthalmoplegia, ataxia)
Thrombocytopenia	● Easy bruising	● Petechiae (tiny purple, red, or brown spots on the skin)

Table 3. Laboratory tests for the differential diagnosis of TTS

Laboratory test	TTS	ITP	TTP	DIC
Platelet count	Usually 20–50 × 10^9/L	Variable thrombocytopenia	Variable thrombocytopenia	Moderate to severe thrombocytopenia
Haemoglobin	Normal	Reduced if there is bleeding	Normal	Reduced
Peripheral blood smear	Normal/schistocytes	Normal/large platelets	Schistocytes	Normal/Schistocytes
Microangiopathic haemolytic anaemia/haemolysis markers	No	No	Yes	Yes (depending on the cause of DIC, usually non-diagnostic)
Anti-PF4 antibodies	Yes	No	No	No
PT	Normal or slightly prolonged	Normal	Normal	Prolonged
APTT	Normal or slightly prolonged	Normal	Normal	Prolonged
Fibrinogen (Clauss method, if available)	Initially increased, then decreased	Normal	Normal	Decreased
D-dimer	Increased (>4 times ULN)	Normal	Normal to increased	Increased
Other			ADAMST13 <10%	

TTS: thrombosis with thrombocytopenia syndrome; IPT: immune thrombocytopenic purpura; TTP: thrombotic thrombocytopenic purpura; DIC: disseminated intravascular coagulation; PT: prothrombin time; APTT: activated partial thromboplastin time; ULN: upper limit of normality; ADAMST13: *a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, also known as von Willebrand factor-cleaving protease.*

Table 4. Other conditions that should be included in the differential diagnosis of thrombosis/disseminated thrombosis and thrombocytopenia

Conditions	Key elements
Autoimmune heparin-induced thrombocytopenia	Heparin use during the past 4-30 days.
Atypical haemolytic uremic syndrome	Decreased complement factor H, and decreased complement factors 3 and 4 (C3, C4)
Catastrophic phospholipid antibody syndrome	Positive antiphospholipid antibodies, including cardiolipin antibodies, beta 2-glycoprotein I antibodies or lupus anticoagulant
Haemophagocytic syndrome	Decreased fibrinogen, extremely high concentration of ferritins (>10 000 µg/L) and high concentration of lipids
Drug-induced thrombotic microangiopathy	Prior treatment with **quinine**, ticlopidine (currently infrequently used), clopidogrel, trimethoprim-sulfamethoxazole, alendronate, vancomycin, pentostatin, chemotherapy (mitomycin, cyclosporine, tacrolimus, gemcitabine, carmustine, cytarabine, Taxotere), illicit drugs (cocaine, ecstasy)

Table 5. Specific diagnostic methods that provide results that may be consistent or suggestive of thrombosis/thromboembolism

Specific diagnostic methods consistent with confirmed thrombosis/thromboembolism	• Ultrasound – Doppler • CT scan – contrast/angiography • Magnetic resonance venography or arteriography • Echocardiogram • Perfusion V/Q scan • Conventional angiography/digital subtraction angiography
Procedures consistent with the presence of a thrombus	• Surgery • Thrombectomy
Pathologic examination that confirmed the presence of a thrombus	• Biopsy • Autopsy
Specific diagnostic modalities supportive of the presence of thrombosis/thromboembolism	• Chest radiography • Echocardiogram • CT scan without contrast • D-dimer (elevated above upper limit of normal for age)

Table 6: Optimal and alternative investigational tests used in TTS workups and typical diagnostic findings

Suspected thrombosis	Optimal tests	Alternative tests	Investigational findings

Cerebral venous sinus thrombosis	Contrast brain CT with CT venogram
	MRI brain with MR with contrast/MR venogram
		Non-contrast CT
		Brain MRI with T1, T2, SWI, GRE, time-of-flight venogram for those who cannot receive MR gadolinium contrast
		Fundoscopy
			• **CT/MR venogram**: Filling defect in sinus (empty delta sign)
			• **Non- contrast CT**:
			  ○ Hyperdense vein or sinus (cord sign)
			  ○ Venous infarcts: Parenchymal hypodensity in a non-arterial territory, typically in a parasagittal distribution
			  ○ Brain oedema signs: compression, obliteration of the basal cisterns, effacement of cerebral sulci, midline shift, optic nerve tortuosity, flattening of the posterior sclera, intraocular protrusion of the optic nerve hea
			  ○ Intracranial haemorrhage with early oedema in parasagittal locations
			• **Fundoscopy**: papilledema

Splanchnic thrombosis	CT angiography	Doppler ultrasound	• **Doppler ultrasound**: intraluminal echogenic material, absent signal on colour doppler consistent with thrombosis • **Ultrasound**: Hepatic enlargement, hepatic hypo echogenicity • **Contrast-CT**: filling defects • **Non-contrast CT**: Non-enhanced hypo-attenuation suggesting infarct
Deep vein thrombosis	Doppler ultrasound	MRI	• **Doppler ultrasound**: intraluminal echogenic material, absent signal on colour doppler consistent with thrombosis. Increased flow in superficial veins • **Ultrasound**: increased venous diameter, non-compressible venous segment with intraluminal material, loss of phasic flow on Valsalva manoeuvre or with calf squeeze.
Pulmonary thrombosis	CTPA	Echocardiogram Plain chest radiograph ECG V/Q scan	• **CTPA**: filling defects, thrombus surrounding by rim of contrast • **Echocardiogram**: signs of right ventricular dysfunction, thrombus-in-transit, flattening or dyskinesis of the interventricular septum • **Chest radiography**: Enlarged pulmonary artery, peripheral wedge of airspace opacity, regional oligemia, pleural effusions, enlarged right pulmonary artery, dilated right descending pulmonary artery with sudden cut-off • **ECG**: Tachycardia, S1Q3T3 pattern • **V/Q scan**: even distribution of radionuclide through both lungs (normal ventilation) with perfusion defects
Myocardial infarction	ECG, Echocardiogram, Coronary artery angiography		• **ECG:** ST segment elevation or depression, abnormal Q wave, T wave abnormalities • **Echocardiogram:** left ventricle ejection fraction, wall motion abnormalities
Ischemic stroke	Non-contrast head CT	MRI, perfusion CT, angio-CT, doppler ultrasound	• **CT:** loss of grey-white matter differentiation, hypoattenuation of deep nuclei, cortical hypodensity, gyral effacement

CT: computerized tomography; CTPA: computerized tomography pulmonary angiography; GRE: gradient echo sequences; MR: magnetic resonance; MRI: magnetic resonance imaging; SWI: susceptibility-weighted imaging; V/Q scan: ventilation–perfusion scan.

Table 7: Examples of non-heparin anticoagulant treatments for TTS

(based on available guidelines, indirect evidence from autoimmune heparin-induced thrombocytopenia and the first case series that have been published so far (103-150)).

Drug	Dose and mode of administration	Monitoring	Treatment duration
Argatroban IV	0.5-2 µg/kg/min (continuous IV infusion)	APTT monitoring (therapeutic range: 1.5-3) Argatroban should ideally be monitored by a direct thrombin inhibitor assay, if available, e.g., HEMOCLOT™ as APTT correlates poorly with the Argatroban effect due to the high concentrations of factor VIII	≤14 days
Bivalirudin IV	0.75 mg/kg bolus and continuous IV infusion 1.75 mg/kg/h	APTT monitoring (therapeutic range: 1.5-3)	Up to 3 months or until switch to oral anticoagulation
Fondaparinux SC	5mg/24h (<50kg) 7.5mg/24h (51-99kg) 10 mg/24 h (SC) >100kg	50% dose in case of platelet count <30 000/µL Reduce dosing with severe renal impairment	Up to 3 months or until switch to oral anticoagulation
Danaparoid SC or IV	500 IU/kg /12h 1x2 (<50 kg) or 750 IU/kg / 12h (SC) IV bolus < 60 kg 1500IU 60-75 kg 2250 IU 75-90 kg 3000 IU >90 kg 3750 IU. Infuse: 400 IU/h for 4h->300 IU/h for 4h-maintenance dose 200 IU/h, if GFR (<50ml/min) 150 IU/h and anti-FXa activity target (0.3-0.5 IU/ml, or trough with SC dosing), SC: 750-1250 IU/8-12 h Prophylactic dose: 750 IU / 12h (SC)	Measure anti-FXa activity, if available.	Up to 3 months or until switch to oral anticoagulation
Rivaroxaban PO	15 mg/12 h	To be considered in less severe patients, with no active bleeding and platelet count >50 000/µL	From day 22: 20 mg/24 h once daily Adjust the dose in case of renal impairment
Apixaban PO	10 mg/12 h	To be considered in less severe patients, with no active bleeding and platelet count >50 000/µL	From day 8: 5 mg/12 h Adjust the dose in case of renal impairment
Dabigatran PO	110 mg/12 h or 150 mg/12 h	CSVT or DVT or PE	According to GFR and patient's weight

APTT: activated partial thromboplastin time; GFR: glomerulus filtration rate; IV: intravenous; PO: per os (oral); SC: subcutaneous

Annex 5: Methods for guideline development

Summary

The present guideline has been developed by a group of multidisciplinary experts and members of the WHO secretariat representing different departments. The scientific approach has been based on PICO questions and the existing literature has been systematically searched, reviewed and summarized. The treatment recommendations have been derived through GRADE methodology.

Methodology

On 13April 2021 the COVID-19 subcommittee of the Global Advisory Committee on Vaccine Safety (GACVS) of the World Health Organization (WHO) recommended the creation of an expert group for advice and guidance on the clinical diagnosis and case management of thrombosis with thrombocytopenia syndrome (TTS) following vaccination with a COVID-19 non-replicant adenovirus vector-based vaccine. A Guideline Development group (GDG) comprising 18 expert specialists in internal medicine, epidemiology, haematology, immunology, neurology, neurosurgery and pharmacology, was established, ensuring gender, age and geographic balance, and LMIC representation. Experts and a subject matter expert contracted by WHO had to sign a confidentiality agreement as well as undergo conflict of interest assessment. Experts with conflicts were excluded from the GDG. The WHO secretariat included staff from different departments: Immunizations, Vaccines and Biologicals (IVB), Quality Norms and Standards (QNS), Regulation and Prequalification (RPQ), Mental Health and Substance use (MSD), Noncommunicable Diseases (NCDs) and WHO Health Emergencies Programme (WHE). Key questions were formulated, a literature search was performed and identified studies were reviewed, and distributed to the GDG members, to provide comments and feedback. These were collated in a zero draft by the subject matter expert. The work of the group was coordinated by the Vice Chairperson of the GACVS. The treatment guidelines section was overseen by a Guideline Methodologist. The guideline was developed between Tuesday 27 April and Friday 4 June 2021.

Guideline development

Based on the expertise of members, four subgroups (coordinated by a secretariat focal person) were established to develop four main sections of the document that were merged and handed over to the subject matter expert (SME) (David Garcia Azorin –Hospital Clínico Universitario de Valladolid, Valladolid, Spain) for harmonization of the different sections and presented to the GDG as a single document. The subgroups were:

- Subgroup 1: Epidemiology, risk factors and pathophysiology (Kim Mulholland (GDG), Huyun Tran (GDG), David Garcia Azorin (SME), Adwoa Bentsi-Enchill (Secretariat focal point)
- Subgroup 2: Manifestation of TTS after COVID19 vaccination (Georgy Genov (GDG), Prasanna Kumar (GDG), Viola Macolic Sarinic (GDG), Huyun Tran (GDG), Nicoline Schiess, (Secretariat focal point)
- Subgroup 3: Case definition, clinical features and laboratory diagnosis. (Dale Nordenberg (GDG), Doris Oberle (GDG), Prasanna Kumar (GDG), Riitta Lissala (GDG), Tom Solomon (GDG), Kameshwar Prasad (GDG), Annick Janin (Secretariat focal point)
- Subgroup 4: Clinical case management, including review of drug treatment and other therapeutics Kameshwar Prasad (GDG), D.S Akram (GDG), Imo J. Akpan (GDG), Kiran Thakur (GDG), Ayeesha Kamal (GDG), Ushma Mehta (GDG), Julio Resendiz (GDG), NK Arora (GDG), Claudia Patricia Vaca Gonzalez (GDG), Madhava Rao Balakrishnan (Secretariat focal point)

Full affiliations of all members are provided in the Acknowledgements section.

A timeline for the work and the process were discussed and developed as described below. Then each subsection was developed independently by the respective subgroups, coordinated by a secretariat focal person, and then merged. The merged document was harmonized by the subject matter expert (SME) before being posted on a dedicated SharePoint for review by all the GDG members.

Stage	Milestone
Week 0	Identification of experts, contact and clearance
Week 0	Subgroups defined
Week 1	Literature review to identify published studies by WHO Rapid Review Group Team Members* from WHO's COVID-19 database, PubMed and Global Index Medicus (GIM) (search terms and the flowchart of the identified, screened and included studies are available in Search strategies for bibliographic databases: PubMed, WHO COVID-19 Database and Global Index Medicus). The selected studies were categorized as ***A. Studies on patients vaccinated with a COVID-19 vaccine (40 citations)*** • studies describing clinical characteristics; • studies describing health services; • studies describing physiopathology of TTS; • studies describing other aspects of TTS, e.g., diagnosis, prognosis; • studies describing epidemiology of TTS. ***B. Studies on patients with COVID-19 without vaccination (107 citations)***
Week 2	Research questions developed and focus areas clearly defined by each subgroup
Week 3	First zero draft for each of the four subsections developed
Week 4	Updated literature review by the WHO Rapid Review Group Team Members using a new search strategy based on the comments/ and recommendations from the clinical experts Only studies that included patients having received ≥1 dose of a COVID-19 vaccine and that met the eligibility criteria were included. • studies describing clinical characteristics (12 records) • studies describing treatment (3 records) • studies describing diagnosis of TTS (4 records) • studies describing epidemiology of TTS (5 records) • studies describing prognosis of patients with TTS (2 records) • studies describing physiopathology of TTS (1 record) • studies describing health services (1 record)
Week 4	All subgroups submit a zero draft with feedback from the meeting on 17 May
Week 5	Zero draft harmonized and posted on SharePoint for feedback from all GDG members
Week 6	Expert discussion

*WHO Science Division

A rapid review of the literature, based on the key questions, was performed on 6 May 2021 and updated on 22 May 2021 to identify existing publications on TTS in patients with COVID-19 and those who had been vaccinated with a COVID-19 vaccine. The search strategies were developed by members of the WHO secretariat. (The search terms are available in Search strategies for bibliographic databases: PubMed, WHO COVID-19 Database and Global Index Medicus). Three electronic databases were searched, WHO's COVID-19 database, PubMed and the Global Index Medicus (GIM) database. Screening of these results to select potential studies was performed by two reviewers, members of the WHO secretariat.

The first strategy, which included COVID-19 populations with and without vaccination, retrieved 404 citations. A total of 257 were excluded by screening the titles and abstracts. After the second screen of the full text, 40 studies in COVID-19-vaccinated populations and 107 studies in non-vaccinated COVID-19 populations.

The second search strategy, which only included COVID-19-vaccinated populations, retrieved 381 citations. A total of 353 were excluded after screening the titles and abstracts. After the second screen of the full text, 28 were included.

In total, 785 citations were retrieved. References from the included studies were also reviewed and the 'similar articles' function in PubMed was used to identify additional records, leading to a total of 804 citations retrieved that were screened by the subject matter expert, who reviewed and extracted the information and summarized the evidence. The documents created by the experts were collated, and all the comments and changes were tracked.

Treatment guidelines

Rapid review for treatment

For the treatment guidelines, two PICO questions were formulated:

1) Should heparin-based anticoagulants (I) versus no heparin or other anticoagulants (C) be administered to individuals diagnosed with TTS following COVID-19 vaccination (P)?
2) Should specific drugs or procedures (intravenous immunoglobulin (IVIG), steroids, anticoagulants excluding heparin-based anticoagulants, blood products transfusion) (I) versus none or other drugs or procedures (C) be administered to individuals who present with TTS following COVID-19 vaccination (P)?

The outcomes of interests were: recovery, death and intracranial haemorrhage.

The risk of bias in the treatment guidelines was assessed for each study included using the GRADE Handbook1. Biases specific to observational studies were analysed, including failure to develop and apply appropriate eligibility criteria, presence of control populations, flawed measurement of both exposure and outcome, failure to adequately control confounding and incomplete follow-up. The data from the included studies were analysed descriptively and the certainty of evidence, which included assessment of the limitations of study design or execution, inconsistency of results, indirectness of evidence, imprecision and publication bias, was evaluated using GRADEPro2.

Three outcomes were considered to be critical; according to the GRADE approach (i.e., most important to the patient who will be affected by the recommendations):

1) recovery, defined as the explicit mention of discharge of the patient in a recovered status;
2) mortality, including all-cause mortality and not just treatment-specific mortality; and
3) intracranial haemorrhage.

Patients with uncertain outcomes were not classified as recovery or death. Studies and patients in the studies with valid information for each of the different outcomes were analysed. The outcome rates were calculated for all patients in the individual studies regardless of treatment received and only for patients who had received the treatment of interest. The outcome rates for all patients were calculated by dividing the total number of patients with the outcome by the total number of patients included in each study. The outcome rates for each specific treatment were calculated by dividing the total of patients who received the treatment with outcome of interest by the total number of patients in the studies that provided valid data for the treatment. Only the data available in the publications were analysed, and no attempt to find missing information was made. Summary of findings (SoF) are presented in GRADE Evidence Profile Tables (Evidence tables)

Recommendation formulation

Two virtual meetings were held via Zoom on 7 and 8 June 2021. The meetings were chaired by a member of the GDG approved by the group in advance of the first meeting. An experienced guidelines methodologist facilitated the evidence-to-decision-making (EtD) process as described in the WHO Handbook for Guideline Development.3 Although the original aim was to base all decisions on a consensus, at the beginning of the meeting the GDG members agreed that if any decisions required a vote, the vote would need to be carried by a 60% majority.

The GDG reviewed the evidence contained in the systematic reviews and in the GRADE EtD tables, and discussed the topics under consideration, facilitated by the guideline methodologist. The GRADE EtD communicate the GDG's judgements about several factors as well as their judgements on the benefits and harms and their certainty. These factors include:

- the values and preferences of end-users;
- resource use, including costs and cost-effectiveness;
- potential impact on human rights and equity; and
- acceptability and feasibility.

1 GRADE Handbook, available from https://gdt.gradepro.org/app/handbook/handbook.html#h.ged5uqebmir9. Accessed 30 May 2021.

2 GRADEpro GDT, available from https://gradepro.org/. Accessed 28 June 2021.

3 World Health Organization. WHO handbook for guideline development – 2nd ed, available from https://www.who.int/publications/i/item/9789241548960. Accessed 28 June 2021.

During the virtual meets, care was taken to ensure that all members of the GDG gave their responses through regular unofficial votes and use of the chat function to gain an initial hint about the GDG members' views on the direction of each recommendation (i.e., for or against an intervention), and on the strength of each recommendation (strong or conditional). The methodologist also asked participants to raise their hands to show support for each separate option. Although there was no formal vote system, this approached enabled the methodologist and the chair to assess the distribution of opinions and to prompt further discussion with the aim of reaching consensus.

The strength of the recommendation was established by considering both the certainty of the evidence and the availability and feasibility of the interventions. A recommendation **for** an intervention indicates that it should be implemented and a recommendation **against** an intervention indicates that it should not be implemented. The strength of a recommendation, which was either 'strong' or 'conditional', reflects the degree of confidence the GDG had that the desirable effects of the recommendation outweighed the undesirable effects for a positive recommendation, or the reverse (that the undesirable effects outweigh the desirable effects) for a negative recommendation.

The final wording of each recommendation, including an indication of its direction and strength, was confirmed by consensus between all GDG members and each member of the GDG was asked to express their decision verbally. The judgements made by the GDG related to each recommendation are summarized in the section Evidence to decision tables framework.

As new data continues to emerge, these observations and recommendations may change. Therefore, these guidelines have a shelf-life of 3-6 months and clinicians should be aware of this.

External review

Seven external reviewers (from Argentina, China, Ghana, Iraq, Morocco, Myanmar and Russia), were invited to review the pre-final version of the guidelines to ensure readability and geographic relevance. All experts signed a confidentiality agreement and were assessed for any conflict of interest.

Limitations

The main limitations of the present guidelines are related to the quality and quantity of the existing evidence., often based on single cases or case series. The degree of certainty was affected by the presence of biases that should be better managed in future studies. In addition, the number of patients represented in the analysis of the different treatment modalities was not balanced and in some cases was significantly low. Evidence regarding some treatment options for LMIC settings was also limited and should be properly evaluated, ideally with well-designed observational studies or with randomized-controlled trials.

The GDG did not include patient representatives, which should be addressed in future updates.

Annex 6: Supplementary information on literature review methods and results

Thrombosis with thrombocytopenia syndrome (TTS) in the context of post-COVID-19 vaccination

Rapid Review Group Team Members: Mónica Ballesteros, Jesús López Alcalde, Kavita Kothari.
Last Date of Search: 22 May 2021
Date of Report: 23 May 2021

1. Objective:

To update the evidence available on TTS in the context of post-COVID-19 vaccination.

2. PICOs:

The unique component of PICO structure included was population because the clinical questions require a comprehensive literature search strategy.

Population (in the context of post COVID-19 vaccination): Adult population who have received a COVID-19 vaccine with clinical signs and symptoms of thrombosis and associated novel thrombocytopenia within 4 to 28 days of vaccination. In this updated version, we have included the following terms without being associated with a new episode of thrombocytopenia: cerebral venous sinus thrombosis (CVST), Splanchnic vein thrombosis (intra-abdominal), deep vein thrombosis (DVT), disseminated intravascular coagulation (DIC), pulmonary embolism (PE), stroke and myocardial infarction.

3. Methods

3.1 Search methods for study identification

This updated search strategy was developed by a WHO librarian taking into account comments and recommendations from clinical experts. More details can be found in the section Search strategies for bibliographic databases: PubMed, WHO COVID-19 Database and Global Index Medicus.

3.2 Electronic databases:

- WHO COVID-19 database
- PubMed
- Global Index Medicus

3.3 Methods for screening search results

The screening was performed by two reviewers (MB, JLA), who classified the results according to the type of data present in the publication (Table S1).

Table S1. Classification of studies by data type

Data type	Definition
Epidemiology	Study describes burden of disease or describes the distribution of disease
Mechanism	The study describes the possible underlying causes of TTS or its physiopathology
Clinical characteristics	Study describes clinical characteristics of inpatients or outpatients
Diagnosis	Study describes clinical signs and symptoms, laboratory tests, imaging examinations, or any other test to establish the diagnosis of TTS
Prognosis	The study provides data on the predicted prognosis of an outcome of interest
Treatment	The study describes the assessment of a treatment or management intervention for TTS (e.g., treatment, maximize comfort, alleviate symptoms or side effects).
Health services	The study describes the assessment of the delivery, processes, management, organization, or financing of healthcare.

Selected PICO questions used in the rapid review of evidence

Selected PICO questions used in the rapid review of evidence on TTS indications and treatment in the context of post-COVID-19 vaccination: a rapid review of evidence. Search Strategy Development.

05 May 2021

- What is the global aetiology, baseline epidemiology of TTS in adults post-COVID-19 vaccination?

- What is the clinical presentation of TTS in patients post-COVID-19 vaccination?

- Is an internationally agreed case definition for patients presenting with TTS post vaccination emerging in the literature? What definitions are currently available, including clinical features and laboratory diagnosis?

- **What algorithm is most suitable for triaging and case management of patients with Covid-19 vaccine-related TTS and thrombosis/thromboembolism after vaccination with an adenovirus vector-based COVID-19 vaccine?**

- The main component of the PICO question was population, because the clinical questions required a comprehensive literature search strategy.

- The second search strategy included the following terms: Cerebral Venous Sinus Thrombosis (CVST), splanchnic vein thrombosis (intra-abdominal), deep vein thrombosis (DVT), disseminated intravascular coagulation (DIC), pulmonary embolism (PE), stroke and myocardial infarction.

Search strategies for bibliographic databases: PubMed, WHO COVID-19 Database and Global Index Medicus

Population	Patients with vaccine-related **TTS. Similar pathophysiology as that for heparin–-induced thrombocytopenia**
Intervention	Non-heparin-based anticoagulant (NHAC)s, high-dose intravenous immunoglobulin (IVIG), and prednisolone, thromboprophylaxis, steroids, folate, platelet transfusion, splenectomy Platelet count <50 000/µL
Full PubMed search strategy	(("Heparin induced thrombocytopenia"[tiab] OR ((prothrombotic OR thrombosis[tiab] OR thrombotic[tiab] OR thromboembolism[tiab]) AND ("thrombocytopenia"[tiab] OR thrombocytopenic[tiab]))) AND systematic[sb]) **(157)** **((prothrombotic OR thrombosis[tiab] OR thrombotic[tiab] OR thromboembolism[tiab]) AND ("thrombocytopenia"[tiab] OR thrombocytopenic[tiab])) AND (2020:2021[pdat]) AND (Coronavirus*[TIAB] OR corona?virus[Tiab] OR "Coronavirus"[Mesh] OR coronavirinae[tiab] OR coronaviridae[tiab] OR betacoronavirus[tiab] OR "corona pandemic"[tiab] OR COVID OR Covid19[TW] OR NCov[Tiab] OR 2019ncov[TW] OR (SARS AND COV) OR SARS2 OR "CoV 2"[tiab] OR CoV2[tiab] OR "Coronavirus Infections"[Mesh] OR NCOV19[tiab] OR "solidarity trial"[tiab]) (142)**
	OR "Vaccine Induced Thrombocytopenia Syndrome"[tiab] OR "vaccine-induced prothrombotic immune thrombocytopenia"[tiab] OR "Vaccine-Induced Immune Thrombotic Thrombocytopenia"[tiab] OR " inflammatory and thrombotic response to vaccination"[tiab] OR "Rare thromboembolic syndrome"[tiab] OR VIPIT[tiab] OR (("Cerebral venous sinus thrombosis"[tiab] OR " Cerebral venous thrombosis"[tiab] OR " Sinus Thrombosis, Intracranial"[tiab] OR "cerebral sinovenous thrombosis"[tiab] OR "cerebral vein thrombosis"[tiab] OR "cerebral venous and sinus thrombosis"[tiab] OR "cerebral vein and dural sinus thrombosis"[tiab] OR " cavernous sinus thrombosis"[tiab] OR CVST[tiab] OR "Anti PF4 antibodies") AND ("thrombocytopenia"[tiab] OR thrombocytopenic[tiab]))
Full String WHO COVID-19 Database	"Heparin induced thrombocytopenia" OR ((prothrombotic OR thrombosis OR thrombotic OR thromboembolism) AND ("thrombocytopenia" OR thrombocytopenic)) OR "Vaccine Induced Thrombocytopenia Syndrome" OR "vaccine-induced prothrombotic immune thrombocytopenia" OR "Vaccine-Induced Immune Thrombotic Thrombocytopenia" OR " inflammatory and thrombotic response to vaccination" OR "Rare thromboembolic syndrome" OR VIPIT OR (("Cerebral venous sinus thrombosis" OR " Cerebral venous thrombosis" OR " Sinus Thrombosis, Intracranial" OR "cerebral sinovenous thrombosis" OR "cerebral vein thrombosis" OR "cerebral venous and sinus thrombosis" OR "cerebral vein and dural sinus thrombosis" OR " cavernous sinus thrombosis" OR CVST OR "Anti PF4 antibodies") AND ("thrombocytopenia" OR thrombocytopenic))
WHO Database Link	https://search.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/?output=site&lang=en&from=0&sort=&format=summary&count=20&fb=&page=1&skfp=&index=tw&q=%22Heparin+induced+thrombocytopenia%22+OR+%28%28prothrombotic+OR+thrombosis+OR+thrombotic+OR+thromboembolism%29++AND+%28%22thrombocytopenia%22+OR+thrombocytopenic%29%29+++OR+%22Vaccine+Induced+Thrombocytopenia+Syndrome%22+OR+%22vaccine-induced+prothrombotic+immune+thrombocytopenia%22+OR+%22Vaccine-Induced+Immune+Thrombotic+Thrombocytopenia%22+OR+%22+inflammatory+and+thrombotic+response+to+vaccination%22+OR+%22Rare+thromboembolic+syndrome%22+OR+VIPIT+OR+++%28%28%22Cerebral+venous+sinus+thrombosis%22+OR+%22+Cerebral+venous+thrombosis%22+OR+%22+Sinus+Thrombosis%2C+Intracranial%22+OR+%22cerebral+sinovenous+thrombosis%22+OR+%22cerebral+vein+thrombosis%22+OR+%22cerebral+venous+and+sinus+thrombosis%22+OR+%22cerebral+vein+and+dural+sinus+thrombosis%22+OR+%22+cavernous+sinus+thrombosis%22+OR+CVST%29++AND+%28%22thrombocytopenia%22+OR+thrombocytopenic%29%29&search_form_submit=

Global Index Medicus	tw:("Heparin induced thrombocytopenia" OR ((prothrombotic OR thrombosis OR thrombotic) AND ("thrombocytopenia" OR thrombocytopenic)) OR "Vaccine Induced Thrombocytopenia Syndrome" OR "vaccine-induced prothrombotic immune thrombocytopenia" OR "Vaccine-Induced Immune Thrombotic Thrombocytopenia" OR " inflammatory and thrombotic response to vaccination" OR "Rare thromboembolic syndrome" OR vipit OR (("Cerebral venous sinus thrombosis" OR " Cerebral venous thrombosis" OR " Sinus Thrombosis, Intracranial" OR "cerebral sinovenous thrombosis" OR "cerebral vein thrombosis" OR "cerebral venous and sinus thrombosis" OR "cerebral vein and dural sinus thrombosis" OR " cavernous sinus thrombosis" OR cvst) AND ("thrombocytopenia" OR thrombocytopenic))) AND (type_of_study:("guideline" OR "policy_brief" OR "systematic_reviews"))
GIM LINK	https://pesquisa.bvsalud.org/gim/?output=site&lang=en&from=0&sort=&format=summary&count=20&fb=&page=1&filter%5Btype_of_study%5D%5B%5D=guideline&filter%5Btype_of_study%5D%5B%5D=policy_brief&filter%5Btype_of_study%5D%5B%5D=systematic_reviews&index=tw&q=tw%3A%28%22Heparin+induced+thrombocytopenia%22+OR+%28%28prothrombotic+OR+thrombosis+OR+thrombotic%29++AND+%28%22thrombocytopenia%22+OR+thrombocytopenic%29%29+++OR+%22Vaccine+Induced+Thrombocytopenia+Syndrome%22+OR+%22vaccine-induced+prothrombotic+immune+thrombocytopenia%22+OR+%22Vaccine-Induced+Immune+Thrombotic+Thrombocytopenia%22+OR+%22+inflammatory+and+thrombotic+response+to+vaccination%22+OR+%22Rare+thromboembolic+syndrome%22+OR+vipit+OR++%28%28%22Cerebral+venous+sinus+thrombosis%22+OR+%22+Cerebral+venous+thrombosis%22+OR+%22+Sinus+Thrombosis%2C+Intracranial%22+OR+%22cerebral+sinovenous+thrombosis%22+OR+%22cerebral+vein+thrombosis%22+OR+%22cerebral+venous+and+sinus+thrombosis%22+OR+%22cerebral+vein+and+dural+sinus+thrombosis%22+OR+%22+cavernous+sinus+thrombosis%22+OR+cvst%29++AND+%28%22thrombocytopenia%22+OR+thrombocytopenic%29%29%29+AND+%28+type_of_study%3A%28%22guideline%22+OR+%22policy_brief%22+OR+%22systematic_reviews%22%29%29&search_form_submit=

Rapid Review Group	Research Topic
	Thrombosis with Thrombocytopenia Syndrome

WHO COVID-19 Database
https://search.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/

Concept	Search string	Results 22.05.2021
#1- TTS in COVID-19	((prothrombotic OR thrombosis OR thrombotic OR thromboembolism OR embolism OR thrombus OR D-dimer OR "Splanchnic vein" OR SVT OR CVST OR DVT OR "Disseminated Intravascular coagulation" OR "consumptive coagulopathy" OR "disseminated intravascular coagulopathy" OR "defibrination syndrome" OR "defibrinogenation syndrome" OR "acquired afibrinogenemia" OR Stroke OR "cerebrovascular accident" OR "CVA" OR "cerebral infarct" OR "ischemic infarctions" OR "CNS infarction" OR "Myocardial Infarction" OR "coronary infarction") AND ("thrombocytopenia" OR thrombocytopenic OR "Anti PF4 antibodies" OR "platelet factor 4" OR "low platelet")) OR "Vaccine Induced Thrombocytopenia Syndrome" OR "vaccine-induced prothrombotic immune thrombocytopenia" OR "Vaccine-Induced Immune Thrombotic Thrombocytopenia" OR " inflammatory and thrombotic response to vaccination" OR "Rare thromboembolic syndrome" OR VIPIT OR "Heparin induced thrombocytopenia"	353
# 2 – Thrombosis after COVID-19 vaccination	("Cerebral venous sinus thrombosis" OR " Cerebral venous thrombosis" OR "Sinus Thrombosis, Intracranial" OR "cerebral sinovenous thrombosis" OR "cerebral vein thrombosis" OR "cerebral venous and sinus thrombosis" OR "cerebral vein and dural sinus thrombosis" OR " cavernous sinus thrombosis" OR "deep venous thrombosis" OR "deep vein thrombosis" OR "diffuse intravascular thrombosis" OR "arterial thrombosis" OR CVST OR DVT OR "Disseminated Intravascular coagulation" OR "consumptive coagulopathy" OR "disseminated intravascular coagulopathy" OR "defibrination syndrome" OR "defibrinogenation syndrome" OR "acquired afibrinogenemia" OR "splanchnic vein" OR SVT OR "intra-abdominal thrombosis" OR "intra-abdominal venous thrombosis" OR "intra-abdominal vein thrombosis" OR "abdominal thrombosis" OR "venous thromboembolism" OR "pulmonary embolism" OR "pulmonary thromboembolism" OR Stroke OR "cerebrovascular accident" OR "CVA" OR "cerebral infarct" OR "ischemic infarctions" OR "CNS infarction" OR "myocardial infarction" OR "coronary infarction") AND (Innoculation* OR Immuniz* OR Vaccin* OR BNT162b2 OR "comirnaty" OR "mRNA-1273" OR CoviShield OR AZD1222 OR "Sputnik V" OR CoronaVac OR "BBIBP-CorV" OR "Ad26.CoV2.S" OR "JNJ-78436735" OR Ad26COVS1 OR VAC31518 OR EpiVacCorona OR Convidicea OR Ad5-nCoV OR Covaxin OR CoviVac OR ZF2001 OR "NVX-CoV2373" OR "ZyCoV-D" OR CIGB 66 OR "CVnCoV" OR "INO-4800" OR "VIR-7831" OR "UB-612" OR BNT162 OR "Soberana 1" OR "Soberana 2" OR Pzifer OR Moderna OR "Pzifer/bioNtech" OR AstraZeneca OR Gamaleya OR Sinovac OR Sinopharm OR "johnson & Johnson" OR Janssen OR "CanSino Biologics" OR "Bharat Biotech" OR "wuhan institute" OR Chumakov OR "Longcom Biopharmaceutical" OR "Finlay Institute of Vaccines" OR Novavax OR "Zydus Cadila" OR "Center for Genetic Engineering and Biotechnology" OR CureVac OR "University of Melbourne" OR "Murdoch Children's Research Institute" OR "Radboud University Medical Center" OR "Faustman Lab" OR "Inovio Pharmaceuticals" OR Dynavax OR ImmunityBio OR NantKwest OR COVAXX OR "adenovirus vector")	256

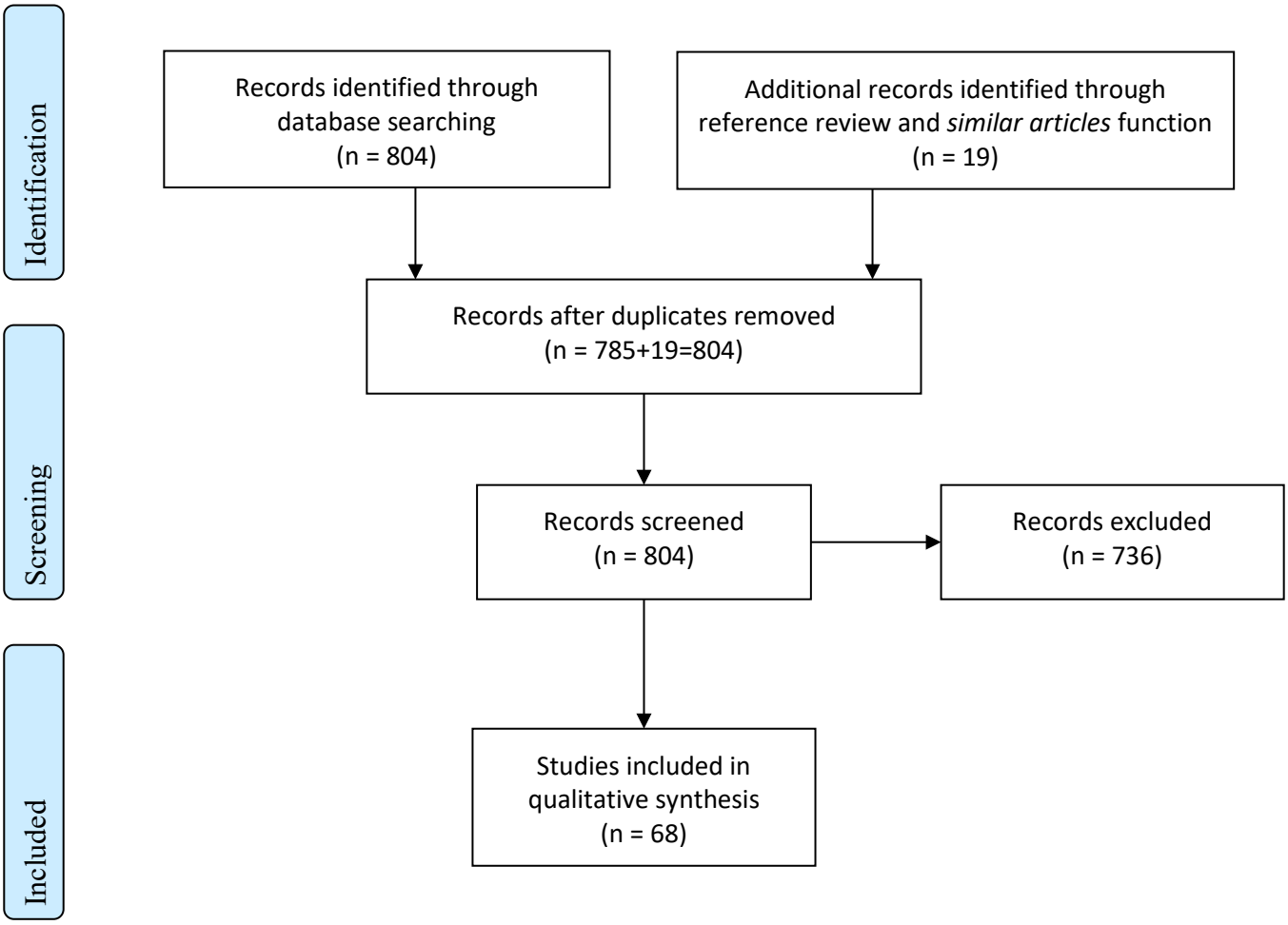
Pubmed
https://pubmed.ncbi.nlm.nih.gov/

Concept	Search string	Results
#3 - SR on TTS-like syndromes	"Heparin induced thrombocytopenia"[tiab] OR "Vaccine Induced Thrombocytopenia Syndrome"[tiab] OR "vaccine-induced prothrombotic immune thrombocytopenia"[tiab] OR "Vaccine-Induced Immune Thrombotic Thrombocytopenia"[tiab] OR " inflammatory and thrombotic response to vaccination"[tiab] OR "Rare thromboembolic syndrome"[tiab] OR VIPIT[tiab] OR ((prothrombotic[tiab] OR thrombosis[tiab] OR thrombotic[tiab] OR thromboembolism[tiab] OR embolism[tiab] OR thrombus[tiab] OR D-dimer[tiab] OR "Splanchnic vein"[tiab] OR SVT[tiab] OR CVST[tiab] OR DVT[tiab] OR "Disseminated Intravascular coagulation"[tiab] OR "consumptive coagulopathy"[tiab] OR "disseminated intravascular coagulopathy"[tiab] OR "defibrination syndrome"[tiab] OR "defibrinogenation syndrome"[tiab] OR "acquired afibrinogenemia"[tiab] OR Stroke[tiab] OR "cerebrovascular accident"[tiab] OR "CVA"[tiab] OR "cerebral infarct"[tiab] OR "ischemic infarctions"[tiab] OR "CNS infarction"[tiab] OR "Myocardial Infarction"[tiab] OR "coronary infarction"[tiab]) AND ("thrombocytopenia"[tiab] OR thrombocytopenic[tiab] OR "Anti PF4 antibodies"[tiab] OR "platelet factor 4"[tiab] OR "low platelet"[tiab])) AND systematic[sb]	181

Global Index Medicus
https://pubmed.ncbi.nlm.nih.gov/

Concept	Search string	Results
#4 - SR on TTS-like syndromes	"Heparin induced thrombocytopenia" OR "Vaccine Induced Thrombocytopenia Syndrome" OR "vaccine-induced prothrombotic immune thrombocytopenia" OR "Vaccine-Induced Immune Thrombotic Thrombocytopenia" OR " inflammatory and thrombotic response to vaccination" OR "Rare thromboembolic syndrome" OR VIPIT OR ((prothrombotic OR thrombosis OR thrombotic OR thromboembolism OR embolism OR thrombus OR D-dimer OR "Splanchnic vein" OR SVT OR CVST OR DVT OR "Disseminated Intravascular coagulation" OR "consumptive coagulopathy" OR "disseminated intravascular coagulopathy" OR "defibrination syndrome" OR "defibrinogenation syndrome" OR "acquired afibrinogenemia" OR Stroke OR "cerebrovascular accident" OR "CVA" OR "cerebral infarct" OR "ischemic infarctions" OR "CNS infarction" OR "Myocardial Infarction" OR "coronary infarction") AND ("thrombocytopenia" OR thrombocytopenic OR "Anti PF4 antibodies" OR "platelet factor 4" OR "low platelet")) AND (type_of_study:("guideline" OR "policy_brief" OR "systematic_reviews"))	14

Flowchart of the identified, screened and included studies.

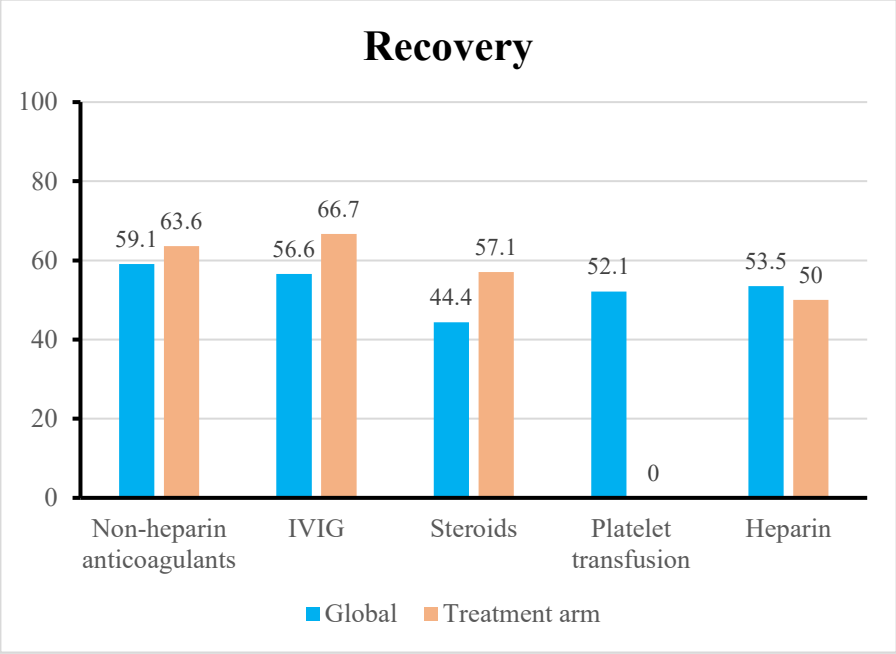


Evidence to decision tables framework[4]

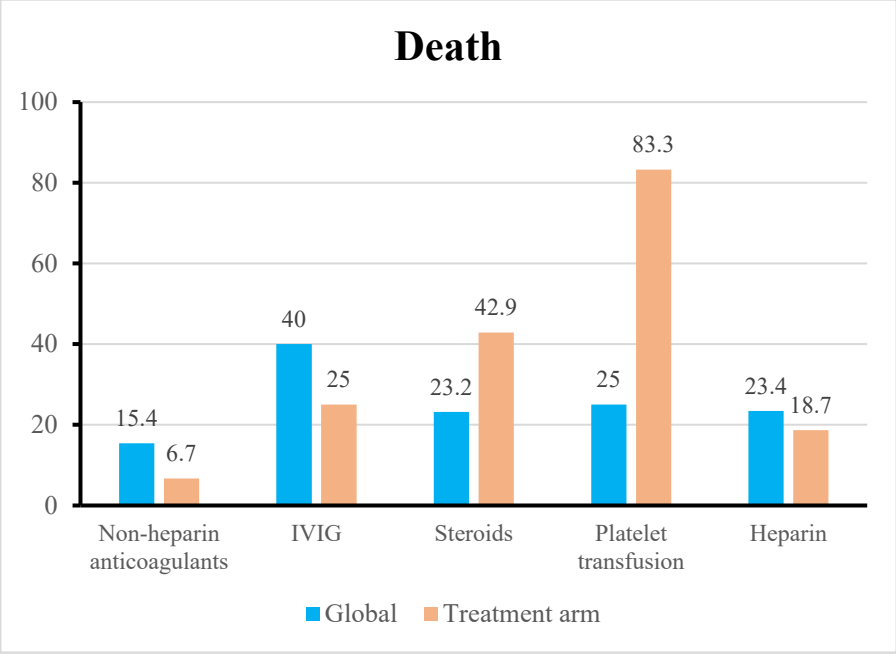
Question	Judgement
1. Is the problem a priority?	No, Yes, Varies, Uncertain
2. How substantial are the Benefits?	Large, Moderate, Small, Trivial, Varies, Uncertain
3. How substantial are the Harms?	Large, Moderate, Small, Trivial, Varies, Uncertain
4. What is the overall certainty of the evidence?	High, Moderate, Low, Very Low
5. What is the balance between benefits and harms?	Favours intervention, Against intervention
6. How do people value treatment for TTS?	Degree of Variability or Uncertainty
7. How large are the resource requirements (costs)?	Large, Moderate, Negligible costs or savings
8. What is the certainty of the evidence for the costs?	High, Moderate, Low, Very Low
9. Are treatments for TTS cost-effective?	Favours intervention, Against intervention
10. What would the impact be on health equity?	Reduced, Increased, Varies, Uncertain
11. Are treatments for TTS acceptable to all stakeholders?	No, Yes, Varies, Uncertain
12. Are treatments for TTS feasible to implement?	No, Yes, Varies, Uncertain

4 GRADE Handbook. Available from: https://gdt.gradepro.org/app/handbook/handbook.html. Accessed 1 July 2021

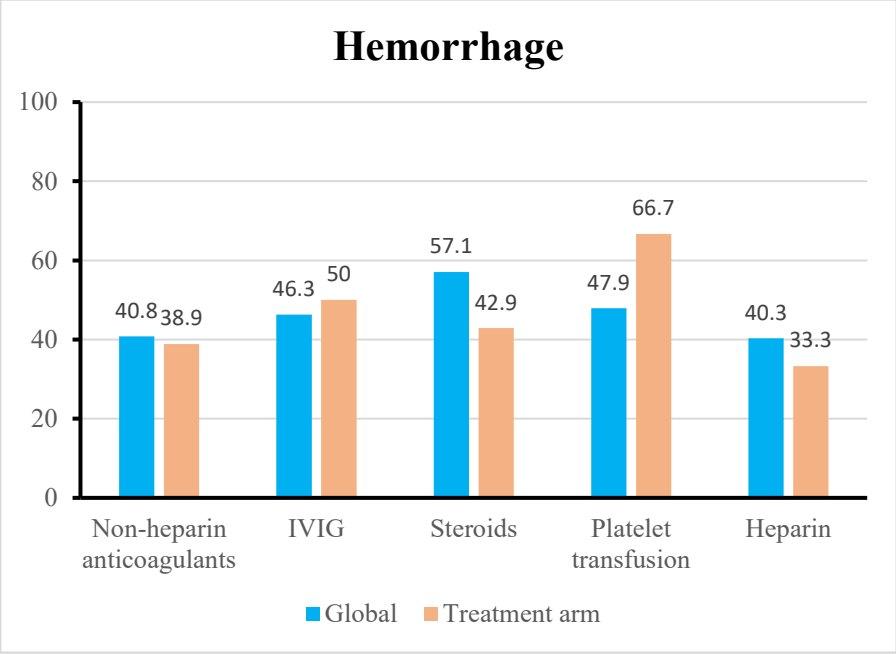
Summary of findings



Drug	Number of studies	Global rate	Number of studies with valid data	Treated rate
Non-heparin anticoagulants	6	65/110	5	28/44
IVIG	7	64/113	4	6/9
Steroids	4	4/9	4	4/7
Platelet transfusion	5	25/48	3	0/6
Heparin	8	68/127	4	7/14



Drug	Number of studies	Global rate	Number of studies with valid data	Treated rate
Non-heparin anticoagulants	6	17/110	4	1/15
IVIG	8	22/55	5	4/16
Steroids	6	19/82	4	3/7
Platelet transfusion	5	12/48	3	5/6
Heparin	9	30/128	5	6/32



Drug	Number of studies	Global rate	Number of studies with valid data	Treated rate
Non-heparin anticoagulants	5	20/49	4	7/18
IVIG	7	25/54	5	5/10
Steroids	5	12/21	4	3/7
Platelet transfusion	5	23/48	3	4/6
Heparin	8	27/67	6	7/21

Possible research gaps for future prophylactic management:

- Should hydroxychloroquine be used in patients having TTS?
- Genetic markers for 'at risk' patients.
- Risk factors for TTS and the role of other thrombotic risk factors.
- Role of point-of-care devices and diagnostics like the thromboelastography (TEG), that gives an indication of fibrinogen levels, or point-of-care ultrasound (POCUS) which would enable frequent testing of coagulation/bleeding profiles.
- Duration and persistence of anti-PF4 antibodies in COVID-19 vaccine-related TTS. In patients with HIT, the median time to antibody clearance is 50 days for platelet activation assays and 85 to 90 days by immunoassays. Immunoassays may remain positive in ~35% of patients for up to 1 year, with levels decreasing over time. Hence patients may be at risk for thrombosis because of circulating anti-PF4/heparin antibodies.
- Duration of immunosuppressive treatments.
- Management of treatment-resistant cases.
- Possibility of organ donation in TTS-deceased patients.
- Consider rapid literature search to identify the most common cause of hypercoagulability.
- Research on increase in hypercoagulopathy in patients with a previous of COVID-19 infection, given any COVID-19 vaccine.
- Randomized controlled trials (RCTs) to determine the safety of proposed anticoagulation, alternate anticoagulants and the role for anti-platelets, such as Ticagrelor, in patient management.
- RCTs to evaluate the efficacy of using new oral anticoagulants (NOACS) as prophylaxis in patients recovering from TTS after COVID-19 vaccine? (If anti-PF 4 antibodies are present).

Evidence tables

Question: **Heparin compared with no heparin or other anticoagulants for patients diagnosed with TTS following COVID-19 vaccination Outcome: death (all-cause)**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Death (All-cause)									
9	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 9 studies providing valid data [7 case series and 2 case reports], including 128 patients, 46 of them treated with heparin. Global death rate was 30/128 (23.4%). Information about death in patients treated with heparin was available in 5 studies including 32 patients. Death rate in patients treated with heparin was 6/32 (18.7%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med. 2021;384(22):2092-2101.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. N Engl J Med. 2021;384(20):1964-1965.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
6. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
7. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
8. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.
9. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after "COVID-19 Vaccine AstraZeneca" exposure. J Clin Med. 2021;10(8):1599.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Heparin compared with no heparin or other anticoagulants for patients diagnosed with TTS following COVID-19 vaccination** **Outcome: intracranial haemorrhage**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Intracranial haemorrhage									
8	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 8 studies providing valid data [6 case series and 2 case reports], including 67 patients, 34 of them treated with heparin. Global intracranial hemorrhage rate was 27/67 (40.3%). Information about intracranial hemorrhage rate in patients treated with heparin was available in 6 studies, and was 7/21 (33.3%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med. 2021;384(22):2092-2101.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. N Engl J Med. 2021;384(20):1964-1965.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
6. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
7. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.
8. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after "COVID-19 Vaccine AstraZeneca" exposure. J Clin Med. 2021;10(8):1599.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Heparin compared with no heparin or other anticoagulants for patients diagnosed with TTS following COVID-19 vaccination – Outcome: recovery**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Recovery									
8	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 8 studies providing valid data [7 case series and 1 case report], including 127 patients, 45 of them treated with heparin. 68/127 (53.5%) patients were recovered by the time the study was published. Information about recovery in patients treated with heparin was available in 4 studies, including 14 patients. Recovery rate was 7/14 (50%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med. 2021;384(22):2092-2101.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
4. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
5. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
6. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
7. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.
8. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after "COVID-19 Vaccine AstraZeneca" exposure. J Clin Med. 2021;10(8):1599.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: Non-heparin-based anticoagulants compared with heparin or no anticoagulation for patients diagnosed with TTS following COVID-19 vaccination **Outcome:** death (all-cause)

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Death (all-cause death)									
6	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 6 studies providing valid data, all case series, including 110 patients, 77 of them treated with non-heparin anticoagulants. Global death rate was 17/110 (15.4%). Information about death in patients treated with non-heparin anticoagulants was available in 4 studies including 15 patients. Death rate in patients treated with non-heparin anticoagulants was 1/15 (6.7%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
2. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
3. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
4. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
5. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.
6. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after "COVID-19 Vaccine AstraZeneca" exposure. J Clin Med. 2021;10(8):1599.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Non-heparin-based anticoagulants compared with heparin or no anticoagulation for patients diagnosed with TTS following COVID-19 vaccination – Outcome: intracranial haemorrhage**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Intracranial haemorrhage									
5	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 5 studies providing valid data, all case series, including 49 patients, 44 of them treated with non-heparin anticoagulants. Global haemorrhage rate was 20/49 (40.8%). Information about haemorrhage in patients treated with non-heparin anticoagulants was available in 4 studies including 18 patients. Death rate in patients treated with non-heparin anticoagulants was 7/18 (38.9%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
2. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
3. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
4. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.
5. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after "COVID-19 Vaccine AstraZeneca" exposure. J Clin Med. 2021;10(8):1599.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: Non-heparin-based anticoagulants compared with heparin or no anticoagulation for patients diagnosed with TTS following COVID-19 vaccination – Outcome: recovery

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Recovery									
6	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 6 studies providing valid data, all case series, including 110 patients, 77 of them treated with non-heparin anticoagulants. Global recovery rate was 65/110 (59.1%). Information about recovery in patients treated with non-heparin anticoagulants was available in 5 studies including 44 patients. Recovery rate in patients treated with non-heparin anticoagulants was 28/44 (63.6%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
2. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
3. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
4. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
5. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.
6. Wolf ME, Luz B, Niehaus L, Bhogal P, Bäzner H, Henkes H. Thrombocytopenia and intracranial venous sinus thrombosis after "COVID-19 Vaccine AstraZeneca" exposure. J Clin Med. 2021;10(8):1599.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: IVIG compared with no treatment or other drugs or procedures for patients diagnosed with TTS following COVID-19 vaccination Outcome: death (all cause)

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Death (all-cause)									
8	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 8 studies reporting use of IVIG, [3 case reports and 5 case series], including 114 patients, 55 of them treated with IVIG. Global death rate was 22/55 (40.0%). Information about death in patients treated with IVIG was available in 5 studies including 16 patients. Death rate in patients treated with IVIG was 4/16 (25.0%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. N Engl J Med. 2021;384(20):1964-1965.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
6. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
7. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
8. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **IVIG compared with no treatment or other drugs or procedures for patients diagnosed with TTS following COVID-19 vaccination – Outcome: intracranial haemorrhage**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Intracranial haemorrhage									
7	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 7 studies reporting use of IVIG, [3 case reports and 4 case series], including 54 patients, 35 of them treated with IVIG. Global haemorrhage rate was 25/54 (46.3%). Information about haemorrhage in patients treated with IVIG was available in 5 studies including 10 patients. Haemorrhage rate in patients treated with IVIG was 5/10 (50.0%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. N Engl J Med. 2021;384(20):1964-1965.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
6. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
7. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **IVIG compared with no treatment or other drugs or procedures for patients diagnosed with TTS following COVID-19 vaccination – Outcome: Recovery**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Recovery									
7	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 7 studies reporting use of IVIG, [2 case reports and 5 case series], including 113 patients, 55 of them treated with IVIG. Global recovery rate was 64/113 (56.6%). Information about recovery in patients treated with IVIG was available in 4 studies including 9 patients. Recovery rate in patients treated with IVIG was 6/9 (66.7%).	⊕◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. N Engl J Med. 2021;384(20):1964-1965.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
6. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.
7. Tiede A, Sachs UJ, Czwalinna A, Werwitzke S, Bikker R, Krauss JK, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. Blood 2021:blood.2021011958. doi: 10.1182/blood.2021011958.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Platelet transfusion compared with no platelet transfusion for patients diagnosed with TTS following COVID-19 – Outcome: death (all-cause)**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Mortality (all-cause)									
5	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 5 studies providing valid data, 2 case reports and 3 case series, including 48 patients, 16 of them treated with platelet transfusions. Global mortality rate was 12/48 (25.0%). Information about mortality in patients treated with platelet transfusions was available in 3 studies including 6 patients. Mortality rate in patients treated with platelet transfusions was 5/6 (83.3%).	⊕◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Franchini M, Testa S, Pezzo M, Glingani C, Caruso B, Terenziani I, et al. Cerebral venous thrombosis and thrombocytopenia post-COVID-19 vaccination. Thromb Res. 2021;202:182-183.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Platelet transfusion compared with no platelet transfusion for patients diagnosed with TTS following COVID-19 – Outcome: intracranial haemorrhage**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Intracranial haemorrhage									
5	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 5 studies providing valid data, 2 case reports and 3 case series, including 48 patients, 16 of them treated with platelet transfusions. Global intracranial haemorrhage rate was 23/48 (47.9%). Information about intracranial haemorrhage in patients treated with platelet transfusions was available in 3 studies including 6 patients. Haemorrhage rate in patients treated with platelet transfusions was 4/6 (66.7%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Franchini M, Testa S, Pezzo M, Glingani C, Caruso B, Terenziani I, et al. Cerebral venous thrombosis and thrombocytopenia post-COVID-19 vaccination. Thromb Res. 2021;202:182-183.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Platelet transfusion compared with no platelet transfusion for patients diagnosed with TTS following COVID-19 – Outcome: recovery**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Recovery									
5	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 5 studies providing valid data, 2 case reports and 3 case series, including 48 patients, 16 of them treated with platelet transfusions. Global recovery rate was 25/48 (52.1%). Information about recovery in patients treated with platelet transfusions was available in 3 studies including 6 patients. Recovery rate in patients treated with platelet transfusions was 0/6 (0%).	⊕◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Franchini M, Testa S, Pezzo M, Glingani C, Caruso B, Terenziani I, et al. Cerebral venous thrombosis and thrombocytopenia post-COVID-19 vaccination. Thromb Res. 2021;202:182-183.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

b. The dose was not described in studies. Duration of follow-up was different. There is heterogeneity clinically (different treatment paradigms, location and severity of thromboses) and methodologically between studies, and there is limited certainty regarding that the differences explain the observed differences in effects.

c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Steroids compared with no steroids or other treatments for patients diagnosed with TTS following COVID-19 vaccination – Outcome; death (all cause)**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Death (all-cause)									
6	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 6 studies providing valid data, 4 case reports and 2 case series, including 82 patients, 14 of them treated with steroids. Global death rate was 19/82 (23.2%). Information about death in patients treated with steroids was available in 4 studies including 7 patients. Death rate in patients treated with steroids was 3/7 (42.9%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. George G, Friedman KD, Curtis BR, Lind SE. Successful treatment of thrombotic thrombocytopenia with cerebral sinus venous thrombosis following Ad26.COV2.S vaccination. Am J Hematol. 2021. doi: 10.1002/ajh.26237.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
4. Schultz JB, Berlit P, Diener HC, Gerloff C, Greinacher A, Klein C, et al. COVID-19 vaccine-associated cerebral venous thrombosis in Germany. medRxiv doi: https://doi.org/10.1101/2021.04.30.21256383; version posted May 13, 2021.
5. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
6. Yocum A, Simon EL. Thrombotic thrombocytopenic purpura after Ad26.COV2-S vaccination. Am J Emerg Med. 2021:S0735-6757(21)00376-4. doi: 10.1016/j.ajem.2021.05.001.

Explanations

a. Selection bias: Fatal cases were more likely to be quickly published as case reports (range 5-8 days) than large case series. Not all confounding factors influencing the prognosis were analyzed (age, time between symptom onset and treatment, other treatments, presence of multiple organ thrombosis), incomplete follow-up in most cases

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c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Steroids compared with no steroids or other treatments for diagnosed with TTS following COVID-19 vaccination – Outcome: intracranial haemorrhage**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Intracranial haemorrhage									
5	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 5 studies providing valid data, 4 case reports and 1 case series, including 21 patients, 10 of them treated with steroids. Global haemorrhage rate was 12/21 (57.1%). Information about haemorrhage rate in patients treated with steroids was available in 4 studies including 7 patients. Haemorrhage rate in patients treated with steroids was 3/7 (42.9%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Franchini M, Testa S, Pezzo M, Glingani C, Caruso B, Terenziani I, et al. Cerebral venous thrombosis and thrombocytopenia post-COVID-19 vaccination. Thromb Res. 2021;202:182-183.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US case reports of cerebral venous sinus thrombosis with thrombocytopenia after Ad26.COV2.S vaccination, March 2 to April 21, 2021. JAMA. 2021;325(24):2448-56.
4. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
5. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.

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c. The total number of patients included is less than the number of patients that should be included according to a sample size calculation for a single adequately powered trial

d. Outcome was not available at the moment of publication in many cases.

Question: **Steroids compared with no steroids or other treatments for patients diagnosed with TTS following COVID-19 vaccination – Outcome: recovery**

Certainty assessment							Impact	Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
Recovery									
4	observational studies	very serious [a]	serious [b]	not serious	very serious [c]	publication bias strongly suspected [d]	There were 4 studies providing valid data, all case reports, including 9 patients, 7 of them treated with steroids. Global recovery rate was 4/9 (44.4%). Information about recovery in patients treated with steroids was available in 4 studies including 7 patients. Recovery rate in patients treated with steroids was 4/7 (57.1%).	⨁◯◯◯ VERY LOW	CRITICAL

Bibliography:

1. Franchini M, Testa S, Pezzo M, Glingani C, Caruso B, Terenziani I, et al. Cerebral venous thrombosis and thrombocytopenia post-COVID-19 vaccination. Thromb Res. 2021;202:182-183.
2. Mehta PR, Apap Mangion S, Benger M, Stanton BR, Czuprynska J, Arya R, et al. Cerebral venous sinus thrombosis and thrombocytopenia after COVID-19 vaccination - A report of two UK cases. Brain Behav Immun. 2021;95:514-517.
3. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N Engl J Med. 2021;384(22):2124-2130.
4. Centers for Disease Control and Prevention. National Center for Immunization & Respiratory Diseases. Update: Thrombosis with thrombocytopenia syndrome (TTS) following COVID-19 vaccination Advisory Committee on Immunization Practices (ACIP) May 12, 2021. Available at: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-05-12/07-COVID-Shimabukuro-508.pdf, accessed 30 May 2021.

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Xeroderma Pigmentosum and Bone Marrow Aplasia: A Rare Association

Abstract

Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder characterized by hypersensitivity to ultraviolet rays and predisposition to cutaneous malignancies. Hematological disorders associated with XP include myelodysplastic syndrome and acute leukemia. We report a 7-year-old child with XP along with squamous cell carcinoma and bone marrow aplasia.

Keywords: *Aplastic anemia, pancytopenia, squamous cell carcinoma, xeroderma pigmentosum*

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Introduction

Xeroderma pigmentosum (XP) is a rare inherited autosomal recessive disorder characterized by extreme hypersensitivity to ultraviolet rays predisposing to early cutaneous malignancies. Other abnormalities include neurological and ocular diseases.[1,2]

The incidence of XP varies across geographic areas, one in one hundred thousand in Europe and the US to one in 22,000 in Japan. The incidence in India has not been studied so far but is believed to be less when compared to the Western countries.[2,3]

There is no sex predilection. Pathophysiology of this inherited disorder is the defect in the nucleotide excision repair mechanism, and hence, the DNA damage caused by ultraviolet (UV) rays is unrepaired resulting in genomic instability which leads to predisposition to oculo-cutaneous malignancies as well as other internal malignancies. The other manifestations of XP include hyperpigmentation, premature aging, and neurological dysfunction. Photosensitivity and burning sensation after exposure to the sun precede the dryness (xeroderma) and pigmentation of skin and ocular manifestations such as photophobia and keratitis.[4,5]

Hematological abnormalities reported include myelodysplastic syndrome, which is characterized by refractory anemia with excess blast cells and leukemias.[6] A single case of XP in association with bone marrow failure has also been reported.[7]

Bone marrow aplasia in XP is very rare when compared to its presence in other DNA repair mechanism defects such as Fanconi anemia, Bloom syndrome, and ataxia telangiectasia.

We present here an interesting case of XP associated with bone marrow aplasia.

Case Report

A 7-year-old male child, a known case of XP, presented with a swelling in the right infraorbital region of short duration associated with pain and discharge from the lesion.

The diagnosis of XP was made based on the clinical findings in the previous admission when the child presented with dermatological symptoms, photosensitivity, itching over the face, and hyperpigmentation. Pigmentation progressed with age. Erythematous scaly plaques with pigmented macules were seen over the areas exposed to the sun. The child was born of a nonconsanguinous marriage and attained normal developmental milestones. There were no neurological manifestations.

In the current admission, complete hemogram showed pancytopenia (white blood cell count - 3.5 × 10^3/μl, red blood cell count - 1.01 × 10^6/μl, and platelet count - 21 × 10^3/μl). Peripheral smear revealed a macrocytic blood picture. There were no atypical cells in the smear [Figure 1].

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Bone marrow aspiration was done. The smears were paucicellular with adipocyte fragments and scant hematopoietic elements. There were no features of dyspoiesis in any lineage [Figure 2].

Bone marrow trephine was markedly hypocellular with fatty marrow and minimal hematopoietic tissue. There was no evidence of an infiltrative disease [Figure 3].

Wide-local excision of the infraorbital swelling showed a moderately differentiated squamous cell carcinoma [Figure 4].

Discussion

XP is a rare genodermatosis caused by defects in the normal repair of DNA damaged by exposure to sunlight. There are eight complementation groups of XP (XP-A to G) caused by defective nucleotide excision repair, corresponding to mutations in the genes encoding XPA, XPB, XPC, XPD, XPE, XPF, XPG, and XP dominant. The incidence and the age at presentation vary, ranging from early infancy to adulthood. XP has been reported to be common with consanguineous marriages.[8]

There are a 1000-fold increased risk of development of cutaneous malignancies, especially in those diagnosed under the age of 2 years and a 10-20-fold increased risk of developing noncutaneous malignancies.[9]

The commonly reported cutaneous malignancies include squamous cell carcinoma followed by melanoma and basal cell carcinoma.

Our patient had squamous cell carcinoma of the face.

In addition, the patient had pancytopenia which suggested the possibility of an infiltrative disorder of bone marrow. Bone marrow metastasis of malignant melanoma presenting as pancytopenia has already been reported in cases with XP.[10] In our patient, bone marrow aspirate and trephine were consistent with aplastic anemia.

Review of the literature revealed that the common hematological disorders associated with XP are myelodysplastic syndrome and acute myeloid leukemia.[6]

Marrow aplasia in XP is very rare and has been reported so far in only a single case of XP.[7] However, marrow

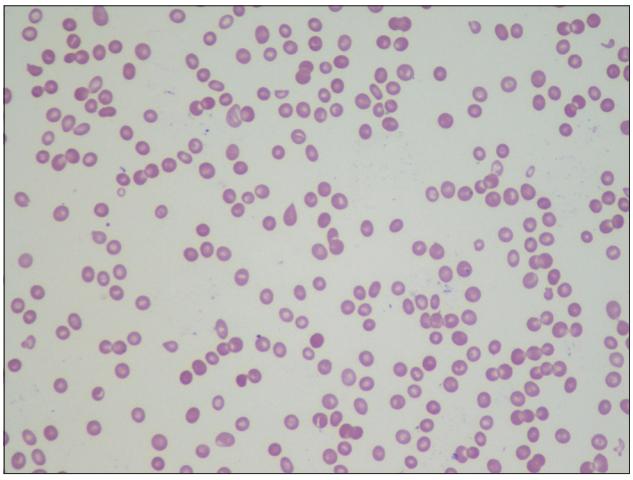


Figure 1: Peripheral smear showing pancytopenia (×40)

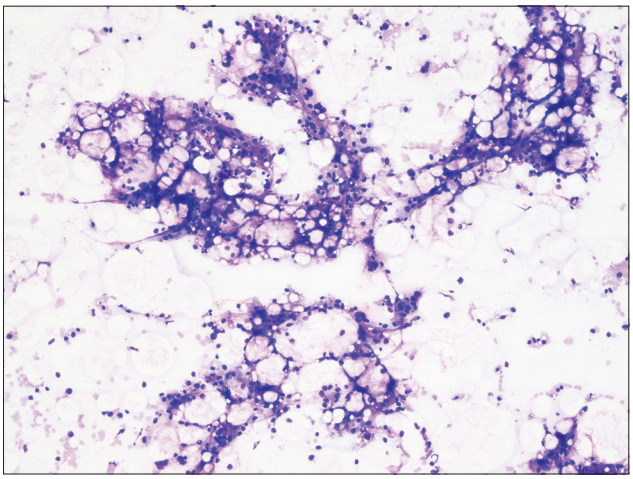


Figure 2: Bone marrow aspirate showing hypocellular marrow particles (×10)

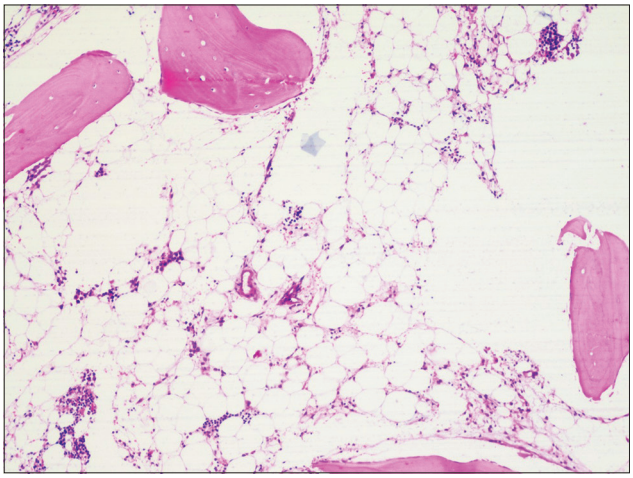


Figure 3: Bone marrow trephine showing fatty marrow (×10)

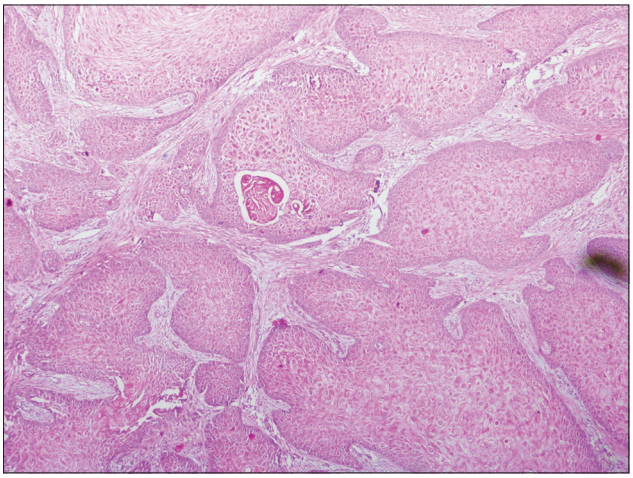


Figure 4: Biopsy showing squamous cell carcinoma (×10)

aplasia is quite common in other inherited DNA damage repair diseases such as Fanconi anemia. The patient did not have any features of Fanconi anemia. Even though skin hyperpigmentation has been reported in Fanconi anemia, it is not restricted to sun-exposed areas unlike in XP.

Aplastic anemia in children is generally attributed to drugs and viral infections. However, 90% of these cases are idiopathic in nature.[7]

The association of XP with leukemias and other internal malignancies shows that DNA damage repair mechanism is essential in repairing non-UV-mediated mutagenesis as well.

The association of aplastic anemia and XP as a new variant of this disease has not yet been proven.[7] The clonal evolution of aplastic anemia to leukemia has been reported.[7] Considering the foregoing discussion, it is tempting to suggest that this patient under reference can be considered to be at the increased risk of developing leukemia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bilodi AK, Gangadhar MR. Xeroderma pigmentosa - A rare clinical entity. Indian J Med Case Rep 2013;2:21-4.
2. Feller L, Wood NH, Motswaledi MH, Khammissa RA, Meyer M, Lemmer J. Xeroderma pigmentosum: A case report and review of the literature. J Prev Med Hyg 2010;51:87-91.
3. Tamhankar PM, Iyer SV, Ravindran S, Gupta N, Kabra M, Nayak C, *et al.* Clinical profile and mutation analysis of xeroderma pigmentosum in Indian patients. Indian J Dermatol Venereol Leprol 2015;81:16-22.
4. Pandey A, Kuldeep K, Koul A, Tyagi M, Singh P, Sharma P, *et al*. Xeroderma pigmentosa with ocular association: Case report. Case Rep Clin Med 2013;2:466-9.
5. Lopes-Cardoso C, Paes da Silva Ramos Fernandes LM, Ferreira-Rocha J, Teixeira-Soares C, Antônio-Barreto J, Humberto-Damante J. Xeroderma pigmentosum – A case report with oral implications. J Clin Exp Dent 2012;4:e248-51.
6. Berbis P, Beylot C, Noe C, Doutre MS, Broustet A, Privat Y. Xeroderma pigmentosum and refractory anaemia in two first cousins. Br J Dermatol 1989;121:767-71.
7. Salob SP, Webb DK, Atherton DJ. A child with xeroderma pigmentosum and bone marrow failure. Br J Dermatol 1992;126:372-4.
8. Chaudhary M, Jajoo SN, Agarwal R. Xeroderma pigmentosum: A case report of two siblings. J Immunodefic Disord 2012;1:2.
9. Rahman T, Sharma JD, Krishnatreya M, Kataki AC. Basaloid squamous carcinoma of skin associated with xeroderma pigmentosum in an 8-year-old child: A rare entity. Indian J Dermatol 2014;59:632.
10. Spiller SE, Hawkins DS, Finn LS, Sze RW, Sybert V. Metastatic malignant melanoma presenting as pancytopenia in a three-year-old boy. Pediatr Blood Cancer 2005;45:60-3.

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Training Pediatric Hematologists / Oncologists for Capacity Building in Ethiopia

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Background: Human resources are essential to the sustainability and scalability of health interventions. A barrier to global cancer efforts has been the scarcity of trained pediatric hematology/oncology (PHO) professionals in low- and middle-income countries (LMIC). Studies highlight this inequity and the need to develop a global PHO workforce through allocation of financial resources, strengthening of healthcare infrastructure, and development of innovative training programs. Ethiopia is the second most populous country in Africa. Because of recent reduction in infant mortality and improved treatment of Malaria and HIV in Ethiopia, non-communicable diseases contribute an increasing proportion of childhood mortality.

The annual incidence of pediatric cancer in Ethiopia is estimated to be 6000-8000 cases. Prior to 2013, no dedicated PHO programs existed in Ethiopia, and there was no formal process for training local physicians in PHO. The following intervention aimed to increase local capacity for treating childhood cancer through the creation of a formal two-year PHO fellowship.

Strategy: The PHO fellowship program was created by The Aslan Project, a US non-profit led by a group of experts in PHO, pediatric oncology nursing, and pathology with experience practicing in LMIC, in collaboration with Addis Ababa University (AAU). The goal was to provide a robust educational experience for fellows within the existing resource-constrained clinical environment. Tikur Anbessa Specialized Hospital (TASH) was the initial clinical site (in 2013) for the training program with a second at Jimma University Medical Center (JUMC) in 2016. An Aslan clinical director was present throughout the first year at each location. Visiting faculty from the US and Canada provided clinical supervision, on-site didactics, and professional mentorship. The second year of training included a six-month rotation at a high-functioning cancer institution in India. Fellows were expected to complete a scholarly activity, pass a certification exam, and serve as pediatric hematologist-oncologists in Ethiopia for a minimum of two years following training.

Outcomes: Since 2013, four physicians completed PHO fellowship based in Ethiopia, with extensive support of local healthcare leadership and visiting faculty. One additional fellow left training prior to completion and one fellow currently is in training. Twenty-three faculty members from 18 Universities made 51 trips to Ethiopia for onsite training of fellows from 2013-2019. Each fellow completed a rotation in India in his/her second year (Tata Memorial Hospital, Mumbai, or TMC Kolkata). Fellowship projects included a comprehensive pediatric cancer unit assessment, safe chemotherapy practices, and the role of diagnostic pathology. The four subspecialty-trained physicians have remained local, two at TASH, one at JUMC, and one returned to Gertrude's Children's Hospital in Nairobi, Kenya. JUMC now supports a 22 bed PHO unit running at 90% capacity with over 300 new diagnoses since August 2016. TASH supports a 26 bed inpatient unit and 16 additional inpatient beds at a nearby oncology center, treating over 600 new patients annually. The fellowship structure has succeeded in training subspecialty physicians to establish PHO care in Ethiopia.

Discussion: Developing specialized care in LMIC requires a multifaceted approach, including nursing training, social support, health system buy-in, diagnostic expertise and facility, pharmacy services, and subspecialty physicians. Focusing on physician training for PHO in Ethiopia, we designed a training structure and curriculum to 1) teach resource appropriate medical care, 2) provide sustained clinical mentorship, 3) develop health system leadership skills, and 4) retain physicians to support local pediatric oncology units. Onsite training by visiting faculty was augmented by sending fellows to a mature, middle-income country program and the support of full-time clinical faculty/mentorship for a large portion of the

program. Challenges included fellow recruitment, pathologic accuracy, chemotherapy access, nutritional support, and treatment retention. Formal subspecialty PHO training can be implemented in LMIC without established subspecialty physicians, but requires ongoing commitment of administrators, visiting faculty, local universities, and a multidisciplinary team of health professionals.

Disclosures

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Author notes

*Asterisk with author names denotes non-ASH members.

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Impact of a Comprehensive Early Clinical Exposure Program for Preclinical Year Medical Students

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Abstract

Purpose: To study the impact of an early clinical exposure program designed to provide a wide variety (cognitive, affective and psychomotor) of learning experiences for the preclinical year students.
Method: One hundred and fifty preclinical students were posted in small groups to selected departments – Transfusion medicine, Catheterization lab, Simulation lab, Radiology, Neurology, Nephrology, Respiratory medicine and General surgery. Each student had atleast ten hours of clinical exposure under this program. The program was evaluated through a series of pre and post-test questionnaires, which were designed based on the learning objectives of each session. Students who wished to participate in the program evaluation gave informed consent, took up the pre / post test and were also asked to give their written open comments about the program.
Results: There was a significant increase in the post-test scores (ranging from 9.14 ± 2.67 to 36.65 ± 6.62) when compared to the pre-test scores (ranging from 7.94 ± 2.31 to 28.69 ± 6.11) for all the sessions (p value < 0.001, $n = 144$). Analysis of the open feedback showed that the program had significant impact on the cognitive, psychomotor and affective domains. "Application of basic sciences in clinical practice", "motivation to learn", "got familiar with various specialties", "insight about what the patient undergoes" were the themes identified from the open comments.

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Conclusion: The innovative use of early clinical exposure program to teach/learn clinical skills like phlebotomy and Basic Life Support had been well appreciated by the students. The present design involving a variety of learning experiences has been successful in introducing the various dimensions of medical profession like scientific, ethical, interpersonal, professional and social to the new entrants in addition to enhancing their motivation to learn.
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Keywords: Attitude; Learning; Simulation lab; Medical education; Curriculum

1. Introduction

In India, students enter medical schools at an early age with little priming towards the profession. Entry of students in most of the medical colleges is entirely based on their academic performance with no weighting for humanistic attitude and communication skills.[1,2] Due to lack of adequate exposure, most students do not realize what to expect in the medical profession. A few enter the medical college purely due to parental pressure.[3] Large classes, intense competition, exploding vocabulary and voluminous content delivered over a short time are some of the challenges faced by the first year MBBS students.[3,4] Insufficient level of guidance and problems pertaining to transition from school to college life can also be included to this list of problems faced by the students during this period. Some students find the abrupt shift of medium of instruction from vernacular language to English difficult to cope. All these, along with the traditional curriculum, where the students are engaged in long hours of classroom and laboratory teaching with no exposure to the clinical setup, plays a major role in dousing the enthusiasm of the upcoming doctor. Under these circumstances, students often find their preclinical year to be tough, dreary and most importantly, they fail to understand the relevance of basic sciences in the clinical setting.

There has been an urgent call for curricular reforms with emphasis towards vertical and horizontal integration and competency based training.[5] Many parts of the world have responded to these needs by shifting away from discipline-based curriculum. In line with these changes, Medical Council of India (MCI) developed the 'Vision 2015' document. MCI vision 2015 aims to produce a new generation of medical graduates of global standards through curricular reforms. "The roles of this new age doctor are recognized as clinician, communicator, life-long learner, team leader and professional".[6] Required curricular changes begin early in the form of a foundation course; early clinical exposure; properly designed integrated teaching and programs to develop the correct attitude and communication skills (ATCOM module).[6]

Early clinical exposure (ECE) needs to be a coordinated effort by the preclinical, paraclinical and clinical faculty. If implemented effectively, it has the potential to improve motivation for learning and promote deep learning, better understanding and longer retention of the knowledge. It can facilitate the students to understand the application of basic sciences in clinical practice and aid in effective learning of clinical skills. It could also serve as a platform for students to improve their communication skills and get initiated into inculcating professionalism at a very early and impressionable stage of their medical education.[7] Above all, it provides an opportunity for them to see the illness from the patient's perspective. All this can foster the development of a holistic doctor who will be competent, communicative, humanistic and empathetic towards the patients.

To offer this experience, 'Clinical observership' program was started in PSG Institute of Medical Sciences and Research (PSGIMSR) in the year 2000. In this program, the first year MBBS students had the opportunity to spend half a day in the following selected specialties in small groups: Cardiology, Gastroenterology, Radiology, Nephrology and Transfusion medicine. This initiative was highly appreciated by the students.[8] This program has now been modified and expanded to include more departments and implemented readily to suit the mandates from MCI as early clinical exposure. The aim of this study was to evaluate the impact of an organized program of early clinical exposure for the preclinical students in enhancing their motivation to learn basic science in relevance to clinical medicine.

2. Methods

2.1. Participants

One hundred and fifty first year MBBS students participated in early clinical exposure program conducted in the year 2015–16. Students were divided into

three batches (A, B, C) based on their roll numbers. Each batch was further divided into five small teams comprising of ten students. Each team spent an hour (from 4 pm to 5 pm) in the clinical departments every day of the week in turns, ensuring that there were no more than ten students in an ECE session. Last hour of the working day was selected for this program without interfering the conventional teaching sessions. Keeping the program at the end of the day also gave an opportunity to extend the session beyond the allotted hour when required.

Though attendance for the ECE program was compulsory as per MCI and University guidelines, participating in the program evaluation and giving feedback was strictly on voluntary basis. Informed consent was obtained from the participants. The study was approved by the Institutional Human Ethics committee.

2.2. Materials

The program was implemented in two cycles of three weeks duration. Five clinical departments were covered in each cycle. The first cycle of ECE sessions were conducted in the months of Nov-Dec 2015 and included rotations in Clinical simulation laboratory (for phlebotomy training), blood bank, and departments of neurology, radiology and surgery. During the second rotation of the program, which was conducted in March 2016, the students were posted once again in the Clinical simulation laboratory (for Basic Life Support training) and Radiology in addition to the Departments of Nephrology, Respiratory medicine and Cardiology (Catheterization laboratory).

Selection of departments was done with an intention to expose the students to a variety of learning experiences involving all the three domains of learning- cognitive, psychomotor and affective. The program also included super specialty departments like Cardiology, Nephrology and Neurology for which the students would not have the opportunity to be exposed in their regular curriculum. The departments to which the students were posted in each rotation were selected based on the subject matter covered in basic sciences until the time of the program. For example, the first rotation concurred with the completion of lower limb in Anatomy, hence visiting the Surgery department to see a case of varicose veins/ peripheral vascular disease was more pertinent than visiting Catheterization lab. Catheterization lab was reserved for the second rotation, which was scheduled when the students were learning or just finished learning thorax and abdomen in Anatomy.

Table 1
Comparison of pretest and posttest scores of ECE sessions.

ECE Session (Maximum score)	Pretest score ($n=144$)	Post test score ($n=144$)	P value
Radiology (50)	28.69 ± 6.11	36.65 ± 6.62	< 0.001
Neurology (50)	21.44 ± 5.77	30.14 ± 7.55	< 0.001
Surgery (50)	22.57 ± 5.93	34.31 ± 7.76	< 0.001
Simulation Lab (Phlebotomy) (30)	15.54 ± 4.75	24.06 ± 4.60	< 0.001
Blood Bank (15)	7.94 ± 2.31	9.14 ± 2.67	< 0.001
Nephrology (40)	16.33 ± 2.84	28.51 ± 5.11	< 0.001
Respiratory Medicine (35)	15.3 ± 12.59	23.27 ± 4.74	< 0.001
Simulation lab (Basic Life Support) (40)	16.11 ± 5.87	29.19 ± 5.93	< 0.001
Cardiology (40)	18.08 ± 5.44	29 ± 5.33	< 0.001

2.3. Process

An orientation session for the students was conducted by the program coordinator prior to starting the rotations, wherein the objectives of the program, clear directives regarding the conduct of the sessions and the expected goals of this innovative venture were highlighted. Clinical

Table 2
Summary of open written comments.

General	'Knew about various departments/ specialties in the hospital, their functioning & infrastructure'
	'Made us familiar with various fields'
Knowledge	'Learnt a lot, better way of learning'
	'Able to correlate theory with practical work'
	'Learnt the importance of basic science and its application in clinical medicine'
Psychomotor domain	'CPR - empowered us'
	'Learnt and taught CPR to my family members'
	'Observed live procedures, saw instruments, drugs'
Affective domain	'Insight about what patient undergoes'
	'Learnt about the duties of a doctor'
	'Learnt the importance of human life'
	'Learnt how to handle a patient'
	'Learnt to behave kindly towards patients'
Motivating	'Interesting motivating inspiring useful worthy awesome'
	'Invoked self interest'
	'Made us feel proud to be a doctor'
	'My dream to walk into the hospital as doctor made true'
Suggestions	'Program timing can be increased'
	'One more cycle'
	'Timing can be shifted to morning hours'
	'Can include all departments in hospital especially cardiothoracic surgery, oncology, gynecology, clinical lab and opportunity to interact with patients and staff'

department postings were coordinated by a faculty in each department. The designate faculty drew out the learning objectives for this session based on the student's prior knowledge and availability of clinical materials. This arrangement was kept uniform for the entire class.

The learning objectives for each session were chosen carefully in view of student's prior knowledge, the availability of clinical material and relevance. In departments like Nephrology, Radiology, Simulation lab, Blood bank the content was fixed and all students were uniformly exposed. For example in nephrology, the students witnessed a patient undergoing dialysis. The principle of dialysis, biochemical alterations in the blood before and after dialysis was discussed. In other departments like Respiratory medicine, Surgery, Neurology the experience was more impromptu, based on the availability of cases. In Cardiology, the objective was to give the students an experience of observing coronary angiogram followed by a discussion on the relevant anatomical aspects. If there were no patients scheduled for angiogram at the time of ECE session they were allowed to view the recorded procedure.

Based on the learning objectives, questionnaires were formulated for all the sessions and used as pre-test and post-test for the students. Students who wished to participate in the program evaluation gave informed consent, took part in the pre-test and post-test and were also encouraged to give their written open comments anonymously.

2.4. Data collection and analysis

The questionnaires were designed in a five point Likert scale with 6–10 items to assess the effectiveness of ECE sessions. The scores used were 1 = not aware, 2 = not sure, 3 = know vaguely, 4 = know with reasonable clarity and 5 = know confidently. The questionnaire was meant to assess the impact of the program on the students. This was achieved by comparing the scores obtained by the students in pre- and post-tests. The scores were expressed as mean ± standard deviation and were compared by paired't' test using IBM SPSS software version 19.

The open comments regarding the program were analyzed through inductive approach. [9] Specific themes were identified and the comments were assigned to the appropriate theme.

3. Results

Among the one hundred and fifty participants, one hundred and forty four participants submitted their filled in pre and post test questionnaires. There was a significant increase in the post-test scores when compared to the pretest scores for all ECE sessions (Table 1).

One hundred and eight students had given their written open comments anonymously. The review of the open comments has been tabulated in Table 2.

4. Discussion

Regulatory bodies of the medical education have spelled out clearly that the medical education needs to be geared to train professionals capable of providing holistic care to patients with compassion.[6] Early clinical exposure, if implemented effectively could very well initiate the changes in the medical education system in the right direction to achieve the stipulated goal. There have been no clear directives to implement this program in terms of content, duration and mode of delivery. This has left the challenging responsibility of designing ECE entirely to the individual institutions, and the resultant programs would certainly depend upon the resources and initiatives available. Reports on the details of the conduct of ECE are scanty.[11–13] Interactive lectures, case scenarios, case based learning and occasional hospital visits have been implemented by a few of the institutions, as part of ECE.[11–13] In PSG Institute of Medical Sciences and Research (PSGIMSR), this program has been implemented as postings to selected departments of not less than an hour's duration per session. Each student gets an exposure of at least ten hours of this experience. The posting involves a variety of experiences like observing an interventional or investigative procedure, seeing a patient in ward or observing the doctor-patient interaction in the outpatient clinics, observing the demonstration of clinical signs, learning and practicing skills like phlebotomy or Basic Life Support (BLS) on manikins in the Clinical simulation laboratory.

Most students appreciated the ECE experience as 'inspiring', 'motivating', 'interesting' 'awesome', 'useful', 'good way of learning', 'evoked self interest'. They felt it helped them see the relevance of basic science in clinical practice. Interested students also contacted the clinicians in the specialty of their choice later and pursued their interest in the subject. For students who entered the medical profession without prior exposure, this program provided a bird's eye view of the opportunities that lay ahead of them as evidenced from their comments like 'Knew about various departments/ specialties in the hospital, their functioning & infrastructure' 'Made us familiar with various fields'.

In the Clinical simulation laboratory students had instructions in Phlebotomy in the first rotation and hands-on training in BLS in the second rotation. BLS enables a person to recognize and support people in life threatening emergencies until full medical care can be provided. Any trained person can perform this if the need arises. Introducing BLS training as early as possible is considered essential and may help to increase their eagerness to learn and passion for the profession.[10,14] ECE could be the ideal platform to introduce BLS training to the medical students. Students in their feedback expressed that learning BLS empowered them and made them feel more confident.

The program seemed to have helped students to develop a humanistic attitude towards the patients as evidenced by their comments like –'developed an insight about what the patient undergoes', 'learnt how to behave kindly with patients', 'learnt the importance of human life'. They also commented that they gained an insight about the various roles/duties of a doctor. Students were of the opinion that more departments need to be incorporated in the program and the duration of the program should be increased. Authors fully endorse these views and feel that time factor could probably be a major restriction for expanding this program as per the students' wishes.

5. Conclusion

ECE can serve as a platform for providing the preclinical year students a cosmic variety of experiences involving all the three domains of learning. It can also be adapted as the teaching strategy to introduce the various dimensions of medical profession like scientific, ethical, interpersonal, professional and social. This program if implemented effectively has the potential to be the ideal first step in the making of a holistic doctor.

Disclosure

The ethical clearance has been granted from the Institutional Human Ethics Committee. (Ref number 15/343, dated 6th November 2015).

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Other disclosures

None.

Conflict of Interest

None.

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References

1. Aggarwal S, Sharma V. The problems of medical education in a developing economy: the case of India. *Ann Trop Med Public Health* 2012;5:627–629.
2. Solanki A, Kashyap S. Medical education in India: current challenges and the way forward. *Med Teach* 2014;36: 1027–1031.
3. Mandal A, Ghosh A, Sengupta G, Das N, Mukherjee S, Bera T. Factors affecting the performance of undergraduate medical students: a perspective. *Indian J Community Med* 2012;37:126–129.
4. Swaminathan A, Viswanathan S, Gnanadurai T, Ayyavoo S, Manickam T. Perceived stress and sources of stress among first-year medical undergraduate students in a private medical college – Tamil Nadu. *Natl J Physiol Pharm Pharmacol* 2016;6:9–14.
5. Irby DM, Cooke M, O'Brien BC. *Calls for reform of medical education by the Carnegie Foundation for the Advancement of Teaching: 1910 and 2010. Irby Acad Med*, 85; 220–227.
6. Vision 2015 Medical Council of India.
7. Yardley S, Littlewood S, Margolis, S, et al. What has changed in the evidence for early experience? Update of a BEME systematic review. *Med Teach* 2010;32:740–746.
8. Kumar PA, Kumar PN. Clinical observership: a desirable adjunct to preclinical training. *AMJ* 2011;4:294–299.
9. Burnard P, Gill P, Stewart K, Treasure E, Chadwick B. Analysing and presenting qualitative data. *BDJ* 2008;204: 429–432.
10. Das M, Elzubeir M. First aid and basic life support skills training early in the medical curriculum: curriculum issues, outcomes, and confidence of students. *Teach Learn Med* 2001;13:240–246.
11. Tayade MC, Bhimani N, Kulkarni NB, Dandekar KN. The impact of early clinical exposure on first M.B.B.S. students. *IJHBR* 2014;2:176–181.
12. Rawekar A, Jagzape A, Srivastava T, Gotarkar S. Skill learning through early clinical exposure: an experience of indian medical school. *JCDR* 2016;10:JC01–JC04.
13. Sathishkumar S, Thomas N, Tharion E, Neelakantan N, Vyas R. Attitude of medical students towards Early Clinical Exposure in learning endocrine physiology. *BMC Med Educ* 2007;7: 30. http://dx.doi.org/10.1186/1472-6920-7-30.
14. Athipathy V, Kumar PA, Tolstoy R, Jeyakrishnan P, Mohanasundaram P, Reddy A. Basic life support through early clinical exposure: students' perspective. *AMJ* 2016;9:319–323.

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GLOBAL ONCOLOGY: RESEARCH ARTICLE

Training pediatric hematologist/oncologists for capacity building in Ethiopia

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Partial results of this research were presented in abstract form entitled "Training Pediatric Hematologists/Oncologists for Capacity Building in Ethiopia" at the American Society of

Abstract

Purpose: A considerable barrier to global pediatric oncology efforts has been the scarcity and even absence of trained professionals in many low- and middle-income countries, where the majority of children with cancer reside. In 2013, no dedicated pediatric hematology-oncology (PHO) programs existed in Ethiopia despite the estimated annual incidence of 6000-12000 cases. The Aslan Project initiative was established to fill this gap in order to improve pediatric cancer care in Ethiopia. A major objective was to increase subspecialty PHO-trained physicians who were committed to practicing locally and empowered to lead programmatic development.

Methods: We designed and implemented a PHO training curriculum to provide a robust educational and clinical experience within the existing resource-constrained

Abbreviations: AAU, Addis Ababa University; ACSH, Ayder Comprehensive Specialized Hospital; FMOH, Federal Ministry of Health; HIC, high-income country; JU, Jimma University; JUMC, Jimma University Medical Center; LMIC, low- and middle-income countries; NGO, nongovernmental organization; PHO, pediatric hematology-oncology; TAPCCO, Tesfa Addis Parents Childhood Cancer Organization; TASH, Tikur Anbessa Specialized Hospital; UGH, University of Gondar Hospital

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environment in Ethiopia. Education relied on visiting PHO faculty, a training attachment abroad, and extraordinary initiative from trainees.

Results: Four physicians have completed comprehensive PHO subspecialty training based primarily in Ethiopia, and all have remained local. Former fellows are now leading two PHO centers in Ethiopia with a combined capacity of 64 inpatient beds and over 800 new diagnoses per year; an additional former fellow is developing a pediatric cancer program in Nairobi, Kenya. Two fellows currently are in training. Program leadership, teaching, and advocacy are being transitioned to these physicians.

Conclusions: Despite myriad challenges, a subspecialty PHO training program was successfully implemented in a low-income country. PHO training in Ethiopia is approaching sustainability through human resource development, and is accelerating the growth of dedicated PHO services where none existed 7 years ago.

KEYWORDS
hematology, oncology, pediatrics, resource-limited, training

1 | INTRODUCTION

Human resources for health care are one of the core building blocks of health systems, and are essential to the sustainability and scalability of health interventions.[1] A considerable barrier to global pediatric hematology-oncology (PHO) efforts has been the scarcity and even absence of trained professionals in many low- and middle-income countries (LMIC), where the majority of children with cancer reside. Studies highlight this inequity[2] and the need to develop a global oncology workforce through allocation of financial resources, strengthening of health care infrastructure, and development of innovative training programs.[3] The 2015 United Nations Sustainable Development Goal 3, "Ensure healthy lives and promote well-being for all at all ages," includes a call to reduce by one-third premature mortality from noncommunicable diseases through prevention and treatment, which provides financial and policy support for cancer control programs in LMIC. Even with this international support, competing priorities for child health, including neonatal care, nutrition, and infection control, have raised concerns about the practicality of focusing efforts on PHO in LMIC. However, the potential for success of PHO clinical care in LMIC has been established for decades,[4] and recent efforts have demonstrated both the cost-effectiveness[5,6] and the international momentum for such programs.[7] The World Health Organization Global Initiative for Childhood Cancer was established in 2018 to help coordinate program development for childhood cancer in LMIC.

Ethiopia is the second most populous country in Africa with a population exceeding 110 million persons spanning 426 400 square miles. As noted in the 2019 UN Human Development Index, more than 83% of Ethiopia's population lives in multidimensional poverty, unable to withstand additional health care expenditures associated with cancer care,[8] with a skilled labor force of 6.8% and a gross national income per capita of $1782. Nearly 80% of the population lives in rural areas, far from urban settings, which can influence access to health care and challenge assessment of disease burden.[9] Current health expenditures constitute 4% of the country's GDP, and the childhood mortality rate (less than 5 years old) is 58.5 per 1000 live births.[10]

Because of the improved treatment of malaria and HIVand other infectious disease in Ethiopia, noncommunicable diseases contribute an increasing proportion of childhood mortality. The annual incidence of pediatric cancers in Ethiopia has been estimated between 6000 and 12 000 cases in children aged 0-14 years,[11,12] who as of 2019 constitute 40% of the population,[13] more than twice the percentage of the population of 0-14 years living in the United States. Prior to 2013, no dedicated PHO programs existed in Ethiopia, and there was no formal process for local PHO subspecialty physician training. To address this gap, we established an organization with the objective to develop sustainable local capacity in Ethiopia to treat pediatric blood disorders and cancers through creation of a formal 2-year PHO fellowship program in tandem with the development of specialized allied health and supportive care services. In 2015, the Ethiopian Federal Ministry of Health (FMOH) developed the first National Cancer Control Plan.[14] While the plan does not specifically address PHO care, support services and palliative care for children are mentioned in the plan. The implementation of the National Cancer Control Plan thus demonstrated a national commitment to cancer care, and provided momentum to our efforts to develop local PHO capacity. Herein, we describe our approach, successes, and challenges to PHO fellowship training in Ethiopia.

2 | METHODS

2.1 | Strategy

The PHO fellowship program has been led by The Aslan Project, a US nonprofit organization consisting of experts in PHO, pediatric

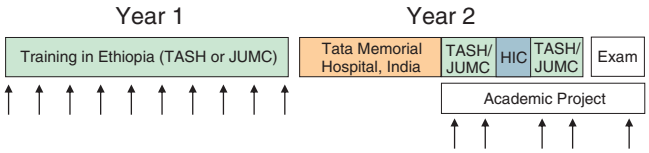


FIGURE 1 Schedule of training through 2 years of subspecialty fellowship. Abbreviations: ↑, visiting faculty - lectures, clinical teaching, clinical consultation, fellow evaluation; TASH, Tikur Anbessa Specialized Hospital; JUMC, Jimma University Medical Center; HIC, high-income country

oncology nursing, and pathology with experience in LMIC.[12] While most faculty are affiliated with academic institutions, this PHO training effort was not a direct collaboration with a specific high-income country (HIC) academic center. Sited initially in 2013 at Tikur Anbessa Specialized Hospital (TASH)—the location of the first comprehensive Ethiopian pediatric cancer program that Aslan developed in collaboration with the FMOH and Addis Ababa University (AAU)—the goal of the fellowship program was to increase subspecialty PHO-trained physicians committed to practicing locally and empowered to lead programmatic development throughout the country. To that end, we sought to provide a robust educational and clinical experience for fellows within the existing resource-constrained environment, and to equip them with the tools to become strong leaders, administrators, and advocates with ownership of the direction of PHO in Ethiopia. To expand capacity to major national hospitals serving other significant catchment areas in the country, we established a second pediatric oncology unit and PHO fellowship program at Jimma University Medical Center (JUMC) in southwestern Ethiopia in 2016. In March 2019, at the direction of the FMOH, we relocated the fellowship program back to TASH to consolidate training programs for PHO at a single center.

2.2 | Curriculum

In developing the fellowship program, we considered a variety of training models, including training abroad, installation of a full-time external PHO specialist, and short focused coursework to share PHO skills with interested pediatricians. To optimize available organizational and local resources, we chose a hybrid model in which the majority of training occurred in Ethiopia with consistent teaching and clinical training from external experts in PHO through onsite faculty visits (Figure 1). Because neither TASH nor JUMC had specialized PHO physicians, one of two Aslan-supported clinical directors—practicing pediatric hematologist-oncologists with significant clinical experience in LMIC—was present throughout the first year at each location.

The fellowship curriculum was based on international standards to cover medical expertise, scholarship, professionalism, communication, management, and health advocacy (Figure 2). Experiential training blocks included 18 months of PHO service in Ethiopia with inpatient, outpatient, and consultation responsibilities. To augment onsite training by visiting faculty, we sponsored fellows for a 6-month attachment at a high-functioning cancer institution in a middle-income country and, as noted, the support of full-time onsite clinical faculty/mentorship for the early stages of the TASH and JUMC programs.[15–17] We initially attempted to supplement onsite training through a tele-lecture series, but use of that tool was not feasible due to poor internet access. Fellows were expected to complete a scholarly activity. Fellows were cosponsored by their home institution and The Aslan Project. Prior to starting fellowship, fellows signed an agreement to serve as PHOs in Ethiopia or their home country in Africa for a minimum of 2 years following training. AAU and Jimma University (JU) approved the PHO fellowship curriculum for TASH and JUMC, respectively.

2.3 | Evaluations and subspecialty certification

Fellows were evaluated on a continuous basis. Following each onsite training visit, the visiting faculty member completed a written evaluation assessing the fellow(s) based on performance, skill level, attendance, and other measurements approved by the academic institution (AAU or JU). Written evaluations were submitted to the academic institution. Additional means of evaluation included the fellow's personal portfolios, case logs, or mini-clinical encounters.

An interim multiple-choice examination was administered at the completion of the fellow's first year of training solely for purposes of assessing skills. At the end of the 2-year program, fellows demonstrating excellent performance were approved to sit for a final qualifying examination, developed by the Aslan Medical Advisory Board, with practical, written, hematopathologic, and clinical skill components. Fellows who fulfilled the above requirements and obtained an aggregate score of 75% in the final qualifying examination were awarded a "Subspecialty Certificate in Clinical Pediatric Hematology/Oncology" by the academic institution, and subsequently endorsed by governing bodies in Ethiopia and Kenya.

2.4 | Partnership development

From the outset, we enlisted and relied upon strong governmental, institutional, and nongovernmental organization (NGO) partnerships in building Ethiopia's pediatric cancer health system. Both the pediatric oncology unit and the PHO fellowship programs at TASH were developed pursuant to agreements with TASH, AAU, and the FMOH, and both JU and JUMC have been key partners in the development of pediatric oncology unit and fellowship program there. Moreover, as the vast majority of pediatric cancer patients in Ethiopia come from extreme poverty, a critical component of our work at TASH and JUMC has been the establishment of and funding support for a local NGO, Tesfa Addis Parents Childhood Cancer Organization (TAPCCO), to provide psychological, social, accommodation, nutritional, and other health and financial support to the pediatric cancer patients and their families. TAPCCO has directly contributed to fellowship training through supporting longitudinal patient care experiences, and teaching fellows the critical components of patient care that exist outside health care

Teaching Blocks

- Block 1: Emergency pediatric hematology/oncology (7 LH)
- Block 2: General aspects of hematology and immunology (2 LH)
- Block 3: Basic principles of chemotherapy (4 LH)
- Block 4: Disorders of hemostasis and management (4 LH)
- Block 5: Anticoagulants and their effects on hemostasis (1 LH)
- Block 6: Supportive care needs in pediatric hematology/oncology (4 LH)
- Block 7: Life course perspectives in pediatric hematology/oncology (2 LH)
- Block 8: Consultative hematology (2 LH)
- Block 9: Transfusion medicine (3 LH)
- Block 10: Redblood cell disorders (6 LH)
- Block 11: Leukocytes, lymph nodes and spleen (2 LH)
- Block 12: Approach to pediatric oncology (4 LH)
- Block 13: Acuteleukemia (4 LH)
- Block 14: Myeloproliferative neoplasms (3 LH)
- Block 15: Malignant lymphomas (4 LH)
- Block 16: Solidtumors (6 LH)
- Block 17: CNS tumors and retinoblastoma (4 LH)
- Block 18: Histiocytic disorders (2 LH)
- Block 19: Infectious diseases and pediatric hematology/oncology (3 LH)
- Block 20: Laboratory technology (4 LH)
- Block 21: Scholarly development (Research and Teaching) (7 LH)
- Block 22: Quality assurance and management practices (4 LH)

Teaching Schedule

Month	PHO Faculty	Education Goals and Programs to be Developed	Other Faculty
1	Hematologic Malignancies	Acute leukemias Emergencies/supportive care	Pharmacy faculty
2	Hematologic Malignancies	Acute leukemias Supportive/Palliative care	Infectious disease faculty
3	Hematologic Malignancies	Lymphomas CML, Supportive/Palliative care	
4	Hematologic Malignancies	Lymphomas Histiocytic disorders Supportive/Palliative care	
5	Solid Malignancies	Bone and soft tissue sarcomas Imaging, Path., Radiation, Surgery	Surgery faculty
6	Solid Malignancies	Bone and soft tissue sarcomas Imaging, Path., Radiation, Surgery	
7	Solid Malignancies	Retinoblastoma, Neuroblastoma Wilms tumor Imaging, Path., Radiation, Surgery	Pathology faculty
8	Solid Malignancies	Brain tumors Imaging, Path., Radiation, Surgery	Radiation oncology faculty
9	Hematology	Red blood cell disorders Hemopathology, Lab medicine	
10	Hematology	Red blood cell disorders Hemopathology, Lab medicine	Pathology/Lab medicine faculty
11	Hematology	Coagulation and hemostasis Hemopathology, Lab medicine	
12	Hematology	Other hematologic disorders Hemopathology, Lab medicine	

FIGURE 2 Curriculum block and samples training schedule throughout the first year of fellowship. The specific schedule was adjusted based upon available traveling faculty, but we required every teaching block to be covered. Abbreviation: LH, lecture hours

settings. Additional program development components required to support fellowship training included establishment of dedicated PHO units, supportive care and cancer treatment guidelines, training for pharmacy, nursing, infection control, and data collection, and partnership creation with blood banks, subspecialty physicians, radiologists, and pathologists.

3 | RESULTS

3.1 | Process

Committed visiting faculty with significant experience in low-resource settings led onsite training to cover the blocks of the approved curriculum. Twenty-five faculty members from 21 universities made 53 trips to Ethiopia between 2013 and 2019. Faculty included PHO clinicians, infectious disease clinicians, pathologists, pediatric oncology nurses, and oncology pharmacists based in Cameroon, Canada, Egypt, England, India, Israel, Pakistan, South Africa, Switzerland, and the United States. Faculty members provided didactic lectures, clinical teaching, professional mentoring, and advocacy during their 1-4-week site visits. In the second fellowship year, fellows completed a 6-month specialized training rotation in India at either Tata Memorial Hospital, Mumbai or Tata Medical Center, Kolkata. In Mumbai and Kolkata, fellows actively performed inpatient and outpatient clinical care alongside world-renowned specialists in PHO, infectious diseases, and pathology. Through this experience, they gained not only the skills and knowledge that came from intensive training, but also the experience of working in comprehensive cancer care centers with access to a full complement of diagnostic technology, therapeutic opportunities, and supportive care resources as well as a patient population similar to children with cancer in Ethiopia.

3.2 | Capacity development

Four physicians have completed PHO fellowship training in Ethiopia, with extensive support of local health care leadership and visiting faculty. An additional fellow left training prior to completion and two fellows currently are in training. The four subspecialty-trained physicians have remained local, two at TASH, one at JUMC, and one returned to her home hospital, Gertrude's Children's Hospital in Nairobi, Kenya, where she has established a PHO unit as the second Kenyan medical board-recognized PHO specialist.

The fellowship structure has succeeded in training subspecialty physicians to establish PHO care in Ethiopia. TASH supports a 26-bed inpatient unit and 16 additional inpatient beds at a nearby oncology center, treating over 650 new patients annually. JUMC likewise supports a 22-bed PHO unit running at 90% capacity with over 340 new diagnoses from August 2016 to November 2019. Moreover, each fellowship graduate is actively leading education and training along with clinical, research, and administrative leadership responsibilities, exponentially expanding the capacity of PHO services at their institutions. In addition to the four fellowship graduates, one additional PHO consultant at TASH, one PHO consultant at Ayder Comprehensive Specialized Hospital (ACSH) in Mekele, one PHO consultant at St Paul's Hospital, and one PHO consultant at the University of Gondar Hospital (UGH) in Gondar were recently trained through other international initiatives. With the development of a cadre of trained PHO clinicians and increased capacity for supporting children with cancer, the Ethiopian

FMOH has recently adopted its first ever National Control Plan for Pediatric Cancer.

3.3 | Challenges

Success of this training effort was not guaranteed. A primary unexpected challenge was fellow recruitment in Ethiopia. The PHO subspecialty was and remains relatively unknown in the country, and thus was not a popular choice for graduating pediatrics residents. As a result, most fellows transitioned from full-time work as practicing pediatricians, leading to a significant pay decrease during fellowship. While subspecialty-credentialing leads to small increase in reimbursement, pay-related challenges continue for the PHO consultants at public hospitals where the PHO units are located, in comparison to salary for pediatricians in the private sector. In addition, the multitude of services required to develop a PHO unit and critical to trainee experiences were sometimes lacking. Key pharmaceutical deficiencies early in the program were chemotherapy and antimicrobial acquisition, supply chain logistics, drug preparation, and safe administration. Additional medical services—diagnostic pathology tools, imaging capabilities, ophthalmology, radiation oncology, surgical availability, infection prevention, and blood product support—were inconsistent or incomplete at times, further complicating efforts to train PHO physicians. Moreover, despite sustained efforts to train pediatric oncology nurses at TASH and JUMC, the frequency of staffing rotations made it difficult to establish and retain a skilled pediatric oncology nursing workforce. Treatment abandonment is also a substantial challenge, which as noted we are addressing through development of an array of social services through TAPCCO.

3.4 | Transition

PHO services in Ethiopia are now in operation with varying levels of capacity at TASH, JUMC, ACSH, and UGH, with a unit opening soon at St Paul's Hospital Millennium Medical College in Addis Ababa (Figure 3). Leadership from each center is actively collaborating on initiatives designed to further the development of Ethiopia's pediatric cancer health system. The PHO unit leaders, the FMOH, and visiting Aslan partners consistently advocate for capacity building beyond fellowship training, including continued pediatric oncology pharmacy and nursing specialization, treatment guideline and registry development, quality improvement monitoring, patient psychosocial support services, and comprehensive social services for families.

4 | DISCUSSION

Models of subspecialty training in low-resource settings have expanded in the past decade alongside the increased development of global health workforce capacity.[18] Initial efforts appropriately focused on infectious disease care; in recent years, workforce development has followed the epidemiologic shift from communicable to noncommunicable diseases (including cancer) and injury as increasingly significant causes of morbidity and mortality.[19]



FIGURE 3 Map of Ethiopia with locations of the four established pediatric oncology units. Blue stars represent the locations of sites that have been used for training. Red stars represent the additional active pediatric hematology-oncology (PHO) units, each supported by a single PHO-trained physician

Early models of subspecialty training existed primarily in the broad categories of training abroad,[20] short-term training,[21] or full time relocation of international health care providers from a HIC to a LMIC setting.[22] However, each of these approaches has significant limitations. Fellowship training abroad presents potential negative consequences as these efforts remove talented physicians for multiple years and can increase the permanent emigration from LMIC.[23–26] Additionally, many of the skills learned in HIC may not be immediately applicable to LMIC settings. Short-term skillset-based observerships or brief coursework can be beneficial for procedural services and increasing funds of knowledge, but these approaches do not provide comprehensive subspecialty development. Subspecialty training can occur through attaching international experts from HIC on a full-time basis to LMIC institutions for extended periods to set up clinical services, administrative structures, and an educational curriculum for complete in-country subspecialty training.[22] While this model has been successful, it has the potential to interfere with the development of local leadership and is not a scalable solution. More recently, researchers have emphasized hybrid approaches focused on local education and physician retention without sacrificing comprehensive training knowledge and skill-based training, including at a program similar to ours in Ethiopia.[27,28] We successfully developed such a hybrid model with limited attachment of full-time physicians from HIC and monthly external faculty visits to anchor the training in Ethiopia, while also supporting meaningful clinical training in a middle-income country setting.

Subspecialty oncology training programs in LMIC remain a recent development[17] and specific attempts at PHO training are just beginning.[29,30] Modern pediatric oncology care requires myriad robust systems to be in place. Because health care infrastructures in LMIC can be fragile or in early stages of strengthening, access to consistent services and specialized support is essential in the following areas: diagnostic pathology, chemotherapy, specialized nursing support, imaging capabilities, ophthalmology, general surgery, neurosurgery, radiotherapy, infection prevention programs, pediatric oncology pharmacists, blood banks, and psychosocial and palliative care support services. This is what makes caring for children and adolescents with cancer so challenging. However, as these health systems improve, other patients also benefit.

Pediatric oncology is a highly demanding subspecialty, and managing immunosuppressed patients in low-resource health care settings is particularly difficult. Through the establishment of two POUs, the implementation of fellowship, pathology, pediatric oncology nursing, and pharmacy trainings, and the partnership with TAPCCO over the past 7 years, we have reached several fundamental conclusions. *First*, it is critical that the newly specialized pediatric oncology workforce in Ethiopia is protected from burnout and work-related compassion fatigue. These pioneer physicians are managing large patient populations while simultaneously training pediatric residents and future fellows. Their daunting workload requires continuing remote and in-person support from visiting faculty. As the number of Ethiopian pediatric hematologist-oncologists increases and their professional organization matures and partners with the MOH, it is foreseeable that external support will no longer be required. At that time, Ethiopia will have a strong network of collaborating pediatric oncology programs capable of addressing the growing burden of childhood cancer going forward.

Second, we have become acutely aware of the interdependence of each service that impacts pediatric cancer care. Without diagnostic pathology, treatment cannot be accurately prescribed; without chemotherapy access or preparation, no hope can be given; without blood bank support, chemotherapy is too toxic; without housing services, patients and families will abandon. Wherever a PHO training program is considered, detailed needs assessment must be performed to assess capacity of ancillary services and to address parallel development needs. Thus, in addition to fellowship training, over the past 7 years we have provided opportunities for international training and conference attendance for TASH and JUMC health professionals who partner in PHO care, including nurses, pharmacists, ophthalmologists, and pathologists. In express recognition of both this interdependence and continuing resource limitations, we were directed in 2018 by the FMOH to centralize PHO fellowship training in Ethiopia at TASH. As the primary teaching and referral hospital in the country, TASH handles the largest patient volume, has access to broad supportive care services, and has the most PHO subspecialty-trained faculty working full-time. TASH currently has two fellows in training, with the capacity to add an additional two-three fellows each year. Together with the three attending PHO consultants, fellows handle a significant patient load, upwards of 7000 inpatient and outpatient visits a year.

Third, in spite of the success in developing PHO services at TASH, JUMC, ACSH, UGH, and soon St Paul's, an estimated 80% of children with cancer in Ethiopia are still not being diagnosed or referred to a PHO treatment center. Accordingly, our experience dictates that PHO workforce training must remain a priority of capacity development in Ethiopia, not just at TASH but at the additional centers as well. Communication technology advances alongside improved internet access in in Ethiopia may facilitate the scaling up of fellowship programs to institutions beyond TASH, particularly the use of interactive remote learning interfaces to conduct much of the required didactic teaching. Our increased use of virtual platforms to continue our fellowship lecture series during the recent SARS-CoV-2 pandemic, for example, highlights how such training can expand in scope to include active participation, not just by physicians in our fellowship program but also trained through other domestic and international initiatives. Moreover, with the further development of reliable remote learning and the PHO workforce capacity now in place in Ethiopia, we anticipate a steady transition of leadership for training, administrative support, and advocacy away from external Aslan staff and faculty and into the hands of PHO-trained Ethiopian physicians.

Development of oncology clinical care and training required an unbalanced amount of our effort. However, we have consistently committed one-third of the didactic curriculum to nonmalignant hematology and offered hematology-focused rotations in HIC. Fellows performed mentored consults and provided direct patient care in Ethiopia for patients with thrombosis, bleeding disorders, red blood cell disorders, bone marrow failure syndromes, and inflammatory conditions. We expect that the growing workforce of PHO subspecialty-trained physicians in Ethiopia will increasingly support critical research and comprehensive clinical care for nonmalignant hematologic conditions.

In summary, developing specialized PHO care in LMIC requires governmental and institutional buy-in and a multifaceted approach, including PHO workforce training and retention, social support, diagnostic expertise and facility, pharmacy services, and subspecialty physician support. Focusing on physician training for PHO in Ethiopia, we designed a training structure and curriculum to: (a) teach resource-appropriate medical care, (b) provide sustained clinical mentorship, (c) develop health system leadership skills, and (d) retain physicians to staff local pediatric oncology units. Challenges included fellow recruitment, chemotherapy access, diagnostic accuracy, retention of pediatric oncology nurses and oncology pharmacists following their training, infection control, blood product availability, and treatment abandonment. Locally sustainable subspecialty PHO training can be implemented in LMIC without previously established subspecialty care, but requires the relentless commitment of government officials, local and international NGOs, local academic and health institutions and faculty, external faculty, trainees, and a multidisciplinary team of health professionals.

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Medical Center. We are thankful for the dedication of faculty members, the commitment of the fellows, the support of governmental and institutional leaders, and, most importantly, the patients and their families.

CONFLICT Of INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. World Health Assembly 2016. *Global Strategy on Human Resources for Health: Workforce 2030*. http://apps.who.int/gb/ebwha/pdf_files/WHA69/A69_R19-en.pdf?ua=1&ua=1. Accessed June 16, 2019.
2. Fadelu T, Shulman LN. Health policy: towards greater equity in the global oncology workforce. *Nat Rev Clin Oncol*. 2018;15(5):270-272.
3. Fundytus A, Sullivan R, Vanderpuye V, et al. Delivery of global cancer care: an international study of medical oncology workload. *J Glob Oncol*. 2018;4(4):1-11.
4. Israels T, Challinor J, Howard S, Arora RH. Treating children with cancer worldwide–challenges and interventions. *Pediatrics*. 2015;136(4):607-610.
5. Bhakta N, Martiniuk AL, Gupta S, Howard SC. The cost effectiveness of treating paediatric cancer in low-income and middle-income countries: a case-study approach using acute lymphocytic leukaemia in Brazil and Burkitt lymphoma in Malawi. *Arch Dis Child*. 2013;98(2):155-160.
6. Fung A, Horton S, Zabih V, Denburg A, Gupta S. Cost and cost-effectiveness of childhood cancer treatment in low-income and middle-income countries: a systematic review. *BMJ Glob Health*. 2019;4(5):e001825.
7. Atun R, Bhakta N, Denburg A, et al. Sustainable care for children with cancer: a Lancet Oncology Commission. *Lancet Oncol*. 2020;21(4):e185-e224.
8. Kasahun GG, Gebretekle GB, Hailemichael Y, Woldemariam AA, Fenta TG. Catastrophic healthcare expenditure and coping strategies among patients attending cancer treatment services in Addis Ababa, Ethiopia. *BMC Public Health*. 2020;20(1):984.
9. Poletti P, Parlamento S, Fayyisaa T, et al. The hidden burden of measles in Ethiopia: how distance to hospital shapes the disease mortality rate. *BMC Med*. 2018;16(1):177.
10. *United Nations Development Programme: Human Development Reports*. 2020. http://hdr.undp.org/en/countries/profiles/ETH. Accessed July 6, 2020.
11. Ward ZJ, Yeh JM, Bhakta N, Frazier AL, Atun R. Estimating the total incidence of global childhood cancer: a simulation-based analysis. *Lancet Oncol*. 2019;20(4):483-493.
12. Shad A, Challinor J, Cohen ML. Paediatric oncology in Ethiopia: an INCTR-USA and Georgetown University Hospital twinning initiative with Tikur Anbessa Specialized Hospital. *Cancer Control*. 2013:108-112.
13. The World Bank. *World Development Indicators*. http://wdi.worldbank.org/table/2.1. Accessed April 30, 2020.
14. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Abate D, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. *JAMA Oncol*. 2019;5(12):1749-1768.
15. Meshkat N, Teklu S, Hunchak C, Taaac EM, Global Health Emergency Medicine organization at the Division of Emergency Medicine University of Toronto. Design and implementation of a postgraduate curriculum to support Ethiopia's first emergency medicine residency training program: the Toronto Addis Ababa Academic Collaboration in Emergency Medicine (TAAAC-EM). *BMC Med Educ*. 2018;18(1):71.
16. Cancedda C, Cotton P, Shema J, et al. Health professional training and capacity strengthening through international academic partnerships: the first five years of the human resources for health program in Rwanda. *Int J Health Policy Manag*. 2018;7(11):1024-1039.
17. Rosen B, Itsura P, Tonui P, Covens A, van Lonkhuijzen L, Orang'o EO. Development of a comprehensive and sustainable gynecologic oncology training program in western Kenya, a low resource setting. *Gynecol Oncol Rep*. 2017;21:122-127.
18. Collins FS, Glass RI, Whitescarver J, Wakefield M, Goosby EP. Public health. Developing health workforce capacity in Africa. *Science*. 2010;330(6009):1324-1325.
19. Girum T, Mesfin D, Bedewi J, Shewangizaw M. The burden of noncommunicable diseases in Ethiopia, 2000-2016: analysis of evidence from global burden of disease study 2016 and global health estimates 2016. *Int J Chronic Dis*. 2020;2020:3679528.
20. Zar HJ, Vanker A, Gray D, Zampoli M. The African Pediatric Fellowship Training Program in pediatric pulmonology: a model for growing African capacity in child lung health. *Ann Am Thorac Soc*. 2017;14(4):500-504.
21. Fish M, Parkes J, Dharsee N, et al. POETIC (Program for Enhanced Training in Cancer): an initial experience of supporting capacity building for oncology training in sub-Saharan Africa. *Oncologist*. 2019;24(12):1557-1561.
22. Haglund MM, Warf B, Fuller A, et al. Past, present, and future of neurosurgery in Uganda. *Neurosurgery*. 2017;80(4):656-661.
23. Guilbert JJ. The World Health Report 2006: working together for health. *Educ Health (Abingdon)*. 2006;19(3):385-387.
24. Tankwanchi AB, Ozden C, Vermund SH. Physician emigration from sub-Saharan Africa to the United States: analysis of the 2011 AMA physician masterfile. *PLoS Med*. 2013;10(9):e1001513.
25. Duvivier RJ, Burch VC, Boulet JR. A comparison of physician emigration from Africa to the United States of America between 2005 and 2015. *Hum Resour Health*. 2017;15(1):41.
26. Kelly CM, Vins H, Spicer JO, et al. The rapid scale up of medical education in Ethiopia: medical student experiences and the role of e-learning at Addis Ababa University. *PLoS One*. 2019;14(9):e0221989.
27. Wondimagegn D, Pain C, Baheretibeb Y, et al. Toronto Addis Ababa Academic Collaboration: a relational, partnership model for building educational capacity between a high- and low-income university. *Acad Med*. 2018;93(12):1795-1801.
28. Sherman CB, Carter EJ, Braendli O, Getaneh A, Schluger NW. The East African Training Initiative. a model training program in pulmonary and critical care medicine for low-income countries. *Ann Am Thorac Soc*. 2016;13(4):451-455.
29. Lubega J, Airewele G, Fruge E, et al. Capacity building: a novel pediatric hematology-oncology fellowship program in sub-Saharan Africa. *Blood Adv*. 2018;2(Suppl 1):11-13.
30. Slone JS, Slone AK, Wally O, et al. Establishing a pediatric hematology-oncology program in Botswana. *J Glob Oncol*. 2018;4:1-9.

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Anaplastic Multiple Myeloma- a Morphological Challenge – A Case Series

Abstract

Anaplastic multiple myeloma (AMM) is a rare and aggressive variant of plasma cell neoplasm. It is relatively treatment resistant when compared to conventional myeloma. It can arise *de novo* or transform from a preexisting plasma cell myeloma. Morphologically, AMM is recognized by the presence of immature plasma cells and pleomorphic, multilobated cells. Here, we present three cases of AMM.

Keywords: *Anaplastic myeloma, anaplastic variant of multiple myeloma, bizarre plasma cells, multilobated plasma cells*

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Introduction

Anaplastic multiple myeloma (AMM) is a very rare morphological variant of multiple myeloma. It has an aggressive clinical course and poor prognosis. It is relatively more treatment resistant when compared to conventional myeloma. It can arise *de novo* or transform from a preexisting plasma cell myeloma.[1,2]

Morphologically, AMM is recognized by the presence of immature plasma cells and pleomorphic, multilobated cells. These cells can be mistaken for carcinoma or lymphoma cells or even at times megakaryocytes. Here, we present three cases of AMM.[3]

Case Reports

Case 1

An 85-year-old male who was diagnosed to have chronic kidney disease presented with the symptoms of anemia and back pain. Laboratory investigations showed anemia (hemoglobin [Hb]: 6.6 g/dl), erythrocyte sedimentation rate (ESR) of 120 mm, and elevated renal parameters (blood urea – 87 mg/dl and serum creatinine – 4.38 mg/dl). Serum albumin was 3.1 g/dl and globulin was 4.8 g/dl. Peripheral smear showed normocytic anemia with exaggerated rouleaux formation [Figure 1].

Bone marrow aspiration showed hypercellular marrow with significant plasmacytosis (plasma cells −72%). Also seen were many large cells with eccentrically placed hyperchromatic nuclei exhibiting marked nuclear lobulations and indentations. Subsequently, serum protein electrophoresis was done which demonstrated a discrete M band.

Case 2

A 61-year-old male, a diagnosed case of chronic kidney disease, presented with severe fatigue and bony tenderness. Blood investigations showed elevated renal parameters (blood urea – 95 mg/dl and serum creatinine – 7.42 mg/dl). There were significant albumin–globulin ratio reversal (serum albumin – 2.1 g/dl and globulin – 12.3 g/dl) and markedly elevated ESR (109 mm/h). Peripheral smear showed normocytic anemia (Hb – 4.4 g/dl), thrombocytopenia (87,000/mm^3), white blood cell count of 4400/mm^3, increased rouleaux formation, and presence of 4% atypical cells [Figure 2].

Serum protein electrophoresis showed a significant fall in albumin level and a steep increase in the gamma globulin region suggestive of "M band." Bone marrow aspiration showed hypercellular marrow particles with pleomorphic plasmacytoid cells with lobated nuclei along with a few mature, binucleate and multinucleate plasma cells. Hypercellular marrow spaces with sheets and singly scattered pleomorphic cells were observed on trephine biopsy. Plasmacytoid morphology was focally discernible [Figure 3]. The differentials

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included lymphoma versus myeloma. Immunohistochemical markers were done. The cells expressed CD138 and were negative for CD20 immunostain [Figures 4 and 5].

Case 3

A 70-year-old male presented with severe fatigue, loss of appetite, and weight loss. He was a known case of multiple myeloma diagnosed 6 years back. He was in remission following treatment. Peripheral smear showed pancytopenia with increased rouleaux formation and presence of plasma cells. Bone marrow studies were done to rule out recurrence. Bone marrow aspirate showed bizarre plasma cells [Figures 6 and 7]. The marrow spaces showed diffuse infiltration by plasmacytoid cells and bizarre cells with multilobated nuclei. The cells expressed membranous staining for CD138. The bone marrow features were consistent with the anaplastic transformation of a myeloma.

Discussion

Multiple myeloma is a common hematological malignancy of the middle aged and elderly. AMM was originally used to describe high-grade transformation occurring as a terminal event in the malignant plasma cell clones having pleomorphic morphology and immature plasma cells.[3,4] It has been referred by various names such as plasma cell sarcoma, aggressive-phase myeloma, and dysplastic myeloma. It is more commonly reported in younger adults, with the median age being 53 years, associated with predisposition to extramedullary sites and immunoglobulin A isotype.[2] Cases of anaplastic myeloma with poorly differentiated cells having extensive involvement of intra-abdominal and retroperitoneal sites have been reported in literature. AMM has morphological and flow cytometry features which are distinct from those of the conventional myeloma.[4]

Historically, this disease has been very resistant to chemotherapy. It has been suggested that this aggressive phase could result from a clonal evolution of the original malignant cell and not considered as the development of an independent new neoplasia.

All the three cases reported were seen in adults. None of them had any evidence of extramedullary disease. Two

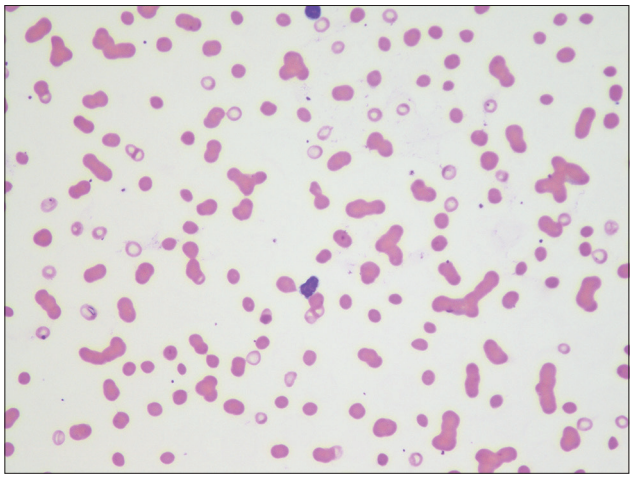


Figure 1: Case 1: Peripheral smear with exaggerated rouleaux formation (×40)

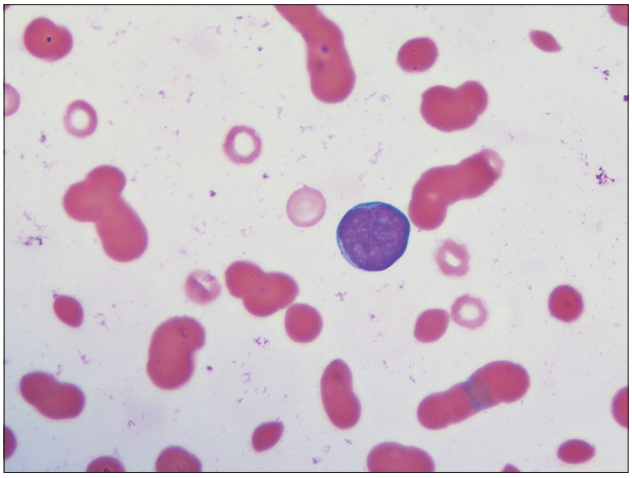


Figure 2: Case 2: Peripheral smear with a blast cell (×100)

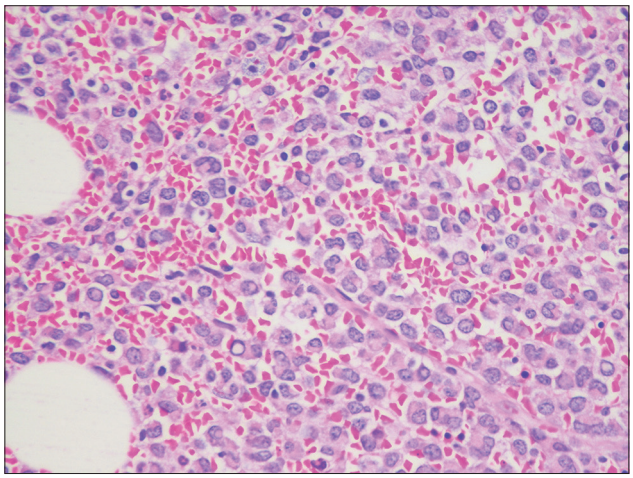


Figure 3: Case 2: Bone marrow trephine showing sheets of plasma cells, anaplastic cells, and Dutcher bodies (H and E, ×40)

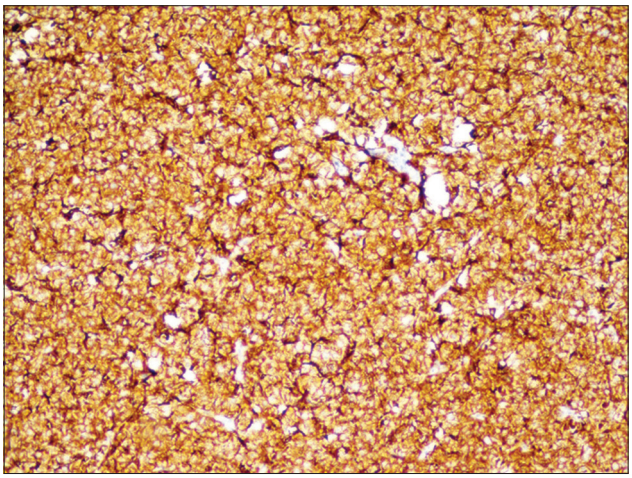


Figure 4: Case 2: Bone marrow trephine with cells expressing membranous CD138 (immunohistochemistry × 40)

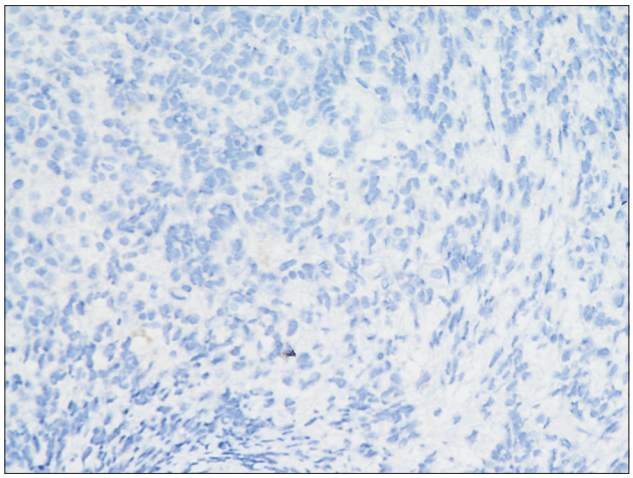


Figure 5: Case 2: Bone marrow trephine showing the neoplastic cells negative for CD20 (immunohistochemistry ×40)

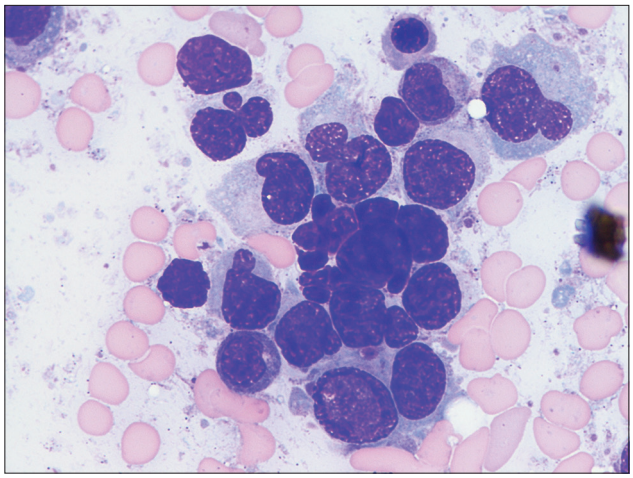


Figure 6: Case 3: Bone marrow aspirate with bizarre plasma cells (×100)

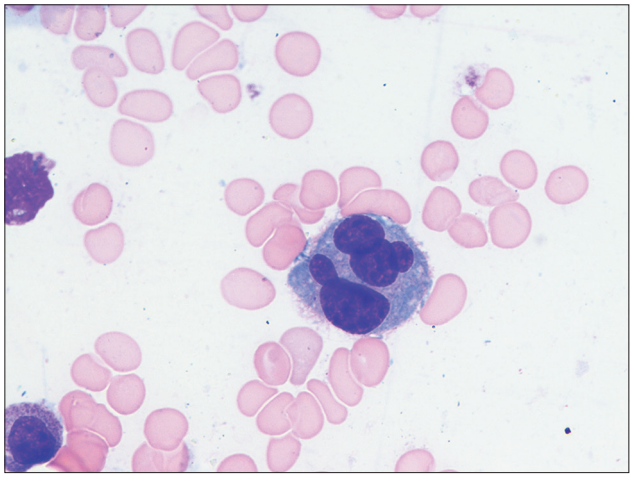


Figure 7: Case 3: Bone marrow aspirate with a plasma cell showing abnormal nuclear lobulation (×100)

cases presented *de novo*, whereas the other was anaplastic transformation seen in a patient in remission after treatment.

Multiple myeloma exhibits a broad morphological variation ranging from mature plasmacytic to highly anaplastic variants. The anaplastic variant poses a morphological diagnostic challenge as it mimics carcinoma and lymphomas, especially when presenting *de novo*. The presence of large cells with nuclear budding may mimic a megakaryocyte in bone marrow samples.[5,6]

Case 2 reported here had a very anaplastic morphology, and the diagnosis was made with the aid of immunohistochemical staining with CD138.

Diagnosing a case as anaplastic myeloma without ancillary techniques is difficult, especially when the histology shows primitive cells. One of our cases had an undifferentiated morphology.

Bone marrow findings varied among the three cases.

Case 1 showed 72% plasma cells with the presence of few anaplastic cells. Whereas, case 2 showed predominantly large cells with pleomorphic morphology and scattered plasma cells.

Case 3 showed plasmacytoid cells with anaplastic morphology and occurred as a recurrence.

AMM, irrespective of presenting *de novo* or as progression, has been reported to have poor response to chemotherapy.[4]

Conclusion

Here, we present a series of three cases of anaplastic myeloma for the rarity of this entity and also for the fact that these cases were considerably different from the previously reported cases in having no evidence of extramedullary disease and presenting at an older age. Anaplastic myeloma needs to be considered in bone marrow aspirates with pleomorphic morphology in a patient with other supportive evidence of plasma cell dyscrasia.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Rodriguez C, Strowd RE, Grier DD. Anaplastic myeloma arising from myeloma: Successful management of two diseases in an individual. Blood 2017;130:5410.

2. Sethi S, Miller I. Plasma cell myeloma with anaplastic transformation. Blood 2016;128:2106.
3. Harankhedkar S, Gupta R, Rahman K. Pleomorphic multinucleated plasma cells simulating megakaryocytes in an anaplastic variant of myeloma. Turk J Haematol 2018;35:150-1.
4. Ammannagari N, Celotto K, Neppalli V, Lee K, Holstein SA. Anaplastic multiple myeloma: An aggressive variant with a poor response to novel therapies. Clin Lymphoma Myeloma Leuk 2016;16:e129-31.
5. Rao S, Kar R, Pati HP. Anaplastic myeloma: A morphologic diagnostic dilemma. Indian J Hematol Blood Transfus 2008;24:188-9.
6. Fujimi A, Nagamachi Y, Yamauchi N, Kanisawa Y. Morphological transformation of myeloma cells into multilobated plasma cell nuclei within 7 days in a case of secondary plasma cell leukemia that finally transformed as anaplastic myeloma. Case Rep Hematol 2017;2017:5758368.

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Original Article

Immunohistochemical Profile and Distribution of Non-Hodgkin and Hodgkin Lymphoma – An Experience in a Medical College Hospital in Tamil Nadu

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Abstract

Context: The subtype distribution of non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) shows distinct pattern across geographic regions. This study analyzes the subtype distribution of lymphomas based on the WHO 2017 Classification using immunohistochemistry (IHC) and also investigates the IHC profile of various lymphomas. **Subjects and Methods:** Fifty consecutive cases of lymphoma diagnosed in the department of pathology in a medical college hospital were included in the study. The cases were subcategorized using panels of IHC based on the light microscopic findings. **Results:** The ratio of NHL to HL was 4.5:1. B-NHL formed 78.5% (n = 32), while T-cell NHL formed 21.5% (n = 9). Among the B-NHL cases, small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (34.4%) was the most common, followed by diffuse large B-cell lymphoma, NOS (DLBCL NOS) (31.2%), follicular lymphoma (FL) (25%), and then by Burkitt's lymphoma (BL) constituting 9.4%. Peripheral T-cell lymphoma, NOS (PTCL NOS) (14.1%) (n = 6) was the most common T-cell NHL, followed by angioimmunoblastic T-cell lymphoma (4.8%), and then by lymphoblastic lymphoma (T-LL) (2.4%). Among Hodgkin lymphomas, mixed cellularity (MC) accounted for 55.6% (n = 5), lymphocyte depleted type (LD) being 22.2%, while nodular lymphocyte predominant and lymphocyte rich type each constituted 11.1%. **Conclusion:** SLL/CLL was the most common lymphoma reported in the study followed by DLBCL which is different from the other Indian studies where DLBCL was most common. CD5 expression was noted in 21% of FLs. PTCL-NOS constituted the most common T-cell lymphoma and MC, the most frequent subtype of classical HL. Further replication studies including larger sample size will be needed to understand the difference in subtype distribution in our population.

Keywords: *CD5, Hodgkin lymphoma, immunohistochemistry, Lennert's lymphoma, non-Hodgkin lymphoma, small lymphocytic lymphoma*

Introduction

There has been a rising incidence of malignant lymphoma. The incidence varies among the different countries as well as within various regions of a country.[1,2] Age-adjusted incidence of non-Hodgkin lymphoma (NHL) in India is 5 / 100,000.[1] NHL accounts for 5.1% of all malignancies and Hodgkin lymphoma (HL) constitutes 9.5% of cancers in men and 5.5% in women.[3,4]

With the advent of changes in lymphoma therapy, the accurate diagnosis of lymphoma and prognostication wherever possible becomes critical. Similarly, the classification of lymphomas has evolved with the incorporation of various novel immunohistochemical markers in the diagnosis and prognostication.[5-7]

Immunohistochemistry (IHC) has become an integral part in the lymphoma diagnosis, histology being the gold standard. The utility of IHC has led to a better understanding of pathogenesis. The panel of IHC markers was chosen based on the morphological findings in light microscopy. The markers include CD45, CD20, and CD3 to differentiate T- and B-cell lymphomas and CD15 and CD30 for HLs. Other markers used for subtyping were CD10, CD5, CD23, BCL2, Cyclin D1, TdT, ALK, and proliferation marker KI67. There are many studies from the various states of the country reporting on the frequency and distribution of malignant lymphomas. Majority of them are from tertiary referral centers.[8-13]

Literature search reveals that the subtype distribution of malignant lymphomas varies

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among countries and also across the geographic regions within the countries, the reason for which remains largely unknown. Mondal *et al*. had reported the frequency of follicular and mantle cell lymphoma to be lower when compared to the Western population.[8] Among HLs, mixed cellularity (MC) was the most common subtype reported by them.[8] DLBCL was the most common B-NHL in most of the Indian studies.[8-13] Arora *et al*. had found the frequency of peripheral T-cell/NK-cell lymphoma to be higher than Western countries but less than that of the other Asian countries.[9] They had reported peripheral T-cell lymphoma, NOS (PTCL NOS) as the most common T-NHL in contrast to other Indian studies,[10] while Devi *et al*. had reported lymphoblastic lymphoma (LL) as the most common subtype of T-NHL.[12] Sandhu *et al*.[11] and Sharma *et al*.[12] had found that the incidence of follicular and mantle cell lymphoma was lower in India than the USA and Europe. T-cell LL and anaplastic large T/null cell lymphomas are more prevalent in India according to the study by Naresh *et al*.[13]

The study was undertaken with the aim of analyzing the subtype distribution of lymphomas in our population using IHC according to the World Health Organization (WHO) 2017 Classification in the patients visiting the medical college hospital. As a secondary aim, this study also analyzed the immunohistochemical profile of various lymphomas.

Subjects and Methods

Between August 2011 and August 2013, 50 consecutive cases diagnosed as lymphoma in the histopathology department in a medical college hospital were included in the study. The study was approved by the Institutional Human Ethics committee. Patients of all ages and either gender with both primary and extranodal lymphomas (treatment naive) were included in the study. For histopathological and immunohistochemical studies, the tumor samples were fixed in 10% neutral buffered formalin, and after tissue processing, they were embedded in a paraffin block. The diagnosis was made by histopathological examination using hematoxylin and eosin. For immunohistochemical studies, 4-μm thick sections were taken in specially coated slides using chrome alum and gelatin. Antigen retrieval was done by microwaving. Various panels of markers were chosen based on histological diagnosis. They include CD20, CD3, CD45, CD15, CD30 CD5, CD10, CD23, BCL2, CYCLIN D1, KI67, TDT, and ALK. These antigens are bound with mouse monoclonal antibody (BioGenex) and then detected by the addition of secondary antibody conjugated with horseradish peroxidase polymer and diaminobenzidine substrate. The slides were counterstained with hematoxylin and mounted.

NHL was further categorized morphologically as small-, medium-, or large-cell lymphomas. Accordingly, the panel of markers was chosen as follows.

For HL – CD45, CD20, CD15, CD30, and CD 3.

For NHL small cell type – CD20, CD5, CD23, CD10, CYCLIN D1, and BCL2.

For NHL medium cell type – CD20, CD3, CD5, CD23, CD10, CYCLIN D1, TDT, KI67, and BCL2.

For NHL large cell type – CD20, CD3, CD5, CD23, CD10, CYCLIN D1, TDT, KI67, CD30, ALK, and BCL2.

All the cases were interpreted by experienced pathologists in the field of lymphoma diagnosis.

Results

During the study period, there were 50 cases of lymphomas which met the inclusion and exclusion criteria. Two cases in which there was no material for IHC workup were excluded from the study. Forty-one cases (82%) were diagnosed with NHL and 9 cases (18%) were diagnosed with HL.

Of the 41 cases of NHL, 61% (n = 25) were male and 39% (n = 16) were female. Of the nine cases of HL, 55.6% (n = 5) were male and 44.4% (n = 4) were female. In both the groups, males outnumbered females (male-to-female ratio being 1.5:1). About 44% (n = 22) cases were in the age group of 41–60 years, 32% (n = 16) were >60 years, and 24% (n = 12) were <40 years.

Nodal lymphomas (88%, n = 44) were more common than extranodal lymphomas (12%, n = 6). The extranodal sites included the gastrointestinal tract (n = 2), pleura, pericardium, skin, and breast (one each). Cervical lymphadenopathy (n = 26, 59.2%) was the common presentation among the nodal group [Table 1].

B-NHL formed 78.5% (32 cases), while T-cell NHL formed 21.5% (9 cases).

Based on the pattern and cellular composition of lymphoma, the lymphomas are classified into small-cell, medium–cell, and large cell type and various panels of immunohistochemical markers were used. The expression of various immunohistochemical markers is tabulated in Table 2.

Small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (n = 11,34.4%) was the most common B-NHL followed by diffuse large B-cell lymphoma (DLBCL NOS) (31.5%, n = 10), follicular lymphoma (FL) (25%, n = 8), and Burkitt's lymphoma (BL) constituting 9.4% (n = 3).

Among the 11 cases of SLL/CLL, peripheral smear findings and bone marrow aspiration studies were available in eight cases. Five cases of SLL/CLL showed bone marrow infiltration, three of which had diffuse infiltration and the remaining two had nodular infiltration. Only three of them showed features of CLL.

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Table 1: Clinical profile of non-Hodgkin lymphoma

Diagnosis	Number of cases	Nodal/extranodal	Bone marrow involvement (number of cases)
B-NHL			
SLL	11	Nodal	5
DLBCL	9	8 nodal and one extranodal (Jejunum)	-
FL	9	Nodal	3
BL	3	Extranodal	-
T-NHL			
T -lymbhoblastic lymphoma	1	Extranodal - pericardium	1
Peripheral T cell lymphoma, NOS	6	5 nodal and 1 extranodal (skin)	-
Angioimmunoblastic T cell lymphoma	2	Nodal	-

NHL: Non-Hodgkin lymphoma, NOS: Not otherwise specified, FL: Follicular lymphoma, SLL: Small lymphocytic lymphoma, DLBCL: Diffuse large B-cell lymphoma, BL: Burkitt's lymphoma

Table 2: Immunohistochemical profile of B non-Hodgkin lymphoma

Subtype of NHL	Positive markers	Negative markers	Other findings
SLL	CD5, CD23	CD3, CD10, Cyclin D1, Bcl2	Weak membranous expression of CD20
FL	CD10, CD20, BCL2, Ki67 variable	CD3, Cyclin D1 and CD5	5 were CD5 positive. 2 cases of Grade III FL showed decreased BCL2 expression
DLBCL	CD 20, CD79a, CD10	BCL2, CD3, ALK	
Burkitt lymphoma	CD20, CD10, KI67 95-100%	CD3, BCL2, TdT	

SLL: Small lymphocytic lymphoma, DLBCL: Diffuse large B-cell lymphoma, FL: Follicular lymphoma, ALK: Anaplastic lymphoma kinase, NHL: Non-Hodgkin lymphoma

Of the nine cases of FL, one was Grade III. The case showed a predominant follicular pattern (85%–90%), with a diffuse area (10%–15%) that was centrocyte rich. There were no sheets of centroblasts in the diffuse areas, and hence, the case was diagnosed as FL Grade III A. Six cases were of Grade II morphology, while the other belonged to Grade I category. Bone marrow involvement was seen in three cases of FL (two were Grade II and the other was Grade III A).

Of the ten cases of DLBCL NOS, nine cases presented with cervical lymphadenopathy, while one case presented as an intestinal mass. Morphological variants included seven cases of centroblastic type, two cases of anaplastic, and one case of immunoblastic category. None of them showed bone marrow involvement at diagnosis.

All the three cases of BL were extranodal lymphomas, of which one case presented with intussusception in a 3-year old child. There was also a rare case of BL presented with breast mass.

PTCL-NOS (14.1%, n = 6) was the most frequent T-cell NHL, followed by angioimmunoblastic T-cell lymphoma (AITL) (4.8%, n = 2), T-LL constituting 2.4% (n = 1). All except one case of PTCL NOS presented with nodal enlargement. LL presented with a pericardial mass.

Among HLs, MC formed 55.6% (n = 5), lymphocyte-depleted type (LD) formed 22.2% (n = 2), and nodular lymphocyte predominant HL (NLPHL) and lymphocyte rich (LR) type each constituted 11.1% (n = 1). The age of the patients ranged from 19 to 78 years. All the nine patients were seronegative for HIV. Both the cases of HL, LD, occurred in elderly males.

Immunohistochemical analysis

All the 11 cases of SLL/CLL were CD5 and CD23 positive with weak expression of CD20 [Figure 1].

Of the 10 cases of DLBCL, CD10 positivity was noted in 5(50%) cases [Figure 2]. All the cases showed diffuse expression of CD20 and CD79a. Ki67 index varied from 76 to 90%.

All the cases of FL expressed CD10, BCL2, and CD20. Grade III FL showed less intense CD10 expression. Three of the nine cases of FL showed positivity for CD5.

All the cases of T-NHL expressed CD3. Ki67 was a variable ranging from 20% to 90%.

Eight cases of classic HL expressed CD30, of which five cases also expressed CD15 [Figure 3].

One case of NLPHL was positive for CD20 and negative for both CD15 and CD30.The background polymorphous cells showed a variable expression of CD3 and CD20.

Discussion

Geographic variations in the incidence as well as distribution of histological subtypes of lymphoma are

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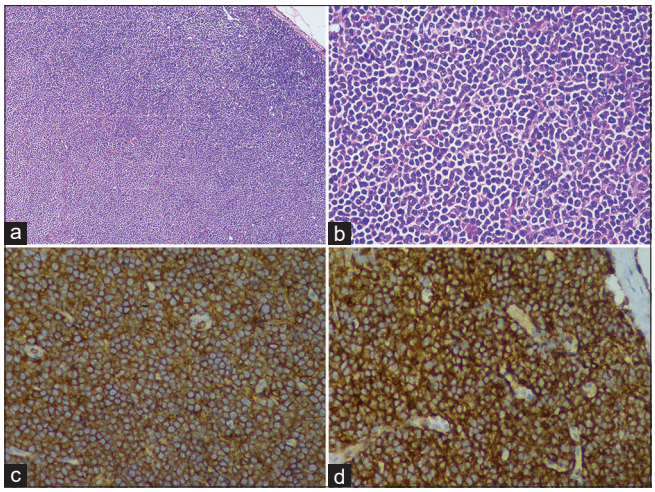


Figure 1: (a) Small lymphocytic lymphoma/chronic lymphocytic leukemia sheets of small lymphoid cells replacing lymph node (H and E, ×10). (b) Cells with scant cytoplasm and coarse chromatin of nucleus (H and E, ×40). (c) Cells expressing CD5 (Immunohistochemistry, ×40). (d) Cells expressing CD23 (Immunohistochemistry, ×40)

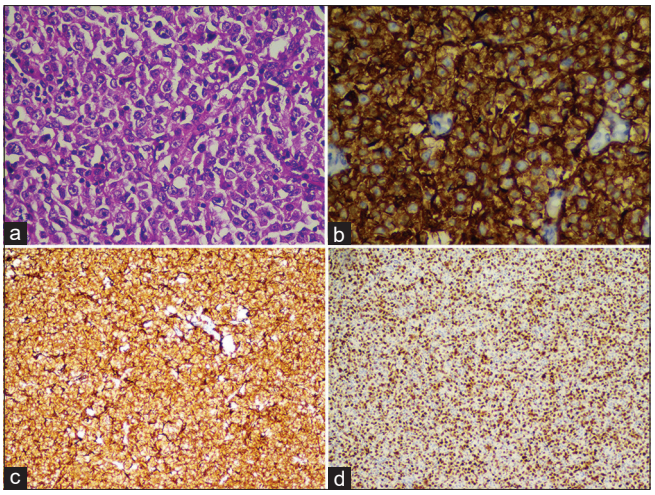


Figure 2: (a) Diffuse large B-cell lymphoma – Sheets of large cells with prominent nucleoli, (H and E, ×40). (b) Large cells with strong membranous expression of CD20 (Immunohistochemistry, ×40). (c) Large cells expressing CD10 (Immunohistochemistry, ×10). (d) High proliferation index with Ki67 staining (Immunohistochemistry, ×10)

well-documented. A higher rate of nasal T-cell lymphomas has been reported in China. BL occurs in endemic form in Africa. Gastric lymphomas and adult T-cell leukemias are reported to be more frequent in Italy and Japan, respectively.[1] The incidence rates for NHL in India are quite high in urban cities when compared to rural centers.[1] The incidence rate is one-quarter of what is reported in America and Europe.[1]

The male-to-female ratio is 1.6 in Asia compared to 1.2 and 1.1 in North America and Europe, respectively. The present study also reports a male preponderance. The median age was 50 years which is higher when compared to Eastern India in which the median age was 39.4 years.[8]

The ratio of NHL to HL was 4.5:1 which is higher when compared to another South Indian study which had reported the ratio of NHL to HL to be 3.6:1.[9]

In line with most other studies in India, the most common mode of presentation in the present study was lymphadenopathy constituting to 86%.[8-11] The most commonly involved lymph node groups were cervical (n = 22,59.5%) followed by inguinal (18.2%, n = 8) and axillary (11.3%, n = 5), Which is similar to that reported by Devi *et al*.[11] Extranodal presentation was seen in 14% of cases in the present study, the frequency of which was much lower than that reported in Northern and Eastern India (44.2 and 27.7%, respectively).[10,11] This could be attributed to the smaller sample size of the present study.

The extranodal lymphomas in the present study were exclusively Non-Hodgkin's lymphomas. The predominant subtype was BL which constituted for about 50%, which is in contrast to the other regions of India and other Asian countries where DLBCL was the most frequent subtype.[11-13] The gastrointestinal tract was the most common site for extranodal lymphomas which is consistent with other Indian studies.[12-14] [Table 3].

Bone marrow involvement was seen in 22% (n = 9) of NHL. The reported percentage varied from 20 to 40% in various western studies and 30.3%–63% in Indian studies.[8-10] Bone marrow biopsy studies were available only in 28 cases.

B-cell non-Hodgkin lymphoma

The ratio between B-cell and T-cell NHL was 3.5:1. SLL formed the major proportion of B-cell NHL constituting 34.4% in contrast to most other studies where DLBCL NOS was the most frequent lymphoma.[15-17] This could be possibly due to higher number of elderly patients found in our study. Extensive literature search did not find any Indian study reporting SLL/CLL to be more frequent.[11-17] Currently, available studies on the distribution of lymphomas are from tertiary referral centers where aggressive lymphomas are likely to be referred. Our study population being the patients attending a teaching hospital, they can show a distribution much different from those studies. However, one cancer registry-based European study done by Marcos *et al*., which had analyzed 5-year survival rate in patients with lymphoid neoplasms, had observed a greater number of SLL/CLL cases during their study period of 7 years.[18] All the 11 cases of SLL showed weak expression of CD20 similar to that observed by Veronika *et al*. in which it was shown that of all mature B-cell neoplasms, SLL/CLL has the dim expression of CD20.[17]

SLL/CLL was followed in frequency by DLBCL constituting to 28%, which is in contrast to majority of other Indian studies reporting the incidence to be between 30% and 58%.[10-14,17] However, Roy *et al*. and Kalyan

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Table 3: Comparison of distribution of non-Hodgkin lymphoma among various zones of India

Lymphoma characteristics	Eastern India Mondal *et al*. (*n*=455), *n* (%)	Punjab Manisha *et al*. (*n*=47), *n* (%)	North East DeviAA *et al*. (*n*=100), *n* (%)	Mumbai Naresh *et al*. (*n*=2773), *n* (%)	Present study (*n*=50), *n* (%)
Male-to-female ratio	3.2:1	1.5:1	1.2:1	-	1.5:1
Median age (years)	39.4	40	54	-	50
B -NHL	257 (74.1)	42 (89.3)	66 (66.0)	79.1	32 (78.5)
T-NHL	90 (25.9)	5 (10.7)	23 (23.0)	15.2	9 (21.5)
SLL	19 (5.5)	8 (17)	0	155 (5.6)	11 (26.8)
FL	67 (19.3)	3 (6.4)	5	316 (11.4)	9 (21.9)
DLBCL	122 (35.2)	22 (46.8)	45	937 (33.8)	9 (21.9)
BL	20 (5.8)	0	6	50 (1.8)	3 (7.3)
PTCL-U	6 (1.7)	0	0	53 (1.9)	6 (14.6)
AITL	5 (1.4)	1 (2.1)	5	28 (1.0)	2 (4.8)
T-ALL	30 (8.6)	3 (6.4)	0	168 (6.0)	1 (2.4)

NHL: Non-Hodgkin lymphoma, AITL: Angioimmunoblastic T-cell lymphoma, PTCL: Peripheral T-cell lymphoma, BL: Burkitt's lymphoma, DLBCL: Diffuse large B-cell lymphoma, FL: Follicular lymphoma, SLL: Small lymphocytic lymphoma, T-ALL: T-cell acute lymphoblastic leukemia

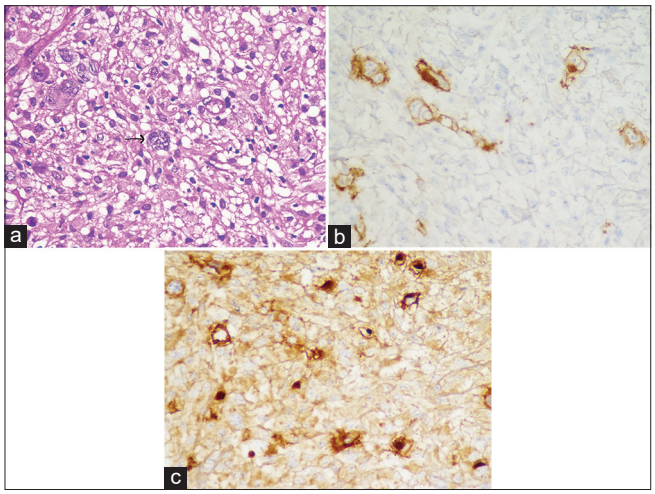


Figure 3: (a) Pleomorphic Reed–Sternberg cells (H and E, ×40). (b) RS cells expressing CD30 (Immunohistochemistry, ×10). (c) RS cells expressing CD15 (Immunohistochemistry, ×10)

et al. had reported the incidence to be 29.3% and 26%, respectively,[19,20] which were not much different from the present study.

DLBCL NOS was the most common of aggressive NHL observed in the current study. The most common mode of presentation was cervical lymphadenopathy except one which presented with an intestinal lymphoma. None of them showed any bone marrow involvement at diagnosis. In contrast, bone marrow involvement had been reported to be varying from 4 to 20% in the Indian population.[21] CD10 positivity was noted in 5 (50%) cases which is similar to that reported by Diwivedi *et al*.[22]

Among the nine cases of FLs, we observed five of them to express CD5 constituting to 21% of CD5-positive lymphomas in our study which was similar to the observation made by Dong *et al*., in which FL accounted for 24% of cases positive for both CD5 and CD10.[23] The case of Grade III FL showed decreased expression of BCL2 which is similar to the other studies.[24,25]

All the three cases of BL were extranodal lymphomas, of which one case presented with intussusception in a 3-year-old child. The presentation was similar to other studies which had shown abdomen, particularly the ileocecal region as the most common site of involvement.[14] We also found an unusual presentation of BL with breast lump in a 25-year-old female. Histology showed that the typical morphology of BL and Ki67 proliferation index was 97%. Primary BL of the breast is much less common than the other types of lymphoma. Primary breast lymphomas are extremely rare, and the most common histologic type is DLBCL.[26]

T-cell non-Hodgkin lymphoma

T-NHL constituted for 24% (*n* = 9) which is similar to other Indian studies.[11-17,27] The frequency of T-NHL is higher in India when compared to Western countries but less than what is reported in other East Asian countries. PTCL NOS was the most common subtype of T-NHL in the present study similar to that reported by Arora *et al*. and Nair *et al*.[9,28] Sharma *et al*. had reported T-LL to be more common followed by anaplastic large cell lymphoma (ALCL) and AITL accounting for 6.4, 2.1, and 2.1% of all NHL, respectively.[27] Naresh *et al*. had reported the incidence of T-LL and ALCL to be 6% and 4.3%, respectively.[13] Although ALCL was the most common PTCL in Eastern India, it was not identified in our study, the reason for which is unknown.

We also found a rare variant of PTCL, Lennert's lymphoma in a patient who presented with bilateral cervical lymphadenopathy. Morphologically, it showed microgranulomas typical of the lymphoepithelioid lymphoma. All the neoplastic cells expressed CD3.

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Hodgkin lymphoma

HL included eight cases of classic HL and one case of NLPHL HL. The mean age of presentation in HL was 34 years which is almost similar to studies in South and Eastern India where the mean age reported was 33 and 31.3 years, respectively.[8,9] All the cases of HL presented with peripheral lymphadenopathy. Extranodal presentation and bone marrow involvement were not seen in any of these cases. The other studies in India too have not reported any extranodal presentation. In contrast, bone marrow involvement was reported to be 14.3% in HL.[8,9]

The most common subtype found in the study was MC (n = 5, 55.6%), followed by LD. Reed–Sternberg cells in LD HL showed strong expression of both CD15 and CD30 [Figure 2]. Other Indian studies had also reported MC to be the most common subtype of HL, followed by nodular sclerosis subtype, which is in contrast to Western countries where NS is much more frequent than other subtypes.[29,30] LD subtype was found in elderly adults.

One case each of LR and NLPHL HL were identified in this study. CD15 expression was seen in 5 (62.1%) of eight cases of classic HL. This is slightly higher than reported in a study which has reported that 49.11% of the cases expressed CD15.[29]

Limitations

One of the limitations of the study is a smaller sample size, yet there was a significant difference in subtype distribution. Follow-up data were not available to assess the value of prognostic markers used in the IHC panel.

Conclusion

The distribution of lymphoma subtypes differs from other regions of India. SLL/CLL was the most common NHL reported in our study, followed by DLBCL which differs from other studies. About 21% of FL expressed CD5 and CD10. PTCL NOS was the most common T-NHL in the study. MC was the most frequent HL. CD15 expression was more frequent in HL. IHC is an essential diagnostic aid in categorization. Further replication studies including larger samples are essential to understand the difference in subtype distribution in our population.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Nair R, Arora N, Mallath MK. Epidemiology of non-Hodgkin's lymphoma in India. Oncology 2016;91 Suppl 1:18-25.
2. Boffetta P. Epidemiology of adult non-Hodgkin lymphoma. Ann Oncol 2011;22:27-31.
3. Yeole BB. Trends in the incidence of non-Hodgkin's lymphoma in India. Asian Pac J Cancer Prev 2008;9:433-6.
4. Parkin D M, Bray F, Ferlay J. Global cancer statistics. Cancer J Clin 2005;55:74-108.
5. Morton LM. Dissecting lymphoma incidence to inform epidemiologic and clinical research. Leuk Lymphoma 2013;54:1575-6.
6. Chan JK. The new World Health Organization classification of lymphomas: The past, the present and the future. Hematol Oncol 2001;19:129-50.
7. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pilleri SA, Stein H, *et al*.., editors. World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissue. Lyon: IARC Press; 2008.
8. Mondal SK, Mandal PK, Samanta TK, Chakaborty S, Roy SD, Roy S. Malignant lymphoma in Eastern India: A retrospective analysis of 455 cases according to World Health Organization classification. Indian J Med Paediatr Oncol 2013;34:242-6.
9. Arora N, Manipadam MT, Nair S. Frequency and distribution of lymphoma types in a tertiary care hospital in South India: Analysis of 5115 cases using the World Health Organization 2008 classification and comparison with world literature. Leuk Lymphoma 2013;54:1004-11.
10. Sandhu DS, Sharma A, Kumar L. Non-Hodgkin's lymphoma in Northern India: An analysis of clinical features of 241 cases. Indian J Med Paediatr Oncol 2018;39:42-5.
11. Devi AA, Sharma TD, Singh YI, Sonia H. Clinicopathological profile of patients with non-hodgkin's lymphoma at a regional cancer center in Northeast India. J Sci Soc 2017;44:140-4.
12. Sharma M, Mannan R, Madhukar M, Navani S, Manjari M, Bhasin TS, *et al*. Immunohistochemical (IHC) analysis of non-Hodgkin's Lymphoma (NHL) spectrum according to WHO/REAL classification: A single centre experience from Punjab, India. J Clin Diagn Res 2014;8:46-9.
13. Naresh KN, Srinivas V, Soman CS. Distribution of various subtypes of nonHodgkin's lymphoma in India: A study of 2773 lymphomas using R.E.A.L. And WHO classifications. Ann Oncol 2000;11:637.
14. Chakrabarti S, Sarkar S, Goswami BK, Mondal S, Roy A, Das S. Hodgkin's and Non-Hodgkin's lymphomas in an Indian rural medical institution: Comparative clinicopathologic analysis. Asian Pac J Cancer Prev 2010;11:1605-8.
15. Mushtaq S, Akhtar N, Jamal S, Mamoon N, Khadim T, Sarfaraz T, *et al*. Malignant lymphomas in Pakistan according to the WHO classification of lymphoid neoplasms. Asian Pac J Cancer Prev 2008;9:229-32.
16. Prakash G, Sharma A, Raina V, Kumar L, Sharma MC, Mohanti BK, *et al*. B cell non-Hodgkin's lymphoma: Experience from a tertiary care cancer center. Ann Hematol 2012;91:160311.
17. Prevodnik VK, Lavrenčak J, Horvat M, Novakovič BJ. The predictive significance of CD20 expression in B-cell lymphomas. Diagn Pathol 2011;6:33.
18. Gragera MR, Allemani C, Tereanu C, De Angelis R, Capocaccia R, Maynadie M, *et al*. HAEMACARE Working Group (2011) Survival of European patients diagnosed with lymphoid neoplasms in 2000-2002: Results of the HAEMACARE project. Haematologica 2011;96:720-8.
19. Roy A, Kar R, Basu D, Badhe BA. Spectrum of histopathologic diagnosis of lymph node biopsies: A descriptive study from a tertiary care center in South India over 5½ years. Indian J Pathol Microbiol 2013;56:103-8.
20. Kalyan K, Basu D, Soundararaghavan J. Immunohistochemical typing of non-Hodgkin's lymphoma-comparing working

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formulation and WHO classification. Indian J Pathol Microbiol 2006;49:203-7.
21. Babu SM, Garg S, Kanakasetty GB, Kuntegowdanahalli LC, Dasappa L, Rao SA. Diffuse large B-cell lymphoma: A retrospective study from a regional care center in South India. Indian J Cancer 2018;55:66-9.
22. Dwivedi A, Mehta A, Solanki P. Evaluation of immunohistochemical subtypes in diffuse large B-cell lymphoma and its impact on survival. Indian J Pathol Microbiol 2015;58:453-8.
23. Dong HY, Gorczyca W, Liu Z, Tsang P, Wu CD, Cohen P, *et al*. B-cell lymphomas with coexpression of CD5 and CD10. Am J Clin Pathol 2003;119:218-30.
24. Rao IS. Role of immunohistochemistry in lymphoma. Indian J Med Paediatr Oncol 2010;31:145-7.
25. Higgins RA, Blankenship JE, Kinney MC. Application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. Arch Pathol Lab Med 2008;132:441-61.
26. Janbabai G, Kayedimajd S, Alian S, Naghshvar F, Rashidi M, Farazmandfar T. Bilateral breast swelling in a 23-year-old woman with Burkitt lymphoma. J Res Med Sci 2012;17:1188-91.
27. Lakshmanan A, Sikri D, Patil S, Kurian A, Annapurneswari S, Nair S. Frequency, distribution, and immunomorphologic characteristics of peripheral T-cell lymphoma, not otherwise specified in a tertiary care center in Southern India. Indian J Pathol Microbiol 2018;61:204-8.
28. Nair RA, Vasudevan JA, Jacob PM, Sukumaran R. Profi ling of peripheralT-cell lymphomas in Kerala, South India: A 5-year study. Indian J Pathol Microbiol 2017;60:206-8.
29. Bidyut Krishna Goswami BK, Sarkar S, Chakrabarti S, Mondal S, Roy A, Deb AR. Clinico-pathologic profile of Hodgkin's lymphoma in a rural medical college. Indian J. Hematol. Blood Transfus 2008;24:166-9.
30. Patkar N, Mehta J, Kulkarni B, Pande R, Advani S, Borges A. Immunoprofile of Hodgkin's lymphoma in India. Indian J Cancer 2008;45:59-63.

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Original Article

Pathology Section

Histomorphological Spectrum of Mediastinal Masses with Special Emphasis on Rare Lesions

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ABSTRACT

Introduction: Mediastinal lesions are not frequently observed in routine clinical practice. A wide variety of disease process can occur within the mediastinum. Tissue from needle biopsies forms the major diagnostic material. There is need for detailed studies to make pathologists aware of the wide histological spectrum and to know the unusual lesions that can be encountered during diagnosis.

Aim: To describe the histomorphological spectrum of various mediastinal lesions and to highlight the rare lesions that posed a diagnostic challenge.

Materials and Methods: This was a retrospective study done on all mediastinal masses over a period of two years from January 2016 to December 2018. The clinical data and imaging findings were collected from the test request forms and patients' files for all the cases. Haematoxylin and eosin stained slides of all cases and immunohistochemistry markers and cytochemical stains wherever done were reviewed and analysed. Mean, median and percentage were used for statistical analysis.

Results: Thirty-four cases of mediastinal lesions were included in the study. Of these, 28 cases (82.4%) presented as anterior mediastinal masses, three cases (8.8%) each in the middle and posterior mediastinum. Thymoma (n=15) followed by lymphoma (n=4) were the most frequent neoplasms reported. There were five lesions which were rare in the mediastinum with unusual presentation that posed diagnostic difficulties. These lesions included Primitive neuroectodermal tumour in an adult, Primary Mediastinal B Cell Lymphoma (PMBCL), Malignant Peripheral Nerve Sheath Tumour (MPNST) with nodal metastasis, Seminoma deposits in mediastinal node occurring as late relapse and dedifferentiation of thyroid carcinoma in mediastinal node metastasis without an obvious primary.

Conclusion: Primary mediastinal tumours were more frequent than metastasis. Although thymomas and lymphomas are dealt extensively in literature, this article highlights the fact that pathologists must be aware of those lesions that don't display the classical histological and/or clinical features as observed in our study.

Keywords: Malignant peripheral nerve sheath tumour, Mediastinum, Mediastinal B cell lymphoma, Morphological spectrum, Primitive neuroectodermal tumour

INTRODUCTION

Primary mediastinal tumours are rare. Anterior mediastinal masses constitute >50% of mediastinal lesions and majority of them involve thymus. Middle mediastinal lesions include congenital cyst, (pericardial and bronchogenic cyst) while neurogenic tumours form the majority of posterior mediastinal masses [1-3]. Lymphomas can arise in any of these three compartments [4]. Aggarwal R et al., in their study of mediastinal lesions have reported 70% of lesions confined to anterior mediastinum, of which thymic lesions were the most frequent, constituting to 45.7% [1]. Posterior, superior and middle mediastinal lesions were reported to be 19, 6 and 4%, respectively. Lymphomas were the second most common tumour (11.2%) after thymoma. Sarcoma was seen in 6.9% cases and showed random compartment localisation. In their study, primary lesions were more common when compared to secondary deposits.

Dixit R et al., in their evaluation of 144 cases of mediastinal lesions have reported metastasis to be the most common, contributing to 37.4% of malignant lesions followed by lymphoma [5]. The clinical findings with radiological findings can narrow the differential diagnosis. However, advent of new techniques has made it easier to obtain tissue material from this site [5,6]. Hence, it is important for a pathologist to be familiar with the wide variety of lesions in the mediastinum since an accurate early tissue diagnosis directs therapy. There are variations in the existing literature regarding the frequency of individual lesions encountered in mediastinum. Hence the study was undertaken to analyse the histopathological spectrum of mediastinal lesions in our population and to highlight the rare lesions.

MATERIALS AND METHODS

This was a retrospective study done on all mediastinal masses over a period of two years from January 2016 to December 2018. The data was retrieved from the archives of Pathology department of PSG Institute of Medical Sciences and Research. Institutional Human ethics committee clearance was obtained before conducting the study (vide letter 20/108).

Needle biopsies and surgical resections were included in the study. A total of 36 cases were received during the study period. Of these cases, two cases showed only necrosis and hence were excluded from the study. The remaining 34 cases were included.

Relevant demographic and clinical data including the presenting symptoms, past history relevant to the lesions and examination findings was collected from patient files in all the cases. Laboratory investigations such as serum markers like carcinoembryonic antigen, CA125, alpha fetoprotein wherever available were obtained from test request forms and hospital information system. Computed Tomography findings including the location, size and solid or cystic nature of the lesion were recorded. Haematoxylin and eosin stained slides of all cases and Immunohistochemistry (IHC) markers and cytochemical stains like periodic acid, Ziehl Neelsen wherever done were reviewed and analysed. Immunohistochemical markers included cytokeratin (Mouse, polyclonal, AE1/AE3), CD99 (Mouse, 12E7) S100 (rabbit, polyclonal, 504),Thyroid transcription factor (Mouse, 056), napsin (EP205), CD45 (Mouse, EP273), CD3 (rabbit, polyclonal, 503), CD20 (Mouse, L26), CD15 (Mouse, 273), CD30 (Mouse, BER-H2), CD117 (IB 077) and PLAP (mouse, 376). IHC was done on paraffin embedded tissue sections using heat induced antigen

retrieval. Diaminobenzidene was the chromogen used. The cases which posed diagnostic challenge or had an unusual presentation were extensively studied. Mean, median and percentage were used for statistical analysis. The analysis was done using SPSS software version 20.0.

RESULTS

Thirty four cases of mediastinal lesions were included in the study. Age of the patients ranged from 14 to 75 years. Majority of the lesions were in females with male to female ratio being 1:1.5. The presenting symptoms included chest pain, cough and breathlessness in 26 cases. A total of 24 cases were the Primary mediastinal tumours while metastatic lesions were two in number. About Twenty eight (82.4%) cases presented as anterior mediastinal masses, and three cases each in the middle and posterior mediastinum. The majority of lesions were involving the thymus (n=21) constituting to 67.8% [Table/Fig-1]. The frequency of malignancy was higher in females (6 cases).

S. No.	Diagnostic category	Anterior 82.4% (n=28)	Middle 8.8% (n=3)	Posterior 8.8% (n=3)	Total % (n)
1	Non-neoplastic				
	Retrosternal goitre	1		1	23.5 (8)
	Sarcoidosis	1	1		
	Thymic cyst	2			
	Thymolipoma	2			
2	Neoplastic, Benign				
	Thymoma	15			47 (16)
	Teratoma		1		
3	Neoplastic, Malignant				
	Thymic carcinoma	2			
	Lymphoma	3	1		23.5 (8)
	Primitive neuroectodermal tumour			1	
	Malignant peripheral nerve sheath tumour			1	
4	Neoplastic, metastatic	2			6 (2)

[Table/Fig-1]: Distribution of mediastinal lesions.

The variants of thymoma included four cases each of type A, B1 and B2 and three cases of type AB thymoma. Both the cases of thymic carcinoma occurred in females, one of which occurred in a young female (age 28 years).

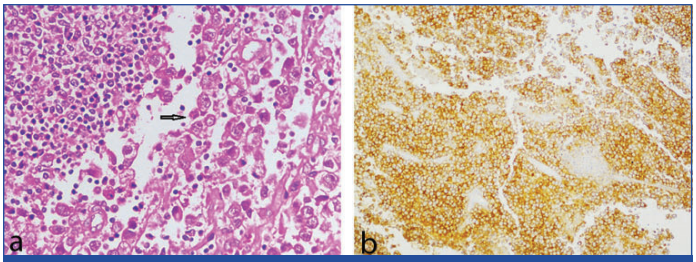
The two cases of Non-Hodgkin Lymphoma (NHL) reported were precursor T-lymphoblastic lymphoma in adolescent patients. The other NHL was Primary Mediastinal B Cell Lymphoma (PMBCL). The two cases of Hodgkin Lymphoma (HL) included one case each of Nodular Sclerosis and Mixed cellularity HL.

Of the 34 cases, 13 (38.2%) were diagnosed on guided biopsies and 21 (61.8%) on excision biopsies. In all the guided biopsies, a confirmatory diagnosis was made only after immunohistochemistry.

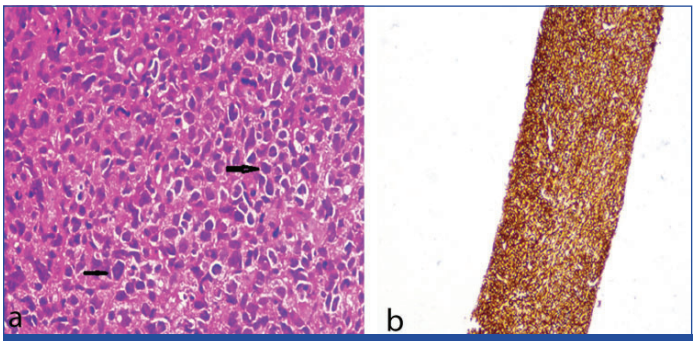
The rare tumours encountered are described below [Table/Fig-2]. Case 1 presented with multiple lymph nodes involvement suggesting the possibility of lymphoma. Seminoma relapse was not initially considered for this case. The diagnosis of seminoma metastasizing to lymph node was made only with the help of immunohistochemistry [Table/Fig-3]. Case 2 (PMBCL) was a rare lesion occurring in a young male, but had the typical presentation. Differential diagnosis entertained in this case were PNET and thymoma based on the morphology [Table/Fig-4]. Case 3 (MPNST) occurred in a patient with Neurofibromatosis. Cytology of pleural fluid also showed atypical cells. Based on the morphology, a provisional diagnosis of high-grade sarcoma was made. The diagnosis of MPNST with nodal metastasis was made with immunohistochemistry (S100 positive and Ki67 90%) [Table/Fig-5]. Case 4 (PNET/Ewing's sarcoma) occurred in an adult female (65 years) which is rare [Table/Fig-6]. Case 5 (dedifferentiated thyroid carcinoma in mediastinal nodes) occurred in an adult female who did not have any history of thyroidectomy. Histopathological examination showed an anaplastic tumour suggesting the possibility of sarcoma/carcinoma. Extensive sampling revealed tiny foci of conventional papillary thyroid carcinoma which was supported by IHC markers (Thyroid transcription factor positive, Napsin negative, Cytokeratin positive in anaplastic areas). Lung carcinoma was ruled out by napsin negativity [Table/Fig-7]. Normal thyroid parenchyma was not identified in the tissue and thus the possibility of aberrant

Case No.	Clinical details	Imaging findings (CT Scan)	Histology and IHC	Diagnosis and unusual finding
1	48/M, operated for testicular seminoma 5 years back and had defaulted surveillance	Hypodense, soft tissue mass of size 5.5×2.9 cm in anterior mediastinum. Mediastinal lymphadenopathy +. Beta HCG-420 mIU/mL LDH-1820IU/L	Lymph node with sheets of large cells having pleomorphic vesicular nuclei and prominent nucleoli separated by fibrous septa rich in lymphocytes. There was no other germ cell tumour elements. CD117 and PLAP positive.	Seminoma metastasing to mediastinal lymph nodes. Late relapse presenting with generalised lymphadenopathy
2.	21/M, anorexia, night sweats, significant loss of weight for two months. No organomegaly or superficial lymphadenopathy	large anterior mediastinal mass of size 6×7 cm invading superior vena cava.	Monomorphous population of blue round cells separated by thick fibrous septa. CD20 and CD45 positive Neuron Specific enolase, Cytokeratin negative	Primary Mediastinal B Cell Lymphoma (PMBCL). Rare lesion (incidence-2 to 3% of NHL) [1]
3.	35/M, known case of Neurofibromatosis presented with a complaint of dry cough associated with breathlessness	Multiple nodules in both lungs and pleura. Multiple enlarged mediastinal, para-aortic, celiac lymph nodes suggesting metastasis	Oval to spindle neoplastic cells exhibiting marked pleomorphism arranged in sheets. Vimentin, S100 positive. Ki67 index-90% Desmin and CD34 negative	MPNST with nodal metastasis. Rare presentation
4	65/F, chest pain, breathlessness and fever	intrathoracic mass measuring 15×12 cm extending into posterior mediastinum, encasing and lifting aorta, inferior vena cava and causing diaphragmatic inversion.	Small round cells with scant cytoplasm, uniform hyperchromatic nuclei with perivascular accentuation and surrounded by fibrous septa. Differential diagnoses entertained were lymphoma, Ewing's sarcoma and rhabdomyosarcoma. CD99 positive, CD45, Tdt and Myogenin negative	PNET/Extraskeletal Ewing's sarcoma in adult. (incidence -1.8%) [27]
5	69/F, chest pain, breathlessness and intermittent fever and inspiratory stridor	Large heterogeneously enhancing mass measuring 5×4×5 cm in the right upper posterior mediastinum encasing the esophagus in the left lateral aspect. Few small nodules in the right lung suggestive of metastasis were seen. A small nodule was noted in the thyroid. Thyroidectomy could not be done in this patient.	An anaplastic tumour composed of multinucleated giant cells and highly pleomorphic cells with extensive areas of coagulative necrosis. Tiny foci of conventional papillary thyroid carcinoma. No normal thyroid tissue identified. (strong nuclear expression of TTF1 in conventional papillary thyroid carcinoma. The anaplastic component was positive for Cytokeratin and lacked TTF 1 expression. Napsin-negative (rules out lung carcinoma)	Dedifferentiated thyroid carcinoma metastasis with no obvious lesion in thyroid.

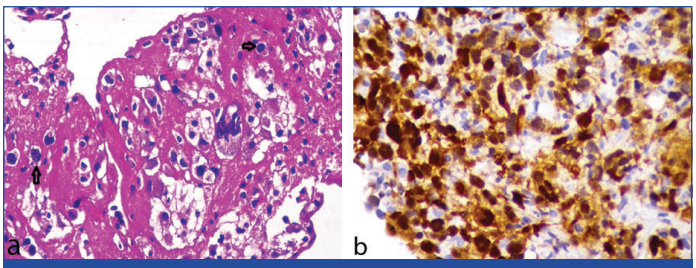
[Table/Fig-2]: Unusual features of the reported rare cases.
MPNST: Malignant peripheral nerve sheath tumour; PNET: Primitive neuroectodermal tumour



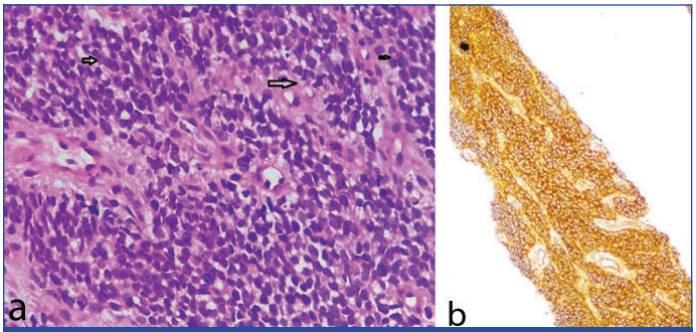
[Table/Fig-3]: a) Large neoplastic cells separated by fibrous septa rich in lymphocytes, Seminoma (H&E,40X). b) Neoplastic cells with strong membranous expression of PLAP (IHC-40X).



[Table/Fig-4]: a) Discohesive pleomorphic large round cells (arrow), PMBCL (H&E, 40X).b) Neoplastic cells with strong membranous CD20 expression (IHC, 10X).



[Table/Fig-5]: a) Pleomorphic tumour cells, MPNST (H&E, 40X). b) Tumour cells expressing S100 (IHC 40X).



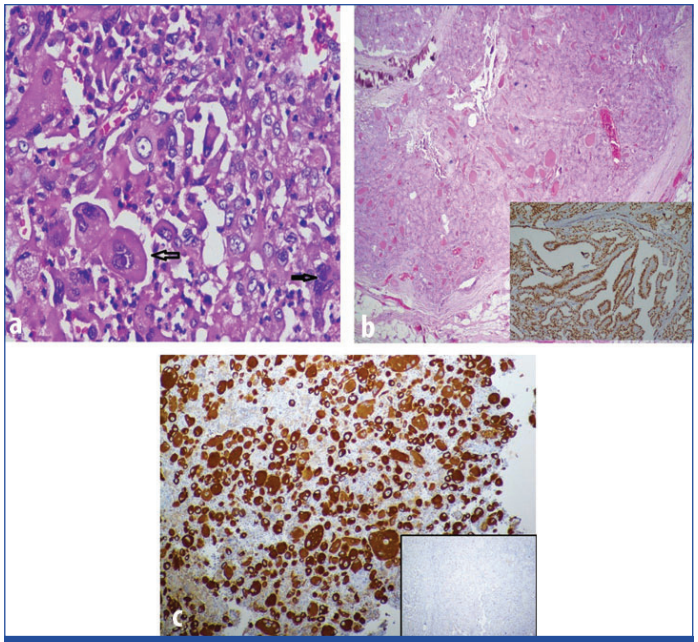
[Table/Fig-6]: a) Small round cell tumour, PNET (H&E, 10X). b) Tumour cells expressing membranous staining for CD99 (IHC 10X).

thyroid and retrosternal extension were ruled out. Small nodule identified in thyroid by Computed Tomography of neck could not be evaluated since thyroidectomy could not be done on this patient.

DISCUSSION

In the present study, the majority of the tumours were primary in origin (88.2%) except for two cases. Literature review also showed that primary mediastinal lesions are more common than metastatic malignancies [3,4,6-9]. There was a female predominance in the current study which contrasts with the previous studies [5-9].

In keeping with other studies [3-10], most of the lesions were in the anterior compartment (82.4%) and benign lesions (47%) were more frequent than malignant tumours (23.5%). However, there are studies reporting malignant lesions to be more commoner constituting about 60% of lesions [11,12].



[Table/Fig-7]: a) Sheets of pleomorphic cells with multinuleation and hyperchromatic nuclei (H&E- 40X).b) Conventional papillary carcinoma component. (IHC-10X). Inset TTF1 expression (IHC 10X). c) Anaplastic carcinoma with CK expression (IHC-40X). Inset TTF 1 negative (IHC 10X).

Thymoma was the most frequent neoplasm in the present study which is similar to other studies [3-9]. However, Vaziri M et al., in their study had reported lymphomas to be more common than thymoma in a series of 105 cases [11]. In the current study, thymic lesions constituted 61.7% of anterior mediastinal masses which is higher when compared to the studies by Aggarwal R et al., and Dasgupta S et al., [1,3].

Thymic carcinomas are reported to be rare malignancies occurring in the age group of 30 to 60 years. Suster S, in his study have reported a slight male predilection (1.5:1) [13]. In the present study both the cases of thymic carcinoma occurred in females.

Retrosternal goitres are usually located anteriorly, in the superior or anterior mediastinum. Posterior mediastinum is rarely involved. Their incidence in the general population is about 1:5000 [14]. The case described in the study also occurred in posterior mediastinum. In the present study, there were five cases which were either rare or had unusual presentations that posed diagnostic difficulties [Table/Fig-2].

Primary mediastinal Seminomas originating from extragonadal germ cells are rare [15]. In general, the relapse occurs within 2 to 3 years. It has been reported in inguinal lymph nodes, contralateral testis, retroperitoneum, mediastinum and lung [16-18]. The case described here presented with seminoma deposits in mediastinal lymph nodes along with multiple abdominal lymphadenopathy 5 years after orchidectomy. Hence, the possibility of any mediastinal germ cell tumour representing a metastasis from testicular primary should always be investigated. Considering the above facts, this case is unusual since it presented as a late relapse.

PMBCL is a subtype of Diffuse Large B Cell Lymphoma (DLBCL) of thymic B cell origin affecting adolescents and young adults and having a slight female preponderance. Of the cases reported, 75% present with bulky anterior mediastinal mass greater than 10 cm that grows rapidly and nearly always present with superior vena cava obstruction [19]. Bone marrow involvement is reported to be extremely rare. It shares the biological and clinical characteristics with Nodular Sclerosis type of Hodgkin Lymphoma (NSHL) [19]. The characteristic histologic findings include clearing of cytoplasm of cells and alveolar fibrosis. Because of the presence of compartmentalisation of

cells divided by fibrous septa, it mimics thymoma, NSHL and germ cell tumours. Hence, immunohistochemistry becomes an essential aid in diagnosis. All the cases of PMBCL express CD20 and a proportion of cases express CD30. PMBCL has been reported to have better prognosis when compared to DLBCL with secondary mediastinal lymph node involvement [19,20]. The case presented here had the typical presentation of PMBCL with superior vena cava obstruction and absence of superficial lymphadenopathy and organomegaly but its occurrence in a young male is rare.

Intrathoracic presentation of MPNST is rare. There are nearly 20 such cases reported in literature, majority of them in the posterior mediastinum [21]. A 20 -30% of MPNST occur in the setting of Neurofibromatosis. Those cases show an increased recurrence and risk of distant metastasis [22,23]. Lung, bone and pleura are the most common sites of metastasis. Metastasis to lymph nodes is reported in less than 10% of patients and is usually seen as a part of widespread metastasis. In the case presented here, there was mediastinal lymph node metastasis from MPNST. Since these tumours show a weak expression of S100 in majority of cases, it becomes difficult in cases of unusual presentation like nodal metastasis, especially in small biopsies to diagnose [24]. The challenge faced in this case was that the presence of neurofibromatosis in this patient was not available at the time of diagnosis.

PNET is a rare and aggressive tumour of neuroectodermal origin most frequently occurring in children and young adults. There are only a few published series of PNET in adults [25]. Primary mediastinal PNET is very rare constituting 1.8% of mediastinal tumours. In a study by Applebaum MA et al., it was found that Extraskeletal Ewing sarcoma had a higher mean age when compared to those with skeletal tumours [26,27]. Metastasis was frequent in the cases with extraskelatal Ewings sarcoma/ PNET [25,28]. The case presented here also occurred in an adult female (65 years).

PTCs are known for anaplastic transformation at the metastatic sites [29] and have been reported in retroperitoneum, liver and lungs. This has been proven even with papillary microcarcinoma [29,30]. The cases reported previously were postsurgery for papillary carcinoma which occurred as a recurrence, many years after treatment for papillary carcinoma [29]. The case presented here did not have any history of proven PTC nor did the patient present with thyroid mass. This could probably represent an occult papillary microcarcinoma in thyroid with mediastinal metastasis of papillary thyroid carcinoma that has undergone dedifferentiation. The diagnosis of dedifferentiated thyroid carcinoma was not made on the initial sections. This case re-emphasises the need for extensive sampling in a case of anaplastic tumour to look for a well differentiated tumour to accurately diagnose even a remote possibility.

Limitation(s)

The smaller sample size in this study precludes generalisation of study results considering the frequency and distribution of mediastinal tumours. Further studies including larger sample size and studies aiming at analysing correlation with clinical and radiological findings are needed to estimate the subtype distribution of mediastinal lesions.

CONCLUSION(S)

To summarise, benign tumours were more common than malignant ones. Primary mediastinal tumours were more frequent than metastasis. Although thymomas and lymphomas are dealt extensively in literature, this article highlights the fact that pathologists must be aware of those lesions that don't display the classical histological and/or clinical features as observed in our study.

REFERENCES

[1] Aggarwal R, Rao S, Chopra P, Bhalla S, Vijay CL, Asaf BB et al. Morphological spectrum of mediastinal lesions with special emphasis on evaluation of needle biopsy: An experience from a tertiary care hospital. Indian J Med Res. 2016;144(4):544-51.

[2] Shrivastava CP, Devgarha S, Ahlawat V. Mediastinal tumours: A clinicopathological analysis. Asian Cardiovasc Thorac Ann. 2006;14:102-04.

[3] Dasgupta S, Bose D, Bhattacharyya NK, Saha M, Biswas K, Biswas PK. A clinicopathological study of mediastinal masses operated in a tertiary care hospital in Eastern India in 3 years with special reference to thymoma. Indian J Pathol Microbiol. 2016;59:20-24.

[4] Prasanth G, Kapoor A, Bajpai J, Agarwal S, Thulkar S, Kuma L. Mediastinal masses- The bad, the ugly and the unusual. Indian Journal of Medical & Paediatric Oncology. 2007;28:11-16.

[5] Dixit R, Shah NS, Goyal M, Patil CB, Panjabi M, Gupta RC, et al. Diagnostic evaluation of mediastinal lesions: Analysis of 144 cases. Lung India. 2017;34:341-48.

[6] Takeda S, Miyoshi S, Akashi A, Ohta M, Minami M, Okumura M, et al. Clinical spectrum of primary mediastinaltumours: A comparison of adult and pediatric populations at a single Japanese institution. J Surg Oncol. 2003;83:24-30.

[7] Sharma P, Jha V, Kumar N, Kumar R, Mandal A. Clinicopathological analysis of mediastinal masses: a mixed bag of non-neoplastic and neoplastic etiologies. Turk Patoloji Derg. 2017;33(1):37-46.

[8] Duwe BV, Sterman DH, Musani AL. Tumours of the mediastinum. Chest. 2005;128:2893-909.

[9] Karki S, Chalise S. Analysis of mediastinal lesions: A study of 27 cases. J Pathol Nepal. 2011;1:114-17.

[10] Aroor AR, Prakasha SR, Seshadri S, Teerthanath S, Raghuraj U. A study of clinical characteristics of mediastinal mass. J Clin Diagn Res. 2014;8:77-80.

[11] Vaziri M, Pazooki A, Zahedi-Shoolami L. Mediastinal masses: Review of 105 cases. Acta Med Iran. 2009;47:297-300.

[12] Adegboye VO, Ogunseyinde AO, Obajimi MO, Ogunbiyi O, Brimmo AI, Adebo OA. Presentation of primary mediastinal masses in Ibadan. East Afr Med J. 2003;80:484-87.

[13] Suster S. Thymic carcinoma: Update of current diagnostic criteria and histologic types. Semin Diagn Pathol. 2005;22(3):198-212.

[14] Samokhvalova A, Loberantb N, Makhoul N. Posterior mediastinal goiters: Report of two cases and literature reviewRespiratory Medicine Case Reports. 2012;5:65-68.

[15] Ota Y, Iihara K, Ryu T, Morikawa T, Fukayama M. Metastatic seminomas in lymph nodes: CD10 immunoreactivity can be a pitfall of differential diagnosis. Int J Clin Exp Pathol. 2013;6(3):498-502.

[16] Warde P, Specht L, Horwich A, Oliver T, Panzarella T, Gospodarowicz M, et al. Prognostic factors for relapse in stage I seminoma managed by surveillance: A pooled analysis. J Clin Oncol. 2002;20(22):4448-52.

[17] Cummins S, Yau T, Huddart R, Dearnaley D, Horwich A. Surveillance in stage I seminoma patients: A long-term assessment. Eur Urol. 2010;57(4):673-78.

[18] Fukushima T, Noguchi T, Kobayashi T, Sekiguchi N, Ozawa T, Koizumi T, et al. Late and rapid relapse in mediastinum from testicular germ cell tumour Stage I over 13 years after surgery. Case Rep Oncol. 2019;12(2):500-05.

[19] Aggarwal R, Rao S, Dhawan S, Bhalla S, Kumar A, Chopra P. Primary mediastinal lymphomas, their morphological features and comparative evaluation. Lung India. 2017;34(1):19-24.

[20] Rogowitz E, Babiker HM, Krishnadasan R, Jokerst C, Miller TP, Bookman M. Heart of lymphoma: Primary mediastinal large B-cell lymphoma with endomyocardial involvement. Case Rep Oncol Med. 2013;2013:81-84.

[21] Kawaguchi T, Kawai N, Watanabe T, Yasukawa M, Morita K, Ohbayashi C, et al. Primary intrathoracic malignant neurogenic tumour: Report of three cases and comparison with benign neurogenic tumours resected at our institution. Surg Case Rep. 2015;1:6.

[22] Ralli M, Singh S, Hasija S, Verma R. Intrathoracic malignant peripheral nerve sheath tumour: Histopathological and immunohistochemical features. Iran J Pathol. 2015;10(1):74-78.

[23] Godfrey GJ, Farghaly H. Lymph node metastasis of malignant peripheral nerve sheath tumour in the absence of widespread disease five years after diagnosis: A rare finding. Int J Clin Exp Pathol. 2010;3(8):812-14.

[24] Shreshtha S. Malignant peripheral nerve sheath tumour with lymph node metastasis: An uncommon presentation. Int Surg J. 2017;4:1138-40.

[25] El Weshi A, Allam A, Ajarim D, Al Dayel F, Pant R, Bazarbashi S, et al. Extraskeletal Ewing's sarcoma family of tumours in adults: Analysis of 57 patients from a single institution. Clin Oncol (R CollRadiol). 2010;22(5):374-81.

[26] Koc U, Duman E. A case of primary mediastinal Ewing's sarcoma/primitive neuroectodermal tumour presenting with chest pain. Turk J Emerg Med. 2016;17(2):77-78.

[27] Applebaum MA, Worch J, Matthay KK, Goldsby R, Neuhaus J, West DC, et al. Clinical features and outcomes in patients with extraskeletal Ewing sarcoma. Cancer. 2011;117:3027-32.

[28] Kalkan KE, Bilici A, Selcukbiricik F, Unver N, Yuksel M. Thoracic primitive neuroectodermal tumour: An unusual case and literature review. Case Rep Pulmonol. 2013;2013:326871.

[29] Sotome K, Onishi T, Hirano A, Nakamaru M, Furukawa A, Miyazaki H, et al. A rare case of anaplastic transformation within the metastatic site of the retroperitoneal region in a patient 17 years after total thyroidectomy for papillary carcinoma of the thyroid beginning with multiple bone metastases. Thyroid. 2007;17:1309-11.

[30] Abe T, Suzuki M, Shimizu K, Shinagawa N, Oizumi S, Matsuno Y, et al. Anaplastic transformation of papillary thyroid carcinoma in multiple lung metastases presenting with a malignant pleural effusion: A case report. J Med Case Rep. 2014;8:460.

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Miliary Sarcoidosis: does it exist? A case series and systematic review of literature

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Abstract. *Background and Objectives:* Sarcoidosis typically presents with peribronchovascular and perilymphatic nodules on high-resolution computed tomography (HRCT); a miliary pattern is reported but not well described. *Design, setting:* We describe four patients with miliary sarcoidosis and results of a systematic review of all previously reported cases from 1985 onwards. *Results:* We identified only 27 cases of "miliary" sarcoidosis in the HRCT era. These patients were older (85.2% older than 40 years), had more co-morbidities (72.7%) and were symptomatic compared to "typical" sarcoidosis. Respiratory symptoms were present in 61.9% at diagnosis. Hypercalcemia was seen in 28.5%. On review of HRCT images, only 34.6% (9/26) had a "true miliary" pattern without fissural nodules. In our series, prominent perivascular granulomas were seen on histopathology in all. 44.4% (12/27) had tuberculosis preceding or concurrent to miliary sarcoidosis. Of the eight true associations, tuberculosis preceded sarcoidosis by 52 (median, IQR 36) weeks in six and occurred concurrently in another two. The diagnosis of tuberculosis was clinical in all with concurrent diagnosis of tuberculosis and sarcoidosis. Treatment with steroids had 100% response and 14.2% relapse. *Conclusions:* A true miliary pattern in the HRCT era is very rare in sarcoidosis and subtle perilymphatic pattern is nearly always seen; this should be labeled "pseudo-miliary". Prominent perivascular granulomas are associated with true miliary pattern. Miliary sarcoidosis patients are older and symptomatic, needing treatment at diagnosis. "Miliary" sarcoidosis may follow treatment for tuberculosis; concurrent cases possibly indicate the difficulty in differentiating both or a "tuberculo-sarcoid" presentation. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (1): 53-65)*

Key words: sarcoidosis, miliary, micronodules, miliary tuberculosis

Introduction

Miliary sarcoidosis has been described as a rare presentation of sarcoidosis and was reported in <1% of all cases in the pre-computed tomography (CT) era on chest radiography (1). With the advent of high-resolution-CT (HRCT), peribronchovascular and perilymphatic nodules in relation to the secondary pulmonary lobule are recognized as classic of sarcoidosis (2). However, random micronodules (miliary pattern) have been described and sarcoidosis is included in the differential diagnosis of a "miliary" pattern on HRCT (3, 4). There are no well-described series or systematic reviews of "miliary" sarcoidosis in the high-resolution CT (HRCT) era. On the other hand, tuberculosis continues to be a global pandemic with an estimated 10 million cases worldwide in 2016 (5). Miliary tuberculosis accounts for 1-2%

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of all cases of new cases of tuberculosis and 8% of all extra-pulmonary tuberculosis (6). The relationship between tuberculosis and sarcoidosis continues to be enigmatic; tuberculosis can precede, follow or present concurrently with sarcoidosis (7). In patients with miliary tuberculosis, the clinical symptoms of night sweats, weight loss and fatigue are non-specific and overlap markedly with sarcoidosis. Respiratory symptoms are often minimal in either forms of miliary disease and microbiological evidence of *M.tuberculosis* is only present in 20-30% of miliary tuberculosis (8). Sarcoidosis has a characteristic perilymphatic pattern on histopathology and radiology (2); the co-relation of random nodules seen on radiology with pathology in "miliary" sarcoidosis is unclear. We describe a series of patients with miliary sarcoidosis and the results of our systematic review on miliary sarcoidosis in the HRCT era.

Materials and methods

We describe four patients with miliary nodules and proven sarcoidosis that was managed in the authors' institution over three years and performed a systematic search of literature for miliary sarcoidosis.

Systematic search of literature

Two of the authors (R.S and R.K) conducted a systematic search of literature in PUBMED independently using the MeSH term "miliary" *AND* "sarcoidosis" limited to title/abstract and restricted the search from the year 1985 onwards (9). The choice of the year was defined by the first description of the current HRCT protocols and detailed description. This was further supplemented by search of IndMed using the same terms, the references of retrieved articles, our personal records and the Internet search engine GOOGLE. These articles were screened without blinding, by title and abstract review to identify relevant studies. Any discrepancy was resolved by consensus. Full texts or abstracts were then retrieved to identify the type of lung involvement in reported cases with miliary sarcoidosis. Only those articles that included patients with HRCT according to established protocols (9), included representative images or detailed descriptions and had a histological diagnosis compatible with sarcoidosis (10) were included for analysis. For the purpose of this systematic review, we included all descriptions that were accepted as "miliary" in published cases but reanalyzed the images independently. The diagnosis of sarcoidosis was according to previous published guidelines (10).

Data extraction

Data was abstracted from selected cases in a predefined data extraction form (Supplementary Table).

Statistical analysis

Continuous data are presented as mean (standard deviation (SD), median [range or Interquartile Range (IQR)] and categorical data as percentages and proportions. All statistical analysis was done using the Statistical software SPSS Version 14.

Ethics

The Human Ethics Committee of PSG IMS&R, Coimbatore, approved the study. All data was anonymized to maintain patient confidentiality.

Case reports

Case 1

A 65-year-old lady presented with cough, night sweats and exertional breathlessness of two months' duration. There was no history of weight loss or fever. Her past history was significant for hypertension and non-productive cough two years before that was evaluated at another center. CT had shown right paratracheal and bilateral hilar lymphadenopathy along with upper lobe-predominant micronodules. Tuberculin skin testing was negative and endobronchial ultrasound-guided needle aspiration (EBUS-FNA) and transbronchial lung biopsy (TBLB) were performed. EBUS-FNA showed few non-caseating granulomas and TBLB was non-contributory. A presumptive diagnosis of tuberculosis was made and four-drug anti-tuberculosis treatment (ATT) was administered for six months with improvement in cough. Examination was unremarkable during current evaluation. There was no exposure to pets, drugs

or occupational dusts. Chest radiography was normal. Spirometry was normal and diffusion capacity showed mild reduction. HRCT showed profuse random micronodules without lymphadenopathy and an upper-lobe and peripheral predominance that was visualized better with maximum intensity projection (MIP, Figure 1). Renal function tests, serum calcium and angiotensin-converting enzyme (ACE) levels were normal. Fibreoptic bronchoscopy (FOB) was done with bronchoalveolar lavage (BAL) and TBLB; BAL Fluid was negative for acid-fast bacilli and Xpert MTB/RIF and TBLB showed interstitial inflammation only. Surgical lung biopsy was performed and showed extensive perivascular non-caseating granulomas (Figure 2). BAL mycobacterial cultures were subsequently reported as negative. She was started on steroids with resolution in cough and breathlessness by second month of treatment. She remains asymptomatic and off steroids after one year of treatment.

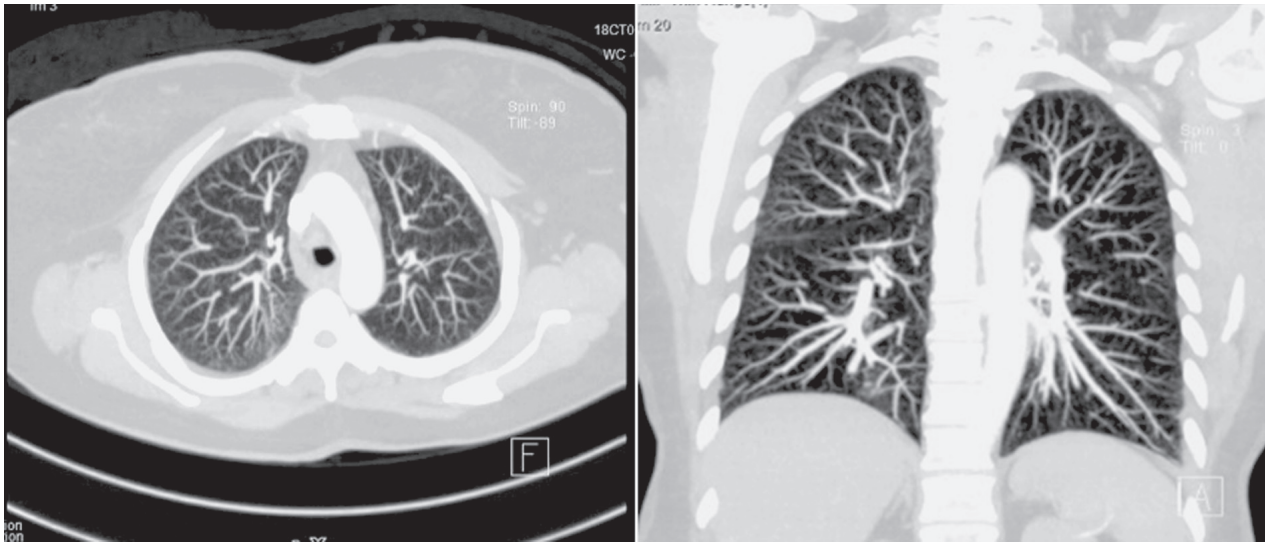


Fig. 1. Composite image of Maximal Intensity Projection images of high-resolution computed tomography (HRCT) of the thorax from patient 1 showing upper-lobe and peripheral-predominant miliary micronodules

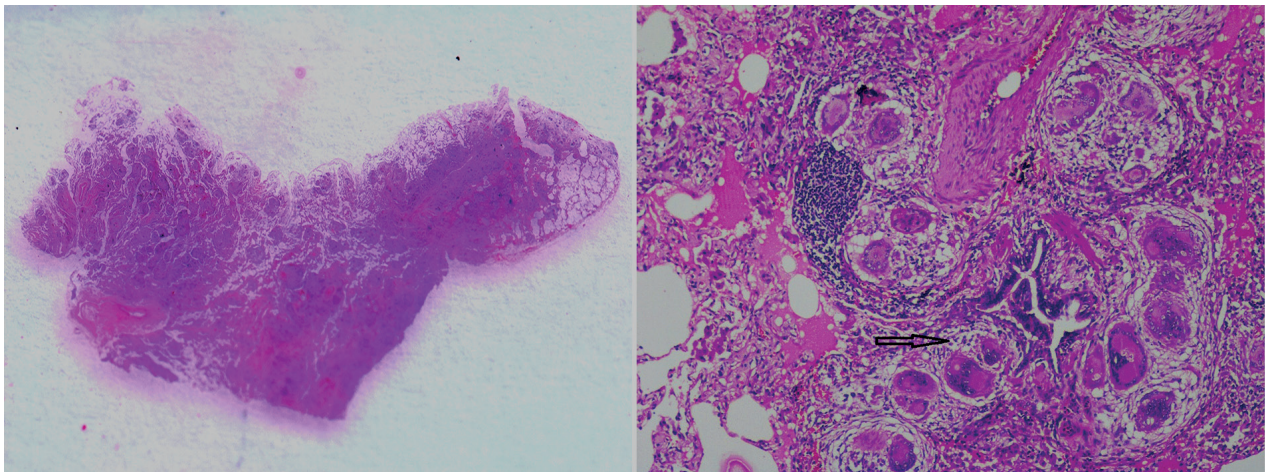


Fig. 2. Composite image of photomicrographs of surgical lung biopsy specimen of Patient 1 with left [Gross Pathological specimen, transverse plane] showing subpleural sparing and (right, Hematoxylin and Eosin stain (H & E), x 4) showing peribronchial and perivascular granulomas (arrow)

Case 2

A 40-year old gentleman presented with polyuria, fatigue and nausea of 20 days duration. There was also history of unquantified weight loss and exertional dyspnea. He did not smoke and did not raise pets, abuse drugs or have exposure to occupational dusts. Physical examination showed raised papulonodular skin lesions over hands and legs. Renal function tests showed serum creatinine of 2.65 mg/dL (normal <0.8 mg/dL); Urine microscopy showed 2+ albumin with bland sediments. Hemoglobin was 10.9 g/dL with normal leukocyte and platelet counts. Ultrasound showed normal kidney size with preserved corticomedullary distinction. Chest radiographs showed miliary nodules. Serum ionized calcium was 1.8 mg/dL (normal 1.1-1.3 mg/dL) with suppressed parathyroid hormone (1.9 pg/mL, normal 15-65) and normal vitamin D levels (23.6 ng/mL). ACE levels were elevated (98 U/mL, normal 8-53). Tuberculin skin testing was negative. CT-Chest showed random micronodules without zonal preponderance and bilateral hilar lymphadenopathy (Figure 3, left). FOB was done with BAL and TBLB; BAL was negative for acid-fast bacilli and Xpert MTB/RIF. TBLB showed perivascular non-caseating granulomas with Schaumann and asteroid bodies (Figure 4). Punch biopsy of the skin also showed non-caseating granulomas in the dermis**.** He was started on steroids with resolution of hypercalcemia and normalization of renal function. His subsequent course was complicated by steroid-aggravated diabetes presenting with hyperosmolar coma; methotrexate was added and steroids were tapered. He remained asymptomatic with near-complete radiologic clearing (Figure 3, right) after one year of stopping methotrexate but relapsed by 18 months and remains on methotrexate.

Case 3

A 55-year old gentleman with moderately controlled diabetes mellitus presented with exertional breathlessness, cough, right-sided shoulder pain and weight loss of one month's duration. There was no anorexia or expectoration. He was a farmer by occupation and did not have exposure to poultry or occupational dusts and chemicals. Physical examination showed fine bilateral crepitations without clubbing. Renal function tests, serum calcium, urine microscopy, ACE levels and hemogram were normal. Chest radiographs showed miliary nodules (Figure 5, left). Human Immunodeficiency virus was negative by enzyme-linked sorbent assay. Tuberculin skin testing was strongly positive (18 mm, 1 TU). CT-Chest showed random micronodules without lymphadenopathy (Figure 6, left). FOB was done with BAL

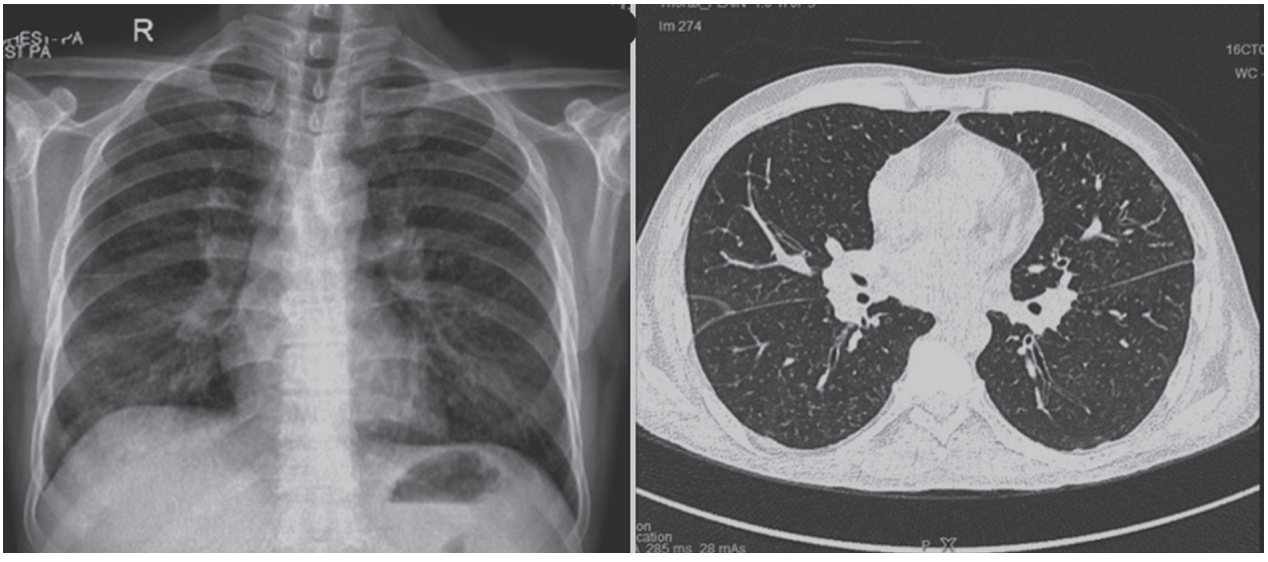


Fig. 3. Composite images of chest radiography of patient 2 showing miliary nodules (left) and HRCT images (major fissure level) showing upper lobe-predominant random nodules. No involvement of the fissure or "beading" of the fissure is seen

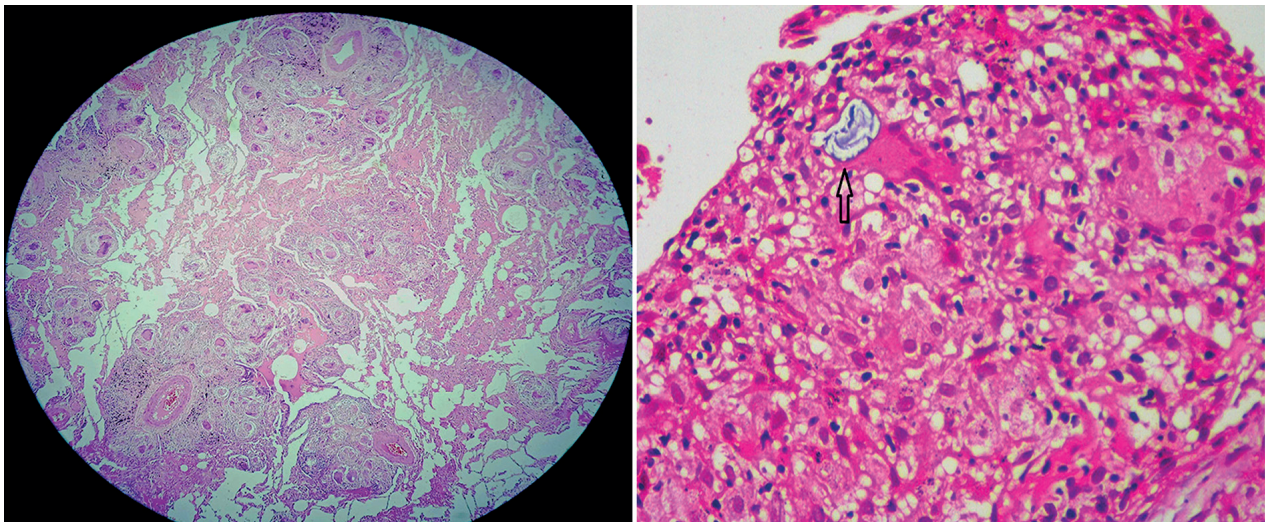


Fig. 4. Composite image of Photomicrographs of lung biopsy sample of Patient 2 with left, [Hematoxylin and Eosin stain (H & E), x scanner power] showing multiple non-necrotizing perivascular granulomas, and right, [H & E stain, x 40) showing a Schaumann body within a giant cell (arrow)

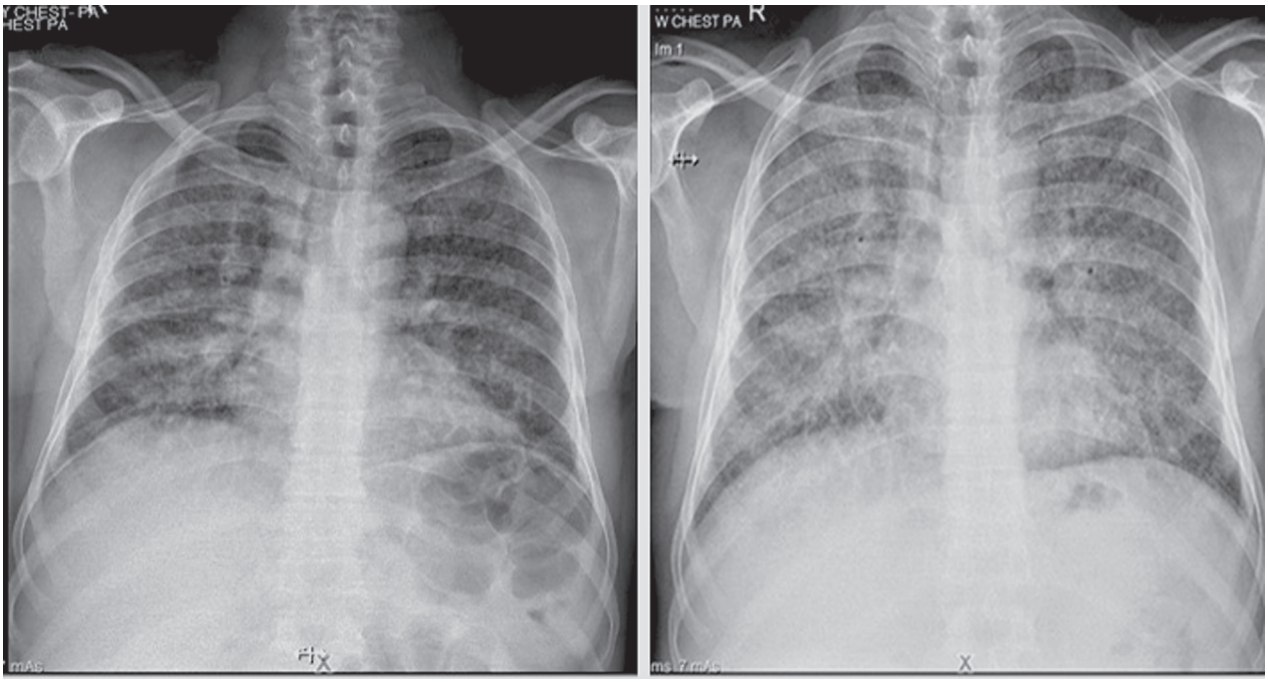


Fig. 5. Composite image of the Chest Radiographs of Patient 3 at initial evaluation (left) showing extensive miliary nodules. Subsequent Chest radiograph (right) after six weeks of anti-tuberculosis treatment alone show profusion of nodules with diffuse ground-glass opacification. Worsening hypoxemia was clinically noted

and TBLB; BAL was negative for acid-fast bacilli and Xpert MTB/RIF. TBLB showed well-formed perivascular granulomas with Langhans' giant cells, occasional fibrinoid necrosis and lymphocytes rimming (Figure 6, right). A diagnosis of miliary tuberculosis was made and four-drug weight-based ATT was started. However, his symptoms continued to worsen with worsening nodules and development

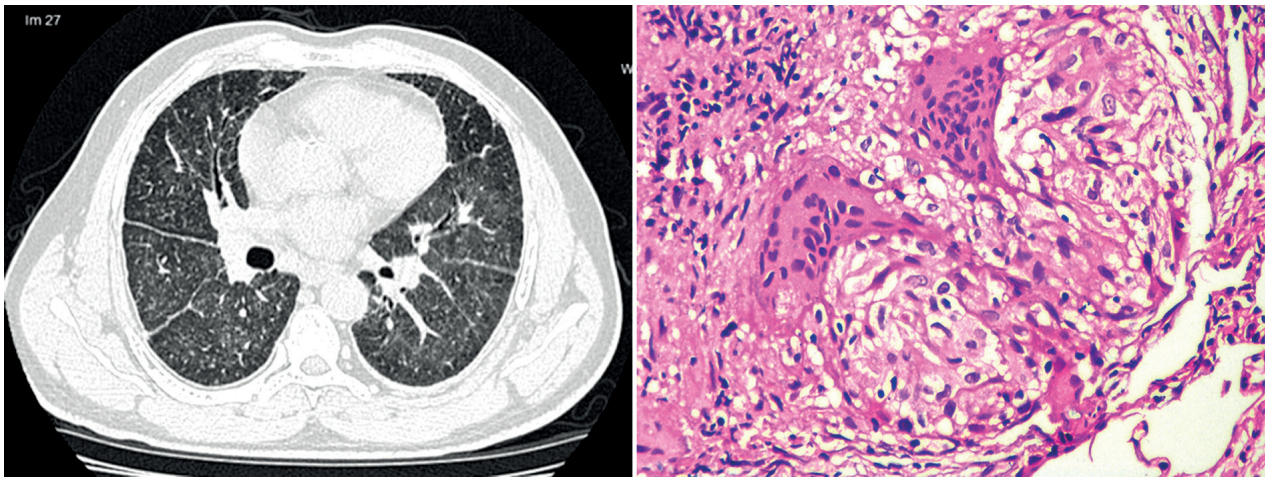


Fig. 6. Composite image of the HRCT image of Patient 3 at the level of major fissure during worsening hypoxemia corresponding to right-side of Figure 5 (left), showing random nodules with fissural prominence, with beading in both oblique fissures; some interlobular septal thickening and diffuse ground glass opacity can be seen and right, (Hematoxylin and Eosin stain, x 40) showing a non-necrotizing granuloma with peripheral lymphocyte cuffing

of hypoxemia needing oxygen at four weeks (Figure 5, right). BAL mycobacterial cultures were negative in the meantime. Steroids were added with a presumptive diagnosis of immune reconstitution syndrome, with resolution of hypoxemia and symptoms. Symptoms recurred after stopping steroids at four weeks; right-sided vision loss due to uveitis occurred. After a clinico-pathological review, a possibility of concurrent miliary tuberculosis with sarcoidosis was considered. ATT was continued and low-dose tapering steroids and methotrexate (in view of poorly controlled diabetes) were added, with complete resolution of symptoms by eight weeks. He remains asymptomatic after two years of cessation of ATT and methotrexate.

Case 4

A 48-year-old gentleman presented with fatigue for two weeks. There was no history of night sweats, weight loss or fever. He did not smoke, did not raise pets, abuse alcohol or drugs and did not report exposure to occupational dusts. Physical examination was normal. Renal function tests, urine microscopy and hemogram were normal. Serum calcium was elevated (1.43 mg/dL, normal 1.1-1.3). Chest radiographs showed miliary nodules (Figure 7, left). ACE levels were elevated (76 U/mL, normal 8-53). Tuberculin skin testing was negative. CT-Chest showed upper lobe predominant random micronodules and lower-lobe interlobular septal thickening (Figure 8, left & right). FOB was done with BAL and TBLB; BAL was negative for mycobacterial cultures and Xpert MTB/RIF. TBLB showed prominent perivascular non-necrotizing epitheloid granulomas with asteroid bodies (Figure 7, right). A diagnosis of miliary sarcoidosis was made and steroids were initiated. His symptoms and hypercalcemia resolved by four weeks and steroids were tapered over the next nine months. He remains asymptomatic on follow-up.

Results of the systematic search

Our systematic search identified 27 reports of miliary sarcoidosis in 21 reports (3, 11-30); this included 16 men and 9 women (Table 1, supplementary Table). This cohort of "miliary sarcoidosis" was older, was mostly symptomatic and had high co-morbidity burden. The mean age of the included patients was 47.5±13.2 years; 85.2% were older than 40 years. 72.7% reported at least one associated co-morbidity (11, 12, 16, 18, 21, 27, 30).

The median time to presentation was 8 (IQR 12) weeks. The majority had symptoms at the time of presentation (90.9%, 20/22); only two patients recognized by surveillance transbronchial lung biopsies post-lung transplantation were asymptomatic

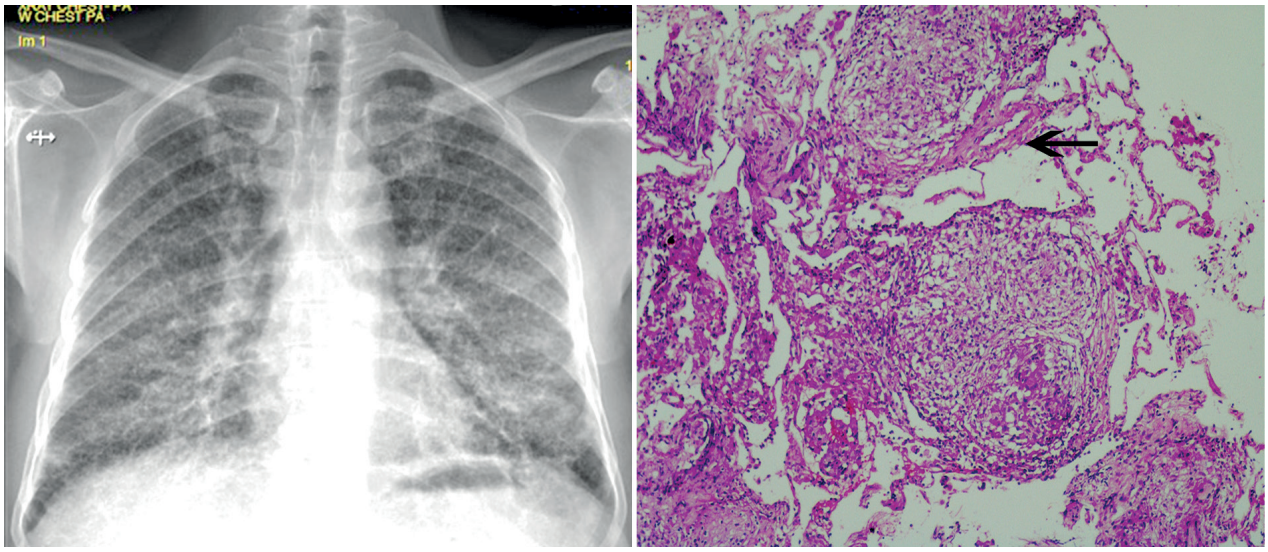


Fig. 7. Composite image of chest radiography images (left) of Patient 4 showing miliary nodules at diagnosis and photomicrographs of the lung biopsy specimen [right, Hematoxylin and Eosin stain (H & E) x 4] showing multiple perivascular granulomas (arrow)



Fig. 8. Composite image of the HRCT image of Patient 4 showing (left, upper lobes) random nodules with perilymphatic predominance, beading of the right oblique fissure and perivascular nodules and (right, lower lobes) marked interlobular septal thickening in the lower lobes associated with random nodules

at diagnosis (11, 12). Respiratory symptoms, however, were present in 63.6% only at diagnosis (13, 14, 16, 20, 21, 23-25, 28-30). In those with respiratory symptoms, breathlessness and cough were present in less than 50% (45.4%). Fever (27.3%) (13, 21-23, 29), night sweats (11.1%) (22, 27) and weight loss (22.7%)(18, 22, 27) were all seen in a significant percentage at diagnosis.

Symptomatic multi-systemic disease in association with miliary sarcoidosis was seen in 36.4%.(17, 19, 20, 23, 30) Hypercalcemia was seen in 27.3% (19, 20, 23, 27) and acute kidney injury was seen in 9.1% (19) at diagnosis. None needed dialysis but chronic kidney disease occurred in association with nephrocalcinosis (19). Ocular (13.6%, 3/22) involvement (25), bone (4.5%, 1/22) disease (17), sicca syndrome

Table 1. Summary of clinical features of all reported cases of thoracic sarcoidosis with miliary nodules on High-resolution Computed Tomography (N=27) [$]

Characteristic	Description
Age, Mean ± S.D, N=24	47.5±13.2 years; Age >40 years 85.2%
Gender (Male: Female), N=25	16:9
	In those >40 years, Male: Females O.R 0.26, p=1.00 (61.9% vs 75%)
Country of description	India (10/26), United States (7/26), Greece (5/26), Japan (3/26), Germany (1/26)
Symptoms at presentation	
Any symptoms, N=22	None, asymptomatic (9.1%, 2/22)
Symptom duration prior to presentation, Median, IQR N=19	8, (12) weeks
Symptoms	Any respiratory symptom (63.6%, 14/22)
	Dyspnea (45.4%, 10/22), cough (45.4%, 10/22)
	Fatigue (30.4%, 7/23), fever (27.3%, 6/22), weight loss (22.7%, 5/22), night sweats (11.1%, 3/22)
Acute kidney injury	9.1%, 2/22
Hypercalcemia	27.3%, 6/22
Co-morbidities, N=22	Any 72.7% (16/22)
	Smoking (18.2%, 4/22), diabetes (13.6%, 3/22), hypertension (18.2%, 4/22), lung transplantation (9.1%, 2/22), dust exposure (13.6%, 3/22)
Symptomatic disseminated sarcoidosis	8/22 (36.4%)
Other site involvement	Ocular (13.6%, 3/22), pleural effusion (9.1%, 2/22), bone (4.5%, 1/22), sicca syndrome (4.5%, 1/22), pancreas (4.5%, 1/22)
Radiological findings (N=26)	
True miliary pattern on HRCT	17/26 (65.4%) only
Perilymphatic nodules on HRCT in miliary CT	8/17 (47.1%); Truly random nodules total 9/26 (34.6%)
Zonal predominance seen	Upper & middle (7/26, 26.9%), central (7.7%, 2/26), peripheral (19.2%, 5/26)
Fissural nodules	65.4% (17/26)
Peripheral subpleural predominance	69.2% (18/26)
Interlobular septal thickening	23.1% (6/26)
Intralobular septal thickening	11.5% (3/26)
Ground glass opacity	11.5% (3/26)
Mediastinal adenopathy	Yes (50%, 13/26); Right paratracheal (23.1%, 6/23), bilateral hilar (23.1%, 9/23), others (8.7%, 2/23)
Generalized lymphadenopathy	8.7% (2/23)
Steroid treatment	Yes 21/22 (95.2%), Response 100% (21/21)
Relapse	14.2% (3/21)
Tuberculosis and miliary sarcoidosis	
Both tuberculosis and sarcoidosis	Both tuberculosis and sarcoidosis 44.4% (12/27)
	Definite or probable tuberculosis followed by sarcoidosis 29.7% (8/27)
	Probable misdiagnosis of tuberculosis 33.3%, (4/12)
	Microbiologically proven 37.5% (3/8) of all cases only
	Sequential (87.5%, 7/8), concurrent (25%, 2/8)[@@]

(continued on next page)

Characteristic	Description
Time to sequential miliary sarcoidosis in those with tuberculosis preceding sarcoidosis, (Median, IQR) N=7	52 (36) weeks
Site of tuberculosis	Pulmonary 87.5% (7/8), spinal 12.5% (1/8)
Yield of TBLB, N=21	85.7% (18/21)
Miliary tuberculosis preceding or concurrent with miliary sarcoidosis	5/8 (62.5%), 2/5 with miliary TB preceding miliary sarcoidosis
ATT-induced hepatitis	2/8 (25%)

Abbreviations: S.D standard deviation, IQR Inter-quartile range, HRCT High-resolution Computed Tomography, TBLB transbronchial lung biopsy, ATT Anti-tuberculosis treatment
[@@]One patient had both sequential and concurrent diagnosis of tuberculosis and sarcoidosis

(4.5%, 1/22) (20) and pancreatic mass (4.5%, 1/22) (28) were the other manifestations seen. Pleural effusion was seen in association in 9.1% (2/22) (24, 28).

On review of the HRCT images, a "true miliary" pattern was seen in 65.4% (17/26) of the reported cases only. In these cases, fissural nodules and prominence could still be seen in 47.1% (8/17); only 34.6% (9/26) of reported cases could be classified as random micronodules on radiologic review (12-14, 17, 18, 24, 26, 28, 30). An upper and middle lobe distribution of nodules (11-13, 15, 16, 25-27) was seen in 26.9% (7/26) and a peripheral distribution was seen in 69.2% (11, 12, 15, 24, 28). Perilymphatic clustering of nodules (3, 11-18, 22, 24-26, 28) or a perivascular dominance (3, 13, 15, 19, 22, 26-28) of nodules was seen in 65.4% and 42.3% respectively. Interlobular septal thickening, often lower-lobe in distribution, was seen in 23.1% (Table 1) (12, 14, 15, 18). Mediastinal adenopathy was observed in 50% in association with micronodules and generalized systemic lymphadenopathy was seen in 8.7% of patients (19, 20).

44.4% (12/26) of the reported patients had tuberculosis preceding or concurrent to the diagnosis of miliary sarcoidosis (13-17, 20-22, 29). In four of these patients, this was likely a misdiagnosis (13, 14, 30). In eight patients, tuberculosis occurred preceding (15-17, 21, 22) sarcoidosis in six (62.5%), concurrently in one (37.5%) (29) and both in one (23). In those with tuberculosis preceding sarcoidosis, a microbiological confirmation was made in 42.9% (15-17, 22); Others were diagnosed based on pathology and clinical response (21). In patients with concurrent diagnosis of tuberculosis and sarcoidosis, the diagnosis of tuberculosis was clinical in all; in all these patients, clinical and pathological findings, including caseous necrosis, were consistent with tuberculosis but clinical resolution occurred on initiation of steroids only (23, 29). The time to subsequent diagnosis of miliary sarcoidosis after treatment for tuberculosis was a median of 52 (IQR, 36) weeks. Miliary tuberculosis followed or concurrent with miliary sarcoidosis was reported in four patients (Table 2) (15, 16, 23, 29).

TBLB was the most common modality used for diagnosis and was reported as diagnostic in 85.7%. As most patients were symptomatic, treatment with steroids was offered in 95.2%, with 100% response. Methotrexate was administered in two patients for steroid-related severe adverse effects, with good response in both. Three patients (14.2%) relapsed after steroid taper; prompt response was noted on re-institution without further relapse.(21)

Discussion

The description of "miliary" refers to small, well-defined, 1 to 3 mm nodules on chest radiography and is derived from Latin miliarius, meaning millet seed (8). This pattern implies hematogenous dissemination of disease and is classically associated with hematogenous spread of tuberculosis, fungal infections and malignancy (31). However, more than 40 diseases have been associated with this pattern on chest radiography (Table 2). The importance of quickly establishing the diagnosis, especially in high prevalence areas of tuberculosis, is that miliary tuberculo-

Table 2. Reported causes of miliary nodules on High-resolution Computed Tomography

Classification	Cause of miliary Nodules	Site of micronodules in SPL
Infectious	Mycobacterium tuberculosis	Random
	Disseminated Bacillus-Calmette-Guerin infection	Random
	Bacterial: Mycoplasma pneumoniae, Hemophilus influenzae	Centrilobular
	Fungal: Histoplasmosis, Coccidioidomycosis, hematogenous Candida infection	Random
	Viral: Varicella-zoster, cytomegalovirus	Centrilobular
	Parasitic: Tropical pulmonary eosinophilia	Centrilobular
Immunologic	Pneumoconiosis: Silicosis	Perilymphatic
	Pneumoconiosis: Coal worker's pneumoconiosis	Perilymphatic
	Hypersensitivity pneumonitis	Centrilobular
	Sarcoidosis	Perilymphatic
	Idiopathic pulmonary hemosiderosis	Centrilobular
	Pulmonary alveolar microlithiasis	Centrilobular
	Diffuse panbronchiolitis	Centrilobular
	Allergic bronchopulmonary aspergillosis	Centrilobular
Malignancy	Secondaries from bronchogenic carcinoma	Perilymphatic
	Others: melanoma, papillary thyroid carcinoma, renal, breast, Trophoblastic tumor, osteosarcoma	Random
	Pulmonary Langerhans' cell histiocytosis	Random
	Metastasizing leimyoma	Random
	Lymphoma	Perilymphatic
Substance abuse	Excipient lung disease	Centrilobular

Abbreviations: SPL secondary pulmonary lobule

Table 3. High-resolution computed tomography findings in Thoracic Sarcoidosis (Modified from Criado et al)

Typical features	
Lymphadenopathy	Site (hilar, right paratracheal), bilateral, symmetrical, well defined. May show "cluster of black pearls" sign. If calcified, patchy or egg-shell
Nodules	Micronodules 2-4 mm, bilateral, well-defined, upper and middle zone and peri-hilar predominant. Peri-bronchovascular, subpleural, interlobular septal (peri-lymphangitic pattern)
Fibrotic changes	Interlobular septal thickening, Reticular opacities, architectural distortion, traction bronchiectasis
Atypical features	
Lymphadenopathy	Unilateral, isolated, anterior or posterior mediastinal
Nodules	Miliary
	Lower-lobe predominant, unilateral, "Halo" sign or "Atoll" sign
Consolidation	Mass-like opacities, conglomerate masses, solitary pulmonary nodules, confluent alveolar opacities and ground-glass opacities
Linear opacities	Intra-lobular septal thickening
Airway	Mosaic attenuation, trachea-bronchial abnormalities, atelectasis
Pleural	Effusion, chylothorax, pneumothorax, pleural thickening, calcification, plaque-like lesions

sis is the most common cause, has 25-30% mortality in adults and is invariably fatal if untreated (8).

The finding of a miliary pattern on chest radiographs was noted in early roentgenologic series of thoracic sarcoidosis; 1.3% (2/150) had "miliary" disease (1). Thoracic sarcoidosis has been reported to have several typical and atypical findings on HRCT; miliary is classified as an atypical finding (Table 3) (3). The description of micronodules by their distribution in relation to the "secondary pulmonary lobule" on HRCT along with the clarity of margins and attenuation further helps in narrowing the differential diagnosis of diffuse pulmonary micronodules. Micronodules can be accurately classified as having a centrilobular, perilymphatic or random distribution with their characteristic disease associations (Table 2) (4). Miliary nodules have a random distribution and can be seen in relation to pleural surfaces, small vessels and interlobular septa but do not have a consistent or predominant relationship to any of these (31). Given the reported prevalence of sarcoidosis varies from 4.5-36 per 100,000, the very small number of cases of "miliary" sarcoidosis reported in literature in the HRCT era (Table 1) suggests that this is a very rare occurrence. Most series of computed tomographic findings in sarcoidosis and a review on atypical findings in thoracic sarcoidosis did not report "miliary" sarcoidosis (32, 33).

Our systematic review suggests that this small reported number is an overestimate as a true miliary pattern was seen only in 34.6% (9/26) of the published cases of "miliary" sarcoidosis; and subtle perilymphatic pattern is nearly always seen and the term "pseudo-miliary" may be more appropriate. Distinct perilymphatic clustering of nodules or a perivascular dominance of nodules was seen in 65.4% and 42.3%; fissural nodules leading to the "beaded fissure sign" was prominent and seen in 65.4% of cases. Further, unlike true miliary nodules that lack any zonal predominance, an upper lobe and peripheral clustering was seen in 30.8% and 19.2% respectively. In all our patients with random micronodules, we observed a prominent perivascular distribution of micronodules on histopathology (Figures 2, 4, 6 and 8); two of these had associated "beaded fissure sign" on HRCT. Pathologically, sarcoidosis causes perilymphatic clustering of granulomas; hematogenous or airway spread has not been postulated (34). Profuse clustering of micronodules along the centrilobular and perilymphatic compartments gives rise to an appearance of random nodules in the majority with a "pseudo-miliary" appearance. In the few patients with a true random pattern, prominent perivascular granulomas or drainage into the pulmonary lymphatics and subsequent dissemination by hematogenous route could be postulated.

The link between mycobacteria and sarcoidosis continues to be an enigma (7, 35). In the original descriptions, about 5% of sarcoidosis had tuberculosis preceding them. Polymerase chain reaction analyses of lung biopsies of sarcoidosis have shown mycobacterial DNA in 26.4%.(35) Tuberculosis may present concurrently and confound the diagnosis and management of these patients (23, 36, 37). It has been hypothesized that the immunological responses in sarcoidosis are analogous to "tuberculoid" or "pauci-bacillary" leprosy (38); persisting immunological responses to mycobacterial antigen can explain the extraordinarily high rates of tuberculosis preceding the diagnosis of pseudo-miliary sarcoidosis in the present systematic review.

The diagnosis of sarcoidosis rests on a constellation of clinical, radiological, histopathological and laboratory findings (39). Constitutional features including significant weight loss can occur in up to 25% of patients (Table 2). Fibrinoid necrosis and rarely, caseation can occur in sarcoidosis on histopathology (40, 41). Caseation may sometimes be absent in tuberculosis (41). Radiology can help in the differentiation of sarcoidosis from tuberculosis (26). The presence of hypercalcemia, skin involvement, negative tuberculin skin-testing, upper and middle-lobe distribution of nodules, a peripheral distribution of nodules when present and interlobular septal thickening all point to the diagnosis of "pseudo-miliary" sarcoidosis alone (42). However, sarcoidosis can precede, occur concurrent with or follow tuberculosis and differentiating tuberculosis from sarcoidosis remains a major challenge, especially in high prevalence areas of tuberculosis (43, 44). When the constellation of findings are non-conclusive, a trial of anti-tuberculosis treatment is appropriate in high prevalence areas given the very small number of true miliary sarcoidosis reported in literature. Steroids could be added when there is no clear response at 4-6 weeks (39, 43).

Conclusion

A true miliary pattern in the HRCT era is very rare in sarcoidosis and subtle perilymphatic pattern is nearly always seen; this pattern should be labeled "pseudo-miliary". Prominent perivascular granulomas are associated with true miliary pattern. Miliary sarcoidosis patients are older and symptomatic and mostly need treatment at diagnosis. "Miliary" sarcoidosis may follow treatment for tuberculosis; concurrent cases possibly indicate difficulty in diagnosis or a "tuberculo-sarcoid" presentation. Transbronchial lung biopsy had a good yield for diagnosis. Most patients were symptomatic and treated with steroids, with 100% response and 14.4% relapse.

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Drs. R.S and Dr. S.S take responsibility for the content of the manuscript, including the data and analysis

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References

1. Kirks DR, McCormick VD, Greenspan RH. Pulmonary sarcoidosis. Roentgenologic analysis of 150 patients. Am J Roentgenol Radium Ther Nucl Med. 1973;117(4):777-86. PubMed PMID: 4266919.
2. Koyama T, Ueda H, Togashi K, Umeoka S, Kataoka M, Nagai S. Radiologic manifestations of sarcoidosis in various organs. Radiographics : a review publication of the Radiological Society of North America, Inc. 2004;24(1):87-104. doi: 10.1148/rg.241035076. PubMed PMID: 14730039.
3. Criado E, Sanchez M, Ramirez J, Arguis P, de Caralt TM, Perea RJ, et al. Pulmonary sarcoidosis: typical and atypical manifestations at high-resolution CT with pathologic correlation. Radiographics : a review publication of the Radiological Society of North America, Inc. 2010;30(6):1567-86. Epub 2010/11/13. doi: 10.1148/rg.306105512. PubMed PMID: 21071376.
4. Raoof S, Amchentsev A, Vlahos I, Goud A, Naidich DP. Pictorial essay: multinodular disease: a high-resolution CT scan diagnostic algorithm. Chest. 2006;129(3):805-15. doi: 10.1378/chest.129.3.805. PubMed PMID: 16537886.
5. Global tuberculosis report 2018. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO2018 10/11/2018.
6. Sharma SK, Mohan A, Sharma A. Challenges in the diagnosis & treatment of miliary tuberculosis. Indian J Med Res. 2012;135(5):703-30. PubMed PMID: 22771605; PubMed Central PMCID: PMCPMC3401706.
7. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Sarcoidosis and tuberculosis: the same disease with different manifestations or similar manifestations of different disorders. Curr Opin Pulm Med. 2012;18(5):506-16. doi: 10.1097/MCP.0b013e3283560809. PubMed PMID: 22759770.
8. Sharma SK, Mohan A, Sharma A, Mitra DK. Miliary tuberculosis: new insights into an old disease. Lancet Infect Dis. 2005;5(7):415-30. doi: 10.1016/S1473-3099(05)70163-8. PubMed PMID: 15978528.
9. Zerhouni EA, Naidich DP, Stitik FP, Khouri NF, Siegelman SS. Computed tomography of the pulmonary parenchyma. Part 2: Interstitial disease. Journal of thoracic imaging. 1985;1(1):54-64. PubMed PMID: 3843414.
10. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 1999;16(2):149-73. PubMed PMID: 10560120.
11. Kazerooni EA, Jackson C, Cascade PN. Sarcoidosis: recurrence of primary disease in transplanted lungs. Radiology. 1994;192(2):461-4. Epub 1994/08/01. doi: 10.1148/radiology.192.2.8029415. PubMed PMID: 8029415.
12. Kazerooni EA, Cascade PN. Recurrent miliary sarcoidosis after lung transplantation. Radiology. 1995;194(3):913. Epub 1995/03/01. doi: 10.1148/radiology.194.3.7863002. PubMed PMID: 7863002.
13. Gupta D, Kumar S, Jindal SK. Bilateral miliary mottling without hilar lymphadenopathy: A rare presentation of sarcoidosis. Lung India. 1996;14(2):87-8.
14. Chugh IM, Agarwal AK, Arora VK, Shah A. Bilateral miliary pattern in sarcoidosis. The Indian journal of chest diseases & allied sciences. 1997;39(4):245-9. PubMed PMID: 9654821.
15. Voloudaki AE, Tritou IN, Magkanas EG, Chalkiadakis GE, Siafakas NM, Gourtsoyiannis NC. HRCT in miliary lung disease. Acta radiologica (Stockholm, Sweden : 1987). 1999;40(4):451-6. Epub 1999/07/08. PubMed PMID: 10394878.
16. Hatzakis K, Siafakas NM, Bouros D. Miliary sarcoidosis following miliary tuberculosis. Respiration; international review of thoracic diseases. 2000;67(2):219-22. Epub 2000/04/25. doi: 10.1159/000029492. PubMed PMID: 10773799.
17. Kline MY, Leibert EM. A case of pulmonary tuberculosis and miliary sarcoidosis. Chest2005. p. 480S-1S.
18. Stroebe K, Haddadin H. Bilateral miliary sarcoidosis. Chest2012. p. 1036A.
19. Hodges HK, Lee PY, Hausmann JS, Teot LA, Sanford EL, Levin KW. Hypercalcemia and miliary sarcoidosis in a 15-year-old boy. Arthritis and rheumatism. 2013;65(8):2112. Epub 2013/05/21. doi: 10.1002/art.38019. PubMed PMID: 23686651.
20. Kumar P, Jaco MJ, Pandit AG, Shanmughanandan K, Jain A, Rajeev, et al. Miliary sarcoidosis with secondary Sjogren's syndrome. The Journal of the Association of Physicians of India. 2013;61(7):505-7. Epub 2014/04/30. PubMed PMID: 24772762.
21. Bostantzoglou C, Samitas K, Gkogkou C, Zervas E, Gaga M. Mediastinal widening and miliary chest radiograph pattern in a middle aged man: could it be sarcoidosis? BMJ case reports. 2014;2014. Epub 2014/07/19. doi: 10.1136/bcr-2014-204884. PubMed PMID: 25035447; PubMed Central PMCID: PMCPMC4112298.
22. Luetkens JA, Zoghi S, Rockstroh JK, Naehle CP. Pulmonary sarcoidosis shortly after spinal tuberculosis infection: a diagnostic challenge. BMJ case reports. 2014;2014. Epub 2014/04/15. doi: 10.1136/bcr-2013-203333. PubMed PMID: 24728896; PubMed Central PMCID: PMCPMC3987564.

23. Mandal SK, Ghosh S, Mondal SS, Chatterjee S. Coexistence of pulmonary tuberculosis and sarcoidosis: a diagnostic dilemma. BMJ case reports. 2014;2014. Epub 2014/12/21. doi: 10.1136/bcr-2014-206016. PubMed PMID: 25527682; PubMed Central PMCID: PMCPMC4275725.
24. Enomoto Y, Yokomura K, Suda T. Bilateral pleural effusion associated with miliary sarcoidosis. American journal of respiratory and critical care medicine. 2015;191(4):474-5. Epub 2015/02/14. doi: 10.1164/rccm.201411-2022IM. PubMed PMID: 25679105.
25. Taki M, Ikegami N, Konishi C, Nakao S, Funazou T, Ariyasu R, et al. Pulmonary Sarcoidosis Presenting with Miliary Opacities. Internal medicine (Tokyo, Japan). 2015;54(19):2483-6. Epub 2015/10/02. doi: 10.2169/internalmedicine.54.4681. PubMed PMID: 26424308.
26. Bhalla AS, Das A, Naranje P, Goyal A, Guleria R, Khilnani GC. Dilemma of diagnosing thoracic sarcoidosis in tuberculosis endemic regions: An imaging-based approach. Part 1. Indian J Radiol Imaging. 2017;27(4):369-79. doi: 10.4103/ijri.IJRI_200_17. PubMed PMID: 29379230; PubMed Central PMCID: PMCPMC5761162.
27. Chaddha U, English R, Daniels J, Walia R, Mehta AC, Panchabhai TS. A 58-Year-Old Man With Fatigue, Weight Loss, and Diffuse Miliary Pulmonary Opacities. Chest. 2017;151(6):e131-e4. Epub 2017/06/11. doi: 10.1016/j.chest.2016.11.015. PubMed PMID: 28599946.
28. Matsuura S, Mochizuka Y, Oishi K, Miyashita K, Naoi H, Mochizuki E, et al. Sarcoidosis with Pancreatic Mass, Endobronchial Nodules, and Miliary Opacities in the Lung. Internal medicine (Tokyo, Japan). 2017;56(22):3083-7. Epub 2017/09/26. doi: 10.2169/internalmedicine.8916-17. PubMed PMID: 28943576; PubMed Central PMCID: PMCPMC5725865.
29. Arar O, Boni F, Meschi T, Tana C. Pulmonary sarcoidosis presenting with miliary opacities. Current Medical Imaging Reviews. 2018. doi: 10.2174/1573405614666180806141415.
30. Joshi V, S. Jain, V. Sharma, N. Khippal, Chaturvedi A. Granulomatous Hepatitis with Miliary Mottling: A Rare Cause. Journal of The Association of Physicians of India. 2018;66:97-8.
31. Andreu J, Mauleon S, Pallisa E, Majo J, Martinez-Rodriguez M, Caceres J. Miliary lung disease revisited. Current problems in diagnostic radiology. 2002;31(5):189-97. PubMed PMID: 12419999.
32. Kuhlman JE, Fishman EK, Hamper UM, Knowles M, Siegelman SS. The computed tomographic spectrum of thoracic sarcoidosis. Radiographics : a review publication of the Radiological Society of North America, Inc. 1989;9(3):449-66. doi: 10.1148/radiographics.9.3.2727355. PubMed PMID: 2727355.
33. Cozzi D, Bargagli E, Calabro AG, Torricelli E, Giannelli F, Cavigli E, et al. Atypical HRCT manifestations of pulmonary sarcoidosis. La Radiologia medica. 2018;123(3):174-84. doi: 10.1007/s11547-017-0830-y. PubMed PMID: 29124658.
34. Spagnolo P, Rossi G, Trisolini R, Sverzellati N, Baughman RP, Wells AU. Pulmonary sarcoidosis. Lancet Respir Med. 2018;6(5):389-402. doi: 10.1016/S2213-2600(18)30064-X. PubMed PMID: 29625772.
35. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis. Eur Respir J. 2007;30(3):508-16. doi: 10.1183/09031936.00002607. PubMed PMID: 17537780.
36. Smith-Rohrberg D, Sharma SK. Tuberculin skin test among pulmonary sarcoidosis patients with and without tuberculosis: its utility for the screening of the two conditions in tuberculosis-endemic regions. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2006;23(2):130-4. PubMed PMID: 17937109.
37. Shah JR, Hede J, Mathur RS. Diagnostic criteria of tuberculous sarcoidosis. Lung India. 2009;26(3):86-8. doi: 10.4103/0970-2113.53232. PubMed PMID: 20442843; PubMed Central PMCID: PMCPMC2862513.
38. Agarwal R, Gupta D. Tuberculous sarcoidosis: Is it a separate entity? Lung India. 2009;26(3):61-2. doi: 10.4103/0970-2113.53225. PubMed PMID: 20442837; PubMed Central PMCID: PMCPMC2862507.
39. Jindal SK, Gupta D, Aggarwal AN. Sarcoidosis in India: practical issues and difficulties in diagnosis and management. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2002;19(3):176-84. PubMed PMID: 12405486.
40. Karkhanis V, Joshi JM. All that caseate is not TB. Lung India.2007(24):100-1.
41. Danila E, Zurauskas E. Diagnostic value of epithelioid cell granulomas in bronchoscopic biopsies. Internal medicine (Tokyo, Japan). 2008;47(24):2121-6. doi: 10.2169/internalmedicine.47.1452. PubMed PMID: 19075536.
42. Bhalla AS, Das A, Naranje P, Goyal A, Guleria R, Khilnani GC. Dilemma of diagnosing thoracic sarcoidosis in tuberculosis-endemic regions: An imaging-based approach. Part 2. Indian J Radiol Imaging. 2017;27(4):380-8. doi: 10.4103/ijri.IJRI_201_17. PubMed PMID: 29379231; PubMed Central PMCID: PMCPMC5761163.
43. Guleria R, Mahashur A, Ghoshal AG, Thomas PK, Raghu G, Baughman RP. Challenges in diagnosing Sarcoidosis in tuberculosis endemic regions: Clinical scenario in India. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2016;33(4):381-4. PubMed PMID: 28079850.
44. Jain R, Mohan A, Guleria R. Sarcoidosis vs tuberculosis: Diagnostic mystery still unresolved. Indian J Tuberc. 2017;64(4):243-5. doi: 10.1016/j.ijtb.2017.09.001. PubMed PMID: 28941846.

ORIGINAL RESEARCH ARTICLE

A Histopathological Review of Nephrectomy Specimens Received in a Referral Center in South Tamil Nadu

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ABSTRACT

Objectives Simple and radical nephrectomies are done for benign and malignant lesions of the kidney respectively. This study was conducted to find out the histopathological spectrum of diseases that were encountered in those received nephrectomy specimens and compare it with other studies.

Methods Nephrectomy specimens both simple and radical that were received during our study period were included. Age, sex and gross measurements of the tumor were retrieved from the records. The slides were reevaluated by a panel of pathologists, classified, graded and staged and detailed discussion of those cases was done.

Results Nephrectomy specimens constituted around 0.5% of all the specimens. Mean age at which nephrectomy was done was 28.7 years with a female preponderance. As our center is a tertiary referral center of south Tamilnadu, this value has a demographic significance. The mean age at which benign lesions were seen was 32 years with M: F: 1:9. Chronic pyelonephritis was the most common benign lesion seen in our study followed by benign cystic lesions. The mean age in the malignant tumor category was 25 years with M: F: 0.8:1. Wilm's tumor in pediatric age group and renal cell carcinoma in the adult age group were commonly seen in the malignant category.

Conclusion Histopathological spectrum seen among the nephrectomy specimens correlated with those available in the literature. Mucinous cystadenoma of the pelvis and clear cell papillary carcinoma – two rare tumors were diagnosed during our study period. Chronic pyelonephritis incidence had some regional variance.

KEYWORDS chronic pyelonephritis, cysts, malignant tumors

INTRODUCTION

Nephrectomy is done for both benign and malignant lesions of the kidney. Simple nephrectomy is done for benign lesions and radical nephrectomy is done for malignant lesions. Under the benign category, nonfunctioning kidney is the most common reason for which nephrectomy is done followed by hydronephrosis. Under malignant tumors, renal cell carcinoma in adults and Wilms tumor in children are most commonly encountered in the nephrectomy specimens[1]. Spontaneous regression, primary tumor harboring metastasis from other tumors and paraneoplastic syndromes are unique features observed among renal tumors[2,3].

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Funding: None.

This study was conducted to evaluate the benign and malignant lesions encountered in nephrectomy specimens that were received in our department for one year period.

MATERIALS AND METHODS

This study was conducted during one year period from August 2015 to September 2016. Nephrectomy specimens both simple and radical were included in our study. Age, sex and gross measurements of the tumor were retrieved from the records. These parameters were compared with those available in the literatures. The slides were reevaluated by a panel of pathologists. Histopathological examination was done and the specimens were categorized into benign and malignant. Malignant tumors were graded using Furhams nuclear grading and WHO staging was used to stage adultrenal tumors. North American National Wilms' tumor study group staging system for renal tumors was followed to stage Wilms' tumor. Detailed discussion of the histopathology, molecular markers and immunohistochemical markers were analyzed with reference to the global literatures.

RESULTS

Out of 3790 histopathological specimens, nineteen nephrectomy specimens were received during our study period.

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Nephrectomy specimens constituted around 0.5% of all the specimens. Out of that, 52.6% were benign lesions. The rest were malignant tumors. Mean age at which nephrectomy was done was 28.7 years. M: F was 1:2.8 (Table 1). As our hospital is a tertiary referral center for south Tamilnadu, these parameters can be taken as a representative of their respective incidences in our area.

Benign lesions were distributed along all age groups. The mean age at which benign lesions were seen was 32 years with M: F: 1:9. Chronic pyelonephritis was the commonest lesion seen among the benign category. The mean age in the malignant tumor category was 25 years with M: F: 0.8:1. A malignant tumor was seen more in the pediatric age group in our study. Wilms' tumor was the commonest tumor seen in our study with the mean age of 2.7 years with M: F: 0.7:1. One case was associated with WAGR syndrome- Wilms' tumors, bilateral undescended testis, mitral regurgitation and aniridia. Among the adults, renal cell carcinoma was most commonly encountered (Table 2). Mean age among adults with malignant renal tumor was 51.75 years with M: F: 1:1

When stage wise stratification was done, Wilms' tumor was seen in the advanced stage in our study (Table 3). Fuhrman nuclear grading was followed only in clear cell renal cell carcinoma and papillary renal cell carcinoma (Table 4).

TABLE 1 Demographic details associated with nephrectomy specimens.

Age	1–10 years	11–20 years	21–30 years	31–40 years	41–50 years	51–60 years	61–70 years
No. of persons	6	2	2	2	3	3	1
Male	2	–	–	–	1	2	–
Female	4	2	2	2	2	1	1
Benign	1	2	2	1	2	2	–
Malignant	5	–	–	1	1	1	1

DISCUSSION

Kidney can be affected by both neoplastic and neoplastic conditions that require nephrectomy. According to Ghalayini's study, the mean age at which nephrectomy was done for benign condition was 38.4 years and for malignant tumor it was 46.7 years[4]. It was 32 years and 25 years for benign and malignant lesions respectively in our study group which was in correlation with their study. When the sex incidence of nephrectomy from our study group was compared with that of Aiman et al., M: F was 1:2.8 in our study group and was found to be similar with their study group [M: F: 1:1.05] and with Rafque M's study group[5,6]. In our study majority of the cases detected in the nephrectomy specimens [52.6%] were benign lesions in accordance with Aiman et al. and Ghalayini study[4,5]. Benign and malignant lesions which were encountered in our study are discussed below.

TABLE 3 Stage wise stratification of the renal tumor cases encountered in our study.

Stage	T1a	T1b	T2a	T2b	T3a	T3b	T3c	T4
Wilm's Tumor	–	2	2	–	1	–	–	–
Clear cell papillary renal cell carcinoma	–	1	1	–	–	–	–	–
Clear cell renal cell carcinoma	–	–	1	–	–	–	–	–
Papillary carcinoma	–	–	–	1	–	–	–	–

TABLE 4 Fuhrman grading of the renal tumor cases encountered in our study.

Grade	Grade I	Grade II	Grade III	Grade IV
Clear cell papillary renal cell carcinoma	–	2	–	–
Clear cell renal cell carcinoma	1	–	–	–
Papillary carcinoma	1	–	–	–

TABLE 2 Age and sex wise stratification of various tumors encountered in our study.

HPE findings	Benign			Malignant				
	Simple cyst	Multi cystic renal dysplasia	Mucinous cystadenoma of the pelvis	Chronic pyelonephritis	Wilm's tumor	Clear cell papillary renal cell carcinoma	Clear cell renal cell carcinoma	Papillary renal cell carcinoma
Numbers	1	2	1	6	5	2	1	1
Male	–	–	–	1	2	1	–	1
Female	1	2	1	5	3	1	1	–
Age								
1–10 years	–	1	–	–	5	–	–	–
11–20 years	–	1	–	1	–	–	–	–
21–30 years	–	–	–	2	–	–	–	–
31–40 years	–	–	1	1	–	–	1	–
41–50 years	1	–	–	–	–	1	–	–
51–60 years	–	–	–	2	–	–	–	1
61–70 years	–	–	–	–	–	1	–	–

Chronic pyelonephritis [CPN] was the commonest benign lesion seen in our study which correlated with Popat et al. and Adamson et al.[7,8] There was a bimodal age related peaks in our study group, 20–30 years and 50–60 years respectively. This was in accordance with Popat et al.[7] CPN may be due to non-obstructive pathology secondary to vesicoureteral reflux or due to obstructive pathology[9]. Gross picture varies according to the etiology. In CPN due to reflux there will polar scars as compound papillae are involved in reflux. This result in blunting and loss of pyramids in the polar regions. Conversely, in obstructive CPN there will diffuse involvement of pyramids. Microscopically in both obstructive and non-obstructive CPN, there is interstitial inflammatory cell infiltrates, tubular atrophy, thyroidization of the tubules, peri-glomerular fibrosis, global glomerular sclerosis and interstitial fibrosis (Fig. 1).

The incidence of nephrectomies for benign conditions such as chronic pyelonephritis is decreasing and nephrectomies for malignant lesions are increasing in the developed countries[10,11]. The reason for this change in trend being early detection and better treatment modalities that prevent the need for surgeries are available for inflammatory lesions of the kidney in the developed countries compared with developing nations.

Cystic lesions of the kidney were classified into four types by potter[12]. Type I- Autosomal recessive polycystic kidney disease. Type IIa -Multicystic renal dysplasia. Type IIb -Aplastic renal dysplasia. Type III -Autosomal dominant polycystic kidney disease, focal renal dysplasia, dysplasia in hereditary syndromes and autosomal recessive polycystic kidney disease. Type IV- Dysplasia associated with lower tract obstruction. The incidence of cystic lesions was 40% in our study which included multicystic renal dysplasia, simple cyst and mucinous cystadenoma of the renal pelvis. Cystic lesions were seen in 12.5% of the cases in Salma et al. which was lower than our study group[13].

In our study group, among the cystic lesions multicystic renal dysplasia was the predominant etiology for which nephrectomy was done. Multicystic renal dysplasia present with varied histological findings. Primitive or dysplastic ducts lined by columnar epithelium and surrounded by the collars of spindle cell and fetal or immature-appearing cartilage derived from the blastema and aberrantly formed glomeruli are the histopathological features commonly seen in multi cystic dysplasia (Fig. 2)[14].

Presence of immature cartilage is diagnostic of multi cystic renal dysplasia according to Rosdon RA[15]. In our study multi cystic dysplasia cases were seen in 20% of the cases with female preponderance. In Amina et al., the incidence of multi cystic renal dysplasia was 10% with a female preponderance which is in correlation with our study[16]. In contrast to our study group [mean age incidence 9.25 years] the age incidence in their study was less than 5 years[16].

Simple cyst is the commonest cystic lesion encountered in the kidney. Microscopically, the cysts are lined by cuboidal epithelium (Fig. 3). A fifty year old female patient had a simple cyst in our study which was in agreement with Marumo K. et al's study[17]. The incidence of simple cyst increase as the age advances according to Kissane and Smith[18], which was also noticed in our study group.

Primary adenocarcinoma of the renal pelvis accounts for <1% of the renal pelvic tumors. The histogenesis of this tumor is metaplastic transformation of multipotent transitional epithelium of the renal pelvis secondary to chronic irritation from the calculi, hydronephrosis and pyelonephritis[19]. Few cases of its benign counterpart were reported in the literature especially in India. Ross and

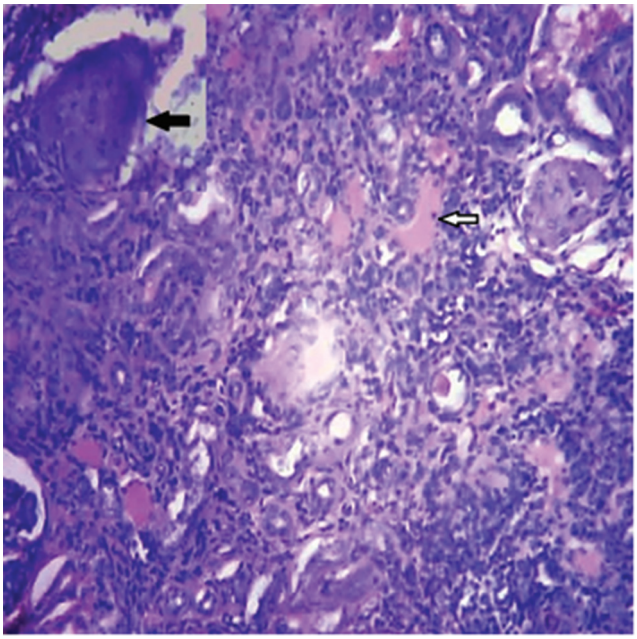


Fig. 1 Shows thyroidization of the tubules [White arrow] with interstitial inflammatory cell infiltrates. Inset shows global glomerulosclerosis [Black arrow] – Chronic pyelonephritis [H&E, 40x].

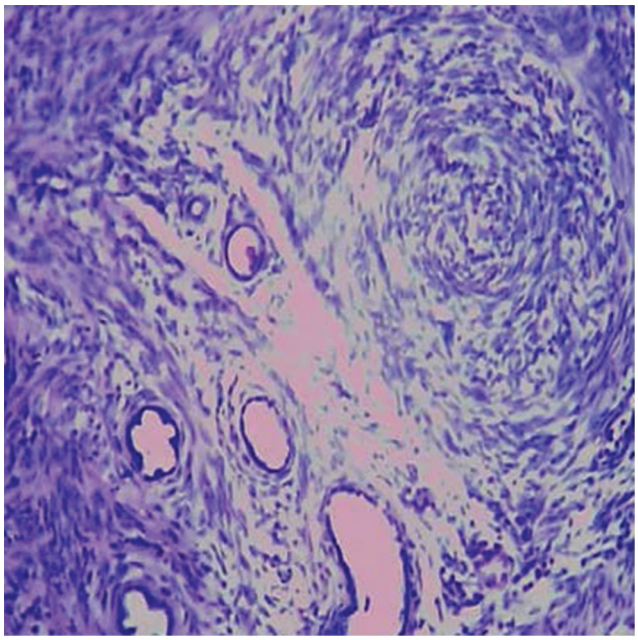


Fig. 2 Shows collars of spindle cell surrounding a primitive tubule- Multi cystic renal dysplasia [H&E, 40x].

D'Amato had reported papillary mucinous cystadenoma in a horseshoe kidney and had suggested the same criteria that were used to categorize ovarian mucinous tumors apply to renal mucinous tumor as well[20]. Mucinous cystadenoma of the pelvis, a rare tumor was seen during our study period. It was diagnosed in a forty year old female. It was radiologically diagnosed as a hydatid cyst attached to the renal pelvis. Histopathologically, the cyst was lined by mucin secreting single layer of columnar epithelium replacing the ureter and pelvis. There were no atypia or pleomorphism or invasion seen (Fig. 4). Tumors with borderline malignant potential and malignant transformation have been reported[21,22]. Its malignant counterpart has a poor prognosis. As borderline and in situ changes cannot be assessed radiologically, radical nephrectomy with total ureterectomy is the treatment of choice. Intra operative frozen section diagnoses invasive and in situ changes thus can help in the treatment planning.

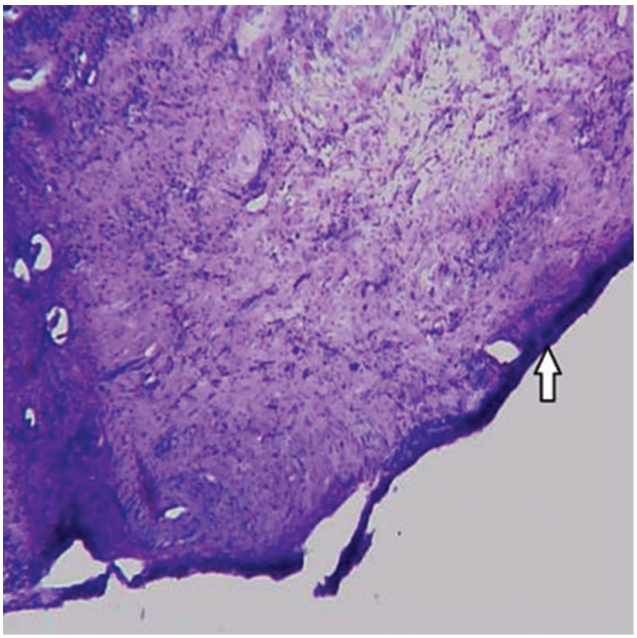


Fig. 3 Shows a cyst lined by flattened cuboidal epithelium [Arrow]- Simple cyst [H&E,10x].

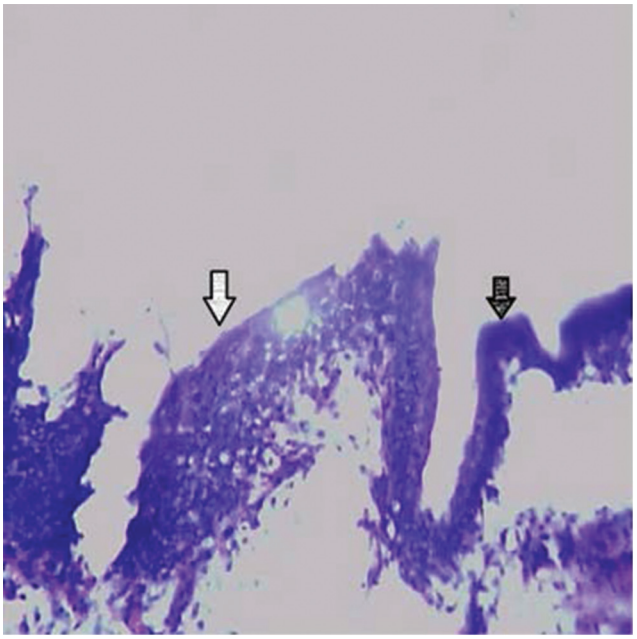


Fig. 4 Shows transitional epithelium [White arrow] replaced by benign mucinous epithelium [Black arrow] – Mucinous cystadenoma of the pelvis [H&E,10x].

Among the malignant tumors, Wilms' tumor was the most reported tumor in our study. It is the most common childhood malignant renal tumors with an incidence of 85%[23]. The incidence of Wilms' tumor in Salma et al. was 18.19%, in Amina et al. was 28.2%, whereas it was 55.56% in our study group[13,16]. The mean age at which it occurred was 3 years in Lemerle et al. and 3.5 years in Amina et al. which were in correlation with our study [2.7 years mean][16,24]. Female predominance seen in our study was in concordance with Abdulkareem et al.[25] Histologically, the tumor is composed of three elements blastema, epithelium and stroma in varying proportions (Figs. 5 and 6). No anaplasia was noted in our study group. Biphasic and monophasic Wilms' have also been reported. Triphasic Wilms' predominated in our study [60%] which was in concordance with Amina et al.[16] The WT1 gene located in chromosome11p13 is altered by germline deletions in the WAGR syndrome[26]. One such case was reported during our study period. This tumor responds to chemotherapy. Immuno histochemically, this tumor show nuclear positivity for WT1. Diffuse anaplasia and secondary carcinoma or sarcoma arising within nephroblastoma is associated with resistance to chemotherapy.

Renal cell carcinoma [RCC] is the most commonly reported malignant tumor among the adults. The incidence of renal cell carcinoma was 44.44% in our study.

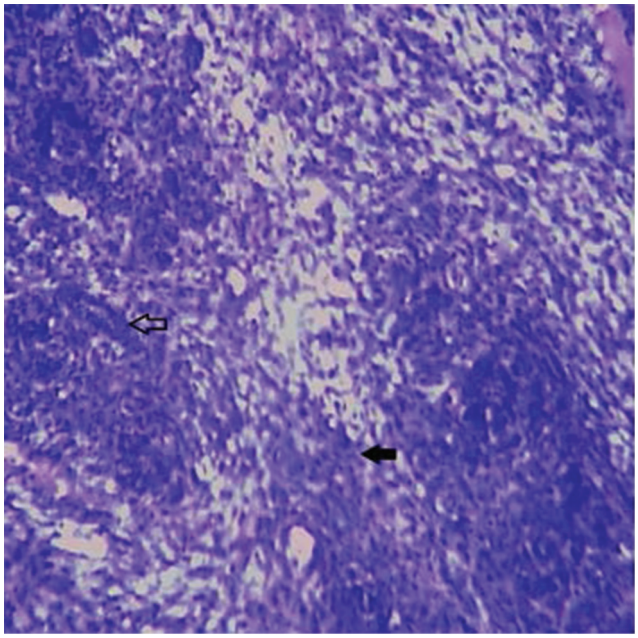


Fig. 5 Shows malignant epithelial cells [Empty black arrow] and mesenchymal component [Dark black arrow] – Wilms' tumor [H&E, 40x].

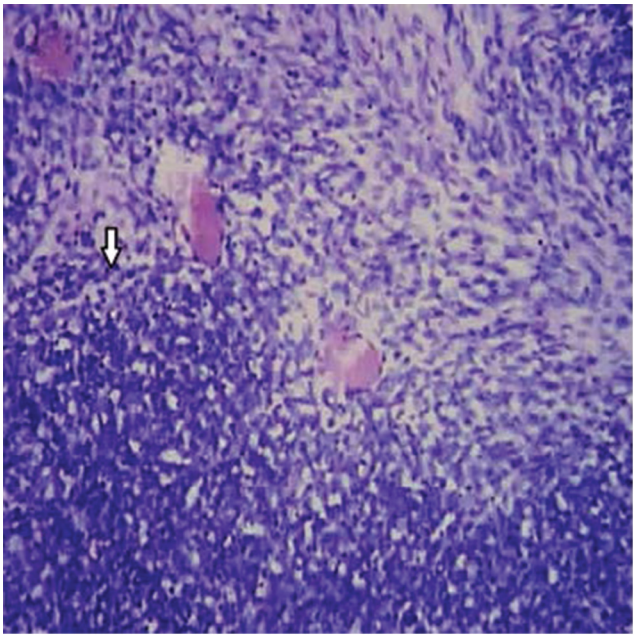


Fig. 6 Shows blastemal component- Wilms' tumor [H&E, 40x].

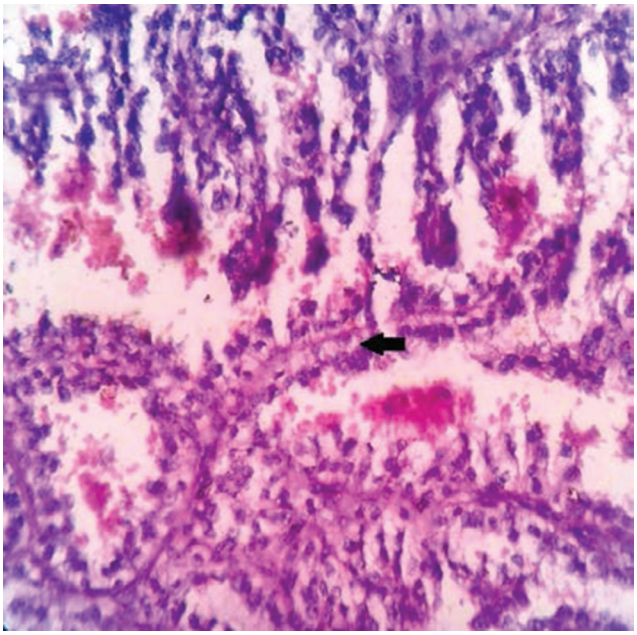


Fig. 7 Show clear cells arranged in a papillary core with the nucleus situated away from the basement membrane with sub nuclear vacuoles [Arrow]- clear cell papillary renal cell carcinoma [H&E, 40x].

The incidence of renal cell carcinoma was 80–85% according to Motzer et al.[27] Clear cell papillary renal cell carcinoma had the highest incidence among the adult renal tumors during our study period. It is a recently characterized tumor initially reported in patients with end stage renal disease[28]. Histologically, this tumor consists of papillary structures lined by clear cells with the nucleus oriented away from the basement membrane. Clear cell renal cell carcinoma areas are also seen (Fig. 7). Low nuclear Fuhrman grading is noted. It runs an indolent course. 3p deletion seen in clear cell RCC, trisomy7/17 seen in papillary RCC and VHL mutation

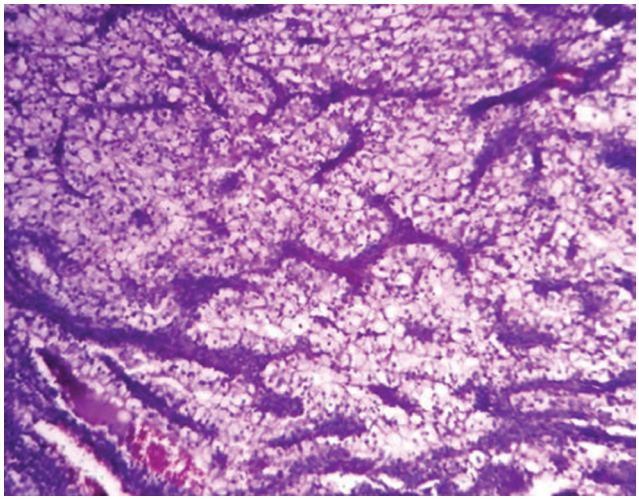


Fig. 8 Show clear cells arranged in nests separated by a fibrovascular septa – Clear cell renal cell carcinoma [H&E, 40x].

are all absent in clear cell papillary renal cell carcinoma[29]. HIF pathway is activated by VHL independent pathway in clear cell papillary renal cell carcinoma. Liddell et al. in their case study had reported it in a forty two-year-old female[30]. In contrast, in our study group there was an equal sex incidence. Immuno histochemically, these tumors are positive for CK7 [Papillary RCC marker], CA 1X [Clear cell RCC marker] and negative for CD10 [Clear cell RCC marker] and AMACR [Papillary RCC marker] thus exhibiting overlapping features of both clear cell RCC and papillary RCC.

Clear cell renal cell carcinoma constituted 11% of all the malignant tumors reported during our study period. In contrast, its incidence was 97% Rafique M' study, it was 51% in Lopez et al., 80% in Aiman et al. and 76% in Eggener et al.[5,6,31,32] Microscopically, tumor cells are arranged in sheets, nests, alveolar, acinar and microcystic or macrocystic structures, separated by thin-walled blood vessels. Individual cells have distinct cell membranes and clear cytoplasm (Fig. 8). Presence of tumor necrosis is a poor prognostic factor. Fuhrman's nuclear grade was grade I in our study in contrast to Aiman et al. and Popet et al.[5,7] This tumor is positive for EMA, RCC ma, CA IX and CD10 immunohistochemical markers.

Papillary renal cell carcinoma was seen in 11% of the cases in our study group. Papillary renal cell carcinoma was seen in 16% of the cases in Stîngă et al.[33] Microscopically, papillary renal cell carcinoma is composed of papillary and tubular structures lined by a single layer of tumor cells. The stalks contain fibrovascular cores, infiltrated by foamy macrophages–Type I tumor (Fig. 9). Type II tumors have thick papillary core lined by cells with eosinophilic cytoplasm. Type I tumors have a better prognosis than Type II tumors. Fuhrman's nuclear grade of this tumor was grade one in our study in contrast to Popat et al.[7] Immunohistochemical markers that are positive in this tumor include AMACR, CK7 and CD15.

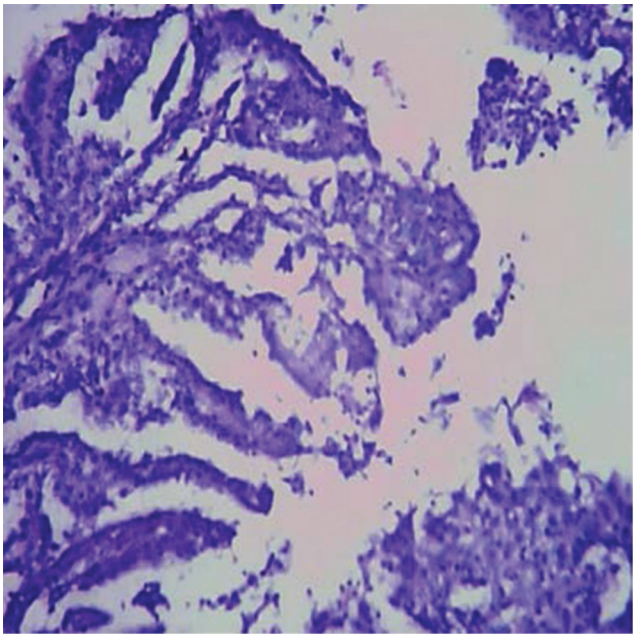


Fig. 9 Show tumor cells arranged in a papillary pattern with a thin fibrovascular core [H&E,10x].

CONCLUSION

Both benign and malignant tumors were seen in the received nephrectomy specimens during our study period. Chronic pyelonephritis was the commonest benign lesion. Wilms' tumor was the commonest pediatric tumor and renal cell carcinoma was the commonest adult tumor encountered in our study. This histopathological spectrum correlated well with the available literature studies except the fact that the incidence of chronic pyelonephritis had declined in the developed countries.

REFERENCES

1. Ozen H, Colowick A, Freiha FS. Incidentally discovered solid renal masses: what are they? Br J Urol. 1993;72:274–6.
2. Singh EO, Benson RC Jr, Wold LE. Cancer-to-cancer metastasis. J Urol. 1984;132:340–343.
3. Chisholm GB, Roy RR. The Systematic effects of malignant renal tumors. Br J Urol. 1971;93:687–700.
4. Ghalayini IF. Pathological spectrum of nephrectomies in a general hospital. Asian J Surg. 2002;25:163–9.
5. Aiman A, Singh K, Yasir M. Histopathological spectrum of lesions in nephrectomy specimens: A five-year experience in a tertiary care hospital. J Sci Soc. 2013;40:148–54.
6. Rafique M. Nephrectomy: Indications, complications and mortality in 154 consecutive patients. J Pak Med Assoc. 2007;57:308–11.
7. Popat VC, Kumar MP, Udani D, Mundra MP, Vora DN, Porecha MM. A study on culprit factors ultimately demanding nephrectomy. Internet J Urol. 2010;7.
8. Adamson AS, Nadjmaldin AS, Atwell JD. Total nephrectomy in children: A clinicopathological review. Br J Urol. 1992;70:550–3.
9. Hodson CJ, Edwards O. Chronic pyelonephritis and vesicoureteral reflux. Clin Radiol. 1960;11:219–231.
10. Beisland C, Medby PC, Sander S, Beisland HO. Nephrectomy-indications, complications and post-operative mortality in 646 consectutive patients. Eur Urol. 2000;37:58–64.
11. Kubba AK, Hollins GW, Deane RF. Nephrectomy: changing indications, 1960–1990. Br J Urol. 1994;74:274–8.
12. Potter EL. Normal and abnormal development of the kidney. Chicago: Year Book Medical Publishers. 1972.
13. Salma M, Kouser T, Nasar AM. A histopathologic study of 50 nephrectomy specimens. Indian Journal of Pathology and Oncology. 2016; 3:421–426.
14. Maizals M, Simpson SB. Primitive ducts of renal dysplasia induced by culturing ureteral buds denuded of condensed renal blastema. Science. 1983;219:509–510.
15. Rosdon RA. Renal Dysplasia part 1 clinico pathological study of 76 cases. Part II A necroscopy study of 41 cases. J Clin Pathol. 1971;24:57.
16. Amina NA, Paib P, Upadhyaya K. A Histopathological Spectrum Of Nephrectomy Specimens In A Tertiary Hospital In Southern India. Int J Biol Med Res. 2015;6:5173–78.
17. Marumo K, Horiguchi Y, Nakagawa K, Oya M, Ohigashi T, Asakura H, et al. Incidence and growth pattern of simple cysts of the kidney in patients with asymptomatic hematuria. International J Urol. 2003;10:63–7.
18. Kissane JM, Smith MG. Pathology of infancy & childhood Ed. 2. St. Louis C. V. Mosby. 1975:587.
19. Mardi K, Sharma J, Mahajan P. Mucinous cystadenoma of the renal pelvis with malignant transformation: A case report. Indian J Pathol Microbiol. 2006;49:595–6.
20. Ross DG, D'Amato NA. Papillary mucinous cystadenoma of probable renal pelvic origin in a horseshoe kidney. Arch Pathol Lab Med. 1985;109: 954–955.
21. Toyoda H, Mabuchi T, Fukuda K. Mucinous cystadenoma with malignant transformation arising in the renal pelvis. Pathol Int. 1997;47:174–178.
22. Arakawa M, Jimi A, Ootomi M. A mucin-producing cystadenoma (borderline malignancy) of the renal pelvis and ureter: a case report. Jpn J Cancer Clin. 1989;35:499–504.
23. Bell ET. Renal diseases, 2nd edn. Philadelphia: Lea & Febiger, 1950.
24. Lemerle J, Tournade MF, Marchant RG, Flamant R, Sarrazin D, Flamant F, et al. Wilms' tumor: natural history and prognostic factors: a retrospective study of 248 cases treated at the Institut Gustave-Roussy 1952–1967. Cancer. 1976;37:2557–66.
25. Abdulkareem SA, Hassawi AB, Ahmed Z. Nephrectomy. A clinicopathological study. Journal of American Science. 2015;11:97–101.
26. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell. 1990;60:509–520.
27. Motzer RJ, Bancer NH, Nanus DM. Renal cell carcinoma. N Engl J Med. 1996;355:865–75.
28. Tickoo SK, dePeralta-Venturina MN, Harik LR, Worcester HD, Salama ME, Young AN, et al. Spectrum of epithelial neoplasms in end-stage renal disease: an experience from 66 tumor-bearing kidneys with emphasis on histologic patterns distinct from those in sporadic adult renal neoplasia. Am J Surg Pathol. 2006;30:141–53.
29. Aydin H, Chen L, Cheng L, Vaziri S, He H, Ganapathi R, et al. Clear cell tubulopapillary renal cell carcinoma: a study of 36 distinctive low-grade epithelial tumors of the kidney. Am J Surg. Pathol. 2010;34: 1608–1621.
30. Liddell H, Mare A, Heywood S, Bennett G, Chan HF. Clear Cell Papillary Renal Cell Carcinoma: A Potential Mimic of Conventional Clear Cell Renal

Carcinoma on Core Biopsy. Case Reports in Urology. 2015;2015:423908: 1–4.
31. López JI, Moreno V, García H, Antón I, Robles A, Oñate JM, et al. Renal cell carcinoma in young adults: a study of 130 cases and a review of previous series. Urol Int. 2010;84:292–300.
32. Eggener SE, Rubenstein JN, Smith ND, Nadler RB, Kontak J, Flanigan RC, et al. Renal tumors in young adults. J Urol. 2004;171:106–10.
33. Stîngă AC, Stîngă SA, Simionescu C, Mărgăritescu C, Cruce M. Histopathological Study of Renal Cell Carcinoma. Current Health Sciences Journal. 2009;35:50–55.

Case Report

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Pathology Section

Pancreatic Plasmacytoma Misdiagnosed as Neuroendocrine Tumour: A Pathologist's Dilemma

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ABSTRACT

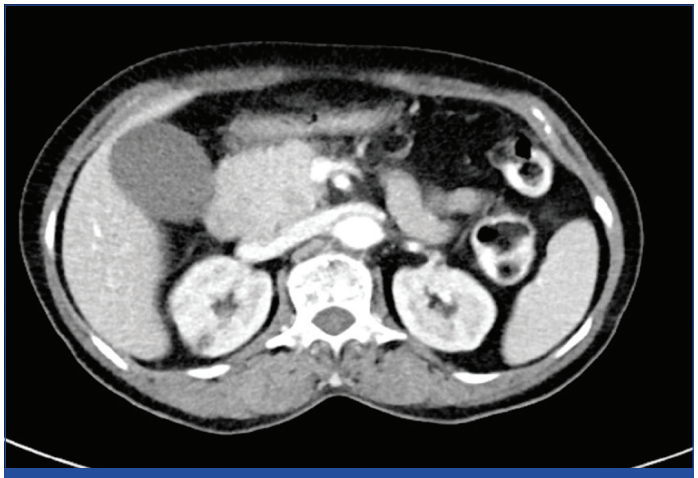
Extramedullary plasmacytomas are rare, constituting to about 5% of plasma cell neoplasms. Pancreatic involvement is uncommon constituting to about 2.3%. Localised solitary plasmacytomas has a good response to radiation. When they are associated with pre-existing multiple myeloma, their prognosis is poor. A case of plasmacytoma of pancreas presenting with obstructive jaundice preceding plasma cell leukaemia is presented here. The lesion was misdiagnosed by fine needle aspiration cytology as neuroendocrine neoplasm. The patient did not present with symptoms of myeloma initially. The present case highlights the significance of considering other causes of pancreatic mass than primary carcinomas.

Keywords: Extramedullary plasmacytoma, Pancreatic neoplasm, Plasma cell leukaemia

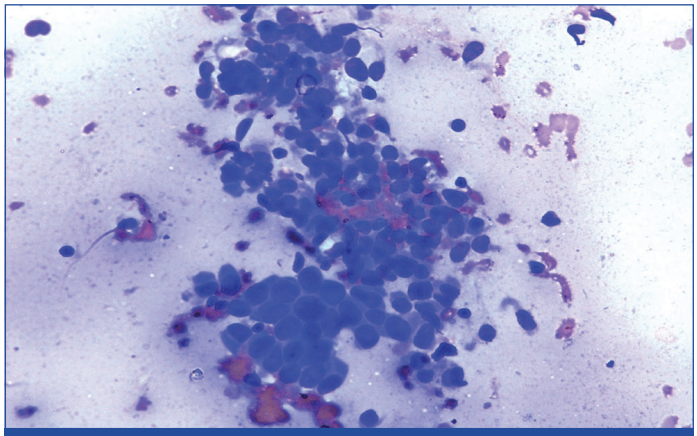
CASE REPORT

A 62-year-old female presented with complaints of fever on and off for 15 days, a vague, non-radiating, intermittent right upper quadrant, paraumbilical pain and vomiting for five days. There was no history of gastrointestinal bleed, loss of weight and loss of appetite. She was a known hypertensive and diabetic for five years and was on treatment with oral hypoglycemics and antihypertensives. Her vitals were within normal limits and there was no organomegaly.

Ultrasound abdomen showed a hypoechoic mass in the pancreatic head dilating the main pancreatic duct. Computed Tomography (CT) revealed an isodense mass lesion with well defined borders in the head of pancreas measuring 4.8×5.5 cm [Table/Fig-1]. Acoustic Radiation Force Impulse (ARFI) elastography showed mean shear velocity of 3.6. The provisional diagnosis included primary pancreatic tumour. Endoscopic Ultrasound Guided Fine Needle Aspiration Cytology (EUS-FNAC) was planned for the evaluation of the pancreatic mass. EUS showed a 3×3.1 cm iso and hypoechoic mass in the head of pancreas with dilated common bile duct measuring 9 mm in the suprapancreatic portion. Dilated main pancreatic duct (6.5 mm) distal to the mass in the pancreas was identified. Visualised tail of pancreas and portal vein appeared normal. Cytological examination of the mass showed sheets and scattered plasmacytoid cells suspicious of a neuroendocrine neoplasm [Table/Fig-2].



[Table/Fig-1]: CT scan showing isodense lesion in head of pancreas.



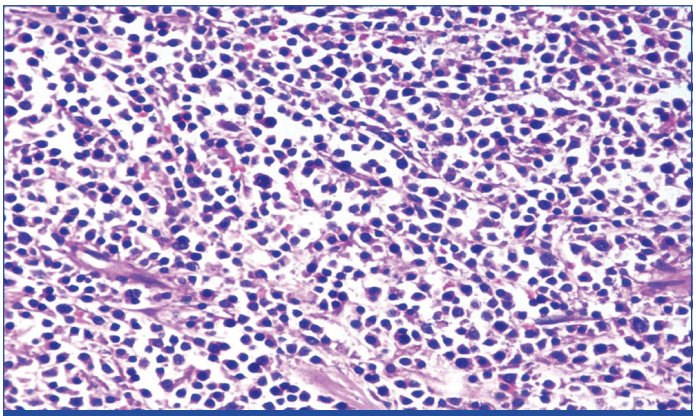
[Table/Fig-2]: Cytology smears showing plasmacytoid cells (40x).

Whipple resection was done with a clinical and cytological diagnosis of a pancreatic neuroendocrine tumour. Pancreas showed an ill-defined mass occupying the head [Table/Fig-3]. Haematoxylin & Eosin (H&E) sections of the mass showed a diffusely infiltrating neoplasm composed of monotonous, plasmacytoid cells infiltrating the acini and ducts [Table/Fig-4]. Few binucleate and multinucleate plasma cells and lymphocytes were present. Morphological differential diagnosis included plasmacytoma and lymphoplasmacytic lymphoma. Immunohistochemical staining was done for confirmation of diagnosis. The tumour cells showed strong membranous positivity for CD138 [Table/Fig-5], CD45 and selective Kappa staining [Table/Fig-6]. They were negative for CD20 and Lambda immunostains. A diagnosis of Plasmacytoma was made and radiological and biochemical workup for multiple myeloma was suggested. Protein electrophoresis was normal. Urine for Bence Jones Protein was negative. Serum calcium and renal parameters were normal. CT spine survey did not reveal any osteolytic lesions. The case was treated as a solitary extramedullary plasmacytoma and suggested follow-up. A month later, the patient developed severe anaemia (Hb-6.8 g/dL). Total count was elevated and peripheral smear showed 40% plasma cells suggestive of plasma cell leukaemia [Table/Fig-7]. Reviewing the patient's peripheral smear and complete haemogram taken a month back, there was normocytic normochromic anaemia and no demonstrable plasma cells in peripheral smear. ESR was elevated (93 mm/hr). Bone marrow aspiration done showed infiltration by sheets of immature

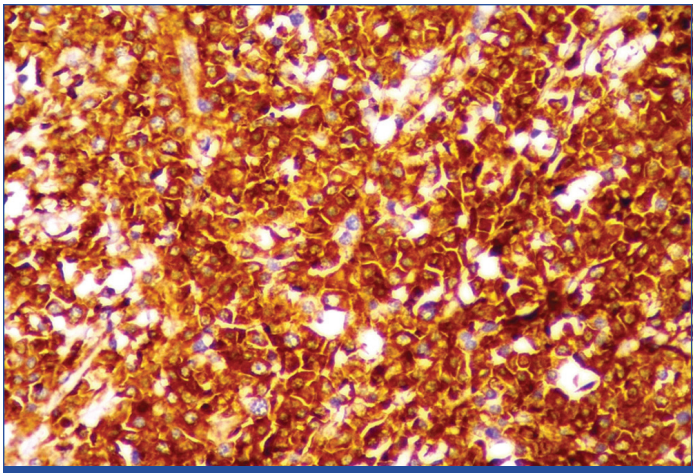
and mature plasma cells. On immunohistochemistry, these cells showed kappa restriction. A diagnosis of plasma cell leukaemia was made. Investigations done at presentation and a month later are tabulated [Table/Fig-8]. The patient was referred to higher centre for further treatment.



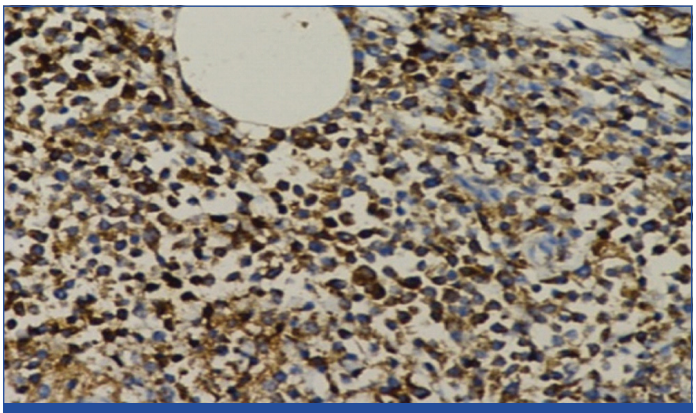
[Table/Fig-3]: Whipple's specimen showing ill-defined lesion in pancreas.



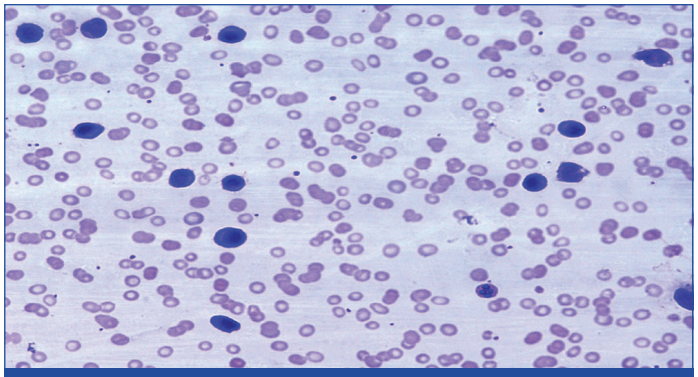
[Table/Fig-4]: Diffuse infiltration by plasma cells (H&E, 40x).



[Table/Fig-5]: Strong membranous expression of CD138 by tumour cells (IHC, 40x).



[Table/Fig-6]: Neoplastic cells with kappa expression (IHC, 40x).



[Table/Fig-7]: Peripheral smear with plasma cell leukaemia (40X).

Parameters	At initial presentation	A month later
Serum urea	42 mg/dL	81 mg/dL
Serum creatinine	1.06 mg/dL	3.82 mg/dL
Direct bilirubin	3.3 mg/dL	1.8 mg/dL
Haemoglobin	9.5 g/dl	6.8 g/dL
Total WBC count	4.3×10^3/µL	30.5×10^3/µL
Peripheral smear	Normocytic anaemia	Plasma cell leukaemia
Serum calcium	1.116 mmol/L	1.178 mmol/L

[Table/Fig-1]: Biochemical and haematological investigations.

DISCUSSION

Plasma cell neoplasms include Plasma Cell Myeloma (PCM) which includes smouldering myeloma, non-secretory myeloma, plasma cell leukaemia and plasmacytoma which includes solitary bone and extraosseous plasmacytoma. In majority of the patients with PCM, features of end organ damage in the form of one or more of the following, hypercalcaemia, Renal insufficiency, Anaemia, and Bone lesions (CRAB) are present. The clonal proliferation usually begins in bone marrow. Renal failure occurs due to tubular damage caused by excessive synthesis of monoclonal immunoglobulins [1]. It constitutes 10 to 15% of haematological malignancies. Median age at diagnosis is 66 years [2]. The clinical spectrum varies from asymptomatic to highly aggressive.

Extramedullary Plasmacytomas (EMPs) are localised proliferation of monoclonal plasma cells forming solitary lesions outside the bone marrow. EMPs can be a manifestation of advanced multiple myeloma or can represent a solitary plasmacytoma without involving the bone marrow [3]. EMPs are uncommon and represent 5% of all plasma cell tumours. The most frequent site of involvement being upper respiratory tract followed by gastrointestinal tract and reticuloendothelial system [2].

Primary pancreatic plasmacytoma is rare constituting 2.3% of EMPs. Majority of the reported pancreatic plasmacytoma are secondary [3]. EMPs have the median age of presentation at 55 years (A decade younger than patients with multiple myeloma) and the majority (three-fourths) are males [4].

Most of these lesions are located in the pancreatic head. Diffuse enlargement and presenting as mass in body (4 cases) [5] and tail of the pancreas have also been reported. The typical presenting symptoms include abdominal pain and obstructive jaundice from bile duct compression when there is involvement of head. Diffuse lesions are picked up rather late as they tend to be asymptomatic [6]. The case presented here also had obstructive jaundice raising a concern for primary pancreatic carcinomas [7].

Radiologically plasmacytoma is characterised by a homogenously enhancing multilobular, solid mass on CT, which is non-specific and resemble that of neuroendocrine tumours or carcinoma [6]. The case described presented as an isodense lesion in the pancreatic head. Differentiating plasmacytomas radiologically from other pancreatic tumours is challenging.

Endoscopic Ultrasound (EUS) is a well recognised technique for confirmation of pancreatic masses by histology. EUS- FNAs are reported to have an accuracy of 78-95% in the diagnosis of pancreatic masses. Neuroendocrine tumour presents as a, homogeneous, isoechoic or hypoechoic mass on EUS while plasmacytoma shows heterogenecity with uneven margins. NETs are hypervascular [5,8].

When FNA was done, the lesion was morphologically misdiagnosed as neuroendocrine neoplasm since both share plasmacytoid morphology. In a rare site, one would be more likely to suspect a primary lesion with plasmacytoid features, particularly endocrine neoplasm. Islet cell tumours have been reported to be a great mimic of plasmacytoma with regards to clinical, cytological and radiological appearance [9]. EUS guided core biopsy from the lesion and immunophenotyping with cytokeratin for carcinoma and CD138, kappa and lambda for identifying monoclonality of plasma cells would have helped in arriving at the diagnosis. EUS guided FNA also serves as a platform for obtaining material for flow cytometry.

The patient described here did not present with any symptoms pertaining to myeloma prior to surgery. The patient progressed to plasma cell leukaemia within a short time (one month) which is evident from the investigations [Table/Fig-8]. Plasma cell leukaemias are primary in 60-70% of cases and secondary to multiple myeloma in 30-40 % cases [10,11].

To our knowledge, this is the first case of plasmacytoma of pancreas being the initial manifestation of a plasma cell leukaemia. Two other cases of plasma cell leukaemia have been reported in association with gastric plasmacytoma. One case reported also had a normal WBC and platelet count with normocytic normochromic anaemia three weeks prior to the diagnosis of gastric plasmacytoma. The authors had hypothesised that secondary plasma cell leukaemia would have occurred as a result of rapid transformation of possibly underdiagnosed light chain multiple myeloma. The present case could also represent a similar rapid transformation from a non-secretory myeloma presenting with an extramedullary plasmacytoma [12].

From the previous reports it is clear that in any mass lesion of a solid organ in a patient with multiple myeloma the possibility of secondary plasmacytoma is to be considered [3,4,7]. Present case in addition highlights the fact that plasmacytoma is to be considered when plasmacytoid cells are identified in EUS-FNAs even when previous diagnosis of myeloma or symptoms pertaining to myeloma is not there at presentation. Immunocytochemistry can be used as an ancillary technique to diagnose with certainty in such cases. Presurgical diagnosis is essential as the management varies between primary pancreatic carcinoma and plasmacytoma.

CONCLUSION(S)

This case highlights the importance of considering other causes of pancreatic head mass than a primary carcinoma in a patient with obstructive jaundice. It also emphasises the fact that pathologists must be aware of the existence of pancreatic plasmacytoma as initial manifestation of myeloma despite its rarity, a high index of suspicion being essential.

REFERENCES

[1] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). IARC: Lyon 2017.
[2] Pallavi R, Ravella PM, Popescu MA. An unusual pancreatic mass: A case report and literature review. Transl Gastrointest Cancer. 2014;3:106-10.
[3] Hue SSS, Azhar K. Plasmacytoma of the pancreas: An unusual manifestation of multiple myeloma. Singapore Med J. 2013;54:105-07.
[4] Padda MS, Milles T, Adeniran AJ, Mahootic S, Aslaniana HR. Pancreatic and gastric plasmacytoma presenting with obstructive jaundice, diagnosed with endoscopic ultrasound-guided fine needle aspiration. Case Rep Gastroenterol. 2010;4:410-15.
[5] Lu T, Pu H, Zhao G. Primary pancreatic plasmacytoma: A rare case report. BMC Gastroenterol. 2017;17:167.
[6] Smith A, Hal H, Frauenhoffer E. Extramedullary plasmacytoma of the pancreas: A rare entity. Case Reports in Radiology. 2012;2012:798264.
[7] Williet N, Dumas O, Rinaldi L. Difficult endoscopic diagnosis of a pancreatic plasmacytoma: Case report and review of literature. World Journal of Clin Oncolog. 2017;8:91-95.
[8] Kim JH, Paik WH, Joo M, Kim JG, Kim JW, Bae WK, et al. Extramedullary plasmacytoma mimicking pancreatic cancer: A case report and literature review. Endosc Ultrasound. 2017;6:269-72.
[9] Dodd LG, Evans DB, Symmans F, Katz RL. Fine-needle aspiration of pancreatic extramedullary plasmacytoma: Possible confusion with islet cell tumour. Diagn Cytopathol. 1994;10:371-75.
[10] Singh S, Rath A, Yadav S. Primary plasma cell leukaemia-Case report and review of the literature. Sultan Qaboos University Med J. 2018;18(3):397-401.
[11] Jain AG, Faisal-Uddin M, Khan AK, Wazir M, Shen Q, Manoucheri M. Plasma cell leukaemia-one in a million: A case report. World J Clin Oncol. 2019;10(3):161-65.
[12] Puria A, Laua M, Zhaoa X, Chana K, Badera K. A rare case of a minimally secretory pasma cell leukaemia with a haemorrhagic gastric plasmacytoma. Leukaemia Research Reports. 2018;9:23-27.

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Morphological Spectrum of Peripheral T Cell Lymphomas and Utility of Immunohistochemistry in the Diagnosis

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Abstract

Context: Peripheral T cell lymphomas (PTCLs) are uncommon subtypes of non Hodgkin lymphoma (NHL). The diagnosis is often challenging and not infrequently misdiagnosed. *Aims:* To analyse the morphological and immunohistochemical profile of Peripheral T cell lymphoma. *Settings and Design:* This study was carried out at the Department of Pathology, where cases were recruited using consecutive sampling method. *Methods and Material:* Immunohistochemical staining was performed in all cases of lymphoma and results were analysed. *Statistical Analysis Used:* Frequency distribution of the diagnoses was studied. *Results:* There were six casesof Peripheral T cell lymphoma – unspecified (CD3+, CD20-), two cases of Angioimmunoblastic T cell lymphoma (AITL)(CD3+, CD10+, CD23+), one case of Lennert's lymphoma(CD3+,CD20-) and a hepatosplenic T cell lymphoma(CD3+,CD2+). *Conclusions:* PTCLs constituted for 20% of NHLs. Immunohistochemistry was helpful in diagnosis when differentiation from Hodgkin lymphoma was difficult morphologically.

Keywords: Angioimmunoblastic T Cell Lymphoma; Lennert's Lymphoma; Lymphoma; Non Hodgkin Lymphoma; Peripheral T Cell Lymphoma.

Introduction

The World Health Organization (WHO), 2008 [1] classifies mature T cell / NK cell neoplasm into four categories (nodal, extranodal, cutaneous and leukemic). Nodal group includes peripheral T cell lymphoma unspecified, angioimmunoblastic T cell and anaplastic large cell lymphoma. PTCLs constitute 4 to 12% in western [2,3] and 12-26 % in eastern countries [4]. Indian studies have shown a frequency of 9 to 12% [5]. PTCLs show a higher morphological and immunological diversity than any other lymphoma. Hence the diagnosis of PTCL remains a challenge to the histopathologist.Thus, this study aims to analyze the morphological and immunohistochemical features of PTCL, for better understanding of this rare variant.

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Key Messages

Immunohistochemistry (CD3, CD20, CD15, CD30, CD23, and CD5) is essential in differentiating peripheral T cell lymphoma from its mimics (Hodgkin lymphoma and reactive conditions) because of its varied morphological pattern on routine hematoxylin and eosin stained sections

Subjects and Methods

Between August 2011 and August 2013, 52 consecutive cases diagnosed as lymphoma in the histopathology department in a tertiary care hospital were included in the study.

Inclusion Criteria

Patients of all ages and both genders with both primary and/or extranodal lymphomas (treatment naïve) were included in the study.

Exclusion Criteria

Samples inadequate for light microscopy and

immunohistochemistry were excluded. Similarly, patients who were on treatment for lymphoma were excluded.

For histological and immunohistochemical studies, the samples were received in neutral buffered formalin. Routine Hematoxylin and eosin staining was done to study the pattern, cellular morphology and presence of key diagnostic features. A panel of immunohistochemical markers was chosen based on the histological differential diagnosis. For immunohistochemistry, 4μ sections were taken in specially coated slides. Slides were then deparaffinised and rehydrated in ethanol and xylene. Antigen retrieval was done by microwaving using two methods 1) by incubating sections with a Tris-EDTA solution, pH 9.0 for 20 minutes before staining with antibodies against CD3, CD15, CD30, CD2, TDT, CD23 and CD10 (mouse, monoclonal, biogenex), and 2) by incubating sections with 0.01 M citrate buffer solution, pH 6.0, for 20 min before staining with antibodies against CD45, CD20 (mouse, monoclonal, biogenex). The slides were then incubated under humid conditions at room temperature for 90 minutes.

The antibodies bound to antigens were detected by addition of secondary antibody conjugated with horse radish peroxidase polymer and diaminobenzidine substrate. The slides were counterstained with hematoxylin. Appropriate positive controls for the primary antibodies as per the manufacturer's instruction manual (biogenex) were used. The immunohistochemically stained slides were analyzed for the presence/absence of reaction and cellular localization. The positive reaction in the neoplastic cells was assessed. This study was conducted with approval of the Institutional ethical committee, in accordance with the ICMR guidelines for human research.

Statistical Analysis

Frequency distribution of gender and diagnoses were studied.

Results

Of the 52 cases of lymphoma, ten were peripheral T cell lymphomas. These ten cases included seven males and three females. The age of the patients ranged from four years to 90 years. All except one, presented with peripheral lymphadenopathy while the other presented with hepatosplenomegaly.

The diagnosis was made based on World Health Organization (WHO) 2008 classification. Six cases were diagnosed as Peripheral T cell lymphoma-unspecified (PTCL-U). These cases showed a heterogenous population of cells which were small to large with pleomorphic nuclei with irregular nuclear contours and variable amount of cytoplasm (Figure 1). Reactive population of cells included plasma cells, lymphocytes and eosinophils. Vascular channels were prominent in five cases. Occasional binucleated cells were seen in two cases (Table 1). The differential diagnosis included Hodgkin lymphoma as the reactive population of cells was seen in all the six cases and T cell rich B cell lymphoma in two cases as there were large neoplastic cells admixed with small lymphoid cells. Immunohistochemistry demonstrated absence of CD15, CD30 and CD20 staining in large cells. The neoplastic cells demonstrated membrane and cytoplasmic staining for CD3 (Figure 2) and CD2 (Table 2).

Two cases were diagnosed as angioimmunoblastic T cell lymphoma. Of the two cases one showed complete effacement of lymph node architecture by diffuse proliferation of small to medium sized lymphoid cells with scattered eosinophils and few histiocytes. The other showed partially preserved lymph node architecture with follicular hyperplasia and paracortical expansion by the polymorphic infiltrate of cells with few cells having clear cytoplasm (Figure 3 and 4). Pale eosinophilic areas comprising of cells with eosinophilic cytoplasm and indistinct cell membrane were observed in one case. Numerous branching and arborizing vascular channels lined by plump endothelial cells and few immunoblast like cells were noted in both the cases. Immunohistochemistry demonstrated CD 3 (Figure 5) and CD10 positivity. CD23 staining highlighted the extrafollicular dendritic cell network (Table 3) (Figure 6).

One case showed loss of nodal architecture by diffuse proliferation of small lymphoid cells and confluent clusters of epithelioid cells (microgranulomas). Scattered large atypical cells and few reactive cells composed of eosinophils and plasma cells were seen. There were no classical Reed Sternberg cells. This was diagnosed as Lennert's lymphoma. The differential diagnosis included reactive lymphadenitis and Hodgkin lymphoma. Immunohistochemistry showed absence of CD15 and CD30 in the large atypical cells, thus excluding Hodgkin lymphoma (Table 3).

In the single extranodal case, the site of biopsy was liver as the patient (four years old female) presented with hepatosplenomegaly without any lymphadenopathy. Histology showed sinusoidal

expansion by dense infiltration of monotonous small to medium sized lymphoid cells. Periportal involvement was also noted. Hepatocytes appeared normal. This was diagnosed as Hepatosplenic T Cell Lymphoma with a differential diagnosis of acute lymphoblastic leukemia. On immunohistochemistry, the lymphoid cells were positive for CD3, CD2 and negative for Terminal deoxynucleotidase(TdT) and thus Lymphoblastic leukemia was ruled out because of TdT negativity (Table 3).

Table 1: Morphological features of PTCL- U

Morphological Features	Distribution of the features (N = 6)
Effacement of architecture	6 (100%)
Heterogenous population of cells	6 (100%)
Reactive population of cells	5 (83%)
Reed Sternberg like cells	2 (33%)
Prominent vascular channels	2 (33%)

Table 2: Immunhistochemical profile of PTCL-U

IHC	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
CD45	+	+	+	+	+	+
CD3	+	+	+	+	+	+
CD2	ND	+	+	+	+	+
CD20	+ in reactive cells	-	-	-	Focally +	-
CD15	-	-	-	-	-	-
CD 30	-	-	-	-	-	-

ND – Not done, IHC- Immunohistochemistry

Table 3: Morphological and immunohistochemical features of other PTCLs

	Clinical features	Morphology	IHC	Diagnosis
1	70/M cervicalnode	Microgranulomas and small lymphoid infiltrates	Positive for CD45,CD3,CD2 and negative for CD20, CD15,CD30	Lennerts lymphoma
2	50/F cervical node	Arborizing vascular channels , polymorphic infiltrates and immunoblast like cells	Positive for CD3, CD10 AND CD23. Negative for CD20, CD15, CD30.	Angioimmunoblastic T cell lymphoma
3	50/M inguinal node			
4	4/F, liver biopsy	Sinusoidal expansion by atypical lymphoid cells	Positive for CD3, CD2. Negative for CD20, TdT.	Heptosplenic T cell lymphoma

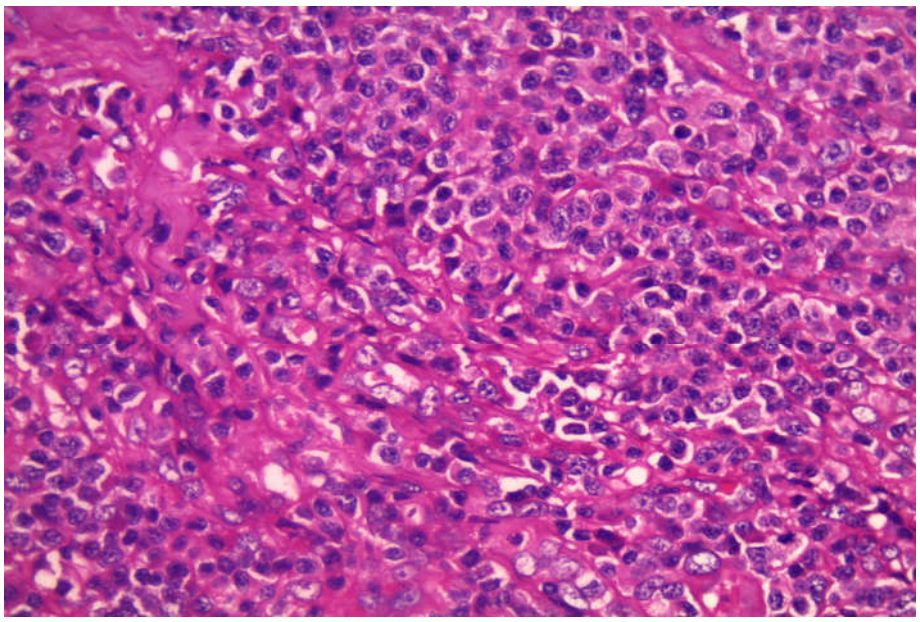


Fig. 1: Peripheral T cell lymphoma–U: heterogeneous population of cells with few reactive cells (40X, original magnification)

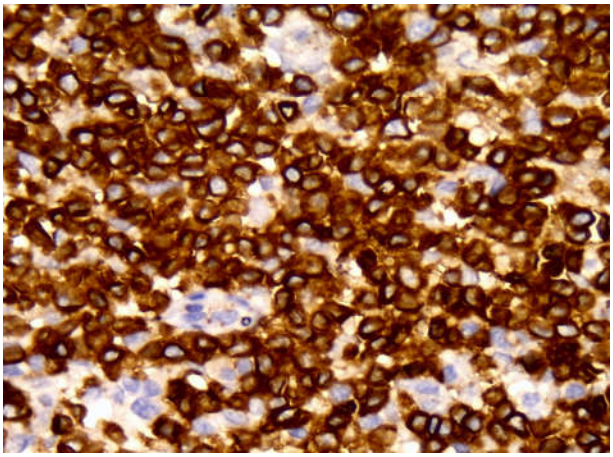


Fig. 2: *Peripheral T cell lymphoma–U:* CD3 positive tumour cells (40X, original magnification)

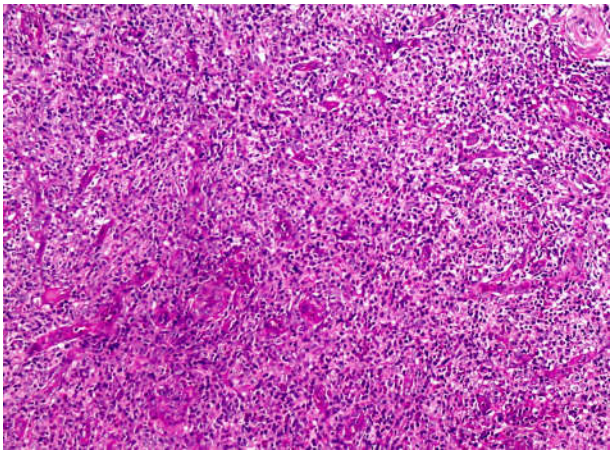


Fig. 3: *Angioimmunoblastic T cell Lymphoma:* arborizing vascular channels, effacement of architecture (4X, original magnification)

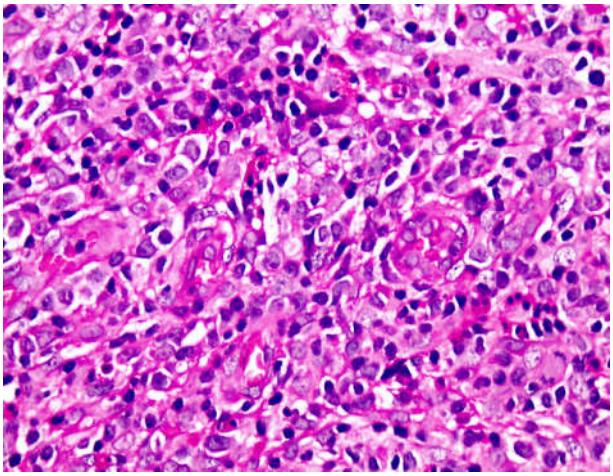


Fig. 4: *Angioimmunoblastic T cell Lymphoma:* neoplastic cells (few with clear cytoplasm), prominent vascular channels and eosinophils. (40X, original magnification)

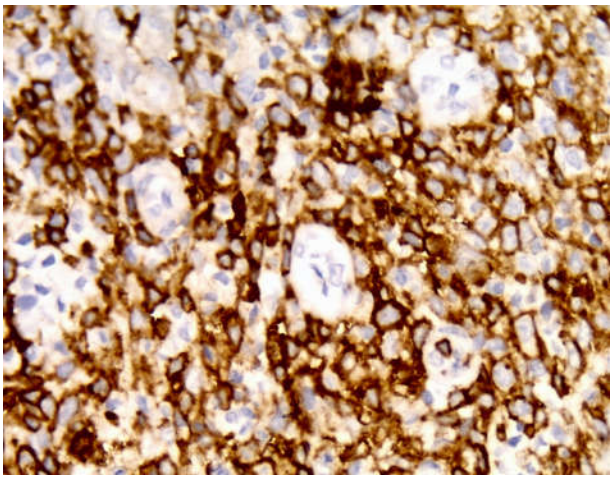


Fig. 5: *Angioimmunoblastic T cell Lymphoma:* CD3 positive neoplastic cells. (40X, original magnification)

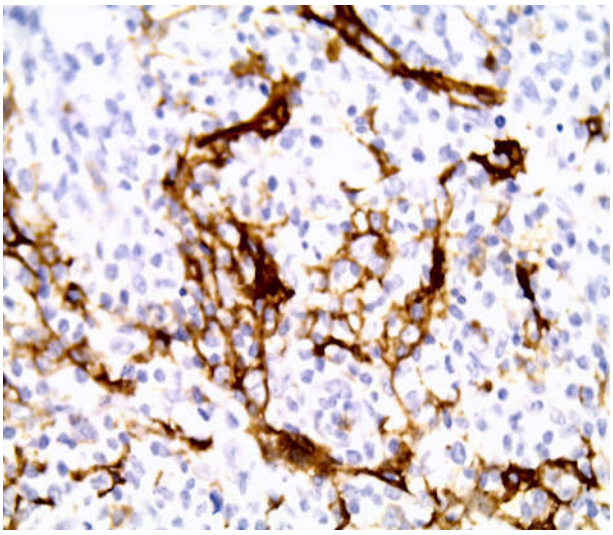


Fig. 6: *Angioimmunoblastic T cell Lymphoma:* Extrafollicular follicular dendritic cell network highlighted by CD 23 staining (40X, original magnification)

Discussion

In the present study, PTCL constitutes 20 % of NHL. This is much higher when compared to other Indian studies [5]. In our study, Nodal PTCLs accounted for about 90% of PTCLs.

PTCL U is a heterogenous group of lesions which do not fit into any of the subtypes of peripheral T cell lymphoma and thus is a diagnosis of exclusion. It is the most common subtype of Mature T cell lymphoma, typically occurring in adults at sixth decade. Lymphadenopathy is the usual presentation. In this study PTCL- U accounts for about 60% of PTCLs which is comparable to other studies [5,6]. As found in other studies, there was a male predominance and 90% of cases had lymphadenopathy. The immuno-histochemistry demonstrated positivity for T cell markers CD3 and CD2 and also showed abberant loss of CD5 expression.

Lennert's lymphoma (lymphoepithelioid lymphoma) is a variant of PTCL-U. It is a rare entity displaying monomorphic small tumor cells, few scattered immunoblasts, epithelioid cells in clusters and absence of high endothelial venules and follicular dendritic cells (FDC) networks [7,8]. In our study, the patient presented with bilateral cervical lymphadenopathy and hepatosplenomegaly. The lymph node showed the unique histological features characterized by epithelioid cell clusters and infiltrates of small lymphoid cells. Immunoblastic cells were noted in this case. CD30 positive cells were not identified.

Angioimunoblastic T cell lymphoma forms approximately 15-20% of PTCLs [9,10]. In the present study, it constituted about 20 % of PTCLs. AITL is a systemic disease clinically characterized by lymphadenopathy, hepatosplenomegaly and skin rash. Polyclonal hypergammaglobulinemia and hemolytic anemias are other associations [10]. Both the patients in the present study presented with lymphadenopathy and hepatosplenomegaly while one patient also had skin rash. The presence of hemolytic anemia and hypergammaglobulinemia were not evaluated. However they showed the classical morphological findings which include arborizing high endothelial venules, polymorphic infiltrates and presence of clear cells. The vascularity was more pronounced in AITL when compared to other PTCLS. Extrafollicular follicular dendritic cell proliferation was evident in one case. CD 23 staining confirmed the presence of this follicular dendritic cell meshwork. In the other case, where extrafollicular dendritic cell network was not evident on H & E staining, immunostaining with CD23 showed the presence of these cells.

Hepatosplenic T cell lymphoma is a rare and aggressive subtype of extranodal peripheral T cell lymphoma. HSTL accounts for less than 1% of NHL and about 3% of T cell lymphoma [11]. It occurs more frequently in immunocompromised patients. It occurs predominantly in adolescents and young adults. However in this study, the patient was a 4 year old female who presented with abdominal distension and fever without any history of immunosuppression. The diagnosis was made based on morphology and immunohistochemistry (CD 3 positivity). TCR rearrangement analysis could have helped in analyzing the variant.

We acknowledge that the sample size was small to generalize our results, but in view of rarity of this Peripheral T Cell lymphoma, our study will help in differentiating PTCL from its mimics like Hodgkin lymphoma and thus by itself may guide further treatment modalities.

Conclusion

In our study, the frequency of Peripheral T cell lymphomas is higher when compared to other Indian studies. Knowledge about the various morphological patterns and cellular composition of PTCL is essential to select the panel of immunohistochemistry markers. In our study we found that morphology when combined with immunohistochemistry is more effective in differentiating PTCL from Hodgkin lymphoma and T cell rich B cell lymphoma and also to classify various PTCLs. We by this study, conclude that immunohistochemistry becomes essential in the diagnosis of PTCL.

References

1. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: Evolving concepts and practical applications. Blood. 2011; 117: 5019-32.
2. Vose J, Armitage J, Weisenburger D; International T-Cell Lymphoma Project. International peripheral T-cell and natural killer/T-cell lymphoma study: Pathology findings and clinical outcomes. J Clin Oncol. 2008; 26: 4124-30.
3. Au WY, Ma SY, Chim CS, Choy C, Loong F, Lie AK, *et al*. Clinicopathologic features and treatment outcome of mature T-cell and natural killer-cell lymphomas diagnosed according to the World Health Organization classification scheme: A single center experience of 10 years. Ann Oncol. 2005; 16: 206-14.
4. The world health organization classification of malignant lymphomas in japan: Incidence of recently recognized entities.Lymphoma Study Group of Japanese Pathologists. PatholInt. 2000; 50: 696-702.
5. Burad DK, Therese MM, Nair S. Peripheral T-cell lymphoma: Frequency and distribution in a tertiary referral centerin South India. Indian J Pathol Microbiol. 2012; 55: 429-32.
6. Niitsu N, Okamoto M, Nakamine H, Aoki S, Motomura S, Hirano M. Clinico-pathologic features and outcome of Japanese patients with peripheral T-cell lymphomas. Hematol Oncol. 2008; 26: 152-8.
7. Lennert K. Histopathology of non-Hodgkin lymphomas (based on the Kiel classification). Berlin: Springer; 1981.
8. Parimal S, Pai R, Manipadam MT, Nair S. Lennert's lymphoma: Clinicopathological profile of fi ve cases. Indian J Pathol Microbiol. 2013; 56: 248-51.

9. Ferry JA. Angioimmunoblastic T-cell lymphoma. AdvAnat Pathol. 2002; 9: 273-79.
10. Attygalle AD, Kyriakou C, Dupuis J, Grogg KL, Diss TC, Wotherspoon AC, *et al*. Histologic evolution of angioimmunoblastic T-cell lymphoma in consecutive biopsies: clinical correlation and insights into natural history and disease progression. Am J Surg Pathol. 2007; 31: 1077-88.
11. Weidmann E. Hepatosplenic T cell lymphoma. A review on 45 cases since the first report describing the disease as a distinct lymphoma entity in 1990. Leukemia. 2000; 14(6): 991–997.

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Xeroderma Pigmentosum and Bone Marrow Aplasia: A Rare Association

Abstract

Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder characterized by hypersensitivity to ultraviolet rays and predisposition to cutaneous malignancies. Hematological disorders associated with XP include myelodysplastic syndrome and acute leukemia. We report a 7-year-old child with XP along with squamous cell carcinoma and bone marrow aplasia.

Keywords: *Aplastic anemia, pancytopenia, squamous cell carcinoma, xeroderma pigmentosum*

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Introduction

Xeroderma pigmentosum (XP) is a rare inherited autosomal recessive disorder characterized by extreme hypersensitivity to ultraviolet rays predisposing to early cutaneous malignancies. Other abnormalities include neurological and ocular diseases.[1,2]

The incidence of XP varies across geographic areas, one in one hundred thousand in Europe and the US to one in 22,000 in Japan. The incidence in India has not been studied so far but is believed to be less when compared to the Western countries.[2,3]

There is no sex predilection. Pathophysiology of this inherited disorder is the defect in the nucleotide excision repair mechanism, and hence, the DNA damage caused by ultraviolet (UV) rays is unrepaired resulting in genomic instability which leads to predisposition to oculo-cutaneous malignancies as well as other internal malignancies. The other manifestations of XP include hyperpigmentation, premature aging, and neurological dysfunction. Photosensitivity and burning sensation after exposure to the sun precede the dryness (xeroderma) and pigmentation of skin and ocular manifestations such as photophobia and keratitis.[4,5]

Hematological abnormalities reported include myelodysplastic syndrome, which is characterized by refractory anemia with excess blast cells and leukemias.[6] A single case of XP in association with bone marrow failure has also been reported.[7]

Bone marrow aplasia in XP is very rare when compared to its presence in other DNA repair mechanism defects such as Fanconi anemia, Bloom syndrome, and ataxia telangiectasia.

We present here an interesting case of XP associated with bone marrow aplasia.

Case Report

A 7-year-old male child, a known case of XP, presented with a swelling in the right infraorbital region of short duration associated with pain and discharge from the lesion.

The diagnosis of XP was made based on the clinical findings in the previous admission when the child presented with dermatological symptoms, photosensitivity, itching over the face, and hyperpigmentation. Pigmentation progressed with age. Erythematous scaly plaques with pigmented macules were seen over the areas exposed to the sun. The child was born of a nonconsanguinous marriage and attained normal developmental milestones. There were no neurological manifestations.

In the current admission, complete hemogram showed pancytopenia (white blood cell count - 3.5 × 10^3/μl, red blood cell count - 1.01 × 10^6/μl, and platelet count - 21 × 10^3/μl). Peripheral smear revealed a macrocytic blood picture. There were no atypical cells in the smear [Figure 1].

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Bone marrow aspiration was done. The smears were paucicellular with adipocyte fragments and scant hematopoietic elements. There were no features of dyspoiesis in any lineage [Figure 2].

Bone marrow trephine was markedly hypocellular with fatty marrow and minimal hematopoietic tissue. There was no evidence of an infiltrative disease [Figure 3].

Wide-local excision of the infraorbital swelling showed a moderately differentiated squamous cell carcinoma [Figure 4].

Discussion

XP is a rare genodermatosis caused by defects in the normal repair of DNA damaged by exposure to sunlight. There are eight complementation groups of XP (XP-A to G) caused by defective nucleotide excision repair, corresponding to mutations in the genes encoding XPA, XPB, XPC, XPD, XPE, XPF, XPG, and XP dominant. The incidence and the age at presentation vary, ranging from early infancy to adulthood. XP has been reported to be common with consanguineous marriages.[8]

There are a 1000-fold increased risk of development of cutaneous malignancies, especially in those diagnosed under the age of 2 years and a 10-20-fold increased risk of developing noncutaneous malignancies.[9]

The commonly reported cutaneous malignancies include squamous cell carcinoma followed by melanoma and basal cell carcinoma.

Our patient had squamous cell carcinoma of the face.

In addition, the patient had pancytopenia which suggested the possibility of an infiltrative disorder of bone marrow. Bone marrow metastasis of malignant melanoma presenting as pancytopenia has already been reported in cases with XP.[10] In our patient, bone marrow aspirate and trephine were consistent with aplastic anemia.

Review of the literature revealed that the common hematological disorders associated with XP are myelodysplastic syndrome and acute myeloid leukemia.[6]

Marrow aplasia in XP is very rare and has been reported so far in only a single case of XP.[7] However, marrow

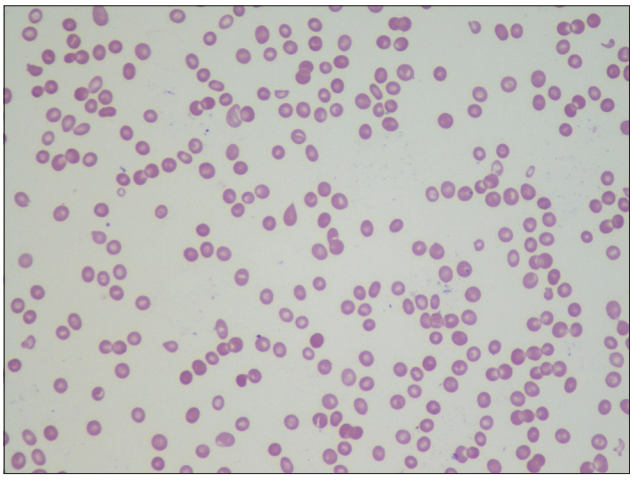


Figure 1: Peripheral smear showing pancytopenia (×40)

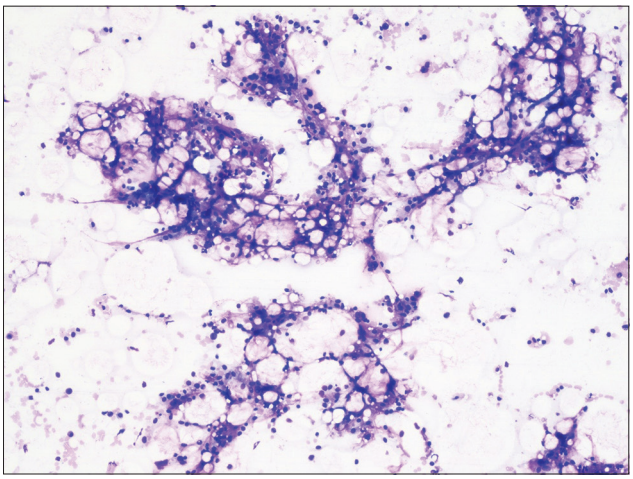


Figure 2: Bone marrow aspirate showing hypocellular marrow particles (×10)

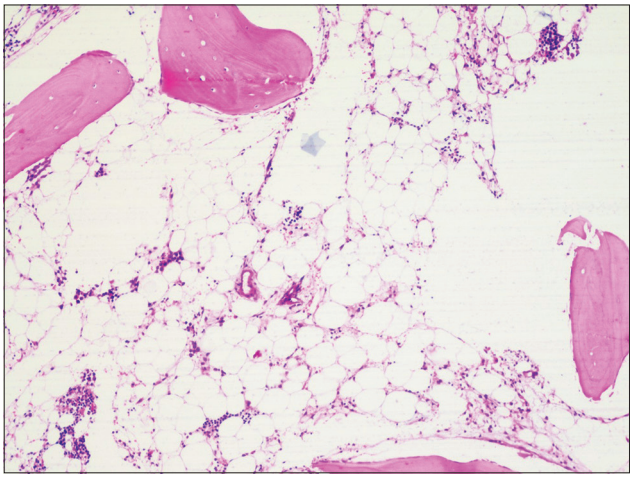


Figure 3: Bone marrow trephine showing fatty marrow (×10)

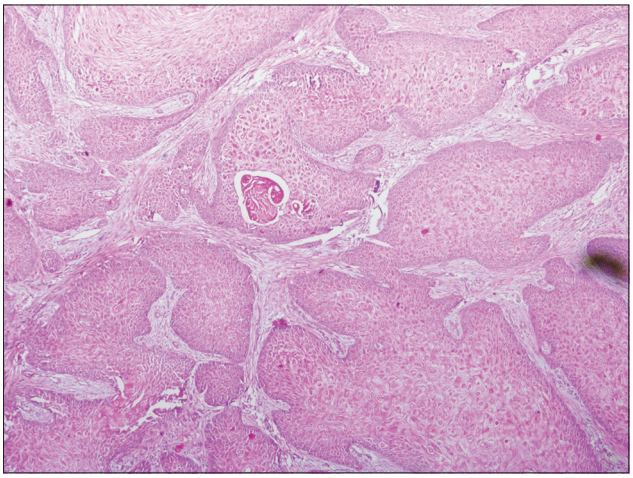


Figure 4: Biopsy showing squamous cell carcinoma (×10)

aplasia is quite common in other inherited DNA damage repair diseases such as Fanconi anemia. The patient did not have any features of Fanconi anemia. Even though skin hyperpigmentation has been reported in Fanconi anemia, it is not restricted to sun-exposed areas unlike in XP.

Aplastic anemia in children is generally attributed to drugs and viral infections. However, 90% of these cases are idiopathic in nature.[7]

The association of XP with leukemias and other internal malignancies shows that DNA damage repair mechanism is essential in repairing non-UV-mediated mutagenesis as well.

The association of aplastic anemia and XP as a new variant of this disease has not yet been proven.[7] The clonal evolution of aplastic anemia to leukemia has been reported.[7] Considering the foregoing discussion, it is tempting to suggest that this patient under reference can be considered to be at the increased risk of developing leukemia.

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Conflicts of interest

There are no conflicts of interest.

References

1. Bilodi AK, Gangadhar MR. Xeroderma pigmentosa - A rare clinical entity. Indian J Med Case Rep 2013;2:21-4.
2. Feller L, Wood NH, Motswaledi MH, Khammissa RA, Meyer M, Lemmer J. Xeroderma pigmentosum: A case report and review of the literature. J Prev Med Hyg 2010;51:87-91.
3. Tamhankar PM, Iyer SV, Ravindran S, Gupta N, Kabra M, Nayak C, *et al.* Clinical profile and mutation analysis of xeroderma pigmentosum in Indian patients. Indian J Dermatol Venereol Leprol 2015;81:16-22.
4. Pandey A, Kuldeep K, Koul A, Tyagi M, Singh P, Sharma P, *et al*. Xeroderma pigmentosa with ocular association: Case report. Case Rep Clin Med 2013;2:466-9.
5. Lopes-Cardoso C, Paes da Silva Ramos Fernandes LM, Ferreira-Rocha J, Teixeira-Soares C, Antônio-Barreto J, Humberto-Damante J. Xeroderma pigmentosum – A case report with oral implications. J Clin Exp Dent 2012;4:e248-51.
6. Berbis P, Beylot C, Noe C, Doutre MS, Broustet A, Privat Y. Xeroderma pigmentosum and refractory anaemia in two first cousins. Br J Dermatol 1989;121:767-71.
7. Salob SP, Webb DK, Atherton DJ. A child with xeroderma pigmentosum and bone marrow failure. Br J Dermatol 1992;126:372-4.
8. Chaudhary M, Jajoo SN, Agarwal R. Xeroderma pigmentosum: A case report of two siblings. J Immunodefic Disord 2012;1:2.
9. Rahman T, Sharma JD, Krishnatreya M, Kataki AC. Basaloid squamous carcinoma of skin associated with xeroderma pigmentosum in an 8-year-old child: A rare entity. Indian J Dermatol 2014;59:632.
10. Spiller SE, Hawkins DS, Finn LS, Sze RW, Sybert V. Metastatic malignant melanoma presenting as pancytopenia in a three-year-old boy. Pediatr Blood Cancer 2005;45:60-3.

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Deep Angiomyxoma of the Vulva: A Case Report and Review of Literature

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Abstract

Deep Angiomyxoma is very rare, slowly growing, and benign tumor of mesenchymal origin which affects women in perimenopausal age group and is associated with a high risk of local recurrence. A case of a 42-year-old female is presented, with a large firm gelatinous mass on the right labia majora, measuring 5.5 × 6 cm. The patient underwent wide local excision of the tumor. Histopathological examination showed a hypocellular neoplasm composed of uniformly distributed stellate cells in an abundant myxoid matrix and variably sized vessels suggestive of deep Angiomyxoma. 1 year followup showed no recurrance. Deep angiomyxoma of the vulva has to be distinguished from benign myxoid and malignant myxoid neoplasm. Wide local excision of the tumour is the treatment of choice. Long term follow up is indicated in view of local recurrence.

Key Words: Angiomyxoma, India, Rare, Vulva

Introduction

Deep angiomyxoma is a very rare, slowly growing, and benign tumor of mesenchymal origin, presenting in women of reproductive age, which has high risk for local relapse[1]. Deep Angiomyxoma was first described by Steeper and Rosai in 1983. It is a very rare local mesenchymal tumor of unknown etiology usually affecting vulva, labia major, buttocks, or pelvis of women in reproductive age[3]. Very few cases reports have also been described in men, which affect the scrotum [2]. Pathophysiology is unclear, but estrogen and progesterone influence may also play a role [4]. Women are affected predominantly in early reproductive years Adult men are less commonly affected. Female to male ratio is 6:1. It is Rare in children [4]. Controversy exists regarding management in view of local recurrence. It is a mesenchymal lesion, angiomyxoma mimics disorders which range from non neoplastic vulval hypertrophy and lymphedema to malignant tumours like myxoid liposarcoma.

Case Report

A 42-year-old female is presented, with a large firm gelatinous mass on the right labia majora, measuring about 5.5 × 6 cm with duration of 2 years and increased in size for 6 months.(Fig.1a) It was painless, soft, spongy in consistency, and non-tender. Menstrual cycles were regular with a normal flow. Examination of the cervix, external urethral meatus and vagina were unremarkable. Bilateral inguinal lymph nodes were not enlarged. Ultrasound of the abdomen and pelvis was normal. Cystoscopy was done in view of mass close to

the external urethral meatus, which showed normal urethral meatus, urethra and bladder. She underwent wide local excision of the lesion (Fig.1b). There was moderate bleeding during the procedure but the postoperative course was uneventful. Histopathologically, lesion showed a neoplasm covered by stratified squamous epithelium. The neoplasm was paucicellular (Fig.2a) composed of uniformly distributed spindle to stellate cells with ill-defined cell borders, moderate amount of eosinophilic cytoplasm and an elongated bland nucleus. Interspersed were thin fascicles of smooth muscle bundles and variably sized numerous thin and thick walled blood vessels (Fig.2b, 2c). These cells were set in an abundant myxoid and collagenous stroma(Fig.2d). Differential diagnosis at this stage included fibroepithelial polyp and, angiomyofibroblastoma both being commonly reported in Vulva. Because of the larger size, ill defined margins and absence of predominantly plump cells with perivascular accentuation, Angiomyofibroblastoma was ruled out. Angiomyofibroblastoma is usually a well circumscribed, smaller neoplasm with hypercellular and hypocellular areas with more plump cells with perivascular accentuation. Lack of bizarre stomal cells ruled out fibroepithelial polyp. There were no atypical cells or increased mitosis. With the above morphological features, it was diagnosed as Deep Angiomyxoma. 1 year follow-up revealed no recurrance.

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Deep angiomyxoma is the benign tumor commonly presents as an asymptomatic mass in the genital area of women in their reproductive life. The term “deep” denotes its propensity for local aggression and recurrence after excision. Approximately 70% - 80 % of the cases have recurrence after a period of 5 years postoperatively and it has been reported even 20 years after surgery. Local recurrences are treated with reoperation with wide local excision [1]. Some controversy exists with regard to need chemotheraphy. Some studies advocate that tumour cells are positive for estrogen and progesterone receptors[2,3]; hence hormonal therapy like tamoxifen and gonadotropin-releasing hormone analogue will reduce the tumor size and helps to make complete excision in large tumors and in the treatment of recurrence [5, 6]. Some studies also conclude that there is No role in Chemotherapy and radiotherapy in view of low grade mitotic figures. However most studies have consensus for Long term follow up with local examination and MRI pelvis for local recurrence [1]. Here we observed that wide local excision of the tumour even without chemotheraphy, with good follow- up can prevent local recurrence as observed in some of the studies. Angiomyxoma is a mesenchymal lesion. Immunohistochemically, these tumors stain for vimentin and desmin. The tumor cells also show some expression for muscle actin. Superficial angiomyxoma, angiomyofibroblastoma, and smooth muscle tumors like leiomyoma, hamartoma, liposarcoma also need to be considered in the differential diagnosis of a solid smooth mass in the perineum [2]. We observed that a wide range of vulvar mesenchymal lesions exist and angiomyxoma has to be differentiated from the mimics which range from non neoplastic vulval hypertrophy and lymphedema to malignant tumours like myxoid liposarcoma.

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Deep Angiomyxoma is rare entity in the Vulva, especially when it is painless lesion, particularly in premenopausal women in their third to fourth decades of life. CT/MRI pelvis is advocated in this situation for the extent of the mass. A wide range of vulvar mesenchymal lesions exist and angiomyxoma has to be differentiated from the mimics which range from non neoplastic vulval hypertrophy and lymphedema to malignant tumours like myxoid liposarcoma. Wide local excision is the treatment of choice for deep angiomyxoma of vulva with low recurrence rate. Long term follow up is required with genital examination and imaging studies.

1. Aggressive angiomyxoma of the vulva: An uncommon entity Mahendra M. Kura, Saurabh R. Jindal, and Usha N. Khemani Indian Dermatol Online J. 2012 May-Aug; 3(2): 128–130.
2. Angiomyofibroblastoma of the vulva Jung-Won Seo,1 Kyoung-A Lee,1 Na-Ra Yoon,2 Jeong-Won Lee, 1 Byoung-Gie Kim,1 and Duk-Soo Bae1. Obstet Gynecol Sci. 2013 Sep; 56(5): 349–351
3. An aggressive angiomyxoma of vulva - A rare entity - A case report Bishnu Prasad Das, Dipankar Baruah,1 Kaberee Bhuyan Medhi,1 and Bharat Talukder J Midlife Health. 2016 Jul-Sep; 7(3): 140–143.
4. Nagarajan P. Aggressive angiomyxoma. PathologyOutlines.comwebsite.http://www.pathologyoutlines.com/topic/vulvaaggressiveangiomyxoma.html. Accessed May 6th, 2019
5. McCluggage WG, Jamieson T, Dobbs SP, Grey A. Aggressive angiomyxoma of the vulva: Dramatic response to gonadotropin-releasing hormone agonist therapy. Gynecol Oncol. 2006; 100:623–5
6. Shinohara N, Nonomura K, Ishikawa S, Seki H, Koyanagi T (2004) Medical management of recurrent aggressive angiomyxoma with gonadotropin releasing hormone agonist. Int J Urol 11: 432-435.

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Original Article

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CD5 Positive Follicular Lymphomas- A Diagnostic Dilemma in a Resource Restricted Laboratory Setting

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ABSTRACT

Background: Follicular lymphoma is an indolent B Non Hodgkin lymphoma which has a characteristic follicular growth pattern and immunohistochemical expression. The neoplastic cells express CD20, CD10, and BCL2 and are negative for CD5, CD23 and Cyclin D1. CD5 is a useful marker in the differentiation of various B cell lymphomas. Small cell lymphoma and mantle cell lymphoma express CD5. Some of the follicular lymphomas express CD5 which results in diagnostic confusion

Methods: 52 cases of lymphoma were diagnosed over a period of 2 years in a tertiary care centre in south India. Among these, 9 cases were diagnosed as follicular lymphoma based on the morphological and immunohistochemical study. A panel of antibodies was chosen based on the morphological diagnosis. The panel used for small cell lymphomas include CD20, CD3, CD5 CD23, Cyclin D1, CD10, BCL2 and Ki67.

Result: Of the nine cases of follicular lymphoma, three were CD5 positive. Two showed bone marrow involvement. There was no significant morphological difference between CD5 positive and CD5 negative follicular lymphoma.

Conclusion: The expression of CD5 by follicular lymphomas could be problematic when the follicular lymphoma as such lacks its characteristic follicular pattern andwhen there are no molecular studies available. Even though rare, follicular lymphoma is to be considered in the differential diagnosis of CD5 positive lymphomas.

Keywords: *Follicular Lymphoma, CD 5 Positive, Indolent Lymphomas, B Cell NHL, Immunohistochemistry*

Introduction

Follicular lymphoma (FL) is the second most common Non Hodgkin lymphoma (NHL) in India. It is an indolent lymphoma derived from germinal centre derived B cells. FL has a characteristic morphology with effaced nodal architecture, follicular growth pattern recapitulating germinal centres. Genetically, the hallmark of FL is t(14;18)q(32;q21) which has been detectable in a significant number of cases accounting to about 80%. This genetic alteration is responsible for the deregulated expression of BCL2 which can be detected using immunohistochemistry. FL has characteristic immunophenotypic expression, positive for pan B cell markers, CD20, CD19, germinal centre associated antigens CD10, BCL6 and BCL2. FL do not express CD5, a T cell marker that has a key role in the differential diagnosis of small B cell Non Hodglkin lymphomas. [4-6]

CD5 is a glycoprotein expressed on mature T cells and a small subset of B cells. CD5 positive cells in a lymph node are present in the follicular mantles. [7] Among the lymphomas, CD5 is considered a marker for small lymphocytic (SLL) and mantle cell lymphoma (MCL). However a small subset of diffuse Large B cell lymphoma, Burkitt lymphoma and extranodal marginal zone lymphoma are found to have expressed CD5.[8,9] And also, cases of CD5 positive follicular lymphomas have been reported in small numbers in the studies performed even before the advent of molecular studies. [10-12]

FL is basically a morphological diagnosis. Immunohistochemistry is used as an ancillary tool in the confirmation of diagnosis. CD5 expression however would result in diagnostic confusion when the morphology is not characteristic and flow cytometric and translocation studies cannot be done. In the present study, we describe morphologic and immune histochemical features of 3 such cases of CD5 positive FL.

Materials and Methods

Fifty two cases of lymphoma were diagnosed over a period of 2 years in a tertiary care centre in south India. The lymphomas were reported based on the WHO classification of tumours (2008 classification). Among these, 9 cases were diagnosed as follicular lymphoma

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based on the morphological and immunohistochemical study. Histology was evaluated in formalin-fixed, paraffin-embedded (FFPE) and H&E-stained sections. Grading of FLs was based on the number of centroblasts per high-power field. A panel of antibodies were chosen based on the morphological diagnosis. The panel used for small cell lymphomas include CD20, CD3, CD5 CD23, Cyclin D1, CD10, BCL2 and Ki67.

For immunohistochemistry, 4µ sections were taken in specially coated slides. Slides were then deparaffinised and rehydrated in ethanol and xylene. Antigen retrieval was done by microwaving using two methods 1) by incubating sections with a Tris-EDTA solution, pH 9.0 for 20 minutes before staining with antibodies against CD10, CD23, BCL2, CD5, CyclinD1, CD3 (mouse, monoclonal, biogenex), and 2) by incubating sections with 0.01 M citrate buffer solution, pH 6.0, for 20 min before staining with antibodies against CD45, CD20 (mouse, monoclonal, biogenex). The slides were then incubated under humid conditions at room temperature for 90 minutes.

The antibodies bound to antigens were detected by addition of secondary antibody conjugated with horse radish peroxidase polymer and diaminobenzidene substrate. The slides were counterstained with hematoxylin. Appropriate positive controls for the primary antibodies as per the manufacturer's instruction manual (biogenex) were used. The immunohistochemically stained slides were analyzed for the presence/absence of reaction and cellular localization. The positive reaction in the neoplastic cells was assessed. This study was conducted with approval of the Institutional ethical committee, in accordance with the ICMR guidelines for human research.

Result

Of the nine cases of FL, 3 were CD5 positive. All the three patients were males with the age range of 37 to 53 presenting with lymphadenopathy.

Histologically, all the three cases showed predominant follicular growth pattern, while one of them showed diffuse component accounting to less than 50 %.(fig 1 and 2). Histological grading was based on the number of centroblasts per high power field. On immunohistochemical study the cells were found to express CD20, CD10 and BCL2. (Fig 3 and 4) BCL2 was expressed in the follicular centres. Approximately 70% of the cells were CD5 positive. CD3 immunostaining was included in the panel to assess the proportion of T cells in follicles. As the T cells in the follicular centre usually express BCL2, CD3 immunostaining was compared with BCL2 before interpreting BCL2 expression in order to avoid misinterpretation. The follicular centre also had CD3 positive T cells which also expressed CD5 (fig5). Cyclin D1 was negative in all the three cases ruling out mantle cell lymphoma. (Table 1)

In case 2 there was weak expression of CD20 and CD5. Bone marrow trephine biopsies were subsequently received for these three cases. Two of the three cases showed bone marrow involvement, one was paratrabecular while the other aws diffuse. There was no peripheral blood involvement. Extranodal involvement was not detected in any of the three cases.

The other six cases which have not been described here showed typical histological and immunohistochemical features of lymphoma.

Discussion

Follicular lymphoma is a mature B cell lymphoma which accounts for 20% of NHL.It is usually a disease of adults having a slight female preponderance. FL predominantly involves the lymph nodes, involvement of bone marrow and other extranodal sites can happen in the setting of widespread disease. Primary Extranodal FL can also occur. [1]

FL is a heterogenous neoplasm having a variable immunoarchitectural pattern. Typically the involved lymph nodes show complete effacement of nodal architecture by uniform sized follicles composed of centrocytes and centroblasts. These neoplastic follicles lack polarization and evidence of apoptotic activity (tangible body macrophages). The growth pattern can be follicular, follicular and diffuse and predominantly diffuse. CD23 immunostaining which highlights the follicular dendritic cells aids in assessing the extent of follicular component. [13, 14]

The varied architectural patterns and variants lead to confusion with other small B cell lymphomas which include small lymphocytic lymphoma (SLL) and marginal

Table 1: Immunohistochemical feature of Follicular Lymphoma.

	Histology	CD20	CD10	CD23	CD5	BCL2
1.	Follicular	+	+	+ in FDC	+ (70 % of cells)	+
2.	Diffuse > 50%	+ (weak)	+	_	+ (70% of cells)	+
3.	Follicular	+	+	+ in FDC	+ (50% of cells)	+

FDC- follicular dendritic cell.

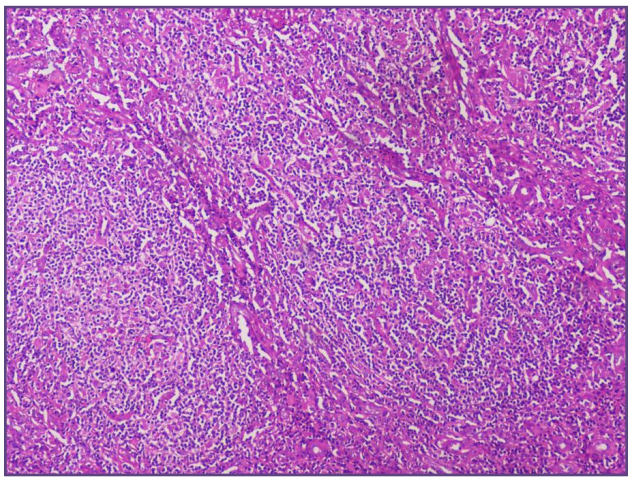


Fig. 1: Follicular growth pattern H& E, 10X magnification.

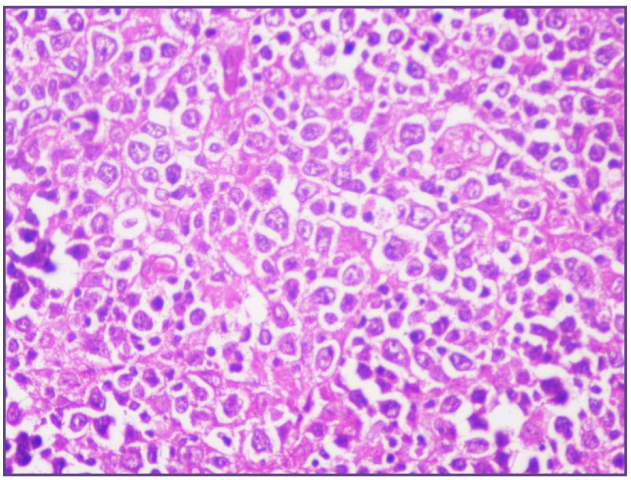


Fig. 2: showing follicular centres H&E, 40X magnification.

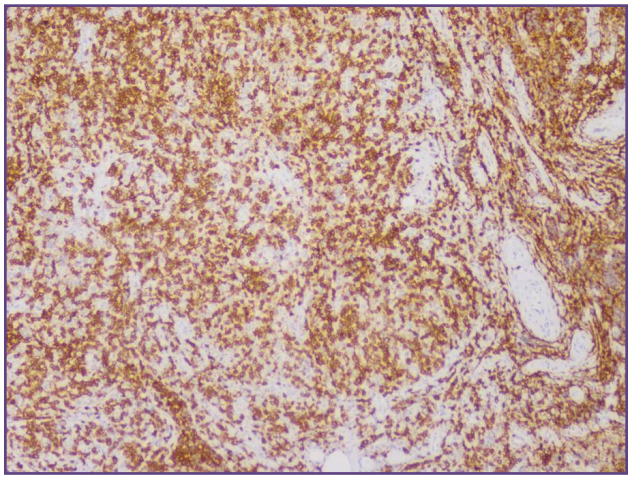


Fig. 3: Follicles expressing CD10 (IHC), 10X magnifiction.

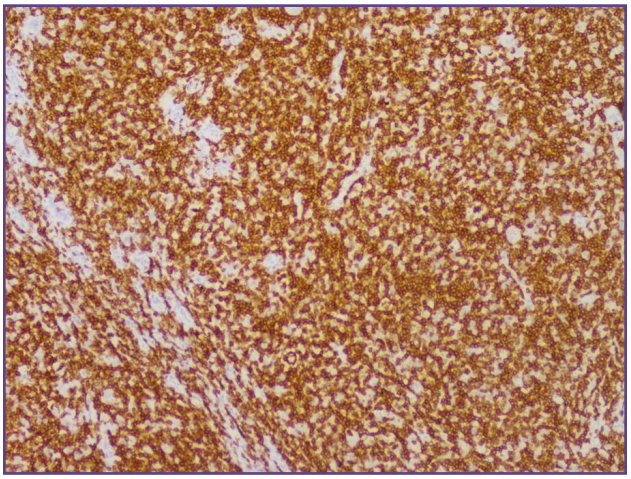


Fig. 4: Follicles expressing BCL2 (IHC), 10X magnification.

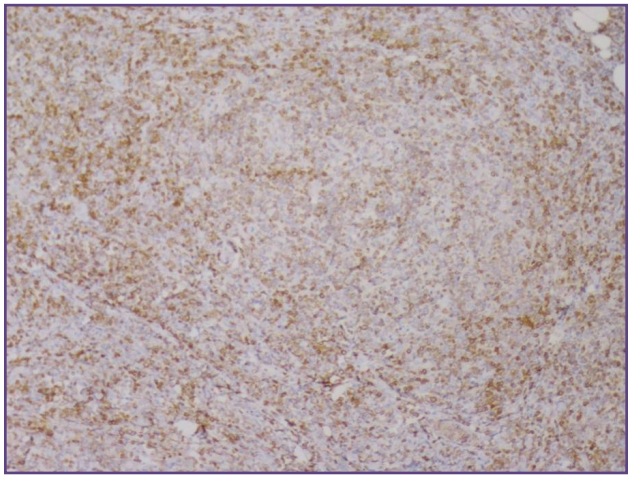


Fig. 5: Neoplastic cells expressing CD5 (IHC) 10X magnification.

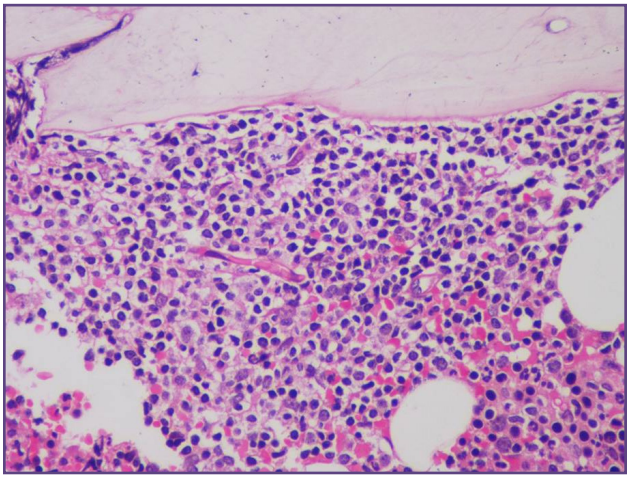


Fig. 6: Bone marrow trephine showing paratrabecular infiltrate of lymphoid cells.10X magnification.

zone lymphoma. The typical immunoprofile of FL includes expression of CD10, BCL6, BCL2 and CD20. The expression of these markers are stronger in the follicles when compared to the interfollicular cells. [15]

CD10 is a germinal centre marker expressed both in reactive and neoplastic follicles. IT is also expressed in Burkitt and Diffuse Large B cell lymphoma. [15] BCL2 is an antiapoptotic protein which is negative in reactive germinal centres and overexpressed in FL, the expression of which is strong in grade 1 and 2 FL. It is a reliable marker for differentiating follicular lymphoma from follicular hyperplasia. T cells present within the follicular centres also express BCL2. [13]

CD5 is involved in T- and B cell receptor signaling. CD5 is a valuable marker in the differentiation of non Hodgkin B cell lymphomas. However CD5 is a mature T cell marker, but is expressed in a subset of B cell lymphomas (SLL and MCL). CD5 along with CD23 helps in the differentiation of small lymphocytic and mantle cell lymphoma. It has also been found to be expressed in marginal zone and diffuse large B cell lymphoma. [1-6] CD5 is one of the negative markers for follicular lymphoma. But CD5 positive follicular lymphomas have been reported to be a rare phenomenon. In the present study, 3 out of 9 cases of FL were CD5 positive, which appears more frequent when compared to the previous published studies.

They have been found to be associated with younger age, male predominance and grade 3 morphology. [15, 16] It has also been found to be expressed in follow up biopsies of FL which initially did not express.

In the present study, the age ranged from 37 to 53. All the three cases were males. Morphologically, all the three cases had follicular growth pattern except for one which showed a predominant diffuse pattern. Two of the cases were grade 2, while the other was grade 3. CD5 positive FL has been reported to be associated with advanced disease and Extranodal involvement. One of the grade 2 and the grade 3 case showed bone marrow involvement. Bone marrow involvement was paratrabecular in grade 2 FL (figure 6) and diffuse in grade 3 case. None of them had any evidence of Extranodal disease at presentation.

In the case 2 there was >50% diffuse component and immunohistochemically, there was weak expression of CD20. This weak expression of CD20 is characteristic of small lymphocytic lymphoma. This weak expression of CD20 along with CD5 positivity made SLL a strong possibility. However CD23 was negative in that case. Hence it was diagnosed as follicular lymphoma. CD5 positive follicular lymphomas reported so far has been confirmed with translocation and flow cytometric studies. These investigations were not conducted in the present study.

All the three cases showed morphological features of classical FL. No specific variants were found to comment on the association between CD5 positivity and any particular variant subtype. The other six cases of FL had wide age range (13 to 68), varied morphology, (4 of them grade 2 and the remaining two cases corresponding to grade 3).

The final diagnosis obtained was based on morphological and immunohistochemical study. Flow cytometry and cytogenetics were not performed to identify the translocation t (14, 18) characteristic of FL. Follow up data was not available to understand the prognostic significance of CD5 expression in a follicular lymphoma.

Conclusion

CD5 positive FL can pose a diagnostic problem especially when the morphology is not characteristic including the presence of diffuse component and when genetic studies are not available. However the use of panel of markers can aid in the diagnosis.

In summary CD5 positive Follicular lymphomas had the same morphological and immunophenotypic expression as that of CD5 negative FL. Eventhough the frequency is very low, we need to consider the possibility of follicular lymphoma in the evaluation of CD5 positive B NHL.

Reference

1. Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J,Vardiman JW (eds). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC: Lyon, 2008; 220–226.
2. Horsman DE, Gascoyne RD, Coupland RW et al. Comparison of cytogenetic analysis, southern analysis,and polymerase chain reaction for the detection of t(14;18) in follicular lymphoma. Am J Clin Pathol 1995; 103:472–478
3. Stamatopoulos K, Kosmas C, Belessi C, et al. Molecular insights into the immunopathogenesis of follicular lymphoma. Immunol Today. 2000; 21:298-305.
4. Dogan A, Bagdi E, Munson P, et al. CD10 and bcl-6 expression in paraffin sections of normal lymphoid tissue and B-cell lymphomas. Am J Surg Pathol. 2000; 24:846-852.
5. Dorfman DM, Shahsafaei A. Usefulness of a new CD5 antibody for the diagnosis of T-cell and B-cell lymphoproliferative disorders in paraffin sections. Mod Pathol. 1997; 10:859–863.
6. Yin CC, Lin P, Carney DA et al. Chronic lymphocytic leukemia/small lymphocytic lymphoma associated with IgM paraprotein. Am J Clin Pathol. 2005; 123: 594–602.

7. Leon ED, Alkan S, Huang JC, et al. Usefulness of an immunohistochemical panel in paraffin-embedded tissues for the differentiation of B-cell non-Hodgkin's lymphomas of small lymphocytes. Mod Pathol. 1998; 11:1046-1051.
8. Sundeen JT, Longo DL, Jaffe ES. CD5 expression in B-cell small lymphocytic malignancies: correlations with clinical presentation and sites of disease. Am J Surg Pathol. 1992; 16:130-137.
9. Dong HY, Gorczyca W, Liu Z et al. B-cell lymphomas with coexpression of CD5 and CD10. Am J Clin Pathol. 2003; 119:218–230.
10. Sekiguchi Y, Imai H, Wakabayashi M et al. CD5- positive follicular lymphoma: a case report and literature review. Intern Med. 2011; 50:899–904.
11. Mayson E, Saverimuttu J, Cartwright K. CD5-positive follicular lymphoma: prognostic significance of this aberrant marker? Intern Med J. 2014; 44:417–422.
12. Takata K, Miyata-Takata T, Sato Y, Yoshino T. Pathology of Follicular Lymphoma. J Clin Exp Hematop. 2014; 54(1): 3-9.
13. Fouad-Younes S, Beck A, Lossos IS, et al. Immunoarchitectural Patterns in Follicular Lymphoma: Efficacy of HGAL and LMO2 in the Detection of the Interfollicular and Diffuse Components. Am J Surg Pathol. 2010; 34(9): 1266–76.
14. Rizzo K, Nassiri M. DiagnosticWorkup of Small B cell Lymphomas: A Laboratory Perspective. Hindawi Publishing Corporation Lymphoma. 2012;
15. Barry TS, Jaffe ES. CD5+ Follicular Lymphoma A Clinicopathologic Study of Three Cases. Am J Clin Pathol 2002; 118:589-598.
16. Yu Li, Shimin Hu, Zhuang Zuo, Ming Hong. CD5-positive follicular lymphoma: clinicopathologic correlations and outcome in 88 cases. Modern Pathology 2015;28, 787–798.

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COEXISTENCE OF STRUMA OVARII AND BRENNER - A CASE REPORT

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Abstract :
Struma ovarii is a rare ovarian tumour. It is a monodermal variant of teratoma which is characterized by presence of more than 50 thyroid tissue. It constitutes about 1percent of ovarian tumours and 2.7 percent of teratomas. It usually occurs in pure form and rarely in association with Brenner and dermoid cyst. Most of struma is benign. Only 5 to 10 percent are malignant. Herein presented is a report of 65 year old female with benign struma ovarii with Brenner tumour.

Keyword : Struma ovarii, Brenner, cystic struma, monodermal teratoma

Introduction:

Struma ovarii is a germ cell tumour. It is a monodermal variant of teratoma which usually occurs in pure form and in association with dermoid, very rarely with Brenner and mucinous tumour. Struma ovarii is usually seen in fourth to sixth decade of life. There is risk of malignancy and thyroid hyperactivity. Hyperthyroidism is seen in 8 % of cases. Preoperative diagnosis is very difficult. Histopathological examination is the confirmatory.

Case report: 65 year old female presented with abdominal distension. Gynecological examination and imaging techniques revealed a complex right ovarian cyst. Ultrasonogram diagnosis was a malignant ovarian cyst possibly serous cystadenocarcinoma. Patient underwent hysterectomy with removal of right ovarian mass and left salphingo oophorectomy.

Gross findings:

Ovarian cyst measured 16 x 9 x 9 cm with smooth external surface (Figure 1). Ovarian cyst was unilocular filled with solidified light brown material (Figure 2). Few solid areas and few cystic spaces were seen. The solid areas were firm and yellow (Figure 3). Uterus, fallopian tubes and the left sided ovary were grossly normal.





Figure 1 gross picture of ovarian cyst
Figure 2 unilocular cyst with light brown material



Figure 3 showing solid yellow areas

Histopathological examination:
The cyst wall was composed of normofollicular and macrofollicular thyroid follicles filled with colloid representing struma ovarii (Figure 4 &5). Few areas of transitional epithelial nests representing Brenner component were also noted (Figure 6). Solid areas revealed features of mature teratoma. No atypia was seen in any of the components. No malignant transformation was noted. These components were present separately present and there was no merge among them. The left ovary and uterus showed no remarkable pathology.

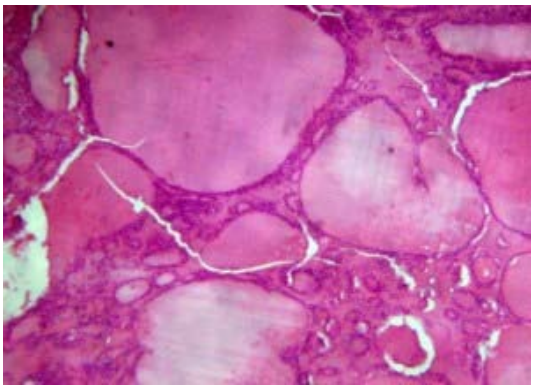
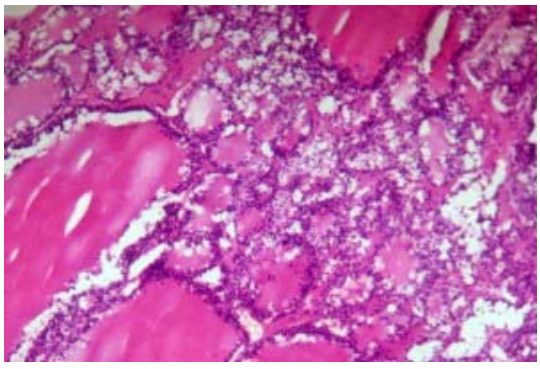


Figure 4 HPE showing thyroid follicles filled with colloid (10X) Figure5 HPE showing thyroid follicles filled with colloid (10X)



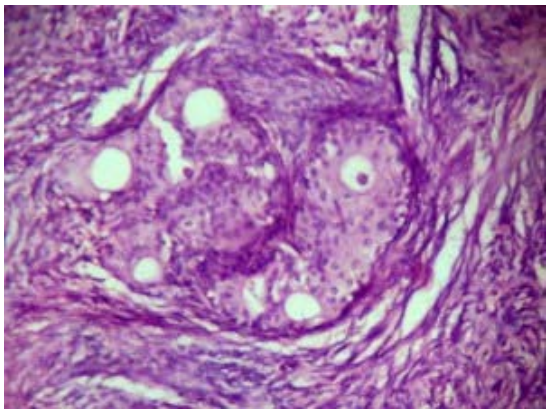


Figure 6 : HPE showing transitional cell nests in a fibrous stroma (40 X)

Discussion:

We hereby had presented a rare coexistence of Struma Ovarii & Brenner tumour. Struma ovarii was first recognized by Ludwig Pick to be composed of thyroid tissue and was labeled as ovarian goiter, which was actually teratoma with predominance of thyroid tissue.[1,2] Later, Plaut showed that the thyroid tissue in Struma ovarii was morphologically, biochemically, and pharmacologically identical to that of the normal thyroid gland. In the World Health Organization classification, Struma ovarii and malignant thyroid tumours arising within Struma ovarii are included in the thyroid tumour group under the category of monodermal teratomas (ICD 9090/ 0) [3,4] and somatic-type tumours associated with dermoid cysts.

As per the existing literature [1,3,5,6,7]. Struma ovarii is generally a unilateral tumour, usually occuring in pure form, less commonly with other germ cell tumours like dermoid, carcinoid and very rarely with epithelial tumours like Brenner, mucinous and serous neoplasms[5]. The tumour is usually solid, some may be cystic. Cut surface of the tumor is composed entirely of light tan, glistening thyroid tissue. Hemorrhage , necrosis, and foci of fibrosis may be present.In our case, struma ovarii was associated with Brenner component and was predominantly cystic**.** Struma ovarii usually shows normal thyroid tissue, but may represent thyroid adenoma and carcinomas[7,8]. In the present case, neither thyroid adenoma nor carcinoma were seen, rather thyroid tissue was normal.[1] Brenner is an epithelial tumour accounting less than 5% of ovarian neoplasms. They are small and mostly incidentally discovered and are 90% unilateral[9]. Brenner may be associated with mucinous tumour or dermoid cyst. Most tumors are well circumscribed, firm and rubbery with a grey white to yellow cut surface[10]. In the present case, the Brenner component exhibited the above typical morphological features. The pathogenesis of this type of ovarian tumor composed of Brenner's tumor and struma ovarii is uncertain. Brenner tumor is generally thought to be derived from surface epithelial cells while struma ovarii is a monodermal teratoma (germ cell tumor). In most cases where there is coexistence of Brenner and struma, both the components express thyroglobulin[11]. While there is strong evidence that pure Brenner tumors originate mostly from the ovarian surface, at least Brenner tumors associated with teratomatous elements may have a germ cell origin which could be confirmed by immunophenotyping.

Conclusion:

Coexistence of struma ovarii with Brenner is a rare entity which is highly unlikely to be diagnosed preoperatively. Struma ovarii has an overall better survival. Histopathology is the confirmatory. Histogenesis of Brenner associated with struma ovarii could be confirmed by further studies like immunophenotyping

and molecular studies.

References:

1 Hemant deshpande, nilesh,struma ovarii a case report Int J pharmBiomed Sci 2012,3 (4) 152-154.

2 Elemenoglou A, Zizi-Serbetzoglou A, Trihia H, Vasilakaki T, Boumia E. Mixed ovarian neoplasm composed of struma ovarii and Brenner tumor: report of a case. Eur J Gynecol Oncol. 1994; 15 :138–141.

3 World health organization classification of tumours,tumors of breast and female genital organs,163 – 171.

4 Blaustein, pathology of female genital tract,sixth edition, 890 – 897.

5Tadashi Terada and Kazuhiro Tateoka. Ovarian cystic tumor composed of Brenner tumor and struma ovariiInt. J Clin Exp Pathol. 2012; 5(3): 274–277.

6 Dardik RB, Dardik M, Westra W, Montz FJ.Malignant struma ovarii : two case reports and areview of literature.Gynecologic oncology [1999 Jun; 73 (3):447-51]

7 Porpora MG, Pallante D, Ferro A, Alò PL, Cosmi EV Asymptomatic struma ovarii: a case report. 2005;32(3):197-8

8 M.A.Costa et al Struma ovarii: a rare form of presentation and clinical rview2005, Vol. 84, No. 8 .

9 Yoshida M, Okabayashi C, Tachibana M, Minami R. Coexisting Brenner tumor and struma ovarii in the right ovary: case report and review of the literature. Pathol Int. 2004; 54:793 –797.

10 Takeuchi K, Ohbayashi C, Kitazawa S,Ohara N, Maruo T. Coexistence of brenner tumor and struma ovarii: case report.Eur J Gynaecol oncol. 2005;26(1):109-10.

11 Burg, Jürgen et al, Mature Cystic Teratoma of the Ovary with Struma and Benign Brenner Tumor: A Case Report with Immunohistochemical Characterization .International Journal of Gynecological Pathology: Januart 2002 - volume 21 - Issue 1-pp 74-77

1	TGA, Pulmonary atresia, DORV	Dysmorphic features, abdominoschisis	Amniotic band syndrome
2	Aortic arch hypoplasia	Hypoplastic nails, clitoromegaly, widely spaced nipples, telangiectatic nevi	Turner syndrome
3	TOF, Right aortic arch	Club foot, atresia of rectum and anus, renal ectopia with hydronephrosis	VACTERL association (vertebral, anal, cardiac, tracheo-esophageal, renal, limb)
4	Hypoplastic aorta and ductus	Bladder outlet obstruction due to urethral stenosis, hydronephrosis, absent corpus callosum	
5	TOF, Right aortic arch, absent ductus	Thymic aplasia	Di George syndrome
6	AVSD, common atrium	Postaxial polydactyly	Ellis Van Creveld syndrome
7	Rhabdomyoma	Subependymal nodules in brain, angiomatoid proliferation in kidney, adrenal rest in test	Tuberous sclerosis
8	Dextrocardia, large Ductus arteriosus	Tracheomalacia, diaphragmatic hernia, lung hypoplasia	
9	ASD	Glottic atresia, tracheomalacia. Pulmonary hyperplasia	Laryngeal atresia (CHAOS) Congenital High airway obstruction syndrome
10	ASD	Craniofacial anomalies, agenesis of corpus callosum, leukodystrophy, nesidioblastosis,	? Zellweger? Smith Lemli Opitz

		liver hemosiderosis	syndrome
11	AVSD, PLSVC, tubular hypoplasia of aorta	Low set ears, cystic hygroma, neck Hypoplasia of thymus, lungs, kidneys, adrenals	Down syndrome
12	AVSD, common atrium	Post axial polydactyly, epispadias, pyloric stenosis	Ellis-Van Creveld Syndrome
13	AVSD, malposition of great arteries, right atrial isomerism, TAPVC, pulmonary atresia	absent spleen, bilateral trilobed lungs.	Ivemark syndrome
14	HLHS	Bladder outlet obstruction	
15	AVSD, Common atrium	postaxial polydactyly, rhizomesomelic shortening in all four limbs short ribs	Ellis-Van Creveld Syndrome
16	VSD, COA	renal cortical cysts with hydrouretronephrosis, olfactory bulb agenesis, corpus callosum agenesis	Fryns syndrome
17	VSD, overriding of aorta	Cleft lip, cleft palate Absent thymus	Di George syndrome
18	VSD	deformed upper limb with single forearm bone oligodactyly, bifid uvula, trilobed left lung	Holt Oram syndrome
19	Hypoplastic aortic arch,	imperforate anus, anal atresia distal urethral obstruction	
20	HLHS	Cleft polydactyly.	
21	HLHS	Diaphragm hernia, rhizomelic hypomelia, single umbilical artery,pulmonary Hypoplasia and agenesis of corpus callosum.	Fryn syndrome

22	TGA, VSD, pulmonary atresia	Dysmorphic features	
23	HLHS	Occipital encephalocele, lung hypoplasia, mild hepatic fibrosis Bilateral Autosomal recessive polycystic kidney disease (ARPCKD)	
24	HLHS	Lung hypoplasia Depressed nasal bridge, anteverted nares, high arch palate.	
25	HRHS, VSD	Micrognathia, rocker bottom foot, thymic hypoplasia	
26	HRHS	low set ears, bilateral talipes (Potter sequence) - Bilateral autosomal recessive polycystic kidney disease (ARPCKD) ureteral dilatation, obstructive uropathy, bilateral severe lung hypoplasia	CAKUT (congenital heart defects with congenital anomalies of the kidney and urinary tract)
27	Tricuspid valve dysplasia	Dysmorphic features	
28	TGA	Situs inversus, polysplenia	Heterotaxy syndrome
29	AVSD, malposition of arteries, TAPVC, right atrial isomerism, pulmonary atresia	Dysmorphism, situs inversus, polysplenia	Heterotaxy syndrome

TGA- Transposition of great arteries, DORV - Double outlet right ventricle , TOF - Tetralogy of Fallot , AVSD- atrio ventricular septal defect , ASD - Atrial septal defect, PLSVC- Persistent left superior vena cava, HLHS- Hypoplastic left heart syndrome, VSD- ventricular septal defect , COA – Coarctation of aorta, HRHS- Hypoplastic right heart syndrome, TAPVC- Total anomalous pulmonary venous return.

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Original Article

Pathologist's role in identifying cardiac defects—a fetal autopsy series ☆

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ABSTRACT

Aims: Major cardiac abnormalities can lead to miscarriages and is also an important indication of medical termination of pregnancy. The present study aims: (1) To determine the spectrum of cardiac anomalies in fetal autopsies, (2) To correlate the autopsy findings with prenatal imaging studies.

Methods: Fetal autopsies with cardiac anomalies were retrospectively analyzed over a period of five years. Autopsy diagnoses were correlated with prenatal ultrasound and echocardiogram findings.

Results: Of the 177 fetal autopsies, 40 (22.5%) cases with cardiac defects were identified. Among these cases, 11 (27.5%) showed only cardiac defects and associated extracardiac malformations were seen in 29 cases (72.5%). Septal defects (45%) were the most frequent cardiac defect followed by left ventricular outflow tract obstruction. Agreement with prenatal imaging and autopsy findings was seen in 65% of cases. There was 100% concordance between autopsy and echocardiogram wherever available.

Conclusion: Cardiac malformation forms a significant subset of fetal congenital anomalies. Meticulous examination and following sequential segmental analysis during autopsy are essential in identifying complex cardiac and additional extra cardiac defects in the fetuses. Fetal autopsy plays a vital role in confirming and/or refuting the imaging findings and could be the steppingstone in understanding the pathogenesis of cardiac abnormalities.

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1. Introduction

Congenital malformations are a common cause of perinatal deaths and account for 10%-15% in India [1], the majority of which can be identified by fetal sonogram. Fetal medicine being emerging field, there is a rapid improvement in the detection rate of prenatal ultrasound screening for fetal abnormalities, which is further complemented by technological advancement in imaging [2]. But fetal autopsy remains the gold standard procedure to confirm the abnormalities and to detect additional findings if present, which can contribute to the final diagnosis. It also serves as a quality indicator for imaging evaluations and fetal medicine specialist [3]. While some authors recommend performing autopsy following termination of pregnancy (TOP) for fetal abnormalities, a few others recommend postmortem examination when an abnormal karyotype is detected [4].In either way, autopsy plays a pivotal role in diagnosing the anomalies and guide the clinicians in the effective management of subsequent pregnancies.

Among the congenital malformations, central nervous system defects are reported to be the most common, followed by cardiac defects accounting for 2.7%-8.8% [5]. The sophisticated imaging techniques to identify the cardiac abnormalities in- utero has allowed better understanding of pathology. But still there remain few unidentified or misdiagnosed lesions, which can be attributed to the pathology per se or the physician investigating it [6]. Decline in the autopsy rates fail to investigate the case completely. Cardiac abnormalities form a significant subset of malformation either as isolated lesions or as a component of syndrome. The present study aims to analyze the cardiac malformations in the present scenario in Indian subcontinent. The objective of the present study was to evaluate the spectrum of cardiac anomalies in routinely performed fetal autopsies and to correlate prenatal ultrasonogram (USG) with autopsy findings.

Abbreviations: TOP, termination of pregnancy; USG, ultrasonogram; IUD, intrauterine death; CNS, central nervous system; LVOTO, left ventricular outflow tract obstruction; RVOTO, right ventricular outflow tract obstruction; DORV, double outlet right ventricle; VSD, ventricular septal defect; TGA, transposition of great arteries; TOF, tetrology of Fallot; AVSD, atrioventricular septal defect; TAPVC, total anomalous pulmonary venous return; HLHS, hypoplastic left heart syndrome; HRHS, hypoplastic right heart syndrome; PLSVC, persistent left superior vena cava; ASD, atrial septal defect.

☆ Conflict of interest: The authors declare that they have no competing interests.

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2. Materials and methods

All the fetal autopsies performed in the department of Pathology during the study period of January 2014 to December 2018 were retrospectively analyzed. The study was conducted after approval from Institutional Human ethics committee.

Fetuses of gestational age greater than 12 weeks were included in the study. Informed written consent was obtained from either of the parents before performing the autopsy. Detailed maternal history including parity, previous pregnancy outcome, consanguinity, medical illness, gestational age, sonographic findings, and mode of delivery was collected and recorded.

All the fetuses were examined using the protocol recommended by Royal college of Pathologists autopsy practice guidelines [7]. This includes whole body radiograph (anteroposterior and lateral view), photography (supine, prone, and lateral positions), external and internal examination.

After removing the chest plate, a systematic in situ examination of all organs, inclusive of heart was done, followed by evisceration with “en bloc” removal of organ systems.

External examination of pericardium, epicardium, cardiac situs [8], anterior interventricular groove, atrial appendages, relationship of great vessels and pulmonary venous connections.

Sequential segmental analysis was performed for internal examination using inflow outflow method. The first entry into the heart is through the caval veins by a lateral cut from inferior to superior vena cava. A perpendicular cut was made in the right atrium exposing the atrial septum. The patency of foramen ovale was assessed. The next cut was made posteriorly from atrium into the ventricle and extending the cut to the apex. The atrioventricular inflow connection was examined and documented. The next cut was from the apex through the outflow with the cutting angle maintained away from the heart and arterial valve. The great artery is cut across the arterial valve and the findings were documented. The left heart was examined by entering the left atrium through pulmonary veins and atrial appendage. The atrial morphology was assessed. Extending the cut posteriorly along the ventricular inflow tract and observation of atrioventricular connection was made. The outflow cut was made from apex to cut through the arterial appendage on the anterior aspect of heart. A spiraling cut around the base of artery was made. The morphology of valve, ventricle and infundibulum were studied and documented to assess the atrio ventricular concordance. The diameter of great arteries and ductus was measured and documented. Photographs were taken for documentation [9].

Rest of the organ systems were dissected, separated, and weighed individually. Representative sections were submitted for microscopic examination. Placenta if received was also examined and relevant tissue sections including cord and membrane roll were given. All the autopsies were performed by two trained Pathologists in the field of fetal and perinatal autopsies.

The cardiac defects were divided into two categories: isolated (single defect) and complex (two or more defects in the same heart). These defects were subcategorized based on the following terminologies: Malposition and isomerism, septal defects, left ventricular outflow tract obstruction (LVOTO), right ventricular outflow tract obstruction (RVOTO), conotruncal anomaly, connection anomaly, anomalous great vessels, and others [10]. These cases were further stratified based on the presence/ absence of extracardiac malformations involving single organ or more than one organ. The flow diagram for analysis is depicted in Fig. 1.

Prenatal ultrasound and fetal echocardiogram details were retrieved from the archives. The prenatal ultrasound examination was done by radiologists /obstetrician trained in fetal medicine. The autopsy findings were correlated with prenatal ultrasound findings. The cases were classified as having complete agreement, major agreement with additional findings and complete disagreement.

Data analysis was done using SPSS statistical software version 20. Comparison of variables was done using chi-square test.

3. Results

There were 177 fetal autopsies during the study period of which 40 (22.5%) cases showed cardiac anomalies inclusive of an intrauterine cardiac tumor, rhabdomyoma. The distribution of gestational age of fetuses when submitted for autopsy ranged from 12 to 37 weeks. Twenty-five (62.5%) were males and 14(37%) were females and one case had ambiguous genitalia. The indication for autopsy was TOP due to an abnormality detected in the sonogram in 32 cases and intrauterine death (IUD) in 8 cases. Considering the indication for TOP in 32 cases, 15 were due to cardiac anomaly, 6 cases due to hydrops, 5 each due to extracardiac anomaly and syndrome complex and a single case due to chromosomal abnormality (Turners syndrome).Of the eight cases of IUD, 5 could be attributable to cardiac defects identified on autopsy while 3 cases could not be directly attributed to cardiac anomalies.

3.1. Morphological findings

3.1.1. Isolated and complex cardiac defects

The distribution of cardiac defects included 20 cases each of isolated and complex defects, the details of which are summarized in Table 1 and the complex cardiac defects are described in Table 2. Eleven cases (27.5%) showed only cardiac defects and the rest of them were associated with extracardiac malformations.

Among all the cardiac defects regardless of being isolated or complex, septal defects were common, constituted 18 cases, followed by LVOTO (11), connection anomaly (8), conotruncal anomaly (4) and right ventricular outflow obstruction (RVOTO) in 5 cases. Of all these cases, coarctation of aorta, double outlet right ventricle, anomalous pulmonary venous return, and anomalous great vessels occurred only as a complex with other defects.

Of the 20 cases of isolated cardiac defects, LVOTO was the most frequent, accounted for 50% (n = 10) and this category occurring as an isolated defect rather than as part of a complex was found to be statistically significant ($P = .012$).

Septal defects occurring as part of a complex rather than isolated defect was statistically significant ($P= .003$). Ventricular septal defect (VSD) was the most common septal defect (n = 10). When considering the subtypes of VSD, there were 8 perimembranous, 1 muscular and 1 subaortic VSD. VSD occurring as a part of Tetrology of Fallot (TOF) (Fig. 2) was not categorized under septal defect and this included perimembranous type.

Two unusual cases of complex cardiac anomalies were observed. One case included atrioventricular septal defect, malposition of great arteries, total anomalous pulmonary venous connection (TAPVC), right atrial isomerism, pulmonary atresia, while the other showed atrioventricular septal defect, hypoplastic left heart, hypoplastic right heart, and right sided aortic arch.

Histological examination of heart in all the fetal autopsy cases did not reveal any nonstructural cardiac anomalies like myocarditis and cardiomyopathy.

3.1.2. Cardiac defects associated with extra cardiac/syndromic findings

These cases were categorized based on single or multiple organs involvement. Among the 29 cases, single organ and multiple organ involvement was found in 31% (n = 9) and 68.9% (n = 20) of cases, respectively (Fig. 3). When comparing the distribution of single organ and multiple organ involvement, there was no statistical significance ($P = .689$) between isolated and complex cardiac

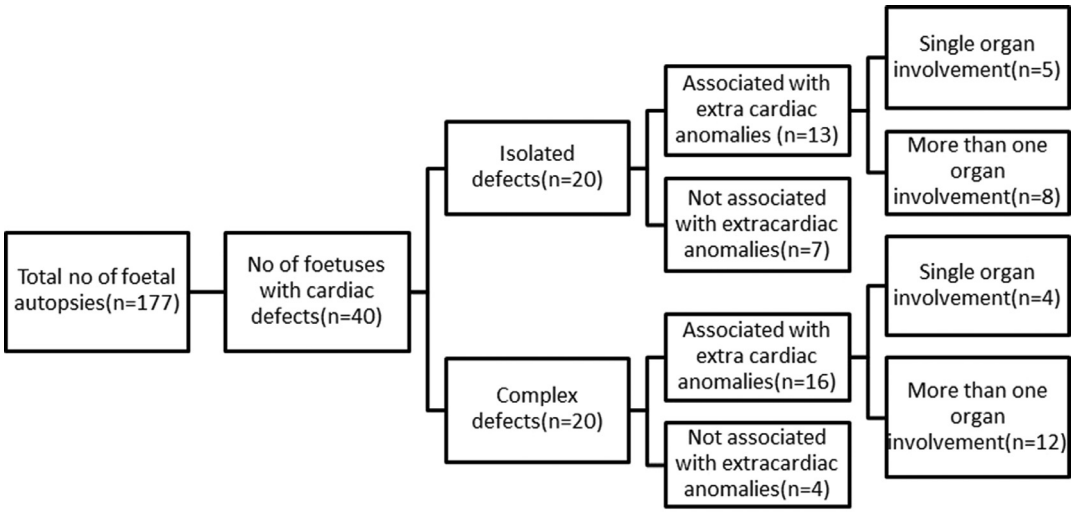


Fig. 1. . Flow diagram illustrating analysis of cardiac defects.

Table 1
Distribution of cardiac defects

S. No	Category	Number	Isolation	Complex	*P* value
1.	Septal defects	18			.003‡
	ASD	2	2	0	
	VSD	10	1	9	
	AVSD	6	0	6	
2.	Left ventricular outflow tract obstruction	11			.012‡
	HLHS	6	6	0	
	Coarctation of aorta*	1	0	1	
	Aortic arch hypoplasia	3	3	0	
	Mitral valve dysplasia	1	1	0	
3.	Right ventricular outflow tract obstruction	5			.082‡
	HRHS	3	0	3	
	Tricuspid valve dysplasia	1	1	0	
	Pulmonary valve atresia with intact ventricular septum	1	1	0	
4	Conotruncal anomaly	5			.66‡
	Truncus arteriosus	1	0	1	
	Tetralogy of Fallot	3	1	2	
	Double outlet right ventricle*	1	0	1	
5	Connection anomaly	8			.75‡
	Transposition of great arteries	7	2	5	
	Anomalous pulmonary venous connection	1	0	1	
6	Anomalous great vessels*	8			
	Persistent left superior vena cava	2	0	2	
	Vascular ring	1	0	1	
	Right aortic arch	5	0	5	
7	Others	2			
	Rhabdomyoma	1	1	0	
	Premature closure of ductus	1	1	0	
8.	Malpositions and isomerism*				
	Dextrocardia	1	0	1	
	Right atrial isomerism	1	0	1	

ASD, atrial septal defect; AVSD, atrio ventricular septal defect; HLHS, hypoplastic left heart syndrome; HRHS, hypoplastic right heart syndrome; VSD, ventricular septal defect.
The occurrence of a cardiac defect to occur in isolation rather than in association with complex was compared using chi-square test. *P* value is significant only for septal defects.
* These defects occurred only as a complex and hence *P* value could not be obtained. ‡- p value obtained using chi square test.

defects. The most common extracardiac malformation was dysmorphism (46.2%) followed by genitourinary tract (41.3%), central nervous system (24%), musculoskeletal (20.6%), respiratory tract (20.6%) and gastrointestinal tract. The details are summarized in Supplementary Table 1 and illustrated in Fig. 4.

Overall, the fetal autopsy yielded additional cardiac defects in 40% of cases and extracardiac anomalies in 45% when compared with prenatal ultrasound.

3.2. Correlation between prenatal ultrasound and autopsy findings

The prenatal ultrasound details were available for all the 40 cases and fetal echocardiogram in 7 of them. Cardiac abnormalities were detected in 30 cases. There was complete agreement in 25% (n = 10) cases, major agreement with additional findings in 40% (n = 16) cases. Complete disagreement was seen in 10% (n = 4) cases.

Table 2
Description of cases classified in the category of complex cardiac defects (n = 20)

S. No	Complex cardiac anomalies
	AVSD, common atrium
	AVSD, PLSVC, hypoplasia of aortic arch
	AVSD, common atrium, PLSVC
	AVSD, TAPVC, right atrial isomerism, pulmonary atresia
	AVSD, common atrium
	AVSD, pulmonary stenosis, right heart hypoplasia, left heart hypoplasia, right aortic arch
	VSD, coarctation of aorta
	VSD, overriding of aorta
	VSD, TGA, pulmonary atresia
	VSD, truncus arteriosus, right aortic arch, vascular ring
	Dextrocardia, large ductus arteriosus
	HRHS, VSD, overriding of aorta
	HRHS, VSD
	HRHS, VSD
	TGA, DORV, pulmonary atresia
	TGA, common atrium
	TGA, VSD
	TGA, VSD, right aortic arch
	TOF, right aortic arch, patent ductus arteriosus
	TOF, right aortic arch, absent ductus, aberrant subclavian artery

AVSD, atrio ventricular septal defect; DORV, double outlet right ventricle; HRHS, hypoplastic right heart syndrome; PLSVC, persistent left superior vena cava; TAPVC, total anomalous pulmonary venous connection; TGA, transposition of great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

In the remaining ten cases where, there were no cardiac anomaly detected prenatally the indication for autopsy was hydrops and IUD in 8 and 2 cases respectively. The cardiac defects identified in these cases were hypoplastic left heart syndrome (HLHS) (n = 5), followed by septal defects (n = 3) then by hypoplastic right heart syndrome and premature closure of ductus. The cases with major agreement and disagreement are described in Tables 3 and 4, respectively.

All the isolated cases had concordance with prenatal USG except for a single case of premature closure of ductus arteriosus in which no abnormality was detected. Fetal echocardiogram was available in seven cases and showed 100% concordance with autopsy.

4. Discussion

The rate of autopsy is declining in general. This is true with fetal autopsies as well, the main reason being the advancement in prenatal imaging techniques followed by declining interest in the field of autopsy pathology due to time consumption and the expertise required. In the current scenario, false positive diagnosis in prenatal USG resulting in TOP is extremely rare. However, associated malformations may be missed by USG and the detection of which might alter the diagnosis with varied recurrence risk. This was evident in the present study. The current study details the spectrum of cardiac abnormalities in prenatal autopsy and correlating these findings with prenatal imaging studies.

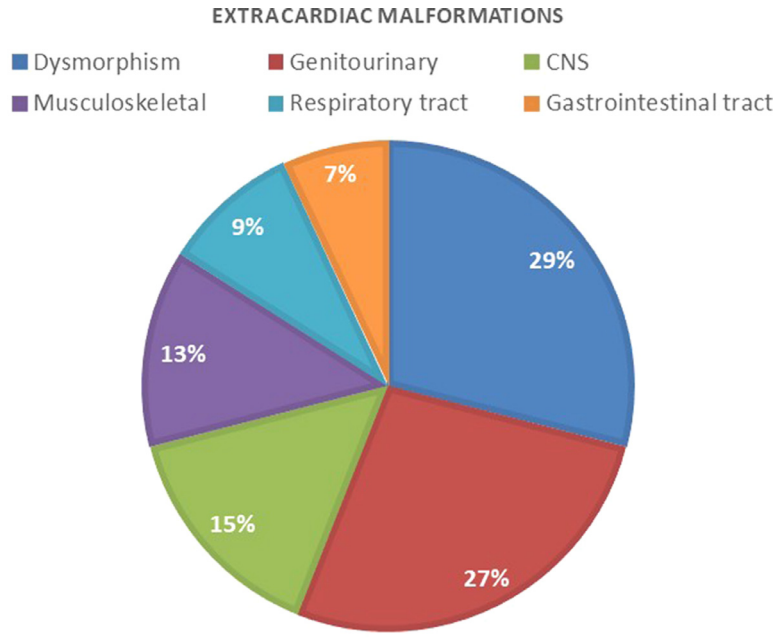


Fig. 3. . Distribution of associated extracardiac malformations.



Fig. 2. . Tetralogy of Fallot (A) Main pulmonary artery is stenosed with right ventricular hypertrophy (B) Perimembranous VSD with overriding of aorta (50%). (C) Right aortic arch.

Cardiac defects were the second most frequent congenital malformations in our institution following central nervous system malformations. The frequency of cardiac malformations (22.6%) was significantly higher in current study which is comparable to that of Taweevisit et al [11], while a few other studies report a lower frequency [12-14]. The higher frequency could be attributed either to increased rate of detection of cardiac anomalies on ultrasound thus leading to TOP or reflect a higher incidence in the given population. Male predominance (1.7:1) observed in this study was similar to majority of the other studies [14–17].

We found that 70% cases had an associated structural extra cardiac anomaly. Autopsy has enabled to identify additional cardiac defects in those fetuses terminated for other structural anomalies. This emphasizes the need for meticulous cardiac examination even in those fetuses.

Cardiac dissection in fetus is laborious and strenuous especially during the first and early second trimester, since the fetus itself is small in size. Usage of dissection microscope if available or loop glasses (in resource poor setting) will aid in qualitative analysis of cardiac defects in the tiny fetuses. A step by step approach by doing inspection, probing, assessing, documenting by taking pictures, dissecting and finally by cutting the heart is the best method.

Slight modifications in the dissection methods can be done in certain congenital heart diseases.

1) Cross-sectioning (transverse) of the heart starting from the apex, parallel to the atrioventricular groove up to the anterior papillary muscle (bread-loaf method) in a case of suspected rhabdomyoma is beneficial. It gives a better visualization for regional lesions confined to one or more walls. Hypoplasia and/or

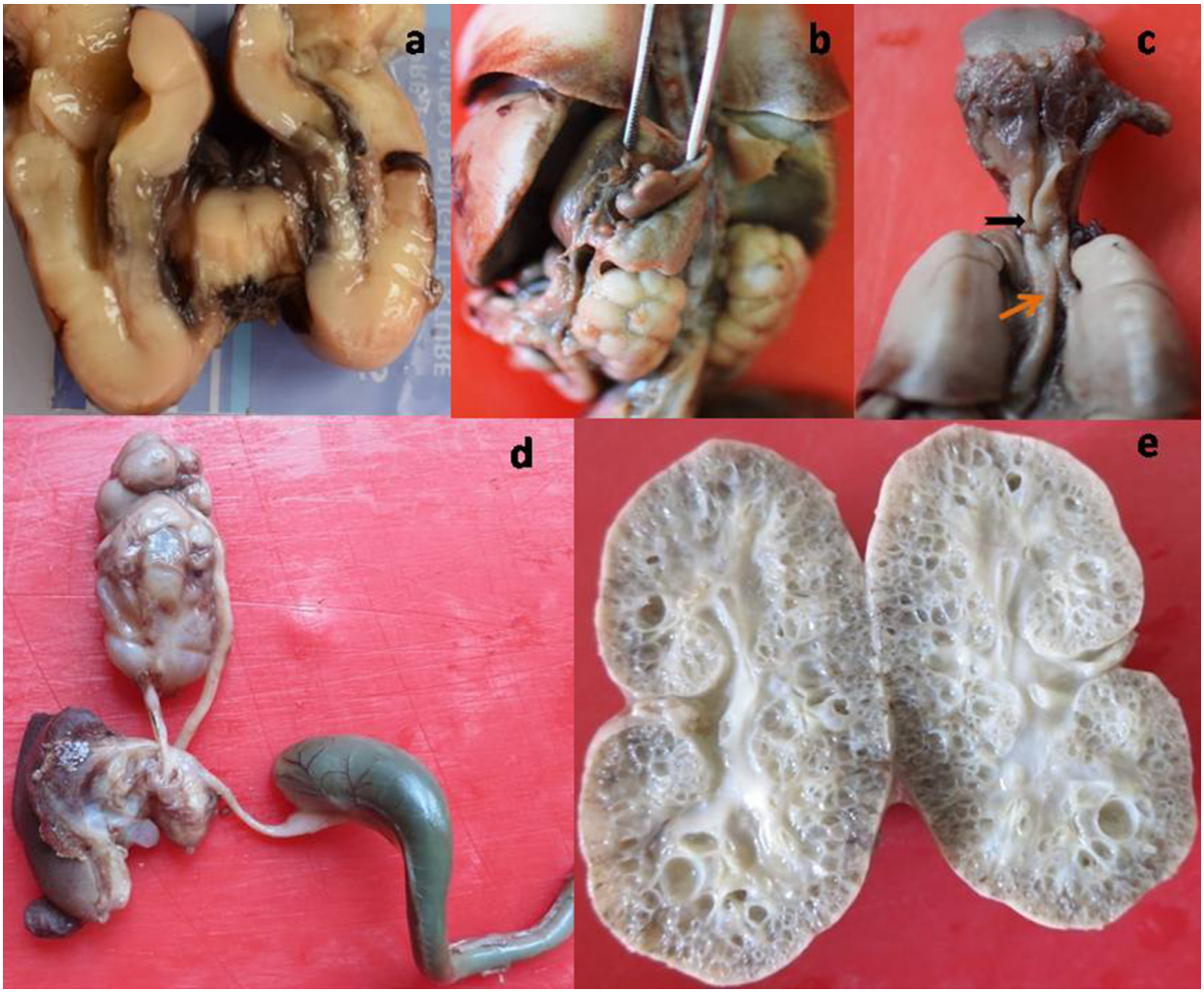


Fig. 4. . Extracardiac abnormalities (A) Coronal section of the brain shows absence of corpus callosum. (B) Splenenculi, there are small multiple brown nodules in peripancreatic region and gastrosplenic ligament. (C) Type C Tracheoesophageal fistula: atresia of proximal esophagus (⟶) with lower esophagus connected to tracheal carina (↗) (D) Urorectal septal malformation: atretic analcanal and ureters are draining into common pouch (cloaca) with crossed fused renal ectopia (E) Polycystic kidney disease.

Table 3
Cases with major agreement and additional findings in autopsy

S. No	Prenatal diagnosis	Additional autopsy findings
	TOF	Right aortic arch
	Cardiac outflow tract anomaly	TGA with pulmonary atresia, DORV
	TOF	TOF, aberrant subclavian artery right aortic arch, absent Ductus
	Dextrocardia	Dextrocardia, large ductus arteriosus
	Right atrial isomerism	Right atrial isomerism, AVSD, TAPVC, malposition of great arteries, pulmonary atresia
	AVSD	AVSD, common atrium
	VSD	VSD with coarctation of aorta
	Overriding of aorta and pulmonary atresia	TOF
	Multiple cardiac anomalies	Hypoplastic aortic arch, absent ductus
	Subaortic VSD, interrupted aortic arch	Hypoplastic left ventricle, Hypoplastic aortic arch, No VSD
	HRHS, pulmonary atresia	HRHS, Tricuspid atresia, muscular VSD
	VSD, overriding of aorta, tricuspid atresia	VSD, overriding of aorta, tricuspid atresia,Pulmonary atresia, hypoplastic right ventricle
	Dysplastic mitral valve, critical aortic stenosis	Mitral valve dysplasia, aortic stenosis Hypertrophied left ventricle
	TGA, VSD with hypoplastic right ventricle.	Congenitally corrected TGA, atrioventricular discordance
	Right atrial and ventricular dilatation with tricuspid regurgitation	Right atrial and ventricular dilatation with tricuspid regurgitation
	AVSD, DORV	VSD, hypoplastic right and left heart, right sided aortic arch

ASD, atrial-septal defect; AVSD, atrio-ventricular septal defect; DORV, double outlet right ventricle; HRHS, hypoplastic right heart syndrome; TGA, transposition of great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

Table 4
Cases with complete disagreement with prenatal ultrasound

S. No	Prenatal diagnosis	Indication for autopsy	Autopsy finding
1	Atrioventricular septal defect	TOP due to the cardiac anomaly detected	Congenitally corrected Transposition of great arteries
2	Intracardiac echogenic focus	TOP due to other system anomalies	Secundum atrial septal defect
3	Tricuspid dysplasia, hypoplastic right ventricle and pulmonary artery	TOP due to the cardiac anomaly detected	Pulmonary valve atresia with intact ventricular septum
4	Hypoplastic left heart syndrome	Intrauterine fetal demise	Ventricular septal defect, overriding of aorta

asymmetry of ventricular chambers can also be assessed by this method.

2) Normally, we reach the aorta from the left ventricle by cutting through the anterior mitral leaflet. In case of doubtful mitral valve pathology, the aorta can be reached by cutting the left ventricular anterior papillary muscle without disturbing the valve.
3) The heart can be bissected longitudinally to visualize all the four chambers for asymmetry.

Septal defects were the commonest abnormality in the present series, dominated by VSD and seen as a part of other cardiac or extracardiac abnormalities similar to that of Ramalho et al some studies had observed VSD as the most commonly identified isolated defect [12,16,18–20]. This difference may be attributed to the sensitivity of prenatal ultrasound in identifying complex cases.

Atrial isomerism and persistent left superior vena cava (PLSVC) were only rarely encountered in the present series unlike the previous studies [12,21,22]. In identifying isomerism and atrio-ventricular and ventriculo-arterial concordance, the intricate knowledge of morphology of atrial appendages, ventricular trabeculations and outflow tracts is very crucial. Both the cases of PLSVC were found as part of a complex cardiac and extracardiac anomaly. The higher percentage of associated anomaly in PLSVC implies developmental abnormality rather than a variation. The findings also reemphasize the fact that PLSVC is a marker of cardiac and extracardiac anomaly as identified in previous studies [21,22] and when identified should prompt an extensive search for other associations. One of the indirect indicators of PLSVC is absence of left innominate vein on dissecting the thymus.

Hydrops fetalis resulting due to cardiac defect was identified in 20%, findings consistent with other studies that showed the frequency between 4 and 27.5% [23].

Autopsy helps to reach the diagnosis in majority of the cases either by confirming the prenatal diagnosis, or refine the diagnosis by means of identifying additional findings. Additional findings in the present series were identified in 45% of cases which altered the diagnosis in 30% of them. This was comparable to the previous studies [1,24]. Majority of these were syndrome complexes due to chromosomal abnormalities. Even though, karyotyping was not available for all these cases, autopsy has enabled to suggest the possibility of these syndromes by the presence of classic dysmorphic features and organ involvement in majority of the cases. Definitive diagnosis was possible in 95% of cases which is similar to that of other studies [24-26].

In two of these cases, the definitive diagnosis of syndrome complex was not possible. One fetus showed multiple anomalies including agenesis of the corpus callosum, Pierre Robin sequence and cardiac defects. Such association can be seen in multiple congenital malformation syndromes. In the present case, a diagnosis of Zellweger syndrome or Smith Lemli Opitz syndrome was offered in view of the presence of hemosiderosis of the liver and cerebral leukodystrophy. The second case showed heart defects with phenotypic features suggesting the possibility of aneuploidy which needs the help of karyotyping for a definitive diagnosis.

4.1. Extracardiac anomalies

Multiple organ involvement was more frequent than single organ involvement. Dysmorphism being the commonest is similar to some of the studies [11,14]. This was followed by genitourinary system malformations which are in contrast to the previous studies. This could be because of the technique of autopsy followed in our institution which includes meticulous dissection of external genitalia and lower urethra in all the cases. This has enabled us to identify majority of the lower urinary tract obstructions which otherwise could have been missed in both prenatal ultrasound and autopsy. Identification of these extracardiac anomalies is essential in arriving at a diagnosis of syndromes and/or association which has an impact in the management of subsequent pregnancies.

The organs which need attention are thymus, spleen (polysplenia, asplenia), gastrointestinal tract and excretory system. Polysplenia can present as very tiny nodules in gastrosplenic ligament and peripancreatic tissue which could be missed in prenatal imaging. Submission of suspected nodules for histological examination is essential.

In the present study, a high proportion of fetuses with HLHS predominantly had coexistent genitourinary, followed by central nervous system malformations. It is a well-known fact that HLHS fetuses experience in-utero hypoxia with dysregulated cerebrovascular hemodynamics [26]. But the exact mechanism of this association with genitourinary system defects is not known. It could also be related to early vascular insult with impaired autoregulation. The potential pathogenesis has to be explored.

4.2. Correlation of prenatal ultrasound and autopsy findings

The overall agreement found in the study was 65% which is slightly lower when compared to the majority of existing literature [25,27,28].

Complete disagreement was seen in 10% of cases and included lesions like Pulmonary valve atresia with intact ventricular septum, congenitally corrected transposition of great arteries and septal defects. Usage of five chambered view on ultrasound (the long axis view of heart highlighting the outflow tract in addition to four chamber view) could have been helpful in the accurate identification of some of these lesions [29]. 25% of cardiac lesions were missed on ultrasound, HLHS being the commonest followed by septal defects. These observations concur with that of Trivedi et al [27].

In spite of the improved accuracy of prenatal USG owing to the technical advancement in radiological techniques, some abnormalities can still be missed. These have been attributed to oligohydramnios, maternal obesity, position of fetus and expertise of the sonologists. In our series of autopsy, 32 cases were performed by expert sonologists experienced in fetal USG. However, some of the

previous studies had not found any statistically different detection rate of congenital heart disease between primary physician (77.9%) and pediatric cardiologist (85.0%) [29,30]. The other factors responsible for missed diagnosis in prenatal USG could not evaluated in the present study.

There was 100% correlation between fetal autopsy and echocardiogram wherever available. This imaging modality could be used as a supplement in fetuses with cardiac defects detected on ultrasound.

5. Strengths of the study

The study was done on all the fetal autopsies performed in our institution including a wider gestational age. Majority of the ultrasound diagnosis was made by expert radiologists and obstetrician trained in fetal medicine. All the autopsies were done by two pathologists with expertise in the field of perinatal and fetal autopsy using a standardized protocol and sequential segmental analysis of heart.

6. Limitations of the study

The exact detection rate of prenatal sonogram cannot be ascertained since the study is retrospective and includes only the cases with congenital heart defects identified in fetal autopsy. Hence the false positivity in prenatal imaging could not be analyzed. Secondly karyotyping was not available in majority of the cases.

7. Conclusion

A higher incidence of heart defects with significant proportion of associated extra cardiac malformations in our series highlights the importance of 1. Sequential segmental analysis of heart to identify even minor defects in all the cases irrespective of prenatal ultrasound findings and 2. modification of technique in certain conditions to demonstrate the lesion.

In the age of advanced imaging modalities with high sensitivity index, autopsy continues to be the gold standard in providing optimal diagnosis thereby guiding appropriate management in future pregnancies. Ensuring proper training of autopsy pathologists is essential to avoid the loss of valuable information in autopsy which could result from suboptimal approach. With a few centers performing routine fetal autopsies, more studies are anticipated to understand the epidemiology of heart malformations in the Asian subcontinent and pave new pathway in exploring the pathogenesis of rare associations.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpath.2020.107312.

References

[1] Sankar VH, Phadke SR. Clinical utility of fetal autopsy and comparison with prenatal ultrasound findings. J Perinatol 2006;26:224–9.
[2] Kinare SG, Sharma S. Congenital heart disease in first year of life (an autopsy study of 270 cases). Indian J Pediatr. 1981;48:745–51.
[3] Şorop-Florea M, Ciurea RN, Ioana M, Stepan AE, Stoica GA, Tanase F, et al. The importance of perinatal autopsy. Review of the literature and series of cases. Rom J Morphol Embryol. 2017;58(2):323–37.
[4] Rossi AC, Prefumo F. Correlation between fetal autopsy and prenatal diagnosis by ultrasound: a systematic review. Eur J Obstet Gynecol Reprod Biol 2017;210:201–6.
[5] Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol 2002;39(12):1890–900.
[6] Ernst LM. A pathologist ′s perspective on the perinatal autopsy. Semin Perinatol 2015;39:55–63.
[7] The Royal College of Obstetricians and Gynecologists and Royal College of Pathologists Fetal and Perinatal Pathology: Report of a Joint Working Party. London: RCOG Press; 2001.
[8] Erickson LK. An approach to the examination of the fetal congenitally malformed heart at autopsy. J Fetal Med 2015;2(3):135–42.
[9] Anderson RH, Shirali G. Sequential segmental analysis. Ann Pediatr Cardiol 2009;2(1):24–35.
[10] Gilbert-Barness E, Debich-Spicer D. Congenital malformations of the heart. In: Gilbert-Barness E, editor. Potter's Pathology of the Fetus, Infant and Child. Philadelphia PA: Mosby Elsevier; 2007. p. 987–1024.
[11] Taweevisit EK, Thorner P. Cardiac findings in fetal and pediatric autopsies: a 15-year retrospective review. Fetal Pediatr Pathol 2019;38(1):14–29.
[12] Ramalho C, Brandao O, Monterroso J, Matias A, Montenegro N. Cardiac findings in routine fetal autopsies: more than meets the eye? Eur J Obstet Gynecol Reprod Biol. 2012;163:142–7.
[13] Tennstedt C, Chaoui R, Korner H, Dietel M. Spectrum of congenital heart defects and extracardiac malformations associated with chromosomal abnormalities: results of a seven-year necropsy study. Heart 1999;82:34–9.
[14] Grant M, Evans MJ. Cardiac findings in fetal and pediatric autopsies: a five-year retrospective review. Pediatr Dev Pathol 2009;12:103–10.
[15] Bakker MK, Bergman JEH, Krikov S, Amar E, Cocchi G, Cragan J, et al. Prenatal diagnosis and prevalence of critical congenital heart defects: an international retrospective cohort study. BMJ Open 2019;9(7):e028139. doi:10.1136/bmjopen-2018-028139.
[16] Mohan H, Bhardwaj S, Bal A. Congenital visceral malformations–role of perinatal autopsy in diagnosis. Fetal Diagn Ther 2004;19(2) 131-13.
[17] Boyd PA, Tondi F, Hicks NR, Chamberlain PF. Autopsy after termination of pregnancy for fetal anomaly: retrospective cohort study. BMJ 2004;328:137–40.
[18] Açikalin A, Bağir EK, Torun G, Ates BT, Erdogan S, Uguz A, et al. Perinatal autopsy evaluation of 2150 autopsies in the Çukurova region of Turkey. Turk Patoloji Derg 2014;30(3):189–94.
[19] Isaksen CV, Eik-Nes SH, Blaas HG, Tegnander E, Torp SH. Comparison of prenatal ultrasound and postmortem findings in fetuses and infants with congenital heart defects. Ultrasound Obstet Gynecol 1999;13(2):117–26.
[20] Andola US, Am A, Ahuja M, Andola SK. Congenital malformations in perinatal autopsies - a study of 100 cases. J Clin Diagn Res 2012;6(10):1726–30.
[21] Hakverdı S, Güzelmansur I, Güngören A, Toprak S, Yaldiz M, Hakverdı AU. Evaluation of fetal autopsy findings in the Hatay region: 274 cases. Turk Patoloji Derg 2012;28(2):154–61.
[22] Ramakrishnan D, Chidambarathanu S, Murli L, Micheal J, Jagadeesh S, Suresh I, et al. Persistent left superior vena cava in fetuses: an autopsy series. Fetal Pediatr Pathol 2017;36(4):304–10.
[23] Yuan SM. Cardiac etiologies of hydrops fetalis. Z Geburtshilfe Neonatol 2017;221(2):67–72.
[24] Phadke SR, Gupta A. Comparison of prenatal ultrasound findings and autopsy findings in fetuses terminated after prenatal diagnosis of malformations: an experience of a clinical genetics center. J Clin Ultrasound 2010;38(5):244–9.
[25] Ramalho C, Matias A, Brandao O, Montenegro N. Critical evaluation of elective termination of pregnancy in a tertiary fetal medicine center during 43 months: correlation of prenatal diagnosis findings and postmortem examination. Prenat Diagn 2006;26(11):1084–8.
[26] Kinnear C, Haranal M, Shannon P, Jaeggi E, Chitayat D, Mital S. Abnormal fetal cerebral and vascular development in hypoplastic left heart syndrome. Prenat Diagn 2019;39(1):38–44.
[27] Trivedi N, Levy D, Tarsa M, Anton T, Hartney C, Wolfson T, et al. Congenital cardiac anomalies: prenatal readings versus neonatal outcomes. J Ultrasound Med 2012;31(3):389–99.
[28] Dolk H, Loane M. Garne E; European Surveillance of Congenital Anomalies (EUROCAT) Working Group. Congenital heart defects in Europe: prevalence and perinatal mortality, 2000 to 2005. Circulation. 2011;123(8):841–9.
[29] Bravo-Valenzuela NJ, Peixoto AB, Araujo Júnior E. Prenatal diagnosis of congenital heart disease: a review of current knowledge. Indian Heart J 2018;70(1):150-164.
[30] Nayak SR, Garg N. Determination of antepartum fetal death. J Obstet Gynecol India 2010;60:494–7.

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ORIGINAL ARTICLE

WILEY

The combination of ACR-Thyroid Imaging Reporting and Data system and The Bethesda System for Reporting Thyroid Cytopathology in the evaluation of thyroid nodules—An institutional experience

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Abstract

Background: The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is widely utilised by cytopathologists. The American College of Radiology (ACR) has also proposed a thyroid imaging reporting and data system (TIRADS) to classify thyroid nodules and guide their selection for fine needle aspiration (FNA). The current study aimed to analyse the usefulness of TBSRTC in thyroid cytology reporting, to examine its histological correlation with TIRADS, and to compare the management of lesions in each cytological category in our institute to the TBSRTC recommendations.

Materials and methods: A retrospective study was performed on all thyroid FNAs from a 2-year period at a tertiary care centre. Histological correlations of TBSRTC and ACR TIRADS were examined for cases with surgical follow-up, and the risk of malignancy in each TBSRTC category, and sensitivity and specificity of FNA and TIRADS, were calculated.

Results: A total of 337 thyroid FNAs were examined, with histological follow-up in 99 cases. Risk of malignancy in categories I-VI was 9.5%, 2.3%, 0.0, 8%, 87.5% and 100.0%, respectively. The sensitivity, specificity, positive predictive value, and negative predictive value of FNA were 60.0%, 99.0%, 85.7%, and 97.5%, respectively, while those values for thyroid ultrasound were 90.3%, 72.2%, 92.9% and 65%, respectively.

Conclusion: The study substantiates the usefulness of TBSRTC in arriving at a more precise diagnosis. Hurthle cell lesions with atypia were the common cause of misdiagnosis. The combination of TIRADS and TBSRTC aids in better stratification of thyroid nodules and in decision making for management of lesions.

KEYWORDS

atypia of undetermined significance, papillary carcinoma, the Bethesda system of reporting thyroid cytopathology, thyroid nodules

Shanmugasundaram Sakthisankari and Sreenivasan Vidhyalakshmi contributed equally to the work.

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1 | INTRODUCTION

Thyroid cancer is the most common endocrine malignancy and constitutes 0.1%-0.2% of all cancers in India. Fine needle aspiration (FNA) of the thyroid gland plays a vital role in the evaluation of thyroid lesions. It is a safe, cost-effective procedure to guide the management of thyroid lesions.[1] With the routine use of FNA, the number of surgeries being performed for benign lesions has been significantly reduced.[2] Most of the centres perform both palpation-guided and ultrasound-guided (USG) FNA.[3] Before the inception of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), various terminologies were in use which often led to confusing risk assessments and unclear clinical management. TBSRTC has established a uniform, tiered reporting system for thyroid FNA specimens with an implied malignancy risk and recommendations for management, and is widely accepted by cytopathologists and clinicians.[4] But unfortunately, a significant subset of cytology interpretation yields indeterminate results (25%), as reported by Valderrabano et al.[5]

The use of ultrasound criteria in differentiating benign and malignant nodules of the thyroid gland has been the focus of research and much of the published literature has confirmed that ultrasound is highly sensitive but less specific in the detection of malignant thyroid nodules. A practical thyroid imaging reporting and data system (TIRADS) for thyroid nodules has been proposed by the American College of Radiology (ACR) to classify nodules of the thyroid gland and to solve the problem of nodule selection for FNAC.[6,7] Shen et al compared the effectiveness of the American Thyroid Association (ATA) classification, the ACR TIRADS, the TIRADS of the European Thyroid Association (EU-TIRADS), and a TIRADS developed by Kwak et al (Kwak-TIRADS) in a large cohort of patients, and concluded that all four risk-stratification systems had good diagnostic performance.[8] The combination of ultrasound imaging criteria using a TIRADS with TBSRTC in the risk stratification of thyroid nodules has not been explored extensively. The present study was carried out to establish the utility of such a combination of TIRADS with TBSRTC in the diagnostic and therapeutic approach to thyroid nodules.

The recent WHO classification has included new variants of thyroid carcinoma such as non-invasive follicular neoplasm with papillary-like nuclear features (NIFTP) and well differentiated thyroid tumour of uncertain malignant potential (WDT-UMP).[9] It is recommended that the risk of malignancy in each category be evaluated by every institution following TBSRTC for reporting as a tool for quality improvement. The current study aims (1) to analyse the diagnostic utility of TBSRTC in thyroid cytology reporting, (2) to establish the histological correlation of thyroid ultrasound using ACR TIRADS and (3) to analyse the adherence to TBSRTC recommendations of management for each category at our Institute.

2 | MATERIALS AND METHODS

This was a retrospective study which involved all patients who underwent FNAC of thyroid lesions over a period of two years (January 2018 to December 2019). The study was approved by the Institute's Human Ethics Committee (20/142). MGG- and PAP-stained smears and cytology reports of thyroid lesions were retrieved from the archives of the Department of Pathology at our Institute, a tertiary care centre. All of these cases were reviewed and diagnosed based on TBSRTC categories I to VI, as follows.

I. Non-diagnostic or unsatisfactory (ND/UNS)

II. Benign

III. Atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS)

IV. Follicular neoplasm/suspicious for follicular neoplasm (FN/SFN)

V. Suspicious for malignancy

VI. Malignant

The probable diagnostic possibilities for categories V and VI were given wherever possible.

In cases with surgical excision, histopathology was considered the final diagnosis and correlated with cytology. Cases with histopathological diagnosis of follicular lesion, NIFTP, were reviewed for adherence to strict criteria for diagnosis. In cases where the cytology and histopathology were discordant, the cytology slides were reanalysed in a blindfolded technique to eliminate subjective bias. The sensitivity and specificity of FNAC in thyroid lesions and probable reasons for diagnostic errors in cytology were analysed.

A retrospective review of thyroid ultrasound features for those patients who underwent thyroidectomy in our institution was performed. The TIRADS score was given by an expert radiologist who was blinded to the histological diagnoses. The ACR TIRADS score was based on the evaluation of composition, echogenicity, shape, margin, and echogenic foci of the lesion, with each feature assigned 0-3 points. The total score is further stratified into categories ranging from 1 (benign) to 5 (highly suspicious). In cases with multiple nodules having different TIRADS scores, the highest score was considered for analysis. Risk of malignancy (ROM) for each TIRADS category was calculated based on the histological diagnosis. For calculating the sensitivity of the TIRADS in predicting malignancy, cross tabulation of the TIRADS score and the histological diagnosis was done. TIRADS categories 1, 2, and 3 were grouped as benign and 4, 5 as suspicious for malignancy.

For each category reported, the recommended management guidelines of TBSRTC and the management done for these cases were compared.

Microsoft Excel 2010 (Microsoft corporation) was used for data entry and statistical analysis was performed using SPSS version 20 (IBM Corporation).

3 | RESULTS

Three hundred and thirty-seven cases of thyroid FNAs were retrieved from the archives of the Department of Pathology. The distribution of the diagnostic categories as per TBSRTC is presented in Table 1.

TABLE 1 Distribution of diagnostic categories of The Bethesda System for Reporting Thyroid Cytopathology

Diagnostic categories	Subcategories	Number of cases (% per category)	Total number of cases (%) n = 337
I. ND/US	Cyst fluid	19	21 (6.2)
	Acellular specimen	2	
II. Benign	Benign follicular nodule	157 (60.6)	259 (76.9)
	Adenomatoid nodule	62 (24)	
	Lymphocytic (Hashimoto) thyroiditis in the proper clinical context	40 (15.4)	
III. AUS		9	9 (2.6)
IV. FN/SFN	Suspicious for follicular neoplasm	22 (88)	25 (7.4)
	Suspicious for Hurthle cell neoplasm	3 (22)	
V. Suspicious for malignancy	Suspicious for papillary thyroid carcinoma	11 (73.3)	15 (4.5)
	Suspicious for medullary carcinoma	2 (13.3)	
	Suspicious for malignancy, not otherwise specified	2 (13.3)	
VI. Malignant	Papillary thyroid carcinoma	3 (37.5)	8 (2.4)
	Anaplastic carcinoma	2 (25)	
	Medullary carcinoma	2 (25)	
	Non-Hodgkin lymphoma	1 (12.5)	

Abbreviations: AUS, atypia of undetermined significance; FN/SFN, follicular neoplasm/suspicious for follicular neoplasm; ND/US, non-diagnostic or unsatisfactory.

The most frequent interpretation was category II of TBSRTC, constituting 76.9% of the total. Among these, benign follicular nodule was the most common interpretation (60.6% of category II), followed by adenomatoid nodule (24%), and Hashimoto thyroiditis (15.4%). Among the category IV lesions, Hurthle cell lesions constituted 22.2% of the category. In both category V and VI lesions, papillary thyroid carcinoma (PTC) was the commonest. Subsequent histopathological examination was possible for 99 cases, and hence the rate of surgical excision constituted 29.4% of cases. The type of surgery depended on the cytological interpretation and radiology findings, and included 11 cases of lobectomy, 62 hemithyroidectomy, and 26 total thyroidectomies. The histological diagnoses for these cases are summarised in Table 2. There were 19 cases of malignancy (excluding three cases of NIFTP and a single case of well-differentiated follicular tumour of uncertain malignant potential), and 79 benign cases which included 10 cases of follicular adenoma. Partially or totally encapsulated nodules showing an admixture of micro- and macrofollicular growth pattern in the background of multiple nodules were interpreted as adenomatoid nodules. Solitary encapsulated nodules consisting of microfollicles were diagnosed as follicular adenoma. Papillary thyroid carcinoma was the commonest malignancy (78.9%). The surgical yield of malignancy in the study was 19.2%. There was a rare coexistence of papillary thyroid carcinoma and calcitonin negative neuroendocrine tumour of the thyroid. The preoperative cytological diagnosis of this case was category VI. The cytological and histological features of category III, IV, and V are shown in Figures 1 and 2. The risk of malignancy for each diagnostic category was calculated excluding NIFTP and WDT-UMP and summarised in Table 2. Risk of neoplasia (RON) was calculated including NIFTP, WDT-UMP and follicular adenoma.

Pre-FNA thyroid ultrasound was available for 90 cases that had histological follow-up. Most of the thyroid nodules belonged to TIRADS categories 2 and 3 on imaging, constituting 67.7% of the total. The distribution of TIRADS categories with the calculated ROM values is tabulated in Table 3. The sensitivity, specificity, positive and negative predictive values of thyroid ultrasound were 90.3%, 72.2%, 92.9% and 65.0% respectively.

The management recommendations as per TBSRTC for each category and the management of patients in the current study are outlined in Table 4. Repeat FNA under ultrasound guidance for ND/UNS category I lesions was done in 19% of cases, of which two showed cystic macrophages, while the remaining two were categorised as benign. Repeat FNA was not performed in any of the Category III lesions. Surgical resection was performed in 23.8%, 25.1%, 22.2%, 60.0%, 60.0%, and 37.5% of categories I, II, III, IV, V, and VI respectively. Molecular testing was not performed in any of these cases.

Of the cases with surgical excision, 3 (4.6%) excluding 2 cases of papillary microcarcinoma had a false-negative diagnosis (these cases were categorised as benign in FNAC but turned out to be PTC in histology), and 2 (22.2%) had a false-positive diagnosis (these two cases were categorised as suspicious for malignancy in FNAC and histology showed adenomatoid nodule). A review of the cytological smears of the 5 cases diagnosed as PTC in histopathology that had been assigned to category II in the cytology was performed. Two cases were papillary microcarcinoma, and no features of PTC were discernible cytologically. In the other three cases, the presence of nuclear grooves and pale nuclei were underdiagnosed as part of a cystic change in colloid goitre. There were no nuclear pseudoinclusions in any of these cases. On reviewing the

TABLE 2 Details of cases with cytohistological correlation and calculated risk of malignancy (ROM)

Category	No of cases with surgery	Diagnostic category	No of cases n (%)	ROM %	ROM as per TBSRTC%	RON%
I	5	Nodular goitre	2 (40.0%)	9.5[a]	5-10	14.2
		Hurthle cell adenoma	1 (20.0%)			
		Papillary thyroid carcinoma	2 (40.0%)			
II	65	Hashimoto thyroiditis	30 (46.1%)	7.6[b]	0-3	9.2
		Nodular colloid goitre	18 (27.6%)			
		Adenomatous nodule	11 (16.9%)			
		NIFTP	1 (1.5)			
		Papillary thyroid carcinoma	5 (7.6%)			
III	2	NIFTP	1 (50.0%)	0	10-30	50.0
		Adenomatous nodule	1 (50.0%)			
IV	15	Hashimoto thyroiditis	2 (13.3%)	13.3[b]	25-40	66.6
		Adenomatous nodule	3 (20%)			
		Follicular adenoma	6 (40%)			
		NIFTP	1 (6.6%)			
		Follicular carcinoma	1 (6.6%)			
		WDT-UMP	1 (6.6%)			
		Papillary microcarcinoma	1 (6.6%)			
V	9	Adenomatous nodule	1 (11.1%)	77.7[b]	50-75	88.8
		Hurthle cell nodule	1 (11.1%)			
		Papillary thyroid carcinoma	6 (66.6%)			
		Medullary carcinoma	1 (11.1%)			
VI	3	Papillary carcinoma and neuroendocrine tumour, Diffuse large B cell lymphoma Anaplastic carcinoma	1 (33.3%) 1 (33.3%) 1 (33.3%)	100[b]	97-99	100

Abbreviations: NIFTP, non-invasive follicular neoplasm with papillary-like nuclear features; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology; WDT-UMP, well differentiated thyroid tumour of uncertain malignant potential.

[a]ROM was calculated using the total number of cases that were assigned category I on cytology.; [b]ROM was calculated using the number of cases that had surgical follow up. RON- Risk of neoplasia.

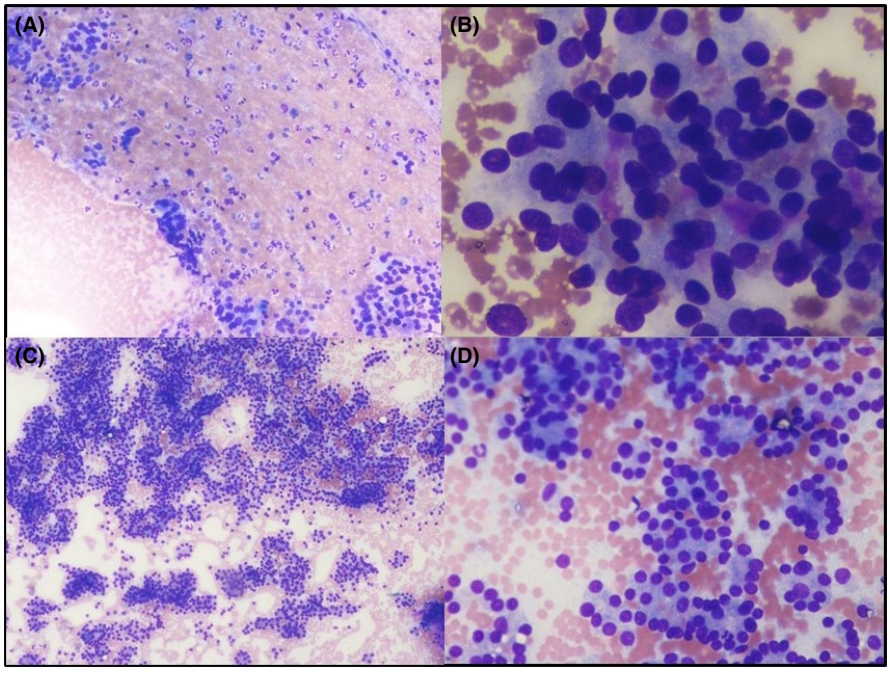


FIGURE 1 Atypia of undetermined significance (category III)-architectural atypia with cells entrapped in blood clot. (MGG,10X) Fig. 1.b. Microfollicles with minimal overlapping (MGG,40X) Fig. 1. c. Follicular neoplasm - a highly cellular aspirate of uniform follicular cells in crowded clusters and microfollicles (MGG 10X) Fig. 1. d Follicular cells show slight size variation and open chromatin (MGG 40X)

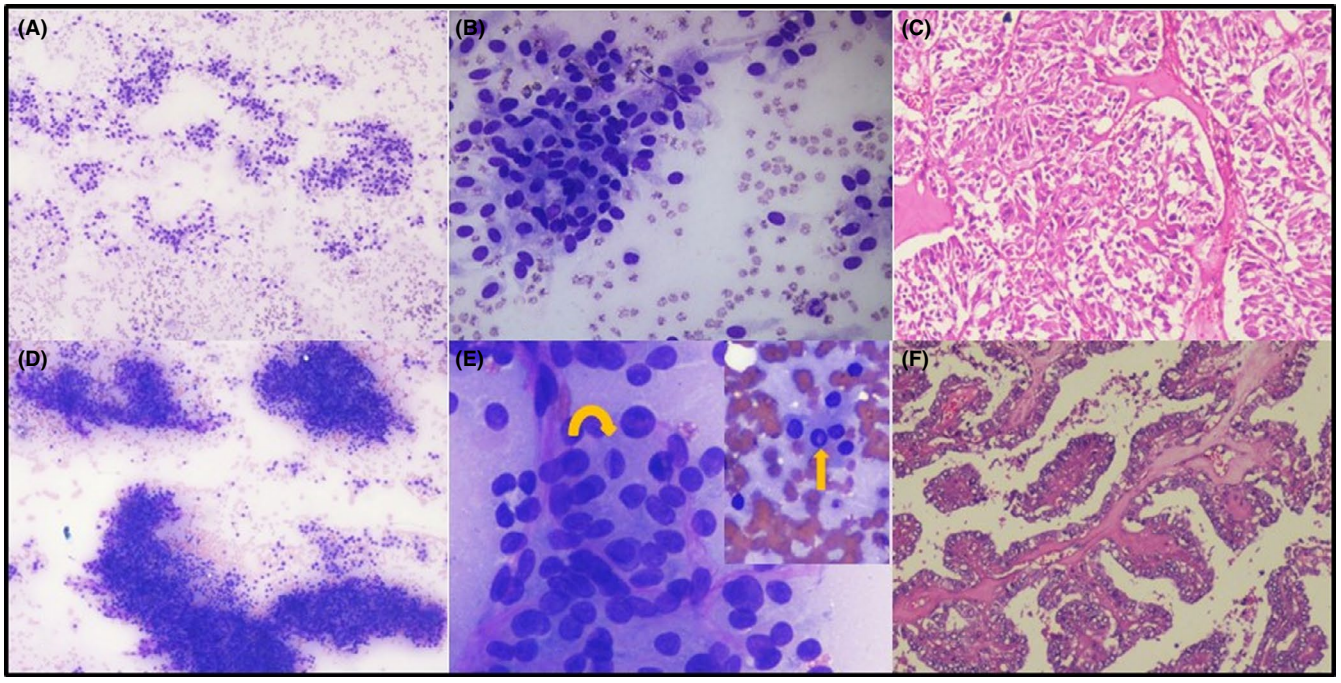


FIGURE 2 (A-C) Medullary carcinoma: (A) Cellular aspirate with crowded groups (MGG, 10×); (B) Plasmacytoid cells with oval nuclei and granular chromatin (MGG, 40×); (C) Lobules of neoplastic cells separated by amyloid (H&E, 10×). (D, E) Papillary thyroid carcinoma: (D) Papillary fragments (MGG, 10×); (E) Cellular overlapping, nuclear grooves (arrow) and nuclear inclusions (arrow in inset). (F) Papillae with fibrovascular core lined by neoplastic cells (H&E, 10×)

TABLE 3 Correlation of TIRADS score with cytology and histology and calculated ROM (n = 90)

	TBSRTC category						Histopathology				
ACR TIRADS score	I	II	III	IV	V	VI	Benign	Malignant	ROM in the study%	Recommended ROM%	RON%
1. (n = 9, 10.0%)		9					9	0	0	0	0
2. (n = 31, 34.4%)	1	26	0	3	1	0	29	2	6.6	<2	16.12
3. (n = 30, 33.3%)	1	20	0	7	2	0	27	3	10	5	20
4. (n = 15, 16.7%)	1	5	2	3	3	1	7	8	53.3	5-20	73.3
5. (n = 5, 5.6%)	1	0	0	0	2	2	0	5	100	>20	100
Total	4	60	2	13	8	3	72	18			27

Abbreviations: ACR, American College of Radiology; ROM, risk of malignancy; TIRADS, thyroid imaging reporting and data system; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology.

TABLE 4 Comparison of management guidelines by TBSRTC and management followed in the study

Diagnostic categories	No of cases	Management recommendations by Bethesda	Management in the study cases
I	21	Repeat FNA under guidance	Repeat FNA in 4 cases (19%) Surgery in 5 cases.
II	259	Clinical and sonographic follow-up	Clinical and sonographic follow-up in 78 cases. Hemithyroidectomy in 22 and total thyroidectomy in 43 cases.
III	9	Repeat FNA, molecular testing, or lobectomy	Lobectomy in 2 cases. Repeat FNA in none of these cases.
IV	25	Molecular testing, lobectomy	Hemithyroidectomy in 7 cases and total thyroidectomy in 8 cases.
V	15	Near-total thyroidectomy or lobectomy	Total thyroidectomy in 9 cases.
VI	8	Near-total thyroidectomy or lobectomy	Total thyroidectomy in 3 cases.

Abbreviations: FNA, fine needle aspiration; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology.

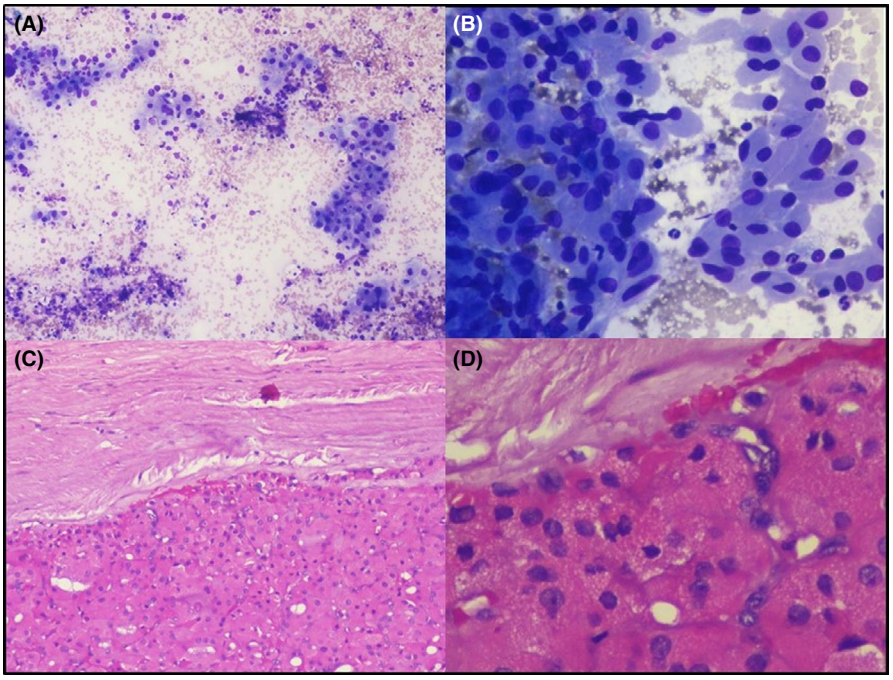


FIGURE 3 Follicular neoplasm, Hurthle cell type. (A) Cellular aspirate, crowded groups and isolated cells (MGG, 10×). (B) Exclusively Hurthle cells with marked anisonucleosis (MGG 40×). (C, D) Hurthle cell adenoma with a thick capsule (H&E, 10× and 40×)

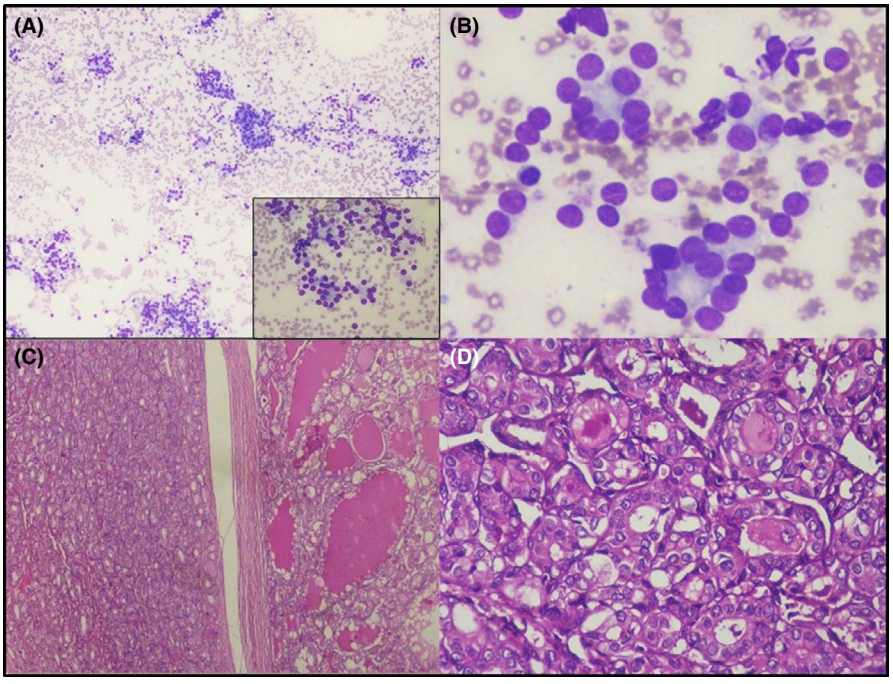


FIGURE 4 Non-invasive follicular neoplasm with papillary like nuclear features. (A) Cells arranged in repetitive microfollicular pattern (MGG, 10×). (B) Cells with nucleomegaly and occasional nuclear grooves (MGG, 40×). (C) Encapsulated nodule with no capsular invasion (H&E, 10×). (D) Closely packed follicles lined by cells exhibiting optically clear nuclei and grooves (MGG, 40×)

cytology smears of cases with a false-positive diagnosis on FNAC, in 1 case the presence of numerous aggregates of Hurthle cells exhibiting atypia was overestimated as features suspicious for malignancy. The cytological and histopathology images are shown in Figure 3. In the other case, hyperplastic changes with cells exhibiting occasional nuclear grooves, and follicular cells entrapped in blood clots with pale chromatin, were misinterpreted as features of malignancy.

The cytological features of WDT-UMP included repetitive microfollicular pattern, mild nuclear enlargement, occasional nuclear grooves, and no pseudoinclusions.

NIFTP constituted 3% of surgically resected thyroid lesions. The histopathology slides of the NIFTP cases were reviewed for the presence of the diagnostic criteria, which include complete encapsulation, follicular growth pattern, absence of invasion in extensive sampling, and optically clear nuclei as in Figure 4.

4 | DISCUSSION

The foremost advantage of TBSRTC is maintaining uniformity in the interpretation of thyroid FNA and guiding the clinicians to plan

TABLE 5 Comparison of the distribution of the diagnostic categories of the present report with other studies

Study, Country	Anand India	Thewzitcha Thailand	Zhu China	Wu USA	Sarkis Australia	Bongiovanni Europe	Present study
Number of FNAC (cases with surgery)	646 (100)	2735 (188)	2781 (1122)	25 445 (6362)	2076 (425)	3724 (1358)	337 (99)
Categories % (ROM %)							
I	13.8 (0)	21.1 (20)	14.8 (57.1)	12.9 (16.8)	12.8 (4.2)	3 (32)	6.2 (9.5)
II	75.9 (8.5)	66.6 (4.2)	17.1 (38.9)	59.3 (3.7)	74.7 (0.26)	55.4 (2.5)	76.9 (7.6)
III	1.2 (66.7)	4.7 (9.4)	15.8 (50)	9.6 (15.9)	4.7 (9.3)	6.7 (14.4)	2.6 (0)
IV	3.7 (63.6)	2.4 (23.5)	2.3 (31)	10.1 (26.1)	4.7 (15.3)	23.8 (32.1)	7.4 (13.3)
V	2.6 (100)	1.8 (57.1)	11.6 (78)	2.7 (75.2)	0.8 (79)	6.0 (74.9)	4.5 (77.7)
VI	2.8 (100)	3.3 (90.3)	38.5 (99)	5.4 (98.6)	2.3 (100)	5.1 (99.9)	2.4 (100)
Surgery	15.4%	9%	40.3%	25%	20.4%	36.5%	29.3%

Abbreviations: FNAC, fine needle aspiration cytology; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology.

appropriate therapy. The preoperative diagnosis of thyroid lesions in the present study using TBSRTC was comparable with other studies as summarised in Table 5.[10-14] The ND/UNS category still challenges accurate diagnosis. Despite the different expertise of cytologists in obtaining aspirates in a teaching hospital, the fraction of the ND/UNS category was well within the limits proposed by TBSRTC and other Indian[10,15] and Western studies.[13,16] This is in part due to the departmental protocol for rapid on-site evaluation (ROSE) in all cases of FNAC to assess for adequacy.

Benign lesions constituted the vast majority of FNA interpretation, which is similar to most other Asian studies[10,11] except a single study in the Chinese population,[8] where category VI was the most frequent interpretation. In Western studies, the reported proportion of the benign category ranges from 59% to 74.7%.[13,16] Regional variation may explain the difference in the distribution for this category.

The current study reports a lower rate of the AUS/FLUS category (2.6%), which is well within the preferred limit of 7% recommended by TBSRTC[4] and comparable to most Asian literature,[10,11,17,18] while some Western studies[9,12] have observed a higher percentage. The lower rate of this category could be attributed to the routine practice of obtaining an intradepartmental second opinion for all the indeterminate cases before assigning a category and hence the reasonable use of the AUS category.

As per the existing literature, the proportion of cases undergoing surgery varies among different institutions and ranges from 11.8% to 45.1%.[19,20] The malignancy rate of 19.2% in the present study was significantly lower when compared to the meta-analysis data by Bongiovanni et al (33.8%)[21] and Dawish et al (33.6%).[22,23] However, this malignancy rate does not reflect the true incidence in the population, because quite a few cases of thyroid malignancy were reported on histopathology and frozen section and did not have a cytological diagnosis before resection. Hence these cases were not included in the study. These resections were decided based on the clinical and sonographic findings.

4.1 | Risk of malignancy in each category

The risk of malignancy was comparable to that proposed by TBSRTC and other published literature for most of the categories except for ND/UNS and FN/SFN.[24] In the present study, the ROM in the ND/UNS category was 9.5%. Inadequate samples as the most common cause for false-negative diagnosis in FNA have been reported by Bakhos et al.[25] The risk of malignancy was calculated assuming that the unresected cases were benign. Hence the ROM across various studies ranges from 0% to 63%.[26,27] This increased risk of reported malignancy emphasises the importance of reaspirating all ND/UNS category lesions for cytology under guidance to rule out malignancy. Another pitfall that results in false-negative interpretation is the exclusive presence of cystic macrophages without cellular fragments, which could represent a cystic variant of PTC. In our study, there were two cases of PTC. The actual ROM in the AUS category is difficult to determine since only a subset of patients with clinical suspicion undergo surgical resection, thus resulting in selection bias. There were no malignancies in the AUS category with histological follow-up in the present data while other Asian studies report a higher ROM.[10-12,15] This could be explained by the fact that most of the cases showed architectural atypia rather than cytological atypia. Cytological atypia have been reported to be associated with a higher ROM as per the available evidence.[4] In cases of AUS, a repeat aspiration under guidance and correlation with clinical and radiological findings would lower the proportion of cases reported as AUS. The prevalence of NIFTP varies across the population and is reported to be between 5% and 13%.[28-30] In the present study, there was a much lower incidence at 3%. The cases of NIFTP showed a wide cytological spectrum spanning categories II to IV. However, the presence of diffuse cytological features of PTC and the absence of papillary fragments should prompt surgical resection to rule out the possibility of NIFTP.

In the recent years, various TIRADS systems have emerged and have been in use to stratify thyroid nodules as benign or malignant

based on ultrasound features and to decide on FNAC. The Kwak system is a simple classification based on suspicious ultrasound features. However, importance is not given to independent risk factors, for example extrathyroidal extension. Also, microcalcification, presence of solid area, and hypoechogenecity are given equal scores, whereas microcalcification generally has a higher malignancy risk.[31] ACR TIRADS is more complicated than other risk-stratification systems. Another drawback to this system is that malignant nodules exhibiting mixed echo patterns are given a lower score. Yet ACR TIRADS is an objective and comprehensive method for the evaluation of the thyroid nodules. The EU TIRADS is simpler than ACR TIRADS, and recommends FNA for mildly hypoechoeic nodules measuring greater than 1.5 cm.[8] However, it does not include solidity as an independent risk factor. The pitfall of the ATA guidelines is the classification of suspicious ultrasound features that have a variety of clinical significance into the same risk stratification with no independent categorisation.[8] In the present study ACR TIRADS was used for risk stratification. The sensitivity of ACR TIRADS observed in the current study is similar to that of other published studies.[6,32,33]

A similar study carried out by Fernandez et al has reported the percentage of malignancy as 77% in moderately and highly suspicious categories (TIRADS score ≥ 4) versus 65% in the current study.[32] ROM values for TIRADS scores except for score 2 are in consensus with most other published articles except for score 3, where a higher ROM (10%) was observed. Even though some malignant nodules were mislabelled as benign and some benign nodules were mistaken for malignant, the overall sensitivity was better.

While exploring the causes for a false-positive diagnosis in cytology, it was observed that cellular smears with a predominance of Hurthle cells exhibiting atypia, and the presence of focal nuclear features of PTC in the absence of nuclear inclusions, were overdiagnosed as features of malignancy. On the other hand, cases with diffuse cytological atypia even with focally observed pseudoinclusions are worth considering as suspicious for malignancy. Analysing the causes for the false negatives in FNA, papillary microcarcinoma being missed by sampling error in FNA has been reported previously. Under these circumstances, USG-guided FNA would have been useful.

4.2 | Adherence to TBSRTC recommendations for management

There exists a heterogeneity in approaches to the management of thyroid nodules despite the recently published guidelines for clinical management. TBSRTC recommends reaspiration under USG guidance as the preferred method for evaluation of ND aspirates unless the nodule is purely cystic. The majority of the ND/UNS lesions in the data consisted only of cystic macrophages, reflecting the lower rate of repeat aspiration in the present study.

The management decision for category II is determined predominantly by radiological findings, since there is a possibility of sampling error with FNA in larger nodules that can hinder detection of the underlying malignancy. However, a vast subset of patients undergo thyroidectomy for cosmetic reasons/pressure symptoms rather than for suspected malignancy.

The treatment for category III and IV lesions is controversial. While surgery is advised for category IV lesions, close follow-up with repeat FNA, and molecular analysis for BRAF and RAS gene mutations are recommended for the AUS category.[4] Chirayath et al[34] in their study in the Indian population have found, however, that mutational analysis for BRAF as an indicator of malignancy in AUS lesions has limited utility, in line with the results of many other studies.[35,36] Even though there was no ROM for the AUS category in the present study, in a country where molecular tests are not very feasible, a multidisciplinary approach based on clinical and radiological findings, together with patient preference, should guide the management.

Surgery as the treatment modality for category III and IV lesions was seen in 22% and 60% of cases, respectively. The resection rate in indeterminate categories is much lower than that reported by Western studies (22% in the current study vs 57.6%).[37] The lower rate could be explained by the loss of follow-up of these patients. Or et al[38] in their study have reported that for cases with indeterminate cytology, 77.4% of clinicians opted for repeat FNA and in case of repeat FNA being indeterminate, 50% opted for surgery.

The authors acknowledge the limitations of this study which include the retrospective design and a small proportion of cases with surgical follow up.

5 | HIGHLIGHTS

The reporting of AUS/FLUS lesions with a supplementary note describing the presence of architectural and/or cytological atypia may be helpful in treatment decisions, since cytological atypia could suggest the possibility of NIFTP or malignancy that would require surgical excision.

In the current study, it was found that there is higher interobserver variability in interpreting Hurthle cell atypia. There is a higher chance for misinterpretation as suspicious for malignancy . A further study analysing the various cytological parameters and cytohistological correlation of Hurthle cell lesions might help in achieving a more accurate cytological diagnosis and lower the rate of false positives in FNAC. The TIRADS score in conjunction with TBSRTC cytological diagnosis can aid in better stratification of thyroid nodules and aid in decision making.

6 | CONCLUSION

This study substantiates the usefulness of TBSRTC in thyroid cytology in arriving at a more precise diagnosis and guiding clinicians on management. The study reports a lower proportion of the AUS category. Hurthle cell lesions with atypia were the common cause

for misdiagnosis observed in the current study. The presence of nuclear features of papillary carcinoma with the presence of numerous microfollicles in the aspirate should prompt the diagnosis of NIFTP. Periodic cytohistological correlation and further studies on Hurthle cell lesions can aid in improving the sensitivity and specificity of FNA. In institutions where TBSRTC in reporting is practised, the clinicians should be aware of the implied risk of malignancy in each category. There was a good correlation between TIRADS, TBSRTC, and histology especially in benign nodules. However, further replication studies adapting a prospective design with a larger sample size are essential to validate the utility of thyroid ultrasound especially for indeterminate nodules.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Sakthisankari S: Conceptualisation, data curation, formal analysis, software, and writing (original draft, review, and editing). Vidhyalakshmi S: Conceptualisation, methodology, analysis, project administration, and writing (review and editing). Shanthakumari S: Conceptualisation, analysis, writing (review and editing). Devanand B: Methodology, resources, analysis, writing (review and editing). Nagul U: Methodology, analysis.

ETHICS APPROVAL AND PATIENT CONSENT STATEMENT

This retrospective study has been approved by Institutional Human Ethics Committee. Patient consent has been waived off.

DATA AVAILABILITY STATEMENT

Data has not been shared and is not available in the repository.

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REFERENCES

1. Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. *Am J Clin Pathol*. 2009;132:658-665.
2. Cibas ES. Fine-needle aspiration in the work-up of thyroid nodules. *Otolaryngol Clin North Am*. 2010;43:257-271.
3. National Centre for Disease Informatics and Research, National Cancer Registry Programme Indian Council of Medical Research. *Three-year report of population-based cancer registries 2012-2014*. Bengaluru, India: NCDIR-NCRP; 2016.
4. Ali SZ, Cibas ES. *The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria and Explanatory Notes*. Berlin, Germany: Springer; 2010.
5. Valderrabano P, McIver B. Evaluation and management of indeterminate thyroid nodules: the revolution of risk stratification beyond cytological diagnosis. *Cancer Control*. 2017;24(5):e107327481772923.
6. Jabar ASS, Koteshwara P, Andrade J. Diagnostic reliability of the Thyroid Imaging Reporting and Data System (TI-RADS) in routine practice. *Pol J Radiol*. 2019;84:e274-e280.
7. Barbosa TLM, Junior COM, Graf H, et al. ACR TI-RADS and ATA US scores are helpful for the management of thyroid nodules with indeterminate cytology. *BMC Endocr Disord*. 2019;19(1):112.
8. Shen Y, Liu M, He J, et al. Comparison of different risk-stratification systems for the diagnosis of benign and malignant thyroid nodules. *Front Oncol*. 2019;9:378.
9. Kakudo K, Bychkov A, Bai Y, Li Y, Liu Z, Jung CK. The new 4th edition World Health Organization classification for thyroid tumors, Asian perspectives. *Pathol Int*. 2018;68:641-664.
10. Anand B, Ramdas A, Ambroise MM, Kumar NP. The Bethesda system for reporting thyroid cytopathology: a cytohistological study. *J Thyroid Res*. 2020;2020:e8095378.
11. Thewjitcharoen Y, Butadej S, Nakasatien S, et al. Incidence and malignancy rates classified by The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) - An 8-year tertiary center experience in Thailand. *J Clin Transl Endocrinol*. 2018;16:e100175.
12. Zhu Y, Song Y, Xu G, Fan Z, Ren W. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC): a report of 2,781 cases in a Chinese population. *Chin J Cancer Res*. 2020;32(2):140-148.
13. Wu HH, Rose C, Elsheikh TM. The Bethesda System for Reporting Thyroid Cytopathology: an experience of 1,382 cases in a community practice setting with the implication for risk of neoplasm and risk of malignancy. *Diagn Cytopathol*. 2012;40:399-403.
14. Sarkis LM, Norlen O, Aniss A, et al. The Australian experience with the Bethesda classification system for thyroid fine needle aspiration biopsies. *Pathology*. 2014;46:592-595.
15. Mondal SK, Sinha S, Basak B, Roy DN, Sinha SK. The Bethesda system for reporting thyroid fine needle aspirates: a cytologic study with histologic follow-up. *J Cytol*. 2013;30:94-99.
16. Guo A, Kaminoh Y, Forward T, Schwartz FL, Jenkinson S. Fine needle aspiration of thyroid nodules using the Bethesda System for Reporting Thyroid Cytopathology: an institutional experience in a rural setting. *Int J Endocrinol*. 2017;2017:e9601735.
17. Mehra P, Verma AK. Thyroid cytopathology reporting by the Bethesda system: a two-year prospective study in an academic institution. *Patholog Res Int*. 2015;2015:e240505.
18. Kapila K, Qadan L, Ali RH, Jaragh M, George SS, Haji BE. The Bethesda system for reporting thyroid fine-needle aspiration cytology: a Kuwaiti experience - a cytohistopathological study of 374 cases. *Acta Cytol*. 2015;59(2):133-138.
19. Theoharis CG, Schofield KM, Hammers L, Udelsman R, Chhieng DC. The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. *Thyroid*. 2009;19:1215-1223.
20. Bohacek L, Milas M, Mitchell J, Siperstein A, Berber E. Diagnostic accuracy of surgeon-performed ultrasound-guided fine-needle aspiration of thyroid nodules. *Ann Surg Oncol*. 2012;19:45-51.
21. Bongiovanni M, Crippa S, Baloch Z, et al. Comparison of 5-tiered and 6-tiered diagnostic systems for the reporting of thyroid cytopathology: a multi-institutional study. *Cancer Cytopathol*. 2012;120(2):117-125.
22. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. *Acta Cytol*. 2012;56:333-339.
23. Al Dawish MA, Robert AA, Muna A, et al. Bethesda System for Reporting Thyroid Cytopathology: a three-year study at a tertiary care referral center in Saudi Arabia. *World J Clin Oncol*. 2017;8(2):151-157.
24. Agarwal S, Jain D. Thyroid cytology in India: contemporary review and meta-analysis. *J Pathol Transl Med*. 2017;51(6):533-547.

25. Bakhos R, Selvaggi SM, DeJong S, et al. Fine-needle aspiration of the thyroid: rate and causes of cytohistopathologic discordance. *Diagn Cytopathol*. 2000;23(4):233-237.
26. Ke J, Jianyong L, Ying L, et al. The use of The Bethesda System for Reporting Thyroid Cytopathology in a Chinese population: an analysis of 13 351 specimens. *Diagn Cytopathol*. 2019;47(9):876-880.
27. Park JH, Yoon SO, Son EJ, Kim HM, Nahm JH, Hong S. Incidence and malignancy rates of diagnoses in the Bethesda system for reporting thyroid aspiration cytology: an institutional experience. *Korean J Pathol*. 2014;48(2):133-139.
28. Lau RP, Paulsen JD, Brandler TC, Liu CZ, Simsir A, Zhou F. Impact of the Reclassification of "noninvasive encapsulated follicular variant of papillary thyroid carcinoma" to "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" on the Bethesda System for Reporting Thyroid Cytopathology: a large academic institution's experience. *Am J Clin Pathol*. 2017;149:50-54.
29. Maletta F, Massa F, Torregrossa L, et al. Cytological features of "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" and their correlation with tumor histology. *Hum Pathol*. 2016;54:134-142.
30. Paniza ACJ, Mendes TB, Viana MDB, et al. Revised criteria for diagnosis of NIFTP reveals a better correlation with tumor biological behavior. *Endocr Connect*. 2019;8(11):1529-1538.
31. Kwak JY, Han KH, Yoon JH, et al. Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. *Radiology*. 2011;260(3):892-899.
32. García-Moncó Fernández C, Serrano-Moreno C, Donnay-Candil S, Carrero-Alvaro J. Acorrelation study between histological results and thyroid ultrasound findings. The TI-RADS classification. *Endocrinol Diabetes Nutr*. 2018;65(4):206-212.
33. Periakaruppan G, Seshadri KG, Vignesh Krishna GM, Mandava R, Sai VPM, Rajendiran S. Correlation between ultrasound based TIRADS and Bethesda System for Reporting Thyroid-cytopathology: 2-year experience at a tertiary care center in India. *Indian J Endocrinol Metab*. 2018;22:651-655.
34. Chirayath SR, Pavithran PV, Abraham N, et al. Prospective study of Bethesda categories III and IV thyroid nodules: outcomes and predictive value of BRAFV600E mutation. *Indian J Endocrinol Metab*. 2019;23:278-281.
35. Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan MG. BRAF V600E mutation in papillary thyroid carcinoma: significant association with node metastases and extra thyroidal invasion. *Endocr Pathol*. 2012;23:83-93.
36. Nair CG, Babu M, Biswas L, et al. Lack of association of B-type Raf kinase V600E mutation with high-risk tumor features and adverse outcome in conventional and follicular variants of papillary thyroid carcinoma. *Indian J Endocrinol Metab*. 2017;21:329-333.
37. Vuong HG, Ngo HTT, Bychkov A, et al. Differences in surgical resection rate and risk of malignancy in thyroid cytopathology practice between Western and Asian countries: a systematic review and meta-analysis. *Cancer Cytopathol*. 2020;128(4):238-249.
38. Or K, Benbassat C, Koren S, et al. Adherence to ATA 2015 guidelines in the management of unifocal non-invasive papillary thyroid cancer: a clinical survey among endocrinologists and surgeons. *Eur Arch Otorhinolaryngol*. 2018;275(11):2851-2859.

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RESEARCH ARTICLE

HIRSCHSPRUNG'S DISEASE – OUR EXPERIENCE TO DETECT IT ON TABLE USING RAPID ACETYLCHOLINE ESTERASE STAINING METHOD IN A RESOURCE RESTRICTED TERTIARY CARE HOSPITAL SETUP

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ABSTRACT

Background and objective: Hirschsprung's disease is a disease that occurs in a newborn child with an incidence of one in 5000 live births with a male predominance. In our setup for the diagnosis of Hirshcsprung's disease, haematoxylin and eosin staining in a formalin fixed tissue was routinely practiced. As a novel diagnostic procedure, rapid on table acetylcholine esterase stain was tried. It is a diagnostic accuracy test done with the following objectives: 1.To perform acetylcholine esterase and rapid hematoxylin and eosin staining in the frozen section of rectal biopsy as a part of intra operative consultation. 2 To correlate routine formalin fixed, hematoxylin and eosin staining results with rapid methods. 3. Feasibility of these procedures in our setup.

Method: All infants who had not passed meconium since birth and who had symptoms of intestinal obstruction were screened by pediatric surgeons. As a part of intra-operative consultation, both frozen section and formalin fixation were done in the received specimen. In the frozen section, acetylcholine esterase staining and rapid haematoxylin and eosin staining was done. In the formalin fixed specimen routine haematoxylin and eosin was done.

Results: All the three stains- acetylcholine esterase, rapid hematoxylin and eosin and hematoxylin and eosin in a formalin fixed specimens gave equal results. When rapid methods were compared with the routine formalin fixed specimen haematoxylin and eosin staining method the turnaround time was very much reduced. By rapid staining, intra-operative consultation facilitated single stage Duhamel pull- through. Repeat surgery was avoided as both the diagnosis and the level of colon having ganglion cells were assessed simultaneously. As a result, hospital stay was reduced.

Conclusion: In this diagnostic study done on twenty cases of suspected Hirschsprung's disease, the diagnostic efficacy of rapid methods correlated with that of the routinely processed tissue sections stained with haematoxylin and eosin. The turnsaround time for the rapid tests were less. This facilitated on table diagnosis, reduced the need for repeat surgery, improved the outcome of surgery and, there by reduced the duration of hospital stay.

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INTRODUCTION

Hirschsprung's disease (HSD) is a multi-genetic neurocrisopathic disorder of newborns and children. The incidence is estimated to be one in 5000 newborns, according to Badner *et al* and Parisi & Kapur (Badner *et al*., 1990; Kapur, 2001). But it varies between the racial groups. In Asians the incidence ratio is 2.8 per 10,000 live births (Torfs, 1998). There is a male predominance, male to female ratio being 4:1. This is less evident in patients with long segment aganglionosis and Down syndrome. Hirschsprung's disease has a sporadic occurrence. In about 20% of the sporadic cases, RET genetic mutation is present (Edery *et al*., 1994). The gold standard in the diagnosis of HSD is the demonstration of absent ganglion cells in the histopathological sections of rectal biopsy (Natarajan *et al*., 2002).

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In 80% of the cases the aganglionosis involves the recto-sigmoid colon, in 11 to 26% the left colon, and in 5 to 15% the entire colon (Bax K. Duhamel, 2006). Usually, full thickness rectal biopsy specimen is examined for the evaluation of ganglion cells. The alternative is suction biopsy of the rectal mucosa, sectioning with cryostat and rapid acetylcholine esterase [ACHE] staining. ACHE stains on frozen sections had given best results in diagnosing HSD according to Coffin CM, *et al*. Meier-Ruge W *et al* and Qualman *et al*. (Coffin CM, *et al*., 2005 Meier-Ruge *et al.,*1972; Qualman *et al.,* 1999). Increase in acetylcholine esterase in the affected tissue is the pathophysiology of this disease (Schofield *et al*., 1990; Bagdzevicius *et al*., 2007; Ghosh and Griffiths, 1998). And acetylcholine esterases do not stain the normal nerve fiber. This concept is used in identifying this disease (Kleinhaus *et al*., 1979).

Before this study was attempted, the procedure followed in our hospital for patients suspected of Hirschsprung's disease were colostomy and formalin fixed full thickness rectal biopsy for ascertaining the diagnosis. Which of course takes time for the processing and later, after confirmation, Duhamel pull through was done. Frozen section and acetylcholine esterase staining was introduced in our histopathological lab in order to know the feasibility of this technique to diagnose accurately and to reduce the turnaround time of the rectal biopsy specimens received. Both confirmations of the diagnosis and the presence of ganglion cells from the cut ends were to be made at the same time. This study was done to evaluate the diagnostic accuracy of rapid on table staining method.

MATERIALS AND METHODS

During our study period (November2011-2013), twenty rectal mucosal biopsies were received from the Department of Paediatric surgery. Rectal biopsy from the children aged 4 days to 12 years suspected to have Hirschsprung's disease were received (Table 1).

Inclusion criteria

- All infants with symptoms suggestive of Hirschsprung's disease – Late passage of meconium, abdominal distension, bilious vomiting, and Hirschsprung's-associated entero-colitis were included.
- When investigated with contrast enema, they should reveal features of the narrow rectal segment, transition zone and proximal bowel dilation.
- Patients should be screened and selected by the Department of Pediatric surgery and their rectal biopsies sent to the Department of Pathology for evaluation
- Biopsy specimens should be taken 2-3cm proximal to dentate line.

Exclusion criteria

- If biopsy specimens contain epithelium of the anal canal.
- Inadequate specimen.
- Specimens that were not received in saline.

We were pre- informed and the biopsy specimens were transported to the laboratory in a saline soaked gauze or in saline. Specimens from the rectal mucosa 2-3 cm above the dentate line and specimens from various levels of the intestinal segment for assessment of ganglion cells were sent. They were labeled accordingly. Specimens were accompanied by a requisition form with detailed history, investigation and levels from where the biopsies were taken. The specimen was then washed to remove the blood, as red cells would take up the ACHE stain and cause background staining. Frozen sections of 10 µm thickness were taken after labeling the slides. Slides were stained with acetylcholine esterase and with rapid haematoxylin and eosin (H&E) method. Only part of the tissues were used for frozen section. The rest of the tissues were fixed in formalin and it was processed routinely and stained with haematoxylin and eosin. Acetylcholine esterase gives dark green color to the nerves when seen under light microscopy.

The principle behind the ACH stain

The thiocholine product produced react with ferricyanide and reduces it to ferrocyanide.It precipitates with copper to form copper ferrocyanide. This produces the dark green color according to Karnowsky and Roots (Karnowsky and Roots, 1964).

Interpretation of the ACHE stains

Presence of hypertrophic nerve bundles and absent ganglion cells are the criteria used for the diagnosis.

- Nerve fibers are stained greenish black
- Ganglion cells are negatively stained, (Fig 1)

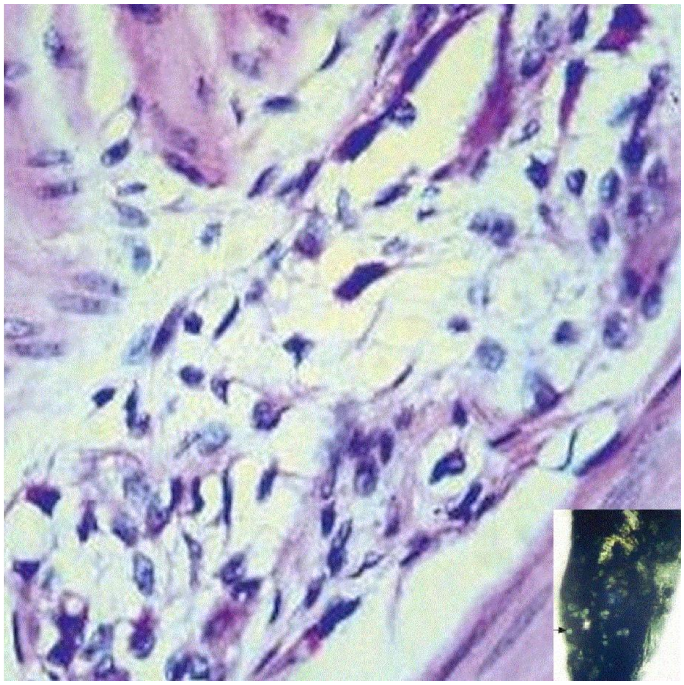


Fig. 1. Shows ganglion cells [H&E,40x].Inset shows ganglion cells are negatively stained with acetylcholine esterase[40x]

Four patterns are observed with ACHE staining based on level of stained nerve fibers. Nerve fibers and red blood cells provide the positive internal control.

Pattern A: Nerve plexus between the crypts in lamina propria, submucosa and muscularis mucosa stain positive with acetylcholine esterase, (Fig 2 & 3).

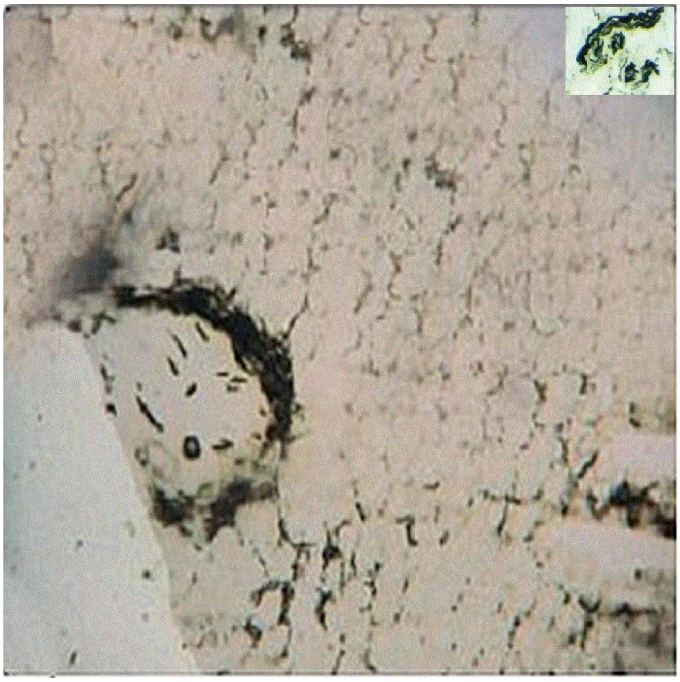


Fig. 2. Shows nerve plexus between the crypts in lamina propria, submucosa and muscularis mucosa stained positive with acetylcholine esterase- Pattern A.[4x].Inset shows hypertrophic nerve bundle [ACH,40x]

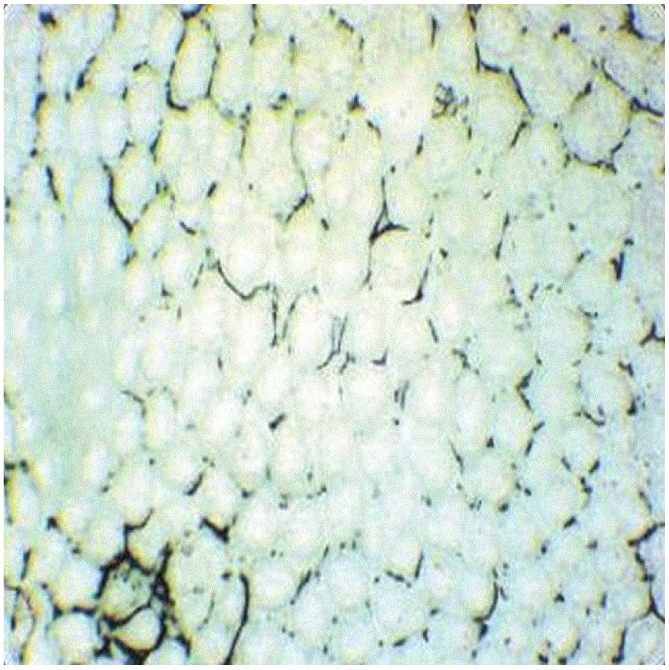


Fig. 3. Shows nerve plexus between the crypts in lamina propria, submucosa and muscularis mucosa stained positive with acetylcholine esterase- Pattern A[40x]

Pattern B: Nerve plexus at the base of the crypts, submucosa and muscularis mucosa stain positive with acetylcholine esterase, (Fig 4).

Equivocal pattern: Nerve fibers in the submucosa alone stained by acetylcholine esterase and absent ganglion cells.

Negative pattern: No staining identified

Interpretation of results of H & E stained sections

Positive for Hirschsprung's disease: Presence of hypertrophic nerve bundles in the submucosa and muscularis propria and absent ganglion cells, (Fig 5 & 6- Rapid H & E and Routine H&E respectively).

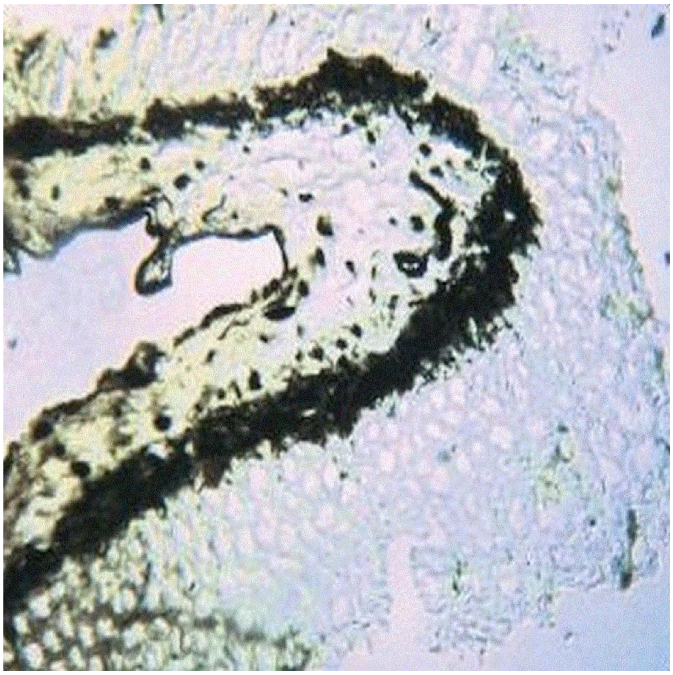


Fig. 4. Shows nerve plexus at the base of the crypts, submucosa and muscularis mucosa stained Positive with acetylcholine esterase- Pattern B[4x]

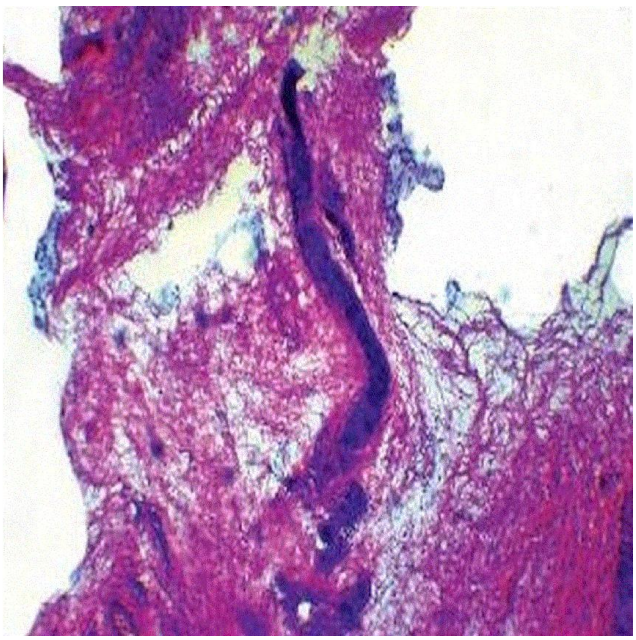


Fig. 5. Shows presence of hypertrophic nerve bundles in the muscularis propria and absent ganglion cells,[Rapid H&E, 40x]

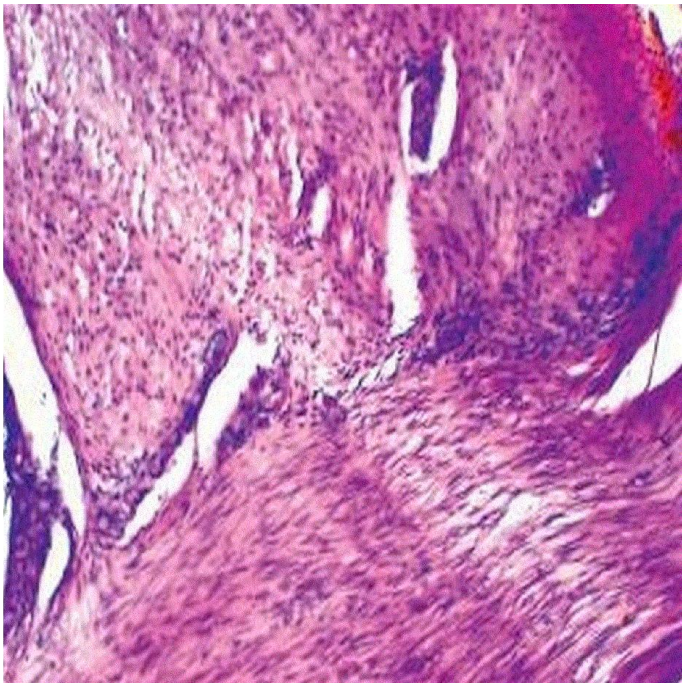


Fig. 6. Shows Presence of hypertrophic nerve bundles in the muscularis propria and absent ganglion cells,[Routine H&E,40x]

Transition zone: Ganglion cells are distributed unevenly along the circumference with hypertrophic nerve bundles. Negative for Hirschsprung's disease: Ganglion cells are present.

RESULTS

Over a two-year study period(November 2011-2013), twenty rectal mucosal biopsies from the children aged 4 days to 12 years suspected of Hirschsprung's disease were received from the Department of Pediatric surgery (Table 1).

Table 1. Age and sex distribution of patients suspected with Hirschsprung's disease

Age group(Years)	Male	Female
0-1	10(50%)	2(10%)
1-5	6(30%)	1(5%)
6-12	1(5%)	Nil

Positive results were obtained in all the three methods, namely, acetylcholine esterase stained sections, rapid haematoxylin and eosin stained sections and in those sections that were formalin fixed, routinely processed and stained with haematoxylin and eosin stain (Table2).

Table 2. Cases positive with ACH, rapid H&E and routine H&E stains

Positive with ACH stain	Positive with rapid H&E	Positive with routine H&E
16	16	16

There was no difference in the diagnostic sensitivity within these three methods used. Ghosh and Griffithshad used both haematoxylin and eosin and acetylcholine esterase to diagnose Hirschsprung's disease (Ghosh and Griffiths, 1998). When analyzing the results of their sensitivity, all the stains were 100% sensitive with 95% confidence interval being 79.41% to 100.00%. Specificity was 100%, 95% confidence interval being 39.76% to 100.00%. Disease prevalence was 80%, 95% confidence interval being 56.34% to 94.27%. Positive predictive value was 100% and negative predictive value was also 100%. When Cohens Kappa value was calculated to analyze the agreement between the observed values of ACHE stain and H&E stains, the number of observed agreements between them were 32 (80.00% of the observations). The Kappa= 0.600 SE of kappa value was 0.126 95% with confidence interval 0.352 to 0.848.The strength of agreement was good according this calculation.

Although the routine H & E and rapid staining methods had an equal sensitivity and specificity, rapid methods score over the routine method with reduced turnaround time The turnaround time was compared between routinely fixed and haematoxylin and eosin stained sections with acetylcholine esterase stain and rapid haematoxylin and eosin stain. The time taken for rapid haematoxylin and eosin was ten minutes. The time taken for acetylcholine esterase stain was about fifty minutes and for routinely stained sections it took about 48 hours (Table 3).

Table 3. Turnaround time noted with ACHE, rapid H&E and routine H&E

Acetylcholine esterase	Rapid H&E	Routine H&E
50 minutes	10 minutes	48 hours

All though rapid haematoxylin and eosin took lesser time than ACH stain, certain patterns that were recognized with the acetylcholine esterase stain were not appreciated with rapid haematoxylin and eosin stain. Two different patterns were observed according to Moore *et al*. (Moore and Johnson, 2005). Different patterns of stained nerve fibers observed with acetylcholine esterase stain with age wise stratification were analyzed. Acetylcholine esterase staining in the nerve plexus between the crypts in lamina propria, submucosa and muscularis mucosa was diagnosed as having pattern A-positive Hirschsprung's disease.

Acetylcholine esterase staining the nerve plexus in the base of the crypts, submucosa and muscularis mucosa was considered as pattern B positive -Hirschsprung's disease. Only one case with equivocal pattern and the hypertrophic nerve bundle was observed, which was also a positive Hirschsprung's disease pattern. Pattern A (9 cases) was more commonly observed than pattern B (6 cases) (Table 4). Aganglionosis could not be assessed perfectly using haematoxylin and eosin stain as ganglion cells normally were scattered and were difficult to find when present in the submucosa.

Table 4. Different patterns observed in ACH stain with age wise stratification

Age	Pattern A	Pattern B	Equivocal with hypertrophic nerves	Negative
1-6months	5	4	1	-
7months – 1 year	-	-	-	1
1-5 years	4	2	-	2
6-12 years	-	-	-	1

And when the hypertrophic nerve twigs formed were very sparse, especially in early infancy, diagnostic difficulty was ensued when H&E was used. Hypertrophic nerve bundles even if it was minimal were highlighted using the acetylcholine esterase, which was difficult when rapid haematoxylin and eosin was used. False positive results were rare, according to the study of Blisard, *et al*. (Blisard and Kleinman, 1986). Particularly, difficulty was encountered in the case of young infants. But H & E clearly complimented the acetylcholine esterase stain. Layer *et al*. in their study had questioned the role of acetylcholine esterase staining if there are clear cut evidence of hypertrophic nerves and ganglion cells (Layer and Kaulich, 1991). But in young infants when hypertrophic nerves are less developed and when the ganglion cells are immature ACH stain clearly compliments.Because the rapid technique diagnoses cases as efficiently as routine stain, the rapid diagnostic method was used as an intra -operative consultation method, thereby waiting period was reduced. As ACHE staining was an on table procedure the results were relayed immediately to the waiting surgeons, facilitating single stage pull through surgery.

DISCUSSION

There are four forms of Hirschsprung's disease.

1. Short segment disease (Classic) - Only a small segment of the rectum and sigmoid colon are involved. Distal sigmoid colon and rectum are affected in 75% to 80% of short segment disease.
2. Ultra short segment disease - Very short segment (<2cm) is involved.
3. Long segment disease - The disease extends beyond the splenic flexure. In 10% of the cases, the disease involves bowel segment proximal to the splenic flexure.
4. Total bowel aganglionosis – In total colonic aganglionosis the disease extends from the anal canal to at least the ileocaecal valve, but does not extend more than 50 cm beyond the ileocaecal valve. The incidence of this form is 5%.Total colonic aganglionosis presents outside the neonatal period, even in the adolescent age and early adulthood.

In Hirschsprung's disease, the main finding is the lack of ganglion cells in the narrow segment of the colon. The aganglionic segment starts distally at the anal ring and extends proximally to a variable length. The internal sphincter has a natural scarcity of ganglion cell close to the anus for a length about 10mm above the dentate line. This is the reason for the general rule to perform biopsies 2-3 cm proximal to the dentate line, according to Teitelbaum *et al*. (Teitelbaum and Coran, 2006)**.**

The affected segment has three zones which include the narrow segment, transition zone and a dilated proximal bowel segment. Histologically, in the narrowed segment there are aganglionosis and hypertrophic nerve bundles. The narrowed segment continues as a transition zone. Transitional zone can be few millimeters to varying centimeters in length. Ganglion cells begin to appear in this zone. Both the presence of hypertrophic nerves and presence of ganglion cells is seen in the transition zone. This continues as a dilated ganglionic segment.

Meier-Ruge did extensive research on the histopathological diagnosis of Hirschsprung's disease and other congenital neuronal intestinal malformations (Meier-Ruge *et al*., 1972). The following criteria for the diagnosis of HSD were formulated by him: Hirschsprung's disease is characterized by aganglionosis in both the Meissner and myenteric plexus. Acetylcholine esterase staining activity is increased in the coarse parasympathetic neurofibrils present in the lamina propria mucosae.

Absence of ganglion cells and the hypertrophic nerve bundles that are present in high numbers is the hallmark of HSD. This is always associated with an increase in acetylcholine esterase in the affected tissue (Qualman *et al*., 1999; Gath *et al*., 2001). Acetylcholine esterase does not stain normally innervated intestinal mucosa (Kamijo *et al*., 1953; Niemi *et al*., 19611 Ariel *et al*., 1983). Histological presence of coarse refractile acetylcholine esterase staining nerve fibrils and the absence of ganglion cells of the inter plexuses should be used in the diagnosis of Hirschsprung's disease according to Meier *et al* (Rouzrokh *et al*., 2010; Barr *et al*., 1985).Garrett *et al* demonstrated that severity with which the case presents and degree of obstruction in Hirschsprung's disease correlated with the acetylcholine esterase stained nerves in the aganglionic zone (Garrett and Howard 1969). The reason behind why acetylcholine esterase levels are raised in Hirschsprung's disease is complex. RET gene, which is the predominant gene identified in Hirschsprung's disease is considered as a potential candidate.

The differential diagnosis includes intestinal neuronal dysplasia. It is a clinical differential for HSD which is diagnosed histologically by the presence of giant submucosal ganglia, containing more than eight nerve cells. Segmental dilatation of the colon, a rare motility disorder affecting children is also a differential for HSD. It can be differentiated from HSD by normally distributed ganglion cells in the colon. Other differentials include internal anal sphincter achalasia in which absence of the rectosphincteric reflex on rectal balloon inflation and the clinical presentation is same as that of Hirschsprung's disease. Histopathologically the presence of ganglion cells on rectal suction biopsy helps to rule out HSD. Hypoganglionosis also a differential is characterized by decreased number of ganglion cells and reduced size of ganglia and wider distances between myenteric ganglia. According to the criteria of Meier-Ruge *et al*, less than 10 ganglion cells/mm (normal -14±3.3) and /or less than 2 ganglions/mm (normal:3.3±1.1) is used to diagnose hypoganglionosis. In contrast to the Hirschsprung's disease, hypoganglionosis do not have nerve plexus hyperplasia. Chronic idiopathic intestinal pseudo-obstruction, slow transit constipation and megacolon are also the mimickers, but can be differentiated histopathologically.

When routine haematoxylin and eosin (H&E) stains are used in rectal suction mucosal biopsies, there are many disadvantages. Submucosa should be equal in thickness to the mucosa in the biopsy specimens. But practically this is not possible. Ganglion

cells are fewer and it is scattered in the submucosa. Hence, evaluation of ganglion cells in the submucosal biopsy using H&E is difficult. This was not in a case when acetylcholine esterase staining was used. The turnaround time was also reduced. This study proved that acetylcholine esterase can be used as a rapid diagnostic tool and it has an advantage over H&E.

Conclusion

Acetylcholine esterase staining, rapid haematoxylin and eosin, and routine formalin fixed sections all had the same diagnostic efficacy. But the turnaround time was very much reduced when rapid diagnostic methods were used when compared with routine formalin fixed sections stained with H&E. Although rapid haematoxylin and eosin method took less time and was less expensive when compared with acetylcholine esterase, certain patterns like aganglionosis and hypertrophic nerve bundles seen using the acetylcholine esterase were not highlighted when rapid haematoxylin and eosin alone was used. Particularly, difficulty was encountered in the case of young infants. Immature ganglion cells are difficult to recognize in frozen sections stained with H&E. But rapid H&E clearly complimented the acetylcholine esterase stain. Intra operative consultation was possible in our setup. This reduced the need for a second surgery, improved the outcome of the surgery and reduced the duration of hospital stay.

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Conflict of interest

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REFERENCES

Ariel, I., Vinograd, I., Lernau, O.Z., Nissan, S. and Rosenmann, E. 1983. Rectal mucosal biopsy in aganglionosis and allied conditions. *Hum. Pathol.,* 14(11):991–995

Badner, J.A., Sieber, W.K., Garver, K.L. and Chakravarti A. 1990. A genetic study of Hirschsprung disease. *American Journal of Human Genetics.,* 46(3):568-580.

Bagdzevicius, R., Vaicekauskas, V. and Bagdzeviciūte, S. 2007. Experience of acetylcholinesterase histo-chemistry application in the diagnosis of chronic constipation in children. Medicina (Kaunas), 43(5):376-384

Barr, L.C., Booth, J., Filipe, M.I. and Lawson, J.O. 1985. Clinical evaluation of the histochemical diagnosis of Hirschsprung's disease. *Gut.,* 26(4):393–399

Bax K. Duhamel Lecture. 2006. The Incurability of Hirschsprung's Disease. *European Journal of Pediatric Surgery,* 16(6):380-384.

Blisard, K.S. and Kleinman, R. 1986. Hirschsprung's disease: a clinical and pathologic view. *Human Pathol.,* 17:1189-1191.

Coffin, C.M., Spilker, K., Zhou, H., Lowichik, A., Pysher, T.J. 2005. Frozen section diagnosispediatric surgical pathology: a decade's experience at a children'shospital. *Arch. Pathol. Lab Med.,* 129:1619–1625.

Edery, P., Lyonnet ,S., Mulligan, L.M., Pelet, A., Dow, E., Abel, L., Holder, S., Nihoul-Fékété, C., Ponder, B.A. and Munnich, A. Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature. 1994 Jan 27; 367(64):378–380.

Gath, R. Goessling, A. *et al*. 2001. Analysis of theRET,GDNF,EDN3, andEDNRB genes in patients with intestinal neuronal dysplasia and Hirschsprung disease, Gut., 48:671–675.

Ghosh, A. and Griffiths, D.M. 1998. Rectal biopsy in the investigation of constipation. Arch. Dis. Child, 79:266-268.

Kamijo, K., Hiatt, R.B. and Koelle, G.B. 1953. Congenital megacolon, a comparison of the specific and hypertrophied segments with respect to cholinesterase activities and sensitivities to acetylcholine, DFP and the barium ion. *Gastroenterology,* 24:173

Kapur, R.P. 2001. Neuropathology of paediatric chronic intestinal pseudo-obstruction and related animal models. *J. Pathol.,* 194:277–88.

Karnowsky, M.J. and Roots, L. 1964. A ''direct-colouring'' thiocholine method for cholinesterase. J. *Histochem. Cytochem.,* 12:219.

Kleinhaus, S., Boley, S.J., Sheran, M., Seiber, W.K. 1979. Hirschprung's disease: a survey of the surgical section of American academy of Paediatrics. *J. Paedtr. Surg.,* 14:588-597.

Layer, P.G. and Kaulich, S. 1991. Cranial nerve growth in birds ispreceded by cholinesterase expression during neural crest cellmigration and the formation of an HNK-1 scaffold. *Cell Tissue Res.,* 265(3):393–407

Meier-Ruge, W., Lutterbeck, P., Herzog, B., Morger, R., Moser, R. and Schärli, A. 1972. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. *Journal of Pediatric Surgery,* 7(1):11-17.

Moore, S.W. and Johnson, Æ G. 2005. Acetylcholinesterase in Hirschsprung's disease. *Pediatr Surg. Int.,* 21: 255–263

Natarajan, D., Marcos-Gutierrez, C., Pachnis, V. and deGraaff, E. 2002. Requirement of signalling by receptor tyrosine kinase RET for the directed migration of enteric nervous system progenitor cells during mammalian embryogenesis. Development, 129(22): 5151–5160,

Niemi, M., Kouvalainen, K., Hjeldt, L. 1961. Cholinesterasesand monoamine oxidase in congenital megacolon. *J. Path Bact.,* 82:363

Qualman, S.J., Jaffe, R., Bove, K.E., *et al*. 1999. Diagnosis of Hirschsprung's disease using the rectal biopsy: Multi-institutional survey. *Pediatr. Dev. Pathol.,* 2(6):588-96.

Rouzrokh, M., Khaleghnejad, A., Mohejerzadeh, L., Heydari, A. and Molaei, H. 2010. What is the most common complication after one-stage transanal pull-through in infants with Hirschsprung's disease?. *Pediatr Surg Int.,* 26(10):967-970.

Schofield, D., Devine, W. and Yunis, E. 1990. Acetyl cholinesterase- Stained Suction Rectal Biopsies in the Diagnosis of Hirschsprung's Disease. *Journal of Pediatric Gastroenterology and Nutrition*, 11(2):221-228.

Teitelbaum, D.H. and Coran, A.G. 2006. Hirschsprung's Disease and Related neuromuscular Disorders of the

Intestine. In: Grosfeld, JL, O'Neill, J.A. Jr, Fonkalsrud, E.W., Coran, A.G. Pediatric Surgery.6th Edition, Philadelphia, *Mosby Elsevier,* p. 1514-59.

Torfs, C. 1998. An epidemiological study of Hirschsprung disease in a multiracial California population. Evian, France: The Third International Meeting: Hirschsprung Disease and Related Neurocristopathies.,

Wakely, P.E. Jr, McAdams, A.J. 1984. Acetylcholinesterasehisto chemistry and the diagnosis of Hirschsprung's disease: a 3 1/2-year experience. *Pediatr. Pathol.,* 2(1):35–46

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Caught by Fingerprint: Dermoscopy of Solitary Neurofibroma

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A 61-year-old female presented to our outpatient department with an asymptomatic nodular lesion on the ventral aspect of the right forearm. She gives a history of a slow-growing nodule for the past 10 years. There were no similar lesions elsewhere in her body. On examination, a small 1 × 2 cm nodular lesion was seen on her right forearm [Figure 1]. The lesion was skin colored, firm, nonmobile, and non-tender on palpation. Button-hole sign was positive. Clinical differential diagnosis of Dermatofibroma and Solitary Neurofibroma were made.

Dermoscopy (Dermlite DL4; 10×) of the lesion revealed a round circumscribed lesion with a central white scar-like area and peripheral halo of pigmentation [Figure 2a]. Fingerprint-like pigmentary lines and peripheral pigment network [Figure 2b] were also observed. Histopathology confirmed the diagnosis of Neurofibroma [Figures 3a and b].



Figure 1: A small, firm, non-tender, 1 × 2 cm nodular lesion on right forearm

Slow growing nodular lesions are difficult to diagnose clinically and histopathology usually aids in the diagnosis. In our patient, the dermoscopy features are suggestive of neurofibroma. Zaballos *et al*.[1] described multiple dermoscopic patterns for Dermatofibroma. The central scar-like area and peripheral pigment network can be seen in both conditions, but the fingerprint pattern along with above-mentioned findings is more suggestive of Neurofibroma. In another study, Duman *et al*.[2] described the above-mentioned findings in a series of cases of neurofibromas and defined the fingerprint pattern as brown parallel cord-like structures within the lesions. Fingerprint pattern can occur in other conditions like seborrheic keratosis, but in the background of other dermoscopic findings, it can help in identifying neurofibroma. This case is presented to highlight the dermoscopy findings in Solitary Neurofibroma and its role in differentiating from Dermatofibroma.

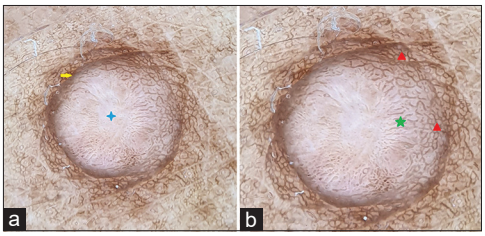


Figure 2: (a): Dermoscopic image showing central white scar like area (blue star) and peripheral halo of hyperpigmentation (yellow arrow) (b): Fingerprint-like pigmentary lines (green star) and peripheral pigment network (red triangle). (Contact polarised light dermoscopy; Dermlite DL4; 10×)

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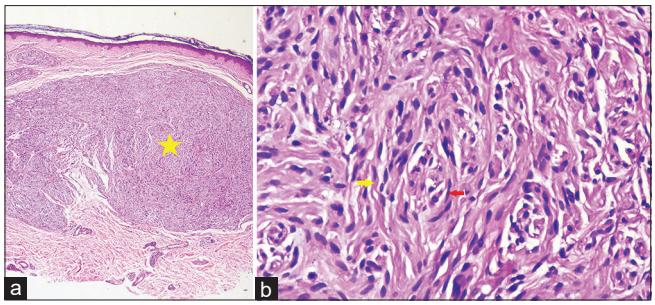


Figure 3: (a): Photomicrograph showing a well-defined lesion in the dermis (yellow star) (H&E, 4×). (b) Spindle shaped cells (yellow arrow) arranged in whorls and fascicles with hyperchromatic, wavy, buckled nuclei (red arrow) (H&E, 40×)

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her names and initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Zaballos P, Puig S, Llambrich A, Malvehy J. Dermoscopy of dermatofibromas: A prospective morphological study of 412 cases. Arch Dermatol 2008;144:75-83.
2. Duman N, Elmas M. Dermoscopy of cutaneous neurofibromas associated with neurofibromatosis type 1. J Am Acad Dermatol 2015;73:529-31.

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ORIGINAL ARTICLE

The utility of immunofluorescence in diagnosing dermatological lesions and its correlation with clinical and histopathological diagnosis in a tertiary health care setup

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ABSTRACT

Context: Immunofluorescence (IF) is an immunological technique which is used for identifying antibodies against antigens that are bound to tissues or those circulating in body fluids. This study is an attempt to evaluate the dermatological lesions such as noninfectious vesiculobullous lesions, connective tissue disorders, vasculitis, and lichen planus using histopathology techniques and direct IF (DIF) studies. Furthermore, an attempt was made to evaluate the diagnostic utility of the IF technique in our hospital. **Aims:** (1) To determine the utility of IF in the diagnosis of skin lesions. (2) To correlate IF with histopathological findings. **Settings and Design:** A cross-sectional, observational study. **Subjects and Methods:** The present study was conducted in the Pathology Department, Coimbatore Medical College, Coimbatore, from August 2012 to August 2013. Fifty cases received during the period were included in our study. Two skin biopsies for each case - one in formalin for routine histopathological examination and other in phosphate buffer solution for IF studies - were received. **Statistical Analysis Used:** Fisher's exact value, mean, and coefficient of mean were calculated. **Results:** The lesions were diagnosed by both light microscopy and IF study. Out of fifty cases, 42 cases were positive with the IF technique and the overall sensitivity of IF was 84% in our study. When histopathological and IF findings were correlated, statistically significant two-tailed $P = 0.0130$ was obtained. Both light microscopic findings and IF findings complemented each other in our study. IF proved to be a necessary supplementary technique to both clinical and histopathological examinations, especially in cases of diagnostic dilemmas. **Conclusions:** IF is a rapid diagnostic procedure. IF was useful in early diagnosis, treatment, and monitoring of various disorders in our study. Positive and negative DIF had prognostic significance in certain cases. Clinicopathological correlation along with DIF studies proved to be a powerful tool in definite diagnosis of the skin lesions.

Key words: Autoimmune, connective tissue disorders, direct immunofluorescence, lichen planus, vasculitis, vesiculobullous

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INTRODUCTION

Skin is the largest organ of the body. As it is the only external organ, skin is examined first. It is a window

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to one's well-being.[1] Skin is also an easily accessible site for biopsy. Not only well-defined *de novo* skin lesions but also many systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and vasculitis can also be easily diagnosed by skin biopsy.[2]

Although histopathology is the gold standard diagnosis for most dermatological lesions, all lesions cannot be diagnosed by histopathology alone. Ancillary techniques such as Tzanck smear, immunofluorescence (IF), serology, and recent advancements such as immunoelectron microscopy and immunoblotting have refined the diagnosis. This study was an attempt to evaluate the dermatological lesions such as vesiculobullous lesions, connective tissue disorders, vasculitis, and lichen planus (LP) using both histopathology and direct IF (DIF) studies and to assess the diagnostic potential of the IF studies.

SUBJECTS AND METHODS

The present study was conducted in the Department of Pathology, Coimbatore Medical College, Coimbatore, from August 2012 to August 2013. All newly diagnosed cases of immunobullous disorders, connective diseases, vasculitis, and LP irrespective of age, sex, and associated disorders were included in the study. Patients treated with steroids in the past 3 weeks and specimens devoid of clinical details and histopathological specimens that were not fixed with formalin were excluded from our study.

Fifty cases with skin lesions were included in our study. The clinical details were collected from the patients. Two punch biopsies of early skin lesions were collected for each case. In case of autoimmune blistering disease (AIBD), perilesional biopsies were taken. In case of connective tissue disorders, LP and vasculitis, lesional biopsies were taken. One was sent in formalin for routine histopathological examination. These specimens were processed routinely and sections were cut and stained routinely using hematoxylin and eosin. The other was sent in phosphate buffer for IF studies. Four-micron-sized sections were cut using cryostat and tissues were transferred to six slides. One slide was used for rapid hematoxylin and eosin staining. The remaining slides were stained with fluorescein isothiocyanate-labeled immunoglobulin G (IgG), IgA, IgM, C3, and fibrinogen (in 1:25 dilution) for an hour and then viewed under a fluorescent microscope. The site (whether dermoepidermal junction [DEJ] or the intercellular spaces [ICSs] of epidermis), nature (IgG, IgA, IgM, C3, fibrinogen), and pattern (linear or granular) of staining were noted.

RESULTS

Our study was an observational one for a period of 1 year from August 2012 to August 2013. During the study period, 8576 specimens were received, of which 264 were skin biopsies. Our study group comprised fifty patients with clinically suspected and newly diagnosed noninfectious vesiculobullous lesions, connective tissue disorders and vasculitis.

In our study group of 50, 26 were men and 24 were women with a sex ratio of 1.08:1 [Table 1]. Most cases were in the age group 41–50 years in both the sexes [Table 1].

Vesiculobullous disorders constituted 0.45% of the total specimens and 14% of all skin specimens [Table 2].

Table 1: Age- and sex-wise distribution of cases

Age group	Sex, *n* (%)		Total (%)
	Male	Female	
0-10	0	0	0
11-20	1 (2)	4 (8)	5 (10)
21-30	1 (2)	2 (4)	3 (6)
31-40	2 (4)	4 (8)	6 (12)
41-50	13 (26)	8 (16)	21 (42)
51-60	4 (8)	3 (6)	7 (14)
61-70	5 (10)	3 (6)	8 (16)
>70	0	0	0
Total	26 (52)	24 (48)	50 (100)

Table 2: Spectrum of distribution of diseases

Final diagnosis	Cases	Percentage
PV	8	16
PF	5	10
IgAP	2	4
PNP	1	2
SCPD	1	2
BP	18	36
DH	2	4
LIBD	2	4
SLE	2	4
DLE	3	6
PSS	1	2
LCV	3	6
EED	1	2
LP	1	2
Total	50	100

PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus

Among the vesiculobullous disorders, bullous pemphigoid (BP) was most frequently diagnosed in our study (18 cases) [Figures 1 and 2]. Histopathological examination confirmed the diagnosis of pemphigus in only 14 cases [Figure 3]. A case each of pemphigus vulgaris (PV) and pemphigus foliaceus (PF) did not show acantholytic cells.[3] Out of the 39 AIBD cases, DIF positivity was seen in 34 cases [Table 3]. PV, PF, and IgA pemphigus showed lace-like pattern in the ICS of the epidermis [Figure 4 and Table 4]. The sensitivity of DIF in diagnosing AIBD was calculated as 89.74% in our study.

Among the connective tissue disorders, three cases of discoid lupus erythematosus (DLE) [Figure 5], two of SLE [Figure 6], and one case of progressive systemic sclerosis (PSS) were diagnosed. However, DIF positivity was seen in five cases and one case was DIF negative (PSS) [Table 3]. The sensitivity of DIF in diagnosing connective tissue disorder was 83.33% in our study.

In the vasculitis category, leukocytoclastic vasculitis (LCV) was diagnosed in three cases and erythema elevatum diutinum in one case. DIF positivity was seen in only two of four histopathologically confirmed cases [Figures 7, 8 and Table 3]. The sensitivity of DIF in diagnosing vasculitis was 50% in our study.

One case of LP was included in our study. DIF was positive and the sensitivity was 100% [Table 3].

The discordant results observed between clinical, histopathological, and IF findings are tabulated in Table 5. In all the discordant cases, IF findings were taken to arrive at the final diagnosis in case of positive IF results.

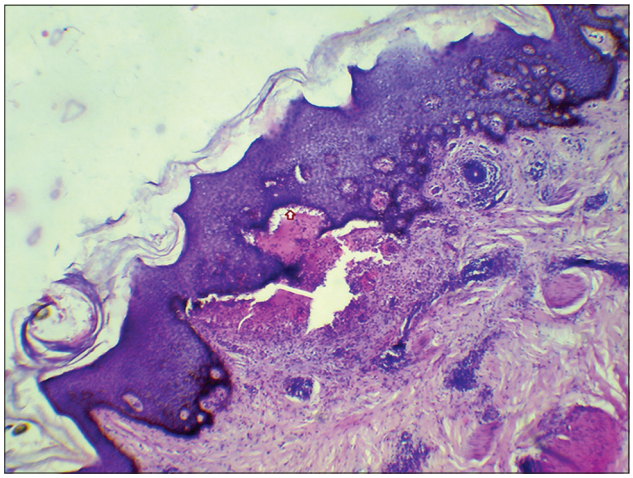


Figure 1: A subepidermal bulla with eosinophilic infiltrates - Bullous pemphigoid (brown arrow, H and E, ×10)

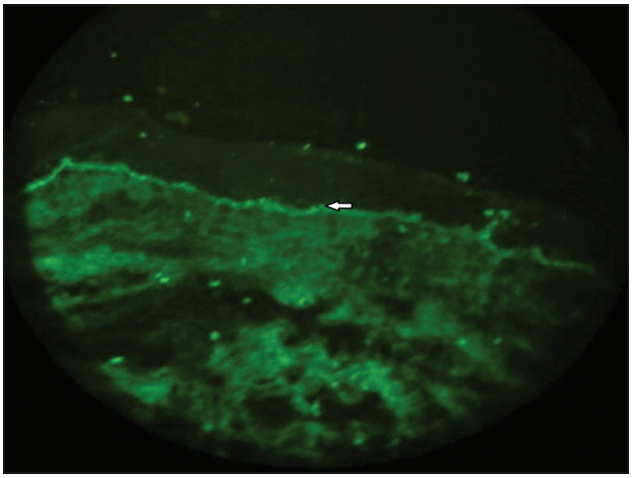


Figure 2: Linear immunoglobulin G deposits along the dermoepidermal junction - Bullous pemphigoid (black arrow, immunofluorescence)

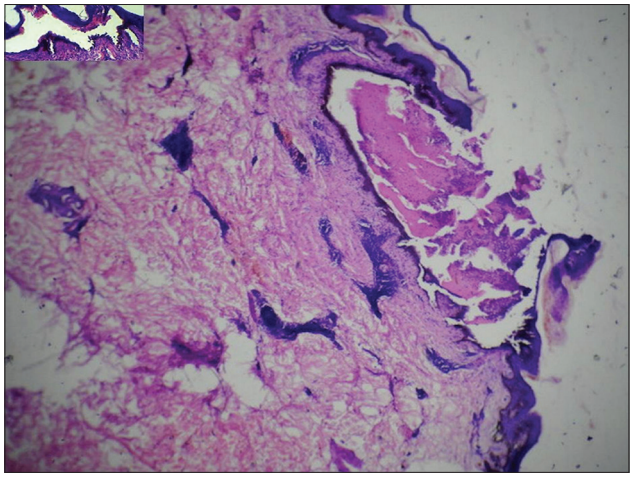


Figure 3: A suprabasal bulla (H and E, ×10) inset shows a bulla with acantholytic cells - Pemphigus vulgaris (H and E, ×40)

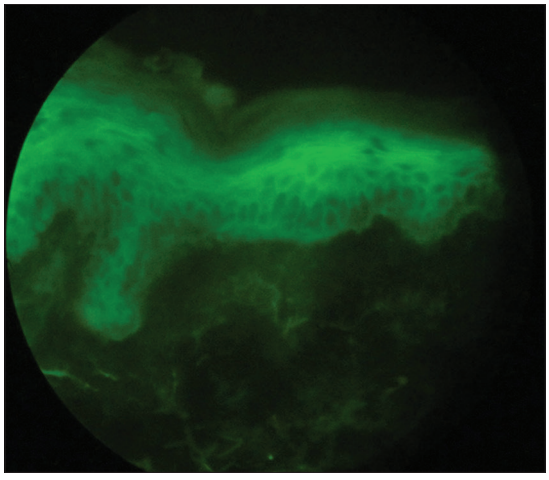


Figure 4: Fish net-like appearance of the epidermis with immunoglobulin G - Pemphigus vulgaris (immunofluorescence)

Table 3: Direct immunofluorescence staining in individual cases

Final diagnosis	DIF findings							
	IgG	IgA	IgM	C3	Fibrinogen	IgG, C3	All	Negative
PV	4					3	0	1
PF	2					2	1	
IgAP		2						
PNP								1
SCPD								1
BP	1			4		11		2
DH		2						
LIBD		2						
SLE						2		
DLE			1		1		1	
PSS								1
LCV					1		1	1
EED								1
LP					1			

DIF: Direct immunofluorescence, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus, ICS: Intercellular space, Ig: Immunoglobulin

Table 4: Pattern of direct immunofluorescence staining

Final diagnosis	Pattern of DIF					
	ICS	DEJ			Dermal vessels	Negative
		Linear	Granular	Shaggy		
PV	7					1
PF	5					
IgAP	2					
PNP						1
SCPD						1
BP		16	0			2
DH			2			
LIBD		2				
SLE			2			
DLE			2	1		
PSS						1
LCV					2	1
EED						1
LP				1		

DIF: Direct immunofluorescence, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus, ICS: Intercellular space, DEJ: Dermoepidermal junction

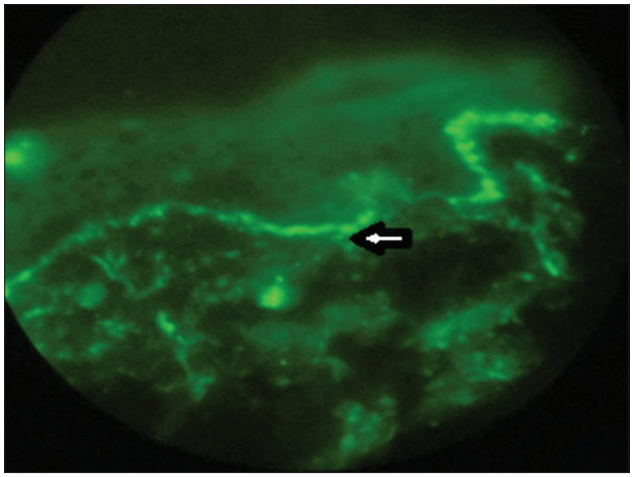


Figure 5: Granular immunoglobulin M deposits along the dermoepidermal junction - Discoid lupus erythematosus (black arrow, immunofluorescence)

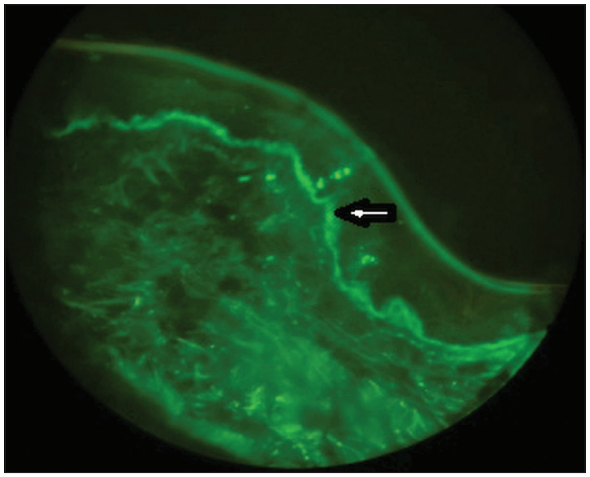


Figure 6: Granular positivity of C3 along the dermoepidermal junction - Systemic lupus erythematosus (black arrow, immunofluorescence)

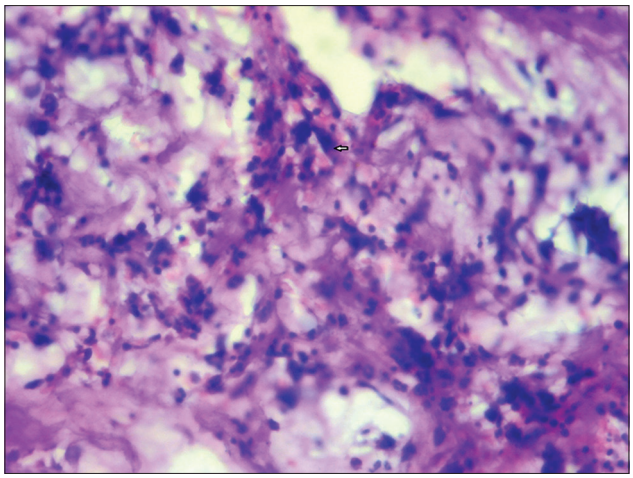


Figure 7: Destruction of the blood vessels - Leukocytoclastic vasculitis (H and E, ×40)

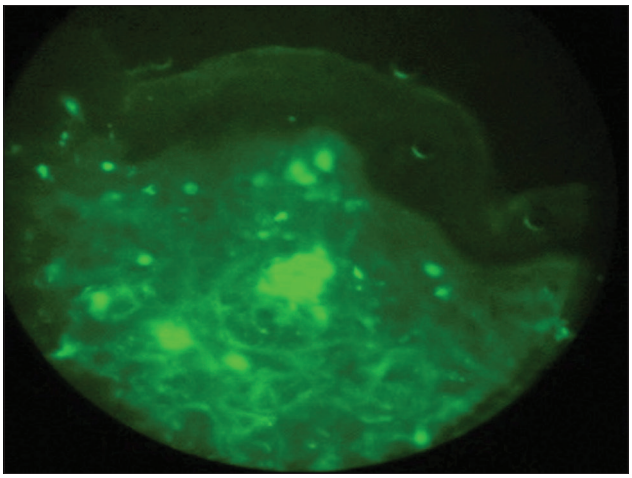


Figure 8: Fibrinogen positivity in the vessels - Leukocytoclastic vasculitis (immunofluorescence)

Statistics

When histopathological and IF findings were correlated, the two-tailed $P = 0.0130$ which was statistically significant [Table 6]. The mean value calculated was 0.50 and the 95% confidence interval of this difference ranged from 0.12 to 0.88. Hence complementary nature of histopathology and IF was proved.

DISCUSSION

Autoimmune vesiculobullous disorders

Vesiculobullous disorders include a wide range of dermatoses, which are characterized by protean manifestations.[1] These disorders have varying etiology, prognostic factors, but united by the formation of blisters either within or beneath the epidermis. These lesions have a striking impact not only on the affected individuals but also for their family members. Histopathological evaluation of vesiculobullous lesions involves the systematic analysis, including the plane of separation of blisters and also the nature of inflammatory infiltrates. These have many differentials which can be sorted out using IF.

Pemphigus is a fatal disease affecting skin and mucosa. Pemphigus was first used by Hippocrates to describe pemphigoid fever as pemphigoides pyertoi. In 1760, De-Sauvages coined the term pemphigus from *pemphix* meaning pustule in Greek.[4] Lever in 1953 described BP and differentiated it from pemphigus.[5-7] This is of great historical significance as BP has a much better prognosis than pemphigus which is commonly fatal.

The most common age group in our study was fourth to fifth decades. This finding was in accordance with

Table 5: Discordance between clinical, histopathological, and immunofluorescence findings

Clinical diagnosis	HPE diagnosis	If diagnosis	Final diagnosis
BP	LIBD/DH	LIBD	LIBD
PV	PF	PF	PF
DH	BP	BP	BP
SCPD	IgAP/PF/SCPD	IgAP	IgAP
DH	PF	PF	PF
IgAP	IgAP/PF/SCPD	IgAP	IgAP
SCPD	IgAP/SCPD	SCPD	SCPD
LIBD	BP/LIBD	LIBD	LIBD
LIBD	BP	BP	BP
Vasculitis	LCV	Negative	LCV
EED	EED	Negative	EED
BP	BP	Negative	BP
BP	BP	Negative	BP
PSS	PSS	Negative	PSS
PNP	PNP	Negative	PNP
PV	PV	Negative	PV

PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus

Kabir *et al*., Kambi *et al*., and Rizvi and Sadiq studies.[8-10] Male:female ratio [1.08:1] was approximately equal in this study, which was similar to Kabir *et al*. and Kambi *et al*. studies.[8,9] However, Anuradha *et al*. and Rizvi and Sadiq's studies showed a male predominance.[10,11]

From Table 1, BP (46.5%) constituted the majority followed by PV (20.5%) in the present study. This was in accordance with Kabir *et al*. and Monsef *et al*. studies.[8,12] Most of the other studies had PV as the predominant disorder. This discordance could be due to variation in the geographical distribution of vesiculobullous lesions. In BP, subepidermal bulla was seen in 77% patients. Other BP cases revealed only numerous eosinophils

Table 6: Clinical, histopathological and direct immunofluorescence correlation

Diagnostic mode	PV	PF	IgAP	PNP	SCPD	BP	DH	LIBD	SLE	DLE	PSS	LCV	EED	LP	Total
Clinical	9	3	1	1	2	17	3	3	2	3	1	3	1	1	50
HP	8	5	3	1	2	14	2	2	2	3	1	3	1	1	48
DIF	7	4	2	-	1	16	2	2	2	3	-	2	-	1	42
Total case diagnosed	8	5	2	1	1	18	2	2	2	3	1	3	1	1	50

DIF: Direct immunofluorescence, HP: Histopathological, PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, IgAP: IgA pemphigus, PNP: Paraneoplastic pemphigus, SCPD: Subcorneal pustular dermatosis, BP: Bullous pemphigoid, DH: Dermatitis herpetiformis, LIBD: Linear IgA bullous disease, SLE: Systemic lupus erythematosus, DLE: Discoid lupus erythematosus, PSS: Progressive systemic sclerosis, LCV: Leukocytoclastic vasculitis, EED: Erythema elevatum diutinum, LP: Lichen planus

in the papillary dermis. This could be due to biopsy of early lesions as prodromal stage shows nonspecific changes such as edematous dermis with abundant eosinophilic infiltration.[13]

The most common intraepidermal disorder in our study was PV. This finding was similar to Kabir *et al.* studies.[8] Suprabasal bulla was seen in 100% of cases and this was in accordance with Kambi *et al*. and Leena *et al*.'s studies.[9,14] In our study, the incidence of PF was 12.82%, which was similar as that of Kambi *et al*. study.[9] All pemphigus lesions show staining in the ICS of epidermis in a fish net-like pattern; hence, they should be interpreted along with histological findings. IgA pemphigus and linear IgA bullous dermatoses can be diagnosed confidently only by immunofluorescent studies. Two cases of dermatitis herpetiformis were seen in this study. Shave biopsy is preferred in dermatitis herpetiformis as IgA is only focally positive in this case. Due to this, DIF should be done at least twice in shave biopsy specimens before awarding a negative DIF diagnosis in DH according to Sontheimer *et al*.s study.[15]

In the present study, lesions such as subcorneal pustular dermatoses (SCPDs) and paraneoplastic pemphigus (PNP) which was not seen in other studies were seen. The five cases were DIF negative in our study which includes two cases of BP, a case of PNP, a case of PV, and a case of SCPD. Grolleau-Rochiccioli *et al*. in their study had mentioned three BP cases that showed negative DIF results.[16] This could be due to subthreshold immunoreactants. DIF negativity or delayed detection with IF technique had been observed in PNP.[17] Mysorekar *et al*. and Buch *et al*. in their study had mentioned about DIF negative PV cases.[18,19] Buch *et al*. in their study had mentioned that IF negative PV meant disease remission.[19] DIF is normally negative in SCPD as it is a nonimmune bullous lesion associated with IgA gammopathy.[20] Our DIF findings in vesiculobullous lesions were in accordance with the Wallach D's study.[20]

Connective tissue disorders

Connective tissue disorders have multisystemic involvement. They have a remarkable diversity, but at the same time, they are clinically similar in many aspects.[20] Cutaneous involvement is an important feature in these diseases. Therefore, skin biopsies play an important role in the classification of these diseases.[20] DIF is helpful in confirming the diagnosis of LE when suspected histologically, clinically, or both. It also helps in classifying the various types of LE as the morphology, site and frequency of immunoreactants deposition vary with various subtypes of LE.

DIF positivity in SLE usually varies with biopsy site, morphology of lesions, treatment profile, and disease activity. In our study, all LE cases were IF positive. Anuradha *et al*. studied four LE cases, of which two belonged to SLE and both showed DIF negativity.[11] In Kabir *et al*. study, seven cases of SLE were included and all were DIF positive which coincides with our study.[21] According to Mutasim and Adams, DIF was more commonly seen in the sun unexposed, oldest, and untreated DLE lesion. Moreover, positivity of combined IgM and IgG deposit was in favor of DLE.[22] In Kabir *et al*. study, of the 3 DLE cases, two showed DIF positivity.[21] Sandra *et al*. study which included 16 LE cases (13 females and 3 males), they had concluded that if DIF was positive in nonlesional skin, there was a greater chance of developing SLE. Hence, those patients required close follow-up.[23] One case of PSS was included in our study. Histopathological examination revealed findings consistent with scleroderma. However, DIF studies were negative. According to Mutasim and Adams, DIF was negative or nonspecific in PSS cases. PSS patients having positive DIF finding seem to have more often overlapping features of SLE, vasculitis, or dermatomyositis.[22] Winkelmann *et al*. had reported ten PSS cases that were DIF negative.[4] Kulthanan *et al*. have mentioned about the inconsistent IF results in PSS cases.[5] Hence, in PSS, clinical examination and histopathology play a more important role than DIF. From this, we conclude that a combination of histopathology, serology and immunology with the background of clinical examination is essential for definite diagnosis of connective tissue disorders.

Vasculitis

Vasculitis refers to necrosis and inflammation of arteries, veins, or both.[1] For confirmation of LCV and Henoch–Schonlein purpura, IF is very useful. In our study, four cases of vasculitis were noted. In Khetan *et al*. study of 61 cases, female predominance was noted unlike our study.[24] In Nandeesh and Tirumalae's study of 198 patients also, a female predominance was noted unlike our study.[25] In vasculitis, the immunoreactants are commonly deposited in the postcapillary venules in the papillary dermis. The pattern could be either fibrillary or granular. It could also be deposited either in the intravascular or extravascular space.[17] In this study, staining was seen in intravascular space of vessels. Henoch–Schonlein purpura was common in other studies, but it was not seen in our study. In Gupta *et al*. study, of fifty cases, LCV was the predominant lesion akin to our study, histopathology gave a conclusive diagnosis in 42 cases (84%) and DIF was positive in 74% cases,.[26] However, similar to this study, in Nandeesh and Tirumalae's study, LCV was the predominant lesion and it had DIF positivity of 54%. However, the overall DIF positivity was 62% in their study.[25] Like our study, LCV was the predominant lesion in Khetan *et al*.'s study (66%). But DIF positivity was seen in 39% and this was lower than our study.[24] Leelavathi *et al*. (85 cases) revealed DIF positivity in 13% cases.[27] DIF provides only supporting evidence in vasculitis. A definite diagnosis of vasculitis should be given after considering clinical, histopathological, laboratory investigations and IF studies. According to Mutasim and Adams, the sensitivity of DIF in vasculitis is limited by both duration and site. Lower limb lesions give false positive DIF results and lesions aged more than a day tend to give false negative test.[22]

Lichen planus

In Kabir *et al*. study, 7 of 12 LP cases were DIF positive showing irregular immunoreactant deposit along the DEJ, constituting 58% positivity.[21]

In Anuradha *et al*. study, all six LP cases showed fibrinogen positivity along the DEJ (100%). The pattern was linear, granular, or shaggy.[11] Sandra *et al*. showed fibrinogen positivity in all their 18 LP cases.[23] According to Mutasim and Adams, DIF was essential only when histopathological and clinical findings were inconclusive and was not necessary in LP as most cases were diagnosed by clinical examination alone.[22]

CONCLUSIONS

IF plays an ancillary role in the definite diagnosis of vesiculobullous disorders, connective tissue disorders and vasculitis and should be interpreted along with clinical features, laboratory workup and histopathology findings.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Burns T, Breathnach S, Cox N, Griffiths C. Rook's Textbook of Dermatology. 8th ed., Vol. 1. New jersey: John Wiley and Sons, Ltd., Publication; 2010. p. 39-41, 50, 51.
2. Minz RW, Chhabra S, Singh S, Radotra BD, Kumar B. Direct immunofluorescence of skin biopsy: Perspective of an immunopathologist. Indian J Dermatol Venereol Leprol 2010;76:150-7.
3. Emmerson RW, Wilson-Jones E. Eosinophilic spongiosis in pemphigus. A report of an unusual hitological change in pemphigus. Arch Dermatol 1968;97:252-7.
4. Winkelmann RK, Carapeto FJ, Jordon RE. Direct immunofluorescence in the diagnosis of scleroderma syndromes. Br J Dermatol 1977;96:231-8.
5. Kulthanan K, Jiamton S, Taiyaitiang C, Pinkaew S, Suthipinittharm P. Direct immunofluorescence study in Thai patients with scleroderma. J Med Assoc Thai 2006;89:1670-6.
6. Singh R, Pandhi RK, Pal D, Kalla G. A clinicopathological study of pemphigus. Indian J Dermatol Venereol Leprol 1973;39:126-32.
7. Ahmed AR, Maize JC, Provost TT. Bullous pemphigoid. Clinical and immunologic follow-up after successful therapy. Arch Dermatol 1977;113:1043-6.
8. Kabir AK, Kamal M, Choudhury AM. Clinicopathological correlation of blistering diseases of skin. Bangladesh Med Res Counc Bull 2008;34:48-53.
9. Kambi DP, Santosh C, Suguna B, Gopal M, Abhishek V. Direct immunofluorescence study in autoimmune bullous disorders of skin. Indian J Biomed Adv Res 2013;4:610-8.
10. Rizvi RS, Sadiq S. Use of direct immunofluorescent microscopy in the diagnosis of vesiculobullous disorders of skin. Pak J Med Sci 2010;26:411-5.
11. Anuradha CH, Malathi N, Anandan S, Magesh KT. Current concepts of immunofluorescence in oral mucocutaneous diseases. Indian J Dermatol Venereol Leprol 2011;15:261-6.
12. Monsef A, Farshchian M, Jafari M, Farshchian M. Immunofluorescence pattern of autoimmune bullous diseases in Iranian patients. Iran J Pathol 2012;7:231-5.
13. Weedon D. Skin Pathology. 3rd ed. Amsterdam: Churchill Livingstone, Elsevier Publication; 2010. p. 38-46, 57-64, 124-68, 201-15.

14. Leena, Vijaya B, Manjunath GV, Sunila. A clinico-pathological study of 22 cases of pemphigus. J Clin Diagn Res 2010;4:2446-51. [Author's initials were not given in the article]
15. Sontheimer RD, Euwer RL, Geppert TD, Cohen SB. Connective tissue diseases. In: Moschella SL, Hurley HJ, editors. Dermatology. 2nd ed. Philadelphia: WB Saunders Company; 1985.
16. Grolleau-Rochiccioli P, Prost C, Bedane C, Oksman F, Intrator L, Bazex J, *et al*. Does bullous pemphigoid with negative direct immunofluorescence exist? Apropos of 3 cases. Ann Dermatol Venereol 1992;119:11-7.
17. Bennett DD, Busick TL. Delayed detection of autoantibodies in paraneoplastic pemphigus. J Am Acad Dermatol 2007;57:1094-5.
18. Mysorekar VV, Sumathy TK, Shyam Prasad AL. Role of direct immunofluorescence in dermatological disorders. Indian Dermatol Online J 2015;6:172-80.
19. Buch AC, Kumar H, Panicker N, Misal S, Sharma Y, Gore CR. A Cross-sectional study of direct immunofluorescence in the diagnosis of immunobullous dermatoses. Indian J Dermatol 2014;59:364-8.
20. Wallach D. Intraepidermal IgA pustulosis. J Am Acad Dermatol 1992;27(6 Pt 1):993-1000.
21. Kabir AN, Das RK, Kamal MD. Direct immunofluorescence test of skin biopsy samples – Results of 2004 cases. Dinajpur Med Coll J 2009;2:8-12.
22. Mutasim DF, Adams BB. Immunofluorescence in dermatology. J Am Acad Dermatol 2001;45:803-22.
23. Sandra A, Bejoy P, Srinivas CR, Shenol SD, Pai S, Dinesh. Immunofluorescence in lichen planus. Indian J Dermatol 1996;41:49. [Author's initial was not provided in the article]
24. Khetan P, Sethuraman G, Khaitan BK, Sharma VK, Gupta R, Dinda AK, *et al*. An aetiological and clinicopathological study on cutaneous vasculitis. Indian J Med Res 2012;135:107-13.
25. Nandeesh B, Tirumalae R. Direct immunofluorescence in cutaneous vasculitis: Experience from a referral hospital in India. Indian J Dermatol 2013;58:22-5.
26. Gupta S, Handa S, Kanwar AJ, Radotra BD, Minz RW. Cutaneous vasculitides: Clinico-pathological correlation. Indian J Dermatol Venereol Leprol 2009;75:356-62.
27. Leelavathi M, Aziz SA, Gangaram HB, Hussein SH. Cutaneous vasculitis: A review of aetiology and clinical manifestations in 85 patients in Malaysia. Med J Malaysia 2009;64:210-2.

The Value of Duodenal Biopsies in the Evaluation of Megaloblastic Anemia

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Abstract

Introduction Megaloblastic anemia is one of the common causes of anemia in India. Duodenal biopsies are routinely performed in the investigation of megaloblastic anemia. The present study was undertaken to analyze the value of duodenal biopsy in megaloblastic anemia and to correlate endoscopic findings with biopsy. As a secondary aim, the study has also analyzed the hematological profile and vitamin B_{12} and folate status of these patients.

Materials and methods All the cases of megaloblastic anemia with bone marrow studies diagnosed at PSG Institute of Medical Sciences and Research during the two year period from January 2016 to December 2017 were retrieved. Clinical and laboratory findings (serum vitamin B_{12} and folate levels) and endoscopic findings were retrieved from hospital records of the patients. Duodenal biopsies of these patients reported in the histopathology department were retrieved and reviewed. Statistical analysis was done using SPSS software 20.0.

Results There were 93 cases of megaloblastic anemia diagnosed on bone marrow biopsies. Tropical sprue was diagnosed in 49.5% of cases, followed by intraepithelial lymphocytosis (17.2%), peptic duodenitis (17.2%), and no significant pathology in 16% of cases. Pancytopenia was present in 54.8% of cases. Isolated vitamin B_{12} deficiency including low levels was present in 48.38% and folate deficiency was seen in 4.3% cases; 34.48% cases had both vitamin B_{12} and folate deficiency.

Conclusion The incidence of tropical sprue in megaloblastic anemia is 49.5% in the study. Duodenal biopsy is valuable in the work up of megaloblastic anemia, irrespective of the endoscopic changes in identifying the etiology.

Keywords
- megaloblastic anemia
- tropical sprue
- vitamin B_{12}
- folate
- pancytopenia
- intraepithelial lymphocytosis

Introduction

Megaloblastic anemia is characterized by the distinctive morphological appearance of developing hematopoietic cells in the bone marrow, resulting from nuclear cytoplasmic asynchrony, occurring as a result of defective deoxyribonucleic acid (DNA) synthesis.[1] The cause is usually nutritional deficiency or malabsorption of either cobalamin (vitamin B_{12}) and/or folate. Malabsorption can result from gastrectomy, tropical and nontropical sprue, intestinal resection, and drugs. It can also occur in defects of metabolism of these vitamins.[2] While pernicious anemia is reported to be the

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common cause for megaloblastic anemia in Western countries, dietary deficiency and malabsorption are the common causes in India.[3,4] Duodenal biopsies are routinely performed in patients with megaloblastic anemia to look for any evidence of malabsorption since tropical sprue (TS) is reported to be more prevalent in India. These biopsies are performed irrespective of the macroscopic endoscopic findings. The present study was undertaken to study the value of duodenal biopsy in patients of megaloblastic anemia and correlate the biopsy findings with endoscopy. As a secondary aim, the study also analyzed the hematological profile of patients with megaloblastic anemia.

Materials and Methods

Approval was obtained from the institutional human ethics committee before the onset of the study. All the cases of megaloblastic anemia with bone marrow studies diagnosed at PSG Institute of Medical Sciences and Research during the period of 2 years from January 2016 to December 2017 were retrieved. Cases with nonmegaloblastic macrocytic anemia, those with other associated neoplastic conditions like myelodysplastic syndrome, and leukemia were excluded from the study. Hematology assays were performed on a LH 780 hematology analyzer (Beckman Coulter, Inc., United States) using volume conductivity and scatter technology. Clinical and laboratory findings (serum vitamin B_{12} and folate levels) were retrieved from hospital records of the patients. Duodenal biopsies of these patients reported in the histopathology department were retrieved. Endoscopic findings of these cases were retrieved from hospital information system and tabulated. Histopathological examination was performed after standard processing and hematoxylin and eosin staining procedures. All the biopsy slides were reviewed and only those that had well-oriented villi were included. Histomorphological features like villous and crypt architecture, intraepithelial lymphocyte (IEL) count per hundred enterocytes, and 20 villous tip enterocytes in 5 villi were studied. Up to 20 IELs per 100 enterocytes was considered normal; 21 to 30 IELs per 100 enterocytes and more than 30 per 100 enterocytes were graded as borderline and severe increase, respectively. Inflammatory cell component in lamina propria—lymphocytes, plasma cells, neutrophils, eosinophils, and epithelioid histiocytes were also analyzed and the severity was graded as mild, moderate, and severe.

Only those with unequivocal partial or complete villous atrophy, intraepithelial lymphocytosis, and increase in inflammatory cell content were diagnosed as TS. Patients with increase in IELs and no abnormality of villous architecture (Marsh I, lesion) were categorized as intraepithelial lymphocytosis. Data were analyzed using SPSS software for windows, version 20.0, IBM Corporation, NY, United States.

Results

There were 93 cases of megaloblastic anemia diagnosed on bone marrow biopsies during the study period. There were 68 (73.2%) males and 25 (26.8%) females. Age ranged from 16 to 85 years, the median age being 42 years. Out of 93 cases, vitamin B_{12} assay was done in all whereas folate levels were available in 73.

According to the World Health Organization (WHO), vitamin B_{12} status in adults is defined by the serum levels of the micronutrient with the following cutoffs and definitions: greater than 221 pmol/L (> 163 pg/mL) is vitamin "B_{12} adequacy"; between 148 and 221 pmol/L (109–163 pg/mL) is "low B_{12}"; and lower than 148 pmol/L (< 109 pg/mL) is "B_{12} deficiency."[5]

In our series we found 59 (63.4%) out of 93 patients to be vitamin B_{12} deficient. 18 (19.3%) had low vitamin B_{12} levels and 16 (17.2%) were categorized as to have adequate B_{12}. Out of the 16 patients with adequate B_{12}, 4 had folate deficiency. Five cases had vitamin B_{12} levels more than 2,000 pg/mL.

Isolated vitamin B_{12} deficiency including low levels was present in 45 (48.38%) patients and folate deficiency (4 ng/mL, reference range being 4.6–34.8 ng/mL) was seen in 4 (4.3%) patients; 32 (34.48%) patients had both vitamin B_{12} and folate deficiency. Twelve (12.9%) patients had normal serum vitamin B_{12} and folate levels.

Considering the hematological parameters, mean hemoglobin and mean corpuscular volume (MCV) of the patients were 6.68 g/dL and 103.9 fL, respectively. MCV was less than 90 fL in five patients (5%). These five patients had coexisting iron deficiency anemia (►**Table 1**).

Fifty-one patients (54.8%) had pancytopenia at presentation (defined by hemoglobin less than 11 g/dL, total WBC count less than 4×10^9/L and platelet count less than 150×10^9/L), 48 of whom had vitamin B_{12} levels < 30 pg/mL. Forty patients had bicytopenia and two had anemia. Peripheral smear and bone marrow morphology are highlighted in ►**Figs. 1** and **2**.

Duodenal biopsies were available for all 93 patients. Out of these, 46 (49.46%) patients who had villous blunting of any degree (mild, moderate, or severe) and intraepithelial lymphocytosis in a decrescendo pattern (more IELs in the crypts) and nucleomegaly were diagnosed as TS (►**Figs. 3** and **4**). Five other cases of TS diagnosed previously did not show unequivocal villous blunting and hence were categorized as intraepithelial lymphocytosis. Sixteen (17.2%) patients had only intraepithelial lymphocytosis, 16 (17.2%) had peptic duodenitis, and 15 (16.12%) had no significant pathology (►**Fig. 5**).

TS was found to be more frequent in the three groups (isolated vitamin B_{12} deficiency, isolated folate deficiency, and combined B_{12} and folate deficiency). TS was less frequent in the patients who had normal vitamin B_{12} and folate levels (►**Table 2**).

Table 1 Hematological profile of patients with megaloblastic anemia

Hematological parameters	Mean	Range
Hemoglobin (g/dL)	6.2 + 2.37	3.0–11.9
Mean corpuscular volume (fL)	103.9 + 15.49	51.8–144.4
Red cell distribution width (%)	19.2	16–28
Platelets ($\times 10^9$/L)	83.8	9–315
Total leucocyte count ($\times 10^9$/L)	3.1	1–18.1

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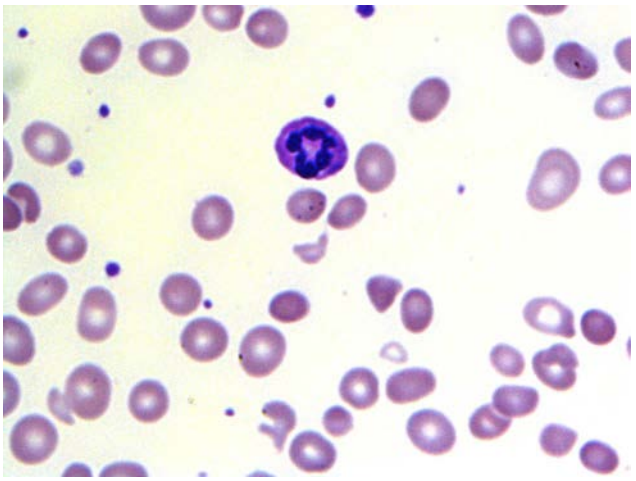


Fig. 1 Peripheral smear with hypersegmented neutrophil and macro-ovalocyte (100×)

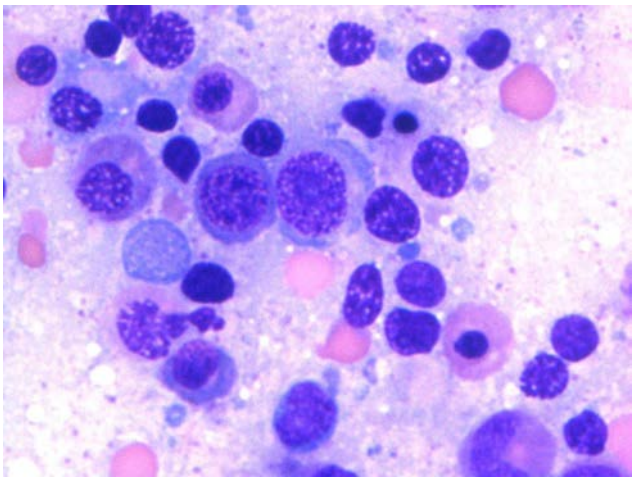


Fig. 2 Bone marrow aspirate showing megaloblastic erythroid hyperplasia (100×)

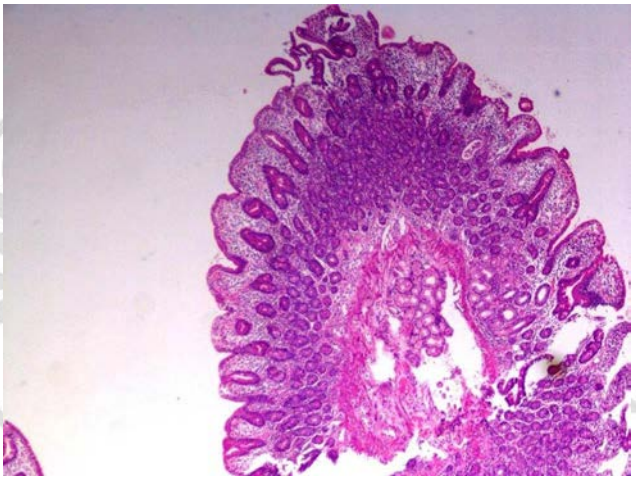


Fig. 3 Duodenal biopsy with villous blunting (4×, Hematoxylin and Eosin)

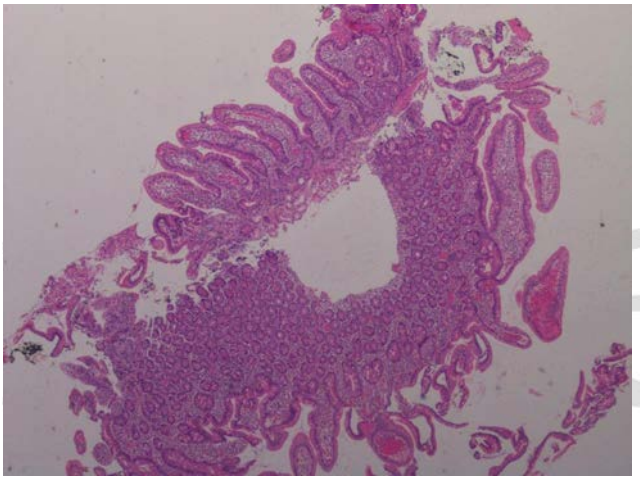


Fig. 4 Duodenal biopsy with variable villous blunting (Hematoxylin and Eosin, 4×)

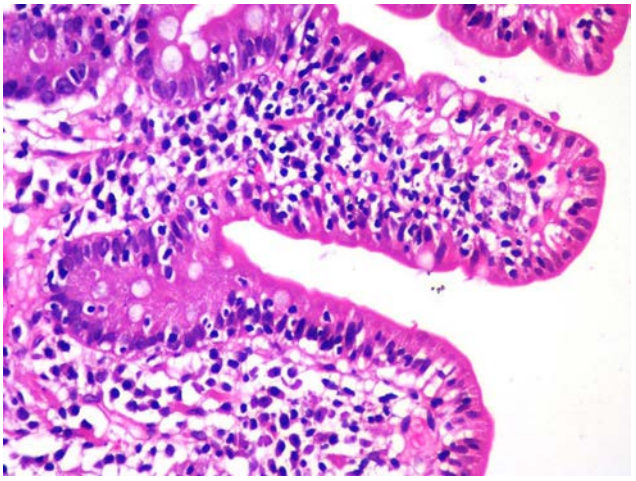


Fig. 5 Duodenal biopsy with marked intraepithelial lymphocytosis (Hematoxylin and Eosin, 40×)

Endoscopic macroscopic changes pertaining to malabsorption like scalloped duodenal and attenuated duodenal folds were identified in 26.8% ($n = 25$) of patients. Of these, the biopsy diagnosis of TS was made in 15 patients (60%). The remaining cases of TS diagnosed on biopsy showed either normal endoscopic appearance or other features like nodularity (►**Table 3**).

Discussion

Megaloblastic anemia is one of the commonest causes for pancytopenia in India. It has a characteristic peripheral smear picture (macrocytosis, ovalocytes, and hypersegmented neutrophils) and low reticulocyte count.[2] Bone marrow examination is done to rule out other neoplastic disorders which can have megaloblastosis, like myelodysplastic syndrome and leukemia. Serum vitamin B_{12} and folate levels will point toward the cause for megaloblastic anemia.[6,7] Duodenal biopsies are performed to identify if there is any evidence of malabsorption. The study reports a male predominance with a ratio of 2.7:1 which is considerably higher when compared with a vast majority of other studies.[8,9] The possible explanation could be the higher population of elderly men in the

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Table 2 Duodenal biopsy findings in patients with vitamin B_{12} and folate deficiency

Number of cases (*N* = 93)	Tropical sprue	Intraepithelial lymphocytosis	Peptic duodenitis	No significant pathology
Vitamin B_{12} deficiency *n* = 45	24 (53.3%)	8 (17.7%)	5 (11.1%)	8 (17.7%)
Folate deficiency *n* = 4	3 (75%)	–	1 (25%)	–
Both *n* = 32	17 (53.1%)	6 (18.75%)	6 (18.75%)	4 (12.5%)
Normal *n* = 12	2 (16.6%)	2 (16.6%)	4 (33.36%)	3 (25%)

Table 3 Endoscopic findings with histological diagnosis

N = 93	Tropical sprue *n* = 46	Intraepithelial lymphocytosis *n* = 16	Peptic duodenitis *n* = 16	No significant pathology *n* = 15
Scalloped duodenal folds *n* = 25	15 (60%)	2 (8%)	3 (12%)	5 (20%)
Nodularity *n* = 25	10 (40%)	6 (24%)	3 (12%)	6 (24%)
Gastroduodenitis *n* = 12	6 (50%)	2 (16.6%)	1 (8.33%)	3 (25%)
No macroscopic abnormality *n* = 31	15 (48.5%)	6 (19.3%)	8 (25.8%)	2 (6.45%)

series in whom vitamin B_{12} deficiency due to intestinal malabsorption are reported to be common.[10]

Cobalamin and folate deficiency are predominantly due to decreased dietary intake or malabsorption in Indian population while pernicious anemia has been reported to be the common etiology in Western population.[11,12] Vitamin B_{12} deficiency has been reported to be the common cause for megaloblastic anemia in our population constituting to 63%, similar to that of Khandhuri et al (68%)[8] and Sarode et al (76%).[9] Combined vitamin B_{12} and folate deficiency was seen in 34% of cases which is slightly higher when compared with 8.8% and 12% reported by Sarode et al and Khandhuri et al, respectively. This is probably because of higher percentage of people with malabsorption in this series where combined micronutrient deficiencies are common. Folate deficiency was seen in 4.3% of patients which is similar to other studies. The cases with vitamin B_{12} levels greater than 2,000 pg/mL could represent cases treated inadvertently with vitamin B_{12} injections prior to investigation.

Pancytopenia and bicytopenia were observed in 54.8% and 43% of the patients, respectively. There are wide variations reported for the frequency of pancytopenia ranging from 17.2 to 43.8%.[13,14] Another study done in South India has reported pancytopenia in 40% of the patients.[15] The higher incidence of pancytopenia in our population can be attributed to severe vitamin B_{12} deficiency in these patients (48 out of 51 had vitamin B_{12} levels < 30 pg/mL). Mean MCV observed in the current study was 103.9 + 15.49 fL, which is lower when compared with that reported by Chidambaram et al (110 fL).[15] MCV was found to be normal in 5.3% of the patients which is lower than another study.[14]

TS was the commonest biopsy diagnosis in the study accounting for 49.5% of patients diagnosed as having megaloblastic anemia, which is similar to most other studies.[16,17] Endoscopic finding of scalloped duodenal folds was identified in 32.6% of TS. In the remaining cases, the diagnosis was possible only with the help of biopsy.

The diagnostic yield of gastrointestinal endoscopy is low in our study. This could be because subtle endoscopic changes were not documented since duodenal biopsies were routinely done in all the patients with megaloblastic anemia. However, Sharma et al[18] in their systematic review had found normal duodenal folds to be more common in TS.

Intraepithelial lymphocytosis without villous abnormality was the second commonest biopsy finding identified in 34.7% of patients. IEL without villous abnormality had been reported in various conditions such as gluten-sensitive enteropathy, infections, autoimmune conditions, drugs, and idiopathic etiology.[19] It has also been reported to be an early morphological feature of sprue.[19,20]

TS and IEL without villous abnormality together constituted 66.6% of cases, indicating malabsorption as the etiology for vitamin B_{12} and folate deficiency in these cases. Hence, biopsy contributes in identifying the etiology in 66.6% of cases.

In the remaining cases, the etiology of megaloblastic anemia could have been dietary deficiency or intrinsic factor deficiency.

Although the guidelines for investigation of megaloblastic anemia recommends serological assays of anti-intrinsic factor antibodies (anti-IFAB)[5] the incidence of pernicious anemia is very low in India. Duodenal biopsies have become a

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routine practice in the workup more based on the evidence of high number of cases of TS being reported. Anti-IFAB levels were not done in the patients in the study.

It was found in the present study that routine duodenal biopsy in cases of megaloblastic anemia yields etiologic information in majority of the cases, irrespective of endoscopy. Hence it is recommended even in the absence of macroscopic endoscopic changes.

Current spectrum of malabsorption in India has found celiac sprue to be increasing in incidence.[19] Since the clinical, endoscopic, and histologic difference between celiac and tropical sprue are not well defined, serum antibodies against tissue transglutaminase and endomysial antibodies are needed in cases where there is no response to therapy for TS.

Limitations

Since this is a retrospective study, follow-up of patients to look for response to therapy for TS is not available. Since the study was conducted in a teaching hospital, endoscopy being done by different endoscopists would have influenced the results. Serological tests for celiac sprue and anti-IFAB antibodies were not done in the study.

Conclusion

Pancytopenia was the commonest presentation of megaloblastic anemia; vitamin B_{12} deficiency and folate deficiency constituted 82% and 4.3%, respectively. The incidence of TS in megaloblastic anemia is as high as 49.5% in the study population. Duodenal biopsy is valuable in the workup of megaloblastic anemia, irrespective of the endoscopic changes in identifying the etiology.

Authors' Contributions

N.N.N. and S.S. contributed to the literature research, data acquisition, and manuscript preparation. All the authors contributed to the development of the concept, design, definition of intellectual content, and manuscript editing and review. S.S. is the guarantor for this article.

Ethical Approval

Ethical approval was obtained from the institutional human ethics committee.

Conflicts of Interest

None declared.

References

1 Hoffbrand V, Provan D. ABC of clinical haematology. Macrocytic anaemias. BMJ 1997;314(7078):430–433

2 Hoffbrand V, Moss PAH, Pettit JE, Megaloblastic anaemias. In: Essential Haematology. 5th ed. Massachussetts: Wiley-Blackwell; 2006 44–57

3 Desai HG, Antia FP. Vitamin B 12 malabsorption due to intrinsic factor deficiency in Indian subjects. Blood 1972;40(5):747–753

4 Allen LH. Causes of vitamin B12 and folate deficiency. Food Nutr Bull 2008;29(2(Suppl):S20–S34, discussion S35–S37

5 Devalia V, Hamilton MS, Molloy AM; British Committee for Standards in Haematology. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. Br J Haematol 2014;166(4):496–513

6 Khanduri U, Sharma A. Megaloblastic anaemia: prevalence and causative factors. Natl Med J India 2007;20(4):172–175

7 Unnikrishnan V, Dutta TK, Badhe BA, Bobby Z, Panigrahi AK. Clinico-aetiologic profile of macrocytic anemias with special reference to megaloblastic anemia. Indian J Hematol Blood Transfus 2008;24(4):155–165

8 Sarode R, Garewal G, Marwaha N, et al. Pancytopenia in nutritional megaloblastic anaemia. A study from Northwest India. Trop Geogr Med 1989;41(4):331–336

9 Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. Natl Med J India 2005;18(4):182–183

10 Holt PR. Intestinal malabsorption in the elderly. Dig Dis 2007;25(2):144–150

11 Singla R, Garg A, Surana V, Aggarwal S, Gupta G, Singla S. Vitamin B12 deficiency is endemic in Indian population: a perspective from North India. Indian J Endocrinol Metab 2019;23(2):211–214

12 Ghoshal UC, Mehrotra M, Kumar S, et al. Spectrum of malabsorption syndrome among adults and factors differentiating celiac disease & tropical malabsorption. Indian J Med Res 2012;136(3):451–459

13 Kumar R, Kalra SP, Kumar H, Anand AC, Madan H. Pancytopenia—a six year study. J Assoc Physicians India 2001;49:1078–1081

14 Sekhar J, Stabler SP. Life-threatening megaloblastic pancytopenia with normal mean cell volume: case series. Eur J Intern Med 2007;18(7):548–550

15 Chidambaram Y, Mambatta AK, Sivara SK. Tropical sprue in megaloblastic anemia. Int J Res Med Sci 2017;5:4133–4137

16 Dutta AK, Balekuduru A, Chacko A. Spectrum of malabsorption in India—tropical sprue is still the leader. J Assoc Physicians India 2011;59:420–422

17 Balasubramanian P, Badhe BA, Ganesh RN, Panicker LC, Mohan P. Morphologic spectrum of duodenal biopsies in malabsorption: a study from Southern India. J Clin Diagn Res 2017;11(7):EC17–EC21

18 Sharma P, Baloda V, Gahlot GP, et al. Clinical, endoscopic, and histological differentiation between celiac disease and tropical sprue: a systematic review. J Gastroenterol Hepatol 2019;34(1):74–83

19 Hammer ST, Greenson JK. The clinical significance of duodenal lymphocytosis with normal villus architecture. Arch Pathol Lab Med 2013;137(9):1216–1219

20 Lauwers GY, Fasano A, Brown IS. Duodenal lymphocytosis with no or minimal enteropathy: much ado about nothing? Mod Pathol 2015;28(Suppl 1) :S22–S29

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ORIGINAL ARTICLE

WILEY

The combination of ACR-Thyroid Imaging Reporting and Data system and The Bethesda System for Reporting Thyroid Cytopathology in the evaluation of thyroid nodules—An institutional experience

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Abstract

Background: The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is widely utilised by cytopathologists. The American College of Radiology (ACR) has also proposed a thyroid imaging reporting and data system (TIRADS) to classify thyroid nodules and guide their selection for fine needle aspiration (FNA). The current study aimed to analyse the usefulness of TBSRTC in thyroid cytology reporting, to examine its histological correlation with TIRADS, and to compare the management of lesions in each cytological category in our institute to the TBSRTC recommendations.

Materials and methods: A retrospective study was performed on all thyroid FNAs from a 2-year period at a tertiary care centre. Histological correlations of TBSRTC and ACR TIRADS were examined for cases with surgical follow-up, and the risk of malignancy in each TBSRTC category, and sensitivity and specificity of FNA and TIRADS, were calculated.

Results: A total of 337 thyroid FNAs were examined, with histological follow-up in 99 cases. Risk of malignancy in categories I-VI was 9.5%, 2.3%, 0.0, 8%, 87.5% and 100.0%, respectively. The sensitivity, specificity, positive predictive value, and negative predictive value of FNA were 60.0%, 99.0%, 85.7%, and 97.5%, respectively, while those values for thyroid ultrasound were 90.3%, 72.2%, 92.9% and 65%, respectively.

Conclusion: The study substantiates the usefulness of TBSRTC in arriving at a more precise diagnosis. Hurthle cell lesions with atypia were the common cause of misdiagnosis. The combination of TIRADS and TBSRTC aids in better stratification of thyroid nodules and in decision making for management of lesions.

KEYWORDS

atypia of undetermined significance, papillary carcinoma, the Bethesda system of reporting thyroid cytopathology, thyroid nodules

Shanmugasundaram Sakthisankari and Sreenivasan Vidhyalakshmi contributed equally to the work.

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1 | INTRODUCTION

Thyroid cancer is the most common endocrine malignancy and constitutes 0.1%-0.2% of all cancers in India. Fine needle aspiration (FNA) of the thyroid gland plays a vital role in the evaluation of thyroid lesions. It is a safe, cost-effective procedure to guide the management of thyroid lesions.[1] With the routine use of FNA, the number of surgeries being performed for benign lesions has been significantly reduced.[2] Most of the centres perform both palpation-guided and ultrasound-guided (USG) FNA.[3] Before the inception of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), various terminologies were in use which often led to confusing risk assessments and unclear clinical management. TBSRTC has established a uniform, tiered reporting system for thyroid FNA specimens with an implied malignancy risk and recommendations for management, and is widely accepted by cytopathologists and clinicians.[4] But unfortunately, a significant subset of cytology interpretation yields indeterminate results (25%), as reported by Valderrabano et al.[5]

The use of ultrasound criteria in differentiating benign and malignant nodules of the thyroid gland has been the focus of research and much of the published literature has confirmed that ultrasound is highly sensitive but less specific in the detection of malignant thyroid nodules. A practical thyroid imaging reporting and data system (TIRADS) for thyroid nodules has been proposed by the American College of Radiology (ACR) to classify nodules of the thyroid gland and to solve the problem of nodule selection for FNAC.[6,7] Shen et al compared the effectiveness of the American Thyroid Association (ATA) classification, the ACR TIRADS, the TIRADS of the European Thyroid Association (EU-TIRADS), and a TIRADS developed by Kwak et al (Kwak-TIRADS) in a large cohort of patients, and concluded that all four risk-stratification systems had good diagnostic performance.[8] The combination of ultrasound imaging criteria using a TIRADS with TBSRTC in the risk stratification of thyroid nodules has not been explored extensively. The present study was carried out to establish the utility of such a combination of TIRADS with TBSRTC in the diagnostic and therapeutic approach to thyroid nodules.

The recent WHO classification has included new variants of thyroid carcinoma such as non-invasive follicular neoplasm with papillary-like nuclear features (NIFTP) and well differentiated thyroid tumour of uncertain malignant potential (WDT-UMP).[9] It is recommended that the risk of malignancy in each category be evaluated by every institution following TBSRTC for reporting as a tool for quality improvement. The current study aims (1) to analyse the diagnostic utility of TBSRTC in thyroid cytology reporting, (2) to establish the histological correlation of thyroid ultrasound using ACR TIRADS and (3) to analyse the adherence to TBSRTC recommendations of management for each category at our Institute.

2 | MATERIALS AND METHODS

This was a retrospective study which involved all patients who underwent FNAC of thyroid lesions over a period of two years (January 2018 to December 2019). The study was approved by the Institute's Human Ethics Committee (20/142). MGG- and PAP-stained smears and cytology reports of thyroid lesions were retrieved from the archives of the Department of Pathology at our Institute, a tertiary care centre. All of these cases were reviewed and diagnosed based on TBSRTC categories I to VI, as follows.

I. Non-diagnostic or unsatisfactory (ND/UNS)

II. Benign

III. Atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS)

IV. Follicular neoplasm/suspicious for follicular neoplasm (FN/SFN)

V. Suspicious for malignancy

VI. Malignant

The probable diagnostic possibilities for categories V and VI were given wherever possible.

In cases with surgical excision, histopathology was considered the final diagnosis and correlated with cytology. Cases with histopathological diagnosis of follicular lesion, NIFTP, were reviewed for adherence to strict criteria for diagnosis. In cases where the cytology and histopathology were discordant, the cytology slides were reanalysed in a blindfolded technique to eliminate subjective bias. The sensitivity and specificity of FNAC in thyroid lesions and probable reasons for diagnostic errors in cytology were analysed.

A retrospective review of thyroid ultrasound features for those patients who underwent thyroidectomy in our institution was performed. The TIRADS score was given by an expert radiologist who was blinded to the histological diagnoses. The ACR TIRADS score was based on the evaluation of composition, echogenicity, shape, margin, and echogenic foci of the lesion, with each feature assigned 0-3 points. The total score is further stratified into categories ranging from 1 (benign) to 5 (highly suspicious). In cases with multiple nodules having different TIRADS scores, the highest score was considered for analysis. Risk of malignancy (ROM) for each TIRADS category was calculated based on the histological diagnosis. For calculating the sensitivity of the TIRADS in predicting malignancy, cross tabulation of the TIRADS score and the histological diagnosis was done. TIRADS categories 1, 2, and 3 were grouped as benign and 4, 5 as suspicious for malignancy.

For each category reported, the recommended management guidelines of TBSRTC and the management done for these cases were compared.

Microsoft Excel 2010 (Microsoft corporation) was used for data entry and statistical analysis was performed using SPSS version 20 (IBM Corporation).

3 | RESULTS

Three hundred and thirty-seven cases of thyroid FNAs were retrieved from the archives of the Department of Pathology. The distribution of the diagnostic categories as per TBSRTC is presented in Table 1.

TABLE 1 Distribution of diagnostic categories of The Bethesda System for Reporting Thyroid Cytopathology

Diagnostic categories	Subcategories	Number of cases (% per category)	Total number of cases (%) n = 337
I. ND/US	Cyst fluid	19	21 (6.2)
	Acellular specimen	2	
II. Benign	Benign follicular nodule	157 (60.6)	259 (76.9)
	Adenomatoid nodule	62 (24)	
	Lymphocytic (Hashimoto) thyroiditis in the proper clinical context	40 (15.4)	
III. AUS		9	9 (2.6)
IV. FN/SFN	Suspicious for follicular neoplasm	22 (88)	25 (7.4)
	Suspicious for Hurthle cell neoplasm	3 (22)	
V. Suspicious for malignancy	Suspicious for papillary thyroid carcinoma	11 (73.3)	15 (4.5)
	Suspicious for medullary carcinoma	2 (13.3)	
	Suspicious for malignancy, not otherwise specified	2 (13.3)	
VI. Malignant	Papillary thyroid carcinoma	3 (37.5)	8 (2.4)
	Anaplastic carcinoma	2 (25)	
	Medullary carcinoma	2 (25)	
	Non-Hodgkin lymphoma	1 (12.5)	

Abbreviations: AUS, atypia of undetermined significance; FN/SFN, follicular neoplasm/suspicious for follicular neoplasm; ND/US, non-diagnostic or unsatisfactory.

The most frequent interpretation was category II of TBSRTC, constituting 76.9% of the total. Among these, benign follicular nodule was the most common interpretation (60.6% of category II), followed by adenomatoid nodule (24%), and Hashimoto thyroiditis (15.4%). Among the category IV lesions, Hurthle cell lesions constituted 22.2% of the category. In both category V and VI lesions, papillary thyroid carcinoma (PTC) was the commonest. Subsequent histopathological examination was possible for 99 cases, and hence the rate of surgical excision constituted 29.4% of cases. The type of surgery depended on the cytological interpretation and radiology findings, and included 11 cases of lobectomy, 62 hemithyroidectomy, and 26 total thyroidectomies. The histological diagnoses for these cases are summarised in Table 2. There were 19 cases of malignancy (excluding three cases of NIFTP and a single case of well-differentiated follicular tumour of uncertain malignant potential), and 79 benign cases which included 10 cases of follicular adenoma. Partially or totally encapsulated nodules showing an admixture of micro- and macrofollicular growth pattern in the background of multiple nodules were interpreted as adenomatoid nodules. Solitary encapsulated nodules consisting of microfollicles were diagnosed as follicular adenoma. Papillary thyroid carcinoma was the commonest malignancy (78.9%). The surgical yield of malignancy in the study was 19.2%. There was a rare coexistence of papillary thyroid carcinoma and calcitonin negative neuroendocrine tumour of the thyroid. The preoperative cytological diagnosis of this case was category VI. The cytological and histological features of category III, IV, and V are shown in Figures 1 and 2. The risk of malignancy for each diagnostic category was calculated excluding NIFTP and WDT-UMP and summarised in Table 2. Risk of neoplasia (RON) was calculated including NIFTP, WDT-UMP and follicular adenoma.

Pre-FNA thyroid ultrasound was available for 90 cases that had histological follow-up. Most of the thyroid nodules belonged to TIRADS categories 2 and 3 on imaging, constituting 67.7% of the total. The distribution of TIRADS categories with the calculated ROM values is tabulated in Table 3. The sensitivity, specificity, positive and negative predictive values of thyroid ultrasound were 90.3%, 72.2%, 92.9% and 65.0% respectively.

The management recommendations as per TBSRTC for each category and the management of patients in the current study are outlined in Table 4. Repeat FNA under ultrasound guidance for ND/UNS category I lesions was done in 19% of cases, of which two showed cystic macrophages, while the remaining two were categorised as benign. Repeat FNA was not performed in any of the Category III lesions. Surgical resection was performed in 23.8%, 25.1%, 22.2%, 60.0%, 60.0%, and 37.5% of categories I, II, III, IV, V, and VI respectively. Molecular testing was not performed in any of these cases.

Of the cases with surgical excision, 3 (4.6%) excluding 2 cases of papillary microcarcinoma had a false-negative diagnosis (these cases were categorised as benign in FNAC but turned out to be PTC in histology), and 2 (22.2%) had a false-positive diagnosis (these two cases were categorised as suspicious for malignancy in FNAC and histology showed adenomatoid nodule). A review of the cytological smears of the 5 cases diagnosed as PTC in histopathology that had been assigned to category II in the cytology was performed. Two cases were papillary microcarcinoma, and no features of PTC were discernible cytologically. In the other three cases, the presence of nuclear grooves and pale nuclei were underdiagnosed as part of a cystic change in colloid goitre. There were no nuclear pseudoinclusions in any of these cases. On reviewing the

TABLE 2 Details of cases with cytohistological correlation and calculated risk of malignancy (ROM)

Category	No of cases with surgery	Diagnostic category	No of cases n (%)	ROM %	ROM as per TBSRTC%	RON%
I	5	Nodular goitre	2 (40.0%)	9.5[a]	5-10	14.2
		Hurthle cell adenoma	1 (20.0%)			
		Papillary thyroid carcinoma	2 (40.0%)			
II	65	Hashimoto thyroiditis	30 (46.1%)	7.6[b]	0-3	9.2
		Nodular colloid goitre	18 (27.6%)			
		Adenomatous nodule	11 (16.9%)			
		NIFTP	1 (1.5)			
		Papillary thyroid carcinoma	5 (7.6%)			
III	2	NIFTP	1 (50.0%)	0	10-30	50.0
		Adenomatous nodule	1 (50.0%)			
IV	15	Hashimoto thyroiditis	2 (13.3%)	13.3[b]	25-40	66.6
		Adenomatous nodule	3 (20%)			
		Follicular adenoma	6 (40%)			
		NIFTP	1 (6.6%)			
		Follicular carcinoma	1 (6.6%)			
		WDT-UMP	1 (6.6%)			
		Papillary microcarcinoma	1 (6.6%)			
V	9	Adenomatous nodule	1 (11.1%)	77.7[b]	50-75	88.8
		Hurthle cell nodule	1 (11.1%)			
		Papillary thyroid carcinoma	6 (66.6%)			
		Medullary carcinoma	1 (11.1%)			
VI	3	Papillary carcinoma and neuroendocrine tumour, Diffuse large B cell lymphoma Anaplastic carcinoma	1 (33.3%) 1 (33.3%) 1 (33.3%)	100[b]	97-99	100

Abbreviations: NIFTP, non-invasive follicular neoplasm with papillary-like nuclear features; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology; WDT-UMP, well differentiated thyroid tumour of uncertain malignant potential.

[a]ROM was calculated using the total number of cases that were assigned category I on cytology.; [b]ROM was calculated using the number of cases that had surgical follow up. RON- Risk of neoplasia.

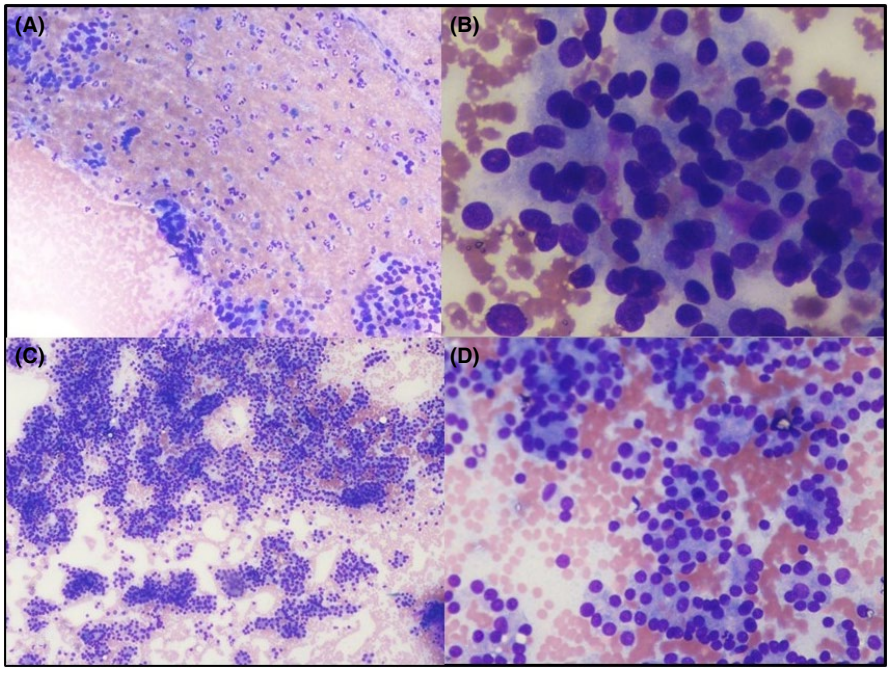


FIGURE 1 Atypia of undetermined significance (category III)-architectural atypia with cells entrapped in blood clot. (MGG,10X) Fig. 1.b. Microfollicles with minimal overlapping (MGG,40X) Fig. 1. c. Follicular neoplasm - a highly cellular aspirate of uniform follicular cells in crowded clusters and microfollicles (MGG 10X) Fig. 1. d Follicular cells show slight size variation and open chromatin (MGG 40X)

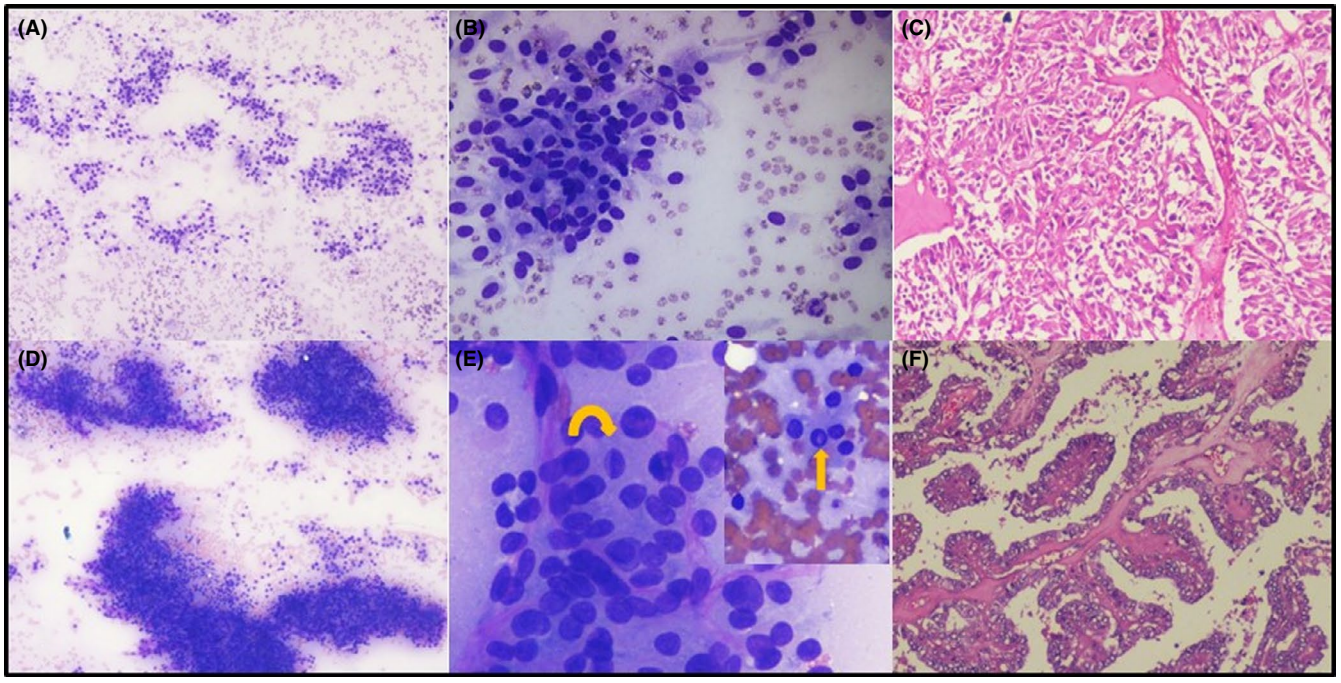


FIGURE 2 (A-C) Medullary carcinoma: (A) Cellular aspirate with crowded groups (MGG, 10×); (B) Plasmacytoid cells with oval nuclei and granular chromatin (MGG, 40×); (C) Lobules of neoplastic cells separated by amyloid (H&E, 10×). (D, E) Papillary thyroid carcinoma: (D) Papillary fragments (MGG, 10×); (E) Cellular overlapping, nuclear grooves (arrow) and nuclear inclusions (arrow in inset). (F) Papillae with fibrovascular core lined by neoplastic cells (H&E, 10×)

TABLE 3 Correlation of TIRADS score with cytology and histology and calculated ROM (n = 90)

	TBSRTC category						Histopathology				
ACR TIRADS score	I	II	III	IV	V	VI	Benign	Malignant	ROM in the study%	Recommended ROM%	RON%
1. (n = 9, 10.0%)		9					9	0	0	0	0
2. (n = 31, 34.4%)	1	26	0	3	1	0	29	2	6.6	<2	16.12
3. (n = 30, 33.3%)	1	20	0	7	2	0	27	3	10	5	20
4. (n = 15, 16.7%)	1	5	2	3	3	1	7	8	53.3	5-20	73.3
5. (n = 5, 5.6%)	1	0	0	0	2	2	0	5	100	>20	100
Total	4	60	2	13	8	3	72	18			27

Abbreviations: ACR, American College of Radiology; ROM, risk of malignancy; TIRADS, thyroid imaging reporting and data system; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology.

TABLE 4 Comparison of management guidelines by TBSRTC and management followed in the study

Diagnostic categories	No of cases	Management recommendations by Bethesda	Management in the study cases
I	21	Repeat FNA under guidance	Repeat FNA in 4 cases (19%) Surgery in 5 cases.
II	259	Clinical and sonographic follow-up	Clinical and sonographic follow-up in 78 cases. Hemithyroidectomy in 22 and total thyroidectomy in 43 cases.
III	9	Repeat FNA, molecular testing, or lobectomy	Lobectomy in 2 cases. Repeat FNA in none of these cases.
IV	25	Molecular testing, lobectomy	Hemithyroidectomy in 7 cases and total thyroidectomy in 8 cases.
V	15	Near-total thyroidectomy or lobectomy	Total thyroidectomy in 9 cases.
VI	8	Near-total thyroidectomy or lobectomy	Total thyroidectomy in 3 cases.

Abbreviations: FNA, fine needle aspiration; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology.

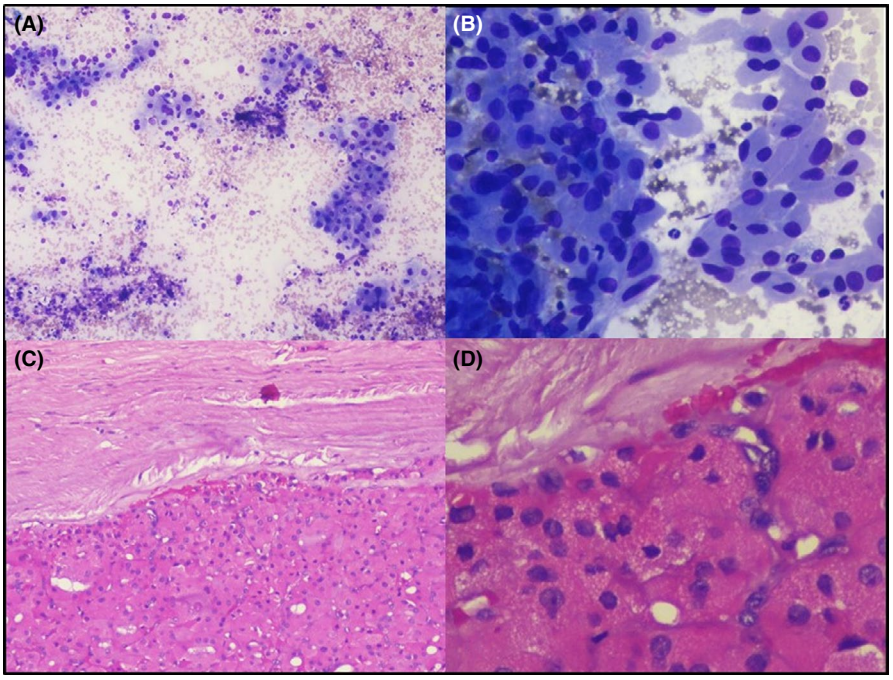


FIGURE 3 Follicular neoplasm, Hurthle cell type. (A) Cellular aspirate, crowded groups and isolated cells (MGG, 10×). (B) Exclusively Hurthle cells with marked anisonucleosis (MGG 40×). (C, D) Hurthle cell adenoma with a thick capsule (H&E, 10× and 40×)

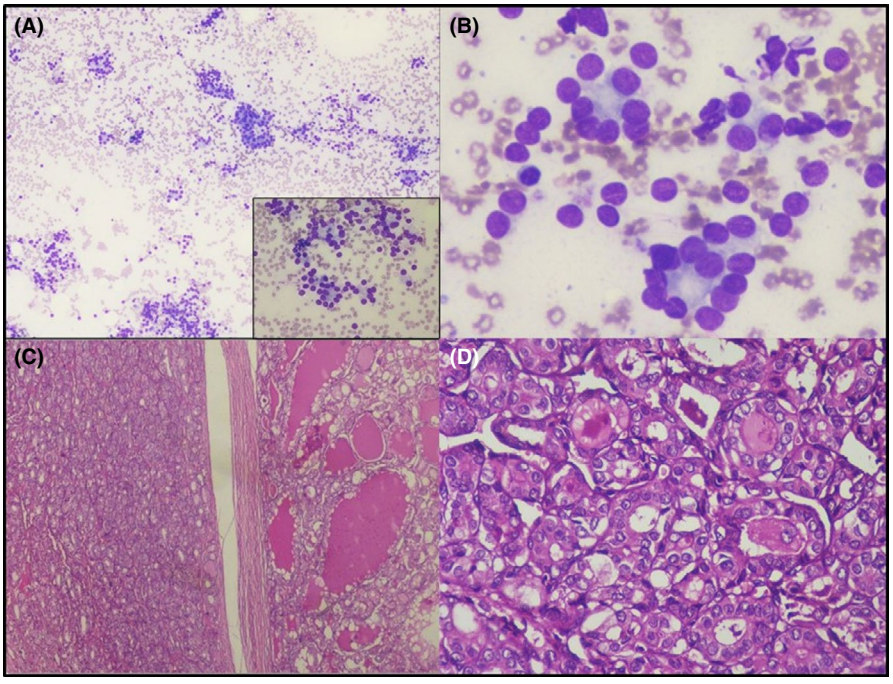


FIGURE 4 Non-invasive follicular neoplasm with papillary like nuclear features. (A) Cells arranged in repetitive microfollicular pattern (MGG, 10×). (B) Cells with nucleomegaly and occasional nuclear grooves (MGG, 40×). (C) Encapsulated nodule with no capsular invasion (H&E, 10×). (D) Closely packed follicles lined by cells exhibiting optically clear nuclei and grooves (MGG, 40×)

cytology smears of cases with a false-positive diagnosis on FNAC, in 1 case the presence of numerous aggregates of Hurthle cells exhibiting atypia was overestimated as features suspicious for malignancy. The cytological and histopathology images are shown in Figure 3. In the other case, hyperplastic changes with cells exhibiting occasional nuclear grooves, and follicular cells entrapped in blood clots with pale chromatin, were misinterpreted as features of malignancy.

The cytological features of WDT-UMP included repetitive microfollicular pattern, mild nuclear enlargement, occasional nuclear grooves, and no pseudoinclusions.

NIFTP constituted 3% of surgically resected thyroid lesions. The histopathology slides of the NIFTP cases were reviewed for the presence of the diagnostic criteria, which include complete encapsulation, follicular growth pattern, absence of invasion in extensive sampling, and optically clear nuclei as in Figure 4.

4 | DISCUSSION

The foremost advantage of TBSRTC is maintaining uniformity in the interpretation of thyroid FNA and guiding the clinicians to plan

TABLE 5 Comparison of the distribution of the diagnostic categories of the present report with other studies

Study, Country	Anand India	Thewzitcha Thailand	Zhu China	Wu USA	Sarkis Australia	Bongiovanni Europe	Present study
Number of FNAC (cases with surgery)	646 (100)	2735 (188)	2781 (1122)	25 445 (6362)	2076 (425)	3724 (1358)	337 (99)
Categories % (ROM %)							
I	13.8 (0)	21.1 (20)	14.8 (57.1)	12.9 (16.8)	12.8 (4.2)	3 (32)	6.2 (9.5)
II	75.9 (8.5)	66.6 (4.2)	17.1 (38.9)	59.3 (3.7)	74.7 (0.26)	55.4 (2.5)	76.9 (7.6)
III	1.2 (66.7)	4.7 (9.4)	15.8 (50)	9.6 (15.9)	4.7 (9.3)	6.7 (14.4)	2.6 (0)
IV	3.7 (63.6)	2.4 (23.5)	2.3 (31)	10.1 (26.1)	4.7 (15.3)	23.8 (32.1)	7.4 (13.3)
V	2.6 (100)	1.8 (57.1)	11.6 (78)	2.7 (75.2)	0.8 (79)	6.0 (74.9)	4.5 (77.7)
VI	2.8 (100)	3.3 (90.3)	38.5 (99)	5.4 (98.6)	2.3 (100)	5.1 (99.9)	2.4 (100)
Surgery	15.4%	9%	40.3%	25%	20.4%	36.5%	29.3%

Abbreviations: FNAC, fine needle aspiration cytology; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology.

appropriate therapy. The preoperative diagnosis of thyroid lesions in the present study using TBSRTC was comparable with other studies as summarised in Table 5.[10-14] The ND/UNS category still challenges accurate diagnosis. Despite the different expertise of cytologists in obtaining aspirates in a teaching hospital, the fraction of the ND/UNS category was well within the limits proposed by TBSRTC and other Indian[10,15] and Western studies.[13,16] This is in part due to the departmental protocol for rapid on-site evaluation (ROSE) in all cases of FNAC to assess for adequacy.

Benign lesions constituted the vast majority of FNA interpretation, which is similar to most other Asian studies[10,11] except a single study in the Chinese population,[8] where category VI was the most frequent interpretation. In Western studies, the reported proportion of the benign category ranges from 59% to 74.7%.[13,16] Regional variation may explain the difference in the distribution for this category.

The current study reports a lower rate of the AUS/FLUS category (2.6%), which is well within the preferred limit of 7% recommended by TBSRTC[4] and comparable to most Asian literature,[10,11,17,18] while some Western studies[9,12] have observed a higher percentage. The lower rate of this category could be attributed to the routine practice of obtaining an intradepartmental second opinion for all the indeterminate cases before assigning a category and hence the reasonable use of the AUS category.

As per the existing literature, the proportion of cases undergoing surgery varies among different institutions and ranges from 11.8% to 45.1%.[19,20] The malignancy rate of 19.2% in the present study was significantly lower when compared to the meta-analysis data by Bongiovanni et al (33.8%)[21] and Dawish et al (33.6%).[22,23] However, this malignancy rate does not reflect the true incidence in the population, because quite a few cases of thyroid malignancy were reported on histopathology and frozen section and did not have a cytological diagnosis before resection. Hence these cases were not included in the study. These resections were decided based on the clinical and sonographic findings.

4.1 | Risk of malignancy in each category

The risk of malignancy was comparable to that proposed by TBSRTC and other published literature for most of the categories except for ND/UNS and FN/SFN.[24] In the present study, the ROM in the ND/UNS category was 9.5%. Inadequate samples as the most common cause for false-negative diagnosis in FNA have been reported by Bakhos et al.[25] The risk of malignancy was calculated assuming that the unresected cases were benign. Hence the ROM across various studies ranges from 0% to 63%.[26,27] This increased risk of reported malignancy emphasises the importance of reaspirating all ND/UNS category lesions for cytology under guidance to rule out malignancy. Another pitfall that results in false-negative interpretation is the exclusive presence of cystic macrophages without cellular fragments, which could represent a cystic variant of PTC. In our study, there were two cases of PTC. The actual ROM in the AUS category is difficult to determine since only a subset of patients with clinical suspicion undergo surgical resection, thus resulting in selection bias. There were no malignancies in the AUS category with histological follow-up in the present data while other Asian studies report a higher ROM.[10-12,15] This could be explained by the fact that most of the cases showed architectural atypia rather than cytological atypia. Cytological atypia have been reported to be associated with a higher ROM as per the available evidence.[4] In cases of AUS, a repeat aspiration under guidance and correlation with clinical and radiological findings would lower the proportion of cases reported as AUS. The prevalence of NIFTP varies across the population and is reported to be between 5% and 13%.[28-30] In the present study, there was a much lower incidence at 3%. The cases of NIFTP showed a wide cytological spectrum spanning categories II to IV. However, the presence of diffuse cytological features of PTC and the absence of papillary fragments should prompt surgical resection to rule out the possibility of NIFTP.

In the recent years, various TIRADS systems have emerged and have been in use to stratify thyroid nodules as benign or malignant

based on ultrasound features and to decide on FNAC. The Kwak system is a simple classification based on suspicious ultrasound features. However, importance is not given to independent risk factors, for example extrathyroidal extension. Also, microcalcification, presence of solid area, and hypoechogenecity are given equal scores, whereas microcalcification generally has a higher malignancy risk.[31] ACR TIRADS is more complicated than other risk-stratification systems. Another drawback to this system is that malignant nodules exhibiting mixed echo patterns are given a lower score. Yet ACR TIRADS is an objective and comprehensive method for the evaluation of the thyroid nodules. The EU TIRADS is simpler than ACR TIRADS, and recommends FNA for mildly hypoechoeic nodules measuring greater than 1.5 cm.[8] However, it does not include solidity as an independent risk factor. The pitfall of the ATA guidelines is the classification of suspicious ultrasound features that have a variety of clinical significance into the same risk stratification with no independent categorisation.[8] In the present study ACR TIRADS was used for risk stratification. The sensitivity of ACR TIRADS observed in the current study is similar to that of other published studies.[6,32,33]

A similar study carried out by Fernandez et al has reported the percentage of malignancy as 77% in moderately and highly suspicious categories (TIRADS score ≥ 4) versus 65% in the current study.[32] ROM values for TIRADS scores except for score 2 are in consensus with most other published articles except for score 3, where a higher ROM (10%) was observed. Even though some malignant nodules were mislabelled as benign and some benign nodules were mistaken for malignant, the overall sensitivity was better.

While exploring the causes for a false-positive diagnosis in cytology, it was observed that cellular smears with a predominance of Hurthle cells exhibiting atypia, and the presence of focal nuclear features of PTC in the absence of nuclear inclusions, were overdiagnosed as features of malignancy. On the other hand, cases with diffuse cytological atypia even with focally observed pseudoinclusions are worth considering as suspicious for malignancy. Analysing the causes for the false negatives in FNA, papillary microcarcinoma being missed by sampling error in FNA has been reported previously. Under these circumstances, USG-guided FNA would have been useful.

4.2 | Adherence to TBSRTC recommendations for management

There exists a heterogeneity in approaches to the management of thyroid nodules despite the recently published guidelines for clinical management. TBSRTC recommends reaspiration under USG guidance as the preferred method for evaluation of ND aspirates unless the nodule is purely cystic. The majority of the ND/UNS lesions in the data consisted only of cystic macrophages, reflecting the lower rate of repeat aspiration in the present study.

The management decision for category II is determined predominantly by radiological findings, since there is a possibility of sampling error with FNA in larger nodules that can hinder detection of the underlying malignancy. However, a vast subset of patients undergo thyroidectomy for cosmetic reasons/pressure symptoms rather than for suspected malignancy.

The treatment for category III and IV lesions is controversial. While surgery is advised for category IV lesions, close follow-up with repeat FNA, and molecular analysis for BRAF and RAS gene mutations are recommended for the AUS category.[4] Chirayath et al[34] in their study in the Indian population have found, however, that mutational analysis for BRAF as an indicator of malignancy in AUS lesions has limited utility, in line with the results of many other studies.[35,36] Even though there was no ROM for the AUS category in the present study, in a country where molecular tests are not very feasible, a multidisciplinary approach based on clinical and radiological findings, together with patient preference, should guide the management.

Surgery as the treatment modality for category III and IV lesions was seen in 22% and 60% of cases, respectively. The resection rate in indeterminate categories is much lower than that reported by Western studies (22% in the current study vs 57.6%).[37] The lower rate could be explained by the loss of follow-up of these patients. Or et al[38] in their study have reported that for cases with indeterminate cytology, 77.4% of clinicians opted for repeat FNA and in case of repeat FNA being indeterminate, 50% opted for surgery.

The authors acknowledge the limitations of this study which include the retrospective design and a small proportion of cases with surgical follow up.

5 | HIGHLIGHTS

The reporting of AUS/FLUS lesions with a supplementary note describing the presence of architectural and/or cytological atypia may be helpful in treatment decisions, since cytological atypia could suggest the possibility of NIFTP or malignancy that would require surgical excision.

In the current study, it was found that there is higher interobserver variability in interpreting Hurthle cell atypia. There is a higher chance for misinterpretation as suspicious for malignancy . A further study analysing the various cytological parameters and cytohistological correlation of Hurthle cell lesions might help in achieving a more accurate cytological diagnosis and lower the rate of false positives in FNAC. The TIRADS score in conjunction with TBSRTC cytological diagnosis can aid in better stratification of thyroid nodules and aid in decision making.

6 | CONCLUSION

This study substantiates the usefulness of TBSRTC in thyroid cytology in arriving at a more precise diagnosis and guiding clinicians on management. The study reports a lower proportion of the AUS category. Hurthle cell lesions with atypia were the common cause

for misdiagnosis observed in the current study. The presence of nuclear features of papillary carcinoma with the presence of numerous microfollicles in the aspirate should prompt the diagnosis of NIFTP. Periodic cytohistological correlation and further studies on Hurthle cell lesions can aid in improving the sensitivity and specificity of FNA. In institutions where TBSRTC in reporting is practised, the clinicians should be aware of the implied risk of malignancy in each category. There was a good correlation between TIRADS, TBSRTC, and histology especially in benign nodules. However, further replication studies adapting a prospective design with a larger sample size are essential to validate the utility of thyroid ultrasound especially for indeterminate nodules.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Sakthisankari S: Conceptualisation, data curation, formal analysis, software, and writing (original draft, review, and editing). Vidhyalakshmi S: Conceptualisation, methodology, analysis, project administration, and writing (review and editing). Shanthakumari S: Conceptualisation, analysis, writing (review and editing). Devanand B: Methodology, resources, analysis, writing (review and editing). Nagul U: Methodology, analysis.

ETHICS APPROVAL AND PATIENT CONSENT STATEMENT

This retrospective study has been approved by Institutional Human Ethics Committee. Patient consent has been waived off.

DATA AVAILABILITY STATEMENT

Data has not been shared and is not available in the repository.

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REFERENCES

1. Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. *Am J Clin Pathol*. 2009;132:658-665.
2. Cibas ES. Fine-needle aspiration in the work-up of thyroid nodules. *Otolaryngol Clin North Am*. 2010;43:257-271.
3. National Centre for Disease Informatics and Research, National Cancer Registry Programme Indian Council of Medical Research. *Three-year report of population-based cancer registries 2012-2014*. Bengaluru, India: NCDIR-NCRP; 2016.
4. Ali SZ, Cibas ES. *The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria and Explanatory Notes*. Berlin, Germany: Springer; 2010.
5. Valderrabano P, McIver B. Evaluation and management of indeterminate thyroid nodules: the revolution of risk stratification beyond cytological diagnosis. *Cancer Control*. 2017;24(5):e107327481772923.
6. Jabar ASS, Koteshwara P, Andrade J. Diagnostic reliability of the Thyroid Imaging Reporting and Data System (TI-RADS) in routine practice. *Pol J Radiol*. 2019;84:e274-e280.
7. Barbosa TLM, Junior COM, Graf H, et al. ACR TI-RADS and ATA US scores are helpful for the management of thyroid nodules with indeterminate cytology. *BMC Endocr Disord*. 2019;19(1):112.
8. Shen Y, Liu M, He J, et al. Comparison of different risk-stratification systems for the diagnosis of benign and malignant thyroid nodules. *Front Oncol*. 2019;9:378.
9. Kakudo K, Bychkov A, Bai Y, Li Y, Liu Z, Jung CK. The new 4th edition World Health Organization classification for thyroid tumors, Asian perspectives. *Pathol Int*. 2018;68:641-664.
10. Anand B, Ramdas A, Ambroise MM, Kumar NP. The Bethesda system for reporting thyroid cytopathology: a cytohistological study. *J Thyroid Res*. 2020;2020:e8095378.
11. Thewjitcharoen Y, Butadej S, Nakasatien S, et al. Incidence and malignancy rates classified by The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) - An 8-year tertiary center experience in Thailand. *J Clin Transl Endocrinol*. 2018;16:e100175.
12. Zhu Y, Song Y, Xu G, Fan Z, Ren W. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC): a report of 2,781 cases in a Chinese population. *Chin J Cancer Res*. 2020;32(2):140-148.
13. Wu HH, Rose C, Elsheikh TM. The Bethesda System for Reporting Thyroid Cytopathology: an experience of 1,382 cases in a community practice setting with the implication for risk of neoplasm and risk of malignancy. *Diagn Cytopathol*. 2012;40:399-403.
14. Sarkis LM, Norlen O, Aniss A, et al. The Australian experience with the Bethesda classification system for thyroid fine needle aspiration biopsies. *Pathology*. 2014;46:592-595.
15. Mondal SK, Sinha S, Basak B, Roy DN, Sinha SK. The Bethesda system for reporting thyroid fine needle aspirates: a cytologic study with histologic follow-up. *J Cytol*. 2013;30:94-99.
16. Guo A, Kaminoh Y, Forward T, Schwartz FL, Jenkinson S. Fine needle aspiration of thyroid nodules using the Bethesda System for Reporting Thyroid Cytopathology: an institutional experience in a rural setting. *Int J Endocrinol*. 2017;2017:e9601735.
17. Mehra P, Verma AK. Thyroid cytopathology reporting by the Bethesda system: a two-year prospective study in an academic institution. *Patholog Res Int*. 2015;2015:e240505.
18. Kapila K, Qadan L, Ali RH, Jaragh M, George SS, Haji BE. The Bethesda system for reporting thyroid fine-needle aspiration cytology: a Kuwaiti experience - a cytohistopathological study of 374 cases. *Acta Cytol*. 2015;59(2):133-138.
19. Theoharis CG, Schofield KM, Hammers L, Udelsman R, Chhieng DC. The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. *Thyroid*. 2009;19:1215-1223.
20. Bohacek L, Milas M, Mitchell J, Siperstein A, Berber E. Diagnostic accuracy of surgeon-performed ultrasound-guided fine-needle aspiration of thyroid nodules. *Ann Surg Oncol*. 2012;19:45-51.
21. Bongiovanni M, Crippa S, Baloch Z, et al. Comparison of 5-tiered and 6-tiered diagnostic systems for the reporting of thyroid cytopathology: a multi-institutional study. *Cancer Cytopathol*. 2012;120(2):117-125.
22. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. *Acta Cytol*. 2012;56:333-339.
23. Al Dawish MA, Robert AA, Muna A, et al. Bethesda System for Reporting Thyroid Cytopathology: a three-year study at a tertiary care referral center in Saudi Arabia. *World J Clin Oncol*. 2017;8(2):151-157.
24. Agarwal S, Jain D. Thyroid cytology in India: contemporary review and meta-analysis. *J Pathol Transl Med*. 2017;51(6):533-547.

25. Bakhos R, Selvaggi SM, DeJong S, et al. Fine-needle aspiration of the thyroid: rate and causes of cytohistopathologic discordance. *Diagn Cytopathol*. 2000;23(4):233-237.
26. Ke J, Jianyong L, Ying L, et al. The use of The Bethesda System for Reporting Thyroid Cytopathology in a Chinese population: an analysis of 13 351 specimens. *Diagn Cytopathol*. 2019;47(9):876-880.
27. Park JH, Yoon SO, Son EJ, Kim HM, Nahm JH, Hong S. Incidence and malignancy rates of diagnoses in the Bethesda system for reporting thyroid aspiration cytology: an institutional experience. *Korean J Pathol*. 2014;48(2):133-139.
28. Lau RP, Paulsen JD, Brandler TC, Liu CZ, Simsir A, Zhou F. Impact of the Reclassification of "noninvasive encapsulated follicular variant of papillary thyroid carcinoma" to "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" on the Bethesda System for Reporting Thyroid Cytopathology: a large academic institution's experience. *Am J Clin Pathol*. 2017;149:50-54.
29. Maletta F, Massa F, Torregrossa L, et al. Cytological features of "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" and their correlation with tumor histology. *Hum Pathol*. 2016;54:134-142.
30. Paniza ACJ, Mendes TB, Viana MDB, et al. Revised criteria for diagnosis of NIFTP reveals a better correlation with tumor biological behavior. *Endocr Connect*. 2019;8(11):1529-1538.
31. Kwak JY, Han KH, Yoon JH, et al. Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. *Radiology*. 2011;260(3):892-899.
32. García-Moncó Fernández C, Serrano-Moreno C, Donnay-Candil S, Carrero-Alvaro J. Acorrelation study between histological results and thyroid ultrasound findings. The TI-RADS classification. *Endocrinol Diabetes Nutr*. 2018;65(4):206-212.
33. Periakaruppan G, Seshadri KG, Vignesh Krishna GM, Mandava R, Sai VPM, Rajendiran S. Correlation between ultrasound based TIRADS and Bethesda System for Reporting Thyroid-cytopathology: 2-year experience at a tertiary care center in India. *Indian J Endocrinol Metab*. 2018;22:651-655.
34. Chirayath SR, Pavithran PV, Abraham N, et al. Prospective study of Bethesda categories III and IV thyroid nodules: outcomes and predictive value of BRAFV600E mutation. *Indian J Endocrinol Metab*. 2019;23:278-281.
35. Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan MG. BRAF V600E mutation in papillary thyroid carcinoma: significant association with node metastases and extra thyroidal invasion. *Endocr Pathol*. 2012;23:83-93.
36. Nair CG, Babu M, Biswas L, et al. Lack of association of B-type Raf kinase V600E mutation with high-risk tumor features and adverse outcome in conventional and follicular variants of papillary thyroid carcinoma. *Indian J Endocrinol Metab*. 2017;21:329-333.
37. Vuong HG, Ngo HTT, Bychkov A, et al. Differences in surgical resection rate and risk of malignancy in thyroid cytopathology practice between Western and Asian countries: a systematic review and meta-analysis. *Cancer Cytopathol*. 2020;128(4):238-249.
38. Or K, Benbassat C, Koren S, et al. Adherence to ATA 2015 guidelines in the management of unifocal non-invasive papillary thyroid cancer: a clinical survey among endocrinologists and surgeons. *Eur Arch Otorhinolaryngol*. 2018;275(11):2851-2859.

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Evaluation of selective laser sintered polyamide/hydroxyapatite composite compositions –*in vitro* and *in vivo*

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Abstract

Objective: To study the suitability of the developed polyamide/hydroxyapatite (PA/HA) composite orthopaedic scaffold in *vitro* and *in vivo*.

Methods: The fabricated scaffolds were seeded with human osteoblast-like cell line (MG 63) and tested for its viability & cytotoxicity, proliferation and calcium mineralization through MTT assay, ALP assay and Alizarin red S staining respectively. Finally, the scaffolds were implanted in non-immunocompromised male wistar rats subcutaneously and pathological tests were carried out.

Results: The results of *in vitro* studies indicate that PA/HA (90:10) composite was non-cytotoxic and supported calcium mineralization relatively better than the other compositions of PA/HA. From the Hematoxylin & Eosin staining, inflammation was observed in the tissues surrounding the scaffold 7 weeks post implantation in rats. However, the scaffolds were found to be incorporated into the subcutaneous tissue at the end of one year without any rejection.

Conclusion: PA/HA (90:10) composite scaffold fabricated using SLS is biocompatible when tested in rats, and could more likely be used for orthopedic applications.

Keywords: Scaffold, orthopedic, biocompatibility, MG 63, rat model.

1. Introduction

Recent reports suggest that among the total injuries due to accidents, 11.5% are bone fractures [18]. Besides fractures, bone replacement is also essential for patients with congenital disorders, tumor resection, and those in a revision surgery to replace the infected implant. This signifies the need for a material that is mechanically strong and biologically compatible to be a good orthopedic scaffold.

Pure HA is a bioactive ceramic known for its osteoinductive property. However, its brittle nature makes it unsuitable to repair the bone defect by itself. Hence, HA is used along with PA. This polymer/ceramic composite yields greater strength [14].

PA and HA are being used as bone scaffolds for many years. Conventional techniques such as freeze-drying and cross-linking [15], thermally induced phase separation [16] and injection-molding technique [17] were used to develop PA/HA scaffolds.

However, Selective laser sintering, a rapid prototyping technique helps in customized development of 3D porous PA/HA composite scaffolds with 100% pore interconnectivity. Here we present the *in vitro* and *in vivo* performance of the 3D porous scaffold of different compositions of PA and HA fabricated using SLS.

PA 66 was used by many researchers [7,8,11,14] for developing the bone scaffold. But in this study, PA 12 is employed. Though PA12 is cost ineffective compared to

PA66, it has the advantage of resistance to corrosion, cracking and chemical degradation [19, 20].

PA/HA scaffolds fabricated using Selective Laser Sintering, an additive manufacturing technique described elsewhere [5] is used in this study.

The aim of this work is to analyze the *in vitro* and *in vivo* performance of the 3D porous scaffold fabricated with different compositions of PA and HA fabricated using SLS, for its use as an orthopedic scaffold.

2. Methods

2.1. Polyamide/hydroxyapatite scaffold

PA 12 was purchased from Duraform 3D systems, and HA was synthesized chemically as per the protocol specified by Sneha *et al* [9]. PA and PA/HA composite scaffolds were fabricated in Selective Laser Sintering machine (Sinterstation DTM 2500 *plus*). The scaffolds have cubical pores of size 800 µm contributing for 70% porosity. 3D porous scaffolds of cubical shape (0.9 x 0.9 x 0.4 cu.mm.) and cylindrical shape (0.9 mm diameter and 0.4 mm thickness) were used for *in vitro* and *in vivo* studies respectively. The images of the fabricated scaffolds are presented in Figure 1.

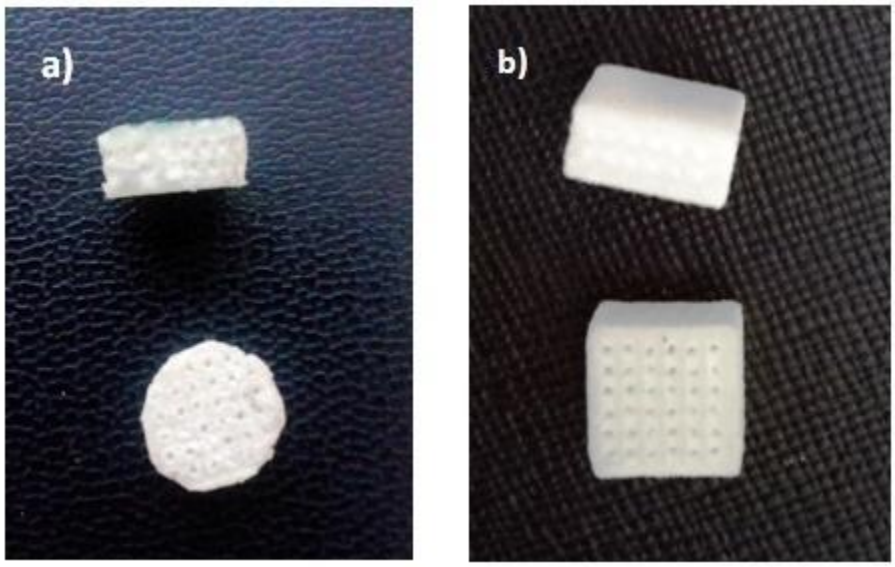


Figure 1: Three dimensional porous scaffolds a) cylindrical shape with 0.9 mm diameter and 0.4 mm height used for *in vivo* testing; b) cubical shape with dimensions 0.9x0.9x0.4 cu.mm used for *in vitro* testing

2.2. Cell culture

The human osteosarcoma cell line, MG-63, (NCCS Pune, India) was cultured in DMEM medium (AL007S, HiMedia, India) supplemented with 200mM L-glutamine, 10% Fetal bovine serum, and 1X of Antibiotic Antimycotic Solution (A002, Himedia, India). The cells were incubated with 5% CO_2 at 37°C. On attaining 80-90% confluency, the cells were trypsinized with trypsin-EDTA. Unless specified, the chemicals were procured from HiMedia, India.

2.3. Cell viability and proliferation studies

MTT assay was employed to assess the cell viability and proliferation of the cells on interacting with the scaffold material. This is a colorimetric assay measuring the reduction of yellow 3-(4, 5-dimethythiazol- 2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) substrate to an insoluble purple formazan product by mitochondrial succinate dehydrogenase enzyme. The MTT enters the cells and passes into the mitochondria where it gets reduced to an insoluble, dark purple colored formazan product. The intensity of the purple color is proportional to the number of live cells.

Each well containing the UV sterilized scaffold was seeded with 1000 µL of medium containing approximately $1x10^4$ cells. The plates were incubated at 37^oC with 5% CO_2. MG 63 cells cultured without any scaffold was used as the control. After 1, 3 and 5 days of incubation, 5mg per ml of freshly prepared MTT (HiMedia, India) solution was added to each well and incubated for 3.5 h at 37^oC with 5% CO_2. After the incubation time, the above solution was discarded and the scaffolds were air dried. The insoluble purple colored formazan crystals formed were solubilized by adding 200 µl of Dimethyl sulphoxide (SD Fine Chemicals). The plate was left in shaking condition for 10-15 min in dark. The solutions from 48-well plate were then transferred to 96-well plate and, the absorbance was read at 570 nm in a multiwell plate reader (Thermo Multiscan Labsystems, Model 352). The absorbance values were plotted in graph using MS Excel.

2.4. Alkaline Phosphatase assay

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from nucleotides, proteins, and alkaloids. Higher levels of ALP activity is observed during the active bone formation, as it is a by-product of the osteoblast activity and, also observed in elevated levels in the cell membrane of undifferentiated pluripotent cells. The alkaline phosphatase assay was carried out using the p-nitrophenol pyrophosphate (N7653 Sigma Aldrich) as the substrate according to the protocol specified by the manufacturer. ALP catalyses the hydrolysis of the colorless ρ-Nitrophenyl phosphate (pNPP) to a colored ρ-Nitrophenol, that has a strong absorbance at 407 nm. The absorbance is a measure of the level of the enzyme activity.

Each well containing a UV sterilized scaffold was seeded with 1000 µL of medium containing approximately $1x10^4$ cells in a 48 well plate, and incubated for 4, 7 and 10 days at 37^{o}C with 5% CO_2. After the incubation, 200µL of p-nitrophenol pyrophosphate solution was added to each well. The plates were then incubated in dark for 30 minutes at room temperature. After 30 minutes, 50µL of 3M NaOH solution was added per 200 µL of the reaction mixture to stop the reaction. The absorbance was then read at 407 nm in a multiwell plate reader (Thermo Multiscan Labsystems, Model 352). Statistical analyses for MTT and ALP assays were carried out using paired t-test using MINITAB 15.0 software. $P<0.005$ were considered to be statistically significant.

2.5. Alizarin Red S staining

Alizarin Red S, an anthraquinone derivative, is used to identify calcium in tissue sections. The reaction is not strictly specific for calcium, since magnesium, manganese, barium, strontium, and iron may interfere. But as these elements usually do not occur in sufficient concentration in this experiment to interfere, the scaffolds were tested for calcium mineralization using Alizarin red S staining. Alizarin Red S powder (A5533 Sigma Aldrich) was used to prepare a fresh solution (pH 4.1-4.2) each time as per the protocol specified by Gregory *et al*, 2004 [3].

Each well containing a UV sterilized scaffold was seeded with 1000 µL of medium containing approximately $1x10^4$ cells in a 48 well plate, and was incubated for 25 and 28 days at 37^{o}C with 5% CO_2. After the incubation period, the media was discarded, and the wells were rinsed gently with 1 mL PBS. The cells were then fixed with 1.0 mL of 10% formalin in each well for 15 minutes at room temperature. Formalin solution was then removed and the wells were washed twice gently with double distilled water. To each well, 500 µL of 40 mM Alizarin red S solution was added and left at room temperature for 20 minutes with gentle shaking. After 20 minutes, the dye solution was removed and the wells were washed four times with 1.0 mL distilled water, replacing the water at each 5 minutes interval. The specimens were then air dried, and images were taken. Mineralized areas appear red in color.

2.6. In vivo Rat model studies

Eight male Wistar rats (*Rattus norvegicus*) each weighing 200-250 g were obtained from the Animal house, PSG Institute of Medical Sciences and Research, Coimbatore. The surgical procedure in the rats was approved by the Institutional Animal Ethics Committee, PSG Institute for Medical Sciences & Research, Registration No. 158/PO/ReBi/SL/99/ CPCSEA. The rats were randomly divided into two groups, each containing two rats as given in Table 1. Two rats per cage were housed in a temperature-controlled facility.

Table 1: Biocompatibility test by subcutaneous implantation of the scaffold in rats

Period/ scaffold material	Polyamide (PA 12)	PA/HA (90:10) composite
7 weeks	2 rats	2 rats
One year	2 rats	2 rats

2.7. Rat Surgical Procedure

Non-immuno compromised eight male Wistar rats were used for this study. Intraperitonial injections of Aneket ® Ketamine (30 mg/kg) and Astra Zeneca ® 2% xylocaine (13 mg/kg) were administered to anesthetize the animals (Figure 2a). The lateral aspect of the abdomen was shaved under aseptic precaution. Using a sterile surgical blade no. 15, an incision of about 3 cm was made (Figure 2b). Skin and subcutaneous tissue was dissected (Figure 2c) and the autoclaved scaffold of dimensions 0.9 mm diameter x 0.4 mm thickness was implanted in the subcutaneous plane (Figure 2d), as given in table 1. Upon implantation of the scaffold in the subcutaneous pouch, the skin was sutured using an Ethicon (5-0) Non-absorbable surgical suture NW 3316 (Jhonson & Jhonson) (Figure 2e). After four days the sutures were found to be eaten off by the rats exposing the scaffolds to outside. The closure was then made using the Glue, N-butyl-2-cyanoacrylate. The animals were fed with rat feed pellets, and the cages were changed every fortnight. Rat surgical procedure was performed according to Subramanian *et al* [12].

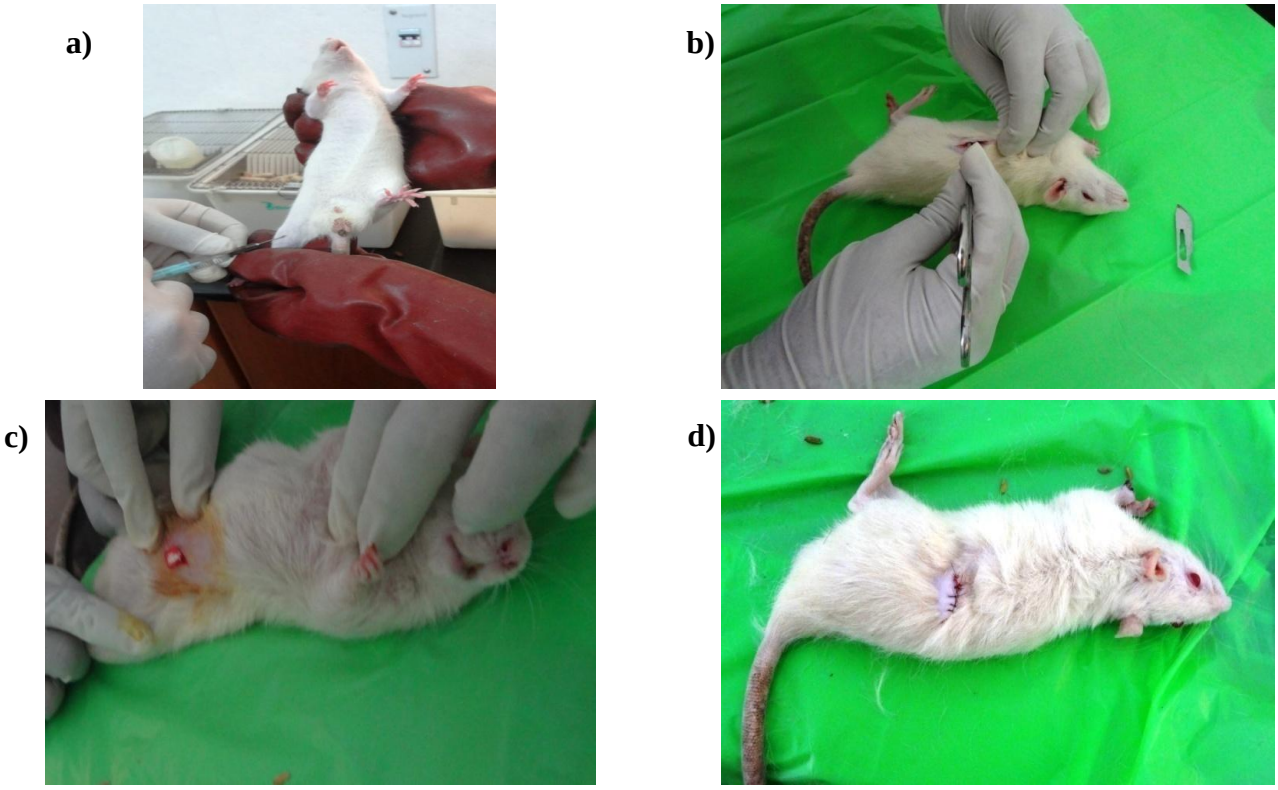


Figure 2. Rat surgical procedure. (a)administration of general anesthesia; (b) creating a subcutaneous pouch; (c)implanting the autoclaved scaffold; (d)sutured using Ethilon 5-0

2.8. Pathological testing

Post-implantation of the scaffold in the rat model, four rats (two in each group) after 7 weeks, and remaining four rats after one year were euthanized with over dose of diethyl ether. Using the previous scar site, incision was made and the scaffold along with the surrounding tissue was removed. The tissues were stored separately in 10% formalin for histopathological analysis.

2.9. Hematoxylin & eosin staining

The samples, with soft tissue cleaned, were stored in 10% formalin for 10 days. The samples were then rinsed in tap water, followed by fixing and dehydration in successive alcohol concentration. The samples were then cleared with xylene and embedded in paraffin wax. After hardening, the sample were cut into 4 µm sections perpendicular to the implants, under cooling water with microtome (Leica RM2255, Germany). The sections were glued onto a plastic support and finally stained with Harris Hematoxylin and Eosin.

3. Results and Discussion

Increasing trauma and injuries in day-to-day life demands orthopedic scaffolds with desirable and reproducible design, adequate strength and biocompatibility. Rapid prototyping is one such fabrication technique that facilitates development of customized scaffolds rather than standardized scaffolds, in a short time. Advantages of custom-made scaffolds of satisfactory size matching the defect area could easily be implanted at the defect site. Besides reducing the time for surgery, custom-made scaffold contributes to the rapid healing of the defects [6]. Selective Laser Sintering uses a high temperature of around 1100^{o}C for fabricating the components. However, the chemical composition of HA and PA were known to remain unmodified even at higher temperatures. Hence, variation in the scaffold properties due to the sintering stays ineffective.

Pores in the bone scaffold aids cellular infiltration and also facilitate the exchange of nutrients and wastes across the bone. The scaffold material becomes more desirable if it supports/enhances cell attachment, proliferation and calcium mineralization in addition to possessing adequate mechanical properties. HA/PA66 composite developed with 75-85% porosity and 500 micron pores yielded the compression strength of 10-20 MPa [14]. Bone screws fabricated using PA/HA without pores yielded bending strength of 200- 300MPa [11]. Porosity is directly proportional to the biological functions of the scaffold, and inversely proportional to the mechanical strength of the scaffold. Hence, fine tailoring of the porosity and pore size without compromising the mechanical property of the scaffold is crucial. The HA/PA12 composite scaffold used in the present study was fabricated with 70% porosity and 800 micron pores using Selective Laser Sintering, discussed

elsewhere [5], yielded a mechanical strength of 25 MPa, which was identified to be adequate to support a person weighing 75 kg.

3.1 In vitro tests

From the MTT assay result shown in Figure 3, it can be observed that there is a gradual increase in the cellular proliferation from day 1 to day 5 in all the scaffold types tested including the control. Incorporation of HA in the composite facilitated better cell attachment and proliferation compared to the polyamide as seen on day 1 and day 5, which could be due to the bioactive nature of the HA. However, it can also be observed that there is no significant difference between the PA/HA (95:5) and PA/HA (90:10) as the percentage increase in minimal. A similar trend was reported by Tang *et al* [13].

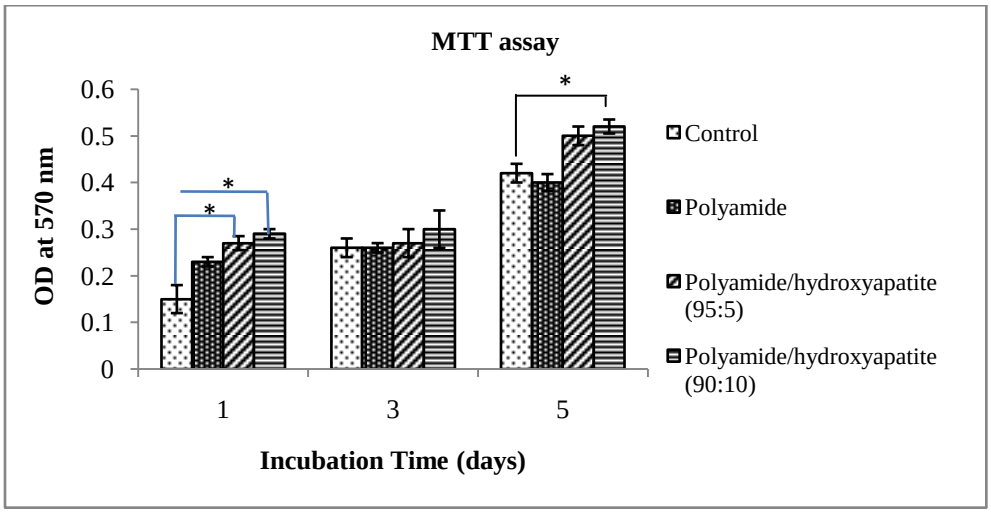


Figure 3: MTT assay results for MG 63 cells cultured on the scaffolds made of polyamide and various compositions of polyamide/Hydroxyapatite

ALP is an early differentiation marker for the osteoblasts. A general increase in the ALP activity is observed from day 4 through day 7 in all the scaffolds tested (Figure 4). ALP level in PA/HA (90:10) was observed to be lower than the control on day 4. However on day 10, the ALP levels of the cells cultured on the PA/HA (90:10) scaffolds were found to be on par with that of the control (cells cultured without scaffold).

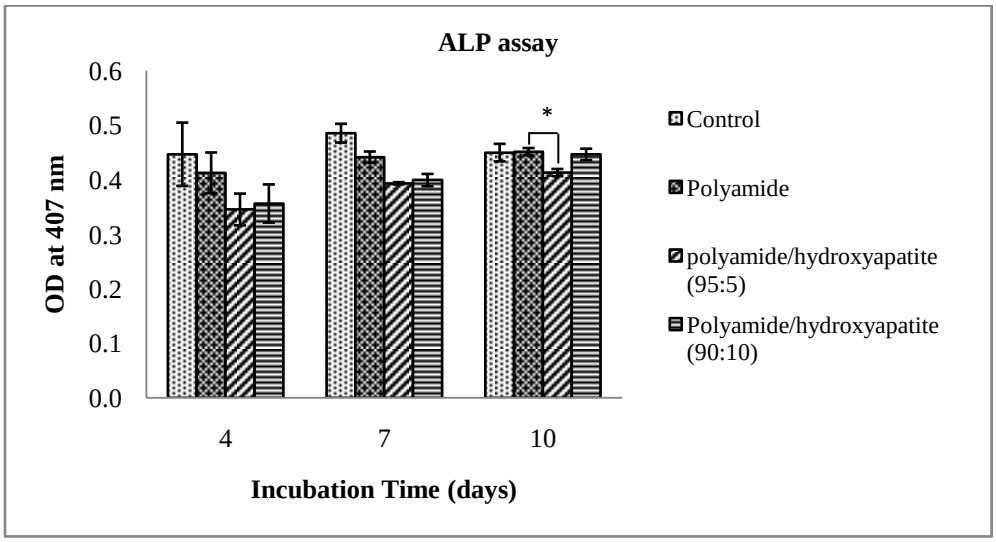


Figure 4: ALP assay results of MG 63 cells cultured on the scaffolds made of polyamide and different compositions of polyamide/hydroxyapatite

Alizarin Red S staining results (Figure 5) shows that the polyamide scaffolds were stained less intense than the scaffolds containing HA. These results suggest that though the polyamide was found to be non-cytotoxic and supporting ALP activity, it lacked the property to support calcium mineralization, which is the desirable criterion for a material to act as a bone scaffold.

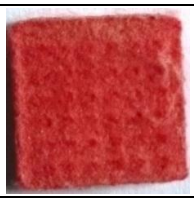
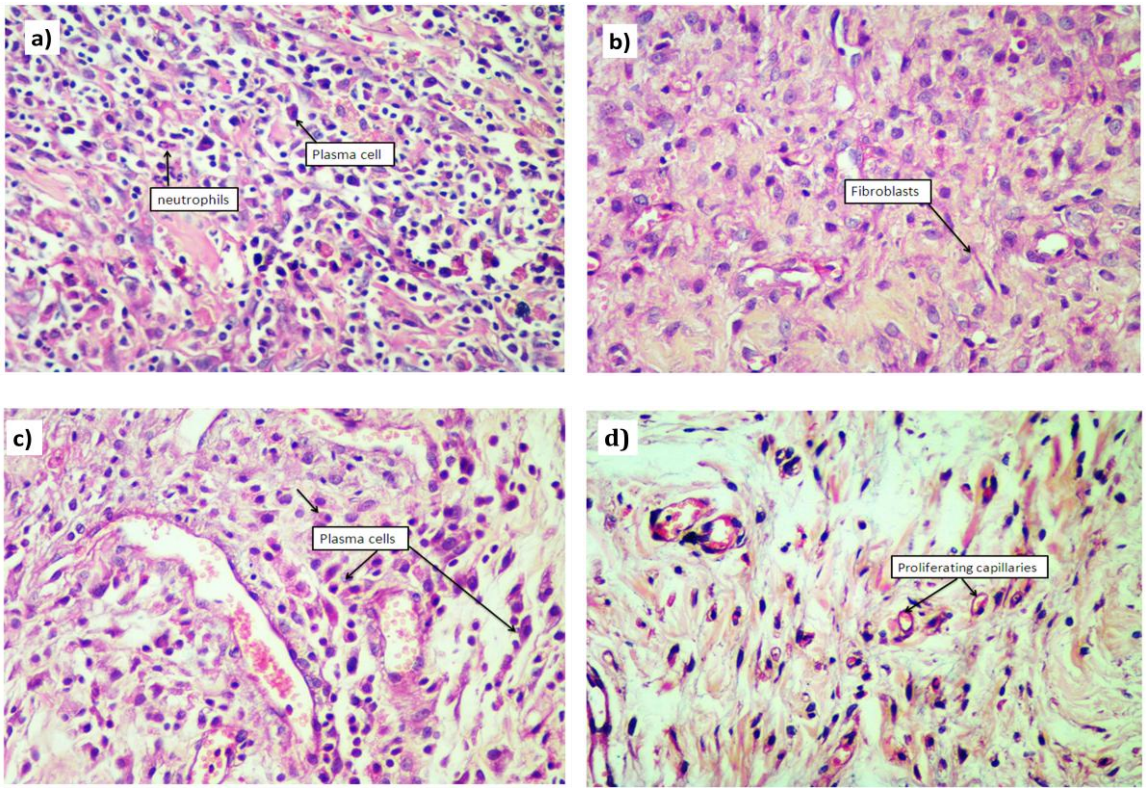
Component	Polyamide	Polyamide/Hydroxyapatite (95:5) composite	Polyamide/Hydroxyapatite (90:10) composite
Day 25			
Day 28			

Figure 5: Images of PA/HA (90:10), PA/HA (95:5) and polyamide scaffolds stained with Alizarin red S stain to identify the mineralized regions

3.2 In vivo study

There was significant difference in the *in vitro* assays observed between PA and PA/HA composite. However, the difference observed between PA/HA (95:5) and PA/HA (90:10) *in vitro* is minimal, as the percentage variation is lesser. Hence, PA and PA/HA (90:10) scaffolds were implanted subcutaneously in the rat models to analyze their performance *in vivo*. Immunological responses were observed after seven weeks and one year post-implantation in the rat models. Scaffold samples implanted in the rat models were stained with H&E after 7 weeks of implantation. Rat implanted with polyamide showed inflammatory response, whilst the H&E staining results of PA/HA (90:10) composite suggests that there is no inflammatory response, and the tissues were in the process of healing (Figure 6). Figure 6a and b are replicates for PA scaffold and, 6c and d are the replicates for the PA/HA (90:10) scaffold seven weeks post-implantation in rat. H&E staining of polyamide scaffold (Figure 6a and b) shows plasma cells, neutrophils, fibroblasts and scattered inflammatory infiltrate, while staining of polyamide/hydroxyapatite (90:10) scaffold. Figure 6c and 6d shows fibroblasts, scattered macrophages, plasma cells and occassional neutrophils. Proliferating vascular channels are also seen.



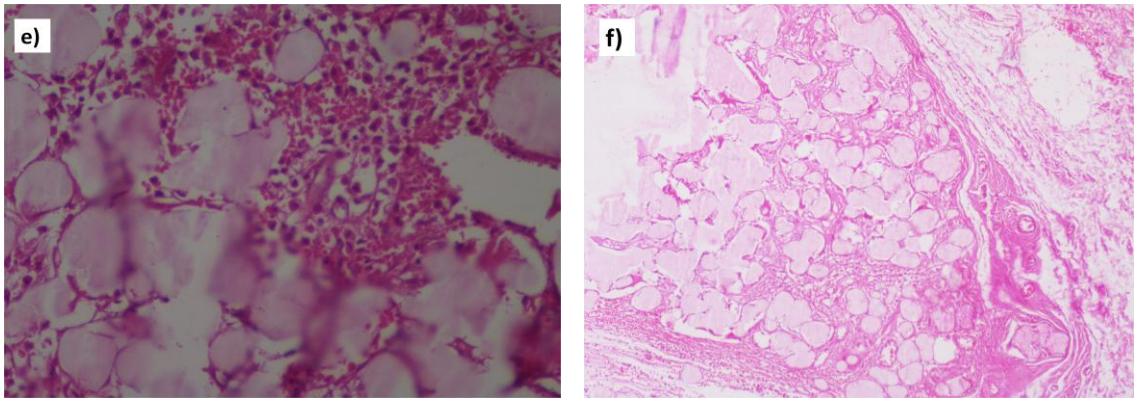


Figure 6: Representative images of Hematoxylin & eosin staining of polyamide (a and b), and PA/HA(90:10) (c and d) scaffolds after 7 weeks of subcutaneous implantation in rats. And Hematoxylin & eosin staining of polyamide (e), and PA/HA(90:10) (f) scaffolds after one year of subcutaneous implantation in rats.

During this course of one year observation post-implantation, of four rats (two with PA and two with PA/HA), a rat with polyamide scaffold was deceased on the 11th month post-implantation due to its age. Meanwhile complete scaffold disintegration was observed in the rat with PA/HA (90:10) scaffold at the end of one year. Consequently, one in each scaffold type were obtained and subjected to pathological analysis, the images of which are presented in Figure 6e & f.

H&E staining of one year old PA scaffold section (Figure 6e) shows fibroblastic reaction, collagenisation and scant inflammatory infiltrate, while PA/HA (90:10) composite scaffold section (Figure 6f) shows sclerosis, mixed inflammatory infiltrate and focal hyalinization. These results suggest that both the scaffold types were biocompatible.

Post-implantation, follow-up for longer durations of one-year was carried out to identify the scaffold failures due to infection, failure of osseintegration or implant loss by mechanical loading [6]. Staffa *et al* suggests a two year follow-up of porous HA cranial prosthesis as per the protocol of Neurosurgicals. This would facilitate the monitoring of osseointeggration. A perfect fit between the implant and the bone allows in-growth of capillaries, perivascular mesenchymal tissue and osteoprogenitor cells from the living bone into the graft. This is crucial to validate the success of the bone implant [10]. Xiong *et al* performed a longer follow-up of rabbit tibial defects fixed with PA/HA composite for internal fixation loosening and joint collapse till 5.3 years [14]. In our study, analysis after one year of implantation was carried out. However, neither any infection nor any inflammation was observed.

Burnett *et al* assessed the safety of using Polyamide and reported to be used in cosmetics and personal care products. And the acute oral LD_{50} for polyamide 12 was reported to be 1g per kg of body weight in rat, mice, guinea pigs and rabbits [2]. Recently, Polyamide is used with nano-hydroxyapatite for developing bone screws [11] and vertebral plates [14]. PA/HA composite has entered the clinical trials for testing in the proximal and distal regions of femur, tibia and humerus in humans [14].

4. Conclusion

3D porous PA and PA/HA scaffolds were tested *in vitro* and *in vivo*. *In vitro* tests such as MTT assay, ALP assay and Alizarin Red S staining carried out using MG 63 cell line reveals that all the three types of scaffolds (PA, PA/HA (95:5) and PA/HA (90:10)) were non-cytotoxic. However, PA/HA (90:10) outperformed the three scaffold types tested. Hence, pure PA and PA/HA (90:10) scaffolds were implanted subcutaneously in the rat models. Pathological tests of 7 week old scaffolds showed that polyamide scaffold showed presence of inflammatory cells, whilst the PA/HA (90:10) composite scaffold showed presence of proliferating capillaries and were in the process of healing. However, pathological testing of scaffolds one year post-implantation showed that both scaffold types were biocompatible and neither showed immune rejection nor infection. The PA/HA (90:10) composite having performed consistently better under *in vitro* and *in vivo* conditions, we conclude that the above composite could more likely be used in orthopedic applications.

Acknowledgment

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Conflict of interest

Authors declare no conflict of interest.

References

[1]. Bougherara H, Bureau M N, Yahia L. Bone remodeling in new biomimetic polymer-composite hip stem. *J Biomed Mater Res Part A.* 2010; 92(1):164-174.

[2]. Burnett C, Heldreth B, Bergfeld W F, Belsito D V, Hill R A, Klaassen C D, Liebler D C, Marks Jr J G, Shank R C, Slaga T J, Snyder P W, Andersen F A. Safety Assessment of Nylon as Used in Cosmetics. *Int. J. Toxicol.* 2014; 33(4) 47S-60S.

[3]. Gregory C A, Gunn W G, Peister A, Prockop D J. An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. *Anal. Biochem.* 2004; 329(1):77-84.

[4]. Henkel J, Woodruff M A, Epari D R, Steck R, Glatt V, Dickinson I C, Choong P F M, Schuetz M A, Hutmacher D W. Bone Regeneration Based on Tissue Engineering Conceptions – A 21st Century Perspective. *Bone Res.* 2013; 1(3): 216-248.

[5]. Kumaresan T, Gandhinathan R, Ramu M, Ananthasubramanian M, Banu Pradheepa K. Design, analysis and fabrication of polyamide/ hydroxyapatite porous structured scaffold using selective laser sintering method for bio-medical Applications. *J. Mech. Sci. Technol.* 2016; 30 (11):5305-5312.

[6]. Mangano F, Macchi A, Shibli JA, Luongo G, Lezzi G, P A, Caprioglio A, Mangano C. Maxillary Ridge Augmentation with Custom-made CAD/CAM scaffolds. A 1-Year Prospective Study on 10 Patients. *J. Oral Implantol.* 2014; 40(5): 561-569.

[7]. Qiao B, Li Lidong, Zhu Q, Guo S, Qi X, Li W, Wu J, Liu Y, Jiang D. Bone plate composed of a ternary nano-hydroxyapatite/polyamide 66/glass fiber composite: biomechanical properties and biocompatibility. *Int. J. Nanomedicine.* 2014; 9: 1423-1432.

[8]. Qu Y, Wang P, Man Y, Zuo Y, Li J. Preliminary biocompatible evaluation of nano-hydroxyapatite/polyamide 66 composite porous membrane. *Int. J. Nanomedicine.* 2010; 2: 429-435.

[9]. Sneha M, Sundaram N M. Preparation and characterization of an iron-oxide-hydroxyapatite nanocomposite for potential bone cancer therapy. *Int. J. Nanomedicine* 2015; 10 (1): 99-106.

[10]. Staffa G, Barbanera A, Faiola A, Fricia M, Limoni P, Mottaran R, Zanotti B, Stefini R. Custom made bioceramic implants in complex and large cranial reconstruction: A two-year follow-up. *J. Cranio Maxillo Fac. Surg.* 2012; 40: 65-70.

[11]. Su B, Peng X, Jiang D, Wu J, Qiao B, Li W, Qi X. In Vitro and In Vivo Evaluations of Nano-Hydroxyapatite/Polyamide 66/Glass Fibre (n-HA/PA66/GF) as a Novel Bioactive Bone Screw. *PLoS ONE* 2013; 8(7): e68342.

[12]. Subramanian A, Krishnan U, Sethuraman S. In vivo Biocompatibility of PLGA-polyhexylthiophene Nanofiber Scaffolds in Rat Model. *Biomed Research International* 2013; Article ID 390518.

[13]. Tang Y, Wu C, Wu Z, Hu L, Zhang W, Zhao K. Fabrication of in vitro biological properties of piezoelectric bioceramics for bone regeneration. *Sci. Rep.* 2017; 7, 43360.

[14]. Xiong Y, Ren C, Zhang B, Yang H, Lang Y, Min Y, Zhang W, Pei F, Yan Y, Li H, Mo A, Tu C, Duan H. Analyzing the Behaviour of a porous nano-hydroxyapatite/polyamide 66 (n-HA/PA66) composite for healing of Bone Defects. *Int. J. Nanomedicine* 2014; 9: 485-494.

[15]. Yao M, Fu M, Liu H, wang x, Sheng X, Gao J. Fabrication and characterization of drug-loaded nano-hydroxyapatite/polyamide 66 scaffolds modified with carbon nanotubes and silk fibroin. *Int. J. Nanomedicine* 2016; 11: 6181-6194.

[16]. You F, Li Y, Zou Q, Zuo Y, Lu M, Chen X, Li L. Fabrication and osteogenesis of a Porous nanohydroxyapatite/polyamide scaffold with an Anisotropic Architecture. *ACS Biomat. Sci. Eng.* 2015; 1(5): 825-833.

[17]. Zhou S, Zhang l, Wang Y, Zuo Y, Gao S, Li Y. Fabrication of hydroxyapatite/Ethylene vinyl Acetate/ Polyamide 66 Composite Scaffolds by Injection-Moulding Method. *Polym. Plast. Technol. Eng.* 2011 Jul 12; 50(10): 1047-1054.

[18]. http://ec.europa.eu/eurostat/statistics-explained/ index.php/Accidents_at_work_statistics #Analysis_by_type_of_injury

[19]. https://www.xpneumatic.com/difference-between-pa6-pa11-and-pa12/

[20]. http://webhotel2.tut.fi/projects/caeds/tekstit/plastics/plastics_POLYAMIDES.pdf

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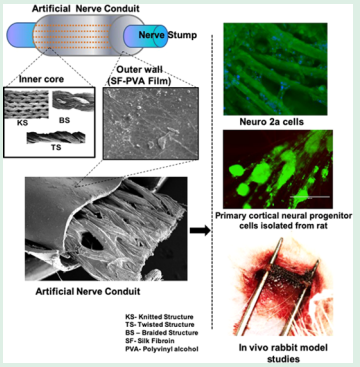
Nanocomposite-Coated Silk-Based Artificial Conduits: The Influence of Structures on Regeneration of the Peripheral Nerve

Mamatha Muraleedharan Pillai, Gopal Sathishkumar, Shadi Houshyar, Rathinasamy Senthilkumar, Anita Quigley, Sivanandam Shanthakumari, Rajiv Padhye, and Amitava Bhattacharyya*

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ABSTRACT: In this study, silk filaments are coated with different concentrations (5, 7.5, and 10% w/w) of carbon nanofibers (CNFs) dispersed in poly-ε-caprolactone. The nanocomposite-coated silk filaments are subjected to knitting, braiding, and twisting. The tubular structures are covered with a silk fibroin/polyvinyl film for the nerve conduit application. Physical characterization of the developed nerve conduits demonstrates suitable mechanical properties comparable to native nerve tissue. Cell proliferation is confirmed through in vitro cell culture studies using Neuro 2a and rat primary cortical neural progenitor cells, which show that the proliferation happens along the interconnected macrochannels of the internal structure of the nerve conduit. The knitted structure presents better biological properties than the nerve conduits with other internal structures. The in vivo sciatic nerve implantation is performed in a rabbit model using the best conduit, i.e., 10% CNF-based nanocomposite-coated silk with a knitted inner structure without any biomolecules or tube filling gels. Regeneration of a 2 cm gap excised sciatic nerve is investigated by immunohistochemistry and histology of implanted nerve conduits removed after 30 days. Results suggest that the CNF-based conducting nanocomposite coating in this well-defined architecture of the conduit helps in signal transmission and neural growth during the regeneration of the transected nerve.



KEYWORDS: *Knitted, braided, twisted, artificial nerve conduit, nanocomposite coating*

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1. INTRODUCTION

There are a large number of incidences of peripheral nerve injury reports each year with a lack of feasible nerve regeneration techniques. Compared to the central nervous system, the peripheral nervous system has an intrinsic ability to regenerate. However, peripheral nerve regeneration is a slow regeneration process and leads to neural and muscle atrophy at the site of denervated area. The present practice of using autografts for peripheral nerve injury repair has limitations like availability, multiple surgeries, sensory loss, and immune suppression.[1] Therefore, the development of bioengineered artificial nerve conduits (ANCs), which mimic the physical and biological characteristics of the native nerve tissue, would be beneficial for suffering patients.[2] Several synthetic and natural-polymer-based ANCs have been successfully developed for short nerve gaps up to 5−10 mm. However, development of the ANC more than 10 mm in length still remains a challenge due to the risk of breakage, lack of continuity, and directional growth.[3] These issues can be overcome by selection of a suitable biomaterial combination as well as structure to enhance the biomechanical property of the ANC and to provide a suitable microenvironment for the cells to proliferate.

Researchers have been using various approaches to develop nerve conduits using different biomaterials[4−6] such as collagen, hyaluronic acid, silk fibroin (SF), polyhydroxybutyrate, poly-D,L-lactide-*co*-glycolic acid (PLGA), poly-ε-caprolactone (PCL), poly-L-lactate (PLA), polyvinyl alcohol (PVA), etc.[7] Among these polymers, SF, PCL, and PVA can be potential polymers for nerve tissue engineering. SF is widely used for peripheral nerve regeneration due to its unique biological and physical properties. SF has the ability to preserve the neuronal physiology, which in turn can promote peripheral nerve regeneration.[8] ANCs based on SF fibers were developed by Xue et al.[7] and successfully bridged the sciatic nerve gap of 30 mm length in a study period of 12 months. Similarly, PCL, a synthetic polymer, also possesses excellent properties such as biocompatibility, slow degradation, and required mechanical properties for nerve tissue engineering application. Panahi-Joo et al.[9] developed 3D tubular aligned PCL nanofibrous scaffolds for peripheral nerve tissue engineering. These scaffolds were found to be promising for PC12, SH-SY5Y, and C6 cell elongation, migration, and proliferation. PCL nanofibrous scaffolds for spinal cord regeneration were developed, and in vitro studies showed the efficacy in Schwann cells and induced pluripotent stem cells.[10] Furthermore, they studied the

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scaffolds' regeneration ability in a rabbit model, and they observed axonal regeneration and re-myelination in the implanted cell-seeded scaffolds. PVA is another biocompatible polymer and possesses prominent features such as nontoxicity and biodegradability, which makes it suitable for nerve tissue engineering. Due to its poor cell affinity and weak antigenicity,[11] PVA is preferably mixed with other polymers such as silk,[12] fibronectin,[13] etc. for enhancing the physical and biological properties of scaffolds.

Regardless of promising results achieved by using non-conducting scaffolds, incorporation of conductive nanoparticles in the scaffolds has been widely investigated to further enhance the electrical and mechanical properties of the scaffold. Nanoparticles such as carbon nanofibers (CNFs),[14,15] graphene,[16] nanosilver,[17] gold,[18] etc. were supplemented within the polymer matrix to enhance the biomaterial−nerve cell interaction followed by regeneration of damaged nerve tissue. Among these nanoparticles, CNF is easy to process and offers very good electrical conductivity with limited toxicity.[2,15]

For the development of ANCs, researchers have been using different techniques such as electrospun nanofibers,[19] multilayer molding,[20] and integration molding.[21] Shrestha et al.[19] developed a nanofibrous nerve conduit using polyurethane/silk fibroin functionalized with multiwalled carbon nanotubes. The developed ANC was found to be efficient for in vitro axonal regeneration and cell proliferation. Qian and his group developed nerve conduits using multilayer molding[20] and integration molding[21] techniques. In the multilayer molding technique, they used a 3D pointed rolling tube mold to coat polydopamine onto the PCL-gold nanocomposite. The in vivo nerve regeneration results were promising for angiogenesis and peripheral nerve repair. In the integration molding technique, they developed tubular molds with concentric rings, and the empty spaces were filled with PCL-graphene oxide combination, and finally, pores were developed using a 3D printer and showed in vivo nerve regeneration potential.[21]

Peripheral nerve conduits are made up of bundles of nerve fibers and surrounding connective tissue with blood vessels. Each bundle of nerve fibers is held together by collagen fibrils.[22] Thus, the natural structure of the peripheral nerve is an intercalated fibrous structure covered with a sheath (epineurium). To mimic these structures rather than a hollow cylinder-based structure, fibrous structures may show a higher biomechanical property as well as regeneration ability. Thus, textile fabrication techniques are used to develop promising scaffolds for nerves, tendons, and ligaments. Recently, knitted, twisted, and braided structures have widely been used for tissue engineering applications due to their excellent mechanical strength, flexibility, precise structure and surface topography, controllable geometry, porosity, and viscoelastic behavior.[2,23] Lu et al.[24] developed microbraided PLA nerve conduits that were found to be promising in regeneration of the rat sciatic nerve (10 mm gap). In another study, Wang et al.[25] developed a braided PLGA catheter with high-density PLGA yarn with chitosan outside and PLGA of low density treated with hydrogen peroxide inside. The developed nerve conduit was found to be efficient in in vitro Schwann cell proliferation. Wang et al.[23] fabricated porous knitted scaffolds using chitosan yarns that exhibited promising results for Neuro 2a cell proliferation along with desirable mechanical properties. A similar study was performed on the efficiency of the twisted (wire-rope) structure for tissue tendon, nerve, and ligament tissue engineering applications.[26]

In this study, different textile structures including knitted (KS), twisted (TS), and braided (BS) were developed using CNF-PCL-based nanocomposite-coated silk fibroin (SF) filaments and analyzed for their potential application in nerve regeneration. Degummed (after removal of surface sericin) SF filaments were subjected to PCL-CNF nanocomposite coatings (5, 7.5, and 10% w/w). The CNF concentrations were selected based on our previous studies.[14,15,27] In our pervious study, we developed a braided conduit structure using degummed silk fibroin coated with PCL and different concentrations of CNF.[28] These conduits' performance in in vitro cell proliferation was satisfactory, which motivated us to study the impact of a defined architecture in the nerve regeneration efficiency. For this, degummed silk fibroin threads were converted to conduit scaffolds using knitting, braiding, or twisting techniques. The final scaffold consists of a knitted, braided, or twisted scaffold as a core structure and SF:PVA (3:1) as an outer layer. The polymer combination selected as the outer layer was based on our previous studies[12,29] to protect the inner core from muscle cell infiltration as well as allow the nutrients to pass inside the inner region. The sample abbreviations are given in Table 1.

Table 1. Different SF-Based Nanocomposite ANCs Developed and Their Abbreviations[a]

		coating		
inner core structure	silk filament	PCL (w/v)	CNF (w/w)	sample code
knitted (KS)	S			S
	S	10%		SP
	S	10%	5%	SPC5
	S	10%	7.5%	SPC7.5
	S	10%	10%	SPC10
twisted (TS)	S			S
	S	10%		SP
	S	10%	5%	SPC5
	S	10%	7.5%	SPC7.5
	S	10%	10%	SPC10
braided (BS)	S			S
	S	10%		SP
	S	10%	5%	SPC5
	S	10%	7.5%	SPC7.5
	S	10%	10%	SPC10
silk-PVA film (outer wall)				SF-PVA

[a]KS: knitted structure, TS: twisted structure, BS: braided structure; S:SF, SP:SF coated with PCL, SPC:SF coated with different concentrations of CNF (5, 7.5, and 10% w/w)-dispersed PCL solution (10% w/v in DMF).

Based on the in vitro cell culture studies using Neuro 2a and rat primary cortical neural progenitor cells, the conduit with a knitted core structure was selected for in vivo sciatic nerve implantation in a rabbit model.

2. EXPERIMENTAL METHODS

2.1. Fabrication of Silk-Fibroin-Based Conducting Nanocomposite ANCs. ANCs developed using silk-based nanocomposites consist of two parts: (i) an inner matrix composed of different inner core structures (KS, TS, and BS) made of silk fibroin (SF) coated with PCL and various concentrations of CNF (5, 7.5, and 10% w/w) and (ii) an outer cover to prevent invasion of unwanted cells into the ANC, consisting of SF:PVA (3:1) thin films.

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For the inner core structure, raw silk threads were purchased from handloom dealers of Mettupalayam, Tamilnadu. The silk threads were degummed to remove sericin by boiling in 0.5 M sodium carbonate solution for 1 h at 60 °C. The degummed silk fibroin threads were coated with PCL and CNF solutions of various concentrations (5, 7.5, and 10% w/w).[15] PCL (10% w/v) was dissolved in *N*′,*N*′-dimethylformamide (DMF) solution, and CNF (diameter: 60−150 nm; length: 30−100 μm, Pyrograf Inc., USA) was added to this solution followed by 2 h of stirring and then 30 min of sonication (Elmasonic S30H, Germany) to form a uniform dispersion of CNF in solution. The PCL-CNF dispersions were used to coat single SF threads using an in-house built coating setup.[30] Finally, the coated SF threads were subjected to knitting, twisting, and braiding. Pure degummed silk threads (S) were used as control for further studies.

For the outer cover, the outer matrix consisted of isolated silk from the *Bombyx mori* cocoon, per our previous protocol.[12] Briefly, SF was dissolved in Ajisawa's reagent and was dialyzed against distilled water followed by concentrating in polyethylene glycol solution (4% v/v). The total weight after degumming was considered as protein weight, and dialyzed SF solution was taken according to the required percentage followed by dissolving PVA in the SF solution in a ratio of 3:1 (v/v) and made up to the final concentration by addition of distilled water. The SF:PVA (3:1 v/v) film was prepared in deionized water, and the solvent casting method was employed to develop a thin film of SF-PVA, and then the film was cross-linked with glutaraldehyde (25%). These films were washed several times to remove unreacted glutaraldehyde and used for fabrication of ANCs by wrapping the inner core and the sides of films by applying a thin layer of SF-PVA solution, cross-linking, and leaving under lab conditions to dry.

Thus, in a complete nerve conduit, the outer cover SF-PVA film is wrapped around the inner tubular textile structures (made of degummed silk threads coated with PCL and CNF) to create a nonporous protective layer for preventing other cellular (mostly muscle cells) migration into the inner core to facilitate nerve cells in their regeneration process.

2.2. Characterization. SF, SP, and SPC10 threads were subjected to Raman spectra and X-ray diffraction analysis (XRD). Raman spectra of samples were recorded using a PerkinElmer Raman Station 400 with a 784 nm laser as the excitation source. XRD patterns of samples were analyzed by using a Bruker D4 Endeavor X-ray diffractometer with copper Kα1 radiation. Surface topography of the ANC was analyzed using a scanning electron microscope (SEM, Carl Zeiss, model EVO18, Germany) at 10 kV after gold coating of the SF filaments. Percentage of porosity of the ANC was calculated using the liquid displacement method with hexane as solvent. Impedance analysis of the ANCs was performed using an impedance analyzer (E4990A, Keysight Technologies, USA) with a Kelvin clip at a distance of 20 mm in a frequency range of 20 Hz to 20 MHz. Normally, the nerve undergoes cyclic expansion and compression for a number of times inside the body. To simulate this condition, dynamic mechanical (DMA) properties of the developed scaffolds were evaluated with a modulated compact rheometer (MCR102, Anton Paar, USA). Universal extension fixture (UXF) mode was used for DMA analysis at 1% initial constant strain with 20 mm length and 37 °C (body temperature).Tensile testing was performed using a universal testing machine (model 3366, Instron, USA) with a 20 mm sample, 100 N load cell, 2 mm/min speed rate, and at room temperature.

2.3. In Vitro Cytotoxicity and Cell Proliferation Studies Using the Neuro 2a Cell Line. To analyze the cell proliferation rate, total DNA estimation was performed using fluorescent DNA binding stain Hoechst as per our previous protocol. Briefly, each scaffold was seeded with 1×10^4 cells and incubated for 10 days. At different time intervals (days 2, 4, 6, 8, and 10), the cell-seeded scaffolds were trypsinized, and the isolated cells were stained with Hoechst, which specifically binds to live cellular DNA. The absorbance was read at wavelengths of 358 (excitation) and 461 nm (emission) using a Fluoroskan Ascent microplate fluorimeter (Thermo Scientific). The evaluation of Neuro 2a cell attachment was performed by SEM and confocal imaging. For SEM, samples were dried using different concentrations of ethanol (70−100%) followed by overnight hexamethyldisilazane (HMDS) treatment. They were placed in a stub with carbon tape followed by sputter coating for 60 s and visualized under SEM. For fluorescence staining, phalloidin and Hoechst were used. For staining, cell-seeded ANCs were removed from the medium and were fixed using 4% glutaraldehyde solution. After that, 10 μL of phalloidin stain was added and incubated for 10 min followed by addition of the Hoechst stain and incubation for 10 min. Further, HMDS was added for complete drying of scaffolds, which is a chemical drying agent and does not alter the ultrastructure of cells. After complete drying, scaffolds were visualized under a confocal microscope.

2.4. In Vitro Live/Dead Microscopic Analysis Using Primary Cortical Neural Progenitor Cells Seeded on Developed Conduits. Primary cortical neural progenitor cells (cortical neurospheres) were isolated from E18 Sprague Dawley rat pups (ethical committee approval: AEC St. Vincent's Hospital Melbourne, Australia Project 015/14r1) and were passaged in neurosphere media (Neurocult NSA, StemCell Technologies), 20 ng/mL EGF (Peprotec), 20 ng/mL basic FGF (Peprotec), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cytocompatibility analysis was performed on KS (SP, SPC5, SPC7.5, and SPC10). Cortical neurospheres were cultured, seeded on the KS scaffolds, and allowed to attach and migrate for 7 days with half medium changes every day. At the end of day 7, KS conduits were stained with calcein AM (Thermo) and ethidium homodimer (Thermo), according to the manufacturer's instruction for the live/dead assay. Images of live, stained cells on developed samples were obtained using an EVOS FL cell imaging system.

2.5. In Vivo Studies. KS, SPC10 was selected for animal studies. Healthy rabbits were selected for this study after getting animal ethical committee approval (approval no. 405/2018/IAEC). Animals were grouped into test and sham animals (three animals per group) and were maintained according to CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines. For nerve regeneration studies, animals were anesthetized using drugs such as ketamine and xylazine. Entire surgery was performed under aseptic conditions. The right thigh sciatic nerve of 2 cm was exposed, clipped on both sides, and excised using microscissors. Further, the distal and proximal nerve stumps were sutured with the SPC10% KS, artificial nerve conduit. After surgery, the wound was closed and rehabilitated under standard conditions. At the end of day 30, the implant was removed, and the nerve stumps were joined together by end-to-end sutures. Similarly, for the control animal, the sciatic nerve was also excised and was used for comparative studies. All animals were observed for recovery of gastrocnemius muscle contraction.

The implanted artificial nerve conduit was subjected to histological and immunohistochemical (IHC) analysis. Histological analysis was performed using hematoxylin and eosin (H&E) and toluidine blue staining (Thermo Fischer kit). The expression levels of S-100, CD 34, and CD 31 were analyzed in the excised regenerated nerve and control nerve tissue (healthy nerve excised from sham). For IHC, Thermo Fischer kits were used, and the experiment was performed according to the manufacturer's instruction. The artificial nerve conduit (SPC10 KS) was further subjected to confocal microscopic imaging to visualize the primary nerve cells attached using Hoechst and phalloidin stains.

All the tests were performed in triplicate, and data are expressed as mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA (Tukey's test) in Origin Pro8.0 software.

3. RESULTS AND DISCUSSION

The main prerequisite for an ideal ANC fabrication is oriented topography with unidirectional fiber arrangement and pore distribution to enhance neurite proliferation and tissue repair.[2,31] In this study, the ANC was assembled with two different components: an inner core wrapped with an outer layer. The outer wall consists of an SF-PVA film wrapped over

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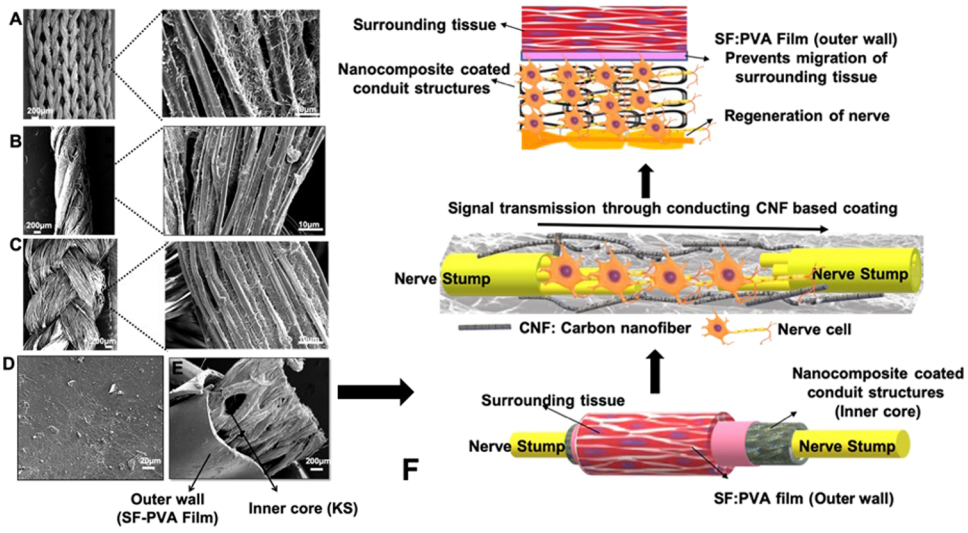


Figure 1. Scanning electron microscopic images of different inner core structures of the (A) KS (magnified image of SPC10), (B) TS (magnified image of SPC10), and (C) BS (magnified image of SPC10). SEM image of the outer wall (D) SF-PVA film and (E) complete nerve conduit with the inner core (knitted structure (KS)) and outer wall SF-PVA film. (F) Combined effect of the CNF and well-defined architecture of the conduit to enhance nerve regeneration.

the inner core; i.e., three different inner core structures were used to investigate the effect of the inner core structure on the mechanical and biological properties of the ANC. The inner core structures included knitted, braided, and twisted scaffolds, which are great options for nerve tissue engineering due to their well-defined architectures, mechanical properties, and controllable porosities (Table1).

3.1. Characterization of Developed ANCs. Figure 1 shows the macroscopic appearance of representative images of the developed ANCs. The KS-ANC with a diameter of 573 ± 19 μm showed well-aligned macrochannels with pores (Figure 1A). A magnified image in Figure 1A reveals the distribution of CNF in the coating layer. The SEM images show very detailed structures with axially oriented fibers and interconnected micropores. Similarly, Figure 1B shows the topographic details of TS. Compared to the KS, the TS is a well-packed wire-rope structure with mere pores that are visible. The average diameter of the TS was 159 ± 13 μm. The fibers in the TS were radially twisted to provide a guided channel for nerve regeneration. A magnified version of the TS shows the dispersed CNF in the PCL coating on silk fiber (right side of Figure 1B). The average thickness of BS is about 273 ± 14 μm (Figure 1C). In the BS-ANC, the fibers were radially arranged with sufficient pores in between the intercalated fibers (Figure 1C). The right side of Figure 1C shows the distribution of PCL-CNF (10%) in the matrix. All the structures exhibited uniform coating of PCL-CNF (5−10%) throughout the matrix. Figure 1D shows the surface topography of the outer wall of the ANC consisting of the SF-PVA film. The average thickness of the film is about 54.2 ± 11 μm with a very small number of pores. The structure of the complete ANC with the inner core (KS) and outer wall (SF-PVA film) is shown in Figure 1E. Figure 1F shows the hypothesis of this study; we hypothesize that (i) addition of conducting CNF in the inner core of the ANC can enhance the nerve cellular signaling and proliferation, (ii) the outer wall of the ANC can prevent invasion of the surrounding cells into the ANC (in vivo) so that undisturbed nerve cell proliferation and regeneration occur, and (iii) effective nerve regeneration depends on the nerve conduit structure and its composition.

SEM images of the developed inner core of the ANC with a well-defined fiber architecture and surface topography are shown in Figure 1. PCL coating with different concentrations of CNF was found to be uniform throughout the matrix. Porosity of the matrix is an important factor to be considered as it has a vital role in passing nutrients and cell percolation. The developed inner core of the ANC in this study showed sufficient porosity to facilitate nutrient transport and cellular migration within the ANC. Further, the outer wall of the ANC, SF-PVA film, showed the least porosity to help in gas and nutrient exchange while preventing migration of tissue cells from outside to inside the conduit.

The Raman spectra and XRD patterns of pure and coated SF threads are given in Figure S1A,B. Raman spectral analysis of the coated and pure SF threads confirmed the presence of SF, PCL, and CNF in the developed threads. The characteristic peaks of SF were at 1665, 1249, and 1334 cm^{-1}, which correspond to amide I, amide III, and CH_2, respectively.[32] SF threads coated with PCL showed the characteristic peaks of SF and PCL. The peaks of different functional groups such as C−COO (826 cm^{-1}), CH_2 (1282 and 1452 cm^{-1}), and C=O (1668 cm^{-1}) confirmed the presence of PCL on SF threads.[33] The presence of G and D bands in the SF threads coated with PCL-CNF confirmed the presence of CNF in the sample (Figure S1A). Diffractograms of coated and uncoated SF filaments showed the characteristic peak of SF at 20°.[34] Two distinct peaks for PCL-coated SF samples were visible at 21.6 and 23.7°, which correspond to the (110) and (200) planes of the PCL orthorhombic crystalline structure, respectively.[35] Similarly, SF filaments coated with PCL and 10% CNF showed a diffraction pattern at 26.5° (002), which reveals the presence of CNF in the coating layer (Figure S1B).[36]

Porosity is an important factor that affects the cellular ingrowth and proliferation. Textile-based scaffolds generally provide adequate porosity for this purpose due to their loose structure. Figure S2A shows the percentages of porosity for developed ANCs in this study. All the developed inner cores

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for ANCs were found to be highly porous, around >90% porosity (Table 2), and this value is 42.3 ± 2.3% for the outer wall. The outer layer with low porosity prevents invasion of outer cells to the inside of the ANC. The electrical impedance profile of the inner cores of ANCs with varying concentrations of CNF showed a significant decrease in the impedance value with increasing CNF concentration (Figure S2B–D). The impedance values at a frequency of 20 MHz for the KS, TS, and BS were 10 ± 5.2, 8.36 ± 3.4, and 171 ± 4.3 Ω, respectively. These values demonstrate the superior electrical properties of ANCs at high CNF concentration irrespective of structural variations (Table 2). The objective of the impedance measurement over a length of 20 mm was to assess the electrical conducting network formation from one end to another end of the conduit. This is expected to help in nerve cell proliferation and repair.[28]

Table 2. Physical Properties of Developed ANCs

no	sample codes	porosity (%)	impedance (Ω at 2 × 10^7 Hz)	storage modulus (MPa at frequency 7 Hz)
KS				
1	S	96.2 ± 3.3		17.7 ± 1.2
2	SP	95.2 ± 4.4		29.8 ± 3.2
3	SPC5	93.3 ± 2.6	732 ± 8.1	31.9 ± 4.2
4	SPC7.5	92.5 ± 4.1	400 ± 6.4	34.8 ± 5.6
5	SPC10	91.6 ± 3.1	10 ± 5.2	42.4 ± 3.2
TS				
6	S	96.2 ± 2.6		715.07 ± 8.2
7	SP	95.2 ± 4.1		795.8 ± 5.3
8	SPC5	92.3 ± 3.2	388.2 ± 3.2	895.8 ± 7.3
9	SPC7.5	91.5 ± 4.5	279.3 ± 4.6	845 ± 6.2
10	SPC10	93 ± 3.6	8.36 ± 3.4	995.8 ± 9.6
BS				
11	S	95.2 ± 4.6		1.2 ± 0.3
12	SP	94.2 ± 3.8		2.2 ± 0.1
13	SPC5	93 ± 2.5	272 ± 4.9	7.2 ± 1.4
14	SPC7.5	92 ± 4.8	189 ± 5.8	17.9 ± 3.2
15	SPC10	91 ± 3.1	171 ± 4.3	29.4 ± 4.5

3.2. Mechanical Properties of Developed ANCs.

Biomimetic scaffolds can provide a good microenvironment for cell proliferation and tissue repair.[37] As reported in the literature, biologically inspired nanoscale topography can be developed in scaffolds by adding nanometer-scale particles such as CNF to enhance the surface area and electrical, mechanical, and biological properties.[37] By incorporation of CNF into the developed inner core of the ANC, the electrical property improved significantly due to its porous nature. As the concentration of CNF was increased, the electrical property was also found to increase with significantly lower impedance. The porous structure of the scaffold with high surface area provided lower impedance, which further enhances the ion penetrating ability into the conducting network of the polymer.

Neural tissue has an anisotropic mechanical behavior due to the epineurium that covers the perineurium and endoneurium.[38] Under normal physiological conditions, nerves are exposed to different mechanical stresses such as compressive, tensile, or combination of stresses.[38] Therefore, understanding the mechanical behavior of the ANC is essential as it has to meet the mechanical requirements in the in vivo environments.

The dynamic mechanical property of the developed inner core of the ANC was studied using a dynamic mechanical analyzer (Figure 2A–C). As the concentration of CNF increased in the inner core of the ANC, the storage modulus increased; the storage modulus values are 42.4 ± 3.2 (KS), 995.8 ± 9.6 (TS), and 29.4 ± 4.5 MPa (BS) at a frequency of 7 Hz. The highest storage modulus was achieved by the TS compared to the KS and BS (Table 2). This proves that the structure has an effect on the mechanical property even using the same material composition. This similar trend was observed in tensile properties of these developed ANCs. The samples showed strains of 112, 172, and 63.3% for the KS, TS,

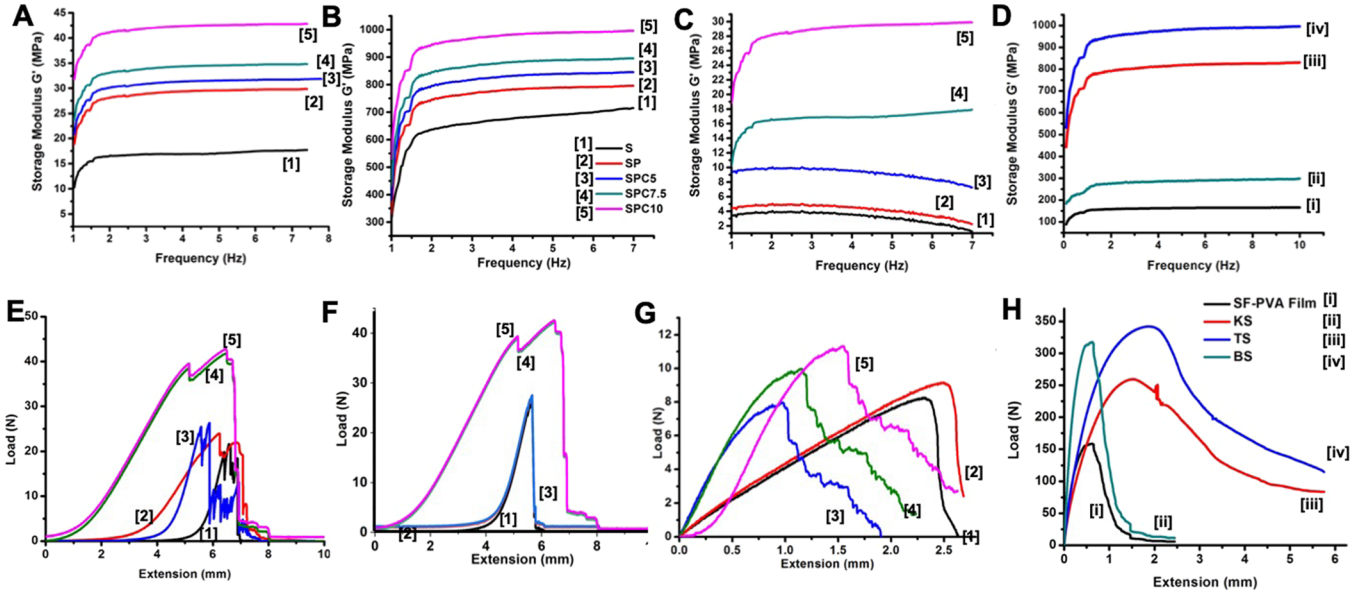


Figure 2. Mechanical characterization of the ANC. Storage moduli of the (A) KS, (B) TS, (C) BS, and (D) assembled nerve conduits (outer coating and 10% CNF-based coated inner structures) and tensile properties of the (E) KS, (F) TS, (G) BS, and (H) assembled nerve conduits (outer coating and 10% CNF-based coated inner structures).

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and BS, respectively, with 10% CNF-based samples (Figure 2D−F).

The mechanical properties of the assembled ANCs including the outer wall and inner cores (10% CNF-based coated structures) are shown in Figure 2D,H. The storage modulus of the outer wall of the ANC was 165 ± 0.4 MPa at a frequency of 10 Hz. The storage modulus was improved significantly compared with inner core structures when assembling ANCs with KS, TS, and BS inner cores and SF-PVA film as an outer layer. The highest storage modulus of the assembled ANC was achieved by TS and KS, 995 ± 0.8 and 885 ± 0.6 MPa, respectively (Figure 2D). The same trend was observed in tensile properties of assembled ANCs (Figure 2H).

The storage modulus was found to be varying in ANCs with various inner core structures. This proves that structural changes in the inner structure of the ANC affect the final properties of the ANC even if they are all made of the same polymer combinations. All the developed ANCs showed comparable mechanical properties to native tissue. However, it is evident from the results that the TS has the highest storage modulus compared to the KS and BS due to the more flexible structure and less interlock between the filaments, which helps in moving filaments without deformation. It means that the strength of the TS is the strength provided by the twisted filaments. Addition of CNF has an effect on storage moduli in the samples. As the concentration of CNF increased, the mechanical properties of samples increased significantly. Figure 2D shows the storage moduli of assembled SF-PVA-wrapped KS, TS, and BS. The impact of SF-PVA wrapping in the different inner core structures on the mechanical property is significant. The storage moduli of TS and KS were found to be highest compared with that of the BS. In summary, this high mechanical property of the assembled ANC can resist muscular contractions and withstand its original shape for a long period after implantation in the body.

Tensile strength is another important mechanical property of the nerve conduit as nerve tissue experiences tensile stress during joint movement, and it was reported that the nerve elongates 1 mm per day, which shows remarkable changes in electro-physiochemical properties of nerve tissue.[38] Femoral lengthening in the range of 0.5−1.5 mm per day reduced the signal transduction ability in sciatic nerves of a rat up to 22−47%.[39] In this scenario, it is very crucial to consider the tensile properties of the inner core in fabricating the assembled ANC. In this study, all ANCs developed with various inner core structures showed remarkable tensile properties. The impact of CNF and structure on the tensile property of the developed ANC is shown in Table 2. The samples with 10% CNF showed the highest tensile property compared to other concentrations irrespective of the inner core structure. Among the different inner core structures studied, the TS showed maximum tensile strength. TS, KS and BS samples with 10% CNF showed maximum loads of 52.4 ± 3.9 (6.7 mm), 40.5 ± 3.2 (6.6 mm), and 12 ± 1.7 N (1.5 mm), respectively, while the tensile property of the human cadaver median nerve was reported to be around 2 N with 7.5 mm extension.[40] Therefore, TS and KS showed superior tensile properties to native tissue. Furthermore, the maximum tearing load is about 343 ± 2.8 N for the TS wrapped with the SF-PVA film compared to all other samples. Thus, the assembled ANC with the TS inner core showed superior mechanical properties.

3.3. In Vitro Cell Culture Studies. In vitro cell culture studies on the developed ANC (inner core) showed promising results. The ANC topography, fiber arrangement, and its microarchitecture had an impact on cell proliferation and growth. The viability of Neuro 2a cells was quantitatively estimated using the MTT assay (Figure S3A). From this study, the nontoxicity of the developed ANC was confirmed. All the developed assembled ANCs showed a viability of >80%, which demonstrates the suitability of SF-PCL-CNF-based ANCs as nerve conduits. The rate of cell proliferation was measured using the DNA estimation method by means of Hoechst staining. To study how Neuro 2a cells proliferate in the developed ANC, cells were allowed to grow on the developed ANC, and at different time intervals, DNA was estimated (Figure S3B−D). The Neuro 2a cellular proliferation profile in the developed ANC increased as the days increased. The effect of CNF on the cellular proliferation was clear in this study: as the concentration of CNF was increased, the cell proliferation rate was also found to be in an increasing trend. At the end of day 10, the SPC10 KS structure showed the highest viability of 115.2 ± 0.3% (Figure S3B). This result was found to be statistically significant for the cell proliferation rate ($p < 0.05$) compared to other structures. In the case of TS and BS, cell viability percentages were around 90.8 ± 0.5 and 105.23 ± 0.6%, respectively. Thus, the results show that the structure of the ANC has an influence on cellular proliferation. Day 10 samples were processed for SEM and confocal microscopic imaging. From the SEM images, it was clear that Neuro 2a cells were able to attach and proliferate on the surface of the developed ANC (Figure S4). The ANC with the KS inner core, arrangement of microchannels, and pores in its architecture can help in guiding the cells to spread in a better way than those with TS and BS structures. KS and TS structures showed promising results in SEM imaging (Figure S4C) compared to SP, SPC, and SPC10 BS. Neuro 2a cells flourished in the interconnected walls of the KS structure giving a thin layer on its surface. Figure S4F shows the morphology of Neuro 2a cells attached on the SPC10 KS structure. From the figure, nerve cell axonal projections are clearly visible. Developed structures were found to be efficient enough for nerve cell differentiation factors or growth factors. These results were in line with phalloidin- and Hoechst-stained confocal images of cells seeded on TS and BS artificial nerve conduits for day 10 samples (Figures S5 and S6, respectively). On day 10, the Neuro 2a cells covered the surface of the ANC with the KS inner core with a thin layer (Figure 3). The SPC10 KS showed interconnected nerve cell proliferation. This provides support for the efficiency of microchanneled structures with sufficient porosity for cell attachment and proliferation. The TS showed the highest mechanical property compared to the KS and BS. However, the cell attachment and proliferation were found to be the best for the KS inner core structure. The sample with 10% CNF showed the highest cell proliferation rate compared to other concentrations.

Primary cortical neural progenitor cells were successfully isolated from the rat and cultured on the KS SPC10 artificial nerve conduit (Figure S7). At the end of day 7, the samples were stained with calcein to stain live cells. Cells were found to attach and proliferate on the artificial nerve conduits, which confirm the cytocompatibility of the ANC with primary neural progenitor cells. Therefore, the KS SPC10 artificial nerve conduit was found to be a potential structure for nerve tissue engineering applications and further studied in vivo.

3.4. In Vivo Animal Studies. Figure 4 shows the total procedure of ANC implantation in a rabbit model and the

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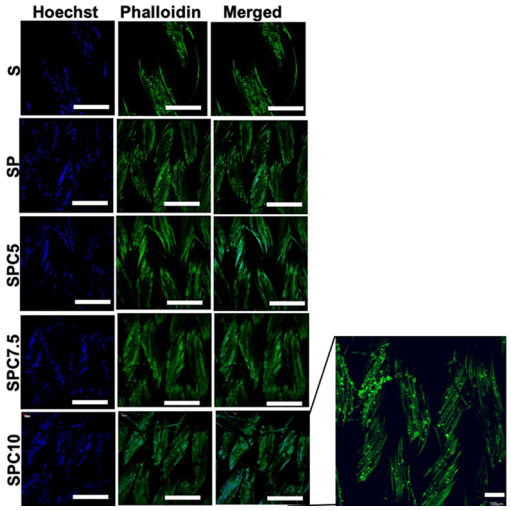


Figure 3. Confocal microscopic images of Hoechst- and phalloidin-stained cells seeded on knitted conduit structures (KS) on day 10 (scale bar − 100 μm).

confocal and SEM images. After implantation, the rabbit's limb movement was gradually improved, and at the end of day 30, the implanted ANC along with regenerated tissue was removed and subjected to microscopic studies, histology, and IHC. After 30 days of implantation, the ANC was found to be intact, and a thin layer of fibrous tissue was covered around the ANC (Figure 4). During removal of the ANC, the gap was found to be bridged by the regenerated nerve. The results from in vivo animal studies confirmed that the KS inner core with 10% CNF (SPC10) nanocomposite-coated silk can be a potential artificial nerve conduit with good nerve regeneration and can bridge the gap.

Confocal microscopic imaging and SEM were performed on the surgically removed implanted conduits to study the in vivo cellular proliferation on the implanted ANC and compared with the healthy control sciatic nerve (Figure 4). Primary nerve cells of the rabbit were found to migrate and proliferate on the inner core of the artificial nerve conduit as shown in Figure 4. The neural continuity was achieved in the neonerve within 30 days compared to the control nerve as seen in the confocal

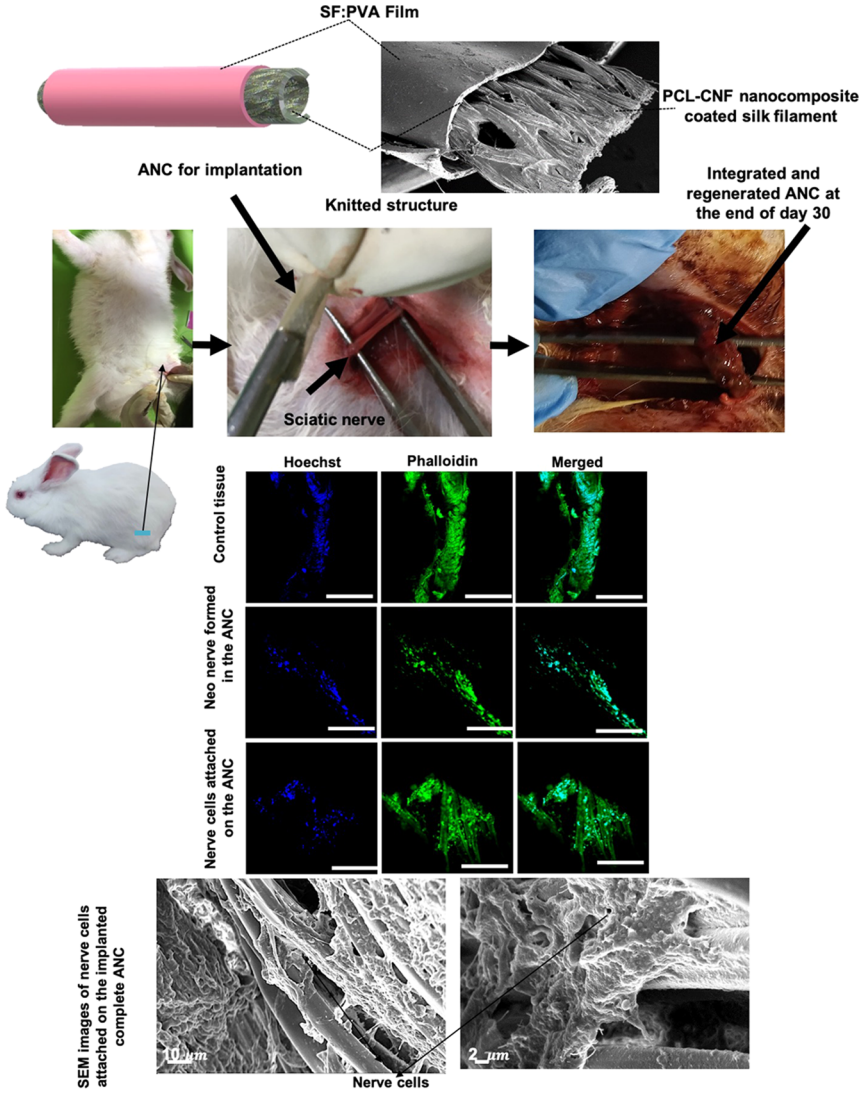


Figure 4. Depicting in vivo rabbit model studies. ANC before implantation in the sciatic nerve and at the end of day 30 of implantation. Confocal images of control tissue, regenerated neotissue from the implanted ANC, and primary cells adhered on the ANC (scale bar − 100 μm). SEM images depict the primary rabbit cells adhered on the ANC.

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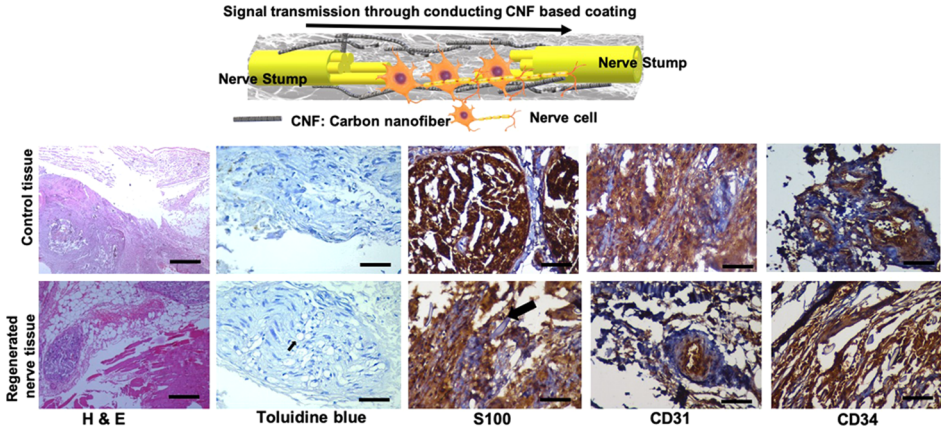


Figure 5. Neural regeneration in the presence of conducting CNF coating. Microscopic images of histology IHC to compare nerve regeneration in control tissue and regenerated nerve using H&E, toluidine blue, S-100, CD 31, and CD34 staining (scale bar − 100 μm).

images. The inside surface of the excised ANC was also subjected to confocal and SEM imaging (Figure 4) that proves the efficiency of developed scaffolds for in vivo nerve regeneration. The cells attach, grow, and proliferate on the inner core of the nerve conduit. The cell attachment and elongated cellular morphology with an extended axon-like structure are clearly visible. CNF plays a major role in signal transmission and helps the subject to move his limb during the regeneration period. The regenerated nerve shows directional growth comparable to the control nerve. Nerve ends are connected at a distance of 2 cm, and almost full diameter of the neonerve was achieved in 30 days.

Further, the histological assessment of removed tissue from the ANC using H&E staining showed the regenerated nerve along with adjacent connective tissue and blood vessels (Figure 5). This was confirmed using toluidine blue staining, which showed the myelinated axons in the regenerated nerve bundle. S-100 staining confirmed the peripheral nerve regeneration with Schwann cell expression. CD 31 and CD 34 depicted the angiogenesis in the regenerated nerve tissue. Histological assessment using H&E and toluidine blue staining of the excised ANC along with regenerated nerve tissue (ANC-implanted nerve) showed promising results as the H&E staining showed the regenerated nerve bundles along with the surrounding tissues. This depicts that the developed ANC was compact enough, outer layer with low porosity, to prevent invasion of connective tissues, which can interfere with the nerve regeneration. The outer wall (SF-PVA film) of the ANC provided an important barrier for the nerve regeneration as well as preventing the surrounding cells to invade the ANC. In toluidine blue staining, myelinated nerve bundles were detected, which confirms the nerve regeneration in the implanted KS-ANC. The toluidine blue stain is a metachromatic stain especially for myelinated nerve bundles. Toluidine blue staining gives clear visuals of the myelinated sheath established by the presence of a circular ring, which is clear in Figure 5 (toluidine blue staining). The circular nerve bundles in the regenerated nerve sample are a remarkable result in a short period of time. In the case of control, it showed mature tissue with collagen bundles. Similar observation was also reported by Iijima et al.[41] and Zhao et al.[42] S-100 is a reliable marker to identify nerve fragments and plays an important role in regeneration of defective nerves as an emergency response to proliferate Schwann cells.[43] In Figure 5, for S-100 staining, closed nerve fragments were visible (labeled with an arrow), which shows the regenerated nerve. Such a fragmented nerve is absent in the control tissue as it is a mature tissue. Thus, the IHC result of S-100 depicts functional recovery of the nerve tissue via bridging the gap by proliferation of Schwann cells. CD 31 and CD 34 positive expression reveals the angiogenesis in the regenerated nerve tissue.[44,45] In CD 31 and CD 34 staining in the case of the regenerated nerve, neovascularization was detected with the appearance of small vessel-like structures. However, in the case of control tissue, such neovascularization was absent. Thus, CD 31 and CD 34 expression proves that the developed ANC is robust enough for angiogenesis.

The KS structure showed good cytocompatibility after seeding with primary cortical neurosphere cells (Figure S7). This confirms that primary mice neurosphere cells were able to proliferate and attach without any additional growth factors, which is a good confirmation for the cytocompatibility of primary cells.

Cytocompatibility studies proved that the developed ANC was nontoxic to Neuro 2a cells (Figure S2A). Furthermore, the biocompatibility of SF:PVA (3:1) nanofibers and cryogels was studied by Pillai et al. and Wang et al.[12,25] and was found to be safe for primary cell attachment and proliferation. From the DNA estimation study, it was observed that CNF has an impact on enhancing cell proliferation rates. It was observed that, as the concentration of CNF increased, the cell proliferation was also found to be in an increasing profile irrespective of the inner core structure (Figure S2B−D). This proves the efficiency of CNF in enhancing the nerve cell communication and signaling process, which may lead to faster cell proliferation. Generally, the nerve has electric potentials of 60 to −100 mV in the resting stage, and during signal transmission, it has potentials of 70 to −55 mV (action potential),[35] which is used to communicate with other cells. Thus, the addition of a conducting material like CNF can enhance electric impulse transmission and can further help in cellular communication, which ultimately leads to cell proliferation. CNF also possesses biomimetic properties that gives nanotopography similar to native tissue and mimics the structure of collagen.[31] Therefore, it can provide an appropriate microenvironment for the cells to proliferate. The cell proliferation in the KS is better than those in other inner core structures in this study. Despite having a high mechanical property, the TS showed comparatively less proliferation ability, which may be due to its compact wire-

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rope structure and reduced cell migration and penetration at the inner region of the developed ANC.

4. CONCLUSIONS

The main aim of this study was to analyze the structural effect of the inner core of the nerve conduit on physical and biological properties of assembled ANCs. The SF-based nanocomposites with various inner structures, including KS, TS, and BS, were developed by wrapping the inner cores with the SF-PVA film to assemble the ANC. Among the three structures, the knitted structured ANC showed excellent properties comparable to native tissue such as low swelling, good porosity, mechanical property, and in vitro cytocompatibility. In vivo studies in New Zealand white rabbits in the transected sciatic nerve demonstrated good rehabilitation with almost normal limb movements within 30 days. The regenerated neonerve was found to be comparable to the control. Thus, without any additional growth factors and tube filling gels, the developed KS structure was found to be promising for regeneration of nerve tissues.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsabm.0c00430.

Raman spectra, XRD analysis, porosity percentage, electrical impedance profile, in vitro cell viability analysis, SEM images, and confocal microscopic of cells seeded on ANCs (PDF)

A video file is also available briefing the entire study and functional recovery of the subject (MP4)

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Author Contributions

M.M.P. conducted in vitro and in vivo experiments and prepared the manuscript. G.S. and R.S. performed physical testing and characterization. S.H. carried out confocal microscopy. A.Q. conducted primary cell culture work. S.S. did the immunohistology tests. R.P. coordinated the Australian part of experiments. A.B. planned and supervised the complete work. All contributed in writing the respective portions and finalizing the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) O'Rourke, C.; Day, A. G. E.; Murray-Dunning, C.; Thanabalasundaram, L.; Cowan, J.; Stevanato, L.; Grace, N.; Cameron, G.; Drake, R. A. L.; Sinden, J.; Phillips, J. B. An allogeneic 'off the shelf' therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells. *Sci. Rep.* **2018**, *8*, 2951.

(2) Houshyar, S.; Bhattacharyya, A.; Shanks, R. Peripheral Nerve Conduit: Materials and Structures. *ACS Chem. Neurosci.* **2019**, *10*, 3349−3365.

(3) Edwards, S. L.; Church, J. S.; Werkmeister, J. A.; Ramshaw, J. A. M. Tubular micro-scale multiwalled carbon nanotube-based scaffolds for tissue engineering. *Biomaterials* **2009**, *30*, 1725−1731.

(4) Sensharma, P.; Madhumathi, G.; Jayant, R. D.; Jaiswal, A. K. Biomaterials and cells for neural tissue engineering: Current choices. *Mater. Sci. Eng., C* **2017**, *77*, 1302−1315.

(5) Benfenati, V.; Stahl, K.; Gomis-Perez, C.; Toffanin, S.; Sagnella, A.; Torp, R.; Kaplan, D. L.; Ruani, G.; Omenetto, F. G.; Zamboni, R.; Muccini, M. Biofunctional silk/neuron interfaces. *Adv. Funct. Mater.* **2012**, *22*, 1871−1884.

(6) Daly, W. T.; Niemier, R.; Dillon, S. T.; Wang, H.; Mirsky, R.; Jessen, K.; Windebank, A.; Pandit, A. Spatial Differences in Cellular and Molecular Responses as a Function of the Material Used in Conduit-Mediated Repair and Autograft Treatment of Peripheral Nerve Injuries. *Adv. Funct. Mater.* **2018**, *28*, 1702170.

(7) Xue, C.; Zhu, H.; Tan, D.; Ren, H.; Gu, X.; Zhao, Y.; Zhang, P.; Sun, Z.; Yang, Y.; Gu, J.; Gu, Y.; Gu, X. Electrospun silk fibroin-based neural scaffold for bridging a long sciatic nerve gap in dogs. *J. Tissue Eng. Regener. Med.* **2018**, *12*, e1143−e1153.

(8) Zou, J.-L.; Liu, S.; Sun, J.-H.; Yang, W.-H.; Xu, Y.-W.; Rao, Z.-L.; Jiang, B.; Zhu, Q.-T.; Liu, X.-L.; Wu, J.-L.; Chang, C.; Mao, H.-Q.; Ling, E.-A.; Quan, D.-P.; Zeng, Y.-S. Peripheral nerve-derived matrix hydrogel promotes Remyelination and inhibits synapse formation. *Adv. Funct. Mater.* **2018**, *28*, 1705739.

(9) Panahi-Joo, Y.; Karkhaneh, A.; Nourinia, A.; Abd-Emami, B.; Negahdari, B.; Renaud, P.; Bonakdar, S. Design and fabrication of a nanofibrous polycaprolactone tubular nerve guide for peripheral nerve tissue engineering using a two-pole electrospinning system. *Biomed. Mater.* **2016**, *11*, No. 025017.

https://dx.doi.org/10.1021/acsabm.0c00430
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

(10) Zhou, X.; Shi, G.; Fan, B.; Cheng, X.; Zhang, X.; Wang, X.; Liu, S.; Hao, Y.; Wei, Z.; Wang, L.; Feng, S. Polycaprolactone electrospun fiber scaffold loaded with iPSCs-NSCs and ASCs as a novel tissue engineering scaffold for the treatment of spinal cord injury. *Int. J. Nanomed.* **2018**, *13*, 6265.
(11) Golafshan, N.; Kharaziha, M.; Fathi, M. Tough and conductive hybrid graphene-PVA: Alginate fibrous scaffolds for engineering neural construct. *Carbon* **2017**, *111*, 752−763.
(12) Pillai, M. M.; Gopinathan, J.; Senthil Kumar, R.; Sathish Kumar, G.; Shanthakumari, S.; Sahanand, K. S.; Bhattacharyya, A.; Selvakumar, R. Tissue engineering of human knee meniscus using functionalized and reinforced silk-polyvinyl alcohol composite three-dimensional scaffolds: understanding the in vitro and in vivo behavior. *J. Biomed. Mater. Res., Part A* **2018**, *106*, 1722−1731.
(13) Mottaghitalab, F.; Farokhi, M.; Zaminy, A.; Kokabi, M.; Soleimani, M.; Mirahmadi, F.; Shokrgozar, M. A.; Sadeghizadeh, M. A biosynthetic nerve guide conduit based on silk/SWNT/fibronectin nanocomposite for peripheral nerve regeneration. *PLoS One* **2013**, *8*, No. e74417.
(14) Gopinathan, J.; Pillai, M. M.; Shanthakumari, S.; Gnanapoongothai, S.; Rai, B. K. D.; Sahanand, K. S.; Selvakumar, R.; Bhattacharyya, A. Carbon nanofiber amalgamated 3D poly-ε-caprolactone scaffold functionalized porous-nanoarchitectures for human meniscal tissue engineering: In vitro and in vivo biocompatibility studies. *Nanomedicine* **2018**, *14*, 2247−2258.
(15) Gopinathan, J.; Quigley, A. F.; Bhattacharyya, A.; Padhye, R.; Kapsa, R. M. I.; Nayak, R.; Shanks, R. A.; Houshyar, S. Preparation, characterisation, and in vitro evaluation of electrically conducting poly (ε-caprolactone)-based nanocomposite scaffolds using PC 12 cells. *J. Biomed. Mater. Res., Part A* **2016**, *104*, 853−865.
(16) Bei, H. P.; Yang, Y.; Zhang, Q.; Tian, Y.; Luo, X.; Yang, M.; Zhao, X. Graphene-based nanocomposites for neural tissue engineering. *Molecules* **2019**, *24*, 658.
(17) Ding, T.; Lu, W. W.; Zheng, Y.; Li, Z. y.; Pan, H. b.; Luo, Z. Rapid repair of rat sciatic nerve injury using a nanosilver-embedded collagen scaffold coated with laminin and fibronectin. *Regener. Med.* **2011**, *6*, 437−447.
(18) Lin, Y.-L.; Jen, J.-C.; Hsu, S.-h.; Chiu, I.-M. Sciatic nerve repair by microgrooved nerve conduits made of chitosan-gold nanocomposites. *Surg. Neurol.* **2008**, *70*, S9−S18.
(19) Shrestha, S.; Shrestha, B. K.; Lee, J.; Joong, O. K.; Kim, B.-S.; Park, C. H.; Kim, C. S. A conducting neural interface of polyurethane/silk-functionalized multiwall carbon nanotubes with enhanced mechanical strength for neuroregeneration. *Mater. Sci. Eng., C* **2019**, *102*, 511−523.
(20) Qian, Y.; Song, J.; Zheng, W.; Zhao, X.; Ouyang, Y.; Yuan, W.; Fan, C. 3D manufacture of gold nanocomposite channels facilitates neural differentiation and regeneration. *Adv. Funct. Mater.* **2018**, *28*, 1707077.
(21) Qian, Y.; Song, J.; Zhao, X.; Chen, W.; Ouyang, Y.; Yuan, W.; Fan, C. 3D fabrication with integration molding of a graphene oxide/polycaprolactone nanoscaffold for neurite regeneration and angiogenesis. *Adv. Sci.* **2018**, *5*, 1700499.
(22) Arslantunali, D.; Dursun, T.; Yucel, D.; Hasirci, N.; Hasirci, V. Peripheral nerve conduits: technology update. *Med. Devices* **2014**, *7*, 405.
(23) Wang, A.; Ao, Q.; Cao, W.; Yu, M.; He, Q.; Kong, L.; Zhang, L.; Gong, Y.; Zhang, X. Porous chitosan tubular scaffolds with knitted outer wall and controllable inner structure for nerve tissue engineering. *J. Biomed. Mater. Res., Part A* **2006**, *79A*, 36−46.
(24) Lu, M.-C.; Huang, Y.-T.; Lin, J.-H.; Yao, C.-H.; Lou, C.-W.; Tsai, C.-C.; Chen, Y.-S. Evaluation of a multi-layer microbraided polylactic acid fiber-reinforced conduit for peripheral nerve regeneration. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, 1175−1180.
(25) Wang, B.; Zhang, P.; Song, W.; Zhao, L.; He, C. Design and properties of a new braided poly lactic-co-glycolic acid catheter for peripheral nerve regeneration. *Text. Res. J.* **2015**, *85*, 51−61.
(26) Naghashzargar, E.; Semnani, D.; Karbasi, S. Improving the mechanical properties of wire-rope silk scaffold by artificial neural network in tendon and ligament tissue engineering. *J. Eng. Fibers Fabr.* **2015**, *10*, 155892501501000.
(27) Gopinathan, J.; Pillai, M. M.; Elakkiya, V.; Selvakumar, R.; Bhattacharyya, A. Carbon nanofillers incorporated electrically conducting poly ε-caprolactone nanocomposite films and their biocompatibility studies using MG-63 cell line. *Polym. Bull.* **2016**, *73*, 1037−1053.
(28) Pillai, M. M.; Kumar, G. S.; Houshyar, S.; Padhye, R.; Bhattacharyya, A. Effect of nanocomposite coating and biomolecule functionalization on silk fibroin based conducting 3D braided scaffolds for peripheral nerve tissue engineering. *Nanomedicine* **2020**, *24*, 102131.
(29) Pillai, M. M.; Gopinathan, J.; Indumathi, B.; Manjoosha, Y. R.; Sahanand, K. S.; Rai, B. K. D.; Selvakumar, R.; Bhattacharyya, A. Silk−PVA hybrid nanofibrous scaffolds for enhanced primary human meniscal cell proliferation. *J. Membr. Biol.* **2016**, *249*, 813−822.
(30) Narayanan, S. C.; Karpagam, K. R.; Bhattacharyya, A. Nanocomposite coatings on cotton and silk fibers for enhanced electrical conductivity. *Fibers Polym.* **2015**, *16*, 1269−1275.
(31) Ghorbani, F.; Zamanian, A. Oriented microstructure in neural tissue engineering: a review. *J Tissue Sci. Eng.* **2016**, *07*, 2.
(32) Monti, P.; Taddei, P.; Freddi, G.; Asakura, T.; Tsukada, M. Raman spectroscopic characterization of Bombyx mori silk fibroin: Raman spectrum of Silk I. *J. Raman Spectrosc.* **2001**, *32*, 103−107.
(33) Kotula, A. P.; Snyder, C. R.; Migler, K. B. Determining conformational order and crystallinity in polycaprolactone via Raman spectroscopy. *Polymer* **2017**, *117*, 1−10.
(34) Jaramillo-Quiceno, N.; Álvarez-López, C.; Restrepo-Osorio, A. Structural and thermal properties of silk fibroin films obtained from cocoon and waste silk fibers as raw materials. *Procedia Eng.* **2017**, *200*, 384−388.
(35) Baji, A.; Wong, S.-C.; Liu, T.; Li, T.; Srivatsan, T. S. Morphological and X-ray diffraction studies of crystalline hydroxyapatite-reinforced polycaprolactone. *J. Biomed. Mater. Res., Part B* **2007**, *81B*, 343−350.
(36) Luhrs, C. C.; Moberg, M.; Maxson, A.; Brewer, L.; Menon, S. IF-WS2/nanostructured carbon hybrids generation and their characterization. *Inorganics* **2014**, *2*, 211−232.
(37) Webster, T. J.; Waid, M. C.; McKenzie, J. L.; Price, R. L.; Ejiofor, J. U. Nano-biotechnology: carbon nanofibres as improved neural and orthopaedic implants. *Nanotechnology* **2003**, *15*, 48.
(38) Pfister, L. A.; Papaloïzos, M.; Merkle, H. P.; Gander, B. Hydrogel nerve conduits produced from alginate/chitosan complexes. *J. Biomed. Mater. Res., Part A* **2007**, *80A*, 932−937.
(39) Topp, K. S.; Boyd, B. S. Structure and biomechanics of peripheral nerves: nerve responses to physical stresses and implications for physical therapist practice. *Phys. Ther.* **2006**, *86*, 92−109.
(40) Millesi, H.; Zöch, G.; Reihsner, R. Mechanical properties of peripheral nerves. *Clin. Orthop. Relat. Res.* **1995**, *314*, 76−83.
(41) Iijima, Y.; Ajiki, T.; Murayama, A.; Takeshita, K. Effect of artificial nerve conduit vascularization on peripheral nerve in a necrotic bed. *Plast. Reconstr. Surg. Global Open* **2016**, *4*, No. e665.
(42) Zhao, Y.; Zhang, Q.; Zhao, L.; Gan, L.; Yi, L.; Zhao, Y.; Xue, J.; Luo, L.; Du, Q.; Geng, R.; Sun, Z.; Benkirane-Jessel, N.; Chen, P.; Li, Y.; Chen, Y. Enhanced peripheral nerve regeneration by a high surface area to volume ratio of nerve conduits fabricated from hydroxyethyl cellulose/soy protein composite sponges. *ACS omega* **2017**, *2*, 7471−7481.
(43) Xu, H.; Yan, Y.; Li, S. PDLLA/chondroitin sulfate/chitosan/NGF conduits for peripheral nerve regeneration. *Biomaterials* **2011**, *32*, 4506−4516.
(44) Mohamadi, F.; Ebrahimi-Barough, S.; Nourani, M. R.; Ahmadi, A.; Ai, J. Use new poly (ε-caprolactone/collagen/NBG) nerve conduits along with NGF for promoting peripheral (sciatic) nerve regeneration in a rat. *Artif. Cells, Nanomed., Biotechnol.* **2018**, *46*, 34−45.

https://dx.doi.org/10.1021/acsabm.0c00430
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

(45) Sidney, L. E.; Branch, M. J.; Dunphy, S. E.; Dua, H. S.; Hopkinson, A. Concise review: evidence for CD34 as a common marker for diverse progenitors. *Stem Cells* **2014**, *32*, 1380−1389.

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Correlation of the Expression of BRAF V600E Mutation With Various Phenotypic Expressions of Thyroid Neoplasms

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Abstract

Aims

We aimed to assess the incidence of the BRAF V600E mutation in thyroid neoplasms at a tertiary care center and its association with various phenotypic features.

Methods and material

We included all cases diagnosed as thyroid neoplasm in the past decade at the Department of Pathology of our institute and obtained their clinical details from the medical records department of the institute after obtaining permission from the authorities and due International Human Epigenome Consortium clearance. We included data on age, sex, clinical presentation, hormone status, and T and N status of the malignant neoplasms. Hematoxylin and eosin (H&E) slides of all cases were evaluated for the type of neoplasm, nuclear features, invasion into the capsule and vascular spaces, extrathyroidal extension, lymph node metastases, mitoses, necrosis, and presence/absence of amyloid. Paraffin blocks of sections with high tumor density and less normal tissue were chosen for evaluation after H&E staining. The slides showing tumors with large areas of hemorrhage, cystic change, or necrosis were excluded. Two primers were used to amplify a 339-bp fragment containing the V600E mutation in exon 15 of BRAF. Tissues were prepared from formalin-fixed paraffin-embedded (FFPE) blocks, and DNA was isolated using a standard protocol BRAF NF and BRAF NR Primer Standardized Protocol For FFPE Tissue DNA. Percentages and tables have been used for data presentation.

Results

Among 47 identified cases, 14 were positive for the BRAF V600E mutation and had papillary carcinoma (n = 9) or follicular neoplasms (n = 5; follicular adenoma, n = 3; follicular carcinoma, n = 2). In the BRAF-positive papillary carcinomas, five cases were aged 20-30 years, eight were female, eight (88.88%) were euthyroid, and one was hypothyroid. Furthermore, 55.55% (5/9 cases) of BRAF-positive cases were stage I, 33.3% (3/9 cases) were stage II, and 0.02% (1/9 cases) were stage III.

Conclusions

In our cohort, 31% of cases of papillary thyroid carcinoma (PTC) and 18.72% of follicular neoplasms expressed the BRAF V600E mutation.

BRAF V600E mutation-positive papillary thyroid carcinomas consistently showed all characteristic nuclear features, such as nuclear crowding, overlapping, and grooves.

Considering the greater prevalence in the younger age group, the importance of mutation surveillance in PTCs for a total thyroidectomy may be warranted in mutation-positive patients.

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Categories: Endocrinology/Diabetes/Metabolism, Pathology, Oncology
Keywords: braf v600e mutation, papillary carcinoma, thyroid gland, thyroid neoplasms, thyroid malignancies

Introduction

Thyroid cancers account for approximately 1% of all malignancies and are one of the most common malignancies of the endocrine system [1,2]. The development of malignancy in the thyroid is influenced by both environmental factors as well as genetic factors. Specifically, the thyroid is vulnerable to genotoxic effects of radioactive iodine and nongenotoxic effects (thyroid-stimulating hormone stimulation) of iodine

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deficiency both of which can lead to neoplastic lesions in the thyroid [3].

Most thyroid tumors can be diagnosed based on morphological assessment and immunohistochemical findings [4,5] As altered gene expression can be discovered before morphological changes, knowledge regarding the genetic background helps in understanding thyroid carcinogenesis and facilitates the planning of appropriate therapeutic strategies and development of drugs that target specific molecules.

Thyroid tumors are classified based on the 2004 World Health Organization guidelines [3]. Recently, the RAS-RAF-MAP kinase signaling pathway has been implicated in many cancers, particularly more frequently in thyroid carcinomas, with the BRAF V600E mutation playing a vital role in papillary thyroid carcinoma. Furthermore, the BRAF mutation is specific for papillary carcinoma, is associated with poorer outcomes, and is frequently noted in high-grade tumors [6-8]

BRAF: an overview

BRAF is a serine-threonine kinase belonging to the family of RAF proteins. It acts upstream of MEK1/2 kinases in response to RAS signals. BRAF mutations are involved in early thyroid carcinogenesis and are somatic genetic alterations rather than germline mutations [9,10]. BRAF mutations occur early and play an important role in the pathogenesis of papillary thyroid carcinoma (PTC). Point mutations of BRAF are the most common types of mutations and occur in approximately 40%-45% of cases [11,12]. Other studies have reported [13,14] BRAF mutation rates of 35%-69% in PTC. The most common BRAF mutation is V600E, which is from missense thymine (T) to adenine (A) transversion at nucleotide 1799 in exon 15. This results in the substitution of valine by glutamate at residue 600. A less common mutation is the K601E mutation found in thyroid cancer [15,16]. Structurally, the RAF protein is divided into two functional domains, namely, the N-terminal and C-terminal regulatory domains, with three conserved regions (CR 1,2, and 3). CR1 and CR2 are present in the N-terminal domain and CR3 in the C-terminal domain [17].

Normally, RAS proteins are attached to the plasma membrane on the cytoplasmic side of the cell, endoplasmic reticulum, and Golgi membrane and are activated by growth factors binding to receptors at the plasma membrane. In an inactive state, RAS proteins bind to guanosine diphosphate (GDP), and upon activation, exchange GDP with guanosine triphosphate (GTP), leading to a conformational change that produces active RAS [2]. This activated RAS binds to the RAS-binding domain in CR1 of RAF, recruits RAF to the membrane, and activates a downstream signaling cascade wherein RAF phosphorylates mitogen-activated protein kinase (MAPK) [17]. Extracellular signal-regulated kinases 1 and 2 (MEK 1 and 2) become activated, which consecutively phosphorylate and activate extracellular signal-regulated kinases 1 and 2 (ERK 1 and 2). Activated ERK migrates to the nucleus where it activates various transcription factors that trigger cell cycle progression and result in cell proliferation and differentiation [8]. The RAF protein has three isoforms, ARAF, BRAF, and CRAF. Among these, BRAF is the most common isoform and most commonly mutated isoform because it has higher basal kinase activity [17] Additionally, BRAF protein expression is higher in hematopoietic cells, neurons, and testicles; is the predominant isoform [18,19] in thyroid follicular cells; and is the most potent activator of the MAPK pathway. Gain-of-function BRAF mutations function as an alternate pathway for aberrant activation of ERK signaling, resulting in constitutive activation of BRAF kinase, i.e., it can phosphorylate MEK as monomers in a RAS-independent manner. Such chronic stimulation of the MAPK pathway results in increased proliferation but reduced survival and differentiation of cells [11]. This signaling cascade is implicated in the etiology of several human cancers including malignant melanoma; thyroid, colorectal, and ovarian carcinomas; and carcinomas of the biliary tract, ovary, and cervix [9,20]

BRAF expression in thyroid carcinomas

Thyroid malignancies are predominantly diagnosed based on morphological assessment alone, and an immunohistochemical study is beneficial in confirming the diagnosis and establishing prognosis if tumor(s) exhibits unusual patterns. A significant improvement has been noted in the knowledge of molecular alterations over the past two decades in all tumors, including thyroid malignancies, and oncogenic BRAF activation represents one of the most prevalent molecular alterations. Reportedly, BRAF mutations are associated with poorer clinicopathological outcomes [21,22]. Among its various subtypes, BRAF most commonly occurred in the tall cell variant of PTC, followed by conventional PTC, and less commonly in the follicular variant of PTC. The tall cell variant is the most aggressive tumor, indicating that BRAF mutations are associated with poorer outcomes [9,23]. Other factors associated with the mutation and aggressive tumor phenotype include older age, extrathyroidal tumor invasion, lymph node, and distant metastasis, higher tumor stage, and poorly differentiated cancer [21,22,24-26].

Frasca et al [27] found that the presence of the BRAF V600E mutation in PTC is associated with aggressive tumor behavior and that tumor aggressiveness is independent of tumor size, suggesting that even small BRAF-positive tumors carry a higher risk of progression and invasiveness than BRAF-negative tumors.

BRAF mutations can be readily tested on thyroid tissue samples obtained by fine-needle aspiration with high preoperative clinicopathological prognostic capabilities in PTC. However, they have limited diagnostic value because [28-30] of low sensitivity in cytologically indeterminate specimens that are mostly non-PTC

and therefore do not harbor the BRAF mutation. Nonetheless, controversies regarding BRAF mutations with poorer clinicopathological outcomes in papillary thyroid cancers have been reported in some studies [31,32]. In the thyroid, apart from papillary carcinomas, BRAF mutations are expressed in anaplastic carcinoma and poorly differentiated carcinoma (prevalence, 20%-30%, and 10%-15%, respectively).

BRAF-mutated, poorly differentiated, anaplastic carcinoma will have a papillary component, suggesting that these tumors progress from BRAF-positive papillary carcinoma [33-36]. The BRAF V600E mutation is not found in follicular thyroid cancers and benign thyroid nodules [23]. However, the other BRAF mutation, K601E, has been detected in follicular adenoma, carcinoma, and follicular variants of PTC. However, Kebebew et al reported a BRAF V600E mutation in one case of follicular carcinoma [22]. Newly developed mitogen extracellular kinase (MEK) inhibitors are particularly promising therapeutic agents for thyroid cancer. With these advances, it has become clearer that BRAF mutation will likely have a significant impact on the clinical management of PTC. Thus, this study aimed to assess the incidence of the BRAF V600E mutation in thyroid neoplasms at a tertiary care center and its association with various phenotypic features.

Materials And Methods

We included all cases diagnosed as thyroid neoplasm in the past decade at the Department of Pathology of our institute and obtained their clinical details from the medical records department of the institute after obtaining permission from the authorities and due International Human Epigenome Consortium clearance (IRB number/Proposal number: 10/098). We included data on age, sex, clinical presentation, hormone status, and T and N status of the malignant neoplasms. Hematoxylin and eosin (H&E) slides of all cases were evaluated for the type of neoplasm, nuclear features, invasion into the capsule and vascular spaces, extrathyroidal extension, lymph node metastases, mitoses, necrosis, and presence/absence of amyloid. Paraffin blocks of sections with high tumor density and less normal tissue were chosen for evaluation after H&E staining. The slides showing tumors with large areas of hemorrhage, cystic change, or necrosis were excluded. Two primers were used to amplify a 339-bp fragment containing the V600E mutation in exon 15 of BRAF. Tissues were prepared from formalin-fixed paraffin-embedded (FFPE) blocks, and DNA was isolated using a standard protocol BRAF NF & BRAF NR Primer Standardized Protocol For FFPE Tissue DNA (Figures *1*-*4*). Percentages and tables have been used for data presentation.

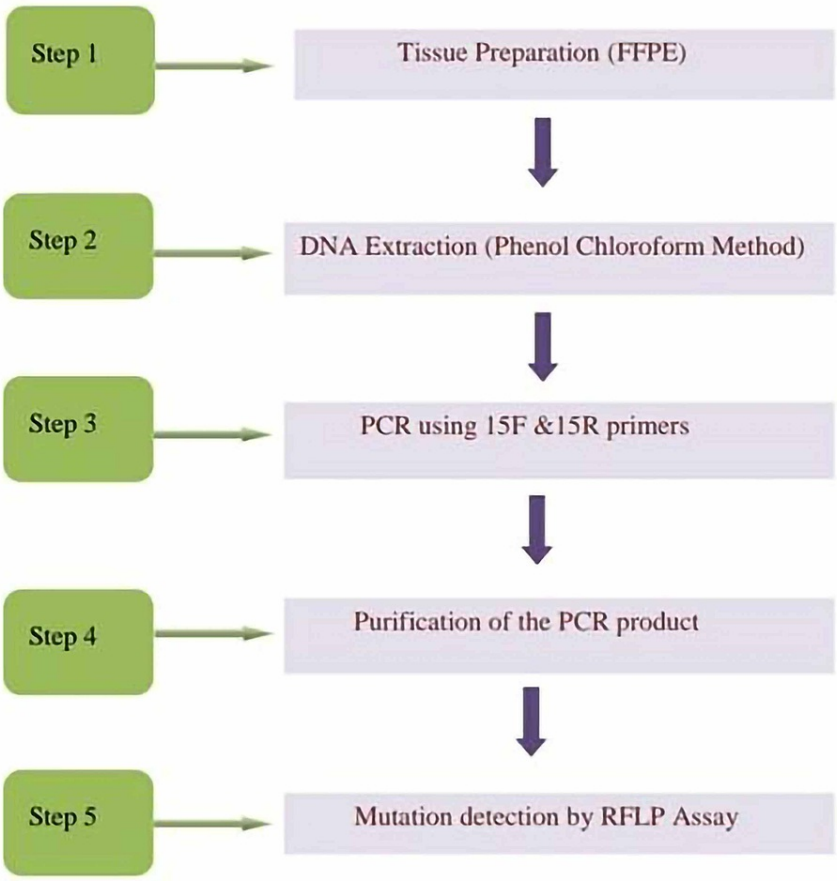


FIGURE 1: Steps in BRAF mutation analysis in various thyroid neoplasms.

FFPE: formalin-fixed paraffin-embedded; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism

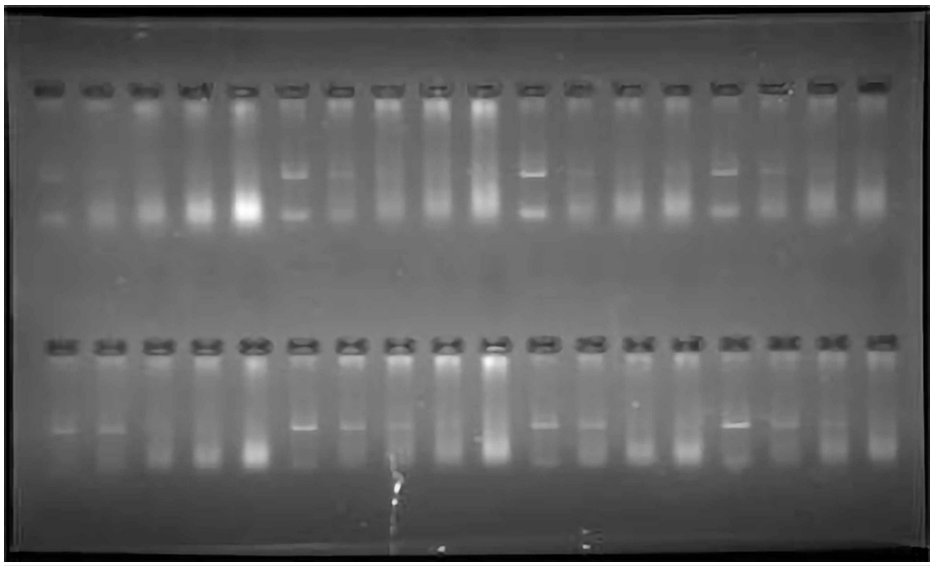


FIGURE 2: Agarose gel electrophoresis: DNA extraction.

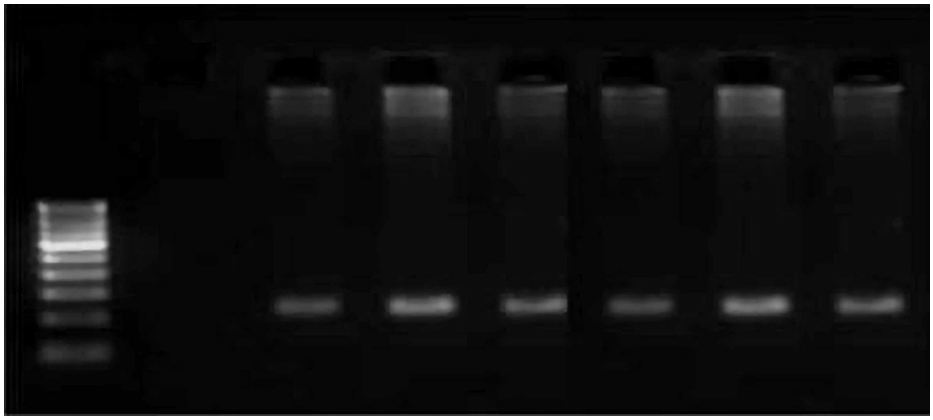


FIGURE 3: Agarose gel electrophoresis: PCR amplification.

PCR: polymerase chain reaction

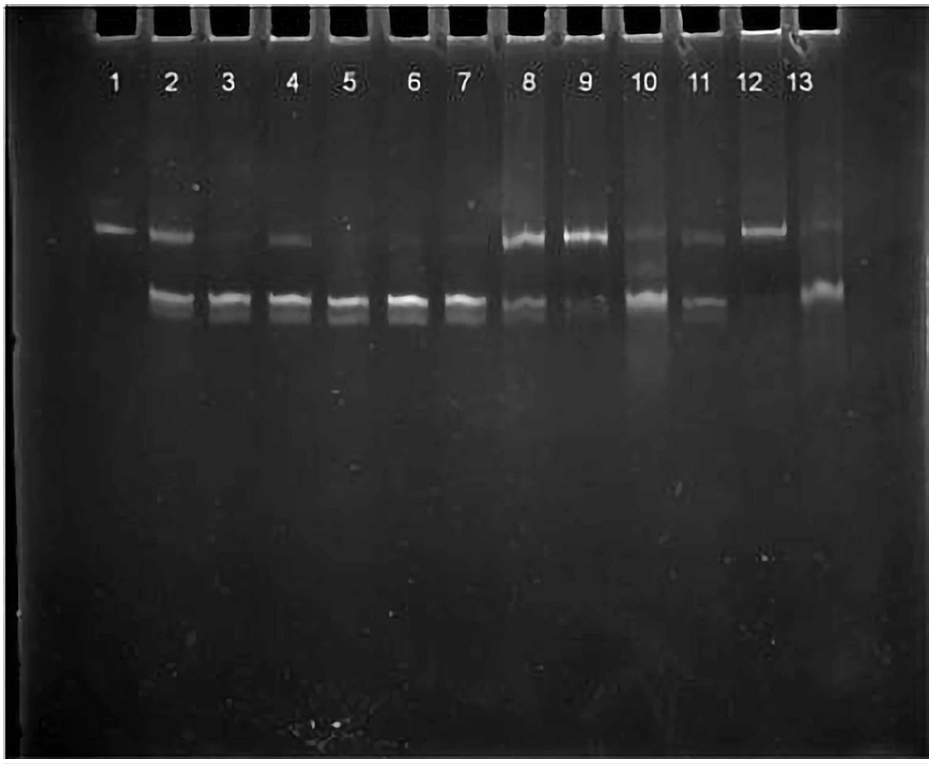


FIGURE 4: Polyacrylamide gel electrophoresis: restriction digestion.

Results

Of the 50 identified cases, DNA could only be extracted from 47 because extraction proved futile in the remaining three cases (Table *1*).

S.No	Age/ Sex	Hormone Status	Size	Other features	Diagnosis	Stage	BRAF
1	48/F	Euthyroid	0.4cm	-	Papillary microcarcinoma	I	Mutant
2	65/F	Hypothyroid	1.3cm	-	Papillary carcinoma	I	Mutant
3	30/F	Euthyroid	0.3cm	-	Encapsulated follicular variant of Papillary carcinoma	I	Mutant

4	28/F	Euthyroid	0.3, 1.4cm	Multifocal, cystic	Papillary carcinoma	I	Mutant
5	32/F	Euthyroid	1cm	Cystic	Papillary carcinoma	I	Mutant
6	48/M	NA	4.2cm	-	Follicular variant of PTC	I	Wild
7	38/M	Euthyroid	0.5cm	-	Papillary microcarcinoma	I	Wild
8	50/F	Euthyroid	0.3-1.0cm	Multifocal	Papillary carcinoma	I	Wild
9	30/F	Euthyroid	0.4cm	-	Papillary microcarcinoma	I	Wild
10	53/M	Euthyroid	2.0cm	-	Papillary carcinoma	I	Wild
11	27/F	Hyperthyroid	0.5cm	-	Papillary microcarcinoma	I	Wild
12	32/F	Euthyroid	1.5cm	-	Papillary carcinoma	I	Wild
13	41/F	Euthyroid	0.3-0.8cm	Multifocal, stromal bone formation, FA	Papillary carcinoma	I	Wild
14	52/F	Euthyroid	0.4-2.3cm	Multifocal	Papillary carcinoma	II	Mutant
15	29/M	Euthyroid	2.5cm	-	Papillary carcinoma	II	Mutant
16	27/F	Euthyroid	2.4cm	-	Papillary carcinoma	II	Mutant
17	49/F	Euthyroid	3.5cm	Multifocal, cystic	Papillary carcinoma	II	Wild
18	51/F	Euthyroid	3cm	Cystic	Papillary carcinoma	II	Wild
19	70/M	Euthyroid	2.0-4.0cm	Multifocal	Papillary carcinoma	II	Wild
20	27/F	Euthyroid	3.0cm	-	Papillary carcinoma	II	Wild
21	45/F	Euthyroid	3.5cm	-	Papillary carcinoma	II	Wild
22	59/M	Euthyroid	1.0-3.0cm	Multifocal	Papillary carcinoma	II	Wild
23	20/F	Euthyroid	3.5cm	Multifocal, capsular and lymph node involvement	Papillary carcinoma	III	Mutant
24	50/F	Euthyroid	NA	Extrathyroidal extension	Papillary carcinoma	III	Wild
25	23/F	Euthyroid	5.5cm	-	Papillary carcinoma	III	Wild
26	46/F	Euthyroid	0.8-5.0cm	Multifocal	Papillary carcinoma	III	Wild
27	24/F	Euthyroid	5.5cm	Cystic	Papillary carcinoma	III	Wild
28	50/M	Euthyroid	2.0cm	Extrathyroidal extension	Papillary carcinoma	III	Wild
29	45/F	NA	3.5cm	lymph node involvement, FA	Papillary carcinoma	III	Wild
30	43/M	Euthyroid	2.5cm	-	Follicular adenoma	-	Mutant
31	58/M	Euthyroid	7.0cm	-	Microfollicular adenoma- signet ring cell type	-	Mutant
32	35/F	Euthyroid	4.0cm	-	Follicular adenoma	-	Mutant
33	32/F	Euthyroid	2.3cm	-	Follicular adenoma	-	Wild
34	35/F	Euthyroid	9.5cm	-	Follicular adenoma	-	Wild
35	34/F	Euthyroid	3.0cm	-	Follicular adenoma	-	Wild
36	43/F	Euthyroid	3.3cm	-	Follicular adenoma	-	Wild
37	36/F	Euthyroid	5.5cm	-	Follicular adenoma	-	Wild

38	45/F	Euthyroid	2.5cm	-	Follicular adenoma	-	Wild
39	34/F	Euthyroid	3.0cm	-	Follicular adenoma	-	Wild
40	35/F	Hyperthyroid	4.5cm	-	Follicular adenoma	-	Wild
41	55/F	Euthyroid	1.0cm	-	Follicular adenoma	-	Wild
42	30/M	Euthyroid	2.6cm	-	Minimally invasive follicular carcinoma	-	Mutant
43	45/F	Euthyroid	6.0cm	-	Hurthle cell neo cap	-	Mutant
44	57/M	Euthyroid	3.0cm	-	FN-LMP	-	Wild
45	30/F	Hypothyroid	3.0cm	-	Minimally invasive follicular carcinoma	-	Wild
46	38/M	Euthyroid	1.8cm	-	Medullary carcinoma	IV A	Wild
47	40/F	Euthyroid	11.5cm	Follicular carcinoma	Anaplastic carcinoma	IVB	Wild

TABLE 1: Master chart showing demographic variables, morphologic subtype of thyroid neoplasms, stage, and mutant status.

PTC: papillary thyroid carcinoma; FN-LMP: follicular neoplasm - low malignant potential

Only 14 cases were positive for the BRAF mutation; of these, nine were of papillary carcinoma (Figure *5*).

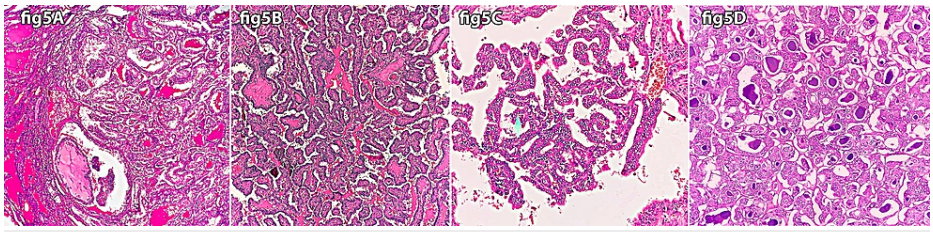


FIGURE 5: Papillary thyroid carcinoma H&E stain (A x10, B x20, C x20, D x20).

A, B, C: Papillary thyroid carcinoma - Showing characteristic papillary architecture, characteristic nuclear features with ground class chromatin, nuclear grooving, nuclear crowding, nuclear overlapping, and intranuclear cytoplasmic pseudo-inclusions.

D: Papillary thyroid carcinoma (follicular variant) - Showing prominent follicular architecture with characteristic nuclear features with ground class chromatin, nuclear grooving, nuclear crowding, nuclear overlapping, and intranuclear cytoplasmic pseudo-inclusions.

Out of the 14 BRAF-positive cases, five were follicular neoplasms, with the latter category comprising three cases of follicular adenomas and two of follicular carcinomas (Tables *1*, *2*) (Figure *6*).

Types of neoplasms	BRAF V600E positive - mutant type (%)	Wild type (%)	Total
Papillary carcinoma: (Conventional papillary thyroid carcinoma - 8 cases) (Encapsulated follicular variant of papillary thyroid carcinoma - 1 case)	9 (31%)	20 (69%)	29
Follicular neoplasm: (Follicular adenoma - 3 cases) (Follicular carcinoma - 2 cases)	5 (31.25%)	22 (68.75%)	16
Medullary carcinoma	0	1 (100%)	1
Anaplastic carcinoma	0	1 (100%)	1
Total	14 (29.8%)	33 (70.2%)	47

TABLE 2: Break up of BRAF V600E-positive cases (n = 47).

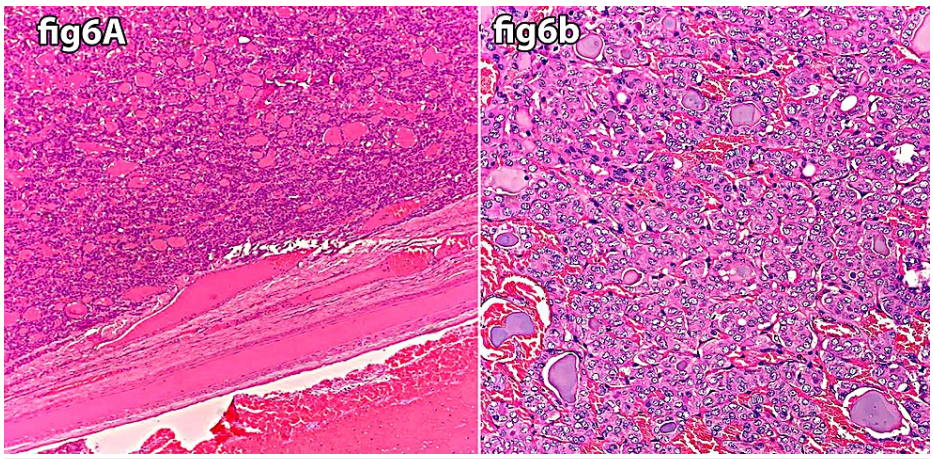


FIGURE 6: Follicular adenoma H&E stain (A x20, B x40).

A: Follicular adenoma showing marked circumscription and encapsulation.

B: Follicular adenoma showing a prominent repetitive microfollicular pattern.

The nine cases of BRAF-positive papillary carcinomas predominantly comprised individuals aged 20-30 years (Tables *1*, *3*), were almost all female (8/9) (Tables *1*, *4*), and were euthyroid (8/9, 88.88%) (Tables *1*, *5*).

Age group	Number of cases
20–30 years	5
31–40 years	1
41–50 years	1
51–60 years	1
61–70 years	1

TABLE 3: Number of BRAF-positive papillary thyroid carcinoma and age distribution.

Male	Female
One case	Eight cases

TABLE 4: BRAF-positive carcinomas and sex distribution.

Number of BRAF-positive cases	Hormonal status
8	Euthyroid
1	Hypothyroid

TABLE 5: BRAF-positive papillary thyroid carcinoma and hormonal status.

Next, eight of the nine cases were euthyroid (88.88%), whereas one was hypothyroid (Tables *1*, *5*). Additionally, 55.55% of BRAF V600E-positive cases were found to be stage I (5/9 cases), 33.33% to be stage II (3/9 cases), and 11.11% to be stage III (1/9 cases) (Tables *1*, *6*).

BRAF-positive papillary thyroid carcinoma staging	Number of cases
Stage I	5
Stage II	3
Stage III	1
Stage IV	0

TABLE 6: BRAF-positive papillary thyroid carcinoma stage at presentation.

Scoring for nuclear and other microscopic features revealed characteristic features compared with other features observed for BRAF-positive papillary carcinomas (Tables *7*, *8*). The scoring was done on a relative qualitative double consensus scale based on mild (Score 1), moderate (Score 2), and severe (Score 3) appearance of the nuclear features.

Histologic nuclear feature	Mild	Moderate	Severe
Nuclear enlargement (NE)	1	2	3
Nuclear irregularity (NI)	1	2	3
Chromatin clearing (CC)	1	2	3
Nuclear crowding (NC)	1	2	3
Nuclear grooves (NG)	1	2	3
Nuclear pseudoinclusions (PI)	1	2	3
Fibrosis (Fib)	1	2	3
Psammoma bodies (PB)	1	2	3

TABLE 7: Relative qualitative double consensus scoring for nuclear features of papillary thyroid carcinoma.

S. No.	Id. No.	NE	NI	CC	NC	NG	PI/10HPF	Fib	PB/10HPF	Inflammatory cell aggregate/10HPF
1	458/06	1	1	2	3	3	0	1	2	1
2	2780/06	2	1	3	3	2	0	0	0	0
3	2874/07	2	1	3	3	2	1	1	0	0
4	1837/08	2	2	1	3	2	2	1	0	0
5	1865/08	2	1	3	2	2	1	1	0	0
6	2222/08	2	1	3	2	1	0	2	2	0
7	229/09	2	0	1	3	2	0	0	0	0
8	1218/09	2	0	1	2	3	0	0	0	0
9	2548/09	3	3	1	1	2	2	3	3	3
Average score		2	1.1	2	2.4	2.1	0.6	1	0.7	0.4

TABLE 8: Scoring for nuclear features and other microscopic features.

NE: nuclear enlargement; NI: nuclear irregularity; CC: chromatin clearing; NC: nuclear crowding; NG: nuclear grooves; PI: nuclear pseudoinclusion; Fib: fibrosis; PB: psammoma bodies; HPF: high-power fields

Discussion

The BRAF V600E mutation is most often expressed in stage III (30%). Importantly, its expression, particularly in the early stages, indicates an aggressive course and warrants a total thyroidectomy, whereas its expression in the later stages is associated with poor outcomes.

In our group of 30 papillary carcinoma cases, DNA could be extracted from 29 and only nine expressed the BRAF V600E mutation. Its prevalence is lower than that reported in the literature, i.e., 40%-45% of PTCs harbor the mutation.

We found that five of the nine cases of BRAF V600E-positive papillary carcinoma were in the 20-30-year age group. Rossella et al found that V600E was expressed more in an older age group [6]. Given the greater prevalence in the younger age group, the importance of mutation surveillance in PTCs for a total thyroidectomy may be warranted in mutation-positive patients.

We found five cases of stage I (55.55%) and three cases of stage II (33.33%) showing BRAF positivity, indicating a significantly higher expression in stages I and II lesions. Therefore, we reanalyzed the reports of PTC.

This high incidence of V600E mutation can be explained by the fact that at a tertiary care hospital, the radiologist and cytopathologist could probably detect PTC at an early stage. Furthermore, the strongest argument against using BRAF mutation as an independent prognostic and predictive factor in PTC is its high prevalence (30%-80%). As the V600E mutation is associated with poor outcomes, these patients require very close follow-up. We found no correlation between nuclear features and BRAF V600E mutations; however, the characteristic nuclear crowding, overlapping, and grooves were consistently observed (Tables *6*, *7*).

The incidence of the BRAF V600E mutation in follicular neoplasms was 31% (five out of 16 cases). In contrast, the literature shows that the V600E mutation is rarely seen in follicular neoplasms, with very few reports that have identified this mutation in follicular neoplasms with minimal incidence [37]. Therefore, a reanalysis of routine H&E slides identified two cases of focal papillary architecture with nuclear features, which might have been overlooked during the morphological assessment. Despite their exclusion, the percentage incidence of the V600E mutation in follicular neoplasm was 18.72% (three out of 16 cases). Nonetheless, papillary carcinoma, particularly the microfollicular type, was possibly missed on routine evaluation as specimens were not extensively sampled, rather were only drawn from the most representative areas of the lesion.

Overall, we show that a significant number of cases of papillary carcinoma (31%; 9/29) and follicular neoplasms (18.7%; 3/16) express the V600E mutation. A larger prospective study, including preoperative cytology specimen and surgically resected specimens of the same patients, is needed to establish the utility of the V600E mutation as a routine diagnostic tool. After development, the primers would be cost-effective

to patients.

An increase in the incidence of papillary carcinoma in areas where there is high iodine intake has been reported [3,38,39] and there is high iodine supplementation to correct iodine deficiency, particularly in the population with an existing V600E mutation. This raises the question of establishing dietary iodine supplementation levels to prevent the incidence of non-neoplastic conditions including multinodular goiter. Furthermore, papillary carcinoma usually remains intra-thyroid and few cases may present with rapid onset of clinical manifestations with enlargement of multiple cervical lymph nodes [40]. Therefore, a larger, multicenter, prospective study that includes cytology specimens collected preoperatively and following surgical resection is essential to establish the relationship between the incidence of papillary carcinoma and V600E mutation in the background of iodine supplementation and deficiency and to prove the utility of BRAF in routine diagnosis. However, the most recent American Thyroid Association (ATA) recommendations do not indicate a routine application of BRAF status for initial risk stratification in differentiated thyroid cancer due to a lack of evident confirmation of a direct influence of mutation on the increase in relapse risk.

Further, considering the increase in the prevalence of the BRAF V600E mutation in follicular neoplasms, a larger study of the V600E mutation in follicular neoplasms is vital to observe changes in the expression patterns of mutations in the Indian population.

Conclusions

In our cohort, 31% of cases of PTC and 18.72% of follicular neoplasms expressed the BRAF V600E mutation.

BRAF V600E mutation-positive PTCs consistently showed all characteristic nuclear features, such as nuclear crowding, overlapping, and grooves.

Considering the greater prevalence in the younger age group, the importance of mutation surveillance in PTCs for a total thyroidectomy may be warranted in mutation-positive patients.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Institutional human ethics committee, PSG institute of medical sciences and research issued approval 10/098. Proposal number / IRB number : 10/098 Project title : A Study of Correlation of the Expression of BRAF V600E Mutation with Various Phenotypic Expressions of the Thyroid Neoplasms. Name of the investigators: Dr H Volga, Dr Subramaniam Ramkumar M.D Name of the guide: Dr Shanthakumari Name of the co-guide: Dr S Ramalingam, Dr Sudha Ramalingam, Dr S Thiagarjan Waiver of consent: Yes Review type: Exempt Date of the meeting: N/A Decision: Approved Approval date: 24.03.2011 Continuing panel review: Not needed. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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References

1. Carcangiu ML: Thyroid gland. Histology for Pathologists, Third Edition. Mills SE (ed): Lippincott Williams & Wilkins, Philadelphia; 2006.
2. DeLellis R, Lloyd RV, Heitz PU, Eng C: World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Endocrine Organs. IARC Press, Lyon; 2004.
3. Abul K, Abbas AM: The endocrine system. Robbins and Cotran Pathological Basis of Disease. Kumar V, Fausto N, Abbas A (ed): Saunders, New York; 2004. 1155-83.
4. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL: Immunohistochemical diagnosis of papillary thyroid carcinoma. Mod Pathol. 2001, 14:338-42. 10.1038/modpathol.3880312
5. Tastekin E, Keskin E, Can N, et al.: CD56, CD57, HBME1, CK19, Galectin-3 and p63 immunohistochemical stains in differentiating diagnosis of thyroid benign/malign lesions and NIFTP. Pol J Pathol. 2019, 70:286-94. 10.5114/pjp.2019.93131
6. Elisei R, Ugolini C, Viola D, et al.: BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. J Clin Endocrinol Metab. 2008, 93:3943-9. 10.1210/jc.2008-0607
7. Al-Salam S, Sharma C, Afandi B, Al Dahmani K, Al-Zahrani AS, Al Shamsi A, Al Kaabi J: BRAF and KRAS mutations in papillary thyroid carcinoma in the United Arab Emirates. PLoS One. 2020, 15:e0231341.

10.1371/journal.pone.0231341
8. Al-Brahim N, Asa SL: Papillary thyroid carcinoma: an overview . Arch Pathol Lab Med. 2006, 130:1057-62. 10.5858/2006-130-1057-PTCAO
9. Xing M: BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev. 2007, 28:742-62. 10.1210/er.2007-0007
10. Singh A, Ham J, Po JW, Niles N, Roberts T, Lee CS: The genomic landscape of thyroid cancer tumourigenesis and implications for immunotherapy. Cells. 2021, 10:1082. 10.3390/cells10051082
11. Nikiforov YE: Molecular diagnostics of thyroid tumors . Arch Pathol Lab Med. 2011, 135:569-77. 10.5858/2010-0664-RAIR.1
12. Qi W, Shi C, Zhang P, Feng L, Wang J, Chen D: Effect of BRAF V600E mutation detection of fine-needle aspiration biopsy on diagnosis and treatment guidance of papillary thyroid carcinoma. Pathol Res Pract. 2020, 216:153037. 10.1016/j.prp.2020.153037
13. Kato MA, Fahey TJ 3rd: Molecular markers in thyroid cancer diagnostics . Surg Clin North Am. 2009, 89:1139-55. 10.1016/j.suc.2009.06.012
14. Ferrari SM, Fallahi P, Ruffilli I, et al.: Molecular testing in the diagnosis of differentiated thyroid carcinomas. Gland Surg. 2018, 7:S19-29. 10.21037/gs.2017.11.07
15. Patel KN, Singh B: Genetic considerations in thyroid cancer . Cancer Control. 2006, 13:111-8. 10.1177/107327480601300205
16. Giordano TJ: Genomic hallmarks of thyroid neoplasia . Annu Rev Pathol. 2018, 13:141-62. 10.1146/annurev-pathol-121808-102139
17. Vakiani E, Solit DB: KRAS and BRAF: drug targets and predictive biomarkers . J Pathol. 2011, 223:219-29. 10.1002/path.2796
18. Al-Humadi H, Zarros A, Al-Saigh R, Liapi C: Genetic basis and gene therapy trials for thyroid cancer . Cancer Genomics Proteomics. 2010, 7:31-49.
19. Laetitia G, Sven S, Fabrice J: Combinatorial therapies in thyroid cancer: an overview of preclinical and clinical progresses. Cells. 2020, 9:830. 10.3390/cells9040830
20. Wan PTC, Garnett MJ, Roe SM, et al.: Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004, 116:855-67.
21. Scheffel RS, de Cristo AP, Romitti M, et al.: The BRAFV600E mutation analysis and risk stratification in papillary thyroid carcinoma. Arch Endocrinol Metab. 2021, 64:751-7.
22. Kebebew E, Weng J, Bauer J, et al.: The prevalence and prognostic value of BRAF mutation in thyroid cancer. Ann Surg. 2007, 246:466-70; discussion 470-1. 10.1097/SLA.0b013e318148563d
23. Kondo T, Ezzat S, Asa SL: Pathogenetic mechanisms in thyroid follicular-cell neoplasia . Nat Rev Cancer. 2006, 6:292-306. 10.1038/nrc1836
24. Nakayama H, Yoshida A, Nakamura Y, et al.: Clinical significance of BRAF (V600E) mutation and Ki-67 labeling index in papillary thyroid carcinomas. Anticancer Res. 2007, 27:3645-9.
25. Chen P, Pan L, Huang W, et al.: BRAF V600E and lymph node metastases in papillary thyroid cancer . Endocr Connect. 2020, 9:999-1008. 10.1530/EC-20-0420
26. Espenbetova M, Krykpayeva A, Zamanbekova Z, Zhumanbaeva Z, Azizov B, Mukanova A, Glushkova N: Analysis of the association of BRAFV600E mutation and Ki-67 overexpression with clinical and pathological characteristics in papillary thyroid cancer. Radiat Environ Biophys. 2021, 60:233-41. 10.1007/s00411-021-00904-y
27. Frasca F, Nucera C, Pellegriti G, et al.: BRAF(V600E) mutation and the biology of papillary thyroid cancer . Endocr Relat Cancer. 2008, 15:191-205. 10.1677/ERC-07-0212
28. Rowe LR, Bentz BG, Bentz JS: Utility of BRAF V600E mutation detection in cytologically indeterminate thyroid nodules. Cytojournal. 2006, 3:10.
29. Zhao CK, Zheng JY, Sun LP, Xu RY, Wei Q, Xu HX: BRAFV600E mutation analysis in fine-needle aspiration cytology specimens for diagnosis of thyroid nodules: the influence of false-positive and false-negative results. Cancer Med. 2019, 8:5577-89. 10.1002/cam4.2478
30. Paek SH, Kim BS, Kang KH, Kim HS: False-negative BRAF V600E mutation results on fine-needle aspiration cytology of papillary thyroid carcinoma. World J Surg Oncol. 2017, 15:202. 10.1186/s12957-017-1266-5
31. Sapio MR, Posca D, Troncone G, et al.: Detection of BRAF mutation in thyroid papillary carcinomas by mutant allele-specific PCR amplification (MASA). Eur J Endocrinol. 2006, 154:341-8. 10.1530/eje.1.02072
32. Costa AM, Herrero A, Fresno MF, Heymann J, Alvarez JA, Cameselle-Teijeiro J, García-Rostán G: BRAF mutation associated with other genetic events identifies a subset of aggressive papillary thyroid carcinoma. Clin Endocrinol (Oxf). 2008, 68:618-34. 10.1111/j.1365-2265.2007.03077.x
33. DeLellis RA: Pathology and genetics of thyroid carcinoma . J Surg Oncol. 2006, 94:662-9. 10.1002/jso.20700
34. Volante M, Lam AK, Papotti M, Tallini G: Molecular pathology of poorly differentiated and anaplastic thyroid cancer: what do pathologists need to know?. Endocr Pathol. 2021, 32:63-76. 10.1007/s12022-021-09665-2
35. Odate T, Oishi N, Kawai M, et al.: Progression of papillary thyroid carcinoma to anaplastic carcinoma in metastatic lymph nodes: solid/insular growth and hobnail cell change in lymph nodes are predictors of subsequent anaplastic transformation. Endocr Pathol. 2021, 10.1007/s12022-021-09674-1
36. Quiros RM, Ding HG, Gattuso P, Prinz RA, Xu X: Evidence that one subset of anaplastic thyroid carcinomas are derived from papillary carcinomas due to BRAF and p53 mutations. Cancer. 2005, 103:2261-8. 10.1002/cncr.21073
37. Soares P, Trovisco V, Rocha AS, et al.: BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene. 2003, 22:4578-80. 10.1038/sj.onc.1206706
38. Harach HR, Escalante DA, Day ES: Thyroid cancer and thyroiditis in Salta, Argentina: a 40-yr study in relation to iodine prophylaxis. Endocr Pathol. 2002, 13:175-81. 10.1385/ep:13:3:175
39. Harach HR, Ceballos GA: Thyroid cancer, thyroiditis and dietary iodine: a review based on the Salta, Argentina model. Endocr Pathol. 2008, 19:209-20. 10.1007/s12022-008-9038-y
40. Jagtap SV, Patil D, Chetan, Gupta SO: Papillary carcinoma thyroid presented with extensive local lymph nodal metastasis. P Arch Cytol Histopathol Res. 2018, 3:113-5. 10.18231/2456-9267.2018.0024

Basidiobolomycosis - Miss Me; It Hurts You

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Abstract

Basidiobolomycosis is a subcutaneous infection of the limbs and trunk caused by *Basidiobolus ranarum*. It is one of the under diagnosed infection which usually occurs in tropical areas. High index of clinical suspicion is needed to diagnose this condition. We hereby report a case of basidiobolomycosis in a 25 year old male who presented to us with a non healing ulcer in left thigh. Biopsy showed granulomatous inflammation with fungal infection suggestive of phycomycosis. Fungal culture grew *Basidiobolus ranarum*. Patient was treated with oral itraconazole. The ulcer resolved completely after 3 months of treatment.

Introduction

Zygomycosis is an infection caused by fungal agents belonging to the phylum Zygomycota. These are saprophytic fungi and are found ubiquitously in the environment. The class Zygomycetes includes two fungal orders: Mucorales and Entomophthorales. They have different pathogenic potentials; the order Mucorales primarily affects the immunocompromised patients causing invasive diseases like rhino cerebral or pulmonary mucormycosis with high mortality, where as the order entomophthorales, which include *Basidiobolus* and *Conidiobolus* genera, cause chronic infection in the subcutaneous tissue in the immune competent individual.[1] Basidiobolomycosis is caused by the fungus *Basidiobolus ranarum*. Here we present a case of subcutaneous basidiobolomycosis which was misdiagnosed as mucormycosis by histopathological examination.

Case Details

A 25 year old male software engineer from Coimbatore was referred to us for intravenous amphotericin B, for the treatment of his non healing ulcer over the anterior aspect of left thigh as biopsy of the lesion was reported as mucormycosis. He had this lesion for the past 3 months which started as a painless nodular swelling (Figure 1A). He had consulted many clinicians earlier and was on multiple antibiotics, but his skin lesion progressively increased in size. He had no co-morbid illness and was apparently normal 3 month back. Ultra sonogram of the thigh was suggestive of abscess in subcutaneous plane. He underwent incision and drainage (I and D) of the abscess which resulted in a deep cavitatory lesion. Culture from the material during I and D was sterile. As the lesion was worsening (Figure 1B) he underwent another debridement. Gram's stain, AFB stain of the tissue didn't reveal any organisms and bacterial culture of the pus was sterile. Biopsy of the ulcer was performed and sent for histopathological examination (HPE). The HPE was reported as extensive ulceration and dense inflammation of dermis and subcutaneous area with fat necrosis and abscess formation composed of neutrophils and eosinophils along with thin walled broad aseptate fungal hyphae branching at right angles and surrounded by fibrosis and dense inflammation with giant cells. PAS stain was positive. Final report was foreign body granuloma with many fungal hyphae resembling mucormycosis. At this point we decided to re-evaluate the patient. On examination, he had a 3×1 cm linear non tender ulcer with surrounding induration on the left thigh (Figure 1C). His complete blood count showed total leukocyte count – 7400 (Neutrophils -50 %, Lymphocytes-34%, Eosinophils-4%, Monocytes-7%), ESR – 24 mm in 1 hour. His blood glucose, renal function test and liver function test were within normal limits. Human Immunodeficiency virus 1 and 2 were non-reactive. Since the history and clinical picture didn't fit with a diagnosis of mucormycosis (the morphology of the lesion, duration of the ulcer), a second opinion of the slide was sought. The histopathology description (Figure 2A, 2B, 2C) included the presence of granulomas in dermis and subcutis associated with the presence of numerous eosinophils. There were also scattered abscesses and eosinophilic material surrounding the fungal hyphae resembling Splendore–Hoeppli phenomenon. A probable diagnosis of subcutaneous phycomycosis was suggested with a comment that fungal culture was required for confirmation. Additional biopsy specimen was collected aseptically for fungal culture from the edge of the lesion. On Sabouraud's Dextrose Agar, furrowed creamy brown, heaped up, radially folded colonies grew after 3 days of incubation (Figure 3). Lactophenol cotton blue wet mount showed aseptate hyphae and smooth walled zygospores with characteristic conjugation beaks which confirmed the fungus to be *Basidiobolus ranarum* (Figure 4). After the diagnosis of basidiobolomycosis was confirmed, the patient was started on oral Itraconazole 200 mg once daily for 3 months. The ulcer healed slowly and complete resolution of the lesion (Figure 1D) was seen after 3 months.

Discussion

Basidiobolomycosis is caused by *Basidiobolus ranarum*, a saprophytic fungus present in soil, decaying fruit and vegetable matter as well as in the gut of amphibians and reptiles.[2] It can cause a variety of clinical manifestations including subcutaneous zygomycosis, gastrointestinal zygomycosis and occasionally an acute systemic illness. The subcutaneous form is a granulomatous infection of the skin and subcutaneous tissues characterized by the formation of firm, painless, disciform nodule generally on the

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Fig. 1: (A) Nodular swelling over thigh; (B) Painless ulcer after I and D; (C) Ulcer with induration after debridement; (D) Healed lesion after treatment

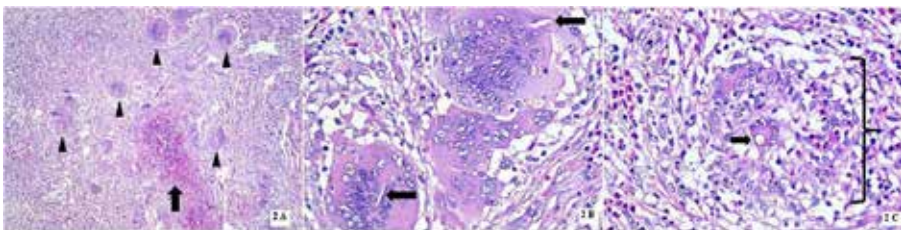


Fig. 2: (A) Low power view showing granulomas with many foreign-body type multinucleated giant cells (arrowheads) and Splendore-hoeppli phenomenon (arrow). H and E; (B) High power view showing giant cells with fragments of broad, aseptate fungal hyphae (arrows) in the cytoplasm. H and E; (C) High power view showing a granuloma (bracket) containing many Eosinophils and a giant cell with cross section of fungal hyphae (arrow) in the cytoplasm. H and E

thighs and buttocks in a "bathing suit" distribution.[1] It mainly affects young, male and immunocompetent persons. It is usually transmitted by minor trauma which may be through insect bite, intravenous catheter, or even intramuscular injection. Clinically it starts as small hardened swelling over the extremities and progressively increases in size to form a painless, non tender swelling. Macroscopically it closely mimics a soft tissue tumor, synovial sarcoma, Burkitt's lymphoma or histopathologically mimics mucormycosis. The other differential diagnosis would be atypical mycobacterial infections, sporotrichosis and parasitic infections like oncocerciais.[3]

In our patient also it started as a small painless swelling and progressively increased in size. Even though the histopathology was reported as mucormycosis, the possibility of mucormycosis could be ruled out by the absence of features such as angio/tissue invasion and necrosis; and slow growing nature of the lesion. Laboratory diagnosis is mainly based on histopathological features and culture of the organism which is the "gold standard". Histologically, basidiobolomycosis is associated with eosinophilic infiltration; with short broad aseptate hyphae.[4] Some may be surrounded by an eosinophilic material which is called as 'Splendore – Hoeppli' phenomenon.[5]

Most patients with basidiobolomycosis respond well to oral itraconazole. The other treatment options are potassium iodide, trimethoprim-sulfamethoxazole. Some strains do not respond to amphotericin B. In the present case also the patient responded well to itraconazole and the ulcer got completely resolved after three months of treatment.

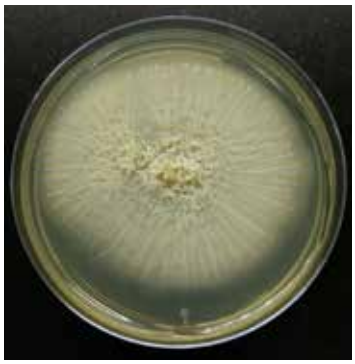


Fig. 3: Growth of basidiobolus ranarum on Sabouraud's dextrose agar appearing yellowish grey rugose and smooth colonies with a waxy texture on the obverse end

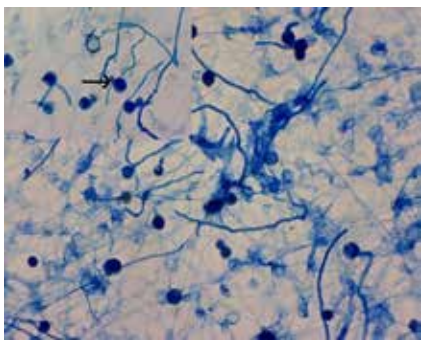


Fig. 4: Lacto phenol cotton blue staining of basidiobolus ranarum. Non septate broad hyphae and zygospores with conjugation beaks seen (Insert - arrow) X 400 magnification

Conclusion

This case is presented to insist on the importance of clinical correlation of histopathology reports along with proper sampling and appropriate microbiological test while treating rare infections.

References

1. Anaparthy UR, Deepika G. A case of subcutaneous zygomycosis. *Indian Dermatol* 2014; 5:51–4.
2. Ellis DH. Subcutaneous zygomycosis. In: Merz WG, Hay RJ, editors. Topley and Wilson's: Microbiology and microbial infections - Medical mycology. 10 th ed. London: Hodder Arnold; 2005:348.
3. Mondal AK, Saha A, Seth J, Mukherjee S. Subcutaneous zygomycosis: A report of one case responding excellently to potassium iodide. *Indian J Dermatol* 2015; 60:500-2.
4. Khan ZU, Khoursheed M, Makar R, Al-Waheeb S, Al-Bader I, Al-Muzaini A, et al. Basidiobolus ranarum as an etiologic agent of gastrointestinal zygomycosis. *J Clin Microbiol* 2001; 39:2360-3.
5. Anand M, Deshmukh SD, Pande DP, Naik S, Ghadage DP. Subcutaneous Zygomycosis Due to Basidiobolus ranarum: A Case Report from Maharastra, India. *Journal of Tropical Medicine* vol. 2010. Article ID 950390, 3 pages, 2010. doi:10.1155/2010/950390.

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Case Report

Erythrokeratoderma En Cocardes – A Rare Phenotype

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Abstract

Erythrokeratodermas are disorders of keratinization with two major types, namely erythrokeratodermia variabilis and symmetrical progressive erythrokeratoderma. Apart from the two main types, rare variants include erythrokeratoderma en cocardes (EEC), erythokeratoderma with erythema gyratum repens-like lesions, erythema annulare centrifugum-like lesions, and reticulate erythrokeratoderma. Herein, we report a rare case of EEC with associated palmoplantar keratoderma, the association which has not been described in previous reports.

Keywords: *En cocardes, erythrokeratoderma, palmoplantar keratoderma*

Introduction

Erythrokeratodermas are disorders of keratinization which encompass two main subtypes, namely erythrokeratodermia variabilis (EKV) and symmetric progressive erythrokeratoderma (SPEK) and other atypical rare variants.[1] Erythrokeratoderma en cocardes (EEC) is a rare variant characterized by polycyclic, concentric, and targetoid lesions along with erythematous plaques.[2]

Case Report

A 4-year-old boy born out of third-degree consanguineous marriage presented with multiple hyperpigmented scaly lesions involving the arms, forearms, back, gluteal region, thighs, and legs since 6 months of his age. There was a history of aggravation of lesions during cold season and improvement during summer. There was no history of atopy and other systemic symptoms. Milestones were achieved normally. There was no history of similar skin lesions in siblings or other family members.

Cutaneous examination revealed multiple scaly plaques with polycyclic borders involving the arms, forearms, thighs, and legs [Figures 1a,b and 2]. In the posterior aspect of the thigh, there were scaly plaques with polycyclic borders sparing of the popliteal fossa [Figure 3]. There were multiple discrete annular scaly plaques with concentric rings involving the forearms and thighs. Anterior aspect of legs showed diffuse ichthyosis extending to the dorsum of the feet [Figure 4]. Palms and soles revealed diffuse keratoderma with fissuring and scaling [Figure 5]. Examination of hair, nails, and teeth was normal. Systemic examination was essentially normal. Hematological examination did not reveal any abnormality. Scrapings of skin for potassium hydroxide mount were negative for fungal elements.

Histopathology revealed hyperkeratosis, focal mild parakeratosis, conspicuous granular layer, and irregular acanthosis. There were mild spongiosis and lymphocytic exocytosis. The papillary and upper dermis showed pigment incontinence, prominent capillaries, and a mild perivascular lymphohistiocytic infiltrate, a picture compatible with erythokeratoderma [Figure 6].

Owing to the distinct clinical morphology with annular lesions, polycyclic borders, and histopathological features, a diagnosis of EEC was entertained.

Discussion

Erythrokeratoderma has two variants: SPEK and EKV. SPEK is characterized by well-demarcated, hyperkeratotic plaques distributed symmetrically over the elbows, knees, dorsal aspect of the hands, feet, and buttocks, sparing the trunk. SPEK has its onset in infancy or early childhood.[3] EKV, on the other hand, manifests as two distinct clinical pictures: with generalized, erythematous, hyperkeratotic plaques in one and localized sharply demarcated

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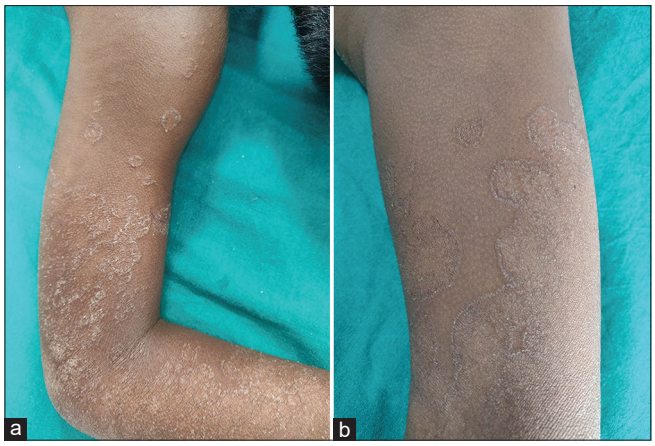


Figure 1: (a) Multiple scaly plaques with polycyclic borders involving the arm and forearm, (b) Scaly plaques with polycyclic borders involving the arm



Figure 2: Scaly plaques with polycyclic borders involving the anterior aspect of thighs

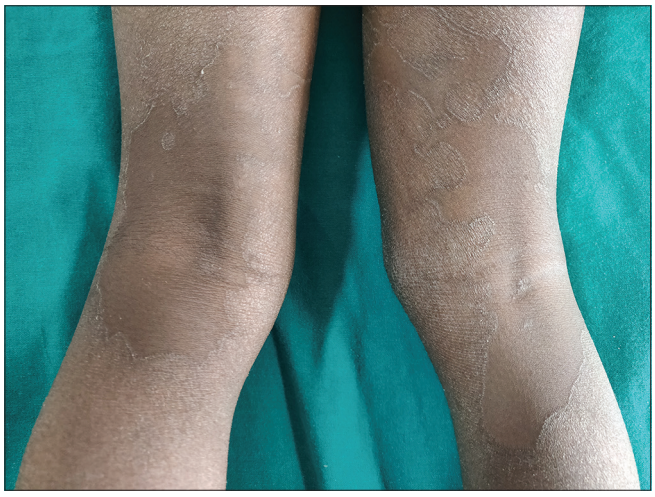


Figure 3: Scaly plaques with polycyclic borders involving the posterior aspect of thighs sparing the popliteal fossa



Figure 4: Diffuse ichthyosis of legs extending to dorsum of feet



Figure 5: Diffuse palmoplantar keratoderma

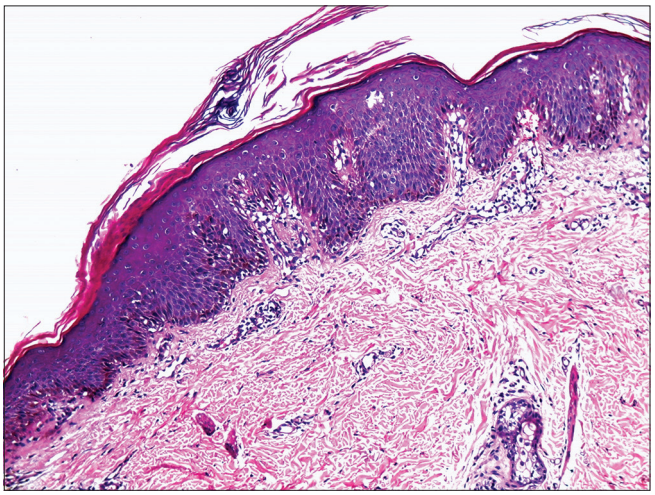


Figure 6: Epidermis with hyperkeratosis, focal mild parakeratosis, conspicuous granular layer, and irregular acanthosis. Dermis with mild perivascular lymphohistiocytic infiltrates.(H and E, ×100)

hyperkeratotic plaques in the other. EKV has its onset in birth or in neonatal period in 50% of patients and within 1st year of life in 90% of patients.[4]

Rare variants of EKV described include EEC, EKV with erythema gyratum repens-like lesions, EKV with

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erythema annulare centrifugum-like lesions, and reticulate erythrokeratoderma.[4,5] Degos *et al*., in 1947, described EEC in a 13-year-old girl, a rare and atypical variant of EKV with polycyclic plaques, with concentric layers of scaling and erythema giving a targetoid, or "en cocardes" appearance.[6]

The index case described by Degos had an onset at birth, remissions and recurrences, desquamation of palm, similar skin lesions in father and paternal uncle pointing to autosomal-dominant inheritance, and improvement of skin lesions during summer.[7] The present case had onset during 6 months of age, without any family history and improvement during summer. Unlike the index case, the cases reported by Rajagopalan *et al*. and López-Ferrer *et al*. did not show improvement of lesions during the summer.[1,2] Furthermore, the present case showed prominent palmoplantar keratoderma, which has not been described in previous reports.[1,2,8]

Apart from EEC, differential diagnoses considered include annular type of epidermolytic ichthyosis, X-linked recessive ichthyosis, and SPEK. Unlike, in annular epidermolytic ichthyosis, there was no history of blisters and erosions at birth, and histopathology did not revealed any epidermolytic hyperkeratosis. X-linked recessive ichthyosis presents with dark brown ichthyotic scales with sparing of popliteal and antecubital fossa with associated cryptorchidism, autism, and deep stromal corneal opacity.[4] Although the present case had ichthyosis of legs with sparing of popliteal fossa, there were no associated systemic abnormalities. Tinea imbricata can be a morphological differential diagnosis, but the presence of skin lesions since 6 months of age and negative test for skin scrapings for fungus preclude the condition.

Histopathology cannot distinguish between the variants of erythrokeratodermas. Genetic studies show mutation in connexin genes in cases of EKV. Connexins are a family of transmembrane proteins that form gap junctions in the cells. These intercellular channels play an important role in cell growth, homeostasis, and epidermal differentiation.[4] Mutations in genes, namely GJB3, GJB4, and rarely GJA1 encoding the connexins, have been identified as the underlying pathogenesis in EKV.[3,9] In SPEK apart from GJB4 mutation, mutations reported include a frameshift mutation in loricrin gene, homozygous loss of function mutation in KRT83 gene, and mutation in KDSR gene which codes for an enzyme involved in ceramide synthesis.[3] López-Ferrer *et al*. have reported a case of EEC with missense mutation R32W in GJB3 gene.[1] Similar mutation in GJB3 gene had been reported in patients with palmoplantar involvement and deafness.[10] The present case had clinical lesions analogous to EEC and palmoplantar involvement but without any hearing impairment. However, genetic study for mutation analysis was not done due to resource constraints.

Treatment options include topical emollients, retinoids, salicylic acid, and corticosteroids. Systemic retinoid such as acitretin and isotretinoin shows good improvement, but with recurrence of lesions once the treatment is stopped.[8] Improvement with retinoids with psoralen and ultraviolet A therapy has been reported.[11] Since the disease is a genodermatoses, parents should be counseled regarding the chronic nature of the disease and the need for long-term follow-up. Treatment plan with acitretin was considered, but the patient did not report for follow-up.

The present case is highlighted for its distinct clinical picture and its association with palmoplantar keratoderma.

Declaration of patient consent

Author certify that they have obtained all appropriate patient consent forms. In the form the patients/parents have given their images and other clinical informations to be reported in the journal. The patients & parents understand that their/their children's names and initials will not be published and due efforts will be made to conceal their identity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. López-Ferrer A, Puig L, Nakamura M, Fernández-Figueras MT, Alomar A. Erythrokeratoderma en cocardes with R32W mutation in GJB3. J Clin Exp Dermatol Res 2010;1:113.
2. Rajagopalan B, Pulimood S, George S, Jacob M. Erythrokeratoderma en cocardes. Clin Exp Dermatol 1999;24:173-4.
3. Richard G, Ringpfeil F. Ichthyoses, Erythrokeratodermas, and Related Disorders. In: Bolognia JL, Schaffer JV, Cerroni L, editors. Dermatology. 4th ed. Philadelphia: Mosby Elsevier; 2018. p. 912-6.
4. Fleckman P, DiGiovanna JJ. The Ichthyoses. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, editors. Fitzpatrick's Dermatology in General Medicine. 8th ed. New York: McGraw Hill; 2012. p. 530-1.
5. Karadag AS, Bilgili SG, Calka O, Bayram I. Erythrokeratodermia variabilis: Two case reports. Indian Dermatol Online J 2013;4:340-3.
6. Degos R, Delzant O, Morival H. Erytheme desquamatif en plaques, congenital et familial (genodermatose nouvelle?). Bull Soc Fr Dermatol Syphiligr 1947;54:442.
7. Cram DL. Erythrokeratoderma variabilis and variable circinate erythrokeratodermas. Arch Dermatol 1970;101:68-73.
8. Srinivas SM, Dhar S. Erythrokeratodermia variabilis and erythrokeratoderma en cocardes: Case series with review of literature. Indian J Paediatr Dermatol 2016;17:202-5.
9. Richard G, Brown N, Smith LE, Terrinoni A, Melino G, Mackie RM, *et al*. The spectrum of mutations in erythrokeratodermias—novel and de novo mutations in GJB3. Hum Genet 2000;106:321-9.
10. Kelsell DP, Wilgoss AL, Richard G, Stevens HP, Munro CS, Leigh IM. Connexin mutations associated with palmoplantar keratoderma and profound deafness in a single family. Eur J Hum Genet 2000;8:469-72.
11. Yüksek J, Sezer E, Köseoğlu D Markoç F, Yildiz H. Erythrokeratodermia variabilis: Successful treatment with retinoid plus psoralen and ultraviolet A therapy. J Dermatol 2011;38:725-7.

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Hemophagocytic Lymphohistiocytosis Complicating Methotrexate Toxicity

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Abstract

Hemophagocytic syndrome or hemophagocytic lymphohistiocytosis (HLH) is a rare but potentially fatal disease caused by dysregulated activation of macrophages against one's blood cells. Major pathologic feature of HLH is hemophagocytosis. We present a case of HLH complicating methotrexate toxicity in a 65-year-old psoriatic patient with history of renal disease. Diagnosis of HLH was established as he fulfilled five out of eight HLH diagnostic criteria. This case report is presented to enlighten clinicians about the clinical entity of HLH and to suspect and recognize this rare and generally fatal disease at the earliest.

Keywords: *Cytopenias*, *hemophagocytic lymphohistiocytosis*, *hemophagocytic syndrome*

Introduction

Hemophagocytic syndrome or hemophagocytic lymphohistiocytosis is a clinical syndrome of hyperinflammation resulting in uncontrolled and ineffective immune response.[1,2] It may be primary or secondary. Primary HLH is a genetic entity. Secondary HLH is associated with infections, malignancies, immune disorders, or drugs.[2,3,4,5] In both types, activated macrophages engulf erythrocytes, leukocytes, platelets, and precursor cells in bone marrow, liver, and lymph nodes.[6] Most severe forms of the disease lead to a sepsis like picture and multiorgan dysfunction. HLH is a disease of rapid progression and has high mortality despite treatment.[3,7,8,9] We report a case of secondary HLH complicating methotrexate toxicity and sepsis.

Case History

A 65-year-old male, a case of chronic plaque psoriasis on intermittent therapy with methotrexate since 15 years and chronic kidney disease since 5 years presented with fever, severe body ache, and ulcerative lesions over lips and trunk. He developed these symptoms 5 days after inadvertent intake of tablet methotrexate 5 mg for 5 consecutive days. Examination showed tender inguinal and axillary lymphadenopathy, hemorrhagic crusting of lips, and necrotic ulcers over psoriatic plaques and as well as normal skin with severe skin tenderness and positive Nikolsky's sign. Since ulcers were present over

psoriatic plaques, methotrexate-induced skin necrosis was suspected. Histopathology findings were skin with detached stratum corneum, large subcorneal pustule [Figure 1a], mild spongiosis, marked regenerative atypia, basal vacuolar degeneration, pigment incontinence, and apoptotic keratinocytes [Figure 1b]. Routine investigations showed bicytopenia with hemoglobin of 9.6 g/dl, WBC of 600 cells/mm^3 with normal platelet count of 120,000 cells/mm^3. His renal function tests were altered with elevated urea (106 mg/dl) and creatinine levels (3.16 mg/dl). The patient was started on antibiotics, leucovorin rescue therapy (folinic acid 10 mg every 6^{th} hourly), and injection Filgrastim (Granulocyte colony-stimulating factor) 300 mg subcutaneously daily. There was worsening pancytopenia and renal function and further developed splenomegaly, features of sepsis. Blood culture was positive for Group B streptococci and methicillin sensitive *staphylococcus aureus* and procalcitonin levels were elevated (24.5 ng/ml). His antibiotics were upgraded and platelet transfusion given in spite of which patient developed fever, tachycardia, hypotension with further decrease in platelet count to 6,000 cells/mm^3 on 5^{th} day. Secondary HLH was suspected and a workup was rapidly initiated based on HLH 2004 diagnostic criteria. Peripheral smear revealed pancytopenia [Figure 2]. Bone marrow aspiration was done which showed megaloblastic erythroid maturation, maturation arrest in the myeloid series, and hemophagocytosis [Figure 3a and b]. Patient had hyperferritinemia (>500 mcg/L) with normal fibrinogen (522 mg/dl) and triglycerides (50 mg/dl) levels. He thus fulfilled 5 out of 8 diagnostic criteria of HLH and was treated as per HLH 2004 protocol with systemic antifungals and injection dexamethasone 10 mg/m^2. Despite all supportive measures, patient developed multiorgan dysfunction and succumbed.

Discussion

HLH is a rare disease characterized by cytokine overproduction and tissue infiltration with cytotoxic lymphocytes and activated macrophages resulting in fever, hepatosplenomegaly, and cytopenias.[1,2,9] A strict diagnostic criteria of HLH was given in 2004 by Histiocyte Society and is as follows.[9,10] Molecular diagnosis consistent with HLH that is the identification of mutations of PRF1, UNC13D, or STX11 or patient should fulfill five out of the eight criteria: fever (>100.4°F), splenomegaly, cytopenia affecting at least two of three lineages: Hb <9 g/dl (in infants <4 weeks: Hb <10 g/100 ml, platelets <100 × 10^9/L, Neutrophils <1 × 10^9/L), hypertriglyceridemia (fasting >265 mg/dl) and or hypofibrinogenemia (≤150 mg/dl), ferritin >500 ng/ml, hemophagocytosis in bone marrow, spleen, or lymph nodes, low or absent NK cell activity, soluble CD25 (soluble IL-2 receptor) >2400 U/ml and in the case of familial HLH there should be no evidence of malignancy.

This patient presented with features of methotrexate toxicity with skin necrosis, bone marrow suppression, and renal failure. His progressive pancytopenia was initially thought to be due to high dose methotrexate. He did not respond to leucovorin rescue therapy or injection Filgrastim and worsened clinically with features suggestive of sepsis due to which antibiotics were upgraded. He was evaluated for HLH as he had progressive pancytopenia unresponsive to treatment and decreased counts in spite of platelet transfusions. The major pathologic feature of HLH is hemophagocytosis as seen in our patient. It is also seen in conditions like sepsis and multiorgan failure.[3] There is considerable clinical and laboratory overlap between sepsis and HLH.[8,9] Diagnosis of HLH was established in our patient as he fulfilled five out of eight diagnostic criteria of HLH. This is a rare presentation of HLH complicating methotrexate toxicity. This case report is presented to enlighten clinicians about HLH inorder to reduce the mortality associated with the disease.

Declaration of patient consent The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest There are no conflicts of interest.

References

1. Janka G. Hemophagocytic lymphohistiocytosis: When the immune system runs amok. Klin Padiatr. 2009;221:278–85. [PubMed: 19707989]

2. Rosado FG, Kim AS. Hemophagocytic lymphocytosis: An update on diagnosis and pathogenesis. Am J Clin Pathol. 2013;139:713–27. [PubMed: 23690113]

3. Gupta S, Weitzman S. Primary and secondary hemophagocytic lymphohistiocytosis: Clinical features, pathogenesis and therapy. Expert Rev Clin Immunol. 2010;6:137–54. [PubMed: 20383897]

4. Mehta RS, Smith RE. Hemophagocytic lymphohistiocytosis (HLH): A review of literature. Med Oncol. 2013;30:740. [PubMed: 24105023]

5. Freeman HR, Ramanan AV. Review of haemophagocytic lymphohistiocytosis. Arch Dis Child. 2011;96:688–93. [PubMed: 20584844]

6. Favara BE. Hemophagocytic lymphohistiocytosis: A hemophagocytic syndrome. Semin Diagn Pathol. 1992;9:63–74. [PubMed: 1561489]

7. Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C. Infections associated with haemophagocytic syndrome. Lancet Infect Dis. 2007;7:814–22. [PMCID: PMC7185531] [PubMed: 18045564]

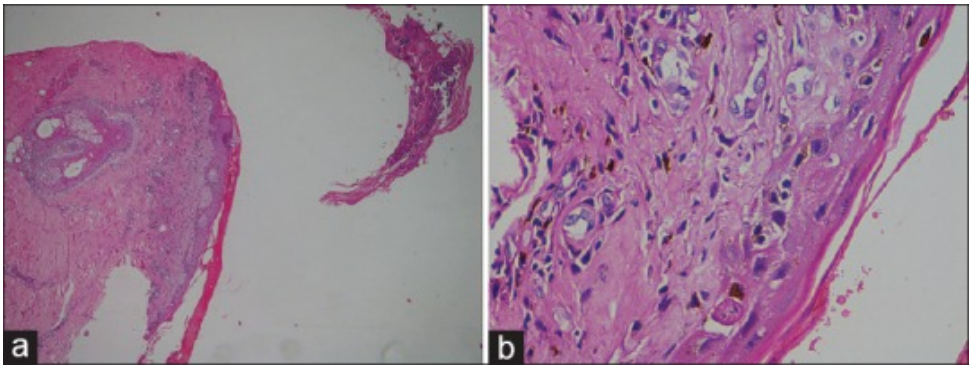
8. Price B, Lines J, Lewis D, Holland N. Haemophagocytic lymphohistiocytosis: A fulminant syndrome associated with multiorgan failure and high mortality that frequently masquerades as sepsis and shock. S Afr Med J. 2014;104:401–6. [PubMed: 25214245]

9. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48:124–31. [PubMed: 16937360]

10. Henter JI, Tondini C, Pritchard J. Histiocyte disorders. Crit Rev Oncol Hematol. 2004;50:157–74. [PubMed: 15157664]

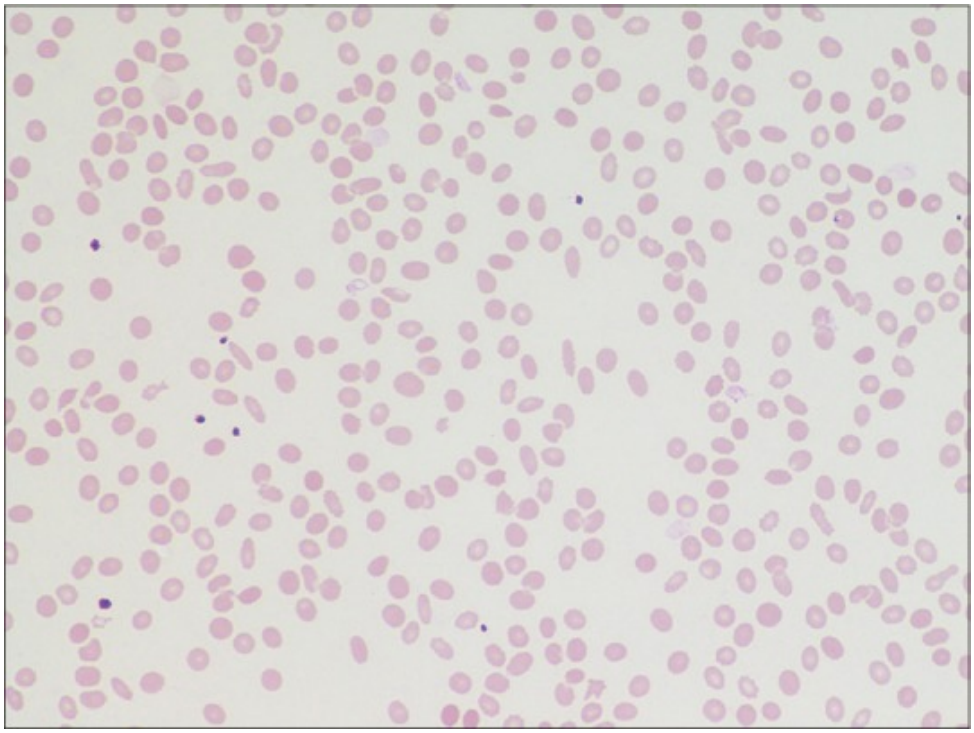
Figures and Tables

Figure 1



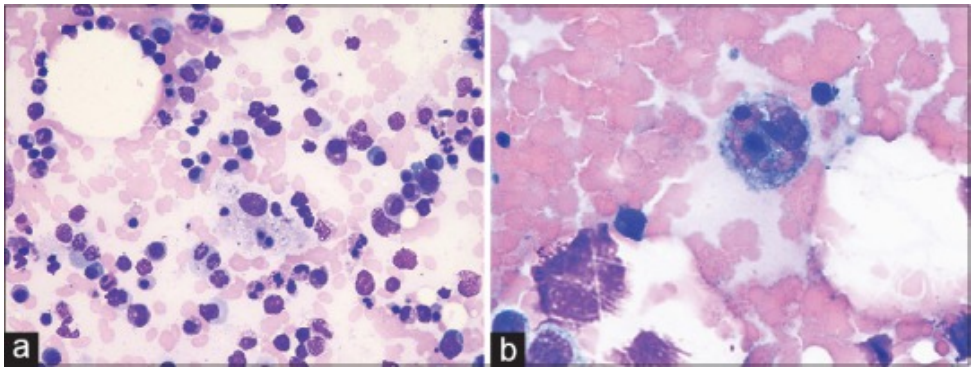
(a) Skin with detached stratum corneum, large subcorneal pustule (H and E, ×4). (b) Mild spongiosis, marked regenerative atypia, basal vacuolar degeneration, pigment incontinence and apoptotic keratinocytes (H and E, ×40)

Figure 2



Peripheral smear showing pancytopenia (Giemsa ×40)

Figure 3



Bone marrow showing hemophagocytosis. (a) Giemsa ×40. (b) Oil immersion ×100)

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Case Report

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Pathology Section

Pancreatic Plasmacytoma Misdiagnosed as Neuroendocrine Tumour: A Pathologist's Dilemma

SAKTHISANKARI SHANMUGASUNDARAM[1], UMAMAHESWARI GURUSAMY[2], PRASANNA N KUMAR[3], BIKU JOSEPH JOHN[4]

ABSTRACT

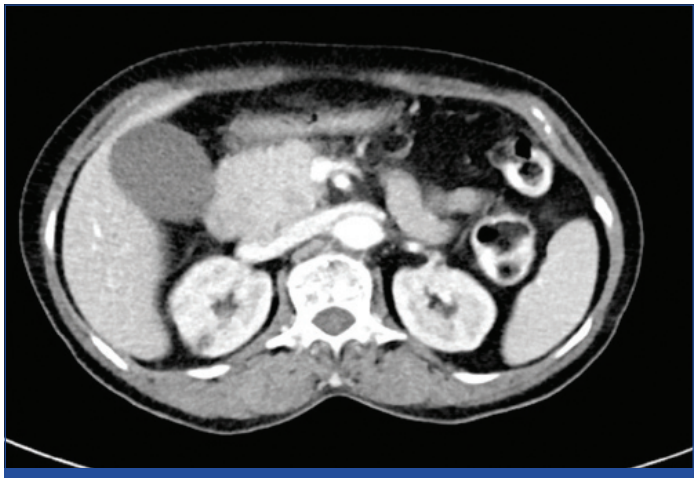
Extramedullary plasmacytomas are rare, constituting to about 5% of plasma cell neoplasms. Pancreatic involvement is uncommon constituting to about 2.3%. Localised solitary plasmacytomas has a good response to radiation. When they are associated with pre-existing multiple myeloma, their prognosis is poor. A case of plasmacytoma of pancreas presenting with obstructive jaundice preceding plasma cell leukaemia is presented here. The lesion was misdiagnosed by fine needle aspiration cytology as neuroendocrine neoplasm. The patient did not present with symptoms of myeloma initially. The present case highlights the significance of considering other causes of pancreatic mass than primary carcinomas.

Keywords: Extramedullary plasmacytoma, Pancreatic neoplasm, Plasma cell leukaemia

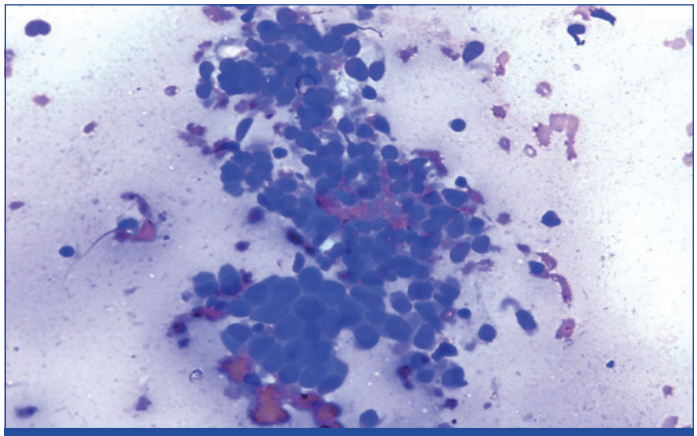
CASE REPORT

A 62-year-old female patient presented with complaints on and off fever from past 15 days. A vague, non-radiating, intermittent paraumbilical pain in right upper quadrant, and vomiting from past five days. There was no history of gastrointestinal bleed, loss of weight and loss of appetite. She was a known hypertensive and diabetic for five years and was on treatment with oral hypoglycemics and antihypertensives. Her vitals were within normal limits and there was no organomegaly.

Ultrasound abdomen showed a hypoechoic mass in the pancreatic head dilating the main pancreatic duct. Computed Tomography (CT) revealed an isodense mass lesion with well defined borders in the head of pancreas measuring 4.8×5.5 cm [Table/Fig-1]. Acoustic Radiation Force Impulse (ARFI) elastography showed mean shear velocity of 3.6. The provisional diagnosis included primary pancreatic tumour. Endoscopic Ultrasound Guided Fine Needle Aspiration Cytology (EUS-FNAC) was planned for the evaluation of the pancreatic mass. EUS showed a 3×3.1 cm iso and hypoechoic mass in the head of pancreas with dilated common bile duct measuring 9 mm in the suprapancreatic portion. Dilated main pancreatic duct (6.5 mm) distal to the mass in the pancreas was identified. Visualised tail of pancreas and portal vein appeared normal. Cytological examination of the mass showed sheets and scattered plasmacytoid cells suspicious of a neuroendocrine neoplasm [Table/Fig-2].



[Table/Fig-1]: CT scan showing isodense lesion in head of pancreas.



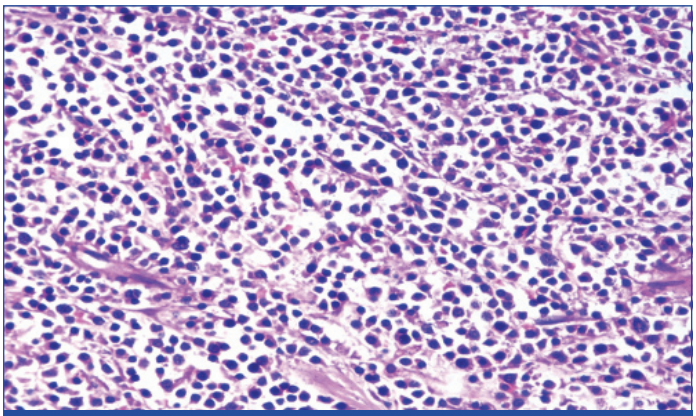
[Table/Fig-2]: Cytology smears showing plasmacytoid cells (40x).

Whipple resection was done with a clinical and cytological diagnosis of a pancreatic neuroendocrine tumour. Pancreas showed an ill-defined mass occupying the head [Table/Fig-3]. Haematoxylin & Eosin (H&E) sections of the mass showed a diffusely infiltrating neoplasm composed of monotonous, plasmacytoid cells infiltrating the acini and ducts [Table/Fig-4]. Few binucleate and multinucleate plasma cells and lymphocytes were present. Morphological differential diagnosis included plasmacytoma and lymphoplasmacytic lymphoma. Immunohistochemical staining was done for confirmation of diagnosis. The tumour cells showed strong membranous positivity for CD138 [Table/Fig-5], CD45 and selective Kappa staining [Table/Fig-6]. They were negative for CD20 and Lambda immunostains. A diagnosis of Plasmacytoma was made and radiological and biochemical workup for multiple myeloma was suggested. Protein electrophoresis was normal. Urine was negative for Bence Jones Protein. Serum calcium and renal parameters were normal. CT spine survey did not reveal any osteolytic lesions. The case was treated as a solitary extramedullary plasmacytoma and follow-up was suggested. A month later, the patient developed severe anaemia (Hb-6.8 g/dL). Total count was elevated and peripheral smear showed 40% plasma cells suggestive of plasma cell leukaemia [Table/Fig-7]. Reviewing the patient's peripheral smear and complete haemogram taken a month back, there was normocytic normochromic anaemia and no demonstrable plasma cells in peripheral smear. ESR was elevated (93 mm/hr). Bone marrow aspiration done showed infiltration by sheets of immature

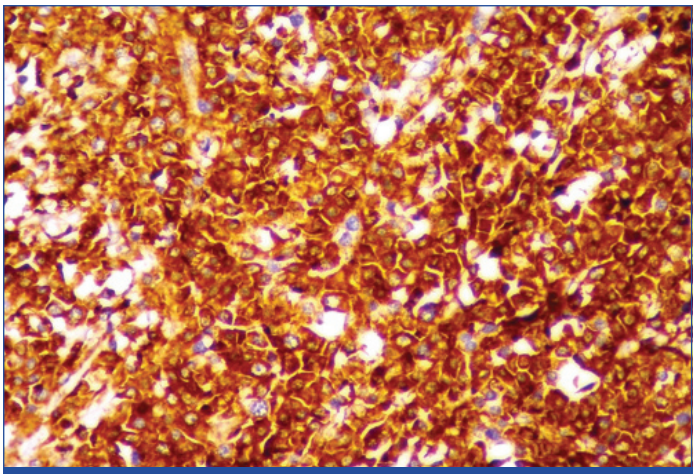
and mature plasma cells. On immunohistochemistry, these cells showed kappa restriction. A diagnosis of plasma cell leukaemia was made. Investigations done at presentation and a month later are tabulated [Table/Fig-8]. The patient was referred to higher centre for further treatment.



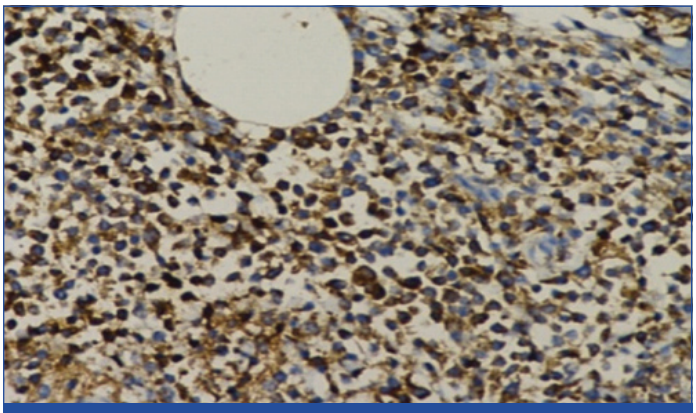
[Table/Fig-3]: Whipple's specimen showing ill-defined lesion in pancreas.



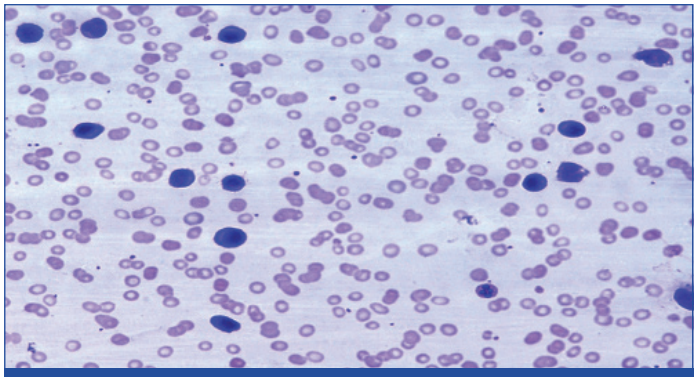
[Table/Fig-4]: Diffuse infiltration by plasma cells (H&E, 40x).



[Table/Fig-5]: Strong membranous expression of CD138 by tumour cells (IHC, 40x).



[Table/Fig-6]: Neoplastic cells with kappa expression (IHC, 40x).



[Table/Fig-7]: Peripheral smear with plasma cell leukaemia (40X).

Parameters	At initial presentation	A month later
Serum urea	42 mg/dL	81 mg/dL
Serum creatinine	1.06 mg/dL	3.82 mg/dL
Direct bilirubin	3.3 mg/dL	1.8 mg/dL
Haemoglobin	9.5 g/dl	6.8 g/dL
Total WBC count	4.3×10^3/µL	30.5×10^3/µL
Peripheral smear	Normocytic anaemia	Plasma cell leukaemia
Serum calcium	1.116 mmol/L	1.178 mmol/L

[Table/Fig-8]: Biochemical and haematological investigations.

DISCUSSION

Plasma cell neoplasms include Plasma Cell Myeloma (PCM) which includes smouldering myeloma, non-secretory myeloma, plasma cell leukaemia and plasmacytoma which includes solitary bone and extraosseous plasmacytoma. In majority of the patients with PCM, features of end organ damage in the form of one or more of the following, hypercalcaemia, Renal insufficiency, Anaemia, and Bone lesions (CRAB) are present. The clonal proliferation usually begins in bone marrow. Renal failure occurs due to tubular damage caused by excessive synthesis of monoclonal immunoglobulins [1]. It constitutes 10 to 15% of haematological malignancies. Median age at diagnosis is 66 years [2]. The clinical spectrum varies from asymptomatic to highly aggressive.

Extramedullary Plasmacytomas (EMPs) are localised proliferation of monoclonal plasma cells forming solitary lesions outside the bone marrow. EMPs can be a manifestation of advanced multiple myeloma or can represent a solitary plasmacytoma without involving the bone marrow [3]. EMPs are uncommon and represent 5% of all plasma cell tumours. The most frequent site of involvement being upper respiratory tract followed by gastrointestinal tract and reticuloendothelial system [2].

Primary pancreatic plasmacytoma is rare constituting 2.3% of EMPs. Majority of the reported pancreatic plasmacytoma are secondary [3]. EMPs have the median age of presentation at 55 years (A decade younger than patients with multiple myeloma) and the majority (three-fourths) are males [4].

Most of these lesions are located in the pancreatic head. Diffuse enlargement and presenting as mass in body (4 cases) [5] and tail of the pancreas have also been reported. The typical presenting symptoms include abdominal pain and obstructive jaundice from bile duct compression when there is involvement of head. Diffuse lesions are picked up rather late as they tend to be asymptomatic [6]. The case presented here also had obstructive jaundice raising a concern for primary pancreatic carcinomas [7].

Radiologically plasmacytoma is characterised by a homogenously enhancing multilobular, solid mass on CT, which is non-specific and resemble that of neuroendocrine tumours or carcinoma [6]. The case described presented as an isodense lesion in the pancreatic head. Differentiating plasmacytomas radiologically from other pancreatic tumours is challenging.

Endoscopic Ultrasound (EUS) is a well recognised technique for confirmation of pancreatic masses by histology. EUS- FNAs are reported to have an accuracy of 78-95% in the diagnosis of pancreatic masses. Neuroendocrine tumour presents as a, homogeneous, isoechoic or hypoechoic mass on EUS while plasmacytoma shows heterogenecity with uneven margins. NETs are hypervascular [5,8].

When FNA was done, the lesion was morphologically misdiagnosed as neuroendocrine neoplasm since both share plasmacytoid morphology. In a rare site, one would be more likely to suspect a primary lesion with plasmacytoid features, particularly endocrine neoplasm. Islet cell tumours have been reported to be a great mimic of plasmacytoma with regards to clinical, cytological and radiological appearance [9]. EUS guided core biopsy from the lesion and immunophenotyping with cytokeratin for carcinoma and CD138, kappa and lambda for identifying monoclonality of plasma cells would have helped in arriving at the diagnosis. EUS guided FNA also serves as a platform for obtaining material for flow cytometry.

The patient described here did not present with any symptoms pertaining to myeloma prior to surgery. The patient progressed to plasma cell leukaemia within a short time (one month) which is evident from the investigations [Table/Fig-8]. Plasma cell leukaemias are primary in 60-70% of cases and secondary to multiple myeloma in 30-40 % cases [10,11].

To our knowledge, this is the first case of plasmacytoma of pancreas being the initial manifestation of a plasma cell leukaemia. Two other cases of plasma cell leukaemia have been reported in association with gastric plasmacytoma. One case reported also had a normal WBC and platelet count with normocytic normochromic anaemia three weeks prior to the diagnosis of gastric plasmacytoma. The authors had hypothesised that secondary plasma cell leukaemia would have occurred as a result of rapid transformation of possibly underdiagnosed light chain multiple myeloma. The present case could also represent a similar rapid transformation from a non-secretory myeloma presenting with an extramedullary plasmacytoma [12].

From the previous reports it is clear that in any mass lesion of a solid organ in a patient with multiple myeloma the possibility of secondary plasmacytoma is to be considered [3,4,7]. Present case in addition highlights the fact that plasmacytoma is to be considered when plasmacytoid cells are identified in EUS-FNAs even when previous diagnosis of myeloma or symptoms pertaining to myeloma is not there at presentation. Immunocytochemistry can be used as an ancillary technique to diagnose with certainty in such cases. Presurgical diagnosis is essential as the management varies between primary pancreatic carcinoma and plasmacytoma.

CONCLUSION(S)

This case highlights the importance of considering other causes of pancreatic head mass than a primary carcinoma in a patient with obstructive jaundice. It also emphasises the fact that pathologists must be aware of the existence of pancreatic plasmacytoma as initial manifestation of myeloma despite its rarity, a high index of suspicion being essential.

REFERENCES

[1] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). IARC: Lyon 2017.
[2] Pallavi R, Ravella PM, Popescu MA. An unusual pancreatic mass: A case report and literature review. Transl Gastrointest Cancer. 2014;3:106-10.
[3] Hue SSS, Azhar K. Plasmacytoma of the pancreas: An unusual manifestation of multiple myeloma. Singapore Med J. 2013;54:105-07.
[4] Padda MS, Milles T, Adeniran AJ, Mahootic S, Aslaniana HR. Pancreatic and gastric plasmacytoma presenting with obstructive jaundice, diagnosed with endoscopic ultrasound-guided fine needle aspiration. Case Rep Gastroenterol. 2010;4:410-15.
[5] Lu T, Pu H, Zhao G. Primary pancreatic plasmacytoma: A rare case report. BMC Gastroenterol. 2017;17:167.
[6] Smith A, Hal H, Frauenhoffer E. Extramedullary plasmacytoma of the pancreas: A rare entity. Case Reports in Radiology. 2012;2012:798264.
[7] Williet N, Dumas O, Rinaldi L. Difficult endoscopic diagnosis of a pancreatic plasmacytoma: Case report and review of literature. World Journal of Clin Oncolog. 2017;8:91-95.
[8] Kim JH, Paik WH, Joo M, Kim JG, Kim JW, Bae WK, et al. Extramedullary plasmacytoma mimicking pancreatic cancer: A case report and literature review. Endosc Ultrasound. 2017;6:269-72.
[9] Dodd LG, Evans DB, Symmans F, Katz RL. Fine-needle aspiration of pancreatic extramedullary plasmacytoma: Possible confusion with islet cell tumour. Diagn Cytopathol. 1994;10:371-75.
[10] Singh S, Rath A, Yadav S. Primary plasma cell leukaemia-Case report and review of the literature. Sultan Qaboos University Med J. 2018;18(3):397-401.
[11] Jain AG, Faisal-Uddin M, Khan AK, Wazir M, Shen Q, Manoucheri M. Plasma cell leukaemia-one in a million: A case report. World J Clin Oncol. 2019;10(3):161-65.
[12] Puria A, Laua M, Zhaoa X, Chana K, Badera K. A rare case of a minimally secretory pasma cell leukaemia with a haemorrhagic gastric plasmacytoma. Leukaemia Research Reports. 2018;9:23-27.

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Second-trimester fetal autopsy: A morphological study with prenatal USG correlations and clinical implications

Chaitra Venkataswamy, Umamaheswari Gurusamy, S. Vidhya Lakshmi

Abstract:

OBJECTIVES: The objective of this study is to analyze the second-trimester fetal autopsies and to reemphasize the role of autopsy by comparing autopsy findings with prenatal ultrasound observations.

MATERIALS AND METHODS: Retrospective analysis of second-trimester fetal autopsies over a period of 7.5 years (January 2009–June 2016). A standard protocol of autopsy procedure was followed, which included external examination with photography, X-ray, internal examination, and histopathological examination. In fetuses with congenital malformations (CMs), the findings of prenatal ultrasonogram and autopsy examination were compared.

RESULTS: We analyzed a total of 66 fetuses, which includes 17 intrauterine fetal death, 49 terminations for CM, and increased risk for chromosomal abnormality. In fetuses with CM, multiple anomalies were more common than a single anomaly. The most common anomalies were seen involving central nervous system (neural tube defect) followed by the genitourinary system. Autopsy confirmed prenatal ultrasound findings in all cases except three. Complete agreement between USG findings and autopsy were seen in 17 cases (39.7%). Additional findings on autopsy were noted in 25 cases (62.2%). Among these, 15 cases had a significant change of recurrence risk due to altered initial ultrasound diagnosis.

CONCLUSION: Fetal autopsy plays an important role in arriving at the final diagnosis and detecting the cause of death. This information is very essential for the clinicians, in genetic counseling of the parents, and management of future pregnancies.

Keywords:

Congenital malformations, fetal autopsy, multiple anomalies, prenatal ultrasound

Introduction

The common indications for second-trimester fetal autopsies are termination of pregnancy (TOP) for congenital anomalies, intrauterine fetal death (IUFD), and inevitable abortion.[1] TOP for congenital anomalies accounts for the majority of cases of second-trimester fetal autopsies as most of the anomalies are detected during 18–20 weeks of gestation by anomaly scan which is an integral part of antenatal screening. These remain the common cause of perinatal deaths both in developed and developing countries and account for 25%–30% and 10%–15%, respectively.[2] The occurrence of these anomalies in subsequent pregnancy varies from negligibly low to 25%, and it depends on the etiology of diagnosis. Hence, it is important to make an accurate diagnosis.

In the present era, Ultrasonographic (USG) examination can accurately diagnose many lesions but has its own limitation in detecting soft-tissue lesions such as malrotation

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of intestines, asplenia/polysplenia, and pulmonary hypoplasia, and the success rates of ultrasound also depend on the equipment used and expertise of the sonologist. Perinatal autopsy either confirms or refute the ultrasound diagnosis and complements USG examination in making a precise diagnosis.

The objectives of the present study are to evaluate the reasons for second-trimester fetal autopsies, to categorize the congenital malformations (CMs) into various systems, syndromes, and complexes, and to compare the autopsy findings with prenatal USG examination.

Materials and Methods

The retrospective analysis of all second-trimester fetal autopsies from January 2009 to June 2016 (7.5 years) was done in the Department of Pathology. Fetuses with gestational age 14–28 weeks were included in the study. Fetuses with gestational age <14 weeks and >28 weeks were excluded from the study. Informed written consent for performing autopsy examination was obtained from either of the parents before the autopsy. All the fetuses were examined on preset protocol based on the recommendation given in guidelines in autopsy practice by Royal College Of the Pathologists,[3] which included photography, fetogram (whole body radiography), and external and internal examination.

Evisceration is performed by starting with a linear skin incision extending from symphysis menti to midline of chest, abdomen, passing to the left side of the umbilicus, and extending up to the symphysis pubis. *In situ* assessment of the intestines was done to rule out malrotation and they were removed from jejunum up to the rectum. Rest of the organs was taken out as "*en bloc*" by freeing the tongue from the floor of the mouth and releasing the attachments of the neck structures. The pelvic organs were cut as low as possible. External genitalia and urethra were dissected in case of suspected urorectal septum malformation. The individual system was thoroughly inspected and dissected for anomalies.

The brain is removed by incising the scalp behind one ear extending to vertex and to the other ear. Skin flaps are reflected anteriorly up to the eyebrows and posteriorly up to the nape of the neck. Skull bones are opened along the suture lines. The brain is delivered by placing the head at a lower level than the body, by releasing the nerve attachments, and falx cerebri, tentorium cerebelli and the upper cervical spinal cord is incised. The removed brain is then fixed by hanging in formalin using gauze.

All the organs are weighed. Histopathological examination of the brain, thyroid, thymus, heart, lung, liver, pancreas, spleen, kidney, adrenal, gonads, and placenta was carried out. Additional sections from the skin, muscle, bladder, pylorus, etc., were also studied when required.

In cases of fetuses terminated for CMs, the findings on autopsy and prenatal ultrasound examination were compared.

Approval was obtained from the institutional Ethical Clearance Committee.

Results

There were 87 fetal autopsies in 7.5 years of which 66 cases (75.8%) were second-trimester fetuses. Causes of second-trimester fetal autopsies are summarized in Figure 1.

In cases of IUFD and abortions, mean maternal age was 27 years (range19–35) and mean gestational age was 22 weeks (range 16–27). An autopsy provided a final diagnosis in 15 (88.2%) out of 17 cases. The causes are summarized in Table 1. In two cases, there was no detectable anomaly in the fetuses and the placental causes could not be excluded as placenta was not available for examination.

Cases terminated for increased risk for chromosomal abnormality include two cases of Down syndrome. One case presented with hydrops fetalis, cystic hygroma neck, common atrioventricular canal defect with persistent left superior vena cava, and hypoplasia of thymus. Other case showed nuchal edema, hydrocephalus, and abnormal left hand, feet, and legs. There was one case of Turner syndrome with partial deletion of short arm of X chromosome. This case showed telangiectatic vascular nevi, hypoplastic nails, widely spaced nipples, clitoromegaly, septate vagina, and aortic arch isthmus hypoplasia. In one case, amniocentesis and FISH showed deletion of short arm of chromosome

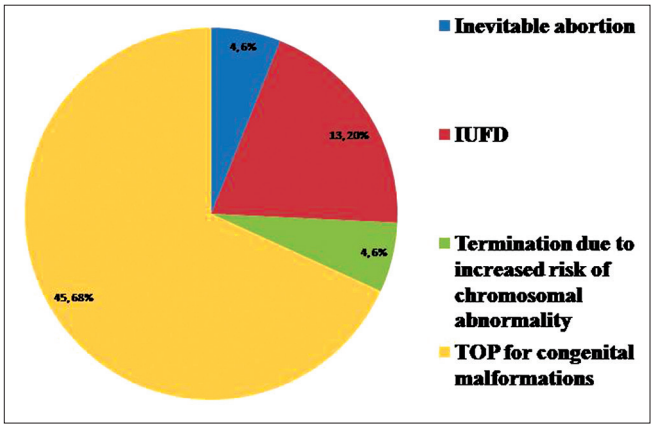


Figure 1: The pie diagram shows causes of fetal autopsies requested

Table 1: Causes of intrauterine fetal death in the second trimester

Causes	Number of cases (*n*=17)
1. Placental causes	10
Cord stricture	6
Hyper coiled cord with fetal vascular obstruction	1
Massive perivillous fibrin deposition	1
Infectious villitis with hydrops fetalis	1
Isolated hydrocephalous with central placenta previa in mother and cord stricture (spontaneous abortion)	1
2. Fetal causes	4
Hypoplastic left heart syndrome with visceral heterotaxy	1
Healed myocardial infarction	1
Hydrops fetalis due to premature ductus closure	1
Bladder outlet obstruction due to distal urethral stenosis (spontaneous abortion)	1
3. Maternal cause	
Maternal thrombophilia	1
4. Diagnosis not possible	2

15. USG examination of fetus showed bilateral mild ventriculomegaly, hypoplastic cerebellum, increased nuchal pad thickness, postaxial polydactyly in both feet, and multiple anomalies. An autopsy confirmed the USG findings and in addition showed focal cerebellar dysplasia. Parental karyotyping was suggested to confirm Prader–Willi/Angelman syndrome.

In fetuses terminated for congenital anomalies, the mean maternal age was 24 years (range: 18–32). The mean gestational age at the time of diagnosis was 21 weeks (13–27). A majority of them showed multiple malformations which are summarized in Tables 2 and 3. Central nervous system (CNS) anomalies were the most common followed by genitourinary malformations. The frequently encountered CNS anomaly was Arnold–Chiari II malformation with myelomeningocele. Two cases of rhombencephalosynapsis characterized by fusion of cerebellar hemisphere with absent cerebellar vermis were seen. There were four cases of hydrops fetalis and the underlying cause was detected in all. In a case of DiGeorge syndrome (DGS), there was hypoplastic thymus in the cervical region which was not detected on ultrasound [Figure 3]. A case of sirenomelia showed single median lower limb. Fetogram revealed single femur, two tibias, and no fibula [Figure 4].

Prenatal USG examination was available in all fetuses terminated due to prenatally diagnosed anomalies. There were a total of 121 anomalies in 45 cases. About 50 (41.3%) out of 121 anomalies were diagnosed only on autopsy. Three anomalies identified on USG were not confirmed on autopsy examination and includes two Dandy–Walker complex (DW) and aqueduct stenosis. One of the cases of DW complex showed additional findings on autopsy, and a diagnosis of Fryns syndrome was offered. Anomalies detected exclusively on autopsy examination include 13 (26%) gastrointestinal (malrotation of intestine), 13 (26%) genitourinary, 5 (10%) cardiovascular, 5 (10%) facial abnormality (cleft palate and micrognathia), four cases of CNS lesions, two cases each of polysplenia, skeletal abnormalities, and ductal plate malformation liver, and one case each of congenital diaphragmatic hernia, omphalocele, choanal atresia, microphthalmia, and nesidioblastosis.

Complete agreement between the USG findings and autopsy results were seen in 17 cases (39.7%). An additional finding in autopsy was noted in 25 (62.2%) out of 45 cases. In 15 cases (34.8%), the additional findings on autopsy changed the initial ultrasound diagnosis [Table 4]. In nine out of 15 cases, the revision of diagnosis led to significant change in recurrence risk (3%–25%) in subsequent pregnancy.

Discussion

With the advent of newer imaging techniques with higher resolution, the rate of autopsy is declining steadily. This is true even in cases of perinatal autopsies.[4] Perinatal autopsy still remains the gold standard in detecting the cause of death in IUFD and confirming the anomalies detected on ultrasound.[1] The present study focuses on second-trimester fetal autopsies and in reemphasizing its value in detecting congenital anomalies.

In the present study, major indication for second-trimester fetal autopsy was TOP for congenital anomalies. In cases of IUFD, fetal malformations were noted in 23.5% of cases, this is slightly higher as compared to the study done by Joó *et al*. where fetal malformation was seen in 13.05% in second-trimester miscarriages.[5] A study done by Peng *et al*. showed that 18.7% cases of IUFD were associated with umbilical cord stricture, hypercoiling or combination of both, most of these demises occurred in the second trimester.[6] The current study showed cord stricture and hypercoiling as a cause of IUFD in 41.1% of cases, significantly high. This highlights the importance of examination of the placenta and umbilical cord in cases with absence of congenital anomalies.

In fetus with deletion in chromosome 15, there were multiple anomalies including cerebellar hypoplasia. Deletion in chromosome 15 is known for phenotypic heterogeneity, with well documented, diverse clinical syndromes. The best known of these are Prader–Willi syndrome and Angelman syndrome, both of which are not associated with generalized cerebellar hypoplasia

Table 2: Postmortem diagnosis in fetuses terminated for congenital malformations (limited to 1 or 2 systems)

Congenital anomalies	Associated anomalies	Number of cases (*n*=27)
1. Central nervous system malformations		9
Arnold–Chiari malformation II with meningomyelocele	Agenesis of the right kidney and fused adrenals (1 case)	3
Hydrocephalous with aqueduct stenosis		1
Rhombencephalosynapsis	Lobar holoprosencephaly, aqueductal stenosis, interhemispheric cyst, TEV (1)	2
	Craniofacial dysmorphism (1)	
Hydrocephalus	Double aortic arch	1
Lumbar meningomyelocele	Kyphoscoliosis spine, ruptured omphalocele	1
Mild ventriculomegaly	Hypoxic ischemic encephalopathy	1
2. Urogenital malformations		8
AR polycystic kidney disease	Ductal plate malformation	2
Bladder outlet obstruction due to the posterior urethral valve	Hydrops fetalis (2 cases)	3
	TEV (1 case)	
Persistent cloaca with persistent Mullerian duct syndrome	Male pseudohermaphroditism (2), Occipital encephalocele, TEV, agenesis right kidney, imperforate anus, and malrotation intestine (1)	2
Persistent Mullerian duct syndrome	Ambiguous genitalia, male pseudohermaphroditism, anorectal atresia, bladder outlet obstruction with bilateral hydronephrosis, and renal obstructive dysplasia SUA	1
3. Cardiovascular system malformation		3
D-transposition of great arteries	Omphalocele (1 case)	2
Congenitally corrected transposition of great arteries		1
4. Respiratory system		2
Congenital high-airway obstruction syndrome	Hydrops fetalis	1
Alveolar capillary dysplasia with misalignment of pulmonary veins		1
5. Congenital diaphragmatic hernia [Figure 2]	Choroid plexus cyst (1 case)	3
	Meckel's diverticulum, bicuspid aortic and pulmonary valves, bicornuate uterus (1 case)	
6. Talipes equinovarus	Accessory tragi	1
7. Hydrops fetalis	Placental mesenchymal dysplasia	1

SUA = Single umbilical artery, TEV = Talipes equinovarus

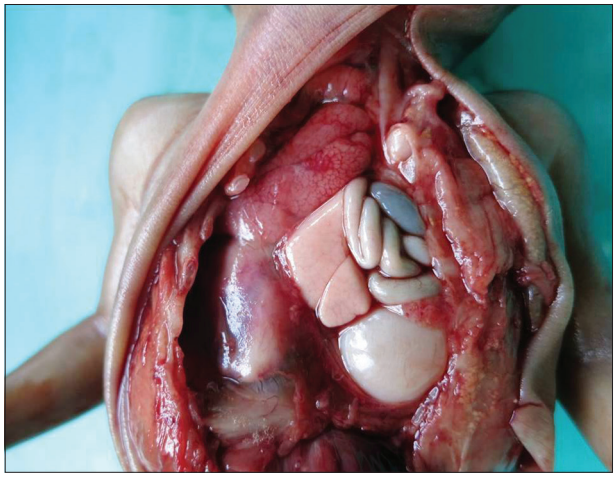


Figure 2: Congenital diaphragmatic hernia. The left hemithorax contains loops of small intestine and stomach pushing the heart and left lung to the right side

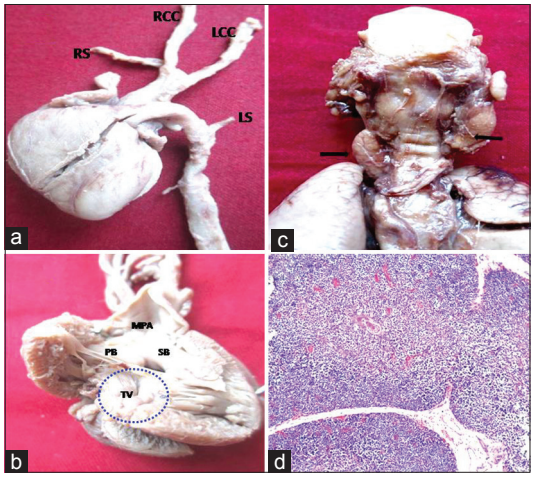


Figure 3: DiGeorge syndrome; (a) Interrupted aortic arch Type-B, (b) ventricular septal defect, perimembranous type, (c) cervical hypoplastic thymus, (d) lobules of thymic tissue composed cortex, medulla with Hassall's corpuscles (H and E, ×100)

as seen in present case. To the best of our knowledge, this association has not been described in literature. It is possible that this might belong to the expanding spectrum of chromosome 15q deletions.[7-9]

In 45 fetuses terminated for congenital anomalies, multiple malformations were seen in 75% of cases which is relatively high when compared to previous studies.[2,10]

CNS malformations were the most common followed by genitourinary anomalies. This is in concordance with the previous studies.[2,9-12] Arnold–Chiari malformation is characterized by downward displacement of cerebellum and brainstem into the spinal cord and is divided into three types. Type II is the most common form; this was true even in our series.[13]

Table 3: Postmortem diagnosis in fetuses terminated for congenital malformations (involving multisystem)

Diagnosis multiple malformations	Number of cases (*n*=17)
1. Syndromes/complexes/association	13
Fraser syndrome	1
Meckel–Gruber syndrome	2
Fryns syndrome	1
DiGeorge syndrome (SUA)	1
DiGeorge/velocardiofacial syndrome	1
Amniotic band syndrome	1
Charge syndrome	1
Zellweger syndrome/Smith–Lemli–Opitz syndrome	1
Sirenomelia with pseudo tail (SUA)	1
VACTERL or VATER Association (SUA)	2
Tuberous sclerosis complex	1
Multiple malformations – no definite syndrome	4
Renal hepatic pancreatic dysplasia with duodenal atresia	
Situs inverses with polysplenia and conotruncal malformation	
Meningocele, Arnold–Chiari malformation, tricuspid atresia, and double-outlet right ventricle	
Neural tube defect, Arnold–Chiari malformation, hydrocephalus with aqueductal stenosis, agenesis of corpus callosum, cerebellar hypoplasia, renal dysplasia, malrotation of small intestine, and polysplenia	

SUA = Single umbilical artery



Figure 4: Mermaid fetus; (a) single median lower limb with pseudo tail in the lumbosacral region, (b) fetogram shows fused pelvic bones, single femur, and absent fibula, (c) hypoplastic descending abdominal aorta continuing as single umbilical artery, (d) meconium filled, blind-ended colon

DGS also known as del22q11.2 is the most common microdeletion. Phenotypic features include heart defects, thymic hypoplasia/agenesis, facial dysmorphism, and developmental delay.[14] Two cases of DGS were diagnosed on prenatal ultrasound examination in view of congenital heart defects and absent thymus. Autopsy examination revealed hypoplastic cervical thymus in one case. Ectopic thymus was noted in 1% of children in a study of 3236 children at autopsy, of these 71% had features of DGS.[15] This might explain the variable immune status in the patients with DGS. Radiologist should be familiar with the locations of ectopic thymus and try to look for them when it is not found in the anterior mediastinum.

There was a case of sirenomelia with atypical skeletal framework. Sirenomelia is a rare CM characterized by varying degree of fusion of the lower limbs, single umbilical artery, and internal malformations related to genitourinary and gastrointestinal systems.[16] Stocker and Heifetz have classified sirenomelia into seven types based on skeletal framework in the lower limbs.[17] The bone pattern in the present case does not fit into any of the subtypes described in the literature. The exact etiological cause for sirenomelia remains unclear, but widely accepted hypothesis include defective blastogenesis and vascular steal hypothesis.[16]

In the present study, fetal autopsy provided definitive diagnosis in 95% of cases and confirmed ultrasound findings in 93% of cases, which is comparable to the previous studies.[2,12,18] An autopsy could not confirm DW complex in two cases. The previous studies have shown a poor correlation between ultrasound and autopsy findings in cases of DW complex with concordance being achieved in 55%, even after standardized reevaluation of sonographic and pathology images.[19] The poor correlation could be attributed either due to the membranous quality of the cyst wall with loss of fluid during anatomical dissection or due to the overdiagnosis of DW complex on ultrasound. Hence, the diagnosis should be reserved to cases which show all the classical findings on ultrasound. In doubtful cases, repeat ultrasound or magnetic resonance imaging (MRI) should be suggested to confirm the diagnosis.[19]

On comparing autopsy and ultrasound findings, our study showed complete agreement in 37.7% (17/45), partial agreement in 22.2% (10/45), and discordance in 33.3% (15/45). Thus, overall agreement was seen in 59.9%. This is in concordance with study done by Yeo *et al*.[20] Table 5 shows comparison of autopsy and ultrasound findings in various studies.[2,10,12,20,21]

Anomalies that were missed on USG examination were related to gastrointestinal, genitourinary, cardiovascular

Table 4: Comparison of ultrasound findings with autopsy findings in cases of discrepancy

Serial number	USG finding with diagnosis	Autopsy findings with diagnosis
1	Sacral meningomyelocele, bifid spine with the hypoplastic right ventricle of the heart	Arnold–Chiari malformation and tricuspid atresia with double-outlet right ventricle
2	Anhydramnios, B/L renal agenesis	Cryptophthalmos, ventriculomegaly, bifid nasal tip, clitoromegaly, B/L renal agenesis, absent vagina, uterus, and proximal fallopian tubes - Fraser syndrome
3	Anhydramnios, cystic dilatation of biliary radicles and common bile duct, a cyst in pancreas, bilateral multicystic dysplastic kidneys, microcephaly and shot bowed tibia - Ivemark syndrome	Bilateral cleft lip, cleft palate, lingual abnormalities and dysplastic epiglottis. Occipital encephalocele, Arnold–Chiari malformation Type III. Holoprosencephaly. Diffuse cystic renal dysplasia congenital hepatic fibrosis (ductal plate malformation), choledochal cyst Todani Type IV. Persistent left SVC, mild atrioventricular valve dysplasia. Ambiguous external genitalia, bilateral testes. Postaxial polydactyly, bow legs Meckel–Gruber syndrome
4	Situs ambiguous, isolated levocardia, VSD, and truncus arteriosus	Situs inversus, isolated levocardia with polysplenia, and conotruncal malformation
5	Cerebellum V-shaped with large cisterna magna. ? partial agenesis of inferior cerebellar vermis- possibility of DW complex	CDH, malrotation of the intestine, B/L cleft palate, ankyloglossia, bifid tongue, and uterus didelphys-Fryns syndrome
6	Short femur, humerus, narrow chest- Possibly achondroplasia	Syndactyly, postaxial polydactyly of hands and feet, dysplasia of epiglottis, nonrotation of intestine, ductal plate malformation of liver, renal dysplasia, X-ray - B/L shortening of long bones of the upper and lower limbs - Meckel–Gruber syndrome-variant
7	Oligohydramnios, B/L echogenic kidneys to R/O CMV	AR PCKD with congenital hepatic fibrosis
8	Increased nuchal translucency, generalized subcutaneous edema, hypertelorism, midline facial cleft, small omphalocele, cardiac outflow tract anomaly. Suggested invasive test to rule out aneuploidy. Patient not willing for amniocentesis	Craniofacial defects with a membranous tag at the head - cranioschisis, absent ears, and nose, bilateral cleft lip and palate, Conotruncal cardiac anomaly? Transposition of great arteries with pulmonary atresia? Double outlet right ventricle with pulmonary atresia, abdominoschisis, renal cystic dysplasia - suggestive of amniotic band syndrome
9	Lumbosacral spina bifida and Arnold–Chiari malformation	Lumbar meningomyelocele, ruptured omphalocele
10	Bilateral presacral kidneys with bilateral hydronephrosis due to pelviureteric junction obstruction, parenchyma of left kidney is dysplastic	Ambiguous genitalia, male pseudohermaphroditism, persistent mullerian ducts Anorectal atresia, bladder outlet obstruction with bilateral hydronephrosis, and renal obstructive dysplasia. SUA
11	SUA, TOF, ectopic hydronephrotic left kidney, left clubfoot with absent 2nd to 5th digits	TOF with the right aortic arch and left-sided patent ductus arteriosus, atresia of distal sigmoid, rectum and anus, fused crossed renal ectopia with hydronephrosis, left clubfoot with a single digit Fetogram-hemivertebra, absent metatarsal l bones-vacterl association
12	Pierre syndrome, left hydronephrosis due to pelviureteric obstruction	Bilateral posterior choanal atresia, lop ears, Micrognathia, Microphthalmia, persistent cloaca, left hydronephrosis - CHARGE syndrome
13	Two echogenic masses suggestive of cardiac rhabdomyoma	Heart: Multiple rhabdomyomas Brain: Subependymal nodules of dystrophic neurons and astrocytes - tuberous sclerosis complex [Figure 5]
14	Complete AVSD Bilateral cleft lip and palate, echogenic focus left ventricle	Congenitally corrected transposition of great arteries Alveolar capillary dysplasia with misalignment of pulmonary veins, bilateral cleft lip and palate, mild pyloric stenosis, bladder outlet obstruction? Vascular dysplasia kidneys and brain

TOF = Tetralogy of Fallot, CDH = Congenital diaphragmatic hernia, SUA = Single umbilical artery, SVC = Superior vena cava, USG = Ultrasonography, CMV = Cytomegalovirus, B/L = Bilateral, DW = Dandy-walker complex, AVSD = Atrio ventricular septal defect, VSD = Ventricular septal defect, R/O = Rule out, AR PCKD= Autosomal recessive polycystic kidney disease

systems, and facial defects. Antenatal detection of these CMs depends on the expertise of sonologist, quality of equipment, maternal habitus, and reduction in liquor volume.[22] In addition, some cardiac, gastrointestinal, and renal abnormalities are not evident at 20 weeks. Few are histopathological diagnosis (e.g.: tuberous sclerosis, PCKD). Oligo- and anhydramnios were noted in some of our cases. All the above factors could explain the shortcomings of routine antenatal USG examination. Other prenatal diagnostic modalities such as three-dimensional ultrasound and MRI will provide better resolution for CNS and soft-tissue abnormalities. Detailed analysis of fetus using these advanced imaging modalities may help in achieving higher rates of complete agreement. Implementing them in routine antenatal screening may not be feasible due to higher cost, lack of expertise, and limited availability in developing countries.

When the parents are reluctant to give consent for full autopsy due to religious reasons, limited, or minimally invasive autopsy may be of value. Minimally

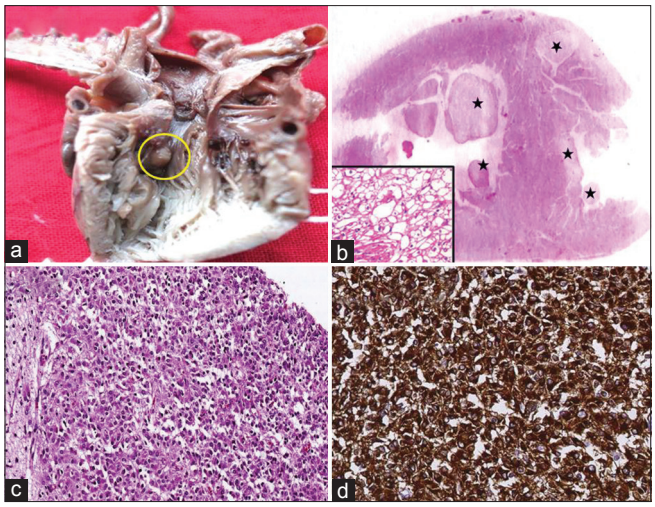


Figure 5: Tuberous sclerosis complex: (a) fleshy, pale brown mass in the left ventricular outflow tract, (b) transverse section of the heart shows multiple paler nodules(*) in subendocardial and intramural location. Inset shows high-power view of the nodule consisting of large vacuolated cells-rhabdomyoma (H and E, ×400), (c) section from medulla shows large subependymal plaque consisting of a cellular admixture of glial cells (H and E, ×100) and (d) these cells strongly express GFAP (IHC, ×400)

Table 5: Comparison of autopsy and ultrasound findings in various studies

Studies	Total number of cases	No change in initial USG diagnosis		Change in initial USG diagnosis (%)
		Complete agreement (%)	Added to USG findings (%)	
Sankar and Phadke[2]	134	41.0	58.0	1.49
Andola *et al*.[10]	39	50	29.54	9.09
Vimercati *et al*.[21]	144	71	35	17
Yeo *et al*.[20]	88	65	35	-
Grover *et al*.[12]	40	32.5	42.5	25.0
Present study	45	37.7	22.2	33.3

USG = Ultrasonography

invasive autopsy includes external examination with photography, placental examination, genetic analysis, and histopathological assessment of open or targeted biopsy of specific internal organs. Imaging studies, such as X-ray, postmortem MRI or micro-computed tomography, and assessment of body cavity fluids may be helpful to a certain extent.

Postmortem MRI can provide the details of structural malformation; however, it is not useful in cases of suspected infection, skeletal dysplasia, and in fetuses with severe maceration. It is not cost-effective and cannot provide details at histopathological levels.

Although minimally invasive autopsy can provide additional information in some cases, clinicians should explain to the concerned parents that complete perinatal autopsy remains the gold standard method for identifying the causes of fetal autopsy and its risk of recurrence.

Conclusions

The abnormalities detected by the fetal autopsy can be minor, major, or additional findings that modify the recurrence risk in subsequent pregnancy from 1%–3% to 25%. Thus, autopsy plays an essential role in accurate diagnosis and appropriate genetic counselling. The present study reemphasizes the need for fetal autopsy even in the era of advanced imaging techniques

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ernst LM. A pathologist's perspective on the perinatal autopsy. Semin Perinatol 2015;39:55-63.
2. Sankar VH, Phadke SR. Clinical utility of fetal autopsy and comparison with prenatal ultrasound findings. J Perinatol 2006;26:224-9.
3. The Royal College of Obstetricians and Gynaecologists and Royal College of Pathologists. Fetal and Perinatal Pathology: Report of a Joint Working Party. London: RCOG Press; 2001.
4. Boyd PA, Tondi F, Hicks NR, Chamberlain PF. Autopsy after termination of pregnancy for fetal anomaly: Retrospective cohort study. BMJ 2004;328:137.
5. Joó JG, Beke A, Berkes E, Papp Z, Rigó J Jr., Papp C, *et al.* Fetal pathology in second-trimester miscarriages. Fetal Diagn Ther 2009;25:186-91.
6. Peng HQ, Levitin-Smith M, Rochelson B, Kahn E. Umbilical cord stricture and overcoiling are common causes of fetal demise. Pediatr Dev Pathol 2006;9:14-9.
7. Cox DM, Butler MG. The 15q11.2 BP1-BP2 microdeletion syndrome: A review. Int J Mol Sci 2015;16:4068-82.
8. Sieg KG. Neurodevelopmental disorders associated with chromosome 15. Jefferson J Psychiatry 2011;8:9-23.
9. Hayashi M, Itoh M, Kabasawa Y, Hayashi H, Satoh J, Morimatsu Y,

et al. A neuropathological study of a case of the Prader-Willi syndrome with an interstitial deletion of the proximal long arm of chromosome 15. Brain Dev 1992;14:58-62.

10. Andola US, Am A, Ahuja M, Andola SK. Congenital malformations in perinatal autopsies – A study of 100 cases. J Clin Diagn Res 2012;6:1726-30.
11. Akgun H, Basbug M, Ozgun MT, Canoz O, Tokat F, Murat N, *et al.* Correlation between prenatal ultrasound and fetal autopsy findings in fetal anomalies terminated in the second trimester. Prenat Diagn 2007;27:457-62.
12. Grover S, Garg B, Sood N, Arora K. Lethal congenital malformations in fetuses-antenatal ultrasound or perinatal autopsy. Fetal Pediatr Pathol 2017;36:220-31.
13. Nejadi MN, Zafarani F, Ahmadi F, Rashidi Z. Arnold-chiari type II malformation: A case report and review of prenatal sonographic findings. Iran J Fuzzy Syst 2008;11:179-82.
14. Besseau-Ayasse J, Violle-Poirsier C, Bazin A, Gruchy N, Moncla A, Girard F, *et al.* A French collaborative survey of 272 fetuses with 22q11.2 deletion: Ultrasound findings, fetal autopsies and pregnancy outcomes. Prenat Diagn 2014;34:424-30.
15. Bale PM, Sotelo-Avila C. Maldescent of the thymus: 34 necropsy and 10 surgical cases, including 7 thymuses medial to the mandible. Pediatr Pathol 1993;13:181-90.
16. Valenzano M, Paoletti R, Rossi A, Farinini D, Garlaschi G, Fulcheri E, *et al.* Sirenomelia. Pathological features, antenatal ultrasonographic clues, and a review of current embryogenic theories. Hum Reprod Update 1999;5:82-6.
17. Stocker JT, Heifetz SA. Sirenomelia. A morphological study of 33 cases and review of the literature. Perspect Pediatr Pathol 1987;10:7-50.
18. Phadke SR, Gupta A. Comparison of prenatal ultrasound findings and autopsy findings in fetuses terminated after prenatal diagnosis of malformations: An experience of a clinical genetics center. J Clin Ultrasound 2010;38:244-9.
19. Phillips JJ, Mahony BS, Siebert JR, Lalani T, Fligner CL, Kapur RP, *et al.* Dandy-walker malformation complex: Correlation between ultrasonographic diagnosis and postmortem neuropathology. Obstet Gynecol 2006;107:685-93.
20. Yeo L, Guzman ER, Shen-Schwarz S, Walters C, Vintzileos AM. Value of a complete sonographic survey in detecting fetal abnormalities: Correlation with perinatal autopsy. J Ultrasound Med 2002;21:501-10.
21. Vimercati A, Grasso S, Abruzzese M, Chincoli A, de Gennaro A, Miccolis A, *et al*. Correlation between ultrasound diagnosis and autopsy findings of fetal malformations. J Prenat Med 2012;6:13-7.
22. Loughna P. Congenital abnormalities: Failure to detect and treat. Obstet Gynaecol 2008;10:33-7.

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Non immune hydrops foetalis: a perinatal autopsy study

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Background: Authors sought to determine the possible factors in the causation of nonimmune hydrops foetalis by perinatal autopsy with placental examination and to reduce the number of cases in which the cause remains elusive.

Methods: Twenty five cases of nonimmune hydrops foetalis were identified in about 200 consecutive perinatal autopsies (including placental examination) performed during a 11 year period. The results were correlated with clinical, laboratory and imaging characteristics in an attempt to establish the aetiology.

Results: Perinatal autopsy and placental examination confirmed the following aetiologies: cardiovascular causes (8) [isolated (4), syndromic (3) and associated chromosomal (1)], placental causes (5), chromosomal (4) [isolated(3) and associated cardiovascular disease (1)], intrathoracic (3), genitourinary causes (3), infections(1),gastrointestinal lesions (1) and idiopathic causes (1). Placental mesenchymal dysplasia was a unique pathology identified among the placental lesions, which constituted the second most common cause of nonimmune hydrops foetalis. Despite careful examination no cause was identified in one case. In more than 50% of studied cases, autopsy examination either refuted or altered the ultrasound diagnosis completely.

Conclusions: The perinatal autopsy in combination with placental study and prenatal imaging represents the most promising tool in the evaluation of aetiology of nonimmune hydrops foetalis. The identification of a cause for nonimmune hydrops foetalis will provide a better correlation with recurrence risk and parental counselling.

Keywords: Aetiology, Nonimmune hydrops foetalis, Perinatal/foetal autopsy, Placental mesenchymal dysplasia, Prenatal imaging

Introduction

Foetal hydrops is a syndrome characterized by generalized skin oedema and abnormal accumulation of fluid in one or more serous cavities of foetuses. It has been broadly classified into immune mediated (materno - foetal blood group incompatibility) and non immune types. There is a steady fall in the incidence of immunologic hydrops foetalis due to the prevention of maternal isoimmunisation and treatment of affected foetuses in utero.[1] This has shifted the focus towards the non immunologic causes of hydrops which now constitutes up to 90% of cases of foetal hydrops.[2]

There are many impressive and ever growing causes of non immune hydrops foetalis (NIHF) and its development is known to be associated with maternal, foetal and placental diseases.[3] The foetal hydrops can be identified anytime throughout the pregnancy. The perinatal mortality rate is generally high. The spectrum of possible causative factors of NIHF include cardiovascular diseases, chromosomal aberrations, haematologic

abnormalities, infections, intrathoracic lesions, lymphatic dysplasias and placental causes including twin-twin transfusion syndrome. The other causes are syndromes, urinary tract malformations, inborn errors of metabolism, extra thoracic tumours, gastrointestinal disorders, miscellaneous and idiopathic.[4]

Improvements in obstetric ultrasound (USG) and foetal echocardiography enable the early and frequent detection of hydrops, resulting in foetal interventions / termination of pregnancy and have altered the incidence of hydrops.[5]

Given the multiple aetiologies of NIHF, we sought to determine the presence of possible factors in its causation by analysing the autopsy with placental material and to reduce the number of cases in which the cause remains elusive.

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Twenty five cases of NIHF were identified in about 200 consecutive perinatal autopsies (including placental examination) performed during a 11 year period from January 2007 to December 2017 at the department of pathology, PSG Institute of Medical Sciences and Research. The institutional ethical review board approval was obtained for the study.

Exclusion criteria

Hydrops foetalis of immunologic origin were excluded from the study on the basis of blood typing, direct and indirect coomb's tests.

The detailed maternal history, antenatal ultrasounds and genetic tests if any were collected. The autopsy was performed after getting informed consent. Photograph of the foetus was routinely taken with special attention to sites where abnormalities were suspected. A thorough systematic examination of foetuses and placenta was done, which included foetogram, foetal weight, anthropometric measurements (head circumference, chest circumference, abdominal circumference, crown rump length, crown heel length, foot length), external and internal examination.

After opening the body cavities, serous fluid collections were measured, and the thoracic and abdominal organs were removed en bloc. All the visceral organs were examined, and the bits were given for microscopic analysis. The brain was removed, inspected, weighed and suspended in a formalin fixative. After fixation, the brain was sliced; examined and relevant bits were taken. Examination of bone, including marrow and spinal cord were performed in selected cases. The placenta and cord were also examined thoroughly, photographed (maternal / foetal surfaces, parenchyma, umbilical cord), cut at 1cm intervals and the parenchyma was assessed for specific lesions. Relevant lesional sections were taken along with 2 sections of membrane roll, 2 sections from the umbilical cord and 3 full thickness sections of the normal parenchyma.

All the histologic sections were stained with haematoxylin and eosin. Special stains such as Grocott-Gomori's methenamine silver (GMS), Giemsa stains for toxoplasma gondii, warthin starry stain for spirochetes and reticulin stain for vascular network were done, when required. Immunohistochemical marker for cytomegalovirus was available, hence done for suspected infectious cases. After the microscopic study, the results were correlated with clinical, laboratory and imaging characteristics and the final results were drawn.

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Out of 200 perinatal autopsies performed during the 10-year period, 25 cases met the criteria for NIHF, representing 12.5%. The gestational age ranged from 13 weeks to 35 weeks. All were dead-born foetuses except two. The two liveborn foetuses were delivered at 35 weeks and survived only a few hours postnatally. There were no cases of multiple gestation.

Clinical findings

Maternal age ranged from 19 to 35 years, of whom 9 were primigravidas. Five cases each of polyhydramnios and oligohydramnios were present. Maternal medical diseases included 2 cases each of preeclampsia and autoimmune diseases and 1 case each of hypothyroidism and rheumatic heart disease. None of the mothers had diabetes. Recurrence of hydrops was found on 5 occasions. Previous history of hydrops was documented in 2 cases of cardiac (complete heart block and transposition of great arteries with ventricular septal defect), one case each of intra thoracic lesion (Congenital pulmonary airway malformation), chromosomal (Down syndrome) and placental mesenchymal dysplasia.

Ultrasound findings

Hydrops foetalis is diagnosed with typical findings during prenatal imaging. The sonographic features include subcutaneous / scalp oedema, fluid accumulation in different serous cavities (Figure 1), polyhydramnios and placentomegaly. Correlation between prenatal USG and autopsy findings in elucidating the causes of hydrops were studied (Table 1). Complete agreement between prenatal USG and autopsy findings were seen in 11 cases (44%), while in 6 cases autopsy examination revealed additional findings (24%). In 8 cases, autopsy findings changed the diagnosis (32%).

Autopsy findings

Foetus

There were 12 males and 13 females with an almost equal gender distribution. Hydrops was noted in <20 weeks (10

cases), 21-28 weeks (10 cases) and >28 weeks (5 cases) of gestation. Aetiological distribution of NIHF is listed in Table 2.



[illegible]

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
<20 weeks (10)	4	3	3
21-28 weeks (10)	3	3	4
>28 weeks (5)	4	0	1

[illegible]

[illegible]	[illegible]
Intrathoracic lesions	3
[illegible]	
Hypertrophic pyloric stenosis	1
Cardiac defects	4
Genitourinary lesions	3
[illegible]	
Down syndrome	3 (1*)
Turner syndrome	1
[illegible]	
Parvo virus	1
Placental causes	5
[illegible]	
Ellis van creveld syndrome	1
Zellweger syndrome	1
Vacterl	1
Idiopathic	1

*Coexistent with congenital heart disease

Cardiovascular diseases (n=8)

Congenital cardiac defects represented the largest aetiological group and were seen in 8 foetuses (32.0%). Among these 8 cases, 7 foetuses had structural malformations and 1 had functional defect. 2 cases of isolated cardiac defects, 3 cases of syndromic association and one case of chromosomal abnormalities were identified (Table 3). Cystic hygroma was noted in 2 instances.

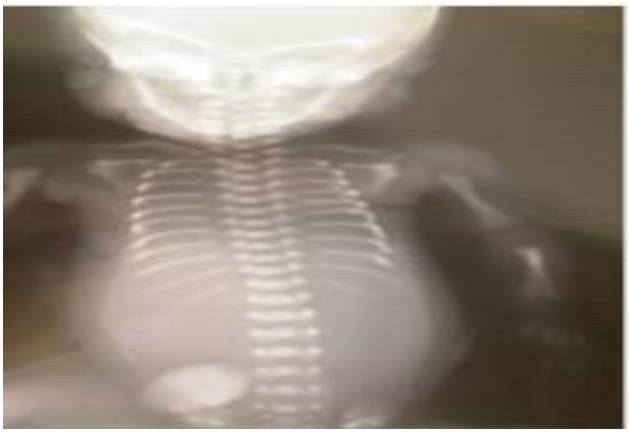
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[illegible]	[illegible]
Premature closure of ductus	
Complete heart block secondary to fibrosis of cardiac conduction system, immune mediated	
TGA with hypoplastic pulmonary arterial trunk	
Subaortic VSD	
TOF with right aortic arch and left sided PDA	VACTERL association
Secundum ASD	Multiple congenital anomaly syndrome -? Zellweger syndrome-? Smith Lemli Opitz syndrome
Common atrium with complete common AVSD	Ellis- Van Creveld syndrome
Common AVSD with persistent left superior vena cava and mild tubular hypoplasia of aortic arch	Down syndrome
TGA and VSD	Partial urorectal septum malformation sequence (Cloacal dysgenesis sequence)

TGA-transposition of great arteries; VSD-ventricular septal defect; TOF-tetralogy of fallot; PDA-patent ductus arteriosus; VACTERL-vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities; ASD-atrial septal defect; AVSD- atrioventricular septal defect.

The most common causes of NIHF in our series were cardiovascular diseases, of which structural anomalies predominate. The most frequently found structural heart lesion with hydrops is atrio ventricular septal defects, and transposition of great vessels. Less common structural cardiac abnormalities observed were premature closure of ductus, tetrology of Fallot and secundum atrial septal defect. There were 2 cases of isolated heart defects. One foetus had premature closure of ductus and the other was a neonate with complete heart block. In the second instance, an association with maternal connective tissue disease was identified. This newborn female with complete heart block also had a sibling delivered at same gestational age with similar hydropic features and died within a few hours of birth. Autopsy was not performed

for the sibling. Our newborn was delivered preterm at 34 weeks of gestational age due to severe bradyarrhythmia. The neonate was hydropic, hypertonic and had intractable bradycardia at birth. Pace maker insertion was not acceptable to the parents. Child expired within 3 hours of birth. Autopsy examination revealed biventricular hypertrophy, dysplasia of atrioventricular valves, fibrosis of sinoatrial and atrioventricular nodes and patchy endocardial fibroelastosis.





In present series, cardiac defects were also seen in association with Ellis-Van Creveld Syndrome (Figure 2), Multiple congenital anomaly syndrome and VACTERL association.

There were 3 cases of Down syndrome, all with typical phenotypic manifestations and one with atrioventricular septal defect (Figure 5). The other significant associated anomaly noted was partial urorectal septum malformation sequence (Cloacal dysgenesis sequence).



Other disorders associated with NIHF

Placental, intrathoracic and genitourinary lesions are summarized in (Table 4).

Placental lesions (n=5)

The placenta was examined in all the cases and showed villous edema, prominent Hofbauer cells and villous capillary erythropoiesis.

Placental causes of hydrops were found in 5 cases. Two cases each of maternal vascular malperfusion and placental mesenchymal dysplasia. The underlying pathology in the fifth case was foetal vascular malperfusion (organising and recanalizing thrombi with avascular villi), villitis of unknown aetiology and acute chorioamnionitis.

Histologically, the cases with maternal vascular malperfusion had multiple parenchymal infarcts, increased syncytial knotting and distal villous hypoplasia. Preeclampsia was noted in both the mothers.

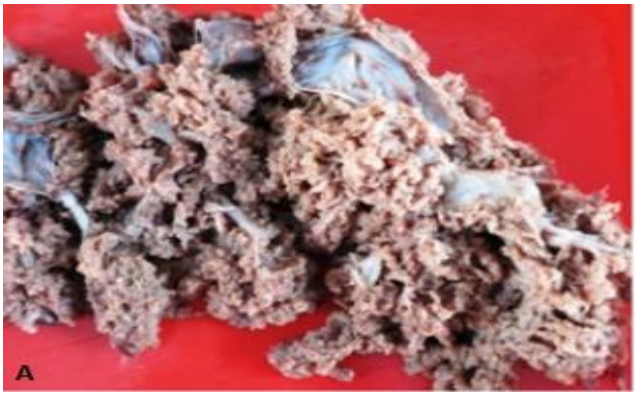


Figure 6: Gross photograph of placenta showing hypercoiled cord, parenchymal disruption with thick, oedematous villi and small vesicles

The placentas with mesenchymal dysplasia grossly showed hypercoiled cord, parenchymal disruption with thick, oedematous villi and small vesicles (Figure 6). Histologically, all villi showed collapsed vesicle with central cystic degeneration with absent trophoblastic proliferation and markedly dilated vascular channels (Figure 7).

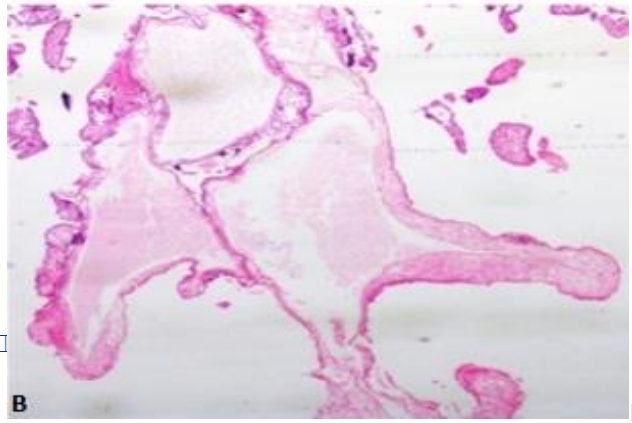


Figure 7: Histology showing collapsed vesicle with central cystic degeneration with absent trophoblastic proliferation and markedly dilated vascular channels

Table 2: Distribution of cases with non-immune hydrops fetalis and associated malformations

Placental causes (N=5)	Case 1: Preeclampsia, Multiple parenchymal infarcts, increased syncytial knotting: MVM
	Case 2: Preeclampsia, infarcts, Distal villous hypoplasia: MVM
	Case 3: The Placental parenchyma is disrupted, fleshy, pulled apart and the villi appear thick. The stem villi oedematous, hypercoiled cord : Placental mesenchymal dysplasia
	Case 4: Small vesicles in the parenchyma, thick stem villi: Placental mesenchymal dysplasia
	Case 5: placenta with FVM.VUE and A/C CAM
Intra thoracic lesions (n=3)	Case 1: Both lungs: dilated airways and lymphatics : CPAM
	Case 2: The right lung appears large and occupies almost the entire thoracic cavity: CPAM
	Case 3: Laryngeal (Glottic) atresia : CHAOS
Genitourinary abnormalities (n=3)	Case 1: BOO due to posterior urethral valve causing dilated posterior urethra, bladder, ureters and bilateral hydronephrosis. Ventriculomegaly, absent corpus callosum
	Case 2: Distal urethral stenosis with dilatation and hypertrophy of the urinary bladder: BOO. Pulmonary hypoplasia
	Case 3: BOO due to posterior urethral valves. Mild dilatation of both ureters and pelvis

CPAM- congenital pulmonary airway malformation; CHAOS- congenital high airway obstruction syndrome; BOO- bladder outlet obstruction; MVM-maternal vascular malperfusion; FVM- foetal vascular malperfusion; VUE- villitis of unknown aetiology; A/C CAM- acute chorioamnionitis

Authors did not find any chorangioma as a cause of NIHF.

Intrathoracic lesions (n=3)

There were 3 instances of intrathoracic lesions causing NIHF. Two cases of congenital pulmonary airway malformation (CPAM) (Figure 8) and one case of congenital high airway obstruction Syndrome (CHAOS) were identified in our series. Mediastinal deviation was noted in all foetuses.

Genitourinary abnormalities (n=3)

Three foetuses with NIHF were complicated by genitourinary causes. Two of them had bladder outlet obstruction (BOO) due to posterior urethral valves, and the third one had distal urethral stenosis with dilatation and hypertrophy of the urinary bladder. However, one

subject with posterior urethral valve also had ventriculomegaly, absent corpus callosum, deep posterior fossa, hypoplastic ductus and pulmonary arteries.



[illegible]

Chromosomal abnormalities (n=4)

Among cases with chromosomal abnormalities, we found one foetus with Turner syndrome, in which cytogenetic study revealed partial deletion of short arm of X chromosome. There were 3 cases of Down syndrome, all with typical phenotypic features and one foetus additionally had cardiac defect.

Miscellaneous causes

Congenital parvovirus B 19 infection was suspected in one instance. The foetus had marked subcutaneous oedema, serous effusions, enlarged liver and spleen. Lymphocytic infiltration of all viscera, predominantly myocardium with no identifiable inclusions were seen. The placenta had no lesion.

One of the foetus had hypertrophic pyloric stenosis which is a relatively uncommon cause of NIHF. The mother had polyhydramnios and autopsy revealed thickened and narrowed pylorus.

The cause of the hydrops foetalis could not be determined in only one case (4%) and it was classified as idiopathic. Subcutaneous oedema, serous effusions, micrognathia, high arched palate and dysmorphic villi were identified, and chromosomal abnormality was suspected. However, the karyotype was normal.

[illegible]

Nonimmune hydrops foetalis is a clinical sequel of wide range of pathology and is not a diagnosis in itself. It is known to be associated with maternal, foetal and placental diseases. In the past, the causes of hydrops were not identified in significant number of cases, even after extensive investigations. Postmortem examination including clinical assessments and targeted investigations can identify the underlying cause in majority of cases.

Cardiovascular diseases

Cardiovascular causes of NIHF accounts for about 20% of cases and includes both structural and functional defects.[6] In the present study cardiac defects represented the largest group and were found in 32% of foetuses.

Association of cardiac lesions with well-defined syndromes, chromosomal aberrations such as Down syndrome, cystic hygroma and additional major congenital anomalies were also noted in some of our cases.

The lone functional cardiac cause for hydrops identified in our study was complete heart block, which had serological evidence of maternal connective tissue disease. Even though sectioning is difficult, and time consuming, microscopic examination is critical in identifying fibrosis of the conduction system.

The cardiogenic hydrops would result from increase in right atrial pressure, reduced preload reserve and congestive cardiac failure.[7]

The immaturity of foetal myocardium (both structural and functional) with reduced relaxation is more vulnerable to any in utero insult leading to decrease in cardiac output.[1]

Hydrops associated with cardiac malformations generally have poor prognosis with high mortality. Recent developments in diagnostic capabilities of prenatal ultrasound along with in utero interventions will probably improve the outcome of foetuses with congenital cardiac lesions.[8]

Placental lesions

Interestingly, in present study group, placental lesions constituted the second most common cause of NIHF. Five of our patients demonstrated exclusive placental lesions significant enough to explain the aetiology of hydrops. Rodriguez et al, demonstrated placental pathology as the cause of hydrops in 10 patients (19.6%). In agreement with their study we too observed a similar incidence.[9]

Notwithstanding its rarity, we found placental mesenchymal dysplasia (PMD) as a unique pathology in two of our cases. PMD is an underreported and under recognized placental vascular anomaly causing hydrops.

It is characterized by placentomegaly (>90th percentile) with hydropic villi and absent trophoblastic proliferation.

The vascular changes observed in PMD might have led to heart failure with hydrops and ultimately resulting in intrauterine foetal demise.[10]

The other causes identified were maternal vascular malperfusion, foetal vascular malperfusion, villitis of unknown aetiology and acute chorioamnionitis.

Intrathoracic lesions

Among the intrathoracic causes (CPAM and CHAOS), an increase in intrathoracic pressure by enlarged lungs leading to impaired venous return could have contributed to foetal hydrops.[11]

Genitourinary abnormalities

Bladder outlet obstruction causing hydrops foetalis have been well documented in the literature. Mechanical obstruction of great vessels due to enlarged urinary bladder with resultant reduced venous return to heart is the underlying pathology.[12] Early onset oligohydramnios secondary to BOO leads to pulmonary hypoplasia, further reducing the survival chances.

Chromosomal abnormalities

In previously reported series of NIHF, chromosomal abnormalities were found in 10% of cases.[4,6,13] In the present study, there was 1 case of Turner syndrome and 3 instances of Down syndrome accounting for 16% of cases. Except for the Turner syndrome, none were confirmed by karyotyping. Among the 4 foetuses, 3 were identified in early gestation and one was associated with cardiac defect and cystic hygroma. Chromosomal analysis should be performed in all cases of NIHF, more so when identified in earlier gestation and when associated with structural anomalies.[14]

Miscellaneous causes

Intrauterine infections can cause foetal hydrops. There was one case of Parvovirus B19 infection in our study which showed hepatosplenomegaly with dense lymphocytic infiltration of myocardium. The underlying mechanisms include foetal anaemia, viral myocarditis and impaired hepatic function.[14,15] High index of suspicion and prenatal screening for intrauterine infections will make the interpretation easier. Incidence of "idiopathic" category varies among studies.[9,16,17] After detailed and systematic examination of foetuses including placenta, we found only one case (4%) of NIHF in which the cause was not identified. This highlights the value of autopsy with accompanying placental examination as the most complete procedure to establish the cause of NIHF.

Ultrasound correlation

Studies comparing the utilisation of foetal autopsy with prenatal USG findings have shown correlation in 98% of cases.[18,19] But these studies did not involve cases with hydrops foetalis. In more than 50% of studied cases, autopsy examination either refuted or altered the USG diagnosis completely. Though it is known that USG might miss some findings due to many reasons like equipment quality, sonographer expertise, maternal body habitus and liquor volume, this gross discrepancy is speculative. This might emphasis the need for autopsy, especially in cases of hydrops to arrive at a final aetiological diagnosis.

Also worthy of mention are the absence / rare diagnosis of inborn errors of metabolism and infective diseases in our series of cases. A better and complete laboratory work up with utilization of immunohistochemistry for lymphatic dysplasia/viral inclusions may be helpful to overcome this low diagnostic yield.

The causes of NIHF is heterogenous and the survival of the foetus depends on the underlying aetiology. The most promising tool in understanding the aetiology of NIHF is systematic evaluation of the foetuses including placenta. Multidisciplinary approach including foetal imaging, laboratory work up, postmortem bacterial / viral culture, genetic study, complete autopsy with utilization of immunohistochemistry (when necessary) will identify the potential causes in foetuses with suspected NIHF. This will determine new strategies for in utero therapy, and thus make a positive impact on perinatal outcome. Knowledge about the aetiology and pathogenesis will provide a better correlation with risk of recurrence. It may also improve parental counselling and prevent similar mishaps in subsequent pregnancies.

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In conclusion, the autopsy provides the final diagnosis and enables us to understand the disease process and its natural history. The decline in clinical autopsies is a matter of great concern to the clinicians and pathologists alike. State of the art imaging devices and molecular biology techniques can complement, but not replace a comprehensive autopsy.

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1. Nagy A, Malcomson RD. Fetal hydrops. In Khong, T. Yee, Malcomson, Roger D. G. (Editors), Keeling's Fetal and Neonatal Pathology, 5th Ed. Springer-Verlag, London, 2015:299-328.

2. Bellini C, Hennekam RC, Fulcheri E, Rutigliani M, Morcaldi G, Boccardo F, et al. Etiology of nonimmune hydrops fetalis: a systematic review. Am J Med Genetics Part A. 2009 May;149(5):844-51.
3. Villaespesa AR, Mier MPS, Ferrer PL, Baleriola IA, Gonzalez JIR. Nonimmunologic hydrops fetalis: an etiopathogenetic approach through the postmortem study of 59 patients. Am J Med Genet. 1990;35:274-9.
4. Bellini C, Hennekam RC. Non-immune hydrops fetalis: a short review of etiology and pathophysiology. Am J Med Genet A. 2012 Mar;158A (3):597-605.
5. Moreno CA, Kanazawa T, Barini R, Nomura ML, Andrade KC, Gomes CP, et al. Non-immune hydrops fetalis: A prospective study of 53 cases. Am J Med Genet Part A. 2013 Dec;161(12):3078-86.
6. Bellini C, Donarini G, Paladini D, Calevo MG, Bellini T, Ramenghi LA, et al. Etiology of non-immune hydrops fetalis: an update. Am J Med Genet. Part A. 2015 May;167(5):1082-8.
7. Yuan SM. Cardiac etiologies of hydrops fetalis. Z Geburtshilfe Neonatol. 2017 Apr 221(2):67-72.
8. Knilans TK. Cardiac abnormalities associated with hydrops fetalis. Semin Perinatol. 1995 Dec;19(6):483-92.
9. Rodríguez MM, Chaves F, Romaguera RL, Ferrer PL, de la Guardia C, Bruce JH. Value of autopsy in nonimmune hydrops fetalis: series of 51 stillborn fetuses. Pediatr Dev Pathol. 2002 Jul-Aug;5(4):365-74.
10. Akbarzadeh-Jahromi M, Sari Aslani F, Parvari S. Placental mesenchymal dysplasia complicated by hydrops fetalis and fetal death: a case report. Arch Iran Med. 2013 Sep; 16(9):551-4.
11. Rodríguez MM, Bruce JH, Jiménez XF, Romaguera RL, Bancalari E, García OL, et al. Nonimmune hydrops fetalis in the liveborn: series of 32 autopsies. Pediatr Developmental Pathol. 2005 Jun 1;8(3):369-78.
12. Faye-Petersen OM, Heller DS. Pathology of the Stillborn Infant for the General Pathologist: Part 2. Adv Anat Pathol. 2015;22:71-93.
13. Machin GA. Hydrops revisited: literature review of 1,414 cases published in the 1980s. Am J Med Genet. 1989 Nov;34(3):366-90.
14. Society of Obstetricians and Gynaecologists of Canada (SOGC) clinical practice guidelines. Investigation and Management of Non-immune Fetal Hydrops J Obstet Gynaecol. 2013;35:e1-e14.
15. Society of Obstetricians and Gynaecologists of Canada (SOGC) clinical practice guidelines. Parvovirus B19 Infection in Pregnancy J Obstet Gynaecol. 2014;36 (12):1107-16
16. Lallemand AV, Doco-Fenzy M, Gaillard DA. Investigation of nonimmune hydrops fetalis: multidisciplinary studies are necessary for diagnosis-review of 94 cases. Pediatr Dev Pathol. 1999 Sep-Oct; 2 (5):432-9.
17. Randenberg AL. Nonimmune hydrops fetalis part II: does etiology influence mortality?. Neonatal Netw. 2010 Nov-Dec;29 (6):367-80.
18. Sankar VH, Phadke SR. Clinical utility of fetal autopsy and comparison with prenatal ultrasound findings. J Perinatol. 2006 Apr;26(4):224.
19. Johns N, Al-salti W, Cox P, Kilby MD. A comparative study of prenatal ultrasound findings and post-mortem examination in a tertiary referral center. Prenat Diag. 2004;24:339-46.

r Ramya T, Umamaheswari G, Chaitra V. Nonimmune hydrops foetalis: value of perinatal autopsy and placental examination in determining aetiology. Int J Res Med Sci 2018;6:3327-34.

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Significance of foetal autopsy in cases of nonimmune hydrops foetalis

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Abstract

Background: Nonimmune hydrops foetalis (NIHF) is a terminal catastrophic event of pregnancy caused by numerous diverse etiology. The aim of this study was to assess the significance of foetal autopsy and to compare the prenatal ultrasound (USG) and foetal autopsy findings in cases of NIHF.

Methods: All perinatal autopsies performed at the department of pathology between March 2011-February 2018 were retrospectively reviewed. Of the received 130 autopsies, twenty cases of NIHF were identified, records of which were collected and correlated with maternal medical history, prenatal imaging and autopsy findings.

Results: The malformations with hydrops foetalis were classified according to the involved organ system. They were cardiothoracic (7/20 cases), genitourinary (3/20 cases), gastrointestinal lesions (1/20 cases), chromosomal (4/20 cases) and multisystem anomaly/syndromic association (5/20 cases). Complete agreement between USG and autopsy was seen in 8 (40%) cases. In 5 (25%) cases autopsy findings were in total disagreement with USG diagnosis. The rest of the 7 (35%) cases, autopsy revealed additional information and changed the recurrence risk in two cases.

Conclusions: Present study demonstrates the high rate of discordancy between USG and autopsy examination in cases complicated by NIHF. Foetal autopsy confirms the USG findings (quality control/audit), adds additional findings or changes the final diagnosis, which helps in redefining the recurrence risk and plausible genetic counselling for future pregnancies. Hence present study underscores the need for autopsy in all cases of NIHF.

Keywords: Nonimmune hydrops foetalis, Perinatal/ foetal autopsy, Prenatal imaging, Ultrasound

Introduction

Hydrops foetalis remains a distressing pregnancy complication with high perinatal mortality. It is characterized by generalized oedema and presence of fluid in pleural, peritoneal and pericardial cavities. Polyhydramnios and abnormally thickened placenta are often associated with hydrops.[1]

Traditionally, hydrops is classified into immune and nonimmune types. There is marked reduction in the incidence of immune hydrops due to effective immunologic prophylaxis for maternal isoimmunisation. Currently, the focus is on nonimmune hydrops foetalis (NIHF), which accounts for >90% of cases of hydrops.[2]

NIHF is associated with multiple aetiology. The various causes include cardiovascular, genitourinary, thoracic lesions, chromosomal aberrations, infections, haematological, genetic/metabolic, placental and idiopathic.[3]

Initial diagnosis of hydrops foetalis is usually made by ultrasound. In most of the cases, an accurate and complete antenatal imaging also points to the cause behind the development of hydrops. Despite having a

high diagnostic accuracy, foetal ultrasonography (USG) has some limitations, especially in cases with hydrops. Foetal autopsy in such instances can help to overcome the challenges.

The goals of this retrospective study were to compare the accuracy and completeness of prenatal ultrasonography with perinatal autopsy in cases of NIHF.

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All perinatal autopsies performed at the department of pathology between March 2011-February 2018, were retrospectively reviewed. It included foetuses of all gestational ages. Causes of deaths were termination of pregnancy for foetal malformations, spontaneous abortions, stillbirths and early neonatal deaths. Of the 130 autopsies, twenty cases of NIHF were identified, records of which were collected and correlated with maternal medical history and prenatal imaging studies.

Immune hydrops foetalis were excluded from the study. Institutional ethical committee approval was obtained. Maternal data collection included maternal age, obstetric history, maternal medical diseases, gestational age of the foetus, and prenatal ultrasound findings.

Sonograms were predominantly performed by radiologists and some by obstetricians trained in obstetric ultrasound examination. The diagnostic criteria for hydrops foetalis were foetal skin oedema >5.0mm with one serous cavity effusion or fluid collection in at least two serous cavities (peritoneal, pleural or pericardial cavities).

The autopsies were performed by pathologists with perinatal experience. Informed written consent was obtained for all cases. A standard autopsy protocol was followed which included foetogram, photography, biometric measurements, external and internal examination. Microscopic examination of relevant organs such as thyroid, thymus, lungs, heart, liver, spleen, pancreas, adrenals, kidneys and ovary (in case of female), testis (in case of male) were done.

The brain was removed and fixed in formalin solution with the help of a gauze in a hanging position, then relevant sections were studied. Spinal cord, bone, muscle were also studied when necessary.

Special stains such as Periodic acid–Schiff (PAS) and Perls' Prussian blue were done if needed. Placental examination was done in all cases, but findings were not considered for the present study.

Placental causes of hydrops were also excluded from the study. Results of karyotype, if known was collected. The final autopsy diagnosis was correlated with prenatal ultrasound findings and results were drawn.

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The maternal age ranged from 19 to 35 years with the mean age of 25.55years. Gestational age ranged from 13 to 35 weeks, with the mean gestational age of 21.45 weeks. There were 18 stillborn foetuses and 2 neonates with the Male: Female ratio of 3:2. Authors did not come across any multiple gestation. Four cases had recurrent hydrops.

Prenatal diagnosis of hydrops was noted in 14 cases and in the remaining 6, diagnosis was made at post-mortem. present data showed prenatal diagnosis of the presence of hydrops in only two-third of the cases.

Subcutaneous oedema and serous cavity effusions were identified in the rest one-third only during autopsy. All of these were second trimester foetuses. Cystic hygroma was identified in five cases.

The malformations with hydrops foetalis were classified according to the involved organ system. They were cardiothoracic (7/20 cases), genitourinary (3/20 cases), gastrointestinal lesions (1/20 cases), chromosomal (4/20 cases) and multisystem anomaly/syndromic association (5/20 cases).

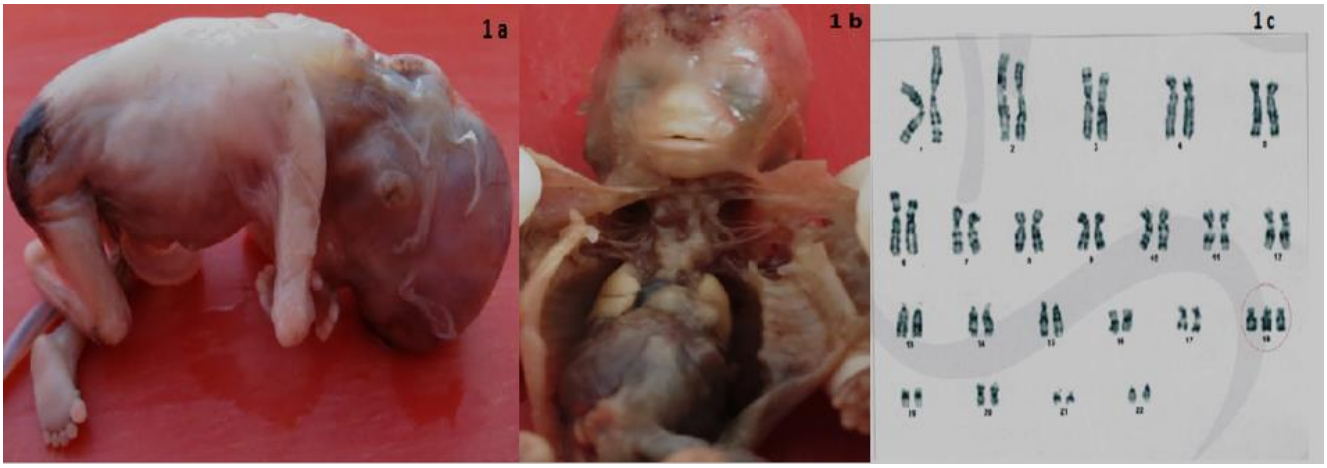
Cardiac lesions (n=4): The cardiovascular malformations included premature closure of ductus (1case), complete heart block(1case), atrioventricular septal defect (AVSD) with persistent left superior vena cava (SVC) with other findings suggestive of Down syndrome(1case) and 1 case of transposition of great arteries (TGA), which also had an associated partial urorectal septum malformations.

Thoracic lesions (n=3): The thoracic lesions were congenital pulmonary adenomatoid malformations (CPAM, 2 cases) and congenital high airway obstruction syndrome (CHAOS, 1 case).

Genitourinary lesions (n=3): Three cases of bladder outlet obstruction (BOO) were included in the group of urogenital malformations, of which one had an associated ventriculomegaly, absent corpus callosum (ACC) with hypoplasia of ductus, right and left pulmonary arteries, distal aortic arch and left subclavian artery

Gastrointestinal lesions (n=1): The gastrointestinal lesions included, a 28weeks foetus with hypertrophic pyloric stenosis.

Chromosomal abnormalities (n=4): Of the 4 cases, two showed phenotypic features of Down syndrome, but karyotype confirmation was not available. Karyotyping was possible in the remaining 2 cases of which one showed partial deletion of short arm of X chromosome (Turner syndrome) and the other showed trisomy 18 (Edwards syndrome) (Figure 1).



[illegible] (Edward's [illegible]

Multisystem anomalies/ syndromic association (n=5): The autopsy findings suggested syndromic associations in five instances. They were one case each of Ellis- Van Creveld syndrome, Multiple congenital anomaly syndrome, VACTERL (vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies and limb abnormalities) association, FRYNS Syndrome and Cerebro-oculo-facio-skeletal syndrome. Karyotype analysis was done for the last two cases and were found to be normal.

Correlation between prenatal ultrasound and autopsy findings: Complete agreement between USG and autopsy was seen in 8 (40%) cases. In 5 (25%) cases, autopsy findings were in total disagreement with USG diagnosis. The rest of the 7 (35%) cases, autopsy revealed additional information.

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
1.	Cardiac	HF	Premature ductus closure.
2.	Thoracic	Large cystic hygroma	CPAM
3.	Chromosomal	HF, oesophageal atresia, absence of stomach	Phenotypic features of foetal trisomy No oesophageal atresia Karyotype-not done
4.	Urogenital	No anomaly	BOO-distal urethral stenosis
5.		HF	BOO-distal urethral stenosis Ventriculomegaly, ACC

HF- hydrops foetalis; BOO- bladder outlet obstruction; CPAM- congenital pulmonary airway malformation; ACC –absent corpus callosum.

Cases with complete disagreement has been shown in Table 1.

Two had bladder outlet obstruction, one each had CPAM and premature closure of ductus.

In all the four cases, there was a complete change in the diagnosis after the autopsy.

The fifth case showed no oesophageal atresia, in contrast to prenatal imaging.

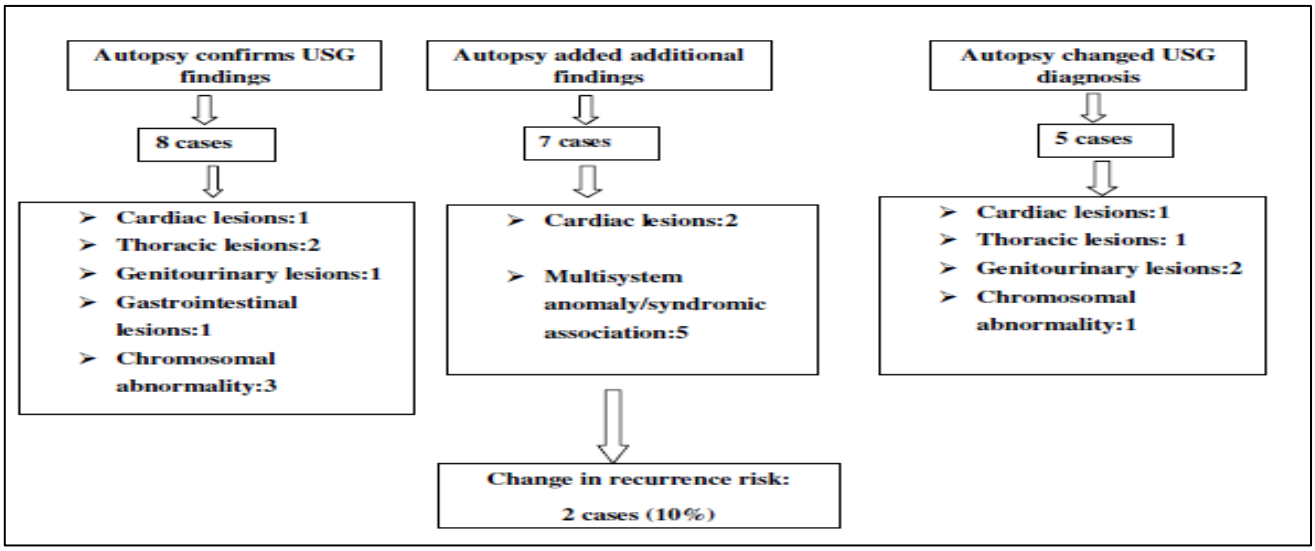
Though this was a significant disagreement, there was no major shift in the final diagnosis as both modalities suggested chromosomal aetiology.

[illegible]

[illegible]	[illegible]	[illegible]
1.	Short long bones, polydactyly, tricuspid atresia with VSD-probably Ellis Van Creveld syndrome	Common atrium with complete AVSD- Ellis-Van Creveld syndrome
2.	Polyhydramnios, HF, micrognathia, hypospadias, cleft soft palate, ACC, cardiomegaly, tricuspid regurgitation	Hypotelorism, depressed nasal bridge, low set ears, Heart – secundum ASD, Liver – haemosiderosis Pancreas – giant islets and nesidioblastosis Multiple congenital anomaly syndrome, Zellweger syndrome, Smith Lemli Opitz syndrome.
3.	SUA, TOF, ectopic hydronephrotic Left kidney, Left club foot with absent 2nd to 5th digits	Hemivertebra, fusion of ribs on the left side, Atresia of distal sigmoid, rectum with absent anal opening VACTERL association
4.	Left diaphragmatic hernia, hypoplastic left heart, short humerus on left side, absent forearm with claw hand on left side; dilated jugulolymphatic sacs: multiple anomalies and with SUA, could be VACTERL association.	Partial ACC, and hydrocephalus Karyotype was normal -Suggestive of FRYNS Syndrome
5.	HF, Foetal akinesia deformation sequence, sloppy forehead, micrognathia, gross bilateral lateral ventriculomegaly	Cerebro-oculo-facio-skeletal syndrome with persistent urogenital sinus
6.	Increased nuchal translucency, cystic hygroma, absent nasal bone	AVSD with persistent left SVC -Suggestive of Down syndrome
7.	Cystic hygroma with TGA and VSD	Partial urorectal septum malformation

VSD-ventricular septal defect; AVSD- atrioventricular septal defect; HF- hydrops foetalis; ACC-agenesis of corpus callosum; ASD-atrial septal defect; SUA-single umbilical artery; TOF-tetralogy of Fallot; VACTERL-vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities; SVC-superior vena cava; TGA-transposition of great arteries

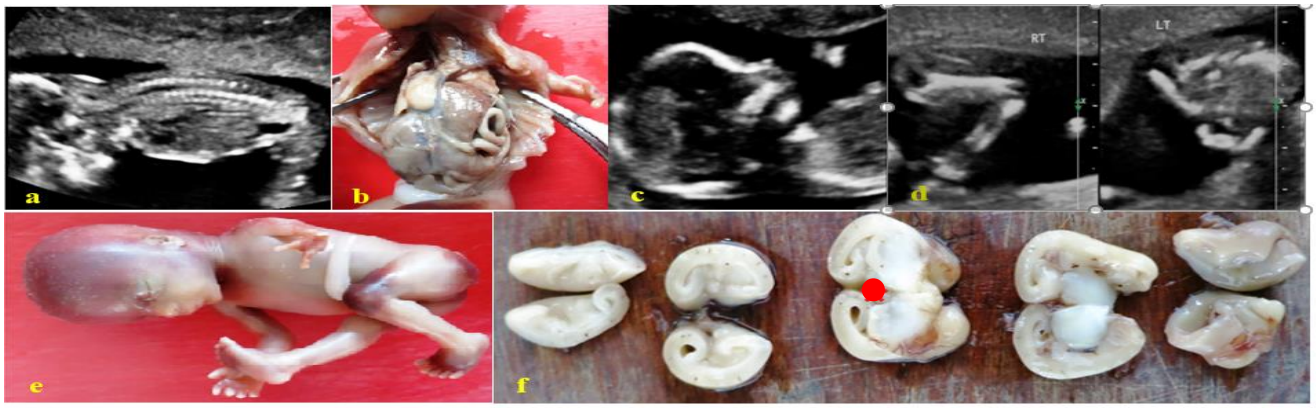
There were seven cases with additional autopsy findings which has been summarized in Table 2. Authors had two cases with cardiac lesions and the rest of the cases showed multisystem involvement. After autopsy, two out of seven cases had a change in recurrence risk (Figure 2).



[illegible]

In cases of multi system involvement, the autopsy finding of hemi vertebra, atresia of distal sigmoid, rectum, anal canal with absent anal opening, completed the diagnosis of VACTERL association (case 3, table 2).

In the same group (case 4, table 2), prenatal diagnosis of VACTERL association was changed to FRYNS syndrome after the identification of central nervous system anomaly (Figure 3).



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Table 3 highlights the cases that showed full agreement between USG and autopsy findings. This included three cases of chromosomal abnormality, two cases of thoracic lesions (CPAM and CHAOS) and one case each from cardiovascular system (complete heart block), genitourinary (BOO) and gastrointestinal group (hypertrophic pyloric stenosis). There were none from the multiple anomaly group.

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
1.	Cardiac	Polyhydramnios, HF, complete heart block	Complete heart block secondary to fibrosis of cardiac conduction system
2.	Thoracic	CHAOS with HF.	Laryngeal atresia (CHAOS)
3.		Polyhydramnios, HF, CPAM with short long bones	CPAM, type 3
4.	Urogenital	Oligohydramnios, bilateral hydronephrosis, distended bladder	BOO - posterior urethral valves.
5.	Gastrointestinal	Polyhydramnios, HF. Hepatomegaly. Stomach visualized	Hypertrophic pyloric stenosis. Hirsutism.
6.	Chromosomal	NT: 3mm. Combined test – increased risk of trisomy 21	Phenotypic features of foetal trisomy Karyotype-not done
7.		NT: 1.75mm Combined test – increased risk of trisomy 21	Phenotypic features consistent with Turners syndrome Karyotyping shows partial deletion of short arm of X chromosome
8.		Cystic hygroma, HF Single outflow with peri membranous VSD, pulmonary atresia	TGA with hypoplastic pulmonary arterial trunk subaortic VSD Cystic hygroma Anteriorly placed anus Hypoplasia of thymus Karyotyping shows trisomy 18.

After extensive search of literature authors came across many studies comparing prenatal USG findings with post-mortem examination. Most of these studies dealt with cases of foetal malformations, stillbirth and neonatal death.[4-8] But, there were only few studies comparing the same in cases of NIHF.[9,10] Hence authors chose to address the accuracy of prenatal USG findings and compare it with perinatal autopsy in cases of NIHF.

Prenatal diagnosis of hydrops was missed in one third of present cases. Fluid collection is initially diagnosed by USG during the first or mid trimester. Significant fluid accumulation is recognized easily, but sometimes the effusions are limited and can escape diagnosis during imaging.

Present data showed complete agreement between USG and autopsy findings in 40% of cases. This was similar to a study by Antonella et al done in 2012.[11] In their study they have compared the USG and autopsy findings of 144 second trimester foetuses. In 49% of cases there were total agreement between the two. Additional information was provided by post-mortem in 34% of cases, while in present study it was 35%. Astonishingly in 13% of their cases, functional anomalies detected by ultrasound could not be confirmed by autopsy. This is due to the fact that ultrasound examination is a dynamic examination, optimum to reveal functional changes like valve insufficiencies, flow reversals and sometimes even cavity effusions. This should not be considered as false positive USG findings, but as an advantage of the imaging modality.

In 7 cases (35%), there were additional findings in autopsy not detected by USG. In two of these cases, the additional findings provided by autopsy redefined the recurrence risk. It seems that the higher proportion of additional findings in the present study might be due to critical identification of minor anomalies at autopsy such as anal atresia, partial agenesis of corpus callosum and urorectal septum malformation.[10]

The identification of these minor anomalies is significant as they might change the diagnosis from an isolated lesion to complex malformation.[4]

All cases in the multiple anomaly had additional findings in autopsy. The identification of partial agenesis of corpus callosum with hydrocephalus in a case with prenatal imaging diagnosis of VACTERL association changed the final diagnosis to be Fryns syndrome. The other anomalies were concordant. The foetus was 16 weeks at the time of diagnosis, and identification of the cranial anomaly at this gestational age is challenging.[12] Fryns syndrome (FS) is the commonest autosomal recessive syndrome in which congenital diaphragmatic hernia (CDH) is a cardinal feature.[13]The autosomal recessive inheritance in FS contrasts with the sporadic inheritance seen in cases of VACTERL anomaly.[14] This underscores the importance of the correct diagnosis for accurate genetic counselling.

Among the cases with cardiovascular lesions, prenatal ultrasound showed increased nuchal translucency, cystic hygroma and absent nasal bone which was all suggestive of trisomy 21. The presence of AVSD, the characteristic heart lesion in these foetuses was made out by post-mortem examination. This more or less confirmed the diagnosis of Down syndrome.

In addition to multiple anomaly, the histological presence of haemosiderosis of the liver with giant islets and nesidioblastosis of the pancreas led to the suggestive diagnosis of multiple congenital anomaly syndrome, Zellweger syndrome, Smith Lemli Opitz syndrome.

One of the study case showed USG findings of Foetal akinesia deformation sequence, with gross bilateral lateral ventriculomegaly. But post-mortem examination also revealed hydranencephaly, lens opacity along with facial and skeletal lesions which led to the diagnosis of cerebro-oculo-facio-skeletal syndrome. Persistent urogenital sinus was noted additionally.

There was total discordance between USG and autopsy in 5 cases of 20(25%). In all 5 cases, though hydrops was diagnosed prenatally, the causative lesion could not be detected by imaging. But autopsy revealed structural anomaly in 4/5 cases. Though all these four lesions (premature closure of ductus, CPAM, BOO) could be identified by antenatal imaging, they were missed. As hydrops foetalis carries a bad prognosis and once it is evident by ultrasound, further careful and complete imaging is overlooked. This could have led to the non-identification of structural abnormality by USG and the discordance with post-mortem examination.

USG as well as autopsy have unavoidable limitations in analysing cases of NIHF. So, they are considered complementary to one another. Both of these modalities depend on the expertise of the personnel, the technological advancement of the ultrasound equipment and the autopsy technique.

The causes for differences in prenatal USG include early gestational age (structures are too tiny to evaluate, respective organs not completed development structurally or functionally), abnormal amount of liquor (oligo/polyhydramnios) which makes visualization of organs difficult, altered maternal habitus (obese, thick abdominal wall) and foetal position. Authors found that additional findings were high in cases of multi system involvement, which was similar to the observation by Rossi et al.[5] This is attributed to the increased focus of the sonographer on severe and more interesting abnormalities, overlooking minor or less lethal abnormalities or not completing the examination once a major/lethal anomaly is detected .[4]

The main limitations of post-mortem examination are longer duration of reporting and the need for appropriate training and expertise in the field of perinatal pathology. There are a few other factors which may hinder the accurate post-mortem examination:

- intrauterine foetal death with long period of foetal retention and superimposed maceration may distort the foetal anatomy
- in foetuses with suspected CNS abnormality, the autopsy findings may be inconclusive due to extreme autolysis
- satisfactory dissection may not be possible in foetuses of very early gestation with small organs (can be overcome by dissecting microscope) and presence of post-mortem artefacts
- functional anomalies can be missed.[10,15-18]

Although present study is a retrospective analysis involving less number of cases, it is the only study to specifically correlate the prenatal USG findings and post-mortem examination of foetuses with nonimmune hydrops. This knowledge and further studies involving large number of cases of NIHF will contribute to improve parental counselling.

NIHF is a terminal catastrophic event of pregnancy caused by numerous diverse aetiology and is known for its low diagnostic yield. Initial recognition of hydrops is usually made by ultrasonographic examination. But the aetiology in many cases remains elusive even after a carefully performed imaging.

In such instances post-mortem study of the foetus including placental examination and other ancillary work up like genetic study, testing for infections and metabolic derangements help in identifying the cause. present study demonstrates a high rate of discordancy between USG and autopsy examination in cases complicated by NIHF.

Foetal autopsy confirms the USG findings (quality control/audit), adds additional findings or changes the final diagnosis. Although, foetuses with nonimmune hydrops carry worse prognosis, it is indispensable to identify the genesis of the hydrops to counsel the couple regarding prognosis, recurrence risk and future pregnancy outcomes. Hence present study underscores the need for autopsy in all cases of NIHF.

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1. Randenberg AL. Nonimmune hydrops fetalis part II: does etiology influence mortality?. Neonatal Netw. 2010 Nov-Dec;29(6):367-80.
2. Bellini C, Hennekam RC. Non-immune hydrops fetalis: a short review of etiology and pathophysiology. Am J Med Genet A. 2012;158A:597-605.
3. Nagy A, Malcomson RDG. Fetal hydrops. In Yee KT, Malcomson RDG, eds. Keeling's Fetal and Neonatal Patholog, 5th ed. London: Springer-Verlag; 2015:299-328.
4. Chescheir NC, Reitnauer PJ. A comparative study of prenatal diagnosis and perinatal autopsy. J Ultrasound Med. 1994 Jun;13(6):451-6.
5. Rossi AC, Prefumo F. Correlation between fetal autopsy and prenatal diagnosis by ultrasound: A systematic review. Eur J Obstet Gynecol Reprod Biol. 2017 Mar;210:201-6.
6. Antonsson P, Sundberg A, Kublickas M, Pilo C, Ghazi S, Westgren M, et al. Correlation between ultrasound and autopsy findings after 2nd trimester terminations of pregnancy. J Perinat Med. 2008;36(1):59-69.
7. Vogt C, Blaas H-GK, Salvesen KA, Eik-Nes SH. Comparison between prenatal ultrasound and postmortem findings in fetuses and infants with developmental anomalies. Ultrasound Obstet Gynecol. 2012;39(6):666-72.
8. Boecking CA, Drey EA, Kerns JL, Finkbeiner WE. Correlation of prenatal diagnosis and pathology findings following dilation and evacuation for fetal anomalies. Arch Pathol Lab Med. 2016 Oct 20;141(2):267-73.
9. Sankar VH, Phadke SR. Clinical utility of fetal autopsy and comparison with prenatal ultrasound findings. J Perinatol. 2006 Apr;26(4):224.
10. Struksnæs C, Blaas HG, Eik-Nes SH, Vogt C. Correlation between prenatal ultrasound and postmortem findings in 1029 fetuses following termination of pregnancy. Ultrasound Obstet Gynecol. 2016 Aug 1;48(2):232-8.
11. Vimercati A, Grasso S, Abruzzese M, Chincoli A, de Gennaro A, Miccolis A, et al. Correlation between ultrasound diagnosis and autopsy findings of fetal malformations. J Prenatal Med. 2012 Apr;6(2):13.
12. Guibert-Trainer F, Piton J, Billerey J. Agenesis of the corpus callosum. J Neuroradiol. 1982;9:135.
13. Slavotinek AM. Fryns syndrome-a review of the phenotype and diagnostic guidelines. Am J Med Genet. 2004;124A:427-33.
14. Solomon BD, Pineda-Alvarez DE, Raam MS, Cummings DA. Evidence for inheritance in patients with VACTERL association. Hum Genet. 2010;127:731-3.
15. Rodriguez MA, Prats P, Rodriguez I, Cusi V, Comas C. Concordance between prenatal ultrasound and autopsy findings in a tertiary center. Prenat Diagn. 2014;34:784-9.

16. Gilbert-Barness E, Debich-Spicer D. Embryo and fetal pathology: color atlas with ultrasound correlation. Cambridge: Cambridge University Press; 2004.
17. Kaasen A, Tuveng J, Heiberg A, Scott H, Haugen G. Correlation between prenatal ultrasound and autopsy findings: A study of second-trimester abortions. Ultrasound Obstet Gynecol. 2006;28:925-33.
18. Amini H, Antonsson P, Papadogiannakis N, Ericson K, Pilo C, Eriksson L, et al. Comparison of ultrasound and autopsy findings in pregnancies terminated due to fetal anomalies. Acta Obstet Gynecol Scand. 2006;85(10):1208-16.

Umamaheswari G, Ramya T, Chaitra V. Nonimmune hydrops foetalis (NIHF): value of fetal autopsy and comparison with ultrasound findings. Int J Reprod Contracept Obstet Gynecol 2018;7:3330-7.

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[illegible]: Under normal circumstances, a woman's uterus sheds a limited amount of blood during each menstrual period (around 80 ml). Bleeding that occurs erratically or excessive menstrual bleeding is called abnormal uterine bleeding (AUB). The causes of AUB are many and varied. Initial investigations include transvaginal ultrasound and histopathologic assessment of the endometrium. Objective of this study was to evaluation of endometrial thickness with trans-vaginal ultrasound and its correlation with histopathology by dilatation and curettage in abnormal uterine bleeding. To determine the efficacy of transvaginal ultrasound in evaluating the endometrial thickness. To correlate the endometrial thickness by transvaginal ultrasound with endometrial histopathology in women with AUB.

M[illegible]: It is a retrospective observational study. All reproductive and perimenopausal age group women who underwent dilatation and curettage for abnormal uterine bleeding during the period June 2014-June 2016 was taken and analyzed and correlated with their endometrial thickness measured with Transvaginal ultrasound.

[illegible]: Around 478 patients who underwent endometrial sampling over a period of two years were analyzed. Maximum number of patients were in the fourth decade and the overweight category 36.6%. Proliferative endometrium was the most common histopathologic picture (44.76%). Detection of precancerous lesions were-5.87% and endometrial cancer was 1.05%.

[illegible]: An ET of 8 mm and above gave 100% sensitivity and negative predictive value for precancerous and cancerous lesions.

[illegible]: Abnormal uterine bleeding, Dilatation and curettage, Endometrial hyperplasia and cancer, Endometrial thickness, Transvaginal ultrasound

[illegible]

Abnormal uterine bleeding is one of the most common gynecological problems in patients approaching the outpatient clinics in the country. These complaints definitely affect the quality of life of the patient. The etiology of AUB varies with the age group of the patient. There is the PALM COEIN classification for AUB by FIGO.[1] There are many diagnostic modalities for evaluating endometrial pathologies in women who have AUB. Transvaginal sonogram (TVS) has permitted the use of higher frequency ultrasound waves at greater proximity of the uterus. It is a relatively cheap and non-invasive diagnostic modality for studying the endometrial pattern and its thickness and at the same time to exclude organic pathology in case of AUB while dilatation and curettage gives us a definitive histopathologic picture. This study attempts to correlate the USG findings and histopathology in patients with AUB. The gold standard

for diagnosing AUB is hysteroscopy and directed biopsy but it is expensive when compared to the simple endometrial sampling. According to the PALM COIEN classification the causes of AUB can be divided into structural and non-structural cause which is given in detail in Table 1.

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Polyps	Coagulopathy
Adenomyosis	Ovulatory dysfunction
Leiomyomas	Endometrial hyperplasia
Malignancy	Iatrogenic
	Not yet specified

Aims and objectives of this study were

- Evaluation of endometrial thickness with trans-vaginal ultrasound and its correlation with histopathology by dilatation and curettage in abnormal uterine bleeding.
- To determine the efficacy of Transvaginal ultrasound in evaluating the endometrial thickness.
- To correlate the endometrial thickness by Transvaginal ultrasound with endometrial histopathology in women with AUB.

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Retrospective observational study included all reproductive and perimenopausal age group women who underwent dilatation and curettage for abnormal uterine bleeding during the period June 2014 to June 2016.

Inclusion criteria

- AUB in all reproductive and perimenopausal women
- Only gynecological indications for dilatation and curettage.

Exclusion criteria

- Unmarried women
- Pregnancy and related causes of bleeding
- Postmenopausal bleeding
- Puberty menorrhagia.

From minor and major OT registers, list of all D and Cs which were done during 2014 June to 2016 June was taken. Cases which satisfied the inclusion and exclusion criteria was taken and analyzed

Statistical analysis

Chi square test was used for the statistical analysis.

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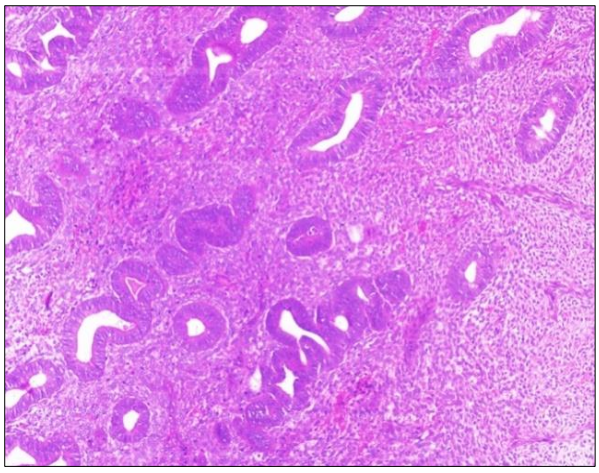
All the patients with abnormal uterine bleeding, fitting the inclusion criteria, who underwent endometrial sampling was taken for analysis. There were around 478 patients. All of them had a premenstrual ultrasound before the procedure. The age distribution, parity, BMI, presenting complaints, the histopathology and the endometrial thickness by TVS was evaluated. The results are given below:

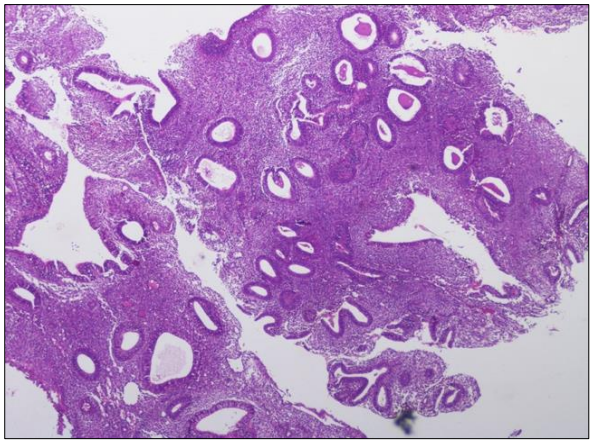
Of the 478 patients, the youngest patient was 22 years old and the oldest was 55 years, the mean age was 38.5 years. Maximum number of patients were in the 41-50 years age group which was 60.25% and the least in the 20-30 years group and was 4.6% (Table 2).

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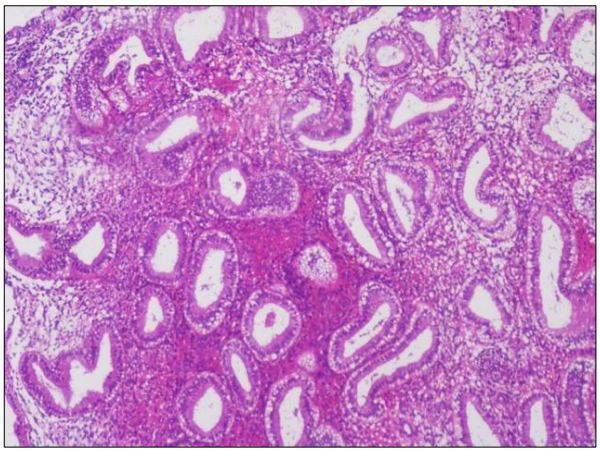
were having proliferative phases (Figure 1), of which 29.7% was disordered proliferative (Figure 2). 30.33% showed secretory changes (Figure 3) endometrial adenocarcinoma (Figure 4) was found in 1.05% of the cases and hyperplasia was seen in 5.86% (Table 5).

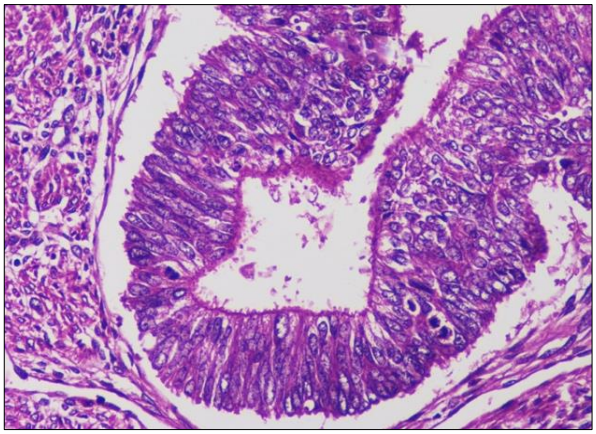
Disordered proliferative	142	29.7%	44.76%
Proliferative	72	15.06%	
Secretory	145	30.33%	
Endometrial hyperplasia	28	5.86%	
Endometrial carcinoma	5	1.05%	
Others	86	17.99%	
Total	478	100%	





Among the hyperplasia, 50% was simple hyperplasia without atypia. Atypical hyperplasia (Figure 5) was found in 12 cases. Complex hyperplasia was found in 13 out of the 28 cases of hyperplasia of which complex atypical was 11 cases (Table 6).



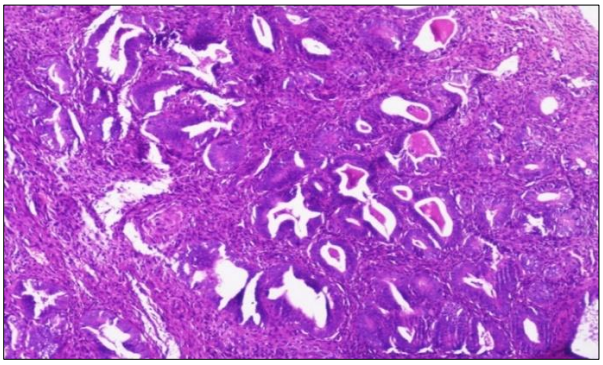


Simple hyperplasia without atypia	14	50%
Simple hyperplasia with atypia	1	3.57%
Complex hyperplasia without atypia	2	7.14%
Complex hyperplasia with atypia	11	39.29%
Total	28	100%

Endometrial thickness was 8mm and above up to 15 mm in majority of cases of AUB-54.81%. ET less than 8mm was found only in 19.3% of the study population.ET 15 mm and above was also less which was 25.5% (Table 7). ET less than 4mm in AUB was very less-only 1.67%. One ET was not measured due to diffuse adenomyosis. Out of the 477 patients whose endometrial thickness were measured, all the five cases of endometrial carcinoma was found with an ET ≥15 mm among the 28 cases of endometrial hyperplasia 12 cases of hyperplasia were also found with ET ≥15 mm and the rest 16 cases had ET ≥8-15 (Table 8). Surprisingly all the five cases of carcinoma was endometrioid adenocarcinoma.

It was found in this study that proliferative and secretory endometrium was maximum when the endometrial

thickness was ≥8-15 mm and least with an ET less than 4 mm (Table 9).



[illegible]

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]
<4	8	1.67%
4-8	85	17.78%
≥8-15	262	54.81%
≥15	122	25.52%
Not measured	1	0.22%
Total	478	100%

If we take 8 mm as the cut off, we can see that in this study population all the cases of hyperplasia and malignancy had an ET ≥ 8 mm. Out of the 384 cases with ET ≥ 8 mm 28 cases had hyperplasia and five patients had endometrial carcinoma i.e. 8.59% (Table 10).

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
≥15	122	12	9.84%	5	4.46%
≥8-15	262	16	6.11%	0	0%
4-8	85	0	0%	0	0%
<4	8	0	0%	0	0%
Total	477	28	5.87%	5	4.46%

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
<4 mm	8	3	3	0	0	2
4 - <8	85	34	29	0	0	22
≥8-15	262	121	86	16	0	39
≥15	122	56	27	12	5	22
Total	477	214	145	28	5	85

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
≥8	384	33	8.59%
<8	94	0	0%

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
≥8	384	33 (a)	351 (b)
<8	94	0 (c)	94 (d)

Sensitivity, specificity, the positive and negative predictive value for an ET of ≥8 mm was calculated. 94 patients with ET less than 8 and 351 patients with ET ≥8 mm did not have carcinoma (Table 11).

- Sensitivity = a/a + c =33/33 = 100%
- Specificity= d/b + d = 94/445 = 21.12%
- Positive predictive value = a/a + b = 33/384 = 8.59%
- Negative predictive value = d/c + d = 94/94 = 100%

[illegible]

Abnormal uterine bleeding is a very common diagnosis in the op population. According to this study the maximum age groups of the patients were in the perimenopausal age of 41-50 years-around 60.25%. This is similar to the study by Shobitha et al -68.1% in the same age group while it was 38% in a study by Mahapatra M et al where the study group was less.[2,3]

The youngest patient was a 22-year-old primary infertility patient with heavy menstrual bleeding for which diagnostic curettage was done and had an ET of 6 mm and secretory endometrium.

In this study, 79.7% were multiparious while 2.93% were nulli, in almost all the studies this is similar. This is in accordance to the study by Nidhi et al, where 88% were multipara and 1.2% were nulliparous.[4] In a study by Betha K et al 81% were multipara.[5]

BMI was in the overweight category in 36.6% and obese in 24.3% in this study which is similar to the study in Iran by Kazamijalezah et al, where it was 33% and also with the study by Sahai A et al, where 56.2% were in overweight category and obese was 25%.[6,7]

There was heavy menstrual bleeding in 98% of patients.

Of these 478 patients only 39 had diabetes as risk factor and out of 39 one had endometrial carcinoma.

When we analyze the histopathologic findings of the 478 cases 44.5% was proliferative endometrium, of this disordered proliferative endometrium seems to be the commonest, in a study by Elavarasan et al, where disordered proliferative endometrium seems to be the commonest pattern observed i.e. 31.1%.[8] The term disordered proliferative endometrium refers to proliferative phase that does not seems to be appropriate for any one time in the menstrual cycle and mimics simple hyperplasia, but the process is focal rather than diffuse. Histologically there shows mild disorganization of architecture, with focal dilated glands, with normal gland to stromal ratio. In this study 30% was secretory endometrium; endometrial hyperplasia was found in 5.8% and endometrial cancer in 1%.

Transvaginal ultrasound was done to assess the endometrial thickness and it was done in the premenstrual phase, of the 478 cases 54.8% had an ET of ≥8-15, 25.5% had ET more than 15 mm. In a study by Jikki et al, 57% had et ≥8 mm which is similar to this study of 54%.[9]

In the study by Jikki et al, the endometrial hyperplasia was seen in 96.4% cases when ET was more than 8 mm and in this study 100% of the patients with endometrial hyperplasia had ET ≥8 mm.[9] In this study 100% cases of endometrial carcinoma occurred with ET ≥15 and all cases of hyperplasia had ET ≥8 mm.

Simple hyperplasia without atypia was 50% in this study which was comparable to 62% in a study by Tabata et al.[10]

Complex endometrial hyperplasia was 14.8% in a study conducted by Idris et al, while their atypical hyperplasia was only 2% whereas atypical hyperplasia was 42.4% in this study.[11] This may be because of the fact that most of the patients in the study group were in the fourth decade (60%) and in their study was in the third decade (80%).

Of the 384 cases with ET ≥8 mm, 33 had endometrial hyperplasia and carcinoma, and 351 did not have e/o hyperplasia and carcinoma, none of the cases below 8mm had any e/o hyperplasia or carcinoma. Sensitivity and negative predictive value was 100% for an ET of ≥8 mm. This is similar to studies by Chatpavit Getpook an ET of 8 mm showed optimal sensitivity and specificity -83.9% and 58.8% respectively and 90.4% negative predictive value for an abnormal endometrium.[12] They concluded that an ET of 8mm or less is less likely to be associated with malignant pathologies. In this study the negative predictive value was 100% and sensitivity was 100% but the specificity was only 21.1%. According to a study by Ozdemir S et al, there was negative predictive value of 95.6%, sensitivity and specificity of 83.6 and 56.4% respectively for an ET of 8 mm.[13]

Kurman et al, classified hyperplasia in to simple and complex and subcategory as atypical.[14] The 2014 revised WHO classification simply separates endometrial hyperplasia into 2 groups-hyperplasia without atypia and atypical hyperplasia.[15] According to the EIN classification all atypical endometrial hyperplasia is classified as endometrial intraepithelial neoplasia.[15] Progesterone was found to cause regression in 90% cases with hyperplasia.

The RCOG cutoff of ET is less than 7 mm below which there is no endometrial hyperplasia in a prospective study of 56 women with pcos.[11,16] In studies by Gianella et al, 11 mm was taken as the cut off.

The most common presenting symptoms of endometrial hyperplasia is abnormal uterine bleeding. According to studies, the risk of progression of simple hyperplasia to carcinoma is 1% and complex hyperplasia with atypia is 29%.[18]

□□□□□□□□□□

The clinical importance of endometrial hyperplasia is because of its progression to endometrial carcinoma. An ET of 8 mm in this study gives a 100% negative predictive value for hyperplasia and carcinoma and can be taken as a cut off for deciding the patients for dilatation and curettage especially in low risk patients.

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□□□□□□□□□□

1. Munro MG, Critchley HO, Broder MS, Fraser IS. FIGO classification system (PALM COIEN) for causes of abnormal uterine bleeding in non-gravid women of reproductive age. FIGO Working Group on Menstrual Disorders. Int J Gynaecol Obstet. 2011;113:3-13.
2. Shobhitha GL, Kumari VI, Priya PL, Sundari BT. Endometrial study by TVS and It's correlation with

histopathology in abnormal uterine bleeding. J Dental Med Sci. 2015;14(4):21-32.
3. Mahapatra M, Mishra P. Clinicopathological evaluation of abnormal uterine bleeding. J Health Res Rev. 2015;2:45-9
4. Meena N, Meena S. Correlation between histology and bleeding pattern in a case of AUB and risk factors analysis of AUB in reproductive age group. JMSCR. 2017;5(7):24629-34.
5. Betha K, Malvatu L, Talasani S Distribution of causes of abnormal uterine bleeding using new FIGO classification system-PALM COEIN: a rural tertiary-based study. Int J Reprod Contracept Obstet Gynecol. 2017;6(8):3523-7.
6. Kazemijaliseh H, Ramezani Tehrani F, Behboudi-Gandevani S, Khalili D, Hosseinpanah F, Azizi F. A Population-based study of the prevalence of abnormal uterine bleeding and its related factors among Iranian reproductive-age women: An updated data. Arch Iran Med. 2017;20(9):558-63.
7. Sharma AS, Gupta S. Analysis of body mass index in patients with abnormal uterine bleeding. Inter J Clin Obstet Gynaecol. 2019;3(1):92-5
8. Puvitha RD, Elavarasan T, Shruthi MS, Shylaja S. Histopathological study of endometrium in abnormal uterine bleeding an experience in a tertiary care centre of rural south India. Nat J Basic Med Sci. 2017;8:1.
9. Kalaiselvi PSJ. Thenmozhi G. A study on endometrial thickness by endovaginal sonography with histopathology in perimenopausal women in a tertiary care centre. Indian J Res. 2017;6(10):33-6.
10. Tabata T, Yamawaki T, Yabana T, Ida M, Nishimura K, Nose Y. Natural history of endometrial hyperplasia Arch Gynecol Obstet. 2001;265(2):85-8.
11. Takai IU, Bukar M, Mayun AA, Ugwa EA, Audu BM. Aisha abdurrahman endometrial hyperplasia: a 2 decade retrospective analysis of histopathological pattern at a university teaching hospital in Northern Nigeria Sub-Saharan. Afr J Med. 2016;3:171-5.
12. Getpook C, Wattanakumtornkul S. Endometrial thickness screening in premenopausal women with abnormal uterine bleeding. J Obstet Gynaecol Res. 2006;32(6):588-92 .
13. Özdemir S, Çelik Ç, Gezginç K, Kıreşi D, Esen H. Evaluation of endometrial thickness with transvaginal ultrasonography and histopathology in premenopausal women with abnormal vaginal bleeding. Arch Gynecol Obstet. 2010;282(4):395-9.
14. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia: a long-term study of "untreated" hyperplasia in 170 patients. Cancer. 1985;56:403-12.
15. Palmer JE, Perunovic B, Tidy JA. Endometrial hyperplasia. The Obstet Gynaecol. 2008;10(4):211-6.
16. Cheung AP. Ultrasound and menstrual history in predicting endometrial hyperplasia in polycystic ovary syndrome. Obstet Gynecol. 2001;98:325-31.
17. Giannella L, Cerami LB, Setti T, Bergamini E, Boselli F. Prediction of endometrial hyperplasia and cancer among premenopausal women with abnormal uterine bleeding. BioMed Res Inter. 2019;2019.
18. No GT. Management of Endometrial Hyperplasia. 2016. Available at: ttps://www.rcog.org.uk/globalassets/documents/guidelines/green-top-guidelines/gtg_67_endometrial_hyperplasia.pdf. Accessed on 01st February 2020.

Mathew CT, Maheswari U, Shanmugam K. Co-relation of endometrial thickness by transvaginal sonogram with histopathology pattern in abnormal uterine bleeding: a study from south India. Int J Reprod Contracept Obstet Gynecol 2020;9:xxx-xx.

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One year retrospective analysis of PAP smears-from a tertiary hospital in Tamil Nadu

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Abstract
Introduction: Cervical cancer is one of the leading causes of maternal mortality in India and worldwide, it usually takes 3–7 years for high-grade changes in cervical cells to become cancer. There are many effective screening tests for cervical cancer. PAP smear is one such test
Aim: To analyse the pap smears done in our hospital for a period of one year
Objectives: 1). To determine the percentage of abnormal smears detected 2). To analyse the myriad of findings in a PAP smear.
Materials and methods: It is a Retrospective Observational study, Data was collected from the PAP smears taken in the Gynaecology department outpatients and master health check-up of our hospital. Data was collected for a period of one year from January 2017 to December 2017.
Results: Around 2333 PAP smears were analyzed over a period of one year, of it 885 was from our OPD and 1448 was from the master health check-up, the detection of precancerous lesion was 1.2%, in addition to detecting pre-cancerous lesions, additional information showed a variety of infections like a Candida, Trichomonas, Actinomyces, etc.
Conclusion: Cytology based screening is one of the most cost effective and easy method of cervical cancer screening.

Keywords: Ca cervix, Pap smear, screening, pre malignant lesions

Introduction
Cervical cancer is one of the leading causes of morbidity and mortality in women worldwide. Cervical cancer is the fourth most frequent cancer in women with an estimated 570,000 new cases in 2018 representing 6.6% of all female cancers. The high mortality rate from cervical cancer globally could be reduced through a comprehensive approach that includes prevention, early diagnosis, effective screening and treatment programmes [1]. The screening methods range from VIA (visual inspection with acetic acid) and VILI (visual inspection with Lugol's Iodine in low resource setup where pathologist is not available, to the PAP smear and liquid base cytology with or without HPV testing and sophisticated colposcopy in high resource centres.

Materials and Methods
This is a retrospective study conducted in PSGIMSR. Data was collected from the PAP smears taken in the Gynaecology department outpatients and master health check-up.
Of our hospital. Data was collected for a period of one year from January 2017 to December 2017

Inclusion criteria
1) All PAP smears taken in our OP
2) All PAP smears taken as a part of master health check-up in our hospital

Exclusion criteria
1) Women without sexual exposure

Procedure
Cells from ectocervix and endocervix are collected simultaneously with the broom like spatula. The central bristles of the broom are inserted into the endocervical canal until the lateral bristles bend fully against the ectocervix. The sampling device is rotated 360º in the same direction five

times while maintaining gentle pressure. The broom is removed and with a single paint stroke motion the cellular sample is transferred along the long axis of the labelled surface of the slide. Minimum of two slides are taken except for vault smears where a single slide is taken. The slide is rapidly fixed by immersion in 95%ethanol and ether mix in a Koplik's jar and transferred to the cytology lab [2].

Slides are analysed by the pathologists and reported based on Bethesda classification.

Bethesda classification (2014) [3].

Specimen Type
Indicate conventional Pap smear vs. liquidbasedpreparation vs. other

Specimen Adequacy
Satisfactory fore valuation /Unsatisfactory for evaluation

Interpretation/Result
Negative for intraepithelial lesion or malignancy Non-neoplastic findings (optional to report*)*

Organisms
Trichomonas vaginalis/ *Candida* spp/ Bacterial vaginosis/ *Actinomyces* spp. /Herpes simplex virus/Cytomegalovirus

Other Endometrial cells

Epithelial Cell Abnormalities
Squamous Cell
Atypical squamous cells of undetermined significance (ASC-US) cannot exclude HSIL (ASC-H) low grade squamous intraepithelial lesion (LSIL) High-grade squamous intraepithelial lesion (HSIL) Squamous cell carcinoma

Glandular Cell
Atypical
- Endocervical cells NOS /favour neoplastic
- Endometrial cells NOS *or specify in comments*
- Glandular cells (NOS /*favour neoplastic*)

Endocervical adenocarcinoma in situ
Adenocarcinoma
Endocervical/endometrial /extra uterine/not otherwise specified

Other Malignant Neoplasms: *(specify)*
Adjunctive Testing
Computer-Assisted Interpretation of Cervical Cytology

Results
Around 2333 PAP smears were analysed over a period of one year from Jan 2017 to Dec 2017, of it 885 was from our OPD and 1448 was from the master health check-up. The age distribution, the indications for taking a smear and the cytology report was analysed and is put up in the tabular column.

Table 1: Age distribution of Pap smear

Age of the patient in years	Number of patients in MHC	Number of patients in OPD	Total	Percentage
20-29	37	68	105	4.50
30-39	221	274	495	21.22
40-49	398	340	738	31.63
50-59	493	143	636	27.26
60-69	256	45	301	12.90
70-79	37	15	52	2.23
80-89	6	0	6	0.26
Total	1448	885	2333	100%

Table 2: Number of vault smear

Number of vault smears in master health checkup	215
Number of vault smears in OPD	25
Total	240
Percentage of vault smears	10.29%

Table 3: Indications for Pap smear

Indication	No of patients in MHC	No of patients in OPD	Total	Percentage
AUB		102	102	4.37%
Leucorrhoea		107	107	4.59%
Itching		6	6	0.26%
Mass P/V		1	1	0.04%
Pain Abdomen		36	36	1.54%
Post-Menopausal Bleeding		17	17	0.73%
Screening	1448	604	2052	87.96%
Strawberry vagna		1	1	0.04%
Post Ca Follow Up			11	0.47%
Post Ca Ovary		1		
Post Ca endometrium		5		
Post ca fallopian tube		1		
Post Lletz		2		
Post Ca cervix		2		
Total	1448	885	2333	100%

Table 4: Analysis of cytology report

Report	Number in MHC	Number in OPD	Total	Percentage	Total
Negative for IEL/Malignancy	849	413	1262	54.10%	Niel 2280 (97.73%)
Inflammation	406	319	725	31.07%	
Organisms					
Candida	93	62	155	6.64%	
BV	71	52	123	5.27%	
TV	4	9	13	0.56%	
Actinomycosis	0	2	2	0.09%	
Epithelial cell abnormalities			24	1.03%	Abnormal 24 (1.03%)
Squamous cells					
ASC-US	4	4	8		
ASC-H	2	2	4		
LSIL	2	2	4		
HSIL	0	4	4		
Glandular Cells					
AGUS	0	4	4		
Other Malignant Cells	0	0	0	0	
Unsatisfactory	17	12	29	1.24%	29 (1.24%)
Total	1448	885	2333	100%	2333(100%)

Table 5: Agewise Analysis of Abnormal Smears

AGE	Ascus	ASC-H	AGUS	LSIL	HSIL	SCC	Total Smears	
21-30					1		1	O.04%
31-40	1		2		1		4	O.17%
41-50	4	3	1		1		9	O.39%
51-60	1		1	4	1		7	0.30%
61-70	1	1					2	O.O9%
71-80	1						1	O.O4%
Total	8	4	4	4	4		24	1.03%
	O.34%	O.17%	O.17%	O.17%	O.17%	0	1.03%	

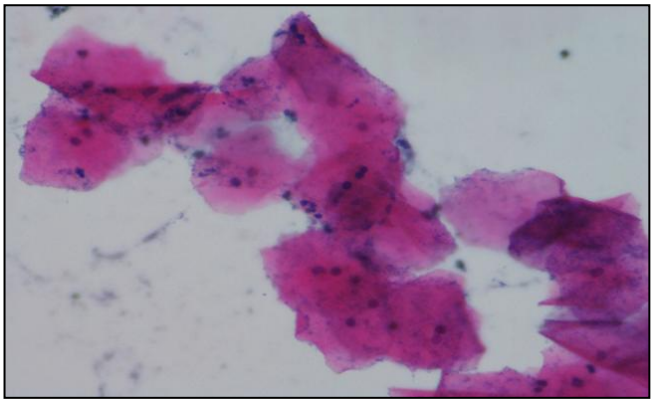


Fig 1: Bacterial Vaginosis

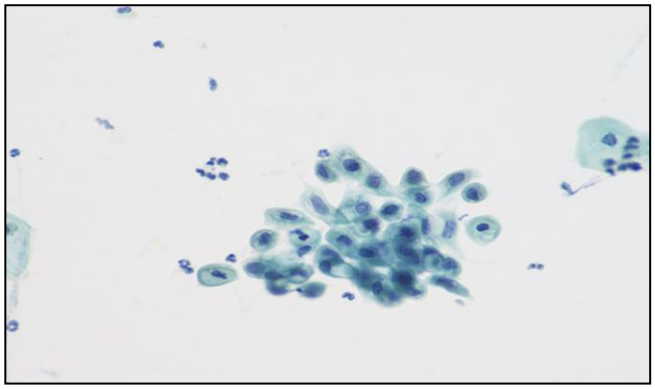


Fig 3: LSIL

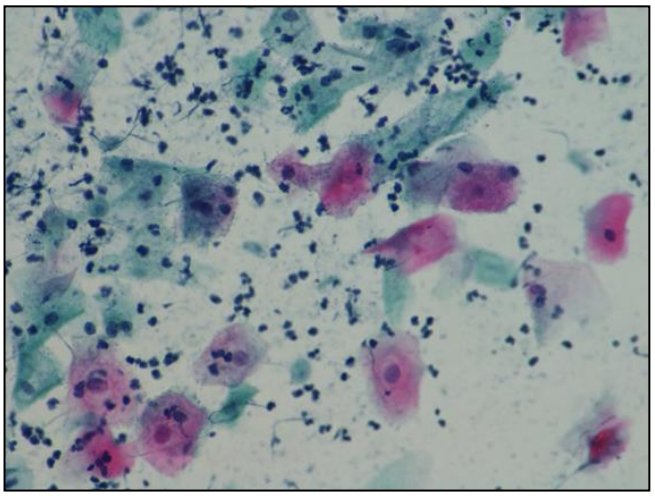


Fig 2: Trichomonas Vaginalis

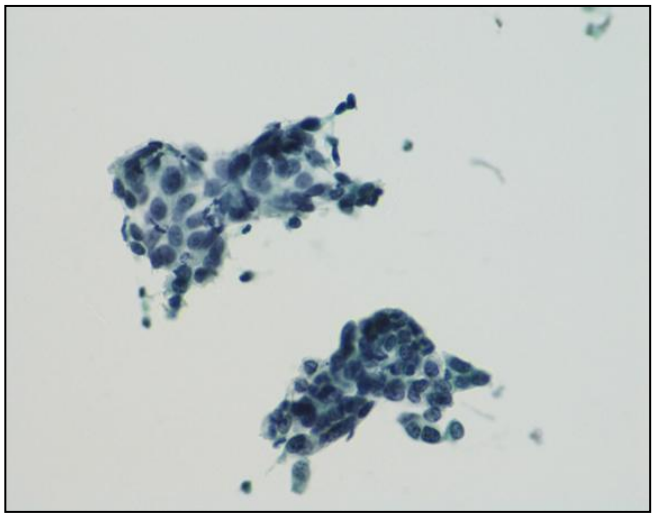


Fig 4: HSIL

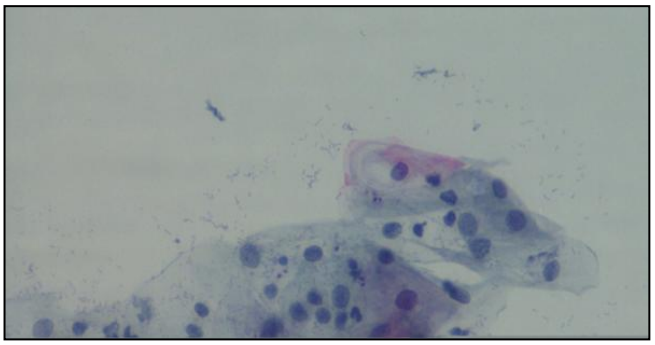


FIG 5: Candida

Discussion

PAP smears are analytical tools for screening of cervical cancer. It is a cost effective way for detecting the pre-invasive cervical lesions. In addition it also gives supplementary data on a variety of infections like Candidiasis, Bacterial vaginosis etc.

Of the 2333 pap smears taken over a period of one year, the maximum number of patients was of the age group 40-49 years accounting for 31.63%. The lowest age of the patient was 20 years –a married woman with severe itching and who's Pap smear showed inflammation. The oldest lady was 84 years old who came for a routine screening and the report was negative for IEL. According to a study by Kalyani *et al*. [4] the rate was 28.7%in the same age group and 32.5% in the 30-39year group which was maximum. According to Vijayalakshmi *et al.* [5], the maximum number of patients was in the age45-55 age group which was 34% The most common indication for Pap smear was for routine screening, accounting for 87.96% of the smears, Leucorrhea was the next common indication in those patients in the OPD- 4.59% with AUB being close behind - 4.37%. There were 0.4% of post Malignancy surgery follow up. Post ca endometrium being the commonest. The most common indication was leukorrhea accounting for 30% in a study by Vijayalakshmi *et al.* [5] while the routine screening was around 23%. The high rate of screening in our study might be due to increased awareness among the city population. Around 10.29% where vault smears.

Of the 2333 smears analyzed 2280 were negative for malignancy though they detected inflammation and infections. We had a detection rate of 1.03% (24 cases) of abnormal PAP smears. of the 24 cases there were 4 each of HSIL, LSIL, ASC-H AND AGUS and 8 of ASC-US, only 50% of these patients underwent colposcopy and directed biopsies and except two LSIL all the biopsy reports confirmed the lesions. This abnormal reports-1.03% in our study is close to the study conducted by Tamrakar *et al*. [6] which was 1.7%, but was less than the international and Indian studies done by Sherpa *et al.* [7]. (3.6%) and Sankaranarayanan *et al.* [8] (3.4%) respectively. There were around 1.24% of unsatisfactory smears were opinion was not possible which iscomparable to the study by Crasta *et al.* [5] which is 1.36%, The rate of unsatisfactory smear was high in the study by Kalyani *et al.*, [5] i.e., 17.8%.

Among the negative smears -Inflammmatory smears were found in 54.1% which was comparable to the study by Patel *et al.* [10] (51.5%).In our study, the various organisms detected where candida in 6.44% followed by bacterial vaginosis in 5.27%.in the study by Kalyani *et al.* [8] the inflammatory smears where 83.1%.. candida was found in 2.03% and bacterial vaginosis in 9.8%.while Candida was 3.71% in the study by Patel *et al.* [10]. In our analysis there were two cases of Actinomycosis also and 13cases of Trichomonas Vaginalis of which one patient had strawberry vagina. The comparison of the unsatisfactory and the pathological reports in the various studies are put in a tabular column below:

Table 6: The comparison of the unsatisfactory and the pathological reports in the various studies

Diagnosis	Kalyani *et al.* [5]	Crasta *et al.* [9]	Narasimha *et al.* [3]	Sankaranarayanan *et al.* [8]	Our study
Unsatisfactory	17.80%	1.36%	24.42%	4.10%	1.24%
ASCUS	1.46%	0.37%	4.14%	8.80%	0.34%
LSIL	0.24%	0.19%	2.70%	6.20%	0.17%
HSIL	0.41%	0.61%	2.50%	1.60%	0.17%

Conclusion

PAP smear examination is an economic method for screening forcervical pre-malignant lesions and help us to deal with the global scare of cervical cancer. Mass educational and awareness programs should be conducted to increase the detection rate and

Reference

1. https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/1https://www.cytopathology.org/specimen-collection adequacy-requisition/shaws textbook
2. The Pap test and Bethesda 2014. FullText-Acta Cytologica 2015. https://www.karger.com/article/fulltext/381842
3. Solomon D. Foreword; in Nayar R, Wilbur DC (EDS): The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes, ed 3. New York, Springer 2015. National Cancer Institute Workshop: The 1988 Bethesda System for reporting cervical/ vaginal cytologic diagnoses, 2015.
4. Kalyani R, Sharief N, Shariff S. A Study of Pap Smear in a Tertiary Hospital in South India. J Cancer Biol Res. 2016; 4(3):1084.
5. P Vijaya Lakshmi, SR Sree Gouri. Study and analysis of two hundred cervical PAP smears in our hospital. International Journal of Contemporary Medical Research. 2016; 3(9):2787-2789.
6. SR Tamrakar. Kathmandu University School of Medical Sciences, Nepal, Charul Chawla. A Clinical Audit of Pap Smear Test for Screening of Cervical Cancer Article in Nepal Journal of Obstetrics and Gynaecology· 2014
7. Sherpa AT, Clifford GM, Vaccarella S, Shrestha S, Nygård M, Karki BS *et al*. Human papillomavirus infection in women with and without cervical cancer in Nepal. Cancer Causes Control. 2010; 21:323-330.
8. Sankaranarayanan R, Thara S, Sharma A, Roy C, Shastri S, Mahé C *et al*. Accuracy of conventional cytology: results from a multicentre screening study in India. J Med Screen. 2004; 11:77-84.
9. Crasta JA, Chaitra V, Simi C, Correa M. An audit of cervicovaginal cytology in a teaching hospital: Are atypical glandular cells under-recognised on cytological screening? J Cytol. 2009; 26:69-73.
10. Hemali J Tailor, Patel RD, Prashant R Patel, Vasudha M. Bhagat Study of cervical pap smears in a tertiary care hospital of south Gujarat, India, international journal of research in medical sciences. 2016; 4(1):286-288.
11. Aparna Narasimha *et al*. An audit of Pap smear cytology Journal of south Asian federation of obstetrics and gynecology sep-dec. 2011; 3(3):121-124.

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Original Article

Pathology Section

Hair Loss in Paediatric and Adolescent Age Group: A Clinico-Pathological Analysis in a Tertiary Health Care Centre

UMAMAHESWARI GURUSAMY[1], CHAITRA VENKATASWAMY[2]

ABSTRACT

Introduction: Hair loss in children causes deep distress to both the child and the parent. The most common causes of paediatric alopecia are tinea capitis, alopecia areata, traction alopecia and trichotillomania. Timely diagnosis and treatment are indeed required to reverse these conditions.

Aim: To categorize paediatric alopecia into non-scarring, scarring and miscellaneous causes and to describe the histopathological features of each entity.

Materials and Methods: This retrospective study was carried out for duration of 10 years (July 2006 to July 2016) in the Department of Pathology. Scalp biopsies from patients aged 0-18 years with the history of hair loss were included in the study.

Results: There were 35 cases, which included non-scarring 22 (63%) cases, scarring 6 (17%) cases and miscellaneous causes 7 (20%) cases. Rare genetic causes such as Vitamin D resistant rickets associated with alopecia and epidermolysis bullosa were seen among the miscellaneous causes. Alopecia areata was the leading cause of hair loss in children followed by trichotillomania. The histopathological features of non-scarring and scarring alopecia were similar to that described in adults. KFSD, rarely seen in females, showed dilated infundibulum with follicular plugging, polytrichia, perifollicular lymphocytic infiltration and fibrosis on histology. Alopecia in patients with vitamin D resistant rickets showed reduction in number of hair follicles, irregular epithelial structures and epithelial cysts.

Conclusion: The commonest cause of paediatric alopecia seen in this study was alopecia areata. A holistic approach including history, clinical examination and histopathology will be essential for correct diagnosis.

Keywords: Alopecia areata, Non-scarring alopecia, Scarring alopecia

INTRODUCTION

Hair loss in children can cause immense psychological and emotional stress to the patients as well as the parents. While most adults with a history of alopecia are able to reconcile themselves to a bald head, the situation in children is different. In children it can be very traumatic and is a common cause of low self-esteem and difficult socio-cultural adjustments. Alopecia in children may be one of the manifestations of a systemic disease or a disease entity itself. Several classification schemes for hair loss in children have been proposed, but none of these are perfect [1,2]. Most forms of alopecia demonstrate clinical and histological overlap. This blurs the distinction between diseases and makes classification difficult. The best approach would be to group diseases according to their aetiology, but this is impossible since the causes of many forms of hair loss are unknown.

The most widely accepted classification divides paediatric alopecia into congenital and acquired [3,4]. Hair loss in children can be subclassified according to: 1) abnormality in initiation of hair growth (genetic); 2) hair shaft abnormalities presenting with hair breakage; 3) abnormal cycling (anagen/telogen loss); 4) focal/diffuse alopecia (scarring/non scarring) [5].

The commonly encountered causes of alopecia in children include, tinea capitis, alopecia areata, traction alopecia, telogen effluvium, trichotillomania and all these are reversible if diagnosed early [6,7]. A thorough clinical history with careful examination (local and systemic), microbiological studies, trichogram and scalp biopsy will be helpful for the evaluation of paediatric alopecia. This holistic approach will be rewarded with correct diagnosis.

There are many clinical studies on paediatric alopecia, but limited studies on histopathological features [6-8]. The present study was carried out to describe histopathological features of alopecia in paediatric and adolescent age groups and categorize them into non scarring, scarring alopecias and miscellaneous causes.

MATERIALS AND METHODS

The retrospective study was carried out for duration of 10 years (July 2006 to July 2016) in the Department of Pathology, PSG Institute of Medical Sciences and Research. Scalp biopsies from children aged 0 to 18 yrs with history of hair loss were included. Biopsies less than 2 mm in size and poorly fixed tissues were excluded from the study. The punch biopsies were kept in 10% formalin and processed in an automatic tissue processor (LEICA TP 1020) prior to paraffin embedding. Both vertical and transverse sections were taken and stained with haematoxylin and eosin. Special stains such as Verhoeff van Gieson (VVG) and Alcian blue/periodic acid-Schiff (AB/PAS) were used as required. The former was used to analyse the loss of elastic fibres in cases of scarring alopecia. The AB/PAS stain was utilised to assess thickening of the basement membrane and increase in dermal and perifollicular mucin.

RESULTS

Over a period of 10 years, 130 scalp biopsies were received in the histopathology lab. A total of 35 children with hair loss (27%) from age group 0 months to 18 years were selected. Except for 2 biopsies that were 2 mm in diameter, the rest were 4 mm punch biopsies.

The distribution of these cases included 22 (63%) non-scarring alopecia, 6 (17%) scarring alopecia and 7 (20%) miscellaneous causes.

Details of non-scarring and scarring alopecia with histopathological features are summarized in [Table/Fig-1,2] respectively.

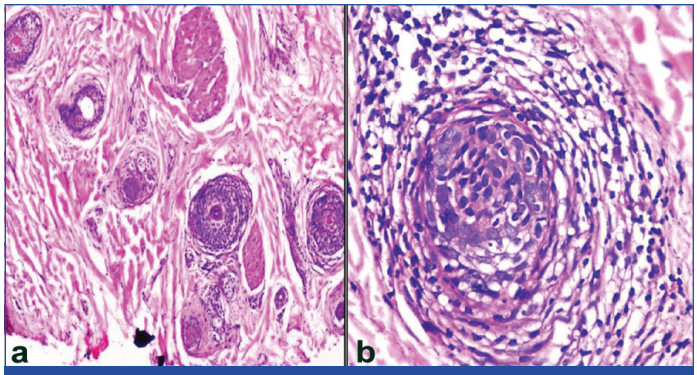
Among the non-scarring alopecia, there were 12 (54.5%) cases of alopecia areata [Table/Fig-3], 8 cases of trichotillomania [Table/Fig-4] and one each of traction alopecia and androgenetic alopecia.

Diagnosis	Sex		Age range	Clinical features	Histopathologic features
	Female	Male			
Alopecia areata	10	2	10 month-18 year	Localized form-10 Alopecia universalis-2	Peribulbar lymphocytic infiltration, miniaturization, increased stelae, telogen follicles and nanogen follicles.
Trichotillomania	8	-	8-18year	No clinical history suggestive of mood and anxiety (obsessive compulsive) disorders	Distorted follicular anatomy with collapse of the inner root sheath, pigment casts, trichomalacia, variably increased telogen follicles and intra follicular haemorrhage.
Traction alopecia	1	-	15 year	Patchy hair loss with broken hairs and follicular prominence-since two yrs H/O traumatic hair styling	Increased number of telogen/ catagen hair follicles, stelae with no significant inflammation. An occasional hair follicle shows distorted anatomy and pigment cast.
Androgenetic alopecia	1	-	18 months	Diffuse hair loss of three months duration	Miniaturization, altered T:V and A:T ratios, slightly increased fibrous stelae and telogen hair follicles

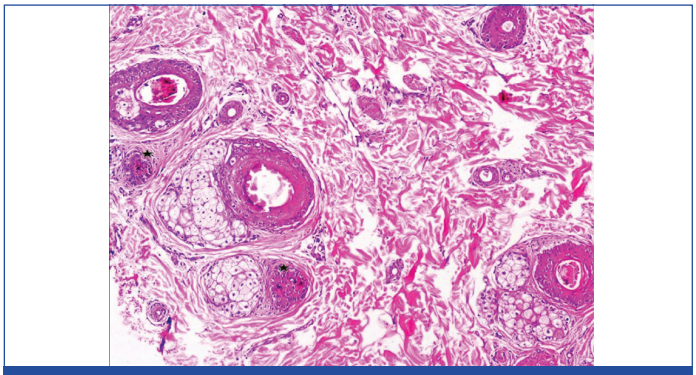
[Table/Fig-1]: Clinical and histopathological features of non-scarring alopecias.

Diagnosis	No of cases	Sex	Age	Clinical features	HP Features
KFSD#	2	female	8 and 13 years	Diffuse hair loss over the scalp, trunk and limbs with papular lesion Similar complaints were seen in female sibling of one case. No ocular lesions in both cases.	Follicular plugging dilated infundibulum, perifollicular inflammation and fibrosis. Polytrichia was noted in one case
Tinea capitis	1	female	14 years	Boggy swelling in the scalp with scaly patches of hair loss	Superficial and deep perifollicular, suppurative and granulomatous inflammation. Fungal spores (endothrix) within the hair follicle.
DLE†	1	female	16 year	Hair loss with pruritic pigmented lesions in frontal region and face	Perivascular, perifollicular and perieccrine lymphoplasmacytic infiltrate, BMZ‡ thickening, increased dermal mucin, full thickness scarring Direct immunofluorescence was positive
Scleroderma	1	female	18 years	Diagnosed case of connective tissue disorder with patchy hair loss over the scalp	Hair bulbs and follicles were surrounded by a thick sheath of hyalinized fibrous sheath, epidermal atrophy, homogenization of the upper dermis with loss of elastic fibres.
ESSA*	1	female	15 years	Presented with bald patches of five yrs duration	Marked reduction in the number of hair follicles with eccentric epithelial atrophy and increased stelae. Atrophic sebaceous glands and many blank spots were present.

[Table/Fig-2]: Clinical and histopathological features of scarring alopecia.
*ESSA-End stage scarring alopecia. #KFSD- Keratosis follicularis spinulosa decalvans
†DLE-Discoid lupus erythematosus. ‡BMZ – Basement membrane zone



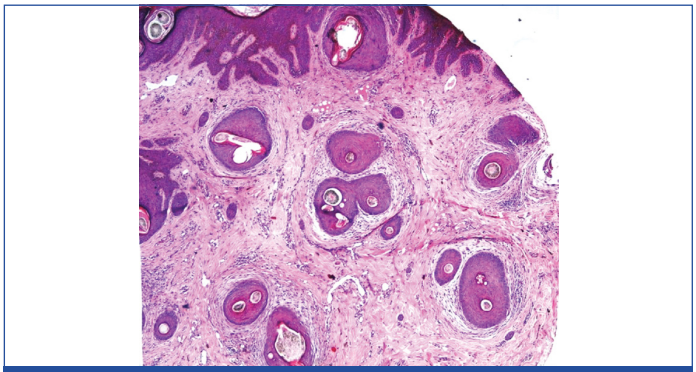
[Table/Fig-3]: Alopecia areata. Transverse section shows a) increase in telogen hair follicles with miniaturization (H&E, 10X). b) Peribulbar lymphocytic infiltration (H&E, 40X).



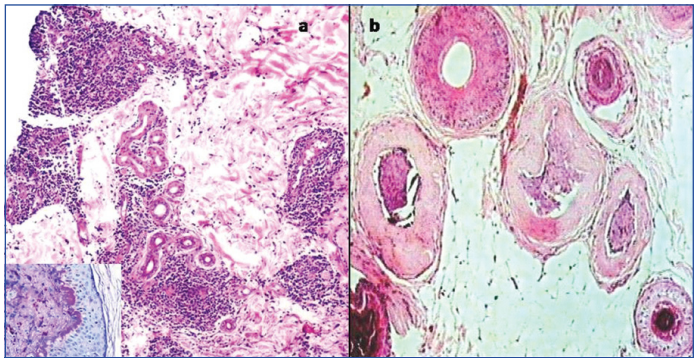
[Table/Fig-4]: Trichotillomania. Transverse section shows hair follicles with Pigment casts, collapse of inner root sheath and telogenization (*). (H&E, 10X).

The group of scarring alopecia consisted of two cases of Keratosis Follicularis Spinulosa Decalvans (KFSD) [Table/Fig-5], and one case each of discoid lupus erythematosus (DLE) [Table/Fig-6a], scleroderma [Table/Fig-6b], tinea capitis and End Stage Scarring Alopecia (ESSA) [Table/Fig-7].

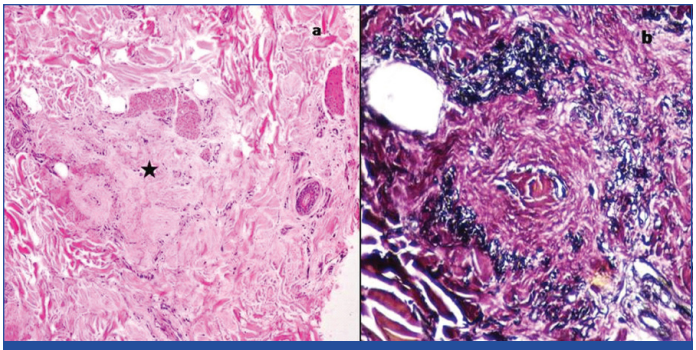
Genetic causes such as vitamin D resistant rickets associated alopecia (two cases), and epidermolysis bullosa (one case) were



[Table/Fig-5]: KFSD. Transverse section shows polytrichia, perifollicular fibrosis and lymphocytic infiltration (H&E, 40X).



[Table/Fig-6]: a) Perieccrine and perifollicular lymphoplasmacytic infiltrate in a case of DLE (H&E, 10X). Inset shows thickened basement membrane (AB-PAS,40X); b) Transverse section shows thick hyalinized sheath around the hair follicles in scleroderma (H&E, 10X).



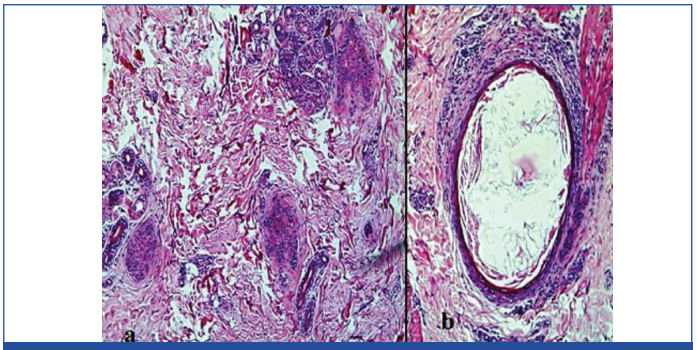
[Table/Fig-7]: End stage scarring alopecia; a) Scarred follicular unit: blank spot (*) (H&E, 10X); b) Loss of elastic fibres in areas of scar (VVG, 40X).

included. Apart from the above causes there were three cases of short anagen syndrome and one case of nevus sebaceus.

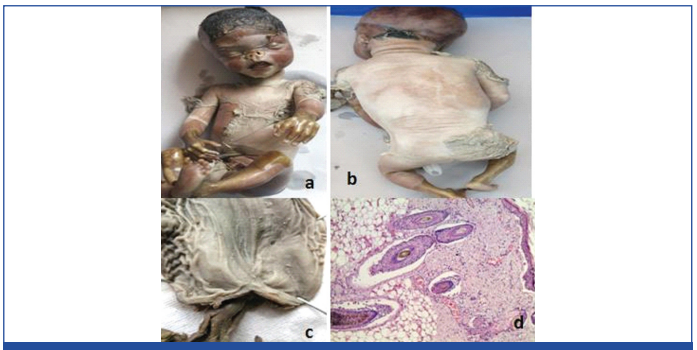
Clinical and histological features of miscellaneous causes are presented in [Table/Fig-8-11]. Epidermolysis bullosa: A neonate born to a 25-year-old G3P2L0 from third degree consanguineous marriage at 35 weeks of gestation presented with extensive bullous lesions and areas of skin peeling in most parts of the body. The child succumbed to death on day one and was submitted for autopsy examination. External examination showed extensive peeling of skin with diffuse hair loss over the occipital and temporal regions [Table/Fig-10a, b]. Internal examination revealed pyloric atresia, horse shoe kidney and urethral stenosis with dilated ureters and bladder [Table/Fig-10c]. Histopathological examination of scalp biopsy showed cleft at dermoepidermal junction and around hair follicles [Table/Fig-10d]. A diagnosis of Junctional Epidermolysis Bullosa (JEB) type was offered in view of severity of disease and involvement of internal organs. Previous two children had similar history and died on day one and three respectively. Genetic testing was suggested for parents to confirm the diagnosis and for selective embryo transfer in future pregnancy.

Diagnosis	Number of cases	sex	Age range	Clinical features	Histopathological features
Vitamin D resistant rickets associated alopecia	2	female	4 and 6 years	Clinically diagnosed Vitamin D resistant rickets presented with hair loss, which was patchy to start with, and progressed to become diffuse over a period of one year	Reduced number of hair follicles, irregular epithelial structures and infundibular type epithelial cyst
Epidermolysis bullosa	1	Male	Neonate	Extensive bullous lesions, skin peeling, diffuse hair loss, pyloric atresia, horse shoe kidney and urethral stenosis. Died on day one.	Cleft at dermo-epidermal junction and around the hair follicles
Short anagen syndrome	3	female	3 to 6 years	Sparse hair growth with short fine brown hairs	Increased telogen hair follicles with reversal of anagen: telogen ratio, miniaturization of hair follicles with irregular and angulated outlines.
Nevus sebaceus	1	male	4 years	Single hairless patch over vertex, noticed since six months of age. Slowly increasing in size	Hyperkeratosis with irregular acanthotic epidermis. Immature hair follicles with dilated infundibulum and abundant sebaceous glands

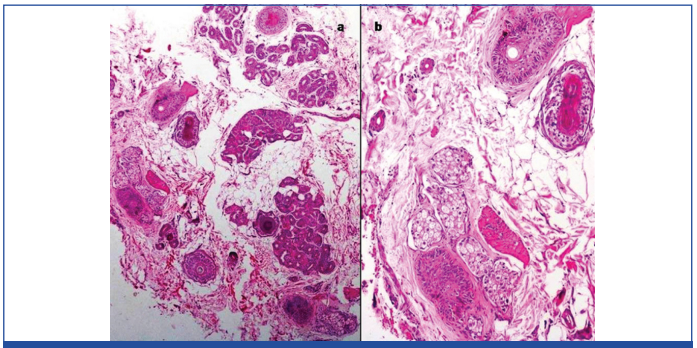
[Table/Fig-8]: Clinical and histopathological features of miscellaneous causes of alopecia.



[Table/Fig-9]: Alopecia associated with Vitamin D dependant rickets; a) Irregular epithelial structures of hair follicles (H&E, 4X); b) Keratinizing epithelial type of infundibular cyst (H&E, 10X).



[Table/Fig-10]: Epidermolysis bullosa: a,b) Generalized peeling off skin over face, chest, abdomen and back with diffuse loss of hair in occipital region; c) Specimen of stomach laid open showing pyloric atresia; d) sections from skin shows sub epidermal and perifollicular clefting. (H&E, 40X).



[Table/Fig-11]: Short anagen syndrome: a) Scanner; and b) low power view showing increased telogen hair follicles (H&E, 40X and 10X).

DISCUSSION

Alopecia is an unpleasant diagnosis at any age group. When it occurs in children, it is highly distressing to the parents, a challenge for the treating physician and has a negative psychosocial impact on the growing child. Elucidating accurate aetiology becomes a crucial step towards educating parents about available treatment options and prognosis.

Hair loss in paediatric age group could be congenital or acquired. Still it would be simpler to classify them into scarring and non scarring alopecia like in adults. There are a few conditions in children which cannot be categorized into either scarring or non scarring and these can be grouped under miscellaneous causes as done in the present study. Thorough clinical details, family history and histological findings are essential in making a right diagnosis. The common causes of paediatric alopecia are acquired and reversible. They include: alopecia areata, tinea capitis, trichotillomania, traction alopecia and androgenic alopecia.

In the present study, we have categorized causes of alopecia into non scarring, scarring and miscellaneous causes and will be discussed accordingly.

Non Scarring Alopecia

They accounted for majority of cases 22 (63%) cases. Among these causes, alopecia areata was the commonest followed by trichotillomania.

Alopecia areata (AA) was more frequently seen in female patients as in previous studies [7,8]. Female predominance can be explained because of its autoimmune basis, as autoimmune diseases are more prevalent in females. Laboratory evaluation of thyroid function, iron deficiency, blood sugar, HbA1c, serological markers of autoimmune diseases and testing for hyperandrogenemia are useful in the evaluation of patients presenting with alopecia. Though AA has bimodal age distribution (paediatric and adult) it is less likely to occur in children below 2 years of age [1,6,7]. In the present study, there were three patients (25%) below two years of age and the earliest onset of disease was in a 10 month old child. Diffuse AA, alopecia universalis and totalis are more frequent in children and in our study we diagnosed two children with alopecia totalis, accounting for 20% of cases of AA [6]. Histological features were similar to that of adult cases. Clinically, the localized form of AA can be confused with tinea capitis and trichotillomania and diffuse type with telogen effluvium and diffuse tinea capitis. This clinical challenge can be resolved by careful histopathological examination of the scalp biopsy [7].

Trichotillomania was the second most common cause of paediatric alopecia in our study and was noted to occur only in females. Trichotillomania is an impulse control disorder characterised by a long term urge that results in pulling out of one's own hair. It is often associated with anxiety and may co-occur with other disorders of the obsessive compulsive spectrum [1]. It is more frequent in females as in our study. The classic histological features include collapse of the inner root sheath, pigment casts, intrafollicular haemorrhage and these were noted in most of our cases [10].

Although, predominantly a disease of adults, children are less commonly affected by androgenetic alopecia. However, some studies have reported androgenic alopecia as the second most common cause of hair loss in children and leading cause in adolescent age group [11,12]. Its occurrence in an eight month old girl child was an unusual feature of our study. The youngest case described in the literature is that of a six year old child [13].

Scarring Alopecia

Keratosis Follicularis Spinulosa Decalvans is a rare genodermatosis with X-linked and autosomal dominant pattern of inheritance. It can also be sporadic in onset [14,15]. KFSD manifests as scarring alopecia mainly in male patients (females being carriers). In our study, two girls aged 8 and 13 years manifested with full blown disease, which has been reported in the literature [14,15].

Tinea capitis is common in children of tropical countries. It has varied clinical presentation such as hyperkeratosis of scalp, seborrhoea, alopecia and broken hair. Hair loss is non scarring type and reversible if treated early. When inflammation is severe, it can progress to scarring alopecia [16]. Though it is a common cause of hair loss, we had a single case of tinea capitis causing scarring alopecia. This is because most of the cases are clinically diagnosed with the help of Potassium Hydroxide (KOH) stained smears of hair shafts and are not biopsied. Other causes of scarring alopecia in the present study included connective tissue disorders such as Discoid Lupus Erythematosus (DLE) and scleroderma. When biopsy is taken from scarred area or in late stages of the disease the diagnosis cannot be ascertained as was seen in one of our cases (end stage scarring alopecia).

Miscellaneous Causes

Vitamin D is essential for bone mineralization. Few studies have shown various cutaneous manifestations of vitamin D deficiency like psoriasis, atopic dermatitis, vitiligo, ichthyosis, keloid and autoimmune diseases and its role in treating the above [17]. Few experiments have demonstrated its role in hair growth signalling. The histological features are similar to that seen in atrichia with papular lesion and include absence of hair shafts, irregular epithelial structures and epithelial cysts [18]. Similar findings were observed in two of our cases. A good clinico-pathological correlation is essential to arrive at correct diagnosis.

Epidermolysis bullosa (EB) causing alopecia is a rare manifestation and it was diagnosed in one of our case. EB is an inherited bullous disorder that can manifest as milder form to fatal disease. It is classified based on the level of separation within the cutaneous basement membrane zone into EB simplex, junctional EB and dystrophic EB. Blistering involving basement membrane zone of hair follicle is the cause of hair loss. Other manifestations include mild hair shaft abnormalities, cicatricial alopecia and alopecia universalis [19].

We had three cases of Short Anagen Syndrome (SAS). There are very few case reports of SAS in literature and one case was reported from India [20,21]. SAS is a recently described entity characterized by persistent short hair due to shorter anagen phase. It has characteristic clinical presentation i.e. short smooth hair since birth and diagnosed based on reversal of anagen: telogen ratio on trichogram, the normal ratio being 9:1. Scalp biopsy also shows increased telogen hair follicles with a mild perifollicular lymphocytic infiltrate and non-scarring type of alopecia [22]. A few reports from the literature revealed, SAS can have autosomal dominant pattern of inheritance and has a self-limiting course with spontaneous resolution at puberty [23,24].

There are some unique findings in present study: 1) unusual age at presentation (<1 year) in cases of alopecia areata and androgenetic alopecia 2) a full blown phenotypic expression of KFSD in females, who are generally carriers 3) rare causes of alopecias such as Vitamin D resistant rickets associated alopecia and short anagen syndrome were identified.

LIMITATION

The pitfall of the present study is the limited number of cases, though the study was carried out for 10 year duration. This is because not all cases of alopecia are biopsied routinely. Diagnosis is established based on its clinical presentation and ancillary techniques like KOH preparation, trichogram, and hair pull test. Biopsies are performed, when the diagnosis is not arrived on clinical grounds or the patient is not responding to treatment.

CONCLUSION

To conclude, alopecia areata is the commonest cause of hair loss in children. Good clinico-histopathological correlation is necessary to arrive at a correct diagnosis. Based on this, parents can be counselled about appropriate treatment options and prognosis.

REFERENCES

[1] Verbov J. Hair loss in children. Arch dis child 1993;68(5):702-06.
[2] Mandt N, Vogt A, Blume-peytavi U. Differential diagnosis of hair loss in children. J Dtsch Dematol Ges. 2004;2(6):399-411.
[3] Bennàssar A, Ferrando J, Grimalt R. Congenital atrichia and hypotrichosis. World J. Pediatr. 2011;7(2):111–17.
[4] Goddard TA. Hair loss in an adolescent. J. Pediatr. Health Care. 2011;25(4):261–65.
[5] Elise A. Olsen MD. Hair Disorders. In: Alan D. Irvine, Peter H. Hoeger and Albert C. Yan. eds. Harper's Textbook of Pediatric Dermatology, Volume 1, 2, Third Edition. Blackwell Publishing Ltd, 2011; pages 148.1–148.35.
[6] Atton AV, Tunnessen WW. Alopecia in Children: The Most Common Causes. Pediatrics in Review. 1990;12(1):25-30.
[7] Castelo-Soccio L. Diagnosis and management of alopecia in children. Pediatr Clin North Am. 2014;61(2):427-42.

[8] Al-Refu K. Hair loss in children: Common and uncommon causes; clinical and epidemiological study in Jordan. Int J Trichology. 2013;5(4):185-89.
[9] Sharma VK, Kumar B, Dawn G. A clinical study of childhood alopecia areata in Chandigar, India. Pediatr Dermatol. 1996;13(5):372-7.
[10] Sellheyer K, Bergfeld WF. Histopathologic evaluation of alopecias. Am J Dermatopathol. 2006;28(3):236-59.
[11] Gonzalez ME, Francis CJ, Orlow SJ. Androgenic alopecia in the paediatric population: a retrospective review of 57 patients. Br J Dermatol. 2010;163(2):378-85.
[12] Price VH. Androgenic alopecia in adolescents. Cutis 2003;71(2):115-22.
[13] Tosti A, Iorizzo M, Pirraccini BM. Androgenetic Lopecia in Childern: Report of 20 cases. Br J Dermatol. 2005;152(3):556-9.
[14] Bellet JS, Kaplan AL, Selim MA, Olsen EA. Keratosis follicularis spinulosa decalvans in a family. J Am Acad Dermatol. 2008; 58(3):499-502.
[15] Maheswari UG, Chaitra V, Mohan SS. Keratosis follicularis spinulosa decalvans: A rare cause of scarring alopecia in two young Indian girls. Int J Trichology. 2013;5(1):29-31.
[16] Grover C, Arora P, Manchanda V. Tinea capitis in paediatric population: A study from north India. Indian J Dermatol Venereol Leprol. 2010;76(5):527-32.
[17] Wadhwa B, Relhan V, Goel K, Kochhar AM, Garg VK. Vitamin D and skin diseases: A review. Indian J Dermatol Venerol Leprol. 2015; 81(4):344-55.
[18] Bergman R, Schein-Goldshmid R. The alopecias associated with vitamin D-dependent rickets type IIA and with hairless gene mutations. Arch Dermatol. 2005;141(3):343-51.
[19] Tosti A, Duque-Estrada B, Murrell DF. Alopecia in epidermolysis bullosa. Dermatol clin. 2010;28(1):165-69.
[20] Dhepe NV, Naik AS. Short Anagen Syndrome in an Indian Woman with its Impact on Quality-of-Life. Int J Trichol. 2012;4(4):271-272.
[21] Herskovitz I, de Sousa ICVD, Simon J, Tosti A. Short Anagen Hair Syndrome. International Journal of Trichology. 2013; 5(1):45-46.
[22] Antaya RJ, Sideridou E, and Olsen EA. Short anagen syndrome. J Am Acad Dermatol. 2005;53(2):130-34.
[23] Barraud-Klenovsek MM, Trueb RM. Congenital hypotrichosis due to short anagen. Brit J Dermatol. 2000;143(3):612-17.
[24] Fernandez MA, Camacho Martínez FM. Short Anagen Syndrome: A Case Study. J Cosmetics, Dermatol Sci Appli. 2012;2:14-15.

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Morphologic Spectrum of Undetermined Causes of Hemoptysis- A Pathologist's Role

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ABSTRACT

Objective: Hemoptysis is the expectoration of blood or blood-streaked sputum from the tracheobronchial tree. The etiology may derive from nonneoplastic conditions such as infections, chronic pulmonary diseases, and vasculitis or neoplastic causes. Sometimes a definitive cause for hemoptysis cannot be found after ample diagnostic workup. The role of biopsy in such cases is to help the clinician in arriving at the final diagnosis. Diffuse alveolar hemorrhage is the main histopathological finding in hemoptysis and it appears with diffuse chest infiltrates radiologically.

Material and Method: A retrospective study of 2 years duration was conducted to identify the morphological spectrum of diseases presenting with hemoptysis. A total of 243 lung biopsies obtained by various methods were retrieved in this study period and 20 cases with hemoptysis of undetermined etiology were detected.

Results: Based on imaging and histopathology findings, the etiological causes of hemoptysis were divided into hemoptysis with and without capillaritis or due to tumor/tumor-like lesions and due to miscellaneous conditions. The most common etiology was vasculitis followed by infections.

Conclusion: Histopathology helps to detect the etiology, particularly in cases of hemoptysis due to non-immunologic causes. In immunologic cases, histopathological findings may support the diagnosis in correlation with the clinical/imaging features.

Key Words: Hemoptysis, Immunologic, Diffuse alveolar hemorrhage, Capillaritis

INTRODUCTION

Hemoptysis is the expectoration of blood from the pulmonary parenchyma or the tracheobronchial tree below the level of the glottis. The expectoration of blood can vary from a small amount of blood-streaking sputum to massive bleeding with life-threatening consequences. The clinical diagnostic algorithm should aim to differentiate and identify the etiology of hemoptysis, since massive hemoptysis may result in a fatal outcome (1, 2).

The common causes of hemoptysis include pulmonary tuberculosis (TB), malignancy, bronchiectasis, chronic bronchitis, pneumonia, and fungal infections. The other rare causes are vasculitis due to collagen vascular diseases and hereditary hemorrhagic telangiectasia, essential cryoglobulinemia, Behcet's disease, acute lung transplantation rejection, drug-induced (e.g., chemotherapeutic agents, propylthiouracil), idiopathic pulmonary hemosiderosis, and toxin or inhalation injury (3).

About 30% of cases attending respiratory clinics and presenting with hemoptysis pose a problem in clinical diagnosis, and the histopathology plays a vital role in diagnosing these conditions. The condition can be localized or diffuse depending upon the underlying etiology (4). Intra alveolar hemorrhage, fibrin deposition, and hemosiderin-laden macrophages associated with or without capillaritis are the common findings observed in cases of diffuse alveolar hemorrhage (DAH) (5). Radiologically, DAH shows patchy/ diffuse bilateral areas of lung consolidation mainly involving the lower lobes and perihilar regions (6).

A literature search revealed only a few reports on using histopathological interpretation in diagnosing unknown causes of hemoptysis. This is vital to diagnose and treat the condition earlier, as it prevents disease progression, and constituted the reason for conducting the current study. This study aims to identify the various uncommon aetiologies of hemoptysis based on histopathological features and the pathologist's approach in diagnosing this condition.

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MATERIAL and METHODS

All lung biopsies, including lobectomy specimens received in the department of pathology, between Jan 2018 to Jan 2020 (2 years duration) were retrospectively reviewed. Of the received 243 specimens, 20 cases had hemoptysis with undetermined causes. The obtained specimens were derived from various procedures and included 117 transbronchial lung biopsies (TBLB), 62 endo bronchial lung biopsies (EBLB), 28 guided (Ultra sonogram /CT) biopsies, 19 thoracoscopic/Video-assisted (VATS) biopsies, and 17 lobectomies. The cases with a history of hemoptysis associated with common causes such as tuberculosis (TB), carcinoma, bronchitis, bronchiectasis, and interstitial lung diseases (ILD) were excluded from the study. The clinical data obtained were age, sex, presenting complaints, clinical diagnosis/differential diagnosis, and significantly associated co-morbidities. Laboratory investigations such as autoimmune workup (ANCA, ANA, etc.), sputum for culture & acid-fast bacilli (AFB), bronchoalveolar lavage (BAL) for culture and cytology, and other relevant information were collected. The findings of imaging (high-resolution computerized tomography - HRCT) and bronchoscopic findings were recorded if available (not always performed).

Ethical approval for this study was obtained from PSG Institute of Medical Sciences & Research (Approval number: 20/117, Date: 27.05.2020).

RESULTS

Out of 243 lung biopsies received, a total of 20 cases with symptoms of hemoptysis due to undetermined etiology were included in our study.

Histopathological Findings

Based on imaging and histopathological findings, the etiology of hemoptysis was divided into localized and diffuse/bilateral pulmonary involvement (Table I). Depending upon the histological findings, the causes of hemoptysis were further divided into

- Hemoptysis with capillaritis (Table II)
- Hemoptysis without capillaritis (Table III)
- Unusual tumors and tumor-like lesions (Table IV)
- Miscellaneous uncommon causes

Miscellaneous uncommon causes are listed below:

Pulmonary Alveolar Micro Lithiasis (n=1)

HRCT of a 34-year-old male who presented with hemoptysis showed bilateral ground-glass densities with septal calcification -? Interstitial lung disease. TBLB sections showed distended alveoli containing concentric lamellated, calcific material with few hemosiderin-laden macrophages.

Pulmonary Endometriosis (n=1)

A 43-year-old man with a history of cirrhosis secondary to hepatitis 'C' had recurrent episodes of hemoptysis. Bronchoscopy revealed a mucosal white patch in the left lingular segment. The biopsied lesion showed lung tissue intimately admixed with a focus of endometrial glands and stroma. CD 10 immunostaining of the stroma, as well as estrogen and progesterone receptor staining of the glandular component, were both consistent with endometriosis (Figure 1A-D).

Table I: Etiological classification based on imaging & histopathological findings.

Causes of hemoptysis			
Localized	**Number of cases**	**Diffuse /bilateral**	**Number of cases**
Pulmonary hamartoma	1	GPA	3
Aspergillosis	1	Silicosis	3
Myelolipoma	1	EGPA	2
Mucor	1	Invasive aspergillosis	1
Pulmonary endometriosis	1	Invasive mucor+aspergillosis	1
Mucoepidermoid carcinoma	1	Multiple metastases from a leiomyosarcoma	1
Probably benign mesenchymal neoplasm	1	PAM	1
		Pulmonary intravascular hemangioma	1

GPA: Granulomatosis with Polyangiitis / Wegener's, **EGPA:** Eosinophilic granulomatosis with polyangiitis / Churg-Strauss syndrome, **PAM:** Pulmonary alveolar microlithiasis.

Table II: Causes of hemoptysis with capillaritis.

	Granulomatosis with Polyangiitis (GPA)			**Eosinophilic granulomatosis with polyangiitis (EGPA)**	
	Case - 1	**Case-2**	**Case-3**	**Case 1**	**Case 2**
Age, sex	40, M	46, F	51, F	43, M	42, M
Complaints	Cough, hemoptysis, and dyspnoea				
Serology	Anti-CCP, PR3-ANCA&RF: positive	PR3-ANCA positive	PR3-ANCA positive	RF: positive	PR3-ANCA: weakly positive
Imaging studies	Bilateral nodules	Ground glass changes, calcified nodule	Bilateral consolidation with few nodules	Bilateral nodules	Bilateral nodular and cavitating lesions.
Type of biopsy	EBLB	CT guided biopsy	CT guided biopsy	TBLB	VATS lung biopsy
HPE findings: **Vascular changes:**	• Capillaritis, venulitis • Fibrinoid necrosis (Figure 2A)	Vague outlines of thrombosed blood vessels	Vasculitis and Fibrin thrombi (Figure 2C- D)	Eosinophilic infiltration of the vessel wall	• Necrosis of the vessel walls with inflammatory cells infiltration (Figure 3A-C) • Organizing, recanalizing and fibrin thrombi
Parenchymal changes	• Granulomatous inflammation with MNG cells (Figure 2B) • Interstitial inflammation	• Necrosis, karyorrhexis, hematoxyphilic nuclear dust • Neutrophilic micro abscess	• Necrosis, karyorrhectic debris • Granulomatous response with MNG cells • Neutrophilic micro abscess	• Eosinophilic microabscess • Palisading granuloma	• Intraalveolar hemorrhage (Figure 3D) • Eosinophilic microabscess, parenchymal necrosis • Perl's stain highlights the hemosiderin-laden macrophages (Figure 4)

MNG cells: Multinucleate giant cells, **HPE:** Histopathological examination, **EBLB:** Endobronchial lung biopsy, **CT:** Computed tomography, **VATS:** Video-assisted thoracoscopic surgery, **TBLB:** Transbronchial lung biopsy.

Table III: Causes of hemoptysis without capillaritis.

	Silicosis			**Fungal infection**			
	Case -1	**Case-2**	**Case-3**	**Case-1**	**Case-2**	**Case-3**	**Case-4**
Age, sex	61, M	54, M	58, M	28, M	56, M	45, F	15, F
Complaints	Cough, hemoptysis and dyspnoea.			Cough, hemoptysis, and dyspnoea			
Another relevant history	Exposure to silicon dust +			Post Koch's sequelae	DM, CKD	Post TB, DM.	DM, DKA^{+}, Mild PAH
Imaging studies	Bilateral lung nodules&hilar nodes	Calcified nodes	B/L nodules, calcification	NA	Cavitatory consolidation	B/L subpleural nodules	B/L cavitating consolidations
Type of biopsy	TBLB			Right upper lobe segmentectomy	EBLB	Right lower lobectomy	TBLB
HPE Findings	• Nodules of epithelioid and pigment laden histiocytes, predominantly around the vascular channels (Figure 5A,B)			• Dilated bronchi forming a cavity containing the fungal ball • Tissue, angioinvasion & infarct • Fibrosis, chronic granulomatous inflammation • Oxalate crystals (Figure 6A,B)			
Special study	Polarizer: birefringent crystals (Figure 5B inset)			GMS & PAS stains: septate, acute-angled branching hyphae of aspergillus	PAS & GMS stains: broad, aseptate and wide-angle branching hyphae of mucor	PAS & GMS stains: Tangles of fungal elements, consistent with aspergillus and mucor	

DM: Diabetes Mellitus, **CKD:** Chronic kidney disease, **TB:** Tuberculosis, **NA:** Not available, **DKA:** Diabetic ketoacidosis, **PAH:** Pulmonary artery hypertension, **B/L:** Bilateral, **HPE:** Histopathological examination, **EBLB:** Endobronchial lung biopsy, **TBLB:** Transbronchial lung biopsy, **GMS:** Grocott Methenamine Silver Stain.

Table IV: Unusual tumors and tumor-like lesions of the lung.

Diagnosis	Pulmonary hamartoma (n=1)	High-grade Spindle cell sarcoma - metastatic LMS* (n=1)	Benign mesenchymal neoplasm (n=1)	Pulmonary myelolipoma (n=1)	Endobronchial mucoepidermoid carcinoma, high grade (n=1)
Age, sex	46, F	59, M	51, M	65, M	39, F
Complaints	Cough, hemoptysis, and dyspnoea				
Imaging studies	Solitary pulmonary nodule.	Nodular masses in both lungs - suggested metastases.	-	Endobronchial nodules -? TB/ sarcoidosis	-
Bronchoscopy	NA	NA	Irregularisation in left lingula? a blood clot? growth	-	Left main bronchus tumor with a possible endobronchial carcinoid.
Type of biopsy	Lobectomy	EBLB	EBLB	EBLB	Left Pneumonectomy
Gross	Glistening lobulated mass of 3x2.5 cm	-	-	-	Endobronchial ulcerated solid, yellow-white polypoidal mass
HPE findings	Circumscribed lesion of mature cartilage interspersed by adipose tissue, smooth muscle, and fibrovascular tissue. Foci of ossification, chondromyxoid area.	• Pleomorphic spindle cells • Coagulative necrosis. Positive for SMA$^{\pm}$and desmin.	• Polypoidal lesion • Fascicles of spindle cells • Cellular myxoid stroma. • Express Vimentin and SMA$^{\pm}$	• Circumscribed lesion of thin trabecular bone enclosing adipocytes and hematopoietic elements • MPO-highlighting the myeloid cells (Figure 7A-C).	• Infiltrating neoplasm, • Solid nests, cribriform pattern, and mucin filled acini. • Squamoid morules (Figure 8A,B). • Positive for Pan CK and P63

LMS: Leiomyosarcoma, **NA:** Not available, **SMA:** Smooth muscle actin, **MPO:** Myeloperoxidase, **HPE:** Histopathological examination, **EBLB:** Endobronchial lung biopsy, **TB:** Tuberculosis.

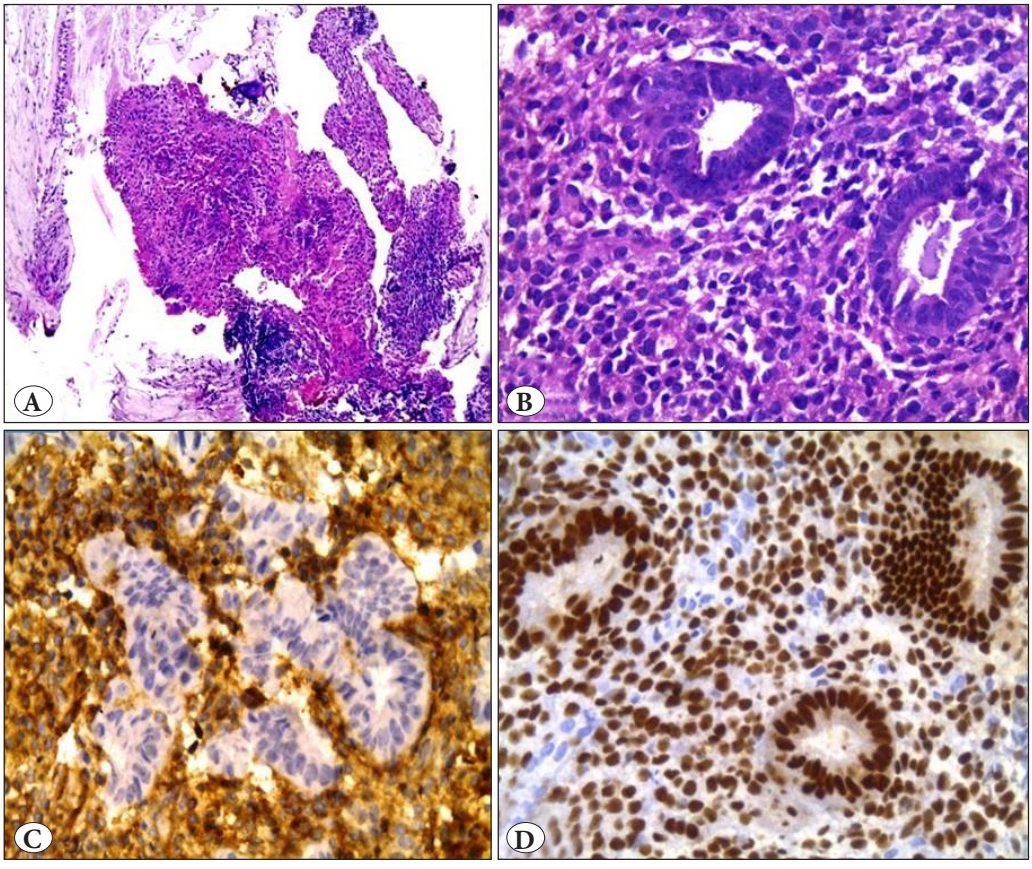


Figure 1: Bronchial endometriosis. **A)** Bronchial mucosa with a focus of endometrial glands and stroma (H&E; x4). **B)** High power view (H&E; x40). **C)** CD10 positive stromal cells (IHC; x40). **D)** ER positive epithelial & stromal cells (IHC; x40).

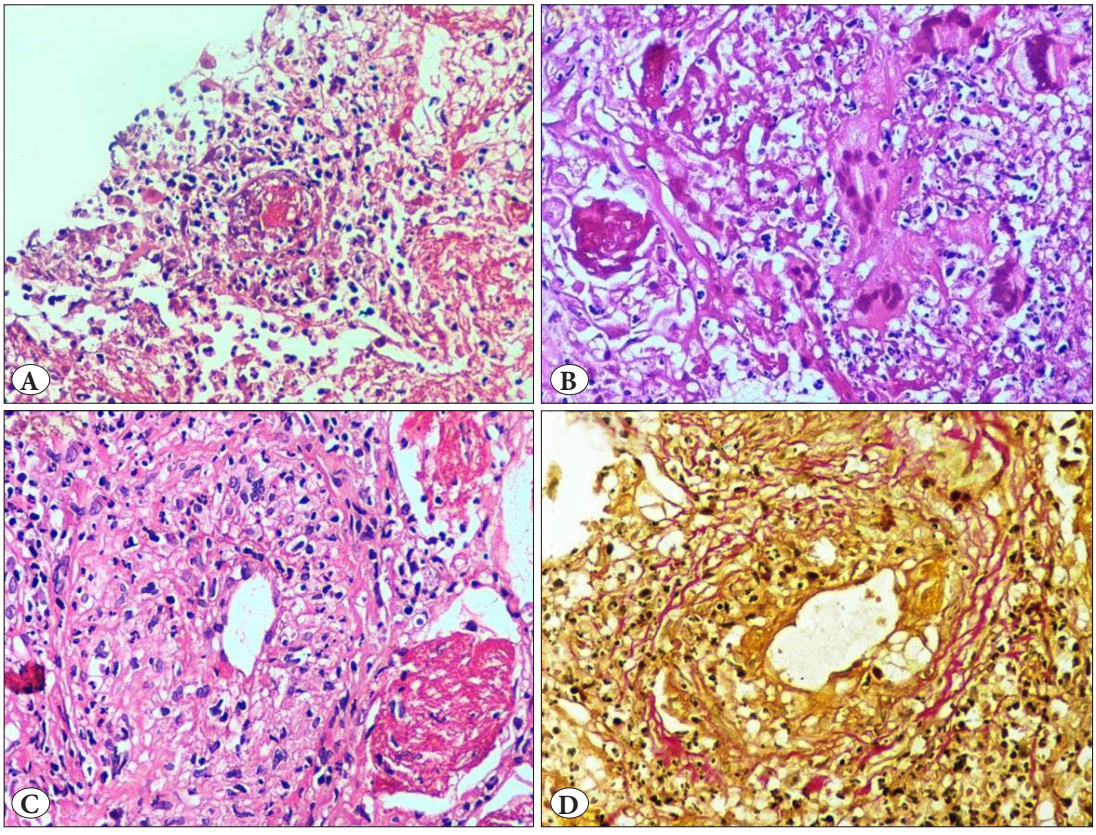


Figure 2: Granulomatosis with polyangiitis. **A)** Capillaritis with fibrin thrombi (H&E; x10). **B)** Scattered multinucleated giant cells, karyorrhectic debris & parenchymal necrosis (H&E; x40). **C)** Medium-sized vessel wall with neutrophilic infiltration and destruction (H&E; x10). **D)** Verhoeff-Van Gieson (VVG) stain highlights the outline of a vessel wall (VVG; x40).

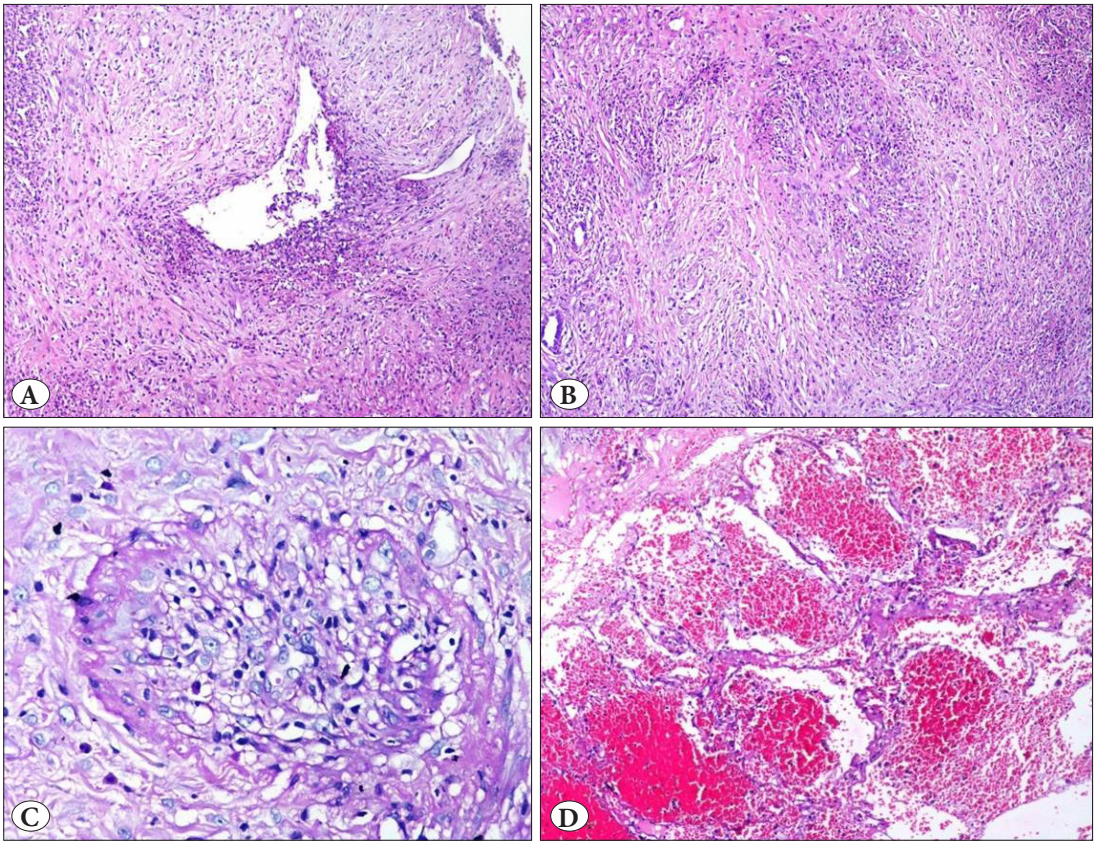


Figure 3: Eosinophilic granulomatosis with polyangiitis. **A-B)** A medium-sized artery infiltrated by eosinophils and histiocytes with partial destruction of a vessel wall (H&E; x10 & x20). **C)** (PAS stain; x40). **D)** Intra-alveolar hemorrhage (H&E; x10).

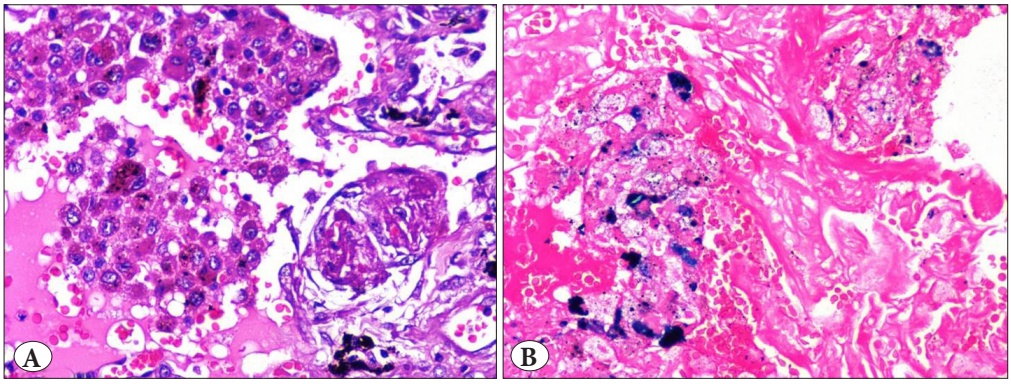


Figure 4: Hemosiderin-laden macrophages **A)** (H&E; x40). **B)** (Perl's stain; x40).

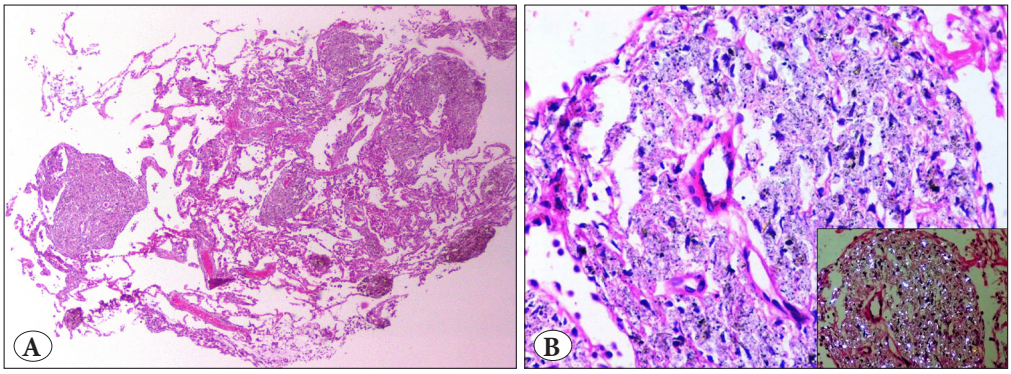


Figure 5: Silicosis. **A)** Multiple, patchy silicotic nodules (H&E; x10). **B)** Perivascular nodular collection of pigmented histiocytes (Inset-birefringent crystals under polarized light) (H&E; 40).

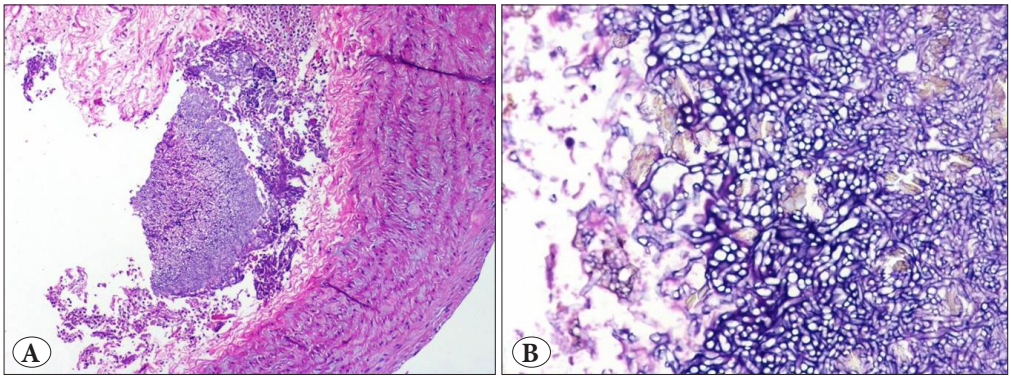


Figure 6: Fungal infection. **A)** Intravascular plugs of fungal organisms (H&E; x4). **B)** Septate, acute angle branching hyphal fungal organisms consistent with aspergillus with oxalate crystals (PAS; x10).

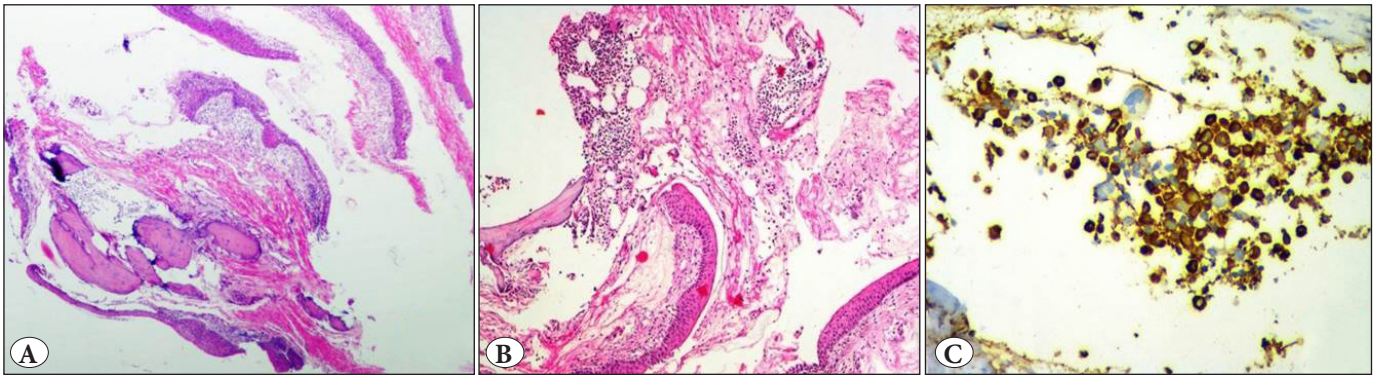


Figure 7: Myelolipoma. **A)** Bronchial mucosa with squamous metaplasia and a fairly circumscribed lesion composed of adipocytes admixed with hematopoietic elements (H&E; x4). **B)** High power view (H&E; x10). **C)** Myeloperoxidase stain (IHC; x10).

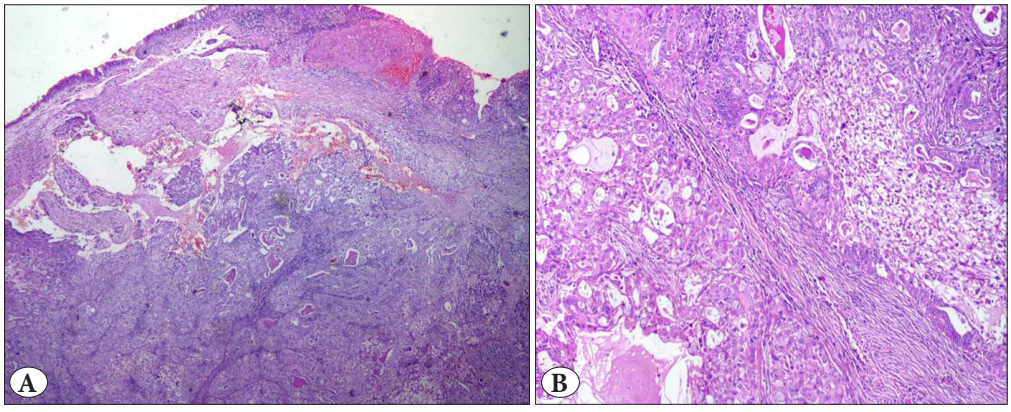


Figure 8: Endobronchial mucoepidermoid carcinoma. **A)** Showing admixture of squamous and glandular elements (H&E; x4). **B)** High power view (H&E; x10).

Pulmonary Intravascular Haemangioma (n=1)

HRCT of an elderly male showed multiple small nodules in both the lungs and a lobulated enhancing soft tissue lesion attached to the anterior wall of the right atrium-? Malignancy. TBLB sections showed lung parenchyma with medium to large-sized vessels containing an intraluminal, adherent lesion of tubular vascular spaces lined by endothelial cells. CD31 & CD34 immunostaining highlighted the endothelial cells. Since the right atrial mass could not be biopsied, the clinical/imaging findings were correlated and the final HPE diagnosis made was intravascular capillary haemangioma/?fragmented from cardiac haemangioma.

DISCUSSION

Haemoptysis is considered to be a serious medical condition and has a broad differential diagnosis. All cases with microscopic evidence of hemorrhage do not present with hemoptysis clinically. Similarly, all patients who presented with hemoptysis will not show alveolar hemorrhage histologically (4).

Haemoptysis with Capillaritis (Granulomatosis with Polyangiitis & Eosinophilic Granulomatosis with Polyangiitis)

Pulmonary vasculitis is characterized by inflammation of the small and medium-sized vessel wall, causing pulmonary hemorrhage. Imaging studies show multiple lung cavitary nodules/masses, which may mimic metastasis. Also in some conditions, the lung infiltrates are transitory making the diagnosis a challenging one (7).

Schnabel et al. stated that histopathological findings may vary depending upon the type of biopsy. They found out that the yield of guided lung biopsy is high when compared to TBLB since gross lesions are easily visible. Similarly, most of our cases had undergone guided biopsies and were adequate (8).

Travis et al. suggested that capillaritis is a morphologic marker of DAH in their study. They concluded that capillaritis and necrotizing granulomatous inflammation with mixed inflammatory cell infiltrate were pathognomonic features of GPA. Parenchymal necrosis, extravasated fibrin, and scattered multinucleate giant cells in a biopsy could suggest the diagnosis of a GPA even in the absence of granuloma. The destruction of the vessel wall can be highlighted by VVG elastic stains. However, correct sampling is essential for the maximum yield (9, 10).

The diagnosis of EGPA can be made if the biopsy shows granulomatous vasculitis with extravasated eosinophils and evidence of eosinophilic pneumonia (11). Correspondingly, three cases in our series showed capillaritis and the remaining two were diagnosed based on relevant clinical and imaging findings.

Haemoptysis without Capillaritis

Fungal Infection: Alveolar hemorrhage resulting from fungal infection is infrequent. The possible pathogenesis of hemoptysis in fungal infection could be 1) due to chronic inflammation as the granulation tissue lining the cavity is rich in capillaries, 2) these new vessels are rather fragile and can rupture easily, and 3) erosion of the blood vessels adjacent to the cavity (12). The presence of calcium oxalate crystals in a patient with a cavitary lesion will raise suspicion of a fungal infection and the pathologist should search for the same in the adjacent area (13). Likewise, our cases also had similar histology which would have led to hemoptysis.

Silicosis: An autopsy case study by Kyeong et al. revealed that unlike silicosis which affects mainly the lung parenchyma, silico - tuberculosis also affects the pulmonary vasculature causing hemoptysis (14). Our cases did not have evidence of tuberculosis. Streak-type hemoptysis seen in our cases could be explained by the destruction of small-sized blood vessels by fibrosis and chronic inflammation (15).

Unusual Tumors and Tumor-Like Lesions of the Lung

Primary Pulmonary Mesenchymal Tumors: Accounts for less than 1% of all lung malignancy. These tumors show lineage differentiation and can be diagnosed in the lung with similar histological and immunological criteria as in other sites (soft tissue) (16). Pulmonary hamartoma is the most common benign mesenchymal tumor of the lung, and is predominantly seen in older males**.** Geramizadeh et al. and Ahmed et al. in their study stated that the occurrence of hemoptysis in hamartoma is very rare and results from giant or endobronchial tumors causing erosion of the vessels (17, 18). In contrast to their findings, our patient was a middle-aged female with a small peripherally located pulmonary hamartoma.

According to Hashimoto et al., it is important to identify and differentiate metastatic mesenchymal tumors correctly, since most mesenchymal tumors presented as occult/unknown primary with pulmonary metastatic foci (19). Identically, one of our cases had metastatic LMS with a known diagnosis of primary LMS of the thigh.

Mucoepidermoid Carcinoma (MEC): MEC is a rare salivary gland type endobronchial tumor with mucous secreting cells, squamous cells, and intermediate cells. A definitive diagnosis requires bronchoscopy and representative biopsy as this tumor exhibits morphological heterogeneity. It can be misdiagnosed as primary non-small cell lung carcinoma if the biopsy is inadequate (20, 21). The diagnosis of this rare neoplasm was possible since ours was a pneumonectomy specimen.

Myelolipoma: Pulmonary myelolipoma is distinctly unusual and rarely presents with hemoptysis. Embolic origin, reticuloendothelial cell metaplasia, and proliferation of hematopoietic stem cells are possible mechanisms of histogenesis. Tumour arising adjacent to the bronchial cartilage may show bony trabeculae and this explains the possible histogenesis of the metaplastic theory (22). Our case also revealed a similar histology.

Miscellaneous Uncommon Causes

Pulmonary Endometriosis: Pulmonary endometriosis is a rare form of extra pelvic endometriosis, occasionally reported in men. Cases of male endometriosis have typically been linked to increased circulating estrogen levels. Peripheral conversion of androstenedione and testosterone to the circulating estrogens was noted in men with cirrhosis of the liver (23). Our patient had a history of cirrhosis, possibly leading to an altered hormonal state that interacted with a reactive/metaplastic process.

Pulmonary Alveolar Microlithiasis (PAM): PAM is a rare autosomal recessive lung disease characterized by the accumulation of concentrically laminated calcospherites within the alveolar spaces. Lauta in his study stated that there is a striking clinico-radiologic disparity in this condition (24). Since our patient did not have other system involvement, the possibility of PAM over secondary calcification was favored by correlating the clinical and imaging features.

Pulmonary Intravascular Haemangioma: The co-existence of cardiac and pulmonary haemangioma is very rare and very few reports of such an association have been described so far. Imaging studies may help in diagnosing cardiac haemangioma while histopathology is essential for confirmation. Complete excision is the treatment of choice in resectable cardiac haemangioma and solitary pulmonary haemangioma (25). Since the general condition of the patient was poor, resection was not possible in our case. Fragmented emboli from a cardiac haemangioma could also be considered as a differential.

In conclusion, in immunologic causes of hemoptysis, histopathological findings may support the diagnosis in correlation with the clinical/imaging features. However, in cases of non-immunologic causes of hemoptysis, a histopathologic examination is mandatory over clinical/imaging studies. The cases presented here are incredibly rare diseases with an uncommon presentation. The diagnosis is often missed or delayed, due to a low index of suspicion. Histopathological examination is imperative in unexplained cases of hemoptysis as it plays a vital role in therapy decisions.

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CONFLICT of INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Haro Estarriol M, Vizcaya Sanchez M, Jimenez Lopez J, Tornero Molina A. Etiology of hemoptysis: Prospective analysis of 752 cases. Rev Clin Esp. 2001;201:696-700

2. Lee BR, Yu JY, Ban HJ, Oh IJ, Kim KS, Kwon YS, Kim YI, Kim YC, Lim SC. Analysis of patients with hemoptysis in a tertiary referral hospital. Tuberc Respir Dis (Seoul). 2012;73:107-14.
3. Quadrelli S, Dubinsky D, Solis M, Yucra D, Hernandez M, Karlen H, Brigante A. Immune diffuse alveolar hemorrhage: Clinical presentation and outcome. Respir Med. 2017;129:59-62.
4. Colby TV, Fukuoka J, Ewaskow SP, Helmers R, Leslie KO. Pathologic approach to pulmonary hemorrhage. Ann Diagn Pathol 2001;5:309-19.
5. Park MS. Diffuse alveolar hemorrhage. Tuberc Respir Dis (Seoul). 2013;74:151-62.
6. Castaner E, Alguersuari A, Gallardo X, Andreu M, Pallardo Y, Mata JM, Ramírez J. When to suspect pulmonary vasculitis: Radiologic and clinical clues. Radiographics. 2010;30:33-53.
7. Lynch JP, Leatherman JW. Alveolar hemorrhage syndromes. In: Fishman, editor. Pulmonary Diseases and Disorders. New York: McGraw Hill; 1998. 1193-1210.
8. Schnabel A, Holl-Ulrich K, Dalhoff K, Reuter M, Gross WL. Efficacy of transbronchial biopsy in pulmonary vasculitides. Eur Respir J 1997;10:2738-43.
9. Travis WD, Colby TV, Lombard C, Carpenter HA. A clinicopathologic study of 34 cases of diffuse pulmonary hemorrhage with lung biopsy confirmation. Am J Surg Pathol. 1990;14:1112-25.
10. Travis WD, Hoffman GS, Leavitt RY, Pass HI, Fauci AS. Surgical pathology of the lung in Wegener's granulomatosis. Review of 87 open lung biopsies from 67 patients. Am J Surg Pathol. 1991;15:315-33.
11. Suster S, Moran CA. Biopsy interpretation series. Biopsy interpretation of lung. 1st ed. Epstein JI, editor. Philadelphia: Lippincott Williams and Wilkins; 2013.
12. Corrin B, Nicholson AG. Pathology of lungs. 3rd ed. Churchill Livingstone: Elsevier; 2011.
13. Mukhopadhyay S. Nonneoplastic pulmonary pathology. An algorithmic approach to histologic findings in the lung. 1st ed. Erika D, editor. United Kingdom: Cambridge University Press; 2016.
14. Kyeong HE, Cheong H, Kim HJ, Choi YS. Pulmonary hemorrhage with progressive massive fibrosis in a silicosis patient: An autopsy case. Korean J Leg Med 2012;36:186-9.
15. Anna-Luise A. Katzenstein MD. Diagnostic Atlas of Non-Neoplastic Lung Disease: A Practical Guide for Surgical Pathologists. 1st ed. David DA, editor. New York: Springer; 2016.
16. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of Tumours of the Lung, Pleura, Thymus, and Heart. 4th ed. International Agency for Research on Cancer: Lyon; 2015.
17. Geramizadeh B, Mottavvas M, Zeyaian B, Amirian A. Giant hamartoma of lung presented with massive hemoptysis: A rare case report and review of the literature. Rare Tumors. 2019; 11:1-4.
18. Ahmed S, Arshad A, Mador MJ. Endobronchial hamartoma; A rare structural cause of chronic cough. Respir Med Case Rep. 2017;22:224-7.
19. Hashimoto H, Tsugeno Y, Sugita K, Inamura K. Mesenchymal tumors of the lung: Diagnostic pathology, molecular pathogenesis, and identified biomarkers. J Thorac Dis. 2019;11:9-24.
20. du Toit-Prinsloo L, Bunn BK. Massive hemoptysis due to primary mucoepidermoid carcinoma of the lung in a 12-year-old. Forensic Sci Med Pathol. 2016;12:380-3.
21. El-Sameed YA, Al Marzooqi SH. Primary mucoepidermoid carcinoma of the lung. J Bronchology Interv Pulmonol. 2012;19:203-5.
22. Sato K, Ueda Y, Katsuda S, and Tsuchihara K: Myelolipoma of the lung: A case report and brief review. J Clin Pathol. 2007;60: 728-30.
23. Jabr FI, Mani V. An unusual cause of abdominal pain in a male patient: Endometriosis. Avicenna J Med. 2014;4:99-101.
24. Lauta VM. Pulmonary alveolar microlithiasis: An overview of clinical and pathological features together with possible therapies. Respir Medi. 2003;97:1081-85.
25. Wang C, Chen H, Sun L, Mei Y. Cardiac cavernous haemangioma coexisting with pulmonary cavernous hemangiomas and giant hepatic hemangioma. Ann Thorac Surg. 2017;103:149-52.

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[illegible]

[illegible] The purpose of this study was to evaluate gross umbilical cord abnormalities and to examine its relationship with placental pathological features and perinatal outcome.

M [illegible] We retrospectively collected 101 singleton placentas with gross umbilical cord abnormalities over 3-year period (delivered on or after 20 weeks of gestational age). Association between gross umbilical cord abnormalities such as hypercoiled cords, hypocoiled cords, thin cords, true knot, strictures, excessively long cord, and abnormal cord insertions were reviewed and evaluated for placental histology, clinical parameters and perinatal outcome.

[illegible] The most common umbilical cord abnormality (UCA) observed was coiling abnormality (hypercoiling, hypocoiling and strictures). Gross UCAs were associated with maternal factors (preeclampsia, oligohydramnios and gestational diabetes), foetal factors (intrauterine foetal death, SGA and intrapartum complications such as NRFHR tracing and low Apgar scores) and histologic placental features (maternal vascular malperfusion, foetal vascular malperfusion, villous capillary lesions and inflammation). Cords with multiple abnormalities were significantly correlated with histologic evidence of foetal vascular obstruction and intrauterine foetal death.

[illegible] Gross UCAs, especially when multiple were associated with clinically significant placental findings and adverse perinatal outcome. Therefore, our study reinforces that all placentas with gross UCAs should be submitted for examination with complete (full length of the) umbilical cord.

[illegible] Coiling abnormality perinatal outcome, Foetal vascular malperfusion, Maternal vascular malperfusion, Placental histology, Umbilical cord abnormalities

[illegible]

Umbilical cord (UC) is a vital structure which acts as a conduit between the developing foetus and placenta. It carries nutrients, oxygen and fluids necessary for the intrauterine life. This unique lifeline needs optimal protection which is provided by Wharton's jelly, amniotic fluid and coiling of the umbilical vessels.[1] The fully developed umbilical cord is pearly white, 50-60cms long with an average diameter of 1-2cm. It contains 2 arteries and a vein which course through the Wharton's jelly in a helical fashion with one coil/twist every 5 cms of cord. The cord normally inserts on the placental surface, more often near or at the center.[2]

Conditions such as abnormal cord length, abnormal coiling, knots, entanglements, constrictions, velamentous vessels, single umbilical artery (SUA) and thrombosis

may lead to cord compression and consequent reduction of blood flow in the umbilical vessels and have been associated with adverse foetal outcome and intrauterine foetal demise (IUFD).[3]

The purpose of this study was to evaluate gross patterns of umbilical cord abnormalities and determine correlations with placental histology and perinatal outcome.

M□□□□□□□

This retrospective study was carried out from January 2014 to December 2016 in the Departments of Obstetrics and Gynaecology and Department of Pathology.

101 Placentas with gross umbilical cord abnormalities delivered on or after 20 weeks of gestational age were taken for study consideration. Clinical indications for histological examination of the placenta were stillbirth, foetal growth restriction, preterm premature rupture of membranes, severe pre- eclampsia, diabetes, gross abnormalities of the placenta, clinical suspicion of infection or abruption and compromised neonatal outcome.

Umbilical cord abnormalities (UCA) included in the study were hypercoiled cord (>3 coils/10cm segment of umbilical cord), hypocoiled cord (< 1 coil per 10 cm), thin /narrow cord due to diminished Wharton's jelly (maximum cord diameter <0.8 cm), strictures (focal narrowing/thinning in relation to the remaining UC), true knot(TK), SUA, abnormal cord insertion-marginal (< 1cm from the true disc margin-MUC) / velamentous (membranous insertion-VEL) / furcate (umbilical vessels splitting and leaving Wharton's jelly prior to reaching the chorionic plate surface).[4] Excessively long umbilical cord (ELUC) was defined as those UCs measuring approximately 2 standard deviations (SD) above the mean or ≥ 70cms in length.[4,5]

Clinical details obtained along with the placental specimens were maternal age, gravidity and parity, gestational age, medical conditions complicating pregnancies, presence of IUFD, foetal growth restriction (FGR), non-reassuring foetal heart rate (NRFHR), meconium stained liquor, Apgar scores at 1 and 5 minutes and birth weight as recorded by the obstetricians. Infants with birth weight <10th percentile and >90th percentile of the gestational age were termed as small for gestational age (SGA) and large for gestational age (LGA) respectively. The rest were considered as appropriate for gestational age (AGA).[6]

Umbilical cord and placental assessment were based on gross and microscopic examinations. An entire placenta was available from all 101 patients and were fixed in 10% formalin. Placentas were weighed after the membranes and umbilical cord were trimmed off and compared with the reference weights for trimmed singleton placentas.[7]

Umbilical cord was examined for length, diameter, colour, insertion site, number of twists and vessels. Photography of the placentas were routinely taken after examining foetal, maternal surfaces and parenchyma. Relevant lesional sections were taken along with 2 sections of membrane roll, 2 or 3 sections from the UC at the stricture and non-strictured area and 3 full thickness sections of the normal parenchyma. All the histologic sections were stained with haematoxylin and eosin.

Umbilical cord abnormalities were correlated with gross, histologic placental characteristics and foetal outcome.

□□□□□□□□

Hundred and one cases were identified with gross UCAs. There were 48 cases of hypercoiling (Figure 1), 31 cases of hypocoiling (Figure 2) and 11 cases each of thin cords and strictures (Figure 3). Abnormal cord insertions were seen in 7 cases each of MUC, VEL (Figure 4) and 2 cases of furcate. Other less commonly seen abnormalities were 2 cases each of TK (Figure 5), ELUC and SUA. Of the 101 cases of analysed placentas 20 had more than one umbilical cord abnormality.

Isolated UCAs were each analyzed for association with clinical and histologic findings (Tables 1 and Table 2).

Clinical characteristics

The most common UCA identified was hypercoiling, followed by hypocoiling. Stillbirth was observed in 42 cases across all cord abnormalities but was by far most frequently associated with abnormal coiling.

Preeclampsia, oligohydramnios and gestational diabetes were the commonest maternal characteristics observed in UCAs. Infections, PPROM, polyhydramnios and autoimmune diseases were other maternal features identified in altered structure of UCs.

NRFHR, low Apgar and Meconium stained liquor were common in coiling abnormalities, more so in the group with hypercoiling. Fifty percent of hypercoiled and one third of hypocoiled cords had birth weights of < 10th percentile.

Placental findings

More than 50% of the placentas with UCAs were small for gestational age. Significant histologic features identified were maternal vascular malperfusion (MVM-35/101), foetal vascular malperfusion (FVM-26/101) and villous capillary lesions (19/101). The other microscopic features noted were distal villous immaturity (DVI), massive perivillous fibrin deposition (MPVFD), and inflammatory lesions.

Table 3: Clinical and histologic findings in cases with multiple UCAs

Parameter	Cases with multiple UCAs (n= 31)	IUFD (n=11)	Live born (n=20)	SGA (n=11)	AGA (n=20)	Hyper coiling (n=20)	Hypo coiling (n=20)	Strictures (n=20)	Thin cord (n=20)
Placental weight									
SGA	31	19	10	0	4	1	2	4	5	1
MVM	21	8	4	0	1	0	1	3	2	1
Infarcts	13	10	4	0	1	0	0	1	2	0
Retroplacental haematoma	3	5	1	0	0	0	0	0	1	0
Intervillous thrombus	9	8	2	0	0	0	0	1	1	1
MPVFD	1	1	0	0	2	1	0	0	0	0
FVM	8	10	5	0	6	0	2	3	1	0
Chorionic plate/stem villous vessel thrombus	5	7	2	-	6	0	2	3	1	0
Avascular villi	8	8	5	-	6	0	2	3	0	0
Villous Stromal Karyorrhexis	4	5	4	-	2	0	1	0	0	0
Nucleated RBCs	2	3	-	-	1	0	1	1	0	0
Inflammation	8	9	3	1	2	0	1	1	4	1
Acute chorioamnionitis	2	5	0	1	1	0	0	0	2	0
VUE	6	4	3	0	1	0	1	1	2	1
Villous capillary/vascular lesions	14	3	3	2	2	1	0	0	0	0
Chorangiosis	9	1	1	1	1	0	0	0	0	0
Chorangiomatosis	3	2	2	0	1	1	0	0	0	0
Villous congestion	2	0	0	1	0	0	0	0	0	0
DVI	5	4	1	0	0	0	0	2	1	0

Infarcts, intervillous thrombus, retroplacental haematoma and MPVFD (histologic markers of MVM) were observed in cases with gross UCAs. The other features noted were decidual vasculopathy, distal villous hypoplasia, increased syncytial knotting, cell islands, villous agglutination, and multinucleate trophoblastic giant cells.

The histologic features of chronic FVM such as chorionic plate /stem villous vessel thrombus, avascular villi, villous stromal karyorrhexis and nucleated red blood cells were significantly more common in cords with hyper, hypo coiling and strictures. Ectatic vessels were also noted in many of the chorionic plate /stem villous vessels of FVM.

Villous vascular lesions such as chorangiosis (increased number of capillaries - more than 10 capillaries/terminal villi, in 10 villi) in many areas of the placental parenchyma, patchy and occasional multifocal chorangiomatosis (excessive capillaries with surrounding pericytes in immature intermediate / stem villi) and villous congestion were found to be associated with hypercoiled cords.

Multiple UCAs

Placentas with multiple UCAs and their associations with clinical and histologic findings were also analysed. IUFD was significantly associated with the presence of multiple UCAs (13/20). In the remaining live born cases the clinical outcome was SGA (6/20) and AGA (1/20). The most common maternal obstetric complication was hypertension and the commonest placental histology was foetal vascular malperfusion (FVM) (Figure 6). These findings are summarized in (Table 3).

Discussion

The coiling pattern of the UC largely affects umbilical blood flow that is vital for sustaining foetal growth and development and its pathophysiological significance has been studied by many authors. UCAs often result in mechanical compression leading to venous stasis, endothelial damage and loss of perfusion to a large area of placental gas exchanging villi and ultimately resulting in foetal distress and hypoxia.[8-10] This study analyzed the UCAs such as hypercoiling, hypocoiling, strictures, thin cord, true knot, SUA, ELUC and abnormal insertions in

relation to gross, histologic placental changes as well as clinical parameters and foetal outcome.

Table 2: Correlation between umbilical cord coiling abnormalities and clinical parameters and foetal outcome

Parameters	Total number of cases	Total number of hypercoiled cords	Total number of hypocoiled cords	Total number of normal coiled cords	Total number of IUFD	Total number of FGR (<10th percentile)	Total number of NRFHR tracing	Total number of low Apgar score (<7 at 1 min)	Meconium stained liquor	Total number of preterm births (<37 weeks)
Gestational age										
<28 weeks	8	6	5	0	4	0	0	1	3	1
>28 weeks	40	25	6	2	7	2	2	6	4	1
Maternal factors										
HT	15	6	2	0	1	0	0	2	3	0
GDM	8	0	0	1	1	0	0	1	0	1
Rh Negative	1	0	0	0	0	0	0	1	0	0
Oligo	10	6	2	0	0	0	0	1	3	0
PPROM	3	6	2	0	0	0	0	0	1	0
Polyhydramnios	1	1	0	0	0	0	1	0	0	0
Auto immune	1	1	2	0	1	0	0	1	1	0
Infections	4	1	1	0	1	0	0	0	0	0
Foetal factors										
SGA	21	9	5	0	1	0	0	5	2	1
AGA	10	8	1	0	1	0	0	0	0	0
LGA	3	0	0	1	0	0	0	0	0	0
IUFD	14	14	5	1	9	2	2	2	5	1
NRFHR	15	2	1	0	1	0	0	2	1	1
Liquor										
<4 @1 mt	6	1	0	0	0	0	0	1	0	0
<7 @ 1 mt	7	2	1	0	1	0	0	1	0	0
Thick meconium stained liquor	4	1	0	0	0	0	0	0	0	0

Consistent with prior studies, our investigation showed that UCA has significant association with IUFD, SGA and intrapartum complications such as NRFHR tracing and low Apgar scores.[8-10] Meconium stained liquor was not a consistent finding in our study.

Umbilical cord coiling abnormality

Little is known about factors determining cord coiling. It is generally believed that coiling is established very early in gestation and is thought to be the result of foetal activity. Lack of coiling may then reflect foetal inactivity, which is supported by the fact that coiling is reduced in cases of restricted foetal movements due to various causes.1 The prenatal ultrasonographic evaluation of umbilical cord is usually limited to vessel number and umbilical artery Doppler. Predanic et al demonstrated a strong correlation between mid-trimester umbilical coiling index (UCI) and post-natal coiling index.[11]

Among 101 studied placentas, abnormal coiling was the commonest UCA, which accounted for 80%. The frequency and clinical correlations of hypercoiled cords were FGR (43%), IUFD (29%), NRFHR (31%). For hypocoiled cords, the frequencies of adverse outcomes were IUFD (45%) and FGR (29%). Our results were in contrast to the study by Machin et al who observed more IUFDs among hypercoiled cords. Our data support the finding that abnormal coiling may cause chronic umbilical cord obstruction and unfavourable foetal outcome.[12]

Among hypercoiled umbilical cords, specific gross patterns of coiling were recognized with histologic evidence of foetal vascular malperfusion and stillbirth.[8]

The placental weight was small for gestational age in more than two thirds of the studied cases. Placental histologic findings associated with UC coiling abnormalities include villous capillary congestion, chorangiosis, chorionic plate / stem villous vessel thrombi, avascular villi, MVM, inflammatory lesions and DVI. Placental infarcts and intervillous thrombus are the conspicuous lesions observed among coiling abnormality cases in our series.

They represent deficient maternal circulation (maternal vascular malperfusion) leading to parts of placental atrophy or infarction in areas of poor nutrition. However,

the association between MVM and cord coiling abnormalities appear speculative.

Hypercoiling+ Stricture	4	SGA	A) congestion around cord insertion	A) FVM	A) NIL	IUFD
		SGA	B) NIL	B) vascular involution, patchy mild villitis	B) post varicella	IUFD
		SGA	C) marginal atrophy and patchy	C) FVM	C)NIL	IUFD
		SGA	firm pale brown areas D) NIL	D) FVM	D)NIL	IUFD
Hypocoiling+ Thin cord	4	SGA	NIL	DVI, decidual arteriopathy, laminar necrosis	A) PPROM	AGA
		SGA	Uneven thickness of	FVM	B) Oligo,FGR	SGA
		SGA	parenchyma Intervillous thrombus, infarcts	MVM, FVM	C)preeclampsia	IUFD
		SGA	NIL	Villous hydrops,FVM,mild intervillositis,viral/thrombophilia possibilities	D)Fever	IUFD
Hypercoiling+ Thin cord	3	A)SGA	Infarcts	FVM, MVM, mild chronic villitis, obliterative vasculopathy	a)Systemic hypertenion, GDM	SGA
		B)SGA	Marginal thinning,uneven thick, yellow brown lesion(infarcts)	extensive multifocal Chorangiomatosis, MVM	b)FGR	SGA
		C)SGA	C subchorionic dark brown congested area	C MVM, Multifocal chorangiomatosis-treated SLE	c)autoimmune disease, PE, FGR, PPROM	SGA
Hypercoiling + Marginal Insertion	2	A)SGA	A) infarcts, intervillous thrombus	MVM	A)PE	IUFD
		B)SGA	B) Basal yellow brown nodule	Infarcts, laminar necrosis, DVI	B) Autoimmune disease, PPROM	SGA
Hypocoiling+ Marginal	1	SGA	Focal Atrophy, Subchorionic Hematoma	Large transmural hematoma occupying >50% parenchyma, recanalizing thrombi (stem vill)-FVM, MVM	Systemic hypertension	IUFD
Hypocoiling+ Stricture	1	SGA	Wedge, pale yellow INFARCT	SMALL, hypocoiled cord with a stricture, MVM	FGR	SGA
Hypocoiling+ SUA	1	SGA	Edematous, congested cord, parenchymal marginal atrophy	FVM	Polyhydramnios	IUFD
VEL + stricture	1	SGA	Uneven thickness of parenchyma	Small placenta with umbilical cord stricture and a velamentous vessel with thrombus FVM vascular involution following foetal death with ? Temporal heterogeneity	Autoimmune disease	IUFD
VEL+Furcate	1	SGA	succenturiate, furcate cord	Chronic histiocytic intervillositis	Fever with rash	IUFD
ELUC+Nuchal+Stricture	1	SGA	maternal surface-plaque like thickening >25%,6 firm pale-yellow areas in parenchyma	MPVFD, focal chorangiomatosis	NIL	IUFD
ELUC+Nuchal+True knot	1	AGA	NIL	DVI, FVM	NIL	Term IUFD

We wondered whether the less placental reserve due to deficient intervillous circulation can affect foetal outcome if cord coiling abnormalities arise additionally.

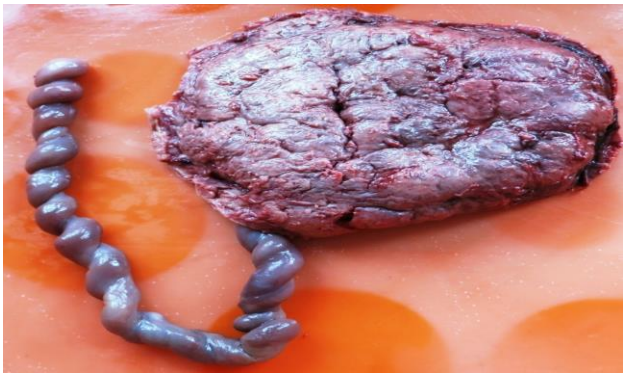


Figure 1: Hypercoiled cord

Presence of avascular villi, thrombosis of foetal vasculature, VSK and NRBCs reflect foetal blood flow obstruction and hypoxia. In our study 25% of the abnormal coiling cases tended to have FVM suggesting that these lesions would have caused chronic vascular obstruction.[10]

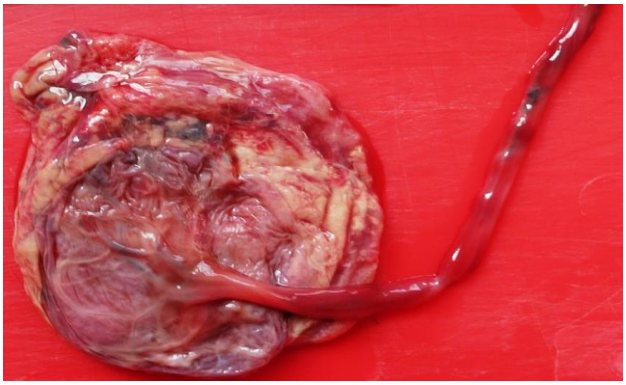


Figure 2: Hypocoiled cord



Figure 3: Hypocoiled cord

Chorangiosis, multifocal chorangiomatosis are overlapping villous capillary lesions believed to be related to hypoxia. Benirschke observed an association between chorangiosis and cord problems.[13] It is an adaptive mechanism to overcome hypoxia occurring in coiling abnormalities. Our observations were also similar.[14] Some of the cases also showed distal villous immaturity, one other lesion known to be associated with coiling abnormalities.



Figure 4: Hypercoiled cord with avascular villi and thrombosis

There was some correlation between inflammatory lesions such as VUE and, acute chorioamnionitis with individual UCA.



Figure 5: Hypercoiled cord with avascular villi and thrombosis

Cord strictures

Most investigators considered strictures and hypercoiling to be similar or related entities. In our study we observed a total of 11 cases with cord strictures. Four among the cases were associated with hypercoiling; one each with hypocoiling, velamentous cord insertion and ELUC. 4 cases had isolated stricture. This data suggested that cord stricture is a distinctive entity as agreed upon by Hong Qi Peng et al in their study.[15] We have also noted a high incidence (more than 80%) of foetal demise associated with cord stricture, coiling abnormalities or a

combination of both. Foetal vascular malperfusion was common when strictures and hypercoiling were coexistent. This implies that UC strictures and hypercoiling could decrease the blood flow with possible predisposition to development of thrombosis.

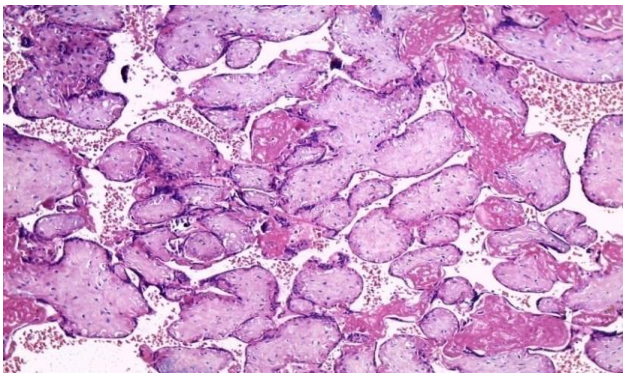


Figure 6: Photomicrograph of placental villi showing avascular hyalinised villi, villous stromal karyorrhexis and nucleated RBCs suggesting that these lesions would have caused prolonged obstruction (H&E, 10×)

Thin cord, SUA

It has been suggested that diameter of the umbilical cord is determined by the water content of Wharton's jelly. Previous studies have found an association of thin cord with FGR and other poor foetal outcome.[16,17]

A Wharton's jelly reductions have also been recognized as a possible cause of foetal death in the presence of single umbilical artery, which is the most common abnormality of the umbilical cord. These associations were confirmed in our study. According to Heifetz SA, non-malformed SUA infants can have an increased mortality rate, although SUA with associated malformations are the common cause of the high perinatal mortality.[18]

True knot, ELUC

Interestingly, both cases of true knot were associated with LGA. The case with a tight TK ended up in IUFD. Common associations like multi gravida and ELUC were also observed in our study. Like earlier studies we also found an association between ELUC and other multiple UCAs.[5] Both cases of ELUC were associated with multiple UCAs such as nuchal cord, TK and stricture and expectantly both resulted in IUFD.

Abnormal cord insertions

Marginal and velamentous insertions are suggested to result from disturbances of implantation and considered a marker of placental insufficiency.[19] We found that non-reassuring foetal status, SGA and IUFD were significantly associated with abnormal insertion which may reflect intrauterine hypoxia.

Multiple UCAs

Any UCA can sufficiently reduce placental function, when multiple predispose the foetus to placental circulatory stasis lesions and thrombosis, thus leading to vascular obstruction and unfavourable clinical sequelae.[3]

In our study the majority of multiple UCA cases tended to have extensive rather than focal FVM with admixture of avascular hyalinised villi, villous stromal karyorrhexis and nucleated RBCs suggesting that these lesions would have caused prolonged obstruction. Infants with multiple UCAs where FVM is extensive were found to have brain imaging abnormalities and/or abnormal neurological follow-up.

There were some limitations in our data. First, as a retrospective study, the clinical information was available only for the immediate neonatal period and further follow up could not be ascertained. Second, not all placentas and complete length of the UC (that remained attached to the neonate) were available for examination. Finally, different patterns of hypercoiling were not studied.

Conclusions

Gross UCAs, especially when multiple was associated with clinically significant placental findings and adverse perinatal outcome. Therefore, our study reinforces that all placentas with gross UCAs should be submitted for examination with complete (full length of the) umbilical cord. Coiling index with patterns of coiling should be a part of routine examination. A prospective study that also includes the follow up of these neonates could address the clinical effects of UCAs.

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References

1. De Laat MW. The umbilical coiling index in complicated pregnancy. Eur J Obstet Gynecol. Reprod Biol. 2007;130(1):66-72.
2. Rebecca N. Baergen. Umbilical Cord Pathology. Surgical pathol. 2013;6:61-85.
3. Chan JS, Baergen RN. Gross umbilical cord complications are associated with placental lesions of circulatory stasis and fetal hypoxia. Pediatr Dev Pathol. 2012;15(6):487-94.
4. T. Yee Khong. Sampling and Definitions of Placental Lesions. Amsterdam Placental Workshop

Group Consensus Statement. Arch Pathol Lab Med. 2016;140:698-713.
5. Benirschke K, Burton GJ, Baergen RN. Pathology of the Human Placenta. Sixth edition. New York, NY: Springer; 2012.
6. Amy Heerema-McKenney, MD, Monique E De Paepe, MD, MSc and Edwina J Popek, DO. Diagnostic Pathology: Placenta. Published by AMIRSYS; 2015.
7. Almog B. Placenta weight percentile curves for singleton and twins deliveries. Placenta. 2011;32:58e62.
8. Ernst LM, Minturn L, Huang MH, Curry E, Su EJ. Gross patterns of umbilical cord coiling: correlations with placental histology and stillbirth. Placenta. 2013;34(7):583-8.
9. Baergen RN. Cord abnormalities, structural lesions, and cord "accidents". Semin Diagn Pathol. 2007;24 (1):23-32.
10. Tantbirojn P, Saleemuddin A. Gross Abnormalities of the Umbilical Cord: Related Placental Histology and Clinical Significance. Placenta. 2009;30:1083-8.
11. Verkleij CPM. Evaluation of antenatal umbilical coiling index at 16–21 weeks of gestation as a predictor of trisomy 21and other chromosomal defects. Ultrasound Obstet Gynecol. 2013;42:545–52.
12. Diwakar RK, Naik MM, Jindal MM. Umbilical cord coiling: case report and review of literature. BJR Case Rep. 2017;2:20150152.
13. Ryan WD, Trivedi N, Benirschke K. Placental Histologic Criteria for Diagnosis of Cord Accident: Sensitivity and Specificity. Pediatr Dev Pathol. 2012;15:275-80.
14. Tihana Rumboldt, Paul T Eberts. Disorders of Fetal Vascular Development Chorangioma, Localised Chorangiomatosis, Chorangiosis, and Diffuse Multifocal Chorangiomatosis, Pathology Case Reviews. 2008;13:236-40.
15. Shun-Jen Tan. Fetal demise by umbilical cord around abdomen and stricture. Archives Gynecol bstetr. 2010;281:137.
16. Waldo Sepulveda, MD et al. Improving Sonographic Evaluation of the Umbilical Cord at the Second-Trimester Anatomy Scan. J Ultrasound Med. 2009;28:831-5.
17. Kulkarni ML. Absence of Wharton's jelly around the umbilical arteries. Indian J Pediatr. 2007;74(8):787-9.
18. Hyeong Ju Kim. Association of isolated single umbilical artery with perinatal outcomes: Systemic review and meta-analysis. Obstet Gynecol Sci. 2017;60(3):266-73.
19. Pathak S, Sebire NJ, Hook L, Hackett G, Murdoch E, Jessop F, et al. Relationship between placental morphology and histological findings in an unselected population near term. Virchows Arch. 2011;459(1):11-20.

Gurusamy U, Thangavelu R, Venkataswamy C. Abnormalities of the umbilical cord: correlation with placental histology and perinatal outcome. Int J Res Med Sci 2017;5:5009-16.

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Placental morphology and perinatal outcome in gestational hyperglycemia on diet and medication

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Abstract

Background: To evaluate the placental morphology and perinatal outcome in patients with gestational hyperglycemia on diet and medication.

Methods: Placental examinations performed at the Department of Pathology between August 2016 to August 2018 were retrospectively reviewed. Of the received 140 placentas, 35 of gestational diabetes (GDM) and pre gestational diabetes were identified and segregated into hyperglycemia on diet and on medication. The clinical details, placental findings and perinatal outcome of patients in both the groups (gestational hyperglycemia on diet and medication) were collected and analyzed.

Results: Among the 35 cases, there were 24 cases of mild gestational hyperglycemia controlled with diet and 11 cases of hyperglycemia on medication (oral hypoglycemic drugs ± insulin).

Most of the placentae in both the groups weighed less than tenth centile. The cord abnormalities such as hyper coiling, velamentous /marginal insertion and furcate cord were observed more in women with GDM on diet. There was no significant gross placental lesion in those on medication. Placental histological features most consistently associated with both the groups include, disturbances of villous maturation (DVM), Derangements in uteroplacental / foetoplacental circulation and villous capillary lesions. Small for gestational age and intrauterine foetal death were found in both the groups, but more commonly in patients with hyperglycemia on medication.

Conclusions: Villous maturation defects, uteroplacental / foetoplacental malperfusion are the essential placental changes which can result in adverse perinatal outcomes in women with hyperglycemia irrespective of the diabetic control.

Keywords: Gestational diabetes, Gestational hyperglycemia, Perinatal outcome, Placental malperfusion, Placental pathology

Introduction

India has the second largest diabetic population in the world after China,with 8.7% of the total population in the age group 20-70 years being diabetic.[1] A cross sectional study among 15 states showed an estimated prevalence of 7.3%.[2] Diabetes in pregnancy is a growing health concern among the antenatal mothers in India. A recent meta-analysis shows the pooled estimate for mean prevalence of gestational diabetes to be 10.1% with a rising trend from 0.53% to 27.3%.[3-5]

Gestational diabetes is defined as glucose intolerance with onset or first recognition during pregnancy and is associated with a multitude of maternal and foetal complications.[6] There is a higher incidence of spontaneous miscarriages, congenital malformations, macrosomia, intrauterine death, operative deliveries, birth injuries, neonatal respiratory distress, neonatal hypoglycaemia in diabetic pregnancies and increased incidence of type 2 diabetes in offspring. Maternal complications include a higher incidence of preeclampsia, polyhydramnios, obstructed labour, caesarean sections, postpartum haemorrhage and infections.

Placenta is the organ which serves as a means of communication at the maternal foetal interface, meanwhile acting as a selective barrier between both the circulations. Changes in the glycemic status are known to affect the placenta and the resulting histopathological findings are categorised based on the time of onset of hyperglycemia.[7,8] Earlier onset of altered glycemic profile results in structural alterations whereas later onset causes functional impairment. Common changes observed include villous immaturity, villous fibrinoid necrosis, chorangiosis, and increased angiogenesis.

Hence the placenta may be regarded as a route map guiding us in our understanding of the pathological basis of the foetal complications encountered in diabetic pregnancies.

We sought to determine the placental morphological changes in diabetic pregnancies and to correlate them with neonatal outcome.

M□□□□□□

In this retrospective study, 35 singleton placentas from mothers with altered/abnormal glucose profile were studied. Institutional ethical committee approval was obtained. The study materials were archived from the departments of Pathology, Obstetrics and gynaecology and from medical record department. The study period was between August 2016 to August 2018 (2 years).

Inclusion criteria

- Placentas from patients with the clinical history of gestational diabetes / hyperglycemia on diet and medication irrespective of associated comorbidities

Exclusion criteria

- Unfixed/poorly fixed placental specimen and Placentas with no clinical details will be excluded

Clinical data collected were patient's age, gestational age, obstetric score, co-morbidities and types of glucose intolerance. All the antenatal women were routinely screened for gestational diabetes during 24 to 28 weeks of gestation by the DIPSI OGCT (75 grams glucose load in non-fasting state followed by a 2-hour plasma glucose).[9] Women with 2 hour plasma glucose more than 140mg/dl were categorized as gestational diabetes. They were categorized into 2 groups: Mild gestational hyperglycemia (on diet), diabetes on medication (pregestational /gestational).

Perinatal factors observed were mode of delivery, pregnancy outcome [intrauterine foetal death (IUD), neonatal death], birth weight, Apgar scores and NICU (neonatal intensive care unit) admission. According to the birth weight, the babies are classified as small for gestational age/ foetal growth restriction (SGA/FGR), appropriate for gestational age (AGA) and large for gestational age (LGA).

The placental tissues were fixed in 10% buffered formalin. The gross parameters observed were trimmed disc weight, measurements, cord (color, length, diameter, number of vessels, twists, insertion site, true/false knots), membranes (color, insertion and rupture site), foetal surface, maternal surface and parenchyma (color, sponginess, location & size of any lesion, calcification, etc.).[10]

After taking photographs of the foetal and maternal surfaces, 2 bits were taken each from the membrane roll and umbilical cord. The placental parenchyma was then cut into thin slices with the interval of 1.0cm (bread loaf method). Picture of the parenchyma was taken for all the placentas. 3 bits were taken from the normal looking central parenchyma and few more from the lesional area (if present grossly). Haematoxylin and eosin stained slides were reviewed, and significant changes were noted.

Since it was a descriptive study with limited numbers, statistical analysis for comparison was not attempted.

□□□□□□□

The 35 cases were identified with hyperglycemia in pregnancy. There were 24 cases of mild gestational hyperglycemia controlled with diet and 11 cases of hyperglycemia on medication, which included gestational and pre gestational diabetes.

Mild gestational hyperglycemia on diet (n=24)

Clinical characteristics

The age distribution of the patients whose placentae were examined ranged from 23 to 40 years with the mean age of 30.83 years. The earliest gestational age was 25 weeks and the maximum was 40 weeks. Fourteen patients were primigravida and ten were multigravida. Half of the placentas were from preterm pregnancies and half from term pregnancies. Associated maternal conditions were analysed with few cases showing comorbidities such as oligohydramnios (n=3),hypertension /preeclampsia (n=2),

antiphospholipid antibody (n=2), anemia (n=2), abruption (n=2) and one each of familial hypercholesterolemia, placenta previa, polyhydramnios and hypothyroidism.

Foetal outcome

The birth weights of nine (37.5%) of the neonates were appropriate for gestational age (AGA). Among the remaining, 8 (33%) were small for gestational age (SGA), 3 (12.5%) were large for gestational age (LGA) and 4 (16.6%) of the pregnancies resulted in intrauterine foetal demise (IUFD). Table 1 highlights the perinatal outcome in both the groups.

Placental findings

Weight: Of the 24 studied placentas, 11 weighed less than the 10th centile (11/24). This is in correlation with the growth restriction in 6 foetuses (6/11) and IUD in 1 case (1/11). The remaining small placentas (4/11) were of foetuses with normal weights.

The weights of nine of the placentas(9/24) were in the normal range

Gestational and pre gestational diabetes on medication (n=11)

Clinical characteristics

The age distribution of the patients whose placentae were examined ranged from 25 to 42 years with the mean age of 32.9 years.

The earliest gestational age was 30 weeks and the maximum was 39 weeks. Four patients were primigravida and seven were multigravida. Three of the placentas were from term pregnancies and eight from preterm pregnancies. Associated maternal conditions included were three cases each of gestational hypertension/preeclampsia and hypothyroidism and one each of anemia and immune thrombocytopenic purpura.

Foetal outcome

Five (45%) of the neonates were SGA, 2(18%) were AGA and 1(9%) was LGA. The remaining 3 (27%) were IUFD (Table 1).

Table 1: Perinatal outcome in GDM on medication and GDM on diet

Perinatal outcome	SGA	AGA	LGA	IUFD
GDM on diet (n=24)	8	3	9	4
GDM on medication (n=11)	5	1	2	3

Placental findings

Weight: 7/11 placentas weighed less than 10th centile. Four of them were associated with small foetuses and 2 each were associated with AGA and IUD respectively.

Gross features

Table 2 highlights the gross placental findings including umbilical cord.

Microscopic findings

Table 3 displays the microscopic features of placenta in both the groups.

Apart from the tabulated findings, calcification, perivillous fibrin deposition, increased NRBCs, subchorionic cysts and mild focal villitis were also observed.

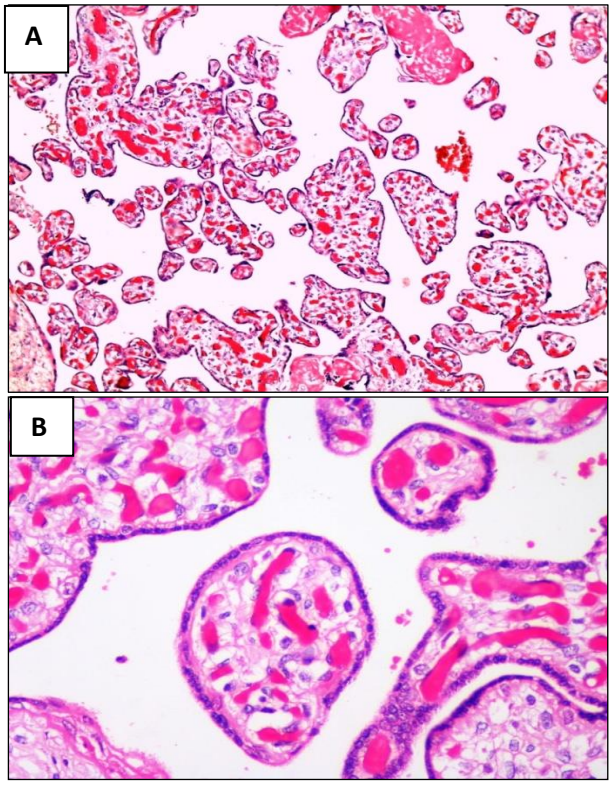
Table 2: Gross placental findings in GDM on medication and GDM on diet

Gross placental findings	GDM on diet (n=24)				GDM on medication (n=11)			
	SGA (n=8)	AGA (n=3)	LGA (n=9)	IUFD (n=4)	SGA (n=5)	AGA (n=1)	LGA (n=2)	IUFD (n=3)
Succenturiate lobe	1	-	-	1	-	-	-	-
Meconium staining	-	1	-	-	-	-	-	1
Subchorionic hemorrhage	-	-	-	-	-	-	-	1
Retroplacental hematoma	-	2	-	-	-	-	-	-
Marginal atrophy	1	3	-	1	-	1	1	2
Cord: 1) Hypercoiled	2	1	3	-	1	-	-	-
2) Hypocoiled	1	-	-	1	-	1	-	-
3) Velamentous insertion	1	-	-	1	-	-	-	-
4) Marginal insertion	2	-	-	-	-	-	-	-
5) Furcate cord	1	-	-	1	-	-	-	--
6) True knot	-	-	1	-	-	-	-	-

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	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
DVI (Fig .1)	5	4	2	2	4	2	0	1
Vascular ectasia	2	2	2	1	3	0	0	0
Villous capillary lesions (chorangiosis)	3	5	2	1	4	1	1	2
Chorangioma	0	1	1	0	0	0	0	0
MVM (infarcts+IVT)	4	6	0	0	5	0	0	2
FVM (Fig .2)	3	2	0	1	3	0	0	1
Laminar necrosis	2	4	1	1	0	0	1	0
A/C CAM (Fig.3)	1	1	1	1	0	1	0	0
Increased syncytial knotting	2	6	0	0	3	1	1	3
Increased cell islands	3	4	1	1	2	2	1	2
CHIV	0	0	0	1	0	0	0	0

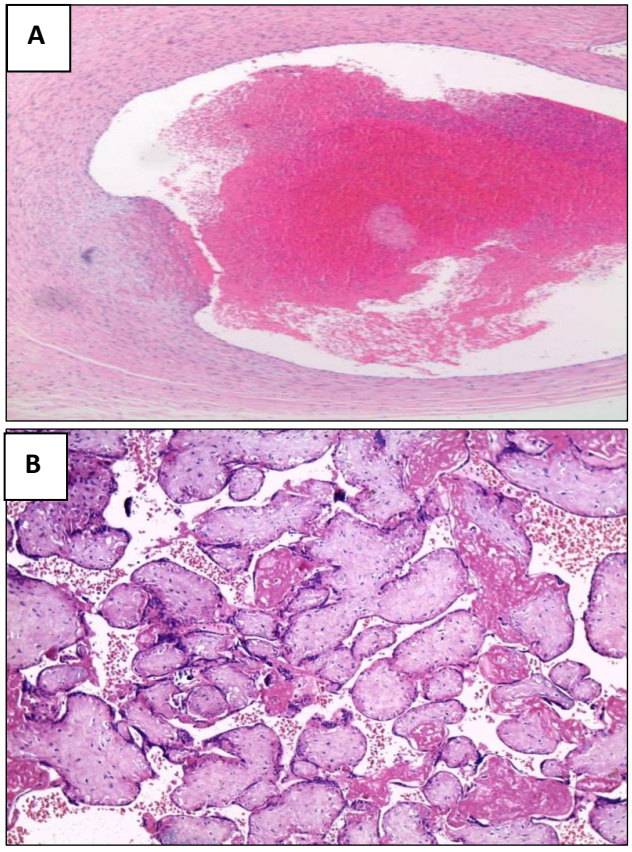
DVI, Distal villous immaturity; MVM,maternal vascular malperfusion; IVT, intervillousthrombus; FVM, foetal vascular malperfusion; A/C CAM, acutechorioamnionitis; CHIV, chronic histiocytic intervillositis



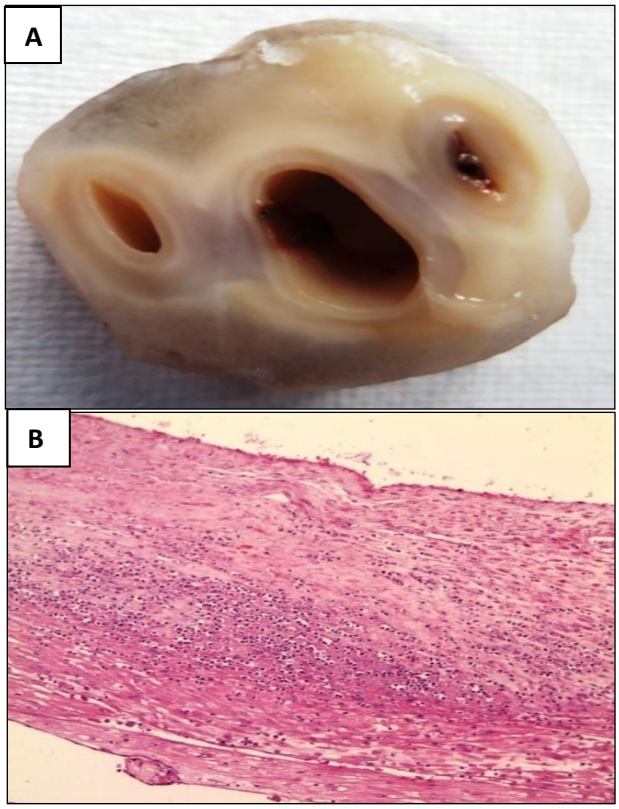
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In the present study, we analyzed the placental morphology and clinical outcome in pregnant women with mild hyperglycemia on diet and Gestational and pre-gestational diabetes on medication.



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There have been studies on placentas of diabetic women which have reported large babies and higher placental weights.[11-15] In contrast to that, we encountered more number of small placentas which belonged to small to normally grown babies and was comparable with Edu et al.[16]

Risk of foetal loss is increased in diabetic pregnancy irrespective of glycemic control.[17] Similarly, incidence of intrauterine death was higher in women in both the groups.

There was no significant increase in gross placental lesions among GDM on medication.[18] The cord abnormalities such as hypercoiling, velamentous/marginal insertion and furcate cord were observed more in women with GDM on diet.[19] Single umbilical artery is generally associated with gestational diabetes, but there were no such instances in our study.

Placental histologic changes

Disturbances of villous maturation (DVM)

DVM also known as distal villous immaturity were found in more than half of the cases in both groups. In contrast to the findings of Huynh et al, we did not find any differences between the two groups.[20]

Generally, in the last trimester of pregnancy, there is accelerated differentiation into terminal villi with increase in vasculosyncytial membrane formation. However, in pregnancies complicated by diabetes, this terminal differentiation is inhibited due to growth factor like activity of foetal insulin. Additionally, this is amplified by coexistent hypercoiling of the cord as seen in our study.[21]

As a result of defective villous maturation, there is reduced placental reserve leading to poor tolerance to acute hypoxia resulting in late intra uterine foetal loss. Since, this entity has a 10% risk of recurrence in subsequent pregnancies, it has been recommended to rescue the foetus by early delivery.[22]

Derangements in uteroplacental circulation/Maternal vascular malperfusion (MVM)

In our study, we observed greater incidence of features of MVM such as, infarcts, intervillous thrombus and increased syncytial knotting in both the groups. As described in literature, decidual vasculopathy was the prominent finding in women with coexistent preeclampsia/APLA as compared to isolated GDM.[23] Retroplacental hematoma with blood dissecting into the intraparenchymal villous space and subsequent haemorrhagic necrosis was also noted in few cases of GDM on diet.

Derangements in foetoplacental circulation/foetal vascular malperfusion (FVM)

FVM frequently goes undetected antenatally and is commonly diagnosed with pathologic examination of placenta. Among the multitude of factors associated with FVM, maternal hyperglycemia plays a significant role.[24]

Pregnancy per se is a thrombogenic state. When it is combined with maternal hyperglycemia (prothrombotic tendency) and hypercoiling of the cord (thereby causing vascular stasis and ischemia), it results in thrombosis in the foetal vascular tree, leading to foetal growth restriction and increased perinatal mortality and morbidity.[25,26]

The following findings were seen as part of FVM in our study population: chorionic plate/ stem villous vessel thrombi, vascular ectasia, groups of avascular, fibrotic to hyalinized villi and villous stromal karyorrhexis.

Villous capillary lesions

A gamut of villous capillary lesions encompassing chorangiosis, chorangiomatosis and chorangiomas believed to be caused by preplacental hypoxia.[27]

GDM in pregnancy is commonly associated with chorangiosis, which is hypothesized to be caused by insulin/IGF-1 receptor mediated angiogenesis.[25]

In our series, we predominantly encountered chorangiosis and an occasional case of microscopic chorangioma, but none of the cases showed chorangiomatosis.

Inflammatory lesions

Acute chorioamnionitis was detected in a few cases of maternal hyperglycemia, of which more than half were complicated by preterm labour /preterm rupture of membranes. Severe maternal inflammatory response and early foetal inflammatory response were noted.[28]

Mild chronic villitis and chronic histiocytic intervillositis (CHIV) were also observed in some cases. Both the lesions have strong association with maternal autoimmune diseases.

Chronic histiocytic intervillositis is an uncommon placental lesion, characterized by a dense lymphohistiocytic infiltrate confined to the intervillous space. CHIV has a tendency for causing recurrent pregnancy loss due to the presence of persistent increase in memory T cells in subsequent pregnancies.[29,30]

Subchorionic pseudocysts, increased cell islands, foetal nucleated red blood cells and vascular ectasia are a known histologic sign of hypoxic lesions and found more in cases of GDM on diet.[31]

The strengths of our study include that all the women were booked at our hospital, the investigations, follow up and delivery was in the same institution and there was no loss to follow up and hence no attrition of data.

The limitations of our study include, smaller sample size and retrospective nature. Not all placentas with GDM were sent for pathological examination. Only those cases with maternal clinical conditions warranting special attention and those with adverse perinatal outcomes were sent for examination.

To summarize, Villous maturation defects, uteroplacental / fetoplacental malperfusion and villous capillary lesions are the essential placental changes which can result in adverse perinatal outcomes in women with hyperglycemia irrespective of the diabetic control.Further prospective studies with large sample size including all GDM placentas with exclusion of confounding factors would help in better characterization of placental lesions in different levels of glycemic control. Understanding the placental pathogenesis will help to develop protocols for prevention and treatment strategies to improve perinatal outcomes.

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1. World Health Organization – Diabetes country profiles, 2016. Available at: https://www.who.int/diabetes/country-profiles/ind_en.pdf
2. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK, et al. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR–INDIAB population-based cross-sectional study. lancet Diabe Endocrinol. 2017;5(8):585-96.
3. Nguyen CL, Pham NM, Binns CW, Duong DV, Lee AH. Prevalence of gestational diabetes mellitus in eastern and southeastern Asia: a systematic review and meta-analysis. J Diabet Res. 2018;6536974:1-10.
4. Ramachandran A, Snehalatha C, Shyamala P, Vijay V, Viswanathan M. Prevalence of diabetes in pregnant women- a study from southern India. Diabet Res Clinic Pract. 1994;25(1):71-4.
5. Nallaperumal S, Bhavadharini B, Mahalakshmi MM, Maheswari K, Jalaja R, Moses A, et al. Comparison of the world health organization and the International association of diabetes and pregnancy study groups criteria in diagnosing gestational diabetes mellitus in South Indians. Ind J Endocrinol Metabol. 2013;17(5):906.
6. Government of India. Maternal and Health Division, Diagnosis and Management of Gestational Diabetes Mellitus: Technical and Operational Guidelines. New Delhi, India: Ministry of Health & Family Welfare, New Concept Inform Syst; 2018.
7. Jarmuzek P, Wielgos M, Bomba-Opon D. Placental pathologic changes in gestational diabetes mellitus. Neuro Endocrinol Lett. 2015;36(2):101-5.
8. Gabbay-Benziv R, Baschat AA. Gestational diabetes as one of the great obstetrical syndromes–the maternal, placental, and fetal dialog. Best Pract Res Clinic Obstetr Gynaecol. 2015;29(2):150-5.
9. Seshiah V, Das AK, Balaji V, Joshi SR, Parikh MN, Gupta S. Gestational diabetes mellitus-guidelines. JAPI. 2006;54:622.
10. Benirschke K, Burton GJ, Baergen RN. Early development of the human placenta. In Pathology of the human placenta 2012:41-53. Springer, Berlin, Heidelberg.
11. Singer DB. The placenta in pregnancies complicated by diabetes mellitus. Perspect Pediatr Pathol. 1984 Fall;8(3):199-212.
12. Ramos A, Caimari F, Pujol IM, García-Patterson A, Ginovart G, Adelantado JM, Corcoy R. In women with gestational diabetes mellitus factors influencing growth have a larger effect on placental weight than on birth weight. Euro J Obstetr Gynecol Reproduc Biol. 2016;202:60-5.
13. Kucuk M, Doymaz F. Placental weight and placental weight-to-birth weight ratio are increased in diet-and exercise-treated gestational diabetes mellitus subjects

but not in subjects with one abnormal value on 100-g oral glucose tolerance test. J Diabet its Complicat. 2009;23(1):25-31.
14. Taricco E, Radaelli T, De Santis MN, Cetin I. Foetal and placental weights in relation to maternal characteristics in gestational diabetes. Placenta. 2003;24(4):343-7.
15. Baptiste-Roberts K, Salafia CM, Nicholson WK, Duggan A, Wang NY, Brancati FL. Maternal risk factors for abnormal placental growth: the national collaborative perinatal project. BMC pregnancy and childbirth. 2008;8(1):44.
16. Edu A, Teodorescu C, Dobjanschi CG, Socol ZZ, Teodorescu V, Matei A, Albu DF, Radulian G. Placenta changes in pregnancy with gestational diabetes. Rom J Morphol Embryol. 2016;57(2):507-12.
17. Stillbirth Collaborative Research Network Writing Group. Association between stillbirth and risk factors known at pregnancy confirmation. JAMA: J Am Med Assoc. 2011;306(22).
18. Fox H. Pathology of the placenta in maternal diabetes mellitus. Obstetr Gynecol. 1969;34(6):792-8.
19. Ezimokhai M, Rizk DE, Thomas L. Maternal risk factors for abnormal vascular coiling of the umbilical cord. Am J perinatol. 2000;17(08):441-6.
20. Huynh J, Yamada J, Beauharnais C, Wenger JB, Thadhani RI, Wexler D, Roberts DJ, Bentley-Lewis R. Type 1, type 2 and gestational diabetes mellitus differentially impact placental pathologic characteristics of uteroplacental malperfusion. Placenta. 2015;36(10):1161-6.
21. Redline RW. Distal villous immaturity. MiniSymposium: Placental and Trophoblastic pathology. Diagnost Histopathol. 2012;18(5):189-94.
22. Paciencia M, Dolley P, Jeanne-Pasquier C, Jacob B, Sadfi A, Leseigneur P, et al. Acute-placental dysfunction by villous-maturation defect and late-fetal mortality. J Gynecol Obstet Biol Reprod. 2008;37(6):602-7.
23. Kovo M, Granot Y, Schreiber L, Divon M, Ben-Haroush A, Bar J. Pregnancy outcome and placental pathology differences in term gestational diabetes with and without hypertensive disorders. J Maternal-Fetal Neonat Med. 2016;29(9):1462-7.
24. Redline RW, Ravishankar S. Fetal vascular malperfusion, an update. Apmis. 2018;126(7):561-9.
25. Raymond W. Redline, Theonia K. Boyd, Drucilla J. Roberts. Diagnostic Pediatric Pathology: Placental and Gestational Pathol. Cambridge Uni Press; 2018:93-102.
26. Gorar S, Alioglu B, Ademoglu E, Uyar S, Bekdemir H, Candan Z, et al. Is there a tendency for thrombosis in gestational diabetes mellitus?. J Lab Physici. 2016;8(2):101.
27. Ogino S, Redline RW. Villous capillary lesions of the placenta: distinctions between chorangioma, chorangiomatosis, and Chorangiosis. Human Pathol. 2000;31(8):945-54.
28. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Development Pathol. 2003;6(5):435-48.
29. Boyd TK, Redline RW. Chronic histiocytic intervillositis: a placental lesion associated with recurrent reproductive loss. Human pathol. 2000;31(11):1389-96.
30. Ramya T, Chaitra V, Umamaheswari G. Chronic histiocytic intervillositis a rare placental cause of poor obstetric outcome: a clinicopathological study and literature review. Int J Reprod Contracept Obstet Gynecol. 2014 Dec;3(4):1146-1149.
31. Stanek J, Weng E. Microscopic chorionic pseudocysts in placental membranes: a histologic lesion of in utero hypoxia. Pediatr Development Pathol. 2007;10(3):192-8.

Natarajan L, Maheswari GU. Gestational hyperglycemia on diet and medication: impact on placental pathology and pregnancy outcomes. Int J Reprod Contracept Obstet Gynecol 2019;8:3350-6.

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Sir,

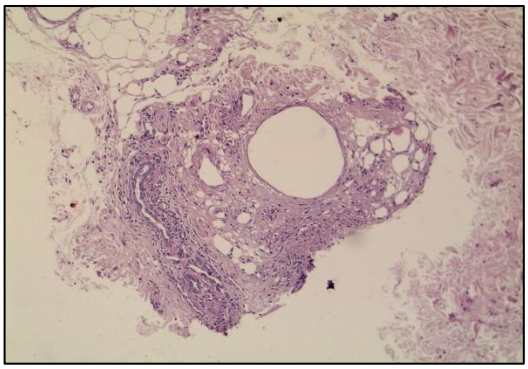
Idiopathic localized involutional lipoatrophy (ILIL) is a rare form of localized lipoatrophy that arises spontaneously without inflammation.

A 6 year old boy presented with depressed skin lesion involving trunk since 3 years. No history of previous trauma, drug intake, injections or prior inflammatory conditions. On examination, well demarcated, non-tender, non-indurated, depressed pigmented plaque seen over centre of abdomen of size 10x7 cm, right side of chest of size 5x4 cm (Figure 1) and over back of size 6x4 cm. A differential diagnosis of morphea and lipoatrophy was made. Routine hematological, biochemical investigations, fasting blood sugar, serum triglycerides, serum TSH and ANA were normal. Ultrasonography of depressed skin lesions showed thinning of subcutaneous plane with increased echoes in right posterior thorax.



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Biopsy from the depressed lesion showed epidermis exhibiting basket weave keratin, mild papillomatosis and acanthosis. Papillary and upper dermis showed mild perivascular lymphocytic infiltrate with occasional eosinophils. Dermal collagen fibres are normal, pilo sebaceous gland appear small with other adnexal structures. There is loss of subcutis with shrunken fat globules, within the lobule, adipocytes appear reduced in size, number, with some dilated fat spaces and many tiny adipocytes. Fibrosis, mild infiltrate of lymphocytes, histiocytes, foamy macrophages observed around fat (Figure 2). As there was no history suggestive of preceeding inflammatory process, normal laboratory findings and classical histopathological involutional changes, a final diagnosis of idiopathic localized involutional lipoatrophy was made.



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ILIL is characterized by selective loss of subcutaneous fat without exudative reaction or fibrosis. It is more common in young females, usually solitary and very few childhood cases have been reported. Pathogenesis is unclear and literature suggests impairment of adipocyte differentiation, adipocyte apoptosis, and mitochondrial dysfunction.[1] Activated macrophages trigger immunologic disorders by secreting variety of cytokines such as fibroblast growth factor-2, platelet-derived growth factor, interleukin-1, tumor necrosis factor-α, and transforming growth factor-β, resulting in involution of subcutaneous fat tissue.[2]

Clinically localized involutional lipoatrophy, presents as an asymptomatic, well demarcated, localized, non-inflamed atrophic depression affecting only one site and commonly involve arms, buttocks, thighs and abdomen. In our case multiple sites were involved. Histologically as seen in our case, ILIL lesions show well developed involutional changes, exhibiting shrunken fat lobules with retraction of whole fat lobules from surrounding collagenous septa. Within each affected fat lobule, lipocytes are shrunken and retracted resembling embryonic fat tissue.[3,4] ILIL was differentiated from morphea which exhibits pronounced inflammatory infiltrate in subcutaneous fat, and the trabeculae dividing the subcutaneous fat are thickened because of inflammation and deposition of new Collagen.[4] Majority

of cases resolve spontaneously. Anti-inflammatory agents such as steroids, calcineurin inhibitors, and antimalarials can be used in the initial phase when the lesion is expanding.[5] In long-standing lesions, localized fat transplantation may be performed.

We report this case for its rarity, as the child presented with ILIL involving multiple sites. Although skin lesions can resolve spontaneously without treatment, currently in our case the lesions persisted without progression.

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1. Giralt M, Domingo P, Villarroya F. Adipose tissue biology and HIV-infection. Best Pract Res Clin Endocrinol Metab. 2011; 25(3):487-99.
2. Sharma RK, Gupta M, Negi L. Idiopathic Localized Involutional Lipoatrophy: A Retrospective Study of 12 Cases. Indian Dermatol Online J. 2019;10(2):149-52.
3. Fung MA, Requena L. Inflammatory diseases of subcutaneous fat. In: Elenitsas R, Rosenbach M, Murphy GF, Rubin AI, Xu X, editors. Lever's histopathology of the skin. 11th ed. Wolters Kluwer: Philadelphia. 2015;610-58.
4. Chang CH, Fang KT, Hong SJ. Morphea-like localized involutional lipoatrophy—a case report associated with family history. Dermatol Sinica. 2010;28:113-6.
5. Pai K, Pai S. Localised involutional lipoatrophy: a case report. Our Dermatol Online. 2013;4(3):356-7.

Nithya VPS, Rai R, Umamaheswari G. Idiopathic localised involutional lipoatrophy in a child involving multiple sites. Int J Res Dermatol 2021;7:753-4.

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Cystic adrenal lymphangioma

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An 18 year old female presented with vaginal bleeding at 2 months of gestation. Ultrasonography revealed a large retroperitoneal cyst. Histopathological examination of the excised cyst showed features suggestive of a cystic adrenal lymphangioma. This case is reported because of its rarity and detection during pregnancy.

Keywords: Retroperitoneal cyst, adrenal cystic lymphangioma

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Introduction

Adrenal lymphangioma is a rare and benign lesion most often found incidentally during abdominal imaging, surgery or autopsy.

Case report

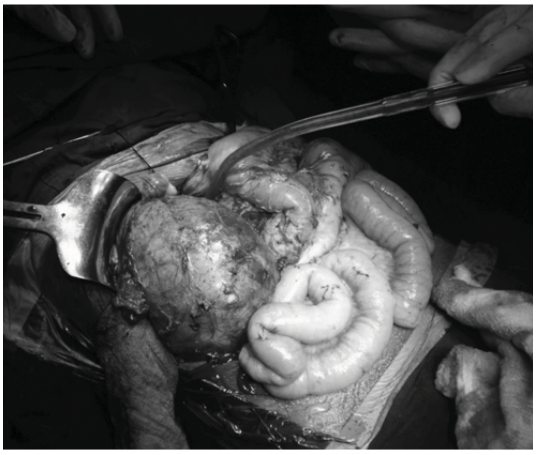
An 18-year old female presented with vaginal bleeding at 2 months of gestation (i.e. at 8 weeks of pregnancy). Abdominal ultrasonography was done to check for the status of embryo. The uterine cavity was empty suggesting a diagnosis of missed abortion. However a retroperitoneal cyst was found incidentally while doing ultrasonography. Computed tomography confirmed the presence of retroperitoneal cyst, which measured 16 x 10 x 10 cms and was seen displacing the right kidney. Surgical excision of the cyst was done 3 months after initial evaluation of the retroperitoneal mass by USG and CT examination.

On exploratory laparotomy, the cyst was identified in the retroperitoneal location and was found to contain straw colored fluid (Figure 1). The cyst was excised and sent for histopathological examination.

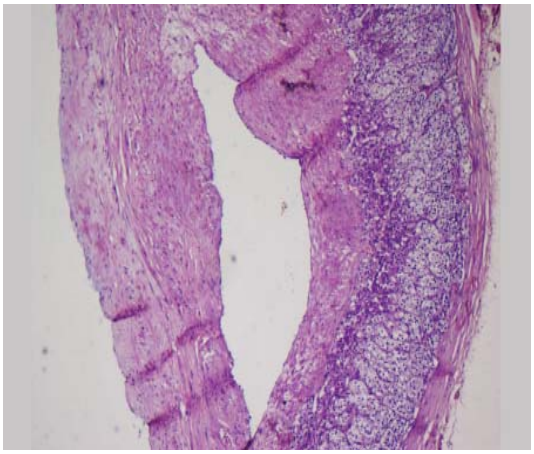
Gross examination showed a grey-white cystic mass weighing 15 grams with yellowish areas admixed with congested areas. The cut surface showed focally thickened areas with nodular yellow tissue. Histologically the cyst was partially lined by endothelial cells overlying fibro-collagenous tissue with areas of hyalinization, large dilated lymphatic channels, with smooth muscle hyperplasia, foci of ceroid laden macrophages and islands of compressed adrenal tissue in the wall (Figure 2). The endothelial cells were immunoreactive for CD34. A diagnosis of adrenal cystic lymphangioma was made.

Discussion

Adrenal cysts are usually asymptomatic and are discovered incidentally at autopsy[1]. Currently, due to increased use of imaging, increased numbers of adrenal cysts are reported[1]. They occur at all ages, with a peak in the 3rd to 6th decades, a definite female preponderance, and bilaterality in 10% of cases[1].



□□□□□ **1**□ Intra-operative picture: cystic mass mobilized below liver.



□□□□□**2**□ Photomicrograph of cyst lined by endothelium and wall showing compressed adrenal tissue (H&E stain, 40X magnification).

Small adrenal cysts are clinically silent. Large ones may cause abdominal discomfort, loin pain, gastro-intestinal disturbances or acute abdomen due to intracystic hemorrhage, rupture or infection. Peripheral and curvilinear calcification is present in 15% of adrenal cysts[1].

Adrenal cysts are histologically classified as parasitic, epithelial, endothelial or pseudocysts; with endothelial cysts being the most common[1]. They can be lymphangiomatous or angiomatous in origin.

Adrenal lymphangioma is a benign cystic tumor composed of dilated lymphatic vessels with a lymphoid stroma and a smooth endothelial lining[2]. It results from faulty development or ectasia of lymphatic vessels[2]. The cyst may contain serous or chylous fluid.

In our case, the patient presented with vaginal bleeding and the cyst was large. The diagnosis of cystic lymphangioma was made only after histopathological examination of the cyst.

The treatment of adrenal cysts depends on symptoms, size and complications. Malignant transformation is extremely rare[3]. The prognosis is generally good after removal of the cyst[3].

1. Khoda J, Hertzanu Y, Sebbag G, Lantsberg L, Barky Y. Adrenal cysts: Diagnosis and therapeutic approach. Int. Surg. 1993; Jul-Sep;78(3):239-42.
2. Trojan J, Schwarz W, Zeuzem S, Dietrich CF. Cystic adrenal lymphangioma: Incidental diagnosis on abdominal sonography. AJR Am.J.Roentgenol. 2000; Apr;174(4):1164-5.
3. Guinier D, Denue PO, Mantion GA. Intra-abdominal cystic lymphangioma. Am.J.Surg. 2006; May;191(5):706-7.

Case report

Intradiploic epidermoid cyst - a pearl within the skull

V Lakshmi[1], KS Thirumurthy[2], S Rabia[1], V Chaitra[1]

Introduction

Epidermoid cysts are quite uncommon in the craniofacial region and more so in the intradiploic region within the skull. They are benign and slow-growing. Some attain great size, producing major neurological signs. A high index of suspicion with an accurate radiological assessment followed by complete removal of the tumour and its capsule are essential for good long-term prognosis. We report an interesting case of an epidermoid cyst occurring in the skull of a young male patient with a short review of the relevant literature.

Case report

A 31-year-old male patient presented to the outpatient unit of the neurosurgery department with complaints of giddiness. The symptoms were present intermittently and no relevant past medical history or family history could be elicited.

On examination, the patient had a GCS (Glasgow Coma Scale) of 15/15 and his vital signs were stable. Computed tomographic scan (CT Scan) showed an intradiploic expansile cystic lesion with a sclerotic rim in the left temporo-parietal region. The patient underwent craniotomy for excision of the cyst. A small piece of the lesion was sent for frozen section study, which showed anucleate squames and keratin debris. The cyst was excised and the residual bone defect was covered with a titanium mesh, fixed with titanium screws. Bone cement was applied to solidify. Post-operatively, the patient remained stable. A course of antibiotics and anticonvulsants were prescribed.

The specimen was sent to the histopathology laboratory in 10% neutral buffered formalin. An elliptical piece of bone with the inner surface showing a fleshy mass was received. Cut surface of the growth appeared friable with gritty areas. (Fig. 1)

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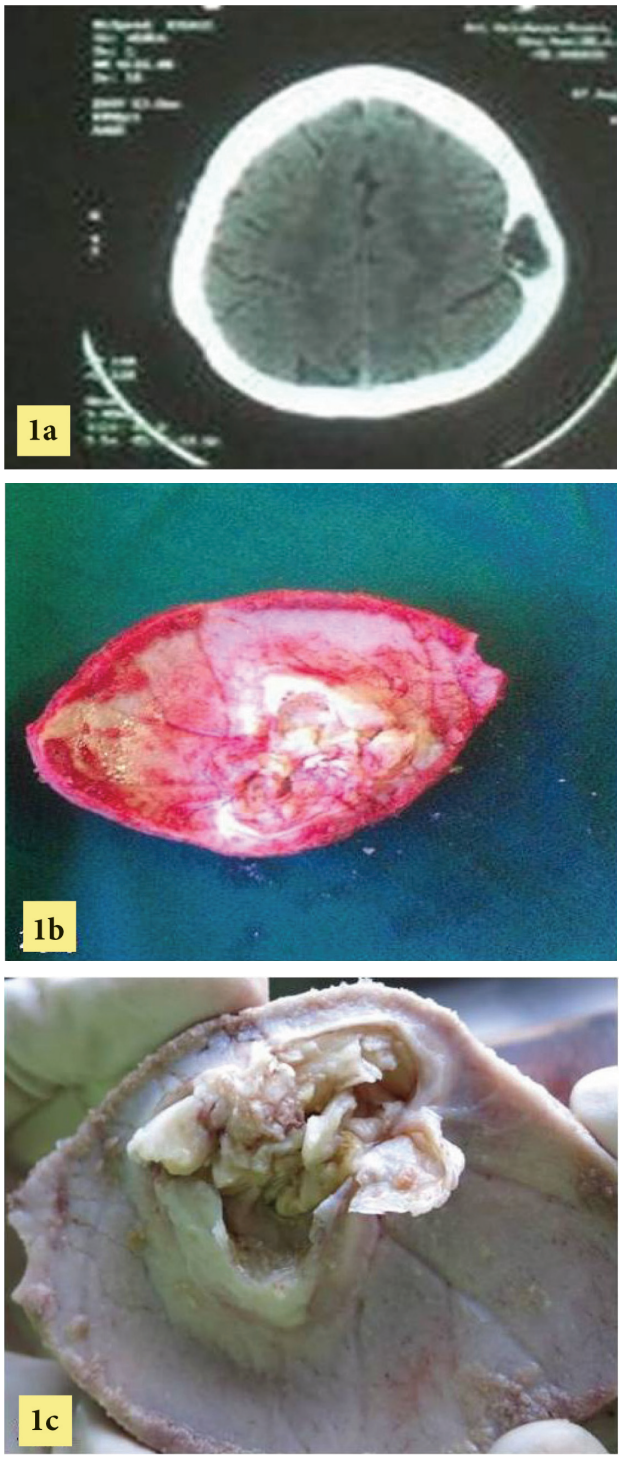


Fig. 1. 1a. Computed tomographic (CT) scan showing an intradiploic expansile cystic lesion with a sclerotic rim in the left tempero-parietal region. 1b. Complete excision of cyst with cranioplasty. 1c. Gross image of inner surface of skull showing a fleshy mass causing ulceration of the dura.

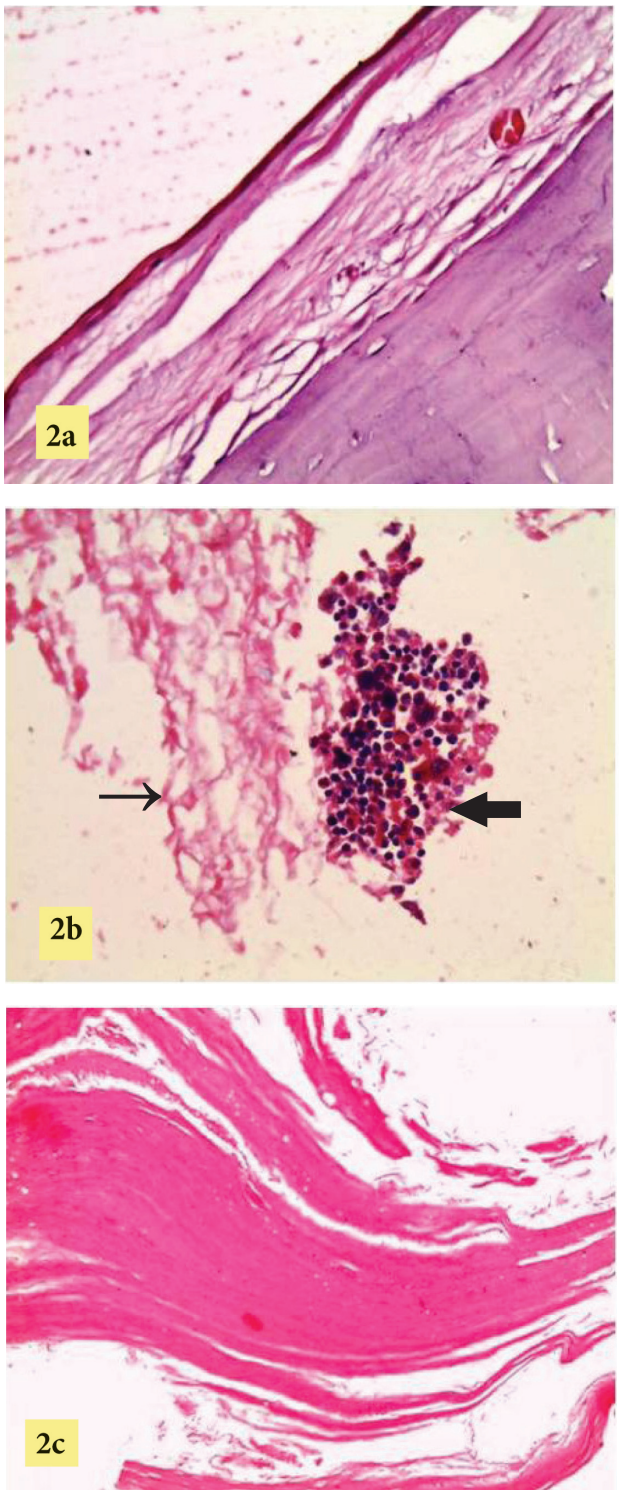


Fig.2. 2a Degenerated bony spicules with a cyst lined by stratified squamous epithelium H&Ex400. 2b. Anucleate squames (arrow) and hematopoietic elements (block arrow). 2c. Keratin flakes; H&E x400.

On histological examination, degenerated fragments of bone were seen with a lesion composed of a lining stratified squamous epithelium overlying lamellated flakes of keratin, keratinous debris and occasional anucleate squames. (Fig.2) Other adenexal structures were not seen. On follow up the patient showed a significant improvement of his symptoms.

Discussion

Epidermoid cysts are common in routine histological practice and they are usually located in hair-bearing areas of the skin. Location within the cranial cavity are rare. They represent 0.04 to 0.7% of intracranial neoplasms (1). Intradiploic dermoid cyst was first described by Cushing in 1922.

During neural tube formation, sequestration of ectodermal elements can be seen within the cranial cavity due to defective separation of the neuroectoderm (2). This results in the formation of epidermoid cysts which can remain asymptomatic even after attaining a large size. The presence of cheesy, keratinous debris within the cyst lumina imparts a "pearly" appearance and hence these tumours have also been referred to as "pearly tumours" in literature (3). The intradiploic location is far less common than the intradural location (4).

CT imaging (5) is of greater value than plain radiography as CT allows better assessment of both skull involvement as well as intracranial extension. Intraparenchymal extension of intradiploic epidermoids is known to occur. Imaging appearance depends on the ratio of keratin to cholesterol in the lesion. Typically epidermoids are hypodense on CT.

Although they remain asymptomatic for long periods, erosion of the bone and involvement of the brain parenchyma can occur. Rupture of the cyst with release of contents can occur into the subarachanoid space. Chronic granulomatous arachnoiditis can result from irritation of the meninges due to released keratin.

Epidermoid cysts are mostly seen in the third and fourth decades of life with a male predominance. The lesions may extend and present as a subcutaneous swelling of the scalp. In contrast, dermoid cysts are typically associated with the suture lines, commonly occur in orbital region and in the midline and usually present in childhood. (6). Histology of an epidermoid cysts shows a keratin-filled cyst lined by stratified squamous epithelium (7). The cyst wall characteristically lacks dermal elements.

Other differential diagnoses of epidermoid intradiploic cysts include bone-destroying lesions affecting the orbit, eosinophilic granulomas, giant cell granulomas, cholesterol granulomas, cavernous hemangioma, and osteolytic intradiploic metastases (8,9).

Giant extradural epidermoids with profound deformation of the brain and extensive lytic skull lesions may allow a normal life without any significant restrictions (10). Individual risk-benefit assesment is mandatory in the discussion of a specific treatment. In our case, the surgical excision was done because the patient was symptomatic and the size of the lesion was significant, causing compressive symptoms.

We report this case because of its characteristic radiological and histopathological appearance and stress the importance of considering this entity in the differential diagnosis of skull lesions.

References

1. Vega R.A., Hidlay D.T., Tye G.W., Fuller C.E., Rhodes J.L., Intradiploic Dermoid Cyst of the Lateral Frontotemporal Skull: Case Report and Review of the Literature. *Pediatric Neurosurgery* 2013; 49:232-235

2. Mehra S, Chandra GU, Kumar S. Intradiploic epidermoid tumor of temporal bone X-ray, CT, MR Imaging. *Indian Journal of Otolarygology and Head and Neck Surgery* [serial online] 2012 [cited 2014 Nov 11]; 18:98-102.

3. S. Gaivas, D. Rotariu, G. Dumitrescu, B. Iliescu. Intradiploic epidermoid cyst of the skull. Case report. *Romanian Neurosurgery* XVIII (2) 2011.

4. Brandon Miner, DO; Paul Aanderud; Steven K. Grekin. Giant Intradiploic Epidermoid Cyst of the Skull: A Case Report and Management of Large Cystic Scalp Masses. *Cosmetic Dermatology* 2009, vol. 22(7)

5. Shibani Mehra, Garga Umesh Chandra, Suresh Kumar. Intradiploic epidermoid tumor of temporal bone X-ray, CT, MR Imaging. *Indian Journal of Otolaryngology and Head and Neck Surgery* 2012; 18:98-102.

6. Narlawar R S, Nagar A, Hira P, Raut A A. Intradiploic epidermoid cyst. *Journal of Postgraduate Medicine* 2002;48:213

7. Bikmaz K, Cosar M, Bek S, Gokduman CA, Arslan M, Iplikcioglu. Intradiploic epidermoid cysts of the skull: a report of four cases. *Clinical Neurology and Neurosurgery.* 2005 Apr;107(3):262-7

8. Aldo F. Berti, MD, FACS, FAAP, Gregory C. Lovaas, MD, FACS, Alejandro Santillan. Primary Intradiploic Pterional Epidermoid Cyst. *Southern Medical Journal.* 2010; 103(1):87-89.

9. Russel DS, Rubinstein LJ. Tumors and tumorlike conditions of maldevelopmental origin. Russel DS Rubeinstein LJ, editors. Pathology of tumors of nervous system. 5 th ed. London: Edward Arnold; 1989.p. 693-5.

10. Wolfgang Krupp, Alexander Heckert, Heidrun Holland. Giant intradiploic epidermoid cyst with large osteolytic lesions of the skull: a case report. *Journal of Medical Case Reports* 2012, 6:85

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RESEARCH ARTICLE

THE ROLE OF BODY MASS INDEX (BMI) AS AN INDEPENDENT RISK FACTOR AND PROGNOSTIC FACTOR IN BREAST CANCER – A HOSPITAL BASED EPIDEMIOLOGICAL STUDY

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ABSTRACT

Objectives: To study the role of Body Mass Index as an independent risk factor and prognostic factor in breast cancer.

Methods: A hospital based epidemiological study was conducted in a tertiary care hospital in India involving 50 patients with a diagnosis of breast cancer who visited the Medical Oncology outpatient department for a period of 3 months. 50 age matched controls who had no detectable breast cancers were selected as controls. Their body mass index was calculated. Data including stage of breast cancer, lymph node status and presence of metastasis, histological type, grade of the tumour and hormone receptor status. (ER, PR and Her 2 neu) were tabulated. Additional information regarding menstrual, menopausal status, co-morbidities like Diabetes mellitus, hypertension and epilepsy was also gathered.

Results: The mean age of patients was 53.8 years and that of controls was 49.08 years. 56 % of cases and 44% of controls were post menopausal. 20 patients and 10 controls were diabetic; 14 patients and 9 controls were hypertensive and 2 patients and 2 controls had hypothyroidism. The odds ratio which quantifies the risk of association of breast cancer and obesity is 0.339 in premenopausal women and 0.045 in post menopausal women. The number of triple negative breast cancers was more in I and II classes of body mass index. Obesity grade 2 and 3 were also associated with advanced disease, including larger tumor size, positive lymph nodes, regional and/or distant stage and deaths after breast cancer.

Conclusion: There is a statistically significant association between obesity and breast cancer in post menopausal women but the same is not applicable in pre menopausal women.

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INTRODUCTION

Obesity is increasingly becoming a major public health problem in developing countries. It is defined as an accumulation of adipose tissue that is of sufficient magnitude to impair health. Excess weight is best assessed by body mass index or BMI, which is a surrogate marker for underlying obesity. The normal BMI range is 18.5 to 25 kg/m^2 (Torre *et al*., 2015). According to World Health Organization (WHO) people can be divided into the following categories based on BMI. Approximately 7% of cancers in women are associated with obesity (van den Brandt *et al*., 2000). In women, a BMI greater than 25kg/m^2 correlated strongly with an increased incidence of carcinomas of esophagus, endometrium, gall bladder, breast and kidneys. A proposed hypothesis for such an association is explained by the presence of hyperinsulinemia and peripheral insulin resistance in obese individuals (Roberts *et al*., 2010). Insulin at high concentrations has multiple effects on cell growth.

i. Activation of phosphatidyl inositol 3 kinase, β catenin and RAS
ii. Hyperinsulinemia causes increase in insulin like growth factor 1, which is a mitogenic and anti apoptotic agent, which activates many of the cell growth pathways.
iii. Obesity increases the synthesis of estrogen from androgen precursors through an effect on adipose tissue aromatases.
iv. Insulin increases androgen synthesis in ovaries and enhances estrogen availability in obese persons by inhibiting production of sex hormone binding globulin in liver.

BMI	Classification
< 18.5	underweight
18.5–24.9	normal weight
25.0–29.9	overweight
30.0–34.9	class I obesity
35.0–39.9	class II obesity
≥ 40.0	class III obesity

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Obese women under the age of 40 have a decreased risk as a result of anovulatory cycles and lower progesterone levels. In contrast, post menopausal obese women have an increased risk, which is attributed to the synthesis of estrogen in fat deposits (Schmidt *et al*., 2015).

MATERIALS AND METHODS

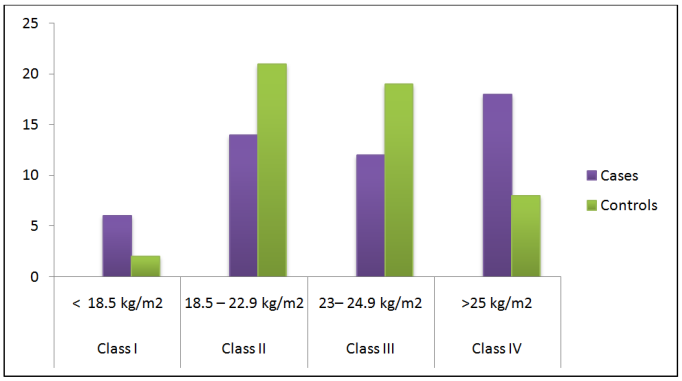
A case control study was carried out at our tertiary care hospital. This was a single institution study drawn from patients who attended the medical oncology outpatient department over a period of three months. The women accompanying the patients and a few voluntary women who had no detectable breast cancers were selected as controls. The study protocol was approved by the Institutional Human Ethics Committee. Informed consent was obtained from every patient and control and the patients were asked to enter the details provided in a questionnaire. Results of their heights and weights were entered. As a relative indicator of body weight, Body Mass Index was calculated as weight in kilograms divided by square of height in meters. The patients and controls were categorized into 4 classes based on BMI.

Class I – BMI < 18.5 kg/m^2
Class II – BMI 18.5 – 22.9 kg/m^2
Class III – BMI 23 – 24.9 kg/m^2
Class IV – BMI > 25 kg/m^2

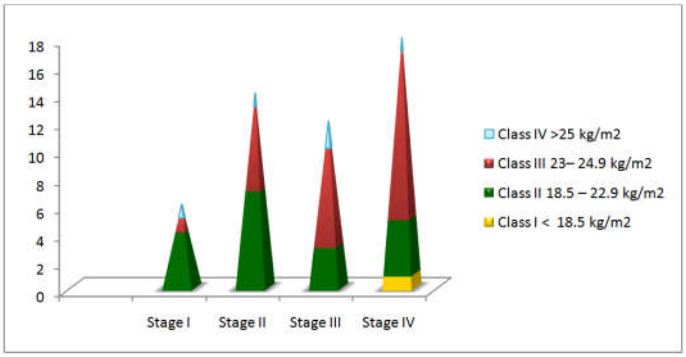
Data including stage of breast cancer, lymph node status and presence of metastasis was obtained from the case records of the patient. The histopathology reports were analyzed for recording the histological type, grade of the tumour and hormone receptor status (ER, PR and Her 2 neu).

Additional information regarding menstrual, menopausal status, co-morbidities like Diabetes mellitus, hypertension and epilepsy was also gathered. The results were tabulated and analyzed using SPSS (Statistical Package for the Social Sciences). The relationship between body mass index and risk of breast cancer was evaluated using odds ratio and the p value was calculated.

Bar diagram showing the distribution of cases and controls among different classes of body mass index



Bar diagram depicting the relation between stages of breast cancer and classes of body mass index



Body mass index in premenopausal women – Cross tabulation

Group	BMI ≤ 24.9 kg/m^2	BMI > 25.0 kg/m^2	P value	Confidence interval	Odds ratio
Cases	15 (68.2%)	7 (31.8%)			
Controls	23 (82.1%)	5 (17.9%)	0.23	0.131-0.877	.339

Table 1. Age distribution of cases and controls in various classes of BMI

Age Range	Class I < 18.5 kg/m^2		Class II 18.5 – 22.9 kg/m^2		Class III 23– 24.9 kg/m^2		Class IV >25 kg/m^2	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
21-30	0	0	0	1	0	1	0	0
31-40	1	0	1	6	2	5	2	3
41-50	1	1	4	6	1	2	5	2
51-60	2	1	5	4	4	6	9	1
61-70	1	0	0	3	5	4	2	3
71-80	1	0	3	0	0	0	0	0
>80	0	0	0	2	0	0	0	0

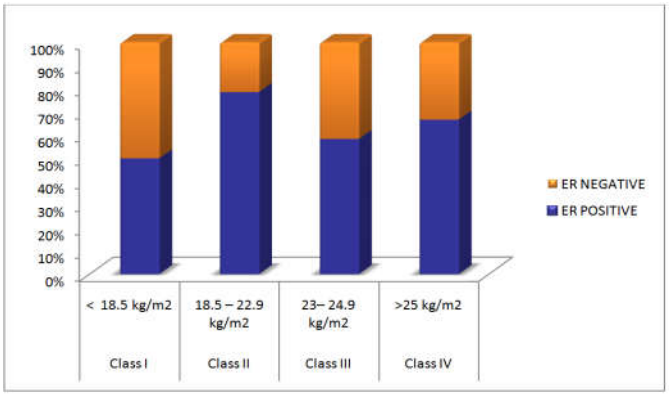
RESULTS AND OBSERVATION

50 women diagnosed with breast cancer during a period of 3 months were taken as cases and 50 women without a history of breast cancer were taken as controls. The mean age of patients was 53.8 years and that of controls was 49.08 years. 56 % of cases and 44% of controls were post menopausal. 20 patients and 10 controls were diabetic; 14 patients and 9 controls were hypertensive and 2 patients and 2 controls had hypothyroidism.

Body mass index in post menopausal women – Cross tabulation

Group	BMI ≤ 24.9 kg/m^2	BMI > 25.0 kg/m^2	P value	Confidence interval	Odds ratio
Cases	17 (60.7%)	11 (39.3%)			
Controls	19 (86.4%)	3 (13.6%)	0.045	0.058-1.024	.045

Histogram showing estrogen receptor expression in various classes of BMI



The odds ratio which quantifies the risk of association of breast cancer and obesity is .339 in premenopausal women and .045 in post menopausal women.

DISCUSSION

Overweight and obesity are current escalating public health issues. The prevalence of obesity in females has accelerated over the past decade in many developing countries. Obesity is an established risk factor for cancer and cancer related deaths, including that of breast cancers. Several studies show that a significantly stronger association is obvious between increased body mass index (BMI) and higher breast cancer incidence. Increased levels of estrogens due to excessive aromatization activity of the adipose tissue, over expression of pro-inflammatory cytokines, insulin resistance and hyper activation of insulin-like growth factors (IGFs) pathways, adipocyte-derived adipokines, hypercholesterolemia and excessive oxidative stress contribute to the development of breast cancer in obese women. Lopez *et al*. (2017) conducted a cross-sectional study of 849 women diagnosed with breast cancer in Columbian patients. Participant's body weight, height, and body mass index were collected at the time of diagnosis. Patients were classified following the Center for Disease control's (CDC) criteria: underweight (BMI $<$ 18.5 kg/m2), normal (18.5 $\leq$ BMI $<$ 25 kg/m2), overweight (25 $\leq$ BMI $<$ 30 kg/m2), and obese (BMI $\geq$ 30 kg/m2). Their data revealed that an association between BMI and menopausal status was not statistically significant in the study population. In the current study, the odds ratio of association was .339 with a p value of 0.23 in premenopausal women, which is not significant. However, the odds ratio in post menopausal women was .045 with a p value of .045 which is significant. Obese breast cancer patients are often associated with advanced stage disease, larger tumors, and axillary lymph node-positive status, partly due to delayed diagnoses and technical difficulties in palpation of the tumors. In a study conducted by Turkos *et al*. (2013), obese patients compared with normal-weight women were older at diagnosis and more often had high grade tumor with lymphovascular invasion. According to the molecular subtypes, overall survival and disease free survival were significantly shorter in obese patients with triple negative breast cancer. Obesity and lymphovascular invasion were found to be independent prognostic factors for TNBC mortality. In our study also we found that more number of obese breast cancer patients presented with stage III or IV disease, although the association was not statistically significant.

The study done by Sahin *et al*. (2017) BMI was an independent factor in patients with BC, with an association indicating a decreased incidence for luminal-like subtype and increased incidence for triple-negative subtype among premenopausal patients. However, this significance was not found in postmenopausal patients. Accordingly, a plausible etiological heterogeneity in BC might play a role among immunohistochemical subtypes in every life stage of women. Makama *et al*. (2017) published an association study of established breast cancer reproductive and lifestyle risk factors with tumour subtype defined by the prognostic 70-gene expression signature. Using prognostic gene expression profiles, they found that specific reproductive factors like higher parity was associated with low-risk (good prognosis) tumours. Also, longer duration of breastfeeding was associated with high-risk (poor prognosis) tumours. Neuhouser *et al*. (2015) performed a randomized control study and detected that A BMI of 25.0 or higher was strongly associated with risk for estrogen receptor-positive and progesterone receptor-positive breast cancers but was not associated with estrogen receptor-negative cancers. In our study we found that there was no significant difference in the distribution of estrogen receptor positive tumours and Her 2 neu positive tumours in obese and non obese patients. However, the number of triple negative breast cancers was more in I and II classes of body mass index. Obesity grade 2 and 3 were also associated with advanced disease, including larger tumor size, positive lymph nodes, regional and/or distant stage and deaths after breast cancer. An article by Schmitz *et al*. (2013) proposes that obesity and related co morbid conditions pose an increased risk for common adverse treatment effects, including breast cancer–related lymphedema, fatigue, poor health–related quality of life, and worse functional health. But we did not notice any significant difference in the post treatment morbidity among obese and non obese individuals.

Limitations of our study

The sample size of our study is limited to derive a statistically significant association of obesity and malignancy. The follow up was for a period of 6 months to one year and so the response of obese and non obese patients to the various treatment strategies could not be compared.

Conclusion

There is a valid association between obesity and breast cancer in post menopausal women but the same is not applicable in pre menopausal women. There is no significant difference in the size, histological type and immunohistochemical profile of breast cancers in obese and non obese patients, although the incidence of triple negative cancers was high in class I and class II BMI individuals. However obese patients presented with more advanced stages, possibly due to delay in detection.

REFERENCES

Cuello-López, J., Fidalgo-Zapata, A. and Vásquez-Trespalacios, E. 2017. Obesity and Prognostic Variables in Colombian Breast Cancer Patients: A Cross-Sectional Study. *International Journal of Breast Cancer,* 1-7.

Makama, M., Drukker, C., Rutgers, E., Slaets, L., Cardoso, F., Rookus, *et al*. 2017. An association study of established breast cancer reproductive and lifestyle risk factors with tumour subtype defined by the prognostic 70-gene

expression signature (MammaPrint®). *European Journal of Cancer, 75*, 5-13.

Neuhouser ML, Aragaki AK, Prentice RL, *et al*. 2015. Overweight, Obesity and Postmenopausal Invasive Breast Cancer Risk. *JAMA Oncology*, 1(5):611-621.

Roberts DL, Dive C, Renehan AG. 2010. Biological mechanisms linking obesity and cancer risk: new perspectives. *Annu Rev Med*., 61: pp. 301.

Sahin, S., Erdem, G. U., Karatas, F., Aytekin, A., Sever, A. R., Ozisik, Y. and Altundag, K. 2017. The association between body mass index and immunohistochemical subtypes in breast cancer. *The Breast, 32*, 227-236.

Schmidt S, Monk JM, Robinson LE, Mourtzakis M. 2015. The integrative role of leptin, oestrogen and the insulin family in obesity-associated breast cancer: potential effects of exercise. *Obesity Reviews*, 16(6):473-487.

Schmitz, K. H., Neuhouser, M. L., Agurs-Collins, T., Zanetti, K. A., Cadmus-Bertram, L., Dean, L. T. and Drake, B. F. 2013. Impact of Obesity on Cancer Survivorship and the Potential Relevance of Race and Ethnicity. *JNCI Journal of the National Cancer Institute*, 105(18), 1344–1354. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. 2015. Global cancer statistics, 2012. *CA Cancer J Clin*., 65: 87–108.

Turkoz F. P., Solak M., Petekkaya I. *et al*. 2013. The prognostic impact of obesity on molecular subtypes of breast cancer in premenopausal women. *Journal of BUON*, 18:335–341.

van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR *et al*. 2000. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. *Am J Epidemiol*., 152: 514–52

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Case Study

BONE MARROW BIOPSY PICTURE IN A PATIENT WITH MARFANOID HABITUS AND ANOREXIA NERVOSA

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ABSTRACTS

Gelatinous marrow transformation (GMT) is a rare disorder of bone marrow characterized by deposition of extra cellular gelatinous material. There is atrophy of the marrow adipocytes and loss of hematopoietic elements. It is also called as osseous atrophy of the bone marrow. It is not a specific disease but it is seen associated with chronic illness like malnutrition, anorexia nervosa, HIV infection and administration of cytotoxic drugs. Here, we report a case of gelatinous transformation of bone marrow in a 17 year old female presented with severe weight loss and anemia.

KEYWORDS: Gelatinous Marrow Transformation, Bone Marrow Biopsy

INTRODUCTION

Gelatinous transformation of the bone marrow was first reported in 1898. The condition is defined by hypo cellularity of bone marrow and deposition of extra cellular gelatinous material (hyaluronic acid). This is associated with various diseases and malnutrition is assumed to be the basic causative factor. We report a patient with unexplained weight loss who had GMT.

CASE REPORT

A 17-year-old female came to the gastroenterology department with history of significant weight loss and amenorrhea for the past one year and vomiting, since 6 months. She had no fever, abdominal pain, diarrhea, jaundice or hematamesis. There was no co morbid illness. Physical examination revealed that she was very emaciated with loss of buccal pad of fat. She had Marfanoid habitus with positive wrist and thumb sign, arachnodactyly and high arched palate. Secondary hair and skin changes, Seborrheic dermatitis and Gottron's papules were present. Her height was 167cm and weight was 27kgs (BMI- 9.68).

Her blood glucose levels, serum urea, creatinine, Bilirubin and liver enzymes were all within normal limits. Serum electrolytes including calcium and magnesium levels were normal.

Urine examination revealed no significant pathology. Her hemoglobin was 13gm/dl with Mean corpuscular volume (MCV) of 98.9fl and packed cell volume of 37.2%. The Erythrocyte sedimentation rate was 5mm/hr. The total WBC count and differential count were within normal limits.

Peripheral smear showed a normocytic to mild macrocytic, normochromic blood picture with no significant findings in leucocytes and platelets

Serology testing for HIV, Hepatitis B and C and VDRL testing did not yield any positive results. Mantoux test was negative. Serum IgG was 1.63g/L, IgA was 6.01g/L and IgM was 1.29 g/L. Stool examination did not reveal any ova or cyst.

Serum Lactate dehydrogenase (LDH) was 212 units/L. Serum B12 level was 469pg/ml and serum folate was > 20.0ng/ml. Serum C3 level was 52mg/dl and C4 level was

17mg/dl. Serum prolactin and TSH levels were within normal limits.

Ultrasound abdomen was normal. Ultra sonogram of the pelvis revealed a small size uterus and the ovaries could not be visualized. Upper GI endoscopy revealed severe esophageal candidiasis, severe antral gastritis with flattened duodenal folds. A dermatology consultation was sought for and their diagnosis was Seborrheic dermatitis with Icthyosis keratosis pilaris, suggestive of nutritional deficiency.

The working clinical diagnosis of a connective tissue disorder was made. Further workup was done to exclude a collagen vascular disease. Antinuclear antibody (ANA) profile including anti SS, Smith, Anti mitochondrial antibody, PCNA, dsDNA, Ro52 nRNP/Sm, Rib were all negative.

Bone marrow aspiration and imprint study showed mild megaloblastic changes with deposition of a proteinaceous material in the background. The bone marrow trephine biopsy sections showed hypo cellular marrow particles with erythroid hyperplasia and scattered megaloblasts. The myeloid and the megakaryocytic lineages showed normal maturation. There was deposition of an eosinophilic material which stained positively with Alcian Blue at pH 2.5 and Periodic Acid Schiff (PAS). The findings were consistent with gelatinous transformation of the bone marrow.

The patient was referred to psychiatry department with the diagnosis of anorexia nervosa. She was discharged with the prescription of Tab.Fluconazole, T.Olanzepine and T.Folic acid. On follow up after 2 weeks, she reported improvement of appetite and showed a weight gain of 1.5 kg.

DISCUSSION

Gelatinous transformation of bone marrow, also called serous atrophy is defined as loss of hematopoietic cells with atrophy of fat cells and deposition of extra cellular gelatinous material in the bone marrow.

The gelatinous material in the marrow space consists of acid mucopolysacharides, mainly of hyaluronic acid staining positively with Alcian Blue at pH of 2.5. According to previous case reports in literature [1], the incidence of Gelatinous marrow transformation (GMT) is more common in males around 20 to 30 years of age.

Bohm [2] and his colleagues state that the spectrum of underlying diseases in gelatinous transformation varies with age. In young adults (<40 years of age), anorexia nervosa in women and AIDS in men were associated with GMT, whereas in the age group of 40 to 50 years alcoholism and age group more than 60 years, lymphomas, carcinomas and chronic heart failure were the most common causes. However it is well documented that chronic malnutrition is the source of gelatinous marrow transformation as in anorexia and starvation. A recent study from South India. [3], published that the underlying clinical diagnosis was HIV positivity in 5 cases, 2 with coexistent disseminated tuberculosis (TB), 1 with cryptococcal meningitis, and 1 with oral candidiasis; disseminated TB in 1 case; pyrexia of unknown origin (PUO) in 2 cases; Hodgkin's lymphoma (HD) in 1 case; acute lymphoblastic lymphoma (ALL) with maintenance chemotherapy in 1 case; and alcoholic pancreatitis in 1 case.

Severe metabolic diseases like diabetes mellitus and hypothyroidism and severe infectious diseases like AIDS also may induce gelatinous transformation. Amos et al in 1990 reported that GMT also occurs in patients in intensive care units. In a study involving 33[4] patients, GMT was almost always seen in men and associated with weight loss and anemia.

The deposited gelatinous material was found to consist of randomly aggregated non-amyloid fibrillar and granular material. Cotta [5] suggested that hyaluronic acid replaces fat cells in the marrow once they are lost.

Hutter [6] et al observed that changes of the peripheral blood cell count in patients with Anorexia Nervosa are a frequent observation but the peripheral blood cell count cannot predict the severity of bone marrow atrophy.

Abella [7] et al concluded that changes in stereo logic bone marrow parameters of adipocytes might appear soon in anorexia nervosa.

A large study was conducted in 2002, which involved the analysis of bone marrow changes in 44 consecutive patients diagnosed with Anorexia Nervosa. The marrow was classified as normal in 11%, hypo plastic or aplastic in 39%, with partial or focal gelatinous degeneration in 30%, or with complete gelatinous degeneration of the bone marrow (GDBM) in 20%.

Starvation of carbohydrate and calories cause hypoplasia and reduction of adipose tissue in the marrow [8], which adversely affects hematopoiesis. This is not a fibrotic process and is found to be reversible with the reestablishment of adequate nutritional intake.[9]The spectrum of peripheral blood changes and bone marrow correlates with the amount of weight loss rather than the clinical findings [10]

In our case, the patient is a young female with Marfanoid habitus presenting with severe weight loss and vomiting. She was immunocompetent with no co-morbid illness. Elaborate investigations did not reveal any evidence of a connective tissue disorder. Despite severe gelatinous transformation of the marrow, her peripheral blood picture and the bone marrow aspiration study were relatively normal.

CONCLUSION

This case is reported because of the rarity of association of gelatinous transformation of marrow in anorexia nervosa occurring in a young female with Marfanoid habitus. Bone marrow trephine biopsy should be carried out in any patient

with unexplained weight loss to rule out a gelatinous transformation.

REFERENCES

[1] Osgood E, Muddassir S, Starvation marrow - gelatinous transformation of bone marrow J Community Hosp Intern Med Perspect. 2014; 4(4): 10.

[2] Böhm, Joachim. Gelatinous Transformation of the Bone Marrow: The Spectrum of Underlying Diseases. American Journal of Surgical Pathology. 24(1):56, January 2000.

[3] Sreeya Das, Pritinanda Mishra, Rakhee Kar, Debdatta Basu. Gelatinous marrow transformation: A series of 11 cases from a tertiary care centre in South India. Turk J Haematol. Jun 2014; 31(2): 175–179.

[4] Rafiullah, Islam R, Mahmood R, Sitwala KV. Gelatinous bone marrow transformation secondary to unusual eating habits and drastic weight loss. BMJ Case Rep. 2013 Jul 16;2013

[5] Cotta CV. Gelatinous transformation. Blood.2012 Sep 13; 120(11):2166.

[6] Hütter G, Ganepola S, Hofmann WK. The hematology of anorexia nervosa. Int J Eat Disord. 2009 May; 42(4):293-300.

[7] E. Abella, E. Feliu, Granada. Bone marrow changes in anorexia nervosa are correlated with the amount of weight loss and not with other clinical findings. Am J Clin Pathol 2002; 118:582- 588.

[8] Mehler PS, Howe SE. Serous fat atrophy with leucopenia in severe anorexia nervosa. Am J Hematol 1995;49:171-2

[9] Shih-Hsiang Chen, Iou-Jih Hung, Tang-Her Jaing. Gelatinous Degeneration of the Bone Marrow in Anorexia Nervosa. Chang Gung Med J 2004;27:845-9

[10] Muhajir Mohamed,Alhossain Khalafallah. Gelatinous transformation of bone marrow in a patient with severe anorexia nervosa. International Journal of Hematology. 2013, Vol 97, Issue 2, pg 157-158.

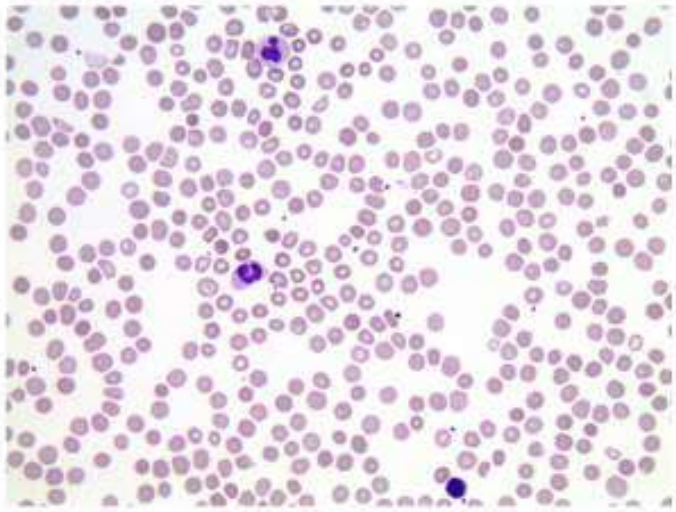


Figure : 1. Peripheral smear showing mild macrocytic normochromic blood picture(Leishman 400X)

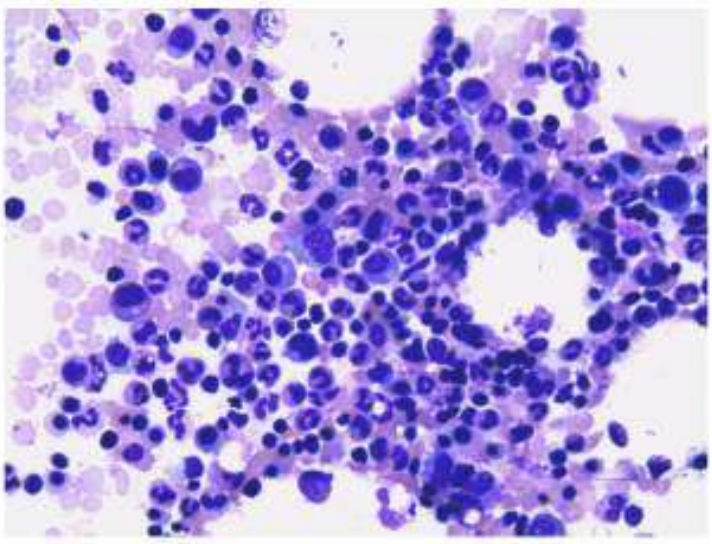


Figure : 2. Bone marrow aspiration smear showing trilineage hematopoiesis with mild megaloblastic change. (Leishman 400X)

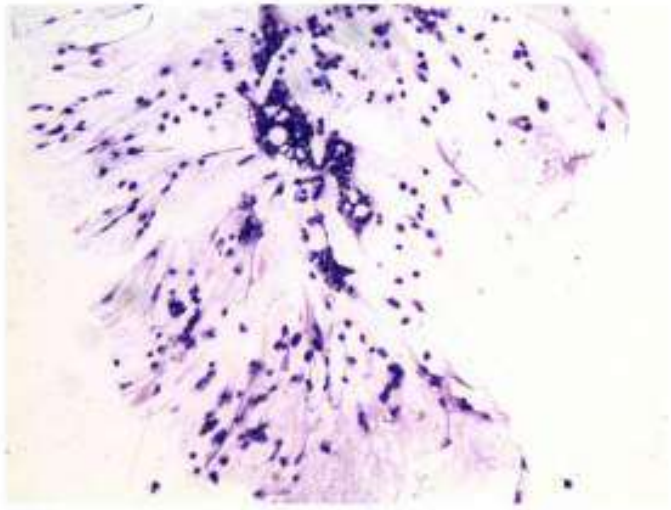


Figure : 3. Bone marrow imprint smear showing proteinaceous material. (Leishman 200X)

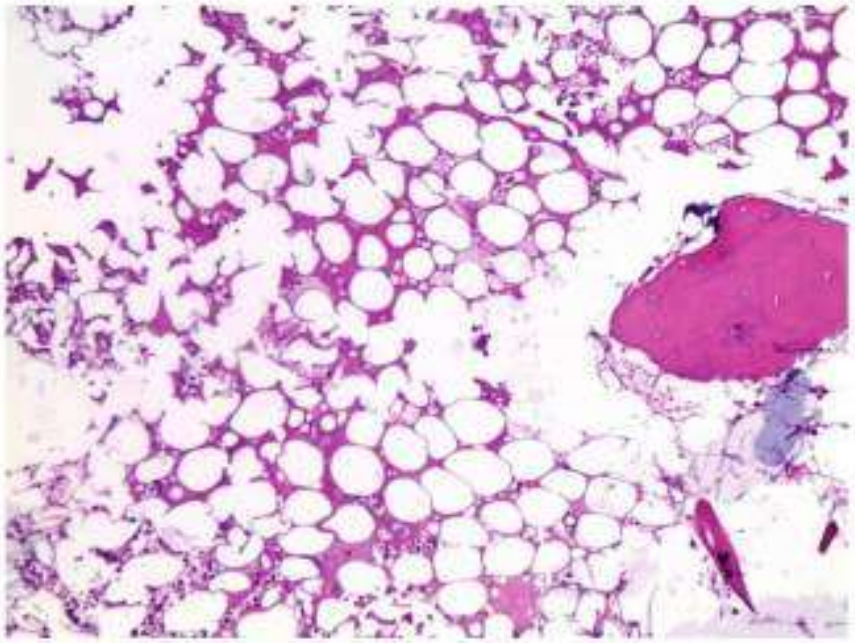


Figure : 4. Bone marrow trephine biopsy showing hypocellular marrow with deposition of eosinophilic gelatinous material (H & E 200 X)

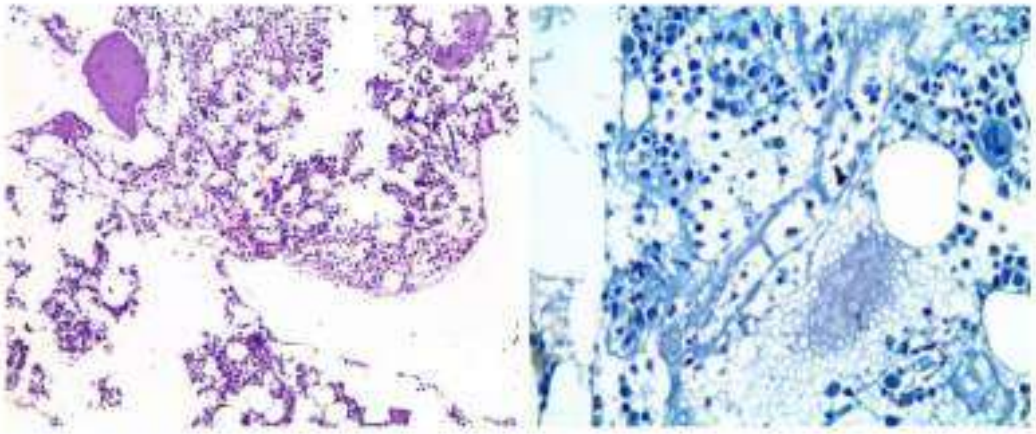
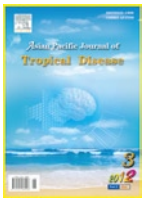


Figure : 5 (a). Trephine biopsy showing Periodic acid Schiff positive material. (PAS 100X)
5 (b) Trephine biopsy showing Alcian Blue positive material. (Alcian Blue 100X)

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Document heading

Histology as a diagnostic tool for intestinal isosporiasis in immunocompromised patients

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ABSTRACT

Isospora belli is an opportunistic protozoan causing wasting diarrhea especially in patients with an immunocompromised status. Diagnosis is usually established by demonstrating the oocyst of the organism on stool examination, which however can often be inconclusive. Serological tests for isosporiasis are currently not available. In such a scenario, biopsy often provides evidence for a confirmatory diagnosis. We describe two such cases, in which intestinal biopsy was the only diagnostic evidence of isosporiasis as the cause for chronic diarrhea.

1. Introduction

An increasing number of zoonotic infections in humans are found to be associated with a declining immunocompetent status of patients. Isosporiasis is a diarrheal illness seen in immunocompromised individuals in the tropics. It was first described by Virchow in 1860. The causative agent *Isospora belli* (*I. belli*) is closely related to *Cryptosporidium* species. Transmission is by ingestion of oocyst contaminated food and person to person contact.

2. Case report

Case 1: A 41-year-old man presented with complaints of watery diarrhea for a duration of 8 months. Peripheral smear showed mild eosinophilia. Ultrasound abdomen, upper gastrointestinal endoscopy and colonscopy were normal. Stool examination was negative for ova and cysts. The patient was subsequently found to be retropositive. Duodenal biopsy was performed to ascertain the cause of diarrhea.

Case 2: A postrenal transplant, diabetic patient on immunosuppressive therapy, presented with diarrhea of 1 year duration. Stool examination was negative for any ova or cysts. On follow up, upper gastrointestinal endoscopy was normal. Colonoscopy showed an erythematous cecal patch. Biopsies were taken from multiple sites in the cecum, ileum, duodenum.

2.1. Histopathological findings and follow up

Light microscopic evaluation of haematoxylin and eosin stained sections of duodenum in the first case and the ileum in the second case, showed relatively normal small intestinal mucosa with focal villous flattening and crypt distortion. Some of the enterocytes on the villous surface epithelium, showed elongated, fusiform organisms suggestive of *Isospora* merozoites in maturing schizonts (Figure 1). The lamina propria showed lymphoplasmacytic infiltrate and congested vessels. The duodenal and cecal biopsies of the second patient did not show any organism. The first patient responded well to trimethoprim-sulfamethoxazole therapy.

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On follow up, the second patient's diarrhea had subsided without any treatment. On enquiry, he was found to have been noncompliant with immunosuppressive therapy during that period, perhaps explaining his recovery. Subsequent intestinal biopsies did not reveal any parasite.

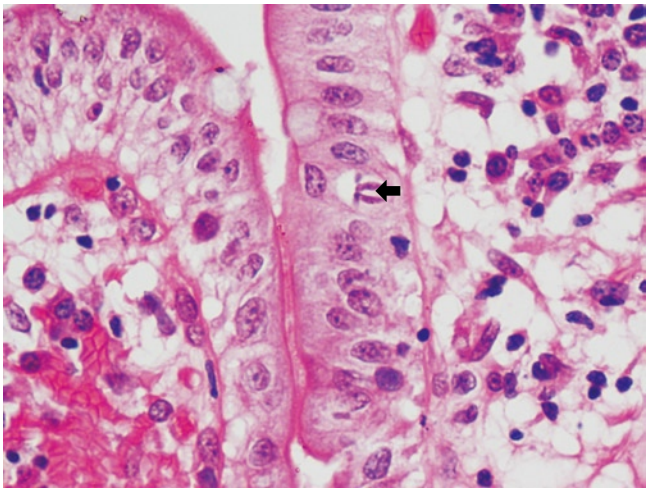


Figure 1. Microphotograph of *I. belli* organism in enterocyte (arrow). H & E × 400.

3. Discussion

I. belli is an obligate intracellular coccidian, spore forming parasite with a prevelance of 0.3%-0.5% in healthy individuals[1]. Human Isosporiasis is caused by *I. belli*, though rarely, it can be caused by *Isospora natalensis*. It may cause acute, self limiting diarrhea in immunocompetent individuals or severe, chronic diarrhea in the immunocompromised[2,3]. Stool examination may be unrewarding, as was noted in both cases. Diagnosis based on stool examination for oocysts is difficult, as these are scanty even in the presence of severe diarrhea. Incubation of the fecal specimen for 2 days with zinc flotation techniques often proves useful[4].

I. belli, like cryptosporidium occurs in the small intestine. Cryptosporidium, however, appears to multiply only in the brush borders of the intestinal epithelial cells whereas *Isospora* invades them. Brandborg *et al*[4] have elucidated, at length, the characteristic morphologic features of these organisms on light microscopy. Identification of any of the several sexual forms, gametocytes and merozoites in the subnuclear cytoplasm of the superficial epithelium and not in the crypts of the small intestine, is diagnostic of the disease. The organisms are rounded to banana shaped, pale staining[1]. The sporozoite first colonizes the epithelial cell, and becomes a rounded trophozoite. These enlarge to mature into schizonts with numerous merozoites. Merozoites infest the adjoining epithelial cell and either become schizonts or sexual gametocyte. The macrogametocyte when fertilized, becomes the oocyst which is passed in the feces. It can also exist as unizoite tissue cysts with uninucleated zoites located in the centre with a vacuole around it[4]. Non diagnostic minimal histological alterations including mild shortening of villi, crypt hyperplasia, increased inflammatory cells especially eosinophils in the lamina propria of the infected intestinal mucosa may be observed. Hence, diligent search for these organisms under high power objective in an hematoxylin and eosin stained slide can prove to be rewarding. Special stains used to identify other organisms like Giardia, cryptosporidia and microsporidia may not be particularly useful in identifying *Isospora*. Besides the intestine, the organism has also been reported in lymph nodes and spleen in disseminated cases[2]. The structure of the sporulated oocyst helps in deciding the species.

Sho Ogata and colleagues diagnosed an isolated case of intestinal cryptosporidiosis by endoscopic biopsy and have stressed the utility of histopathology in diagnosing such cases[5]. No such reports however, have come to light of isosporiasis being diagnosed primarily on the basis of a biopsy. The diagnosis of isosporiasis is important, as patients respond promptly to treatment.

In conclusion, light microscopic examination of intestinal biopsy plays a crucial role in diagnosing intestinal parasitic infestation and can be instrumental in initiating prompt and specific treatment. Medical professionals treating immunosuppressed individuals should be aware of the role of biopsy in diagnosing this curable cause of diarrhea, especially in cases where fecal examination and endoscopy are inconclusive.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Serra S, Jani PA. An approach to duodenal biopsies. *J Clin Pathol* 2006; **59**: 1133-1150.

[2] Velasquez JN, Carnevale S, Mariano M, Kuo LH, Caballero A, Chertcoff A, et al. Isosporosis and unizoite tissue cysts in patients with acquired immunodeficiency syndrome. *Hum Pathol* 2001; **32**(5): 500-505.

[3] Resiere D, Vantelon JM, Bouree P, Chachaty E, Nitenberg G, Blot F. *Isospora belli* infection in a patient with non-Hodkin's Lymphoma. *Clin Microbiol Infect* 2003; **9**(10): 1065-1067.

[4] Brandborg LL, Goldberg SB, Beidenbach WC. Human coccidiosis-A possible cause of malabsorption. The life cycle in small bowel mucosal biopsies as a diagnostic feature. *N Engl J Med* 1970; **283**(24): 1306-1313.

[5] Ogata S, Sagunama T, Okada C, Inoue K, Kinoshita A, Sato K. A case of sporadic intestinal cryptosporidiosis diagnosed by endoscopic biopsy. *Acta Med Okayama* 2009; **63**(5): 287-291.

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Original Article

Pathology Section

Histomorphological Spectrum of Mediastinal Masses with Special Emphasis on Rare Lesions

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ABSTRACT

Introduction: Mediastinal lesions are not frequently observed in routine clinical practice. A wide variety of disease process can occur within the mediastinum. Tissue from needle biopsies forms the major diagnostic material. There is need for detailed studies to make pathologists aware of the wide histological spectrum and to know the unusual lesions that can be encountered during diagnosis.

Aim: To describe the histomorphological spectrum of various mediastinal lesions and to highlight the rare lesions that posed a diagnostic challenge.

Materials and Methods: This was a retrospective study done on all mediastinal masses over a period of two years from January 2016 to December 2018. The clinical data and imaging findings were collected from the test request forms and patients' files for all the cases. Haematoxylin and eosin stained slides of all cases and immunohistochemistry markers and cytochemical stains wherever done were reviewed and analysed. Mean, median and percentage were used for statistical analysis.

Results: Thirty-four cases of mediastinal lesions were included in the study. Of these, 28 cases (82.4%) presented as anterior mediastinal masses, three cases (8.8%) each in the middle and posterior mediastinum. Thymoma (n=15) followed by lymphoma (n=4) were the most frequent neoplasms reported. There were five lesions which were rare in the mediastinum with unusual presentation that posed diagnostic difficulties. These lesions included Primitive neuroectodermal tumour in an adult, Primary Mediastinal B Cell Lymphoma (PMBCL), Malignant Peripheral Nerve Sheath Tumour (MPNST) with nodal metastasis, Seminoma deposits in mediastinal node occurring as late relapse and dedifferentiation of thyroid carcinoma in mediastinal node metastasis without an obvious primary.

Conclusion: Primary mediastinal tumours were more frequent than metastasis. Although thymomas and lymphomas are dealt extensively in literature, this article highlights the fact that pathologists must be aware of those lesions that don't display the classical histological and/or clinical features as observed in our study.

Keywords: Malignant peripheral nerve sheath tumour, Mediastinum, Mediastinal B cell lymphoma, Morphological spectrum, Primitive neuroectodermal tumour

INTRODUCTION

Primary mediastinal tumours are rare. Anterior mediastinal masses constitute >50% of mediastinal lesions and majority of them involve thymus. Middle mediastinal lesions include congenital cyst, (pericardial and bronchogenic cyst) while neurogenic tumours form the majority of posterior mediastinal masses [1-3]. Lymphomas can arise in any of these three compartments [4]. Aggarwal R et al., in their study of mediastinal lesions have reported 70% of lesions confined to anterior mediastinum, of which thymic lesions were the most frequent, constituting to 45.7% [1]. Posterior, superior and middle mediastinal lesions were reported to be 19, 6 and 4%, respectively. Lymphomas were the second most common tumour (11.2%) after thymoma. Sarcoma was seen in 6.9% cases and showed random compartment localisation. In their study, primary lesions were more common when compared to secondary deposits.

Dixit R et al., in their evaluation of 144 cases of mediastinal lesions have reported metastasis to be the most common, contributing to 37.4% of malignant lesions followed by lymphoma [5]. The clinical findings with radiological findings can narrow the differential diagnosis. However, advent of new techniques has made it easier to obtain tissue material from this site [5,6]. Hence, it is important for a pathologist to be familiar with the wide variety of lesions in the mediastinum since an accurate early tissue diagnosis directs therapy. There are variations in the existing literature regarding the frequency of individual lesions encountered in mediastinum. Hence the study was undertaken to analyse the histopathological spectrum of mediastinal lesions in our population and to highlight the rare lesions.

MATERIALS AND METHODS

This was a retrospective study done on all mediastinal masses over a period of two years from January 2016 to December 2018. The data was retrieved from the archives of Pathology department of PSG Institute of Medical Sciences and Research. Institutional Human ethics committee clearance was obtained before conducting the study (vide letter 20/108).

Needle biopsies and surgical resections were included in the study. A total of 36 cases were received during the study period. Of these cases, two cases showed only necrosis and hence were excluded from the study. The remaining 34 cases were included.

Relevant demographic and clinical data including the presenting symptoms, past history relevant to the lesions and examination findings was collected from patient files in all the cases. Laboratory investigations such as serum markers like carcinoembryonic antigen, CA125, alpha fetoprotein wherever available were obtained from test request forms and hospital information system. Computed Tomography findings including the location, size and solid or cystic nature of the lesion were recorded. Haematoxylin and eosin stained slides of all cases and Immunohistochemistry (IHC) markers and cytochemical stains like periodic acid, Ziehl Neelsen wherever done were reviewed and analysed. Immunohistochemical markers included cytokeratin (Mouse, polyclonal, AE1/AE3), CD99 (Mouse, 12E7) S100 (rabbit, polyclonal, 504),Thyroid transcription factor (Mouse, 056), napsin (EP205), CD45 (Mouse, EP273), CD3 (rabbit, polyclonal, 503), CD20 (Mouse, L26), CD15 (Mouse, 273), CD30 (Mouse, BER-H2), CD117 (IB 077) and PLAP (mouse, 376). IHC was done on paraffin embedded tissue sections using heat induced antigen

retrieval. Diaminobenzidene was the chromogen used. The cases which posed diagnostic challenge or had an unusual presentation were extensively studied. Mean, median and percentage were used for statistical analysis. The analysis was done using SPSS software version 20.0.

RESULTS

Thirty four cases of mediastinal lesions were included in the study. Age of the patients ranged from 14 to 75 years. Majority of the lesions were in females with male to female ratio being 1:1.5. The presenting symptoms included chest pain, cough and breathlessness in 26 cases. A total of 24 cases were the Primary mediastinal tumours while metastatic lesions were two in number. About Twenty eight (82.4%) cases presented as anterior mediastinal masses, and three cases each in the middle and posterior mediastinum. The majority of lesions were involving the thymus (n=21) constituting to 67.8% [Table/Fig-1]. The frequency of malignancy was higher in females (6 cases).

S. No.	Diagnostic category	Anterior 82.4% (n=28)	Middle 8.8% (n=3)	Posterior 8.8% (n=3)	Total % (n)
1	Non-neoplastic				
	Retrosternal goitre	1		1	23.5 (8)
	Sarcoidosis	1	1		
	Thymic cyst	2			
	Thymolipoma	2			
2	Neoplastic, Benign				
	Thymoma	15			47 (16)
	Teratoma		1		
3	Neoplastic, Malignant				
	Thymic carcinoma	2			
	Lymphoma	3	1		23.5 (8)
	Primitive neuroectodermal tumour			1	
	Malignant peripheral nerve sheath tumour			1	
4	Neoplastic, metastatic	2			6 (2)

[Table/Fig-1]: Distribution of mediastinal lesions.

The variants of thymoma included four cases each of type A, B1 and B2 and three cases of type AB thymoma. Both the cases of thymic carcinoma occurred in females, one of which occurred in a young female (age 28 years).

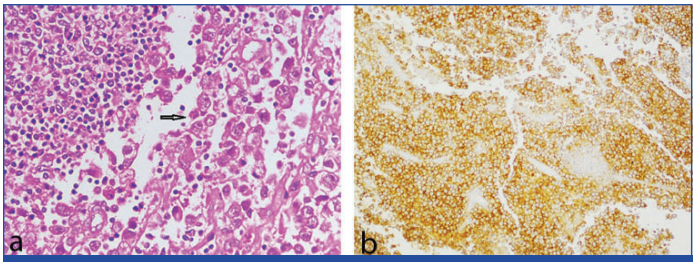
The two cases of Non-Hodgkin Lymphoma (NHL) reported were precursor T-lymphoblastic lymphoma in adolescent patients. The other NHL was Primary Mediastinal B Cell Lymphoma (PMBCL). The two cases of Hodgkin Lymphoma (HL) included one case each of Nodular Sclerosis and Mixed cellularity HL.

Of the 34 cases, 13 (38.2%) were diagnosed on guided biopsies and 21 (61.8%) on excision biopsies. In all the guided biopsies, a confirmatory diagnosis was made only after immunohistochemistry.

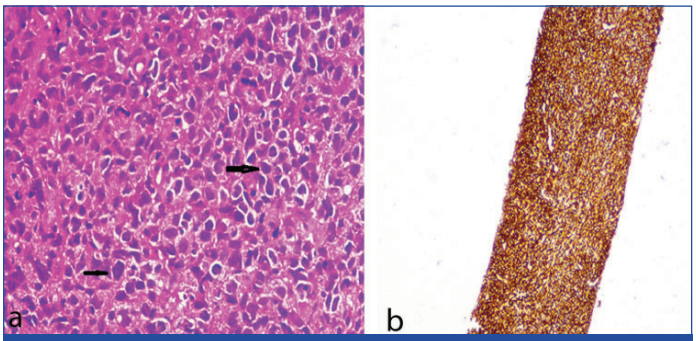
The rare tumours encountered are described below [Table/Fig-2]. Case 1 presented with multiple lymph nodes involvement suggesting the possibility of lymphoma. Seminoma relapse was not initially considered for this case. The diagnosis of seminoma metastasizing to lymph node was made only with the help of immunohistochemistry [Table/Fig-3]. Case 2 (PMBCL) was a rare lesion occurring in a young male, but had the typical presentation. Differential diagnosis entertained in this case were PNET and thymoma based on the morphology [Table/Fig-4]. Case 3 (MPNST) occurred in a patient with Neurofibromatosis. Cytology of pleural fluid also showed atypical cells. Based on the morphology, a provisional diagnosis of high-grade sarcoma was made. The diagnosis of MPNST with nodal metastasis was made with immunohistochemistry (S100 positive and Ki67 90%) [Table/Fig-5]. Case 4 (PNET/Ewing's sarcoma) occurred in an adult female (65 years) which is rare [Table/Fig-6]. Case 5 (dedifferentiated thyroid carcinoma in mediastinal nodes) occurred in an adult female who did not have any history of thyroidectomy. Histopathological examination showed an anaplastic tumour suggesting the possibility of sarcoma/carcinoma. Extensive sampling revealed tiny foci of conventional papillary thyroid carcinoma which was supported by IHC markers (Thyroid transcription factor positive, Napsin negative, Cytokeratin positive in anaplastic areas). Lung carcinoma was ruled out by napsin negativity [Table/Fig-7]. Normal thyroid parenchyma was not identified in the tissue and thus the possibility of aberrant

Case No.	Clinical details	Imaging findings (CT Scan)	Histology and IHC	Diagnosis and unusual finding
1	48/M, operated for testicular seminoma 5 years back and had defaulted surveillance	Hypodense, soft tissue mass of size 5.5×2.9 cm in anterior mediastinum. Mediastinal lymphadenopathy +. Beta HCG-420 mIU/mL LDH-1820IU/L	Lymph node with sheets of large cells having pleomorphic vesicular nuclei and prominent nucleoli separated by fibrous septa rich in lymphocytes. There was no other germ cell tumour elements. CD117 and PLAP positive.	Seminoma metastasing to mediastinal lymph nodes. Late relapse presenting with generalised lymphadenopathy
2.	21/M, anorexia, night sweats, significant loss of weight for two months. No organomegaly or superficial lymphadenopathy	large anterior mediastinal mass of size 6×7 cm invading superior vena cava.	Monomorphous population of blue round cells separated by thick fibrous septa. CD20 and CD45 positive Neuron Specific enolase, Cytokeratin negative	Primary Mediastinal B Cell Lymphoma (PMBCL). Rare lesion (incidence-2 to 3% of NHL) [1]
3.	35/M, known case of Neurofibromatosis presented with a complaint of dry cough associated with breathlessness	Multiple nodules in both lungs and pleura. Multiple enlarged mediastinal, para-aortic, celiac lymph nodes suggesting metastasis	Oval to spindle neoplastic cells exhibiting marked pleomorphism arranged in sheets. Vimentin, S100 positive. Ki67 index-90% Desmin and CD34 negative	MPNST with nodal metastasis. Rare presentation
4	65/F, chest pain, breathlessness and fever	intrathoracic mass measuring 15×12 cm extending into posterior mediastinum, encasing and lifting aorta, inferior vena cava and causing diaphragmatic inversion.	Small round cells with scant cytoplasm, uniform hyperchromatic nuclei with perivascular accentuation and surrounded by fibrous septa. Differential diagnoses entertained were lymphoma, Ewing's sarcoma and rhabdomyosarcoma. CD99 positive, CD45, Tdt and Myogenin negative	PNET/Extraskeletal Ewing's sarcoma in adult. (incidence -1.8%) [27]
5	69/F, chest pain, breathlessness and intermittent fever and inspiratory stridor	Large heterogeneously enhancing mass measuring 5×4×5 cm in the right upper posterior mediastinum encasing the esophagus in the left lateral aspect. Few small nodules in the right lung suggestive of metastasis were seen. A small nodule was noted in the thyroid. Thyroidectomy could not be done in this patient.	An anaplastic tumour composed of multinucleated giant cells and highly pleomorphic cells with extensive areas of coagulative necrosis. Tiny foci of conventional papillary thyroid carcinoma. No normal thyroid tissue identified. (strong nuclear expression of TTF1 in conventional papillary thyroid carcinoma. The anaplastic component was positive for Cytokeratin and lacked TTF 1 expression. Napsin-negative (rules out lung carcinoma)	Dedifferentiated thyroid carcinoma metastasis with no obvious lesion in thyroid.

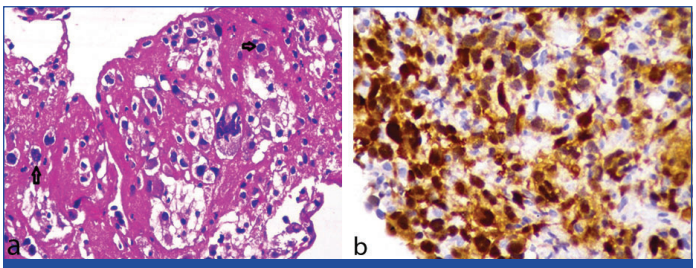
[Table/Fig-2]: Unusual features of the reported rare cases.
MPNST: Malignant peripheral nerve sheath tumour; PNET: Primitive neuroectodermal tumour



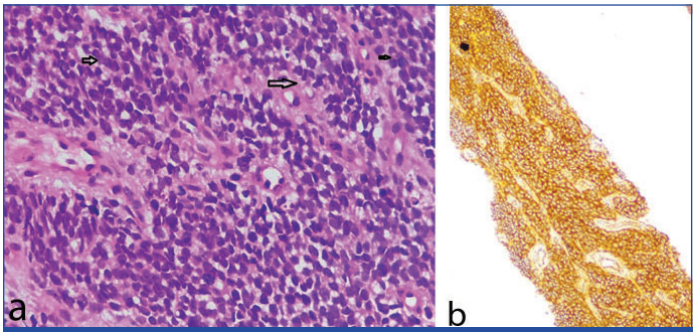
[Table/Fig-3]: a) Large neoplastic cells separated by fibrous septa rich in lymphocytes, Seminoma (H&E,40X). b) Neoplastic cells with strong membranous expression of PLAP (IHC-40X).



[Table/Fig-4]: a) Discohesive pleomorphic large round cells (arrow), PMBCL (H&E, 40X).b) Neoplastic cells with strong membranous CD20 expression (IHC, 10X).



[Table/Fig-5]: a) Pleomorphic tumour cells, MPNST (H&E, 40X). b) Tumour cells expressing S100 (IHC 40X).



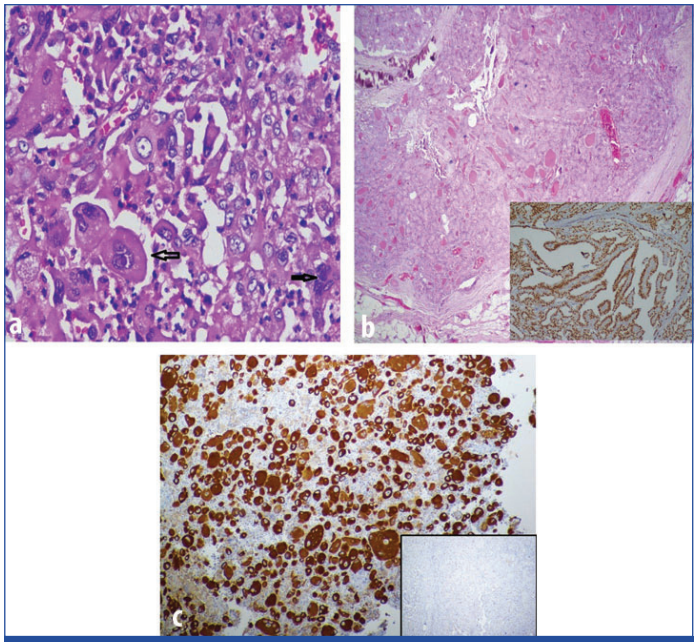
[Table/Fig-6]: a) Small round cell tumour, PNET (H&E, 10X). b) Tumour cells expressing membranous staining for CD99 (IHC 10X).

thyroid and retrosternal extension were ruled out. Small nodule identified in thyroid by Computed Tomography of neck could not be evaluated since thyroidectomy could not be done on this patient.

DISCUSSION

In the present study, the majority of the tumours were primary in origin (88.2%) except for two cases. Literature review also showed that primary mediastinal lesions are more common than metastatic malignancies [3,4,6-9]. There was a female predominance in the current study which contrasts with the previous studies [5-9].

In keeping with other studies [3-10], most of the lesions were in the anterior compartment (82.4%) and benign lesions (47%) were more frequent than malignant tumours (23.5%). However, there are studies reporting malignant lesions to be more commoner constituting about 60% of lesions [11,12].



[Table/Fig-7]: a) Sheets of pleomorphic cells with multinuleation and hyperchromatic nuclei (H&E- 40X).b) Conventional papillary carcinoma component. (IHC-10X). Inset TTF1 expression (IHC 10X). c) Anaplastic carcinoma with CK expression (IHC-40X). Inset TTF 1 negative (IHC 10X).

Thymoma was the most frequent neoplasm in the present study which is similar to other studies [3-9]. However, Vaziri M et al., in their study had reported lymphomas to be more common than thymoma in a series of 105 cases [11]. In the current study, thymic lesions constituted 61.7% of anterior mediastinal masses which is higher when compared to the studies by Aggarwal R et al., and Dasgupta S et al., [1,3].

Thymic carcinomas are reported to be rare malignancies occurring in the age group of 30 to 60 years. Suster S, in his study have reported a slight male predilection (1.5:1) [13]. In the present study both the cases of thymic carcinoma occurred in females.

Retrosternal goitres are usually located anteriorly, in the superior or anterior mediastinum. Posterior mediastinum is rarely involved. Their incidence in the general population is about 1:5000 [14]. The case described in the study also occurred in posterior mediastinum. In the present study, there were five cases which were either rare or had unusual presentations that posed diagnostic difficulties [Table/Fig-2].

Primary mediastinal Seminomas originating from extragonadal germ cells are rare [15]. In general, the relapse occurs within 2 to 3 years. It has been reported in inguinal lymph nodes, contralateral testis, retroperitoneum, mediastinum and lung [16-18]. The case described here presented with seminoma deposits in mediastinal lymph nodes along with multiple abdominal lymphadenopathy 5 years after orchidectomy. Hence, the possibility of any mediastinal germ cell tumour representing a metastasis from testicular primary should always be investigated. Considering the above facts, this case is unusual since it presented as a late relapse.

PMBCL is a subtype of Diffuse Large B Cell Lymphoma (DLBCL) of thymic B cell origin affecting adolescents and young adults and having a slight female preponderance. Of the cases reported, 75% present with bulky anterior mediastinal mass greater than 10 cm that grows rapidly and nearly always present with superior vena cava obstruction [19]. Bone marrow involvement is reported to be extremely rare. It shares the biological and clinical characteristics with Nodular Sclerosis type of Hodgkin Lymphoma (NSHL) [19]. The characteristic histologic findings include clearing of cytoplasm of cells and alveolar fibrosis. Because of the presence of compartmentalisation of

cells divided by fibrous septa, it mimics thymoma, NSHL and germ cell tumours. Hence, immunohistochemistry becomes an essential aid in diagnosis. All the cases of PMBCL express CD20 and a proportion of cases express CD30. PMBCL has been reported to have better prognosis when compared to DLBCL with secondary mediastinal lymph node involvement [19,20]. The case presented here had the typical presentation of PMBCL with superior vena cava obstruction and absence of superficial lymphadenopathy and organomegaly but its occurrence in a young male is rare.

Intrathoracic presentation of MPNST is rare. There are nearly 20 such cases reported in literature, majority of them in the posterior mediastinum [21]. A 20 -30% of MPNST occur in the setting of Neurofibromatosis. Those cases show an increased recurrence and risk of distant metastasis [22,23]. Lung, bone and pleura are the most common sites of metastasis. Metastasis to lymph nodes is reported in less than 10% of patients and is usually seen as a part of widespread metastasis. In the case presented here, there was mediastinal lymph node metastasis from MPNST. Since these tumours show a weak expression of S100 in majority of cases, it becomes difficult in cases of unusual presentation like nodal metastasis, especially in small biopsies to diagnose [24]. The challenge faced in this case was that the presence of neurofibromatosis in this patient was not available at the time of diagnosis.

PNET is a rare and aggressive tumour of neuroectodermal origin most frequently occurring in children and young adults. There are only a few published series of PNET in adults [25]. Primary mediastinal PNET is very rare constituting 1.8% of mediastinal tumours. In a study by Applebaum MA et al., it was found that Extraskeletal Ewing sarcoma had a higher mean age when compared to those with skeletal tumours [26,27]. Metastasis was frequent in the cases with extraskelatal Ewings sarcoma/ PNET [25,28]. The case presented here also occurred in an adult female (65 years).

PTCs are known for anaplastic transformation at the metastatic sites [29] and have been reported in retroperitoneum, liver and lungs. This has been proven even with papillary microcarcinoma [29,30]. The cases reported previously were postsurgery for papillary carcinoma which occurred as a recurrence, many years after treatment for papillary carcinoma [29]. The case presented here did not have any history of proven PTC nor did the patient present with thyroid mass. This could probably represent an occult papillary microcarcinoma in thyroid with mediastinal metastasis of papillary thyroid carcinoma that has undergone dedifferentiation. The diagnosis of dedifferentiated thyroid carcinoma was not made on the initial sections. This case re-emphasises the need for extensive sampling in a case of anaplastic tumour to look for a well differentiated tumour to accurately diagnose even a remote possibility.

Limitation(s)

The smaller sample size in this study precludes generalisation of study results considering the frequency and distribution of mediastinal tumours. Further studies including larger sample size and studies aiming at analysing correlation with clinical and radiological findings are needed to estimate the subtype distribution of mediastinal lesions.

CONCLUSION(S)

To summarise, benign tumours were more common than malignant ones. Primary mediastinal tumours were more frequent than metastasis. Although thymomas and lymphomas are dealt extensively in literature, this article highlights the fact that pathologists must be aware of those lesions that don't display the classical histological and/or clinical features as observed in our study.

REFERENCES

[1] Aggarwal R, Rao S, Chopra P, Bhalla S, Vijay CL, Asaf BB et al. Morphological spectrum of mediastinal lesions with special emphasis on evaluation of needle biopsy: An experience from a tertiary care hospital. Indian J Med Res. 2016;144(4):544-51.

[2] Shrivastava CP, Devgarha S, Ahlawat V. Mediastinal tumours: A clinicopathological analysis. Asian Cardiovasc Thorac Ann. 2006;14:102-04.

[3] Dasgupta S, Bose D, Bhattacharyya NK, Saha M, Biswas K, Biswas PK. A clinicopathological study of mediastinal masses operated in a tertiary care hospital in Eastern India in 3 years with special reference to thymoma. Indian J Pathol Microbiol. 2016;59:20-24.

[4] Prasanth G, Kapoor A, Bajpai J, Agarwal S, Thulkar S, Kuma L. Mediastinal masses- The bad, the ugly and the unusual. Indian Journal of Medical & Paediatric Oncology. 2007;28:11-16.

[5] Dixit R, Shah NS, Goyal M, Patil CB, Panjabi M, Gupta RC, et al. Diagnostic evaluation of mediastinal lesions: Analysis of 144 cases. Lung India. 2017;34:341-48.

[6] Takeda S, Miyoshi S, Akashi A, Ohta M, Minami M, Okumura M, et al. Clinical spectrum of primary mediastinaltumours: A comparison of adult and pediatric populations at a single Japanese institution. J Surg Oncol. 2003;83:24-30.

[7] Sharma P, Jha V, Kumar N, Kumar R, Mandal A. Clinicopathological analysis of mediastinal masses: a mixed bag of non-neoplastic and neoplastic etiologies. Turk Patoloji Derg. 2017;33(1):37-46.

[8] Duwe BV, Sterman DH, Musani AL. Tumours of the mediastinum. Chest. 2005;128:2893-909.

[9] Karki S, Chalise S. Analysis of mediastinal lesions: A study of 27 cases. J Pathol Nepal. 2011;1:114-17.

[10] Aroor AR, Prakasha SR, Seshadri S, Teerthanath S, Raghuraj U. A study of clinical characteristics of mediastinal mass. J Clin Diagn Res. 2014;8:77-80.

[11] Vaziri M, Pazooki A, Zahedi-Shoolami L. Mediastinal masses: Review of 105 cases. Acta Med Iran. 2009;47:297-300.

[12] Adegboye VO, Ogunseyinde AO, Obajimi MO, Ogunbiyi O, Brimmo AI, Adebo OA. Presentation of primary mediastinal masses in Ibadan. East Afr Med J. 2003;80:484-87.

[13] Suster S. Thymic carcinoma: Update of current diagnostic criteria and histologic types. Semin Diagn Pathol. 2005;22(3):198-212.

[14] Samokhvalova A, Loberantb N, Makhoul N. Posterior mediastinal goiters: Report of two cases and literature reviewRespiratory Medicine Case Reports. 2012;5:65-68.

[15] Ota Y, Iihara K, Ryu T, Morikawa T, Fukayama M. Metastatic seminomas in lymph nodes: CD10 immunoreactivity can be a pitfall of differential diagnosis. Int J Clin Exp Pathol. 2013;6(3):498-502.

[16] Warde P, Specht L, Horwich A, Oliver T, Panzarella T, Gospodarowicz M, et al. Prognostic factors for relapse in stage I seminoma managed by surveillance: A pooled analysis. J Clin Oncol. 2002;20(22):4448-52.

[17] Cummins S, Yau T, Huddart R, Dearnaley D, Horwich A. Surveillance in stage I seminoma patients: A long-term assessment. Eur Urol. 2010;57(4):673-78.

[18] Fukushima T, Noguchi T, Kobayashi T, Sekiguchi N, Ozawa T, Koizumi T, et al. Late and rapid relapse in mediastinum from testicular germ cell tumour Stage I over 13 years after surgery. Case Rep Oncol. 2019;12(2):500-05.

[19] Aggarwal R, Rao S, Dhawan S, Bhalla S, Kumar A, Chopra P. Primary mediastinal lymphomas, their morphological features and comparative evaluation. Lung India. 2017;34(1):19-24.

[20] Rogowitz E, Babiker HM, Krishnadasan R, Jokerst C, Miller TP, Bookman M. Heart of lymphoma: Primary mediastinal large B-cell lymphoma with endomyocardial involvement. Case Rep Oncol Med. 2013;2013:81-84.

[21] Kawaguchi T, Kawai N, Watanabe T, Yasukawa M, Morita K, Ohbayashi C, et al. Primary intrathoracic malignant neurogenic tumour: Report of three cases and comparison with benign neurogenic tumours resected at our institution. Surg Case Rep. 2015;1:6.

[22] Ralli M, Singh S, Hasija S, Verma R. Intrathoracic malignant peripheral nerve sheath tumour: Histopathological and immunohistochemical features. Iran J Pathol. 2015;10(1):74-78.

[23] Godfrey GJ, Farghaly H. Lymph node metastasis of malignant peripheral nerve sheath tumour in the absence of widespread disease five years after diagnosis: A rare finding. Int J Clin Exp Pathol. 2010;3(8):812-14.

[24] Shreshtha S. Malignant peripheral nerve sheath tumour with lymph node metastasis: An uncommon presentation. Int Surg J. 2017;4:1138-40.

[25] El Weshi A, Allam A, Ajarim D, Al Dayel F, Pant R, Bazarbashi S, et al. Extraskeletal Ewing's sarcoma family of tumours in adults: Analysis of 57 patients from a single institution. Clin Oncol (R CollRadiol). 2010;22(5):374-81.

[26] Koc U, Duman E. A case of primary mediastinal Ewing's sarcoma/primitive neuroectodermal tumour presenting with chest pain. Turk J Emerg Med. 2016;17(2):77-78.

[27] Applebaum MA, Worch J, Matthay KK, Goldsby R, Neuhaus J, West DC, et al. Clinical features and outcomes in patients with extraskeletal Ewing sarcoma. Cancer. 2011;117:3027-32.

[28] Kalkan KE, Bilici A, Selcukbiricik F, Unver N, Yuksel M. Thoracic primitive neuroectodermal tumour: An unusual case and literature review. Case Rep Pulmonol. 2013;2013:326871.

[29] Sotome K, Onishi T, Hirano A, Nakamaru M, Furukawa A, Miyazaki H, et al. A rare case of anaplastic transformation within the metastatic site of the retroperitoneal region in a patient 17 years after total thyroidectomy for papillary carcinoma of the thyroid beginning with multiple bone metastases. Thyroid. 2007;17:1309-11.

[30] Abe T, Suzuki M, Shimizu K, Shinagawa N, Oizumi S, Matsuno Y, et al. Anaplastic transformation of papillary thyroid carcinoma in multiple lung metastases presenting with a malignant pleural effusion: A case report. J Med Case Rep. 2014;8:460.

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Second-trimester fetal autopsy: A morphological study with prenatal USG correlations and clinical implications

Chaitra Venkataswamy, Umamaheswari Gurusamy, S. Vidhya Lakshmi

Abstract:

OBJECTIVES: The objective of this study is to analyze the second-trimester fetal autopsies and to reemphasize the role of autopsy by comparing autopsy findings with prenatal ultrasound observations.

MATERIALS AND METHODS: Retrospective analysis of second-trimester fetal autopsies over a period of 7.5 years (January 2009–June 2016). A standard protocol of autopsy procedure was followed, which included external examination with photography, X-ray, internal examination, and histopathological examination. In fetuses with congenital malformations (CMs), the findings of prenatal ultrasonogram and autopsy examination were compared.

RESULTS: We analyzed a total of 66 fetuses, which includes 17 intrauterine fetal death, 49 terminations for CM, and increased risk for chromosomal abnormality. In fetuses with CM, multiple anomalies were more common than a single anomaly. The most common anomalies were seen involving central nervous system (neural tube defect) followed by the genitourinary system. Autopsy confirmed prenatal ultrasound findings in all cases except three. Complete agreement between USG findings and autopsy were seen in 17 cases (39.7%). Additional findings on autopsy were noted in 25 cases (62.2%). Among these, 15 cases had a significant change of recurrence risk due to altered initial ultrasound diagnosis.

CONCLUSION: Fetal autopsy plays an important role in arriving at the final diagnosis and detecting the cause of death. This information is very essential for the clinicians, in genetic counseling of the parents, and management of future pregnancies.

Keywords:

Congenital malformations, fetal autopsy, multiple anomalies, prenatal ultrasound

Introduction

The common indications for second-trimester fetal autopsies are termination of pregnancy (TOP) for congenital anomalies, intrauterine fetal death (IUFD), and inevitable abortion.[1] TOP for congenital anomalies accounts for the majority of cases of second-trimester fetal autopsies as most of the anomalies are detected during 18–20 weeks of gestation by anomaly scan which is an integral part of antenatal screening. These remain the common cause of perinatal deaths both in developed and developing countries and account for 25%–30% and 10%–15%, respectively.[2] The occurrence of these anomalies in subsequent pregnancy varies from negligibly low to 25%, and it depends on the etiology of diagnosis. Hence, it is important to make an accurate diagnosis.

In the present era, Ultrasonographic (USG) examination can accurately diagnose many lesions but has its own limitation in detecting soft-tissue lesions such as malrotation

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of intestines, asplenia/polysplenia, and pulmonary hypoplasia, and the success rates of ultrasound also depend on the equipment used and expertise of the sonologist. Perinatal autopsy either confirms or refute the ultrasound diagnosis and complements USG examination in making a precise diagnosis.

The objectives of the present study are to evaluate the reasons for second-trimester fetal autopsies, to categorize the congenital malformations (CMs) into various systems, syndromes, and complexes, and to compare the autopsy findings with prenatal USG examination.

Materials and Methods

The retrospective analysis of all second-trimester fetal autopsies from January 2009 to June 2016 (7.5 years) was done in the Department of Pathology. Fetuses with gestational age 14–28 weeks were included in the study. Fetuses with gestational age <14 weeks and >28 weeks were excluded from the study. Informed written consent for performing autopsy examination was obtained from either of the parents before the autopsy. All the fetuses were examined on preset protocol based on the recommendation given in guidelines in autopsy practice by Royal College Of the Pathologists,[3] which included photography, fetogram (whole body radiography), and external and internal examination.

Evisceration is performed by starting with a linear skin incision extending from symphysis menti to midline of chest, abdomen, passing to the left side of the umbilicus, and extending up to the symphysis pubis. *In situ* assessment of the intestines was done to rule out malrotation and they were removed from jejunum up to the rectum. Rest of the organs was taken out as "*en bloc*" by freeing the tongue from the floor of the mouth and releasing the attachments of the neck structures. The pelvic organs were cut as low as possible. External genitalia and urethra were dissected in case of suspected urorectal septum malformation. The individual system was thoroughly inspected and dissected for anomalies.

The brain is removed by incising the scalp behind one ear extending to vertex and to the other ear. Skin flaps are reflected anteriorly up to the eyebrows and posteriorly up to the nape of the neck. Skull bones are opened along the suture lines. The brain is delivered by placing the head at a lower level than the body, by releasing the nerve attachments, and falx cerebri, tentorium cerebelli and the upper cervical spinal cord is incised. The removed brain is then fixed by hanging in formalin using gauze.

All the organs are weighed. Histopathological examination of the brain, thyroid, thymus, heart, lung, liver, pancreas, spleen, kidney, adrenal, gonads, and placenta was carried out. Additional sections from the skin, muscle, bladder, pylorus, etc., were also studied when required.

In cases of fetuses terminated for CMs, the findings on autopsy and prenatal ultrasound examination were compared.

Approval was obtained from the institutional Ethical Clearance Committee.

Results

There were 87 fetal autopsies in 7.5 years of which 66 cases (75.8%) were second-trimester fetuses. Causes of second-trimester fetal autopsies are summarized in Figure 1.

In cases of IUFD and abortions, mean maternal age was 27 years (range19–35) and mean gestational age was 22 weeks (range 16–27). An autopsy provided a final diagnosis in 15 (88.2%) out of 17 cases. The causes are summarized in Table 1. In two cases, there was no detectable anomaly in the fetuses and the placental causes could not be excluded as placenta was not available for examination.

Cases terminated for increased risk for chromosomal abnormality include two cases of Down syndrome. One case presented with hydrops fetalis, cystic hygroma neck, common atrioventricular canal defect with persistent left superior vena cava, and hypoplasia of thymus. Other case showed nuchal edema, hydrocephalus, and abnormal left hand, feet, and legs. There was one case of Turner syndrome with partial deletion of short arm of X chromosome. This case showed telangiectatic vascular nevi, hypoplastic nails, widely spaced nipples, clitoromegaly, septate vagina, and aortic arch isthmus hypoplasia. In one case, amniocentesis and FISH showed deletion of short arm of chromosome

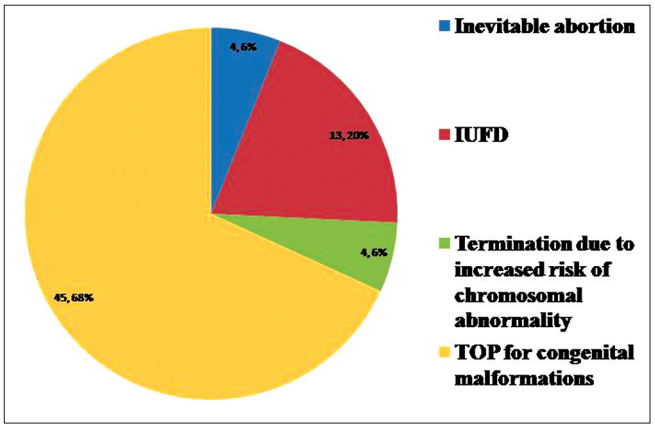


Figure 1: The pie diagram shows causes of fetal autopsies requested

Table 1: Causes of intrauterine fetal death in the second trimester

Causes	Number of cases (*n*=17)
1. Placental causes	10
Cord stricture	6
Hyper coiled cord with fetal vascular obstruction	1
Massive perivillous fibrin deposition	1
Infectious villitis with hydrops fetalis	1
Isolated hydrocephalous with central placenta previa in mother and cord stricture (spontaneous abortion)	1
2. Fetal causes	4
Hypoplastic left heart syndrome with visceral heterotaxy	1
Healed myocardial infarction	1
Hydrops fetalis due to premature ductus closure	1
Bladder outlet obstruction due to distal urethral stenosis (spontaneous abortion)	1
3. Maternal cause	
Maternal thrombophilia	1
4. Diagnosis not possible	2

15. USG examination of fetus showed bilateral mild ventriculomegaly, hypoplastic cerebellum, increased nuchal pad thickness, postaxial polydactyly in both feet, and multiple anomalies. An autopsy confirmed the USG findings and in addition showed focal cerebellar dysplasia. Parental karyotyping was suggested to confirm Prader–Willi/Angelman syndrome.

In fetuses terminated for congenital anomalies, the mean maternal age was 24 years (range: 18–32). The mean gestational age at the time of diagnosis was 21 weeks (13–27). A majority of them showed multiple malformations which are summarized in Tables 2 and 3. Central nervous system (CNS) anomalies were the most common followed by genitourinary malformations. The frequently encountered CNS anomaly was Arnold–Chiari II malformation with myelomeningocele. Two cases of rhombencephalosynapsis characterized by fusion of cerebellar hemisphere with absent cerebellar vermis were seen. There were four cases of hydrops fetalis and the underlying cause was detected in all. In a case of DiGeorge syndrome (DGS), there was hypoplastic thymus in the cervical region which was not detected on ultrasound [Figure 3]. A case of sirenomelia showed single median lower limb. Fetogram revealed single femur, two tibias, and no fibula [Figure 4].

Prenatal USG examination was available in all fetuses terminated due to prenatally diagnosed anomalies. There were a total of 121 anomalies in 45 cases. About 50 (41.3%) out of 121 anomalies were diagnosed only on autopsy. Three anomalies identified on USG were not confirmed on autopsy examination and includes two Dandy–Walker complex (DW) and aqueduct stenosis. One of the cases of DW complex showed additional findings on autopsy, and a diagnosis of Fryns syndrome was offered. Anomalies detected exclusively on autopsy examination include 13 (26%) gastrointestinal (malrotation of intestine), 13 (26%) genitourinary, 5 (10%) cardiovascular, 5 (10%) facial abnormality (cleft palate and micrognathia), four cases of CNS lesions, two cases each of polysplenia, skeletal abnormalities, and ductal plate malformation liver, and one case each of congenital diaphragmatic hernia, omphalocele, choanal atresia, microphthalmia, and nesidioblastosis.

Complete agreement between the USG findings and autopsy results were seen in 17 cases (39.7%). An additional finding in autopsy was noted in 25 (62.2%) out of 45 cases. In 15 cases (34.8%), the additional findings on autopsy changed the initial ultrasound diagnosis [Table 4]. In nine out of 15 cases, the revision of diagnosis led to significant change in recurrence risk (3%–25%) in subsequent pregnancy.

Discussion

With the advent of newer imaging techniques with higher resolution, the rate of autopsy is declining steadily. This is true even in cases of perinatal autopsies.[4] Perinatal autopsy still remains the gold standard in detecting the cause of death in IUFD and confirming the anomalies detected on ultrasound.[1] The present study focuses on second-trimester fetal autopsies and in reemphasizing its value in detecting congenital anomalies.

In the present study, major indication for second-trimester fetal autopsy was TOP for congenital anomalies. In cases of IUFD, fetal malformations were noted in 23.5% of cases, this is slightly higher as compared to the study done by Joó *et al*. where fetal malformation was seen in 13.05% in second-trimester miscarriages.[5] A study done by Peng *et al*. showed that 18.7% cases of IUFD were associated with umbilical cord stricture, hypercoiling or combination of both, most of these demises occurred in the second trimester.[6] The current study showed cord stricture and hypercoiling as a cause of IUFD in 41.1% of cases, significantly high. This highlights the importance of examination of the placenta and umbilical cord in cases with absence of congenital anomalies.

In fetus with deletion in chromosome 15, there were multiple anomalies including cerebellar hypoplasia. Deletion in chromosome 15 is known for phenotypic heterogeneity, with well documented, diverse clinical syndromes. The best known of these are Prader–Willi syndrome and Angelman syndrome, both of which are not associated with generalized cerebellar hypoplasia

Table 2: Postmortem diagnosis in fetuses terminated for congenital malformations (limited to 1 or 2 systems)

Congenital anomalies	Associated anomalies	Number of cases (*n*=27)
1. Central nervous system malformations		9
Arnold–Chiari malformation II with meningomyelocele	Agenesis of the right kidney and fused adrenals (1 case)	3
Hydrocephalous with aqueduct stenosis		1
Rhombencephalosynapsis	Lobar holoprosencephaly, aqueductal stenosis, interhemispheric cyst, TEV (1)	2
	Craniofacial dysmorphism (1)	
Hydrocephalus	Double aortic arch	1
Lumbar meningomyelocele	Kyphoscoliosis spine, ruptured omphalocele	1
Mild ventriculomegaly	Hypoxic ischemic encephalopathy	1
2. Urogenital malformations		8
AR polycystic kidney disease	Ductal plate malformation	2
Bladder outlet obstruction due to the posterior urethral valve	Hydrops fetalis (2 cases)	3
	TEV (1 case)	
Persistent cloaca with persistent Mullerian duct syndrome	Male pseudohermaphroditism (2), Occipital encephalocele, TEV, agenesis right kidney, imperforate anus, and malrotation intestine (1)	2
Persistent Mullerian duct syndrome	Ambiguous genitalia, male pseudohermaphroditism, anorectal atresia, bladder outlet obstruction with bilateral hydronephrosis, and renal obstructive dysplasia SUA	1
3. Cardiovascular system malformation		3
D-transposition of great arteries	Omphalocele (1 case)	2
Congenitally corrected transposition of great arteries		1
4. Respiratory system		2
Congenital high-airway obstruction syndrome	Hydrops fetalis	1
Alveolar capillary dysplasia with misalignment of pulmonary veins		1
5. Congenital diaphragmatic hernia [Figure 2]	Choroid plexus cyst (1 case)	3
	Meckel's diverticulum, bicuspid aortic and pulmonary valves, bicornuate uterus (1 case)	
6. Talipes equinovarus	Accessory tragi	1
7. Hydrops fetalis	Placental mesenchymal dysplasia	1

SUA = Single umbilical artery, TEV = Talipes equinovarus

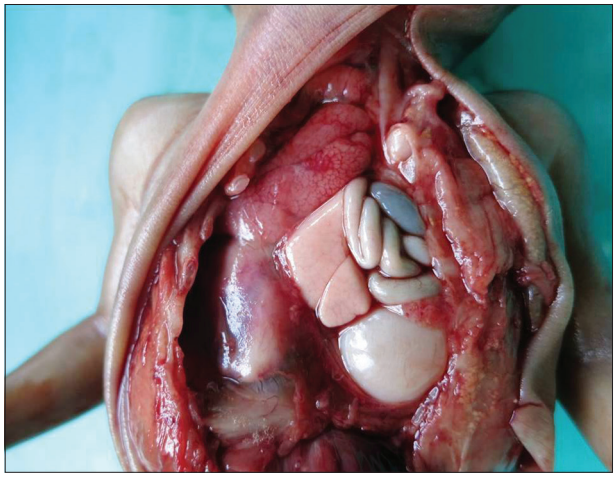


Figure 2: Congenital diaphragmatic hernia. The left hemithorax contains loops of small intestine and stomach pushing the heart and left lung to the right side

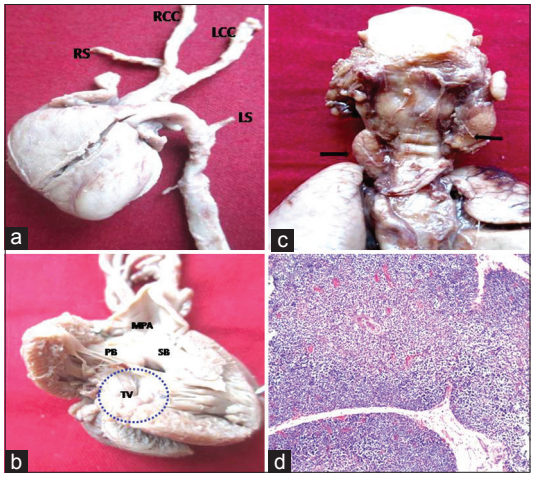


Figure 3: DiGeorge syndrome; (a) Interrupted aortic arch Type-B, (b) ventricular septal defect, perimembranous type, (c) cervical hypoplastic thymus, (d) lobules of thymic tissue composed cortex, medulla with Hassall's corpuscles (H and E, ×100)

as seen in present case. To the best of our knowledge, this association has not been described in literature. It is possible that this might belong to the expanding spectrum of chromosome 15q deletions.[7-9]

In 45 fetuses terminated for congenital anomalies, multiple malformations were seen in 75% of cases which is relatively high when compared to previous studies.[2,10]

CNS malformations were the most common followed by genitourinary anomalies. This is in concordance with the previous studies.[2,9-12] Arnold–Chiari malformation is characterized by downward displacement of cerebellum and brainstem into the spinal cord and is divided into three types. Type II is the most common form; this was true even in our series.[13]

Table 3: Postmortem diagnosis in fetuses terminated for congenital malformations (involving multisystem)

Diagnosis multiple malformations	Number of cases (*n*=17)
1. Syndromes/complexes/association	13
Fraser syndrome	1
Meckel–Gruber syndrome	2
Fryns syndrome	1
DiGeorge syndrome (SUA)	1
DiGeorge/velocardiofacial syndrome	1
Amniotic band syndrome	1
Charge syndrome	1
Zellweger syndrome/Smith–Lemli–Opitz syndrome	1
Sirenomelia with pseudo tail (SUA)	1
VACTERL or VATER Association (SUA)	2
Tuberous sclerosis complex	1
Multiple malformations – no definite syndrome	4
Renal hepatic pancreatic dysplasia with duodenal atresia	
Situs inverses with polysplenia and conotruncal malformation	
Meningocele, Arnold–Chiari malformation, tricuspid atresia, and double-outlet right ventricle	
Neural tube defect, Arnold–Chiari malformation, hydrocephalus with aqueductal stenosis, agenesis of corpus callosum, cerebellar hypoplasia, renal dysplasia, malrotation of small intestine, and polysplenia	

SUA = Single umbilical artery



Figure 4: Mermaid fetus; (a) single median lower limb with pseudo tail in the lumbosacral region, (b) fetogram shows fused pelvic bones, single femur, and absent fibula, (c) hypoplastic descending abdominal aorta continuing as single umbilical artery, (d) meconium filled, blind-ended colon

DGS also known as del22q11.2 is the most common microdeletion. Phenotypic features include heart defects, thymic hypoplasia/agenesis, facial dysmorphism, and developmental delay.[14] Two cases of DGS were diagnosed on prenatal ultrasound examination in view of congenital heart defects and absent thymus. Autopsy examination revealed hypoplastic cervical thymus in one case. Ectopic thymus was noted in 1% of children in a study of 3236 children at autopsy, of these 71% had features of DGS.[15] This might explain the variable immune status in the patients with DGS. Radiologist should be familiar with the locations of ectopic thymus and try to look for them when it is not found in the anterior mediastinum.

There was a case of sirenomelia with atypical skeletal framework. Sirenomelia is a rare CM characterized by varying degree of fusion of the lower limbs, single umbilical artery, and internal malformations related to genitourinary and gastrointestinal systems.[16] Stocker and Heifetz have classified sirenomelia into seven types based on skeletal framework in the lower limbs.[17] The bone pattern in the present case does not fit into any of the subtypes described in the literature. The exact etiological cause for sirenomelia remains unclear, but widely accepted hypothesis include defective blastogenesis and vascular steal hypothesis.[16]

In the present study, fetal autopsy provided definitive diagnosis in 95% of cases and confirmed ultrasound findings in 93% of cases, which is comparable to the previous studies.[2,12,18] An autopsy could not confirm DW complex in two cases. The previous studies have shown a poor correlation between ultrasound and autopsy findings in cases of DW complex with concordance being achieved in 55%, even after standardized reevaluation of sonographic and pathology images.[19] The poor correlation could be attributed either due to the membranous quality of the cyst wall with loss of fluid during anatomical dissection or due to the overdiagnosis of DW complex on ultrasound. Hence, the diagnosis should be reserved to cases which show all the classical findings on ultrasound. In doubtful cases, repeat ultrasound or magnetic resonance imaging (MRI) should be suggested to confirm the diagnosis.[19]

On comparing autopsy and ultrasound findings, our study showed complete agreement in 37.7% (17/45), partial agreement in 22.2% (10/45), and discordance in 33.3% (15/45). Thus, overall agreement was seen in 59.9%. This is in concordance with study done by Yeo *et al*.[20] Table 5 shows comparison of autopsy and ultrasound findings in various studies.[2,10,12,20,21]

Anomalies that were missed on USG examination were related to gastrointestinal, genitourinary, cardiovascular

Table 4: Comparison of ultrasound findings with autopsy findings in cases of discrepancy

Serial number	USG finding with diagnosis	Autopsy findings with diagnosis
1	Sacral meningomyelocele, bifid spine with the hypoplastic right ventricle of the heart	Arnold–Chiari malformation and tricuspid atresia with double-outlet right ventricle
2	Anhydramnios, B/L renal agenesis	Cryptophthalmos, ventriculomegaly, bifid nasal tip, clitoromegaly, B/L renal agenesis, absent vagina, uterus, and proximal fallopian tubes - Fraser syndrome
3	Anhydramnios, cystic dilatation of biliary radicles and common bile duct, a cyst in pancreas, bilateral multicystic dysplastic kidneys, microcephaly and shot bowed tibia - Ivemark syndrome	Bilateral cleft lip, cleft palate, lingual abnormalities and dysplastic epiglottis. Occipital encephalocele, Arnold–Chiari malformation Type III. Holoprosencephaly. Diffuse cystic renal dysplasia congenital hepatic fibrosis (ductal plate malformation), choledochal cyst Todani Type IV. Persistent left SVC, mild atrioventricular valve dysplasia. Ambiguous external genitalia, bilateral testes. Postaxial polydactyly, bow legs Meckel–Gruber syndrome
4	Situs ambiguous, isolated levocardia, VSD, and truncus arteriosus	Situs inversus, isolated levocardia with polysplenia, and conotruncal malformation
5	Cerebellum V-shaped with large cisterna magna. ? partial agenesis of inferior cerebellar vermis- possibility of DW complex	CDH, malrotation of the intestine, B/L cleft palate, ankyloglossia, bifid tongue, and uterus didelphys-Fryns syndrome
6	Short femur, humerus, narrow chest- Possibly achondroplasia	Syndactyly, postaxial polydactyly of hands and feet, dysplasia of epiglottis, nonrotation of intestine, ductal plate malformation of liver, renal dysplasia, X-ray - B/L shortening of long bones of the upper and lower limbs - Meckel–Gruber syndrome-variant
7	Oligohydramnios, B/L echogenic kidneys to R/O CMV	AR PCKD with congenital hepatic fibrosis
8	Increased nuchal translucency, generalized subcutaneous edema, hypertelorism, midline facial cleft, small omphalocele, cardiac outflow tract anomaly. Suggested invasive test to rule out aneuploidy. Patient not willing for amniocentesis	Craniofacial defects with a membranous tag at the head - cranioschisis, absent ears, and nose, bilateral cleft lip and palate, Conotruncal cardiac anomaly? Transposition of great arteries with pulmonary atresia? Double outlet right ventricle with pulmonary atresia, abdominoschisis, renal cystic dysplasia - suggestive of amniotic band syndrome
9	Lumbosacral spina bifida and Arnold–Chiari malformation	Lumbar meningomyelocele, ruptured omphalocele
10	Bilateral presacral kidneys with bilateral hydronephrosis due to pelviureteric junction obstruction, parenchyma of left kidney is dysplastic	Ambiguous genitalia, male pseudohermaphroditism, persistent mullerian ducts Anorectal atresia, bladder outlet obstruction with bilateral hydronephrosis, and renal obstructive dysplasia. SUA
11	SUA, TOF, ectopic hydronephrotic left kidney, left clubfoot with absent 2nd to 5th digits	TOF with the right aortic arch and left-sided patent ductus arteriosus, atresia of distal sigmoid, rectum and anus, fused crossed renal ectopia with hydronephrosis, left clubfoot with a single digit Fetogram-hemivertebra, absent metatarsal l bones-vacterl association
12	Pierre syndrome, left hydronephrosis due to pelviureteric obstruction	Bilateral posterior choanal atresia, lop ears, Micrognathia, Microphthalmia, persistent cloaca, left hydronephrosis - CHARGE syndrome
13	Two echogenic masses suggestive of cardiac rhabdomyoma	Heart: Multiple rhabdomyomas Brain: Subependymal nodules of dystrophic neurons and astrocytes - tuberous sclerosis complex [Figure 5]
14	Complete AVSD Bilateral cleft lip and palate, echogenic focus left ventricle	Congenitally corrected transposition of great arteries Alveolar capillary dysplasia with misalignment of pulmonary veins, bilateral cleft lip and palate, mild pyloric stenosis, bladder outlet obstruction? Vascular dysplasia kidneys and brain

TOF = Tetralogy of Fallot, CDH = Congenital diaphragmatic hernia, SUA = Single umbilical artery, SVC = Superior vena cava, USG = Ultrasonography, CMV = Cytomegalovirus, B/L = Bilateral, DW = Dandy-walker complex, AVSD = Atrio ventricular septal defect, VSD = Ventricular septal defect, R/O = Rule out, AR PCKD= Autosomal recessive polycystic kidney disease

systems, and facial defects. Antenatal detection of these CMs depends on the expertise of sonologist, quality of equipment, maternal habitus, and reduction in liquor volume.[22] In addition, some cardiac, gastrointestinal, and renal abnormalities are not evident at 20 weeks. Few are histopathological diagnosis (e.g.: tuberous sclerosis, PCKD). Oligo- and anhydramnios were noted in some of our cases. All the above factors could explain the shortcomings of routine antenatal USG examination. Other prenatal diagnostic modalities such as three-dimensional ultrasound and MRI will provide better resolution for CNS and soft-tissue abnormalities. Detailed analysis of fetus using these advanced imaging modalities may help in achieving higher rates of complete agreement. Implementing them in routine antenatal screening may not be feasible due to higher cost, lack of expertise, and limited availability in developing countries.

When the parents are reluctant to give consent for full autopsy due to religious reasons, limited, or minimally invasive autopsy may be of value. Minimally

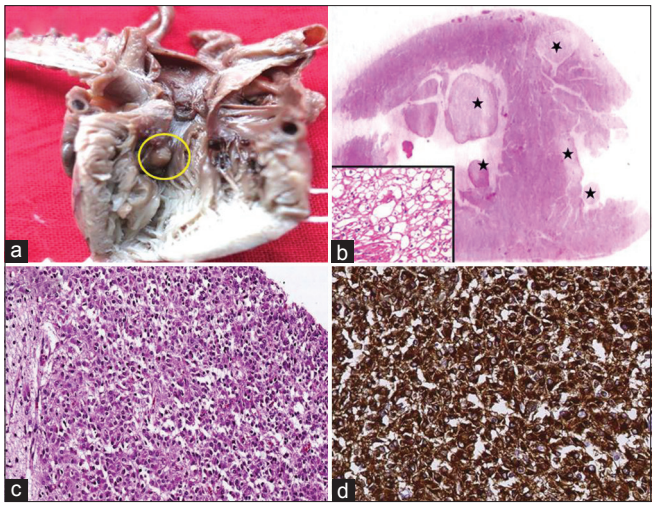


Figure 5: Tuberous sclerosis complex: (a) fleshy, pale brown mass in the left ventricular outflow tract, (b) transverse section of the heart shows multiple paler nodules(*) in subendocardial and intramural location. Inset shows high-power view of the nodule consisting of large vacuolated cells-rhabdomyoma (H and E, ×400), (c) section from medulla shows large subependymal plaque consisting of a cellular admixture of glial cells (H and E, ×100) and (d) these cells strongly express GFAP (IHC, ×400)

Table 5: Comparison of autopsy and ultrasound findings in various studies

Studies	Total number of cases	No change in initial USG diagnosis		Change in initial USG diagnosis (%)
		Complete agreement (%)	Added to USG findings (%)	
Sankar and Phadke[2]	134	41.0	58.0	1.49
Andola *et al*.[10]	39	50	29.54	9.09
Vimercati *et al*.[21]	144	71	35	17
Yeo *et al*.[20]	88	65	35	-
Grover *et al*.[12]	40	32.5	42.5	25.0
Present study	45	37.7	22.2	33.3

USG = Ultrasonography

invasive autopsy includes external examination with photography, placental examination, genetic analysis, and histopathological assessment of open or targeted biopsy of specific internal organs. Imaging studies, such as X-ray, postmortem MRI or micro-computed tomography, and assessment of body cavity fluids may be helpful to a certain extent.

Postmortem MRI can provide the details of structural malformation; however, it is not useful in cases of suspected infection, skeletal dysplasia, and in fetuses with severe maceration. It is not cost-effective and cannot provide details at histopathological levels.

Although minimally invasive autopsy can provide additional information in some cases, clinicians should explain to the concerned parents that complete perinatal autopsy remains the gold standard method for identifying the causes of fetal autopsy and its risk of recurrence.

Conclusions

The abnormalities detected by the fetal autopsy can be minor, major, or additional findings that modify the recurrence risk in subsequent pregnancy from 1%–3% to 25%. Thus, autopsy plays an essential role in accurate diagnosis and appropriate genetic counselling. The present study reemphasizes the need for fetal autopsy even in the era of advanced imaging techniques

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ernst LM. A pathologist's perspective on the perinatal autopsy. Semin Perinatol 2015;39:55-63.
2. Sankar VH, Phadke SR. Clinical utility of fetal autopsy and comparison with prenatal ultrasound findings. J Perinatol 2006;26:224-9.
3. The Royal College of Obstetricians and Gynaecologists and Royal College of Pathologists. Fetal and Perinatal Pathology: Report of a Joint Working Party. London: RCOG Press; 2001.
4. Boyd PA, Tondi F, Hicks NR, Chamberlain PF. Autopsy after termination of pregnancy for fetal anomaly: Retrospective cohort study. BMJ 2004;328:137.
5. Joó JG, Beke A, Berkes E, Papp Z, Rigó J Jr., Papp C, *et al.* Fetal pathology in second-trimester miscarriages. Fetal Diagn Ther 2009;25:186-91.
6. Peng HQ, Levitin-Smith M, Rochelson B, Kahn E. Umbilical cord stricture and overcoiling are common causes of fetal demise. Pediatr Dev Pathol 2006;9:14-9.
7. Cox DM, Butler MG. The 15q11.2 BP1-BP2 microdeletion syndrome: A review. Int J Mol Sci 2015;16:4068-82.
8. Sieg KG. Neurodevelopmental disorders associated with chromosome 15. Jefferson J Psychiatry 2011;8:9-23.
9. Hayashi M, Itoh M, Kabasawa Y, Hayashi H, Satoh J, Morimatsu Y,

et al. A neuropathological study of a case of the Prader-Willi syndrome with an interstitial deletion of the proximal long arm of chromosome 15. Brain Dev 1992;14:58-62.

10. Andola US, Am A, Ahuja M, Andola SK. Congenital malformations in perinatal autopsies – A study of 100 cases. J Clin Diagn Res 2012;6:1726-30.
11. Akgun H, Basbug M, Ozgun MT, Canoz O, Tokat F, Murat N, *et al.* Correlation between prenatal ultrasound and fetal autopsy findings in fetal anomalies terminated in the second trimester. Prenat Diagn 2007;27:457-62.
12. Grover S, Garg B, Sood N, Arora K. Lethal congenital malformations in fetuses-antenatal ultrasound or perinatal autopsy. Fetal Pediatr Pathol 2017;36:220-31.
13. Nejadi MN, Zafarani F, Ahmadi F, Rashidi Z. Arnold-chiari type II malformation: A case report and review of prenatal sonographic findings. Iran J Fuzzy Syst 2008;11:179-82.
14. Besseau-Ayasse J, Violle-Poirsier C, Bazin A, Gruchy N, Moncla A, Girard F, *et al.* A French collaborative survey of 272 fetuses with 22q11.2 deletion: Ultrasound findings, fetal autopsies and pregnancy outcomes. Prenat Diagn 2014;34:424-30.
15. Bale PM, Sotelo-Avila C. Maldescent of the thymus: 34 necropsy and 10 surgical cases, including 7 thymuses medial to the mandible. Pediatr Pathol 1993;13:181-90.
16. Valenzano M, Paoletti R, Rossi A, Farinini D, Garlaschi G, Fulcheri E, *et al.* Sirenomelia. Pathological features, antenatal ultrasonographic clues, and a review of current embryogenic theories. Hum Reprod Update 1999;5:82-6.
17. Stocker JT, Heifetz SA. Sirenomelia. A morphological study of 33 cases and review of the literature. Perspect Pediatr Pathol 1987;10:7-50.
18. Phadke SR, Gupta A. Comparison of prenatal ultrasound findings and autopsy findings in fetuses terminated after prenatal diagnosis of malformations: An experience of a clinical genetics center. J Clin Ultrasound 2010;38:244-9.
19. Phillips JJ, Mahony BS, Siebert JR, Lalani T, Fligner CL, Kapur RP, *et al.* Dandy-walker malformation complex: Correlation between ultrasonographic diagnosis and postmortem neuropathology. Obstet Gynecol 2006;107:685-93.
20. Yeo L, Guzman ER, Shen-Schwarz S, Walters C, Vintzileos AM. Value of a complete sonographic survey in detecting fetal abnormalities: Correlation with perinatal autopsy. J Ultrasound Med 2002;21:501-10.
21. Vimercati A, Grasso S, Abruzzese M, Chincoli A, de Gennaro A, Miccolis A, *et al*. Correlation between ultrasound diagnosis and autopsy findings of fetal malformations. J Prenat Med 2012;6:13-7.
22. Loughna P. Congenital abnormalities: Failure to detect and treat. Obstet Gynaecol 2008;10:33-7.

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ORIGINAL PAPER

Design and development of whole-body rodent inhalation chamber for exposure to Aroclor 1232 in Swiss albino mice

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Abstract

The development of whole-body inhalational chambers for acute toxicity risk assessment of aerosols in rodents is of key importance in clinical and occupational hazard evaluations. The key aim of study is the design, development, optimization, and validation of an inhalational chamber using water aerosol, vehicle, and Aroclor 1232. The second goal has been to apply this chamber in the risk assessment of Aroclor 1232. Broncho-alveolar Lavage fluid cytology and chromatography, and lung histopathology were used to supplement the validation process. PCB concentrations were uniformly distributed across different places inside the chamber and a MMAD generation of 4.3 µm ± 1.7 µm GSD was suggestive of lower airway atomization of PCB particles. The chamber environmental parameters were within acceptable limits for rodents during the experimental procedures. Mouse behavioral alterations were minimal during water aerosolization. Acute exposure to Aroclor 1232 showed reproducibility of behavioral scores. Benchmark dose for further studies using Aroclor 1232 was derived at 4 mg/cu.m. Its toxicities could be verified by correlating the behavioral scores, neurobehavioral functions with concentrations in plasma and BAL fluid samples from Swiss albino mice. The rodent chamber can be utilized for risk assessment of occupational hazards such as complex pollutant mixtures and particulate matter aerosols. The inhalational chamber possesses the versatility to enable assessment of toxicological mixtures across sub-acute toxicity timelines.

Keywords Inhalation toxicity · Acute toxicity test · Whole-body chamber · Rodent toxicology · Behavioral toxicology · Respiratory system · Aerosol toxicity · Aroclor 1232 · Biphenyl · Polychlorinated biphenyl · Broncho-alveolar Lavage

Introduction

The first line of defense in any organism exposed to such intoxicants can be the skin (dermal exposures) or the gastrointestinal tract (oral exposures). But an especially important route of exposure is inhalation which should bypass the respiratory tract to produce toxicity in the organism. The dosing of inhaled toxicants and pathophysiologic responses to inhaled toxicants and mechanisms behind their toxicities are key determinants of current and future human health, due to various reasons. Currently pollution rates and pollutants levels in the environment including the atmosphere are increasing. Also, the increasing incidence and prevalence of respiratory disorders including occupational diseases and substance abuse through inhalation route could be considered as important justifications to carry out research in inhalation toxicology. The results of inhalation toxicity studies can then be used to extrapolate to further improve health and quality of life of patients. Clinical and pre-clinical studies of this nature make use of inhalation chamber models

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to evaluate the toxicology of complex inhaled intoxicants. Based on the design and the exposure protocols, inhalation chambers can be used to evaluate the toxic effects of general exposures like whole-body chambers, or more specific exposures, e.g., nose-only or head-only chambers. They can also be utilized to assess health effects after even more localized exposures like whole-lung or partial lung chambers in animal models or human participants (Pauluhn and Mohr 2000; Colli Neto et al. 2014). Being the oldest models described in inhalation toxicology, whole body chambers are widely used, much like the utility of nose-only chambers.

The utility of inhalation chambers for pharmacological and toxicological risk evaluations cannot be undermined at the cost of their limitations, like difficulty in experimental extrapolations to real-life situations, ethical issues in exposure methods, and the presence of concurrent routes of exposures and other confounders apart from the inhalation route. Though few research evaluations have utilized exposure chambers in modeling inhalation toxicities in the past, their applications are of importance in regulatory policy making (Pauluhn 2003; Raju et al. 2014).

Standard environment exposure systems exist for inhalation exposures of animals like the BuxCo® system used to evaluate the effects of printer emitted particles (Pirela et al. 2014) and the Madison whole body chamber in fungal inhalant evaluations (Springer et al. 2013), though they may not be suited to deliver semi-volatile chemical and bioactive mixtures with potent but undetermined toxicities. Secondly, these chemical mixtures and congeners are complex and persistent, which makes it prudent to afford for safe elimination after the experiment without much provision to cause pollution and accumulation.

Aroclor 1232 is a semi-volatile PCB mixture which contains many individual Polychlorinated biphenyl, PCB congeners. Of the representative PCB congeners, we selected PCB 77, a tetrachlorobiphenyl, and PCB 180, a heptachlorobiphenyl, both being of toxicological significance (ATSDR, 2000). PCB 77 is a dioxin-like PCB, whereas PCB 180 is a highly lipophillic, biomagnifiable PCB congener.

There are only a handful of studies reporting the successful development and design of inhalation chambers in avian and rodent models of exposure (Kumamoto et al. 2013; Shaheen et al. 2016).

We describe the design and development of a whole-body rodent inhalation chamber for sub-acute inhalational toxicity testing, from its conception to its utility in evaluation of neurobehavioral parameters in mouse models. Since nose-only chambers usually do not simulate the occupational exposure better than whole-body chambers, we have applied the latter in toxicity assessment of a PCB mixture in rodents (Swiss albino mice). We have used water, water plus methanol, and Aroclor 1232 (PCB mixture) aerosols for validation of the chamber. We have also quantified PCB 77 and PCB 180 concentrations in plasma and BAL fluid of mice, after dosing Aroclor 1232 at dose increments, and evaluated using behavioral responses. Lung histopathology was used to supplement these results. The research was carried out at PSG Center for Molecular Medicine & Therapeutics laboratory, from March 2015 to July 2018.

Materials and methods

Chemicals and reagents

The PCB mixture, Aroclor 1232 was obtained from Sigma-Aldrich Inc., India. PCB 77, PCB 180 HPLC-grade standards were obtained from Sigma-Aldrich Inc., India. Acetonitrile of HPLC grade was purchased from Sigma-Aldrich, India. HPLC grade of methanol (carbinol) and Methyl tert-butyl Ether were purchased from Hi-Media, India. Ultrapure water (18.2 mΩ cm) was obtained from Millipore (Milford, USA) using MilliQ water purification system. Disodium EDTA calcium hydrate crystals and Sodium Hydroxide crystals were purchased from Sigma-Aldrich Inc., India.

Animals

Swiss albino mice were procured and quarantined at the PSG Animal Facility. They were housed under ambient light–dark cycle and optimum environmental conditions with water *ad-libitum*. Prior Institutional Animal Ethics Committee (IAEC) approval was obtained for study execution. All mice were of age 8–12 weeks with body weights ranging from 17 to 28 g at initiation of experiments. The Animal Facility is accredited by the local Regulatory Authority, the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA 2003).

Structural design of the inhalation chamber

The inhalation chamber is a whole-body exposure chamber meant for small laboratory animals including laboratory rodents. The development process of the chamber has been undertaken in two stages viz., design-and-development, and characterization-cum-optimization.

Design and development

The whole-body inhalation exposure chamber consists of three different mechanical units. The units have been divided based on their utility and role in the function of the chamber—the inlet system, the central chamber system, and the outlet system. Additionally, the environment of the chamber system, and the connections of the systems are also included in the chamber construction (Fig. 1a).

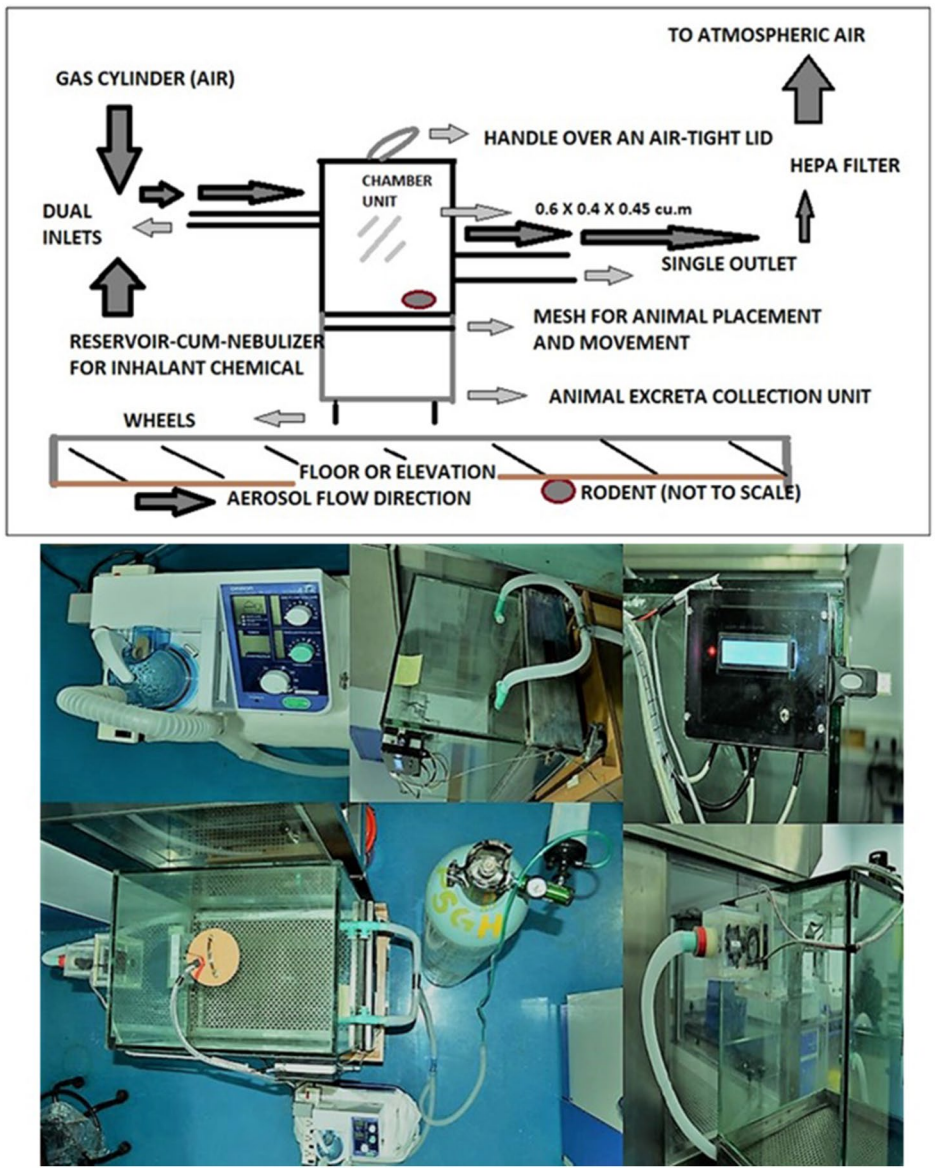


Fig. 1 Chamber Design—Line blueprint depicting chamber design (**a**) and photograph (**b**) depicting the key elements for inhalant exposures

Before initiation of the experimental procedure, the chemical mixture is prepared as per the dilution and dosing protocol of the experiment, for delivery of the test substance as aerosol particles. In order to achieve the desired flow rates of the aerosol particles by nebulization, air is used to forcibly propel the chemical mixture into the chamber after generation from the gas source (Air cylinder with control gauge and valve). The chemical pumping system (Ultrasonic nebulizer, Omron Inc.) is used to deliver the chemical aerosol of the test substance into the inhalation chamber. The central chamber core system is a glass transparent cuboid measuring 600 mm × 400 mm × 450 mm in dimensions. The base of this chamber comprises of another subunit made of stainless-steel mesh grid, below which a compartment is located to collect and dispose of the animal wastes. The roof of the chamber has a 10-cm diameter hole which can be sealed off or opened to place or retrieve the experimental animals. The wall along a breadth side has two inlet openings, each with a diameter of 10 cm, located about 10 cm from the base of the wall, which are connected to the aerosol pumping-cum-delivery system. The base compartment can be removed like the lid covering the roof hole, both of which are air-tight, then sealed to avoid gas or aerosol particles from escaping into the external environment. The internal environment of the chamber is controlled by five different sensors connected to a display panel through wires and a printed circuit board (pressure, temperature, relative humidity, oxygen level, and flow rate). This panel is then connected to a computer software (Serial Input–Output interface, SIOW) console for recording and storing the readings of different atmospheric variables. The outlet is meant for maintaining a uniform circulation and pressure of the aerosol particles in the chamber

atmosphere by continuously removing the exhaled gases and particles from the chamber. To achieve this purpose, the outlet system, consisting of a single circular hole, is located on the contralateral wall of the chamber about 40 cm from the base of that wall, with a diameter of 10 cm. A fan is encased behind the outlet opening for providing continuous ventilation to animals and rotates with a mean speed of 3400 rpm to achieve uniformity of flow rates inside the chamber. The terminal end of the outlet opens onto a Class II Type A_1 Biohazard workplace system with HEPA filters to eliminate the aerosols after circulation and recirculation inside the chamber system.

Five different sensors were pre-calibrated and fitted onto the chamber. These sensors were installed to continuously detect relative humidity, pressure, temperature, oxygen concentration and flow rate inside the chamber. We measured Mass Median Aerodynamic Diameter (MMAD) in the room wherein the chamber was housed during the experiment on three occasions using an impact cascade method. The Geometric Standard Deviation (GSD) of MMAD was calculated using the following formula:

$$\mathrm{GSD} = \frac{84.1\%\ \text{mark}}{50\%\ \text{mark}}$$

Allowed limits for the chamber environment parameters must not exceed 20%, and oxygen concentration must not fall below 19% in the chamber. The time to chamber equilibrium, $t\text{-}_{95\%}$ was calculated using the following formula:

$$t - 95\% = 3 \times \frac{\text{Chamber Volume}}{\text{Air flow rate in chamber}}$$

Periodical and regular variations in the chamber environmental conditions were recorded and visualized onto a display panel and computer console using Serial Input and Output software (SIOW, software v 1.2, hardware v 1.1, CCS, Inc.).

Characterization and optimization

Experiment 1 We checked for hourly variations in chamber atmosphere (Pressure, temperature, Relative humidity, flow rate and oxygen content) in response to water nebulization. The experiment was carried out water aerosol (350 mL daily dosing volume) for 4 h/day for two weeks (five days / week).

Experiment 2 The procedure consisted of standardization of methanol and water ratio using 54 animals inside for assessing any behavioral effects on animals. For the initial tests, a dose ranging from 0.1 to 1000 ppm of water aerosol in methanol was used in different combinations with dosing durations (1 s, 3 min, 5 min, 10 min, 30 min). Any acute signs of intoxication or mortality were noted later at each of these dose levels using 6 mice, with equal sex ratio. Animals were randomized using a Latin square design to various combinations of dose levels and dosing durations based on a c x t protocol. The behavior was measured periodically using Irwin scale (Table 1, Irwin, 1968).

Experiment 3 To assess the uniformity of aerosol distribution inside the chamber, PCB concentrations were measured after 4 mg/cu.m of Aroclor 1232 for 6 h-duration was introduced into the chamber without animals inside. The floor of the chamber interior was divided into 3×3 equal spatial circles. In each of these circular divisions, a 5 mL glass beaker was placed with 2 mL of 1:1 methanol: water inside. The analytes (PCB 77 and PCB 180) were quantified by Ultra-high performance liquid chromatography with Diode array detection (UPLC-DAD) after 6 h of exposures in each of the beakers (Ramanujam et al. 2017).

Risk assessment of PCB mixture (single duration exposure)

Experiment 4: Aroclor 1232 (1 mg/cu.m) in methanol–water aerosol at 1 ppm was used to ensure reproducibility of chamber conditions with animals. This was performed on three different days for assessment of replicability of acute 4-h behavioral toxicity. The behavior was measured periodically, and the respiratory physiological parameters were calculated, using the following formulae:

$$\text{volume of dissolved oxygen}\left(\frac{\text{ml}}{\text{dl}}\right) = 0.003 \times P02$$

Experiment 5 Three male and three female Swiss albino mice were exposed to 1 mg/cu.m of Aroclor 1232 for 6 h to assess gender-differences in behavioral scores. Irwin scale was used to assess behavioral scores across all eleven subdomains, and forty-seven functions from six physiological systems in Swiss albino mice.

Broncho-alveolar lavage (BAL) analysis

Experiment 6 Twelve female Swiss albino mice (12–14 weeks age) were assigned to four cages (three mice per cage), weighed and acclimatized. Isotonic (0.65%) sterile BAL ringer was prepared 6.5 g NaCl in 700 ml double distilled water with citric acid as buffer, till p H is adjusted to 7.0–7.2 at temperature of 4 °C. Methanol 1 ppm was used for exposure in the vehicle control group. Aroclor 1232 at 2, 4, and 16 mg/cu.m was used for test group exposures, with

Table 1 Table depicting Irwin behavioral checklist and normative scores for behavioral functions

Behavioral domain	Behavioral sub-domain	Normative score
Awareness		8
	Alertness	4
	Visual placing	4
	Passivity	0
	Stereotypy	0
Mood		4
	Grooming	4
	Vocalization	0
	Restlessness	0
	Irritability/aggression	0
	Fearfulness	0
Motor activity		16
	Reactivity to environment	4
	Spontaneous activity	4
	Touch response	4
	Pain response	4
CNS excitation		0
	Startle response	0
	Straub tail	0
	Tremors	0
	Twitches	0
	Convulsions	0
Posture		8
	Body posture	4
	Limb position	4
Motor incoordination		0
	Staggering gait	0
	Abdominal gait	0
	Righting reflex	0
Muscle tone		16
	Limb tone	4
	Grip strength	4
	Body sag	0
	Body tone	4
	Abdominal tone	4
Reflexes		12
	Pinna	4
	Corneal	4
	IFR (Ipsilateral flexor reflex)	4
Autonomic		20
	Writhing	0
	Pupil size	4
	Palpebral opening	4
	Exophthalmos	0
	Urination	0
	Salivation	0
	Piloerection	0
	Hypothermia	0
	Hypothermia	0
	Skin color	4
	Heart rate	4
	Respiratory rate	4
Miscellaneous		4
	Exploratory activity	2
	Hostility	0
	Social behavior	2
Lethality/dead	Acute	0
	Delayed	0
Total score (normative)		88

three mice per group. Exposures were carried out for 6 h per group, and tracheostomy was performed after termination of exposure under Ketamine (75 mg/kg I.P) – Xylazine (3 mg/kg I.P) using 25 G needle. After tracheostomy was performed, a 40 n. thread was inserted under trachea, 21 G needle with long (25 cm) tube inserted to its other end, and using 1 m L syringe, 500 mcL of BAL fluid was pushed through plunger and aspirated thrice, finally being collected onto 2.5 mcL microcentrifuge tubes (Tarsons Inc.) for centrifuge at 300 g for 5 min till storage at 4 °C, and further analysis after freeze-thawing. Analysis was carried out for cytology (Giemsa stain for Total and differential counts), and UPLC-DAD estimation of PCB 77 and PCB 180; the analysis was carried out using a pre-defined protocol (Ramanujam et al. 2017).

Lung histopathology

Experiment 7 Ice cold PBS perfusate was prepared overnight and after tracheostomy was performed, the trachea and lungs were irrigated with the perfusate. This was followed by dissection of the carinae and / or lung parenchyma for storage in 8% Formalin solution for histopathological analysis at 40× in H&E stain.

Applicability to behavioral toxicology risk assessment: acute toxicity

Experiment 8 An acute Aroclor 1232 toxicity study was used to determine the sex differences and reproducibility

of behavioral scores on three days in response to 4 mg/cu.m exposure dose level for 6 h. For this objective, three male and three female mice behavior were compared for their variability. To ascertain the constancy of animal concentrations from different spatial co-ordinates inside the chamber, all animals were anesthetized for retro-orbital sampling of plasma. PCB 77 and PCB 180 were then quantified in these plasma samples using UPLC-DAD method in these six mice (three males and three females) after 6 h of exposure to 4 mg/cu.m of Aroclor 1232 (Ramanujam et al. 2017).

Benchmark dose derivation

Experiment 9 Acute toxicity study with control, 1, and, 4 mg/cu.m of Aroclor 1232 in 8 female Swiss albino mice each with a pre-defined Bench Mark Response Rate (BMR) from control of ± 50% change in basal behavioral scores and subsequent benchmark dose modeling in the BMDS version 2.4 (BMD Software, US-EPA) using polynomial model (Fig. 2):

$$y\,(\text{dose}) = \text{control} + \text{slope} * \text{dose}^{\text{power}}$$

Applicability to behavioral toxicology risk assessment: repeated dose neurotoxicity

Experiment 10 The optimization was further enhanced by addition of operant or instrumental assessments of mice exposed to Aroclor 1232 aerosols (4 mg/cu.m) for two-week occupational protocol. Briefly the mice were exposed to water aerosols (control group) via inhalational route, followed by washing of chamber thrice with 20% Isopropyl alcohol in methanol. This was followed by exposure to Aroclor 1232 at benchmark dose in similar manner. Occupational exposure was defined as 6 h a day (9 am to 3 pm), 5 days per week for two weekly exposures for this dosing group. Assessments were performed for neuro-cognitive endpoints a baseline, after one week and at termination for each dose level, on Y-maze, elevated plus maze and open field box in both groups. (Narayanan et al. 2017).

Results and discussion

Chamber (Fig. 1b) characterization, validation and optimization protocols were successful in yielding uniform and reproducible results in animal behavior and survival, within acceptable variability limits.

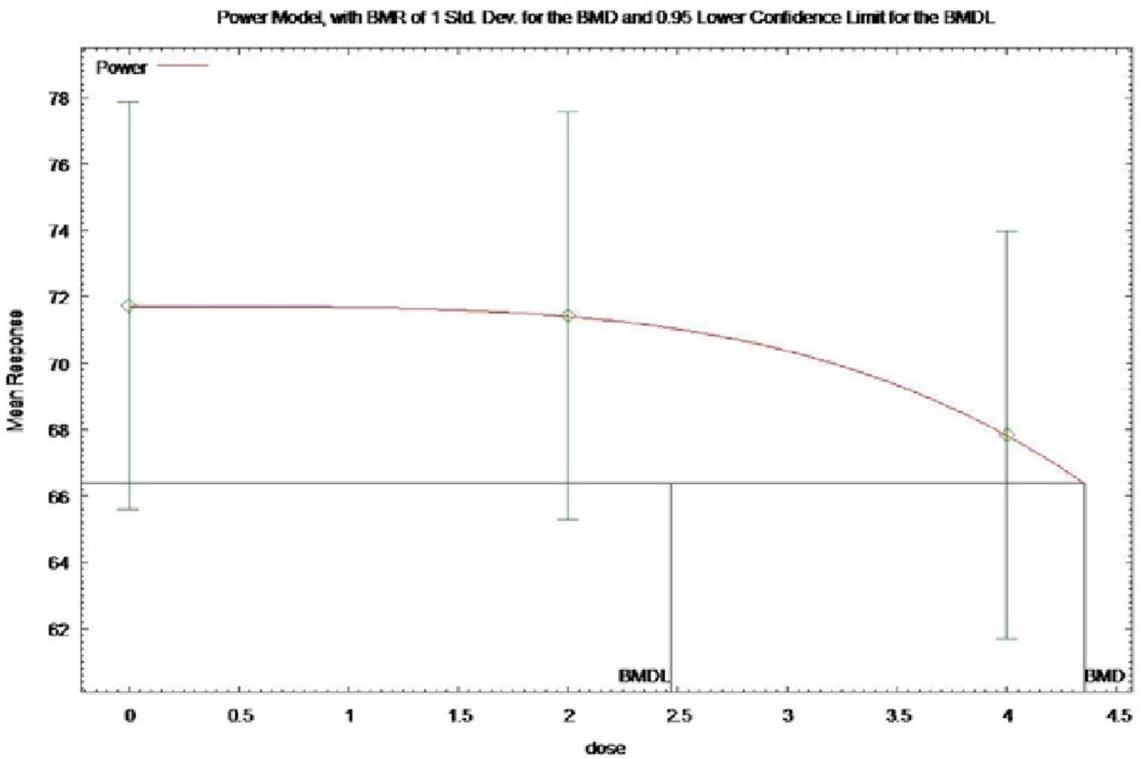


Fig. 2 Dose Response graph depicting the Bench-Mark Dose (BMD) with 95% Limit (BMDL) from the US-EPA BMDS Version 2.6, using Power modeling

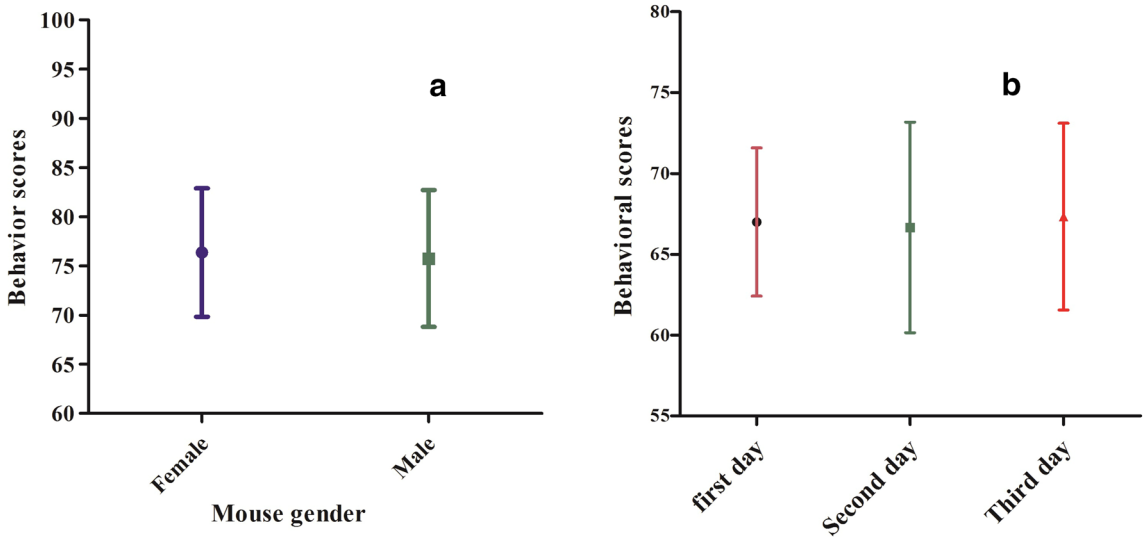


Fig. 3 **a** Chamber optimization for behavioral toxicity. **b** The effects are reproducible acutely. 3-male and 3-female mice were used for all three tests, at 1 ppm dose of water aerosol

Development

The chamber dimensions are well within the CPCSEA-regulatory limit of cage size for laboratory mouse (CPCSEA 2003). We optimized the air flow and PCB flow patterns inside the chamber in-silico using different permutations of inlet and outlet and the physicochemical properties of PCBs. Based on the simulation experiments, the best inlet–outlet configuration was determined to be with two-inlet and single-outlet chamber design (Fig. 3). The inlets were placed below the plane of the contralateral outlet, for optimum flow and uniform particle distribution in the chamber (Table 2). As per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, the minimum requirement for one mouse, each weighing up to 25 g, is 77.4 sq. cm per mouse with a height of 12 cm (cage) (CPCSEA 2003). Using a cascade impactor, we estimated the MMAD to be well within the Organization for Economic Co-operation and Development (OECD) toxicity guidelines (OECD 2009). The MMAD with GSD of the particle sizes was 4.3 µm ± 1.7 µm (Table 3). The time to 95% equilibrium ($T\text{-}_{95}$) in the aerosol under the chamber conditions was found to be 7.27×10^{-1} s. Then after the apparent completion of $T\text{-}_{95}$ as theoretically calculated, the inhalation exposure to test aerosol was initiated at pre-calibrated flow rates and other nebulization parameters.

Characterization

We have analyzed the environmental conditions in the chamber using five different sensors connected to a panel and a software console. Similarly, hourly atmospheric variations did not exceed 20%, which was preserved in our limit test and the sighting study over two-week exposures (Table 4). Oxygen saturation in the chamber was well at 21%.

The optimum ratio of methanol–water was 1:1 with optimum dosing duration of ≥ 90 min, based on minimal behavioral disruptions achieved for this protocol, compared to other methanol to water ratio x dosing duration permutations. The exposure duration for optimization of vehicle-solvent (methanol: water) ratio was below the 4 h as per OECD requirements

Table 2 Table depicting chamber optimization parameters: chamber environment variables assessed using pre-calibrated sensors

Environment variable	Mean ± SD
Flow rate (L/min)	16 ± 1
Oxygen (%)	21 ± 0
Pressure (mm Hg)	735 ± 8
Temperature (°C)	25 ± 0.2
Relative humidity (%)	50 ± 3.2

Table 3 Optimum particle size distribution conditions obtained from MMAD measurements inside the chamber

Particle size (mcm)	Particle distribution (Σm^3)
0.3	16,461,620
0.5	1,219,929
1	94,982
3	6996
5	1696
10	0

for acute inhalation toxicity. Based on this experiment, the behavioral scores did not vary much across different dosing combinations of methanol to water (77 ± 6). However, the variation was minimum with a 1:1 ratio of methanol to water for all durations tested in this phase.

Optimization

After 6-h exposure to 4 mg/cu.m of Aroclor 1232, the entire nine beakers (3 × 3 equal spatial circles) containing samples were quantified for PCB 77 and 180 using UPLC-DAD. PCB 77 and PCB 180 concentrations in the all the beakers were found to be 1795.65 ± 6.41 and 1964.42 ± 18.24 ng/mL. Hence, this indicates uniform distribution of test aerosols across the chamber floor and its vicinity.

No significant change in behavioral scores between male and female mice was observed after exposure to 4 mg/cu.m × 6 h of Aroclor 1232 (Fig. 4).The results of behavioral assessment of Aroclor 1232 acute exposures on three similar occasions with the exposure duration of four hours as shown in Fig. 4. The mean behavioral scores for water aerosol-treated animals were 65 ± 15.

The mean plasma PCB 77 and PCB 180 concentrations were 144.00 ± 16.51 ng/mL and 323.03 ± 34.95 ng/mL, respectively, at 6 h of exposure to 4 mg/cu.m in six (1:1: Male: Female) Swiss albino mice. The minimum variability of concentrations between animals in the group may be due to their movement across different parts of the inhalational chamber and the resultant uniform distribution of the PCB mixture at the bottom of the chamber during the exposures, as shown by the simulation results.

BAL fluid analysis and lung histopathology

The cytological analysis of BAL fluid samples showed dose-dependent leukocytosis with lymphocytes occupying majority of the WBCs in low power under Giemsa stain (Fig. 5a). The control (Methanol) group exhibited minimal signs of alveolar edema in the lung parenchymal specimen (Fig. 5a). There was dose-dependent increase in alveolar inflammation, mild interstitial fibrosis, alveolar edema, tracheitis and eosinophilic and mast cell infiltrates in the treatment groups (Fig. 5b–d). HPLC-FLD analysis of BAL-fluid showed dose-dependent increase in PCB 77 and PCB 180 concentrations (Fig. 5b).

Benchmark dose

The median scores of behavior in control (water aerosol), 2 mg/cu.m, and 4 mg/cu.m Aroclor 1232 dose levels were nearly 72, 71, and 68, respectively, at 2–4 h of single 6 h exposures, respectively (Fig. 4). The change in scores was more than 50% (behavioral depression) in the 2 mg/cu.m versus 4 mg/cu. group, as compared to control versus 2 mg/cu.m group comparison. Using a polynomial model,

Table 4 Normative values for neuro-cognitive indices assessed during the validation of the chamber using water aerosolization for 2 weeks at 1 ppb dose

Paradigm	Index	Week-0	Week-1	Week-2	*P* Value for the temporal interactions
Y-Maze novelty preference Test	NOR index (%)	60.72	56.29	47.30	No significant temporal interaction
Y-Maze alternation test	Alternation index (%)	31.83	17.42	25.28	No significant temporal interaction
Elevated plus maze anxiety test	Transfer latency (s)	31.33	23.00	22.50	No significant temporal interaction
Open field box test	Spontaneous motor activity index	21.95	22.57	25.61	No significant temporal interaction

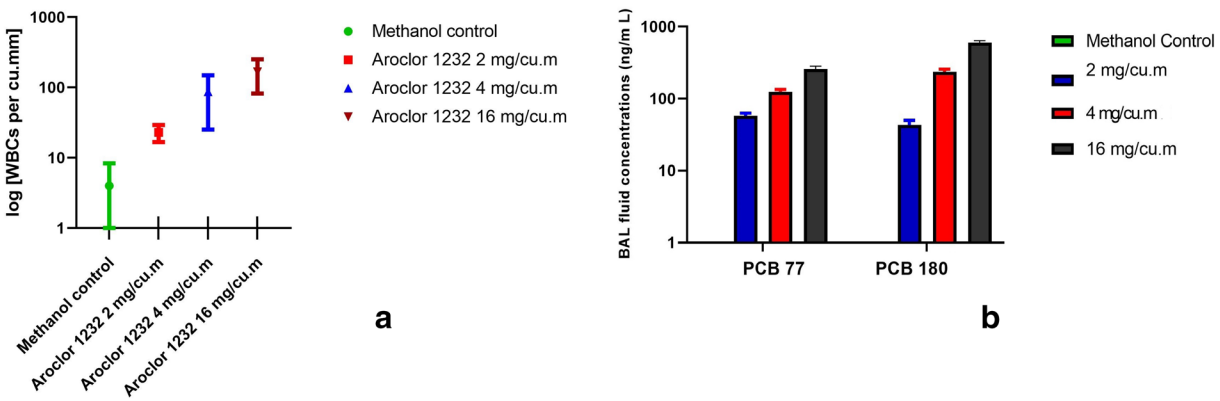


Fig. 4 **a** Bar chart depicting dose-dependent leukocytosis in the BAL-fluid cytology. **b** Bar chart depicting the dose-dependent PCB 77 and PCB 180 concentrations in the BAL-fluid aspirates

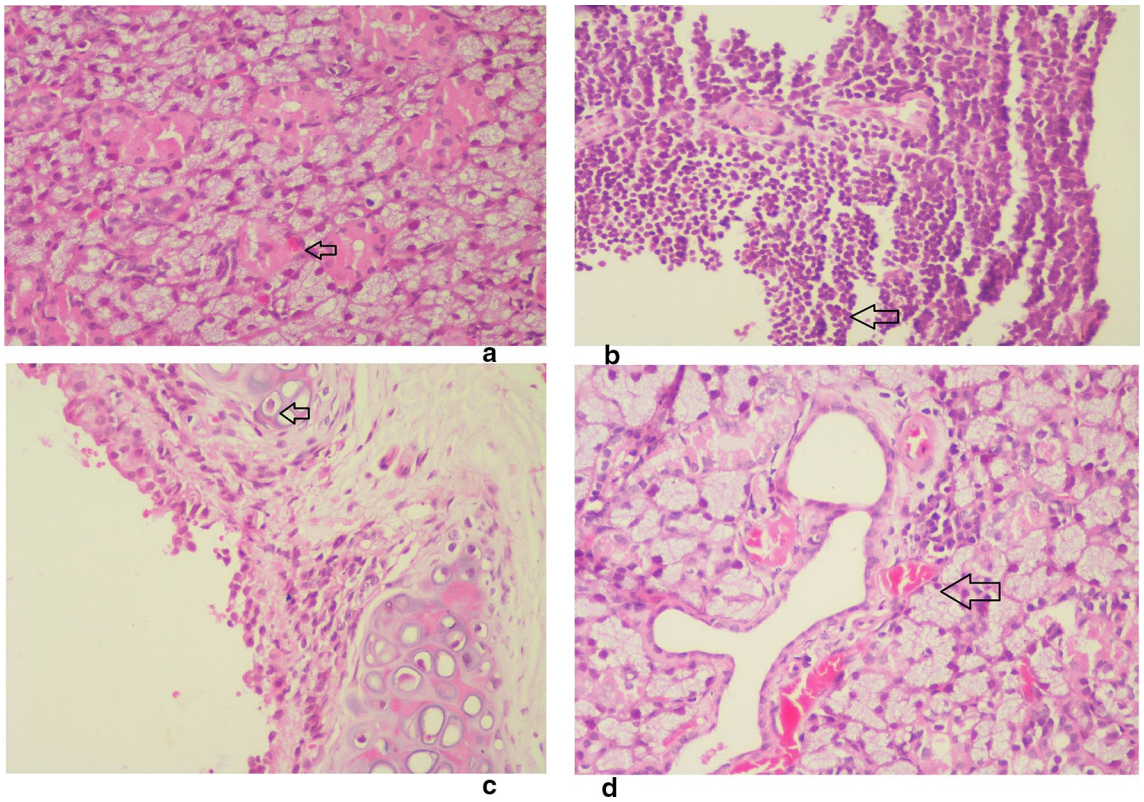


Fig. 5 **a** H&E stain (40x) showing alveolar edema in the methanol group. **b** H&E stain (40x) showing tracheitis in the low dose group. **c** H&E stain (40x) showing dense eosinophilic infiltrates in the intermediate dose group. **d** H&E stain (40x) showing mast cells and alveolar inflammation in high dose group

calculations on the BMD (Bench Mark Dose) Software yielded a BMD of nearly 4.35 mg/cu.m [BMDL = 2.46 mg/cu.m, 95% Confidence Interval for risk of deviation of control values from SD of test dose values, log-likelihood test, $p < 0.003$] for single 6 h exposures.

Sub-acute risk assessment

The neuro-cognitive indices across three time points over two-week water exposure at 1 ppm of water aerosol show preservation of the normative ranges, as shown by the two-way ANOVA (Table 4), for temporal interactions of each mean value. Hence, the chamber can be used for neurobehavioral efficacy or toxicity testing of chemicals by aerosols with minimum temporal variations. Also, the mean behavioral scores showed minimal inter-day and intra-group variations, which correlated well the neuro-cognitive indices of mice.

Discussion

We describe the successful designing, development, and characterization of a whole-body inhalation chamber for exposing mouse to aerosols and pollutant mixtures (Fig. 1b). Inhalation chambers can be used in animals and less commonly in human participants to deliver physical, chemical, or even biological agents to the animal model via the inhalation route. For example, inhalation of smoke from e-cigarettes have shown to decrease phagocytosis and other host defenses in mice following infection with respiratory bacteria and viruses in a whole-body chamber (Sussan et al. 2015). Similarly, using an inhalation chamber, pregnant mice exposed to diesel exhaust particles on daily basis exhibited developmental abnormalities in the off spring as determined by altered brain DNA methylation markers independent of -CpG- islands. Chemical agents like Acrolein have also been delivered to the respiratory tract and detected in the circulatory systems of animal models using inhalation chambers and gas cylinder systems (Tully et al. 2014). Fungal agents

like *Aspergillus conidia* have also been delivered to the respiratory tract of mice as a part of model development using inhalation chambers (Buskirk et al. 2014). Likewise, to evaluate the teratogenic effects of alcohol and alcohol spectrum disorders in mice, vapor chambers were designed and used successfully to deliver alcohol vapors(Morton et al. 2014). Studies assessing inhalation risk with vapor measurements in the upper respiratory tract and silicon dioxide, design of Aluminum chambers for rodent exposures(O'Shaughnessy et al. 2003), histopathological assessment after exposures to quartz exist in publication databases, with provision for risk assessment of diverse xenobiotic exposures.

Although the nose-only exposure chambers for rodents utilize or allow less durations of exposures (Duch et al. 2014) than whole-body chambers because of the differences in the portal of entry of aerosols, the chamber we developed was successfully tested at lower durations also. Unlike earlier semi-open inhalation chambers which were used for chronic dosing at high concentrations, the chamber we have developed is versatile and could be used for dosing low to high concentration aerosols and vapors from acute to chronic protocols. However, we also agree that our chamber can be used only for small laboratory animals including laboratory rodents (OECD 2009). It cannot be used for large animals like rabbits and monkeys which are more closely related to humans than rodents, making extrapolations of research data to humans more reliable in the latter.

Acute or even repeated dose toxicity studies in short term (sub-acute and sub chronic durations) do not affect the histology of lung of mice adversely as reported recently (Umezawa et al. 2013), which was taken as a rationale for acute testing of mice in ours study in the inhalation chamber. Although lung-only or nose-only exposure systems (Weber et al. 2010) would directly deliver the inhalant to the lungs than a whole-body inhalation chamber, they either do not reach lower airways, or do more damage if at all they reach the lower airways, and are better suited for vapors than aerosols. Since the conditions in the chamber were normobaric with Pressures ranging from 731–737 mm Hg, we are certain that the flow rates used will not affect the mouse respiratory physiology adversely.

Though the inhalation chamber was developed to evaluate the effects of pollutant aerosols with complex chemistry as described previously by others (Mainelis et al. 2013; McIntosh-Kastrinsky et al. 2013), the chamber can also be applied for a range of utilities ranging from cardio-respiratory assessments to chronic respiratory and occupational toxicological evaluations of pollutant mixtures. Indeed, it took nearly 0.7 s for the chamber's internal environment to equilibrate, which is very quick; preventing cross-contamination. Recently such assessments have been reported elsewhere using simple exposure systems (Weber et al. 2010; Gatzioufas et al. 2013). Many of these address the designing of nose-only inhalational chamber meant for short-term exposure durations of drug aerosols (Kaur et al. 2020). Nanotoxicology research inputs have also been used previously in the characterization of inhalation chambers (Taquahashi et al. 2013; Yi et al. 2013). Usually, respirable fraction via dry powder inhaler for rodents ranges from 47–54% and that via nebulizer ranges from 34–73% in previous studies(Sinha and Mukherjee 2012). Although nose-only inhalation is preferred to whole-body exposures to deliver drugs to target tissue specifically, whole body chambers simulate occupational exposures more naturally in rodents than nose-only chambers though the deposition of particles in lungs may be similar by both types of inhalation chambers (Pauluhn 2006; Oyabu et al. 2016). Hence, though nose-only inhalation usually achieves greater respirable concentrations and is specific for delivery of aerosol mixtures into the alveoli than whole-body chambers, the mode of aerosol generation which is reflected upon by time to equilibrium ($T\text{-}_{95\%}$) is more important determinant of total body concentrations of test mixtures than the method of aerosol exposure.

Although physical parameters in the chamber like humidity and oxygen saturation are key confounding determinants of the health of animal housed and exposed (Springer et al. 2013; Reis et al. 2013), we have maintained the chamber environment under strict allowed limits to ensure safety of the animals exposed in our study and avoid variations in the study results.

We conclude with certain limitations of our study such as the requirement of aerosol particles to be within the limits set by regulatory authorities may be difficult to meet with due to the complex chemical and unknown physical properties of the inhaled intoxicants and the non-generic nature of our chamber in that it can house small rodents only. We, however, have simulated the chamber conditions and ensured the safety of the housed animals with the uniformity of the results as incorporated in actual chamber development and testing (Olsgard and Smits 2008).

A novel technique of a whole-body inhalation chamber optimization has been reported and this type of biologically-accountable chamber can be used for inhalation neurological or neurotoxicity studies, climatic simulation experiments with animals and pharmacology and physiology studies after dosing drugs via inhalational route, with strict compliance to standard regulatory guidelines. In conclusion, the inhalational chamber developed in the above-mentioned manner is versatile and robust; it could cause minimum neuro-cognitive and behavioral alterations during the sub-acute exposure timelines, in small rodents. This inhalation chamber can be applied in regulatory pharmacology studies (e.g. aerosol/drug nebulization studies), toxicology studies (e.g., pollutants' inhalational risk assessment in rodents), and in biohazard studies (e.g., suspended viral hazards—risk assessments in rodents). It

can be specifically curated to neuropharmacology and neurotoxicity efficacy and toxicity studies in rodents via inhalational route.

Conclusion

The whole-body inhalation chamber has been constructed, developed, optimized, and validated for use in rodents to assimilate the Aroclor 1232 toxicity data at acute and subacute timelines. To achieve this objective and outcome, the in-vitro validation was confirmed with uniformity studies of PCB concentration across different chamber floor quadrants, followed by in vivo penetrability and neurobehavioral risk assessment of PCBs. To this end, Irwin scale and interoceptive memory and higher functional battery of tests were used to evaluate the risk hazard of Aroclor 1232 as an indoor and industrial occupational hazard as per regulatory guidelines for neurotoxicity studies in mice. The environmental and analytical optimization parameters hence developed can be applied to higher rodent models including rats and guinea pigs as per standard animal housing guidance available world-wide. The final output, i.e. the inhalational chamber hence developed could be utilized for risk assessment of complex chemical mixtures, including Aroclors, and related dioxins, via inhalational route, in regulatory toxicology studies assessing persistent environmental pollutants as occupational hazards. However, despite limitations like non-applicability of this inhalational chamber for risk assessment of non-volatile, non-hydrophilic, and non-air pollutant chemicals in rodents, these applications can be further explored, in addition to the currently broad scope of this chamber. Further, the BAL assessments and MMAD measurements confirm lower airway penetrability of PCBs, as a novel regulatory platform, confirming the chamber's robustness and working mechanism.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13762-021-03515-8.

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Author contributions RN: Contributed to the study design and execution, apart from manuscript writing. SM: Contributed to study execution and inputs on chromatography, apart from manuscript editing. VLS: Contributed to BAL-fluid analysis for cytology and lung histopathology. KR: Contributed to chamber design and development. GB: Gave inputs on chamber design, validation, and construction. RS: Expert inputs on chamber design and optimization and in manuscript editing.

Declarations

Conflict of interest The author(s) do not have any conflict(s) of interest(s) to declare.

Ethical approval The study has been performed with incorporation and in compliance with Indian ethical standards for animal research (CPCSEA Guidelines, 2005), and international regulatory standards for toxicological research (OECD Guidance on Inhalational toxicity studies). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted and ethical approval was obtained from the PSG Institutional Animal Ethics Committee, PSG-IAEC Approval Nos. 207/2013/IAEC, 207/2014/IAEC, 207/2015/IAEC [158/PO/ReBi/SL/99/CPCSEA] and PSG-IAEC Approval No. 392/2018/IAEC [158/PO/ReBi-S/Re-L/99/CPCSEA].

References

Buskirk AD, Green BJ, Lemons AR et al (2014) A murine inhalation model to characterize pulmonary exposure to dry Aspergillus fumigatus conidia. PLoS ONE 9:e109855. https://doi.org/10.1371/journal.pone.0109855

Colli Neto JA, Zen Júnior JH, Del Negro A et al (2014) Tobacco experimental model to induce urinary bladder neoplasms. Rev Col Bras Cir 41:56–60. https://doi.org/10.1590/s0100-69912014000100011

CPCSEA (2003) CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol 35:257–274

Duch P, Nørgaard AW, Hansen JS et al (2014) Pulmonary toxicity following exposure to a tile coating product containing alkylsiloxanes. A clinical and toxicological evaluation. Clin Toxicol Phila Pa 52:498–505. https://doi.org/10.3109/15563650.2014.915412

Gatzioufas Z, Hafezi F, Kopsidas K, Thanos S (2013) Dysfunctional uveoscleral pathway in a rat model of congenital glaucoma. J Physiol Pharmacol off J Pol Physiol Soc 64:393–397

Kaur R, Kaushik A, Singh KK et al (2020) An efficient and cost-effective nose-only inhalational chamber for rodents: design optimization and validation. AAPS PharmSci Tech 21:82. https://doi.org/10.1208/s12249-019-1608-4

Kumamoto T, Tsukue N, Takano H et al (2013) Fetal exposure to diesel exhaust affects X-chromosome inactivation factor expression in mice. J Toxicol Sci 38:245–254. https://doi.org/10.2131/jts.38.245

Mainelis G, Seshadri S, Garbuzenko OB et al (2013) Characterization and application of a nose-only exposure chamber for inhalation delivery of liposomal drugs and nucleic acids to mice. J Aerosol Med Pulm Drug Deliv 26:345–354. https://doi.org/10.1089/jamp.2011-0966

McIntosh-Kastrinsky R, Diaz-Sanchez D, Sexton KG et al (2013) Photochemically altered air pollution mixtures and contractile parameters in isolated murine hearts before and after ischemia. Environ Health Perspect 121:1344–1348. https://doi.org/10.1289/ehp.1306609

Morton RA, Diaz MR, Topper LA, Valenzuela CF (2014) Construction of vapor chambers used to expose mice to alcohol during the equivalent of all three trimesters of human development. J vis Exp JoVE. https://doi.org/10.3791/51839

Narayanan R, Gilbert B, Ravichandran S, Bhuvaneswari K (2017) Psychopharmacological characterization of effects of Ferula asafoetida Linn formulation in mouse on a Y-maze, EPM, and open field apparatus. Int J Basic Clin Pharm 6:2254–2258

O'Shaughnessy PT, Achutan C, O'Neill ME, Thorne PS (2003) A small whole-body exposure chamber for laboratory use. Inhal Toxicol 15:251–263. https://doi.org/10.1080/08958370304504

OECD (2009) GD 39, OECD guidance document on acute inhalation toxicity testing. The Environment, Health and Safety Division. Organization for Economic Cooperation & Development, EU

Olsgard ML, Smits JEG (2008) The design, construction, and operation of a whole-body inhalation chamber for use in avian toxicity studies. Inhal Toxicol 20:191–197. https://doi.org/10.1080/08958370701821367

Oyabu T, Morimoto Y, Izumi H et al (2016) Comparison between whole-body inhalation and nose-only inhalation on the deposition and health effects of nanoparticles. Environ Health Prev Med 21:42–48. https://doi.org/10.1007/s12199-015-0493-z

Pauluhn J (2003) Overview of testing methods used in inhalation toxicity: from facts to artifacts. Toxicol Lett 140–141:183–193. https://doi.org/10.1016/s0378-4274(02)00509-x

Pauluhn J (2006) Mosquito coil smoke inhalation toxicity. Part I: validation of test approach and acute inhalation toxicity. J Appl Toxicol JAT 26:269–278. https://doi.org/10.1002/jat.1140

Pauluhn J, Mohr U (2000) Inhalation studies in laboratory animals–current concepts and alternatives. Toxicol Pathol 28:734–753. https://doi.org/10.1177/019262330002800514

Pirela SV, Pyrgiotakis G, Bello D et al (2014) Development and characterization of an exposure platform suitable for physico-chemical, morphological and toxicological characterization of printer-emitted particles (PEPs). Inhal Toxicol 26:400–408. https://doi.org/10.3109/08958378.2014.908987

Raju SV, Tate JH, Peacock SKG et al (2014) Impact of heterozygote CFTR mutations in COPD patients with chronic bronchitis. Respir Res 15:18. https://doi.org/10.1186/1465-9921-15-18

Ramanujam N, Sivaselvakumar M, Ramalingam S (2017) Fast and parallel determination of PCB 77 and PCB 180 in plasma using ultra performance liquid chromatography with diode array detection: a pharmacokinetic study in Swiss albino mouse. Biomed Chromatogr BMC. https://doi.org/10.1002/bmc.4000

Reis RB, Nagato AC, Nardeli CR et al (2013) Alterations in the pulmonary histoarchitecture of neonatal mice exposed to hyperoxia. J Pediatr (rio J) 89:300–306. https://doi.org/10.1016/j.jped.2012.10.003

Shaheen HM, Onoda A, Shinkai Y et al (2016) The ceramide inhibitor fumonisin B1 mitigates the pulmonary effects of low-dose diesel exhaust inhalation in mice. Ecotoxicol Environ Saf 132:390–396. https://doi.org/10.1016/j.ecoenv.2016.06.025

Sinha B, Mukherjee B (2012) Development of an inhalation chamber and a dry powder inhaler device for administration of pulmonary medication in animal model. Drug Dev Ind Pharm 38:171–179. https://doi.org/10.3109/03639045.2011.592532

Springer DJ, Saini D, Byrnes EJ et al (2013) Development of an aerosol model of Cryptococcus reveals humidity as an important factor affecting the viability of Cryptococcus during aerosolization. PLoS ONE 8:e69804. https://doi.org/10.1371/journal.pone.0069804

Sussan TE, Gajghate S, Thimmulappa RK et al (2015) Exposure to electronic cigarettes impairs pulmonary anti-bacterial and anti-viral defenses in a mouse model. PLoS ONE 10:e0116861. https://doi.org/10.1371/journal.pone.0116861

Taquahashi Y, Ogawa Y, Takagi A et al (2013) Improved dispersion method of multi-wall carbon nanotube for inhalation toxicity studies of experimental animals. J Toxicol Sci 38:619–628. https://doi.org/10.2131/jts.38.619

Tully M, Zheng L, Acosta G et al (2014) Acute systemic accumulation of acrolein in mice by inhalation at a concentration similar to that in cigarette smoke. Neurosci Bull 30:1017–1024. https://doi.org/10.1007/s12264-014-1480-x

Umezawa M, Sekita K, Suzuki K-I et al (2013) Effect of aerosol particles generated by ultrasonic humidifiers on the lung in mouse. Part Fibre Toxicol 10:64. https://doi.org/10.1186/1743-8977-10-64

Weber WM, Kracko DA, Lehman MR et al (2010) Inhalation exposure systems for the development of rodent models of sulfur mustard-induced pulmonary injury. Toxicol Mech Methods 20:14–24. https://doi.org/10.3109/15376510903483730

Yi J, Chen BT, Schwegler-Berry D et al (2013) Whole-body nanoparticle aerosol inhalation exposures. J vis Exp JoVE. https://doi.org/10.3791/50263

DOI: 10.14260/jemds/2014/3991

ORIGINAL ARTICLE

AN ANALYSIS OF CHANGING TRENDS IN THE EPIDEMIOLOGICAL, PATHOLOGICAL AND RECEPTOR EXPRESSION OF BREAST CANCERS TREATED IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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Vidhya Lakshmi S, Seyed Rabiya. "An Analysis of Changing Trends in the Epidemiological, Pathological and Receptor Expression of Breast Cancers Treated in a Tertiary Care Hospital in South India". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 69, December 11; Page: 14225-14234,
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ABSTRACT: Carcinoma breast is the commonest malignancy of females all over the world and second leading cause of death due to cancer among females. The incidence of breast carcinoma in India is on the rise. The prognosis of tumors depends upon the size of the tumour at presentation, histological type, grade, marginal clearance and lymph node status. The prevalence of hormone receptor-positive breast cancer in Asian countries has been found to be lower than the western world where more than 50% tumours express hormone receptors. The study aims at determining the incidence and prevalence of hormonal receptors and Her-2-neu in various subtypes of breast carcinoma and the distribution of expression among the epidemiological subgroups of patients of different age groups, parity, reproductive status etc. All cases of breast cancers attending the oncology OPD in a tertiary care hospital in South India during a 2 year period (2012-2013) were taken up for our study. The criteria of selection of patients included availability of histopathological diagnosis, hormone receptor studies, Her-2-neu expression status and follow up till the study period. Breast carcinomas tend to occur more frequently in older and post-menopausal females. However the incidence of breast carcinoma in females below 40 is on the rise. Greater awareness regarding self-examination of breast, screening tests like mammogram and ultrasound and fine needle aspiration cytology should be advocated for an early diagnosis and better prognosis. Risk factors of developing carcinoma breast include obesity, diabetes, estrogen exposure and nulliparity. Most tumors belong to Grade III, probably because patients present to the hospital a late stage of cancer. The prevalence of estrogen receptor positivity is 43.3%, progesterone receptor is 30% and Her 2 neu over expression is 56.6%. Hormone receptor status depends on size, histological type and grade of the tumour. In our study we found that there is no significant association between hormone receptor status and age, menopausal status or number of axillary lymph nodes showing tumor deposits.

KEYWORDS: Breast cancers, tumour grading, hormone receptors.

INTRODUCTION: Carcinoma breast is the commonest malignancy of females all over the world and second leading cause of death due to cancer among females. The incidence of breast carcinoma in India is on the rise. The risk factors of breast carcinoma include long estrogen exposure, nulliparity, obesity and family history of breast cancer. The incidence of breast carcinoma differs in various age groups of patients. Most cancers are diagnosed when patients find a lump during self-examination of their breasts. Mammograms are done as a screening test in many centers and the finding of micro calcifications in a breast lump alerts the treating physician that there is a high index of suspicion of malignancy.

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Fine needle aspiration of the tumour can be done as an out-patient procedure to confirm malignancy. Neo adjuvant chemotherapies before surgeries have improved the over-all prognosis of operable breast cancers. The surgeries for a malignant breast swelling range from lumpectomy to modified radical mastectomy. Nevertheless, breast conservation surgery followed by chemotherapy and hormonal therapy produce almost equal results as radical surgery, thereby ensuring better quality of life after surgery.

The prognosis of tumors depends upon the size of the tumour at presentation, histological type, grade, marginal clearance and lymph node status. Immunohistochemical evaluation of estrogen receptor (ER) and progesterone receptor (PR) status of breast carcinoma has become a routine investigation to predict the response to adjuvant endocrine therapy. The prevalence of hormone receptor-positive breast cancer in Asian countries has been found to be lower than the western world where more than 50% tumours express hormone receptors.

HER-2/neu is the human homologue of the neu gene, called HER-2 or c-erbB-2, which shares extensive homology with epidermal growth factor receptor (EGFR). It is considered one of the poor prognostic factors in breast cancer. The introduction of Herceptin {Trastuzumab}, which is a monoclonal antibody which binds to Her 2 neu receptor has brought significant changes in the prognosis and survival of breast cancer patients.

The study aims at determining the incidence and prevalence of hormonal receptors and Her-2-neu in various subtypes of breast carcinoma and the distribution of expression among the epidemiological subgroups of patients of different age groups, parity, reproductive status etc. The hormonal status has definite prognostic implications and so enables the clinicians to initiate various ancillary modalities of therapy.

AIMS AND OBJECTIVES:

To identify the risk factors of breast Carcinomas:

1. To classify breast carcinomas based on histopathological features
2. To document the estrogen and progesterone receptor (ER & PR) status and Her-2-neu expression of breast cancers
3. To correlate the steroid receptor status of breast cancer with all relevant patient and tumour characteristics.

MATERIALS AND METHODS: All cases of breast cancers attending the oncology OPD in a tertiary care hospital in South India during a 2 year period (2012-2013) were taken up for our study. The criteria of selection of patients included availability of histopathological diagnosis, hormone receptor studies, Her-2-neu expression status and follow up till the study period. The clinical data regarding age, family history, reproductive status, exogenous hormone administration and parity were gathered from the hospital case records. Special care was taken to note the presence of Diabetes mellitus, hypertension, History of tobacco, alcohol and use of oral contraceptives. An approval from the Institutional Human Ethics Committee was obtained prior to our study.

The clinical presentation of the tumour, the size of the swelling, axillary lymph node status, the presence of sub cutaneous lymphatic involvement, ulceration and adherence to the chest wall etc were noted.

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A detailed record of all pre-operative investigations like mammogram, ultrasound, Fine needle aspiration cytology and true cut biopsies was made. Some patients had received pre-operative neo adjuvant chemotherapy and the complete record of it was made too.

The details of surgery performed like radical mastectomies, breast conservation surgeries or toilet mastectomies and presence of any post-operative complications were noted.

The gross specimen reports were reviewed to note the size of the tumour, marginal clearance, involvement of the nipple and areola and presence of ulceration. The number of axillary lymph nodes dissected was recorded in each case.

The histopathological slides were reviewed and the tumour was classified into the following histological types namely infiltrating ductal carcinoma, lobular carcinoma, colloid or mucinous carcinoma, medullary carcinoma, tubular carcinoma and other rarer type of tumors like sarcomas.

The tumours were graded according to the Nottingham modification of Scarff Bloom Richardson (SBR) grading system into Grade I, II and III based on tubule formation, mitotic activity and nuclear pleomorphism.

The margin clearance status, necrosis and lymphovascular invasion were observed. The exact number of axillary nodes showing tumor deposits was recorded.

The paraffin blocks were taken up for immunohistochemical studies using the Avidin Biotin immunoassay method. The markers for Estrogen and Progesterone receptors and Her-2-neu over expression were used. Evaluation of the staining and assigning a score was performed. The staining was evaluated on the invasive component only. Best-preserved and best-stained areas of the sections were assessed. Nuclear staining was assessed for ER and PR while membrane staining was assessed for Her 2.

A score for the proportion of stained cells (0 = no nuclear staining, 1 = <1% nuclear staining, 2 = 1-10% nuclear staining, 3 = 11-33% nuclear staining, 4 = 34-66% nuclear staining and 5 = 67-100% nuclear staining) and the intensity of staining (0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining) were assigned to each tumour. Tumors that showed strong complete membrane staining in >10% of the tumour cells were considered positive.

The observations in each of these were tabulated and critically analyzed. Graphs and charts were made to study the expression of the hormone receptors and Her-2-neu among different histological types, high & low grade tumors. These results were compared with other high risk epidemiological factors.

OBSERVATIONS AND RESULTS: 30 patients were included in our study. The age of the patients varied from 27 to 78. The median age was 54. The distribution of the tumour in various age groups is given below.

7 patients were pre-menopausal and 23 of them were post-menopausal. 2 patients gave a positive history of breast cancer in mother and maternal aunt respectively.

The average parity of the patients was 2.8. Only one patient was nulliparous and she had a grade III tumour with metastatic deposits in the liver. All the 30 patients denied use of tobacco or alcohol. 5 patients had been sterilized and none of them had taken oral contraceptives.

The tumours were classified based on size into 3 groups; < 5cm, 5 to 10 cm and > 30cm. There were 11, 13 and 6 cases in each of these sub groups respectively.

The size of the tumour did not correlate with the grade or receptor status.

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There were 24 cases of infiltrating ductal carcinoma, 2 cases of invasive lobular carcinoma, 1 case of tubulolobular carcinoma, 2 cases of colloid/mucinous carcinoma and 1 case of ductal carcinoma in situ.

Grading of the tumours was done using the Nottingham modification of Scarff Bloom Richardson grading system and tumours were grouped in to 3 categories based on tubule formation, mitotic rates and nuclear pleomorphism. 5 tumours were graded as Grade I, 11 as grade II and 14 as grade III.

Grade of the tumour	Total cases	ER +	PR +	Her 2 neu +
I	5	5	3	4
II	11	4	2	7
III	14	4	4	6

The number of axillary nodes showing metastasis ranged from 0 to 8.

13 patients received neoadjuvant chemotherapy prior to surgery. 20 of them received chemotherapy after surgery also. The patients with estrogen receptor positive tumours received Tamoxifen.

The details of follow up of all these 30 patients were obtained from the hospital case files. 3 patients developed metastases in the liver within a period of 1 year. 1 patient had a cystic metastasis in the brain. 3 of these patients had a primary grade III tumour whereas one had grade II tumour.

3 patients developed a contra lateral tumour in the opposite breast. Interestingly, a patient with ductal carcinoma in situ had carcinomatous deposits in all 20 lymph nodes detected and in due course she developed invasive tumour in the opposite breast.

DISCUSSION: In India there is concern about the rising incidence of breast cancers in younger women. Premenopausal women comprised 50% of the cohort in an urban study conducted by Raina et al.[1] The median age at presentation in their study was 47 years. In our study only 7 patients were premenopausal. The median age of presentation in our study was 54. However 3 patients were below the age of 35. Primary breast carcinomas arising before the age of 40 are far more aggressive and the likelihood of metastasis is higher than those arising in older patients regardless of the hormone status. These issues raise the concern about the lifestyle, use of exogenous hormone administration and the need to implement screening mammograms at an early age.

As mammographic facilities are not widely available here and there is no nation-wide breast-screening program, the commonest mode of presentation remains a lump in the breast, 96% of the patients in the present series presented with a breast lump.

In our study we found that modified radical mastectomy was the most preferred surgery followed by lumpectomy. Raina et al[1] proposed that patient preference for mastectomy is an important reason for the under-utilization of breast conservation therapy. The advanced stage and lack of facility for radiotherapy provoke the treating physicians to opt for radical surgeries. The majority of patients included in the present study had high-grade tumors, which explains the low prevalence of hormone receptor expression. It is often observed that high-grade tumors tend to be negative for hormone receptor expression.

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Thriveni et al[2] proposed that grade of the tumour was directly proportional to the number of axillary lymph nodes. But in our study, there was no significant association between the grade of the tumor and number of lymph nodes.

Estrogen receptor is a well-established predictive and prognostic factor in breast cancer. In the recent International consensus on the treatment of early breast cancer, the panel affirmed that the first consideration was endocrine responsiveness of the tumour and suggested categorization of the tumours into endocrine responsive, endocrine non-responsive and tumours of uncertain endocrine responsiveness. Therefore testing for estrogen and progesterone receptors status is critical to plan optimal treatment in breast cancers.

According to Desai et al[3] the receptor positivity rates are lower in Indian population than in Western countries. This is probably due to the high prevalence of high-grade tumors among the study population. The Nottingham grade and the mitotic count have a significant inverse correlation with the Quick Score of hormone receptor expression. They reported ER and PR positivity rates of 32.6% and 46.1% respectively. Additionally, not all patients in India undergo hormone testing due to financial constraints. In our study ER positivity was found in 43.3% of the cases and PR positivity in 30% of the cases.

Chlebowski et al[4] published a data indicating a different pattern of association for most factors with ER-positive disease than with ER-negative disease. For ER-negative cancers, odds ratios for age, family history, age at menopause, greater parity, smoking, and alcohol use were all close to 1.0. The association between breast cancer risk and chronologic age, race or ethnicity, and age at menopause differed statistically significantly across ER disease subtypes. Age at menarche was not statistically significantly associated with either ER-positive or ER-negative breast cancer.

Our study is in concordance with the above data.

PR^- breast cancers, even if they are ER^+, are more aggressive than the PR^+ phenotype and are more likely to be of high grade than PR^+ tumours. ER and PR were inversely related to HER-2/neu over expression, whereas tumour grade was positively associated with HER-2/neu over expression according to the study undertaken by Huang et al.[5] In our study also, the PR^- tumours were all grade III and 2 of them showed muscle invasion and lymphovascular invasion. Unlike ER, there was only a low tendency for PR to be associated with smaller carcinomas and with older patients, but this tendency was not statistically significant.

Almasri et al[6] said that ER expressing breast carcinomas were, on average, 1.6 cm smaller than carcinomas lacking ER expression. Similarly, 57% of T2 tumors were ER positive as opposed to only 30% of T3 tumors. Our study did not show any significant relationship between ER expression and size of the tumour.

The presence of necrosis and lymphovascular invasion showed an inverse relationship with ER and PR reactivity according to Desai et al.[3] In our study, 2 tumors showed perineural/ lymphovascular invasion and both of these were negative for ER and PR but showed strong membrane positivity for Her 2 neu.

Swain et al[7] declared that whether contralateral breast cancer represents a second primary versus recurrent or metastatic disease clearly has implications for treatment. For example, if contralateral breast cancer represents a second, biologically distinct primary cancer, treatment with Tamoxifen may be beneficial for women with an ER-negative primary cancer who are at risk for

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developing contralateral disease. In our project, only one of the tumours which metastasized to the opposite breast was ER positive and she was treated with Tamoxifen.

Naeem et al[8] reported that Her 2 neu over expression tends to decrease with age. But in our study there was no significant difference in Her 2 neu over expression among patients of different age groups.

Various studies have shown that Her 2 neu over expression is significantly associated with ER and PR negativity. In our study, Her 2 neu was positive in 13.3% cases with positive ER receptor status.

Almasri et al[6] observed that Her 2 neu over expression is directly proportional to the size of the tumour and the number of positive axillary lymph nodes. But in our study we found that there was no relation between the Her 2 neu expression status and the number of positive axillary lymph nodes. Also Her 2 neu over expression was more prevalent in grade II tumours.

In the present study, the overall grade of the tumour correlated well not only with the proportion of cells stained but also the intensity of staining, the two components of the Quick Score that justify the assessment of the intensity of staining in giving a score in an article that was published by Lakmini et al.[9]

Onody et al[10] suggested The HER-2/neu gene status may be useful not only as a prognostic factor in determining clinical outcome, but also as a predictive factor in projecting the response to adjuvant therapies. In our study, most of the Her 2 neu positive cases were given neo adjuvant chemotherapy and were disease free during our follow up period.

Dujaily et al[11] reported that most of the ductal carcinoma cases were purely invasive ductal carcinomas of nonspecific type (74.4%) that showed significant HER-2/neu over expression (65.6%). This provides more evidence of the hypothesis that aggressive tumors seem to show significant HER-2/neu over expression and demonstrates the association between the nature of the biological expression of HER-2/neu by the tumor and its degree of malignancy since it has been argued that nonspecific type ductal carcinomas are the most aggressive variants of breast cancer. The current study demonstrated that 58.3% of invasive ductal carcinoma cases exhibited HER-2/neu expression whereas 100% of invasive lobular carcinomas were Her2 neu positive.

The present study found no significant association between HER-2/neu over expression and age of the patients, which is in concordance with the publication of Dujaily et al.[11]

Li et al[12] proved that lobular and ductal/lobular tumours tended to be diagnosed at a more advanced stage and to be both >5.0 cm and node positive, but they were also much more likely to be hormone receptor positive. In contrast, come do and medullary carcinomas were less likely to have an advanced stage at diagnosis and to be node positive, but more likely to be hormone receptor negative and to have a high grade. Our study reproduced similar inferences.

Guarneri et al[13] proposed that the molecular classification of breast cancer represents the foundation of treatment selection for early and advanced disease. The more clinically useful classification recognizes three subtypes: HR positive (ER and/or PR ≥10%), HER-2 positive (IHC 3+, irrespective of HR expression), and triple-negative tumors (ER, PR, and HER-2 negative). Endocrine manipulation and anti–HER-2 agents represent the foundation of treatment in cases of HR-positive and HER-2–positive tumors, respectively, while, so far, chemotherapy is the only available option in triple-negative tumors. The practice of obtaining biopsies of metastatic lesions varies considerably across centers; therefore, the clinical management of the majority of patients is still based on the

initial assessment. In our study all the 3 tumours which metastasized to the liver were triple negative, whereas the tumour that produced a cystic metastasis in the brain was strongly positive for ER and PR.

Hawkins et al[14] suggested that tumour type, soluble protein content, type of surgery, tumour stage, type of systemic therapy and tumour size (clinical/pathological)] appeared to relate to risk of any recurrence. Eight of these factors also appeared to relate by univariate analysis to risk of distant metastases. And seven (ER concentration, node status, tumour grade, clinical and pathological tumour size, type of surgery and soluble protein content) appeared to relate to risk of death. For risk of local recurrence, however, only tumour grade and ER concentration appeared important. The tumors which metastasized to liver and brain in our study were all grade II/III, size above 5cm and triple negative (except one). But there was no difference in the type of therapy or surgery given to these patients. This was similar to the study done by Sambasivaiah et al [15] in 2007.

CONCLUSION: Breast carcinomas tend to occur more frequently in older and post-menopausal females. However the incidence of breast carcinoma in females below 40 is on the rise. Greater awareness regarding self-examination of breast, screening tests like mammogram and ultrasound and fine needle aspiration cytology should be advocated for an early diagnosis and better prognosis. Risk factors of developing carcinoma breast include obesity, diabetes, estrogen exposure and nulliparity:

- Infiltrating ductal carcinoma is the commonest histological type of breast carcinoma.
- Most tumors belong to Grade III, probably because patients present to the hospital a late stage of cancer.
- The prevalence of estrogen receptor positivity is 43.3%, progesterone receptor is 30% and Her 2 neu over expression is 56.6%
- Hormone receptor status depends on size, histological type and grade of the tumour. In our study we found that there is no significant association between hormone receptor status and age, menopausal status or number of axillary lymph nodes showing tumor deposits.
- Her 2 neu over expression status is inversely proportional to hormone receptor positivity. Poorly differentiated tumours, larger tumours and tumours with a more aggressive histological type express stronger Her 2 neu membrane staining.
- There is no significant association between Her 2 neu over expression and parameters like age, menopausal status and number of axillary lymph nodes.
- Widely metastasizing tumours are usually negative for hormone receptors and Her 2 neu genes.
- The introduction of neo adjuvant chemotherapy has brought about significant improvement in the prognosis of operable breast cancers.
- A regular follow up and better patient awareness will bring about drastic reduction in mortality due to breast carcinoma.

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SUMMARY:

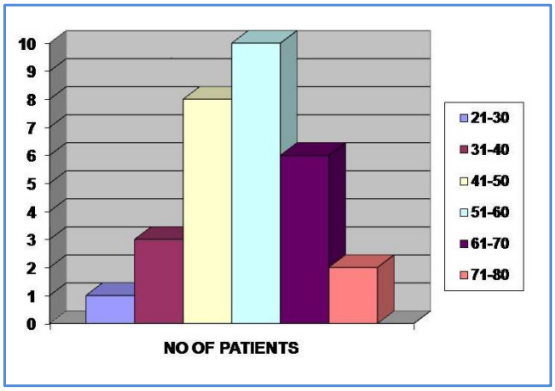
Clinical features (Figures in parenthesis show percentage)	
Age (Median)	54 years
Range	(27–78)
Grade	
I	5 (16.6)
II	11 (36.6)
III	14 (46.6)
Metastatic	4 (13.3)
Bilateral Breast Ca	3 (10.0)
Axillary Lymph node	
Positive	17 (56.6)
Negative	13 (43.4)
Estrogen Receptor	
Negative	13(43.3)
Positive	17 (56.6)
Progesterone Receptor	
Positive	9 (30.0)
Negative	21 (70.0)
Her 2 neu	
Positive	17(56.6)
Negative	13 (43.3)
Chemotherapy	
Neoadjuvant	13
Post-surgery	20

REFERENCES:

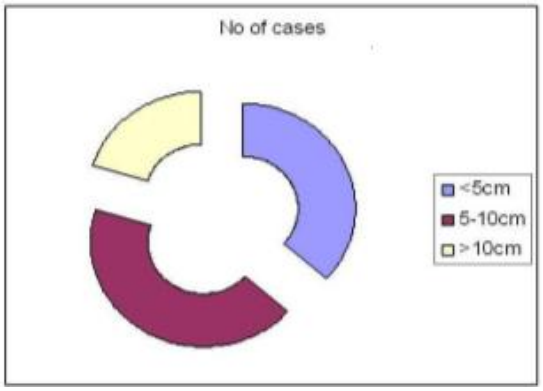
1. Raina V, Bhutani M, Bedi R, Sharma A, Deo SV, Shukla NK, Mohanti BK, Rath GK: Clinical features and prognostic factors of early breast cancer at a major cancer center in North India. Indian J Cancer 2005, 42:40-45.
2. Thriveni K, Vijayalakshmi Deshmane, P.P.Bapsy.Clinical utility of human epidermal growth factor receptor-2 neu detection in breast cancer patients. Indian J of Med Res: 125, 2007, pp 137-142.
3. Desai SB, Moonim MT, Gill AK, Punia RS, Naresh KN, Chinoy RF. Hormone receptor status of breast cancer in India: A study of 798 tumours. Breast 2000; 9: 267-70.
4. Rowan T. Chlebowski, Garnet L. Anderson, Dorothy S. Lane, Predicting Risk of Breast Cancer in Postmenopausal Women by Hormone Receptor Status. Oxford journal 2007:99; 1695-1705.
5. H J Huang, P Neven, M Drijkoningen. Association between tumour characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. J Clin Pathol 2005; 58: 611-616.

DOI: 10.14260/jemds/2014/3991

6. Nidal M Almasri and Mohammad Al Hamad. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. Breast cancer research 2005: 7; 598-694.
7. Sandra M. Swain, John W. Wilson, Eleftherios P. Mamounas. Estrogen Receptor Status of Primary Breast Cancer Is Predictive of Estrogen Receptor Status of Contralateral Breast Cancer, Oxford journal 2004: 96 (13); 1041.
8. Mohammad Naeem, Amir Nasir, Zahid Aman, Tariq Ahmad, Ambreen Samad. Frequency of her-2/neu receptor positivity and its association with other features of breast cancer. J Ayub Med Coll Abbottabad 2008; 20(3).
9. Mudduwa LK. Quick score of hormone receptor status of breast carcinoma: Correlation with the other clinicopathological prognostic parameters. Indian J Pathol Microbiol 2009;52:159-63
10. Peter Onody, BS, Françoise Bertrand, MD, Françoise Muzeau, BS, Ivan Bièche, PhD, and Rosette Lidereau, PhD. Fluorescence In Situ Hybridization and Immunohistochemical Assays for HER-2/neu Status Determination; Application to Node-Negative Breast Cancer. Archives of Pathology & Laboratory Medicine: Vol. 125, No. 6, pp. 746-750.
11. AL-Dujaily EA, Al-Janabi AA, Pierscionek T, Yasseen AA. High prevalence of HER-2/neu overexpression in female breast cancer among an Iraqi population exposed to depleted uranium. J Carcinog 2008;7.
12. C I Li, D J Uribe, and J R Daling Clinical characteristics of different histologic types of breast cancer. British Journal of cancer 2005. 93; 1046-56.
13. Valentina Guarneri, Simona Giovannelli, Guido Ficarra. Comparison of HER-2 and Hormone Receptor Expression in Primary Breast Cancers and Asynchronous Paired Metastases: Impact on Patient Management. The Oncologist 2008. Vol. 13, No. 8, 838-844.
14. RA Hawkins1, AL Tesdalel, ME Killen. Prospective evaluation of prognostic factors in operable breast cancer. Br J Cancer. 1996 November; 74(9): 1469–1478.
15. Sambasivaiah Kuraparthy, Kumaraswamy M Reddy, Lakshmi Amancharla Yadagiri. Epidemiology and patterns of care for invasive breast carcinoma at a community hospital in Southern India. World Journal of Surgical Oncology 2007, 5:56.

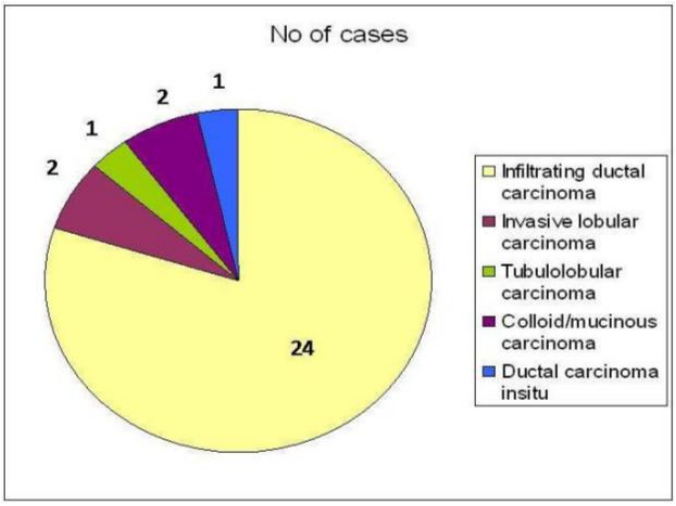


Slide 1

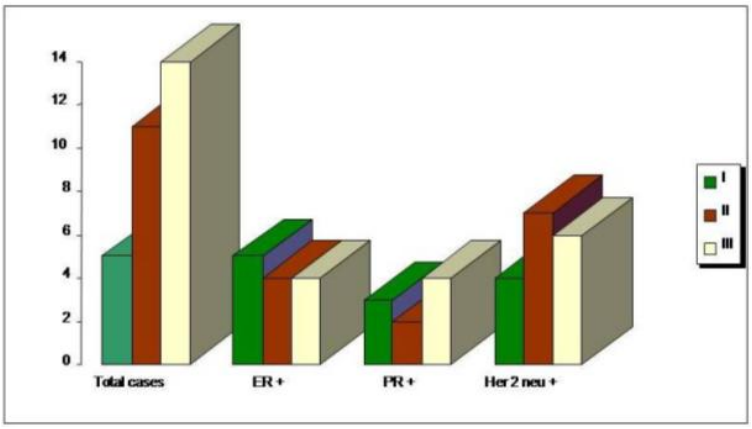


Slide 2

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Slide 3



Slide 4

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Original Research Article

THE EXPRESSION OF BASAL CYTOKERATINS IN BREAST CANCERS

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Abstract-

Introduction: Treatment for breast cancer is based on the expression of the immunomarkers such as ER, PR, and HER2/neu. Cases which are negative to all the three immunomarkers are called Triple Negative Breast Cancers (TNBC) and they have a poor prognosis. Recent studies have shown that some of the TNBCs express cytokeratins CK 5/6 (subcategorizing them as basal-like breast cancers) and these respond well to anthracycline-based chemotherapy.

Aim and Objectives: To study the expression of basal cytokeratins CK 5/6 in breast carcinomas reported in our center and to correlate with histological type, grade, size, clinical features and ER, PR and HER2/neu status.

Methods: Tissues of 44 cases of breast carcinoma diagnosed between June 2009 and May 2014 were retrieved. Immunohistochemical staining for CK 5/6 was done and it was correlated with parameters such as histopathological type, grade, size, invasion and ER, PR and HER2/neu status.

Results: Eight of the breast carcinomas (18%) were categorized as Triple Negative Breast Cancers (TNBC) as they were negative for ER, PR, and HER2/neu. Four of the TNBCs (50%), were positive for CK 5/6. The significant statistical correlation was observed between the size of a tumor and positive CK 5/6 expression. All CK 5/6 positive cases were of high grade.

Conclusion: The routine use of CK 5/6 is recommended in all cases of TNBCs, as 50% of them are positive for these markers. Patients in this subcategory could benefit from anthracycline-based chemotherapy.

Keywords- Triple Negative Breast Cancers, Cytokeratin 5/6, basal-like breast carcinoma

Introduction

Breast cancers are a diverse group of diseases that vary remarkably in terms of clinical presentation, histology, behavior and genetic characteristics [1]. There has been a steady increase in the incidence of breast

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cancers worldwide and especially in the developing countries, mainly attributable to globalization causing adaptation of western lifestyle and improved access to diagnostic modalities. As per the International agency for research on cancer, the number of new cases of female breast cancers in India in the year 2012 was 144,937. [2] The mortality rate in the Indian cohort was 50% compared to that in the USA, where only one woman out 5-6 patients die of breast cancer.

Breast cancers that express Estrogen and Progesterone receptors can be treated by hormonal manipulation [3]. Targeted therapy towards HER2 neu has great success and Trastuzumab has been introduced as an adjuvant drug in those showing overexpression of Her 2 neu [4]. A subset of breast cancers has been found to show no expression of any of the above-mentioned markers. These have been labeled as Triple Negative Breast Cancers (TNBCs). Though hormonal manipulation is of no use in this subset, they have found to show expression of other markers such as basal Cytokeratins and EGFR. They have greater sensitivity to anthracycline-based chemotherapy despite poor pathologic complete response [5].

This study focuses on identifying the cases of breast cancer at our center, performing immunohistochemical studies of the basal Cytokeratin CK5/6 in them and studying their expression and correlation with various clinicopathological parameters.

Materials and Methods

Cases of breast carcinomas diagnosed between the years 2009 and 2014 were included in our study. The study was performed after getting approved by the Institutional Human Ethics Committee (IHEC). A few of the cases were rejected owing to the absence of sufficient clinical information, ER/PR studies or if blocks were unavailable. The requisition form sent by the operating surgeon was used for deriving information such as age, size, nodal status, and other gross findings. Hematoxylin and eosin stained slides from representative sections of the breast tumors were used for grading and assessing the histological type of a tumor, evidence of lymphovascular invasion, perineural invasion and skin involvement. Immunohistochemical staining for Estrogen Receptor, Progesterone Receptor, Her2neu and CK5/6 was performed on these sections after antigen retrieval in pressure cooker followed by EDTA buffer at an alkaline pH (pH of 9).

The antibody reagent clones were Clone EP1 by DAKO, Clone PgR 636 by DAKO, Anti-v-erbB-2 Clone CB11 by Biogenix and FLEX Monoclonal Mouse Anti-human Cytokeratin5/6 (Clone D5/16B4) for ER, PR, Her2neu and CK5/6 respectively. A two-stage process involving binding of the primary antibody to the targeted epitope; second step by identifying a secondary antibody bound to a dextran polymer with the help of horseradish peroxidase enzyme attached to a chromogen.

The various parameters analyzed were age, histological type, size of a tumor, grade, skin, lymphovascular and perineural invasions, number of axillary lymph nodes showing metastasis and staining properties of ER, PR, Her2neu, and CK5/6.

Based on studies conducted by Rakha et al [1], Laakso et al [6] an arbitrary scoring system was drawn up for quantifying the expression of CK5/6. (Table: 1)

Score	Description
0	Less than 1 % positivity
1 +	1-10% tumour cells are positive

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2 +	10-50% tumour cells are positive
3 +	More than 50% of tumor cells are positive

Results

44 cases of female breast cancer were under study. The ages ranged between 33 and 67 years. The age group that had the most number of cases was 41 to 50 years. The commonest histological grade in our study was Grade 2, with 24 cases and 9 cases were grade 3(Fig:1).

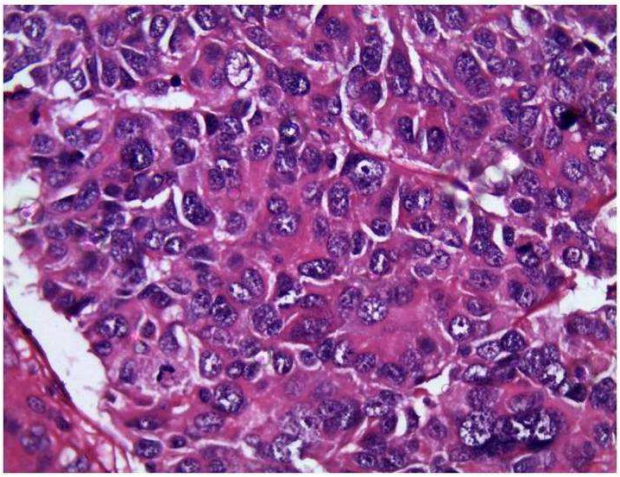


Fig 1 - Invasive ductal carcinoma NOS, H&E 400x

88% of the tumors were of the infiltrating ductal carcinoma, not otherwise specified (NOS). Two cases of micropapillary carcinoma, a case of metaplastic carcinoma and papillary carcinoma were included. Most cases (22) were of sizes between 2cm-5 cm. Lymphovascular invasion was seen in 20 out of 44 cases, with most cases belonging to Grade 2. Perineural invasion (Fig: 2) was seen in only two cases.

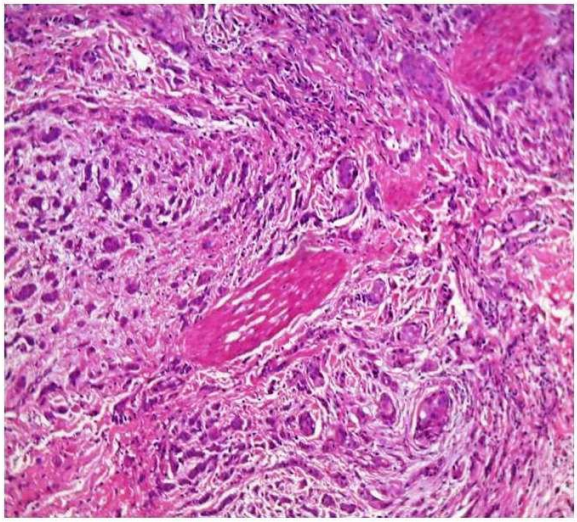


Fig 2 - Invasive ductal carcinoma with perineural invasion, 400x

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"The expression of basal cytokeratins in breast cancers."
Estrogen receptor expression was seen in around 45% of cases and 52% of the cases showed Her 2 neu overexpression. (Fig 3)

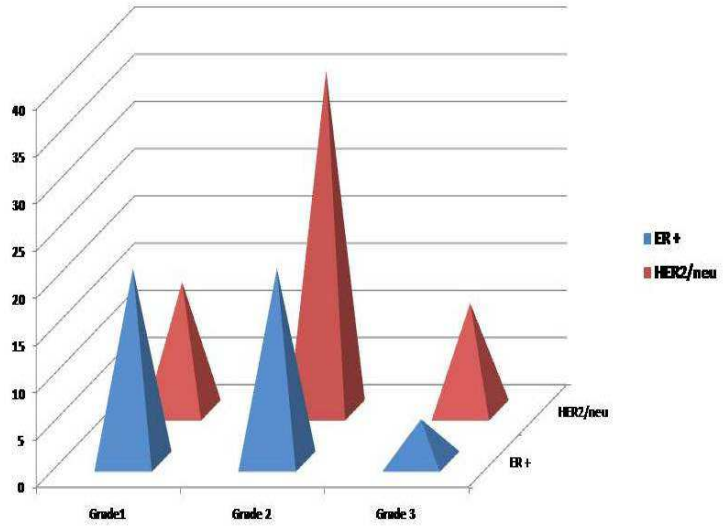


Fig 3 - Histogram showing the distribution of ER/Her 2 neu & grade
K 5/6 positivity. Larger tumors had a greater incidence of CK 5/6 positivity with the largest t Size of a tumor statistically correlated with C a tumour being 13.5 cm in size. (Fig 4)

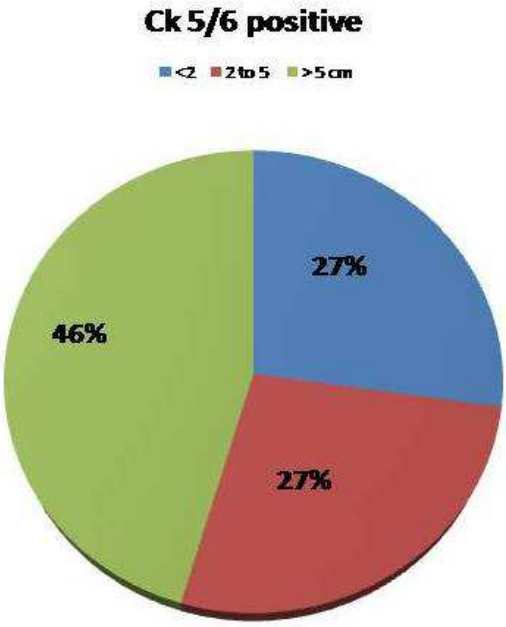


Fig 4 – Pie chart showing CK5/6 positive cases and size of tumors

Correlation between CK 5/6 with invasive and prognostic features was performed and we observed that the tumors with higher grade, lymphovascular, perineural invasion, and extensive lymph node metastases showed greater CK5/6 expression than their respective counterparts. (Fig 5).

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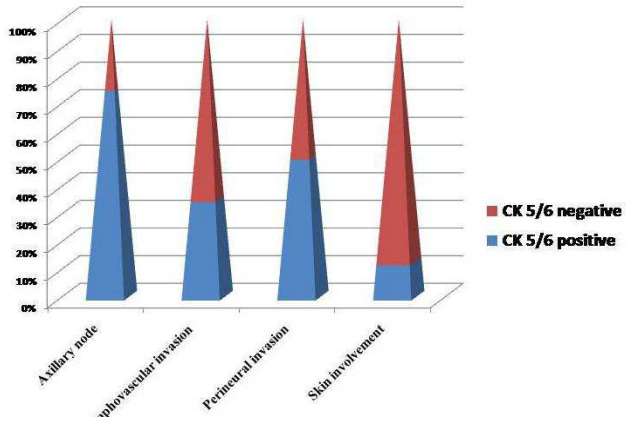


Fig 5 - Histogram showing prognostic parameters vs CK5/6 positivity

Discussion

Breast carcinomas have emerged as the most common malignancy in Indian women. Not only is their incidence high, but the fatality rate of these cases exceeds those of the western population [6]. The cure rates, quality, and length of life have improved in these women after the development of targeted therapy. Glass et al [7] observed quantitative and qualitative trends in breast cancer incidence. There has been a tremendous increase, particularly in ER-positive tumors. The reason for the exponential rise has been attributed to the use of post-menopausal hormone replacement therapy and widespread utilization of mammography.

Breast cancers have been subclassified into 4 molecular subtypes.

1. **Luminal A** – a high expression of luminal cytokeratins and hormone receptors. ER/PR-positive.HER2/neu- negative.
2. **Luminal B** - Expression of luminal cytokeratins are seen. ER/PR-positive.HER2/neu expression-variably positive. Higher grade and proliferation than Luminal A
3. **HER2/neu** - a Low expression of ER. High expression of HER2/neu and 17q12. ER/PR negative. HER2/neu positive. High grade, TP53 mutations present and a higher likelihood of nodal metastasis
4. **Basal-like** - A High expression of basal cytokeratins and epithelial genes. Low expression of hormone receptors. Triple-negative (ER, PR, HER2/neu). TP53, BRCA1 mutations seen.

Treatment response to endocrine therapy is good in 'Luminal type A tumors. The response in 'Luminal B' tumors is not as satisfactory as in Luminal A tumors. Her 2 neu class of tumors responds to Trastuzumab. The basal-like tumors do not respond to endocrine therapy or Trastuzumab

The subsets of breast carcinoma which are not susceptible to conventional therapy, have a paradigm shift in molecular genetics and immunohistochemical expression. These are the Basal-like breast carcinomas and the Triple Negative Breast Cancers.

The overall percentage of ER-positive cases in our study was 45%, lesser when compared with western literature. (Fig 6). This is consistent with findings in a study conducted by Ambroise et al [8], which concluded by saying that hormonal expression is lesser in the south Indian population. We noted that 54% of ER-positive tumors were node positive and most ER positive neoplasms [9] were less than 2 cm in size.

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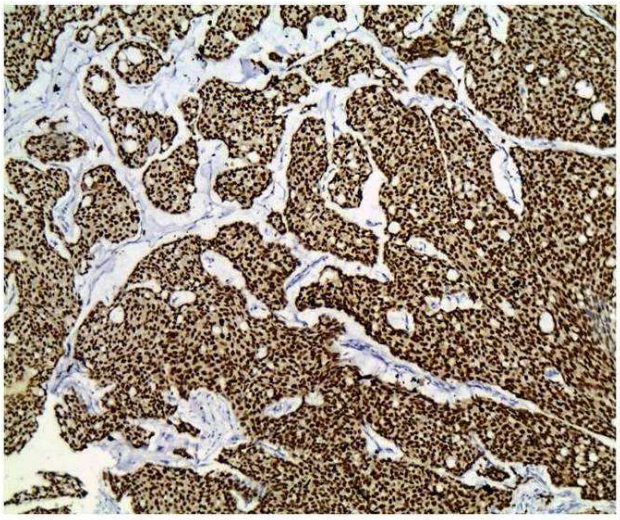


Fig 6 - 100% cells showing strong nuclear staining for Estrogen Receptor

We inferred that due to the large size of the tumors in our study, there was increased nodal metastasis. Almost all the women with ER-positive cases belonged to the 35 to 65 year age group.

Normalization technique was introduced for standardization of results and to avoid discordance between immunohistochemistry and FISH results. There is improved accuracy of HER2 studies using a subtraction scoring system in which a signal score of non-neoplastic breast epithelium is subtracted from that of a tumor [10]. Using this system, the proportion of HER2 positive tumors in our study is 63%. (Fig 7). This stresses the need to look into other markers and their routine use in South Indian cohorts.

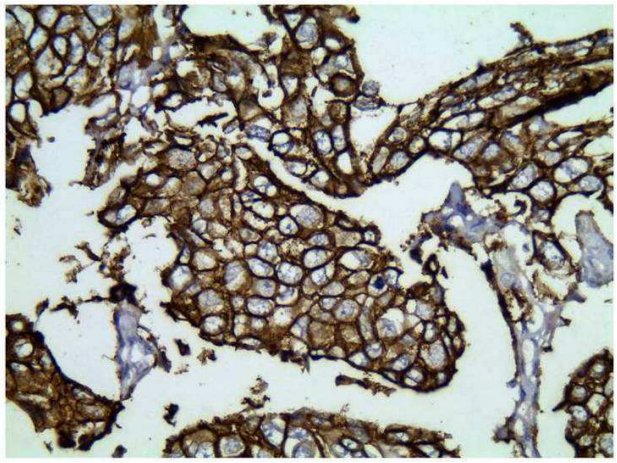


Fig 7 - Cells showing strong and complete membrane staining for Her2neu

Dolle et al [11] inferred that Triple Negative Breast Cancers (TNBCs) are breast cancer subtypes associated with high mortality rate and resistance to hormonal manipulation and Herceptin. Since these tumors are negative for ER, PR, and HER2, newer markers are to be identified for this subtype. These tumors have been seen with increased incidence in younger women (aged 45 years or younger). Our study

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showed close correlation, with the mean age of women with TNBCs being 46 years and nearly a third of the women were 40 or younger.

The purpose of our study was to identify a newer basal marker and observe the expression and clinic-pathological in cases of breast cancer at our center. The basal marker that we selected for our study was CK 5/6[12]. Clark et al suggested that CK5 is positive in breast progenitor cells, which are believed to be the cell of origin in basal-like breast cancers (Rakha) [1]. In our study, 25% of the cases were basal-like, with all of these tumors falling into either Grade 2 or Grade 3. Thus CK 5/6 was positive in 50 % of TNBCs (Fig: 8). The only case of the metaplastic tumor was negative for basal markers.

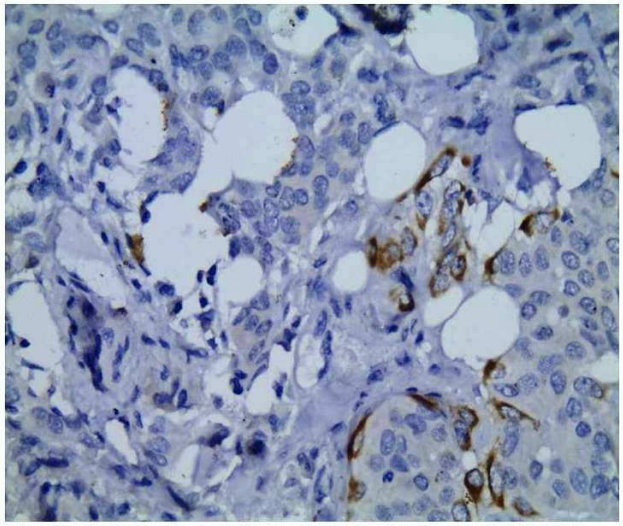


Fig 8 - < 10% cells showing staining with CK5/6 IHC marker, IHC 100 x

Nodal positivity in basal Cytokeratin positive cases in our study was nearly 80%. Vascular emboli were also prominent in these tumors, consistent with their highly invasive nature (Fig: 9). These malignancies are associated with poor prognosis and have a distinctive response to chemotherapy.

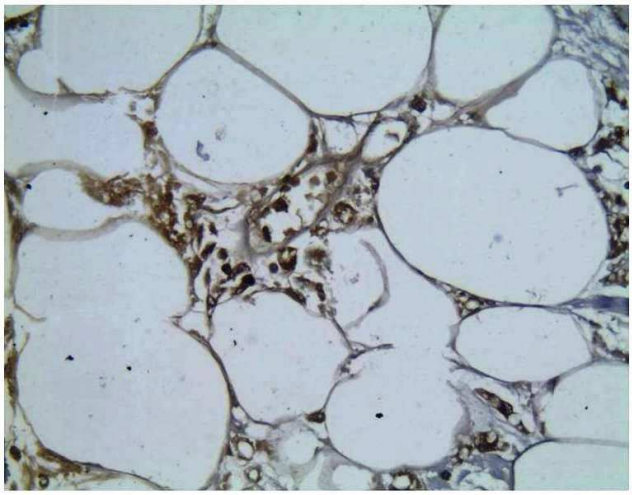


Fig 9 - > 50% cells showing staining with CK5/6 IHC marker, IHC 100 x

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Nielson et al [13] observed that a panel consisting of ER, HER2 and CK 5/6 to identify the basal-like subset was useful as this immunohistochemical combination had a 76% sensitivity and 100% specificity rate when compared with genetic microarray analysis.

The conclusions drawn from our study is that CK5/6 positivity was seen in tumors of larger size and higher grades.

References

1. Rakha E, Reis-Filho JS. Basal-like breast carcinoma-From expression profiling to routine practice. Archives of Pathology & Laboratory Medicine 2009; 133:860-868.
2. http://globocan.iarc.fr/old/factsheet.asp
3. Heatley M, Maxwell P, Whiteside C, Toner. Cytokeratin intermediate filament expression in benign and malignant breast disease. Journal of Clinical Pathology 1994; 48:26-32.
4. Barnes DM, Hanby A M. Oestrogen and Progesterone receptors in breast cancer: past, present, and future. Histopathology 2001; 38:271-274.
5. Glass AG, Lacey J V, Carreon J D, Hoover R N. Breast cancer incidence 1980-2006: Combined roles of menopausal hormone therapy, screening mammography, and Estrogen receptor status. Journal of the National Cancer Institute 2007; 99(15): 1152-61.
6. Laakso M, Tanner M, Nilsson J. Basoluminal carcinoma: a new biologically and prognostically distinct entity between basal and luminal breast cancer. Clin Cancer Res 2006; 12: 4185-91
7. Sarrio D, Rodriguez-Pinilla SM, Hardisson D. Epithelial-Mesenchymal Transition in Breast cancer relates to the basal-like phenotype. Cancer Res 2008; 68:989-997.
8. Doval DC, Sharma A, Sinha R, Kumar K, Dewan AK, Chaturvedi H et al. Immunohistochemical Profile of Breast Cancer Patients at a Tertiary Care Hospital in South India. Asian Pacific J Cancer Prev, 12, 625-629
9. Harvey JM, Clark GM, Osbourne CK and Allred DC. Estrogen receptor status by immunohistochemistry is superior to ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. Journal Of Clinical Oncology 1999; 17(5): 1474-81.
10. Gown AM. Current issues in ER and HER2 testing by IHC in breast cancer. Modern Pathology 2008; 21: S8-S15.
11. Dolle, J. M., Daling, J. R., White, E., Brinton, L. A., Doody, D. R., Porter, P. L et al. Risk Factors for Triple-Negative Breast Cancer in Women under Age 45. Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology, 18(4), 1157–1166.
12. Clark SE, Warwick J, Carpenter R, Bowen RL, Duffy SW, Jones JL. Molecular subtyping of DCIS: heterogeneity of breast cancer reflected in pre-invasive disease. Br J Cancer 2011; 104: 120-7.
13. Torsten O. Nielsen, Forrest D. Hsu, Kristin Jensen, Maggie Cheang, Gamze Karaca, Zhiyuan Hu, Tina Hernandez-Boussard et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004; 10: 5367-74.

Histological Evaluation Of Articular Cartilage Damage Following Radiofrequency Chondroplasty

Vidhya Lakshmi, Rufus V Raj, and Santosh Sahanand

***Abstract*— *Background*: Radiofrequency probes have been used extensively in various arthroscopic procedures. Measurable cartilage damage occurs after radiofrequency probe application. However, the histological changes of cartilage damage following radiofrequency chondroplasty have not been extensively studied.**

***Materials & Methods*: Articular cartilage specimens were obtained from patients undergoing total knee replacement surgeries. Radiofrequency probes were applied at 1, 3 and 5 seconds and samples were sent to histopathology laboratory. The histological changes of cartilage damage were analyzed using the International Cartilage Repair Society (ICRS) assessment scale. Safranin O staining was done to assess the proteoglycan content.**

***Results*: Histologically measurable articular cartilage damage occurs after radiofrequency probes**

***Conclusions*: The time of application of radiofrequency probes should be optimized so as to minimize cartilage damage.**

***Index Terms*— Articular cartilage, damage, Radiofrequency Chondroplasty,**

I. Introduction

In Chondroplasty, Radiofrequency energy (RFE) creates a smooth cartilage surface at the expense of collagen denaturation & dose dependent chondrocyte death especially in the superficial layer. Despite the popularity and promising short term outcomes, histological damage to articular cartilage after Radiofrequency (RF) technology has not yet been extensively studied. The safety profile of RF energy and long term prognosis after treatment with this technology remains a concern for both surgeons and patients. The proposed hypothesis is that at minimal settings, radiofrequency probes cause cell death in measurable areas when applied to human articular cartilage. This article aims at studying the degree of articular cartilage damage using radiofrequency chondroplasty in humans by histological evaluation.

II. Materials and Methods

A controlled laboratory study was performed on articular cartilage specimen samples that were obtained from patients undergoing total knee replacement surgeries after obtaining due informed consent. The study was carried out after clearance from Institutional Ethics Committee.

Cartilage was classified into 4 grades:

- Grade I - softening of the cartilage
- Grade II - partial-thickness fibrillation and fragmentation of the cartilage
- Grades III and IV - extensive fibrillation down to the bone with much of the cartilage worn away.

Only fresh, healthy appearing Grade ≤ II cartilage from the distal posterior femoral condyle was included in this study. To simulate an arthroscopic procedure, samples were placed in normal saline maintained at 37°C in a large metal container. RF probe placed on the cartilage at 1, 3 and 5 seconds at different places.

A total of 90 samples of cartilage (30 cases – 1, 3 and 5 seconds after radiofrequency probes) were received in the department of Pathology. Representative bits were taken from these samples and were subjected to decalcification using nitric acid for 1 day(short DC). The specimens were processed using Leica automatic tissue processor and 3 micron sections were cut using the microtome. The slides were stained using routine Hematoxylin and Eosin stains. All these sections were also stained with Safranin O.

Safranin O is a basic dye that stains growth plate cartilage and articular cartilage (proteoglycans, chondrocytes and type II collagen) in varying shades of red. The intensity of Safranin O stain is proportional to the proteoglycan content in the cartilage tissue. Fast green was used for counter staining to provide contrast. Full thickness, diffuse strong staining is optimal and considered as 100%.

International Cartilage Repair Society (ICRS II) developed a scoring system to evaluate the quality of the repair tissue. ICRS visual assessment scale assesses 6 components – matrix, cell distribution, Subchondral bone & surface, cell population viability and cartilage mineralization.

Overall assessment – Fibrous tissue is indicative of poor quality of the repaired tissue. Articular cartilage is indicative of ideal repair/ regeneration. Fibro cartilage should be graded in the midrange of the scale (average).

III. Results

A. Histological Assessment Of Human Cartilage Repair

Histological assessment may provide an indication of the quality of structural repair because the organization of the tissue as well as of the components present is being evaluated. Articular cartilage is a tissue with specific organization for low friction, efficient load bearing and distribution. The major constituents are chondrocytes embedded in an extra cellular matrix along with collagen fibers and proteoglycans. Changes in structure and

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composition of cartilage lead to abnormal function. The lack of blood vessels and lymphatic supply and the presence of extra cellular matrix restrict the natural repair of cartilage. There is a clear need for a reliable, sensitive, objective and reproducible method to assess the quality of repair tissue.

In 2003, International Cartilage Repair Society (ICRS II) developed a scoring system to evaluate the quality of the repair tissue. ICRS visual assessment scale assesses 6 components – matrix, cell distribution, Subchondral bone & surface, cell population viability and cartilage mineralization.

The following 14 parameters were analyzed based on the scoring criteria proposed by ICRS

1. Tissue morphology – hyaline, fibrous or mixed. Hyaline is glass like without any collagen fibers and hence indicates good quality repair. Fibrous indicates presence of collagen fibers which means poor healing.
2. Matrix staining – the metachromasia indicates the proteoglycan content, i.e. load bearing qualities of the cartilage. Absence of metachromatic staining using Safranin O is considered poor. Full thickness diffuse strong staining is optimal
3. Cell morphology – cells associated with hyaline cartilage are oval with pericellular lacuna. Cells associated with fibro cartilage are elongated and spindle shaped.
4. Chondrocytes clustering – chondrocyte cluster is defined as a group of at least 4 cells. Presence of chondrocyte clustering may indicate remodeling of cartilage during healing.
5. Surface architecture – Smooth surface is indicative of ideal repair/ regeneration. Delamination, loosening and disruptions indicate poor repair.
6. Basal Integration – Integration to subjacent bone is an important feature of cartilage repair.
7. Formation of tide mark – "Tidemark" separates the lower radial zone of cartilage from the underlying zone of calcified cartilage. A single tidemark is a feature of normal healthy cartilage The lack of a visible interface between radial zone and calcified cartilage indicates poor quality
8. Subchondral bone abnormalities/marrow fibrosis – presence of an advanced/irregular Subchondral plate with or without infiltration by fibroblasts/ vascularity (marrow fibrosis) into the subchondral bone marrow is considered as poor tissue repair. Abnormal subchondral bone interface is seen in fractures, sclerosis, disruption and intense remodeling causing fibro vascular infiltration into the marrow space.
9. Inflammation – presence of lymphocytes or plasma cells in repaired cartilage is a negative feature.
10. Abnormal calcification/ ossification within the cartilage repair tissue – indicative of functional impairment
11. Vascularization (within the repair tissue) – No blood vessels in normal articular cartilage. Vascularization seen in fibrous tissue is considered as a negative feature in cartilage repair
12. Surface/ superficial assessment – upper 3rd of cartilage tissue should be intact with elongated cells and collagen fibers parallel to the surface in the most superficial zone. Severely disrupted cartilage with no obvious collagen fiber organization suggest poor healing
13. Mid/Deep zone assessment – lower 2/3rd of tissue – hyaline cartilage should be rich in proteoglycans with no detectable collagen fibers.
14. Overall assessment – Fibrous tissue is indicative of poor quality of the repaired tissue. Articular cartilage is indicative of ideal repair/ regeneration. Fibro cartilage should be graded in the midrange of the scale (average).
15. Safranin O staining – full thickness , diffuse strong staining is optimal and considered as 100%.

These histological parameters and Safranin O staining (intensity and percentage of cells) after radiofrequency probe application for 1, 3 and 5 seconds in the 30 cartilage samples are given below in Table I.

TABLE I: HISTOLOGICAL PARAMETERS AND SAFRANIN O STAINING AFTER RADIOFREQUENCY PROBE APPLICATION

Parameter	**A** **RF- 1sec**	**B** **RF- 3secs**	**C** **RF- 5secs**
Fibrosis of cartilage	2/30	4/30	13/30
Chondrocyte clustering	0/30	7/30	16/30
Basal integration	18/30	11/30	7/30
Lack of interface	2/30	12/30	12/30
Abnormal calcification/vascularity	0/30	2/30	6/30
Safranin O staining < 50%	2/30	5/30	20/30

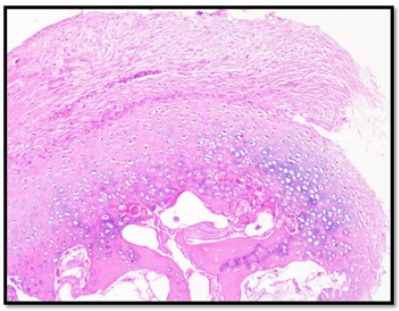


Fig. 1. Normal cartilage (H&E 20x)

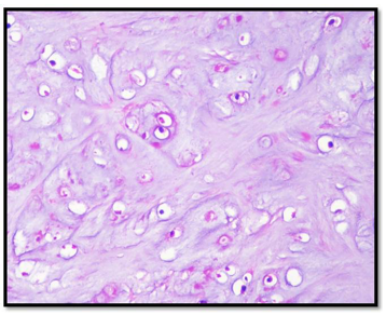


Fig. 2. Fibro-hyaline cartilage (H&E 40x)

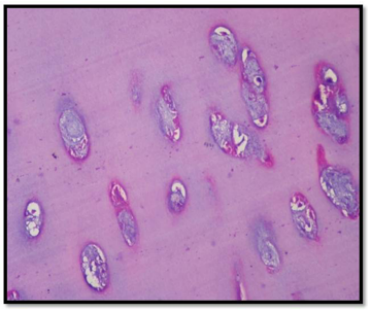


Fig. 3. Fibrous cartilage(H&E 20x)

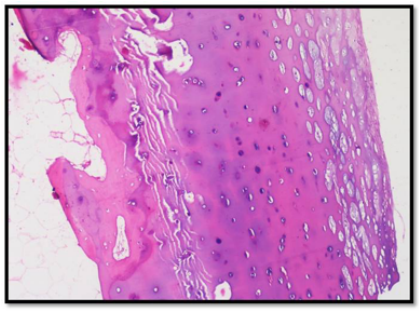


Fig. 4. Lack of interface between radial zone and calcified cartilage (H & E 20 x)

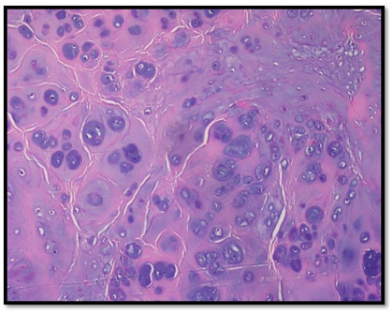


Fig. 5. Clustering of chondrocytes. (H&E 40x)

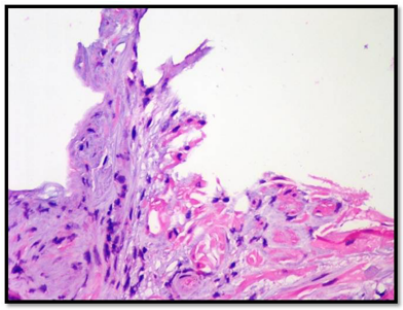


Fig. 6. Increased infiltration by fibroblasts within the marrow space. (H & E 20 x)

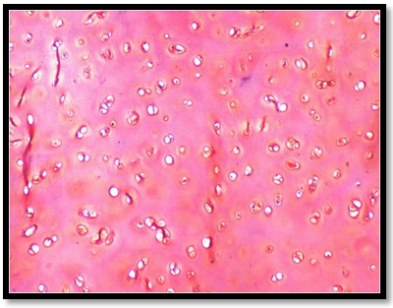


Fig. 7. Safranin O staining (100%) , 40x

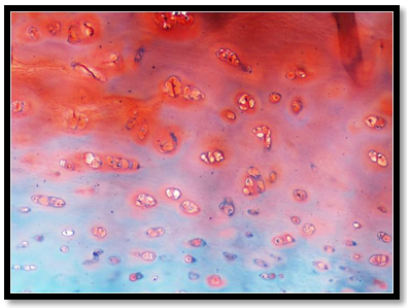


Fig. 8. Safranin O staining (50%) , 40x

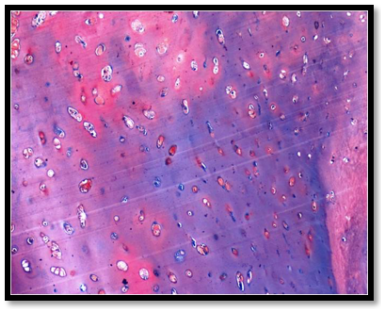


Fig. 9. Safranin O staining (25%) , 40x

IV. Discussion

Radiofrequency probe is increasingly used for arthroscopic procedures. The harmful effects of this probe on cartilage need to be studied to avoid any iatrogenic cartilage damage. Intact articular cartilage provides a smooth, lubricated gliding surface that distributes the loads of articulation in a joint. Injury and disease can alter the mechanical and biochemical properties of articular cartilage. Cartilage injury response varies depending on the extent and type of injury. The assessment of cartilage damage was based only on clinical response of the patients in earlier studies. The present study is unique in that the histological parameters of the cartilage damage were assessed using a scoring system and Safranin O staining of proteoglycan content. The damage to articular cartilage in the form of chondrocyte clustering, lack of interface and abnormal calcification increases with increasing time of application of RF probes. Also there is decrease in Safranin O positive proteoglycan content as time of probe application increases.

V. Conclusion

Histologically measurable articular cartilage damage occurs after radiofrequency probes. Considering the effects of iatrogenic damage to the cartilage, the time of application of RF probes has to be optimized.

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References

[1] Turner AS, Tippett JW, Powers BE, Dewell RD, Mallinckrodt CH. Radiofrequency (electrosurgical) ablation of articular cartilage: a study in sheep. Arthroscopy. 1998;14:585-91

[2] Lu Y, Edwards RB, Nho S, Heiner JP, Cole BJ, Markel MD. Thermal chondroplasty with bipolar and monopolar radiofrequency energy:

effect of treatment time on chondrocyte death and surface contouring. Arthroscopy. 2002; 18:779-88.

[3] Kaplan L, Uribe JW. The acute effects of radiofrequency energy in articular cartilage: an in vitro study. Arthroscopy. 2000; 16:2-5.

[4] Edwards RB, Lu Y, Nho S, Cole BJ, Markel MD. Thermal chondroplasty of chondromalacic human cartilage: an ex vivo comparison of bipolar and monopolar radiofrequency devices. Am J Sports Med.2002;30:90-97

[5] Lu Y, Edwards RB, Kalscheur VL, Nho S, Cole BJ, Markel MD. Effect of bipolar radiofrequency energy on human articular cartilage: comparison of confocal laser microscopy and light microscopy. Arthroscopy. 2001;17:117-23

[6] Stein DT, Ricciardi CA, Viehe T. The effectiveness of the use of electrocautery with chondroplasty in treating chondromalacic lesions: a randomized prospective study. Arthroscopy. 2002;18:190-93.

[7] Owens BD, Stickles BJ, Balikian P, Busconi BD. Prospective analysis of radiofrequency versus mechanical debridement of isolated patellar chondral lesions. Arthroscopy. 2002; 18:151-55.

[8] Lawrence Rosenberg. Chemical basis for the Histological use of Safranin O in study of articular cartilage. J Bone Joint Surg Am, 1971 Jan; 53 (1): 69 -82.



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Incidence, characteristics and outcome of ICU-acquired candidemia in India

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For the SIHAM Candidemia Network.

Take home message: This multicentric study from India on ICU-acquired candidemia highlights the unique epidemiology of this country with its vast spectrum of *Candida* species and high rate of *C. tropicalis* isolation. The disease occurred comparatively early after ICU admission, even in patients with less severe physiology scores.

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-014-3603-2) contains supplementary material, which is available to authorized users.

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Abstract *Purpose:* A systematic epidemiological study on intensive care unit (ICU)-acquired candidemia across India. *Method:* A prospective, nationwide, multicentric, observational study was conducted at 27 Indian ICUs. Consecutive patients who acquired candidemia after ICU admission were enrolled during April 2011 through September 2012. Clinical and laboratory variables of these patients were recorded. The present study is an analysis of data specific for adult patients. *Results:* Among 1,400 ICU-acquired candidemia cases (overall incidence of 6.51 cases/1,000 ICU admission), 65.2 % were adult. Though the study confirmed the already known risk factors for candidemia, the acquisition occurred early after admission to ICU (median 8 days; interquartile range 4–15 days), even infecting patients with lower APACHE II score at admission (median 17.0; mean ± SD 17.2 ± 5.9; interquartile range 14–20). The important finding of the study was the vast spectrum of agents (31 *Candida* species) causing candidemia and a high rate of isolation of *Candida tropicalis* (41.6 %). Azole and multidrug resistance were seen in 11.8 and 1.9 % of isolates. Public sector hospitals reported a significantly higher presence of the relatively resistant *C. auris* (8.2 vs. 3.9 %; $p = 0.008$) and *C. rugosa* (5.6 vs. 1.5 %; $p = 0.001$). The 30-day crude and attributable mortality rates of candidemia patients were 44.7 and 19.6 %, respectively. Logistic regression analysis revealed significant independent predictors of mortality including admission to public sector hospital, APACHE II score at admission, underlying renal failure, central venous catheterization and steroid therapy. *Conclusion:* The study highlighted a high burden of candidemia in Indian ICUs, early onset after ICU admission, higher risk despite less severe physiology score at admission and a vast spectrum of agents causing the disease with predominance of *C. tropicalis.*

Keywords Candidemia · Intensive care unit · *Candida tropicalis* · Risk factor · Mortality

Introduction

Candida bloodstream infection (candidemia) is a life-threatening affliction in intensive care unit (ICU) patients. Its incidence varies from 0.24 to 34.3 patients/1,000 ICU admissions [1–4]. With a high mortality rate of 35–75 % [2], early antifungal treatment is essential for survival. ICU-acquired candidemia patients characteristically have several underlying medical and surgical risk factors, and are frequently exposed to high-risk medications [5]. Furthermore many regions of the world are witnessing a surge in non-*albicans Candida* species [6, 7], which have diverse virulence and susceptibility profiles [8]. Geographic variation in causative species, ethnically diverse at-risk population and varying diagnostic and management capabilities of ICUs further add to the complexity. As there are very few multicentric studies on candidemia from Asian countries, we undertook this study to comprehensively elucidate the disease burden, epidemiology, microbial circulation, resistance pattern and management

challenges of adult ICU-acquired candidemia in India. We aim to utilize this information for better management and survival of these patients.

Methods

Study design

This was a prospective, multicentric, observational study undertaken from April 2011 to September 2012 at 27 medical and surgical ICUs across India. The Postgraduate Institute of Medical Education and Research, Chandigarh, India was the coordinating centre. Ethics approval was obtained from each institution's review board. Being an observational study, it entailed no additional risk to the patients. Prior to commencing work, the study was registered as "SIHAM (Society for Indian Human and Animal Mycologists) Candidemia Network: an observational study" (identifier NCT01281345) with ClincalTrials.gov.

Definitions

A patient older than 18 years of age was considered an adult. Candidemia was defined as the isolation of *Candida* species from blood cultures. A case was defined as ICU-acquired candidemia if it occurred more than 48 h after ICU admission or less than 48 h after discharge from ICU. Patients already diagnosed with candidemia before ICU admission or those transferred from another ICU with a positive culture for any yeast infection were excluded from the study. A patient was defined to have contracted more than one episode of *Candida* bloodstream infection if the subsequent episode occurred more than 30 days after the previous one. Date of onset of candidemia was taken as the date when the first positive blood culture was drawn from the patient. A surgery or invasive procedure undertaken within 30 days of contracting candidemia was considered a potential risk factor. Outcome was determined at the end of 30 days after onset of candidemia and cure was considered complete microbiological resolution. A death attributed to candidemia was ascertained by the treating physician independently on the basis of clinical judgement and microbiology reports.

Data collection

All consecutive patients who contracted ICU-acquired candidemia during April 2011 through September 2012 were enrolled after informed consent. A total of 398 variables were recorded for every patient in standardized proforma. These included demographic details; clinical presentations at admission; severity scores including Acute Physiology and Chronic Health Evaluation II (APACHE II) and Glasgow Coma Scale (GCS); comorbid medical conditions; surgical and invasive procedures; exposure to antibiotics, corticosteroids, anticancer chemotherapeutics, prior antifungals; secondary bacterial infections; candidemia-associated complications; antifungal treatment instituted in the ICU; treatment adverse effects; *Candida* species isolated; antifungal susceptibility and final outcome. All data from participating centres were collected and analysed at the coordinating centre.

Microbiological methods

Candida isolates were identified and tested for antifungal susceptibility at the coordinating centre. Isolates were identified by a Vitek YBC system, and ITS2 and D1/D2 region sequencing was performed when concordance was less than 98 %. Antifungal susceptibility was performed using the Clinical and Laboratory Standards Institute's (CLSI) broth microdilution M27-A3 method [9]. MIC breakpoints were interpreted from the CLSI M27-S4 supplement for yeasts [10]. Isolates with a MIC greater than 1 μg/ml for amphotericin B were considered resistant. Multidrug resistance (MDR) was defined as resistance to two or more classes of antifungals.

Statistical analysis

Descriptive analysis is presented as frequencies and confidence intervals (CI); medians and range; or means and standard deviation (SD), as appropriate. Pearson's Chi square test and Fisher's exact test were used to compare categorical variables, while the Student *t* test, ANOVA, Mann–Whitney or Kruskal–Wallis tests were employed for continuous data. A predictive model for 30-day mortality was developed using logistic regression. Its predictive power was evaluated by the Hosmer–Lemeshow goodness-of-fit test and area under the receiver operating characteristic (ROC) curve. Kaplan–Meier statistics was used for survival analysis. Two-tailed p values <0.05 were taken as significant.

Results

Demography and burden

A total of 215,112 patients were admitted to 27 ICUs from April 2011 to September 2012. Of these 1,400 patients contracted ICU-acquired candidemia, amounting to a burden of 6.51 cases/1,000 ICU admissions (95 % CI

6.18–6.86). Eleven ICUs were from north, three each from east and west, four from central and six from south India (Fig. 1). Eleven were public sector institutions, while 16 were private/corporate hospitals. The incidence varied significantly across regions ($p < 0.001$), with the highest burden from the north (8.95/1,000 ICU admissions; 95 % CI 8.29–9.66) and lowest from west (3.61/1,000 admissions; 95 % CI 2.82–4.63). Of 1,400 patients, 913 (65.2 %) were adults with a median age of 50.0 years (interquartile range 34–63; mean ± SD 49.7 ± 17.7). Further analysis pertains only to adult patients.

ICU presentation

The majority of our adult patients were non-neutropenic (98.7 %) and were admitted to medical ICUs ($n = 464$; 50.8 %). The median duration of onset of candidemia in ICU was 8.0 (interquartile range 4–15) days. Adult candidemia patients had a median APACHE II score of 17.0 (interquartile range 14–20; mean ± SD 17.2 ± 5.9) at admission. GCS was measured in 34 patients with a median score of 15.0 (interquartile range 7–15).

Circulating *Candida* species

The majority of patients experienced only a single episode ($n = 855$; 93.6 %) of candidemia, though 58 (6.4 %) suffered from more than one episode and five (0.5 %) of these were infected with two different *Candida* species during different candidemia episodes. Of the 918 *Candida* strains thus isolated, *Candida tropicalis* ($n = 382$; 41.6 %) was the most prevalent followed by *Candida albicans* ($n = 192$; 20.9 %) and *Candida parapsilosis* ($n = 100$; 10.9 %) (Electronic supplementary material Fig. 1). Eight species caused 92.8 % (852) of the infections while the remaining 7.2 % were inflicted by 22 less common species. Non-*albicans Candida* infections affected 79.1 % (726) of cases. While *C. tropicalis* was significantly higher (44.8 % vs. 37.7 %; $p = 0.035$) in private/corporate ICUs, *C. auris* (8.2 % vs. 3.9 %; $p = 0.008$) and *C. rugosa* (5.6 % vs. 1.5 %; $p = 0.001$) were significantly more common in public sector ICUs. There was no significant difference between times of onset of candidemia when all species were compared. The emerging multidrug-resistant *C. auris* was isolated from 19 of 27 ICUs and comprised 5.2 % of all *Candida*



Fig. 1 Map of India depicting the 27 intensive care units which participated in this study. The *red dot* represents the coordinating centre for this study

isolates. The species was also significantly more common in patients with central venous catheterization (6.8 % vs. 2.9 %; $p = 0.035$).

Antifungal resistance

The antifungal susceptibility profiles of the commonest species are summarised in Table 1. Overall 46.6 % of isolates were sensitive to all antifungals. Resistance to amphotericin B was noted in 19 (2.1 %), azoles 108 (11.8 %), and echinocandin 63 (6.9 %) isolates, respectively. MDR was noted in 17 (1.9 %) isolates, with three (0.3 %) pan-resistant to all antifungal classes. Common MDR isolates were *C. tropicalis* ($n = 4$; 23.5 %), *C. auris* ($n = 4$; 23.5 %) and *C. krusei* ($n = 3$; 17.6 %).

Underlying diseases and risk factors

Underlying respiratory illness was noted in 228 (25.0 %) patients. Pneumonia ($n = 75$; 32.9 %), acute respiratory distress syndrome ($n = 41$; 17.9 %) and chronic obstructive pulmonary disease (COPD) ($n = 35$; 15.4 %) were the major respiratory afflictions. Underlying renal disease was equally prevalent ($n = 209$; 22.9 %), with the majority in acute ($n = 128$; 61.2 %) or chronic ($n = 63$; 30.1 %) renal failure. Malignancy was present in 117 (12.8 %) cases, 82.9 % being solid organ malignancies and 17.1 % haematological. Nearly half of the malignancies (47.9 %) were gastrointestinal while 60.7 % were intraperitoneal. More than a third ($n = 341$; 37.3 %) of patients had undergone one or more surgical procedures in the 30 days or less prior to the onset of candidemia. Nearly half ($n = 165$; 48.4 %) of these comprised gastrointestinal, hepatobiliary and pancreatic surgeries (Electronic supplementary material Table 1). Central venous catheterization was instituted in 74.0 % of patients for a median duration of 8.0 (interquartile range 7–26) days. Parenteral nutrition was instituted in 122 (13.4 %) patients for a median of 9.0 (interquartile range 5–16) days before the onset of candidemia. Median duration of any dialysis performed prior to development of candidemia was 8.0 (interquartile range 2–15) days.

Nearly all candidemia patients were receiving antibiotics ($n = 849$; 93.0 %) with the majority on broad-spectrum agents ($n = 784$; 92.3 %) before the onset of candidemia. The median duration of any antibiotic therapy prior to development of candidemia was 16.0 (interquartile range 7–36) days and that for broad-spectrum agents was 11.0 (interquartile range 4–23) days (Electronic supplementary material Table 1). Corticosteroids were given to 164 cases (18.0 %), at a median dose of 50.0 mg per day (interquartile range 25–100) and median duration of 7.0 (interquartile range 4–12) days before the onset of candidemia.

Antifungal exposure before onset of candidemia was recorded in 143 (15.7 %) cases for a median duration of 8.0 (interquartile range 5–13) days (Electronic supplementary material Table 1). Prior antifungal exposure significantly increased the propensity for subsequent non-*albicans Candida* infection (85.5 vs. 77.9 %; $p = 0.045$) and azole-resistant *Candida* infection (7.1 vs. 3.3 %; $p = 0.045$).

Treatment

Antifungal therapy after definitive diagnosis of ICU-acquired candidemia could be instituted in 59.9 % (547) cases with a median duration of 9.0 (interquartile range 5–15) days (Electronic supplementary material Table 2). Azoles ($n = 394$; 72.0 %) were preferred, followed by echinocandins ($n = 100$; 18.3 %) and amphotericin B (including dexoycholate and lipid preparations) ($n = 79$; 14.4 %). While amphotericin B deoxycholate was used more commonly in public sector ICUs (11.5 vs. 5.3 %; $p < 0.001$), azole (47.1 vs. 37.7 %; $p = 0.006$) and echinocandin (13.4 vs. 7.4 %; $p = 0.005$) use was more common in private/corporate hospitals. Antifungal regimen was altered during the course of therapy in 81 (14.8 %) patients, regimen escalation and antifungal resistance being the major reasons. Only eight (1.5 %) patients on treatment suffered any adverse reactions (Electronic supplementary material Table 2). Central venous catheter (CVC) was removed from 32.1 % (217/676) catheterized patients, with the majority (54.4 %) withdrawn within 48 h of diagnosis. Kaplan–Meier analysis revealed that CVC removal significantly increased 30-day survival (mean ± standard error 19.0 ± 0.9 vs. 15.9 ± 0.6 days; $p = 0.002$). Regression models of the effect of the antifungal therapy and CVC removal on the outcome after adjusting for the comorbidities (APACHE II) showed significant survival ($p = 0.016$) only when treated with antifungal agents with simultaneous removal of the catheter within 48 h (Table 2). Survival was poor in patients treated at public sector hospitals with respiratory and renal disease, invasive ventilation, CVC, dialysis, corticosteroid and polymyxin therapy (Fig. 2).

Outcome and mortality predictors

Candidemia microbiologically resolved in 251 (27.5 %) cases. The 30-day crude mortality was 44.7 % (408/913) and 30-day attributable mortality was 19.6 % (179/913) (Electronic supplementary material Table 1). Univariate analysis between survivors and non-survivors revealed 31 key factors associated with 30-day crude mortality (Electronic supplementary material Table 3). Logistic regression was used to construct a predictive model for 30-day crude mortality ($R^2 = 0.399$, $p < 0.001$,

Table 1 Antifungal susceptibility profile of highest burden *Candida* species circulating among adult candidemia intensive care patients from India, based on the CLSI M27-S4 (2012) guidelines

Antifungal	AFST	All species ($n = 918$)	*C. tropicalis* ($n = 382$)	*C. albicans* ($n = 192$)	*C. parapsilosis* ($n = 100$)	*C. auris* ($n = 52$)	*C. glabrata* ($n = 65$)	*C. rugosa* ($n = 29$)	*C. krusei* ($n = 16$)	*C. guilliermondii* ($n = 16$)
Amphotericin B	MIC_{50} (μg/ml)	–	0.50	0.25	0.50	1.00	0.25	0.25	0.50	0.25
	MIC_{90} (μg/ml)	–	1.00	1.00	1.00	2.00	1.00	1.00	1.00	1.00
	Resistant (%)	2.1 %	4 (1.0)	1 (0.5)	2 (2.0)	7 (13.5)	2 (3.1)	1 (3.4)	0 (0.0)	0 (0.0)
	MIC percentile (25–75)		0.25–1	0.12–1	0.25–1	0.25–1	0.12–0.5	0.12–0.5	0.25–0.7	0.22–1
Fluconazole	MIC_{50} (μg/ml)	–	0.50	0.50	1.00	8.00	0.50	0.50	6.00	0.50
	MIC_{90} (μg/ml)	–	2.00	2.00	4.00	64.00	2.00	8.00	8.00	4.00
	Resistant (%)	6.2 %	10 (2.6)	10 (5.2)	4 (4.0)	16 (30.8)	1 (1.5)	0 (0.0)	16 (100.0)	0 (0.0)
	SDD (%)	11.0 %	9 (2.4)	8 (4.2)	9 (9.0)	7 (13.5)	64 (98.5)	1 (3.4)	0 (0.0)	0 (0.0)
	MIC percentile (25–75)		0.25–1	0.12–1	0.25–1	1–64	0.25–1	0.5–2	3.25–8	0.5–1
Itraconazole	MIC_{50} (μg/ml)	–	0.06	0.06	0.06	0.03	0.03	0.03	0.12	0.12
	MIC_{90} (μg/ml)	–	0.12	0.25	0.12	0.50	0.12	0.12	0.50	1.00
	Resistant (%)	1.2 %	1 (0.3)	1 (0.5)	1 (1.0)	2 (3.8)	0 (0.0)	0 (0.0)	1 (6.3)	1 (6.3)
	SDD (%)	9.3 %	27 (7.1)	22 (11.5)	2 (2.0)	11 (21.2)	4 (6.2)	2 (6.9)	4 (25.0)	4 (25.0)
	MIC percentile (25–75)		0.03–0.12	0.03–0.12	0.03–0.06	0.03–0.18	0.03–0.06	0.03–0.06	0.05–0.25	0.03–0.25
Posaconazole	MIC_{50} (μg/ml)	–	0.03	0.03	0.03	0.06	0.03	0.03	0.12	0.06
	MIC_{90} (μg/ml)	–	0.25	0.25	0.12	0.50	0.12	0.25	0.50	0.25
	MIC percentile (25–75)		0.03–0.12	0.03–0.06	0.03–0.06	0.03–0.18	0.03–0.06	0.03–0.12	0.03–0.12	0.03–0.15
Voriconazole	MIC_{50} (μg/ml)	–	0.12	0.06	0.06	0.50	0.06	0.06	0.25	0.06
	MIC_{90} (μg/ml)	–	0.50	0.50	0.25	1.00	0.50	1.00	0.50	1.00
	Resistant (%)	5.6 %	31 (8.1)	15 (7.8)	3 (3.0)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	SDD (%)	22.9 %	128 (33.5)	58 (30.2)	18 (18.0)	1 (1.9)	1 (1.5)	2 (6.9)	0 (0.0)	0 (0.0)
	MIC percentile (25–75)		0.06–0.25	0.03–0.25	0.03–0.12	0.12–1	0.03–0.12	0.03–0.25	0.12–0.25	0.03–0.07
Anidulafungin	MIC_{50} (μg/ml)	–	0.03	0.03	0.25	0.12	0.03	0.12	0.06	0.12
	MIC_{90} (μg/ml)	–	0.25	0.25	1.00	1.00	0.25	2.00	0.50	2.00
	Resistant (%)	1.7 %	8 (2.1)	2 (1.0)	0 (0.0)	0 (0.0)	4 (6.2)	0 (0.0)	0 (0.0)	0 (0.0)
	Intermediate (%)	1.6 %	8 (2.1)	3 (1.6)	0 (0.0)	–	3 (4.6)	–	1 (6.3)	0 (0.0)
	MIC percentile (25–75)		0.03–0.06	0.03	0.06–1	0.06–0.04	0.03	0.03–0.5	0.03–0.09	0.03–0.62
Caspofungin	MIC_{50} (μg/ml)	–	0.25	0.25	0.50	0.50	0.25	0.50	0.50	0.50
	MIC_{90} (μg/ml)	–	0.50	0.50	1.00	2.00	0.50	2.00	1.00	1.00
	Resistant (%)	5.6 %	16 (4.2)	7 (3.6)	0 (0.0)	4 (7.7)	15 (23.1)	2 (6.9)	3 (18.8)	0 (0.0)
	Intermediate (%)	10.1 %	50 (13.1)	19 (9.9)	0 (0.0)	–	19 (29.2)	–	5 (31.3)	0 (0.0)
	MIC percentile (25–75)		0.12–0.25	0.12–0.25	0.25–0.5	0.25–1	0.12–0.25	0.5–1	0.25–0.5	0.25–0.62
Micafungin	MIC_{50} (μg/ml)	–	0.03	0.03	0.25	0.12	0.03	0.06	0.05	0.12
	MIC_{90} (μg/ml)	–	0.12	0.25	1.00	1.00	0.12	2.00	0.50	1.00
	Resistant (%)	1.7 %	5 (1.3)	2 (1.0)	0 (0.0)	0 (0.0)	4 (6.2)	1 (3.4)	0 (0.0)	0 (0.0)
	Intermediate (%)	2.2 %	11 (2.9)	4 (2.1)	1 (1.0)	–	3 (4.6)	–	1 (6.3)	0 (0.0)
	MIC percentile (25–75)		0.03	0.03	0.06–0.5	0.03–0.25	0.03	0.03–0.5	0.03–0.12	0.3

AFST antifungal susceptibility testing, *MIC* minimum inhibitory concentration, *SDD* susceptible dose dependent

Table 2 Logistic regression model depicting the effect of antifungal therapy and catheter removal (within 48 h of diagnosis) on the outcome of the candidemia patients

Treatment modalities	OR	*P*	95 % CI	
			Lower	Upper
No antifungal + no catheter removal	1.000	–	–	–
No antifungal + catheter removal	1.006	0.996	0.125	8.064
Antifungal + no catheter removal	0.655	0.171	0.358	1.200
Antifungal + catheter removal	0.389	0.016	0.180	0.839
APACHE II at admission	1.117	<0.001	1.058	1.180

OR odds ratio, *CI* confidence intervals, *APACHE II* Acute Physiology and Chronic Health Evaluation II

predictive accuracy = 69.5 %, area under ROC curve = 0.75). The most significant independent predictors of 30-day crude mortality include admission to a public sector hospital, APACHE II score at admission, underlying renal failure, central venous catheterization and steroid therapy (Table 3).

Discussion

This is the first and largest multicentre observational study of ICU-acquired candidemia from India. We found an overall incidence of 6.51 cases per 1,000 ICU admissions, though the incidence varied significantly across the country. The variation may be due to difference in patient groups and management protocol of respective ICUs. In addition, significant variation in the rate of candidemia between private/corporate and public sector hospitals was noted. A large number of patients attend public sector hospital (in the coordinating centre 10,000 new patients attend outpatient departments every working day) as the hospital charges are subsidized, which may lead to compromise in the standard of the health care and higher rate of candidemia. In comparison to the nationwide survey reports from other countries, the incidence in the present study approaches the higher end of values from Australia, France, Germany and the EPIC II study (0.24–6.9/1,000 ICU admission) [1, 11–13], yet is much lower than reported in Spain and Argentina (34.3/1,000 ICU admission) [2].

Our adult candidemia patients were considerably younger (mean 49.7 years) than in other countries (mean 59.0–66.2 years) [2, 14]. Notably our patients contracted ICU-acquired candidemia significantly earlier (8 days) than in other series (11–15 days) ($p = 0.03$) [1, 15]; however, Playford et al. from Australia and Leroy et al. from France have also reported similar early onset of infection [11, 16]. Our patients had lower mean APACHE II scores (17.2) than studies from Spain (20.1), Argentina (20.1) and the USA (18.6) [2, 17]. The reasons for such younger and comparatively less serious patients acquiring candidemia may be due to prior exposures to broad-spectrum antibiotics and steroids in large numbers of our patients and compromised health care.

Our study revealed high prevalence of *C. tropicalis* (41.6 %) while *C. albicans* and *C. parapsilosis* affected only 20.9 and 10.9 % of cases, respectively. This is in contrast to the developed world, where *C. tropicalis* is uniformly less common (5.6–12.0 %) [11, 17–20], and *C. albicans* (45.0–74.0 %) and *C. glabrata* (16.7–22.6 %) are more prevalent [1, 15–17, 20]. We encountered *C. glabrata* candidemia in only 7.1 % of patients. The rise in *C. glabrata* in the Western world can be linked to increased use of azole prophylaxis [1, 11, 15, 21]. Though our patients had a significantly higher propensity of developing candidemia due to non-*albicans Candida* species after azole exposure, such a link is not possible in our patients with *C. tropicalis* candidemia. Prior antifungal exposure ($p = 0.09$), prior azole therapy ($p = 0.24$) or prior fluconazole therapy ($p = 0.26$) was not significantly high in *C. tropicalis* candidemia. Moreover, the majority of our *C. tropicalis* isolates were sensitive to amphotericin B ($n = 378$, 99.0 %), azoles ($n = 344$, 90.1 %), fluconazole ($n = 372$, 97.4 %) and echinocandins ($n = 360$, 94.2 %), thereby indicating interplay of other undetermined factors for their high prevalence. In the present study, we did not attempt to find the possible reasons for the high prevalence of *C. tropicalis* candidemia as it was purely an observational study. A study form Paris and surrounding areas noted pre-exposure to the fluconazole as an independent risk factor for acquiring *C. tropicalis* candidemia [22], whereas the present study did not find this to be an independent risk factor for *C. tropicalis* candidemia. A detailed case–control and environmental study is essential to clarify the reason for the high prevalence of *C. tropicalis* candidemia in India. In an earlier study at the coordinating centre, 82 % of health care providers were found to carry yeast on their hands and 80 % were *C. tropicalis* [23]. Therefore, horizontal transmission and compromise of infection control systems are distinct possibilities for the high rate of *C. tropicalis* candidemia in India. The high rate of *C. tropicalis* candidemia was observed in other Asian countries as well [24]. But no study has evaluated the exact reasons for the high rate of *C. tropicalis* candidemia and hand carriage in these countries. We noted a low prevalence of *C. glabrata* despite prior exposure to antifungal agents in 15.7 % of patients. The higher rate of non-*albicans Candida* infections in patients exposed to prior antifungals was influenced by significantly the higher prevalence of *C. auris* (32.7 vs. 14.8 %, $p = 0.002$) and *C. krusei* (37.5 vs. 15.4 %, $p = 0.029$). In another study Fournier et al. reported a significantly higher proportion of *C. parapsilosis* due to prior exposure to caspofungin [25]. In contrast, Blot et al. reported no change in *Candida* species

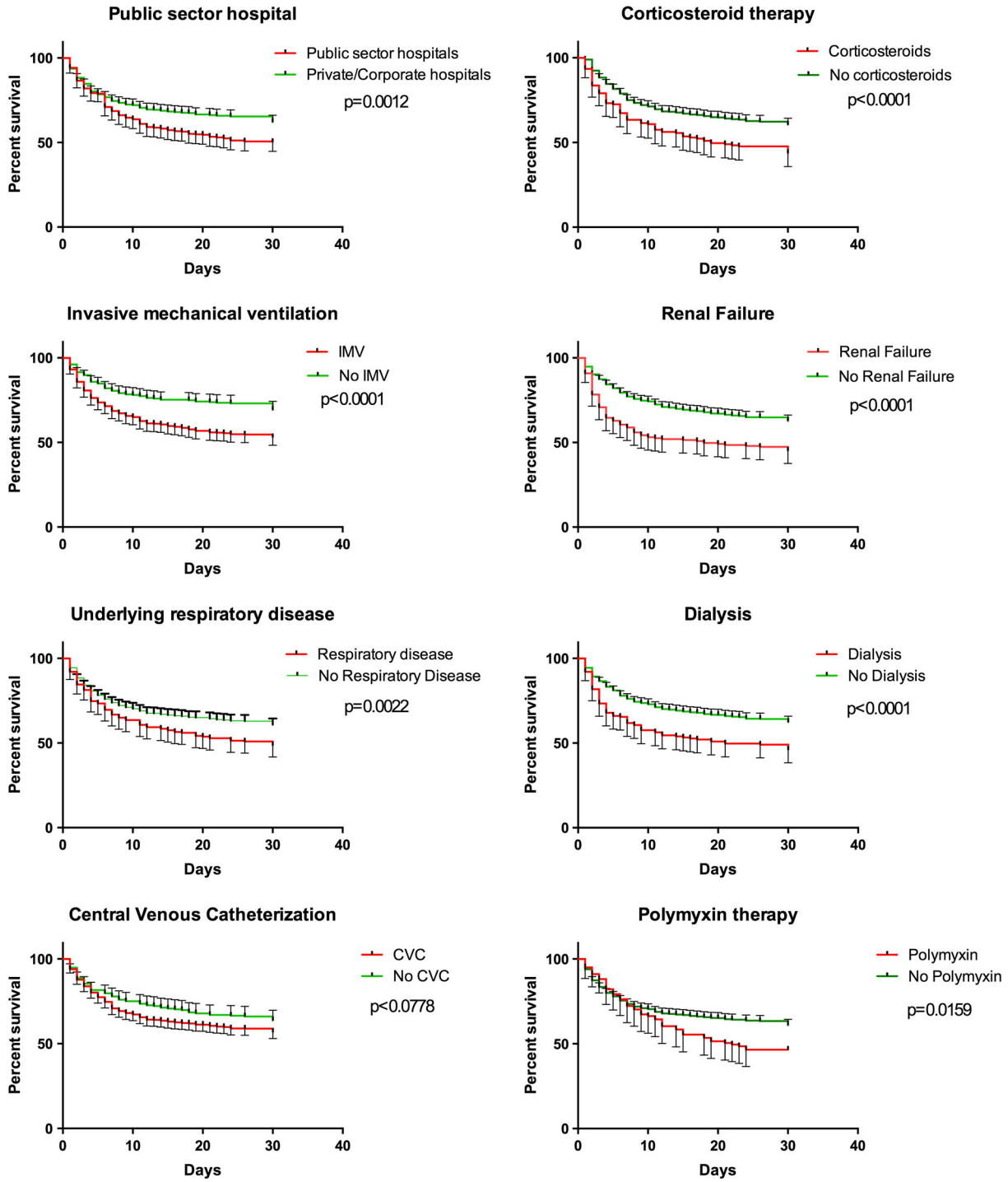


Fig. 2 Kaplan–Meier survival curves (with confidence intervals) according to risk factors for mortality. Comparison of the survival curve was performed by Log-rank (Mantel–Cox) test

distribution despite long-term exposure to fluconazole [26]. Therefore, more studies are required to understand the interplay of the factors responsible for any shift in *Candida* species distribution. In our study, *C. tropicalis* candidemia was more common in private/corporate sector ICUs, while *C. auris* and *C. rugosa* were more prevalent in public sector ones. The emergence of MDR *C. auris* in India is a matter of concern, as this fungus was isolated

Table 3 Logistic regression predictive model summarizing the risk factors for 30-day mortality in adult candidemia intensive care patients

Predictor	OR	*P*	95 % CI	
			Lower	Upper
Public sector hospital	2.062	0.006	1.235	3.446
APACHE II at admission	1.114	<0.001	1.061	1.169
Renal failure	2.237	0.016	1.161	4.312
Central venous catheterization	2.343	0.003	1.330	4.129
Steroid therapy	5.425	<0.001	2.627	11.201
Antifungals treatment for candidemia[a]	0.494	0.007	0.295	0.827

OR odds ratio, *CI* confidence intervals, *APACHE II* Acute Physiology and Chronic Health Evaluation II

[a] Therapy instituted after definitive microbiological diagnosis of candidemia in the ICU

from 19 of 27 ICUs and comprised 5.2 % of all *Candida* isolates in the present study.

Overall, 46.6 % of our isolates were susceptible to all antifungals. Species-specific resistance rates against fluconazole were 2.6 % in *C. tropicalis*, 5.2 % in *C. albicans*, 4.0 % in *C. parapsilosis* and 1.5 % in *C. glabrata*. Corresponding figures from across the globe include *C. tropicalis* (4.5–14.3 %), *C. albicans* (1.4–4.4 %), *C. parapsilosis* (2.7–10.5 %) and *C. glabrata* (5.9–93.8 %) [11, 14, 16, 20]. Likewise, resistance to voriconazole (1.2–5.9 %), itraconazole (4.7 %) and echinocandins (0.3–2.2 %) from international studies are different from our isolates [14, 20]. Low resistance of our *C. glabrata* isolates emphasizes the need to compare our isolates with MDR isolates from other centres, as cryptic species are already known under *C. glabrata* [27]. In contrast to *C. glabrata*, we observed more MDR *C. tropicalis*, *C. auris* and *C. krusei*.

Co-morbidities of our patients are similar to a certain extent to earlier reports. Like in our study, underlying respiratory, renal and gastrointestinal illnesses were significant in other studies as well. But they have also reported sizable proportions of diabetes (10.7–28.0 %), cardiovascular (15.8–48.3 %) and neurological (13.2 %) illnesses in their patients, which were much less common in our cohort, 0.1 %, 15.1 % and 19.5 %, respectively [2, 11–16, 19]. Underlying malignancy was present in 12.8 % of our patients, compared to 24.6–36.1 % in other studies [13, 14]. HIV/AIDS (0.4 %) and neutropenia (1.3 %) were rare, unlike in other series, 4.0–6.0 % and 6.6–19.7 %, respectively [15–17, 19]. A third of our patients (37.3 %) had undergone recent surgery as compared to 44.7–66.1 % seen in other series [2, 11]. Among types of surgery, abdominal surgery is considered a leading risk factor for candidemia [28]. Half of our surgeries (18.1 % of total) also were gastrointestinal, similar to other series (17.0–31.6 %) [2, 12, 28]. Central venous catheterization (74.0 %), invasive mechanical ventilation (52.9 %), urinary catheterization (75.9 %), haemodialysis (17.3 %) and total parenteral nutrition (13.4 %) prior to onset of candidemia were also encountered at lower proportions in our cohort as compared to other series (88.5–100 % [2, 13], 72.1–97.4 % [2, 13], 86.7–97.4 % [2, 16], 17.5–32.5 % [11, 15] and 43.7–71.1 % [2, 11]).

Antifungal therapy after microbiological diagnosis of ICU-acquired candidemia could be evaluated in only 59.9 % of cases because of delayed diagnosis and early death of our patients unlike other studies (74.5–94.1 %) [4, 16–18]. In certain situations the patient left for another hospital or could not afford the therapy. The delay in diagnosis at a few centres was due to use of conventional biphasic media for *Candida* isolation instead of commercial systems. Removal of CVCs and start of targeted antifungal therapy within 48 h of onset of candidemia improved 30-day survival in our patients ($p = 0.016$) like other reports [2, 16, 29, 30], yet the catheter was removed only in one-third of our patients. The reason is difficult to explain. We did not interfere with the existing protocol of any ICU and some critical care specialists believe those studies where a lack of benefit of CVC removal was demonstrated [4, 30].

Our 30-day crude mortality (44.7 %) is similar to the EPIC II international series (42.6 %) [13]. Mortality rates vary across the globe (35–75 %) [2]. Candidemia-attributable mortality also varies widely (5.0–49.0 %) between centres [2, 14, 18, 31] possibly because of lack of standardized criteria. Lower attributable mortality (10.9–21.7 %) has been reported in some studies [3, 20, 32], as compared to the present one (19.6 %).

Comparison of independent mortality predictors modelled in our and other series highlight common and divergent patterns, though the large number (398) of variables studied might act as a limitation of the study by increasing the likelihood of false positive results. However, we have factored in the higher alpha error expected out of multiple testing owing to the large number of variables tested. A stringent Bonferroni's test was used to filter out only those significant variables which stood the test. APACHE II scores in our study proved useful for developing a robust predictive model as has been previously described [30]. Other severity scores like SAPS II and SOFA, central venous catheterization, corticosteroid therapy, total parenteral nutrition, antibiotic use, sepsis/shock and antifungal treatment are common predictors [15–18, 33]. In contrast, admission to a public sector ICU and renal failure appear additional significant predictors for our patients. Similarly, increasing age, underlying diabetes, immunosuppression, neutropenia, *C. glabrata* and *C. parapsilosis* infection, mechanical ventilation, haemodialysis, gastrointestinal surgery and underlying malignancy were unique to other countries, but did not stand out in our study [4, 15–18, 33].

Conclusions

This is the largest prospective multicentre study of ICU-acquired candidemia from India. The overall incidence of ICU-acquired candidemia is high and marked by an early onset infection after ICU admission. Our patients are comparatively young and have lower APACHE II scores at admission. *C. tropicalis* is the leading pathogen in our patients, though its predominance is not explained by higher exposure to prior azoles or antifungals. Emergence of MDR *C. auris* in the majority of ICUs is a matter of concern. Our 30-day mortality prediction model can help triage and manage high-risk candidemia patients better.

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References

1. Meyer E, Geffers C, Gastmeier P et al (2013) No increase in primary nosocomial candidemia in 682 German intensive care units during 2006–2011. Euro Surveill 18(24):20505
2. Gonzalez de Molina FJ, Leon C, Ruiz-Santana S et al (2012) Assessment of candidemia-attributable mortality in critically ill patients using propensity score matching analysis. Crit Care 16(3):R105
3. Nolla-Salas J, Sitges-Serra A, Leon-Gil C et al (1997) Candidemia in non-neutropenic critically ill patients: analysis of prognostic factors and assessment of systemic antifungal therapy. Study group of fungal infection in the ICU. Intensive Care Med 23(1):23–30
4. Charles PE, Doise JM, Quenot JP et al (2003) Candidemia in critically ill patients: difference of outcome between medical and surgical patients. Intensive Care Med 29(12):2162–2169
5. Guery BP, Arendrup MC, Auzinger G et al (2009) Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: part I. Epidemiology and diagnosis. Intensive Care Med 35(1):55–62
6. Lass-Florl C (2009) The changing face of epidemiology of invasive fungal disease in Europe. Mycoses 52(3):197–205
7. Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20(1):133–163
8. Maubon D, Garnaud C, Calandra T et al (2014) Resistance of *Candida* spp. to antifungal drugs in the ICU: where are we now? Intensive Care Med 40(9):1241–1255
9. CLSI (2008) Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard. CLSI document M27-A3, 3rd edn. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA
10. CLSI (2012) Reference method for broth dilution antifungal susceptibility testing of yeasts, fourth informational supplement. CLSI document M27-S4. Clinical and Laboratory Standards Institute, Wayne, PA
11. Playford EG, Marriott D, Nguyen Q et al (2008) Candidemia in nonneutropenic critically ill patients: risk factors for non-albicans *Candida* spp. Crit Care Med 36(7):2034–2039
12. Leroy G, Lambiotte F, Thevenin D et al (2011) Evaluation of *Candida* score in critically ill patients: a prospective, multicenter, observational, cohort study. Ann Intensive Care 1(1):50
13. Kett DH, Azoulay E, Echeverria PM et al (2011) *Candida* bloodstream infections in intensive care units: analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med 39(4):665–670
14. Bassetti M, Merelli M, Righi E et al (2013) Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol 51(12):4167–4172
15. Chow JK, Golan Y, Ruthazer R et al (2008) Risk factors for albicans and non-albicans candidemia in the intensive care unit. Crit Care Med 36(7):1993–1998
16. Leroy O, Gangneux JP, Montravers P et al (2009) Epidemiology, management, and risk factors for death of invasive *Candida* infections in critical care: a multicenter, prospective, observational study in France (2005–2006). Crit Care Med 37(5):1612–1618
17. Pappas PG, Rex JH, Lee J et al (2003) A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis 37(5):634–643
18. Chen S, Slavin M, Nguyen Q et al (2006) Active surveillance for candidemia, Australia. Emerg Infect Dis 12(10):1508–1516
19. Leroy O, Mira JP, Montravers P et al (2010) Comparison of albicans vs. non-albicans candidemia in French intensive care units. Crit Care 14(3):R98
20. Pfaller MA, Messer SA, Moet GJ et al (2011) *Candida* bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in intensive care unit (ICU) and non-ICU settings in the SENTRY antimicrobial surveillance program (2008–2009). Int J Antimicrob Agents 38(1):65–69

21. White MH (1997) The contribution of fluconazole to the changing epidemiology of invasive candidal infections. Clin Infect Dis 24(6):1129–1130
22. Lortholary O, Renaudat C, Sitbon K et al (2014) Worrisome trends in incidence and mortality of candidemia in intensive care units (Paris area, 2002–2010). Intensive Care Med 40(9):1303–1312
23. Chakrabarti A, Chatterjee SS, Rao KL et al (2009) Recent experience with fungaemia: change in species distribution and azole resistance. Scand J Infect Dis 41(4):275–284
24. Mootsikapun P, Hsueh PR, Talwar D et al (2013) Intravenous anidulafungin followed optionally by oral voriconazole for the treatment of candidemia in Asian patients: results from an open-label phase III trial. BMC Infect Dis 13:219
25. Fournier P, Schwebel C, Maubon D et al (2011) Antifungal use influences *Candida* species distribution and susceptibility in the intensive care unit. J Antimicrob Chemother 66(12):2880–2886
26. Blot S, Janssens R, Claeys G et al (2006) Effect of fluconazole consumption on long-term trends in candidal ecology. J Antimicrob Chemother 58(2):474–477
27. Lockhart SR, Messer SA, Gherna M et al (2009) Identification of *Candida* nivariensis and *Candida* bracarensis in a large global collection of *Candida* glabrata isolates: comparison to the literature. J Clin Microbiol 47(4):1216–1217
28. Leon C, Ostrosky-Zeichner L, Schuster M (2014) What's new in the clinical and diagnostic management of invasive candidiasis in critically ill patients. Intensive Care Med 40(6):808–819
29. Blot SI, Vandewoude KH, Hoste EA et al (2002) Effects of nosocomial candidemia on outcomes of critically ill patients. Am J Med 113(6):480–485
30. Nucci M, Anaissie E, Betts RF et al (2010) Early removal of central venous catheter in patients with candidemia does not improve outcome: analysis of 842 patients from 2 randomized clinical trials. Clin Infect Dis 51(3):295–303
31. Gudlaugsson O, Gillespie S, Lee K et al (2003) Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis 37(9):1172–1177
32. Das I, Nightingale P, Patel M et al (2011) Epidemiology, clinical characteristics, and outcome of candidemia: experience in a tertiary referral center in the UK. Int J Infect Dis 15(11):e759–e763
33. Colombo AL, Guimaraes T, Sukienik T et al (2014) Prognostic factors and historical trends in the epidemiology of candidemia in critically ill patients: an analysis of five multicenter studies sequentially conducted over a 9-year period. Intensive Care Med 40(10):1489–1498

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RESEARCH ARTICLE

Coexistence of Metallo Beta Lactamase Resistant Gene Variants Among Clinical Isolates in Tertiary Care Hospital

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Abstract: ***Background*:** Carbapenem-resistant Enterobacteriaceae (CRE) has emerged as a global threat with mortality risk ranging from 48%-71% worldwide. The emergence of MBL resistance is threatening as carbapenem is one of the last line antibiotics. A total 24 variants of NDM resistance raises a concern to the clinicians and epidemiologists worldwide.

***Objective*:** The study aims at identifying MBL resistance (NDM, IMP, VIM, GIM, SPM, and SIM) and its coexistence in clinical isolates in a single tertiary care center.

***Methodology*:** Forty five clinical isolates characterized phenotypically for Carbapenem resistance obtained from PSG Institute of Medical Science and Research (PSG IMSR), Coimbatore, between February to March 2018 were taken for analysis.

***Results*:** Out of the 45 Clinical isolates, 38 isolates (84%) were detected as MBL carriers. VIM, NDM, GIM, and SPM were the predominant resistance genes, with detection rates of 48.8%, 28.8%, 24.4%, and 22.2% respectively. Fifteen isolates were observed to harbor more than one MBL gene in coexistence. Two isolates - U42 and R714 (K. pneumoniae) were found to harbor all 5 MBL variants in combination.

***Conclusion*:** 33% of clinical isolates harboring multiple MBL variants is a concern in clinical settings. The presence of SPM and GIM gene amongst isolates in this geographical location within India is an indicator demanding continuous monitoring of these resistance determinants.

Keywords: Clinical isolates, carbapenem resistance, enterobacteriaceae, metallo beta lactamase, multidrug resistance, MBL variants.

1. INTRODUCTION

There is a global concern due to the threat posed by a significant increase in infection caused by Multidrug Resistant (MDR) gram negative bacteria in recent years. Greater challenges prevail in countries like India. According to the 2013 estimate, around 58,000 newborns die in India each year due to sepsis from antibiotic resistant bacteria [1]. Carbapenems are powerful broad-spectrum antibiotics that are the last-line antibiotics by the clinicians particularly for critically ill MDR patients [2]. Carbapenemase (carbapenem hydrolyzing enzymes) are differentiated into two major groups by their hydrolytic mechanism in the active site. The first group of enzyme, serine carbapenemases contain a serine in the active site and the second group of enzyme which contains one or more zinc atoms at the active site represents Metallo beta lactamases (MBL) belonging to the class B carbapenemases [3].

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MBL was discovered in the 1960's with low pathogenic potential. In the1990's, MBL genes found to be associated with mobile elements gained clinical importance with the dissemination of carbapenem-resistant *Enterobacteriaceae* worldwide [4]. The denoted variants of the most clinically paramount MBL groups, VIM (Verona Integron-encoded Metallo-β-lactamase), IMP (Imipenemase), and NDM (New Delhi Metallo-β-lactamase), whereas the other groups like GIM (German imipenemase), SIM (Seoul imipenemase), SPM (Sao Paulo Metallo-β-lactamase), AIM (Adelaide imipenemase) are rare [5]. Carbapenem resistance levels for MBL-producing strains vary and mortality rate ranges from 18% to 67% [6]. The IMP and VIM type MBLs are currently reported as a serious threat. The MBL, SPM-1 identified in 1997 is widespread in Brazil but is restricted to *Pseudomonas aeruginosa* until recently [7]. The GIM variant was reported in a non transferable plasmid in Germany (2002) [8] and SIM was discovered in Class 1 integron in Korea (2005). These variants are endemic in nature [9]. Detection and spread of NDM in 2007 raised a serious concern towards these resistance genes [6].

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South Indian reports revealed the presence of independent MBL genes from tertiary care centers. The present study was focused on detecting the presence of MBL gene(s) (and its variants) and to assess its coexistence in a variety of clinical samples. Detection was done through Direct PCR amplification rather than a conventional PCR approach. Further, Multiplex PCR strategy has been optimized to detect the co existing clinically relevant MBL genes.

2. MATERIALS AND METHODS

Forty five Carbapenem resistant clinical samples were identified from the PSG Institute of Medical Science and Research (PSG IMSR), Coimbatore. These isolates were selected to suit the diversity of sources from the regular antibiotic screening procedure followed in the center between February- March. The isolates were obtained from Respiratory (R), Blood (B), Urine (U) and Miscellaneous (M) samples. The clinical records of the samples, species, source of infection, for antibiotic resistance, antibiotic sensitivity were obtained from PSG IMSR and are listed in the order of samples selected for analysis (Table **1**).

2.1. Confirmation for MBL Genes

Colony PCR and Direct PCR were used for molecular detection of the MBL resistance. PCR is one of the gold standards for pathogen identification.

2.1.1. Colony PCR

The colony PCR was performed as per the protocol described by a study [10]. The single bacterial colony was added in eppendorf tube containing 25µl of TE buffer. Then, the tube was heated in the water bath for about 10 mins at 95°C. The mixture was centrifuged for 2mins at maximum speed. The supernatant was taken for PCR analysis.

2.1.2. Direct PCR

One loop full of the clinical sample (Sputum/ Tracheal aspirate) was taken and added in eppendorf tube containing 50µl of TE buffer. Then, it was subjected to soft vortexing followed by incubation in a water bath for 10 mins at 65°C. 2µl of the sample was directly used as a template for PCR. In the case of Urinary sample, 10µl of sample was taken and processed in the same way [11].

2.1.3. Multiplex PCR

After the independent amplification of MBL genes using Colony PCR and Direct PCR, Multiplex PCR (mPCR) was standardized with specific primer combinations to identify MBL genes.

The PCR conditions were as follows: 94°C /5 min; 36 cycles of 94°C /30 s, 55°C/40 s (depend on specific primer pair) and 72°C/50 s and a final elongation step at 72°C/5min. Primer combinations used are listed in Table **2** [12-15].

3. RESULTS AND DISCUSSION

The present study was carried out to detect the Metallo beta lactamase genes *viz.,* IMP, VIM, SIM, SPM, GIM and NDM in clinical isolates by performing PCR (using 16s rDNA as internal control). Out of the 45 samples collected from varied sections of the hospital, 22 were *K. pneumoniae*, 16 were *Acinetobacter spp*, 4 from *P. aeruginosa*, 2 from *E. coli* and only one from *Enterobacter spp*. The carbapenem resistant isolates taken for the study included, 26 respiratory specimens (57%), 10 urine specimens (22%), 5 from miscellaneous section (11%) (like pus, wounds etc) and 4 from blood cultures (8%). In the current study, the clinical isolates taken for the analysis carried resistance to the commonly used antibiotics (Table **1**). Colistin (84.5%) and tigecycline (62.2%) were the only drugs found to be active against the analyzed clinical isolates. High resistance was observed for imipenem (95.6%), meropenem (95.6%), ceftazidime (82.2) and gentamicin (80%). A study by Bhat et al, 2016 stated that *Acinetobacter* and *K. pneumoniae* strains were resistant to cephalosporins, fluoroquinolones, gentamicin and carbapenems greater than 50% [16] and a similar pattern was observed in the isolates obtained for the study.

3.1. MBL Occurrence

Among the 45 clinical isolates appraised, 38 isolates (84%) including tracheal aspirate, urine, pus, sputum and blood samples were found to be MBL producers. MBL resistance was predominant among *Acinetobacter spp* (87%), obtained majorly from tracheal aspirate samples followed by *K. pneumoniae* (77%) predominately from tracheal aspirate and sputum isolates (Table **3**). In India, 59% of MBL resistance was reported from Lucknow [17] and 82% from Chennai particularly in *P.aeruginosa* [18]. Turkey reported 5.8% of carbapenam resistant *K.pneumoniae* [19], 92.4% in *Acinetobacter spp* involving 73 clinical isolates [20]. Fig. (**1**) represents the MBL genes identified through Colony PCR.

Multiplex detection for MBL genes was also attempted successfully for these genes. Direct PCR amplification was attempted using crude samples (Sputum and Urine) for six isolates (R794, R797, R872, R877 and R906 (Respiratory samples) and only one sample from Urinary section (U1417)). The presence of the VIM gene was observed in 3 respiratory samples and 2 respiratory isolates harbored NDM gene. The results were concurrent with colony PCR detection. Fig. (**2**) represents the Direct PCR amplification of MBL genes.

Amongst the MBL resistance genes; the occurrence of VIM resistance (48%) was detected to be higher followed by NDM in 13 isolates (28%), GIM in 11 isolates (24%) and SPM in 10 isolates; whereas SIM and IMP occurrence were lesser than 10% (Table **1**). VIM and NDM resistant gene was predominantly detected in *K. pneumoniae* followed by *A. baumannii*. 42.5% NDM resistance in association with Insertion Sequence (IS) like ISAba125, ISEc33 and ISSen4 (among 120 isolates analyzed) was reported previously from the same geographical location [21]. VIM occurrence was not observed in *E.coli* and *Enterobacter spp* in the sample study as against reports from China and Italy. GIM-1 has not been reported outside Germany since 2002. Wendel et al., 2013 showed the presence of the GIM gene in 50 isolates from the suburbs of Germany [22]. The controlled spread had been attributed to the absence of mobile elements associated with this gene when it was identified in Germany [23]. However, an endemic MBL gene like GIM was identified in 13 isolates from sputum sample. Six isolates of *A. baumannii* and three isolates of *K. pneumoniae* considered in

Table 1. Details of clinical isolates analyzed.

Sample No.	Register ID	Date of Collection	Organisms	Patient Ward	Patient Age	Patient Sex	Sample Source	Sensitive Antibiotics	Resistant Antibiotics	MBL Gene Occurrence					
										NDM	IMP	VIM	SIM	GIM	SPM
1	U42	02-01-2018	*K. pneumoniae*	S3	62	M	Urine	Col,Tg	Ac, Ak, Ax, Ca, Cf, Cfs, Ci, Cot, Cpm, Cu, G, I,Netil, Nf, Pt, Tb	+	-	+	+	+	+
2	B1051	09-01-2018	*P. aeruginosa*	2W	55	M	Blood	Col	Ac, Ak, Ax, Ca, Cf, Cfs, Ci, Cot, Cpm, Cu, G, I,Netil, Nf, Pt, Tb	-	-	+	-	-	-
3	B1518	09-01-2018	*P. aeruginosa*	S4	45	M	Blood	Col, Nt	Ak, Am, Ca, Cfs, Cpm,Cu, I, Le, Mr, Of, Pt, Pi,Tb	-	-	+	-	-	-
4	U1075	09-01-2018	*K. pneumoniae*	OP	52	F	Blood	Col, Tg	Am, Amp, Cfs, Cl, Cot, Cpm, Cfs, Etp, G, I, Mr, Netil, Nt	+	-	-	-	-	+
5	U1062	09-01-2018	*K. pneumoniae*	3W	62	M	Blood	Col	Am, Amp, Cf, Cfs, Cpm, Etp, G, I, Mr, Netil, Pt, Tb, Tg	+	-	+	-	-	-
6	R771	11-02-2018	*A. baumannii*	Gastro	44	M	Tracheal Aspirate	Col, Tg, Co	Ak, Ca, Cfs, Do, G, I, Mr, Nt, Pt, Pi, Tb	+	-	-	-	-	-
7	R770	11-02-2018	*K. pneumoniae*	Pulmo	71	M	Tracheal Aspirate	Col	Ak, Amp, Ca, Cf, Cfs, Ci, Cpm, Cu, Do, Etp, I, Mr, Nt, Pt, Tb	-	-	-	-	-	-
8	R764	10-02-2018	*K. pneumoniae*	RM/3E	63	M	Sputum	Col	Ak, Amp, Cfs, Ci, Cl, Co, Cpm, Cu, Etp, I, M, Mr, Nt, Of, Pt, Tb	-	+	+	-	-	+
9	R714	10-02-2018	*K. pneumoniae*	NSICU	24	F	Sputum	Col, Tg	Ac, Ak, Ca, Cf, Cfs, Ci, Co, Cpm, Etp, G, I, Mr, Nt, Pt,Tb	+	+	+	-	+	+
10	R706	08-02-2018	*P. aeruginosa*	MICU	70	M	Tracheal Aspirate	Col	Ac, Ak, Am, Ca, Cf, Cfs, Cpm, G, I, Mr, Nt, Of, Pi, Pt,Tb	-	-	+	-	-	+
11	R702	07-02-2018	*A. baumannii*	RM/3E	71	M	Sputum	Col	Ac, Ak, Ca, Cf, Cfs, Co, Do, G, I, Mr, Nt, Of, Pi, Pt,Tb	-	-	+	-	-	-
12	R672	06-02-2018	*A. baumannii*	MICU/ Pulmo	71	M	Tracheal Aspirate	Col, Of, Tg	Am, As, Ca, Cf, Cfs, Cpm, Co,Do, G, I, Le, Mr, Nt, Pt, Tb	+	-	+	-	+	-
13	R797	10-02-2018	*P. aeruginosa*	G1	50	M	Tracheal Aspirate	Col,Tgc, As	Am,Pi, Pt, Ca, Cfs, I, Mr, Ak, G, Nt, Tb, Le, Cf, Of	-	-	+	-	-	+
14	R794	12-02-2018	*A. baumannii*	Spl	50	M	Tracheal Aspirate	Col,Tgc, As	Ak, Ca, Ce, Cf, Cfs, Cpm, Do, G, I, Le, Mr, Nt, Of, Pt, Tb	-	-	+	-	-	-
15	R837	15-02-2018	*A. baumannii*	Neuro	30	M	Tracheal Aspirate	Of, G, Tg, Col,Cat	Ak, Am, Ca, Cf, Cfs, Cpm, Do, I, Mr, Nt, Pt, Tb	+	-	-	+	-	-
16	R849	15-02-2018	*K. pneumoniae*	M6/IW	54	M	Tracheal Aspirate	Tg, G	Ac, Ak, Ca,Ce, Cf, Cfs, Ci, Cl, Cu, Do, Etp, I, Le, Mr, Nt, Of, Pt, Tb	-	-	+	-	-	-
17	R872	17-02-2018	*K. pneumoniae*	Female Ortho	24	F	Tracheal Aspirate	Do	Ac, Ak, Ca, Ci, Co, Cpm, Cu, Etp, G, I, Mr, Nt, Of, Tb	+	-	+	-	-	+
18	R877	17-02-2018	*A. baumannii*	GW	32	F	Sputum	Col, Tg, Nt, Tet	Ak, Ca, Cf, Co, G, I, Le, Mr, Pi, Ptm	-	+	+	-	-	+
19	U1417	21-02-2018	*K. pneumoniae*	2W	75	F	Urind MS	Col	Ac, Ak, Ca, Ce, Cf, Cfs, Ci, Co,Cpm, Cu, Etp, G, Mr, Nt, Nx, Pt, Tb, Tg	-	-	-	-	-	-
20	R911	19-02-2018	*A. baumannii*	MICU	54	M	Tracheal Aspirate	Col,Tg	Ak, As, Ca, Cfs, Co, Do, G, I, Le, Mr, Nt, Pt, Pi, T, Tb,	-	-	-	-	+	-
21	R906	19-02-2018	*K. pneumoniae*	Spl ward	62	M	Sputum	Col, Tg	Ak, Amp, Ca, Cf, Cfs, Ci, Co, Cpm, Cu, Etp, I, G, Mr, Nt, Pt, Tb	+	-	+	-	-	-
22	R899	19-02-2018	*K. pneumoniae*	MICU	46	M	Sputum	G	Ak, Amp, Ce, Cf, Cfs, Ci, Co, Cpm, Cu, Do, I, Mr, Nt, Of, Pt,Tb	-	-	-	-	+	-
23	R889	18-02-2018	*K. pneumoniae*	-	45	M	Tracheal Aspirate	Col, Tg	Ak, Amp, Ce, Cf, Cfs, Ci, Co, Cpm, Cu, Do, Etp, G, I, Le, Mr, Nt, Of, Pt,Tb	-	-	+	-	-	-
24	M789	25-02-2018	*A. baumannii*	BW/Ped	5	F	Wound Swab	Col, Tg	Ak, Co, Cfs, Cl, Do, G, I, Le, Mr, Pi, Pt, Tb	-	-	-	-	+	-
25	M811	27-02-2018	*K. pneumoniae*	O4-8	28	F	Pus Swab	Col, Tg	Ac, Ak, Amp, Ca, Ce, Cf, Cfs, Ci, Cpm, Cu, Do, G, Mr, Nt, Pt	-	-	+	-	-	-
26	U1667	27-02-2018	*K. pneumoniae*	MICU	41	M	Urine	Col, Tg	Ak, Amp, Ca, Cf, Cfs, Ci, Cpm, Cu, Do, Etp,G, I, Le, Mr, Netil, Nf, Nx, Pt	-	-	+	-	-	-
27	U1675	28-02-2018	*K. pneumoniae*	514	69	M	Urine Mid	Col	Ak, Amp, Ca, Cf, Cfs, Ci, Cpm, Cu, Do, Etp,G, I, Le, Mr, Nb, Netil, Nx, Pt	+	-	-	-	-	-
28	M871	03-03-2018	*A. baumannii*	BW/Ped	5	F	Wound Swab	Col, Tg, Nt, Le	Ak, Am, Ca, Cf, Cfs, Do, I, Mr, Nt, Pi, Pt, Tb	-	-	+	-	-	-

(Table 1) Contd…

Sample No.	Register ID	Date of Collection	Organisms	Patient Ward	Patient Age	Patient Sex	Sample Source	Sensitive Antibiotics	Resistant Antibiotics	MBL Gene Occurrence					
										NDM	IMP	VIM	SIM	GIM	SPM
29	M900	05-03-2018	*K. pneumoniae*	-	59	M	Liver abscess	Col, Tg, Do	Ac, Ak, Amp, Ca, Ce, Cf, Cfs, Ci, Co, Cu, Etp, G, I, Le, Mr, Nt, Of, Pt, Tb	-	-	-	+	-	+
30	U1816	05-03-2018	*E. coli*	S5/Spl	83	M	Urine	Col,Tg	Ac, Amp, Ca, Ce, Cfs, Ci, Co, Cu, Etp, G, I, Mr, Nx, Pt,Tb	-	-	-	-	+	-
31	R1428	19-03-2018	*K. pneumoniae*	MICU/Neuro	65	M	Tracheal Aspirate	Col	Ac, Ak, Ca, Cf, Cfs, Ci, Co, Cpm, Cu, Etp, G, I, Le, Mr, Nt, Of, Pt	-	-	+	-	-	-
32	R1419	19-03-2018	*A. baumannii*	Neuro	52	F	Tracheal Aspirate	Col, Tg, Of, Le	Ak, Ca, Ce, Cfs, Do, G, M, Mr, Nt, Pb, Pi, Pt	-	-	-	-	+	-
33	U1827	05-03-2018	*E. coli*	Spl ward	1	M	Urine	Col, AmK, Tg, Nt, Tb	Ac, Amp, Ca, Ce, Cf, Cfs, Ci, Co, Cu, Do, G, I, Mr, Nx, Pt	+	-	-	-	-	+
34	B3276	17-03-2018	*K. pneumoniae*	SW	35	F	Femoral	No	Ac, Ak, Amp, Ca, Ce, Cf, Cfs, Ci, Co, Cpm, Cu, Etp, G, I, Le,Mr, Nf, Nt, Nx, Pt,Tb, Tg	-	-	-	-	-	-
35	B3277	17-03-2018	*K. pneumoniae*	SW	35	F	CVP	No	Ac, Ak, Amp, Ca, Ce, Cf, Cfs, Ci, Co, Cpm, Cu, Etp, G, I, Le,Mr, Nf, Nt, Nx, Pt,Tb, Tg	-	-	-	-	-	-
36	M1118	20-03-2018	*A. cinetobacter spp*	SU/FSW	67	F	Wound Swab	Col	Ak, As, Ca, Cf, Cfs, Cpm, Do, G, I, Mr, Nt, Pt, Tb	+	-	-	-	-	-
37	U2403	24-03-2018	*K. pneumoniae*	MICU/Mid	33	M	Urine Mid	Tg	Ac, Ak, Amp, Ca, Cfm, Cfs, Ci, Ct, Cu,Etp, G, I, Mr, Netil, Nt, Nx, Of, Pt, Tb	-	-	+	-	-	-
38	U2398	24-03-2018	*Enterobacter spp*	N-I	61	F	Urine Catheter	Col,Tg, Ak, G, Netil, Nt, Cot	Ac, Amp, Ca, Ci, Cf, Cfm, Cfs, Cu, I, Mr, Nx, Pt	-	-	-	-	+	-
39	R1479	22-03-2018	*A.baumannii*	Spl/Pulmo	70	M	Sputum	Tg, Col	Ak, Cf, Cfs, Ci, Co, Cpm, Do, I, Le, Mr, Nt, Of, Pe, Pt, Tb	-	-	-	-	+	-
40	R1488	23-03-2018	*Acinetobacter spp*	Gastro	41	M	Tracheal Aspirate	Col,Tg, AS, Of, Le, Nt, Tb	Am, Cf, Cfs, Co, Cpm, Cu, G, I, Mr, Pc, Pt, T	-	-	-	-	-	-
41	R1492	23-03-2018	*K.pneumoniae*	MSW-SI	69	M	Sputum	Col, Do	Ak, Amp, Ca, Ce, Cfs, Ci, Co, Cpm, Cu, Etp, G, I, Mr, Nt, Of, Pt, Tb, Tg	-	-	-	-	-	-
42	R1512	24-03-2018	*A.baumannii*	BAC	41	M	Tracheal Aspirate	NT, Tb, As, Tg, Col, Cfs	Am, Ca, Cf, Cpm, G, I, Mr, Pt, Tb	-	-	-	+	-	-
43	R1513	24-03-2018	*K.pneumoniae*	NS/NSICU	58	M	Tracheal Aspirate	Col, Nt	Ac, Ak, Amp, Ca, Ce, Ci, Cpm, Cu, Etp, G, I, Of, Mr, Pt	+	-	+	-	-	-
44	R1539	26-03-2018	*Acinetobacter spp*	-	52	F	Sputum	Col, Tg	Ak, Am, Ca, Cfs, Co, Do, G, I, Le, Mr, Nt, Pi, Pt, Tb	-	-	-	-	-	-
45	R1524	26-03-2018	*A.baumannii*	MICU	58	F	Tracheal Aspirate	Nt, Tb, Tg	Ca, Cfs, Co, Cpm, G, I, Mr, Pt	-	-	-	-	+	-
Total No. of MBL Positive Isolates										**13**	**3**	**22**	**4**	**11**	**10**

Table 2. Primers used for PCR amplification.

Gene	-	Primer Sequence	Annealing Temp	Product Size	References
NDM	FP	GGGCAGTCGCTTCCAACGGT	55°C	475bp	[12]
	RP	GTAGTGCTCAGTGTCGGCA			
IMP	FP	GGAATAGAGTGGCTTAAYTCTC	52°C	188bp	[13]
	RP	CCAAACYACTASGTTATCT			
VIM	FP	GATGGTGTTTGGTCGCATA	52°C	390bp	[13]
	RP	CGAATGCGCAGCACCAG			
GIM	FP	TCGACACACCTTGGTCTGAA	52°C	477bp	[13]
	RP	AACTTCCAACTTTGCCATGC			
SPM	FP	CCTACAATCTAACGGCGACC	52°C	670bp	[14]
	RP	TCGCCGTGTCCAGGTATAAC			
SIM	FP	TACAAGGGATTCGGCATGG	52°C	570bp	[13]
	RP	TAATGGCCTGTTCCCATGTG			
16s rDNA	FP	AGAGTTTGATCCTGGCTCAG	55°C	1500bp	[15]
	RP	ACGGCTACCTTGTTACGACTT			

Table 3. Results consolidated for Colony PCR amplification.

Organisms	NDM	IMP	VIM	SIM	GIM	SPM
K. pneumoniae	8	2	13	2	3	6
Acinetobacter spp.	4	1	5	2	6	1
P. aeruginosa	0	0	4	0	0	2
E. coli	1	0	0	0	1	1
Enterobacter spp.	0	0	0	0	1	0
Total	**13**	**3**	**22**	**4**	**11**	**10**

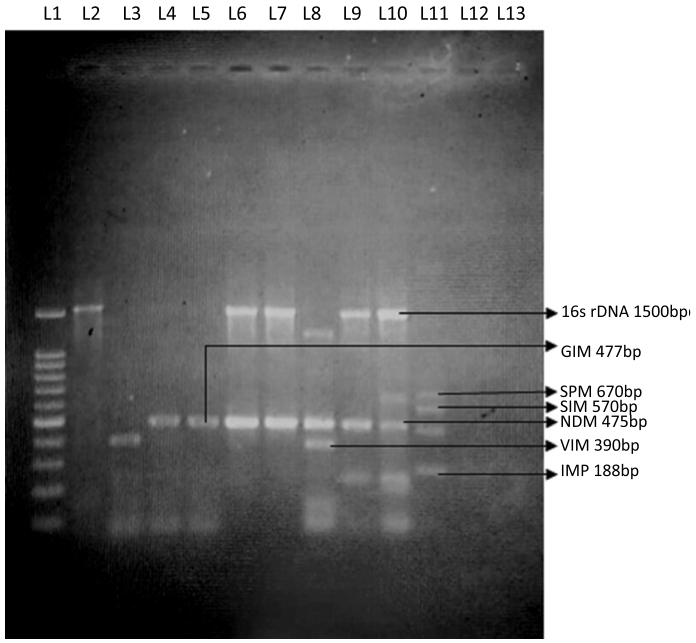


Fig. (1). Colony PCR Amplification for MBL Genes (Individual and Multiplex).

Colony PCR based detection of MBL genes:
L1 – 100bp ladder, L2 – U1062 (16s rDNA), L3 – R906 (blaVIM), L4 – R672 (blaNDM), L5 – R877 (blaGIM), L6 – M1118 (blaNDM & 16s rDNA), L7 – R771 (blaGIM & 16s rDNA), L8 – R714 (blaNDM & blaVIM), L9 – R872 (blaNDM, blaIMP & 16s rDNA), L10 – U1075 (blaIMP, blaNDM, blaSPM & 16s rDNA), L11 – U42 (blaSPM, blaSIM, blaIMP & blaNDM), L12 – U1417 (Negative control), L13 – Blank.

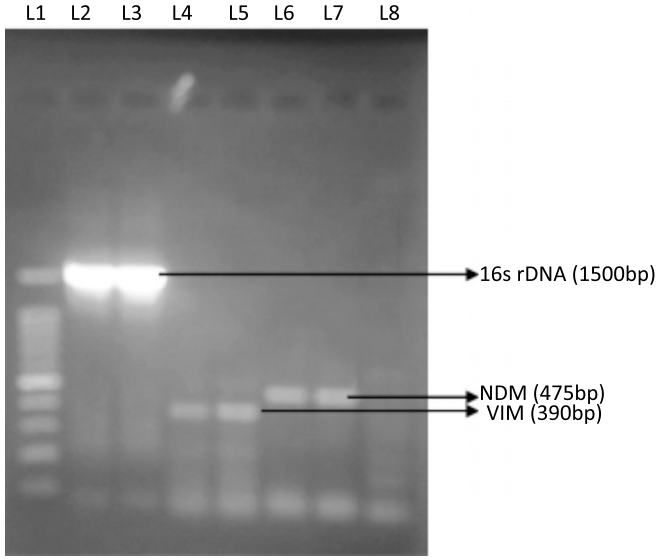


Fig. (2). Direct PCR amplification for MBL genes.

Direct PCR based detection of MBL genes:
L1 – 100bp ladder, L2 & L3 – 16s rDNA (R797 &U1417), L4 & L5– blaVIM (R794 & R877), L6 & L7– blaNDM (R872 & R906), L8 – Blank.

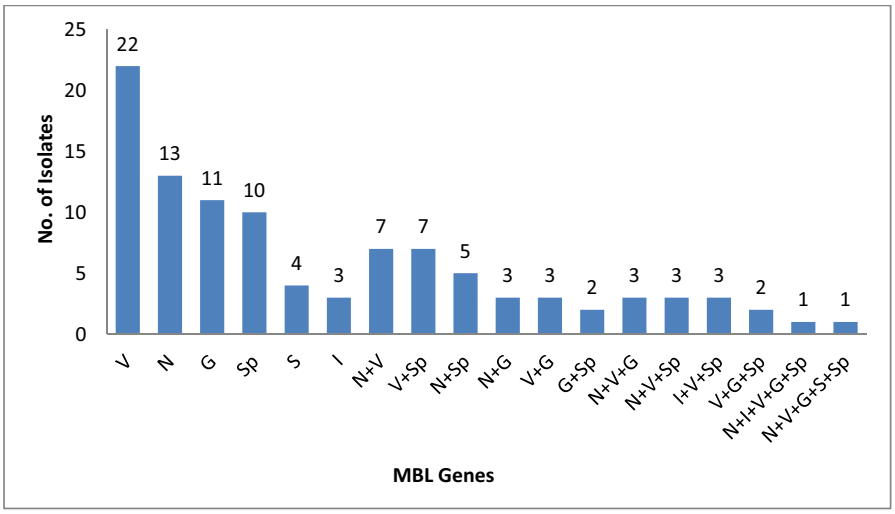


Fig. (3). MBL co resistance profile. V –VIM, I – IMP, N – NDM, S –SIM, SP –SPM, G-GIM. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

this study were detected positive for GIM gene. The isolates were found to be colistin and tigecycline sensitive.

This investigation has also revealed the occurrence of the SIM gene in four clinical isolates (two each in *K. pneumoniae* and *Acinetobacter spp*); ten isolates positive for the SPM gene and three for the IMP gene. The isolates which harbor SIM, SPM and IMP genes were found to be resistant to various antibiotics and showed sensitivity to colistin and tigecycline. A study by Mohanam *et al.,* 2017 in Chennai had no report on SPM, SIM and GIM amongst 213 *P. aeruginosa* isolates analyzed [18]. However study by Abaza *et al*., 2017 in Egypt has analyzed 19 out of 30 *P. aeruginosa* isolates possessing SPM gene and 11 for IMP gene; although the occurrence of VIM was not reported [24].

3.2. Coexistence of MBL Genes

Optimization of mPCR amplification was done for MBL genes like NDM, VIM and IMP since these genes were found to be coexisting in the samples analyzed. mPCR was also performed for endemic MBL genes like SIM and SPM along with NDM by using 16s rDNA as an internal control. Multiplexing aids in the rapid detection of these resistance genes.

Of the 45 isolates, 15 (35.5%) were positive for more than one carbapenemase gene. The MBL genes like NDM and SPM were coexisting in 5 different isolates whereas GIM and VIM combination was seen in 3 isolates. The coexistence of VIM and NDM and VIM and SPM was detected in 7 different clinical isolates, majorly isolated from respiratory isolates (Fig. **3**). Two isolates, U42 and R714 (*K. pneumoniae*) was found to harbor all 5 MBL genes in combination. Four isolates from Respiratory section (R872 and R764 - *K. pneumoniae*; R672 and R877 - *A. baumannii*) carried 3 MBL genes in combination (Table **4**). Results indicating the coexistence of five MBL genes in a single isolate is a significant finding from this study; predicting restricted diagnostic and treatment options for the stated clinical conditions. Adwan *et al*., 2017 had discussed a similar scenario in Palestine [25]. A study by Mushi *et al*., 2014 revealed 15 out of 80 isolates had multiple genes coding for carbapenem resistance especially in *E. coli* and *P. aeruginosa* in Germany [26].

Twenty two out of 26 respiratory samples were found to have a single MBL gene; whereas 9 isolates possessed multiple MBL gene(s). Four isolates from urinary infection showed multiple resistance determinants yet they were sensitive to colistin and tigecycline. All the 10 MICU (Medical Intensive Critical Care) isolates (100%) taken for analysis showed the presence of at least one MBL gene. Three out of 10 isolates were found to have 3 MBL genes in a single isolate. Airborne transmission could contribute to the spread of MBL genes especially in respiratory specimens [27]. A similar study by Gladstone et al., 2005 detected the presence of carbapenem resistance (12.2%) among 460 NFGNB (Non Fermenting Gram-Negative Bacilli) isolated from ICU patients on a ventilator over a one-year period [28].

3.3. Risk Factors

In the current study, the minimum age of MBL instance was an infant (12 months) and a maximum of 83 years. The average age of positive samples was 50 years. Twenty seven males and 11 females reported to harbor MBL genes out of the sample group of 30 males, 15 females. Whereas 55% of people belonged to the age group between 50 to 85 years while 26% were younger adults and 6% were pediatric isolates found to have the presence of atleast one MBL gene. The study by Viehman et al., 2014 stated that carbapenem resistance is related to age of affected patients: it was present in 60-64% of all isolates in the elderly (age >65) and 46–51% in younger adults, while only in 13-17% of pediatric isolates [29]. The presence of VIM and NDM gene occurred in age groups between 40 to 80 years; whereas 6 out of 10 SPM positive isolates were between the age group of 50 to 60 years (Fig. **4**). A distinct result was seen in Abaza et al., 2017 study which reported that more than half of the patients who had *P. aeruginosa* isolates with IMP gene and SPM gene (54.5% and 47.4% respectively) were aged between 20 $\leq$ 30 years old [24]. Therefore, age related clinical complications could result in significant control challenges for the clinicians.

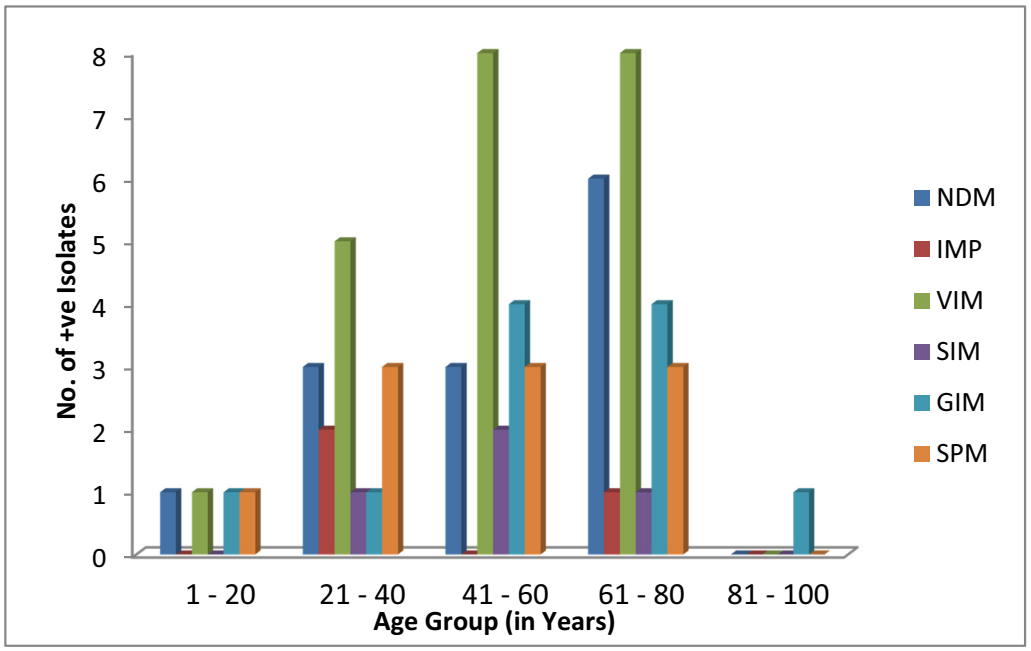


Fig. (4). MBL gene resistance occurrence with respect to patient's age group. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

CONCLUSION

The present study analysed 45 samples chosen randomly from the list of isolates identified phenotypically as resistant to carbapenams from 10 - 15 resistant samples evaluated on a daily basis by the hospital between February – March 2018. The results confirm the presence and coexistence of MBL resistance gene(s) at the tertiary care center. The salient findings call for attention and further investigation on the prevalence and spread of these gene(s) at the center and there off in the geographical vicinity. *A. baumannii* and *K. pneumonia*e are currently potent carriers of MBL resistance in the sample set from the tertiary care centre. A rare MBL variant including SIM, SPM and GIM has been observed as a maiden case. The direct PCR amplification and multiplex detection methods prove enhanced and rapid detection for these gene(s) in the clinical setup. The sample size though limited due to the time period involved (Feb- March 2018), exhibits diversity on source and species that carry the resistant gene(s). The small sample size eliminates any outbreak incidents at the tertiary center. The report focuses attention on the prevalence of multiple MBL resistance gene(s) and warrants molecular methods for identifying the same to facilitate rapid, authentic diagnostic means to aid clinicians with cure and control spread. Future attempts to gain insights on the clonal diversity of the isolates taken for analysis will highlight the means of origin and spread. Further investigations in associated clinical set ups around the geographical limits can reveal substantial significance to these MBL variants reported from this analysis. This report could assure new insight for extensive, improved and rapid approaches for detecting MBL resistance.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

[1] Dixit, A.; Kumar, N.; Kumar, S.; Trigun, V. Antimicrobial resistance: Progress in the decade since emergence of New Delhi metallo-β-lactamase in India. *Indian J. Community Med.,* **2019**, *44*(1), 4-8.
PMID: 30983704

[2] Zilahi, G.; Artigas, A.; Martin-Loeches, I. What's new in multidrug-resistant pathogens in the ICU? *Ann. Intensive Care,* **2016**, *6*(1), 96.
http://dx.doi.org/10.1186/s13613-016-0199-4 PMID: 27714706

[3] Drawz, S.M.; Bonomo, R.A. Three decades of beta-lactamase inhibitors. *Clin. Microbiol. Rev.,* **2010**, *23*(1), 160-201. http://dx.doi.org/10.1128/CMR.00037-09 PMID: 20065329

[4] Cornaglia, G.; Giamarellou, H.; Rossolini, G.M. Metallo-β-lactamases: a last frontier for β-lactams? *Lancet Infect. Dis.,* **2011**, *11*(5), 381-393. http://dx.doi.org/10.1016/S1473-3099(11)70056-1 PMID: 21530894

[5] Walsh, T.R.; Toleman, M.A.; Poirel, L.; Nordmann, P. Metallo-beta-lactamases: the quiet before the storm? *Clin. Microbiol. Rev.,* **2005**, *18*(2), 306-325. http://dx.doi.org/10.1128/CMR.18.2.306-325.2005 PMID: 15831827

[6] Nordmann, P.; Naas, T.; Poirel, L. Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg. Infect. Dis.,* **2011**, *17*(10), 1791-1798. http://dx.doi.org/10.3201/eid1710.110655 PMID: 22000347

[7] Gupta, V. Metallo beta lactamases in Pseudomonas aeruginosa and Acinetobacter species. *Expert Opin. Investig. Drugs,* **2008**, *17*(2), 131-143. http://dx.doi.org/10.1517/13543784.17.2.131 PMID: 18230049

[8] Hong, D.J.; Bae, I.K.; Jang, I.H.; Jeong, S.H.; Kang, H.K.; Lee, K. Epidemiology and Characteristics of Metallo-β-Lactamase-Producing Pseudomonas aeruginosa. *Infect. Chemother.,* **2015**, *47*(2), 81-97. http://dx.doi.org/10.3947/ic.2015.47.2.81 PMID: 26157586

[9] Shalley, D.; Pooja, S.; Uma, C.; Bijender, S. *A Review. Int J Adv Health Sci,* **2015**, *2*(4)

[10] Lee, J.J.; Lee, J.H.; Kwon, D.B.; Jeon, J.H.; Park, K.S.; Lee, C.R.; Lee, S.H. Fast and Accurate Large-Scale Detection of β-Lactamase Genes Conferring Antibiotic Resistance. *Antimicrob. Agents Chemother.,* **2015**, *59*(10), 5967-5975. http://dx.doi.org/10.1128/AAC.04634-14 PMID: 26169415

[11] Ben, A. A.; Oueslati, S.; Mliki, A. Universal direct PCR amplification system: a time- and cost-effective tool for high-throughput applications *3 Biotech,* **2017**, *7*(4), 246.

[12] Shenoy, K.A.; Jyothi, E.K.; Ravikumar, R. Phenotypic identification & molecular detection of bla (ndm-1) gene in multidrug resistant Gram-negative bacilli in a tertiary care centre. *Indian J. Med. Res.,* **2014**, *139*(4), 625-631. PMID: 24927351

[13] Ellington, M.J.; Kistler, J.; Livermore, D.M.; Woodford, N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. *J. Antimicrob. Chemother.,* **2007**, *59*(2), 321-322. http://dx.doi.org/10.1093/jac/dkl481 PMID: 17185300

[14] Moosavian, M.; Rahimzadeh, M. Molecular detection of metallo-β-lactamase genes, bla IMP-1, bla VIM-2 and bla SPM-1 in imipenem resistant Pseudomonas aeruginosa isolated from clinical specimens in teaching hospitals of Ahvaz, Iran. *Iran. J. Microbiol.,* **2015**, *7*(1), 2-6. PMID: 26644866

[15] Fouad, A.F.; Barry, J.; Caimano, M.; Clawson, M.; Zhu, Q.; Carver, R.; Hazlett, K.; Radolf, J.D. PCR-based identification of bacteria associated with endodontic infections. *J. Clin. Microbiol.,* **2002**, *40*(9), 3223-3231. http://dx.doi.org/10.1128/JCM.40.9.3223-3231.2002 PMID: 12202557

[16] Bhat, V.; Gupta, S.; Kelkar, R.; Biswas, S.; Khattry, N.; Moiyadi, A.; Bhat, P.; Ambulkar, R.; Chavan, P.; Chiplunkar, S.; Kotekar, A.; Gupta, T. Bacteriological profile and antibiotic susceptibility patterns of clinical isolates in a tertiary care cancer center. *Indian J. Med. Paediatr. Oncol.,* **2016**, *37*(1), 20-24. http://dx.doi.org/10.4103/0971-5851.177010 PMID: 27051152

[17] Saxena, S.; Banerjee, G.; Garg, R.; Singh, M.; Verma, S.K.; Kushwahar, A.S. ESBL, MBL AND AMP C-β lactamases produced by superbugs: an emerging threat to clinical therapeutics. *Int. J. Pharm. Pharm. Sci.,* **2015**, *7*, 353-356.

[18] Mohanam, L.; Menon, T. Coexistence of metallo-beta-lactamase-encoding genes in *Pseudomonas aeruginosa. Indian J. Med. Res.,* **2017**, *146*(Suppl.), S46-S52. http://dx.doi.org/10.4103/ijmr.IJMR_29_16 PMID: 29205195

[19] Celikbilek, N.; Unaldi, O.; Kirca, F.; Gozalan, A.; Acikgoz, Z.C.; Durmaz, R. Molecular Characterization of Carbapenem-Resistant *Klebsiella pneumoniae* Species Isolated From a Tertiary Hospital, Ankara, Turkey. *Jundishapur J. Microbiol.,* **2017**, *10*(10) http://dx.doi.org/10.5812/jjm.14341

[20] Pournajaf, A.; Rajabnia, R.; Razavi, S.; Solgi, S.; Ardebili, A.; Yaghoubi, S.; Khodabandeh, M.; Yahyapour, Y.; Emadi, B.; Irajian, G. Molecular characterization of carbapenem-resistant Acinetobacter baumannii isolated from pediatric burns patients in an Iranian hospital. *Trop. J. Pharm. Res.,* **2018**, *17*(1), 135-141. http://dx.doi.org/10.4314/tjpr.v17i1.19

[21] Jose, J.; Santhiya, K.; Jayanthi, S.; Ananthasubramanian, M. Insertion sequence based analysis of clinical isolates with NDM (NDM-1) resistance. *Indian J. Biotechnol.,* **2017**, *16*(2), 182-188.

[22] Wendel, A.F.; Brodner, A.H.; Wydra, S.; Ressina, S.; Henrich, B.; Pfeffer, K.; Toleman, M.A.; Mackenzie, C.R. Genetic characterization and emergence of the metallo-β-lactamase GIM-1 in Pseudomonas spp. and Enterobacteriaceae during a long-term outbreak. *Antimicrob. Agents Chemother.,* **2013**, *57*(10), 5162-5165. http://dx.doi.org/10.1128/AAC.00118-13 PMID: 23877696

[23] Castanheira, M.; Toleman, M.A.; Jones, R.N.; Schmidt, F.J.; Walsh, T.R. Molecular characterization of a beta-lactamase gene, blaGIM-1, encoding a new subclass of metallo-beta-lactamase. *Antimicrob. Agents Chemother.,* **2004**, *48*(12), 4654-4661. http://dx.doi.org/10.1128/AAC.48.12.4654-4661.2004 PMID: 15561840

[24] Abaza, A.F.; El Shazly, S.A.; Selim, H.S.A.; Aly, G.S.A. Metallo-Beta-Lactamase Producing Pseudomonas aeruginosa in a Healthcare Setting in Alexandria, Egypt. *Pol. J. Microbiol.,* **2017**, *66*(3), 297-308. http://dx.doi.org/10.5604/01.3001.0010.4855 PMID: 29319510

[25] Adwan, G.; Shtayah, A.; Adwan, K.; Al-Sheboul, S.; Othman, S. Prevalence and Molecular Characterization of P. aeruginosa isolates in the West Bank-Palestine for ESBLs, MBLs and Integrons. *J. Appl. Life Sci. Int,* **2016**, *8*(2), 1-11. http://dx.doi.org/10.9734/JALSI/2016/29259

[26] Mushi, M.F.; Mshana, S.E.; Imirzalioglu, C.; Bwanga, F. Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. *BioMed Res. Int.,* **2014**, *2014*303104 http://dx.doi.org/10.1155/2014/303104 PMID: 24707481

[27] de Jager, P.; Chirwa, T.; Naidoo, S.; Perovic, O.; Thomas, J. Nosocomial Outbreak of New Delhi Metallo-β-Lactamase-1-Producing Gram-Negative Bacteria in South Africa: A Case-Control Study. *PLoS One,* **2015**, *10*(4) e0123337 http://dx.doi.org/10.1371/journal.pone.0123337 PMID: 25909482

[28] Gladstone, P.; Rajendran, P.; Brahmadathan, K.N. Incidence of carbapenem resistant nonfermenting gram negative bacilli from patients with respiratory infections in the intensive care units. *Indian J. Med. Microbiol.,* **2005**, *23*(3), 189-191. http://dx.doi.org/10.4103/0255-0857.16593 PMID: 16100428

[29] Viehman, J.A.; Nguyen, M.H.; Doi, Y. Treatment options for carbapenem-resistant and extensively drug-resistant Acinetobacter baumannii infections. *Drugs,* **2014**, *74*(12), 1315-1333. http://dx.doi.org/10.1007/s40265-014-0267-8 PMID: 25091170

Comparison of Weil Felix Test and ELISA with the Gold Standard IFA for the Diagnosis of Scrub Typhus in Clinically Suspected Cases of Scrub Typhus at a Tertiary Care Hospital in Tamil Nadu

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ABSTRACT

BACKGROUND

Scrub typhus is a febrile disease, the clinical diagnosis of which is difficult due to its vague symptoms. Scrub typhus is diagnosed mainly via serological tests. Sensitivity of Weil Felix was found to be poor, but the specificity of Weil Felix was variable in different studies. Enzyme linked immunosorbent assay (ELISA) IgM is known to be a sensitive test, but its specificity was variable in different studies. Therefore, in this study we have compared these two tests with the gold standard IFA.

METHODS

The study was performed as a cross-sectional study. Among the suspected scrub typhus cases, fifty consecutive IFA scrub typhus positive and fifty consecutive IFA negative samples were taken for comparison of Weil Felix and ELISA tests. The indirect fluorescent antibody test (IFA) was taken as the gold standard.

RESULTS

The sensitivity of ELISA and Weil Felix at a titre of 1 : 160 was found to be 96 % and 40 % respectively. The specificity of ELISA and Weil Felix at 1 : 160 and was found to be 88 % and 94 % respectively.

CONCLUSIONS

Scrub typhus should be kept in mind as a possible diagnosis in acute febrile illnesses. Weil - Felix was found to have a good specificity but low sensitivity. Hence, if the results by Weil Felix are negative, scrub typhus cannot be ruled out and may require further testing. ELISA showed good sensitivity making it a preferable screening test but is not highly specific and confirmation with IFA may be required when feasible.

KEYWORDS

Scrub Typhus, ELISA, Weil Felix, IFA, Laboratory Diagnosis

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BACKGROUND

Scrub typhus presents as an acute undifferentiated febrile illness and could become life threatening. It is caused by an obligate intracellular bacterium called *Orientia tsutsugamushi* that is transmitted by the bite of the larval trombiculid mite, which is the reservoir of the agent and the only life stage that feeds on a vertebrate host.[1,2] Recent reports from several parts of India, including South India, indicate that there has been a resurgence of the disease.[3]

The vague clinical symptoms and lack of a definitive protocol for its diagnosis often results in scrub typhus going undiagnosed. The diagnosis of scrub typhus depends largely on the laboratory diagnosis of which serology is the main stay. The oldest test is the Weil-Felix OX K agglutination reaction which is inexpensive, easy to perform and results are available overnight.[4] In Weil Felix test agglutinating antibodies are detectable 5 – 10 days following the onset of symptoms and the antibodies detected are mainly of IgM type.[5] ELISA for the detection of IgM antibodies against *Orientia tsutsugamushi* offers advantages of being able to test large number of samples at a time and can be automated. Indirect fluorescent antibody assay is the gold standard for the serological detection of antibodies in scrub typhus.[5,6]

All the serological tests that are currently available for scrub typhus have its limitations of which the clinician needs to be aware. Generally, a $\geq$ fourfold increase in antibody titre between two consecutive samples taken with a gap of 10 – 14 days is diagnostic, but this is usually retrospective and cannot guide in treatment. Serological diagnosis based on a single acute serum sample requires a cut off antibody titre depending on the endemic titre and could vary from 1 : 10 to 1 : 400.[6]

Diagnosis and surveillance of scrub typhus is challenging particularly in the absence of advanced diagnostic techniques. The availability and cost of other serological methods are a major problem in India.[7]

The purpose of this study was to evaluate the serological diagnostic methods: Weil Felix and ELISA against Indirect Fluorescent antibody assay (IFA) which is the gold standard for the diagnosis of scrub typhus.

METHODS

At the start of the study, approval was obtained from the Institutional Ethics committee and was renewed periodically during the study period which took place over a span of 2 years from October 2012 to August 2014. The study was performed as a cross sectional study. Considering the specificity of ELISA to be 94 % from previous studies with an absolute precision of 5 %, the minimum sample size was calculated to be 90. We tested Weil Felix, ELISA and IFA on a total of 100 samples collected from clinically suspected cases of scrub typhus during the period of study i.e., patients with acute undifferentiated illness of 5 days or more with or without eschar.

Weil Felix test was done using the PROGEN antigen suspension kit (Tulip Diagnostics (p) LTD, Goa). Titres of $\geq$ 1 : 160 was taken as significant. The ELISA test was done using the scrub typhus detect™ IgM ELISA kit (In Bios International Inc., USA). Samples showing values above the cut off titre according to manufacturer's instructions was considered as positive. *Orientia tsutsugamushi* IFA IgM antibody kit (Fuller Labs, California, USA) was used for IFA testing. Bright staining (at least 1+) of short pleomorphic rod forms in any of the 4 antigen areas was taken as a positive reaction. Each field was compared with the positive and negative control reactions with respect to the size, appearance, and density. If the reactivity pattern was different from the positive controls, it was considered non-specific. If the slides show positivity at a dilution of 1 : 64, it was considered positive and if negative at 1 : 64 dilution, it is reported as negative.

RESULTS

The results of Weil Felix, ELISA and IFA were entered via excel sheet and the data was analysed using the IBM SPSS software. The test parameters and McNemar's test were calculated with IFA as gold standard. Sensitivity of a test is defined as the ability to correctly identify the infected individuals and specificity is the ability to correctly identify the uninfected individuals.

Negative predictive value is the proportion of those with a negative test result who are uninfected and positive predictive value as the proportion of those with a positive test result who are infected.

		IFA +ve	IFA -ve	Total
WF 1 : 80 positive		27	4	**31**
WF 1 : 80 negative		23	46	**69**
Total		**50**	**50**	**100**
Chi- Square Tests			Value	Exact Sig.
	McNemar Test			<.001a
	N of valid cases		100	

Table 1.1 Comparison of Results of Weil Felix at a Titre of 1 : 80 and IFA

		IFA +ve	IFA -ve	Total
Weil Felix titre 1 : 160 +ve		20	3	**23**
Weil Felix titre 1 : 160 -ve		30	47	**77**
Total		**50**	**50**	**100**
Chi- Square Tests			Value	Exact Sig.
	McNemar Test			<.001a
	N of valid cases		100	

Table 1.2 Comparison of Results of Weil Felix at a Titre of 1 : 160 and IFA

	IFA +ve	IFA -ve	Total
ELISA +ve	48	6	**54**
ELISA -ve	2	44	**46**
Total	**50**	**50**	**100**
		Value	Exact Sig.
McNemar Test			<.289a
N of valid cases		100	

Table 1.3. Comparison of Results of ELISA and IFA

Of the 50 IFA positive cases, 20 were Weil Felix positive (at a titre of 1 : 160) and 48 ELISA positive, and 20 were both Weil Felix (at a titre of 1 : 160) and ELISA positive. Of the 50 IFA negative cases, 47 were Weil Felix (titre less than 1 : 160) negative and 44 were ELISA negative and 40 were both together negative. The parameters of the test obtained are given in the table 2.

	Weil Felix at 1:160	Weil Felix at 1:80	ELISA
Sensitivity	40.0 %	54.0 %	96.0 %
Specificity	94.0 %	92.0 %	88.0 %
PPV	87.0 %	87.1%	88.9 %
NPV	61.0 %	66.1%	95.7 %

Table 2. Parameters of Weil Felix and ELISA

Out of the six ELISA tests which were false positives, two were positive for dengue, one had raised Widal titres of 1 : 160 for *S. typhi* H antigen. More details are given in table 3.

Sl. No. of Cases	Coinfection / Infectious Focus
1	*Salmonella typhi* H 1:160 others less than 1:20
2	Secondary dengue positive hepatitis A positive urine culture – Pseudomonas aeruginosa (10^5)
3	Urine culture – Escherichia coli (10^5)
4	Blood culture - Staphylococcus aureus
5	Nil
6	Secondary dengue positive

Table 3. Co Infections Observed in ELISA Positive and IFA Negative Cases (6 Cases)

DISCUSSION

Diagnosis of scrub typhus is challenging specially in the presence of co-endemic diseases like dengue, leptospirosis, and typhoid. The Weil Felix test is the oldest serological test for the diagnosis of Scrub typhus and non rickettsial antigens are used. Our study has reported good specificity for the diagnosis of scrub typhus as is reported in other studies[8,9] though in the latter study[9] a titre 1 : 80 was taken as the cut off. In the study by Pradutkanchana J et al.[8] the Weil Felix test at a break point of 1 : 160 gave a sensitivity of 52.1 % when compared to the gold standard IFA. A study from Sri Lanka reported low specificity with Weil Felix and a high Weil Felix titre of 1 : 320 in 54 % of healthy volunteers and 62 % of non-rickettsial fever patients.[10] In contrast in an unpublished data from our hospital a titre of $\leq$ 1 : 20 was recorded in healthy volunteers. The grossly insensitive results for Weil Felix in this study are in concordance with the other studies[8,9] and is attributed to the late appearance of antibody, suppression of their production by antibiotic treatment or due to its absence in re-infections that occurs frequently in endemic areas.[8]

Earlier ELISA with native *O. tsutsugamushi* were used either individually for Karp, Kato, Gilliam strains or pooled but now due to safety issues associated with the culturing of live rickettsia, the recombinant 56- kDa immunodominant protein from *O. tsutsugamsuhi* is used to develop serological ELISA for scrub typhus. Large quantities of this can be prepared using *E.coli* without needing BSL 3 facilities.[11] The r56 ELISA when compared with indirect immunoperoxidase showed a sensitivity of 86 % in one study[11] and 93 % in another study,[12] whereas in our study a sensitivity of 96 % was obtained with IFA taken as the gold standard. The specificity in our study was 84 % and it showed good correlation with one study[11] but showed 94 % specificity in another study.[12]

Our study observed false positive reaction for IgM ELISA in cases of dengue, typhoid fever and one case of blood culture positive for *Staphylococcus aureus*. False - positive reactions were observed in patients with falciparum malaria, pulmonary tuberculosis, *S. viridans* septicaemia and typhoid fever using IgM ELISA in other studies.[13]

CONCLUSIONS

We have observed good specificity but a low sensitivity with Weil Felix test. Therefore, if the results by Weil Felix are negative, scrub typhus cannot be ruled out and further testing may be needed. The good specificity we have observed could also be because of a low endemic titre in the local population. ELISA showed good sensitivity thus is the preferable screening test but is not highly specific and may require confirmation with IFA when feasible.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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REFERENCES

[1] Traub R, Wisseman CL Jr. The ecology of chigger-borne rickettsiosis (scrub typhus). J Med Entomol 1974;11(3):237-303.
[2] Burgdorfer W. Ecological and epidemiological considerations of Rocky Mountain spotted fever and scrub typhus. In: Walker DH, Peacock MG, eds. Biology of rickettsial diseases. Vol. 1. Boca Raton, FL: CRC Press 1988:p. 33-50.
[3] Mathai E, Rolain JM, Varghese GM, et al. Outbreak of scrub typhus in southern India during the cooler months. Ann NY Acad Sci 2003;990:359-364.
[4] Kelly DJ, Wong PW, Gan E, et al. Comparative evaluation of the indirect immunoperoxidase test for the serodiagnosis of rickettsial disease. Am J Trop Med Hyg 1988;38(2):400-406.
[5] La Scola B, Raoult D. Laboratory diagnosis of Rickettsiosis: current approaches to diagnosis of old and new rickettsial diseases. J Clin Microbiol 1997;35(11):2715-2727.
[6] Blacksell SD, Bryant NJ, Paris DH, et al. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: a lack of consensus leads to a lot of confusion. Clin Infect Dis 2007;44(3):391-401.
[7] Mahajan SK, Kashyap R, Kanga A, et al. Relevance of Weil-Felix test in diagnosis of scrub typhus in India. J Assoc Physicians India 2006;54:619-621.
[8] Pradutkanchana J, Silpapolakul K, Paxton H, et al. Comparative evaluation of four serodiagnostic tests for scrub typhus in Thailand. Trans R Soc Trop Med Hyg 1997;91(4):425-428.
[9] Isaac R, Varghese GM, Mathai E, et al. Scrub typhus: prevalence and diagnostic issues in rural Southern India. Clin Infect Dis 2004;39(9):1395-1396.

[10] Kularatne SAM, Gawarammana IB. Validity of the Weil-Felix test in the diagnosis of acute rickettsial infections in Sri Lanka. Trans R Soc Trop Med Hyg 2009;103(4):423-424.
[11] Land MV, Ching WM, Dasch GA, et al. Evaluation of a commercially available recombinant-protein enzyme linked immunosorbent Assay for the detection of antibodies produced in scrub typhus rickettsial infections. J Clin Microbiol 2000;38(7):2701-2705.
[12] Coleman RE, Sandkasuwan V, Suwanabun N, et al. Comparative evaluation of selected diagnostic assays for the detection of IgG and IgM antibody to Orientia tsutsugamushi in Thailand. Am J Trop Med Hyg Nov 2002;67(5):497-503.
[13] Prakash JAJ, Abraham OC, Mathai E. Evaluation of tests for serological diagnosis of scrub typhus. Trop Doct 2006;36(4):212-213.

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Phenotypic and Genotypic Characterization of *Enterococci* from Clinical Isolates in a Tertiary Care Hospital

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A B S T R A C T

Enterococci mainly being commensals in human faeces are now considered as an important cause of nosocomial infections. Of the infections, the commonly observed are urinary tract infections, abdominal infections followed by bacteremia, endocarditis and meningitis rarely. Speciation of *Enterococcus* aids in effective management of the infections caused by them. The initial treatment for enterococcal infections has become challenging due to development of resistance. The resistance has been observed mainly against aminoglycosides due to the presence of bi-functional gene. VRE strains are mostly isolated from patients with recurrent bacteremia, endovascular infections leading to increase deaths in the patients. To isolate and speciate enterococcal isolates from clinical samples, study the antibiotic susceptibility pattern and the genotype associated with the aminoglycoside and vancomycin resistance. *Enterococcus* isolates obtained from various sections were characterized by conventional phenotypic methods. The antibiotic susceptibility was studied by disc diffusion method. The resistant strains were further confirmed by MIC using automated methods following which the genotypic analysis was done. The incidence of *Enterococci* was 2.5% in which around 86.4% were obtained as pure isolates. Among the species *E. faecalis* was found to be the maximum. The urinary isolates exhibited sensitivity of around 92.3% to nitrofurantoin and among the non - urinary isolates the maximum sensitivity was for linezolid. The molecular study showed van A to be most common gene with vancomycin resistance (71.42%), and bifunctional gene among the aminoglycoside resistance (96%). *Enterococcus faecalis* was the commonest species isolated. The maximum number of isolates was obtained from urinary samples. The increase incidence of resistance gene to high level gentamicin could result in failure to the synergy treatment of gentamicin in combination with penicillin group of antibiotics. The emergence of vancomycin resistant strains among the clinical isolates makes treatment options more challenging.

Keywords

Enterococci, Speciation, Antibiotic resistance, High level gentamicin, Vancomycin, Bifunctional gene.

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Introduction

Enterococci are gram positive organisms having the property to grow at 6.5% NaCl concentration, pH of 9.6 and hydrolyze bile esculin. Initially they were grouped under group D Streptococcus but later with the help of DNA studies they are characterized under the genus *Enterococcus* in 1960. They were considered as harmless pathogens formerly, but in the recent years have been emerging as the most important causative agents in nosocomial infections (Murray *et al.*, 1990).

Enterococci being the commonest micro flora of both humans and animals share their source

of origin. They have been isolated from variety foods including cheese, fish, sausages, beef and pork. Studies have been reported that *Enterococci* have been isolated from various clinical and environmental samples all over the world. Among the isolates obtained clinically it has been observed that *Enterococcus faecalis* has been the predominant species clinically (Lancefield *et al.,* 1933; Cetinkaya *et al.,* 2000).

Enterococcal infections have been referred to as tough, tenacious and troublesome infections (Marothi *et al.,* 2005).In the past years there has been an increase in the prevalence of enterococcal infections in hospitals and of particular concern is the emergence of antimicrobial resistant strains. Being second commonest organism behind the abdominal and pelvic infections, third in causing blood stream infections, cases have also been reported in causing CNS and neonatal infections.

Reports have been confirmed with their association with clinical conditions including respiratory tract infections, osteomyelitis and cellulitis (Fischer *et al.,* 2009). *Enterococci* were initially regarded as the disease causing agent in the early nineties. But now various reports have been established reporting them as the second commonest pathogen (Kuhn *et al.,* 2003).

Species identification becomes essential for investigating outbreaks in case of nosocomial infections and also susceptibility pattern varies accordingly which is also helpful in clinical management of infections due these agents (Gordon *et al.,* 1992).

Infections caused by enterococcal pathogens were initially being treated with the combined treatment of cell wall active agents as penicillins and aminoglycoside as the synergistic effect was effective. But now due to the development of antibiotic resistance as high level aminoglycoside resistance, beta lactamase production and also resistance to vancomycin have led to serious concern in management of these nosocomial pathogens (Mendiratta *et al.,* 2008). Vancomycin resistant *Enterococcus* was initially reported in 1980s, after which there was a increase in the incidence of its spread. The main phenotypes associated with its spread are Van A, Van B and Van C. Van A and Van B are mainly associated with *E. faecalis* and *E. faecium*. Van C is noted in *E. gallinarum* and *E. casseliflavus*. Among the phenotypes Van A is predominantly associated in the resistant strains.

VRE strains are mostly isolated from patients with recurrent bacteremia, endovascular infections leading to increase deaths in the patients. Vancomycin resistance and penicillin resistance are often seen co-existing which makes the treatment harder (Murray *et al.,* 1990).

Resistance development in *Enterococcus* against glycopeptide antibiotics gets transferred from them to other bacterial pathogens which include *Staphylococcus aureus, Streptococci, Listeria monocytogenes* (Gold *et al.,* 1996).

Thus due to the recent emergence in association with significant clinical infections and development of resistance, *Enterococci* have gained importance. Studies are thus required especially in tertiary care hospitals for appropriate isolation, identification and speciating them.

Antibiograms should also be followed up in accordance to the species. Emergence of VRE needs to be checked and limit its spread in hospital environment. CDC also stresses the control of VRE in hospitals by educating the staff in detecting VRE early, reporting them and action plan taken promptly.

Our study aims to isolate, speciate the *Enterococcus* in a simplified manner and also to study the drug sensitivity and resistance patterns among the isolates obtained.

Materials and Methods

The Institutional Human Ethical clearance was obtained before the commencement of the study.

Type of study

Experimental study

Confidentiality

Confidentiality of the reports were maintained

Sample size estimation

A total of 250 isolates of *Enterococci* obtained from outpatient and inpatient samples from blood section, miscellaneous and urine section were processed for the species identification, antimicrobial sensitivity followed by molecular study.

Specimen processing: Specimens from various sections including urine, pus and blood for cultures were collected and processed in the laboratory.

Pus/wound swabs

The swabs were streaked in blood agar and MacConkey agar and were incubated at 37^0C for 24 hours.

Urine

A loopful of the uncentrifuged specimen was examined as a wet mount for the presence of pus cells and bacteria. One loopful of specimen was inoculated onto blood agar and Mac Conkey agar. Plates were incubated at 37^0 C for 24 hours.

Growth in sheep blood agar

Following overnight incubation of the samples, colonies of *Enterococcus* were seen as grey colonies of about 0.5-1mm in diameter with alpha, beta or gamma hemolysis.

Growth in Mac Conkey agar

Colonies were about 0.5- 1mm in size with a smooth surface and convex margins, tiny magenta colored following 18-24 hour growth.

Catalase test

A drop of the catalase reagent (30% hydrogen peroxide) was added to the slide.

The growth was further applied to it with an applicator stick. Appearance of bubbles was taken as positive test.

Enterococcal colonies showed a negative test due to the absence of bubbles.

Gram stain

Smears made from the colony showed Gram positive cocci arranged singly, in pairs or in short chains.

Storage

If the isolate was catalase negative, gram staining showing the presence of gram positive cocci in pairs or short chains and growth in MacConkey agar as tiny lactose fermenting colonies, they were sub cultured and pure growth was stocked in Robertson's cooked meat medium in tubes.

The tubes were sealed and stored at 4-8^0C for 3-6 months. When the test was to be done they were further sub cultured.

Identification tests

Growth in media with pH – 9.6

Isolated colonies of *Enterococcus* was streaked in a media with a pH 9.6 and incubated at 37^0C overnight. The presence of growth was taken as positive.

Tolerance with 6.5% NaCl

The colonies were inoculated in a broth with 6.5% NaCl, and incubated overnight. The next day the tubes were checked for any increase in turbidity. This was further confirmed by sub culturing onto the agar plates. Growth confirmed the presence of organism. Whereas absence of turbidity indicated organism was unable to grow in this concentration.

Heat tolerance test

Enterococcus was inoculated in Todd-Hewitt broth. It was later incubated in water bath set at 45^oC overnight and 60^0 C for half an hour, and then sub cultured. *Enterococci* survived both the temperature showing positive reaction.

Bile esculin test

Colonies from 24 hour culture were inoculated in media containing 40% bile. Blackening of the medium confirmed positive reaction for *Enterococci* and non-*Enterococci*.

PYR test

The colonies were inoculated in PYR broth and incubated for a period of 4 hours. Later 3 drops of PYR reagent was added to it and change of colour to red was confirmed positive indicating *Enterococci* whereas absence of colour change indicates negative test.

Motility test

Stab culture of the colony was done in a semi-solid media and motility was indicated by the spread of the organism into the media. The strains which did not diffuse into the media were considered non-motile. Tubes were incubated for up to 7 days for confirming motility.

Arginine test

A well isolated colony of *Enterococcus* from the culture was inoculated in Moeller's decarboxylase media with arginine as the amino acid and overlaid with mineral oil. After 24 hours of incubation the colour of the tube was changed to purple indicating a positive reaction. Yellow colour indicated only acid production only and no deamination.

Potassium tellurite reduction test

Colonies were streaked in media containing 0.004%potassium tellurite. *E. faecalis* formed black colonies with the reduction of tellurite to tellurium.

This test was used to differentiate *E. faecalis* from *E. faecium.*

Pyruvate test

When inoculated in a media containing pyruvate with bromothymol blue as indicator *E. faecalis* showed a colour change from green to yellow color after overnight incubation. Absence of colour change ruled out *E. faecalis.*

Pigment production

Ability to produce pigment was observed by a few species of *Enterococci*. This was tested by sub culturing colonies in nutrient agar.

Any color produced was visible and also further confirmed by touching the colony with a sterile swab.

Sugar fermentation tests

Species identification was further done by sugar utilization by inoculating the organism in peptone with 1% sugars with Andrade indicator. The sugars used were mannitol, arabinose, raffinose, sorbose, sorbitol, lactose and sucrose. Any change in colour from colourless to pink indicated the positive sugar fermentation test. Absence of colour change was taken as negative.

Antimicrobial susceptibility test: (CLSI 2015)

The 250 test isolates of *Enterococci* were checked for the antimicrobial susceptibility pattern using Kirby-Bauer disc diffusion method. Three or four pure colonies were inoculated in peptone water and incubated for about 3 hours. The turbidity of the growth was matched to 1 of Mc Farlands. A lawn culture of the broth with a sterile swab was done in Muller Hinton agar and the discs were placed at a distance of 24 mm.

About 6 discs were kept on each plate. The discs used were Penicillin (10U), Ampicillin (10µgm), Amoxicillin Clavulanic acid (20/10), Erythromycin (15µgm), Vancomycin (30µgm), High level gentamicin (120µgm), Linezolid (30µgm), Tetracycline (30µgm). For isolates from urine section Norfloxacin (10µgm) and Nitrofurantoin (300µgm) was kept. All discs were bought from HIMEDIA.

Zones of inhibition were measured and recorded and the organism was interpreted as sensitive or resistant as per the recommendations from CLSI guidelines. The quality Control used was *Enterococcus faecalis* ATCC 29212.

Screening for high level gentamicin resistance (CLSI 2015)

Screening media for high level gentamicin resistance was prepared. Screening for high level gentamicin resistance was done by agar dilution method. BHI agar with gentamicin at a concentration of 500µgm/ml was added and the plates were prepared. Inoculum of 10µL of 0.5 Mc Farland was spot inoculated. Presence of even 1 colony was interpreted as a resistant strain. These strains were collected for molecular studies.

Detection of beta lactamase (Marothi *et al.*, 2005)

The detection of beta lactamase test was done based on the chromogenic cephalosporin method. Nitrocefin disc was obtained commercially from BD. The disc was moistened with sterile saline and pure isolated colony was applied on it. The reaction was read within 5 min. Isolates producing beta lactamase produced a red color.

Molecular study of isolates

A representative sample of 50 high level gentamicin resistant isolates was selected for genomic analysis. The DNA was extracted using the QIAGEN kit. Followed by extraction, the isolates were analysed for the presence of Bifunctional resistant geneaac (6') le aph (2") la. The vancomycin resistant isolates were checked for the presence of van A gene simultaneously.

Detection of aac (6') le aph (2") la gene

The primers for HLGR with a base pair of 348 bp were amplified. The sequence was obtained from the studies done previously[11]. PCR Master mix: The master mix was prepared as follows: 2.5µl of PCR buffer,2.5µl of MgCl2, 2.5µl of DNTPs,

10.2µl of Mili Q H2O, 1µl of forward and reverse primers and 0.3µl of Taq Polymerase was added to eppend off. From the above mixture 18µl was taken and 5µl of the extracted DNA was added.

Primers for aac (6')Ie- aph (2")la with base pair of 348 bp (Vakulenko *et al.*, 2003):

F (5'CAGAGCCTTGGGAAGATGAAG3')
R (5'CCTCGTGTAATTCATGTTCTGGC3')

PCR program

The program consisted of initial denaturation step at 95^0C for 10 minutes followed by 30 cycles of denaturation at 94^0C for 300 seconds, annealing at 56^0C for 1 minute and extension at 72^0C for a period of 1 minute. The program had a holding temperature of 72^0C for 10 minutes.

Analysis

PCR products were analyzed by running gel electrophoresis with 1.5% agarose gel in Tris Boric acid buffer. The samples along with the controls obtained by running ladder were analysed.

The gel was stained with ethidium bromide and the bands were obtained were visualized under UV light and also in automated GEL DOC viewer.

Detection of vanA gene

The primers with a base pair of 732bp for detection of vanA were obtained from the studies done previously.

Primers for van A with base pair of 732bp (Dutka-Malen *et al.*, 1995):

F (5' GGGAAAACGACAATTGC-3')
R (5'GTACAATGCGGCCGTCGTTA-3')

PCR program

This program had a denaturation step at 95^0C for 5 minutes followed by 30 cycles of DNA denaturation at 95^0C for 1 minute, annealing at 54^0C for 45 seconds and primer extension at 72^0C for 45 seconds. This was followed by a holding temperature of 72^0C for 10 minutes.

Analysis

PCR products were analyzed by running gel electrophoresis with 1.5% agarose gel in Tris Boric acid buffer. The samples along with the controls obtained by running ladder were analysed. The gel was stained with ethidium bromide and the bands were obtained were visualized under UV light and in automated GEL DOC viewer.

Results and Discussion

All the 250 isolates of *Enterococcus* from various clinical samples including urine, pus, wound swab, blood and CSF were processed.

The maximum number of isolates was obtained from urine section (184) followed by wound swabs (24), blood section (14), 4 from peritoneal samples, 1 from CSF (Figure 1). The majority of the isolates were obtained as pure growth (216) and 34 was obtained as mixed from the samples processed. Figure 2 depicts the speciation of enterococcal isolates in which *E. faecalis* was the predominant species (157), succeeded by *E. faecium* (79), *E. raffinosus* (7), and *E. durans* (5) and *E. casseliflavus* (2).

Illustration 1 shows the genus specific biochemical tests used in the identification.

The antibiotic susceptibility pattern of the enterococcal isolates by disc diffusion method revealed that out of the 250 isolates, about 69.2% were ampicillin sensitive, 49.2% were

high level gentamicin sensitive, 89.3% were sensitive to erythromycin. The maximum number of isolates was found to be sensitive to vancomycin (97.2%) in our study (Table 1). Among the urinary isolates maximum sensitivity was noticed to nitrofurantoin (92.3%) (Table 2) and norfloxacin (83.1%).

The maximum sensitivity was observed for linezolid followed by vancomycin, teicoplanin, ampicillin and high level gentamicin. Among the non-urinary isolates erythromycin sensitivity was observed to be the highest.

The chromogenic nitrocefin disc was used to screen the isolates for the presence of beta lactamase. In our study none of the isolates were found to be positive for beta lactamase.

Figure 3 shows the presence of Bifunctional gene aac (6')1eaph(2")1a among the high level gentamicin resistant strains. Out of the 50 randomly selected resistant strains for genotype study, 48 had the gene.

Illustration 2 shows the presence of bands of size 348 bp during the gel electrophoresis.

In our study 7 out of the 250 isolates were found to be resistant to vancomycin. The genotypic analysis was done among these resistant strains using the van A gene. The results showed that 5 of the 7 strains were found to be positive for van A gene (Figure 4).

Illustration 3 shows the presence of van A gene of base pair 732, confirming its presence among the resistant strains.

Enterococcus has been noted to cause a majority of urinary tract infections. This being the most common followed by bacteremia, sepsis and even case reports of meningitis has been reported. In our study out of 250 samples studied the majority of the isolates were obtained from urinary tract infections (73.6%) followed by wound swabs (9.6%) and blood (5.6%). This correlates well with other studies in which *Enterococci* was obtained from urine isolates maximally (Karmarkar *et al.,* 2004, Mathur *et al.,* 2003). According to the studies *Enterococci* are considered as the commonest urinary pathogen justifying the increase in the rate of isolation from urinary samples (Murray *et al.,* 1990).

Among the various species, in our study *E. faecalis* was the most common isolate accounting for 62.8% followed by *E. faecium, E. durans, E. raffinosus* and *E. casseleflavus.* Similar findings were reported from other studies done in Bangalore in which *E.faecalis* was 59%, *E. faecium* was 38 % (Golia *et al.,* 2014).

Enterococci show intrinsic resistance to cotrimoxazole, clindamycin, cephalosporins and normal aminoglycosides. Thus these antibiotics are not tested (Mendiratta *et al.,* 2008).

The antibiotic susceptibility pattern of the isolates in our study showed the following:

The sensitivity to linezolid was maximum (98.8%) followed by vancomycin (97.2%), erythromycin (89.3%), teicoplanin (75.2%), ampicillin (69.2%), high level gentamicin (49.2%) and penicillin (25%).

Among the urinary isolates maximum sensitivity was noted in Nitrofurantoin (92.3%) followed by Norfloxacin (83.15%). Studies done in North India showed a similar susceptibility pattern with increased resistance to penicillin and aminoglycosides. Also the sensitivity percentage to linezolid and vancomycin was higher compared to other antibiotics (Mulla *et al.,* 2012).

Fig.1 Distribution of *Enterococcus* isolates among various samples (N=250)

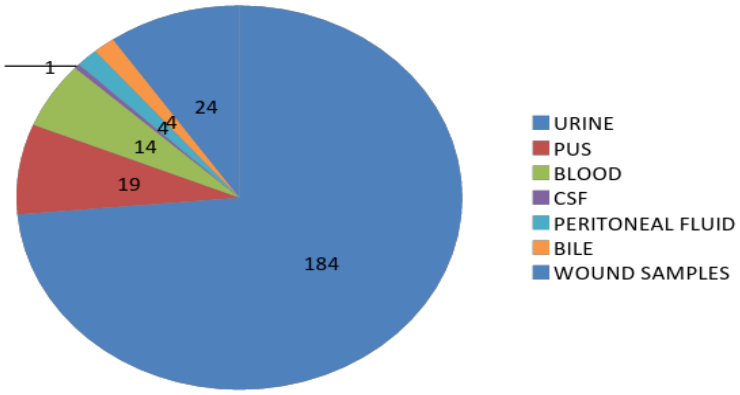
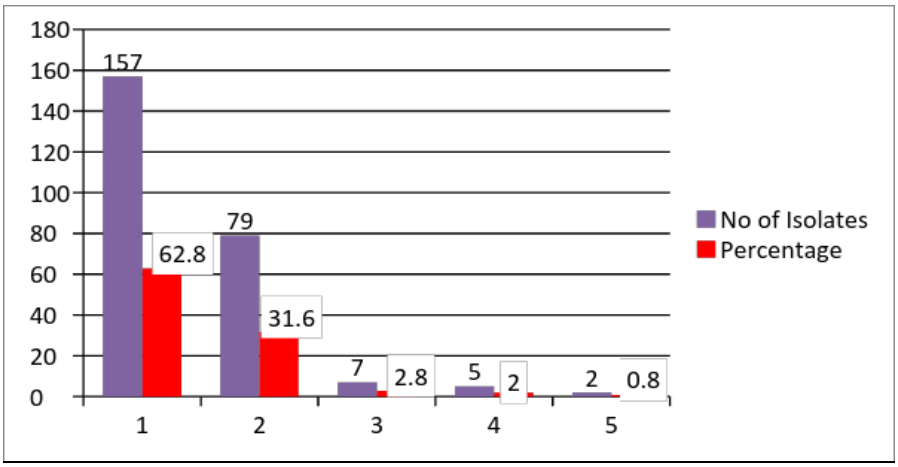


Fig.2 Speciation of *Enterococcus* from clinical isolates N=250



E.faecalis	1
E.faecium	2
E.raffinosus	3
E.durans	4
E.casseliflavus	5

Fig.3 Detection of the presence of aac (6') le aph (2") la gene N = 50

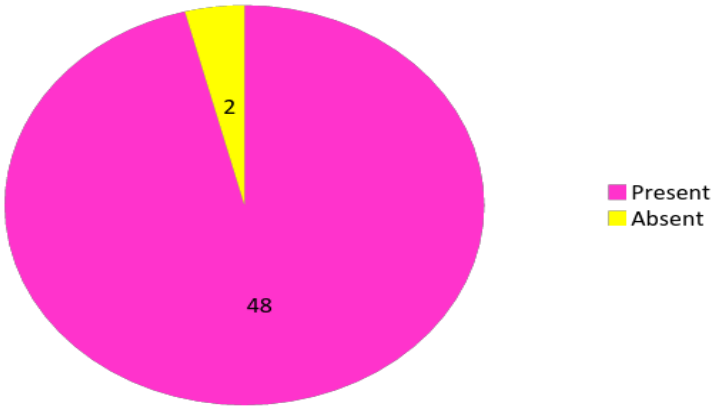


Fig.4 Detection of van A gene among the VRE isolates (N=7)

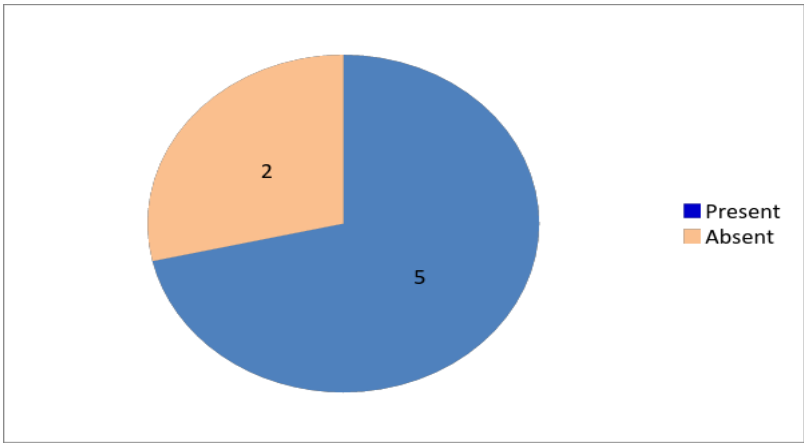


Table.1 Antibiotic susceptibility pattern of Enterococcal isolates

N = 250

RESISTANCE	SENSITIVE	ANTIBIOTICS
77	173	AMPICILLIN
30.8	69.2	%
127	123	HIGH LEVEL GENTAMICIN
50.8	49.2	%
7	243	VANCOMYCIN
2.8	97.2	%
3	247	LINEZOLID
1.2	98.8	%
62	188	TEICOPLANIN
24.8	75.2	%

Table.2 For urinary isolates

N = 184

RESISTANCE	SENSITIVE	ANTIBIOTICS
31	153	NORFLOX
16.85	83.15	%
14	170	NITROFURANTOIN
7.61	92.39	%
37	147	TETRACYCLINE
20.11	79.89	%

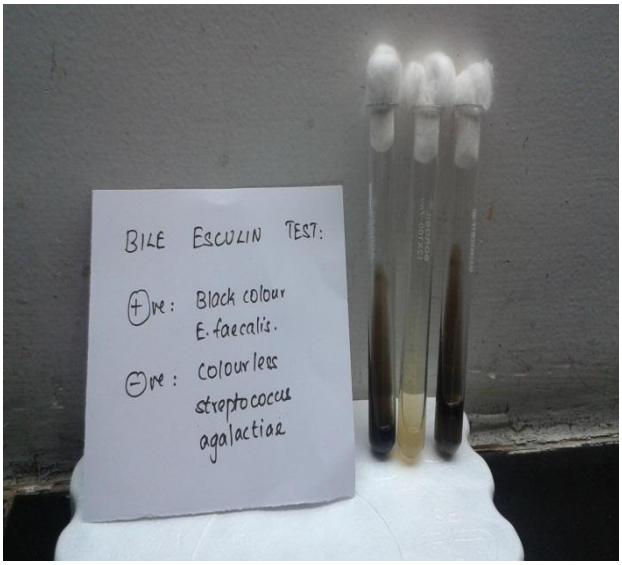
Table.3 For non urinary isolates

N = 66

RESISTANCE	SENSITIVE	ANTIBIOTICS
49	17	PENICILLIN
74.25	25.75	%
7	59	ERYTHROMYCIN
10.7	89.3	%

Illustration.1 Genus specific test

A) Bile-Esculin Test



B) PYR Test



C) 6.5% NaCl test



Illustration.2 PCR showing aac(6')-aph(2") BAND OF SIZE 348 bp

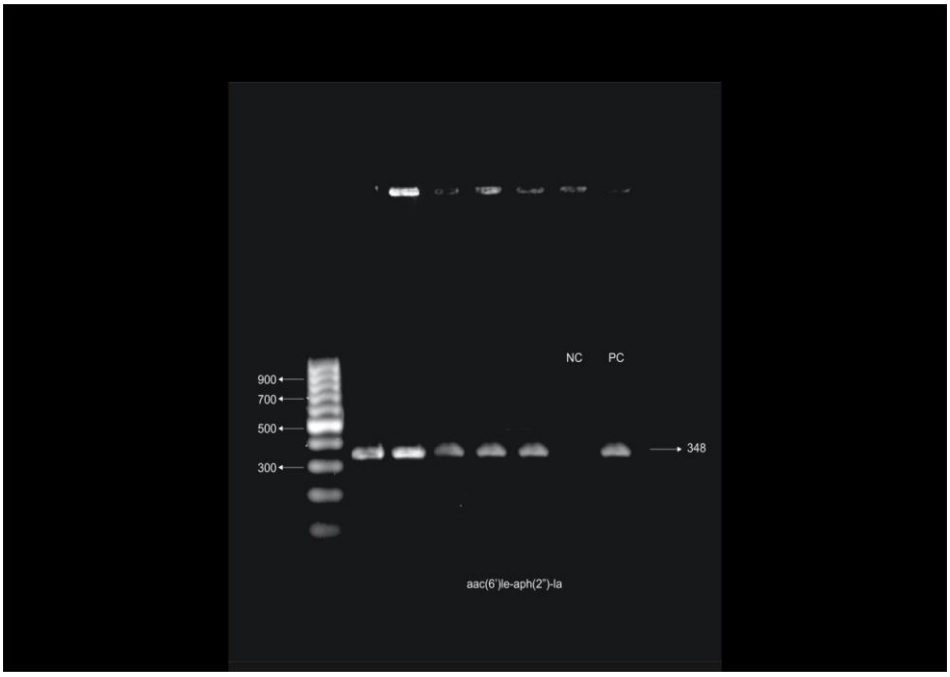
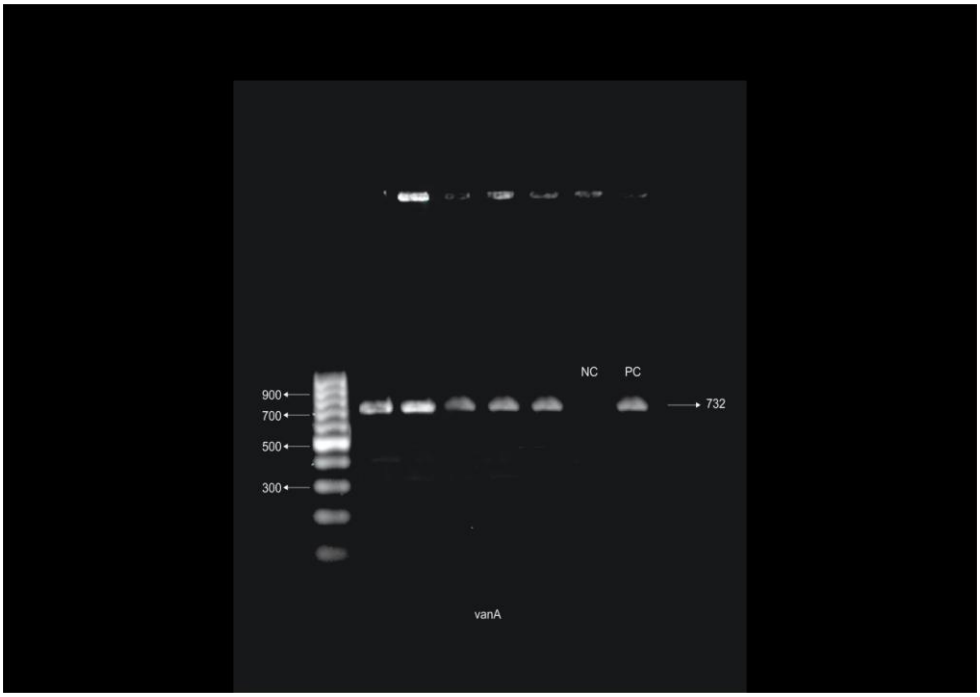


Illustration.3 PCR showing van A gene of band size 732bp



Another study showed the percentage of high level gentamicin resistance to be 68% (Randhawa *et al.*, 2004). Reports from South India showed the resistance percentage of high level aminoglycoside to be more than 50% (Sreeja *et al.*, 2012).

The detection of beta lactamase production by the chromogenic nitrocefin disc method showed that none of the isolates had beta lactamase enzyme. Studies done in Delhi and in Maharashtra also showed similar results (Jain *et al.,* 2011; Mendiratta *et al.,* 2008). The resistance to beta lactams though common, the mechanism could be due to alteration in penicillin binding protein PBP 5 which is more common than beta lactamase production (Murray *et al.,* 1992).

The molecular study done to detect the presence of the resistant gene confirmed the presence of the bifunctional gene aac (6') le-aph (2") la of base pair 348 to be present in 48 (96%) out of the 50 randomly selected resistant strains.

Studies done in Chennai have showed the presence of this gene in 38.2% of the isolates (Padmasini *et al.,* 2014) whereas there are other studies in which all the resistant isolates possessed this bifunctional gene (Hasani *et al.,* 2012). The resistance to high level gentamicin indicates resistance to all the aminoglycosides except streptomycin (CLSI 2015). The combination effect of aminoglycoside with beta lactam antibiotic is not helpful in the treatment of patients possessing such resistant strains.

Out of the 250 isolates the vancomycin resistant isolates obtained in our study was 2.8%. Studies have shown the prevalence of vancomycin resistance to be less in India ranging around 1.7-20% (Sreeja *et al.,* 2012).

Molecular studies revealed the presence of van A gene in 5 (71.42 %) isolates. Studies have also been reported showing van A to be the most common genotype isolated among the vancomycin resistant strains (Praharaj *et al.,* 2013)

Out of the seven isolates, five were *E. faecium* and two were *E. casseliflavus*. The remaining two isolates were *E. casseliflavus* which would have exhibited intrinsic resistance to vancomycin with van C gene (Praharaj *et al.,* 2013).

The incidence of *Enterococci* from the clinical isolates in our study was 2.5%.

The incidence of the pure isolates was 86.4% in comparison to the mixed isolates which was found to be 13.6%.

Among the isolates of *Enterococci*, the predominant species was *E. faecalis* (62.8%) followed by *E. faecium* (31.6%), *E. raffinosus* (2.8%), *E. durans* (2%) and *E. casseliflavus* (0.8%).

The antibiotic sensitivity pattern of the urinary isolates showed 92.3% sensitive to nitrofurantoin and 83.15% sensitive to norfloxacin. Among the non-urinary isolates maximum sensitivity was observed in linezolid which was 98.8% followed by vancomycin (97.2%), erythromycin (89.3%), teicoplanin (75.2%), ampicillin (69.2), high level gentamicin sensitivity (HLG) (49.2%) and pencillin (25%) (Table 3).

The vancomycin resistance was found to be 2.8% in *E. faecium*.

All the isolates were found to be beta lactamase negative by chromogenic (nitrocefin) method proving the alteration in PBP5 to be the more common mechanism involved in penicillin resistance.

The molecular characterization of HLGR(high level gentamicin resistant) strains, 50 isolates selected randomly out of 127, showed the presence of bifunctional gene aac(6')le-aph(2")la to be around 96%. (Primers of base pair 348 - F (5'CAGAGCCTTGGGAAGATGAAG3') R (5'CCTCGTGTAATTCATGTTCTGGC3')

The presence of van A gene was found to be 71.42% in our study.

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References

Cetinkaya *et al.,* (2000) Vancomycin-resistant *Enterococci*. *Clinical microbiology reviews* 13, no: 686-707.

CLSI. Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial susceptibility testing. CLSI document M100-524.Wayne, PA: Clinical and Laboratory standard Institute 2014.

Dutka-Malen *et al.,* (1995) "Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant *Enterococci* by PCR." *Journal of clinical microbiology* 33, no. 1: 24-27.

Fisher *et al.,* (2009) "The ecology, epidemiology and virulence of *Enterococcus*." *Microbiology* 155, no. 6: 1749-1757.

Gold *et al.,* (1996). "Antimicrobial-drug resistance. *"New England Journal of Medicine* 335, no. 19:1445-1453.

Golia *et al.,* (2014) "Isolation and speciation of *Enterococci* from various clinical samples and their antimicrobial susceptibility pattern with special reference to high level aminoglycoside resistance. *"International Journal of Medical Research and Health Sciences* 3, no. 3: 526-529.

Gordon *et al.,* (1992) "Antimicrobial susceptibility patterns of common and unusual species of *Enterococci* causing infections in the United States. Enterococcal Study Group." *Journal of clinical microbiology* 30, no. 9: 2373-2378.

Hasani *et al.,* (2012). "Molecular screening of virulence genes in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical specimens in Northwest Iran." *Indian journal of medical microbiology* 30, no. 2: 175.

Jain *et al.,* (2011) "Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. *"International Journal of Applied and Basic Medical Research* 1, no. 2: 80.

Karmarkar *et al.,* (2004) "Enterococcal infections with special reference to phenotypic characterization and drug resistance." *Indian Journal of Medical Research* 119: 22-25.

Kühn *et al.,* (2003) "Comparison of enterococcal populations in animals, humans, and the environment-a European study. "*International journal of food microbiology* 88, no. 2: 133-145.

Lancefield *et al.,* (1930). "A serological differentiation of human and other groups of hemolytic streptococci." *The Journal of experimental medicine* 57, no. 4: 571-595.

Marothi *et al.,* (2005) "Enterococcal resistance-an overview." *Indian journal of medical microbiology* 23, no. 4: 214.

Mathur *et al.,* (2003) "Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of northern India." *Indian Journal of Medical Research* 118: 25-28.

Mendiratta *et al.,* (2008). "Status of high level aminoglycoside resistant *Enterococcus faecium* and *Enterococcus faecalis* in a

rural hospital of central India." *Indian Journal of Medical Microbiology* 26, no. 4 : 369.

Mulla *et al.,* (2012)"Prevalence of *Enterococci* with higher resistance level in a tertiary care hospital: a matter of concern." *therapy* 1, no. 2: 3.

Murray *et al.,* (1992). "Beta-lactamase-producing *Enterococci*." *Antimicrobial agents and chemotherapy* 36, no. 11: 2355.

Murray *et al.,* (1990)"The life and times of the *Enterococcus*." *Clinical microbiology reviews* 3, no.1: 46-65.

Padmasini *et al.,* (2014) "High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of *Enterococcus* species in Chennai, India." *The Scientific World Journal* 2014.

Praharaj *et al.,* (2013) "Phenotypic and genotypic characterization of vancomycin resistant *Enterococcus* isolates from clinical specimens." *The Indian journal of medical research* 138, no. 4: 549.

Randhawa *et al.,* (2004) "Aminoglycoside resistance in *Enterococci* isolated from paediatric septicaemia in a tertiary care hospital in north India." *Indian Journal of Medical Research* 119: 77-79.

Sreeja *et al.,* (2012). "The prevalence and the characterization of the *Enterococcus* species from various clinical samples in a tertiary care hospital." *Journal of clinical and diagnostic research: JCDR* 6, no. 9: 1486.

Vakulenko *et al.,* (2003) "Multiplex PCR for detection of aminoglycoside resistance genes in *Enterococci*." *Antimicrobial agents and chemotherapy* 47, no. 4: 1423-1426.

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Molecular Detection and Subtyping of New Delhi Metallobetalactamases (NDM-1) in Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* in a Tertiary Care Hospital, South India

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A B S T R A C T

Enterobacteriaceae are inhabitants of human, animal gastrointestinal tract and various other environmental sites. *Escherichia coli* and *Klebsiella pneumoniae* initially responded to betalactam antibiotics but there was an increase in resistance to betalactam antibiotics due to beta lactamase produced by them. Resistance to carbapenems has now become more prevalent due to production of carbapenemases. NDM-1 is a novel metallo betalactamase that is capable of hydrolysing all beta lactam antibiotics except Aztreonam. It was aimed for Molecular detection and subtyping of NDM-1 in *Escherichia coli* and *Klebsiella pneumoniae*. Hundred carbapenem resistant isolates (42 isolates of *Escherichia coli* and 58 isolates of *Klebsiella pneumoniae*) were selected randomly for Molecular detection and sequencing of subtypes. A total of 82% strains were found to be NDM-1 positive yielding 475bp amplicon by molecular methods. Nine samples were sequenced for subtypes, of which five were NDM-1, two were NDM-4, and remaining two were NDM-3, and NDM-5.Sequencing and finding out of the subtypes may help to select new drug targets and design novel antibiotics.

Introduction

Enterobacteriaceae are inhabitants of wide variety of niches, including the human, animal gastrointestinal tract and various other environmental sites. The family Enterobacteriaceae includes a large number and diversity of genera.

During the past decade, there has been an emergence of carbapenems-resistant *Enterobacteriaceae* that produce carbapenamases, enzymes that efficiently hydrolyze carbapenems, as well as most β-lactam drugs (Queenan *et al*., 2007). New Delhi Metallo-beta-lactamases-1 (NDM-1) are one such carbapenamase that makes bacteria resistant to a broad range of beta-lactam antibiotics. These include the antibiotics of the carbapenems family, which are a mainstay for the treatment of antibiotic-resistant bacterial infections. Bacteria that produce carbapenamases are often referred to in the news media as "superbugs" because infections caused by them are difficult to treat.

Such bacteria are usually susceptible only to polymyxins and tigecycline. NDM-1 was first detected in a *Klebsiella pneumoniae* isolate from a Swedish patient of Indian origin in 2008. It was later detected

in bacteria in India, Pakistan, the United Kingdom, the United States, Canada, and Japan (Kumarasamy *et al*., 2010).

Materials and Methods

Study population

After Ethical clearance from the institution, the study was conducted between April 2014 and August 2015 on various clinical samples. Two thousand and forty two isolates of *Escherichia coli* and thousand hundred and ninety two isolates of *Klebsiella pneumoniae*, were identified by IMViC tests. Of which Five hundred and sixty isolates of *Klebsiella pneumoniae* (47%) and hundred and forty eight isolates of *Escherichia coli* (7%) were resistant to carbapenems. Among them hundred isolates were selected randomly for the study (42 isolates of *Escherichia coli* and 58 isolates of *Klebsiella pneumoniae*)

Isolation of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae*

The isolates were screened for the production of Carbapenam resistance as per CLSI 2014, using Ertapenem 10ug and Merapenam 10ug disc. The zone diameter of inhibition less than 19-21mm for Ertapenem and less than 16-21mm for Merapenam indicates Carbapenemase production. As per CLSI 2014 Ertapenem non susceptibility was the most sensitive indicator of Carbapenem resistance. Isolates were also tested by automated identification system (VITEK 2) for carbapenem resistance, based on Minimal Inhibitory Concentration. Among them a total of 100 isolates (42 isolates of *Escherichia coli* and 58 isolates of *Klebsiella pneumoniae*) were selected randomly for molecular study.

DNA extraction

The isolates were incubated overnight at 35ºC in Luria broth. About 2-3 ml of inoculum was centrifuged at 5000rpm for 10 minutes. After centrifugation the supernatant was discarded and the pellet was taken in a sterile Eppendorf tube. The pellets were resuspended in distilled water and kept in a water bath at 95ºC for 20 minutes. It was then cooled and centrifuged at 5000 rpm for 10 minutes. The supernatant was the extracted DNA and is stored at -20ºC.

Polymerase chain reaction

The extracted DNA was mixed with forward, reverse primers for NDM-1, master mix and PCR water and amplified in step one AB applied Bio system Real time PCR machine.PCR cycle consists of the following steps (Jeremiah *et al*., 2014). Initial denaturation at 94°C for 3 min, Denaturation at 94°C for 30 s Annealing at 60°C for 25 Extension at 72°C for 30 s, Final extension at 72°C for 3 min for 30 cycles. The amplified products are stored at -20ºC. Gel Electrophoresis was run. The images of the gel were captured by Gel doc and viewed. The presence of the NDM-1 gene was confirmed by the formation of bands at 475bp.

Gene sequencing and blasting

The amplied products which were confirmed for the presence of NDM-1 were sent for gene sequencing to Eurofins Genomics Bangalore. The sequenced genes were blasted using BIO EDIT software and results were interpreted in the NCBI (National centre for Biotechnology Information) website.

Results and Discusion

Two thousand and forty two isolates of *Escherichia coli* and thousand hundred and ninety two isolates of *Klebsiella pneumoniae*, were identified in various clinical samples during the study period, of which Five hundred and sixty isolates of *Klebsiella pneumoniae* (47%) and hundred and forty

eight isolates of *Escherichia coli* (7%) were resistant to carbapenems. Among them Forty two isolates of *Escherichia coli* and Fifty eight isolates of *Klebsiella pneumoniae* were selected for molecular study.

Polymerase chain reaction

Among the hundred isolates, eighty two (82%) were found to be NDM-1 positive (475bp) by PCR. Gel pattern of NDM-1 is depicted (Fig 1). Thirty seven (88%) of *Escherichia coli* and Forty five *Klebsiella pneumoniae* (77%) were NDM-1 producers.

Subtypes of NDM-1

Nine samples of amplified DNA (25ul) were sent randomly for sequencing at Euro fin genomics India pvt Ltd in Bangalore. Among them four were *Escherichia coli* and five were *Klebsiella pneumoniae*. The results send by the Eurofins Genomics were subjected to gene blasting using the BIO EDIT software and the sequence results were interpreted in NCBI (National centre for Biotechnology and Information) web site as shown in figures 2 and 3. On interpretation it was found that Amplicon-1, Amplicon -2, Amplicon -4, Amplicon -6 and 8 had amino acid sequences of NDM-1 and Amplicon -3 was NDM-5 and Amplicon -5 was NDM-3 and Amplicon -7 and Amplicon -9 were NDM-4. NDM-5 showed a point mutation variant differing from NDM-1 having a Met to Leu (M-L) substitution at 154 and Val to Leu (V-L)substitution at 88. NDM-3 showed a point mutation variant differing from NDM-1 having substitution of Asp to Asn (D-N) at 95.NDM-4 differs from NDM-1 by a single amino acid substitution (M-L) at 154 (Met to Leu) which is shown in table 1.

Gram negative bacteria belonging to the family of Enterobacteriaceae are the most frequently isolated organisms from clinical specimens. They are incriminated in virtually any type of infectious diseases and recovered from any specimen received in the laboratory. *Escherichia coli* and *Klebsiella pneumoniae* cause sepsis, endotoxic shock, urinary tract infections, meningitis in neonates and pneumonia in immune compromised.

They are normally treated based on the antibiotic sensitivity, site of infection and co-morbidity conditions (Longo *et al*., 2011). *Escherichia coli* and *Klebsiella* infections initially responded to betalactam antibiotics, but there was a dramatic increase in resistance to beta lactam antibiotics due to betalactamases produced by them. This resistance was superseded by use of second and third generation cephalosporins and combination of betalactam antibiotics with betalactamases inhibitors (Nosocomial burn wound infections, 2004).

Further with the emergence of ESBLs and Amp C resistance, carbapenems became the drug of choice. There has been a rapid increase in carbapenems resistance in Enterobacteriaceae in the recent years. The carbapenems resistance may be due to carbapenamases production, Efflux pump and Amp C enzyme production with membrane impermeability. However, carbapenamases (betalactamases) were found to be the predominant mechanism of this resistance. Among the newly emerged carbapenamases, New Delhi Metallo betalactamases (NDM-1) represents a war between the bugs and drugs.NDM-1 producing organisms are called super bugs (BorA *et al*., 2012).

NDM-1 was first reported in 2008 in *Klebsiella pneumoniae* and *Escherichia coli*. Both of them were recovered from a Swedish patient of Indian origin who was previously admitted in a hospital in New Delhi, India. Thereafter, NDM-1 has increasingly been reported from India and from several other parts of the world.

Besides this, most of these NDM-1 positive bacteria are also resistant to a wide variety of Antimicrobials like aminoglycosides, flouroquinolones, macrolides and sulphonamides and carry several additional resistance mechanisms, leaving only few or no therapeutic options (Krishna, 2010). In our study period carbapenem resistance was observed in 47% of *Klebsiella pneumoniae* and 7% of *Escherichia coli* in our hospital.

As per CLSI 2014 Ertapenem is a good marker for detection of Carbapenems resistance as others do not predict this resistance well. We randomly selected 42 *Escherichia coli* followed by 58 *Klebsiella pneumoniae* which were carbapenems resistant for molecular analysis. Molecular tests are the most accurate and usually taken as Gold standard. In our study we used the following primers (475bp) to detect NDM-1 by conventional PCR. NDM Forward: 5'-GGGCAGTCGCTTCC AACGGT and

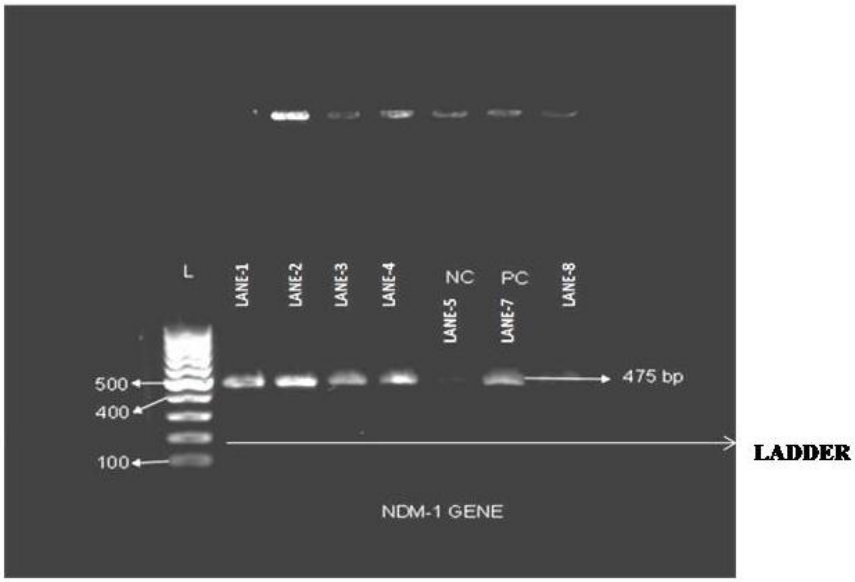
NDM Reverse: 5'- GTAGTGCTCAGTGTC GGCAT

Amplification, sequencing and subsequent blasting were performed to identify the subtypes. Eighty-two (82%) strains were found to be NDM-1 positive (475bp) of which Thirty seven (45%) were *Escherichia coli* and Forty five were *Klebsiella pneumoniae* (54%). The presence of NDM-1 has also been reported in Bangladesh, Australia, Netherlands, USA, Canada, Japan, and China indicating that blaNDM-1is widely spread all over the world (Berrazeg *et al*., 2014).

Table.1 DNA sequencing results of NDM-1 carbapenamase genes studied

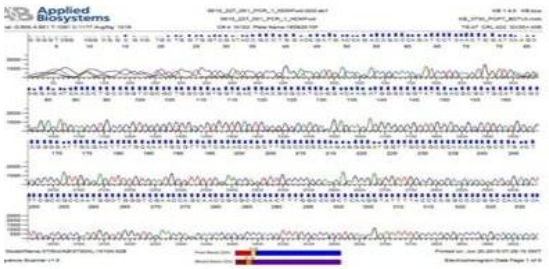
Amplicon	Aminoacid Substitution	Interpretation
Amplicon-I	Aminoacid sequences of NDM-1	NDM-1
Amplicon-2	Aminoacid sequences of NDM-1	NDM-1
Amplicon-3	Met to Leu (M-L) substitution at 154 and Val to Leu (V-L)substitution at 88.	NDM-5
Amplicon-4	Aminoacid sequences of NDM-1	NDM-1
Amplicon-5	Substitution of Asp to Asn (D-N) at 95.	NDM-3
Amplicon-6	Aminoacid sequences of NDM-1	NDM-1
Amplicon-7	Single amino acid substitution (M-L) at 154 (Met to Leu).	NDM-4
Amplicon 8	Aminoacid sequences of NDM-1	NDM-1
Amplicon 9	Single amino acid substitution (M-L) at 154 (Met to Leu).	NDM-4

Fig.1 Gel electrophoretic analysis of amplified NDM-1 gene products



LANE-1&2-Escherichia coli (+ve for NDM-1);LANE 3&4 –Klebsiellapneumoniae(+ve for NDM-1)

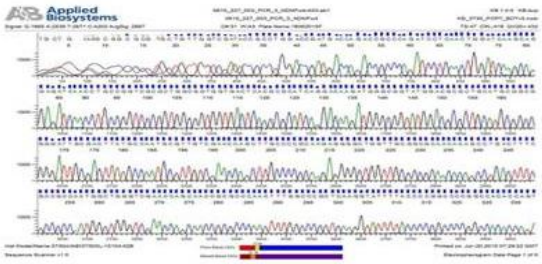
Fig.2 DNA sequencing results of amplified NDM-1



>0615_227_14_AMPLICON_1
AACGATTGGCCTTGCTGTCCTTGATCAGGCAGCCACCAAAAGCGATGT
CGGTGCCGTCGATCCCAACGGTGATATTGTCACTGGTGTGGCCGGGGC
CGGGGTAAAATACCTTGAGCGGGCCAAAGTTGGGCGCGGTTGCTGGTT
CGACCCAGCCATTGGCGGCGAAAGTCAGGCTGTGTTGCGCCGCAACCA
TCCCCTCTTGCGGGGCAAGCTGGTTCGACAACGCATTGGCATAAGTCG
CAATCCCCGCCGCATGCAGCGCGTCCATACCGCCCATCTTGTCCTGAT
GCGCGTGAGTCACCACCGCCAGCGCGACCGGCAGGTTGATCTCCTGCT
TGATCCAGTTGAGGATCTGGGCGGTC

AMINOACID BLAST
IVRDGGRVLVVDTAWTDDQTAQILNWIKQEINLPVALAVVTHAHQDKM
GGMDALHAAGIATYANALSNQLAPQEGMVAAQHSLTFAANGWVEPATA
PNFGPLKVFYPGPGHTSDNITVGIDGTDIAFGGCLIKDS

NDM-1

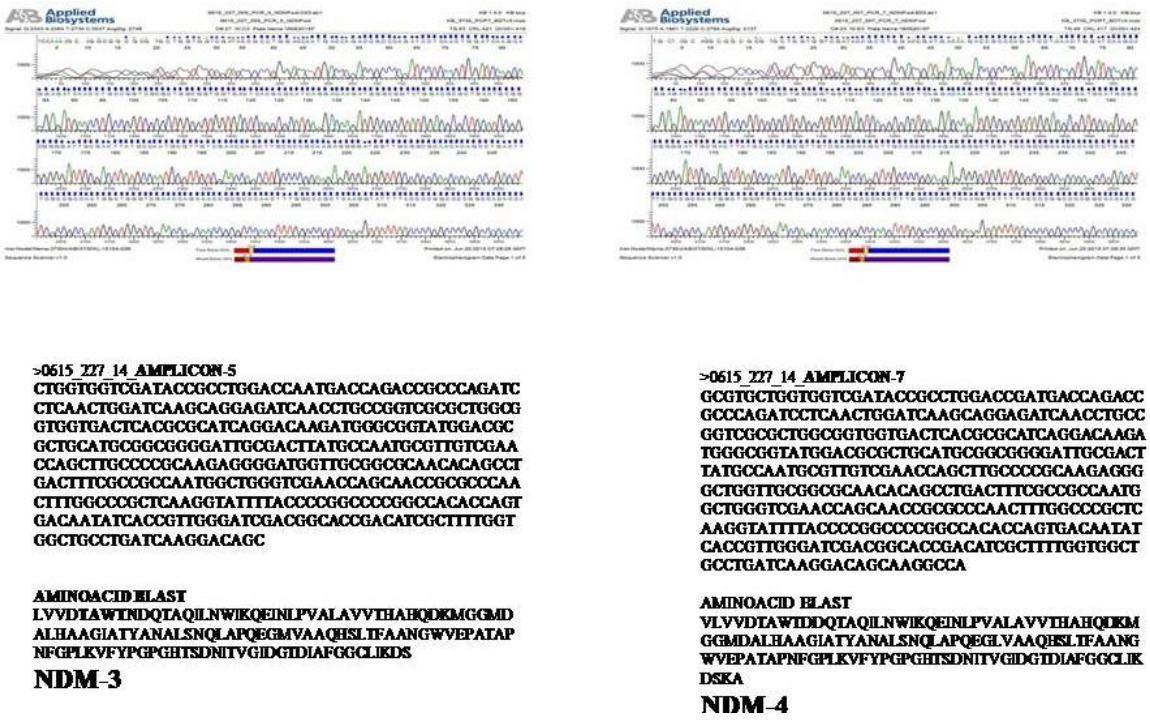


>0615_227_14_AMPLICON-3
TTTTGCTGTTGGTCGATACCGCCTGGACCGATGACCAGACCGCCCAGA
TCCTCAACTGGATCAAGCAGGAGATCAACCTGCCGGTCGCGCTGGCGG
TGGTGACTCACGCGCATCAGGACAAGATGGGCGGTATGGACGCGCTG
CATGCGGCGGGGATTGCGACTTATGCCAATGCGTTGTCGAACCAGCTT
GCCCCGCAAGAGGGGCTGGTTGCGGCGCAACACAGCCTGACTTTCGCC
GCCAATGGCTGGGTCGAACCAGCAACCGCGCCCAACTTTGGCCCGCTC
AAGGTATTTTACCCCGGCCCCGGCCACACCAGTGACAATATCACCGTT
GGGATCGACGGCACCGACATCGCTTTTGGTGGCTGCCTGATCAAGGAC
AGCAAGGC

AMINOACID BLAST
3LLVDTAWTDDQTAQILNWIKQEINLPVALAVVTHAHQDKMGGMDALH
AAGIATYANALSNQLAPQEGLVAAQHSLTFAANGWVEPATAPNFGPLKV
FYPGPGHTSDNITVGIDGTDIAFGGCLIKDSKA

NDM-5

Fig.3 DNA DNA sequencing results of amplified NDM-1



In a similar study from south India, NDM-1 was detected in 75% of K. pneumoniae and 66% of E. coli by PCR and the remaining isolates were assumed to posses other mechanisms of resistance like efflux pumps, other carbapenamases, Amp C production and membrane impermeability (Nagaraj *et al*., 2012). DNA and protein sequencing were started in the 1970s, when the virus Lambda (50,000 nucleotides) was sequenced by Sanger *et al*., DNA sequencing was initially carried out for small genomes such as viruses and organelles, and complete sequencing of a bacterial genome were started later on with the improvements in sequencing technologies. DNA mutations has resulted in changes in the amino acid sequences and produced an ever increasing range of subtypes or variants in each of carbapenamase.

Fifteen variants of NDM-1 have been identified. These variants were identified after sequencing and blasting. They differ by various aminoacid substitutions. We found NDM-1 in 5 strains (56%), NDM-4 in 2 strains (22%) NDM-3and NDM-5 in one strains each (11%). Further characterization of the 18 strains (5 were *Escherichia coli* and 13 *Klebsiella pneumoniae* which were negative for NDM-1 gene) for the mechanism of resistance was not done in our study. These isolates probably have other carbapenemases like VIM or it may have efflux changes or Amp C beta lactamase production with outer membrane impermeability.

Presently NDM-1 producing bacterial infections can be managed with Colistin and Tigecycline combination. Colistin should not be given alone as it provokes resistance in vivo due to hetero resistant strains. Fosfomycin may also serve as an option for combination therapy. Detection of NDM-1 like carbapenemases in members of Enterobacteriaceae will help in the management and prevention of spread of such

bacteria in hospital and community which in turn will save the patients and valuable drugs. Besides epidemiological surveillance sequencing would help to identify new drug targets, which can be used in the design of novel antibiotics.

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I thank Dr. S. Ramalingam and Dr. B. Appalaraju for permitting me to carry out the work in the Department of Microbiology, PSG IMS&R, Coimbatore. I acknowledge the kind help rendered by the supervisor Dr. B. Appalaraju for having guided at every level.

References

Berrazeg, M., S. Diene, L. Medjahed, P. Parola, M. Drissi, Didier Raoult, and J. Rolain. 2014. "New Delhi Metallo-beta-lactamase around the world: an eReview using Google Maps." *Euro Surveill.*, 19, no. 20.

BorA, Arijit, and GiA Suddin AhMed. 2012. "Detection of NDM-1 in Clinical Isolates of *Klebsiella pneumoniae* from Northeast India." *J. Clin. Diag. Res.*, 6, no. 5, 794-800.

Bush, Karen, and George A. Jacoby. 2015. "Updated Functional Classification of B-Lactamases" *Antimicrobial Agents and Chemotherapy*, 54(3): 969–976.

Deshpande, P., Rodrigues, C., Shetty, A., Kapadia, F., Hedge, A., Soman, R. 2010. New Delhi Metallo-beta lactamase (NDM-1) in Enterobacteriaceae: Treatment options with carbapenems compromised. *J. Assoc. Physicians India*, 58: 147-9.

Jeremiah, S.S., V. Balaji, S. Anandan, and R.D. Sahni. 2014. A possible alternative to the error prone modified Hodge test to correctly identify the carbapenemase producing Gram-negative bacteria. *Indian J. Med. Microbiol.*, 32, no. 4: 414.

Krishna, B.V. 2010. New Delhi metallo-beta-lactamases: a wake-up call for microbiologists. *Indian J. Med. Microbiol.*, 8(3): 265-6.

Kumarasamy, K.K., Toleman, M.A., Walsh, T.R., *et al*. 2010. "Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study". *Lancet Infect. Dis.*, 10(9): 597–602.

Longo, Dan, Anthony Fauci, Dennis Kasper, and Stephen Hauser. 2011. *Harrison's Principles of Internal Medicine 18th edition*.McGraw-Hill Professional, 18 th edition p 1251-54.

Nagaraj, S., S.P. Chandran, P. Shamanna, and R. Macaden. 2012. "Carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital in south India." *Indian J. Med. Microbiol.*, 30, no. 1: 93.

Nosocomial burn wound infections. 2004. In: Hospital Epidemiology and Infection Control, 4th ed.Mayhall CG, ed. Lippincott Williams and Wilkins, Philadelphia, 495-497.

Queenan, A.M., Bush, K. 2007. Carbapenemases, The versatile β-lactamases, *Clin. Microbiol. Rev.*, 20: 440–58.

Sanger, Frederick, Steven Nicklen, and Alan R. Coulson. 1977. "DNA sequencing with chain-terminating inhibitors."*Proceedings of the National Acad. Sci.*, 74, no. 1: 5463-5467.

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Clinico-mycological and Antifungal Susceptibility Profiles of Candiduria in A Tertiary Care Hospital From South India

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Original Article

Microbiology Section

Clinico-mycological and Antifungal Susceptibility Profiles of Candiduria in A Tertiary Care Hospital From South India

DINOOP KOROL PONNAMBATH, SWARAN KUMAR M, APPALARAJU BOPPE, KARTHIKEYAN SHANMUGAM

ABSTRACT

Introduction: *Candida* is one of the common causative agent of Urinary Tract Infections (UTI) worldwide. The most common reported species causing UTI is *Candida albicans*. Incidence of UTI due to non-albicans *Candida* species. is on rise in recent years because of their better adaptability and increased resistance to antifungals. Susceptibility profile reports of various *Candida* species. to newer azoles like voriconazole and beta-glucan inhibitors (e.g., caspofungin) are deficient in India, since the reference broth microdilution method is not widely utilized. In this study, a rapid reliable and easier alternative, VITEK 2 compact system was utilized to determine the antifungal susceptibility profile.

Aim: To analyse the clinical and mycological profile with determination of drug susceptibility pattern of *Candida* isolates from urine samples.

Materials and Methods: This observational study was conducted in PSG Institute of Medical Sciences and Research, during April to September 2015. *Candida* isolated with a colony count of ≥103 CFU/ml of urine from clinically suspected cases of UTI were included in the study. Urine samples (n=3821) from clinically suspected UTI cases (n=3821) were subjected to microscopic examination and semi quantitative estimation of yeast culture obtained by inoculated of calibrated volume of urine onto blood, Mac-conkey and HiCrome UTI agar. Clinical parameters of the cases were obtained for analysis. Speciation of *Candida* was performed using germ tube test, observation of morphology in corn-meal agar and pigment production in HiChrome *Candida* differential agar. Confirmation of the species identification and anti-fungal susceptibility profile were obtained using VITEK-2 compact system.

Results: Total 101 patients were identified with significant candiduria. Community-Acquired Candiduria (CAC) was seen in 11 (10.8%) of the cases. 23 (22.7%) cases of candiduria were associated with pyuria. Concomitant candidemia was observed in 4 (3.9%) cases of candiduria The most common species identified was *Candida* albicans (n=53, 52.4%). The commonest non-albicans *Candida* species causing significant candiduria cases were *C. glabrata* and *C. tropicalis* (n=18, 17.8% each). The antifungal susceptibility testing revealed an overall 94% susceptibility to amphotericin B, 89% susceptibility to flucytosine, 84% & 88% susceptibility to azoles, fluconazole and voriconazole respectively with 90% susceptibility to caspofungin. Significant high linear correlation existed between fluconazole and voriconazole resistance for *C. glabrata*, *C. albicans* and *C.tropicalis*. [Pearson's correlation coefficient, *C. glabrata* [r =1.00 (p<0.001)], *C. albicans* [r=0.91 (p<0.001)] and *C.tropicalis* [r=0.79 (p<0.001)].

Conclusion: *C. albicans* was found to the commonest species in our Tertiary Care Center in contrast to the reporting of non-*albicans Candida* species as the commonest species in most other recent studies. Determination of the antifungal susceptibility profile showed increasing trend of resistance to most antifungal agents, particularly azoles.

Keywords: Antibacterial agents, Antibiotics, Immunosuppression

INTRODUCTION

Candida species. has been reported as one of the common etiological agent of UTI, especially in a hospital setting. Advances in critical care management, immunosuppression and widespread use of antibacterial agents have led to their increased prevalence. Common risk factors associated with candiduria are extremes of age, diabetes mellitus, females in reproductive age group, structural abnormalities of the urinary tract, broad-spectrum antibiotic use, immunosuppression, indwelling catheters, abdominal surgeries, admission in ICU etc., [1]. Identification and susceptibility testing of the yeast helps targeted therapy of the infection and yields epidemiological

data for assessment of the prevalence and resistance pattern. Fungal UTI are mostly asymptomatic, occasionally can present with upper or lower urinary tract symptoms. Despite the lack of symptoms, increased colonization burden may predispose to invasive candidiasis particularly in debilitated individuals. It necessitates differentiation of asymptomatic and symptomatic candiduria since unnecessary treatment, catheter changes, and hospital prolongation can be avoided, since hospital cost burden and patient morbidity increases substantially. The scenario of presence of yeast in microscopic examination or isolation in culture needs to be evaluated with the clinical picture to determine its relevance and the decision on the commencement of antifungal therapy. Semi-quantitative/ quantitative urine culture is performed in evaluation of significant candiduria and a colony count of ≥1000 CFU/ml of urine, obtained in culture media in most situations represents significant candiduria. Significant candiduria occurs as 2 clinical presentations, asymptomatic and symptomatic candiduria. Patients with symptomatic candiduria need to be started on antifungal therapy, whereas asymptomatic candiduria needs to be interpreted with care. The significance of asymptomatic candiduria arises when the patient is severely debilitated/immunosuppressed, or is planned for urological instrumentation/procedures. Asymptomatic candiduria can also represent a picture of sample contamination (inappropriate collection) or colonization (in catheterized patients). In these cases, evaluating a repeat urine sample rules out contamination and colonization when the repeat sample has been collected after removal of indwelling catheter [2]. The most common reported species causing UTI is *Candida albicans* [3]. Incidence of Non-*albicans Candida* species. (e.g.: *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis* etc.,) causing UTI have been increasingly reported due to enhanced adaptation potential and increased resistance to antifungal agents [4]. In the past few years, there has been emergence of drug-resistance in *Candida* species causing clinical infections, leading to high morbidity and mortality in hospitalized patients [5]. Testing for antifungal drug susceptibility in clinically significant *Candida* species isolates guides prompt treatment thereby improving the patient's outcome. Susceptibility profile reports of various *Candida* species. to newer azoles like voriconazole and beta-glucan inhibitors (eg: caspofungin) are deficient in India, since disc diffusion interpretation guidelines are not available for all antifungals and the reference broth microdilution method is not widely practiced. The VITEK 2 antifungal drug susceptibility determination system has emerged as an easier and an expeditious alternative to the reference broth microdilution method (CLSI and EUCAST) [6]. Studies report 95-100% essential agreement between the results of VITEK 2 and the reference methods [7,8]. This study was undertaken to analyse the clinical and mycological profile with determination of drug susceptibility pattern of *Candida* isolates from urine.

MATERIALS AND METHODS

This was an observational study conducted in PSG Institute of Medical Sciences and Research (PSGIMS&R) during the period April 2015 - September 2015 (6 months). The Institute's ethics committee approval was obtained before the conduct of the study. (IHEC approval number: 15/039).

Inclusion criteria: *Candida* being isolated with a colony count of ≥1000 CFU/ml of urine either in pure culture or as one among the 2 isolates obtained from urine samples collected from clinically suspected cases of urinary tract infection (UTI)/ screening prior to urological instrumentation/intervention of all ages and both sex.

Exclusion criteria: If growth of *Candida* with a colony count of <1000 CFU/ml of urine or when >3 types of growth was obtained.

Urine samples were received in sterile screw capped leak-proof container, immediately transported from the collection center to the microbiology laboratory and stored in refrigerator until further investigations were undertaken. Urine wet mount examination was performed to look for the presence of pus cells, epithelial cells, red blood cells, casts, crystals, budding yeast cells with/without pseudohyphae. The urine samples were inoculated onto blood, mac-conkey and chromogenic media (HiCrome UTI agar, HiMedia Laboratories) by a calibrated wire loop standardized to deliver 5µl for semi-quantitative estimation of yeast growth. The inoculated culture plates were incubated aerobically at 37°C for 24-48 hours. *Candida* isolates with colony count of ≥1000 CFU/ml of urine were considered significant and included in the study. Clinical parameters of the cases were obtained in the case study form which included age, sex, details of admission (outpatient/ ward/ICU), presence/absence of any risk factors like extremes of age (elderly and pre-mature neonates), diabetes mellitus, structural abnormalities of the urinary tract, broad-spectrum antibiotic use in preceeding 30 days, immunosuppression (neutropenia, HIV etc.,), indwelling catheters, abdominal surgeries and admission in ICU etc. Significant candiduria was classified as hospital-acquired and community-acquired. CAC was defined as candiduria observed in outpatients with no prior hospitalization or within 48 hours of hospitalization. When the onset of candiduria is >48 hours of hospitalization, it was classified as Hospital-Acquired Candiduria (HAC) [9]. Preliminary speciation of *Candida* was performed using Germ Tube Test (GTT), observation of morphology in corn-meal agar and pigment production in chromogenic medium (HiChrome *Candida* differential agar, HiMedia Laboratories) [10]. Confirmation of the species identification and anti-fungal susceptibility profile were obtained using VITEK-2 compact system (YST ID and AST card, bioMerieux, Marcy-l'Etoile, France) for the following antibiotics: amphotericin B, flucytosine, fluconazole, voriconazole and caspofungin. The VITEK 2 cards were stored at 2-8°C until testing was performed. An inoculum turbidity equivalent to 2.0 McFarland was prepared in sterile saline as per the manufacturer's instructions using the DensiChek instrument, for antifungal susceptibility testing [11]. *Candida albicans* ATCC 10231 was used as the control

strain for the antifungal susceptibility testing.

STATISTICAL ANALYSIS

The data obtained were analyzed using SPSS software version 19. Fischer' exact/Chi-square test were used for comparative analysis and a p value of ≤0.05 was considered significant. Association/correlation between variables were analysed using Pearson's correlation coefficient (r).

RESULTS

During the study period of April - September 2015 (6 months), 101 patients were identified with significant candiduria. The mean age of the study population was 55.9±16.9 years (Median age-60 years). The age distribution of the study population ranged between 12-89 years and the majority of the candiduria cases (76%) were observed in the 41-80 years age group with a peak observed in the 61-70 years age group [Table/Fig-1]. In the study population, males (n=56, 55.4%) were more commonly affected than females (n=45, 44.6%). Candiduria was observed more commonly in the in-patient wards (n=58, 57.4%), followed by intensive care units (n=32, 31.8%) and outpatient department (n=11, 10.8%).

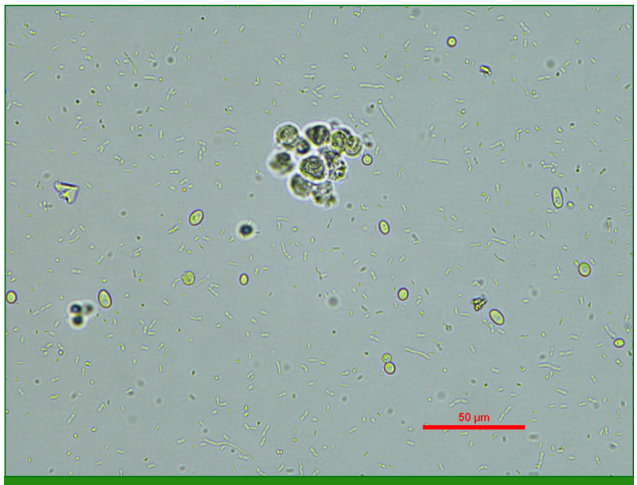
Age Group (in years)	Number (n)
0-10	0
11-20	4
21-30	9
31-40	9
41-50	10
51-60	22
61-70	30
71-80	15
81-90	2

[Table/Fig-1]: Age distribution of the patients with significant candiduria.

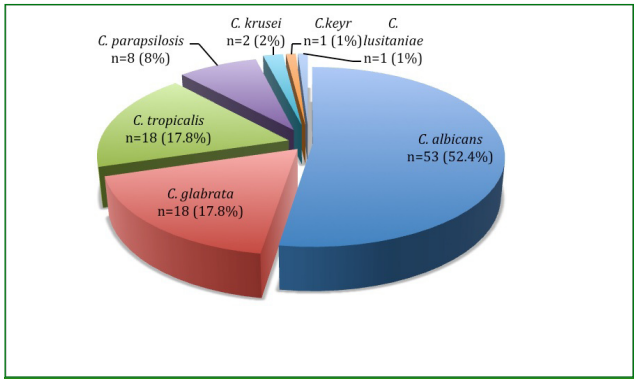
Among the risk factors, the commonest cause was broad-spectrum antibiotic usage (67.3%) followed by indwelling foley's catheterisation (56.4%), diabetes mellitus (16.8%), immunosuppression (13.8%), previous abdominal surgeries (1.9%) and urinary tract structural abnormality (0.9%) [Table/Fig-2]. Among the broad spectrum antibiotics, cephalosporin (68%) and fluroquinolone (32%) were the common antibiotics observed to be used in patients with significant candiduria, in the preceeding 30 days. Pyuria was observed in 23 (22.7%) cases of significant candiduria [Table/Fig-3], while concomitant candidemia was observed in 4 (3.9%) cases. The various species of *Candida* identified in the candiduria cases are depicted in [Table/Fig-4]. The most common species of *Candida* identified was *Candida albicans* (52.4 %). A case of significant candiduria had both *C. albicans* and *Trichosporon* species. The commonest non-*albicans Candida* species causing significant candiduria cases were *C. tropicalis* and *C. glabrata* (18% each).

Risk Factors	Number (n)	Percentage (%)
Diabetes Mellitus	17	16.8
Structural abnormalities of Urinary Tract	1	0.9
Broad Spectrum Antibiotic usage	68	67.3
Immunosuppressive Conditions	14	13.8
Indwelling Foley's Catheter	57	56.4
Underwent Abdominal Surgeries	2	1.9

[Table/Fig-2]: Distribution of risk factors in patients with significant candiduria.



[Table/Fig-3]: Wet mount examination of urine from showing pus cells and budding yeast cells (Magnification – 400X).



[Table/Fig-4]: Frequency of *Candida* species in the study population.

The antifungal susceptibility testing revealed an overall 94% susceptibility to amphotericin B, 89% susceptibility to flucytosine, 84% and 88% susceptibility to azoles, fluconazole and voriconazole respectively with 90% susceptibility to caspofungin. Susceptible-Dose Dependent (SDD) for fluconazole was observed in 5 isolates (9.4%) of *C. albicans*, 2 isolates of *C. glabrata* and *C. tropicalis* (11.1% each). The species-based susceptibility profiles to the antifungal agents tested are provided in [Table/Fig-5]. A significant high linear correlation existed between fluconazole and voriconazole

Candida species	Sensitive									
	Amphotericin B		Flucytosine		Fluconazole		Voriconazole		Caspofungin	
	n	%	n	%	N	%	N	%	N	%
C. albicans	50	94.3	47	88.6	46*	86.7	47	86.7	48	90.5
C. glabrata	17	94	16	88.8	15*	83.3	15	83.8	17	94
C. tropicalis	17	94	16	88.8	15*	83.3	16	88.8	14	77.7
C. parapsilosis	7	87.5	7	87.5	7	87.5	7	87.5	8	100
C. krusei	2	100	2	100	1	50	2	100	2	100
C. kefyr	1	100	1	100	1	100	1	100	1	100
C. lusitaniae	1	100	1	100	1	100	1	100	1	100
All species	95	94	90	89	85	84	89	88	91	90

[Table/Fig-5]: Antifungal susceptibility pattern of the isolated *Candida* species.
* includes susceptible-dose dependent (SDD) isolates

resistance for *C. glabrata* [Pearson correlation coefficient, r =1.00 (p<0.001)], *C. albicans* [r=0.91 (p<0.001)] and *C. tropicalis* [r=0.79 (p<0.001)].

DISCUSSION

Candiduria is a common nosocomial phenomenon and large scale observational surveys reveal *Candida* as the third most common organism isolated from urine in hospitalized patients [12]. During the study period, a total of 101 *Candida* species were isolated from cases of significant candiduria. Candiduria cases proportionally increased with age and were predominantly observed in the 41-80 years age group (76.2%). This observation could be due to the increased prevalence of co-morbid conditions like diabetes mellitus, benign prostatic hypertrophy, immunosuppression, frequent use of broad-spectrum antibiotics and foley's catheter etc. seen in the elderly age group. The median age and age distribution was similar to observational study reports by Nayman Alpat S et al., and Bobade O et al., [13,14].

Males and females were almost equally affected in our study population, whereas most reports from literature show the incidence of candiduria being more common in females [14,15]. Significant candiduria were observed commonly in hospitalized patients (inpatient and ICU settings) probably because of the presence of risk factors like broad spectrum antibiotic use, long term indwelling catheters and also to presence of other debilitating illness like uncontrolled diabetes commonly in the inpatient population than the outpatients. The broad-spectrum antibiotics implicated in cases of significant candiduria cases in our study population were cephalosporins (68%) and fluoroquinolones (32%) use in the preceding 30 days. There are documented reports of use of broad-spectrum antibiotics like cephalosporins attributing to causation of candiduria [16]. CAC was seen in 10.8% of the cases and the risk factors associated were diabetes mellitus, pregnant women and chronic indwelling catheters. Similar risk factors for CAC have been reported by Colodner R et al., [9].

Pyuria is defined as the presence of ≥3 leucocytes/hpf of unspun urine or ≥10 leucocytes per cubic mm of urine or positive dipstick test for leucocyte esterase [17]. In our study, 23 (22.7%) cases of candiduria were associated with pyuria. Pyuria is an important sign of candiduria yet lacks sensitivity and doesn't differentiate colonization from infection (lacks specificity) [18]. Concomitant candidemia was observed in 4 (3.9%) cases of candiduria. Studies report that approximately 1-8% of patients with candiduria particularly ICU admissions may have concomitant candidemia [3,12,19].

The commonest *Candida* species identified in our study was *Candida albicans* (52.4%), while non-*albicans Candida* species attributed to the remaining 47.6% of cases of significant candiduria. Among the non-*albicans Candida* species, *C. glabrata* and *C. tropicalis* were the common species isolated. Earlier multicentric reports on candiduria had similar findings of *Candida albicans* being the commonest species yet recent studies report the emergence of non-*albicans Candida* species as the commonest cause of significant candiduria due to the widespread use of antifungal agents for antifungal prophylaxis and treatment, particularly increasing incidence of *C. glabrata* and *C. krusei* infections which are intrinsically less susceptible to the commonly used azole drug, fluconazole [14,20,21]. Our report suggests relatively lower prevalence of non-*albicans Candida* species, in contrast to reports from other centers in India [22,23].

Analysis of the antifungal susceptibility pattern showed that most isolates were susceptible to amphotericin B. Resistance to the polyene drug, amphotericin B was observed in 6 isolates (6%) (3-*C. albicans*, 1-*C. tropicalis*, 1-*C. glabrata* and 1-*C. parapsilosis*). Increasing resistance was observed for azole group of drugs particularly fluconazole in non-*albicans Candida* species (16.7% for both *C. glabrata* and *C. tropicalis*, 50% for *C. krusei*) when compared to *C. albicans* (13.3%). These drugs (fluconazole and voriconazole) belong to triazole group of azoles and exert their action by inhibiting cytochrome P450-dependent enzyme lanosterol 14 alpha-demethylase. Voriconazole have better activity against *Candida* species (16-32 fold potent than fluconazole) due to effective binding to the cytochrome P450 enzyme. Yet, cross-resistance between fluconazole and voriconazole have been increasingly reported

in *Candida* species, particularly *C. glabrata* [24]. Pfaller et al, (2007) had reported the use of fluconazole as a surrogate marker to predict voriconazole susceptibility in resource-poor settings [25]. In our study, high cross-resistance correlation between azoles was observed in *C. albicans*, *C. glabrata* and *C. tropicalis*. One *C. glabrata* isolate was resistant to three classes of drugs (Polyene, azoles and β-glucan inhibitors). This is of high-concern because azoles and β-glucan inhibitors are increasingly recommended for prophylaxis, thereby leading to selection of drug-resistant non-*albicans Candida* strains [26,27]. The echinocandin, caspofungin had excellent activity against most *Candida* isolates, while 5 isolates of *C. albicans* (9.5%) and 4 isolates of *C. tropicalis* (22.3%) were found to be resistant. This observation predicts that echinocandins like caspofungin and micafungin may emerge in the recent future as the drug of choice for prophylaxis and treatment of cases with symptomatic candiduria.

LIMITATION

The limitation of our study was the lack of genomic characterization of the resistant isolates with MIC correlation and follow-up assessment of clinical/microbiological outcome.

CONCLUSION

This study describes the clinical profile and the distribution of various *Candida* species causing significant candiduria, where *C. albicans* was found to the commonest species in our Tertiary Care Center, in contrast to the reporting of non-*albicans Candida* species as the commonest species in most other recent studies. Although, *C. albicans* was the predominant cause of candiduria, increasing trend towards non-*albicans Candida* species causing significant candiduria was observed. Most health care centers in India do not routinely test for antifungal susceptibility for the clinical isolates due to the laborious phenotypic procedures, lack of expertise and lack of availability of updated interpretative guidelines, thereby leading to lack of drug-resistance data for fungal infections. Newer automated technologies like VITEK 2 compact system have emerged as an effective and promising alternative in determination of antifungal susceptibility thereby guiding prompt treatment and early detection of emerging drug resistance. In our study, antifungal susceptibility profile showed increased resistance to fluconazole and associated cross-resistance to voriconazole in commonly isolated *Candida* species., probably due to their increased usage for prophylaxis and treatment. Newer antifungal drugs like caspofungin and micafungin (echinocandins) are emerging as promising agents for prophylaxis and treatment of symptomatic candiduria. In addition, strengthening the hospital antimicrobial stewardship programs would taper this scenario of widespread drug resistance similar to Multi-Drug Resistant (MDR) bacterial isolates.

Declaration: A part of this study has been presented as a poster at the MICROCON 2015 conference organized by Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Puducherry. (Abstract available at http://microcon2015.co.in/abstracts/free_ePosters/Abstracts/abs-1153.html).

REFERENCES

[1] Nayman Alpat S, Özguneş I, Ertem OT, Erben N, Doyuk Kartal E, Tözun M, et al. Evaluation of risk factors in patients with candiduria. Mikrobiyol Bul. 2011;45(2):318-24.
[2] Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, Edwards JE Jr, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(5):503-35.
[3] Achkar JM, Fries BC. *Candida* infections of the genitourinary tract. Clin Microbiol Rev. 2010;23(2):253-73.
[4] Deorukhkar SC, Saini S, Mathew S. Non-*albicans Candida* infection: an emerging threat. Interdisciplinary Perspectives on Infectious Diseases, vol. 2014, Article ID 615958, 7 pages, 2014.
[5] Shor E, Perlin DS. Coping with stress and the emergence of multidrug resistance in fungi. PLOS Pathog 2015;11(3):e1004668.
[6] Bourgeois N, Dehandschoewercker L, Bertout S, Bousquet P-J, Rispail P, Lachaud L. Antifungal susceptibility of 205 *Candida* species. isolated primarily during invasive candidiasis and comparison of the Vitek 2 System with the CLSI Broth Microdilution and Etest Methods. J Clin Microbiol. 2010;48(1):154-61.
[7] Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the VITEK 2 yeast susceptibility test with the CLSI broth microdilution reference method for testing fluconazole against *Candida* species. J Clin Microbiol. 2007;45(3):796-802.
[8] Melhem M, Bertoletti A, Lucca H, Silva R, Meneghin F, Szeszs M. Use of the VITEK 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species. Braz J Microbiol. 2013;44(4):1257-66.
[9] Colodner R, Nuri Y, Chazan B, Raz R. Community-acquired and hospital-acquired candiduria: comparison of prevalence and clinical characteristics. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2008;27(4):301-05.
[10] Kaup S, Sankarankutty J, Balasubrahmanya HV, Kulkarni S, Nirmala M. Speciation of *Candida* using HiCrome *candida* differential agar. Int J Curr Microbiol Appl Sci. 2016;5(7):267-74.
[11] Cuenca-Estrella M, Gomez-Lopez A, Alastruey-Izquierdo A, Bernal-Martinez L, Cuesta I, Buitrago MJ, et al. Comparison of the Vitek 2 antifungal susceptibility system with the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution reference methods and with the sensititre yeast one and E-test techniques for in vitro detection of antifungal resistance in yeast isolates. J Clin Microbiol. 2010;48(5):1782-86.
[12] Bouza E, San Juan R, Muñoz P, Voss A, Kluytmans J.Co-operative Group of the European Study Group on Nosocomial Infections. A European perspective on nosocomial urinary tract infections I. Report on the microbiology workload, etiology and antimicrobial susceptibility (ESGNI-003 study). European Study Group on Nosocomial Infections. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2001;7(10):523-31.
[13] Nayman Alpat S, Özguneş I, Ertem OT, Erben N, Doyuk Kartal E, Tözun M, et al. Evaluation of risk factors in patients with candiduria. Mikrobiyol Bul. 2011;45(2):318-24.

[14] Bobade O, Waghmare M, Chhabrani P, Kaur I. Species distribution and antifungal susceptibility profile of *Candida* isolated from urine samples. Int J Med Sci Public Heal. 2013;2(4):1.
[15] Sobel JD, Kauffman CA, McKinsey D, Zervos M, Vazquez JA, Karchmer AW, et al. Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis Off Publ Infect Dis Soc Am. 2000;30(1):19-24.
[16] Paul N, Mathai E, Abraham OC, Michael JS, Mathai D. Factors associated with candiduria and related mortality. J Infect. 2007;55(5):450-55.
[17] Longo DL, Wise GJ, Schlegel PN. Sterile pyuria. N Engl J Med. 2015;372(11):1048-54.
[18] Kauffman CA, Fisher JF, Sobel JD, Newman CA. *Candida* urinary tract infections-diagnosis. Clin Infect Dis. 2011;52(Suppl 6):S452-56.
[19] Bougnoux ME, Kac G, Aegerter P, d' Enfert C, Fagon J-Y, CandiRea Study Group. Candidemia and candiduria in critically ill patients admitted to intensive care units in France: incidence, molecular diversity, management and outcome. Intensive Care Med. 2008;34(2):292-99.
[20] Kauffman CA, Vazquez JA, Sobel JD, Gallis HA, McKinsey DS, Karchmer AW, et al. Prospective multicenter surveillance study of funguria in hospitalized patients. Clin Infect Dis. 2000;30(1):14-18.
[21] Yashavanth R, Shiju MP, Bhaskar UA, Ronald R, and Anita KB. Candiduria: prevalence and trends in antifungal susceptibility in a tertiary care hospital of Mangalore. J Clin Diagn Res. 2013;7(11):2459-61.
[22] Mohandas V, Ballal M. Distribution of *Candida* Species in different clinical samples and their virulence: Biofilm formation, proteinase and phospholipase production: A study on hospitalized patients in Southern India. J Glob Infect Dis. 2011;3(1):4.
[23] Rathor N, Khillan V, Sarin S. Nosocomial candiduria in chronic liver disease patients at a hepatobilliary center. Indian J Crit Care Med. 2014;18(4):234.
[24] Pfaller MA, Messer SA, Boyken L, Rice C, Tendolkar S, Hollis RJ, et al. Use of Fluconazole as a Surrogate Marker To Predict Susceptibility and Resistance to Voriconazole among 13,338 Clinical Isolates of *Candida* species. Tested by Clinical and Laboratory Standards Institute-Recommended Broth Microdilution Methods. J Clin Microbiol. 2007;45(1):70-75.
[25] Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ. Activities of fluconazole and voriconazole against 1,586 recent clinical isolates of *Candida* species determined by Broth microdilution, disk diffusion, and Etest methods: report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. J Clin Microbiol . 2003;41(4):1440-46.
[26] Pelz RK, Hendrix CW, Swoboda SM, Diener-West M, Merz WG, Hammond J, et al. Double-blind placebo-controlled trial of fluconazole to prevent *candida*l infections in critically ill surgical patients. Ann Surg. 2001;233(4):542-48.
[27] Ostrosky-Zeichner L, Shoham S, Vazquez J, Reboli A, Betts R, Barron MA, et al. MSG-01: A randomized, double-blind, placebo-controlled trial of caspofungin prophylaxis followed by preemptive therapy for invasive candidiasis in high-risk adults in the critical care setting. Clin Infect Dis Off Publ Infect Dis Soc Am. 2014;58(9):1219-26.

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Letter to Editor

Microbiology Section

Prevalence of Vancomycin Resistant Enterococci Carriers from Rectal Swabs among High Risk Patients in a Tertiary Care Hospital

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Keywords: Chromogenic media, *Enterococcus faecium*, *Enterococcus faecalis*, Urinary tract infections

Dear Editor,

Enterococci with acquired vancomycin resistance is a major concern amongst hospital. These resistant species have the ability to survive for prolonged periods and they can get transmitted easily. The infections caused by these organisms range from complicated Urinary Tract Infections (UTI) to life threatening Central Nervous System (CNS) infections making the treatment challenging [1]. The rate of Vancomycin Resistant Enterococci (VRE) colonisation in Northern India is nearly 34.5%, with *van A* genotype being the most common [2]. Studies have reported that active screening through rectal swabs is a definite tool in preventing complications in high risk patients by early identification and treatment of VRE carriers [3]. The conventional method of screening with bile esculin azide agar supplemented with 6 µg/mL of Vancomycin/ml is reasonably sensitive but requires confirmation by molecular techniques [4]. The molecular methods in the detection are limited to the referral laboratories. Development of highly specific chromogenic substrates in the VRE selective media help in effective screening of colonisers. The sensitivity and specificity of the chromogenic media was 100% and 99%, respectively [4]. This was in accordance to present study as well. This study was undertaken to screen the high risk patients in critical care for VRE colonisation from stool samples and identify the genotype. Early identification of the carrier state in high risk patients is helpful in planning the management strategy of these patients.

This was an observational study done over the period of three months from January to March 2016 at PSGIMS&R, Coimbatore, Tamil Nadu, India. The Institutional Human Ethical clearance number obtained was 14/118. Informed consent was obtained before collecting the rectal swabs. The criteria for high risk patients were defined as hospitalisation >7 days, chronic haemodialysis, use of multiple antibiotics and associated chronic disease status. Rectal swabs were obtained from 100 patients (no repeat isolates and the mean age group was 60 years 75% were males) following the identification as high risk as per the criteria explained above. The rectal swabs were streaked on chromogenic ChromID VRE media (Biomerieux) and incubated at 37°C for 24 hours. The presence of violet colour colonies indicated *Enterococcus faecium* and blue colour colonies indicated *Enterococcus faecalis*. Isolates with intrinsic resistance to vancomycin as *Enterococcus casseliflavus* and most of the other gram positive organisms were selectively inhibited by this media. Conventional biochemical tests as bile esculin, mannitiol, argininine and PYR (Pyrrolidonyl Arylamidase) were done for confirmation at the species level. The antibiotic sensitivity was also performed by Kirby Bauer method as per the CLSI guidelines [5]. The antibiotic susceptibility pattern of the 100 isolates showed the maximum sensitivity to linezolid (98%) followed by vancomycin (97%), erythromycin (89%), teicoplanin (75%), ampicillin (69.2%), high level gentamycin (49.2%) and penicillin (25%). The Minimum Inhibitory Concentration (MIC) of the resistant isolates was confirmed by using commercial E-strips. Molecular characterisation of the resistant isolates for the specific genotype was done by performing conventional PCR. The DNA from the resistant isolates was extracted using the QIAGEN kit. The primers of base pair 732bp for detection of *van A* were used. The PCR products were analysed by running gel electrophoresis and visualised under UV light in automated GEL DOC viewer. Primers for *van A* used were:

F (5' GGGAAAACGACAATTGC-3')

R (5'GTACAATGCGGCCGTCGTTA-3') [6]

Out of the 100 patients screened, three were found to be positive for VRE carriage (3%). All the three were *Enterococcus faecium*. The positive sample was used as the control strain. Analysis of the predisposing risk factors among the three patients revealed the association of chronic renal impairment, immunocompromised status (retroviral infection) and presence of co-morbid conditions such as diabetes mellitus along with prolonged hospital stay.

The VRE carriers can pose a risk to the patients harboring these pathogens as they can invade the host and result in bacteremia complicating the management. In such patients, treatment of the VRE carriage aids in better prognosis. Isolation and cohorting of these patients prevents the transmission and risk of infection in other patients, especially in healthcare setups. The choice of VRE screening can aid in proper infection control measures.

Thus, chromogenic VRE media was found to be a simple and rapid method for identifying VRE carriage.

Declaration: The present study was presented as poster presentation in 39th National Conference of the Indian Association of Medical Microbiologists MICROCON in 2015.

REFERENCES

[1] Zhanel GG, Laing NM, Nichol KA, Palatnick LP, Noreddin A, Hisanaga T, et al. Antibiotic activity against Urinary Tract Infection (UTI) isolates of Vancomycin-Resistant Enterococci (VRE): Results from the 2002 North American Vancomycin Resistant Enterococci Susceptibility Study (NAVRESS). Journal of Antimicrobial Chemotherapy. 2003;52(3):382-88.

[2] Banerjee T, Anupurba S, Filgona J, Singh DK. Vancomycin-resistance enterococcal colonisation in hospitalised patients in relation to antibiotic usage in a tertiary care hospital of North India. Journal of Laboratory Physicians. 2015;7(2):108.

[3] Humphreys H. Controlling the spread of vancomycin-resistant enterococci. Is active screening worthwhile? Journal of Hospital Infection. 2014;88(4):191-98.

[4] Cuzon G, Naas T, Fortineau N, Nordmann P. Novel chromogenic medium for detection of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. Journal of Clinical Microbiology. 2008;46(7):2442-44.

[5] CLSI. Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial Susceptibility testing. CLSI document M100-524.Wayne, PA: Clinical and Laboratory standard Institute 2020.

[6] Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 1995;33:24-27.

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Haemophilus influenzae in Community Acquired Infections

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A B S T R A C T

Haemophilus influenzae is strictly a human pathogen, responsible for many diseases like community acquired pneumonia, meningitis, sinusitis, epiglottitis and otitis media. *Haemophilus* type b (Hib) was the most common strain responsible for all the infections. Non type b strains were at an emerging risk. Ampicillin was commonly used but resistance due to TEM-1gene is being reported. It is aimed to find out whether Hib continues to be isolated from childhood cases of pneumonia and its susceptibility to the drugs of choice and possible mechanisms of resistance. All purulent respiratory samples were processed for isolation of *Haemophilus influenzae*. They were identified based on its X&V factor requirement and a negative Porphyrin synthesis test. Biotyping and Serotyping was done using standard methods. Ampicillin resistance was characterized by phenotypic and genotypic methods. From the results, out of 1540 purulent respiratory samples, *Haemophilus influenzae* was isolated from 103 cases. Among the 103 isolates, 69 were Nontype b and 34 were type b *Haemophilus influenzae*. All the Hib isolates were from those above 20 years of age. Among 15 Ampicillin resistant isolates, 13 were positive for TEM-1 gene. Finally, Nontype b isolates were predominant and Hib was isolated only from adult age group.

Introduction

Haemophilus influenzae is strictly a human pathogen responsible for many diseases like community acquired pneumonia, meningitis, sinusitis, epiglottitis and otitis media (Mac Neil *et al.,* 2011). *Haemophilus influenzae* is a short pleomorphic Gram negative bacillary rod which is fastidious in nature. It is mainly recovered from the human respiratory tract and very rarely from other sites. The mode of spread of the infection is mainly airborne droplets (Puig *et al.,* 2014). Among the 6 capsulated strains (a, b, c, d, e, f) type b remains the most common cause of invasive diseases (Puig *et al.,* 2014; Jain *et al.,* 2006). Type b H. influenzae colonizes the respiratory tract of children at a rate of 2- 4% and this has shown substantial decrease after the introduction of the conjugate vaccine. Non typeable strains account for 25-35% of otitis media in children less than 5 years of age and exacerbations of chronic obstructive pulmonary disease in adults (Urwin *et al.,* 1996).

Haemophilus influenzae is confirmed by performing satellitism, in which the colony grows near a streak of Staphylococcus aureus. *Haemophilus influenza* is a fastidious organism which requires two important factors, namely, factor X (hemin factor) and factor V (Nicotinamide adenine nucleotide factor) for its growth. The detection of

porphyrins produced from b-Aminolevulinic acid is evaluated for use in speciation of the *Haemophilus* genus (Lund *et al.,* 1977). Porphyrin synthesis is positive for the non hemin requiring *H. parainfluenzae* and is negative for *Haemophilus influenzae* which requires both hemin and NAD factor for its growth.

Haemophilus influenzae can be classified in to eight biotypes based on three biochemical tests indole, Ornithine, urease and this has been used as an epidemiological marker for carriage in the upper respiratory tract. Of these biotypes, type b falls mostly in biotype I (Campos *et al.,* 2004).

Infections caused by *H. influenzae* were initially being treated with cell wall active agents like Ampicillin and protein synthesis inhibitors like Chloramphenicol. Now the favoured regimen is Ceftrioxzone, Tetracycline and Azithromycin (Barbour *et al.,* 1996; Guevara *et al.,* 2008).Some of the *Haemophilus influenzae* shows resistance to ampicillin which is mainly due to beta lactamase production. Beta lactamase may be due to two genes, TEM-1 and ROB-1 genes, but the most commonly isolated gene is the TEM-1 gene. Both phenotypic and genotypic methods were used to detect Ampicillin resistance (CDC, 1998).

The aim of this study is to identify the serotype predominance in childhood and adult cases of pneumonia and to know the whether the vaccination against Hib has had any effect on the serotype predominance in different age groups and whether the isolates are susceptible to the drugs of choice like Ceftrioxzone, tetracycline and Azithromycin. The study also characterizes resistance to Ampicillin, which was used as an empirical treatment by the clinicians in earlier days for upper respiratory tract infections.

Materials and Methods

Study population

The study was done during the period from March 2014 to August 2015. A total of 1540 purulent respiratory samples from patients suspected of having community acquired pneumonia were processed in our microbiology laboratory.

Isolation of *Haemophilus influenzae*

All samples as indicated above were inoculated on Chocolate agar, Fildes agar, Chocolate agar with antibiotics (Vancomycin, Bacitracin and Clindamycin), and Blood agar with Staphylococcus streak.

Blood agar (MacNeil *et al.*, 2011)

Blood agar was streaked with the sample along with Staphylococcus aureus and it was incubated at 37^0 C in the presence of 5-10% co_2 in a candle extinction jar for 24 hours. They form low, translucent, convex, pinpoint colonies around the staphylococcus which provides the factor V for its growth. The size of the colony decreases as it goes away from the staphylococcus streak. This is the satellite phenomenon shown by *Haemophilus influenzae*.

Chocolate agar (Kilian, 1976)

Heated blood agar or the Chocolate agar which provides both the hemin and NAD factor was streaked with the sample and it was incubated at 37^0 C in the presence of 5-10% co_2in a candle extinction jar for 24 hours. *Haemophilus influenzae* shows a shiny, tiny, smooth, flat colony which smells like wet fur.

Fildes agar (Campos *et al.*, 2004)

Fildes enrichment is an enzymatic digest of blood used to enhance the growth of fastidious organisms. The resultant digest is rich in growth factors including X and V factor required by *Haemophilus influenzae*. To the BHI chocolate agar of 100 ml, 5ml of Fildes enrichment broth was added and allow the media to set. The sample is streaked and incubated at 37^0C in the presence of 5-10% co_2in a candle extinction jar for 24 hours. Colony morphology is very similar to that on chocolate agar.

Chocolate agar with VBC (Harrison *et al.*, 2009)

The CHOC-VBC media was made by preparing chocolate agar and the following antibiotics were added in concentrations of 5 microgram/ml Vancomycin, 300 microgram/ml of Bacitracin, 1 microgram/ml of clindamycin 16.After that the media is allowed to dry and the samples are directly streaked on to the plates and incubated at 370C in the presence of 5-10% co_2in a candle extinction jar for 24 hours. *Haemophilus influenzae* shows a shiny, tiny, smooth, flat, slightly yellow colony which smells like wet fur.

Antibiotic susceptibility testing (Nag *et al.*, 2001)

Antibiotic susceptibility testing was done on chocolate agar for susceptibility to Ceftrioxzone, Azithromycin, Aztreonam, ciprofloxacin, ampicillin, tetracycline, Cotrimoxazole and Meropenem based on 2014 CLSI guidelines. Ampicillin resistant strains were then tested for beta lactamase production by acidimetric method. One of the genes responsible for beta lactamase production (TEM-1) was identified by conventional PCR. The following primers are used for detection of TEM-1 gene:-

5’TGGGTGCACGAGTGGGTTAC3’ and 5’TTATCCGCCTCCATCCAGTC3’

Porphyrin synthesis test (Campos *et al.*, 2004)

This test indicates the absence of requirement of X factor. If α-Amino levulinic acid is provided to the bacterium, it synthesizes it and excretes porphobilinogen and other porphyrins. This test is carried out by using kovac’s indole reagent (Para dimethyl amino Benzaldehyde) and formation of pinkish red colour in the lower water phase indicates the synthesis of porphyrin and absence of X factor requirement.

X and V requirement (Madore, 1996)

Make a lawn culture in the Muller Hinton agar plate and keep the commercially available discs X and V from HIMEDIA laboratories. The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6 mm. If both X and V factors are

Typing

i) Biotyping was done using the biochemical tests as follows: - Indole production, Ornithine decarboxylation and urease production (Campos *et al.*, 2004).
ii) Serotyping was done using –Becton-Dickinson --- Kit.
iii)BD Difco catalogue no. Type b-222361
iv)Type a-222501
v) Type f-227921

Results and Discussion

Among the 1540 cases of suspected pneumonia, the purulent respiratory samples yielded. *Haemophilus influenzae* were noted in 103 cases. Out of 103 isolates, 84 were

from inpatient samples and 19 were from outpatient samples. Among 84 inpatient cases, 33% were associated with COPD, 26.2% with Bronchial asthma and 18.44% were provisionally diagnosed as pyrexia of unknown origin.

Distribution of *Haemophilus influenzae* in various age groups

Among the 103 *Haemophilus influenzae* isolates, 95 (92%) of them were isolated from patients above 20 years of age and 8 (7.7%) from below 20 years. Among the 95 strains of *Haemophilus influenzae* isolated from adults, 63 (61.2%) were in males and 40(38.8%) in female patients. The strains isolated were more during winter months of October to January (Figure 1).

Serotype

All the isolates of *Haemophilus influenzae* were serotyped with type b, type a and type f antiserum. A total of 34(33%) were type b and the remaining 69 were non-type b. Among the 69 non type b strains, 46.6% were other serotypes, 10.7% were type a and 9.7% were type f. (Figure 2). Comparison of *Haemophilus influenzae* serotype b in different age groups showed that all isolates of type b were from those above 20 years, whereas no type b were isolated from below 20 years and this is statistically significant ($p < 0.05$).

Biotypes

Haemophilus influenzae can be divided in to eight biotypes based on three biochemical tests, namely, Indole, Ornithine and Urease production. Out of the 103 *Haemophilus influenzae* isolates, strains belonging to all 8 biotypes were detected and 47 strains belonged to biotype I (Table 1).

Antibiotic sensitivity pattern

Antibiotic sensitivity testing of 103 isolates of *Haemophilus influenzae* showed that 91.2% were sensitive to Ceftrioxzone, 96.1% to Azithromycin, 99.02% to Aztreonam, 94.2% to ciprofloxacin, 85.4% to Ampicillin, 96.1% to Meropenem, 97.08% to Tetracycline and 65.04% to Cotrimoxazole.

Detection of Ampicillin resistance

Among the 103 isolates of *Haemophilus influenzae* isolated, 15(14.6%) strains showed Ampicillin resistance. Out of the 15 Ampicillin resistant strains, all 15 produced beta lactamases by acidimetric method and 13 showed presence of TEM-1 (Figure 3).

Haemophilus influenzae causes a variety of community acquired respiratory infections, including sinusitis, acute otitis media, bronchitis and community acquired pneumonia. Earlier, these infections were mainly caused by *Haemophilus influenzae* serotype b (Hib), but after the introduction of vaccines, the incidence of *Haemophilus influenzae* type b has markedly reduced.In our study, Non type b *Haemophilus influenzae* has emerged as an increasing cause of the pneumonia (67%) as compared to type b and this is similar to other studies also where it is isolated in 70-90% cases (Mac Neil *et al.*, 2011; Puig *et al.*, 2014) and Jain *et al.*, (2006).

Earlier studies done during the period from 1993-1997, show a high rate (86%) of isolation of Hib amongst children below 5 years of age (Lund *et al.*, 1977). Studies between 1996 and 2004 have reported lower isolations of 26-28% among children (Urwin *et al.*, 1996; Campos *et al.*, 2004 and Barbour (1996).

Among children in Asia and central European countries a considerable decrease has been demonstrated from 21 cases per 1, 00,000 populations per year in 1994 to 5 cases per 1, 00,000 populations per year by 2013 (MacNeil *et al.,* 2011; Puig *et al.,* 2014). A comparative study in Costa Rica have also reported a significant decline in the incidence of type b *H. influenzae* when the data from 1992 -1997 was compared with that between 1999 and 2004 (Guevara *et al.,* 2008). By June 2011, Hib vaccine was included in the National Immunization program of 170 countries in all regions of the world. After that the incidence of Hib invasive disease and oropharyngeal carriage in young children has drastically decreased to 0.3-0.7 cases per 1, 00,000 populations per year wherever vaccination programs have been implemented (Campos *et al.,* 2004; Barbour, 1996 and Madore, 1996). In our study none of the isolates were from cases below 20 years of age.

The *H. influenzae* isolates in this study were more commonly seen among male patients (61%), which are similar to other studies (Urwin *et al.,* 1996). In contrast, a study reports higher prevalence among female elderly patients, and a lower prevalence in female children (Mac Neil *et al.,* 2011). This infection has always shown a seasonal variation. Isolation of *H. influenzae* has shown higher incidence during winter months across many countries (Kilian, 1976; Zhu *et al.,* 2015). In our study also 61% were isolated during winter months. An increase in isolations during rainy season is also observed in some studies (Guevara *et al.,* 2008; Zhu *et al.,* 2015 and Leibovitz *et al.,* 2004).

In this study, strains belonging to all known biotypes were isolated and 44% were Biotype I whereas in other studies 60-70% belonged to this biotype (Landgraf *et al.,* 1993). COPD was an important risk factor for the development of *Haemophilus influenzae* pneumonia and their association was 25 to 30% percent in many studies (Jain *et al.,* 2006; Urwin *et al.,* 1996), when compared to 33% in our study. Association of *Haemophilus influenzae* with bronchial asthma cases were 26.2% in our study which correlated well with many studies where 28% association is noted (Jain *et al.,* 2006; Urwin *et al.,* 1996).

Ceftrioxzone being the drug of choice for invasive *H. influenzae* infections, 91% of our isolates were susceptible, and this correlated well (95%-97%) with other studies in both developed and developing countries (Zhu *et al.,* 2015; Harrison *et al.,* 2009). Infections caused by *Haemophilus influenzae*, particularly sinusitis and otitis media are now frequently treated with fluroquinolones and their sensitivity were 94% in our study which is comparable to other studies (Farrell *et al.,* 2005).

A susceptibility of 94.5% in 2012 as compared to 54% in the year 2000 was observed with tetracycline and this remarkable increase in susceptibility is reportedly due to decrease in its usage in the past 20 years (Bennett *et al.,* 2014). In this study also 97% of isolates were sensitive to tetracycline.

Azithromycin is an acid stable orally administered Macrolides antimicrobial drug that appears to be more active against many Gram-positive pathogens and several other Gram negative pathogens, notably *Haemophilus influenzae*. After oral administration, serum concentrations of Azithromycin are lower reflecting the rapid and extensive movements of the drug from the circulation into intracellular compartments resulting in increased tissue concentrations (Puig *et al.,* 2015). In our study 99% were susceptible to Azithromycin, which was similar to other studies (Appelbaum *et al.,* 2003; Jacobs *et al.,* 1999; Doern *et al.,* 1997).

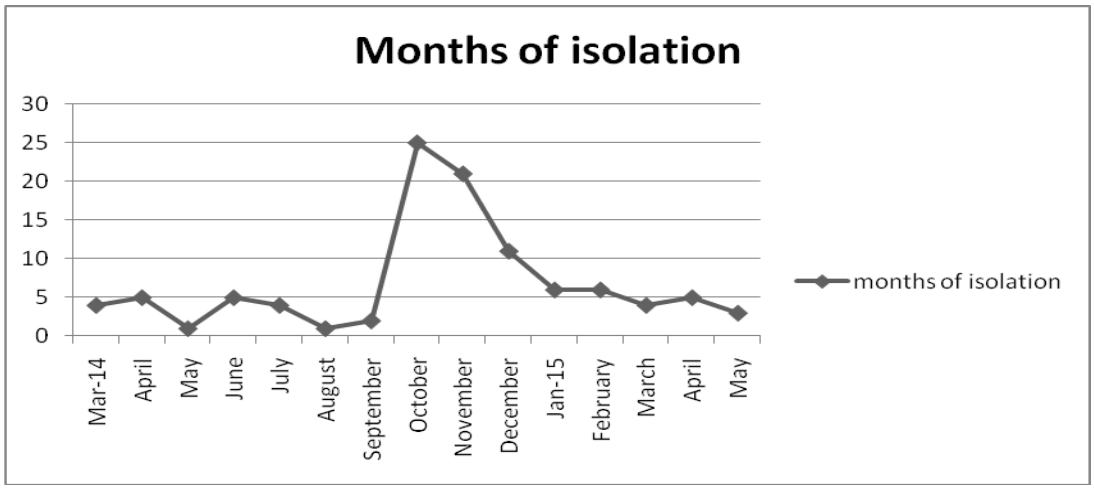
High level resistances to Macrolides in *H. influenzae* (0.5%-2%) are rare as is reported in many studies (Appelbaum *et al.,* 2003; Jacobs *et al.,* 1999; Doern *et al.,* 1997). Macrolides are of particular use in children who are allergic to beta lactams (Jacobs *et al.,* 1999). There was no difference in susceptibility between type b and non-type b isolates to Ceftrioxzone, tetracycline, Azithromycin and ciprofloxacin (Zhu *et al.,* 2015; Nag *et al.,* 2001).

Table.1 Biotype distribution among different isolates of *Haemophilus influenza*

Biotypes	Number of isolates showing different biotypes	Percentage
I	47	45%
II	6	6%
III	5	5%
IV	1	1%
V	2	2%
VI	5	5%
VII	4	4%
VIII	33	32%

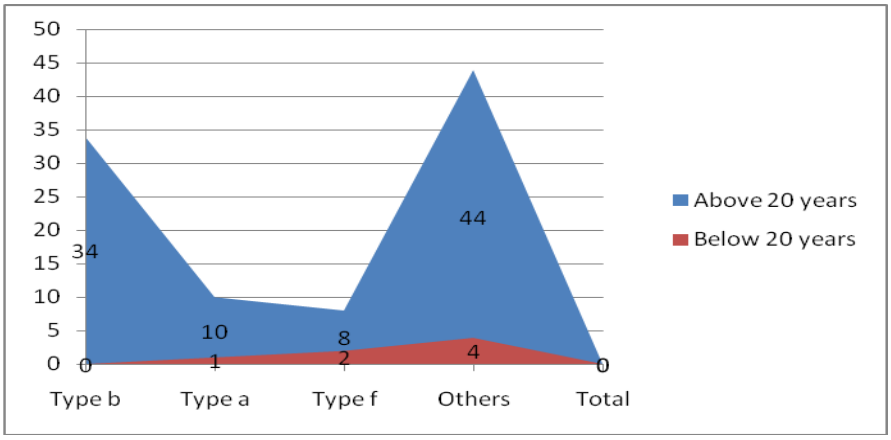
Haemophilus influenzae isolates, strains belonging to all 8 biotypes were detected and 47 strains belonged to biotype I (Table 1).

Fig.1 Seasonal variation of *Haemophilus influenzae*



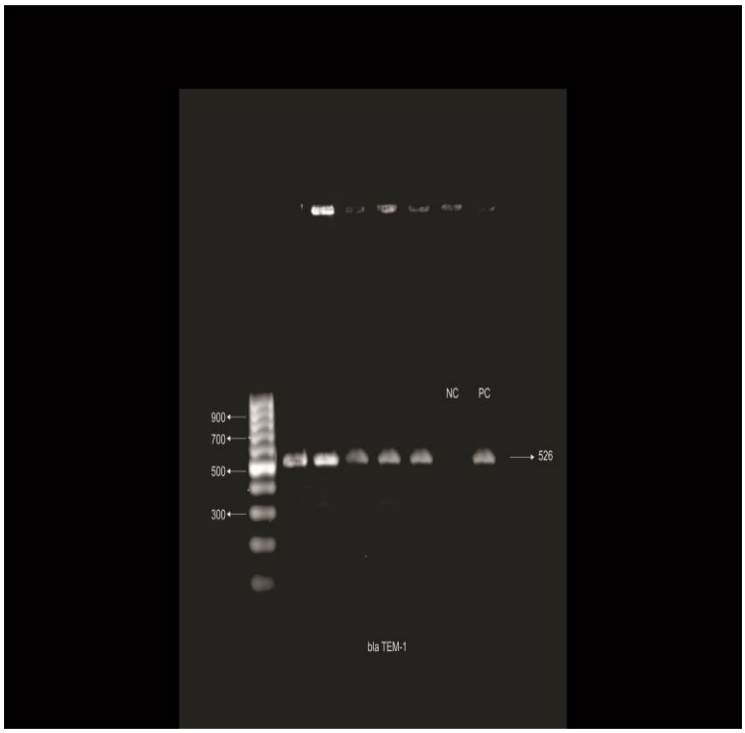
The strains isolated were more during winter months of October to January

Fig.2 Serotypes in different age groups



No type b was isolated from below 20 years and this is statistically significant ($p < 0.05$)

Fig.3 TEM-1 gene detection by PCR



Beta lactamase mediated resistance was seen in 14.6% isolates in this study, whereas in multicentre studies done in US between 1984-1999 it was 15% to 36 % (Nag *et al.*, 2001) and in recent studies done in China, the percentage of Ampicillin resistant strains progressively increased from 4% in the year 2000 to 29% in 2012 (Zhu *et al.*, 2015). The resistance detected was higher among type b (60%) strains when compared to non type b strains (40%) which is similar to study by Nag *et al.*, (30% and 20%) suggesting that the strains that are invasive is showing a higher ampicillin resistance (Nag *et al.*, 2001).

All the ampicillin resistant isolates produced beta-lactamase enzyme when screened by the phenotypic acidimetric method, but when

analyzed by molecular method, only 13 isolates (86%) were positive for TEM-1 gene which is similar to many studies where the detection of TEM-1 gene (93%) is more when compared to the ROB-1gene (5%) detection (Hasegawa *et al.,* 2003; Farrell *et al.,* 2005 and Kuvat *et al.,* 2015).

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References

Appelbaum, P.C., Peric, M., Bozdogan, B., Jacobs, M.R. 2003. 'Effects of an Efflux Mechanism and Ribosomal Mutations on Macrolides Susceptibility of *Haemophilus influenzae* Clinical Isolates'. *Antimicrobial Agents Chemother.,* p. 1017–1022.

Barbour, M.L. 1996. 'Conjugate vaccines and the carriage of *Haemophilus influenzae* type b'. *Emerg Infect. Dis.,* 2(3): 176.

Bennett, J.E., Dolin, R., Blaser, M.J. 2014. 'Principles and practice of infectious diseases'. *Elsevier Health Sci.*

Campos, J., Hernando, M., Roman, F., Perez Vazquez, M., Aracil, B., Oteo, J., Lazaro, E., de Abajo, F. 2004. Analysis of invasive *Haemophilus influenzae* infections after extensive vaccination against *H. influenzae* type b'. *J Clin. Microbiol.,* 42(2): 524-9.

Centers for Disease Control and Prevention. 1998. 'Progress toward eliminating *Haemophilus influenzae* type b disease among infants and children'--United States, 1987-1997. MMWR. *Morbidity and Mortality Weekly Report,* 47(46): 993.

Doern, G.V., Brueggemann, A.B., Pierce, G., Holley, H.P., Rauch, A. 1997. 'Antibiotic resistance among clinical isolates of *Haemophilus influenzae* in the United States in 1994 and 1995 and detection of beta-lactamase-positive strains resistant to amoxicillin-Clavulanate: results of a national multicenter surveillance study'. *Antimicrobial Agents and Chemother.,* 41(2): 292-7.

Farrell, D.J., Morrissey, I., Bakker, S., Buck ridge, S., Felmingham, D. 2005. 'Global distribution of TEM-1 and ROB-1 β-lactamases in *Haemophilus influenzae*'. *J. Antimicrobial Chemother.,* 56(4): 773-6.

Guevara, S., Soley, C., Arguedas, A., Porat, N., Dagan, R. 2008. 'Seasonal distribution of Otitis media pathogens among Costa Rican children'. *The Pediatric Infect. Dis. J.,* 27(1): 12-6.

Hasegawa, K., Yamamoto, K., Chiba, N., Kobayashi, R., Nagai, K., Jacobs, M.R., Appelbaum, P.C., Sunakawa, K., Ubukata, K. 2003. 'Diversity of Ampicillin-resistance genes in *Haemophilus influenzae* in Japan and the United States'. *Microbial Drug Resistance,* 9(1): 39-46.

Harrison, C.J., Woods, C., Stout, G., Martin, B., Selvarangan, R. 2009. 'Susceptibilities of *Haemophilus influenzae*, *Streptococcus pneumoniae,* including serotype 19A, and *Moraxella catarrhalis* paediatric isolates from 2005 to 2007 to commonly used antibiotics'. *J. Antimicrobial Chemother.*

Jacobs, M.R., Bajaksouzian, S., Zilles, A., Lin, G., Pankuch, G.A., Appelbaum, P.C. 1999. 'Susceptibilities of Streptococcus pneumoniae and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 US surveillance study'. *Antimicrobial Agents and Chemother.,* 43(8): 1901-8.

Jain, A., Kumar, P., Awasthi, S. 2006. 'High Ampicillin resistance in different biotypes and serotypes of *Haemophilus influenzae* colonizing the nasopharynx of healthy school-going Indian children'. *J. Med. Microbiol.,* 55(2): 133-7.

Kilian, M. 1976. 'A taxonomic study of the

genus Haemophilus, with the proposal of a new species'. *J. Clin. Microbiol.*, 93(1): 9-62.

Kuvat, N., Nazik, H., Berkiten, R., Ongen, B. 2015. 'TEM-1 and ROB-1 presence and Antimicrobial Resistance in *Haemophilus influenzae* Strains, Istanbul, Turkey'. *The Southeast Asian J. Trop. Med. Public Health*, 46(2): 254-61.

Landgraf, I.M., Vieira, M.F. 1993. 'Biotypes and serotypes of *Haemophilus influenzae* from patients with meningitis in the city of Sao Paulo, Brazil'. *J. Clin. Microbiol.*, 31(3): 743-5.

Leibovitz, E., Jacobs, M.R., and Dagan, R. '*Haemophilus influenzae*: a significant pathogen in acute Otitis media'. *The Pediatric Infect. Dis. J.*, 23(12): 1142-52.

Lund, M.E., Blazevic, D.J. 1977. Rapid speciation of Haemophilus with the porphyrin production test versus the satellite test for X.' *J. Clin. Microbiol.*, 5(2): 142-144.

Mac Neil, J.R., Cohn, A.C., Farley, M., Mair, R., Baumbach, J., Bennett, N., Gershman, K., Harrison, L.H., Lynfield, R., Petit, S., Reingold, A. 2011. 'Current epidemiology and trends in invasive *Haemophilus influenzae* disease'—United States, 1989–2008. *Clin. Infect. Dis.*, 53(12): 1230-1236.

Madore, D.V. 1996. 'Impact of immunization on *Haemophilus influenzae* type b disease'. *Infectious Agents and Dis.*, 5(1): 8-20.

Nag, V.L., Ayyagari, A., Venkatesh, V., Ghar, M., Yadav, V., Prasad, K.N. 2001. 'Drug resistant *Haemophilus influenzae* from respiratory tract infection in a tertiary care hospital in north India'. *Indian J. Chest Dis. Allied Sci.*, 43(1): 13-8.

Puig, C., Grau, I., Marti, S., Tubau, F., Calatayud, L., Pallares, R., Liñares, J., Ardanuy, C. 2014. 'Clinical and molecular epidemiology of *Haemophilus influenzae* causing invasive disease in adult patients'. *PLoS one*, 9(11): e112711.

Puig, C., Tirado-Velez, J.M., Calatayud, L., Tubau, F., Garmendia, J., Ardanuy, C., Marti, S., Adela, G., Liñares, J. 2015. 'Molecular characterization of fluroquinolones resistance in nontypeable *Haemophilus influenzae* clinical isolates'. *Antimicrobial Agents and Chemother.*, 59(1): 461-466.

Urwin, G., Krohn, J.A., Robinson, K.D., Wenger, J.D., Farley, M.M. 1996. *Haemophilus influenzae* Study Group.' Invasive disease due to *Haemophilus influenzae* serotype f: clinical and epidemiologic characteristics in the *H. influenzae* serotype b vaccine era'. *Clin. Infect. Dis.*, 22(6): 1069-76.

Zhu, H., Wang, A., Tong, J., Yuan, L., Gao, W., Shi, W., Yu, S., Yao, K., Yang, Y. 2015. 'Nasopharyngeal carriage and antimicrobial susceptibility of *Haemophilus influenzae* among children younger than 5 years of age in Beijing, China'. *BMC Microbiol.*, 15(1): 1.

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Original Research Article

Prevalence and the factors influencing soil transmitted helminths among school age children of 5 to 14 years in a rural area of Coimbatore district

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ABSTRACT

Background: Highest intensity of soil transmitted helminthiasis (STH) is seen among school age children. The objective of this study is to find out the prevalence and factors associated with STH infection among school age children of 5-14 years in a rural area of Coimbatore district.
Methods: After getting ethical clearance, five of the fourteen villages of Vedapatti were selected by cluster sampling method. Totally 819 participated in the survey conducted between November 2015-July 2016 in the field practice area. Consent from parents and assent from child were obtained. Structured questionnaire was used to collect information. Totally 610 gave one adequate stool sample. Early morning samples were collected and transported to the laboratory within four hours. Formal ether concentration method was performed and examination was done. Data analysis was done with SPSS-19 software. Prevalence is expressed in percentage with 95% confidence interval (CI). Univariate and Multivariate analysis were performed. Strength of association was expressed in terms of odds ratio and adjusted odds ratio with 95% confidence interval. P value <0.05 was considered as statistically significant.
Results: The prevalence of STH was 7.70% (95% CI: 5.58- 9.82). Ascaris lumbricoides was highly prevalent 6.9% (4.89% to 8.91%) followed by hook worm 0.2(0.15% to 0.55%) and Trichuris trichura 0.7%(0.04% to 1.36%). Uni variate and Multivariate logistic regression analysis showed that pucca houses offered protection against STH.
Conclusions: STH is still a public health problem in Coimbatore. When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent re infection of STH, a long-term impact can be achieved.

Keywords: Soil transmitted helminthiasis, Prevalence, School age children, Coimbatore

INTRODUCTION

The soil-transmitted helminthes (STH) causing major illness among human beings are *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale*.[1] STH are considered Neglected Tropical Diseases (NTDs). [2] STH affects more than one fourth of the world's population, and it is reported more in tropical and subtropical areas.[3] The greatest numbers of cases are occurring in sub-Saharan Africa, the Americas, China and East Asia.[4] In India the reported prevalence of STH infection varied from 7.8% to 92.6%.[5-9] The reported prevalence varies according to the place and time of the study conducted.

The highest intensity of soil transmitted helminthes occur in school age children.[10] Children in their schooling age do not fully understand the connections between illness and behaviour.[11] It has been demonstrated that children often than adults tend to go barefoot, resulting in more contact with soil, and they often fail to use sanitary facilities even when they are present.[12] STH infections are considered as an important cause of sickness absenteeism. They are accounting for loss of 12.3% and 11.4% of disability adjusted life years (DALY) in girls and boys respectively .[13] Over 270 million preschool-age children and over 600 million school-age children live in areas where sanitation is poor and these parasites are intensively transmitted.[14] In India more than 241 million children are at risk of parasitic worm infections. Ministry of Health and Family Welfare (MoHFW), Government of India launched the National Deworming Day (NDD) on Feb 10, 2015. NDD aims to de-worm all children between the ages of 1 to 19 years.[15] The tablets were supplied to children through Government, Government-aided and private schools, and Anganwadi centers.

When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent re-infection, a long-term impact can be achieved Epidemiological studies are needed to identify risk factors which may represent critical control points. There is no recently published article on prevalence of soil transmitted helminthes in Coimbatore, where open air defecation is a common practice. As per 2011 census, 46% of the households in Coimbatore district lack toilets. Hence this cross sectional study was conducted with the objective of finding the prevalence and risk factors associated with soil transmitted helminth infections in school age children of 5-14 years in a rural area of Coimbatore district.

METHODS

The study was conducted in the field practice area of the Rural Health Training Centre (RHTC) Vedapatti, Coimbatore. RHTC caters to a total population of 23,841 distributed in 14 villages of Thondamuthur and Madukarai block of Coimbatore district. The total number of school aged children of 5–14 years is 3266.

Ethical clearance was obtained from IHEC, in 2014. The sample size was calculated to be 819 assuming 34.5% as prevalence , relative precision of 15%, design effect of 2, and non-response rate of 20%.[16] Five out of fourteen villages were selected by cluster sampling method. House to house survey was conducted in the five villages during November 2015 to July 2016. All school-age children (aged 5–14 years) who were permanent residents of Vedapatti field practice area of RHTC were included in the study. Children who were not available on three visits within a week time were excluded from the study.

At the home setting, after getting consent from the parent and assent from the child any of the parents of the child was interviewed in the evening hours of the day. A structured questionnaire was used to collect information on demographic, socioeconomic and environmental information like availability of type of drinking water and availability of functioning sanitary toilet etc. Few questions were asked regarding the knowledge and attitude of the parents on STH. Respondent was asked to give comment on the personal hygiene practices of childlike washing hands with soap before taking food and after defecation, eating fallen foods on the floor, wearing foot wear outside the house and intake of de-worming tablets in the last three months. Observations were also made regarding the personal hygiene of the children related to STH like clipped nails etc. Knowledge and attitude score was generated from the correct responses for the knowledge and attitude questions. The correct responses were given a score of one, and maximum score one could get was nine. Score five and above was considered as adequate knowledge and attitude score.

At the end of survey a screw-capped plastic container with identification of the child was given to collect the stool sample of the child on the next morning. Instructions regarding stool collection were given. The stool samples were transported to microbiology lab within four hours of sample collection. Stool samples were examined by saline wet mount and Iodine wet mount .[17] Iodine preparations was done for better visualization of morphological details of ova. All Samples were re-examined after concentrating with WHO recommended.[18] Formalin-Ether sedimentation method (FES). To reduce human error, duplicate slides (both direct and after concentration method) were prepared from each sample and the slides were read by two different Microbiologists and the opinion of Chief Microbiologist was considered as final. [19]

Statistical analysis

Data entry was made in the Microsoft Excel software in codes and analysis was done using SPSS-19 computer package. The Presence of ova of *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale* was considered as dependent variable. Prevalence is expressed in percentage with 95% confidence interval (CI). Uni-variate analysis and logistic regression were performed to find the associations between independent variables and dependent variables. Strength of association was expressed in terms of odds ratio and adjusted odds ratio with 95% CI and p value <0.05 was considered as statistically significant.

RESULTS

The results of this study are based on only 610 children, who gave one adequate stool sample. Totally 819 children participated in the survey, hence the non-response rate was 24.42%. Analysis showed that more number of risk group (29.13%) were found among the non-responders when compared to younger children (22.72%, p=0.02).

The distribution of study population and their risk factors is shown in (Table 1 and 2). The mean age of the study population was 8.92±2.68. Among them Children below ten year, female participants (52.1%), children belonged to nuclear family (62.1%) and children belonged to low socio economic status (52.3%) class IV and V according to modified Prasad classification were found in majority. Illiteracy was found among 20.2% of mothers 16.1% of fathers. Open air defecation was found among 33.4% of the children. Regarding the toilet usage only 50.7% of the children were using the toilets in the home. Around 66% did not wash their hands with soap before taking food. And 44.6% did not wash their hands with soap after defecation around 63 % children regularly used foot wear and around 70% of the children had trimmed nail during the visit. Nearly half of the parents of children were having adequate knowledge on STH. Only 59% reported intake of de-worming tablets in the last three months.

Table 1: Association of STH with socio economic and demographic factors by univariate and multivariate analysis.

Factors	Category	STH		Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	P value
		Yes	No			
Age (years)	≥10	24	229	1.52 (0.80-2.90)	1.45 (0.78-2.7)	0.24
	<10	23	334	1	1	
Sex	Male	24	268	1.15 (0.60-2.18)	1.07 (0.58-2.00)	0.82
	Female	23	295	1	1	
Total number of family members	Less than 4	22	253	1.08 (0.56- 2.05)	1.21 (0.64- 2.27)	0.55
	Four and above	25	310	1		
Type of house	Pucca house	4	115	0.36 (0.09-1.03)	0.34 (0.10- 0.91)	0.05
	Others (semipucca, kutcha house)	43	448	1	1	
Mother's educational status	Illiterate	10	113	1.08 (0.46-2.29)	1.03 (0.43- 2.30)	0.94
	Literate	37	450	1	1	
Socio economic status (modified prasad scale)	Lower SES (Class IV,V)	26	293	1.14 (0.60-2.19)	1.10 (0.56-2.20)	0.78
	High SES (Class I,II,III)	21	270	1	1	

Table 2: Association of STH with Risk factors by univariate and multivariate analysis.

Factors	Category	STH		Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)	P value
		Yes	No			
Open air defecation	Yes	16	188	1.03 (0.51-2.00)	1.10 (0.56-2.20)	0.68
	No	31	375	1	1	
Type of safe drinking water	Pipe water	36	427	1.04 (0.50-2.33)	0.93 (0.45-2.06)	0.86
	Others (UV/ RO/ boiled water)	11	136	1	1	
Habit of eating unwashed vegetables	Yes	13	168	0.90 (0.42-1.80)	0.82 (0.38-1.72)	0.61
	No	34	395	1	1	
Habit of eating foods fallen on ground	Yes	1	21	0.56 (0.01-3.66)	0.62 (0.03-3.30)	0.65
	No	46	542	1	1	
Washing with soap before eating	No	27	313	1.08 (0.57-2.08)	0.89 (0.40-1.93)	0.76
	Yes	20	250	1	1	
Washing with soap after Toilet	No	24	248	1.32 (0.70-2.52)	1.57 (0.71-3.51)	0.27
	Yes	23	315	1	1	
Regular foot wear usage	No	16	207	0.89 (0.44-1.72)	0.92 (0.46-1.79)	0.81
	Yes	31	356	1	1	
Nail clipping	No	11	175	0.68 (0.30-1.40)	0.63 (0.28-1.34)	0.25
	Yes	36	388	1	1	
Deworming during past three months	No	22	228	1.29 (0.68-2.45)	1.43 (0.76-2.66)	0.26
	Yes	25	335	1	1	
Knowledge attitude score regarding STH	Inadequate	25	296	1.02 (0.54-1.96)	0.90 (0.47-1.72)	0.75
	Adequate	22	267	1	1	

The prevalence of STH among 610 children who had given one adequate stool sample and whose parents participated in survey was 7.70% (95% CI: 5.58-9.82). Among all STH, *Ascaris lumbricoides* was highly prevalent 6.9% (95% CI: 4.89-8.91) followed by hook worm 0.2% (95% CI: 0.15-0.55) and *Trichuris trichura* 0.7% (95% CI: 0.04-1.36).

There was no significant association found between STH and factors influencing STH by univariate analysis. Since none of the factors came as significant in univariate analysis, all independent variables were subjected to multivariate logistic regression analysis. Only pucca houses offered protection OR 0.36 [95% CI: 0.09-1.03] against STH, by multivariate analysis.

DISCUSSION

Relatively high non response rate (24.42%) was noted in this study. It is also evident from a similar study by Shubha et al that surveys involving bio samples shows high non response rate (30.9%) when compared to simple questionnaire based surveys.[20]

The prevalence of STH in the study population is 7.7% (95% CI 5.58- 9.82). Results of current study were lesser than other studies conducted in the nearby areas.

The explanations for low prevalence are more number of risk group (29.13%) were found among the non-responders compared to younger children (22.72%, p=0.02). The study results were based on single stool sample, Modified Formol-ether concentration (FEC) method with low sensitivity (63.1%) which could have estimated the low prevalence. [21]The other probable explanations are Mass Drug Administration under National Deworming Day (NDD) might have changed the scenario.[5-9] However there is no sufficient data from to compare prevalence before de-worming in this area to support this statement. Coimbatore first round of National De-worming Day was just completed and second round was on- going at the time of the survey (Febraury 2016). Kattula et al also explains the reason for low prevalence of STH (7.8%) in Vellore area is due to Albendazole supplied in Mass Drug Admininistation (MDA) along with Diethylcarbamazine since 2004.[8] Deworming using Albendazole rapidly declines the overall prevalence of STH, and this is evident from Mani et al study on efficacy of Albendazole in Tamilnadu.[22] However there are other studies reported with higher prevalence in nearby areas. Kaliappan et al explains the reason for the increased prevalence (39%) in Thiruvanamalai District in the year 2013 could be due to five stool samples collected among tribal population where open air defecation and bare foot walking were common practice.[23] Ragunathan et al explains the reason for higher prevalence (34.56%) reported in Pudhuchery, a coastal area using FEC method in year 2006 is due to poor hygienic practices of the participants.[16]

This study showed the most common soil transmitted helminth as *Ascaris lumbricoides* with the prevalence of 6.9% (95% CI 4.89-8.91) followed by Hook worm 0.7% (95% CI 0.04%-1.36%) and *Trichuris Trichura* 0.2% (95% CI 0.15-0.55). In general studies showed that *Ascaris* and *Trichuris* were more common among urban children and hookworm infestation among rural children.[24]

Regarding the factors influencing STH pucca houses offered protection OR 0.36 [95% CI 0.09-1.03] against STH, by multivariate analysis. A cross sectional study by Kattula et al showed that children living in Hut/ Kutcha houses, are at higher risk for STH.[8]

Kattula et al also showed that children of more than 10 years of age, living in households without toilet, open air defecation, unhygienic practices like keeping untrimmed nails are important risk factors for STH.[8] Kattula showed that consumption of deworming tablets offered protection. However there was no significant association between STH and factors influencing STH noted in this study.[8]

The major limitations of the study are, the results were based on single stool sample which could have estimated the low prevalence. The newer techniques with higher sensitivity would have given higher prevalence. Sample size was not adequate to comment on factors influencing STH. This is a cross-sectional study; hence the cause-effect relationship cannot be determined

In Asia, several countries, notably Japan, South Korea and Taiwan, showed that they have achieved sustained and successful control of STH infections over the last forty years (WHO, 1996). More recently, Sri Lanka has reduced prevalence to less than five percent.[25]

CONCLUSION

It is evident from our results that soil-transmitted helminth (STH) infections can be controlled by improving the coverage of NDD, and NDD should be evaluated for further control. Open air defecation is a biggest concern (33.4%), hence more private, public toilets should be built in this area. The children should be trained to use toilet facilities either private or public. It is also very important to raise the awareness about these infections, hence parents of children with inadequate knowledge should be given Health education. Regular deworming if accompanied by appropriate sanitation and hygiene activities designed to prevent reinfection, a long-term impact can be achieved.

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REFERENCES

1. World Health Organization, Focusing on STH: towards an integrated approach for STH control. Available at http://www.who.int/intestinal_worms/en/http://whqlibdoc.who.int/publications/2012/9789241503129_eng.pdf?ua=1. Accessed 10 September 2019.
2. Statement regarding Soil Transmitted Helminths, by CDC. Available at http://www.cdc.gov/globalhealth/ntd/. Accessed 10 September 2019.
3. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasites Vectors. 2014;7:37.
4. WHO: 2012. Eliminating soil-transmitted helminthiasis as a public health problem in children soil-transmitted helminthiasis progress report; 2001-2010.
5. Ramesh GN, Malla N, Raju GS, Sehgal R, Ganguly NK, Mahajan RC, et al. Epidemiological study of parasitic infections in lower socio-economic group in Chandigarh (north India). Indian J Med Res. 1991;93:47-50.
6. Singh P, Gupta ML, Thakur TS, Vaidya NK. Intestinal parasitism in Himachal Pradesh. Indian J Med Sci. 1991;45:201-4.
7. Singh S, Raju GV, Samantaray JC. Parasitic gut flora in a north Indian population with gastrointestinal symptoms. Trop Gastroenterol. 1993;14:104-8.
8. Kattula D, Sarkar R, Ajjampur SS, Minz S, Levecke B, Kang G. Prevalence and risk factors for soil transmitted helminth infection among school children in south India. Indian J Med Res. 2014;139:76-82.
9. Naish S, McCarthy J, Williams GM. Prevalence, intensity and risk factors for soil-transmitted helminth infection in a South Indian fishing village. Acta Trop. 2004;91:177-87.
10. World Bank. The global burden of disease. Appendix B. In: World development report. Investing in health-world development indicators. New York: World Bank and Oxford University Press; 1993;213-25.
11. Richmond JB, Kotelchuck M. Personal health maintenance for children. West J Med. 1984;141:816–23.
12. Abera B, Alem G, Yimer M, Herrador Z. Epidemiology of soil transmitted helminths, Schistosomamansoni and haematocrit values among schoolchildren in Ethiopia. J Infect Dev Ctries. 2013;7(3):253-60.
13. Curtale F, Pezzotti P, Sharbini AL, al Maadat H, Ingrosso, Saad YS. Knowledge, perceptions and behaviour of mothers toward intestinal helminths in Upper Egypt: implications for control. Health Policy Plan. 1998;13:423-32.
14. World health organisation: 1998. Control of Tropical Disease Geneva. Available at http://www.who.int/neglected_diseases/en/. Accessed 10 September 2019.
15. Information regarding National Deworming Day in India from NHM website. Available at http://nrhm.gov.in/nrhm-components/rmnch-a/child-health-immunization/national-deworming-day-august-2016.html. Accessed 10 September 2019.
16. Ragunathan L, Kalivaradhan SK, Ramadass S, Nagaraj M,Ramesh K. Helminthic infections in school childrensPudhuchery, South India. Microbiol Immunol Infect. 2010;43(3):228–32.
17. Manual of basic techniques for a health laboratory, Second Edition: World Health Organization, Geneva 2003.
18. World Health Organization; Geneva: 1994. Bench Aids for the Diagnosis of Intestinal Parasites. Available at http://apps.who.int/iris/handle/10665/37323. Accessed 10 September 2019.
19. Chatterjee KD. Text book of Parasitology 13th edition. CBS publishers and distributors. 2015.
20. Shubha D, Fatima F. A coprological survey for assessing intensity of parasitic infection in school children: cross-sectional study. Trop Parasitol. 2011;1(2):88-93.
21. Yimer M, Hailu T, Mulu W, Abera B. Evaluation performance of diagnostic methods of intestinal parasitosis in school age children in Ethiopia. BMC Research Notes. 2015;8:820.
22. Mani TR, Rajendran R, Munirathinam A, Sunish IP, Md Abdullah S, Augustin DJ, et al. Efficacy of co-administration of albendazole and diethyl-carbamazine against geohelminthiases: a study from South India. Trop Med Int Health. 2002;7:541-8.
23. Kaliappan SP, George S, Francis MR, Kattula D, Sarkar R, Minz S, et al. Prevalence and clustering of soil-transmitted helminth infections in a tribal area in southern India. Trop Med Int Health. 2013;18(12):1452–62.
24. Fernandez MC, Verghese S, Bhuvaneswari R, Elizabeth SJ, Mathew T, Anitha A, et al. A comparative study of the intestinal parasites prevalent among children living in rural and urban settings in and around Chennai. J Commun Dis. 2002;34:35–9.
25. WHO (World Health Organization) Report of the WHO Informal Consultation on the use of chemotherapy for the control of morbidity due to soil-transmitted nematodes in humans. Geneva: WHO; 1996. (WHO/ CTD/ SIP/ 96.2). Available at http://apps.who.int/iris/handle/10665/63331. Accessed 10 September 2019.

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In vitro activity of a novel antibacterial agent, levonadifloxacin, against clinical isolates collected in a prospective, multicentre surveillance study in India during 2016–18

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Background: Levonadifloxacin is a novel antibiotic belonging to the benzoquinolizine subclass of fluoroquinolones with potent activity against MRSA and quinolone-resistant *Staphylococcus aureus*. IV levonadifloxacin and its oral prodrug alalevonadifloxacin have recently been approved in India for the treatment of acute bacterial skin and skin structure infections (ABSSSIs) including diabetic foot infections.

Objectives: To investigate the *in vitro* activity of levonadifloxacin against contemporary clinical isolates collected from multiple tertiary care hospitals across India in the Antimicrobial Susceptibility Profiling of Indian Resistotypes (ASPIRE) surveillance study.

Methods: A total of 1376 clinical isolates, consisting of staphylococci ($n = 677$), streptococci ($n = 178$), Enterobacterales ($n = 320$), *Pseudomonas aeruginosa* ($n = 140$) and *Acinetobacter baumannii* ($n = 61$), collected (2016–18) from 16 tertiary hospitals located across 12 states in India, were included in the study. The MICs of levonadifloxacin and comparator antibiotics were determined using the reference agar dilution method and broth microdilution method.

Results: Levonadifloxacin exhibited potent activity against MSSA ($MIC_{50/90}$: 0.5/1 mg/L), MRSA ($MIC_{50/90}$: 0.5/1 mg/L) and levofloxacin-resistant *S. aureus* ($MIC_{50/90}$: 1/1 mg/L) isolates. Similarly, potent activity of levonadifloxacin was also observed against CoNS including MDR isolates ($MIC_{50/90}$: 1/2 mg/L). Against *Streptococcus pneumoniae*, levonadifloxacin ($MIC_{50/90}$: 0.5/0.5 mg/L) showed superior activity compared with levofloxacin ($MIC_{50/90}$: 1/2 mg/L). Among levofloxacin-susceptible Enterobacterales, 80.6% of isolates were inhibited at $\leq$2 mg/L levonadifloxacin.

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Conclusions: Levonadifloxacin displayed potent activity against contemporary MRSA and fluoroquinolone-resistant staphylococcal isolates, thus offering a valuable IV as well as an oral therapeutic option for the treatment of ABSSSIs. Furthermore, levonadifloxacin exhibited a broad-spectrum activity profile as evident from its activity against streptococci and levofloxacin-susceptible Gram-negative isolates.

Introduction

Levonadifloxacin is a broad-spectrum anti-MRSA antibiotic belonging to the benzoquinolizine subclass of fluoroquinolones. IV levonadifloxacin (WCK 771) and its oral prodrug alalevonadifloxacin (WCK 2349) are being developed for the treatment of acute bacterial skin and skin structure infections (ABSSSIs), hospital-acquired bacterial pneumonia (HABP) and community-acquired bacterial pneumonia (CABP). Both WCK 771 and WCK 2349 have completed a Phase 3 clinical study (Clinical Trial Registry India, CTRI/2017/06/008843) and have been recently approved in India for the indication of ABSSSIs. The *in vitro* activity of levonadifloxacin against staphylococcal and streptococcal clinical isolates collected from India, the USA and Europe[1–4] has been reported earlier. Levonadifloxacin has also been comparatively evaluated against 12 474 clinical isolates as a part of the 2014 global SENTRY surveillance programme.[5] These studies established potent activity of levonadifloxacin against diverse resistotypes, such as MRSA, quinolone-resistant *Staphylococcus aureus* (QRSA), macrolide- or penicillin-resistant streptococci and quinolone-susceptible Gram-negative bacteria. These studies also showed that, similar to other fluoroquinolones, levonadifloxacin lacks therapeutically relevant activity against quinolone-resistant Gram-negative bacteria.

Though India is known for a high level of antibiotic resistance burden, large-scale hospital-based surveillance data encompassing diverse regions of the country are scarce. Most epidemiological studies are limited to a single or a few hospitals and report antibiotic susceptibilities based on disc diffusion testing. For instance, the only pan-India surveillance data reported by the Indian Council for Medical Research involves a limited number of centres with susceptibility rates reported solely based on disc diffusion testing.[6] In order to generate antimicrobial susceptibility data based on MIC determination, a nationwide surveillance study [Antimicrobial Susceptibility Profiling of Indian Resistotypes (ASPIRE)], with the participation of 16 medical centres across India, was initiated in 2016. The objective of the present study was to assess the *in vitro* activity of levonadifloxacin against contemporary Indian clinical isolates. Antimicrobial susceptibility testing was performed using the reference agar dilution method and broth microdilution method to assess the activities of levonadifloxacin and 20 comparator antibiotics (vancomycin, linezolid, ciprofloxacin, moxifloxacin, levofloxacin, penicillin G, oxacillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefepime, imipenem, meropenem, clarithromycin, azithromycin, clindamycin, gentamicin, amikacin, tobramycin, tigecycline and colistin) against contemporary clinical isolates collected between January 2016 and June 2018.

Materials and methods

Bacterial isolates

A total of 1376 clinical isolates, consisting of staphylococci ($n = 677$), streptococci ($n = 178$), levofloxacin-susceptible Enterobacterales ($n = 320$), *Pseudomonas aeruginosa* ($n = 140$) and *Acinetobacter baumannii* ($n = 61$), collected from January 2016 to June 2018 as part of the ASPIRE surveillance study, were included in this study. Levofloxacin-susceptible isolates accounted for 31.5% of total Gram-negative isolates collected in the ASPIRE surveillance study against which levonadifloxacin activity is presented. Sixteen tertiary care hospitals, located in 12 Indian states, participated in this study (Figure S1, available as Supplementary data at *JAC* Online). The inclusion criterion for participant patients was a minimum of 2 days of hospitalization in any of the following hospital departments: surgery, medicines, burns, ICUs, transplantation units and gynaecology. Only one isolate per patient (episode) was included in the study. Bacterial species were confirmed at a central laboratory (Metropolis Healthcare Ltd, Mumbai, India) using MALDI-TOF-based VITEK® MS (bioMérieux). Pure cultures were dispatched to Wockhardt Research Centre (Aurangabad, India) for MIC-based susceptibility testing. Prior to MIC determination studies, cultures were revived and passaged twice in tryptone soya agar (HiMedia, India) medium.

Antimicrobial susceptibility testing

MICs of antibiotics were mostly determined using the reference agar dilution method as described by CLSI.[7] The broth microdilution method was used for determination of MICs of colistin and tigecycline as recommended by CLSI.[7] Levonadifloxacin, clarithromycin, linezolid and clavulanic acid were synthesized at Wockhardt Research Centre. Penicillin G, piperacillin and oxacillin were procured from Sigma, USA. Other antibiotics were recovered from their respective commercial preparations and purity was ascertained by HPLC analyses. Since the final breakpoints have yet to be assigned, the proportions of strains inhibited by levonadifloxacin at 4 mg/L (proposed pharmacokinetic/pharmacodynamic breakpoint) have been reported. Susceptibility to other antibiotics was interpreted based on EUCAST (v 9.0) breakpoints.[8] *Escherichia coli* ATCC 25922, *S. aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 were included in each run as quality control strains.

Results

The clinical indication-wise distribution of Gram-positive and Gram-negative isolates is provided in Table S1. Isolates were recovered from documented ABSSSIs ($n = 501$), HABP ($n = 296$), urinary tract infections ($n = 180$), bloodstream infections ($n = 195$), intra-abdominal infections ($n = 50$) and other infection sources ($n = 154$).

Table 1 provides the cumulative distribution of levonadifloxacin MICs for the various resistant subsets. Table 2 presents MIC ranges, MIC_{50} values, MIC_{90} values and percentage susceptibilities for levonadifloxacin (percentage inhibition at 4 mg/L) and comparator antibiotics for *S. aureus* ($n = 390$). Based on susceptibility interpretive criteria for oxacillin, 49% of *S. aureus* were designated as MRSA phenotypes and, based on resistance to levofloxacin, 79% were QRSA. Levonadifloxacin exhibited potent activity against *S. aureus* including MSSA, MRSA and QRSA phenotypes, with MIC_{50} values being 0.5, 0.5 and 1 mg/L, while MIC_{90} values were 1, 1 and 1 mg/L, respectively. Notably, the MICs of levonadifloxacin for these groups of isolates were 4 times and 8–32 times lower than those of

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Table 1. Summary of the *in vitro* activity of levonadifloxacin against Gram-positive and Gram-negative clinical isolates

Organism group	No. of isolates (cumulative percentage) inhibited by levonadifloxacin [MIC (mg/L)]										MIC_{50} (mg/L)	MIC_{90} (mg/L)
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8		
All *S. aureus* (*n* = 390)	12 (3.1)	30 (10.8)	10 (13.3)	26 (20)	46 (31.8)	108 (59.5)	137 (94.6)	14 (98.2)	2 (98.7)	5 (100)		0.5	1
MSSA (*n* = 199)	5 (2.5)	15 (10.1)	3 (11.6)	9 (16.1)	24 (28.1)	58 (57.3)	76 (95.5)	5 (97.9)	1 (98.5)	3 (100)		0.5	1
MRSA (*n* = 191)	7 (3.7)	15 (11.5)	7 (15.2)	17 (24.1)	22 (35.6)	50 (61.8)	61 (93.7)	9 (98.4)	1 (99)	2 (100)		0.5	1
LVX-S *S. aureus* (*n* = 82)	11 (13.4)	28 (47.6)	8 (57.3)	2 (59.8)	14 (76.8)	17 (97.6)	1 (98.8)	1 (100)				0.06	0.5
LVX-R *S. aureus* (*n* = 308)	1 (0.3)	2 (0.97)	2 (1.6)	24 (9.4)	32 (19.8)	91 (49.3)	136 (93.5)	13 (97.7)	2 (98.4)	0 (98.4)	5 (100)	1	1
All CoNS (*n* = 287)	9 (3.1)	20 (10.1)	13 (14.6)	5 (16.4)	33 (27.9)	66 (50.9)	118 (91.9)	14 (96.9)	1 (97.2)	2 (97.9)	6 (100)	0.5	1
MS-CoNS (*n* = 83)	2 (2.4)	6 (9.6)	5 (15.7)	1 (16.8)	11 (30.1)	18 (51.8)	34 (92.7)	5 (98.8)	0 (98.8)	0 (98.8)	1 (100)	0.5	1
MR-CoNS (*n* = 204)	7 (3.4)	14 (10.3)	8 (14.2)	4 (16.1)	22 (26.9)	48 (50.5)	84 (91.7)	9 (96.1)	1 (96.6)	2 (97.6)	5 (100)	0.5	1
LVX-S CoNS (*n* = 84)	9 (10.7)	20 (34.5)	13 (50)	2 (52.4)	17 (72.6)	22 (98.8)	1 (100)					0.06	0.5
LVX -R CoNS (*n* = 203)			3 (1.5)	16 (9.4)	44 (31)	117 (88.7)	14 (95.6)	1 (96)	2 (97)	6 (100)		1	2
S. pneumoniae (*n* = 62)			1 (1.6)	0 (1.6)	29 (48.3)	29 (95.2)	0 (95.2)	0 (95.2)	2 (98.4)	0 (98.4)	1 (100)	0.5	0.5
S. pyogenes (*n* = 58)		2 (3.4)	0 (3.4)	2 (6.9)	17 (36.2)	19 (68.9)	15 (94.8)	0 (94.8)	0 (94.8)	3 (100)		0.5	1
Viridans group streptococci (*n* = 25)		1 (4)	2 (12)	1 (16)	8 (48)	4 (64)	0 (64)	0 (64)	0 (64)	1 (68)	8 (100)	0.5	>8
Group B and C streptococci (*n* = 33)			2 (6)	0 (6)	21 (69.6)	5 (84.8)	1 (87.8)	0 (87.8)	0 (87.8)	4 (100)		0.25	8
All Enterobacterales (*n* = 320)			16 (5)	19 (10.9)	61 (30)	68 (51.3)	43 (64.7)	51 (80.6)	33 (90.9)	8 (93.4)	21 (100)	0.5	4
E. coli (*n* = 54)			3 (5.6)	3 (11.1)	4 (18.5)	3 (24.1)	3 (29.6)	28 (81.5)	4 (88.9)	0 (88.9)	6 (100)	2	>8
Klebsiella pneumoniae (*n* = 65)				1 (1.5)	14 (23.1)	29 (67.7)	5 (75.4)	5 (83.1)	6 (92.3)	2 (95.4)	3 (100)	0.5	4
P. aeruginosa (*n* = 140)			2 (1.4)	0 (1.4)	1 (2.1)	5 (5.7)	18 (18.6)	64 (64.3)	46 (97.1)	2 (98.6)	2 (100)	2	4
Stenotrophomonas maltophilia (*n* = 38)			1 (2.6)	0 (2.6)	1 (5.3)	8 (26.3)	13 (60.5)	10 (86.8)	2 (92.1)	1 (94.7)	2 (100)	1	4
A. baumannii (*n* = 61)			3 (4.9)	10 (21.3)	29 (68.9)	16 (95.1)	2 (98.4)	0 (98.4)	0 (98.4)	0 (98.4)	1 (100)	0.25	0.5

LVX-S, levofloxacin-susceptible; LVX-R, levofloxacin-resistant; MS, methicillin-susceptible; MR, methicillin-resistant.

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Table 2. *In vitro* activity of levonadifloxacin and other antibiotics against *S. aureus* isolates

					Percentage susceptibility (EUCAST)		
Antibiotic	Organism group	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	S	I	R
Levonadifloxacin	*S. aureus*	0.015–8	0.5	1	NA	NA	NA
	MSSA	0.015–8	0.5	1	NA	NA	NA
	MRSA	0.015–4	0.5	1	NA	NA	NA
	LVX-S *S. aureus*	0.015–0.25	0.06	0.5	NA	NA	NA
	LVX-R *S. aureus*	0.015–8	1	1	NA	NA	NA
Moxifloxacin	*S. aureus*	0.03 to >32	2	4	18.9	NA	81
	MSSA	0.03–16	2	4	18.9	NA	85.8
	MRSA	0.03 to >32	2	4	19.9	NA	80.1
	LVX-S *S. aureus*	0.03–4	0.06	0.5	79.2	NA	20.7
	LVX-R *S. aureus*	0.03 to >32	2	4	2.9	NA	97.1
Levofloxacin	*S. aureus*	0.12 to >32	8	16	21.1	NA	79
	MSSA	0.12 to >32	8	8	20.5	NA	84.2
	MRSA	0.12 to >32	8	32	22.5	NA	77.5
	LVX-S *S. aureus*	0.12–1	0.25	1	100	NA	0
	LVX-R *S. aureus*	2 to >32	8	16	0	NA	100
Oxacillin	*S. aureus*	0.03 to >32	2	>32	51.1	NA	49
	MSSA	0.03–2	0.5	2	100	NA	0
	MRSA	4 to >32	32	>32	0	NA	100
	LVX-S *S. aureus*	0.12 to >32	0.5	4	47.5	NA	52.4
	LVX-R *S. aureus*	0.03 to >32	4	>32	51.9	NA	48.1
Vancomycin	*S. aureus*	0.25–2	1	1	100	NA	0
	MSSA	0.25–2	1	1	100	NA	0
	MRSA	0.25–2	1	1	100	NA	0
	LVX-S *S. aureus*	0.5–2	1	1	100	NA	0
	LVX-R *S. aureus*	0.25–2	1	1	100	NA	0
Clarithromycin	*S. aureus*	0.12 to >32	8	>32	33.8	3.8	62.3
	MSSA	0.12 to >32	16	>32	34.7	4.2	65.8
	MRSA	0.12 to >32	32	>32	34.5	3.6	61.8
	LVX-S *S. aureus*	0.12 to >32	0.25	>32	54.8	6.1	39.1
	LVX-R *S. aureus*	0.12 to >32	8	>32	28.2	3.2	68.5
Azithromycin	*S. aureus*	0.03 to >32	>32	>32	29.7	2.3	67.9
	MSSA	0.03 to >32	16	>32	28.9	3.7	72.1
	MRSA	0.03 to >32	>32	>32	31.9	1.1	67.1
	LVX-S *S. aureus*	0.5 to >32	1	>32	47.5	4.8	47.5
	LVX-R *S. aureus*	0.03 to >32	>32	>32	25	1.6	73.4
Clindamycin	*S. aureus*	0.015 to >32	0.12	0.12	79.5	1.1	19.5
	MSSA	0.015–8	0.12	0.12	84.2	1.1	19.5
	MRSA	0.015 to >32	0.12	0.12	31.9	1.1	67.1
	LVX-S *S. aureus*	0.06–0.25	0.12	0.12	74.4	0	25.6
	LVX-R *S. aureus*	0.015 to >32	0.12	0.12	80.8	1.3	17.8
Linezolid	*S. aureus*	0.5–2	2	2	100	NA	0
	MSSA	0.5–2	2	2	100	NA	0
	MRSA	0.5–2	2	2	100	NA	0
	LVX-S *S. aureus*	1–2	2	2	100	NA	0
	LVX-R *S. aureus*	0.5–2	2	2	100	NA	0
Tigecycline	*S. aureus*	0.06–1	0.25	0.25	95.6	NA	4.4
	MSSA	0.06–1	0.25	0.5	97.4	NA	2.6
	MRSA	0.06–1	0.25	0.25	93.7	NA	6.3
	LVX-S *S. aureus*	0.015–1	0.12	0.5	96.3	NA	3.6
	LVX-R *S. aureus*	0.06–2	0.25	0.25	95.4	NA	4.5
Gentamicin	*S. aureus*	0.12 to >32	8	>32	42.6	NA	57.4
	MSSA	0.12 to >32	0.5	8	48.9	NA	55.8
	MRSA	0.12 to >32	16	>32	38.2	NA	61.8
	LVX-S *S. aureus*	0.12–128	0.25	4	78.1	NA	21.9
	LVX-R *S. aureus*	0.12 to >32	4	>32	33.1	NA	66.8

S, susceptible; I, intermediate; R, resistant; LVX-S, levofloxacin-susceptible; LVX-R, levofloxacin-resistant; NA, not applicable.

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Table 3. *In vitro* activity of levonadifloxacin and other antibiotics against CoNS

					Percentage susceptibility (EUCAST)		
Antibiotic	Organism group	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	S	I	R
Levonadifloxacin	CoNS	0.015–8	0.5	1	NA	NA	NA
	MS-CoNS	0.015–8	0.5	1	NA	NA	NA
	MR-CoNS	0.015–4	0.5	1	NA	NA	NA
	LVX-S CoNS	0.015–0.5	0.06	0.5	NA	NA	NA
	LVX-R CoNS	0.06–8	1	2	NA	NA	NA
Moxifloxacin	CoNS	0.03–32	2	4	26.8	NA	73.2
	MS-CoNS	0.03–4	1	4	31.5	NA	68.5
	MR-CoNS	0.03–32	2	4	24.8	NA	75.8
	LVX-S CoNS	0.03–4	0.12	0.5	83.3	NA	16.7
	LVX-R CoNS	0.25–32	2	4	3.5	NA	96.5
Levofloxacin	CoNS	0.03 to >32	8	32	29.3	NA	70.7
	MS-CoNS	0.03 to >32	4	16	28.6	NA	71.4
	MR-CoNS	0.03 to >32	8	32	28.8	NA	71.2
	LVX-S CoNS	0.03–1	0.25	1	100	NA	0
	LVX-R CoNS	2 to >32	16	32	0	NA	100
Oxacillin	CoNS	0.03 to >32	4	>32	31.0	NA	69
	MS-CoNS	0.03–0.25	0.12	0.25	100	NA	0
	MR-CoNS	0.5 to >32	16	32	0	NA	100
	LVX-S CoNS	0.03 to >32	4	>32	32.1	NA	67.9
	LVX-R CoNS	0.03 to >32	4	>32	30.5	NA	69.5
Vancomycin	CoNS	0.06–4	1	2	100	NA	0
	MS-CoNS	0.06–4	1	2	100	NA	0
	MR-CoNS	0.25–4	2	2	100	NA	0
	LVX-S CoNS	0.25–4	1	2	100	NA	0
	LVX-R CoNS	0.25–4	2	2	100	NA	0
Clarithromycin	CoNS	0.015 to >32	32	>32	30.3	3.5	66.2
	MS-CoNS	0.015 to >32	16	>32	31.5	4.5	64
	MR-CoNS	0.06 to >32	32	>32	29.8	3	67.2
	LVX-S CoNS	0.015 to >32	8	>32	40.5	5.9	53.6
	LVX-R CoNS	0.06 to >32	16	>32	26.1	2.5	71.4
Azithromycin	CoNS	0.03 to >32	>32	>32	26.8	3.8	69.3
	MS-CoNS	0.03 to >32	32	>32	29.2	2.2	68.5
	MR-CoNS	0.03 to >32	>32	>32	25.8	4.5	69.7
	LVX-S CoNS	0.03 to >32	16	>32	36.9	5.9	57.1
	LVX-R CoNS	0.03 to >32	>32	>32	22.6	2.9	74.4
Clindamycin	CoNS	0.015 to >32	0.12	>32	73.9	0.7	25.4
	MS-CoNS	0.015 to >32	0.12	>32	78.6	1.1	20.2
	MR-CoNS	0.03 to >32	0.12	>32	72.2	0.5	27.3
	LVX-S CoNS	0.015 to >32	0.12	8	75.0	1.2	23.8
	LVX-R CoNS	0.03 to >32	0.25	>32	73.9	0.5	25.6
Linezolid	CoNS	0.12 to >32	1	2	96.1	NA	3.8
	MS-CoNS	0.12–8	1	2	98.8	NA	1.1
	MR-CoNS	0.5 to >32	1	2	94.9	NA	5
	LVX-S CoNS	0.12–2	1	2	100	NA	0
	LVX-R CoNS	0.25 to >32	1	2	94.5	NA	5.4
Tigecycline	CoNS	0.015–1	0.25	0.5	95.5	NA	4.5
	MS-CoNS	0.015–1	0.25	0.5	95.5	NA	4.5
	MR-CoNS	0.06–1	0.25	1	95.4	NA	4.5
	LVX-S CoNS	0.015–1	0.25	0.5	97.6	NA	2.4
	LVX-R CoNS	0.03–1	0.25	1	94.6	NA	5.4
Gentamicin	CoNS	0.015 to >32	16	>32	55	NA	44.9
	MS-CoNS	0.015 to >32	0.25	>32	60.7	NA	39.3
	MR-CoNS	0.03 to >32	32	>32	52.5	NA	47.5
	LVX-S CoNS	0.015 to >32	0.25	32	84.5	NA	15.5
	LVX-R CoNS	0.06 to >32	32	>32	42.8	NA	57.1

S, susceptible; I, intermediate; R, resistant; MS, methicillin-susceptible; MR, methicillin-resistant; LVX-S, levofloxacin-susceptible; LVX-R, levofloxacin-resistant; NA, not applicable.

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Table 4. *In vitro* activity of levonadifloxacin and other antibiotics against streptococci

Organism group	Antibiotic	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Percentage susceptibility (EUCAST)		
					S	I	R
S. pneumoniae (*n* = 62)	levonadifloxacin	0.06–16	0.5	0.5	NA	NA	NA
	levofloxacin	0.5–16	1	2	95.2	NA	4.8
	vancomycin	0.06–0.5	0.5	0.5	100	NA	0
	penicillin G	0.003–8	1	4	27.4	53.2	19.4
	amoxicillin/clavulanate	0.003–8	1	8	38.7	38.7	22.58
	clarithromycin	0.015 to >32	8	>32	24.2	3.2	72.6
	azithromycin	0.015 to >32	8	>32	24.2	0	75.8
	clindamycin	0.007 to >32	0.06	>32	58	NA	41.9
S. pyogenes (*n* = 58)	levonadifloxacin	0.03–8	0.5	1	NA	NA	NA
	levofloxacin	0.03 to >8	1	2	94.8	NA	5.2
	vancomycin	0.03–0.5	0.03	0.5	100	NA	0
	penicillin G	0.003–0.12	0.007	0.015	100	NA	0
	clarithromycin	0.007 to >32	2	>32	34.5	0	65.5
	azithromycin	0.007 to >32	8	>32	31	0	69
	clindamycin	0.007–0.5	0.06	0.25	100	NA	NA
Viridans group streptococci[a] (*n* = 25)	levonadifloxacin	0.03 to >8	0.5	>8	NA	NA	NA
	levofloxacin	0.03–16	1	>8	IE[c]	IE	IE
	vancomycin	0.03–2	1	1	100	NA	0
	penicillin G	0.003 to >8	0.06	2	64	28	8
	clarithromycin	0.007 to >32	0.25	>32	IE	IE	IE
	azithromycin	0.007 to >32	0.5	>32	IE	IE	IE
	clindamycin	0.007 to >32	0.03	1	88	NA	12
Group B and C streptococci[b] (*n* = 33)	levonadifloxacin	0.06–8	0.25	8	NA	NA	NA
	levofloxacin	0.5 to >8	1	8	87.9	NA	12.1
	vancomycin	0.06–1	0.5	1	100	NA	0
	penicillin G	0.007–0.5	0.03	0.06	97	NA	3
	clarithromycin	0.007 to >32	0.03	4	66.7	0	33.35
	azithromycin	0.03 to >32	1	>32	48.5	0	51.5
	clindamycin	0.007 to >8	0.03	0.12	94	NA	6

S, susceptible; I, intermediate; R, resistant; NA, not applicable.
[a]Viridans group streptococci: *Streptococcus mitis/oralis* (*n* = 19), *Streptococcus salivarius* ssp. *thermophilus* (*n* = 1), *S. salivarius* (*n* = 1), *Streptococcus sanguinis* (*n* = 1), *Streptococcus infantarius* ssp. *coli* (*n* = 1) and *Streptococcus constellatus* (*n* = 2).
[b]Group B and C streptococci: *Streptococcus agalactiae* (*n* = 27) and *Streptococcus dysgalactiae* (*n* = 6).
[c]IE indicates that there is insufficient evidence that the organism or group is a good target for therapy with the agent as mentioned in EUCAST (v 9.0) breakpoints.

moxifloxacin and levofloxacin, respectively. At a levonadifloxacin pharmacokinetic/pharmacodynamic breakpoint of 4 mg/L, 98.7%, 99% and 98.4% of all *S. aureus*, MRSA and QRSA were inhibited, respectively. There was little or no resistance to vancomycin, linezolid and tigecycline among *S. aureus*.

Despite 71.1% methicillin resistance and 70.7% levofloxacin resistance, levonadifloxacin displayed potent activity against CoNS with MIC_{50} and MIC_{90} values of 0.5 and 1 mg/L, respectively (Table 3). CoNS (*n* = 287) isolates consisted of *Staphylococcus haemolyticus* (*n* = 135), *Staphylococcus hominis* (*n* = 55), *Staphylococcus epidermidis* (*n* = 74) and other CoNS species (*n* = 23). For these isolates, levonadifloxacin was 4 times more active than moxifloxacin and 16–32 times more active than levofloxacin. At 4 mg/L levonadifloxacin, 97.2% of CoNS isolates were inhibited (Table 1).

Table 4 provides the activity of levonadifloxacin and other antibiotics against streptococci. Against *S. pneumoniae* (*n* = 62), levonadifloxacin demonstrated significantly lower MICs with an MIC_{90} of 0.5 mg/L, which was four times lower than that of levofloxacin. Considering a levonadifloxacin-susceptible breakpoint of 4 mg/L, 98.4% of these isolates were found to be susceptible to levonadifloxacin (Table 1). The *S. pneumoniae* isolates showed 75.8% azithromycin resistance and 41.9% clindamycin resistance. The isolates exhibited a meagre susceptibility rate to penicillin G of 27.4%. Levonadifloxacin exhibited potent activity against *Streptococcus pyogenes* (*n* = 58) with an MIC_{90} value of 1 mg/L, which was one dilution less than that of levofloxacin. Against viridans group streptococci (*n* = 25), levonadifloxacin showed limited activity. Levonadifloxacin showed good activity against group B and C streptococci (*n* = 33), with MIC_{50} and MIC_{90} values of 0.25 and 8 mg/L, respectively (Table 4).

Levonadifloxacin was also tested against levofloxacin-susceptible Enterobacterales (*n* = 320). Against this set of isolates,

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Table 5. Activity of levonadifloxacin and other antibiotics against levofloxacin-susceptible Gram-negative isolates

				Percentage susceptibility (EUCAST)		
Antibiotic	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	S	I	R
All Enterobacterales (*n* = 320)						
levonadifloxacin	0.06 to >32	0.5	4	NA	NA	NA
levofloxacin	0.06–0.5	0.12	0.5	100	0	0
ciprofloxacin	0.06 to >32	0.12	0.5	78.9	11.9	9.1
cefepime	0.015 to >256	0.06	8	81.9	5.6	12.5
imipenem	0.06–128	0.25	1	96.9	0.6	2.5
meropenem	0.015–64	0.03	0.06	98.4	0.04	1.5
piperacillin/tazobactam	0.06 to >256	1	8	92.8	1.9	5.3
amikacin	0.06 to >256	4	8	94.4	1.2	4.4
tobramycin	0.06 to >128	1	8	88.9	0.66	10.4
tigecycline	0.03 to >8	0.5	2	65.7	NA	34.3
colistin	0.06 to >128	0.5	32	87	NA	13
P. aeruginosa (*n* = 140)						
levonadifloxacin	0.06 to >32	2	4	NA	NA	NA
levofloxacin	0.06–1	1	1	100	NA	0
ciprofloxacin	0.06–2	0.25	0.5	97.1	NA	2.9
cefepime	0.015–128	2	8	95.7	NA	4.3
imipenem	0.06–256	2	4	92.9	NA	7.1
meropenem	0.015–128	0.25	1	95	4.3	0.7
piperacillin/tazobactam	0.25–256	4	16	92.9	NA	7.1
amikacin	1 to >256	4	8	95	2.9	2.1
gentamicin	0.25 to >32	2	8	88.6	NA	11.4
tobramycin	0.25 to >32	1	2	97.1	NA	2.9
colistin	0.06 to >32	2	4	80	NA	20
A. baumannii (*n* = 61)						
levonadifloxacin	0.06 to >32	0.25	0.5	NA	NA	NA
levofloxacin	0.06–0.5	0.12	0.25	100	0	0
ciprofloxacin	0.12–32	0.25	0.5	0	98.6	1.6
cefepime	0.06 to >256	2	8	NA	NA	NA
imipenem	0.06–256	0.25	2	90.2	0	9.8
meropenem	0.03 to >256	0.25	2	90.2	0	9.8
piperacillin/tazobactam	0.25–256	0.5	32	NA	NA	NA
amikacin	1 to >256	4	16	86.8	3.2	9.8
gentamicin	0.25 to >32	1	4	90.1	NA	9.8
tobramycin	0.25 to >32	1	2	97.1	NA	2.8
tigecycline	0.03–32	0.12	0.5	NA	NA	NA
colistin	0.06 to >32	0.5	1	96.7	NA	3.3

S, susceptible; I, intermediate; R, resistant; NA, not applicable.
Levofloxacin-susceptible isolates are selected based on the levofloxacin EUCAST susceptible breakpoint. EUCAST breakpoints are used for all other antibiotics.

ciprofloxacin susceptibility was 78.9% (Table 5), whereas 90.9% of strains were inhibited by levonadifloxacin at the proposed breakpoint of 4 mg/L with MIC_{50} and MIC_{90} values being 0.5 and 4 mg/L (Table 1), respectively. Disturbingly, against Enterobacterales isolates, last-resort agents such as tigecycline and colistin showed susceptibilities of 65.7% and 87%, respectively, when EUCAST susceptibility breakpoints were applied. In the case of levofloxacin-susceptible *P. aeruginosa* isolates (*n* = 140), levonadifloxacin MIC_{50} and MIC_{90} values were 2 and 4 mg/L, respectively, with 97.1% susceptibility based on the pharmacokinetic/pharmacodynamic breakpoint (Table 1), which was similar to ciprofloxacin (Table 5). Against levofloxacin-susceptible *A. baumannii* (*n* = 61) isolates, levonadifloxacin MIC_{50} was 0.25 mg/L and MIC_{90} was 0.5 mg/L. Levonadifloxacin inhibited 98.4% of isolates at 1 mg/L, while the potency (in terms of MIC_{90}) of levonadifloxacin was comparable to that of ciprofloxacin.

Discussion

The present study aimed to assess the *in vitro* activity of levonadifloxacin against contemporary nosocomial Gram-positive and Gram-negative isolates collected from 16 medical centres across

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India. The study showed a high level of quinolone resistance (79%) among *S. aureus* isolates, while MRSA prevalence of 49% was noted. Previously, the Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group reported an overall MRSA prevalence rate of 41%–47% in India during 2008–09.[9] A recent report from Rajkumar *et al.*[10] showed MRSA rates in the range of 21%–45%. Thus, MRSA rates encountered in this study are broadly in agreement with previous studies. The present study also substantiated the prevalence of high fluoroquinolone resistance in *S. aureus* reported by the Center for Disease Dynamics, Economics & Policy (CDDEP), wherein a 79%–90% fluoroquinolone resistance rate was observed in 2014 while 71% resistance was reported in the year 2017.[11] Despite a high prevalence of quinolone and methicillin resistance in *S. aureus*, levonadifloxacin displayed a therapeutically relevant antibacterial activity with >90% isolates inhibited at $\leq$1 mg/L. The quinolone resistance rate among CoNS was 70.7%, while the methicillin resistance rate was 71.1%, against which levonadifloxacin showed MIC_{50} and MIC_{90} values of 0.5 and 1 mg/L, respectively.

In conjunction with several previous studies undertaken over the last 10 years, the present study reaffirmed an unchanged levonadifloxacin MIC_{90} of 1 mg/L for MRSA even in the face of increasing fluoroquinolone resistance.[1–4] The stable activity of levonadifloxacin is attributed to its differentiated mechanism of action based on preferential targeting of DNA gyrase in *S. aureus*, an attribute distinct from topoisomerase IV-targeting levofloxacin and moxifloxacin.[12] This differentiated mechanism of action possibly emanates from its distinctive structural feature of a tricyclic core with the absence of the conventional amine at the C-8 position of the side chain.[13] Further, it was demonstrated that the DNA gyrase-targeting feature of levonadifloxacin helps overcome trovafloxacin and moxifloxacin resistance in *S. aureus*, which is invariably mediated by multiple mutations in topoisomerase IV.[12] A similar observation was apparent in the present study, which showed four times greater activity of levonadifloxacin compared with moxifloxacin against *S. aureus*.

Against streptococci, activity of levonadifloxacin was found to be superior to levofloxacin. The improved activity of levonadifloxacin against streptococci points towards a favourable interaction with bacterial topoisomerases in this pathogen as well. However, for viridans group streptococci, levonadifloxacin showed comparable activity to that of levofloxacin.

The potent anti-MRSA activity of levonadifloxacin observed in this study supports its therapeutic utility for MRSA-dominated indications such as ABSSSIs, HABP and bone and joint infections. In particular, availability of an oral administration option in the form of alalevonadifloxacin would offer the convenience of outpatient treatment for indications requiring longer or stepdown therapy for early hospital discharge. Promising activity against *S. pneumoniae* and other respiratory pathogens, including atypical pathogens, demonstrated in previous studies[14–16] points towards its potential as an empirical oral therapy option for lower respiratory tract infections. Furthermore, Rodvold *et al.*[17] studied intrapulmonary pharmacokinetics of levonadifloxacin following oral administration of alalevonadifloxacin, which showed higher unbound plasma and epithelial lining fluid (ELF) exposures (AUC_{0-24} =345.2 mg·h/L) compared with moxifloxacin. Considering an MIC_{90} of 0.5 and 1 mg/L for streptococci and MRSA, respectively, high ELF levels are likely to bestow a significant pharmacokinetic/pharmacodynamic advantage on levonadifloxacin in the treatment of CABP and HABP.

In summary, the study conducted on the contemporary pan-India isolates obtained in the ASPIRE study highlights the potent activity of levonadifloxacin against fluoroquinolone- and methicillin-resistant staphylococci, macrolide-resistant streptococci and quinolone-susceptible Gram-negative organisms.

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Transparency declarations

Prashant Joshi, Ashish Mane, Rishi Jain and Sachin Bhagwat are employees of Wockhardt Ltd, India (please note that Wockhardt Research Centre is the research unit of Wockhardt Ltd, India). Sachin Bhagwat is a shareholder of Wockhardt Ltd, India. Hemant Khande was an employee of Wockhardt Ltd, India. All other authors: none to declare.

Supplementary data

Figure S1 and Table S1 are available as Supplementary data at *JAC* Online.

References

1 Patel MV, De Souza NJ, Gupte SV *et al*. Antistaphylococcal activity of WCK 771, a tricyclic fluoroquinolone, in animal infection models. *Antimicrob Agents Chemother* 2004; **48**: 4754–61.

2 Al-Lahham A, De Souza NJ, Patel M *et al*. Activity of the new quinolones WCK 771, WCK 1152 and WCK 1153 against clinical isolates of *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *J Antimicrob Chemother* 2005; **56**: 1130–3.

3 Jacobs MR, Bajaksouzian S, Windau A *et al*. In vitro activity of the new quinolone WCK 771 against staphylococci. *Antimicrob Agents Chemother* 2004; **48**: 3338–42.

4 Bhagwat SS, McGhee P, Kosowska-Shick K *et al*. In vitro activity of the quinolone WCK 771 against recent U.S. hospital and community-acquired *Staphylococcus aureus* pathogens with various resistance types. *Antimicrob Agents Chemother* 2009; **53**: 811–3.

5 Flamm RK, Farrel DJ, Sader HS *et al*. *In vitro* activity of WCK 771, a benzoquinolizine fluoroquinolone (levonadifloxacin) when tested against contemporary Gram-positive and -negative bacteria from a global surveillance program. *ASM Microbe 2016, Boston, MA, USA*. Sunday-456.

6 Walia K, Ohri V, Singh H *et al*. *Annual Report Antimicrobial Resistance Surveillance Network Jan 2018 - Dec 2018*. AMR Surveillance Network Indian Council of Medical Research, 2019.

7 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Tenth Edition: M07*. 2015.

8 EUCAST. *Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 9.0*. 2019. http://www.eucast.org.

9 Indian Network for Surveillance of Antimicrobial Resistance (INSAR). Methicillin resistant *Staphylococcus aureus* (MRSA) in India: prevalence & susceptibility pattern. *Indian J Med Res* 2013; **137**: 363–9.

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10 Rajkumar S, Sistla S, Manoharan M *et al*. Prevalence and genetic mechanisms of antimicrobial resistance in *Staphylococcus* species: a multicentre report of the indian council of medical research antimicrobial resistance surveillance network. *Indian J Med Microbiol* 2017; **35**: 53–60.

11 Gandra S, Joshi J, Trett A *et al*. *Scoping Report on Antimicrobial Resistance in India*. Center for Disease Dynamics, Economics & Policy, 2017.

12 Bhagwat SS, Mundkur LA, Gupte SV *et al*. The anti-methicillin-resistant *Staphylococcus aureus* quinolone WCK 771 has potent activity against sequentially selected mutants, has a narrow mutant selection window against quinolone-resistant *Staphylococcus aureus*, and preferentially targets DNA gyrase. *Antimicrob Agents Chemother* 2006; **50**: 3568–79.

13 de Souza NJ, Gupte SV, Deshpande PK *et al*. A chiral benzoquinolizine-2-carboxylic acid arginine salt active against vancomycin-resistant *Staphylococcus aureus*. *J Med Chem* 2005; **48**: 5232–42.

14 Xue G, Crabb DM, Xiao L *et al*. *In vitro* activities of the benzoquinolizine fluoroquinolone levonadifloxacin (WCK 771) and other antimicrobial agents against mycoplasmas and ureaplasmas in humans, including isolates with defined resistance mechanisms. *Antimicrob Agents Chemother* 2018; **62**: e01348-18.

15 Kohlhoff S, Huerta N, Hammerschlag MR. *In vitro* activity of levonadifloxacin (WCK 771) against *Chlamydia pneumoniae*. *Antimicrob Agents Chemother* 2019; **63**: e01048-19.

16 Dubois J, Dubois M. *In vitro* intracellular activity of levonadifloxacin against *Legionella pneumophila. Twenty-Ninth European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, The Netherlands, 2019*. Abstract P1986.

17 Rodvold KA, Gotfried MH, Chugh R *et al*. Intrapulmonary pharmacokinetics of levonadifloxacin following oral administration of alalevonadifloxacin to healthy adult subjects. *Antimicrob Agents Chemother* 2018; **62**: e02297-17.

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Research Article

Incidence of infections with extended spectrum beta Lactamase (ESBL)-producing gram-negative bacteria among patients admitted in medical intensive care unit of tertiary care hospital

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Abstract

Introduction: Extended spectrum beta-lactamases (ESBLs) are beta-lactamases capable of conferring bacterial resistance to the Penicillins, first, second and third generation cephalosporins, and Aztreonam (but not the Cephamycins or Carbapenems). Clinicians, Microbiologists and hospital epidemiologists are concerned about ESBL-producing Gram negative bacteria because of increased incidence of such infections, limitations of effective antimicrobial therapy and adverse patient outcomes. **Aims and Objectives:** To find the incidence of infections caused by ESBL producing Gram negative bacteria among patients admitted in Medical ICU of tertiary care hospital. To compare the antimicrobial susceptibility patterns of the ESBL producing Gram negative isolates with ESBL non-producing isolates. **Materials and Methods:** Clinical samples were collected from patients admitted in Medical ICU & processed as per standard protocol. Antibiotic susceptibility was performed by Kirby-Bauer disc diffusion method. Screening for ESBL production was done as per CLSI guidelines. Presence of ESBL was detected by Phenotypic confirmatory disc diffusion method using discs containing 30µg of Ceftazidime with and without 10µg of Clavulanic acid on Mueller-Hinton Agar. **Results:** A total of 50 samples were collected. Of these, majority (38%) of samples were urine followed by sputum (36%). Of the 50 samples, majority of the isolates were *Klebsiella* spp and *E.coli*, 14(28%) each. Among 50 samples, 42(84%) isolates were ESBL producers. Of the 42 ESBL producing organisms, majority(13(30.9%)) of them were *Klebsiella* spp. **Conclusion:** In the present study, we found an alarming number (84%) of ESBL producing Gram negative isolates in Medical ICU patients. Strict antibiotic policies and measures to limit indiscriminate use of Cephalosporins should be undertaken to minimize the emergence of such resistance.

Key words: ESBL, Gram negative bacteria, Medical ICU.

Introduction

Extended spectrum beta-lactamases (ESBLs) are beta-lactamases capable of conferring bacterial resistance to the Penicillins, first, second and third generation cephalosporins, and Aztreonam (but not the Cephamycins or Carbapenems) [1]. Typically, they derive from genes TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these beta-lactamases. This extends the spectrum of beta-lactam antibiotics susceptible to hydrolysis by these enzymes [2]. ESBL are most commonly produced by Klebsiella spp and E.coli.

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However, Enterobacter, Salmonella, Proteus, Citrobacter, Morganella,Serratia,Shigella, Pseudomonas and Burkholderia spp also produce them [3]. Clinicians, Microbiologists and hospital epidemiologists are concerned about ESBL-producing Gram negative bacteria because of increased incidence of such infections, limitations of effective antimicrobial therapy and adverse patient outcomes.

Special efforts should be undertaken by Clinical microbiology laboratories as recommended by the Clinical and Laboratory Standards Institute (CLSI) for ESBL detection [4,5]. ESBL detection is not commonly carried out in laboratories in developing countries due

to lack of awareness and facilities to conduct ESBL identification [6]. Detection of ESBL producing organism from samples is important because this represents an epidemiologic marker of colonization and therefore there is potential for transfer of such organisms to other patients [7]. Within countries, hospital-to-hospital variability in ESBLs may also be marked [8].

This prospective Cross-sectional study is conducted to find and compare incidence of infections with ESBL producing Gram negative bacteria among patients admitted in Medical intensive care unit of Karnataka Institute of Medical Sciences, Hubballi. Besides it is important to understand the extent of spread of these isolates in the community so as to formulate corrective measures.

Aims and Objectives

To find the incidence of infections caused by ESBL producing Gram negative bacteria among patients admitted in Medical ICU of tertiary care hospital. To compare the antimicrobial susceptibility patterns of the ESBL producing Gram negative isolates with ESBL non-producing isolates.

Materials and Methods

- The study has been carried out after obtaining the clearance of Institutional ethical committee.
- **Study design:** Prospective Cross-sectional study
- **Study site:** Medical Intensive care unit of KIMS Hospital, Hubballi.
- **Duration of study**: June 1st to July 31st, 2014.
- **Sample size:** 50

Inclusion criteria

- Patients admitted in Medical ICU during study period were included.
- Only Gram negative isolates were included.

Exclusion criteria

- Isolates other than Gram negative bacteria
- Repeat isolates from same patient.

Sample collection and processing: Clinical samples were collected from patients admitted in Medical ICU & processed as per standard protocol.

Methodology

Antibiotic susceptibility was performed by Kirby-Bauer disc diffusion method. Screening for ESBL production was done as per CLSI guidelines [9]. Presence of ESBL was detected by Phenotypic confirmatory disc diffusion method using discs containing 30µg of Ceftazidime with and without 10µg of Clavulanic acid on Mueller-Hinton Agar. ESBL production was inferred if the inhibition zone increases by 5mm towards Ceftazidime plus clavulanic acid in comparison to Ceftazidime disc alone [9].

Statistical Analysis was done by Chi-square test using SPSS software.



Fig 1: Phenotypic confirmatory test showing ESBL positive isolate



Fig 2: Phenotypic confirmatory test showing ESBL negative isolate

Observations and Results

The present study was carried out in the Department of Microbiology, Karnataka Institute of Medical Sciences (KIMS), Hubballi.

Table-1: Specimen-wise distribution of isolates.

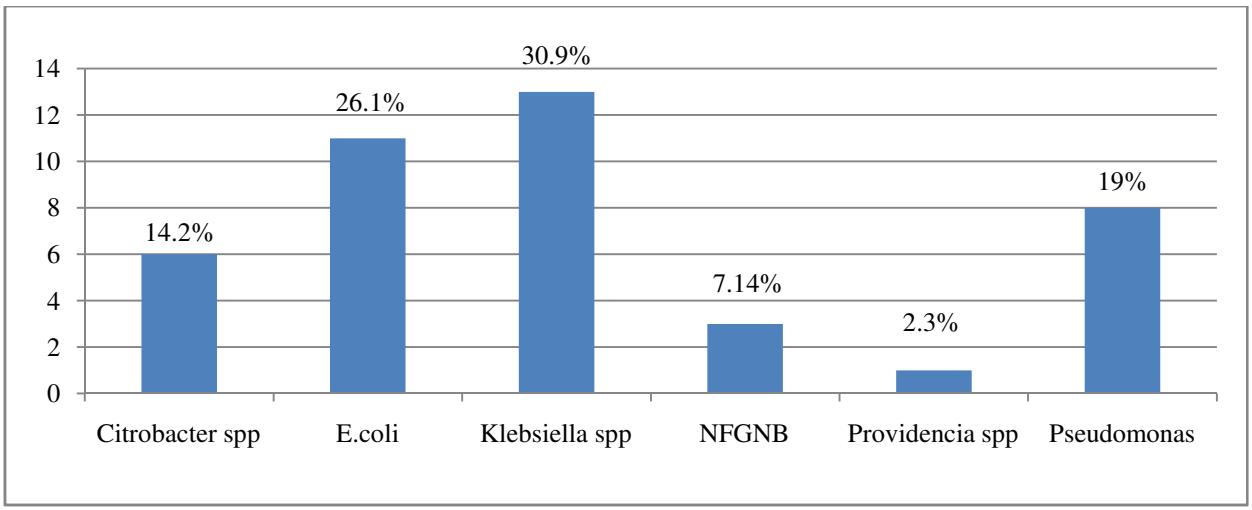
Specimen	Number (n=50)	Percentage
Ascitic fluid	3	6%
CSF	4	8%
ET tube secretion	1	2%
Pleural fluid	4	8%
Pus	1	2%
Sputum	18	36%
Urine	19	38%
Total	**50**	**100%**

Majority (38%) of samples were urine followed by sputum (36%).

Table-2: Distribution of isolates.

Isolate	Number	Percentage(%)
E.coli	14	28%
Klebsiella spp.	14	28%
Pseudomonas spp	9	18%
Citrobacter spp.	7	14%
NFGNB	5	10%
Providencia spp.	1	2%

Of the 50 samples, majority of isolates were *Klebsiella* spp and *E.coli,*14(28%) each.



Graph-1: Distribution of isolates with ESBL

Of the 42 ESBL producing organisms, majority of them were *Klebsiella* spp which was 13 (30.9%).

Table-3: Comparison of antibiotic resistance pattern of ESBL positive and ESBL negative isolates.

Antibiotics	ESBL positive n=42		ESBL negative n=8		p value	Significance
	Resistant	%	Resistant	%		
Amikacin	11	26.19	1	12.5	0.017	S
Netilmicin	13	30.95	0	0	0.567	NS
Amoxy-clavulanic acid	24	57.14	1	12.5	0.009	S
Ceftazidime	5	11.90	1	12.5	0.738	NS
Cefepime	28	66.66	2	25	0.042	S
Cefaperazone	6	14.28	1	12.5	0.896	NS
Cefotaxime	27	64.28	2	25	0.002	S
Cefoxitin	21	50	4	50	0.882	NS
Ciprofloxacin	3	7.14	0	0	0.436	NS
Levofloxacin	8	19.04	0	0	0.191	NS
Norfloxacin	11	26.19	0	0	0.188	NS
Ofloxacin	7	16.66	1	12.5	0.402	NS
Pefloxacin	18	42.85	1	12.5	0.179	NS
Imipenem	6	14.28	0	0	0.007	S
Pip-tazobactam	10	23.8	2	25	0.029	S
Tetracycline	25	59.52	2	25	0.058	NS

Difference was statistically significant for Amikacin, Amoxy-clavulanic acid, Cefepime, Cefotaxime, Imipenem and Piperacillin+tazobactam. ESBL producing isolates were found to show higher rates of resistance when compared to non ESBL producers.

Discussion

A total of fifty isolates from various clinical samples from medical ICU were screened for ESBL Production. Screening for ESBL production is done as per CLSI guidelines. Of these 50 isolates, 42(84%) were ESBL producers. Of these, majority of the isolates were from urine and sputum, 16(38%) each.

In India, ESBL presence rate varies in different situations from 24 to 84% [10]. A study from Coimbatore, Tamil Nadu, presence of ESBLs to be 40%, while a study done from Nagpur showed it as 50% [11,12]. Another study in 2005, from New Delhi, showed 68.78% of the strains of Gram negative bacteria to be ESBL producers [13]. In our study, presence rate of ESBL was found to be 84%. This must be due to injudicious usage of Cephalosporins in Medical ICU patients.

Klebsiella and *E.coli* were the predominant ESBL producers (28% each) lower than the study done by Neelam Taneja et al who observed 51.2% *Klebsiella* spp followed by 40.2% *Escherichia coli* [14].

The incidence of ESBL was found to be 84% higher than in the study done by Kumar D, Singh AK, Ali MR, Chander Y to determine the antimicrobial sensitivity profile of ESBL producing *E. coli* isolates from various clinical samples such as pus, urine, blood, cerebrospinal fluid (CSF), stool, sputum, swabs, and different body fluids which was 55.5% [15].

ESBL producing isolates were found to show higher rate of resistance to Cefipime (66.6%), Cefotaxime (64.28%) and Amoxy-clavulanic acid (57.14%). Imipenem (14.28%), Piperacillin-Tazobactam (23.8%), and Ciprofloxacin (7.14%) were found to be the effective antibiotic in ESBL producing isolates. It is statistically significant for Amikacin, Amoxy-clavulanic acid, Cefepime, Cefotaxime, Imipenem and Piperacillin+tazobactam antibiotics.

Detection of ESBL production is of paramount importance in hospital settings as these strains are probably more prevalent than currently recognized; these enzymes constitute a serious threat to currently available antibiotics. Institutional outbreaks are increasing because of selective pressure due to indiscriminate use of expanded- spectrum Cephalosporins and lapses in effective control measures [16].

Screening of ESBL in Gram negative bacteria poses considerable therapeutic challenges in critical care patients. The infections which are caused by ESBL producing Gram negative bacteria have been reported with an increasing frequency in the intensive care units and they are associated with a significant morbidity and mortality [17].

One of the areas of interest in infections in ICU is in the trend in the antibiotic susceptibility patterns (AST) of common pathogens in ICU. This is important as antibiotic guidelines are commonly made based on them and changes in the AST patterns need o be reflected in regularly updated antibiotic guidelines [18]. Strategies to keep a check on the emergence of such drug resistant microbes by hospital environmental surveillance and laboratory monitoring should form an important aspect of Hospital Infection Control policy guidelines [3].

Conclusion

In the present study, we found an alarming number of ESBL producing Gram negative isolates. The laboratories should have the capacity to detect ESBL producers, so that appropriate therapy can be chosen for patient management. The report must also include a note that ESBL producer may result in therapeutic failure with antimicrobials such as Penicillin, Aztreonam and Cephalosporin.

Considering the gravity of the implication of wrong therapy in critical care, looking for ESBL producers must be made mandatory in all reporting in Microbiology laboratories and clinicians also educated on the issue. Strict antibiotic policies and measures to limit indiscriminate use of Cephalosporins should be undertaken to minimize the emergence of such resistance.

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References

1. Idowu OJ, Onipede AO, Orimolade AE, Akinyoola LA, Babalola GO. Extended-spectrum beta-lactamase orthopedic wound infections in Nigeria. J Global Infect Dis 2011; 3:211-5.

2. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005 Oct;18(4):657-86.

3. Grover CN, Sahni AK, Bhattacharya CS. Therapeutic challenges of ESBL and AmpC beta-lactamase producers in a tertiary care center. Indian medical journal of armed forces 2013;69(1):4-10.

4. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005 Oct;18(4):657-86.

5. Nathisuwan S, Burgess DS, Lewis JS 2nd. Extended-spectrum beta-lactamases: epidemiology, detection, and treatment. Pharmacotherapy. 2001 Aug;21(8):920-8.

6. Tsering DC, Das S, Adhiakari L, Pal R, Singh TS. Extended Spectrum Beta-lactamase Detection in Gram-negative Bacilli of Nosocomial Origin. J Glob Infect Dis. 2009 Jul;1(2):87-92. doi: 10.4103/0974-777X.56247.

7. Rupp ME, Fey PD. Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. Drugs. 2003;63(4):353-65.

8. Babini GS, Livermore DM. Antimicrobial resistance amongst Klebsiella spp. collected from intensive care units in Southern and Western Europe in 1997-1998. J Antimicrob Chemother 2000; 45:183-9.[PUBMED]

9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 18th informational supplement 2012.

10. Das A, Ray P, Garg R, Kaur B. Extended spectrum beta-lactamase production in Gram negative bacterial isolates from cases of septicemia. Proceedings of the Silver Jubilee Conference of IAMM. New Delhi: All India institute Of medical Sciences; 2001.

11. Babypadmini S, Appalaraju B. Extended spectrum -lactamases in urinary isolates of Escherichia coli and

Klebsiella pneumoniae - prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004 Jul-Sep;22(3):172-4.

12. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res. 2004 Dec;120 (6): 553-6.

13. Mohanty S, Singhal R, Sood S, Dhawan B, Das BK, Kapil A. Comparative in vitro activity of beta-lactam/beta-lactamase inhibitor combinations against gram negative bacteria. Indian J Med Res. 2005 Nov; 122(5):425-8.

14. Taneja N, Rao P, Arora J, Dogra A. Occurrence of ESBL & Amp-C beta-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. Indian J Med Res. 2008 Jan;127(1):85-8.

15. Shashwati N, Kiran T, Dhanvijay AG. Study of Extended spectrum β- lactamase producing Enterobacteriaceae and antibiotic co-resistance in a tertiary care teaching hospital. J Nat Sci Biol Med. 2014; 5(1): 30-5.

16. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of extended spectrum beta-lactamase producing uropathogens and their antibiotic resistance profile in patients visiting a tertiary care hospital in central India: Implications on empiric therapy. Indian J Pathol Microbiol. 2014 Jul-Sep;57(3):407-12. doi: 10. 4103/0377-4929.138733.

17. Itokazu GS, Quinn JP, Bell-Dixon C, Kahan FM, Weinstein RA. Antimicrobial resistance rates among aerobic gram-negative bacilli recovered from patients in intensive care units: evaluation of a national postmarketing surveillance program. Clin Infect Dis. 1996 Oct;23(4):779-84.

18. Kaul S, Brahmadathan KN, Jagannati M. one year trends in the Gram negative bacterial antibiotic susceptibility patterns in a Medical ICU in South India. 2007; 25(3): 230-235.

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Letter to Editor

Microbiology Section

Prevalence of Vancomycin Resistant Enterococci Carriers from Rectal Swabs among High Risk Patients in a Tertiary Care Hospital

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Keywords: Chromogenic media, *Enterococcus faecium*, *Enterococcus faecalis*, Urinary tract infections

Dear Editor,

Enterococci with acquired vancomycin resistance is a major concern amongst hospital. These resistant species have the ability to survive for prolonged periods and they can get transmitted easily. The infections caused by these organisms range from complicated Urinary Tract Infections (UTI) to life threatening Central Nervous System (CNS) infections making the treatment challenging [1]. The rate of Vancomycin Resistant Enterococci (VRE) colonisation in Northern India is nearly 34.5%, with *van A* genotype being the most common [2]. Studies have reported that active screening through rectal swabs is a definite tool in preventing complications in high risk patients by early identification and treatment of VRE carriers [3]. The conventional method of screening with bile esculin azide agar supplemented with 6 µg/mL of Vancomycin/ml is reasonably sensitive but requires confirmation by molecular techniques [4]. The molecular methods in the detection are limited to the referral laboratories. Development of highly specific chromogenic substrates in the VRE selective media help in effective screening of colonisers. The sensitivity and specificity of the chromogenic media was 100% and 99%, respectively [4]. This was in accordance to present study as well. This study was undertaken to screen the high risk patients in critical care for VRE colonisation from stool samples and identify the genotype. Early identification of the carrier state in high risk patients is helpful in planning the management strategy of these patients.

This was an observational study done over the period of three months from January to March 2016 at PSGIMS&R, Coimbatore, Tamil Nadu, India. The Institutional Human Ethical clearance number obtained was 14/118. Informed consent was obtained before collecting the rectal swabs. The criteria for high risk patients were defined as hospitalisation >7 days, chronic haemodialysis, use of multiple antibiotics and associated chronic disease status. Rectal swabs were obtained from 100 patients (no repeat isolates and the mean age group was 60 years 75% were males) following the identification as high risk as per the criteria explained above. The rectal swabs were streaked on chromogenic ChromID VRE media (Biomerieux) and incubated at 37°C for 24 hours. The presence of violet colour colonies indicated *Enterococcus faecium* and blue colour colonies indicated *Enterococcus faecalis*. Isolates with intrinsic resistance to vancomycin as *Enterococcus casseliflavus* and most of the other gram positive organisms were selectively inhibited by this media. Conventional biochemical tests as bile esculin, mannitiol, argininine and PYR (Pyrrolidonyl Arylamidase) were done for confirmation at the species level. The antibiotic sensitivity was also performed by Kirby Bauer method as per the CLSI guidelines [5]. The antibiotic susceptibility pattern of the 100 isolates showed the maximum sensitivity to linezolid (98%) followed by vancomycin (97%), erythromycin (89%), teicoplanin (75%), ampicillin (69.2%), high level gentamycin (49.2%) and penicillin (25%). The Minimum Inhibitory Concentration (MIC) of the resistant isolates was confirmed by using commercial E-strips. Molecular characterisation of the resistant isolates for the specific genotype was done by performing conventional PCR. The DNA from the resistant isolates was extracted using the QIAGEN kit. The primers of base pair 732bp for detection of *van A* were used. The PCR products were analysed by running gel electrophoresis and visualised under UV light in automated GEL DOC viewer. Primers for *van A* used were:

F (5' GGGAAAACGACAATTGC-3')

R (5'GTACAATGCGGCCGTCGTTA-3') [6]

Out of the 100 patients screened, three were found to be positive for VRE carriage (3%). All the three were *Enterococcus faecium*. The positive sample was used as the control strain. Analysis of the predisposing risk factors among the three patients revealed the association of chronic renal impairment, immunocompromised status (retroviral infection) and presence of co-morbid conditions such as diabetes mellitus along with prolonged hospital stay.

The VRE carriers can pose a risk to the patients harboring these pathogens as they can invade the host and result in bacteremia complicating the management. In such patients, treatment of the VRE carriage aids in better prognosis. Isolation and cohorting of these patients prevents the transmission and risk of infection in other patients, especially in healthcare setups. The choice of VRE screening can aid in proper infection control measures.

Thus, chromogenic VRE media was found to be a simple and rapid method for identifying VRE carriage.

Declaration: The present study was presented as poster presentation in 39th National Conference of the Indian Association of Medical Microbiologists MICROCON in 2015.

REFERENCES

[1] Zhanel GG, Laing NM, Nichol KA, Palatnick LP, Noreddin A, Hisanaga T, et al. Antibiotic activity against Urinary Tract Infection (UTI) isolates of Vancomycin-Resistant Enterococci (VRE): Results from the 2002 North American Vancomycin Resistant Enterococci Susceptibility Study (NAVRESS). Journal of Antimicrobial Chemotherapy. 2003;52(3):382-88.

[2] Banerjee T, Anupurba S, Filgona J, Singh DK. Vancomycin-resistance enterococcal colonisation in hospitalised patients in relation to antibiotic usage in a tertiary care hospital of North India. Journal of Laboratory Physicians. 2015;7(2):108.

[3] Humphreys H. Controlling the spread of vancomycin-resistant enterococci. Is active screening worthwhile? Journal of Hospital Infection. 2014;88(4):191-98.

[4] Cuzon G, Naas T, Fortineau N, Nordmann P. Novel chromogenic medium for detection of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. Journal of Clinical Microbiology. 2008;46(7):2442-44.

[5] CLSI. Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial Susceptibility testing. CLSI document M100-524.Wayne, PA: Clinical and Laboratory standard Institute 2020.

[6] Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 1995;33:24-27.

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Phenotypic and Genotypic Characterization of *Enterococci* from Clinical Isolates in a Tertiary Care Hospital

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A B S T R A C T

Enterococci mainly being commensals in human faeces are now considered as an important cause of nosocomial infections. Of the infections, the commonly observed are urinary tract infections, abdominal infections followed by bacteremia, endocarditis and meningitis rarely. Speciation of *Enterococcus* aids in effective management of the infections caused by them. The initial treatment for enterococcal infections has become challenging due to development of resistance. The resistance has been observed mainly against aminoglycosides due to the presence of bi-functional gene. VRE strains are mostly isolated from patients with recurrent bacteremia, endovascular infections leading to increase deaths in the patients. To isolate and speciate enterococcal isolates from clinical samples, study the antibiotic susceptibility pattern and the genotype associated with the aminoglycoside and vancomycin resistance. *Enterococcus* isolates obtained from various sections were characterized by conventional phenotypic methods. The antibiotic susceptibility was studied by disc diffusion method. The resistant strains were further confirmed by MIC using automated methods following which the genotypic analysis was done. The incidence of *Enterococci* was 2.5% in which around 86.4% were obtained as pure isolates. Among the species *E. faecalis* was found to be the maximum. The urinary isolates exhibited sensitivity of around 92.3% to nitrofurantoin and among the non - urinary isolates the maximum sensitivity was for linezolid. The molecular study showed van A to be most common gene with vancomycin resistance (71.42%), and bifunctional gene among the aminoglycoside resistance (96%). *Enterococcus faecalis* was the commonest species isolated. The maximum number of isolates was obtained from urinary samples. The increase incidence of resistance gene to high level gentamicin could result in failure to the synergy treatment of gentamicin in combination with penicillin group of antibiotics. The emergence of vancomycin resistant strains among the clinical isolates makes treatment options more challenging.

Keywords

Enterococci, Speciation, Antibiotic resistance, High level gentamicin, Vancomycin, Bifunctional gene.

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Introduction

Enterococci are gram positive organisms having the property to grow at 6.5% NaCl concentration, pH of 9.6 and hydrolyze bile esculin. Initially they were grouped under group D Streptococcus but later with the help of DNA studies they are characterized under the genus *Enterococcus* in 1960. They were considered as harmless pathogens formerly, but in the recent years have been emerging as the most important causative agents in nosocomial infections (Murray *et al.*, 1990).

Enterococci being the commonest micro flora of both humans and animals share their source

of origin. They have been isolated from variety foods including cheese, fish, sausages, beef and pork. Studies have been reported that *Enterococci* have been isolated from various clinical and environmental samples all over the world. Among the isolates obtained clinically it has been observed that *Enterococcus faecalis* has been the predominant species clinically (Lancefield *et al.,* 1933; Cetinkaya *et al.,* 2000).

Enterococcal infections have been referred to as tough, tenacious and troublesome infections (Marothi *et al.,* 2005).In the past years there has been an increase in the prevalence of enterococcal infections in hospitals and of particular concern is the emergence of antimicrobial resistant strains. Being second commonest organism behind the abdominal and pelvic infections, third in causing blood stream infections, cases have also been reported in causing CNS and neonatal infections.

Reports have been confirmed with their association with clinical conditions including respiratory tract infections, osteomyelitis and cellulitis (Fischer *et al.,* 2009). *Enterococci* were initially regarded as the disease causing agent in the early nineties. But now various reports have been established reporting them as the second commonest pathogen (Kuhn *et al.,* 2003).

Species identification becomes essential for investigating outbreaks in case of nosocomial infections and also susceptibility pattern varies accordingly which is also helpful in clinical management of infections due these agents (Gordon *et al.,* 1992).

Infections caused by enterococcal pathogens were initially being treated with the combined treatment of cell wall active agents as penicillins and aminoglycoside as the synergistic effect was effective. But now due to the development of antibiotic resistance as high level aminoglycoside resistance, beta lactamase production and also resistance to vancomycin have led to serious concern in management of these nosocomial pathogens (Mendiratta *et al.,* 2008). Vancomycin resistant *Enterococcus* was initially reported in 1980s, after which there was a increase in the incidence of its spread. The main phenotypes associated with its spread are Van A, Van B and Van C. Van A and Van B are mainly associated with *E. faecalis* and *E. faecium*. Van C is noted in *E. gallinarum* and *E. casseliflavus*. Among the phenotypes Van A is predominantly associated in the resistant strains.

VRE strains are mostly isolated from patients with recurrent bacteremia, endovascular infections leading to increase deaths in the patients. Vancomycin resistance and penicillin resistance are often seen co-existing which makes the treatment harder (Murray *et al.,* 1990).

Resistance development in *Enterococcus* against glycopeptide antibiotics gets transferred from them to other bacterial pathogens which include *Staphylococcus aureus, Streptococci, Listeria monocytogenes* (Gold *et al.,* 1996).

Thus due to the recent emergence in association with significant clinical infections and development of resistance, *Enterococci* have gained importance. Studies are thus required especially in tertiary care hospitals for appropriate isolation, identification and speciating them.

Antibiograms should also be followed up in accordance to the species. Emergence of VRE needs to be checked and limit its spread in hospital environment. CDC also stresses the control of VRE in hospitals by educating the staff in detecting VRE early, reporting them and action plan taken promptly.

Our study aims to isolate, speciate the *Enterococcus* in a simplified manner and also to study the drug sensitivity and resistance patterns among the isolates obtained.

Materials and Methods

The Institutional Human Ethical clearance was obtained before the commencement of the study.

Type of study

Experimental study

Confidentiality

Confidentiality of the reports were maintained

Sample size estimation

A total of 250 isolates of *Enterococci* obtained from outpatient and inpatient samples from blood section, miscellaneous and urine section were processed for the species identification, antimicrobial sensitivity followed by molecular study.

Specimen processing: Specimens from various sections including urine, pus and blood for cultures were collected and processed in the laboratory.

Pus/wound swabs

The swabs were streaked in blood agar and MacConkey agar and were incubated at 37^0C for 24 hours.

Urine

A loopful of the uncentrifuged specimen was examined as a wet mount for the presence of pus cells and bacteria. One loopful of specimen was inoculated onto blood agar and Mac Conkey agar. Plates were incubated at 37^0 C for 24 hours.

Growth in sheep blood agar

Following overnight incubation of the samples, colonies of *Enterococcus* were seen as grey colonies of about 0.5-1mm in diameter with alpha, beta or gamma hemolysis.

Growth in Mac Conkey agar

Colonies were about 0.5- 1mm in size with a smooth surface and convex margins, tiny magenta colored following 18-24 hour growth.

Catalase test

A drop of the catalase reagent (30% hydrogen peroxide) was added to the slide.

The growth was further applied to it with an applicator stick. Appearance of bubbles was taken as positive test.

Enterococcal colonies showed a negative test due to the absence of bubbles.

Gram stain

Smears made from the colony showed Gram positive cocci arranged singly, in pairs or in short chains.

Storage

If the isolate was catalase negative, gram staining showing the presence of gram positive cocci in pairs or short chains and growth in MacConkey agar as tiny lactose fermenting colonies, they were sub cultured and pure growth was stocked in Robertson's cooked meat medium in tubes.

The tubes were sealed and stored at 4-8^0C for 3-6 months. When the test was to be done they were further sub cultured.

Identification tests

Growth in media with pH – 9.6

Isolated colonies of *Enterococcus* was streaked in a media with a pH 9.6 and incubated at 37^0C overnight. The presence of growth was taken as positive.

Tolerance with 6.5% NaCl

The colonies were inoculated in a broth with 6.5% NaCl, and incubated overnight. The next day the tubes were checked for any increase in turbidity. This was further confirmed by sub culturing onto the agar plates. Growth confirmed the presence of organism. Whereas absence of turbidity indicated organism was unable to grow in this concentration.

Heat tolerance test

Enterococcus was inoculated in Todd-Hewitt broth. It was later incubated in water bath set at 45^oC overnight and 60^0 C for half an hour, and then sub cultured. *Enterococci* survived both the temperature showing positive reaction.

Bile esculin test

Colonies from 24 hour culture were inoculated in media containing 40% bile. Blackening of the medium confirmed positive reaction for *Enterococci* and non-*Enterococci*.

PYR test

The colonies were inoculated in PYR broth and incubated for a period of 4 hours. Later 3 drops of PYR reagent was added to it and change of colour to red was confirmed positive indicating *Enterococci* whereas absence of colour change indicates negative test.

Motility test

Stab culture of the colony was done in a semi-solid media and motility was indicated by the spread of the organism into the media. The strains which did not diffuse into the media were considered non-motile. Tubes were incubated for up to 7 days for confirming motility.

Arginine test

A well isolated colony of *Enterococcus* from the culture was inoculated in Moeller's decarboxylase media with arginine as the amino acid and overlaid with mineral oil. After 24 hours of incubation the colour of the tube was changed to purple indicating a positive reaction. Yellow colour indicated only acid production only and no deamination.

Potassium tellurite reduction test

Colonies were streaked in media containing 0.004%potassium tellurite. *E. faecalis* formed black colonies with the reduction of tellurite to tellurium.

This test was used to differentiate *E. faecalis* from *E. faecium.*

Pyruvate test

When inoculated in a media containing pyruvate with bromothymol blue as indicator *E. faecalis* showed a colour change from green to yellow color after overnight incubation. Absence of colour change ruled out *E. faecalis.*

Pigment production

Ability to produce pigment was observed by a few species of *Enterococci*. This was tested by sub culturing colonies in nutrient agar.

Any color produced was visible and also further confirmed by touching the colony with a sterile swab.

Sugar fermentation tests

Species identification was further done by sugar utilization by inoculating the organism in peptone with 1% sugars with Andrade indicator. The sugars used were mannitol, arabinose, raffinose, sorbose, sorbitol, lactose and sucrose. Any change in colour from colourless to pink indicated the positive sugar fermentation test. Absence of colour change was taken as negative.

Antimicrobial susceptibility test: (CLSI 2015)

The 250 test isolates of *Enterococci* were checked for the antimicrobial susceptibility pattern using Kirby-Bauer disc diffusion method. Three or four pure colonies were inoculated in peptone water and incubated for about 3 hours. The turbidity of the growth was matched to 1 of Mc Farlands. A lawn culture of the broth with a sterile swab was done in Muller Hinton agar and the discs were placed at a distance of 24 mm.

About 6 discs were kept on each plate. The discs used were Penicillin (10U), Ampicillin (10µgm), Amoxicillin Clavulanic acid (20/10), Erythromycin (15µgm), Vancomycin (30µgm), High level gentamicin (120µgm), Linezolid (30µgm), Tetracycline (30µgm). For isolates from urine section Norfloxacin (10µgm) and Nitrofurantoin (300µgm) was kept. All discs were bought from HIMEDIA.

Zones of inhibition were measured and recorded and the organism was interpreted as sensitive or resistant as per the recommendations from CLSI guidelines. The quality Control used was *Enterococcus faecalis* ATCC 29212.

Screening for high level gentamicin resistance (CLSI 2015)

Screening media for high level gentamicin resistance was prepared. Screening for high level gentamicin resistance was done by agar dilution method. BHI agar with gentamicin at a concentration of 500µgm/ml was added and the plates were prepared. Inoculum of 10µL of 0.5 Mc Farland was spot inoculated. Presence of even 1 colony was interpreted as a resistant strain. These strains were collected for molecular studies.

Detection of beta lactamase (Marothi *et al.*, 2005)

The detection of beta lactamase test was done based on the chromogenic cephalosporin method. Nitrocefin disc was obtained commercially from BD. The disc was moistened with sterile saline and pure isolated colony was applied on it. The reaction was read within 5 min. Isolates producing beta lactamase produced a red color.

Molecular study of isolates

A representative sample of 50 high level gentamicin resistant isolates was selected for genomic analysis. The DNA was extracted using the QIAGEN kit. Followed by extraction, the isolates were analysed for the presence of Bifunctional resistant geneaac (6') le aph (2") la. The vancomycin resistant isolates were checked for the presence of van A gene simultaneously.

Detection of aac (6') le aph (2") la gene

The primers for HLGR with a base pair of 348 bp were amplified. The sequence was obtained from the studies done previously[11]. PCR Master mix: The master mix was prepared as follows: 2.5µl of PCR buffer,2.5µl of MgCl2, 2.5µl of DNTPs,

10.2µl of Mili Q H2O, 1µl of forward and reverse primers and 0.3µl of Taq Polymerase was added to eppend off. From the above mixture 18µl was taken and 5µl of the extracted DNA was added.

Primers for aac (6')Ie- aph (2")la with base pair of 348 bp (Vakulenko *et al.*, 2003):

F (5'CAGAGCCTTGGGAAGATGAAG3')
R (5'CCTCGTGTAATTCATGTTCTGGC3')

PCR program

The program consisted of initial denaturation step at 95^0C for 10 minutes followed by 30 cycles of denaturation at 94^0C for 300 seconds, annealing at 56^0C for 1 minute and extension at 72^0C for a period of 1 minute. The program had a holding temperature of 72^0C for 10 minutes.

Analysis

PCR products were analyzed by running gel electrophoresis with 1.5% agarose gel in Tris Boric acid buffer. The samples along with the controls obtained by running ladder were analysed.

The gel was stained with ethidium bromide and the bands were obtained were visualized under UV light and also in automated GEL DOC viewer.

Detection of vanA gene

The primers with a base pair of 732bp for detection of vanA were obtained from the studies done previously.

Primers for van A with base pair of 732bp (Dutka-Malen *et al.*, 1995):

F (5' GGGAAAACGACAATTGC-3')
R (5'GTACAATGCGGCCGTCGTTA-3')

PCR program

This program had a denaturation step at 95^0C for 5 minutes followed by 30 cycles of DNA denaturation at 95^0C for 1 minute, annealing at 54^0C for 45 seconds and primer extension at 72^0C for 45 seconds. This was followed by a holding temperature of 72^0C for 10 minutes.

Analysis

PCR products were analyzed by running gel electrophoresis with 1.5% agarose gel in Tris Boric acid buffer. The samples along with the controls obtained by running ladder were analysed. The gel was stained with ethidium bromide and the bands were obtained were visualized under UV light and in automated GEL DOC viewer.

Results and Discussion

All the 250 isolates of *Enterococcus* from various clinical samples including urine, pus, wound swab, blood and CSF were processed.

The maximum number of isolates was obtained from urine section (184) followed by wound swabs (24), blood section (14), 4 from peritoneal samples, 1 from CSF (Figure 1). The majority of the isolates were obtained as pure growth (216) and 34 was obtained as mixed from the samples processed. Figure 2 depicts the speciation of enterococcal isolates in which *E. faecalis* was the predominant species (157), succeeded by *E. faecium* (79), *E. raffinosus* (7), and *E. durans* (5) and *E. casseliflavus* (2).

Illustration 1 shows the genus specific biochemical tests used in the identification.

The antibiotic susceptibility pattern of the enterococcal isolates by disc diffusion method revealed that out of the 250 isolates, about 69.2% were ampicillin sensitive, 49.2% were

high level gentamicin sensitive, 89.3% were sensitive to erythromycin. The maximum number of isolates was found to be sensitive to vancomycin (97.2%) in our study (Table 1). Among the urinary isolates maximum sensitivity was noticed to nitrofurantoin (92.3%) (Table 2) and norfloxacin (83.1%).

The maximum sensitivity was observed for linezolid followed by vancomycin, teicoplanin, ampicillin and high level gentamicin. Among the non-urinary isolates erythromycin sensitivity was observed to be the highest.

The chromogenic nitrocefin disc was used to screen the isolates for the presence of beta lactamase. In our study none of the isolates were found to be positive for beta lactamase.

Figure 3 shows the presence of Bifunctional gene aac (6')1eaph(2")1a among the high level gentamicin resistant strains. Out of the 50 randomly selected resistant strains for genotype study, 48 had the gene.

Illustration 2 shows the presence of bands of size 348 bp during the gel electrophoresis.

In our study 7 out of the 250 isolates were found to be resistant to vancomycin. The genotypic analysis was done among these resistant strains using the van A gene. The results showed that 5 of the 7 strains were found to be positive for van A gene (Figure 4).

Illustration 3 shows the presence of van A gene of base pair 732, confirming its presence among the resistant strains.

Enterococcus has been noted to cause a majority of urinary tract infections. This being the most common followed by bacteremia, sepsis and even case reports of meningitis has been reported. In our study out of 250 samples studied the majority of the isolates were obtained from urinary tract infections (73.6%) followed by wound swabs (9.6%) and blood (5.6%). This correlates well with other studies in which *Enterococci* was obtained from urine isolates maximally (Karmarkar *et al.,* 2004, Mathur *et al.,* 2003). According to the studies *Enterococci* are considered as the commonest urinary pathogen justifying the increase in the rate of isolation from urinary samples (Murray *et al.,* 1990).

Among the various species, in our study *E. faecalis* was the most common isolate accounting for 62.8% followed by *E. faecium, E. durans, E. raffinosus* and *E. casseleflavus.* Similar findings were reported from other studies done in Bangalore in which *E.faecalis* was 59%, *E. faecium* was 38 % (Golia *et al.,* 2014).

Enterococci show intrinsic resistance to cotrimoxazole, clindamycin, cephalosporins and normal aminoglycosides. Thus these antibiotics are not tested (Mendiratta *et al.,* 2008).

The antibiotic susceptibility pattern of the isolates in our study showed the following:

The sensitivity to linezolid was maximum (98.8%) followed by vancomycin (97.2%), erythromycin (89.3%), teicoplanin (75.2%), ampicillin (69.2%), high level gentamicin (49.2%) and penicillin (25%).

Among the urinary isolates maximum sensitivity was noted in Nitrofurantoin (92.3%) followed by Norfloxacin (83.15%). Studies done in North India showed a similar susceptibility pattern with increased resistance to penicillin and aminoglycosides. Also the sensitivity percentage to linezolid and vancomycin was higher compared to other antibiotics (Mulla *et al.,* 2012).

Fig.1 Distribution of *Enterococcus* isolates among various samples (N=250)

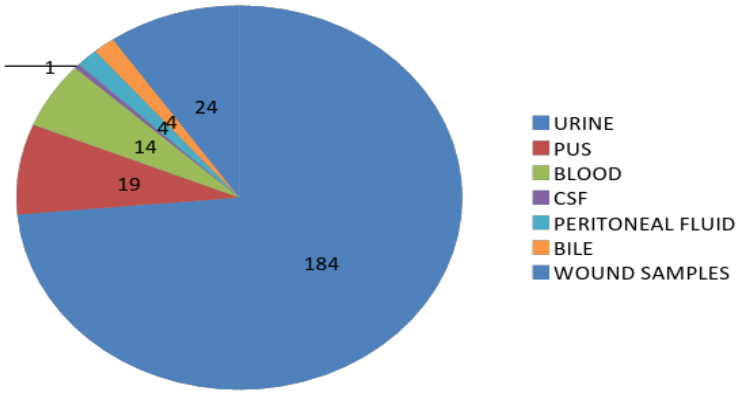
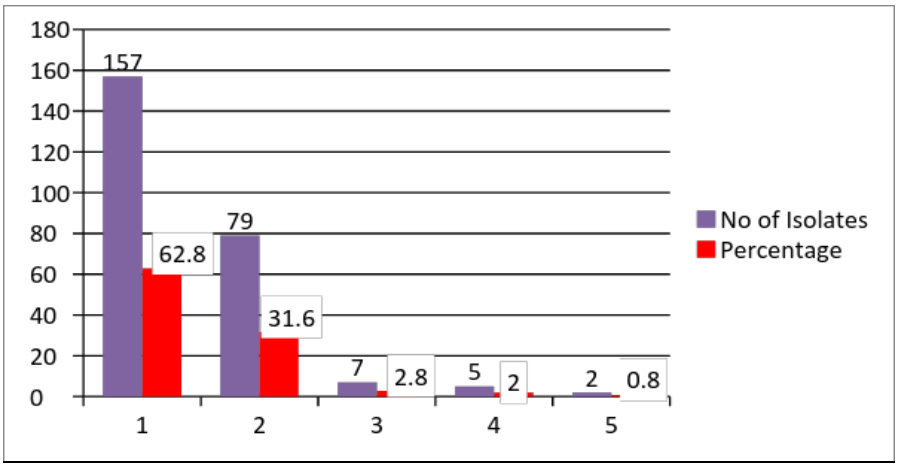


Fig.2 Speciation of *Enterococcus* from clinical isolates N=250



E.faecalis	1
E.faecium	2
E.raffinosus	3
E.durans	4
E.casseliflavus	5

Fig.3 Detection of the presence of aac (6') le aph (2") la gene N = 50

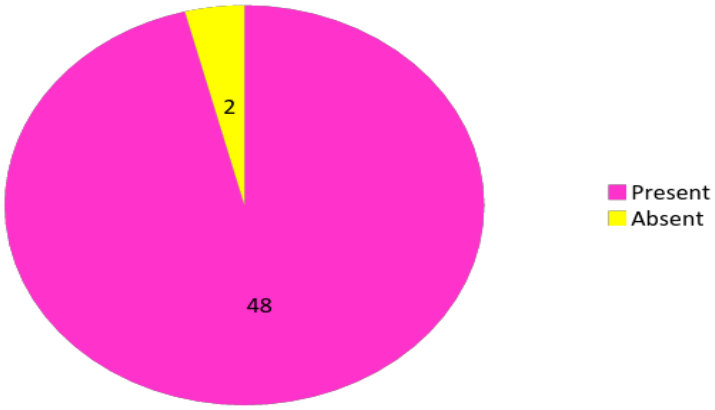


Fig.4 Detection of van A gene among the VRE isolates (N=7)

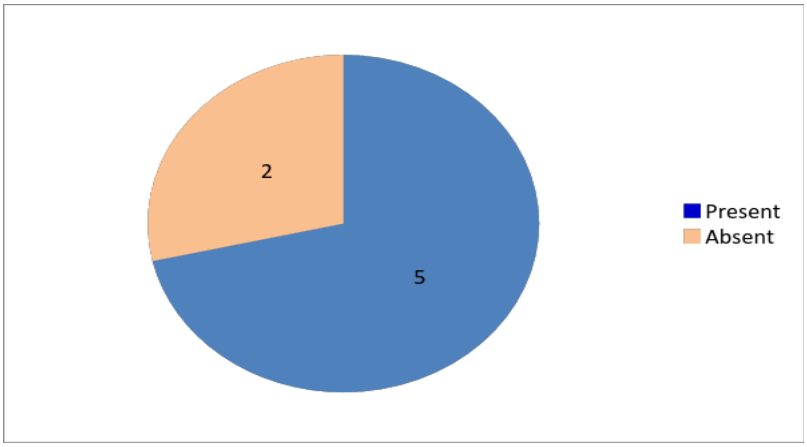


Table.1 Antibiotic susceptibility pattern of Enterococcal isolates

N = 250

RESISTANCE	SENSITIVE	ANTIBIOTICS
77	173	AMPICILLIN
30.8	69.2	%
127	123	HIGH LEVEL GENTAMICIN
50.8	49.2	%
7	243	VANCOMYCIN
2.8	97.2	%
3	247	LINEZOLID
1.2	98.8	%
62	188	TEICOPLANIN
24.8	75.2	%

Table.2 For urinary isolates

N = 184

RESISTANCE	SENSITIVE	ANTIBIOTICS
31	153	NORFLOX
16.85	83.15	%
14	170	NITROFURANTOIN
7.61	92.39	%
37	147	TETRACYCLINE
20.11	79.89	%

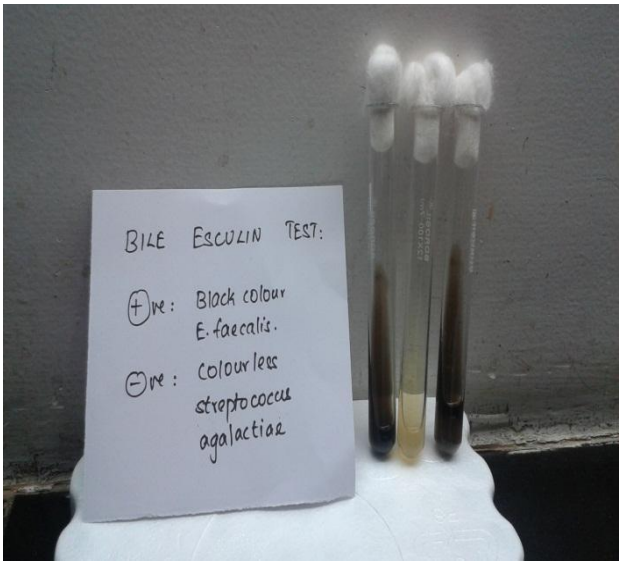
Table.3 For non urinary isolates

N = 66

RESISTANCE	SENSITIVE	ANTIBIOTICS
49	17	PENICILLIN
74.25	25.75	%
7	59	ERYTHROMYCIN
10.7	89.3	%

Illustration.1 Genus specific test

A) Bile-Esculin Test



B) PYR Test



C) 6.5% NaCl test



Illustration.2 PCR showing aac(6')-aph(2") BAND OF SIZE 348 bp

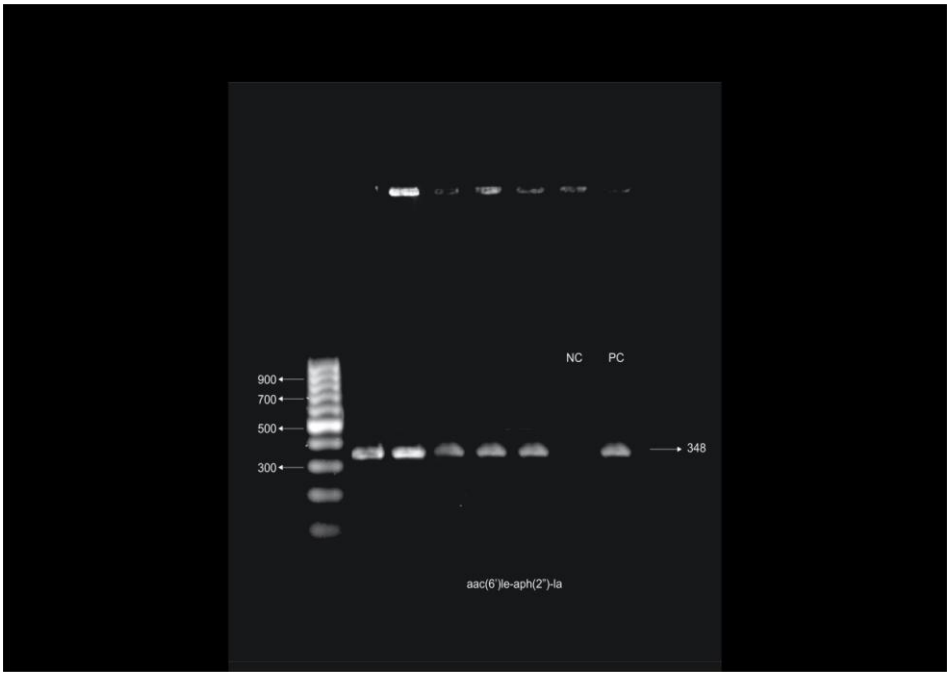
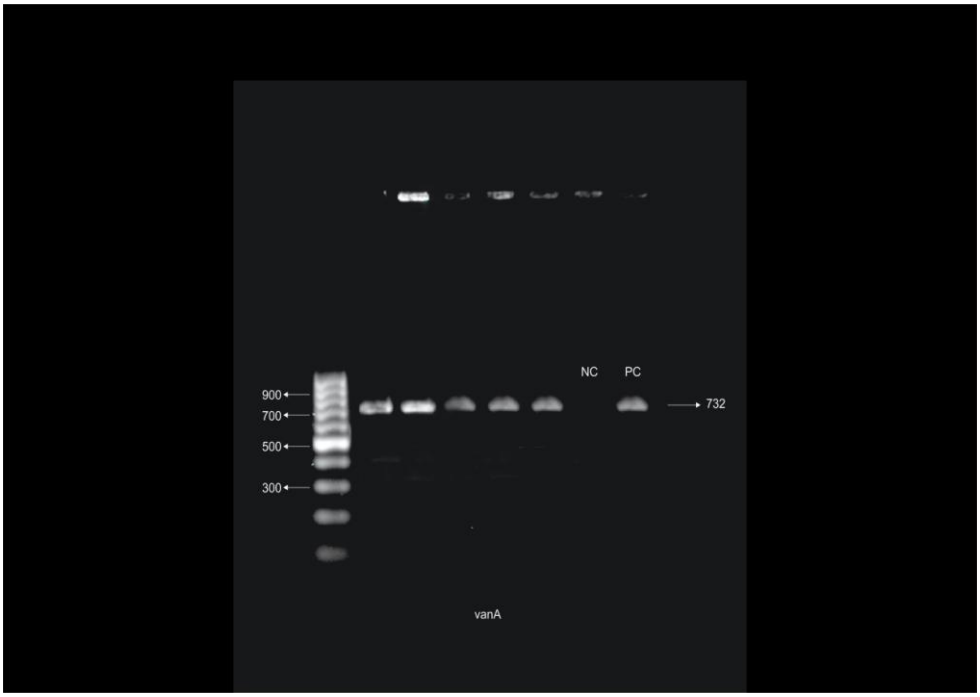


Illustration.3 PCR showing van A gene of band size 732bp



Another study showed the percentage of high level gentamicin resistance to be 68% (Randhawa *et al.*, 2004). Reports from South India showed the resistance percentage of high level aminoglycoside to be more than 50% (Sreeja *et al.*, 2012).

The detection of beta lactamase production by the chromogenic nitrocefin disc method showed that none of the isolates had beta lactamase enzyme. Studies done in Delhi and in Maharashtra also showed similar results (Jain *et al.,* 2011; Mendiratta *et al.,* 2008). The resistance to beta lactams though common, the mechanism could be due to alteration in penicillin binding protein PBP 5 which is more common than beta lactamase production (Murray *et al.,* 1992).

The molecular study done to detect the presence of the resistant gene confirmed the presence of the bifunctional gene aac (6') le-aph (2") la of base pair 348 to be present in 48 (96%) out of the 50 randomly selected resistant strains.

Studies done in Chennai have showed the presence of this gene in 38.2% of the isolates (Padmasini *et al.,* 2014) whereas there are other studies in which all the resistant isolates possessed this bifunctional gene (Hasani *et al.,* 2012). The resistance to high level gentamicin indicates resistance to all the aminoglycosides except streptomycin (CLSI 2015). The combination effect of aminoglycoside with beta lactam antibiotic is not helpful in the treatment of patients possessing such resistant strains.

Out of the 250 isolates the vancomycin resistant isolates obtained in our study was 2.8%. Studies have shown the prevalence of vancomycin resistance to be less in India ranging around 1.7-20% (Sreeja *et al.,* 2012).

Molecular studies revealed the presence of van A gene in 5 (71.42 %) isolates. Studies have also been reported showing van A to be the most common genotype isolated among the vancomycin resistant strains (Praharaj *et al.,* 2013)

Out of the seven isolates, five were *E. faecium* and two were *E. casseliflavus*. The remaining two isolates were *E. casseliflavus* which would have exhibited intrinsic resistance to vancomycin with van C gene (Praharaj *et al.,* 2013).

The incidence of *Enterococci* from the clinical isolates in our study was 2.5%.

The incidence of the pure isolates was 86.4% in comparison to the mixed isolates which was found to be 13.6%.

Among the isolates of *Enterococci*, the predominant species was *E. faecalis* (62.8%) followed by *E. faecium* (31.6%), *E. raffinosus* (2.8%), *E. durans* (2%) and *E. casseliflavus* (0.8%).

The antibiotic sensitivity pattern of the urinary isolates showed 92.3% sensitive to nitrofurantoin and 83.15% sensitive to norfloxacin. Among the non-urinary isolates maximum sensitivity was observed in linezolid which was 98.8% followed by vancomycin (97.2%), erythromycin (89.3%), teicoplanin (75.2%), ampicillin (69.2), high level gentamicin sensitivity (HLG) (49.2%) and pencillin (25%) (Table 3).

The vancomycin resistance was found to be 2.8% in *E. faecium*.

All the isolates were found to be beta lactamase negative by chromogenic (nitrocefin) method proving the alteration in PBP5 to be the more common mechanism involved in penicillin resistance.

The molecular characterization of HLGR(high level gentamicin resistant) strains, 50 isolates selected randomly out of 127, showed the presence of bifunctional gene aac(6')le-aph(2")la to be around 96%. (Primers of base pair 348 - F (5'CAGAGCCTTGGGAAGATGAAG3') R (5'CCTCGTGTAATTCATGTTCTGGC3')

The presence of van A gene was found to be 71.42% in our study.

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References

Cetinkaya *et al.,* (2000) Vancomycin-resistant *Enterococci*. *Clinical microbiology reviews* 13, no: 686-707.

CLSI. Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial susceptibility testing. CLSI document M100-524.Wayne, PA: Clinical and Laboratory standard Institute 2014.

Dutka-Malen *et al.,* (1995) "Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant *Enterococci* by PCR." *Journal of clinical microbiology* 33, no. 1: 24-27.

Fisher *et al.,* (2009) "The ecology, epidemiology and virulence of *Enterococcus*." *Microbiology* 155, no. 6: 1749-1757.

Gold *et al.,* (1996). "Antimicrobial-drug resistance. *"New England Journal of Medicine* 335, no. 19:1445-1453.

Golia *et al.,* (2014) "Isolation and speciation of *Enterococci* from various clinical samples and their antimicrobial susceptibility pattern with special reference to high level aminoglycoside resistance. *"International Journal of Medical Research and Health Sciences* 3, no. 3: 526-529.

Gordon *et al.,* (1992) "Antimicrobial susceptibility patterns of common and unusual species of *Enterococci* causing infections in the United States. Enterococcal Study Group." *Journal of clinical microbiology* 30, no. 9: 2373-2378.

Hasani *et al.,* (2012). "Molecular screening of virulence genes in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical specimens in Northwest Iran." *Indian journal of medical microbiology* 30, no. 2: 175.

Jain *et al.,* (2011) "Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. *"International Journal of Applied and Basic Medical Research* 1, no. 2: 80.

Karmarkar *et al.,* (2004) "Enterococcal infections with special reference to phenotypic characterization and drug resistance." *Indian Journal of Medical Research* 119: 22-25.

Kühn *et al.,* (2003) "Comparison of enterococcal populations in animals, humans, and the environment-a European study. "*International journal of food microbiology* 88, no. 2: 133-145.

Lancefield *et al.,* (1930). "A serological differentiation of human and other groups of hemolytic streptococci." *The Journal of experimental medicine* 57, no. 4: 571-595.

Marothi *et al.,* (2005) "Enterococcal resistance-an overview." *Indian journal of medical microbiology* 23, no. 4: 214.

Mathur *et al.,* (2003) "Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of northern India." *Indian Journal of Medical Research* 118: 25-28.

Mendiratta *et al.,* (2008). "Status of high level aminoglycoside resistant *Enterococcus faecium* and *Enterococcus faecalis* in a

rural hospital of central India." *Indian Journal of Medical Microbiology* 26, no. 4 : 369.

Mulla *et al.,* (2012)"Prevalence of *Enterococci* with higher resistance level in a tertiary care hospital: a matter of concern." *therapy* 1, no. 2: 3.

Murray *et al.,* (1992). "Beta-lactamase-producing *Enterococci*." *Antimicrobial agents and chemotherapy* 36, no. 11: 2355.

Murray *et al.,* (1990)"The life and times of the *Enterococcus*." *Clinical microbiology reviews* 3, no.1: 46-65.

Padmasini *et al.,* (2014) "High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of *Enterococcus* species in Chennai, India." *The Scientific World Journal* 2014.

Praharaj *et al.,* (2013) "Phenotypic and genotypic characterization of vancomycin resistant *Enterococcus* isolates from clinical specimens." *The Indian journal of medical research* 138, no. 4: 549.

Randhawa *et al.,* (2004) "Aminoglycoside resistance in *Enterococci* isolated from paediatric septicaemia in a tertiary care hospital in north India." *Indian Journal of Medical Research* 119: 77-79.

Sreeja *et al.,* (2012). "The prevalence and the characterization of the *Enterococcus* species from various clinical samples in a tertiary care hospital." *Journal of clinical and diagnostic research: JCDR* 6, no. 9: 1486.

Vakulenko *et al.,* (2003) "Multiplex PCR for detection of aminoglycoside resistance genes in *Enterococci*." *Antimicrobial agents and chemotherapy* 47, no. 4: 1423-1426.

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Comparative Evaluation of Performance of Qualitative and Semiquantitative Slide Widal Test versus Quantitative Tube Widal Test in Diagnosing Enteric Fever

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A B S T R A C T

Enteric fever still remains as an important cause for morbidity and mortality throughout the world and the causative organism for Typhoid and paratyphoid fever are *Salmonella typhi* and *Salmonella paratyphi A, B* and *C* respectively. Objective of the study is to compare and evaluate the performance of Qualitative and semiquantitative Slide Widal test and Quantitative Tube Widal test for diagnosis of enteric fever. A cross sectional study was undertaken and about 400paired serum samples were obtained from the patients and were subjected to both slide and tube widal tests. The results were noted for further statistical analysis. Prevalence of Enteric fever by tube agglutination and slide agglutination tests were found to be 23% (92 out of 400 cases) and 13% (52 out of 400 cases) respectively. Though blood culture remains gold standard, its sensitivity is high only during the first week of fever. So, Widal test is the preferred test used by most clinicians especially during second week of fever. The sensitivity and specificity of tube widal test is much remarkable and so it can definitely be used as a diagnostic test. Slide Widal test can be used as supplementary test to Tube Widal test so as to fetch the results earlier and to facilitate early and appropriate management.

Introduction

Enteric fever has been an important cause of morbidity and mortality throughout the world for centuries. The causative organism for Enteric fever viz., Typhoid and paratyphoid fever are *Salmonella typhi* and *Salmonella paratyphi A, B* and *C* respectively (John *et al.*, 2016). Though the overall incidence and prevalence has decreased significantly, infection still remains endemic in most of the developing countries. Global prevalence of Typhoid and paratyphoid fever was 10-11.7% and 2.2-2.5% in 2016, respectively. Prevalence of Typhoid fever and paratyphoid fever in India was 31.5-36% and 8-9% in 2016, respectively (Dandona *et al.*, 2017). Poor sanitation largely contributes to the

persistence of infection. Improvement in hygiene and timely diagnosis coupled with appropriate treatment ensuring complete cure of cases and carriers remains the keystone in controlling the infection. Culturing the causative bacteria from various body fluids like blood, bone marrow, stool, bile and urine and detection of anti-Salmonella antibodies through serology remains the most common diagnostic tests. Isolation of Salmonella species using Wilson and Blair medium (selective media), which remains the gold standard investigation till date, is greatly influenced by the time duration of illness and appropriateness of the sample chosen for culture (Olopoenia and King, 2000; Mengist and Tilahun, 2017).

Yield of a positive blood culture is very high during the first week of the illness, followed by a marked decrease in its sensitivity. The habit of patients getting started on few doses of antibiotics before the culture has made the investigation lesser sensitive. Hence serological test-Tube Widal test is the most preferred choice by the physicians. It is found more useful in case of culture negative but clinically suspected cases. Widal was first introduced by Georges Fernand Widal in 1896. This test has evolved since then. With the introduction of commercial kits, it has becoming one of the easily available and performable tests.

The quicker result issued in one day, compared to culture (may need 7 days) makes it more useful investigation to decide on the treatment. Recent advancements like Slide Widal test have made the procedure much simpler and very rapid which offers results in one minute. The literature available on its diagnostic ability is however scanty (Olopoenia and King, 2000; Keddy *et al.,* 2011). Hence this study is undertaken to see if the performance of Slide widal is as efficacious as tube widal test.

Study rationale

Widal test works on the principle of agglutination and it is the only serological test available to diagnose enteric fever. If the performance of semi quantitative slide widal test in estimating the titres is found satisfactory, then turn around time for diagnosis of enteric fever and start of treatment can be reduced from 24 hours to 10 minutes.

Since slide widal test does not require additional glassware/instruments or involve complex procedure as in tube widal, moderately skilled technician can perform the test in small resource limited settings. This will benefit remote areas of the country where sophisticated lab facilities are absent. Also cost incurred on inventories and performance of widal can be largely reduced.

The main aim of this study, Comparison of performance of qualitative and semiquantitative slide widal test and quantitative tube widal test in diagnosis of enteric fever.

Also the main objectives of this study include, to compare the performance of qualitative slide Widal test against the semi-quantitative tube Widal test. And to compare the performance of semiquantitative slide Widal test against the semi-quantitative tube Widal test.

Inclusion criteria

In patients and out patients with probable Enteric fever attending various clinical departments of our hospital

Patient presenting with fever for 1 to 2 weeks.

Exclusion criteria

Chronic enteric fever.

Recurrent enteric fever.

Recently vaccinated with TAB vaccine.

Patients with Bleeding disorders.

Patient who did not give informed written consent for the study.

Cross infection with Proteus, Rickettesial infection etc…

Materials and Methods

A Cross sectional study was conducted after obtaining Institutional Human Ethics Committee Clearance (IHEC), and patient's informed written consent was obtained prior to the commencement of the study. A cross sectional study was undertaken from April 2018 to October 2018 and was conducted in the Department of Microbiology, Karpagam Faculty Of Medical Sciences and Research. About 400 serum samples were obtained from the patients with probable Enteric fever, who constituted the study population. Serum samples received in the Serology section of Diagnostic Microbiology Division, Central Service Laboratory of our Hospital were subjected to both qualitative and semi quantitative analysis using slide and tube widal test respectively as per Manufacturer's Instructions (Span Diagnostic Kit).

Serum samples were evaluated for the presence of Genus specific antibodies to poly 'O' Antigen *(Salmonella typhi)*, and also for the presence of species specific antibodies against 'H' Antigen *(Salmonella typhi)*, 'AH' Antigen (*Salmonella paratyphi A)*, 'BH' Antigen *(Salmonella paratyphi B)* respectively. Standard tube widal agglutination test was performed by making serial dilutions of the serum by using isotonic normal saline and 0.5ml of each antigen suspension (O, H, AH, BH) were added to the corresponding tubes (6 tubes), Comprising different dilutions viz., 1:20, 1:40, 1:80, 1:160, 1:320, 1:640. Antigen control to detect Auto-agglutination is also done simultaneously (7^{th} tube). Dreyers tube (round bottom) was used for demonstrating antibodies against 'O' Antigen and Felix tube (conical bottom) are used to demonstrate antibodies to 'H', 'AH', 'BH' antigens respectively.

Then these tubes were incubated at 37^{o}C for 24 hours and the reading of the tubes was done on the next day. Tubes showing 50% agglutination viz., for 'O' antigen – purple granular deposits along with clearing of the tube $\geq 2/3^{rd}$ of the suspension was considered positive. And for 'H', 'AH', 'BH' antigen – cotton wooly clumps along with $\geq 2/3^{rd}$ clearing of the suspension in the tube is considered as positive.

In case of semi-quantitative slide widal and tube widal agglutination test, titres of $\geq$ 1:80 is considered as significant titre for Antibodies to 'O' antigen, $\geq$ 1:160 is considered as significant titre for antibodies to 'H', 'AH', 'BH' antigens respectively. Qualitative widal agglutination tests are done using one drop (50µl) of patients serum and one drop (50µl) of desired antigen (O, H, AH, BH). Presence or absence of visible agglutination –granular clumps, at the end of a minute gentle rotatory shaking is interpreted as positive and negative respectively. Quality control was done using positive and negative quality control given along with the kit as per manufacturer's instruction. Four fold rise or decrease in titres between acute phase sera and convalescent phase sera is considered as significant titre for this serological test. And finally the results were tabulated for further statistical analysis using SPSS version 23.0 statistical software.

Results and Discussion

Out of 400 paired serum samples, Prevalence of Enteric fever by Tube agglutination and slide agglutination test are 23% (92 out of 400

cases) and 13% (52 out of 400 cases) respectively. Incidence of Typhoid fever, paratyphoid 'A' fever and paratyphoid 'B' fever were found to be 13% (52 out of 400 cases), 0 (0 out of 400 cases) and 0 (0 out of 400 cases) respectively by slide widal test. The incidence of Typhoid, paratyphoid 'A' fever and paratyphoid 'B' fever was found to be 23% (92 out of 400 cases), 0 (0 out of 400 cases), 0 (0 out of 400 cases) respectively by tube widal test. *Chi–square test* was applied, the results obtained by the Qualitative and Semi quantitative slide and tube widal test was found to be statistically significant. (X^2=113.31), df=1 ($p<0.0001$)

Enteric fever is one of the significant health problems in the community because of its mortality and morbidity, the diagnosis of Enteric fever can be made by testing the patients stool, blood, urine and other body fluids. Among which the Gold standard for diagnosing enteric fever is by isolation of bacteria by culture method but it takes almost a week to obtain the results, so then tube widal is the next best option which takes 24 hours to obtain the result, and recently slide widal was subsequently introduced for diagnosing enteric fever which almost gives results within Minutes. Among 400 samples tested for salmonella species, the sensitivity and specificity of the slide widal test is 80% and 85% respectively. Sensitivity and specificity of the tube widal is 84% and 96% respectively. Positive and Negative predictive value of the slide and tube widal test was 82% and 85% respectively.

Our results Matched the study done elsewhere, Thelma E. Tupasi *et al.,* in their study from various developing countries endemic to typhoid fever, found the sensitivity and specificity of tube Widal ranging from 61-90% and 88-100% respectively and that of slide agglutination method to be 53-80% (sensitivity) and 57-90% (specificity) (Tupasi *et al.,* 1991). Keddy H Karen *et al.,* in their study concluded that the semiquantitative slide agglutination test had shorter turnaround times than the Widal tube test and poor specificity and hence should not be used as diagnostic tool (Keddy *et al.,* 2011). John Wain *et al.,* stated that the quantitative slide agglutination test used instead of standard Widal test should always be interpreted with reference to clinical data (Wain John *et al.,* 2005).

Endemic titre of *Salmonella typhi* among healthy population was reported to be 1:40 and 1:80, respectively. And the Endemic titre of *Salmonella paratyphi A* and *B* were found to be 1: 40 and 1:80, respectively (Rucha Patki *et al.,* 2017). Although blood culture remains the gold standard test for diagnosing enteric fever, it may not be available in many rural areas. In that case tube widal can be used, which is relatively skill based, time consuming, inexpensive and Non-invasive. And when compared with the slide widal test, sensitivity and specificity of tube widal test is much remarkable than the slide widal test, so it can be indefinitely used as a diagnostic test.

Limitations of the Study

Time duration of the study is short. Sample size is less to arrive at a definitive conclusion. Cost effectiveness is a factor.

Post-centrifugation Hemolysis (practical issues).

Widal test is used to detect antibodies against *Salmonella typh*i, *Salmonella paratyphi A and B* only but no other *Salmonella* species, which are also human pathogens of medical importance but rare.

Table.1 Interpretation of Tube widal test

Interpretation of Tube Widal test	O	H	AH	BH
Positive for *Salmonella typhi* (Typhoid) fever	≥ 1:80	≥ 1:160	≤ 1:20	≤ 1:20
Positive for *Salmonella paratyphi A* (paratyphoid 'A' fever)	≥ 1:80	≤ 1:160	≥ 1:160	< 1:160
Positive for *Salmonella paratyphi B* (paratyphoid 'B' fever)	≥ 1:80	≤ 1:160	<1 :160	≥ 1:160
Negative for Enteric fever	< 1:80	<1: 160	<1:160	<1:160

Table.2 Incidence of Enteric fever

Study population (N=400)	SLIDE WIDAL (N, %)	TUBE WIDAL (N, %)
Incidence of *Salmonella typhi*	**(52, 13%)**	**(92, 23%)**
Incidence of *Salmonella paratyphi A*	(0, 0)	(0, 0)
Incidence of *Salmonella paratyphi B*	(0, 0)	(0, 0)
Total number of Enteric fever cases	52 out of 400 cases	92 out of 400 cases
Negative for Enteric fever	(348, 87%)	(308, 77%)

Anamnestic response due to recent vaccination and cross infection

Prozone phenomenon of antibody excess.

Recommendations

Hospital antibiotic policy to be adhered and revised periodically. Serological profile of salmonella infection would help us to understand type prevalence and can be a potential tool for Epidemiological study and infection control. Molecular methods like polymerase chain reaction, DNA probes could be the future scope.

Large number of samples can be subjected for the comparison between the slide and tube widal test to make the results still authoritative. Comparison of effectiveness between commercially available different widal kits. Comparison of results of widal test between the patients who have and have not consumed antibiotics prior to the widal test. Prospective study can be conducted to find the clinical correlation of the patient, who was positive for widal test.

Prevalence of Enteric fever evaluated by slide and tube widal agglutination tests were found to be 13% and 23% respectively. Though blood culture is the Gold standard for diagnosis of Enteric fever, the sensitivity is better only during first week of fever. Especially during second week of fever we could use widal test as an alternative or supplementary test of choice. We conclude our study with the fact that the performance of tube widal is better than the slide widal test.

Hence slide widal test alone cannot be used for diagnosis of Enteric fever especially during second week of fever. Though slide widal test is easy to perform and gives rapid results than the tube widal test, slide widal could only be considered as a supplementary test along with tube widal agglutination test.

Diagnosis of enteric fever earlier is very important, because of the prescription of irrational antibiotics where the results tends to be negative, so in that case tube widal test plays a major role in diagnosis of enteric fever. But now at present slide widal has been introduced in the diagnosis of the enteric fever. So this research is done to compare the qualitative and semi quantitative results of both the test, of which the results came satisfactory with tube widal which is highly superior in diagnosing the disease when compared to slide widal test, which can be applied as the diagnostic test.

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References

Dandona R, Kumar *et al.,* Nations within a nation: variations in epidemiological transition across the states of India, 1990–2016 in the Global Burden of Disease Study. India State-level Disease Burden Initiative Collaborators. *The Lancet*, 2017; 390 (10111): 2437-60.

John J, Van Aart C. J, Grassly N. C. The burden of typhoid and paratyphoid in India: systematic review and meta-analysis. PLoS Negl. *Trop.Dis*, 2016; 10 (4): e0004616. 10.1371/journal. pntd.0004616.

Keddy H Karen *et al.,* Sensitivity and Specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two Sub-saharan African sites. *Bulletin of WHO*, 2011; 89: 640-47.

Mengist HM, and Tilahun K. Diagnostic Value of Widal test in the Diagnosis of Typhoid Fever: A Systematic Review. *J Med Microb Diagn*, 2017; 6: 248. doi: 10.4172/2161-0703.1000248.

Olopoenia LA, and King AL. Widal agglutination test - 100 years later: Still plagued by controversy. *Postgrad Med J*, 2000; 76: 80-4.

Rucha Patki *et al.,* Baseline antibody titre against Salmonella enterica in Healthy individuals, India. 2017: 9042125.

Tupasi E. Thelma *et al.,* Clinical Application of the widal test. Phil J Microbiol Infect Dis 1991; 20 (1): 23-6.

Wain John *et al.,* The laboratory diagnosis of enteric fever. J infect Developing countries. 2005; 421-25.

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Bacteriological Profile and Antibiogram of Catheter Related Blood Stream Infections (CR-BSIs) among Chronic Hemodialysis Patients Attending Tertiary Care Medical College Hospital

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A B S T R A C T

Central Venous Catheter (CVC) remains a suitable form of vascular access for patients with chronic hemodialysis and has been estimated that almost 30-40% of patients are dependent on CVCs for their vascular access. Aim of the study is to determine the prevalence of Catheter Related Blood Stream Infections (CRBSIs). A Cross sectional study was undertaken after ethical committee approval, Blood samples (5ml-10ml) were collected from catheter site under sterile precautions and Blood Culture (Bac'T'Alert) performed and isolates grown were identified by routine methods as per standards. Antibiotic Susceptibility Testing was also performed and results noted. Out of the 60 samples processed 24 samples (40%) were "Culture Positives" and 36 samples (60%) were sterile and termed "Culture Negatives" after 5 days of incubation. The predominant Gram positive organism observed in our study was MRCONS followed by MSCONS. The prevalence of CRBSIs among chronic hemodialysis patients with central venous catheter in- situ was found to be 40%. Improving basic infection control measures and increasing the awareness by educating the health care providers could significantly reduce the occurrence of bacteremia could possibly aid in early diagnosis.

Introduction

Catheter Related Blood Stream Infections (CRBSIs) refers to bloodstream infection attributed to an intravascular catheter by quantitative culture of the catheter tip or by the difference in growth between catheter and peripheral venipuncture blood culture specimens (Harshal Shah *et al.,* 2013). Traditional classification of blood stream infections constitutes two variants viz., a. Community acquired, b. Health care associated or Hospital- acquired or Nosocomial infections (Friedman *et al.*, 2002). Central Venous Catheter (CVC) remains a suitable form of vascular access for patients with chronic hemodialysis and has been estimated that almost 30-40% of patients with

chronic hemodialysis are dependent on Central Venous Catheters (CVCs) for their vascular access. Nosocomial infections are the major cause for repeated hospitalization and death in hemodialysis patients in many countries (Masashi Suzuki *et al.,* 2016). The prevalence of Catheter Related Blood Stream Infections ranges between 10-30% in hemodialysis patients which occurs at a rate of 0.5-47/1000 catheter days (Ramasubramanian *et al.,* 2014). Catheter Related Infections are associated with substantial morbidity and mortality. Therefore, it is a potential menace and clinical challenge for Nephrologists and Physicians to diagnose CRBSIs early and to initiate appropriate management to prevent further complications. Strict adherence to Catheter care and Universal Safety Precautions by the health care providers will reduce the incidence of CRBSIs to a significant level.

Catheter Related Infections results from migration of skin commensals along the catheter into the blood stream or by contamination and or by colonization of catheter lumens by pathogenic microbes. Prevention strategies are directed at decreasing the growth and adherence of pathogens to catheter hub and or to the surface (Abhilash Chandra *et al.,* 2016). Recently, promising techniques like Antibiotic Lock Therapy was introduced where antimicrobial solutions are instilled into the lumen of the catheter between the hemodialysis sessions in order to address intra luminal sources of infection (Jun Zhang *et al.,* 2016). Antimicrobial solutions (Vancomycin-heparin (2.5mg/ml), Cefazolin-heparin (5mg/ml), Gentamicin–Sodium citrate (40mg/ml)) can prevent biofilm formation (Lars Skov Dalgaard *et al.,*).

Bio film acts as an important source of bacteremia and also a key factor favoring bacterial lodgement and in turn results in bacterial resistance thereby limiting the physician's antibiotic options for therapeutic management of CRBSIs. At the same time, concerns about the toxicity of antimicrobial lock therapy in case of over fill, especially at higher concentrations must be overlooked (Jun Zhang *et al.,* 2016). The risk factors for CRBSIs in chronic hemodialysis patients includes: i) Septicemia, ii) Stenosis, iii) Atheromatous plaques, iv) Aneurysm dilatation, v) Low serum albumin level, vi) Low BMI, vii) Diabetes, viii) infections, ix) High BUN (Tarek *et al.,* 2016). The specific aim of this study is to determine the incidence rate of Catheter Related Blood Stream Infections among chronic hemodialysis patients with central venous catheters in- situ. The objectives of our study are to evaluate the bacteriological profile, antibiotic susceptibility pattern and to screen for specific drug resistance among the culture isolates if any.

A study conducted by Masashi Suzuki *et al.,* on Bacteremia in hemodialysis patients showed that the risk of bacteremia was higher and the common causative organisms resulting in bacteremia were found to be Gram- positive bacteria (Masashi Suzuki *et al.,* 2016). Intricate details of review regarding Intravascular Catheter-Related Blood Stream Infections viz., epidemiology, pathogenesis and microbiology of CRBSIs and current guidelines for the diagnosis, management and prevention of these infections are very well elucidated by Harshal Shah *et al.,* in their study done elsewhere (Harshal Shah *et al.,* 2013). Deborah Friedman *et al.,* developed a new classification scheme for CRBSIs that distinguishes community- acquired CRBSI and nosocomial acquired CRBSI (Friedman *et al.,* 2002). The most common risk factor for bacteremia in hemodialysis patients is the use of central venous catheters. Previous hospitalization in turn serves as a second leading risk factor for CRBSIs. Membrane reuse was also found to be an important risk

factor for septicemia. Likewise, Diabetes mellitus and Hypoalbuminemia (<3.5mg/dl) were also found to be important risk factor for recurrent bacteremia. Analysis of "United States Renal Data System" reported 50% higher risk of septicemia in hemodialysis patients who were on temporary catheter. (Abhilash Chandra *et al.,* 2016) Bacteremia may be the result of either extra luminal or intra luminal catheter surface infections.

Extra luminal catheter surface infections are associated with percutaneous catheters. Following catheter hub contamination, infection spreads by exit site infection with subsequent migration into the blood stream along the extra luminal surface. Other unusual causes reported were intrinsic contamination of infusates and hematogenous seeding from distant infections (Harshal Shah *et al.,* 2013).

According to the guidelines for the diagnosis and management of the intravascular catheter-related infection by the Infectious Diseases Society of America (IDSA), a definitive diagnosis of CRBSI require/s the following: (a) a set of peripheral blood cultures; (b) blood cultures from the peripheral vein and from a culture of the catheter tip; or (c) cultures from an arterial and venous catheter hub that meet differential time-to-positivity criteria (Masashi Suzuki *et al.,* 2016). In this study, blood cultures from the peripheral vein and from a culture of the catheter tip were taken into account for diagnosis. In addition to this, Catheter Related Blood Stream Infections were suspected in a patient with an intravascular catheter who developed the signs of septicemia viz., fever, dyspnoea, edema and were also considered to have CRBSIs. Extensive research data are available which reports Antibiotic lock therapy significantly reduces the incidence of CRBSIs. Commonly used antibiotics for antibiotic lock therapy are Gentamicin, Cefotaxime, Cefazolin, Minocycline and Vancomycin. Generally the treatment of bacteremia includes administration of systemic antibiotics and care of the catheter site (Masashi Suzuki *et al.,* 2016).

The main aim of this study to determine the prevalence of Catheter Related Blood Stream Infections (CRBSIs) among chronic hemodialysis patients with central venous catheters in-situ attending dialysis unit of our hospital.

The objectives of this study include, to evaluate the bacteriological profile of Catheter Related Blood Stream Infections in chronic hemodialysis patients with central venous catheter in- situ. To detect the Antibiotic susceptibility pattern of the clinical isolates identified. And to screen for the specific drug resistance patterns among clinical isolates.

Inclusion criteria

Chronic kidney disease patients (age ≥ 18 years) with tunneled central venous catheter in-situ for hemodialysis presenting with/without the signs and symptoms of septicemia.

Exclusion criteria

Patients who had not given informed written consent.

Patients positive for Hepatitis B, HCV, HIV were excluded.

Patients who had taken antibiotics in the last 1 month.
Chronic kidney disease Patients on non-tunneled catheters.

Materials and Methods

A Cross sectional study was undertaken after obtaining clearance from Institutional Human Ethics Committee (IHEC). Informed written consent was obtained from the study

participants viz., In-patients and Out-patients clinically diagnosed to have chronic kidney disease from Medicine department constitutes the study population. Blood samples (5ml-10ml) were collected from catheter site and also from peripheral vein under sterile and aseptic conditions in a blood culture bottle (BacT/ALERT^{R} FA plus 410851, Automated Blood Culture System) (Liesa *et al.,* 2016). Second blood sample was collected from the same patient at a time interval of 10-15 minutes (Wasse *et al.,* 2007) and were transported immediately to the Microbiology laboratory. The specimens obtained were processed by performing routine diagnostic tests like Aerobic culture, appropriate biochemical tests to aid in the identification of bacteria. The Antibiotic Susceptibility Testing was done and susceptibility patterns observed, analyzed and recorded as per Standard Operating Procedures.

The blood samples after inoculation were incubated in an automated blood culture system (BacT/ALERT^{R}) for a period of 5 days. In the meantime, a beep alert was considered as positive after which the bottles were taken out and Semi-quantitative blood cultures were done with 84µL volume in a disposable 5ml sterile plastic syringe on routine plating media viz., Blood Agar Plate (BAP), Mac Conkey Agar Plate (MAP), Chocolate Agar Plate (CAP) and were then incubated aerobically at 37^{o}C for 24-48 hours (Dubey *et al.,* 2010). The culture plates were read for presence or absence of bacterial growth. In case of positive cultures, Gram stain was performed and gram reactions were recorded. The culture isolates were identified by routine methods as per standard guidelines by interpretation of colonial morphology, gram reaction and routine Bio-chemical reactions and also with some other special tests like catalase, oxidase, nitrate, motility and etc. Interpretation of "culture sterile" was given as preliminary report after 2 days and as final report after 5 days of incubation in case of no growth.

Antibiotic Susceptibility Test (AST) were done on Mueller-Hinton Agar using by Kirby Bauer's disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines with 0.5 and 1.0 Mac Farland turbidity standard of Gram-negative bacteria and Gram-positive bacteria respectively. A lawn culture of test organism was made on Mueller Hinton Agar Plate (MHAP) and commercially available antibiotic discs of fixed antibiotic concentrations were placed individually (5 to 6 antibiotic discs were kept at a distance of 1.5 cm between each disc on standard 90mm Petri plate). MHAP was incubated at 37^{o}C under aerobic incubation for a period of 16-24 hours and Antibiotic Susceptibility Patterns were noted by reading the zone of inhibition around the antibiotic disc (Sahu *et al.,* 2012). Specific drug resistance patterns viz., MRSA, VRE, CRE, AmpC, ESBL, K1 enzyme production were identified by combination or double disk potentiation method and etc., as per CLSI 2018 guidelines. Quality Control for Culture and AST were done using standard reference strains like *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 standard reference strains. Data entry and Statistical analysis were done using IBM SPSS 23.0 Statistical Analysis software.

Results and Discussion

A total of 60 paired blood samples were collected from the study populations during the two months period. The male and female ratio was 1.07:0.93 with slight male preponderance (male 31 out of 60 (51.66%) & female 29 out of 60 (48.33%)). Out of the 60 samples processed for culture and sensitivity, nearly 24 samples (40%) were "Culture Positives" and 36 samples (60%) were sterile and termed "Culture Negatives" after 5 days of incubation.

Among culture positives, 19 out of 24 (79.16%) were identified as "Gram positive bacteria" namely Methicillin Resistant Coagulase Negative Staphylococcus (MRCONS, 8 out of 24, 33.33%), Methicillin Sensitive Coagulase Negative Staphylococcus (MSCONS, 3 out of 24, 12.5%), Methicillin Resistant *Staphylococcus aureus* (MRSA, 2 out of 24, 8.33%), Enterococcus (1 out of 24, 4.16%), Micrococci (2 out of 24, 8.33%), Aerobic spore bearing gram positive bacilli (3 out of 24, 12.5%).

About 5 out of 24 (20.83%) culture positive isolates were identified as "Gram negative bacteria" namely Enterobacter species (3 out of 24, 12.5%), *Escherichia coli* (2 out of 24, 8.33%).

The predominant Gram positive bacteria observed in our study was MRCONS followed by MSCONS, MRSA and Micrococci.

The predominant Gram negative bacteria that were isolated from the study population was found to be Enterobacter species followed by *Escherichia coli*.

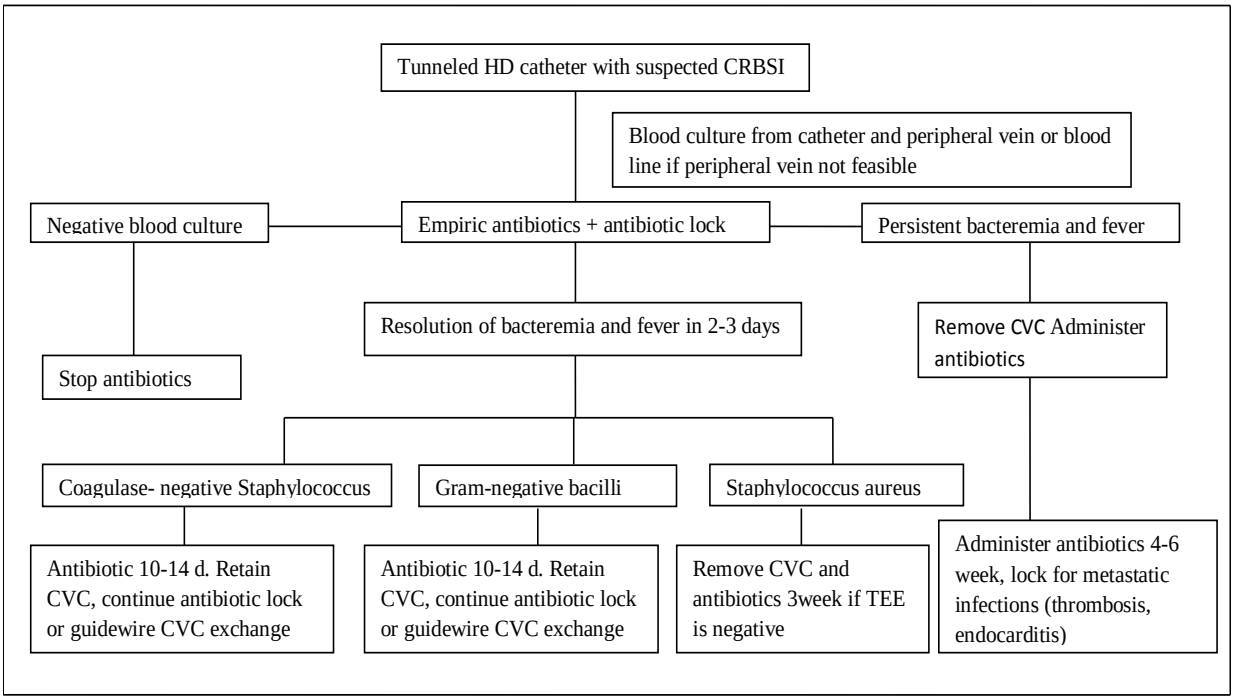
MRCONS culture isolates exhibited resistance to Erythromycin and Penicillin G. MSCONS manifested resistance to Erythromycin. MRSA manifested resistance to Gentamicin and Penicillin G. Among Enterococcal isolates, Gentamicin and Doxycycline showed resistance. In our study, all the Gram positive bacteria were found to be susceptible to Vancomycin, Linezolid, Doxycycline and Gentamicin. Among Gram negative isolates Enterobacter species showed resistance to Amoxycillin Clavulanate, Ampicillin and Ciprofloxacin. This isolate was susceptible to Tigecycline, Amikacin, Imipenem and Tobramycin. *Escherichia coli* isolates displayed resistance to Ampicillin, Amikacin and were susceptible to Ceftazidime, Cefotaxime, Piperacillin and Tazobactam.

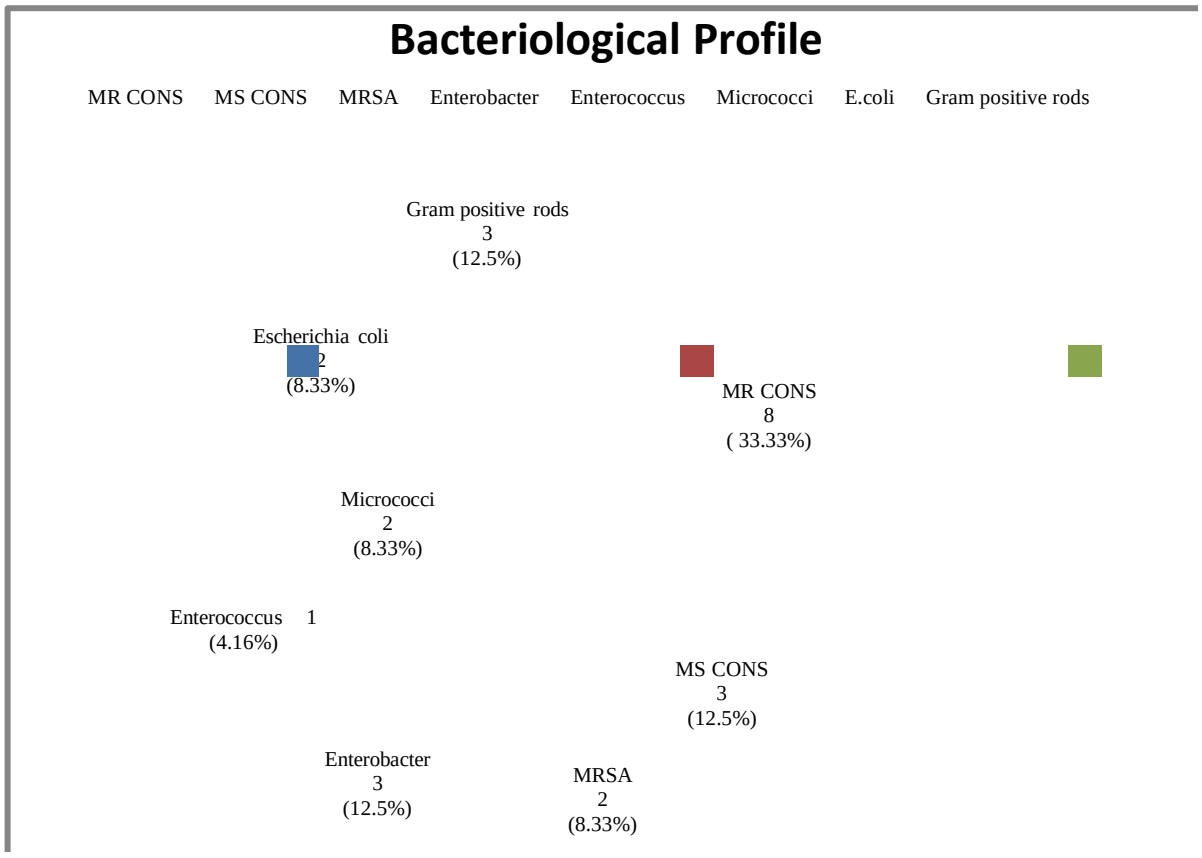
In our study, the prevalence of Catheter Related Blood Stream Infections among chronic hemodialysis patients with central venous catheter in- situ was found to be 40%. Gram positive bacteria comprised 3/4th of the cases while the remaining 1/4th were Gram negative bacteria.

Most of the studies showed that prevalence rate of bacteremia due to MRSA (Masashi Suzuki *et al.,* 2016) as high whereas in our study prevalence rate of MRCONS (33.33%) was higher than that of MRSA (8.33%).

Recommended practices for prevention of catheter related bloodstream infections include: a) To limit the insertion of catheter to the trained personnel; b) To use > 0.5% chlorhexidiene plus alcohol or povidone-iodine for preparation of skin; c) Strict adherence to maximum sterile barrier precautions like head cap, face mask, sterile gown, sterile glove and sterile full body drape while insertion of catheter and also during guidewire exchange; d) Complete hand hygiene prior to insertion, assessment or changing the dressing is mandatory; e) To use subclavian vein instead of internal jugular or femoral vein; f) To scrub catheter hubs with appropriate antiseptics after cap is removed and before accessing; g) To replace gauze dressings every 2 days once and to replace semi-permeable transparent dressing every 7 days once; h) To remove intravascular catheter when it is no longer required for patient care (recommended) (Harshal Shah *et al.,* 2013).

Fig.1 Management guidelines for CRBSIs in chronic hemodialysis patients on Central Venous Catheter





The potential limitations of this study are: a) The Sample size is considerably low to understand the epidemiology of CRBSIs. b) Follow-up of patients was difficult. c) Anaerobic bacteriological profile was not done. d) Antibiotic consumption by patients during the last 1 month could have had been the reason for culture negatives in cases of unreliable drug history given by the patients. e) Lack of hospital antibiotic policy exempted as to correlate right prescription of antibiotics by Physician according to the culture & sensitivity report given by the Clinical Microbiologist. f) Drug resistant pathogens identified were not subjected for molecular confirmation by PCR and other special confirmatory tests. g) Lack of hospital environment surveillance system to screen for prevailing nosocomial pathogens creates concerns about the understanding of epidemiology of CRBSIs. h) Data about the prevalence of nasal MRSA carrier state among health care providers (doctors, nurses, medical students and sanitary workers) and other specific drug resistance pathogenic bacteria are unavailable in our hospital.

Implications of the study

The study will give a first-hand research experience to the principal investigator in data collection, sample collection and performing special laboratory tests and co-relating their results with clinical outcome of the patients.

Details like Prevalence of CR-BSI's, Bacteriological profile, antibiogram of susceptible and drug resistant bugs will help understand the nosocomial pathogens causing these infections and in turn would help us revise the Hospital Antibiotic Policy. To Plan and execute periodic Environmental surveillance for Nosocomial pathogens. Designing good preventive measures in the form of practical, low cost, low-technology system that is feasible to implement.

To conclude, when we encounter hemodialysis patients with catheter as vascular access presenting with fever, the first diagnosis to be considered is CRBSIs (Abhilash Chandra *et al.,* 2016). The prevalence of CRBSIs among chronic kidney disease patients receiving hemodialysis was found to be 40%. Evidence- based guidelines is available to plan appropriate antibiotic treatment and holistic catheter management when infection occurs. Risk of CRBSI can be reduced by optimizing catheter insertion and maintenance and by also removing catheters when they are no longer needed. Improving basic infection control measures viz., hand hygiene and other universal safety precautions. Increasing the awareness and educating the health care providers and patients, could significantly reduce the occurrence of bacteremia and could possibly aid in early diagnosis and prompt therapeutic management of CRBSIs. Although this is difficult because the blood streams of these patients frequently exposed to extracorporeal devices (Masashi Suzuki *et al.,* 2016).

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References

Harshal Shah, MBBS[1], Wendelyn Bosch, MD[1], Kristine M. Thomson, MD[2], and Walter C. Hellinger, MD[1]. Intravascular Catheter-Related Bloodstream Infection. The Neurohospitalist. 2013; 3(3):144-151.

Friedman ND[1], Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, Lamm W,

Clark C, MacFarquhar J, Walton AL, Reller LB, Sexton DJ. Health care--associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. 2002 Nov19; 137(10): 791-7.

Masashi Suzuki, Nobuhiko Satoh, Motonobu Nakamura, Shoko Horito, George Seki, Kyoji Moriya. Bacteremia in hemodialysis patients, World Journal of Nephrology, 2016; Vol. 6; 5(6): 489-496.

Ramasubramanian, V., Vivek Iyer, Sandeep Sewlikar and Anish Desai. Epidemiology of healthcare acquired infection-An Indian perspective on surgical site infection and catheter related blood stream infection. Indian Journal of Basic and Applied Medical Research, 2014; Vol-3, Issue-4, P. 46-63.

Abhilash Chandra, Anupam Das, Manodeep Sen, Divya Srivastava. Hemodialysis catheter related blood stream infection in ESRD patients: incidence, outcome and antibiogram of the isolated organisms, International Journal of Advances in Medicine, 2016; Vol-3, Issue-4, P. 912-919.

Jun Zhang, Rong-Ke Li, Kee-HsinChen, Long Ge, Jin-Hui Tian. Antimicrobial local solutions for the prevention of catheter-related infection in patients undergoing hemodialysis: study protocol for network meta-analysis of randomised controlled trials. BMJ Open 2016; e010264.

Lars Skov Dalgaard, Mette Norgaard, Bente Jespersen, Soren Jensen-Fangel, Lars Jorgen Ostergaard, Henrik Carl Schonheyder, Ole Schmeltz Sogaard. Risk and Prognosis of Bloodstream Infections among Patients on Chronic Hemodialysis: A Population-Based Cohort Study, PloS ONE 10(4): e0124547.

Tarek A. Ghonemy, Salama E. Farag, Sameh A. Soliman, Essam M. Amin, Amal A. Zidan. Vascular access complications and risk factors in haemodialysis patient: A single centre study, Alexandria Journal of Medicine, 2016; Vol-52, Issue-1, P. 67-71.

Liesa M. Miller, Edward Clark, Christiane Dipchand, Swapnil Hiremath, Joanne Kappel, Mercedeh Kiaii, Charmaine Lok, Rick Luscombe, Louise Moist, Matthew Oliver and Jennifer MacRae. Hemodialysis Tunneled Catheter-Related Infections: Canadian Journal of Kidney Health and Disease; 2016: Vol-3, P. 1-11.

Wasse, H, Kutner N, Zhang R, Huang Y. Association of initial hemodialysis vascular access with patient-reported health status and quality of life. Clin Journal Am Soc Nephral 2007; Vol-2, P.708-714.

Dubey D, Rath S, Sahu MC, Padhy RS. Culture methods for gram negative bacilli. 2010; 13:766-789.

Sahu MC, Rath S, Dubey D, Debata MK, Padhy. Antibiotic Susceptibility Test to determine resistance of bacilli. 2012; 16: 313-322.

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Letter to Editor

Microbiology Section

Prevalence of Vancomycin Resistant Enterococci Carriers from Rectal Swabs among High Risk Patients in a Tertiary Care Hospital

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Keywords: Chromogenic media, *Enterococcus faecium*, *Enterococcus faecalis*, Urinary tract infections

Dear Editor,

Enterococci with acquired vancomycin resistance is a major concern amongst hospital. These resistant species have the ability to survive for prolonged periods and they can get transmitted easily. The infections caused by these organisms range from complicated Urinary Tract Infections (UTI) to life threatening Central Nervous System (CNS) infections making the treatment challenging [1]. The rate of Vancomycin Resistant Enterococci (VRE) colonisation in Northern India is nearly 34.5%, with *van A* genotype being the most common [2]. Studies have reported that active screening through rectal swabs is a definite tool in preventing complications in high risk patients by early identification and treatment of VRE carriers [3]. The conventional method of screening with bile esculin azide agar supplemented with 6 µg/mL of Vancomycin/ml is reasonably sensitive but requires confirmation by molecular techniques [4]. The molecular methods in the detection are limited to the referral laboratories. Development of highly specific chromogenic substrates in the VRE selective media help in effective screening of colonisers. The sensitivity and specificity of the chromogenic media was 100% and 99%, respectively [4]. This was in accordance to present study as well. This study was undertaken to screen the high risk patients in critical care for VRE colonisation from stool samples and identify the genotype. Early identification of the carrier state in high risk patients is helpful in planning the management strategy of these patients.

This was an observational study done over the period of three months from January to March 2016 at PSGIMS&R, Coimbatore, Tamil Nadu, India. The Institutional Human Ethical clearance number obtained was 14/118. Informed consent was obtained before collecting the rectal swabs. The criteria for high risk patients were defined as hospitalisation >7 days, chronic haemodialysis, use of multiple antibiotics and associated chronic disease status. Rectal swabs were obtained from 100 patients (no repeat isolates and the mean age group was 60 years 75% were males) following the identification as high risk as per the criteria explained above. The rectal swabs were streaked on chromogenic ChromID VRE media (Biomerieux) and incubated at 37°C for 24 hours. The presence of violet colour colonies indicated *Enterococcus faecium* and blue colour colonies indicated *Enterococcus faecalis*. Isolates with intrinsic resistance to vancomycin as *Enterococcus casseliflavus* and most of the other gram positive organisms were selectively inhibited by this media. Conventional biochemical tests as bile esculin, mannitiol, argininine and PYR (Pyrrolidonyl Arylamidase) were done for confirmation at the species level. The antibiotic sensitivity was also performed by Kirby Bauer method as per the CLSI guidelines [5]. The antibiotic susceptibility pattern of the 100 isolates showed the maximum sensitivity to linezolid (98%) followed by vancomycin (97%), erythromycin (89%), teicoplanin (75%), ampicillin (69.2%), high level gentamycin (49.2%) and penicillin (25%). The Minimum Inhibitory Concentration (MIC) of the resistant isolates was confirmed by using commercial E-strips. Molecular characterisation of the resistant isolates for the specific genotype was done by performing conventional PCR. The DNA from the resistant isolates was extracted using the QIAGEN kit. The primers of base pair 732bp for detection of *van A* were used. The PCR products were analysed by running gel electrophoresis and visualised under UV light in automated GEL DOC viewer. Primers for *van A* used were:

F (5' GGGAAAACGACAATTGC-3')

R (5'GTACAATGCGGCCGTCGTTA-3') [6]

Out of the 100 patients screened, three were found to be positive for VRE carriage (3%). All the three were *Enterococcus faecium*. The positive sample was used as the control strain. Analysis of the predisposing risk factors among the three patients revealed the association of chronic renal impairment, immunocompromised status (retroviral infection) and presence of co-morbid conditions such as diabetes mellitus along with prolonged hospital stay.

The VRE carriers can pose a risk to the patients harboring these pathogens as they can invade the host and result in bacteremia complicating the management. In such patients, treatment of the VRE carriage aids in better prognosis. Isolation and cohorting of these patients prevents the transmission and risk of infection in other patients, especially in healthcare setups. The choice of VRE screening can aid in proper infection control measures.

Thus, chromogenic VRE media was found to be a simple and rapid method for identifying VRE carriage.

Declaration: The present study was presented as poster presentation in 39th National Conference of the Indian Association of Medical Microbiologists MICROCON in 2015.

REFERENCES

[1] Zhanel GG, Laing NM, Nichol KA, Palatnick LP, Noreddin A, Hisanaga T, et al. Antibiotic activity against Urinary Tract Infection (UTI) isolates of Vancomycin-Resistant Enterococci (VRE): Results from the 2002 North American Vancomycin Resistant Enterococci Susceptibility Study (NAVRESS). Journal of Antimicrobial Chemotherapy. 2003;52(3):382-88.

[2] Banerjee T, Anupurba S, Filgona J, Singh DK. Vancomycin-resistance enterococcal colonisation in hospitalised patients in relation to antibiotic usage in a tertiary care hospital of North India. Journal of Laboratory Physicians. 2015;7(2):108.

[3] Humphreys H. Controlling the spread of vancomycin-resistant enterococci. Is active screening worthwhile? Journal of Hospital Infection. 2014;88(4):191-98.

[4] Cuzon G, Naas T, Fortineau N, Nordmann P. Novel chromogenic medium for detection of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. Journal of Clinical Microbiology. 2008;46(7):2442-44.

[5] CLSI. Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial Susceptibility testing. CLSI document M100-524.Wayne, PA: Clinical and Laboratory standard Institute 2020.

[6] Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 1995;33:24-27.

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Phenotypic and Genotypic Characterization of *Enterococci* from Clinical Isolates in a Tertiary Care Hospital

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A B S T R A C T

Enterococci mainly being commensals in human faeces are now considered as an important cause of nosocomial infections. Of the infections, the commonly observed are urinary tract infections, abdominal infections followed by bacteremia, endocarditis and meningitis rarely. Speciation of *Enterococcus* aids in effective management of the infections caused by them. The initial treatment for enterococcal infections has become challenging due to development of resistance. The resistance has been observed mainly against aminoglycosides due to the presence of bi-functional gene. VRE strains are mostly isolated from patients with recurrent bacteremia, endovascular infections leading to increase deaths in the patients. To isolate and speciate enterococcal isolates from clinical samples, study the antibiotic susceptibility pattern and the genotype associated with the aminoglycoside and vancomycin resistance. *Enterococcus* isolates obtained from various sections were characterized by conventional phenotypic methods. The antibiotic susceptibility was studied by disc diffusion method. The resistant strains were further confirmed by MIC using automated methods following which the genotypic analysis was done. The incidence of *Enterococci* was 2.5% in which around 86.4% were obtained as pure isolates. Among the species *E. faecalis* was found to be the maximum. The urinary isolates exhibited sensitivity of around 92.3% to nitrofurantoin and among the non - urinary isolates the maximum sensitivity was for linezolid. The molecular study showed van A to be most common gene with vancomycin resistance (71.42%), and bifunctional gene among the aminoglycoside resistance (96%). *Enterococcus faecalis* was the commonest species isolated. The maximum number of isolates was obtained from urinary samples. The increase incidence of resistance gene to high level gentamicin could result in failure to the synergy treatment of gentamicin in combination with penicillin group of antibiotics. The emergence of vancomycin resistant strains among the clinical isolates makes treatment options more challenging.

Keywords

Enterococci, Speciation, Antibiotic resistance, High level gentamicin, Vancomycin, Bifunctional gene.

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Introduction

Enterococci are gram positive organisms having the property to grow at 6.5% NaCl concentration, pH of 9.6 and hydrolyze bile esculin. Initially they were grouped under group D Streptococcus but later with the help of DNA studies they are characterized under the genus *Enterococcus* in 1960. They were considered as harmless pathogens formerly, but in the recent years have been emerging as the most important causative agents in nosocomial infections (Murray *et al.*, 1990).

Enterococci being the commonest micro flora of both humans and animals share their source

of origin. They have been isolated from variety foods including cheese, fish, sausages, beef and pork. Studies have been reported that *Enterococci* have been isolated from various clinical and environmental samples all over the world. Among the isolates obtained clinically it has been observed that *Enterococcus faecalis* has been the predominant species clinically (Lancefield *et al.,* 1933; Cetinkaya *et al.,* 2000).

Enterococcal infections have been referred to as tough, tenacious and troublesome infections (Marothi *et al.,* 2005).In the past years there has been an increase in the prevalence of enterococcal infections in hospitals and of particular concern is the emergence of antimicrobial resistant strains. Being second commonest organism behind the abdominal and pelvic infections, third in causing blood stream infections, cases have also been reported in causing CNS and neonatal infections.

Reports have been confirmed with their association with clinical conditions including respiratory tract infections, osteomyelitis and cellulitis (Fischer *et al.,* 2009). *Enterococci* were initially regarded as the disease causing agent in the early nineties. But now various reports have been established reporting them as the second commonest pathogen (Kuhn *et al.,* 2003).

Species identification becomes essential for investigating outbreaks in case of nosocomial infections and also susceptibility pattern varies accordingly which is also helpful in clinical management of infections due these agents (Gordon *et al.,* 1992).

Infections caused by enterococcal pathogens were initially being treated with the combined treatment of cell wall active agents as penicillins and aminoglycoside as the synergistic effect was effective. But now due to the development of antibiotic resistance as high level aminoglycoside resistance, beta lactamase production and also resistance to vancomycin have led to serious concern in management of these nosocomial pathogens (Mendiratta *et al.,* 2008). Vancomycin resistant *Enterococcus* was initially reported in 1980s, after which there was a increase in the incidence of its spread. The main phenotypes associated with its spread are Van A, Van B and Van C. Van A and Van B are mainly associated with *E. faecalis* and *E. faecium*. Van C is noted in *E. gallinarum* and *E. casseliflavus*. Among the phenotypes Van A is predominantly associated in the resistant strains.

VRE strains are mostly isolated from patients with recurrent bacteremia, endovascular infections leading to increase deaths in the patients. Vancomycin resistance and penicillin resistance are often seen co-existing which makes the treatment harder (Murray *et al.,* 1990).

Resistance development in *Enterococcus* against glycopeptide antibiotics gets transferred from them to other bacterial pathogens which include *Staphylococcus aureus, Streptococci, Listeria monocytogenes* (Gold *et al.,* 1996).

Thus due to the recent emergence in association with significant clinical infections and development of resistance, *Enterococci* have gained importance. Studies are thus required especially in tertiary care hospitals for appropriate isolation, identification and speciating them.

Antibiograms should also be followed up in accordance to the species. Emergence of VRE needs to be checked and limit its spread in hospital environment. CDC also stresses the control of VRE in hospitals by educating the staff in detecting VRE early, reporting them and action plan taken promptly.

Our study aims to isolate, speciate the *Enterococcus* in a simplified manner and also to study the drug sensitivity and resistance patterns among the isolates obtained.

Materials and Methods

The Institutional Human Ethical clearance was obtained before the commencement of the study.

Type of study

Experimental study

Confidentiality

Confidentiality of the reports were maintained

Sample size estimation

A total of 250 isolates of *Enterococci* obtained from outpatient and inpatient samples from blood section, miscellaneous and urine section were processed for the species identification, antimicrobial sensitivity followed by molecular study.

Specimen processing: Specimens from various sections including urine, pus and blood for cultures were collected and processed in the laboratory.

Pus/wound swabs

The swabs were streaked in blood agar and MacConkey agar and were incubated at 37^0C for 24 hours.

Urine

A loopful of the uncentrifuged specimen was examined as a wet mount for the presence of pus cells and bacteria. One loopful of specimen was inoculated onto blood agar and Mac Conkey agar. Plates were incubated at 37^0 C for 24 hours.

Growth in sheep blood agar

Following overnight incubation of the samples, colonies of *Enterococcus* were seen as grey colonies of about 0.5-1mm in diameter with alpha, beta or gamma hemolysis.

Growth in Mac Conkey agar

Colonies were about 0.5- 1mm in size with a smooth surface and convex margins, tiny magenta colored following 18-24 hour growth.

Catalase test

A drop of the catalase reagent (30% hydrogen peroxide) was added to the slide.

The growth was further applied to it with an applicator stick. Appearance of bubbles was taken as positive test.

Enterococcal colonies showed a negative test due to the absence of bubbles.

Gram stain

Smears made from the colony showed Gram positive cocci arranged singly, in pairs or in short chains.

Storage

If the isolate was catalase negative, gram staining showing the presence of gram positive cocci in pairs or short chains and growth in MacConkey agar as tiny lactose fermenting colonies, they were sub cultured and pure growth was stocked in Robertson's cooked meat medium in tubes.

The tubes were sealed and stored at 4-8^0C for 3-6 months. When the test was to be done they were further sub cultured.

Identification tests

Growth in media with pH – 9.6

Isolated colonies of *Enterococcus* was streaked in a media with a pH 9.6 and incubated at 37^0C overnight. The presence of growth was taken as positive.

Tolerance with 6.5% NaCl

The colonies were inoculated in a broth with 6.5% NaCl, and incubated overnight. The next day the tubes were checked for any increase in turbidity. This was further confirmed by sub culturing onto the agar plates. Growth confirmed the presence of organism. Whereas absence of turbidity indicated organism was unable to grow in this concentration.

Heat tolerance test

Enterococcus was inoculated in Todd-Hewitt broth. It was later incubated in water bath set at 45^oC overnight and 60^0 C for half an hour, and then sub cultured. *Enterococci* survived both the temperature showing positive reaction.

Bile esculin test

Colonies from 24 hour culture were inoculated in media containing 40% bile. Blackening of the medium confirmed positive reaction for *Enterococci* and non-*Enterococci*.

PYR test

The colonies were inoculated in PYR broth and incubated for a period of 4 hours. Later 3 drops of PYR reagent was added to it and change of colour to red was confirmed positive indicating *Enterococci* whereas absence of colour change indicates negative test.

Motility test

Stab culture of the colony was done in a semi-solid media and motility was indicated by the spread of the organism into the media. The strains which did not diffuse into the media were considered non-motile. Tubes were incubated for up to 7 days for confirming motility.

Arginine test

A well isolated colony of *Enterococcus* from the culture was inoculated in Moeller's decarboxylase media with arginine as the amino acid and overlaid with mineral oil. After 24 hours of incubation the colour of the tube was changed to purple indicating a positive reaction. Yellow colour indicated only acid production only and no deamination.

Potassium tellurite reduction test

Colonies were streaked in media containing 0.004%potassium tellurite. *E. faecalis* formed black colonies with the reduction of tellurite to tellurium.

This test was used to differentiate *E. faecalis* from *E. faecium.*

Pyruvate test

When inoculated in a media containing pyruvate with bromothymol blue as indicator *E. faecalis* showed a colour change from green to yellow color after overnight incubation. Absence of colour change ruled out *E. faecalis.*

Pigment production

Ability to produce pigment was observed by a few species of *Enterococci*. This was tested by sub culturing colonies in nutrient agar.

Any color produced was visible and also further confirmed by touching the colony with a sterile swab.

Sugar fermentation tests

Species identification was further done by sugar utilization by inoculating the organism in peptone with 1% sugars with Andrade indicator. The sugars used were mannitol, arabinose, raffinose, sorbose, sorbitol, lactose and sucrose. Any change in colour from colourless to pink indicated the positive sugar fermentation test. Absence of colour change was taken as negative.

Antimicrobial susceptibility test: (CLSI 2015)

The 250 test isolates of *Enterococci* were checked for the antimicrobial susceptibility pattern using Kirby-Bauer disc diffusion method. Three or four pure colonies were inoculated in peptone water and incubated for about 3 hours. The turbidity of the growth was matched to 1 of Mc Farlands. A lawn culture of the broth with a sterile swab was done in Muller Hinton agar and the discs were placed at a distance of 24 mm.

About 6 discs were kept on each plate. The discs used were Penicillin (10U), Ampicillin (10µgm), Amoxicillin Clavulanic acid (20/10), Erythromycin (15µgm), Vancomycin (30µgm), High level gentamicin (120µgm), Linezolid (30µgm), Tetracycline (30µgm). For isolates from urine section Norfloxacin (10µgm) and Nitrofurantoin (300µgm) was kept. All discs were bought from HIMEDIA.

Zones of inhibition were measured and recorded and the organism was interpreted as sensitive or resistant as per the recommendations from CLSI guidelines. The quality Control used was *Enterococcus faecalis* ATCC 29212.

Screening for high level gentamicin resistance (CLSI 2015)

Screening media for high level gentamicin resistance was prepared. Screening for high level gentamicin resistance was done by agar dilution method. BHI agar with gentamicin at a concentration of 500µgm/ml was added and the plates were prepared. Inoculum of 10µL of 0.5 Mc Farland was spot inoculated. Presence of even 1 colony was interpreted as a resistant strain. These strains were collected for molecular studies.

Detection of beta lactamase (Marothi *et al.*, 2005)

The detection of beta lactamase test was done based on the chromogenic cephalosporin method. Nitrocefin disc was obtained commercially from BD. The disc was moistened with sterile saline and pure isolated colony was applied on it. The reaction was read within 5 min. Isolates producing beta lactamase produced a red color.

Molecular study of isolates

A representative sample of 50 high level gentamicin resistant isolates was selected for genomic analysis. The DNA was extracted using the QIAGEN kit. Followed by extraction, the isolates were analysed for the presence of Bifunctional resistant geneaac (6') le aph (2") la. The vancomycin resistant isolates were checked for the presence of van A gene simultaneously.

Detection of aac (6') le aph (2") la gene

The primers for HLGR with a base pair of 348 bp were amplified. The sequence was obtained from the studies done previously[11]. PCR Master mix: The master mix was prepared as follows: 2.5µl of PCR buffer,2.5µl of MgCl2, 2.5µl of DNTPs,

10.2µl of Mili Q H2O, 1µl of forward and reverse primers and 0.3µl of Taq Polymerase was added to eppend off. From the above mixture 18µl was taken and 5µl of the extracted DNA was added.

Primers for aac (6')Ie- aph (2")la with base pair of 348 bp (Vakulenko *et al.*, 2003):

F (5'CAGAGCCTTGGGAAGATGAAG3')
R (5'CCTCGTGTAATTCATGTTCTGGC3')

PCR program

The program consisted of initial denaturation step at 95^0C for 10 minutes followed by 30 cycles of denaturation at 94^0C for 300 seconds, annealing at 56^0C for 1 minute and extension at 72^0C for a period of 1 minute. The program had a holding temperature of 72^0C for 10 minutes.

Analysis

PCR products were analyzed by running gel electrophoresis with 1.5% agarose gel in Tris Boric acid buffer. The samples along with the controls obtained by running ladder were analysed.

The gel was stained with ethidium bromide and the bands were obtained were visualized under UV light and also in automated GEL DOC viewer.

Detection of vanA gene

The primers with a base pair of 732bp for detection of vanA were obtained from the studies done previously.

Primers for van A with base pair of 732bp (Dutka-Malen *et al.*, 1995):

F (5' GGGAAAACGACAATTGC-3')
R (5'GTACAATGCGGCCGTCGTTA-3')

PCR program

This program had a denaturation step at 95^0C for 5 minutes followed by 30 cycles of DNA denaturation at 95^0C for 1 minute, annealing at 54^0C for 45 seconds and primer extension at 72^0C for 45 seconds. This was followed by a holding temperature of 72^0C for 10 minutes.

Analysis

PCR products were analyzed by running gel electrophoresis with 1.5% agarose gel in Tris Boric acid buffer. The samples along with the controls obtained by running ladder were analysed. The gel was stained with ethidium bromide and the bands were obtained were visualized under UV light and in automated GEL DOC viewer.

Results and Discussion

All the 250 isolates of *Enterococcus* from various clinical samples including urine, pus, wound swab, blood and CSF were processed.

The maximum number of isolates was obtained from urine section (184) followed by wound swabs (24), blood section (14), 4 from peritoneal samples, 1 from CSF (Figure 1). The majority of the isolates were obtained as pure growth (216) and 34 was obtained as mixed from the samples processed. Figure 2 depicts the speciation of enterococcal isolates in which *E. faecalis* was the predominant species (157), succeeded by *E. faecium* (79), *E. raffinosus* (7), and *E. durans* (5) and *E. casseliflavus* (2).

Illustration 1 shows the genus specific biochemical tests used in the identification.

The antibiotic susceptibility pattern of the enterococcal isolates by disc diffusion method revealed that out of the 250 isolates, about 69.2% were ampicillin sensitive, 49.2% were

high level gentamicin sensitive, 89.3% were sensitive to erythromycin. The maximum number of isolates was found to be sensitive to vancomycin (97.2%) in our study (Table 1). Among the urinary isolates maximum sensitivity was noticed to nitrofurantoin (92.3%) (Table 2) and norfloxacin (83.1%).

The maximum sensitivity was observed for linezolid followed by vancomycin, teicoplanin, ampicillin and high level gentamicin. Among the non-urinary isolates erythromycin sensitivity was observed to be the highest.

The chromogenic nitrocefin disc was used to screen the isolates for the presence of beta lactamase. In our study none of the isolates were found to be positive for beta lactamase.

Figure 3 shows the presence of Bifunctional gene aac (6')1eaph(2")1a among the high level gentamicin resistant strains. Out of the 50 randomly selected resistant strains for genotype study, 48 had the gene.

Illustration 2 shows the presence of bands of size 348 bp during the gel electrophoresis.

In our study 7 out of the 250 isolates were found to be resistant to vancomycin. The genotypic analysis was done among these resistant strains using the van A gene. The results showed that 5 of the 7 strains were found to be positive for van A gene (Figure 4).

Illustration 3 shows the presence of van A gene of base pair 732, confirming its presence among the resistant strains.

Enterococcus has been noted to cause a majority of urinary tract infections. This being the most common followed by bacteremia, sepsis and even case reports of meningitis has been reported. In our study out of 250 samples studied the majority of the isolates were obtained from urinary tract infections (73.6%) followed by wound swabs (9.6%) and blood (5.6%). This correlates well with other studies in which *Enterococci* was obtained from urine isolates maximally (Karmarkar *et al.,* 2004, Mathur *et al.,* 2003). According to the studies *Enterococci* are considered as the commonest urinary pathogen justifying the increase in the rate of isolation from urinary samples (Murray *et al.,* 1990).

Among the various species, in our study *E. faecalis* was the most common isolate accounting for 62.8% followed by *E. faecium, E. durans, E. raffinosus* and *E. casseleflavus.* Similar findings were reported from other studies done in Bangalore in which *E.faecalis* was 59%, *E. faecium* was 38 % (Golia *et al.,* 2014).

Enterococci show intrinsic resistance to cotrimoxazole, clindamycin, cephalosporins and normal aminoglycosides. Thus these antibiotics are not tested (Mendiratta *et al.,* 2008).

The antibiotic susceptibility pattern of the isolates in our study showed the following:

The sensitivity to linezolid was maximum (98.8%) followed by vancomycin (97.2%), erythromycin (89.3%), teicoplanin (75.2%), ampicillin (69.2%), high level gentamicin (49.2%) and penicillin (25%).

Among the urinary isolates maximum sensitivity was noted in Nitrofurantoin (92.3%) followed by Norfloxacin (83.15%). Studies done in North India showed a similar susceptibility pattern with increased resistance to penicillin and aminoglycosides. Also the sensitivity percentage to linezolid and vancomycin was higher compared to other antibiotics (Mulla *et al.,* 2012).

Fig.1 Distribution of *Enterococcus* isolates among various samples (N=250)

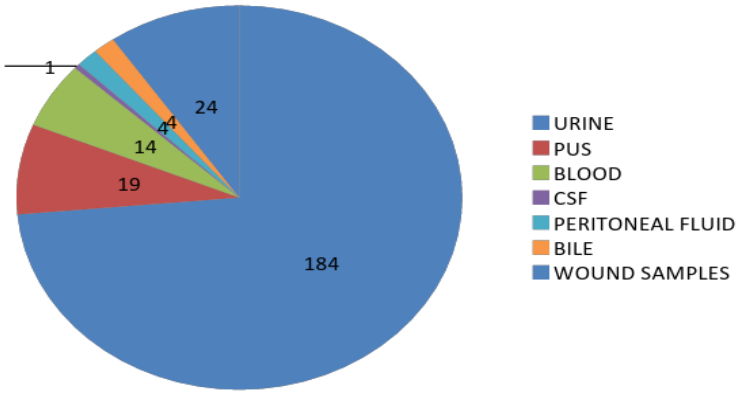
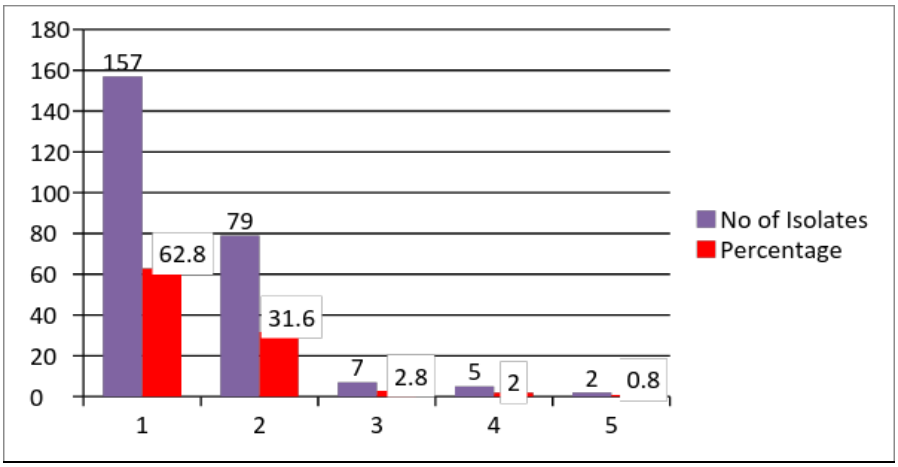


Fig.2 Speciation of *Enterococcus* from clinical isolates N=250



E.faecalis	1
E.faecium	2
E.raffinosus	3
E.durans	4
E.casseliflavus	5

Fig.3 Detection of the presence of aac (6') le aph (2") la gene N = 50

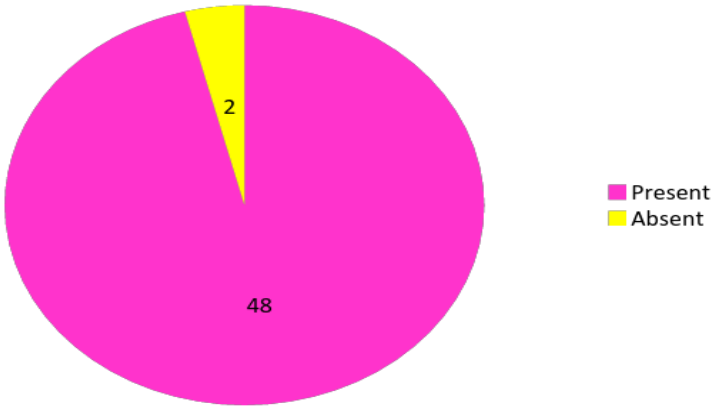


Fig.4 Detection of van A gene among the VRE isolates (N=7)

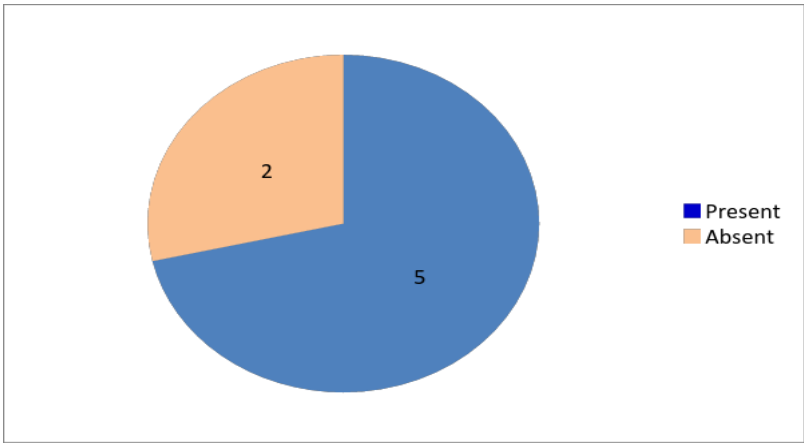


Table.1 Antibiotic susceptibility pattern of Enterococcal isolates

N = 250

RESISTANCE	SENSITIVE	ANTIBIOTICS
77	173	AMPICILLIN
30.8	69.2	%
127	123	HIGH LEVEL GENTAMICIN
50.8	49.2	%
7	243	VANCOMYCIN
2.8	97.2	%
3	247	LINEZOLID
1.2	98.8	%
62	188	TEICOPLANIN
24.8	75.2	%

Table.2 For urinary isolates

N = 184

RESISTANCE	SENSITIVE	ANTIBIOTICS
31	153	NORFLOX
16.85	83.15	%
14	170	NITROFURANTOIN
7.61	92.39	%
37	147	TETRACYCLINE
20.11	79.89	%

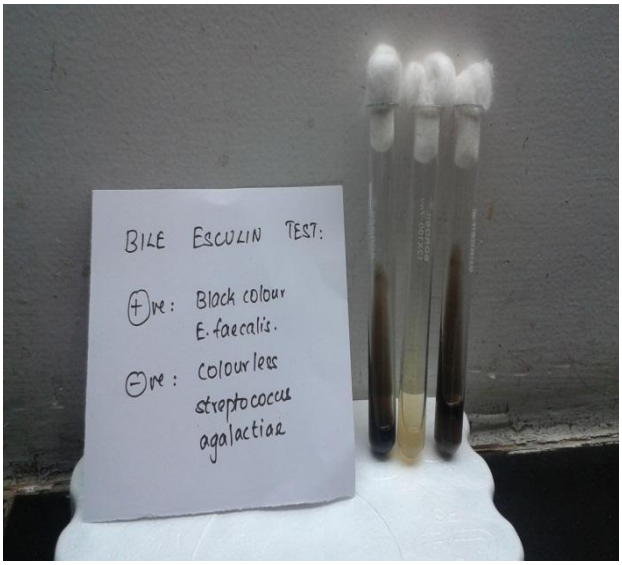
Table.3 For non urinary isolates

N = 66

RESISTANCE	SENSITIVE	ANTIBIOTICS
49	17	PENICILLIN
74.25	25.75	%
7	59	ERYTHROMYCIN
10.7	89.3	%

Illustration.1 Genus specific test

A) Bile-Esculin Test



B) PYR Test



C) 6.5% NaCl test



Illustration.2 PCR showing aac(6')-aph(2") BAND OF SIZE 348 bp

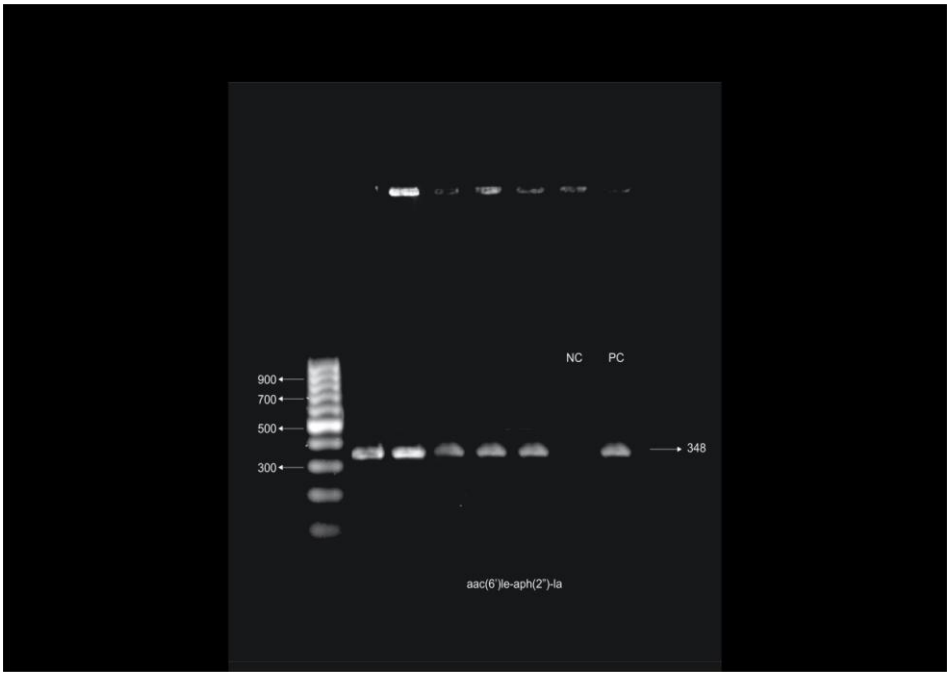
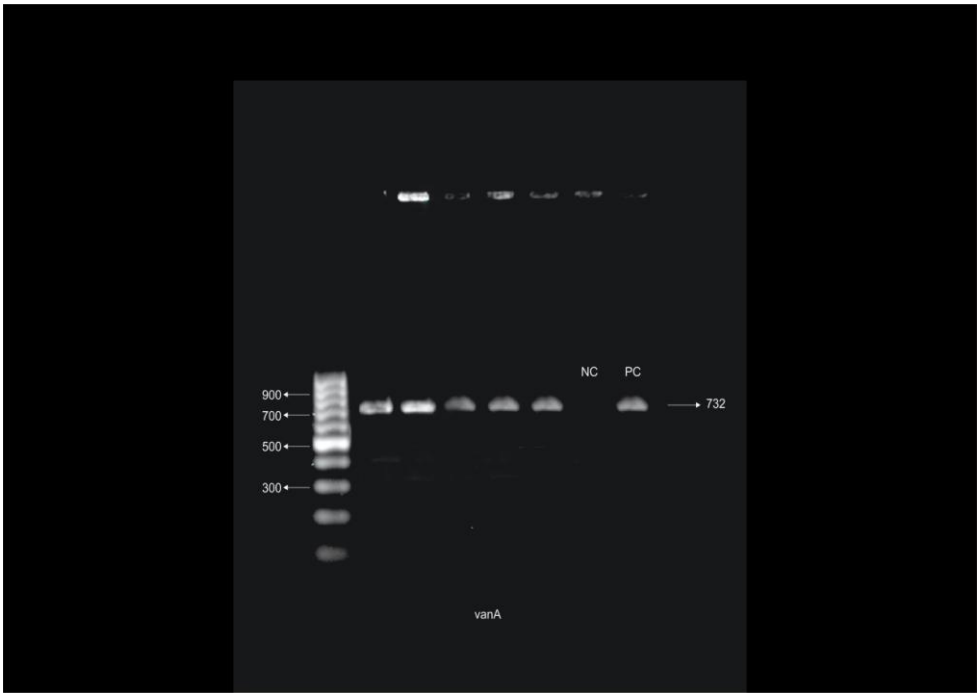


Illustration.3 PCR showing van A gene of band size 732bp



Another study showed the percentage of high level gentamicin resistance to be 68% (Randhawa *et al.*, 2004). Reports from South India showed the resistance percentage of high level aminoglycoside to be more than 50% (Sreeja *et al.*, 2012).

The detection of beta lactamase production by the chromogenic nitrocefin disc method showed that none of the isolates had beta lactamase enzyme. Studies done in Delhi and in Maharashtra also showed similar results (Jain *et al.,* 2011; Mendiratta *et al.,* 2008). The resistance to beta lactams though common, the mechanism could be due to alteration in penicillin binding protein PBP 5 which is more common than beta lactamase production (Murray *et al.,* 1992).

The molecular study done to detect the presence of the resistant gene confirmed the presence of the bifunctional gene aac (6') le-aph (2") la of base pair 348 to be present in 48 (96%) out of the 50 randomly selected resistant strains.

Studies done in Chennai have showed the presence of this gene in 38.2% of the isolates (Padmasini *et al.,* 2014) whereas there are other studies in which all the resistant isolates possessed this bifunctional gene (Hasani *et al.,* 2012). The resistance to high level gentamicin indicates resistance to all the aminoglycosides except streptomycin (CLSI 2015). The combination effect of aminoglycoside with beta lactam antibiotic is not helpful in the treatment of patients possessing such resistant strains.

Out of the 250 isolates the vancomycin resistant isolates obtained in our study was 2.8%. Studies have shown the prevalence of vancomycin resistance to be less in India ranging around 1.7-20% (Sreeja *et al.,* 2012).

Molecular studies revealed the presence of van A gene in 5 (71.42 %) isolates. Studies have also been reported showing van A to be the most common genotype isolated among the vancomycin resistant strains (Praharaj *et al.,* 2013)

Out of the seven isolates, five were *E. faecium* and two were *E. casseliflavus*. The remaining two isolates were *E. casseliflavus* which would have exhibited intrinsic resistance to vancomycin with van C gene (Praharaj *et al.,* 2013).

The incidence of *Enterococci* from the clinical isolates in our study was 2.5%.

The incidence of the pure isolates was 86.4% in comparison to the mixed isolates which was found to be 13.6%.

Among the isolates of *Enterococci*, the predominant species was *E. faecalis* (62.8%) followed by *E. faecium* (31.6%), *E. raffinosus* (2.8%), *E. durans* (2%) and *E. casseliflavus* (0.8%).

The antibiotic sensitivity pattern of the urinary isolates showed 92.3% sensitive to nitrofurantoin and 83.15% sensitive to norfloxacin. Among the non-urinary isolates maximum sensitivity was observed in linezolid which was 98.8% followed by vancomycin (97.2%), erythromycin (89.3%), teicoplanin (75.2%), ampicillin (69.2), high level gentamicin sensitivity (HLG) (49.2%) and pencillin (25%) (Table 3).

The vancomycin resistance was found to be 2.8% in *E. faecium*.

All the isolates were found to be beta lactamase negative by chromogenic (nitrocefin) method proving the alteration in PBP5 to be the more common mechanism involved in penicillin resistance.

The molecular characterization of HLGR(high level gentamicin resistant) strains, 50 isolates selected randomly out of 127, showed the presence of bifunctional gene aac(6')le-aph(2")la to be around 96%. (Primers of base pair 348 - F (5'CAGAGCCTTGGGAAGATGAAG3') R (5'CCTCGTGTAATTCATGTTCTGGC3')

The presence of van A gene was found to be 71.42% in our study.

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References

Cetinkaya *et al.,* (2000) Vancomycin-resistant *Enterococci*. *Clinical microbiology reviews* 13, no: 686-707.

CLSI. Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial susceptibility testing. CLSI document M100-524.Wayne, PA: Clinical and Laboratory standard Institute 2014.

Dutka-Malen *et al.,* (1995) "Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant *Enterococci* by PCR." *Journal of clinical microbiology* 33, no. 1: 24-27.

Fisher *et al.,* (2009) "The ecology, epidemiology and virulence of *Enterococcus*." *Microbiology* 155, no. 6: 1749-1757.

Gold *et al.,* (1996). "Antimicrobial-drug resistance. *"New England Journal of Medicine* 335, no. 19:1445-1453.

Golia *et al.,* (2014) "Isolation and speciation of *Enterococci* from various clinical samples and their antimicrobial susceptibility pattern with special reference to high level aminoglycoside resistance. *"International Journal of Medical Research and Health Sciences* 3, no. 3: 526-529.

Gordon *et al.,* (1992) "Antimicrobial susceptibility patterns of common and unusual species of *Enterococci* causing infections in the United States. Enterococcal Study Group." *Journal of clinical microbiology* 30, no. 9: 2373-2378.

Hasani *et al.,* (2012). "Molecular screening of virulence genes in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical specimens in Northwest Iran." *Indian journal of medical microbiology* 30, no. 2: 175.

Jain *et al.,* (2011) "Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. *"International Journal of Applied and Basic Medical Research* 1, no. 2: 80.

Karmarkar *et al.,* (2004) "Enterococcal infections with special reference to phenotypic characterization and drug resistance." *Indian Journal of Medical Research* 119: 22-25.

Kühn *et al.,* (2003) "Comparison of enterococcal populations in animals, humans, and the environment-a European study. "*International journal of food microbiology* 88, no. 2: 133-145.

Lancefield *et al.,* (1930). "A serological differentiation of human and other groups of hemolytic streptococci." *The Journal of experimental medicine* 57, no. 4: 571-595.

Marothi *et al.,* (2005) "Enterococcal resistance-an overview." *Indian journal of medical microbiology* 23, no. 4: 214.

Mathur *et al.,* (2003) "Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of northern India." *Indian Journal of Medical Research* 118: 25-28.

Mendiratta *et al.,* (2008). "Status of high level aminoglycoside resistant *Enterococcus faecium* and *Enterococcus faecalis* in a

rural hospital of central India." *Indian Journal of Medical Microbiology* 26, no. 4 : 369.

Mulla *et al.,* (2012)"Prevalence of *Enterococci* with higher resistance level in a tertiary care hospital: a matter of concern." *therapy* 1, no. 2: 3.

Murray *et al.,* (1992). "Beta-lactamase-producing *Enterococci*." *Antimicrobial agents and chemotherapy* 36, no. 11: 2355.

Murray *et al.,* (1990)"The life and times of the *Enterococcus*." *Clinical microbiology reviews* 3, no.1: 46-65.

Padmasini *et al.,* (2014) "High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of *Enterococcus* species in Chennai, India." *The Scientific World Journal* 2014.

Praharaj *et al.,* (2013) "Phenotypic and genotypic characterization of vancomycin resistant *Enterococcus* isolates from clinical specimens." *The Indian journal of medical research* 138, no. 4: 549.

Randhawa *et al.,* (2004) "Aminoglycoside resistance in *Enterococci* isolated from paediatric septicaemia in a tertiary care hospital in north India." *Indian Journal of Medical Research* 119: 77-79.

Sreeja *et al.,* (2012). "The prevalence and the characterization of the *Enterococcus* species from various clinical samples in a tertiary care hospital." *Journal of clinical and diagnostic research: JCDR* 6, no. 9: 1486.

Vakulenko *et al.,* (2003) "Multiplex PCR for detection of aminoglycoside resistance genes in *Enterococci*." *Antimicrobial agents and chemotherapy* 47, no. 4: 1423-1426.

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Original Article

Persistent organic pollutants-environmental risk factors for diabetes mellitus? – A population-based study

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Abstract

Background: Globally, type-2 diabetes mellitus is increasing in epidemic proportions. A major cause of concern in India is the increasing incidence of cases, especially troubling is the observed increase in younger age groups with no risk factors. New evidence suggests that many environmental factors, such as air pollution, persistent organic pollutants (POPs), and environmental estrogens are implicated as risk factors for type-2 diabetes mellitus. Animal and human epidemiological studies have shown ubiquitous lipophilic substances, including POPs, are frequently associated with type-2 diabetes mellitus. Such studies have not been undertaken in Indian youth. **Methods:** This is a cross-sectional study that explored the association between POPs and type-2 diabetes mellitus in Indian urban and rural population. About 7 ml of venous blood was collected from all consenting patients and serum was separated immediately and was transported to the lab for further analysis. Serum levels of POPs, including organochlorine (OC) compounds and organophosphorus pesticides, were estimated using sample gas chromatography-mass spectrometry (GC-MS). The fasting blood sugar values and the serum levels of POPS were tested using Pearson correlation coefficient. The magnitude of increase in blood sugar corresponding to increase in POPs was analyzed using linear regression analysis. The odds ratios (ORs) were expressed at 95% confidence intervals (CIs). **Results:** Three OC pesticides and one organophosphate pesticide were strongly associated with increasing blood sugar levels after adjusting for age, sex, and body mass index – lindane (OR 4.95, 95% CI 1.03–23.73), DDT o, p' (OR 3.50, 95% CI 1.04–11.73), dimethoate (OR 19.31, 95% CI 4.22–88.37), and dichlorvas (OR 6.33, 95% CI 1.28–31.18).

Keywords: DDT, dimethoate, health effects, persistent organic pollutants, pesticides, type- 2 diabetes mellitus.

Introduction

Incidence of diabetes is rapidly increasing worldwide.[1] The dominant risk factors include obesity, sedentary lifestyle, poor diet, old age, ethnicity, family history of diabetes, and dyslipidemia. Prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and expected to rise to 4.4% by 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.[1] Type-2 diabetes is an epidemic in Asia, characterized by a rapid increase in onset at relatively young ages and low body mass index (BMI),[2] and environmental estrogens implicated in the rise of the same.[3] Persistent organic pollutants are a class of compounds characterized by their ability to persist in the environment, due to their low water, high lipid solubility and bio-magnification in the food chain.[4] POPs include polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls (PCB), hexachlorobenzene, p, p'-dichlorodiphenyldichloroethylene (DDE), and several organochlorine (OC) mixtures. These have been studied by international organizations for their impact on environment and humans alike and have been implicated in several adverse effects on human health especially with impaired glucose metabolism and prevalence of diabetes mellitus.[4,5] India, a highly populated middle-income country, is subjected to environmental contamination of POPs from several activities

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and sources like production units, illegal imports as well as stockpiles of obsolete pesticide.[6] Except for DDT seven other pesticide POPs listed in the Stockholm Convention are banned for manufacture but are in wide use because large stockpiles are still available. In a nationwide food survey in 2001, 75% of the samples had detectable levels of DDT with about 10–15% of the samples having levels of DDT that exceeded the levels prescribed by the Food and Agricultural organization and World Health Organization. Aldrin and dieldrin were also frequently detected pesticides in foodstuffs.[7,8] Racial and ethnic differences in responses to POPs are well known.[9] POPs may be associated with T2DM with a higher magnitude in Asians, contributing to the epidemic. Hence, prevention and control of diabetes is a top public health priority in Asian populations.[10]

Many studies based on self-reported diabetes and mortality rate analysis indicate an association between pollutants and diabetes/prediabetes.[11,12] Many POPs act as endocrine disrupting agents in animals and humans.[13] Concentrations of most of these pollutants have been diminishing in the environment, food chain, and human body over recent decades in Europe and the United States. However, there are subgroups within these populations that still show an elevated body burden because of dietary habits and current or past exposures.[11] Diabetes has been associated with dioxin-like chemicals, nondioxin like PCBs, DDE, and/or other OCs in several cross-sectional investigations.[4,11,14,15] Altered glucose transport, lipid metabolism, and modifications in the insulin signaling pathways are potential mechanisms that might be involved in the association between POPs and diabetes.[11] The Jørgensen group showed that POPs may affect insulin secretion rather than insulin resistance.[16]

POPs metabolism, particularly low-level exposures, warrants investigations using a cohort study design.[17] There have been few studies that have used fasting blood glucose levels (≥126 mg/dL or 7.0 mmol/L), HbA1c, or random glucose level (≥200 mg/dL or 11.1 mmol/L) to diagnose diabetes.[4,14] The Morgan lab at University of Iowa College of Medicine, Iowa observed that workers with incident diabetes had significantly elevated levels of DDE+DDT in their serum compared to workers without diabetes.[18] Vasiliu *et al*.[12] found that PCBs were associated with incident diabetes in women but not in men and remained associated when cases encountered during the first 15 years of follow-up were excluded, suggesting that reverse causality was an unlikely explanation for the relationship. Investigations on Operation Ranch Hand Veterans, who were exposed to 2,3,7,8- tetra chlorodibenzo-p-dioxin (TCDD) through application of Agent Orange in Vietnam, were found to have incident diabetes associated with TCDD exposure.[19] Also, diabetes was associated with DDE exposure but not with mono-ortho PCB-118, total PCBs, or years of sport fish consumption.[5] In another case-control study it was confirmed that p, p'- DDE exposure could be a potential risk factor for T2DM.[11] A cross-sectional survey conducted in a heavily polluted area of eastern Slovakia demonstrated increasing serum concentrations of individual POPs considerably increased prevalence of prediabetes and diabetes in a dose-dependent manner.[20] Based on the current literature, there is a strong implied linkage between POPs exposure in humans and type-2 diabetes. There have been many studies analyzing the presence of POPs in Indian population, but none connecting the link and establishing association between POPs and diabetes.[21] Hence in this study, we investigated the association of blood sugar with the serum concentrations of POPs in south Indian population.

Methods

This is a cross-sectional study conducted at the rural and urban outreach centers affiliated to the PSG Institute of Medical Sciences and Research, Coimbatore, India. The study was conducted with approval from Institutional Human Ethics Committee at PSGIMS&R. A written informed consent was obtained from all eligible participants. All participants who consented were recruited in the study. Details about the study were informed by health workers from the center by house to house visit in addition to distribution of pamphlets which included details of the study and requirement of 8 h fasting for the collection of samples. Both male and female of age above 18 were included based on convenience sampling. Diabetics were confirmed as evidenced by fasting blood sugar above 126 mg/dL or HbA1c above 6.4% or use of hypoglycemic medications. Participants with type-1 diabetes and terminally ill conditions were excluded from the analysis. Basic demographic details, socioeconomic status, family history of diabetes, hypertension, general health, and medical history were elicited using a questionnaire. Anthropometric measurements were recorded and blood pressure was recorded in the sitting posture in the right arm. Age, gender, body weight, and height were recorded for each patient. Body mass index

Table 1: Demographics of participants enrolled in the study

Demographic characteristics of the participants (n=186)						
Gender (n)	**Male**	**56**	**Female**	**130**		
	Min	**Mean**	**25%**	**Median**	**75%**	**Max**
Age (year)	32	58.46	50.25	60	67	86
Height (cm)	137	154.3	149.2	152.5	158	184
Weight (kg)	31	61.75	54	61	68	126
BMI (kg/m^2)	16.44	25.86	22.35	25.43	28.95	46.28

n: number of participants

Table 2: Correlation between POPs (ng/mL) and serum blood sugar levels

Compound	Min	Mean	Median	Max	SD	r^2
Lindane	1.02	10.92	4.50	38.57	9.26	0.22 *
Dichlorvas	1.05	17.98	18.40	47.92	8.54	0.19 *
DDT o, p'	1.05	17.89	20.13	42.76	10.25	0.16 *
Dimethoate	15.98	27.17	25.35	75.68	9.44	0.36

P<0.05; SD – Standard deviation; r^2 – Pearson correlation coefficien

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Table 3: Correlation between POPs (ng/mL) and BMI

Compound	Mean	Median	SD	r^2		
				Normal	Overweight	Obese
Dichrotophos	7.50	7.22	7.30	0.70 *	NA	0.42
Monochrotophos	71.22	52.68	78.63	0.07	-0.07	0.19 *
1,1 Biphenyl, 3,3',4 Trichloro	1.34	1.13	0.41	-0.67 *	NA	-0.34
1,1 Biphenyl, 2,2',4,4',5,5' Hexachloro	1.47	1.37	0.29	NA	0.9 *	NA

*P<0.05; SD – Standard deviation; r^2 – Pearson correlation coefficient

Table 4: Correlation between POPs (ng/mL) and age (years)

Compound	Min	Mean	Median	Max	SD	r^2
BHC alpha isomer	1.02	9.5	11.78	24.5	5.89	- 0.4*
Parathion methyl	3.32	142.7	28.51	2324.91	314.7	- 0.1*
DDE, p, p'	1.50	36.59	10.99	484.98	71.15	- 0.1*
DDD, o, p'	1.11	43.92	16.77	1160.8	124.67	- 0.2*
1,1 Biphenyl, 2,4,4' Trichloro	1.06	6.60	5.10	21.35	5.29	0.1*

*P<0.05; SD – Standard deviation; r^2 – Pearson correlation coefficient

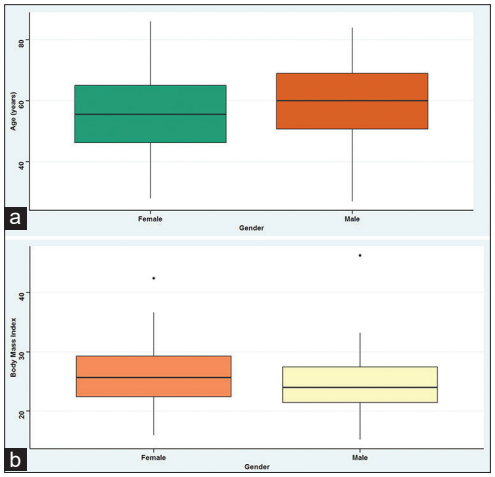


Figure 1: Box plots for (a) Age and (b) BMI categorized as per gender

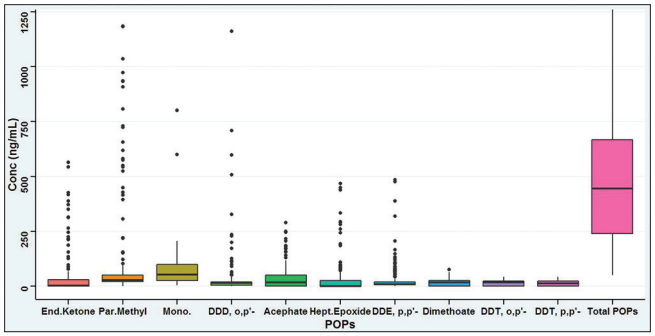


Figure 2: Concentrations of major POPs compounds in the serum of the participants. End.Ketone - Endrin Ketone; Par.Methyl - Parathion methyl; Mono - Monocrotophos; Hept.Epoxide – Heptachlor Epoxide

was calculated as weight in kilograms divided by height in meter squared. Blood was drawn after at least 8 h overnight fasting. Fasting blood sugar (FBS) was determined by hexokinase method which is a standard laboratory procedure. Serum POPs were determined by method described by Koc and Karakus.[22] Briefly, OC pesticide residues were determined using florisil packed-columns and GC-MS. Linear response between FBS and POPs were measured by the Pearson correlation method. The association between increasing sugar level and POPs was calculated by linear regression after adjusting for age, sex and BMI. All statistical analyses were conducted using R version 3.5.1 (R Development Core Team, http://www.r-project.org).

Results

The total numbers of participants in our study was 191, out of which FBS was not available for five participants. The demographic characteristics for the remaining 186 participants are given in Table 1. Box plots for age and BMI stratified as per gender are given in Figure 1a and 1b. Of the 44 compounds, 11 of them contributed to 93% of total POPs load [Figure 2]. The serum levels of the following four compounds – lindane, dichlorvas, DDT o p', and dimethoate were significantly correlated with increasing blood sugar levels [Table 2]. Among the remaining compounds 31 were not comparable and seven were below limit of detection. Correlation statistics among selected compounds is illustrated in Figure 3, nonsignificant correlation indicated by cross mark, positive correlation by blue solid circles, and negative correlation by red solid circles. Size the circle indicates the magnitude of correlation. Figure 4a-d illustrates the trend analysis by Pearson method showing increasing linear relationship as sugar level increases. Since POPs are obesogenic, we checked its correlation between different strata of BMI [Table 3] and with age [Table 4]. The participants were categorized as normal, overweight, and obese based on the height and weight. The odds ratio (OR) and 95% confidence intervals (CIs) of POPs showed significant risks for compounds like lindane (OR 4.95, 95% CI 1.03–23.73),

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DDT o, p' (OR 3.50, 95% CI 1.04–11.73), dimethoate (OR 19.31, 95% CI 4.22–88.37), dichlorvas (OR 6.33, 95% CI 1.28–31.18). Similar compounds are found to be significantly associated with blood sugar after adjusting for age, sex, and BMI [Table 5].

Discussion

Our study demonstrated that environmental exposure to various OC and organophosphate pesticides are associated with prevalent type-2 diabetes in Indian population. Our findings are supported with published literature, which implicate OC pesticides in risk of diabetes among general population.[14,15] Benzene Hexachloride (BHC) beta isomer was shown to be a strongly correlated with age in our study. One possible mechanistic pathway for BHC isomers is via pancreatic and extra-pancreatic actions, i.e. caspase 3 activation due to reactive oxygen species generation causing membrane destabilization and mitochondrial dysfunction of the beta cell.[15,20,23,24] Other OCs like DDD and DDT were also moderately associated with sugar levels, their ORs a little above 1.00. Epidemiological evidence also substantiates the positive risk DDT and its metabolites can have on diabetes.[20,24] DDT and its metabolites are inducers of cytochrome P450 (CYP 2BA and CYP 4A1), enzymes involved in lipid and steroid metabolism.[25,26] Moreover, these compounds can inhibit aromatase, an enzyme that metabolizes steroids like cortisol.[27] As a result of aromatase inhibition, steroidal compounds tend to accumulate leading to anti-estrogenic effects, although at a receptor level, they are pro-estrogenic in action.[28] These

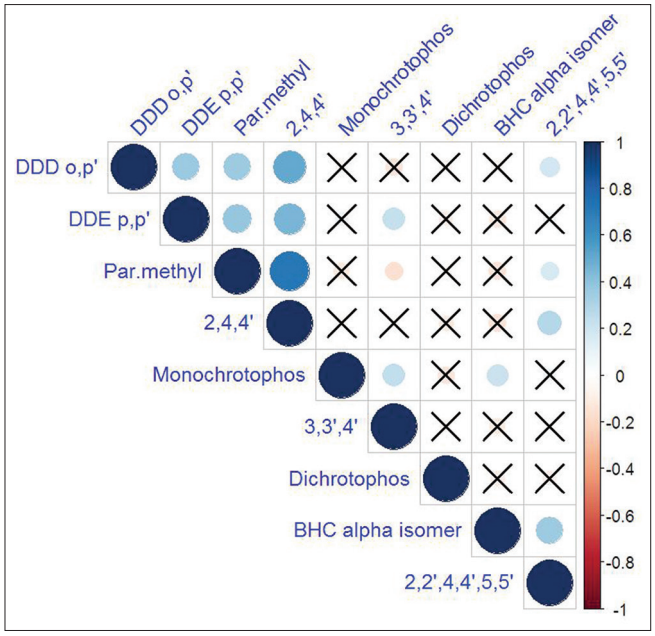


Figure 3: Correlogram for POPs. Non-significant correlation indicated by cross mark, positive correlation by blue solid circles and negative correlation by red solid circles

Table 5: Risks of increasing sugar levels and POPs

Compound	Parameters	Odds ratio	95% CI		*P*
			Lower	Upper	
Lindane	POPs	4.9585	1.0357	23.7392	0.0491 *
	Age	1.5382	0.4401	5.3757	0.5023
	BMI	0.8169	0.028	23.804	0.9068
	Gender	NA	NA	NA	NA
DDT, o, p'-	POPs	3.5041	1.046	11.7382	0.044 *
	Age	2.3531	0.8923	6.2056	0.086
	BMI	0.3416	0.0236	4.9413	0.4321
	Gender	NA	NA	NA	NA
Dimethoate	POPs	19.3124	4.2205	88.3709	2.00E -04*
	Age	2.1495	0.6246	7.3971	0.228
	BMI	0.5344	0.0211	13.5535	0.7049
	Gender	NA	NA	NA	NA
Dichlorvas	POPs	6.3379	1.2882	31.1826	0.025 *
	Age	3.2142	1.0804	9.562	0.0381 *
	BMI	1.0741	0.0507	22.7355	0.9635
	Gender	NA	NA	NA	NA

*P<0.05; CI – confidence interval

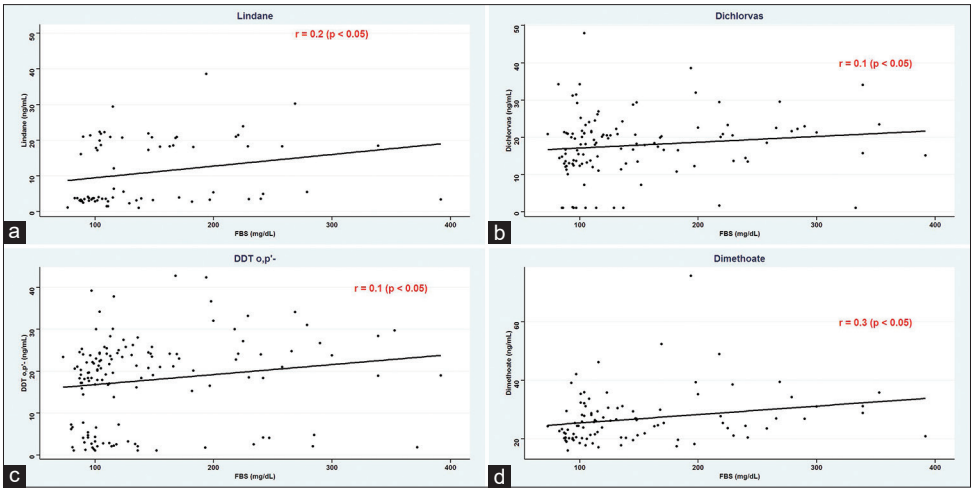


Figure 4: Trend plots for compounds showing significant correlation (a) Lindane (b) Dichlorvas (c) DDT (d) Dimethoate. (FBS – Fasting Blood Sugar)

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Table 6: Epidemiological and animal studies substantiating our study

Compounds	Odds ratio (CI)	Exposure contrast	Reference
p, p'- DDT	2.52 (1.26-5.02)	20.8-26.6 vs. ≤20.7 (ng/g lipid adj.)	Everett and Matheson[24]
	1.96 (1.29-2.98)	≥20.7 vs. ≤20.7 (ng/g lipid adj.)	Everett and Matheson[24]
	1.84 (1.03-2.27)	QU3 vs. QU1 (ng/g lipid adj.)	Ukropec *et al.*[20]
p, p'- DDD	3.6 (0.8-16.3)	T3 vs. T1 (ng/g lipid std.)	Son *et al.* [31]
o, p'- DDT	12.3 (1.3-113.2)	T3 vs. T1 (ng/g lipid std.)	Son *et al.* [31]
β- HCH	2.3 (1-4.3)	≥ 1 vs.<1 ng/g ww	Cox *et al.*[15]
	2.67 (1.59-4.49)	≥ 9.35-< 9.35 ng/g lipid adj.	Everett and Matheson[24]
	1.08 (0.59-1.97)	QU3 vs. QU1 (ng/g lipid adj.)	Ukropec *et al.*[20]
	0.8 (0.3-2.2)	Q4 vs. Q1 pg/g	Lee *et al.*[4]
	8.2 (1.3-53.4)	T3 vs. T1 ng/g lipid adj.	Son *et al.*[31]
Lindane	2.02 (0.88-4.65)	Not available	Zhang *et al.*[30]
Dichlorvas	1.21 (0.98-1.49)	Not available	Montgomery *et al.*[32]
Dimethoate	Study suggested Omethoate could potentially cause insulin resistance.		Zhang *et al.*[30]

effects coupled with cross talk with catecholamines in the adrenal gland lead to endocrine disruption. These compounds are also known to inhibit expression of Dio-2, low-density lipoprotein (LDL), Protein kinase B (AKT), extracellular-signal-regulated kinase (ERK), Patatin-like phospholipase domain-containing protein (PNPLA), and Glut-4 genes in target tissues.[29] Although we could not find epidemiological evidence for dimethoate even after PubMed search with the following terms "Dimethoate" [MESH] AND "Diabetes" [MESH], we were able to find animal studies implicating omethoate (a break-down product of dimethoate) with insulin resistance.[30] The epidemiological evidences are summarized in Table 6. Major limitation of the study is the cross-sectional nature, which might not have captured the cause-effect relationship.

Conclusion

This exploratory study found that serum levels of several OCs and organophosphate pesticides were associated with type-2 diabetes in a south Indian population. This is the first study in the Indian population to report the association of POPs and type-2 diabetes mellitus. Our finding complement other studies on Asian groups providing evidence for adverse effects of POPs, which explains the current epidemic of type-2 diabetes in Asia.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. Magliano DJ, Sacre JW, Harding JL, Gregg EW, Zimmet PZ, Shaw JE. Young-onset type 2 diabetes mellitus-implications for morbidity and mortality. Nat Rev Endocrinol 2020;16:321-31.
3. Bonini M, Sargis R. Environmental toxicant exposures and type 2 diabetes mellitus: Two interrelated public health problems on the rise. Curr Opin Toxicol2017;7:52-9. doi: 10.1016/j.cotox. 2017.09.003.
4. Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, *et al*. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: Results from the national health and examination survey 1999-2002. Diabetes Care 2006;29:1638-44.
5. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of great lakes sport fish consumers. Environ Health Perspect 2009;117:1076-82.
6. Syed J, Malik R, Liu D, Xu Y, Wang Y, Li J, *et al*. Organochlorine pesticides in air and soil and estimated air–soil exchange in Punjab, Pakistan. Sci Total Environ 2013;444:491-7.
7. Adeleye A, Sosan M, Oyekunle J. Dietary exposure assessment of organochlorine pesticides in two commonly grown leafy vegetables in South-western Nigeria. Heliyon 2019;5:e01895.
8. Olutona G, Aderemi M. Organochlorine pesticide residue and heavy metals in leguminous food crops from selected markets in Ibadan, Nigeria. Legume Sci 2019;1:e3.
9. Nebert DW, Zhang G, Vesell ES. Genertic risk prediction: Individualized variability in susceptibility to toxicants. Ann Rev Pharmacol Toxicol 2013;53:355-75.
10. Chan JC, Schymura MJ, Negoita S, Rej R, Carpenter DO. Diabetes in asia: Epidemiology, risk factors, and pathophysiology. J Am Med Assoc 2009;301:2129-40.
11. Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type-2 diabetes mellitus. Hum Exp Toxicol 2007;26:447-52.
12. Vasiliu O, Cameron L, Gardiner J, Dequire P, Karmaus W. Polybrominated biphenyls, polychlorinated biphenyls, body weight, and incidence of adult-onset diabetes mellitus. Epidemiology 2006;17:352-9.
13. McLachlan JA. Environmental signaling: What embryos and evolution

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teach us about endocrine disrupting chemicals. Endocrinol Revs 2001;22:319-41.
14. Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO, The Akwesasne Task Force on the Environment. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult native americans. Environ Health Perspect 2007;115:1442-7.
15. Cox S, Niskar AS, Venkat Narayan KM, Marcus M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among mexican americans: Hispanic health and nutrition examination survey, 1982-1984. Environ Health Perspect 2007;115:1747-52.
16. Jørgensøn ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance mong greenland inuit. Diabetologia 2008;51:1416-22.
17. Longnecker MP. Pharmacokinetic variability and the miracle of modern analytical chemistry. Epidemiology 2006;17:350-1.
18. Morgan DP, Lin LI, Saikaly HH. Morbidity and mortality in workers occupationally exposed to pesticides. Arch Environ Contamin Toxicol 1980;9:349-82.
19. Michalek JE, Ketchum NS, Tripathi RC. Diabetes mellitus and 2,3,7,8-tetrachlorodibenzo-p-dioxin elimination in veterans of operation ranch hand. Toxicol Environ Health Part A 2003;66:211-21.
20. Ukropec J, Radikova Z, Huckova M, Koska J, Kocan A, Sebokova E, *et al*. 2010. High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. Diabetologia 2010;53:899-906.
21. Tomar LR, Agarwal MP, Avasthi R, Tyagi V, Mustafa M, Bannerjee BD. Serum organochlorine pesticide levels in patients with metabolic syndrome. Indian J Endocrinol Metab 2013;17(Suppl 1):S342–4.
22. Koc F, Karakus E. Determination of organochlorinated pesticide residues by gas chromatography-mass spectrometry after elutio in a florisil column. Kafkas Univ Vet Fak Derg 2011;17:65-70.
23. Michalowicz J, Mokra K, Rosiak K, Sicińska P, Bukowska B. Chlorobenzenes, lindane and dieldrin induce apoptotic alterations in human peripheral blood lymphocytes (*in vitro* study). Environ Toxicol Pharmacol 2013;36:979-88.
24. Everett CJ, Matheson EM. Biomarkers of pesticide exposure and diabetes in the 1999-2004 national health and nutrition examination study. Environ Int 2010;36:398-401.
25. Kitamura S, Shimizu Y, Shiraga Y, Yoshida M, Sugihara K, Ohta S. Reductive metabolism of *p, p'*-ddt and *o, p'*-ddt by rat liver cytochrome p450. Drug Metabol Dispos 2002;30:113-8.
26. Sierra-Santoyo A, Hernandez M, Albores A, Cebrian ME. Sex-dependent regulation of hepatic cytochrome p450 by ddt. Toxicol Sci 2000;54:81-7.
27. Wójtowicz AK, Milewicz T, Gregoraszczuk EŁ. Ddt and its metabolite dde alter steroid hormone secretion in human term placental xplants by regulation of aromatase activity. Toxicol Lett 2007;173:24-30.
28. Ndebele K, Graham B, Tchounwou PB. Estrogenic activity of coumestrol, ddt, and tcdd in human cervical cancer cells. Int J Environ Res Public Health 2010;7:2045-56.
29. La Merrill M, Karey E, Moshier E, Lindtner C, La Franco MR, Newman JW, *et al*. Perinatal exposure of mice to the pesticide ddt impares energy expenditure and metabolism in adult female offspring. PLoS One 2014;9:e103337.
30. Zhang Y, Ren M, Li J, Wei Q, Ren Z, Lv J, *et al*. Does omethoate have the potential to cause insulin resistance? Environ Toxicol Pharmacol 2014;37:284-90.
31. Son HK, Kim SA, Kang JH, Chang YS, Park SK, Lee SK, *et al*. Strong associations between low-dose organochlorine pesticides and type-2 diabetes in korea. Environ Int 2010;36:410-4.
32. Montgomery M, Kamel F, Saldana T, Alavanja M, Sandler D. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural health study, 1993-2003. Am J Epidemiol 2008;167:1235-46.

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ORIGINAL PAPER

Design and development of whole-body rodent inhalation chamber for exposure to Aroclor 1232 in Swiss albino mice

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Abstract

The development of whole-body inhalational chambers for acute toxicity risk assessment of aerosols in rodents is of key importance in clinical and occupational hazard evaluations. The key aim of study is the design, development, optimization, and validation of an inhalational chamber using water aerosol, vehicle, and Aroclor 1232. The second goal has been to apply this chamber in the risk assessment of Aroclor 1232. Broncho-alveolar Lavage fluid cytology and chromatography, and lung histopathology were used to supplement the validation process. PCB concentrations were uniformly distributed across different places inside the chamber and a MMAD generation of 4.3 µm ± 1.7 µm GSD was suggestive of lower airway atomization of PCB particles. The chamber environmental parameters were within acceptable limits for rodents during the experimental procedures. Mouse behavioral alterations were minimal during water aerosolization. Acute exposure to Aroclor 1232 showed reproducibility of behavioral scores. Benchmark dose for further studies using Aroclor 1232 was derived at 4 mg/cu.m. Its toxicities could be verified by correlating the behavioral scores, neurobehavioral functions with concentrations in plasma and BAL fluid samples from Swiss albino mice. The rodent chamber can be utilized for risk assessment of occupational hazards such as complex pollutant mixtures and particulate matter aerosols. The inhalational chamber possesses the versatility to enable assessment of toxicological mixtures across sub-acute toxicity timelines.

Keywords Inhalation toxicity · Acute toxicity test · Whole-body chamber · Rodent toxicology · Behavioral toxicology · Respiratory system · Aerosol toxicity · Aroclor 1232 · Biphenyl · Polychlorinated biphenyl · Broncho-alveolar Lavage

Introduction

The first line of defense in any organism exposed to such intoxicants can be the skin (dermal exposures) or the gastrointestinal tract (oral exposures). But an especially important route of exposure is inhalation which should bypass the respiratory tract to produce toxicity in the organism. The dosing of inhaled toxicants and pathophysiologic responses to inhaled toxicants and mechanisms behind their toxicities are key determinants of current and future human health, due to various reasons. Currently pollution rates and pollutants levels in the environment including the atmosphere are increasing. Also, the increasing incidence and prevalence of respiratory disorders including occupational diseases and substance abuse through inhalation route could be considered as important justifications to carry out research in inhalation toxicology. The results of inhalation toxicity studies can then be used to extrapolate to further improve health and quality of life of patients. Clinical and pre-clinical studies of this nature make use of inhalation chamber models

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to evaluate the toxicology of complex inhaled intoxicants. Based on the design and the exposure protocols, inhalation chambers can be used to evaluate the toxic effects of general exposures like whole-body chambers, or more specific exposures, e.g., nose-only or head-only chambers. They can also be utilized to assess health effects after even more localized exposures like whole-lung or partial lung chambers in animal models or human participants (Pauluhn and Mohr 2000; Colli Neto et al. 2014). Being the oldest models described in inhalation toxicology, whole body chambers are widely used, much like the utility of nose-only chambers.

The utility of inhalation chambers for pharmacological and toxicological risk evaluations cannot be undermined at the cost of their limitations, like difficulty in experimental extrapolations to real-life situations, ethical issues in exposure methods, and the presence of concurrent routes of exposures and other confounders apart from the inhalation route. Though few research evaluations have utilized exposure chambers in modeling inhalation toxicities in the past, their applications are of importance in regulatory policy making (Pauluhn 2003; Raju et al. 2014).

Standard environment exposure systems exist for inhalation exposures of animals like the BuxCo® system used to evaluate the effects of printer emitted particles (Pirela et al. 2014) and the Madison whole body chamber in fungal inhalant evaluations (Springer et al. 2013), though they may not be suited to deliver semi-volatile chemical and bioactive mixtures with potent but undetermined toxicities. Secondly, these chemical mixtures and congeners are complex and persistent, which makes it prudent to afford for safe elimination after the experiment without much provision to cause pollution and accumulation.

Aroclor 1232 is a semi-volatile PCB mixture which contains many individual Polychlorinated biphenyl, PCB congeners. Of the representative PCB congeners, we selected PCB 77, a tetrachlorobiphenyl, and PCB 180, a heptachlorobiphenyl, both being of toxicological significance (ATSDR, 2000). PCB 77 is a dioxin-like PCB, whereas PCB 180 is a highly lipophillic, biomagnifiable PCB congener.

There are only a handful of studies reporting the successful development and design of inhalation chambers in avian and rodent models of exposure (Kumamoto et al. 2013; Shaheen et al. 2016).

We describe the design and development of a whole-body rodent inhalation chamber for sub-acute inhalational toxicity testing, from its conception to its utility in evaluation of neurobehavioral parameters in mouse models. Since nose-only chambers usually do not simulate the occupational exposure better than whole-body chambers, we have applied the latter in toxicity assessment of a PCB mixture in rodents (Swiss albino mice). We have used water, water plus methanol, and Aroclor 1232 (PCB mixture) aerosols for validation of the chamber. We have also quantified PCB 77 and PCB 180 concentrations in plasma and BAL fluid of mice, after dosing Aroclor 1232 at dose increments, and evaluated using behavioral responses. Lung histopathology was used to supplement these results. The research was carried out at PSG Center for Molecular Medicine & Therapeutics laboratory, from March 2015 to July 2018.

Materials and methods

Chemicals and reagents

The PCB mixture, Aroclor 1232 was obtained from Sigma-Aldrich Inc., India. PCB 77, PCB 180 HPLC-grade standards were obtained from Sigma-Aldrich Inc., India. Acetonitrile of HPLC grade was purchased from Sigma-Aldrich, India. HPLC grade of methanol (carbinol) and Methyl tert-butyl Ether were purchased from Hi-Media, India. Ultrapure water (18.2 mΩ cm) was obtained from Millipore (Milford, USA) using MilliQ water purification system. Disodium EDTA calcium hydrate crystals and Sodium Hydroxide crystals were purchased from Sigma-Aldrich Inc., India.

Animals

Swiss albino mice were procured and quarantined at the PSG Animal Facility. They were housed under ambient light–dark cycle and optimum environmental conditions with water *ad-libitum*. Prior Institutional Animal Ethics Committee (IAEC) approval was obtained for study execution. All mice were of age 8–12 weeks with body weights ranging from 17 to 28 g at initiation of experiments. The Animal Facility is accredited by the local Regulatory Authority, the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA 2003).

Structural design of the inhalation chamber

The inhalation chamber is a whole-body exposure chamber meant for small laboratory animals including laboratory rodents. The development process of the chamber has been undertaken in two stages viz., design-and-development, and characterization-cum-optimization.

Design and development

The whole-body inhalation exposure chamber consists of three different mechanical units. The units have been divided based on their utility and role in the function of the chamber—the inlet system, the central chamber system, and the outlet system. Additionally, the environment of the chamber system, and the connections of the systems are also included in the chamber construction (Fig. 1a).

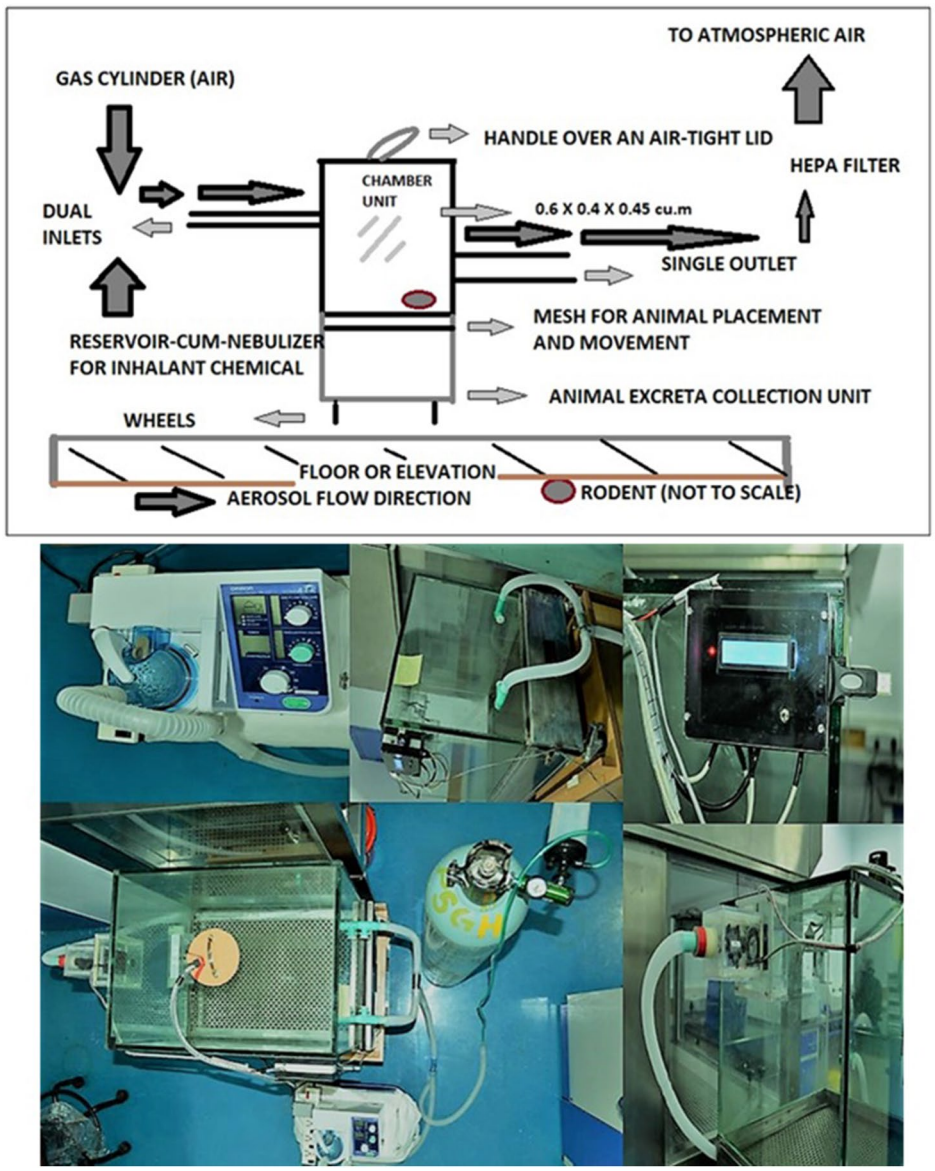


Fig. 1 Chamber Design—Line blueprint depicting chamber design (**a**) and photograph (**b**) depicting the key elements for inhalant exposures

Before initiation of the experimental procedure, the chemical mixture is prepared as per the dilution and dosing protocol of the experiment, for delivery of the test substance as aerosol particles. In order to achieve the desired flow rates of the aerosol particles by nebulization, air is used to forcibly propel the chemical mixture into the chamber after generation from the gas source (Air cylinder with control gauge and valve). The chemical pumping system (Ultrasonic nebulizer, Omron Inc.) is used to deliver the chemical aerosol of the test substance into the inhalation chamber. The central chamber core system is a glass transparent cuboid measuring 600 mm × 400 mm × 450 mm in dimensions. The base of this chamber comprises of another subunit made of stainless-steel mesh grid, below which a compartment is located to collect and dispose of the animal wastes. The roof of the chamber has a 10-cm diameter hole which can be sealed off or opened to place or retrieve the experimental animals. The wall along a breadth side has two inlet openings, each with a diameter of 10 cm, located about 10 cm from the base of the wall, which are connected to the aerosol pumping-cum-delivery system. The base compartment can be removed like the lid covering the roof hole, both of which are air-tight, then sealed to avoid gas or aerosol particles from escaping into the external environment. The internal environment of the chamber is controlled by five different sensors connected to a display panel through wires and a printed circuit board (pressure, temperature, relative humidity, oxygen level, and flow rate). This panel is then connected to a computer software (Serial Input–Output interface, SIOW) console for recording and storing the readings of different atmospheric variables. The outlet is meant for maintaining a uniform circulation and pressure of the aerosol particles in the chamber

atmosphere by continuously removing the exhaled gases and particles from the chamber. To achieve this purpose, the outlet system, consisting of a single circular hole, is located on the contralateral wall of the chamber about 40 cm from the base of that wall, with a diameter of 10 cm. A fan is encased behind the outlet opening for providing continuous ventilation to animals and rotates with a mean speed of 3400 rpm to achieve uniformity of flow rates inside the chamber. The terminal end of the outlet opens onto a Class II Type A_1 Biohazard workplace system with HEPA filters to eliminate the aerosols after circulation and recirculation inside the chamber system.

Five different sensors were pre-calibrated and fitted onto the chamber. These sensors were installed to continuously detect relative humidity, pressure, temperature, oxygen concentration and flow rate inside the chamber. We measured Mass Median Aerodynamic Diameter (MMAD) in the room wherein the chamber was housed during the experiment on three occasions using an impact cascade method. The Geometric Standard Deviation (GSD) of MMAD was calculated using the following formula:

$$\mathrm{GSD} = \frac{84.1\%\ \text{mark}}{50\%\ \text{mark}}$$

Allowed limits for the chamber environment parameters must not exceed 20%, and oxygen concentration must not fall below 19% in the chamber. The time to chamber equilibrium, $t\text{-}_{95\%}$ was calculated using the following formula:

$$t - 95\% = 3 \times \frac{\text{Chamber Volume}}{\text{Air flow rate in chamber}}$$

Periodical and regular variations in the chamber environmental conditions were recorded and visualized onto a display panel and computer console using Serial Input and Output software (SIOW, software v 1.2, hardware v 1.1, CCS, Inc.).

Characterization and optimization

Experiment 1 We checked for hourly variations in chamber atmosphere (Pressure, temperature, Relative humidity, flow rate and oxygen content) in response to water nebulization. The experiment was carried out water aerosol (350 mL daily dosing volume) for 4 h/day for two weeks (five days / week).

Experiment 2 The procedure consisted of standardization of methanol and water ratio using 54 animals inside for assessing any behavioral effects on animals. For the initial tests, a dose ranging from 0.1 to 1000 ppm of water aerosol in methanol was used in different combinations with dosing durations (1 s, 3 min, 5 min, 10 min, 30 min). Any acute signs of intoxication or mortality were noted later at each of these dose levels using 6 mice, with equal sex ratio. Animals were randomized using a Latin square design to various combinations of dose levels and dosing durations based on a c x t protocol. The behavior was measured periodically using Irwin scale (Table 1, Irwin, 1968).

Experiment 3 To assess the uniformity of aerosol distribution inside the chamber, PCB concentrations were measured after 4 mg/cu.m of Aroclor 1232 for 6 h-duration was introduced into the chamber without animals inside. The floor of the chamber interior was divided into 3×3 equal spatial circles. In each of these circular divisions, a 5 mL glass beaker was placed with 2 mL of 1:1 methanol: water inside. The analytes (PCB 77 and PCB 180) were quantified by Ultra-high performance liquid chromatography with Diode array detection (UPLC-DAD) after 6 h of exposures in each of the beakers (Ramanujam et al. 2017).

Risk assessment of PCB mixture (single duration exposure)

Experiment 4: Aroclor 1232 (1 mg/cu.m) in methanol–water aerosol at 1 ppm was used to ensure reproducibility of chamber conditions with animals. This was performed on three different days for assessment of replicability of acute 4-h behavioral toxicity. The behavior was measured periodically, and the respiratory physiological parameters were calculated, using the following formulae:

$$\text{volume of dissolved oxygen}\left(\frac{\text{ml}}{\text{dl}}\right) = 0.003 \times P02$$

Experiment 5 Three male and three female Swiss albino mice were exposed to 1 mg/cu.m of Aroclor 1232 for 6 h to assess gender-differences in behavioral scores. Irwin scale was used to assess behavioral scores across all eleven subdomains, and forty-seven functions from six physiological systems in Swiss albino mice.

Broncho-alveolar lavage (BAL) analysis

Experiment 6 Twelve female Swiss albino mice (12–14 weeks age) were assigned to four cages (three mice per cage), weighed and acclimatized. Isotonic (0.65%) sterile BAL ringer was prepared 6.5 g NaCl in 700 ml double distilled water with citric acid as buffer, till p H is adjusted to 7.0–7.2 at temperature of 4 °C. Methanol 1 ppm was used for exposure in the vehicle control group. Aroclor 1232 at 2, 4, and 16 mg/cu.m was used for test group exposures, with

Table 1 Table depicting Irwin behavioral checklist and normative scores for behavioral functions

Behavioral domain	Behavioral sub-domain	Normative score
Awareness		8
	Alertness	4
	Visual placing	4
	Passivity	0
	Stereotypy	0
Mood		4
	Grooming	4
	Vocalization	0
	Restlessness	0
	Irritability/aggression	0
	Fearfulness	0
Motor activity		16
	Reactivity to environment	4
	Spontaneous activity	4
	Touch response	4
	Pain response	4
CNS excitation		0
	Startle response	0
	Straub tail	0
	Tremors	0
	Twitches	0
	Convulsions	0
Posture		8
	Body posture	4
	Limb position	4
Motor incoordination		0
	Staggering gait	0
	Abdominal gait	0
	Righting reflex	0
Muscle tone		16
	Limb tone	4
	Grip strength	4
	Body sag	0
	Body tone	4
	Abdominal tone	4
Reflexes		12
	Pinna	4
	Corneal	4
	IFR (Ipsilateral flexor reflex)	4
Autonomic		20
	Writhing	0
	Pupil size	4
	Palpebral opening	4
	Exophthalmos	0
	Urination	0
	Salivation	0
	Piloerection	0
	Hypothermia	0
	Hypothermia	0
	Skin color	4
	Heart rate	4
	Respiratory rate	4
Miscellaneous		4
	Exploratory activity	2
	Hostility	0
	Social behavior	2
Lethality/dead	Acute	0
	Delayed	0
Total score (normative)		88

three mice per group. Exposures were carried out for 6 h per group, and tracheostomy was performed after termination of exposure under Ketamine (75 mg/kg I.P) – Xylazine (3 mg/kg I.P) using 25 G needle. After tracheostomy was performed, a 40 n. thread was inserted under trachea, 21 G needle with long (25 cm) tube inserted to its other end, and using 1 m L syringe, 500 mcL of BAL fluid was pushed through plunger and aspirated thrice, finally being collected onto 2.5 mcL microcentrifuge tubes (Tarsons Inc.) for centrifuge at 300 g for 5 min till storage at 4 °C, and further analysis after freeze-thawing. Analysis was carried out for cytology (Giemsa stain for Total and differential counts), and UPLC-DAD estimation of PCB 77 and PCB 180; the analysis was carried out using a pre-defined protocol (Ramanujam et al. 2017).

Lung histopathology

Experiment 7 Ice cold PBS perfusate was prepared overnight and after tracheostomy was performed, the trachea and lungs were irrigated with the perfusate. This was followed by dissection of the carinae and / or lung parenchyma for storage in 8% Formalin solution for histopathological analysis at 40× in H&E stain.

Applicability to behavioral toxicology risk assessment: acute toxicity

Experiment 8 An acute Aroclor 1232 toxicity study was used to determine the sex differences and reproducibility

of behavioral scores on three days in response to 4 mg/cu.m exposure dose level for 6 h. For this objective, three male and three female mice behavior were compared for their variability. To ascertain the constancy of animal concentrations from different spatial co-ordinates inside the chamber, all animals were anesthetized for retro-orbital sampling of plasma. PCB 77 and PCB 180 were then quantified in these plasma samples using UPLC-DAD method in these six mice (three males and three females) after 6 h of exposure to 4 mg/cu.m of Aroclor 1232 (Ramanujam et al. 2017).

Benchmark dose derivation

Experiment 9 Acute toxicity study with control, 1, and, 4 mg/cu.m of Aroclor 1232 in 8 female Swiss albino mice each with a pre-defined Bench Mark Response Rate (BMR) from control of ± 50% change in basal behavioral scores and subsequent benchmark dose modeling in the BMDS version 2.4 (BMD Software, US-EPA) using polynomial model (Fig. 2):

$$y\,(\text{dose}) = \text{control} + \text{slope} * \text{dose}^{\text{power}}$$

Applicability to behavioral toxicology risk assessment: repeated dose neurotoxicity

Experiment 10 The optimization was further enhanced by addition of operant or instrumental assessments of mice exposed to Aroclor 1232 aerosols (4 mg/cu.m) for two-week occupational protocol. Briefly the mice were exposed to water aerosols (control group) via inhalational route, followed by washing of chamber thrice with 20% Isopropyl alcohol in methanol. This was followed by exposure to Aroclor 1232 at benchmark dose in similar manner. Occupational exposure was defined as 6 h a day (9 am to 3 pm), 5 days per week for two weekly exposures for this dosing group. Assessments were performed for neuro-cognitive endpoints a baseline, after one week and at termination for each dose level, on Y-maze, elevated plus maze and open field box in both groups. (Narayanan et al. 2017).

Results and discussion

Chamber (Fig. 1b) characterization, validation and optimization protocols were successful in yielding uniform and reproducible results in animal behavior and survival, within acceptable variability limits.

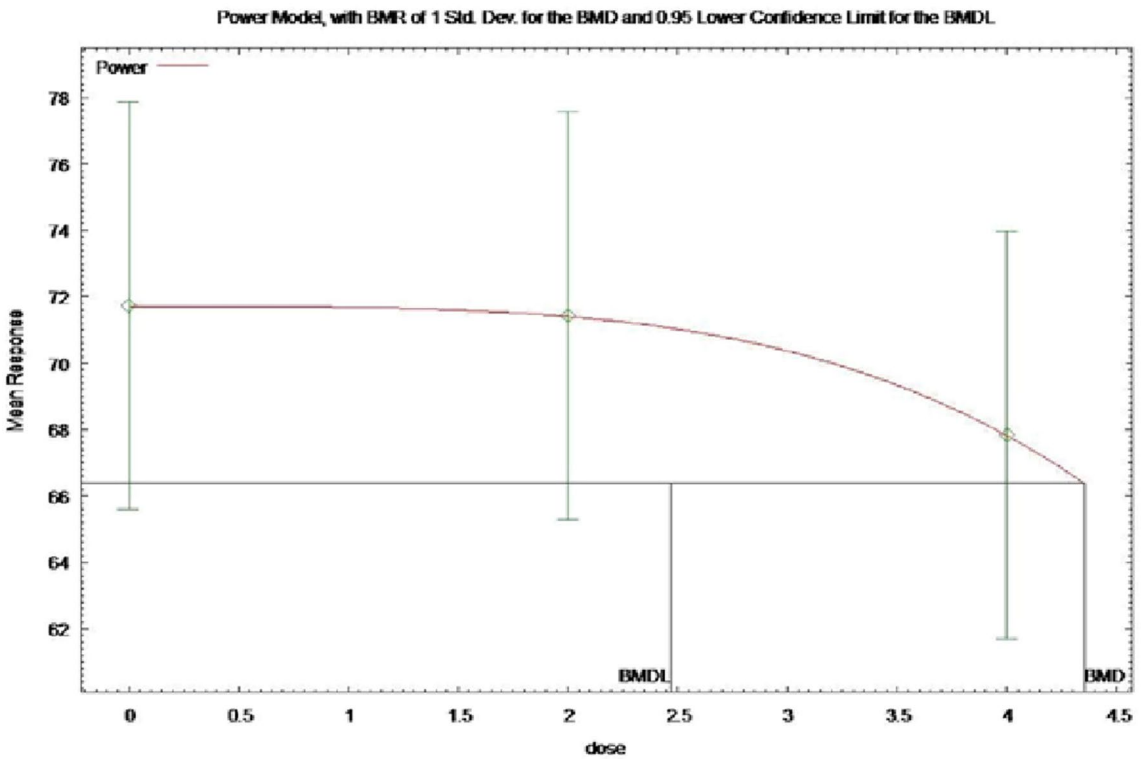


Fig. 2 Dose Response graph depicting the Bench-Mark Dose (BMD) with 95% Limit (BMDL) from the US-EPA BMDS Version 2.6, using Power modeling

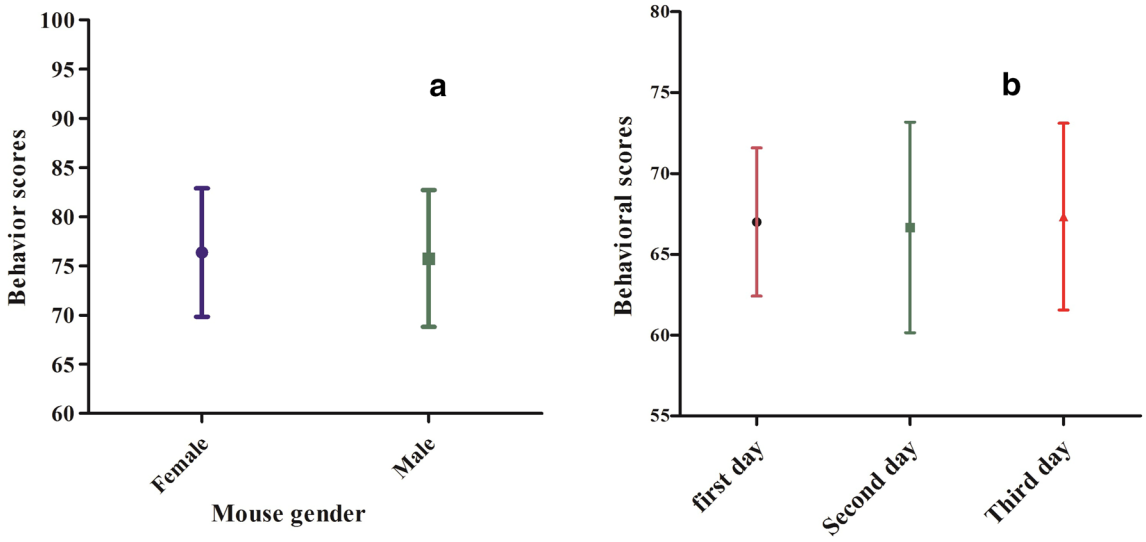


Fig. 3 **a** Chamber optimization for behavioral toxicity. **b** The effects are reproducible acutely. 3-male and 3-female mice were used for all three tests, at 1 ppm dose of water aerosol

Development

The chamber dimensions are well within the CPCSEA-regulatory limit of cage size for laboratory mouse (CPCSEA 2003). We optimized the air flow and PCB flow patterns inside the chamber in-silico using different permutations of inlet and outlet and the physicochemical properties of PCBs. Based on the simulation experiments, the best inlet–outlet configuration was determined to be with two-inlet and single-outlet chamber design (Fig. 3). The inlets were placed below the plane of the contralateral outlet, for optimum flow and uniform particle distribution in the chamber (Table 2). As per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, the minimum requirement for one mouse, each weighing up to 25 g, is 77.4 sq. cm per mouse with a height of 12 cm (cage) (CPCSEA 2003). Using a cascade impactor, we estimated the MMAD to be well within the Organization for Economic Co-operation and Development (OECD) toxicity guidelines (OECD 2009). The MMAD with GSD of the particle sizes was 4.3 µm ± 1.7 µm (Table 3). The time to 95% equilibrium ($T\text{-}_{95}$) in the aerosol under the chamber conditions was found to be 7.27×10^{-1} s. Then after the apparent completion of $T\text{-}_{95}$ as theoretically calculated, the inhalation exposure to test aerosol was initiated at pre-calibrated flow rates and other nebulization parameters.

Characterization

We have analyzed the environmental conditions in the chamber using five different sensors connected to a panel and a software console. Similarly, hourly atmospheric variations did not exceed 20%, which was preserved in our limit test and the sighting study over two-week exposures (Table 4). Oxygen saturation in the chamber was well at 21%.

The optimum ratio of methanol–water was 1:1 with optimum dosing duration of ≥ 90 min, based on minimal behavioral disruptions achieved for this protocol, compared to other methanol to water ratio x dosing duration permutations. The exposure duration for optimization of vehicle-solvent (methanol: water) ratio was below the 4 h as per OECD requirements

Table 2 Table depicting chamber optimization parameters: chamber environment variables assessed using pre-calibrated sensors

Environment variable	Mean ± SD
Flow rate (L/min)	16 ± 1
Oxygen (%)	21 ± 0
Pressure (mm Hg)	735 ± 8
Temperature (°C)	25 ± 0.2
Relative humidity (%)	50 ± 3.2

Table 3 Optimum particle size distribution conditions obtained from MMAD measurements inside the chamber

Particle size (mcm)	Particle distribution (Σm^3)
0.3	16,461,620
0.5	1,219,929
1	94,982
3	6996
5	1696
10	0

for acute inhalation toxicity. Based on this experiment, the behavioral scores did not vary much across different dosing combinations of methanol to water (77 ± 6). However, the variation was minimum with a 1:1 ratio of methanol to water for all durations tested in this phase.

Optimization

After 6-h exposure to 4 mg/cu.m of Aroclor 1232, the entire nine beakers (3 × 3 equal spatial circles) containing samples were quantified for PCB 77 and 180 using UPLC-DAD. PCB 77 and PCB 180 concentrations in the all the beakers were found to be 1795.65 ± 6.41 and 1964.42 ± 18.24 ng/mL. Hence, this indicates uniform distribution of test aerosols across the chamber floor and its vicinity.

No significant change in behavioral scores between male and female mice was observed after exposure to 4 mg/cu.m × 6 h of Aroclor 1232 (Fig. 4).The results of behavioral assessment of Aroclor 1232 acute exposures on three similar occasions with the exposure duration of four hours as shown in Fig. 4. The mean behavioral scores for water aerosol-treated animals were 65 ± 15.

The mean plasma PCB 77 and PCB 180 concentrations were 144.00 ± 16.51 ng/mL and 323.03 ± 34.95 ng/mL, respectively, at 6 h of exposure to 4 mg/cu.m in six (1:1: Male: Female) Swiss albino mice. The minimum variability of concentrations between animals in the group may be due to their movement across different parts of the inhalational chamber and the resultant uniform distribution of the PCB mixture at the bottom of the chamber during the exposures, as shown by the simulation results.

BAL fluid analysis and lung histopathology

The cytological analysis of BAL fluid samples showed dose-dependent leukocytosis with lymphocytes occupying majority of the WBCs in low power under Giemsa stain (Fig. 5a). The control (Methanol) group exhibited minimal signs of alveolar edema in the lung parenchymal specimen (Fig. 5a). There was dose-dependent increase in alveolar inflammation, mild interstitial fibrosis, alveolar edema, tracheitis and eosinophilic and mast cell infiltrates in the treatment groups (Fig. 5b–d). HPLC-FLD analysis of BAL-fluid showed dose-dependent increase in PCB 77 and PCB 180 concentrations (Fig. 5b).

Benchmark dose

The median scores of behavior in control (water aerosol), 2 mg/cu.m, and 4 mg/cu.m Aroclor 1232 dose levels were nearly 72, 71, and 68, respectively, at 2–4 h of single 6 h exposures, respectively (Fig. 4). The change in scores was more than 50% (behavioral depression) in the 2 mg/cu.m versus 4 mg/cu. group, as compared to control versus 2 mg/cu.m group comparison. Using a polynomial model,

Table 4 Normative values for neuro-cognitive indices assessed during the validation of the chamber using water aerosolization for 2 weeks at 1 ppb dose

Paradigm	Index	Week-0	Week-1	Week-2	*P* Value for the temporal interactions
Y-Maze novelty preference Test	NOR index (%)	60.72	56.29	47.30	No significant temporal interaction
Y-Maze alternation test	Alternation index (%)	31.83	17.42	25.28	No significant temporal interaction
Elevated plus maze anxiety test	Transfer latency (s)	31.33	23.00	22.50	No significant temporal interaction
Open field box test	Spontaneous motor activity index	21.95	22.57	25.61	No significant temporal interaction

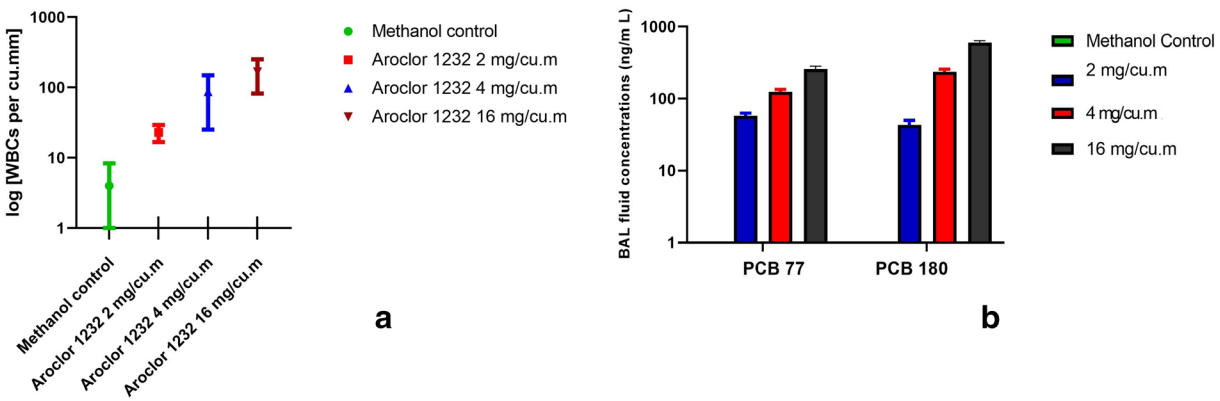


Fig. 4 **a** Bar chart depicting dose-dependent leukocytosis in the BAL-fluid cytology. **b** Bar chart depicting the dose-dependent PCB 77 and PCB 180 concentrations in the BAL-fluid aspirates

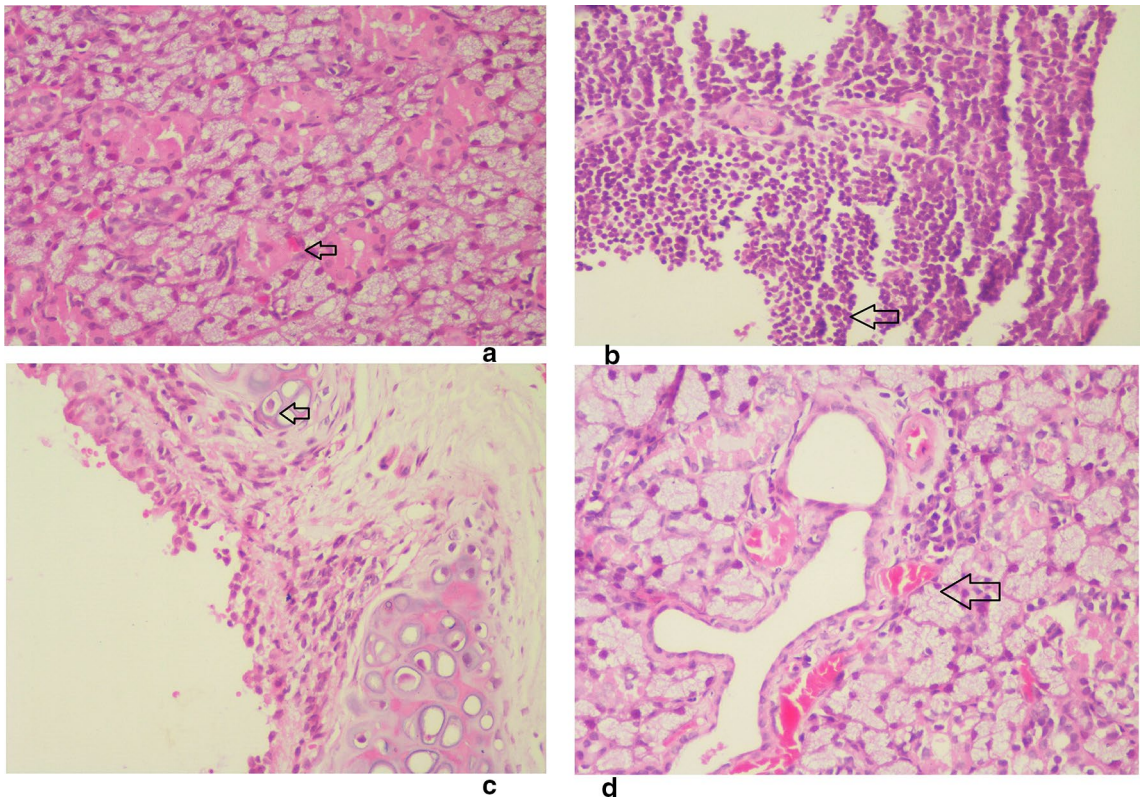


Fig. 5 **a** H&E stain (40x) showing alveolar edema in the methanol group. **b** H&E stain (40x) showing tracheitis in the low dose group. **c** H&E stain (40x) showing dense eosinophilic infiltrates in the intermediate dose group. **d** H&E stain (40x) showing mast cells and alveolar inflammation in high dose group

calculations on the BMD (Bench Mark Dose) Software yielded a BMD of nearly 4.35 mg/cu.m [BMDL = 2.46 mg/cu.m, 95% Confidence Interval for risk of deviation of control values from SD of test dose values, log-likelihood test, $p < 0.003$] for single 6 h exposures.

Sub-acute risk assessment

The neuro-cognitive indices across three time points over two-week water exposure at 1 ppm of water aerosol show preservation of the normative ranges, as shown by the two-way ANOVA (Table 4), for temporal interactions of each mean value. Hence, the chamber can be used for neurobehavioral efficacy or toxicity testing of chemicals by aerosols with minimum temporal variations. Also, the mean behavioral scores showed minimal inter-day and intra-group variations, which correlated well the neuro-cognitive indices of mice.

Discussion

We describe the successful designing, development, and characterization of a whole-body inhalation chamber for exposing mouse to aerosols and pollutant mixtures (Fig. 1b). Inhalation chambers can be used in animals and less commonly in human participants to deliver physical, chemical, or even biological agents to the animal model via the inhalation route. For example, inhalation of smoke from e-cigarettes have shown to decrease phagocytosis and other host defenses in mice following infection with respiratory bacteria and viruses in a whole-body chamber (Sussan et al. 2015). Similarly, using an inhalation chamber, pregnant mice exposed to diesel exhaust particles on daily basis exhibited developmental abnormalities in the off spring as determined by altered brain DNA methylation markers independent of -CpG- islands. Chemical agents like Acrolein have also been delivered to the respiratory tract and detected in the circulatory systems of animal models using inhalation chambers and gas cylinder systems (Tully et al. 2014). Fungal agents

like *Aspergillus conidia* have also been delivered to the respiratory tract of mice as a part of model development using inhalation chambers (Buskirk et al. 2014). Likewise, to evaluate the teratogenic effects of alcohol and alcohol spectrum disorders in mice, vapor chambers were designed and used successfully to deliver alcohol vapors(Morton et al. 2014). Studies assessing inhalation risk with vapor measurements in the upper respiratory tract and silicon dioxide, design of Aluminum chambers for rodent exposures(O'Shaughnessy et al. 2003), histopathological assessment after exposures to quartz exist in publication databases, with provision for risk assessment of diverse xenobiotic exposures.

Although the nose-only exposure chambers for rodents utilize or allow less durations of exposures (Duch et al. 2014) than whole-body chambers because of the differences in the portal of entry of aerosols, the chamber we developed was successfully tested at lower durations also. Unlike earlier semi-open inhalation chambers which were used for chronic dosing at high concentrations, the chamber we have developed is versatile and could be used for dosing low to high concentration aerosols and vapors from acute to chronic protocols. However, we also agree that our chamber can be used only for small laboratory animals including laboratory rodents (OECD 2009). It cannot be used for large animals like rabbits and monkeys which are more closely related to humans than rodents, making extrapolations of research data to humans more reliable in the latter.

Acute or even repeated dose toxicity studies in short term (sub-acute and sub chronic durations) do not affect the histology of lung of mice adversely as reported recently (Umezawa et al. 2013), which was taken as a rationale for acute testing of mice in ours study in the inhalation chamber. Although lung-only or nose-only exposure systems (Weber et al. 2010) would directly deliver the inhalant to the lungs than a whole-body inhalation chamber, they either do not reach lower airways, or do more damage if at all they reach the lower airways, and are better suited for vapors than aerosols. Since the conditions in the chamber were normobaric with Pressures ranging from 731–737 mm Hg, we are certain that the flow rates used will not affect the mouse respiratory physiology adversely.

Though the inhalation chamber was developed to evaluate the effects of pollutant aerosols with complex chemistry as described previously by others (Mainelis et al. 2013; McIntosh-Kastrinsky et al. 2013), the chamber can also be applied for a range of utilities ranging from cardio-respiratory assessments to chronic respiratory and occupational toxicological evaluations of pollutant mixtures. Indeed, it took nearly 0.7 s for the chamber's internal environment to equilibrate, which is very quick; preventing cross-contamination. Recently such assessments have been reported elsewhere using simple exposure systems (Weber et al. 2010; Gatzioufas et al. 2013). Many of these address the designing of nose-only inhalational chamber meant for short-term exposure durations of drug aerosols (Kaur et al. 2020). Nanotoxicology research inputs have also been used previously in the characterization of inhalation chambers (Taquahashi et al. 2013; Yi et al. 2013). Usually, respirable fraction via dry powder inhaler for rodents ranges from 47–54% and that via nebulizer ranges from 34–73% in previous studies(Sinha and Mukherjee 2012). Although nose-only inhalation is preferred to whole-body exposures to deliver drugs to target tissue specifically, whole body chambers simulate occupational exposures more naturally in rodents than nose-only chambers though the deposition of particles in lungs may be similar by both types of inhalation chambers (Pauluhn 2006; Oyabu et al. 2016). Hence, though nose-only inhalation usually achieves greater respirable concentrations and is specific for delivery of aerosol mixtures into the alveoli than whole-body chambers, the mode of aerosol generation which is reflected upon by time to equilibrium ($T\text{-}_{95\%}$) is more important determinant of total body concentrations of test mixtures than the method of aerosol exposure.

Although physical parameters in the chamber like humidity and oxygen saturation are key confounding determinants of the health of animal housed and exposed (Springer et al. 2013; Reis et al. 2013), we have maintained the chamber environment under strict allowed limits to ensure safety of the animals exposed in our study and avoid variations in the study results.

We conclude with certain limitations of our study such as the requirement of aerosol particles to be within the limits set by regulatory authorities may be difficult to meet with due to the complex chemical and unknown physical properties of the inhaled intoxicants and the non-generic nature of our chamber in that it can house small rodents only. We, however, have simulated the chamber conditions and ensured the safety of the housed animals with the uniformity of the results as incorporated in actual chamber development and testing (Olsgard and Smits 2008).

A novel technique of a whole-body inhalation chamber optimization has been reported and this type of biologically-accountable chamber can be used for inhalation neurological or neurotoxicity studies, climatic simulation experiments with animals and pharmacology and physiology studies after dosing drugs via inhalational route, with strict compliance to standard regulatory guidelines. In conclusion, the inhalational chamber developed in the above-mentioned manner is versatile and robust; it could cause minimum neuro-cognitive and behavioral alterations during the sub-acute exposure timelines, in small rodents. This inhalation chamber can be applied in regulatory pharmacology studies (e.g. aerosol/drug nebulization studies), toxicology studies (e.g., pollutants' inhalational risk assessment in rodents), and in biohazard studies (e.g., suspended viral hazards—risk assessments in rodents). It

can be specifically curated to neuropharmacology and neurotoxicity efficacy and toxicity studies in rodents via inhalational route.

Conclusion

The whole-body inhalation chamber has been constructed, developed, optimized, and validated for use in rodents to assimilate the Aroclor 1232 toxicity data at acute and subacute timelines. To achieve this objective and outcome, the in-vitro validation was confirmed with uniformity studies of PCB concentration across different chamber floor quadrants, followed by in vivo penetrability and neurobehavioral risk assessment of PCBs. To this end, Irwin scale and interoceptive memory and higher functional battery of tests were used to evaluate the risk hazard of Aroclor 1232 as an indoor and industrial occupational hazard as per regulatory guidelines for neurotoxicity studies in mice. The environmental and analytical optimization parameters hence developed can be applied to higher rodent models including rats and guinea pigs as per standard animal housing guidance available world-wide. The final output, i.e. the inhalational chamber hence developed could be utilized for risk assessment of complex chemical mixtures, including Aroclors, and related dioxins, via inhalational route, in regulatory toxicology studies assessing persistent environmental pollutants as occupational hazards. However, despite limitations like non-applicability of this inhalational chamber for risk assessment of non-volatile, non-hydrophilic, and non-air pollutant chemicals in rodents, these applications can be further explored, in addition to the currently broad scope of this chamber. Further, the BAL assessments and MMAD measurements confirm lower airway penetrability of PCBs, as a novel regulatory platform, confirming the chamber's robustness and working mechanism.

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Author contributions RN: Contributed to the study design and execution, apart from manuscript writing. SM: Contributed to study execution and inputs on chromatography, apart from manuscript editing. VLS: Contributed to BAL-fluid analysis for cytology and lung histopathology. KR: Contributed to chamber design and development. GB: Gave inputs on chamber design, validation, and construction. RS: Expert inputs on chamber design and optimization and in manuscript editing.

Declarations

Conflict of interest The author(s) do not have any conflict(s) of interest(s) to declare.

Ethical approval The study has been performed with incorporation and in compliance with Indian ethical standards for animal research (CPCSEA Guidelines, 2005), and international regulatory standards for toxicological research (OECD Guidance on Inhalational toxicity studies). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted and ethical approval was obtained from the PSG Institutional Animal Ethics Committee, PSG-IAEC Approval Nos. 207/2013/IAEC, 207/2014/IAEC, 207/2015/IAEC [158/PO/ReBi/SL/99/CPCSEA] and PSG-IAEC Approval No. 392/2018/IAEC [158/PO/ReBi-S/Re-L/99/CPCSEA].

References

Buskirk AD, Green BJ, Lemons AR et al (2014) A murine inhalation model to characterize pulmonary exposure to dry Aspergillus fumigatus conidia. PLoS ONE 9:e109855. https://doi.org/10.1371/journal.pone.0109855

Colli Neto JA, Zen Júnior JH, Del Negro A et al (2014) Tobacco experimental model to induce urinary bladder neoplasms. Rev Col Bras Cir 41:56–60. https://doi.org/10.1590/s0100-69912014000100011

CPCSEA (2003) CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol 35:257–274

Duch P, Nørgaard AW, Hansen JS et al (2014) Pulmonary toxicity following exposure to a tile coating product containing alkylsiloxanes. A clinical and toxicological evaluation. Clin Toxicol Phila Pa 52:498–505. https://doi.org/10.3109/15563650.2014.915412

Gatzioufas Z, Hafezi F, Kopsidas K, Thanos S (2013) Dysfunctional uveoscleral pathway in a rat model of congenital glaucoma. J Physiol Pharmacol off J Pol Physiol Soc 64:393–397

Kaur R, Kaushik A, Singh KK et al (2020) An efficient and cost-effective nose-only inhalational chamber for rodents: design optimization and validation. AAPS PharmSci Tech 21:82. https://doi.org/10.1208/s12249-019-1608-4

Kumamoto T, Tsukue N, Takano H et al (2013) Fetal exposure to diesel exhaust affects X-chromosome inactivation factor expression in mice. J Toxicol Sci 38:245–254. https://doi.org/10.2131/jts.38.245

Mainelis G, Seshadri S, Garbuzenko OB et al (2013) Characterization and application of a nose-only exposure chamber for inhalation delivery of liposomal drugs and nucleic acids to mice. J Aerosol Med Pulm Drug Deliv 26:345–354. https://doi.org/10.1089/jamp.2011-0966

McIntosh-Kastrinsky R, Diaz-Sanchez D, Sexton KG et al (2013) Photochemically altered air pollution mixtures and contractile parameters in isolated murine hearts before and after ischemia. Environ Health Perspect 121:1344–1348. https://doi.org/10.1289/ehp.1306609

Morton RA, Diaz MR, Topper LA, Valenzuela CF (2014) Construction of vapor chambers used to expose mice to alcohol during the equivalent of all three trimesters of human development. J vis Exp JoVE. https://doi.org/10.3791/51839

Narayanan R, Gilbert B, Ravichandran S, Bhuvaneswari K (2017) Psychopharmacological characterization of effects of Ferula asafoetida Linn formulation in mouse on a Y-maze, EPM, and open field apparatus. Int J Basic Clin Pharm 6:2254–2258

O'Shaughnessy PT, Achutan C, O'Neill ME, Thorne PS (2003) A small whole-body exposure chamber for laboratory use. Inhal Toxicol 15:251–263. https://doi.org/10.1080/08958370304504

OECD (2009) GD 39, OECD guidance document on acute inhalation toxicity testing. The Environment, Health and Safety Division. Organization for Economic Cooperation & Development, EU

Olsgard ML, Smits JEG (2008) The design, construction, and operation of a whole-body inhalation chamber for use in avian toxicity studies. Inhal Toxicol 20:191–197. https://doi.org/10.1080/08958370701821367

Oyabu T, Morimoto Y, Izumi H et al (2016) Comparison between whole-body inhalation and nose-only inhalation on the deposition and health effects of nanoparticles. Environ Health Prev Med 21:42–48. https://doi.org/10.1007/s12199-015-0493-z

Pauluhn J (2003) Overview of testing methods used in inhalation toxicity: from facts to artifacts. Toxicol Lett 140–141:183–193. https://doi.org/10.1016/s0378-4274(02)00509-x

Pauluhn J (2006) Mosquito coil smoke inhalation toxicity. Part I: validation of test approach and acute inhalation toxicity. J Appl Toxicol JAT 26:269–278. https://doi.org/10.1002/jat.1140

Pauluhn J, Mohr U (2000) Inhalation studies in laboratory animals–current concepts and alternatives. Toxicol Pathol 28:734–753. https://doi.org/10.1177/019262330002800514

Pirela SV, Pyrgiotakis G, Bello D et al (2014) Development and characterization of an exposure platform suitable for physico-chemical, morphological and toxicological characterization of printer-emitted particles (PEPs). Inhal Toxicol 26:400–408. https://doi.org/10.3109/08958378.2014.908987

Raju SV, Tate JH, Peacock SKG et al (2014) Impact of heterozygote CFTR mutations in COPD patients with chronic bronchitis. Respir Res 15:18. https://doi.org/10.1186/1465-9921-15-18

Ramanujam N, Sivaselvakumar M, Ramalingam S (2017) Fast and parallel determination of PCB 77 and PCB 180 in plasma using ultra performance liquid chromatography with diode array detection: a pharmacokinetic study in Swiss albino mouse. Biomed Chromatogr BMC. https://doi.org/10.1002/bmc.4000

Reis RB, Nagato AC, Nardeli CR et al (2013) Alterations in the pulmonary histoarchitecture of neonatal mice exposed to hyperoxia. J Pediatr (rio J) 89:300–306. https://doi.org/10.1016/j.jped.2012.10.003

Shaheen HM, Onoda A, Shinkai Y et al (2016) The ceramide inhibitor fumonisin B1 mitigates the pulmonary effects of low-dose diesel exhaust inhalation in mice. Ecotoxicol Environ Saf 132:390–396. https://doi.org/10.1016/j.ecoenv.2016.06.025

Sinha B, Mukherjee B (2012) Development of an inhalation chamber and a dry powder inhaler device for administration of pulmonary medication in animal model. Drug Dev Ind Pharm 38:171–179. https://doi.org/10.3109/03639045.2011.592532

Springer DJ, Saini D, Byrnes EJ et al (2013) Development of an aerosol model of Cryptococcus reveals humidity as an important factor affecting the viability of Cryptococcus during aerosolization. PLoS ONE 8:e69804. https://doi.org/10.1371/journal.pone.0069804

Sussan TE, Gajghate S, Thimmulappa RK et al (2015) Exposure to electronic cigarettes impairs pulmonary anti-bacterial and anti-viral defenses in a mouse model. PLoS ONE 10:e0116861. https://doi.org/10.1371/journal.pone.0116861

Taquahashi Y, Ogawa Y, Takagi A et al (2013) Improved dispersion method of multi-wall carbon nanotube for inhalation toxicity studies of experimental animals. J Toxicol Sci 38:619–628. https://doi.org/10.2131/jts.38.619

Tully M, Zheng L, Acosta G et al (2014) Acute systemic accumulation of acrolein in mice by inhalation at a concentration similar to that in cigarette smoke. Neurosci Bull 30:1017–1024. https://doi.org/10.1007/s12264-014-1480-x

Umezawa M, Sekita K, Suzuki K-I et al (2013) Effect of aerosol particles generated by ultrasonic humidifiers on the lung in mouse. Part Fibre Toxicol 10:64. https://doi.org/10.1186/1743-8977-10-64

Weber WM, Kracko DA, Lehman MR et al (2010) Inhalation exposure systems for the development of rodent models of sulfur mustard-induced pulmonary injury. Toxicol Mech Methods 20:14–24. https://doi.org/10.3109/15376510903483730

Yi J, Chen BT, Schwegler-Berry D et al (2013) Whole-body nanoparticle aerosol inhalation exposures. J vis Exp JoVE. https://doi.org/10.3791/50263

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ORIGINAL ARTICLE

Association of Gastroesophageal Reflux Disease with Anxiety, Depression, and Sleep Disorders

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Background: Gastroesophageal reflux disease (GERD) is commonly associated with impaired quality of life. Chronic symptoms in this highly prevalent disorder could potentially lead to psychological manifestations such as anxiety, depression, and sleep disorders. **Aim:** The purpose of the study was to evaluate the magnitude of association of GERD symptoms based on health-related quality of life (GERD-HRQL) with anxiety, depression, and sleep quality using Hospital Anxiety and Depression Scale (HADS) and Pittsburgh Sleep Quality Index (PSQI). **Methods:** This was a cross-sectional study done at a tertiary care hospital in 241 participants. Cases were patients diagnosed with GERD, and the control group was participants who did not have GERD, devoid of all gastrointestinal symptoms according to GERD-HRQL. Data on age, gender, body weight, smoking/alcohol intake, and medication history were obtained from 98 cases and 143 matched control subjects. The three questionnaires, namely, GERD-HRQL, HADS, and PSQI questionnaires were administered by a trained blinded interviewer. **Results:** There was a statistically significant difference ($P < 0.001$) in the mean score of cases in the HADS depression scale (7.35 ± 3.65); the HADS anxiety scale (9.33 ± 4.51) and the mean global PSQI sleep score (4.62 ± 1.96) compared to the controls. Using Pearson's correlation, heart burn ($P = 0.036$), and regurgitation scores ($P = 0.026$), including the global quality of life score ($P = 0.003$), correlated significantly with the anxiety score. We found a statistically significant correlation between heart burn and poor sleep ($P = 0.012$) but not for regurgitation ($P = 0.772$). **Conclusions:** This study highlights the increased risk of anxiety, depression, sleep disorder in GERD, and the significant correlation between HRQL, especially heartburn with anxiety and poor sleep quality. This enlightens that specific screening and treatment strategies targeting such psychological manifestations are imperative for overall improved quality of life in GERD patients.

Key words: Mood disorders, sleep quality, Hospital Anxiety and Depression Scale, Pittsburgh Sleep Quality Index, gastroesophageal reflux disease-health related quality of life

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic disorder characterized by reflux of the stomach contents, which can give rise to symptoms and complications. Typical GERD symptoms affect patients' quality of life and overall work productivity.[1] In addition, it also contributes to enormous health-care costs. It is a highly prevalent disorder, and a recent systematic review showed that the prevalence of GERD is 18.1%–27.8% in North America, 8.8%–25.9% in Europe, 2.5%–7.8% in East Asia, 8.7%–33.1% in the Middle East, 11.6% in Australia, and 23.0% in South America.[2] Although conventionally considered as a disease affecting middle age and the older population, recent studies have shown that there is a significant increase in the proportion of the disease in the younger age group.[3]

Patient's quality of life is increasingly being considered as a medical outcome index in the evaluation of the impact of GERD symptoms on patients' health status. The impact of

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GERD on quality of life can be evaluated by a detailed symptom analysis which typically includes heartburn, acid regurgitation and epigastric pain in the majority of the patients using GERD health-related quality of life (HRQL) questionnaire.[4]

Studies have shown that chronic conditions like GERD could secondarily result in psychological manifestations of anxiety, depression, and insomnia. Few studies have also demonstrated that anxiety and depression could decrease the threshold for perception of visceral stimuli, thereby exacerbating the risk of developing functional gastrointestinal (GI) disorders, thus elucidating a bidirectional cause and effect relationship.[5-7] The current research focuses on the interplay between the psychological manifestations of anxiety, depression, altered sleep, and the symptomatology of GERD with the objective to evaluate the magnitude of the correlation between the quality of life and the psychological status of patients.

Literature evidence reveals that there is a paucity of studies evaluating psychosocial impairment due to GERD symptoms in the Indian population. Hence, this study was aimed to evaluate the magnitude of association of GERD symptoms with anxiety, depression and sleep quality by assessing the correlation of the GERD-HRQL scores with that of anxiety, depression and sleep quality using the respective Hospital Anxiety and Depression scale (HADS) and Pittsburgh Sleep Quality Index (PSQI) scale in Indian patients with GERD. In addition, the study was also designed to analyze whether anxiety, depression, and sleep scores were affected by comorbidities in GERD patients.

METHODS

This was a hospital-based cross-sectional questionnaire study done at tertiary care teaching hospital after the approval by PSG Institute of Medical Sciences and Research-Institutional Human Ethics Committee (Approval no: 16/435 dated January 10, 2017). Patients attending Gastroenterology and Medicine outpatient department and diagnosed with GERD were included for the study after obtaining written informed consent. GERD was diagnosed based on the presence of the typical clinical presentation along with the endoscopic features of GERD. The control group were subjects who did not have GERD and were devoid of all GI symptoms, according to GERD-HRQL. GERD-HRQL questionnaire, a disease-specific tool, was used to measure the symptomatic outcomes and therapeutic effects in patients with GERD. The scale had 16 items that focused on heartburn symptoms, dysphagia, medication effects, and the patient's present health condition. Each item was scored from 0 to 5, with a higher score indicating a better quality of life. Two scores, namely heartburn score and regurgitation score, were obtained by the summation of the scores for questions 1–6 and 10–15 respectively in addition to the total score derived by summing all the 15 score values. The 16th question was a patient's satisfaction question based on a 3 point scale.

Velanovich *et al*. had examined the psychometric properties of the GERD-HRQL in GERD patients and reported that the Cronbach's α coefficient for each subscale ranged from 0.89 to 0.94 demonstrating a high test– retest reliability and internal consistency.[4]

The presence of anxiety and depression levels was assessed using the Anxiety and Depression Scale. The HADS, a widely used scale that had been designed for detecting depression and anxiety in out-patient clinic setting, was used to measure symptoms of anxiety and depression and consisted of 14 items, seven items for the anxiety subscale, and seven for the depression subscale. HADS Anxiety (HADS A) focused on the symptoms of generalized anxiety disorder, and HADS depression (HADS D) was focused on anhedonia, the main symptom of depression. Each item was scored on a response-scale with four alternatives ranging between 0 and 3. The overall score value 8 or higher was considered the cutoff value for the existence of anxiety and depression.[8]

The PSQI, an effective instrument was used for measuring the quality and patterns of sleep based on examination of seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the past month. Scoring of responses ranged from 0 to 3, and all the seven components were summed together with scores of 5 or greater indicating poor sleep quality.[9]

The subjects included in this study were >20 years of age, either newly diagnosed or on proton-pump inhibitor (PPI) therapy. Patients who had concomitant diabetes, hypertension, liver, pancreatic, or chronic renal disease were also included in the study. The participants included were either non-smokers or those who were current or ex-smokers with a smoking index <100 and also non-alcoholic. However, patients who had serious comorbidities such as liver failure, renal failure, morbid obesity, patients with heart disease on antiplatelet therapy, osteoporosis patients and those on long-term nonsteroidal anti-inflammatory drugs (NSAIDs) or other chronic medications which cause gastric reflux and those with documented history of psychiatric illnesses including anxiety disorders, depression or bipolar illness and sleep disorders were excluded. Subjects aged <20 years, pregnant and lactating women were also excluded from the study.

A total of 98 cases by consecutive sampling were recruited from January 2018 to May 2018. Data on age, gender, body weight, alcohol intake, smoking, NSAID intake, concomitant GI medications other than PPI, including their daily dose were obtained. Similarly, data were also obtained from 143

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control subjects who were matched so that they represented the same proportion of subjects with age, sex, and smoking status as in the case group. The three questionnaires, namely, GERD-HRQL, HADS, PSQI questionnaires, were administered by a trained interviewer blinded to the group to which the participant belongs.

Statistical analysis

All data were entered in excel, and the statistical analyses were performed using SPSS version 24 (IBM Corporation, United states). The mean HADS and PSQI scores of patients and controls were compared using an independent sample *t*-test to detect statistical significance. Similarly, Student's *t*-test was also used to analyze if the mean difference in the scores of the patients with and without comorbidities were significant. In addition, the correlation of the GERD-HRQL scores with anxiety, depression, and sleep quality scores in patients with GERD was performed using Pearson's correlation.

RESULTS

We recruited a total of 241 participants after obtaining written informed consent, among which 98 subjects were GERD patients and recruited to the case group, while the remaining 143 participants were the non-GERD control population. A total of 84 cases were on proton-pump inhibitor therapy, accounting for a percentage of 85.71%. The mean age of the cases was 46 years, whereas the mean age in the control group was 50 years. Among the cases, 55.10% were <50 years of age and 44.90% were in the age group of 50 years and above. Also, 67.35% cases were males and the rest were females with male: female ratio approximating 2:1. The analysis revealed that there was no difference in the mean scores between the cases in the age group <50 years and those who were aged 50 years and above. Among the cases, an independent sample *t*-test demonstrated that the mean score for anxiety was significantly higher in the female population compared to the males among the cases, but no such significant difference was evident for the depression or the sleep score [Table 1].

The mean score of the cases in the HADS D scale (7.35 ± 3.65) was significantly higher ($P < 0.001$) compared to the mean score of the control population (4.60 ± 3.34) using independent sample *t*-test. Likewise, the mean anxiety score of the cases (9.33 ± 4.51) was greater in comparison to the mean score of the controls (6.35 ± 3.74) using HADS A, and this difference was also statistically significant ($P < 0.001$). A similar significance in the PSQI sleep score between cases and controls was also obtained using Student's *t*-test [Table 2 and Figure 1]. A Chi-square test revealed that the proportion of cases found to have depression (50%); anxiety (66.32%), and sleep disorder (45.91%) were significantly higher compared to the control population [Table 3].

Table 1: Comparison of scores across age and gender groups using independent sample *t*-test

Scale	Age (years)/sex	Mean score	SD	*t*	*P**
GERD-HRQL	<50	24.24	11.92	0.472	0.638
	≥50	23.20	9.82		
HADS D	<50	6.88	3.47	1.399	0.165
	≥50	7.93	3.81		
HADS A	<50	9.72	4.20	0.921	0.359
	≥50	8.86	4.87		
PSQI	<50	4.77	2.10	0.883	0.379
	≥50	4.43	1.77		
GERD-HRQL	Male	23.56	10.68	0.268	0.790
	Female	24.21	11.76		
HADS D	Male	6.92	3.32	1.572	0.122
	Female	8.25	4.17		
HADS A	Male	8.66	4.26	2.067	0.043
	Female	10.71	4.76		
PSQI	Male	4.46	2.05	1.175	0.244
	Female	4.93	1.74		

*$P<0.05$ is statistically significant. SD=Standard deviation; GERD=Gastroesophageal reflux disease; HRQL=Health-related quality of life; HADS=Hospital anxiety and depression scale; HADS D=HADS depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

Table 2: Comparison of depression, anxiety, and sleep quality scores between cases and controls using independent sample *t*-test

Scale	Case/control	Mean score	SD	*t*	*P**
HADS D	Case	7.35	3.65	5.934	<0.001
	Control	4.60	3.34		
HADS A	Case	9.33	3.51	5.387	<0.001
	Control	6.35	3.74		
PSQI	Case	4.62	1.96	5.076	<0.001
	Control	3.33	1.89		

*Statistically significant. SD=Standard deviation; HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

Analysis of the GERD-HRQL scores revealed that among the 98 cases in the study population, 35 patients (35.7%) had symptomatic heartburn, whereas 45 patients (45.9%) were symptomatic for regurgitation. On the satisfaction scale, 66.35% were satisfied with their present condition and 26.50% recorded a neutral response, while 7.15% were found to be dissatisfied with their current clinical status [Figure 2]. A comparative analysis of whether the mean scores vary

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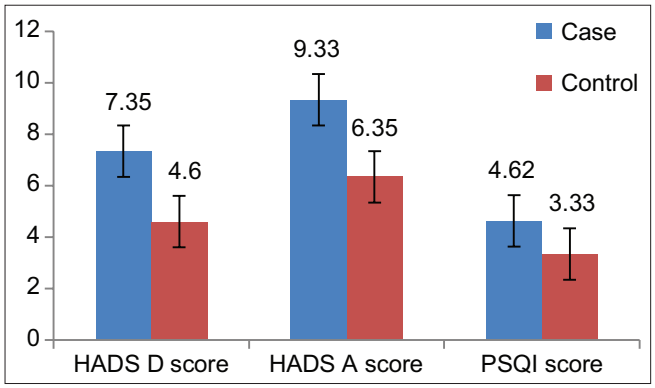


Figure 1: Chart representing mean ± standard deviation of the scores between cases and controls. The error bars represent standard deviation

Table 3: Comparison of the proportion of cases and control with and without depression, anxiety and sleep disturbances using Chi-square test

	Depression		Anxiety		Sleep disorder	
	Yes	No	Yes	No	Yes	No
Cases	49	49	65	33	45	53
Controls	35	108	58	85	26	117
Chi-square statistic	16.684		15.449		21.526	
*P**	<0.001		<0.001		<0.001	

*Statistically significant

significantly among the patients with asymptomatic (scores of ≤12 with each individual question not exceeding 2 for questions 1–6 of the GERD-HRQL indicate heartburn elimination) and symptomatic heartburn with scores of >12, it was found that the though the mean depression and anxiety scores were higher in the symptomatic patients, there was no statistical significance between the mean difference in the scores detectable. However, patients with symptomatic heartburn had a statistically significant higher mean score for sleep disturbance ($P = 0.034$) compared to the asymptomatic group. However, when a similar analysis was performed for the regurgitation, there was no significant mean difference between those with scores >12 and those with lower scores in all the three scales [Table 4].

Comparison of the mean scores of the cases with and without comorbidities, there was no statistical significance in the mean scores obtained for those with and without diabetes, hypertensive and normotensive patients, those with other comorbidities such as liver disease, pancreatic, or chronic renal disease [Table 5].

Analyzing the correlation of heartburn, regurgitation and satisfaction components of the quality of life scores with the depression, anxiety and sleep quality scores, it was found that neither the heartburn nor the regurgitation score correlated

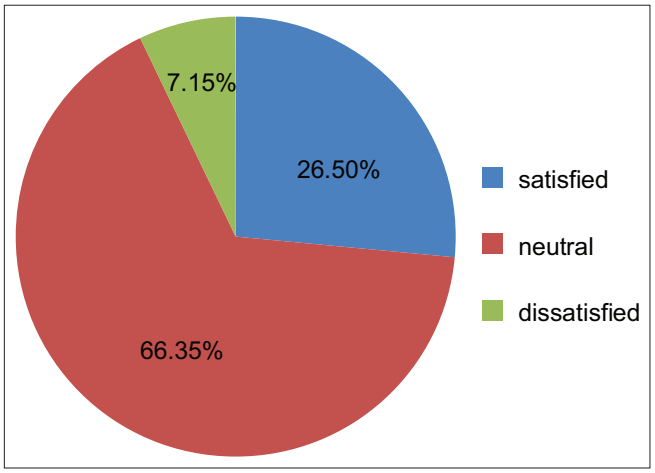


Figure 2: Chart representing satisfaction score in Gastroesophageal Reflux Disease-Health Related Quality of life Scale

with depression score whilst there was a correlation of the patient satisfaction with the depression score. However, both the heartburn and the regurgitation scores correlated with the anxiety score, including the global quality of life score. Interestingly, we found that there was a statistically significant correlation between heartburn and poor sleep, which was not detectable for regurgitation. In addition, it was found that the patients unsatisfied with their present clinical status were more likely to have depression and poor sleep quality rather than anxiety [Table 6 and Figure 3].

DISCUSSION

A statistically higher mean score for all the three clinical conditions, namely depression, anxiety, and sleep disturbance was demonstrable in the GERD cases compared to the control group indicating a high risk of these psychological manifestations in patients with reflux disease. Conceivably, impairment in quality of life was also elicited in the cases compared to the control population using GERD-HRQL.

Our study is one of the few studies evaluating the interplay between the HRQL changes in GERD with the various psychological manifestations such as anxiety, depression, and sleep manifestations in a South Indian population. Considering the cut off value of 8 for the diagnosis of anxiety and depression using the respective components of the HADS questionnaire, the study has effectively demonstrated that a high probability of anxiety disorder existed in the GERD population with a mean anxiety score of 9.33 ± 3.51 unlike with respect to depression wherein the mean score was marginally below the cut off value. A similar result was also obtained in another study done in 225 patients by Kessing *et al.*[10] However, our result was in contrast to a previous study

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Table 4: Comparison of depression, anxiety, and sleep quality scores with gastroesophageal reflux disease-health related quality of life scores of the cases

Scale	Heart burn score ≤12	Heart burn score >12	Regurgitation score ≤12	Regurgitation score >12
HADS D	7.17±3.92	7.68±3.14	7.18±3.79	7.55±3.51
HADS A	8.85±4.42	10.20±4.61	8.96±3.98	9.77±5.08
PSQI	4.26±1.56*	5.25±2.41*	4.71±2.04	4.51±1.87

**P* value significant at 0.05 level using independent sample *t*-test. HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

Table 5: Comparison of depression, anxiety, and sleep quality scores of cases with and without comorbidities

Scale	Comorbidities	*n*	Mean score	SD	*t*	*P**
HADS D	With comorbidities	70	7.04	3.25	1.181	0.245
	No comorbidities	28	8.14	4.47		
	Diabetes	22	8.68	3.83	1.872	0.070
	No diabetes	76	6.97	3.53		
	Hypertension	17	8.64	4.66	1.310	0.205
	No hypertension	81	7.08	3.37		
	Liver disease	35	7.65	2.74	0.674	0.502
	No liver disease	63	7.19	4.08		
	Pancreatic and renal disease	36	7.52	4.32	0.325	0.746
	No pancreatic and renal disease	62	7.25	3.23		
HADS A	With comorbidities	70	9.25	4.12	0.244	0.808
	No comorbidities	28	9.53	5.44		
	Diabetes	22	9.40	5.34	0.075	0.941
	No diabetes	76	9.31	4.28		
	Hypertension	17	9.58	5.88	0.203	0.842
	No hypertension	81	9.28	4.21		
	Liver disease	35	9.48	4.27	0.249	0.804
	No liver disease	63	9.25	4.67		
	Pancreatic and renal disease	36	10.02	4.99	1.105	0.273
	No pancreatic and renal disease	62	8.93	4.20		
PSQI	With comorbidities	70	4.42	1.72	1.351	0.185
	No comorbidities	28	5.10	2.42		
	Diabetes	22	5.00	2.48	0.856	0.399
	No diabetes	76	4.51	1.78		
	Hypertension	17	5.11	2.20	1.042	0.309
	No hypertension	81	4.51	1.90		
	Liver disease	35	4.57	2.01	0.189	0.851
	No liver disease	63	4.65	1.94		
	Pancreatic and renal disease	36	4.66	1.92	0.171	0.865
	Pancreatic and renal disease	62	4.59	1.99		

**P*<0.05 is significant. SD=Standard deviation; HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

in which depression was found to be one of the most prevalent comorbidities in patients with GERD.[11] Our research revealed that a high proportion of cases (71.42%) had comorbidities amongst which concomitance of liver diseases recorded the highest. However, none of the comorbidities were significantly associated with the HADS or the sleep scores.

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Table 6: Correlation between gastroesophageal reflux disease-health related quality of life score with depression, anxiety, and sleep quality scores of cases

Scale	GERD-HRQL score		Heart burn score		Regurgitation score		Satisfaction score	
	Pearson correlation	*P**	Pearson correlation	*P**	Pearson correlation	*P**	Pearson correlation	*P**
HADS D score	0.157	0.122	0.071	0.488	0.150	0.141	0.244	0.016
HADS A score	0.293	0.003	0.212	0.036	0.225	0.026	0.177	0.081
PSQI score	0.161	0.113	0.252	0.012	0.030	0.772	0.267	0.008

*Correlation is significant if $P<0.05$ (two-tailed significance). GERD=Gastroesophageal reflux disease; HRQL=Health-related quality of life; HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

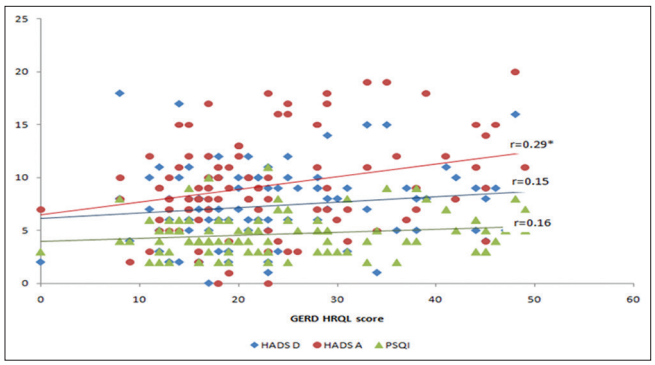


Figure 3: Chart representing the correlation of Gastroesophageal Reflux Disease-Health Related Quality of Life score with depression, anxiety, and sleep quality scores of cases. *Correlation coefficient *r* value is significant

Although the literature evidence strongly supports the association of sleep disorder manifestations with GERD[7,12] the mean global PSQI score of cases in our study did not achieve the significant cut-off level of 5 indicative of poor quality sleep despite the mean score being significantly higher than the control population. This could be potentially because a large majority of patients (85.71%) were on treatment with PPI. A systematic review has delineated that a statistically significant improvement in sleep quality occurred after initiation of PPI therapy in GERD patients though it may not be completely successful in normalizing the sleep quality to achieve a global PSQI score that is not statistically different from the control population.[13] The large proportion of cases on PPI therapy precluded a between-group analysis of the depression, anxiety, and sleep scores with the patients not on PPI in our research. Symptomatic relief with PPI therapy can consequentially alleviate the psychological manifestations in GERD though evidences support that persistent symptoms despite PPI treatment is quite common which can impede the improvement in severity of the psychological parameters.

Studies have supported that chronic and mild inflammation in the GI tract due to GERD could result in up-regulation of the central nervous system (CNS) cytokine production which in turn can affect the CNS functions, ultimately resulting in psychiatric manifestations such as depressive and anxiety disorders.[14-16] Studies in animal models have also demonstrated that chronic peripheral inflammation induces central pro-inflammatory markers like interleukin IL-1β as well as tumor necrosis factor alpha-α;[17] the long term elevation of which significantly correlate with the occurrence of psychological manifestations supporting the hypothesis.[18]

In addition, the symptom complex of GERD enables envisioning the intertwined and bidirectional nature of the relationship between sleep disorders and GERD. GERD results in heartburn, acid reflux, sinusitis or sinus problems, and frequent dry cough necessitating throat-clearing that awakens the patient during sleep. Frequent arousal during sleep activates neuroendocrine systems, including the vagal nerve and triggers bronchoconstriction. Frequent bronchoconstriction narrows the diameter of the respiratory tract and arouses patients from sleep, intensifying sleep disorders.[19-21] Furthermore, GERD-related micro-aspirations inducing bronchial exudative mucosal reaction also adds to the narrowing in diameter of the respiratory tract, accelerating sleep disorder.[22] The flip side of the coin is that for several reasons, GERD symptoms aggravate during sleep. Anti-reflux mechanisms such as swallowing rate, upper and lower esophageal sphincter pressure, gastric emptying, and the awareness of heartburn have all been found to be depressed during sleep. Also, while recumbent, the force of gravity encourages the flow of gastric content towards the esophagus. Thus, current literature supports the fact that sleep disorder has a dual cause and effect relationship with GERD.[13,23]

A differential effect of the two predominant symptoms, namely heartburn and regurgitation analyzed using GERD-HRQL, illustrated that heartburn patients had a significantly lower mean PSQI score and a positive correlation with anxiety and poor sleep quality whilst regurgitation only correlated with anxiety score. A similar result has been documented by Lee *et al*. whose study disclosed that patients with heartburn had more severely impaired daily activity, including sleep interruption compared to those with a predominant regurgitation.[24] Likewise, increased anxiety level has been found to be associated with greater heartburn but not regurgitation.[10] This discrepancy perhaps stems from the

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differences in physico-pathological mechanism underlying the key clinical presentations of GERD. Reflux episodes inducing regurgitation has been shown to have a higher proximal extent of the liquid component, but less esophageal pH drop, than episodes inducing heartburn.[25]

In the current study, a significant positive correlation between the GERD-HRQL and anxiety was demonstrable but not for depression and sleep derangements. A similar study using a different scale for evaluating depression, anxiety and HRQL was able to prove that there existed a significant correlation of the quality of life with both anxiety and depression.[26] The difference in results could be attributed to the fact that our study was done in a smaller subset of patients with GERD and that most patients were on treatment which also precluded a between-group analysis of untreated patients with those on treatment. Furthermore, the categorization of cases into the erosive and nonerosive disease was not done, and these were our study limitations.

However, the study has clearly brought out the need to understand the increased risk of psychological manifestations including depression, anxiety, and sleep disorders in GERD patients including those on treatment, which necessitates addressal by periodic assessments and redressal by specific planned psychiatric interventions, both pharmacological and behavioral, as a part of the comprehensive treatment plan.

CONCLUSIONS

The study highlights that GERD patients constitute a vulnerable population for psychological disturbances such as anxiety, depression, and sleep disorder, which may not be completely eliminated by the treatment of the underlying reflux disease. Hence, periodic evaluation and specific treatment strategies for such manifestations would be beneficial in improving the quality of life in these patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Tack J, Becher A, Mulligan C, Johnson DA. Systematic review: The burden of disruptive gastro-oesophageal reflux disease on health-related quality of life. Aliment Pharmacol Ther 2012;35:1257-66.
2. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: A systematic review. Gut 2014;63:871-80.
3. Yamasaki T, Hemond C, Eisa M, Ganocy S, Fass R. The changing epidemiology of gastroesophageal reflux disease: Are patients getting younger? J Neurogastroenterol Motil 2018;24:559-69.
4. Velanovich V, Vallance SR, Gusz JR, Tapia FV, Harkabus MA. Quality of life scale for gastroesophageal reflux disease. J Am Coll Surg 1996;183:217-24.
5. You ZH, Perng CL, Hu LY, Lu T, Chen PM, Yang AC, *et al*. Risk of psychiatric disorders following gastroesophageal reflux disease: A nationwide population-based cohort study. Eur J Intern Med 2015;26:534-9.
6. Chen JH, Wen SH, Hsu CS, Yi CH, Liu TT, Chen CL, *et al*. Clinical characteristics and psychosocial impact of different reflux time in gastroesophageal reflux disease patients. J Formos Med Assoc 2017;116:123-8.
7. Kimura Y, Kamiya T, Senoo K, Tsuchida K, Hirano A, Kojima H, *et al*. Persistent reflux symptoms cause anxiety, depression, and mental health and sleep disorders in gastroesophageal reflux disease patients. J Clin Biochem Nutr 2016;59:71-7.
8. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res 2002;52:69-77.
9. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213.
10. Kessing BF, Bredenoord AJ, Saleh CM, Smout AJ. Effects of anxiety and depression in patients with gastroesophageal reflux disease. Clin Gastroenterol Hepatol 2015;13:1089-950.
11. Moraes-Filho JP, Navarro-Rodriguez T, Eisig JN, Barbuti RC, Chinzon D, Quigley EM. Comorbidities are frequent in patients with gastroesophageal reflux disease in a tertiary health care hospital. Clinics (Sao Paulo) 2009;64:785-90.
12. Iwakura N, Fujiwara Y, Shiba M, Ochi M, Fukuda T, Tanigawa T, *et al*. Characteristics of sleep disturbances in patients with gastroesophageal reflux disease. Intern Med 2016;55:1511-7.
13. Regenbogen E, Helkin A, Georgopoulos R, Vasu T, Shroyer AL. Esophageal reflux disease proton pump inhibitor therapy impact on sleep disturbance: A systematic review. Otolaryngol Head Neck Surg 2012;146:524-32.
14. Salim S, Chugh G, Asghar M. Inflammation in anxiety. Adv Protein Chem Struct Biol 2012;88:1-25.
15. Bakunina N, Pariante CM, Zunszain PA. Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology 2015;144:365-73.

[Downloaded free from http://www.jmedscindmc.com on Thursday, February 4, 2021, IP: 103.196.31.66]

16. Ranjbaran Z, Keefer L, Stepanski E, Farhadi A, Keshavarzian A. The relevance of sleep abnormalities to chronic inflammatory conditions. Inflamm Res 2007;56:51-7.
17. Lampa J, Westman M, Kadetoff D, Agréus AN, Le Maître E, Gillis-Haegerstrand C, *et al*. Peripheral inflammatory disease associated with centrally activated IL-1 system in humans and mice. Proc Natl Acad Sci U S A 2012;109:12728-33.
18. Kivimäki M, Shipley MJ, Batty GD, Hamer M, Akbaraly TN, Kumari M, *et al*. Long-term inflammation increases risk of common mental disorder: A cohort study. Mol Psychiatry 2014;19:149-50.
19. Jansson C, Nordenstedt H, Wallander MA, Johansson S, Johnsen R, Hveem K, *et al*. A population-based study showing an association between gastroesophageal reflux disease and sleep problems. Clin Gastroenterol Hepatol 2009;7:960-5.
20. Dubois RW, Aguilar D, Fass R, Orr WC, Elfant AB, Dean BB, *et al*. Consequences of frequent nocturnal gastro-oesophageal reflux disease among employed adults: Symptom severity, quality of life and work productivity. Aliment Pharmacol Ther 2007;25:487-500.
21. Jung HK, Choung RS, Talley NJ. Gastroesophageal reflux disease and sleep disorders: Evidence for a causal link and therapeutic implications. J Neurogastroenterol Motil 2010;16:22-9.
22. Demeter P, Pap A. The relationship between gastroesophageal reflux disease and obstructive sleep apnea. J Gastroenterol 2004;39:815-20.
23. Khoury RM, Camacho-Lobato L, Katz PO, Mohiuddin MA, Castell DO. Influence of spontaneous sleep positions on nighttime recumbent reflux in patients with gastroesophageal reflux disease. Am J Gastroenterol 1999;94:2069-73.
24. Lee SW, Lien HC, Lee TY, Yang SS, Yeh HJ, Chang CS. Heartburn and regurgitation have different impacts on life quality of patients with gastroesophageal reflux disease. World J Gastroenterol 2014;20:12277-82.
25. Bredenoord AJ, Weusten BL, Curvers WL, Timmer R, Smout AJ. Determinants of perception of heartburn and regurgitation. Gut 2006;55:313-8.
26. Yang XJ, Jiang HM, Hou XH, Song J. Anxiety and depression in patients with gastroesophageal reflux disease and their effect on quality of life. World J Gastroenterol 2015;21:4302-9.

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[illegible] Incomplete and erroneous prescriptions affect the effective management of patients. The undergraduates of today are the physicians of tomorrow. Hence, the need for inculcating good prescription writing practice from undergraduate level to improve quality of healthcare. The objective of this study was to assess the impact of a sensitization program on the effective prescription writing skills of second year MBBS students.

[illegible] This prospective study was conducted between January and November 2017 among 149 second year medical students. The prescriptions by students were assessed prior to and after a sensitization program which emphasized on the importance of each component of the prescription, need for generic prescribing and rational use of drugs.

[illegible] After the sensitization program, there was significant improvement in mentioning of all components ($p<0.05$) except for route of administration, instructions, and health advise which were still deficient. The rationality in prescribing was analyzed by scoring vital drug related information on a scale of 12. Majority of inscriptions (72%; n=107) were rational after the program. There existed a weak positive correlation ($r=0.15$) between students' knowledge of drugs assessed by theory examinations and scores of prescriptions assessed in practical examinations.

[illegible] The sensitization program served as an effective strategy in improving knowledge regarding every component of the prescription and enhanced the prescription writing skills at the undergraduate level. This could help prevent deficiencies in health care arising from errors in prescribing.

[illegible] Prescription writing, Medical students, Sensitization program, Rational prescribing

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The prescription is a written order from the physician to the pharmacist for dispensing medications and plays a key role in the management of patients. Proper decision making and appropriate transcribing are integral for an ideal prescription.[1] Prescribing is a complex skill that requires a thorough understanding of the pathophysiology of a diagnosis, the pharmacology of an individual medication and how an individual's response may be affected by the pharmacokinetic and pharmacodynamic properties of a particular medication.[3] There is no global standard for prescriptions and every country has its own regulations. The most important requirement is that the prescription be clear. It should be legible and indicate precisely what should be given.[4] The parts of a prescription includes doctor and patient details, the superscription or heading with the symbol 'R' or 'Rx', which stands for the word recipe (in Latin, to take); the inscription, which focuses on the rational use of medicines; the subscription or directions for compounding the drug; and the signature of the prescriber.

In recent years, medical researchers observe deficiencies in health care occurring due to many prescribing errors, which arise because of two factors. One could be due to decision making and the other due to defect in the art of writing prescriptions. The factors related to the former could be inappropriate prescription, irrational prescription, under prescribing, or over prescribing. Those related to the latter is purely because of inappropriately writing the prescription.[5] Incomplete and erroneous prescriptions affect the well-being of patient adversely. Suboptimal or irrational prescription writing skills results in treatment failure, adverse clinical outcomes, wastage of our resources, and economical harm not only to the patients but also to the community.[2]

The second year MBBS Pharmacology curriculum includes training medical students in prescription writing. The students will incorporate this skill for the rest of their medical career. The results of a study of how well final years medical students performed in a prescribing exercise suggested that the root cause of prescribing errors was lack of a knowledge base that integrated scientific knowledge with clinical know-how.[6] Many studies opine that once the newly qualified doctors are exposed to workplace prescription writing, they retrospectively feel that there was insufficient emphasis on practical aspects of writing prescription in undergraduate curriculum.[15] Hence, prescription writing skill is an important target area for improvement. This study focuses on the prescription writing skills of Second MBBS students taking into consideration the completeness of the prescription, rational use of drugs and translational of theoretical knowledge to prescribing. This will enable to assess the prescription writing training provided by the department of pharmacology and determine the possible scope for improvement, if exists.

The objective of this study was to assess the prescription writing skills of second year MBBS students thereby reflecting the quality of learning pharmacology during their undergraduate period in the department of pharmacology.

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The present study was conducted in the Department of Pharmacology at PSG IMS & R, Coimbatore. Based on convenient sampling, the sample size was determined to be 149 students doing second year MBBS. After obtaining IHEC approval and permission from the HOD, department of pharmacology, data collection was initiated. The study period was between January to August 2017.

Prescription Order writing is a skill that is a part of the second MBBS curriculum and students are trained in this skill during their practical session at the department of pharmacology. In addition to the regular classes on prescription writing, a sensitization program emphasizing the importance of each component of the prescription, rational use of drugs and their clinical relevance was held at the department using suitable clinical examples and scenarios. In addition to the completeness of the prescription, students were insisted to apply 'rational use of medicine concept'- right drug (in generic name - in capital letters) in the right dose for the right duration at the right frequency. Data was collected from internal assessment exam (IAE) papers that comprises of a section on prescription order writing for a clinical scenario. Data was analyzed and interpreted for improvement in quality of prescription writing skills.

Statistical analysis

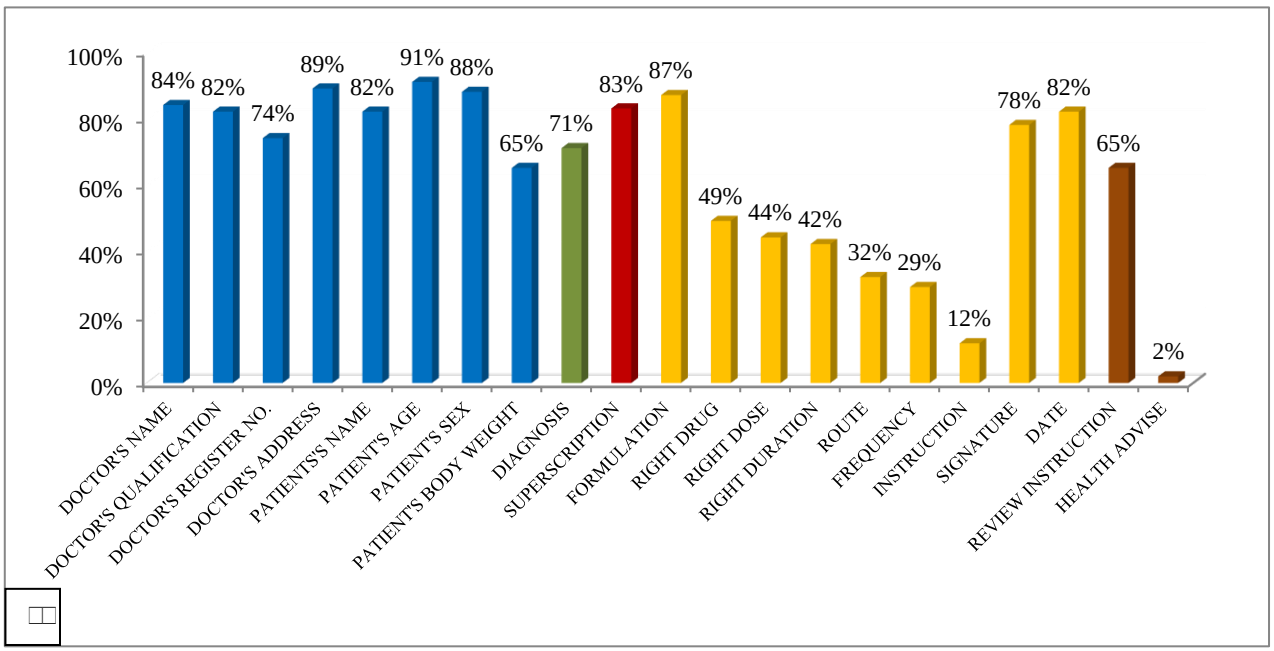
Data of all the 149 students were included in the statistical analysis. Analysis was performed using IBM SPSS software (version 20 SPSS Inc., Chicago, IL, USA). All categorical data were represented by frequency distribution while descriptive statistics was used for numerical data. To determine statistical significance between the difference in proportions chi-square test was used. The statistical significance was set at 5% i.e., $p<0.05$. Pearson correlation analysis was used to analyze the correlation between theory and prescription score.

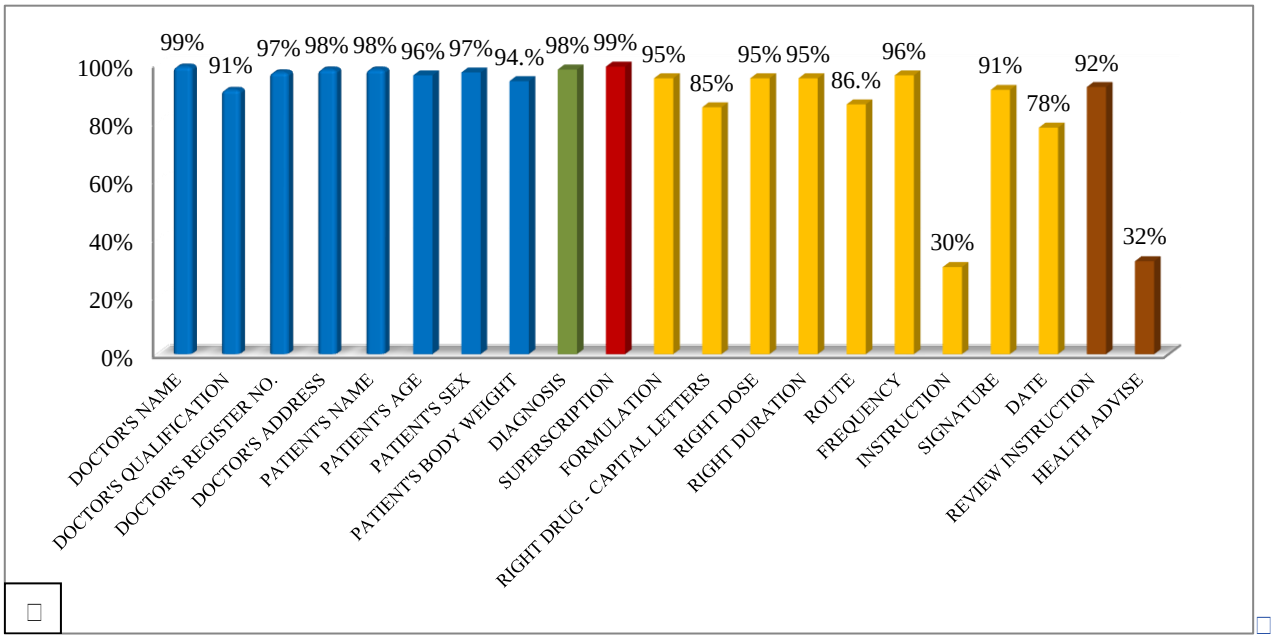
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Prescriptions of students were analyzed for completeness in prescribing (n=149) (Figure 1) with reference to a standard format of prescription set by the department of pharmacology at PSGIMSR. In all the prescriptions 21 components as per the standard format were checked for presence or absence.

The basic demographic parameters of the prescription that included doctor's information and patient's information were initially analyzed. Before the sensitization program it was observed that doctor's information (doctor's name, qualification, register number, address) was provided by 125 (84%), 122 (82%), 110 (74%), 133 (89%) whereas after the sensitization program analysis revealed 147 (99%), 136 (91%), 144 (97%), 146 (98%) of students had provided the same information respectively. This difference following the sensitization session was statistically significant ($p<0.05$) for all components related to doctor's information (Table 1).

Patient's information (patient's name, age, sex, body weight) was provided by 122 (82%), 136 (91%), 131 (88%), 97 (65%) and following the sensitization program it was provided by 146 (98%), 143 (96%), 145 (97%), 140 (94%) of the students respectively (Figure 1 a and b). After the sensitization program the improvement in all patient related components was statistically significant except for the mention of patient's age which was mentioned by most students even before the program (Table 1).





On analyzing the prescriptions for presence of diagnosis it was found that before the sensitization program only 105 (71%) had mentioned it, while the proportion increased after the program to 146 (98%). This was a statistically significant improvement (Table 1). Rx symbol was provided by 124 (83%) of students which also increased after the program to 146 (98%) students and was analyzed to be statistically significant (Figure 1 and Table 1).

Analysis of the inscription part of the prescription containing drug related information and instruction (formulation, right drug - in capital letters, right dose, right duration, route of administration, frequency, instructions for intake, signature and date) revealed that prior to the program these information was provided by 130 (87%), 73 (49%), 66 (44%), 63 (42%), 48 (32%), 43 (29%), 18 (12%), 116 (78%), 122 (82%) respectively. Following the sensitization program the proportions increased to 142 (95%), 127 (85%), 142 (95%), 142 (95%), 39 (86%), 143 (96%), 43 (30%), 136 (30%), 116 (78%) of students respectively (Figure 1 a and b). There was statistically significant improvement with regard to all components of the inscription except for mention of date which had declined after the program, however this decline was not statistically significant (Table 1).

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□ □□□□r □□□□□□□ □□	125 (84	147 (99)	15	21.480***	p<0.0001
Doctor's qualification □	122 (82)	136 (91)	9	5.150*	p<0.05
Doctor's register □□□ □□r □	110 (74)	144 (97)	23	31.682***	p<0.0001
Doctor's address □	133 (89)	146 (98)	9	9.896**	p=0.0017
□□□□□□□□□□□□□ □□□	122 (82)	146 (98)	16	21.120***	p<0.0001
□□□□□□□□□□□□□	136 (91)	143 (96)	5	3.054	p=0.0805
□□□□□□□□□□□□	131 (88)	145 (97)	9	8.669**	p=0.0032
□□□□□□□□□□□d □□□ □□□□□□	97 (65)	140 (94)	29	38.315***	p<0.0001
□ □□□□□□□□	105 (71)	146 (98)	27	41.327***	p<0.0001
□ □□□□□ □□□	124 (83)	146 (98)	15	19.432***	p<0.0001
□□r □ □□□□□□□□□	130 (87)	142 (95)	8	5.802**	p<0.05
□ □□□□dr □□□□□□□□□□□□□□□□ □□□□□r □□	73 (49)	127 (85)	36	43.522***	p<0.0001
□ □□□□d □□□□	66 (44)	142 (95)	51	91.107***	p<0.0001
□ □□□□d □r □□□□□□	63 (42)	142 (95)	53	93.660***	p<0.0001
□ □□□□□□□□ □d □ □□□□r □□□□□	48 (32)	39 (86)	54	89.505***	p<0.0001
□r □□□□□□□□	43 (29)	143 (96)	67	142.212***	p<0.0001
□□□r □□□□□ □□□r □ □□□□□□□	18 (12)	43 (30)	18	14.501**	p=0.0001
□□□□□□r □□□	116 (78)	136 (91)	13	9.581**	p=0.0020
□ □□□□	122 (82)	116 (78)	4	0.742	p=0.3889
□ □□□□□ □□	97 (65)	137 (92)	27	32.071***	p<0.0001
□ □□□□□□d □□□□	3 (2)	47 (32)	30	47.360***	p<0.0001

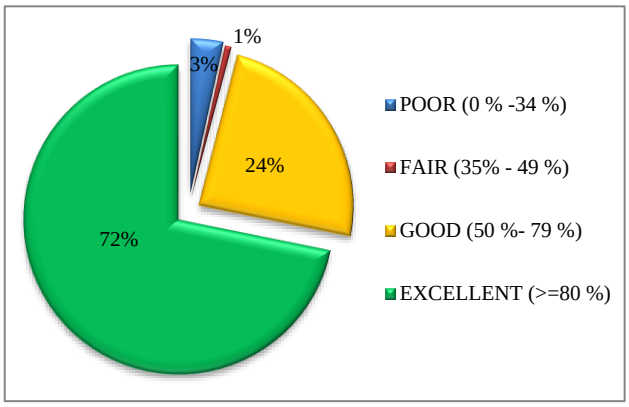
*p<0.05, **p<0.01, ***p<0.001.

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On analyzing for information on review and health advise it was observed that before the program only 97 (65%) and 3 (2%) of students respectively had mentioned it whereas after the program 137 (92%) and 47 (32%) of students respectively had provided relevant information (Figure 1). This improved was also found to be statistically significant (Table 1).

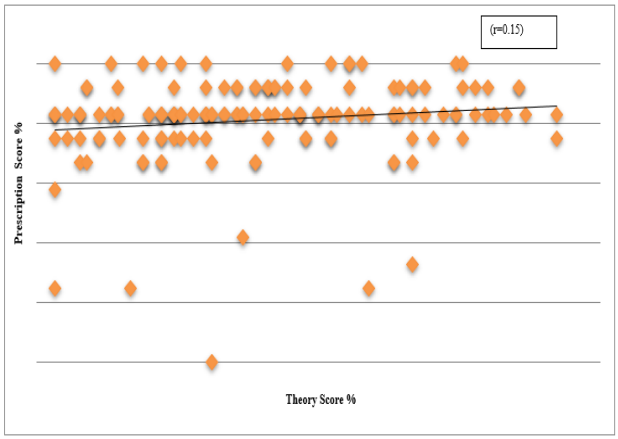
The rationality in prescribing was analyzed by scoring the 9 parameters included in the inscription that contains drug related information and instruction (formulation, right drug - in capital letters, right dose, right duration, route of administration, frequency, instructions for intake, signature and date). The scoring was done on a scale of 12 and percentage of the scores were calculated. Based on which rationality in prescribing was grouped as poor score 0%-34%; fair score 35%-49%; good score 50%-79% and excellent score ≥80%.

On analysis 3% of students (n=5) were grouped as poor, 1 (1%) were grouped as fair, 36 (24%) were grouped as good and 107 (72%) as excellent. Among the 72% of students - 9 % of the students (n=13) provided 100% complete prescription as per the standard format (Figure 2).



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In order to assess the application of theoretical knowledge in writing a prescription, the theory score for a question related to the same diagnosis as that of the prescription audited was analyzed for correlation (Figure 3). The median prescription score (assessed for a maximum score of 12) was 10±6.4 and the median theory question score (assessed for a maximum score of 30) was 10.7±6.4. On analysis it was found that there was a weak positive correlation (r=0.15) between theory and prescription scores.



□□□r □□□□□ □rr □□□□□□□□□□□□r □□□d □r □□r □□□□□□□□
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Prescribing drugs for common ailments are an important core competency of medical undergraduate.[8,9] The existing medical undergraduate curriculum includes prescription training in the second year under pharmacology. Once poor prescribing skills are habitualized, changing them during postgraduate life is more difficult and usually requires different education strategies, which are expensive and time consuming.[10,11]

Babar et al, conducted a study on 206 prescriptions to assess the quality of prescription writing, patients name and age was present in 180 (87%) and 115 (55%) prescriptions respectively.[12] In the current study we observed that the percentage of students completing the doctor's information (doctor's name, qualification, register number, address), patients information (patient's name, age, sex, body weight) and percentage of students mentioning diagnosis and superscription following the sensitization program was satisfactory with reference to the standards set by the department. A study conducted by James et al among third- and fourth-year medical students found that the 79.6% were able to correctly select the drugs, 69.4% and 68.2% were able to correctly mention duration and formulation of drug to be dispensed.[13]

In the current study it was observed that even after the sensitization program only 78% of students had mentioned the date which is a vital component of the prescription. In view of the fact that the prescriptions audited were those written in an exam setting rather than a clinical one, it is possible that the students have not attached due significance in mentioning it within the prescription as they had already mentioned it at the beginning on the exam paper.

Similarly, in spite of the program only 30 % of students had mentioned instructions pertaining to drug intake (with respect to food or specific time of the day) and only 32% of students had mentioned health advice which could be attributed to their beginning phase of clinical exposure. Probably more bedside observation of prescriptions with relevant instructions would positively reinforce the clinical importance of drug related instructions and health advises.

Although the percentage of students mentioning the right drug (in capital letters) and route of administration was above the expected standards, it can be further improved as it is the integral part of the prescription. In most instances students assumed route of administration to have been understood even without mentioning (e.g., Tablets are assumed to be taken only orally) which they probably would acquire by a deeper learning approach and better clinical exposure wherein they would be able to integrate learning with practical experience.[14]

Rational use of medicines is based on individualizing therapy and drugs, requiring an in-depth understanding of the mechanisms of drugs, their adverse effects and contraindications in addition to bedside learning. Among the parts of the prescription, the inscription containing drug related information reflects understanding by students of the rational use of medicine concept. The inscription scores and subsequent grouping showed that majority of students, 107 (72%) where able to understand the rational use of medicine concept although there is still a scope for improvement.

The limitations of the study were that, this study was done to determine the quality of prescription orders written by II MBBS students who have limited clinical exposure. Since the prescription orders that are assessed in the exam are same as that taught in the practical session there may be a possibility that students memorized the prescription order format that is taught in the practical session.

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The sensitization program served as an effective strategy in improving knowledge regarding every component of the prescription resulting in better completeness of prescriptions. The program also enhanced rational prescribing at the undergraduate level by promoting better correlation of theoretical knowledge with clinical scenarios. Such programs could help prevent deficiencies in health care arising from incomplete and erroneous prescriptions.

Recommendations

Sensitization programs coupled with bedside training of prescription order writing by virtue of its reinforcing nature could further improve the quality of prescribing in terms of completeness and rationality. Periodic monitoring of prescriptions of interns by clinical pharmacologists with feedbacks to interns might improve

the quality of prescriptions issued by the fresh outgoing graduates.

My sincere thanks to the entire department of pharmacology at PSG IMS & R, Coimbatore and the second year MBBS students of PSG IMS & R without whom this study would not have been possible.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Human Ethics Committee - PSGIMS and R. Project no.17/332 dated 30th October 2017

1. Akram A, Mohamad N, Meerah TSM, Zam ZR, Abdullah D. A pilot study-an action research to acquire clinical skill. Procedia Soc Behav Sci. 2012;60:236-40.
2. Kumar J, Shaik M. Appraisal of prescription writing skills of preclinical undergraduate students in a medical institute of Nepal. JCMS Nepal. 2010;6(4):7-13.
3. Aronson JK. A prescription for better prescribing. Br J Clin Pharmacol. 2006;61(5):487-91.
4. World Health Organization. How to investigate drug used in health facilities: selected drug use indicators. World Health Organization, Geneva; 1993.
5. Calligaris L, Panzera A, Arnoldo L, Londero C, Quattrin R, Troncon MG, et al. Errors and omissions in hospital prescriptions: A survey of prescription writing in a hospital. BMC Pharmacol Toxicol. 2009;9:9.
6. Boreham NC, Mawer GE, Foster RW. Medical students' errors in pharmacotherapeutics. Med Educ. 2000;34:188-93.
7. Thenrajan P, Murugan PR. Impact of patient-based teaching in improving prescription writing skills of II MBBS students. Int J Applied Basic Med Res. 2016;6(3):174.
8. Heaton A, Webb DJ, Maxwell SRJ. Undergraduate preparation for prescribing: the views of 2413 UK medical students and recent graduates. Br J Clin Pharmacol. 2008;66(1):128-34.
9. Kripalani S, Jones BJ, Katz MG, Genao I. A prescription for cultural competence in medical education. J Gen Intern Med. 2006;21(10):1116-20.
10. Grand LA, Hogerzeil HV, Ruskamp HFM. Intervention research in rational use of drugs: a review. Health Policy Plan. 1999;14(2):89-102.
11. Hogerzeil HV. Promoting rational prescribing: an international perspective. Br J Clin Pharmacol. 1995;39:1-6.
12. Babar HS, Hussain S, Maqsood Z, Dad HA, Khan M, Rahman AA, et al. Adherence to prescription format and compliance with WHO core prescribing indicators. J Pharm Sci Res. 2014;6(4):195-99.
13. Khaja KA, Handu SS, James H, Mathur VS, Sequeira RP. Int J Clin Pharmacol Ther. 2005;43(9):429-35.
14. Maxwell S, Walley T. BPS Clinical Section Committee, Teaching safe and effective prescribing in UK medical schools: A core curriculum for tomorrow's doctors. Br J Clin Pharmacol. 2003;55:496-503.
15. Heaton A, Webb DJ, Maxwell SRJ. Undergraduate preparation for prescribing: the views of 2413 UK medical students and recent graduates. Br J Clin Pharmacol. 2008;66(1):128-34.

Ramakrishnan S, Krishnamurthy B, Anbarasu U. Impact of a sensitization program on prescription order writing skills of second year MBBS students: a prospective study. Int J Basic Clin Pharmacol 2020;9:906-11.

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Lipid Lowering Effect of Teneligliptin in Comparison to Simvastatin in Diet Induced Hyperlipidemic Rats

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Gliptins are drugs that inhibit the enzyme dipeptidyl peptidase-4 and are one of the current treatment options for type 2 diabetes mellitus. The study aimed to compare the lipid lowering effects of teneligliptin with simvastatin in diet induced hyperlipidemic rats.22 Sprague Dawley male rats were administered high fat diet over a period of 4 weeks to induce hyperlipidemia, after which, 10 rats each in groups 1 and 2wereorally gavaged with simvastatin (standard) and teneligliptin (treatment) respectively for 2 weeks. 2 rats in group 3 were vehicle controls. Blood samples were collected at baseline, at the end of 4 weeks and 6 weeks to determine the levels of total cholesterol, triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol. Histopathology of liver and aorta of 2 control animals and 3 animals each from the groups 1 and 2 was done at the end of the study.A paired t test showed a statistically significant reduction in mean total cholesterol ($p<0.001$), triglycerides ($p<0.001$) and low density lipoprotein cholesterol ($p=0.003$), but no significant change in mean high density lipoprotein cholesterol ($p=0.796$) in teneligliptin group at the end of 6 weekscompared to the pre-treatment values. There was no statistically significant difference between the simvastatin and teneligliptin groups in all the four lipid parameters studied. Compared to baseline, there was a rise in body weight after induction and a reduction post-treatmentin both groups which did not achieve statistical significance. Histopathology of liver in both groups 2 and 3 demonstrated reversal of the congestion and mild fatty changes which were apparent in the control group while sections of aorta failed to reveal any significant changes even in the control group.The lipid lowering property of teneligliptin demonstrated in this study will be therapeutically beneficial in type 2 diabetic patients with dyslipidemia.

Keywords: Dipeptidyl Peptidase4 Inhibitors; Dyslipidemia; Hypolipidemic Activity; Teneligliptin.

Hyperlipidemia is a metabolic disorder that involves abnormally increased levels of lipids and lipoproteins in the blood. Most dyslipidemias (80%) are related to diet and lifestyle, although familial disorders (20%) are important as well. The American Heart Association estimates a prevalence

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of 11.9% which is around28.5 million adults =20 years of age having serum total cholesterol levels =240 mg/dl, which is considered a high level necessitating treatment.[1] A study conducted by Indian Council of Medical Researchshows a high prevalence in India with approximately 79% of the 16607 study subjects manifesting abnormalities inat least one of the lipid parameters.[2]

Hyperlipidemia is one of the major risk factors that causearteriosclerosis, cerebral stroke, coronary heart disease, myocardial infarction and renal failure. Statins are widely used to lower cholesterol levels because of their inhibitory effects on the 3-hydroxyl-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the rate-limiting step in cholesterol biosynthesis. The most important adverse effects of statins are increased concentrations of liver enzymes, myopathy, rhabdomyolysis and increased risk of diabetes.[3,4] In addition, reports of an increase in the potential risk of cognitive impairment with statins have been documented.[5]

Teneligliptin is a dipeptidylpeptidase-4 inhibitor that inhibits the enzyme dipeptidyl peptidase-4 (DPP-4) and is a potent treatment option for type 2 diabetes (T2DM) either as monotherapy or in combination with other hypoglycemic agents. Gliptins are used for the treatment of T2DM when patients fail to show adequate glycemic control after dietary changes and exercise or with a combination of lifestyle modification and metformin /sulfonylurea treatment.[6,7] They have relatively lesser adverse effects, weight neutral and do not cause any significant hypoglycemia.[7,8] Dyslipidemia is an important risk factor for cardiovascular complications in type 2 diabetic patients. It is unclear whether gliptins favorably improve lipid profile in type 2 diabetic patients as there are contradicting evidences from clinical studies.[9,10]

The DPP-4 inhibitors are also currently being evaluated for their effects on obesity and other metabolic traits.[11,12] In addition, their role in chronic liver diseases including non-alcoholic steatohepatitis is also under investigation.[13, 14]

In animal studies, a beneficial effect on blood lipids has been detected in type 2 diabetic animal models like Zucker fatty rats. studies indicate a potential lipid lowering effect of teneligliptinin T2DM, the efficacy of the drug as an independent anti-hyperlipidemic agent is not yet determined.Thus the aim of this study was to evaluate the lipid lowering effect of teneligliptin in high fat diet rat model as there is paucity of studies assessing the hypolipidemic action of gliptins in diet induced hyperlipidemia model. So, in this study, we compared the effect of gliptins(teneligliptin) to that of statins (simvastatin) on hyperlipidemia induced in ratsusing high fat diet.

MATERIALS AND METHODS

After the approval of the Institutional Animal Ethics Committee (Approval number:288/2015/IAEC),the study was done in the department of Pharmacology at a Medical college and research institute in Coimbatore. 22 healthy adult Sprague Dawley male rats of weight 180 g to 250g and age more than 2 months were chosen for the study. They were housed in polypropylene cages at normal ambient temperature and 12-hour light dark cycle. The animals were administered high cholesterol diet over a period of 4 weeks to induce hyperlipidemia after which,with 2 rats as controls, 10 rats were randomly allotted to each of the groups 1 and 2 following which they were administered the drug/vehicle during the last two weeks (5th,6th) along with normal standard chow diet.

Standard group (1): 10 rats given simvastatin(10mg/kg/day)

Treatment group (2): 10 rats given teneligliptin (20 mg/kg/day)

Control group (3): 2 rats given vehicle (sterile water)

The composition of high-lipid diet consisted of 1% (w/w) cholesterol, 10% (w/w) fat lard, 0.2% propylthiouracil, 5% yolk and 1% sodium tauroglycocholate.15The rats were allowed the high fat diet and water ad . The drugs simvastatin at a dose of 10 mg/kg and teneligliptin at a dose of 20 mg/kg were dissolved in sterile water and the control animals were given the vehicle (sterile water) all of which were administeredby oral gavage.

Blood samples (1.5 ml) were collected from tail vein of each rat using tail snip method under anesthesia. Blood sample collection was done at baseline, at the end of 4th week and 6th

week. Lipid profiling was done to determine the levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). The LDL-C, HDL-C and (TC) estimations were done in auto-analyzer using GenX lipid parameter kits from Proton Biologicals and the (TG) was estimated in auto-analyzer using Coral clinical systems kit.

At the end of the study, the two control animals and three animals from the group 1 (standard) and group 2 (treatment) were sacrificed for collection of liver and aorta specimens for histopathology examination.Tissue sections of liver and aorta were preserved in 10% neutral buffered formalin later processed and stained using H&E stain.The slides were observed under microscope for fatty degeneration in liver and sub-intimal fat deposition in aorta.

Statistical Analysis

The changes in lipid parameters within the groupswere analyzed using paired t-test to detect statistically significant difference. One way ANOVAwith post-hocTukey test was done to evaluate the statistically significant difference between the treatment and standard groups. The results were expressed as mean ± SD. A value of $p<0.05$ was considered statistically significant. The histopathological changes were analyzed descriptively.

RESULTS

All the three groups exhibited a rise in mean TG after induction which was statistically significant compared to the baseline. Following treatment with teneligliptin, the mean TG was

Table 1. Comparison of mean ± standard deviation of the body weight and lipid parameters after treatment (at the end of 6 weeks) between the three groups

Parameters	Group 1	Group 2	Group 3
Body weight (g)	267.62 ± 18.91	258.37 ± 37.20	324.33 ± 19.66
Triglycerides (mg/dl)	46.21±16.05	57.51 ± 16.00	77.25 ± 10.08
Total cholesterol (mg/dl)	127.21 ± 24.86	122.21 ± 28.75	168.65 ± 22.58
LDL(mg/dl)	44.96 ± 7.61	40.70 ± 10.62	62.90 ± 4.49
HDL(mg/dl)	36.82 ± 6.61	33.08 ± 6.68	30.55 ± 3.34

Table 2. Comparison of lipid parameters within groups using paired t test

Parameter	Group	Pair	Mean Paired difference	Std. Deviation	Std. Error Mean	p value*
Triglycerides	Group1	BaselineInduction	-54.20	10.83	4.42	<0.001
	Group1	Induction Treatment	24.71	18.11	7.39	0.021
	Group2	BaselineInduction	-39.51	8.92	3.64	<0.001
	Group2	Induction Treatment	28.70	7.61	3.10	<0.001
Total cholesterol	Group1	BaselineInduction	-181.80	28.64	10.82	<0.001
	Group1	Induction Treatment	132.95	23.57	8.90	<0.001
	Group2	BaselineInduction	-129.35	31.98	10.66	<0.001
	Group2	Induction Treatment	66.85	35.02	11.67	<0.001
LDL	Group1	BaselineInduction	-35.03	8.51	3.47	<0.001
	Group1	Induction Treatment	21.06	8.82	3.60	0.002
	Group2	BaselineInduction	-31.30	6.79	2.77	<0.001
	Group2	Induction Treatment	21.13	9.72	3.97	0.003
HDL	Group1	BaselineInduction	11.88	5.49	2.24	0.003
	Group1	Induction Treatment	-3.16	10.07	4.11	0.476
	Group2	BaselineInduction	14.05	5.21	2.12	0.001
	Group2	Induction Treatment	-1.01	9.11	3.72	0.796

*$p<0.05$ is significant

57.51 ± 16.00 mg/dl while that in the simvastatin group was 46.21 ± 16.05 mg/dl (Table 1) and the mean reduction of TG (28.70 ± 7.61 mg/dl) in group 2 was statistically different (p< 0.001) as that of the mean reduction in group 1(24.71 ± 18.11 mg/dl) with p = 0.021 (Table 2).However there was no statistical significance in the mean difference in TG levels between the two groups at the end of 6 weeks with p = 0.318(Table 3).

Likewise, serum TC level which increased significantly due to induction with high cholesterol diet demonstrated a reduction following treatment both in standard as wellas the treatment groups (Table 2). There was no statistically significant difference (p= 0.923) between the mean TC group 2 (122.22 ± 28.75 mg/dl) compared to that of group 1(127.21± 24.86 mg/dl) after treatment (Table 1 & 3).

In addition, at the end of 6 weeks, serum LDL-C also revealed a parallel reduction in both groups with the mean of group 1 (44.96±7.61 mg/dl) and 2(40.70 ± 10.62 mg/dl) at the end of intervention beingstatistically significant compared to that of mean serum after induction with p=0.002 & p=0.003 for groups 1 and 2 respectively using paired t test (Table 1 & 2). A statistical significance in mean LDL-C levels could not be detected (p = 0.735) between the two groups using ANOVA (Table 3). However, a similar trend was not evident for serum HDL-C. A fall in HDL-C level could be noted in both groups with induction compared to the baseline. The mean HDL-C value after induction for group 1 (33.65 ± 4.41 mg/dl) and group 2 (32.06 ± 4.27 mg/dl) revealed a rise with the standard (36.82 ± 6.61 mg/dl) as well as

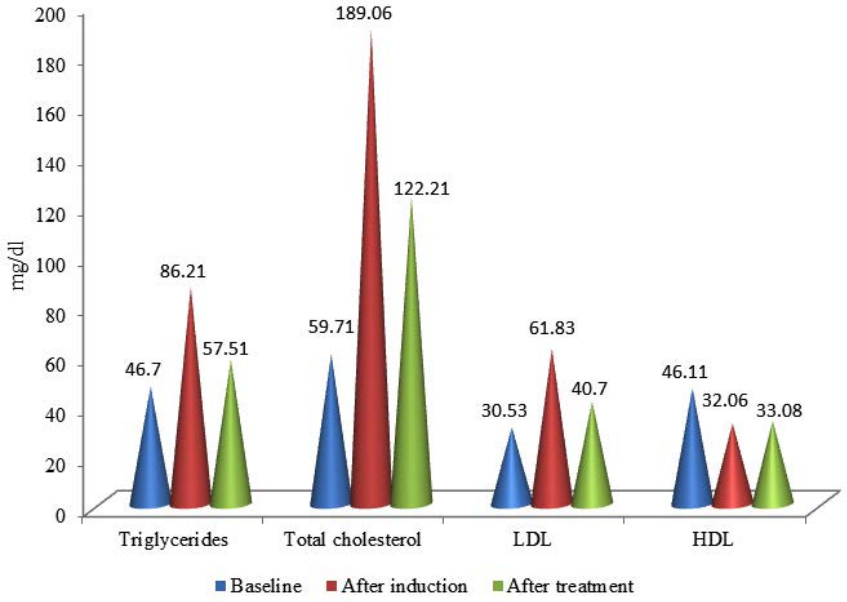


Fig. 1. Comparison of means of lipid parameters (mg/dl) of group 2 treated with teneligliptin

Table 3. Comparison between groups using one way ANOVA

Parameters	Mean difference between group 1 and 2	Standard error	Significance (p value)
Body weight(g)	9.25	14.67	.805
Triglycerides(mg/dl)	11.30	9.53	.318
Total cholesterol(mg/dl)	4.99	13.11	.923
LDL(mg/dl)	4.26	5.64	.735
HDL(mg/dl)	3.73	3.32	.516

*p<0.05 is significant

the treatment drug (33.08 ± 6.68 mg/dl) but the increase produced by either of the drugs was not statistically significantwith p=0.476 & p=0.796 for groups 1 and 2 respectively (Table 1 & 2).

Thus the net effect of teneligliptin evident was a statistically significant reduction in TC (p<0.001), TG (p<0.001) and LDL-C (p=0.003), but not the HDL-C (p=0.796) (Table 2 & Fig.1).A between group analysis using one way ANOVA revealed that there was no statistically significant difference between the groups treated with simvastatin and teneligliptin in all the four lipid parameters studied (Table 3).

Compared to baseline mean body weight of group 1(273.75±34.13 g) and group 2 (274.62±37.20 g), there was a rise in body weight in both standard (286.62±21.59 g) and treatment groups (280.87±40.99 g)after induction though the increase was not statistically significant. In addition, a reduction in body weight was discernable in the study animals of both groups with treatment using standard/drug, yet the fall in mean body weight was also not statistically significant in both groups. At the end of 6th week, the mean body weight of the standard group (267.62±18.91 g) did not differ significantly (p=0.805) from that of the treatment group(258.37± 37.20 g)(Table 1 & 3).

Histopathologic examination using H&E stain revealed a normal liver section in groups 1 and 2 while mild fatty changes in the form of intracytoplasmic vacuolation with occasional areas of congestion could be detected in the control group. This indicates therapy with both simvastatin and teneligliptin had reversed the mild fatty change induced by the disease model. Sections of aorta on histopathology examination failed to reveal any significant changes in all three groups including the control group indicating that induction for a period of 4 weeks withhigh fat diet had not caused any significant atherosclerotic changes in the aorta of our study animals (Fig.2).

Fig.2 shows normal liver section in groups 1 and 2 while mild fatty changes in the form of intracytoplasmicvacuolation with occasional areas

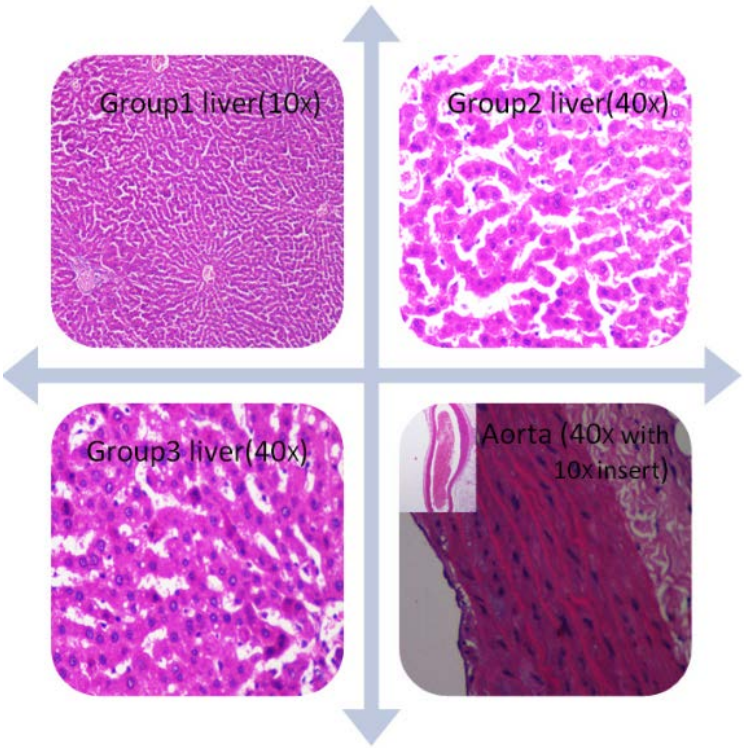


Fig. 2. Histopathological examination of the sections of liver under H&E staining

of congestion in the control group. Section of aorta of group3 reveals normal histology

DISCUSSION

This study which was done in a rat model of diet induced hyperlipidemia has explicitly brought out the lipid lowering property of teneligliptin. This study has demonstrated that there is no statistical significance in the reduction in blood lipids between simvastatin and teneligliptin including serum TC, TG, LDL-C. In addition, though the change in mean serum HDL-C revealed a greater rise with simvastatin compared to teneligliptin, the rise was not statistically significant in both groups implying that the beneficial effect of teneligliptin on HDL-C levels closely paralleled that of simvastatin though had lesser magnitude to be statistically significant.

Teneligliptin, classified as a class III DPP-4 inhibitor, has a unique structural feature that provides strong binding to DPP-4 enzymes compared with other gliptins and additional pleiotropic benefits[6]. The mechanism of action of teneligliptin which is used as a hypoglycemic agent in the treatment of T2DM is to amplify the levels of incretins namely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide in response to meal intake, in turn resulting in augmented insulin secretion and attenuated glucagon secretion.[6,7]

The hypolipidemic effect of teneligliptin is probably due to increased lipid mobilization from the adipose tissue and its oxidation by sympathetic activation facilitated through GLP-1 receptor activation, thereby reducing blood lipid levels as demonstrated by Boschmann *et al*.[16]

Also, the administration of teneligliptin has been shown to reduce the body weight in six-weeks-old C57BL/6N mice fed on a high-fat diet. The study has also established that teneligliptin increased oxygen consumption by 22% apart from proving that adipocyte hypertrophy and hepatic steatosis induced by a high-fat diet were suppressed by teneligliptin. In addition, a reduction in mean adipocyte size and hepatic TG content were demonstrable in this study.[12]

Another study investigating the effectiveness of teneligliptin in a mouse model of non-alcoholic steatohepatitis revealed that DPP4 inhibitors, by amplifying GLP-1 induced AMPK activation in hepatocytes inhibits hepatic lipogenesis, thus consequentially inhibiting cholesterol and TG biosynthesis. The AMPK activation also promotes fatty acid ß-oxidation and reduction of free fatty acid.14Thus it is obvious that multiple pathophysiological mechanisms are potentially contributory to the hypolipidemic effect of teneligliptin.

However two clinical studies in Japanese population have yielded conflicting results on teneligliptin's effect on lipid profile. But both these studies were done in a small sample size which could be one of the reasons in discordance in their results.[9,10] Yet another large clinical study done in a Korean population wherein about 1732 patients on any of the six other gliptins including alogliptin, gemigliptin, sitagliptin, vildagliptin, saxagliptin and linagliptin were switched over to teneligptin, the effects of which were recorded at the end of 12 weeks.This study demonstrated that the weight decreased significantly by a mean of 0.4 kg from baseline in addition to a statistically significantdecrease in the mean BMI by 0.1 kg/ m2. Among the serum lipid parameters, TC and LDL-C levels decreased from baseline to week 12 ($p <?0.05$). Though a favorable trend in the change from mean baseline values were noted for TG and HDL-C they were not statistically significant.[17]

The greater potency and additional beneficial effects of teneligliptin could be attributed to the fact that it binds more tightly to the DPP-4 enzyme compared to other gliptins because of the "J-shaped" structure formed by five rings.[18] Thus the long-duration and strong binding property of teneligliptin could potentially yield greater clinically meaningful metabolic benefits.

CONCLUSION

This study has delineated the hypolipidemic activity of teneligliptin which will be of potential clinical value in the therapeutic use of teneligliptinin type 2 diabetic patients especially in the face of increased concomitance in the occurrence of T2DM and hyperlipidemia.

REFERENCES

1. Benjamin EJ, Virani SS, Callaway CW, *et al.* Heart Disease and Stroke Statistics — 2018 update: a report from the American Heart

Association. *Circulation*. **137**(12):e67-e492 (2018).
2. Joshi SR, Anjana RM, Deepa M, Pradeepa R, Bhansali A, Dhandania VK *et al*. Prevalence of dyslipidemia in urban and rural India: the ICMR-INDIAB study. *PLoS One*. **9**(5):e96808 (2014).
3. Naci H, Brugts J, Ades T. Comparative tolerability and harms of individual statins: a study-level network meta-analysis of 246 955 participants from 135 randomized, controlled trials. Circulation: *Cardiovascular Quality and Outcomes.* **6**(4):390-9 (2013).
4. Newman CB, Preiss D, Tobert JA, Jacobson TA, Page RL, Goldstein LB, Chin C, Tannock LR, Miller M, Raghuveer G, Duell PB. Statin safety and associated adverse events: a scientific statement from the American Heart Association. *Arteriosclerosis, thrombosis, and vascular biology.* **39**(2):e38-81 (2019).
5. Schultz BG, Patten DK, Berlau DJ. The role of statins in both cognitive impairment and protection against dementia: a tale of two mechanisms. *Translational neurodegeneration.* **7**(1):5 (2018).
6. Sharma SK, Panneerselvam A, Singh KP, Parmar G, Gadge P, Swami OC. Teneligliptin in management of type 2 diabetes mellitus. Diabetes, metabolic syndrome and obesity: targets and therapy. **9**: 251-260(2016).
7. Kishimoto M. Teneligliptin: a DPP-4 inhibitor for the treatment of type 2 diabetes. Diabetes, metabolic syndrome and obesity: targets and therapy. **6**: 187-195 (2013).
8. Singh AK. Efficacy and safety of teneligliptin. *Indian journal of endocrinology and metabolism*. **21**(1): 11-17(2017).
9. Kutoh E, Hirate M, Ikeno Y. Teneligliptin as an initial therapy for newly diagnosed, drug naive subjects with type 2 diabetes. *Journal of clinical medicine research.* **6**(4): 287-94 (2014).
10. Kusunoki M, Sato D, Nakamura T, Oshida Y, Tsutsui H, Natsume Y, Tsutsumi K, Miyata T. DPP-4 inhibitor teneligliptin improves insulin resistance and serum lipid profile in Japanese patients with type 2 diabetes. *Drug research*. **65**(10): 532-4 (2015).
11. Martin KA, Mani MV, Mani A. New targets to treat obesity and the metabolic syndrome. *European journal of pharmacology.***763**: 64-74 (2015).
12. Fukuda-Tsuru S, Kakimoto T, Utsumi H, Kiuchi S, Ishii S. The novel dipeptidyl peptidase-4 inhibitor teneligliptin prevents high-fat diet-induced obesity accompanied with increased energy expenditure in mice. *European journal of pharmacology.* **723**: 207-15 (2014).
13. Itou M, Kawaguchi T, Taniguchi E, Sata M. Dipeptidyl peptidase-4: a key player in chronic liver disease. *World journal of gastroenterology.* **19**(15): 2298-2306 (2013).
14. Ideta T, Shirakami Y, Miyazaki T, Kochi T, Sakai H, Moriwaki H, Shimizu M. The dipeptidyl peptidase-4 inhibitor teneligliptin attenuates hepatic lipogenesis via AMPK activation in non-alcoholic fatty liver disease model mice. *International journal of molecular sciences.* **16**(12): 29207-18 (2015).
15. Zhang Q, Wang GJ, Ji-ye A, Wu D, Zhu LL, Ma B, Du Y. Application of GC/MS-based metabonomic profiling in studying the lipid-regulating effects of Ginkgo biloba extract on diet-induced hyperlipidemia in rats. *Acta Pharmacologica Sinica*. **30**(12):1674-87 (2009).
16. Boschmann M, Engeli S, Dobberstein K, Budziarek P, Strauss A, Boehnke J, Sweep FC, Luft FC, He Y, Foley JE, Jordan J. Dipeptidyl-peptidase-IV inhibition augments postprandial lipid mobilization and oxidation in type 2 diabetic patients. *The Journal of Clinical Endocrinology & Metabolism.* **94**(3): 846-52 (2009).
17. Kim HJ, Kim YS, Lee CB, Choi MG, Chang HJ, Kim SK, Yu JM, Kim TH, Lee JH, Ahn KJ, Kim K. Efficacy and Safety of Switching to Teneligliptin in Patients with Type 2 Diabetes Inadequately Controlled with Dipeptidyl Peptidase-4 Inhibitors: A 12-Week Interim Report. *Diabetes Therapy*. **10**(4): 1271-1282 (2019).
18. Nabeno M, Akahoshi F, Kishida H, Miyaguchi I, Tanaka Y, Ishii S, *et al*. A comparative study of the binding modes of recently launched dipeptidyl peptidase IV inhibitors in the active site. *Biochem Biophys Res Commun.* **434**(2): 191–196 (2013).

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Original Article

A Study to Identify the Effect of *Momordica Charantia* on Smooth Muscle Activity in Isolated Guinea Pig Ileum

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Abstract

Background: *Momordica charantia (M. charantia),* bitter melon is commonly used in traditional medicine for its wide variety of medicinal properties.

Aim and objectives: To study the effect of successive extract of *M. charantia* on smooth muscle contractility of the isolated guinea pig ileum compared to that of standard drugs and to evaluate the receptors mediating the effects of *M. charantia* on intestinal smooth muscle using appropriate antagonists.

Materials and methods: An adult albino male guinea pig of weight 700 g was euthanased and the ileum was dissected out for isolated tissue experiment in organ bath using tyrode as physiological salt solution, aerated with air, maintained at pH of 7.4 and temperature 37°C. Successive extract of *M. charantia* was prepared in soxhlet apparatus using petroleum ether, methanol, ethanol and water. 100 mg of this extract was diluted in 10 ml of water to get a stock solution of 10 mg/ml w/v which was used after appropriate dilutions to obtain the working standard solutions. Experimental procedures were done in accordance to the standard principles of isolated tissue experiments.

Results: Contractile response was noted *for M. charantia* and its dose response curve was obtained following those of standard drugs, acetylcholine and histamine. The receptor actions were studied using atropine (muscarinic antagonist) and pheneramine maleate (histamine antagonist) and adrenaline (adrenergic agonist) which are gut smooth muscle relaxants and we found that all the three drugs did not alter the response of *M. charantia* proving that none of these three receptors systems, muscarinic, histaminic or adrenergic were involved in mediating the contractile actions of *M. charantia*.

Conclusion: This study has evaluated the contractile effect and the underlying receptor mechanisms contributing to this effect of *M. charantia* on the intestinal smooth muscle of guinea pig using isolated tissue experiment.

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Introduction

Momordica charantia, commonly known as bitter gourd or bitter melon is a medicinal plant belonging

to the family Cucurbitaceae and commonly cultivated in many tropical and subtropical countries. Bitter melon is known for its wide variety of medicinal properties (1). It has an array of biologically active chemicals including saponins, steroids, alkaloids, triterpens, proteins and flavonoids due to which the fruit is shown to possesses anti-bacterial, anti-fungal, anti-viral, anti-parasitic, hypoglycemic and anti-carcinogenic properties (2-5). It is especially used in the treatment of diabetes due to its effect in reducing blood glucose, enhancing insulin sensitivity and preserving the islet cell function (6). It is also used in the treatment of rheumatism, gout and diseases of liver and spleen (7).

A study done using the methanolic extract of *M. charantia* has demonstrated its antidepressant and anxiolytic properties in mice and it has been proven that the antidepressant-like effect is dependent on the agonistic activity at central serotonergic (5-HT2 receptor), noradrenergic (α1 and α2 adrenoceptors), dopaminergic (D2 receptor), and muscarinic cholinergic systems (8). The effect of *M. charantia* on the peripheral tissues like the intestinal smooth muscles expressing such receptors have not been studied. Additionally, several studies have documented a laxative effect (9, 10, 11) with the use of *M. charantia* and the mechanism underlying this effect has not been investigated. So the current study aimed to identify the change in intestinal smooth muscle contractile tone by *M. charantia* with progressively increasing doses of successive extract *M. charantia* compared to that of the spasmogens like acetylcholine and histamine in isolated tissue experiment using guinea pig ileum and to interpret the receptor mechanisms underlying such actions by using relaxants like adrenergic agonist as well as receptor antagonists of muscarinic and histamine receptors.

Materials & Methods

The study protocol was approved by the institutional ethics committee (proposal number: 324/2016/IAEC). The *M. charantia* was procured from the local market. It was identified by a botanist from the PSG Institutions and a vocher sample of *M. charantia* fruit is preserved in the department of Pharmacology (Pha/ 03/ 2014).

Preparation of M. charantia successive extract (12):

250 g of *M. charantia* fruit was shade dried and grounded to fine powder. 200 g of the powder was weighed and subjected to extraction in a soxhlet apparatus at room temperature using petroleum ether, methanol, ethanol and water successively. Before extraction with the next solvent, the powder was air dried to remove the adhering solvent and was evaporated to dryness. The final extract was weighed after open dish evaporation to ensure removal of the solvents and stored at 4°C in refrigerator till further use.

Preparation of standard drugs for comparison:

Acetylcholine (1 mg/ml in 5% w/v NaH_2PO_4), Histamine (10 mg/ml), Adrenaline (0.1 mg/ml), Atropine (1 mcg/ml), Pheniramine (1 mg/ml) and stock solution of *M. charantia* successive extract was prepared by diluting 100 mg of the extract in 10 ml of water to obtain 10 mg/ml w/v which was mixed using magnetic stirrer at 300 rpm for 15 minutes to enhance the solubility. These were used for the isolated tissue experimental procedures after appropriate dilutions to working standard solutions.

Preparation of isolated guinea pig ileum tissue:

An adult male albino guinea pig (*Cavia porcellus*) aged 14 months and weighing 700 g was obtained from the animal house and fasted overnight. The animal was sacrificed by overdose of pentobarbitone (100 mg/kg) injected intraperitoneally followed by cervical dislocation for confirmation of euthanasia. The ileum was identified and dissected out. The ileal tissue was divided into two segments and mounted in two separate organ baths using Tyrode as the physiological salt solution (adjusted to pH = 7.4) provided with air for aeration and temperature maintained at 37°C. One end of the each tissue segment was tied securely to the tissue holder while the other end was tied to a frontal writing lever, adjusted for suitable tension and magnification. The ink-writing kymograph drum (INCO E8 recording drum using 220V/50 Hz power) wrapped with paper was run at a speed of 0.12 mm/sec to record the

contractions. The responses were thus recorded on the paper fixed to the travelling surface of kymograph drum.

Determination of the response of intestinal smooth muscle to Successive extract of M. charantia and receptors involved in mediating the effect:

Following 40 min of relaxation of the mounted tissue, graded dose responses were recorded for the standards namely acetylcholine, histamine and then for the successive extract of *M. charantia* preparation. Since graded contractions of the smooth muscle were obtained for *M. charanti*a successive extract, we proceeded to study the effect of antagonists/relaxants on the contraction induced by the extract on the intestinal smooth muscle.

Competitive antagonists for the receptors expressed in the smooth muscles of intestine which mediate contraction, namely atropine for muscarinic M2, 3 and pheniramine maleate for histamine H1 were utilized for receptor antagonism evaluation. Antagonism of the response to a single dose of *M. charantia* by these two antagonists was tested by adding increments of 10 times higher dose of each of the antagonists individually along with the agonist (*M. charantia* successive extract 8 mg) consequentially in 3 cycles to the isolated tissue preparation for determining antagonism by any of these doses on the smooth muscle contraction and if so, whether the antagonism signified competitive type by recording further responses keeping the dose of antagonist eliciting such reduction in response of *M. charanti* as constant and increasing the dose of *M. charantia* in geometric progression. In the event of antagonism not being demonstrable, ascertaining the nature of antagonism would not be performed and the protocol was to proceed to elucidating the role of adrenergic antagonism with a similar evaluation of the change in the contractile effect of the extract by adrenergic agonist adrenaline in order to delineate if anti-adrenergic action of successive extract on the gut smooth muscle was responsible for the stimulant effect of *M. charantia.*

Results

The graded dose responses were obtained for acetylcholine, histamine and the recordings ensured that the tissue was viable and sensitive. The threshold dose for acetylcholine was determined to be 0.1 µg and the maximal response was obtained for 3.2 µg (Fig. 1). Likewise for histamine, the first response was recorded for the dose of 0.2 µg and the maximal response was seen at 3.2 µg (Fig. 2). For the successive extract of *M. charantia*, the intestinal smooth muscle responses were found to be contractile and the threshold response was evident at 1mg dose. The doses were added to the inner organ bath in geometric progression as per the standard procedure. It was found that the magnitude of responses also showed progressive increase with increasing doses until the maximal response for *M. charantia* extract being recorded at 32 mg (Fig. 3).

Log dose response curves (LDRCs) were plotted (Fig. 4). The log dose response curves analyzed using Microsoft Excel 2007 revealed that the slope of the successive extract (4.740) differed significantly from those of acetylcholine (2.782) and histamine (6.389) (Fig. 5).

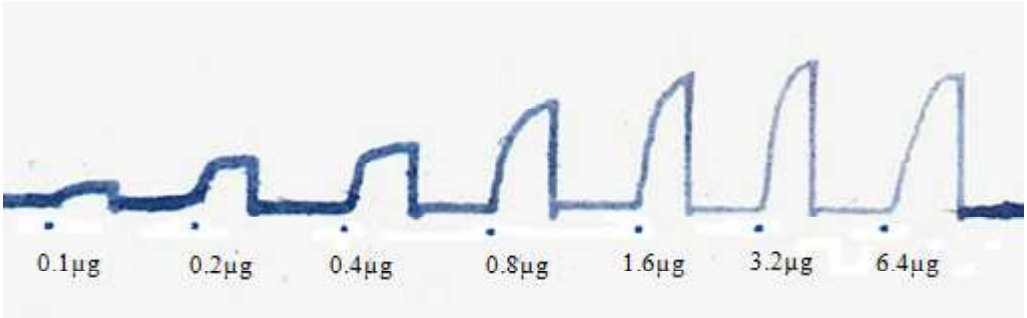


Fig. 1: Graded dose response curve for acetylcholine (Stock = 1 mg/ml) using guinea pig ileum.

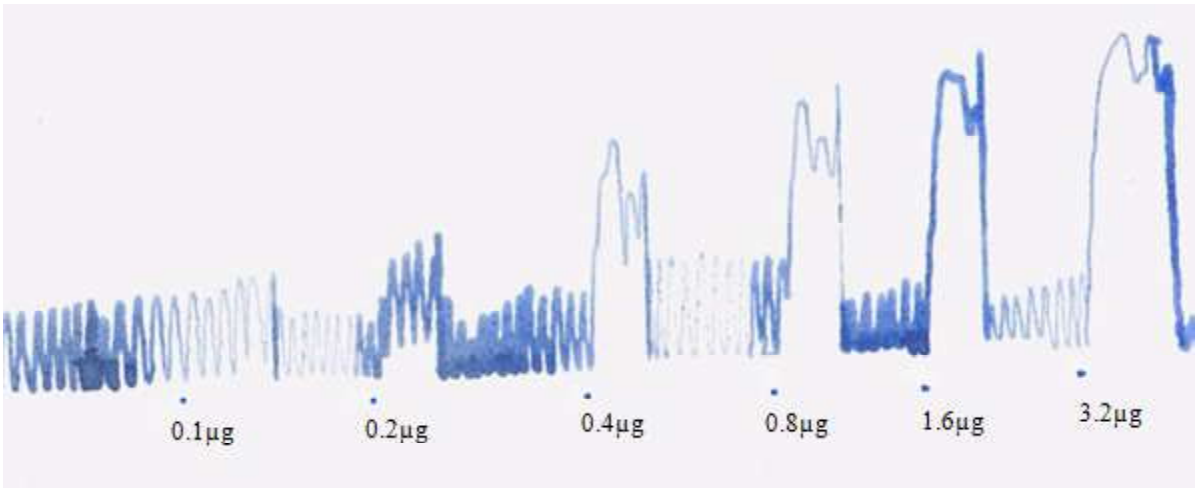


Fig. 2 : Graded dose response curve for histamine (Stock = 10 mg/ml) using guinea pig ileum.

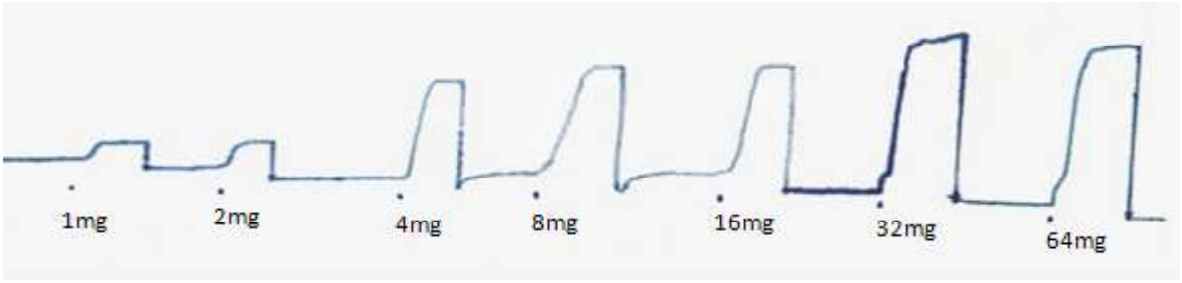


Fig. 3 : Graded dose response curve for successive extract of *M.charantia* (Stock = 10 mg/ml) using guinea pig ileum.

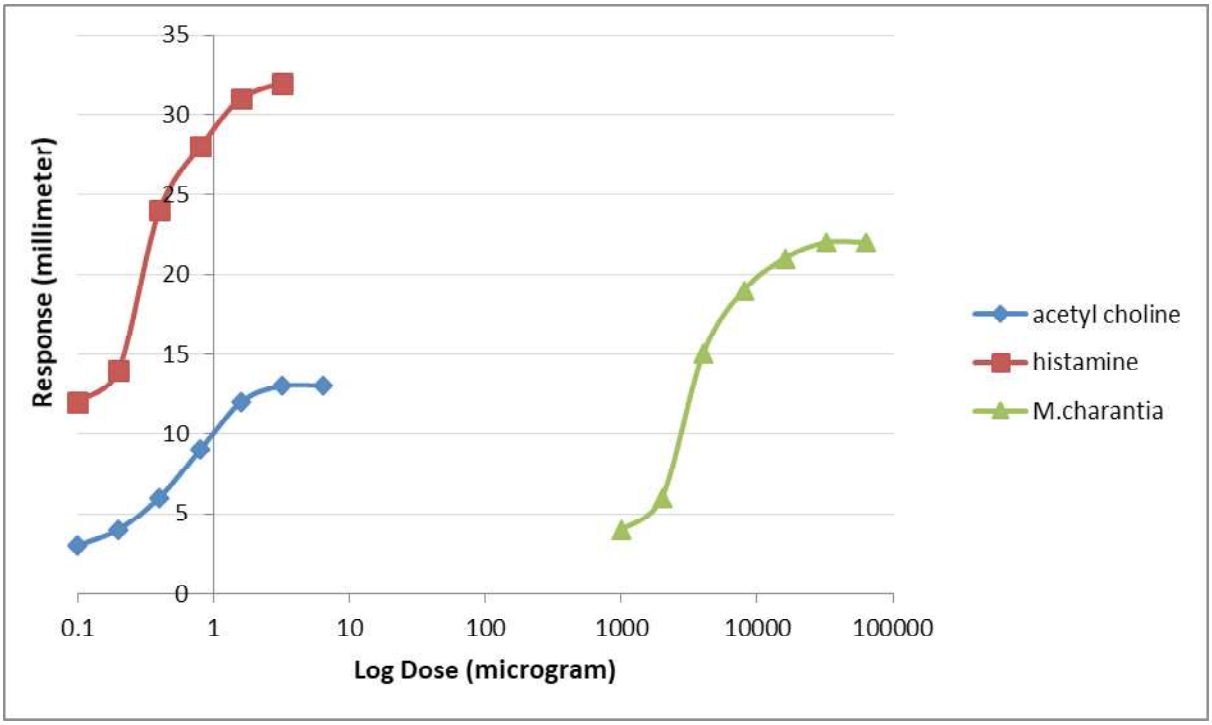


Fig. 4 : Comparison of log dose response curves of acetylcholine, histamine and *M.charantia*.

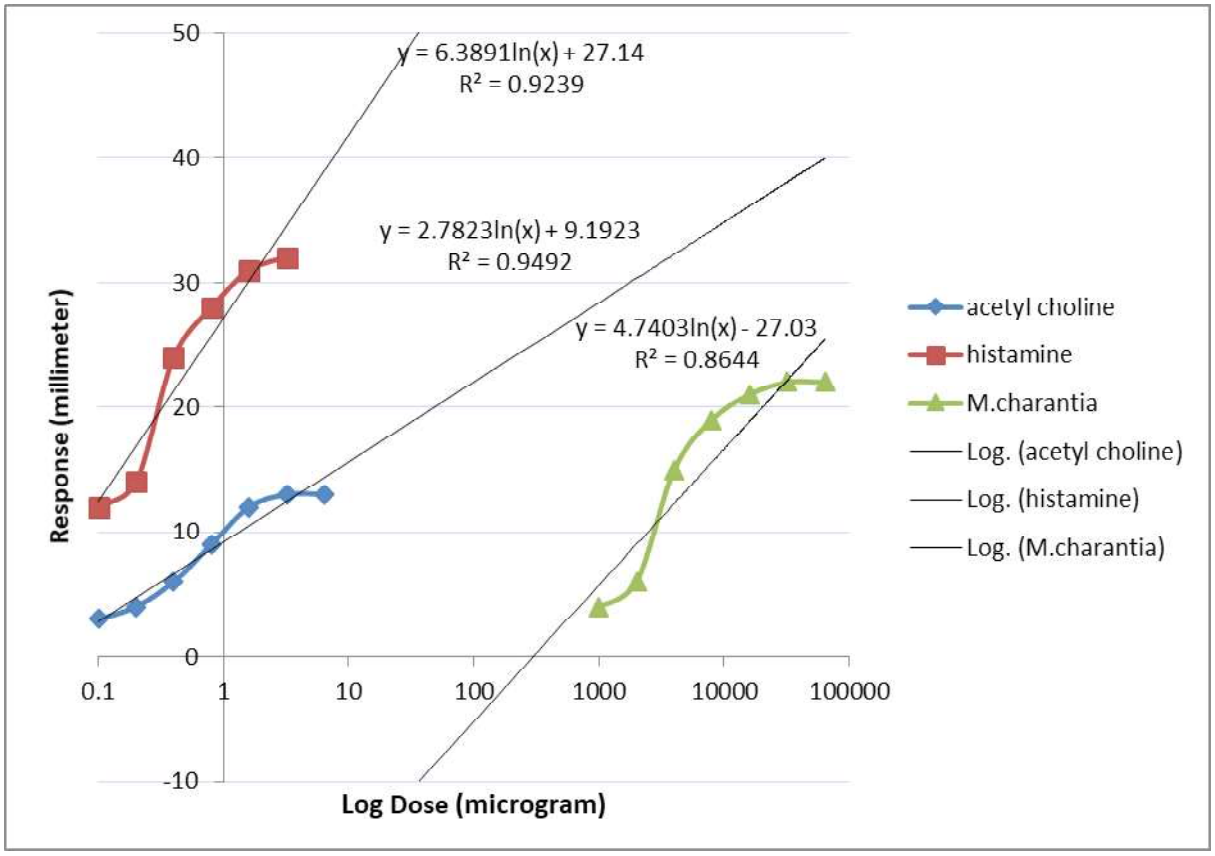


Fig. 5 : Comparison of slope of log dose response curves with trendlines of acetylcholine, histamine and *M.charantia.*

The change in contractile responses of *M. charantia* was then tested in presence of antagonist for the cholinergic receptors namely atropine and the histamine antagonist, pheniramine maleate. The dose of *M. charantia*, 8 mg was chosen to study the effect of increasing concentrations of antagonist. The choice of this dose was essentially to avoid higher doses which on repetitive administration, may contribute to bias by reducing the response of the tissue and thus affect the sensitivity of the tissue.

Interestingly, the study of antagonists revealed that the height of response to the successive extract of *M. charantia* was neither altered by atropine at the three dose levels studied (0.01 µg, 0.1 µg, 1 µg) nor by that of histamine antagonist Pheniramine maleate (0.01 mg, 0.1 mg, 1 mg). Hence we proceeded to evaluate the effect of adrenaline, an adrenergic agonist (0.01 mg, 0.1 mg) which is an intestinal smooth muscle relaxant on the contractile response of *M. charantia* and to this end, we noticed a similar lack of change in the contraction induced by successive extract of *M. charantia* indicating that adrenoreceptors were not involved in the contractions of the smooth muscles of the ileum in response to *M. charantia* successive extract (Fig. 6).

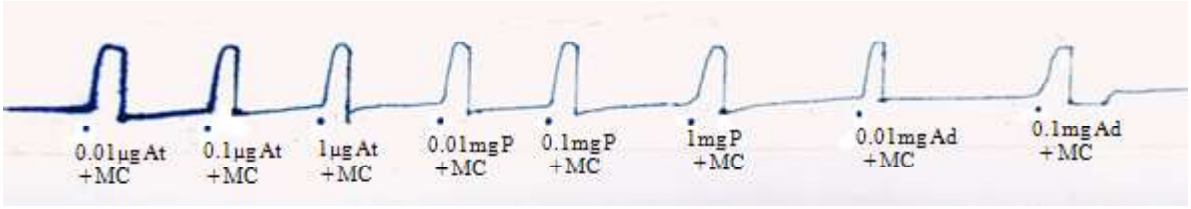


Fig. 6 : Study of antagonism of *M.charantia* response in guinea pig ileum using smooth muscle relaxants. Atropine – At; Pheniramine – P; Adrenaline – Ad; *M.charantia* - MC (4 mg)

Discussion

This isolated tissue animal experimentation study has brought out a very important effect of one of the commonly used natural products in food and traditional medicine. A contractile action of *M. charantia* on the intestinal smooth muscles of guinea pig ileum was evident and it was demonstrable that the height of responses increased with doses administered in geometric progression indicating that the magnitude of contractile responses was a function of the dose of *M. charantia* extract with R^2=0.864 (Fig. 5).Similar to the graded responses obtained for acetylcholine (R^2 = 0.949) and histamine(R^2 = 0.923), there was a dose dependent increase in the responses deductable up to the maximal dose, beyond which the supra-maximal dose did not show any further increase in response height; thus a complete graded dose response curve (GDRC) was obtained. The GDRC was thus demonstrative of the fact that the intestinal smooth muscle contractions induced by *M. charantia* extract were not a mere erratic phenomenon exhibited at certain high doses of the extract; on the other hand, proved to be a standard response that could be assayed on a standard tissue like the guinea pig ileum in accordance to the principles governing bioassay.

Additionally, logarithmic conversion of the dose response curve revealed a sigmoid dose response relationship akin to that of the standard spasmogens like acetylcholine and histamine. Moreover, it was evident that potency of the *M. charantia* extract was more than 1000 times lower in comparison to acetylcholine or histamine (Fig. 4). This essentially explains that it is safe for human consumption without undue spasmodic effect except at very high doses.

Literature exists for the laxative action of *M. charantia* especially at high doses and the effect illustrated in our study explicates the mechanism contributory to the increased intestinal motility with *M. charantia*. Few studies have proved that inhibition of glucose absorption in the gut is one of the mechanisms by which *M. charantia* exerts the well known anti-hyperglycemic effect and these studies have also shown that there is inhibition of intestinal fluid transport secondary to reduced transport of glucose and aminoacids in the rat everted gut sacs in vitro (11, 13). Such an effect could be potentially additive to increased contractility to produce a laxative effect.

Deciphering the receptors likely to be responsible for the pharmacological response of *M. charantia* elucidated in our study was done using the principles of antagonism. The change in response with addition of antagonists of the two major receptors, muscarinic and histaminergic, involved in intestinal smooth muscle contractility namely atropine, pheniramine maleate respectively were investigated apart from adrenaline, agonist for the adrenoreceptor causing gut smooth muscle relaxation. However, failure to reduce or abolish the contractile response to *M. charantia* extract was observed with all the three relaxants indicating that none of these receptors were involved in mediating the increased contractility of *M. charantia* extract (Fig. 6). The plausible explanation for this could be attributed to non-receptor mediated actions of *M. charantia,* acting directly on the ion channels like calcium or potassium ion channels. A potential increase in conductance of calcium channels or alternatively a decreased K^+ ion conductance could be the likely contributory mechanism for this activity. Another probable mechanism could be activation of the 5HT3 or 5HT4 receptors on the intrinsic primary afferents of the enteric nervous system, which however were not evaluated in this isolated tissue model and is therefore considered as a limitation of the study.

Moreover, literature evidence states that agonists whose log dose response curves (LDRCs) are not parallel are suggestive of acting through different receptors and hence said to have varying mechanism of action despite the same resultant effect (14). In our study, the slope of LDRC of *M. charantia* extract was significantly different from that of acetylcholine and histamine. Hence the LDRCs of all three weren't parallel, indirectly indicating that the mechanisms eliciting the stimulant response on the intestinal smooth muscle were diverse. This mandates the need for further molecular studies like receptor binding and ion channel conductance studies to confirm the results of our study.

Thus the study has delineated the effect of increased contractility of gut smooth muscle by the successive

extract of *M. charantia* which can be utilized therapeutically for conditions affecting intestinal motility like diabetic gastroparesis. Its effectiveness in such diabetic complication, which if confirmed by clinical studies, would be dually beneficial especially in the face of paramount importance of *M. charantia* in management of diabetes patients and the huge burden of disease that this metabolic disorder contributes to, owing to the sheer high proportion of population it affects.

Conclusion

This is the first study done to identify the smooth muscle contractile effect of *M. charantia* in isolated intestinal smooth muscle of guinea pig ileum. This study based on the concept of reverse pharmacology has ascertained one of the salient pharmacological actions of *M. charantia* and the potential underlying mechanism, enabling future studies in further evaluating the therapeutic benefit of this effect of *M. charantia* in clinical conditions associated with reduced gut motility including diabetic gastrointestinal dysmotility disorders.

Conflict of interest

The authors have none to declare.

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References

1. Chao CY, Sung PJ, Wang WH, Kuo YH. Anti-inflammatory effect of Momordica charantia in sepsis mice. *Molecules* 2014; 19(8): 12777–12788.
2. Alam S, Asad M, Asdaq SM, Prasad VS. Antiulcer activity of methanolic extract of Momordica charantia L. in rats. *Journal of Ethnopharmacology* 2009; 123(3): 464–469.
3. Beloin N, Gbeassor M, Akpagana K, Hudson J, de Soussa K, Koumaglo K, Arnason JT. Ethnomedicinal uses of Momordica charantia (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. *Journal of Ethnopharmacology* 2005; 96(1): 49–55.
4. Joseph B, Jini D. Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. *Asian Pacific Journal of Tropical Disease* 2013; 3(2): 93–102.
5. Grover JK, Yadav SP. Pharmacological actions and potential uses of Momordica charantia: a review. *Journal of Ethnopharmacology* 2004; 93(1): 123–132.
6. Chaturvedi P. Antidiabetic potentials of Momordica charantia: multiple mechanisms behind the effects. *J Med Food* 2012; 15(2): 101–107.
7. Ng TB, Chan WY, Yeung HW. Proteins with abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plants. General Pharmacology: The vascular system 1992; 23(4): 575–590.
8. Ishola IO, Akinyede AA, Sholarin AM. Antidepressant and anxiolytic properties of the methanolic extract of Momordica charantia Linn (Cucurbitaceae) and its mechanism of action. *Drug Research* 2014; 64(07): 368–376.
9. Kumar DS, Sharathnath KV, Yogeswaran P, Harani A, Sudhakar K, Sudha P, Banji D. A medicinal potency of Momordica charantia. *International Journal of Pharmaceutical Sciences Review and Research* 2010; 1(2): 95–100.
10. Grover JK, Yadav SP. Pharmacological actions and potential uses of Momordica charantia: a review. *Journal of Ethnopharmacology* 2004; 93(1): 123–132.
11. Mahomoodally MF, Fakim AG, Subratty AH. Momordica charantia extracts inhibit uptake of monosaccharide and amino acid across rat everted gut sacs in-vitro. *Biological and Pharmaceutical Bulletin* 2004; 27(2): 216–218.
12. Roopashree TS, Dang R, Rani RS, Narendra C. Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. *International Journal of Applied Research in Natural Products* 2008; 1(3): 20–28.
13. Mahomoodally MF, Gurib-Fakim A, Subratty AH. Effect of exogenous ATP on Momordica charantia Linn. (Cucurbitaceae) induced inhibition of D-glucose, L-tyrosine and fluid transport across rat everted intestinal sacs in vitro. *J Ethnopharmacol* 2007; 110(2): 257–263.
14. Tallarida R, Jacob LS. The Dose-Response Relation in Pharmacology. Springer Science & Business Media; 2012 Dec 6.

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Research Article

MOMORDICA CHARANTIA NANO DRY POWDER ORAL FORMULATION IN CONTROLLING HISTAMINE-INDUCED BRONCHOSPASM

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ABSTRACT

Objective: *Momordica charantia* (MC) (bitter gourd) is known traditionally to possess medicinal values. This edible vegetable is known for its anti-inflammatory properties and has been studied for its bronchospasm preventive action. With the recent development in nanoformulations, this study was done to identify the effectiveness of MC nanoformulation in comparison to crude MC formulation in protecting animal from histamine-induced bronchospasm.

Methods: The optimized MC nanoformulation was prepared with polyethylene glycol and soy phosphatidylcholine. The MC crude powder was prepared by drying in hot air oven and grinding in a blender. Eight guinea pigs of both sexes were given once daily MC nanoformulation per oral by mixing with water for 7 days. Evaluation of the formulation was done on the 2nd, 4th, and 6th h on day 1 and day 7 after administration of MC for protection in developing pre-convulsive dyspnea due to histamine-induced bronchospasm.

Results: An analysis of variance test revealed statistically significant results in the protection of animals from developing bronchospasm after treatment with the MC nanoformulation in comparison to the control values. About 75% of animals were protected from developing bronchospasm on day 7 attributing to the cumulative effect of nanoformulation.

Conclusion: The study proved that the MC nanoformulation could effectively prevent histamine-induced bronchospasm in guinea pigs.

Keywords: *Momordica charantia*, Nanoformulation, Anti-inflammatory.

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INTRODUCTION

Momordica charantia (MC) also known as bitter gourd is a common edible vegetable, known to possess medicinal values [1]. It is traditionally known for its medicinal properties such as anti-diabetic, anticancer, anti-inflammation, antivirus, and for cholesterol-lowering effects. It contains many phenol compounds that may have the potential as antioxidant and anti-mutagen [2]. The fruit, stems, leaves, and roots of MC have all been used in traditional medicine to help treat ailments such as hyperlipidemia, digestive disorders, microbial infections, and menstrual problems [3]. Studies have also shown that bitter melon has anticarcinogenic properties and can be used as a cytotoxic agent [4]. Many studies had confirmed the electrolyte balancing property, anti-inflammatory, and antioxidant property of MC with cytokine-inhibiting activity [5,6]. The anti-inflammatory activity of MC has been confirmed by its action through, interleukin 1 (IL)-1, IL-2, IL-6, IL-8, tumor necrosis factor-α, and nuclear factor kappa β normalization both in animal and human studies.

Asthma is a chronic inflammatory disorder of the airways and needs long-term use of anti-inflammatory drugs to control symptoms [7]. MC is known for its anti-inflammatory action and previous done have proven its property to prevent histamine-induced bronchospasm. However, no studies have been done on the nanoformulation of MC. This study was proposed as an attempt to prove the benefitting properties of MC nanoformulation pre-clinically in guinea pigs for its anti-inflammatory potent action in preventing histamine-induced bronchospasm.

The purpose of this study was to maximize the pharmacological property of the MC nanoformulation by making it available for maximum systemic absorption. As there is variation in the various extracts and powders of MC, there is probably variation in the formulation we provide to the patients. One such recent advance is the nanocapsule formulation [8]. They are considered as weapons of new drug delivery system. Hence, this study was proposed to identify the effectiveness of MC nanoformulation in protecting guinea pigs from histamine-induced bronchospasm in whole animal model.

METHODS

The study was done in two parts. First, the nanoformulation of MC and crude dry powder formulation of MC were prepared and shelf life of the two compounds was tested for 180 days. The second part of the study dealt with administration of the nanoformulation of MC per oral in guinea pigs and its efficacy in preventing histamine-induced bronchospasm was evaluated.

Preparation of nanoformulation of MC

MC crude powder was prepared by drying in hot air oven and grinding in a blender. Nanosuspension of crude bitter gourd powder was prepared by probe sonication and lyophilization technique [9,10]. Briefly, sufficient quantity (10 g) of crude bitter gourd powder was weighed and added to the aqueous phase (water) containing surfactant soy phosphatidylcholine. The aqueous mixture was sonicated using a bath sonicator for 15 min to get a homogeneous mixture. The resulting homogeneous mixture was sonicated using an ultrasonic probe sonicator (Vibra Sonics) for 30 min at 20,000 Hz to produce bitter gourd nanosuspension. This sample of nanosuspension particle size was measured using Malvern Zetasizer (Nano ZS90, Malvern Instruments) at 25°C and measured 291.3 diameter nanometer (d. nm). To improve the bioavailability of this MC nanoproduct, we added polyethylene glycol (PEG) and deep freezing was done at −80°C for 4 h following this, the sample was again freeze-dried in a lyophilizer (Lyodel) at a temperature and pressure of −50° and −1 bars, respectively, for 48 h to form powder.

The dry nanoparticles were, hence, ready and the final particle size noted was 536.4 d. nm [11].

Following this, a 180 days stability test was done on both the formulations of MC, filled in gelatin capsules and found that both were stable in room temperature (25°) and high temperature (45°). Stability was tested for its color, shape, consistency, pH, weight, and disintegration in acid buffers.

Protection of histamine-induced bronchospasm by MC nanopowder (Fig. 1)

Eight adult guinea pigs of both sexes were chosen and the animals were kept in rooms under standard conditions. The animals were provided with ambient temperature of 25°C and 55% relative humidity, 12 h light period. Granular mixed feed and drinking water were available for all animals *ad libitum* throughout the experiment. The Institutional Animal Ethics Committee approved the study and the study was conducted strictly with guidelines of ICMR and CPCSEA.

On day 1, all the eight guinea pigs were weighed and animal dose of MC nanoformulation at 600 mg/kg and was administered orally after dissolving in drinking water. After 2 h, the animals were exposed to 2% histamine aerosol for 120 s using histamine chamber. Preconvulsive dyspnea (PCD) was noted in seconds [12,13]. The same experiment was repeated after 4 h from the time of MC nanoformulation administration and the PCD values were noted in seconds.

Following this, all the eight guinea pigs were given MC nanoformulation at 600 mg/kg/day (standardized animal dose) OD from day 2 to day 7. On day 7, the animals were again exposed to bronchospasm induction with 2% histamine aerosol chamber for 120 s from 2 h of MC nanoformulation administration. Bronchospasm protection activity for both the formulations of MC was observed. All the three sets of values to develop PCD (control value, day 1 value, and day 7 value) were tabulated for statistical analysis. The mean value for developing PCD in a control animal (before giving drug) was found to be 61.10 s [14].

RESULTS

The animals were administered with MC nanoformulation from day 1 to day 7 every day at 8 am. Protection for bronchospasm was observed on day 1 and day 7. The time to develop PCD was as follows:

Table 1: Particle size, polydispersion unit, and zeta potential of the formulation of MC

Formulations	Particle size	PDI (polydispersion index)	Zeta potential
MC crude dry powder	3953 d. nm	1.000	−6.01
MC nano dry powder (SPL+PEG)	534.6 d. nm	0.612	−36.23

MC: *Momordica charantia*, PEG: Polyethylene glycol

On day 1, after 4 h from nanoformulation of MC administration, it was observed that 5 (62.5%) animals were completely protected from PCD and three animals had prolonged duration in developing PCD compared with the previous exposure at 2 h. (Further, exposure to histamine not done as >50% of animals were protected at the end of 4 h).

On day 7, six animals (75%) were completely protected from developing PCD, and hence, further, exposure to histamine was not done.

Statistical analysis

Analysis of variance (ANOVA) test was applied to compare the time difference to develop PCD in the animals in seconds between the three values (Day 1: 2 h and 4 h and day 7: 2 h).

ANOVA test done between the three values has effectively demonstrated that all the animals were significantly protected from developing bronchospasm on day 1 after 4 h of MC nanodrug formulation ($p<0.01$)*. Similarly, the animals were significantly protected from developing bronchospasm on day 7 after 2 h of drug administration ($p<0.01$)*.

Post-study animals were observed for a period of 2 months and no drug reactions or fatal effects were observed.

DISCUSSION

The aim of this study was to compare the efficacy and potency of MC nano dry formulation in contrast to its raw form as MC dry powder and MC fresh juice demonstrated by Bhuvaneswari *et al*. in animal model [14]. MC nanopowder was prepared and the particle size measurement showed 75% reduction in size of the particles in comparison to MC crude dry powder (Table 1). The nanoformulation was proved stable in high and low temperature after a 180 days stability testing (Table 2).

The mean of the control group was found to be 61.10 s [14]. Comparison was done for the same group animals on three different timings of histamine exposure post nanodrug administration and all the three sets of values were compared with ANOVA test (Table 3).

Guinea pigs being most sensitive for its histamine receptor sensitivity were chosen for this study [15]**.** Fig. 2 has clearly shown the number of animals protected from developing PCD. A normal control animal can hold from developing PCD for 61.1 s and animals treated with standard drug Montelukast could hold from developing PCD for 120 s, evinced from the previous studies [14].

Table 4 has illustrated that 50% of animals were able to attain protection from developing bronchospasm after 2 h of MC nano dry powder formulation administration. Following this, almost 100% of animals showed prolongation of duration from developing PCD in comparison to the control, hence, proving the bronchospasm protective activity of MC nanodrug. On the same day, 62.5% of animals were completely protected from developing PCD after 4 h of MC nanodrug administration on day 1 and further exposure to histamine

Table 2: Physical properties of individual capsules from day 0 to day 180 at room temperature and high temperature

Capsules	Color	Odor	Shape	Consistency
MC crude dry powder	Yellow+red capsule color was not altered	No odor	Shape maintained	Dry capsules
MC nano dry powder (SPL+PEG)	Yellow+red capsule color was not altered	No odor	Shape maintained	Dry capsules

Table 3: ANOVA test between the time to develop PCD on day 1 at 2 h and 4 h and day 7 at 2 h with control value

S. No.	Group	Mean	Control (mean value)	SD of group	*Post hoc* ANOVA between group and control
1.	Day 1 (2 h)	69	61	20.1	0.496
2.	Day 1 (4 h)	113.5	61	9.5	0.000* ($p<0.01$)
3.	Day 7 (2 h)	116.6	61	6.3	0.000* ($p<0.01$)

ANOVA: Analysis of variance, PCD: Preconvulsive dyspnea

Table 4: Time to develop PCD on day 1 after 2 h and 4 h and day 7 after 2 h of MC nanoformulation administration

S. No.	Guinea pig	Weight (g)	PCD (s) after 2 h on day 1	PCD (s) after 4 h on day 1	PCD (s) after 2 h on day 7
1	G1	775	67	104	120
2	G2	700	95	120	120
3	G3	600	60	96	79
4	G4	500	43	108	104
5	G5	775	53	120	120
6	G6	800	58	120	120
7	G7	678	78	120	120
8	G8	850	100	120	120
Average PCD time			69.25 s	113.5 s	116.6 s

PCD: Preconvulsive dyspnea, MC: *Momordica charantia*

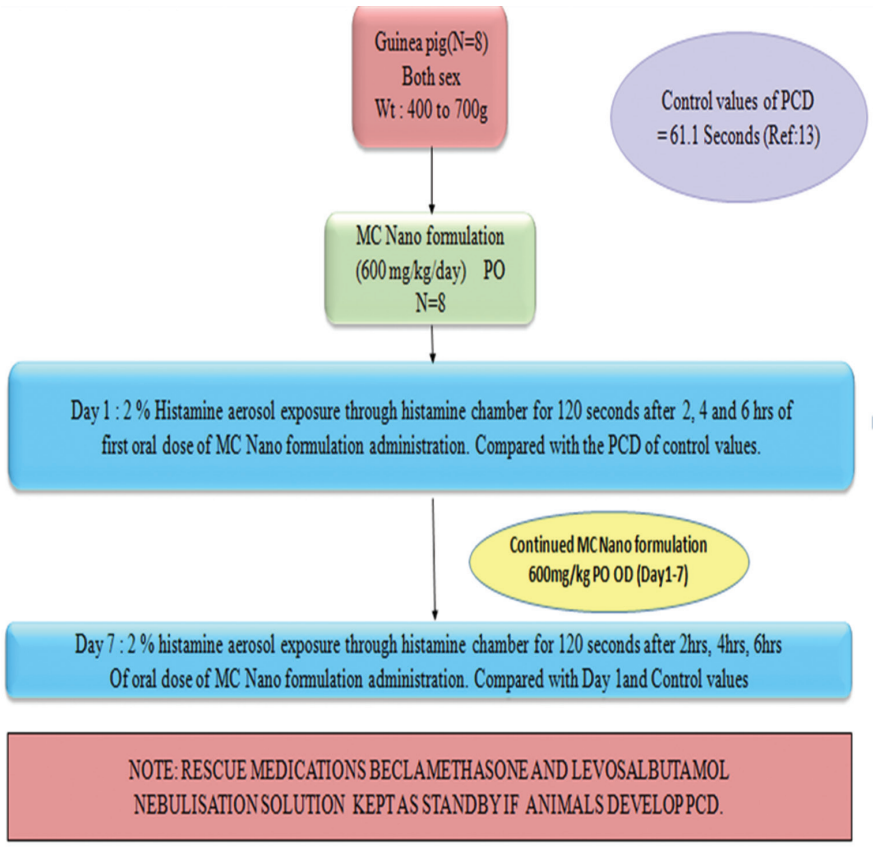


Fig. 1: Study method to assess the potency of *Momordica charantia* dry powder and nano dry powder in whole animal model

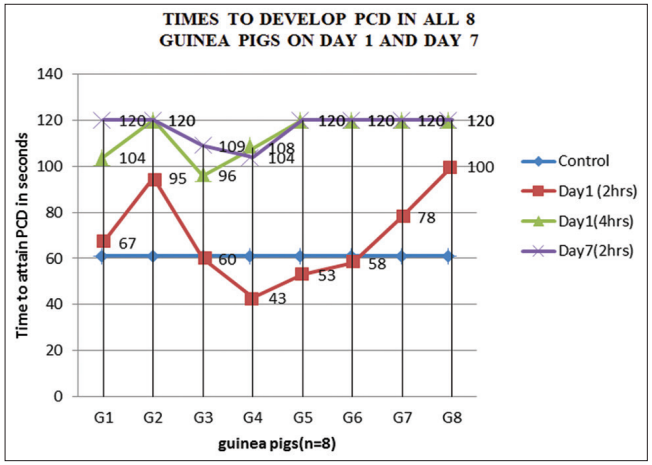


Fig. 2: Time to develop preconvulsive dyspnea on day 1 after 2 h and 4 h and day 7 after 2 h of *Momordica charantia* nanoformulation administration

was terminated on day 1. Tables 3 and 4 also illustrate cumulative doses of nanoformulation of MC given from day 1 to day 7 evidently showed that the effect was observed early. On day 7, 75% of animals were completely protected from PCD after 2 h of the nanoformulation administration (Fig. 2).

Statistical significance is shown with ANOVA test for a collective comparison between day 1 values at 2 h and 4 h and day 7 values at 2 h. *Post hoc* ANOVA comparison was done with control values and three values, respectively. This demonstrated the statistical significance ($p<0.01$)* of animal protection from developing PCD at 4th h value of day 1 and 2nd h value at day 7 (Table 3).

CONCLUSION

MC nanoformulation is found to be more efficient and potent in bringing pharmacological effect and protecting the animal from bronchospasm in comparison to MC dry powder and fresh juice. Nanoformulation is obtaining wide popularity in the pharmacotherapy for its better bioavailability, potency, and efficacy. MC being a natural product together with the optimization of the product into nanoparticles with

PEG and soy phosphatidylcholine has proved to be a potent formulation than the crude dry powder. Hence, this study can become a future platform to compare MC nanoformulation as an adjuvant management in the standard care of treatment for bronchial asthma after effective clinical trials.

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AUTHORS' CONTRIBUTIONS

Bhuvaneswari *et al.*, the corresponding author designed and supervised the study. Karthika P and Balachander R, the Principle investigator and coprinciple investigator, respectively, carried out the experimental work and drafted the manuscript. All the authors were involved in manuscript editing and finalization. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

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REFERENCES

1. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: A review. J Ethnopharmacol 2004;93:123-32.
2. Sathishsekar D, Subramanian S. Antioxidant properties of *Momordica charantia* (bitter gourd) seeds on streptozotocin induced diabetic rats. Asia Pac J Clin Nutr 2005;14:153-8.
3. Senanayake GV, Maruyama M, Shibuya K, Sakono M, Fukuda N, Morishita T, *et al.* The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats. J Ethnopharmacol 2004;91:257-62.
4. Cunnick JE, Sakamoto K, Chapes SK, Fortner GW, Takemoto DJ. Induction of tumor cytotoxic immune cells using a protein from the bitter melon (*Momordica charantia*). Cell Immunol 1990;126:278-89.
5. Srivastava AK, Mishra A, Gautam S, Pal S, Mishra A, Kumar AR, *et al*. Effect of *Momordica charantia* fruits on streptozotocin-induced diabetes mellitus and its associated complications. Int J Pharm Pharm Sci 2015;7:356-63.
6. Navya G, Shirisha Y, Girija P, Venkateshwarlu K, Sirisha K. Effect of *Momordica charantia* and *Syzygium cumini* extract on serum electrolytes in alloxan induced diabetic rats. Int J Pharm Pharm Sci 2018;11:24-7.
7. Frossard N, Freund V, Advenier C. Nerve growth factor and its receptors in asthma and inflammation. Eur J Pharmacol 2004;500:453-65.
8. Vllasaliu D, Fowler R, Stolnik S. PEGylated nanomedicines: Recent progress and remaining concerns. Expert Opin Drug Deliv 2014;11:139-54.
9. Singh U, Sagar VR. Effect of drying methods on nutritional composition of dehydrated bitter gourd (*Momordica charantia*). Indian agricultural research institute, New Delhi-12. Agric Sustain Dev 2013;1:83-86.
10. Tiruwa R. A review on nanoparticles-preparation and evaluation parameters. Indian J Pharm Biol Res 2016;4:27-8.
11. Pinzaru I, Coricovac D, Dehelean C, Moacă EA, Mioc M, Baderca F, *et al.* Stable PEG-coated silver nanoparticles a comprehensive toxicological profile. Food Chem Toxicol 2018;111:546-56.
12. Singh S, Agrawal SS. Anti-asthmatic and anti-inflammatory activity of *Ocimum sanctum*. Int J pharm 1991;29:306-10.
13. Sridevi G, Gopkumar P, Ashok S, Shastry C. Pharmacological basis for antianaphylactic, antihistaminic and mast cell stabilization activity of *Ocimum sanctum*. Internet J. Pharmacol 2009;7:22-5.
14. Bhuvaneswari K, Swarna RM, Amudhan A. Anti asthmatic effect of *Momordica charantia* and its comparison with montelukast an *in vitro* and *in vivo* model. Int J Basic Clin Pharmacol 2017;6:1810-5.
15. Ichinose M, Barnes PJ. Histamine H3-receptors modulate nonadrenergic noncholinergic neural bronchoconstriction in guinea-pig *in vivo*. Eur J Pharmacol 1989;174:49-55.

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Research Article

EFFECT OF *EMBLICA OFFICINALIS* FRUIT DRY POWER ON BEHAVIOR CHANGES AND ANTIOXIDANT LEVELS: AN *IN VITRO* AND *IN VIVO* STUDY

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ABSTRACT

Objective: This study aim was to compare the *in vitro* antioxidant property of fresh juice and dry powder of *Emblica officinalis* (EO) by chemical testing and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay and also to observe the *in vivo* effect of EO fruit dry powder on food intake, body weight, behavior changes, and antioxidant levels in restraint stress rat model.

Methods: *In vitro* study, EO fresh juice 1% v/v and its dry powder form were analyzed for chemical testing and DPPH assay initially. In the *in vivo* study, 12 adult male Wistar rats were obtained. Initially, baseline parameters (Body wt, food intake, behavior changes, blood levels of superoxide dismutase, and malondialdehyde) and control values were assessed. Later 12 animals were divided into two treatment groups (1 and 2) and pre-treatment was given with dry powder of EO at a dose of 250 mg/kg and 500 mg/kg orally once a day, respectively. Animals from both the groups were restrained for 2 h/day and the same parameters were analyzed at the end of 24 h and 7 days of stress induction.

Results: *In vitro* study, fresh juice and dry powder preparations of EO were found to be positive for all the four chemical testing. In DPPH assay, dry powder showed better antioxidant activity (97%) than the fresh juice form (57%). *In vivo* study, significant antioxidant activity was seen at the dose of 500 mg/kg after day 1 and 7 days of stress induction.

Conclusion: Dry powder of EO has brought out the reversal of restraint stress-induced behavioral changes and reduced food intake, body weight, and antioxidant levels.

Keywords: Antioxidant property, 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay, *Emblica officinalis*, Restraint stress, Rats.

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INRODUCTION

Oxidative stress is the result of an imbalance between oxidants and antioxidants. Physical or psychological stress induces a change in the hypothalamic–pituitary–adrenal axis which releases glucocorticoids and chemical mediators including adrenocorticotropic hormone, norepinephrine, serotonin, dopamine, and acetylcholine. The metabolism of these mediators leads to more production of free radicals and reactive oxygen species (ROS), which later causes an imbalance between oxidants and antioxidants. Acute stress in any organism will produce compensatory responses to restore previous stability. However, in chronic stress, ROS put forth negative effects and becomes a major causative factor implicated in several pathologies – cardiovascular, immunological, neurodegenerative, or neuro behavior changes [1]. Antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase play important roles in scavenging free radicals and preventing cell injury. When the mechanism of antioxidant protection becomes unbalanced in the human body, an antioxidant supplement may be used to help reduce oxidative damage [2]. Synthetic antioxidants are commercially available. By considering potential health risks and toxicity of synthetic antioxidants (butylhydroxytoluene and butylhydroxyanisole), researchers have drawn their attention toward the natural occurring antioxidants (plant materials) [3,4].

Studies have demonstrated that plant phenolics as a major source of natural antioxidants. The fruit of Indian Gooseberry (*Emblica officinalis* [EO]) is rich in Vitamin C and it acquires maximum antibacterial and antioxidative activities [5]. The unique antioxidants such as Gallic acid, Vitamin C, ß-carotene, and SOD enzyme have been reported to be present in the fruit. Various studies have shown the antioxidant property of EO in various forms of its extractions (ethanol and methanol) [5,6].

Stress is well known to change body weight and food intake in animal models. Many studies have shown that restraint stress suppresses body weight gain and food intake in rodents [7]. No studies have explored the *in vivo* effects of EO fruit dry powder on a combination of food intake, body weight, behavior changes, and antioxidant levels in a whole animal model. Hence, the study was designed to compare the *in vitro* antioxidant property of fresh juice and dry powder preparations of EO by chemical testing and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay and also to observe the *in vivo* effect of EO fruit dry powder on food intake, body weight, general behavior changes, and antioxidant levels (SOD), malondialdehyde (MDA) in an adult male Wistar rat model after restraint stress induction.

MATERIALS AND METHODS

Fresh fruits of EO were obtained from the local market and washed thoroughly in running water. Under sterile condition, the fruits were cut into pieces and seeds were removed and weighed. Then dried in a hot air oven at 60°C aseptically and grinded to get a fine powder form. EO fresh juice 1% v/v was prepared. Both the preparations were used for DPPH assay and chemical testing.

Chemical testing

Detection of phenolic compounds

- Ferric chloride test: Juice and the dry powder forms of EO were added to 5% $FeCl_3$ reagent and the formation of deep blue color was observed
- Lead acetate test: Juice and the dry powder forms of EO were added to 10% lead acetate solution and mixed. Formation of white precipitate was observed [8].

Detection of flavonoids

Aqueous sodium hydroxide test: Juice and the dry powder forms of EO were treated along with aqueous NaOH solution. Formation of yellow-orange color was observed.

H_2SO_4 test: Juice and the dry powder forms of EO were added to concentrate H_2SO_4 and observed for the formation of the orange color [8].

DPPH assay

The dry powder and fresh juice of EO were prepared in 1% w/v and 1% v/v solutions, respectively. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of samples (dry powder and Fresh juice), 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacted with an antioxidant compound, which donated hydrogen and it was reduced. The change in color (from deep violet to light yellow) was read (Absorbance [Abs]) at 517 nm after 100 min of reaction using an ultraviolet-visible spectroscopy spectrophotometer. The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Reference standard compound being used was ascorbic acid 50 ng/ml. Lower Abs of the reaction mixture indicated higher free radical activity [9-12]. Percentage inhibition was calculated by.

DPPH scavenging effect (%) = A0-A 1/A0 × 100.

Where A0 was the Abs of control reaction and A1 was the Abs in the presence of test or standard sample (control = 0.856).

In vivo

After getting approval from the Institutional Animal Ethics Committee, 12 adult male Wistar rats weighing 150–250 g were obtained and divided into two groups of six each. Initially, six animals were assessed for the baseline values of the parameters (body weight, food intake, behavior changes, blood levels of SOD, and MDA) and then those six animals were used as control (without treatment) and subjected to psychological stress by restraining for 2 h and the above said parameters were assessed at the end of 24 h and after 7 days of stress induction. This method was aimed toward reducing animal usage in the study. Then, 2 weeks interval period was allowed to acclimatize the animals back to its normal laboratory environment and to overcome the stress induction. Later then, total of 12 animals were randomly divided into two treatment groups. For Groups 1 and 2, pre-treatment with EO dry powder at a dose of 250 mg/kg body weight and 500 mg/kg body weight orally [13,14] was given once daily. Pre-treated animals from both groups were restrained for 2 h/day, and the same tests were repeated at the end of 24 h and after 7 days of stress induction. The body weight of the animals was measured in each group at the end of 24 h and after 7 days. Food intake in grams was measured for each animal in both groups at the end of 24 h and after 7 days using Oxylet system. Food intake in dark/light phase cycle was measured and compared with controls.

Behavior changes

Open field test: locomotor activity (quiet room)

On the day of testing, rats were allowed to adapt to the environment for 1 h before testing. After 5 min of acclimatization in the open field apparatus, each animal from the respective treatment group was placed individually in the center of the open field and number of squares crossed, number of rearings (number of times the animal stood on its hind legs), grooming (duration of time the animal spent licking or scratching itself while stationary), and fecal pellets were observed for a period of 5 min [15].

SOD and MDA levels

End of 24 h and on the 8th day, 1 ml of blood was withdrawn from rat tail vein for the analysis of SOD and MDA levels.

Estimation of SOD

Reagents used were Tris buffer – 50 ml (containing 50 mM of Tris and 1 mM of ethylenediaminetetraacetic acid), hydrochloric acid – adjust PH at 8.5, and Pyrogallol – 25 mg added to 10 ml of distilled H2O (20 mM conc.). For blank: 2.9 ml Tris + 0.1 ml Pyrogallol– Mixed well. Abs A was read at 420 nm using colorimeter at 1 min 30 s and Abs B at 3 min 30 s. For Sample: 2.8 ml Tris + 0.1 ml sample and 0.1 ml Pyrogallol were added and mixed well, and the Abs were read at 420 nm after 1 min 30 s (Abs A) and after 3 min 30 s (Abs B) [16].

SOD levels (in Units) were calculated using the formula: Abs (A-B)/Abs A × 50

Estimation of MDA

Reagents used were trichloroacetic acid (TCA) – 40% (40 g in 100 ml), thiobarbituric acid (TBA) – 0.67% (0.67 g in 100 ml). Then, 0.5 ml sample + 0.5 ml TCA + 2 ml TBA were added very slowly. The test tubes were kept in a boiling water bath at 90–100°C for 10 min. After 10 min, the tubes were brought back to the room temperature. Samples were centrifuged for 10 min at 3000 rpm. Separated the supernatant and readings were taken at 540 nm using colorimeter [16].

MDA levels (in units) were calculated using the formula: Abs at 532 nm × 105/1.56

Statistics

Data from the control and two treatment groups were analyzed by ANOVA in SPSS software version 24.

RESULTS

***In vitro* study**

Chemical testing

Fresh juice and dry powder preparations of EO were found to be positive for all the four chemical testing.

DPPH FREE RADICAL ASSAY

In DPPH assay, dry powder of EO showed 97% inhibition of free radical scavenging activity compared to its fresh juice preparation and standard (ascorbic acid), which were about 57% and 76%, respectively.

***In vivo* study**

In this study, after the 1st day of stress induction, rats showed changes in the mean values of body weight, food intake pattern, behavior changes, and SOD and MDA levels in both the treatment group of EO (250 mg/kg and 500 mg/kg) in comparison to the control group (without treatment) and their baseline values. More statistically significant values were seen in the EO 500 mg/kg treated group for body weight (*p=0.01), behavior changes (*p<0.01), and SOD (*p=0.01) (Figs. 1-3).

Similarly, after 7 days of repeated stress induction, rats showed, change in the mean values of body weight, food intake, behavior changes, and

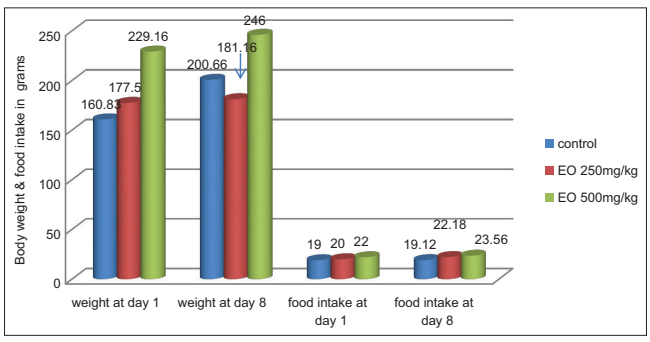


Fig. 1: Mean ± standard deviation values of body weight (*p<0.05) and food intake pattern after stress induction

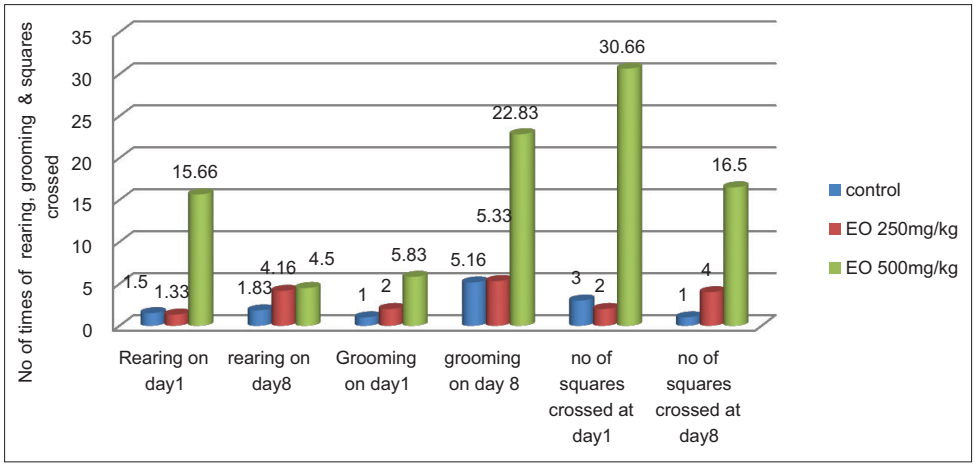


Fig. 2: Mean ± SD values of behavior parameters in open field test after stress induction (*p<0.01)

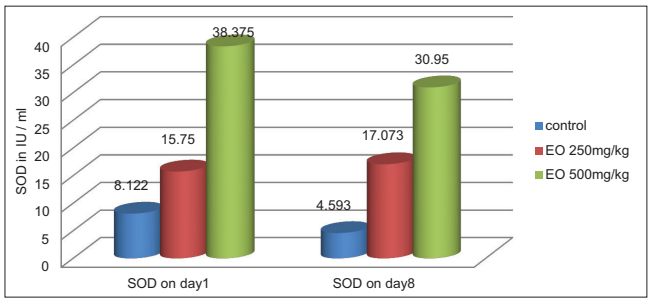


Fig. 3: Mean ± SD values of superoxide dismutase in IU/ml after stress induction (*p<0.05)

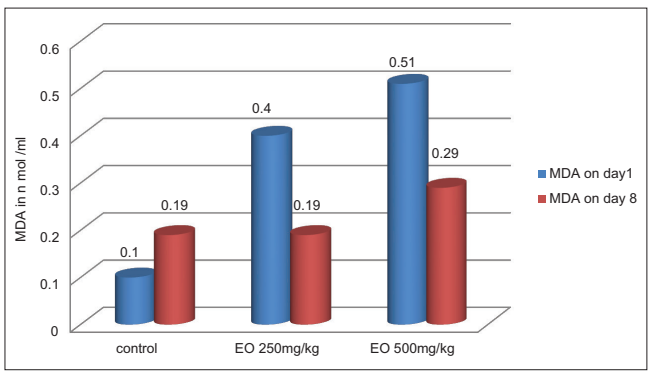


Fig. 4: Mean ± SD values of malondialdehyde levels after stress induction (*p<0.05)

SOD and MDA levels in both the treatment groups. However, statistically significant changes were seen in the body weight (*p<0.01), SOD (*p=0.01), and MDA (*p=0.04) levels only in the treatment group of EO 500 mg/kg (Figs. 1, 3 and 4).

Mean values of baseline parameters were weight–160.833±11.58 g, food intake–19.260±10.00 g, rearing–3.500±4.0866, grooming–13.500±7.7136, squares crossed–5.333±11.2190, SOD–11.007±10.45 IU/ml, and MDA–1.0596 ± 1.1341 IU/ml.

DISCUSSION

In vitro study

In this study, both the dry powder and the fresh juice of EO tested positive for flavonoids and phenolic compounds (Table 1). In terms of chemical testing, these results were similar to the studies which showed the presence of flavonoids and phenolic compounds in the aqueous methanolic fruit extract of EO [17]. In DPPH assay, dry powder of EO showed 97% inhibition of free radical scavenging activity compared to its fresh juice preparation, which was about 57% (Table 2). In the previous DPPH assay, studies of EO showed that the maximum antioxidant activity was found to be in the methanolic extract [6,17].

In vivo study

Stress has identified to modify body weight and food intake in animal models. Especially the restraint stress model effectively depicted the physical and psychological stress and used as an animal model of depression [1]. Many studies have shown that restraint stress suppresses body weight gain and food intake in rodents [18]. In the present study, mean values of body weight and food intake were increased in both the treatment groups after the 1st and the 7 days of repeated stress induction compared to the control group (without treatment) in which there was a reduction in the mean values of body weight and food intake pattern after repeated stress induction. This showed that there was reduction in the body weight and food intake pattern in restraint stress animals whereas, in the treatment groups (EO 250 mg/kg and 500 mg/kg) the animals had overcome the stress induction and they have followed the normal physiological increase in the body weight and food intake pattern (Fig. 1).

In case of behavior changes and blood levels of SOD and MDA, there were reduction in the mean value of rearing, grooming, number of square crossed and decreased level of SOD and increased level of MDA values in the control group (without treatment) compared to its baseline values indicating a state of psychological depression immediately after the 1st day and the 7 days of repeated stress induction. The two groups treated with the EO (250 mg/kg and 500 mg/kg) showed a better increase in mean values of rearing, grooming, number of squares crossed in parallel to increase in SOD, and decreased in MDA values after the 1st day and the 7 days of repeated stress induction (Figs. 2-4) This ensures that the treatment with EO had overcome the changes of stress induction, including antioxidant levels, which were comparable to the results of a few previous studies wherein the antioxidant property of EO was due to the presence of polyphenols in the fruit [19,20].

The results of the study clearly revealed the antioxidant effect of EO in overcoming stress induction. Improved changes in the body weight, food intake, and behavior might depict the antidepressant and antianxiety property of *E. officinalis*. These results were proven by the previous studies, by forced swim test, tail suspension test, elevated plus maze, and open field method with standard drugs. Apart from increased antioxidant levels, no other clear mechanism could be elicited behind the improved changes in food intake and body weight after repeated stress induction. Therefore, the proposed pharmacological mechanism behind these effects could be the involvement of monoaminergic neurotransmitters [19,21,22].

Table 1: Results of chemical testing

Preparations	Phenolic compound		Flavonoids	
	Ferric chloride test	Lead acetate test	Aqueous sodium hydroxide test	Sulphuric acid test
Fresh juice of EO	+	+	+	+
Dry powder of EO	+	+	+	+

EO: *Emblica officinalis;* +: Presence/Test positive

Table 2: Percentage inhibition of free radical scavenging activity in 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay

Samples	Absorbance	Percentage inhibition
Ascorbic acid (50 ng/ml)	0.206	76
EO (dry powder 1% w/v)	0.024	97
EO (fresh juice 1% v/v)	0.364	57

EO: *Emblica officinalis*

The previous studies also confirmed that the present of phytochemicals in the EO such as tannic acid, gallic acid, flavonoids, and ascorbic acid might contribute to increase the levels of monoaminergic neurotransmitters and neurotropic action in the hypothalamus [21,22]. The monoamine receptors such as 5-hydroxytryptamine (5HT1A) and 5HT2C have their involvement in the appetite and satiety center in the brain. Hence, further studies could look into the involvement of 5HT, behind the effects of EO.

CONCLUSION

In vitro study, both the chemical testings' and DPPH assay showed maximum antioxidant activity in the dry powder preparation of EO compared to its fresh juice. *In vivo* study has brought out the reversal of restraint stress-induced behavioral changes and reduced antioxidant levels in a rat model. Further studies might focus to elucidate the various possible molecular mechanisms of action of EO in overcoming stress induction.

AUTHORS' CONTRIBUTIONS

Umamaheswari Anbarasu design the study executed the assays and participated in the writing of the entire manuscript. Bhuvaneswari K initiated the hypothesis and designed the study. Muthuraman PL participated in the animal handling and performance of *in vitro* and *in vivo* assays.

CONFLICT OF INTEREST

The authors report no conflicts of interest regarding this manuscript.

REFERENCES

1. Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behav Brain Res 2005;161:45-59.
2. Badhani S, Kainth A, Kabra A, Parashar B. Evaluation of antioxidant activity of *Benincasa hispida* fruit extracts. Am J Pharm Tech Res 2013;3:336-45.
3. Abdullah N, Kamarudin W, Samicho Z, Zulkifli KS, Saidatul SW. Study on antioxidant capacity and phenolic content of various parts of wax gourd (*Benincasa hispida*). World Appl Sci J 2012;19:1051-6.
4. Bharathi S, Kumaran S, Suresh G, Ramesh B, Sundari MN. Phytosynthesis of silver nanoparticles using Hygrophila auriculata leaf extract and assessment of their antibacterial and antioxidant properties. Int J Appl Pharm 2019;10:112-8.
5. Nadheesha M, Bamunuarachchi A, Edirisinghe EM, Weerasinghe WM. Studies on antioxidant activity of Indian gooseberry fruit and seed. J Sci Univ 2007;3:83-92.
6. Luqman S, Kumar R. Correlation between scavenging property and antioxidant activity in the extracts of *Emblica officinalis* Gaertn.,syn. *Phyllanthus emblica* L. Fruit. Ann Phytomed 2012;1:54-61.
7. Bhatnagar S, Vining C, Iyer V, Kinni V. Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. J Neuroendocrinol 2006;18:13-24.
8. Elangovan M, Dhanarajan M, Rajalakshmi A, Jayachitra A, Mathi P, Bhogireddy N, *et al*. Analysis of phytochemicals, antibacterial and antioxidant activities of *Moringa oleifera* Lam. Leaf extract an *in vitro* study. Int J Drug Dev Res 2014;6:173-80.
9. Patel RM, Patel NJ. *In vitro* antioxidant activity of coumarin compounds by DPPH, superoxide and nitric oxide free radical scavenging methods. J Adv Pharm Educ Res 2011;1:52-68.
10. Garcia EJ, Oldoni TL, Alencar SM, Reis A, Loguercio AD, Grande RH, *et al*. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Braz Dent J 2012;23:22-7.
11. Melanathuru V, Rengarajan S, Thangavel N. Comparative study of antioxidant and anticancer activity of *Alpinia calcarata* and *Alpinia galanga*. Int J Pharm Pharm Sci 2017;9:186-93.
12. Balakrishnan BB, Krishnasamy K. Evaluation of free radical screening and antioxidant potential of *Moringa concanensis* Nimmo a medicinal plant used in Indian traditional medication system. Int J Pharm Pharm Sci 2018;10:91-7.
13. Govind P, Pandey SP. Phytochemical and toxicity study of *Emblica officinalis*. Int Res J Pharm 2011;2:270-2.
14. Tasanarong A, Kongkham S, Itharat A. Antioxidant effect of Phyllanthus emblica extract prevents contrast-induced acute kidney injury. BMC Complement Altern Med 2014;14:138.
15. Bolivar VJ, Caldarone BJ, Reilly AA, Flaherty L. Habituation of activity in an open field: A survey of inbred strains and F1 hybrids. Behav Genet 2000;30:285-93.
16. Todorova I, Simeonova G, Kyuchukova D, Dinev D, Gadjeva V. Reference values of oxidative stress parameters (MDA, SOD, CAT) in dogs and cats. Comp Clin Pathol 2005;13:190-4.
17. Sadoni H, Sharma P, Waheed SM, Singh S. Phytochemical analyses and evaluation of antioxidant, antibacterial and toxic properties of *Emblica officinalis* and terminalia bellirica fruit extracts. Asian J Pharm Clin Res 2016;9:96-102.
18. Jeong JY, Lee DH, Kang SS. Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice. Endocrinol Metab (Seoul) 2013;28:288-96.
19. Kumar CP. Effect of *Emblica officinalis* on stress induced biochemical and psychological changes in mice. Glob J Med Res 2014;13:25-8.
20. Pal R, Gulati K, Chakraborti A, Banerjee B, Ray A. Role of free radicals in stress-induced neurobehavioural changes in rats. Indian J Exp Biol 2006;4:816-20.
21. Dhingra D, Joshi P, Gupta A, Chhillar R. Possible involvement of monoaminergic neurotransmission in antidepressant-like activity of *Emblica officinalis* fruits in mice. CNS Neurosci Ther 2012;18:419-25.
22. Pemminati S, Gopalakrishna HN, Shenoy AK, Sahu SS, Mishra S, Meti V, *et al*. Antidepressant activity of aqueous extract of fruits of *Emblica officinalis* in mice. Int J Appl Biol Pharm Technol 2010;1:448-54.

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A Cross Sectional Study on the Status of Inflammatory Markers in Polycystic Ovary Syndrome (PCOS) in Indian Population

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Polycystic Ovarian Syndrome (PCOS) is a common disease exclusively seen in women of reproductive age group. World Health Organization reports that Polycystic Ovarian Syndrome approximately affects 5-10% of the reproductive female population worldwide. Women with PCOS have chronic inflammation. This in turn leads to increased incidence of non-communicable diseases like cardiovascular disease and diabetes[2]. Chronic inflammation in PCOS patients can be identified using serum markers such as C - reactive protein [CRP], Interleukin-6 [IL-6] and Interleukin-18 [IL-18].So the main objective of the study was to assess the status of inflammation in patients with Polycystic Ovary Syndrome (PCOS) and to assess the influence of drugs prescribed for PCOS on inflammation. Patients were divided into three groups as healthy volunteers, newly diagnosed PCOS patients, already on treatment patients for PCOS. High sensitivity CRP (hs-CRP), IL-6 and IL-18 were assessed. There was an increase in the hs-CRP, IL-6 and IL-18 compared to healthy volunteers in both newly diagnosed and already diagnosed groups. But there was no statistical significance (hs-CRP=0.975, IL-6=0.746, IL-18 = 0.148) between them. The mean value has decreased in the already on treatment group as duration of treatment increases. There was no statistical significance between them. There was an inversely proportional relationship between the mean values of inflammatory markers and duration of treatment.

Keywords: Polycystic ovarian syndrome, Insulin Resistance, Inflammation, inflammatory markers, hs-CRP, IL-6, IL-18.

Polycystic Ovarian Syndrome (PCOS) is a common disease exclusively seen in women of reproductive age group.World Health Organization reports that Polycystic Ovarian Syndrome approximately affects 5-10% of the reproductive female population worldwide. A study by Ridhi R *et al*. shows it affects the Indian female adolescents approximately by 9.13%[1].

Women with PCOS have chronic inflammation. This in turn leads to increased incidence of non-communicable diseases like cardiovascular disease and diabetes[2]. **Chronic inflammation** in PCOS patients can be identified using serum markers such as C - reactive protein [CRP], Interleukin-6 [IL-6] and Interleukin-18 [IL-18].

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Insulin resistance[IR] and its associated hyperinsulinemia leads to a sequence of events collectively called as insulin resistant syndrome. The sequence of events includes cardiovascular disease, hypertension, polycystic ovary syndrome and non-alcoholic steato hepatitis[3]. Insulin resistant syndrome and diabetes are inter-related but are different entities. All insulin resistant individuals need not be diabetic, but develops diabetes only if he is not able to make up the degree of hyperinsulinemia needed to overcome the insulin resistance. Even though an insulin resistant individual need not be diabetic the risk of developing the consequences of insulin resistance syndrome are high. Patients with insulin resistance syndrome have increased concentrations of plasminogen activator inhibitor-1, C-reactive protein and increased white cell count. Hence these patients are at increased risk of developing PCOS.

Low grade inflammation which is of chronic is related to obesity, IR syndromes such as polycystic ovary syndrome, and an increased risk of cardiovascular disease[4]. In obese patients enlargement of adipose tissue causes adipocyte hypertrophy and hyperplasia and this huge adipocytes outshine the local supply of oxygen causing the cell hypoxia independently with stimulation of cellular stress pathway[5]. This leads to independent inflammation of the cells and cytokines and otherpro-inflammatory signals to get released. As a fragment of this chronic inflammatory process, locally released chemokine draws pro-inflammatory macrophages into the adipose tissue. These then produce structures similar to crown round the huge lifeless or dying adipocytes. These tissue macrophages release cytokines which then trigger the inflammatory process in adjacent adipocytes, aggravating inflammation and insulin resistance.

There is also genetic basis for the inflammation observed in PCOS[6].Variants in the gene encoding TNFá[7], type 2 TNF receptor and IL-6[8-10] and its signal transducer[11] have been reported in association with PCOS in European population. These findings are in conceptual agreement with a common evolutionary background for PCOS and metabolic disorders.Interleukin-6 is an endocrine cytokine produced by mononuclear cell and adipose tissue that is directly responsible for stimulating hepatic CRP synthesis[12-15]. CRP in turn has emerged as a major predictor of metabolic dysfunction in asymptomatic individuals, and is also produced by adipose tissue[16, 17]. Hence **C-reactiveprotein** is the most consistent circulating marker of low- grade chronic inflammation in PCOS. Like C-reactive protein and IL-6, IL-18 is also a proinflammatory cytokine which promotes secretion of TNF-á[18], which further stimulates the production of IL-6.

So this study was basically carried out to see if there is any association between inflammation and PCOS. If the serum inflammatory markers are higher then there is an association between inflammation and PCOS in Indian Population.

In addition to it we would also like to see drugs used in PCOS have any influence over the inflammation in PCOS.Numerous therapies have been projected for the treatment modalities of polycystic ovarian syndrome, but the ideal treatment for infertile women with polycystic ovarian syndrome has not yet been clear. Drug treatment comprises clomiphene citrate, insulin sensitizing agents, gonadotropins and gonadotropin- releasing hormone (GnRH) analogs. Surgical treatments include laparoscopic ovarian drilling and the application of assisted reproduction techniques. Besides this loss of weight, diet and physical exercise have been shown to develop hyper insulinemia, menstruation abnormalities and ovulatory rates. Even though these many drugs and modalities of treatment were available for PCOS, only few drugs targeted in reduction of inflammation.Studies showed that there was a decrease in interleukin-6 and interleukin-18 level after metformin treatment for three months in polycystic ovary syndrome patients. This shows metformin decreases the inflammatory markers and have beneficial effect on inflammatory process of PCOS[19]. Many earlier studies spoke about metformin and their anti-inflammatory properties. There were no studies demonstrating the anti-inflammatory effect of other drugs used to treat PCOS in practise. So we thought to see any other drugs used to influence the inflammation.

MATERIALS AND METHODS

This study was a prospective cross sectional study. The study population was from all the patients who attended the outpatient Department of Obstetrics and Gynecology in PSGIMSR

Hospital, Coimbatore. We included all the PCOS patients according to Rotterdam consensus criteria with age group 18-45 years and excluded those patients presenting with a history of inflammatory diseases or intake of inflammatory drugs and also pregnant women. Healthy Volunteers of age group 18- 45 years women with normal reproductive cycle were included and those who present with history of inflammatory drugs or diseases are excluded. Further those who have hypertension or diabetes are also excluded from the healthy volunteers.

The overview of the study design is given in the figure below. The patients were divided into three groups.

Group 1: Healthy volunteers

Group 2: Newly diagnosed PCOS

Group 3: Already on treatment for PCOS

Sample size estimated according to previous literature search.30 participants in each group was taken.The study design was submitted to the Institute Human Ethics Committee (IHEC) stating its purpose preceding the study. After its approval the study was initiated. The details and the usefulness of the study design were described to every participant individually and clarified their doubts before getting the informed consent forms. The informed consent forms provided to the participants were either in English or in Tamil. The participants, who gave written informed consent, came under the inclusion criteria were enrolled for the study. Healthy volunteers were also given the informed consent. The patients who declined to give informed consent were also excluded from the study.

PCOS patients were selected according to Rotterdam Consensus Criteria 2003 which is given below.

• Polycystic ovaries(12 or more follicles in each ovary, each follicle measuring 2-9 mm in diameter and/or ovarian volume > 10 ml, one polycystic ovary is sufficient for diagnosis)

• Oligo-/anovulation; clinical diagnosed as Oligo-/amenorrhea (menstrual cycles longer than 35 days or less than 10 menstruation/year)

• Hyperandrogenism (clinical or biochemical)

Basic demographic data like name, age and measurements like Height, weight, body mass index were recorded. History of the patient and if the patient is on already treated group then their treatment history were noted.

Our study was to observe if there were abnormalities in High sensitivity C - reactive protein, Interleukin-6 and Interleukin-18 in PCOS patients and to compare those with the healthy volunteers.

The high sensitivity CRP was tested in the Clinical Biochemistry lab using theCobas Integra C - reactive protein (Latex). This machine works by the methodology of "Particle enhanced turbidimetric assay".

The Interleukin-6 and Interleukin-18 were

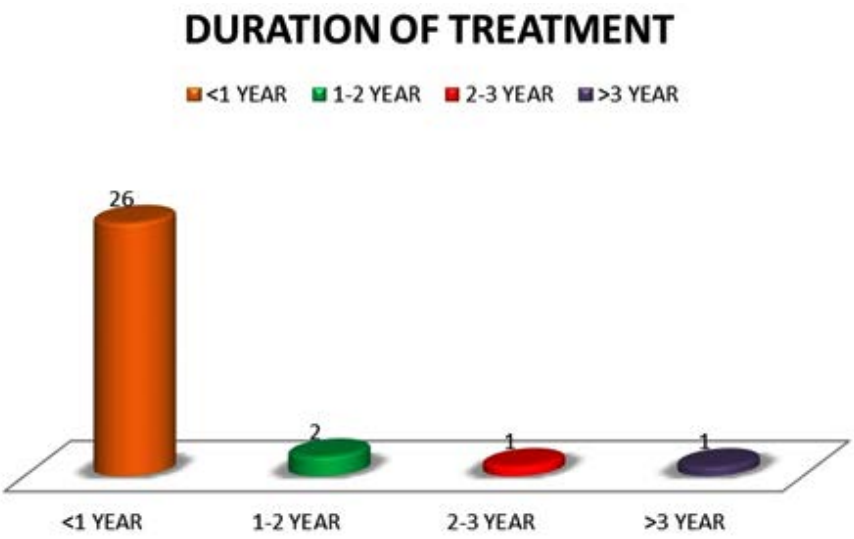


Fig. 1. Duration of treatment

estimated in the Pharmacology laboratory using Krishgen Biosystems, imported from California, USA and read by an Elisa reader which is a Bio-rad system at an absorbance of 405 and 450 nM.

Statistical analysis was performed by using SPSS software version 19. The results were analyzed using one way ANOVA and post hoc analysis was done using LSD.

RESULTS AND DISCUSSION

Our study was intended to evaluate the status of inflammation, by measuring hs-CRP, IL-6 and IL-18, in PCOS patients in Indian population. It was also aimed to assess the influence of drugs prescribed in PCOS patients on the inflammation.

Polycystic Ovary Syndrome is an endocrine disorder which is very common in the female population in the reproductive age group[20]. Most women shows the features of metabolic syndrome. It is also concomitant with hypertension, diabetes, dyslipidaemia, gestational type 2 diabetes[21].

PCOS is associated with obesity and also increased incidence of insulin resistance[22]. There is also increased risk of cardiovascular complications. The inflammation which is of chronic low grade is related to PCOS. The C-reactive protein levels are an acute phase reactant and is circulating marker of cardiovascular events. CRP > 3mg/L in a patient is equal for metabolic syndrome as in ATP III guidelines. IL-6, TNF-á, IL-18 concentrations in serum is the predictors of cardiovascular events[1]. So, in this study we proposed to measure the IL-6, IL-18 and hs-CRP levels in PCOS patients in Indian population. There are various studies showing the

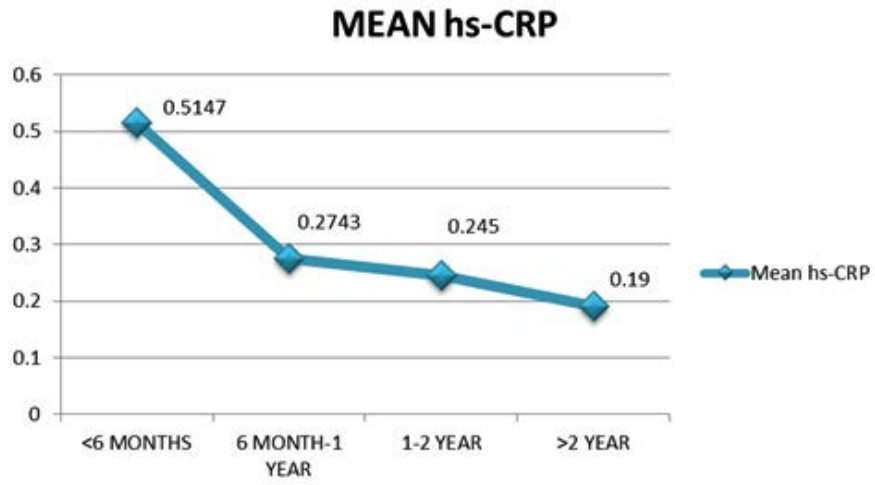


Fig. 2. Meanhs-CRP levels

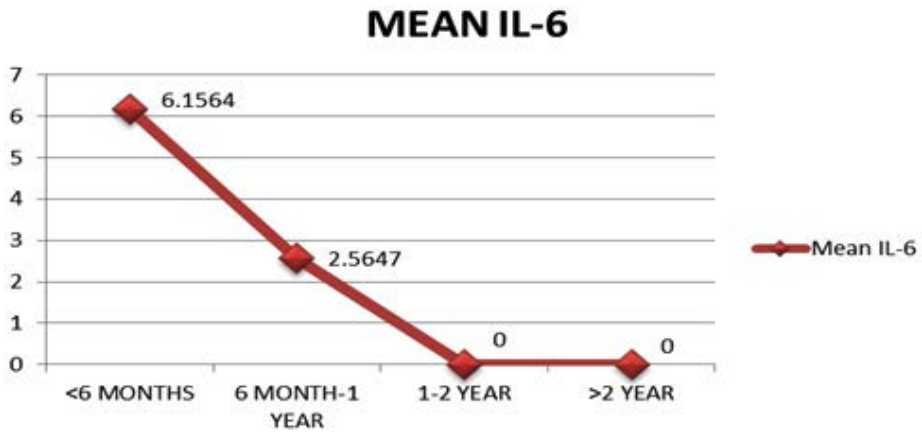


Fig. 3. MeanIL-6 levels

significance of IL-6, IL-18 and hs-CRP in PCOS in other countries. There are no studies showing this significance in Indian Population.

There were many therapies of polycystic ovarian syndrome including drugs as well as surgical therapies and life style modifications. In previous studies drugs like metformin and thioazolidinones like pioglitazone, rosiglitazone decreases insulin resistance and also known to decrease the inflammation. Other drugs like myoinositol, clomiphene citrate improves ovulation alone. Their role in inflammation was not known in PCOS.

Ninety participants were recruited in our study. These participants were divided into three groups as normal controls, newly diagnosed patients and already diagnosed on treatment patients. Low grade chronic inflammation is associated with Polycystic Ovary Syndrome and it can be assessed using inflammatory markers like IL-6, IL-18 and hs-CRP. These are strong predictors of cardiovascular complications. Kelly *et al*. reported a study exhibiting an increase in CRP which shows a low grade chronic inflammation in PCOS patients. In this study they showed a mean value of PCOS patients was greater than normal controls[2].

In our study there was no significant increase in the CRP levels (p=0.975).There was no significant difference between the newly diagnosed and already diagnosed patients suggesting that hs-CRP didn't rise significantly in already diagnosed group as they were on treatment (Table 2&3).

In the same way there was also no significant changes in the IL-6 levels (p=0.746). There was an increase in their mean values when compared to normal controls. Mean value for normal controls was 34.35±93.88 whereas for newly diagnosed patients it was 90.58±308.62 and for already diagnosed it was 91.41±476.6 suggesting there was a rise in IL-6 levels in PCOS when compared to normal controls but that was not significant. Between newly diagnosed patients and already diagnosed patients there was no much difference between the levels (Table 2&3).

Another study by Hector *et al* showed there was an increase in IL-18 independent of obesity[23]. In our study the results showed there was an increase in the mean values of IL-18 in PCOS patients when compared to normal controls. The mean value for Normal control was 131.4±224.17 whereas for newly diagnosed

Table 1. Represents the combinations of drug

Drugs	Percentage
Only Metformin	46.67
Metformin + Myoinositol	13.33
Myoinositol + Clomiphene citrate	10
Metformin+ Clomiphene citrate	6.67
Myoinositol + HCG + Clomiphene citrate	6.67
Myoinositol + Metformin + Clomiphene citrate	6.67
Myoinositol + HCG	3.33
Metformin + HCG	3.33
Myoinositol + HCG + LOD	3.33

Table 2. One way ANOVA

	hs-CRP		IL-6		IL-18	
	F	p-value	F	p-value	F	p-value
Between groups	0.025	0.975	0.293	0.746	1.954	0.148
Within groups						

Table 3. Post Hoc (LSD) analysis

	hs-CRP		IL-6		IL-18	
Between Groups Comparison	Mean Difference	P-value	Mean Difference	P-value	Mean Difference	P-value
I and II	0.00667	0.963	56.23703	0.746	133.417	0.114
I and III	0.02433	0.867	57.06563	0.506	150.75323	0.074
II and III	0.03100	0.831	0.82860	0.992	17.33650	0.836

and already diagnosed group of patients it was 264.86±316.33 and 282.2±403.92 respectively. The difference was not statistically significant (p=0.148) (Table 2 &3). There were also, as in IL-6, no difference in newly diagnosed and already diagnosed patients.

When we considered the treatment prescribed to the patients in already diagnosed group, most of the patients were in Metformin combination. Nearly 70% of patients were in metformin combination. Metformin was given along with myoinositol, clomiphene citrate, Human Chorionic Gonadotropins (HCG). Only 46.67% patients were on monotherapy of metformin (Table 1). The other common drug prescribed was myoinositol combination. It was also given along with metformin, clomiphene citrate and HCG. Metformin along with myoinositol was given to 13.3% of patients.

86.67% of patients were on these treatments for less than 1 year and only 13.33% of patients were on these therapies for more than 1 year (Figure 1).

Mekkawi *et al* stated that there was a statistical significant change in the Interleukin-6 and Interleukin-18 levels before and after

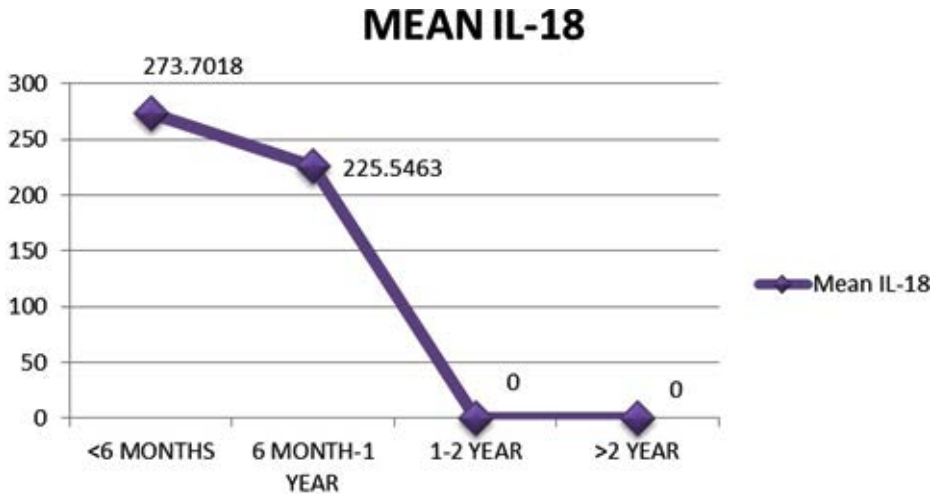


Fig. 4. MeanIL-18 levels

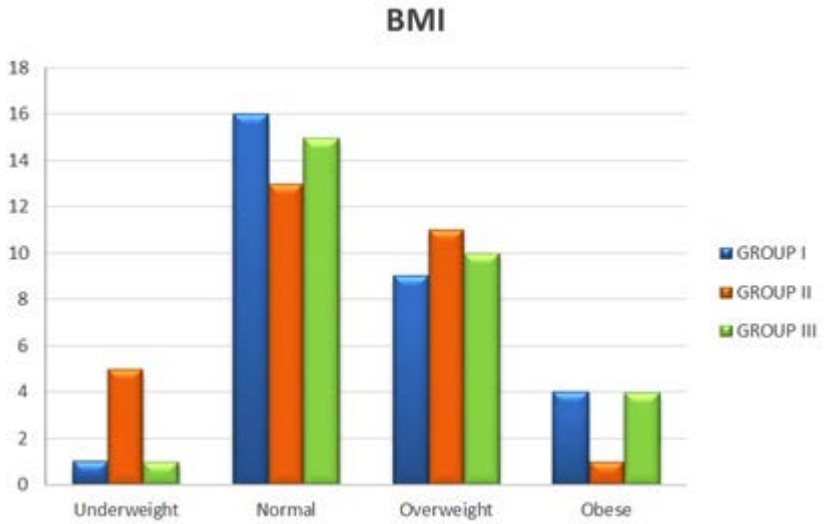


Fig. 5. Body Mass Index of the study population

metformin therapy showing metformin decreases the inflammatory markers in PCOS patients[19]. In previous studies it was known that there was an increase in the nuclear factor ê B (NFêB) in PCOS. Metformin inhibits NFêB which inhibits IL-1â and inhibits the production of pro inflammatory gene[24].

In our study, the mean ± SD for hs-CRP, IL-6,IL-18 were decreased between the patients as there was an increase in the duration of treatment (Figure 2,3,4).

Our results were consistent with Mohlig *et al*. who showed there was no substantial decrease in CRP and IL-6 levels. In their study these levels were increased in both normal and PCOS obese patients compared to the lean patients. There was a positive relationship between the obesity, insulin resistance and inflammatory markers than the PCOS itself. When a linear regression was done in their study it showed obesity was the one which increases the inflammatory marker than the insulin resistance[9]. Another study by Escobar-Morreale *et al*. also showed obesity causes an increase in the inflammatory marker and not the disease itself[25].

Our study had only 13.3% of obese study population in the already diagnosed and on treatment group and 3.33% in the newly diagnosed PCOS group(Figure 5).

Also in our study there were about 86.7% of already diagnosed group of patients' on treatment for less than one year(Figure 2). So even though there was a fall in the mean values of inflammatory markers there was no statistical significance in them.

The lack of statistical significance which may be due to small sample size in each group and wide range of values between the patients. The duration of treatment which was less than one year in most of the patients and hence the effect of drugs on the inflammatory markers could not be assessed. As this was a cross sectional study and blood samples were taken at one time the difference in the inflammatory markers could not be studied for the same group of patients. The influence of drugs prescribed was also in combination for most of the patients and so they could not be studied individually.

CONCLUSIONS

Though there are various pharmacological drugs available to treat PCOS, only few drugs targeted in reduction of inflammation. There were various studies, as we quoted earlier, demonstrating the effect of inflammation on PCOS. These studies were all done in other parts of the world. There were very less number of studies done in India. So we proposed to study the status of inflammation on PCOS in Indian population in particular South Indian population.

The pro inflammatory cytokines were produced when NFêB was stimulated. Metformin inhibits this NFêB and thus reduces the inflammation. Other drugs used in PCOS in practise like myoinositol, clomiphene citrate, hormones target at the disease per se and not on the inflammation produced by the disease.

Our study has shown that mean values of inflammatory markers were increased in Polycystic Ovary Syndrome (PCOS) in newly diagnosed and already on treatment for PCOS. 86.7% of the patients who were on already treatment for PCOS group were on treatment for less than one year. It has shown that there was an inversely proportional relationship between the mean values of inflammatory markers and duration of treatment.

We suggest larger follow up studies with longer duration of treatment so that influence of drugs on the inflammatory markers can be studied exhaustively in Indian population. Further studies are needed to demonstrate the effects of drugs for PCOS targeting on the inflammatory pathway so that these drugs not only brings down the severity of PCOS but also reduces the cardiovascular risk factors associated with it.

Table 2 and 3 represents the comparison of hs-CRP, IL-6 and IL-18 for the three groups.

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REFERENCES

1. Nidhi R, Padmalatha V, Nagarathna R, Amritanshu R. Prevalence of polycystic ovarian syndrome in Indian adolescents. *Journal of pediatric and adolescent gynecology.*; **24**(4):223-7 (2011).
2. Chris C.J.Kelly, Helen Lyall, John R.Petrie *et al*. Low grade chronic Inflammation in Women with Polycystic Ovarian Syndrome. *The Journal of Clinical Endocrinology & Metabolism.,* 2453-2455 (2001).
3. ACE position statement on the Insulin Resistance Syndrome, *Enocr Pract*, **9**(3); (2003).
4. Laure Morin-Papunen, Katrina Rautio, Aimo Ruokonen *et al*. Metformin Reduces Serum C-Reactive Protein Levels in Women with Polycystic Ovary Syndrome. *J Clin Endocrinol Metab,* **88**(10): 4649-4654 (2003).
5. Carl de Luca, Jerrold M. Olefsky. Inflammation and insulin resistance. FEBS [Federation of European Biohemical Societies] *Letters* **582**: 97-105 (2008).
6. Escobar-Morreale HF, Batella. Carretero JI, Alvarez- Blasco F *et al*. The Polycystic Ovary syndrome associated with morbid obesity may resolve after weight loss induced by bariatric surgery. *J Clin Endocrinol Metab.;* **90**: 6364-6369 (2005).
7. Escobar-Morreale HF, Calvo RM, Sancho J *et al*. TNF-alpha and hyperandrogenism: a clinical, biochemical and molecular genetic study. *J Clin Endocrinol Metab*.; **86**: 3761-3767 (2001).
8. Villuendas G, San Millan JL, Sancho J *et al*. The -597 G>A and -174 G>C polymorphism in the promoter of the IL-6 gene are associated with hyperandrogenism. *J Clin Endocrinol Metab.;* **87**: 1134-1141 (2002).
9. Mohling M, Spranger J, Osterhoff M *et al*. The polycystic ovary syndrome per se is not associated with increased chronic inflammation. *Eur J Endocrinol.;* **150**: 525-532 (2004).
10. Erdogan M, Karadeniz M, Berdeli A, *et al*. The relationship of interleukin-6- 174 G>C gene polymorphism with oxidative stress markers in Turkish polycystic ovary syndrome patients. *J Endocrinol Invest.*; **31**: 624-629 (2008).
11. Escobar-Morreale HF, Calvo RM, Villuendas G, *et al*. Association of the polymorphism in the interleukin-6 receptor complex with obesity and hyperandrogenism. *Obes Res.;* **11**: 987-996 (2003).
12. Jones TH. Interleukin-6 an endocrine cytokine. *Clin Endocrinol* (Oxf).; **40**: 703-713 (1994).
13. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissue of obese subjects release interleukin-6:depot difference and regulation of glucocorticoid. *J Clin Endocrinol Metab;* **83**: 847-850 (1998).
14. Purohit A, Ghilehik MW, Duncan L, *et al*. Aromatase activity and interleukin-6 production by normal and malignant breast tissues. *J Clin Endocrinol Metab*; **80**: 3052-3058 (1995).
15. Moshage HJ, Roelofs HM, van Pelt JF, *et al*. The effect of interleukin-1, interleukin-6 and its interrelationship on the synthesis of serum amyloid A and C-reactive protein in primary cultures of adult human hepatocytes. *Biochem Biophys Res Commun;* **155**: 112-117 (1988).
16. Ridker PM, Hennekens CH, Buring JE, *et al*. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*.; **342**: 836-843 (2000).
17. Ouchi N, Kihara S, Funahashi T *et al*. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation.;* **107**: 671-674 (2003).
18. Okamura H, Tsutsui H, Kashiwamura S *et al*. Interkeukin-18: a novel cytokine that augments both innate and acquired immunity. *Adv Immunol* **70**: 281-312.
19. ELMekkawi S.F., ELHosseiny A.S. Effect of Metformin Therapy on Serum Interleukin-6 and Interleukin-18 Levels in Patients with Polycystic Ovary Syndrome. *Nature and Science*.; **8**(9):23-26 (2010).
20. Knochenhauer ES, Key TJ, Kahsar-Miller M, *et al*. Prevalence of the polycystic ovary syndrome in unselected Black and White Women of the Southeastern United States: A prospective study. *J Clin Endocrinol Metab* ; **83**: 3078-3082 (1998).
21. Wild S, Pierpoint T, *et al*. Long term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. *Human Fertility.*; **3**(2): 101-105 (2000).
22. Barber T.M., McCarthy M.I., *et al*. Obesity and plycystic ovary syndrome. *Clinical Endocrinology.;* **65**(2): 137-145 (2006).
23. Hector F., Escobar-Morreale, *et al*. Serum Interleukin-18 Concentrations are increased in the polycystic ovary syndrome: Relationship to insulin resistance and to obesity. *The Journal of Clinical Endocrinology & metabolism;* **89**: 806-811 (2004).
24. Isoda K, Young JL,*et al*. Metformin inhibits

proinflammatory responses and nuclear factor-ê B in human vascular wall cells. Arterioscler. *Thromb. vasc. Biol*. **26**: 611-617 (2006).

25. Escobar-Morreale H.F., Villuendas G, *et al*. Obesity, and not insulin resistance, is the major determinant of serum inflammatorycardiovascular risk marker in premenopausal women. *Diabetologia.*; **46**: 625-633 (2003).

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Effects of *Momordica Charantia* (Bitter gourd) on Oxidative Stress and Pro-inflammatory marker in Metabolic Syndrome Using a High-fructose Diet Induced Rat Model

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To understand the biological basis of the effect of *Momordicacharantia* and also it's effectiveness as a mono-therapy for metabolic syndrome. Thirty four adult male Sprague-Dawley rats weighing were divided into Groups I, II, III and IV having 10, 10, 8 and 6 animals respectively. Pellets with 66% fructose were fed to 28 animals [Groups I, II, III] while 6 normal control animals [Group IV] were fed with standard rat chow diet for 6 weeks. Later, for next 6 weeks, animals in Groups I, II and III were treated with *M.charantia* 300mg/kg/day, and600mg/kg/day and Standard treatment respectively. Serial measurements of body weight, BMI, fasting blood sugar, noninvasive blood pressure, serum lipid profile, LDA, SOD and serum NF-êB were assessed at the base line, at the end of 6 weeks of fructose diet and following treatment (12 weeks). Following induction, there was statistically significant increase in body weight, BMI, FBS, serum triglycerides, total cholesterol, LDL, lipid derived aldehydes and serum NF-êB while there was reduction in HDL cholesterol and SOD compared to baseline values. Following treatment Group II and III shown reversal of the parameters to the level of normalization. *M.charantia* 600mg/kg/day had exclusively matched the therapeutic efficacy of the standard therapy given in combination. The biological basis of the effects of *M.charantia* was by inhibiting the inflammatory pathway in metabolic syndrome both at the levels of ROS generation and transcription of pro-inflammatory marker NF-êB.

Keywords: Metabolic syndrome, *Momordicacharantia*, Serum NF-êB, Lipid derived aldehydes.

'Metabolic syndrome'[1] is a constellation of metabolic derangements such as, abdominal obesity, hypertension, insulin resistance, hyperinsulinemia, impaired glucose tolerance, dyslipidemia, proinflammatory and prothrombotic states as well. It is the disorder reaching epidemic proportions worldwide in recent times[2]. The recently anticipated global prevalence of metabolic syndrome is approximately 16%.[3] It was also found that prevalence as 23.7%[4] in United States heeding to third National Health and Nutrition Examination Survey (NHANES III). The overall prevalence of cardio-metabolic syndrome in India is estimated to be ranging from 11% to 41%.[5]

The annual direct healthcare cost for diabetes and its associated diseases worldwide of age 20 to 79 years is estimated to be around 286 billion dollars or even more. And it is predicted that this figure would rise to 396 billion dollars by 2025 accounting 13% - 40% of global health care budget due to perpetual inflation of prevalence.[6]

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There is no well clear cut-off point in treating metabolic syndrome, as of now, individual components are treated in addition to the risk factors of the patients and moreover, no single drug is available to treat all the individual components of the syndrome altogether. On the other hand, *Momordicacharantia*(bitter gourd) tends to possess anti-diabetic, anti-hypertensive, hypolipidemic and anti-inflammatory properties individually as a sole agent.

With this background, our study aimed to study the effectiveness of *Momordicacharantia,* as a mono-therapy for metabolic syndrome. In addition our study also aimed at understanding the biological basis of the effect of *Momordicacharantia*in the treatment of metabolic syndrome.

MATERIALS AND METHODS

Institutional Animal Ethics Committee (IAEC) approval was obtained, and 34 adult, male Sprague-Dawley rats weighing 150-200g aged 3 months were included in our study.

Animals were randomly allocated into 4 groups;

- Group I: Treatment group 1 (*M.charantia* extract-300 mg/kg/day) -(10)
- Group II: Treatment group 2 (*M.charantia* extract-600 mg/kg/day)-(10)
- Group III: Standard treatment group (Metformin-180mg/kg/day + Telmisartan-2.5mg/kg/day + Rosuvastatin -2.5mg/kg/day)-(8)
- Group IV: Normal control-(6)

The animals were group housed, 4 rats per cage at a constant ambient temperature on a 12 - h light, 12 - h dark cycle. Pellet diet containing 66% fructose[177] made from D- fructose [purchased from Lobachemie laboratories] was given to 28 animals and standard rat chow diet to 6 controls and water were provided *ad libitum* out of 34 for 6 weeks. Then serial measurements of body weight, BMI, fasting blood sugar were done at the baseline and every week thereafter, whereas noninvasive blood pressure, serum lipid profile, LDA, SOD and serum NF-êBwere assessed at the base line and at the end of 6 weeks (i.e., following induction). Then at the end of induction, 28 animals of 3 groups, which were fed with fructose were provided *M.charantia* extract-300 mg/kg/day, *M.charantia* extract- 600 mg/kg/day and standard treatment respectively. And those animals were provided with standard chow and water *ad libitum* all through the 6 weeks of treatment. Then serial measurements of all the parameters were assessed at the end of 6 weeks (i.e., following treatment).Finally, at the end of the study 2 rats from each of the groups were sacrificed for histopathological examination of liver and heart to seek for pathological changes developed during induction and magnitude of the reversal of changes following treatment [Figure 1].

Tail cut bleeding (tail snip method)[7] was the method of blood collection used in our study,BMI[8] was determined from body weight and body length (nose-to-anus length) using the formula; BMI (Body mass index) = body weight (g) / length2 (cm2). We followed the method of blood glucose estimation with glucometer, according to the Institutional animal care and use committee (IACUC)[9] standard procedure for glucose monitoring of blood in rats and mice. LE 5002, non-invasive blood pressure meter (Panlab-Harvard apparatus) was used for measuring the blood pressure in rats. The lipid parameters estimated using GenX lipid parameters estimation kit, manufactured by Proton Biologicals India Pvt. Ltd (Bangalore) were serum total cholesterol, triglycerides and HDL (high density lipoprotein) cholesterol. LDL cholesterol was estimated using Friedewald's formula;[10] LDL = TC – HDL – $[TG/5]^2$.The principle behind the superoxide dismutase estimation done by us was inhibition of pyrogallol's auto-oxidation by superoxide dismutase. Then, the superoxide dismutase levels are analyzed from the 2 absorbance values (A,B) measured at two minutes interval using spectrometric method,

SOD (in Units) = [Absorbance (A-B) X 50/ Absorbance A]

Estimated malondialdehyde from the supernatant of the centrifuged samples by colorimeter,

Malondehyde (in Units) = [Absorbance at 532nm X 105/1.56]

Rat nuclear factor-kappa-B (NF-êB) assay was a quantitative sandwich enzyme immunoassay technique done by ELISA kit [Cusabio,China]. The microplate provided was pre-coated with NF-êB

specific antibody. NF-êB present on the standards and samples when pipetted into the wells would bind to antibody that was immobile. After removal of the substance that were not bound, a biotin-conjugated antibody specific for NF-êBwould be pipetted into the wells. Subsequently, wells were washed and addition of Horseradish Peroxidase (HRP) done. An addition of substrate solution to the wells would lead on to color development that would be in proportion to bound NF-êB, and the color intensity was measured.

The animals to be sacrificed for histopathological examination were anaesthetized with ether before the procedure. Once the level of anaesthesia is certain, the rats were dissected and the organs liver and heart were collected separately in a promptly labeled sterile container with formalin. Those specimens were immediately sent for histopathological examination.

Paraffin blocks were made and 4m thick sections were made from the chosen blocks for routine haematoxylin and eosin staining. Reagents required for this staining were Harris haematoxylin and eosin.The analysis done was Paired-t-test for analyzing the paired data i.e., parameters before and after induction and also for comparing data at the end of treatment with that following induction. One-way ANOVA and post hoc LSD were done to analyze the difference between groups.

RESULTS

Body mass index [BMI]

An increase in BMI was evidenced after a period of 6 weeks in animals [N=28] on 66% fructose diet in comparison to the normal control animals with a mean difference of 0.132 g/cm^2.

Following treatment for a period of 6 weeks, when compared with induced animals, Group I rats on *M.charantia* 300 mg/kg/day, Group II rats on *M.charantia* 600 mg/kg/day and Group III rats on Standard treatment showed a statistically significant reduction [P =0.021,P =0.001 and P=0.020 respectively] in BMI (Table 1). In contrast to all the treatment groups, the untreated normal control animals showed a statistically significant increase in BMI [P <0.001].ANOVA (Analysis of variance) assessed at baseline there was no statistical significant difference in BMI between groups. Following induction with 66% fructose all the three groups showed a statistically significant increase in body mass index compared to normal control [P <0.001]. Further, following treatment, though all the three groups showed reduction in body mass index, there was no statistically significant difference when compared to control [P=0.441,0.064,0.651 respectively] indicating normalization of BMI.

Fasting blood sugar

Change in fasting blood sugar in individual groups on induction

All the 3 groups showed a statistically significant increase in fasting blood sugar [P <0.001] from the baseline on induction of the disease been illustrated in the chart.

The difference in fasting blood sugar between groups was analyzed using ANOVA showed that a statistically significant increase [P <0.001] in all the 3 groups when compared to normal control on induction. Following treatment, Group II and III had reduced fasting blood sugar to a level similar to normal control [P=0.250,0.457 respectively], while reduction of the fasting blood sugar by *M.charantia* low dose (Group I) was not to the extent of that of normal control.

While following treatment of the animals for a period of 6 weeks all the three groups [Groups I,II and III] a paired t test detected a statistically significant reduction in fasting blood sugar [P <0.001], while the normal control group did not show significant change in blood sugar [P =0.610] (Table 1).

The difference in fasting blood sugar between groups was analyzed using ANOVA showed that a statistically significant increase [P <0.001] in all the 3 groups when compared to normal control on induction. Following treatment, Group II and III had reduced fasting blood sugar to a level similar to normal control [P=0.250,0.457 respectively], while reduction of the fasting blood sugar by *M.charantia* low dose (Group I) was not to the extent of that of normal control.

Mean arterial pressure[MAP]

Change in MAP in individual groups on treatment following induction:

Though all the 3 groups of animals fed on 66% fructose diet showed an increase in Mean arterial pressure (MAP) from the baseline within6 weeks, the induction was not homogeneous among all the groups with maximum rise in standard

treatment group [Figure-5].

A paired-t-test showed a statistically significant increase [P=0.006] only in the animals assigned to standard treatment group in comparison to baseline values. A paired-t-test was done to compare the difference in MAP following treatment found that the reduction though evident, was statistically significant in none of the groups [P =0.707, 0.173, 0.065] from the period of induction (Table 1).

The difference in MAP between groups was analyzed using ANOVA, at baseline and there was no statistical significant difference between groups indicating homogeneity of the animals while on induction, only Group III animals showed a statistically significant increase [P=0.002] compared to normal control. Further, on treatment, all the 3 groups of animals had demonstrated a reduction of MAP to reach the level of normal control rats.

Serum triglycerides on induction and treatment of metabolic syndrome

As shown in Table 2, all the 3 groups showed a statistically significant increase in triglycerides [P <0.001] from the baseline following induction of metabolic syndrome with 66% fructose diet for a period of 6 weeks [Figure-6].

Following treatment period for 6 weeks, all the 3 groups showed a statistically significant reduction in triglycerides [P <0.001] from the period of induction when a paired t test was used to test significance.

ANOVA (Analysis of variance) was done to assess the significant change in triglycerides levels between the 4 groups of animals. At baseline there was no statistically significant difference in triglycerides between groups indicating homogeneity of the animals. Following induction, all the three groups [Group I,II and III] showed a statistically significant increase in triglycerides compared to normal control [P =0.001,0.33,0.000 respectively]. Further, following treatment, all the three groups showed reduction in triglycerides, not statistically significant from that of control, [P=0.177,0.355,0.123 respectively] indicative of normalization of triglycerides levels.

Total cholesterol levels

Similar to triglycerides, total cholesterol of all the 3 groups showed a statistically significant increase [P <0.001] from the baseline following induction [Figure-7]. Compared to the normal control, Groups II and III on treatment showed a statistically significant [P=0.001] reduction in total cholesterol, while the reduction in Group I rats on *M.charantia*300 mg/kg/day (low dose) failed to demonstrate statistical significance [P=0.085] (Table 2).

ANOVA done to evaluate the significant change in total cholesterol between groups showed a significant increase in total cholesterol [P <0.001] compared to normal control following induction and after treatment, all the 3 treatment groups showed reduction in total cholesterol, in addition to which the mean total cholesterol in Groups II and III were not statistically significant compared to normal control rats indicating normalization of cholesterol level in these groups at the end of treatment.[P=0.126, 0.282].

LDL cholesterol levels

Following induction and treatment of metabolic syndrome

As shown in Table 2, all the 3 groups showed a statistically significant increase in LDL [P <0.001] from the baseline following induction of metabolic syndrome with 66% fructose diet for a period of 6 weeks [Figure-8]. Following treatment period of 6 weeks, Group II and III showed a statistically significant reduction in LDL [P <0.001] from the period of induction while Group I animals did not show significant reduction in LDL [P=0.366].

ANOVA (Analysis of variance) was done to assess the significant change in LDL levels between the 4 groups of animals at three time zones namely baseline, following induction and following treatment. At baseline, there was no statistical significant difference in LDL levels between groups. Following induction all the three groups [Group I,II and III] showed a statistically significant increase in LDL levels compared to normal control [P < 0.001].

Further, following treatment, Group II and III, when compared to control showed a statistically significant reduction in LDL [P=0.476,0.130 respectively] indicative of normalization of LDL levels while Group I animals on *M.charantia* low dose did not show significant reduction in LDL to the level of normal control.

HDL cholesterol levels

Following induction and treatment of metabolic syndrome

Comparison of serum HDL levels done among all the 3 groups showed a statistically significant reduction [P <0.001] at the time of disease induction to baseline level.

A paired-t-test showed a statistically significant increase in mean HDL levels [P =0.001, 0.001, and 0.003 respectively (Table 2)] at the end of treatment when compared to the mean following the period of induction, while normal control did not show statistically significant change in HDL levels [P=0.161].

ANOVA done to evaluate the significant change in HDL levels between groups showed a significant reduction in HDL levels [P <0.001] compared to normal control following induction and after treatment, there was an increase in HDL levels all the 3 treatment groups, despite this increase the mean HDL cholesterol in Groups II was not statistically significant compared to normal control rats indicating normalization of HDL level in this group at the end of treatment [P=0.808].

Thio-barbituric acid reactive substance levels

As shown in Table 3, a paired-t-test had shown a statistically significant increase in Thio-barbituric acid reactive substance, Malondialdehyde (MDA) in all the 3 groups [P=0.007,0.001,0.003] [Table-3] from the baseline following induction of metabolic syndrome with 66% fructose diet over a 6 weeks period [Figure-10].

Following treatment period of 6 weeks, a paired t test was done to detect the difference between groups found that though all the three groups had reduced the TBARS levels, it was only II and III showed a statistically significant reduction of malondialdehyde levels [P=0.024, 0.001] respectively.While the group I animals on low dose *M.charantia* did not show significant reduction [P=0.066] in malondialdehyde in comparison to the other groups.

ANOVA done to assess the significant change in MDA levels between groups revealed a statistically significant rise in the levels of MDA [P =0.002,<0.001, <0.001 respectively] compared to normal control following induction. Following treatment, the MDA levels in all the 3 treatment groups, was not statistically significant different from that of normal controls indicating normalization of MDA level all the groups at the end of treatment [P=0.078,0.743,0.454 respectively].

Superoxide dismutase [SOD]

SOD following induction and treatment of metabolic syndrome

All the 3 groups showed a statistically significant reduction in SOD levels [P=0.001, 0.005, 0.000 respectively] from the baseline following induction of metabolic syndrome with 66% fructose diet [Figure-11] for a period of 6 weeks when tested using paired-t-test.

Following treatment period for 6 weeks, all the 3 groups though showed a increase in SOD levels from the period of induction, but was not of statistical significance when a paired t test was used to test the significance [P=0.087,0.107,0.214 respectively (Table 3)].

When ANOVA was done to evaluate the significant change in SOD levels between groups following induction, a statistically significant reduction in SOD levels [P=0.020, 0.001, 0.000 respectively] was detected compared to that of normal control. After treatment, there was an increase in SOD levels in all the 3 treatment groups, with the increase in the mean SOD cholesterol in Groups I and II not being statistically significant compared to normal control rats indicating normalization of SOD level in this group at the end of treatment.

Nuclear factor kappa-B [NF-êB]

NF-êB following induction and treatment of metabolic syndrome

The mean serum NF-êB levels of the normal control animals in our study at baseline was established as 22.033±5.657 pg/ml [Figure-12].All the 3 groups showed a increase in NF-êB levels, but not of statistical significance [P=0.303, 0.270 and 0.327 respectively] from the baseline following induction when a paired-t-test was used (Table 3).

In comparison to the normal control values in their individual groups, all the 3 groups showed a statistically significant reduction in serum NF-êB levels [P=0.043, 0.001 and 0.027 respectively].

ANOVA done to analyze the difference between groups had found that there was a reduced serum NF-êB levels, which is not statistically significant when compared to normal control rats indicating normalization of serum NF-êB levels

in groups I and III at the end of treatment, while group III animals showed a statistically significant difference with that of normal control indicating non-achievement on normalization.

Histopathological examination

Histopathological changes in liver

On comparing the liver of treated animals with that of normal control, all the 3 groups showed fatty infiltration [micro &macrovesicular steatosis]. Group II and III animals showed fatty changes of grade 2+ while the fatty liver was graded as 3+ in Group I animals treated with low dose *M.charantia* (Figure 13).

Histopathological changes in heart

On comparing the histopathology of heart of the treatment groups with that of normal control, it was evident that all the 3 groups showed hypertrophy of heart due to fructose diet. The grade of hypertrophy was found to be homogenous in Groups I, II and III and did not any show any changes related to reversal of the hypertrophied heart. But the fatty change that is observed in the disease control is not seen in sections of Groups I, II and III heart (Figure 14).

DISCUSSION

The aim of our work was to study the biological basis of effects of *Momordicacharantia*in metabolic syndrome by measuring serum NF-êB, lipid derived aldehydes and superoxide dismutase levels. And we also focused to identify the therapeutic advantage, of *M.charantia*as a sole agent in the treatment of all the entities of metabolic syndrome with respect to available standard treatment, by ascertaining the changes in body mass index, fasting blood sugar, lipid profile and blood pressure.

Body mass index showed a statistically significant [P <0.001] increase in all the groups of animals after an induction period and also in the control animals compared to baseline values. The increase in BMI noted in normal control can be explained as due to the linear growth that is normally observed.[11] Following treatment, all the treated groups of animals showed a statistically significant reduction. Inspite of not achieving statistical significance in body weight reduction in the Groups I and III, BMI showed a significant reduction and this disparity can be accounted by the fact that the length and the thoracic circumference would become static at around 4-5 months and thus, signifying the advantage of BMI over body weight, as diet induced obesity could be easily estimated from the BMI in rats in addition to being a predictor of dyslipidemia and oxidative stress.[12]

Fasting blood sugar was estimated every week for a period of 12 weeks showed a statistically significant increase in all the groups [P <0.001] from the baseline to induction. It is well established that the enzyme fructokinase, would phosphorylate the surplus fructose absorbed from the intestine. And also, the metabolism pertaining to fructose is not similar to that of glucose, the difference being fructose-1-phosphate that is formed from fructose would be converted to several 3-carbon molecules, like glyceraldehyde, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate and they are considered as the potential source for gluconeogenesis, and thereby raise blood glucose levels.[13]

Following treatment all the three groups [Groups I,II and III] showed a statistically significant reduction in fasting blood sugar. A study which was done earlier to analyze the anti-diabetic effect of *M.charantia* with reference to glibenclamide found that all the doses used in the study from lower to higher doses [250 mg/kg, 500 mg/kg, 750 mg/kg] showed a statistical significant reduction in blood sugar similar to that resulted from our study.[14]

On comparison of the mean of treated groups to normal age control at the end of treatment, it was evident that, though *M.charantia*low dose had reduced the blood sugar significantly but not to the extent of normalization. The probable reason for this could be due to shorter duration of treatment. There are numerous studies demonstrating hypoglycemic activity of *M.charantia*, apart from the observation that the active component being structurally similar to insulin. Accordingly, it is also shown to exhibit insulin secretagogue action with additional activity of inhibiting two key gluconeogenic enzymes fructose 1, 6-diphosphatase and glucose-6-phosphatase.[15,16]

Our study animals though have demonstrated an increase in Mean arterial pressure in response to high fructose diet, the rise was not identical in all the groups of animals and there was inter and intra group variation with statistically

significant [P=0.006] rise in animals grouped for standard therapy (Group III). Though studies had demonstrated the induction of hypertension by fructose diet, contradictorily, there are also studies demonstrating inconsistent elevation of blood pressure.[17,18] A study done in male Sprague-Dawley rats with high fructose diet showed a demonstrable rise in systolic blood pressure but not mean arterial pressure (MAP) which was rationalized by the variability between animals and also the scale of metabolic dysfunction not being sufficient to defer an effect on blood pressure.[17] The probable reason for the increased MAP response in Group III rats unlike the other groups in our study, would have been due to the associated rise in serum triglycerides. Similar observation was also made in a previous study.[19]

Subsequently after the treatment period of 6 weeks, all the 3 groups of animals [groups I, II and III] showed a reduction in mean arterial pressure from the period of induction though not statistically significant.

When the difference between groups were analyzed at the end of treatment, a constructive finding illustrated in our study was that groups on high dose *M.charantia* and standard therapy showed a reduction in MAP to the level of normalization, while low dose *M.charantia*in contrast showed a rise in blood pressure following a fall indicative of its inconsistency in treating hypertension.

There exists an analogous pattern of MAP changes in the groups II and III in the line chart indicating a potentially similar mechanism of BP lowering by *M.charantia* and telmisartan. This could be explained by a recent study signifying the existence of a peptide named VY-7 in *M.charantia*that possesses antihypertensive property by inhibiting angiotensin converting enzyme,[20] and in turn reducing aldosterone production. In addition, naturally, it has high potassium content and both could be contributory to increased natriuresis and thereby a fall in blood pressure. Telmisartan, an angiotensin receptor antagonist, also acts similarly by reducing aldosterone production. Yet, another homology that exists between telmisartan and *M.charantia* is that both possess PPAR ã agonistic activity.

Our study evinced a statistically significant increase in triglyceride levels at induction of metabolic syndrome as consistent with another study. [21] It had been documented earlier with studies that mechanism of fructose induced gluconeogenesis and hypertriglyceridemia were interlinked. The analogy that exists is that 3 carbon molecules formed from fructose-1-phosphate could be utilized eventually in the formation of fatty acids and glycerol, which on esterification would form triglycerides. There could be few other mechanistic contributions, for instance, either up regulation of lipogenic pathway or down regulation of PPAR-á activity involved in catabolism of triglycerides.[21]

Subsequently after treatment, all the 3 groups showed a statistically significant reduction in triglycerides, it was evident that, *M.charantia*low, high doses and standard treatment had reduced the triglycerides to the extent of normalization. Yet, another study evaluating the hypolipidemic effects of *M.charantia*showed a similar reduction including low dose of 300 mg/kg as with our study.[22] The milieu of reduction in triglyceride levels caused by bitter melon was due to increased expression of PPAR-á activity in the liver. As a sequel of increased activity of PPAR-á, the key receptor in down regulation of apolipoprotein C-III involved in hydrolysis of triglycerides by lipoprotein lipase (LPL) contributed to the lipid-lowering effect of *M.charantia.*[23]

All the 3 groups of animals included in our study had attained a statistically significant increase in total cholesterol levels following induction same as that observed in previous studies with high fructose diet induction.[24] The reason behind the elevation of total serum cholesterol level by fructose as a retort to the increased weight and insulin resistance which would lead on to activation of the enzymes HMG CoA reductase and fatty acid synthase along with increased expression of hepatic sterol response element binding protein (SREBP).[25]

Compared to the normal control, group II and group III animals on showed a statistically significant reduction in total cholesterol, and further the results of a study done on off springs of rats fed with 66% fructose diet showed that bitter melon had significantly reduced the total cholesterol as seen with our study. [26] On comparing the groups with that of normal animals at the end of treatment period revealed the reversal of serum cholesterol level to the degree of normality in those animals on high dose *M.charantia*and standard treatment. The

probable mechanism as demonstrated by an earlier study is inhibition of the bile acid reabsorption, thereby leading on to increased elimination of bile acids in feces. In addition, it is also known to regulate cholesterol 7á-hydroxylase (CYP7A1) by increasing the level of hepatic CYP7A1 mRNA expression contributing to increased conversion of cholesterol to bile acids.[27]

This study also elucidated a statistically significant increase in LDL levels in all the 3 groups from the baseline, and a similar effect was produced earlier in another study. The perception behind the occurrence of increased LDL with fructose is that at large amounts it would undergo glycation rather than fructation, which is considered to be detrimental to the cellular proteins by the production advanced glycation end products (AGEs),[28] thereby furnishing oxidative damage. Once the LDL is glycated, they are poorly recognized by the lipoproteins and scavenging receptors, which consequentially leads on to increased LDL levels.[29]

After treatment, Group II and Group III animals showed a statistically significant reduction in LDL from the period of induction to normalization. This action of *M.charantia*could be justified by its antiglycating activity proven by a study that demonstrated the percentage inhibition of AGE as 40% at 1 mg/ml concentration by AGE fluorescence. [30] Furthermore, Momordin (charantin), the active component of bitter melon, by promoting PPARä activity decreases LDL, with subsidiary action of decreased primary lipoprotein of LDL apolipoprotein B (Apo B) production, and apolipoprotein C- III expression on VLDL.[31]

Comparison of serum HDL levels among all the groups at induction, showed a statistically significant reduction and this result

Table 1. Illustration of body weight, body mass index, fasting blood sugar and mean arterial pressure in three different groups

Parameters	Groups	Pairs	Mean	Sd	P
Body Weight	I	Baseline-Induction	-134.8	33.31	<0.001
		Induction-Treatment	5.81	16.26	0.288
	II	Baseline-Induction	-147.9	23.53	<0.001
		Induction-Treatment	11.10	10.91	0.011
	III	Baseline-Induction	- 125.8	11.44	<0.001
		Induction-Treatment	0.50	12.14	0.899
BMI	I	Baseline-Induction	-0.24	0.97	<0.001
		Induction-Treatment	0.07	0.08	0.021
	II	Baseline-Induction	-0.28	0.59	<0.001
		Induction-Treatment	0.06	0.03	<0.001
	III	Baseline-Induction	-0.23	0.39	<0.001
		Induction-Treatment	0.04	0.05	0.020
FBS	I	Baseline-Induction	-95.30	16.93	<0.001
		Induction-Treatment	51.37	18.23	<0.001
	II	Baseline-Induction	-89.60	23.09	<0.001
		Induction-Treatment	73.12	25.19	<0.001
	III	Baseline-Induction	-93.25	18.20	<0.001
		Induction-Treatment	73.12	25.19	<0.001
MAP	I	Baseline-Induction	-34.63	51.93	0.101
		Induction-Treatment	-8.41	60.85	0.707
	II	Baseline-Induction	-14.25	38.16	0.326
		Induction-Treatment	28.71	53.59	0.173
	III	Baseline-Induction	-57.50	41.31	0.006
		Induction-Treatment	49.83	64.57	0.065

BMI=Body mass index, FBS=Fasting blood sugar, MAP=Mean arterial pressure, SD= Standard deviation

was homogenous to that of former studies.[32] It has been well entrenched by studies that caveolins are found to be involved in the export of bile salts from the liver, and there exists a concordant relationship between exportation of bile salts and HDL levels i.e., any reduction or increment in the earlier would lead on to reduction or increase of the HDL levels respectively. Based on this mechanism, ingestion of high fructose diet would lower the HDL levels by reducing the bile salt export.[33]

Following treatment, all the 3 groups showed a statistically significant increase as observed in earlier study. [34] Revealed that the restoration of serum HDL level to the degree of normality in those animals on high dose *M.charantia*only. In adipocytes, the enzyme lipoprotein lipase was responsible for both release of fatty acid and conversion of VLDL to cholesterol-rich LDL. Normally, macrophages embedded in the vessel wall would take up VLDL and ox-LDL actively and excess cholesterol would then be fluxed out through the HDL pathway. As demonstrated by studies that the balance between lipid influx and efflux is carried out by PPAR-ã in humans and PPAR-ä in lower animals. Any agent with the PPAR agonistic activity would increase HDL levels by decreasing LDL and *M.charantia* is one such agent with PPAR-ä agonistic activity thus reducing HDL levels.[31]

The estimated levels of thiobarbituric acid reactive substance malondialdehyde (MDA) showed a statistically significant rise. It was well recognized by studies that the animals fed on high fructose diet were known to produce remarkable changes in serum triglyceride levels, which in turn would increase the activity of fatty-acyl-coA oxidase leading on to â-oxidation. Further, excess usage of non-esterified fatty acid (NEFA) for â-oxidation would lead on to the down-regulation of malonyl-CoA, and the reduced NEFA

Table 2. Illustration of triglyceride, total cholesterol, low density lipoprotein and high density lipoprotein in three different groups

Parameters	Groups	Pairs	Mean	Sd	P
TGL	I	Baseline-Induction	-158.35	89.83	0.002
		Induction-Treatment	176.16	95.14	0.001
	II	Baseline-Induction	-125.68	35.45	<0.001
		Induction-Treatment	132.99	29.69	<0.001
	III	Baseline-Induction	-202.05	73.85	<0.001
		Induction-Treatment	235.78	77.08	<0.001
TC	I	Baseline-Induction	-107.05	42.89	0.001
		Induction-Treatment	25.61	32.87	0.085
	II	Baseline-Induction	-119.13	24.45	<0.001
		Induction-Treatment	78.77	35.81	0.001
	III	Baseline-Induction	-108.05	10.13	<0.001
		Induction-Treatment	70.80	28.12	0.001
LDL	I	Baseline-Induction	-104.97	26.43	<0.001
		Induction-Treatment	28.98	78.38	0.366
	II	Baseline-Induction	-135.78	20.11	<0.001
		Induction-Treatment	80.62	29.74	<0.001
	III	Baseline-Induction	-113.04	13.82	<0.001
		Induction-Treatment	32.09	9.38	<0.001
HDL	I	Baseline-Induction	32.03	9.87	<0.001
		Induction-Treatment	-10.84	3.25	<0.001
	II	Baseline-Induction	41.46	1.83	<0.001
		Induction-Treatment	-23.56	10.09	<0.001
	III	Baseline-Induction	45.85	4.31	<0.001
		Induction-Treatment	-2.29	1.80	0.003

TGL=Triglyceride, TC=Total cholesterol, LDL=Low density lipoprotein, HDL=high density lipoprotein, SD= Standard deviation

transport via CPT-1 across mitochondria thereby increasing oxidation at endoplasmic reticulum and peroxisomes. In addition, oxidation of organelles in the cytoplasm releases ROS, which when formed in excess, damages the mitochondrial membrane rich in polyunsaturated fat thus serving as a source for lipid peroxidation derived aldehydes. However, the process does not terminate there, as those systemically released MDA would inhibit the defensive anti-oxidant enzymes SOD and catalase.[35]

Following treatment Groups II and III showed a statistically significant decrease in lipid derived aldehydes. Analysis done to scale the difference between groups revealed no statistically significant difference between the treated groups and normal control indicating the normalization of lipid peroxidation. Ours is the first study to demonstrate the effect of *M.charantia* on Lipid derived aldehydes in metabolic syndrome model even though the mechanism contributing to the effect has been studied.

Superoxide dismutase estimation done in our study revealed a statistically significant decline in their levels during the span of induction with fructose. It was recognized that ROS plays a key role in regulating various cellular functions but at higher concentration are responsible for disruption of structural integrity of cells and thereby damage the proteins, DNA and lipid. Any condition that disrupts this balance could lead on to oxidative stress by causing profound reduction in the enzyme superoxide dismutase, one such state is hyperglycemia induced by high fructose diet.[36]

The subsequent determination of enzyme levels following the treatment period though endowed ascend in their serum levels did not achieve statistical significance when compared to the values at induction. When we analyzed the difference in mean superoxide dismutase levels between the treated and age control rats, there was no statistically significant difference indicative of its acceleration towards normal with both the doses of *M.charantia*. While the standard therapy did not favorably usher the free radical scavenger, superoxide dismutase towards the expected magnitude, the reason could be credibly due to the mean reduction in their level was huge at the time of induction with respect to other two groups.

In our study, serum NF-KB levels were

Table 3. Illustration of thio-barbituric acid reactive substance levels, super oxide dismutase and nuclear factor kappa-B in three different groups

Parameters	Groups	Pairs	Mean	Sd	P
TBARS	I	Baseline-Induction	-2.59	1.95	0.007
		Induction-Treatment	1.24	1.62	0.066
	II	Baseline-Induction	-3.19	1.75	0.001
		Induction-Treatment	2.71	2.67	0.024
	III	Baseline-Induction	-3.47	2.14	0.003
		Induction-Treatment	2.98	1.49	0.001
SOD	I	Baseline-Induction	0.70	0.39	0.001
		Induction-Treatment	-0.34	0.48	0.087
	II	Baseline-Induction	0.52	0.37	0.005
		Induction-Treatment	-0.46	0.70	0.107
	III	Baseline-Induction	1.24	0.45	<0.001
		Induction-Treatment	-0.35	0.73	0.214
NFκB	I	Baseline-Induction	-8.22	19.30	0.303
		Induction-Treatment	16.12	16.70	0.043
	II	Baseline-Induction	-6.95	15.13	0.270
		Induction-Treatment	22.15	8.76	0.001
	III	Baseline-Induction	-3.53	8.76	0.327
		Induction-Treatment	14.35	13.02	0.027

TBARS=Thio-barbituric acid reactive substance levels, SOD=Super oxide dismutase
NFκB=Nuclear factor kappa-B, SD= Standard deviation

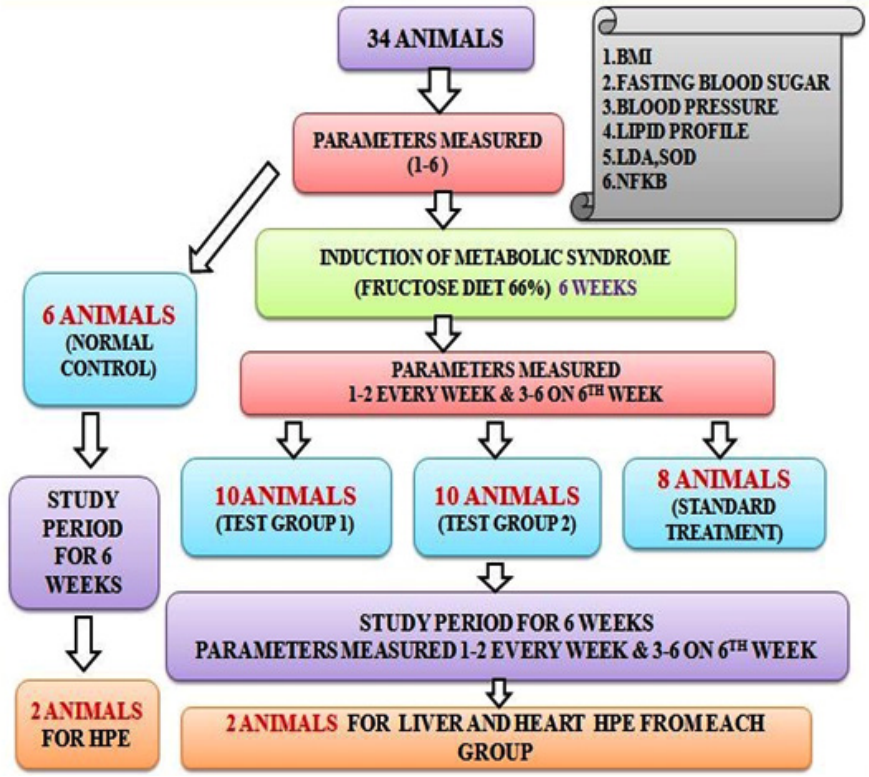


Fig. 1. Flow chart of study design

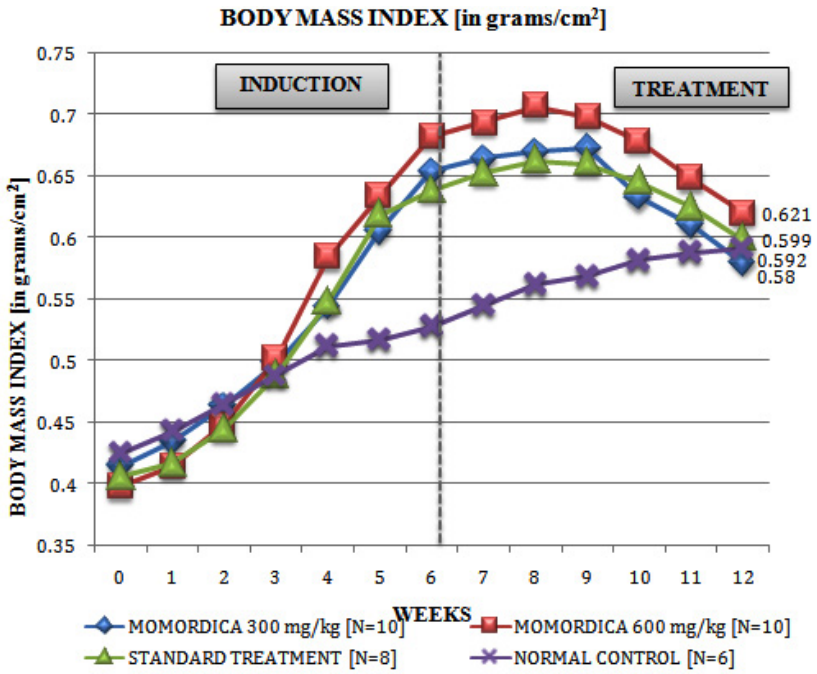


Fig. 2. BMI in individual groups after treatment

estimated using ELISA kit at the baseline(0 week), on induction (6 week) and following treatment (12 week). Though there are studies on tissue expression of NF-êB, subsequent to infections, inflammation and malignancies, there is only sparse literature available on serum levels of NF-êBtill date. A study done in rats with mucositis on inflammatory markers including serum NF-

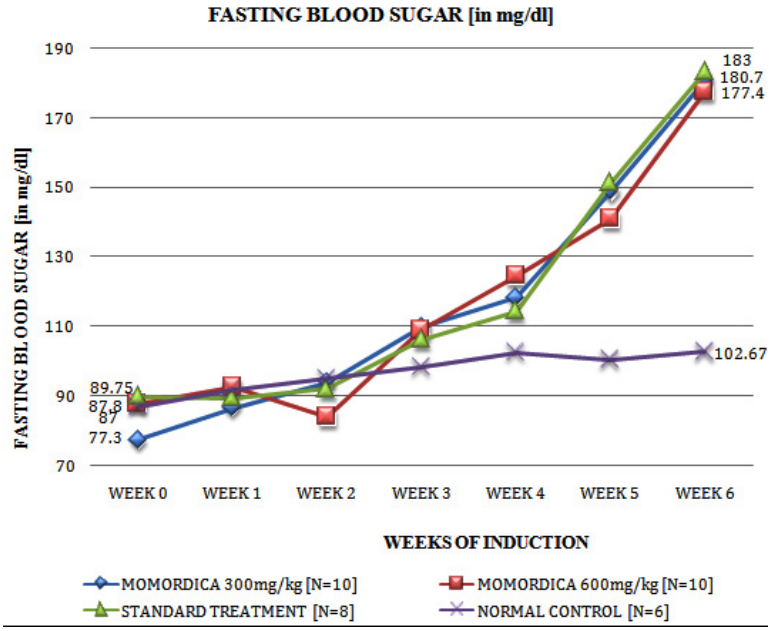


Fig. 3. Change in fasting blood sugar during induction

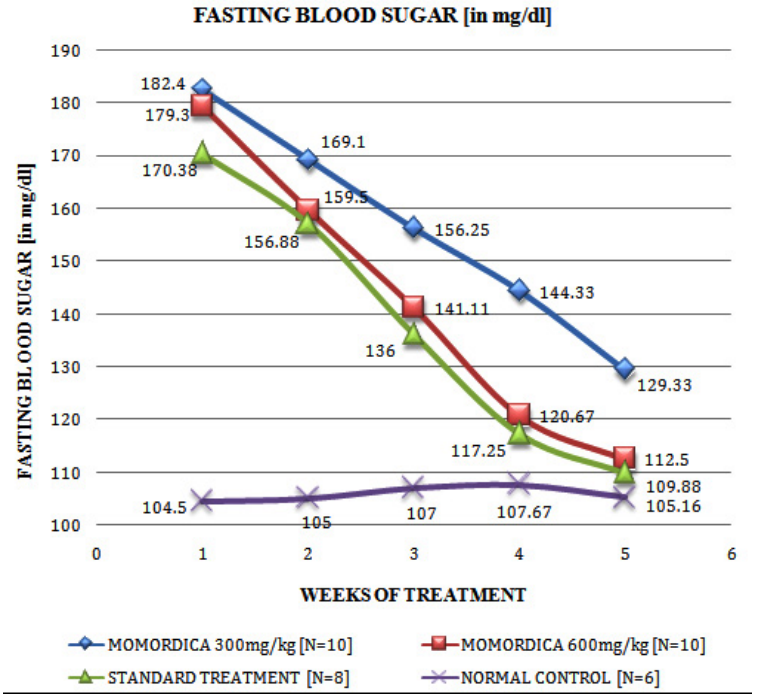


Fig. 4. Change in fasting blood sugar during treatment

êBfound that there is difference between groups at baseline as in concordance with our study.[37] The mean serum level at baseline was established as 22.033±5.657 pg/ml in our study. All the 3 groups of animals showed an increase in the levels from that of baseline but were not to the level of significance.

Following treatment all the 3 groups of

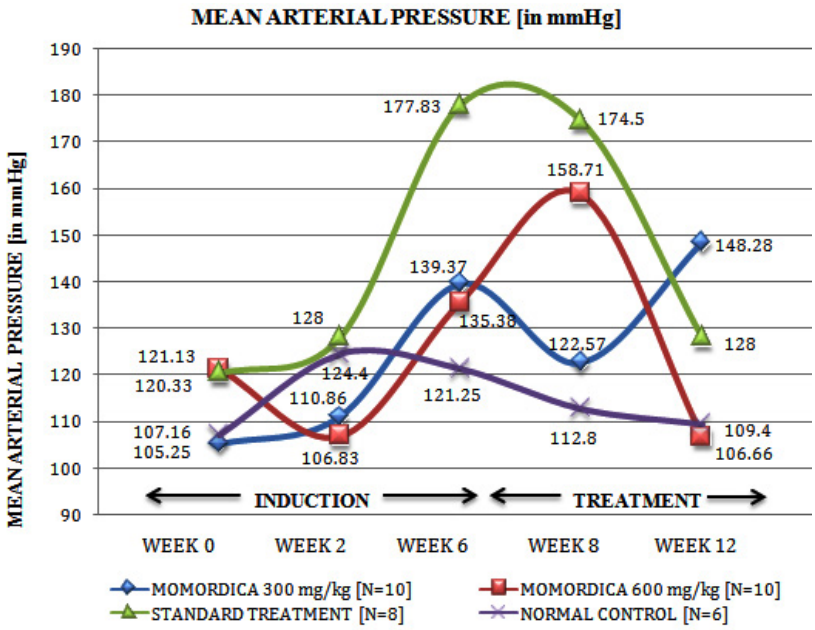


Fig. 5. Change in MAP during induction and treatment

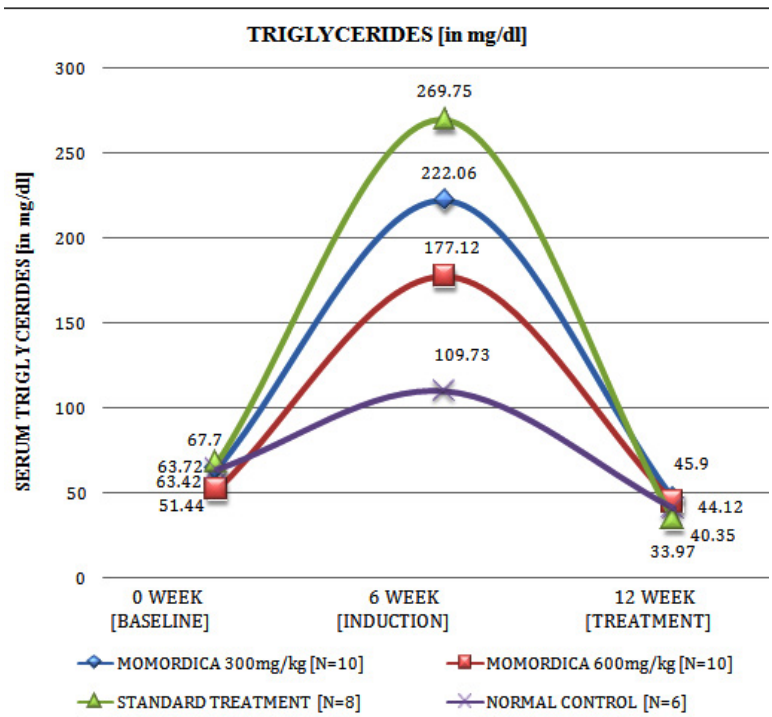


Fig. 6. Change in triglycerides levels on induction and treatment

animals revealed a statistically significant reduction in serum NF-êBlevels [P=0.043, 0.001, 0.027]. As of now, there are only 3 studies done with the objective of establishing the role of *M.charantia*on pro-inflammatory marker NF-êB, one being the work done to demonstrate the inhibitory activity of *M.charantia* as one of the tested compounds on NF-êBtranscriptional activation in HepG2

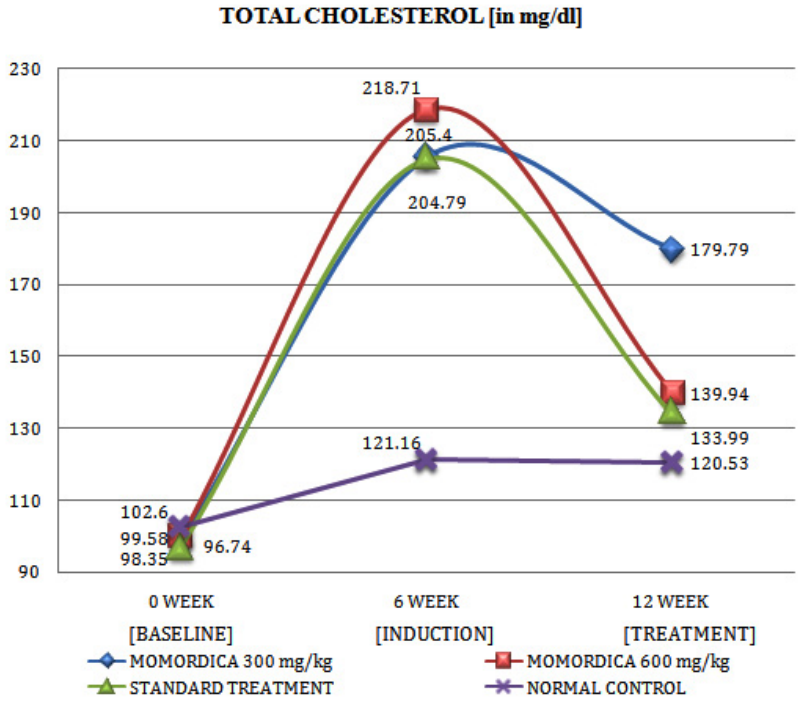


Fig. 7. Change in total cholesterol following induction and treatment

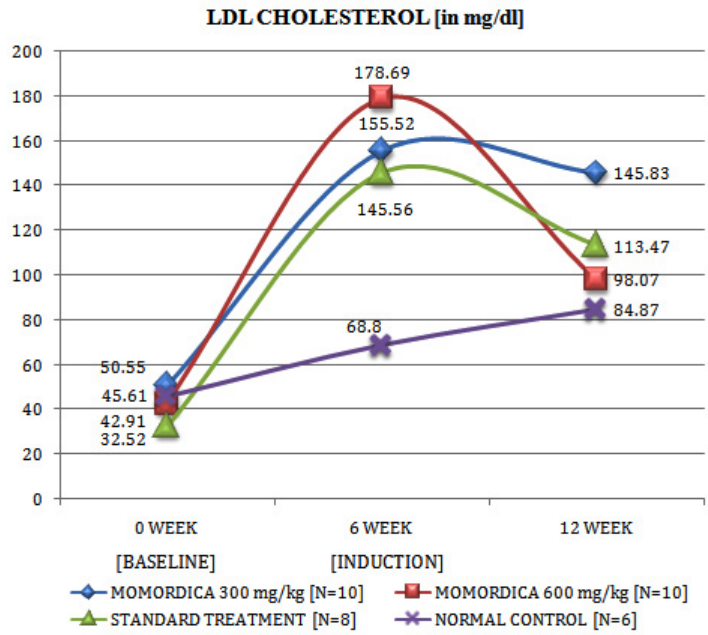


Fig. 8. Change in LDL levels following induction and treatment

cells (human hepato-carcinoma) in Dulbecco's modiûed Eagle's medium had proven its inhibitory effect.[38] Another was an m-RNA study on heart tissue of the obese Zucker rats demonstrated that down-regulation of nuclear factor-êB (NF-êB), and interferon-ã by *M.charantia*.[39] The third

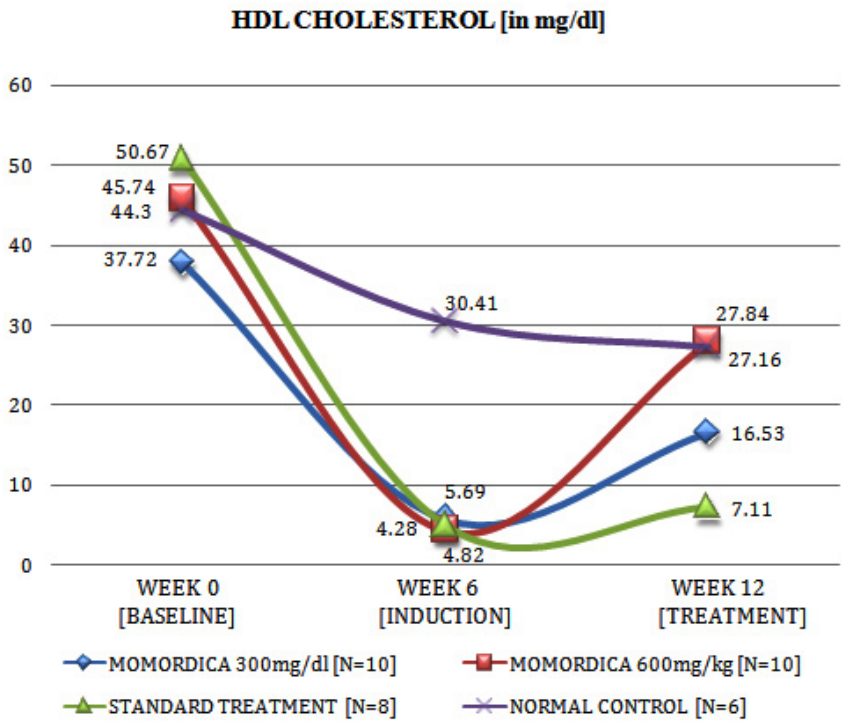


Fig. 9. Changes in HDL levels following induction and treatment

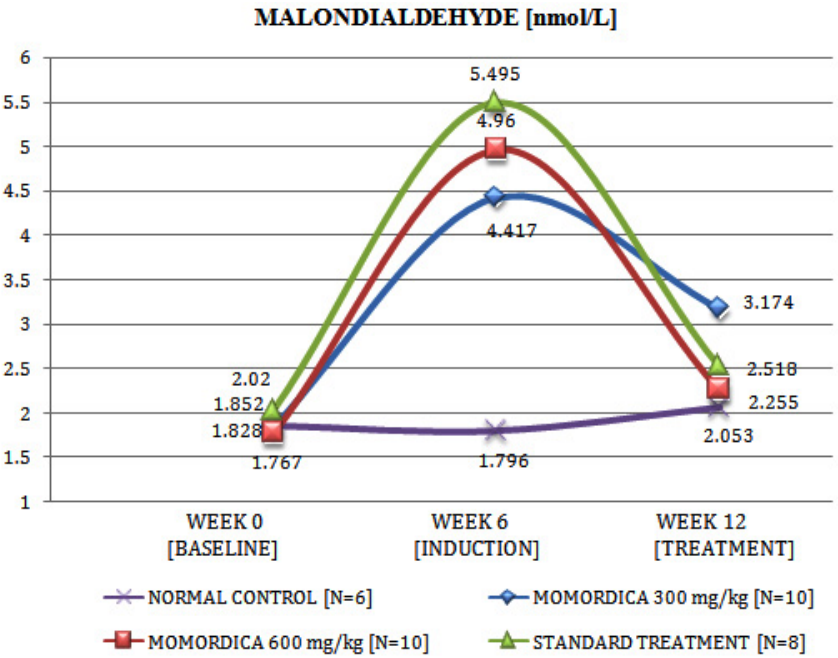


Fig. 10. TBARS during induction and treatment of metabolic syndrome

being an electrophoretic mobility shift assay with the ethanol extracts and the hot aqueous extracts of *M.charantia*showing inhibition of NF-êB activation.[40] But so far no research is done to assess the effect of *M.charantia*extract in vivo on serum NF-êB levels.

The histopathological examination of the liver and heart was done in our study. Due to liability of ethical concern, we have taken the pictures of diseased liver and heart in response to fructose intake from standard research articles. In comparison to normal control all the liver showed fatty infiltration, both microvesicular and macrovesicular steatosis that was in ordinance to

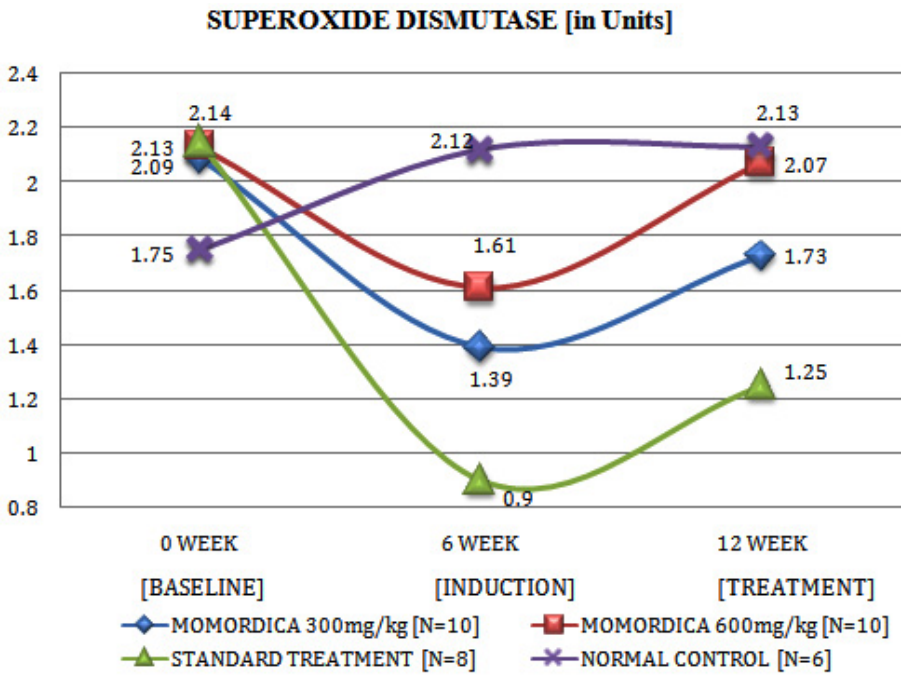


Fig. 11. Changes in SOD levels on induction and treatment

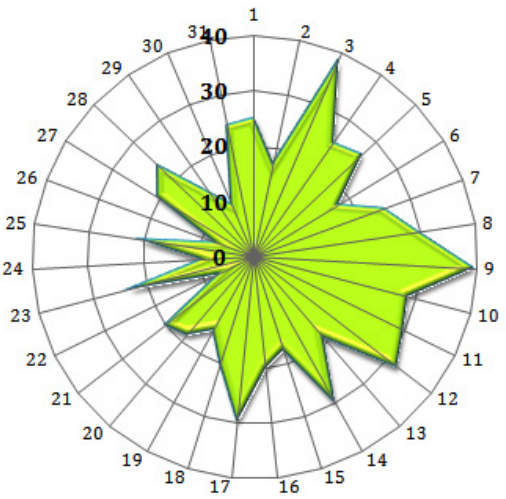


Fig. 12. Normal serum NF-êB levels [in pg/ml] observed in our study

high fructose diet as apparent in prior studies. In addition, the changes in histopathology of the liver and heart by *M.charantia*unlike that of pancreas, has not been studied in literature till date.

In distinction, the fatty change that was observed in these groups in comparison to the diseased picture revealed a significantly minimal change. There also exists a difference in fatty changes among groups, HPE of liver of Group I showed maximum change of grade 3+ while that of Groups II and III were presented with only grade 2+ changes.

So far there are no studies dealt on the role of *M.charantia* on the fatty changes precipitated on consumption of the fructose. So ours is the first work done to learn the changes, and we obtained an affirmative result that the changes in both *M.charantia* high dose and standard treatment

groups were homogenous and the fatty change in these groups were of a lower degree in comparison to the low dose *M.charantia* group.

In comparison to normal control all the heart HPE sections showed hypertrophy that was in accordance to previous studies with reference to high fructose diet.

The cardiac hypertrophy was highly

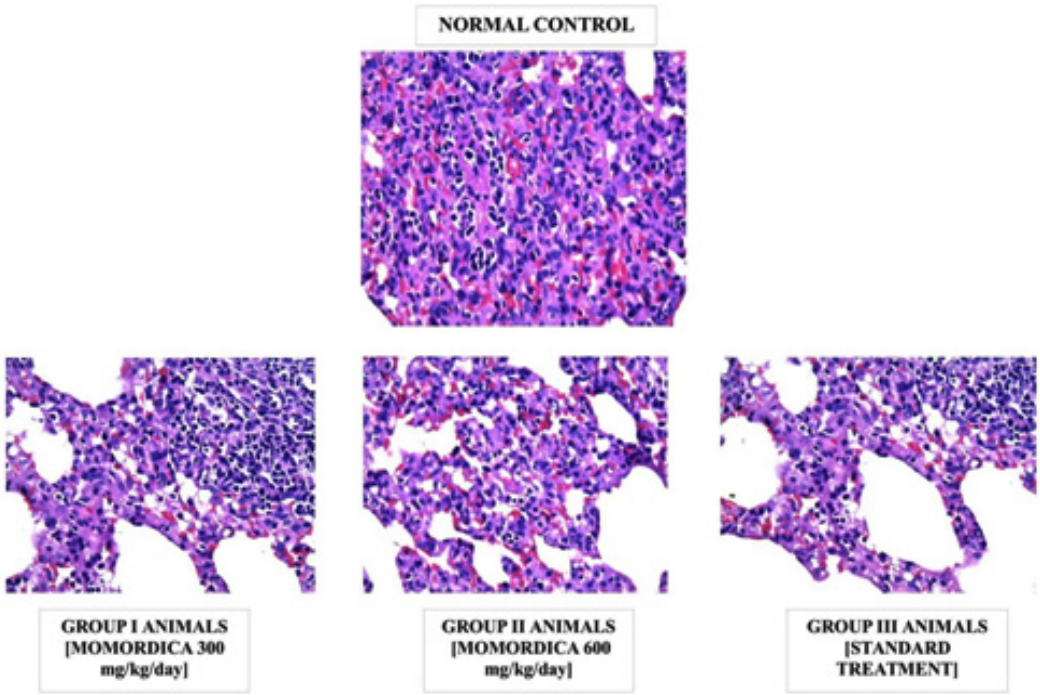


Fig. 13. Histopathological examination of Liver [H&E, 40X]

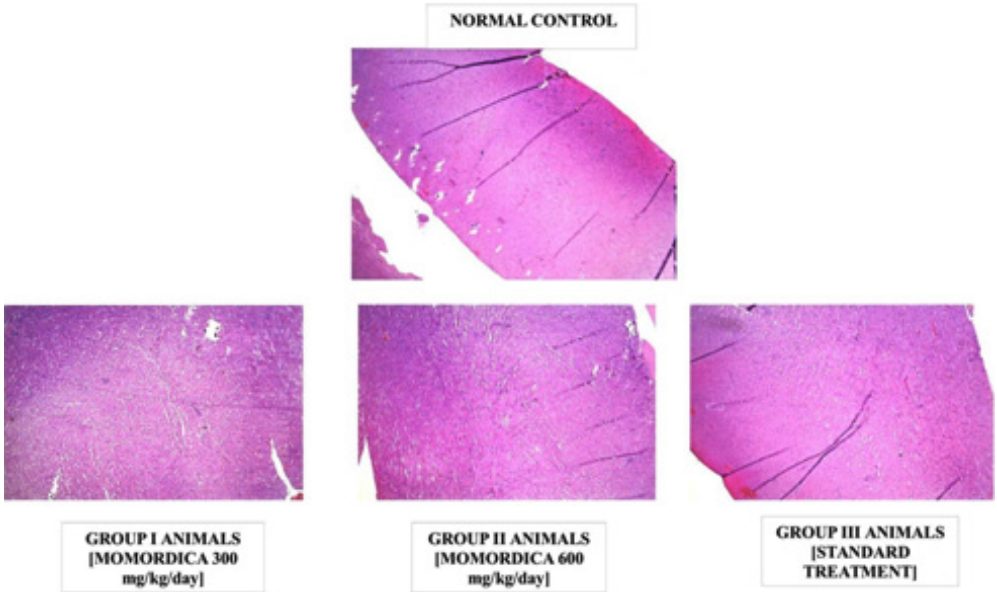


Fig. 14. Histopathological examination of Heart [H&E, 40X]

evident but observations made in these 3 groups were homogenous with no difference among them. The reason for this could duly be explained by the relatively brief span of treatment was not sufficient to bring about the scale of expected changes in the hypertrophied myocytes. But the positive finding illustrated was that the fatty change observed in the picture of disease control was not evident in the HPE sections of all three groups of treated animals. So, further studies pertaining to this could be done in future to assess the effect of *Momordica* on progression of cardiac hypertrophy.

CONCLUSION

High fructose diet (66%)was affirmatively found to be the animal model appropriate for inducing metabolic syndrome to study the effect of drugs as the model effectively reproduced all the metabolic changes [BMI,FBS,BP, Lipid parameters]seen in the syndrome. We included two different doses of *M.charantia*, of which 600mg/kg/day had exclusively matched the therapeutic efficacy of the standard therapy given in combination. *M.charantia's*efficacy in a chronic inflammatory disease like metabolic syndrome is successfully demonstrated in our study with respect to reduction of inflammation and oxidative stress evidenced by a decline in serum NF-êB and LDA along with increased free radical scavenger, SOD. The biological basis of the effects of *M.charantia* by inhibiting the inflammatory pathway in metabolic syndrome both at the levels of ROS generation and transcription of pro-inflammatory marker NF-êB was proven. The histopathological examination findings has imparted that metabolic syndrome is a multi-organ inflammatory disease condition inducing detectable pathological changes in various organs like liver and heart and that *M.charantia* is a potentially effective agent to reverse such significant changes.

REFERENCES

1. Reaven GM, Banting, Role of insulin resistance in human disease. *Diabetes*; **37**:1595-607 (1988).
2. Organization WHO: Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: Diagnosis and classification of diabetes mellitus. Edited by: WHO. *World Health Organization.*1999; 1-28.
3. Wild S.H, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030 - Response to Rathman and Giani. *Diabetes Care*; **27**:2569 (2004).
4. Thaman R.G, Arora, Metabolic Syndrome: definition And Pathophysiology -the Discussion Goes On! *J PhyPharm,Adv*; **3**(13):48-56 (2013).
5. Prasad DS, Kabir Z, Dash AK, Das BC, Prevalence and risk factors for metabolic syndrome in Asian Indians: A community study from urban Eastern India, *J Cardio vasc Dis Res,* **3**(3):204-11 (2012).
6. Diabetes Atlas, second edition, International Diabetes Federation, (2003).
7. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals *Journal of Pharmacology & Pharmacotherapeutics;* **1**(2):87-93 (2010).
8. E L B Novelli, Y S Diniz, C M Galhardi, G M X Ebaid, H G Rodrigues, F Mani *et al*., Anthropometrical parameters and markers of obesity in rats, *Lab Anim,* **41**: 111-19 (2007).
9. Institutional animal care and use committee (IACUC)standard procedure Glucose Monitoring of Blood in Rats and Mice.
10. Prabhat Kumar Nigam, Calculated Low Density Lipoprotein-Cholesterol: Friedewald's Formula versus Other Modified Formulas *International Journal of Life Science and Medical Research.;* **4**(2): 25-31.
11. Kanarek, R. and Orthen-Gambil, N., Differentional effects of sucrose, fructose and glucose on carbohydrate-indused obesity in rats. *JN The Journal of Nutrition,* **112**: 1546-54 (1982).
12. E L B Novelli, Y S Diniz, C M Galhardi, G M X Ebaid, H G Rodrigues , F Mani , A A H Fernandes, A CCicogna J L V B NovelliFilho. Anthropometrical parameters and markers of obesity in rats *Laboratory Animals;* **41**:111-19 (2007).
13. Angela C. Rutledge, KhosrowAdeli, Fructose and the Metabolic Syndrome: Pathophysiology and Molecular Mechanisms. *Nutrition Reviews.*; **65**(6):13-23 (2007).
14. M.A. Hossain, M. Mostofa, D. Debnath, A. K. M. R. Alam, Z. Yasmin,N. F. Moitry,Antihyperglycemic and Antihyperlipidemic of Karala (Momordicacharantia) Fruits in Streptozotocin Induced Diabetic *Rats.J.Environ.Sci.&Natural Resources,* **5**(1):29-37 (2012).
15. Platel, K.; Srinivasan, K. Effect of dietary intake of freeze-dried bitter gourd (Momordicacharantia) in streptozotocin induced diabetic rats. *Nahrung,*

39:262-68 (1997).

16. Day.C, Cartwright.T, Provost.J, Bailey C. J. Hypoglycaemic effects of Momordicacharantia extract. *Planta Med.,* **56**: 426-429 (1990).
17. D Angelo, Ahmed A. Elmarakby, David M. Pollock, David W. Stepp. Fructose Feeding Increases Insulin Resistance but Not Blood Pressure in Sprague-Dawley Rats Gerard, *Hypertension.*; **46**: 806-11 (2005).
18. R.M.N. Bezerra, M. Ueno, M.S. Silva, D.Q. Tavares, C.R.O. Carvalho, M.J.A. Saad, J.A.R. Gontijo.A high-fructose diet induces insulin resistance but not blood pressure changes in normotensive rats. *Braz J Med Biol Res,*; **34**(9): 1155-60 (2001).
19. Zvi Ackerman, MorOron-Herman, Maria GrozovskiTalma Rosenthal, Orit Pappo, Gabriela Link, Ben-Ami Sela. Fructose-Induced Fatty Liver Disease Hepatic Effects of Blood Pressure and Plasma Triglyceride Reduction. *Hypertension*.; **45**:1012-18 (2005).
20. Priyanto AD, Doerksen RJ, Chang CI, Sung WC, Widjanarko SB, Kusnadi J, Lin YC, Wang TC, Hsu JL. Screening, discovery, and characterization of angiotensin-I converting enzyme inhibitory peptides derived from proteolytichydrolysate of bitter melon seed proteins. *J Proteomics*; **128**:424-35 (2015).
21. Suzan A Darwish ,TarekAbd El Aziem , Nagwa M Nour El Din , AblaAbouZied, NihadToussoun. Fructose induced metabolic syndrome in rats, a role for glitazones, fibrates and statins. *Bull. Alex. Fac. Med.*; **42**(4) (2006).
22. Ibraheem Mohammady, SamahElattar, Sanaa Mohammed, MadehaEwais. An Evaluation of Anti-Diabetic and Anti-Lipidemic Properties of Momordicacharantia (Bitter Melon) Fruit Extract in Experimentally Induced Diabetes *Life Science Journal,* **9**(2):363-74 (2012).
23. Chih-hao Lee, Peter Olson, Ronald M Evans. Minireview: Lipid Metabolism, Metabolic Diseases, and Peroxisome Proliferator-Activated Receptors Endocrinology, **144**(6):2201–07 (2003).
24. Renata Juliana da Silva,NathaliaBernardes,Janaina de O. Brito,IrisCalladoSanches,Maria Claudia Irigoyen,Katia De Angelis. Simvastatin-induced cardiac autonomic control improvement in fructose-fed female rats. *Clinics* ; **66**(10):1793-96 (2011).
25. Nawal Al-Rasheed,Nouf Al-Rasheed, Yieldez Bassiouni, Laila Fadaah, Azza M Mohamad, Potential Protective Effects of Nigella Sativa and Allium Sativum Against Fructose-Induced Metabolic Syndrome in Rats. *Journal of Oleo Science.* **63**(8):839-48 (2014).
26. P. V. Nerurkar, Y. K. Lee, M. Motosue, K. Adeli, and V. R. Nerurkar, *Momordicacharantia*(bitter melon) reduces plasma apolipoprotein B-100 and increases hepatic insulin receptor substrate and phosphoinositide-3 kinase interactions, *British Journal of Nutrition,* **100**(4):751-59 (2008).
27. MatsuiS, YamaneT, TakitaT, OishiY,Kobayashi K The Nhypocholesterolemic activity of Momordicacharantia fruit is mediated by the altered cholesterol- and bile acid-regulating gene expression in rat liver. *Nutr Res.;* **33**(7): 580-5 (2013).
28. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med*; **318**: 1315-21 (1988).
29. Zimmermann R, Panzenböck U, Wintersperger A, *et al*. Lipoprotein lipase mediates the uptake of glycated LDL in fibroblasts, endothelial cells, and macrophages. *Diabetes*; **50**: 1643-53 (2001).
30. MeghaSaraswat, Yadagiri Reddy, Muthenna, Bhanuprakash Reddy. Prevention of non-enzymicglycation of proteins by dietary agents: prospects for alleviating diabetic complications. *British Journal of Nutrition.* ; **101**: 1714-21 (2009).
31. D. Sathish Kumar, K. VamshiSharathnath, P. Yogeswaran, A. Harani, K. Sudhakar, P. Sudha, David BanjiA Medicinal potency of momordicacharantia, *International Journal of Pharmaceutical Sciences Review and Research;* **1**(2):95-100 (2010).
32. H.F. Modulation of metabolic and cardiac dysfunctions by swimming in overweight rats on a high cholesterol and fructose diet: Possible role of adiponectin. *Journal of physiology and pharmacology*; **64**(2):231-40 (2013).
33. Stephanie Seneff, Glyn Wainwright, Luca Mascitelli.Is the metabolic syndrome caused by a high fructose, and relatively low fat, low cholesterol diet? *Arch Med Sci.;* **7**(1):8-20 (2011).
34. Chaturvedi P. Role of Momordicacharantia in maintaining the normal levels of lipids and glucose in diabetic rats fed a high-fat and low-carbohydrate diet. *Br J Biomed Sci.;* **62**(3):124-6 (2005).
35. Jose Diego Botezelli, Lucieli Teresa Cambri, Ana Carolina Ghezzi, Rodrigo Augusto Dalia,FabricioAzevedoVoltarelli ,Maria Alice Rostom de Mello.Fructose-rich diet leads to reduced aerobic capacity and to liver injury in rats Lipids in Health and Disease; **11**(78):1-9 (2012).
36. Young-HeePyo,Kyung-WonLee, Preventive Effect of *Monascus*-Fermented Products Enriched with Ubiquinones on Type 2 Diabetic

Rats Induced by a High-Fructose Plus High-Fat Diet. *J Med Food.*; **17**(7): 826–829 (2014).

37. Richard M. Logan, Andrea M. Stringer,Joanne M. Bowen,Rachel J. Gibson,Stephen T. Sonisand Dorothy M.K. Keefe.Serum levels of NFêB and pro-inflammatory cytokines following administration of mucotoxic drugs. *Cancer Biology & Therapy*; **7**(7):1139-45 (2008).
38. Nguyen XuanNhiem, *et al* Inhibition of Nuclear Transcription Factor-jBand Activation of Peroxisome Proliferator-Activated Receptors in HepG2 CellsbyCucurbitane-Type Triterpene Glycosides from Momordicacharantia *J Med Food* **15**(4): 369–377 (2012).
39. Gadang V *et al*,Dietary bitter melon seed increases peroxisome proliferator-activated receptor-ã gene expression in adipose tissue, down-regulates the nuclear factor-êB expression, and alleviates the symptoms associated with metabolic syndrome, *J Med Food.;* **14**(1-2):86-93 (2011).
40. Lii CK *et al*, Suppressive effects of wild bitter gourd (Momordicacharantia Linn. var. abbreviata ser.) fruit extracts on inflammatory responses in RAW264.7 macrophages *J Ethnopharmacol.;* **122**(2):227-33 (2009).

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To Compare the Anti-Inflammatory Effect of Oral Hypoglycemic Drugs in Type 2 Diabetes Mellitus

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Insulin resistance and endothelial dysfunction which shares multiple signaling pathways likehyperinsulinemia, glucotoxicity and inflammation in type 2 Diabetes Mellitus (DM) leads to several micro and macrovascular complications. Studies have shown the anti-inflammatory effects of certain oral hypoglycemic agents which will be helpful in preventing the impact of diabetes related complications. The study aimed to compare the anti-inflammatory effects of Sitagliptin and Acarbose in combination with Metformin and Sulfonylurea in Type2DM patients by using Anti-inflammatory markers Interleukin-6 (IL6), high sensitive C-reactive protein (hsCRP) and also to compare the clinical outcome between these two groups by using the parameters Fasting blood sugar (FBS), Post prandial blood sugar (PPBS), hemoglobin A1c (HbA1C), Plasma Insulin. In this open labeled prospective parallel group clinical study 30 type 2 diabetes patients on Metformin and Sulfonylurea combination, with HbA1c value >/=7.5 were recruited in tertiary care hospital and divided into two groups based on their HbA1C levels and were added on either Acarbose or Sitagliptin along with Metformin Sulfonylurea combinations and were followed for 3 months. Parameters like FBS, PPBS, HbA1c, Plasma Insulin hs CRP, IL-6were measured before and after the study. In the study the mean value of FBS, PPBS, HbA1c, Plasma Insulin, Insulin Resistance, hsCRP were reduced in both Sitagliptin and Acarbose group, which were similar to the results of previous studies except IL6 which got reduced in Sitagliptin group but increased in Acarbose group. The study had showed the synergism of Sitagliptin with Metformin Sulfonylurea combinationin reducing inflammation however; still long term studies are required to confirm their anti-inflammatory effects.

Keywords: Acarbose, Cardiovascular risk, high sensitive C-reactive protein, Interleukin-6, Sitagliptin.

Diabetes mellitus is one of the non communicable diseases which have become a major global health problem[1] This is mainly due to insulin resistance or decreased production of insulin. Insulin resistance, one of the key factor common to obesity and type 2 DM, is associated with endothelial dysfunction and increases the cardiovascular risk[2] by sharing multiple signaling pathways which include hyperinsulinemia, glucotoxicity, lipotoxicity and inflammation[2]

In 2010, it was found that 285 million people around world have diabetes, of which 80% are from developed countries. The prevalence of diabetes is expected to reach about 438 million by 2030[1, 3]

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Type 2 DM is characterized by improper secretion of insulin, resistance to insulin, increased glucose production in liver, and abnormal lipid metabolism. Most studies say that insulin resistance occurs before the insulin secretory defect and type 2 diabetes develops only when insulin secretion become less. In the earlier stages of diabetes, glucose tolerance is found to be normal, because pancreatic beta cell gives a compensatory increase in insulin output which further leads to insulin resistance after a certain period of time leading to a state called Impaired Glucose Tolerance (IGT), where there will be high postprandial glucose level. Later reduction in secretion of Insulin and high hepatic glucose formation leads to overt diabetes with increasing blood glucose values, indicating the failure of beta cells[4]

The obesity in diabetes increases the levels of fat cell products like, nonesterified free fatty acids, retinol-binding protein 4, leptin, Tumor Necrosis Factor (TNF)-á, resistin, adiponectin and free fatty acids (FFA) in circulation. Adipokines modulate the sensitivity of insulin plus increase in FFA production, causes resistance of insulin in hepatic and skeletal muscle. Products of adipocyte ground a state of inflammation and in turn they involve in the pathogenesis of insulin resistance and endothelial dysfunction[5-11]

Diabetes -Inflammation - Cardiovascular risk

The hypotheses propose that hyperglycemia and insulin resistance increases FFA and forms diacylglycerol leading to stimulation of protein kinase C (PKC). PKC modifies the gene transcription of endothelial cells[12] Endothelial dysfunction presents in patients with insulin resistance in stages prior to the development of impaired glucose tolerance (IGT) and type 2 DM. This suggests that insulin resistance is key factor and endothelial dysfunction is not simply a result of hyperglycemia.

Rise in FFA stimulates toll like receptor (TLR) and Nuclear Factor-kB (NF-kB) and subsequently promotes the genes of inflammation like TNF-α and Interleukin-6 (IL-6), which is followed by PKC activation and decreases insulin sensitivity. Impaired Insulin sensitivity in endothelium of the blood vessels promotes Reactive oxygen species (ROS) formation, FFA oxidation, which subsequently stimulates PKC activation leading to advanced glycated end products (AGE)synthesis, and down-regulates Prostacyclin(PGI2). Finally endothelial nitric oxide synthatase(eNOS) activity is impaired leading to endothelial dysfunction[12-14]

Obese diabetic patients treated with Metformin have proven to lower the levels of hsCRP, expression of TNF-á and TLR 2/4[15] Previous studies proved that Sitagliptin apart from stimulating the Adenosine Mono phosphate (AMPK)phosporylation, it also inhibit the activation of Mitogen activated protein kinase (MAPK) including p38 and Extracellular signal regulated kinase(ERK)and arrest the progression of atherosclerosis leading to decreases in adhesion molecules and inflammatory cytokines like hsCRP, IL-6 and FFA levels after 12 weeks of therapy[16-19] It concludes the anti-inflammatory action of Sitagliptin and its effect in inhibition of atherosclerosis[20] The results from the previous studies revealed, that the Acarbose treatment showed hopeful benefits in cardiovascular disease by decreasing the proinflammatory transcription factor (NFê B) activity and hsCRP levels[21-24]

Based on this background information, the study targeted the oral hypoglycemic agents like Acarbose and Sitagliptin to look for their anti-inflammatory action.Hence the study aimed to compare the anti-inflammatory effects of Metformin sulfonylurea and Sitagliptin combination with Metformin Sulfonylurea and Acarbose combination in Type2DM patients by using Anti-inflammatory markers (IL6, hsCRP) and also to compare the clinical outcome between these two groups by using the following parameters like FBS, PPBS, HbA1C, and Plasma Insulin.

MATERIALS AND METHODS

This study was an open labeled prospective parallel group clinical study. After getting approval from the Institute Human Ethics Committee (IHEC) (proposal no: 12/282), 30 type 2 diabetic patients (convenient sample) attending Endocrinology and Medicine OPD in a Tertiary care hospital Coimbatore were recruited. We included all type 2 diabetic patients in an age group of 25-65 years, who were on conventional treatment of Metformin Sulfonylurea combination with HbA1C level $\geq$ 7.5%.Patients with diabetes other than type 2, history of cardiac, renal and

cerebrovascular diseases, patients on treatment with statins and steroids, history of altered liver function and pancreatitis, known history of alcohol intake and smoking were excluded from the study. The details of the study were explained to each participant individually before getting the informed consent. Basic data's like name, age and height, weight were recorded and comorbid conditions were noted.

Then the clinical examination and the base line investigations (Fasting blood glucose, postprandial blood glucose, HbA1C, hsCRP, IL6, Plasma Insulin) were done before starting the treatment. Later those 30 participants were divided into two groups based on their HbA1C values with 15 patients in each group. One group was on Sitagliptin, and the other group was on Acarbose treatment along with their conventional Metformin and Sulphonylurea combination. Sitagliptin was started on 50mg OD and then titrated to 100mg according to the patient's blood glucose level by the physician. In the same way, Acarbose was started on 25mg BD initially and later it was titrated to 50mg BD or 25mg TDS according to the patient's blood glucose level .The study participants were advised to come for review after two weeks for titration of the drug dosage and followed for 3 months. In between the 3 months of study period, the participant's FBS, PPBS levels were noted in the case file.

Finally at the end of three months the clinical examination and the investigations (FBS, PPBS, HbA1C, IL6, hsCRP and Plasma insulin) were done again and the study was completed.

The results of the parameters were analyzed in the hospital laboratory for FBS, PPBS, HbA1c, hsCRP, Plasma Insulin. By using the formula of Homa Index, HOMA-IR = [Fasting glucose (nmol/L) * insulin (μU/mL)/22.5]Insulin Resistance for all the 30 patients were calculated. The Interleukin-6 (Krishgen Biosystems) was estimated in the Pharmacology laboratory by enzyme immunoassay which wasprogrammed for quantification of human interleukin-6 and analyzed.

Statistical analysis

Data was analyzed using SPSS soft ware version 19.0. Values between the two groups were interpreted using independent sample *t*-test and before and after values within the same groups with paired t test.

RESULTS

Among the total number of 30 patients 77% of study population were under 55 years of age and 23% were between 55 to 65 years of age. In that 57% were male and 43% were female patients.

Regarding the duration, 50% were in between 0-5 years , 37% of patients were between 6-10 yrs, and 13% of patients were beyond 11years of duration of DM .This mainly implied the chronicity and progression of disease pattern in those study participants.

Results of BMI in Acarbose and Sitagliptin groups showed no statistical significance even though there was greater reduction in Sitagliptin group in which mean value decreased from 27.02 ± 4.64 SD to 26.32 ± 4.81 SD (p value 0.033) (Table 1). End values of FBS in the study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 183.71 ±44.99 SD to 142.92 ±33.60 SD, 178.07 ± 46.73 SD to 138.84 ±25.13 SD with p value 0.007, 0.020 respectively(Table 1). Regarding PPBS it showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 272.35 ± 64.08 SD to 204.85 ± 57.97 SD , 263.92 ± 101.60 SD to 194.23 ± 44.50 SD with p value 0.006, 0.025 respectively(Table 1).

Results of HbA1c in the study showed statistical reduction in mean value in both Acarbose and Sitagliptin group ranging from 10.01 ± 1.51 SD to 8.17 ± 1.28 SD , 9.69 ±1.53 SD to 7.75 ± 1.32 SD (p value of 0.001, 0.004) respectively(Table 1). Results of Plasma Insulin in the study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 18.71 ± 16.42 SD to 14.18 ± 6.71 SD , 27.72 ±25.98 to 22.58 ± 17.68 SD respectively. This was of no statistical significance because of p value = 0.327, 0.324 respectively (Table 1). End values of IR in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 9.00 ± 8.38 SD to 5.12 ± 3.14 SD , 11.49 ±9.42 SD to 7.63 ± 5.78 SD respectively (p value = 0.100 , 0.101) (Table 1) but no statistical significance.

Results of hsCRP in this study showed reduction in mean value in both Acarbose and Sitagliptin group ranging from 0.58 ± 0.93 SD to 0.30 ± 0.30 SD, 0.45 ± 0.43 SD to 0.41 ± 0.39 SD respectively. This was of no statistical significance (p value = 0.265, 0.671 respectively) (Table 1).

Results of IL-6 in this study showed reduction in mean value of Sitagliptin group ranging from 136.32 ± 241.81 SD to 57.679 ± 78.55 SD (p value =0.314) but in case of Acarbose group there was slight increase in IL-6 levels with the mean value ranging from 120.01 ± 203.3 SD to 174.08 ± 492.1 SD .(p value=0.591) (Table 1).

At the end of three months there was no significant difference seen in the reduction of FBS (p value = 0.725), PPBS (p value = 0.600), HbA1C (p value =0.403), Plasma Insulin (p value =0.110), Insulin Resistance (p value =0.170), hsCRP (p value =0.451) IL-6 (p value = 0.408) on comparing the after values of Acarbose and Sitagliptin group.

DISCUSSION

It is a well known fact that, the risk of cardiovascular disorder is high in both type I and type II Diabetes Mellitus .In case of type I DM risk of CVD arise after some decades but in type II DM, it sets very early in the asymptomatic period of hyperglycemia and even by the time of diagnosis these patients are at potential risk. This is mainly due to the Insulin Resistance, which emerges at an early stage of type II DM. Obesity in diabetes along with inflammation leads to endothelial dysfunction and IR which then subsequently causes platelet aggregation, and all this process collectively leads to atherogenesis and cardiovascular disease ultimately. It has been proven that increase in the inflammatory markers would predict the risk of cardiovascular complications at the earliest.

Previous studies states that drugs like Metformin, Sitagliptin and Acarbose effectively reduces the inflammatory markers TNF-α, IL-6,

Table 1. Mean values of the test parameters

	Week 0 (baseline) mean ± SD	Week 12 mean ± SD	Mean difference	P value
BMI				
Acarbose	25.20±2.84	25.25±2.83	-.056+.79	0.796
Sitagliptin	27.02±4.64	26.32±4.81	0.69±1.04	0.033
FBS				
Acarbose	183.71±44.99	142.92±33.60	40.78±47.75	0.007
Sitagliptin	178.07±46.73	138.84±25.13	39.23±52.50	0.020
PPBS				
Acarbose	272.35±64.08	204.85±57.97	67.50±76.83	0.006
Sitagliptin	263.92±101.60	194.23±44.50	69.69±98.3	0.025
HbA1c				
Acarbose	10.01±1.51	8.17±1.28	1.83±1.67	0.001
Sitagliptin	9.69±1.53	7.75±1.32	1.94±1.99	0.004
Plasma Insulin				
Acarbose	18.71±16.42	14.18±6.71	4.52±16.60	0.327
Sitagliptin	27.72±25.98	22.58±17.68	5.13±17.9	0.324
Insulin Resistance				
Acarbose	9.00±8.38	5.12±3.14	3.88±8.19	0.100
Sitagliptin	11.49±9.42	7.63±5.78	3.86±7.84	0.101
hsCRP				
Acarbose	0.58±0.93	0.30±0.3	0.27±0.89	0.265
Sitagliptin	0.45±0.43	0.41±0.39	0.48±0.40	0.671
IL6				
Acarbose	120.01±203.3	174.08±492.1	-54.07±36.7	0.591
Sitagliptin	136.32±241.81	57.67±78.55	78.64±269.97	0.314

hsCRP and NF-kB levels in diabetes patients and facilitate the prevention of endothelial dysfunction and atherosclerosis[15,20, 24,25]

In this study the results of BMI showed greater reduction in the Sitagliptin group with mean value ranging from 27.02 ±4.64 SD to 26.32 ± 4.81SD, p value = 0.033 compared to the Acarbose group. These were similar to the results of previous studies. [20]

Regarding the mean values of FBS, PPBS, HbA1c, Plasma Insulin, Insulin Resistance, both Sitagliptin and Acarbose combination groups showed good reduction in their mean value, which were again similar to the results of previous studies. [20, 22, 27].

Then mean levels of hsCRP at the starting of study in Acarbose and Sitagliptin groups were found to be 0.58 ±0.93 SD and 0.45 ± 0.43 SD respectively. At the end of the three months levels of hsCRP were reduced in both Acarbose and Sitagliptin groups to the mean value of 0.30±0.3 SD (p value= 0.265) and 0.41 ± 0.39 SD (p value= 0.671) (Table 1) respectively. Though there was greater reduction in mean values of hsCRP

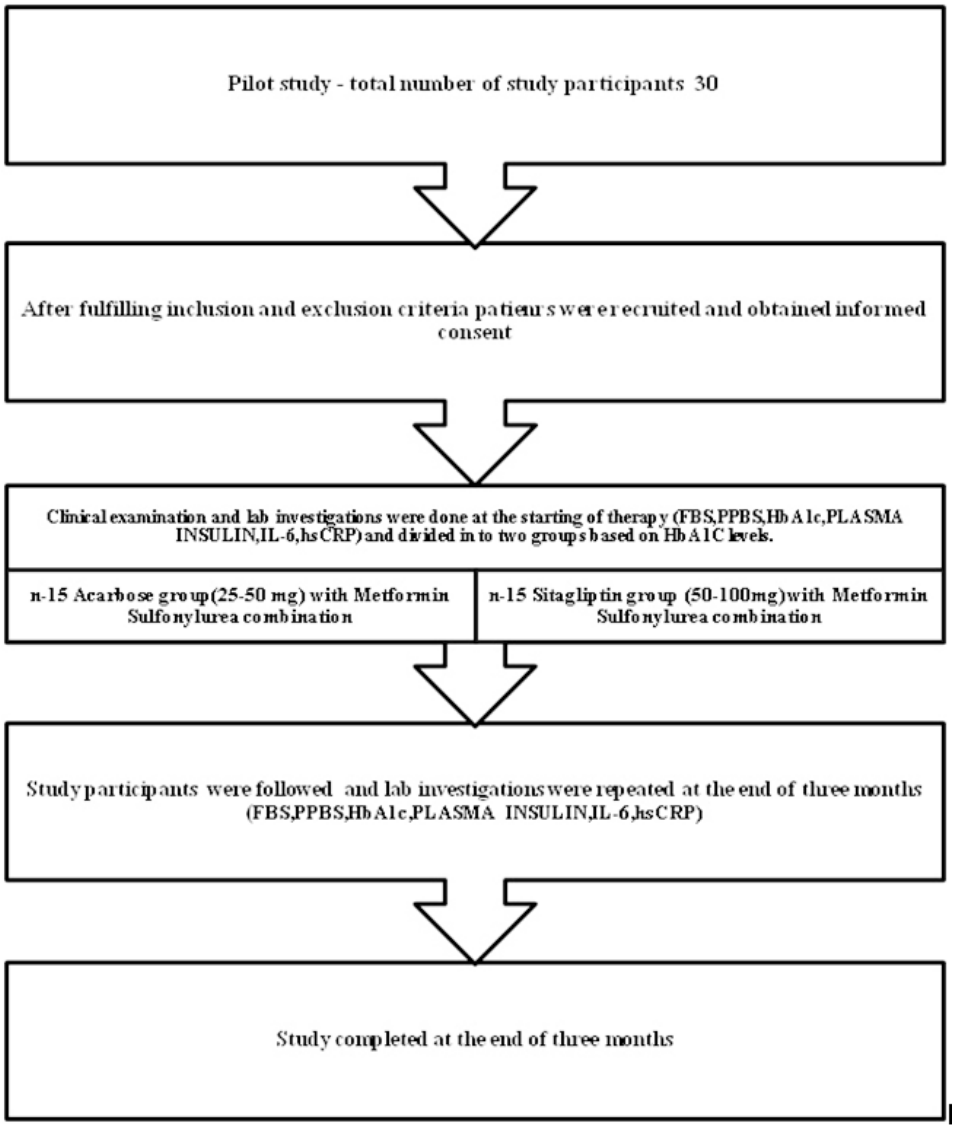


Fig. 1. Methodology of anti-inflammatory effect of oral hypoglycemic drugs

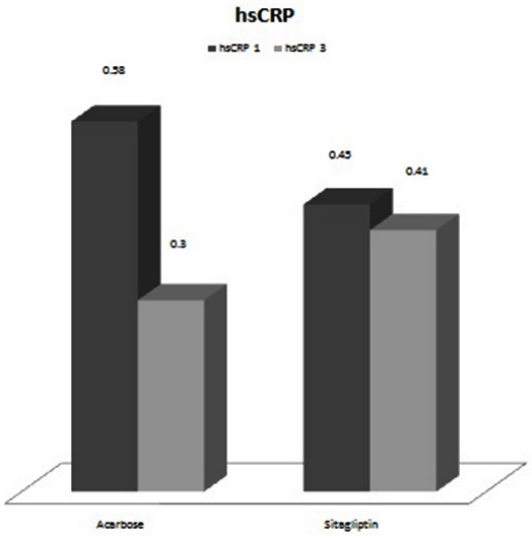


Fig. 2. hcCRP status in Acarbose and Sitagliptin groups at 1 month and at the end of 3 month

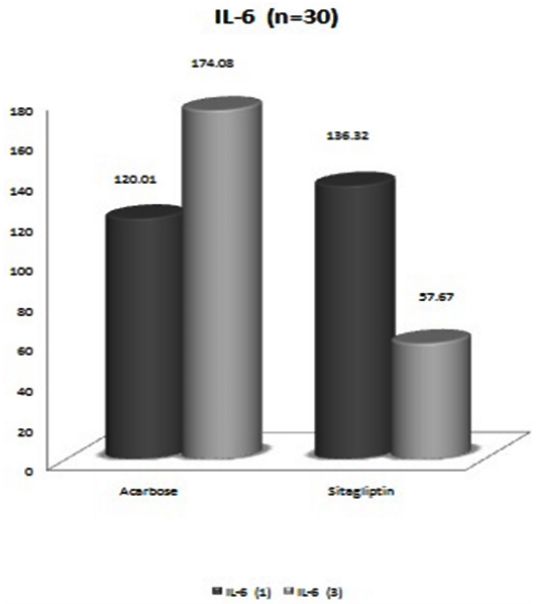


Fig. 3. IL-6 status in Acarbose and Sitagliptin groups at 1 month and at the end of 3 month

levels which were consistent with the results of previous studies [20, 25, 26] these results were not statistically significant. When the values of hsCRP were compared between Acarbose and Sitagliptin groups, at the end of the three months there were no statistical significant reduction in hsCRP values (p value=0.451).

Next inflammatory marker analyzed was the IL-6 levels. The mean values in the starting of treatment were 120.01 $\pm$ 203.3 SD in Acarbose group and 136.32 $\pm$ 241.81 SD in Sitagliptin group. At the end of the three months the mean values of IL-6 in Acarbose group was found to be increased to the mean value of 174.08 $\pm$ 492.1 SD but in Sitagliptin group it was found to be reduced to 57.69 $\pm$ 78.55 SD.When the before and after values of Sitagliptin group was analyzed, it showed reduction in the mean values of IL-6 levels at the end of the three months. But there was no statistical significance (p value =0.314) (Table 1).

When comparing the after values of IL-6 in Acarbose and Sitagliptin groups, greater reduction was seen in the Sitagliptin group after 3 months of therapy. Thus the findings in the study were consistent with the previous studies which showed the anti- inflammatory effect of Sitagliptin therapy. [20,21] But this is again not statistically significant (p value = 0.408).

Concerning the results of the anti-inflammatory markers in Sitagliptin treated group with metformin sulphonylureas combination showed a definite reduction in the mean values of both hsCRP, IL-6 levels after the three months treatment, but still it was not statistically significant. This may be due to less sample size, and the patients were followed only for 3 months. A long term follow up and a bigger sample size ismandatory to get a statistically significant results.Sitagliptin has proven to have a definite role in reducing chronic inflammation. Based on this study finding, fixed dose combination of Sitagliptin and Metformin might be helpful in preventing the progress of diabetes.

When the results of inflammatory markers in Acarbose group were analyzed and compared with previous studies,[22-24] Acarbose also reduced the inflammatory markers moderately in this study. This study showed a greater reduction in the mean value of hsCRP levels but not in IL-6, this might be because of small sample size, poor compliance of patients and the patients were followed only for 3 months. Hence long term follow up and large sample size may be required to see the reduction in the inflammatory markers and the role of these drugs in the insulin resistant states. During the

study period no adverse reactions were noted among the study participants.

CONCLUSION

This study demonstrated the additive anti-inflammatory effect of Sitagliptin, and Acarbose as these groups of drugs are now in trend and commonly been prescribed either as base line therapy or as add on therapy based on the HbA1C values. When such anti inflammatory property is pronounced with Sitagliptin, it can be sure that addition of Sitagliptin with Metformin will be a beneficial combination at the initial therapy to the patients in preventing the progression of diabetic related cardio vascular complications[26]

Limitations

In this study the patient sample size was small and the study duration was 3 months which is again one of the limitations to assess the anti-inflammatory effects of these drugs on long term effects. Hence long term follow up and bigger sample size may be mandatory in future to get the statistically significant reduction in the inflammatory markers.

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REFERENCES

1. Roglic G, Editor. World Diabetes Foundation summit for Latin America.2010 Estimates of the global and regional burden of diabetes [Internet]. World Health Organization,Salvador (Brazil) [cited 2014 Sep 15].Available from: https://www.worlddiabetesfoundation.org/sites/default/files/WDF_Brazil_Summit_Report_2010.pdf
2. Del Turco S, Gaggini M, Daniele G, Basta G, Folli F, Sicari R, Gastaldelli A. Insulin resistance and endothelial dysfunction: a mutual relationship in cardiometabolic risk. *Current pharmaceutical design.*; **19**(13):2420-31 (2013).
3. Unwin N, Whiting D, Gan D, Jacqmain O, Ghyoot G, editors. IDF diabetes atlas. International Diabetes Federation; 2009.
4. Fauci AS, Braunwald E, editors. Harrison's Principles of Internal Medicine. 17th ed. New York: McGraw-Hill2008; p. 2275
5. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.;* **259**(5091):87-91 (1993).
6. Maachi M, Pieroni L, Bruckert E, Jardel C, Fellahi S, Hainque B, Capeau J, Bastard JP. Systemic low-grade inflammation is related to circulating and adipose tissue TNFá, leptin and IL-6 levels in obese women. *International journal of obesity.;* **28**(8):993-7 (2004).
7. Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Silvennoinen O. Signaling through the hematopoietic cytokine receptors. *Annual review of immunology.*; **13**(1):369-98 (1995).
8. Mooney RA, Senn J, Cameron S, Inamdar N, Boivin LM, Shang Y, Furlanetto RW. Suppressors of cytokine signaling-1 and-6 associate with and inhibit the insulin receptor A potential mechanism for cytokine-mediated insulin resistance. *Journal of Biological Chemistry.;* **276**(28):25889-93 (2001).
9. Rieusset J, Bouzakri K, Chevillotte E, Ricard N, Jacquet D, Bastard JP, Laville M, Vidal H. Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes.;* **53**(9):2232-41 (2004).
10. Kroder G, Bossenmaier B, Kellerer M, Capp E, Stoyanov B, Mühlhöfer A, Berti L, Horikoshi H, Ullrich A, Häring H. Tumor necrosis factor-alpha-and hyperglycemia-induced insulin resistance. Evidence for different mechanisms and different effects on insulin signaling. *Journal of Clinical Investigation.*; **97**(6):1471 (1996).
11. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link?. *Atherosclerosis.;* **148**(2):209-14 (2000).
12. Du X, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M. Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. *Journal of Clinical Investigation.*; **116**(4):1071 (2006).
13. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *European heart journal.*; **34**(31):2436-43 (2013).
14. Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl

RA, Warnholtz A, Meinertz T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circulation research.*; **88**(2):e14-22 (2001).

15. Andrews M, Soto N, Arredondo M. Effect of metformin on the expression of tumor necrosis factor-á, Toll like receptors 2/4 and C reactive protein in obese type-2 diabetic patients. *Revistamedica de Chile.;* **140**(11):1377-82 (2012).
16. Choi MK, Jin QR, Ahn SH, Bae MA, Song IS. Sitagliptin attenuates metformin-mediated AMPK phosphorylation through inhibition of organic cation transporters. *Xenobiotica.;* **40**(12):817-25 (2010).
17. Lenski M, Kazakov A, Marx N, Böhm M, Laufs U. Effects of DPP-4 inhibition on cardiac metabolism and function in mice. *Journal of molecular and cellular cardiology.;* **51**(6):906-18 (2011).
18. Ikeda T, Kumagai E, Iwata S, Yamakawa A. Soluble CD26/dipeptidyl peptidase IV enhances the transcription of IL-6 and TNF-á in THP-1 cells and monocytes. *PLoS One.* **8**(6):e66520 (2013).
19. Erdogdu Ö, Eriksson L, Xu H, Sjöholm Å, Zhang Q, Nyström T. Exendin-4 protects endothelial cells from lipoapoptosis by PKA, PI3K, eNOS, p38 MAPK, and JNK pathways. *Journal of molecular endocrinology.*; **50**(2):229-41 (2013).
20. Makdissi A, Ghanim H,Vora M,*et al*.Sitagliptin Exerts an Ant inflammatory Action**.** *J Clin Endocrinol Metab.*; **97**(9):3333–41 (2012).
21. Hanefeld M, Cagatay M, Petrowitsch T, Neuser D, Petzinna D, Rupp M. Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta-analysis of seven long-term studies. *European heart journal.;* **25**(1):10-6 (2004).
22. Rudofsky Jr G, Reismann P, Schiekofer S, Petrov D, Von Eynatten M, Humpert PM, Isermann B, Müller-Hoff C, Thai TP, Lichtenstein S, Bärtsch U. Reduction of postprandial hyperglycemia in patients with type 2 diabetes reduces NF-êB activation in PBMCs. *Hormone and metabolic research.* **36**(09):630-8 (2004).
23. Tschoepe D. Decreased fibrinogen by treatment with the alpha-glucosidase inhibitor acarbose. *Diabetes.*; 53:A189 (2004).
24. Wang X, Lu J, Pan C. Comparison of serum C-reactive protein level in different glucose tolerance subjects and the change in serum CRP level in IGT subjects with acarbose [abstract 1634]. *Chin J EndocrinolMetab.;* **19**: 254-6 (2003).
25. Rudofsky Jr G, Reismann P, Schiekofer S, Petrov D, Von Eynatten M, Humpert PM, Isermann B, Müller-Hoff C, Thai TP, Lichtenstein S, Bärtsch U. Reduction of postprandial hyperglycemia in patients with type 2 diabetes reduces NF-êB activation in PBMCs. *Hormone and metabolic research.;* **36**(09):630-8 (2004).
26. Reasner C, Olansky L, Seck TL, Williams Herman DE, Chen M, Terranella L, Johnson Levonas AO, Kaufman KD, Goldstein BJ. The effect of initial therapy with the fixed dose combination of sitagliptin and metformin compared with metformin monotherapy in patients with type 2 diabetes mellitus. *Diabetes, Obesity and Metabolism.*; **13**(7):644-52 (2011).

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ROLE OF FRESH JUICE AND DRY POWDER PREPARATIONS OF *BENINCASA HISPIDA* AND *LAGENARIA SICERARIA* FRUITS IN INHIBITING CELLULAR STRESS BY MEASURING DPPH ASSAY

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ABSTRACT

Introduction: During the past few decades, the drugs from natural products have gained importance in the field of medicine. Many plants and their products exhibit marked pharmacological activities. Benincasa Hispida and Lagenaria siceraria fruits are one of those important natural plants which are used in the treatment of various disorders. The present study was designed to analyze the antioxidant property of fresh juice and dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* fruits in inhibiting cellular stress by chemical testing and DPPH assay. **Materials and Methods:** Fresh fruits were obtained from the local market and washed thoroughly in the running water. Then the dry powder and fresh juices of *Benincasa hispida* and *Lagenaria siceraria* were made by using hot air oven at 60 degree Celsius made and used for DPPH assay and chemical testing of Phenolic and Flavonoid compounds. **Results:** Only dry powder of *Benincasa hispida* fruit showed positive for both phenolic and flavonoid compounds. In *Lagenaria siceraria*, both dry powder and fresh juice revealed positive only for flavonoid compounds. In DPPH assay, dry powder preparartions of *Benincasa hispida* and *Lagenaria siceraria* proved better free radical scavenging activity compared to their fresh juice forms. **Conclusion:** Enhanced antioxidant property of *Benincasa hispida* and *Lagenaria siceraria* dry powders possess good therapeutic potential and can be useful as an effective antioxidant in clinical use of various diseases.

KEYWORDS: Antioxidant property, Dry powder, Fresh juice, *Benincasa Hispida*, *Lagenaria Siceraria*.

INTRODUCTION

The fruit of *Benincasa hispida*, commonly called as ash guard, belonging to cucurbitaceae family. Scientific studies disclose its anti-inflammatory, diuretic, anti-Alzheimer, antidiarrheal, antiulcer, anorectic, antihistaminic activities and its antioxidant activity.[1,2] Several studies have shown the antioxidant property of Benicia *hispida* fruit in various forms of its extraction (ethanol, methanol).[3]

Lageneria siceraria commonly called as Bottle guard is a rich source of vitamin B & C and used by the cardiac patients to prevent atherosclerosis. It is an edible fruit and has got many medicinal values. Many studies in the past have shown the antioxidant property of *Lagenaria siceraria* in different forms of its extraction (ethanol, Acetone).[4,5] No studies have analyzed the antioxidant property of fresh juice and dry powder preparations of *Benincasa Hispida and Lagenaria Siceraria* fruits.

Since these fruit are edible, easily available, and have got lot of medicinal values, it becomes essential to see their antioxidant property in fresh juice and dry powder forms.

AIM

To analyze the property of fresh juice and dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* fruits in inhibiting cellular stress by chemical testing and DPPH assay.

MATERIALS AND METHOD

The fresh fruits were obtained from the local market and washed thoroughly in the running water. Then under sterile condition seeds were removed and the pulp of the fruits were cut into pieces and weighed. After weighing, both the fruit pieces were grinded separately and filtered using sterile cloth to extract juice from both the fruits. From this half quantity of the juices were used for dry powder preparations and the remaining were stored for chemical test analysis in the refrigerator. For dry powder preparation, both the juices were measured and filled in

the separate bowls and kept in hot air oven at 60 degree Celsius under sterile condition till all the water content of the juices get evaporated and the fine particles get settled. Now the settled powder particles were been dried, collected and weighed again. Finally, the dry powder and the fresh juice of both the fruits were used for the chemical testing and DPPH assay.

Chemical Testing[6]

Detection of phenolic compounds

a. Ferric chloride test: Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were added to 5% FeCl3 reagent. Formation of deep blue colour was not appeared in juice and dry powder preparations of both fruits.

b. Lead acetate test: Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were added to 10% lead acetate solution and mixed. Formation of white precipitate was present in dry powder preparation but not in the juice of *Benincasa hispida*. Whereas, in *Lagenaria siceraria* white precipitate was not present in both dry powder and juice forms. Thus the test was positive only for the dry powder preparation of *Benincasa hispida.*

Detection of flavonoids

a. Aqueous sodium hydroxide test: The Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were treated along with aqueous NaOH solution. Formation of yellow-orange colour was not found in juice but present in dry powder preparation of *Benincasa hispida* and the test was found to be positive in both juice and the dry powder preparation of *Lagenaria siceraria.*

b. H2SO4 test: The Juice and the dry powder forms of *Benincasa hispida* and *Lagenaria siceraria* were added to concentrate H2SO4 and observed for the formation of orange colour. Only the dry powder preparation of *Benincasa hispida* was found to be positive for the presence of flavanoids and not in the fresh juice form. In *Lagenaria siceraria,* the test was positive in both juice and the dry powder preparation.

Table No. 1: Results of the chemical testing.

		Phenolic compound		Flavonoids	
Sr.No	**Test substances**	**Ferric chloride test**	**Lead acetate test**	**Aqueous sodium hydroxide test**	**Sulphuric acid test**
1	*Benincasa hispida* Fresh juice	-	-	-	-
2	*Benincasa hispida* Dry powder	-	+	+	+
3	*Lagenaria siceraria* Fresh juice	-	-	+	+
4	*Lagenaria siceraria* Dry powder	-	-	+	+

DPPH Assay

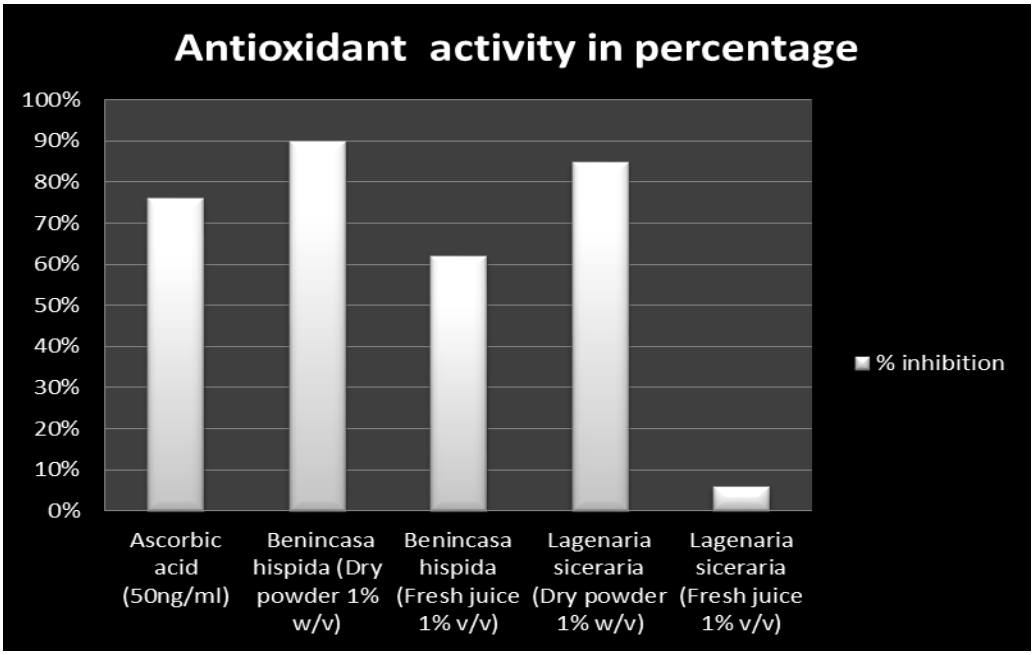
The percentage of antioxidant activity (AA %) of fresh juice and dry powder preparation of *Benincasa hispida* and *Lagenaria siceraria* were assessed by DPPH free radical assay. The dry powder and the fresh juice of *Benincasa hispida* and *Lagenaria siceraria* were prepared in 1% w/v and 1% v/v solutions. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of samples (Dry powder and Fresh juice), 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV VIS spectrophotometer. The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Reference standard compound being used was ascorbic acid 50 ng/ml. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.[7,9] Percentage inhibition was calculated by,

DPPH scavenging effect (%) = A0 - A 1 / A0 × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample. (Control =0.856).

Table No. 2: Absorbance and % inhibition of DPPH assay.

Samples	Absorbance	% inhibition
Ascorbic acid (50ng/ml)	0.206	76%
Benincasa hispida (Dry powder 1% w/v)	0.088	90%
Benincasa hispida (Fresh juice 1% v/v)	0.322	62%
Lagenaria siceraria (Dry powder 1% w/v)	0.131	85%
Lagenaria siceraria (Fresh juice 1% v/v)	0.808	6%



"Fig. 1: Antioxidant activity of fresh juice and dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* in percentage."

DISCUSSION

In this study, the dry powder of *Benincasa hispida* showed positive for both flavonoids and phenolic compounds by the chemical testing. These results were similar to the studies which showed the presence of flavonoids and phenolic compounds in the methanolic fruit extract of *Benincasa hispida*.[10] In DPPH assay, dry powder of *Benincasa hispida* revealed 90% inhibition of free radical scavenging activity compared to its fresh juice preparation which was about 62%. In the previous DPPH assay studies of *Benincasa hispida* proved that the maximum antioxidant activity was found to be in the methanolic extract compared to the alcoholic extract.[1,3]

In this study, both the dry powder and the fresh juice of *Lageneria siceraria* showed positive for flavonoids and negative for phenolic compounds by the chemical testing. These results were similar to the studies which showed the presence of flavonoids in the ethanolic fruit extract of *Legenaria siceraria*.[11] In DPPH assay, dry powder of *Lageneria siceraria* showed 85% inhibition of free radical scavenging activity compared to its fresh juice preparation which was about 6%. In the previous DPPH assay studies of *Lageneria Siceraria* showed that the maximum antioxidant activity was found to be in the acetone extract compared to the alcoholic extract.[4,5]

CONCLUSION

In this study, both the chemical testing and DPPH assay showed maximum antioxidant activity in the dry powder preparations of *Benincasa hispida* and *Lageneria siceraria* compared to their fresh juice preparations. The result of enhanced antioxidant property of *Benincasa hispida* and *Lagenaria siceraria* dry powder possess good therapeutic potential and can be clinically useful as an effective antioxidant in various disease conditions after appropriate preclinical studies and clinical trials.

REFERENCES

1. Mandana B, Russly A. R, et al. Antioxidant activity of winter melon (*BenincasaHispida)* seeds using conventional soxhlet extraction technique. International Food Research Journal, 2012; 19(1): 229-34.
2. Abdullah N, KamarudinW, et al. Study on Antioxidant Capacity and Phenolic Content of Various Parts of Wax Gourd (*Benincasa hispida*). World Applied Sciences Journal, 2012; 19(7): 1051-56.
3. Badhani S, Kainth A, et al. Evaluation of Antioxidant Activity of *Benincasa hispida* Fruit Extracts. Am. J. Pharm Tech Res., 2013; 3(2): 336-45.
4. Deshpande JR, Mishra MR, Meghre VS. Free radical scavenging activity of Lageneria siceraria (Mol.) Standl. fruit. Natural Product Radiance., 2007; 6(2): 127-30.
5. Sharma N, Yadav P, et al. In vitro antioxidant activity of *lagenaria siceraria* leaves. Malaysian journal of pharmaceutical sciences, 2013; 11: 1–11.
6. Elangovan M, Dhanarajan M, Rajalakshmi A, et al. Analysis of Phytochemicals, Antibacterial and Antioxidant activities of Moringa oleifera Lam. Leaf extract- an in vitro study. Int. J. Drug Dev. & Res., 2014; 6(4): 173-80.
7. Patel, R.M, Patel, N.J. In vitro antioxidant activity of coumarin compounds by DPPH, superoxide and nitric oxide free radical scavenging methods. Journal of advanced pharmacy education & research, 2011; 1: 52-68.

8. 17Koleva, I.I, Beek, T.A.V, et al, Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods. Phytochemical analysis, 2002; 13: 8-17.
9. Garcia E J, Oldoni T L C, Alencar S M, et al. Antioxidant Activity by DPPH Assay of Potential Solutions to be applied on Bleached Teeth. Braz Dent J., 2012; 23(1): 22-7.
10. Sheemole M.S, Antony V T, Kala K, Saji A. Phytochemical Analysis of Benincasa hispida(Thunb.) Cogn. Fruit Using LC-MS Technique. Int. J. Pharm. Sci. Rev. Res., 2016; 36(1): 244-48.
11. Mutalib LY, Nuraddin SM, Aka ST. Phytochemical screening, antibacterial and antibiofilm evaluation of Lagenaria siceraria fruit growing in Kurdistan Region\Iraq. Journal of Pharmacognosy and Phytochemistry, 2015; 4(1).

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A study comparing cognitive function assessment in type-2 diabetes mellitus using Rowland Universal Dementia Assessment Scale and Mini Mental State Examination

S Shanmugapriya, N Dhandapani[1], T Saravanan[1]

Abstract:

BACKGROUND: Diabetes mellitus is a metabolic disorder which can predispose to cognitive impairment. It is important to screen diabetic patients for cognitive dysfunction in the routine clinical management. Our study aimed at comparative evaluation of commonly used cognition assessment tools such as Mini Mental State Examination (MMSE) and Rowland Universal Dementia Assessment Scale (RUDAS) in diabetes patients.

METHODS: The study was conducted as a prospective case–control design in a tertiary care teaching hospital from March to October 2015. After obtaining written informed consent, the data on demographic details, duration of diabetes, and glycemic control marked by glycated hemoglobin were recorded. A total of 101 previously diagnosed type-2 diabetes patients and an equal number of age-, gender-, and literacy-matched controls without diabetes were administered the MMSE and RUDAS cognition scales in a one-to-one interview by a trained diabetic educator who was blinded to the groups.

RESULTS: The study revealed that there was a significant difference in cognition scores between the diabetic patients with short duration (<5 years) and those with a longer history of diabetes (≥5 years) only in the MMSE scale using independent sample *t*-test ($P = 0.018$), unlike the RUDAS in which the difference was insignificant ($P = 0.235$). Similarly, there was also a significant correlation between glycemic control and cognition scores in the MMSE ($P = 0.03$), but not in the RUDAS scale ($P = 0.083$).

CONCLUSION: MMSE scale has been proved to be advantageous over the RUDAS as a screening tool for cognition assessment in diabetes patients.

Keywords:

Cognition score, diabetes duration, glycemic control, screening

Introduction

Diabetes mellitus is a metabolic disorder due to dysregulation of glucose homeostasis. The world prevalence of diabetes in adults has been predicted to increase from 285 million in 2010 to 439 million by 2030.[1] It is well established that diabetes is associated with significant morbidity, including neurological disability. Although it is known that diabetes can lead to cognitive deficits in the long term, elucidation of the effects on the central nervous system is still incomplete, unlike other complications such as nephropathy, retinopathy, and peripheral neuropathy. It is estimated that in 2010, 35.6 million people lived with dementia worldwide, with numbers expected to almost double every

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20 years, to 65.7 million by 2030 and 115.4 million by 2050.[2] In addition to this, the scenario of type-2 diabetes predisposing to and accelerating cognitive impairment would further increase the burden of disease. With increasing prevalence of type-2 diabetes in India, the magnitude of cognitive impairment that the disease contributes to and the factors affecting should be studied.

Studies from various populations have consistently shown that diabetes is associated with cognitive deficits and dementia; both in elderly and younger patients.[3] Various pathophysiological mechanisms have been proposed. A process similar to that of peripheral neuropathy such as alterations in Na^+K^+ATPase activity and the ensuing reduction in myoinositol and sorbitol metabolism has been suggested. These may trigger biochemical abnormalities at the neuronal level, resulting in structural and functional changes, primarily in the white matter.[4] Chronic hyperglycemia, atherosclerosis, and hemodynamic changes may lead to microinfarcts which in turn to cognitive impairment. Diabetes-induced microvascular changes, such as disruption of the blood–brain barrier, and the resultant derangement of neurovascular coupling could be partly related to cognitive dysfunction.[5-7] Hyperglycemia may also be directly toxic to the neuron, leading to its degeneration[8] and consequentially the hippocampal as well as global atrophy.[5] Thus, diabetes is considered as one of the important contributory factors in the development of cognitive deficits.

Undiagnosed cognitive impairment can lead to reduced health status,[9] implying that screening for cognitive deficits should be an integral part of patient management in diabetes. Among the neuropsychological tests for dementia, the Mini Mental State Examination (MMSE), devised by Folstein *et al*., is one of the most widely used tools for the assessment of cognitive impairment.[10] The Rowland Universal Dementia Assessment Scale (RUDAS) is yet another screening test, designed as a multicultural cognitive assessment scale relatively unaffected by gender, education, and first language, as it can easily be converted for implementation in the local community.[11] Although there are studies comparing the accuracy and reliability of these two scales in geriatric population with and without dementia,[12-15] a thorough literature review reveals a paucity of studies directly comparing the two in a diabetic population. Hence, our study aimed at evaluating the effectiveness of the two scales as tools in assessing the cognitive decline in type-2 diabetes patients by comparing the correlation of cognitive score with the glycemic control and duration of diabetes.

Methods

This was a single-center, hospital-based case–control study conducted by collecting data from study participants from March to October 2015. Approval from the Institutional Human Ethics Committee was obtained before the start of the study.

Inclusion criteria

All patients willing to give consent, both males and females aged between 30 and 60 years with previously diagnosed type-2 diabetes mellitus (case) and without diabetes mellitus (control), with or without hypertension were included for the study.

Exclusion criteria

Patients with type-1 diabetes, stroke, alcohol intake or smoking habits, total blindness in both eyes, or complete loss of hearing in both ears; and patients who were previously diagnosed with and were known case of mental retardation, psychiatric disorders, and psychoactive drug use in addition to pregnant and lactating women were excluded.

The sample size calculation was done using "Epi tools," with a prevalence of 7% which was based on the previous data, and desired precision of 0.05 at 95% confidence interval revealed the sample size estimate of 101.

After obtaining written informed consent, the data on age, sex, literacy, current fasting and postprandial blood glucose (done by enzymatic reference method with hexokinase), and glycated hemoglobin (HbA1c) values (done by immunoturbidimetry), along with history on duration of diabetes and duration of hypertension were collected. The study was conducted on a sample of 101 diabetic cases and 101 non-diabetic controls matched for age, sex, and literacy to ensure homogeneity of the sample and to avoid bias in the results, owing to these parameters. In this study, patients who exhibited the ability to both read and write the colloquial language and/or English were included as literates.

MMSE and RUDAS scales were administered in a one-to-one interview with the study participant by a trained diabetic educator who was blinded to the group to which the patient belonged to. MMSE tested global cognition function with items assessing orientation, word recall, registration, attention, language abilities, and visuospatial ability and was scored out of 30. As per the MMSE scale, 21–23, 11–20, and ≤10 scores were indicative of mild, moderate, and severe cognitive dysfunction, respectively, while a score of 24 or higher was considered normal.[10] According to the recommendations of Crum *et al*., the MMSE scores were corrected for the literacy status. Based on these criteria, a score <24 was considered as impaired cognition for literates and that <22 for illiterates.[16] RUDAS scale measured memory, gnosis, praxis, visuospatial skills, judgment, and language. The maximum RUDAS score

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was 30, for which the recommended cut point ≤22 was used to indicate cognitive impairment.[11]

Statistical analyses was done using Pearson's correlation test to analyze the correlation between mean MMSE/RUDAS score with parameters such as duration of diabetes and glycemic control. Student's *t*-test was used to identify significant difference in the mean scores between cases and controls based on age, sex, literacy, and comorbidity.

Results

The data were entered into Excel and analyzed using SPSS version 19. Among 101 cases, 4% had mild and 1% had moderate cognitive dysfunction using MMSE, whereas cognition deficit was identified in 5% cases using RUDAS. 1% controls, according to RUDAS score, had cognitive impairment while MMSE scoring revealed that 3% controls had cognitive dysfunction which was mild.

The baseline characteristics of age, sex, and literacy in both groups were matched [Table 1]. There were a higher proportion of females in both the case and the control group, with a male to female ratio of 36:65. Percentage of literates was high (94%) in both groups. The cases were arbitrarily categorized based on the observed HbA1c value as those with good control and poor control. An HbA1c value of 8.5% was taken as the cutoff value; those with <8.5% were included in the good control group and those with HbA1c values ≥8.5% were grouped as poor controllers of glycemic level. 44.6% of cases had good control, while 54.4% were included in the poor control group. The mean score was found to be less in patients who had HbA1c level ≥8.5% using both the scales. An independent sample *t*-test showed that the mean difference between the scores of patients with good and poor control was statistically significant in MMSE scale ($P = 0.046$), while in RUDAS scale, the mean difference was not statistically significant ($P = 0.372$) [Figure 1].

Cases in our study population were divided into those with duration of diabetes <5 years and those with ≥5 years history of diabetes. Accordingly, it was found that 60.4% cases had <5 years of diabetes history and the rest had ≥5 years duration. The mean scores were found to be less in people who had diabetes ≥5 years using both scales and was statistically significant in MMSE scale ($P = 0.018$) but not in RUDAS scale ($P = 0.235$) [Figure 2]. Furthermore, using Pearson's correlation test, a statistically significant negative correlation between duration of diabetes and cognitive levels ($P = 0.015$) in addition to a significant positive correlation between glycemic control and cognitive score ($P = 0.03$) was detected in testing with MMSE scale in the diabetes patients. In contrast, no significant correlation between duration of diabetes or glycemic control with cognitive dysfunction was evident while using RUDAS scale [Table 2].

In both MMSE and RUDAS scales, an independent sample *t*-test revealed that there was no statistically significant difference between the cases and controls in both categories of <50 years of age and those with ≥50 years. The difference in cognition level between the cases and controls in the two gender groups was similarly tested using independent sample *t*-tests, and it was found that there was no statistically significant difference in the mean scores based on gender using either of the scales [Table 3]. Another significant observation was that the mean score of females, in both cases and controls, were lower than that of males, even though the difference was not statistically significant, except in the diabetic group assessed using RUDAS scale, wherein the mean score

Table 1: Baseline characters of study population

Parameters	Mean±SD	
	Cases, 101 (diabetic)	Controls, 101 (non-diabetic)
Age	49.75±7.1	49.57±7.0
Sex (%)		
Male	36 (35.6)	36 (35.6)
Female	65 (64.4)	65 (64.4)
Literacy (%)		
Literate	6 (5.9)	6 (5.9)
Illiterate	95 (94.1)	95 (94.1)
MMSE	28.09±2.3	28.01±2.13
RUDAS	27.80±2.4	28.11±1.65
Duration of diabetes	5.67±5.38	-
HBA1c	9.36±2.34	-

SD = Standard deviation, MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, HBA1c = Hemoglobin A1c

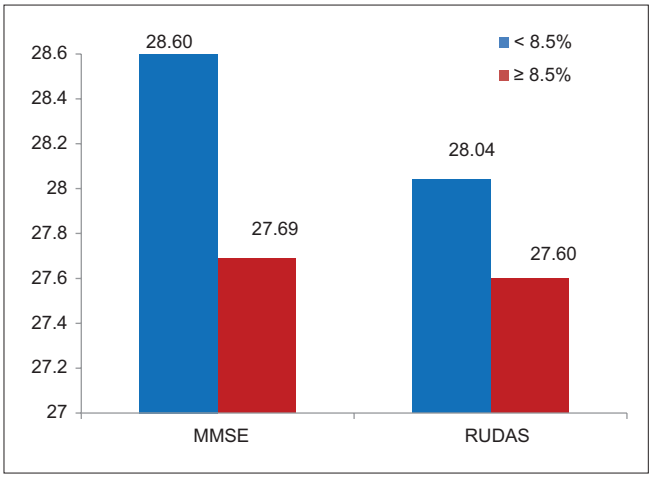


Figure 1: Association between mean cognition score expressed as mean ± standard deviation and glycated hemoglobin (glycemic control) using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

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of females was significantly higher than that of males [Figure 3].

It was found using Student's *t*-test that the illiterates had a mean score lower than that of the literates, though a statistically significant difference was observed only with the MMSE scale (P = 0.001) but not in the RUDAS scale (P = 0.085). Ninety-five percent of the study population were literates, and this precluded performing a between-group analysis for cases and controls [Figure 4].

An independent sample *t*-test revealed that there was no statistically significant difference between the mean scores of cases and controls with hypertension. Similarly, there was no statistically significant difference between the mean scores of those with diabetes alone and those with diabetes and hypertension in the case group using both MMSE and RUDAS [Table 4], indicating that coexisting hypertension did not significantly affect cognition score in patients with type-2 diabetes.

Table 2: Correlation of duration of diabetes and glycemic control with cognitive levels using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

Scale	Parameter	Correlation value	*P*
MMSE	Duration of diabetes	0.240	0.015
	HbA1c	−0.216	0.030
RUDAS	Duration of diabetes	0.163	0.103
	HbA1c	−0.173	0.083

MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, HBA1c = Hemoglobin A1c

Table 3: Independent sample *t*-test for comparing the mean scores of cases and controls across age and gender groups using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

Scale	Parameter	Study population	Mean±SD	*t*	*P*
MMSE	<50 years	Cases	28.23±2.162	0.291	0.772
		Controls	28.10±2.141		
	≥50 years	Cases	27.98±2.483	0.082	0.935
		Controls	27.94±2.138		
RUDAS	<50 years	Cases	27.76±2.496	0.065	0.073
		Controls	28.54±1.530		
	≥50 years	Cases	27.83±2.339	0.187	0.852
		Controls	27.76±1.677		
MMSE	Male	Cases	28.11±2.148	−0.634	0.528
		Controls	28.39±1.517		
	Females	Cases	28.09±2.447	0.653	0.515
		Controls	27.82±2.391		
RUDAS	Male	Cases	27.08±2.970	1.300	0.197
		Controls	28.36±1.533		
	Females	Cases	28.20±1.930	−1.594	0.250
		Controls	27.98±1.709		

MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, SD = Standard deviation

Discussion

The MMSE is a cognitive scale used for detecting cognitive impairment. Having developed in the West, it has serious limitations due to cultural and educational

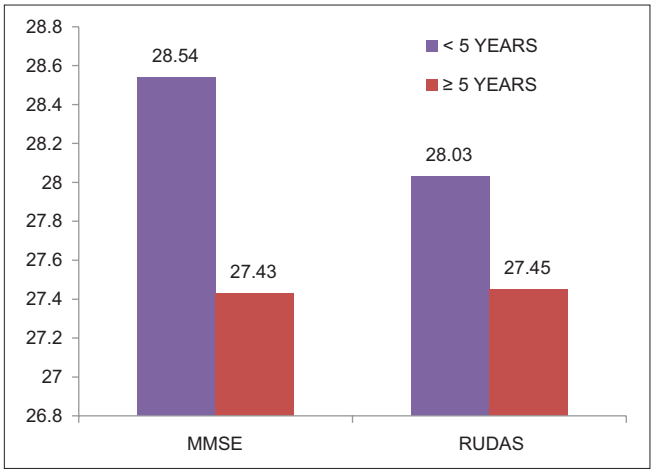


Figure 2: Association between mean cognition score expressed as mean ± standard deviation and duration of diabetes using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

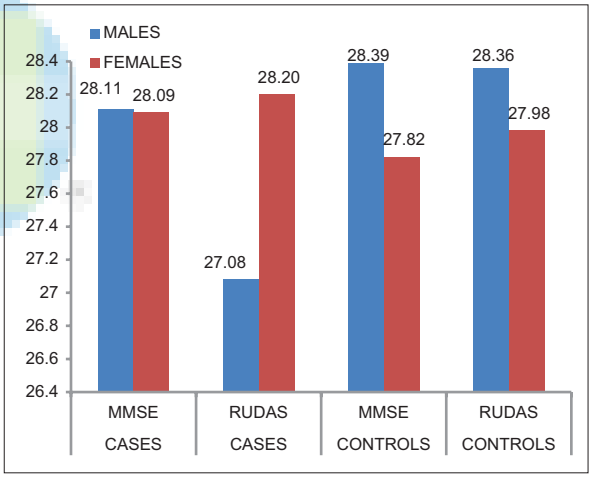


Figure 3: Comparison of mean cognition score expressed as mean ± standard deviation between males and females using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

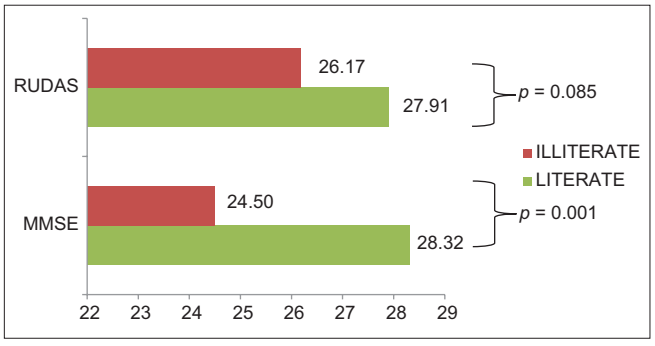


Figure 4: Association between cognition level and literacy using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

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Table 4: Independent sample *t*-test for comparing the difference in mean cognition scores in those with and without hypertension using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

Scale	Parameter	Mean±SD	*t*	*P*
MMSE	Cases with hypertension	27.81±2.212	−0.676	0.504
	Controls with hypertensive	27.17±1.472		
RUDAS	Cases with hypertension	27.55±2.908	0.091	0.928
	Controls with hypertensive	27.67±3.011		
MMSE	Diabetes with hypertension	27.81±2.212	0.837	0.405
	Diabetes only	28.23±2.391		
RUDAS	Diabetes with hypertension	27.55±2.908	0.705	0.483
	Diabetes only	27.91±2.152		

MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, SD = Standard deviation

bias.[16,17] It is one of the commonly used tools for cognitive assessment consisting of 11-item testing with sensitivity of 87% and specificity of 82%.[18] The RUDAS is a newer screening test developed in Australia. It is a brief six-item screening test which is short and easy to administer. It is claimed to be culturally and educationally unbiased and developed in view of application in multicultural setting.[11,12,19] It assesses body orientation, praxis, drawing, judgment, memory, and language. It has the additional advantage of being capable of assessing the impairment in executive function. With a cutoff score of 23/30, it has 88% sensitivity and 90% specificity.[11,18]

It has been well proven that age, in addition to ethnicity and education, is an important factor affecting the MMSE score. In our study, there was no statistically significant difference in the mean score between patients aged above and below 50 years. In a study done using MMSE scale, there was no quantitative difference in cognition scores above and below 70 years of age, but a significant difference between diabetic and non-diabetic patients was detected.[3] Their result indicated that in diabetic patients, diabetes could be a greater risk factor for cognitive impairment than age. However, our study results did not replicate such a significant difference between cases and controls in both the age groups studied [Table 3]. The reason attributable to this inference is that most of the earlier studies were done in a population with higher mean age,[5,20] but our study included a much younger population and that may also account for the lower percentage of cognitive deficits detected. A similar result has been demonstrated in a study with middle-aged diabetic population.[4]

The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men.[21] In our study sample, the proportion of females was much higher than the males. Using MMSE, the males scored higher than females though not statistically significant and this was in concordance with earlier studies.[3] Assessed using RUDAS, the female cases scored significantly higher than male cases, but such an observation was not seen in the control group and the reason for the result could not be explained.

Our study demonstrated that the magnitude of the difference in the mean score between literates and illiterates was significantly higher in the MMSE score as compared to the RUDAS scale, indicating that our study has also proven that RUDAS scale is free of educational barrier as described in the previous studies.[11,12] However, former studies done in Thailand and South Indian Malayalam population showed that cultural, linguistic, and education barriers were not fully overcome with the use of RUDAS scale.[13,15]

The cutoff values for glycemic control and duration of diabetes have been chosen arbitrarily and do not depend on the conventional cutoff adopted internationally for the management of clinical condition. The reason being that the duration of diabetes and the glycemic control beyond which cognitive dysfunction occurs, in comparison to other complications of diabetes, is less defined and less well understood. Hence, using the same clinical cutoff criteria used for management would obviously result in false-negative correlation. In the diabetic population studied, the association of mean cognition score with the glycemic control marked by HbA1c level was found to be statistically significant, in addition to a significant positive correlation which was evident only in the MMSE but not the RUDAS scale. Our study thus reflected the results obtained in few previous studies which have highlighted the positive association between poor glycemic control and poor cognition using MMSE,[22,23] but no such correlation detected using RUDAS.[24] Another study done in middle-aged adults with type-2 diabetes has demonstrated that there is predominantly psychomotor slowing with poor metabolic control, even though learning, memory, and problem-solving skills appear to be largely intact in such patients.[4] On the contrary, few other studies have established that the magnitude of glycemic control does not have significant correlation with the cognitive score using MMSE scale.[3,25,26] The potential explanation for the contradictory results between correlations of cognitive dysfunction with glycemic control is that only the recent HbA1c values representing short-term control were included in most analyses and not the time trend in HbA1c, indicative of long-term control as the measure to categorize patients with good and bad glycemic control.

Evaluating the association between the duration of diabetes and cognitive scores revealed a significant negative correlation. Those with a longer duration (≥5 years) of diabetes exhibited a lower mean score than those with shorter duration of diabetes (<5 years)

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using the MMSE, but not with the RUDAS. Our results were consonant with results obtained in few similar studies.[3,22,25,27] Thus, most studies have proven a definitive correlation between cognitive decline and duration of diabetes than with glycemic control.

In addition, our study has shown that there was a statistically significant difference neither between the mean score of cases with and without hypertension nor between the scores of hypertensive cases and controls using both scales. Literature evidence states that although hypertension alone may not be associated with significant cognitive decline, along with diabetes, it increases the risk of cognitive impairment.[28]

With enormously increasing prevalence of diabetes and diabetes being an important risk factor for cognitive impairment, it is the need of the hour to incorporate cognitive assessment in the routine management of diabetes patients. Ours is the first study that compared MMSE with RUDAS head-to-head in a diabetic population, and the study has proved that MMSE has a definitive advantage over RUDAS for screening diabetes-induced cognitive deficit. In patients with type-2 diabetes, increase in memory deficits with reduction in psychomotor speed and reduced executive function are strongly supported by the findings of earlier studies though there are also other cognitive domains such as verbal fluency, complex motor function, attention, and recall which are shown to be negatively affected. Hence, it is clear that diabetes indeed has composite neurocognitive effects, rather than a single one and difficulty in diagnosis of such multiple neurocognitive function deficits is well recognized.[29] RUDAS tests the executive function unlike MMSE, yet RUDAS failed to demonstrate statistical significance which could be potentially attributed to the relatively high rate of intrasubject variability which is inherent to cognitive testing, thus impairing its ability to identify executive function deficits, especially those which are mild.[29]

In our study, the actual differences in the mean scores between those with good and poor glycemic control (0.91) and those with shorter and longer duration of diabetes (1.11) in the MMSE were small despite achieving statistical significance. Since score differences in the range of 2–4 or greater in MMSE are necessary to conclude a clinically relevant difference, the clinical significance of our interpretation has to be guarded and requires further investigation involving a larger sample size.

Another limitation of the study is that a large proportion of participants were literates and this could be a potential source of bias in interpreting the results. Hence, we recommend that the effectiveness of MMSE scale between literates and illiterates should be compared in a diabetic population before it can be emphasized that the MMSE scale is best suited for screening cognitive deficit in routine clinical examination of diabetic patients for early identification of cognitive impairment.

Conclusion

Our study has truly delineated that cognition deficits based on MMSE scoring correlates with the magnitude of glycemic control as well as the duration of diabetes. The results signify that in the general population, screening for dementia may preferably be done with RUDAS scale, given the higher sensitivity, specificity and particularly as it is efficient to overcome the literacy barrier in a multicultural multi-linguistic population like the one in India. However, in type-2 diabetes patients, it is the MMSE scale that has been proved to be useful in detecting early cognitive impairment, especially in patients with poor glycemic control and longer history of diabetes.

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Conflicts of interest

There are no conflicts of interest.

References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
2. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP, *et al*. The global prevalence of dementia: A systematic review and metaanalysis. Alzheimers Dement 2013;9:63-7500.
3. Alencar RC, Cobas RA, Gomes MB. Assessment of cognitive status in patients with type 2 diabetes through the mini-mental status examination: A cross-sectional study. Diabetol Metab Syndr 2010;2:10.
4. Ryan CM, Geckle MO. Circumscribed cognitive dysfunction in middle-aged adults with type 2 diabetes. Diabetes Care 2000;23:1486-93.
5. Korf ES, White LR, Scheltens P, Launer LJ. Brain aging in very old men with type 2 diabetes: The Honolulu-Asia aging study. Diabetes Care 2006;29:2268-74.
6. Mogi M, Horiuchi M. Neurovascular coupling in cognitive impairment associated with diabetes mellitus. Circ J 2011;75:1042-8.
7. Arvanitakis Z, Schneider JA, Wilson RS, Li Y, Arnold SE, Wang Z, *et al*. Diabetes is related to cerebral infarction but not to AD pathology in older persons. Neurology 2006;67:1960-5.
8. Klein JP, Waxman SG. The brain in diabetes: Molecular changes in neurons and their implications for end-organ damage. Lancet Neurol 2003;2:548-54.
9. Koekkoek PS, Biessels GJ, Kooistra M, Janssen J, Kappelle LJ, Rutten GE, *et al*. Undiagnosed cognitive impairment, health status and depressive symptoms in patients with type 2 diabetes. J Diabetes Complications 2015;29:1217-22.
10. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
11. Storey JE, Rowland JT, Basic D, Conforti DA, Dickson HG.

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The Rowland universal dementia assessment scale (RUDAS): A multicultural cognitive assessment scale. Int Psychogeriatr 2004;16:13-31.
12. Rowland JT, Basic D, Storey JE, Conforti DA. The Rowland universal dementia assessment scale (RUDAS) and the Folstein MMSE in a multicultural cohort of elderly persons. Int Psychogeriatr 2006;18:111-20.
13. Iype T, Ajitha BK, Antony P, Ajeeth NB, Job S, Shaji KS, *et al.* Usefulness of the Rowland universal dementia assessment scale in South India. J Neurol Neurosurg Psychiatry 2006;77:513-4.
14. Pang J, Yu H, Pearson K, Lynch P, Fong C. Comparison of the MMSE and RUDAS cognitive screening tools in an elderly inpatient population in everyday clinical use. Intern Med J 2009;39:411-4.
15. Limpawattana P, Tiamkao S, Sawanyawisuth K. The performance of the Rowland universal dementia assessment scale (RUDAS) for cognitive screening in a geriatric outpatient setting. Aging Clin Exp Res 2012;24:495-500.
16. Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the mini-mental state examination by age and educational level. JAMA 1993;269:2386-91.
17. Tombaugh TN, McIntyre NJ. The mini-mental state examination: A comprehensive review. J Am Geriatr Soc 1992;40:922-35.
18. Velayudhan L, Ryu SH, Raczek M, Philpot M, Lindesay J, Critchfield M, *et al.* Review of brief cognitive tests for patients with suspected dementia. Int Psychogeriatr 2014;26:1247-62.
19. Naqvi RM, Haider S, Tomlinson G, Alibhai S. Cognitive assessments in multicultural populations using the Rowland universal dementia assessment scale: A systematic review and meta-analysis. CMAJ 2015;187:E169-75.
20. Gregg EW, Yaffe K, Cauley JA, Rolka DB, Blackwell TL, Narayan KM, *et al.* Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of osteoporotic fractures research group. Arch Intern Med 2000;160:174-80.
21. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
22. Ebady SA, Arami MA, Shafigh MH. Investigation on the relationship between diabetes mellitus type 2 and cognitive impairment. Diabetes Res Clin Pract 2008;82:305-9.
23. Reaven GM, Thompson LW, Nahum D, Haskins E. Relationship between hyperglycemia and cognitive function in older NIDDM patients. Diabetes Care 1990;13:16-21.
24. Iype T, Shaji SK, Balakrishnan A, Charles D, Varghese AA, Antony TP, *et al.* Cognition in type 2 diabetes: Association with vascular risk factors, complications of diabetes and depression. Ann Indian Acad Neurol 2009;12:25-7.
25. Cosway R, Strachan MW, Dougall A, Frier BM, Deary IJ. Cognitive function and information processing in type 2 diabetes. Diabet Med 2001;18:803-10.
26. Karan NS. Assessment of the cognitive status in diabetes mellitus. J Clin Diagn Res 2012;6:1658-62.
27. Ruis C, Biessels GJ, Gorter KJ, van den Donk M, Kappelle LJ, Rutten GE, *et al.* Cognition in the early stage of type 2 diabetes. Diabetes Care 2009;32:1261-5.
28. Hassing LB, Hofer SM, Nilsson SE, Berg S, Pedersen NL, McClearn G, *et al.* Comorbid type 2 diabetes mellitus and hypertension exacerbates cognitive decline: Evidence from a longitudinal study. Age Ageing 2004;33:355-61.
29. Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. Endocr Rev 2008;29:494-511.

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Original Article

Drug prescription pattern of outpatients in a tertiary care teaching hospital in Tamil Nadu

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Abstract

Purpose: Drug use prescribing indicators advocated by the World Health Organization (WHO) are important tools for assessing the degree of polypharmacy, use of generic medicines, and to evaluate if there is inappropriate use of antibiotics or parenteral medications besides estimating the adherence to the essential drugs list. This study aimed to assess the WHO prescribing indicators in prescriptions given at the medical outpatient department (OPD) in a private medical college hospital in South India.

Materials and Methods: The study was done prospectively from patients when they presented for consultation at the medical OPD at our tertiary care center. Prescriptions were randomly chosen to be analyzed for the WHO prescribing indicators from September 2016 to April 2017.

Results: A total of 700 prescriptions were analyzed and the average number of drugs per prescription was 2.955 ± 1.32. 32.57% of prescriptions had fixed drug combinations and a similar value of 36% was obtained for prescriptions containing more than one drug for the same indication. Amongst the prescribing indicators, generic prescribing was appallingly low (6.42%). In contrast, antibiotic prescribing and prescription of injections showed an appreciably rational trend with 15.42% and 8.14%, respectively. Furthermore, the prescription of the drugs enlisted in the essential drugs list was determined to be 90.67%.

Discussion: The need for increase in generic prescribing and augmenting the adherence of prescriptions to the essential drugs list has been identified. This can be accomplished by multimodal approach that includes regulatory changes, conducting educational programs directed at attitudinal change among current doctors and imparting modifications in medical curriculum so as to inculcate the culture of abiding by the best prescription practices among budding doctors.

Conclusion: This study has delineated the requisite for pertinent changes in current prescribing trends in a tertiary care teaching private colleges.

Keywords: Generic drugs, polypharmacy, prescribing indicators, rational prescribing

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INTRODUCTION

Evaluation of drug prescription pattern is an important aspect of patient care, which also serves as a measure of the quality of care provided. A recent systematic analysis has ascertained that prescribing quality is a dimension requiring constant evaluation.[1] The rational use of drugs is imperative for an effective and efficient health-care system. However, irrational drug use, considered as a global menace

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is especially prevalent in the developing countries due to irrational prescribing, dispensing, and administration of medications.[2] Such irrational prescribing is unethical and greatly decrease the standard of drug therapy in addition to resulting in widespread health hazards such as increased incidence of adverse effects, drug interactions, and emergence of drug resistance, especially with antimicrobial therapy.[3] The World Health Organization (WHO) has reported that more than half of all medicines are prescribed, dispensed, or sold inappropriately.[4]

Prescription pattern monitoring studies are tools for assessing the prescribing, dispensing, and distribution of medicines prevailing in a particular locale. The main aim of such studies is to facilitate rational use of medicines.[5] The drug use indicators have been developed by the WHO in coordination with international network for rational use of drugs as an endeavor to measure the extent of rational prescribing.[5,6] According to the WHO, core drug use indicators are divided into three categories, namely, the prescribing indicators, patient care indicators, and the quality of care indicators. These are highly standardized indicators which do not need national adaptation. Although they do not measure all aspects of drug utilization which require intensive methodologies, extensive and varied sources of data, the core drug use indicators provide a simple tool for quickly and reliably assessing a few critical aspects of pharmaceuticals use in health care.

The drug use indicators collected in a cross-sectional survey or measured at different points in time to assess the change in performance are typically measured within a defined geographic or administrative area, either to describe drug use at a given point in time or to monitor changes over time. This study was designed to assess the drug prescribing practices at the medical outpatient department at our tertiary care center which is a teaching medical college hospital, using the five WHO prescribing indicators which include the average number of drugs per patient encounter, percentage of drugs prescribed by generic name, percentage of encounters with an antibiotic prescribed, percentage of encounters with an injection prescribed, and percentage of drugs prescribed from essential drugs list or formulary.[6] These WHO indicators would be instrumental in identifying the degree of polypharmacy, extent of generic prescribing, whether usage of antibiotics and parenteral medications are appropriate besides estimating the adherence to the essential drugs list.[6,7]

Treatment of diseases by the use of essential drugs, prescribed by their generic names, has been emphasized by the WHO and the National Health Policy of India. It has been highlighted that the prescription monitoring studies are imperative to bridge the areas such as rational use of drugs, pharmacovigilance, pharmacoeconomics, pharmacogenetics, and evidence-based medicine.[5] In India, it is currently essential to conduct studies on prescribing patterns in every state and utilize the data generated by such studies to improve the quality of patient care with the primary aim of promoting rational use of drugs.

Appraisal of the WHO prescribing indicators in a private health facility is less common compared to public sector hospitals. Moreover, the level of health care, wherein this study is undertaken adds to its significance. Unlike most Indian studies, the current study is aimed to analyze the entire list of prescribing indicators advocated by the WHO in a teaching medical college hospital. In such a scenario, not only are the prescribers and patients but also the medical students from one of the stakeholders who imbibe the culture of rational prescribing. Thus, this study would throw light on deficiencies which require appropriate and sustained interventions to avoid being carried onto the next generation.

Noteworthy, most studies encompass prescription monitoring of a particular group of drugs such as antiepileptics, antimicrobials, antiasthmatics, and antihypertensive drugs[5] rather than evaluating all the prescribing indicators irrespective of the diagnosis like our study, which would enable capturing a wider picture of the current trends. Medicine is an ever-evolving science and it is important to understand that conducting such prescription monitoring studies periodically as a measure to ensure rational drug use is imperative.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Ethics Committee (Approval number: 16/261), The research was conducted as a cross-sectional study in the outpatient medical department (OPD) of our tertiary care teaching hospital in South India. Based on the WHO guideline of including at least 600 patient encounters, we chose a sample size of 700. This study was done prospectively from the patients when they presented for consultation at the OPD after obtaining a written informed consent. Prescriptions were randomly chosen to be analyzed for the WHO prescribing indicators over 8 months from September 2016 to April 2017. Two well-trained clinical pharmacists were involved in collecting data on prescribing indicators. Each prescription was regarded as single-patient encounter for calculation of the required parameters. Prescriptions of patients attending medical OPD and treated on outpatient

basis for their ailments were included irrespective of the comorbidities. Data were collected on the demographic details of age, gender, diagnosis, and the treatment prescribed which were mentioned in the prescription. The prescribing indicators were studied in accordance to the standard guideline advocated by the WHO.[6]

1. Average number of drugs per encounter: Average was calculated by dividing the total number of different drug products prescribed, by the number of encounters surveyed. Whether the patient actually received the drugs was not considered relevant in calculating this indicator
2. Percentage of drugs prescribed by generic name: percentage was calculated by dividing the number of drugs prescribed by generic name, by the total number of drugs prescribed and expressed as a percentage
3. Percentage of encounters with an antibiotic prescribed: Percentage was calculated by dividing the number of patient encounters during which an antibiotic was prescribed, by the total number of encounters surveyed and expressed as a percentage
4. Percentage of encounters with an injection prescribed: percentage was calculated by dividing the number of patient encounters during which an injection was prescribed, by the total number of encounters surveyed, multiplied by 100
5. Percentage of drugs prescribed from essential drugs list or formulary: percentage was calculated by dividing the number of products prescribed which were on the essential drugs list or local formulary, by the total number of products prescribed and multiplied by 100.

RESULTS

The data were entered in Microsoft Excel 2007 and analyzed as frequency distributions and percentages to assess the prescribing indicators. A total of 700 prescriptions were analyzed over 8 months from September 2016 to April 2017. The demographic distribution of patients mirrored a rising trend with increasing age as a higher proportion of patients (295) were 60 years and above (42.1%). Among the rest, those aged 40 years and above were 36.7% (257) and 21.2% (148) were <40 years. Both males and females were almost equal in proportion with 357 males and 343 females. Diagnosis were multiple and diverse. Hence, categorization into communicable and non-communicable diseases showed that majority (68.6%) had non-communicable diseases contrary to those with communicable diseases (31.4%). It was found that a total number of 2069 drug products had been prescribed in the 700 patient encounters, and thus, the average number of drugs per prescription was 2.955 and the standard deviation was 1.32. Moreover, the median number of drugs per prescription was 3, which unlike the mean, would not be unduly influenced by the outliers. Overall, the study revealed a higher value for this indicator than the reference standard [Table 1]. Analyzing the patient encounters lacking the data for specified variables, it was found, 647 (92.42%) prescriptions did not have any generic names, whereas only 17 patient encounters lacked at least one drug from essential drugs list. It was evident that 84.57% prescriptions did not have any antibiotics and likewise, a high percentage of 91.85% did not have any injections prescribed.

It was interesting to find that among the 2069 drug products prescribed, the highest percentage (10.95%) of prescribed drugs were supplements which ranked first followed by the antidiabetic, antiplatelet, hypolipidemic, and anti-hypertensive drugs, whereas the overall percentage of antibiotics prescribed was only 15.42%, and hence lower in comparison to the former groups of drugs [Table 2].

The number of prescriptions with fixed drug combinations was 228 accounting for 32.57% [Figure 1]. The most common one being prescribed was analgesic combination (paracetamol with ibuprofen followed by tramadol with paracetamol) for pain relief. A similar value of 36% (252 out of 700) was obtained for prescriptions containing more than one drug for the same indication. Interestingly, prescriptions analyzed showed that patient encounters with two drugs (27.73%) were equal in proportion to those that included three drugs (27.28%) accounting for a total of 55% prescriptions falling under either of these two categories. An analogous high percentage (23.98%) was also recorded for prescriptions with four drugs in the treatment regimen [Figure 2].

With respect to generic prescribing, this study recorded a very low percentage of 6.42% indicating that current prescribing pattern of clinicians favored prescribing by

Table 1: Comparison of the World Health Organization prescribing indicators observed with standard reference range

Indicator	*n*	Average/percentage (SD)	Standard reference range/optimal value
Average number of drugs per patient encounter	2069	2.955 (1.32)	1.6-1.8
Percentage drugs prescribed by generic names	133	6.42% (0.34)	100%
Percentage patient encounters with an antibiotic	108	15.42% (0.38)	20.0%-26.8%
Percentage patient encounters with an injection	57	8.14% (0.27)	13.4%-24.1%
Percentage drugs from essential drugs list	1876	90.67% (1.31)	100%

SD=Standard deviation

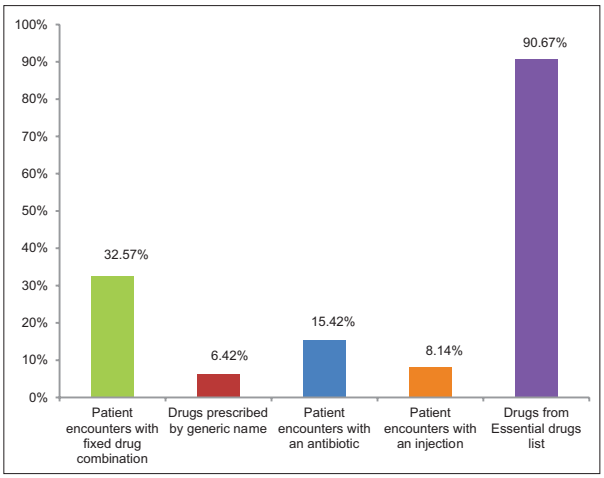


Figure 1: Measured percentages of prescribed fixed-drug combinations and the World Health Organization prescribing indicators

Table 2: Frequency distributions of commonly prescribed drugs

Name of the drug groups commonly prescribed	Name of drugs commonly prescribed	Sum (%)
Antidiabetic drugs	Metformin	161 (7.78)
Antiplatelet drugs	Aspirin	52 (2.51)
NSAIDS	Paracetamol	86 (4.15)
Supplements	Calcium and Vitamin D3	60 (2.89)
	Iron and folic acid	112 (5.41)
	Multivitamins and folic acid	55 (2.65)
Antiulcer drugs	Pantoprazole	99 (4.78)
	Ranitidine	73 (3.52)
Antihypertensive drugs	Amlodipine	36 (1.73)
	Telmisartan	21 (1.01)
Antibiotics	Levofloxacin	10 (0.48)
	Trimethoprim + sulfamethoxazole	8 (0.38)
	Azithromycin	7 (0.33)
	Amoxicillin + clavulanate	7 (0.33)
	Cefpodoxime proxetil	6 (0.28)

NSAIDS=Nonsteroidal anti-inflammatory drugs

brand names rather than using the generic ones. The percentage of patient encounters in which an antibiotic was prescribed was only 108 (15.42%) [Figure 1]. The highest prescribed antibiotic was levofloxacin followed by co-trimoxazole with azithromycin, amoxicillin-clavulanate, and cefpodoxime proxetil being next to the quinolone and the sulfomethoxazole-trimethoprim combination which were widely prescribed at our center during the study period [Table 2]. The most common indication for the antibiotic use was found to be a variety of respiratory infections ranging from pharyngitis, sinusitis to pneumonia.

About 8.14% of patient encounters had an injection being prescribed, and an overall 90.67% of adherence to essential drugs list was evident [Figure 1].

DISCUSSION

It is well known that the indicators of prescribing practices measure the performance of health-care providers in several key dimensions related to rational use of drugs. The indicators were assessed in our study based on a sample of 700 patient encounters that took place at the OPD in our hospital. The data that were collected prospectively by analyzing the prescriptions demonstrated that in our teaching hospital, the average number of drugs prescribed per encounter was 2.955. Comparison to the standard range advocated by the WHO for this indicator which estimates the degree of polypharmacy revealed that the measured average was much higher than the reference range of 1.6–1.8 considered as ideal.[6] This is also reflected in the high percentage of fixed drug combinations prescribed in addition to the use of combination of different drugs for a single indication in one patient encounter. In a similar study done in Goa,[8] the average number of drugs prescribed was 1.8 which was found to be lower than our finding and that obtained in other Indian studies on prescribing indicators by Upadhyay *et al.* (3.76) and Raj *et al.* (4.98).[9,10] In contrast, similar studies of drug use pattern in other countries such as Sudan (1.4), Zimbabwe (1.3), and Ethiopia (1.9)[11] have shown that the average number of drugs per encounter estimated in our study was higher.

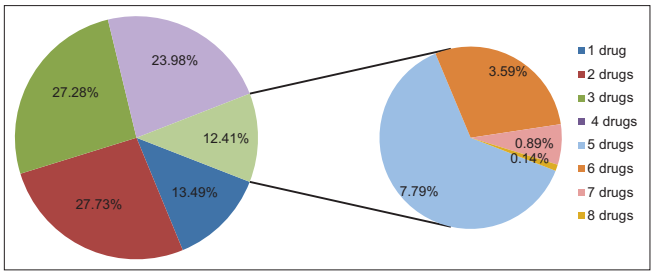


Figure 2: Percentage distribution of number of drugs in the prescriptions

The high average number of drug products per prescription exceeding the WHO reference range is demonstrative of a high degree of polypharmacy prevalent in our study locale. The reasons that could be attributed to this is the change in the epidemiological trend of rampantly increased prevalence of non-communicable diseases such as diabetes, hypertension, dyslipidemia, and coronary artery disease which are often coexistent contributing to the need for treating multiple disease entities in the same patient simultaneously.[12] Concomitance of such cardiometabolic conditions in a single patient further entails the requisite for prescribing more than a single drug for a given clinical indication which is often resistant to treatment in the face of multiple diseases presenting concurrently. Our study also stands testimony to this fact since a high proportion of participants (68.6%) had the diagnosis of non-communicable disease with diabetes ranking highest with 34.4%

Another rationalization for the high percentage of polypharmacy lies in the fact that currently there is a demographic shift with an increase in the aging population in our country.[13] This epidemiological transition is further responsible for perpetuating the current scenario prevailing. About 42.1% of the study population also was aged >60 years signifying that the greater proportion of aged population contributed to the high polypharmacy.

However, the high prescription rates for supplements recorded in our study remain unjustified and arise from two important factors potentially contributing to the irrational prescribing practice. First, the influence of pharmaceutical industries which vigorously promote supplementary products among doctors by providing biased information supporting the need for prescribing such products. Second, patients misconceptions on the health-promoting effects of supplements resulting in their persuasion and demand for such products in their prescriptions. However, the best approach toward such patients is to educate them rather than giving in to their false ideations.[3]

The percentage of drugs prescribed by generic name in our study was 6.42% which is too low compared with the standard derived to serve as ideal, which is 100%.[6] Wang *et al*. found that higher the doctor's education and training experience, the proportion of drugs that they prescribed by generic names showed a decrease.[14] Moreover, attitudinal differences have also been shown to exist among consultants in low- and middle-income countries compared to those in high-income countries.[15]

The likely explanation for the low percentage of generic prescribing could be due to repeated and persuasive promotion of the propriety products by pharmaceutical companies and in certain instances, clinicians are compelled to concede to the insistence of affluent patients demanding innovator drugs for treatment.[16] Another plausible cause for this stems from the presumed belief among a subset of prescribing physicians that the bioavailability differences between generic and brand drugs could adversely affect the therapeutic outcomes.[17] Such prejudices could unfavorably affect the tendency of prescribing generic drugs. Finally, the role played by industries in hindering generic prescribing by offering financial perks to prescribers cannot be underestimated.[18] Literature evidence states that generic prescribing is better in public centers in comparison to that in private sector hospitals.[16]

It is important to increase awareness on generic prescribing considering the high treatment costs incurred by the practice of prescribing by brand names. Nicolosi and Gray investigated the cost impact of generic and proprietary prescribing among chronic disease patients in South Africa and their findings indicated that "all generics were more than 40% cheaper, per defined daily dose per month, than the brand version."[19] An analysis of facility-based medicines price data from 17 countries by Cameron and Laing,[20] found that an average of 9%–89% could be saved by switching from brand to generic equivalents. Shift in the trend favoring generic prescribing is warranted and this can be implemented by an integrated approach of training the medical students who are future prescribers on the pharmacoeconomic significance of this practice in addition to conducting continuing medical education programs for clinicians with the focus of alleviation of their apprehension on bioequivalence regarding the use of generic drugs. Moreover, a variety of strategies have been recommended by experts to overcome the barriers to genetic prescribing and the most vital of these include enforcing statutory obligations, setting clear guidelines for generic prescribing, and legally de-incentivizing prescribing by propriety name.[21]

Interestingly, we found that the number of antibiotics prescribed (15.42%) as well as the number of encounters with an injection (8.14%) was low, indicating a positive trend toward reduction in indiscriminate use of antibiotics and unnecessary injections. An obviously rational prescribing pattern is noted for both these indicators in our tertiary care center. Noteworthy, our study duration was 8 months, and hence, the study would not have comprehensively captured the seasonal variation in antibiotic prescription pattern due to differential incidence rates of infections at different times of the year.

About 90.67% of drugs prescribed conform to the WHO essential drugs list which is lower than that obtained in other studies in India (99.6%), Tanzania (96%), and South Ethiopia (96.6%), whereas higher in comparison to that in countries like Nepal (88%). This could be attributable to the lack of sensitization of the prescribers and lack of rules enforced to mandate prescribing from the essential drugs list. Akin to generic prescribing, this indicator also has been demonstrated to vary between the private and public sectors[22] indicating the need for the practice of prescribing from essential drugs list to be more widely adopted especially in private sector hospitals.

One of the strengths of the study is that it was done prospectively using the patient prescriptions attending the OPD rather than the data being collected retrospectively from case records which is often the design adopted in prescription pattern studies as it is much easier to

collect information from patient records. In addition, the prospective design of this study positively ensures eliminating duplication of data, while retrospective analyses cannot be claimed to be completely devoid of bias such as exclusion of incomplete data from poor record keeping unlike prospective evaluation.

A major limitation of our study is that it was done in a single center which is a private medical college hospital with limited sample size, and hence, the data cannot be extrapolated to other centers lacking similar characteristics like public or government hospitals and health-care centers.

This study implies the need for implementing interventions such as programs, workshops, and symposia to impart awareness on rational prescribing among the medical fraternity. Thus this research has not only identified the pitfalls in current prescription practices, but in addition, has recommended the potential solutions to weed out irrational prescribing trends encountered.

CONCLUSION

Our study on prescribing indicators has clearly delineated that the prescribing practices for antibiotics and injections are appropriate and rational; conformity to essential drugs list is considerably good, though scope for improvising is certainly evident. However, the degree of polypharmacy is higher than the standard. Generic prescribing is also an element that needs to be vastly improved on. Therefore, appropriate measures to reduce polypharmacy and increase generic prescribing by clinicians have to be implemented by the administrative team and policymakers to ensure rational and safe prescribing.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Song J, Zhang L, Li Y, Zeng L, Hu D, Liang Y, *et al*. Indicators for assessing quality of drug use: A systematic literature review. J Evid Based Med 2017;10:222-32.
2. Enato EF, Chima IE. Evaluation of drug utilization patterns and patient care practices. West Afr J Pharm 2011;22:36-41.
3. Garg M, Vishwakarma P, Sharma M, Nehra R, Saxena KK. The impact of irrational practices: A wake up call. J Pharmacol Pharmacother 2014;5:245-7.
4. World Health Organization Medicine Strategy, 2008-2013. Geneva: WHO; 2008.
5. Jain S, Upadhyaya P, Goyal J, Kumar A, Jain P, Seth V, *et al*. A systematic review of prescription pattern monitoring studies and their effectiveness in promoting rational use of medicines. Perspect Clin Res 2015;6:86-90.
6. World Health Organization. How to Investigate Drug use in Health Facilities: Selected Drug use Indicators – EDM Research Series No. 007. WHO/DAP/93.1. Geneva: World Health Organization; 1993.
7. Hogerzeil HV, Bimo, Ross-Degnan D, Laing RO, Ofori-Adjei D, Santoso B, *et al*. Field tests for rational drug use in twelve developing countries. Lancet 1993;342:1408-10.
8. Chandelkar UK, Rataboli PV. A study of drug prescribing pattern using WHO prescribing indicators in the state of Goa, India. Int J Basic Clin Pharmacol 2014;3:1057-61.
9. Upadhyay DK, Palaian S, Ravi Shankar P, Mishara P, Sah AK. Prescribing pattern in diabetic outpatients in a tertiary care teaching hospital in Nepal. J Clin Diagn Res 2007;1:248-55.
10. Raj K, Kamlesh K, Kajal HL. A study of drug prescribing pattern and cost analysis among diabetic patients in a tertiary care teaching institute in North India. J Drug Deliv Ther 2013;3:56-61.
11. Desalegn AA. Assessment of drug use pattern using WHO prescribing indicators at Hawassa University Teaching and Referral Hospital, South Ethiopia: A cross-sectional study. BMC Health Serv Res 2013;13:170.
12. Mohan V, Venkatraman JV, Pradeepa R. Epidemiology of cardiovascular disease in type 2 diabetes: The Indian scenario. J Diabetes Sci Technol 2010;4:158-70.
13. Marasinghe KM, Lapitan JM, Ross A. Assistive technologies for ageing populations in six low-income and middle-income countries: A systematic review. BMJ Innov 2015;1:182-95.
14. Wang H, Li N, Zhu H, Xu S, Lu H, Feng Z, *et al*. Prescription pattern and its influencing factors in Chinese county hospitals: A retrospective cross-sectional study. PLoS One 2013;8:e63225.
15. Hassali MA, Wong ZY, Alrasheedy AA, Saleem F, Mohamad Yahaya AH, Aljadhey H, *et al*. Perspectives of physicians practicing in low and middle income countries towards generic medicines: A narrative review. Health Policy 2014;117:297-310.
16. Ofori-Asenso R, Brhlikova P, Pollock AM. Prescribing indicators at primary health care centers within the WHO African region: A systematic analysis (1995-2015). BMC Public Health 2016;16:724.
17. Kesselheim AS, Eddings W, Raj T, Campbell EG, Franklin JM, Ross KM, *et al*. Physicians' trust in the FDA's use of product-specific pathways for generic drug approval. PLoS One 2016;11:e0163339.
18. Qian J, Hansen RA, Surry D, Howard J, Kiptanui Z, Harris I, *et al*. Disclosure of industry payments to prescribers: Industry payments might be a factor impacting generic drug prescribing. Pharmacoepidemiol Drug Saf 2017;26:819-26.
19. Nicolosi E, Gray A. Potential cost savings from generic medicines-protecting the prescribed minimum benefits. S Afr Fam Pract 2009;51:60-3.
20. Cameron A, Laing R. Cost Savings of Switching Private Sector Consumption from Originator Brand Medicines to Generic Equivalents; World Health Report (2010) Background Paper, 35. Geneva: World Health Organization; 2010.
21. Drozdowska A, Hermanowski T. Solution-driven approaches to generic substitution challenges – A survey among international experts. Fundam Clin Pharmacol 2015;29:592-603.
22. Mohlala G, Peltzer K, Phaswana-Mafuya N, Ramlagan S. Drug prescription habits in public and private health facilities in 2 provinces in South Africa. East Mediterr Health J 2010;16:324-8.

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Original Article

Pharmacology Section

Comparison of the Effects of Zonisamide and Flupirtine on Paclitaxel Induced Peripheral Neuropathy in Rats

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ABSTRACT

Introduction: Many peripheral and central causes lead to neuropathic pain disorders. Effective treatment of neuropathic pain is not completely manageable and hence, it becomes necessary to evaluate the application of Zonisamide as a sodium channel and T-type calcium (Ca^{+}) current inhibitors and Flupirtine by activation of potassium (K) channel through N-methyl-D-aspartate (NMDA) receptor inhibition in the treatment of neuropathic pain.

Aim: To analyze the analgesic effect of Zonisamide and Flupirtine in paclitaxel induced neuropathic pain model in rats by hot plate and cold allodynia test.

Materials and Methods: Total of six groups of animals, each with six rats were given with single intraperitoneal (i.p.) injection of 1 mg/kg of paclitaxel on four alternate days (day 0, 2, 4, and 6). Drugs Zonisamide (50 mg/kg daily for group 2, 100 mg/kg daily for group 3, Flupirtine (10 mg/kg, daily for group 4, 20 mg/kg daily for group 5) and Gabapentin daily for group 6 were administered in the dosages per group from days 0 to 7. Nociceptive tests were done for all animals on days 0, 7, 14, 21, 28 to assess the pain threshold. Student's t-test was used to analyze the statistical significance.

Results: In our study, on the 21st day of testing we observed that 100 mg/kg dosage of Zonisamide group has shown a significant increase in reaction time suggesting analgesic effect. Prominent increase in the reaction time was also observed that on day 14 of testing, both the Gabapentin and Flupirtine groups showed an earlier analgesic effects when compared with the Zonisamide group.

Conclusion: Zonisamide and Flupirtine showed anti-nociceptive activity in the Paclitaxel model of peripheral neuropathy compared with the standard treatment of Gabapentin.

Keywords: Analgesic action, Effect of ion channels, Neuropathic pain, Reaction time

INTRODUCTION

Neuropathic pain is a disabling condition affecting about 7-8% of the population [1]. The Health Related quality of life for individuals with neuropathic pain is rated low compared to those with coronary artery disease, diabetes mellitus and recent myocardial infarction [2]. Opioid analgesics have a role in the treatment of neuropathic pain, but they have been questioned in regard to their safety profile. This is especially important in treatment of the elderly who have an increased prevalence of neuropathic pain [3,4]. In a country like India, with an estimated 62 million people living with diabetes mellitus, treatment of neuropathic pain effectively is important [5]. The first line treatment for neuropathic pain includes Gabapentin, Anticonvulsants, Topical Lidocaine and Capsaicin - they have achieved a total treatment satisfaction of only 50% according to one study done in diabetic patients [6]. Thus, it becomes necessary to evaluate the application of other drugs in the treatment of neuropathic pain.

Flupirtine is a centrally acting, non-opioid, NMDA receptor antagonist that causes analgesic effect by opening Kv7 potassium channels. It is effective in alleviating several types of pain especially chronic musculoskeletal pain and post-surgical pain [7]. It has been demonstrated that Flupirtine has significant analgesic property in several animal pain models including in diabetic neuropathic pain model, carrageenan-induced hyperalgesia and ciguatoxin induced pain in rats [8,9].

Zonisamide is an antiepileptic drug that has been tried previously for the treatment of chronic pain with inconclusive results [10]. It has been evaluated in the Streptozotocin induced Peripheral Neuropathy model previously and has shown to have an effect in reducing the pain induced [11]. In the published literature, it was found that Flupirtine provided significant pain relief in patients with small fiber neuropathy within a week when the other first line drugs like gabapentin and pregabalin have failed. Furthermore, studies have shown it to be useful in neuropathic pain only in conjunction with opioids and there isn't any literature evidence with head-head comparison of the drug with the currently available first line drugs [12]. In a recent review in Cochrane library on Zonisamide in neuropathic pain, there was only one study done on 25 patients with painful diabetic neuropathy (13 in Zonisamide vs 12 in placebo) which showed that three out of 11 responders had ≥50% reduction in pain intensity which may be due to one of its diverse mechanisms of action. There was no study directly comparing the effect the drug with that of the first line drugs [13].

Since, the data available regarding the modulation of pain in the Paclitaxel-induced neuropathic pain in rat model, by Zonisamide and Flupirtine is insufficient this study evaluated Zonisamide and Flupirtine for their pain relieving effects in the Paclitaxel Model of Peripheral Neuropathy in rats.

MATERIALS AND METHODS

This experimental study was carried out after obtaining ethical clearance from the Institutional Animal Ethics Committee of PSG Institute of Medical Sciences and Research, Coimbatore, India during May 2014 to July 2014.

All the animals were procured from the Animal House at the Institution. All the groups were housed in different cages with the male and female rats separated. They were allowed to acclimatize to the ambient temperature, following with they were tamed before each injection to facilitate easy handling.

Paclitaxel Model of Peripheral Neuropathy in Rats

Paclitaxel induced peripheral neuropathy in rats has been evaluated as

a robust model for studying the antinociceptive effects of various drugs [14, 15]. This model involves administration of single intraperitoneal (i.p.) injection of 1 mg/kg of paclitaxel on four alternate days (0, 2, 4, and 6). The volume of injection is kept constant at 1 mL/kg. This model typically presents sensory neuropathy on the 14th day.

Test Groups

This consisted of six groups of animals, each with 6 wistar rats (weight 200-250 g). Three male and three female in each group.

Group-1: Paclitaxel alone

Group-2: Paclitaxel + Zonisamide 50 mg/kg

Group-3: Paclitaxel + Zonisamide 100 mg/kg

Group-4: Paclitaxel + Flupirtine 10 mg/kg

Group-5: Paclitaxel + Flupirtine 20 mg/kg

Group-6: Paclitaxel + Gabapentin

Nociceptive tests were done for all animals on days 0, 7, 14, 21, 28 to assess the pain threshold.

The dosages of drugs were chosen based on the previous published literature in similar rat models [8,9]. In these studies the dosages considered included 50 mg/kg, 100 mg/kg, and 150 mg/kg of orally administered Zonisamide. These were found to be the optimum dosages in which there was a significant analgesic effect obtained.

Drugs Zonisamide, Flupirtine and Gabapentin were administered everyday orally in the dosages as per the group from days 0 to 7.

Outcome Parameters

Hot plate test: The hot plate test involves placing the rat in a transparent glass cylinder on a preheated plate at 55°C. After 60 minutes of corresponding test drugs administration, per group rats were placed on hot plate to measure the latency period with cutoff time 60 seconds was allowed to each animal to prevent injury. The time duration of latency is the time interval between placing the rat on the hot plate and the time for the rat to perform two behavioural components, namely, paw licking and jumping. The average latency time period in different groups was 17-21 seconds [Table/Fig-1]. Both of these responses are supra-spinal integrated responses [16,17].

Cold allodynia test: This was performed by immersing the tip of the tail in cold water at four degrees. The time taken for a withdrawal reflex is the time of latency considered. A cut of latency of 20 seconds was kept to avoid any injury [18,19]. Both the tests were done for all the animals on days 0, 7, 14, 21, and 28.

STATISTICAL ANALYSIS

Data obtained from the study were entered in SPSS software version 19.0. Student's t-test was done to analyze the statistical significance.

RESULTS

Paclitaxel-induced neuropathy was seen on day seven by Hot plate test and on day 14 by cold allodynia test. Decrease in latency was observed in hot plate and cold allodynia tests and it was persisted till 28 days in the control group. No other abnormal behavioural response was seen.

Hot Plate Test

Latency period was significantly delayed for the paclitaxel group on Day 7 (p=0.01) and Day 14 (p=0.01) whereas Gabapentin treated group, both Flupirtine treated groups 10 mg/kg (p=0.02) showed significant delay in latency time on day 14. However, Zonisamide showed significant delay (p=0.02) in latency time only for the dose 100 mg/kg on the day 21 compared to Gabapentin and control groups [Table/Fig-1].

Cold Allodynia Test

The latency time for a reaction is reduced in the paclitaxel group significantly from day 14 (p=0.01 and still reduction seen on day 28 (p=0.02) when compared to Day 0. Also, there was a significant delay in reaction time in the 100 mg/kg Zonisamide group (p=0.02) on Day 14, Gabapentin group (p=0.01) and both the 10 mg/kg (p=0.01), 20 mg/kg (p=0.01) of Flupirtine groups and showed delay in latency time for a reaction in on day 14 [Table/Fig-1].

Day	Paclitaxel alone (Control) group			
	Cold allodynia test		Hot plate Test	
	Avg. Time (sec)	p-value	Avg. Time (sec)	p-value
Day 28	25.67	0.02*	10.74	0.01*
Day 21	26.59	0.02*	11.04	0.01*
Day 14	23.35	0.01*	11.44	0.01 *
Day 7	31.33	0.12*	13.97	0.01*
Day 0	36.76	NA	18.92	NA
	Paclitaxel + Zonisamide 50 mg/Kg			
Day 28	31.16	0.16#	13.27	0.16#
Day 21	31.59	0.03#	11.75	0.46#
Day 14	32.46	0.01#	13.96	0.13#
Day 7	34.53	0.31#	12.53	0.24#
Day 0	38.50	NA	21.63	NA
	Paclitaxel + Zonisamide 100 mg/Kg			
Day 28	36.92	0.02#	19.96	0.01#
Day 21	37.32	0.02#	18.84	0.02#
Day 14	31.40	0.02#	16.69	0.03#
Day 7	30.32	0.50#	12.11	0.19#
Day 0	38.77	NA	18.60	NA
	Paclitaxel + Flupirtine 10mg/kg			
Day 28	34.13	0.02#	18.83	0.01#
Day 21	36.71	0.03#	19.68	0.01#
Day 14	37.53	0.01#	19.12	0.02#
Day 7	36.37	0.16#	13.04	0.47#
Day 0	37.26	NA	19.88	NA
	Paclitaxel + Flupirtine 20mg/kg			
Day 28	38.05	0.02#	19.72	0.01#
Day 21	39.84	0.01#	21.74	0.01#
Day 14	36.63	0.01#	20.10	0.01#
Day 7	36.82	0.10#	13.27	0.47#
Day 0	41.78	NA	17.86	NA
	Paclitaxel + Gabapentin			
Day 28	31.05	0.07#	15.88	0.01#
Day 21	32.23	0.05#	16.73	0.01#
Day 14	32.91	0.01#	16.72	0.01#
Day 7	34.15	0.30#	13.99	0.98#
Day 0	37.15	NA	18.27	NA

[Table/Fig-1]: p-value of both Cold allodynia test and Hot plate test.
*p-value compared to Day 0, (NA-Not applicable), #p-value (0.01) compared to paclitaxel group(significant p value p≤0.05).

DISCUSSION

The Paclitaxel induced peripheral neuropathy model in rats has shown to be an established indicator which mimics neuropathic pain in humans. It has been used to evaluate the neuropathic effect of other anticonvulsants [5].

The results show that neuropathic pain was induced by the 14th day from the starting of Paclitaxel in group 1, identified by the decrease in latency time. This is in keeping with the Paclitaxel model of

peripheral neuropathy. On examining the difference in groups, we find that the Gabapentin group showed delayed latency period from day 14, i.e., less neuropathic pain. This cements the proven potent analgesic effect of Gabapentin [10].

In our study, on the 21st day of testing we observed that the group with the 100 mg/kg dosage of Zonisamide has shown a significant increase in reaction time suggesting analgesic effect.

Similar prominent increase in the reaction time was observed for the Gabapentin and both the Flupirtine groups earlier, on day 14 of testing, showing that Gabapentin and Flupirtine act producing earlier analgesic effects when compared to the Zonisamide group. Thus, in our study both Zonisamide and Flupirtine produce analgesic effects comparable to the established Gabapentin.

Zonisamide, an anticonvulsant, has different mechanism of action, one of which includes inhibition of the T-type Calcium channels [20,21]. These channels have been studied and they have been implicated in the modulation of chronic pain [22]. They are emerging targets for treatment of neuropathic pain [23]. Lercanidipine, an antihypertensive, also acts via modulation of the T-type Ca^{++} channels in the body. This drug has been shown to be effective in treating neuropathy in this Paclitaxel model [19]. Previous studies have shown that Zonisamide has a potential ability to treat neuropathic pain [24]. There is literature on evaluation of the analgesic property of Zonisamide on chronic pain models [25]. On the other hand, a recently published Cochrane analysis has shown that Zonisamide has not been efficacious enough to advocate routine use in the treatment of peripheral neuropathy [10].

Flupirtine is a K(V) seven channel activator with GABA-A receptor modulating action [26]. It has central nervous system activity causing analgesic effects [27]. Flupirtine has not undergone extensive clinical trials but there is evidence of analgesic activity as shown in previously evaluated animal pain models [28]. One study has demonstrated its analgesic activity on prostate bone metastasis in rats [29]. Flupirtine has also been studied previously in combination with an Opioid in Streptozotocin Induced Peripheral neuropathy model in rats showing analgesic properties [9]. There had been no published reports of the analgesic effects being evaluated in the Paclitaxel model.

Gabapentin has been evaluated extensively in the treatment of peripheral neuropathy and has been placed as one of the first line drugs used in the treatment of peripheral neuropathy [30].

Zonisamide and Flupirtine showing anti-nociceptive activity in the Paclitaxel model of peripheral neuropathy, evaluated alongside a treatment standard of Gabapentin, provide robust evidence of their analgesic activity.

Gabapentin is used as a first line drug in patients with neuropathic pain. Since, many of these patients have comorbidities it becomes vital to choose a drug that has minimal adverse reactions [31]. Gabapentin is associated with adverse reactions such as sedation, nausea and vomiting, which makes it cumbersome to use in debilitated patients [32]. The other drugs which have been used in neuropathic pain including opioids and sedatives have significant adverse effects [33].

Flupirtine has a unique mechanism of action and studies have shown that it causes a anti-nociceptive action without producing sedation [9]. Flupirtine has also shown to have neuroprotective and muscle relaxing properties [34,35]. Further since Zonisamide and Flupirtine are non-opioid drugs and tolerance does not develop in most cases they have minimal abuse potential [36,37]. Flupirtine has also shown shown to be effective in refractory neuropathic pain [38].

LIMITATION

In this study only two methods (cold allodynia, hot plate) of evaluation were used. A nerve conduction study would have given better results.

Further only two doses of Zonisamide have been tested. Other dosages to evaluate the most effective dosage have to be evaluated.

CONCLUSION

Zonisamide and Flupirtine, thus can be used as adjunctive drugs and reduce the dose related side effects of conventional therapy. They also work by a different, novel mechanism of action when used for the treatment of neuropathic pain, making them a potential choice for complementary therapy.

Thus, further studies evaluating the antinociceptive property of Zonisamide and Flupirtine in humans may further their usage in pain pathways.

REFERENCES

[1] Bennett MI, Rayment C, Hjermstad M, Aass N, Caraceni A, Kaasa S. Prevalence and aetiology of neuropathic pain in cancer patients: a systematic review. Pain. 2012;153(2):359-65.

[2] Epidemiology of Neuropathic Pain [Internet]. 1st ed. International Association for the Study of Pain; 2014 [cited 2016 Nov 2]. Available from: http://iasp.files.cms-plus.com/AM/Images/GYAP/Epidemiology%20of%20Neuropathic%20Pain.pdf

[3] Mendlik MT, Uritsky TJ. Treatment of Neuropathic Pain. Curr Treat Options Neurol. 2015;17:50.

[4] Gallagher HC, Gallagher RM, Butler M, Buggy DJ, Henman MC. Venlafaxine for neuropathic pain in adults. Cochrane Database Systematic Reviews. [Internet]. 2015 [cited 2016 Jan 10]; Issue 8. Art. No.: CD011091 DOI: 10.1002/14651858.CD011091.pub2.

[5] Kaveeshwar S. The current state of diabetes mellitus in India. Australasian Medical Journal. 2014;7(1):45-48.

[6] Dulipsingh L, Zailskas S, Goldsby T, McInnis T, Marotta A. Assessment of pain and treatment satisfaction in patients with painful diabetic peripheral neuropathy. Conn Med. 2013;77(9):523-7.

[7] Friedel HA, Fitton A. Flupirtine. A review of its pharmacological properties, and therapeutic efficacy in pain states. Drugs. 1993;45(4):548-69.

[8] Zimmermann K, Deuis JR, Inserra MC, Collins LS, Namer B, Cabot PJ, et al. Analgesic treatment of ciguatoxin-induced cold allodynia. Pain. 2013;154(10):1999-2006.

[9] Goodchild C, Kolosov A, Tucker A, Cooke I. Combination Therapy with Flupirtine and Opioid: Studies in Rat Pain Models. Pain Med. 2008;9(7):928-38.

[10] Moore R, Wiffen PJ, Derry S, Lunn MPT. Zonisamide for neuropathic pain in adults. Cochrane Database of Systematic Reviews 2015, Issue 1. Art. No.: CD011241. DOI: 10.1002/14651858.CD011241.pub2.

[11] Tanabe M, Murakami T, Ono H. Zonisamide Suppresses Pain Symptoms of Formalin-Induced Inflammatory and Streptozotocin-Induced Diabetic Neuropathy. J Pharmacol Sci .2008 Jun 10; 107(2):213-20.

[12] Mishra S, Choudhary P, Joshi S, Bhatnagar S. Successful use of Flupirtine in refractory neuropathic pain due to small fiber neuropathy. American Journal of Hospice and Palliative Medicine. 2013 Feb; 30(1):91-93.

[13] Moore RA, Wiffen PJ, Derry S, Lunn M. Zonisamide for neuropathic pain in adults. Cochrane Database Syst Rev.[Internet]. 2015 [cited 2016 Jan 2]; Issue 1. Art.No.CD011241 DOI: 10.1002/14651858.CD011241.pub2

[14] Jaggi AS, Jain V, Singh N. Animal models of neuropathic pain. Fundam Clin Pharmacol. 2011;25(1):01-28.

[15] Polomano RC, Mannes AJ, Clark US, Bennett GJ. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. Pain. 2001;94(3):293-304.

[16] Zhuravlev BV, Murtazina EP, Pertsov SS. Behavioral Indexes of Thermal Nociceptive Sensitivity in Rats after Melatonin Administration. Bull Exp Biol Med. 2015;160(2):179-82.

[17] Yamamoto T, Nozaki-Taguchi N, Chiba T. Analgesic effect of intrathecally administered orexin-A in the rat formalin test and in the rat hot plate test. British Journal of Pharmacology. 2002;137(2):170-76.

[18] Allchorne AJ, Broom DC, and Woolf CJ. Detection of cold pain, cold allodynia and cold hyperalgesia in freely behaving rats. Molecular Pain. 2005;1:36.

[19] Saha L, Hota D, Chakrabarti A. Evaluation of Lercanidipine in Paclitaxel-Induced Neuropathic Pain Model in Rat: A Preliminary Study. Pain Research and Treatment. 2012;143579.

[20] Goyal S, Singla S, Kumar D, Menaria G. Comparison of the effects of zonisamide, ethosuximide and pregabalin in the chronic constriction injury induced neuropathic pain in rats. Ann Med Health Sci Res 2015;5(3):189-96.

[21] Leppik IE. Zonisamide: chemistry, mechanism of action, and pharmacokinetics. Seizure. 2004;13(Suppl 1):S5-S9.

[22] Flatters SJL. T-type calcium channels: a potential target for the treatment of chronic pain. Drugs Fut. 2005;30(6):573.

[23] Snutch, TP, David, LS. T-type calcium channels: an emerging therapeutic target for the treatment of pain. Drug Dev Res. 2006;67(4):404-15.

[24] Hord AH, Denson DD, Chalfoun AG, Azevedo MI. The effect of systemic zonisamide (Zonegran) on thermal hyperalgesia and mechanical allodynia in rats with an experimental mononeuropathy. Anesth Analg. 2003 Jun; 96(6):1700-06.

[25] Bektas N, Arslan R, Ozturk Y. Zonisamide. Anti-hyperalgesic efficacy, the role of serotonergic receptors on efficacy in a rat model for painful diabetic neuropathy.

Life Sci. 2014;95(1):09-13.
[26] Szelenyi I. Flupirtine, a re-discovered drug, revisited. Inflamm Res. 2013;62(3):251-58.
[27] Raffa RB, Pergolizzi JV. The evolving understanding of the analgesic mechanism of action of flupirtine. J Clin Pharm Ther. 2012;37(1):04-06.
[28] Devulder J. Flupirtine in pain management: pharmacological properties and clinical use. CNS Drugs. 2010;24(10):867-81.
[29] Kolosov A, Goodchild CS, Williams ED, Cooke I. Flupirtine enhances the anti-hyperalgesic effects of morphine in a rat model of prostate bone-metastasis. Pain Med. 2012;13(11):1444-56.
[30] Rao RD, Michalak JC, Sloan JA, Loprinzi CL, Soori GS, Nikcevich DA, et al. Efficacy of gabapentin in the management of chemotherapy-induced peripheral neuropathy: a phase 3 randomized double-blind, placebo-controlled, crossover trial (N00C3). Cancer. 2007;110(9):2110-18.
[31] Woo J, Stern T, Maytal G. Clinical Challenges to the Delivery of End-of-Life Care. The Primary Care Companion to the Journal of Clinical Psychiatry. 2006;08(06):367-72.
[32] Doleman B, Heinink TP, Read DJ, Faleiro RJ, Lund JN, Williams JP. A systematic review and meta-regression analysis of prophylactic gabapentin for postoperative pain. Anaesthesia. 2015;70(10):1186-204.
[33] Haanpää M, Cruccu G, Nurmikko TJ, McBride WT, Docu Axelarad A, Bosilkov A, et al. Capsaicin 8% patch versus oral pregabalin in patients with peripheral neuropathic pain. Eur J Pain. 2016;20(2):316-28.
[34] Francesca B, Annunziato L, Taglialatela M. "Retigabine And Flupirtine Exert Neuroprotective Actions In Organotypic Hippocampal Cultures". Neuropharmacology. 2006;51(2):283-94.
[35] Su TR, Zei WS, Su CC, Hsiao G, Lin MJ. The Effects of the KCNQ Openers Retigabine and Flupirtine on Myotonia In Mammalian Skeletal Muscle Induced By A Chloride Channel Blocker. Evidence-Based Complementary and Alternative Medicine. 2012;803082.
[36] Preston KL, Funderburk FR, Liebson IA, Bigelow GE. Evaluation of the Abuse Potential of the Novel Analgesic Flupirtine Maleate. Drug Alcohol Depend. 1991;27(2):101-13.
[37] Zonegran. European Medicines Agency, 2013. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Scientific_Discussion/human/000577/WC500052398.pdf. Access Date: 2 Nov. 2016.
[38] Mishra S, Choudhary P, Joshi S, Bhatnagar S. Successful Use of Flupirtine in Refractory Neuropathic Pain Due To Small Fiber Neuropathy. American Journal of Hospice and Palliative Medicine. 2013;30(1):91-93.

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Original article

A study on the effectiveness of teaching the principles and methods of clinical audit to medical postgraduates

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ABSTRACT

Background: Clinical audit is based on the concept of quality assurance in health care. It is important for all health care providers especially specialists and consultants to be aware of the principles governing clinical audit in addition to the methods in conducting such an audit.
Objectives:
- To assess the knowledge and attitude of medical postgraduate students on clinical audit.
- To evaluate the effectiveness of an interventional program conducted to educate the principles and methods of conducting clinical audit to medical postgraduates.

Methods: The study was done in 92 postgraduate medical students in our institute using a pre-test and post-test questionnaire, evaluating their knowledge on different perspectives of medical audit and also assessing the change in their attitude towards audit after an educative interventional program. The questionnaire consisted of 13 knowledge questions and 3 attitude questions. Each question was scored and the overall maximum score was 35.
Results: The mean post-test score (24.45 ± 3.21) was significantly higher than the mean pre-test score (16.18 ± 3.41) using paired *t*-test ($p < 0.001$) indicating that the educational intervention was effective in improving the understanding of the candidates on clinical audit.
Conclusion: It is imperative for all medical postgraduates to realize the importance of clinical audit in addition to acquiring the skill of conducting a meaningful and successful clinical audit. Attainment of such a competency can be achieved through planned repeated interventions and help usher in an era of best quality of patient care.
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1. Introduction

It is important for all clinicians to realize the growing importance of the clinical audits. A clinical audit is considered as an integral part of the clinical practice. It is a quality improvement process that seeks to improve patient care and outcomes.[1,2] Evaluation of patient outcomes in the field of medicine have been conducted since as early as 1853.[3] The aim of the clinical audit process is to ensure adequate quality of care in the management of patients. With the current day and age of advanced medical care, it is crucial to review and constantly improve the standard of care provided. This requires sustained efforts in the unrelenting strive towards providing the best possible quality of care to patients.

The "Quality Initiative" considered forerunner of clinical audit, was launched by the Royal College of General Practitioners in 1983 to define specific objectives for patient care and monitor the extent to which those objectives were met.[4] Later, the National Institute for health and Clinical Excellence (NICE) published the paper "Principles for Best Practice in Clinical Audit", which defined clinical audit as "a quality improvement process that seeks to improve patient care and outcomes through systematic review of care against explicit criteria and the implementation of change".[2]

Hence it is necessary to understand and be familiar with the underlying principles and methods in conducting a clinical audit. This is especially important from the perspective of the medical

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post-graduate who is not only the future specialist and consultant but an important stakeholder in ascertaining whether quality care is offered to all patients in a particular health care unit. It is therefore essential for all medical postgraduates in various disciplines to have first-hand knowledge of clinical audit. It is also being increasingly recognized by the academic sectors that postgraduates should be trained to conduct clinical audit conceptualizing it as an essential component of evidence based medicine.[5,6]

Accordingly, our study was designed to assess the knowledge of postgraduate students on the principles and methods of clinical audit in our tertiary care teaching hospital and also to evaluate the effectiveness of an interventional training program in medical audit which was conducted specifically with the objective of sensitizing and imparting knowledge on how to conduct a clinical audit.

2. Materials and methods

The study protocol was approved by the institutional human ethics committee before the start of the study (Approval Number: 13/289). The study was done prospectively in a tertiary care teaching medical college hospital using a pre-test and post-test questionnaire that was validated by a team of three experts from the hospital clinical audit committee and institutional quality control committee. After obtaining a written informed consent, the questionnaire was administered to the postgraduate students at the beginning and at the end of an educational program on how to conduct clinical audit by audit experts. The questions framed evaluated the knowledge and attitude of medical postgraduates about clinical audit before and after the program. The data was collected from the participants of two consecutive programs conducted in two successive years. Each participant attended only one of the two consecutive sessions. The questionnaire consisted of 16 questions out of which 13 were knowledge and three were attitude questions.

2.1. Statistical analysis

The questions were scored and statistical difference in the mean pre-test and post-test score was analyzed using paired *t*-test. The results of the survey was also analyzed qualitatively and expressed as frequency distribution of percentages.

3. Results

A total of 92 postgraduates participated in the study. The study which was done at a single centre in two consecutive years involving two different batches of 2nd year postgraduates from various specialties, 39 students participated in the first year and the remaining 43 in the next successive session in the following year.

The first question on the need for an audit evoked diverse array of responses both in pre and post-test. The four options which were included were (1) audit has to be an integral part of clinical practice, (2) done to evaluate adverse patient outcomes, (3) as academic program in medical education and (4) administrative evaluation of patient care. Though all the four choices may be apparently appropriate, doing an audit as a mere administrative evaluation is unjustified as the objective of any clinical audit is betterment of patient care, based on which, the first three options are considered the correct responses. At the end of the session, it was observed that about 60% had clearly excluded administrative evaluation though only 20% of them had acquired complete knowledge on the need for clinical audits in contrast to only 8% who were aware of this before the program as made obvious by pre-test analysis. It was apparent that the term 'audit' had been deeply entrenched with the belief of being a tool for administrative evaluation, as made evident from a statistical comparison of pre-test and post-test scores ($p = 0.904$) (Table 1).

Answering the question on types of clinical audit which can be standard based, adverse occurrence screening or critical incident monitoring and peer reviewed, only 50% in the pre-test agreed that all three were indeed the audit types while the post-test revealed that 82.6% accepted "all the above" as the appropriate response.

In assessing the understanding of the students on the types of evaluation, design of the audit and the stages in the audit cycle, it could be demonstrated that there was definitive acquisition of knowledge on these aspects of audit based upon the increase in percentage of the responders opting for the correct answer in the post-test when compared to the pre-test (Figs. 1 and 2).

On the ethics approval perspective, a shift in the responses favoring the need for ethics committee approval before publication of the results (54.3%) rather that at the beginning of the audit (9.8%) was evident in the post-test, contrary to the impression of most students in the pre-test that ethics committee's approval had to be obtained before the start of an audit (59.8%). A considerable increase in the proportion of participants who indicated that there

Table 1
Comparison of pre-test and post-test scores on knowledge questions in clinical audit.

Knowledge/Attitude on clinical audit	Pre-test score Mean ± SD	Post-test score Mean ± SD	Mean difference of the paired sample ± SD	P value
Need for audit	0.47 ± 0.68	0.49 ± 0.68	−0.02 ± 0.85	0.904
Types of audit	2.78 ± 1.48	3.51 ± 1.11	−0.72 ± 1.85	<0.001
Types of evaluation	0.67 ± 0.95	1.37 ± 0.93	−0.69 ± 1.12	<0.001
Design	1.52 ± 0.85	1.96 ± 0.29	−0.43 ± 0.82	<0.001
Steps in audit cycle	0.44 ± 0.83	1.82 ± 0.56	−1.38 ± 1.01	<0.001
Ethical approval process	0.53 ± 0.79	1.42 ± 0.69	−0.89 ± 1.02	<0.001
Who can conduct audit	3.64 ± 0.97	3.87 ± 0.61	−0.22 ± 1.01	0.034
Who can make recommendations	1.28 ± 1.05	1.63 ± 1.06	−0.34 ± 1.50	0.029
Tool for prioritizing topic	0.61 ± 0.92	1.24 ± 0.97	−0.63 ± 1.14	<0.001
Defining aims	0.93 ± 1.00	1.52 ± 0.85	−0.58 ± 1.09	<0.001
Criteria selection	0.54 ± 0.89	1.35 ± 0.94	−0.80 ± 1.15	<0.001
Standard definition	0.76 ± 0.97	1.50 ± 0.87	−0.73 ± 1.35	<0.001
Commonest method involving service users	0.58 ± 0.49	0.78 ± 0.41	−0.20 ± 0.56	0.01
Audit is not research	0.51 ± 0.50	0.91 ± 0.28	−0.40 ± 0.51	<0.001
Professional responsibility	0.71 ± 0.45	0.82 ± 0.38	−0.11 ± 0.50	0.041
Achievement of same performance level as in research indicates lag in quality	0.20 ± 0.39	0.26 ± 0.44	−0.06 ± 0.48	0.203
Overall score	16.18 ± 3.41	24.45 ± 3.21	−8.28 ± 4.02	<0.001

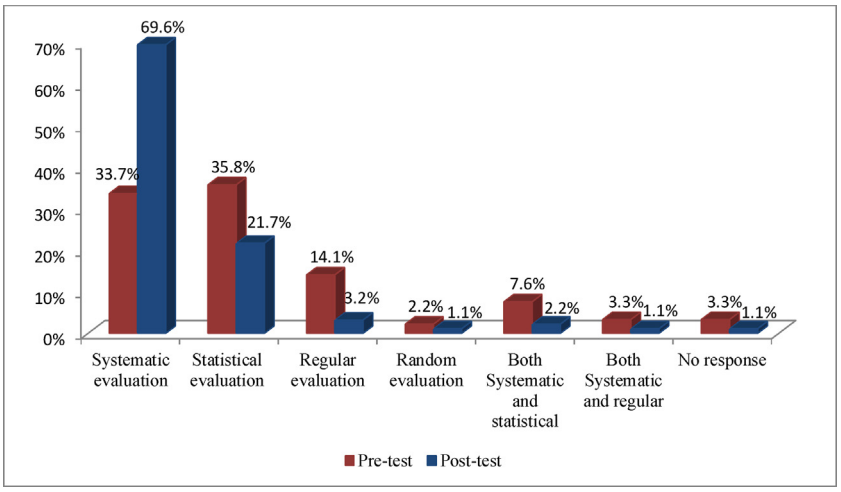


Fig. 1. Percentge of responses to types of evaluation for structure, processes and outcomes of care in clinical audit.

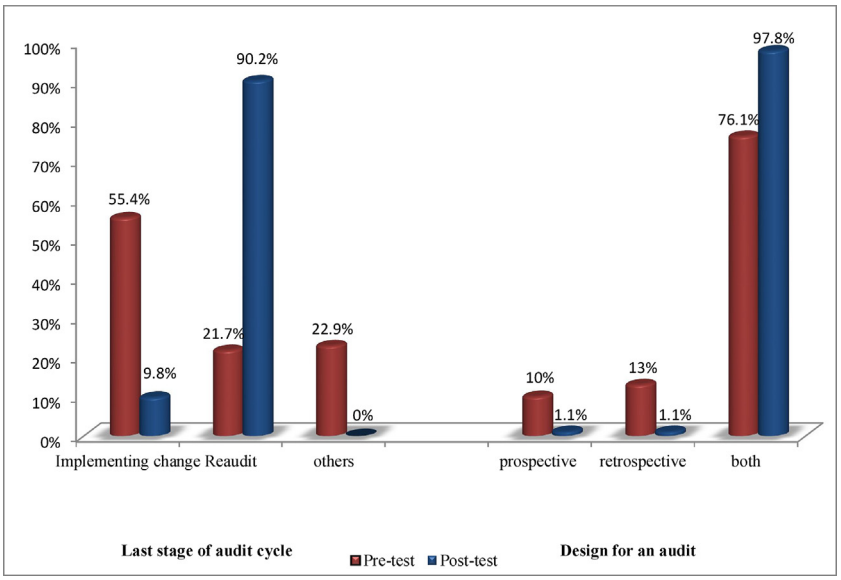


Fig. 2. Percentage of responses to steps in an audit cycle and the design for an audit.

was no need for ethics approval for the conduct of a clinical or therapeutic audit was also noted in the post-test (33.7%).

Appraisal of the postgraduate students' knowledge on who can conduct an audit and who can make recommendations based on an audit result, drafted as two separate questions made it evident that the session had enlightened 97.8% on the fact that any medical professional including an administrator can conduct an audit whilst the question on recommendation which was structured as dual correct response one, namely the PI (principal investigator) and the hospital audit team, only 5.5% responded with completely correct answer in contrast to 72.8% who had chosen only one of the correct responses at the end of the session though it was obvious that there was an increase in the percent of single correct response compared to the pre-test results (59.8%).

Knowledge on the tool for prioritizing the audit topic showed an increase, demonstrated by the rise in percentage of students opting the right response in the post-test (69.6%) compared to the pre-test (30.4%) (Fig. 3). Likewise their understanding on defining the aims, criteria selection and comprehending the standard revealed augmentation, manifested by selection of the correct choice indicating an improved knowledge on these concepts by large majority of participants (Fig. 4).

Attitude questions patterned on yes/no response ascertained that the students considering audit as yet another research was high before the program (40.2%) but they were able to discern the difference between an audit and research subsequent to the intervention (91.30%). In addition, a high proportion of participants were able to appreciate that auditing forms part of their professional accountability at the end of the program (82.6%). However, it was clear that the study subjects had not undoubtedly understood that failure to achieve the same performance level as that in research was often not possible in unselected patient populations in a practical clinical scenario; only 60.9% had chosen the right response in the post-test (Fig. 5).

Evaluating the candidates' knowledge on the commonest method of audit involving patients, 85.9% rightly agreed that it was satisfaction survey in the post-test contrary to only 57.6% who were in favor of it in the pre-test. Each question was scored with maximum total score being 35. A paired *t*-test was done to assess if the difference in mean score for post-test (24.45 ± 3.21) was statistically higher compared to that of the pretest (16.18 ± 3.41)

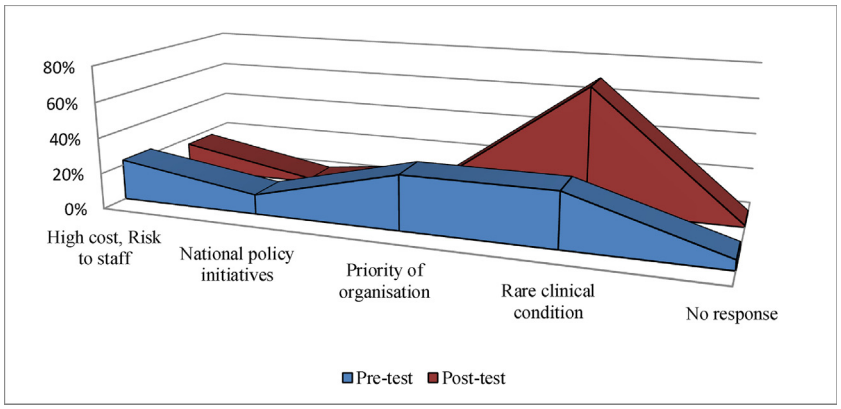


Fig. 3. Frequency distribution of responses to "the tool for prioritizing the topic for audit are all except" expressed in percentages.

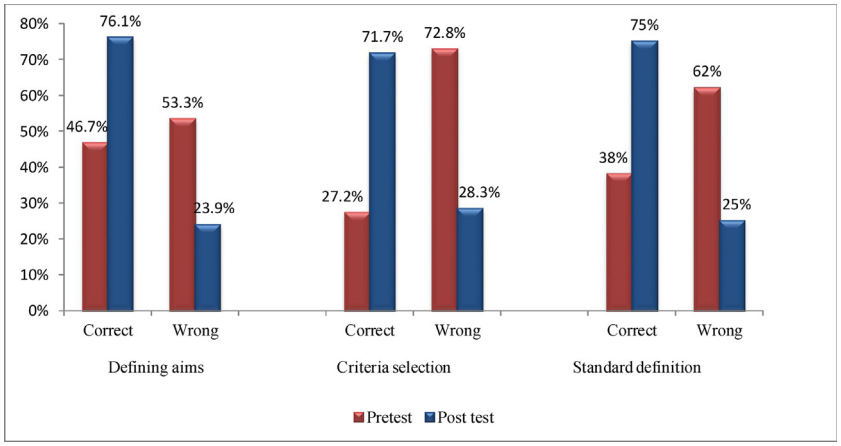


Fig. 4. Frequency distribution of the percentage of correct and wrong responses to the parameters of aims, criteria and standard in an audit.

and it was found that a statistically higher ($p < 0.001$) mean post-test score was achieved (Table 1).

4. Discussion

This study involving 92 postgraduate students in a tertiary care teaching hospital revealed that the knowledge and understanding on the principles and concepts were quite limited as was evident from the pretest analysis. However the planned intervention in the form of an educational session was effective in conveying the basic fundamentals regarding clinical audit to the participants. This was discernible from the statistically greater mean score attained in the post-test compared to the pre-test (Table 1).

In analyzing the individual components, it was demonstrable that elements such as types of audit, types of evaluation, design and stages, ethics committee approval for an audit were well comprehended by the participating candidates following the intervention. Likewise their understanding on the prioritizing tool,

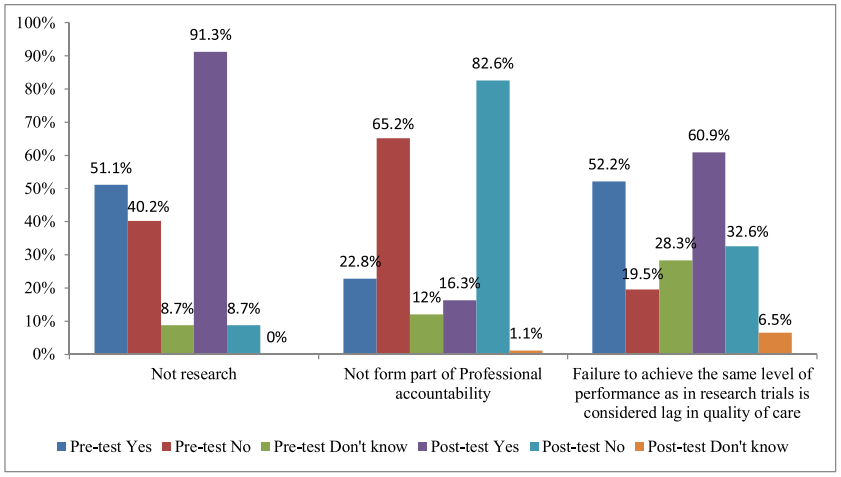


Fig. 5. Percentage distribution of responses to the attitudinal aspects of an audit.

defining aims, criteria selection and the meaning of standard showed statistically significant improvement ($p < 0.001$). A fairly good knowledge was also gained on who can conduct ($p = 0.034$) and who can make recommendations following an audit ($p = 0.029$). The participants were able to appreciate the role played by the PI in making recommendations proved by the shift in the choice of responses from the hospital audit team in the pre-test to the PI in the post-test.

An equal magnitude of improvement was not seen in the understanding on the need for an audit because a considerable majority of participants, despite the program, erroneously believed that the conduct of clinical audit was purely on administrative motive; this was made obvious by the post-test score on this element (Table 1). Also, their attitude towards achieving the same performance level as in research to ensure the best quality of care was rather unaltered (Fig. 5). Such misbelieves and misconceptions seem to be deeply rooted signifying the need for repetitive and sustained measures such as continuing medical education programs, symposia and workshops in clinical audit to enable better understanding as well as need for an attitudinal change towards such clinical audit facets.

Introspecting on the need for such programs targeting the medical postgraduates reveals that this measure not only provides a good learning experience but also would ensure ready involvement of these future consultants in the quality improvement process, as rightly pointed out by Walshe that the outcomes of an audit depend on 'who did it and how it was done'.[7] In addition, enhanced understanding of the audit concepts would pave the way for weeding out the lack of commitment, poor record keeping and inadequate data entries which are the deterrents in the path of quality improvement. Assuming the role of audit lead to facilitating teamwork are the positive attributes that would stem from the in-depth knowledge thus gained. It would not be an exaggeration to say that incorporating the competency of participation in medical audit as a module in postgraduate medical curriculum would be a step forward in implementing evidence based medicine in clinical care, since it is understandable that practicing medicine does not merely stop with updating on current research evidence of best practice but being able to show that such new standards of care is achieved in one's own practice through the clinical audit findings. Thus postgraduate training should encompass clinical audit which is considered as an integral part of clinical governance, for ascertaining the best clinical care to patients.[8]

5. Conclusion

This study has highlighted that educational programs on clinical audit with the objective to impart knowledge and improve understanding of the principles, concepts and methods should be designed and conducted for the postgraduate medical students of diverse specialties for enabling them to acquire the skill of conducting clinical audit which would ensure sustained quality control in patient care.

Authors contributions

The first author was involved in concept, design, data acquisition, interpretation, analysis, drafting and approval of manuscript.

The second author was involved in concept, design, data analysis, drafting and approval of manuscript.

Conflict of interest

The authors have none to declare.

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References

1. Dixon N. *Good Practice in Clinical Audit: A Summary of Selected Literature to Support Criteria for Clinical audit*. National Centre for Clinical Audit; 1996.
2. National Institute for Clinical Excellence (Great Britain). *Principles for Best Practice in Clinical Audit*. Radcliffe Publishing; 2002.
3. Lokuarachchi S. Clinical audit-what is it and how to do it? *Galle Med J*. 2009;11:41–4310.4038/gmj.v11i1.1122.
4. Chen M. Clinical audit. *J Clin Exp Ophthalmol*. 2012;3:1000107.
5. Glasziou P, Burls A, Gilbert R. Evidence based medicine and the medical curriculum. *BMJ (Clini Res Ed)*. 2008;337:704–705.
6. Rhodes M, Ashcroft R, Atun RA, Freeman GK, Jamrozik K. Teaching evidence-based medicine to undergraduate medical students: a course integrating ethics, audit, management and clinical epidemiology. *Med Teach*. 2006;28:313–317.
7. Walshe KM. Principles for best practice in clinical audit. *Qual Saf Health Care*. 2002;11:392.
8. Straus SE, Ball C, Balcombe N, Sheldon J, McAlister FA. Teaching evidence-based medicine skills can change practice in a community hospital. *J Gen Intern Med*. 2005;20:340–343.

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Original Article

Pharmacology Section

Knowledge and Attitude Towards the Translation of Medical Research to Clinical Benefit Amongst Researchers in A Tertiary Care Hospital

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ABSTRACT

Introduction: One of the primary goals of any biomedical researcher should be practical applicability or the translation of scientific discoveries to clinical care. Research that lacks potential for translation contributes to ineffective use of resources, time and manpower. Hence, it is important for researchers to be aware that the clinical significance should not be forgotten in the quest for a significant p-value.

Aim: To assess the knowledge of researchers in ascertaining the practical applicability of his/her own research, to ascertain their motives in planning research and to evaluate the attitude of researchers towards the ethics of doing research that is less likely to translate into clinical benefit.

Materials and Methods: This cross-sectional study was conducted at a tertiary care medical college hospital in South India. Teaching faculty from clinical, pre and para-clinical departments were administered a questionnaire which included 14 questions. The questionnaire consisted of five knowledge and nine attitude questions. The responses were analysed qualitatively and expressed as frequency distributions.

Results: A total of 60 researchers were included and given the questionnaire. The respondent rate was 32 (53.3%). Only 3 (9.4%) were completely aware of "translational research" while about 11 (34.4%) knew that research done by pre & para-clinical sciences could be both basic and translational. Also, 17 (53.1%) of participants were willing to do research that did not result in either immediate or direct clinical benefit. A small minority of 4 (12.5%) admitted that the focus of their research was to achieve statistical significance. About 10 (31.3%) of researchers surveyed and felt that research offered an opportunity for free treatment to patients and 5 (15.6%) felt that therapeutic misconception was acceptable.

Conclusion: This study identified the gap between knowledge and understanding of the practical applicability of research and its ethical perspectives amongst researchers and hence, indicates the need for increasing awareness on the importance of assessing the translatability of one's research.

Keywords: Clinical significance, Ethics, Practical applicability, Statistical significance

INTRODUCTION

In the current era, there is a substantial increase in the number of researches done and published. The current system of publication in biomedical research provides a distorted view of the data generated [1]. There is too much focus on statistical significance, with parameters more important than statistics fading into oblivion [2]. The primary goal of any biomedical researcher should be practical applicability which refers to the translation of the scientific discoveries to clinical care [3]. Translational research refers to transfer of knowledge from bench to bedside [4].

Biomedical research tends to be skewed as most data generated are positive and mushroom around a particular topic at a particular point in time [1]. When a researcher looks to find solutions for his or her own clinical problems, research is done with a practical end point in view. However, when there is no room for this, research tends to lose quality.

Improper study designs waste resources and generate results that are not strong enough to translate to clinical practice. Small effect sizes are chosen to generate statistical significance more readily [5]. However, statistical significance is not the same as clinical significance and this tends to be forgotten in the quest for a 'significant' p-value [2]. From an epidemiologist's perspective, the relevant question is whether the study's hypothesis is true i.e. the probability of the hypothesis being greater than 0.05? For clinicians, the relevant question is whether a particular strategy can be followed in an individual patient or a subset of similar patients [6].

We believe that it is also unethical to expose the patient or healthy volunteer to any risk, however, small or theoretical, if it does not have the potential to benefit him and/or society. So, this study was designed to evaluate the knowledge of the researchers in assessing the practical applicability of his or her research in future patient care. Secondly, the study also aimed to ascertain the motives of the researcher towards doing research and to evaluate the attitude of the researcher towards the ethics of doing research which is less likely to translate into clinical benefit.

MATERIALS AND METHODS

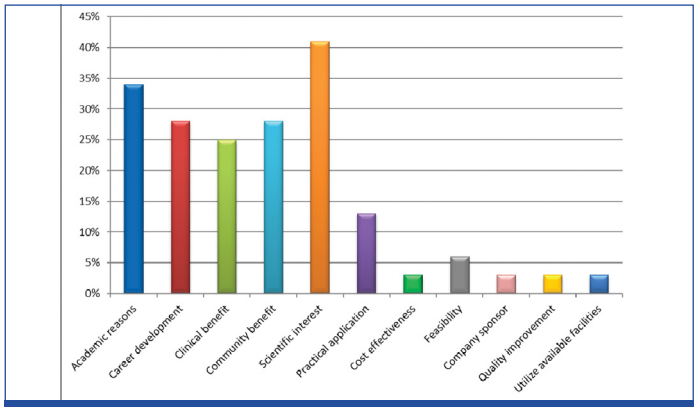
This cross-sectional study was done after the approval of Institutional Human Ethical Committee (Approval number: 13/250) for six months from November 2014 to April 2015. Teaching faculty of our tertiary care and teaching hospital in Coimbatore, South India was surveyed with a questionnaire to evaluate their knowledge on assessing the practical applicability of their research and their attitude towards the ethics of research lacking the potential for translation. The study participants recruited were teaching faculty who had involved in research while postgraduates, residents and faculty who had not done any research were excluded.

The questionnaire consisted of 14 questions, of which five were knowledge questions and the remaining were attitude questions. The questionnaire was drafted to be self-administered and it was validated by a team of three members who were researchers belonging to the three levels namely, Assistant professor, Associate professor and Professor; thus representing three different strata with reference to both the quantum and experience in research. Reliability analysis done using SPSS software revealed a cronbach's alpha value of 0.797. After obtaining a written informed consent from each participant, the questionnaire was administered. The responses were qualitatively analysed and expressed as frequency distribution of percentages. Detailed statistical analysis was deferred to enable meaningful expression and lucidity of inferences from the qualitative data.

RESULTS

Out of 60 researchers, who were included for the study and given the questionnaire, only 32 responded by returning the completed questionnaire. Out of 32 teaching faculty who participated in the study, 16 (50%) were Professors, 8 (25%) Associate Professors and 8 (25%) were Assistant Professors. The rest either returned an unfilled questionnaire or failed to give it back. Thus, the respondent rate for the study was 32 (53.3%) and the reasons offered by most for non-participation was lack of time and interest.

There were varied qualitative responses from the participants to the first question on three motives for research. The research intentions were categorized based on the responses specified into 11 categories. It was found that majority had indicated scientific interest 13 (40.6%) and academic endeavours which



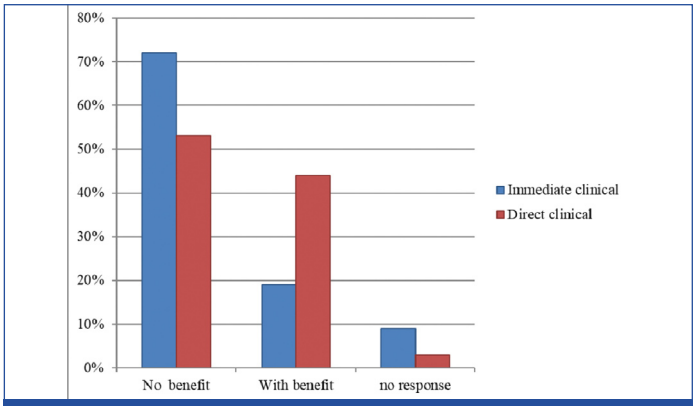
[Table/Fig-1]: Motives indicated for research (%).

included guideship responsibilities, interdisciplinary research, or mandatory involvement in clinical audits 11 (34.4%) as the driving forces in their research involvement [Table/Fig-1].

It is fundamental for the researchers to be familiar with the term "translational research" which denotes translating knowledge from basic sciences to clinical studies as well as translating clinical trials to everyday practice. On this perspective, it was found that the understanding of what translational research means was incomplete; 24 (75%) of participants were partially aware in contrast to mere 3 (9.4%) with complete knowledge about this term. The rest were totally unaware of translational research indicated by their lack of response.

In addition, assessment of the knowledge on research done in preclinical and paraclinical departments, structured as a dichotomous true/false question, revealed that only 11 (34.4%) of participants were aware that research done in pre and para-clinical sciences could be both basic and translational.

Also, 17 (53.1%) of participants were willing to do research that did not result in direct clinical benefit and 23 (71.9%) of them

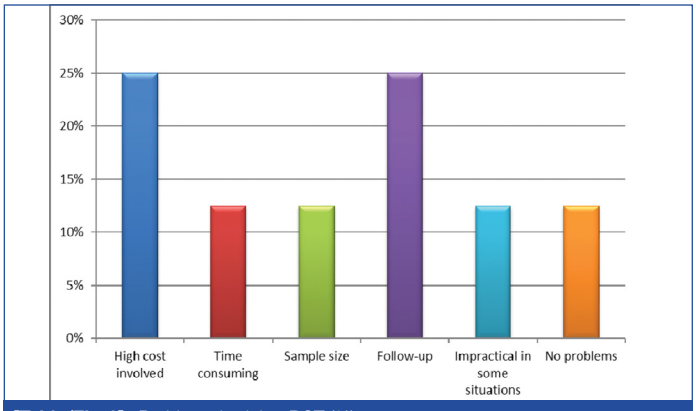


[Table/Fig-2]: Attitude towards doing research and its clinical benefit (%).

opined that their research would not have immediate clinical benefit [Table/Fig-2]. In appraising the attitude of the researchers towards the outcome of testing their hypothesis, a small minority of about 4 (12.5%) admitted that the focus of their research was to achieve statistical significance whilst the rest ascribed that their primary aim in research was to confer greater importance to the clinical significance 10 (31.3%), practical applicability 11 (34.4%) while 7 (21.9%) were in favour of analysing the results irrespective of the statistical significance.

A couple of knowledge questions on sample size and Randomized Clinical Trial (RCT) design revealed that 24 (75%) were aware that smaller the sample size in a study, more likely the research findings cannot be relied upon. On the other hand, the remaining 8 (25%) were unaware of the fact that the sample size could influence the research outcome, out of which 1 (3%) had opted not to answer the question on sample size.

An estimate of only 8 (25%) had the experience of being involved in a RCT and only to this fraction of the study population was the question regarding experiential knowledge on the problems faced during the RCT was directed. The difficulties that they had enumerated included cost, time, achieving the required sample size, follow up of the participants and the impracticality of



[Table/Fig-3]: Problems in doing RCT (%).

addressing certain hypothesis using RCT design. Amongst these responses, cost and follow-up issues ranked highest with 25% each [Table/Fig-3].

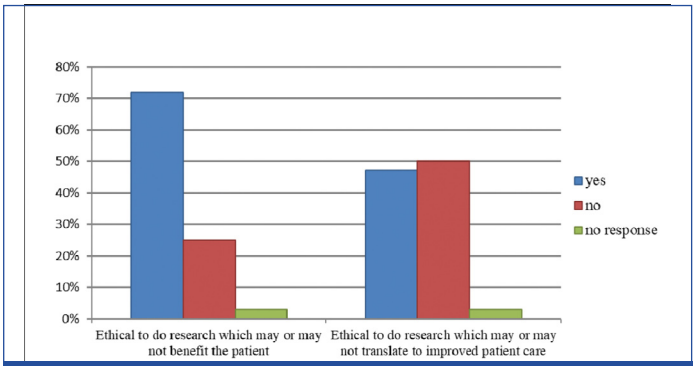
In assessing the attitude of those not having done any RCT 24 (75%), the reasons offered by a greater percentage of participants were that RCT design was not applicable to their research 3 (12.5%) and that they were apprehensive on the difficulties in the conduct of a randomized study 3 (12.5%) whilst 4 (16.7%) revealed that they had not such an opportunity. Two (8.3%) attributed it to practical constraints. The rest indicated lack of motivation.

A rating scale to assess the frequency with which the researchers evaluated the practical application of their research, before

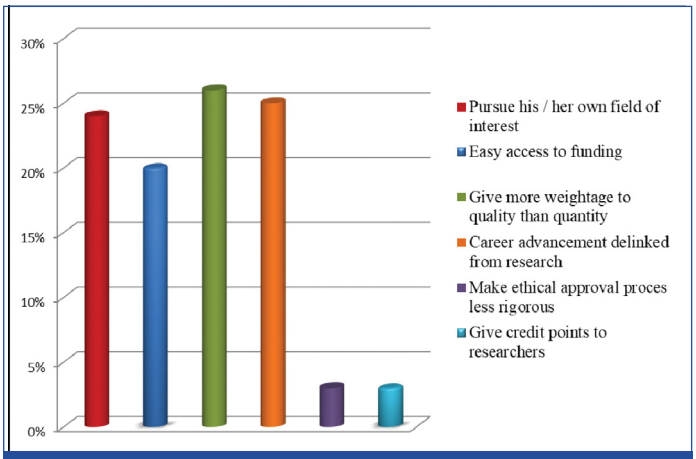
formulating their hypothesis demonstrated that 11 (34.4%) did so "always" and an equal percentage admitted that they did such an evaluation "most of the times."

Interrogating on the ethical aspects, 23 (71.9%) researchers divulged that it was ethical to do research which may or may not directly benefit the patient. Surprisingly, 15 (46.9%) agreed that it was ethical to do research which may or may not translate to clinical benefit [Table/Fig-4].

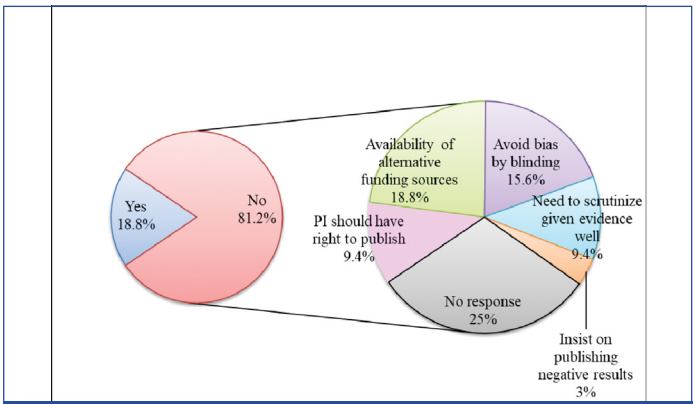
A large majority of study participants 25 (78.1%) opined that the current "publish or perish" situation was unhealthy for translational research. A valid list of strategies identified for improving translational research which most voted for, were



[Table/Fig-4]: Attitude towards research ethics (%).



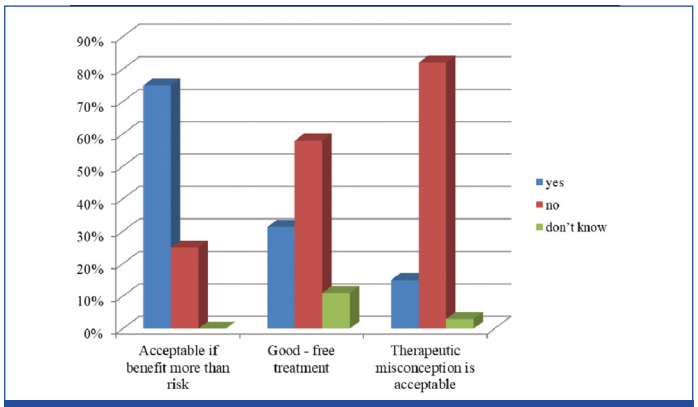
[Table/Fig-5]: Strategies to improve translational research (%).



[Table/Fig-6]: Research in collaboration with drug companies is always healthy (%).
PI - Principal Investigator

to give greater significance to quality than to the quantity of research, allowing one to pursue his/her own interest, delinking research from carrier progression and enabling access to easy funding [Table/Fig-5].

Twenty five (81.2%) were convinced that research in collaboration with drug companies was unhealthy due to a variety of reasons [Table/Fig-6]. Those who felt otherwise 6 (18.8%) did so because such collaboration enabled research, offered funding source, provided easy compensation and facilitated achieving a larger sample size. Twenty four (75%) opined that research which attributed some risk to an individual research subject or a group of research subjects was acceptable only if the possible benefit



[Table/Fig-7]: Questions regarding attitude towards certain aspects of research (%).

to the community was large. It was shocking to discover that 10 (31.3%) felt that clinical research offered an opportunity for free treatment to patients who enrolled themselves in the trial and 5 (15.6%) felt that therapeutic misconception was acceptable [Table/Fig-7].

DISCUSSION

Translation of basic medical research refers to the transfer of new understanding of disease mechanisms gained in the laboratory into clinical care [4]. Though, there are studies on various perspectives of translational research, ours is the first study done to assess the knowledge and attitude of researchers towards translational value of their research findings. The study has brought out that there is only incomplete understanding on translational research and in addition, a high percentage of study subjects were unaware that research in pre and para-clinical departments could be both basic and translational.

It is important for all academic researchers to be aware of "translational research" terminology [7] as they are one of the important stakeholders who are committed not only in identifying and confirming novel concepts through appropriate research, but are also involved in validating the potential of new discoveries to translate to clinical application and improving therapeutic outcomes, thereby culminating in better patient care [8].

In spite of the fact majority of participants have indicated scientific interest as the motive for research, an equally high percentage have revealed that their research endeavours, was part of the academic requirement and was on account of career advancement [Table/Fig-1]. Linking career advancement with the number of publications is indeed a double edged weapon since it motivates the basic scientists and clinicians to undertake more research, thereby promoting research activities; however, the same may not always be true from a translational perspective. This is reflected by a high proportion of study participants disfavouring the "publish or perish" standpoint prevailing as it is unhealthy for translational research.

Clinical evidence of efficacy relies on the dissemination of research results, usually by publication in medical journals which is a critical step that would impact further progression of the research findings [9]. A recent review identified 101 articles published in six top basic science journals which had an apparent promise for development as a major clinical application and yet

only five of these promising advances has been licensed for clinical use over a period of 20 years; out of which only one had created a major impact on medical practice. Three quarters of the promised interventions from basic science papers do not proceed to randomized trial. The article also reported that the strongest predictor of moving to randomized experimentation was industry involvement in the original basic science publication [10].

Science is subject to great uncertainty. An empirical evaluation of the 49 most-cited papers on the effectiveness of medical interventions, published in highly visible journals in 1990–2004, showed that a quarter of the randomized trials and five of six non-randomized studies had already been contradicted or found to have been exaggerated by 2005 [11].

The plethora of scientific information gained through research, promoted largely by academic requisite for career advancement, coupled to limited venues for publication (journals with sufficiently high impact) are factors which deter research in the translation path. Moreover, the current system of publication further perpetuates the publication bias [1]. The more extreme, spectacular results indicating large treatment effects are preferentially published as is evident from the paucity of negative data which are more difficult to get published [12]. The abundance of positive findings published in scientific journals marks the tell-tale effect of selective publishing which not only hampers translation of research findings but also questions the discriminating value of the statistical significance [12-14].

In-depth knowledge of the researchers on the sample size and the relevant design for the research hypothesis is warranted. A study done analysing 215 two arm parallel group RCT of superiority with a single primary outcome published in six high impact factor general medical journals between 1st January 2005 and 31st December 2006 revealed that 5% of reports did not have any sample size calculation and the difference between the sample size reported in the article and the replicated sample size calculation was greater than 10% in 30% of the 157 reports that gave enough data to recalculate the sample size [15]. Thus, every component of a RCT is important for its success; if the design or sample size is inappropriate, then the results of the study will be unreliable, however, well the study is conducted [16].

As it has been rightly highlighted by many participants, there are a number of constraints for conducting RCT which is considered as the gold standard for interventional studies. Apart from implementation issues, complexity involved in understanding the level of clinically meaningful improvement, the expected variation of improvement in the sample, high dropout rates during follow-up are other disadvantages which hinder the choice of RCT design by researchers [17].

The study has made it obvious that researchers are apprehensive about industrial collaboration despite some advantages like the easy access to funding for the conduct of the trial, alleviation of difficulties in providing compensation, use of high quality methods [18] and potential for publication in high impact journals [19]. Nevertheless, industry funding and collaboration in trial design, data collection and analysis have been found to be associated with an increased likelihood of reporting a positive primary outcome and decreased prospect of reporting of trial limitations. Collaborative trials more commonly use surrogate primary endpoints which may, in part, explain why these trials are more likely to have positive primary outcomes [20]. Thus, our results signify that researchers are concerned with interference and influence of the study results and its dissemination by the sponsoring company.

Yet another remarkable finding established is that there is inadequate understanding on the ethical concepts of research as well. Though, ethical challenges for translational research remains the same as clinical research, it should be realized that considerable difference do exists as clinical research is regarded to be ethically distinct from medical care, while translational research emphasizes the links between the two, showing how both need to be grounded in the ethics of doctor-patient relationship [3]. Thus, there is requisite for a different approach towards translational research ethics which reaches farther beyond the research ethics and medical ethics [21]. This study has implied that it is clearly essential to impart ethical concepts to researchers to augment their understanding on ethical principles governing research.

LIMITATION

The major limitation of this study was the small sample size and limited experience of the researchers in RCT designs or trials with industry collaboration. A multi-centric design involving a large sample size from different tertiary centers and medical colleges across India will enable to capture a broader picture of the challenges to translational research.

CONCLUSION

The study has delineated the need for rigorous implementation of educative programs for all researchers to enhance their understanding on the significance of ensuring the practical applicability or translatability of research in addition to improving their knowledge on ethical perspectives. Ultimately, research should be aimed at finding solutions to the everyday clinical problems inorder to have a greater practical end point in view.

REFERENCES

[1] Young NS, Ioannidis JP, Al-Ubaydli O. Why current publication practices may distort science. PLoS Med. 2008;5(10):e201.
[2] Ioannidis JPA. Why most published research findings are false. PLOS Med. 2005;2(8):e124.
[3] Petrini C. Ethical issues in translational research. Perspect Biol Med. 2010;53(4):517-33.
[4] Sung NS, Crowley WF Jr, Genel M, Salber P, Sandy L, Sherwood LM, et al. Central challenges facing the national clinical research enterprise. JAMA. 2003;289(10):1278-87.
[5] Shrier I. Power, reliability, and heterogeneous results. PLoS Med. 2005;2(11):e386.
[6] Pauker S. The clinical interpretation of research. PLoS Med. 2005; 2(11):e395.
[7] Woolf SH. The meaning of translational research and why it matters. JAMA. 2008;299(2):211-13.
[8] Wang X, Marincola FM. A decade plus of translation: what do we understand? Clin Transl Med. 2012;1(1):3.
[9] Douet LJ, Preedy D, Thomas V, Cree IA. An exploratory investigation of the influence of publication on translational medicine research. J Transl Med. 2010;8:62.
[10] Contopoulos-Ioannidis DG, Alexiou GA, Gouvias TC, Ioannidis JP. Medicine. Life cycle of translational research for medical interventions. Science. 2008;321(5894):1298-99.
[11] Ioannidis JP. Contradicted and initially stronger effects in highly cited clinical research. JAMA. 2005;294(2):218-28.
[12] Kavvoura FK, Liberopoulos G, Ioannidis JP. Selection in reported epidemiological risks: an empirical assessment. PLoS Med. 2007;4(3):e79.
[13] Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Almost all articles on cancer prognostic markers report statistically significant results. Eur J Cancer. 2007;43(17):2559-79.
[14] Turner EH, Matthews AM, Linardatos E, Tell RA, Rosenthal R. Selective publication of antidepressant trials and its influence on apparent efficacy. N Engl J Med. 2008;358(3):252-60.
[15] Charles P, Giraudeau B, Dechartres A, Baron G, Ravaud P. Reporting of sample size calculation in randomised controlled trials: review. BMJ. 2009;338:b1732.
[16] Morley R, Farewell V. Methodological issues in randomized controlled trials. Semin Neonatol. 2000;5(2):141-48.

[17] Levin KA. Study design VII. Randomised controlled trials. Evid Based Dent. 2007;8(1):22-23.
[18] Linker A, Yang A, Roper N, Whitaker E, Korenstein D. Impact of industry collaboration on randomised controlled trials in oncology. Eur J Cancer. 2017;72:71-77.
[19] Sun GH, Houlton JJ, MacEachern MP, Bradford CR, Hayward RA. Influence of study sponsorship on head and neck cancer randomized trial results. Head Neck. 2013;35(10):1515-20.
[20] Roper N, Zhang N, Korenstein D. Industry collaboration and randomized clinical trial design and outcomes. JAMA Intern Med. 2014; 174(10):1695-96.
[21] Hostiuc S, Moldoveanu A, Dascalu MI, Unnthorsson R, Johannesson OI, Marcus I. Translational research-the need of a new bioethics approach. J Transl Med. 2016;14:16.

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WILEY Biomedical Chromatography

RESEARCH ARTICLE

Fast and parallel determination of PCB 77 and PCB 180 in plasma using ultra performance liquid chromatography with diode array detection: A pharmacokinetic study in Swiss albino mouse

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Abstract

A simple, sensitive and reproducible ultra-performance liquid chromatography (UPLC) method has been developed and validated for simultaneous estimation of polychlorinated biphenyl (PCB) 77 and PCB 180 in mouse plasma. The sample preparation was performed by simple liquid–liquid extraction technique. The analytes were chromatographed on a Waters Acquity H class UPLC system using isocratic mobile phase conditions at a flow rate of 0.3 mL/min and Acquity UPLC BEH shield RP_{18} column maintained at 35°C. Quantification was performed on a photodiode array detector set at 215 nm and PCB 101 was used as internal standard (IS). PCB 77, PCB 180, and IS retention times were 2.6, 4.7 and 2.8 min, respectively, and the total run time was 6 min. The method was validated for specificity, selectivity, recovery, linearity, accuracy, precision and sample stability. The calibration curve was linear over the concentration range 10–3000 ng/mL for PCB 77 and PCB 180. Intra- and inter-day precisions for PCBs 77 and 180 were found to be good with CV <4.64%, and the accuracy ranged from 98.90 to 102.33% in mouse plasma. The validated UPLC method was successfully applied to the pharmacokinetic study of PCBs 77 and 180 in mouse plasma.

KEYWORDS

mouse plasma, PCB 180, PCB 77, pharmacokinetics, UPLC-dad

1 | INTRODUCTION

Polychlorinated biphenyls (PCBs) are a chlorinated class of chemicals that are divided into 209 congeners grouped across 10 homologs (US-EPA, 1989). They are emerging as persistent pollutants in the environment although their production has been restricted in many countries owing to their high lipophilicity and ubiquitous nature (UNEP, n.d.). Two such biphenyl congeners, viz. PCB 77 and PCB 180, have contrasting and unique physicochemical and toxicological characteristics. PCB 77 is a coplanar dioxin like tetra-chlorobiphenyl congener, while PCB 180 is a nondioxin-like congener with greater lipophilicity. PCB 77 is low chlorinated congener (Agency for Toxic Substances and Disease Registry, 2000), whereas, PCB 180 is heptachlorobiphenyl with more chlorination of biphenyl nucleus (Viluksela, Heikkinen, van der Ven, Rendel, & Roos, 2014). Since PCB 77 is planar with a small dihedral angle (Dasary, Saloni, Fletcher, Anjaneyulu, & Yu, 2010), it is an endocrine disrupter. Moreover as it is non-*ortho*-substituted dioxin-like congener, it is unique among endocrine disrupters, and also forms polar hydroxylated metabolites (Grimm et al., 2015). PCB 180, on the other hand, is not an endocrine disrupter as it lacks dioxin activity and is more lipophilic (Maisano et al., 2016; Matthews & Dedrick, 1984; Saija et al., 2016). This congener forms reactive metabolites in addition to hydroxylated compounds (Grimm et al., 2015). In summary, PCB 77 and PCB 180 differ from a toxicological perspective – PCB 77 is a low chlorinated neurotoxic endocrine disrupter that is more polar than the other congener, PCB 180, which is a high chlorinated nondioxin-like lipophilic persistent pollutant. PCBs and other chlorinated compounds can be estimated by isotope dilution high-resolution gas chromatography–mass spectrometry in tissues (Sather, Newman, & Ikonomou, 2003). PCBs can also be estimated in other matrices like air samples (Muir & Sverko, 2006), aroclor standard mixtures (Pietrogrande, Benvenuti, & Previato, 2000), rat liver microsomes (Zhai, Wu, Lehmler, & Schnoor, 2013), groundwater samples (Letcher, Li, &

ABBREVIATIONS: PCB, polychlorinated biphenyl

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Chu, 2005) and blood plasma (Mathews, 2014) by various gas chromatographic detection techniques.

Most studies on PCB 77 concentrations in environmental and biological samples have used gas chromatographic techniques with detection methods including mass spectrometry (Eske et al., 2014; Ghosh et al., 2010; Wang et al., 2013) and nonspectrometric methods (Brieger & Hunter, 1993; Chu et al., 1995). Studies employing PCB 180 as the analyte of interest have evaluated its concentrations in matrices using gas chromatography with electrochemical detector (ECD) detection (Bogdevich & Cadocinicov, 2001; Cossu, Ledda, & Chessa, 2013; Tian et al., 2016; Viluksela et al., 2014). Only one study (Dmitrovic, Chan, & Chan, 2002) which has evaluated both the congener serum levels has employed GC–MS. To date, there are only a handful of studies that have employed liquid chromatography for assessment of PCB concentrations (Mathews, 2014; Pietrogrande et al., 2000). None of these studies have assessed PCB 77 and PCB 180, congeners with toxicological significance and uniqueness, simultaneously, in a cost-effective and time-efficient manner employing UPLC or HPLC.

In this study, we report such a method development and validation for PCB congeners, viz. PCB 77 and PCB 180 using a novel ultra-high-performance liquid chromatographic method for simultaneous estimation of these two PCB congeners in mouse plasma samples. We have optimised a cost-effective and time-efficient method of routine estimation of these congeners in plasma samples. Additionally, one of the objectives of this study is to estimate the pharmacokinetic parameters of these congeners in plasma, after occupational exposure in mouse using a whole body inhalational chamber.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The reference standards of PCB 77, PCB 180 and PCB 101 (IS) were purchased from Sigma-Aldrich, India. Acetonitrile and methanol of HPLC grade was purchased from Sigma-Aldrich, India. Methyl *tert*-butyl ether of HPLC grade was obtained from Himedia, India. Ultrapure water (18.2 MΩ cm) was obtained from a MilliQ water purification system from Millipore (Milford, USA). All other chemicals used were analytical grade.

2.2 | Animals

Thirty-two Swiss albino mice were procured from PSG animal facility and quarantined. All animals were housed in standard housing conditions with ambient light–dark cycle (10:14 h) and environment (temperature of 24–26°C) with feed and water *ad-libitum*. The animals were weighed and grouped into cages randomly. All mice were females with 18–25 g body weight. The mice were acclimatized in the animal house for 3 days followed by acclimatization in the laboratory for 2 days. Prior approval for the study was obtained from the Institutional Animal Ethics Committee. All experiments were carried out as per regulatory guidelines like the Organisation for Economic Co-operation and Development (EU), Committee for the Purpose of Control and Supervision of Experiments on Animals (India) and Indian Council for Medical Research Guidelines for Biomedical Research (India).

2.3 | Instrumentation and chromatography conditions

The UPLC instrument consisted of a Waters Acquity H class UPLC system equipped with a quaternary pump and 96-vial autosampler coupled with a diode array UV detector (Waters, Milford, MA, USA). The chromatographic separation was performed on an Acquity UPLC BEH C_{18} column from Waters (2.1 × 100 mm; 1.7 µm). The column temperature was set at 35°C and the autosampler was kept at 15°C. The mobile phase was composed of a mixture of acetonitrile (85%, *v*/v) and water (15%, *v*/v) at a flow rate of 0.3 mL/min. Before analysis the mobile phase was filtered through a 0.22 µm membrane filter and degassed by ultra-sonication. A 5 µL injection of each sample was loaded onto the system and total analysis time was 6 min. The diode array detector (DAD) was set at 215 nm. The sampling needle was washed with 300 µL of strong wash (ACN–water, 65:35) to reduce carry-over and 300 µL of weak wash (ACN–water, 30:70). Data acquisition was done using Empower 3 software version 1.0 (Waters, USA).

2.4 | Preparation of stock and working standard solution

PCB 77 and PCB 180 stock solutions of 1 mg/mL were prepared by dissolving suitable amounts of the single drug in methanol. Mixtures of stock solutions of PCB 77 and PCB 180 (100 µg/mL) were prepared in methanol. The IS stock solution of 200 µg/mL was prepared in methanol. The stock solutions of PCB 77, PCB 180 and IS were stable at 4°C for 1 month. Further dilution was made in methanol–water (50:50, *v*/v) to produce working stock solutions for the calibration standards and quality control (QC) standards. The IS working solution (5 µg/mL) was prepared in methanol–water (50:50 *v*/v). Calibration curve samples were prepared in drug-free mouse plasma with the appropriate mixture of working solution of PCB 77 and PCB 180 on the day of analysis. All the samples were stored together at −80 ± 10°C until analysis.

2.5 | Sample preparation

Sample preparation was carried out by the liquid–liquid extraction procedure. Into a 100 µL of aliquot of plasma, 10 µL of IS working solution was mixed for 30 s on a spinix vortex shaker (Tarsons, India). To this, 3.0 mL of *tert*-butylmethylether was added and this was vortex mixed for 5 min. This was centrifuged at 10,000 rpm for 5 min at 4°C on an Eppendorf 5810R centrifuge (Eppendrof AG, Hamburg, Germany). The clear supernatant organic layer (2.5 mL) was transferred into 5 mL polypropylene tubes and evaporated to dryness at 35°C using a nitrogen evaporator (Turbovap®, Biotage, USA). The residue was reconstituted in 100 µL of the mobile phase, vortex mixed for 1.0 min and centrifuged at 10,000 rpm for 5 min. Finally, 90 µL of the clear supernatant was transferred into glass micro-vials and 5 µL was injected onto the UPLC system for analysis.

2.6 | Method validation

The method was validated for selectivity, specificity, recovery, linearity, accuracy, precision and stability using US Food and Drug Administration guidelines (US DHHS et al., 2001) for the assay in plasma.

2.7 | Carry-over and limit of quantification

Carry-over effect was assessed by injecting the highest calibration standard just before blank plasma. The limit of quantitation (LOQ) was defined as the first point of the calibration standard with lowest concentration (signal-to-noise ratio ≥ 10:1) that can be calculated with an acceptable precision and accuracy.

2.8 | Selectivity and specificity

The selectivity of the method was assessed by analyzing pooled drug-free mouse plasma injected at the beginning of the validation and the potential interferences at the LC peak region for analytes and IS were investigated. At least four out of six lots should have a response <5 times the lower limit of quantitation (LLOQ) level response in the same matrix.

2.9 | Recovery

The recoveries of PCB 77 and PCB 180 were assessed by analyses of the three various concentrations (30, 500 and 2250 ng/mL) whereas the recovery of the IS was determined at a single concentration of 5 µg/mL. Recovery was calculated as the extracted spiked plasma peak area response compared with the response obtained for neat standard solution at equivalent concentrations by liquid–liquid extraction process. The analytes responses from the extracted samples at known concentrations were compared with responses of un-extracted standards.

2.10 | Linearity

Linearity was evaluated by linear regression analysis with the use of working standard solutions and spiked plasma samples containing the drugs of interest at different concentrations within the range 10–3000 ng/mL for PCB 77 and PCB 180. The calibration curves were constructed by plotting the ratio of the peak area of each analyte to the peak area of internal standard vs the nominal drug concentration. The slopes and intercept were calculated with least square linear regression analysis of the data with the use of a $1/x^2$ (x-concentration) weighting factor. The acceptance criteria for each back-calculated standard concentration were ±15% deviation from the nominal value except at the LLOQ, which was set as ±20% (US DHHS et al., 2001).

2.11 | Accuracy and precision

The validation method evaluations of the accuracy and precision in the experiments were performed with four independent series (including ruggedness) in plasma. The intra- and inter-day assay accuracy and precision were estimated by analyzing six replicates containing analytes at three different QC levels, that is, 10, 30, 500 and 2250 ng/mL. Accuracy represents the closeness of agreement between the mean values obtained from the series of measurements by the method and the actual value. The accuracy should be within 85–115% of the nominal value, except at the LLOQ, where it should not deviate more than 20% (US DHHS et al., 2001). Precision represents the closeness of agreement among a series of measurements obtained from multiple samplings and was estimated with variances of repeatability (intra-day variances) and intermediate precision (sum of intra-day and inter-day variances). The precision should not exceed ±15% relative standard deviation (RSD), except for the LLOQ, where it should be within ±20% of RSD (US DHHS et al., 2001).

2.12 | Stability

Stability tests were performed to evaluate the stability of analytes in plasma samples under different conditions. The bench-top stability study assessed the stability of analytes in plasma at room temperature for 9 h, whereas a post-preparative stability study assessed the stability of analytes in the treated samples (autosampler stability for 24 h at 15°C) at low (30 ng/mL) and high (2250 ng/mL) QC concentration. Freeze–thaw stability was determined after three freeze and thaw cycles and long-term stability was determined by testing QC samples stored at −80°C for 30 days.

2.13 | Pharmacokinetic study

Animals were exposed to PCB 77 and 180 via inhalation at a dose of 4 mg/m^3 in water for 6 h in a whole body inhalation chamber. Blood samples (150 µL) were obtained using lithium tubes with heparin, using retro-orbital venipuncture at 0.0 (before exposure) followed by 2.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 h after the initiation of exposure. Whole blood samples were centrifuged at 3500 rpm for 10 min and plasma was separated and stored at −80°C until use. The areas under the plasma concentration–time curve (AUC_{0-inf} and AUC_{0-t}) were used to estimate maximum plasma concentration and time taken to reach this concentration (C_{max} and T_{max}) using trapezoidal rule. The half-life of elimination ($T_{1/2}$) was determined using linear regression with sum of least squares method. WinNonlin software 5.1 was used to calculate the pharmacokinetic parameters by noncompartmental model (Pharsight Corporation, CA, USA).

3 | RESULTS AND DISCUSSION

3.1 | Optimization of the chromatographic conditions

We optimised a reverse-phase liquid chromatographic method for simultaneous estimation of PCB 77 and PCB 180 in mouse plasma samples using diode array detection. LC method development was carried out focusing on achieving sufficient resolution of target drugs and endogenous interferences in matrix within a short run time. The method is novel and additionally has certain unique advantages over previously used methods for quantification of chlorinated biphenyl pollutants. First this method has optimum sensitivity in determination of PCB levels in mouse plasma with sparse samples. Adequate specificity has also been

demonstrated in this method for detection of PCB 77 and PCB 180 using an internal standard which has intermediate chemistry.

The method was developed and optimized using a mobile phase with simple composition and a reverse-phase column. The mobile phase and solvent usage are cost-effective compared with the gas chromatography employed elsewhere, and the run time is less time consuming such that a number of samples can be estimated quickly in the prescribed time duration. The method is also simple and can be used for rapid estimation of these congeners in plasma samples during experimental sampling procedures. Finally, the fact that this is one of the first reports of a rapid simultaneous method that has been developed and successfully optimized for quantification of two distinct PCB congeners in plasma cannot be overemphasized.

3.2 | Carry-over and limit of quantification

No carry-over was observed when the highest calibration standard was analyzed just before the blank plasma. This indicated that flow through needle configuration of the injector and the washing step of the column at the end of the chromatography separation were adequate to remove potential residues of the analytes. The LOQ on the calibration curve was 10.0 ng/mL for all of the compounds and the signal-to-noise ratio in plasma was ≥10.

3.3 | Selectivity and specificity

Selectivity was evaluated by comparing the chromatograms of pooled plasma and pooled plasma spiked with IS with analytes (10 ng/mL) and IS. A kinetic study sample was obtained after 4.0 h of inhalation at a dose of 4 mg/m^3 (PCB 77 and PCB 180). As shown in Figure 1, no interfering peaks from endogenous compounds were observed at the retention times of analytes and IS. The total chromatographic run time was 6 min.

3.4 | Recovery

Liquid–liquid extraction technique gave adequate recovery and clean samples. The results of the evaluation of neat standards vs plasma extracted standards were estimated for PCB 77 and PCB 180 (30, 500 and 2250 ng/mL) and the mean recoveries were 90.10 ± 1.34 and 89.51 ± 1.52%, respectively. The recovery of IS at 5 µg/mL concentration was 87.11 ± 2.70%.

3.5 | Linearity

The calibration curves exhibited excellent linearity with regression correlation coefficient (r^2) >0.996 over the concentration range of 10–3000 ng/mL for all of the drugs in mouse plasma. The standard calibration curve had a consistent reproducibility over the standard concentrations across the calibration range. A typical regression equation was prepared by determining the best fit of peak-area ratio (peak area analyte/peak area IS) vs concentration, and fitted to $y = mx + c$ using a weighting factor ($1/x^2$). The lowest concentration with the RSD <20% was taken as the LLOQ and was found to be 10.0 ng/mL for the PCB 77 and PCB 180. The percentage accuracies observed for the mean of back-calculated concentration for four calibration curves for the entire drugs were within 96.10–102.50%.

3.6 | Accuracy and precision

Accuracy and precision of the method evaluated intra-day (repeatability) and inter-day (intermediate) for mouse plasma for all of the drug samples are summarized in Table 1. The intra- and inter-day precisions were <4.64% and the accuracy ranged from 98.90 to 102.33% at all levels. All of the values were within the accepted range and the method was accurate and precise.

3.7 | Stability

The stabilities of PCB 77 and PCB 180 in mouse plasma were investigated under a variety of storage and process conditions, in plasma at room temperature for 9 h, in the autosampler for 24 h at 15°C, after three freeze–thaw cycles and after long-term storage at −80°C for 30 days, and are summarized in Table 2. The results indicated that PCB 77 and PCB 180 were stable under the conditions described above.

3.8 | Applicability of the validated method

In this study, we report a rapid simultaneous liquid chromatographic method for quantifying PCB 77 and PCB 180 in mouse plasma. For

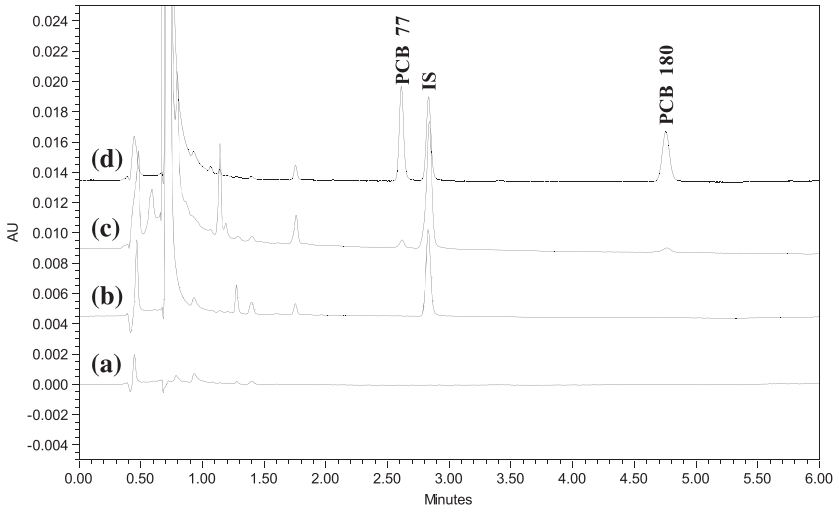


FIGURE 1 Representative UPLC chromatograms of polychlorinated biphenyl (PCB) 77, PCB 180 and IS in mouse plasma. (a) blank plasma sample; (b) blank plasma sample spiked with IS; (c) blank plasma spiked with analytes (10 ng/mL) and IS; and (d) pharmacokinetic plasma samples collected from mice at 4.0 h time point following inhalation exposure to PCBs

TABLE 1 Intra- and inter-day precision and accuracy of polychlorinated biphenyl (PCB) 77 and PCB 180 in mouse plasma

Nominal concentration (ng/mL)	Intra-day (n = 6)			Inter-day (n = 18)		
	Measured concentration (mean ± SD, ng/mL)	CV (%)	Accuracy (%)	Measured concentration (mean ± SD, ng/mL)	CV (%)	Accuracy (%)
PCB 77						
10.89	10.77 ± 0.50	4.64	98.90	10.83 ± 0.25	2.31	99.45
31.12	31.10 ± 0.39	1.25	99.94	30.98 ± 0.38	1.23	99.55
497.88	493.88 ± 5.56	1.13	99.20	494.21 ± 7.74	1.57	99.26
2243.52	2265.67 ± 7.24	0.32	100.99	2295.72 ± 3.40	2.32	102.33
PCB 180						
11.00	10.95 ± 0.21	1.92	99.55	11.16 ± 0.27	2.42	101.45
31.43	31.46 ± 0.62	1.97	100.10	31.32 ± 0.40	1.28	99.65
502.94	503.07 ± 5.00	0.99	100.03	502.15 ± 4.60	0.92	99.84
2266.32	2283.80 ± 6.21	1.39	100.77	2257.64 ± 9.74	0.85	99.62

TABLE 2 Stability of PCB 77 and PCB 180 in mouse plasma under different conditions (n = 6)

Nominal concentration (ng/mL)	Bench-top (room temperature for 9 h)		Autosampler (8°C for 24 h)		Freeze–thaw cycles		Long-term stability (−80°C for 30 days)	
	Measured concentration (mean ± SD, ng/mL)	Bias (%)	Measured concentration (mean ± SD, ng/mL)	Bias (%)	Measured concentration (mean ± SD, ng/mL)	Bias (%)	Measured concentration (mean ± SD, ng/mL)	Bias (%)
PCB 77								
31.12	31.51 ± 0.65	−1.25	30.79 ± 0.54	1.06	31.46 ± 0.82	−1.09	31.10 ± 0.70	2.54
2243.52	2267.05 ± 2.84	−1.05	2221.77 ± 5.45	0.97	2276.17 ± 3.27	−1.46	2225.60 ± 9.24	0.80
PCB 180								
31.43	31.89 ± 0.47	−1.46	31.09 ± 0.50	1.08	31.71 ± 0.64	2.55	30.99 ± 0.54	1.40
2266.32	2323.15 ± 9.90	−2.51	2257.08 ± 8.75	0.41	2289.96 ± 6.57	−0.89	2300 ± 5.28	−1.52

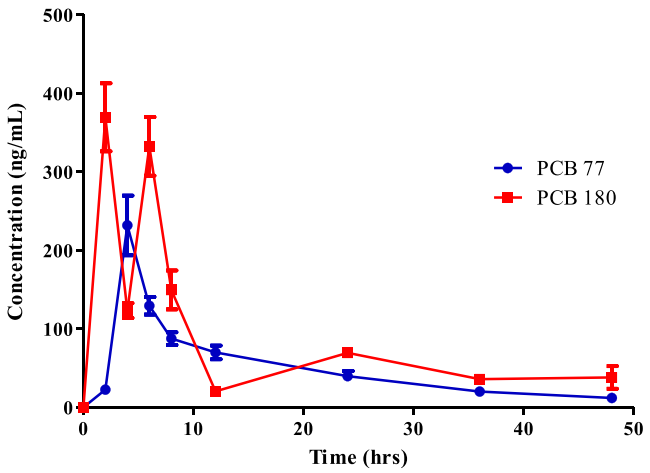


FIGURE 2 Mean plasma concentration–time profile of PCB 77 and PCB 180 in mouse plasma after inhalation of PCBs at 4 mg/m^3 dose

PCB 77, the AUC_{0-inf} was 2640.37 ± 300.92 ng h/mL, as compared with 4855.76 ± 733.19 ng h/mL for PCB 180. Similarly, the AUC_{0-t} was 2387.12 ± 280.64 ng h/mL for PCB 77 which is less than the value of 3761.24 ± 375.65 ng h/mL obtained for PCB 180. The C_{max} for PCB 77 was 231.76 ± 37.90 ng/mL. The C_{max} for PCB 180 was greater as compared with that of PCB 77, which was 369.47 ± 43.24 ng/mL. The T_{max} for PCB 77 was 4 h, which was twice that for PCB 180. The half-life of elimination of PCB 77 from plasma was 14.11 ± 1.94 h, which was less than the value obtained for PCB 180, i.e. 19.34 ± 7.26 h. The profile of mean plasma concentration of PCB 77 and PCB 180 vs time is shown in Figure 2.

4 | CONCLUSION

A simple, sensitive, and reliable UPLC method has been developed and validated for simultaneous determination of PCB 77, and PCB 180 in mouse plasma. This UPLC method has significant advantages, including good resolution between peaks and adequate extraction recovery with shorter chromatographic run time. The current method has been optimized for estimation of PCB congeners of diverse chemical properties, with high precision and accuracy. It is also highly cost and time effective, while not compromising the recovery and yield. We have developed a quick and reliable method to estimate PCB congeners in mouse plasma samples, which could be applied in regular measurement of these pollutants in occupational hazard risk assessment. Finally, this method is comparable to if not better to gas chromatographic methods reported previously, in terms of analytical validity and practicability.

REFERENCES

Agency for Toxic Substances and Disease Registry (2000). Toxicological profile of polychlorinated biphenyls. Available from: https://www.atsdr.cdc.gov/ToxProfiles/index.asp (accessed 25 February 2017).

Bogdevich, O. P., & Cadocinicov, O. P. (2001). PCBs determination in transformer oil by SPE and gas chromatography analysis. *Moldavian Journal of the Physical Sciences*, *3*, 226–235.

Brieger, G., & Hunter, R. D. (1993). Uptake and depuration of PCB 77, PCB 169, and hexachlorobenzene by zebra mussels (*Dreissena polymorpha*). *Ecotoxicology and Environmental Safety*, *26*(2), 153–165.

Chu, I., Villeneuve, D. C., Yagminas, A., Lecavalier, P., Håkansson, H., Ahlborg, U. G., … Seegal, R. F. (1995). Toxicity of PCB 77 (3,3′,4,4′-tetrachlorobiphenyl) and PCB 118 (2,3′,4,4′5-pentachlorobiphenyl) in the rat following subchronic dietary exposure. *Fundamental and Applied Toxicology*, *26*(2), 282–292.

Cossu, M., Ledda, G., & Chessa, G. (2013). Validation and applications of a GC-ECD method for the determination of polychlorinated biphenyls in fish and seafood. *Monatshefte für Chemie – Chemical Monthly*, *144*, 1597.

Dasary, S. S., Saloni, J., Fletcher, A., Anjaneyulu, Y., & Yu, H. (2010). Photodegradation of selected PCBs in the presence of Nano-TiO_2 as catalyst and H_2O_2 as an oxidant. *International Journal of Environmental Research and Public Health*, *7*(11), 3987–4001.

Dmitrovic, J., Chan, S. C., & Chan, S. H. (2002). Analysis of pesticides and PCB congeners in serum by GC/MS with SPE sample cleanup. *Toxicology Letter*, *134*(1–3), 253–258.

Eske, K., Newsome, B., Han, S. G., Murphy, M., Bhattacharyya, D., & Hennig, B. (2014). PCB 77 dechlorination products modulate pro-inflammatory events in vascular endothelial cells. *Environmental Science and Pollution Research*, *21*(10), 6354–6364.

Ghosh, S., De, S., Chen, Y., Sutton, D. C., Ayorinde, F. O., & Dutta, S. K. (2010). Polychlorinated biphenyls (PCB-153) and (PCB-77) absorption in human liver (HepG2) and kidney (HK2) cells *in vitro*: PCB levels and cell death. *Environment International*, *36*(8), 893–900.

Grimm, F. A., Hu, D., Kania-Korwel, I., Lehmler, H. J., Ludewig, G., Hornbuckle, K., & Duffel, M. A. (2015). Metabolism and metabolites of polychlorinated biphenyls. *Critical Reviews in Toxicology*, *45*(3), 1–28.

Letcher, R. J., Li, H. X., & Chu, S. G. (2005). Determination of hydroxylated polychlorinated biphenyls (HO-PCBs) in blood plasma by high-performance liquid chromatography–electrospray ionization–tandem quadrupole mass spectrometry. *Journal of Analytical Toxicology*, *29*(4), 209–216.

Maisano, M., Cappello, T., Oliva, S., Natalotto, A., Giannetto, A., Parrino, V., … Mauceri, A. (2016). PCB and OCP accumulation and evidence of hepatic alteration in the Atlantic bluefin tuna, *T. thynnus*, from the Mediterranean Sea. *Marine Environmental Research*, *121*, 40–48.

Mathews, S. (2014). Biodegradation of polychlorinated biphenyls (PCBs), Aroclor 1260, in wastewater by isolate MD2 (*Pseudomonas aeruginosa*) from wastewater from Notwane sewage treatment plant in Gaborone, Botswana. *Journal of Bioremediation & Biodegradation*, *5*, 266.

Matthews, H. B., & Dedrick, R. L. (1984). Pharmacokinetics of PCBs. *Annual Review of Pharmacology and Toxicology*, *24*, 85–103.

Muir, D., & Sverko, E. (2006). Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: A critical appraisal. *Analytical and Bioanalytical Chemistry*, *386*(4), 769–789.

Pietrogrande, M. C., Benvenuti, A., & Previato, S. (2000). HPLC analysis of PCBs on porous graphitic carbon: Retention behavior and gradient elution. *Chromatographia*, *52*, 425.

Saija, E., Mangano, V., Casale, K. E., La Torre, G. L., Dugo, G., & Salvo, A. (2016). Determination and quantification of PCBs, POCs and PAHs in *Thunnus thynnus* from the straits of Messina (Italy). *Data in Brief*, *7*, 129–134.

Sather, P. J., Newman, J. W., & Ikonomou, M. G. (2003). Congener-based Aroclor quantification and speciation techniques: A comparison of the strengths, weaknesses, and proper use of two alternative approaches. *Environmental Science & Technology*, *37*(24), 5678–5686.

Tian, L., Han, F., Cai, Y., Kong, C., Shi, Y., Wang, Y., … Huang, D. (2016). Determination of 7 indictor polychlorinated biphenyls (PCBs) residues in porphyra by ultrasonic extraction and gas chromatography (GC). *Journal of Agricultural Chemistry and Environment*, *5*, 1–5.

UNEP (n.d.). *United Nations Environment Programme – United Nations Stockholm Fund*. Available from: http://web.unep.org/, accessed 25 February 2017.

US DHHS, FDA, & CDER (2001). *Guidance for Industry: Bioanalytical Method Validation* (pp. 2001). US Department of Health and Human Services, Food and Drug Administration and Center for Drug Evaluation and Research and Center for Veterinary Medicine: Rockville, MD.

US-EPA (1989). Agency for Toxic Substances and Disease Registry United States-environment protection Agency, toxicological profiles for selected PCBs. Available from: https://www.atsdr.cdc.gov/ToxProfiles/index.asp (accessed 25 February 2017).

Viluksela, M., Heikkinen, P., van der Ven, L. T., Rendel, F., & Roos, R. (2014). Toxicological profile of ultrapure 2,2′,3,4,4′,5,5′-heptachlorbiphenyl (PCB 180) in adult rats. *PloS One*, *9*(8), e104639.

Wang, L., Zhang, S., Lin, R., Li, L., Zhang, D., Li, X., & Liu, S. (2013). PCB-77 disturbs iron homeostasis through regulating hepcidin gene expression. *Gene*, *532*(1), 146–151.

Zhai, G., Wu, X., Lehmler, H. J., & Schnoor, J. L. (2013). Atropisomeric determination of chiral hydroxylated metabolites of polychlorinated biphenyls using HPLC-MS. *Chemistry Central Journal*, *7*(1), 183.

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Original research Article

Sim Man 3G – HIGH FIDELITY SIMULATION: A METHOD TO FACILITATE PHARMACOLOGY LEARNING BY MBBS STUDENTS

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ABSTRACT

***Introduction*:** Many students in the II MBBS curriculum find difficult to learn Pharmacology. II MBBS students do not have many opportunities to observe the clinical effects of the drugs as they read about, making learning experience largely theoretical. To improve the learning experience and to facilitate recall by providing an opportunity to observe the clinical effects of drugs using simulation a practical way of teaching method in Pharmacology would improve student understanding of the role of drugs in a given clinical scenario and it would improve their prescription writing skills. ***Methods*:** 29 II MBBS students attended a lecture class on drugs used in bronchial asthma and underwent a pre-test, followed by demonstration of management of acute exacerbation of bronchial asthma in a simulation model, in smaller groups. Later, their individual management of the simulated patient was assessed by observation & they were asked to write a prescription for maintenance therapy for their patient. A post-test was conducted one month later. ***Results*:** All students improved their post-test scores, and this was especially marked in those who had scored less than 50% in their pre-test. 70% of students were able to revert bronchospasm within 15 minutes and all students wrote a correct prescription for maintenance therapy. ***Conclusions*:** Thus, simulation mode of teaching improved the learning skill and outcome, with an added advantage of knowing to interpret clinical data to select the appropriate drugs to solve the clinical problem.

KEYWORDS: Bronchial asthma, II MBBS learning, novel pharmacology practical method, novel teaching method.

INTRODUCTION

Undergraduate medical students learn Pharmacology through regular lectures and few types of practical methods. Even though they have early clinical postings in various clinical departments to observe /understand the basic concepts like history taking, value of interpretation of lab reports to the related clinical situation and treatment given, there is less chances to observe and interpret /visualize various effects of drugs other than animal experiments, Computer assisted learning, video based teaching, problem based teaching /learning which are used currently in traditional method as an informative session to improve their

knowledge. This many time overload the students with more information with knowledge gain and may or may not be creating interest in students to practice reflective thinking and better understanding.**(1,2)** Hence, to understand pharmacology in the 2nd professional MBBS program, to acquire good prescription writing skill, the undergraduate MBBS students need some exercises with application based assessment example - learning with need for writing correct , effective prescription individually than making them to recall theory and writing in a paper as simple practical assessment. . In present scenario, students need imagination and memorizing skills, since majority of the assessment in II MBBS program is recall type and do not have much scope for rational thinking.

Pharmacology is a science in the MBBS Curriculum where students are given a complete knowledge of drugs and their role in therapeutics. They are expected to write a complete and rational prescription for any given condition at the end of the year. Teaching is primarily lecture-based and students do not have many opportunities to observe or visualize the effects of the drugs. **(1, 2)** As part of our continuous commitment to improve quality, to observe and interpretation of drug effects in a realistic model like simulation would positively influence the undergraduate medical students self learning habits and enable them to learn therapeutics effectively during MBBS training to value the rational prescription skills**.(3,4,5)**
Simulation, when used as an adjunct in the II MBBS lecture-based program helps the students to bridge the theory –practice gap. **(6)** Hence we planned this study to evaluate the use of simulation as a teaching method in Pharmacology. Our objectives were to investigate if the use of SimMan 3G high fidelity manikin, a wireless human patient simulator represents an average-size adult patient useful in teaching core skills like airway, breathing, cardiac, and circulation management with attached patient monitor that displays vitals, labs, and X-ray. This high fidelity manikin is effective in recognizing, identify drugs and dose with programmable response as simulation based learning environment a practical teaching method to improve student understanding of the role of drugs in a given clinical scenario with specific route and dose of administration and whether it would improve their prescription writing skills.

MATERIALS AND METHODS:

The study was conducted at the Department of Pharmacology and Centre for Clinical Simulation and Research, in a private Medical College after obtaining ethical approval from the Institutional Human Ethics Committee.

A. *Pre and Post-test model*

Second MBBS student volunteers (n=30) were participated in this observational study after signing written informed consent using one group pre and post-test model. All students were listened to a preliminary lecture class on the Pathophysiological approach to the pharmacotherapy of bronchial asthma and the management of acute exacerbations of chronic asthma. They were provided with learning material and after a month, were asked to attend a pre-test, a short test with multiple choice questions on the management of acute exacerbation of bronchial asthma and the interpretation of clinical parameters related to assessment of severity of the exacerbation. After the pre-test, smaller groups of student participants were taught the management of acute exacerbation of bronchial asthma, with stepwise therapy according to the severity of symptoms in a simulation model. They were assessed individually, within a month of their demonstration exercise, by observation of their

management of acute exacerbation of bronchial asthma in the simulation model (NAEP guidelines, ERP-3, 2007 Bethesda US).**(7)** This was followed by post-test with the same pre-validated multiple choice questions, one month later. Learning outcome of each student, who had voluntarily participated in this study with variable learning potential was compared before and after the simulation method of learning.

*Methodology for SimMan 3G High fidelity simulator way of learning **(8)***

To determine whether the students would be able to select and administer the appropriate drug/s to relieve bronchoconstriction in an acute exacerbation of chronic asthma model using SimMan 3G – High Fidelity simulator with in ½ hour to 1 hour, the following methodology (Figure -1) was used.

B. *Learning assessment after exposure to Simulation Model*

I- *Quantitative Assessment*:

A questionnaire was administered to each student after he/she completed the exercise on the simulation model. The first part of the questionnaire was for quantitative assessment, and questions were meant to assess the students' practical knowledge gained through their performance in the asthmatic simulation model. Students were asked to name the drug that had the fastest onset of action and its route of administration. They were also asked to write a prescription-on-discharge for maintenance therapy of the patient they had managed in the simulation model. A Questionnaire with three questions was used in this study mainly to know how the students were able to tackle the problem given to them using problem solving ability in simulation method, with an intention to rate the difficulty level of this simulation method by the students. Parameters like interpretation of the clinical investigations like PO_2, HR, RR etc, to choose the drug to reduce the symptoms based on the clinical parameters, to select and apply appropriate drug with route to reduce symptoms using pathophysiological approach were incorporated in the questionnaire. All the students were requested to rate this parameters with likert scale where 0 is no difficulty level, 1 is 20% difficulty and 5 is 100% difficulty. This is to check the friendliness of simulation technique students were asked to rate their difficulty in solving various aspects of the clinical problem they had encountered.

II -*Qualitative assessment:*

The students were asked to describe their opinion in learning through this mode of teaching and whether it improved their ease of recollection of pharmacological principles through a questionnaire. They were asked to describe the barriers to learning, if any, in the simulation mode of teaching and to rate their confidence in handling a similar clinical situation.

RESULTS:

Out of 30 volunteers students, 29 students completed the study and were included in statistical analysis. One of them dropped out as she was unable to attend the initial pre-test. 29 students completed the study and were included in statistical analysis. The pre-test and post test scores on comparison by paired-t test showed a statistical significance (P=0.001) with SPSS version 19.

There was improvement in post-test scores compared to pre-test scores and this improvement was especially marked in those students who had scored less than 50% in the pre-test (Figures 2 , 3 & 4). The vast majority of students (70%) were able to revert bronchospasm

in the acute asthma model in SimMan-3G in less than 15 minutes by timely and appropriate intervention with drugs. Only 10% of students required more than 20 minutes (Figure 5).

Quantitative Analysis

Most of the students found it very easy to interpret clinical parameters as part of the management of bronchial asthma in the simulation. Only 13% (n=4) of the study participants (n=29) felt a difficulty level of 20%. Almost all students were able to correctly identify the drug for producing effective bronchodilation with immediate improvement in oxygen saturation in the simulation model. Only 7% (n=2) of the study participants failed to identify an appropriate bronchodilator. All the students were able to write a correct prescription for 10 days maintenance therapy after discharge of the simulated patient, and all of them were able to write the correct dose and route of drug administration.

Qualitative Analysis

All the student participants were asked to answer few open ended questions to assess parameters like any barrier to choose drugs, able to give appropriate prescription for maintenance therapy, simulation mode of learning whether improved their confidence, facilitate recollecting pharmacological principles, able to solve clinical problem using simulation. All the student participants were able to confidently manage the clinical scenario through this learning method and felt that it was easy to clinically apply the pharmacological principles of each drug used. All the students were also confident of giving an appropriate prescription for maintenance therapy after managing the acute exacerbation of asthma and did not encounter any barriers in the choice of appropriate drugs. Simulation improved the student's self-confidence and helped them to recollect the pharmacological principles and apply these principles rationally in therapeutics.

DISCUSSION:

We had carried out a questionnaire-based survey to understand the efficacy of our teaching methods in the students' ability to apply their theoretical knowledge of the pharmacological concepts of drugs in clinical application. We surveyed II MBBS, Final MBBS (end of course) and CRRI (end of internship) to analyze if the training imparted during the Pharmacology course was sufficient for clinical application of pharmacological concepts, according to their current requirement. We identified the need for an active teaching method to enable students to think, apply and reinforce their prescription-writing skills. Because this simulation based exercise is related with assessment and students has commitment to solve given clinical situation using simulation surprised us, that students would have an improved learning experience if they were given the opportunity to observe the clinical effects of drugs in a simulated environment and that this would improve their self-learning and practical skills.**(3,4)**. Present medical undergraduate curriculum-pharmacology teaching under MCI guidelines, facilitate medical students to require good prescription skills with the knowledge on pharmacoeconomics, effective dose calculation, value of drug interactions, appropriate selection of drugs with appropriate Pharmacotherapeutic reasoning, adverse drug reaction(ADR) impact in clinical setup and the need to report ADR to Pharmacovigilance centre, importance of rational use of medicine in clinical practice (RUM) through practical's and in theory classes. Even though all the students are taught and assessed with suitable assessment method, how many students are really carrying the core principles of

pharmacology to clinical practice is not clear and difficult to judge.**(5)**

Pharmacotherapeutic knowledge can be suitably kindled with case based discussion, problem solving exercises to improve the quality of RUM and to develop good prescription writing skills during undergraduate training program. Unless there is an active demonstration of these training, students may tend forget these practical concepts before they become trained medical professional to undergo internship with minimum theory knowledge. **(5)** Majority of the practical pharmacological assessment are carried out in writing like recall, except very few exercises with OSPE format, may be one of the factor which can induce lack of importance about the applied aspect of the subject pharmacology by the students.

In this study, when students were assessed with pretest after routine theory class on bronchial asthma with pathophysiological way of approaching the disease, to provide good clinical care with clinical Pharmacotherapeutic knowledge, students were scored less than 50%(Figur2-IA), but the same set of students expressed better assessment after witnessing the clinical problem through simulation and the students' scores were more than 50% (Figur2-IA) clearly exhibits the need of clinical way of leaning pharmacology in undergraduate curriculum.**(5)** This might improve the value of learning pharmacology to self-improve good prescription skills during medical undergraduates training.

Simulation provides more chances to create realistic scenarios and to undergo training, retraining with many times possible. But with patients, medical undergraduate students have little limitation to provide treatment and allowed to observe the treatment procedures, which may not improve the real skill or competency expected to treat the similar clinical situation. Even they make mistakes during problem solving skills training, it's correctable and with their own pace students can work with simulated patients which is not possible with real patients. These type of training will help the student to develop required skill personally whether real patients available or not. Though this model is very expensive and need special preparation, training and time consuming, when it's used as a learning tool to visualize drug effects during undergraduate training program will definitely improve the quality of training and competencies like problem solving, decision making and interpersonal, drug selection, prescription writing skills expected in the medical undergraduate training.

This study also demonstrated that, pharmacology can be learned in a realistic way using simulated patients not only for one clinical reason but also when there is a change in patient based information's or monitoring the patients vitals for achieving the treatment goal effectively with rationale prescription/ selection of drugs which in turn to value the students responsibility as a future physician. This may motivate the students to learn pharmacology in a systematic way by reading the text books appropriately not only with motive to pass university exam purpose but also equip personally with pharmacological knowledge to solve clinical problems with good drug selection /application skills. by reading text book appropriately.**(6)** In this study after managing the simulated asthma patient, students were also asked to name the drug with the fastest action in relieving bronchospasm, with its route of administration, based on their experience in the simulated patient, to rate their difficulty in choice of drug and in understanding the clinical parameters used to assess the severity of the clinical problem of the simulated patient and students comfort level (quantitative and

qualitative assessment) revealed students improved organizational skills to solve the given simulated patient problem.**(6)**

Initially 30 students volunteered to participate in the study, but one withdrew. There was a significant improvement in post-test scores for all participants (Figures 3 and 4), but this improvement was especially marked in those students who had scored less than 50% in the initial pre-test (Figure 2). Overall 70% student participants were able to revert bronchospasm within 15 min and 30% took more than 15 min (Figure 5). This method of study closely followed *Kirkpatrick's four levels of assessment* of students learning through simulation method. **(9)**

Level 1: *Assess the simulation training and participant's reaction:*

Initial theory class followed by observing the simulated patient, responsibility to select appropriate drugs in suitable route of administration to reduce the seriousness of the problem with well appreciated learning environment by all the students (Qualitative Analysis)

Level 2: *Improvement in knowledge*. Comparison of pretest and posttest scores (Figures 2, 3 & 4) after students were exposed to simulation , a method to self understand the clinical importance of the given clinical problem solving attitude showed a remarkable shift in the learning outcome in both poor and good performers alike. When pre and posttest scores were compared for poor performers alone, the scores had almost doubled (Figure 2) expressed the need of clinical way of learning pharmacology will provide better understanding of the subject than by memorizing all the Pharmacotherapeutic concepts.**(2)**

Level 3: *Behavioural change or knowledge application in a clinical setting:*

By answering open ended questions, all the student participants were confident, able to recollect pharmacological principles in the applied format as per the situation or clinical requirement and were able to give a correct prescription (Qualitative analysis).

Level 4: *Impact of simulation training in terms of result*:

Simulation mode improved the learning skills (qualitative analysis) and outcome in terms of marks /knowledge gained (Figures 2, 3 and 4) with an improvement in interpretation of clinical data related to clinical scenario (integrated approach) to select the appropriate drugs to solve the clinical problem (Figure 5).

Studies express that if students are provided with an opportunity to learn in a simulated environment, their learning experience is improved. When they are given an opportunity to observe the clinical effects of the drugs they read about, both their self-learning and their practical skills improve, as a result of moving the students' emotions associated with learning in a positive direction.

High fidelity simulation adopted in this study depends on the type of outcome to be achieved with respect to student's level of learning.(10) This study facilitated the 2nd professional MBBS student participants to understand and interpret the effects of anti-asthmatic drugs using SimMan 3G – High Fidelity stimulator.

This study also exhibited similarity with *Kolb's learning cycle* and professional competency in learning process as an outcome (Figure - 4, 5) by:

a) Students experienced the actual medical problem (acute exacerbation of chronic asthma model) given to them through simulation method

b) Students were able to explore the reasons for the observed clinical problem through recall of their theory exposure

c) Students were able to recall their knowledge to interrelate and execute plans to stop/ overcome the problem exposed to them through simulation

d) Students were able to apply solutions to overcome/limit the clinical problem given to them using simulation method.

CONCLUSION:

Hence, this study clearly exhibited the positive role of simulations on changing learning outcome in MBBS professional teaching and training process and showed an improvement in knowledge gained after simulation (Figure 4) with their learning objective.**(10,11)**Through this study, simulation mode of teaching and learning environment has proved a definite influence on the student mindset for self -learning and kindled interest to teach/learn the subject effectively to achieve the long term goal of clinical application of pharmacological science in undergraduate medical students training. Small sample size of the study participants and questionnaire (quantitative analysis) which is not prevalidated were the limitations of this study.

ACKNOWLEDGMENT:

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REFERENCES

1. Choy SC, Oo PS. Reflective Thinking and Teaching Practices: A Precursor for Incorporating Critical Thinking into the Classroom? Online Submission. 2012; 5(1):167-82.

2. Papanna KM, Kulkarni V, Tanvi D, Lakshmi V, Kriti L, Unnikrishnan B,et al. Perceptions and preferences of medical students regarding teaching methods in a Medical College, Mangalore India. African health sciences. 2013; 13(3):808-13.

3. Fink LD. Creating significant learning experiences: An integrated approach to designing college courses. John Wiley & Sons; 2013 Jul 31.

4. Hertel JP, Millis BJ. Using simulations to promote learning in higher education: An introduction. Stylus Publishing, LLC. 2002.

5. Desai MK, Panchal JR, Shah S, Iyer G. Evaluation of impact of teaching clinical pharmacology and rational therapeutics to medical undergraduates and interns. International Journal of Applied and Basic Medical Research 2016 Jul; 6(3):205.

6. Seropian M, Dillman D, Lasater K, Gavilanes J. Mannequin-based simulation to reinforce pharmacology concepts. Simulation in Healthcare 2007; 2(4):218-23.

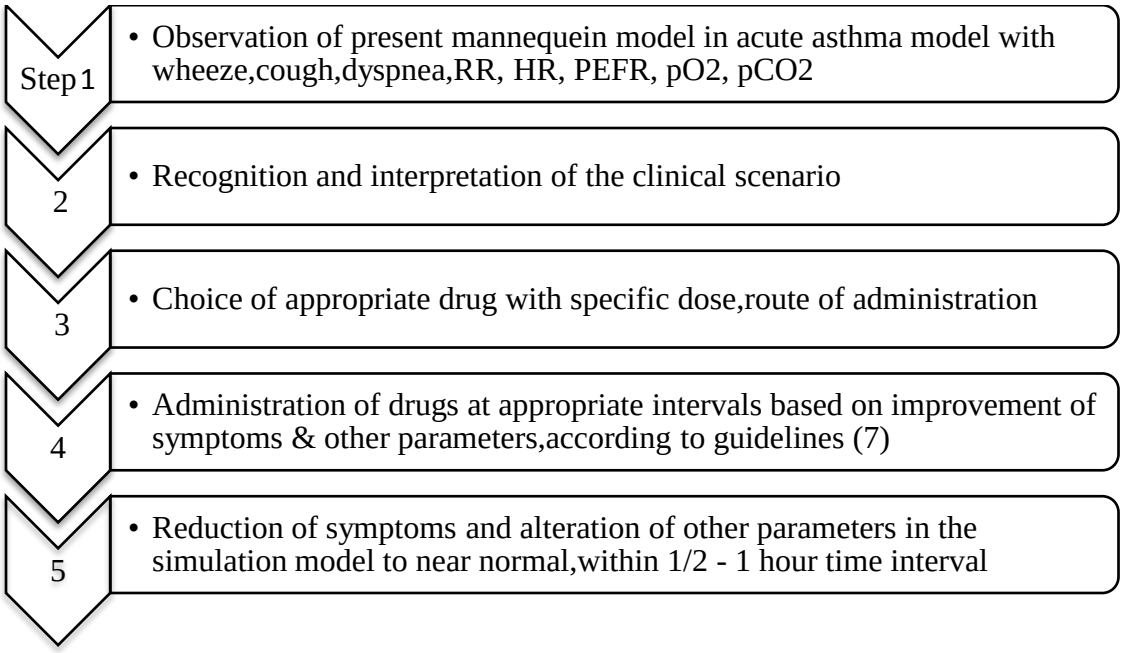
7. National Heart, Lung, and Blood Institute. National asthma education and prevention program. Expert panel report. 2007; 3:1-417

8. Munshi F, Lababidi H, Alyousef S. Low-versus high-fidelity simulations in teaching and assessing clinical skills. Journal of Taibah University Medical Sciences 2015; 10(1):12-5.

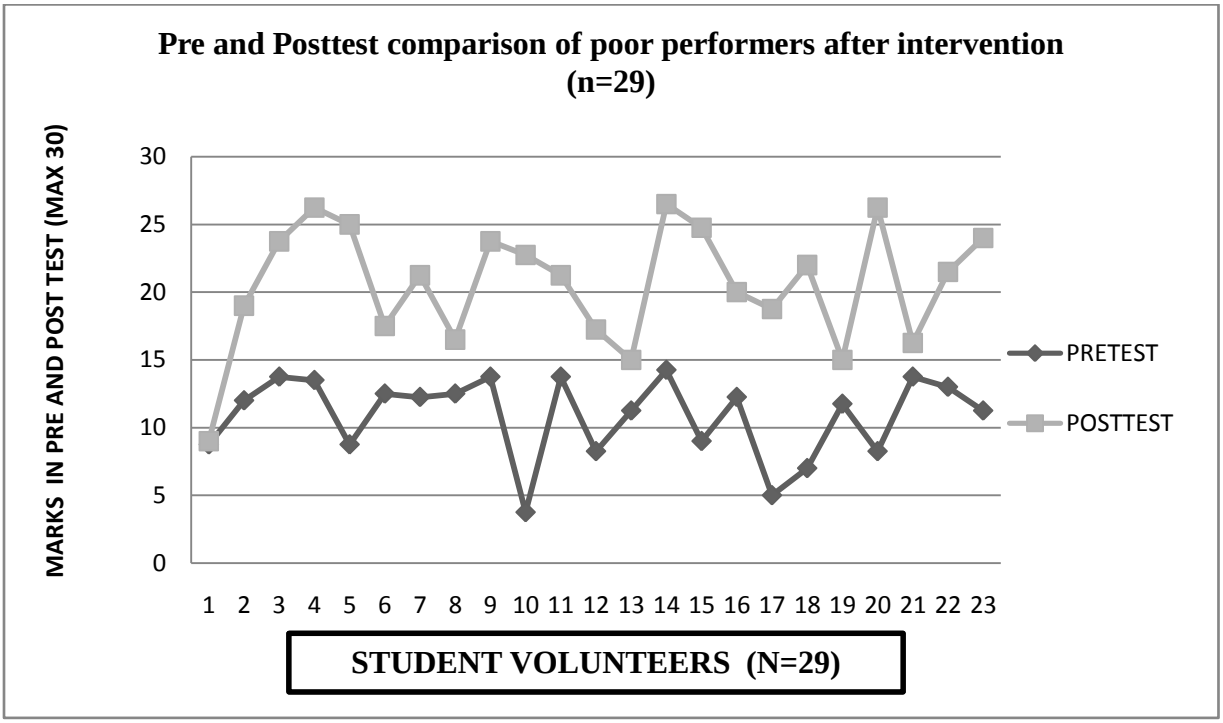
9. Bates R. A critical analysis of evaluation practice: the Kirkpatrick model and the principle of beneficence. Evaluation and Program Planning 2004; 27(3): 341 -347

10. Rodgers DL, Securro Jr S, Pauley RD. The effect of high-fidelity simulation on educational outcomes in an advanced cardiovascular life support course. Simulation in Healthcare. 2009; 4(4):200-6.

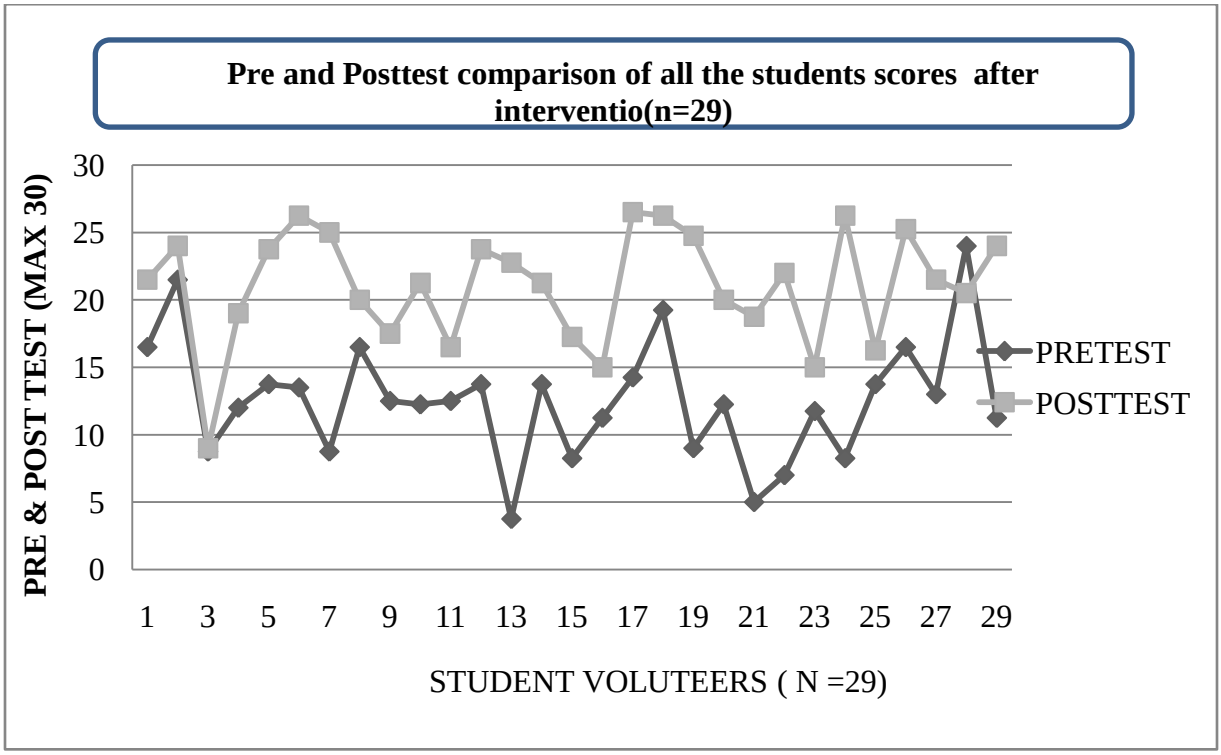
11. Curtin LB, Finn LA, Czosnowski QA, Whitman CB, Cawley MJ. Computer-based simulation training to improve learning outcomes in mannequin-based simulation exercises. American journal of pharmaceutical education. 2011; 75(6):113.



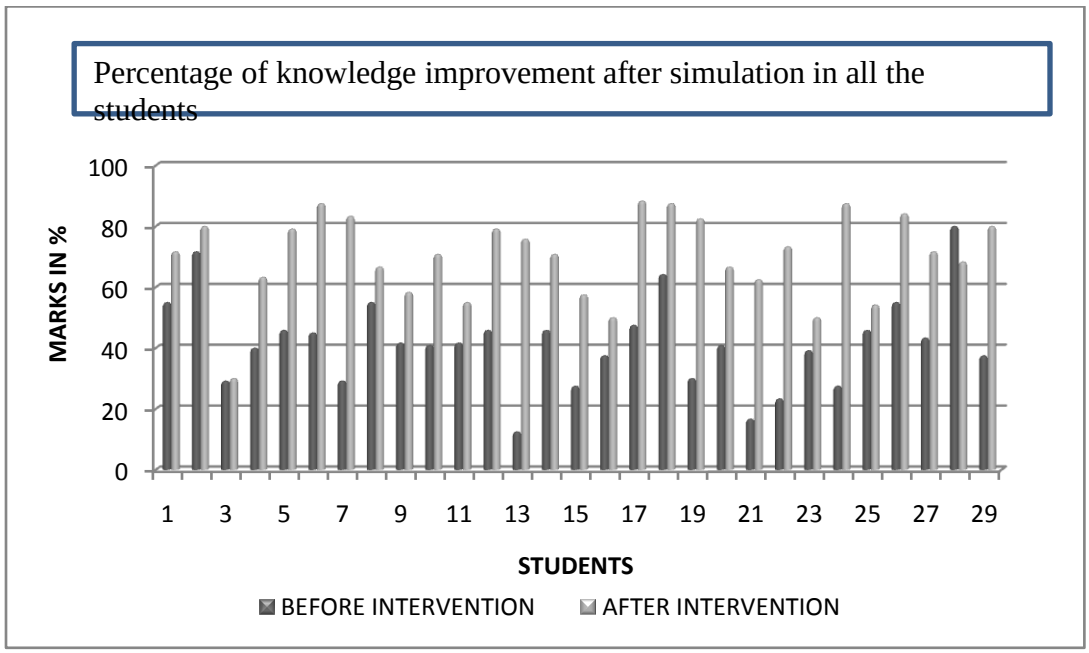
"Figure 1: Computerized case management scenario for Simulator way of Learning"



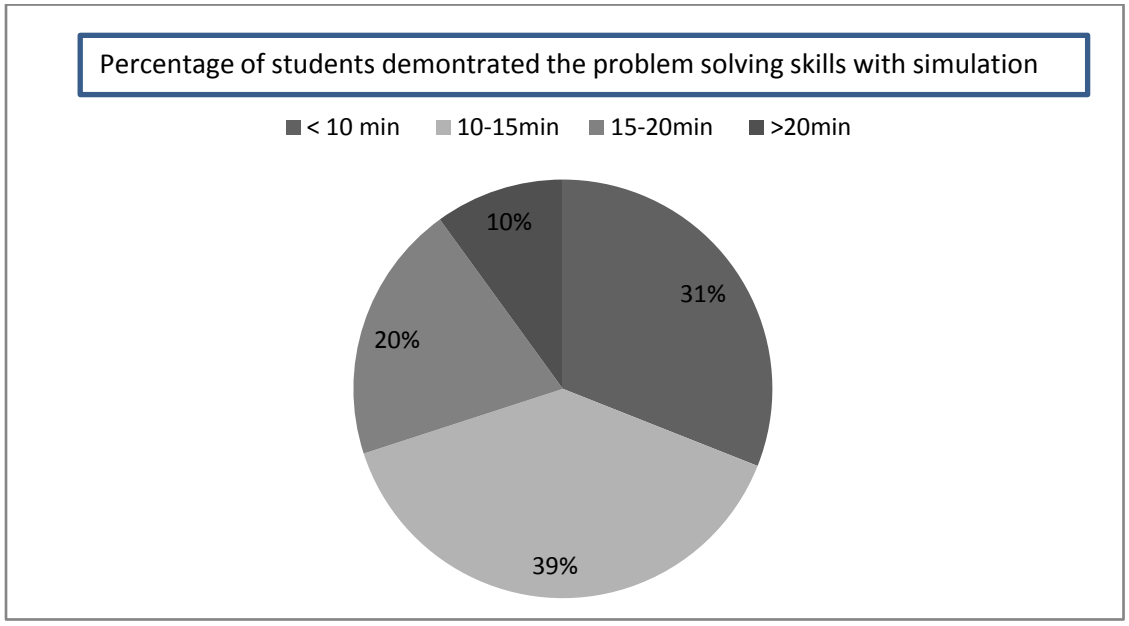
"Figure 2: Simulation influence on learning outcome in students performed with less than 50% in pretest (n=29) [IA]"



"Figure 3: Simulation influence on learning outcome in all the students appeared for pretest (N=29) [IB]"



"Figure 4: Impact of simulation in learning pattern as individual students [IC]"



"Figure 5: Student performance using SimMan 3G-high fidelity simulator]"

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Medication Practices of School Teachers in Coimbatore

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ABSTRACT

Knowledge regarding use of medicine to school teachers is an important component of the overall health care delivery system of any country. School teachers need to be aware of rational use of medicines because health education provided by them may have an immediate effect on students, their parents and also to the community. This study is aimed to evaluate the medication practices of school teachers in Coimbatore including the both city and rural areas. It is a Cross sectional study using a preformed questionnaire conducted from October 2014 to January 2015 among 400 school teachers working in both government and private schools in Coimbatore (urban and rural areas). After IHEC approval, permissions from the school authority were obtained. Written informed consent was obtained from the participants. Through preformed study Questionnaire data was collected from the participants and results were analyzed. Among the voluntarily participated participants it was found 86.5% follow doctor's advice for their personal ailments and nearly 76% of teachers do not visit physician for basic complaints. About 44.5% give medications to children without doctor's prescription or advice. Around 73% had agreed upon the importance of full course of medicines as per the prescription. This study revealed a wide gap in the knowledge of health habits and medication practices, which can be overcome in future with personal or group discussion in the form of workshop to alleviate the doubts in their minds, since we understand that teachers are the best medium to disseminate the concept of rational use of medicines to the society.

Keywords: Knowledge, Medication practices, School teachers.

INTRODUCTION

Appropriate use of medicines plays an important role in clinical practice. Both the health professionals and the public need to be aware of the rational drug usage and the consequences of inappropriate or irrational use of medicines having ill effects on health. Almost half of all medicines are used irrationally all over the world according to WHO. Globally up to 80% of illness episodes are self-treated with modern pharmaceuticals[1].This can have severe outcomes like drug resistance, adverse drug reactions, prolonged illness and even death. Lack of access to medicines and high financial cost are some of the common reasons particularly found in developing countries like India.

Knowledge regarding use of medicine to school teachers is an important component of the overall health care delivery system of any country[2]. School teachers need to be aware of rational use of medicines because health education provided by them may have an immediate effect on students, their parents and also to the community. This study is aimed to evaluate the medication practices of school teachers in Coimbatore including the both city and rural areas.

The World Health Organization has defined Rational Use of Drugs as follows: "Patients receive medicines appropriate to their clinical needs, in doses that meet their own individual requirements, for an adequate period of time, and at the lowest

cost to them and the community" [3]. Irrational use of medicines includes overtreatment of a mild illness, inadequate treatment of a serious illness, misuse of anti-infective drugs, over-use of injections, self-medication of prescription drugs and premature interruption of treatment[4].

People have several reasons for irrational use of medicines like lack of knowledge or skills, unrestricted availability of medicines, overwork of health personnel, inappropriate promotion of medicines and profit motives from selling medicines. There are twelve core interventions by WHO to promote more rational use of medicines and public education is one among them. Without sufficient knowledge about the risks and benefits of using medicines and when and how to use them,

People will often not get the expected clinical outcomes and may suffer adverse effects. This is true for prescribed medicines, as well as medicines used without the advice of health professionals[3].

Children spend a considerable part of their life in school exposed to a variety of environmental, physical, emotional and social influences. School is the place where the child learns how to interact, behave with others and to give and accept respect and the school teachers are the one who are creative, caring, dedicated, determined and have the ability to shape up the students to shine as a responsible person in the community. As teachers play an important role in educating their students and are regarded as learned and respected persons in local communities it is important that they have proper knowledge about medicines[5, 6]. This knowledge can be transferred to their students, their parents and members of the community.

A study done in Kuwait among school children found that self-medication among adolescents shown to be high and teachers considered teaching their students about the proper use of medicines so important that it should be included in the national curriculum of health education[7]. Similarly, another study done in Poland illustrated that appropriate informational devices implemented in primary and secondary schools could play an influential role in reducing the strain of community-acquired infections. The resource improves knowledge about microorganisms, hygiene and antimicrobial agents, and can be targeted at pupils, teachers and parents[8]. A pre-post comparison of a community intervention in Nepal has also shown the seriousness and gain of educating school teachers and their impact on students[9]. Another questionnaire based study done before and after

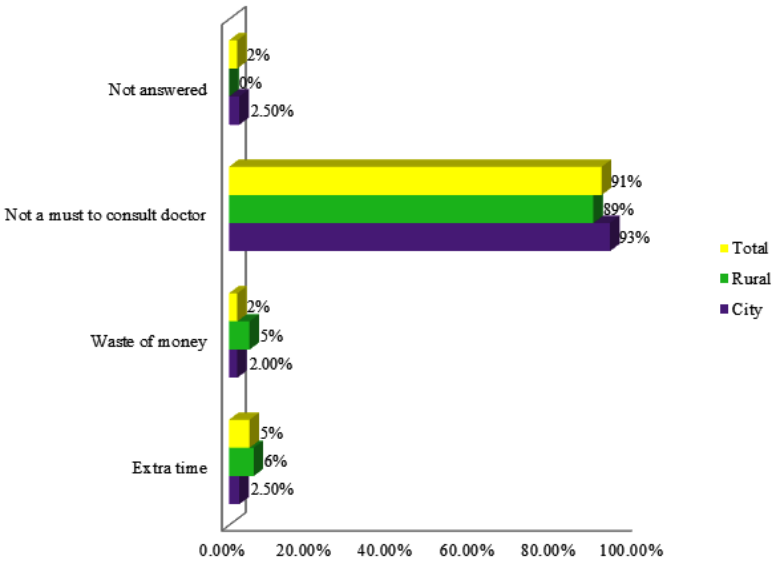


Fig. 1: Reasons for not visiting doctor for basic complaints

the educational intervention in Nepal regarding the knowledge, attitude and practice towards medicines among school teachers showed overall improvement in total scores post-intervention [10]. Similarly a study done among literate consumers in Dehradun found the habitual stocking of medicines, awareness regarding the expiry period of medicines and their self-perception in usage of medicines in addition to the doctor's advice[11].

In developing countries like India where the morbidity and mortality rates are high, awareness on rational medication usage is necessary to the society. At schools students are taught different subjects including topics like human body, health and nutrition, environmental health and personal hygiene. Teachers who teach the students should have at least a basic knowledge in using medicines which can be transferred to students and through them to parents and the community.

Table 1: Knowledge of school teachers in percentage for the given questionnaire

S.No	Questions	City (%)		Rural (%)		Total (%)	
		Yes	No	Yes	No	Yes	No
1	Do you visit doctor for a basic complaint (eg. headache, body pain)?	22	78	27	73	24	76
2	Do you purchase medicines from pharmacy without a doctor's prescription?	19	81	11	89	15	85
3	Do you follow the instructions given by the doctors / pharmacists to use drugs appropriately (eg. antacids in empty stomach)?	88.5	11.5	84.5	15.5	86.5	13.5
4	Would you agree purchasing full course of medicines as a must as per the prescription?	71	29	74.5	25.5	73	27
5	Do you have the habit of checking expiry date before intake of medicine?	97.5	2.5	94.5	5.5	96	4
6	Do you give adult medications to children?	6	94	2	98	4	96
7	Do you use the same prescription given by the doctor for a particular health complaint once again without consulting him?	35	65	26	74	31	69
8	Do you have the habit of using your family member's medications for your health complaints?	10.5	89.5	13.5	86.5	12	88
9	Are you regularly maintaining first aid box in school?	93.5	6.5	97	3	95	5
10	Do you have the habit of giving medications to children without doctor's prescription/advice? (Eg. fever-paracetamol, pain-buprufen, cough-cough syrup, diarrhea-oral rehydration powder)	48	52	41	59	44.5	55.5
11	Have you shared fellow staff member /family members with your medicines?	19.5	80.5	16.5	83.5	18	32
12	Do you have the habit of stocking medications at home to use in case of emergency?	66	34	64.5	35.5	65	35

Coimbatore is the second largest city in Tamil Nadu state in India which is surrounded by the Western Ghats and known for its pleasant climate. It is credited as the "Manchester of South India" due to its cotton production and textile industries. Coimbatore has a population of 1,601,438 with an average literacy rate of 82.43%[12].Though the city is known for its traditions; heavy industrialization has resulted in the growth of trade unions. There are various ethnic groups settled here as a result of increased economic growth and job opportunities. So this study is planned to evaluate the medication practices of school teachers in the city and rural areas of Coimbatore.

Aim & objective

To evaluate the medication practices of school teachers in the city and rural areas of Coimbatore.

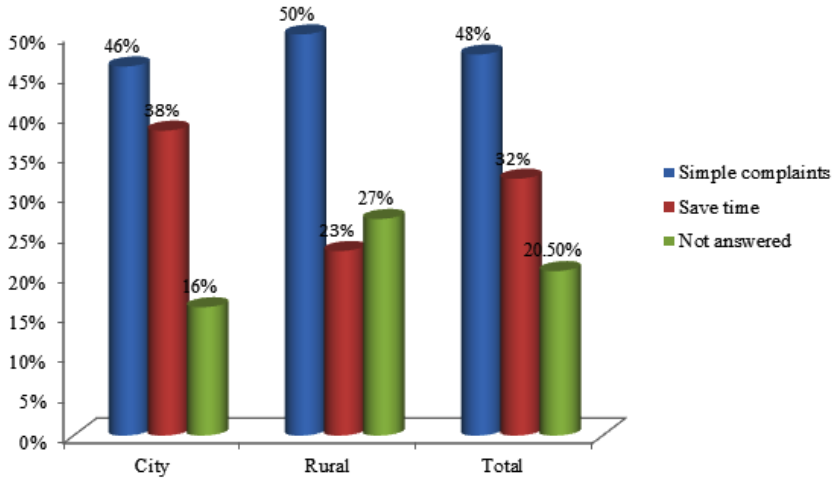


Fig. 2: Purchasing medicines without prescrption - Reasons

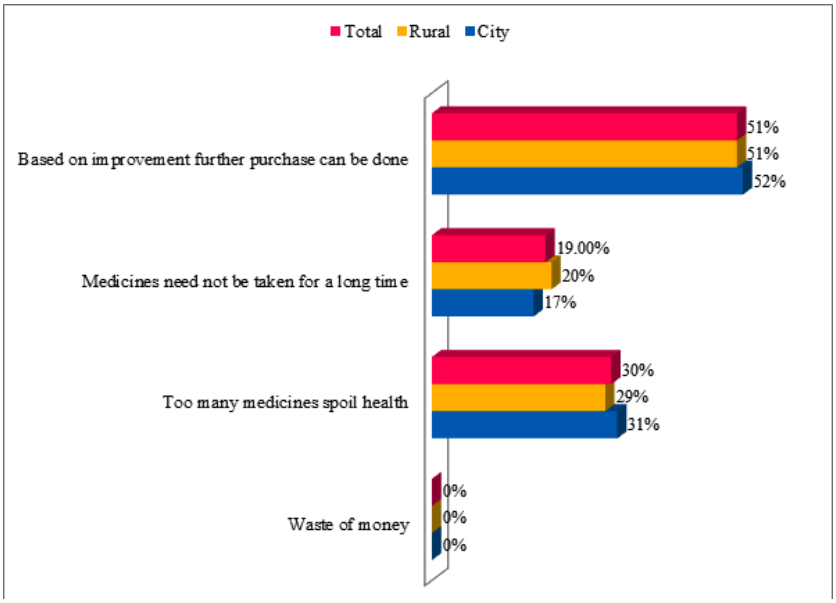


Fig. 3: Reasons for not purchasing full course of medicines

Methodology

A Cross sectional study was done using a preformed questionnaire during the period of October 2014 to January 2015. There were four hundred school teachers working in both government and private schools situated at urban and rural areas of Coimbatore district voluntarily participated in this study.

After getting approval from IHEC, then. Permissions from School management were obtained. Written informed consent from the participants and the Questionnaire was filled by them. Finally, the results were analyzed in percentage (frequency distribution) and the study was completed.

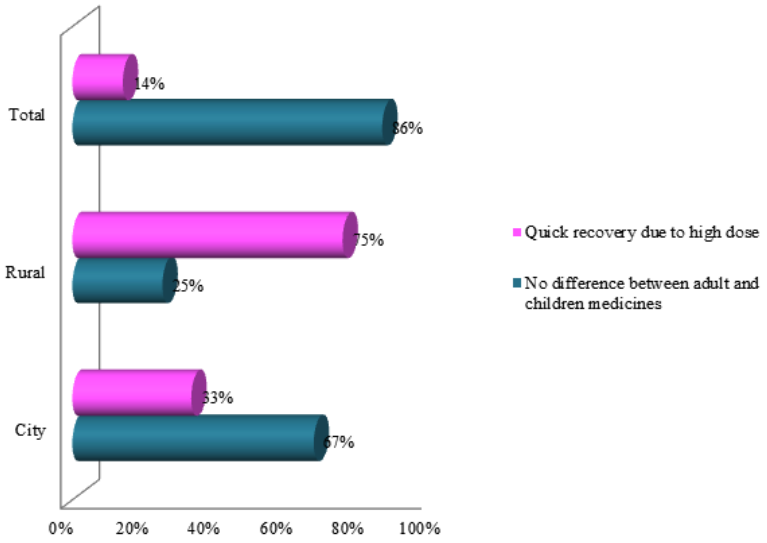


Fig. 4: Reasons for giving adult medications to children

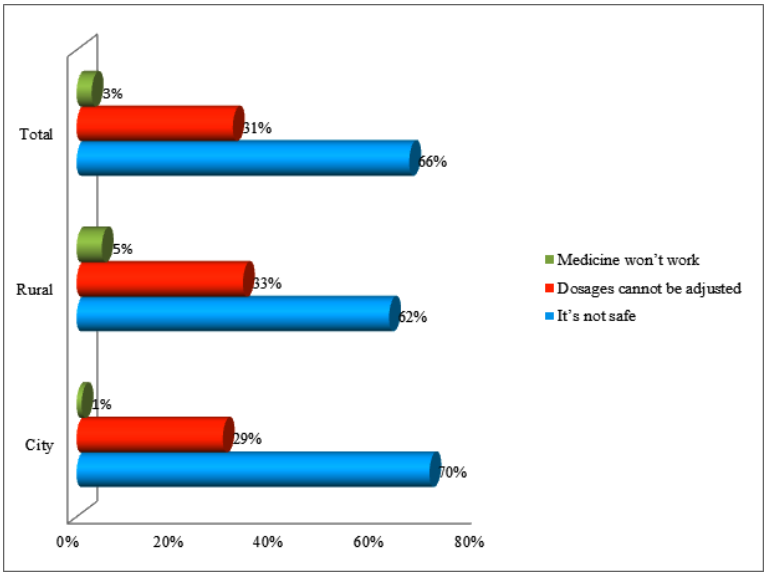


Fig. 5: Reasons for not giving adult medications to children

RESULTS

Among the School teachers who voluntarily participated in this study, we found 86.5% follow doctor's advice for their personal ailments. However, nearly 76% of teachers does not visit physician for basic complaints. About 44.5% give medications to children without doctor's prescription / advice. Around 73% agree the importance of full course of medicines as per the prescription. (Table 1)

DISCUSSION

This cross sectional study using a preformed questionnaire was done to evaluate the medication practices of school teachers in the city and rural areas of Coimbatore district. Over 400 school teachers working in both government and private schools including primary, middle and higher secondary schools in Coimbatore district after obtaining permission through proper channel from the school management participated in the study. The teachers were given a brief explanation about the study and after obtaining informed consent they were asked to fill up the questionnaire.

In this study only 22% from city, 27% from rural areas visit doctor for basic complaints (Table.1) and most of them (93%) consider that it is not necessary to visit doctor for simple / basic complaints (Fig.1).

Around 81% from city and 89% from rural areas said that they won't purchase medicines without doctor's prescription (Table.1)[11]. About half of the study population said that they purchase medicines without prescription for minor or simple complaints. Others have mentioned that it takes lot of time to consult a doctor and in order to save time they purchase by themselves (Fig.2).

Most of the school teachers from both city (88.50%) and rural (84.50%) areas have said that they follow the instructions given by the doctors / pharmacists to use drugs appropriately [eg. taking antacids in empty stomach] (Table.1).

In this study around 71% of school teachers from city and 74.50% of school teachers from rural area (Table.1) purchase full course of medicines as per prescription given by the doctor. Remaining

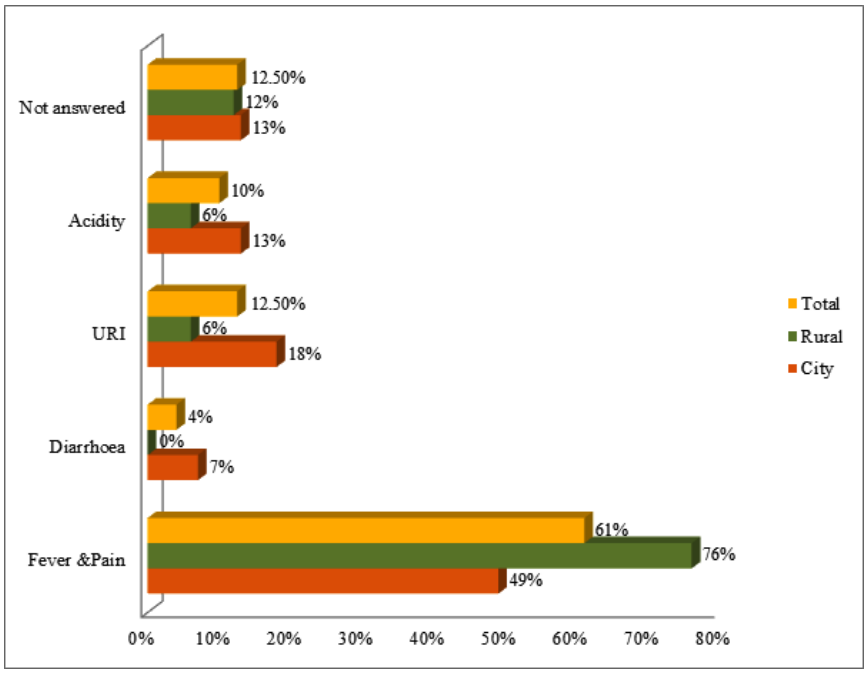


Fig. 6: Health problems for sharing your medicines with others

teachers have answered that based on improvement of symptoms further purchase could be done (around 50%), some teachers answered that medicines need not be taken for a long time and others said that too many medicines would spoil their health (Fig.3).

Among the 400 school teachers, around 96% (97.50% from city and 94.50% from rural areas) have answered that they would check the expiry date before intake of medicine (Table.1).

Most of the teachers participated in the study answered that they would not give adult medications to children (Table.1). The most common reason was that it is not safe to give adult medicines to children (70% in city % 62% in rural area) followed by dosages cannot be adjusted for children and medicines given for adult won't work for children (Fig.5). Very few teachers answered that they give adult medications to children because there is no difference between adult and children medicines and quicker recovery occurs due to high dose as given for adults (Fig.4).

Around 35% from city and 26% from the rural areas have answered that they use the same prescription again and again for getting the drugs from pharmacy without consulting a doctor (Table.1).

Only 10.50% from the city and 13.50% from rural areas use their family members medications for their health complaints (Table.1).The reasons they have mentioned for using their medicines were that they use only for simple complaints or they have similar complaints as them. Most of them did not use their family members' medicines as they considered them to be not safe or they have different health complaints not related to them.

Regarding the maintenance of first aid box in the schools, around 93.50% from the city and 97% from the rural areas have answered that they regularly maintain the first aid box in their schools (Table.1).

In this study it was found that almost half of the school teachers (48% from city and 41% from rural) give medications to children without doctor's advice or prescription (Table.1).

Regarding sharing of their medicines with the staffs or family members, only about 19.50% from city and 16.50% from rural areas share their medicines with others (Table.1). They mainly used to share medicines for fever, pain, acidity, diarrhea and respiratory infections (Fig .6).

In this study it was found that around 66% from city and 64.50% from rural areas were stocking medications at home (Table.1).Only some of the teachers have mentioned the drugs that they stock at home. They used to stock analgesics, cough syrups, antacids and anti diarrheal medications.

CONCLUSION

From the above results it was found three fourth of school teachers practice self-medication for basic complaints and one third teachers share their medicines with family members. Nearly half of participants administer medicines to their children without a prescription

This study revealed wide gap in the knowledge of health habits and medication practices, which can be overcome in future with personal / group discussion in the form of workshop to alleviate the doubts in their minds, since we understand that teachers are the best medium to disseminate the concept of rational use of medicines to the society.

REFERENCES

1. Fresle, D.A. and Wolfheim C. Public education in rational drug use: a global survey. Action Programme on Essential Drugs. World Health Organization. (1997).
2. Hameen-Anttila K, Juvonen M, Ahonen R, *et al.* What School children should Be Taught about Medicines: Combined Opinions of Children and Teachers? *Health Education.,* **105**(6): 424-436 (2005).
3. World Health Organization. Promoting rational use of medicines: core components.
4. World Health Organization Media Centre:

Promoting rational use of Medicines save lives and money, WHO experts say. http://www.who.int/mediacentre/news/notes/2004/np9/en/.

5. Bozoni K, Kalmanti M, Koukouli S. Perception and knowledge of medicines of primary schoolchildren: the influence of age and socioeconomic status. *European journal of pediatrics.,* **165**(1): 42-49 (2006).
6. Ofovwe, G. E. and Ofili A.N. Knowledge, attitude and practice of school health programme among head teachers of primary schools in Egor Local Government Area of Edo State, Nigeria. *Annals of African medicine*., **6**(3):99 (2007).
7. Abahussain E. A, Matowe L. K, Nicholls P. J. Self-reported medication use among adolescents in Kuwait. *Medical principles and practice.;* **14**(3):161-164 (2005).
8. Olczak-Pienkowska A. and Grzesiowski P. Progress towards implementing the e-Bug Project in Poland. *Journal of Antimicrobial Chemotherapy*.; **66**: 77-79 (2011).
9. Kafle K.K, Karkee S.B, Shrestha N, *et al*. Community intervention to improve knowledge and practices on commonly used drugs. *Kathmandu University Medical Journal*., **8**(1): 29-34 (2010).
10. Jha N, Bajracharya O. and Shankar P.R. Knowledge, attitude and practice towards medicines among school teachers in Lalitpur district, Nepal before and after an educational intervention. *BMC public health.*, **13**(1): 1 (2013).
11. Thawani V.R, Gharpure K.J. and Sontakke S.D. Impact of medicine-related information on medicine purchase and use by literate consumers. Indian journal of pharmacology., **46**(4): 420 (2014).
12. Coimbatore, https://en.wikipedia.org/w/index.php?title=Coimbatore&oldid=743263747

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Research Article

A STUDY ON THE EFFECTS OF AGOMELATINE ON FOOD INTAKE AND BODY WEIGHT IN RESTRAINT STRESS MODEL IN ADULT SWISS ALBINO MICE

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ABSTRACT

Objectives: Agomelatine is a novel melatonin (MT) receptor agonist at MT 1 and 2, serotonin receptor antagonist and an effective chronobiotic agent. The study was designed to evaluate the effects of agomelatine on body weight and food intake in restraint stress model in adult Swiss albino mice.

Methods: After the approval of Institutional Animal Ethics Committee, 40 male Swiss albino mice were randomly divided into four groups of 10 animals each; two were treatment groups which received 25 mg/kg (low dose) agomelatine, 50 mg/kg (high dose) agomelatine, standard group given trazodone and the control group administered the vehicle (1% hydroxyethyl cellulose [HEC]) intraperitoneally for the last 14 days in the 3 weeks study period. Chronic restraint stress was given for 4 hrs per day for all groups starting from day 0 to 21.

Results: Using paired t-test, both 12 hrs ($p=0.011$) and 24 hrs ($p<0.001$) food intake in the high dose agomelatine group were significantly increased. Between groups using ANOVA test showed a statistically significant increase in food intake for this group when compared to the control group. Unlike the low dose agomelatine group ($p=0.205$), the mean body weight in the group treated with high dose agomelatine revealed a statistically significant rise compared to that of the control ($p=0.001$) in ANOVA test.

Conclusion: High dose agomelatine was effective in antagonizing the body weight lowering effect of restraint stress in addition to amelioration of reduced food intake. The study has potentially brought out the additional therapeutic benefit of agomelatine in improving the altered feeding and body weight changes when used in the treatment of the depression.

Keywords: Agomelatine, Body weight, Food intake, Satiety, Circadian rhythm.

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INTRODUCTION

Depression is a common illness worldwide, with an estimated 350 million people affected; constituting a major portion of mental health disorders [1]. A meta-analysis reported that the worldwide prevalence rate of depressive disorders in elderly population between 4.7% and 16% with a comparatively higher prevalence of 21.9% in India [2]. A study conducted in North India has shown that the prevalence of depression in the elderly was high even in a rural community with an estimate of 14.4% [3]. Hamilton Rating Scale for depression states that key features of depression include weight loss and reduced appetite [4]. Core symptoms according to diagnostic and statistical manual of mental disorders-V also includes eating too much/little. Moreover a U-shaped association between body weight and depression has been described [5].

Agomelatine is a novel melatonin (MT) receptor agonist at MT1 and MT2 receptors, serotonin receptor 5-hydroxytryptamine ($5HT_3$2C) antagonist and an effective chronobiotic agent. It is found to be an effective antidepressant and an anxiolytic agent [6,7]. It has also been proved to improve sleep in a depressed patient by synchronizing the circadian rhythm [7].

Agomelatine, in addition to being a chronobiotic agent, has been shown to be effective in restraint stress induced depression in animal models [8]. Chronic restraint stress induces depressive-like behavior by increasing immobility time in the forced swimming test and cognitive deficit by producing memory impairment in the object recognition testing in animal models [9]. Restraint stress also affects the food intake and body weight of the animals [10]. There is a paucity of studies evaluating the effects of agomelatine on body weight and food intake in depression. Therefore the study was designed to evaluate the effects of agomelatine on body weight and food intake in restraint stress (immobility induced) model in adult Swiss albino mice.

METHODS

The Institutional Animal Ethics Committee approved the protocol of the study (Approval number: 241/2014/IAEC). Adult, male Swiss albino mice weighing 25-40 g and aged 5-10 months were housed in constant ambient temperature under 12 hrs light, 12 hrs dark cycle. They were group housed as 3-4 mice per cage in polypropylene cages. Pellet diet and tap water were provided *ad libitum*. The animals were randomly allocated into four groups of 10 mice per group.

- Group 1 - (treatment Group 1) low dose agomelatine was administered - 25 mg/kg/day
- Group 2 - (treatment Group 2) high dose agomelatine was administered - 50 mg/kg/day
- Group 3 - (standard group) trazodone 10 mg/kg/day
- Group 4 - control group given 1% HEC.

The vehicle HEC is cellulose derived hydrophilic polymer [11]. The test, standard and vehicle (1% HEC) were given intraperitoneally (single dose per day) to the animals in the respective groups for the last 14 days in the 3 weeks period. Chronic restraint stress was given for 4 hrs per day starting from day 0 to day 21 for all groups.

The body weight and food intake (both 24 hrs and 12 hrs dark phase) were measured at 0, 7, 14, 21 days in each group [12]. After acclimatizing to individual housing for12 hrs, food intake namely the 24 hrs and 12 hrs dark phase food intake were measured by keeping pre-weighed amount of food in each of the cages, in which the animals

were individually housed and measuring the left-over food at the end of light phase (12 hrs) and dark phase (12 hrs). An accurate measurement was made by returning the spilled food at the bottom of the cage before measuring the weight of left-over food. Each animal was weighed and the body weight was recorded before the measurement of food intake on 0, 7, 14, and 21 days (Fig. 1).

RESULTS

All data ware entered into excel and analyzed using SPSS software version 19. At baseline, a one way ANOVA test done showed that there was no significant difference in either the body weight (p=0.08) or the food intake, both the 12 hrs (p=0.53) and 24 hrs (p=0.05) in the four groups of animals indicating that there was no significant difference at baseline among the animals which were randomized to the four groups.

The food intake, both 12 hrs and 24 hrs in both the treatment groups (low dose and high dose) as well as the standard trazodone group showed an increase when compared between the 7th day intake and 21st day intake demonstrating the effects of drug treatment (Figs. 2 and 3). Using paired t-test, both 12 hrs (p=0.011) and 24 hrs (p<0.001) food intake in the high dose agomelatine group were found to be significantly increased. In addition, between groups analyses using ANOVA test showed a statistically significant increased food intake only in group 2 treated with high dose agomelatine when compared to control, but not the other two groups (Table 1).

A paired t-test done at the end of 7 days showed that there was a statistically significant reduction in weight in Groups 1-3 (Fig. 4) which were subsequently treated with low dose agomelatine, high dose agomelatine and trazodone respectively. The reduction in weight was induced as a result of the chronic restraint stress in this animal model.

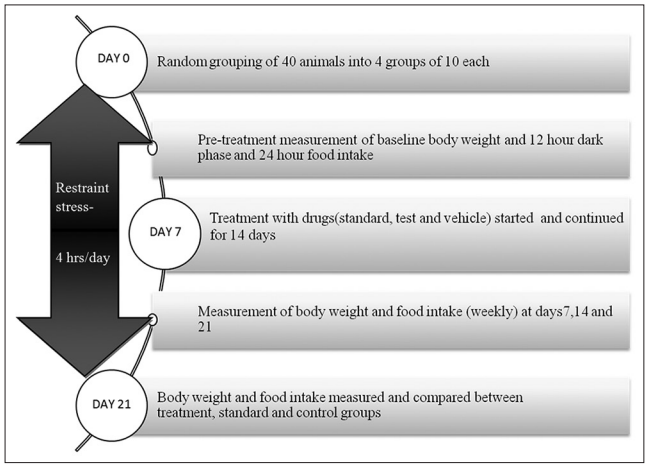


Fig. 1: Methodology of the study

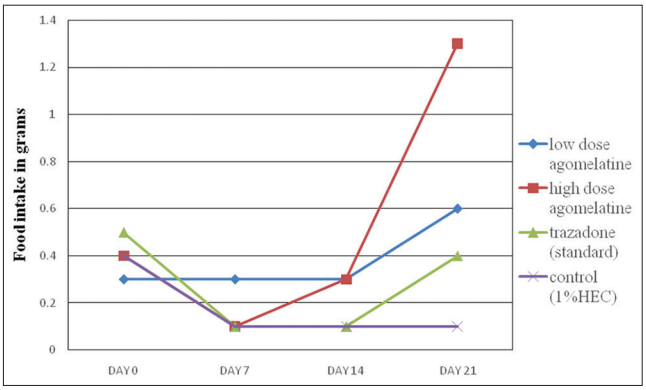


Fig. 2: Comparison of 12 hrs mean food intake among groups

A paired t-test between the body weight measurements on day 7 and day 21 was done to detect a statistically significant change in mean body weight when agomelatine/trazodone was given along with the restraint stress. The low dose agomelatine group showed a statistically significant reduction in body weight (p=0.026). However, using ANOVA test, the changes in mean body weight were not significant in the groups which were treated with low dose agomelatine or trazodone compared to the control group (Fig. 4 and Table 1). The rise in mean body weight in the group treated with high dose agomelatine (p=0.001) when compared to control, was statistically significant indicating that agomelatine at 50 mg/kg was effective in antagonizing the body weight lowering effect of restraint stress. This was also mirrored in the statistical analysis of food intake between the three groups done at the end of 21 days (Table 1).

DISCUSSION

Agomelatine is a MT receptor agonist, serotonin receptor 5HT2C antagonist and an effective chronobiotic agent. It has been found to be an effective antidepressant and an anxiolytic [6]. Agomelatine synchronizes the circadian rhythm and also ameliorates stress-related changes like depressive symptoms by modulating the cytoskeleton micro tubular dynamics and synaptic markers such as brain derived neurotrophic factor in amygdala and hippocampus [13,14]. Agomelatine antagonizes serotonin 2C receptor which is an important mediator of satiety [15]. It is well established from studies conducted earlier that restraint stress model in mice induces depression such as state in addition to the reduction in body weight and food intake by altered expression of food related genes like decreased ghrelin and increased Pro-opiomelanocortin [16,17].

A statistically significant increase in food intake was demonstrated only in the group treated with high dose agomelatine when compared

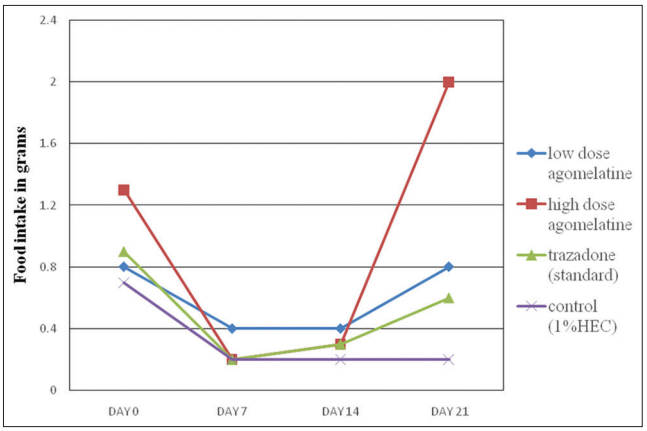


Fig. 3: Comparison of 24 hrs mean food intake among groups

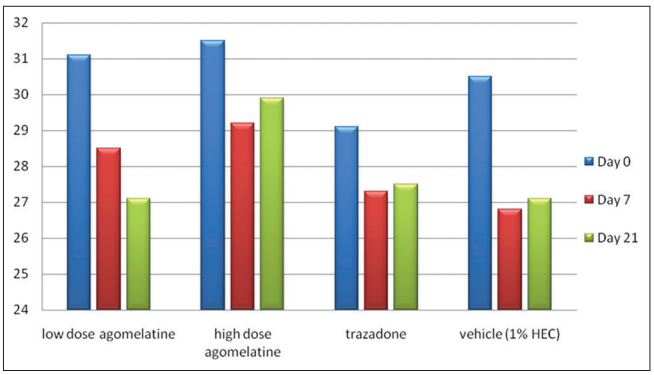


Fig. 4: Comparison of body weight (grams) among groups

Table 1: Between-groups analysis using one-way ANOVA

Groups	12 hrs food intake		24 hrs food intake		Body weight	
	F statistic	p value	F statistic	p value	F statistic	p value
Group 1 (low dose agomelatine)	7.091	0.073	7.606	0.140	10.051	0.205
Group 2 (high dose agomelatine)		0.000		0.000		0.001
Group 3 (trazodone)		0.275		0.321		0.176

to control, but not in groups which were treated with low dose agomelatine and trazodone (Table 1). This has confirmed that though there was an increased food intake evident in all the three groups, it was the high dose agomelatine which has significantly increased the food intake higher than that of the control group at the end of the treatment period, thereby effectively neutralizing the effect of restraint stress on the body weight. This also explains why the high dose agomelatine group had mean body weight significantly higher compared to that of control in contrary to the low dose agomelatine and trazodone groups.

The reasons for the effects of agomelatine on body weight and food intake are twofold. The first being the antidepressant activity of the drug, responsible for the therapeutic effect on the animals in depression-like state induced by chronic restraint stress which secondarily has led to the changes in body weight and improved food intake in the stressed animals. Second, this can be explained as due to the agomelatine's action at the serotonin receptor 5HT2C, which is a serotonin receptor subtype that is involved in normal satiation following a meal. Agomelatine antagonizes the 5HT2C receptor, and thereby it interferes with the satiety, increasing the food intake in the treated animals. The effect was well manifested in the group treated with high dose agomelatine. In contrast, the low dose agomelatine group did not show a similar response which probably could have been due to inadequate dosage to demonstrate such effects on weight and food intake. Thus, this study has delineated the effects of agomelatine on food intake and body weight when used as an antidepressant.

CONCLUSION

This study highlights the additional therapeutic benefit of agomelatine in the treatment of depression as it improves the altered feeding and body weight changes. The study clearly indicates the role of agomelatine in normalizing food intake and body weight in depressed patients with weight loss from poor feeding. These beneficial effects can be explained by its synchronizing action on circadian rhythm and antagonism of 5HT2C receptor, an important mediator of satiety.

REFERENCES

1. Reddy MS. Depression - The global crisis. Indian J Psychol Med 2012;34(3):201-3.
2. Barua A, Ghosh MK, Kar N, Basilio MA. Prevalence of depressive disorders in the elderly. Ann Saudi Med 2011;31:620-4.
3. Pilania M, Bairwa M, Khurana H, Kumar N. Prevalence and predictors of depression in community-dwelling elderly in rural Haryana, India. Indian J Community Med 2017;42(1):13-8.
4. Worboys M. The Hamilton rating scale for depression: The making of a 'gold standard' and the unmaking of a chronic illness, 1960-1980. Chronic Illn 2013;9(3):202-19.
5. de Wit LM, van Straten A, van Herten M, Penninx BW, Cuijpers P. Depression and body mass index, a u-shaped association. BMC Public Health 2009;9:14.
6. Guaiana G, Gupta S, Chiodo D, Davies SJ, Haederle K, Koesters M. Agomelatine versus other antidepressive agents for major depression. Cochrane Database Syst Rev 2013;12:CD008851.
7. Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML. MT1 and MT2 melatonin receptors: A therapeutic perspective. Annu Rev Pharmacol Toxicol 2016;56:361-83.
8. Reagan LP, Reznikov LR, Evans AN, Gabriel C, Mocaër E, Fadel JR. The antidepressant agomelatine inhibits stress-mediated changes in amino acid efflux in the rat hippocampus and amygdala. Brain Res 2012;1466:91-8.
9. Budni J, Zomkowski AD, Engel D, Santos DB, dos Santos AA, Moretti M, *et al*. Folic acid prevents depressive-like behavior and hippocampal antioxidant imbalance induced by restraint stress in mice. Exp Neurol 2013;240:112-21.
10. Jiang SZ, Eiden LE. Activation of the HPA axis and depression of feeding behavior induced by restraint stress are separately regulated by PACA Pergic neurotransmission in the mouse. Stress 2016;19(4):374-82.
11. Arafat M. Approaches to achieve an oral controlled release drug delivery system using polymers: A recent review. Int J Pharm Pharm Sci 2015;7(7):16-21.
12. Shanmugapriya S, Senthurselvi R, Bhuvaneswari K. A study comparing the anorectic activity of hot and cold aqueous extracts of *Dolichos biflorus* Linn. Seeds in freely feeding rats. Int J Pharm Pharm 2016;8(7):336-9.
13. Ladurelle N, Gabriel C, Viggiano A, Mocaër E, Baulieu EE, Bianchi M. Agomelatine (S20098) modulates the expression of cytoskeletal microtubular proteins, synaptic markers and BDNF in the rat hippocampus, amygdala and PFC. Psychopharmacology (Berl) 2012;221(3):493-509.
14. Conboy L, Tanrikut C, Zoladz PR, Campbell AM, Park CR, Gabriel C, *et al*. The antidepressant agomelatine blocks the adverse effects of stress on memory and enables spatial learning to rapidly increase neural cell adhesion molecule (NCAM) expression in the hippocampus of rats. Int J Neuropsychopharmacol 2009;12(3):329-41.
15. de Bodinat C, Guardiola-Lemaitre B, Mocaër E, Renard P, Muñoz C, Millan MJ. Agomelatine, the first melatonergic antidepressant: Discovery, characterization and development. Nat Rev Drug Discov 2010;9(8):628-42.
16. Haque Z, Akbar N, Yasmin F, Haleem MA, Haleem DJ. Inhibition of immobilization stress-induced anorexia, behavioral deficits, and plasma corticosterone secretion by injected leptin in rats. Stress 2013;16(3):353-62.
17. Jeong JY, Lee DH, Kang SS. Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice. Endocrinol Metab (Seoul) 2013;28(4):288-96.

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Original Research Article

Knowledge and awareness of community pharmacists towards rational use of medicine in and around Coimbatore

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Abstract

Background: Achievement of goals of rational use of medicine (RUM) in the community is not possible without participation of community pharmacists. Pharmacists must know that they are the main tool to educate and spread information on rational use of medicine in the community. This background enabled this study to observe the knowledge, awareness and attitude of the community pharmacists towards the rational use of medicine. Aim and Objectives were to assess the knowledge and awareness among community pharmacists about rational drug use in and around Coimbatore.

Methods: It was a cross sectional observational study using a preformed questionnaire. Study participants were 215 Community Pharmacists (those are registered pharmacist running standalone pharmacy stores not affiliating to any institutions /hospitals/not a part of a large chain of stores) inside (115 Pharmacist) and around (100 Pharmacist) Coimbatore city. Questionnaire was given to them and results were analyzed and expressed in percentage at the end of the study.

Results: Comparing the awareness about RUM in the community pharmacists in and around Coimbatore was found with the results which was almost equal or less in terms of dispensing medicines without prescription, issuing medicines to outdated prescription, educating the public about ADR etc.

Conclusions: This study ensure the need of the community pharmacist participation for the development of a clear educational policy on promoting rational use of medicine involving all segments of health care system to ensure the community benefit and safety.

Keywords: Attitude, Awareness, Knowledge, Rational use of medicine, Pharmacist

Introduction

Rational uses of medicines (RUM) play an important role in clinical practice. The clinicians, pharmacists and the public should have an idea about rational use of medicine to prevent adverse drug reaction due to inappropriate use of medicine which will produce ill effects on health. The goal in rational use of medicine can be achieved only by participation of community pharmacists. The pharmacists form the bridge between the clinicians and patients. The reach of the pharmacists is extensive both in the community and hospital.

Community pharmacists are the first contact with public in terms of many common illnesses. Public have faith and confidence in community pharmacists because they were belonging to the same location and can be easily approached. Pharmacists in rural areas educate the patients about their illness and even guide them which clinician to consult for their illness. Its beyond medicine supply, community pharmacists have cordial relationship with the public. Community pharmacists form the key in unlocking the patient's ignorance towards rational use of medicine. In western world, it is documented that appropriate pharmacy practice by the pharmacist will lead us to reach the goal in rational use of medicine.

World Health Organization has described RUM as "The meaning of rational use of medicine is that the patient gets appropriate medicine according to their clinical needs, in doses needed for their requirements for an adequate period and at a reasonable cost for both the patient and their community."

In a publication done by WHO only the rules and regulations of RUM was enumerated. Very few studies were conducted among the pharmacists. This study emphasises the vital role of pharmacists in concept of rational use of medicine and provide necessary education to them. Many pharmacists are aware of the consequences of irrational use of medicine. Pharmacists must know they are the main tool to educate patients about advantages of rational use of medicine.[1]

In another study, the participants were general practitioners and community pharmacists with patients diagnosed as upper respiratory infection where general practitioners were better than the pharmacists in concept of using antibiotics when compared to the pharmacists.[2]

One study was done with 756 community pharmacists. The pharmacists lacked public concern and were not keen about the RUM concept. The main reason for this was their diversities in education, influence by the management to get attached to drug policies and finally the most important was the financial needs.[3]

The patients must educate about various drugs, drug interactions and adverse effect due to irrational use of medicine. In another study 66% pharmacists explained ADR, 41% checking prescription validity, 87% maintains professional relationship, 12% consult other pharmacist and 81% explaining medicines effects.[4]

This was the first study to compare community pharmacists in urban and rural area of Coimbatore.

Objectives

1. To assess the knowledge and awareness among community pharmacists about rational drug use in and around Coimbatore.
2. To know the dispensing attitude of community pharmacist regarding rational drug use.

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The design of this study was 1 year, non-interventional, a Prospective cross sectional observational study was initiated using a preformed questionnaire.

Inclusion criteria

Community Pharmacists (those are registered pharmacist running standalone pharmacy stores not affiliating to any institutions /Hospitals/not a part of a large chain of stores).

Exclusion criteria

Pharmacists in nursing home, chains of pharmacy stores, multispecialty hospitals, and medical college hospitals, Government hospitals were excluded from this study.

After obtaining written informed consent, 215 Community Pharmacists (115 Pharmacist - inside Coimbatore city) and (100 Pharmacist outside Coimbatore city) were participated in this study. Study period was from October 2014 to February 2015. Questionnaire was filled by the study participants, and then the results were analyzed and expressed in percentage at the end of the study.

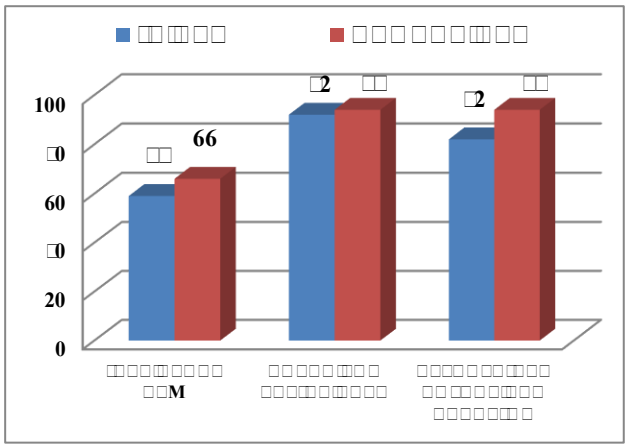
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Comparing the knowledge, awareness and attitude about RUM of both the groups of pharmacists in Coimbatore was done. While comparing the knowledge about RUM in community pharmacists in and around Coimbatore, was found to be 59% and 49% respectively (Table 1).

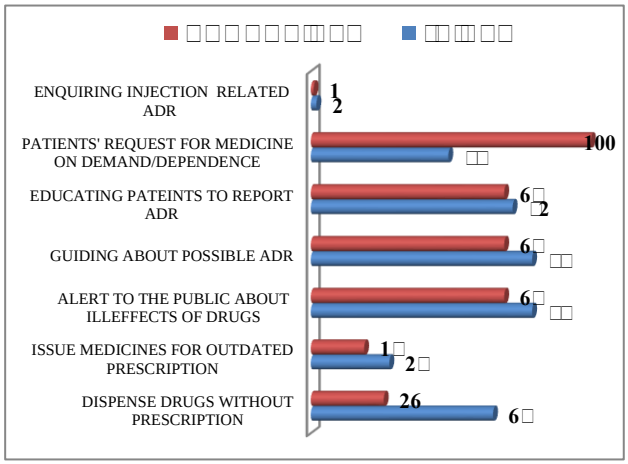
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Awareness about RUM concepts	59	41	49	51
Demanding prescription before dispensing the drug	65	35	80	20
Issued medicines for same / old prescriptions	72	28	81	19
Alert to the public about the ill effects of drugs	79	21	69	31
Guiding patient about the possible ADR	79	21	69	31
Guiding patients to report about ADR (if any)	72	28	69	31
Dispensed medicines with appropriate dosage and duration	82	18	51	49
Check expiry date of the drug before dispensing	92	8	94	6
Any observation with repeated request to issue medicine on demand /dependence to drugs	49	51	71	29
Observed any injections related ADR (injection abscess, hepatitis etc)	2	98	1	99

Community pharmacists were dispensing medicines without prescription was 65 % in Coimbatore and was 80% around Coimbatore. The reasons for dispensing medicines without prescription by both groups were compared and the major reason identified was patient's request (Figure 1). 6% of community pharmacists inside Coimbatore city are dispensing medicines without prescription because of emergency. Community pharmacists in and around Coimbatore were issuing drugs over the counter and the percentage was 38 % and 41 % respectively and 3 % had other reasons for dispensing medicines without prescription.



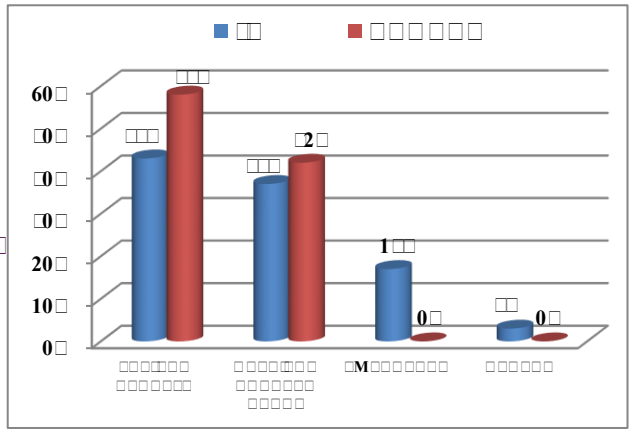
The next comparison between the community pharmacists were those issuing medicines for old prescriptions, which was72 % and 81 % respectively. (Table 1) The number of times medicines issued for the old prescriptions was analysed (Figure 2). About 56% and 74 % of community pharmacists in both the groups are dispensing medicine for the old prescription more than once. Some community pharmacists in and around Coimbatore are issuing medicine for the old prescription only once around 11% and 1% respectively.

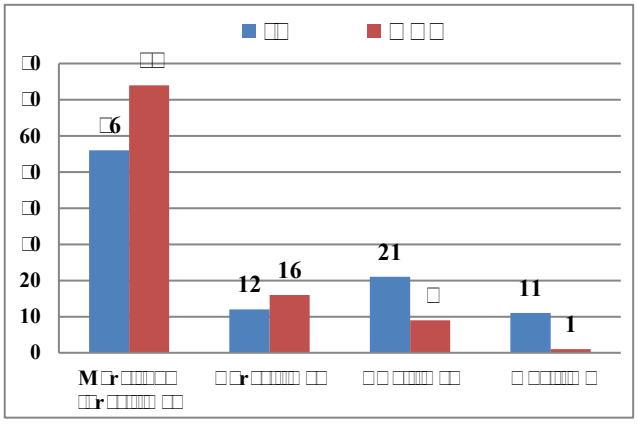


There was a comparison between the two groups whether these pharmacists were provided alert to the public about the ill effects of drugs. Community pharmacists in and around Coimbatore were all most equal in giving alert to the public about the ill effects of drugs (70%-80%) (Table 1) when compared to international study [4] this study showed a good indicator.

Regarding the Pharmacovigilancereduction program, whether the community pharmacists in and around Coimbatore guided the patients to report the ADRs was found to be almost equal around 65%-75% (Table 1).

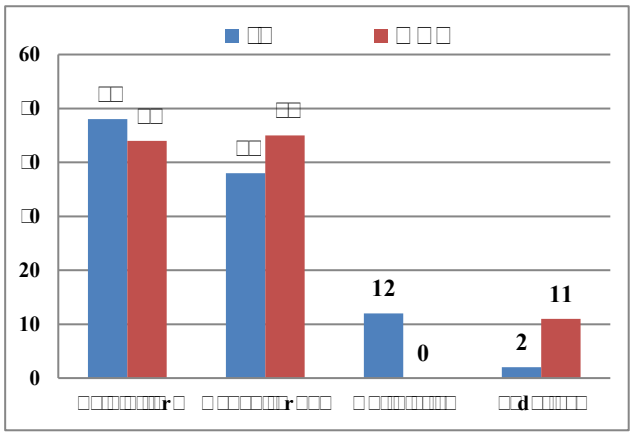
A RUM concept of dispensing medicine for appropriate dosage and duration were compared between the two groups of the community pharmacists in and around Coimbatore was found that 82% and 51% of community pharmacists in and around Coimbatore respectively were dispensing medicines in appropriate dosage and duration (Table 1).

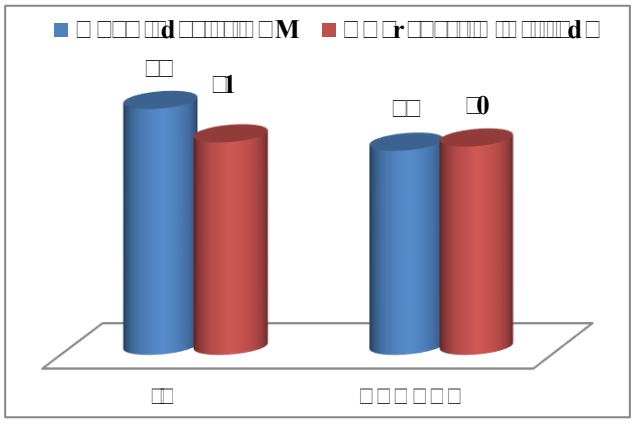




92%-94% of in and around Coimbatore Community pharmacists were checking expiry date before dispensing the drugs (Table 1) compared to an international study

which was 41%.[4] This study could not observe any injection related ADR in both groups. Patients repeatedly requesting to issue medicine without any prescriptions were 49% and 71% respectively (Table 1). Medicines which were given repeatedly without prescription were pain killers and it was 45-50% in both the groups. Cough syrups were the second commonly issued medicine with repeated request by the patients which was around 35-45% in both the groups. The community pharmacists in Coimbatore were expressed that 12% of patients are repeatedly requesting to issue antibiotics, while community pharmacists around Coimbatore report that 11% of patients are repeatedly requesting to issue sedatives. The number of patients repeatedly requesting to issue sedatives in Coimbatore is only 2% (Figure 3).





Discussion

This study was the first kind study India which tried to assess the knowledge, attitude and awareness of community pharmacists in and around Coimbatore (metro population city) towards rational use of medicine. Community pharmacies are the part of the healthcare system throughout the world. Community pharmacists act as a first-line treatment source for most of the population diversity in their distribution and setups make them an easily accessible. Community pharmacists are a quick source of advice, referral, dispensing medicines and information to the patient's. The way drugs are procured, stored and dispensed and the information given by the dispensers indicates the quality of their practice leading to rational or irrational use of medication.

This study found that only around 55% of community pharmacists have the idea about RUM (Table 1). A similar study was done in another country found that community pharmacists lacked awareness and knowledge about RUM.[5,6]

While comparing the reasons for issuing drugs without prescription, in this study majority of the community pharmacists were issued the medicines at the patient's request and also, they issued medicines over the counter without prescription (Figure 3). In an international study it was expressed that no need for the prescription for chronic and CNS diseases and also they were dispensed medicines without prescription because patient asked for the drug by name.[6]

This study found that community pharmacists dispensed medicines for old prescriptions. A similar finding was seen in another study, where medicines were issued for old prescriptions for months.[7]

In this study it was found that the number of community pharmacists provided alert to the public about the ill effects of drugs was 79% and 69% respectively in and around Coimbatore. There was a study expressing that only 40-50% community pharmacists provide alert the public about the ill effects of drugs.[8]

In another study it was found that community pharmacists guiding patients to report ADR was deficient.[4,9] But in this study, it was found that community pharmacists guiding patients for possible ADRs was 79% - and 69% and provided guidance to report ADR 72% and 69% respectively in and around Coimbatore.

It was found that, 82% and 51% of community pharmacists in and around Coimbatore was dispensing medicines in the appropriate dosage and duration. But a study done in another country had found that 97% of community pharmacists were dispensing antibiotics irrationally.[6]

In this study, it was confirmed that, almost all the community pharmacists check expiry date before dispensing the drugs and only 1.3% were enquired and observed ADRs related to injection.

This study also bring out that, the community pharmacists issued medicine without demanding prescriptions was 35% and 20% in and around Coimbatore compared to another international study which had expressed about the

patients repeated request to issue medicine without any prescriptions.[10]

Knowledge and attitude of the community pharmacists on drug safety and related aspects could greatly influence their dispensing behavior and thereby positively contribute to the patient safety. Majority of the community pharmacists had low acceptable knowledge on drug safety related aspects for specific drugs and a good number of them were not aware of certain practical aspects.[11]

In many places it was observed that, the practice of irrational drug use like self-medication, antibiotic prescribing, and sale of less-than-prescribed quantity of drugs specially antibiotics, and finally poor drug storage practices. Global research provides the evidence that it's possible to improve and regulate the practice of community pharmacists through a combination of interventions, education to achieve the goal of promoting RUM. Changing their knowledge and practices would be a slow, difficult and challenging procedure, but if the intervention once done it efficiently improves the skills even in the most resource-deprived settings.

Educational interventions with interactive training sessions if provided would be useful in improving prescribing practices of the community pharmacists like proper drug storage, record-keeping, reading doctor's prescription, medication counselling, discouraging sale of antibiotics without prescription, promoting quality dispensing of medicine etc may improve the practices in a possible way through appropriate and repeated interventions which are sustained.[12] There is a compelling need for the development of a clear policy on promoting rational use of medicine involving all segments of health care system such as physicians, community pharmacists, drug companies and government agencies. This will ensure that rational use of medicines which will benefit the community can be achieved.[12]

There is a definite need for the development of a clear educational policy on promoting rational use of medicine involving all segments of health care system such as physicians, community pharmacists, drug companies and government agencies to ensure that rational use of medicines benefits in the community.[12] Community Pharmacist require continues educational services like frequent update, scientific meeting, creating and providing good communication activities with physicians, training pharmacy staffs, adhering treatment principles, RUM practices, skill development opportunities, technical support, and pharmacy education may reduce the gap between health care system and community.[13,14]

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The World Health Organization has recognized few factors like patient demands, drug advertising etc as limitations towards achieving rational use of medicine based on various research evidence. If an appropriate intervention applied in the needy area may be helpful to improve RUM practices in the community where the pharmacist's services are very important segment in the health care system of many countries.

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Ethical approval: The study was approved by the Institutional Human Ethics Committee (14 /331)

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1. WHO. Promoting rational use of medicines: core components WHO Policy Perspectives on Medicines. September 2002.
2. Alabid AH, Ibrahim MI, Hassali MA. Antibiotics Dispensing for URTIs by Community Pharmacists (CPs) and General Medical Practitioners in Penang, Malaysia: A Comparative Study using Simulated Patients (SPs). J Clin Diagn Res. 2014;8(1):119-23.
3. Toklu HZ, Hussain A. The changing face of pharmacy practice and the need for a new model of pharmacy education. J Young Pharm. 2013 Jun;5(2):38-40.
4. Rasool BK, Fahmy SA, Abu-Gharbieh EF, Ali HS. Professional practices and perception towards rational use of medicines according to WHO methodology in United Arab Emirates. Pharm Pract. 2010 Jan;8(1):70-6.
5. Hussain A, Ibrahim MI, Baber ZU. Using the potentials of community pharmacies to promote rational drug use in Pakistan: an opportunity exists or lost? J Pak Med Assoc. 2012 Nov;62(11):1217-22.
6. Al-Mohamadi A, Badr A, Bin Mahfouz L, Samargandi D, Al Ahdal A. Dispensing Medications without prescription at Saudi community pharmacy: Extent and perception. Saudi Pharm J. 2013 Jan;21(1):13-8.
7. Kolhatkar A, Cheng L, Chan FK, Harrison M, Law MR. The impact of medication reviews by community pharmacists. J Am Pharm Assoc. 2016 Sep-Oct;56(5):513-20.
8. Cohen MR, Smetzer JL, Westphal JE, Comden SC, Horn DM. Risk models to improve safety of dispensing high-alert medications in community pharmacies. J Am Pharm Assoc. 2012 Sep-Oct;52(5):584-602.
9. Ting KN, Stratton-Powell DM, Anderson C. Community pharmacists' views on adverse drug reactions reporting in Malaysia: a pilot study. Pharm World Sci. 2010 Jun;32(3):339-42.
10. Tommasello AC. Substance abuse and pharmacy practice: what the community pharmacist needs to know about drug abuse and dependence. Harm Reduct J. 2004 Apr20;1(1):3.
11. Jose J, Jimmy B, Al-Ghailani AS, Al Majali MA. A cross sectional pilot study on assessing the knowledge, attitude and behavior of community pharmacists to adverse drug reaction related aspects in the Sultanate of Oman. Saudi Pharm J. 2014 Apr;22(2):163-9.
12. Hussain A, Ibrahim MI, Baber ZU. Using the potentials of community pharmacies to promote

rational drug use in Pakistan: an opportunity exists or lost? J Pak Med Assoc. 2012 Nov; 62(11):1217-22.
13. Dosea AS, Brito GC, Santos LM, Marques TC, Balisa-Rocha B, Pimentel D, et al. Establishment, Implementation, and Consolidation of Clinical Pharmacy Services in Community Pharmacies: Perceptions of a Group of Pharmacists. Qualitative health research. 2017 Feb; 27(3):363-73.
14. Puspitasari HP, Aslani P, Krass I. Challenges in the management of chronic no communicable diseases by Indonesian community pharmacists. Pharmacy Practice. 2015 Jul-Sep;13(3):578.

Bhuvaneswari K, Jeyalakshmi D, Umamaheswari A. Knowledge, awareness and attitude towards rational use of medicines by community pharmacists in and around Coimbatore, India. Int J Basic Clin Pharmacol 2017;6:2422-7.

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Original Research Article

A study of antibiotic drug sensitivity patterns in urinary tract infections in a tertiary care hospital

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Abstract

Background: Urinary tract infection (UTI) is one of the most common bacterial illnesses and hence one of the most important indications for antibiotic treatment. Current knowledge of the common organisms implicated in causing UTI in the local community and surveillance to monitor the changes in susceptibility of uropathogens are imperative to ensure appropriate therapy. The study objectives were to assess the proportion of UTI caused by each of the common urinary pathogens, to study the antibiotic drug sensitivity patterns by analyzing the culture and sensitivity reports and to identify the drugs which would be potentially favourable candidates for empirical therapy in the study locale.

Methods: This hospital based cross sectional study was done by collecting the data from case records of patients who attended OPD or were admitted with diagnosis of UTI during the study period.

Results: *Escherichia coli* was the single most important cause of UTI, accounting for 70% of the infection among the studied subjects, amongst which 55% was extended spectrum beta-lactamase producing organisms. A pattern of increased susceptibility to higher antibiotics like ertapenem, tigecycline and decreased sensitivity to fluroquinolones was evident. In addition, nitrofurantoin was found to be one of the antibiotics to which most of the organisms were susceptible (85%) favouring its use in empirical therapy of UTI.

Conclusions: The pattern of accentuated susceptibility to higher antibiotics in the face of declining trend in the sensitivity to commonly used antibiotics necessitates promoting awareness on the need for rational prescribing of antibiotics among clinicians.

Keywords: Antibiotics, Culture and sensitivity, *Escherichia coli*, Urinary tract infection

Introduction

Urinary tract infections (UTI) remain one of the commonest infections diagnosed in out-patients and hospitalized patients. An in-depth knowledge on antimicrobial susceptibility pattern is essential for appropriate therapy, particularly with increasing resistance resulting from repeated use of the same antibiotic.[1] It is also essential to be aware of the changing patterns of antibiotic resistance in a locale.[2] Published data may not always represent the scenario prevailing in a particular location as the studies done elsewhere could vary in the pattern of microorganisms involved in causation, their sensitivity and resistance to different antibiotics which in turn depends on the antibiotic prescribing pattern in that region. This indicates that there is a need for studying the existing antibiotic sensitivity patterns in the local population.[3] Resistance significantly varies by region and the highest antibiotic resistance has been observed in developing countries. Similarly a significant rise in resistance over time is seen in studies reporting on community acquired *E. coli* UTI.[3]

Hence this study was designed to evaluate the drug sensitivity patterns to some of the commonly used antimicrobials in UTI like the beta lactams, fluroquionolones and aminoglycosides. In addition, the

antibacterial drugs commonly used in UTI like nitrofurantoin, cotrimoxazole were also studied for the sensitivity patterns. Thus, the study was done to provide an insight into the drug sensitivity patterns of common urinary pathogens to antibiotic therapy by analyzing the culture and sensitivity reports.

Our study was designed to identify the proportion of UTI caused by each of the urinary pathogens prevalent in the study locale and the likely drug candidates with the potential of being used as empirical therapy in community acquired pathogens by evaluating their sensitivity pattern, thereby enabling identification of the drugs which can effectively be used in the management of UTI.

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This was a hospital based study done at a tertiary care teaching hospital after the approval from Institutional Human Ethics Committee. The study was a cross sectional one, done using case records. The data from case records of the patients who attended OPD or admitted with diagnosis of UTI during the study period were included for the study based on inclusion and exclusion criteria.

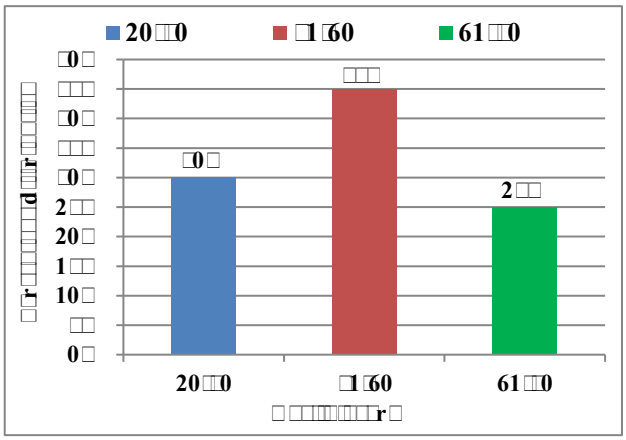
The data from the case records of adult patients above the age of 20 years, of both sexes, diagnosed with UTI were included for the study. Patients with asymptomatic bacteriuria, those with complicated UTI, upper UTI or known malformations, paediatric and pregnant patients, immunocompromised patients like patients with HIV, on corticosteroids or immunosuppressant therapy, organ transplant recipient were excluded.

The culture and *in vitro* sensitivity testing report data were collected in addition to demographic data, co morbid conditions, symptoms and duration of symptoms and drug therapies. In addition, data on history of hospitalizations, catheterizations and frequency of recurrences were also obtained from the case sheets. Also, the details recorded on the review date regarding cure or continuing symptoms, repeat culture reports, if any, was made note of from the case record.

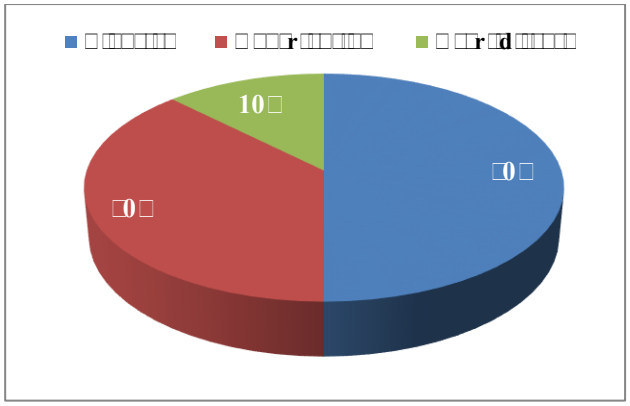
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A total of 20 patients whose case records indicated that they had been diagnosed with UTI were included for the study. All these patients were urine culture positive. In our study 30% of patients were in the age group 20 -40 years and a large majority of about 45% were in the age group of 41-60 years and 25% of the patients were aged 61 -80 years (Figure 1). Male female ratio in our study population was 65:35.

Catheterization was done in 20% of the study subjects. The duration of catheterization was variable with one patient having a history of prolonged catheterization of 12 days. Concomitant renal disease was found in a high proportion of the UTI patients. Amongst the studied population, 10% had renal failure, 10% nephritis and 5% pyelonephritis with renal calculi. One patient manifested with lupus nephritis as she was a known case of systemic lupus erythematosis. One patient was a case of neurogenic bladder. About10% patients had presented with concomitant prostatitis and a similar 10% with UTI had history of recurrent episodes (Figure 2).



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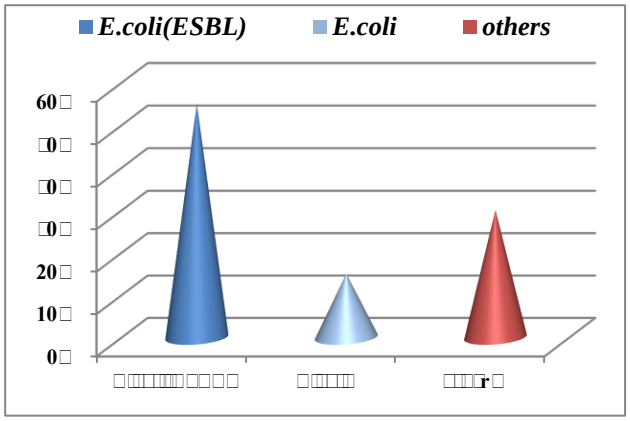
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Escherichia coli was the single most important microorganism found to implicated in causation of UTI, accounting for 70% of the infection among the studied subjects (Table 1).

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Escherichia coli (ESBL)	55%
Escherichia coli	15%
Pseudomonas aeroginosa	10%
Enterococcus fecium	10%
Enterococcus fecalis	5%
Klebsiella pneumoniae	5%
Infection with dual organisms	10%

Organisms grown in urine culture in the study population revealed a high proportion of *E. coli* being Extended spectrum beta-lactamase (ESBL) producing organisms. Next in frequency was *Pseudomonas aeroginosa* and *Enterococcus fecium*, responsible for 10% each whilst *Enterococcus fecalis and Klebsiella* contributing 5% each. Co-infection with two of these organisms (dual infection) was seen in 10% (Figure 4).

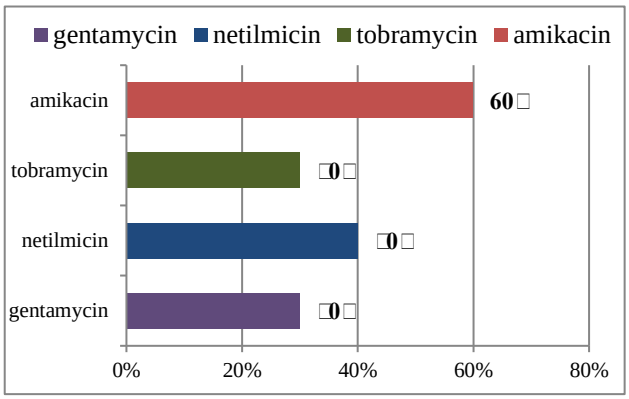


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The sensitivity of the cultured organisms was also evaluated based on their susceptibility to different groups of antibiotics.

Aminoglycosides

The sensitivity to amikacin was found to be highest (60%) followed by netilmycin (40%) and the sensitivity for tobramycin and gentamycin were equal, each with 30% (Figure 5).



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cefaperazone in combination with sulbactam and the next highest being piperacillin tazobactum combination with 45% and a slightly lower percentage of 40% was recorded for amoxicillin clavulanate (Figure 7).

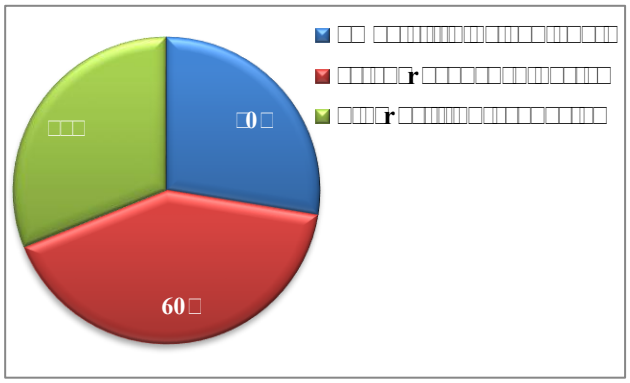


Figure 7. Pie chart showing the percentage of sensitivity of the organisms to the antibiotics in the amino-penicillin group

Amino-penicillins

The aminopenicillins were one of the drug groups studied for bacterial sensitivity apart from ureidopencillins in the penicillin group of beta-lactam antibiotics and among the two amino-pencillins used commonly, organisms showed a marginally higher sensitivity to ampicillin (20%) as compared to amoxicillin (10%).

Cephalosporins

In the cephalosporin group, cefaperazone in combination with sulbactum was the antibiotic to which most organisms (60%) were sensitive. Apart from it, the other cephalosporins found to be useful in 10 -20% of infections based on culture and sensitivity pattern are cefepime, ceftriaxone, cephalothin and cefuroxime respectively with 30%, 20%, 15% and 10%. It was evident that sensitivity for the first and second generation was lower in comparison to third and fourth generations of cephalosporins (Figure 8).

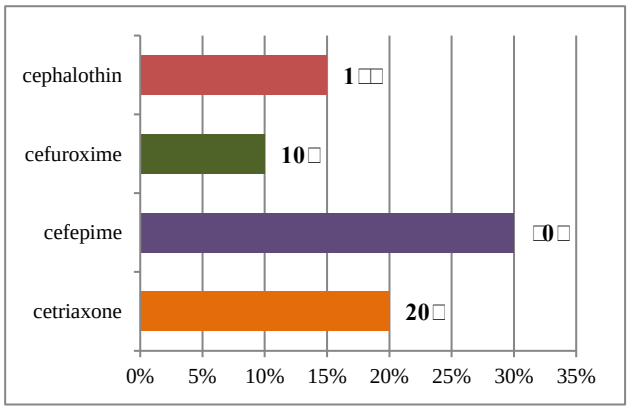


Figure 8. Bar chart showing the percentage of sensitivity of the organisms to the cephalosporin group of antibiotics

Carbapenems

Among carbapenems, greatest sensitivity was recorded for ertapenem (70%) (Figure 9).

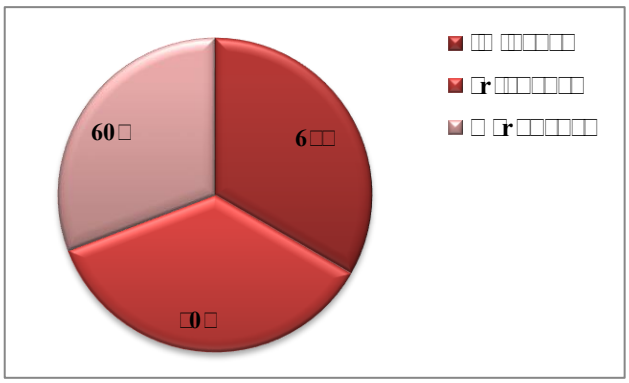


Figure 9. Pie chart showing the percentage of sensitivity of the organisms to the carbapenem group of antibiotics

Tetracycline and glycylcycline

Tigecycline was found to be the antibiotic to which 60% of the organisms studied were susceptible to and it matched that of cefaperazone combined with sulbactum in achieving the same percentage of sensitivity (Figure 10).

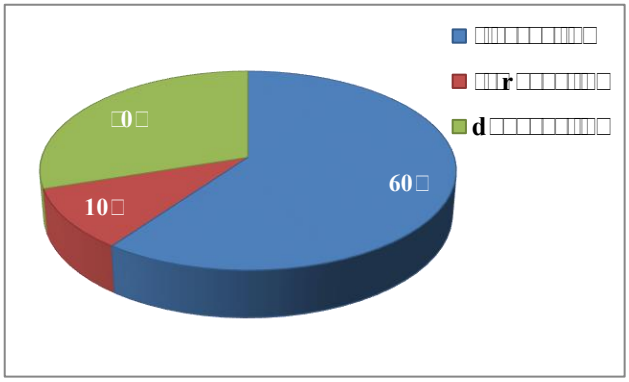


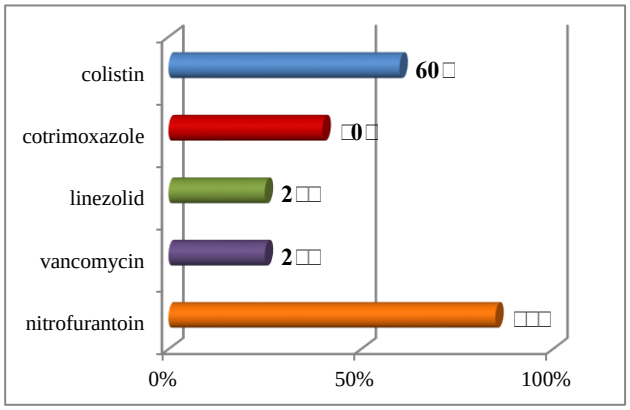
Figure 10. Pie chart showing the percentage of sensitivity of the organisms to the tigecycline and cefaperazone sulbactam combination

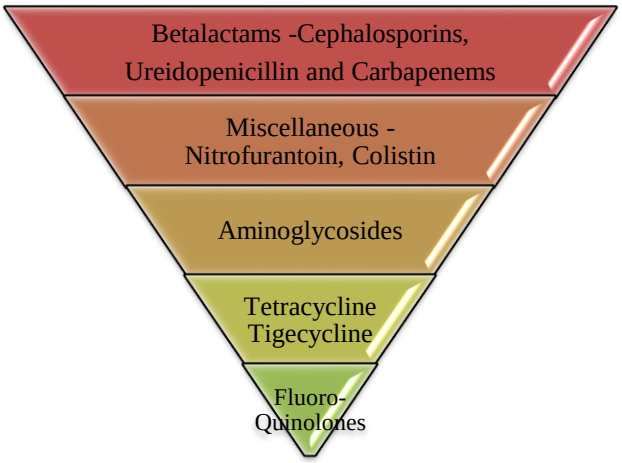
Miscellaneous antibacterials

Interestingly, 85% of the cultures showed sensitivity to nitrofurantoin. Also, colistin was another antibiotic found to have similar percentage of sensitivity comparable with tigecycline and cefaperazone sulbactam combination. Sensitivity to cotrimoxazole was 40% and that for vancomycin and linezolid, with 20% each (Figure 11).

The pattern of antibiotic sensitivity of the UTI causing organisms was analyzed. Comparing the various drug groups with differential sensitivity percentages, the highest *in-vitro* sensitivity was to the beta lactam group followed by nitrofuantoin and colistin of the

miscellaneous antibacterial group and the least sensitivity was demonstrated by the fluoroquinolones (Figure 12).





Discussion

Urinary tract infections (UTI) is the most common infection experienced by human after respiratory and gastrointestinal infections, accounting for 8.6 million visits (84% by women) in 2007 in the United States.[4,5] UTI is said to occur more commonly in females than in males; in males it is uncommon, but often associated with structural abnormality.[6] But in our sample, we found a greater percentage of males than females.

The highest co-morbidity noted in our study population was diabetes and hypertension. Diabetes plays a definitive role in increasing the occurrence of UTI because of the changes in immunological function and thereby increasing the susceptibility to UTI. Studies have demonstrated that the epidemiology of urinary tract infections (UTIs) among men and women with diabetes is similar to the epidemiology of those without diabetes with women having greater risk than men.[7] However, the bacteriology and antibiotic susceptibility patterns also do not, in general, differ from those without diabetes.[7,8] Our study results are well in line with these findings. In addition, a study has revealed that the virulence factors of *E. coli* affecting the diabetic individuals also remains the same as in the general population.[8]

Worldwide, the most frequent etiology is *Escherichia coli* and this is also reflected in our study results.[9] However the sensitivity and resistance patterns of *E. coli* strains causing UTI varies considerably between regions and countries.[10] In this study, we found that most *E. coli* isolated in culture were susceptible to cefaperazone-sulbactum, ertapenem, tigecycline, nitrofurantoin, colistin and amikacin. Among these nitrofurantoin and colistin are the drugs administered orally and the rest have to be administered by parenteral route. Interestingly, we found a decreasing sensitivity to fluoroquinolones which have been regarded as one of the first line drugs in UTI and therefore rampantly used which consecutively would have lead to a high degree of fluoroquinolone resistance in our population.

The study highlights the increasing trend of resistant Gram negative organism in culture necessitating the need for higher antibiotics. A study has highlighted that among tetracyclines, tigecycline has best in vitro susceptibility against ESBL producing Gram negative rods.[11] Likewise, a high cure rate, both clinical and microbiological has been recorded for ertapenem also, particularly for ESBL infections.[12] The indiscriminate use of such higher antibiotics like ertapenem and tigecycline will foster development of drug resistance, further complicating the management of urinary tract infection.

The vicious cycle wherein widespread use of newer antibiotics leading on to the organisms developing resistance to them and that in sequence, further necessitating the development of newer antibiotics ultimately results in difficult to treat infections. Such a situation can be overcome by implementing rational prescribing practices like the use of lower antibiotics with limited spectrum to which the organism is susceptible rather than resorting to prescribe higher antibiotics with a wide spectrum.[13]

This study has clearly delineated the emphatic role of nitrofurantoin in urinary tract infections. Our findings are in concordance with currently emerging trend in the use of nitrofurantoin as a key oral antibiotic option in the treatment of acute uncomplicated cystitis due to multi-drug resistant Gram negative bacilli causing UTI.[14] Studies also have acknowledged that the efficacy of nitrofurantoin for the empirical treatment is supportable from a public health perspective in an attempt to decrease uropathogen resistance.[15] Thus the current scenario based on drug sensitivity justifies the use of nitrofurantoin in the empiric management of UTI, not only due to the high susceptibility of *E. coli* to nitrofurantoin and the ease of oral administration but also owing to the fact that it lacks

cross-resistance with commonly prescribed antibiotics for UTI. However, nitrofurantoin is contraindicated in renal disease since most of it is really excreted.

Limitation

The limitation of the study is the small sample size. A larger study with a larger sample is needed to confirm the drug sensitivity pattern of UTI causing organisms in the study locale established through our study results.

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The study highlights that *E. coli* contributes to the majority of the urinary tract infections diagnosed in our tertiary care hospital. In addition, the current drug sensitivity pattern signifies the need for higher antibiotics and this mandate implementing strategies of rational prescribing to avert forging resistance to these agents also. Nitrofurantoin, with good activity against a high percentage of the cultured organisms causing UTI, rationalizes its use for empirical treatment of UTI.□

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Ethical approval: The study was approved by the Institutional Human Ethics Committee (Approval number: 16/158)

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1. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital, Aligarh, India. Ann Clinical Antimicrob, 2007 Mar 23;6:4.
2. Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxocin resistance in community and hospital acquired Escherichia coli urinary tract infections: a systematic review and meta analysis of observational studies. BMC Infec Dis, 2015 Nov 25;15(1):545.
3. Cullen IM, Manecksha RP, McCullagh E, Ahmad S, O'Kelly F, Flynn RJ, et al. The changing pattern of antimicrobial resistance within 42,033 Escherichia coli isolates from nosocomial, community and urology patient-specific urinary tract infections, Dublin, 1999-2009. BJU Int. 2012 Apr;109(8):1198-206.
4. Najar MS, Saldanha CL, Banday KA. Approach to urinary tract infections. Indian J Nephrol. 2009 Oct;19(4):129-39.
5. Hooton TM. Clinical practice. Uncomplicated urinary tract infection. N Engl J Med. 2012;366:1028-37.
6. Ferdosi-Sh E, Javanian M, Moradian KM. Resistance patterns of Escherichia coli causing urinary tract infection Caspian J Intern Med. 2015;6(3):148-51.
7. De Lastours V, Foxman B. Urinary tract infection in diabetes: epidemiologic considerations. Curr Infect Dis Rep. 2014 Jan;16(1):389.
8. Harwalkar A, Gupta S, Rao A, Srinivasa H. Prevalence of virulence factors and phylogenetic characterization of uropathogenic Escherichia coli causing urinary tract infection in patients with and without diabetes mellitus. Trans R Soc Trop Med Hyg. 2015 Dec;109(12):769-74.
9. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015 May;13(5):269-84.
10. Ferdosi-Shahandashti E, Javanian M, Moradian-Kouchaksaraei M, Yeganeh B, Bijani A, Motevaseli E, et al. Resistance patterns of Escherichia coli causing urinary tract infection. Caspian J Intern Med. 2015;6(3):148-51.
11. Gill MM, Usman J, Hassan A, Kaleem F, Shaheen N. In vitro susceptibility pattern of extended spectrum β-lactamase producing gram negative bacilli against tetracyclines. J Ayub Med Coll Abbottabad. 2015 Oct-Dec;27(4):788-90.
12. Veve MP, Wagner JL, Kenney RM, Grunwald JL, Davis SL. Comparison of fosfomycin to ertapenem for outpatient or step-down therapy of extended-spectrum β-lactamase urinary tract infections. Int J Antimicrob Agents. 2016 Jul;48(1):56-60.
13. Beveridge LA, Davey PG, Phillips G, McMurdo ME. Optimal management of urinary tract infections in older people. Clin Interv Aging. 2011;6:173-80.
14. Cunha BA, Cunha CB, Lam B, Giuga J, Chin J, Zafonte VF, Gerson S. Nitrofurantoin safety and effectiveness in treating acute uncomplicated cystitis (AUC) in hospitalized adults with renal insufficiency: antibiotic stewardship implications. Eur J Clin Microbiol Infect Dis. 2017 Feb 2.
15. Hooton TM. The current management strategies for community-acquired urinary tract infection. Infect Dis Clin North Am. 2003 Jun;17(2):303-32.

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Original Research Article

Effects of *Ferula asafoetida linn* in Mouse Models of Memory and Motor Activity

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Abstract

Background: Asafoetida is an herbal food supplement that has numerous peripheral pharmacological actions. Its central nervous system effects have not yet been completely elucidated in both animals and human participants. The study focuses on deciphering the effects of this Herbaceutical with that of Donepezil and vitamin C in mouse models of short term novelty object recognition memory and open field test.

Methods: The study was conducted in two stages - a pilot stage which assessed the most effective dose of asafoetida on cognition, followed by a post-pilot study phase wherein its effects at that dose were compared with those of Donepezil and vitamin C. Novelty Preference Test (NPT) on y-maze and Open field test on Open field box were used to evaluate the effects of the formulation on memory and motor activity in mice.

Results: A dose of 400mg/day of asafoetida orally was found to improve memory significantly in comparison to 200mg/day in mice. Asafoetida showed a greater potency in improving memory than Donepezil and vitamin C. After 11 days of daily treatment with Asafoetida commercial powder, more than fifty percent of the mice showed an increase in recognition index of 0.7-0.8 as compared to 0.55 at baseline.

Conclusions: Asafoetida powder has nootropic efficacy in mouse model - this has to be explored mechanistically and pharmaceutically to enable the psychopharmacological characterization of the active principles and its pharmacological effects in the central nervous system.

Keywords: Asafoetida, Donepezil, Elevated plus maze, Memory, Novelty object recognition memory, Open field test, Vitamin C, Y-Maze

Introduction

Asafoetida is a commercial form of dried latex extracted from the tap root of *Ferula asafoetida linn*. Plant, that is used in some countries as food supplement and flavouring agent. Although it has been widely used in Chinese and Indian medicine systems for thousands of years, its pharmacological effects have yet to be completely elucidated.

Different mechanism for augmentation of cognitive deficits exist in different pathophysiological states and disease- for example, Donepezil is a cholinesterase inhibitor which improves memory even in severe cognitive impairment seen in dementia by increasing acetylcholine levels in the cortical regions of the brain.[1] Other mechanisms have also been proposed, including antioxidant actions of vitamins and minerals on free radicals and glutamate excitotoxicity prevention.[2] We propose to evaluate for the first time, the mechanism of nootropic action of a commercial preparation of the oleoresin of Ferula spp., although few studies have addressed the research question in other ways. For example, studies on its amnesia reversal in galactose and sodium nitrate induced deficits in rats and assessment of its cognitive effects using passive avoidance tests have been published with positive evidence of its central

actions, although it has widespread effects in both preclinical and clinical models peripherally.[3,4]

The most commonly evaluated active principle of this herb is ferulic acid.[5] For example, it contains sesquiterpenes, coumarins and sulphur-rich components and phenolics including ferulic acids.[6-8]

The present study aims to evaluate the most efficacious dose of asafoetida's nootropic effect and then to compare its cognitive effects with two probe drugs acting by different mechanisms viz., vitamin C and donepezil. This has been accomplished using a two-phase study evaluation approach.

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Animals

Prior animal ethics committee approval was obtained from the Institutional Animal Ethics Committee (IAEC) for thirty Swiss albino mice. After procurement from breeder and quarantine, these thirty mice were housed in groups of six, after separating them into two divisions, the first randomly selected twelve mice for pilot stage and the remaining eighteen mice for the post pilot efficacy study. The first twelve mice were initially acclimatized in the laboratory and then randomly reallocated after one week into two cages for the pilot study execution.

Drugs

Commercially available asafoetida powder is obtained and dissolved in water and administered by oral gavage in mice daily till a specific criterion is met with. Donepezil tablets are ground and dissolved in water and administered by intraperitoneal syringes daily. Vitamin C is obtained as tablets and dissolved in aqueous solvent for oral gavage (pH = 7.0). The specific efficacy criterion is that treatment is continued in all groups till at least 50% of one either group exhibits a 50% improvement in baseline NOR index earlier.

Drug treatment

The study comprised of two phases - an initial pilot phase and a second efficacy phase. The objective of the pilot study phase was to ascertain the more effective of two doses of asafoetida for meeting a predefined criterion of cognitive improvement, along with the standard drug Donepezil. The objective of the next phase was to compare the cognitive improvement of asafoetida with two standard drugs acting by different mechanism, using surrogate behavioural markers.

The mice in the first cage were administered 200mg/kg/day of a commercial preparation of asafoetida powder in solution by oral gavage, whilst the other cage mice were given the same formulation at a dose of 400mg/kg/day. Each mouse was also co-administered Donepezil 2mg/kg/day intraperitoneal, till at least 50% of the rodents given either combination exhibited a 15-20% improvement in novelty recognition indices from baseline. At this cut-off, treatment was stopped in both groups and an interim analysis was performed to ascertain which of the two doses is more potent in improving novelty object recognition memory on a y-maze.

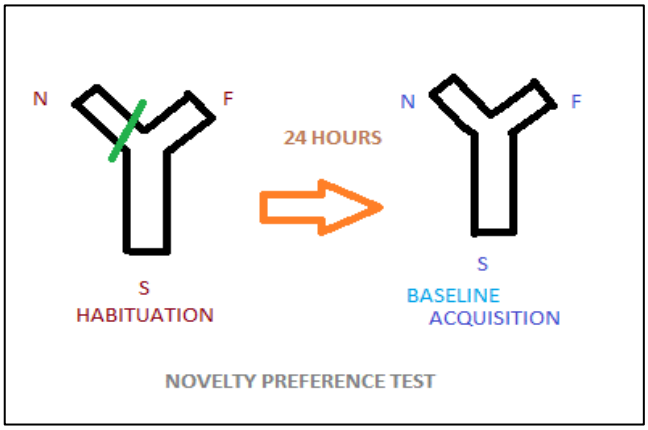
The efficacy study phase was executed using eighteen mice which, after acclimatization, were divided into three groups - first group received Asafoetida 400mg/kg/day, the second group mice were administered Donepezil 2 mg/kg/day intraperitoneal, and the six mice of the third group were given vitamin C 125mg/kg/day (pH = 7.0) orally.

The Y-Maze

The y-maze novelty preference test using the elevated y-maze for mouse evaluates short term spatial novelty-object recognition (NOR) memory. The elevated y-maze consists of three arms in a horizontal plane that is elevated from the floor by 50 centimetres. The three equally long arms are placed at 120° to each other, separated by a triangular central platform. Each arm measures about 100 centimetres. In length and is enclosed by walls whose height is about 50 centimetres. One of the arms has a provision for closure at the junction with the central platform, with a guillotine door - this is the Novel (N) arm. The other two arms which do not have such doors are the Start (S) and the Familiar (F) arms. The NOR index calculated from the number of arm entries of a single mouse (NAE) into each arm, with ¾ths of its body inside that arm, is as follows:

$$NOR\ INDEX\ (\%) = \frac{NAE\ (N)}{NAE\ (N) + NAE\ (F)} \times 100$$

$$Familiarization\ index = \frac{NAE\ (F)}{NAE\ (S)} \geq 1.0$$



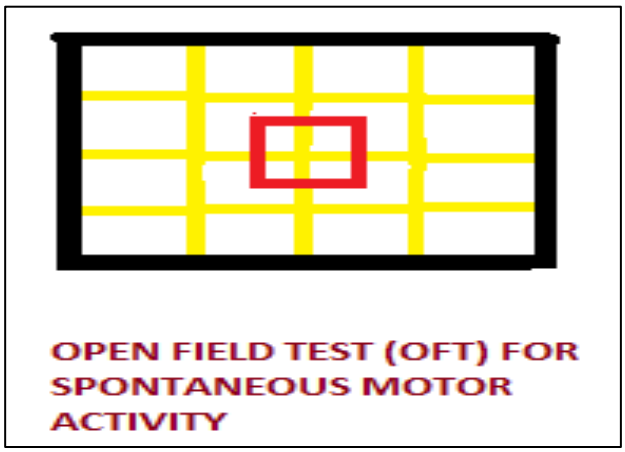
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The level of NOR memory acquisition and retention is directly proportional to the above index. The rodents are allowed to explore all three arms freely for 8 minutes in the y-maze followed by four successive habituation trials, in which the novel arm is closed. The habituation trials are meant for the rodent to familiarize with the familiar arm with novel arm closed. Thirty minutes after drug treatment, each animal can explore the novel arm relative to familiar arm, after placing it in the start arm for 5 minutes. NOR index is then measured for each animal (Figure 1).

The open field test

The open field box measures spontaneous motor activity (SMA) in rodents.[4] It is a cubical box measuring 90 cm^3 volume with an arena or floor, that is divided into 16 smaller yellow-bordered squares (Figure 2). The central portion of this arena is marked by a similar red-bordered square. The whole arena is divided into 4 white-bordered quadrants for purposes of software-acquisition of activity trials. The Open Field test measures SMA activity in 3-minute trials per mouse. The SMA index as a measure of motor activity is calculated as follows:

$$SMA\ INDEX = \frac{\sum Peripheral\ yellow\ square\ entries}{\sum Central\ red\ square\ entry}$$

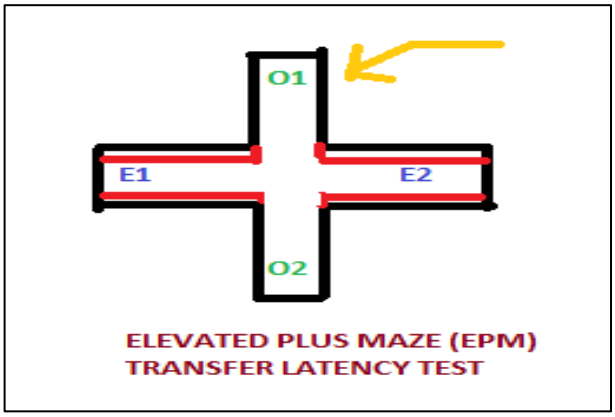


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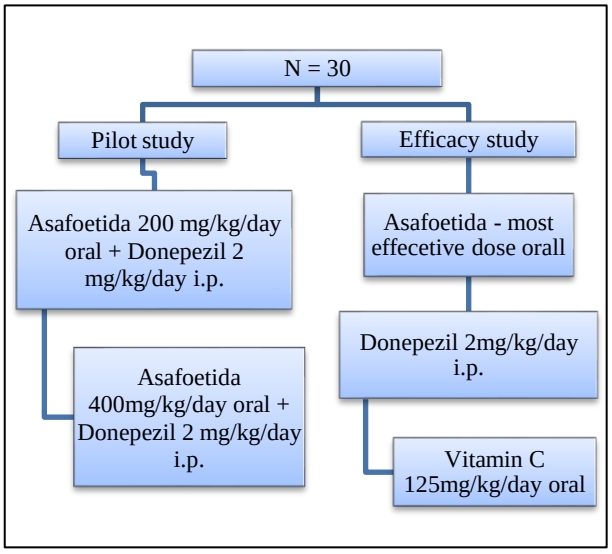
Elevated plus maze: The Elevated Plus Maze (EPM) is a plus like maze used to assess effects on interventions on anxiety like behaviour (Figure 3). It consists of four arms, two of which are enclosed on all three sides by walls. The other two arms which are on opposite end of the maze are not enclosed by walls. The whole maze is elevated on the ground at a height of 50cm. The transfer latency is the time the mouse takes to move from the open arm to the enclosed arm in a single daily ninety second trial. Additionally, the ratio of open to enclosed arm entries was also calculated as a measure of anxiolytic index.

The study design (Figure 4) is such that the more potent of two doses of asafoetida (with donepezil) is chosen based on greater / earlier nootropic activity followed by an efficacy phase, that aims to compare this dose of asafoetida with vitamin C and Donepezil-induced activities on y maze.

Statistical analysis: All statistical analysis was performed using Graphpad Prism v 4.0 and SPSS v 16.0. Analysis of variance (1 way and 2 way) with post hoc Bonferroni test and Pearson correlation along with Levenne test were performed for hypothesis testing.



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There was a statistically significant difference between the groups as determined by one-way ANOVA after 7 days ($F(3,21) = 3.581$, $p = 0.031$) and after 14 days of drug treatment ($F(3,21) = 7.996$, $p = 0.001$). A post hoc Bonferroni test with Y Maze Recognition index as the dependent variable across all groups of both phases taken together revealed a statistical significance between control

and high dose asafoetida group (p = 0.002). The improvement with high dose (400mg/kg/day) asafoetida was noted in 10 days of treatment even earlier compared to Donepezil in the efficacy study phase. After 11 days of daily treatment with Asafoetida commercial powder, more than fifty percent of the mice showed an increase in recognition index of 0.7-0.8 as compared to 0.55 at baseline. A two-way ANOVA for determination of factors affecting the repeated measures of Y maze NOR Index revealed statistical significant for the influence of both days of exposure (p <0.0001) and drug treatment (p = 0.007). Pairwise group comparison across all four treatment groups revealed significant interaction of drug treatment over days – for example Asafoetida high dose was more nootropic than Donepezil on days 10 and 11, and vitamin C on day 11, in the Bonferroni test (Figure 5).

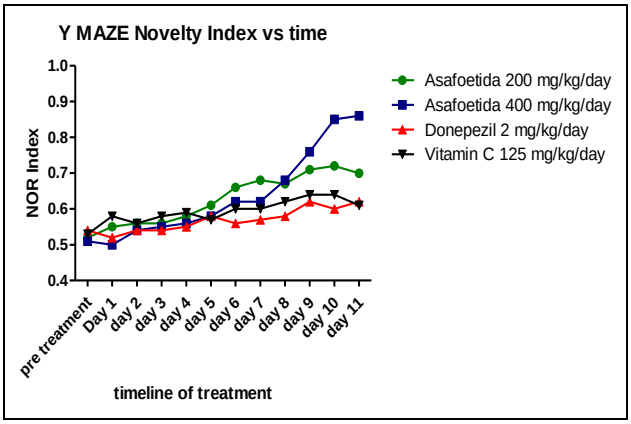


Figure 5: Y maze NOR index vs time in days for Asafoetida, Donepezil and Vitamin C treated groups

The one-way ANOVA of open to enclosed arm entries on the EPM revealed statistical significance between Donepezil and high dose Asafoetida group (p = 0.017, 95% CI: -137.17 to -7.16), with the mean ratios greater in the asafoetida 400 mg/kg/day group (p = 0.024), as revealed by a Bonferroni test. The percentage of open to enclosed arm entry ratios on days 7 and 14 across all the groups did not yield statistical significance using Levenne test (p>0.05).

Intergroup comparison of the ratio of peripheral to red square ratios on open field on days 7 and 14 across all the treated groups revealed no statistical significance using 1-way ANOVA (p>0.05). Also, the number of red and peripheral square entries on open field test on days 7 and 14 did not yield significant differences among all groups.

The Pearson correlation coefficient between y maze recognition index and the ratio of peripheral to central square entries on open field showed significant correlations on day 7 (R^2 = 0.8), but not on day 14, and both the correlations were statistically insignificant. A similar result was obtained for correlation between recognition index and transfer latency.

DISCUSSION

A novel effect of Asafoetida on spatial memory on y-maze which was earlier and greater than even Donepezil has been reported in this study. The mechanism of cognitive improvement seems to correlate more with cholinergic pathway improvement with Donepezil than with vitamin C effects. Also, this nootropic effect of Donepezil was higher at high dose with minimal motor and anxiogenic side effects as noted by results of open field and elevated plus maze tests.

Vitamin C exerts neuroprotective effects after its transport into neurons through sodium-dependent ascorbate transporters.[1] Donepezil is a cerebroselective acetyl-cholinesterase inhibitor that increases neurogenesis in the central nervous system, especially the hippocampus, and improves cognition.[2]

Although only a couple of studies exist to prove asafoetida nootropic effects,[3,4] our study reports that it affects spatial memory with minimal motor and anxiety adverse effects, which parallels more with Donepezil mechanism than antioxidant pathway, from the recognition indices parallelism.

Derivatives of sesquiterpenes are known to have chemo preventive effects in cancers and coumarins have anticoagulant activity. [5-7] Ferulic acid have also been used to improve haemostatics during silk weaving.[8,9] Phenolic acids which includes about half a dozen chemicals in Ferula spp. possess emotional improving and antidepressant activities. However, its effects in the central nervous system on higher cognitive properties have not been deciphered and evaluated completely.

Limitations

A small sample size and absence of biochemical confirmation; but it will serve as a pilot study for enabling further studies on this important nutraceutical.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

REFERENCES

1. Hansen SN, Tveden-Nyborg P, Lykkesfeldt J. Does Vitamin C Deficiency Affect Cognitive Development and Function? Nutrients. 2014;6(9):3818-46.
2. Jacobsen FM, Comas-Díaz L. Donepezil for psychotropic-induced memory loss. J Clin Psychiatry. 1999 Oct;60(10):698-704.
3. Bagheri SM, Dashti-R MH. Influence of asafoetida on prevention and treatment of memory impairment

induced by d-galactose and NaNO2 in mice. Am J Alzheimers Dis Other Demen. 2015 Sep;30(6):607-12.
4. Vijayalakshmi, Adiga S, Bhat P, Chaturvedi A, Bairy KL, Kamath S. Evaluation of the effect of Ferula asafoetida Linn. gum extract on learning and memory in Wistar rats, Ind J Pharmacol. 2012;44(1):82-7.
5. Augustine A, Sreeraj G. Biological activities and medicinal properties of Asafoetida: A review, Journal of Traditional and Complementary Medicine; 2016. ISSN 2225-4110. Available at: http://dx.doi.org/10.1016/j.jtcme.2016.11.004.
6. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin): A review. Journal of ethnopharmacology. 2011 Mar 8;134(1):1-0.
7. Kogure K, Yamauchi I, Tokumura A, Kondou K, Tanaka N, Takaishi Y, et al. Novel antioxidants isolated from plants of the genera Ferula, Inula, Prangos and Rheum collected in Uzbekistan. Phytomedicine. 2004 Nov 25;11(7):645-51.
8. Singh UP, Singh DP, Maurya S, Maheshwari R, Singh M, Dubey RS, et al. Investigation on the phenolics of some spices having pharmacotherapeutic properties. Journal of herbal pharmacotherapy. 2004 Jan 1;4(4):27-42.
9. Wang S, Gao Z, Chen X, Lian X, Zhu H, Zheng J, et al. The anticoagulant ability of ferulic acid and its applications for improving the blood compatibility of silk fibroin. Biomedical Materials. 2008 Nov 25;3(4):044106.

Ramanujam N, Bindu G, Santoshini R, Bhuvaneswari K. Psychopharmacological characterization of effects of *Ferula asafoetida linn*. formulation in mouse on a Y-maze, EPM, and open field apparatus. Int J Basic Clin Pharmacol 2017;6:2254-8.

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Hepato-Protective Effect of Folic Acid and Vitamin B12 in Comparison to N-acetylcysteine in Experimentally Induced Acetaminophen Toxicity in Rats

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ABSTRACT

To compare the effectiveness of vitamin B12 and folic acid in comparison to N-acetylcysteine (NAC) as hepato-protective agent against liver damage in experimentally induced acute acetaminophen toxicity in Sprague dawley rats. 18 male Sprague dawley rats were randomly divided into three groups of six each. Group A was administered NAC, group B - vitamin B12 and group C - folic acid intraperitoneally one hour after an acute over-dosage of acetaminophen 3mg/kg. After 48 hours, 1.5 ml of blood was withdrawn and the animals were sacrificed for histopathological examination of liver. The mean AST and ALT of vitamin B12 group did not differ statistically from that of the NAC group. The folic acid group had higher mean AST though not statistically significant whilst the mean ALT was significantly higher compared to NAC group. The reduction in the mean ALP and GGT with vitamin B12 showed statistically significant reduction whereas the folic acid group did not. Histopathology of liver revealed that the tissue architect was totally disturbed with portal tract appearing hemorrhagic and congested in addition to centrilobular necrosis in the folic acid group. In vitamin B12 group, the architecture of tissue was preserved with only mild inflammation. Vitamin B12, unlike folic acid has proved to be an efficacious hepato-protective agent in animal model of acute acetaminophen toxicity.

Keywords: Acetaminophen toxicity, Folic acid, Vitamin B12, Liver enzymes, Histopathology.

INTRODUCTION

Acetaminophen or paracetamol is one of the commonly used non-steroidal anti-inflammatory drugs. Though considered as safe analgesic-antipyretic, an overdose can lead to hepatotoxicity. Each year in the United States, approximately 6% of adults are prescribed acetaminophen doses of more than 4 g/day and 30,000 patients are hospitalized for acetaminophen toxicity.[1] Acetaminophen overdose is clinically significant as it is the most common cause of drug-induced liver failure.[2] Liver

injury occurs in 17% of adults with unintentional acetaminophen overdose.[1]Acute tubular necrosis is yet another manifestation of acute acetaminophen toxicity and it can occur either isolated or along with hepatotoxicity.[3]

N-acetylcysteine (NAC) is used as the treatment of choice for acetaminophen overdosage.[4, 5] Intravenous and oral formulations of NAC are available for the treatment of acetaminophen overdose. Though the current standard of care involves NAC, the use of this drug has many adverse effects like allergy, anaphylaxis, angioedema, bronchospasm.These reactions are dose-dependent and preceded by release of histamine. Hypotension can occur due to the vasodilatory effect and in severe cases, there can be consequent oliguria which may lead on to the issue of pre-renal pathology for an acute renal failure.[6]Animal studies and previous case reports strongly suggest that massive NAC overdose can cause cerebral edema and seizures.[7]

Though it has been well established that NAC can reduce mortality in acute overdose toxicity of acetaminophen, it is important to understand that the risk of adverse reactions to NAC do exists as is the risk of its overdosage.[6, 8] Noteworthy, the preparation of NAC for intravenous administration is quite complex.[6] Cases of iatrogenic NAC overdose have been reported in literature and it is important as this could even lead to death of the patient due to progressive hemolysis, thrombocytopenia and acute renal failure.[6, 7]

A high incidence of more than 60% patients treated with NAC has been reported to develop ADR.[8] Vitamin B12 and folic acid have been demonstrated to possess hepato-protective effect in other models of liver injury. Though one animal study indicating the effectiveness of folic acid in liver injury induced by acetaminophen has been reported in literature,[9] there are no studies evaluating the protective role of vitamin B12 in this animal model and in addition, this is the first study comparing the hepato-protective activity of vitamin B12 with folic acid in experimentally induced acute acetaminophen toxicity in rats in order to identify the potentially less toxic alternative to NAC by evaluating the effects of vitamin B12 and folic acid on liver enzymes and histopathology of liver tissue in comparison to that of NAC.

MATERIALS AND METHODS

The institutional animal ethics committee approved the protocol of the study (Approval number: 289/2015/IAEC). 18 Adult, male Sprague dawley rats weighing 200-300g were housed at constant ambient temperature in 12hr light, 12hr dark cycle. They were group housed as 3 animals per cage in polypropylene cages. Pellet diet and tap water were provided *ad libitum*. The animals were randomly allocated into the following three groups of 6 rats per group.

Group A: 6 rats in which acetaminophen at a dose of 3g/kg was given and NAC at a dose of 150mg/kg once after 1 hour of acetaminophen toxic dose.
Group B: 6 rats in which acetaminophen at a dose of 3g/kg was given and vitamin B12 at a dose 10mg/kg once after 1 hour of acetaminophen toxic dose.
Group C: 6 rats in which acetaminophen at a dose 3g/kg was given and folic acid 8mg/kg administered once after 1 hour of acetaminophen toxic dose.

The animals are weighed and the drugs were orally gavaged based on the body weight. 48 hours later, 1.5ml of blood was collected from the tail vein of the rats and liver function test (LFT) was performed. Quantitative analyses of liver enzymes namely, aspartate-aminotransferase (AST) or serum glutamic-oxaloacetic transaminase (SGOT), alanine-aminotransferase (ALT) or serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and ã-glutamyl transpeptidase (GGT) were performed. The values of these enzymes thus estimated were indicative of the severity of liver damage and were statistically analyzed.

All the rats were then sacrificed and liver specimens were taken, fixed in 10% formaldehyde and embedded in paraffin. Sections were cut from the tissue blocks, mounted on slides, and stained with Van Gieson's stain. They were examined under microscope for liver cell damage and disruption of normal architecture. [Figure1]

Statistical analysis: The data was entered in Excel and analyzed using SPSS software version

19. Comparison of the mean of liver enzymes was done using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test. The difference between the groups was considered statistically significant if $p<0.05$. Histopathological changes between groups were qualitatively analyzed.

RESULTS

The mean AST (260.2 ± 54.7) of vitamin B12 group was lower compared to that of the group which received NAC with mean AST (319.8 ± 57.6) and though the mean ALT for vitamin B12 group (114.3 ± 32.4) was higher than that of NAC

Table 1: Comparison of liver enzymes between groups using one way ANOVA

Parameter	Between groups comparison	Mean difference	p value
AST	Groups A and B	59.667	0.103
	Groups A and C	-30.167	0.393
ALT	Groups A and B	-27.167	0.348
	Groups A and C	-207.333*	0.000
ALP	Groups A and B	119.167*	0.001
	Groups A and C	-378.000*	0.000
GGT	Groups A and B	-0.400	0.372
	Groups A and C	-1.650*	0.002

*The mean difference is significant at the 0.05 level.

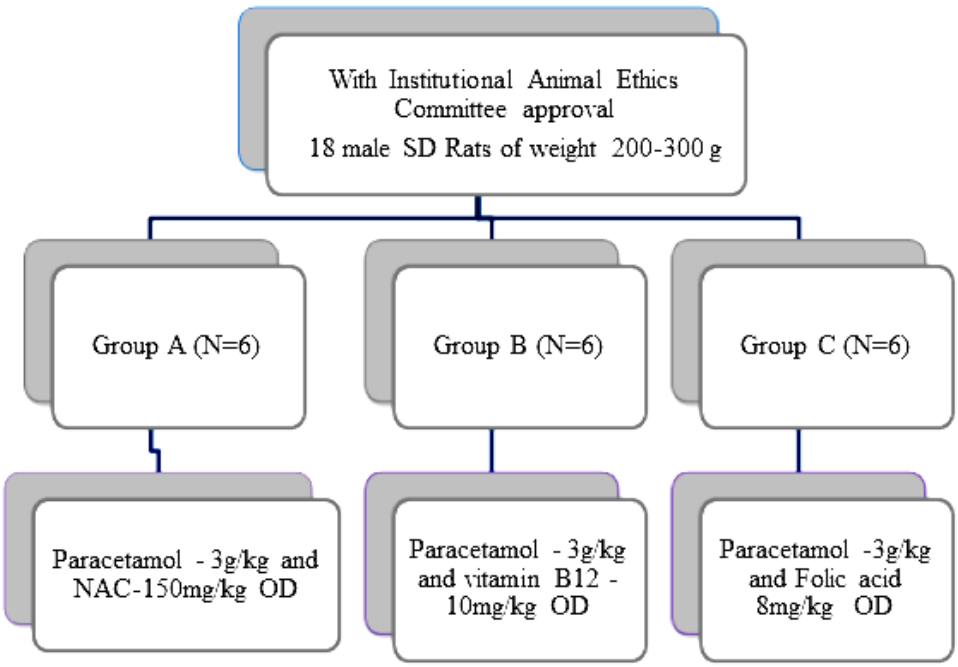


Fig. 1: Methodology

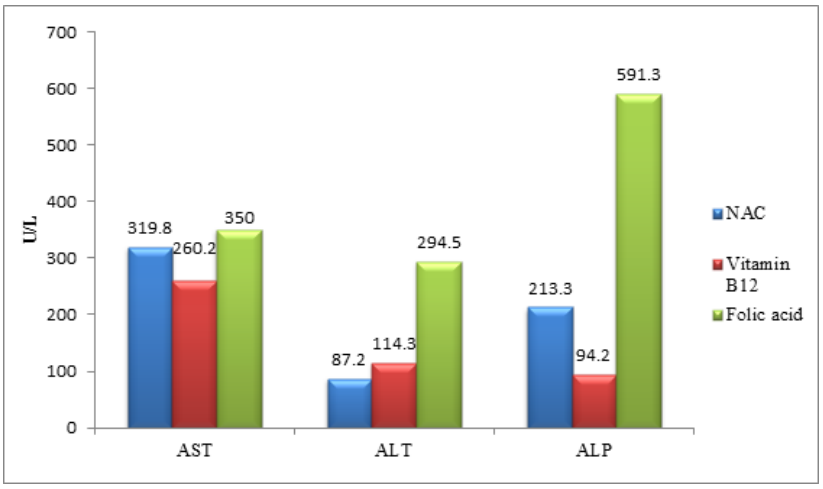


Fig. 2: Comparison of mean AST, ALT, ALP between groups

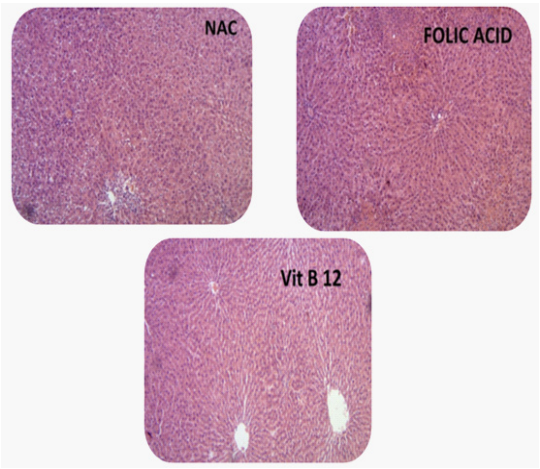


Fig. 3: Histopathology picture of the study groups

group (87.2 ± 41.5), [Figure2] the difference was not statistically significant by ANOVA with *post-hoc* LSD test either for AST (p=0.103) or ALT (p=0.348) between the two groups. [Table1]

On the contrary, folic acid group demonstrated a higher mean AST (350 ± 65.4) and mean ALT (294.5 ± 65.5) than NAC group. Comparison of the folic acid group with the group that received standard NAC treatment using ANOVA revealed that there was no statistical significance for the difference in the mean AST (p= 0.393) unlike that for mean ALT (p< 0.001). In addition, the vitamin B12 group had a significantly lower AST with mean difference of -89.8 ± 34.3 (p= 0.019) as well as for ALT with mean difference of -180.2 ± 28.0(p< 0.001) when compared to the group given folic acid. [Figure2 and table1]

Interestingly, there was a statistically greater reduction in mean ALP for vitamin B12 (94.2 ± 61.2) compared to NAC group (213.3 ± 48.2) whereas folic acid group (591.3 ± 44.8) had a statistically higher mean ALP (p< 0.001) indicating that folic acid did not demonstrate ALP reduction analogous to vitamin B12. [Table1]

Though mean GGT was slightly higher for the vitamin B12 group (1.18 ± 0.77) compared to the NAC group (0.78 ± 0.68), there was no statistical significance (p=0.372) for the difference in the mean values. However GGT for folic acid group (2.43 ± 0.79) mirrored the ALP wherein there was a statistically significant higher mean GGT (p=0.002) observed for folic acid group compared to the NAC group. [Table1]

Histopathological examination of the liver specimens revealed interface hepatitis, focal hepatic necrosis and thinning of sinusoidal plate in group A. In contrast, the group which received folic acid demonstrated tissue architect being totally disturbed with portal tract appearing hemorrhagic and congested in addition to centrilobular necrosis. Group B or vitamin B12 group had the architecture of tissue preserved with only mild inflammation. [Figure3]

DISCUSSION

This animal study has brought out the effective lowering of the liver enzymes by vitamin B12 in acute acetaminophen toxicity. The protective efficacy of vitamin B12 in reducing the liver damage after acetaminophen overdose is also evident from the histopathological changes observed in the study. It has been well established that acetaminophen induced liver damage causes centrilobular necrosis associated with a marked increase in AST, ALT activity.[10, 11, 12] Also, the increase in serum ALP activities is supported by the findings of earlier studies that, acetaminophen overdose is also accompanied by an increase in ALP activity due to hepatocellular injury. [10, 13] The reactive oxygen species-sensitive transcription factor namely nuclear factor-kappaB (NF-kappaB) is said to play a vital role in genesis of acetaminophen induced hepatotoxicity[12] and it has been reported that oxidative stress mediated by the metabolite N-acetyl-P-benzoquinone-imine (NAPQI) is considered to be the main cause of hepatic injury which involves depletion of cellular ATP, reduced Ca^{+2} ATPase activity with increased cytosolic Ca^{+2} levels, apoptosis and lipid peroxidation mediated by reactive oxygen species associated with a concomitant increase in AST, ALT, ALP.[14,15] It has also been hypothesized that reactive oxygen species induced lipid peroxidation generates peroxynitrite, a powerful oxidant and nitrating agent. Peroxynitrite can consequentially modify cellular macromolecules, aggravating mitochondrial dysfunction and ATP depletion, thus leading to cellular necrosis in hepatocytes and sinusoidal endothelial cells.[16]

The effectiveness of vitamin B12 parallels that of NAC since there is no significant difference in the mean AST, ALT and GGT values between the two groups [Table1]. In addition, the reduction in mean ALP was statistically greater for the vitamin B12 group than the NAC group. In comparison, folic acid did not demonstrate a similar effectiveness either in reduction of raised hepatic enzymes or reversal of histopathological changes induced by acute administration of high dose acetaminophen. [Figue3] This is in contrast to an earlier study which indicated lowering of liver enzymes and improvement in hepatocellular damage histopathologically following administration of folic acid in acetaminophen induced toxicity in rats. Folic acid, though has plausible hepato-protective effect attributed to its property of eliminating the changes that are related to the oxidative stress such as lipid peroxidation and oxidative DNA damage, [9, 17] our study did not replicate a similar efficacy and this can be ascribed to a lower dose of acetaminophen for induction of toxicity in the previous studies and disparity in the time duration of blood withdrawn for estimation of liver enzymes. Such hepato-protective property of folate supplementation regimens after induction of liver toxicity using either chromium in rabbits or folate deprivation with copper exposure in rats have been reported in literature.[18, 19] The contradiction in findings between different studies probably stems from the fact that there is some inherent variation in the pathways and diversity in the intensity of oxidative stress induced by different models.

On the other hand, our study has delineated a significant hepato-protective effect of vitamin B12 demonstrating its efficacy in acute acetaminophen toxicity rat model.[Table1 and Figure3] The potential mechanisms contributory to this beneficial effect is the ability of vitamin B12 to maintain the sulphydryl level under oxidative conditions despite lack of radical scavenging property.[20] Moreover, vitamin B12 has been shown to protect rat primary hepatocytes from hepatotoxin-induced cell death in *in-vitro* experiments.[20] Evidence suggests that the activity of glutathione reductase is significantly lower in B12 deficient liver[21] and that the interaction between vitamin B12 and glutathione could protect against disease related to vitamin B12 deficiency.[22] The presence of cobalt complex in vitamin B12 has been shown to effectively inhibit the hepatic inflammation and fibrosis.[20] Besides, protective effect of vitamin B12 in experimental liver injury by carbon tertrachloride[23, 24] has been well documented in literature wherein the beneficial effect has been primarily hypothesized as owing to the improvement in mitochondrial integrity and this could be another contributory factor in the hepato-protection in acetaminophen toxicity amelioration also.

CONCLUSION

This study highlights that vitamin B12 exhibits significant hepato-protective effect and hence can be considered as a potential therapeutic

agent, alternative to N-acetyl cysteine in acute acetaminophen over dosage after appropriate human trials.

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REFERENCES

1. Blieden M, Paramore LC, Shah D, Ben-Joseph R. A perspective on the epidemiology of acetaminophen exposure and toxicity in the United States. *Expert Rev Clin Pharmacol.* **7**(3):341-8 (2014).
2. Yaman H, Isbilir S, Cakir E, Uysal B. Current issues with paracetamol induced toxicity. *J Exp Integr Med.* **1**(3):165-6 (2011).
3. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. *J Med Toxicol*. **4**(1):2-6 (2008).
4. Prescott LF, Illingworth RN, Critchley JA, Stewart MJ, Adam RD, Proudfoot AT. Intravenous N-acetylcystine: the treatment of choice for paracetamol poisoning. *Br Med J.* **2**(6198): 1097-100 (1979).
5. Heard K, Green J. Acetylcysteine therapy for acetaminophen poisoning. *Curr Pharm Biotechnol,* **13**(10): 1917-23 (2012).
6. Mahmoudi GA, Astaraki P, Mohtashami AZ, Ahadi M. N-acetylcysteine overdose after acetaminophen poisoning. *Int Med Case Rep J.* **8**:65-9 (2015).
7. Heard K, Schaeffer TH. Massive acetylcysteine overdose associated with cerebral edema and seizures. *ClinToxicol* (Phila). **49**(5):423-5 (2011).
8. Zyoud SH, Awang R, Syed Sulaiman SA, Sweileh WM, Al-Jabi SW. Incidence of adverse drug reactions induced by N-acetylcysteine in patients with acetaminophen overdose. *Hum Exp Toxicol.*; **29**(3):153-60 (2010).
9. AL-Sowyan NS. Efficacy and safety of folic acid during toxic hepatitis induced by acute overdose of paracetamol. *International Journal of Pharmacology.* **5**: 208-214 (2009).
10. Abdel Salam OM, Baiuomy AR, El-Shenawy SM, Hassan NS. Effect of pentoxifylline on hepatic injury caused in the rat by the administration of carbon tetrachloride or acetaminophen. *Pharmacol Rep.* **57**(5):596-603 (2005).
11. Janbaz KH, Saeed SA,Gilani AH. Studies on the protective effects of caffeic acid and quercetin on chemical-induced hepatotoxicity in rodents. *Phytomedicine.* **11**(5):424-30 (2004).
12. Oz HS, McClain CJ, Nagasawa HT, Ray MB, de Villiers WJ, Chen TS. Diverse antioxidants protect against acetaminophen hepatotoxicity. *J Biochem Mol Toxicol.;* **18**(6):361-8 (2004).
13. Shivashangari KS, Ravikumar V, Devaki T. Evaluation of the protective efficacy of Asteracantha longifolia on acetaminophen-induced liver damage in rats. *J Med Food.* **7**(2):245-51 (2004).
14. Ferret PJ, Hammoud R, Tulliez M, Tran A, Trébéden H, Jaffray P, Malassagne B, Calmus Y, Weill B, Batteux F. Detoxification of reactive oxygen species by a nonpeptidyl mimic of superoxide dismutase cures acetaminophen-induced acute liver failure in the mouse. *Hepatology.* **33**(5):1173-80 (2001).
15. Sener G, Sehirli O, Cetinel S, Yegen BG, Gedik N, Ayanoglu-Dulger G. Protective effects of MESNA (2-mercaptoethane sulphonate) against acetaminophen-induced hepatorenal oxidative damage in mice. *J Appl Toxicol;* **25**(1):20-9 (2005).
16. Jaeschke H, Knight TR, Bajt ML. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. *Toxicol Lett.;* **144**(3):279-88 (2003).
17. Ghosh A, Sil PC. Anti-oxidative effect of a protein from Cajanus indicus L against acetaminophen-induced hepato-nephro toxicity. *J Biochem Mol Biol.;* **40**(6):1039-49 (2007).
18. El-Demerdash FM, Yousef MI, Elaswad FA. Biochemical study on the protective role of folic acid in rabbits treated with chromium (VI). . *J Environ Sci Health B.* **41**(5):731-46 (2006).

19. Lin HL, Yu CC, Chou YF, Huang RF. Folate deprivation and copper exposure potentiate reactive oxygen species generation and chromosomal DNA loss but not mitochondrial DNA deletions in rat hepatocytes. *Int J Vitam Nutr Res.;* **76**(5):332-40 (2006).
20. Isoda K, Kagaya N, Akamatsu S, Hayashi S, Tamesada M, Watanabe A, Kobayashi M, Tagawa Y, Kondoh M, Kawase M, Yagi K. Hepatoprotective effect of vitamin B12 on dimethylnitrosamine-induced liver injury. *Biol Pharm Bull.* **31**(2):309-11 (2008).
21. Biswas DK, Johnson BC. Glutathione reductase and dehydrogenase activity in vitamin b12 deficiency. *Arch Biochem Biophys.* **104**: 375-80 (1964).
22. Watson WP, Munter T, Golding BT. A new role for glutathione: protection of vitamin B12 from depletion by xenobiotics. *Chem Res Toxicol;* **17**(12):1562-7 (2004).
23. Kasbekar DK, Lavate WV, Rege DV. Sreenivasan A. A study of vitamin B12 protection in experimental liver injury to the rat by carbon tetrachloride. *Biochem J.* **72**:384-9 (1959).
24. Antelava NA, Gogoluari MI, Gogoluari LI, Pirtskhalaishvili NN, Okudzava MV. Efficacy and safety of heptral, vitamin B6 and folic acid during toxic hepatitis induced by CCL4. *Georgian Med News.* **150**:53-6 (2007).

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Pre-convulsive dyspnea in Guinea pig model induced by *Momordica Charantia* and its role in asthma

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Abstract

Background: Previous studies on *Momordica charantia* (MC) (bitter gourd) have shown the inhibition of NF-κβ and Leukotrienes expression in many inflammatory pathological conditions. Based on ist anti-inflammatory action this study aimed to Identify MC fruit dry powder (MCp) and MC fresh juice (MCj) action on airway inflammation in Guinea Pig model and also to find out the presence of alkaloids and flavonoides.

Methods: 18 adult Guinea pigs of both sexes (excluding mating animals) were randomly divided into three groups with six animals in each namely Montelukast, MCj and MCp groups respectively. They were given with OD oral administration of concerned drugs for 1 -7 days. 6 control animals were exposed to 2% histamine aerosol for 120 seconds using histamine chamber prior to treatment with Montelukast, MCj and MCp to observe the normal Pre-Convulsive Dyspnea (PCD) in seconds. After six hours of daily OD oral dose of test drugs administration, all the groups' were exposed to 2% Histamine aerosol one by one for 120 sec on day1 & 7 to observe PCD. The MCj and MCp were also tested with chemical assay, TLC to confirm the presence of alkaloids, flavonoides.

Results: ON DAY 1 and 7: MCj and MCp showed significant decrease in PCD occurrence and it is statistically significant compared to the control. PCD blocking action of MCj group was effective in DAY 1. TLC and Chemical Assay were not supportive for bronchodilator action.

Conclusions: MCj and MCp have promising preventive role in asthma. This study had expressed the positive protective role of *Momordica charantia* in asthmatic condition based on its known anti-inflammatory action.

Keywords: Guinea pig, *Momordica charantia*, Pre-convulsive dyspnea

Introduction

Asthma is a controllable chronic inflammatory disorder of the airways with high difficulty to cure completely.[1] Long term use of anti-inflammatory drugs is the treatment of chronic asthma. Most potent and consistent anti-inflammatory agent available is Corticosteroids and it's not free of adverse effects even in the inhaled form.

As a result, there is continuous search for an alternative especially from natural sources. *Momordica Charantia* (bitter gourd), a common edible vegetable, is known traditionally to possess medicinal values.[2,3] Many studies had confirmed the anti-inflammatory property of *Momordica Charantia* with cytokine inhibiting activity.[4,5] It inhibited the expression of inflammatory genes like IL-1,IL-2,IL-6,IL-8,TNF-α and Matrix Metallo Proteinases (MMPs) in animal studies.[6] Like Montelukast a Leukotrienes receptor antagonist used in asthma, *Momordica Charantia* fruit also inhibits NF-Kβ expression.[7]

Very few animal studies were done on its anti-inflammatory property utilizing the prostaglandins and inflammatory cytokine inhibition by *Momordica Charantia.* So far, no study has been done to prove its anti-inflammatory effect in asthma. Hence this study has

aimed to understand the bronchodilator property of *Momordica Charantia* as an agent to reduce airway inflammation in a whole animal model using Montelukast as a comparative standard.

M□□□□□□□

Method I: Animal study-histamine induced bronchospasm in guinea pig model

Animal which used for the study were adult guinea pigs with weight 400-700gram of both sex excluding animals used for breeding.

Period of the study was 15 days to 2 months including animal selection.

Total number of animals were 18 as 3 groups with 6 animals in each.

Study parameters were PCD (Pre-convulsive Dyspnea) per 120 seconds.

Histamine chamber with nebulizer instrument was used with standard technique to apply.

Momordica fruit dry powder and fresh juice

Momordica Charantia was bought from local farm after getting botanical certification. Then it was used to prepare fresh juice and dry powder after removing seeds.

Fresh juice

Fresh juice was prepared by using fresh 500gram of *M. Charantia* unripe fruits. Then it was cut into small pieces after removal of the seeds. These fruits were crushed with a commercial blender and juice was obtained after filtration through soft muslin cloth.

Dry powder

500gram of *M. Charantia* unripe fruits were cut into small pieces after removal of the seeds. These pieces were allowed to dry in hot air oven at 60 degrees for 24 hrs.[8] Once the fruit pieces were dried enough, they were made into fine powder using blender. Powder thus prepared was passed through a sieve and fine particles was obtained and stored in air tight containers.

IAEC

The animals were maintained at ambient temperature 25±2^{0}C and 55% relative humidity. The Institutional Animal Ethics Committee of PSG and IMSR approved the study and the study was conducted strictly with guidelines of ICMR and CPCSEA. 6 animals out of 18, randomly selected to observe the control values for PCD per 120 seconds. Control was exposed to 2% Histamine aerosol for 120 seconds.[7] These values were compared with Montelukast, MCp (*Momordica Charantia* dry powder) and MCj (*Momordica Charantia* fresh juice) exposure to understand the protective effect on histamine induced Bronchospasm.

If any animals showed severe bronchospasm in any of these groups were immediately exposed to normal air and Inhalational corticosteroid + salbutamol aerosol using nebulizer to revert the histamine induced bronchospasm. After completion of the study, all the animals were observed for a period of 1 week in their own environment with the normal water, food for any reactions.□

Method II

A. Chemical analysis for alkaloid material[9]

Detection of alkaloids

Dry powder and Fresh Juice were dissolved individually in dilute Hydrochloric acid was heated for 30 min, allowed to cool and filtered.

- Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).
- Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).
- Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide).
- Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution).
- Marquis test was performed with both dry powder and fresh juice.[10]

1 drop reagent made up of 8-10 drops (approx. 0.25ml) of 37% formaldehyde solution to10 ml of glacial acetic acid. Then added 3 drops of concentrated sulfuric acid

Results are present in Table 4.

- Chen-Kao Test: Test for ephedrine and pseudoephedrine.[10]
  - Reagent 1: Added 1 ml of glacial acetic acid to 100 ml of water (=1% (v/v) aqueous acetic acid solution).
  - Reagent 2: Dissolved 1 g of copper (II) sulphate in 100 ml of water (=1% (w/v) aqueous CuSO4 solution).
  - Reagent 3: Dissolved 8 g of sodium hydroxide in 100 ml of water (=2N aqueous sodium hydroxide solution).

Procedure

Placed a small amount (1-2 mg of powder, or 1-2 drops of a liquid) of the suspected material in a depression on a spot plate.

- Added 2 drops of Reagent 1.
- Added 2 drops of Reagent 2 and then added 2 drops of Reagent 3 and stirred. Results were shown in Table 5.

B. Thin layer chromatography[11]

MC Dry powder and fresh juice were subjected to thin layer chromatography (TLC) with silica gel F254 plates using Chloroform-Methanol solvent was used and the extract showed spots on Merck silica gel (MSG) pre-coated TLC plate of thickness of about 0.2mm.

The plates were saturated in chromatography tank and spots were detected following drying of the solvent. Band of separated fractions was detected under UV light. The retention fraction (Rf) of each fraction or band was calculated and recorded.

Statistical analysis

Analysis was done by comparing the before and after values, and also between groups student t- test was done for comparing before and after values, whereas ANOVA was used in comparing between all three groups. Analysis was done using SPSS version 19.

On day 1

MCj and MCp were showed significant decrease in PCD occurrence and it was statistically significant compared to the control. MCj group PCD blocking action was significant, when all the three groups were compared (Table 1, 2 and 3).

Table 1: Mean PCD inhibition in control and after 120 minutes in MC powder and juice treated groups

Mean PCD inhibition	120 minutes
Control	61.10±9.74
Day 1	79.16±16.21
Day 7	87.35±21.16
Momordica dry powder (MCp)	
Control	61.10±9.74
Day 1	93.47±12.36
Day 7	93.47±10.52
Momordica fresh juice (MCj)	
Control	61.10±9.74
Day 1	73.46±16.48
Day 7	100.00±0.00

On day 7

MCj and MCp showed significant decrease in PCD occurrence statistically, compared to the control. TLC and chemical assay were not supportive for bronchodilator action (Table 1, 2, 3).

Table 2: Comparison between control and day 1 and day 7 in MC powder and juice treated groups

		't'	
PCD (Pre convulsive dyspnoea)			
Montelukast	Control and day 1	2.225	0.077
	Control and day 7	2.521	0.053
	Day 1 and day 7	0.955	0.383
MC powder	Control and day 1	4.187	0.009
	Control and day 7	5.194	0.003
	Day 1 and day 7	0.000	1.000
MC juice	Control and day 1	2.733	0.041
	Control and day 7	7.950	0.001
	Day 1 and day 7	3.176	0.034

Table 3: Comparison between MCj and MCp in day 1 and day 7

Day 1	MCj is significant over MCp	P =0.037
Day 7	No Significance between all 3 groups	P >0.05

Table 4: Chemical tests for alkaloid and ephedrine

1. Mayer's test	Positive	Positive
2. Wagner's test	Positive	Positive
3. Dragendroff's test	Negative	Negative
4. Hager's test	Positive	Positive

Table 5: Chemical tests for ephedrine and pseudoephedrine by *Marquis* reagent

Ephedrine	Pale yellow	-	-
Pseudoephedrine	Pale yellow (greenish	-	-
Norephedrine	Pale yellow (brownish)	-	-
Norpseudo-ephedrine	Yellow (brownish)	-	-
Norpseudo-ephedrine	Yellow (brownish)	-	-
N-methylephedrine	Brownish red		
Chloropseudo-ephedrine	no change	-	-

- Chemical tests: Test for alkaloid and ephedrine were revealed in tabulation (Table 4, 5).
- Chen-Kao test: Test for ephedrine and pseudoephedrine11 - Both dry powder and fresh juice didn't show violet color positive reaction.

Thin layer chromatography[11]

- Solvent System: Chloroform-Methanol (9:1) Detection at UV: 366nm.
- Color of bands: Reddish spots

Results were shown in the Table 6 and Table 7.

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Dry powder (MCp)	1	0.84
Fresh juice (MCj)	2	0.51
		0.64

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which proved that the dry powder of *Momordica Charantia* was effective in delaying PCD in Histamine exposed guinea pigs significantly.

MCp group's were compared for the difference between PCD on Day 1 and Day 7. The mean PCD was found to be 93.47±12.36 on Day 1 against 93.47±10.52 on day 7. This was not statistically significant with P value of 1.000 as there was not much difference in the average value of PCD.

MCj group were compared the baseline Day 1 control value the mean was found to be 73.46±16.42 which was more than the control mean of 61.1%. Also this difference in PCD was statistically significant with P value of 0.041 which shows Fresh juice was effective in delaying PCD. MCj group compared for the Day 7 values, the mean was found to be 100.00±0.00. This too was statistically significant with P value of 0.001. This proved that the fresh juice of *Momordica Charantia* was significantly effective in delaying PCD in Histamine exposed guinea pigs.

MCj group compared for the difference between PCD on Day 1 and Day 7, the mean PCD was found to be 73.46±16.42 on Day 1 against 100.00±0.00 on day 7. This was statistically significant with P value of 0.034 which justifies Momordica was effective in both short term and long-term management.

Further analysis of both study groups of Momordica dry powder and fresh juice with student t-test. On day 1 in dry powder group the mean was found to be 93.47±12.36 and in fresh juice group it was found to be 73.46±16.42. This was statistically significant with P value of 0.038; which showed the dry powder was better on Day 1 of treatment with quick response. On day 7 in dry powder group the mean was found to be 93.47±10.52 whereas in fresh juice group it was 100.00±0.00. This was not statistically significant as both groups had similar protective effect on day 7. The average was more in fresh juice group compared to the dry powder group on day 7.

When ANOVA was used to analyze the difference between the groups, (Table 2) on Day 1, MCp was significantly better than MCj with P value of 0.037 whereas it was not statistically better than Montelukast. On day 7, there was no statistical significance between all 3 groups since all were effective in abolishing PCD occurrence.

These results showed that, there was a significant improvement in both groups of *Momordica* after starting treatment, whereas there was no much difference between groups. It's a pilot study and each group strength was six may be the limitation of this study.

Momordica charantia had been extensively studied for various metabolic, antihypertensive and its role in Diabetes with its anti-inflammatory background and not much significant reference for the direct effect of MC on smooth muscle activity.[12-15]

[illegible]

This study had expressed the positive protective role of *Momordica charantia* in asthmatic condition based on its known anti-inflammatory action. Further, MC both dry powder and fresh juice protecting role on histamine aerosol induced bronchospasm as an anti asthmatic action may be explored and proved in clinical situation using suitable models to ensure the usefulness and effectiveness in chronic asthma treatment compared to standard care of management in future.

[illegible]

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[illegible]

1. Global Initiative for Asthma. Global stargery for asthma management and prevention, 2017. Available from: www.ginasthma.org [Accessed 1st May 2017]
2. Pharmacological actions and potential uses of Momordica charantia: a review 9 jEthnopharmacol. 2004;93(1):123-32.
3. Kumar SKP, Bhowmik D. Traditional Medicinal Uses and Therapeutic Benefits of Momordica Charantia Linn. International Journal of Pharmaceutical Sciences Review and Research. 2010;4(3):23-8.
4. Mariko M, Ryo T, Teruko N. Induction of Anti-inflammatory Responses by Dietary Momordica charantia L. (Bitter Gourd) Biosci. Biotechnol. Biochem. 2003;67(12):2512-7.
5. Nguyen, Pham, Nguyen, Tran, Phan, Chau. Inhibition of Nuclear Transcription Factor-jBand Activation of Peroxisome Proliferator-Activated Receptors in HepG2 Cells by Cucurbitane-Type Triterpene Glycosides from Momordica Charantia. J Med Food. 2012;15(4):369-77.
6. Chao C, Sung P, Wang W, Kuo Y. Anti-Inflammatory Effect of Momordica Charantia In Sepsis Mice. Molecules. 2014;19:12777-88.
7. Sridevi G, Gopkumar P, Ashok S, Shastry C. Pharmacological Basis For Antianaphylactic,

Antihistaminic and Mast Cell Stabilization Activity of Ocimum Sanctum. The Internet J Pharmacol. 2009;7(1).
8. Singh U, Sagar VR. Effect of drying methods on nutritional composition of dehydrated bitter gourd (*Momordica Charantia*.). Indian Agricultural Research Institute, New Delhi-12. Agriculture for Sustainable Development. 2013;1(1):83-6.
9. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia. 2011 Mar;1(1):98-106.
10. Nagy G, Szöllősi I, Szendrei K. Colour Tests for Precursor Chemicals of Amphetamine-Type substancesthe Use of Colour Tests for Distinguishing between Ephedrine-Derivatives un scientific and technical notes. Scitec; 2005:16-17.
11. Abalaka ME, Inabo HI, Onaolapo JA, Olonitola OS. Chromatographic Analysis and Antibacterial Effects of the Ethanolic Extracts of *Momordica Charantia*. Afr J Med Phy, Biomed Eng and Sc. 2010;2:14-20.
12. Tsai CH, Chen EC, Tsay HS, Huang CJ. Wild bitter gourdimproves metabolic syndrome: a preliminary dietary supplementationtrial. Nutr J. 2012;11:4.
13. Klomann SD, Mueller AS, Pallauf J, Krawinkel MB. Antidiabeticeffects of bitter gourd extracts in insulin-resistant db/db mice. BrJ Nutr. 2010;104:1613-20.
14. Virdi J, Sivakami S, Shahani S, Suthar AC, Banavalikar MM, Biyani MK. Antihyperglycemic effects of three extracts from *Momordica Charantia*. J Ethnopharmacol. 2003;88:107-11.
15. Fuangchan A, Seubnukarn T, Jungpattanawadee D, Sonthisombat P, Ingkaninan K, Plianbangchang P, et al. Retrospective study on the use of bitter melon for type 2 diabetes at Dansai Crown Prince Hospital, Thailand. Srinagarind Med J. 2009;24:332-8.

r Bhuvaneswari K, Swarna RMPL, Amudhan A. Anti asthmatic effect of *Momordica Charantia* and its comparison with montelukast an in vitro and in vivo model. Int J Basic Clin Pharmacol 2017;6:1810-5.

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Original article

Audit to evaluate the completeness of adverse drug reporting in an ADR monitoring centre in Tamilnadu

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ABSTRACT

Objectives: The study was aimed to evaluate the completeness of suspected adverse drug reaction (ADR) forms in order to identify the areas lacking complete information, to assess the proportion of the ADR forms received with incomplete data and to evaluate the completeness score of the individual case safety reports.

Methods: This cross sectional study was done as an audit using data in the ADR forms collected and Individual Case Safety Reports (ICSRs) submitted in VigiFlow in the previous two years from January 2015 to December 2016 at our ADR Monitoring centre (AMC).

Results: The total number of ADR forms audited was 1650. Seriousness, action taken and outcomes and concomitant drugs were three areas identified to be lacking complete information. The mean completeness score was 0.95 for 2015 and 0.97 for 2016, indicative of a high completeness score of the ICSRs uploaded in Vigiflow maintained by the AMC.

Conclusion: The audit has analyzed various parameters of ADRs reported for completeness and has demonstrated that, though some improvement in completeness of reporting is evident in the year 2016 compared to 2015, there is an obvious need for increasing the awareness on reporting of ADRs with complete information amongst reporters.

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1. Introduction

Adverse drug reactions (ADRs) are inevitable consequences of pharmacotherapy. It is well known that all drugs carry the potential to produce both desirable and undesirable effects. No drug is absolutely safe under all circumstances and ADRs can occur even if a drug is correctly selected and dosed [1]. It has been reported that the number of patients dying because of adverse effects of drugs per year has increased up to 2.6-fold from the past [2]. Moreover, rates of hospitalization of patients and polypharmacy have increased the risk of ADRs. Thus, it becomes challenging for the physician, health care providers, World Health Organization (WHO) and pharmaceutical industries to address the problem of ADRs. Documented details are required to identify the ADRs in patients.

Pharmacovigilance plays a very important role in the surveillance of adverse drug reactions. Adverse drug reactions are documented in suspected ADR reporting forms [issued in India by Indian Pharmacopeia Commission (IPC) at National Coordination Centre (NCC), Ghaziabad] under the Pharmacovigilance program of India (PvPI) which strives towards ensuring drug safety in patient care [3,4]. ADR monitoring centres (AMC) play a vital role in the collection and follow up of ADR reports. Currently there are 197 AMCs functioning across India [5]. They are responsible for collecting the adverse reaction information and reporting it to the IPC, functioning under the Ministry of Health, Government of India. The ADR information is in turn forwarded to the WHO and the online database, VigiFlow provides the platform for reporting the ADR data [6]. Hence reported information in the ADR forms plays a vital role in the interpretation of drug reactions and the ensuing drug safety measures implemented.

Incomplete reporting of essential data is a very important factor that hinders effective interpretation of the ADR forms. Hence ensuring completeness of the ADR forms plays an indispensible role in success of Pharmacovigilance program. The recommendation by PvPI is that the reporter has to ascertain entering the information on patient's initials, age, gender and weight, date on which the suspected reaction appeared, description of event, name, dose, dosing frequency, the route of administration of the suspected medicine and concomitant drugs including the details

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on batch number, expiry date and the indication for the use of the medication. In addition similar data on vaccines and biological products, de-challenge and re-challenge results, causality assessment, relevant laboratory investigations, relevant past medical history and action taken for the ADR are required to be filled in while reporting [5]. Missing information or incomplete data is a deterrent not only in analyzing the ADR reports and assigning causality but also affects the quality of information that serves as the source of input for the international database [7,8].

The vigiGrade completeness score is a numerical score assigned to each individual case safety report (ICSR) entered in VigiFlow based on the completeness of information provided. The maximum score is 1 for each complete report calculated from the information provided according to a structured algorithm. Based on the algorithm, a field score is assigned to specific data fields, such as patient gender, indicating whether the field has been filled or not. In addition to the field scores, scores for pieces of information derived from multiple fields is generated. The completeness score is calculated for each drug- ADR combination separately and the mean of these scores is the Report Completeness Score. Reports with overall score >0.8 out of 1 are considered well documented [9]. Overall, only one report in eight provides the desired level of information, but much higher proportions are observed for individual countries [7,8].

Hence this study was done to evaluate the completeness in suspected ADR reporting forms to identify the areas lacking complete information, to assess the proportion of the ADR forms received with incomplete data and to evaluate the completeness score in the ADR forms reported over the past two calendar years 2015 and 2016 at our AMC.

2. Materials and methods

After the approval of the institutional human ethics committee (Approval number:17/010, dated 13th January 2017), the study was done as a cross sectional one using data in the ADR forms collected over the previous two years (January 2015 to December 2016) at the ADR monitoring centre in our tertiary care teaching hospital in Coimbatore. The audit was done as a retrospective record based study during January to March 2017. All the ADR forms from January 2015 till December 2016 and likewise all ICSRs submitted in VigiFlow and stored as soft copy during the same period were analyzed for the completeness score in accordance to the guideline provided by vigiGrade.

The areas lacking complete information were analyzed under ten categories namely report title, source (name, qualification), patient information (patient age, sex, initials), seriousness, free text (laboratory investigation & past medical history), outcome, drug details, action taken, indication and concomitant drugs.

Percentage of forms with incomplete information in each of the parameters analyzed from ADR reporting forms were recorded. The parameters showing a considerable difference in the percentages observed in the year 2016 compared to that in 2015 was analyzed for statistical significance using the Z test- social science statistics software and p value <0.05 was considered significant.

The completeness score of each ICSR reported in both years were calculated using the formula $C = \pi^{n}_{i=1}((1\text{-}w_i) + (w_i{*}f_i))$ where, C is the Completeness score, i is the Field included in the score, w_i is the Field weight and f_i is the Field score, n is total number of Drug-ADR combination [8,9]. The mean completeness score for the two years were estimated.

3. Results

All Suspected ADR forms submitted to the ADR monitoring centre from January 2015 to December 2016 were audited. The total number of audited forms were 797 from the year 2015 and 853 from the year 2016 accounting for a total of 1650 ADR forms. The report title was complete in all the ADR forms in both years and hence 100% complete. The source of the report was analyzed for two sub-parameters which included the name of the reporter and secondly, the qualification of the reporter. Name of reporter was complete in all the forms and thus 100% complete. On the other hand, qualification of reporter was incomplete in 274 (34%) forms out of 797 in the year 2015 and 226 forms out of 853 in the year 2016 accounting for 26% incompleteness. (Table 1)

Patient information which encompassed the details on patient's age, sex and initials was complete in 100% forms in both years. Seriousness was not filled in a considerable proportion of the ADR forms. 296 out of 797 forms were incomplete contributing to 37% incomplete seriousness data in 2015 and 252 out of 853 forms accounted for 29% of incompletion in 2016. It was found that a total of 92 serious ADRs in 2015 and 135 in 2016 were reported. Free text data analyzed which included the relevant laboratory investigation results and past medical history was found to be 100% complete in both the years. (Table 1)

The outcomes column was incomplete in 56 out of 797 forms in 2015 and 40 out of 853 in 2016 amounting to a fraction of 7% and 4% in 2015 and 2016 respectively. The other parameters audited were drug details such as name of brand, dose, route and frequency of administration. 45 out of 797 forms were incomplete in 2015 attributing 5% being incomplete as against 2% (18 out of 853 forms) in 2016. (Table 1) The area most frequently incomplete was frequency and route of administration.

Action taken column was incomplete in majority of the forms; in 2015, 581 out of 797 forms accounting for 72% and comparatively, a lower value of 42% (364 out of 853 forms) were revealed to be incomplete in 2016. In addition, 6% (52 out of 797

Table 1
Percentage of incomplete forms in the years 2015 and 2016.

Year	2015 n = 797			2016 n = 853		
Parameter	Complete	Incomplete	Percentage of Incomplete forms	Complete	Incomplete	Percentage of Incomplete forms
Report title	797	0	0%	853	0	0%
Source	523	274	34%	627	226	26%
Seriousness	296	501	37%	601	252	29%
Patient information	797	0	0%	853	0	0%
Free text	797	0	0%	853	0	0%
Outcome	741	56	7%	813	40	4%
Drug details	752	45	5%	835	18	2%
Action taken	216	581	72%	489	364	42%
Indication	745	52	6%	850	3	0.30%
Concomitant drug	783	14	1%	841	12	1%

forms) in 2015 and a meager 0.3% (3 out of 853 forms) in 2016 were found to lack the indication. (Table 1) In the concomitant drugs information, only 14 out of 797 forms were incomplete amounting to 1% in 2015 and an exactly equal one percentage (12 out of 853) were identified to be incomplete in the reported forms in 2016. (Table 1)

A detailed breakup of ADRs from various departments could not be done since specific mentioning of the department is not mandated in either the ADR form or for the entry in Vigiflow. Hence we did not include it as a criterion for assessing completeness. However all ADRs reported were drug related and no AEFI (adverse event following immunization) were reported to our AMC.

We estimated the statistical significance of the mean change in percentage of incomplete forms with respect to the parameters namely source, seriousness, outcome, drug details and action taken which had a considerable difference in the values obtained for 2015 and 2016. Analysis was done using the Z test- social science statistics software revealed that the reduction in percentage of incompleteness were not significant for the parameters- source ($p = 0.186$), seriousness ($p = 0.197$), outcome ($p = 0.307$) and drug details ($p = 0.218$) whilst p value for the action taken was statistically significant. (Fig. 1)

The mean completeness score was 0.95 for 2015 and 0.97 for 2016 implying that a high completeness score of the ICSRs uploaded in VigiFlow was maintained by the AMC.

4. Discussion

Amongst the 1650 forms audited, a high degree of completeness was maintained with reference to the report title and patient information. With regards to the source which represented spontaneous reporting from the clinicians, the Pharm D interns and Pharmacology postgraduates, the majority of incomplete forms on qualification of the reporter were from the postgraduates of Pharmacology. This lack of complete information could be attributed to the fact that they were known to the department of Pharmacology functioning as the AMC, which could have precluded them from giving complete information on qualification. However, the incomplete reporting with respect to the source has shown a decline of 8% in 2016 (26%) compared to 2015 (34%) though the difference was not statistically significant ($p = 0.186$)

It is noteworthy that a lack of clarity exists among the reporters in labeling an ADR as serious or not. It is possible that most of the reporters are unfamiliar with assessing the seriousness of the reaction. Categorizing those reactions that can prolong the hospital stay which is one of the seriousness indicators, cannot be easily discerned, as in a practical clinical scenario, it is difficult to attribute whether the reaction itself or the disease *per se* was responsible for the extended hospital stay of the patient. It was found that the ADR forms were complete in seriousness data when the reaction was definitely a life threatening one like the anaphylaxis. Also, a significant finding was that a notable improvement in the percentage of seriousness data entry in the forms in 2016 was evident compared to 2015, though the difference was not statistically significant.

100% completeness in free text information was illustrated based on the audit and with reference to the drug details; potential for improvement was delineated especially with the frequency and route of administration. Outcomes parameter referring to the recovery information can be rather considered as a grey area due to lack of follow up information regarding the patient's status as recovery may take much longer and often beyond the date of reporting [10].

The action taken with regards to the changes in the regime of the offending drug was not stated in a high proportion of the ADR forms reported. Nevertheless, the information on how the reaction was treated was indicated in majority of the forms. The action taken column in the ADR forms had to be filled with the data on whether the suspected drug was withdrawn or the dose increased/changed/reduced. In such situations, wherein the offending drug

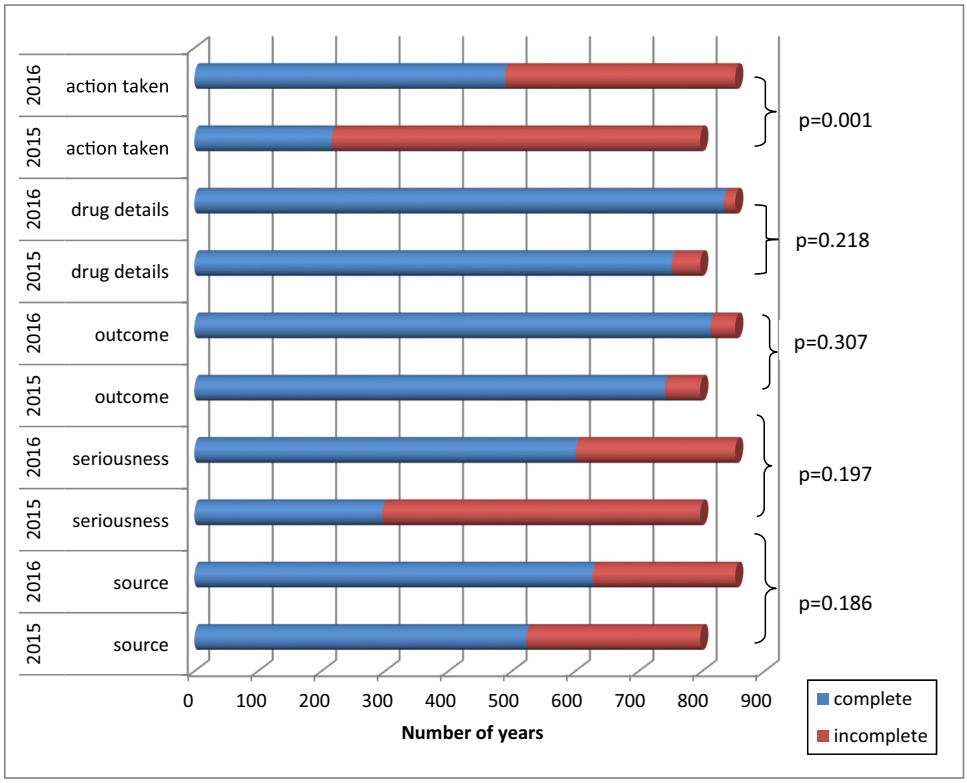


Fig. 1. Comparison of parameters showing difference in percentage of completeness in the years 2015 and 2016.

had been withdrawn or dose modification done, it was obviously mentioned in the form. On the contrary, if the suspected drug was continued in the same dosage despite the reaction, the column on action taken was left incomplete indicating the reporter's attitude of not attaching significance to that information or unwillingness to reveal it. In such cases, the option of "not applicable" which needs to be chosen remained unselected and this could be deciphered from the fact that the "date stopped" column of the suspected medication was also left blank simultaneously. Remarkably, a statistically significant improvement in the action taken data ($p = 0.001$) was demonstrated in the forms reported in 2016 compared to that in previous year denoting gradually increasing awareness about pharmacovigilance among health care professionals [9,11].

A meager percentage of reporters have failed to specifically mention the indication for which the offending drug was administered. This might have resulted from the attitude that the indication of the particular drug for a given diagnosis is widely known. The indication columns were obviously better filled in forms reported in 2016 (0.3%) in comparison to 2015 (6%). Nonetheless, this large variation precluded testing for statistically significance which could not be performed due to the very small value of 0.3%.

The list of concomitant drugs was largely complete in both years and this apparently high percentage is due to the standard operating procedure followed by the AMC personnel to retrospectively collect the information on concomitant drugs, if missing/incomplete either from the consultants or case records [10]. This information is imperative for a meaningful causality analysis and therefore the completeness of concurrently administered drugs information is ensured by the AMC workforce to the maximum extent possible.

Interestingly, we found that ADRs to newer drugs reported were primarily for the anti-diabetic drugs (voglibose, vildagliptin) and cardiology related drugs namely ticagrelor (antiplatelet), lercarnidipine and cilnidipine (antihypertensive drugs). In addition, certain chemotherapeutic drugs like tigecycline, the antifungal drug- caspofungin and antifibrotic drug – pirfenidone used for idiopathic pulmonary fibrosis were among other newer drugs to be implicated in causing ADR. This is quite reflective of the trends in use of new drugs indicating the prompt use of such newly discovered agents for treatment of clinical conditions like diabetes, hypertension and heart disease.

It is important to furnish complete data on the ADR reported in order to ensure the utility of the data for patient safety. The study which was conducted as an audit has highlighted the areas with a definite scope for improvement. Seriousness, action taken, outcomes and concomitant drugs are four such critical areas identified. The major setback in the seriousness entry is lack of certainty on the part of reporters in assigning the seriousness category. This can be improved by periodic contact programs educating them on the various criteria for deciding the seriousness of the reaction report. The completeness of information on action taken and outcomes can be improved if there are ways to ensure the reporter contacting the AMC personnel to share the follow up information. The study has also brought out the need for increasing the awareness amongst reporters that a comprehensive list of concomitant drugs is essential for causality assessment. This can be emphasized through appropriate education programs and providing feedback to the reporters [12–14].

A high completeness score of the ICSRs had been maintained by the AMC. A similar value of 0.94 was recorded in a study for ICSRs received by IPC during July 2011 to September 2014 [9]. This is primarily owing to the programming of VigiFlow software which is designed such that the mandatory columns are filled before submission through appropriate pop up message alerts and stepped data entry which consequentially averts low scoring of ICSRs submitted.

Key message

The trend of increasing awareness on pharmacovigilance is evident from the audit and a definite scope for further improvement has also been identified. Initiation of appropriate programs in view of inculcating the culture of ensuring completeness in ADR reporting amongst healthcare professionals will be instrumental in improving the quality of reported data.

5. Conclusion

The audit has analyzed various parameters of ADRs reported for completeness and has demonstrated that there is increasing awareness on reporting of ADRs with complete information as is evident from the progress in completeness of reporting in 2016 compared to 2015. This can be further improved by structured educative programs with objectives targeting achievement of 100% complete reporting with respect to all the parameters. This would envision an era of best practices for ensuring patient safety.

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None.

Conflicts of interest

The authors have none to declare.

References

[1] Jose J, Rao PG. Pattern of adverse drug reactions notified by spontaneous reporting in an Indian tertiary care teaching hospital. Pharmacol Res 2006;54(3):226–33.
[2] Sahu RK, Yadav R, Prasad P, Roy A, Chandrakar S. Adverse drug reactions monitoring: prospects and impending challenges for pharmacovigilance. Springerplus 2014;3:695.
[3] Olsson S, Pal SN, Dodoo A. Pharmacovigilance in resource-limited countries. Expert Rev Clin Pharmacol 2015;8(4):449–60.
[4] Swamy S, Mourya M, Kadhe G, Mane A, Sawant S. Safety reporting through a comprehensive and pragmatic pharmcovigilance process for India and emerging markets: an industry perspective. Expert Opin Drug Saf 2015;14(9):1409–20.
[5] Kalaiselvan V, Thota P, Singh GN. Pharmacovigilance Programme of India: Recent developments and future perspectives. Indian J Pharmacol 2016;48(6):624–8.
[6] Ploen M, Wallberg M, Olsson S. User-driven development of a web-based tool for patient reporting of drug-related harm. Drug Saf 2015;38(4):359–64.
[7] Durrieu G, Jacquot J, Mege M, Bondon-Guitton E, Rousseau V, Montastruc F, et al. Completeness of spontaneous adverse drug reaction reports sent by general practitioners to a regional pharmacovigilance centre: a descriptive study. Drug Saf 2016;39(12):1189–95.
[8] Bergvall T, Noren GN, Lindquist M. vigiGrade: a tool to identify well-documented individual case reports and highlight systematic data quality issues. Drug Saf 2014;37(1):65–77.
[9] Vivekanandan K, Rishi K, Prasad T, Arunabh T, Singh GN. Status of documentation grading and completeness score for Indian individual case safety reports. Indian J Pharmacol 2015;47(3):325–7.
[10] Kheloufi F, Default A, Rouby F, Laugier-Castellan D, Boyer M, Rodrigues B, et al. Informativeness of patient initial reports of adverse drug reactions. Can it be improved by a pharmacovigilance centre? Eur J Clin Pharmacol 2017(April 8), doi:http://dx.doi.org/10.1007/s00228-017-2254-y (in press).
[11] Gupta SK, Nayak RP, Shivaranjani R, Vidyarthi SK. A questionnaire study on the knowledge, attitude, and the practice of pharmacovigilance among the healthcare professionals in a teaching hospital in South India. Perspect Clin Res 2015;6(1):45–52.
[12] Bhagavathula AS, Elnour AA, Jamshed SQ, Shehab A. Health professionals' knowledge, attitudes and practices about pharmacovigilance in India: a systematic review and meta-analysis. PLoS One 2016;11(3):e0152221.
[13] Pimpalkhute SA, Jaiswal KM, Sontakke SD, Bajait CS, Gaikwad A. Evaluation of awareness about pharmacovigilance and adverse drug reaction monitoring in resident doctors of a tertiary care teaching hospital. Indian J Med Sci 2012;66(3–4):55–61.
[14] Datta S, Sengupta S. An evaluation of knowledge, attitude, and practice of adverse drug reaction reporting in a tertiary care teaching hospital of Sikkim. Perspect Clin Res 2015;6(4):200–6.

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Original Article

A STUDY ON THE EFFECT OF LOW MOLECULAR WEIGHT ON POTASSIUM HOMEOSTASIS IN PATIENTS ADMINISTERED HEPARIN FOR THROMBOPROPHYLAXIS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: Low molecular weight heparin (LMWH) is an indirect thrombin inhibitor used clinically as an anticoagulant for thromboprophylaxis of patients at risk of deep vein thrombosis. The study was done to assess the magnitude of rise in serum potassium after administration of low molecular weight heparin comparing enoxaparin with dalteparin and to evaluate the frequency of clinically significant hyperkalemia in the population studied.

Methods: The study was done as a prospective non-randomized observational study in a population of 32 patients started on heparin for thromboprophylaxis for deep venous thrombosis, pulmonary thromboembolism or stroke.

Results: A statistically significant elevation in serum potassium was seen in patients treated with LMWH ($p = 0.007$). The magnitude of rise in potassium was significantly higher in enoxaparin ($p = 0.008$) than the dalteparin group ($p = 0.447$). A clinically relevant hyperkalemia of>5 mEq/l was seen in 25% of the population studied. Other important associations detected from the study were that the advancing age and increasing dose could be potential risk factors contributory to an accentuated rise in serum potassium which may culminate in clinically significant hyperkalemia.

Conclusion: The study has highlighted that the likelihood of rise in potassium levels during LMWH therapy necessitates monitoring of serum potassium.

Keywords: Serum potassium, Enoxaparin, Dalteparin, Hyperkalemia

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INTRODUCTION

Heparin is a parenteral anticoagulant that acts as indirect thrombin inhibitor and is extensively used for treatment and prophylaxis of several thromboembolic conditions in clinical practice. Heparin is a heterogeneous mixture of sulfated mucopolysaccharides whose biologic activity is dependent upon the endogenous anticoagulant antithrombin. Antithrombin inhibits clotting factor proteases, especially thrombin (IIa), IXa, and Xa by forming equimolar stable complexes with them.

Hyperkalemia is an electrolyte abnormality associated with the use of heparin therapy in patients. It is a condition wherein the serum potassium is elevated above the normal range (Normal: 3.5–5 mEq/l). The incidence of heparin-induced hyperkalemia in hospitalized patients is estimated to be 1-10% [1]. The risk of heparin-induced hyperkalemia is increased many folds in patients with renal insufficiencies and in the elderly population [2]. Thus it is important for the physician to be aware of this complication and ensure appropriate monitoring of serum potassium levels during heparin therapy.

Unfractionated heparin (UFH) has a high molecular weight in the range of 5000–30,000 Daltons. In contrast, low molecular weight heparin (LMWH) is shorter chain fractions which inhibit activated factor X with a lesser effect on thrombin than the UFH. LMWH is as efficacious as UFH with more predictable therapeutic effect and lesser adverse effects. LMWH offers many advantages such as weight-based dosing, lower frequency of injections, eliminating the need for laboratory monitoring in addition to a decreased risk of heparin-induced thrombocytopenia and osteoporosis. Therefore LMWH preparations are more commonly used in the current clinical scenario.

Heparin therapy can induce hypoaldosteronism by inhibiting the enzyme involved in the synthesis of angiotensin ll receptors in the adrenal gland. This, in turn, produces natriuresis and hyperkalemia. Heparin can produce hyperkalemia within 4-7 d of beginning treatment [3]. The risk of hyperkalemia is increased in patients with chronic renal failure, diabetes mellitus, and elderly people [2]. While most physicians are aware of heparin-induced thrombocytopenia, heparin-induced hyperkalemia is less commonly recognised [4].

Though there are many case reports on unfractionated heparin-induced hyperkalemia in literature, there are very few prospective studies evaluating the effect of LMWH on serum K^+ levels. Therefore our study was designed to evaluate the frequency of occurrence of a heparin-induced increase in serum K^+ levels in hospitalized patients receiving LMWH for thromboprophylaxis. We chose to compare the change in K^+ electrolyte levels due to enoxaparin with that of dalteparin as enoxaprin and dalteparin are the two commonly used low molecular weight heparins in patient care.

MATERIALS AND METHODS

This was a hospital based prospective open-label, non-randomized study. The institutional human ethics committee (IHEC) approved the protocol of the study. (Approval number: 13/361). The study was done in 32 patients started on low molecular weight heparin in our tertiary care teaching hospital. Patients who were admitted and started on LMWH, either enoxaparin or dalteparin were included for the study, based on inclusion and exclusion criteria.

All patients, aged>30 y, both males and females with normal baseline potassium value and started on low molecular weight heparin, either enoxaparin or dalteparin for thromboprophylaxis in ICU or inpatient care were included for the study after obtaining a written informed consent. Patients with chronic renal insufficiencies or altered renal function tests, on pre-existing potassium supplementation or receiving drugs which can increase K^+ level like spironolactone, ACE inhibitors, angiotensin receptor blockers, NSAIDS including cyclooxygenase 2 inhibitors, trimethoprim, amiloride, triamterene, pentamidine, nafamostat, renin inhibitors, digoxin and related glycosides, mannitol, beta-blockers, succinylcholine and other drugs known to alter the potassium levels

were excluded by a detailed evaluation of the list of concomitant drugs. Children, pregnant and lactating women were also excluded from the study. In addition, patients whose baseline potassium value was not estimated or missing were not included in the study.

After obtaining a written informed consent, data on demographic details, the diagnosis, the indication for heparin, other co-administered drugs and the serum electrolyte levels were obtained. The baseline K^+ level was collected from the case record of the patient. Another K^+ level estimation was done at the end of day 4 of starting heparin. Blood collection was done meticulously by a trained lab technician using standard venipuncture technique; taking care to avoid tourniquet, fist-clenching, inappropriate needle diameter, excessive force during aspiration and other maneuvers which could result in hemolysis or falsely elevated potassium values. Potassium estimation was done in the biochemistry laboratory by ion selective electrode method using potentiometry. Any documentation of symptoms pertaining to hyperkalemia during heparin therapy recorded in the case record was also noted. Data regarding the patients who developed clinically significant hyperkalemia requiring a change in therapy or other measures taken control the hyperkalemia was also collected. The data collected was entered into Excel and statistical analysis was done using SPSS software version 19.

Table 1: Baseline demographic data of the participants

S. No.	Parameter	Measured values
1	Mean Age	58.5 y
2	Male female ratio	14:18
3	Patients treated with LMWH	32
4	Patients treated with Enoxaparin	17(53%)
5	Patients treated with Dalteparin	15(47%)

Table 2: Paired t-test to compare the means of the serum potassium before and after administration of heparin across different age groups

Groups (n= no of patients)	Serum potassium level (mEq/l)				Paired difference of before and after values		p-value
	Before		After		Mean	±Standard deviation	
	Mean	±SD	Mean	±SD			
31-60 y (n=13)	3.87	0.436	4.20	0.794	-0.329	0.526	0.065
61-80 y (n=19)	3.83	0.736	4.13	0.669	-0.295	0.430	0.015

n=32; Data expressed as mean±SD

RESULTS

A total number of 32 patients participated in this study. The mean age of the study participants was found to be 58.5±14.5 y (table 1). About 59% of the patients were in the age group between 61-80 y and the rest (41%) were 31-60 y aged. The occurrence of serum potassium elevation, according to the study, was found to be significantly higher in the patients of 61 to 80 y age group, unlike the 31-60 y age group subjects in whom the rise in potassium level after treatment was not statistically significant (table 2).

Amongst the study participants, 44% were males and 56% were females (table 1). Among the patients started on low molecular weight heparin therapy, around 53% received enoxaparin while 47% of patients received dalteparin (fig. 1). The mean potassium level done at the end of 4 d after starting treatment with low molecular weight heparin, showed a demonstrable increase compared to baseline and a paired t-test revealed a statistically significant difference (p = 0.007) in the serum potassium before and after treatment with heparin (fig. 2).

Considering the change in serum potassium levels in the enoxaparin-treated group and the group treated with dalteparin, it was observed that both groups had a definite increase in the mean potassium levels after commencing treatment. However, it was found using paired t-test that a statistically significant increase in the serum potassium level was demonstrable only for the enoxaparin group (p =0.008), but not for the dalteparin group (p = 0.447) (table 3).

In addition, a correlation between increases in serum potassium with that of the dose administered could be identified for both groups as shown in the graphical representations fig. 3, 4.

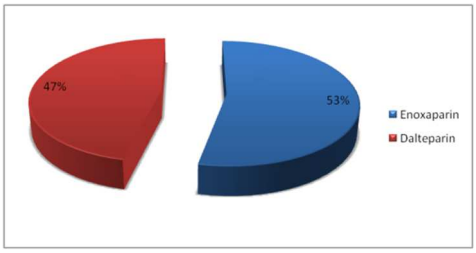


Fig. 1: Frequency distribution of the patients started on low molecular weight (enoxaparin, dalteparin)

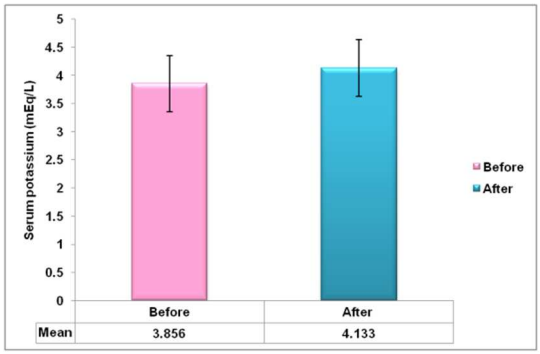


Fig. 2: Serum Potassium expressed as mean±SD before and after treatment with low molecular weight heparin

Table 3: Paired t-Test to show the statistically significant difference in the serum potassium values before and after treatment

Groups (n= no of patients)	Serum potassium level (mEq/l)				Paired difference of before and after values		p-value
	Before		After		Mean	±Standard deviation	
	Mean	±SD	Mean	±SD			
LMWH (n=32)	3.89	0.667	4.12	0.707	-0.232	0.391	0.007
Enoxaparin (n=17)	3.81	0.556	4.10	0.602	-0.292	0.396	0.008
Dalteparin (n=15)	4.06	0.31	4.16	0.332	-0.106	0.373	0.447

Data expressed as mean±SD

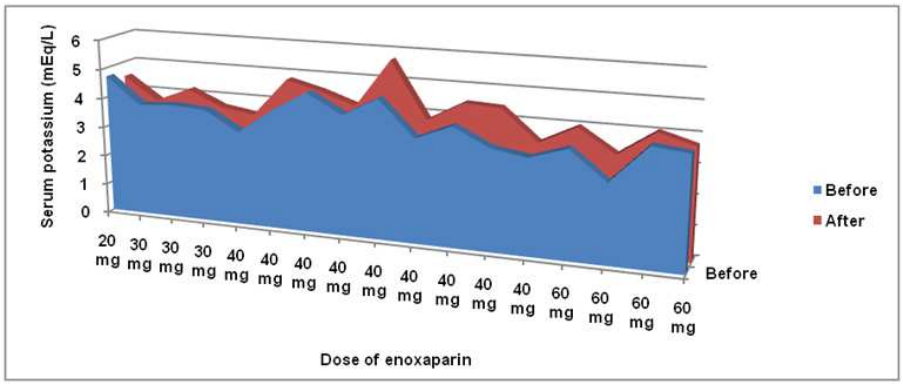


Fig. 3: Chart representing the rise in serum potassium levels across various doses of enoxaparin

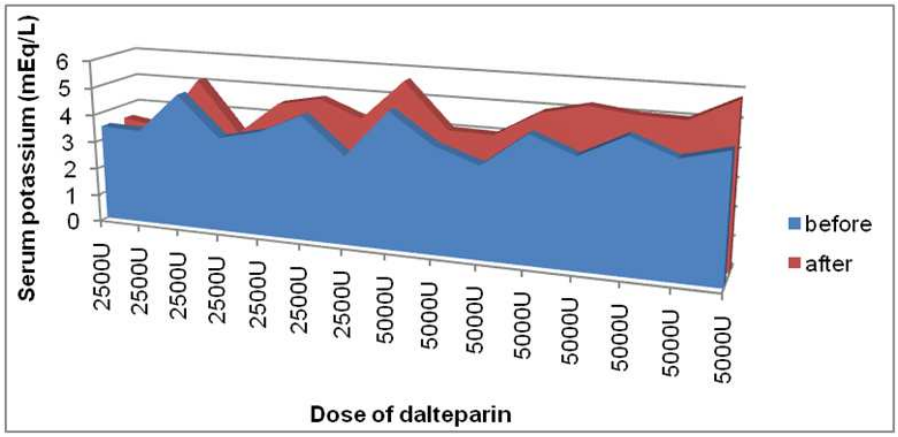


Fig. 4: Chart representing the rise in serum potassium levels across various doses of dalteparin

Clinically relevant hyperkalemia (more than 5mEq/l) was noted in 8 (25%) patients out of the 32 participants. Among these patients, 5(16%) were treated with dalteparin and 3(9%) were in the group treated with enoxaparin (fig. 5). Though around 1/4th of the patients in the study manifested with clinically relevant hyperkalemia, none of them was symptomatic or needed any specific measures to correct the electrolyte abnormality.

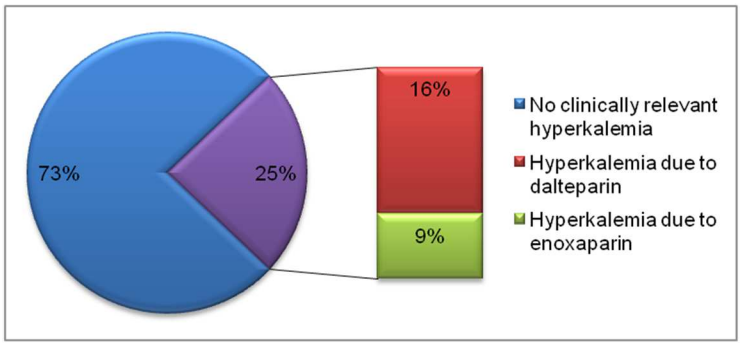


Fig. 5: Chart representing the frequency of patients who developed clinically significant hyperkalemia (>5 mEq/l)

DISCUSSION

In our study done in 32 patients started on low molecular weight heparin, there were 46% aged 31-60 y whereas a larger proportion of patients, ie, 59% were in the age group of 61-80 y. This emphasises that there is an increased risk of thromboembolic disease as the age advances. This has been demonstrated in earlier studies. [5, 6] This is probably attributed to the increase in the prevalence of the risk factors for thromboembolic diseases with ageing.

In addition, it was established through the study results that increasing age is also associated with an increased risk of hyperkalemia with heparin therapy. This is evident from the statistically significant increase in serum potassium in the 61-80 y age group (p = 0.065), but not in the age group of 31-60 y (p= 0.015) (table 3). The male-female ratio was 14: 18 indicating that a higher number of female patients had received heparin therapy during the study duration. Noteworthy as most of the female patients who participated in the study were in the postmenopausal age group but none of them was on any hormonal treatment.

Using paired t-test, a statistically significant rise in serum potassium was demonstrated in the study population treated with LMWH (p = 0.007). In the current clinical scenario, UFH has been largely replaced by the LMWH. This is quite understandable, particularly since there are plenty of advantages for LMWH over UFH like equal efficacy, increased bioavailability, and less frequent dosing. Also, monitoring of activated partial thromboplastin time is not required. It has been well brought out in this study that the low molecular weight heparin is also implicated in increasing the serum potassium levels like UFH.

The primary mechanism of heparin-induced hyperkalemia has been well established and it is due to suppression of aldosterone synthesis. This is mediated by a reversible effect on aldosterone through blockade of an enzymatic step in the synthesis and also the angiotensin II receptors in the adrenal gland. The most important mechanism of heparin-induced hypoaldosteronism involves a reduction in both number and affinity of angiotensin II receptors in the zona glomerulosa. Prolonged administration of heparin causes a marked reduction in the width of adrenal zona glomerulosa thereby leading to hyperkalemia and natriuresis. This reversible suppression of aldosterone occurs within a few days of initiation of therapy and is independent of either the anticoagulant effect or the route of administration. It has been reported that reduced aldosterone levels may be evident as early as 4th day of initiation of heparin therapy [3]. Thus LMWH has effects similar to that of UFH on aldosterone [7, 8] and hence LMWH is also associated with increasing the risk of hyperkalemia in treated patients. This has also been brought out by our study result which is well in concordance with previous studies reported in the literature [9, 10].

It was found that among the LMWH, enoxaparin (53%) was more commonly used than dalteparin for thromboprophylaxis (47%). The definitive reason for this cannot be offered, especially because an extended literature search has shown multiple studies and metanalyses comparing UFH with LMWH, but head to head comparisons of the different LMW heparins are few in number and these have claimed similar compliance, health status, deep venous thrombosis, and bleeding rates with both [11] while some studies have brought out that switching patients from enoxaparin to dalteparin was cost-effective [12]. However the DETECT trial has established the hypothesis that dalteparin 5,000 units subcutaneous (SC) daily may not be clinically non-inferior to enoxaparin 30 mg SC twice daily for venous thromboembolism prophylaxis in high-risk population and until an adequately powered, prospective noninferiority trial is performed, enoxaparin is supported by level 1 evidence and should be the prophylactic agent of choice [13].

In our study, a statistically significant increase in the potassium level was seen only in the group treated with enoxaparin (p = 0.008) but not the dalteparin group (p = 0.447). Though the study infers a statistically higher risk of serum potassium elevation with enoxaparin in comparison to dalteparin, this cannot be translated to a definite clinical significance with certainty owing to two reasons. First is the lack of sufficient sample size and secondly, on account of the remarkable finding that a higher proportion of patients in dalteparin group (15%) had clinically relevant hyperkalemia (>5 mEq/l) compared to 9% in the enoxaparin group. An explanation for more number of patients manifesting hyperkalemia in the dalteparin group is probably due to the higher mean baseline value (4.06±0.31) compared to that of enoxaparin group (3.81±0.55). This is in accordance to a study which has shown that baseline potassium level reflects pretreatment aldosterone level and hence its value has a predictive role for assessing the susceptibility to develop hyperkalemia [10].

Interestingly, the results of the study also revealed an association of a larger rise in potassium levels being associated with higher doses of both the low molecular heparins studied. This is substantiated by the fact that the higher doses of enoxaparin 40 and 60 mg produced a greater elevation of serum potassium levels as compared to the lower doses of 20, 30 mg and this observation was replicated in the groups on dalteparin 2500U versus 5000U as well (fig. 3,4). Thus the adverse effect is found to be dose dependent and the possible explanation of this observation stems from an accentuated aldosterone suppression by higher doses of heparin leading to an augmented rise in serum potassium levels.

Thus the study has clearly delineated that significant elevation of serum K+is seen in patients treated with low molecular weight heparin and that the magnitude of the rise in potassium to clinically relevant hyperkalemia (>5mEq/l) occurs in approximately one-quarter of the patients studied. This signifies the need for adequate monitoring of the serum K+while on LMWH for thromboprophylaxis.

The limitation of the study is the small sample size. Therefore it is recommended that the study results have to be confirmed in larger trials in order to enable extrapolation of the results to the larger patient population treated with heparin clinically.

CONCLUSION

The study has highlighted the magnitude of changes in potassium levels in patients receiving low molecular weight heparin for thromboprophylaxis. In addition to the known risk factors like renal failure and concurrently administered drugs, the study has made it evident that advancing age and increasing dose could potentially contribute to the development of hyperkalemia with low molecular weight heparin therapy. Thus the study results help the clinicians to potentially predict the patients likely to experience this electrolyte disturbance; stratify their risk and manage them appropriately.

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CONFLICT OF INTERESTS

There are no conflicts of interest

AUTHORS CONTRIBUTION

Author 1 was involved in concept, design, data collection, analysis and interpretation and author 2 were involved in concept, design and data interpretation. The third author was involved in the design, data collection and analysis of the research project.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Kuijvenhoven MA, Haak EA, Gombert-Handoko KB, Crul M. Evaluation of the concurrent use of potassium-influencing drugs as risk factors for the development of hyperkalemia. Int J Clin Pharm 2013;35:1099-104.
2. Bacon NC. Heparin-induced disturbance of potassium homeostasis. Q J Med 1997;90:725.
3. Thomas CM, Thomas J, Smeeton F, Leatherdale BA. Heparin-induced hyperkalemia. Diabetes Res Clin Pract 2008;80:e7-8.
4. Torres OH, Hernandez N, Francia E, Barcelo M, Mateo J, Ruiz D. Effect of prophylactic treatment with low-molecular-weight heparin bemiparin sodium on serum potassium levels: a prospective observational study. Drugs Aging 2010;27:399-406.
5. Olson NC, Cushman M, Lutsey PL, McClure LA, Judd S, Tracy RP, *et al*. Inflammation markers and incident venous thromboembolism: the REasons for geographic AND racial differences in the stroke (REGARDS) cohort. J Thromb Haemost 2014;12:1993-2001.
6. Aronow WS, Schoenfeld MR, Gutstein H. Frequency of thromboembolic stroke in persons greater than or equal to 60 y of age with extracranial carotid arterial disease and/or mitral annular calcium. Am J Cardiol 1992;70:123-4.
7. Gheno G, Savarino C, Vellar S, Cinetto L. Heparin-induced life-threatening hyperkalemia. Ann Ital Med Int 2002;17:51–3.
8. Gonzalez-Martin G, Díaz-Molinas MS, Martínez AM, Ortiz M. Heparin-induced hyperkalemia: a prospective study. Int J Clin Pharmacol Ther Toxicol 1991;29:446–50.
9. Gheno G, Cinetto L, Savarino C, Vellar S, Carraro M, Randon M. Variations of serum potassium level and risk of hyperkalemia in inpatients receiving low-molecular-weight heparin. Eur J Clin Pharmacol 2003;59:373–7.

10. Bengalorkar GM, Sarala N, Venkatrathnamma PN, Kumar TN. Effect of heparin and low-molecular-weight heparin on serum potassium and sodium levels. J Pharmacol Pharmacother 2011;2:266–9.
11. Chiou-Tan FY, Garza H, Chan KT, Parsons KC, Donovan WH, Robertson CS, et al. Comparison of dalteparin and enoxaparin for deep venous thrombosis prophylaxis in patients with spinal cord injury. Am J Phys Med Rehabil 2003;82:678-85.
12. Krotenberg R, Adler U, Pomeranz B, Miller JD, Russell MW. Dalteparin vs. enoxaparin as prophylaxis for deep-vein thrombosis after total hip or knee arthroplasty: a retrospective analysis. Am J Phys Med Rehabil 2001;80:889-95.
13. Slavik RS, Chan E, Gorman SK, de Lemos J, Chittock D, Simons RK, *et al*. Dalteparin versus enoxaparin for venous thromboembolism prophylaxis in acute spinal cord injury and major orthopedic trauma patients: 'DETECT' trial. J Trauma 2007;62:1075-81.

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Background: To assess the attitude of second year medical students towards the teaching Principles in Pharmacology didactic lecture classes.

Methods: It was a descriptive epidemiological and questionnaire based study. Second year students who were attending pharmacology lecture classes for a period of one month (nearly 8 lectures) were given questionnaire and ask to fill it by assessing the theory classes which was conducted throughout that month. Each question was graded with mark and finally total score was converted to 50 marks. Five-level Likert's criteria were used to analyze few questions in the questionnaire. Data were analyzed by frequency distribution.

Results: Out of 115 students who attended the questionnaire session, 61% students were having a very good and better attitude towards the faculty talking Pharmacology lecture classes and remaining students have a few lacking areas in the teaching mode which should be analyzed.

Conclusions: Students have good attitude towards the faculty taking didactic lectures and the lacking areas can be future improved by efficient planning of interactive and interesting lecture methods for the usefulness of students as well as the teacher.

Keywords: Attitude, Second year medical students, Pharmacology lectures

Pharmacology is the study of drugs. In pharmacology teaching will be a combination of lectures and practical sessions.[1,2] Lecture is a traditional and didactic method of teaching. It is the most common method of teaching which is used to provide knowledge and information to a vast number of medical students but there is negligible student involvement in these types of teaching process.[3] Medical teachers are working hard to implement diverse teaching techniques to educate medical students who were formerly thought only by blackboard and slide projectors. Now day's audio visual aids such as power point have been used as one of the newer methods. The optimum use of audio visual aids is essential for their learning. Traditional didactic lecture is an inactive method in nature and less effective as compared with active learning methods, such as problem-based learning. However, a well-efficient lecture remains one of the most powerful ways to integrate and provide information from numerous sources on scientific topics.[4,5] Hence this study aims to analyze student's attitude towards the medical education teaching skills and methods.

Aim of the study was to evaluate the attitude of second year medical students towards the teaching principles in Pharmacology didactic lectures.

Objectives

- To evaluate students' perception regarding the motivation, teaching, communication and linguistic aspects of the faculty during the lecture classes.
- To evaluate the effectiveness of teaching with regard to content and teaching methods.

- To analyze students' level of satisfaction about the faculty with respect to teaching, insisting on newer concepts, including research ideas.
- To know whether introduction, objectives, summary, take home message of a particular topic are covered by the faculty during the lectures.

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Pharmacology topics by pharmacology teachers to 2nd year MBBS students for a period of one month in 8 lecture classes.□

Inclusion criteria

- Second MBBS students who give consent to participate in my research study
- Second MBBS students who are present during the questionnaire study

Exclusion criteria

- Second MBBS students who are not giving consent to participate in the study
- Second year MBBS students those who are absent during the questionnaire Study
- All other MBBS students in the college excluding second year MBBS students
- Partially filled or unfilled questionnaire even after giving consent

Type of study was descriptive epidemiological study.

Duration of the study was one month from 1st July 2015 to 31st July 2015.

Study tool□□

Feedback assessment form

Study was carried out at Department of Pharmacology, Private Medical College, Coimbatore.

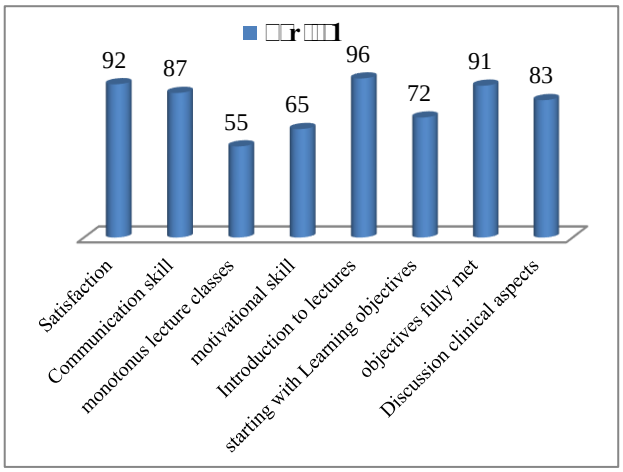
A total of 145 students studying second year MBBS from a private medical college was included in the study. Informed consent was obtained and the study was conducted by providing a planned questionnaire for assessing the attitude of medical students towards professional teaching skills and methods. An assessment was set with a maximum of 50 marks to evaluate the students' attitude towards facilitator and the presentation. Each question was graded with mark and finally total score was converted to 50 marks and no negative marks were awarded to questions unanswered. Five-level Likert's criteria were used to analyze few questions in the questionnaire.[6] Based on the marks obtained, the attitude of students towards faculty was classified into 6 categories. Later the data were analyzed followed by tabulation of results and interpretation was done.

Statistical analysis

Data were analyzed statistically using frequency distribution

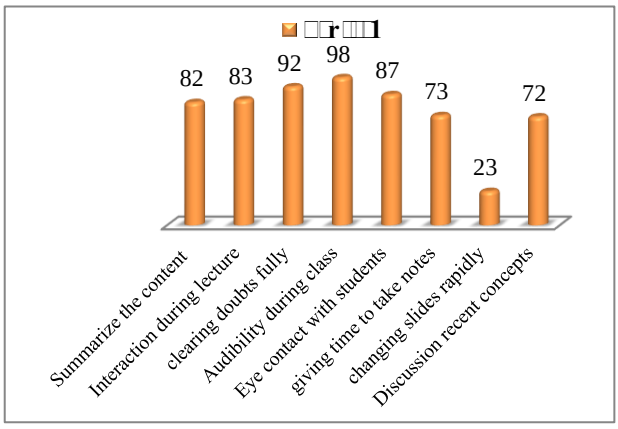
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96% of students said that faculties give introduction to the lectures in an interesting manner, 92 % said that they were satisfied with the teaching methods and 91% said that the objectives of the classes were fully achieved (Figure 1).



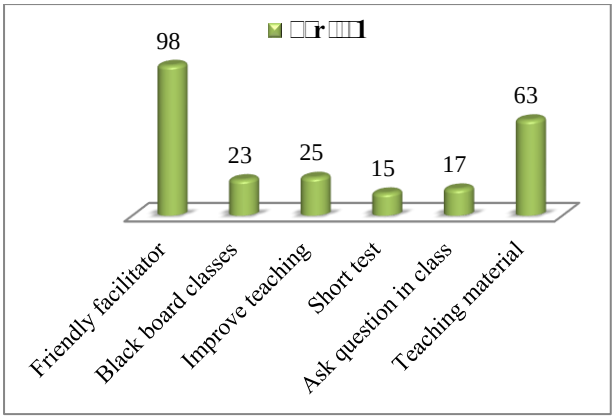
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98% of students said that faculties teaching was audible clearly and 92% said that they also clear the doubts then and there (Figure 2).

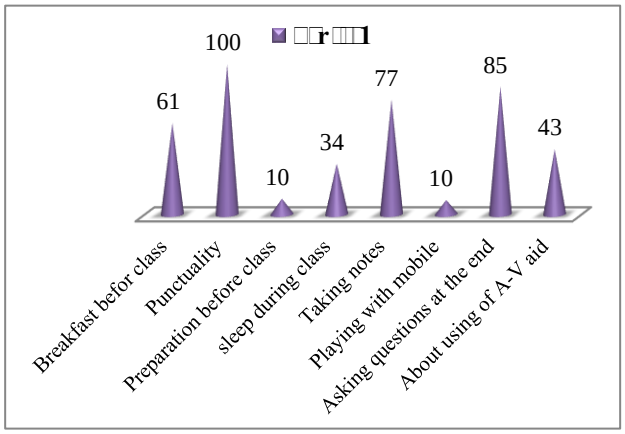


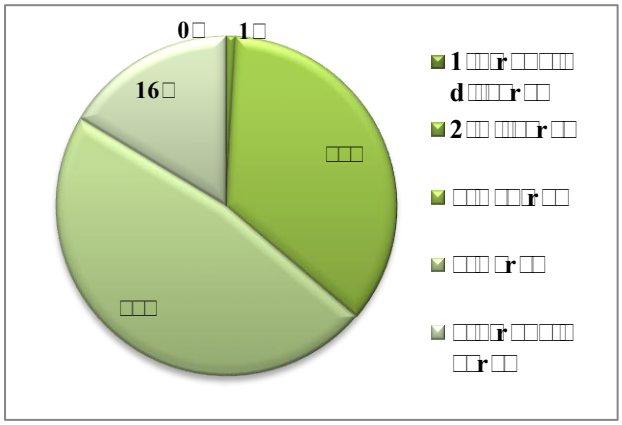
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98% of students suggest that all faculties should be student friendly, 63% suggested providing teaching material one week before the class so that they can read the concepts in better way (Figure 3).

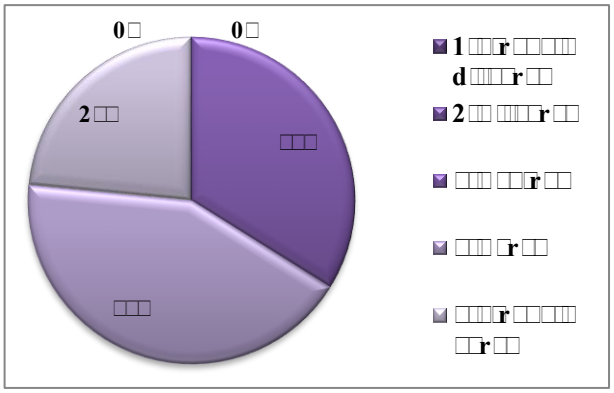


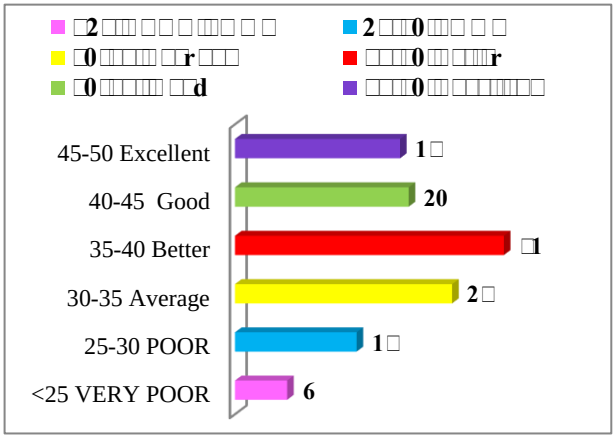
In students' aspect 85% told that they ask doubts in the class, 70% of the students said that they take notes during class (Figure 4).





Out of 115 students who attended the questionnaire session,61% students were having a very good and better attitude towards the faculty talking Pharmacology lecture classes and remaining students have a few lacking areas in the teaching mode which should be analyzed (Figure 5, 6 and 7).





Discussion

Out of 145 second year medical students, only 115 students participated in the research study by filling the questionnaire after giving informed consent. Around 100% students have told that the teachers were punctual to lecture classes. 96% of students accepted that the faculty gave introduction, stressed upon learning objectives and reinforced at the end of the class after summarizing. 92% of students were satisfied with the faculty teaching. This satisfaction is important because it shows that at least half of the student's population tries to understand the concepts. 91% of students had stated that the objectives of the class were completely met. 65% was satisfied with the faculty's motivation skills.

87% of students felt that the linguistic skills of faculty were good. 83% of students felt that the faculty gave enough clinical background concerned to the theory topic. These results showed that the faculties were efficient in disseminating the relevant concepts and that

they could also give real life examples for the clinical cases which would make a good impact on the topic by the students.[4]

83% and 92% of students said that the teaching staffs interacted and cleared doubts regularly during the lecture hours. Approximately 45% of students agreed that the teaching aids used by faculty were good. 87% of students told that faculties have good eye contact with them during lectures. 98% of students said that faculty teaching was clearly audible during the lecture. 73% of students felt the faculty had given enough time for taking notes. Students who took notes during the lecture regularly were found to be 77%. Earlier studies have also stated that in order to improve the understanding of the students and for their future referencing, enough time to take notes has to be given which will in-turn improve the learning skill of the students.[4]

Regarding pitfalls during teaching was that, 23% felt that the faculty changed the slides rapidly. 25% of students felt that the faculty could further improve the way of teaching. 55% of students felt that lecture classes were monotonus. In the previous studies, they have mentioned that breaking the monotony for every 20 minutes by directing questions towards the students and also by showing some slides unrelated to the theory topic (E.g. Vacation slides) will be helpful and prevent the students from getting bored.[7,8]

Merely 10% of students said that they come prepared for the theory classes. It is good to come prepared for the theory classes because they could understand the entire concepts clearly and also clarify the doubts in the theory class itself. 30% of students used to sleep occasionally during the class hours. It shows that faculties should take few more steps to make the theory classes much more interesting, as we discussed earlier by giving some real-life examples and by breaking the monotony.[4,7-9] 10% of students stated that they would play with mobiles during the lecture classes. Institutions should make some arrangements to restrict the mobiles at least during the academic hours so that the student's distractions can be reduced. It can also be minimized by modifying the theory class into active and interactive learning sessions which involves both the students and the teacher actively taking part in the teaching learning process.

Now considering the role of small group teaching and problem based learning etc., we can incorporate these methods in between the theory classes to make the topic much more interesting. Studies have also shown better results by use of these methods in teaching.[5,10,11]

Regarding the students' suggestions, 98% of the students wanted the faculty to be a friendly facilitator, so that they can easily approach the faculty whenever it's needed. Studies have also shown that by incorporating Postgraduates in Under graduates teaching program, it will improve the imparting of knowledge and learning skill in the students.[12] Merely 23% of students wanted the class to be taken purely in a black board. Black board teaching is necessary but still it wouldn't be completely sufficient to ensure that all scientific concepts to be effectively delivered through this approach. So we can also use Audio visual aids and hand models to describe the topic. 63% of students wished to get the teaching material one week before the class.15% of students suggested to keep short tests at periodic intervals.

Out of 115 students 70 (61%) students were having a very good and better attitude towards the faculty taking Pharmacology lecture classes. So instead of giving monotonus lecture classes alone, we can also incorporate multiple modes of teaching methods as we have discussed above to improve the knowledge and learning skills in the students.

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From the results of the study conducted regarding the attitude of students towards the didactic lectures, it was found that overall students' satisfaction was good. However, faculty could further improve their skills in certain areas like motivating the students, communication skill, usage of needed teaching aids, interactive teaching class which include recent advances and discussion of clinical points. Regarding the suggestions of the students, they expect every faculty to be a friendly facilitator, in addition to periodic short tests to evaluate their understanding of the subject. Thus, continuous monitoring of faculty and student performance by means of assessment is mandatory.

The final expectation from every faculty in a medical college is to bring out a knowledgeable and skilful Indian medical graduate which is an achievable process. Suggestions from the students must be taken in to consideration to implement better teaching methods than before. This can be achieved by the efficient planning of interactive and interesting lecture methods for the usefulness of students as well as the teacher.

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Ethical approval: Not required

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1. Sudha J. Graduate training programmes in pharmacology in India. Health Adm. 2006;19(1):88-91.

2. Mohan L, Shankar PR, Kamath A, Manish MS, Eesha BR. Students' attitudes towards the use of audio visual aids during didactic lectures in pharmacology. J Clin Diagn Res. 2010;4(6):3363-8.
3. Tisonova J, Hudec R, Szalayova A. Experience with problem oriented teaching in pharmacology. Bratisl Lek Listy. 2005;106(2):83-7.
4. Richardson D. Don't dump the didactic lecture; fix it. Adv Physiol Educ. 2008;32(1):23-4.
5. Rathnakar UP, Gopalakrishna HN, Pai PG, Ullal SD. Didactic lecture and interactive sessions in small groups: A comparative study among undergraduate students of pharmacology in India. Journal of Diagnostic and Clinical Research. 2010;4(2):2260-4.
6. Uebersax JS. Likert scales: dispelling the confusion. Statistical Methods for Rater Agreement website. 2006. Available from: http://john-uebersax.com/stat/likert.htm.Accessed:July 25, 2015.
7. Michael JA, Modell H. Active Learning in Secondary and College Scienc Classrooms. Mahwah, NJ: Erlbaum; 2003.
8. Myers C, Jones T. Promoting Active Learning: Strategies for the College Classrom. San Francisco, CA: Jossey-Bass; 1993.
9. Angelo TA, Cross KP. Classroom Assessment Techniques. A Handbook for College Teachers (2nd ed.). San Francisco, CA: Jossey-Bass; 1993.
10. Washington ET, Tysinger JW, Snell LM, Palmer LR. Implementing Problem based Learning in a Family Medicine Clerkship. Fam Med. 1998;30(10):720-6.
11. Goshtasebi A, Zarifi A, Tarami B, Ghorbani A. Small group teaching in epidemiology courses. Journal of Medical Education. 2006;9(1):11-5.
12. Kalra R, Modi J, Vyas R. Involving postgraduate's students in undergraduate small group teaching promotes active learning in both. Int J Appl Basic Med Res. 2015;5(1):S14-7.

Umamaheswari A, Umamageswari MS, Vinotha SAT. Assessment of medical students' attitude towards didactic lectures taken in pharmacology in a private medical college. Int J Basic Clin Pharmacol 2017;6:1451-5.

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A Study of Bony and Cartilaginous Neck Structures in Deaths Due to Suicidal Hanging at New Delhi

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ABSTRACT

The present prospective study was done at Lady Hardinge Medical College, New Delhi. In this study the incidences of the bony and cartilaginous injuries in 100 cases of deaths due to hanging were analysed with the gender, age, socio economic status, place of hanging, the type of suspension, the location of the knot, type of the knot, the type of hanging. 76(76%) of cases were males and the majority of the cases were from 21-30 age group, 94(94%) of the victims belonged to the low socioeconomic status, 97(97%) of the cases hanged themselves indoor, 2(2%) had committed suicide by hanging at their workplace, 81(81%) cases had complete type of hanging and 64(64%) had atypical type of hanging. In our study there were 42 (42%) cases had some injuries in their bony and cartilaginous neck structures. Hyoid bone fractures were found in 6(6%) cases, thyroid cartilage injury was seen in 39(39%) cases among which 3 cases had both hyoid and thyroid cartilage fracture in combination. All the above injuries occurred only in cases with complete type of hanging. The incidence of such injuries was more frequent in males who had typical and complete type of hanging.

Keywords: *Hanging, injuries, hyoid bone, thyroid cartilage.*

INTRODUCTION

Death due to hanging is a form of asphyxia caused with a ligature which encircles and constricts the neck, in such a manner that the weight of the whole or a part of the body acts as a constricting force. The external and internal findings in the neck in such cases are well documented. But for unknown reasons there are major variations in the bony and cartilaginous injuries reported in various studies. This study was undertaken to establish a strong correlation with the various factors that influence the pattern of injuries to the bony and cartilaginous neck structures in such cases.

MATERIALS AND METHOD

The study was done in 100 cases of suicidal hanging brought for medico-legal autopsies to the Department of Forensic medicine and toxicology, Lady Hardinge medical college, New Delhi. Only those cases with a well documented type of suspension, an intact ligature material were included in this study. A detailed history with photographs of the crime scene was mandated in the inclusion criteria. A standard autopsy procedure with special emphasis on the fine dissection of neck structures was done. Provided, cases only with evidential proven ante mortem injuries were alone considered in our study.

RESULTS

1. Among all the 100 cases of suicidal hanging, 76(76%) were males, 24(24%) were females.

2. The age groups ranged from 18-59 years, Majority of them (i.e. 60% of the entire study group) were from 21-30 years, 94(94%) of the victims were from low socio economic status, 97(97%) of the cases had hanged themselves indoors, 2(2%) of the cases had hanged themselves at their work place.

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3. Ropes were used as the ligature material in 52(52%) of the cases and others preferred saris, scarf, bed sheets, wires and plastic tapes.

4. 81(81%) cases had a complete type of hanging and 19(19%) of them were partially hanged.

5. In 36 (36%) cases the knot was at the nape of the neck thus constituting typical type of hanging. 64 (64%) cases had atypical type of hanging.

6. About 37 (37%) cases had contused sternocleidomastoid muscles. And the internal carotid artery showed transverse tears in 19 (19%) cases.

7. In 42 (42%) cases there were some injuries either in the hyoid bone or the thyroid cartilage only. Among which 3 cases had both hyoid and thyroid cartilage injury.

8. Unilateral fracture of greater horn of hyoid bone was present in 6 (6%) cases.

9. Fracture of the greater horn of thyroid cartilage was present in 39 (39%) cases, in which 33 individuals had unilateral fracture of the superior horn and 6 individuals had bilateral superior horn fractures.

10. The frequency of fractures in our study was higher among the males who were in the elderly age group and those who had a complete and typical type of hanging.

DISCUSSION

The present study was done to establish a strong correlation with the various factors that influence the pattern of injuries to the bony and cartilaginous neck structures in such cases.

With regard to the gender the male female ratio in our study was 3:1 with 76 (76%) and 24 females (24%). More than half of the males i.e. 38 out of 76 males and 4 out of 24 females had some injury in their bony and cartilaginous structures. Similar results were shown in other studies [1,2].

The age distribution ranged from 18 years to 59 years. Almost half of the cases were from 21-30 age groups. Various other studies also have reported the same age distributions [2,3,4]. The relations of the frequencies of fractures with respect to the gender and age distribution are as given (Table 1).

Table 1

In our study the fracture of the hyoid bone and the thyroid cartilage frequented in elderly aged victims.

There were 81 (81%) victims had a complete

Age	Total no. of cases n=100		No. of cases with fracture n=42	
	Males	Females	Males	Females
11-20	9	3	0	0
21-30	47	10	24	0
31-40	6	7	5	1
41-50	9	3	4	2
51-60	5	1	5	1
>60	0	0	0	0
Total	76	24	38	4

hanging and 19(19%) victims were partially hanged and with regard to the location of the knot there were 36 (36%) cases who had their knot on their nape of the neck thus constituting typical hanging. In 64 (64%) cases had atypical type of hanging among which 42 cases had their knot on the right side of the neck, 21 cases had their knot on their left side of the neck and one of the victims had the knot under the chin. 94% of the victims were from low socio economic status, 97% of the cases had preferred hanging indoors and 2% of victims had committed suicide at their workplace.

More than half of the cases 52% preferred ropes as the ligature material. Other ligature materials were chunni (a long scarf), sari and bed sheets. Similar findings are being reported in other studies[5,6].

The external and internal soft tissue findings were similar to other case studies reported [5,7,8,9].

In our study 42(42%) cases had some injury in their hyoid bone and thyroid cartilage. In our entire study there were 6 (6%) cases of hyoid bone fracture in their greater horn and 39 (39%) cases had their superior horn of thyroid cartilage fractured. No other bony injuries were detected on the neck.

With regard to the type of suspension all the injuries to the hyoid bone and the thyroid cartilage occurred only in those victims who had a complete type of hanging. And the incidence of such injuries was higher in those cases with typical type of hanging.

The frequency of the fracture with respect to the type of suspension and hanging are as given (Table 2).

Table 2

	Type of suspension		Total	Type of hanging		Total
	Complete	Partial		Typical	Atypical	
Total no. of cases	81	19	100	36	64	100
No. of cases with injury.	42	0	42	24	18	42

Thus the incidence of injuries to the hyoid and thyroid cartilage is highest in victims who had a complete typical type of hanging.

REVIEW OF LITERATURE

Regarding the frequency of the hyoid bone fracture Polson and Ghee[10] reported that hyoid bone fractures in 25% of their cases. Davidson and marshall[11] showed that 26.7% of their 101 cases had associated hyoid bone fracture. R.Clement et[12] al in their retrospective study claim that incidence of hyoid fracture is about 1% of their 206 cases. In India Sharma BR et al[13] and Th.Meera et al[14] had reported hyoid bone fractures in 21% and 3.5% of their case series respectively. Weintraub[15] stated that hyoid bone was fractured in a seventh of all hangings and a fifth of victims aged over 40 years, irrespective of the type of hanging. Interestingly, Hyoid bone fractures were never reported in 83 cases studied by VJM DiMaio[16]. Smith and Fiddes[17] also claimed that hyoid bone injuries never occurred in such deaths.

Fracture of superior horn of thyroid cartilage is the only cartilaginous injury in various studies. According to Polson and Ghee[10] fractures of superior horn of thyroid were found in almost 50% of their cases. Davidson and Marshall[11] showed that 26.7% of their 101 cases showed thyroid cartilage fractures. Eventually VJM DiMaio 12% of his 83 cases of hanging had fractures of thyroid cartilage[16]. Then Camps and Hunt[18] in their study claimed that the fractures of neck structures were directly related to the age of the individual. Luke et al[19], Simonsen J[20] and P.Betz et al[21] related the incidences of the neck injuries to the type of hanging. But Slobadan Nikolic[22] et al contradicted the above fact in their study. Suarez Penaranda[23] et al in their series of 228 cases correlated the bony and cartilaginous injuries to the gender and claimed that there were no correlation between the side of the fracture and the location of the knot. Bowen[24] in his retrospective series of 201 cases did not report any such injuries at all.

CONCLUSION

The present study shows that unilateral fracture in greater cornu of hyoid bone occurred in 6 out of 100 cases (6%). Hyoid bone fractures occurred exclusively in males who were aged more than 30 years of age. The hyoid bone fractures occurred only in complete type of hanging.

In our study fracture of the superior horn of thyroid cartilages were the only cartilaginous injuries found in 39 out of 100 cases (39%). Among these 39 cases 33 had unilateral and 6 cases had bilateral superior horn fractures. Thyroid cartilage fractures occurred more in males and exclusively in those victims who had a typical and complete type of hanging. The incidence of fracture of thyroid cartilage increased in elderly victims.

Conflict of Interest: The authors declare that there is no conflict of interest in this study.

Ethical Clearance: This study was cleared by the Institutional ethical committee as a dissertation topic at Delhi University.

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REFERENCES

1. Kocic S, Radovanovic , VAasiljevic D, Milosavljevic M, Dordevic G, Zivanovic S, Sex as suicidal risk factor. Med Pregl 2012; 65(9-10): 415-20.
2. Dogan KH, Demirci S, Gunaydin G,Buken B, Homicide-Suicide in Konya, Turkey between 2000-2007, J For Sci 2010;55(1):110-5.
3. Karbeyaz K, Ayranci O, Balciy, Gunduz.T, Cattle-Caused fatalities in a province of western Turkey1996-2010 autopsy results. J Forensic Sci 2013; 58(3):697-9.
4. Bastia BK, Kar N, A psychological autopsy study

of suicidal hanging from Cuttack, India; Focus of stressful life situations. Arch Suicide Res 2009; 13(1):100-4.

5. James R, Silcocks P, Suicidal hanging in Cardiff a 15 year retrospective study. Forensic Sci Int 1992; 56(2):167-75.
6. Cooke CT, Cadden GA, Margolius KA, Death by hanging in Western Australia. Pathology 1995; 27(3):21-9.
7. Dean DE, Kohler IJ, Sterbenz GC, Gillespe PJ, Gonzanga NS, Bauer IJ, et.al. Observed characteristics of suicidal hanging: a 11 year retrospective review. J Forensic Sci 2012; 57(5): 1226-30.
8. Uzun I, Buyuk Y, Gurpinar K, Suicidal hanging fatalities in Istanbul retrospective analysis of 761 autopsy cases, J Forensic leg Med 2007 Oct:14(7): 406-9.
9. Simonsen J. Patho-anatomic findings in neck structures in asphyxiation due to hanging: a survey of 80 cases. Forensic Sci Int. 1998; 38:83-91.
10. Polson CJ, Gee DJ, Knight B. The essentials of forensic medicine.4th ed. Oxford: Pergamon Press, 1985. P.351-420.
11. Davidson A, Marshall T. Hanging in Northern Ireland- a survey. Med Sci Law 1986; 26(1):23-8.
12. Clement R, Guay JP, Sauvageau A. Fracture of neck structures in suicidal hangings: A retrospective study on contributing variables. Forensic Sci Int. 2011; 207:122-6.
13. Sharma BR, Harish D, Sharma A, Sharma S, Singh H. Injuries to neck structures in deaths due to constriction of neck, with a special reference to hanging. J Forensic Leg Med 2008; 15(5):298-305.
14. Meera Th, Bapin Kumar Singh M. Pattern of neck findings in suicidal hanging – A study in Manipur. JIAFM. 2011; 33(4):352-4.
15. Weintraub CM. Med-leg.J. (Camb.), 21, 209-16.
16. Di Maio VJ, Di Maio D. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001. p. 245-69.
17. Smith SS, Fiddes FS. Forensic medicine – A Textbook for students and practitioners. 10th ed. J&A Churchill; 1955. p. 252-6.
18. F.E.Camps. Gradwohl's Legal Medicine.3rd ed.Bristol: John Wright & Sons ltd.:1976. p. 326-36.
19. Luke JL, Reay DT, Eisele JW,Bonnell HJ. Correlation of circumstances with pathological findings in asphyxia deaths by hanging: A prospective study of 61 cases form Seattle, WA.J Forensic Sci 1985; 30:1140-7.
20. Simonsen J. Patho-anatomic findings in neck structures in asphyxiation due to hanging: a survey of 80 cases. Forensic Sci Int. 1998; 38:83-91.
21. Betz P, Eisenmenger W. Frequency of throat-skeleton fractures in hanging. Am J Forensic Med Pathol 1996; 17:191-3.
22. Nikolic S, Micic J, Atanasijevic T, et al. Analysis of neck injuries in hanging. Am J Forensic Med Pathol. 2003; 24:179-182.
23. Suárez-Peñaranda JM, Alvarez T, Miguéns X, Rodríguez-Calvo MS, de Abajo BL, Cortesão M, Cordeiro C, Vieira DN, Muñoz JI. Characterisation of lesions in hanging deaths. J Forensic Sci. 2008 May; 53(3):720-3.
24. Bowen DAL. Hanging: A review. Forensic Sci Int.1982; 20:247-9.

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Crucial Factors Frequently Overlooked in Deaths due to Hanging: A Prospective Study

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ABSTRACT

For long time forensic investigators have considered Hyoid and laryngeal injuries as important findings in cases of deaths due to strangulation. Further the incidence of injuries to the neck structures in such cases being related to various factors like the age, gender, the type of the hanging based on suspension and the position of the knot are well documented. Our study highlights the impact of other crucial factors which are usually overlooked, but having strong association in the deaths due to strangulations in general and hanging in particular. The study was undertaken in Central Delhi in 100 cases of hanging, where we studied the association between the hyoid and laryngeal fractures with other variables like type of the ligature material used, the number of turns around the neck, the width of the ligature mark, the type of the knot (fixed/running noose), the duration of suspension and the Body Mass Index of the individuals. In our study 42% of cases had hyoid/laryngeal injuries. These injuries were more common in those who had used hard ligature material with more than one turn around their neck and with a broader ligature mark on their neck. Hyoid and laryngeal injuries were also more common in victims who had a running noose and a prolonged duration of suspension.

Keywords: *Hanging, ligature material, Number of turns, width of the ligature mark, type of knot, duration of suspension.*

INTRODUCTION

Death due to suicidal hanging is a major cause of death worldwide[1]. Hanging is a common method to commit suicide in India[2]. Investigating authorities consider the injuries in the hyoid bone and the laryngeal cartilages to be significant findings in such deaths. In our study, factors like the type of the ligature material (soft/hard), number of turns around the neck, the width of the ligature mark, the type of the knot (fixed knot/running noose), the duration of suspension (with evidential Postmortem findings) and the Body Mass Index of the individuals were compared and associated with the hyoid and the laryngeal injuries.

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MATERIALS AND METHOD

This prospective study was done in 100 cases of suicidal hanging at Central Delhi, which were brought to Lady Hardinge Medical College for medico-legal autopsy. Only the cases which satisfied our mandated inclusion criteria i.e. A meticulous inquest with a well documented approximate time since suspension which correlates with the postmortem findings, photographs of the crime scene, an intact ligature material, weight of the body in kilograms and length of the body in centimeters (To calculate the body mass index), were taken up for analysis. A standard autopsy procedure with meticulous examination of neck structures was performed. The nature of the ligature mark (soft/hard ligature), the numbers of turns around the neck made by the ligature, the width of the ligature mark and the body mass index of the individuals were considered. The structured Performa was used for data collection.

RESULTS

1. In our study of 100 cases, the fracture of the greater horn of hyoid bone and fracture of the superior horn of thyroid cartilage were the only injuries found.

2. 42 cases had some injury in their neck structures. 39 cases had only thyroid cartilage fracture, 3 cases had only hyoid bone fracture among which 3 cases had both hyoid bone and thyroid cartilage fractures.

3. 50% of the victims who had used a hard ligature material like ropes, wires had injuries in their hyoid bone and laryngeal cartilages.

4. 46% among those who had multiple wraps around their neck showed some bony and cartilaginous injuries.

5. The incidence of Hyoid and cartilaginous injuries were greatest in those who had a hard ligature with more number of turns around the neck.

6. 56% of those victims who had a broader ligature mark had injuries in their hyoid bone and laryngeal cartilage.

7. Hyoid and laryngeal injuries were present in every two victims who had a running noose around their neck.

DISCUSSION

Incidence of injuries in relation to the type of the ligature material used and its number of turns around the neck:

The nature of ligature materials used was broadly classified into hard and soft type. 52 of them had a soft type of ligature material like blankets, chunni (a long scarf) and sari and 48 of 100 victims had a hard ligature material like nylon ropes, coir ropes, electrical wires and plastic tapes. Chunni was the commonest material used in 33 cases followed by the nylon rope in 29 cases. Similar results were shown by Jayprakash and Sreekumari[3], Sharma et al.[4]

Regarding the number of turns around the neck, 61 of 100 victims in our study group had only a single turn and 39 of them had more than one turn of ligature material around their neck. (Table. 1)

Table No 1: Incidence of fractures with respect to the Ligature material and the number of turns

S.No.	Type of ligature and no. of turns.	Total no. of cases.	No. of cases with bony or cartilaginous injury.
1.	Hard ligature with single turn.	21	9
2.	Hard ligature with more than one turn.	27	15
3.	Soft ligature with a single turn.	40	15
4.	Soft ligature with more than one turn.	12	3
Total		**100**	**42**

Conspicuously the incidence of fracture was highest in those who had a hard ligature with more than one turn around their neck. Similar results were reported by Sharma et al, that injuries were more frequent in victims who had tough materials as the ligature[5]. But Luke et al. in their study reported that there was no association of the fractures with the composition and number of wraps around the neck[6]. Paparo and Siegel in their study also did not find that the type of ligature had any role on the incidence of the fractures[7].

Incidence of fractures with respect to the width of the ligature mark:

The ligature mark was measured in all the cases. Based on the width of the ligature mark all the cases were grouped into narrow, medium and broad ligature marks. 18 cases had narrow ligature mark (<2cm), 38 cases had medium ligature mark (2-4cm) and 44 cases had broad ligature mark (>4cm). The incidence of fracture with respect to the width of the ligature mark is as given in the Table 2.

Table No 2: Incidence of fractures with respect to the width of the ligature mark

S.No.	Width of the ligature mark	Total no. of cases	No. of cases with bony or cartilaginous injury.
1.	Narrow (<2cm)	18	6
2.	Medium (2-4cm)	38	11
3.	Broad (>4cm)	44	25
Total		100	42

In our study 56% among those victims who had broader ligature mark had neck injuries. Similar results were shown by G.Feigin in his study and he related the neck injuries directly to the width of the ligature mark[8]. Luke et al. in their study did not derive any association with the width of the ligature mark[6].

Incidence of fractures with respect to the type of the knot:

87 cases had a fixed knot and 13 had a running noose around their neck. 35 cases out of 87 and 7 out of 13 cases had injuries in their neck bony and cartilaginous structures. Thus the incidence of the neck injuries was higher in victims who had a running noose (Table No. 3). Similar results were shown by Sharma BR et al. in their study[4].

Table No 3: Incidence of fractures with respect to the type of the knot:

S.No	Type of knot	No. of cases	No. of cases with bony and cartilaginous injury.
1.	Fixed knot	87	35
2.	Running noose	13	7
		100	42

Incidence of fractures with respect to the Duration of suspension:

An approximate duration of suspension was estimated from postmortem changes and the police inquest as mandated in our inclusion criteria. There were 24 victims who were suspended for 0-2 hours, 36 were suspended from 2-6 hours and 39 were suspended for more than 6 hours (Table No. 4).

Table No 4: Incidence of fractures with respect to the Duration of suspension:

S.No.	Duration of suspension (hours).	No. of cases	No. of cases with bony and cartilaginous injury.
1.	0-2	24	3
2.	2-6	37	9
3.	>6	39	30
Total		100	42

The study showed that the incidence of the fracture of the hyoid and the thyroid cartilage increased proportionately with the prolonged duration of suspension. Similar results were claimed in the study by I Morild which showed that higher frequencies of fractures were found in longer suspension time[9].

Frequency of the fractures with respect to the Body mass index of the victim:

The weight and length of the corpse was measured as the part of the autopsy procedure. BMI was calculated with the standard formula for all the victims (Weight in kg/Height in m^2). The BMI of the entire study group fell within the normal narrow range with an average of 23.5 kg/m^2 among males and 21.6 kg/m^2 among females[10]. Hence it was difficult to derive a definite cut-off point to relate the body mass index with the frequency of the neck injuries. R Clement et al. in their study related that the incidence of fractures increases with the weight of the individuals[11].

CONCLUSION

In our prospective study it was apparent that, the injuries to the hyoid bone and the neck cartilage

occurred more in the victims who used a hard ligature material with more number of turns around their neck with a broader ligature mark and a prolonged duration of suspension. Hence it is recommended that these factors which are usually overlooked may also be evaluated along with the other variables like the age, gender, type of hanging, location of the knot to make the forensic investigation complete. We have prepared a pro-forma containing checklist that can be used before conducting autopsy, so that the frequently overlooked factors can be avoided (Table No 5)

1. Name :
2. Age :
3. Gender:
4. Brief history:
5. Place of death:
6. When the person was last seen alive:
7. Time of the last meal:
8. Time of his last urination:
9. Time of recovery:
10. Time since suspension (Based on Post mortem findings):
11. Type of suspension (Complete/Partial):
12. Type of the knot (Fixed/Running noose):
13. Position of the knot (On the nape of the neck/ side of the neck):
14. Type of the ligature material (Hard/Soft):
15. Distance of the drop:
16. Number of turns around the neck (Single/more than one):
17. Width of the ligature mark on the neck (<2cm/ 2-4 cm/>4cm):
18. Length of body (cm):
19. Weight of the body (kg):
20. BMI (Weight in KGs/Height in M^2):

Table No:5 Checklist in hanging cases

Conflict of Interest: The authors declare that there is no conflict of interest in this study.

Ethical Clearance: This study was cleared by the Institutional ethical committee prior to MD Dissertation Thesis at Delhi University.

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REFERENCES

1. Mohanty S, Sahu G, Mohanty MK. Suicide in India- a four year retrospective study. J Forensic Leg Med. 2007; 14(2): 185-9.
2. Ambade VN, Keoliya AN, Wankhede AG. Availability of means of suicides. Int JMed Toxicol Leg Med 2012:14:83-9.
3. Jayaprakash S, Sreekumari K. Pattern of injuries to neck structures in hanging- an autopsy study. Am J Forensic Med Pathol 2012 Dec; 33(4):395-9.
4. Sharma BR, Harish D, Sharma A, Sharma S, Singh H. Injuries to neck structures in deaths due to constriction of neck, with a special reference to hanging. J Forensic Legal Med 2008 Jul: 15(5); 298-305.
5. B.R.Sharma, V.P.Sing, D.Harish, Neck structure injuries in hanging: Comparing retrospective and prospective studies. Med. Sci. Law. 2005; 45:321-30.
6. Luke JL, Reay DT, Eisele JW,Bonnell HJ. Correlation of circumstances with pathological findings in asphyxia deaths by hanging: A prospective study of 61 cases form Seattle, WA.J Forensic Sci 1985; 30:1140-7.
7. G.P.Paparo. H.Seigel, Neck markings and fractures in suicidal hangings, J Forensic Sci. Int. 1984; 24: 27-35.
8. G.Feigin, Forensic of neck organ fractures in hanging. Am. J. Forensic Med.Pathol. 1999; 20: 128-30.
9. Morild I. Fractures of neck structures in suicidal hanging. Med Sci Law 1996; 36:80-4.
10. WHO Expert Consultation. Appropriate body mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004; 363: 157-63.
11. Clement R, Guay JP, Sauvageau A. Fracture of neck structures in suicidal hangings: A retrospective study on contributing variables. Forensic Sci Int. 2011; 207:122-6.

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Stroke education focusing on recognition and response to decrease pre-hospital delay in India: Need of the hour to save hours

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ABSTRACT

Background: Pre-hospital delays contribute to late arrival for acute ischemic stroke (AIS) treatment in the emergency department and are an important barrier preventing people from receiving thrombolytic therapy. This study looked into the factors causing pre-hospital delay in a tertiary care centre in South India with the aim of identifying measures to decrease pre-hospital delay.

Methods: Consecutive cases of AIS from October 2018 to June 2019 were included. A structured questionnaire was used to collect data regarding demographic details, presenting symptoms, time of stroke onset and arrival, mode of transport and knowledge about stroke and thrombolysis. Pre-hospital delay was defined as $>$ 4 h (Considering our door-to-needle time of 30 min) between stroke onset or the time point of wake-up and the time of arrival.

Results: Overall, 505 patients were enrolled. Prehospital delay was observed in 352patients (69.7%). The median pre-hospital delay was 9.5 h. In the multivariable analysis, the odds of early arrival were increased in patients who perceived the initial symptoms as serious (OR: 45.57; 95%CI, 11.43–181.63), those with prior knowledge of stroke symptoms (OR: 4.64; 95% CI, 1.86–11.55), those using ambulance services (OR: 3.11; 95% CI, 1.39–6.95), and those developing hemiparesis (OR: 2.50; 95% CI, 1.25–4.97). The odds of early arrival were lower in those visiting their family doctor after the stroke (OR: 0.17; 95%CI, 0.05–0.51).

Conclusions: Modifiable factors related to stroke recognition and response behavior continue to cause significant pre-hospital delay in India. There is an immediate need for a strong education campaign focusing on stroke specific knowledge to decrease pre-hospital delay.

1. Introduction

India is a low middle-income country with a cumulative incidence of stroke ranging from 105 to 152/100,000 persons per year which is higher than that of high income countries [1]. We have an estimated adjusted prevalence rates of between 334 and 424 strokes per 100,000 persons in urban areas and between 84 and 262 strokes per 100,000 persons in rural areas [2]. When compared to the Western countries, Asian countries have greater mortality and morbidity from stroke than from coronary heart disease due to a higher prevalence of hypertension and a lower level of serum total cholesterol [3].

Many randomized and placebo-controlled multicentre studies have clearly shown that intravenous thrombolysis is an effective treatment for acute ischemic stroke (AIS) [4–6]. Thrombolysis is generally performed within 3·0 h to 4.5 h after the onset of symptoms in AIS and thus its effectiveness could be limited by this narrow therapeutic time window. The problem of underuse of thrombolysis is common in many countries as patients come late for consideration of this treatment resulting in pre-hospital delay (PHD). In a developing country scenario like ours, hurdles can be expected in the efforts to improve PHD. First, very little robust data is available addressing this issue in India [7–10]. Second, very few corrective measures would have been possible since India spends only 3.66% of its Gross Domestic Product (GDP) on healthcare [11].

We initiated this single centre study and aimed to look into the reasons of delayed arrival of patients with acute ischemic stroke and identify measures to decrease the delay.

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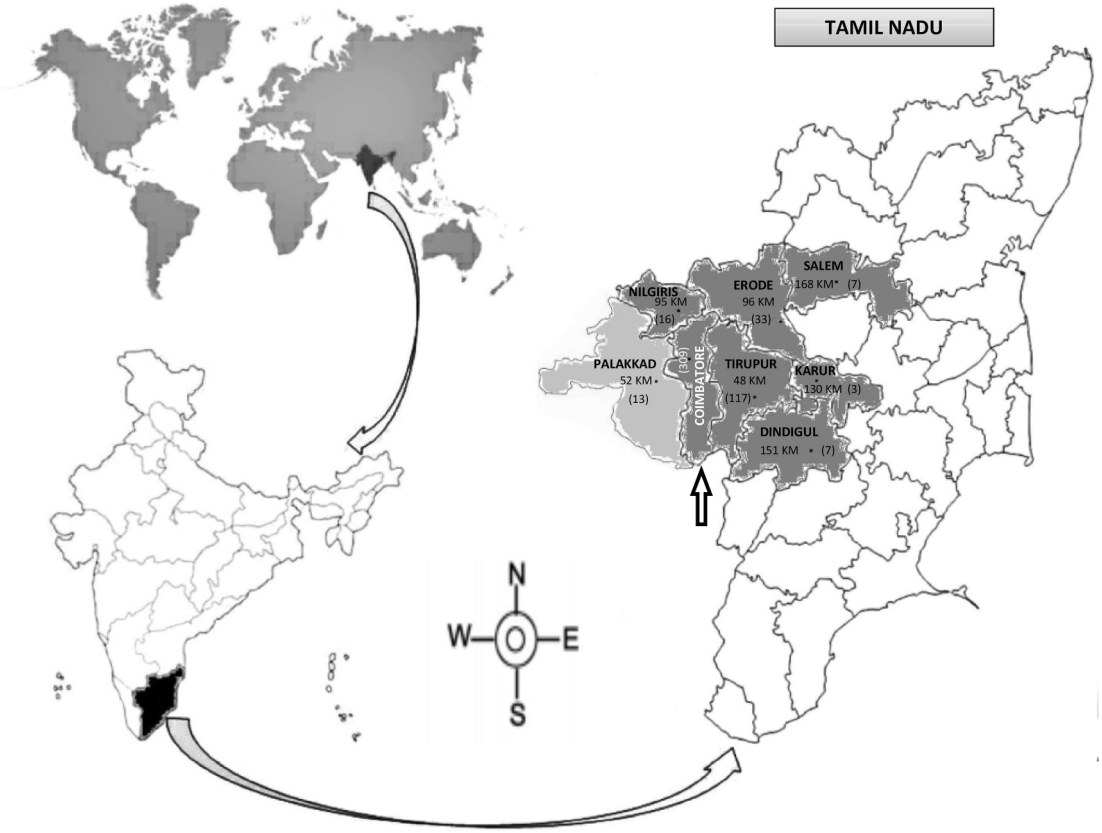


Fig. 1. Location of the study (arrow) and the distribution of patients with the distance to our centre in kilometers.

2. Materials and methods

2.1. Setting

This study was conducted by our institute, PSG Institute of Medical Sciences and research, located at Coimbatore, Tamil Nadu. Coimbatore is a district in the state of Tamil Nadu occupying its western border with the neighboring state of Kerala. (Fig. 1) Coimbatore caters to around 3.5 million people which includes the local population and those from the neighboring districts of Tamil Nadu as well as the Palghat district of Kerala.

2.2. Standard protocol approvals, registrations, and patient consents

The institutional ethics committee approved the study protocol. Written informed consent was obtained from all the participants in the study. Data not published within the article will be shared by request from any qualified investigator.

2.3. Patients

All the patients who came to our Emergency medicine department (EMD) presenting with the symptoms of AIS between October 2018 and June 2019 were included in the study. All hemorrhagic and venous infarcts were excluded.

2.4. Data collection

The patients were subjected to detailed clinical examination on arrival by one of three on-duty neurologists enrolling patients into the study. The time when the patient noted the first neurological symptom of stroke was considered the time of onset. The time the patient was examined by a physician at the EMD (available round the clock in the EMD and hence no physician delays) was considered the time of arrival. The time gap between the time of onset and the time of arrival was taken as the onset-to-door time (OTD). In wake-up strokes, the time of awakening was considered the time of onset (last seen normal was not taken since the aim was to look into the pre-hospital delay) since that represents the time when one could possibly seek medical attention. The OTD time was categorized into $<$ 1 h, 1–3 h, 3–4 h, 4–6 h, 6–24 h and $>$ 24 h. Those who arrived within 4 h of the stroke onset were considered to have come within the window period (WWP) and the rest out of window period (OWP). The time of 4 h was taken considering the door-to-needle time of 30 min in our hospital. This treatment time is made possible by the dedicated stroke team aided by a dedicated CAT scanner situated close to the emergency room. A structured questionnaire was then completed by interviewing either the patient or the caregiver. This questionnaire was designed after reviewing similar studies and following consultations with the medical and neurological societies (Supplement 1) Patients were interviewed in the hospital wards by the three clinical pharmacists (SK, NS & AAS) who underwent an orientation to the questionnaire for 2 weeks. In case of difficulties caused by neurological deficits, the caregivers were interviewed. The interview was conducted in the local Tamil language within 24 h of admission to minimize the possibility of stroke education happening during the stay in the hospital. Except for some instances of late arrival in the night and lack of caregiver availability, the interview was completed within the first few hours of arrival.

The questionnaire had queries addressing various demographic details including age, gender, urban or non-urban living and whether they stay with family or friends. Education was categorized into those with college education ($>$12th) and those without ($\leq 12^{th}$). Information regarding prior knowledge of stroke symptoms and thrombolysis along with details of the mode of transport of the patient to our hospital was collected. Whether they visited their family doctor or the local hospital before coming to our centre was noted. Knowledge of stroke symptoms

Table 1
Demographic and clinical variables of 505 patients presenting with acute ischemic stroke.

Age (years)	60.76 ± 13.71 (28–85)[a]
≤40 years	82 (16.2)[b]
>40 years	423 (83.8)[b]
Gender	
Male	345 (68.3)[b]
Female	160 (31.7)[b]
Mode of living	
Family	489 (96.8)[b]
Friends	16 (3.2)[b]
Place of residence	
Urban	223 (44.2)[b]
Non - urban	282 (55.8)[b]
Distance from hospital	
≤20 km	305 (60.4)[b]
>20 km	200 (39.6)[b]
Education	
≤12th grade	389 (77.0)[b]
>12th grade	116 (23.0)[b]
Stroke onset time	
Night (8 pm- 8 am)	145 (28.7)[b]
Day (8 am – 8 pm)	360 (71.3)[b]
Visit to family doctor/nearby hospital	
Yes	154 (30.5)[b]
No	351 (69.5)[b]
Mode of transport	
Ambulance	279 (55.2)[b]
Private vehicles	226 (44.8)[b]
Time of arrival	
Within window period	153 (30.3)[b]
Out of window period	352 (69.7)[b]
Previous history of stroke	
Yes	90 (17.8)[b]
No	415 (82.2)[b]
Family history of stroke	
Yes	65 (12.9)[b]
No	440 (87.1)[b]
Circulation of brain involved	
Anterior	453 (89.7)[b]
Posterior	52 (10.3)[b]
NIHSS	8.72 ± 3.92 (2–26)[a]
≤15	482 (95.5)[b]
>15	23 (4.5)[b]

a = Mean ± SD (range).
b = N(%).

was assessed based on whether the patient could attribute the presenting symptom to stroke. Whether they perceived the initial symptoms as serious requiring urgent care was asked. The presence of previous stroke and family history of stroke was enquired into. The National Institute of Health Stroke Scale (NIHSS) was used to document the stroke severity. The stroke onset time was divided into day (8am–8 pm) and night (8 pm–8am). The circulation of brain, anterior or posterior which was predominantly affected, was noted with imaging assistance. We assumed that people staying within a 20-km radius from the hospital would be able to reach within the 4-hour window period and categorized them into groups staying within or beyond 20 km from the hospital. It takes more than an hour to cover the distance of 20 km in the busy Indian roads. This doubles during peak hours. Details of the clinical presentation including the various risk factors and clinical features at presentation were also noted.

2.5. Statistical analysis

Statistical package of social sciences (SPSS) version 24 was used to analyze the data. Descriptive statistics, mean and standard deviation were calculated for continuous variables whereas frequency distribution and percentage were used for categorical or ordinal variables. The variables were compared between people who came WWP and OWP using the Chi-square test to evaluate the statistical significance of associations. Statistical significance was set at $P < 0.05$. The significant variables identified by univariate analysis were further subjected to binary logistic regression analysis to identify significant predictors of prehospital delay.

3. Results

There were 505 patients who presented with AIS during the study period of six months. No patient opted out of the study. Demographic data of the patients is given in Table 1. A total of 309 patients (61.2%) were from Coimbatore. (Fig. 1**)** Majority of the remaining (163 out of 505 = 32.3%) was from the neighboring districts of Tirupur, Erode and Palghat from where the travelling time to our hospital can vary between 1 and 1.5 h. Ambulance services were utilized by 279 patients (55.2%) whereas the remaining came in private vehicles.

Overall 153 out of 505 patients came WWP of four hours (30.3%) whereas the remaining 352 (69.7%) came OWP. The mean time to reach the hospital after stroke onset was 14.6 h (median 9.5 h, range 30 min-36 h). The OTD of our patients is depicted in Fig. 2. Around 154 (30.5%) patients visited a nearby clinic or hospital before reaching our hospital of which 110 (21.7%) had done so within the window period of four hours. Only 22 (4.3%) of them could finally reach our centre WWP.

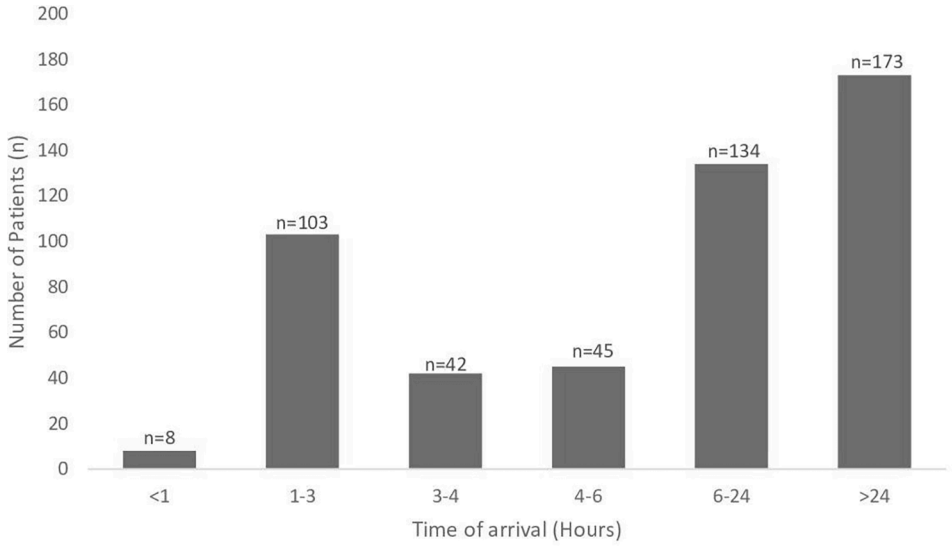


Fig. 2. Graph depicting the onset-to-door time of our study population.

Table 2
Comparison of variables between patients (n = 505) presenting within and out of window period. N (%).

Variable	Within window period (n = 153)	Out of window period (n = 352)	p value	Variable	Within window period (n = 153)	Out of window period (n = 352)	p value
Demographic & clinical factors				**Family history of stroke**			
				Yes			
					6 (3.9)	59 (16.8)	0.000
Age				**Prior Knowledge of symptoms**			
≤40 years		53 (15.1)	NS				
>40 years	29 (19.0)	299 (84.9)		Present	102 (66.7)	44 (12.5)	0.000
	124 (81.0)			Absent	51 (33.3)	308 (87.5)	
Gender				**Prior Knowledge of thrombolysis**			
Male	109 (71.2)	236 (67.0)	NS				
Female	44 (28.8)	116 (33.0)		Yes	78 (51.0)	14 (4.0)	0.000
Mode of living				No	75 (49.0)	338 (96.0)	
Family	145 (94.8)	344 (97.7)	NS	**Perceived symptoms as serious**			
Friends	8 (5.2)	8 (2.3)					
Place of residence				Yes	150 (98.0)	159 (45.2)	0.000
Urban	109 (71.2)	114 (32.4)	0.000	No	3 (2.0)	193 (54.8)	
Non-urban	44 (28.8)	238 (67.6)					
Distance				**Mode of transport**			
≤20 km	119 (77.7)	186 (52.8)	0.000	Ambulance	130 (85.0)	149 (42.3)	0.000
>20 km	34 (22.3)	166 (47.2)		Private vehicle	23 (15.0)	203 (57.7)	
Education				**NIHSS Score**			
≤12 grade	84 (54.9)	305 (86.6)	0.000	0 – 15	135 (88.2)	347 (98.6)	0.000
>12 grade	69 (45.1)	47 (13.4)		16–42	18 (11.8)	5 (1.4)	
Visit to family doctor				**Risk factors**			
Yes	23 (15.0)	131 (37.2)	0.000				
No	130 (85.0)	221 (62.8)		Diabetes mellitus	68 (44.4)	155 (44.0)	NS
Stroke onset time				Hypertension	103 (67.3)	201 (57.1)	0.031
Night (8 pm- 8 am)	44 (28.8)	101 (28.7)	NS	Dyslipidemia	32 (20.9)	87 (24.7)	NS
Day (8 am – 8 pm)	109 (71.2)	251 (71.3)		Coronary artery disease	17 (11.1)	42 (11.9)	NS
Predominant circulation involved				**Stroke symptoms**			
Anterior	151(98.7)	302(85.7)	0.000				
Posterior	2(1.3)	50(14.3)		Hemiparesis	97 (63.4)	183 (52.0)	0.016
				Facial Deviation	102 (66.7)	49 (13.9)	0.000
Previous history of stroke				Loss of consciousness	53 (34.6)	120 (34.1)	NS
				Speech disturbances	87 (56.9)	148 (42.0)	0.002
Yes	32 (20.9)	58 (16.5)	NS	Visual disturbances	68 (44.4)	155 (44.0)	NS
No	121 (79.1)	294 (83.5)		Vertigo	2 (1.3)	44 (12.5)	0.000
				Headache	104 (68.0)	200 (56.8)	0.019
				Gait disturbances	105 (68.6)	203 (57.7)	0.020
				Seizures at stroke onset	1 (0.7)	22 (6.3)	0.006

The results of univariate analysis are shown in Table 2. In the multivariable analysis, the odds of early arrival were increased in patients who perceived the initial symptoms as serious (OR: 45.57; 95%CI, 11.43–181.63), those with prior knowledge of stroke symptoms (OR: 4.64; 95% CI, 1.86–11.55), those using ambulance services (OR: 3.11; 95% CI, 1.39–6.95), and those developing hemiparesis (OR: 2.50; 95% CI, 1.25–4.97). The odds of early arrival were lower in those visiting their family doctor after the stroke (OR: 0.17; 95%CI, 0.05–0.51).

4. Discussion

Despite India keeping pace with the developed nations in terms of availability of advanced medical facilities, the proportion of our population getting access to thrombolysis remains low. The problem of PHD continues to be a major stumbling block as evidenced by the failure of nearly 70% of our study population to reach before 4 h. Four major modifiable factors and one non-modifiable factor were the major determinants of PHD. The modifiable factors were knowledge about stroke symptoms (stroke recognition), perception of urgency at stroke onset and use of ambulance services which facilitated early arrival and visit to family doctor which resulted in PHD (stroke response behavior). The non-modifiable factor was the development of hemiparesis which facilitated an early arrival.

The use of ambulances for transportation resulted in early arrival of our patients. Fifty five percent of our population were transported in the ambulances of the emergency response services of Tamil Nadu which is comparable to the 38–65% use of Emergency Medical Services (EMS) in the developed nations [12,13]. Use of EMS has been clearly shown to be crucial in reducing PHD [14,15]. From the time of its inception in 1985, EMS services have progressed slowly in India till they made a quantum leap in the last decade [16]. Ambulances were made available on calling a single number 108. The sub-optimal response time and unattended calls along with difficult access to rural areas are some of the issues with our EMS which could explain increased PHD of our patients despite good ambulance utilization. Similarly improper patient response (not calling EMS) and system response (non-availability) could be responsible for some of them resorting to private transport.

Patients who came WWP were more aware of the stroke warning signs and recognized stroke. Prior knowledge about stroke symptoms and thrombolysis has been shown to be an important determinant of early arrival to hospital in various studies [8,17]. In contrast to the knowledge of risk factors, knowledge about warning symptoms of stroke is less worldwide. Only 29% of our study population knew about warning symptoms. Knowledge about thrombolysis, was noted in eighteen percent of our study population which is an improvement over the previously reported knowledge of 0% in a population based study from Tamil Nadu and 2% among stroke patients in South India [18,19]. Of particular interest is the fact that despite this low percentage of patients who understood the signs and symptoms of stroke, the ambulance utilization remained high. This discrepancy is because many people in non-urban areas are necessarily dependent on ambulance services for emergency transport especially in the night. Their response though delayed would still be to call the ambulance.

People who perceived the symptoms as requiring urgent attention

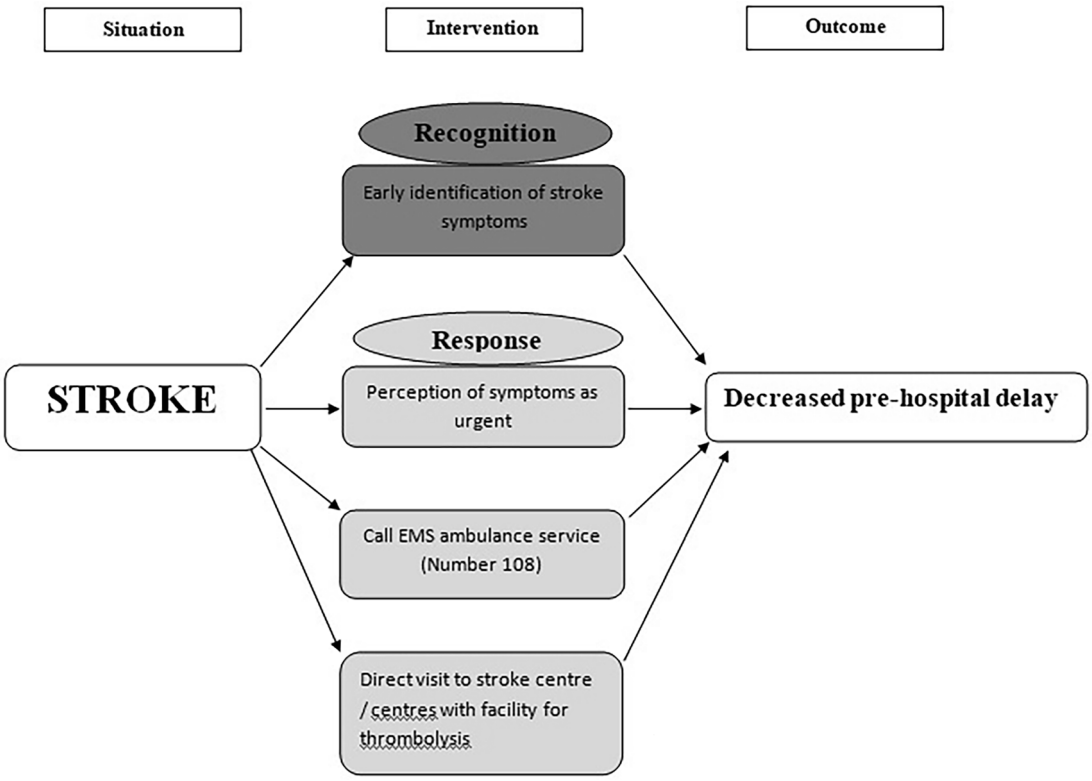


Fig. 3. Schematic diagram showing the suggested interventions to decrease pre-hospital delay.

could avoid PHD. Severity of stroke could be the clinical factor contributing to this perception since people with higher NIHSS score reached early. Even increased stroke awareness may not necessarily translate into a rapid response and early arrival to the hospital [20]. We found that a previous history of stroke was not associated with decreased PHD. Also, knowing somebody in the family with stroke was associated with increased PHD. That neither personal experience of stroke nor knowing somebody with stroke is capable of improving the response demonstrates the dissociation between knowledge and behavior [21]. In sharp contrast to the West, living together also did not help in improving PHD which reflects on the poor response behavior of the caregivers. Education campaigns across the world have failed to increase the emergency response of patients despite improving stroke awareness [22]. One reason could be the fading memory of the public as experienced by Canadian researchers who noticed that the benefits of increased awareness after a television based campaign lasted only for 5 months [23]. Self-efficacy is suggested as an important determinant of behavior and hence promotion of stroke recognition and response self efficacies could also help narrow the recognition-response gap [24].

Visit to the family doctor resulted in significant PHD. Similar observations have been made by other studies [10,20]. The health seeking behavior of our population includes visit to the nearby clinic or the family doctor who would decide the further course of treatment. Eighty eight patients (17.4%) missed out on the treatment opportunity of thrombolysis because of the time lost during local clinical visit. In a study conducted among 109 general practitioners (GPs) of Tamil Nadu, only 24% opted for thrombolysis as the best treatment for AIS and 17% were aware of the therapeutic time window for thrombolysis [25]. Hence there is an urgent need to educate them about stroke and thrombolysis. Since CAT scan facilities are currently available in both the private and Government sectors in rural Tamil Nadu, the local physicians can be encouraged to drip-and-ship the patient. Smartphone-based telestroke services can be arranged in a few clinics to facilitate the process. This was successfully implemented by the state of Himachal Pradesh [26]. Tenecteplase, after its recent approval in India for stroke thrombolysis can become a good option considering its lower cost and ease of administration. The GPs and their staff can discourage patients seeking appointment over phone for stroke symptoms from meeting them and advise them to contact EMS to reach the nearest centre with stroke treatment facilities.

Similar modifiable factors and increased PHD have been observed in the available studies from India [7–10]. They continue to be our bane preventing people from benefitting from thrombolysis. We need a strong public education campaign to address the modifiable factors and improve the PHD. The 'Act FAST' campaign launched in the UK in 2009 also served as a template for similar campaigns in other European countries [27]. The FAST-Television campaign in UK resulted in the remarkable fall in the PHD in 2009 which was sustained till 2013 [28]. Studies published from Europe on PHD after 2010 generally show median delay times of 1–3 h [29–33]. The European nations have benefitted by addressing the modifiable factors and brought down the median PHD below 3 h. The problems which remain are more of the non-modifiable ones like age and ethnicity, stroke related factors like severity, circulation involved, onset time and insurance related issues.

Our study has few limitations. First is the possibility of underestimation of the prevalence of PHD in a hospital based study because of selection bias. Many patients in the rural setup would not have ventured for treatment resulting in infinite delay. Second, is the possibility of recall bias due to unintentional reporting errors from the caregivers of patients.

5. Conclusion

Modifiable factors pertaining to stroke recognition and response behavior continue to cause PHD in India. The need of the hour is a strong patient education campaign to help recognize stroke and respond quickly and appropriately. We suggest the following interventions to improve recognition and response behavior as outlined in Fig. 3.

1. Patient education regarding warning symptoms of stroke.

2. The availability of dedicated ambulance services especially in the rural areas should be strengthened and the public should be informed to contact 108. People operating EMS services should also be educated periodically about the need to respond immediately in cases of stroke.
3. General practitioners should avoid appointments for people with symptoms of stroke. They should inform them about the nearest center with facilities and encourage them to call EMS.
4. Target practical aspects to facilitate a positive behavior in case of an acute stroke. The need for immediate attention irrespective of the perceived urgency based on stroke severity should be emphasized.

6. Disclosures

Dr. Josy Vallippalam reports no disclosures
Dr. Saloni Krishna reports no disclosures
Dr. Nitya Suresh reports no disclosures
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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.inat.2021.101309.

References

[1] S. Kamalakannan, A.S.V. Gudlavalleti, V.S.M. Gudlavalleti, S. Goenka, H. Kuper, Incidence & prevalence of stroke in India: a systematic review, Indian J. Med. Res. 146 (2) (2017) 175–185.
[2] T.K. Banerjee, S.K. Das, Epidemiology of stroke in India, Neurol. Asia 11 (2006) 1–4.
[3] H. Ueshima, A. Sekikawa, K. Miura, T.C. Turin, N. Takashima, Y. Kita, M. Watanabe, A. Kadota, N. Okuda, T. Kadowaki, Y. Nakamura, T. Okamura, Cardiovascular disease and risk factors in Asia: a selected review, Circulation 118 (25) (2008) 2702–2709.
[4] The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke, N. Engl. J. Med. 333 (1995) 1581–1587.
[5] W. Hacke, M. Kaste, E. Bluhmki, M. Brozman, A. Dávalos, D. Guidetti, V. Larrue, K. R. Lees, Z. Medeghri, T. Machnig, D. Schneider, R. von Kummer, N. Wahlgren, D. Toni, for the ECASS Investigators. Thrombolysis with alteplase 3 to 4·5 hours after acute ischemic stroke, N. Engl. J. Med. 359 (13) (2008) 1317–1329.
[6] K.R. Lees, E. Bluhmki, R. von Kummer, T.G. Brott, D. Toni, J.C. Grotta, G.W. Albers, M. Kaste, J.R. Marler, S.A. Hamilton, B.C. Tilley, S.M. Davis, G.A. Donnan, W. Hacke, for the ECASS, ATLANTIS, NINDS, and EPITHET rt-PA Study Group Investigators. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials, Lancet 375 (9727) (2010) 1695–1703.
[7] A.K. Mishra, R. Iyadurai, Prehospital and hospital delays for stroke patients treated with thrombolysis: access to health care facility – still a bottle neck in stroke care in developing nation, Austral. Emerg. Care 22 (4) (2019) 227–228.
[8] N. Arulprakash, M. Umaiorubahan, Causes of delayed arrival with acute ischemic stroke beyond the window period of thrombolysis, J. Family Med. Prim. Care 7 (6) (2018) 1248, https://doi.org/10.4103/jfmpc.jfmpc_122_18.
[9] SijuV Abraham, SVimal Krishnan, F. Thaha, JayarajMymbilly Balakrishnan, T. Thomas, BabuUrumese Palatty, Factors delaying management of acute stroke: an Indian scenario, Int. J. Crit. Illn. Inj. Sci. 7 (4) (2017) 224, https://doi.org/10.4103/IJCIIS.IJCIIS_20_17.
[10] V.V. Ashraf, A.S. Girija, M. Maneesh, R. Praveenkumar, K. Saifudheen, Factors delaying hospital arrival of patients with acute stroke, Ann. Indian Acad. Neurol. 18 (2) (2015) 162, https://doi.org/10.4103/0972-2327.150627.
[11] "Health expenditure, total (% of GDP)". World Bank. Retrieved 5th April 2020 https://data.worldbank.org/indicator/SH.XPD.CHEX.GD.ZS.
[12] J. Kwan, P. Hand, P. Sandercock, A systematic review of barriers to delivery of thrombolysis for acute stroke, Age Ageing 33 (2004) 116–121.
[13] F. Harraf, A.K. Sharma, M.M. Brown, K.R. Lees, R.I. Vass, Kalra L.A multicentre observational study of presentation and early assessment of acute stroke, BMJ 325 (2002) 17.
[14] J.L. Saver, E.E. Smith, G.C. Fonarow, M.J. Reeves, X. Zhao, D.M. Olson, L. H. Schwamm, on behalf of the GWTG-Stroke Steering Committee and Investigators. The "golden hour" and acute brain ischemia: presenting features and lytic therapy in >30 000 patients arriving within 60 minutes of stroke onset, Stroke 41 (7) (2010) 1431–1439.
[15] D.L. Morris, W. Rosamond, K. Madden, C. Schultz, S. Hamilton, Prehospital and emergency department delays after acute stroke: the Genentech Stroke Presentation Survey, Stroke 31 (11) (2000) 2585–2590.
[16] S. Das, R. Desai, Emergence of EMS in India: India's budding EMS infrastructure is a model for the region despite challenges moving forward, JEMS 42 (4) (2017 Apr) 55–61.
[17] B. Jiang, X. Ru, H. Sun, H. Liu, D. Sun, Y. Liu, J. Huang, L.i. He, W. Wang, Pre-hospital delay and its associated factors in first-ever stroke registered in communities from three cities in China, Sci. Rep. 6 (1) (2016), https://doi.org/10.1038/srep29795.
[18] K. Lakshmi, K. Viswanath, C.E. Ze, D. Sam Marconi, S.N. David, R. Isaac, Stroke care challenges in rural India: awareness of causes, preventive measures and treatment options of stroke among the rural communities, Ind. J. Comm. Health 26 (4) (2014) 349–355.
[19] S. Badachi, T. Mathew, A. Prabhu, R. Nadig, G.R. Sarma, Hurdles in stroke thrombolysis: Experience from 100 consecutive ischemic stroke patients, Ann. Indian Acad. Neurol. 18 (4) (2015) 415–418.
[20] J. Fladt, N. Meier, S. Thilemann, A. Polymeris, C. Traenka, D.J. Seiffge, R. Sutter, N. Peters, H. Gensicke, B. Flückiger, K. de Hoogh, N. Künzli, B. Bringolf-Isler, L. H. Bonati, S.T. Engelter, P.A. Lyrer, G.M. De Marchis, Reasons for prehospital delay in acute ischemic stroke, Am. Heart Assoc. 8 (20) (2019), https://doi.org/10.1161/JAHA.119.013101.
[21] M.A. Ritter, S. Brach, A. Rogalewski, R. Dittrich, R. Dziewas, B. Weltermann, P. U. Heuschmann, D.G. Nabavi, Discrepancy between theoretical knowledge and real action in acute stroke: self-assessment as an important predictor of time to admission, Neurol. Res. 29 (5) (2007) 476–479.
[22] J. Lecouturier, H. Rodgers, M.J. Murtagh, M. White, G.A. Ford, R.G. Thomson, Systematic review of mass media interventions designed to improve public recognition of stroke symptoms, emergency response and early treatment, BMC Public Health 10 (2010) 784.
[23] C. Hodgson, P. Lindsay, F. Rubini, Can mass media influence emergency department visits for stroke? Stroke 38 (7) (2007) 2115–2122.
[24] L.O. Wilhelm, P. Gellert, M. White, V. Araujo-Soares, G.A. Ford, J.E. Mackintosh, H. Rodgers, F.F. Sniehotta, R.G. Thomson, S.U. Dombrowski, The recognition-response gap in acute stroke: examining the relationship between stroke recognition and response in a general population survey, J. Stroke Cerebrovasc. Dis. 29 (2) (2020) 104499, https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.104499.
[25] Aaron S, Alexander M, Maya T, Mathew V, Goyal M. Treatment of acute ischemic stroke: Awareness among general practitioners. Neurol. India 58, 441–442.
[26] S. Sharma, M.V. Padma, A. Bhardwaj, A. Sharma, N. Sawal, S. Thakur, Telestroke in resource-poor developing country model, Neurol. India 64 (5) (2016) 934, https://doi.org/10.4103/0028-3886.190243.
[27] J. Harbison, O. Hossain, D. Jenkinson, J. Davis, S.J. Louw, G.A. Ford, Diagnostic accuracy of stroke referrals from primary care, emergency room physicians, and ambulance staff using the face arm speech test, Stroke 34 (1) (2003) 71–76.
[28] F.J. Wolters, N.L.M. Paul, L. Li, P.M. Rothwell, Oxford Vascular Study. Sustained impact of UK FAST-test public education on response to stroke: a population-based time-series study, Int. J. Stroke 10 (7) (2015) 1108–1114.
[29] R. Soto-Cámara, J. González-Santos, J. González-Bernal, A. Martín-Santidrian, E. Cubo, J.M. Trejo-Gabriel-Galán, Factors associated with shortening of prehospital delay among patients with acute ischemic stroke, J. Clin. Med. 8 (10) (2019) 1712.
[30] P. Dimitriou, K. Tziomalos, K. Christou, S. Kostaki, S.-M. Angelopoulou, M. Papagianni, E. Ztriva, G. Chatzopoulos, C. Savopoulos, A.I. Hatzitolios, Factors associated with delayed presentation at the emergency department in patients with acute ischemicstroke, Brain Inj. 33 (9) (2019) 1257–1261.
[31] Simonsen, Morten Andresen, Lene Michelsenet, et al. Evaluation of pre-hospital transport time of stroke patients to thrombolytic treatment. Scand. J. Trauma Resuscit. Emerg. Med. 22 (2014) 65.
[32] K.W. Faiz, A. Sundseth, B. Thommessen, O.M. Rønning, Factors related to decision delay in acute stroke, J. Stroke Cerebrovasc. Dis. 23 (3) (2014) 534–539.
[33] S. Vidale, E. Beghi, F. Gerardi, C. De Piazza, S. Proserpio, M. Arnaboldi, G. Bezzi, G. Bono, G. Grampa, M. Guidotti, P. Perrone, D. Porazzi, D. Zarcone, A. Zoli, E. Agostoni, Time to Hospital Admission and Start of Treatment in Patients with Ischemic Stroke in Northern Italy and Predictors of Delay, Eur. Neurol. 70 (5-6) (2013) 349–355.

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Original Article

Profile of hematological abnormalities and its correlation with absolute CD4 count and human immunodeficiency virus viral load in human immunodeficiency virus-infected patients in a tertiary care hospital

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Abstract

Background: A variety of hematological manifestations are seen at every stage of human immunodeficiency virus (HIV) infection, and they often pose a great challenge in the comprehensive management of acquired immunodeficiency syndrome. Anemia is the most common hematological abnormality associated with HIV infection. The severity and the incidence of cytopenia are usually correlated with the stage of the disease and underlying immune status if interpreted cautiously, especially if the patient is on regular follow-up. The primary objective of the present study was to understand the spectrum of hematological abnormalities in HIV-infected patients, whereas the secondary objective was to evaluate the correlation of hematological abnormalities with absolute CD4 count and HIV viral load. **Materials and Methods:** The present cross-sectional descriptive study was conducted on 100 patients, aged 18 years and above, diagnosed with HIV infection and confirmed by Western blot or ELISA method. Both inpatients and outpatients at our tertiary care hospital were included in the study. **Results:** Individuals with high viral load and low CD4 count had a higher prevalence of anemia. There was a statistically significant and directly proportionate decrease in the absolute CD4 count as the hemoglobin levels decreased ($P = 0.004$). In the present study, normocytic normochromic blood picture and anemia of chronic disease blood picture were more prevalent among the study participants. Individuals with high viral load and CD4 count <200 cells/mm^3 had a higher rate of occurrence of coinfections. The correlation of absolute neutrophil count and thrombocytopenia with absolute CD4 count and HIV viral load was not statistically significant. **Conclusions:** Complete blood counts and peripheral smear observations were significantly correlated with high HIV viral load and lower absolute CD4 cell counts and therefore can be suggested as economical alternatives for the evaluation of the status of HIV disease stage and its progression.

Key words: Absolute CD4 count, anemia, cytopenia, hematological profile, HIV viral load

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INTRODUCTION

A variety of hematological manifestations are seen at every stage of human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS), and they often pose a great challenge in the comprehensive management of AIDS. Hematological abnormalities and their complications are the most common causes of mortality and morbidity in HIV-infected individuals.[1] They hinder the treatment directed at HIV and the opportunistic infections and malignancies of AIDS. The hematological abnormalities have also been reported to augment the risk of bacterial infections and affect the quality of life.

Anemia is the most common hematological abnormality associated with HIV infection. This, in turn, is attributed to the abnormal cytosine expression and alteration in bone marrow microenvironment.[2] On the other hand, thrombocytopenia occurs by immune-mediated destruction of the platelets, in addition to inadequate platelet production. Other causes of cytopenia in these patients include treatment-related adverse events or secondary to the opportunistic infections or neoplasms or preexisting or coexisting medical issues. HIV-infected patients with cytopenia require bone marrow examination to determine the cause of cytopenia and also to direct appropriate therapy. The severity and the incidence of cytopenia are usually correlated with the stage of the disease.[1,3,4] These manifestations also reflect the underlying immune status if interpreted cautiously, especially if the patient is on regular follow-up. Hematological abnormalities can be the initial presentation of HIV infection. Patients might be asymptomatic due to the HIV infection and the reason for referral to a physician is usually the abnormal blood count or lymphoid disorders. By considering the possibility of HIV infection, there is an opportunity to diagnose and treat the patients earlier and prevent the transmission of infection. Hematological abnormalities might be the direct result of HIV infection or due to secondary infections, neoplasms, and side effects of the therapy. Early diagnosis and treatment for hematological abnormalities are key factors that contribute to reduction in the morbidity and mortality. Despite the availability of numerous reports on HIV in India, the focus on the hematological manifestations appeared to be limited. Most of the available data are from the West, which might not be directly applicable to the Indian population. Hence, the present study was conducted with the primary objective to understand the spectrum of hematological abnormalities in HIV patients among the Indian population, whereas the secondary objective was to evaluate the correlation of hematological abnormalities with absolute CD4 count and HIV viral load.

MATERIALS AND METHODS

The present cross-sectional study was conducted on 100 patients, aged 18 years and above, diagnosed with HIV infection and confirmed by Western blot or ELISA method. Written informed consent was obtained from all the participants who expressed their willingness to participate in the study. Both inpatients and outpatients at the institution (PSG Institute of Medical Sciences and Research) were included in the study. Patients who refused to give consent and did not have a complete profile of investigations were excluded from the study.

All patients were investigated with a complete hemogram that included estimation of hemoglobin level, red cell indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration), red cell distribution width, total leukocyte count, differential leukocyte count, platelet count, reticulocyte count, and peripheral smear examination. Further to determine the cause of hematological abnormality, indirect bilirubin level, serum iron, total iron-binding capacity, fasting serum Vitamin B12, serum folic acid, stool occult blood, upper gastrointestinal endoscopy, colonoscopy, and bone marrow examination were done accordingly.

Statistical analysis

The data were analyzed using Microsoft Excel 2010 and Statistical Package for the Social Sciences version 19.0 (Armonk, NY: IBM Corp.). A predictive value of <0.05 was considered statistically significant.

RESULTS

Complete hemogram

In the present study conducted on 100 patients, 81 patients were treatment naive and 19 were on antiretroviral therapy (ART). Individuals with high viral load and low CD4 count <200 cells/mm^3 had a higher prevalence of anemia. Table 1 shows the comparison of absolute CD4 count and HIV viral load of groups of patients of varying hemoglobin levels. Individuals with high viral load and low CD4 count <200 cells/mm^3 had a higher prevalence of anemia. Severe anemia was noted in about 71.4% of individuals with CD4 count <200 cells/μL, 28.6% among CD4 count 200–500 cells/mm^3, and 26% among CD4 count >500 cells/mm^3 [Table 1]. There was a statistically significant and directly proportionate decrease in the absolute CD4 count as the hemoglobin levels decreased ($P = 0.004$).

Likewise, the comparison of HIV viral load in groups of patients of varying hemoglobin levels showed a statistically significant increase in the viral load as the hemoglobin levels decreased ($P = 0.000$). Severe anemia was noted in about 85.7% of individuals among HIV viral load >1000 copies/ml [Table 1].

In the present study, there were 81 treatment-naive individuals. Hence, the data analysis was done separately

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for the treatment-naive patients. Treatment-naive individuals with high viral load and low CD4 count <200 cells/mm^3 had a higher prevalence of anemia. Severe anemia was noted in about 66.7% of individuals among CD4 count <200 cells/mm^3 and 33.3% among CD4 count 200–500 cells/mm^3. Severe anemia was noted in 83.3% of participants with HIV viral load >1000 copies/ml. Severe anemia was not reported in treatment-naive patients with CD4 count >500 cells/mm^3 and HIV viral load <200 copies/ml [Table 1]. The differences observed in hemoglobin count in treatment-naive patients between different groups of HIV viral load (P = 0.000) and absolute CD4 count (P = 0.008) were statistically significant.

Comparison of platelet count and absolute neutrophil counts in varying categories of absolute CD4 count and HIV viral load showed insignificant associations in both total study population and treatment-naive patients [Tables 2 and 3].

Type of anemia

In the present study, normocytic normochromic blood picture was noted in majority of the patients. Among the types of anemia, anemia of chronic disease blood picture (35.8%) was more prevalent among the study participants. Iron-deficiency and Vitamin B12-deficiency anemia were noted in about 8.6% and 2% of the study participants, respectively. Hemolytic anemia was not observed. Nearly ten patients presented with pancytopenia. Among the individuals on ART, the patients who were on zidovudine-based therapy had normocytic normochromic anemia with macrocytosis.

Bone marrow biopsy

In the current study, bone marrow biopsy was done among five patients. The bone marrow picture in three participants was erythroid hyperplasia with mild dyserythropoiesis, megakaryocytic hyperplasia, and mild increase in plasma cells which were suggestive of HIV infection. In the other two participants, immune thrombocytopenia was observed.

Coinfections

Comparison of occurrence of coinfections in various categories of absolute CD4 count and HIV viral load showed statistically significant differences (P = 0.000). In individuals with high viral load and low CD4 count <200 cells/mm^3, a higher rate of occurrence of coinfections was observed.

Table 1: Comparison of absolute CD4 count and human immunodeficiency virus viral load in groups of patients with varying hemoglobin level

Hemoglobin count	Absolute CD4 count (%)				HIV viral load (%)			
	>500 cells/mm^3	200-500 cells/mm^3	<200 cells/mm^3	*P*	>200 copies/ml	200-1000 copies/ml	<1000 copies/ml	*P*
Treatment naïve (g/dl)								
>12	17 (39.5)	13 (30.2)	13 (30.2)	0.008**	29 (67.4)	1 (2.3)	13 (30.2)	0.000**
10-12	3 (15.8)	3 (15.8)	13 (68.4)		6 (31.6)	0	13 (68.4)	
8-10	0	3 (23.1)	10 (76.9)		0	0	13 (100.0)	
<8	0	2 (33.3)	4 (66.7)		0	1 (16.7)	5 (83.3)	
On HAART (g/dl)								
>12	21 (39.6)	14 (26.4)	18 (34.0)	0.004**	38 (71.7)	1 (1.9)	14 (26.4)	0.000**
10-12	5 (22.7)	4 (18.2)	13 (59.1)		9 (40.9)	0	13 (59.1)	
8-10	0	3 (16.7)	15 (83.3)		1 (5.6)	0	17 (94.4)	
<8	0	2 (28.6)	5 (71.4)		0	1 (14.3)	6 (85.7)	

**P<0.05 is considered to be statistically significant. HAART=Highly active antiretroviral therapy; HIV=Human immunodeficiency virus

Table 2: Comparison of absolute CD4 count and human immunodeficiency virus viral load in groups of patients with varying platelet level

Platelet count	Absolute CD4 count (%)				HIV viral load (%)			
	>500 cells/mm^3	200-500 cells/mm^3	<200 cells/mm^3	*P*	>200 copies/ml	200-1000 copies/ml	<1000 copies/ml	*P*
Treatment naïve (cells/mm^3)								
50,000-100,000	20 (25.0)	21 (26.3)	39 (48.8)	0.595	35 (43.8)	2 (2.5)	43 (53.8)	0.653
20,000-500,000	0	0	0		0	0	0	
<20,000	0	0	1 (100.0)		0	0	1 (100.0)	
On HAART (cells/mm^3)								
50,000-100,000	26 (27.7)	22 (23.4)	46 (48.9)	0.511	47 (50.0)	2 (2.1)	45 (47.9)	0.560
20,000-50,000	0	0 (o)	1 (100.0)		0	0	1 (100.0)	
<20,000	0	1 (20)	4 (80.0)		1 (20)	0	4 (80.0)	

HAART=Highly active antiretroviral therapy; HIV=Human immunodeficiency virus

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Table 3: Comparison of absolute CD4 count and human immunodeficiency virus viral load in groups of patients with varying absolute neutrophil count levels

Absolute neutrophil count	Absolute CD4 count (%)				HIV viral load (%)			
	>500 cells/mm³	200-500 cells/mm³	<200 cells/mm³	*P*	>200 copies/ml	200-1000 copies/ml	<1000 copies/ml	*P*
Treatment naïve (cells/mm³)								
1000-1500	20 (27.0)	20 (27.0)	34 (45.9)	0.345	35 (47.3)	2 (2.7)	37 (50.0)	0.168
500-1000	0	1 (16.7)	5 (83.3)		0	0	6 (100.0)	
<500	0	0	1 (100.0)		0	0	1 (100.0)	
On HAART (cells/mm³)								
1000-1500	26 (28.3)	22 (23.9)	44 (47.8)	0.266	48 (52.2)	2 (2.2)	42 (45.7)	0.069
500-1000	0	1 (16.7)	5 (83.3)		0	0	6 (100.0)	
<500	0	0	2 (100.0)		0	0	2 (100.0)	

HAART=Highly active antiretroviral therapy; HIV=Human immunodeficiency virus

Among the coinfections, pulmonary tuberculosis was predominant and it was about 25% of the total infections. The other coinfections observed were extrapulmonary tuberculosis (11%), central nervous system toxoplasmosis (4 individuals), oral candidiasis (8%), and esophageal candidiasis (5%). Other coinfections (<5%) noted were *Pneumocystis jiroveci* infection, nocardiosis, and herpes simplex infection. Hepatitis B coinfection was noted in about 2% of the total population.

DISCUSSION

Many studies have been conducted across India on HIV manifestations and various aspects have been addressed. However, the focus on the hematological manifestations was very limited. Most of the available data are from the Western studies and may not be directly applicable to the Indian population. Hence, the present study was an attempt to understand the profile of hematological abnormalities in HIV infection in a South Indian population. The study looked into the hematological manifestations and their correlation with viral markers.

HIV is a retrovirus affecting immunity, impairing the immune function, thereby resulting in opportunistic infections and malignancies. The hallmark of HIV infection is decreasing the activity and number of CD4+ T lymphocytes. Furthermore, it impedes other cell lineages and tissues. Cytopenias have been noted even without highly active antiretroviral therapy (HAART) or opportunistic infections and malignancies, proving that HIV infection is directly associated in causing these hematological derangements.

The present study among HIV-infected individuals from South India showed the prevalence of anemia to be 47%. The incidence and severity of anemia, thrombocytopenia, and neutropenia reflect the underlying immune status, if interpreted cautiously. It will be of great benefit, especially if the patient is on regular follow-up. Cytopenias are the leading cause of morbidity and mortality in HIV patients. Hence, it is necessary to identify and treat for hematological abnormalities to reduce the morbidity and mortality.

Studies done worldwide reveal that the prevalence of anemia in HIV-infected persons has been found to be as high as 63%–95%; they also reveal that anemia is more common than thrombocytopenia or leukopenia. In a US-based study, Mildvan *et al*. noted the prevalence of anemia in 9690 HIV-infected individuals and showed that 39.5% (1721) of patients receiving no ART and 35.5% (7252) of patients receiving HAART were anemic. They found that anemia was more prevalent among men and patients with CD4 <200 cells/mm³.[5] Reasons for anemia were found to be decreased production because of associated infections, ART drugs, low erythropoietin levels, or HIV infection *per se*. Furthermore, red blood cell lysis due to autoimmune hemolytic anemia, disseminated intravascular coagulation or thrombotic microangiopathy, and nutritional deficiencies were causes for anemia.[6] The most common reason for anemia in HIV patients is the anemia of chronic disease. In the Multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project, Sullivan *et al*. analyzed cytopenia in 32,867 individuals affected by HIV infection. They showed that anemia occurrence increased with the clinical severity of the disease.[7] About 3% of HIV patients developed anemia at 1-year follow-up, compared to 12% among those who had immunologic AIDS that is CD4 count <200/mm³ and 37% among those with clinical AIDS.[8] Among cytopenia, anemia in an HIV-infected individual was significantly correlated with higher mortality.[1] However, on the contrary, decreased risk of death was noted in those who recovered from anemia. Although the levels of serum erythropoietin may be high in these patients, the response to anemia was blunted compared to those with uncomplicated iron-deficiency anemia of comparable severity.[9]

Drug treatment and the subsequent complications were also the common reasons for anemia among HIV patients. Zidovudine is the most common among the ART drug to cause anemia. It inhibits the *in vitro* erythroid colony

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formation. It does it in a dose-dependent manner.[10] Following zidovudine intake, macrocytosis develops within weeks. Macrocytosis in these patients also acts as a useful marker of drug compliance. It was noted that serum Vitamin B12 levels were often low in HIV-infected patients. However, only few patients presented with true Vitamin B12 deficiency. *Mycobacterium avium* complex, *Mycobacterium tuberculosis*, and *Histoplasma capsulatum* caused anemia in AIDS patients by bone marrow infiltration.

In the current study, anemia was more prevalent in individuals with high HIV viral load and low absolute CD4 count. The present finding was concurrent with numerous studies conducted globally.[5,11,12] Low hemoglobin count was observed in both treatment-naive HIV patients and patients on HAART therapy.[11,12] Low hemoglobin levels were also found to correlate significantly with the increased severity of the disease and poor prognosis as in other studies.[1,3,4,13] Normocytic normochromic anemia was the common finding among the blood picture of HIV patients in the present study and was concurrent with the report of Evans and Scadden.[14] The severity of anemia was significantly correlated with high HIV viral load and low absolute CD4 count. The abovementioned findings were consistent with the study done by Volberding *et al*., who reported that more severe levels of anemia are found among HIV-positive patients presenting with low CD4 counts.[15]

Neutropenia was also found in majority of patients with high viral load and low CD4 count.[16] Among leukopenia, neutropenia was more prevalent in HIV patients, ranging from 10% to 30% at later stages of the disease. HIV infection leads to bone marrow suppression causing decreased granulocyte colony-stimulating factor levels, thereby causing low granulocyte–macrophage lineage. This resulted in leukopenia and neutropenia in HIV patients. Bone marrow suppressive drugs or opportunistic infections might also contribute to leukopenia. Moreover, HIV infection *per se* results in lymphopenia at later stages of disease, causing low CD4+ lymphocytes. In HIV patients, lower values of absolute neutrophil counts were due to granulopoiesis inhibition by the HIV virus itself or by bone marrow infiltration by infections. Leukopenia was also attributed to malignancies, adverse drug reactions, autoimmunity, and hypersplenism.

Various studies from developing nations showed a marked decline in mortality in HIV patients following HAART therapy. An Indian study showed a dramatic decline in mortality from 25 to 5 deaths per 100 person years in HIV patients between 1997 and 2003 after starting HAART therapy.[16] Factors such as older age, baseline low CD4 counts, tuberculosis infection at any point of time, and ART-naive status had significant associations with mortality.

Thrombocytopenia was noted during the early stages of the disease and it was not associated with poor prognosis. Thrombocytopenia in HIV infection is due to immune complex-mediated peripheral destruction and antiplatelet antibodies. Interestingly, thrombocytopenia is noted as the first clinical manifestation in otherwise asymptomatic HIV-infected individuals, whereas neutropenia and anemia were mostly noted in the later stages of HIV disease. The possible mechanisms considered for thrombocytopenia are increased destruction of platelets by immune complexes in circulation which nonspecifically get deposited on the platelets. Furthermore, thrombocytopenia can be attributed to the existence of specific antiplatelet antibodies, as well as direct action on megakaryocytic by HIV *per se* causing ineffective platelet production. The incidence of thrombocytopenia among HIV patients was about 40%, and in 10% of HIV patients, it might be the first sign of AIDS. Thrombocytopenia was related to increased morbidity and mortality, by accelerating the deterioration of CD4 counts and leading to progression to AIDS.

Among all the study participants, anemia was more common than thrombocytopenia or neutropenia. In treatment-naive individuals and individuals on ART, thrombocytopenia and neutropenia were more prevalent among high HIV viral load and low CD4 count <200 cells/mm^3. Immune thrombocytopenic purpura remains the most common cause of low platelet count in HIV patients.[17]

Abnormalities in the bone marrow were noted at various stages of the HIV disease and more during the later stages of the disease. HIV infection in the mesenchymal stem cells of bone marrow leads to various bone marrow abnormalities.[2] HIV infection leads to a histiocytic reaction and further resulting in hemophagocytic syndrome and severe pancytopenia. HIV also causes decrease in progenitor cells in the bone marrow.[18] As per Paradela *et al*.'s study, it was reported that bone marrow defects in HIV-infected individuals mainly showed features of increased cell counts, granulomatous changes, and some amount of dysplasias.[19] Studies conducted *in vitro* showed the direct influence of HIV on mesenchymal cells and hematopoietic cell activity.[2]

India has the third largest HIV population with 2 million people affected, more than 60,000 deaths due to AIDS-related illnesses, and only about 43% receiving proper HAART from health sectors. The government spends a huge sum of its health budget over HIV treatment and prevention. Our study can be considered as a pilot study and should be extrapolated to a larger study with case and control group selected within the sample, for instance with low and high absolute CD4 count and HIV viral load.

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CONCLUSIONS

Most of the available data on HIV infections and hematological profiles are from the Western studies, which might not be directly applicable to the Indian population. The present study looked into the hematological manifestations and their correlation with viral markers of HIV patients from South India. HIV viral load and absolute CD4 count are the most essential biomarkers to identify the stage of HIV disease and its progression. However, these tests are expensive in both developed and developing countries. The results of the present study suggest that complete blood counts and peripheral smear can be economical alternatives to HIV viral load and absolute CD4 cell counts in staging the disease and assessing the prognosis.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, Colebunders R, *et al*. Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. AIDS 1999;13:943-50.
2. Wang L, Mondal D, La Russa VF, Agrawal KC. Suppression of clonogenic potential of human bone marrow mesenchymal stem cells by HIV type 1: Putative role of HIV type 1 tat protein and inflammatory cytokines. AIDS Res Hum Retroviruses 2002;18:917-31.
3. Moyle G. Anaemia in persons with HIV infection: Prognostic marker and contributor to morbidity. AIDS Rev 2002;4:13-20.
4. Mekonnen Y, Dukers NH, Sanders E, Dorigo W, Wolday D, Schaap A, *et al*. Simple markers for initiating antiretroviral therapy among HIV-infected Ethiopians. Ethiop Med J 2003;41 Suppl 1:61-5.
5. Mildvan D, Creagh T, Leitz G; Anemia Prevalence Study Group. Prevalence of anemia and correlation with biomarkers and specific antiretroviral regimens in 9690 human-immunodeficiency-virus-infected patients: Findings of the Anemia Prevalence Study. Curr Med Res Opin 2007;23:343-55.
6. Antelman G, Msamanga GI, Spiegelman D, Urassa EJ, Narh R, Hunter DJ, *et al*. Nutritional factors and infectious disease contribute to anemia among pregnant women with human immunodeficiency virus in Tanzania. J Nutr 2000;130:1950-7.
7. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ward JW. Epidemiology of anemia in human immunodeficiency virus (HIV)-infected persons: Results from the multistate adult and adolescent spectrum of HIV disease surveillance project. Blood 1998;91:301-8.
8. Remacha AF, Cadafalch J. Cobalamin deficiency inpatients infected with the human immunodeficiency virus. Semin Hematol 1999;36:75-87.
9. Spivak JL, Barnes DC, Fuchs E, Quinn TC. Serum immune reactive erythropoietin in HIV-infected patients. JAMA 1989;261:3104-7.
10. Dainiak N, Worthington M, Riordan MA, Kreczko S, Goldman L. 3'-Azido-3'-deoxythymidine (AZT) inhibits proliferation *in vitro* of human haematopoietic progenitor cells. Br J Haematol 1988;69:299-304.
11. Abrams DI, Steinhart C, Frascino R. Epoetin alfa therapy for anaemia in HIV-infected patients: Impact on quality of life. Int J STD AIDS 2000;11:659-65.
12. Grossman HA, Goon B, Bowers P, Leitz G; 010 Study Group. Once-weekly epoetin alfa dosing is as effective as three times-weekly dosing in increasing hemoglobin levels and is associated with improved quality of life in anemic HIV-infected patients. J Acquir Immune Defic Syndr 2003;34:368-78.
13. Mofenson LM, Harris DR, Moye J, Bethel J, Korelitz J, Read JS, *et al*. Alternatives to HIV-1 RNA concentration and CD4 count to predict mortality in HIV-1-infected children in resource-poor settings. Lancet 2003;362:1625-7.
14. Evans RH, Scadden DT. Haematological aspects of HIV infection. Baillieres Best Pract Res Clin Haematol 2000;13:215-30.
15. Volberding PA, Baker KR, Levine AM. Human immunodeficiency virus hematology. Hematology Am Soc Hematol Educ Program 2003;1:294-313.
16. Kumarasamy N, Solomon S, Chaguturu SK, Cecelia AJ, Vallabhaneni S, Flanigan TP, *et al*. The changing natural history of HIV disease: Before and after the introduction of generic antiretroviral therapy in southern India. Clin Infect Dis 2005;41:1525-8.
17. Nardi M, Karpatkin S. Antiidiotype antibody against platelet anti-GPIIIa contributes to the regulation of thrombocytopenia in HIV-1-ITP patients. J Exp Med 2000;191:2093-100.
18. Leiderman IZ, Greenberg ML, Adelsberg BR, Siegal FP. A glycoprotein inhibitor of *in vitro* granulopoiesis associated with AIDS. Blood 1987;70:1267-72.
19. Paradela A, Rivas C, Fernández-Guerrero M, Román A. Histopathology of bone marrow biopsy in patients with human immunodeficiency virus infection. Rev Clin Esp 1996;196:9-15.

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Affordability, availability and tolerability of anti-seizure medications are better predictors of adherence than beliefs: Changing paradigms from a low resource setting

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ABSTRACT

Objectives: Anti-seizure medication (ASM) non-adherence contributes to treatment gap and increases mortality and morbidity associated with epilepsy. Beliefs about medications are considered better predictors of ASM non-adherence than clinico-demographic factors. We aimed to look into ASM non-adherence rates among adults with epilepsy (AWE), identify the contributing barriers and determine whether medication beliefs were more powerful predictors than clinico-demographic factors.

Methods: This was a cross-sectional study of AWE receiving ASMs. Participants (n = 304) were assessed by validated questionnaires, for non-adherence (8-item Morisky Medication Adherence Scale) and perceptions of ASMs (Beliefs about Medicines Questionnaire) along with clinico-demographic details.

Results: Our group with high literacy and low-income had a high non-adherence rate (55 %) despite having positive beliefs (Mean necessity-concern differential [NCD] = 2.86). Among the beliefs, ASM non-adherence was significantly associated with ASM-concern ($t = 4.23$, $p < 0.001$) and NCD ($t = -4.11$, $p < 0.001$). Stepwise multiple linear regression analysis showed that non-adherence was significantly associated with per-capita income ($\beta -0.215$, $p < 0.001$), ASM side effects ($\beta 0.177$, $p = 0.001$), high seizure frequency ($\beta 0.167$, $p = 0.002$), ASM availability ($\beta -0.151$, $p = 0.004$), ASM costs ($\beta -0.134$, $p = 0.013$ and NCD ($\beta -0.184$, $p = 0.001$). NCD accounted for 2.9 % of the variance in non-adherence whereas the other clinico-demographic variables together accounted for 14.6 %.

Conclusion: We describe a paradigm shift in AWE with high non-adherence to ASMs, wherein clinico-demographic variables emerge as better predictors of non-adherence than beliefs. High literacy facilitates the perception of need for ASMs whereas costs and side effects hamper adherence.

1. Introduction

Epilepsy is one of the most common neurological disorders estimated to affect around 70 million people worldwide [1]. Anti-seizure medications (ASM) form the mainstay of treatment and help in achieving good seizure control in 70 % of patients with epilepsy (PWE). Medication non-adherence magnifies the morbidity and causes a threefold increase in mortality [2,3]. Medication adherence is defined as "the extent to which a person's behaviour including taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider" [4]. Adherence to long term treatment is around 50 % in developed countries whereas its is much less in the developing nations [4].

The prevalence of epilepsy in India is estimated to be 3.0–11.9 per 1000 populations, which is similar to the developed countries [5]. Treatment gap is a well known obstacle in developing countries, which ranges from 22 % among urban, middle-income people to 90 % in villages in India [6]. Medication non-adherence is one of the main causes of treatment gap which leads to poorer outcomes and increased utilisation and strain of the health care system of a resource poor nation like India.

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Hence, identification of barriers followed by implementation of targeted methods to improve adherence is essential to fix the treatment gap and improve outcomes and decrease mortality in our PWE.

Medication adherence is a dynamic process and not a stable patient characteristic [7]. In addition to the various clinical factors, specific and general medication beliefs are known to impact adherence to medications. This is operational as a theoretical necessity-concern framework (NCF) where the perception of need for ASMs is balanced by the concern about their adverse effects [8]. Negative beliefs wherein the patients perceive low necessity and high concern about ASM use are associated with non-adherence. Medication beliefs were found to be more powerful predictors of adherence compared to clinical factors in epilepsy as well as in other chronic illnesses [9,10]. In a metaanalysis of 94 articles, Horne et.al demonstrated the strong association of medication adherence with the NCF in many chronic disorders including epilepsy [11]. This association remained significant irrespective of the sample size, country of research and the type of adherence measure used. Very few studies show contradictory results. Shallcross et al. [12] and Kemp et al. [13] found no correlations between beliefs about medicines and ASM adherence. The sample sizes in these studies were very small and hence not sufficiently powered to draw conclusions. Nakhutina et al. [14] found nonsignificant association between beliefs and adherence with the Morisky scale but significant associations with participant self rating scale. The Morisky scale had low internal reliability in their sample.

Very little data is available from India about the prevalence and causes of non-adherence to ASMs. Hence we conducted this study in our centre located in the South Indian district of TamilNadu. We presumed that the changing socio-economic scenario of increasing literacy rate (>80 % vs the national literacy rate of 74 %) and unemployment rate (7.6 % vs the national rate of 6.1 %) [15] in our state would have a bearing on the predictors of ASM non-adherence. We aimed to (1) determine ASM non-adherence rates among adults with epilepsy (AWE) and identify the demographic and clinical barriers that contribute to ASM non-adherence (2)determine whether medication beliefs influenced non-adherence and if so whether they were more powerful predictors than the demographic and clinical factors.

2. Methodology

2.1. Study site and subjects

This prospective cross-sectional study was conducted at the PSG Comprehensive Epilepsy Care Centre located at Coimbatore, South India. The study was approved by the ethics committee of the hospital. Patients were recruited from the out-patient department of our epilepsy clinic from June 2019 to December 2019. **A**ll the patients are seen by the epilepsy specialist (RSI).The inclusion criteria was as follows (1) diagnosis of epilepsy according to the International League Against Epilepsy (ILAE) [16] and on ASMs (2) age ≥18 years (3) minimum 6 months of ASM therapy; (4) No major cognitive impairment; and (5) can read and write. The exclusion criteria consisted of (1) presence of psychogenic nonepileptic seizures and (2) any surgery for epilepsy. Our sample size was calculated using a single population proportion formula assuming 95 % confidence level, 5 % margin of error and 50 % medication adherence rate.

2.2. Questionnaire

After obtaining written informed consent, information was collected regarding the demographic details, epilepsy and ASM therapy using a questionnaire. The patients then completed few validated self-reported assessments with the assistance of a bilingual research assistant as required.

2.3. Demographic details

Age, sex, urban or rural residence, number of members in the house, marital status and family income details were collected from the participants. An age ≤30 years (median age) was considered young. Family percapita income (PCI) was calculated as total family income divided by the number of members in the house. Occupation was noted as currently employed or not. Educational status was considered as formal if they had completed tenth grade or more and informal if completed less than tenth grade.

2.4. Disease related details

Age at onset, duration and family history of epilepsy along with the presence of generalized tonic clonic seizures (GTCS) over the past six months were noted. Seizure frequency over the past six months was classified using the Engel seizure frequency scoring system [17]. They were divided into 2 groups of low seizure frequency or well-controlled epilepsy with score ≤4 (seizure free or those with auras only or non-disabling nocturnal seizures) and high seizure frequency or poorly controlled epilepsy with score >4. The epilepsy type according to the latest classification by ILAE was noted [16].

2.5. ASM related details

Details regarding ASM consumption were collected. ASMs were divided into old or conventional being marketed before 1980, and new. Thus, carbamazepine, clonazepam, diazepam, phenobarbital, phenytoin, primidone and valproate were defined as old, the remainder as new ASMs. Taking single ASM was considered monotherapy and more than one as polytherapy. Frequency of dosing was categorized into once or twice a day. Any drug intake other than ASMs was noted. Details of side effects and monthly costs of ASMs were collected. Participants were directly queried for adherence as to whether they ever missed ASMs and if so the reasons thereof. Whether they are dependent atleast on few occasions on reminders from family members was noted. The ease of availability of ASMs was obtained.

2.6. Measurement scales

We used the following scales: Morisky Medication Adherence Scale (MMAS) [18] and the Beliefs about Medicines Questionnaire (BMQ) [19]. The BMQ holds two sections: BMQ specific and BMQ general. BMQ specific contains questions regarding beliefs about medicines specific for a disease condition whereas BMQ general involves questions on general beliefs about pharmacotherapy. BMQ Epilepsy specific scale consisting of the ASM-Necessity and ASM-Concern scales and BMQ General scale consisting of the General-harm and General-overuse scales were used. These scales are well validated and used extensively in English language. However translated versions in Indian languages are not available. Two bilingual translators independently translated these scales from English to Tamil and another translator did the reverse translation. The forward and backward translated versions were compared and inconsistencies resolved by consensus. It was tested for content validity in a pilot study. Adequate internal reliability was indicated by post hoc Cronbach's alpha for MMAS (0.76), ASM-Necessity (0.74), ASM-Concern (0.71), General-harm(0.69) and General-overuse(0.72) scales.

2.7. Morisky – 8 item medication adherence questionnaire

An 8-item Morisky medication adherence questionnaire was used to assess patients' adherence to ASMs [18,20]. It consists of 8 questions, with the first seven items having a dichotomous answer(yes/no) indicating adherent or non-adherent behaviour. Scoring was reversed with the "no" answer getting a score of 0 and "yes" answer a score of 1, except for the fifth question which was scored as no = 1 and yes = 0 [21]. For

item 8, a patient can choose an answer on a 5-point Likert scale, expressing how often happens that he/she does not take the medications. The answer "never" was scored as 0 and the rest were taken as 1. MMAS-8 scores can range from 0 to 8 points. Adherence was considered high (score = 0), medium (score = 1) and low (≥2). Patients were dichotomized for further analysis as adherent (high adherence) and non-adherent (medium and low adherence).

2.8. BMQ

The BMQ has been validated in the general population as well as across different patient groups [22,23]. It has high internal consistency, allows assessment of self efficacy and has good test-retest reliability [24–26]. BMQ-epilepsy specific scale comprises of two 5-item factors assessing perceptions about the necessity of ASMs (ASM-Necessity) and concerns about prescribed medication based on beliefs about the danger of dependence and long-term toxicity of ASMs (ASM-Concerns). The BMQ general has two 4-item sections, one looking into the perceptions of patients towards the adverse effects caused by medications in general (General-Harm) and the other focussing on the patient perceptions of physician's approach including overuse of medicines and adequacy of time spent with them(General-Overuse).The response for each question was scored on a five-point Likert type scale (1=strongly agree, 2=agree, 3=uncertain, 4=disagree, and 5=strongly disagree). Scores were reverse coded (score 1 becomes 5 and vice versa) so that higher scores indicate higher and lower scores indicate lower necessity, concern, harm and overuse. Each individual item score was added and the total score calculated for each of the four sections.

2.9. Necessity-Concerns Differential (NCD)

This was calculated by subtracting ASM-Concerns scores from ASM-Necessity scores. The NCD score is an indicator of the personal need for ASMs felt by the patients in relation to their concerns about their side effects. It ranges from -20 to +20, where positive scores indicate that patient perceived benefits outweigh their perception of risk and hence considered as positive belief about ASMs.

2.10. Attitudinal groups

Based on the scoring of necessity and concern, patients were divided into four groups of attitudes towards medications. Patients were dichotomized depending on their score below and above the midpoint of 15. Patients with higher score (≥15) were considered to have high necessity and high concern and those scoring <15, low necessity and low concern. Patients were then categorized into 4 groups with regard to their perception of ASM treatment; Accepting (high necessity & low concern), Indifferent (low necessity and low concern), skeptical (low necessity and high concern) and ambivalent (high necessity and high concern) [27]. For statistical comparison, accepting group was considered to have positive attitude and the remaining three together to have negative attitude.

2.11. Statistical analysis

Statistical package of social sciences (SPSS) version 24 was used to analyze the data. For descriptive statistics, mean and standard deviation were calculated for continuous variables whereas frequency distribution and percentage were used for categorical or ordinal variables. Independent samples t-tests were used to test for differences between adherence and non-adherence groups on perceptions of ASM (ASM Necessity, ASM Concerns, NCD, General Overuse and General Harm). To compare the size of these differences, standardized effect sizes (Cohen's d) were calculated. Stepwise linear regression analysis was used to identify factors associated with treatment non-adherence (dependent variable) by including all demographic and clinical variables and NCD

Table 1
Clinico-demographic details of adults with epilepsy (n = 304).

Age(years)	33.36 ± 15.59 (18–82)[a]	ASMs	
≤30 years	173 (56.9)[b]	New	245 (80.6)[b]
>30 years	131 (43.1)[b]	Old	21 (6.9)[b]
		New & old	38 (12.5)[b]
Gender			
Male	185 (60.9)[b]	**Use of individual ASMs**	
Female	119 (39.1)[b]	Oxcarbazepine	136 (44.7)[b]
		Levetiracetam	95 (31.3)[b]
Education		Clobazam	91(30)[b]
Informal	109 (35.9)[b]	Divalproex	63(20.7)[b]
Formal	195 (64.1)[b]	Lamotrigine	29 (9.5)[b]
		Lacosamide	17 (5.6)[b]
Occupation		Carbamazepine	29 (9.5)[b]
Unemployed	166 (54.6)[b]	Phenytoin	21(7)[b]
Employed	138 (45.4)[b]	Phenobarbitone	6 (2)[b]
Place of residence		**Number of ASMs**	
Urban	195 (64.1)[b]	Mono therapy	151 (49.7)[b]
Non-urban	109 (35.9)[b]	Poly therapy	153 (50.3)[b]
Marital Status		**Frequency of administration**	
Married	143 (47)[b]	Once daily	86(28.3)[b]
Unmarried / Separated / Divorced	161 (53)[b]	Twice daily	218 (71.7)[b]
Per capita income (INR)	7757.99 ± 13928.62[a]	**Cost of ASMS (INR)**	1042.84 ± 1000.67[a]
Age of onset (years)	20.1 ± 16.11[a]		
Duration of Epilepsy (years)	13.97 ± 13.06[a]	**Side effects**	
		Present	91 (29.9)[b]
Family History of Epilepsy		Absent	213 (70.1)[b]
Present	60 (19.7)[b]		
Absent	244 (80.3)[b]	**Patient perceived side effects**	
		Drowsiness	37 (12.2)[b]
Epilepsy type		Weight gain	31 (10.2)[b]
Focal Epilepsy	247 (81.3)[b]	Forgetfulness	10 (3.3)[b]
Generalized Epilepsy	49 (16.1)[b]	Fatigue	9 (3)[b]
Combined Focal & Generalized Epilepsy	8 (2.6)[b]	Mood changes	1 (0.3)[b]
Seizure frequency		**Adherence**	
Low	179 (58.9)[b]	Low	112 (36.8)[b]
High	125 (41.1)[b]	Medium	57 (18.8)[b]
		High	135 (44.4)[b]
GTCS			
Present	112 (36.8)[b]	**Perceived reasons for non-adherence**	132 (43.4)[b]
Absent	192 (63.2)[b]	Forgetfulness	62 (20.4)[b]
		Non-availability of ASMs	24 (7.9)[b]
Family reminds medicine		Belief	21 (7)[b]
Yes	71 (23.4)[b]	Fear about side effects	9 (3)[b]
No	233 (76.6)[b]	Cost	9 (3)[b]
		Lack of benefit	1 (0.3)[b]
Drugs other than ASMs			
Yes	85 (28)[b]	**Availability of ASMs**	
No	219 (72)[b]	Good	211 (69.4)[b]
		Poor	93 (30.6)[b]

a = Mean ± SD b = n(%).
ASM: Anti-seizure medications, GTCS = Generalized tonic clonic seizure.

(independent variables). This linear regression model was used to assess the relative strength of medication beliefs (NCD scores) vis-à-vis clinical and demographic factors in predicting non-adherence by stepwise entry and removal method. Stepwise linear regression analysis was again used to compare the attitudinal groups wherein negative attitude was used as dependent variable and all clinical and demographic factors and adherence were used as independent variable. Statistical significance was set at $P < 0.05$.

3. Results

Our study included 304 patients. The demographic, disease and ASM related variables are depicted in Table 1.

3.1. Demographic variables

The mean age of the study population was 33.3 years and consisted of 185(60.9 %) males and 119(39.1 %) females. Majority were young (56.9 %) and unemployed (54.6 %). Around 195(64.1 %) of them were staying in urban areas and had formal education whereas 143(47 %) were married. The mean per-capita income of the study group was 7758 INR. The PCI was significantly more in the adherent group than the nonadherent group (7228.77 INR vs 5525.88 INR p = 0.004).

3.2. Disease related variables

The mean age of onset of epilepsy was 20.1 years and the mean duration 13.97 years. Seizure frequency was high in 125(41.1 %) and low in 179(58.9 %) patients. Around 247(81.3 %) had focal epilepsy and 49(16.1 %) generalized epilepsy syndromes whereas 112 (36.8 %) had atleast one GTCS over the past six months.

3.3. ASM related variables

Around half of our population was on monotherapy. Eighty six of them (28.3 %) were on once daily dosing and the remaining on twice daily dosing. The average cost of ASMs was around 1042.84 INR per month. The average ASM costs were the same in the adherent and non-adherent groups. (1105.53INR vs 992.75INR p = 0.33). Only 132 AWE (43.4 %) perceived non-adherence. Forgetfulness and non-availability were the most common reasons of nonadherence. For 211(69.4 %) patients, ASMs were freely available whereas the remaining 93(30.6 %) had difficulty in the local availability of ASMs. Overall 91(29.9 %) patients reported side effects which were predominantly drowsiness and weight gain. Newer ASMs were used exclusively in 245(80.6 %) patients. Oxcarbazepine, clobazam and levetiracetam were the most frequently used newer ASMs.

3.4. Belief variables

Descriptive statistics about the belief variables is given in Table 2. The overall belief about medications is positive with 70 % showing high necessity beliefs and positive NCD scores and 55 % showing low concern beliefs. Similarly 47 % showed low overuse beliefs and 76.7 % had low harm beliefs.

3.5. ASM economics

This is displayed in Table 3. The average PCI of our study group was 73.6 % of the national PCI (10,534 INR). Corresponding values for the adherent and non-adherent groups were 90 % and 60.5 % respectively. Average monthly cost of ASMs for the group was equivalent to 4 days of their PCI or 5.8 days of wages of the lowest paid labour in India in 2019 (178 INR). Corresponding values for the adherent and non-adherent groups were 3.5 days PCI / 6.2 days of daily wages and 4.7 days PCI / 5.6 days of daily wages respectively. An additional cost of 1–4 days of PCI or daily wages is incurred for the monthly purchase of originator brands of newer ASMs when compared to the commonly available generic brands. For the older ASMs, the difference in costs between the originator and generic brands is very negligible.

Table 3
ASM economics of our study group.

	Income (%)[a]	Expenditure[b]
Overall (n = 304)	73.6	(4, 5.8)
Adherent group (n = 135)	90	(3.5, 6.2)
Non-adherent group (n = 169)	60.5	(4.7, 5.6)
Cost of ASMs		
Antiseizure medication (DDD)	**Generic[b]**	**Brand[b]**
Oxcarbazepine (1200 mg)	(1.8, 2.6)	(4.6, 6.7)
Clobazam (20 mg)	(0.6, 0.87)	(1.5, 2.2)
Levetiracetam (1500 mg)	(1.9, 2.8)	(4.6, 6.7)
Divalproate (1000 mg)	(1.2, 1.8)	(1.4, 2)
Phenytoin (300 mg)	(0.15, 0.22)	(0.46, 0.67)
Carbamazepine (1000 mg)	(0.7, 1)	(0.83, 1.26)
Phenobarbitone (100 mg)	(0.12, 0.16)	(0.51, 0.75)

DDD = Defined daily dose.
[a] Per capita income (PCI) as a proportion of the national PCI.
[b] Mean number of day's income required for one month of ASM treatment as a proportion of (PCI of the study group, minimum daily wages in India [2019]).

Table 2
Beliefs about medications in the study group and in both adherence groups.

Variable	Mean(SD)	Median (range)	People above the midpoint[d]	Adherent (n = 135) Mean(SD))	Non-adherent (n = 169) Mean(SD))	Cohen's d (95 %CI)	t (304)	p-value
ASM necessity[a]	17.26 (4.69)	18 (5–25)	213 (70)	17.77 (4.94)	16.86 (4.46)	0.19 (-0.03, 0.42)	1.68	NS
ASM concern[a]	14.41 (4.76)	14 (5–25)	137 (45)	13.15 (4.85)	15.41 (4.45)	−0.48 (-0.71, -0.25)	−4.23	<0.001
ASM NCD[b]	2.86 (6.86)	3 (-17 to 19)	211 (69.4)	4.62 (6.76)	1.44 (6.62)	0.47 (0.24, 0.7)	4.11	<0.001
General overuse[c]	18.74 (3.02)	19(4–18)	161 (53)	12.72 (3.19)	12.75 (2.89)	−0.01 (-0.23, 0.21)	−0.09	NS
General harm[c]	16.22 (3.22)	16(4–20)	71 (23.3)	10.12 (3.6)	10.3 (2.89)	−0.05 (-0.28, 0.17)	−0.49	NS

[d]N(%) Midpoint is 15 in case of necessity and concern, 0 in case of NCD and 12 for overuse and harm. NS = non significant, M (SD) = Mean (standard deviation).
[a] Scale from 5 to 25 where high scores indicate high necessity and high concern towards anti-seizure medication use.
[b] Scale from –20 to 20 where positive scores indicate that patient perceived benefits outweigh risks.
[c] Scale from 4 to 20 where low scores indicate positive attitude towards medicines.

Table 4
Stepwise multiple linear regression model for reported non-adherence to ASMs.

Predictors	β	p	Adjusted R^2 (%variance)
Per capita income	−0.215	<0.001	0.046 (4.6)
ASM side effects	0.177	0.001	0.084 (3.8)
Seizure frequency	0.167	0.002	0.108 (2.4)
ASM availability	−0.151	0.004	0.131 (2.3)
Cost of ASMs	−0.134	0.013	0.146 (1.5)
NCD	−0.184	0.001	0.175 (2.9)

3.6. Adherence to ASMs

MMAS scores were dichotomized into an adherent group (n = 135, 44.4 %) and a nonadherent group (n = 169, 55.6 %).

a) Differences in the adherence groups on perception of ASMs:
This is displayed in Table 2. The necessity, overuse and harm scores were distributed equally between the two groups of adherence. ASM-concern scores and NCD were significantly high in the non-adherent and adherent groups respectively ($p < 0.0001$). Both the factors accounted equally for the largest differences between the adherence groups when effect sizes were compared using Cohen's d.
b) Multivariate analysis for non-adherence:
Stepwise entry and removal of the clinical, demographic, and belief variables resulted in a linear regression model explaining 17.5 % of the variance in reported non-adherence to medication (Table 4). Higher non-adherence rates were associated with PCI (β -0.215, $p < 0.001$), ASM side effects (β 0.177, $p = 0.001$), high seizure frequency (β 0.167, $p = 0.002$), ASM availability (β -0.151, $p = 0.004$), ASM costs (β -0.134, $p = 0.013$ and patients' beliefs represented by NCD (β -0.184, $p = 0.001$). NCD accounted for 2.9 % of the variance in non-adherence whereas the other clinico-demographic variables together accounted for 14.6 % of the variance.

3.7. Attitudinal outcome

This is depicted in Fig. 1. Majority of our patients were either "Accepting" of their ASMs and having positive attitude (n = 114; 37.5 %) or were "Ambivalent" (n = 108; 35.5 %). Very few were "Skeptical" (n = 43; 14.1 %) or "Indifferent" (n = 39; 12.8 %). The non-adherence rate in the various groups was as follows: Accepting (57/114; 50 %), Ambivalent (63/108; 58.3 %), Skeptical (31/43; 72 %) and Indifferent (18/39; 46.1 %). The respective contributions to the overall non-adherence (n = 169) of the four attitudinal groups were 33.7 %, 37.2 %, 18.3 % and 10.6 % respectively. Multiple linear regression analysis with negative attitude as the dependent variable and the various clinico-demographic factors and adherence as independent variables showed 3 predictors of negative attitude; education (β 0.173, $p < 0.001$), positive family history(β 0.156, $p = 0.002$), and ASM side effects(β 0.134, $p = 0.005$).

4. Discussion

Our centre receives more refractory cases and this explains why more than 80 % of our patients were suffering from symptomatic focal epilepsy. Our study group is predominantly urban and educated. Majority is unemployed and the average PCI is 73.6 % of the average national PCI. We involve our patients in all decisions including choice of ASMs and counsel them at every visit regarding side effects and drug compliance. Our patients generally express more concern about potential adverse effects than the cost of ASMs and hence newer ASMs were used in more than 80 % of them. The commonly used ones were oxcarbazepine, clobazam and levetiracetam. We use ASMs either once daily or twice daily to facilitate adherence. Since conventional sodium valproate is administered thrice daily, we use only the divalproex or chrono preparations.

We found that non adherence to ASMs is still very common and seen in 55 % of our population of AWE. Our study demonstrated one demographic (PCI) and four clinical (high seizure frequency, presence of side effects, low costs and poor availability of ASMs) predictors of non adherence. The PCI was significantly high in the adherent group than the non-adherent group whereas the ASM costs in both the groups were similar. This would mean that lower ASM costs would improve affordability and hence improve adherence. However regression analysis showed a reversal in the expected relationship between ASM costs and non-adherence. This is explained by a possible correlation between the predictor variables, low R-squared value and a small sample size. The sixth predictor was related to patient perception wherein those with low

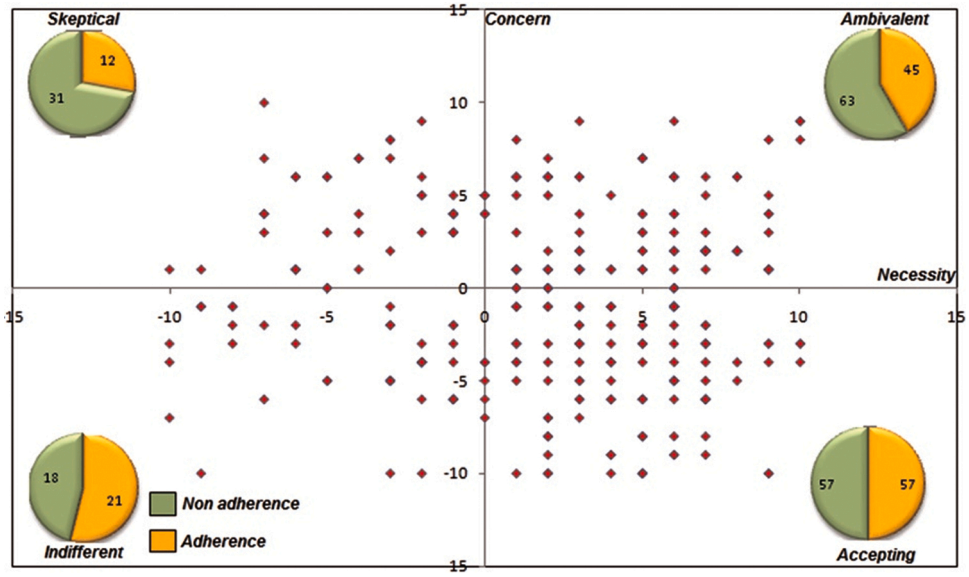


Fig. 1. Scatter diagram showing the four attitudinal groups and their adherence to ASMs. The midpoint (0,0) indicates (15,15) of the necessity and concern scores. Every point score more or less than 15 will have a similar point added or subtracted from 0 so that +10 on the graph represents a score of 25 and -10 represents a score of 5.

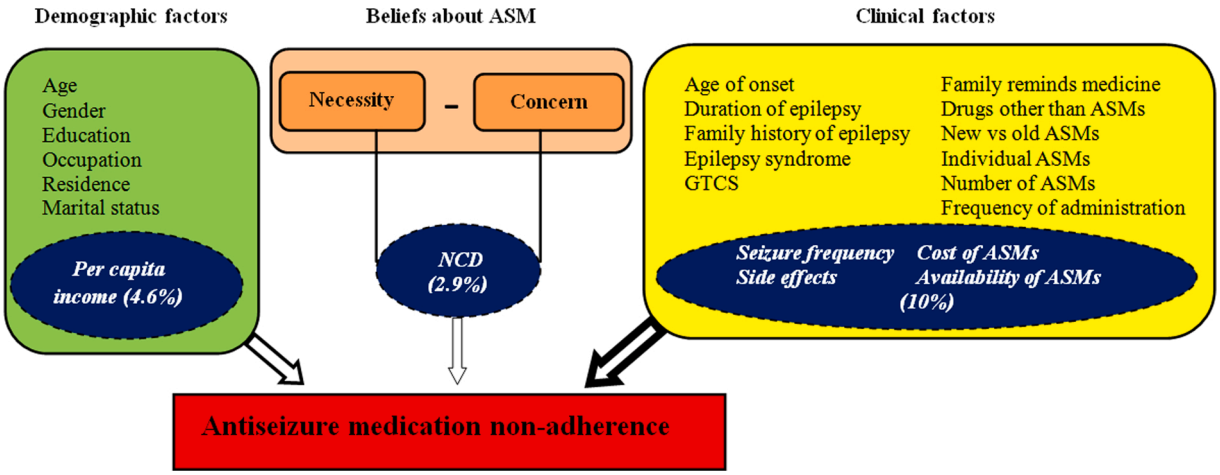


Fig. 2. Schematic diagram showing the various clinico-demographic variables and beliefs. The predictors and their contribution (%) to the variance of non-adherence are shown in dotted oval circles.

NCDs had higher non-adherence.

The two key factors deciding adherence, availability and affordability of ASMs are poor in low- and middle-income countries. Newer ASMs are better available in the private sector compared to the public sector. Data for five conventional ASMs from multiple low- and middle-income countries including India has shown that the average availability of generic ASMs in the public sector was <50 % and 40–70 % in the private sector [28]. Our hospital is in the private sector and patients pay for medicines. Initially they buy ASMs for 2–4 weeks from our pharmacy and subsequently depend on the local pharmacies. Thirty six percent of the study group was from the non-urban or rural setup where there are problems with the availability of ASMs. Many a time, the originator brand ASMs prescribed by us are not available in the rural pharmacies. Some of them use lower-priced generics which may not be quality-assured resulting in breakthrough seizures. Similar problems exist with regard to the ASM costs. The average PCI of our non-adherent group was 30 % less than the adherent group whereas they were spending 1.2 days of PCI more for the monthly purchase of ASMs. For the more commonly used newer ASMs, oxcarbazepine and levetiracetam, an additional 3 days of PCI was needed when compared to their respective generics to meet the monthly requirements. The above data suggest that use of generic medicines in our setup would improve affordability. However we are generally reluctant to switch from brand to generic ASMs for fear of loss of seizure control. Ensuring the supply of quality generic ASMs with good average bioequivalence should improve ASM prescribability for those being treated for the first time.

Around 30 % of our population of AWE reported side effects to ASMs which contributed to non adherence. Drowsiness and weight gain were the most common side effects. The presence of polytherapy in about half of the population has probably contributed towards adverse effects. Studies have shown the negative association of adverse effects of ASMs with adherence [29]. The non-adherence rate in our study goes against the common belief that newer ASMs result in better adherence due to low potential for adverse effects. Recent studies show that despite the increasing use of newer ASMs as the initial therapy, the overall tolerability has not changed [30].

The relationship between seizure outcome and ASM non-adherence is not linear [31]. That non adherence leads to poor seizure control is well known. Early educational and behaviour interventions to improve adherence improve seizure frequency and seizure outcome [32,33]. The paradoxical effect of uncontrolled seizures contributing to non adherence is also well documented in the literature [34–36]. The time since the last seizure has been shown to determine adherence. The presence of seizures within 30 days or 6 months result in more non adherence while absence of seizures for more than a year result in better adherence [36, 37]. The high non-adherence rate would have contributed to high seizure frequency observed in 41 % of our patients. Since our centre deals with more refractory cases, the paradoxical response to uncontrolled seizures of non adherence, arising out of increasing concern is also a possibility. While the relationship between non adherence and poor seizure control could be established by drug level monitoring, the relationship between illness perception and non adherence needs more exploration and understanding.

That the clinico-demographic factors emerged as better predictors accounting for 14.6 % of the explained variation in adherence in contrast to beliefs which accounted for 2.9 % of the variation was not necessarily expected as it was contradictory to the existing observations about medication adherence in epilepsy [10]. Neither was this surprising considering the socioeconomic situation of the state which is reflected in our study group. The overall belief about medications of our group has been positive. A good proportion of those with negative attitude were still adherent, demonstrating that negative attitude doesn't necessarily result in non-adherence. The ASM-related side effects seem to make them develop a negative attitude but being better educated has helped them feel the need for the medications. The human adherence behaviour depends more on the cognitive representation of the beneficial effects of a drug than on the concern regarding its adverse effects [38]. It is possible therefore that high literacy of our group acted as a protective factor against non-adherence by contributing to increased perception of necessity, whereas unemployment with its resultant poverty made the ASM economics more significant contributor to non-adherence.

We acknowledge the following limitations of our study. First, is the use of self-reported adherence scale which is subjected to retrospective and recall bias and usually underestimates non-adherence by 20 % [39]. Direct measures of adherence are impractical in our population. We preferred an ordinal rather than an interval scale of assessment of adherence and gained confidence from our patients by a non-confrontational consultation and ensured the confidentiality of their responses. We also considered both medium and low adherence as non adherent to minimize underestimation of non adherence. Second, is the problem of low adjusted R-squared value. This could be due to the non-inclusion of other potentially important predictors like depression and anxiety, health care related factors and support systems. Any study trying to predict human behaviour is also expected to have low R-squared value. Regardless of the R-squared, the presence of significant

coefficients will help us draw important conclusions about how changes in the predictor value will affect the response value. Third, we recognize that adherence is dynamic and hence best understood by prospectively looking at trajectories rather than by cross-sectional point estimates. Fourth, we did not try to differentiate between unintentional and intentional non-adherence. Intentional non-adherence is more likely to be associated with perceptions of need and concern. Fifth, our findings are not generalizable and could be different in scenarios of low literacy, high dependence on traditional healers and medicines etc.

Despite its limitations, this study demonstrates a high degree of non-adherence and identifies the factors linked to non-adherence to ASMs in our relatively well literate, low income group of adults with chronic epilepsy. The clinico-demographic variables of PCI, ASM related side effects, ASM costs, availability and seizure frequency along with medication beliefs were the predictors identified. Improving the affordability, availability and tolerability of ASMs would facilitate adherence more than interventions to improve beliefs. Making available good quality generic ASMs will be an important step in this direction. Older ASMs may be initiated as monotherapy. Newer ASMs could be limited to those who could afford them, those who develop side effects to older ASMs and also in special situations and to prevent drug interactions. It is imperative to look for side effects during each visit and take corrective steps including proper counselling of patients. We describe a paradigm shift in the relative roles of medication beliefs and clinical factors wherein the latter emerges as better predictor of adherence than beliefs. The closing knowledge gaps due to improving literacy rates and various accesses to epilepsy specific education would mean that our study could herald a changing trend rather than remain an isolated finding. Further studies on adherence to ASMs focussing on various demographic and geographic groups are required to support our observation.

5. Conclusion

The findings of our study are summarized in Fig. 2. We found a high degree of non-adherence to ASMs of 55 % in our study group. We provide novel insights into the predictors of medication adherence from a robust sample of low-income and high literacy. Though medication beliefs predicted non-adherence, the clinico-demographic factors of ASM cost, availability and tolerability proved to be better predictors.

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Declaration of Competing Interest

The authors report no declarations of interest.

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References

[1] Ngugi AK, Kariuki SM, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Incidence of epilepsy: a systematic review and meta-analysis. Neurology 2011;77:1005–12.
[2] Manjunath R, Davis KL, Candrilli SD, Ettinger AB. Association of antiepileptic drug nonadherence with risk of seizures in adults with epilepsy. Epilepsy Behav 2009;14(2):372–8.
[3] Faught E, Duh MS, Weiner JR, Guérin A, Cunnington MC. Nonadherence to antiepileptic drugs and increased mortality: findings from the RANSOM study. Neurology 2008;71:1572–8.
[4] World Health Organization. Adherence to long-term therapies: evidence for action. 2003. http://www.who.int/chron ic_conditions/en/adherence_report.pdf.
[5] Amudan S, Gururaj G, Satishchandra P. Epilepsy in India I: epidemiology and public health. Ann Indian Acad Neurol 2015;18:263–77.
[6] Meyer AC, Dua T, Ma J, Saxena S, Birbeck G. Global disparities in the epilepsy treatment gap: a systematic review. Bull World Health Organ 2010;88(4):260–6.
[7] Smithson WH, Hukins D, Colwell B, Mathers N. Developing a method to identify medicines non-adherence in a community sample of adults with epilepsy. Epilepsy Behav 2012;24:49–53.
[8] Horne R. Treatment perceptions and self-regulation. In: Cameron LD, Leventhal H, editors. The self-regulation of health and illness behaviour. London: Routledge; 2003. p. 138–53.
[9] Horne R, Weinman J. Patients' beliefs about prescribed medicines and their role in adherence to treatment in chronic physical illness. J Psychosom Res 1999;47:555–67.
[10] Chapman SCE, Horne R, Eade R, Balestrini S, Rush J, Sisodiya S. Applying a perceptions and practicalities approach to understanding nonadherence to antiepileptic drugs. Epilepsia 2015;56(9):1398–407.
[11] Horne R, Chapman SCE, Parham R, Freemantle N, Forbes A, Cooper V. Understanding patients' adherence-related beliefs about medicines prescribed for longterm conditions: a meta-analytic review of the Necessity Concerns Framework. PLoS One 2013;8(12):e80633.
[12] Shallcross AJ, Becker AB, Singh A, Friedman D, Jurd R, French JA, et al. Psychosocial factors associated with medication adherence in ethically and socioeconomically diverse patients with epilepsy. Epilepsy Behav 2015;46:242–5.
[13] Kemp S, Feely M, Hay A, Wild H, Cooper C. Psychological factors and use of antiepileptic drugs: pilot work using an objective measure of adherence. Psychol Health Med 2007;12(1):107–13.
[14] Nakhutina L, Gonzalez JS, Margolis SA, Spada A, Grant A. Adherence to antiepileptic drugs and beliefs about medication among predominantly ethnic minority patients with epilepsy. Epilepsy Behav 2011;22(3):584–6.
[15] "Periodic Labour Force Survey (2019-2020)" (PDF). Ministry of Labour and Employment. p. 212. Retrieved 3 May 2020.
[16] Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:512–21.
[17] Engel Jr J, Van Ness PC, Rasmussen TB, et al. Outcome with respect to epileptic seizures. In: Engel Jr J, editor. Surgical treatment of the epilepsies. 2nd edition. New York: Raven Press; 1993. p. 609–21.
[18] Morisky DE, Ang A, Krousel-Wood M, Ward HJ. Predictive validity of a medication adherence measure in an outpatient setting. J Clin Hypertens 2008;10(5):348–54.
[19] Horne R, Weinman J, Hankins M. The beliefs about medicines questionnaire: the development and evaluation of a new method for assessing the cognitive representation of medication. Psychol Health 1999;14:1–24.
[20] gou Yi, Ding X-Y, Lu R-Y, Shen C-H, Ding Y, Wang S, et al. Depression and anxiety are associated with reduced antiepileptic drug adherence in Chinese patients. Epilepsy Behav 2015;50:91–5.
[21] Fadare JO, Sunmonu TA, Bankole IA, Adekeye KA, Abubakar SA. Medication adherence and adverse effect profile of antiepileptic drugs in Nigerian patients with epilepsy. Neurodegener Dis Manag 2018;8(1):25–36.
[22] Andersson Sundell K, Jönsson AK. Beliefs about medicines are strongly associated with medicine-use patterns among the general population. Int J Clin Pract 2016;70:277–85.
[23] Sjölander M, Eriksson M, Glader E. The association between patients' beliefs about medicines and adherence to drug treatment after stroke: a cross-sectional questionnaire survey. BMJ Open 2013;3:e003551.
[24] Salgado T, Marques A, Geraldes L, Benrimoj S, Horne R, Fernandez-Llimos F. Cross-cultural adaptation of the beliefs about medicines questionnaire into Portuguese. Sao Paulo Med J 2020;131(2):88–94.
[25] Lavsa SM, Holzworth A, Ansani NT. Selection of a validated scale for measuring medication adherence. J Am Pharm Assoc 2011;51(1):90–4.
[26] Alhalaiqa F, Masa'Deh, Batiha R, Deane K. Validity of arabic version of beliefs about medication questionnaire. Clin Nurs Res 2015;24:539–55.
[27] Aikens Je, Nease Jr De, Nau Dp, Klinkman MS, Schwenk TL. Adherence to maintenance phase antidepressant medication as a function of patient beliefs about medication. Ann Fam Med 2005;3:23–30.
[28] Cameron A, Bansal A, Dua T, Hill SR, Moshe SL, Mantel-Teeuwisse AK, et al. Mapping the availability, price, and affordability of antiepileptic drugs in 46 countries. Epilepsia 2012;53(6):962–9.
[29] Martins HH, Alonso NB, Guilhoto LMFF, Guaranha MsBittar, Yacubian EMT. Adherence to treatment in patients with juvenile myoclonic epilepsy: correlation with quality of life and adverse effects of medication. JECN 2009;15:192–6.
[30] Alsfouk BAA, Brodie MJ, Walters M, Kwan P, Chen Z. Tolerability of antiseizure medications in individuals with newly diagnosed epilepsy. JAMA Neurol 2020;77(5):574–81.
[31] Modi AC, Wu YP, Rausch JR, Peugh JL, Glauser TA. Antiepileptic drug nonadherence predicts pediatric epilepsy seizure outcomes. Neurology 2014;83:2085–90.
[32] Li J, Si Y, Hu J, Liu L, Deng Y, He J, et al. Enhancing medical compliance of patients with convulsive epilepsy in rural community: a randomized intervention trial. Epilepsia 2013;54(11):1988–96.
[33] Tang F, Zhu G, Jiao Z, Ma C, Chen N, Wang B. The effects ofmedication education and behavioral intervention on Chinesepatients with epilepsy. Epilepsy Behav 2014;37:157–64.
[34] Hovinga CA, Assato MR, Manjunath R, Wheless JW, Phelps SJ, Sheth RD, et al. Association of non-adherence to antiepileptic drugs and seizures, quality of life, and productivity: survey of patients with epilepsy and physicians. Epilepsy Behav 2008;13:316–22.
[35] Jones RM, Butler JA, Thomas VA, Peveler RC, Prevett M. Adherence to treatment in patients with epilepsy: associations between seizure control and illness beliefs. Seizure 2006;15(7):504–8.

[36] Ferrari CMM, Cardosa de Sousa RM, Castro LHM. Factors associated with treatment non-adherence in patients with epilepsy. Seizure 2013;27(3):384–9.
[37] Smithson WH, Hukins D, Buelow JM, Allgar V, Dickson J. Adherence to medicines and self-management of epilepsy: a community-based study. Epilepsy Behav 2013; 26(2):109–13.
[38] Schuz B, Marx C, Wurm S, Warner LM, Ziegelmann JP, Schwarzer R, et al. Medication beliefs predict medication adherence in older adults with multiple illnesses. J Psychosom Res 2011;70(2):179–87.
[39] Haynes Rb, Taylor Dw, Sackett Dl, Gibson ES, Bernholz CD, Mukherjee J. Can simple clinical measures detect patient noncompliance? Hypertension 1980;2: 757–64.

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Dental Health Status among College Students and the Treatment Needs

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ABSTRACT

Aim: The aim of this cross-sectional study was to investigate the oral health status and normative need of college students both from urban –rural population of age group 17-23.

Materials and method: A descriptive cross-sectional study was done among 3312 college students belonging to urban –rural population of age group 17-23 in Coimbatore district, Tamil Nadu using purposive sampling method. Data entered in Microsoft excel sheet and analyzed using statistical package for social science (SPSS) version 24. Chi-square test was used for computing statistical significance

Result: Overall dental caries prevalence accounted to 70% (rural-72.12% urban-68.43%) with a mean DT of (1.14) in the rural population than urban population (1.09) and majority of the student's required one surface restoration. Periodontal status of the students as measured by community periodontal index (CPI) showed that majority of the student participant (32.66%) had calculus necessitating the need for oral prophylaxis, with the mean CPI index of (1.13) rural than (0.94) Urban.

Conclusion: In this study the dental caries prevalence and the periodontal problem was more in the rural population than the urban population with the one surface restoration and oral prophylaxis as the predominant treatment need.

Key words: College students, Oral health, Urban, Rural, Dental caries, Periodontal disease

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INTRODUCTION

Oral diseases are the most common of the chronic diseases and are important in public health problems because of their prevalence, their impact on individuals, society, and the expense of their treatment [1]. On September 6, 2016, a new definition of oral health was approved by the FDI (World Dental Federation General Assembly) [2]. The new definition acknowledges the multifaceted nature and attributes of oral health. In developing countries were the resources are sparse it is of utmost importance to plan primordial preventive strategies rather than treating oral diseases and data regarding the prevalence of oral diseases gives us comprehensive picture to identify the target group and impart preventive modalities and there is a paucity of such data of this age group (17-23) in Coimbatore, Tamil Nadu, India.

College life is a crucial period of transition with personal responsibility. Students in this period can be targeted for preventing dental diseases and building future oral health [3] voluminous literature pertaining to oral health status of school children [4,5] is available, but data pertaining to young adults, especially to college students are sparse. Thus, analysing epidemiological data of age groups not customarily studied [world health organisation (WHO) index age groups] give the exact picture of the current oral health status and normative needs of the population. The aim of this study was to accesses the oral health status among and the normative needs among college students in Coimbatore in Tamil Nadu.

MATERIALS AND METHOD

Study Design: Descriptive cross-sectional

study. A health screening camp of dental, ophthalmology and general medicine was conducted for students in a private college in Coimbatore from 22-1-2018 to 22-2-2018 and about 3500 students were screened by a team of doctors from PSGUHTC. Two examiners were involved in this research study, examiner A and examiner B. The study was carried out for a period of 28 days approximately examining 125 students per day. Demographic data including name, age, gender, and permanent address of the candidate were recorded. The Inclusion criteria were subjects with permanent dentition, with no remaining deciduous dentition, subjects with age group of 17-23, Students present during the period of oral examination. The students taken for the study were 3312. The study protocol was submitted to the institutional ethical committee [PSGIMSR] and clearance was obtained Project proposal number-18/223 August 10th 2018.

Oral examination was performed by two trained and calibrated examiners. Before the survey, both the examiners and recording clerks were participated in a training and clinical calibration program in the department. Following this training, 10% of the students were examined by each of the two investigators to assess inter-examiner reliability and Kappa Values of 0. 87 and 0.88 were found, respectively. The interview and examination of a single study subject took 3 to 4 minutes. The study was done using probability sampling method and the sample size of calculated reference to Punitha et al. [6] using prevalence of caries as 36.7%. Sample size was calculated using formula $n=4 \times pq/d^2$ and found to be n=372.

Method of registration

Oral examination was done using the WHO Basic Oral Health Assessment [7] form with plane mouth mirror and WHO periodontal probe. Clinical examination was conducted using natural light with the participant sitting on a chair with a head rest. The examination was performed in a classroom. The examination area was arranged in such a way to allow one person at a time apart from the examiner and the recorder. The recorder was made to stand close to the examiner so that the instructions and the code could be easily heard, and the examiner could see that the findings were being recorded correctly. The data were coded and analyzed using the SPSS version 24 software the level of statistical significance was kept at $p<0.05$. The descriptive statistics were calculated. Chi-square test was used.

RESULT

A total of 3312 (urban-1888; 57%, rural -1424; 43%) participated in this study. The average DMFT among the target group was 1.92 with urban having DMFT score of 1.95 rural with 1.80 (Table 1).

In this study the highest score as measured by CPI showed that a total of 21.13% of the student had a bleeding as their highest score, 32.66% had calculus indicating the need for oral hygiene instruction and oral prophylaxis and 5.07% had a pocket of 4-5mm necessitating surgical interventions (Table 2). The community–wise distribution according to the highest score as measured by CPI showed that35.74% rural and 30.34% urban had calculus as the highest score.

Mean number of sextants with bleeding was higher in urban (0.21), whereas the mean number of sextants with calculus and shallow pocket was more in rural (0.35) compared to urban (0.30) (Table 3).

Association of CPI Index with community (urban/rural) shows that rural population has increased periodontal diseases than urban, and it is statistically significant $p < .000$ with 95% CI (0.255-0.121) (Table 4).

Loss of attachment according to the highest score showed that loss of attachment of 0-3mm was seen in 94.41%, 4-5mm in 3.38%, 6-8mm in 1.20% of the population (Table 5).

Mean number of sextants with score 1 was more in rural than urban (0.04; 0.02) (Table 6).

Association of Loss of attachment with community (urban/rural) shows that rural population has increased Loss of attachment than urban and it is statistically significant $p < .003$ with 95% CI (0.048-0.010) (Table 7).

Among the study participant 0.3% had prosthesis in the upper arch and 0.1% in the lower arch, out of which 0.3% had bridge denture and 0.1% had both bridge and partial denture.

Table 1: Distribution of study participants according to dental caries status.

	Urban	Rural	Total	P value	95% Confidence interval
Caries prevalence	1292 (68.43%)	1027 (72.12%)	2319 (70%)	-	-
DT	1.09 ± 0.92	1.14 ± 0 .90	1.11 ± 0.91	0.094	-0.117-0.009
MT	0.27 ± 0.86	0.24 ± 0.86	0.26 ± 0.86	0.226	-0.023-0.096
FT	0.59 ± 1.36	0.5 ± 1.26	0.55 ± 1.32	0.051	0.000-0.182
DMFT	1.95 ± 1.97	1.80 ± 1.86	1.92 ± 1.92	0.271	-0.058-0.205
DMFT-decayed, missed and filled teeth; DT-decayed teeth; FT-filled teeth; MT-missing teeth.					

Table 2: Periodontal status according to the highest score as measured by CPI.

	Urban	Rural	Total
Dentate	1888	1424	3312
Score 0	836 (44.27%)	521 (36.58%)	1357 (40.97%)
Score 1	402 (21.29%)	298 (20.92%)	700 (21.13%)
Score 2	573 (30.34%)	509 (35.74%)	1082 (32.66%)
Score 3	77 (4.07%)	91 (6.39%)	168 (5.07%)
Score 4	0 (0%)	5 (0.35%)	5 (0.15%)
[Score 0- Normal, score 1- Bleeding gums, score 2-Calculus, Score 3-4-5 mm pocket depth] [Chi square value- 34.191, P value-.000(Significant)]			

Table 3: Distribution according to mean number of sextants with highest score as measured by CPI.

	Urban	Rural	Total
Score 0	0.44	0.36	0.41
Score 1	0.21	0.2	0.21
Score 2	0.3	0.35	0.32
Score 3	0.04	0.06	0.05
Score 4	-	0.003	0.001

Table 4: Association of CPI Index with community (urban/rural).

Distribution	Number	Mean (SD)	F-value	P-value	95% Confidence interval
Urban	1888	0.94 ± 0.952	8.31	0	0.255- 0.121
Rural	1424	1.13 ± 0.997			

Table 5: Loss of attachment according to the highest score.

	Urban	Rural	Total
Dentate	1888	1424	3312
Score 0	1821 (96.45%)	1339 (94.03%)	3160 (94.41%)
Score 1	48 (2.54%)	64 (4.49%)	112 (3.38%)
Score 2	19 (1%)	21 (1.47%)	40 (1.20%)
Chi square value-11.119, P value-0.004 (Significant)			

Table 6: Loss of attachment according to the mean number of sextants with the highest score.

	Urban	Rural	Total
Score 0	0.96	0.94	0.95
Score 1	0.02	0.04	0.03
Score 2	0.01	0.01	0.01

Table 7: Association of Loss of attachment with community (urban/rural).

Distribution	Number	Mean (SD)	F-value	P-value	95% Confidence interval
Urban	1888	0.05 ± 0.252	33.52	0.003	0.048- 0.010
Rural	1424	0.07 ± 0.314			

DISCUSSION

Oral health is an essential component of general health and overall well-being of an individual. Oral cavity and its surrounding structures that are free of any diseases are indicative of good oral health. This not only makes a person look and feel good, it is equally relevant in maintaining oral function [8-10]. Oral disease is one of the most common public health issues worldwide with significant socio-economic impacts, and yet it is frequently neglected in public health policy [11].

Schools and colleges play an immense role in the growth of young minds not only for learning new information but also by having long-lasting effects [12]. In India despite improvements in infrastructure and dental manpower, oral health care remains to be neglected entity as it is not considered to be life threatening [13].

As there is no national level oral health policy and fee for services is still the most common mode of payment for dental service preventive strategies hold priority as the most economical way for improving oral health of the community. To advocate preventive measures, it is essential to assess the complete presence of disease burden in the community.

This cross-sectional study aims at finding the percentage and prevalence of dental caries, periodontal diseases in students of age group 17-23 from urban and rural population in a college in Coimbatore district, Tamil Nadu State, India.

Overall caries percentage in the study conducted by DharV et al. [14], Saravanan S et al. [15], Niyanta Joshi et al. [16], Farooqi FA et al. [17], Kumar S et al. [18] was found similar to the present study around 70%

The mean DMFT of the study conducted by theFarooqi et al. [17], Kumar et al. [18] is like the present study (1.94 ± 2.0) and (1.94).

The study conducted by Mohammed A.AL-Rafee et al. [19], Sriailapanan P, et al. [20], Soroye et al. [21], Sanadhya et al. [22], Akinyamoju et al. [23], showed rural population had more caries prevalence which is supporting to the present study.

The study conducted by Maserejian NN et al. [24], Patro et al. [25], David J et al [26] showed Urban population to have more Dental caries prevalence contradicting to the present study.

The study conducted by Kalsbeek H et al. [27] (24%) (Score-1), Hannan et al [28] (33.5%) (score-2) periodontal index was like the present study.

The study conducted by Arvidson et al. [29], Baiju et al. [30], Vadiakas et al. [31] concluded that rural population had more periodontal diseases than urban population like the present study.

The study conducted by Popoola et al. [32], Sane [33] had mean plaque index (1.12 ± 0.41) which was like the present study (1.13 ± 0.997). The study conducted by Zhang S et al. [34] showed that only 7% of the population had normal periodontium which was contradicting to the present study. The limitations of this study are inherent to cross-sectional studies, which do not allow establishing a relationship of cause and effect, and it only addresses the prevalence and severity of the disease without looking at sociodemographic variables such as parental education, economic status or family size. Furthermore, the current paper only reports the dental caries and the periodontal status and does not examine the oral hygiene practice or other individual reasons for the above dental morbidity.

CONCLUSIONS

The caries prevalence and the periodontal diseases severity were higher in students from rural areas than the students from the urban area. The DMFT index was higher in students from urban area than the students from rural area; this is because the FT value was higher in the urban area indicating the awareness and availability of dental care.Dental health care needs are high in rural areas than in urban areas.

RECOMMENDATION

It is recommended that continuous scientific monitoring, design, and effective implementation of preventive and restorative programs to be carried out by dental surgeons and included in national wide program like RBSK (RASHTRIYA BAL SWASTHYA KARYAKRAM).

REFERENCES

1. Sheiham A. Oral health, general health and quality of life. Bull World Health Organ. 2005; 83:644.
2. Glick M, Williams DM, Kleinman DV, et al. A new definition for oral health developed by the FDI world dental federation opens the door to a universal definition of oral health. J Public Health Dent 2017; 77:3–5.
3. Sharda AJ, Shrtty S. Relationship of periodontal status and dental caries status with oral health knowledge, attitude and behaviour among professional students in India. Int J oral Sci 2009; 1:196-206.
4. Rodrigues JS, Damle SG. Prevalence of dental caries and treatment need in 12-15-year-old municipal school children of Mumbai. Indian Soc Pedod Prev Dent 1998; 16:31-36.
5. Kundu H, Patthi B, Singla A, et al. Dental caries scenario among 5,12 and 15 –year-old children in India-A retrospective analysis. J Clin Diagn Res 2015; 9:1-5.

6. Punitha VC, Amudhan A, Sivaprakasam P, et al. Role of dietary habits and diet in caries occurrence and severity among urban adolescent school children. J Pharm Bioallied Sci 2015; 7:296–300.
7. World Health Organisation. Oral health surveys–Basic methods. 4th Edn Geneva: World Health Organisation: 1997.
8. Kumar H, Behura SS, Ramachandra S, et al. Oral health knowledge, attitude, and practices among dental and medical students in eastern India-A comparative study. J Int Soc Prev Community 2017; 7:58-63.
9. Abdollahi M, Radfar M. A review of drug-induced oral reactions. J Contemp Dent Pract 2003; 4:10-31.
10. Puy CL. The role of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Oral Cir Bucal 2006; 11:449-455.
11. Jin LJ, Lamster IB, Greenspan JS, et al. Global burden of oral diseases: Emerging concepts, management and interplay with systemic health. Oral Dis 2016; 22:609–619.
12. Dileep CP, Basavaraj P, Jaayaprakash K. Survey on Knowledge, attitude and practice about the hygiene among teachers in the Ksnpurcity. J Indian Assoc Public Health Dent 2006; 8:57-59.
13. Ahuja NK, Parmar R. Demographics and current scenario with respect to dentist, dental institutions and dental practices in India. Indian J Dent Sci 2011; 3:8-11.
14. Dhar V, Jain A, Van Dyke TE, et al. Prevalence of dental caries and treatment needs in the school-going children of rural areas in Udaipur district. J Indian Soc Pedod Prev Dent. 2007; 25:119–121.
15. Saravanan S, Kalyani V, Vijayarani MP, et al. Caries prevalence and treatment needs of rural school children in Chidambaram Taluk, Tamil Nadu, South India. Indian J Dent Res 2008; 19:186–190.
16. Joshi N, Sujan SG, Joshi K, et al. Bhavna dave prevalence, severity and related factors of dental caries in school going children of Vadodara city– An epidemiological study. J Int Oral Health 2013; 5:40-48.
17. Farooqi FA, Khabeer A, Moheet IA, et al. Prevalence of dental caries in primary and permanent teeth and its relationship with tooth brushing habits among schoolchildren in eastern Saudi Arabia. Saudi Med J 2015; 36:737–742.
18. Kumar S, Tadakamadla J, Duraiswamy P, et al. Dental caries and its socio-behavioral predictors-An exploratory cross-sectional study. J Clin Pediatr Dent 2016; 40:186–192.
19. Al-Rafee MA, AlShammery AR, AlRumikan AS, et al. A comparison of dental caries in urban and rural children of the Riyadh Region of Saudi Arabia. Front Public Health 2019; 7:195.
20. Srisilapanan P, Nirunsittirat A, Roseman J. Trends over time in dental caries status in urban and rural Thai children. J Clin Exp Dent 2017; 9.
21. Soroye MO, Adegbulugbe CI. Oral health status, knowledge of dental caries aetiology, and dental clinic attendance: A comparison of secondary school students in the rural and urban areas of Lagos. Port Harcourt Med J 2016; 10:42-49.
22. Sanadhya S, Aapaliya P, Jain S, et al. Assessment and comparison of clinical dental status and its impact on oral health-related quality of life among rural and urban adults of Udaipur, India: A cross-sectional study. J Basic Clin Pharmacy 2015; 6:52-58.
23. Akinyamoju CA, Dairo DM, Adeoye IA, et al. Dental caries and oral hygiene status: Survey of schoolchildren in rural communities, Southwest Nigeria. Nigerian Postgrad Med J 2018; 25:239-245.
24. Maserejian NN, Tavares MA, Hayes C, et al. Rural and urban disparities in caries prevalence in children with unmet dental needs: The New England Children's Amalgam Trial. J Public Health Dent 2008; 68:7-13.
25. Patro BK, Kumar RB, Goswami A, et al. Prevalence of dental caries among adults and elderly in an urban resettlement colony of New Delhi. Indian J Dent Res 2008; 19:95-98.
26. David J, Wang NJ, Astrøm AN, et al. Dental caries and associated factors in 12-year-old schoolchildren in Thiruvananthapuram, Kerala, India. Int J Paediatr Dent 2005; 15:420-428.
27. Kalsbeek H, Helderman WHP. Oral health in 13–15-year-old schoolchildren in Palmyra, Syrian Arab Republic. Leiden, The Netherland: TNO Prevention and Health 2004.
28. Hannan MA, Chowdhury MTH, Khan MAI, et al. Prevalence of gingivitis, plaque accumulation and decayed, missing and filled teeth among slum population in Bangladesh. Med Res Counc Bull 2014; 40:47-51.
29. Arvidson-Bufano UB, Holm AK. Dental health in urban and rural areas of central and western Bangladesh. Trpoical Dent J 1990; 13:81-86.
30. Baiju RM, Peter E, Nayar BR, et al. Prevalence and predictors of early periodontal disease among adolescents. J Indian Society Periodontol 2019; 23:356-361.
31. Vadiakas G, Oulis CJ, Tsinidou K, et al. Oral hygiene, and periodontal status of 12 and 15-year-old Greek adolescents: A national pathfinder survey. Eur Archives Paediat Dent 2012; 13:11-20.
32. Popoola BO, Dosumu EB, Ifesanya JU. Periodontal status and treatment need among adolescents in Ibadan, Southwestern Nigeria. Braz J Oral Sci 2015; 14:117-121.
33. Sane AS, Nikhbakht-Nasrabadi A. Periodontal health status and treatment needs in Iranian adolescent population. Arch Iran Med 2005; 8:290-294.
34. Zhang S, Xu B, Liu J, et al. Dental and periodontal status of 12-year-old Dai school children in Yunnan Province, China: A cross-sectional study. BMC Oral Health 2015; 15:117.

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Depression among elderly diabetics at Peripheral health centres attached to a private medical college in Coimbatore: a cross sectional study

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ABSTRACT

Introduction; Depression is an illness that may affect and be affected by diabetes mellitus. The clinical guidelines for older adults with diabetes mellitus have recognized this by recommending screening for and management of depression in diabetic elderly people. The aim of this study was to find out the prevalence of depression using PHQ -9 questionnaire among elderly diabetics in peripheral primary health care centres attached to a Private medical college. **Objectives:** 1. To find out the prevalence of depression among elderly diabetics 2. To find out the factors associated with depression among elderly diabetics **Methodology:** This cross sectional study was conducted among known diabetics above 60 yrs of age attending the NCD clinic of Peripheral health centres at Peelamedu, Vedapatti, Neelambur during December 2017 – April 2018. The socio demographic factors and morbidity factors such as fasting blood sugar, duration of diabetes, history of hypertension, past history of diabetes related complications, hours of sleep were included to find out the association with levels of depression. **Results:** Among the study population, 40.4 % were having the signs of depression in which the majority have only minimal symptoms of depression. The factors such as Female Gender, Fasting blood sugar >126 mg/dl and hours of sleep < 7 hours found to be statistically significant association with depression. **Conclusion:** The prevalence of depression using PHQ 9 is 40.4% and the factors such as female gender, fasting blood sugar more than 127 mg/dl and sleep at night less than 7 hours were found to have statistically significant association with depression.

Key word: Depression, PHQ-9, Screening, Prevalence, Diabetes, Elderly.

INTRODUCTION

Depression is an illness that may affect and be affected by diabetes mellitus. Depression is an independent risk factor for the onset of type 2 diabetes mellitus. It negatively affects the course of diabetes and is associated with increased risk of complications, hyperglycemia, and mortality. Depression may exert its negative effect through hormonal, neuronal, or immune system changes that directly affect the body's ability to produce or use insulin. or, the effect of depression may be indirect, by resulting in poor self-care behaviours, such as overeating, drinking alcohol, not exercising, skipping medications, or failing to keep medical appointments. Thus, identifying and treating depression in diabetes is strongly recommended.[1]

Evidence suggests that depression may relate to poorer glycaemic control.[2] and that poorer control predicts a poorer course of depression in adult samples.[3]There is some evidence that depression does not prospectively predict worsening of blood glucose control in the elderly, but more research needs to be done to understand this relationship in this group.

In interactions with the health care system, the presence of depression may influence decisions made by health care providers concerning medical outcomes (e.g., blood glucose and blood pressure). For example, providers may set goals that are easier to achieve, yet are less stringent, when they believe that depression will interfere with a patient's ability to adhere to a stricter regimen. Depression can also have an effect on patients' own goals and preferences for treatment, because depressed patients may feel too hopeless or helpless to embark on the lifestyle changes that are most beneficial for diabetes self-care.

Thus, in many different ways, diabetes may become more difficult to manage for elderly people who are also depressed, and depression may become more difficult to resolve in those who have diabetes, resulting in higher health care utilization and medical costs for those with this comorbidity.[4] The clinical guidelines for older adults with diabetes mellitus have recognized this by recommending screening for and management of depression in diabetic elderly people.

Tools such as the short form of the Geriatric Depression Scale, the Patient Health Questionnaire-9, and the newly

developed Depression and Suicide Screen are useful. [5] The present study used PHQ – 9 questionnaire to arrive at a provisional diagnosis of depression as patients with minimal symptoms (score 5-9), minor depression (score 10-14), moderate Major depression (score 15-19), severe major depression (score >20).

The objectives of this study were to find out the prevalence of depression using PHQ -9 questionnaire among elderly diabetics in peripheral primary health care centres attached to a Private medical college and to find out the factors associated with depression.

METHODOLOGY

Department of community medicine of PSG Institute of Medical sciences and Research has one Urban (Peelamedu) and Two Rural health training centres (Vedapati and Neelambur) to deliver primary health care to people. These centres have a basic laboratory services to manage Non communicable diseases (NCD). All these three centres run NCD clinic on a particular day of the week and maintain NCD register. This cross sectional study was conducted among known diabetics above 60 years of age attending the NCD clinic of Peripheral health centres at Peelamedu, Vedapatti, Neelambur during December 2017 – April 2018. By purposive sampling, 104 elderly diabetics those came for review during the study period at NCD clinic were selected. All registered diabetics above 60 years of age attending the NCD clinic included and those who were on treatment for pre-existing major and minor mental illness excluded.

After obtaining approval from Institutional Human Ethics Committee, informed consent obtained from the patients at NCD clinics of peripheral health centres. Study Questionnaire with PHQ-9 administered to the patients till the sample size of 100 reached. Questionnaire contains the following items i.e. Socio demographic variables, fasting blood sugar, presence of hypertension, complications related to diabetes and hours of sleep at past night to find out the association of above factors on depression among the study population.

Those patients found to be having depression will be followed up based on degree of depression at the peripheral health centres. Proportions, test of statistical significance (Chi square) and odds ratio with 95% confidence interval (CI) using SPSS version 24 were calculated.

RESULTS

Table 1: Distribution of study population at Peripheral Health Centres

Peripheral Health centres	Study population	Percent (%)
UHTC (Peelamedu)	46	44.2
VHTC (Vedapatti)	29	27.9
NHTC (Neelambur)	29	27.9
Total	104	100

The present study intended to find out the prevalence of depression among elderly diabetics and the factors associated with depression. There were 104 elderly

Table2: Prevalence of depression among the study population

Depression	Study population	Percent (%)
NO DEPRESSION	62	59.6
DEPRESSION	42	40.4
Total	104	100

Table 3: PHQ 9 Grading of Depression among the study population

PHQ 9 Grading	Study population	Percent (%)
NO DEPRESSION	62	59.6
MINIMAL SYMPTOMS	25	24
MINOR DEPRESSION	9	8.7
MODERATE MAJOR DEPRESSION	5	4.8
SEVERE MAJAOR DEPRESSION	3	2.9
Total	104	100

Table 4: Socio Demographic and co-morbidity variables of the study population

Variables	Category	Study population (n=104)	Percent (%)
Age in years	60-69	73	70.2
	70-79	27	26
	>80	4	3.8
Gender	Male	47	45.2
	Female	57	54.8
Educational status	Illiterate	32	30.8
	Primary	39	37.5
	Secondary	26	25
	Intermediate	4	3.8
	College	3	2.9
Marital status	Married	76	73.1
	Divorced	1	1
	Widow	27	26
Socio-economic status (modified Prasad scale August 2017)	CLASS I Rs>5910	22	21.2
	CLASS II Rs 2960 - 5909	34	32.7
	CLASS III Rs 1770-2959	16	15.4
	CLASS IV Rs 890-1769	22	21.2
	CLASS V Rs >890	10	9.6
Duration of Diabetes	<10 YEARS	81	77.9
	>11 YEARS	23	22.1
Fasting blood sugar level	FBS <126 mg/dl	31	29.8
	FBS >127 mg/dl	73	70.2
Hours of sleep	<7 hours	83	79.8
	>8 hours	21	20.2
Hypertension	Yes	53	51
	No	51	49
Complications	Yes	47	45.2
	No	57	54.8

Table 5: Factors associated with depression in the study population by univariate analysis

Study variables	Category	Depression	No depression	Unadjusted Odds Ratio 95% CI	P Value
Age	60-70 years	32(39.5%)	49(60.5%)	1.178	
	>71 years	10(43.5%)	13(56.5%)	(0.46-3.00)	0.732
Gender	Male	12(25.5%)	35(74.5%)	3.241	
	Female	30(52.6%)	27(47.4%)	(1.40-7.48)	**0.005***
Educational status	Up to Primary	30(42.3%)	41(57.7%)	0.781	
	Secondary and above	12(36.4%)	21(63.6%)	(0.33-1.83)	0.569
Marital status	Married	31(40.8%)	45(59.2%)	0.939	
	Divorced/widow	11(39.3%)	17(60.7%)	(0.38-2.27)	0.89
Socioeconomic status	Class I,II & III	27(37.5%)	45(62.5%)	1.471	
	Class IV& V	15(46.9%)	17(53.1%)	(0.63-3.41)	0.368
Duration of Diabetes	<10 YEARS	33(40.7%)	48(59.3%)	0.935	
	>11 YEARS	9(39.1%)	14(60.9%)	(0.36-2.41)	0.544
Fasting blood sugar level	FBS <126 mg/dl	8(25.8%)	23(74.2%)	2.506	
	FBS >127 mg/dl	34(46.6%)	39(53.4%)	(0.99-6.33)	**0.038***
Hours of sleep	<7 hours	38(45.8%)	45(54.2%)	0.279	
	>8 hours	4(19.0%)	17(81.0%)	(0.08-0.89)	**0.028***
Hypertension	Yes	18(34.0%)	35(66.0%)	0.579	
	No	24(47.1%)	27(52.9%)	(0.26-1.27)	0.174
Complications	Yes	16(34.0%)	31(66.0%)	0.615	
	No	26(45.6%)	31(54.4%)	(0.27-1.36)	0.231

* Statistically significant (p< 0.05)

diabetics (Table. 1) included in the study. Among the study population, 40.4 % were having the signs of depression (Table.2), of which 24% have only minimal symptoms of depression (Table.3).

The socio demographic factors i.e. age, gender, education, income per capita per month and morbidity factors such as fasting blood sugar, duration of diabetes, history of hypertension, past history of diabetes related complications, hours of sleep and PHQ -9 score are shown in Table.4.

Each of the above variables were categorized into two to find out the statistically significant (p< 0.05) association with depression among the study population using chi square test (Table. 5) in SPSS version 24. The factors such as Female Gender, fasting blood sugar >126 mg/dl and hours of sleep < 7 hours found to be statistically significant association with depression in the present study.

DISCUSSION

In the present study the prevalence of depression among elderly with diabetes mellitus was found to be 40.4%. The study conducted by SA Black et al showed that 31.1% of the older diabetic individuals reported high levels of depressive symptoms. [6] Prevalent major depressive episodes were present in 15.4% of the subjects with diabetes, which was significantly higher than in nondiabetic subjects in a study conducted among community-dwelling elderly persons with diabetes mellitus by P. de Jonge et. al. [7] In a study conducted by Manjiri D Pawaskar et al. among Medicare enrolled elderly population with type 2 diabetes mellitus, 17% had depressive symptoms. [8] In a study conducted by Kee-Lee Chou et al, on Prevalence of depression among elderly Chinese with diabetes, 26% of elderly diabetics reported elevated levels of depressive symptoms. [9] Elderly female diabetics are more among depressed (OR 3.2, 95%CI: 1.40-7.48) than men in this study which is statistically significant. Finkelstein EA et al reported diagnosed annual prevalence rates of major depression more among elderly female diabetics. In our study there is no significant association between the subjects with duration of diabetes less than 10 years and more than 10 years whereas in a study done by Yu-Ling Bai et al. a significant difference was found for depression scores between subjects with diabetes for <1 year and those with diabetes for >10 years.[10] Elderly with high Fasting blood sugar >127 mg/dl have higher risk of depression (OR 2.5, 95%CI: 0.99-6.33) than those with Fasting blood sugar <126 mg/dl in the present study. PJ Lustman et al reported in the meta analytic review of 24 studies that Depression is associated with hyperglycemia in patients with type 1 or type 2 diabetes3. However, association of depression with hyperglycemia in elderly diabetics is not documented. Elderly diabetics who slept more than 8 hours at night had lesser risk of depression (OR 0.279, 95%CI: 0.08-0.89) than those who slept less than 7 hours at night in this study. The factors such as age, education,

income, marital status, past history of diabetes related complications and hypertension were not found to be significant in this study. This study has few limitations like very small sample size, FBS is taken as an indicator for glycemic control instead of Hb A1C and PHQ 9 administered by different doctors at three health centres.

Conclusion:

This cross sectional study among elderly diabetics in primary health centre setting of a private medical college revealed that prevalence of depression using PHQ 9 is 40.4% and the factors such as female gender, fasting blood sugar more than 127 mg/dl and sleep at night less than 7 hours were found to have statistically significant association with depression.

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REFERENCES

1. Trief PM. Depression in elderly diabetes patients. Diabetes spectrum. 2007 Apr 1;20(2):71-5
2. Finkelstein EA, Bray JW, Chen H, Larson MJ, Miller K, Tompkins C, Keme A, Manderscheid R: Prevalence and costs of major depression among elderly claimants with diabetes. Diabetes Care 2003Feb 1:26:415–420
3. Lustman PJ, Anderson RJ, Freedland KE, DeGroot MK, Carney RM, Clouse RE: Depression and poor glycemic control: a meta-analytic review of the literature. Diabetes Care 2000 23:934 –942,
4. Finkelstein EA, Bray JW, Chen H, Larson MJ, Miller K, Tompkins C, Keme A, Manderscheid R: Prevalence and costs of major depression among elderly claimants with diabetes. Diabetes Care 2003Feb 1:26:415–420,
5. Hoyl MT, Alessi CA, Harker JO, Josephson KR, Piertruszka FM, Koelfgen M, Mervis JR, Fitten LJ, Rubenstein LZ: Development and testing of a five-item version of the Geriatric Depression Scale. J Am Geriatr Soc 1999 47 :873–878,
6. S.A.Black, Increased health burden associated with comorbid depression in older diabetic Mexican Americans. Results from the Hispanic Established Population for the Epidemiologic Study of the Elderly survey; Diabetes Care 1999 Jan;22(1):56-64.
7. De Jonge P, Roy JF, Saz P, Marcos G, Lobo A. Prevalent and incident depression in community-dwelling elderly persons with diabetes mellitus: results from the ZARADEMP project. Diabetologia. 2006 Nov 1;49(11):2627-33
8. Pawaskar MD, Anderson RT, Balkrishnan R. Self-reported predictors of depressive symptomatology in an elderly population with type 2 diabetes mellitus: a prospective cohort study. Health and quality of life outcomes. 2007 Dec;5(1):50
9. Chou KL, Chi I. Prevalence of depression among elderly Chinese with diabetes. International Journal of Geriatric Psychiatry. 2005 Jun;20(6):570-5
10. Bai YL, Chiou CP, Chang YY, Lam HC. Correlates of depression in type 2 diabetic elderly patients: A correlational study. International journal of nursing studies. 2008 Apr 1;45(4):571-9.

OPEN

A Prospective Cohort Study on the Safety of Infant Pentavalent (DTwP-HBV-Hib) and Oral Polio Vaccines in Two South Indian Districts

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Background: Safety of pentavalent (DTwP-HBV-Hib) vaccine has been a public concern in India and other countries. This study attempted to document the association of serious adverse events following immunization (AEFI, including hospitalizations and deaths of all causes) with the 3 doses of pentavalent and oral poliovirus (OPV) vaccines.

Methods: A cohort of 30,688 infants in 2 south Indian districts were enrolled and followed-up between October 2014 and May 2016, following their first vaccination with DTwP-HBV-Hib and OPV at public health facilities. During weekly follow-ups, by telephone or home visits, the serious AEFIs (hospitalizations and deaths) occurring any time after each vaccination until 4 weeks after third dose were documented. The incidence risk ratios (IRRs) of serious AEFIs in the first (days 0–6) and fourth weeks (days 21–27) after the vaccine doses were compared using the poisson regression analysis.

Results: Of the 30,688 infants enrolled, 30,208 received their third doses of vaccines. During the 4-week periods following each vaccination, there were 365 hospitalizations and 17 deaths. Adjusted incidence risk ratio of 3 doses combined for post-vaccination serious AEFIs during the first week compared with fourth week was 0.8 [95% confidence interval: 0.6–1.0].

Conclusions: There was no increased risk of a serious AEFIs during the first week after any of the 3 doses of pentavalent and OPV vaccination compared with the fourth week. In the absence of any temporal clustering, mortality and hospitalization rates observed in vaccinated infants probably reflects the natural occurrence of such events.

Key Words: pentavalent vaccine, oral polio vaccine, vaccine safety, serious adverse events, hospitalization, death

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Vaccination has been one of the major success stories in global health, leading to the eradication of deadly diseases like smallpox and possibly the virtual disappearance of some diseases from several regions.[1] Over the last 25 years, effective vaccination programs have resulted in a >90% decrease in the global disease burdens for diphtheria, pertussis, and tetanus, including India.[2–4] However, in spite of the extensive evidence of their benefits, concerns and controversies about the value and safety of vaccines continue to impact adversely their acceptance in diverse contexts. Public concerns over reports linking autism with measles, mumps, and rubella vaccine in the United Kingdom had negative impact on vaccine coverage causing several disease outbreaks,[5] which spread to other countries, such as the United States, France, Italy, and the Netherlands.[6–9] This misinformation has spread globally. The rumor mongering through social media considerably affected vaccine coverage during the MR campaigns in south India.[10] The growing global challenge of vaccine hesitancy relates largely to vaccine safety concerns, leading to public confidence erosion, which in turn dampens the vaccine coverage.[11] The Global Advisory Committee on Vaccine Safety emphasized the need for thorough investigation and causality assessment of serious adverse events following immunization (AEFI) as a quality assurance mechanism for immunization programs.[12]

The decreased disease burdens in the developed countries have largely been due to the successful implementation of immunization programs using multivalent combination vaccines. The vaccines against diphtheria, tetanus, and pertussis combined (DTwP) are in use for several decades under universal immunization program. Recently the HBV and *Haemophilus influenza* type b (Hib) have been added to DTwP, known as the pentavalent (DTwP-HBV-Hib) vaccine. Over the past 15 years Global Alliance for Vaccines and Immunizations has supported the introduction of pentavalent vaccine in developing countries for protection against invasive Hib disease in universal immunization program.[13] However, there were temporary interruptions and introduction delays in Sri Lanka,

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Bhutan, Vietnam[14] and Pakistan[15] during 2011–2016 due to reports of hospitalizations and deaths following vaccination after national launch of the pentavalent vaccine. Temporal association of vaccinations and sudden infant death syndrome (SIDS) has been the subject of extensive speculation, despite several studies having refuted any causal association,[16–18] particularly after the diphtheria-pertussis-tetanus immunization.[19]

In India, the reports of 32 infant deaths following the pentavalent vaccination as part of the national AEFI surveillance between 2011 and 2013 raised concerns, although no causal association could be identified.[20] Some professionals and civil society groups, however, appealed before the Supreme Court of India to ban the pentavalent vaccine.[21,22] Although the safety of pentavalent vaccine has been documented in the pre-licensure studies,[23,24] these studies were too small to identify rare adverse events. Thus, it was deemed necessary to carry out further investigations on this issue. This study was designed in consultation with all key stakeholders including the Ministry of Health and Family Welfare, Government of India and WHO (advised by Global Advisory Committee on Vaccine Safety), to assess the risk of serious AEFIs (hospitalizations and deaths) following pentavalent vaccination in an infant cohort from 2 Indian states, where pentavalent vaccine was first introduced.

MATERIALS AND METHODS

The pentavalent vaccine (manufactured by Serum Institute of India Ltd., Pune, India) was first introduced in the states of Kerala and Tamil Nadu in December 2011 with a 6, 10 and 14 weeks schedule for 3 primary doses. Both states have efficient health systems with high vaccination coverage[25] and low infant-mortality rates (IMR): 12 and 21 per 1000 live births in 2013 in Kerala and Tamil Nadu, respectively, compared with an overall IMR of 40 across India.[26] This study was conducted in 2 districts, Kollam (Kerala State) and Coimbatore (Tamil Nadu State).

Study Design

This prospective self-controlled cohort study enrolled infants who received their first dose of pentavalent and oral poliovirus (OPV) vaccine from the public health system between October 2014 and November 2015 in Kollam district, and between May 2015 and February 2016 in Coimbatore district.

Recruitment and Data Collection

Infants who received their first dose of pentavalent and OPV vaccines at all public health facilities in the 2 districts (District, Special and Taluk Hospitals, Community Health Centers, Primary Health Centers, Sub-centers) and out-reach points of rural and urban areas were traced based on the contact details recorded in the immunization registers. Home visits were made to confirm eligibility (received first dose of vaccine, expected to stay in the district for the 6 months follow-up period and contactable by telephone/mobile) and to enroll infants after obtaining informed written consent. Data on maternal care, infant gender, date of birth, birth weight, pregnancy-related and neonatal illnesses were collected at enrollment. The parent(s) were contacted weekly by telephone or home visit to document the health of the infant including any illness, hospitalization or death. The follow-up contact was continued even if the second and third vaccine doses were delayed due to any reason. Study staff confirmed receipt of the second and third vaccine doses from the vaccination card and the health facility register. If infants received their second and third vaccine doses at private facilities, a copy of the vaccination card was collected as proof of date of exposure. For each dose, the vaccine batch number, date and place of administration was documented. Recruited infants were followed up to 4 weeks after the third dose of the vaccines or death, whichever was earlier. Data were collected on tablets using a customized program developed with open source platforms (Android, PHP and MySQL version 5.1) and data including other materials uploaded, synchronized in the server and stored/deposited in a central data repository. An independent International Advisory Group (IAG) with vaccine safety experts and Brighton Collaboration representation supervised the study implementation including field visits. The IAG reanalyzed the data for validation.

Outcomes

This study captured the serious AEFIs which included both hospitalizations and deaths due to any cause during the follow-up period. The details about the events such as clinical features, diagnosis, treatment, period and place of hospitalization were captured. For hospitalizations, the study team visited the hospital for confirmation and followed till outcome to document the information. Hospitalization episodes of at least 24-hours as inpatient were further categorized as "appropriate," "inappropriate" and "indeterminate" using the Pediatric Appropriateness Evaluation Protocol–India criteria. The PAEP-India tool for child and newborn were adapted from the available tools and validated in hospitalized patients from multiple hospitals in India.[27] Each case was assessed by 2 independent pediatricians using the PAEP-India tool for child and categorized the case based on checklist criteria. A third pediatrician was consulted in cases of mismatch between the 2 reviewers. For deaths, the study confirmed the event by home visit, followed by verbal autopsy by senior investigator 4 weeks later. Causes of death were determined independently by 3 pediatricians trained in review of verbal autopsies and also referred to available hospital records.

Data Analysis

The 4 weeks following every dose of vaccination were considered to be the observation period with weekly intervals post-vaccination as separate risk periods (Figure, Supplemental Digital Content 1, http://links.lww.com/INF/D758). As almost all the serious AEFI (deaths and hospitalizations) reported in the passive national AEFI surveillance program occurred during the first week after any vaccine dose, the first week was considered as the high-risk period and the fourth week as the comparison period. It was hypothesized that, the risks of serious AEFI due to any cause, during the first and fourth weeks post-vaccination remain similar for each dose of the vaccines.

Person-time of observation was calculated based on the time from vaccine administration and the date of occurrence of an event, the date of administration of the next vaccine dose (if <28 days), the last contact date (if lost to follow-up), date of death or the first 28 days after vaccination when the next dose was delayed, according to the recommended schedule, whichever came first. Follow-up was defined by weekly risk windows after each vaccine dose split into first (0–6 days), second (7–13 days), third (14–20 days) and fourth (21–27 days) weeks. If a subsequent dose was given before the end of the fourth week, the follow-up time was censored and follow-up period was assigned to the subsequent dose. If the next dose was delayed, the person-time beyond 27 days after each dose were excluded from the analysis. Poisson regression analysis was used to estimate the incidence rates (IRs) and incidence rate ratios (IRRs) for serious AEFIs (hospitalization and all-cause deaths) during the 4-consecutive weekly risk periods after each dose separately and for all 3-doses combined. IRs were calculated by dividing the number of events by the corresponding person-time denominator and IRRs were calculated by dividing the IR in the risk period of interest by the IR in the fourth week. In a multivariate model, the IRR

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was adjusted for age at start of risk period (continuous, in days) and seasonality (quarterly; January–March, April–June, July–September, and October–December). To assess the potential of healthy vaccinee bias, we performed a sensitivity analysis to compare the risks in the first week after vaccination following the second and third doses with the risks in the fourth weeks of the preceding doses. All statistical analyses were performed using Stata version 15.0 (StataCorp LLC, TX). In addition, all deaths documented were reviewed by the national AEFI committee for causality assessment according to WHO published methodology.[20]

Sample Size

Considering the IMR in the study states to be 12–21 per 1000 live births and a neonatal mortality rate (0–28 days) of 6–15 per 1000 live births,[26] the post-neonatal mortality (29–365 days) was expected to be 6 per 1000 live births. This translated to ~0.125 infant deaths per 1000 live births per week. As there was no information about the hospitalization:death ratio in this age group from India, we assumed that for every infant death there would be ~4 hospitalizations. The required sample size was 27,422 infants to detect an IRR for hospitalizations and or deaths of 1.8 in the analyses pooling the different doses, assuming a 5% significance level ($\alpha = 0.05$) and 80% power ($\beta = 0.2$).[28] With the available sample (30,688), the observed results show that between the risk periods, we are able to detect a risk-ratio of 2.5 and 1.5 (at a 5% significance level and 80% power) of serious AEFIs (hospitalization and deaths), for individual doses and all 3-doses combined, respectively.

Ethical Approvals

The study protocol and related documents were approved by ethics committees of participating institutes. The infants were recruited after obtaining informed written consent from parents or legally authorized representatives. The study protocol and related documents were approved by Institute Ethics committees at The INCLEN Trust International (INCLEN) (vide IIEC-021 dated October 14, 2013), Government Medical College, Thiruvananthapuram (vide 01/01/2014 dated January 17, 2014), PSG Institute of Medical Sciences and Research, Coimbatore (vide 13/351 dated March 5, 2014) and World Health organization, Geneva (vide RPC625 dated January 22, 2014).

RESULTS

During the study period, 34,914 infants received their first dose of pentavalent and OPV vaccines at the public health facilities. Of these, 1377 (3.9%) infants could not be traced, 2818 (8.1%) were not eligible (1287 were not staying in the district for 6 months, and 1531 were from other districts) and consent was refused for 31 (0.1%) infants. Of the 30,688 infants recruited, 30,208 (98.4%) received their third vaccine dose and 29,728 (96.9%) completed the scheduled follow-up (Fig. 1). Of 479,523 scheduled weekly follow-up contacts (including the contacts during the intervals between the different vaccine doses till completion of 4 weeks after third dose), 89.9% were completed with averages of 4.4 (dose 1), 4.8 (dose 2) and 4.9 (dose 3) contacts for the vaccine doses (data not shown).

Baseline characteristics of the cohort are summarized in Table 1. No age or sex differences were observed across the districts. The Kollam population had a higher proportion of low and median socio-economic status and joint or extended families. Median ages of infants were 48, 83 and 118 days at the times of the first, second and third vaccine doses, respectively. A total of 622 hospitalizations and 32 deaths were documented during the follow-up period.

Within the 4 weeks after the 3 vaccine doses (per protocol study risk periods), a total of 365 hospitalizations (120, 137 and 108 after doses 1, 2 and 3, respectively) were documented. Of these 365 hospitalizations, 288 were classified as appropriate (96, 106 and 86 after doses 1, 2 and 3, respectively). A total of 257 hospitalizations (92, 136 and 29 after doses 1, 2 and 3, respectively) occurred after fourth weeks of the first and second doses of vaccination (Fig. 1). Pneumonia/acute respiratory infection (70.1%) was the leading cause followed by diarrhea (6.4%), fever (4.3%) and urinary tract diseases (3.1%) during the 4 week and post-4 week periods after the vaccine doses (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759).

Of the 32 total deaths observed, 17 deaths (8,7 and 2 after doses 1, 2 and 3, respectively) occurred within 4 weeks after 3 vaccine doses, while 15 deaths (11, 3 and 1 after doses 1, 2 and 3, respectively) occurred during post-4 weeks period, (Fig. 1). The leading causes of death included infections (31.3%), congenital malformations (28.1%), congenital heart diseases with infections (15.6%), SIDS (15.6%) and other causes (9.4%) (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759). Almost all congenital malformations were diagnosed prior to death. The SIDS was made based on Brighton Collaboration Criteria.[29] Causality assessment by the National AEFI committee classified all deaths as coincidental. Differentials in causes of hospitalization and death during the first 4-weeks (0–27 days) and post-4 weeks (28+ days) after vaccination were small (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759). Figures 2 and 3 show the IRs of serious AEFIs (hospitalization and deaths) by age and seasonality. Higher serious AEFI (hospitalization and deaths) rates were observed during 91 and 146 days of age (Fig. 2); between October and December, with a peak in December due to higher prevalence of respiratory illnesses (Fig. 3).

Serious AEFIs (Hospitalization and Deaths) Within 4 Risk-periods

The occurrence of serious AEFIs (hospitalization and deaths) across the risk-periods for the 3 doses are summarized in Table 2. Within the 4 weeks after the 3 vaccine doses, a total of 375 serious AEFIs (365 hospitalizations, 17 deaths and 7 hospitalization and deaths) occurred (125, 140 and 110 after doses 1, 2 and 3, respectively) (Fig. 1). For all-3 doses combined, the adjusted IRRs of serious AEFIs within the first week compared with the fourth week were 0.8 [95% confidence interval, (CI): 0.6–1.0]. Compared with the fourth week, the adjusted IRRs of serious AEFIs for the first week after first, second and third doses were 1.2 (95% CI: 0.8–1.9), 0.9 (95% CI: 0.6–1.5) and 0.5 (95% CI: 0.3–0.9), respectively. IRRs for serious AEFIs were not significantly higher in any of the other risk periods (second or third weeks) either for the 3 doses separately or for all doses pooled. Analyses of hospitalizations according to appropriateness did not show any increased risk in any week compared with the fourth week after any dose (Tables, Supplemental Digital Content 3, http://links.lww.com/INF/D760, and Supplemental Digital Content 4, http://links.lww.com/INF/D761). The sensitivity analysis comparing the rates of serious AEFI in the first week after the second or third doses with the fourth week after the first or second doses did not show any differences, suggesting the absence of a healthy vaccinee effect (Table 2).

DISCUSSION

This is the first large study to examine the association between administration of routine infant pentavalent and OPV vaccines and serious AEFIs (all-cause hospitalizations and/or deaths) in India, using a self-controlled prospective cohort design. We have shown that the unadjusted and adjusted risks of hospitalizations

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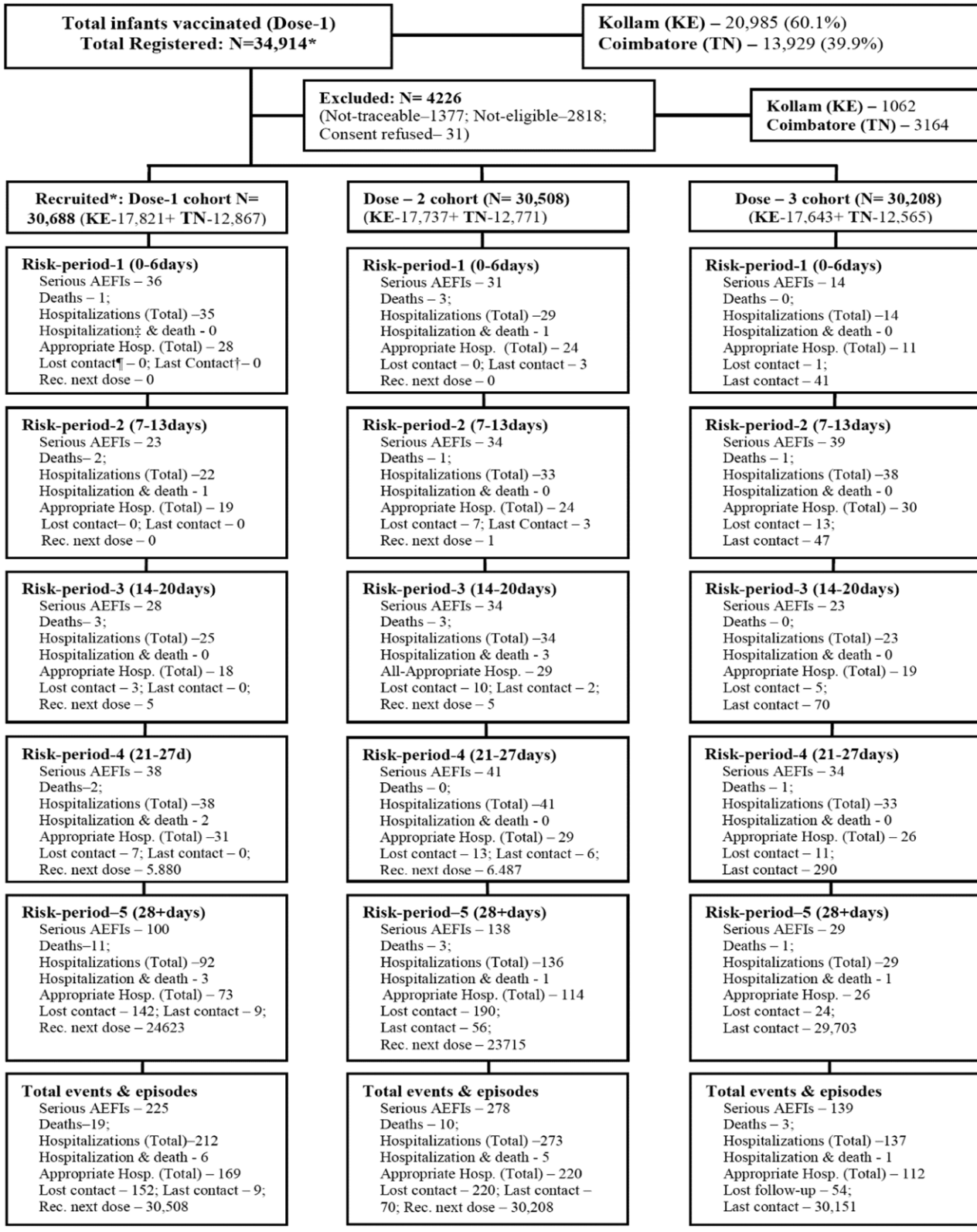


FIGURE 1. Selection and follow-up of the study population in the study districts and distribution of events. *Received first dose at public health facilities; ¶ lost contact is "Lost contact during the period"; †last contact defined as "Received respective vaccination and time period at last follow-up contact"; ‡appropriate hospitalizations as per expert opinion and identified using PAEP criteria. KE-Kerala; TN-Tamil Nadu.

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TABLE 1. Characteristics of Study Infants and Their Households

Characteristic	Kollam (N = 17,821)	Coimbatore (N = 12,867)	Combined (N = 30,688)*
Sex of the child (%)			
Males	50.3	50.4	50.3
Females	49.7	49.6	49.7
Median age at vaccination in days (P25, P75)			
Dose 1	47 (45, 50)	49 (46, 53)	48 (45, 51)
Dose 2	83 (79, 88)	83 (77, 90)	83 (78, 89)
Dose 3	118 (113, 127)	117 (109, 127)	118 (111, 127)
Birth order (%)			
1	58.7	49.4	54.8
2	37.0	43.0	39.5
≥3	4.3	7.6	5.7
Household standard of living index (%)			
Low	26.0	7.7	18.3
Middle	56.8	45.3	52.0
High	17.2	47.0	29.7
Type of family (%)			
Nuclear/elementary family	32.7	57.0	42.9
Joint/extended	67.3	43.0	57.1
Age of mother in years (mean ± SD)	25.9±4.3	24.9±4.2	25.5±4.3
Education of mother (%)			
Illiterate	0.1	2.0	0.9
Literate	99.9	98.0	99·1
Mean ± SD (in years)	12.9±2.7	11.1±3.3	12·1±3.1
Age of father in years (Mean ± SD)	32.2±4.5	30.2±4.7	31.4±4.7
Education of father (%)			
Illiterate	0.1	3.0	1.3
Literate	99.9	97.0	98.7
Mean ± SD (in years)	11.8±2.7	10.5±3.4	11.1±3.3
Received any ANC care (%)	98.9	92.5	96.2

*Total includes a small number of cases information missing for type of family, standard of living index (2 each in Kollam); Age of mother (20 in Kollam and 12 in Coimbatore); education of mother (14 in Kollam and 6 in Coimbatore); education of father (36 in Kollam and 16 in Coimbatore) and birth order (53 in Kollam and 40 in Coimbatore, respectively). Standard of living index (a proxy to household socio-economic status), constructed based on household assets and durable goods.

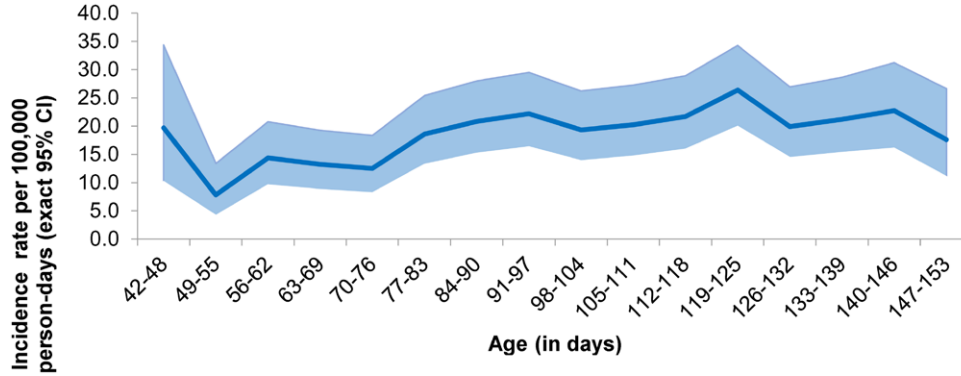


FIGURE 2. Serious AEFI (hospitalization and all-cause deaths) incidence rates (per 100,000 person-days with exact 95% CI as shaded area) by age (independent of vaccination windows). Incidence per 100,000 person-days (with exact 95% CI as shaded area); Week defined as 6-days period, starting on day-0; Person-time starts from age at first dose of vaccination and duration until the first and last day of the age period or until the Serious AEFI (hospitalization/death) events (succeeding vaccinations not considered). The hospitalization incidence presented in the figure is between 42 and 153 days of age, where 98.6% [(3,402,860/3,451,027)*100] of follow-up calls/visits were made. full color online

and deaths after any of the 3 scheduled doses of routine vaccines were similar during the first and fourth weeks post-vaccination, for either each dose separately, or all doses combined. Due to low death rates,we could not perform analyses for each individual dose but the combined analysis and analyzing the first dose only did not indicate any death clusters following vaccination.

In a highly immunized population, self-controlled study designs with defined risk windows for outcome assessment are best suited to assess possible effects during a specific time period as they are not susceptible to control selection.[30] Activation of the immune response to different vaccines occurs at different time points according to the nature of the antigen.[18,31–34] Hence, in the current study, we considered the first week after vaccination to represent the period of highest risk and the fourth week to be the comparison period. The findings of our study refute the concern of an association between serious adverse events and the pentavalent vaccine.

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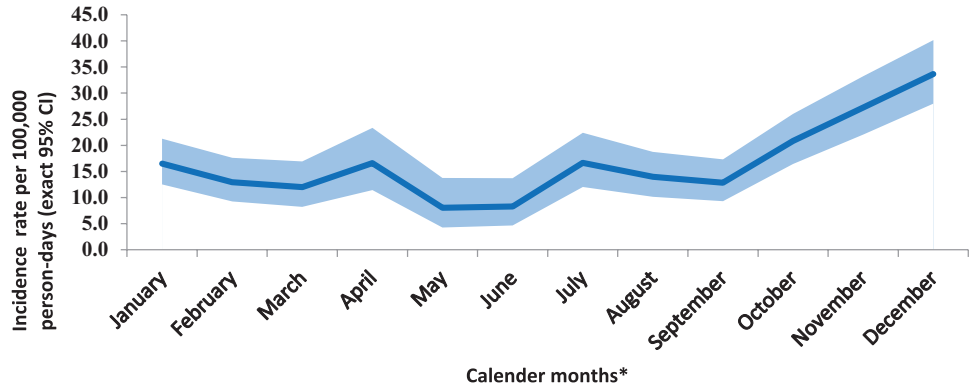


FIGURE 3. Serious AEFIs (hospitalization and all-cause deaths) incidence rates (per 100,000 person-days with exact 95% CI) by calendar months. Incidence per 100,000 person-days (with exact 95% CI); *Calendar month with 30-days period, irrespective of study year period; Person-time starts from the first day of the month and until the last day of the month period or until the Serious AEFI events (hospitalization/death). full color online

TABLE 2. Serious AEFIs (Hospitalization and All-Cause Deaths) IRs and IRRs (Unadjusted and Adjusted) After Pentavalent and Oral Polio Vaccines: Combined for Both Districts, Kollam (Kerala) and Coimbatore (Tamil Nadu), India

Vaccine dose/risk period (in days)*	Total number of		Incidence-rate (IR, with exact 95% CI)	IRRs, with 95% CI			
	Events	Person-time (in days)		Unadjusted	*P*	Adjusted†	*P*
Dose-1							
0–6 days	36	214,702	16.8 (11.7–23.2)	0.9 (0.6–1.5)	0.808	1.2 (0.8–1.9)	0.407
7–13 days	23	214,740	10.7 (6.8–16.1)	0.6 (0.4–1.0)	0.056	0.7 (0.4–1.2)	0.206
14–20 days	28	214,693	13.0 (8.7–18.9)	0.7 (0.5–1.2)	0.216	0.8 (0.5–1.3)	0.368
21–27 days‡	38	214,158	17.7 (12.6–24.4)	Reference		Reference	
Dose-2							
0–6 days	31	213,472	14.5 (9.9–20.6)	0.8 (0.5–1.2)	0.233	0.9 (0.6–1.5)	0.740
7–13 days	34	213,400	15.9 (11.0–22.3)	0.8 (0.5–1.3)	0.409	0.9 (0.6–1.5)	0.811
14–20 days	34	213,306	15.9 (11.0–22.3)	0.8 (0.5–1.3)	0.411	0.9 (0.6–1.4)	0.592
21–27 days‡	41	212,523	19.3 (13.8–26.2)	Reference		Reference	
Dose-3							
0–6 days	14	211,294	6.6 (3.6–11.1)	0.4 (0.2–0.8)	0.005	0.5 (0.3–0.9)	0.026
7–13 days	39	210,847	18.5 (13.2–25.3)	1.1 (0.7–1.8)	0.582	1.3 (0.8–2.0)	0.294
14–20 days	23	210,418	10.9 (6.9–16.4)	0.7 (0.4–1.1)	0.142	0.7 (0.4–1.2)	0.207
21–27 days‡	34	209,168	16.3 (11.3–22.7)	Reference		Reference	
All 3 doses							
0–6 days	81	639,468	12.7 (10.1–15.7)	0.7 (0.5–0.9)	0.020	0.8 (0.6–1.0)	0.068
7–13 days	96	638,987	15.0 (12.2–18.4)	0.8 (0.6–1.1)	0.226	0.9 (0.7–1.2)	0.374
14–20 days	85	638,417	13.3 (10.6–16.5)	0.7 (0.6–1.0)	0.044	0.8 (0.6–1.0)	0.062
21–27 days‡	113	635,849	17.8 (14.7–21.4)	Reference		Reference	
Sensitivity analyses							
0–6 days of dose-2	31	213,472	14.5 (9.9–20.6)	0.8 (0.5–1.3)	0.408	0.7 (0.4–1.1)	0.117
21–27 days of dose-1‡	38	214,158	17.7 (12.6–24.4)	Reference		Reference	
0–6 days of dose-3	14	211,294	6.6 (3.6–11.1)	0.3 (0.2–0.6)	0.001	0.3 (0.2–0.6)	0.001
21–27 days of dose-2‡	41	212,523	19.3 (13.8–26.2)	Reference		Reference	

*Risk period corresponds to date of hospitalization (considered as at least 24-hours stay in the hospital) or all-cause death after administration of vaccination (in days); The day of vaccination defined as day-0.

†Adjusted for seasonality (January–March, April–June, July–September, October–December); age at start of risk period (in days), using Poisson regression model.

‡Twenty-one to 27 days as reference category; IR: Number of events per 100,000 person-days of follow-time, calculated as the ratio of the hospitalizations of the person-time at risk.

The main strength of the study is the representativeness of the study cohort with respect to the population in the study area due to the high rates of participation and completion of follow-up. The socioeconomic status of the population enrolled in the study was highly representative of the overall population in the 2 districts. Use of electronic data collection enabled timely follow-up and accurate data capture with real-time monitoring. Documentation of hospitalization from both participants and hospitals allowed confirmation of causes of hospitalizations and deaths. In addition, the availability of source documents and vaccination records ensured accurate confirmation of vaccine exposure.

The main limitation was possible selection bias, as infants received first vaccine dose in the private system were not represented in the study. However, according to recent National Family Health Survey (NFHS-4, 2015–2016) data, 78% and 86% of children 12–23 months of age in Kerala and Tamil Nadu, received their vaccines from a public health facility.[25] We could not trace 3.9% infants due to inaccurate address information and 8.1% could not

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be included due to moving out or residing outside of the 2 districts. To control for confounding by contraindication to vaccination, pre-vaccination time intervals were not included in the control periods in the main analysis. Sensitivity analyses performed to assess the potential of a healthy vaccinee bias did not suggest presence of this. The study was also limited in detecting small changes for deaths, despite the large sample size.

The study found that 128 serious adverse events (120 hospitalizations and 8 deaths) occurred among 30,000 infants during the 4 weeks after receiving their first dose of pentavalent vaccine. Calculating based on Black et al[35] and projected on a 26 million annual Indian birth cohort, it is estimated that with national coverage of 100% at a minimum 26,500 infants per year will suffer a serious health event within 1 week of receiving their first dose of pentavalent vaccine, or there will be at least 21,200 events with the current 3-dose coverage of ~80% for pentavalent vaccine. These events reflect prevailing morbidities and mortality that would have occurred regardless of vaccination as documented in 2 Indian districts with low infant mortality. Investigating these events to ensure that the vaccine product or its administration are safe remains a fundamental function of the vaccine pharmacovigilance system. This highlights the importance of continued monitoring of common serious health problems in the infant population to measure the health burden and provide new epidemiologic information on the true causes of morbidity and mortality in this group.

The study did not find any increased risk of serious AEFIs (all-cause hospitalizations or deaths) in the first week following pentavalent (and OPV) vaccination and the occurrence of these AEFIs were just coincidental. That is, in the absence of temporal clustering, mortality and hospitalization rates observed in vaccinated children reflect the natural occurrence of such events in that age range in this part of India. The study findings should help to address the vaccine safety concerns and controversies around the pentavalent vaccine in India and other low- and middle-income countries, and so boost public confidence in the program. Suspicion about a possible relationship between the vaccination and serious AEFIs among parents, communities and the health providers may not only threaten the success of immunization programs but also potentially hinder introduction of newer vaccines.

CONCLUSIONS

Close monitoring of over 30,000 infants during and after receiving their first 3 doses of DTwP-HBV-Hib and OPV did not identify any relationship between the vaccinations and the occurrence of serious AEFIs (hospitalization and/or death). The study did document the huge number of serious coincidental events that can be expected even when vaccines are properly administered. In view of the recent inclusion of several new vaccines into the immunization program in India, the findings of this study will be very useful in addressing vaccine hesitancy and safety concerns to increase public acceptance and coverage.

APPENDIX

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REFERENCES

1. Roush SW, Murphy TV; Vaccine-Preventable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA*. 2007;298:2155–2163.
2. Kyu HH, Mumford JE, Stanaway JD, et al. Mortality from tetanus between 1990 and 2015: findings from the global burden of disease study 2015. *BMC Public Healt*. 2017;17:179.

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3. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375:1969–1987.
4. India State-Level Disease Burden Initiative Collaborators. Nations within a nation: variations in epidemiological transition across the states of India, 1990–2016 in the Global Burden of Disease Study. *Lancet*. 2017;390: 2437–2460.
5. Fitzpatrick M. MMR: risk, choice, chance. *Br Med Bull*. 2004;69:143–153.
6. Rosen JB, Arciuolo RJ, Khawja AM, et al. Public health consequences of a 2013 measles outbreak in New York City. *JAMA Pediatr*. 2018;172: 811–817.
7. Béraud G, Abrams S, Beutels P, et al. Resurgence risk for measles, mumps and rubella in France in 2018 and 2020. *Euro Surveill*. 2018;23 :pii=1700796.
8. Siani A. Measles outbreaks in Italy: a paradigm of the re-emergence of vaccine-preventable diseases in developed countries. *Prev Med*. 2019;121: 99–104.
9. Lisowski B, Yuvan S, Bier M. Outbreaks of the measles in the Dutch Bible Belt and in other places - new prospects for a 1000 year old virus. *Biosystems*. 2019;177:16–23.
10. Rao M, Govindarajan V. WhatsApprumours about vaccinations hamper India's drive to halt measles and rubella, The government plans to extend the campaign by a week to achieve targets. scroll.in Media report, [Internet]. Feb 24 2017; Available from: https://scroll.in/pulse/830129/rumours-about-measles-rubella-vaccine-hit-coverage.
11. Larson HJ, de Figueiredo A, Xiahong Z, et al. The state of vaccine confidence 2016: global insights through a 67-country survey. *EBioMedicine*. 2016;12:295–301.
12. World Health Organization. *Global Manual on Surveillance of Adverse Events Following Immunization*. Geneva: World Health Organization; 2014.
13. Centers for Disease Control and Prevention (CDC). Progress toward introduction of Haemophilusinfluenzae type b vaccine in low-income countries--worldwide, 2004-2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:148–151.
14. World Health Organization. Global Advisory Committee on Vaccine Safety (GACVS), Report of meeting 12–13 June 2013. *ReleveEpidemiolHebd*. 2013;88:301–312.
15. TVR Staff (Media report). Three infants in Pakistan die after receiving polio vaccine. The Vaccine Reaction [Internet]. May 13, 2018; Available from: https://thevaccinereaction.org/2018/05/three-children-die-after-receiving-polio-vaccine-in-pakistan/#_edn1.
16. Chen RT, Shimabukuro TT, Martin DB, et al. Enhancing vaccine safety capacity globally: a lifecycle perspective. *Vaccine*. 2015;33(suppl 4): D46–D54.
17. Vennemann MM, Höffgen M, Bajanowski T, et al. Do immunisations reduce the risk for SIDS? A meta-analysis. *Vaccine*. 2007;25:4875–4879.
18. Kuhnert R, Schlaud M, Poethko-Müller C, et al. Reanalyses of case-control studies examining the temporal association between sudden infant death syndrome and vaccination. *Vaccine*. 2012;30:2349–2356.
19. Walker AM, Jick H, Perera DR, et al. Diphtheria-tetanus-pertussis immunization and sudden infant death syndrome. *Am J Public Health*. 1987;77:945–951.
20. Ministry of Health and Family Welfare (MoHFW),Government of India. Results of causality assessment for reported AEFIs [Internet]. New Delhi: Ministry of Health and Family Welfare, Government of India; 2018. Available from: https://mohfw.gov.in/node/107.
21. Moneylife Digital Team Media reports. Stop unnecessary vaccination: eminent doctors, scientists to the PM. [Internet]. Dec 1, 2014; Available from: https://www.moneylife.in/article/stop-unnecessary-vaccination-eminent-doctors-scientists-to-the-pm/39645.html.
22. Nair H, Hazarika I, Patwari A. A roller-coaster ride: introduction of pentavalent vaccine in India. *J Glob Health*. 2011;1:32–35.
23. Sharma HJ, Yadav S, Lalwani SK, et al. Immunogenicity and safety of an indigenously manufactured reconstituted pentavalent (DTwP-HBV+Hib) vaccine in comparison with a foreign competitor following primary and booster immunization in Indian children. *Hum Vaccin*. 2011;7:451–457.
24. Dutta AK, Verghese VP, Pemde HK, et al. Immunogenicity and safety of a pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Haemophilus influenzae type B conjugate combination vaccine (Pentaxim) with hepatitis B vaccine. *Indian Pediatr*. 2009;46:975–982.
25. International Institute for Population Sciences (IIPS) and ICF. National Family Health Survey (NFHS-4), 2015-16: India [Internet]. Mumbai: IIPS; 2017. Available from: http://rchiips.org/nfhs/NFHS-4Reports/India.pdf.
26. Ministry of Home Affairs, Government of India. SRS Statistical Report 2013, Report No. 1 of 2014:75-76 [Internet]. Ministry of Home Affairs, Government of India; 2014. Available from: http://www.censusindia.gov.in/vital_statistics/SRS_Reports_2013.html.
27. Das MK, Arora NK, Poluru R, et al. Pediatric Appropriate Evaluation Protocol for India (PAEP-India): tool for assessing appropriateness of pediatric hospitalization. *Indian Pediatr*. 2018;55:1041–1045.
28. Lwanga S, Lemeshow S. *Sample Size Determination in Health Studies: A Practical Manual*. Geneva: World Health Organization; 1991.
29. Jorch G, Tapiainen T, Bonhoeffer J, et al.; Brighton Collaboration Unexplained Sudden Death Working Group. Unexplained sudden death, including sudden infant death syndrome (SIDS), in the first and second years of life: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*. 2007;25:5707–5716.
30. Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. *Epidemiol Infect*. 2011;139:1805–1817.
31. Gold M, Dugdale S, Woodman RJ, et al. Use of the Australian Childhood Immunisation Register for vaccine safety data linkage. *Vaccine*. 2010;28:4308–4311.
32. Cameron JC, Walsh D, Finlayson AR, et al. Oral polio vaccine and intussusception: a data linkage study using records for vaccination and hospitalization. *Am J Epidemiol*. 2006;163:528–533.
33. Stowe J, Andrews N, Ladhani S, et al. The risk of intussusception following monovalent rotavirus vaccination in England: a self-controlled case-series evaluation Ref. No: JVAC-D-16-01124. *Vaccine*. 2016;34:6115.
34. Patel MM, López-Collada VR, Bulhões MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med*. 2011;364:2283–2292.
35. Black S, Eskola J, Siegrist C-A, et al. Importance of background rates of disease in assessment of vaccine safety during mass immunisation with pandemic H1N1 influenza vaccines. *Lancet*. 2009;374:2115–2122.

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Usage of personal protective equipments among workers of a foundry in South India: interventional study

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ABSTRACT

Introduction: Occupational hazards exist in every industry. Along with engineering and administrative measures, usage of personal protective equipments (PPE) by the workers is equally important in protecting the workers. However a high level of non-compliance to PPE usage among the workers is commonly noted. South India has several foundries, giving employment to many workers. The objectives of the study were to find out the level of PPE usage among the foundry workers, before and after an interventional program and to assess the relative effectiveness of different strategies of the program in bringing out the improved PPE compliance among them. **Materials and methods**: After obtaining ethical clearance, the study was conducted among 78 permanent workers of a foundry in Coimbatore. First the demographic details of the subjects and their level of usage of PPE were recorded, in the pre-test. Then a comprehensive three faceted interventional program, consisting of strategies such as health education to the workers, peer motivation by co-workers, and posters in the working environment, was implemented simultaneously among all subjects. After three months of implementation of the program, the post-test was conducted and the data were analyzed to measure the effectiveness of the intervention. **Results**: After the interventional program, the workers showed significant improvement in PPE usage ($p<0.001$) from the 'pre-test' level. Regarding their perception about which of the three strategies relatively contributed to bring out the improvement, most of the workers rated the strategy of peer motivation as 'high' effectiveness, health education- 'average' effectiveness and posters in working environment- 'low' effectiveness. **Conclusion**: Interventional program using multiple strategies in an integrated manner has synergistically enhanced the behaviour change among the workers.

Key Words: Foundry, Personal protective equipment, Intervention.

INTRODUCTION

Foundry[1] is an industry which produces metal castings that are made by pouring molten metal into precise moulds. After the metal has cooled, the casting is removed from the mould and the process of 'fettling' involves cleaning and finishing procedures. Occupational hazards[2] exist in every industry and the hazards in the foundry are heat, electricity, machinery, vibration, noise, dust & fumes, heavy objects, pointed objects, chemicals like formaldehyde and amines etc. Along with engineering and administrative measures, usage of personal protective equipments (PPE) by the workers is equally important in protecting the workers. Various PPE are expected to be used in foundry, such as helmet, goggles, ear plugs, mask, face-shield, gloves, boots, etc.

However, a high level of non-compliance for PPE usage among the workers is commonly noted possibly because of ignorance about benefits, or it may cause discomfort. Research studies[3-6] conducted on non-compliance to PPE usage among industrial workers, found that it is a global problem. A study published by Foundry Management & Technology, USA[3] recorded high level of non-compliance to PPE usage. An Egyptian study[4] among industrial workers found similar findings. A cross-sectional study from Nepal[5] detected that only 47.7% of workers use at least one kind of PPE during work. A study conducted among factory workers in India[6] noted that most workers were aware of the benefits of PPE, but only some workers were using PPE regularly.

Similarly various other studies have been done on the effectiveness of interventional program in bringing out improvement in PPE usage among industrial workers. A study in USA[7] demonstrated statistically significant difference in the use of PPE before and after an interventional training program. Similarly a Nigerian study[8] found significant increase in the usage of PPE, after the intervention. Another multi-faceted interventional study done among foundry workers in Italy[9] showed significant decrease in occupational injuries, after intervention.

Regarding the motivation of workers, Malawi study[10] noted that the workers viewed their high-ability coworkers as a source of motivation. According to WHO[11], one of the components essential to community based program and prevention strategies, is that it should

be an integrated program. Each element of the program should support the other elements and the program should be integrated into the settings where people work (e.g. work-sites) or live their lives.

Since behavior change is difficult to sustain unless a good supporting environment is created, we designed an intervention among foundry workers which went beyond just conducting an awareness program. Our design was guided by the Ecological Approach[11] to Behaviour Change. This approach addresses the multiple determinants that influence health and health behavior through synergy of multi-pronged interventions. Accordingly a comprehensive interventional program which had three strategies integrated together was adopted, to bring out a synergy of effect. The first strategy was health education to the workers (guided by Health Belief Model[11] for behavior change) to create the immediate awareness about the hazards that accrue due to non-usage of PPE. The second one was peer motivation by co-workers and role-models (guided by Social Cognitive Theory[11]) that will help to translate awareness into practice and to use the peer pressure to ensure compliance with PPE usage. The third strategy was posters in the working environment as constant reminders of the statuary nature of the need to use PPE at the workplace and benefits of usage from the health point of view.

The Objectives of the study were to find out the level of PPE usage among the foundry workers, before and after an interventional program and to assess the relative effectiveness of different strategies of the program in bringing out the improved PPE compliance among them.

MATERIAL AND METHODS

The study was conducted in a foundry at Coimbatore, after obtaining the permission from the relevant foundry authorities and ethical clearance from Institutional Human Ethics Committee. There were two categories of workers, permanent and temporary, in the various sections of production/ manufacturing division of the foundry. It was decided to conduct the study among the permanent workers only, because the temporary workers used to get salary in the week-end and they might go elsewhere in the next week. There were 81 permanent workers in the production division. Those workers, who were missed during a visit, were contacted during the second or third visits. Two workers could not be contacted even after three visits and one worker was not willing to participate in the study. So those 3 workers were excluded and the remaining 78 workers were included in the study.

An interview questionnaire was prepared, translated to local (Tamil) language, and back translated. A pilot study was done among the temporary workers, which helped to refine the interview questionnaire. The finalized questionnaire was then used for the study.

Pre-test: After getting informed written consent, the subjects were interviewed. For each subject, the general personal information and frequency of usage of PPE were recorded.

Interventional program: After the pre-test, all of the following three strategies of the program were started and implemented simultaneously among the subjects.

Strategy 1: 'Health education' sessions were conducted for the workers in batches, each batch consisting about 12-15 workers. Thus six sessions were conducted. Power point presentation was used for health education. During the session, it was explained how the different equipments safe-guard the workers from different hazards. 'Question and answer' method was also used which encouraged the workers to involve and pay attention. Thus every subject received health education once.

Strategy 2: 'Peer-motivaton by co-workers' was performed as follows: Some of the workers volunteered to become 'role-models'. These role-models wore PPE throughout the eight hours of work. Apart from that, for the next three months, they talked with other workers once every week, and motivated them for PPE usage. If any worker was found to be resistant to adopt PPE, the role-models talked with them more often and motivated them.

Strategy 3: 'Posters' illustrating the usage of PPE were prepared, laminated and put-up in various prominent places of the working environment. Whenever the workers would see the posters, the posters would remind them to wear PPE.

Post-test: At the end of three months, each subject was interviewed again. Along with the previous questionnaire, some more questions were added to assess the level of effectiveness of each strategy in bringing out the improvement in PPE usage.

Analysis of data: First the data were entered into Microsoft excel sheets and then transferred through SPSS package (Version 19) for analysis. For descriptive analyses, the categorical variables were analyzed by using percentages and the continuous variables were analyzed by calculating mean ± Standard Deviation. Chi-square test was used to ascertain the association between the independent and dependent variables. A 'p' value of <0.05 was regarded as statistically significant.

RESULTS

The interventional study was conducted among 78 permanent workers of a foundry.

Demography of the study subjects: Table-1 shows the general demography of the study subjects.

Table 1: General demography of the study subjects.

Demographic details	Group	Frequency (N = 78)	Mean ± SD
Gender	Men	78 (100%)	–
Literacy status	Literate	78 (100%)	–
Training on PPE usage.	All subjects got training.	78 (100%)	–
Age (in years)	< 40 years	1 (1.3 %)	48.38
	40 – 49 years	46 (59.0%)	± 5.49
	50 – 57 years	31 (39.7%)	
	Total	78 (100%)	
Work experience (in years)	5 – 9 years	2 (2.6 %)	25.77
	10 & more years	76 (97.4%)	± 7.44
	Total	78 (100%)	

All workers were men. All were literate. All were of middle age (Range: 38 – 57 years. Mean ± SD = 48.38 ± 5.49). All workers had work experience of more than 5 years (Range: 7 – 40 years; Mean ± SD = 25.77±7.44). All subjects got trained in the usage of PPE, at the time of joining the foundry. However the training was not repeated.

Table 2: Usage of PPE by the subjects during the work time.

	Frequency level of usage of PPE during the work time					
	Very low	Below average	Average	Above average	Very high	**Total**
Pre-test	7 (9.0)	16 (20.5)	38 (48.7)	11 (14.1)	6 (7.7)	**78 (100)**
Post-test	0	6 (7.7)	17 (21.8)	26 (33.3)	29 (37.2)	**78 (100)**

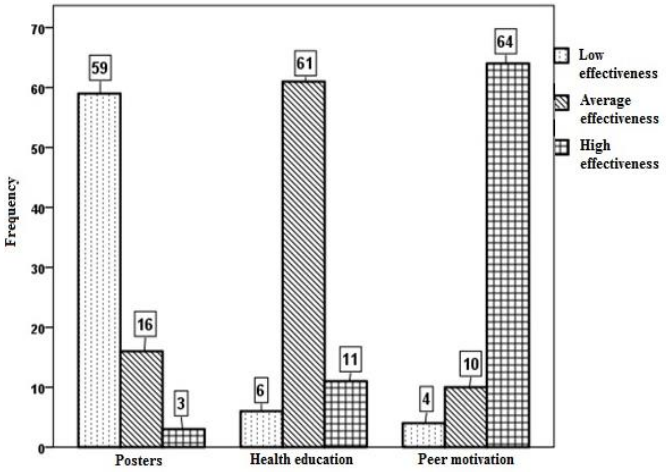
Figures in parentheses are percentage values.

Table 3: Effectiveness of intervention on the workers' usage of PPE during the work time

	Frequency of usage of PPE during the work time (n=78)			X^2	'p'
	Low	Average	High	**value**	**Value**
Pre-test	23 (29.5)	38 (48.7)	17 (21.8)	38.04	**<0.001***
Post-test	6 (7.7)	17 (21.8)	55 (70.5)		

Figures in parentheses are percentage values; 'p' <0.05 is statistically significant*

Figure 1: Perception of workers on the effectiveness of each strategy in bringing out the improvement in PPE usage.



Usage of PPE during the work time, before and after intervention:

During the pre-test, each subject was enquired about his own practice and frequency of usage of PPE during the work time. For this purpose, each subject was asked to select any one of the following five responses viz., very low, below average, average, above average and very high. The response of each subject was recorded. Again during the post-test, the responses for this question were noted. Table-2 reveals the usage of PPE by the subjects during the work time.

In the post-test, all of the 78 (100%) workers reported improvement in the frequency level of usage of PPE. During the pre-test, 7 workers reported that their usage of PPE during the work time as 'very low'. During the post-test, 6 of them reported that their usage of PPE as 'below average' and the remaining one worker reported as 'average'. Similarly the improvement was noted in all categories. Even the six workers who reported in the pre-test 'very high' usage of PPE, reported in the post-test 'still higher level of usage'. However they were included in the same category of 'very high'.

To measure the effectiveness of the intervention, the above table-2 was condensed as follows: The middle category 'Average' was kept as it was. The first two categories were combined together to form one category and named as 'low'. The last two categories were combined together and named as 'high'. Table-3 illustrates the effectiveness of intervention in bringing out PPE compliance by the workers, during the work time.

In the pre-test, 21.8% of subjects reported 'high' level of usage of PPE and 29.5% reported 'low' level of usage. In the post test, 70.5% of workers reported 'high' level of usage of PPE and only 7.7% of workers reported 'low' level of usage. This improvement in PPE usage was statistically significant (Chi square test; $p<0.001$), with substantial improvement in the effect size (70.5 minus 21.8 = 48.7). This shows that a comprehensive approach is very effective in bringing out the change in behavior.

Evaluation of the workers' perception on effectiveness of each strategy:

The subjects informed at the time of post-test, that all three strategies complemented each other in bringing out the improvement in PPE usage, thus validating the synergy of effect described by the Ecological approach to behavior change.

However the subjects were compelled to evaluate their perception of the relative role of each strategy's 'level of effectiveness' in contributing to PPE compliance. For this, they were asked to select any one of the following responses, viz., 'low' 'average', or 'high'. Their responses are shown in Figure 1.

Regarding the role of posters in the working environment, 59 workers (75.6%) felt that it had 'low' level of effectiveness in bringing out the improvement in PPE usage. On the role of health education, 61 workers (78.2%) felt that it had 'average' effectiveness. However, 64 workers (82.1%) felt that the strategy of peer-motivation by co-workers had 'high' level of effectiveness.

DISCUSSION

The present study found that the intervention program of three strategies integrated together resulted in significant improvement in PPE usage among the workers. This is in line with the Ecological Approach to Behaviour Change which posits the need to address the multiple determinants that influence health and health behavior and so there is synergy of effect in multi-pronged interventions since they address multiple determinants of health behavior.

We wanted to know the workers' perception of the relative role played by each strategy, so that we can address any misconception that required remedial action. Our study findings revealed that the workers rightly recognized the strategy of peer-motivation as having

'high' level of effectiveness. This is in line with Social Cognitive Theory which posits that people not only learn through their experiences but also by observing the action of others and the results of that action. In the present study, the 'role-model volunteers' who were fellow workers, had overcome the commonly perceived inconvenience and discomfort in PPE usage and showed by example that it is possible to practice the good behavior in their work environment. Peer-pressure then forced the other workers to fall in line, to avoid being socially rejected as deviant from the expected norm in behavior. That health education had 'average' effectiveness and posters in working environment had 'low' effectiveness goes to show that such interventions they had experienced in the past without leading to increased usage of PPE.

Other interventional studies conducted in various countries had also recorded similar improvement in PPE usage among the workers. A study on the effect of a training program among laborers in USA[7] found significant changes in the usage of PPE. It was a participatory, peer-facilitated training program. Most workers (66%) shared the information of the program with their co-workers and friends. A study in Nigeria[8] also found significant increase in the usage of PPE after intervention. In Italy[9], a multi-faceted intervention study **was done among the workers in two foundries.** The decrease in occupational injuries was significant ($P < 0.01$). Another interventional study in Nigeria[12] revealed that the usage of PPE among the workers in the interventional group were significantly higher than the control group. A study conducted in Behara[13]concluded that the intervention was effective and the results had noticeable improvement in the workers' compliance with PPE usage. An interventional program among factory workers in Iran[14] was found to be very effective in improving the usage of PPE. An interventional study in Hamedan[15] revealed that integration of motivational interviewing principles into the safety program had improved the safe behaviors of the workers in the motivational group and resulted in significant change in PPE usage among them. Another study in Iran[16] observed that the intervention program using peers was effective in promoting safe practices of the workers.

Conclusion:

Most workers perceived that the strategy of peer-motivation had 'high' level of effectiveness, health education had 'average' effectiveness, and posters in the working environment had 'low' effectiveness. However, when the intervention program consisting of three strategies integrated together, it synergistically resulted in significant improvement in PPE usage among the foundry workers because each strategy addresses a different factor that facilitates behavior change.

Limitations:We did not do a repeat study after 3 months to see whether the benefit in terms of increased PPE usage was sustained.

Recommendations: 1. Training program on the usage of PPE and health education should be conducted periodically for the workers, to ensure reinforcement of messages as well as to remove barriers to behavior change.

2. The administration should enforce that the workers should wear PPE during the work time, through promoting the peer workers as role-models and putting up posters at prominent places of the work place, where the work exposure to hazards due to non-usage of PPE take place.

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REFERENCES

[1]. McBarron M, NathanailP, Nathanail J, Morley JG. Risk-based management of Land contamination at foundry sites. Castings Technology International, England. 2004. [cited 2013 Aug 11]. Available from:

http://www.castingstechnology.com/public/documents/000000000000312.pdf

[2]. Queensland Government. Foundry. [cited 2013 Aug 12]. Available from: http://www.deir.qld.gov.au/workplace/business/manufacturing/foundry/index.htm#.U9CKFx03s4U

[3]. Foundry Management & Technology. FMT. Safety survey reveals shockingly high noncompliance for PPE Usage. [cited 2013 Aug 12]. Available from: http://foundrymag.com/feature/safety-survey-reveals-shockingly-high-noncompliance-ppe-usage

[4]. Kamal AA, Sayed GM, Hassan MH, Massoud AA. Usage of personal protective devices among Egyptian industrial workers. *American Journal of Industrial Medicine.*1988; 13 (6): 707-16.

[5]. Budhathoki SS, Singh SB, Sagtani RA, Niraula SR, Pokharel PK. Awareness of occupational hazards and use of safety measures among welders: a cross-sectional study from eastern Nepal. BMJ Open. 2014; 4:e004646. doi: 10.1136/bmjopen-2013-004646 [cited 2015 Nov 12]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24889850

[6]. Parimalam P, Kamalamma N, Ganguli AK. Knowledge, attitude and practices related to occupational health problems among garment workers in Tamil Nadu. Journal of Occupational Health. 2007; 47: 528-534. [cited 2013 Aug 25]. Available from: joh.*sanei.or.jp/pdf/E49/E49_6_13.pdf*

[7]. Williams Q, Ochsner M, Marshall E, Kimmel L, Martino C. The impact of a peer-led participatory health and safety training program for Latino day laborers in construction. Journal of Safety Research. 2010*;* 41*:* 253-*2*6.

[8]. Adewoye KR, Awoyemi AO, Babatunde OA, Atoyebi OA, Salami SK, Issa FY. Effect of health education intervention on the awareness and use of personal protective equipments among small scale electric arc welders in Ilorin, Nigeria. Indian Journal of Occupational and Environmental Medicine. 2014; 18: 3-8.

[9]. Porru S, Calza S, Arici C. An effectiveness evaluation of a multifaceted preventive intervention on occupational injuries in foundries: a 13-year follow-up study with interrupted time series

analysis. *International Archives of Occupational and Environmental Health*. 2011; 84: 867-876

[10]. Brune l, Chyn E, Kerwin J. Peers and Motivation at Work: Evidence from a Firm Experiment in Malawi. 2017; [cited 2017 Dec 27]. Available from: *https://www.aeaweb.org/conference/2018/preliminary/paper/QR7K8Yb6*

[11]. WHO. Health education: theoretical concepts, effective strategies and core competencies. 2012; Page.39-40. [Cited 2013 Aug 21]. Available from: http://applications.emro.who.int/dsaf/EMRPUB_2012_EN_1362.pdf

[12]. Umoren QM, Ekanem US, Johnson OE, Olugbemi MO. An assessment of the effect of health education on the use of personal protective equipment among small scale welders (panel beaters) in Akwa Ibom state, Nigeria. International Journal of Community Medicine and Public Health. 2016. Vo.3. No.11. [cited 2017 Mar 14]. Available from: http://www.ijcmph.com/index.php/ijcmph/article/view/143

[13]. Seageldein H. An intervention study to evaluate compliance with personal protective equipment among workers at textile industry. Research Gate. [cited 2014 Sep 21]. Available from: https://www.researchgate.net/publication/282322913

[14]. Solhi M, Saki M, Alimohammadi I, Haghani H. Effect of health education based on BASNEF pattern on use of personal protective respiratory equipment in Ahvaz carbon block factory workers, 2009. Iran Occupational Health Journal. 2012; 9 (2): 50-58
URL: http://ioh.iums.ac.ir/article-1-845-en.html

[15]. Navidian A, Rostami Z, Rozbehani N. Effect of motivational group interviewing-based safety education on Workers' safety behaviors in glass manufacturing. BMC Public Health. 2015 Sep 19; 15:929. doi: 10.1186/s12889-015-2246-8. PMID: 26386556. [cited 2017 Dec 27].

Available from: *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4575785/*

[16]. Aghamolaei S, Rahmani T, Zare M, Ghanbarnejad A. Effect of peer education on safety behaviors among workers of renovation of structures and machines shop in Bandar Abbas Oil Refinery Company. Iran Journal of Health Education and Health Promotion. 2014; 1 (4): 45-56. URL: http://journal.ihepsa.ir/article-1-106-en.html

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Original Research Article

Nutritional status assessment of children aged 0-5 years and its determinants in a tribal community of Coimbatore district

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ABSTRACT

Background: Nutritional assessment in children is very important to prevent nutritional disorders and to decrease the morbidity and mortality. The present study was undertaken to assess the prevalence and determinants of malnutrition among children aged 0-5 years in a tribal community of Coimbatore district. Anaikatty and Palamalai tribal areas of Perianaikenpalayam block (population=347062) was selected for our study.
Methods: A total of 206 children aged 0-5 years were included in the study after implementing the exclusion criteria.
Results: Overall prevalence of malnutrition found to be 51%. These 51% (105) malnourished children consisted of 41.3% underweight, of which 11.2% were severely underweight. Prevalence of stunting was 32.5%, of which 6.3% were severely stunted. About 21.8% children were wasted and 6.8% were severely wasted among them. Socio-demographic factors like total number of family members exceeding four, mother's educational status, father's educational status, mother's occupational status, socio-economic status, alcohol usage by any family member, and mother's nutritional status were found to be significantly associated with malnutrition on univariate analysis.
Conclusions: The problems of low standard of living, hunger, starvation, malnutrition, agricultural illiteracy, poor antenatal care, disease, poor sanitary and housing facilities, etc. has to be improved totally by implementation of policy that already exists.

Keywords: Nutrition, Prevalence, Determinants, Under five aged children

INTRODUCTION

Malnutrition among children in India is a well known public health problem due to inadequate nutrition. According to WHO, malnutrition is defined as a "pathological state resulting from a relative or absolute deficiency or excess of one or more essential nutrients".[1] Malnutrition is the biggest contributor to child mortality where 70%, 26% and 4% of malnourished children live in Asian, Africa and Latin America respectively.[2] The nutritional status of under-five children is causing great concern among social scientists and planners nowadays because child is the principal victim of interaction of nutritional, social, economical and also health related factors that lead to malnutrition. On an average, a child dies every 5 seconds as a direct or indirect result of malnutrition –700 every hour –16,000 each day –6 million every year.[3] Assessment of growth not only serves as a method of evaluating the health and nutritional status of children, but also it provides an alternative method of assessment of the quality of life of the entire community.[4] Death due to common childhood illness is more common in a malnourished child when compared with an adequately nourished child.[5] Under-five children are the most at risk segment in any

population and their nutritional status is a sensitive indicator of their health status and nutrition.[6] Malnourished children are more likely to become malnourished adults and they face increased risks of morbidity and mortality.[7,8] It is essential to pay more attention to correlate between children's nutritional status and their capability to attain the required physical growth and mental development.[9] In South India, only few tribal based studies to determine the prevalence of malnutrition among under-five children has been carried out, with paucity of data regarding prevalence of malnutrition obtained as community based study in tribal areas of Tamilnadu. Hence this community based study was undertaken to assess the prevalence of malnutrition and its association with selected known risk factors among children aged 0-5 years in the tribal hamlets of Periyanaickenpalayam of Coimbatore.

METHODS

Study design

Cross sectional study

Study population

The study included 206 children aged 0-5 years, from 25 tribal hamlets in Anaikatty and Palamalai tribal areas of Perianaikenpalayam block of Coimbatore district, Tamil Nadu. Mothers of all these children were interviewed and anthropometric measurements were taken for the children and their mothers who were included for analysis.

Sample size determination

With an estimated prevalence of 55% from earlier studies[10] and 20% allowable error, sample size was calculated using the formula,

$$n = \frac{(1.96^2)pq}{d^2}$$

$$= \frac{3.84\ x\ 55\ x\ 45}{11\ x\ 11}$$

n=82

Where, n=Number of samples required; p=Prevalence; q=100–p; d=allowable error.

Non response rate = 20%

Sample size = $\frac{82 \times 100}{80}$ = 103

Study period

Home-visits were carried out from December 2013 to August 2014.

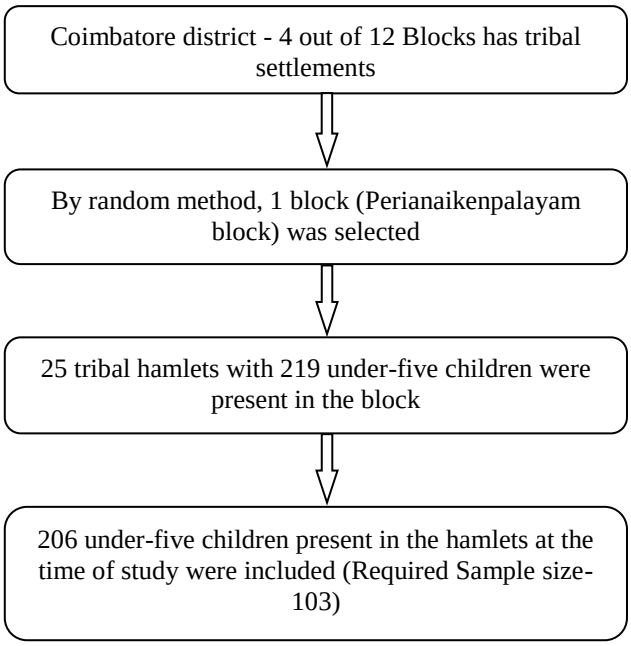


Figure 1: Study protocol.

Inclusion criteria

All the children of age 0-59 months who were permanent residents in the 25 tribal hamlets of Periyanaickenpalayam block.

Exclusion criteria

Under-five children who were not present at home even after three consecutive visits.

Data collection tools

Physical instruments and well-structured questionnaire were used for data collection.

Physical instrument

The physical instrument used in the study included a weighing scale, a Salter scale, retractable metal measuring tape and an infantometer. All the instruments were calibrated by the Department of Biometrics, PSGIMS&R validated through Best Standard Technologies Private Ltd Chennai (Certificate No: BSM-012501-12) prior to the study.

Questionnaire

A Pre-tested semi structured questionnaire was presented in the Department for critical review, following which necessary changes were made in the questionnaire, it was translated into Tamil and back translated to eliminate loss of meaning. Data was collected using this pre-tested semi structured questionnaire by interview technique. The parents of children of age group 0-59 months were

informed about the study and each question was explained to gather the data about socio-demographic factors, environmental factors, birth history and feeding practices, recent illness, availability of health facilities and their utilization. Simultaneously height and weight of the child and mother were measured.

Steps in data collection

After getting approval from the institutional ethics committee, the study was started.

Pilot study

Pilot study was carried out in a rural area, Neelambur, Coimbatore, which is located outside the field practice area of the Rural Health Training Center of the Community Medicine department, PSG IMSR to identify problems in eliciting the data using the prevalidated instruments.

Data collection for study

Consent for the participation in the study was obtained from mother using questionnaire.

Anthropometric measurements

Weight and height of all 206 children and their mothers who participated in the study were measured based on WHO recommended procedure.[11]

Measurement of weight

The weight of the children were measured using the conventional standard weighing scale and Salter spring balance for children aged less than two years. These scales were calibrated before commencement of data collection. These scales are widely used in epidemiological studies. With light clothing and without any footwear, children were asked to stand on the platform of weighing scale with body weight evenly distributed between both feet and weight was measured to nearest 0.5 kg. Zero error was set after each measurement.[11]

Measurement of height

Height was measured using a retractable metal measuring tape. Children whose height has to be measured stood on the floor in an erect position without any foot wear with his / her back against the wall. He / she was asked to put their feet together and move back till their heels touched the bottom of the wall and stretch upwards to the fullest extent with their arms hanging on the side. The head was held in such a way that lower rim of the orbit and auditory canal were in horizontal plane. The height appears in the read off area and measured to nearest 0.5 cm. The supine length was measured in children <than 2 years of age using an infantometer. The child was placed on board with head positioned firmly against the fixed head board, the knees extended by firm pressure and the feet fixed at right angles to the lower legs. Then the up right foot piece was moved to obtain firm contact with the heels and the length was measured to the nearest 0.5 cms.[11]

Assessment of nutritional status using anthropometry method

The 2006 WHO Growth Standards for Preschool Children were used for the determination of nutritional status of preschool children. Standard deviation of scores (Z-scores) for weight-for-age, height-for-age and weight-for-height were calculated. The Z-score (SD score) is calculated as follows. The *Z*-score is defined as the deviation of the value observed for an individual from the median of the reference population, divided by the standard deviation (SD) of the reference population.

Z score = (individual value-median value of reference population)/ SD value of reference population.

For each of the anthropometric indicators of malnutrition a cutoff point of-2 standard deviations (-2 SD) below the median of that of the WHO reference population was used. Deviation of Z-scores less than – 3SD put children in the severe under nutrition category.

24-hour food recall method

It is a retrospective assessment method in which the respondent is prompted to recall and describe all foods and beverages consumed in the preceding 24 hours or the preceding day. Portion size estimating aides were carried to assist the respondent to recall amounts consumed. Total Energy and Proteins in terms of calories and grams consumed was calculated for 24 h period. Percentage of Energy and Proteins consumed in comparison to recommended daily allowance (RDA) by NIN was calculated and the results were analyzed.[12]

Statistical analysis

Body Mass Index (BMI) was computed for all mothers using the following formula:

$$BMI\ (Kg/m^2) = Weight\ (Kg) / Height\ (m^2)$$

The WHO AnthroII. PC software of WHO was used for analyzing the nutritional status of children.

The variables which were found to have statistically significant association ($p<0.05$) with Malnutrition upon univariate analysis were then subjected to multivariate logistic regression analysis. Wald statistics was then used to test the significance of the odds ratio. $P<0.05$ was considered as statistically significant. Data was entered in Microsoft excel and analyzed using SPSS 19.0 version.

RESULTS

Of the 206 children studied, 106 were boys and 100 were girls. The mean (SD) height of the boys was 89.7 cm (12.29) and mean (SD) weight of the boys was 12.2 kg (3.32). Similarly for girls, the mean (SD) height was 89.6 cm (12.72) and mean (SD) weight is 12.2 kg (3.44). The mean (SD) BMI of the mothers was 20.2 (2.69).

Table 1: Hamlet-wise distribution of study subjects.

S.N	Name of Hamlet	Number of children
1.	Alamaramedu	9
2.	Arnatkadu	15
3.	Jammukundi	5
4.	Kandivalli	6
5.	Kilurpathy	5
6.	Kondanur	13
7.	Kondanurpudur	5
8.	Koodanur	13
9.	Kunjurpathy	6
10.	Kuttupulikadu	2
11.	Mangarai	2
12.	Manguli	8
13.	Melurpathy	3
14.	Palamalai	10
15.	Panapalli	13
16.	Pasumani	6
17.	Pasumanipudur	9
18.	Perukupathy	8
19.	Perumpathy	3
20.	Rayarurpathy	12
21.	Sembukarai	3
22.	Thirumalur	31
23.	Thumanur	5
24.	Thuvaipathy	11
25.	Milagayampathy	2
	Total	206

Prevalence of malnutrition

The child is considered as "Malnourished" if he/she is underweight or wasted or stunted. The overall prevalence of malnutrition was found to be 51% and 49% of the children was found to be "Normal" anthropometrically. Of these 105 malnourished children, 41.3% were underweight, 32.5% were stunted and 21.8% had wasting.

Low weight-for-age is termed underweight. This is a composite measure of malnutrition. There is no statistically significant gender difference in the prevalence of underweight in any of the age-groups. (p=0.33) (Table 2).

Low weight-for-height is termed wasting. Wasting reflects a state of acute (short-term) under nutrition. There is no statistically significant gender difference in prevalence of wasting among different age-groups (p=0.75). Prevalence of wasting increases as the age of the children increases.

Low height-for age is termed stunting. Stunting is indicative of chronic (long-term) under nutrition. There is no statistically significant gender difference in the prevalence of stunting among different age-groups (p=0.07). Higher prevalence of malnutrition was observed among the lower socio-economic classes (p<0.001).

The study revealed, 81% of malnourished children are consuming <50% of the RDA of energy. Forty two% of normal children according to anthropometry are consuming <50% 0f the RDA of energy. No child in the malnourished category is consuming >90% of recommended allowance and only 3 children are consuming >70% of the RDA of energy.

Table 2: Age sex distribution of underweight children.

Age group (months)	Male			Female			Total		
	Total	**Moderate (%)**	**Severe (%)**	**Total**	**Moderate (%)**	**Severe (%)**	**Total**	**Moderate (%)**	**Severe (%)**
0-12	9	4 (44.4)	1 (11.1)	10	4 (40)	2 (20)	19	8 (42.1)	3 (15.8)
13-24	16	5 (31.3)	3 (18.8)	16	5 (31.3)	3 (18.8)	32	10 (31.3)	6 (18.8)
25-36	26	10 (30.3)	4 (15.4)	19	7 (36.8)	1 (5.3)	45	17 (37.8)	5 (11.1)
37-48	26	6 (230.1)	2 (7.7)	28	7 (25)	2 (7.1)	54	13 (24.1)	4 (7.4)
49-60	29	8 (27.6)	3 (10.3)	27	6 (22.2)	2 (7.4)	56	14 (25)	5 (8.9)
Total	106	33 (31.1)	13 (12.3)	100	29 (29)	10 (10)	206	62 (30.1)	23 (11.2)

Statistically significant gender difference in the prevalence of underweight is $p \leq 0.33$)

Univariate analysis was done to explore the association of malnutrition with socio-demographic characteristics of the child. The risk factors that were significantly associated with malnutrition were: Parents being illiterate, mother not working outside, total family members >4, child belonging to Class IV and Class V SES according to modified Prasad's classification, The children mainly taken care by any other family member other than mother is also not a significant risk factor for malnutrition (Table 3).

Table 3: Socio-demographic characteristics associated with malnutrition among under-five children.

Factors	Category	Total	Malnourished		Unadjusted odds ratio	95% CI*	P value
			N	%			
Age of child	≤1 year	19 (9.2)	11	57.9	1	0.28 – 1.91	0.5
	>1 year	187 (90.8)	94	50.3	0.735		
Sex	Male	106 (51.5)	54	50.9	1	0.58 – 1.73	0.9
	Female	100 (48.5)	51	51	1.1		
Main care-taker	Mother	203 (98.5)	102	50.2	1	0.27 – 74.64	0.6
	Other	3 (1.5)	3	100	2.94		
Any alcoholic	No	35 (17)	5	14.3	1	3.12 – 22.84	<0.001
	Yes	171 (83)	100	58.5	8.45		
Mother's educational status	Literate	119 (57.8)	46	38.7	1	1.86-5.98	<0.001
	Illiterate	87 (42.2)	59	67.8	3.34		
Father's educational status	Literate	144 (69.9)	49	34	1	7.28-44.94	<0.001
	Illiterate	62 (30.1)	56	90.3	18.09		
Mother's occupation status	Home maker	148 (71.8)	88	59.5	1	0.15 – 0.54	<0.001
	Working	58 (28.2)	17	29.3	0.283		
Total family members	≤4	102 (49.5)	34	33.3	1	2.4 – 7.7	<0.001
	>4	104 (50.5)	71	68.3	4.3		
SES	Class I, II, III	35 (17)	3	8.6	1	4.64 – 33.52	<0.001
	Class IV, V	171 (83)	102	59.6	15.76		
Mother's BMI	Normal and above	145 (70.4)	58	40	1	2.54 – 9.97	<0.001
	Under weight	61 (29.6)	47	77	5.03		

Table 4: Household characteristics associated with malnutrition among under-five children.

Factors	Category	Total	Malnourished		Unadjusted odds ratio	95% CI*	P value
			N	%			
Type of house	Pucca	53 (25.7)	18	34	1	1.20–4.95	<0.01
	Semi pucca	144 (69.9)	80	55.6	2.43		
	Kutcha	9 (4.4)	7	77.8	6.81	1.11–53.41	
Water source	Piped water	196 (95.1)	96	49	1	1.18–101.29	<0.05
	Well water	10 (4.9)	9	90	9.38		
Toilet	Private/public	36 (17.5)	5	13.9	1	3.28–23.90	<0.001
	Open air	170 (82.5)	100	58.8	8.85		

The association of semi-pucca house and kutcha house as risk factors relating the house-hold environment with malnutrition found to be significant compared with pucca house. Source of drinking water other than piped water supply and the practice of open air defecation were also found to be significant risk factors for malnutrition (Table 4).

Mother's age less than 20 years during this child birth, deliveries other than institutional deliveries, preterm delivery of this child, and low birth weight were found to be significant risk factors for malnutrition. Birth order of the child was not a significant risk factor (Table 5).

Correct feeding practices of infant and young child are very essential to the nutritional status of any child. Initiation of breastfeeding more than an hour, breastfeeding the child exclusively for a period less than 6 months, breastfeeding the child for a period less than 1 year, not giving any milk supplements to children above 6 months, consumption of <50% of the RDA of calories and proteins are significant risk factors for malnutrition. More than 90% of the children were fed with colostrum after birth. Avoiding colostrum does not turn out to be a risk factor for malnutrition in our study (Table 6).

It is evident from Table 7 that under-five children who suffered from any illness in the past month and those children who were not treated for those illnesses were more prone for malnutrition.

Table 5: Birth history associated with malnutrition among under-five children.

Factors	Category	Total	Malnourished		Unadjusted odds ratio	95% CI*	P value
			N	%			
Mothers age at child birth	<20	31 (15)	24	77.4	**1**	0.10 – 0.61	<0.05
	≥20	175 (85)	81	46.2	0.25		
Place of birth	Hospital/health centre	184 (46.7)	86	46.7	1	2.06 – 25.23	<0.001
	Home	22 (10.7)	19	86.4	7.21		
Term of delivery	Term	188 (91.3)	91	48.4	1	1.18 – 11.75	<0.05
	Pre term	18 (8.7)	14	77.8	3.73		
Birth weight	Normal	124 (60.2)	41	33.1	1	3.78 – 13.69	<0.001
	LBW	82 (39.8)	64	78	7.19		
Birth order	≤2	191 (92.7)	95	49.7	1	0.66 – 6.13	0.2
	>2	15 (7.3)	10	66.7	2.02		

Table 6: Infant and young child feeding practices associated with malnutrition among under-five children.

Factors	Category	Total	Malnourished		Unadjusted odds ratio	95% CI*	P value
			N	%			
Colostrum given	No	16 (7.8)	11	68.8	1	0.14-1.1.33	0.1
	Yes	190 (92.2)	94	49.5	0.44		
Breast feeding initiation	≤1 hr	171 (83)	71	41.5	1	6.40-98.0	<0.001
	>1 hr	35 (17)	34	97.1	47.88		
Exclusive breast-feeding	6 months	73 (35.4)	19	26	1	2.76-9.73	<0.001
	> or <6 months	133 (64.6)	86	64.7	5.20		
Total breast feeding in children more than 1yr	≥12 months	191 (92.7)	93	48.7	1	1.06-19.48	<0.05
	<12 months	15 (7.3)	12	80	4.22		
Other milk in children >6months	Yes	60 (30.5)	11	18.3	1	3.73-10.74	<0.001
	No	137 (69.5)	89	65	8.26		
Calorie consumption(% of RDA)	≥50	83 (40.3)	24	28.9	1	2.59-8.66	<0.001
	<50	123 (59.7)	81	65.9	4.74		
Protein consumption(% of RDA)	≥50	149 (72.3)	65	61.9	3.04	1.58-5.84	<0.05
	<50	57 (27.7)	40	38.1	1		

Table 7: Illness in the past month associated with malnutrition among under-five children.

Factors	Category	Total	Malnourished		Unadjusted odds ratio	95% CI*	P value
			N	%			
Illness present in the last month	No	110 (53.4)	39	35.5	1	2.23-7.16	<0.001
	Yes	96 (46.6)	66	68.8	4.00		
Illness treated among morbid children	Yes	64 (66.7)	39	60.9	1	1.07-11.83	<0.05
	No	32 (33.3)	27	84.4	3.46		

Table 8 shows the health service availability, its utilization and the association of these factors with malnutrition among the tribal children. Availability of health facility within a radius of 3 km is not associated with malnutrition and so show that it is protective against getting malnourished.

Children who are not immunized appropriate for age are at significant risk of malnutrition. It is interesting to see that children who are not attending the ICDS centers are having better nutritional status according to anthropometric measurements.

Table 8: Health service availability and utilization associated with malnutrition among under-five children.

Factors	Category	Total	Malnourished		Unadjusted odds ratio	95% CI*	P value
			N	%			
Distance of health facility	≤3 kms	76 (36.9)	43	56.6	1	0.39-1.23	0.218
	>3 kms	130 (63.1)	62	47.7	0.70		
Immunization status	Appropriate	187 (90.8)	88	47.1	1	1.53-2.36	<0.001
	Not appropriate	19 (9.2)	17	89.5	1.90		
Attending ICDS	Yes	123 (59.7)	77	62.6	1	0.38-0.75	<0.001
	No	83 (40.3)	28	33.7	0.54		

Table 9: Multivariate logistic regression analysis of determinants of malnutrition among under-five children.

S.No	Factors	Category	Adjusted odds ratio	95% Confidence interval	P value
1.	Father's education	Literate	1	7.98-15.67	<0.001
		Illiterate	10.30		
2.	Mother's education	Literate	1	0.22-1.58	0.49
		Illiterate	0.59		
3.	Mother's occupation	Home maker	1	0.12-0.99	<0.05
		Working	0.35		
4.	Family members	≤4	1	0.65-4.13	0.29
		>4	1.63		
5.	SES	Class I,II,III	1	1.03-13.80	<0.05
		Class IV,V	6.42		
6.	Mother's nutritional status	Underweight	1	0.46-3.53	0.63
		Others	1.28		
7.	Any alcoholic	Yes	1	0.09-3.15	0.49
		No	0.54		
8.	Type of house	Pucca	1	0.15-1.64	0.25
		Semipucca/Kutcha	0.50		
9.	Piped water supply	Yes	1	0.33-63.24	0.25
		No	4.58		
10.	Toilet usage	Toilet	1	0.51-8.30	0.30
		Open air defecation	2.07		
11.	Mother's age at birth	≤20	1	0.42-5.22	0.53
		>20	1.48		
12.	Place of birth	Institutional delivery	1	0.33-10.18	0.48
		Home	1.83		
13.	Term of delivery	Term	1	0.09-3.35	0.52
		Pre-term	0.56		
14.	Birth weight	Normal	1	1.23-9.43	<0.05
		LBW	3.41		
15.	Breast feeding initiation	Within 1 hr	1	2.58-80.44	<0.01
		Above 1 hr	26.92		
16.	Calorie consumption (% of RDA)	≥50	1	2.15-16.41	<0.05
		<50	5.95		
17.	Protein consumption (% of RDA)	≥50	1	0.95-9.90	0.61
		<50	3.07		
18.	Illness in the past month	Present	1	0.49-2.97	0.66
		Not present	1.21		
19.	Attending ICDS	Yes	1	0.68-4.21	0.25
		No	1.70		
20.	Immunization status	Appropriate	1	0.16-6.43	0.97
		Not appropriate	1.02		

Table 9 explored the factors identified from univariate analysis and confirmed those truly associated with malnutrition through multivariate logistic regression analysis. This reveals that:

1. The risk of malnutrition is 10.3 times higher in under-five children of illiterate fathers when compared to literate fathers, but there is no association with the educational status of mother.
2. Occupational status of mothers show significant association with malnutrition of under-five children, where the factor that mothers employed for salary is 65% protective against malnutrition when compared to home-makers.
3. Socio-economic status is significantly associated with malnutrition after logistic regression. Under-five children belonging to Class IV and Class V families according to Modified Prasad's classification have 6.42 times more risk of malnutrition when compared to Class I, Class II and class III families.
4. Low birth weight babies have 3.41 times more risk of suffering from malnutrition when compared to babies who are born normal or overweight.
5. Earlier initiation of breastfeeding, less than an hour after birth is protective for malnutrition in under-five children. Babies who are initiated with breastfeeding after the first hour after birth are 2.9 times more prone for malnutrition.
6. Consumption of calories less than 50% of the RDA proved to be a significant risk factor from the above analysis and protein consumption did not show any statistical association. Children who consume < 50% of recommended calories are nearly 6 times at risk of malnutrition.

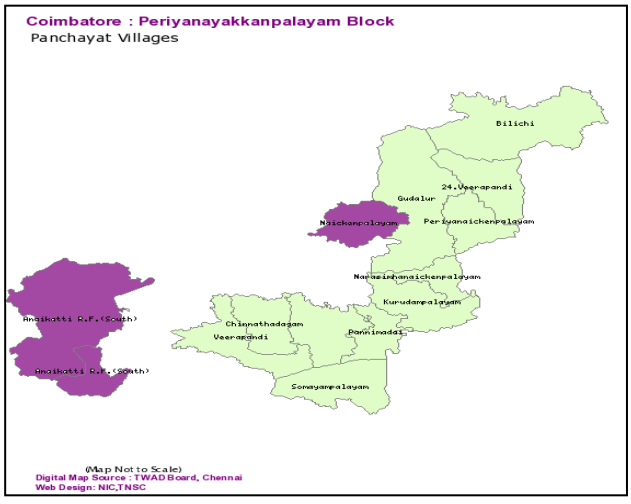


Figure 2: Map showing the tribal areas in Periyanaickenpalayam block.

DISCUSSION

The study is to assess prevalence of malnutrition among 206 under-five children in 25 tribal hamlets of Periyanaickenpalayam block, Coimbatore and to ascertain the determinants of malnutrition among them.

The possible risk factors selected in our study associated with malnutrition are age, sex, number of family members, mother's education, father's education, mother's occupational status, father's occupational status, socio-economic status, alcohol usage by family members, mother's nutritional status, mother's age at pregnancy, place of delivery of the child, term of delivery of the child, birth order of the child, birth weight of the child, time of initiation of breast-feeding, exclusive breast-feeding, total breast-feeding, energy and protein consumption of the child, Immunization status, ICDS utilization by the child, distance of health facility, recent illness, treatment for the illness, type of house, source of drinking water and toilet usage.

Data was collected using Pre tested semi structured Questionnaire by interview technique. The mothers of children of age group 0-59 months were informed about the study and each question was explained to gather the data and simultaneously height and weight of the child and mother were measured. Anthropometric measurements of the children and mothers like height and weight were measured using standardized calibrated instruments as per WHO recommendations.[13]

Prevalence of malnutrition

The study revealed overall prevalence of malnutrition was 51% and 49% of the children studied were "Normal" anthropometrically according to WHO classification. These 105 malnourished children consisted of 41.3% underweight, of which 11.2% are severely underweight. Prevalence of stunting is 32.5%, of which 6.3% are severely stunted. About 21.8% children are wasted and 6.8% are severely wasted among them.

The overall prevalence of underweight, Stunting and Wasting are 55%, 54% and 28% respectively among under-five children of tribal population in India according to NFHS-3 data.[10] Our study showed a lower prevalence of all three indicators of malnutrition when compared with nationwide statistics.

In a study conducted among tribal under-five children in 9 states of India in 2007-08 among a total of 14,587 children by NIN, Hyderabad it was found that the overall prevalence of underweight was about 49%, of which 19% were severely underweight.[14] The extent of overall stunting was about 51%, and of them, about 24% were severely stunted. About 22% of children had wasting, of which 7% had severe wasting. Only the degree of wasting is comparable to our study in this context, which may be due to acute causes of malnutrition.

Meshram et al, in a study conducted among 1751 tribal children of Maharashtra also showed a higher prevalence of underweight, stunting and wasting as 64%, 61% and 29%, respectively.[15]

Rao et al in a study conducted for Regional Medical Research Center for Tribals (ICMR study), Jabalpur, India revealed that 61.6% of preschool children were underweight, 51.6% were stunted and 32.9% were wasted.[16] The study also revealed that severe degree (below -3 SD) of underweight, stunting and wasting in 27.8%, 30.3% and 6.5% children respectively.

According to Bisai et al, in a study conducted among pre-school children of Lodha tribal community of West Bengal, the Overall prevalence of underweight, stunting and wasting was 33.9%, 26.1% and 19.4%, respectively.[17] Of these, 9.1%, 9.7% and 3.6% children were found to be severely underweight, stunted and wasted. These findings show a slightly lower prevalence when compared to our study.

Risk factors for malnutrition

Age of the child and malnutrition

No statistically significant association between different age groups of the under-five children and malnutrition. The findings are in variance to Meshram et al who has reported that there is increased risk of malnutrition in older children when compared with infants, whereas the results of Bisai et al is contradictory showing more risk of malnutrition in early childhood (1-3 years) when compared to older children (3-6 years).[14,17]

Sex of the child and malnutrition

There is no statistically significant difference in prevalence of malnutrition among both the sexes in our study. Meshram et al has concluded that male under-five children are more prone for underweight, stunting and wasting when compared to girl children.[14] He had also reported an increased risk of malnutrition in boys when compared with girls, in a study conducted in Maharashtra.[15]

Number of family members and malnutrition

If the total number of family members exceeds 4 members, it acts as a risk factor for malnutrition in univariate analysis, not so in regression analysis. Meshram et al in India and Mekonnen et al in Ethiopia, the odds for malnutrition is increased in large families (>4 members) when compared to small families (≤4 members).[14,18] Also there is no association between the main care-taker of the child whether it is the child's mother or others in our study, and this finding correlates with the findings of Seetharaman, in a study done among slum under-five children in a part of South India.

Mother's education and malnutrition

The unadjusted odd's ratio for malnutrition and mother's educational status shows that there is significant risk if the child's mother is an illiterate in our study. Nationwide shows that maternal education has a strong inverse relationship with all three measures of nutritional status according to NFHS-3, also Meshram et al al has reported increased risk of malnutrition in children of illiterate mothers.[10,14]

Father's education and malnutrition

Illiteracy state of fathers came as very significant risk for malnutrition in under-five children in our study. The adjusted odds of malnutrition were 10.3 times more for children living with illiterate fathers. The results of our study was consistent with study done by Meshram et al in India and Islam et al in Bangladesh.[14,19]

Mother's occupational status and malnutrition

The factor that mother's working for salary came as a protective factor for malnutrition when compared to home-makers on logistic regression from our study. In our study all the fathers were waged workers and we could not elicit the association of malnutrition with father's occupational status.

Socio-economic status and malnutrition

Children of families belonging to lower SES were at increased risk of malnutrition when compared to children of higher SES. The results were similar to studies done by Harishankar et al and Reddy et al in Uttar Pradesh.[20,21]

Alcohol consumption by any family member and malnutrition

The association of any member being an alcoholic in the family is not a significant risk factor according to the study. But a study done in Nairobi, Kenya involving 170 households has reported that a high percentage (38.1%) of children from alcohol consuming households were underweight, compared to children from non-alcohol consuming households (14.3%).

Mother's nutritional status and malnutrition

Rayhan et al in Bangladesh shows that there is an increased risk of undernutrition in children of mothers belonging to underweight category by BMI classification.[22] In contradiction to their findings our study shows that there is no significant association between mother's nutritional status and children's undernourishment by logistic regression.

Household characteristics and malnutrition

The association of type of the house, source of drinking water supply and usage of toilet factors with malnutrition of under-five children, revealed that Inferior type of the house (semi-pucca/kutcha vs pucca), poor source of water supply (such as dug well water, unprotected well, surface water, unprotected spring, river or dam or lake or ponds or canal, rain water, etc), non-availability and non-usage of available toilet posed as a risk factor for malnutrition

in univariate analysis but it did not turn out to be a risk factor after regression analysis.

A study done by Yadav et al concluded that severe and moderate levels of malnutrition was much higher among those with poor housing conditions even with the same level of dietary intake, whereas in spite of lower dietary intake. Birth order of the child and malnutrition.[23]

No significant association between birth order and malnutrition was observed in our study. According to NFHS-3 data, children of higher birth orders are much more likely to be underweight than children of lower birth orders.[10]

Birth weight of the child and malnutrition

The association of Low Birth Weight with malnutrition is very much significant in our study with an adjusted odd's ratio of 3.41. Our findings are concordant with NFHS-3 which reveals that children with a history of low birth weight (<2.5 kg) are much more likely to be malnourished than other children (≥2.5 kg).[10]

Mother's age at child birth and malnutrition

Mother's age ≤20 during child delivery is not a significant risk factor for malnutrition taking into account of adjusted odd's ratio in our study, but Sonowal, in a study conducted in Maharashtra has reported that prevalence of malnourishment in children delivered by mothers before 20 yrs is high (>12%) when compared to children delivered after 20 years (<8%).[14]

Place of child birth and malnutrition

Home deliveries, when compared to institutional deliveries are reported as a significant risk factor for malnutrition by Biswas et al in a community based study done in West Bengal.[25] Home delivery children had 13 times greater risk to be underweight, 5 times greater risk to be stunted and 6 times greater risk to be wasted than their Institutional delivery counterparts. In our study this factor does not turn out to be a risk factor.

Term of delivery of the child and malnutrition

Under-five children from preterm delivery are not at risk of malnutrition when compared to children from normal or postterm delivery after logistic regression in our study. These results are contradictory to study done by Moster et al in Norway, who has reported that risk of poor physical and social growth is high in preterm children.[26]

Infant and young child feeding practices and malnutrition

Exclusive breast-feeding duration less than 6 months in children aged more than 6 months, total breast feeding duration less than 12 months in children aged more than 12 months, not giving other milk or milk substituent in children >6 months and consumption of <50% of the recommended amount of calories and protein were significant risk factors for malnutrition according to our study.

Hien et al found that duration of exclusive breast feeding <6 months is a significant risk factor to underweight (OR=4.41) and stunting (OR=3.58) and time of initiation of breast feeds was significantly related to underweight (OR=2.54).[27] His results are in consistency with our study. Results regarding total breast feeding duration by Majlesi et al in their study in rural Khorambad Province were also consistent with our study.[28] 10.5% of children breast fed for ≤1 year were underweight whereas only 4.5% of the children breast fed for 1-2 years were underweight as reported by them.

Precedent illness in the child and malnutrition

Suffering from any illness in the past fortnight and not treating the illness, both were significantly associated with under nutrition in our study. Meshram et al from Maharashtra, India reported that children who had suffered any illness in the past fortnight is at more risk for under nutrition.[15]

Immunization status and malnutrition

The factors that the child is not appropriately immunized for age and malnutrition are not significantly associated if we take into account of adjusted odd's ratio in our analysis. Studies done by Devi et al in Andhra Pradesh, India and Mekonnen et al of Fogera District, Northwest Ethiopia has shown that under-five children who are appropriately immunized for age were protected against under nutrition.[18,29]

ICDS attendance and malnutrition

Statistically significant association between of ICDS utilization and malnutrition was obsreved our study, and non- utilization of ICDS services is found to be a protective factor for malnutrition.

Alim et al in a study conducted in Aligarh, Uttar Pradesh, has reported that out of 23.6% children who did not receive supplementary nutrition from ICDS, majority 14.3% of the total children were underweight.[30] On the other hand, out of 76.4% children, who received supplementary nutrition from ICDS majority 62.7% of children were having normal weight for age and these results are in variance to our study.

CONCLUSION

The basic problem of the tribal people is poverty. The problems of low standard of living, hunger, starvation, malnutrition, agricultural illiteracy, poor antenatal care, disease, poor sanitary and housing facilities, etc has to be

improved totally by implementation of policy that already exists.

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REFERENCES

1. Jelliffe DB. The assessment of nutritional status of the community, WHO Monograph. 1966: 53.
2. Yigit EK, Tezcan S. Infant feeding practices and children's and women' nutritional status. Ankara; 2004: 141-155.
3. Food and Agriculture Organization Staff. The State of Food Insecurity in the World, 2002: Food Insecurity: When people Live with Hunger and Fear Starvation. Food and Agriculture Organization of the United Nations 2002; 6.
4. World health organization, global database on malnutrition. Available at: http://www.who.int/nutgrowthdb/en/. Accessed on 3 August 2017.
5. The Silent Emergency. State of World's Children 1998. Focus on Nutrition. UNICEF.
6. Sachdev HPS. Assessing child malnutrition-some basic issues. Bull Nutr Foundations India. 1995;16:1-5.
7. Haq MN. Age at menarche and the related issue: A pilot study on urban school girls. Journal of Youth and Adolescence. 1984;13(6):559–67.
8. Karim A, Chowdhury AKMA, Kabir M. Nutritional status and age at secondary sterility in rural Bangladesh. J Biosocial Sci. 1985;17(4):497–502.
9. Onis MD, Frongillo EA, Blossner M. Is malnutrition declining? An analysis of changes in levels of malnutrition since 1980. Bulletin of WHO, 2000;78 (10):1222-33.
10. National Family Health Survey (NFHS-3), 2005-06, National Report, International Institute of Population Sciences, Mumbai.
11. Deshmukh PR, Dongre AR, Gupta SS, Garg BS. Newly developed WHO growth standards; Implications for demographic surveys and child health programs. Indian J Pediatr. 2007;74;987-90.
12. Dietary Guidelines for Indians - A Manual. National Institute of Nutrition, Hyderabad; 2011: 89-90.
13. Physical status: The use and interpretation of anthropometry – Report of WHO expert committee, Geneva: World Health Organization; 1995: 263-308.
14. Meshram II, Arlappa N, Balakrishna N, Rao M K, Laxmaiah A, Brahmam GN. Trends in the prevalence of undernutrition, nutrient and food intake and predictors of undernutrition among under five year tribal children in India. Asia Pac J Clin Nutr. 2012;21(4):568-76.
15. Meshram II, Arlappa N, Balakrishna N, Mallikharjuna Rao K, Laxmaiah A, Brahmam GN. Prevalence and Determinants of Undernutrition and its Trends among Pre-School Tribal Children of Maharashtra State, India. J Trop Pediatr. 2012;58(2):125-32.
16. Rao VG, Yadav R, Dolla CK, Kumar S, Bhondeley MK, Mahendra U. Under nutrition and childhood morbidities among tribal pre-school children. Indian J Med Res. 2005;122(7):43-7.
17. Bisai S, Bose K, Ghosh A. Prevalence of Undernutrition of Lodha Children Aged 1-14 Years of Paschim Medinipur District, West Bengal, India. Iranian J Pediatr. 2008;18:323-9.
18. Mekonnen HT, Tadesse and Kisi T. Malnutrition and its Correlates among Nutr Disorders Ther S. 2013;12:2161-509.
19. Islam MM, Alam M, Tariquzaman M, Kabir MA, Pervin R, Begum M, et al. Predictors of the number of under-five malnourished children in Bangladesh: application of the generalized poisson regression model. BMC Public Health. 2013;13:1.
20. Harishankar. Nutritional Status of Children under 6 years of Age. Indian J Prev Soc Med. 2004;35:3-4.
21. Reddy DCS, Singh K, Tiwari IC. Epidemiology of Nutritional deficiency disorders among tribal pre-school children around Manikpur, Banda district, Uttar Pradesh. Indian J Prevent Social Med. 1984;15(1-2):57-62.
22. Rayhan MI, Khan MSH. Factors Causing Malnutrition among under Five Children in Bangladesh. Pakistan J Nutr. 2006;5(6):558-62.
23. Yadav RJ, Singh P. Nutritional status and dietary intake in tribal children of Bihar. Indian Pediatr. 1999;36:37-42.
24. Sonowal CJ. Factors Affecting the Nutritional Health of Tribal Children in Maharashtra Ethno Med. 2010;4(1):21-36.
25. Biswas S, Bose K. Association between place of delivery and under nutrition. Nutritional Segment: 2011.
26. Moster D, Lie RT, Markestad T. Long-Term Medical and Social Consequences of Preterm Birth. N Engl J Med. 2008;359:262-73.
27. Hien, Ngoc N, Hoa NN. Nutritional status and determinants of malnutrition in children under three years of age in Nghean, Vietnam. Pakistan J Nutr. 2009;8(7):958-64
28. Majlesi F, Nikpoor B, Golestan B. Growth chart study in children under 5 years old in rural area of Khoramabad province. Iranian J Public Health. 2001;30;(3):107-10.
29. Yasoda DP, Geervani P. Determinants of nutrition status of rural preschool children in Andhra Pradesh, India. Birth. 1994;36:24-6.
30. Alim F, Jahan F. Assessment of Nutritional Status of Rural Anganwadi Children of Aligarh under the ICDS (Integrated Child Development Services) and Rural Health. Stud Home Com Sci. 2012;6(2):95-8.

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Review Article

A Review of Research Projects done by Under-Graduate Medical Students over a Period of Fifteen Years in a Medical College in Coimbatore

Senthil Kumar SK[1], Thanigai Nayaki KP[2], Sivamani M[3]

Abstract

Background: As per Medical Council of India guidelines, Under-graduate medical students must involve in planning, implementation and evaluation of a health education program. Problem Solving for Better Health (PSBH) is an excellent opportunity provided to fulfill this objective. PSBH participants are encouraged to generate new ideas and methods to more effectively utilize the resources that are currently available to them to help solve specific health problems in their areas. Many of the PSBH projects are Health Education Intervention projects and Behavior change is the core idea of Health Education. We intend to assess the projects documenting behavior change following Health Education Intervention.

Methods: Abstract, Objectives, Methodology, Results, Discussion and Questionnaires of the submitted Health Education Intervention projects were reviewed for data on the Knowledge, Attitude, Practice, and Change in Behavior of participants before and after Health Education Intervention. Data entered in Epi-Info 6.0 and percentage projects showing Knowledge, Attitude and Behavioral change were assessed.

Results: Out of the 243 PSBH projects 177(72.83%) were Health Education Intervention projects. Behavioral assessment before intervention was done in 152(85.87%) studies. 88(49.71%) studies have documented the changes in behavior after intervention and only 38 out of these 88 studies have documented the statistical significance for the change in behavior. 2(0.01%) of the studies have used Health Education Models other than Knowledge Attitude Practice (KAP) Model. Convenient sampling is done in all the studies.

Conclusion: Nearly half of the PSBH projects on Health Education Intervention have documented the change in behavior. The use of appropriate Health Education Models, standard sampling methods, proper statistical analysis and documenting change in Behavior will add value for their study.

Keywords: Problem Solving for Better Health (PSBH), Health Education, Behavioral change

Introduction

According to Medical Council of India (MCI) guidelines, under-graduate medical students must involve in planning, implementation and evaluation of a health education programme during their medical course. Health education program can enable students of the health professions to promote better health behavior among the underserved,

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since such a program provides them with an opportunity to meet and work with people from a different culture in a comfortable setting.

Problem Solving for Better Health (PSBH) is an excellent opportunity provided to fulfill this objective. PSBH as a concept was developed by the Dreyfus Health Foundation (DHF), New York. The mission of the PSBH program is to assist in developing small scale, problem solving projects that will directly benefit many people. PSBH participants are encouraged to generate new ideas and methods to more effectively utilize the resources that are currently available to them to help solve specific health problems in their areas.[1] The goals of the program are to stimulate a series of better-health projects based on individual responsibility and action and to create a collaborative network of small, self-sustaining, and lasting health projects.[1] The satisfaction of having completed a research project and seeing it in print is enough motivation for a young student researcher to want to repeat the feat.[2] The steps in PSBH projects involve Identify a Problem, Refining the Problem, Decide on a Solution, Framing a Question, Develop an Action Plan – The Study Protocol, Ethical clearance, Take Action, Write the Report and Share the Experience. The PSG Institute of Medical Sciences & Research, Coimbatore was among the first to join this movement as far back as in 1997. This study is a review of the Health Education Intervention projects done under PSBH in the last fifteen years.

Health Education is a process aimed at encouraging people to want to be healthy, to know how to stay healthy, to do what they can individually and collectively to maintain health, and to seek help when needed.[3] Behavior change is the core idea of Health Education.[4] This study is intended to measure the number of projects demonstrating and documenting behavior change and whether it is statistically significant.

It is important to improve the methodological quality of health education research. This can be done by using controlled, preferably randomized, designs, ensuring adequate sample sizes, examining only objective changes in behavior or, better yet, changes in morbidity or mortality.[5] The limited use of theoretical frameworks, poor statistical analyses, the use of convenient samples and the short post intervention follow-up periods diminish the contribution to research and the formation of strategies to improve the health of communities.[6]

A health behavior theory can be used as a guide to help explain the processes for changing health behavior and the influences of the many forces that affect it, including social and physical environments.[5] Theories and models are among health educator's most useful tools as they tackle the challenges of needs assessment, program planning, program implementation, program evaluation, coordination of services, acting as a resource of health information, and communicating needs, concerns, and resources outlined in the Framework.[7]

Effective health promotion operates in a number of dimensions at the same time to create the conditions for change, provide support to change, and to maintain healthy behaviors.[8] Reviews of the effectiveness of interventions require three components for calculation of effectiveness for reducing health inequalities:

- A valid measure of health status (or change in health status);
- A measure of socio-economic position (or disadvantage); and
- A statistical method for summarizing the magnitude of health differences between people in different groups.[9]

This study intends to measure the effectiveness of interventions in terms of behavioral change and the magnitude of behavioral change in terms of statistical significance. Our objective is to review the Problem Solving for Better Health (PSBH) projects submitted by undergraduate medical students from the year 1998 till 2012 at PSG Institute of Medical Sciences and Research on changes in behavior documented among participants following Health Education Intervention.

Methodology

Our study is a cross-sectional study based on secondary data, conducted over a period of one month. All PSBH projects on Health Education Intervention done by undergraduate students during the period of 1998 – 2012 (210 projects) were included in the study. PSBH projects not involving pre-test and post-test of the Health Education Intervention were excluded.

Collection of data on documented changes of behavior was done. Data of projects measuring Behavior change was analyzed. Statistical significance shown for the Behavior change was also analyzed. A thorough scrutiny of Abstract, Introduction and Justification of the study, Methodology involving Ethical clearance, Sample size calculation, Sampling method, Validated Questionnaire, the change they have recorded in behavior of study participants, results and its statistical significance was done using check list. The discussion and conclusion part were studied for their comments on recommendations and limitations. Data entered in Epi-Info 6.0 and statistical analysis done.

Results and Discussion

Out of the 243 PSBH projects 177(72.83%) were Health Education Intervention projects.

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Table 1.Assessment of Behavioral changes documented in the projects (N=177)

S.No	Variables	n (%)
1	Behavioral assessment before intervention	152(85.87)
2	Change in Behavior documented after intervention	88(49.71)
3	Statistical significance documented for Behavioral change	38(21.47)
4	Health Education Models used	2(0.01)

Behavioral assessment before intervention was done in 152(85.87%) studies. 88(49.71%) studies have documented the changes in behavior after intervention and only 38 out of these 88 studies have documented the statistical significance for the change in behavior. 2(0.01%) of the studies have used Health Education Models other than Knowledge Attitude Practice (KAP) Model. Convenient sampling is done in all the studies.

Table 2.Assessment of Knowledge (K) and Attitude (A) and the Changes documented (N=177)

S.No	Variable	Assessment of K and A before intervention	Change Documented after Intervention for K and A
1	Knowledge(K)	174	159(90%)
2	Attitude(A)	54	37(21%)

Change in Knowledge is documented in most of the projects within the specified project period compared to change in Behavior (mostly attempted in nutrition related projects). Studies involving Nutritional disorder related interventions like Anemia, Obesity, PEM, Vitamin A deficiency and Iodized salt usage have observed and documented significant behavior change.

Studies involving Substance abuse related interventions like Alcohol and Tobacco usage have shown behavior change to a smaller extent

Knowledge change alone will not bring the desired behavioral change in many situations (e.g., smoking, alcoholism) as it needs periodic reinforcing visits and supportive counseling. Most projects on Smoking and Alcoholism were Awareness creation projects directed towards Primordial prevention in schools.

It is possible to bring about change in Behavior within the constraints of time and resources as evidenced by half (50%) of educational interventions being able to demonstrate and document change in behavior indicating the effectiveness of the Health education program. Time constraint of availability of only one semester to complete the PSBH projects influenced the fact that mostly nutritional interventions could demonstrate improved health as an indicator of effectiveness of the health education intervention. Studies mostly used non validated questionnaires. Use of Validated Questionnaires like Global youth tobacco survey in smoking (GYTS) will add value to the project. Many studies measured change in behavior based on self-report / survey. Efforts to assess the behavioral change through observation could be made wherever possible. Appropriate Health Education Models may be used in various contexts.

Our study did not examine the reasons for failure in behavior change or reasons why projects did not attempt measuring behavior change.

Conclusion

Nearly half of the PSBH projects on Health Education Intervention have documented the change in behavior. The use of appropriate Health Education Models, standard sampling methods, proper statistical analysis and documenting change in Behavior will add value for their study.

Conflict of Interest: None

References

1. Problem solving for better health (psbh). Participant's handbook. Dreyfus Health Foundation[Internet]http://www.dhfglobal.org/who/pdf/psbh_handbook.pdf (Accessed on 11.11.2013).
2. Anton N Isaacs. Strengthening Research in Community Medicine. Indian Journal of Community Medicine October 2007;32(4):239-240.
3. K Park, Park's Textbook of Preventive and social medicine,22nd Edition, Bhanarsidas Bhanot, Jabalpur :p-800.
4. Health education: theoretical concepts, effective strategies and core competencies: a foundation document to guide capacity development of health educators/World Health Organization. Regional Office for the Eastern Mediterranean, 19.
5. Levisohn. BP. Health Education interventions in developing countries: A methodological review of published articles.International Journal of Epidemiology1990;19: 788-794.

 ISSN: 2454-325X

6. Louise F. Brown. Research in Dental Health Education and Health Promotion: A Review of the Literature. Health Educ Behav April 1994;21(1): 83-102.
7. Debarr KA Review of current health education theories CJHP2004,2:1,74-87.
8. The prevention paradox .Principles and Practice of Health Promotion: Health Promotion Models and Theories[Internet] http://www.healthknowledge.org.uk/public-health-textbook/disease-causation-diagnostic/2h-principles-health-promotion/prevention-paradox (Accessed on 11.11.2013).
9. Systematic Reviews of Health Promotion and Public Health Interventions. Rebecca Armstrong, Elizabeth Waters on behalf of the. Guidelines for Systematic Reviews in Health Promotion and. Public Health Taskforce..July 2007.p-30.

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A Prospective Cohort Study on the Safety of Infant Pentavalent (DTwP-HBV-Hib) and Oral Polio Vaccines in Two South Indian Districts

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Background: Safety of pentavalent (DTwP-HBV-Hib) vaccine has been a public concern in India and other countries. This study attempted to document the association of serious adverse events following immunization (AEFI, including hospitalizations and deaths of all causes) with the 3 doses of pentavalent and oral poliovirus (OPV) vaccines.

Methods: A cohort of 30,688 infants in 2 south Indian districts were enrolled and followed-up between October 2014 and May 2016, following their first vaccination with DTwP-HBV-Hib and OPV at public health facilities. During weekly follow-ups, by telephone or home visits, the serious AEFIs (hospitalizations and deaths) occurring any time after each vaccination until 4 weeks after third dose were documented. The incidence risk ratios (IRRs) of serious AEFIs in the first (days 0–6) and fourth weeks (days 21–27) after the vaccine doses were compared using the poisson regression analysis.

Results: Of the 30,688 infants enrolled, 30,208 received their third doses of vaccines. During the 4-week periods following each vaccination, there were 365 hospitalizations and 17 deaths. Adjusted incidence risk ratio of 3 doses combined for post-vaccination serious AEFIs during the first week compared with fourth week was 0.8 [95% confidence interval: 0.6–1.0].

Conclusions: There was no increased risk of a serious AEFIs during the first week after any of the 3 doses of pentavalent and OPV vaccination compared with the fourth week. In the absence of any temporal clustering, mortality and hospitalization rates observed in vaccinated infants probably reflects the natural occurrence of such events.

Key Words: pentavalent vaccine, oral polio vaccine, vaccine safety, serious adverse events, hospitalization, death

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Vaccination has been one of the major success stories in global health, leading to the eradication of deadly diseases like smallpox and possibly the virtual disappearance of some diseases from several regions.[1] Over the last 25 years, effective vaccination programs have resulted in a >90% decrease in the global disease burdens for diphtheria, pertussis, and tetanus, including India.[2–4] However, in spite of the extensive evidence of their benefits, concerns and controversies about the value and safety of vaccines continue to impact adversely their acceptance in diverse contexts. Public concerns over reports linking autism with measles, mumps, and rubella vaccine in the United Kingdom had negative impact on vaccine coverage causing several disease outbreaks,[5] which spread to other countries, such as the United States, France, Italy, and the Netherlands.[6–9] This misinformation has spread globally. The rumor mongering through social media considerably affected vaccine coverage during the MR campaigns in south India.[10] The growing global challenge of vaccine hesitancy relates largely to vaccine safety concerns, leading to public confidence erosion, which in turn dampens the vaccine coverage.[11] The Global Advisory Committee on Vaccine Safety emphasized the need for thorough investigation and causality assessment of serious adverse events following immunization (AEFI) as a quality assurance mechanism for immunization programs.[12]

The decreased disease burdens in the developed countries have largely been due to the successful implementation of immunization programs using multivalent combination vaccines. The vaccines against diphtheria, tetanus, and pertussis combined (DTwP) are in use for several decades under universal immunization program. Recently the HBV and *Haemophilus influenza* type b (Hib) have been added to DTwP, known as the pentavalent (DTwP-HBV-Hib) vaccine. Over the past 15 years Global Alliance for Vaccines and Immunizations has supported the introduction of pentavalent vaccine in developing countries for protection against invasive Hib disease in universal immunization program.[13] However, there were temporary interruptions and introduction delays in Sri Lanka,

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Bhutan, Vietnam[14] and Pakistan[15] during 2011–2016 due to reports of hospitalizations and deaths following vaccination after national launch of the pentavalent vaccine. Temporal association of vaccinations and sudden infant death syndrome (SIDS) has been the subject of extensive speculation, despite several studies having refuted any causal association,[16–18] particularly after the diphtheria-pertussis-tetanus immunization.[19]

In India, the reports of 32 infant deaths following the pentavalent vaccination as part of the national AEFI surveillance between 2011 and 2013 raised concerns, although no causal association could be identified.[20] Some professionals and civil society groups, however, appealed before the Supreme Court of India to ban the pentavalent vaccine.[21,22] Although the safety of pentavalent vaccine has been documented in the pre-licensure studies,[23,24] these studies were too small to identify rare adverse events. Thus, it was deemed necessary to carry out further investigations on this issue. This study was designed in consultation with all key stakeholders including the Ministry of Health and Family Welfare, Government of India and WHO (advised by Global Advisory Committee on Vaccine Safety), to assess the risk of serious AEFIs (hospitalizations and deaths) following pentavalent vaccination in an infant cohort from 2 Indian states, where pentavalent vaccine was first introduced.

MATERIALS AND METHODS

The pentavalent vaccine (manufactured by Serum Institute of India Ltd., Pune, India) was first introduced in the states of Kerala and Tamil Nadu in December 2011 with a 6, 10 and 14 weeks schedule for 3 primary doses. Both states have efficient health systems with high vaccination coverage[25] and low infant-mortality rates (IMR): 12 and 21 per 1000 live births in 2013 in Kerala and Tamil Nadu, respectively, compared with an overall IMR of 40 across India.[26] This study was conducted in 2 districts, Kollam (Kerala State) and Coimbatore (Tamil Nadu State).

Study Design

This prospective self-controlled cohort study enrolled infants who received their first dose of pentavalent and oral poliovirus (OPV) vaccine from the public health system between October 2014 and November 2015 in Kollam district, and between May 2015 and February 2016 in Coimbatore district.

Recruitment and Data Collection

Infants who received their first dose of pentavalent and OPV vaccines at all public health facilities in the 2 districts (District, Special and Taluk Hospitals, Community Health Centers, Primary Health Centers, Sub-centers) and out-reach points of rural and urban areas were traced based on the contact details recorded in the immunization registers. Home visits were made to confirm eligibility (received first dose of vaccine, expected to stay in the district for the 6 months follow-up period and contactable by telephone/mobile) and to enroll infants after obtaining informed written consent. Data on maternal care, infant gender, date of birth, birth weight, pregnancy-related and neonatal illnesses were collected at enrollment. The parent(s) were contacted weekly by telephone or home visit to document the health of the infant including any illness, hospitalization or death. The follow-up contact was continued even if the second and third vaccine doses were delayed due to any reason. Study staff confirmed receipt of the second and third vaccine doses from the vaccination card and the health facility register. If infants received their second and third vaccine doses at private facilities, a copy of the vaccination card was collected as proof of date of exposure. For each dose, the vaccine batch number, date and place of administration was documented. Recruited infants were followed up to 4 weeks after the third dose of the vaccines or death, whichever was earlier. Data were collected on tablets using a customized program developed with open source platforms (Android, PHP and MySQL version 5.1) and data including other materials uploaded, synchronized in the server and stored/deposited in a central data repository. An independent International Advisory Group (IAG) with vaccine safety experts and Brighton Collaboration representation supervised the study implementation including field visits. The IAG reanalyzed the data for validation.

Outcomes

This study captured the serious AEFIs which included both hospitalizations and deaths due to any cause during the follow-up period. The details about the events such as clinical features, diagnosis, treatment, period and place of hospitalization were captured. For hospitalizations, the study team visited the hospital for confirmation and followed till outcome to document the information. Hospitalization episodes of at least 24-hours as inpatient were further categorized as "appropriate," "inappropriate" and "indeterminate" using the Pediatric Appropriateness Evaluation Protocol–India criteria. The PAEP-India tool for child and newborn were adapted from the available tools and validated in hospitalized patients from multiple hospitals in India.[27] Each case was assessed by 2 independent pediatricians using the PAEP-India tool for child and categorized the case based on checklist criteria. A third pediatrician was consulted in cases of mismatch between the 2 reviewers. For deaths, the study confirmed the event by home visit, followed by verbal autopsy by senior investigator 4 weeks later. Causes of death were determined independently by 3 pediatricians trained in review of verbal autopsies and also referred to available hospital records.

Data Analysis

The 4 weeks following every dose of vaccination were considered to be the observation period with weekly intervals post-vaccination as separate risk periods (Figure, Supplemental Digital Content 1, http://links.lww.com/INF/D758). As almost all the serious AEFI (deaths and hospitalizations) reported in the passive national AEFI surveillance program occurred during the first week after any vaccine dose, the first week was considered as the high-risk period and the fourth week as the comparison period. It was hypothesized that, the risks of serious AEFI due to any cause, during the first and fourth weeks post-vaccination remain similar for each dose of the vaccines.

Person-time of observation was calculated based on the time from vaccine administration and the date of occurrence of an event, the date of administration of the next vaccine dose (if <28 days), the last contact date (if lost to follow-up), date of death or the first 28 days after vaccination when the next dose was delayed, according to the recommended schedule, whichever came first. Follow-up was defined by weekly risk windows after each vaccine dose split into first (0–6 days), second (7–13 days), third (14–20 days) and fourth (21–27 days) weeks. If a subsequent dose was given before the end of the fourth week, the follow-up time was censored and follow-up period was assigned to the subsequent dose. If the next dose was delayed, the person-time beyond 27 days after each dose were excluded from the analysis. Poisson regression analysis was used to estimate the incidence rates (IRs) and incidence rate ratios (IRRs) for serious AEFIs (hospitalization and all-cause deaths) during the 4-consecutive weekly risk periods after each dose separately and for all 3-doses combined. IRs were calculated by dividing the number of events by the corresponding person-time denominator and IRRs were calculated by dividing the IR in the risk period of interest by the IR in the fourth week. In a multivariate model, the IRR

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was adjusted for age at start of risk period (continuous, in days) and seasonality (quarterly; January–March, April–June, July–September, and October–December). To assess the potential of healthy vaccinee bias, we performed a sensitivity analysis to compare the risks in the first week after vaccination following the second and third doses with the risks in the fourth weeks of the preceding doses. All statistical analyses were performed using Stata version 15.0 (StataCorp LLC, TX). In addition, all deaths documented were reviewed by the national AEFI committee for causality assessment according to WHO published methodology.[20]

Sample Size

Considering the IMR in the study states to be 12–21 per 1000 live births and a neonatal mortality rate (0–28 days) of 6–15 per 1000 live births,[26] the post-neonatal mortality (29–365 days) was expected to be 6 per 1000 live births. This translated to ~0.125 infant deaths per 1000 live births per week. As there was no information about the hospitalization:death ratio in this age group from India, we assumed that for every infant death there would be ~4 hospitalizations. The required sample size was 27,422 infants to detect an IRR for hospitalizations and or deaths of 1.8 in the analyses pooling the different doses, assuming a 5% significance level ($\alpha = 0.05$) and 80% power ($\beta = 0.2$).[28] With the available sample (30,688), the observed results show that between the risk periods, we are able to detect a risk-ratio of 2.5 and 1.5 (at a 5% significance level and 80% power) of serious AEFIs (hospitalization and deaths), for individual doses and all 3-doses combined, respectively.

Ethical Approvals

The study protocol and related documents were approved by ethics committees of participating institutes. The infants were recruited after obtaining informed written consent from parents or legally authorized representatives. The study protocol and related documents were approved by Institute Ethics committees at The INCLEN Trust International (INCLEN) (vide IIEC-021 dated October 14, 2013), Government Medical College, Thiruvananthapuram (vide 01/01/2014 dated January 17, 2014), PSG Institute of Medical Sciences and Research, Coimbatore (vide 13/351 dated March 5, 2014) and World Health organization, Geneva (vide RPC625 dated January 22, 2014).

RESULTS

During the study period, 34,914 infants received their first dose of pentavalent and OPV vaccines at the public health facilities. Of these, 1377 (3.9%) infants could not be traced, 2818 (8.1%) were not eligible (1287 were not staying in the district for 6 months, and 1531 were from other districts) and consent was refused for 31 (0.1%) infants. Of the 30,688 infants recruited, 30,208 (98.4%) received their third vaccine dose and 29,728 (96.9%) completed the scheduled follow-up (Fig. 1). Of 479,523 scheduled weekly follow-up contacts (including the contacts during the intervals between the different vaccine doses till completion of 4 weeks after third dose), 89.9% were completed with averages of 4.4 (dose 1), 4.8 (dose 2) and 4.9 (dose 3) contacts for the vaccine doses (data not shown).

Baseline characteristics of the cohort are summarized in Table 1. No age or sex differences were observed across the districts. The Kollam population had a higher proportion of low and median socio-economic status and joint or extended families. Median ages of infants were 48, 83 and 118 days at the times of the first, second and third vaccine doses, respectively. A total of 622 hospitalizations and 32 deaths were documented during the follow-up period.

Within the 4 weeks after the 3 vaccine doses (per protocol study risk periods), a total of 365 hospitalizations (120, 137 and 108 after doses 1, 2 and 3, respectively) were documented. Of these 365 hospitalizations, 288 were classified as appropriate (96, 106 and 86 after doses 1, 2 and 3, respectively). A total of 257 hospitalizations (92, 136 and 29 after doses 1, 2 and 3, respectively) occurred after fourth weeks of the first and second doses of vaccination (Fig. 1). Pneumonia/acute respiratory infection (70.1%) was the leading cause followed by diarrhea (6.4%), fever (4.3%) and urinary tract diseases (3.1%) during the 4 week and post-4 week periods after the vaccine doses (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759).

Of the 32 total deaths observed, 17 deaths (8,7 and 2 after doses 1, 2 and 3, respectively) occurred within 4 weeks after 3 vaccine doses, while 15 deaths (11, 3 and 1 after doses 1, 2 and 3, respectively) occurred during post-4 weeks period, (Fig. 1). The leading causes of death included infections (31.3%), congenital malformations (28.1%), congenital heart diseases with infections (15.6%), SIDS (15.6%) and other causes (9.4%) (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759). Almost all congenital malformations were diagnosed prior to death. The SIDS was made based on Brighton Collaboration Criteria.[29] Causality assessment by the National AEFI committee classified all deaths as coincidental. Differentials in causes of hospitalization and death during the first 4-weeks (0–27 days) and post-4 weeks (28+ days) after vaccination were small (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759). Figures 2 and 3 show the IRs of serious AEFIs (hospitalization and deaths) by age and seasonality. Higher serious AEFI (hospitalization and deaths) rates were observed during 91 and 146 days of age (Fig. 2); between October and December, with a peak in December due to higher prevalence of respiratory illnesses (Fig. 3).

Serious AEFIs (Hospitalization and Deaths) Within 4 Risk-periods

The occurrence of serious AEFIs (hospitalization and deaths) across the risk-periods for the 3 doses are summarized in Table 2. Within the 4 weeks after the 3 vaccine doses, a total of 375 serious AEFIs (365 hospitalizations, 17 deaths and 7 hospitalization and deaths) occurred (125, 140 and 110 after doses 1, 2 and 3, respectively) (Fig. 1). For all-3 doses combined, the adjusted IRRs of serious AEFIs within the first week compared with the fourth week were 0.8 [95% confidence interval, (CI): 0.6–1.0]. Compared with the fourth week, the adjusted IRRs of serious AEFIs for the first week after first, second and third doses were 1.2 (95% CI: 0.8–1.9), 0.9 (95% CI: 0.6–1.5) and 0.5 (95% CI: 0.3–0.9), respectively. IRRs for serious AEFIs were not significantly higher in any of the other risk periods (second or third weeks) either for the 3 doses separately or for all doses pooled. Analyses of hospitalizations according to appropriateness did not show any increased risk in any week compared with the fourth week after any dose (Tables, Supplemental Digital Content 3, http://links.lww.com/INF/D760, and Supplemental Digital Content 4, http://links.lww.com/INF/D761). The sensitivity analysis comparing the rates of serious AEFI in the first week after the second or third doses with the fourth week after the first or second doses did not show any differences, suggesting the absence of a healthy vaccinee effect (Table 2).

DISCUSSION

This is the first large study to examine the association between administration of routine infant pentavalent and OPV vaccines and serious AEFIs (all-cause hospitalizations and/or deaths) in India, using a self-controlled prospective cohort design. We have shown that the unadjusted and adjusted risks of hospitalizations

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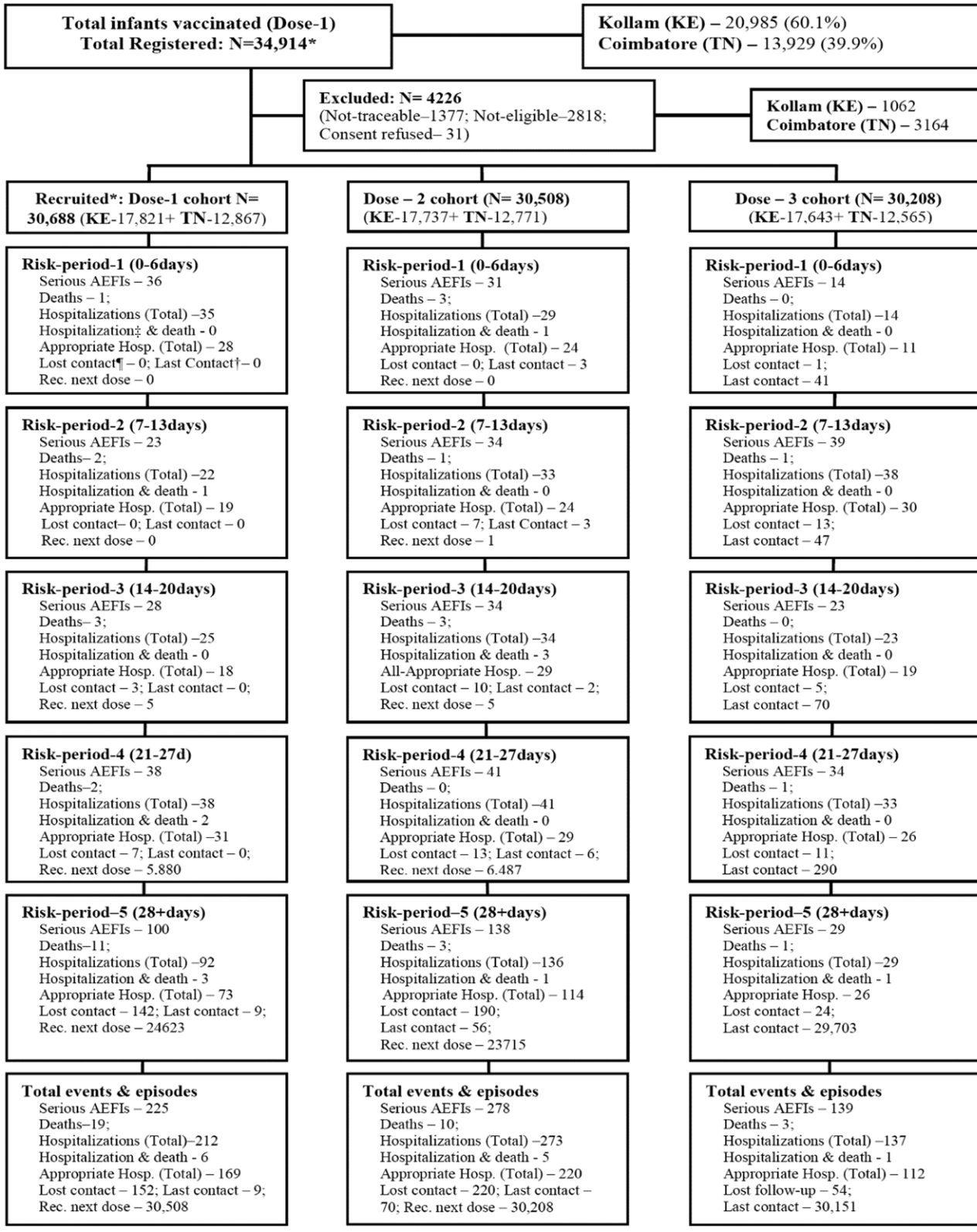


FIGURE 1. Selection and follow-up of the study population in the study districts and distribution of events. *Received first dose at public health facilities; ¶ lost contact is "Lost contact during the period"; †last contact defined as "Received respective vaccination and time period at last follow-up contact"; ‡appropriate hospitalizations as per expert opinion and identified using PAEP criteria. KE-Kerala; TN-Tamil Nadu.

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TABLE 1. Characteristics of Study Infants and Their Households

Characteristic	Kollam (N = 17,821)	Coimbatore (N = 12,867)	Combined (N = 30,688)*
Sex of the child (%)			
Males	50.3	50.4	50.3
Females	49.7	49.6	49.7
Median age at vaccination in days (P25, P75)			
Dose 1	47 (45, 50)	49 (46, 53)	48 (45, 51)
Dose 2	83 (79, 88)	83 (77, 90)	83 (78, 89)
Dose 3	118 (113, 127)	117 (109, 127)	118 (111, 127)
Birth order (%)			
1	58.7	49.4	54.8
2	37.0	43.0	39.5
≥3	4.3	7.6	5.7
Household standard of living index (%)			
Low	26.0	7.7	18.3
Middle	56.8	45.3	52.0
High	17.2	47.0	29.7
Type of family (%)			
Nuclear/elementary family	32.7	57.0	42.9
Joint/extended	67.3	43.0	57.1
Age of mother in years (mean ± SD)	25.9±4.3	24.9±4.2	25.5±4.3
Education of mother (%)			
Illiterate	0.1	2.0	0.9
Literate	99.9	98.0	99·1
Mean ± SD (in years)	12.9±2.7	11.1±3.3	12·1±3.1
Age of father in years (Mean ± SD)	32.2±4.5	30.2±4.7	31.4±4.7
Education of father (%)			
Illiterate	0.1	3.0	1.3
Literate	99.9	97.0	98.7
Mean ± SD (in years)	11.8±2.7	10.5±3.4	11.1±3.3
Received any ANC care (%)	98.9	92.5	96.2

*Total includes a small number of cases information missing for type of family, standard of living index (2 each in Kollam); Age of mother (20 in Kollam and 12 in Coimbatore); education of mother (14 in Kollam and 6 in Coimbatore); education of father (36 in Kollam and 16 in Coimbatore) and birth order (53 in Kollam and 40 in Coimbatore, respectively). Standard of living index (a proxy to household socio-economic status), constructed based on household assets and durable goods.

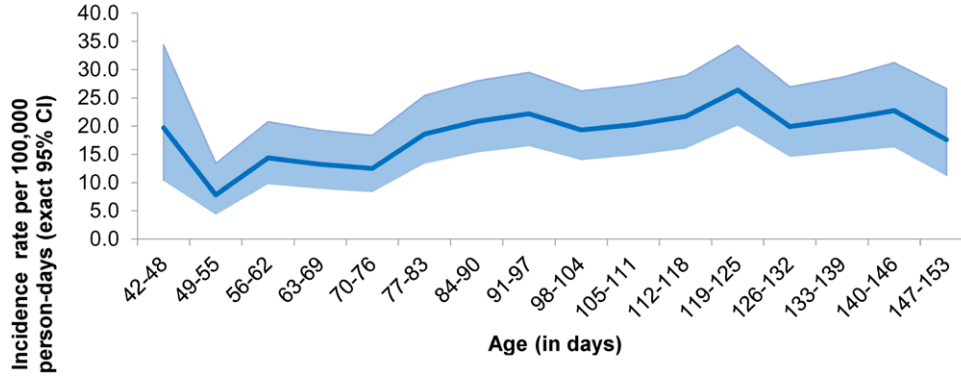


FIGURE 2. Serious AEFI (hospitalization and all-cause deaths) incidence rates (per 100,000 person-days with exact 95% CI as shaded area) by age (independent of vaccination windows). Incidence per 100,000 person-days (with exact 95% CI as shaded area); Week defined as 6-days period, starting on day-0; Person-time starts from age at first dose of vaccination and duration until the first and last day of the age period or until the Serious AEFI (hospitalization/death) events (succeeding vaccinations not considered). The hospitalization incidence presented in the figure is between 42 and 153 days of age, where 98.6% [(3,402,860/3,451,027)*100] of follow-up calls/visits were made. full color online

and deaths after any of the 3 scheduled doses of routine vaccines were similar during the first and fourth weeks post-vaccination, for either each dose separately, or all doses combined. Due to low death rates,we could not perform analyses for each individual dose but the combined analysis and analyzing the first dose only did not indicate any death clusters following vaccination.

In a highly immunized population, self-controlled study designs with defined risk windows for outcome assessment are best suited to assess possible effects during a specific time period as they are not susceptible to control selection.[30] Activation of the immune response to different vaccines occurs at different time points according to the nature of the antigen.[18,31–34] Hence, in the current study, we considered the first week after vaccination to represent the period of highest risk and the fourth week to be the comparison period. The findings of our study refute the concern of an association between serious adverse events and the pentavalent vaccine.

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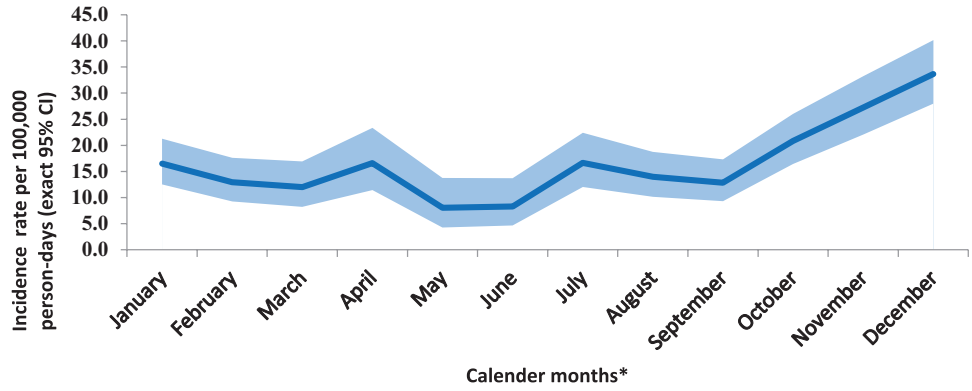


FIGURE 3. Serious AEFIs (hospitalization and all-cause deaths) incidence rates (per 100,000 person-days with exact 95% CI) by calendar months. Incidence per 100,000 person-days (with exact 95% CI); *Calendar month with 30-days period, irrespective of study year period; Person-time starts from the first day of the month and until the last day of the month period or until the Serious AEFI events (hospitalization/death). full color online

TABLE 2. Serious AEFIs (Hospitalization and All-Cause Deaths) IRs and IRRs (Unadjusted and Adjusted) After Pentavalent and Oral Polio Vaccines: Combined for Both Districts, Kollam (Kerala) and Coimbatore (Tamil Nadu), India

Vaccine dose/risk period (in days)*	Total number of		Incidence-rate (IR, with exact 95% CI)	IRRs, with 95% CI			
	Events	Person-time (in days)		Unadjusted	*P*	Adjusted†	*P*
Dose-1							
0–6 days	36	214,702	16.8 (11.7–23.2)	0.9 (0.6–1.5)	0.808	1.2 (0.8–1.9)	0.407
7–13 days	23	214,740	10.7 (6.8–16.1)	0.6 (0.4–1.0)	0.056	0.7 (0.4–1.2)	0.206
14–20 days	28	214,693	13.0 (8.7–18.9)	0.7 (0.5–1.2)	0.216	0.8 (0.5–1.3)	0.368
21–27 days‡	38	214,158	17.7 (12.6–24.4)	Reference		Reference	
Dose-2							
0–6 days	31	213,472	14.5 (9.9–20.6)	0.8 (0.5–1.2)	0.233	0.9 (0.6–1.5)	0.740
7–13 days	34	213,400	15.9 (11.0–22.3)	0.8 (0.5–1.3)	0.409	0.9 (0.6–1.5)	0.811
14–20 days	34	213,306	15.9 (11.0–22.3)	0.8 (0.5–1.3)	0.411	0.9 (0.6–1.4)	0.592
21–27 days‡	41	212,523	19.3 (13.8–26.2)	Reference		Reference	
Dose-3							
0–6 days	14	211,294	6.6 (3.6–11.1)	0.4 (0.2–0.8)	0.005	0.5 (0.3–0.9)	0.026
7–13 days	39	210,847	18.5 (13.2–25.3)	1.1 (0.7–1.8)	0.582	1.3 (0.8–2.0)	0.294
14–20 days	23	210,418	10.9 (6.9–16.4)	0.7 (0.4–1.1)	0.142	0.7 (0.4–1.2)	0.207
21–27 days‡	34	209,168	16.3 (11.3–22.7)	Reference		Reference	
All 3 doses							
0–6 days	81	639,468	12.7 (10.1–15.7)	0.7 (0.5–0.9)	0.020	0.8 (0.6–1.0)	0.068
7–13 days	96	638,987	15.0 (12.2–18.4)	0.8 (0.6–1.1)	0.226	0.9 (0.7–1.2)	0.374
14–20 days	85	638,417	13.3 (10.6–16.5)	0.7 (0.6–1.0)	0.044	0.8 (0.6–1.0)	0.062
21–27 days‡	113	635,849	17.8 (14.7–21.4)	Reference		Reference	
Sensitivity analyses							
0–6 days of dose-2	31	213,472	14.5 (9.9–20.6)	0.8 (0.5–1.3)	0.408	0.7 (0.4–1.1)	0.117
21–27 days of dose-1‡	38	214,158	17.7 (12.6–24.4)	Reference		Reference	
0–6 days of dose-3	14	211,294	6.6 (3.6–11.1)	0.3 (0.2–0.6)	0.001	0.3 (0.2–0.6)	0.001
21–27 days of dose-2‡	41	212,523	19.3 (13.8–26.2)	Reference		Reference	

*Risk period corresponds to date of hospitalization (considered as at least 24-hours stay in the hospital) or all-cause death after administration of vaccination (in days); The day of vaccination defined as day-0.

†Adjusted for seasonality (January–March, April–June, July–September, October–December); age at start of risk period (in days), using Poisson regression model.

‡Twenty-one to 27 days as reference category; IR: Number of events per 100,000 person-days of follow-time, calculated as the ratio of the hospitalizations of the person-time at risk.

The main strength of the study is the representativeness of the study cohort with respect to the population in the study area due to the high rates of participation and completion of follow-up. The socioeconomic status of the population enrolled in the study was highly representative of the overall population in the 2 districts. Use of electronic data collection enabled timely follow-up and accurate data capture with real-time monitoring. Documentation of hospitalization from both participants and hospitals allowed confirmation of causes of hospitalizations and deaths. In addition, the availability of source documents and vaccination records ensured accurate confirmation of vaccine exposure.

The main limitation was possible selection bias, as infants received first vaccine dose in the private system were not represented in the study. However, according to recent National Family Health Survey (NFHS-4, 2015–2016) data, 78% and 86% of children 12–23 months of age in Kerala and Tamil Nadu, received their vaccines from a public health facility.[25] We could not trace 3.9% infants due to inaccurate address information and 8.1% could not

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be included due to moving out or residing outside of the 2 districts. To control for confounding by contraindication to vaccination, pre-vaccination time intervals were not included in the control periods in the main analysis. Sensitivity analyses performed to assess the potential of a healthy vaccinee bias did not suggest presence of this. The study was also limited in detecting small changes for deaths, despite the large sample size.

The study found that 128 serious adverse events (120 hospitalizations and 8 deaths) occurred among 30,000 infants during the 4 weeks after receiving their first dose of pentavalent vaccine. Calculating based on Black et al[35] and projected on a 26 million annual Indian birth cohort, it is estimated that with national coverage of 100% at a minimum 26,500 infants per year will suffer a serious health event within 1 week of receiving their first dose of pentavalent vaccine, or there will be at least 21,200 events with the current 3-dose coverage of ~80% for pentavalent vaccine. These events reflect prevailing morbidities and mortality that would have occurred regardless of vaccination as documented in 2 Indian districts with low infant mortality. Investigating these events to ensure that the vaccine product or its administration are safe remains a fundamental function of the vaccine pharmacovigilance system. This highlights the importance of continued monitoring of common serious health problems in the infant population to measure the health burden and provide new epidemiologic information on the true causes of morbidity and mortality in this group.

The study did not find any increased risk of serious AEFIs (all-cause hospitalizations or deaths) in the first week following pentavalent (and OPV) vaccination and the occurrence of these AEFIs were just coincidental. That is, in the absence of temporal clustering, mortality and hospitalization rates observed in vaccinated children reflect the natural occurrence of such events in that age range in this part of India. The study findings should help to address the vaccine safety concerns and controversies around the pentavalent vaccine in India and other low- and middle-income countries, and so boost public confidence in the program. Suspicion about a possible relationship between the vaccination and serious AEFIs among parents, communities and the health providers may not only threaten the success of immunization programs but also potentially hinder introduction of newer vaccines.

CONCLUSIONS

Close monitoring of over 30,000 infants during and after receiving their first 3 doses of DTwP-HBV-Hib and OPV did not identify any relationship between the vaccinations and the occurrence of serious AEFIs (hospitalization and/or death). The study did document the huge number of serious coincidental events that can be expected even when vaccines are properly administered. In view of the recent inclusion of several new vaccines into the immunization program in India, the findings of this study will be very useful in addressing vaccine hesitancy and safety concerns to increase public acceptance and coverage.

APPENDIX

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REFERENCES

1. Roush SW, Murphy TV; Vaccine-Preventable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA*. 2007;298:2155–2163.
2. Kyu HH, Mumford JE, Stanaway JD, et al. Mortality from tetanus between 1990 and 2015: findings from the global burden of disease study 2015. *BMC Public Healt*. 2017;17:179.

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3. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375:1969–1987.
4. India State-Level Disease Burden Initiative Collaborators. Nations within a nation: variations in epidemiological transition across the states of India, 1990–2016 in the Global Burden of Disease Study. *Lancet*. 2017;390: 2437–2460.
5. Fitzpatrick M. MMR: risk, choice, chance. *Br Med Bull*. 2004;69:143–153.
6. Rosen JB, Arciuolo RJ, Khawja AM, et al. Public health consequences of a 2013 measles outbreak in New York City. *JAMA Pediatr*. 2018;172: 811–817.
7. Béraud G, Abrams S, Beutels P, et al. Resurgence risk for measles, mumps and rubella in France in 2018 and 2020. *Euro Surveill*. 2018;23 :pii=1700796.
8. Siani A. Measles outbreaks in Italy: a paradigm of the re-emergence of vaccine-preventable diseases in developed countries. *Prev Med*. 2019;121: 99–104.
9. Lisowski B, Yuvan S, Bier M. Outbreaks of the measles in the Dutch Bible Belt and in other places - new prospects for a 1000 year old virus. *Biosystems*. 2019;177:16–23.
10. Rao M, Govindarajan V. WhatsApprumours about vaccinations hamper India's drive to halt measles and rubella, The government plans to extend the campaign by a week to achieve targets. scroll.in Media report, [Internet]. Feb 24 2017; Available from: https://scroll.in/pulse/830129/rumours-about-measles-rubella-vaccine-hit-coverage.
11. Larson HJ, de Figueiredo A, Xiahong Z, et al. The state of vaccine confidence 2016: global insights through a 67-country survey. *EBioMedicine*. 2016;12:295–301.
12. World Health Organization. *Global Manual on Surveillance of Adverse Events Following Immunization*. Geneva: World Health Organization; 2014.
13. Centers for Disease Control and Prevention (CDC). Progress toward introduction of Haemophilusinfluenzae type b vaccine in low-income countries--worldwide, 2004-2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:148–151.
14. World Health Organization. Global Advisory Committee on Vaccine Safety (GACVS), Report of meeting 12–13 June 2013. *ReleveEpidemiolHebd*. 2013;88:301–312.
15. TVR Staff (Media report). Three infants in Pakistan die after receiving polio vaccine. The Vaccine Reaction [Internet]. May 13, 2018; Available from: https://thevaccinereaction.org/2018/05/three-children-die-after-receiving-polio-vaccine-in-pakistan/#_edn1.
16. Chen RT, Shimabukuro TT, Martin DB, et al. Enhancing vaccine safety capacity globally: a lifecycle perspective. *Vaccine*. 2015;33(suppl 4): D46–D54.
17. Vennemann MM, Höffgen M, Bajanowski T, et al. Do immunisations reduce the risk for SIDS? A meta-analysis. *Vaccine*. 2007;25:4875–4879.
18. Kuhnert R, Schlaud M, Poethko-Müller C, et al. Reanalyses of case-control studies examining the temporal association between sudden infant death syndrome and vaccination. *Vaccine*. 2012;30:2349–2356.
19. Walker AM, Jick H, Perera DR, et al. Diphtheria-tetanus-pertussis immunization and sudden infant death syndrome. *Am J Public Health*. 1987;77:945–951.
20. Ministry of Health and Family Welfare (MoHFW),Government of India. Results of causality assessment for reported AEFIs [Internet]. New Delhi: Ministry of Health and Family Welfare, Government of India; 2018. Available from: https://mohfw.gov.in/node/107.
21. Moneylife Digital Team Media reports. Stop unnecessary vaccination: eminent doctors, scientists to the PM. [Internet]. Dec 1, 2014; Available from: https://www.moneylife.in/article/stop-unnecessary-vaccination-eminent-doctors-scientists-to-the-pm/39645.html.
22. Nair H, Hazarika I, Patwari A. A roller-coaster ride: introduction of pentavalent vaccine in India. *J Glob Health*. 2011;1:32–35.
23. Sharma HJ, Yadav S, Lalwani SK, et al. Immunogenicity and safety of an indigenously manufactured reconstituted pentavalent (DTwP-HBV+Hib) vaccine in comparison with a foreign competitor following primary and booster immunization in Indian children. *Hum Vaccin*. 2011;7:451–457.
24. Dutta AK, Verghese VP, Pemde HK, et al. Immunogenicity and safety of a pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Haemophilus influenzae type B conjugate combination vaccine (Pentaxim) with hepatitis B vaccine. *Indian Pediatr*. 2009;46:975–982.
25. International Institute for Population Sciences (IIPS) and ICF. National Family Health Survey (NFHS-4), 2015-16: India [Internet]. Mumbai: IIPS; 2017. Available from: http://rchiips.org/nfhs/NFHS-4Reports/India.pdf.
26. Ministry of Home Affairs, Government of India. SRS Statistical Report 2013, Report No. 1 of 2014:75-76 [Internet]. Ministry of Home Affairs, Government of India; 2014. Available from: http://www.censusindia.gov.in/vital_statistics/SRS_Reports_2013.html.
27. Das MK, Arora NK, Poluru R, et al. Pediatric Appropriate Evaluation Protocol for India (PAEP-India): tool for assessing appropriateness of pediatric hospitalization. *Indian Pediatr*. 2018;55:1041–1045.
28. Lwanga S, Lemeshow S. *Sample Size Determination in Health Studies: A Practical Manual*. Geneva: World Health Organization; 1991.
29. Jorch G, Tapiainen T, Bonhoeffer J, et al.; Brighton Collaboration Unexplained Sudden Death Working Group. Unexplained sudden death, including sudden infant death syndrome (SIDS), in the first and second years of life: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*. 2007;25:5707–5716.
30. Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. *Epidemiol Infect*. 2011;139:1805–1817.
31. Gold M, Dugdale S, Woodman RJ, et al. Use of the Australian Childhood Immunisation Register for vaccine safety data linkage. *Vaccine*. 2010;28:4308–4311.
32. Cameron JC, Walsh D, Finlayson AR, et al. Oral polio vaccine and intussusception: a data linkage study using records for vaccination and hospitalization. *Am J Epidemiol*. 2006;163:528–533.
33. Stowe J, Andrews N, Ladhani S, et al. The risk of intussusception following monovalent rotavirus vaccination in England: a self-controlled case-series evaluation Ref. No: JVAC-D-16-01124. *Vaccine*. 2016;34:6115.
34. Patel MM, López-Collada VR, Bulhões MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med*. 2011;364:2283–2292.
35. Black S, Eskola J, Siegrist C-A, et al. Importance of background rates of disease in assessment of vaccine safety during mass immunisation with pandemic H1N1 influenza vaccines. *Lancet*. 2009;374:2115–2122.

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[illegible] Anaemia is almost universal in haemodialysis patients. Here, we evaluated the utility and clinical implications of the commonly employed tests in assessing iron status in haemodialysis patients.

[illegible] In this prospective observational study, we enrolled 100 prevalent haemodialysis patients, and measured transferrin saturation (TSAT) and serum ferritin at enrolment. Patients were categorized to have normal, deficient, overload, or indeterminate status of iron based on KDOQI guidelines. To study anaemia trend, haemoglobin (Hb) level at time of enrolment, as well as levels 3-months before and 3-months after enrolment, were collected. Patient survival outcomes were also obtained till October 2016.

[illegible] Using serum ferritin and TSAT, iron status was determinable in 48% of the patients ie 20%-normal iron status, 25% - iron deficiency, 3% - iron overload. Fifty-two percent of the patients' iron status was indeterminate by current parameters. In spite of being on standard-of-care treatment in our centre, we observed that mean-Hb level of patients in the indeterminate group showed insignificant increases compared to normal- and deficient-groups. Mean delta-Hb levels over 6-months were 0.80±1.54 g/dl in the indeterminate group, compared to 1.87±1.95 g/dl in the deficient group (P=0.03). In our cohort, 57% had died and 5 lost follow-ups during the study (P=0.30 between groups).

[illegible] Serum ferritin and TSAT accurately categorized the iron status of 48% haemodialysis patients in our study. Divergent serum ferritin and TSAT values, or an iron indeterminate state was prevalent in 52% of our urban dialysis cohort. The indeterminate group had inferior increases in Hb over time with current treatment, showing clinical relevance of this finding. Our data suggests the need for more sensitive indices to accurately assess iron status, and improve anaemia management.

[illegible] ESRD patients, Iron status, Serum ferritin, TSAT

[illegible]

Anaemia is frequently found in chronic kidney disease (CKD) patients and is an almost universal finding in patients with end stage renal disease (ESRD).[1] Additionally, anaemia is associated with increased cardiovascular morbidity and mortality in this patient population.[2]

The pathophysiology of anaemia in CKD is multifactorial.[3] The proximate cause of the anaemia is relative deficiency of erythropoietin, a glycoprotein produced by renal tubular cells. Other factors which may cause or contribute to anaemia includes iron deficiency, vitamin B12 and/or folic acid deficiency, decreased erythrocyte survival, severe hyperparathyroidism, aluminium toxicity and hypothyroidism. The use of erythrocyte stimulating agents (ESA) such as recombinant human erythropoietin (EPO) began two decades ago and has revolutionized the treatment of anaemia in ESRD. This has decreased the need for blood transfusions and consequently the incidence of associated complications.[4]

Despite the widespread use of EPO, over 50% of the patients do not reach target haemoglobin levels.[1,5] Resistance to erythropoietin most commonly is due to iron deficiency but chronic inflammation, infection, and/or hyperparathyroidism also contribute.[6,7] Iron deficiency in ESRD is primarily due to impaired dietary intake and impaired gastrointestinal absorption but blood loss during haemodialysis and increased iron demands from ESA use also contribute.[8] Management of iron deficit is crucial in haemodialysis patients. Adequate treatment of iron deficiency is vital to attain target haemoglobin concentration and avoid excessively high EPO doses, which has been associated with increased risk of malignancy.[9] Furthermore, IV iron and blood transfusions both increase the risk of developing hospital acquired infections.[10,11] Therefore effective treatment of anaemia in haemodialysis patients requires continuous assessment of iron status.

Serum ferritin and transferrin saturation (TSAT) are the commonly used tests for the assessment of iron status in ESRD. However, measurement of these parameters in ESRD patients may be altered by inflammation, infection, or nutritional status.[12] Therefore, we conducted this study to evaluate the utility of the common tests, serum ferritin and TSAT, in assessing iron status in haemodialysis patients. In order to examine the clinical utility of iron status as defined by the current guidelines, we evaluated anaemia in this cohort, as well as patient outcomes.

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This study was conducted from August 2011 to March 2012 in a large tertiary care teaching hospital in South India. The study was conducted among the ESRD patients undergoing haemodialysis. All ESRD patients on haemodialysis for at least 3 months were enrolled in the study after the application of the exclusion criteria and after obtaining informed consent. The study was done after obtaining institutional ethical committee clearance. The exclusion criteria were: age less than 18 years old, evidence of acute or chronic infection, trauma within the previous 4 weeks, recent bleeding episodes, blood transfusion within the last one month, malignancy, post-transplant status, liver disease, haemoglobinopathies and other haematological diseases. The diagnostic biochemical tests used to assess the iron status were TSAT and serum ferritin.

Patients were categorized to have normal, deficient, overload, or indeterminate status of iron based on serum ferritin and TSAT values. Based on NKF-DOQI guidelines, iron deficiency is taken as ferritin level less than 200mg/dl and TSAT less than 20%, iron overload as ferritin level more than 500 mg/dl and TSAT more than 50%. The values in between the above ranges (ferritin between 200 and 500 mg/dl; TSAT between 20 and 50 %) are considered as normal iron status, whereas values not conforming to these categories are said to be indeterminate. The combined utility of the above tests in assessing iron status was determined by dividing the number of patients in whom iron status was detected (normal, deficient or overload) by total number of patients and expressed in percentage. The haemoglobin level of all the enrolled patients at time of enrolment was collected. To determine haemoglobin trend within each group, we collected haemoglobin levels 3-months before and 3-months after enrolment from chart review. Patient outcomes (death, alive and lost follow up) were obtained by chart review and telephone-follow up till October 2016.

Statistical analysis

The data were analysed using Statistical Package for Social Science (SPSS) Version 19.0, Graphpad Prism-6 and Microsoft Excel 2010. Non-parametric characteristics between any two groups were compared using Mann-Whitney test, and proportions using Chi-square test. For comparing more than two mean values we have used one-way analysis of variance. P value less than 0.05 was considered as statistically significant.

□□□□□□□□

Demographics of study population

A total of 100 prevalent ESRD patients on haemodialysis were studied. The key demographics of the study cohort are summarized in Table 1. Patients ranged from 18 to 80 years. The mean age of the patients was 48.5 years. Among 100 patients, 63 were male and 37 were female.

Description by iron status

We classified these patients based on their iron status as per the NKF-KDOQI (see methods). Using serum ferritin and TSAT, iron status could be determined in 48% of the patients; 20% had normal iron status, 25% had iron deficiency, 3% had iron overload. The remaining 52% of the patient's iron status was indeterminate (Table 1 and 2). In univariate analysis, patients who were iron indeterminate were nearly indistinguishable from other groups by clinical and epidemiologic variables (as shown

in Table 1). However interestingly, iron indeterminate and overload groups had significantly greater dialysis vintage than the normal/iron-deficient groups.

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	M [illegible]	M [illegible]	M [illegible]	M [illegible]	M [illegible]	
Age (years)	48.5 (13.91)	43.85 (11.75)	48.56 (13.18)	48.66 (23.18)	50.25 (14.48)	0.38
Sex						
Male (%)	63 (63)	12 (60)	17 (68)	1 (33.3)	33 (63.5)	0.68
Female (%)	37 (37)	8 (40)	8 (32)	2 (66.7)	19 (36.5)	
Vintage (duration in months)	21 (18.76)	13.65 (11.96)	15.88 (13.09)	48.00 (41.56)	24.73 (19.75)	0.003
Diabetes mellitus (%)	30 (30)	5 (25)	9 (36)	0 (0)	16 (30.8)	0.57
Systemic Hypertension (%)	84 (84)	16 (80)	24 (96)	2 (66.7)	42 (80.8)	0.26
Cardiac disease (%)	12 (12)	2 (10)	5 (20)	0 (0)	5 (9.6)	0.51
Iron supplements (route and duration)						0.24
Oral >6months	27 (27)	2 (10)	6 (24)	2 (66.7)	17 (32.7)	
Oral < 6months	13 (13)	3 (15)	4 (16)	0 (0)	6 (11.5)	
IV > 6months	28 (28)	6 (30)	4 (16)	0 (0)	18 (34.6)	
IV<6months	16 (16)	6 (30)	5 (20)	0 (0)	5 (9.6)	
No iron supplements	16 (16)	3 (15)	6 (24)	1 (33.3)	6 (11.5)	
Haemoglobin (g/dL)	7.95 (1.74)	8.14 (1.76)	7.23 (1.60)	8.26 (2.39)	8.21 (1.71)	0.12
Ferritin (ng/mL)	423.12 (388.61)	346.40 (86.87)	114.2 (48.45)	1446.00 (549.06)	542.13 (390.31)	<0.001
TSAT (expressed in %)	23.94 (11.58)	27.68 (5.57)	14.69 (4.15)	61.27 (8.94)	24.80 (10.37)	<0.001
Iron (micro g/dL)	59.62 (25.91)	66.45 (20.86)	43.32 (12.83)	106.00 (72.95)	62.15 (23.95)	<0.001
TIBC (micro g/dL)	260.97 (63.31)	240.55 (57.80)	300.20 (71.09)	164.33 (89.01)	255.53 (48.46)	<0.001
Died (%)	57 (57)	9 (45)	14 (56)	3 (100)	31 (59.6)	0.30

TSAT: Transferrin saturation, TIBC: Total iron binding capacity.

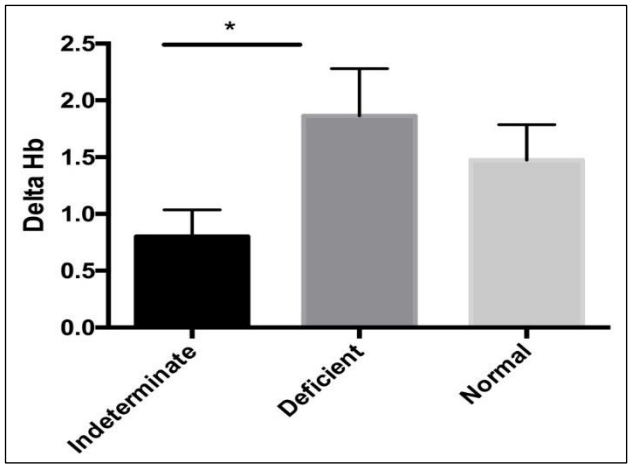
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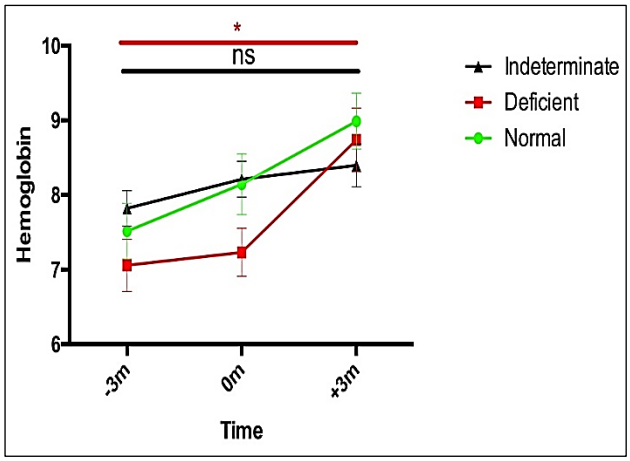
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[illegible]	48	48	23.02	12.81	294.18	346.62
Normal	20	20	27.68	5.57	346.4	86.87
Deficient	25	25	14.69	4.15	114.2	48.45
Overload	3	3	61.27	8.94	1446	549.06
[illegible]	52	52	24.80	10.37	542.13	390.31
High serum ferritin-normal TSAT	22	22	29.54	8.28	805.13	413.67
High serum ferritin-low TSAT	4	4	18.49	2.24	815	195.30
High TSAT- normal ferritin	1	1	67.95		496	
High TSAT-low ferritin	0	0				
Low ferritin-normal TSAT	11	11	25.49	4.35	154.54	40.18
Low TSAT-normal ferritin	14	14	15.53	2.98	358.71	87.84

TSAT: Transferrin saturation.

Outcomes by iron status

Anaemia outcomes: To examine the haemoglobin responses and anaemia, trends of patients according to iron status, we tabulated haemoglobin levels of patients in each group by iron status at enrolment at different time points before- and after- study enrolment (Figure 1 and Figure 2).





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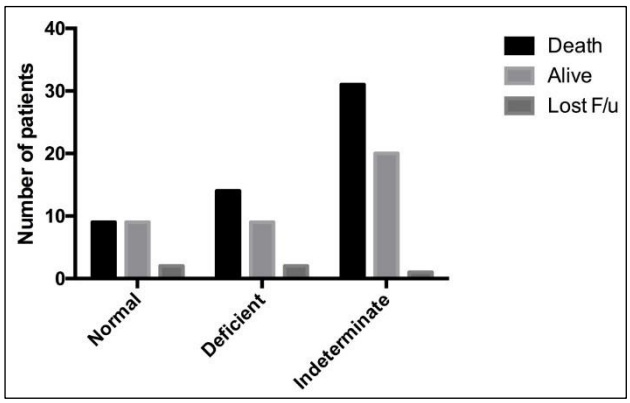
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Haemoglobin 3months before (g/dL)	n=94 7.56(1.64)	n=19 7.51(1.61)	n=23 7.05 (1.67)	n=3 7.46 (1.15)	n=49 7.82 (1.66)
Haemoglobin 0 month (g/dL)	n=100 7.95(1.74)	n=20 8.14(1.76)	n=25 7.23 (1.60)	n=3 8.26 (2.39)	n=52 8.21 (1.71)
Haemoglobin 3 months after (g/dL)	n=90 8.64 (1.91)	n=19 8.98(1.63)	n=23 8.73 (2.00)	n=3 9.56 (3.13)	n=45 8.39 (1.92)

Table 3 summarizes haemoglobin levels and patients (n) in each group at each of the different time points studied. As the overload group consisted of 3 patients only, we excluded these from subsequent analysis. We interestingly observed that the mean haemoglobin level of patients in the Normal and Deficient groups showed significant increases over a 6-month time period, while those in the indeterminate group had insignificant increases in haemoglobin levels in this period (Figure 1). Mean delta haemoglobin levels over 6 months (3-months before enrolment to 3-months after enrolment) were 0.80±1.54 g/dl in the indeterminate group, compared to 1.87±1.95 g/dl in the deficient group (Mann Whitney P=0.03; Figure 2).

Patient outcomes

Overall during the follow up period, 29 patients continued on dialysis, 5 were at outside dialysis units, 8 patients received kidney transplants (4 of whom expired after transplantation). A total of 57 patients in our cohort had died during the follow up period (till October 2016). Five patients were lost to follow up in this time. There were no significant differences detectable in number of deaths between any of the groups by iron status in the duration of our follow-up, although all 3 patients in the overload group had expired (Table 1 and Figure 3).



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Anaemia contributes significantly to the morbidity and mortality in ESRD. ESA and iron supplementation are the most important factors in treatment of anaemia in ESRD patients.[13] Iron deficiency has been identified as a major factor for anaemia in CKD patients, with a 43-90% prevalence in different series.[6,14-16] Hence the periodical monitoring of iron status is crucial in ESRD.

There are two forms of iron deficiency, absolute iron deficiency and functional iron deficiency. Reduction of reticuloendothelial system iron in the bone marrow is referred to as absolute iron deficiency. Absolute iron deficiency may occur from impaired intestinal absorption, blood loss through the dialysis circuit, vascular access surgeries, repeated phlebotomies and increased iron utilization with erythropoietin therapy. In clinical practice, absolute iron deficiency is determined by serum ferritin less than 200ng/ml and TSAT less than 20%.[17] In the non-ESRD population, "functional" iron deficiency is ascribed when iron reserves in the bone marrow are adequate, but iron mobilization is impaired as in states of chronic inflammation.[18]

The current study assesses the utility of combining serum ferritin and TSAT to assess iron status among an Indian haemodialysis population. The evaluation of iron status is straightforward when both serum ferritin and TSAT are either high or low. But when these parameters are divergent, for instance, if the TSAT is low and serum ferritin is high, the accurate interpretation of iron status is difficult. This entity was common in our study where the iron status of only 48% of the patients were clearly determined (20% had normal iron status, 25% had iron deficiency and 3% had iron overload), and was indeterminate in the remaining 52% of patients. Interestingly in our study, the only epidemiologic factor associated significantly with indeterminate status was dialysis vintage. Why patients with indeterminate or overload status appeared to have longer durations of dialysis, is unclear but requires further exploration. Our data suggest that in a significant proportion of haemodialysis patients in a typical urban dialysis unit in India, the conventionally applied parameters were insufficient to accurately categorize iron status. Similar data has emerged from other groups.[19] Further, our data crucially demonstrates that patients with indeterminate status by conventional assessment have significantly inferior increases in haemoglobin levels with current therapy, suggesting the need for development of more specific diagnostic strategies to ascertain iron status in this group, to accurately manage iron status and anaemia.

Most studies have revealed that serum ferritin is very well related with iron stores in the bone marrow is the best surrogate of iron status in healthy individuals.[20-22] However, ferritin is an acute phase reactant increasing in states of chronic inflammation (such as dialysis), malignancy, or systemic disease, and ferritin levels alone may not reflect iron stores available for haematopoiesis. Thirty-six patients had low ferritin (<200) in our study. Only 25 of them had iron deficiency (combined with low-TSAT), whereas ~31% of them were in indeterminate group. Further, 29 patients had high serum ferritin. Only 3 of these had iron overload (combining TSAT), while 4/29 had low TSAT (<20%). Hence, decision to treat these patients with iron supplements based on these parameters would likely be imprecise. Similarly, a transferrin saturation of less than 20% in patients on haemodialysis is considered to be suggestive of iron deficiency whereas other studies have suggested that a transferrin saturation < 20% versus >20% alone may be inaccurate for assessing iron deficiency.[23-28] While most patients with TSAT less than 20% are iron deficient, few patients don't respond to an increase in iron supplements with either increase in haematocrit or maintenance of haematocrit with a decreased ESA requirement. On the other hand, numerous patients with a TSAT greater than 20% have functional iron deficiency respond to increased iron doses with elevations in the haematocrit, or maintenance of their haematocrit at decreased erythropoietin doses.[25-28] In our study, 43 patients had low TSAT (<20%); only 25 of them had iron deficiency (combined with ferritin). We found that 57 patients had TSAT values more than 20%, out of which 11 had low ferritin (<200). TSAT values in these 11 patients may be falsely high because of low total iron binding capacity (TIBC) during inflammation and infection.

The difficulties in the interpretation of iron status using TSAT and serum ferritin in ESRD patients and the consequent hematologic implications we show, warrant the application of other erythrocyte and reticulocyte based indices, such as the percentage of hypochromic red blood cells (Hypo) and the reticulocyte haemoglobin content (CHr). CHr requires a special analyser and is recommended as an alternative iron status indicator as well as an initial measure of the response to iron treatment in haemodialysis population.[29-31] Fishbane et al evaluated the reticulocyte haemoglobin content among 32 haemodialysis population and showed it to have a sensitivity of 100% and specificity of 80%.[29] Mittman et al evaluated reticulocyte haemoglobin content among 364 haemodialysis population receiving EPO and proved it to be an indicator of functional deficiency of iron.[30] Based on these and other data, NKF DOQI recommends a target of CHr greater than 29pg/cell.[32-35] The Hypo is an indicator of the percentage of red blood cells that contain low haemoglobin. Thus, it indicates only the functional iron availability and does not reflect storage iron. A Hypo level of greater than 10% indicates definitive functional iron deficiency. European Best Practice Guidelines stated that Hypo >10% is the most accurate marker for ID.[36] However, there are other studies which suggested lower threshold values for Hypo.[32,37-40] Both these tests are not widely available in India.

Here, we report that the combination of serum ferritin and TSAT accurately categorized the iron status in only 48%

of ESRD patients in our study. When serum ferritin and TSAT values diverged, they become unreliable for accurate interpretation of iron status, signaling an iron indeterminate state that was prevalent in 52% of our urban dialysis cohort. As we show here, this group of iron-indeterminate patients has inferior increases in haemoglobin over time, showing clinical relevance of this finding. Our data suggests that more sensitive indices such as Hypo and/or CHr may need to be employed (along with conventional tests) to accurately assess iron status, and improve management tools. This could lead to more accurate dosing of iron and ESAs, decrease overtreatment of anaemia, and possibly translate to cost savings from medication usage. Future studies on iron markers including both traditional and newer indices are required to improve iron management in ESRD population, especially in Indian population, where iron deficiency is common and similar studies are lacking.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

References

1. KDOQI. Clinical Practice Guidance and Clinical Practice Recommendations for anaemia in Chronic Kidney Disease: 2007 Update of Haemoglobin target. Am J Kidney Dis. 2007;50:471-530.
2. Parfrey PS, Lauve M, Latremouille-Viau D, Lefebvre P. Erythropoietin therapy and left ventricular mass index in CKD and ESRD patients: a meta-analysis. Clin J Am Soc Nephrol. 2009;4:755-62.
3. Sonnweber T, Theurl I, Seifert M, Schroll A, Eder S, Mayer G, et al. Impact of Iron treatment on immune effector function and cellular iron status of circulating monocytes in dialysis patients. Nephrol Dial Transplant. 2011;26:977-87.
4. Feldman HI, Santanna J, Guo W, Furst H, Franklin E, Joffe M, et al. Iron administration and clinical outcomes in haemodialysis patients. J Am Soc Nephrol. 2002;13:734-44.
5. Collins AJ, Li S, Ebben J, Ma JZ, Manning W. Hematocrit levels and associated Medicare expenditures. Am J Kidney Dis. 2000;36:282-93.
6. MacDougall IC, Hutton RD, Cavill I, Coles GA, Williams JD. Poor response to treatment of renal anaemia with erythropoietin corrected by iron given intravenously. BMJ. 1989;299:157-8.
7. Sakiewicz P, Paganini E. The use of iron in patients on dialysis- mistakes and misconceptions. J Nephrol. 1998;11:5-15.
8. Horl WH. Clinical aspects of iron use in the anaemia of kidney disease. J Am Soc Nephrol. 2007;18:382-93.
9. René É, Lazrak HH, Laurin LP, Elftouh N, Vallée M, Lafrance JP. Association of erythropoiesis stimulating agents and the incidence risk of cancer diagnosis among chronic dialysis patients: a nested case-control study. Nephrol Dial Transplant. 2017;32:1047-52.
10. Brewster UC, Perazella MA. Intravenous iron and the risk of infection in end-stage renal disease patients. Semin Dial. 2004;17:57-60.
11. Rohde JM, Dimcheff DE, Blumberg N, Saint S, Langa KM, Kuhn L, et al. Health care-associated infection after red blood cell transfusion: a systematic review and meta-analysis. JAMA. 2014;311:1317-26.
12. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol. 2006;1:S4-8.
13. Nissenson AR. Hyporesponsiveness to erythropoietin: overview, 1996. Perit Dial Int. 1996;16:417-20.
14. Eschabach JW, Abdulhadi MH, Browne JK, Delano BG, Downing MR, Egrie JC, et al. Recombinant human erythropoietin in anaemic patients with end stage renal disease. Results of a phase III multicenter clinical trial. Ann Intern Med. 1989;111:992-1000.
15. Aggarwal HK, Nand N, Singh S, Singh M, Hemant, Kaushik G. Comparison of oral versus intravenous iron therapy in predialysis patients of chronic renal failure receiving recombinant human erythropoietin. J Assoc Physicians India. 2003;51:170-4.
16. Gupta M, Kannan M, Gupta S, Saxena R. Contribution of iron deficiency to anemia in chronic renal failure. Indian J Pathol Microbiol. 2003;46:563-4.
17. Fernandez-Rodriguez AM, Guindeo-Casasus MC, Molero-Labarta T, Domínguez-Cabrera C, Hortal-Casc n L, Pérez-Borges P, et al. Diagnosis of iron deficiency in chronic renal failure. Am J Kidney Dis. 1999;34:508-13.
18. Coyne DW. Hepcidin: clinical utility as a diagnostic tool and therapeutic target. Kidney Int. 2011;80:240-4.
19. Rafi A, Karkar A, Abdelrahman M. Monitoring iron status in end stage renal disease patients on haemodialysis. Saudi J Kidney Dis Transplant. 2007;18:73-8.
20. Caville I. Disorders of iron metabolism. Diagnostic methods. Clin Haematol. 1982;11:259-73.
21. Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. Annu Rev Med. 1993;44:63-74.
22. Cooper MJ, Zlotkin SH. Day-to-day variation of transferrin receptor and ferritin in healthy men and women. Am J Clin Nutr. 1996;64:738-42.

23. Macdougall IC. Monitoring of iron status and iron supplementation in patients treated with erythropoietin. Curr Opin Nephrol Hypertens. 1994;3:620-5.
24. Neff M. Algorithm for the treatment of anaemia in ESRD. Network Forum Quality Assurance Committee; 1995.
25. Fishbane S, Kowalski EA, Imbriano LJ, Maesaka JK. The evaluation of iron status in haemodialysis patients. J Am Soc Nephrol. 1996;7:2654-7.
26. Macdougall IC, Tucker B, Thompson J, Tomson CR, Baker LR, Raine AE. A randomized controlled study of iron supplementation in patients treated with erythropoietin. Kidney Int. 1996;50:1694-9.
27. Silverberg DS, Laina A, Peer G, Kaplan E, Levi BA, Frank N, et al. Intravenous iron supplementation for the treatment of the anemia of moderate to severe chronic renal failure patients not receiving dialysis. Am J Kidney Dis. 1996;27:234-8.
28. Silverberg DS, Blum M, Peer G, Kaplan F, Laina A. Intravenous ferric saccharate as an iron supplement in dialysis patients. Nephron. 1996;72:413-7.
29. Fishbane S, Galgano C, Langley RC Jr, Cranfield W, Maesaka JK. Reticulocyte haemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int. 1997;52:217-22.
30. Mittman N, Sreedhara R, Mushnick R, Chattopadhyay J, Zelmanovic D, Vaseghi M, et al. Reticulocyte haemoglobin content predicts functional iron deficiency in hemodialysis patients receiving EPO. Am J Kidney Dis. 1997;30:912-3.
31. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. Crit Rev Clin Lab Sci. 2000;37:93-130.
32. Cullen P, Söffker J, Höpfl M, Bremer C, Schlaghecken R, Mehrens T, et al. Hypochromic red cells and reticulocyte haemoglobin content as markers of iron-deficient erythropoiesis in patients undergoing chronic haemodialysis. Nephrol Dial Transplant. 1999;14:659-65.
33. Fishbane S, Galgano C, Langley RC Jr, Canfield W, Maesaka JK. Reticulocyte hemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int. 1997;52:217-22.
34. Mittman N, Sreedhara R, Mushnick R, Chattopadhyay J, Zelmanovic D, Vaseghi M, et al. Reticulocyte hemoglobin content predicts functional iron deficiency in hemodialysis patients receiving EPO. Am J Kidney Dis. 1997;30:912-22.
35. KDOQI; National Kidney Foundation. Clinical Practice Guidelines and Clinical Practice Recommendations for Anaemia in Chronic Kidney Disease. Am J Kidney Dis. 2006;47:S11-145.
36. European best practice guidelines for the management of anaemia in patients with chronic renal failure. Nephrol Dial Transplant. 1999;14:S14-15.
37. Braun J, Lindner K, Schreiber M, Heidler A, Hörl WH. Percentage of hypochromic red cells as predictor of erythropoietic and iron response after i.v. iron supplementation in maintenance haemodialysis patients. Nephrol Dial Transplant. 1997;12:1173-81.
38. Jones CH, Richardson D, Ayers S, Newstead CG, Will EJ, Davison AM. Percentage hypochromic red cells and the response to intravenous iron therapy in anaemic haemodialysis patients. Nephrol Dial Transplant. 1998;13:2873-6.
39. Tessitore N, Solero P, Gammaro, Sabato A, Maschio G. Iron status and low dose intravenous Fe gluconate in hemodialysis patients on maintenance erythropoietin (Abstract). J Am Soc Nephrol. 1998;9:272.
40. Tessitore N, Solero GP, Lippi G, Bassi A, Faccini GB, Bedogna V, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. Nephrol Dial Transplant. 2001;16:1416-23.

r Mambatta AK, Alagesan M, Meeran M, Rein JL, Ganesan S, Mathew AC, et al. Evaluation of iron status in patients with end stage renal disease. Int J Adv Med 2017;4:1415-21.

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Original Article

Clinical Efficacy of Levosimendan vs Milrinone in Preventing Low Cardiac Output Syndrome Following Pediatric Cardiac Surgery

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ABSTRACT

Background: Prophylactic milrinone is commonly used to prevent Low Cardiac Output Syndrome (LCOS) after pediatric cardiac surgery. This study compares the use of levosimendan with milrinone when used as the primary inotrope following pediatric cardiac surgery.

Subjects and Methods: Forty infants undergoing corrective surgery for congenital heart disease were recruited during the study and randomized into two groups (group L and group M). During rewarming, a loading dose of levosimendan or milrinone was administered followed by a 24-hour infusion of the chosen inotrope. Echocardiographic variables were measured postoperatively. Statistical analysis was done with SPSS-20 computer package. Association between the variables was found by independent t test. $P < 0.05$ was considered statistically significant.

Results: Mean age and weight of the patient in Group L was 8.55 ± 5.83 months and 6.05 ± 2.09 kgs, while that in group M was 6.85 ± 3.57 months and 5.26 ± 2.11 kgs. 4 patients (20%) treated with levosimendan had LCOS in comparison with 6 (30%) patients in those treated with milrinone. Echocardiographic parameters in both groups L and M were comparable (cardiac index 3.47 ± 0.76 vs 3.72 ± 1.05 L/min/m^2, EF 66.10 ± 7.82% vs 59.34 ± 10.74%, stroke volume index 25.4 ± 6.3 vs 27.74 ± 10.35 mL/m^2). The duration of ventilation, ICU stay and hospital stay were lesser in group L (12.75 ± 9.69, 35.95 ± 12.11, 119.10 ± 46.397 vs 23.60 ± 22.03, 51.20 ± 29.92, 140.20 ± 52.65 hours).

Conclusions: The incidence of LCOS was lesser in those patients treated with levosimendan, when compared with those treated with milrinone. Cardiac index and stroke volume index were comparable between the two groups. Thus, levosimendan provides a non-inferior alternative to milrinone when used as the primary inotrope following pediatric cardiac surgery.

Keywords: Echocardiography, levosimendan, pediatric cardiac surgery

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INTRODUCTION

Low cardiac output syndrome (LCOS) is defined as a reduction in cardiac output and is a common occurrence following cardiac surgery in the pediatric population. It was first defined in the pediatric population by Parr and colleagues in 1975 using the dye dilution technique.[1] Early recognition and treatment of LCOS is paramount due to its adverse impact on perioperative morbidity and mortality.

A myriad of strategies exist for managing low cardiac output syndrome such as optimization of preload, inotropes, afterload reducing agents, and positive pressure

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Table 1: Demographic Data

Parameters	Mean (SD)		*P*
	Simenda Group	Milrinone Group	
Age (Months)	8.55 (5.83)	6.85 (3.57)	0.273
Weight (kgs)	6.05 (2.09)	5.26 (2.11)	0.240
Height (cms)	66.07 (9.95)	65.00 (11.77)	0.757
Body surface area	0.32 (.08)	0.30 (.08)	0.478

Table 2: Risk stratification

RACHS	Simenda Group		Milrinone Group	
	Frequency	Percentage	Frequency	Percentage
2	17	85	18	90
3	2	10	1	5
4	1	5	1	5
Total	20	100	20	100

Table 3: Pump related variables

Variable	Simenda Group		Milrinone Group		*P*
	Mean	SD	Mean	SD	
Lowest temperature	30.40	3.14	29.83	3.99	0.62
Pump Time (min)	89.70	49.91	85.15	46.12	0.76
Clamp Time (min)	61.30	31.61	59.80	34.26	0.88
Urine Output (ml)	14.50	21.26	6.00	11.98	0.12
Ultrafiltration (ml)	1075.00	343.16	1072.50	327.86	0.98
Pump Balance	-17.50	70.315	-35.65	68.128	0.41
Intraoperative Fluid Balance (ml)	122.00	165.406	57.10	103.322	0.14

Table 4: Echocardiographic data

Parameters	Mean (SD)		*P*
	Simenda Group	Milrinone Group	
CO (ICU)	1.14±0.46	1.09±0.37	0.683
CO (24 h)	1.05±0.288	1.11±0.37	0.625
CI (ICU)	3.47±0.76	3.72±1.05	0.400
CI (24 h)	3.33±0.65	3.67±1.16	0.263
EF (ICU)	66.10±7.82	59.34±13.50	0.060
EF (24 h)	64.04±5.68	57.69±10.74	0.025
SV (ICU)	8.36±3.56	8.36±3.61	0.999
SV (24 h)	7.84±2.36	8.84±3.80	0.327
SVI (ICU)	25.40±6.30	27.74±10.35	0.392
SVI (24 h)	24.71±5.68	30.20±12.36	0.079

Table 5: Secondary outcomes

Parameters	Mean (SD)		*P*
	Simenda Group	Milrinone	
Ventilation	12.75±9.689	23.60±22.032	0.051
ICU stay	35.95±12.111	51.20±29.922	0.041
Hospital stay	119.10±46.397	140.20±52.652	0.187

ventilation. Multiple regimens combining an inotrope or inodilator along with vasopressor are utilized in various institutes to prevent LCOS. However, most inotropes currently available cause an undesirable increase in heart rate and therefore myocardial oxygen demand. An inotropic regimen which increases myocardial performance without substantially increasing oxygen consumption is therefore desirable. Prophylactic milrinone is most commonly used to prevent LCOS following pediatric cardiac surgery. Other alternatives available include dobutamine and levosimendan.

Levosimendan is an inodilator with calcium sensitizing properties, as a result of which it has a unique catecholamine sparing property. There is probably more controlled clinical data available on levosimendan than any other inotrope in adult cardiac surgery.[2] In the pediatric population, it has been used mainly as a rescue agent, when other inotropic agents are insufficient to maintain stable hemodynamics.[3] However, studies in the pediatric population are limited. In fact, a meta-analysis of studies using levosimendan in pediatric cardiac surgery, published in the Cochrane Database of Systematic Reviews in 2017, included 5 studies and had a total number of only 212 infants and children.[4]

Therefore, it was decided to conduct a randomized prospective study at our institute, in children under 1 year of age undergoing cardiac surgery, to compare the clinical efficacy of levosimendan with milrinone and to study the incidence of LCOS with both these drugs.

SUBJECTS AND METHODS

Study design

This was a single center, randomized study comparing the efficacy of milrinone and levosimendan in preventing LCOS after congenital heart surgery in infants. This study was conducted in the Department of Cardiothoracic and Vascular Surgery at our hospital.

Study population

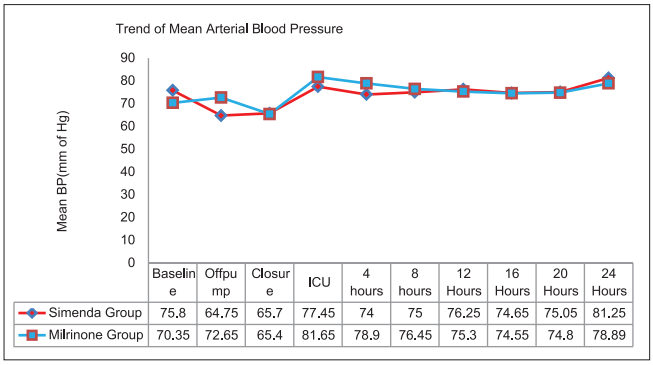
Approval from the institutional ethical committee was obtained following which written informed consent was obtained from the patients parents. 40 infants undergoing corrective open heart surgery were chosen. Patients were randomly assigned using computer- generated randomization into two groups, Group L (levosimendan) and Group M (milrinone). Some types of congenital heart diseases were not included during the study. The exclusion criteria were:

a. Single ventricle physiology
b. RACHS-I score 5, 6
c. Preoperative LCOS requiring inotropic support
d. Preoperative renal failure, cardiac arrest, thrombocytopenia.

Study method

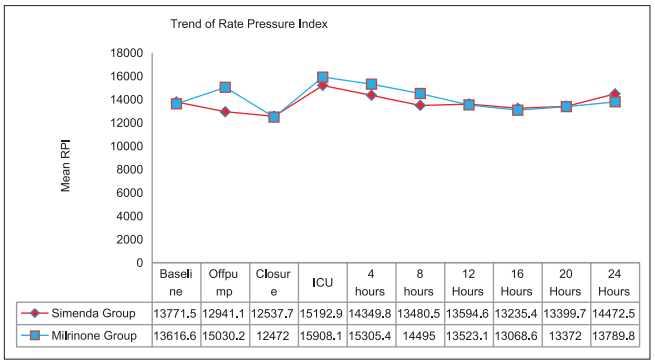
After standard induction and surgical exposure, CPB was initiated. Del Nido cardioplegia was used in all patients to achieve cardiac standstill. Continuous ultrafiltration was used in all patients to ensure zero-fluid balance at the end of CPB. The initial therapeutic intervention comprised of administration of

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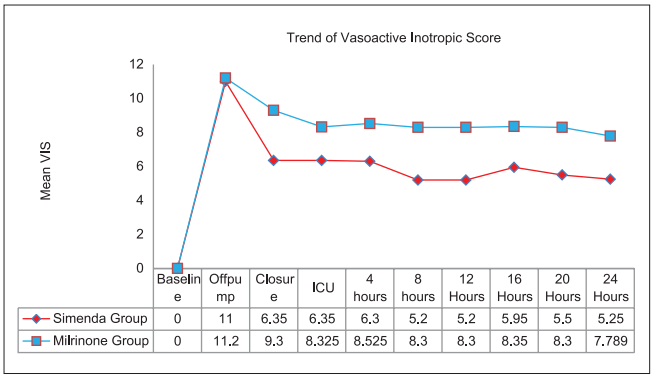
	Baseline	Offpump	Closure	ICU	4 hours	8 hours	12 Hours	16 Hours	20 Hours	24 Hours
Simenda Group	75.8	64.75	65.7	77.45	74	75	76.25	74.65	75.05	81.25
Milrinone Group	70.35	72.65	65.4	81.65	78.9	76.45	75.3	74.55	74.8	78.89

Figure 1: Trend of mean arterial pressure



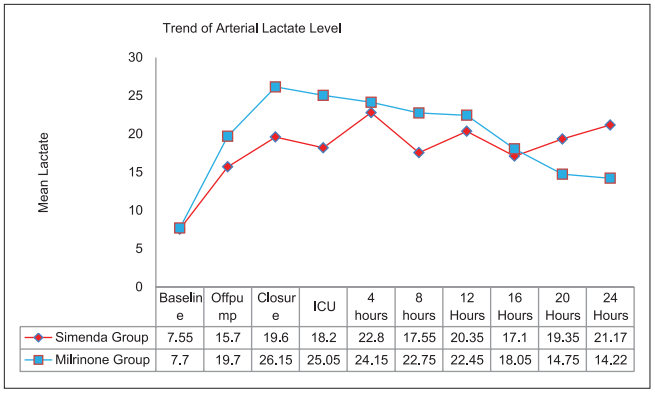
	Baseline	Offpump	Closure	ICU	4 hours	8 hours	12 Hours	16 Hours	20 Hours	24 Hours
Simenda Group	13771.5	12941.1	12537.7	15192.9	14349.8	13480.5	13594.6	13235.4	13399.7	14472.5
Milrinone Group	13616.6	15030.2	12472	15908.1	15305.4	14495	13523.1	13068.6	13372	13789.8

Figure 2: Trend of Rate Pressure Index



	Baseline	Offpump	Closure	ICU	4 hours	8 hours	12 Hours	16 Hours	20 Hours	24 Hours
Simenda Group	0	11	6.35	6.35	6.3	5.2	5.2	5.95	5.5	5.25
Milrinone Group	0	11.2	9.3	8.325	8.525	8.3	8.3	8.35	8.3	7.789

Figure 3: Trend of Vasoactive Inotropic Score



	Baseline	Offpump	Closure	ICU	4 hours	8 hours	12 Hours	16 Hours	20 Hours	24 Hours
Simenda Group	7.55	15.7	19.6	18.2	22.8	17.55	20.35	17.1	19.35	21.17
Milrinone Group	7.7	19.7	26.15	25.05	24.15	22.75	22.45	18.05	14.75	14.22

Figure 4: Trend of arterial lactate level

loading dose of either inotrope over 10 minutes at the time of rewarming during cardiopulmonary bypass. This was followed by a 24-hour infusion of the chosen inotrope. Children in group M, received milrinone, prepared and marketed as MILRON by United Biotech (P) limited. Children in group L received levosimendan, prepared by Gufic Biosciences Limited and marketed as SIMENDA by Lupin Ltd.

Drug dosages

Patients in group L received a loading dose of 10 μg/kg levosimendan followed by a 24-hour infusion at 0.1 μg/kg/min. On the other hand, patients in group M received a loading dose of 50 μg/kg milrinone followed by a 24-hour infusion at 0.5 μg/kg/min. The dose has been extrapolated from adult literature as standard pediatric dosage has not been described. Adrenaline was used to aid separation from cardio-pulmonary bypass in both the groups and was continued in the ICU, if required.

Parameters measured

Hemodynamic parameters like heart rate, blood pressure, saturation, central venous pressure, and urine output were measured at the time of induction, coming off CPB, chest closure, transfer to the ICU and subsequently every 4 hours through 24 hours. Clinical evidence of LCOS and inotropic requirements were recorded during this period.

LCOS was defined as two or more of the following:[4]

a. Arterial lactates >27 mg/dL (or 3 mmoL/L) on two consecutive readings
b. Increase in blood lactate of at least 18 mg/dL (or 2 mmoL/L) from baseline
c. Central venous oxygen saturation <50% in the absence of intracardiac shunts
d. Decrease in central venous oxygen saturation by at least 20% from baseline
e. Urine output <1 mL/kg/hour for two consecutive hours
f. Cardiac index less than 2.2 L/min/m^2.

Inotropic requirements were measured with the Vasoactive Inotropic Score (VIS) for group M and Levosimendan- Vasoactive Inotropic Score (LVIS)[5] for group L. Arterial blood gases along with central venous oxygen saturation were serially monitored. Echocardiographic measurement of cardiac output, cardiac index, ejection fraction, stroke volume, and stroke volume index was done on arrival in the ICU and 24 hours later. Ejection fraction was measured using Simpsons monoplane method, while the Doppler- VTI method was used to derive the cardiac output. Morbidity, duration of ventilation, ICU stay and duration of hospital stay were also recorded.

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Primary outcomes measured were the incidence of LCOS, while the secondary outcomes were hemodynamic parameters, inotropic requirements, duration of mechanical ventilation, ICU stay, and hospital stay.

Statistical analysis

Data entry was made in the Microsoft Excel software in codes and analysis was done with SPSS-20 computer package. Categorical variables are expressed as percentages whereas continuous variables are expressed as mean ± standard deviation. The trend of vitals and biochemical parameters of the study participants were followed and their mean ± standard deviation assessed.

RESULTS

Demographic data

Forty patients received one of the two inotropes during the study and were randomized into two groups. There was no statistically significant difference between the two groups as regards the demographic data. The demographic data in both the groups has been represented in the Table 1.

The RACHS-I scoring in both the groups were comparable. Most of the patients (85% in group L, 90% in group M) enrolled were in the RACHS 2 category of patients [Table 2].

Pump-related variables

Pump-related variables in both the groups were also identical [Table 3]. Patients in both the groups had similar pump times and clamp times. None of the patients required low flows during CPB. We followed a zero-balance continuous ultrafiltration technique to ensure nil balance at the end of cardiopulmonary bypass. However, post- CPB, patients treated with levosimendan had a slightly higher fluid requirement compared with those treated with milrinone. This was indicated by the difference in intraoperative fluid balance between both the groups.

(122 + 165.406 mL in group L vs 57.10 + 103.322 mL in group M).

Primary outcomes

We found that 4 patients (20%) had LCOS in the levosimendan group, in comparison to 6 patients (30%) in those treated with milrinone during the first 24 hours post surgery. However, none of the 40 patients had LCOS by echocardiographic criteria on arrival in the ICU and 24 hours thereafter.

Secondary outcomes

Hemodynamic parameters

The mean arterial pressure in both the groups was comparable at all points of time after arrival into the ICU for the first 24 hours [Figure 1]. None of the patients in the two groups had severe hypotension requiring vasopressor therapy during the study. The heart rate of patients in both the groups was comparable during this time frame. None of the patients in either group had any tachyarrhythmia during the first 24 hours post-surgery. The rate-pressure index (RPI) was used to assess the myocardial oxygen demand. It was found that the RPI was comparable between both groups and was lower during the first 24 hours in those patients treated with levosimendan [Figure 2].

Echocardiographic data

Echocardiography demonstrated a consistently good cardiac function in both the groups [Table 4]. The cardiac index in both the groups were similar at arrival in ICU (3.47 ± 0.765 in group L vs 3.72 ± 1.05 in group M) and 24 hours after surgery (3.33 ± 0.65 in group L vs 3.67 ± 1.16). None of the patients in both the groups had LCOS by echocardiographic criteria at the time of measurement. The ejection fraction in patients treated with levosimendan was similar to those treated with milrinone at the time of arrival into the ICU (66.10 ± 7.82% vs 59.34 ± 13.50) and 24 hours thereafter (64.05 ± 5.68 vs 57.69 ± 10.74). The stroke volume index in both the groups was comparable as well (25.40 + 6.30 vs 27.7 + 10.35).

Inotropic requirements

Only 3 patients in group L required adrenaline to maintain adequate cardiac output on arrival into the ICU. This was in contrast to 11 patients requiring adrenaline in group M. Thus, almost 85% of the patients in group L were discharged from the operation theatre on a single inotrope. One patient in both the groups required high inotropic support (more than 0.1 μg/kg/min adrenaline) at the time of arrival into the ICU. Overall inotropic requirements were much lesser in those patients treated with levosimendan compared to milrinone following surgery [Figure 3].

Lactates and mixed venous oxygen saturation

Lactate levels on arterial blood gases were consistently lower in those patients treated with levosimendan in the immediate post surgical period, until 16 hours. Thereafter, there was a slight increase in lactate levels in group L, which coincided with the timing of diuretic administration [Figure 4]. The mixed venous oxygen saturation was comparable in both groups.

Fluid requirements

The intraoperative fluid requirements was higher in group L, compared with group M. Only 2 patients (10%) in group L had a negative fluid balance at the end of surgery, in

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comparison to 5 patients (25%) in group M. However, at the end of 24 hours, most patients in both the groups (13 in group L vs 11 in group M) had a negative fluid balance. The fluid overload index at 24 hours was comparable in both groups (-0.66 ± 3.53% in group L vs -0.99 ± 2.85% in group M).

Other secondary outcomes

Patients in group L had a shorter (statistically insignificant) duration of ventilation (12.75 ± 9.68 hours in group L vs 23.60 ± 22.03 hours in group M), as well as hospital stay (119.10 ± 46.397 hours in group L vs 140.20 ± 52.65 hours in group M). The duration of ICU stay was also lesser in those patients treated with levosimendan (35.95 ± 12.11 hours) as compared to those treated with milrinone (51.20 ± 29.92 hours). This difference in ICU stay was statistically significant [Table 5].

DISCUSSION

Levosimendan is an exciting inodilator with a unique non-catecholamine-based mechanism of action. It improves cardiac function, hemodynamic performance and survival in critically ill adult patients, but limited data exists on levosimendan use in the pediatric population.[6] In these patients therefore, its use is off-label, where it is mainly used as a rescue agent.[7,8]

Several smaller trials in the past have suggested a beneficial effect with levosimendan in the perioperative scenario. A meta-analysis published in the Cochrane Database of Systematic Reviews in 2017 evaluated studies in the pediatric population and concluded that the current level of evidence is insufficient to judge whether levosimendan prevents LCOS in pediatric patients.[4] In the wake of several neutral or inconclusive trials of late in the adult population, (CHEETAH, LEVO-CTS, LICORN) this study was designed with the purpose of addressing one quintessential question: How effective is levosimendan, when compared with standard inotropic regimens in preventing LCOS in children? We also aimed to study its hemodynamic repercussions and whether it had any outcome on the duration of ICU and hospital stay.

Unlike most other inotropes available today, levosimendan acts independently of the β-adrenergic receptors and cyclic adenosine monophosphate mechanism. It produces its effects by two mechanisms. First, being a calcium sensitizer, it stabilizes the interaction between calcium and troponin C, thereby improving inotropy without adversely affecting lusitropy.[9] Diastolic relaxation remains unhampered as there is no intracellular accumulation of calcium.[10] Second, it is a potassium-channel opener on vascular smooth muscle, which causes hyperpolarization leading to coronary and peripheral vasodilatation.[11] This renders it with several hemodynamic advantages over other conventional inotropes available today. Thus, amongst the wide spectrum of inotropic agents available today, levosimendan offers a unique alternative to prevent LCOS following cardiac surgery in the pediatric population.

In our study, we found that the incidence of low cardiac output syndrome was lower in patients treated with levosimendan compared with those treated with milrinone. This was in conjunction with multiple studies conducted in the pediatric population in the past.[12,13]

LCOS is a common occurrence following pediatric cardiac surgery with an incidence as high as 25%.[14] No stringent diagnostic criteria exist at present for the diagnosis of LCOS in the pediatric population. The existing criteria rely on hemodynamic measurements, rather than an objective measurement of cardiac output. This is in contrast to the adult population, where a definition based on objective measurement of cardiac index exists.[15] Levosimendan offers a better hemodynamic profile compared to standard inotropic therapy owing to its catecholamine independent mechanism of action.[12] This reliance on hemodynamic parameters for diagnosis of LCOS in children, in conjunction with the better hemodynamic profile offered by levosimendan may account for the low incidence of LCOS in these patients.

The mean arterial pressures in both the groups were comparable, as was the rate pressure index. The rate pressure index can be used as a surrogate marker of myocardial oxygen demand and was found to be lower in those patients treated with levosimendan. Our findings were consistent with those of Momeni *et al*.[12]

Fluid requirement in patients treated with levosimendan was higher in the immediate postoperative period. This maybe explained in part due to the fact that we used a loading dose of levosimendan given over 10 minutes at the time of rewarming during cardiopulmonary bypass. Most studies in the past have avoided using the loading dose of levosimendan or milrinone due to fear of hypotension. Therefore, although almost all patients had a zero fluid balance at coming off CPB, those treated with levosimendan required additional fluids to maintain optimal hemodynamics. This difference was in stark contrast to the patients treated with milrinone, whose reliance on fluid therapy was minimal.

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Serum lactate level is considered to be one of the most important biochemical markers of early adverse outcome after congenital cardiac surgery. It is one of the biochemical markers used to support the diagnosis of LCOS. In our study, levosimendan was found to cause a greater reduction in lactate levels between 4-12 hours post surgery, compared with milrinone. Thus, the beneficial effects of levosimendan administration were more pronounced in the typical time frame when LCOS is known to occur. A parallel conclusion was drawn by Ricci *et al*., in his study published in 2012.[16] In our study, the lactate levels in patients treated with levosimendan were consistently lower during the first 16 hours of stay in the ICU. Thereafter, there was a slight increase in lactate levels in these patients, which coincided with the time of diuretic administration (1 mg/kg of furosemide). This may suggest a reliance on optimal preload for levosimendan to maintain adequate cardiac output.[17]

The ejection fraction of patients treated with levosimendan was slightly higher than those treated with milrinone at the time of arrival in ICU. A study published in 2018, found that the inotropic and lusitropic properties of levosimendan and milrinone were comparable at clinically relevant and equipotent infusion rates.[18] This difference in ejection fraction was despite the fact that most of these patients (almost 85%) were on a single inotrope on arrival in the ICU. Thus, levosimendan clearly allowed an early weaning of catecholamines in our patients, ensuring that these patients were on minimal inotropes on arrival in the ICU. This benefit was sustained during the entire length of stay in the ICU. Similar conclusions were drawn in multiple studies published earlier.[8,19] Levosimendan, therefore, reduced the reliance on catecholamine pathways to maintain an optimal cardiac output.[20]

In our study, patients who were supported post cardiac surgery with levosimendan had a shorter duration of ventilation and stay in the ICU. This led to an earlier discharge of these patients from the hospital. None of the patients in both the groups had any sustained arrhythmias or required mechanical circulatory support. There were no in-hospital mortalities in both the groups.

Limitations

Our study had several limitations. Most glaring was the lack of a definitive definition of LCOS and the dependence on clinical parameters for the diagnosis of the same. Also, we excluded patients in the RACHS 5 and 6 category, along with single ventricle physiology. Thus, sicker patients were excluded. The study also does not delve into the different dose ranges of levosimendan to study its effect. The study population, though more or less homogenous, is restricted. Thus, larger, multicenter studies may be warranted.

CONCLUSIONS

We found that the incidence of LCOS was lesser in patients treated with levosimendan, when compared with those treated with milrinone. Also, the hemodynamic profile offered by levosimendan was better, as suggested by the rate-pressure index (RPI). These effects were exerted at a time when the heart most required it and when the incidence of LCOS was highest. However, the cardiac output enhancement by levosimendan was more sensitive to changes in the fluid status of the patient. Also, children treated with levosimendan had a shorter duration of ICU stay and hospital stay. Thus, levosimendan offers a unique, timely, attractive, and equipotent alternative to standard inotropes in use today to prevent LCOS following cardiac surgery in the pediatric population.

Abbreviations used in text

LCOS: Low Cardiac Output Syndrome; RACHS: Risk Adjustment for Congenital Heart Surgery; VIS: Vasoactive Inotropic Score; RPI: Rate Pressure Index.

Abbreviations used in tables and figures

BSA: Body Surface Area; CO: Cardiac Output; CI: Cardiac Index; EF: Ejection Fraction; SV: Stroke Volume; SVI: Stroke Volume Index.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Parr GV, Blackstone EH, Kirklin JW. Cardiac performance and mortality early after intracardiac surgery in infants and young children. Circulation 1975;51:867-74.
2. Rognoni A, Lupi A, Lazzero M, Bongo AS, Rognoni G. Levosimendan: From basic science to clinical trials. Recent Pat Cardiovasc Drug Discov 2011;6:9-15.
3. Suominen PK. Single-center experience with levosimendan in children undergoing cardiac surgery and in children with decompensated heart failure. BMC Anesthesiol 2011;11:18.
4. Hummel J, Rücker G, Stiller B. Prophylactic levosimendan for the

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prevention of low cardiac output syndrome and mortality in paediatric patients undergoing surgery for congenital heart disease. Cochrane Database Syst Rev 2017;3:CD011312.
5. Favia I, Vitale V, Ricci Z. The vasoactive-inotropic score and levosimendan: Time for LVIS? J Cardiothorac Vasc Anesth 2013;27:e15-6.
6. Landoni G, Biondi-Zoccai G, Greco M, Greco T, Bignami E, Morelli A, *et al*. Effects of levosimendan on mortality and hospitalization. A meta-analysis of randomized controlled studies. Crit Care Med 2012;40:634-46.
7. Silvetti S, Silvani P, Azzolini ML, Dossi R, Landoni G, Zangrillo A. A systematic review on levosimendan in paediatric patients. Curr Vasc Pharmacol 2015;13:128-33.
8. Namachivayam P, Crossland DS, Butt WW, Shekerdemian LS. Early experience with Levosimendan in children with ventricular dysfunction. Pediatr Crit Care Med 2006;7:445-8.
9. Ng TM. Levosimendan, a new calcium-sensitizing inotrope for heart failure. Pharmacotherapy 2004;24:1366-84.
10. Haikala H, Nissinen E, Etemadzadeh E, Levijoki J, Lindén IB. Troponin C-mediated calcium sensitization induced by levosimendan does not impair relaxation. J Cardiovasc Pharmacol 1995;25:794-801.
11. Milligan DJ, Fields AM. Levosimendan: Calcium sensitizer and inodilator. Anesthesiol Clin 2010;28:753-60.
12. Momeni M, Rubay J, Matta A, Rennotte MT, Veyckemans F, Poncelet AJ, *et al*. Levosimendan in congenital cardiac surgery: A randomized, double-blind clinical trial. J Cardiothorac Vasc Anesth 2011;25:419-24.
13. Namachivayam P, Crossland DS, Butt WW, Shekerdemian LS. Early experience with Levosimendan in children with ventricular dysfunction. Pediatr Crit Care Med 2006;7:445-8.
14. Wernovsky G, Wypij D, Jonas RA, Mayer JE Jr, Hanley FL, Hickey PR, *et al*. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants. A comparison of low-flow cardiopulmonary bypass and circulatory arrest. Circulation 1995;92:2226-35.
15. Pérez Vela JL, Martín Benitez JC, Carrasco Gonzalez M, de la Cal López MA, Hinojosa Pérez R, Sagredo Meneses V, *et al*. [Summary of the consensus document: "Clinical practice guide for the management of low cardiac output syndrome in the postoperative period of heart surgery"]. Med Intensiva 2012;36:277-87.
16. Ricci Z, Garisto C, Favia I, Vitale V, Di Chiara L, Cogo PE. Levosimendan infusion in newborns after corrective surgery for congenital heart disease: Randomized controlled trial. Intensive Care Med 2012;38:1198-204.
17. Charpie JR, Dekeon MK, Goldberg CS, Mosca RS, Bove EL, Kulik TJ. Serial blood lactate measurements predict early outcome after neonatal repair or palliation for complex congenital heart disease. J Thorac Cardiovasc Surg 2000;120:73-80.
18. Fredholm M, Jörgensen K, Houltz E, Ricksten SE. Inotropic and lusitropic effects of levosimendan and milrinone assessed by strain echocardiography-A randomised trial. Acta Anaesthesiol Scand 2018;62:1246-54.
19. Egan JR, Clarke AJ, Williams S, Cole AD, Ayer J, Jacobe S, *et al*. Levosimendan for low cardiac output: A pediatric experience. J Intensive Care Med 2006;21:183-7.
20. Milligan DJ, Fields AM. Levosimendan: Calcium sensitizer and inodilator. Anesthesiol Clin 2010;28:753-60.

Original Article

Are Body Image Issues Affecting Our Adolescents? A Cross-sectional Study among College Going Adolescent Girls

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Abstract

Background: Body image relates to how people think and feel about their own body. In today's society, with the growing sense of ideal body image, adolescents try to lose or gain body weight to attain that perfect body. Body image perception is still naive, and this research will try to understand these unexplored areas, where there is paucity of body image-related studies. **Objectives:** The objective of the study is to find out the proportion of girls dissatisfied about body image, and the association of various factors with body image dissatisfaction and to ascertain the weight control behaviors adopted by adolescent college girls. **Methodology:** A cross-sectional study was done among 1200 college girls in Coimbatore. A semi-structured questionnaire was used to collect data on various factors associated with body image dissatisfaction. Body mass index (BMI) of the participants was calculated. **Results:** Body image dissatisfaction was there among 77.6% of the girls. It was found that factors such as higher BMI, sociocultural pressure to be thin and depression were all significantly associated with body image dissatisfaction. The most commonly followed weight control behaviors were eating small meals and skipping meals. Improving the appearance and body shape were the main reasons for weight control behaviors. **Conclusion:** This study establishes the fact that body image dissatisfaction is no longer a western concept and affects Indian adolescent girls to a great extent. Hence, effective interventions have to be planned to increase awareness on ideal body weight and protect our young generation from pressures of negative body image.

Keywords: Adolescent, body image dissatisfaction, body weight, college girls, weight control

Introduction

Body image relates to how people think and feel about their own body. It relates to a person's perceptions, feelings, and thoughts about his or her body and is usually conceptualized as incorporating body size estimation, evaluation of body attractiveness, and emotions associated with body shape and size.[1]

Adolescents are particularly vulnerable to body dissatisfaction due to the physiological, social, and psychological changes they are going through.[2] For adolescent girls, a range of body and social changes take place during and following puberty that can strongly influence their body image and become more pronounced by late adolescence.[3]

Perception of overweight and dissatisfaction with body size, rather than actual weight appears to be a potent force behind girls' dieting and weight control behaviors. Such behavior is related to negative physical and psychological changes and can lead to life-threatening conditions including eating disorders.[4]

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With rapid economic development and affluence, there is a progressive shift toward thinness as the ideal body shape and increasing concerns about body weight leading to dissatisfaction.[5]

Factors contributing to body image problems have been studied, and biopsychosocial model of risk factors was developed by Paxton.[6] This model [Figure 1] suggests that biological factors, such as body size, together with individual psychological characteristics such as low self-esteem, depression, and perfectionism, which refers to individuals desire to attain idealistic goals without failing, social influences, and interpersonal interactions, all contributes to the development of body image.

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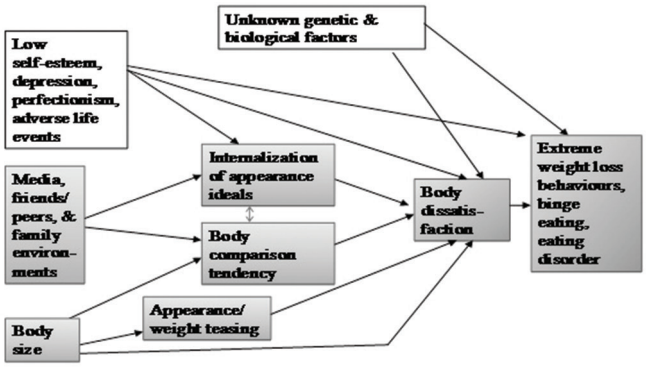


Figure 1: Biopsychosocial model of risk factors for the development of body dissatisfaction by Susan J Paxton

There is a paucity of studies in India and gaps remain in our understanding of the factors responsible for the development of attitudes and behavior related to body weight and appearance among adolescent girls.[7]

Hence, this study aimed to find out the proportion of girls dissatisfied about body image, and the association of various factors with body image dissatisfaction and to ascertain the various weight control behaviors adopted by adolescent college girls.

Methodology

This study was conducted in a college located in an urban area of Coimbatore. With an estimated prevalence of body image dissatisfaction of 33.3% from a study in Mangalore,[8] sample size was calculated to be 978, with the precision of 3.3%, and nonresponse 20%. After approval by the Institutional Human Ethics Committee, 1220 college going adolescent girls in the age group of 18–19 years, were enrolled in the study by convenience sampling. After obtaining informed consent, a semi-structured questionnaire was used to collect the data on body image dissatisfaction and the associated factors namely demographic factors, socioeconomic and media influence, sociocultural pressure, self-esteem, perfectionism, and depression. Furthermore, data regarding weight control measures adopted were collected.

Measuring body image dissatisfaction

Body image dissatisfaction of the adolescents was measured using the Stunkard silhouettes Figure Rating Scale (FRS). It includes drawing of nine silhouettes ranging from very thin (1) to very heavy (9). Students were presented with female versions of the FRS and were asked to identify the figure they currently perceived themselves with, and the figure they would wish to have. Discrepancy scores were calculated based on the difference between the actual and ideal figures chosen. Positive scores indicate a desire to be smaller, while negative scores suggest a desire to be larger. The discrepancies between the current and desired figures were interpreted as an indication of body image dissatisfaction.[9,10] Hence, participants who had discrepancies in the figures chosen were identified as having body image dissatisfaction.

Measuring factors associated with body image dissatisfaction

The factors associated with body image dissatisfaction such as self-esteem, depression, sociocultural pressure, media influence, and perfectionism were measured using previously validated questionnaires and using Likert scale ratings, and score was given.[11-15] Rosenberg Self-Esteem Scale was used to measure Self-esteem with the Likert scales ranging from strongly agree (score 1) to strongly disagree (score 4). Higher scores indicate higher self-esteem.[11] Depression was measured using Center of Epidemiological studies (CES) Depression questionnaire with Likert scale which graded from rarely to mostly with the scoring of 0–3, respectively. Score on the CES-Depression questionnaire range from 0 to 60, with higher scores indicating more severe levels of depressive symptoms.[12]

Socio-cultural pressure was measured with the pressure grading from none (score 1) to a lot of pressure (score 5). The scoring ranges from 10 to 50.[13] Media influence was measured using SATAQ 3 questionnaire with Likert scale grading of completely disagree to completely agree with a scoring of 1–5. The scoring ranges from 14 to 70.[14] Perfectionism was measured using the EDI questionnaire with Likert scale grading from always to never with score from 1 to 6, respectively. The scoring ranges from 6 to 36.[15]

The height and weight of the participants were measured and body mass index (BMI) was calculated. According to the WHO standards in our study, BMI <18.5 kg/m^2 was classified as underweight, a BMI of 18.5–24.9 kg/m^2 was considered normal, overweight is 25–29.9 kg/m^2, and obesity is ≥30 kg/m^2.[16]

The association between the possible risk factors and body image dissatisfaction was assessed by univariate analysis using Chi-square test, *t*-test, and odds ratio was estimated. Those factors found significant by univariate analysis were further subjected to multivariate logistic regression analysis to identify factors that were significantly associated with body image dissatisfaction.

Results

Majority of the study participants (60.6%) were from an urban background. About 67.5% of them belonged to Class I and Class II and 32.5% of them belong to Class III and Class IV socioeconomic status according to modified Prasad classification.[17]

Based on the BMI, about half (53.6%) of them were having normal BMI while 26.7% of them were undernourished, 15.7% were overweight, and 3.9% were obese.

The study showed that 947 (77.6%) girls were dissatisfied with their body image (95% CI: 75.2–79.9). Among the 947 participants who were dissatisfied the mean score of the perceived silhouette figure was 4.0 ± 1.5 (four corresponds to the normal range of BMI), and the mean of the chosen ideal

silhouette was 3.3 ± 0.8 (three corresponds to the lower end of the normal BMI toward the underweight range).

Table 1 shows the distribution of body image dissatisfaction based on the calculated BMI of the participants. It shows that 58.3% of the participants of normal BMI also were dissatisfied with their appearance and wanted to reduce weight. In the underweight category, 23.3% were satisfied, and 7.4% wanted to reduce their weight further showing the tendency of the college students to have a thin appearance. Tables 2 and 3 show the association of body image dissatisfaction with its risk factors by univariate analysis. Higher BMI, higher socioeconomic status, depression, increased sociocultural pressure to be thin, media influence and low self-esteem were found to be significantly associated with body image dissatisfaction.

There was no significant association with the place of residence and perfectionism.

Table 4 shows multivariate logistic regression exploring the association of body image dissatisfaction with those risk factors having statistically significant association with body image dissatisfaction by univariate analysis.

Factors such as higher BMI, higher socioeconomic status, depression, and increased sociocultural pressure to be thin were found to be significantly associated with body image dissatisfaction by multivariate logistic regression analysis.

Among the 1220 students who participated in the current study, 791 (64.8%) of them had undertaken at least one weight control measure in the past 1 year. Of which 157 (19.8%) were satisfied and 634 (80.2%) were dissatisfied with their body image.

Among the multiple options of weight control measures adopted, the most common method was taking small meals (53.2%) followed by skipping meals (42.7%). Extreme measures such as taking laxatives (5.3%) and vomiting after meals (9.7%) were also seen.

Among participants who followed weight control measures, multiple reasons were given by them, of which 61.7% gave improving the appearance and body shape as the main reason for going in for weight control measures and 33.5% said to look better in clothes.

Our study also showed that 67% of participants who were dissatisfied with their body image were adopting one or more weight control measures while in the satisfied group 57.5% have adopted weight control measures. The difference was found to be statistically significant ($P < 0.01$) with an odds ratio of 1.49 (1.13–1.97).

Table 1: Distribution of body image dissatisfaction of study participants based on calculated body mass index (*n*=1220)

BMI status (BMI in kg/m²)	Total number	Frequency of dissatisfaction	
		Increase weight	Reduce weight
Underweight (<18.50)	326 (26.7)	226 (69.3)	24 (7.4)
Normal (18.50-24.99)	654 (53.6)	84 (12.8)	381 (58.3)
Overweight (25.00-29.99)	192 (15.7)	0	185 (96.4)
Obese (≥30.00)	48 (3.9)	0	47 (97.9)
Total	1220 (100)	310 (25.4)	637 (52.2)

BMI: Body mass index

Table 2: Association of body image dissatisfaction with the risk factors (continuous variables) by univariate analysis

Factor	Mean±SD		OR (95% CI)	*P*
	Satisfied	Dissatisfied		
Media influence	37.60±8.96	40.35±8.19	1.04 (1.02-1.05)	<0.001
Depression	15.7±7.38	19.2±9.19	1.05 (1.03-1.06)	<0.001
Sociocultural pressure	15.49±5.57	19.08±7.70	1.08 (1.05-1.10)	<0.001
Perfectionism	19.09±5.53	19.21±6.09	1.00 (0.98-1.02)	0.77
Self esteem	18.65±3.20	17.92±3.30	0.937 (0.90-0.97)	<0.01
BMI	19.92±2.27	21.99±4.40	1.15 (1.111.20)	<0.001

SD: Standard deviation, BMI: Body mass index, CI: Confidence interval, OR: Odds ratio

Table 3: Association of body image dissatisfaction with the risk factors (categorical variables) by univariate analysis

Factors	Category	Total	Body image dissatisfaction (%)	*P*
Socioeconomic class	Class I	53	43 (81.1)	<0.001
	Class II	643	510 (79.3)	
	Class III	380	302 (79.5)	
	Class IV	144	92 (63.9)	
Place of residence	Urban	739	569 (77)	0.52
	Rural	481	378 (78.6)	

Table 4: Association of body image dissatisfaction with the risk factors by multivariate logistic regression

Factor	AOR	95% CI	*P*
Media influence	1.02	1.00-1.03	<0.05
Depression	1.04	1.01-1.05	<0.001
High self esteem	0.98	0.94-1.03	0.411
Increased sociocultural pressure	1.05	1.02-1.07	<0.001
Higher BMI	1.13	1.08-1.18	<0.001
Higher socioeconomic status	1.28	1.06-1.54	<0.001

BMI: Body mass index, AOR: Adjusted odds ratio, CI: Confidence interval

Discussion

Our study showed that body image dissatisfaction was there in 77.6% of the adolescents. This was similar to a study done by Sasi and Maran in Chennai among adolescents above 12 years of age which showed a prevalence of 81%.[18]

Our study also showed that 23% of the underweight people were satisfied with their BMI, and among those who were dissatisfied 7.4% wanted to reduce their weight further. Similarly, 71% of the normal BMI category was dissatisfied

with their appearance of which 58.3% wanted to reduce their weight. This clearly shows the tendency for liking toward thin body shapes.

Socioeconomic status of an individual impacts their body image perceptions and satisfaction/dissatisfaction with the body. Studies have shown that body image dissatisfaction is seen more among higher socioeconomic status.[19] Our study also showed that the proportion of girls having body image dissatisfaction, who belonged to class I socio economic status are slightly higher than other classes and it was statistically significant.

Studies have also shown that urban adolescents have more body image dissatisfaction compared to rural,[20] but our study did not show any significant association.

Our study also showed that increased socio-cultural pressure, depression and higher BMI had significant association with body image dissatisfaction. Literature also shows that these factors are associated with body image dissatisfaction.[21-23]

Our study failed to show any significant association between body image dissatisfaction and media influence, self-esteem, and perfectionism which were also established in other studies.[24-26]

This study also showed that 64.8% of the college students had undertaken at least one weight control measure in the past 1 year. It also showed that girls who were dissatisfied with their appearance tend to do more weight control measures and the most common weight control measures were eating small meals, skipping meals, and avoiding certain foods. It also showed that the majority of students have cited improving the appearance and to look better in clothes as main reasons for going in for weight control measures. This was also found similar to other studies which showed that appearance was a major motivation for dieting practices.[27,28]

Our study was done in a large sample and measured the different factors which were associated with body image dissatisfaction. The study of these factors is crucial for improving our understanding of body image dissatisfaction in adolescent girls which is still naive in our cultural setup.

Limitations

Although the study area was in a college which had both self-financing and government aided divisions which brought together adolescents from different socioeconomic and cultural backgrounds, it was done in only one college - this restriction may limit the generalizability of the findings.

Conclusion

This study establishes the harsh fact that body image perceptions and dissatisfaction is no longer a western concept and affects our adolescent girls to a great extent. There is also a tendency favoring toward thin body figures which is evident in the study.

Recommendations

Effective interventions to improve awareness on ideal body weight, media literacy, and harmful effects of extreme weight control measures have to be planned, and young people both maintain a healthy body weight and are protected from the pressure of negative body image and weight control practices.

Further studies are recommended to establish the cause-effect relationship of the identified factors.

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Conflicts of interest

There are no conflicts of interest.

References

1. Grogan S. Body Image: Understanding Body Dissatisfaction in Men, Women and Children. London: Routledge; 1999. Available from: http://www.art-therapy.gr/images/stories/book_library/new/%CE%92%CE%99%CE%92%CE%9B%CE%99%CE%91/art%20therapy/Body-Image-Understanding-Body-Dissatisfaction-in-Men-Women-a.pdf. [Last accessed on 2016 Oct 15].
2. Clay D, Vignoles VL, Dittmar H. Body image and self-esteem among adolescent girls: Testing the influence of sociocultural factors. J Res Adolesc 2005;15:451-77.
3. Littleton HL, Ollendick T. Negative body image and disordered eating behavior in children and adolescents: What places youth at risk and how can these problems be prevented? Clin Child Fam Psychol Rev 2003;6:51-66.
4. Mann AH, Wakeling A, Wood K, Monck E, Dobbs R, Szmukler G, *et al.* Screening for abnormal eating attitudes and psychiatric morbidity in an unselected population of 15-year-old schoolgirls. Psychol Med 1983;13:573-80.
5. Cash TF, Morrow JA, Hrabosky JI, Perry AA. How has body image changed? A cross-sectional investigation of college women and men from 1983 to 2001. J Consult Clin Psychol 2004;72:1081-9.
6. Paxton SJ. Evidence, Understanding and Policy: A Perspective from Psychology – Prevention, Early Intervention and Treatment of Body Image Problems. Body Image: Evidence, Policy, Action. Report of a Multidisciplinary Academic Seminar on Behalf of the Government Equalities Offi ce. United Kingdom; 2003. Available from: https://www.gov.uk/.../uploads/.../Susan_Paxton_-_Body_Image_Seminar__3_.docx. [Last accessed on 2017 Dec 16].
7. Chugh R, Puri S. Affluent adolescent girls of Delhi: Eating and weight concerns. Br J Nutr 2001;86:535-42.
8. Priya D, Prasanna KS, Sucharitha S, Vaz NC. Body image perception and attempts to change weight among female medical students at Mangalore. Indian J Community Med 2010;35:316-20.
9. Lo WS, Ho SY, Mak KK, Lam TH. The use of Stunkard's figure rating scale to identify underweight and overweight in Chinese adolescents. PLoS One 2012;7:e50017.
10. Bhuiyan AR, Gustat J, Srinivasan SR, Berenson GS. Differences in body shape representations among young adults from a biracial (Black-white), semirural community: The Bogalusa heart study. Am J Epidemiol 2003;158:792-7.
11. Gray-Little B, Williams VS, Hancock TD. An item response theory analysis of the rosenberg Self-esteem Scale. Pers Soc Psychol Bull 1997;23:443-51.
12. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Appl Psychol Meas 1977;1:385-401.

13. Reina SA, Shomaker LB, Mooreville M, Courville AB, Brady SM, Olsen C, *et al.* Sociocultural pressures and adolescent eating in the absence of hunger. Body Image 2013;10:182-90.
14. Thompson JK, van den Berg P, Roehrig M, Guarda AS, Heinberg LJ. The sociocultural attitudes towards appearance scale-3 (SATAQ-3): Development and validation. Int J Eat Disord 2004;35:293-304.
15. Garner DM, Olmstead MP, Polivy J. Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia. Int J Eat Disord 1983;2:15-34.
16. Eveleth PB. Physical status: The use and interpretation of anthropometry. Report of a WHO Expert Committee. Am J Hum Biol 1996;8:786-7.
17. Mangal A, Kumar V, Panesar S, Talwar R, Raut D, Singh S, *et al.* Updated BG Prasad socioeconomic classification, 2014: A commentary. Indian J Public Health 2015;59:42-4.
18. Sasi RV, Maran K. Advertisement pressure and its impact on body dissatisfaction and body image perception of women in India. Glob Med J Indian Ed 2012;3:1-9.
19. Felden EP, Teixeira CS, Gattiboni BD, Bevilacqua LA, Confortin SC, da Silva TR. Body image perception and socioeconomic status in adolescents: Systematic review. Rev Paul Pediatr 2011;29:423-9.
20. Mishra SK, Mukhopadhyay S. Eating and weight concerns among sikkimese adolescent girls and their biocultural correlates: An exploratory study. Public Health Nutr 2011;14:853-9.
21. Calzo JP, Sonneville KR, Haines J, Blood EA, Field AE, Austin SB, *et al.* The development of associations among body mass index, body dissatisfaction, and weight and shape concern in adolescent boys and girls. J Adolesc Health 2012;51:517-23.
22. Stice E, Whitenton K. Risk factors for body dissatisfaction in adolescent girls: A longitudinal investigation. Dev Psychol 2002;38:669-78.
23. Marsella AJ, Shizuru L, Brennan J, Kameoka V. Depression and body image satisfaction. J Cross Cult Psychol 1981;12:360-71.
24. Grabe S, Ward LM, Hyde JS. The role of the media in body image concerns among women: A meta-analysis of experimental and correlational studies. Psychol Bull 2008;134:460-76.
25. Fortes Lde S, Cipriani FM, Coelho FD, Paes ST, Ferreira ME. Does self-esteem affect body dissatisfaction levels in female adolescents? Rev Paul Pediatr 2014;32:236-40.
26. Boone L, Soenens B, Braet C. Perfectionism, body dissatisfaction, and bulimic symptoms: The intervening role of perceived pressure to be thin and thin ideal internalization. J Soc Clin Psychol 2011;30:1043-68.
27. Kalarchian MA, Levine MD, Klem ML, Burke LE, Soulakova JN, Marcus MD. Impact of addressing reasons for weight loss on behavioral weight-control outcome. Am J Prev Med 2011;40:18-24.
28. Brink PJ, Ferguson K. The decision to lose weight. West J Nurs Res 1998;20:84-102.

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Original Research Article

An educational intervention on the risk factors of lifestyle diseases among men aged 30-50 years in an urban slum in Coimbatore, Tamil Nadu, India

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ABSTRACT

Background: Lifestyle diseases like hypertension, atherosclerosis, coronary heart disease (CAD), type –II diabetes mellitus, ischemic stroke are Non -communicable diseases leading to premature sickness, disability and death. To determine the risk factors of lifestyle diseases like hypertension and diabetes mellitus and to impart health education on the risk factors of lifestyle diseases and to determine the impact of health education.
Methods: A educational interventional study was conducted in a randomly selected urban slum in Peelamedu, Coimbatore funded by ICMR STS.100 Men in the age group of 30-50 years were administered questionnaire to determine the risk factors of lifestyle diseases. Health education was given to the respondents regarding risk factors. The same questionnaire was given to the respondents after the health education to determine the impact of health education. Statistical analysis was performed by using SPSS version 20. Prevalence of risk factors was presented in percentage. Paired T test was used to find out the effectiveness of Health Education.
Results: Among the study population, the risk factors for life style diseases in our study are smoking (39%), alcohol intake (27%), lack of physical activity (40%), overweight (36%), obesity (14%). The impact of health education on risk factors of lifestyle disease and it was statistically significant with smoking (p=.000), alcohol (p=.02), physical activity (p=.001) and not significant with BMI (p=.71).
Conclusions: Regular health checks can be done to improve the frequency of preventive care and support for behavior change. Effective implementation of lifestyle modifications behavior like healthy diet intake, avoidance of high caloric foods, promoting physical activity help in preventing future complications as a part of primary preventive strategies at primary care level. The importance of lifestyle modifications should be reinforced.

Keywords: Alcohol, Health education, Lifestyle diseases, Overweight, Physical activity, Smoking

INTRODUCTION

Lifestyle diseases like hypertension, atherosclerosis, Coronary Heart disease (CAD), Type -II Diabetes mellitus, Ischemic stroke are Non -communicable diseases leading to premature sickness, disability and death. Lifestyle diseases now emerge as the most severe health problems in developed and developing countries like India.[1] These diseases take a tremendous toll in premature sickness, disability, death and have a major

economic impact on its victim and health care delivery system. ICMR and WHO have predicted that cardiovascular diseases would be the most important cause of mortality and morbidity in India by the year 2015.[2] India is undergoing a rapid health transition with rising burden of coronary heart disease (CHD).[3] Among adults over 20 years of age, the estimated prevalence of CHD is around 3-4 per cent in rural areas and 8-10 per cent in urban areas, representing a two-fold rise in rural areas and a six-fold rise in urban areas between the years 1960 and 2000.[4]

Several studies conducted in India had shown an increase in the prevalence of many risk factors contributing for chronic diseases and strengthens the needs of interventions to reduce the prevalence of these risk factors.[5] Recent figures released by WHO indicated that approximately 80% of the global deaths due to chronic diseases are occurring in developing countries and only 20% occurs in developed countries. There is thus a widespread scientific and public health consensus that the likelihood of developing chronic diseases linked to the exposure of an individual, community or population to a cluster of behavior risk factors such as tobacco use, unhealthy diets and physical inactivity.[6] At present the health care system do not focus on preventive health services. A highly structured family life, education with appropriate intervention and counseling services can tackle many of these issues. Cardiovascular risk factors can be combated and controlled by adherence to current lifestyle recommendations.[7-9] The INTERHEART study emphasizes the role of behavioral and conventional risk factors in the prediction of CHD risk among Indians.[10] These findings have implication for the health care providers and policy makers in the country due to the fact that all these conventional risk factors are potentially modifiable and are good starting points for prevention. The policy measures by means of legislation and regulatory approaches on food industry or tobacco or physical activity will have large impact on CHD risk factor reduction in the population.[11,12] The development of an effective education program should impact on self-care, lifestyle change and adherence to medication and lead to better control of diabetes, fewer complications and better quality of life. As the prevalence of non communicable disease is increasing, regular interactive awareness programs involving small groups targeting the vulnerable group of the population is the need of the hour.[13,14] Early intervention will be critical to reduce the occurrence of lifestyle diseases and associated complications in the vulnerable population. Hence we took up this study to create awareness on risk factors and measures to combat these diseases among the study subjects.

METHODS

The present study was conducted in a randomly selected urban slum in Peelamedu, Coimbatore among men in the age group of 30-50 years funded by ICMR STS. All Urban slums in Peelamedu, Coimbatore were listed. An Urban slum was selected by simple random method. From the selected urban slum, number of men aged 30-50years was listed from the data already available and among them 100 men who have one of the lifestyle diseases like Diabetes and Hypertension were included in the study was selected by systematic sampling. House to house survey was conducted and 100 men with the above inclusion criteria were selected from the urban slum area and those who have no lifestyle diseases were excluded from the study. After a period of 3 months each participants were made phone calls to ensure their availability for follow-up and everyone responded. The weight and height of the participants were measured by Weighing scale and portable stadiometer which were calibrated and validated by Department of Biometrics prior to the study. Height and weight were measured based on WHO standards with sensitivity of 0.5 cm and 0.1 kg. Zero error was set after every 10 measurements. The participants were made to stand straight with heels, buttocks and back touching the vertical limb of the instrument and stretching upwards to the fullest extent with arms hanging on the side. The head was aligned so that the lower rim of the orbit and the auditory canal were in horizontal plane (Frankfurt plane).Weight was measured without any footwear and with minimal clothing (school uniform) by using Krup's weighing scale. Based on the height and weight BMI were calculated. Ethical clearance was obtained from Institutional Human Ethics Committee.

Pre–tested Questionnaire was administered to find out the level of risk factors of lifestyle diseases. Health education was given simultaneously to the respondents regarding risk factors after administering questions and 1 month after health education was again repeated to the participants. After a period of 3 month impact of health education was analyzed among the same participants.

Statistical analysis was performed by using SPSS version 20. Prevalence of risk factors was presented in percentage. Paired T test was used to find out the impact of health education on risk factors.

RESULTS

The study was conducted to find out the prevalence of risk factors of life style diseases and also to determine the impact of Health education regarding risk factors of life style diseases. A total of 100 Men in the age group of 30-50 years were involved in the study. The findings of the study are as follows:

Table 1 shows 41% were diabetic and 43% hypertensive and 16 % were both. Among them the diabetes (44.1%) is more in the age group 46-50 years, hypertension (59.4%) is more in the age group 36-40years and both diabetes & hypertension (29.4%) is more common in the age group 46-50 years.

Table 2 shows the risk factors of life diseases among the study population. Among Diabetics (n=41) 43.9% were smokers, 66.4% were alcoholic, 31.7% were overweight, 19.5% were obese and 58.5% were not doing any physical activity. Among Hypertensive (n=43) 37.2% were smokers, 23.3% were alcoholic, 44.2% were overweight, 14.0% were obese and 25.6% were not doing any physical activity. Among both Diabetics & Hypertensive (n=16) 31.3% were smokers, 25.5% were alcoholic, 25.0% were overweight and 31.3%were doing any physical activity.

Table 3 shows the Impact of health education on risk factors of lifestyle disease and it was statistically significant with Smoking (p=0.000), Alcohol (p=0.02) and Physical activity (p=.001) and not significant with BMI (p=0.71).

Table 1: Age distribution of the Life style diseases in the study population.

Age in years	Number (%)	Diabetes No (%)	Hypertension No (%)	Diabetes & Hypertension No (%)
30-35	11 (11%)	3 (27.3%)	8 (72.7%)	0
36-40	32 (32%)	13 (40.6%)	19 (59.4%)	0
41-45	23 (23%)	10 (43.5%)	7 (30.4%)	6 (26.1%)
46-50	34 (34%)	15 (44.1%)	9 (26.5%)	10 (29.4%)
Total	100	41	43	16

Table 2: Risk factors of life style diseases.

Life style diseases	Smokers	Alcoholics	BMI		No physical activity
			Overweight	Obese	
Diabetes (n=41)	18 (43.9%)	13 (66.4%)	13 (31.7%)	8 (19.5%)	24 (58.5%)
Hypertension (n=43)	16 (37.2%)	10 (23.3%)	19 (44.2%)	6 (14.0%)	11 (25.6%)
Diabetes & Hypertension (n=16)	5 (31.3%)	4 (25.5%)	4 (25.0%)	0	5 (31.3%)
Total	**39**	**27**	**36**	**14**	**40**

Table 3: Impact of health education on risk factors of life style disease using Paired‘t’ test.

Risk factors		Mean ± SD	P value
Smoking	Pre-test	1.6±**0**.49	0.000
	Post-test	1.73±**0**.44	
Alcohol	Pre-test	1.73±**0**.44	0.02
	Post-test	1.82±**0**.38	
Physical activity	Pre-test	1.29±**0**.45	0.001
	Post-test	1.40±**0**.49	
BMI	Pre-test	25.75±8.4	0.71
	Post-test	25.6±8.2	

DISCUSSION

The most common life style disease in developing nations like India is Diabetes and Hypertension. The common risk factors for both were obesity, decreased physical activity, stress, hereditary, alcohol, and smoking. Identifying these risk factors will help to reduce the incidence of the lifestyle diseases and effective implementation of lifestyle modifications at primary care level also can reduce the mortality and morbidity rates.[15-17] Hence we conducted a study among 100 people with life style disease like diabetes and hypertension to find out the risk factors and to determine the impact of health education regarding risk factors of life style disease given to the study participants.

In our study around <11.0% of the participants are less than 35years of age and >89% were in the age group 36-50 years. In our study 41% are having diabetes, 43% are having hypertension and 16% are having both diabetes & hypertension.

In our study diabetes (44.1%) is more in the age group 46-50 years, hypertension (59.4%) is more in the age group 36-40years and both diabetes & hypertension (29.4%) is more common in the age group 46-50 years. In

our study we have taken participants with known diabetes and hypertension and so the prevalence is high in our study.

In our study among Diabetics (n=41) 43.9% were smokers, 66.4% were alcoholic, 31.7% were overweight, 19.5% were obese and 58.5% were doing any physical activity. Among Hypertensive (n=43) 37.2% were smokers, 23.3% were alcoholic, 44.2% were overweight, 14.0% were obese and 25.6%were doing any physical activity. Among both Diabetics & Hypertensive (n=16) 31.3% were smokers, 25.5% were alcoholic, 25.0% were overweight, 0% were obese and 31.3%were doing any physical activity. The risk factors for life style diseases in our study are smoking (39%) , alcohol intake (27%), lack of physical activity (40%), overweight (36%),obesity (14%). A study conducted by Prasanna D et al in Karnataka identified the presence of various risk factors among noncommunicable disease are smoking (45.4%), alcohol intake (41.8%), lack of physical activity (38.1%), high BMI (49.34%).[18]

In our study the impact of health education on risk factors of lifestyle disease was statistically significant with Smoking (p=.000), Alcohol (p=.02), Physical activity (p=.001) and not significant with BMI (p=.71). Prabhakaran et al study showed that Change in proportion of risk factors in intervention vs. control sites: tobacco use: 39% to 29% vs. 17% to 20%, median physical activity score: 6 to 11 vs. 8 to 6.[19] The limitation of the study was changes in their behavior after health education was assessed subjectively.

CONCLUSION

The prevalence of risk factors for life style diseases in the study is high. The Impact of health education on risk factors of lifestyle disease was statistically significant. Targeting groups who are at risk will help to reduce the burden arising due to lifestyle disease. Implementation of relevant health promoting activities is advised for people who are at risk. Regular health checks can be done to improve the frequency of preventive care and support for behavior change. Effective implementation of lifestyle modifications behavior like healthy diet intake, avoidance of high caloric foods, promoting physical activity help in preventing future complications as a part of primary preventive strategies at primary care level.

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REFERENCES

1. Mane SV, Agarkhedkar SR, Karwa DS, Pande V, Singhania SS, Karambelkar GR. Study of risk factors for lifestyle diseases among adolescents in Western India. Int J Pharm Biomed Sci. 2012;3(4):224-8.
2. Dholpuria R, Raja S, Gupta BK, Chahar CK, Panwar RB, Gupta R, Purohit VP. Atherosclerotic risk factors in adolescents. Indian J Pediatrics. 2007;74(9):823-6.
3. Reddy KS, Shah B, Varghese C, Ramadoss A. Responding to the threat of chronic diseases in India. Lancet. 2005;366(9498):1744-9.
4. Gupta R, Joshi P, Mohan V, Reddy KS, Yusuf S. Epidemiology and causation of coronary heart disease and stroke in India. Heart. 2008;94(1):16-26.
5. McGill HC, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP, Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Origin of atherosclerosis in childhood and adolescence. The American journal of clinical nutrition. 2000;72(5):1307s-15s.
6. US Centers for Disease Control and Prevention. Targeting Tobacco Use at a Glance 2008. Atlanta, GA: US Centers for Disease Control and Prevention; 2008.
7. Vamadevan AS, Prabhakaran D. Coronary heart disease in Indians: Implications of the INTERHEART study. Indian J Medical Research. 2010;132(5):561.
8. Tawa N, Waggie F, Frantz JM. Risk factors for chronic non communicable diseases in Mombasa, Kenya: Epidemiological study using WHO stepwise approach.
9. Hoang VM, Byass P, Dao LH, Nguyen TK, Wall S. Risk factors for chronic disease among rural Vietnamese adults and the association of these factors with sociodemographic variables: findings from the WHO STEPS survey in rural Vietnam, 2005. Preventing chronic disease. 2007;4(2):1-10.
10. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937-52.
11. Laskar A, Sharma N, Bhagat N. Lifestyle disease risk factors in a North Indian Community in Delhi. Indian Journal of Community Medicine. 2010; 35(3):426.
12. Tawa N, Waggie F, Frantz JM. Risk factors for chronic non communicable diseases in Mombasa, Kenya: Epidemiological study using WHO stepwise approach. Afr J Health Sci. 2011:19:24-9.
13. Shah B, Mathur P. Surveillance of cardiovascular disease risk factors in India: The need & scope. The Indian J Medical Research. 2010;132(5):634-42.
14. Sugathan TN, Soman CR, Sankaranarayanan K. Behavioural risk factors for non communicable diseases among adults in Kerala, India. Indian J Medical Research. 2008;127(6):555-63.
15. Villareal DT, Miller BV, Banks M, Fontana L, Sinacore DR, Klein S. Effect of lifestyle

intervention on metabolic coronary heart disease risk factors in obese older adults. Am J clinical nutrition. 2006;84(6):1317-23.
16. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002;2002(346):393-403.
17. Balagopal P, Kamalamma N, Patel TG, Misra R. A community-based diabetes prevention and management education program in a rural village in India. Diabetes care. 2008;31(6):1097-104.
18. Prasanna D, Rajesh V, Satish Kumar BP, Rajveer S, Kumarswamy M. Assessment Of Lifestyle-Related Risk Factors Contributing For Chronic Non Communicable Disease In Patients Visiting Rural Tertiary Care Hospital. Int Res J Pharm. 2013;4(6).
19. Prabhakaran D, Jeemon P, Goenka S, Lakshmy R, Thankappan KR, Ahmed F, Joshi PP, Mohan BM, Meera R, Das MS, Ahuja RC. Impact of a worksite intervention program on cardiovascular risk factors: a demonstration project in an Indian industrial population. J American College of Cardiology. 2009;53(18):1718-28.

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Original Article

Microbiology Section

Clinico-mycological and Antifungal Susceptibility Profiles of Candiduria in A Tertiary Care Hospital From South India

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ABSTRACT

Introduction: *Candida* is one of the common causative agent of Urinary Tract Infections (UTI) worldwide. The most common reported species causing UTI is *Candida albicans*. Incidence of UTI due to non-albicans *Candida* species. is on rise in recent years because of their better adaptability and increased resistance to antifungals. Susceptibility profile reports of various *Candida* species. to newer azoles like voriconazole and beta-glucan inhibitors (e.g., caspofungin) are deficient in India, since the reference broth microdilution method is not widely utilized. In this study, a rapid reliable and easier alternative, VITEK 2 compact system was utilized to determine the antifungal susceptibility profile.

Aim: To analyse the clinical and mycological profile with determination of drug susceptibility pattern of *Candida* isolates from urine samples.

Materials and Methods: This observational study was conducted in PSG Institute of Medical Sciences and Research, during April to September 2015. *Candida* isolated with a colony count of ≥103 CFU/ml of urine from clinically suspected cases of UTI were included in the study. Urine samples (n=3821) from clinically suspected UTI cases (n=3821) were subjected to microscopic examination and semi quantitative estimation of yeast culture obtained by inoculated of calibrated volume of urine onto blood, Mac-conkey and HiCrome UTI agar. Clinical parameters of the cases were obtained for analysis. Speciation of *Candida* was performed using germ tube test, observation of morphology in corn-meal agar and pigment production in HiChrome *Candida* differential agar. Confirmation of the species identification and anti-fungal susceptibility profile were obtained using VITEK-2 compact system.

Results: Total 101 patients were identified with significant candiduria. Community-Acquired Candiduria (CAC) was seen in 11 (10.8%) of the cases. 23 (22.7%) cases of candiduria were associated with pyuria. Concomitant candidemia was observed in 4 (3.9%) cases of candiduria The most common species identified was *Candida* albicans (n=53, 52.4%). The commonest non-albicans *Candida* species causing significant candiduria cases were *C. glabrata* and *C. tropicalis* (n=18, 17.8% each). The antifungal susceptibility testing revealed an overall 94% susceptibility to amphotericin B, 89% susceptibility to flucytosine, 84% & 88% susceptibility to azoles, fluconazole and voriconazole respectively with 90% susceptibility to caspofungin. Significant high linear correlation existed between fluconazole and voriconazole resistance for *C. glabrata*, *C. albicans* and *C.tropicalis*. [Pearson's correlation coefficient, *C. glabrata* [r =1.00 (p<0.001)], *C. albicans* [r=0.91 (p<0.001)] and *C.tropicalis* [r=0.79 (p<0.001)].

Conclusion: *C. albicans* was found to the commonest species in our Tertiary Care Center in contrast to the reporting of non-*albicans Candida* species as the commonest species in most other recent studies. Determination of the antifungal susceptibility profile showed increasing trend of resistance to most antifungal agents, particularly azoles.

Keywords: Antibacterial agents, Antibiotics, Immunosuppression

INTRODUCTION

Candida species. has been reported as one of the common etiological agent of UTI, especially in a hospital setting. Advances in critical care management, immunosuppression and widespread use of antibacterial agents have led to their increased prevalence. Common risk factors associated with candiduria are extremes of age, diabetes mellitus, females in reproductive age group, structural abnormalities of the urinary tract, broad-spectrum antibiotic use, immunosuppression, indwelling catheters, abdominal surgeries, admission in ICU etc., [1]. Identification and susceptibility testing of the yeast helps targeted therapy of the infection and yields epidemiological

data for assessment of the prevalence and resistance pattern. Fungal UTI are mostly asymptomatic, occasionally can present with upper or lower urinary tract symptoms. Despite the lack of symptoms, increased colonization burden may predispose to invasive candidiasis particularly in debilitated individuals. It necessitates differentiation of asymptomatic and symptomatic candiduria since unnecessary treatment, catheter changes, and hospital prolongation can be avoided, since hospital cost burden and patient morbidity increases substantially. The scenario of presence of yeast in microscopic examination or isolation in culture needs to be evaluated with the clinical picture to determine its relevance and the decision on the commencement of antifungal therapy. Semi-quantitative/ quantitative urine culture is performed in evaluation of significant candiduria and a colony count of ≥1000 CFU/ml of urine, obtained in culture media in most situations represents significant candiduria. Significant candiduria occurs as 2 clinical presentations, asymptomatic and symptomatic candiduria. Patients with symptomatic candiduria need to be started on antifungal therapy, whereas asymptomatic candiduria needs to be interpreted with care. The significance of asymptomatic candiduria arises when the patient is severely debilitated/immunosuppressed, or is planned for urological instrumentation/procedures. Asymptomatic candiduria can also represent a picture of sample contamination (inappropriate collection) or colonization (in catheterized patients). In these cases, evaluating a repeat urine sample rules out contamination and colonization when the repeat sample has been collected after removal of indwelling catheter [2]. The most common reported species causing UTI is *Candida albicans* [3]. Incidence of Non-*albicans Candida* species. (e.g.: *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis* etc.,) causing UTI have been increasingly reported due to enhanced adaptation potential and increased resistance to antifungal agents [4]. In the past few years, there has been emergence of drug-resistance in *Candida* species causing clinical infections, leading to high morbidity and mortality in hospitalized patients [5]. Testing for antifungal drug susceptibility in clinically significant *Candida* species isolates guides prompt treatment thereby improving the patient's outcome. Susceptibility profile reports of various *Candida* species. to newer azoles like voriconazole and beta-glucan inhibitors (eg: caspofungin) are deficient in India, since disc diffusion interpretation guidelines are not available for all antifungals and the reference broth microdilution method is not widely practiced. The VITEK 2 antifungal drug susceptibility determination system has emerged as an easier and an expeditious alternative to the reference broth microdilution method (CLSI and EUCAST) [6]. Studies report 95-100% essential agreement between the results of VITEK 2 and the reference methods [7,8]. This study was undertaken to analyse the clinical and mycological profile with determination of drug susceptibility pattern of *Candida* isolates from urine.

MATERIALS AND METHODS

This was an observational study conducted in PSG Institute of Medical Sciences and Research (PSGIMS&R) during the period April 2015 - September 2015 (6 months). The Institute's ethics committee approval was obtained before the conduct of the study. (IHEC approval number: 15/039).

Inclusion criteria: *Candida* being isolated with a colony count of ≥1000 CFU/ml of urine either in pure culture or as one among the 2 isolates obtained from urine samples collected from clinically suspected cases of urinary tract infection (UTI)/ screening prior to urological instrumentation/intervention of all ages and both sex.

Exclusion criteria: If growth of *Candida* with a colony count of <1000 CFU/ml of urine or when >3 types of growth was obtained.

Urine samples were received in sterile screw capped leak-proof container, immediately transported from the collection center to the microbiology laboratory and stored in refrigerator until further investigations were undertaken. Urine wet mount examination was performed to look for the presence of pus cells, epithelial cells, red blood cells, casts, crystals, budding yeast cells with/without pseudohyphae. The urine samples were inoculated onto blood, mac-conkey and chromogenic media (HiCrome UTI agar, HiMedia Laboratories) by a calibrated wire loop standardized to deliver 5µl for semi-quantitative estimation of yeast growth. The inoculated culture plates were incubated aerobically at 37°C for 24-48 hours. *Candida* isolates with colony count of ≥1000 CFU/ml of urine were considered significant and included in the study. Clinical parameters of the cases were obtained in the case study form which included age, sex, details of admission (outpatient/ ward/ICU), presence/absence of any risk factors like extremes of age (elderly and pre-mature neonates), diabetes mellitus, structural abnormalities of the urinary tract, broad-spectrum antibiotic use in preceeding 30 days, immunosuppression (neutropenia, HIV etc.,), indwelling catheters, abdominal surgeries and admission in ICU etc. Significant candiduria was classified as hospital-acquired and community-acquired. CAC was defined as candiduria observed in outpatients with no prior hospitalization or within 48 hours of hospitalization. When the onset of candiduria is >48 hours of hospitalization, it was classified as Hospital-Acquired Candiduria (HAC) [9]. Preliminary speciation of *Candida* was performed using Germ Tube Test (GTT), observation of morphology in corn-meal agar and pigment production in chromogenic medium (HiChrome *Candida* differential agar, HiMedia Laboratories) [10]. Confirmation of the species identification and anti-fungal susceptibility profile were obtained using VITEK-2 compact system (YST ID and AST card, bioMerieux, Marcy-l'Etoile, France) for the following antibiotics: amphotericin B, flucytosine, fluconazole, voriconazole and caspofungin. The VITEK 2 cards were stored at 2-8°C until testing was performed. An inoculum turbidity equivalent to 2.0 McFarland was prepared in sterile saline as per the manufacturer's instructions using the DensiChek instrument, for antifungal susceptibility testing [11]. *Candida albicans* ATCC 10231 was used as the control

strain for the antifungal susceptibility testing.

STATISTICAL ANALYSIS

The data obtained were analyzed using SPSS software version 19. Fischer' exact/Chi-square test were used for comparative analysis and a p value of ≤0.05 was considered significant. Association/correlation between variables were analysed using Pearson's correlation coefficient (r).

RESULTS

During the study period of April - September 2015 (6 months), 101 patients were identified with significant candiduria. The mean age of the study population was 55.9±16.9 years (Median age-60 years). The age distribution of the study population ranged between 12-89 years and the majority of the candiduria cases (76%) were observed in the 41-80 years age group with a peak observed in the 61-70 years age group [Table/Fig-1]. In the study population, males (n=56, 55.4%) were more commonly affected than females (n=45, 44.6%). Candiduria was observed more commonly in the in-patient wards (n=58, 57.4%), followed by intensive care units (n=32, 31.8%) and outpatient department (n=11, 10.8%).

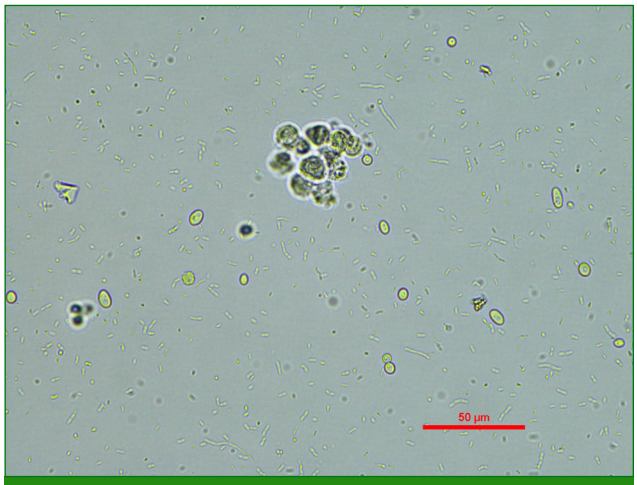
Age Group (in years)	Number (n)
0-10	0
11-20	4
21-30	9
31-40	9
41-50	10
51-60	22
61-70	30
71-80	15
81-90	2

[Table/Fig-1]: Age distribution of the patients with significant candiduria.

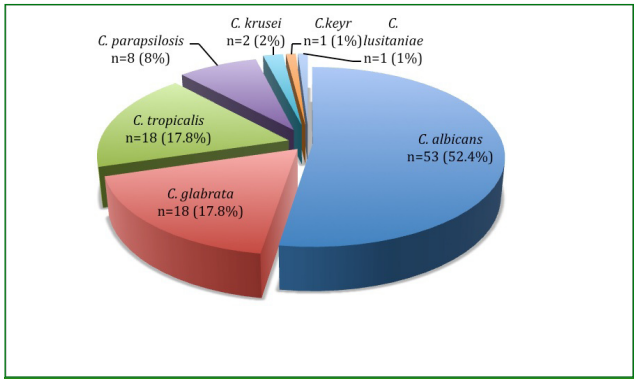
Among the risk factors, the commonest cause was broad-spectrum antibiotic usage (67.3%) followed by indwelling foley's catheterisation (56.4%), diabetes mellitus (16.8%), immunosuppression (13.8%), previous abdominal surgeries (1.9%) and urinary tract structural abnormality (0.9%) [Table/Fig-2]. Among the broad spectrum antibiotics, cephalosporin (68%) and fluroquinolone (32%) were the common antibiotics observed to be used in patients with significant candiduria, in the preceeding 30 days. Pyuria was observed in 23 (22.7%) cases of significant candiduria [Table/Fig-3], while concomitant candidemia was observed in 4 (3.9%) cases. The various species of *Candida* identified in the candiduria cases are depicted in [Table/Fig-4]. The most common species of *Candida* identified was *Candida albicans* (52.4 %). A case of significant candiduria had both *C. albicans* and *Trichosporon* species. The commonest non-*albicans Candida* species causing significant candiduria cases were *C. tropicalis* and *C. glabrata* (18% each).

Risk Factors	Number (n)	Percentage (%)
Diabetes Mellitus	17	16.8
Structural abnormalities of Urinary Tract	1	0.9
Broad Spectrum Antibiotic usage	68	67.3
Immunosuppressive Conditions	14	13.8
Indwelling Foley's Catheter	57	56.4
Underwent Abdominal Surgeries	2	1.9

[Table/Fig-2]: Distribution of risk factors in patients with significant candiduria.



[Table/Fig-3]: Wet mount examination of urine from showing pus cells and budding yeast cells (Magnification – 400X).



[Table/Fig-4]: Frequency of *Candida* species in the study population.

The antifungal susceptibility testing revealed an overall 94% susceptibility to amphotericin B, 89% susceptibility to flucytosine, 84% and 88% susceptibility to azoles, fluconazole and voriconazole respectively with 90% susceptibility to caspofungin. Susceptible-Dose Dependent (SDD) for fluconazole was observed in 5 isolates (9.4%) of *C. albicans*, 2 isolates of *C. glabrata* and *C. tropicalis* (11.1% each). The species-based susceptibility profiles to the antifungal agents tested are provided in [Table/Fig-5]. A significant high linear correlation existed between fluconazole and voriconazole

Candida species	Sensitive									
	Amphotericin B		Flucytosine		Fluconazole		Voriconazole		Caspofungin	
	n	%	n	%	N	%	N	%	N	%
C. albicans	50	94.3	47	88.6	46*	86.7	47	86.7	48	90.5
C. glabrata	17	94	16	88.8	15*	83.3	15	83.8	17	94
C. tropicalis	17	94	16	88.8	15*	83.3	16	88.8	14	77.7
C. parapsilosis	7	87.5	7	87.5	7	87.5	7	87.5	8	100
C. krusei	2	100	2	100	1	50	2	100	2	100
C. kefyr	1	100	1	100	1	100	1	100	1	100
C. lusitaniae	1	100	1	100	1	100	1	100	1	100
All species	95	94	90	89	85	84	89	88	91	90

[Table/Fig-5]: Antifungal susceptibility pattern of the isolated *Candida* species.
* includes susceptible-dose dependent (SDD) isolates

resistance for *C. glabrata* [Pearson correlation coefficient, r =1.00 (p<0.001)], *C. albicans* [r=0.91 (p<0.001)] and *C. tropicalis* [r=0.79 (p<0.001)].

DISCUSSION

Candiduria is a common nosocomial phenomenon and large scale observational surveys reveal *Candida* as the third most common organism isolated from urine in hospitalized patients [12]. During the study period, a total of 101 *Candida* species were isolated from cases of significant candiduria. Candiduria cases proportionally increased with age and were predominantly observed in the 41-80 years age group (76.2%). This observation could be due to the increased prevalence of co-morbid conditions like diabetes mellitus, benign prostatic hypertrophy, immunosuppression, frequent use of broad-spectrum antibiotics and foley's catheter etc. seen in the elderly age group. The median age and age distribution was similar to observational study reports by Nayman Alpat S et al., and Bobade O et al., [13,14].

Males and females were almost equally affected in our study population, whereas most reports from literature show the incidence of candiduria being more common in females [14,15]. Significant candiduria were observed commonly in hospitalized patients (inpatient and ICU settings) probably because of the presence of risk factors like broad spectrum antibiotic use, long term indwelling catheters and also to presence of other debilitating illness like uncontrolled diabetes commonly in the inpatient population than the outpatients. The broad-spectrum antibiotics implicated in cases of significant candiduria cases in our study population were cephalosporins (68%) and fluoroquinolones (32%) use in the preceding 30 days. There are documented reports of use of broad-spectrum antibiotics like cephalosporins attributing to causation of candiduria [16]. CAC was seen in 10.8% of the cases and the risk factors associated were diabetes mellitus, pregnant women and chronic indwelling catheters. Similar risk factors for CAC have been reported by Colodner R et al., [9].

Pyuria is defined as the presence of ≥3 leucocytes/hpf of unspun urine or ≥10 leucocytes per cubic mm of urine or positive dipstick test for leucocyte esterase [17]. In our study, 23 (22.7%) cases of candiduria were associated with pyuria. Pyuria is an important sign of candiduria yet lacks sensitivity and doesn't differentiate colonization from infection (lacks specificity) [18]. Concomitant candidemia was observed in 4 (3.9%) cases of candiduria. Studies report that approximately 1-8% of patients with candiduria particularly ICU admissions may have concomitant candidemia [3,12,19].

The commonest *Candida* species identified in our study was *Candida albicans* (52.4%), while non-*albicans Candida* species attributed to the remaining 47.6% of cases of significant candiduria. Among the non-*albicans Candida* species, *C. glabrata* and *C. tropicalis* were the common species isolated. Earlier multicentric reports on candiduria had similar findings of *Candida albicans* being the commonest species yet recent studies report the emergence of non-*albicans Candida* species as the commonest cause of significant candiduria due to the widespread use of antifungal agents for antifungal prophylaxis and treatment, particularly increasing incidence of *C. glabrata* and *C. krusei* infections which are intrinsically less susceptible to the commonly used azole drug, fluconazole [14,20,21]. Our report suggests relatively lower prevalence of non-*albicans Candida* species, in contrast to reports from other centers in India [22,23].

Analysis of the antifungal susceptibility pattern showed that most isolates were susceptible to amphotericin B. Resistance to the polyene drug, amphotericin B was observed in 6 isolates (6%) (3-*C. albicans*, 1-*C. tropicalis*, 1-*C. glabrata* and 1-*C. parapsilosis*). Increasing resistance was observed for azole group of drugs particularly fluconazole in non-*albicans Candida* species (16.7% for both *C. glabrata* and *C. tropicalis*, 50% for *C. krusei*) when compared to *C. albicans* (13.3%). These drugs (fluconazole and voriconazole) belong to triazole group of azoles and exert their action by inhibiting cytochrome P450-dependent enzyme lanosterol 14 alpha-demethylase. Voriconazole have better activity against *Candida* species (16-32 fold potent than fluconazole) due to effective binding to the cytochrome P450 enzyme. Yet, cross-resistance between fluconazole and voriconazole have been increasingly reported

in *Candida* species, particularly *C. glabrata* [24]. Pfaller et al, (2007) had reported the use of fluconazole as a surrogate marker to predict voriconazole susceptibility in resource-poor settings [25]. In our study, high cross-resistance correlation between azoles was observed in *C. albicans*, *C. glabrata* and *C. tropicalis*. One *C. glabrata* isolate was resistant to three classes of drugs (Polyene, azoles and β-glucan inhibitors). This is of high-concern because azoles and β-glucan inhibitors are increasingly recommended for prophylaxis, thereby leading to selection of drug-resistant non-*albicans Candida* strains [26,27]. The echinocandin, caspofungin had excellent activity against most *Candida* isolates, while 5 isolates of *C. albicans* (9.5%) and 4 isolates of *C. tropicalis* (22.3%) were found to be resistant. This observation predicts that echinocandins like caspofungin and micafungin may emerge in the recent future as the drug of choice for prophylaxis and treatment of cases with symptomatic candiduria.

LIMITATION

The limitation of our study was the lack of genomic characterization of the resistant isolates with MIC correlation and follow-up assessment of clinical/microbiological outcome.

CONCLUSION

This study describes the clinical profile and the distribution of various *Candida* species causing significant candiduria, where *C. albicans* was found to the commonest species in our Tertiary Care Center, in contrast to the reporting of non-*albicans Candida* species as the commonest species in most other recent studies. Although, *C. albicans* was the predominant cause of candiduria, increasing trend towards non-*albicans Candida* species causing significant candiduria was observed. Most health care centers in India do not routinely test for antifungal susceptibility for the clinical isolates due to the laborious phenotypic procedures, lack of expertise and lack of availability of updated interpretative guidelines, thereby leading to lack of drug-resistance data for fungal infections. Newer automated technologies like VITEK 2 compact system have emerged as an effective and promising alternative in determination of antifungal susceptibility thereby guiding prompt treatment and early detection of emerging drug resistance. In our study, antifungal susceptibility profile showed increased resistance to fluconazole and associated cross-resistance to voriconazole in commonly isolated *Candida* species., probably due to their increased usage for prophylaxis and treatment. Newer antifungal drugs like caspofungin and micafungin (echinocandins) are emerging as promising agents for prophylaxis and treatment of symptomatic candiduria. In addition, strengthening the hospital antimicrobial stewardship programs would taper this scenario of widespread drug resistance similar to Multi-Drug Resistant (MDR) bacterial isolates.

Declaration: A part of this study has been presented as a poster at the MICROCON 2015 conference organized by Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Puducherry. (Abstract available at http://microcon2015.co.in/abstracts/free_ePosters/Abstracts/abs-1153.html).

REFERENCES

[1] Nayman Alpat S, Özguneş I, Ertem OT, Erben N, Doyuk Kartal E, Tözun M, et al. Evaluation of risk factors in patients with candiduria. Mikrobiyol Bul. 2011;45(2):318-24.
[2] Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, Edwards JE Jr, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(5):503-35.
[3] Achkar JM, Fries BC. *Candida* infections of the genitourinary tract. Clin Microbiol Rev. 2010;23(2):253-73.
[4] Deorukhkar SC, Saini S, Mathew S. Non-*albicans Candida* infection: an emerging threat. Interdisciplinary Perspectives on Infectious Diseases, vol. 2014, Article ID 615958, 7 pages, 2014.
[5] Shor E, Perlin DS. Coping with stress and the emergence of multidrug resistance in fungi. PLOS Pathog 2015;11(3):e1004668.
[6] Bourgeois N, Dehandschoewercker L, Bertout S, Bousquet P-J, Rispail P, Lachaud L. Antifungal susceptibility of 205 *Candida* species. isolated primarily during invasive candidiasis and comparison of the Vitek 2 System with the CLSI Broth Microdilution and Etest Methods. J Clin Microbiol. 2010;48(1):154-61.
[7] Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the VITEK 2 yeast susceptibility test with the CLSI broth microdilution reference method for testing fluconazole against *Candida* species. J Clin Microbiol. 2007;45(3):796-802.
[8] Melhem M, Bertoletti A, Lucca H, Silva R, Meneghin F, Szeszs M. Use of the VITEK 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species. Braz J Microbiol. 2013;44(4):1257-66.
[9] Colodner R, Nuri Y, Chazan B, Raz R. Community-acquired and hospital-acquired candiduria: comparison of prevalence and clinical characteristics. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2008;27(4):301-05.
[10] Kaup S, Sankarankutty J, Balasubrahmanya HV, Kulkarni S, Nirmala M. Speciation of *Candida* using HiCrome *candida* differential agar. Int J Curr Microbiol Appl Sci. 2016;5(7):267-74.
[11] Cuenca-Estrella M, Gomez-Lopez A, Alastruey-Izquierdo A, Bernal-Martinez L, Cuesta I, Buitrago MJ, et al. Comparison of the Vitek 2 antifungal susceptibility system with the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution reference methods and with the sensititre yeast one and E-test techniques for in vitro detection of antifungal resistance in yeast isolates. J Clin Microbiol. 2010;48(5):1782-86.
[12] Bouza E, San Juan R, Muñoz P, Voss A, Kluytmans J.Co-operative Group of the European Study Group on Nosocomial Infections. A European perspective on nosocomial urinary tract infections I. Report on the microbiology workload, etiology and antimicrobial susceptibility (ESGNI-003 study). European Study Group on Nosocomial Infections. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2001;7(10):523-31.
[13] Nayman Alpat S, Özguneş I, Ertem OT, Erben N, Doyuk Kartal E, Tözun M, et al. Evaluation of risk factors in patients with candiduria. Mikrobiyol Bul. 2011;45(2):318-24.

[14] Bobade O, Waghmare M, Chhabrani P, Kaur I. Species distribution and antifungal susceptibility profile of *Candida* isolated from urine samples. Int J Med Sci Public Heal. 2013;2(4):1.
[15] Sobel JD, Kauffman CA, McKinsey D, Zervos M, Vazquez JA, Karchmer AW, et al. Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis Off Publ Infect Dis Soc Am. 2000;30(1):19-24.
[16] Paul N, Mathai E, Abraham OC, Michael JS, Mathai D. Factors associated with candiduria and related mortality. J Infect. 2007;55(5):450-55.
[17] Longo DL, Wise GJ, Schlegel PN. Sterile pyuria. N Engl J Med. 2015;372(11):1048-54.
[18] Kauffman CA, Fisher JF, Sobel JD, Newman CA. *Candida* urinary tract infections-diagnosis. Clin Infect Dis. 2011;52(Suppl 6):S452-56.
[19] Bougnoux ME, Kac G, Aegerter P, d' Enfert C, Fagon J-Y, CandiRea Study Group. Candidemia and candiduria in critically ill patients admitted to intensive care units in France: incidence, molecular diversity, management and outcome. Intensive Care Med. 2008;34(2):292-99.
[20] Kauffman CA, Vazquez JA, Sobel JD, Gallis HA, McKinsey DS, Karchmer AW, et al. Prospective multicenter surveillance study of funguria in hospitalized patients. Clin Infect Dis. 2000;30(1):14-18.
[21] Yashavanth R, Shiju MP, Bhaskar UA, Ronald R, and Anita KB. Candiduria: prevalence and trends in antifungal susceptibility in a tertiary care hospital of Mangalore. J Clin Diagn Res. 2013;7(11):2459-61.
[22] Mohandas V, Ballal M. Distribution of *Candida* Species in different clinical samples and their virulence: Biofilm formation, proteinase and phospholipase production: A study on hospitalized patients in Southern India. J Glob Infect Dis. 2011;3(1):4.
[23] Rathor N, Khillan V, Sarin S. Nosocomial candiduria in chronic liver disease patients at a hepatobilliary center. Indian J Crit Care Med. 2014;18(4):234.
[24] Pfaller MA, Messer SA, Boyken L, Rice C, Tendolkar S, Hollis RJ, et al. Use of Fluconazole as a Surrogate Marker To Predict Susceptibility and Resistance to Voriconazole among 13,338 Clinical Isolates of *Candida* species. Tested by Clinical and Laboratory Standards Institute-Recommended Broth Microdilution Methods. J Clin Microbiol. 2007;45(1):70-75.
[25] Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ. Activities of fluconazole and voriconazole against 1,586 recent clinical isolates of *Candida* species determined by Broth microdilution, disk diffusion, and Etest methods: report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. J Clin Microbiol . 2003;41(4):1440-46.
[26] Pelz RK, Hendrix CW, Swoboda SM, Diener-West M, Merz WG, Hammond J, et al. Double-blind placebo-controlled trial of fluconazole to prevent *candida*l infections in critically ill surgical patients. Ann Surg. 2001;233(4):542-48.
[27] Ostrosky-Zeichner L, Shoham S, Vazquez J, Reboli A, Betts R, Barron MA, et al. MSG-01: A randomized, double-blind, placebo-controlled trial of caspofungin prophylaxis followed by preemptive therapy for invasive candidiasis in high-risk adults in the critical care setting. Clin Infect Dis Off Publ Infect Dis Soc Am. 2014;58(9):1219-26.

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Intravitreal Bevacizumab for Choroidal Neovascularization Associated with Angioid Streaks: Long-term Results

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Abstract:

PURPOSE: The purpose of the study was to evaluate the long-term efficacy and safety of intravitreal bevacizumab (IVB) in the treatment of choroidal neovascularization (CNV) secondary to angioid streaks (AS).

MATERIALS AND METHODS: Noncomparative, interventional retrospective case series involving ten patients (15 eyes) with a minimum follow-up of 25 months following IVB for AS-associated CNV. Demographic and clinical details at baseline and during follow-up were collected from patient records. Detailed clinical examination was followed by fundus fluorescein angiography and optical coherence tomography to confirm CNV. Both primary and recurrent CNVs were treated with monthly injections of IVB till the lesion stabilized. Primary outcome measures were the percentage of patients with stable or improved visual acuity (VA) and with stable or decreased central retinal thickness (CRT) at the last visit. Secondary outcome measure was the incidence of ocular and systemic complications.

RESULTS: Recurrence was observed in 11 eyes (73.33%) over a mean follow-up of 57.33 months (range: 25–100). A mean number of injections administered was 5.60. VA improved or stabilized in 73.33% and deteriorated in 26.67% of eyes at the final visit. Mean CRT improved from 324.40 μm at baseline to 265.53 μm at final visit, which was statistically significant. Complications observed were ocular hypertension in one patient and thromboembolic event in another patient.

CONCLUSIONS: IVB appears to be a safe and effective option to treat CNV and to preserve vision over a prolonged period. It cannot eliminate the risk of recurrent CNV indicating the need for more effective treatments to arrest this visually debilitating condition.

Keywords:

Angioid streaks, bevacizumab, choroidal neovascularization, long-term efficacy, safety

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Introduction

Angioid streaks (ASs) are reddish brown, jagged lines radiating from peripapillary area into the peripheral fundus, predominantly affecting population in the second to fifth decade. Systemic associations include pseudoxanthoma elasticum (PXE), Ehler-Danlos syndrome, Paget's disease, or hemoglobinopathies.[1,2] Choroidal neovascularization (CNV) complicating AS (prevalence: 72%–86%) is an important cause of legal blindness and financial burden in middle-aged individuals affected by this condition.[3] Treatment of CNV has remained a challenge, and different modalities such as laser photocoagulation, transpupillary therapy, photodynamic therapy (PDT), or surgery usually resulted in poor outcomes.[1,4,5]

Vascular endothelial growth factors (VEGFs), a potent mitogen for endothelial cells, have been implicated in the pathogenesis of CNV.[4] A recent review on the treatment of AS-related CNV has shown Anti-VEGF

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drugs such as bevacizumab and ranibizumab to be the most effective treatment.[1] Anti-VEGF therapy in angioid CNV was first reported in 2006.[6] Subsequently, several case reports and case series have confirmed its efficacy and safety over a period ranging from 6 to 24 months.[4,7-14] A recent prospective multicentric study involving 44 patients with AS-associated CNV also confirmed the efficacy of Anti-VEGF therapy in stabilizing CNV over a period of 12 months.[5] However, AS being a chronic disease affecting young individuals, it is important to understand the long-term benefits of anti-VEGF therapy, and so a retrospective analysis of our ten patients (15 eyes) treated with intravitreal bevacizumab (IVB) and with a minimum follow-up of 25 months (range: 25–100 and mean: 57.33 months) was done. To the best of our knowledge, this is the longest follow-up results reported for this rare condition.

Materials and Methods

This is a retrospective chart review of ten patients (15 eyes) treated for CNV secondary to AS in the Retina Services of our hospital from September 2006 to March 2014 and who had completed a minimum follow-up of 25 months. Data collected from the patient's chart included age, gender, details of clinical evaluation, and treatment at baseline and subsequent visits. A total number of injections given, duration of total follow-up (from the initial treatment visit to the last visit), and duration of follow up after the last intervention and any local or systemic adverse effects of treatment were also noted. Ophthalmic evaluation included checking of best-corrected visual acuity (VA) with Snellen's chart, slit lamp biomicroscopy, applanation tonometry, indirect ophthalmoscopy followed by fundus fluorescein angiography (FFA) and optical coherence tomography (OCT). FFA was performed using Zeiss FF450; Zeiss Oberkochen, Germany, and OCT was done using time domain Stratus OCT or spectral domain Cirrus HD-OCT (Carl Zeiss Meditech, USA). Patients were also evaluated by general physician to look for systemic associations of AS. CNV identified at the time of presentation was labelled as primary CNV and its location and activity were evaluated based on the leakage seen on FFA and the morphological changes on OCT such as presence of CNV, subretinal fluid (SRF), and retinal edema. All patients with active CNV and with no systemic morbidities such as uncontrolled hypertension, cardiovascular, or cerebrovascular events in the previous 6 months were administered IVB 1.25 mg in 0.05 ml (Avastin; Genentech, South San Francisco, CA, USA) under aseptic precautions, as an outpatient procedure. Antibiotic eye drops four times a day for 5 days were prescribed after the injection for all patients. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Ethics Committee. An informed written consent was obtained from all patients.

Patients were followed up at monthly intervals with reinjections on as needed basis till CNV became inactive. At each visit, recording of VA, fundus examination, fundus photography, and OCT was done. FFA was repeated only when a new lesion was suspected or in eyes with poor response to therapy. Improvement or stabilization of VA along with resolution of CNV, retinal edema, and SRF on OCT was considered as a favorable response to therapy. Recurrent CNV was diagnosed if deterioration of vision and/or metamorphopsia was associated with the presence of new hemorrhage or active lesion adjacent to primary CNV or at new foci with the corresponding leakage on FFA and reappearance of SRF or retinal edema on OCT. Additional injections were administered to treat recurrent CNV and monitoring continued at monthly intervals till stabilization was achieved. This was in accordance with the retreatment criteria for neovascular age-related macular degeneration which allows less frequent injections with comparable outcome.[15] Two patients also underwent PDT. This included the first patient in this series who presented with a subfoveal CNV in his left eye (OS) in September 2006, when the standard of care for subfoveal CNV was PDT. IVB was a new option, and its efficacy as monotherapy was not well established. Hence, he was initially treated with a combination of PDT and IVB, but the multiple recurrences in that eye as well as the CNV that developed later in the fellow eye were managed with IVB alone. Similarly, patient 5 initially managed by IVB monotherapy for CNV in the OS, preferred PDT when he was detected later to have bilateral active CNV, to reduce the need for multiple injections in both eyes. Following treatment, OS stabilized with a large subfoveal scar, whereas right eye (OD) developed three more recurrences which were managed by IVB monotherapy.

Primary end points of this study were the percentage of patients with stable or improved VA and with stable or decreased macular thickness at the last visit. Secondary end point was the long-term incidence of ocular and systemic complications in patients with AS who are predisposed to systemic complications. For statistical analysis, Snellen's VAs were converted to logMAR. Any change in VA was defined as improved or worse based on gain or loss of 3 or more lines from baseline VA. If change in vision was within three lines of baseline VA, it was considered as stable. Morphological outcome was analyzed by comparing the mean central retinal thickness (CRT) measured on central macular map on OCT at baseline and at final visit. Data were entered in Excel and analyzed using IBM SPSS software version 19.0. Continuous variables were expressed in mean and standard deviation. Quantitative data were compared

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using Paired "*t*" test, and correlation between baseline and final VA and CRT was done using Pearson correlation. $P < 0.05$ was considered statistically significant.

Results

Fifteen eyes of ten patients treated for AS-associated CNV and with a minimum follow-up of 25 months were analyzed [Table 1]. There were seven males and three females with a mean age of 49.47 ± 4.37 years (range: 46–63 years). Three patients had biopsy-proven PXE, and in others, AS was considered as idiopathic. Follow-up period ranged from 25 to 100 months with a mean of 57.33 ± 21.76 months. Mean follow-up after the last injection was 28.26 ± 23.42 months (range: 0–73). CNV was bilateral in seven patients (70%), of which five had active CNV bilaterally, and two had active CNV in one eye and scarred CNV in the fellow eye. All the three patients with PXE had bilateral CNV, but in two of them, CNV was already scarred in one eye and was not included in the study. All eyes had naïve CNV, of which eight (53.33%) were juxtafoveal and 7 (46.67%) were subfoveal CNV.

A mean number of injections needed to treat primary CNV was 2.26 ± 0.96. Three eyes (20%) stabilized with one injection, seven eyes (46.67%) with two injections, three eyes (20%) with three injections, and two eyes (13.33%) with four injections. Thus, primary CNV resolved with ≤3 injections in 83.67% of eyes. Mean baseline CRT was 283.33 ± 17.21 μm in eyes that responded to one injection compared to 473.50 ± 106.78 μm in eyes which needed four injections. Recurrence was observed in 11 eyes (73.33%) whereas 4 eyes (26.67%) remained inactive throughout follow-up. Most of the recurrences developed adjacent to the primary CNV. Time of detection of the first recurrence ranged from 10 to 26 months. Six eyes had only one recurrence, two eyes had two recurrences, one eye had three recurrences, and two eyes had four recurrences. Number of injections required to obliterate recurrent CNV were 11 injections (in 1 eye), 10 injections (in 1 eye), 5 injections (in 4 eyes), 2 injections (in 4 eyes), and 1injection (in 1 eye) amounting to a total of fifty injections (mean 3.33 ± 3.50). Overall, the mean number of injections administered was 5.60 ± 3.50. There was a statistically significant correlation between the mean number of recurrences to the number of injections given for recurrent CNV ($P = 0.00$) as well as to the total number of injections ($P = 0.00$). All the three eyes treated with PDT had poor outcome, with two of these eyes having the maximum recurrences.

VA improved or stabilized in 73.33% of eyes (11 eyes) and deteriorated in 26.67% of eyes (four eyes). However, the mean logMAR VA worsened from 0.68 ± 0.54 at baseline to 0.71 ± 0.52 at final visit, but the difference was not statistically significant ($P = 0.75$). Mean variation in VA was −0.27 ± 3.63 lines ranging from −6 lines to +9 lines. At baseline, 26.67% (four eyes) had VA 20/40 (logMAR: 0.3) or better which improved to 33.34% (five eyes) at final visit. Similarly, proportion of eyes with VA 20/200 (logMAR: 1.0) or worse also increased from 33.34% at baseline to 40% at final visit. However, no statistically significant conclusions could be derived as the numbers were small. Mean CRT improved from 324.40 ± 88 μm at baseline to 265.53 ± 66.41 μm at the final visit, a difference which approached statistical significance ($P = 0.01$). Following treatment, 14/15 eyes (93.33%) had stable or decreased CRT with inactive CNV. The only exception was the OD of patient 5 (treated with PDT and IVB) which developed multiple recurrences and chronic

Table 1: Demographic data, treatment details and visual and morphological data at baseline and final visit

Patient number	Age/sex	Study eye (*n*=15)	Baseline VA	Baseline CRT	Time of recurrence - months	Number of injections for primary + recurrent CNV	Total follow up (months)	Final VA	Final CRT
1*,†	47/male	OS	0.18	359	10, 24, 56, 87	3+10	100	0.40	208
		OD	0.0	257	15	2+2	25	0.0	251
2	46/male	OD	1.0	294	10, 31, 63	2+5	83	0.10	212
3	47/male	OS	1.80	549		4	79	1.50	349
		OD	0.10	284	15, 44	2+5	52	0.10	257
4*	48/female	OS	0.78	297	24, 53	1+5	77	0.78	240
5†	48/male	OS	0.40	342	20	3+2	68	1.0	237
		OD	0.48	289	12, 17, 42, 48	1+11	48	1.0	409
6	47/male	OS	1.0	232		2	67	1.30	228
7	53/female	OD	1.30	264		1	59	1.3	248
		OS	0.40	298	16	3+1	59	0.30	211
8*	63/female	OS	0.48	204		2	40	0.78	200
9	52/male	OS	0.60	398	12	4+5	39	0.80	269
10	48/male	OD	0.18	359	26	2+2	32	0.0	267
		OS	1.50	440	12	2+2	32	1.30	397

*Patients with PXE, †Patients who underwent photodynamic therapy. VA: Best corrected visual acuity, CRT: Central retinal thickness measured in μm, CNV: Choroidal neovascularization, PXE: Pseudoxanthoma elasticum, OD: Right eye, OS: Left eye

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cystoid macular edema (CME) with worsening of CRT from 289 μm to 409 μm. Serial morphological changes in patients with the best and worst outcomes are shown in Figures 1 and 2, respectively. Overall, ten eyes developed macular scar which was associated with CME in three eyes, foveal thinning in two eyes, and vitreomacular traction in one eye. Thus, despite an improvement in the retinal thickness, majority had poor functional outcome due to degenerative macular changes.

The only ocular complication noted during follow-up was increased intraocular pressure in one eye (patient 1, OS) which was controlled with Timolol eye drops. Patient 3, who did not have PXE but a known diabetic, hypertensive, and cardiac patient under our care since 2008, developed minor cerebrovascular accident in April 2012 and also had to undergo coronary artery bypass graft in February 2014. No other ocular or systemic complications were observed during the follow-up.

Discussion

All the published studies regarding IVB in AS-associated CNV and having a minimum follow-up of 23 months are summarized in Table 2. Alagöz *et al*.[16] who have the largest series achieved improvement or stabilization of VA in 65.2% of their eyes, but 61% (14/23 eyes) continued to have active CNV at the last follow-up. Young patients with resistant CNV and those with PXE required more aggressive treatment. However, there are reports with a lesser number of patients that have demonstrated 100% improvement/stabilization both visually and morphologically over a follow-up period ranging from 23 to 28.6 months.[10,17,18] Finger *et al*.,[19] in their analysis of 14 patients with PXE and AS, found visual improvement in those with early disease and emphasizes the need for early initiation of treatment with regular follow-up and prompt reinjection for recurrence. Maintenance therapy with monthly injections is not necessary considering the cardiovascular risk profile of these patients with PXE and also to minimize the risk of RPE atrophy which can be aggravated by repeated anti-VEGF injections. Myung *et al*.[18] treated nine patients with PXE and AS-related CNV and reported statistically significant improvement in the final VA at 6 months in their preliminary study. However, in their extended study of the same cohort of patients followed up for 28.6 months, overall visual outcome was less favorable due to frequent recurrences and scarring. Similar observations were also made by Iacono *et al*.,[20] who followed up 15 patients with nonsubfoveal CNV secondary to AS and found that the efficacy of IVB in halting the CNV deteriorated from 80% at 12 months to 46% at 36 months. Another recent study by Rosina *et al*.[21] evaluating 16 eyes with a mean follow-up of 52 months states that risk of recurrence increased

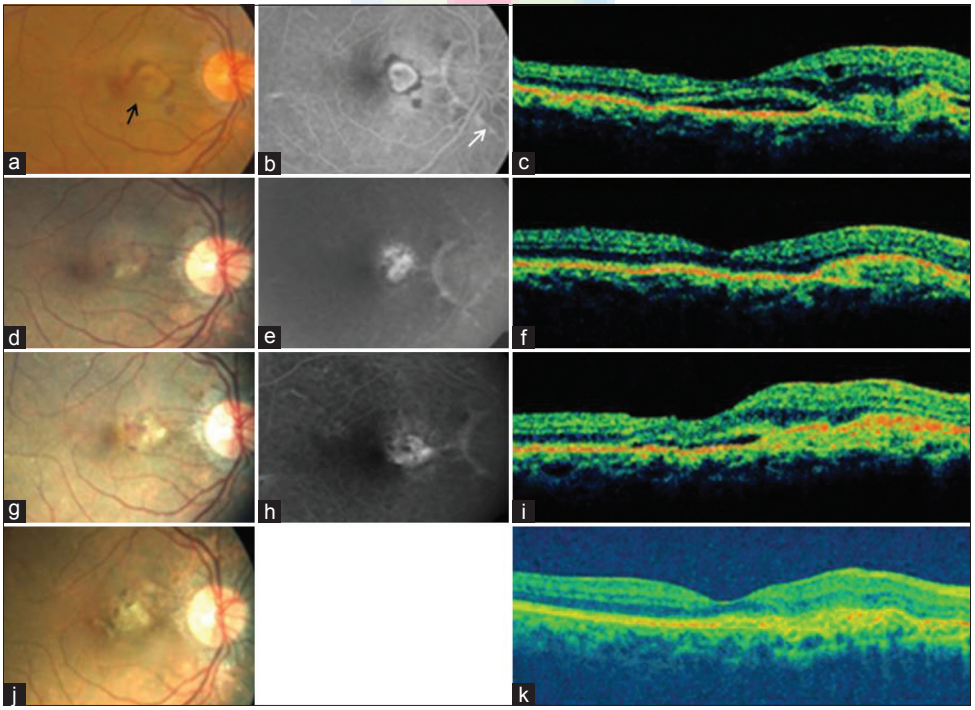


Figure 1: Images of patient 2 who had successful outcome following intravitreal bevacizumab for choroidal neovascularization in the right eye. At presentation, color photograph (CF) (a) and fundus fluorescein angiography (b) showed angioid streak (white arrow) and Juxtafoveal choroidal neovascularization with hemorrhage (black arrow). Optical coherence tomography (c) showed choroidal neovascularization with subretinal fluid, which resolved after treatment as seen on CF (d), fundus fluorescein angiography (e), and optical coherence tomography (f). 10 months later, recurrent choroidal neovascularization was detected based on fresh hemorrhage on CF (g), mottled hyper fluorescence on fundus fluorescein angiography (h), and subretinal fluid on optical coherence tomography (i) and was treated with one more injection. He remained stable at his final visit with a scarred juxtafoveal choroidal neovascularization clinically (j) and on optical coherence tomography (k)

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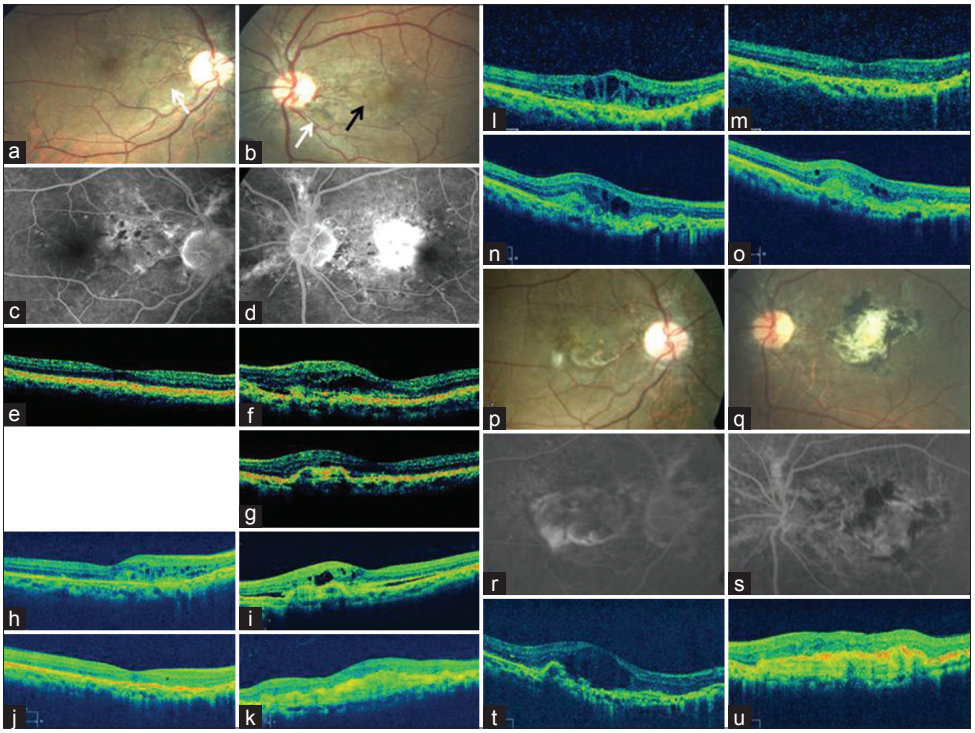


Figure 2: Images of patient five who had the worst outcome. At presentation, color photograph (CF) (a and b), fundus fluorescein angiography (c and d) showed angioid streak (white arrow) in both eyes and choroidal neovascularization (black arrow) in left eye. Optical coherence tomography (e and f) of right eye was normal, left eye had choroidal neovascularization which resolved after treatment (g). 20 months later (h and i), there was new choroidal neovascularization in right eye and reactivation in left eye which stabilized with treatment (j and k). Multiple recurrences in right eye caused chronic edema and scarring as observed in the pre (l and n) and post (m and o) injection scans. CF (p and q), fundus fluorescein angiography (r and s) and optical coherence tomography (t and u) at his final visit showed partially scarred choroidal neovascularization with active inferotemporal edge in right eye and fibrotic scar in left eye

during the first 50 weeks and remains stable afterward. More injections were needed in eyes with increased CNV reactivation, and the final VA is related to the initial VA and not to the number of injections, previous treatments, or baseline CNV size. In a comprehensive review of treatment of AS-related CNV, Gliem *et al*.[1] also support that potential benefit of anti-VEGF therapy correlates best with the structural alterations and functional loss present at baseline. Based on a pooled analysis of 16 studies on the efficacy of bevacizumab or ranibizumab, authors conclude that there is no superiority of one drug over the other and also of fixed versus as needed regimen.

We preferred bevacizumab in our patients due to its affordability and proven efficacy in CNV. In our series with the longest follow-up reported till date (57.33 ± 21.76 months), there was stabilization or improvement in VA in 73.33% and morphological stabilization in 93.33% of patients. These results were achieved with a mean of 5.60 ± 3.50 injections. Our results are comparable to the long-term outcomes with IVB reported by other authors and far superior to the outcome achieved with the treatments in the pre- anti-VEGF era.[1] Recurrent CNV was observed in 73.33% and required more treatment for stabilization (mean of 4.54 injections) than for the primary CNV (mean of 2.26 injections). First recurrence was noted at 10 months and the last recurrence at 87 months in the patient with 100-month follow-up [Figure 3]. Interval between recurrences ranged from 5 months (Patient 5, OD) to 32 months (Patient 1, OS and Patient 2, OD) indicating that reactivation of the CNV can continue to occur at any time and at any location in some eyes in this chronically active disease necessitating long-term monitoring with timely intervention. Here, we differ from Rosina *et al*.,[21] who observed that patients free from disease reactivation showed a smaller recurrence rate. Furthermore, the same authors did not find any difference between those with naïve CNV and those treated with PDT. However, all the three eyes treated with PDT in our study had a recurrence with worse vision at final visit. Abdelkader *et al*. also have reported disappointing results with PDT and recommend anti-VEGF therapy as the treatment of choice at the present time for CNV complicating AS.[5] Since only three patients in our series had PXE, we could not compare the difference in the outcome between those with and without PXE. The reported ocular complications with bevacizumab include lens touch by the needle[12], Bruch's membrane rupture,[22] and arteritic anterior ischemic optic neuropathy.[23] In our series, ocular hypertension was detected in one patient which was well controlled with a single antiglaucoma medication and never developed any progressive glaucomatous optic neuropathy. Besozzi *et al*.[24] have reported acute stoke within 3 days of injection in a patient with PXE and no other systemic illness. Although one patient in our cohort developed two thromboembolic

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Table 2: Published data regarding long-term efficacy of intravitreal bevacizumab in choroidal neovascularization secondary to angioid streaks

Author	Number of eyes/patients	Mean age	Follow up (months)	Mean number of injections	Percentage of eyes with improved/ stable VA	Percentage of eyes with stable CNV/ CRT at last visit	Conclusions
Alagöz *et al*.[16]	23/20	45.7	23	5.1	65.2	39	CNV more aggressive in young, more frequent injections in those with PXE
Neri *et al*.[10]	11/11	46.8	23.8	3.5	100	100	IVB is a promising tool for long-term control of CNV
Teixeira *et al*.[17]	5/5		24±5.2	5.8	100	100	More benefit in those with early symptoms
Finger *et al*.[19]	16/14	55	28	6.5±5.7	81.25	56.25	Preserves vision in advanced disease and improves vision in early disease
Myung *et al*.[18]	9/9	53.5	28.6	8.4	100	100	Effective, continued experience needed to establish long-term efficacy and dosing strategy
Iacono *et al*.[20]	15/15	59.2	36	5.2	NR	NR	Monthly monitoring to detect recurrence and prompt treatment
Rosina *et al*.[21]	16/10	56	52	2.5	75	100	Risk of recurrence increased during first 50 weeks. Final VA related to initial VA
Present study	15/10	49.47±4.37	57.33±21.76	5.60±3.50	73.33	93.33	Effective in stabilizing CNV, more effective options needed to reduce risk of recurrence

IVB: Intravitreal bevacizumab, CNV: Choroidal neovascularization, VA: Visual acuity, CRT: Central retinal thickness, NR: Not reported, PXE: Pseudoxanthoma elasticum

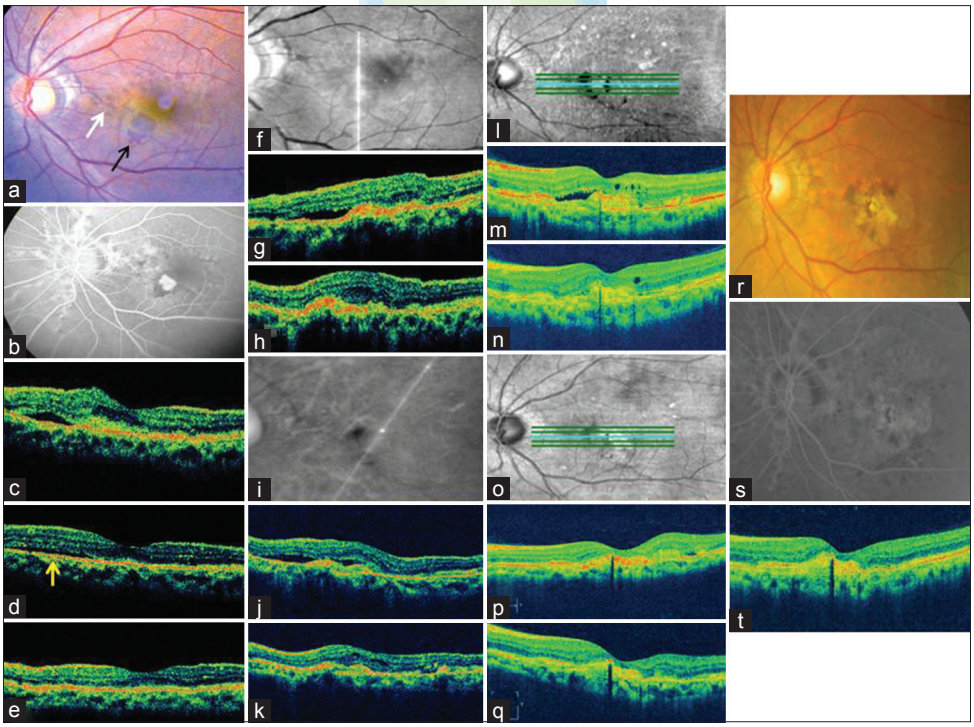


Figure 3: Images of the left eye of patient 1 which had the longest follow-up. At presentation, color photograph (CF) (a) and fundus fluorescein angiography (b) showed angioid streak (white arrow) and juxtafoveal choroidal neovascularization (black arrow). Optical coherence tomography (c) showed choroidal neovascularization with subretinal fluid. Posttreatment Optical coherence tomography (d and e) showed gradual resolution of subretinal fluid (yellow arrow). Serial fundus photographs with optical coherence tomography images pre- and post-treatment of his first recurrence (f-h), second (i-k), third (l-n), and fourth (o-q) demonstrate the progressive macular scarring. He remained stable at his last visit with a scarred choroidal neovascularization clinically (r) and on fundus fluorescein angiography (s). Thinning of neurosensory retina was seen on optical coherence tomography (t)

events, it is difficult to establish a causal relationship with IVB as he had other risk factors such as systemic hypertension and diabetes mellitus. Majority of the authors did not observe any ocular or systemic complications.[3,15,17,19]

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The strength of our study is the long duration of follow-up involving a significant number of eyes which is the longest reported so far, for this relatively rare condition. The limitations include retrospective design and heterogeneous study population. Despite these limitations, our results confirm the long-term efficacy of anti-VEGF therapy to stabilize the CNV and to preserve or even improve vision. It also confirmed the long-term safety of bevacizumab in patients with AS. However, it cannot eliminate the risk of recurrent CNV indicating the need to develop more effective strategies to arrest this visually debilitating condition.

Conclusions

IVB is a safe and effective option in the long-term stabilization of CNV due to AS.

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Conflicts of interest

There are no conflicts of interest.

References

1. Gliem M, Finger RP, Fimmers R, Brinkmann CK, Holz FG, Charbel Issa P, *et al.* Treatment of choroidal neovascularization due to angioid streaks: A comprehensive review. Retina 2013;33:1300-14.
2. Georgalas I, Papaconstantinou D, Koutsandrea C, Kalantzis G, Karagiannis D, Georgopoulos G, *et al.* Angioid streaks, clinical course, complications, and current therapeutic management. Ther Clin Risk Manag 2009;5:81-9.
3. Battaglia Parodi M, Iacono P, La Spina C, Berchicci L, Scotti F, Leys A, *et al.* Intravitreal bevacizumab for nonsubfoveal choroidal neovascularization associated with angioid streaks. Am J Ophthalmol 2014;157:374-7.e2.
4. Ladas ID, Kotsolis AI, Ladas DS, Niskopoulou M, Georgalas I, Papakonstantinou D, *et al.* Intravitreal ranibizumab treatment of macular choroidal neovascularization secondary to angioid streaks: One-year results of a prospective study. Retina 2010;30:1185-9.
5. Abdelkader E, Scott NW, Lois N. Epidemiology of angioid streaks-associated choroidal neovascular membranes in the United Kingdom. Cureus 2013;5:e138.
6. Teixeira A, Moraes N, Farah ME, Bonomo PP. Choroidal neovascularization treated with intravitreal injection of bevacizumab (Avastin) in angioid streaks. Acta Ophthalmol Scand 2006;84:835-6.
7. Bhatnagar P, Freund KB, Spaide RF, Klancnik JM Jr., Cooney MJ, Ho I, *et al.* Intravitreal bevacizumab for the management of choroidal neovascularization in pseudoxanthoma elasticum. Retina 2007;27:897-902.
8. Rinaldi M, Dell'Omo R, Romano MR, Chiosi F, Cipollone U, Costagliola C. Intravitreal bevacizumab for choroidal neovascularization secondary to angioid streaks. Arch Ophthalmol 2007;125:1422-3.
9. Wiegand TW, Rogers AH, McCabe F, Reichel E, Duker JS. Intravitreal bevacizumab (Avastin) treatment of choroidal neovascularisation in patients with angioid streaks. Br J Ophthalmol 2009;93:47-51.
10. Neri P, Salvolini S, Mariotti C, Mercanti L, Celani S, Giovannini A, *et al.* Long-term control of choroidal neovascularisation secondary to angioid streaks treated with intravitreal bevacizumab (Avastin). Br J Ophthalmol 2009;93:155-8.
11. Donati MC, Virgili G, Bini A, Giansanti F, Rapizzi E, Giacomelli G, *et al.* Intravitreal bevacizumab (Avastin) for choroidal neovascularization in angioid streaks: A case series. Ophthalmologica 2009;223:24-7.
12. Sawa M, Gomi F, Tsujikawa M, Sakaguchi H, Tano Y. Long-term results of intravitreal bevacizumab injection for choroidal neovascularization secondary to angioid streaks. Am J Ophthalmol 2009;148:584-90.e2.
13. El Matri L, Kort F, Bouraoui R, Karim B, Chebil A, Chaker N, *et al.* Intravitreal bevacizumab for the treatment of choroidal neovascularization secondary to angioid streaks: One year of follow-up. Acta Ophthalmol 2011;89:641-6.
14. Vadalà M, Pece A, Cipolla S, Monteleone C, Ricci F, Boscia F, *et al.* Angioid streak-related choroidal neovascularization treated by intravitreal ranibizumab. Retina 2010;30:903-7.
15. Heier JS, Brown D, Ciulla T, Abraham P, Bankert JM, Chong S, *et al.* Ranibizumab for choroidal neovascularization secondary to causes other than age-related macular degeneration: A phase I clinical trial. Ophthalmology 2011;118:111-8.
16. Alagöz C, Alagöz N, Özkaya A, Çelik U, Turan MF, Yazici AT, *et al.* Intravitreal bevacizumab in the treatment of choroidal neovascular membrane due to angioid streaks. Retina 2015;35:2001-10.
17. Teixeira A, Mattos T, Velletri R, Teixeira R, Freire J, Moares N, *et al.* Clinical course of choroidal neovascularization secondary to angioid streaks treated with intravitreal bevacizumab. Ophthalmic Surg Lasers Imaging 2010;41:546-9.
18. Myung JS, Bhatnagar P, Spaide RF, Klancnik JM Jr., Cooney MJ, Yannuzzi LA, *et al.* Long-term outcomes of intravitreal antivascular endothelial growth factor therapy for the management of choroidal neovascularization in pseudoxanthoma elasticum. Retina 2010;30:748-55.
19. Finger RP, Charbel Issa P, Schmitz-Valckenberg S, Holz FG, Scholl HN. Long-term effectiveness of intravitreal bevacizumab for choroidal neovascularization secondary to angioid streaks in pseudoxanthoma elasticum. Retina 2011;31:1268-78.
20. Iacono P, Battaglia Parodi M, La Spina C, Bandello F. Intravitreal bevacizumab for nonsubfoveal choroidal neovascularization associated with angioid streaks: 3-year follow-up study. Am J Ophthalmol 2016;165:174-8.
21. Rosina C, Romano M, Cigada M, de Polo L, Staurenghi G, Bottoni F, *et al.* Intravitreal bevacizumab for choroidal neovascularization secondary to angioid streaks: A long-term follow-up study. Eur J Ophthalmol 2015;25:47-50.
22. Sen PR, Rishi P, Sen P, Rishi E, Shroff D. Rapid progression of angioid streaks following intravitreal bevacizumab. Can J Ophthalmol 2009;44:e39-40.
23. Ganssauge M, Wilhelm H, Bartz-Schmidt KU, Aisenbrey S. Non-arteritic anterior ischemic optic neuropathy (NA-AION) after intravitreal injection of bevacizumab (Avastin) for treatment of angoid streaks in pseudoxanthoma elasticum. Graefes Arch Clin Exp Ophthalmol 2009;247:1707-10.
24. Besozzi G, Ferrara A, Epifani E, Intini D, Apruzzese M, Provenzano A, *et al.* Acute stroke after intravitreal bevacizumab to treat choroidal neovascularization due to angioid streaks in pseudoxanthoma elasticum: A severe systemic adverse event after an off-label procedure. Int Ophthalmol 2013;33:181-3.

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Association of ineffective esophageal motility and low lower esophageal sphincter pressure with endoscopic esophagitis in patients with GERD

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Abstract

Background: Abnormal esophageal motility and low Lower Esophageal Pressure (LES) play an integral role among various etiologies implicated in pathogenesis and severity of Gastro Esophageal Reflux Disease (GERD). Delayed clearance of refluxate leads to prolonged mucosal exposure to gastro duodenal contents which promotes esophagitis and its complications. To find out the association of ineffective esophageal motility (IEM) and low Lower Esophageal Sphincter (LES) pressure with endoscopic esophagitis in patients presenting with symptoms of GERD. Settings and designs: Prospective cross sectional study done in a tertiary medical care center in south India from October 2016 to March 2017.

Methods: This cross sectional study was carried out among patients presenting with symptoms of GERD (heart burn and regurgitation,) at least twice per week for past three months. Based on their endoscopy findings patients with GERD are grouped into two having erosive and nonerosive reflux disease. After general and systemic clinical examination, High resolution Manometry was performed, and data was analyzed using Trace 1.2.3a V software. Statistical methods: Data was analysed using SPSS version 19. Chi square test was used to analyze categorical variables and independent 't' test was used for continuous variables.

Results: A total of 66 patients with GERD symptoms were subjected to esophageal manometry. Out of these 66 patients 42 were males and 22 were females with their mean age 40.82±12.86 years. Among 66 patients presented with GERD symptoms 26.7% were found to have ineffective esophageal motility and only 11.7% had low LES pressure. Ineffective esophageal motility was also found to be more common among older age group individuals with their mean age being 44.64 ±14.154. Among the patients with erosive reflux disease (70.21%), 66.7% had low LES pressure and 75 % had IEM; the association was not statistically significant.

Conclusions: The incidence of Ineffective esophageal motility in patients with GERD is found to be higher than that of low LES pressure. There was no significant association observed between IEM and low LES pressure with endoscopic esophagitis in patients with GERD.

Keywords: Chicago classification, Hypotensive sphincter, High Resolution Manometry, Ineffective peristalsis

Introduction

Globally Gastro Esophageal Reflux Disease (GERD) remains a highly prevalent disease with considerable geographic variation; estimated prevalence rate was found to be 18.1-27.8% in US and 8.8-25.9% in Europe, and less than 10% in Asian countries.[1] GERD causes significant pain and discomfort which compromise

patients' quality of living. It is considered as major risk factor for the occurrence of Barrett's oesophagus and oesophageal adenocarcinoma.[2,3]

Among multiple causes which constitute the pathogenesis of GERD abnormal esophageal motility plays a crucial role and also associated with disease progression and severity.[4] Delayed clearance of the refluxate leads to prolonged mucosal exposure to refluxed gastro duodenal contents which in turn promotes esophagitis and its complications. Prevalence of ineffective esophageal peristalsis was found to be 40-50% among GERD patients.[5]

Moreover, pattern of esophageal motility was found to be different in various sub classes of GERD patients. Abnormal motility pattern and low LES pressure observed in GERD patients are more likely to be related to endoscopic esophagitis. Esophageal High-Resolution Manometry (HRM) an advanced technique which gives a picture of change in intraluminal pressure from upper esophageal sphincter to lower one as well as motor activity of the esophagus while swallowing.[6]

Further studies are warranted to elucidate the role of dysmotility in patients with reflux disease. Hence, we proposed this study to find out the association of ineffective esophageal motility and low basal Lower Esophageal Sphincter (LES) pressure with their endoscopic findings in patients with GERD using High Resolution Manometry, which is the gold standard investigation to diagnose and evaluate esophageal motility disorders using new Chicago classification.

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This prospective cross sectional study was conducted among 66 patients of both sexes after obtaining ethical clearance from institutional human ethical committee. Study was conducted during the October 2016-March 2017 for duration of six months. Study participants belonged to the age group ranging from 20-65 years. Patients presented with symptoms of GERD (heart burn or acid regurgitation) at least twice a week for more than 3 months were included in the study after obtaining their informed consent. Patients with achalasia, esophageal or fundic varices, previous gastric or esophageal surgery, esophageal cancer, collagen diseases or previous ingestion of corrosive agents, acute cardiovascular, respiratory, digestive tract or metabolic diseases were excluded from the study.

Patients were asked to discontinue drugs such as nitrates and calcium-channel blockers which affect esophageal motor function 48hours before the manometry. Patients on proton pump inhibitors also were to ask with hold the drug for three weeks. Based on their endoscopic findings patients were categorized into two groups with erosive reflux disease and non-erosive reflux disease. After general and systemic clinical examination manometry was performed 2hours after endoscopy. Manometry was performed with a 16-channel water perfused catheter which has 8 channels placed 1 cm apart at the lower end and the remaining 8 channels were placed 3cm apart (Manufactured by RMH, Victoria, Australia. The data were analyzed using Trace 1.2.3a V software (Geoff Hebbard, Royal Melbourne Hospital, Victoria, Australia).

Manometry procedure

The manometry catheter was introduced by transnasal route. Basal LES pressure was recorded for 1 minute, which is followed by ten 5 ml wet swallows at an interval of 30seconds. 10 wet swallow frames show upper and lower esophageal sphincter relaxation and contraction of esophageal body is represented as isobaric color contour plot. Spatial and temporal analysis of esophageal motor events was made. Tests swallows are characterized based on HRM data using recently evolved Chicago classification.[7] According to Chicago classification it is considered as ineffective esophageal motility, when >50% of swallows are ineffective, that is either failed (DCI* <100 mmHg.cm.sec) or weak (DCI 100-450 mmHg·cm·sec).[8]

*DCI- Distal Contractile Integral- measure of how robust peristalsis is in the smooth muscle of esophagus

Analysis

Data was entered in excel sheet and analyzed using SPSS software version 19. Data was expressed as mean (SD) values for quantitative variables and percentages for continuous variables. Chi square test was used to analyze categorical variables and independent 't' test was used for continuous variables.

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A total of 66 patients with GERD symptoms were subjected to endoscopy and esophageal manometry. Out of these 66 patients 42 were males and 22 were females with their mean age 40.82±12.86. Four of the patients had hiatus hernia. Their clinical presentations were heart burn (48.8%), acid regurgitation (30.2%) chest pain (16.3%) and vomiting and nausea (4.7%). Among 66 patients presented with GERD symptoms 26.7% were found to have ineffective esophageal motility and only 11.7% had low LES pressure. 6% of patients with GERD had both low LES pressure and ineffective esophageal motility. Among 18 patients with ineffective esophageal peristalsis 38.8% had failed peristalsis and 61.1% had weak peristalsis. Ineffective esophageal motility was also found to be more common among older age group individuals with their mean age being 44.64±14.154. On upper GI endoscopy 48 patients had erosive reflux disease and 18 patients had non-erosive reflux disease. In the sub group of patients with erosive reflux disease, 66.7% had low LES pressure and 75 % had IEM and the association was not statistically significant (Table 1).

[illegible]

[illegible]	[illegible]		[illegible]
	[illegible]	[illegible]	
Normal lower esophageal sphincter pressure (%)	16 (26.7)	44(73.3)	0.727
Low Lower esophageal sphincter pressure (%)	2(33.3)	4(66.7)	
Normal esophageal motility (%)	13(28.3)	33(71.7)	0.785
Ineffective esophageal motility (%)	5 (25)	15(75)	

[illegible]

Low basal pressure of LES along with low amplitude and ineffective peristaltic waves in the distal esophagus was found to be a more common finding in patients with GERD.[9] In our present study ineffective esophageal motility was found to be higher when compared to low basal LES pressure. Kruse-Anderson et al also had observed increased fewer and low amplitude propagative peristalsis among patients with esophagitis when compared to controls.[10]

Abnormal Esophago Gastric Junction (EGJ), Transient Lower Esophageal Sphincter Relaxations (TLESRs), hypotensive Lower Esophageal Sphincter (LES), anatomic distortion of EGJ and ineffective esophageal peristalsis are found to be the major contributing factors for reflux of gastric contents into the esophagus. We found that ineffective esophageal motility was found to be the more significant abnormality found among GERD patients than low LES pressure as esophageal peristalsis is an important anti reflux mechanism which governs the clearance of refluxed gastric contents. Savarino et al also stated that abnormally low LES pressure, IEM, and hiatus hernia were found to be more prevalent in GERD patients than patients with functional heart burn and healthy controls.[11] Several studies had reported that GERD and its complications are more commonly observed in scleroderma patients, with failed or absent peristalsis which proved the role of esophageal clearance in the development of GERD.[12,13]

It was reported in recent studies that there occurs impairment of esophageal function such as reflux or dysphagia was found to be more prevalent among older individuals which starts from 40 years onwards.[14] In our study we observed increased incidence of ineffective esophageal motility in older patients with GERD. Gutschow et al in his study conducted among patients with reflux symptoms with and without GERD reported that there was significant decrease in peristaltic function in older patients with GERD than in younger patients.[15]

In our study low, LES pressure was found to be reported higher among patients with erosive reflux disease than in patients with Non-erosive reflux disease. Frazzoni et al in a comparative study conducted among GERD patients had observed lower mean LES pressure in patients with erosive and non-erosive reflux disease compared to controls.[16] Both LES pressure and peristaltic abnormality was found to be affected in reflux esophagitis. Somani et al also in this study described that severity of endoscopic esophagitis is inversely related with amplitudes of contraction in the distal esophagus.[17] In our present study ineffective esophageal motility was found to be reported more among patients with erosive reflux disease when compared to non-erosive reflux disease. Daum et al found peristaltic dysfunction in 56% of Non-Erosive Reflux Disease (NERD) and 76% of Erosive Reflux Disease (ERD), they also had observed that esophageal motility disorder was reported higher in GERD patients using HRM than with conventional manometry.[18] In contrast Lemme et al reported that there was no difference observed in the prevalence of IEM among non-erosive (38%) and erosive (38%) GERD patients.[19] Simren et al, also suggested that IEM had little influence on esophageal clearance during upright acid reflux and only severe esophageal motility disturbances, were associated with prolonged esophageal clearance in those with supine reflux.[20]

[illegible]

Ineffective esophageal motility was found to be higher than low basal LES pressure in patients with GERD. There was no significant association observed between IEM and low LES pressure in patients with endoscopic esophagitis. Hypotensive sphincter and Ineffective esophageal motility may be the cause for GERD and may not always associated with esophagitis.

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1. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2014;63: 871-80.
2. Peters JH, Avisar N. The molecular pathogenesis of Barrett's esophagus: common signaling pathways in embryogenesis metaplasia and neoplasia. J Gastrointest Surg. 2010;14(Suppl 1):S81-7.
3. Theisen J, Peters JH, Fein M, Hughes M, Hagen JA, Demeester SR, Demeester TR, Laird PW. The

mutagenic potential of duodenoesophageal reflux. Ann Surg. 2005;241:63-8
4. Ang D, Blondeau K, Sifrim D, Tack J. The spectrum of motor function abnormalities in gastroesophageal reflux disease and Barrett's esophagus. Digestion. 2009;79:158-68.
5. Diener U, Patti MG, Molena D, Fisichella PM, Way LW. Esophageal dysmotility and gastroesophageal reflux disease. J Gastrointest Surg. 2001;5:260-5.
6. Spechler SJ, Castell DO. Classification of oesophageal motility abnormalities. Gut. 2001;49:145-51.
7. Fox MR, Bredenoord AJ. Oesophageal high-resolution manometry: moving from research into clinical practice. Gut. 2008;57:405-23.
8. Kahrilas PJ, Ghosh SK, Pandolfino JE. Esophageal motility disorders in terms of pressure topography: the Chicago Classification. J Clin Gastroenterol. 2008;42:627-35.
9. Csendes A, Maluenda F, Burdiles P, Bendriquez A, Quesada S,Cesendes P. Prospective study of esophageal motor abnormalities in patients with gastro esophageal disease reflux according to severity of endoscopic esophagitis. Hepatogastroenterol. 1996;43:394.
10. Kruse-Anderson S, Wallin L, Madsen T. Reflux patterns and related esophageal motor activity in gastro esophageal reflux disease. Gut. 1990;31: 633-8.
11. Savarino E, Gemignani L, Pohl D, Zentilin P, Dulbecco P, Assandri L, et al. Oesophageal motility and bolus transit abnormalities increase in parallel with the severity of gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2011;34:476-86.
12. Lemme EM, Abrahão-Junior LJ, Manhães Y, Shechter R, Carvalho BB, Alvariz A. Ineffective esophageal motility in gastroesophageal erosive reflux disease and in nonerosive reflux disease:are they different? J Clin Gastroenterol. 2005;39:224-7.
13. Simrén M, Silny J, Holloway R, Tack J, Janssens J, Sifrim D.Relevance of ineffective oesophageal motility during oesophageal acid clearance. Gut 2003;52:784-90.
14. Gregersen H, Pedersen J, Drewes AM. Deterioration of muscle function in the human esophagus with age. Dig Dis Sci. 2008;53(12):3065-70.
15. Gutschow CA, Leers JM, Schröder W, Prenzel KL, Fuchs H, Bollschweiler E, et al. Effect of aging on esophageal motility in patients with and without GERD. Ger Med Sci. 2011;9:22.
16. Frazzoni M, De Micheli E, Zentilin P, Savarino V. Pathophysiological characteristics of patients with non-erosive reflux disease differ from those of patients with functional heartburn. Aliment Pharmacol Ther. 2004;20:81-8.
17. Somani SK, Ghoshal UC, Saraswat VA, Aggarwal R, Misra A, Krishnani N, et al. Correlation of esophageal pH and motor abnormalities with endoscopic severity of reflux esophagitis. Dis Esophagus. 2004;17(1):58-62.
18. Daum C, Sweis R, Kaufman E, Fuellemann A, Anggiansah A, Fried M, et al. Failure to respond to physiologic challenge characterizes esophageal motility in erosive gastro-esophageal reflux disease. Neurogastroenterol Motil. 2011;23:517-200.
19. Savarino E, Bazzica M, Zentilin P, Pohl D, Parodi A, Cittadini G, et al. Gastroesophageal reflux and pulmonary fibrosis in scleroderma: a study using pH-impedance monitoring. Am J Respir Crit Care Med. 2009;179:408-13.
20. Gemignani L, Savarino V, Ghio M, Parodi A, Zentilin P, de Bortoli N, et al. Lactulose breath test to assess oro-cecal transit delay and estimate esophageal dysmotility in scleroderma patients. Semin Arthritis Rheum. 2013;42:522-9.

r Deepalakshmi K, Vasanthi R, Venkatakrishnan L, Karthikeyan S. Clinical and manometric profile of patients with GERD in a tertiary care hospital. Int J Adv Med 2017;4:1658-61.

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Prevalence of overweight and obesity among children aged 5–15 years in a rural school in Coimbatore

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Research Article

Prevalence of overweight and obesity among children aged 5–15 years in a rural school in Coimbatore

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Abstract

Background: Childhood obesity is a fast emerging problem in developing countries such as India because of lifestyle changes and economic transition.[1] Childhood overweight and obesity are major risk factors for many chronic diseases such as diabetes, hypertension, cardiovascular diseases, and cancer.[1–4] Effective preventive measures will prevent the risk of developing chronic diseases in adult life.

Objective: To study the prevalence of overweight and obesity among school children in a rural school in Coimbatore using the WHO standard reference for age 5–19 years.

Materials and Methods: A cross-sectional study was conducted along with annual school health check up for the children studying in a rural matriculation school located in Neelambur village, Coimbatore district. A total of 890 school children in the age group 5–15 years enrolled in the study were examined for height and weight, and BMI was calculated. The WHO standard reference for age 5–19 years was used to define overweight (>2SD) and obesity (>3SD).

Result: In our study, prevalence of overweight was 8.32% and prevalence of obesity was 4.72%. Among boys, prevalence of overweight and obesity was 8.43% and 6.43%, respectively. Among girls, prevalence of overweight and obesity was 8.20% and 2.96%, respectively. There is no statistical significant difference in the prevalence of overweight/obesity among boys and girls.

Conclusion: A high proportion of Rural School children in our study are having weight more than their normal weight, indicating the need for public awareness and preventive measures on childhood obesity. Intervention in the form of health education should be initiated at all levels of society to stop the progression of the problem.

KEYWORDS: Overweight, childhood obesity, body mass index, school children

Introduction

India is going through an economic and nutritional transition rapidly. In India under nutrition among children is common. Childhood obesity is also increasing in India due to increased purchasing power, decreasing physical activity, change in dietary habits, more comfortable and luxurious living.[1,2] Childhood obesity and overweight are associated with several risk factors for many chronic diseases such as diabetes mellitus, hypertension, cardiovascular diseases, and cancer.[3]

Estimating early childhood overweight and obesity during schooling helps in preventing disease progression into adulthood. Approximately 50%–80% obese children will continue as obese adults.[3] Studies on prevalence of obesity and overweight in Indian cities such as Delhi, Pune, and Chennai showed higher prevalence of obesity and overweight.[4–6] Different studies used different criteria to define childhood obesity and overweight such as International Obesity Task Force classification (IOTF), NCHS classification, Agarwal classification, and WHO classification.[7–10] So the

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prevalence of overweight and obesity varies with each standard classification. Only few studies have been conducted using the WHO growth references.[25] The WHO defines childhood (5–19 years) overweight as >+2SD (equivalent to BMI 25 kg/m2 at 19 years) and obesity as >+3SD (equivalent to BMI 30 kg/m2).[25] This study was conducted to find out the prevalence of obesity and overweight among school children aged 5–15 years in a rural school in Coimbatore using the WHO growth references.[25]

Materials and Methods

This cross–sectional study was conducted along with annual school health check up for the children studying in a matriculation school located in Neelambur village, Coimbatore district. The study protocol was approved by Ethics committee of PSG Institute of Medical Science and Research, Coimbatore. The study was carried out after obtaining prior consent from school authorities, parents, and assent from children. A total of 890 school children in the age group 5–15 years were enrolled in the study.

Their age was verified from school registration records and rounded off to completed years. All anthropometric measurements were taken by medical professionals. Weighing scale and stadiometer were calibrated and validated by Department of Biometrics prior to the study. Height and weight were measured based on the WHO standards with sensitivity of 0.5 cm and 0.1 kg. Zero error was set after every 10 measurements. The children were made to stand straight with heels, buttocks, and back touching the vertical limb of the instrument and stretching upward to the fullest extent with arms hanging on the side. The head was aligned so that the lower rim of the orbit and the auditory canal were in horizontal plane (Frankfurt plane). Weight was measured without any footwear and with minimal clothing (school uniform) using Krup's weighing scale.

BMI was calculated and cut-off values for children as proposed by the WHO growth reference were used for defining overweight and obesity. The WHO defines childhood (5–19 years) overweight as >+2SD (equivalent to BMI 25 kg/m^2 at 19 years) and obesity as >+3SD (equivalent to BMI 30 kg/m^2).[25]

Statistical Analysis

Data entry and analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. All the entries were double checked and verified randomly. Prevalence of obesity and overweight was shown in percentage. χ^2-Test was carried out to find association of obesity and overweight among boys and girls.

Result

In the study, 890 school children were examined of which 451 (50.7%) were boys and 439 (49.3%) were girls [Tables 1 and 2]. In our study, the overall prevalence of overweight/obesity was found to be 13.03%. There is no statistically significant association between prevalence of overweight/obesity among boys and girls within this study [Table 3].

Discussion

In our study the prevalence of overweight and obesity among children in a private rural school was 8.32% and 4.72%, respectively. Prevalence of obesity was greater in boys when compared to girls (6.43% vs. 2.96%). Prevalence of overweight was almost equal in both sexes. There is no statistically significant association between prevalence of overweight/obesity among boys and girls within this study.

The prevalence in our study was higher compared to a study carried out in rural school in Udupi, Karnataka among school children aged 6–15 years using the WHO percentile criterion which showed prevalence of overweight and obesity as 1.5% and 1.3%,[9] respectively and also a study in Puducherry conducted among urban and rural school children using 85th percentile as overweight and 95th percentile as obesity which showed a prevalence of overweight as 4.98% and obesity as 2.24%,[6] respectively. But the prevalence is lower in our study when compared to a study in Pune using International cut-off points which showed prevalence of overweight as 19.9% conducted among affluent school boys[11] and also a study carried out in urban school children in Delhi using International cut-off points which showed a prevalence of overweight as 11.1% and obesity as 14.2%, respectively. [10] A study carried out in Karnataka using the WHO percentile criterion showed a prevalence of obesity in boys with 2.8% and in girls with 2.6%.[9] A study in Punjab using 85th percentile as overweight and 95th percentile as obesity showed prevalence of obesity and overweight was higher in boys when compared to girls (12.4% vs. 9.9%, 15.7% vs. 12.9%).[13] Our study that was conducted among private rural school children in Coimbatore showed a higher prevalence of overweight and obesity similar to urban school children in other studies. This might be because of lifestyle transition and economic improvement in rural area.

Difference in prevalence of overweight/obesity with other studies might be due to different standards used for estimation in other studies. In this study, the prevalence of overweight and obesity was analyzed using standard WHO growth references[25] among 890 school children age group 6–15 years. Not determining the risk factors for higher prevalence of overweight/obesity was a limitation to this study.

Complications of obesity during adult life worsen if obesity starts in childhood. Early prevention of childhood obesity is easier. It is recommended that high fat and junk foods should be avoided by children. Sedentary lifestyle should be discouraged and increased physical activity should be encouraged. Health education should be given to parents, children, and teachers regarding dietary habits and sedentary lifestyle.

Table 1: Age-wise distribution of overweight and obesity among boys

Age	Total	Normal weight *N* (%)	Underweight *N* (%)	Overweight *N* (%)	Obesity *N* (%)
5	16	7(43.75%)	7(43.75%)	1(6.25%)	1 (6.25%)
6	47	28(59.57%)	18(38.30%)	0	1(2.13%)
7	48	18(37.50%)	25(52.09%)	4(8.33%)	1(2.08%)
8	41	20(48.78%)	17(41.46%)	1(2.44%)	3(7.32%)
9	79	36(45.57%)	36(45.57%)	2(2.53%)	5(6.33%)
10	62	29(46.78%)	17(27.42%)	8(12.90)	8(12.90%)
11	45	21(46.67%)	12(26.67%)	10(22.22%)	2(4.44%)
12	33	16(48.49%)	12(36.36%)	1(3.03%)	4(12.12%)
13	31	10(32.26%)	16(51.61%)	3(9.68%)	2(6.45%)
14	21	7(33.33%)	9(42.86%)	4(19.05%)	1(4.76%)
15	28	17(60.72%)	7(25.00%)	3(10.71%)	1(3.57%)
Total	451	209(46.34%)	176(39.02%)	37(8.21%)	29(6.43%)

Table 2: Age-wise distribution of overweight and obesity among girls

Age	Total	Normal weight *N* (%)	Underweight *N* (%)	Overweight *N* (%)	Obesity *N* (%)
5	20	11(55.00%)	8(40%)	1(5.00%)	0
6	45	25(55.56%)	14(31.11%)	2(4.44%)	4(8.89%)
7	52	19(36.54%)	26(50.00%)	6(11.54%)	1(1.92%)
8	39	23(58.97%)	11(28.21%)	5(12.82%)	0
9	49	28(57.14%)	16(32.66%)	3(6.12%)	2(4.08%)
10	55	33(60.00%)	16(29.09%)	4(7.27)	2(3.64%)
11	39	29(74.36%)	10(25.64%)	0	0
12	55	27(49.09%)	22(40%)	4(7.27%)	2(3.64%)
13	41	21(51.22%)	10(24.39%)	8(19.51%)	2(4.88%)
14	17	9(52.94%)	7(41.18%)	1(5.88%)	0
15	27	19(70.37%)	5(18.52%)	3(11.11%)	0
Total	439	244(55.58%)	145(33.03%)	37(8.43%)	13(2.96%)

Table 3: Prevalence of overweight and obesity by sex

	Overweight/obesity			χ2-value	*P*-value
	Present	Absent	Total		
Boys	66(14.63%)	385(85.37%)	451	2.07	0.150
Girls	50(11.39%)	389(88.61%)	439		

Conclusion

The results of the study shows that the prevalence of overweight and obesity among school children is becoming high in rural area which is an alarming signal and calls for an immediate action. Effective implementation of lifestyle modifications behaviors such as healthy diet intake, avoidance of high calorie foods, and promoting physical activity in early school life help in preventing future complications as a part of primordial preventive strategies.[1–4]

References

1. Sheety PS. Nutrition transition in India. Public Health Nutr 2002;5175–82.
2. Popkin BM. The nutrition transition in low-income countries: an emerging crisis. Nutr Rev 1994;52:285–98.
3. Chopra M, Galbraith S, Darnton-Hill I. A global response to a global problem: the epidemic of over nutrition. Bull World Health Organ 2002;80:952–8.

4. Bundred P, Kitchiner D, Buchan I. Prevalence of overweight and obese children between 1989 and 1998: population based series of cross sectional studies. Br Med J 2001;322:326–8.
5. Subramanyam V, Jayashree R, Rafi M. Prevalence of overweight and obesity in affluent adolescent girls in Chennai in 1981 and 1988. Indian Paediatr 2003;40:332–6.
6. Mahajan PB, Purty AJ, Singh Z, Cherian J, Natesan M, Arepally S, et al. Study of childhood obesity among school children aged 6 to 12 years in union territory of Puducherry. Indian J Community Med 2011;36:45–50.
7. Ramachandran A, Snehalatha C, Vinitha R, Thayyil M, Kumar CK, Sheeba L, et al. Prevalence of overweight in urban Indian adolescent school children. Diabetes Res Clin Pract 2002;57:185–90.
8. Guo SS, Chumlea WC. Tracking of body mass index in children in relation to overweight in adulthood. Am J Clin Nutr 1999;70:145S–18.
9. Kamath V, Pattanshetty S, Mithra P, Lina A. Prevalence of overweight and obesity among rural school children in Udupi taluk, Karnataka, South India. Australasian Med J 2010.
10. Kapil U, Singh P, Pathak P, Dwivedi SN. Prevalence of obesity amongst affluent adolescent school children. Indian Pediatr 2001;39:449–52.
11. Khadikar VV, Khadikar AA. Prevalence of obesity in affluent school boys in Pune. Indian Pediatr 2004;41:857–8.
12. Raj M, Kumar RK. Obesity in children and adolescents. Indian J Med Res 2010;132:598–607.
13. Chhatwal J, Verma M, Riar SK. Obesity among preadolescents of a developing country (India). Asia Pac J Clin Nutr 2004;13:231–5.
14. Moayeri H, Bidad K, Zadhoush S, Gholami N, Anari S. Increasing prevalence of iron deficiency in overweight and obese children and adolescents (Tehran Adolescent Obesity Study). Eur J Pediatr 2006 ;165:813–4.
15. Flodmark CE, Marcus C, Britton M. Interventions to prevent obesity in children and adolescents: a systematic literature review. Int J Obes (Lond) 2006;30:579–89.
16. Kelishadi R, Pour MH, Sarraf-Zadegan N, Sadry GH, Ansari R, Alikhassy H, et al. Obesity and associated modifiable environmental factors in Iranian adolescents: Isfahan Healthy Heart Program—Heart Health Promotion from Childhood. Pediatr Int 2003;45: 435–42.
17. Kapil U, Singh P, Pathak P, Dwivedi SN. Prevalence of obesity amongst affluent adolescent school children. Indian Pediatr 2001;39:449–52.
18. Anura VK, Sumathi S, Swarnarekha B. IAP National Task Force for Childhood Prevention of Adult Diseases: the effect of childhood physical activity on prevention of adult diseases. Indian Pediatr 2004;41:37–62.
19. Cole JC, Mary CB, Katherine MF, William HD. Establishing a standard definition for child overweight and obesity worldwide: international survey. Br Med J 2000;20:320.
20. WHO (World Health Organisation). *Obesity: Preventing and Managing the global Epidemic*. Report of WHO Consultation on Obesity. Geneva; WHO, 1998.
21. WHO (World Health Organisation), International Association for the Study of Obesity (IASO) and International Obesity Task Force (IOTF). *The Asia-Pacific Perspective: Redefining Obesity and its Treatment*. Geneva: World Health Organisation, 2000.
22. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999–2000. JAMA 2002;288:1728–32.
23. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025 prevalence, numerical estimates and projection. Diabetes Care 1998;21:1414–31.
24. Dietz WH. Health consequences of obesity in youth: childhood predictors of adult disease. Pediatrics 1998;101:518–25.
25. BMI. Available at: http://www.who.int/growthref/who2007_bmi_for_age/en/index.html (last accessed on November 14, 2015).

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RESEARCH ARTICLE

THE ROLE OF BODY MASS INDEX (BMI) AS AN INDEPENDENT RISK FACTOR AND PROGNOSTIC FACTOR IN BREAST CANCER – A HOSPITAL BASED EPIDEMIOLOGICAL STUDY

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ABSTRACT

Objectives: To study the role of Body Mass Index as an independent risk factor and prognostic factor in breast cancer.

Methods: A hospital based epidemiological study was conducted in a tertiary care hospital in India involving 50 patients with a diagnosis of breast cancer who visited the Medical Oncology outpatient department for a period of 3 months. 50 age matched controls who had no detectable breast cancers were selected as controls. Their body mass index was calculated. Data including stage of breast cancer, lymph node status and presence of metastasis, histological type, grade of the tumour and hormone receptor status. (ER, PR and Her 2 neu) were tabulated. Additional information regarding menstrual, menopausal status, co-morbidities like Diabetes mellitus, hypertension and epilepsy was also gathered.

Results: The mean age of patients was 53.8 years and that of controls was 49.08 years. 56 % of cases and 44% of controls were post menopausal. 20 patients and 10 controls were diabetic; 14 patients and 9 controls were hypertensive and 2 patients and 2 controls had hypothyroidism. The odds ratio which quantifies the risk of association of breast cancer and obesity is 0.339 in premenopausal women and 0.045 in post menopausal women. The number of triple negative breast cancers was more in I and II classes of body mass index. Obesity grade 2 and 3 were also associated with advanced disease, including larger tumor size, positive lymph nodes, regional and/or distant stage and deaths after breast cancer.

Conclusion: There is a statistically significant association between obesity and breast cancer in post menopausal women but the same is not applicable in pre menopausal women.

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INTRODUCTION

Obesity is increasingly becoming a major public health problem in developing countries. It is defined as an accumulation of adipose tissue that is of sufficient magnitude to impair health. Excess weight is best assessed by body mass index or BMI, which is a surrogate marker for underlying obesity. The normal BMI range is 18.5 to 25 kg/m^2 (Torre *et al*., 2015). According to World Health Organization (WHO) people can be divided into the following categories based on BMI. Approximately 7% of cancers in women are associated with obesity (van den Brandt *et al*., 2000). In women, a BMI greater than 25kg/m^2 correlated strongly with an increased incidence of carcinomas of esophagus, endometrium, gall bladder, breast and kidneys. A proposed hypothesis for such an association is explained by the presence of hyperinsulinemia and peripheral insulin resistance in obese individuals (Roberts *et al*., 2010). Insulin at high concentrations has multiple effects on cell growth.

i. Activation of phosphatidyl inositol 3 kinase, β catenin and RAS
ii. Hyperinsulinemia causes increase in insulin like growth factor 1, which is a mitogenic and anti apoptotic agent, which activates many of the cell growth pathways.
iii. Obesity increases the synthesis of estrogen from androgen precursors through an effect on adipose tissue aromatases.
iv. Insulin increases androgen synthesis in ovaries and enhances estrogen availability in obese persons by inhibiting production of sex hormone binding globulin in liver.

BMI	Classification
< 18.5	underweight
18.5–24.9	normal weight
25.0–29.9	overweight
30.0–34.9	class I obesity
35.0–39.9	class II obesity
≥ 40.0	class III obesity

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Obese women under the age of 40 have a decreased risk as a result of anovulatory cycles and lower progesterone levels. In contrast, post menopausal obese women have an increased risk, which is attributed to the synthesis of estrogen in fat deposits (Schmidt *et al*., 2015).

MATERIALS AND METHODS

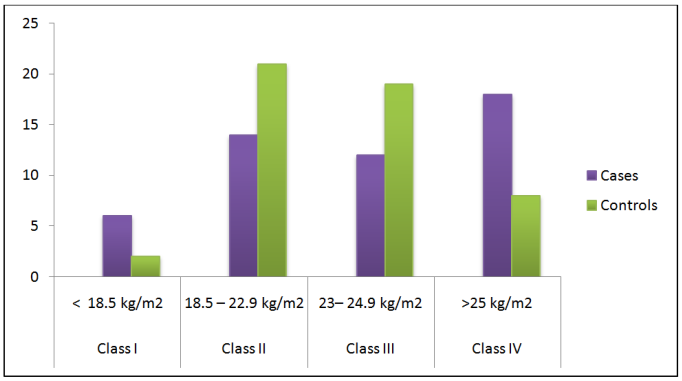
A case control study was carried out at our tertiary care hospital. This was a single institution study drawn from patients who attended the medical oncology outpatient department over a period of three months. The women accompanying the patients and a few voluntary women who had no detectable breast cancers were selected as controls. The study protocol was approved by the Institutional Human Ethics Committee. Informed consent was obtained from every patient and control and the patients were asked to enter the details provided in a questionnaire. Results of their heights and weights were entered. As a relative indicator of body weight, Body Mass Index was calculated as weight in kilograms divided by square of height in meters. The patients and controls were categorized into 4 classes based on BMI.

Class I – BMI < 18.5 kg/m^2
Class II – BMI 18.5 – 22.9 kg/m^2
Class III – BMI 23 – 24.9 kg/m^2
Class IV – BMI > 25 kg/m^2

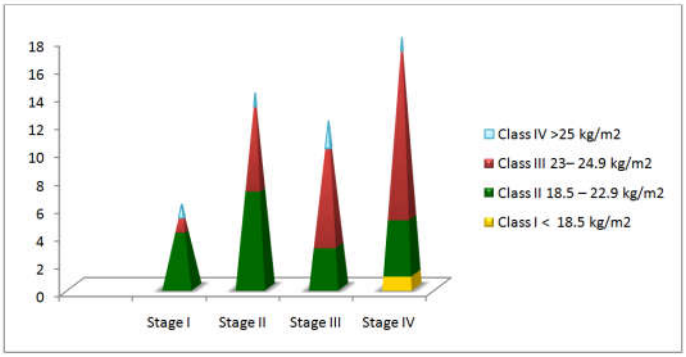
Data including stage of breast cancer, lymph node status and presence of metastasis was obtained from the case records of the patient. The histopathology reports were analyzed for recording the histological type, grade of the tumour and hormone receptor status (ER, PR and Her 2 neu).

Additional information regarding menstrual, menopausal status, co-morbidities like Diabetes mellitus, hypertension and epilepsy was also gathered. The results were tabulated and analyzed using SPSS (Statistical Package for the Social Sciences). The relationship between body mass index and risk of breast cancer was evaluated using odds ratio and the p value was calculated.

Bar diagram showing the distribution of cases and controls among different classes of body mass index



Bar diagram depicting the relation between stages of breast cancer and classes of body mass index



Body mass index in premenopausal women – Cross tabulation

Group	BMI ≤ 24.9 kg/m^2	BMI > 25.0 kg/m^2	P value	Confidence interval	Odds ratio
Cases	15 (68.2%)	7 (31.8%)			
Controls	23 (82.1%)	5 (17.9%)	0.23	0.131-0.877	.339

Table 1. Age distribution of cases and controls in various classes of BMI

Age Range	Class I < 18.5 kg/m^2		Class II 18.5 – 22.9 kg/m^2		Class III 23– 24.9 kg/m^2		Class IV >25 kg/m^2	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
21-30	0	0	0	1	0	1	0	0
31-40	1	0	1	6	2	5	2	3
41-50	1	1	4	6	1	2	5	2
51-60	2	1	5	4	4	6	9	1
61-70	1	0	0	3	5	4	2	3
71-80	1	0	3	0	0	0	0	0
>80	0	0	0	2	0	0	0	0

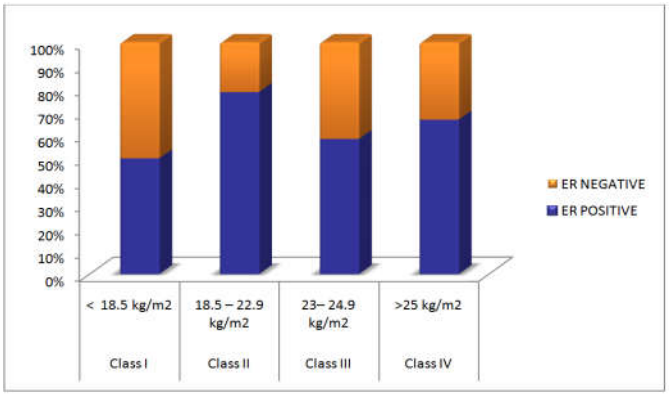
RESULTS AND OBSERVATION

50 women diagnosed with breast cancer during a period of 3 months were taken as cases and 50 women without a history of breast cancer were taken as controls. The mean age of patients was 53.8 years and that of controls was 49.08 years. 56 % of cases and 44% of controls were post menopausal. 20 patients and 10 controls were diabetic; 14 patients and 9 controls were hypertensive and 2 patients and 2 controls had hypothyroidism.

Body mass index in post menopausal women – Cross tabulation

Group	BMI ≤ 24.9 kg/m^2	BMI > 25.0 kg/m^2	P value	Confidence interval	Odds ratio
Cases	17 (60.7%)	11 (39.3%)			
Controls	19 (86.4%)	3 (13.6%)	0.045	0.058-1.024	.045

Histogram showing estrogen receptor expression in various classes of BMI



The odds ratio which quantifies the risk of association of breast cancer and obesity is .339 in premenopausal women and .045 in post menopausal women.

DISCUSSION

Overweight and obesity are current escalating public health issues. The prevalence of obesity in females has accelerated over the past decade in many developing countries. Obesity is an established risk factor for cancer and cancer related deaths, including that of breast cancers. Several studies show that a significantly stronger association is obvious between increased body mass index (BMI) and higher breast cancer incidence. Increased levels of estrogens due to excessive aromatization activity of the adipose tissue, over expression of pro-inflammatory cytokines, insulin resistance and hyper activation of insulin-like growth factors (IGFs) pathways, adipocyte-derived adipokines, hypercholesterolemia and excessive oxidative stress contribute to the development of breast cancer in obese women. Lopez *et al*. (2017) conducted a cross-sectional study of 849 women diagnosed with breast cancer in Columbian patients. Participant's body weight, height, and body mass index were collected at the time of diagnosis. Patients were classified following the Center for Disease control's (CDC) criteria: underweight (BMI $<$ 18.5 kg/m2), normal (18.5 $\leq$ BMI $<$ 25 kg/m2), overweight (25 $\leq$ BMI $<$ 30 kg/m2), and obese (BMI $\geq$ 30 kg/m2). Their data revealed that an association between BMI and menopausal status was not statistically significant in the study population. In the current study, the odds ratio of association was .339 with a p value of 0.23 in premenopausal women, which is not significant. However, the odds ratio in post menopausal women was .045 with a p value of .045 which is significant. Obese breast cancer patients are often associated with advanced stage disease, larger tumors, and axillary lymph node-positive status, partly due to delayed diagnoses and technical difficulties in palpation of the tumors. In a study conducted by Turkos *et al*. (2013), obese patients compared with normal-weight women were older at diagnosis and more often had high grade tumor with lymphovascular invasion. According to the molecular subtypes, overall survival and disease free survival were significantly shorter in obese patients with triple negative breast cancer. Obesity and lymphovascular invasion were found to be independent prognostic factors for TNBC mortality. In our study also we found that more number of obese breast cancer patients presented with stage III or IV disease, although the association was not statistically significant.

The study done by Sahin *et al*. (2017) BMI was an independent factor in patients with BC, with an association indicating a decreased incidence for luminal-like subtype and increased incidence for triple-negative subtype among premenopausal patients. However, this significance was not found in postmenopausal patients. Accordingly, a plausible etiological heterogeneity in BC might play a role among immunohistochemical subtypes in every life stage of women. Makama *et al*. (2017) published an association study of established breast cancer reproductive and lifestyle risk factors with tumour subtype defined by the prognostic 70-gene expression signature. Using prognostic gene expression profiles, they found that specific reproductive factors like higher parity was associated with low-risk (good prognosis) tumours. Also, longer duration of breastfeeding was associated with high-risk (poor prognosis) tumours. Neuhouser *et al*. (2015) performed a randomized control study and detected that A BMI of 25.0 or higher was strongly associated with risk for estrogen receptor-positive and progesterone receptor-positive breast cancers but was not associated with estrogen receptor-negative cancers. In our study we found that there was no significant difference in the distribution of estrogen receptor positive tumours and Her 2 neu positive tumours in obese and non obese patients. However, the number of triple negative breast cancers was more in I and II classes of body mass index. Obesity grade 2 and 3 were also associated with advanced disease, including larger tumor size, positive lymph nodes, regional and/or distant stage and deaths after breast cancer. An article by Schmitz *et al*. (2013) proposes that obesity and related co morbid conditions pose an increased risk for common adverse treatment effects, including breast cancer–related lymphedema, fatigue, poor health–related quality of life, and worse functional health. But we did not notice any significant difference in the post treatment morbidity among obese and non obese individuals.

Limitations of our study

The sample size of our study is limited to derive a statistically significant association of obesity and malignancy. The follow up was for a period of 6 months to one year and so the response of obese and non obese patients to the various treatment strategies could not be compared.

Conclusion

There is a valid association between obesity and breast cancer in post menopausal women but the same is not applicable in pre menopausal women. There is no significant difference in the size, histological type and immunohistochemical profile of breast cancers in obese and non obese patients, although the incidence of triple negative cancers was high in class I and class II BMI individuals. However obese patients presented with more advanced stages, possibly due to delay in detection.

REFERENCES

Cuello-López, J., Fidalgo-Zapata, A. and Vásquez-Trespalacios, E. 2017. Obesity and Prognostic Variables in Colombian Breast Cancer Patients: A Cross-Sectional Study. *International Journal of Breast Cancer,* 1-7.

Makama, M., Drukker, C., Rutgers, E., Slaets, L., Cardoso, F., Rookus, *et al*. 2017. An association study of established breast cancer reproductive and lifestyle risk factors with tumour subtype defined by the prognostic 70-gene

expression signature (MammaPrint®). *European Journal of Cancer, 75*, 5-13.

Neuhouser ML, Aragaki AK, Prentice RL, *et al*. 2015. Overweight, Obesity and Postmenopausal Invasive Breast Cancer Risk. *JAMA Oncology*, 1(5):611-621.

Roberts DL, Dive C, Renehan AG. 2010. Biological mechanisms linking obesity and cancer risk: new perspectives. *Annu Rev Med*., 61: pp. 301.

Sahin, S., Erdem, G. U., Karatas, F., Aytekin, A., Sever, A. R., Ozisik, Y. and Altundag, K. 2017. The association between body mass index and immunohistochemical subtypes in breast cancer. *The Breast, 32*, 227-236.

Schmidt S, Monk JM, Robinson LE, Mourtzakis M. 2015. The integrative role of leptin, oestrogen and the insulin family in obesity-associated breast cancer: potential effects of exercise. *Obesity Reviews*, 16(6):473-487.

Schmitz, K. H., Neuhouser, M. L., Agurs-Collins, T., Zanetti, K. A., Cadmus-Bertram, L., Dean, L. T. and Drake, B. F. 2013. Impact of Obesity on Cancer Survivorship and the Potential Relevance of Race and Ethnicity. *JNCI Journal of the National Cancer Institute*, 105(18), 1344–1354. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. 2015. Global cancer statistics, 2012. *CA Cancer J Clin*., 65: 87–108.

Turkoz F. P., Solak M., Petekkaya I. *et al*. 2013. The prognostic impact of obesity on molecular subtypes of breast cancer in premenopausal women. *Journal of BUON*, 18:335–341.

van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR *et al*. 2000. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. *Am J Epidemiol*., 152: 514–52

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Original Article

Peer Educators as Change Leaders – Effectiveness of Peer Education Process in Creating Awareness on Reproductive Health among Women Workers in Textile Industry

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Abstract

Context: India is one of the leading manufacturers of garments with roughly 80% of the workforce dominated by female migrant workers. Lack of education and access to resources in these women contribute to unsafe sexual behaviors, sometimes leading to unwanted pregnancies or sexually transmitted infections (STIs). Awareness raising, and peer education in particular, is extremely effective in addressing reproductive health issues. **Aims:** This study aims to study the effectiveness of peer health education in improving the awareness on reproductive health among women workers in textile industries. **Settings and Design:** A health educational interventional study for women in garment and spinning mills using peer education process carried out over a period of 18 months. **Subjects and Methods:** The process includes orientation to factory management, health needs' assessment, identifying health topics, peer educators' selection, peer educator trainings followed by outreach sessions, and impact measurement. The impact of peer education on reproductive health has been assessed among 597 participants from 9 factories. **Statistical Analysis Used:** Data were entered in SPSS version 24 and analyzed. Results were expressed as percentages. Chi square test was used where appropriate to find association between categorical variables. **Results:** The mean age of the workers in both the groups is 25.4 (±9.6) years and 25.1 (±8.3) years. In both the groups, most of the workers were single and more than half of them reside within the factory premises. Significant change in menstrual hygiene practices, knowledge in family planning methods, STIs, and cervical cancer has been observed following peer health education. **Conclusions:** A well-designed health educational interventional program using peer educators could be a successful approach to create awareness on sensitive topics.

Keywords: Peer health education, reproductive health, textile industry, women workers

Introduction

India has made a name for itself as a garment manufacturing center of global renown. The textiles and garments industry contributes 16.63% of India's export earnings. However, India's niche in the global garments market has been carved out at the cost of lakhs of workers in this industry's predominantly female and migrant labor force. Roughly 80% of garment workers are women between the ages of 21 and 25. Most are semi-skilled migrant workers and the sole earning members in their families.

These women workers often suffer from anemia, poor hygiene, inadequate pre- and post-natal care, sexual violence, and exposure to infections and illness. Lack of education and access to resources contribute to unsafe sexual behaviors, sometimes leading to unwanted pregnancies or sexually transmitted infections (STIs). They also contribute to high rates of malnutrition, maternal and infant mortality, and the spread of sexually transmitted and other preventable diseases such as HIV/AIDS and tuberculosis.[1]

Facility-based studies done in Chennai[2] and other parts of India[3] have shown that unwanted pregnancies among young

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unmarried women are high and emphasize the need for educating them about contraceptives. Data from subnational study conducted in 2006 have shown that around 80% of young women perceive the need for family life education. The same study has shown that peers are the major sources of information regarding contraception.[4]

Awareness raising, and peer education in particular, is extremely effective in addressing reproductive health issues. Women are grateful for such education focusing on personal and family health, with information they have seldom received before. An environment focused on education rather than behavior change empowers the women to make better health choices for themselves.

Peer education typically involves training and supporting members of a given group to affect change among members of the same group. The peer education model initiated by business for social responsibility for improving health of women at factories has proven to be an effective model that encourages women to share their knowledge with other family and community members extending the benefits well beyond the factory floor.[5,6] A study conducted among women workers in garment industries has shown that peer education approach is effective in raising the awareness on STIs. The study also concludes that it is likely that the health education program may help to reduce the burden of healthcare costs to the community in the long run.[7]

This study looks into the effectiveness of peer health education in improving the awareness on reproductive health among women workers in textile industries.

Subjects and Methods

This study is part of an ongoing health education program done by the Department of Community Medicine for women workers in garment and spinning mills located within 100 kilometer radius from our institution. Pooled baseline and endline assessment data of 597 workers from nine industries which include six spinning mills and three garment factories have been analyzed for the study purpose. The study has been approved by the institution's human ethics committee.

Data were entered and analyzed using statistical package for social sciences (SPSS) version 19 (IBM SPSS statistics Company, IBM corporation). Results are presented as percentages. Chi-square test is done for statistical association wherever appropriate.

Salient steps of the health education program

As part of the program, baseline assessment is conducted by survey method using a prevalidated questionnaire among 10% of women workers chosen by simple random sampling. Health education was then conducted by peer education process. Peer educators were selected by the factory representatives(supervisory level) using the following guidelines: workers who have ability to read and write in local language, working in the factory for at least one year and having exhibited leadership skills. As a measure to ensure sustainability, nurse, female human resource (HR) representatives, and volunteers from staff level were also included. Peer educator strength in each factory ranged from 10 to 20 proportionate to the total strength of the women workforce.

Peer education process

Health education in reproductive health issues focused on menstrual hygiene and reproductive tract infections, STIs including HIV and hepatitis B, family planning and cancers. Health education sessions for the peer educators were conducted once in 2 months. Each session was conducted for over 2 h by interactive lecture aided by flip charts and videos supplemented with demonstrations where appropriate. Participatory methods such as body mapping exercise were used to teach anatomy of reproductive system. Energizers were conducted to break monotony and avoid boredom.

Following the session, peer educators conduct outreach sessions during weekends, lunch breaks, etc., for their coworkers in small groups ranging between 10 and 15. These sessions were facilitated by the HR department in coordination with the nurse. Informal discussions also took place in leisure times in the dormitories. A refresher session was conducted after a month following the main session to reemphasize the important points and clarify any doubts the peer educators might have. An endline assessment at the end of the program was done among 10% of randomly chosen workers.

Results

The pooled data of 597 female workers interviewed during baseline (316) and endline (281) assessment were included for analysis. The mean age of the workers in both the groups is 25.4 (±9.6) years and 25.1 (±8.3) years.

Table 1 shows the distribution of the female workers based on certain sociodemographic variables.

Table 1: Distribution of workers based on certain sociodemographic variables

Sociodemographic variables	Baseline (%) (n=316)	Endline (%) (n=281)
Mean age (years)	25.4±9.6	25.1±8.3
Marital status		
Single	69.3	71.8
Married	29.8	26.4
Divorced/widowed/separated	0.9	1.8
Educational status		
None	7.7	6.8
Primary	27.5	30.0
Secondary	53.8	52.5
Postsecondary	11.0	10.7
Residence		
Within the factory premises	52.2	67.3
Home outside factory	47.8	32.7

In both the groups, most of the workers were single and more than half of them reside within the factory premises.

Table 2 shows the comparison of practices during menstruation before and after intervention. A statistically significant ($P < 0.01$) improvement in frequency of changing pads has been observed before and after intervention. The results also reveal a significant ($P < 0.01$) decrease in menstrual problem-related absenteeism.

A significant increase in knowledge on awareness about family planning and its methods has been observed following the intervention [Table 3].

Knowledge regarding both HIV and hepatitis B has improved significantly among the workers, but very few women (17.1%) had awareness about hepatitis B before intervention compared to HIV where the preintervention knowledge was 74.4% [Table 4].

During baseline assessment, it was observed that only 40% women have heard of cervical cancer, and among them, only 24.6% were aware about the symptoms [Table 5]. Following health education, significant increase in knowledge has been observed.

Discussion

The mean age of the female workers in the textile industry is around 25 (±9 years). Similar age profile was observed in studies conducted among women workers in textile industries in Bangladesh and China.[8,9] Around 30% of the workers in our factories have no or limited literacy. This finding is similar to studies done in other parts of India,[10] but low literacy rate in Chinese workers is only around 2%.[9] More than half of the workers in our study population reside the dormitories within the factories. This is in contrast to situation in others places[8] where most workers come from their residence. Women of younger age together with limited literacy and being away from home warrant need for creating awareness and providing enabling environment for reproductive health issues in industries.

Peer education process is a successful method in bringing about awareness, especially in sensitive health issues. Peer educators could be powerful change agents as evidenced by interventional studies in HIV/AIDS using peer educators.[11,12] Our study has also proven that peer education process has led to significant change in knowledge and practice in reproductive health issues. A significant improvement in menstrual hygiene practices (59.7% vs. 84.2%, $P < 0.05$) and decrease in absenteeism (36.6% vs. 27.2%, $P < 0.05$) during menstruation has been observed. There is slight increase in the proportion of workers using sanitary pads, but this difference is not statistically significant. This could be due to a relatively higher proportion of workers already using sanitary pads.

Although 75% of women have heard about family planning methods, the baseline assessment findings showed that only around a quarter of them could mention few contraceptive methods. Peer educators were able to bring a significant improvement in knowledge on the different contraceptive methods (8.2% vs. 55.6%, $P < 0.05$). This is important considering the age profile of the workers and most of them being away from home. Our study has shown that peer education has been effective in bringing about a significant awareness about various contraceptive methods.

Educational intervention on STI focusing on HIV and hepatitis B has brought a significant increase in awareness, especially in hepatitis B (17% vs. 89%, $P < 0.05$). Many of the workplace based health educational interventions focus mainly on HIV[13] even though the awareness on hepatitis B is very low[14] conducted among women in reproductive age. Our study also showed that the preintervention knowledge was only 17%.

Despite being the leading cause of all cancers among women in India, the awareness of cervical cancer and its symptoms was

Table 2: Comparison of certain practices during menstruation before and after intervention

Parameters	Baseline ($n=303$) (%)	Endline ($n=279$) (%)	*P*
Frequency of changing pads more than two times a day	181 (59.7)	235 (84.2)	0.000
Absenteeism during menstruation	111 (36.6)	78 (27.9)	0.026
Use of sanitary pads during menstruation	277 (91.4)	261 (93.5)	0.748

P<0.05 is considered statistically significant

Table 3: Knowledge regarding family planning and its methods

Parameters	Baseline ($n=316$) (%)	Endline ($n=281$) (%)	*P*
Heard of family planning	231 (74.3)	257 (94.5)	0.000
Can mention at least two methods of family planning	26 (8.2)	176 (55.6)	0.000

P<0.05 is considered statistically significant

Table 4: Knowledge regarding HIV and hepatitis B

Parameters	Baseline ($n=316$) (%)	Endline ($n=281$) (%)	*P*
Heard of HIV	235 (74.4)	260 (92.5)	0.000
Heard of Hepatitis B	54 (17.1)	250 (89.0)	0.000

P<0.05 is considered statistically significant

Table 5: Knowledge regarding cervical cancer and its symptoms

	Baseline (%)	Endline (%)	*P*
Heard of cervical cancer	(n=316) 126 (39.9)	(n=281) 247 (87.9%)	0.000
Heard of cervical cancer symptoms	(n=126) 31 (24.6)	(n=247) 193 (78.1)	0.000

P<0.05 is considered statistically significant

less among our study population. Community-based studies in other parts of the country have shown even higher level of lack of awareness.[15] Our study showed a significant improvement in knowledge of cervical cancer (39.9% vs. 87.9%, $P < 0.05$), and its symptoms were brought about following health education (24.6% vs. 78.1%, $P < 0.05$).

Conclusions

A significant increase in knowledge and practice has been brought about in menstrual hygiene, family planning methods, STIs, and cervical cancer. Our study has shown evidence that peer education approach is effective in bringing about significant change in knowledge in reproductive health issues. A well-designed health educational interventional program using peer educators could be a successful approach to create awareness on sensitive topics.

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Conflicts of interest

There are no conflicts of interest.

References

1. Singh S. India Cheap Labor Garment Export Industry. Fashionable and Famous – At the Garment Worker's Cost. Global Research; 2009. Available from: https://www.globalresearch.ca/india-cheap-labor-garment-export-industry/13530. [Last accessed on 2018 Feb 23].
2. Kalyanwala S, Zavier AJ, Jejeebhoy S, Kumar R. Abortion experiences of unmarried young women in India: Evidence from a facility-based study in Bihar and Jharkhand. Int Perspect Sex Reprod Health 2010;36:62-71.
3. International Institute of Population Sciences and the Population Council. Youth in India: Situation and Needs 2006–2007. Mumbai: International Institute of Population Sciences. Available from: http://www.popcouncil.org/uploads/pdfs/2010PGY_YouthInIndiaReport.pdf. [Last accessed on 2018 Feb 23].
4. Vaidya D, Hussainy D. AIDS Awareness programme in industry. Indian J Occup Environ Med 2004;8:11-5.
5. Investing in Women for a Better World. BSR Report. Investing in Women for a Better World; 2010. HERproject. Available from: https://herproject.org/downloads/BSR_HERproject_Investing_In_Women.pdf. [Last accessed on 2018 Feb 23].
6. Hossain MI, Al Mahmud A, Bajracharya A, Rob U, Reichenbach L. Evaluation of the Effectiveness of the HERhealth Model for Improving Sexual and Reproductive Health and Rights Knowledge and Access of Female Garment Factory Workers in Bangladesh. The Evidence Based project. Population council. 2017. Available from: http://evidenceproject.popcouncil.org/. [last accessed on 2018 Feb 03].
7. Rianon N, Selwyn B, Shahidullah SM, Swint JM, Franzini L, Rasu R. Cost of health education to increase STD awareness in female garment workers in Bangladesh. Int Electron J Health Educ 2009;12:135-49.
8. Barkat A, Majid M, Ara R, Mahiyuddin G, Rahman M, Poddar A, *et al*. Study on Change in Attitudes and Behavior of Garment Owners, Managers and Workers Towards Gender and Reproductive Health Issues. Prepared for UNFPA Bangladesh: December, 2008. Accessed from: http://www.hdrcbd.com/admin_panel/images/notice/1386584302.07.%20change%20in%20attitude%20and%20behaviour%20of%20garment%20owners,%20managers%20and%20workers%20towards%20gender%20and%20rh%20issues.pdf. [Last accessed on 2018 Feb 23].
9. Lu C, Xu L, Wu J, Wang Z, Decat P, Zhang WH, *et al*. Sexual and reproductive health status and related knowledge among female migrant workers in Guangzhou, China: A cross-sectional survey. Eur J Obstet Gynecol Reprod Biol 2012;160:60-5.
10. Saha TK, Dasgupta A, Butt A, Chattopadhyay O. Health status of workers engaged in the small-scale garment industry: How healthy are they? Indian J Community Med 2010;35:179-82.
11. Cornish F, Campbell C. The social conditions for successful peer education: A comparison of two HIV prevention programs run by sex workers in India and South Africa. Am J Community Psychol 2009;44:123-35.
12. Van Rompay KK, Madhivanan P, Rafiq M, Krupp K, Chakrapani V, Selvam D. Empowering the people: Development of an HIV peer education model for low literacy rural communities in India. Hum Resour Health 2008;6:6.
13. Prabhu TR. Legal abortions in the unmarried women: Social issues revisited. J Obstet Gynaecol India 2014;64:184-8.
14. Jha S, Devaliya D, Bergson S, Desai S. Hepatitis B knowledge among women of childbearing age in three slums in Mumbai: A cross-sectional survey. Hepatol Med Policy 2016;1:5.
15. Harsha Kumar H, Tanya S. A study on knowledge and screening for cervical cancer among women in Mangalore city. Ann Med Health Sci Res 2014;4:751-6.

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Original Research Article

Co-relation of endometrial thickness by transvaginal sonogram with histopathology pattern in abnormal uterine bleeding: a study from South India

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ABSTRACT

Background: Under normal circumstances, a woman's uterus sheds a limited amount of blood during each menstrual period (around 80 ml). Bleeding that occurs erratically or excessive menstrual bleeding is called abnormal uterine bleeding (AUB). The causes of AUB are many and varied. Initial investigations include transvaginal ultrasound and histopathologic assessment of the endometrium. Objective of this study was to evaluation of endometrial thickness with trans-vaginal ultrasound and its correlation with histopathology by dilatation and curettage in abnormal uterine bleeding. To determine the efficacy of transvaginal ultrasound in evaluating the endometrial thickness. To correlate the endometrial thickness by transvaginal ultrasound with endometrial histopathology in women with AUB.
Methods: It is a retrospective observational study. All reproductive and perimenopausal age group women who underwent dilatation and curettage for abnormal uterine bleeding during the period June 2014-June 2016 was taken and analyzed and correlated with their endometrial thickness measured with Transvaginal ultrasound.
Results: Around 478 patients who underwent endometrial sampling over a period of two years were analyzed. Maximum number of patients were in the fourth decade and the overweight category 36.6%. Proliferative endometrium was the most common histopathologic picture (44.76%). Detection of precancerous lesions were-5.87% and endometrial cancer was 1.05%.
Conclusions: An ET of 8 mm and above gave 100% sensitivity and negative predictive value for precancerous and cancerous lesions.

Keywords: Abnormal uterine bleeding, Dilatation and curettage, Endometrial hyperplasia and cancer, Endometrial thickness, Transvaginal ultrasound

NTRODUCTION

Abnormal uterine bleeding is one of the most common gynecological problems in patients approaching the outpatient clinics in the country. These complaints definitely affect the quality of life of the patient. The etiology of AUB varies with the age group of the patient. There is the PALM COEIN classification for AUB by FIGO.[1] There are many diagnostic modalities for evaluating endometrial pathologies in women who have AUB. Transvaginal sonogram (TVS) has permitted the use of higher frequency ultrasound waves at greater proximity of the uterus. It is a relatively cheap and non-invasive diagnostic modality for studying the endometrial pattern and its thickness and at the same time to exclude organic pathology in case of AUB while dilatation and curettage gives us a definitive histopathologic picture. This study attempts to correlate the USG findings and histopathology in patients with AUB. The gold standard

for diagnosing AUB is hysteroscopy and directed biopsy but it is expensive when compared to the simple endometrial sampling. According to the PALM COIEN classification the causes of AUB can be divided into structural and non-structural cause which is given in detail in Table 1.

Table 1: PALM COEIN classification for AUB.[1]

Structural	Non structural
Polyps	Coagulopathy
Adenomyosis	Ovulatory dysfunction
Leiomyomas	Endometrial hyperplasia
Malignancy	Iatrogenic
	Not yet specified

Aims and objectives of this study were

- Evaluation of endometrial thickness with trans-vaginal ultrasound and its correlation with histopathology by dilatation and curettage in abnormal uterine bleeding.
- To determine the efficacy of Transvaginal ultrasound in evaluating the endometrial thickness.
- To correlate the endometrial thickness by Transvaginal ultrasound with endometrial histopathology in women with AUB.

METHODS

Retrospective observational study included all reproductive and perimenopausal age group women who underwent dilatation and curettage for abnormal uterine bleeding during the period June 2014 to June 2016.

Inclusion criteria

- AUB in all reproductive and perimenopausal women
- Only gynecological indications for dilatation and curettage.

Exclusion criteria

- Unmarried women
- Pregnancy and related causes of bleeding
- Postmenopausal bleeding
- Puberty menorrhagia.

From minor and major OT registers, list of all D and Cs which were done during 2014 June to 2016 June was taken. Cases which satisfied the inclusion and exclusion criteria was taken and analyzed

Statistical analysis

Chi square test was used for the statistical analysis.

RESULTS

All the patients with abnormal uterine bleeding, fitting the inclusion criteria, who underwent endometrial sampling was taken for analysis. There were around 478 patients. All of them had a premenstrual ultrasound before the procedure. The age distribution, parity, BMI, presenting complaints, the histopathology and the endometrial thickness by TVS was evaluated. The results are given below:

Of the 478 patients, the youngest patient was 22 years old and the oldest was 55 years, the mean age was 38.5 years. Maximum number of patients were in the 41-50 years age group which was 60.25% and the least in the 20-30 years group and was 4.6% (Table 2).

Table 2: Age distribution of patients with AUB.

Age	No. of cases	%
20-30	22	4.60%
31-40	117	24.48%
41-50	288	60.25%
51-60	51	10.67%
Total	478	100%

Majority of the patients were multiparious i.e. 79.71% and 2.93% was nulliparous as seen in (Table 3).

Table 3: Parity of the patients with AUB.

Parity	No. of cases	%
Nullipara	14	2.93%
Primipara	83	17.36%
Multipara	381	79.71%
Total	478	100%

A total 35.8% of the patients under analysis were of the normal BMI and maximum numbers of patients were overweight-36.6% and the least number of patients were underweighted i.e. 3.3% as given in Table 4.

Table 4: BMI of patients with AUB.

BMI	No. of cases	%
Underweight	16	3.3%
Normal	171	35.8%
Overweight	175	36.6%
Obese	116	24.3%
Total	478	100%

A total 98.3% had heavy menstrual bleeding among the 478 patients and 72.4% had undergone tubal ligation. Among the other USG findings, adenomyotic changes were found in 11.1%, fibroids seen in 18.6%, while simple ovarian cysts were found in 11.7% and PCO in 4.2%.

There were 42 cases of hormone induced changes, 25 menstrul endometium, 17 were inadequate and 2 were TB endometritis which were all entered under others.44.7%

were having proliferative phases (Figure 1), of which 29.7% was disordered proliferative (Figure 2). 30.33% showed secretory changes (Figure 3) endometrial adenocarcinoma (Figure 4) was found in 1.05% of the cases and hyperplasia was seen in 5.86% (Table 5).

Table 5: Histopathology findings in study population.

HPR	No. of cases	Percentage	
Disordered proliferative	142	29.7%	44.76%
Proliferative	72	15.06%	
Secretory	145	30.33%	
Endometrial hyperplasia	28	5.86%	
Endometrial carcinoma	5	1.05%	
Others	86	17.99%	
Total	478	100%	

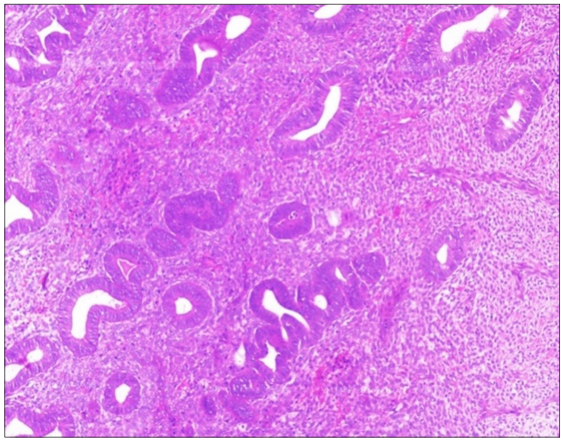


Figure 1: Proliferative endometrium.

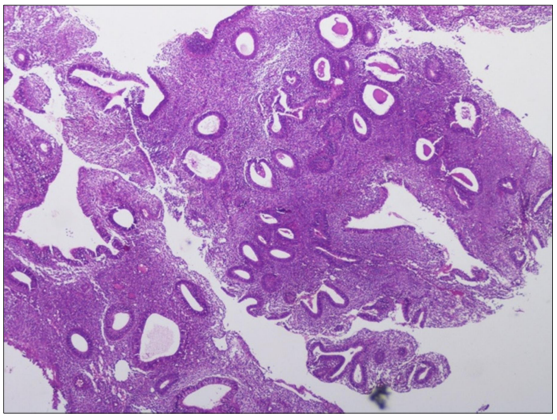


Figure 2: Disordered proliferative.

Among the hyperplasia, 50% was simple hyperplasia without atypia. Atypical hyperplasia (Figure 5) was found in 12 cases. Complex hyperplasia was found in 13 out of the 28 cases of hyperplasia of which complex atypical was 11 cases (Table 6).

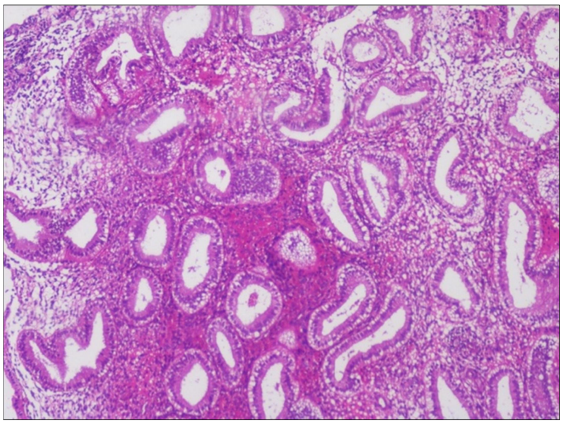


Figure 3: Secretory endometrium.

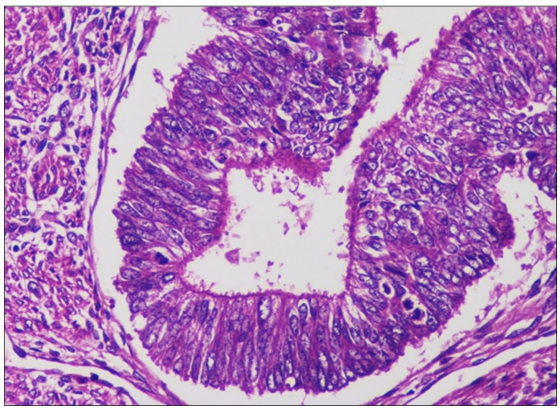


Figure 4: Adenocarcinoma.

Table 6: Cases of endometrial hyperplasia.

Type of hyperplasia	Number	%
Simple hyperplasia without atypia	14	50%
Simple hyperplasia with atypia	1	3.57%
Complex hyperplasia without atypia	2	7.14%
Complex hyperplasia with atypia	11	39.29%
Total	28	100%

Endometrial thickness was 8mm and above up to 15 mm in majority of cases of AUB-54.81%. ET less than 8mm was found only in 19.3% of the study population.ET 15 mm and above was also less which was 25.5% (Table 7). ET less than 4mm in AUB was very less-only 1.67%. One ET was not measured due to diffuse adenomyosis. Out of the 477 patients whose endometrial thickness were measured, all the five cases of endometrial carcinoma was found with an ET ≥15 mm among the 28 cases of endometrial hyperplasia 12 cases of hyperplasia were also found with ET ≥15 mm and the rest 16 cases had ET ≥8-15 (Table 8). Surprisingly all the five cases of carcinoma was endometrioid adenocarcinoma.

It was found in this study that proliferative and secretory endometrium was maximum when the endometrial

thickness was ≥8-15 mm and least with an ET less than 4 mm (Table 9).

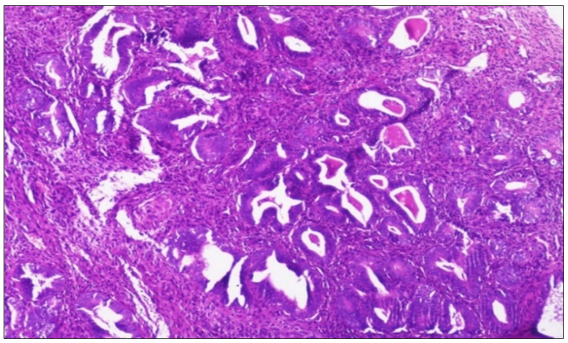


Figure 5: Atypical hyperplasia.

Table 7: Endometrial thicknesses by transvaginal ultrasound.

ET IN mm	Number	%
<4	8	1.67%
4-8	85	17.78%
≥8-15	262	54.81%
≥15	122	25.52%
Not measured	1	0.22%
Total	478	100%

If we take 8 mm as the cut off, we can see that in this study population all the cases of hyperplasia and malignancy had an ET ≥ 8 mm. Out of the 384 cases with ET ≥ 8 mm 28 cases had hyperplasia and five patients had endometrial carcinoma i.e. 8.59% (Table 10).

Table 8: Co-relation of et with endometrial hyperplasia and carcinoma.

ET in mm	Number	Endometrial hyperplasia	%	Endometrioid carcinoma	%
≥15	122	12	9.84%	5	4.46%
≥8-15	262	16	6.11%	0	0%
4-8	85	0	0%	0	0%
<4	8	0	0%	0	0%
Total	477	28	5.87%	5	4.46%

Table 9: Co-relation of et by TVS and histopathology.

ET	Number	Proliferative	Secretory	EH	Carcinoma	Others
<4 mm	8	3	3	0	0	2
4 - <8	85	34	29	0	0	22
≥8-15	262	121	86	16	0	39
≥15	122	56	27	12	5	22
Total	477	214	145	28	5	85

Table 10: Co-relation of endometrial thickness with hyperplasia and malignancy.

ET in mm	Number	Hyperplasia and carcinoma	%
≥8	384	33	8.59%
<8	94	0	0%

Table 11: Co-relation of endometrial thickness with HPR.

ET in mm	Number	Hyperplasia and carcinoma	Not having carcinoma
≥8	384	33 (a)	351 (b)
<8	94	0 (c)	94 (d)

Sensitivity, specificity, the positive and negative predictive value for an ET of ≥8 mm was calculated. 94 patients with ET less than 8 and 351 patients with ET ≥8 mm did not have carcinoma (Table 11).

- Sensitivity = a/a + c =33/33 = 100%
- Specificity= d/b + d = 94/445 = 21.12%
- Positive predictive value = a/a + b = 33/384 = 8.59%
- Negative predictive value = d/c + d = 94/94 = 100%

DISCUSSION

Abnormal uterine bleeding is a very common diagnosis in the op population. According to this study the maximum age groups of the patients were in the perimenopausal age of 41-50 years-around 60.25%. This is similar to the study by Shobitha et al -68.1% in the same age group while it was 38% in a study by Mahapatra M et al where the study group was less.[2,3]

The youngest patient was a 22-year-old primary infertility patient with heavy menstrual bleeding for which diagnostic curettage was done and had an ET of 6 mm and secretory endometrium.

In this study, 79.7% were multiparious while 2.93% were nulli, in almost all the studies this is similar. This is in accordance to the study by Nidhi et al, where 88% were multipara and 1.2% were nulliparous.[4] In a study by Betha K et al 81% were multipara.[5]

BMI was in the overweight category in 36.6% and obese in 24.3% in this study which is similar to the study in Iran by Kazamijalezah et al, where it was 33% and also with the study by Sahai A et al, where 56.2% were in overweight category and obese was 25%.[6,7]

There was heavy menstrual bleeding in 98% of patients.

Of these 478 patients only 39 had diabetes as risk factor and out of 39 one had endometrial carcinoma.

When we analyze the histopathologic findings of the 478 cases 44.5% was proliferative endometrium, of this disordered proliferative endometrium seems to be the commonest, in a study by Elavarasan et al, where disordered proliferative endometrium seems to be the commonest pattern observed i.e. 31.1%.[8] The term disordered proliferative endometrium refers to proliferative phase that does not seems to be appropriate for any one time in the menstrual cycle and mimics simple hyperplasia, but the process is focal rather than diffuse. Histologically there shows mild disorganization of architecture, with focal dilated glands, with normal gland to stromal ratio. In this study 30% was secretory endometrium; endometrial hyperplasia was found in 5.8% and endometrial cancer in 1%.

Transvaginal ultrasound was done to assess the endometrial thickness and it was done in the premenstrual phase, of the 478 cases 54.8% had an ET of ≥8-15, 25.5% had ET more than 15 mm. In a study by Jikki et al, 57% had et ≥8 mm which is similar to this study of 54%.[9]

In the study by Jikki et al, the endometrial hyperplasia was seen in 96.4% cases when ET was more than 8 mm and in this study 100% of the patients with endometrial hyperplasia had ET ≥8 mm.[9] In this study 100% cases of endometrial carcinoma occurred with ET ≥15 and all cases of hyperplasia had ET ≥8 mm.

Simple hyperplasia without atypia was 50% in this study which was comparable to 62% in a study by Tabata et al.[10]

Complex endometrial hyperplasia was 14.8% in a study conducted by Idris et al, while their atypical hyperplasia was only 2% whereas atypical hyperplasia was 42.4% in this study.[11] This may be because of the fact that most of the patients in the study group were in the fourth decade (60%) and in their study was in the third decade (80%).

Of the 384 cases with ET ≥8 mm, 33 had endometrial hyperplasia and carcinoma, and 351 did not have e/o hyperplasia and carcinoma, none of the cases below 8mm had any e/o hyperplasia or carcinoma. Sensitivity and negative predictive value was 100% for an ET of ≥8 mm. This is similar to studies by Chatpavit Getpook an ET of 8 mm showed optimal sensitivity and specificity -83.9% and 58.8% respectively and 90.4% negative predictive value for an abnormal endometrium.[12] They concluded that an ET of 8mm or less is less likely to be associated with malignant pathologies. In this study the negative predictive value was 100% and sensitivity was 100% but the specificity was only 21.1%. According to a study by Ozdemir S et al, there was negative predictive value of 95.6%, sensitivity and specificity of 83.6 and 56.4% respectively for an ET of 8 mm.[13]

Kurman et al, classified hyperplasia in to simple and complex and subcategory as atypical.[14] The 2014 revised WHO classification simply separates endometrial hyperplasia into 2 groups-hyperplasia without atypia and atypical hyperplasia.[15] According to the EIN classification all atypical endometrial hyperplasia is classified as endometrial intraepithelial neoplasia.[15] Progesterone was found to cause regression in 90% cases with hyperplasia.

The RCOG cutoff of ET is less than 7 mm below which there is no endometrial hyperplasia in a prospective study of 56 women with pcos.[11,16] In studies by Gianella et al, 11 mm was taken as the cut off.

The most common presenting symptoms of endometrial hyperplasia is abnormal uterine bleeding. According to studies, the risk of progression of simple hyperplasia to carcinoma is 1% and complex hyperplasia with atypia is 29%.[18]

CONCLUSION

The clinical importance of endometrial hyperplasia is because of its progression to endometrial carcinoma. An ET of 8 mm in this study gives a 100% negative predictive value for hyperplasia and carcinoma and can be taken as a cut off for deciding the patients for dilatation and curettage especially in low risk patients.

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REFERENCES

1. Munro MG, Critchley HO, Broder MS, Fraser IS. FIGO classification system (PALM COIEN) for causes of abnormal uterine bleeding in non-gravid women of reproductive age. FIGO Working Group on Menstrual Disorders. Int J Gynaecol Obstet. 2011;113:3-13.
2. Shobhitha GL, Kumari VI, Priya PL, Sundari BT. Endometrial study by TVS and It's correlation with

histopathology in abnormal uterine bleeding. J Dental Med Sci. 2015;14(4):21-32.
3. Mahapatra M, Mishra P. Clinicopathological evaluation of abnormal uterine bleeding. J Health Res Rev. 2015;2:45-9
4. Meena N, Meena S. Correlation between histology and bleeding pattern in a case of AUB and risk factors analysis of AUB in reproductive age group. JMSCR. 2017;5(7):24629-34.
5. Betha K, Malvatu L, Talasani S Distribution of causes of abnormal uterine bleeding using new FIGO classification system-PALM COEIN: a rural tertiary-based study. Int J Reprod Contracept Obstet Gynecol. 2017;6(8):3523-7.
6. Kazemijaliseh H, Ramezani Tehrani F, Behboudi-Gandevani S, Khalili D, Hosseinpanah F, Azizi F. A Population-based study of the prevalence of abnormal uterine bleeding and its related factors among Iranian reproductive-age women: An updated data. Arch Iran Med. 2017;20(9):558-63.
7. Sharma AS, Gupta S. Analysis of body mass index in patients with abnormal uterine bleeding. Inter J Clin Obstet Gynaecol. 2019;3(1):92-5
8. Puvitha RD, Elavarasan T, Shruthi MS, Shylaja S. Histopathological study of endometrium in abnormal uterine bleeding an experience in a tertiary care centre of rural south India. Nat J Basic Med Sci. 2017;8:1.
9. Kalaiselvi PSJ. Thenmozhi G. A study on endometrial thickness by endovaginal sonography with histopathology in perimenopausal women in a tertiary care centre. Indian J Res. 2017;6(10):33-6.
10. Tabata T, Yamawaki T, Yabana T, Ida M, Nishimura K, Nose Y. Natural history of endometrial hyperplasia Arch Gynecol Obstet. 2001;265(2):85-8.
11. Takai IU, Bukar M, Mayun AA, Ugwa EA, Audu BM. Aisha abdurrahman endometrial hyperplasia: a 2 decade retrospective analysis of histopathological pattern at a university teaching hospital in Northern Nigeria Sub-Saharan. Afr J Med. 2016;3:171-5.
12. Getpook C, Wattanakumtornkul S. Endometrial thickness screening in premenopausal women with abnormal uterine bleeding. J Obstet Gynaecol Res. 2006;32(6):588-92 .
13. Özdemir S, Çelik Ç, Gezginç K, Kıreşi D, Esen H. Evaluation of endometrial thickness with transvaginal ultrasonography and histopathology in premenopausal women with abnormal vaginal bleeding. Arch Gynecol Obstet. 2010;282(4):395-9.
14. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia: a long-term study of "untreated" hyperplasia in 170 patients. Cancer. 1985;56:403-12.
15. Palmer JE, Perunovic B, Tidy JA. Endometrial hyperplasia. The Obstet Gynaecol. 2008;10(4):211-6.
16. Cheung AP. Ultrasound and menstrual history in predicting endometrial hyperplasia in polycystic ovary syndrome. Obstet Gynecol. 2001;98:325-31.
17. Giannella L, Cerami LB, Setti T, Bergamini E, Boselli F. Prediction of endometrial hyperplasia and cancer among premenopausal women with abnormal uterine bleeding. BioMed Res Inter. 2019;2019.
18. No GT. Management of Endometrial Hyperplasia. 2016. Available at: ttps://www.rcog.org.uk/globalassets/documents/guidelines/green-top-guidelines/gtg_67_endometrial_hyperplasia.pdf. Accessed on 01st February 2020.

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Original Article

A study on contraceptive prevalence rate and factors influencing it in a rural area of Coimbatore, South India

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Abstract

Introduction: India is the first country to implement National Family Program in 1952. Acceptance of contraceptive methods is influenced by various factors at the individual, family, and community level with their roots in the socioeconomic and cultural milieu of Indian society. This study was carried to find the contraceptive prevalence rate among eligible couples as well as to find the factors influencing it in a rural area of Coimbatore. **Methodology:** The study was carried out using the data extracted from the family health survey conducted by trained field staff for the purpose of furnishing details regarding people residing in the field practice area of Rural Health Training Centre (RHTC), Vedapatti located in Coimbatore district after obtaining Institutional Human Ethical clearance. All eligible couples were included for the study. Contraceptive prevalence is expressed in percentage with 95% Confidence interval. Univariate analysis (Chi-square test) was done to find the association between contraceptive acceptance and independent variables. Strength of association is determined by odds ratio with 95% Confidence Interval (CI). $P < 0.05$ was considered statistically significant. **Results:** Contraceptive Prevalence Rate among eligible couples was found to be 75% (95% CI: 73.6–76.4). Most commonly used method among the study participants was tubectomy (81.6%), followed by condoms (11.4%), intrauterine devices (6.3%), and oral contraceptive pills (0.7%). Higher age of the women, religion, educational status of the women, socioeconomic status, working status of the women, number of living children, age at the time of marriage, and age at the time of first child birth were found to have statistical significant association with higher usage of contraception. **Conclusion:** The contraceptive prevalence rate was found to be higher in this study population and multiple factors were influencing the contraception usage among the eligible couples.

Keywords: Contraceptive prevalence, eligible couples, rural population

Introduction

Population explosion has been the major problem of India since Independence.[1] Considering the consequences, population stabilization has been identified as an essential prerequisite for the economic development of the nation resulting in improved quality of life.[2] India became the first country in the world to formulate National Family Planning Program in 1952, with the objective of "reducing birth rate to the extent necessary to stabilize the population at a level consistent with requirement of national economy."[2,3] As per the projections by United Nations, India will become the most populous country by the year 2045. One of the important components of family planning program management is to assess the potential demand for contraceptive services so as to mitigate the adverse impact of population growth.[4] Family planning can reduce maternal mortality by reducing the number of pregnancies, abortions, and the proportion of births at high risk.[5]

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Many empirical studies have shown the acceptance of contraceptive methods is influenced by various factors operating at the individual, family, and community level with their roots in the socioeconomic and cultural milieu of Indian society.[6] The contraceptive prevalence rate is "the percentage of women of reproductive age (15–49 years) who are married or in a union: Who are currently using, or whose sexual partner is currently using, at least one contraceptive method, regardless of the method used".[7,8] Contraceptive prevalence rate serves as a proxy measure of access to reproductive health services and is an indicator of health, population, development, and women's empowerment[7,8] that are essential for meeting many of the Sustainable Development Goals (SDG), especially the child mortality and maternal health related goals.[8]

If the population has ready access to good quality services at affordable cost, it will be possible for them to meet all their needs, achieve the desired family size, and enable the country to achieve population stabilization rapidly.[1,9] Majority of India's population (about 70%) live in the rural communities[9] but majority of the health facilities are concentrated in the urban localities leading to a disparity in access to health care. Contraceptive use is influenced by a wide range of factors including demographic, social, and economic characteristics.

There is paucity of community-based rural studies with regard to contraceptive usage. Identifying the prevalence of contraceptive usage and the local determinants could help to plan strategy and make necessary recommendations that would help improve utilization of family planning services. Hence, this study was carried out to determine the contraceptive prevalence rate among eligible couples as well as to study the association of selected social, economic and demographic factors with contraceptive usage in a rural area of Coimbatore.

Methodology

The study was carried in accordance with the ethical standards of PSGIMSR Institutional Human Ethics Committee and Ethical Principles for Medical Research was followed. The Ethics committee approval was obtained prior to carrying out the study. Date of approval : 18/12/2019. The Institute permission was obtained prior to carrying out the study. In the current study, the data were extracted from the family health survey conducted by trained field staff for the purpose of furnishing details regarding people residing in the field practice area of Rural Health Training Centre (RHTC), Vedapatti, a rural area of Tamil Nadu located in Coimbatore district. The RHTC caters to 14 villages with a population of 25,886.

Sample size was calculated based on NFHS IV report- Contraceptive Prevalence Rate of rural Tamil Nadu was 52.3%.[10] Using the formula, $n = 4\,pq/d^2$ where P is 52, q is 100-52, that is, q = 48 and d is the relative precision (5% of p), the sample size required was estimated to be 1,477. Though the required sample size was only 1,477, all 4,438 eligible couples in the field practice area were included for the current study and their data was analyzed.

Various demographic, social, and economic factors like age of women, number of children, age at marriage, age at first child birth, religion, caste, family type, educational status of husband and wife, working status of women, socioeconomic status classification (based on Modified Prasad classification - Class I – ≥Rs. 6,414; Class II – Rs. 3,207–6,413; Class III – Rs. 1,924–3,206; Class IV – Rs. 962–1,923, and Class V - ≤Rs. 961) were included in the study.

Statistical analysis

Data was entered in Microsoft excel and analyzed in SPSS 20.0 version Univariate analysis (Chi-square test) was used to find out the association between the independent variables and the dependent variable. Odds ratio was calculated with 95% Confidence Interval (CI) to find the strength of association. $P < 0.05$ was considered as statistically significant.

Results

Total number of eligible couples enrolled in the study was 4,438.

The demographic profile of the participants is shown in the Table 1. Majority of the participants (41.3%) were in the age group of 35–49 years, 96.3% of women were Hindus. Majority of married women (70.4%) were living in nuclear families, 64% were

Table 1: Demographic profile of the study population

Variable	Number (*n*=4438)	Percentage
Age of Women (completed years)		
15-24	1196	27.0
25-34	1408	31.7
35-49	1834	41.3
Religion		
Hindu	4276	96.3
Christian	133	3
Muslim	29	0.7
Caste		
General	1389	31.3
OBC	2926	65.9
SC/ST	123	2.8
Family Type		
Nuclear	3125	70.4
Extended	1313	29.6
Number of Living Children		
No child	443	10
One child	777	17.5
Two child	2841	64
≥3 children	377	8.5
Age at the Time of Marriage		
<18 years	1152	26
≥18 years	3286	74
Age at the Time of First Child		
<18 years	957	21.6
≥18 years	3481	78.4

having two children, 26% of the women in the study population got married before completion of 18 years and 21.6% women delivered a baby before completion of 18 years.

Table 2 shows that most of the eligible couples were educated up to middle school. Female literacy rate was 84.8%, whereas male literacy rate was 87.3%. Higher proportion (37.4%) belonged to Class IV socioeconomic class and 31.4% women were employed with salary.

Out of 4,438 eligible couples studied, 3,328 were current users of contraception at the time of the survey. Hence, the Contraceptive Prevalence Rate was 75% (95% CI: 73.6–76.4%) in this study population. Table 3 shows the various family planning methods adopted by the eligible couples. Most common preferred method was tubectomy (81.6%), followed by condoms (11.4%), intrauterine devices (IUDs) (6.3%), and oral contraceptive pills (OCPs) (0.7%).

Table 2: Education status and socio-economic class of the study participants

Variable	Number (*n*=4438)	Percentage
Educational status of Women		
Illiterate	675	15.2
Primary	698	15.7
Middle	1237	27.9
High school	688	15.5
Higher secondary	543	12.2
Graduate	597	13.5
Educational status of Husband		
Illiterate	565	12.7
Primary	682	15.4
Middle	1644	37.0
High school	566	12.8
Higher secondary	311	7.0
Graduate	670	15.1
Socio Economic Status (Modified Prasad Classification)		
Class I	309	7.0
Class II	798	18.0
Class III	1333	30.0
Class IV	1660	37.4
Class V	338	7.6
Working status of women		
Working	1394	31.4
Not working	3044	68.6

Table 3: Various family planning methods adopted by eligible couples

Family Planning Method Adopted	Number (Percentage)
Tubectomy	2717 (81.64)
Condom	378 (11.36)
Intra Uterine Device (IUD)	210 (6.31)
Oral Contraceptive Pill (OCP)	22 (0.66)
Vasectomy	1 (0.03)
Total	3328 (100)

The demographic factors influencing the contraception acceptance is shown in Table 4. The significant predictors for contraceptive acceptance are higher age of women, those who are having two living children, women who are married after 19 years of age, and those who had given birth to first child after 19 years of age.

The social and economic factors influencing the contraception acceptance is shown in Table 5. Factors like higher educational level of women, working women, and higher socioeconomic class are significantly associated with adoption of any of the contraceptive method.

Discussion

Contraceptive use and unmet need for family planning are keys to understand profound changes in fertility pattern and also helps to improve reproductive health. By understanding the concept of unmet needs, the gap between women reproductive intentions and contraceptive behavior can be understood. In this study, Contraceptive Prevalence Rate among eligible couples was found to be 75%, which is higher than studies reported by NFHS IV (52.3%),[10] Patel AA (55.3%),[11] Singh *et al.* (68.4%),[1] Makade *et al.* (68.4%),[12] and Gupta *et al.* (71.1%).[13] The contraceptive acceptance is quite high among the study population.

Most common method followed by the eligible couples was permanent method, that is, tubectomy (81.6%) which is higher than other studies by Shree *et al.* (70.9%),[14] Patel AA, *et al.* (33.7%),[11] and Nanda *et al.* (30.5%).[15] In our study, 11.4% couples use condoms which is similar to study done by Chandra *et al.* (11.7%)[3] and higher than Nanda *et al.* (5.1%).[15] Usage of IUDs was 6.3% which is lower than study done by Nanda *et al.* (45.8%)[15] and Shree *et al.* (11.3%).[14] Another important finding as that only one male individual has undergone vasectomy. This clearly portrays that even though permanent sterilization method in male (i.e., vasectomy) is an easier procedure and has lesser postoperative symptoms the responsibility for birth control still traditionally rests with the woman since she has to undergo the physical and psychological burden of unplanned pregnancy.

In our study, contraception usage showed significant association with higher age of the women, religion, education of the women, higher socioeconomic class, working status of the women, presence of two living children, age at the time of marriage as well as first child birth more than 18 years of age. There was no significant association with type of family, educational status of husband and caste.

In this study, the usage of contraception was significantly higher among those who were aged 35–49 years. The contraceptive acceptance was three times higher than those of aged 15–24 years, probably these couples would have completed their family. Similar findings were reported by Patel AA,[11] Valekar SS, *et al.*,[16] and Prateek *et al.*[2] wherein women of age group 35–49 years are using better contraception. The

Table 4: Association of Demographic variables with adoption of family planning methods

Variables	Adoption of Family Planning method		OR	95% CI	*P*
	Yes (*n*=3328) Total (%)	No (*n*=1110) Total (%)			
Age of women completed years					
15-24	742 (62)	454 (38)	1		
25-34	1048 (74.4)	360 (25.6)	1.78	1.5-2.11	<0.001
35-49	1538 (83.9)	296 (16.1)	3.18	2.67-3.78	<0.001
Religion					
Hindu	3220 (75.3)	1056 (24.7)	1		
Muslim	10 (34.5)	19 (65.5)	0.17	0.07-0.39	<0.001
Christian	98 (73.7)	35 (26.3)	0.92	0.61-1.39	0.67
Caste					
SC/ST	90 (73.2)	33 (26.8)	1		
OBC	2192 (74.9)	734 (25.1)	1.10	0.71-1.67	0.66
General	1046 (75.3)	343 (24.7)	1.12	0.72-1.73	0.59
Family Type					
Nuclear	2349 (75.2)	776 (24.8)	1		
Extended	979 (74.6)	334 (25.4)	0.97	0.83-1.13	0.67
Number of Living Children					
≥3 child	343 (91)	34 (9)	1		
2 child	2707 (95.3)	134 (4.7)	2.0	1.33-3.01	<0.001
1 child	275 (35.4)	502 (64.6)	0.05	0.04-0.08	<0.001
No child	3 (0.7)	440 (99.3)	0.0007	0.0002-0.0022	<0.001
Age at the Time of Marriage (Women)					
<18 years	2559 (77.9)	727 (22.1)	1		
≥18 years	769 (66.8)	383 (33.2)	0.57	0.49-0.66	<0.001
Age at the Time of First Child Birth (Women)					
<18 years	2854 (82)	627 (18)	1		
≥18 years	474 (49.5)	483 (50.5)	0.22	0.18-0.25	<0.001

contraceptive usage was significantly low (34.5%) among Muslims than Hindus (75.3%) but the proportion of Muslims was low in this study. This result was consistent with other studies like Singh AK, *et al.*[1] and Chandra *et al.*[3] The reason for lower usage of contraception among the Muslim couples could be because of religious and cultural beliefs on contraception. Hence, religion still plays a predictor role on deciding the choice of contraception.

Education of women leads to a greater awareness and a significant effect on family planning behavior and use of contraception. Our study revealed significantly higher usage of contraception among literates when compared with the illiterates. The literates will be able to understand the pros of contraceptive usage which could have brought the behavioral change. This result was consistent with studies done by Singh AK, *et al.*[1] Patel AA,[11] Gupta *et al.*,[13] Kumar N, *et al.*,[17] and Oppong FB, *et al.*[18] Improving education status can pave way to delay a women's marriage age, she will be able to seek a job and can help to change the attitude toward birth spacing and family size and empowers women to make a better decision.

In our study, contraceptive use was significantly higher among working women as they are more likely to be educated and their socioeconomic status is supposed to be better than the non-working women. Working women are likely to be more knowledgeable; would have wide exposure to media and social contacts which can influence their contraceptive behavior. This finding was similar to study done by Mukherjee *et al.*[19]

In the present study, it was found that as the socioeconomic status decreases, the usage of contraception also decreases. Contraception usage is significantly higher among class I when compared to Class V. This may be due to the fact that the higher socioeconomic status individuals have better accessibility and availability of contraception and they have better awareness regarding contraception. Similar finding was reported by Prateek *et al.*[2]

Number of living children had a significant impact on contraceptive usage. Those women who had completed their family by getting at least two children (small family norm) are using contraception higher than others. This may be due to the fact that many couples decided to complete their family with two children and also female sterilization (after institutional deliveries) was emphasized for women after they bear two children. Government policies and advisory of two child norm and benefits announced could have favored contraception acceptance by these couples. This finding was consistent with study done by Singh *et al.*[1] Shree *et al.*[14] Valekar SS, *et al.*,[16] and Gothwal M, *et al.*[20] Contrastingly, it was also found contraceptive usage was higher among those having two children compared with couples having three or more

Table 5: Association of Social and Economic variables with adoption of family planning methods

Variables	Adoption of Family Planning method		OR	95% CI	*P*
	Yes (*n*=3328) Total (%)	No (*n*=1110) Total (%)			
Educational status of Women					
Illiterate	445 (65.9)	230 (34.1)	1		
Primary	529 (75.8)	169 (24.2)	1.62	1.27-2.06	<0.01
Middle	966 (78.1)	271 (21.9)	1.84	1.49-2.28	<0.01
High school	569 (82.7)	119 (17.3)	2.47	1.9-3.21	<0.01
Higher secondary	405 (74.6)	138 (25.4)	1.52	1.17-1.96	<0.01
Graduate	414 (69.3)	183 (30.7)	1.17	0.92-1.49	0.19
Educational status of Men					
Illiterate	434 (76.8)	131 (23.2)	1		
Primary	501 (73.5)	181 (26.5)	0.84	0.64-1.09	0.17
Middle	1241 (75.5)	403 (24.5)	0.93	0.74-1.17	0.5
High school	431 (76.1)	135 (23.9)	0.96	0.73-1.28	0.79
Higher secondary	225 (72.3)	86 (27.7)	0.79	0.57-1.10	0.14
Graduate	496 (74)	174 (26)	0.86	0.66-1.13	0.26
Socio Economic Status					
Class I	247 (79.9)	62 (20.1)	1		
Class II	608 (76.2)	190 (23.8)	0.80	0.57-1.12	0.18
Class III	998 (74.9)	335 (25.1)	0.75	0.54-1.02	0.06
Class IV	1261 (76)	399 (24)	0.79	0.58-1.08	0.13
Class V	214 (63.3)	124 (36.7)	0.43	0.30-0.63	<0.001
Working status of women					
Not Working	2190 (71.9)	854 (20.1)	1		
Working	1138 (81.6)	256 (18.4)	1.73	1.48-2.03	<0.01

children. This could be due to the fact the need for contraception is not well appreciated by these couples.

Age at the time of marriage and age at time of first child birth also showed significant association with contraceptive usage. Those women who got married or gave birth before the age of 18 years used less contraception when compared with those who got married or gave birth to child after the age of 18 years. This may be because the former individuals are less aware of the contraceptive methods and their maturity levels are not favorable. Hence delaying the age of marriage of women as well as delaying the age of first child birth more than 18 years could have a positive impact on contraceptive acceptance. But studies by Prateek *et al.*[2] and Valekar SS, *et al.*[16] did not show significant association between contraception usage and age at marriage or age at first child birth.

Our study did not reveal association of contraception use and educational status of husband. This result is consistent with study done by Valekar SS, *et al.*[16] But studies by Prateek *et al.*[2] and Shree *et al.*[14] has shown association between educational status of husband and contraception usage. The current study did not show significant association of contraception use with caste and type of family, but a study by Nanda *et al.*[15] has shown significant association of contraception with caste and studies conducted by Singh *et al.*[1] and Valekar SS, *et al.*[16] has shown individuals in nuclear family are accepting the family planning methods higher than those in extended family.

This study has few limitations because other confounders which can influence the contraceptive prevalence like knowledge about contraception, sex preference, female autonomy, and unmet needs of contraception were not studied. Despite the data being retrieved from the routine family health survey, the reliability of the data is not a major concern as it was collected by trained field workers for purpose of family health survey.

Conclusion and Recommendation

From our study, it is obvious that the contraceptive prevalence was high among the study population and epidemiological factors like higher age of the women, religion, education of the women, higher socioeconomic class, working status of the women, presence of two living children, age at the time of marriage, as well as first child birth more than 18 years of age are influencing the contraception acceptance. Measures to sustain the gains of the family planning program should be given priority to sustain population stabilization and to achieve Net Reproductive Rate of one. Hence, on the first-hand targeted health awareness among those who are not using contraception could help to bring about a behavioral change so contraception usage can be maximized. Secondly, outreach contraceptive services should be intensified and expanded for easy access without fear of stigma by the society. Thirdly, reproductive health programs and policies should adequately involve men, by encouraging and motivation to adopt vasectomy as an easy method of family planning.

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Conflicts of interest

There are no conflicts of interest.

References

1. Arvind K, Alok C. Contraceptive Use among Rural Women: A Micro-Regional Study. Asia-Pac. J. Soc. Sci 2012;4:151-73.
2. Prateek S, Saurabh R. Contraceptive practices adopted by women attending an urban health centre. Afr Health Sci 2012;12:416-21.
3. Chandra R, Kandpal S, Negi K. Epidemiological correlates of contraceptive prevalence in rural population of Dehradun district. Indian J Community Med 2005;30:60-2.
4. Evaluation study on family programme [Last accessed 2014 Jul 11]. Available from: http://planningcommission.nic.in/reports/peoreport/cmpdmpeo/volume1/135.pdf.
5. Elizabeth L, Susheela S, Chowdhury, Sadia A. Fertility Regulation Behaviors and Their Costs: Contraception and Unintended Pregnancies in Africa and Eastern Europe & Central Asia. HNP discussion paper series. World Bank, Washington, DC. 2007; © World Bank. https://openknowledge.worldbank.org/handle/10986/13783 License: CC BY 3.0 IGO.
6. Rao A, Somayajulu U. Factors responsible for family planning acceptance with single child: Findings from a study in Karnataka. Demography India. 1999;28:65 73.
7. Organization WH. Sexual and Reproductive Health: Contraceptive Prevalence. Geneva: WHO; 2013.
8. UNDP. Indicators for monitoring the millennium development goals definitions rationale concepts and sources. United Nations; New York, 2003. Available From: https://docplayer.net/21179513-Indicators-for-monitoringthe-millennium-development-goals-definitions-rationale-conceptsand-sources-united-nations.html. [Last accessed 2020 Sep 02].
9. Barman S. Socio-economic and demographic differentials of contraceptive usage in Indian states: A study based on NFHS data. J Hum Ecol 2013;42:53-68.
10. International Institute for Population Sciences (IIPS) and ICF National Family Health Survey (NFHS-4), 2015–16: India Available from: http://mohfwnicin/nfhsfactsheethtm. [Last accessed 2020 Sep 2].
11. Anil A P. Knowledge and practices of contraception among married females of rural Tamil Nadu. Asian J Biomed Pharm Sci. 2015;5:1-4.
12. Makade KG, Padhyegurjar M, Padhyegurjar SB, Kulkarni R. Study of contraceptive use among married women in a slum in Mumbai. Natl J Community Med 2012;3:40-3.
13. Gupta RK, Singh P, Gupta R, Akhtar N, Gupta C, Sharma P. Contraceptive use: Its prevalence, awareness, practices and determinants in a rural population of Northern India. Int J Med Sci Public Health 2017;6:1543-8.
14. Shree V, Prasad R, Kumar S, Sinha S, Choudhary SK. Factors for non-acceptance of contraceptive methods amongst married women of reproductive age group in rural Patna. Int J Community Med Public Health 2017;4:1882-7.
15. Nanda J, Adak DK, Bharati P. Contraceptive practices among adolescent married women in Tamil Nadu, India. Asian Pac J Trop Dis 2011;1:137-41.
16. Valekar S, Chawla P, Tukaram H, Fernandez K, Kalra K. The socio-demographic determinants of contraceptive use among rural women in reproductive age group. J Women's Health Care 2017;6:1.
17. Kumar N, Das V, Agarwal A, Pandey A, Agrawal S, Care P. Pregnancy in women with previous two healthy children, associated factors, and acceptability of contraception among these women: A questionnaire-based, cross-sectional KAP study in world's second most populated country advocating two-child norm. J Family Med Prim Care 2020;9:2899-2906.
18. Oppong FB, Agbedra SY, Adomah AA, Amenyaglo S, Arhin-Wiredu K, Afari-Asiedu S, *et al.* Determinants of contraceptive use among sexually active unmarried adolescent girls and young women aged 15–24 years in Ghana: A nationally representative cross-sectional study. BMJ Open 2021;11:e043890.
19. Mukherjee BN. A comparison of the results of family planning KAP surveys in Haryana and Tamil Nadu, India. Stud Fam Plann 1974;5:224-31.
20. Gothwal M, Tak A, Aggarwal L, Rathore AS, Singh P, Yadav G, *et al.* A study of knowledge, attitude, and practice of contraception among nursing staff in All India Institute of Medical Sciences, Jodhpur, Rajasthan. J Family Med Prim Care 2020;9:706-10.

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RESEARCH

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Evidence for a hyper-reductive redox in a sub-set of heart failure patients

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Abstract

Background: Oxidative stress has been linked to heart failure (HF) in humans. Antioxidant-based treatments are often ineffective. Therefore, we hypothesize that some of the HF patients might have a reductive stress (RS) condition. Investigating RS-related mechanisms will aid in personalized optimization of redox homeostasis for better outcomes among HF patients.

Methods: Blood samples were collected from HF patients (n = 54) and healthy controls (n = 42) and serum was immediately preserved in − 80 °C for redox analysis. Malondialdehyde (MDA; lipid peroxidation) levels by HPLC, reduced glutathione (GSH) and its redox ratio (GSH/GSSG) using enzymatic-recycling assay in the serum of HF patients were measured. Further, the activities of key antioxidant enzymes were analyzed by UV–Vis spectrophotometry. Non-invasive echocardiography was used to relate circulating redox status with cardiac function and remodeling.

Results: The circulatory redox state (GSH/MDA ratio) was used to stratify the HF patients into normal redox (NR), hyper-oxidative (HO), and hyper-reductive (HR) groups. While the majority of the HF patients exhibited the HO (42%), 41% of them had a normal redox (NR) state. Surprisingly, a subset of HF patients (17%) belonged to the hyper-reductive group, suggesting a strong implication for RS in the progression of HF. In all the groups of HF patients, SOD, GPx and catalase were significantly increased while GR activity was significantly reduced relative to healthy controls. Furthermore, echocardiography analyses revealed that 55% of HO patients had higher systolic dysfunction while 62.5% of the hyper-reductive patients had higher diastolic dysfunction.

Conclusion: These results suggest that RS may be associated with HF pathogenesis for a subset of cardiac patients. Thus, stratification of HF patients based on their circulating redox status may serve as a useful prognostic tool to guide clinicians designing personalized antioxidant therapies.

Keywords: Hyper-reductive, Hyper-oxidative, Heart failure, Circulatory redox state, Reductive stress

Background

There is a general consensus that oxidative stress induces various pathophysiological processes including cardiovascular complications [1, 2]; however, counteracting antioxidant supplementations have failed to prevent the progression or curtail disease pathogenesis [3, 4]. At present, it is not clear whether oxidative stress is a cause or consequence in a given cell or organ system exhibiting a chronic disease state. Therefore, it is vital to critically analyze the global redox milieu of patients experiencing chronic illnesses including heart failure (HF). HF is a progressive condition in which the cardiac muscle weakens and becomes inefficient to meet the body's demand for blood and oxygen supply. The etiology of HF is multifaceted as several genetic, biochemical, electrical and inflammatory factors have been shown to underlie the structural and functional remodeling that develops over time [5–8]. Based on the currently available literature, a majority of

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the HF conditions have been correlated with oxidative stress for the past several decades. In particular, ischemic heart disease and/or reperfusion injury have been shown to display a hyper-oxidative state wherein increased reactive oxygen and nitrogen species (ROS/RNS) generation correlates with a worsening of myocardial injury [9–11]. In spite of these observations, supplementation with antioxidants seems to be inefficient to treat such conditions in a failing heart [12–14]. In particular, pre-clinical observations using rodent models have documented that a forced induction of oxidative stress leads to "heart failure" and pre-treatment with potential antioxidants seems to be protective [15–17]. However, these findings were not reproducible in HF patients [18–20].

To the best of our knowledge, all HF studies have focused on documenting the differences between HF patients and healthy control groups, and have not examined the potential for individual variations in the context of redox status among HF patients. Importantly, findings based on a group may not be precise to each individual of that group. Therefore, considering the inconsistent effects of antioxidant trials in human patients, it is worth testing whether all HF patients experience similar redox state. The ROS/RNS produced during basal mitochondrial metabolism (oxygen consumption at resting state) or in response to physical activity are key modulators of cellular motility to maintain a redox homeostasis and preserve the dynamic function of the myocardium [21–24]. However, other factors including genetic or chronic stresses that modulate ROS/RNS may tip the redox milieu towards either direction of the redox spectrum (i.e. reductive or oxidative). Despite several studies demonstrating the futility and/or detrimental effects of antioxidants, there has not been a single study attempting to understand the mechanisms associated with failure of the antioxidants in over six decades of biomedical research. In the present study, we attempt to address this critical gap in knowledge and postulate that some HF patients may either exhibit a hyper-reductive or normal redox state potentially conferring vulnerability and inimical side effects to antioxidant treatment.

In the current study, we determined the circulatory redox state of HF patients by measuring glutathione redox (GSH:GSSG) ratio, lipid peroxidation (MDA) and the activities of key antioxidant enzymes to compare with the healthy control group. Moreover, we utilized the ratio of GSH/MDA as a peripheral redox index and focused on stratifying HF patients according to this measure. Our findings revealed a surprising but meaningful observation in that there appears to be distinct subsets of HF patients exhibiting divergent redox signatures. Therefore, our data indicates that not all HF patients have oxidative stress as traditionally reported. Our pilot observations in this small cohort of HF patients (n = 54) warrant a new redox-based classification in these patients for selecting an appropriate therapy.

Methods

Study population

Our study population included heart failure (HF) patients (n = 54) (with systolic or diastolic dysfunction, dilated cardiomyopathy or biventricular dysfunction) and healthy controls (n = 42) who attended the in-patient and out-patient department of cardiology in PSG Hospitals, Tamil Nadu, India. Their characteristics are tabulated in Table 1. Patients with HF who were diagnosed using Framingham criteria were considered for this study [25]. Patients on dialysis, as well as those exhibiting severe liver diseases, malignancies or consuming antioxidant supplements were excluded from the study. While 85% of patients were admitted to the hospital for the first time, the remaining were presented for follow-up visits. The patients were treated as per the existing guidelines at the time. Briefly, the patients were categorized into those displaying heart failure (HF) with reduced ejection fraction (HFrEF) and preserved ejection fraction (HFpEF). The patients with HFrEF received stage-appropriate medications such as ACE inhibitors or Beta Blockers or ARBS alone or in combination, along with diuretics. Mineralocorticoid Receptor Antagonists and Hydralazine were also added if required. Patients with HRpEF had diuretics as their main stay along with the treatment of underlying causes such as coronary artery disease, chronic kidney disease, HF, atrial fibrillation etc. The study was approved by the PSG Institutional Human Ethics Committee (IHEC) and all patients/subjects completed a written informed consent prior to their participation.

Reagents

Supplies and reagents were purchased from Sisco Research Laboratories (India) unless otherwise specified.

Clinical assessment and echocardiography

Validation and confirmation of HF in patients were made based on clinical and echocardiography assessment. One echocardiogram was selected for each patient at the time of enrollment. Two-dimensional and M-mode echocardiography, color flow and spectral Doppler as well as annular TDI data were obtained from all patients and healthy controls using an ultrasound system (Philips IE33, Netherlands). Standard views, including the parasternal long axis view, short axis at the papillary muscle level and apical four and two chamber views were recorded. Cardiac chamber dimensions, volumes and left ventricular mass were measured according to current recommendations [26]. Left ventricular ejection fraction (LV EF) was

Table 1 Demographic details for heart failure patients and healthy controls

Characteristics of patients	Heart failure patients (n = 54)			Healthy controls
	Hyperreductive (n = 9)	Hyperoxidative (n = 23)	Normal redox (n = 22)	
Age in years: mean age (range)†	50 (32–75)	58 (34–73)	52 (34–75)	36.35 (18–64)
Sex: n (% male)†	9 (100)	23 (100)	21 (95)	27 (64)
Mean height in cm (range)†	164 (151.7–177)	163 (152–173)	163 (143–178)	165.8 (148–192)
Mean weight in kg (range)†	60 (50–70)	62 (41–75)	63 (38–100)	62.7 (38–89)
Body mass index (kg/m^2) (range)†	22 (19.5–24.5)	23 (16.4–30.3)	24 (15.2–35.4)	22.9 (16.1–34.6)
Hemodynamics†				
Heart rate (no. of times/min)	97 (60–100)	94 (74–121)	96 (70–116)	78 (64–98)
Mean systolic blood pressure (mmHg)	129 (80–170)	129 (80–180)	125 (90–170)	119 (90–190)
Mean diastolic blood pressure (mmHg)	79 (60–140)	85 (60–110)	77 (50–110)	76 (50–110)
Comorbidities, %†				
DM and DM associated disorders	5 (55)	15 (65)	10 (45)	–
Systemic hypertension	3 (33)	5 (22)	6 (27)	–
Hypothyroidism	0	4 (17)	1 (4.5)	–
Chronic kidney disease	1 (11)	3 (13)	0	–
Anaemia	0	2 (7)	2 (9)	–
BPH (benign prostatic hyperplasia)	1 (11)	0	1 (4.5)	–
Hepatitis	0	0	1 (4.5)	–
CVA (cerebrovascular accident)	0	1 (4)	0	–
COPD	1 (11)	2 (9)	3 (14)	–
Cardiac disease pathology, %†				
Dilated cardiomyopathy	5 (55)	8 (35)	9 (41)	–
Coronary artery disease	2 (22)	10 (43)	10 (45)	–
Rheumatic heart disease	1 (11)	0	2 (9)	–
Ischemic heart disease	0	1 (4)	1 (4.5)	–
Aortoiliac disease	1 (11)	5 (22)	0	–
Diastolic dysfunction	10 (43)	7 (32)	8 (89)	–
Systolic dysfunction	16 (69.5)	19 (86)	9 (100)	–
Biventricular dysfunction	6 (26)	4 (18)	0	–
Pulmonary artery hypertension (PAH)	12 (52)	10 (45)	4 (44)	–
Medication, %*				
Diuretics	5 (55)	20 (87)	18 (82)	–
β blockers	4 (44)	12 (52)	12 (54)	–
Statins	1 (11)	9 (39)	6 (27)	–
ACE inhibitors	3 (33)	10 (43)	10 (45)	–
Digitalis glycosides	3 (33)	11 (48)	4 (18)	–
Anticoagulant	1 (11)	5 (22)	2 (9)	–
(ARB) angiotensin II antagonists	0	5 (22)	1 (4.5)	–
Antiplatelet medications	0	5 (22)	3 (14)	–

* p = 0.005, statistically significant

† p = ns, nonsignificant

calculated using the teichholz formula [27]. Mitral Doppler signals were recorded in apical 4 chamber view, with Doppler sample volume placed at the tip of the mitral valve leaflets. The following parameters were obtained: early diastolic mitral inflow peak velocity (E), late diastolic mitral inflow peak velocity (A) and their ratio.

Blood sampling

Blood samples were obtained by venipuncture using a 21-gauge needle from the patients and healthy control subjects in plain tubes. Serum was separated by centrifugation at 3000 rpm for 5 min at 4 °C and immediately aliquoted and frozen at − 80 °C. A fraction of the serum was

treated with 10% meta-phosphoric acid (MPA) to remove proteins and the MPA-supernatant was stored for glutathione redox analysis instantly after the blood collection for all the controls and patients. The stored aliquots of serum were used for analyzing redox status and the activities of antioxidant enzymes.

Lipid peroxidation

Serum malondialdehyde levels were measured by the DNPH (2,4-dinitrophenyl hydrazine) derivatization method [28]. Briefly, 125 µl of serum was diluted in 125 µl 1× PBS (pH 7.4), mixed with 50 µl of 6 M NaOH and incubated at 60 °C for 30 min. 125 µl of 35% perchloric acid was then added to the mixture. 250 µl of this mixture was combined with 25 µl of 5 mM DNPH (in 2 M HCl) and incubated in the dark for 30 min. An aliquot of 25 µl of the solution was injected into the HPLC system (Shimadzu, Japan). The mobile phase of 0.2% (v/v) acetic acid and acetonitrile (50:50 v/v) was run at a flow rate of 0.5 ml/min at 25 °C. A C18 column (Agilent, USA) was used and the chromatograms were obtained at 310 nm. Concentration of MDA was measured after comparing with a reference curve using TMP (1,1,3,3-tetramethoxypropane) as a standard.

Reduced glutathione levels

The spectrophotometric based glutathione reductase/5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) recycling assay was used to measure reduced glutathione (GSH) levels as per [29–31] with minor modifications. In short, metaphosphoric acid extracts of serum samples were prepared and treated with triethanolamine (TEAM reagent) to adjust the pH for total GSH quantification according to the manufacturers' protocols (GSH redox Kit #CS0260, Sigma-Aldrich). An aliquot of TEAM treated samples were mixed with 1.0 mM 2-vinyl pyridine and incubated for 1.0 h at room temperature for GSSG measurements. For enzymatic recycling, the processed samples were treated with a reaction mixture containing 0.25 mM dithiobis-nitrobenzoic acid (DTNB), 0.38 U/ml Glutathione reductase and 60 µl of 0.17 mM NADPH in 0.1 M sodium phosphate buffer (pH 7.4) with 5 mM EDTA. The rate of DTNB formation was measured at A412 nm. Similarly, GSH and GSSG standards were treated and measured to obtain a standard graph to extrapolate the values from serum samples. The concentration of reduced glutathione (GSH) was estimated by subtracting the measured oxidized (GSSG) glutathione levels from the measured total (GSH plus GSSG) glutathione. GSH/GSSG ratio was then determined.

Superoxide dismutase (SOD) activity

Superoxide dismutase activity in the serum samples were measured using the pyrogallal auto-oxidation method [32]. This assay was carried out in a microtiter plate and the readings were measured using multimode reader (Varioskan Flash, Thermoscientific, USA). Approximately 50 µl of serum was treated with 25 µl ethanol and 15 µl chloroform. The contents were vortexed and centrifuged at 8000 rpm for 5 min and the supernatant was analyzed for SOD activity by mixing with 50 mM Tris–HCl buffer with 1.0 mM EDTA, pH 8.2 and 2.0 mM pyrogallal (in 50 mM HCl). The rate of autoxidation was measured from the increase in absorbance at 420 nm and the values were expressed as U/ml.

Catalase activity

The activity of catalase in serum of HF and HC samples was measured by H_2O_2 decomposition kinetics using a spectrophotometer [33]. A mixture containing potassium phosphate buffer (50 mM, pH 7.0), 20 mM H_2O_2 and 2.0 µl serum was used to measure the rate of decrease in optical density at 240 nm. Catalase activity was expressed as U/l.

Glutathione reductase activity

Glutathione reductase activity was assayed by monitoring NADPH oxidation linked to GSSG reduction [34] using a spectrophotometer. The assay was performed by suspending 10 µl of the serum in sodium phosphate buffer (0.3 M, pH 6.8), 25 mM EDTA, 20 mM GSSG, 2 mM NADPH and measuring the decrease in absorbance at 340 nm over time. The oxidation of 1 µmol of NADPH/min was used as a unit of glutathione reductase activity. Glutathione reductase activity was expressed as U/l.

Glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was measured by a spectrophotometric assay using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) according to [35] where the oxidation of GSH in the presence of H_2O_2 takes place, and the unused GSH reacts with DTNB whose absorbance was measured at 412 nm. The enzyme assay was performed by incubating the serum (25 µl) in sodium phosphate buffer containing sodium azide (10 mM), GSH (4 mM) and 2.5 mMH_2O_2 for 5 min. Protein was precipitated using 10% trichloroacetic acid, centrifuged at 5000 rpm for 5 min, and the supernatant was assayed. This assay was carried out in a microtitre plate and the readings were measured using multimode reader (Varioskan Flash, Thermoscientific, USA) after mixing with disodium hydrogen phosphate (0.3 M, pH 7.0) and 100 mM DTNB (in 1% sodium citrate) at 412 nm. GPx activity (U/l) was measured as the amount of GSH consumed/min/mg protein after reaction with GPx and DTNB.

Protein estimation

Serum proteins were estimated using Bradford method. A known volume (5 µl) of 1:100 diluted serum was incubated in Bradford reagent (#18004246723, Biorad, USA) and measured the absorbance at 595 nm. The absorbance was extrapolated with the appropriate standard reference curve using bovine serum albumin (0.031–0.5 mg/ml) and the protein concentration was calculated. Specific activity for the antioxidant enzymes were measured after normalizing with the respective protein concentration.

Statistical analysis

For comparing two groups, Mann–Whitney U test was used for non-normal data and for groups with normal distribution, group mean ± standard deviation (SD) were compared, homogeneity variance test (Bartlett's test) followed by one way ANOVA was carried out. A $p > 0.05$ was considered to be statistically significant. Fisher's exact test was performed to analyse statistical significance of EF fraction and E/A ratio among the different groups of HF patients. For data which was not normally distributed, a nonparametric test, Kruskal–Wallis test was carried out to obtain the p value. All the statistical analyses were carried out using GraphPad Prism software version 5.0 Windows (GraphPad Software, USA).

Results

Stratification of heart failure patients based on circulatory redox status

Circulatory redox state (CRS) was assessed by quantifying the levels of reduced glutathione (GSH), a ubiquitous, small molecular antioxidant redox couple and malondialdehyde (MDA), an end-product of lipid peroxidation (Fig. 1). Compared to the healthy controls (HC; n = 42), circulating GSH levels were unchanged in HF patients (n = 54) (0.09207 ± 0.1215 µM in HF patients vs. 0.06374 ± 0.04887 µM in HC; p = NS; Fig. 1a), suggesting a wide range of redox states among the HF patients. In addition to total glutathione levels, the ratio of reduced to oxidized glutathione (GSH:GSSG) did not differ between HF and HC (4.93 ± 7.25 in HF patients vs. 3.01 ± 1.78 in HC; p = NS; Fig. 1c). In contrast, MDA was significantly higher in HF patients vs. HC (4.342 ± 2.103 vs. 1.566 ± 0.3018 µM, respectively; $p < 0.0001$) (Fig. 1b), suggesting that increased lipid peroxidation is coupled with the development of HF. Therefore, we next assessed whether the redox index (reduction vs. oxidation) is significantly different between the groups. Comparisons of GSH/GSSG ratio revealed insignificant differences between the groups, indicating that the HF patients seem to exhibit a wide range of redox levels in relation to HC (4.93 ± 7.25 in HF patients vs. 3.01 ± 1.78 in HC; p = NS; Fig. 1c). Therefore, our approach for stratifying HF patients used CRS (i.e. GSH/MDA) to gain more knowledge and understand the pathogenesis. But, comparisons of GSH/MDA ratios between the HF and HC yielded a statistically significant result (0.02 ± 0.0345 in HF patients vs. 0.04 ± 0.028 in HC; $p < 0.0001$; Fig. 1d). Therefore, we were interested in stratifying the HF patients based on their circulating redox score (CRS) (Fig. 1d). The CRS of healthy subjects was designated as normal redox (NR). A CRS value in any patient lower than the NR displayed by HC was regarded as hyper-oxidative (HO) while a patient exhibiting a GSH/MDA ratio greater than the NR cutoff was classified as hyper-reductive (HR). Surprisingly, our CRS-based stratification revealed three distinct classes within our cohort of HF patients (Fig. 1e). Along these lines, 41% of HF patients were assigned to the normal redox (NR) group whose mean GSH/MDA ratio was similar to HC. Next, 42% of HF patients belonged to the hyper-oxidative (HO) class whose mean GSH/MDA ratio (0.004787 ± 0.002055) was lower than HC group (0.03969 ± 0.02857), and 17% of HF patients exhibited a hyper-reductive (HR) state in which mean GSH/MDA ratio (0.2271 ± 0.03192) was greater than HC group (0.03969 ± 0.02857). Interestingly, the GSH/MDA ratios were statistically significant among all three HF groups ($p < 0.0001$) by one way ANOVA analysis. These novel observations suggest that redox status plays a variable role in the progression of HF and that both extremes (e.g. oxidative and reductive) may contribute to disease pathogenesis.

Activities of antioxidant enzymes were significantly altered in HF vs. healthy controls

To assess the capacity of the endogenous antioxidant defense system in the periphery of HF patients, we further analyzed the activity of circulating antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), catalase, and glutathione peroxidase (GPx) among the various groups of HF patients (Fig. 2). SOD (Fig. 2a), Gpx (Fig. 2b), catalase (Fig. 2c), GR (Fig. 2d) did not show any statistically significant differences among NR, HO, and HR groups of HF patients. While the activities of SOD (6.455 ± 2.30 for HO, 6.646 ± 2.556 for NR, 6.400 ± 1.752 for HR vs. 2.571 ± 0.61 U/ml for HC; Fig. 2a), GPx (527.2 ± 343.4 for HO, 413.3 ± 243.7 for NR, 581 ± 310.2 for HR vs. 312 ± 141.4 U/l for HC; $p < 0.01$; Fig. 2b), and catalase (855.2 ± 776.5 for HO, 787.8 ± 454.9 for NR, 1068 ± 590.3 for HR vs. 689.1 ± 556.8 U/l for HC; $p < 0.05$; Fig. 2c) were significantly increased, GR activity was significantly lower (11.66 ± 5.5 for HO, 14.29 ± 9.86 for NR, 13.32 ± 6.79 for HR vs. 24.70 ± 28.22 U/l for HC; $p < 0.05$; Fig. 2d) in all HF patient groups (HO, NR and HR) relative to healthy controls.

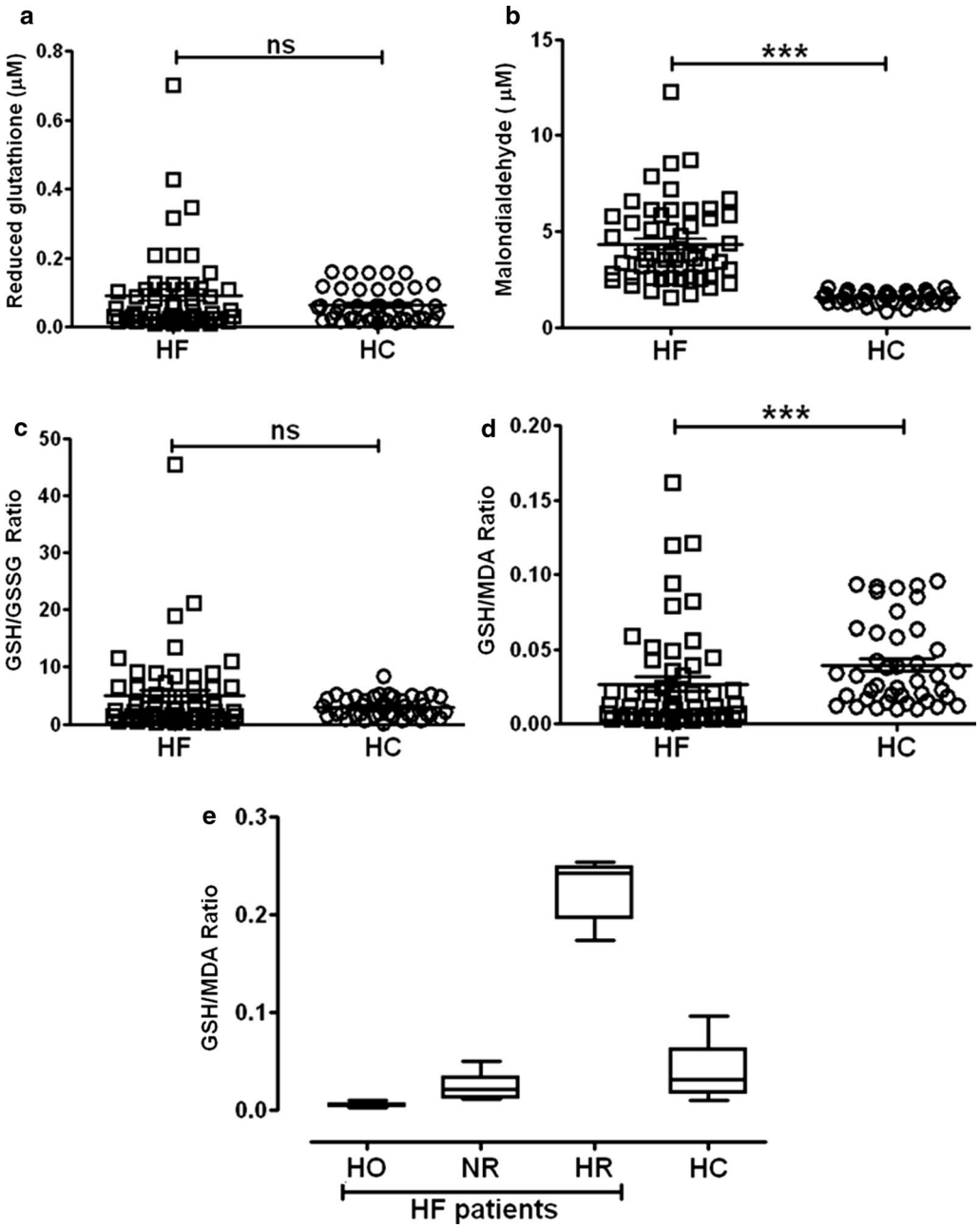


Fig. 1 Stratification of heart failure (HF) patients based on redox status. **a** Comparison of reduced glutathione (GSH) levels showing statistically insignificant GSH levels between HF patients and HC. **b** Significantly higher malondialdehyde levels in HF patients vs. HC. **c** Statistically insignificant GSH/GSSG ratio between HF patients and the healthy controls. **d** Statistically significant, higher GSH/MDA ratio in HC and lower values in HF patients. Mann–Whitney U test was carried out for statistical significance. ***$p < 0.001$. $n = 42$ HC and 54 HF patients. **e** Stratification of HF patients into hyper-oxidative (HO), normal redox (NR) and hyper-reductive (HR) groups based on comparison of GSH/MDA ratio with healthy controls (HC). Remarkably, higher GSH/MDA ratio in HR group, similar GSH/MDA ratio in NR and HC, and a significantly lower GSH/MDA ratio in HO group were observed. One way ANOVA was carried out with $p < 0.0001$ among all the heart failure patient groups ($n = 42$ HC, 23 HO, 22 NR, and 9 HR)

Redox associated changes in cardiac remodeling and systolic function in HF patients

To relate our biochemical redox observations in the distinct subsets of HF patient with myocardial structural and functional remodeling, we performed non-invasive echocardiography analyses to determine fractional shortening, ejection fraction and left ventricular (LV) mass. First, we confirmed that HF patients

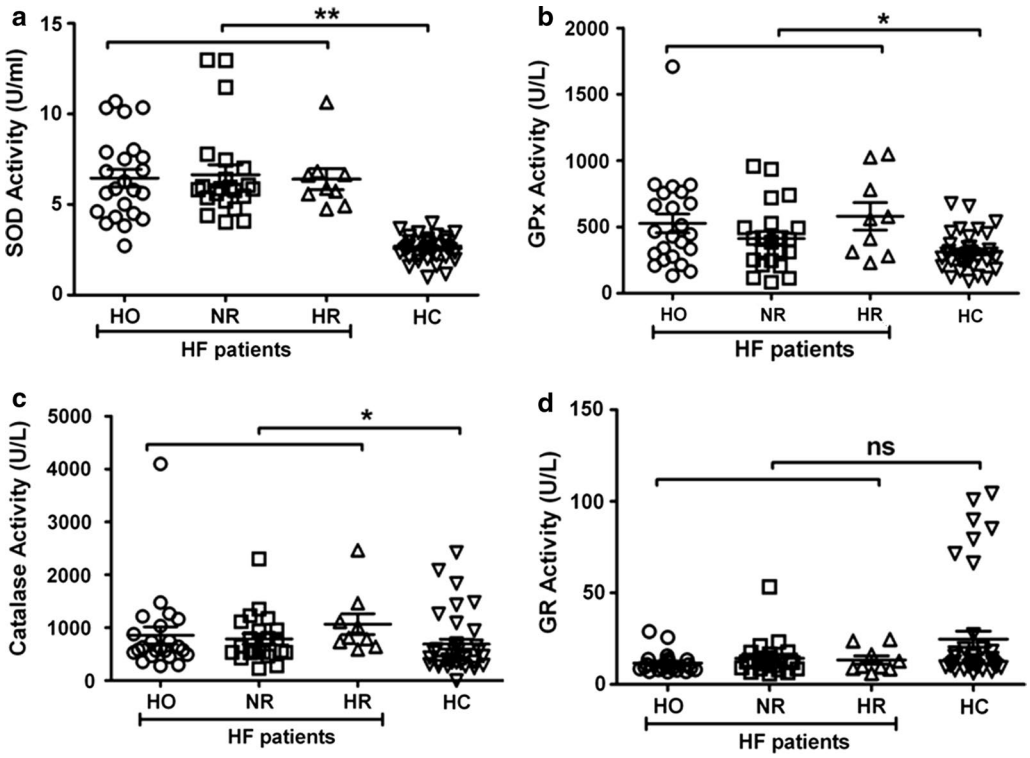


Fig. 2 Comparison of antioxidant enzymes between heart failure (HF) patients and healthy controls. **a** Statistically significant higher superoxide dismutase activity (SOD) in HF patients compared to healthy controls. **b** Statistically significant higher glutathione peroxidase activity (GPx) in HF patients compared to healthy controls. **c** Statistically significant higher catalase activity compared to HC. **d** Statistically significant lower glutathione reductase activity (GR) compared to HC. One way ANOVA was carried out (**$p < 0.001$, *$p < 0.05$) among all the heart failure patient groups (n = , 23 HO, 22 NR, and 9 HR 42 HC except for catalase and GPx, where n = 35 HC because of limited sample volume)

exhibit significantly distinct myocardial mass and systolic function when compared to HC (Fig. 3a–c). Fractional shortening (Fig. 3a), ejection fraction (Fig. 3b) and LV mass (Fig. 3c) did not show any statistically significant differences among the NR, HO, and HR groups of HF patients. However, when compared with HC, all HF patient groups (HO, NR and HR) showed significantly lower fractional shortening (17.03 ± 9.256 for HO, 15.40 ± 5.863 for NR, 16.54 ± 5.185 for HR vs. 36.13 ± 6.020 for HC; $p < 0.01$) and ejection fraction (33.78 ± 17.62 for HO, 32.47 ± 10.41 for NR, 32.57 ± 9.424 for HR vs. 65.46 ± 7.9 for HC; $p < 0.01$), but significantly greater LV mass (309.1 ± 107.9 for HO, 281.1 ± 128.2 for NR, 320.1 ± 103.8 for HR vs. 162.6 ± 13.72 for HC; $p < 0.01$). Representative M-mode echo images for systolic function analysis of NR, HO, HR and healthy control groups are shown in Fig. 3d.

Evidence for a wide range of diastolic abnormalities in HF patients

Primary measurements of trans-mitral inflow including the peak early filling (E wave), late diastolic filling (A wave) velocities and the E/A ratio were performed in HF patients as well as HC (Fig. 4a–c). Although there was no statistically significant difference between HF patients and the HC for ME, MA and ME/A ratio, their measures were widely distributed among the groups compared to the controls (Fig. 4d–f). Representative mitral valve Doppler analysis for diastolic function with graphs for ME/A ratios for HO, NR, HR and healthy control groups are shown in Fig. 4g. M E/A ratio differed according to age and gender [36, 37]. Based on this range, we grouped the HF patients into impaired relaxation and restrictive filling.

Proportion of patients based on systolic (EF) and diastolic (E/A ratio) functions suggest a hyper-reductive condition may be a detrimental factor in HF pathogenesis

Among the HF patients, the HO group (55%) had a higher percentage of patients with ejection fraction $\leq 30\%$ than the NR (44.5%) and HR groups (44.5%). Patients with an EF between 31 and 40% were more frequently assigned to the HR group (33.3%) compared to NR (28%) and HO groups (18%). In contrast, HF patients displaying an EF between 41 and 50% were

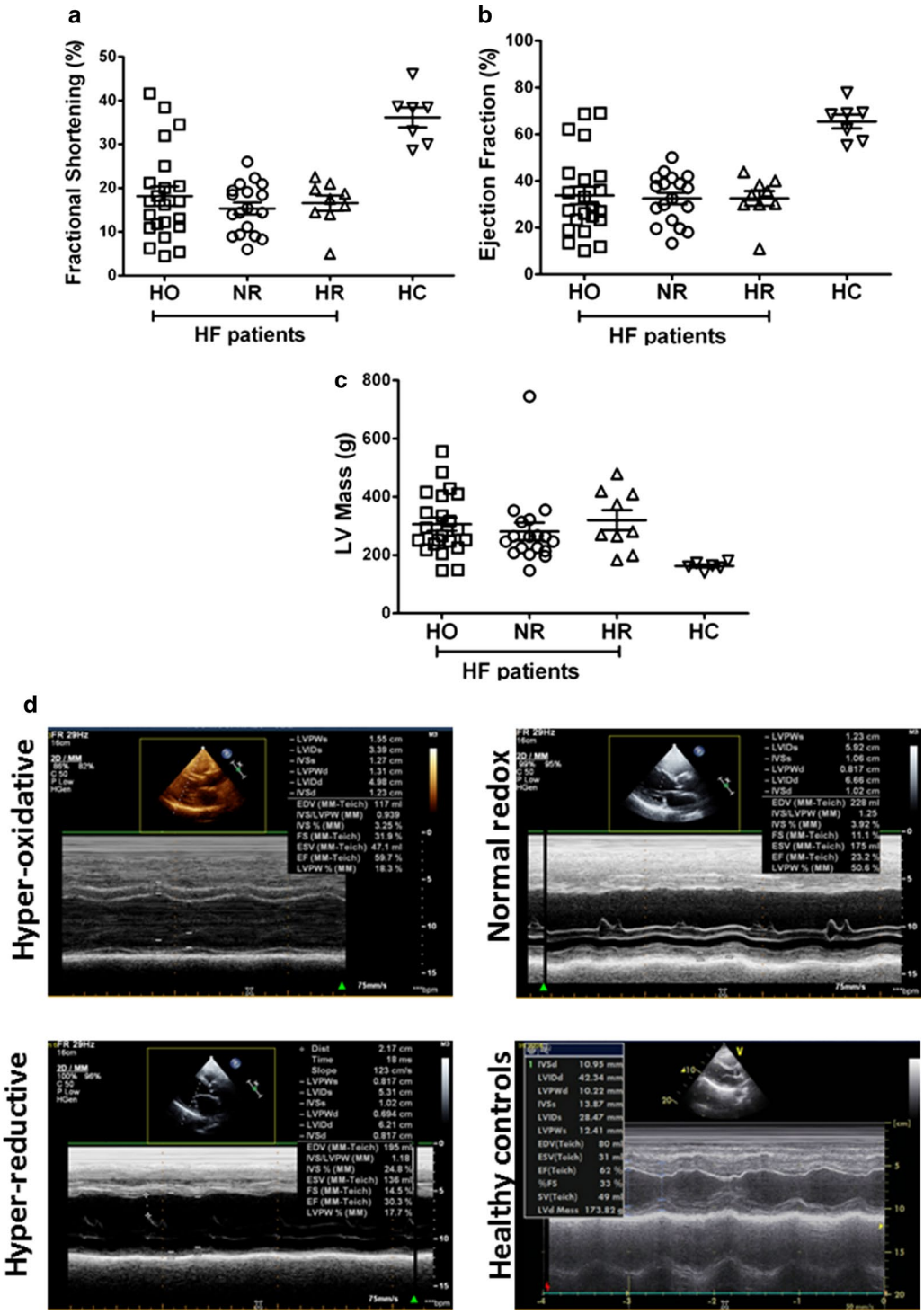


Fig. 3 Echocardiographic parameters for hyper-oxidative (HO), normal redox (NR), hyper-reductive (HR) and healthy controls (HC). **a** Statistically significant lower percentage of fractional shortening (FS) for HO, NR and HR groups of HF patients compared to HC. **b** Statistically significant lower percentage of ejection fraction for HO, NR and HR groups of heart failure patients compared to HC and **c** statistically significant higher LV mass for HO, NR and HR groups of heart failure patients compared to HC. One way ANOVA was carried out with a $p < 0.01$ vs. HC for **a**–**c** $n = 7$ HC, 22 HO, 18 NR and 9 HR. M-mode ECHO related parameters focusing on systolic functions and dimensions with ejection fraction (EF) and FS for **d** representative m-mode images for each group

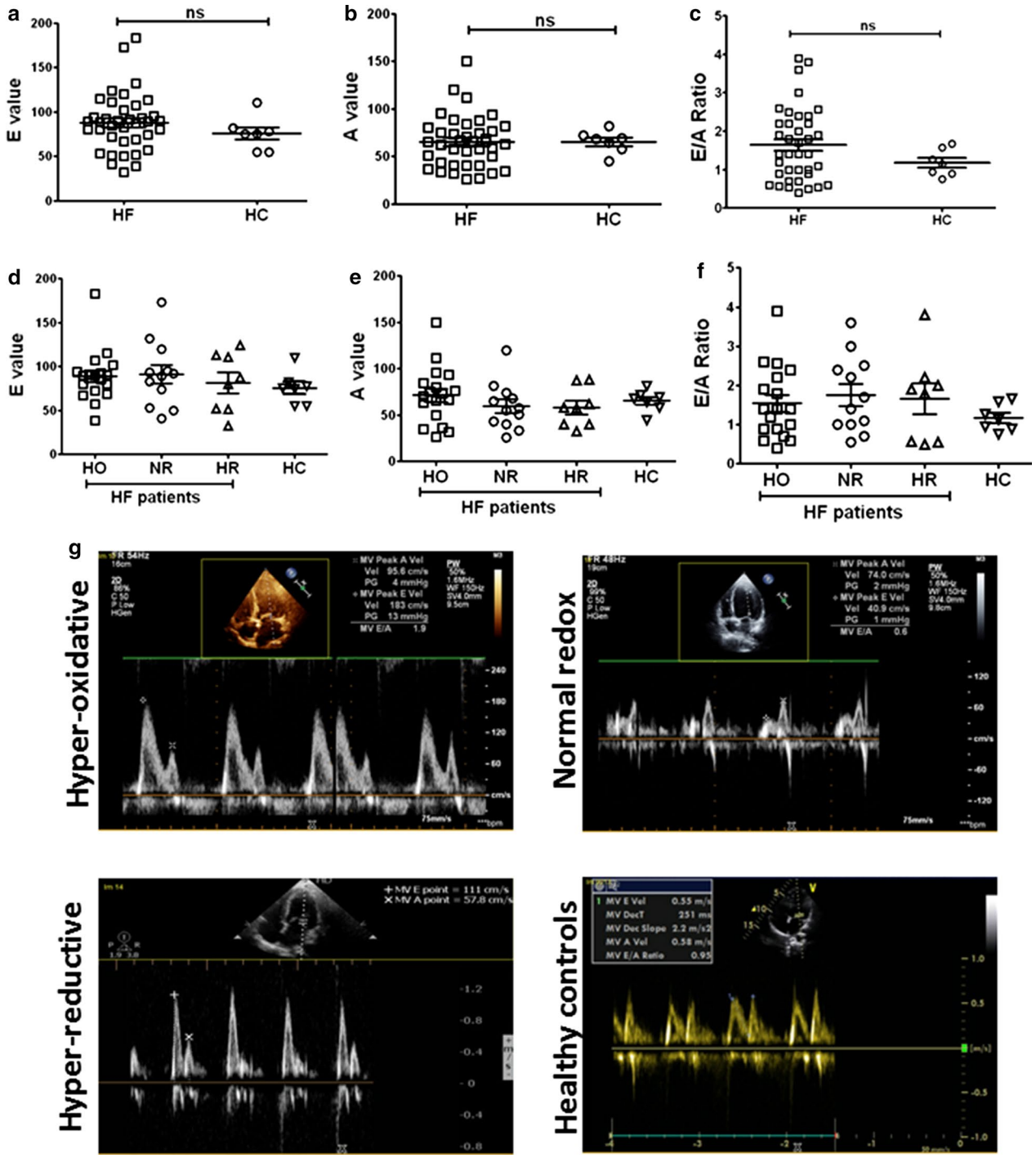


Fig. 4 Comparison of diastolic function abnormalities between HF patients and healthy controls. Similar values between HF patients and healthy controls were observed for **a** Early (E) filling, **b** late (A) filling and **c** E/A ratio. Mann–Whitney U test was carried out with no statistical significance among the groups for **a**–**c**, n = 7 HC and 38 HF patients. **d** Early (E) filling values were slightly higher in HF group patients (HO, NR and HR) than healthy controls. Relative to healthy controls, **e** late (A) filling values and were slightly higher in HO and lower in NR and HR. **f** E/A ratio were slightly higher in HF group patients (HO, NR and HR) than healthy controls. n = 7 HC, 18 HO, 12 NR and 8 HR. One way ANOVA was carried out with no statistical significance among the groups for **d**–**f**. Mitral valve Doppler analysis for diastolic function with graphs for M E/A ratios for **g**. Representative mitral valve Doppler images for each group

less prone to be classified as HO (9%) when compared to the NR (22%) and HR groups (22%). Intriguingly, HF patients with HFpEF were completely absent in HR groups relative to HO (18%) and NR patients (5.5%) (Fig. 5a).

Using the ME/A ratio as an index, diastolic function was compared between different HF groups and healthy controls (Fig. 5b). To our surprise, none of the HR patients exhibited normal diastolic function (E/A ratio 0.76–1.6); however, 41.3% of HO and 33% of NR patients revealed such E/A ratios. While only 17% of NR patients exhibited impaired relaxation (E/A ratio $\leq$ 0.75), the incidence of impaired relaxation in HR and HO patients were 37.5 and 23.5% respectively. Finally, the HR group had higher percentage of patients (62.5%) with restrictive filling (E/A ratio $\geq$ 1.61) as compared with HO (35.2%) and NR patients (50%). Fisher's exact test was carried out with no statistical significance for EF fraction and E/A ratio among the HO, NR, and HR group of HF patients.

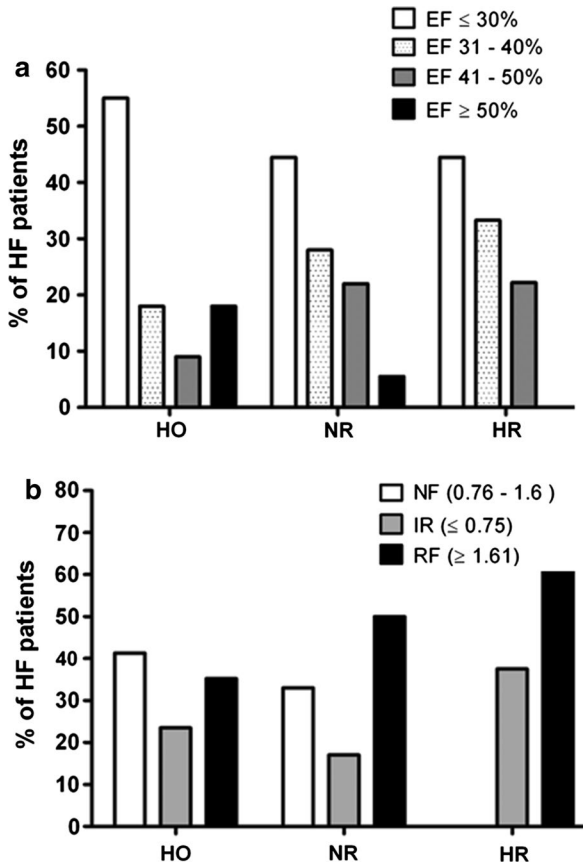


Fig. 5 Comparison of systolic functions (EF) and diastolic functions (E/A ratio) among hyper-oxidative, normal redox and hyper-reductive groups of HF patients. **a** Comparison of ejection fraction (EF) among hyper-oxidative (HO), normal redox (NR) and hyper-reductive (HR) groups of HF patients indicating higher number of HO patients have lowest range of EF $\leq$ 30% and patients with highest range of EF $\geq$ 50% are absent in HR group. **b** Comparison of E/A ratio among HO, NR and HR groups of cardiac failure patients with normal filling (NF), impaired relaxation (IR) and restrictive filling (RF) indicating that the HR group had higher number of patients with RF and HO group had higher patients with NF. Fisher's exact test was carried out with no statistical significance for EF fraction and E/A ratio among the HO, NR, HR group of HF patients

Discussion

The current understanding of redox changes during heart failure (HF) is centered on results obtained from a given group of HF patients compared to healthy controls. Importantly, this experimental approach assumes that redox imbalances during HF progression conform to the oxidative stress paradigm and neglect the potential for observations of individual redox responses in unique subsets of cardiac patients. Here, we attempted to investigate the individual differences of circulating redox biomarkers such as reduced glutathione (GSH), its redox ratio (GSH/GSSG), lipid peroxidation levels (i.e. MDA) and its normalization to reduced glutathione (i.e. GSH/MDA). In particular, we examined whether some HF patients exhibit a unique redox state that is likely to be stretched on either direction of the redox spectrum (i.e. hyper-reductive vs. hyper-oxidative). The development of HF might occur in response to various factors including a chronic oxidative stress/inflammation, infection, ischemic insult, genetic cues and other chronic stresses [38, 39]. Hither to, the redox-based classification of HF has not been implemented.

Generation of reactive oxygen/nitrogen species (ROS/RNS) and oxidative stress has been reported to be associated with the development of HF [40, 41]. However, the evidence for oxidative stress as a causal factor for chronic HF is ambiguous as oxidative stress is absent at the onset of HF in some patients while it appears as a consequence at the later stages of the disease in others. Therefore, we postulate that the oxidative stress may not be solely responsible for the development of HF, and that the other extreme of the redox continuum, reductive stress (RS; a hyper-reductive condition) may play an equally pathological role in certain instances. In the past decade, we and others have suggested that RS might also be an additional mechanism that causes pathological cardiac remodeling leading to heart failure [30, 42–44]. Notably, the existence of RS as a key driver of pathogenesis has been shown in a human mutant protein aggregation cardiomyopathy with augmented antioxidant capacity [30, 45]. Furthermore, other laboratories have reported that augmentation of HSP25, a molecular chaperone that co-regulates glutathione metabolism, promoted RS and resulted in cardiac hypertrophy in transgenic mice expressing Hsp25

[44]. Of late, it has been shown that significant upregulation of NADPH in the heart promotes reductive stress and exacerbates myocardial injury in a mouse model [46]. Nonetheless, such an evidence for RS or a hyper-reductive condition in human HF has not been demonstrated. Our current observations in a small cohort of HF patients (n = 54) have indicated that a distinct subset of HF patients exhibit a hyper-reductive condition in their circulation. Thus, we believe that our novel findings might be useful in stratifying HF patients based on the circulatory redox state (CRS) and in designing appropriate treatment strategies targeting antioxidants.

Based on the CRS (i.e. GSH/MDA), HF patients were stratified into three distinct groups, namely HF patients with (a) hyper-oxidative (HO), (b) normal redox (NR), and (c) hyper-reductive (HR) conditions. Interestingly, while a majority of the HF patients fall into the HO (42%) and NR (41%) categories, a small subset exhibited the HR (n = 17%). Of note, these observations suggest that not all HF patients experience oxidative stress. Therefore, a key question remains as to how and why NR or HR redox conditions contribute to the development of HF remains. Although these analyses seem to be novel and interesting, the mechanisms for NR and/or HR redox conditions in the development of HF are yet to be discovered. Importantly, changes in circulating redox biomarkers in a single group of HF patients appear to be insignificant when compared to healthy controls as the individual levels of CRS have a wide range, which masks the actual scenario in a given HF patient. Hence, our approach for stratifying HF patients based on their CRS will be appropriate to gain more knowledge and understand the pathogenesis of HF in a personalized manner that is likely to enhance the potential of a patient's treatment. Furthermore, we believe that a basic and simple biochemical clinical test for the CRS of a HF patient is a feasible strategy to improve the patient's health and increase their survival through selecting appropriate treatment strategies.

In the majority of the patients (> 85%), our CRS measurements were performed upon the first hospital admission. Critically, we meticulously confirmed that none of the subjects had consumed antioxidant supplements prior to this visit, thereby indicating a clear picture of a hyper-reductive condition associated with HF. In addition to providing the first evidence for an abnormally reductive state in human HF patients, another key finding of our study is the wide heterogeneity of the CRS in response to HF. Along these lines, the prevalence of NR and HR in a considerable number of participants contradicts the expected HO. Considering that ROS/RNS serve as key cellular signaling molecules for basal physiology and responses/adaptations to acute/chronic stress conditions [47–49], patients who exhibit NR or HR may have not gained protection from developing HF. Altogether, these results indicate that in contrast to the common understanding, oxidative stress is not the only factor inducing HF.

Next, our detailed analyses of cardiac structure and function among the 3 classes of HF patients revealed interesting information. Although there is no statistically significant differences among the groups, most of the HO patients displayed severe systolic dysfunction with an ejection fraction $\leq 30\%$ when compared with NR and HR group. It has been established that an over production of ROS/RNS adversely alters cardiac function eventually leading to diastolic and systolic dysfunction [50, 51]. Moreover, left ventricular (LV) dysfunction in HF patients correlates well with the extent of oxidative stress in the myocardium and plasma [50]. In the HR group none of the patients had HFpEF and only 5% in NR group. Hence, both the HR and NR groups predominantly have mild to moderate systolic dysfunction. As such, we speculate that oxidative stress could be a major cause for severe systolic dysfunction rather than reductive stress. In addition to systolic function, we assessed the grade of diastolic dysfunction among the three groups of HF patients utilizing noninvasive Doppler echocardiography mitral inflow (E/A ratio). Surprisingly, the HR group exhibited a higher percentage of patients with a restrictive filling pattern while none for normal filling pattern. However, all other groups had almost equal percentage of patients with normal filling pattern. From our data, it is apparent that patients with RS have severe diastolic dysfunction as compared with normal redox and oxidative stress conditions. Specifically, reductive stress imparts both mild to moderate systolic dysfunction and severe diastolic dysfunction among HF patient groups. There has been accumulating evidence indicating that HF with systolic dysfunction is associated with oxidative stress and nitric oxide (NO) signaling [52] whereas our observations suggest a novel role for reductive stress in the development of diastolic dysfunction.

Conclusion

In this study, we believe that we provided evidence supporting the presence of a hyper-reductive (i.e. reductive stress) condition in a subset of HF patients. Notably, we observed that the HR–HF group had a higher percentage of patients with diastolic dysfunction while the HO–HF group exhibited a higher percentage of patients with systolic dysfunction. Further, individual differences in CRS during the development of HF are apparent suggesting unique contributions of redox disequilibrium across distinct classes of patients. The exact nature of the mechanisms responsible for the heterogeneity in redox responses in the context of HF is presently unknown. We

consider that the wide inter-individual variability for CRS shown here is not limited to the two biomarkers (GSH and MDA) of the redox milieu. Remarkably, the data presented herein emphasize that the mean CRS of a group of HF patients can be misleading. We also acknowledge that this pilot study included a small (but tightly controlled for age and other comorbidities) and male dominant cohort of HF patients. Therefore, our future investigations will focus on larger groups including both genders with specific types of HF such as ischemic disease, hypertrophic cardiomyopathy, dilated cardiomyopathy, and rheumatoid cardiac diseases, and will accentuate differences in diastolic vs. systolic dysfunctions.

Authors' contributions
TS and NSR conceived the idea, performed the experiments and wrote the manuscript. MS performed experiments and collected data. RG, SR and SR provided samples, resources and processed ethical approvals. ANP and SMP critically reviewed and revised the clinical portion of the results. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Consent for publication
Written consent for publication was obtained from the patients.

Ethics approval and consent to participate
The study was approved by the PSG Institutional Human Ethics Committee (IHEC) and all patients/subjects completed a written informed consent prior to their participation.

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References

1. Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. Oxid Med Cell Longev. 2009;2:259–69.
2. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol. 2011;301:H2181–90.
3. Blomhoff R. Dietary antioxidants and cardiovascular disease. Curr Opin Lipidol. 2005;16:47–54.
4. Gotto AM. Antioxidants, statins, and atherosclerosis. J Am Coll Cardiol. 2003;41:1205–10.
5. Ben-Haim S, Gacinovic S, Israel O. Cardiovascular infection and inflammation. Semin Nucl Med. 2009;39:103–14.
6. Gorski PA, Ceholski DK, Hajjar RJ. Altered myocardial calcium cycling and energetics in heart failure—a rational approach for disease treatment. Cell Metab. 2015;21:183–94.
7. Van Linthout S, Tschope C. Inflammation—cause or consequence of heart failure or both? Curr Heart Fail Rep. 2017;14:251–65.
8. Zhou Q, Zhao C, Xie D, Xu D, Bin J, Chen P, Liang M, Zhang X, Hou F. Acute and acute-on-chronic kidney injury of patients with decompensated heart failure: impact on outcomes. BMC Nephrol. 2012;13:51.
9. Bagheri F, Khori V, Alizadeh AM, Khalighfard S, Khodayari S, Khodayari H. Reactive oxygen species-mediated cardiac-reperfusion injury: mechanisms and therapies. Life Sci. 2016;165:43–55.
10. Bilenko MV, Vladimirov IuA, Pavlova SA, Thu Thuy NT, Hai Yen TT. Production of reactive oxygen species by monocyte-derived macrophages from the blood of healthy donors and patients with IHD. Biomed Khim. 2008;54:445–53.
11. Ellidag HY, Eren E, Yilmaz N, Cekin Y. Oxidative stress and ischemia-modified albumin in chronic ischemic heart failure. Redox Rep. 2014;19:118–23.
12. Kritharides L, Stocker R. The use of antioxidant supplements in coronary heart disease. Atherosclerosis. 2002;164:211–9.
13. Saremi A, Arora R. Vitamin E and cardiovascular disease. Am J Ther. 2010;17:e56–65.
14. Tornwall ME, Virtamo J, Korhonen PA, Virtanen MJ, Taylor PR, Albanes D, Huttunen JK. Effect of alpha-tocopherol and beta-carotene supplementation on coronary heart disease during the 6-year post-trial follow-up in the ATBC study. Eur Heart J. 2004;25:1171–8.
15. Akhlaghi M, Bandy B. Mechanisms of flavonoid protection against myocardial ischemia–reperfusion injury. J Mol Cell Cardiol. 2009;46:309–17.
16. Breitbart E, Lomnitski L, Nyska A, Malik Z, Bergman M, Sofer Y, Haseman JK, Grossman S. Effects of water-soluble antioxidant from spinach, NAO, on doxorubicin-induced heart injury. Hum Exp Toxicol. 2001;20:337–45.
17. Qin CX, Williams SJ, Woodman OL. Antioxidant activity contributes to flavonol cardioprotection during reperfusion of rat hearts. Free Radical Biol Med. 2011;51:1437–44.
18. Briancon S, Boini S, Bertrais S, Guillemin F, Galan P, Hercberg S. Long-term antioxidant supplementation has no effect on health-related quality of life: the randomized, double-blind, placebo-controlled, primary prevention SU.VI.MAX trial. Int J Epidemiol. 2011;40:1605–16.
19. Tribble DL. AHA Science Advisory. Antioxidant consumption and risk of coronary heart disease: emphasis on vitamin C, vitamin E, and beta-carotene: a statement for healthcare professionals from the American Heart Association. Circulation. 1999;99:591–5.
20. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet. 2003;361:2017–23.
21. Hare JM, Stamler JS. NO/redox disequilibrium in the failing heart and cardiovascular system. J Clin Invest. 2005;115:509–17.
22. Pagliaro P, Penna C. Redox signalling and cardioprotection: translatability and mechanism. Br J Pharmacol. 2015;172:1974–95.

23. Sugamura K, Keaney JF Jr. Reactive oxygen species in cardiovascular disease. Free Radic Biol Med. 2011;51:978–92.
24. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84.
25. King M, Kingery J, Casey B. Diagnosis and evaluation of heart failure. Am Fam Physician. 2012;85:1161–8.
26. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015;16:233–70.
27. Wilson DJ, North N, Wilson RA. Comparison of left ventricular ejection fraction calculation methods. Echocardiography. 1998;15:709–12.
28. Rezaei Z, Jamshidzadeh A, Sanati E. A rapid and sensitive method for the determination of malondialdehyde as its hydralazine derivative in human plasma using high performance liquid chromatography. Anal Methods. 2013;5:2995–9.
29. Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat Protoc. 2006;1:3159–65.
30. Rajasekaran NS, Connell P, Christians ES, Yan LJ, Taylor RP, Orosz A, Zhang XQ, Stevenson TJ, Peshock RM, Leopold JA, et al. Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. Cell. 2007;130:427–39.
31. Martensson J, Meister A. Glutathione deficiency increases hepatic ascorbic acid synthesis in adult mice. Proc Natl Acad Sci USA. 1992;89:11566–8.
32. Javed M, Ahmad MI, Usmani N, Ahmad M. Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in *Channa punctatus* exposed to heavy metal loaded waste water. Sci Rep. 2017;7:1675.
33. Shangari N, O'Brien PJ. Catalase activity assays. Curr Protoc Toxicol. 2006. https://doi.org/10.1002/0471140856.tx0707s27.
34. Frasier CR, Sloan RC, Bostian PA, Gonzon MD, Kurowicki J, Lopresto SJ, Anderson EJ, Brown DA. Short-term exercise preserves myocardial glutathione and decreases arrhythmias after thiol oxidation and ischemia in isolated rat hearts. J Appl Physiol. 1985;2011(111):1751–9.
35. Noeman SA, Hamooda HE, Baalash AA. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetol Metab Syndr. 2011;3:17.
36. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T, Flachskampf FA, Gillebert TC, Klein AL, Lancellotti P, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American society of echocardiography and the European association of cardiovascular imaging. Eur Heart J Cardiovasc Imaging. 2016;17:1321–60.
37. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T, Flachskampf FA, Gillebert TC, Klein AL, Lancellotti P, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American society of echocardiography and the European association of cardiovascular imaging. J Am Soc Echocardiogr. 2016;29:277–314.
38. Machado FS, Tanowitz HB, Ribeiro AL. Pathogenesis of chagas cardiomyopathy: role of inflammation and oxidative stress. J Am Heart Assoc. 2013;2:e000539.
39. Ribeiro-Samora GA, Rabelo LA, Ferreira ACC, Favero M, Guedes GS, Pereira LSM, Parreira VF, Britto RR. Inflammation and oxidative stress in heart failure: effects of exercise intensity and duration. Braz J Med Biol Res. 2017;50:e6393.
40. Moris D, Spartalis M, Spartalis E, Karachaliou GS, Karaolanis GI, Tsourouflis G, Tsilimigras DI, Tzatzaki E, Theocharis S. The role of reactive oxygen species in the pathophysiology of cardiovascular diseases and the clinical significance of myocardial redox. Ann Transl Med. 2017;5:326.
41. Moris D, Spartalis M, Tzatzaki E, Spartalis E, Karachaliou GS, Triantafyllis AS, Karaolanis GI, Tsilimigras DI, Theocharis S. The role of reactive oxygen species in myocardial redox signaling and regulation. Ann Transl Med. 2017;5:324.
42. Brewer AC, Mustafi SB, Murray TV, Rajasekaran NS, Benjamin IJ. Reductive stress linked to small HSPs, G6PD, and Nrf2 pathways in heart disease. Antioxid Redox Signal. 2013;18:1114–27.
43. Kannan S, Muthusamy VR, Whitehead KJ, Wang L, Gomes AV, Litwin SE, Kensler TW, Abel ED, Hoidal JR, Rajasekaran NS. Nrf2 deficiency prevents reductive stress-induced hypertrophic cardiomyopathy. Cardiovasc Res. 2013;100:63–73.
44. Zhang X, Min X, Li C, Benjamin IJ, Qian B, Zhang X, Ding Z, Gao X, Yao Y, Ma Y, et al. Involvement of reductive stress in the cardiomyopathy in transgenic mice with cardiac-specific overexpression of heat shock protein 27. Hypertension. 2010;55:1412–7.
45. Rajasekaran NS, Varadharaj S, Khanderao GD, Davidson CJ, Kannan S, Firpo MA, Zweier JL, Benjamin IJ. Sustained activation of nuclear erythroid 2-related factor 2/antioxidant response element signaling promotes reductive stress in the human mutant protein aggregation cardiomyopathy in mice. Antioxid Redox Signal. 2011;14:957–71.
46. Yu Q, Lee CF, Wang W, Karamanlidis G, Kuroda J, Matsushima S, Sadoshima J, Tian R. Elimination of NADPH oxidase activity promotes reductive stress and sensitizes the heart to ischemic injury. J Am Heart Assoc. 2014;3:e000555.
47. Afanas'ev I. ROS and RNS signaling in heart disorders: could antioxidant treatment be successful? Oxid Med Cell Longev. 2011;2011:293769.
48. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30:11–26.
49. Yan LJ. Positive oxidative stress in aging and aging-related disease tolerance. Redox Biol. 2014;2:165–9.
50. Munzel T, Camici GG, Maack C, Bonetti NR, Fuster V, Kovacic JC. Impact of oxidative stress on the heart and vasculature: part 2 of a 3-part series. J Am Coll Cardiol. 2017;70:212–29.
51. Munzel T, Gori T, Keaney JF Jr, Maack C, Daiber A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. Eur Heart J. 2015;36:2555–64.
52. Jeong EM, Dudley SC Jr. Diastolic dysfunction. Circ J. 2015;79:470–7.

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Factors Associated with Timely Initiation of Breast Feeding: a Hospital Based Study

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Abstract

Background: Early or timely initiation of breastfeeding, specifically within 1 h of birth, refers to the best practice recommendation by the World Health Organization (WHO). Timely initiation of breastfeeding has the potential to prevent 22 % of neonatal deaths if all infants were breastfed within an hour after birth [1]. The primary objective of the paper is to estimate the prevalence of breast feeding initiation within first hour of life and the clinical correlates and socio demographic factors. **Material and methods:** This study was conducted at PSG Institute of Medical Science and Research, Coimbatore in the month of July 2018. Mothers with the children age below four years attending paediatric outpatient department were included in the study. Multivariate logistic analysis was usedto determine the factors associated with timely initiation breastfeeding practice. **Results:** The prevalence of timely initiation of breastfeeding within one hour was 56.5%. It was observed that the caesarean delivery, preterm delivery, sick condition of the baby, earlier breast surgery, lack of counselling after delivery and lack of skin to skin contact at birth were negatively associated with timely initiation of breastfeeding within one hour. **Conclusion:** The findings clearly indicated that nearly half of the mothers did not initiate breastfeeding within one hour of birth.

Key Words: breastfeeding, prevalence, sociodemographic factors, timely initiation.

Introduction

Timely initiation of breastfeeding is defined as putting the new born to the breast within one hour of birth [1] .Early or timely initiation of breastfeeding, specifically within 1 h of birth, refers to the best practice recommendation by the World Health Organization (WHO) [1]. Few studies revealed that breastfeeding initiation after the first hour of birth doubles the risk of neonatal mortality [2] and the promotion of breast feeding within one hour is a key component in child survival. In addition it has also been shown that a total of 71% of neonatal mortality could be prevented by using antenatal, intra-partum and post natal interventions related to breastfeeding. In addition, many studies reported that the initiation of breast feeding within 24 hours of birth was significantly associated with reduction in 'all cause of mortality'. Recognizing the importance of timely initiation of breastfeeding, the Ethiopian government had developed infant and young child feeding guidelines giving appropriate emphasis to key messages on timely initiation of breastfeeding in 2004. Early initiation of breastfeeding (EIBF) promotes the release of oxytocin that enables contraction of the uterus and decreases postpartum haemorrhage [3]. Though the breast feeding within first hour of life is considered as an indicator of excellence of breast feeding and only 39%

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of the new born in the developing countries are put to breast within one hour of birth. According to national family health survey 3, in India, the rate of early initiation of breast feeding stands low at 24.5% [4]. The characteristics associated with the practice have been little investigated. Hence the current aim is to estimate the prevalence of breastfeeding initiation within first hour of life and examine the factors associated with timely initiation of breastfeeding.

Materials and Methods

Study methods and participants

This study was conducted in the month of July 2018 at PSG Institute of Medical Science and Research, Coimbatore. Ethical clearance from the institution was obtained prior to the study. A total of 527 mothersof children under four years were participated in the study.Mothers with the children age below four years attending paediatric outpatient department were included in the study. Babies with congenital anomalies that interfere with breastfeeding were excluded from the study. A structured interviewer administered questionnaire was used to collect data from mothers about timely initiation of breast feeding related with youngest child. The mothers were asked how long after birth their babies were first put to the breast. Initiating breastfeeding within one hour was categorized as "yes", whereas later than one hour was categorized as "no".The socio-demographic characteristics considered were as education of the mother, age of the mother, occupation of the mother, education of the father, socio economic status, gender of the child, gestational age of the child, birth weight of the child, mode of delivery, condition of the child soon after delivery and counselling for breastfeeding after delivery. Modified BG Prasad socioeconomic scale has been inuse for determining the socio-economic status with people having per-capita income greater than or equal to Rs.6180 is included in class 1, class 2 ranging from Rs.3090-6179, class 3 ranging from Rs.1850-3089, class 4 ranging from Rs.930-1849 and below Rs.930 is included in class 5. The classification is done based on the Consumer Price Index (CPI) value of 279 for Coimbatore [5]. Each study participant was adequately informed about the objectives of the study and obtained consent for ensuring their confidentiality.

Statistical methods

The collected data were checked manually for completeness and entered in to SPSS (23) for analysis. Descriptive statistics was used to summarize and analyse the socio-demographic characteristics of the study participants and the prevalence of timely initiation of breast feeding. Proportions were compared by timely initiation of breast feeding using Chi-square test.To determine the factors associated with timely initiation breastfeeding practice, binary logistic regression analysis carried out. Variables that showed significant association with timely initiation of breastfeeding in the bivariate models were entered in a multivariable logistic model. Strength of association was measured using odds ratio and 95 % confidence intervals. p value < 0.05 was considered as statistically significant.

Results

A total of 527 women were studied. The socio-demographic characteristics of children and families were given in Table 1. The prevalence of timely initiation of breastfeeding within one hour was 56.5% (Table 2).

Table 1: Socio demographic characteristics of infants and Families

Characteristics		Number (%)
Infant characteristics Child's age(months) (Mean ± SD)		15.55 ± 11.92
Education of the Mother	Below 12th standard	138(26.2)
	Above 12th standard	389(73.8)
Mother's age (years)	15-24	108(20.5)
	25-34	388(73.6)
	35-44	31(5.9)

Mother's Occupation	House wife	403(76.5)
	Paid worker	124(23.5)
Education of the Father	Below 12th standard	129(24.5)
	Above 12th standard	398(75.5)
Socio economic status	Class 1 & 2	426(80.8)
	Class 3,4 & 5	101(19.2)
Gender of the child	Female	246(46.7)
	Male	281(53.3)
Gestational age of the child	Term	469(89.0)
	Pre term	58(11.0)
Birth weight of the child (gm)	<=2500	126(23.9)
	>2500	401(76.1)
Mode of delivery	Vaginal delivery	336(63.8)
	Caesarean	191(36.2)
Condition of the child	Well	454(86.1)
	Sick	73(13.9)
Counselling after delivery	Yes	326(61.9)
	No	201(38.1)

Table 2: Timely initiation of Breast Feeding (Hours)

Brest feeding in hours	Number (%)
Within one hour	298 (56.5)
Between 1-4hr	78(14.8)
After 4hr	151 (28.7)
Total	527 (100)

In logistic regression analysis, (Table 3) it was observed that the timely initiation of breast feeding was lower among caesarean delivery compared to vaginal delivery and the difference was statistically significant ($p<0.001$). The timely initiation of breastfeeding was more when the condition of mother was well as compared to those mothers with sick condition ($p<0.05$). Timely initiation of breastfeeding was lower among mothers having low birth weight children than children having normal birth weight ($p<0.001$). Late initiation of breast feeding was higher for pre- term babies than term babies($p<0.001$). It was also observed that the mothers with the well children tend to initiate breastfeeding within one hour than sick children ($p<0.001$). Increased number of antenatal visits had significantly higher prevalence of timely initiation of breastfeeding than less antenatal visits ($p<0.01$). The timely initiation of breastfeeding was less in mothers who had earlier breast surgery compared to mothers who had no earlier breast surgery ($p<0.05$). It was also observed that counselling about timely initiation of breastfeeding after delivery ($p<0.001$) and skin to skin contact at birth ($p<0.001$) were significantly associated with timely initiation of breastfeeding.

Table 3: Factors associated with timely initiation of breast feeding in univariate analysis

Variable		Total	Feeding started within one hour (%)	Feeding started after one hour (%)	odds ratio	p value
Total		527	298(56.5)	229(43.5)		
Age (mean, SD)		27.72,3.95	27.69,4.06	27.75,3.82		
Age of the mother (years)	35-44	31(5.9)	15(5)	16(7)	1.676	0.403
	25-34	388(73.6)	217(72.8)	171(74.7)	1.238	
	15-24	108(70.5)	66(22.2)	42(18.3)	1	
Education of the mother	Above 12th	389(73.8)	226(75.8)	163(71.2)	0.787	0.228
	Below 12th	138(26.2)	72(24.2)	66(28.8)	1	
Occupation of the mother	Employed	124(23.5)	73(24.5)	51(22.3)	0.883	0.550
	House wife	403(76.5)	225(75.5)	178(77.7)	1	
Mode of delivery	caesarean	191(36.2)	46(15.4)	145(63.3)	9.457	P<0.001
	vaginal	336(63.8)	252(84.6)	84(36.7)	1	
Condition of mother	Sick	28(5.3)	11(3.7)	17(7.4)	2.092	P<0.05
	Well	499(94.7)	287(96.6)	212(92.2)	1	
Use of medication	Yes	66(12.5)	35(11.7)	31(13.5)	1.176	0.538
	No	461(87.5)	263(88.3)	198(86.5)	1	
Religion	others	2(0.4)	2(7.0)	0(0)	0.000	0.513
	Muslim	28(5.3)	18(6.1)	10(4.3)	0.704	
	Christian	48(9.1)	27(9.1)	21(9.1)	0.986	
	Hindu	449(85.2)	251(84.2)	198(86.5)	1	
Gender of the baby	female	246(46.7)	150(50.3)	96(41.9)	0.712	0.055
	male	281(53.3)	148(49.7)	133(58.1)	1	
Birth weight of the baby(Kg) (mean, SD)		2.88,0.56	2.97,0.45	2.76,0.65		
Birth weight of the baby(gram)	<=2500	126(23.9)	53(17.8)	73(31.9)	0.462	P<0.001
	>2500	401(76.1)	245(82.2)	156(68.1)	1	
Gestational age at delivery	Pre term	58(11.0)	12(4.0)	46(20.1)	5.991	P<0.001
	Term	469(89.0)	286(96.0)	183(79.9)	1	
Condition of baby	Sick	73(13.9)	26(8.7)	47(20.4)	2.702	p<0.001
	Well	454(86.1)	272(91.3)	182(79.6)	1	
Education of the father	Above 12th	398(75.5)	232(77.9)	166(72.5)	0.750	0.156
	Below 12th	129(24.5)	66(22.1)	63(27.5)	1	
Parity	>=2	162(30.7)	98(32.9)	64(27.9)	.792	0.223
	1	365(69.3)	200(67.1)	165(72.1)	1	
Past experience in breast feeding	No	366(69.4)	200(67.1)	166(72.5)	1.291	0.184
	Yes	161(30.6)	98(32.9)	63(27.5)	1	
Antenatal visits	>8	379(71.9)	231(77.5)	148(64.6)	1	p<0.01
	≤8	148(28.1)	67(22.5)	81(35.4)	1.887	
Earlier Breast surgery	Yes	6(1.1)	1(0.3)	5(2.2)	6.629	p<0.05
	No	521(98.9)	297(99.7)	224(97.8)	1	

Counselling during antenatal visits	No	268(50.9)	146(49)	122(53.3)	1.187	0.330
	Yes	259(49.1)	152(51)	107(46.7)	1	
Counselling after delivery	No	201(38.1)	82(27.5)	119(52)	2.85	p<0.001
	Yes	326(61.9)	216(72.5)	110(48)	1	
skin to skin contact at birth	No	311(59.0)	132(44.3)	179(78.2)	4.502	p<0.001
	Yes	216(41.0)	166(55.7)	50(21.8)	1	
Socio-economic status	Class 5	4(0.8)	1(0.3)	3(1.3)	3.649	0.498
	Class 4	25(4.7)	12(4)	13(5.7)	1.318	
	Class 3	72(13.7)	42(14.1)	30(13.1)	0.869	
	Class 2	180(38.0)	122(36.2)	78(31.4)	0.811	
	Class 1	246(42.5)	135(45.3)	111(48.5)	1	

Inthe multivariate logistic regression analysis (Table 4)it was observed that caesarean delivery (p<0.001), preterm delivery (p<0.01), sick condition of the baby (p<0.01), earlier breast surgery (p<0.01), lack of counselling after delivery (p<0.001) and lack of skin to skin contact at birth (p<0.05) were negatively associated with timely initiation of breastfeeding within one hour.

Table 4: Factors associated with delayed initiation of breast feeding in multivariate analysis

Variables		**Adjusted Odds ratio**	**95% CI**	**p value**
Mode of delivery	caesarean	7.908	4.905-12.748	p<0.001
	vaginal	1		
Condition of mother	Sick	2.189	0.814-5.888	0.121
	Well	1		
Gestational age at delivery	Pre term	3.344	1.447-7.728	p<0.01
	Term	1		
Condition of the baby	Sick	2.943	1.280-4.858	p<0.01
	Well	1		
Antenatal visits	≤8	1.406	0.869-2.275	0.165
	>8	1		
Earlier breast surgery	Yes	20.378	1.962-211.695	p<0.01
	No	1		
Counselling after delivery	No	2.626	1.676-4.115	p<0.001
	Yes	1		
skin to skin contact at birth	No	1.847	1.144-2.983	p<0.05
	Yes	1		
Birth weight of the baby	>2500	0.736	0.428-1.265	0.267
	<=2500	1		

Discussion

Globally, around four million new born die, most from preventable causes each year. Death in the neonatal period accounts for 41 % of all deaths in children below four years. Timely initiation of breastfeeding can help to prevent neonatal deaths caused by infections such as sepsis, pneumonia and diarrhoea[6].In India, a multipronged strategy including large scale programmes, effective capacity building initiatives, strong partnerships, community-

based action, and strategic mass media communication led to an increase in the rates of early initiation of breastfeeding from 24.5% in 2006 to 44.6% in 2014 (i.e. a 1.8-fold increase) [7]. More recently, analyses on a large cohort of almost 100 000 new born from three large trials in Ghana, India and Tanzania has shown that, compared with infants who initiated breast feeding within the first hour of life, the risk of neonatal death among children who initiated breast feeding between 2 and 23 hours after birth was 41% higher (RR 1.41; 95% CI 1.24 to 1.62), and 79% higher among those who initiated breast feeding at 24–96 hours of birth (RR 1.79; 95% CI 1.39 to 2.30) [7].

In thisstudy,caesarean delivery was associated with delayed breastfeeding initiation compared to vaginal delivery.Caesarean delivery is the most consistent predictor of late initiation of breastfeeding reported by many studies conducted in Ethiopia and abroad[8]. Mothers might be uncomfortable to breastfeed due to the pain experienced after surgery and the related factors associated with caesarean delivery.

It was observed that the gestational age of the child is a predictor of timely initiation of breastfeeding. The timely initiation of breastfeeding was significantly lower in preterm children. This result may be explained by the common problems, such as limited oral-motor skills, delayed lactogenesis in prematurely delivered mothers and babies and babies were used to give formula feed from hospital. Thus, effective antenatal care to prevent premature births will contribute to early breastfeeding initiation. The observational prospective study conducted on 500 new born delivered at Department of Rajkiya Mahila Chikitsalaya (RMC), Ajmer and out born section of Department of paediatrics, JLN MC, Ajmer reported that preterm infants had significantly lower rate of early initiation of breastfeeding than term infants[9].

Furthermore, condition of the baby was significantly associated with timely initiation of breastfeeding. It was observed that sick condition of the baby adversely affect the initiation of breastfeeding within one hour. Babies who may have sick at birth include those who experienced a difficult delivery, jaundice after delivery,those with birth defects, those born prematurely and these problems can lead to late initiation of breastfeeding. Fortunately for these babies special care is available. New born babies who need intensive medical attention are often admitted in to Neonatal intensive care unit (NICU) for hours after delivery.so mothers failed to initiate breastfeeding in the first hour after delivery.

Earlier breast surgery was found to be statistically significant with timely initiation of breastfeeding. Women undergo breast surgery for many reasons. Augmentations, reductions, mastectomies, lumpectomies, and biopsies, are often carried out on women of childbearing age. These surgeries can adversely affect timely initiation of breastfeeding and the ability to make healthy breast milk supply for a child.

In addition we have also observed a significant association between timely initiation of breast feeding and counselling about timely initiation after delivery. Counselling about timely initiation has a major impact on timely initiation of breastfeeding. The findings suggest that more efforts are needed to create awareness in providing counselling for the timely initiation during antenatal visits and delivery time. This might be related to the fact that mothers received breastfeeding information/counselling immediately after delivery which is the most appropriate time for delivering key messages about breastfeeding[10].

Baby put on mother's abdomen (skin to skin contact between mother and baby) is found to be an important factor to initiate breast feeding early. WHO recommends that early skin to skin contact between mother and baby helps to initiate early breast feeding.

Birth weight of the child is a predictor in the association of timely initiation of breast feeding in univariate analysis. Socio economic status did not have any association with timely initiation of breast feeding. Previous studies showed that initiations of breastfeeding in low birth weight children were significantly delayed [9]. Also, here educational status of mother did not have any significant association with timely initiation of breast feeding ($p>0.05$). Prior findings showed that education was found to have predominant importance for timely initiation of breastfeeding. Parity and initiation of breast feeding were found to have a lack of association in this study.

This study involves several limitations. One limitation is the recall bias caused by difference in the accuracy or completeness of the recollections retrieved by study participants regarding the breastfeeding practice from the past (retrospective reporting). Another limitation is that the region of study has a generally higher socio-economic status and it was a hospital based study. Community based study program is an approach that emphasize on community empowerment as an important tool in health promotion. Even though the study has several strengths. We have obtained a large number of demographic and clinical data which can influence the association of timely initiation of breastfeeding. All respondents are asked the same basic questions in the same order by the same interviewer tominimize the potential observer bias. Advanced statistical analysis was used to analyse the association

of socio demographic and clinical correlates with timely initiation of breastfeeding.

Conclusion

This study revealed that the prevalence of timely initiation of breastfeeding was significantly lower than the present WHO recommendations [11]. The findings clearly indicate that appropriate strategies are needed to minimize delays in initiation of breastfeeding and for practising better timely initiation of breastfeeding within one hour of birth.

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References

1. World Health Organization, UNICEF." Indicators for assessing infant and young child feeding practices" part 3: country profiles. Geneva: WHO. available at http://apps.who.int/iris/bitstream/handle/10665/44368/%209789241599757_eng.pdf;jsessionid=47902C5018B04906526317E6DB2699D7?sequence=1.
2. AnimutAlebel, GetiyeDejenu, GetachewMullu et al. Timely initiation of breastfeeding and its association with birth place in Ethiopia: a systematic review and Metaanalysis,International Breastfeeding Journal 2017;12-44
3. Khanal V, Scott J, Binns CW. Factors associated with early initiation of breastfeeding in Western Nepal, International journal of environment research and public health. 2015;12(8):9562-9574.
4. Manthan Patel, Shetal Prajapti. A comparative analytical study of knowledge, attitude and practice of breastfeeding in pri,I and multipara women at a tertiary care centre in Gujarat, India,International journal of research in medical science. 2016;4(10):4403-4407.
5. Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers. Available from: http://labourbureaunew.gov.in/UserContent/Linking_factor_CPI_IW.pdf?pr_id. Accessed August 10, 2018.
6. Ekubay M, Berhe A, Yism E. Initiation of breastfeeding within one hour of birth among mothers with infants younger than or equal to 6 months of age attending public health institutions in Addis Ababa, Ethiopia Int Breastfeed J. 2018; 13
7. Víctor M Aguayo, Gagan Gupta, Gayatri Singh et al. Early initiation of breast feeding on the rise in India,BMJ Glob Health2016; 1(2): e000043.
8. Abdul basit Musa Seid. Vaginal Delivery and Maternal Knowledge on Correct Breastfeeding Initiation Time as Predictors of Early Breastfeeding Initiation, Lesson from a Community-Based Cross-Sectional Study. ISRN Epidemiology. 2014, 3–5.
9. NavneetBadaya,Sanjiv Jain, Nayan Kumar. Time of initiation of breastfeeding in various modes of delivery and to observe the effect of low birth weight and period of gestation on initiation of breastfeeding,Int J ContempPediatr 2018;5(4):1509-1517.
10. Gelete BW, Addisu YK, Haile MT. Timely Initiation of Breastfeeding and Its Associated Factors among Mothers in Tiyo Woreda, Arsi Zone, Ethiopia: A Community- Based Cross Sectional Study,Clinics Mother Child Health. 2016;13-221.
11. Tiwari, Bharadva, Yadav, Malik, Gangal, et al. Infant and Young Child Feeding Guidelines, Indian paediatrics. 2016; 53:703-713.

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Factors Associated with Hypertension Among Truck Drivers: A Cross Sectional Study at A Check Post on A National Highway in South India

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ABSTRACT

Background: *Workers in the transport industry are at greater risk for cardio vascular diseases (CVD) due to their increased diet and sedentary behavior.* ***Aims:*** *The aim of the present study is to estimate the prevalence of hypertension among truck drivers and its associated factors.* ***Methods and materials:*** *This study was conducted at Walayar check post which is located on a National highway of South India in August 2016. A detailed physical examination was conducted by a team of medical doctors. Socio demographic information were also elicited.* ***Results:*** *The prevalence of overweight was 50% and the prevalence of hypertension was 40%. In multivariate data analysis, only history of chronic disease was significantly associated with hypertension. Overweight was not significantly associated with hypertension when controlled for age, duration of driving, smoking, marital status, and history of chronic disease (odds ratio=1.987, P=0.054). On further stratified analysis, it was found that higher age (odds ratio=3.160 P<0.001) and longer duration of driving (odds ratio=1.076 P<0.001) were significantly associated with overweight. Overweight (odds ratio=2.210 P<0.05) and history of chronic disease (odds ratio=10.178 P<0.001) were significantly associated with high blood pressure after removing both age and duration of driving from this model. Receiver operating characteristic curve analysis relating age with overweight identified 38 years as the optimum cut off value.* ***Conclusions:*** *The truck drivers must be educated at their younger age preferably early thirties to prevent overweight and thereby to reduce hypertension.*

Keywords: Truck drivers, hypertension, overweight, obesity

INTRODUCTION

The social and economic effect of increased morbidity and mortality associated with cardiovascular diseases (CVD) is a great challenge in both developed and developing countries [1-3]. Many studies have demonstrated that there is a strong correlation between risk factors of cardiovascular diseases and occupational factors [4-7]. Workers in the transport industry are at greater risk of an increased diet and sedentary behavior [8,9]. Truck drivers in India have to travel long distances in their life time on an extensive spread of national and state high ways that range from well-engineered roads to complete absence of concrete roads. Their occupation pre-disposes them to multitude of risk factors such as prolonged sitting and driving, tight running schedules, reduced rest breaks, traffic congestion, the sedentary nature of job and resultant physical, psychological, and behavioral problems [8-10]. Thus, professional drivers in particular have higher mortality, morbidity and absenteeism rates due to obesity. Hypertension is one of the main outcomes of the disease and is common among professional drivers. Considering the inherent risks associated with the profession of truck drivers, the importance of truck drivers to the country's economy and scarcity of studies aimed at evaluating the cardiovascular risk profile among these professionals, the present study is aimed to assess the prevalence of hypertension and the factors associated with hypertension among long distance truck drivers.

METHODS

Study design

This was a cross sectional study conducted at Walayar check post which is located on a National highway of South India on August 2016.

Ethics

Ethical clearance for the study was obtained from Institutional Ethical Committee, PSGIMS&R, Coimbatore.

Sample size: With an expected prevalence of hypertension as 45% [9] and with 20% allowable error, the sample size required for this study was estimated as 122. With a non-response of 20%, the total sample was 153. We totally studied 175 truck drivers.

Inclusion and exclusion criteria

Drivers who came with valid driving license for submission of documents at the check post on the day of survey and those who gave informed consent were included. Drivers with known congenital heart disease and genetic disorders were excluded.

Methodology

A detailed physical examination was conducted by a team of medical doctors. Blood pressure was measured on right arm in sitting position using digital sphygmomanometer. Two readings were taken at 3 minutes interval and the mean of the two was recorded. The following variables were also evaluated: socio demographic characteristics (age, education, family history of CVD, prior diagnosis of chronic disease), physically active, eating patterns, consumption of alcohol or other stimulants and smoking habits.

The following criteria and definitions were used [9]:

- Family history of CVD: when the individual mentions the occurrence of heart disease in a first degree relative.
- History of chronic diseases: truck drivers were asked whether they had been previously being diagnosed with any chronic diseases and what medicines they were taking.
- Subjects were considered physically active if they are performing aerobic (walking, biking, dancing, and racing) or anaerobic activities (body building) at least 150 minutes in a week and moderately active if they do 10 to 150 minutes in a week and insufficient if they do less than 10 minutes in a week.
- Subjects were considered active smokers if they use tobacco daily in any quantity or at quit smoking in the previous 12 months. Subjects were considered non-smokers if they had not used tobacco for the past 12 months.
- For determining the body mass index (BMI) the truck drivers were weighed (in kg) without shoes on a 150 kg mechanical anthropometric scale and height in meter. The BMI levels were classified according to the WHO guidelines [11]; subjects were normal if their BMI was 18.5 kg/m^2 to 24.5 kg/m^2, overweight if their BMI was 25.0 kg/m^2 to 29.9 kg/m^2, grade 1 obese if the BMI was 30.0 kg/m^2 to 34.9 kg/m^2, grade 2 obese if the BMI was 35.0 kg/m^2 to39.9 kg/m^2 and grade 3 obese if the BMI $\geq$ 40.0 kg/m^2.

Statistics

Data were analysed using SPSS (19). Mean (SD) were calculated for continuous variables and percentages were calculated for categorical variables. Logistic regression analysis was done to estimate the odds ratio and its statistical significance. Hypertension was defined as present in those with systolic blood pressure greater than or equal to 140 mm Hg or those with diastolic blood pressure greater than or equal to 90 mm Hg. For statistical analysis, BMI>25 kg/m^2 were considered as overweight. Variables significantly associated ($P<0.2$) with hypertension were subjected to multivariate regression analysis. However, due to multicollinearity, stratified analysis was further done to observe the association. Receiver operating characteristic curve analysis was then done to find the optimum cut off value.

RESULTS

The mean age of the study participants were 39.38 years with the minimum age of 22 years and the maximum of 67

years. The prevalence of overweight was 50% and the prevalence of hypertension was 40% (Table 1). In univariate analysis, positive family history, overweight, history of chronic disease, smoking, longer duration of driving, marital status and older age were significantly associated with hypertension (P<0.2) (Table 2). However, in the multivariate analysis, only history of chronic disease was found to be significant. Overweight was not significantly associated with hypertension when controlled for age, duration of driving, smoking, marital status and history of chronic disease (odds ratio=1.987; P=0.054). On further analysis, it was found that higher age (odds ratio=3.160; P<0.001) and longer duration of driving (odds ratio=1.076; P<0.001) were significantly associated with overweight. Overweight (odds ratio=2.210; P<0.05) and history of chronic disease (odds ratio=10.178; P<0.001) were significantly associated with high blood pressure after removing both age and duration of driving from this model. Receiver operating characteristic curve analysis relating age with overweight identified 38 years as the optimum cut off value. Hence it is concluded that the truck drivers must be educated at their younger age preferably early thirties to prevent overweight and thereby to reduce hypertension.

Table 1 Prevalence of hypertension and obesity among the study population

Condition	Number N=175	%
Hypertension	70	40
Raised systolic blood pressure	48	27.4
Raised diastolic blood pressure	51	29.1
Overweight/Obesity	86	49.1

Table 2 Variables associated with hypertension

Variable	Status	Number	Prevalence	Odds ratio	P value
Positive family history	Yes	73	45.2	1.449	0.234
	No	102	36.3	1	
Overweight	Yes	86	50	2.296	0.008*
	No	89	30.3	1	
Family history of chronic disease	Yes	25	84	10.821	0.000*
	No	150	32.7	1	
Smoking	Yes	98	44.9	1.558	0.136*
	No	77	33.8	1	
Old age	Yes	87	47.1	1.813	0.056*
	No	88	33	1	
Marital status (Married)	Yes	137	45.3	3.1	0.007*
	No	38	21.1	1	
Physical activity (Active)	Yes	49	38.8	0.931	0.837
	No	126	40.5	1	
Duration of driving (hours)	-	-	-	1.072	0.000*
P<0.2					

DISCUSSION

A number of studies have demonstrated that the high prevalence of obesity among workers in the transport industry. According to Moreno, et al. this category of workers has a higher incidence of obesity, physical inactivity, inadequate diet, smoking habits, higher levels of cholesterol and hypertension [12,13]. A study carried out in the United States involving more than six hundred thousand workers found the highest prevalence of obesity to be among male employees who work in the transport services [14]. The worldwide prevalence of hypertension was estimated at 1 billion individuals with approximately 7.1 million deaths occurring per year due to this condition [9]. According to the World Health Organization, systolic blood pressure greater than 115 mm Hg accounts for 62% of cases of cardiovascular disease and 49% of cases of ischemic heart disease. Moreover, arterial hypertension is one of the major risk factors of cerebrovascular accident. In our study, we observed a prevalence of 41%, with a prevalence of raised systolic blood pressure of 27.4% and a prevalence of raised diastolic blood pressure of 29.1% among truck drivers.

We observed that the truck drivers must be educated at their younger age particularly early thirties to prevent overweight and obesity and thereby to reduce hypertension. Young adulthood is a vulnerable period for weight gain and the health consequences of becoming obese during the life span are of serious concern. Unhealthy dieting habits include high sugar sweetened beverage consumption, lower vegetable intake and greater consumption of foods prepared outside

the home, which may place young adult long distance truck drivers at increased risk for overweight and obesity. To improve their nutrition and health, as they progress through the life cycle, it requires approaches specifically targeted to this group. Strategies and programs for obesity prevention of truck drivers should include both individual level and population level approaches. However, the evidence base for prevention of weight gain and halting overweight and obesity in young adulthood truck drivers is currently limited.

CONCLUSION

The data in the present study allow us to draw conclusion that long distance truck drivers are highly vulnerable to develop CVD because of the high prevalence of obesity and hypertension observed. The accumulation and association of risk factors and unique features of this profession indicate that traditional actions could not change the scenario. Appropriate health care actions based on comprehensive policies that focus on the truck drivers work environment and monitoring of these factors must be established. More research into programs tailored to the needs of young truck drivers is warranted.

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REFERENCES

[1] Gersh, Bernard. "The epidemic of cardiovascular disease in the developing world: Global implications." *Circulation* 126.Suppl 21 (2012): A606-A606.

[2] Yusuf, S., et al. "Global burden of cardiovascular diseases-part I: General considerations, the epidemiologic transition, risk factors, and impact of urbanization." *Quarterly Journal of Cardiology* 7.2 (2002): 132-139.

[3] Lopez, Alan D., et al. "Global and regional burden of disease and risk factors, 2001: Systematic analysis of population health data." *The Lancet* 367.9524 (2006): 1747-1757.

[4] Winkleby, Marilyn A., et al. "Excess risk of sickness and disease in bus drivers: A review and synthesis of epidemiological studies." *International Journal of Epidemiology* 17.2 (1988): 255-262.

[5] Albright, Cheryl L., et al. "Job strain and prevalence of hypertension in a biracial population of urban bus drivers." *American Journal of Public Health* 82.7 (1992): 984-989.

[6] Ragland, David R., et al. "Prevalence of hypertension in bus drivers." *AAOHN journal* 37.2 (1989): 71-79.

[7] Bigert, C., et al. "Time trends in the incidence of myocardial infarction among professional drivers in Stockholm 1977-1996." *Occupational and environmental medicine* 61.12 (2004): 987-991.

[8] Udayar, Sharvanan Eshwaran, et al. "Epidemiological study of cardiovascular risk factors among public transport drivers in rural area of Chittoor district of Andhra Pradesh." *International Journal of Community Medicine and Public Health* 2.4 (2017): 415-420.

[9] Hirata, Raquel Pastréllo, et al. "General characteristics and risk factors of cardiovascular disease among interstate bus drivers." *The Scientific World Journal* 2012 (2012).

[10] Sangaleti, Carine Teles, et al. "Prevalence of cardiovascular risk factors among truck drivers in the South of Brazil." *BMC public health* 14.1 (2014): 1063.

[11] Barba, Corazon, et al. "Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies." *The lancet* 363.9403 (2004): 157.

[12] Moreno, C. R. C., et al. "High risk for obstructive sleep apnea in truck drivers estimated by the Berlin questionnaire: Prevalence and associated factors." *Chronobiology international* 21.6 (2004): 871-879.

[13] Moreno, C. R. C., et al. "Short sleep is associated with obesity among truck drivers." *Chronobiology international* 23.6 (2006): 1295-1303.

[14] Caban, Alberto J., et al. "Obesity in US workers: The national health interview survey, 1986 to 2002." *American Journal of Public Health* 95.9 (2005): 1614-1622.

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Genetic Architecture of Parkinson's Disease in the Indian Population: Harnessing Genetic Diversity to Address Critical Gaps in Parkinson's Disease Research

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Over the past two decades, our understanding of Parkinson's disease (PD) has been gleaned from the discoveries made in familial and/or sporadic forms of PD in the Caucasian population. The transferability and the clinical utility of genetic discoveries to other ethnically diverse populations are unknown. The Indian population has been under-represented in PD research. The Genetic Architecture of PD in India (GAP-India) project aims to develop one of the largest clinical/genomic bio-bank for PD in India. Specifically, GAP-India project aims to: (1) develop a pan-Indian deeply phenotyped

clinical repository of Indian PD patients; (2) perform whole-genome sequencing in 500 PD samples to catalog Indian genetic variability and to develop an Indian PD map for the scientific community; (3) perform a genome-wide association study to identify novel loci for PD and (4) develop a user-friendly web-portal to disseminate results for the scientific community. Our "hub-spoke" model follows an integrative approach to develop a pan-Indian outreach to develop a comprehensive cohort for PD research in India. The alignment of standard operating procedures for recruiting patients and collecting biospecimens with international standards ensures harmonization of data/bio-specimen collection at the beginning and also ensures stringent quality control parameters for sample processing. Data sharing and protection policies follow the guidelines established by local and national authorities.We are currently in the recruitment phase targeting recruitment of 10,200 PD patients and 10,200 healthy volunteers by the end of 2020. GAP-India project after its completion will fill a critical gap that exists in PD research and will contribute a comprehensive genetic catalog of the Indian PD population to identify novel targets for PD.

Keywords: Parkinson's disease, genetic diversity, genome-wide association study, common genetic variation, biobank

BACKGROUND

Parkinson's disease (PD) is the second most common neurodegenerative disorder in adults over the age of 60 years (1). According to the Global Burden of Disease study (2018), the worldwide burden of PD has more than doubled over the past two decades from 2.5 million patients in 1990–6.1 million patients in 2016 (2). India is home to nearly 0.58 million persons living with PD as estimated in 2016, with an expected major increase in prevalence in the coming years (2). Despite the large number of people affected with PD, insights into the underlying genetic and environmental risk factors specific to the Indian population are limited. This is in contrast to the Caucasian population in whom easy access to the patient cohort and the population homogeneity have driven initial large scale genome-wide studies (3, 4). Despite the success, the constraints of performing studies in a single homogenous population became apparent as well. This is because the Caucasian population contains only a subset of genetic diversity (5). Populations vary in terms of allele frequency, linkage disequilibrium (LD) patterns, and differences in effect estimates. This provides a scientific rationale that no single population is sufficient to fully uncover the variants underlying disease in all populations, and makes it imperative to pursue genetic research in diverse populations to capture the genetic diversity of a disease.

About 5–10% of PD is monogenic and inherited in an autosomal dominant or recessive manner. The large majority of patients have a sporadic disease. To date, 90 PD loci have been identified explaining a missing heritability in a range of 16–36% (3). It is also increasingly recognized that additional loci with varying degrees of minor allele frequency and effect size remain to be discovered which might account for the remaining missing heritability. Most of the PD loci have been identified in cohorts that are heavily biased toward persons with Caucasian ancestry (3, 4). This generates issues of reproducibility in a global context. For instance, variants in leucine-rich repeat kinase 2 (*LRRK2*), glucocerebrosidase (*GBA*), and alpha-synuclein (*SNCA*) genes identified in the western population have been shown to pose negligible risk to the Indian patients (6–9). Novel variants in the known genes or novel genes may be associated with PD risk in the genetically more diverse Indian population (10). Variations in allele frequency in genetically heterogeneous populations may provide adequate power to GWA studies with smaller sample sizes for the enriched loci. For example, the discovery of an association at a new putative locus at chr1 (PARK16) in the Japanese population for PD underscores the need to study ethnically diverse populations. The associated SNP, rs823128, which was shown to be protective against the development of PD specifically in the Asian population has a minor allele frequency ∼20% in the Japanese population as compared to only 3% in the Caucasian population (11). With this minor allele frequency, individual GWAS in the Caucasian populations had very little power to detect an association, even though the SNP was well-tagged with arrays. The 1,000 Genomes project which uses the combinatorial approach of exome and whole-genome sequencing suggests that individuals from different populations carry different profiles of rare and common variants and that low-frequency variants show substantial geographic differentiation, thus arguing in favor of diversifying genetic research especially in populations which have so far been underrepresented in gene mapping such as the Indian population (12). In addition to the potential for new gene discovery, the inclusion of ethnically diverse cohorts provides an opportunity to cross-validate newly identified loci, which has direct implications for the global applicability and scalability of potential novel therapeutic targets.

We initiated the Genetic Architecture of Parkinson disease in India (GAP-India) project to provide for the first time, a large-scale genetic catalog of the Indian PD population. This paper describes the design of the GAP-India project including the study sites, subject recruitment, clinical assessments, biospecimens processing, plan for data analysis and sharing, capacity building, and the ethical and regulatory frameworks within which we operate.

STUDY DESIGN

GAP-Indiastudy aims to understand the genetic architecture of PD in the Indian population through large scale sample collection and federated data analysis models. The study aims to collect pan-Indian genetic and phenotypic data and will develop one of the largest clinico-genomic PD resources for the scientific community from India. To achieve our objectives, we have formed a trilateral consortium, the Luxembourg-German-Indian Alliance on Neurodegenerative diseases and Therapeutics (Lux-GIANT) (**Figure 1**). The "knowledge-sharing" model aims to build capacity and exchange programs to integrate clinical/genetic centers and harmonize data collection with Luxembourg and German centers. Lux-GIANT follows a decentralized model and based on expertise, different cores have been created (**Figure 2**). For example, the central clinical core in India is established at the Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, Kerala. Given the diversity and vastness of India, apart from the central clinical core, three high volume academic movement disorders centers across India have been identified and established as nodal centers to recruit participants with a pan-India representation. These four clinical nodes are further connected to twenty clinical sub-centers which are spread throughout India. The central clinical core is responsible for coordinating patient recruitment and biospecimen collection with nodal centers. The nodal centers supervise the patient recruitment and biospecimen collection at the sub-centers. Similarly, genomics, functional and bioinformatics cores have been established in Luxembourg, Germany, and India. The functional core at the National Brain Research Center, Manesar, India, is mandated to develop the Lux-GIANT iPSCs biobank. The functional core in India coordinates its activities with the functional cores in Germany and Luxembourg. This has been done to share protocols and develop common functional protocols to perform functional studies. The array processing will be done at the Center for Cellular and Molecular Biology (CCMB), Hyderabad. The central genomics/bioinformatics core at Tubingen will coordinate data generation with the local center and subset of samples will be processed in Germany for quality control purposes.

PROJECTED COHORT AND STUDY SITES

The GAP-India study revolves around a network of clinical sites in India organized in a "hub and spoke" manner. Patient recruitment at each nodal or sub-center will be supervised by a neurologist with specific expertise in Movement Disorders. Subjects will be enrolled at all the sub-centers and the four nodal centers (SCTIMST, Trivandrum; All India Institute of Medical Sciences, New Delhi; National Institute for Mental Health and Neurosciences, Bengaluru and Nizams Institute of Medical Sciences, Hyderabad). The nodal centers are all high-volume academic centers with established movement disorders programs. The sub-centers include additional public sector teaching hospitals, larger multispecialty hospitals, and neurology clinics in the private sector.

Genetic evidence indicates that most Indians descended from a mixture of two divergent populations: Ancestral North Indians (related to Central Asians, Middle Easterners, and Europeans) and Ancestral South Indians (not closely related to other genetic groups) and almost all the current inhabitants are admixtures of these two broad groups to varying extents (13). Within the population, allele frequency changes between subgroups are larger than in European populations, owing to founder effects maintained by a transition to endogamy about 1,900–4,200 years ago (14). The 1,000 genomes project contains about 500 genomes from the Indian subcontinent (including India and geographically neighboring countries), from five diverse linguistic groups, yet the Ancestral North Indian component is prominent in this dataset (15). Within the linguistic groups too, population substructures were evident suggesting that careful matching of cases and controls from within the same ethno-linguistic groups is necessary to avoid false positive associations.

Geographical locations of the enrolling sites in India were chosen to consider this unique population structure and to enable a pan-Indian representation (**Supplementary Figure 1**). The study aims to enroll 10,200 PD patients and 10,200 healthy volunteers over 1 year. Furthermore, GAP-India aims to develop a cohort of 25,000 cases and 25,000 controls by 2024. The four nodal centers will directly enroll about 6,000 patients and the remaining subjects will be enrolled at the sub-centers. The sample size was chosen to take into consideration the statistical power to detect a risk associated variant in GWAS as well as the feasibility of attaining it within the timeframe of the project. The extensive multi-centric nature of the project helps in covering diverse genetic subgroups and meeting recruitment goals within the timelines.

SUBJECT RECRUITMENT

Subjects will be recruited from the Movement Disorder clinics or Neurology clinics run by the PIs of nodal and sub-centers. A detailed history and systemic and general neurological examination will be performed in all subjects. Research staff at all recruiting centers will be trained in the standard operating procedures including clinical assessments and familiarized with online data entry systems before site initiation. Subjects who meet all the following inclusion criteria will be recruited in the patient group: (1) clinical diagnosis of PD as per United Kingdom Parkinson's Disease Society Brain Bank (UKPDSBB) diagnostic criteria (16), (2) age more than 18 years and (3) Asian Indian ethnicity. Subjects meeting any of the following criteria will be

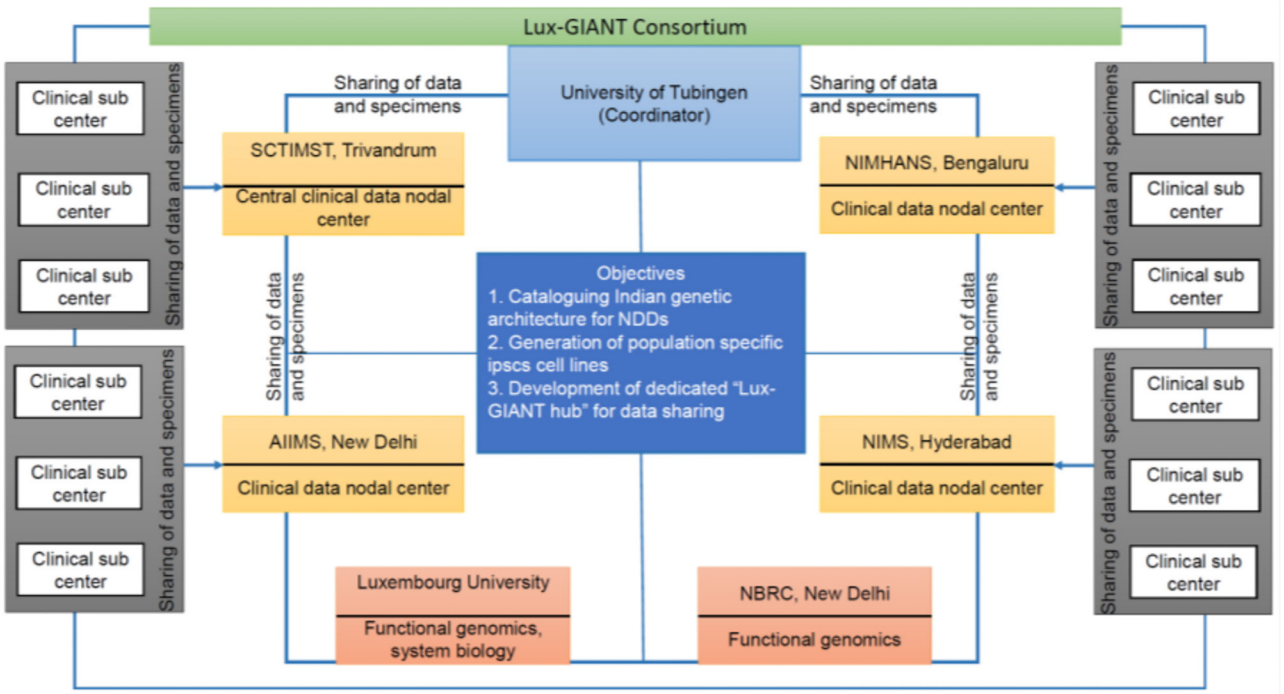


FIGURE 1 | Overall flow chart describing the details of the Lux-GIANT consortium. The consortium follows the "hub-spoke" model. The Lux-GIANT has established three main cores: genomics, clinical, and functional genomics. The University of Tubingen is the coordinator site. Luxembourg site aims to strengthen functional genomics and system biology. Functional core from India, National Brain Research Center, will be responsible for developing and maintaining iPSCs. Four main clinical-nodes capturing most of India are formed. These four nodes are connected to clinical sub-centers which span throughout India. The "central clinical node" aims to streamline the administrative process, which is required for clearance and sample shipment. The different nodes within India are connected with the main "central clinical node" for continuous update of cohort and members.

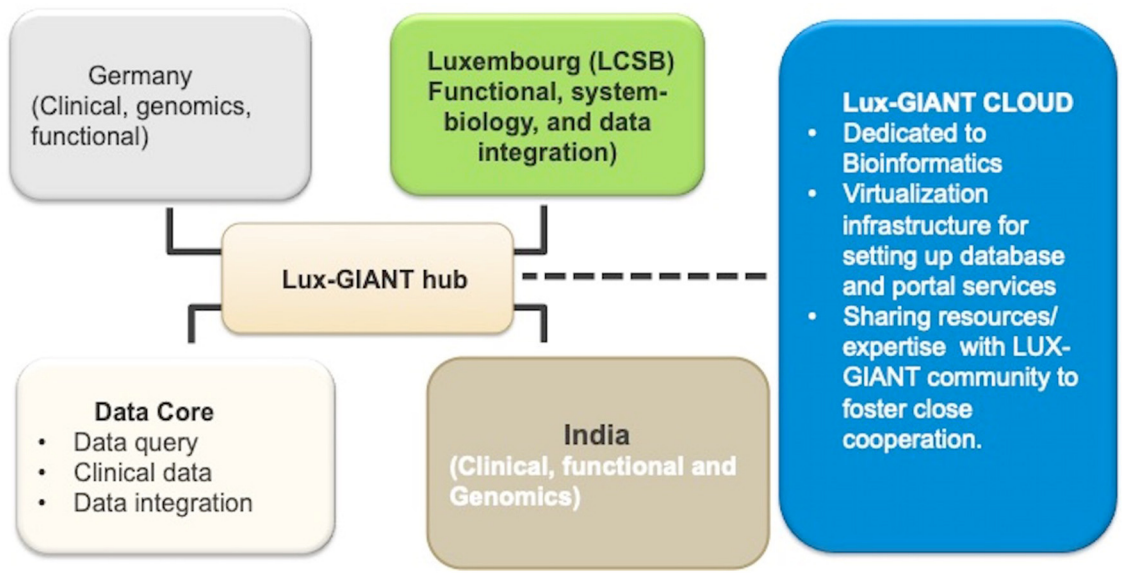


FIGURE 2 | Clinical, genomics, bioinformatics, and functional cores in the Lux-GIANT consortium.

excluded: (1) cognitive or psychiatric dysfunction sufficiently severe enough to impair the patient's ability to provide informed written consent (2) red flags or additional neurological signs raising suspicion of atypical Parkinsonism. Patients who have previously undergone surgical procedures such as pallidotomy, thalamotomy, or Deep Brain Stimulation will not be excluded. Healthy volunteers will be recruited through advertisements displayed on the hospital campus. They will be gender-matched and should belong to the same geographic–ethnic background as the patients. A detailed history and standard neurological examination will be performed before inclusion as controls. Volunteers with a family history of PD or other neurodegenerative diseases will be excluded from the control group. All subjects will be recruited after obtaining written informed consent and with the approval of the Institutional Ethics Committee. Centralized monitoring of recruitment rates and fidelity to operating procedures will be done by the clinical nodal center, SCTIMST.

CLINICAL ASSESSMENTS AND BIOSPECIMEN COLLECTION

Trained personnel at each clinical site will collect clinical and demographic data. The demographic data collected includes information on the geographical origin within India. Structured questions will capture information related to environmental exposures known to be associated with PD including pesticides, fungicides, insecticides, and other chemicals, smoking, caffeine, and head injury. Patients will be asked to report if they ever held a job requiring exposure to pesticides, herbicides, fungicides, insecticides, rodenticides, and fumigants and whether they were exposed to these chemicals at their place of residence through self-use or via another person. Lifetime smoking history of 100 or more cigarettes will be documented. History of head injury or concussion including falls, sporting activities, violence, and car or other accidents in childhood or adulthood will be queried. Patients will also be asked about exposure to caffeinated coffee in quantities more than once per week for 6 months or longer. Years of education will be documented. Furthermore, our environmental exposure data collection will align with the environmental questionnaire from the Genetic-Epidemiology of Parkinson disease (GEoPD) consortium to harmonize the dataset across the ongoing studies. A structured history and clinical examination will be conducted to collect data regarding onset symptoms, motor fluctuations, and dyskinesias, medications, and non-motor symptoms. Non-motor symptoms included in the interview are cognitive impairment, psychosis, depression, sweating abnormalities, seborrhea, sleep disorders including REM behavioral disorder, restless legs, hyposmia, orthostatic hypotension, constipation, dysphagia, and urinary/fecal incontinence. Data from Computerized Tomography (CT), Magnetic Resonance Imaging (MRI), Dopamine Active Transporter- Single Photon Emission Computed Tomography (DaT SPECT) will be collected if available. The family history will be probed for consanguinity and to identify any known relations with PD, dementia, tremor, or other neurological disorders. For patients who have undergone functional neurosurgery, the target, time since surgery and other details will be collected. The motor symptoms at the time of recruitment will be assessed by the Unified Parkinson's Disease Rating Scale (UPDRS Parts I- IV) (17). Subjects will be screened for cognitive dysfunction using the Montreal Cognitive Assessment (MoCA) and for depression using the Beck's Depression Inventory (BDI- II) (18, 19). Validated regional language versions of MoCA will be used for non-English speaking subjects. Demographic and risk factor information will be collected from the control group. Clinical terminologies have been standardized to enable data harmonization with existing research groups and also build a phenotypic information resource for this particular population of PD patients.

The whole blood samples will be collected at each recruiting center 10–15 ml of blood samples collected in EDTA tubes will be processed for DNA extraction using the *salting-out* method. The quantity and quality of DNA will be analyzed using a micro-volume UV/visible spectrophotometer (Nanodrop, Thermo Fischer). Additionally, the quality will also be checked by agarose gel electrophoresis. Those samples with an $A_{260/280}$ ratio of 1.8–2.0 and $A_{260/230}$ ratio of >2.0 will be stored in 1.2 ml screwcap cryovials barcoded with a unique sample ID at SCTIMST Biobank (−80°C), in aliquots. The samples received from the nodal centers and sub-centers will be again checked (using Nanodrop and agarose gel) at SCTIMST to ensure the quality and quantity of DNA that is required for the genotyping. For sequencing, 50 μl of 50–100 ng/μl DNA will be transported in 96-well microtiter plates sealed with peelable heat seals in a waterproof container and dry ice. At the time of collection, specimens will be de-identified by avoiding any personal identifiers on the label. Specimen labels and data collection instruments will be labeled by center-specific serial numbering. No direct personal identifiers will be stored in the online data capture system and quasi-identifiers like date of birth are flagged as such and de-identified by the system before export. Only the site PIs hold identifying information if required for re-identification at a later stage. The central clinical node and other investigators with access to the online database will not have access to direct personal identifiers. Biospecimens collected at the sub-centers will be transported to the nodal centers for DNA isolation. All biospecimens are finally routed to the clinical core at SCTIMST, Trivandrum for storage and in consenting subjects, longer-term bio-banking. In keeping with the existing regulatory framework in India, to promote capacity development, array processing and genotyping will be done at the Center for Cellular and Molecular Biology, Hyderabad. The genetic core at Tübingen will perform the bioinformatics analysis and a subset of specimens will be processed at the Lux-GIANT genotyping core facility in Munich for quality control purposes. All clinical and genetic data will be stored on a shared electronic platform with access restrictions and security protocols in place. Functional validation of putative pathogenic variants including patient-specific induced pluripotent stem cell modeling will be done at the National Brain Research Center, Gurgaon, India. In this way, the study is designed to comprehensively capture clinical and genetic information from a large Indian cohort in

a manner that enables integration with existing international cohorts. DNA isolation from whole blood will be done at the four nodal centers and centralized quality control monitoring at SCTIMST, Trivandrum. DNA specimens from consenting subjects will be maintained in a biorepository at SCTIMST for potential future research.

DATA ANALYSIS

For genetic analysis, a two-stage design will be followed. Currently, the arrays available for genotyping lack in-depth genetic variability information from the Indian population. GAP-India aims to address this issue by performing whole-genome sequencing (WGS) of around 500 subjects covering the north, south, east, and west of India.

Whole-Genome Sequencing

The data generated from whole-genome sequencing will be analyzed using the megSAP pipeline (https://github.com/imgag/megSAP) developed at the Institute of Medical Genetics and Applied Genomics, University Hospital of Tübingen (Tübingen, Germany). In brief, SeqPurge (v. 2020_03) will be used for adapter and quality trimming (20), Burrow-Wheeler Aligner mem (BWA mem (v.0.7.17) for read mapping (21), samblaster (v. 0.1.24) for duplicate removal (22), ABRA2 (v. 2.22) for indel-realignment (23), freebayes (v. 1.2.0) for calling of small variants (24), ClinCNV (v. 1.16.1) for CNV calling (25), Manta (v. 1.6.0) for structural variant calling (26), and Ensembl VEP (v. 96.3) for variant annotation (27). Furthermore, additional tools available from the ngs-bits toolset (https://github.com/imgag/ngs-bits) will be used for data cleaning.

GWAS Analysis

Study Population and Genotyping

The analysis cohort will represent ∼10,200 PD cases and 10,200 controls of Indian ancestry, genotyped with Illumina's Global Diversity Array (GDA) containing neurodegenerative specific content.

Quality Control

GenomeStudio will be used to cluster the genotyping array using the GenCall algorithm and preliminary QC will be implemented in GenomeStudio as described elsewhere (28). Data will be exported in the standard PLINK format and downstream QC procedures and statistical analysis will be conducted using the latest PLINK (http://pngu.mgh.harvard.edu/_purcell/plink) and R software packages (http://www.r-project.org/), installed on a Linux based computation resource (29). The post-GenomeStudio QC will be broadly divided into three main steps comprising of (i) Sample and genetic marker quality (ii) Population structure (iii) Genotyping consistency. Furthermore, QC will be implemented independently in each Indian subpopulation covering north, south, east, and west of India.

Sample and Genetic Marker Quality

Firstly, all samples and SNPs with missing rate>1% will be excluded. Concerning genetic marker quality, we would exclude SNPs with MAF<0.01 and HWE p-value $<1 \times 10^{-10}$ in cases as well as HWE $<1 \times 10^{-6}$ in controls (30). Allele frequencies will be checked with Indian sub-populations represented in the Haplotype Reference Consortium (HRC). Furthermore, allele frequency consistency across different batches of genotyping datasets will be checked to rule out the batch effect.

Population Structure

Individuals deviating ±3 SD from the samples' heterozygosity rate mean will be excluded. Only those males will be included which have an X chromosome homozygosity estimate of more than 0.8. On the other hand, only those females will be included which have an X chromosome homozygosity estimate of less than 0.2. Related samples will be filtered based on identity by descent (IBD) coefficient>0.1 (31). Principal component analysis (PCA) will be used to detect population outliers using the first ten principal components and the outlier samples will be removed. We identified five populations representing the Indian subcontinent in phase 3 1,000 Genomes Project (KGP): two from the northwestern region [Gujarati Indian in Houston, TX (GIH) and Punjabi in Lahore, Pakistan (PJL)], two from Southern region [Indian Telugu in the UK (ITU) and Sri Lankan Tamil in the UK (STU)] and one from Eastern region [Bengali in Bangladesh (BEB)]. The five Indian subcontinent populations marked as South Asain population in the PCA plot of the worldwide population showed a clear demarcation emphasizing the need to diversify the genomic research in under-represented populations to identify population-specific novel genetic loci for complex diseases (**Figure 3**).

Imputation

Imputation will be carried out using the Haplotype reference consortium (HRC) as a reference panel consisting of individuals from more than 26 worldwide populations (32). The SNPs with imputation info score of less than 0.7 will be discarded.

Association Analysis

Post-QC and imputation, association analysis will be conducted for each sub-population using binary logistic regression analysis assuming an additive genetic model adjusting for age, sex, and relevant principal components. A conventional genome-wide significance threshold of 5 × 10–8 will be used to identify the significant SNPs. The fixed meta-analyses inverse-variance weighting of log-ORs will be implemented in METAL to combine summary statistics across all the Indian sub-populations (33). Genome inflation factor λ will be computed using the median χ^2-statistics. Lastly, Manhattan and QQ plots will be constructed to visualize the results. All the summary statistics will be made available publicly. Heterogeneity in allelic effect sizes between different Indian sub-populations contributing to the meta-analysis will be assessed using Cochran's Q statistic.

Polygenic Prediction

We will further use genome-wide complex trait analysis (GCTA) to perform conditional and joint analysis to identify the top variants that account for heritable variation among different loci

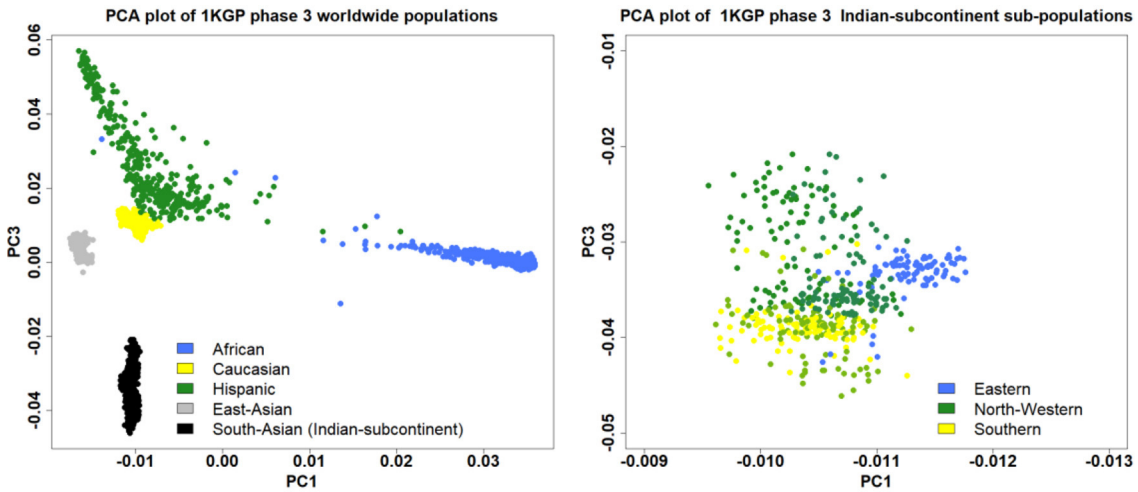


FIGURE 3 | The PCA plot using the phase 3 1,000 Genomes Project showing the distribution of the South Asian population among the worldwide populations (left), and sub-populations from different regions of the Indian subcontinent (right).

(34). Polygenic risk score profiling will be done in a standard weighted allele dose manner (35).

Biological Annotation

We will further integrate our GWAS summary statistics with expression and network data using Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) to perform the tissue specificity and pathway enrichment analysis (36).

The genomics/bioinformatics core in Tubingen will be responsible for data integration and analysis.

Data Management and Sharing

To ensure seamless data exchange among Lux-GIANT partners, we have established a secure data management and analysis platform. At this moment this platform is equipped with REDCap, a state-of-the-art EDC (Electronic Data Capturing) system (https://www.project-redcap.org) widely used in various clinical and translational projects and is hosted at the "clinical core" site, SCTIMST. The Lux-GIANT REDCap instance is aligned with the Genetic Epidemiology of Parkinson disease (GEoPD) consortium minimal dataset Case Report Forms (CRFs). This will ensure uniform clinical data collection across various participating countries in GEoPD that are spread across five continents. This will facilitate cross-study data pooling and analysis for future multinational projects. All Indian nodal centers and sub-centers taking part in this study are collecting pseudonymized data into this secure and access-controlled instance centrally. All the identifiable information of each study participant stored separately at each site in a corresponding hospital system and only authorized clinical people from that site to have access to it. This setup is aligned with European general data protection regulations (GDPR) as well as Personal Data Protection Bill (PDP) 2019. This pseudonymized clinical data, as well as the corresponding molecular data, will be served to all Lux-GAINT partners via the "data core" site established at Tubingen by leveraging the infrastructure established by the German Network for Bioinformatics and Infrastructure (de.NBI, https://cloud.denbi.de). Through the dashboard of the de.NBI Cloud Site Tubingen the allocation of the desired resources (number of virtual CPU cores, number of virtual machines (VMs), amount of storage and RAM) will be covered. Furthermore, to provide secure access to the VMs to researchers, so-called security groups will be created, which can be seen as a VM specific firewall to control incoming and outgoing network traffic connections. To provide an additional layer of security to Lux-GIANT genomics data, a private network will be added to the Lux-GIANT cloud project to protect the network traffic from and to the volume where the genomics data reside. In addition to the network traffic protection, a certificate-based approach will be used to grant specific permissions (read, write) on a per-user base. The certificates will be distributed to the particular person using a state-of-the-art asymmetric encryption technique. The whole process starting with the project application is illustrated in **Figure 4**. For further details, please see (37). The system-infrastructure, as created, will provide a secure environment to handle and process sensitive patient data in a restrictive and responsible way using cloud resources. To complement our cloud-based activities, the "data integration" site, ELIXIR-Luxembourg Node (ELIXIR-LU) will FAIRify this data by making them Findable, Accessible, Interoperable, and Reusable (38). All the meta-data will be shared through the ELIXIR data catalog (https://datacatalog.elixir-luxembourg.org) that facilitates the Findability of the data. Both clinical and associated molecular data will be curated, harmonized, and integrated into a discovery analytics system—Ada (https://ada-discovery.github.io). It will be hosted in the de.NBI cloud Tubingen and will facilitate the data exploration and analysis through intuitive web interface rich with dynamic visual analytics and advanced machine learning (Deep Learning).

GAP-India aims to share data at the end of a 2-year embargo period, consistent with guidelines followed by other consortia's

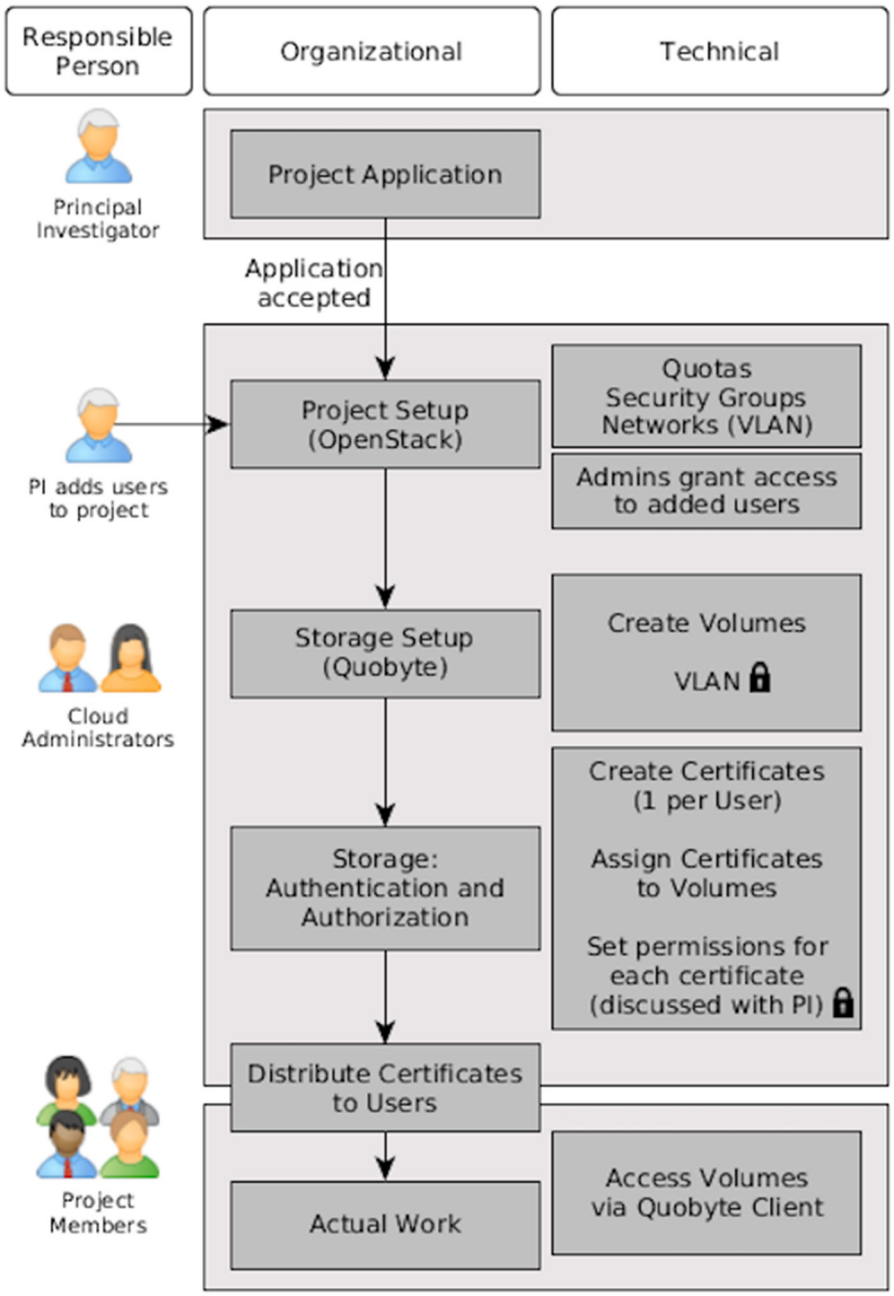


FIGURE 4 | Data management and access workflows in the Lux-GIANT network.

such as H3AfricaConsortium. The purpose of the 2-year embargo period is to give Lux-GIANT researchers a reasonable time-frame to analyze and publish their data before others do. The GAP-India project aims to develop an extensive data sharing plan designed to maximize the utility of its data for the scientific community. Lux-GIANT cloud portal through which GAP-India data will share data fall into two categories: (i) controlled access, and (ii) open access.

The controlled access via the Lux-GIANT portal hosted on the de.NBI Cloud will be given to researchers/institutes who will comply with the data protection and ethical regulations, as described in the GDPR, and PDP 2019. The open-access data which does not require prior ethical clearance will be made available to the scientific community either via the Lux-GIANT Portal or PDgene database.

Regulatory and Ethical Framework

GAP-India project aims to address two main issues: (1) To generate the most comprehensive PD genome-phenome catalog, including iPSC biobank of the Indian PD population, and (2)

to develop scientific and infrastructure capacities in India which have so far lagged in PD genomics research.

One of the major reasons that hinder the collaboration in the genomic era between various research consortia which are primarily led by institutions either in the USA and/or Europe and under-represented population such as India were concerns that data generated from the under-represented population will not be properly represented by the local stakeholders. GAP-India aims to dispel this notion of "scientific imperialism" by developing the "knowledge-sharing" model and also establishing the guidelines which adequately protect the interests of local investigators as well.

The data generated from the GAP-India project follows the strict ethical guidelines, as stipulated by the Indian Medical Council of Research (ICMR)- HMSC for international collaborative research and follows the provisions of ICMR guidelines for biomedical research in India (39). All the clinical recruiting sites obtain ethical approval from their specific ethics committee according to local protocols.

The GAP-India project involves multi-centers across India. There exists a considerable disparity in access to and protocols for regular health care among patients. Therefore, various ethical considerations have arisen during the development stage of the GAP-India project. Specifically, the following issues have been considered. (1) return of genetic results generated from the study, and how they will be received; (2) providing information about genetic findings to patients and care-providers; (3) concerns about stigmatization; and (4) ensuring equity and fairness in collaboration.

As per the Indian guidelines, we are mandated to return actionable results, with the potential to improve the health outcome of the participants. For this, a re-identification process will be followed through the PI of the recruiting center. Genetic counseling and guidance will be offered in case of such a return of results. Incidental findings that are not actionable will not be returned (39).

One of the major spin-offs from this study will create a core network of clinicians and researchers dedicated to PD genetics in India. A long-term biorepository and capacity building in terms of infrastructure and skill upgradation are additional advantages. Taken together, GAP-India aims to develop a dedicated pool of researchers and health care professionals to raise PD awareness in India.

The GAP-India study and the LUX-GIANT network aim to address a critical gap in knowledge regarding the genetic origins of PD, by leveraging the population diversity afforded by as a yet unaddressed population. We expect to generate novel data that may drive targeted therapies and make them applicable on a global scale.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethics Committees of all participating clinical centers. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RR conceived, designed, organized and wrote the first draft, reviewed, and critically revised the manuscript. KD, RMK, and RY conceived the project, reviewed, and critically revised the manuscript. VS, UM, PA, NK, TF, HK, AS, KS, SM, SD, SK, LP, MB, PW, SR, MH, GW, and SP reviewed and critically revised the manuscript. FB, MH, JK, AK-S, SG, PL, MSt, and JR reviewed, and critically revised the manuscript. VB, GC, JS, PS, TG, OR, and VG reviewed and critically revised the manuscript. PP, RK, and RB organized the research project, and reviewed and critically revised the manuscript. KB, NC, RC, CT, MD, CG, HM, NK, SK, PM, CS, AKS, and DW reviewed and critically revised the manuscript. AK and MSh conceived, organized, and executed the research project; designed, executed, reviewed, and wrote the first draft; and reviewed and critically revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00524/full#supplementary-material

REFERENCES

1. de Lau LML, Breteler MMB. Epidemiology of Parkinson's disease. *Lancet Neurol.* (2006) 5:525–35. doi: 10.1016/S1474-4422(06)70471-9
2. Dorsey ER, Elbaz A, Nichols E, Abd-Allah F, Abdelalim A, Adsuar JC, et al. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the global burden of disease study 2016. *Lancet Neurol.* (2018) 17:939–53. doi: 10.1016/S1474-4422(18)30295-3
3. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol.* (2019) 18:1091–102. doi: 10.1016/S1474-4422(19)30320-5
4. Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, van der Brug M, Cai F, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet.* (2017) 49:1511–6. doi: 10.1038/ng.3955
5. International HapMap 3 Consortium, Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, et al. Integrating common and rare genetic variation in diverse human populations. *Nature.* (2010) 467:52–8. doi: 10.1038/nature09298
6. Punia S, Behari M, Govindappa ST, Swaminath PV, Jayaram S, Goyal V, et al. Absence/rarity of commonly reported LRRK2 mutations in Indian Parkinson's disease patients. *Neurosci Lett.* (2006) 409:83–8. doi: 10.1016/j.neulet.2006.04.052
7. Sanyal J, Sarkar B, Ojha S, Banerjee TK, Ray BC, Rao VR. Absence of commonly reported leucine-rich repeat kinase 2 mutations in Eastern Indian Parkinson's disease patients. *Genet Test Mol Biomarkers.* (2010) 14:691–4. doi: 10.1089/gtmb.2010.0054
8. Vijayan B, Gopala S, Kishore A. LRRK2 G2019S mutation does not contribute to Parkinson's disease in South India. *Neurol India.* (2011) 59:157–60. doi: 10.4103/0028-3886.79125
9. Vishwanathan Padmaja M, Jayaraman M, Srinivasan AV, Srikumari Srisailapathy CR, Ramesh A. The SNCA (A53T, A30P, E46K) and LRRK2 (G2019S) mutations are rare cause of Parkinson's disease in South Indian patients. *Parkinsonism Relat Disord.* (2012) 18:801–2. doi: 10.1016/j.parkreldis.2012.02.012
10. Kishore A, Ashok Kumar Sreelatha A, Sturm M, von-Zweydorf F, Pihlstrøm L, Raimondi F, et al. Understanding the role of genetic variability in LRRK2 in Indian population. *Mov Disord.* (2019) 34:496–505. doi: 10.1002/mds.27558
11. Cai M, Liu Z, Li W, Wang Y, Xie A. Association between rs823128 polymorphism and the risk of Parkinson's disease: a meta-analysis. *Neurosci Lett.* (2018) 665:110–6. doi: 10.1016/j.neulet.2017.11.057
12. McClellan J, King M-C. Genetic heterogeneity in human disease. *Cell.* (2010) 141:210–7. doi: 10.1016/j.cell.2010.03.032
13. Reich D, Thangaraj K, Patterson N, Price AL, Singh L. Reconstructing Indian population history. *Nature.* (2009) 461:489–94. doi: 10.1038/nature08365
14. Moorjani P, Thangaraj K, Patterson N, Lipson M, Loh P-R, Govindaraj P, et al. Genetic evidence for recent population mixture in India. *Am J Hum Genet.* (2013) 93:422–38. doi: 10.1016/j.ajhg.2013.07.006
15. Sengupta D, Choudhury A, Basu A, Ramsay M. Population stratification and underrepresentation of Indian subcontinent genetic diversity in the 1000 genomes project dataset. *Genome Biol Evol.* (2016) 8:3460–70. doi: 10.1093/gbe/evw244

16. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. (1992) 55:181–4. doi: 10.1136/jnnp.55.3.181
17. Fahn S, Elton R, UPDRS program members. Unified Parkinsons disease rating scale. In: Fahn S, Marsden CD, Goldstein M, Calne DB, editors. *Recent Developments in Parkinson's Disease.* Florham Park, NJ: Macmillan Healthcare Information (1987). p. 153–63.
18. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatrics Soc.* (2005) 53:695–9. doi: 10.1111/j.1532-5415.2005.53221.x
19. Beck AT, Steer RA, Brown GK. *Manual for the Beck Depression Inventory-II.* San Antonio, TX: Psychological Corporation (1996).
20. Sturm M, Schroeder C, Bauer P. SeqPurge: highly-sensitive adapter trimming for paired-end NGS data. *BMC Inform.* (2016) 17:7. doi: 10.1186/s12859-016-1069-7
21. Li H. *Aligning sequence reads, clone sequences assembly contigs with BWA-MEM. arXiv:1303.3997v1 [q-bio.GN]* (2013).
22. Faust GG, Hall IM. SAMBLASTER: fast duplicate marking and structural variant read extraction. *Bioinformatics.* (2014) 30:2503–5. doi: 10.1093/bioinformatics/btu314
23. Mose LE, Perou CM, Parker JS. Improved indel detection in DNA and RNA via realignment with ABRA2. *Bioinformatics.* (2019) 35:2966–73. doi: 10.1093/bioinformatics/btz033
24. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. *arXiv.* (2012) arXiv:1207.3907 [q-bio.GN]
25. Demidov G, Ossowski S. ClinCNV: novel method for allele-specific somatic copy-number alterations detection. *bioRxiv*. (2019) 837971. doi: 10.1101/837971
26. Chen X, Schulz-Trieglaff O, Shaw R, Barnes B, Schlesinger F, Källberg M, et al. Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. *Bioinformatics*. (2016) 32:1220–2. doi: 10.1093/bioinformatics/btv710
27. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, et al. The ensembl variant effect predictor. *Genome Biol.* (2016) 17:122. doi: 10.1186/s13059-016-0974-4
28. Guo Y, He J, Zhao S, Wu H, Zhong X, Sheng Q, et al. Illumina human exome genotyping array clustering and quality control. *Nat Protoc*. (2014) 9:2643–62. doi: 10.1038/nprot.2014.174
29. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* (2007) 81:559–75. doi: 10.1086/519795
30. Marees AT, de Kluiver H, Stringer S, Vorspan F, Curis E, Marie-Claire C, et al. A tutorial on conducting genome-wide association studies: quality control and statistical analysis. *Int J Methods Psychiatr Res*. (2018) 27:e1608. doi: 10.1002/mpr.1608
31. Reed E, Nunez S, Kulp D, Qian J, Reilly MP, Foulkes AS. A guide to genome-wide association analysis and post-analytic interrogation. *Stat Med*. (2015) 34:3769–92. doi: 10.1002/sim.6605
32. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet*. (2016) 48:1279–83. doi: 10.1038/ng.3643
33. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics.* (2010) 26:2190–1. doi: 10.1093/bioinformatics/btq340
34. Yang Y, Ferreira T, Morris AP, Medland SE, Genetic Investigation of ANthropometric Traits (GIANT) Consortium, DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium, Madden PAF, et al. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat Genet*. (2012) 44:369–75. doi: 10.1038/ng.2213
35. Euesden J, Lewis CM, O'Reilly PF. PRSice: polygenic risk score software. *Bioinformatics.* (2015) 31:1466–8. doi: 10.1093/bioinformatics/btu848
36. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun.* (2017) 8:1826. doi: 10.1038/s41467-017-01261-5
37. Gläßle B, Hanussek M, Bartusch F, Lutz V, Hahn U, Dilling W, et al. A federated and georedundant solution for large scientific data. *In: Proceedings of the 5th bwHPC Symposium.* Freiburg (2017). p. 16–9. doi: 10.15496/publikation-29054
38. Wilkinson MD, Dumontier M, Aalbersberg IJJ, Appleton G, Axton M, Baak A, et al. The FAIR guiding principles for scientific data management and stewardship. *Sci Data.* (2016) 3:160018. doi: 10.1038/sdata.2016.18
39. Mathur R, Swaminathan S. National ethical guidelines for biomedical and health research involving human participants, 2017: a commentary. *Indian J Med Res.* (2017) 148:279–83. doi: 10.4103/0971-5916.245303

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Original Article

Pharmacology Section

Genotype Phenotype Correlation of Genetic Polymorphism of PPAR Gamma Gene and Therapeutic Response to Pioglitazone in Type 2 Diabetes Mellitus- A Pilot Study

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ABSTRACT

Introduction: Pro12Ala polymorphism is a missense mutation at codon 12 in peroxisome proliferator-activated receptor γ gene (PPARG). This polymorphism is known to be associated with increased insulin sensitivity. Pioglitazone, a thiazolidinedione, is an anti-diabetic drug which acts as an agonist at PPAR γ receptor.

Aim: To determine the association between Pro12Ala polymorphism of the PPARG and variation in therapeutic response to the PPARγ agonist, pioglitazone.

Materials and Methods: The study was done as a hospital based pilot project in 30 patients with type 2 diabetes mellitus, on treatment with sulfonylurea or metformin but without adequate glycaemic control. They were started on pioglitazone as add on therapy for a period of 12 weeks. The participants were categorized as responders and non-responders based on the change in HbA1C level after 12 weeks. Pro12Ala polymorphism was analysed by polymerase chain reaction-restriction fragment length polymorphism.

Statistical Analysis: Logistic regression analysis was done to evaluate the associations between age, baseline body weight, BMI, waist circumference, waist-hip ratio and Pro12Ala variants with the response to pioglitazone. The p-value< 0.05 was considered significant.

Results: The frequency distributions of PPAR gamma genotypes were 80% for Pro/Pro and 20% for Pro/Ala in the study population. Among the study participants, 30% were non-responders and 70% responders to pioglitazone. A significantly higher frequency of the polymorphism was detected in the responders (p=0.005) compared to non-responders group.

Conclusion: Our study suggests that there is a potential association between Pro12Ala polymorphism and glycaemic response to pioglitazone.

Keywords: Pioglitazone, Pro12Ala, Pharmacogenetic, Glycaemic response, South India

INTRODUCTION

Type 2 diabetes mellitus is considered as a heterogeneous syndrome of dysregulated glucose homeostasis. The world prevalence of diabetes in adults has been predicted to increase from 285 million in 2010 to 439 million in 2030 [1]. It is also increasingly recognized that adequate management of diabetes by appropriate drug therapy is essential in preventing complications.

The peroxisome proliferator–activated receptor γ gene (PPARG) encodes the PPARγ receptor, a transcription factor that belongs to the family of nuclear receptors and it regulates the carbohydrate and lipid metabolism. Among the two isoforms, PPAR-γ2 is specific for adipose tissue, where it plays a pivotal role in adipogenesis and is an important mediator of insulin sensitivity [2].

Pro12Ala polymorphism is the result of a CCA-to-GCA missense mutation in the codon12 of exon B of the PPARG gene. There is substantial evidence from various genetic studies that the Pro12Ala polymorphism improves insulin sensitivity in humans [3,4]. It is possible that alterations in transcriptional activity of the polymorphic gene in adipocytes primarily enhance insulin's action [4,5]. Pioglitazone is a thiazolidinedione, the pharmacological ligand for PPARγ receptor. The thiazolidinediones act by decreasing the insulin resistance in muscle, liver and adipose tissue.

The effect of Pro12Ala polymorphism (rs1801282) on the therapeutic response to pioglitazone has not yet been studied in the South Indian population. Hence our study was designed to investigate the association of PPARG gene Pro12Ala polymorphism and the therapeutic response to pioglitazone therapy in type 2 diabetes mellitus patients in a South Indian population. The secondary objectives were to determine the influence of patient characteristics like body weight, BMI, waist-hip ratio on glycaemic response to pioglitazone and also to identify the association between Pro12Ala polymorphism and these patient characteristics in the population studied.

MATERIALS AND METHODS

The present study was a hospital based prospective pilot study done in 30 patients. The study protocol was approved by the Institutional Human Ethics Committee before the start of the study. Patients who had already been diagnosed with type 2 diabetes mellitus and on regular treatment were included for the study. As per the current American Diabetes Association (ADA) guidelines, fasting plasma glucose ≥ 126 mg/dl or 2 hour postprandial plasma glucose ≥ 200 mg/dl or HbA1C ≥ 6.5% is the threshold for the diagnosis of diabetes [6].

Diabetic patients aged 30–85 years, of both sexes and already on treatment with sulfonylurea or metformin, but without adequate glycaemic control, as evidenced by HbA1C values of 7.5 to 9.5 % were selected for the study. Only those patients whose diabetic medications were not changed in the previous six months and those who had no previous history of PPAR agonist use were included.

Patients with type1 diabetes and known history of ischemic heart disease or congestive cardiac failure were excluded. Similarly

known cases of liver disease, bladder cancer or previous history of fractures were excluded from the study. Patients already on drugs which increase fluid retention like steroids, NSAIDs as well as pregnant and lactating women were excluded.

Written informed consent was obtained from each of the patients who met the above criteria. Basic demographic data like name, age, sex and anthropometric measurements of height, weight, body mass index, waist circumference, hip circumference and waist hip ratio were recorded. Baseline fasting blood glucose levels (by enzymatic reference method) and HbA1C levels (by immunoturbidimetry) were also determined during the first visit. Pioglitazone 30 mg once daily was added to the existing regimen without changing any other medication. The patients were counseled for self reporting of any adverse events. They were also instructed to maintain the same level of energy intake and physical activity as before. The next visit was scheduled after 12 weeks, during which the body weight was recorded in addition to repeating the fasting blood glucose and HbA1C. There were no significant adverse drug reactions reported by the participants during the study period.

The expected average reduction in HbA1C in type 2 diabetic patients with pioglitazone therapy is in the range of 0.5 to 1.4% [7]. Glycaemic response to pioglitazone was determined by the fall in HbA1C after 12 weeks of add-on pioglitazone therapy. If the absolute decrease in HbA1C was less than 0.5%, they were categorized as non-responders. During the same visit, blood was also drawn for genetic analysis.

Genotyping of the patients was performed to identify the Pro12Ala polymorphism in the PPAR gamma gene. The genomic DNA was first extracted from the blood samples of the study participants. The extracted DNA was rinsed with 70% alcohol and re-suspended in 40 µL of TE buffer (pH 8.0). Pro12Ala polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The DNA was amplified by PCR using a sense primer (5'-GCCAATTCAAGCCCAGTC-3') and an antisense primer (5'-GA TATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3') that flanked the region containing the 12-amino acid site of PPARγ2. The PCR product was 270 bp in length. The PCR conditions were an initial denaturation step at 94ºC for 8 min, followed by 35 cycles of denaturation at 94ºC for 50 sec, annealing at 59ºC for 50 sec, and extension at 72ºC for 1 min, with a final extension of 5 min at 72ºC.

After PCR amplification, the PCR product was subjected to restriction digestion by BstUI/Bsh 1236 I (Fermentas 10units/ µL) enzyme at 37ºC overnight, electrophoresed on a 12% polyacrylamide gel and stained with ethidium bromide. The digested RFLP products after gel electrophoreses was used to identify the study subjects with the Pro12Ala polymorphism. A single 270 bp fragment indicated the presence of CC (Pro12Pro) genotype while three fragments of 270, 227 and 43 bp product size confirmed the presence of GC (Pro12Ala) genotype [Table/Fig-1]. GG (Ala12Ala) genotype would be represented by two fragments of 227 and 43 bp [8]. Inorder to ensure validity, the samples were run in polyacrylamide gel using an internal positive and negative control and replicating 10% of the samples randomly. In addition, the gel was read by two independent blinded observers and the concordance rate was found to be 100%.

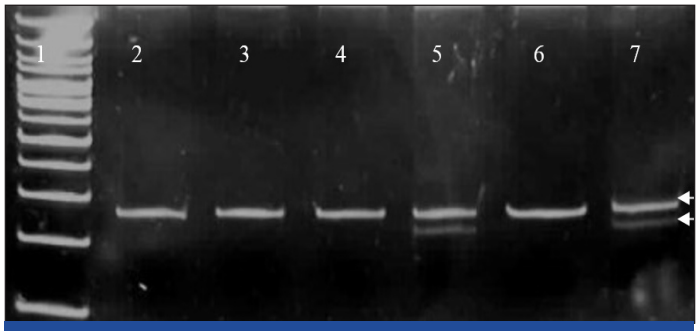
STATISTICAL ANALYSIS

The data was analysed using the SPSS version 19. All data were expressed as mean± standard deviation. Paired t-test was performed to assess the effect of pioglitazone treatment and an independent sample t-test to analyse if there was any significant difference in the body weight, BMI, waist circumference, waist-hip ratio, baseline fasting blood glucose or the baseline HbA1C between wild type (homozygous) and those with Pro12Ala polymorphism (heterozygous). Logistic regression analysis was used to evaluate the associations between age, baseline body weight, BMI, waist circumference, waist-hip ratio and Pro12Ala variants with the response to pioglitazone. The p-value< 0.05 was considered significant.

RESULTS

The mean age of the study participants was 53.2 ± 10.95 years, of which 46% were males and 54% females. At baseline, the mean body weight of the study population was 66.83 ± 10.492 kg and the mean BMI 26.247 ± 3.71. The mean waist circumference and mean waist-hip ratio were 90.50 ± 9.343 cm and 0.9033 ± 0.0649 respectively. Using 'paired t-test', a significant decrease in mean fasting blood glucose and HbA1C levels was seen after 12 weeks of add-on treatment with pioglitazone whereas mean body weight and BMI did not change significantly [Table/Fig-2]. The PPARγ (rs1801282) genotype frequency distributions were 80% for Pro/Pro, 20% for Pro/Ala and 0% for Ala/Ala in the study population. An independent sample t-test [Table/Fig-3] showed that there was no statistically significant difference found in the body weight, BMI, waist circumference, waist-hip ratio, fasting blood glucose or HbA1C between wild type (homozygous) and those with Pro12Ala polymorphism (heterozygous) at baseline. Interestingly, after pioglitazone treatment, the fasting blood glucose between wild type (125.92 ± 21.549) and heterozygous (106.33 ± 5.645) was statistically significant with p-value of 0.03. A similar statistically significant difference in HbA1C between the wild type (7.158 ± 1.007) and heterozygous (6.300 ± .4690) genotypes was found. However, the difference in other parameters after treatment was not statistically significant.

Among the study participants, 30% were non-responders and 70% responders to pioglitazone add-on therapy. At baseline, 22



[Table/Fig-1]: PCR-Restriction fragment length polymorphism detection of Pro12Ala missense mutation.
Lane1 shows the DNA ladder. Lanes 2,3,4,6 show the uncut wild type Pro12Pro genotype with single DNA fragment of product of size 270 bp (base pairs). Lanes 5,7 show the two DNA fragments of size 270 bp (upper arrow) and 227 bp (lower arrow) resulting from the restriction site introduced by the downstream primer due to Proline →Alanine substitution (Pro12Ala polymorphism). The 43bp DNA fragment is not seen.

Parameter	Mean ± Standard deviation	Mean ± Standard deviation (paired difference)	95% CI	t value	p-value
Weight (baseline)	66.83 ± 10.492	-0.300 ± 1.055	-0.694 - 0.094	-1.557	0.13
Weight (after 12 weeks)	67.13 ± 10.536				
BMI (baseline)	26.24 ± 3.774	-0.017 ± 0.7968	-0.3145 - 0.2805	-0.117	0.9
BMI (after 12 weeks)	26.30 ± 3.710				
Fasting blood glucose (baseline)	144.17 ± 13.608	22.167 ± 10.107	18.393 - 25.941	12.013	<0.001
Fasting blood glucose (after 12 weeks)	122.00 ± 20.910				
HbA1C (baseline)	7.770 ± 0.6199	0.7833 ± 0.4348	0.6210 - 0.9457	9.868	<0.001
HbA1C (after 12 weeks)	6.987 ± 0.9825				

[Table/Fig-2]: Paired t-test showing significant difference in fasting and HbA1C before and after 12 weeks pioglitazone treatment.

Characteristics	Pro12Pro	Pro12Ala	p-value
Body weight	68.04 ± 9.976	62.00 ± 12.066	0.2
BMI	26.695 ± 3.7858	24.458 ± 3.0170	0.1
Waist circumference	91.33 ± 8.746	87.17 ± 11.737	0.3
Waist-hip ratio	0.9046 ± 0.0678	0.8983 ± 0.0570	0.8
Fasting blood glucose	146.08 ± 14.222	136.50 ± 7.450	0.1
HbA1C	7.854 ± 0.6527	7.433 ± 0.3141	0.1

[Table/Fig-3]: Comparison of baseline patient characteristics in type 2 diabetes with Pro12Pro and Pro12Ala genotypes.

Variable	Odds ratio	p-value
Age	0.015	0.90
Body Weight	4.933	0.02
BMI	3.199	0.07
Waist circumference	0.985	0.30
Waist hip ratio	2.717	0.09
Pro12Ala (rs1801282)	7.763	0.005

[Table/Fig-4]: Multiple logistic regression analysis for the influence of clinical and genetic factors on the response to pioglitazone treatment.

patients in the study were receiving metformin while the rest were on treatment with sulfonylureas, among which, five patients were on glimepride and three on glibenclamide treatment. Also, it was noted that amongst those with the Pro12Ala polymorphism, five were on pre-existing metformin therapy while only one patient was on glibenclamide therapy.

Logistic regression analysis performed showed that there was no statistically significant association between the age, BMI, waist circumference or waist-hip ratio with the glycaemic response to pioglitazone therapy in the study population. But a statistically significant association was detected both for baseline bodyweight of the patients and PPARγ genotype Pro12Ala with the therapeutic response to pioglitazone [Table/Fig-4]. An analysis based on different pre-existing treatment regimes of the patients was not done for two reasons. First, we had included only those patients whose treatments were not changed in the previous six months implying that any change in their glycaemic response after the addition of pioglitazone was due to the add-on treatment. In addition, the smaller sample in the sulfonylurea group precluded direct comparison with the metformin group.

DISCUSSION

In this study, the Pro12Ala polymorphism has been detected in 20% of the study population. This is similar to the frequency of the polymorphism reported in other studies [9-12] done in the Indian population. The current study has illustrated that there is no significant difference in the body weight between the wild type and heterozygous population [Table/Fig-3]. Likewise, there is no statistically significant association between the polymorphism and parameters of adiposity like BMI, waist circumference and waist hip ratio. Various studies done in different ethnic populations have established that the effect of Pro12Ala polymorphism on body mass index is complex. A higher BMI has been demonstrated in the Pro12Ala heterozygotes in certain populations like the white Americans, Iranians and other Caucasian populations [10,13,14] while few studies have also demonstrated a lower BMI in the Pro12Ala polymorphic individuals [13,15]. Genetic heterogeneity due to differences in ethnicity could be contributory to the discrepancies [13,16].

A metanalysis in British subjects has shown that the Pro12Ala polymorphism has an apparent effect on BMI only in markedly obese individuals. When BMI of the three genotype groups are compared, it is found that the Ala12 homozygotes have significantly higher BMI than heterozygotes and Pro12 homozygotes indicating that the Ala12 allele is associated with increased BMI, particularly in the obese individuals [17].

The effect on BMI can be ascribed to an increased PPAR γ expression in the adipose tissue of obese subjects, but this expression can be down-regulated by low-calorie diet. It has been shown that, when the dietary polyunsaturated fat to saturated fat ratio is low, the BMI in Ala12 carriers is greater than that in Pro12 homozygotes, but when the dietary ratio is high, the opposite is seen. This gene–nutrient interaction may, in part, explain the differential effects of the polymorphism on the body mass index in obese and lean individuals [18].

In addition, studies in the Indian population have largely shown that there are no differences in BMI, waist-hip ratio and waist circumference between the carriers of the polymorphism and the wild-type genotype in contrast to the Caucasians [10,11]. Contradictorily, a higher BMI has also been observed [12]. However, the BMI of individuals with the Pro12Ala genotype did not significantly differ from individuals with the Pro12Pro genotype in the global analysis implying only a modest effect of the polymorphism [9,17].

The first evidence for an association between the Pro12Ala polymorphism in PPARG gene and increased insulin sensitivity was reported by Debb et al., [15]. A reduced transcriptional activity of the PPARG2 gene as a result of Proline to Alanine aminoacid substitution has been demonstrated but however the mechanism by which the reduced transcriptional activity of PPARγ influences insulin sensitivity is still unclear [15].

Moreover, multiple studies have proven that Pro12 Ala polymorphism reduces the risk of type 2 diabetes mellitus [19-21]. In addition, the progression from normal glucose tolerance to clinical type2 diabetes in which insulin resistance plays a major risk factor, is genetically influenced by this polymorphism [20]. Currently it is also recognized that this polymorphism plays an important role in the risk of nephropathy in diabetes patients [21].

On the contrary, studies have also revealed that reduction in the risk of diabetes is not conferred by the polymorphism in the Indian population unlike that seen in Caucasian population [10,12]. It is interesting to note that some studies also refute such differential effects of the polymorphism ascertaining that the increased insulin sensitivity and protective role of the polymorphism in reducing the risk of diabetes is also evident in the Asian Indians [11,16]. The disparate results are attributed to the diversity in ethnicity of the Indian sub-populations [11].

This is the first study in the South Indian population evaluating the effect of the Pro12Ala polymorphism on therapeutic response to pioglitazone, a PPAR gamma agonist. A statistically significant genetic association between Pro12Ala polymorphism and therapeutic response to pioglitazone [Table/Fig-4] was detected implying a potentially significant contribution of the PPARG2 gene in determining the response to the drug. It has been difficult to explain why paradoxically, both reduced transcriptional activity due to Pro12Ala polymorphism and pharmacological activation of PPARγ by thiazolidinediones result in improved insulin sensitivity. Yamauchi et al., have proposed that the supra-physiological activation of PPAR gamma by thiazolidinediones markedly increases the triglyceride content of white adipose tissue, thereby decreasing triglyceride content of liver and muscle [22]. This in turn leads to amelioration of insulin resistance at the expense of obesity whereas a moderate reduction of PPAR gamma activity by Pro12Ala polymorphism decreases triglyceride content of white adipose tissue, skeletal muscle, and liver due to increased leptin expression and fatty acid combustion along with decreased lipogenesis, thereby ameliorating high fat diet induced obesity and insulin resistance. Thus even though different mechanisms appear to be involved, both act to reduce insulin resistance. This additive effect could be the possible mechanism by which the study participants with Pro12Ala polymorphism have demonstrated a better therapeutic response compared to those with Pro12Pro genotype.

Similar studies done in other populations have yielded conflicting evidence [23-25]. These studies were performed in different ethnic populations like the Chinese, Iranian, and German. A study done in thirty, type 2 diabetes patients in a North Indian population revealed a better glycaemic response of those with Pro12Ala polymorphism to pioglitazone monotherapy [26]. A metanalysis of five studies in which the effect of Pro12Ala polymorphism on the pharmacodynamics of pioglitazone was examined, showed that three studies proved a genotype phenotype correlation while the rest did not reveal such association. According to the metanalysis, this disparity in the results could be attributed to factors like heterogeneity of the patient population in the studies included in the metanalysis, their inclusion/ exclusion criteria, different pioglitazone treatment regimens and presence of other anti-diabetic treatments. Thus PPARG2 gene Pro12Ala polymorphism could be contributory to the inter-individual variability observed in therapeutic response to pioglitazone, even though the variability cannot be completely explained by the polymorphism indicating the possible involvement of other genetic and environmental factors [27].

CONCLUSION

The current study has demonstrated a significant association between the Pro12Ala polymorphism and a better glycaemic response to pioglitazone. But the major limitation of the study is small sample size. Therefore the results require confirmation in larger trials involving larger sample before establishing the genotyping for Pro12Ala polymorphism as a predictor of response to pioglitazone therapy in diabetes. Thus the study emphasizes that genotyping could, in future, guide in optimizing diabetes management in individual patients and that pharmacogenetics has an important role to play in achieving the goal of individualized therapy in patient care.

REFERENCES

[1] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*. 2010;87(1):4-14.

[2] Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, et al. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. *Molecular Cell*. 1999;4(4):611–17.

[3] Koch M, Rett K, Maerker E, Volk A, Haist K, Deninger M, et al. The PPAR gamma2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia*. 1999;42(6):758–62.

[4] Stumvoll M, Wahl HG, Loblein K, Becker R, Machicao F, Jacob S, et al. The Pro12Ala polymorphism in the peroxisome proliferator–activated receptor-γ2 gene is associated with increased antilipolytic insulin sensitivity. *Diabetes*. 2001;50(4):876–81.

[5] Stumvoll M, Haring H. Reduced lipolysis as possible cause for greater weight gain in subjects with the Pro12Ala polymorphism in PPARγ2. *Diabetologia.* 2001;45(1):152–53.

[6] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33(Suppl. 1):S62 – 69.

[7] Powers AC, D'Alessio D. Endocrine pancreas and pharmaotherapy of diabetes mellitus and hypoglycemia. In: Brunton LL, Chabner BA, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 12th edn. New York: McGraw Hill Publishers; 2011.

[8] Gupta AC, Chaudhory AK, Sukriti C, Pande C, Sakhuj P, Singh Y, et al. Peroxisome proliferators-activated receptor c2 Pro12Ala variant is associated with body mass index in non-alcoholic fatty liver disease patients. *Hepatol Int*. 2011;5(1):575–80.

[9] Bhatt SP, Misra A, Sharma M, Luthra K, Guleria R, Pandey RM, et al. Ala/Ala genotype of Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-γ2 gene is associated with obesity and insulin resistance in Asian Indians. *Diabetes Technol Ther*. 2012;14(9):828-34.

[10] Radha V, Vimaleswaran KS, Babu HN, Abate N, Chandalia M, Satija P, et al. Role of genetic polymorphism peroxisome proliferator γ activated receptor-gamma2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: evidence for heterogeneity. *Diabetes Care*. 2006;29(5):1046-51.

[11] Sanghera DK, Demirci FY, Been L, Ortega L, Ralhan S, Wander GS, et al. PPARG and ADIPOQ gene polymorphisms increase type 2 diabetes mellitus risk in Asian Indian Sikhs: Pro12Ala still remains as the strongest predictor. *Metabolism*. 2010;59(4):492-501.

[12] Tai ES, Corella D, Deurenberg-Yap M, Adiconis X, Chew SK, Tan CE, et al. Differential effects of the C1431T and Pro12Ala PPARgamma gene variants on plasma lipids and diabetes risk in an Asian population. *J Lipid Res*. 2004;45(4):674-85.

[13] Fornage M, Jacobs DR, Steffes MW, Gross MD, Bray MS, Schreiner PJ. Inverse effects of the PPAR(gamma)2 Pro12Ala polymorphism on measures of adiposity over 15 years in African Americans and whites. The CARDIA study. *Metabolism*. 2005;54(7):910-17.

[14] Mirzaei H, Akrami SM, Golmohammadi T, Doosti M, Heshmat R, Nakhjavani M, et al. Polymorphism of Pro12Ala in the peroxisome proliferator-activated receptor gamma2 gene in Iranian diabetic and obese subjects. *Metab Syndr Relat Disord*. 2009;7(5):453-58.

[15] Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, et al. A Pro12Ala substitution in PPARg2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet*. 1998;20(3):284–87.

[16] Ludovico O, Pellegrini F, Di Paola R, Minenna A, Mastroianno S, Cardellini M, et al. Heterogeneous effect of peroxisome proliferator-activated receptor gamma2 Ala12 variant on type 2 diabetes risk. *Obesity (Silver Spring)*. 2007;15(5):1076-81.

[17] Masud S, Ye S. Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. *J Med Genet*. 2003;40(10):773-80.

[18] Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, et al. Evidence for gene-nutrient interaction at the PPARgamma locus. *Diabetes*. 2001;50(3):686–89.

[19] Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet*. 2000;26(1):76-80.

[20] Dubinina IA, Chistiakov DA, Eremina IA, Brovkin AN, Zilberman LI, Nikitin AG, et al. Studying progression from glucose intolerance to type 2 diabetes in obese children. *Diabetes Metab Syndr*. 2014;8(3):133-37.

[21] Azab MM, Abdel Azeez HA, Zanaty MF, El Alawi SM. Peroxisome proliferator activated receptor γ2 gene Pro12Ala gene polymorphism in type2 diabetes and its relationship with diabetic nephropathy. *Clin Lab*. 2014;60(5):743-49.

[22] Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, et al. The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. *J Biol Chem*. 2001;276(44):41245–54.

[23] Hsieh MC, Lin KD, Tien KJ, Tu ST, Hsiao JY, Chang SJ, et al. Common polymorphisms of the peroxisome proliferator-activated receptor-gamma (Pro12Ala) and peroxisome proliferator-activated receptor-gamma coactivator-1 (Gly482Ser) and the response to pioglitazone in Chinese patients with type 2 diabetes mellitus. *Metabolism*. 2010;59(8):1139-44.

[24] Namvaran F, Azarpira N, Rahimi-Moghaddam P, Dabbaghmanesh MH. Polymorphism of peroxisome proliferator-activated receptor γ (PPARγ) Pro12Ala in the Iranian population: relation with insulin resistance and response to treatment with pioglitazone in type 2 diabetes. *Eur J Pharmacol*. 2011;671(1-3):1-6.

[25] Bluher M, Lubben G, Paschke R. Analysis of the relationship between the Pro12Ala variant in the PPAR-gamma2 gene and the response rate to therapy with pioglitazone in patients with type 2 diabetes. *Diabetes Care*. 2003;26(3):825-31.

[26] Mathur SK, Rathore R, Chandra S, Sharma BB, Sharma L, Mathur P, et al. Primary pioglitazone failure in Asian Indian diabetics is not related to common Pro12Ala polymorph of PPAR-gamma gene. *Indian J Physiol Pharmacol*. 2009;53(2):175-80.

[27] Kawaguchi-Suzuki M, Frye RF. Current clinical evidence in pioglitazone pharmacogenomics. *Front Pharmacol*. 2013;4:147.

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Association of Geriatric Syndromes with Malnutrition Among Elderly

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ABSTRACT

Background: *Malnutrition is a serious and frequent condition among older adults. However, older adults are poorly assessed for malnutrition although it is not an uncommon problem in this population. Age related decline in food intake, is associated with various psychological, physiological, and social problems.* ***Aims:*** *The study was aimed to conduct more comprehensive nutritional assessment tests among malnourished and among normal elders to assess other geriatric syndromes and to investigate the association of geriatric syndromes with malnutrition.* ***Methods and Material:*** *An earlier community survey was conducted at the urban field practice area of PSG Institute of Medical Sciences and Research, Coimbatore on 154 households and 190 elders aged 60 years and above were interviewed. Malnutrition was assessed using mini nutritional assessment test (MNA) in which 37 were found to be malnourished, 47 were found to be at risk of malnutrition and 106 were found to be normal. The present cross sectional study included 30 malnourished elders and 30 normal elders randomly selected from this population. The geriatric syndromes were assessed and compared between malnourished and normal.* ***Results:*** *It was observed that, the presence of visual disturbances (86.71% vs. 53.3% x^2=7.937, P<0.01), dizziness, (60.0% vs. 16.7% x^2=7.937, P<0.01), hearing loss (40.0% vs. 10.0% x^2=7.200, P<0.01), dementia, (30.01% vs. 3.3% x^2=7.680, P<0.01) and depression (60.0% vs.13.8% x^2=13.464, P<0.01) were significantly higher in malnourished compared to normal elderly. The functional dependence mean + standard deviation (for GUGT (2.16+0.83 vs. 1.36+0.49 t=4.530 P<0.001 and Barthel index (83.50+12.18 vs. 90.66+6.91 t=2.801 P<0.01) were also significantly different between malnourished and normal elderly.* ***Conclusion:*** *The presence of various geriatric syndromes was significantly higher in malnourished elderly compared to normal. Thus, the interventional programs for malnutrition among elderly should also identify and address their geriatric syndromes.*

Keywords: Barthel index, elderly, geriatric syndrome, malnutrition

INTRODUCTION

Malnutrition is a serious and frequent condition among older adults [1]. The ageing process is a biological reality which has its own dynamic, largely beyond human control. However, it is also subject to the constructions by which each society make sense of old age. Aging is presumed to be process starting from infancy and continuing until death. On the other hand, being elderly is an unpreventable process that has biological, chronological, and social aspects and problems. Aging is a multidimensional change involving the physical, psychological as well as social aspects for an individual. It can be described as a progressive deterioration of the physical and mental functions resulting in a simultaneous decline in both the capacity of the body to maintain homeostatic balance as well as the adaptability of the individual to various stressors thereby consequently increasing the chances of illness and mortality. Many changes occur as people enter old age and these changes decrease quality of life.

Malnutrition is a condition that results from eating a diet in which nutrients are either not enough or are too much such that the diet causes health problems. It may involve calories, protein, carbohydrates, vitamins, or minerals. Malnutrition is often used specifically to refer to under nutrition where there is not enough calories, protein, or micronutrients. Poor nutritional status and malnutrition in the elderly population are important areas of concern.

Malnutrition and unintentional weight loss contribute to progressive decline in health, reduced physical and cognitive functional status, increased utilization of health care services, premature institutionalization, and increased mortality. Nonetheless, many health care practitioners inadequately address the multi factorial issues that contribute to nutritional risk and to malnutrition. A common assumption is that nutritional deficiencies are an inevitable consequence of aging and disease and that intervention for these deficiencies are only minimally effective [2]. Hence, despite the high prevalence of malnutrition among elderly, nutritional problems were not yet acknowledged by health professionals. The need for nutritional assessments and interventions are particularly crucial in this age group.

To determine the nutritional status, an earlier community survey was conducted at the Urban field practice area of PSG Institute of Medical Sciences and Research, Coimbatore on 154 households and 190 elders aged 60 years and above were interviewed. Malnutrition was assessed using mini nutritional assessment test (MNA) in which 37 were found to be malnourished, 47 were found to be at risk of malnutrition and 106 were found to be normal [1]. The predictive validity of mini nutritional assessment test has been validated in several studies by demonstrating its association with adverse health outcomes, social functioning, mortality, and higher rates of visit to general practitioner. Besides, this test has the advantage that without any laboratory test, nutritional status of elderly can be determined with questions and anthropometric measurements [1].

There were very few studies in which malnutrition is correlated to functional capacity in the elderly. Oliveira, et al. studied nutritional status and functional capacity in hospitalized elderly [3]. Li-Chin, et al. were able to show that MNA assessment could predict activity of daily living (ADL) and instrumental activity of daily living (IADL) status in community-dwelling elderly in Taiwanese population [4]. However, they did not do a comprehensive assessment of other parameters of functional capacity and nutrition. In order to suggest more appropriate measure to improve the health of the elders, further geriatric comprehensive assessment tests are needed to assess other geriatric syndromes. The present study was undertaken to compare the prevalence of other geriatric syndromes among malnourished and normal elders living in a community.

METHODS

Study design

This was a cross sectional study conducted to do an in depth comprehensive assessment among a sample of malnourished and normal elders.

Ethics

We have obtained the institutional ethical committee clearance prior to the study.

Sample size

We have used the sample size calculation for two sample means. With an expected standard deviation of Barthel index in the normal group as 4 and expecting 3 units decrease in the malnourished group, with α=0.05 and β=0.20, the sample size required for this study was 30 in each group.

Inclusion and exclusion criteria

The present study was conducted during June-August 2016 at the urban field practice area of PSG Institute of Medical sciences and Research, Coimbatore. India. The urban health centre of PSG Institute of Medical Sciences and Research has six areas on which three areas were randomly selected. An earlier community survey was conducted in these areas to estimate the prevalence of malnutrition [1]. The selected areas were HUDCO colony, AD colony, and Pattalmman Kovil street. The total number of households in these areas were 762. In 565 houses, there were no elderly people and non-response was obtained in 43 houses. Hence, we surveyed 154 households and 190 elders were examined in which 37 were found to be malnourished, 47 were found to be at risk of malnutrition and 106 were found to be normal [1]. From this study population, 30 malnourished and 30 normal adults were randomly selected.

Methodology

We have conducted a detailed comprehensive geriatric assessment after obtaining informed consent. Dementia was assessed using ‘mini-cog test” which include word recall score with a range of 0 to 3 and clock drawing score with range of 0 to 2. A total score of 0 to 2 indicates positive screen for dementia and 3 to 5 indicates negative screen for dementia [5]. Depression was assessed using validated “Geriatric depression scale” [6,7]. Barthel index test was done to measure the activity of daily living (ADL) [8]. This include a pre fixed score for feeding, bathing, grooming,

dressing, bowels, bladder, toilet use, bed to chair and back transfers and mobility on level surfaces and on stairs. In addition, we have conducted get up and go test (GUGT) [9]. Other established geriatric syndrome studied were visual disturbance, hearing loss, urinary incontinence, insomnia, dizziness, hearing loss and fall.

Statistics

The mean and Standard deviation (SD) of, ADL and GUGT were calculated and compared between malnourished and normal using independent t-test. Similarly, the prevalence of geriatric syndrome between normal and malnourished were compared using Chi square test. The data were analyzed using SPSS. $P<0.05$ was considered as statistically significant.

RESULTS

The mean (SD) of age in this subsample population for malnourished people was 71.33 (7.41) years and normal was 68.67 (5.91) years. The mean (SD) of MNA score for malnourished was 15.38 (1.23) and normal was 24.9 (2.48) (Table 1).

Table 1 Characteristics of the sample population by nutritional status

Variables		Normal (Mean (SD))	Malnourished (Mean (SD))	P value
Age		68.67 (5.91)	71.33 (7.41)	0.129
MNA		24.9 (2.48)	15.38 (1.23)	P<0.001
Sex (%)	Men	7 (23.3%)	12 (40.0%)	0.165
	Women	23 (76.7%)	18 (60%)	-

The presence of various geriatric syndromes was significantly higher in malnourished elderly compared to normal. We observed that the prevalence of visual disturbances (86.71% vs. 53.3% $x^2=7.937$, $P<0.01$), dizziness, (60.0% vs. 16.7% $x^2=7.937$, $P<0.01$), hearing loss (40.0% vs. 10.0% $x^2=7.200$, $P<0.01$), dementia, (30.01% vs.3.3% $x^2=7.680$, $P<0.01$) and depression (60.0% vs. 13.8% $x^2=13.464$, $P<0.01$) were significantly higher in malnourished compared to normal elderly. The functional dependence mean + standard deviation (for GUGT (2.16+0.83 vs. 1.36+0.49 t=4.530 $P<0.001$ and Barthel index (83.50+12.18 vs. 90.66+6.91 t=2.801 $P<0.01$) were also significantly different between malnourished and normal elderly (Table 2).

Table 2 Association of geriatric syndromes with malnutrition

Geriatric syndromes	Normal (N=30)	Malnourished (N=30)	P value
Visual disturbance	16 (53.3%)	26 (86.71%)	P<0.01
Urinary incontinence	1 (3.3%)	2 (6.7%)	0.554
Insomnia	11 (36.7%)	16 (53.3%)	0.194
Dizziness	5 (16.7%)	18 (60.0%)	P<0.01
Deafness	3 (10.0%)	12 (40.0%)	P<0.01
Fall	9 (30.01%)	11 (36.7%)	0.584
Dementia	1 (3.3%)	9 (30.01%)	P<0.01
Depression	4 (13.8%)	18 (60.0%)	P<0.001
Get Up and Go Test (GUGT Score) (Mean + SD)	1.36+0.49	2.16+0.83	P<0.001
Barthel index score (Mean + SD)	90.66+6.91	83.50+12.18	P<0.001

DISCUSSION

The world health organization has stated that aging population will present new challenges for health care [10]. The health of the elderly will be an important issue defining the health status of the population [11]. The mini nutritional assessment scale (MNA) is a widely used international questionnaire to evaluate the nutritional state of elders with high sensitivity (98.9%), specificity (94.3), and diagnostic accuracy (97.2%) [1]. However in India, only very few community studies were conducted to estimate malnutrition among elderly [1]. Both its reliability and validity were assessed by Mathew, et al. and found reliable and valid in Indian context [11,12].

The elderly is the fastest growing segment of the population including in India. Malnutrition has emerged as an important problem among elderly. In our earlier study, we have reported an estimated prevalence of 19.47% in the general population. There are different mechanisms involved in the development of malnutrition in elderly include gastrointestinal and endocrine system disorders, loss of taste and smell, decreased appetite and inadequate dietary

intake that is related to both disease state and psychosocial factors [12]. Many studies reported sarcopenia, immobility, social isolation, and dementia were also associated with decreased oral food intake [13]. The term 'geriatric syndrome' is used to cover functional impairment, cognitive impairment, malnutrition, incontinence, falls, depression, psychosis, insomnia, vertigo, and other related disorders [13].

In our study, nutritional status of malnourished patients was found correlated with the number of established geriatric syndromes. Dementia was one among them which had significantly higher proportion in malnourished group compared to normal group. Many studies reported that cognitive deterioration in the elderly affects daily functional status and instrumental activities which result with disability, dependence and decreased oral intake [14]. Many studies found greater impairment in both simple and instrumental daily living activities and a more severe ideomotor praxis deficit in patients at risk of malnutrition [15]. Activity of daily living measured through GUGT test and Barthel index test was associated with nutritional status in this study. We have also observed a significant association between visual disturbance and malnutrition. Visual activities can lead to fall which can affect the activity of daily living [3]. In an earlier study, it was observed that ADL was shown a relation between dietary quality and geriatric outpatients. Lower self-efficacy, a lower score of attitudes toward health and difficulty with meal preparation were found to be the common problems in many reported studies [2].

Depression has been proven to be a very common cause of weight loss in the older patients [16]. It was observed in some studies that weight loss in depression occurs in 90% in older patients, compared with only 60% in the youngsters [17]. It was also observed that causes of malnutrition in depressed patients mainly involve lack of appetite, loss of interest in self-care, apathy, and physical weakness [17]. In general, inadequate nutritional intake is associated with various conditions of older people, in particular, with subjective conditions such as mood and functional status and with objective situation such as living alone, income and education [18].

However, causal relationships between the factors and the nutritional status were unclear. Another limitation of the study is the collection of data from a small sample population. Large scale studies are needed for confirmation of findings. Another limitation of the study is dementia was assessed using mini cog test which was not followed up by confirmatory MMSE [18] as it is difficult to use in rural illiterate population. Despite these limitations, our study has several strengths. We have used validated tests to assess the geriatric syndrome. Many of these earlier studies were hospital based and not much studies were done at community. In addition, cases and controls were selected through a well-defined sampling procedure.

CONCLUSION

The presence of various geriatric syndromes was significantly higher in malnourished elderly compared to normal. This association between malnutrition and the presence of geriatric syndromes needs further study as either could be causative or a sequel of each other. An understanding of these causes is essential to formulate appropriate treatment strategies. Although an adequate nutrition supply is the key to the treatment of malnutrition, it alone may not necessarily be successful. Other causative factors must also be addressed and treated. In conclusion, the interventional programs for malnutrition among elderly should also identify and address their geriatric syndromes.

REFERENCES

[1] Mathew, Anil Chankaramangalam, et al. "Prevalence and correlates of malnutrition among elderly in an urban area in Coimbatore." *Indian Journal of Public Health* 60.2 (2016): 112.

[2] Ülger, Zekeriya, et al. "Comprehensive assessment of malnutrition risk and related factors in a large group of community-dwelling older adults." *Clinical Nutrition* 29.4 (2010): 507-511.

[3] Oliveira, Maria R. M., Kelly, C. P. Fogaça., and Vânia A. Leandro-Merhi. "Nutritional status and functional capacity of hospitalized elderly." *Nutrition Journal* 8.1 (2009): 54.

[4] Lee, Li-Chin, and Alan C. Tsai. "Mini-nutritional assessment predicts functional decline of elderly Taiwanese: Result of a population-representative sample." *British Journal of Nutrition* 107.11 (2012): 1707-1713.

[5] Borson, Soo, et al. "Improving identification of cognitive impairment in primary care." *International journal of geriatric psychiatry* 21.4 (2006): 349-355.

[6] Rinaldi, Patrizia, et al. "Validation of the five-item geriatric depression scale in elderly subjects in three different settings." *Journal of the American Geriatrics Society* 51.5 (2003): 694-698.

[7] Burke, William J., William H. Roccaforte, and Steven P. Wengel. "The short form of the geriatric depression scale: A comparison with the 30-item form." *Topics in geriatrics* 4.3 (1991): 173-178.

[8] Jacelon, Cynthia S. "The Barthel Index and other indices of functional ability." *Rehabilitation Nursing* 11.4 (1986): 9-11.

[9] Mathias, S., Nayak, U. S., and Bernard Isaacs. "Balance in elderly patients: The "get-up and go" test." *Archives of physical medicine and rehabilitation* 67.6 (1986): 387-389.

[10] World Health Organization. *The world health report 2008: Primary health care*. World Health Organization, 2008.

[11] Joshi, Kamlesh, Rajesh Kumar, and Ajit Avasthi. "Morbidity profile and its relationship with disability and psychological distress among elderly people in Northern India." *International Journal of Epidemiology* 32.6 (2003): 978-987.

[12] Mathew, Anil C., et al. "The reliability of mini nutritional assessment questionnaire in screening malnutrition among elderly aged 60 years and above." *Asian Pacific Journal of Health Sciences* 2.3 (2015): 47-48.

[13] Visvanathan, R. "Under-nutrition in older people: A serious and growing global problem!." *Journal of postgraduate medicine* 49.4 (2003): 352.

[14] Claggett, M. S. "Nutritional factors relevant to Alzheimer's disease." *Journal of the American Dietetic Association* 89.3 (1989): 392-396.

[15] Spaccavento, Simona, et al. "Influence of nutritional status on cognitive, functional and neuropsychiatric deficits in Alzheimer's disease." *Archives of Gerontology and Geriatrics* 48.3 (2009): 356-360.

[16] Hickson, M. "Malnutrition and ageing." *Postgraduate Medical Journal* 82.963 (2006): 2-8.

[17] Blazer, Dan, James R. Bachar, and Dana C. Hughes. "Major depression with melancholia: A comparison of middle-aged and elderly adults." *Journal of the American Geriatrics Society* 35.10 (1987): 927-932.

[18] Folstein, Marshal F., Susan E. Folstein, and Paul R. McHugh. ""Mini-mental state": A practical method for grading the cognitive state of patients for the clinician." *Journal of psychiatric research* 12.3 (1975): 189-198.

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RESEARCH ARTICLE

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Exposure of bisphenol A in breast cancer patients—quantitatively assessed by sensitivity-enhanced high-performance liquid chromatography coupled with fluorescence detection: A case–control study

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Abstract

A simple, reproducible and sensitive liquid chromatography (HPLC) method has been developed and validated for estimation of bisphenol A (BPA) in human urine. A simple liquid–liquid extraction technique was used in BPA sample preparation. The analyte was chromatographed on a Shimadzu Prominence HPLC system using isocratic mobile phase conditions at a flow rate of 0.500 ml/min and a Hypersil Gold C_{18} column maintained at 40°C. Quantification was performed on a fluorescence detector set at excitation 275 nm, emission 313 nm and bisphenol B was used as internal standard. The total run time was 8 min. The method was found to have acceptable sensitivity, selectivity, accuracy (98.82–103.64%), precision (1.17–5.36) and stability in the validation experiment carried out as per the USFDA guidelines. The method sensitivity was as low as 0.50 ng/ml. The applicability of the validated analytical method was established in human patient urine samples. The mean human urine BPA concentrations were 1.18 ± 2.11 ng/ml in the control group and 5.76 ± 6.00 ng/ml in the patient group ($P < 0.001$). Therefore, this method could be considered as an alternative for routine bio-monitoring of BPA which is less expensive and feasible in resource-poor settings.

KEYWORDS
bisphenol a, breast cancer, HPLC–FLD, human urine, patients

1 | INTRODUCTION

Bisphenols are a heterogenous class of phenolic chemical compounds, formed by aromatic condensation of two phenol rings (Rochester, 2013). About 18 bisphenolic compounds have been synthesized and named as per the synthetic functional groups with which the phenols condense. Bisphenol A (BPA) is formed when two phenol groups react with acetone, whereas bisphenol S is formed after sulfur trioxides react with phenols groups. However, the efficiency of their overall radioactive degradation is high (76%) (Rochester, 2013).

Bisphenols have been used as either polycarbonate or epoxy resin polymers in various applications (Peller et al., 2009). For example, epoxy bisphenol resins have been used in floorings, varnishes, printed circuit boards and beverage cans, among many applications, whereas polycarbonate polymers have applications in housing electronics, optical devices and other important industrial uses (Corrales et al., 2015). Many of these industrial applications are due to their favorable chemical properties and their superiority over alternative chemicals. For example, their use in medical equipment as reflectors is unavoidable and no alternatives are available, owing to their favorable optical properties. The use of BPA in dental fillings, brake fluids, flame retardants in electronic equipments, rubber and thermal paper industries is allowed up to a permitted level.

List of Abbreviations: BPA, bisphenol A; FLD, fluorescence detection; HPLC, high-performance liquid chromatography; TBME, *tert*-butyl methyl ether; USFDA, United States Food and Drug Administration.

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Certain bisphenols can enter the body of fishes through contaminated water, and when these fishes are consumed by humans, they can enter the higher levels of the food chain. Bisphenols can also enter the body via food cans when they react with hot water and are consumed. Epoxy resin polymers of bisphenols can also penetrate the body via food when foodstuffs with additives containing phenolic chemicals are consumed. Other industrial chemicals like tetra-bromobisphenol A, which are manufactured in large quantities, are degraded to bisphenolic chemicals in the environment. Bisphenols are known to be toxic and to pollute the environment. Two such bisphenols, BPA and bisphenol S are proven to be endocrine disruptors (Ribeiro et al., 2017). Certain bisphenols like BPA are potent carcinogens, and have been shown to either induce cancers or be associated with the risk of cancer progression in animal and human models (Stossi et al., 2014). This has been attributed to their interaction with the nuclear receptors like aryl hydrocarbon receptors in endocrine tissues. Their etiological role in the causation and progression of hormone-positive cancers like breast cancer has been proven to be because of the ability of the BPA–aryl hydrocarbon receptor complex to induce estrogen-modulated developmental reprogramming (Dang et al., 2007; Fernandez & Russo, 2010). Owing to their toxicological profiles as environmental pollutants, their production, supply and use are regulated by the United Nations Basel and Stockholm Conventions as industrial chemical pollutants (Haffner & Schecter, 2014).

Recent evidence indicates that, more than the glucuronide metabolite levels in urine, the levels of the parent BPA is highly indicative of bisphenolic toxicity profiles, although the urinary levels of the metabolite are generally higher (Foster et al., 2019). Although the estimation of phase II BPA metabolites indicates the source of exposure in humans as compared with urinary BPA levels (Gerona et al., 2016), there may be significant inter-species variation in the metabolite concentrations in urine, leading to variations in both total BPA and metabolite BPA levels in urine (Völkel et al., 2002). Hence, in this study, we hypothesize a probable association of urinary BPA levels in cancer patients with the risk of breast cancer.

The biomonitoring of BPA and its metabolites in urine was evaluated using high-performance liquid chromatography coupled with fluorescence detection (HPLC–FLD) after chemical derivatization with *p*-nitrobenzoyl chloride (Mao et al., 2004). Other methods to estimate these compounds in biological matrices have also been developed as high-throughput alternatives to HPLC, like ultra-high-pressure liquid chromatography–mass spectromentry (UPLC–MS; Anderson et al., 2014), liquid chromatography–tandem mass spectrometry (LC–MS/MS; Lee et al., 2013; Li & Franke, 2015) and SPE-UPLC–MS (Jing et al., 2011). However, these methods are neither cost-effective nor time-efficient, and hence cannot be employed in regular biomonitoring of both environmental and biological samples in a routine manner. While LC–MS/MS methods are beneficial in several ways, the costly instrumentation and restricted accessibility for regular hospital practice and laboratories are their major limitations.

In this study, the association of urinary BPA concentrations and the risk of breast cancer was assessed in patients. To achieve this objective, an HPLC–FLD method for BPA was developed and validated. Additionally, the method was designed using an isocratic mobile phase proportional with a light buffer and better reproducibility with less interference. An LC–MS/MS compatible buffer and chromatographic conditions were used; if required, the method can be easily adapted to suit LC–MS/MS. The method and chromatographic conditions employed are high-throughput, cost-effective and time-efficient for estimating BPA in human urine. The proposed method was successfully applied to estimate the exposure of BPA in human urine samples in breast cancer patients and a comparable control group.

2 | MATERIALS AND METHODS

2.1 | Chemical and reagents

Acetonitrile and methanol HPLC grade were purchased from Sigma-Aldrich, India. Ammonium acetate of HPLC grade was purchased from Fisher Scientific, India. The reference standard BPA and bisphenol B (internal standard, IS) were purchased from Sigma-Aldrich, India. Methyl *tert*-butyl ether (TBME) and acetic acid of HPLC grade were obtained from Himedia, India. Blank urine was obtained from the blood bank services of PSG Hospitals, Coimbatore, India. Ultrapure water (18.2 MΩ cm), used in all experiments, was obtained from a MilliQ water purification system (Millipore, Milford, MA, USA).

2.2 | Instrumentation and chromatography conditions

The bioanalytical method was developed using an HPLC instrument consisting of a Shimadzu Prominence HPLC system equipped with an isocratic solvent delivery unit LC–20A pump and column oven CTO-20A coupled with a fluorescence detector (Shimadzu, RF-20A, Japan). The chromatographic separation was performed on a Thermo Hypersil Gold C_{18} column from Thermo-Fisher (3.0 × 150 mm; 5 μm). The column temperature was set at 40°C. The mobile phase was composed of a mixture of solvent A (80% acetonitrile in water, 60%, v/v) and solvent B (10 mM ammonium acetate, 40%, v/v) at a flow rate of 0.5 ml/min. Before analysis the mobile phase was filtered through a 0.45 μm membrane filter and degassed by ultra-sonication. A 20 μl injection of each sample was loaded onto the system and total analysis time was 8 min. The calibration curve was linear over the concentration range 0.5–100 ng/ml. The fluorescence detector set at excitation 275 nm and emission 313 nm. Data acquisition was done using LC solution software (Shimadzu).

2.3 | Preparation of calibration standard and quality control samples

The BPA and IS stock solutions were prepared in methanol at a concentration of 1,000 μg/ml. The series of BPA working stock solutions

in the concentration range of 10–1,000 ng/ml were obtained by further diluting the stock solution with methanol–water (50:50, v/v). The stock solution of BPA and IS were stored at 4°C until analysis. The calibration standards of BPA in urine with final concentrations of 0.50, 2.0, 5.0, 15.0, 30.0, 50.0, 70.0, 90.0 and 100 ng/ml were prepared by spiking a 450 µl aliquot of blank urine with a 50 µl aliquot of BPA working standard stock solutions. Quality control samples (QC) were prepared at four concentration levels of 0.50 ng/ml (lower limit of quantification, LLOQ), 2.50 ng/ml (low quality control, LQC), 35.0 ng/ml (middle quality control, MQC) and 80.0 ng/ml (high quality control, HQC). These samples were stored at −80 ± 10°C.

2.4 | Sample preparation

Sample preparation was carried out by the liquid–liquid extraction procedure. To a 500 µl of aliquot of urine, 50 µl of IS working solution was added into a 5 ml polypropylene tube and mixed for 30 s on a Spinix vortex shaker (Tarsons, India). To this was added 3.0 ml of TBME and it was vortex mixed for 5 min. The solution was centrifuged at 3,000 rpm for 5 min at 4°C on an Eppendrof 5810R centrifuge (Eppendrof AG, Hamburg, Germany). The clear organic layer was transferred into 5 ml clean glass tubes and evaporated to dryness at 40°C using nitrogen evaporator (Turebovap®, Biotage, USA). The residue was reconstituted in 250 µl of the mobile phase, vortex mixed for 1.0 min and centrifuged at 10,000 rpm for 5 min. Finally, the clear supernatant was injected onto the HPLC system for analysis.

2.5 | Development of an HPLC method

Different buffers, ionic strengths and pHs were used as an aqueous solvent during method development. Acetonitrile and methanol were used at different compositions to optimize and evaluate the organic content of the mobile phase. A C_{18} column was used to attain optimum retention, resolution and good peak shape for BPA and IS.

2.6 | Development of sample extraction method

Different extraction methods were employed, such as protein precipitation and liquid–liquid extraction, to obtain maximum recovery and good peak shape without interferences. Methanol, acetonitrile and trichloroacetic acid were used as the precipitating solvent for protein precipitation. Dichloromethane, hexane, ethyl acetate and TBME solvents were used in the liquid–liquid extraction method with different acidic, basic and neutral conditions.

2.7 | Bioanalytical method validation

The validation procedures were carried out according to US Food and Drug Administration guidelines (USFDA, 2018) as explained previously (Siva Selva Kumar & Ramanathan, 2016). The method was validated for selectivity, specificity, recovery, linearity, accuracy, precision and stability using US Food and Drug Administration (USFDA) guidelines[17] for assay in human urine.

2.8 | Selectivity and specificity

Selectivity of the method was assessed by analyzing 10 separate human urine samples that were collected and injected at the beginning of the validation and the potential interferences at the LC peak region for analytes and IS were subsequently investigated. At least eight out of 10 lots should have a response less than 5 times the lower limit of quantification (LLOQ) level response in the same matrix as per USFDA guidelines (USFDA, 2018).

2.9 | Carryover

Carryover effects were assessed by injecting the HQC samples just before the blank human urine to check for potential interference. As per the USFDA guidelines, carryover assessed by injection of high concentration should be <20% of the area of the LLOQ (USFDA, 2018).

2.10 | Sensitivity

Sensitivity of the method was evaluated by spiked urine samples at LLOQ level injected six times and the area of the BPA peak at LLOQ level should be at least 5 times greater than the blank peak area, as recommended by USFDA guidelines. As per the USFDA guidelines, the injected LLOQ samples accuracy should be ±20% and the precision, expressed as the coefficient of variation, should be <20% (USFDA, 2018).

2.11 | Extraction recovery

The recovery was evaluated to demonstrate the reproducibility of the extraction procedure. The recovery of the BPA in LQC, MQC and HQC samples (six extracted QC samples) was evaluated relative to six extracted blank samples spiked with neat standards containing concentrations equivalent to low, mid and high concentrations of BPA with the intended standard concentration (200 ng/ml) corresponding to 100% recovery.

2.12 | Linearity

Linearity was evaluated by linear regression analysis with the use of working standard solutions and spiked urine samples containing the BPA of interest at different concentrations within the range

0.50–100 ng/ml for BPA. The calibration curves were constructed by plotting the ratio of the peak area of BPA to the peak area of internal standard vs. the nominal BPA concentration. The slopes and intercepts were calculated with least square linear regression analysis of the data with the use of a $1/x^2$ (where x is concentration) weighting factor. The acceptance criteria for each back-calculated standard concentration were ±15% deviation from the nominal value except at the LLOQ, which was set as ±20% (USFDA, 2018).

2.13 | Accuracy and precision

The validation methods for evaluation of the accuracy and precision in the experiments were performed as four independent series (including ruggedness) in urine. The intra- and inter-day assay accuracy and precision were estimated by analyzing six replicates containing analytes at four different QC levels, that is, 0.50, 2.5, 35.0 and 80 ng/ml. The accuracy, which represents the closeness of the agreement between the mean values obtained from the series of measurements by the method and the actual value, should be within 85–115% of the nominal value, except at the LLOQ, where it should not deviate by <20% (USFDA, 2018). Precision, which represents the closeness of agreement among a series of measurements obtained from multiple sampling, was estimated with variances in repeatability (intra-day variances) and intermediate precision (sum of intra- and inter-day variances). The precision should not exceed ±15% relative standard deviation (RSD), except for at the LLOQ, where it should be within ±20% of the RSD (USFDA, 2018).

2.14 | Stability

Stability tests were performed to evaluate the stability of analyte in urine samples under different conditions that might be encountered during processing of patient sample analysis. The bench-top stability study assessed the stability of the analyte in urine at room temperature for 8 h, whereas a post-preparative stability study assessed the stability of the analyte in the treated samples (refrigerator stability for 24 h at 10°C) at low (2.5 ng/ml) and high (80 ng/ml) QC concentrations. Freeze–thaw stability was determined after three freeze–thaw cycles and long-term stability was determined by asessing QC samples stored at −80°C for 30 days against a freshly spiked calibration curve and quality control samples.

2.15 | Participants

The present study was a hospital-based case–control study which included 50 breast cancer cases and 50 healthy control subjects. The study was approved by the Institutional Human Ethics Committee and all consenting participants were included. Clinically confirmed breast cancer subjects were recruited from the Department of Medical Oncology, PSG Hospitals, Coimbatore. The age-matched controls were those who had undergone mammogram screening for a routine health checkup during the same period, and not been diagnosed with breast cancer. Informed consent was obtained for all the participants and during the hospital visit we collected 5 ml of urine from each subject in a sterile container and stored it at −80°C until use.

2.16 | Statistical methods

Data analysis was performed using SPSS (IBM SPSS, version 24.0). Comparisons of BPA urinary concentrations among the groups were conducted using nonparametric procedures, including *t*-tests and Mann–Whitney *U*-test. Statistical significance was set at $P < 0.05$. Data was reported as mean ± SD.

3 | RESULTS AND DISCUSSION

3.1 | Optimization of the chromatographic conditions

Method development was initiated for the determination of BPA in human urine samples using reverse-phase liquid chromatography coupled with fluorescent detection. The HPLC–FLD method development was performed to achieve adequate resolution of BPA and endogenous interferences in matrix with simple buffers and short run time. This method has certain advantages over those which were used earlier for BPA quantification in urine samples. In the method development, different mobile phases comprising a number of combinations of buffers (e.g. acetate buffers and phosphate buffers) and organic solvents (methanol and acetonitrile) along with different flow rates (0.350–0.800 ml/min) were tested to optimize for a successful chromatographic resolution of BPA and IS. In the mobile phase optimization, methanol solvents produced an asymmetric peak shape and long run time. Acetonitrile was used as the organic solvent in the mobile phase, and peaks were sharp and symmetrical and the run time was reduced. The good resolution of peaks attained with isocratic mobile phase consisted of a mixture of 80% acetonitrile in water (60%, v/v) and 10 mM ammonium acetate (40%, v/v) at a flow rate of 0.5 ml/min on a Hypersil Gold C_{18} column. Compared with phosphate buffers, volatile buffers like ammonium acetate in mobile phase give good column life and can be suitable for mass spectrometry.

3.2 | Development of sample extraction method

Poor recovery was observed with precipitation extraction for BPA (23.48%). For liquid–liquid extraction, BPA recovery was 31.88 and 38.28% for dichoromethane and ethyl acetate. TBME was used as the extraction solvent in neutral, acidic and basic conditions. However, a reproducible and good recovery (>90%) was obtained in TBME with neutral conditions for BPA and the IS. Therefore, liquid–liquid

extraction using TBME was the preferred method for human urine sample preparation in this study.

3.3 | Bioanalytical method validation

3.4 | Selectivity and specificity

Selectivity of the method was evaluated by analyzing pooled BPA-free human blank urine, blank urine spiked with IS and with analyte injected at the beginning of the validation, and the possible interferences at the LC peak region for analyte and IS were investigated (Figure 1). At least eight out of 10 lots should have a response <5 times the LLOQ level response in the same matrix.

3.5 | Carryover

There was no peak response observed when the highest calibration standard was analyzed just before the blank urine. This indicated that the washing step of the column at the end of the chromatography separation was adequate to remove potential residues of the analyte. The BPA peak response in blank was <20% of the LLOQ.

3.6 | Sensitivity

The analyte peak area at LLOQ was found to be >5 times higher compared with the peak area of the blank sample. The mean accuracy for BPA for the six LLOQ injections was 101.82% and the precision value for BPA was 3.57 (CV). Hence, the developed method was sensitive at LLOQ level (0.50 ng/ml).

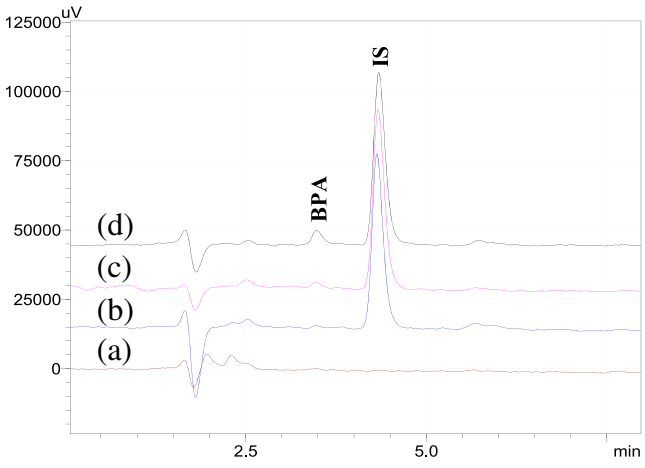


FIGURE 1 Representative HPLC chromatograms of bisphenol a (BPA) and internal standard (IS) in human urine. (a) Blank urine sample; (b) blank urine spiked with IS; (c) blank urine spiked with analyte (0.50 ng/ml) and IS; and (d) sample collected from breast cancer patient

3.7 | Recovery

The liquid–liquid extraction technique gave adequate recovery and cleaner samples. The results of the evaluation neat standard vs. urine extracted standard were estimated for BPA (2.50, 35.0 and 80.0 ng/ml) and the mean recovery was 96.07 ± 8.42%. The recovery of the IS at 200 ng/ml was 79.12 ± 4.45%.

3.8 | Linearity

The calibration curves were constructed using nine calibration standards in human urine and exhibited excellent linearity with regression correlation coefficient (r^2 > 0.996) over the concentration range of 0.50–100 ng/ml for BPA. A typical regression equation was prepared by determining the best fit of peak-area ratio (peak area analyte/peak area IS) vs. nominal BPA concentration. The slopes and intercept were calculated with least square linear regression analysis of the data with use of a $1/x^2$ weighting factor. The lowest concentration with the RSD <20% was taken as the LLOQ and was found to be 0.50 ng/ml for BPA. The percentage accuracy observed for the mean back-calculated concentration for four calibration curves for the BPA was within 95.40–105.30%.

3.9 | Accuracy and precision

The accuracy and precision of the method were evaluated as intra-day (repeatability) and inter-day (intermediate) human urine for BPA samples, as summarized in Table 1. The intra- and inter-day precisions were <5.36% and the accuracy ranged from 98.82 to 103.64% at all levels. All the values were within the accepted range and the method was accurate and precise.

3.10 | Stability

The stability of BPA in human urine was investigated under a variety of storage and process conditions, in urine at room temperature for 8 h, in the refrigerator for 24 h at 10°C, after three freeze–thaw cycles and after long-term storage at −80°C for 30 days, as summarized in Table 2. The results indicated that BPA was stable under the conditions described.

3.11 | Regular practical applicability of the validated analytical method in patient samples

An association study between the prevalence of breast cancer and urine BPA concentrations was successfully undertaken. To achieve this objective, patient samples were analytically processed to estimate the BPA levels in urine and these were later correlated with the patient's phenotype status with emphasis on breast cancer.

TABLE 1 Intra- and inter-day precision and accuracy of bisphenol A (BPA) in human urine

	Intra-day ($n = 6$)			Inter-day ($n = 18$)		
Nominal concentration (ng/ml)	Measured concentration (mean ± SD, ng/ml)	CV (%)	Accuracy (%)	Measured concentration (mean ± SD, ng/ml)	CV (%)	Accuracy (%)
0.55	0.57 ± 0.03	5.26	103.64	0.56 ± 0.03	5.36	101.82
2.54	2.58 ± 0.12	4.65	101.57	2.51 ± 0.10	3.98	98.82
35.28	35.63 ± 0.97	2.72	100.99	35.99 ± 1.12	3.11	102.01
80.19	79.81 ± 1.11	1.39	99.53	79.58 ± 0.93	1.17	99.24

TABLE 2 Stability of BPA in human urine under different conditions ($n = 6$)

	Bench-top (room temperature for 8 h)		Refrigerator (8°C for 24 h)		Freeze–thaw cycles		Long-term stability (−80°C for 30 days)	
Nominal concentration (ng/ml)	Measured concentration (mean ± SD, ng/ml)	Bias (%)	Measured concentration (mean ± SD, ng/ml)	Bias (%)	Measured concentration (mean ± SD, ng/ml)	Bias (%)	Measured concentration (mean ± SD, ng/ml)	Bias (%)
2.54	2.59 ± 0.10	−1.97	2.65 ± 0.13	−4.33	2.50 ± 0.11	1.57	2.63 ± 0.10	−3.54
80.19	82.05 ± 0.98	−2.32	79.82 ± 0.42	0.46	81.25 ± 0.89	−1.32	79.31 ± 1.38	1.10

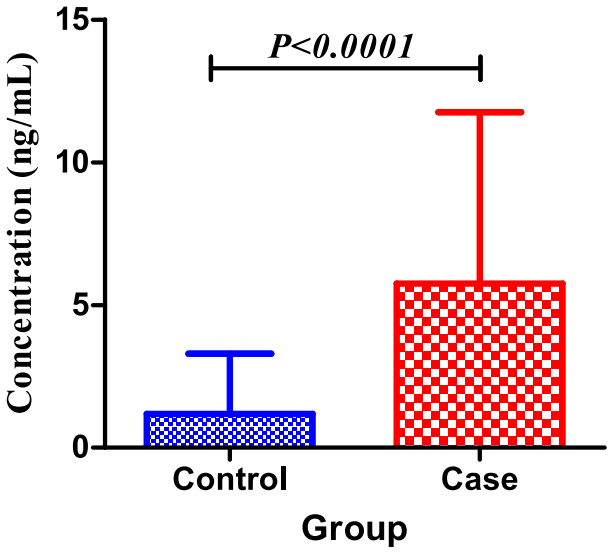


FIGURE 2 The mean urinary BPA levels in control vs. case group

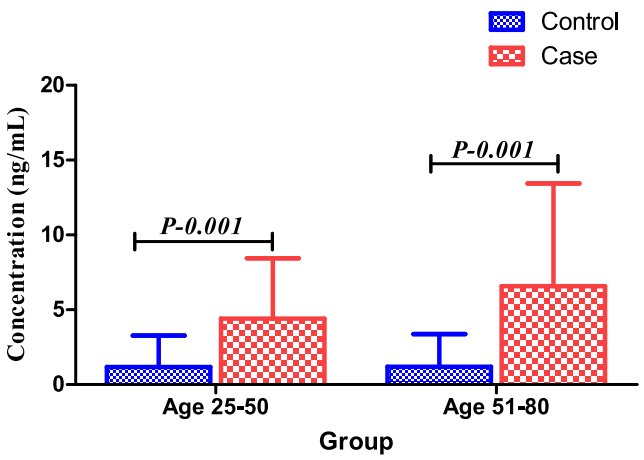


FIGURE 3 The mean urinary BPA levels in control vs. case in the age groups 25–50 and 51–80 years

Moreover, 84% of cases and 64% of controls in our study had detectable levels of BPA in the biological matrix cross-section.

The mean urinary BPA level in the control group was 1.18 ± 2.11 ng/ml, whereas it was 5.76 ± 6.00 ng/ml in the patient group ($P < 0.0001$; Figure 2). The mean ages of the study population were cases, 52.68 ± 10.99 years, and controls, 48.48 ± 10.07 years. The sample of study volunteers was then divided into two age groups for subgroup analysis. In the 25–50 year age group, the mean urinary BPA concentrations in the control and patient groups were 1.18 ± 2.09 and 4.41 ± 4.04 ng/ml ($P < 0.001$; Figure 3). These were 1.19 ± 2.19 and 6.58 ± 6.87 ng/ml in the 51–80 years control and patient group populations ($P < 0.0001$). The data are summarized in Table 3.

3.12 | Current status of BPA biomonitoring in breast cancer patients

Bisphenol A is used as a plasticizer in rubber and chemical manufacturing units, and it is useful as a chemical reagent in the synthesis of epoxy resins and polyvinyl chloride. It is also useful in healthcare industrial sectors, for example in dental extraction units

TABLE 3 Comparisons of BPA concentration and age in case vs. control study in breast cancer patients

Groups	Case (mean ± SD)	*N*	Control (mean ± SD)	*N*	*P*-Value
BPA concentration (ng/ml)					
1	5.76 ± 6.00	50	1.18 ± 2.11	50	<0.001***
2	4.41 ± 4.04	19	1.18 ± 2.09	27	0.001***
3	6.58 ± 6.87	31	1.19 ± 2.19	23	0.001***
Age					
1	52.68 ± 10.99	50	48.48 ± 10.07	50	0.050*
2	41.42 ± 6.37	19	41.22 ± 5.88	27	0.914
3	59.58 ± 6.60	31	57.00 ± 6.66	23	0.163

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(Konieczna et al., 2015). However, it is an environmental pollutant, specifically classified as a Persistent Organic Pollutant by the United Nations Stockholm Convention and as a grade 2B as a possible carcinogen in humans by the International Agency for Research on Cancer (IARC) (Seachrist et al., 2016). However its potential risk as an endocrine disruptor is greater in causing breast cancer and neuroendocrine toxicities. We have investigated the association between prior BPA exposure, cross-sectionally quantified in the urine of patients, and the breast cancer phenotype in different age groups.

Bisphenol A increased the risk of trans-generational epigenetic transfer when Sprague–Dawley rats were fed with graded doses in a high-fructose diet for 2 weeks (Holmes, 2017), which increased breast cancer incidence in female offspring rats. Indeed BPA is a known carcinogen and environmental endocrine disruptor. The carcinogenic ability of BPA is apparently expressed in mice exposed prenatally to ≥0.25 μg/kg b.w./day, on embryonic day 18, by increased chronic inflammatory processes in the breast adipocytes and tissue (Wang et al., 2017). It exhibits carcinogenic potency in breast tissue by increasing the expression of hormone and proliferation of hormone-positive breast tissue cell, both *in-vitro* and *in-vivo*. Since BPA is an estrogenic compound with endocrine disruption potential, it is hypothesized to increase the proliferation of estrogen-positive breast cells, specifically of the MCF-7 lineage (Soto et al., 1995; Vandenberg et al., 2009; Wang et al., 2017). Being structurally similar to estrodiol, BPA increases wet uterine weight and lactoferrin expression and has other effects in estrogen-specific screens (Markey et al., 2001). Being also chemically similar to diethylstilbestrol, it can increase the risk of *in-utero* estrogenic effects in both animal models and humans, causing trans-generational endocrine disruption (Seachrist et al., 2016). Although it can bind to both forms of nuclear receptors for estrogen, its affinity to bind to orphan estrogen-related and GPR-30 receptors stimulates the genomic and nongenomic cascades of cellular proliferation in the breast tissue. Low-dose BPA can also additionally suppress androgen synthesis and thyroid hormone actions, both of which can further contribute to the endocrine disrupting effect of this pollutant.

The geometric mean of BPA concentrations across seven countries was 1.20 ng/ml, and it was found to be the highest in India with a median urinary level of BPA reaching 1.71 ng/ml in the general population (Zhang et al., 2011). Although the range of creatinine-clearance-adjusted BPA levels in urine in volunteer samples was broad (0.31–39.2 μg/g), it was reported to be nearly equal in male and female genders. In this study, the mean BPA urinary concentration in the volunteers was 1.18 ± 2.11 ng/ml, which approximates previously reported exposure levels in the general population. In breast cancer patients, there was a 3- to 4-fold increase in this value (mean urinary level was 5.76 ± 6.00 ng/ml in cases). This was higher than previously reported values in cases with breast cancer (after adjusting for creatinine clearance), approximating nearly 0.71 ± 0.31 ng/g in breast adipocytes (Reeves et al., 2018). The level was higher in the blood samples of cancer patients in another case–control study (mean blood BPA was 13.87 ng/L (Yang et al., 2009), and in the general population (noncancer volunteers) in urine (0.37 ng/g creatinine in rural areas vs. 0.46 ng/g in urban areas) owing to chronic low-dose environmental exposures (Battal et al., 2014).

The current study proposes a biomonitoring method that could be routinely used for urinary estimation of BPA in both control populations and breast cancer patients as a toxicology biomarker. It is a cost effective and less expensive alternative to other methods used for BPA estimation. For example, studies reporting effective methods for BPA estimation like gas chromatography–mass spectrometry, LC–MS/MS (Caban & Stepnowski, 2020; Kato et al., 2005). Most of these reported methods also have used the highly expensive solid-phase extraction in BPA biomonitoring, using environmental samples for research outcomes (Caban & Stepnowski, 2020; Murphy et al., 2019). Moreover, this study reports a less time-consuming method of BPA estimation in patients for the first time in a South Indian population using robust case–control design compared with previous reports (Murphy et al., 2019). Other studies reported in India have developed and applied expensive method alternatives to the one developed, and have reported their application in patient plasma biomonitoring for other diseases (Pednekar et al., 2018). Finally, this method has been validated in 100 healthy volunteers and patients, with a higher degree of precision and extrapolatibility to human biomonitoring, owing to higher statistical confidence. This reported method hence could be used for routine risk assessment and biomonitoring of both environmental samples and patient matrices, with minor variations in method optimization, in a high-throughput, cost-effective and time-gaining manner, in contrast to previous reports in an Indian population.

To conclude, a high-throughput, cost-effective routine alternative to LC–MS (HPLC–FLD) without compromising on the analytical

accuracy was developed as an easier low-cost alternative for screening BPA in urine samples.

4 | CONCLUSION

A reliable, simple and sensitive HPLC method has been developed and validated for BPA analysis and applied to obtain a significant association in breast cancer patients' urinary samples. This method has significant advantages, including adequate recovery and good peak shape with shorter run time.

We have developed a rapid and reliable method to estimate the BPA in human urine and applied it for quick and cost-effective routine biomonitoring in breast cancer patients exposed environmentally to BPA. This method has similar sensitivity to other methods developed using LC–MS/MS.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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REFERENCES

Anderson, D. J., Brozek, E. M., Cox, K. J., Porucznik, C. A., & Wilkins, D. G. (2014). Biomonitoring method for bisphenol a in human urine by ultra-high-performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, *953–954*, 53–61.

Battal, D., Cok, I., Unlusayin, I., Aktas, A., & Tunctan, B. (2014). Determination of urinary levels of bisphenol a in a Turkish population. *Environmental Monitoring and Assessment*, *186*(12), 8443–8452. https://doi.org/10.1007/s10661-014-4015-z

USFDA. (2018). Bioanalytical Method Validation Guidance for Industry. 44. https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf

Caban, M., & Stepnowski, P. (2020). The quantification of bisphenols and their analogues in wastewaters and surface water by an improved solid-phase extraction gas chromatography/mass spectrometry method. *Environmental Science and Pollution Research International*, *27*(23), 28829–28839. https://doi.org/10.1007/s11356-020-09123-2

Corrales, J., Kristofco, L. A., Steele, W. B., Yates, B. S., Breed, C. S., Williams, E. S., & Brooks, B. W. (2015). Global assessment of bisphenol A in the environment: Review and analysis of its occurrence and bioaccumulation. *Dose-Response Publication of International Hormesis Society*, *13*(3), 1559325815598308.

Dang, V. H., Nguyen, T. H., Choi, K.-C., & Jeung, E.-B. (2007). A calcium-binding protein, calbindin-D9k, is regulated through an estrogen-receptor mediated mechanism following xenoestrogen exposure in the GH3 cell line. *Toxicological Sciences: an Official Journal of the Society of Toxicology*, *98*(2), 408–415. https://doi.org/10.1093/toxsci/kfm120

Fernandez, S. V., & Russo, J. (2010). Estrogen and xenoestrogens in breast cancer. *Toxicologic Pathology*, *38*(1), 110–122. https://doi.org/10.1177/0192623309354108

Foster, W. G., Kubwabo, C., Kosarac, I., Gregorovich, S., Aryal, G., & Coleman, K. (2019). Free bisphenol a (BPA), BPA-glucuronide (BPA-G), and total BPA concentrations in maternal serum and urine during pregnancy and umbilical cord blood at delivery. *Emerging Contaminants*, *5*, 279–287. https://doi.org/10.1016/j.emcon.2019.08.002

Gerona, R. R., Pan, J., Zota, A. R., Schwartz, J. M., Friesen, M., Taylor, J. A., Hunt, P. A., & Woodruff, T. J. (2016). Direct measurement of bisphenol A (BPA), BPA glucuronide and BPA sulfate in a diverse and low-income population of pregnant women reveals high exposure, with potential implications for previous exposure estimates: A cross-sectional study. *Environmental Health: Global Access Science Source*, *15*, 50.

Haffner, D., & Schecter, A. (2014). Persistent organic pollutants (POPs): A primer for practicing clinicians. *Current Environmental Health Reports*, *1*(2), 123–131. https://doi.org/10.1007/s40572-014-0009-9

Holmes, D. (2017). Breast cancer: Increased risk with concurrent dietary and EDC exposures. *Nature Reviews. Endocrinology*, *13*(7), 378. https://doi.org/10.1038/nrendo.2017.68

Jing, X., Bing, S., Xiaoyan, W., Xiaojie, S., & Yongning, W. (2011). A study on bisphenol a, nonylphenol, and octylphenol in human urine amples detected by SPE–UPLC–MS. *Biomedical and Environmental Sciences BES*, *24*(1), 40–46. https://doi.org/10.3967/0895-3988.2011.01.005

Kato, K., Silva, M. J., Needham, L. L., & Calafat, A. M. (2005). Determination of 16 phthalate metabolites in urine using automated sample preparation and on-line preconcentration/high-performance liquid chromatography/tandem mass spectrometry. *Analytical Chemistry*, *77*(9), 2985–2991. https://doi.org/10.1021/ac0481248

Konieczna, A., Rutkowska, A., & Rachoń, D. (2015). Health risk of exposure to bisphenol A (BPA). *Roczniki Państwowego Zakładu Higieny*, *66*(1), 5–11.

Lee, S.-Y., Son, E., Kang, J.-Y., Lee, H.-S., Shin, M.-K., Nam, H.-S., Kim, S.-Y., Jang, Y.-M., & Rhee, G.-S. (2013). Development of a quantitative analytical method for determining the concentration of human urinary paraben by LC–MS/MS. *Bulletin of the Korean Chemical Society*, *34*(4), 1131–1136. https://doi.org/10.5012/bkcs.2013.34.4.1131

Li, X., & Franke, A. A. (2015). Improvement of bisphenol a quantitation from urine by LCMS. *Analytical and Bioanalytical Chemistry*, *407*(13), 3869–3874. https://doi.org/10.1007/s00216-015-8563-z

Mao, L., Sun, C., Zhang, H., Li, Y., & Wu, D. (2004). Determination of environmental estrogens in human urine by high performance liquid chromatography after fluorescent derivatization with p-nitrobenzoyl chloride. *Analytica Chimica Acta*, *522*(2), 241–246. https://doi.org/10.1016/j.aca.2004.04.071

Markey, C. M., Michaelson, C. L., Veson, E. C., Sonnenschein, C., & Soto, A. M. (2001). The mouse uterotrophic assay: A reevaluation of its validity in assessing the estrogenicity of bisphenol a. *Environmental Health Perspectives*, *109*(1), 55–60. https://doi.org/10.1289/ehp.0110955

Murphy, L., Mérida-Ortega, Á., Cebrián, M. E., Hernández-Garciadiego, L., Gómez-Ruiz, H., Gamboa-Loira, B., & López-Carrillo, L. (2019). Exposure to bisphenol A and diabetes risk in Mexican women. *Environmental Science and Pollution Research International*, *26*(25), 26332–26338. https://doi.org/10.1007/s11356-019-05731-9

Pednekar, P. P., Gajbhiye, R. K., Patil, A. D., Surve, S. V., Datar, A. G., Balsarkar, G. D., Chuahan, A. R., & Vanage, G. R. (2018). Estimation of plasma levels of bisphenol-A & phthalates in fertile & infertile women by gas chromatography–mass spectrometry. *The Indian Journal of Medical Research*, *148*(6), 734–742. https://doi.org/10.4103/ijmr.IJMR_2077_16

Peller, J. R., Mezyk, S. P., & Cooper, W. J. (2009). Bisphenol a reactions with hydroxyl radicals: Diverse pathways determined between deionized water and tertiary treated wastewater solutions. *Research on Chemical Intermediates*, *35*(1), 21–34. https://doi.org/10.1007/s11164-008-0012-6

Reeves, K. W., Schneider, S., Xue, J., Kannan, K., Mason, H., Johnson, M., Makari-Judson, G., & Santana, M. D. (2018). Bisphenol-A in breast adipose tissue of breast cancer cases and controls. *Environmental Research*, *167*, 735–738. https://doi.org/10.1016/j.envres.2018.08.033

Ribeiro E., Ladeira C., & Viegas S. (2017). Occupational Exposure to Bisphenol A (BPA): A Reality That Still Needs to Be Unveiled. *Toxics*, *5*(3), 22. https://doi.org/10.3390/toxics5030022

Rochester, J. R. (2013). Bisphenol A and human health: A review of the literature. *Reproductive Toxicology Elmsford N.Y.*, *42*, 132–155. https://doi.org/10.1016/j.reprotox.2013.08.008

Seachrist, D. D., Bonk, K. W., Ho, S.-M., Prins, G. S., Soto, A. M., & Keri, R. A. (2016). A review of the carcinogenic potential of bisphenol A. *Reproductive Toxicology Elmsford N.Y.*, *59*, 167–182. https://doi.org/10.1016/j.reprotox.2015.09.006

Siva Selva Kumar, M., & Ramanathan, M. (2016). Concurrent determination of olanzapine, risperidone and 9-hydroxyrisperidone in human plasma by ultra performance liquid chromatography with diode array detection method: Application to pharmacokinetic study. *Biomedical Chromatography BMC*, *30*(2), 263–268. https://doi.org/10.1002/bmc.3545

Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., & Serrano, F. O. (1995). The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. *Environmental Health Perspectives*, *103*(Suppl 7), 113–122.

Stossi, F., Bolt, M. J., Ashcroft, F. J., Lamerdin, J. E., Melnick, J. S., Powell, R. T., Dandekar, R. D., Mancini, M. G., Walker, C. L., Westwick, J. K., & Mancini, M. A. (2014). Defining estrogenic mechanisms of bisphenol A analogs through high throughput microscopy-based contextual assays. *Chemistry & Biology*, *21*(6), 743–753. https://doi.org/10.1016/j.chembiol.2014.03.013

Vandenberg, L. N., Maffini, M. V., Sonnenschein, C., Rubin, B. S., & Soto, A. M. (2009). Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption. *Endocrine Reviews*, *30*(1), 75–95. https://doi.org/10.1210/er.2008-0021

Völkel, W., Colnot, T., Csanády, G. A., Filser, J. G., & Dekant, W. (2002). Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chemical Research in Toxicology*, *15*(10), 1281–1287. https://doi.org/10.1021/tx025548t

Wang, Z., Liu, H., & Liu, S. (2017). Low-dose bisphenol A exposure: A seemingly instigating carcinogenic effect on breast cancer. *Advanced Science Weinheim, Baden-Wurttemberg*, *4*(2), 1600248.

Yang, M., Ryu, J.-H., Jeon, R., Kang, D., & Yoo, K.-Y. (2009). Effects of bisphenol A on breast cancer and its risk factors. *Archives of Toxicology*, *83*(3), 281–285. https://doi.org/10.1007/s00204-008-0364-0

Zhang, Z., Alomirah, H., Cho, H.-S., Li, Y.-F., Liao, C., Minh, T. B., Mohd, M. A., Nakata, H., Ren, N., & Kannan, K. (2011). Urinary bisphenol a concentrations and their implications for human exposure in several Asian countries. *Environmental Science & Technology*, *45*(16), 7044–7050. https://doi.org/10.1021/es200976k

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Background: According to the recommendations from The World Health Organization, exclusive breast feeding (EBF) should be given to infants for the first six months for healthy growth and development. However, developing countries have a wide range of variation in the practice of EBF. From this study, we aim to estimate the duration of EBF and find the impact of socio-demographic characteristics on EBF.

Materials and Methods: This study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore in the month of July 2018. Mothers with children aged below 4 years attending the paediatric outpatient department were included in the study. Kaplan-Meier curve analysis and logistic regression analysis were employed.

Results: Our analysis indicates that the prevalence of EBF was 58.4% among infants up to 6 months of age with mean duration of EBF 4.52 months. It was observed that mothers of a younger age (15-24 years) and with lower socio-economic status were negatively associated with the practice of providing EBF to infants up to six months of age.

Conclusion: The findings clearly indicate that the interventions related to EBF up to six months of age should be targeted at women in the lower socio-economic status and at younger ages.

Keyword: Exclusive breast feeding, socio-economic status, mother's age.

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Introduction

According to the recommendation of the World Health Organization (WHO), exclusive breastfeeding (EBF) should be given to infants for the first six months of life and continued breastfeeding up to two years of age or beyond for the healthy growth and development [1-3]. Recent literatures have suggested exclusive breastfeeding as a cost-effective intervention to reduce infant mortality rate in less developed countries [4, 5]. Globally, it is estimated that the lack of providing exclusive breast feeding to infants in the first 6 months of life results in 1.4 million deaths and 10% of disease in under-five children [6]. On the other hand, poor school performance, reduced productivity and impaired intellectual and social development are reported to be much higher due to the lack of EBF in the first 6 months of life.

In India, a large number of children, nearly 20 million, were not able to receive EBF for the first six months, according to the reports from National Family Health Survey (NFHS) [7]. Over the past several years, India has failed to witness any remarkable achievements in infant feeding practices. Only a small increment was recorded in EBF rates amongst infants in the 0-6 month age group from 41.2% in 1998-99 (NFHS 2) to 46.3% in 2005-2006 (NFHS 3). The mean duration of EBF among last born children is as brief as two months.

In 2016, the Government of India started various initiatives to tackle the health issues among children due to inappropriate breast-feeding practices. The Government of India developed the infant and young child feeding guidelines in 2016 in view of this initiative. However, more studies are needed to explore the factors associated with the low proportion of EBF. The present study aims to estimate the duration of EBF and to find the impact of socio-demographic characteristics influences on EBF practice.

Methodology

Study design and setting

A cross-sectional study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore in the month of July 2018. The mothers of infants less than four years of age, who attended the paediatric outpatient department, were included in the study. The selected mothers were broadly classified into two groups: EBF and non-EBF. Mothers having children aged below 6 months and who were given only breast milk in the 24 hours preceding the survey were considered as EBF and others were in non-EBF group [8].

Sample size estimation

In order to estimate the EBF with the precision of 20% of the expected prevalence of 30%, with 95% CI, we require a minimum sample size of 300 for the study.

Data collection

Data were collected using direct interview method. Mothers were interviewed about the duration of EBF in months with respect to youngest child. The socio-demographic characteristics and breast-feeding related information were elicited from the selected mothers. The characteristics studied were educational status of the mother, age of the mother, working status of the mother, educational status of father, socio-economic status of the household, child's gender, gestational age of the child, birth weight of the child, mode of delivery, condition of the child soon after delivery, and counselling for breastfeeding after delivery. The socio-economic status was calculated using Prasad classification with class 1 as the person having per-capita income greater than or equal to Rs.6180, class 2 ranging from Rs.3090-6179, class 3 ranging from Rs.1850-3089, class 4 ranging from Rs.930-1849, and class 5 as person having per-capita income below Rs.930. The classification was done based on the Consumer Price Index (CPI) value of 279 for Coimbatore [9].

Exclusion criteria

Babies with congenital anomalies that interfered with breastfeeding were not included in the study.

Ethical approval

The study was approved by the Institutional Human Ethics Committee, PSG Institute of Medical Sciences and Research, Coimbatore.

Outcome variable

The outcome variable was the exclusive breast feeding (EBF) status of infants up to six months of age.

Explanatory variables

Mother's age, educational status of mother and father, working status of mother, gender, gestational age and birth weight of the child and socio-economic status of the household were the explanatory variables.

Statistical analysis

To estimate the mean and median duration of exclusive breast feeding among infants under study, the Kaplan-Meier Survival analysis method was applied and conditional probability of survival at monthly interval of time was then obtained. The termination of EBF was considered as outcome of the event of the occurrence. Accordingly, those women who fed their babies exclusively with breast feeding on the date of interview were considered as censored cases. In such cases, the duration of EBF was recorded as it was not known when they would discontinue EBF and start introducing adult diet. The duration of exclusive breastfeeding was sub grouped into smaller time intervals and for each interval all those who have been observed at least that long were used to calculate the probability of a terminal event occurrence in the interval. Then the overall probability of the event occurrence at different time

periods were estimated using the probabilities estimated from each interval. Survival probabilities, standard error, hazard rate, and mean and median survival time have been calculated. Log rank test was applied to compare the statistical significance for the equality of duration of exclusive breast feeding for different groups. In addition, logistic regression analysis was done to find the association of socio-demographic characteristics with EBF categorized as Yes or No. Odds ratio and then 95% confidence intervals were then calculated. Multivariate logistic regression analysis was done for those variables found significantly associated with the outcome variable of interest in the Univariate analysis. $p<0.05$ was considered as statistically significant. The data were analysed using SPSS (Ver. 23).

Results

A total of 527 women were studied. The socio-demographic characteristics of infants and families are given in Table 1. The prevalence of exclusive breast feeding was 58.4% among infants up to 6 months of age with the mean duration of EBF as 4.52 months.

Table 1: Socio-demographic characteristics of infants and families

Characteristics	Number (%)
Child's age (months) (Mean±SD)	15.55 ± 11.92
Mother's education	
Below 12th standard	138 (26.2)
Above 12th standard	389 (73.8)
Mother's age (years)	
15-24	108 (20.5)
25-34	388 (73.6)
35-44	31 (5.9)
Mother's Occupation	
House wife	403 (76.5)
Paid worker	124 (23.5)
Father's education	
Below 12th standard	129 (24.5)
Above 12th standard	398 (75.5)
Socio-economic class	
Class 1 and 2	426 (80.8)
Class 3,4 and 5	101 (19.2)
Child's gender	
Female	246 (46.7)
Male	281 (53.3)
Gestational period	
Term	469 (89.0)
Preterm	58 (11.0)
Birth weight	
<=2500	126 (23.9)
>2500	401 (76.1)
Mode of delivery	
Vaginal delivery	336 (63.8)
Caesarean	191 (36.2)
Child's health	
Well	454 (86.1)
Sick	73 (13.9)
Received breast feeding counselling	
Yes	326 (61.9)
No	201 (38.1)

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Below 12th standard	4.33	5.00	0.89	0.345
Above 12th standard	5.57	6.00		
Mother's age □ □□□□r □□□□				
15-24	4.15	5.00	7.44	P<0.05
25-34	4.55	6.00		
35-44	5.04	6.00		
Mother's Occupation □				
House wife	4.49	6.00	0.19	0.663
Paid worker	4.58	6.00		
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Below 12th standard	4.15	5.00	4.49	P<0.05
Above 12th standard	4.62	6.00		
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Class 1 and 2	4.61	6.00	5.45	P<0.05
Class 3,4 and 5	4.10	5.00		
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Female	4.58	6.00	0.15	0.690
Male	4.44	6.00		
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Term	4.58	6.00	3.88	0.143
Preterm	4.02	6.00		
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≤2500	4.33	6.00	0.11	0.772
>2500	4.58	6.00		
M □d □□□□d □□□□r □□				
Vaginal delivery	4.58	6.00	0.20	0.654
Caesarean	4.41	6.00		
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Well	4.63	6.00	5.07	P<0.05
Sick	3.84	5.00		
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Yes	4.54	6.00	0.05	0.860
No	4.493	6.00		
□□□□□□	4.523	6.00		

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Yes	37 (8.7)	10 (9.9)	0.482
No	389 (91.3)	91 (90.1)	
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Yes	56 (13.1)	21 (20.8)	P<0.05
No	370 (86.9)	80 (79.2)	
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Yes	111 (26.1)	13 (12.9)	P<0.01
No	315 (73.9)	88 (87.1)	
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Yes	303 (80.6)	73 (72.3)	0.818
No	123 (19.4)	28 (27.7)	□

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On testing equality of the duration of EBF, it was observed that the mean duration of EBF was higher in mothers of age group 35-44 years than those aged 15-24 years and 25-34 years ($p<0.05$). The mean duration of EBF was more in class 1 and 2 of the socio-economic status than class 3,4, and 5 of the socio-economic status ($p<0.05$). The mean duration of EBF was more in children whose father's education was above 12th standard than those less than 12th standard ($p<0.05$). It was also observed that the mothers with the sick children tend to do less EBF than those with well children ($p<0.05$). The findings are presented in Table 2.

In the logistic regression analysis (Table 3), the prevalence of EBF was higher among the mothers of age group 25-34 years than those aged 15-24 years ($p<0.05$) and it was significantly higher among class 1 and 2 of the socio-economic status than class 3, 4, and 5 of socio-economic status ($p<0.01$). The multivariate logistic regression analysis also shows that the socio-economic status of class 1 and 2 had significantly higher prevalence of exclusive breast feeding than class 3, 4, and 5 of the socio-economic status ($p<0.01$) with an odds ratio of 1.788 (95% CI: 1.153-2.775). In addition, the prevalence of EBF was higher among the age group 25-34 years than in the aged group 15-24 years ($p<0.05$) with an odds ratio of 1.572 (95% CI: 1.021-2.421).

During the interviews, 47 (8.9%) mothers reported the problem of inverted nipple as a constraint for EBF. Seventy-seven (14.6%) mothers perceived to have the problem of low breast milk and 124 (23.5%) mothers had employment outside home. In addition, 151 (28.6%) mothers had no supportive family members at home (Table 4). It was also observed that there was a statistically significant association between perception of low breast milk and lower socio-economic status ($p<0.05$) (Table 4).

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Breast feeding is universally recognized as the best way of feeding infants which helps in their healthy growth and development. Therefore, several countries, especially developing nations, have started implementing health promotion interventions to support successful breastfeeding by taking into account the factors influencing breast feeding outcomes. For infants till the first six months of life, the WHO strongly recommends EBF without any additional food or water. The WHO also suggests providing complementary diet at 6 months along with breast milk and sustaining breast feeding until the age of 2 years. However, whether an infant is breastfed or formula fed depends on several factors like social, psychological, and environmental scenarios.

Despite several efforts made for EBF up to six months, in our study, 42% of the mothers were still not following EBF up to six months. The mean duration of EBF was 4.52 months which is much lower than the findings reported by Chudsama et al. [5]. Infants not exclusively breast fed up to six months are at risk of higher occurrence of associated infections, malnutrition deficiencies, and under-development [10, 11]. Most of the recent studies regarding EBF come out with the similar fact that the practice of EBF tends to decline and highlight the tremendous progress in the marketing of industrial milk products as well as the lack of awareness of this serious issue among mothers.

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A significantly lower prevalence of EBF was observed among mothers with lower socio-economic status, which is controversial with the result obtained from an Ethiopian demographic health survey that explained a declining trend of EBF with the higher maternal educational and socio-economic

status [12]. Furthermore, our study found that the duration of EBF was significantly lower among mothers with lower socio-economic status. The findings of our study clearly acknowledge socio-economic status of the household as a major influencing factor for longer breastfeeding practice among mothers in a higher income bracket. In addition, we have also observed a significant association between the perception of low breast milk and lower socio-economic status. The findings clearly suggest that interventions should be tailored to target women with lower socio-economic status.

[illegible]

It was observed that age of the mother is a predictor of EBF. The mean duration of EBF was significantly lower in the age group of mothers with 15-24 years. The findings suggest that more efforts are needed to create awareness about the need for EBF in younger aged mothers.

Education and occupation of the parent's and its [illegible]

We could not find statistical significance between the father's education and increased prevalence of EBF in the multivariate analysis though we observed a statistical significance in the univariate analysis. Maternal educational status was also found statistically not significant with EBF, which is a controversial observation with the findings of the Ethiopian demographic health survey which indicated a declining trend of EBF practices with higher maternal educational status but supports the findings observed by Tesfaye Setegn et al. [10].

[illegible]

The findings clearly indicate that the interventions related to EBF up to six months of age should be targeted to women in the lower socio-economic status and at younger ages.

[illegible]

Our study relies on retrospective reporting, which may result in inaccurate recall and this can be point out as a major limitation. However, most of the recent literature also followed the similar maternal recall mode for elucidating breast-feeding practices. Since this was a hospital-based study, the study population was representative of generally higher socio-economic status and which also reflects as a limitation of our work. A community-based cross-sectional study with a large sample size can provide more light in this field. Despite these limitations, the study has several strengths. We have elicited a large number of demographic and clinical data which can influence the association of duration of EBF. The same interviewer collected all information which reduces the inter observer bias. Advanced statistical methods were employed to analyse the association of socio-demographic and clinical correlates with EBF.

[illegible]

Our results can help to create more awareness about the need for EBF in the younger age group and the mothers in lower socio-economic status.

[illegible]

To the best of our knowledge, not many studies were conducted in Tamil Nadu to examine the socio-demographic and clinical correlates of exclusive breastfeeding. Results of this study indicate a need to create awareness to mothers regarding the need of providing EBF to infants up to six months of age.

[illegible]

India has undergone a rapid transition. Contradictory to our expectations, higher socio-economic status mothers are now aware about the need for EBF. More efforts are needed for those mothers in lower socio-economic status and those at younger ages. Secondly, apart from mother's education, father's education also plays a great role in breast feeding practices. In conclusion, it was observed that lower socio-economic status and young age of the mother have negative impact on EBF for six months of age.

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Author's Contribution:

ACM Design of the study, preparation of manuscript and interpretation of data, revising critically for intellectual content and final approval of the version to be published.

JKB, DMP and CD. Data collection, analysis, preparation of the manuscript, drafting the article and final approval of the version to be published.

MJ. Revision of the manuscript

VS, NK and RS. Design of the study, technical guidance and revising critically for intellectual content and final approval of the version to be published.

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All authors have declared that they have no conflict of interest.

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1.World Health Organization: The optimal duration of exclusive breastfeeding: report of an expert consultation. Geneva: World Health Organization, Department of nutrition for health and development and department of child and adolescent health and development; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

2.World Health Organization: Global strategy for infant and young child feeding. The optimal duration of exclusive breastfeeding. Geneva: World Health Organization; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

3.World Health Organization: Infant and young child feeding (IYCF) Model Chapter for textbooks for medical students and allied health professionals. Switzerland: World Health Organization: 2009,pp 19-29.
https://apps.who.int/iris/bitstream/handle/10665/44117/9789241597494_eng.pdf;jsessionid=D51E85BC3FEFDD64E24D3B3CA0B10287?sequence=1

4.Setegn T, Belachew T, Gerbaba M, Deribe K, Deribew A, Biadgilign S. Factors associated with exclusive breastfeeding practices among mothers in Goba district, south east Ethiopia: a cross-sectional study. International Breastfeeding Journal 2012; 7:17.
https://internationalbreastfeedingjournal.biomedcentral.com/articles/10.1186/1746-4358-7-17

5.Chudasama RK, Patel PC, Kavishwar AB. Factors Associated with Duration of Exclusive Breastfeeding. The Internet Journal of Pediatrics and Neonatology 2007; 9(1):1-6.
https://print.ispub.com/api/0/ispub-article/4871

6.Oweis A, Tayem A, Froelicher ES. Breastfeeding practices among Jordanian women. Int J NursPrac 2009; 15:32-40.
https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1440-172X.2008.01720.x

7.Tiwari S, Bharadva K, Yadav B, Malik S, Gangal P, Banapurmath CR, et al. Infant and Young Child Feeding Guidelines.IndianPediatrics 2016; 53:703-713.

8.WHO Division of Child Health and Development. Indicators for assessing breastfeeding practices.Geneva: WHO; 1991. WHO/CDD/SER/91.14.
https://apps.who.int/iris/bitstream/handle/10665/62134/WHO_CDD_SER_91.14.pdf?sequence=1

9.Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers [online] 2018 [cited 2018 Aug 10]. Available from:
URL:http://labourbureaunew.gov.in/UserContent/Linkingfactor_CPI_IW.pdf?pr_id .

10.SetegnT, Gerbaba M, Belachew T. Determinants of timely initiation of breastfeeding among mothers in GobaWoreda. South east Ethiopia: a cross-sectional study. BMC Public Health 2011; 11:217.
https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-11-217

11.Habibi M, Laamiri FZ, Aguenaou H, Doukkali L, Mrabet M, Barkat A. The impact of maternal socio demographic characteristics on breast feeding knowledge and practice: An experience from Casablanca, Morocco. International Journal of Paediatrics and Adolescent Medicine 2018;5: 39-48
https://www.sciencedirect.com/science/article/pii/S2352646717301576

12.Alemayehu T, Haidar J, Habte D. Determinants of exclusive breast feeding practices in Ethiopia. Ethio. J Health Dev 2009; 23: 12-18
https://www.ajol.info/index.php/ejhd/article/viewFile/44832/28326

1.World Health Organization: The optimal duration of exclusive breastfeeding: report of an expert consultation. Geneva: World Health Organization, Department of nutrition for health and development and department of child and adolescent health and development; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

2.World Health Organization: Global strategy for infant and young child feeding. The optimal duration of exclusive breastfeeding. Geneva: World Health Organization; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

3.World Health Organization: Infant and young child feeding (IYCF) Model Chapter for textbooks for medical students and allied health professionals. Switzerland: World Health Organization: 2009,pp 19-29.
https://apps.who.int/iris/bitstream/handle/10665/44117/9789241597494_eng.pdf;jsessionid=D51E85BC3FEFDD64E24D3B3CA0B10287?sequence=1

4.Setegn T, Belachew T, Gerbaba M, Deribe K, Deribew A, Biadgilign S. Factors associated with exclusive breastfeeding

practices among mothers in Goba district, south east Ethiopia: a cross-sectional study. International Breastfeeding Journal 2012; 7:17.
https://doi.org/10.1186/1746-4358-7-17
PMid:23186223 PMCid:PMC3560275
5.Chudasama RK, Patel PC, Kavishwar AB. Factors Associated with Duration of Exclusive Breastfeeding. The Internet Journal of Pediatrics and Neonatology 2007; 9(1):1-6.
https://doi.org/10.5580/734
6.Oweis A, Tayem A, Froelicher ES. Breastfeeding practices among Jordanian women. Int J NursPrac 2009; 15:32-40.
https://doi.org/10.1111/j.1440-172X.2008.01720.x
PMid:19187167
7.Tiwari S, Bharadva K, Yadav B, Malik S, Gangal P, Banapurmath CR, et al. Infant and Young Child Feeding Guidelines. IndianPediatrics 2016; 53:703-713.
https://doi.org/10.1007/s13312-016-0914-0
PMid:27567645
8.WHO Division of Child Health and Development. Indicators for assessing breastfeeding practices.Geneva: WHO; 1991. WHO/CDD/SER/91.14.
https://apps.who.int/iris/bitstream/handle/10665/62134/WHO_CDD_SER_91.14.pdf?sequence=1
9.Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers [online] 2018 [cited 2018 Aug 10]. Available from:
URL:http://labourbureaunew.gov.in/UserContent/Linkingfactor_CPI_IW.pdf?pr_id.
10.SetegnT, Gerbaba M, Belachew T. Determinants of timely initiation of breastfeeding among mothers in GobaWoreda. South east Ethiopia: a cross-sectional study. BMC Public Health 2011; 11:217.
https://doi.org/10.1186/1471-2458-11-217
PMid:21473791 PMCid:PMC3088906
11.Habibi M, Laamiri FZ, Aguenaou H, Doukkali L, Mrabet M, Barkat A. The impact of maternal socio demographic characteristics on breast feeding knowledge and practice: An experience from Casablanca, Morocco. International Journal of Paediatrics and Adolescent Medicine 2018;5: 39-48
https://doi.org/10.1016/j.ijpam.2018.01.003
PMid:30805532 PMCid:PMC6363246
12.Alemayehu T, Haidar J, Habte D. Determinants of exclusive breast feeding practices in Ethiopia. Ethio. J Health Dev 2009; 23: 12-18
https://doi.org/10.4314/ejhd.v23i1.44832

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Characterization of cardiac autonomic function in COVID-19 using heart rate variability: a hospital based preliminary observational study

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Abstract

Objectives: The novel corona virus disease, which was initially reported in China in late 2019, has become a global pandemic affecting 330 million cases. COVID-19 affects predominantly the respiratory system, in addition to other organ systems, mainly the cardiovascular system. One of the hypotheses is that virus entering the target cells by binding to angiotensin converting enzyme 2 affecting hypothalamic pituitary axis could lead to dysautonomia which is measured by heart rate variability (HRV). HRV is a non-invasive measure of autonomic function that facilitates identification of COVID-19 patients at the risk of developing cardiovascular complications. So, we aimed to assess HRV in COVID patients and compare between COVID patients and normal controls.

Methods: In a case control design, we compared 63 COVID-19 infected patients with 43 healthy controls matched for age and gender. Along with clinical characterization, heart rate variability was evaluated using ambulatory 5 min ECG in lead II and expressed in frequency and time domain measures. Statistical analysis was performed using SPSS 17.0.

Results: Mean age of the study population was 49.1 ± 14.2 years and 71 (66.9%) were males. Frequency domain measures high (HF) and low (LF) frequency powers were significantly decreased in COVID-19 patients compared to controls. HF/LF and LF/HF ratios were not different between groups. Time domain measures rMSSD (root mean square of successive RR interval differences) and SDNN (standard deviation of NN intervals) were significantly increased among COVID-19 subjects. COVID-19 infection was associated with increased parasympathetic activity as defined by rMSSD>40 {adjusted odds ratio 7.609 (95% CI 1.61–35.94); p=0.01} and SDNN>60 {adjusted odds ratio 2.620 (95% CI 1.070–6.44); p=0.035} after adjusting for age, gender and comorbidities.

Conclusions: Our study results showed increased parasympathetic tone in COVID patients. Early diagnosis of autonomic imbalance in COVID patients is needed to plan management and limit progression of disease.

Keywords: heart failure; pulmonary hypertension; right heart.

Introduction

In December 2019, a novel coronavirus disease (nCoV) epidemic emerged in Wuhan City, Hubei Province, China. Owing to its high transmissibility and infectivity rate it spread across the world and WHO declared it as a pandemic on March 11, 2020 [1, 2]. As this virus predominantly affects the respiratory system it has been renamed as severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) and the disease caused by this virus has been named as corona virus disease 19 (COVID-19) [3].

COVID-19 shows wide range of clinical presentations ranging from asymptomatic/mild symptoms (fever, cough, dyspnea, myalgia, fatigue, anosmia, dysgeusia and diarrhea) to severe illness like acute respiratory distress syndrome (ARDS), arterial and venous thrombosis, myocarditis and varieties of neurological manifestations [4–7]. A report from WHO says that “80% of infections are mild or asymptomatic, 15% are severe infections and 5% are critical infections” [8]. Co-morbid conditions like obesity, type 2 diabetes mellitus, coronary artery disease, chronic liver disease, chronic kidney disease and chronic respiratory disease are known to be risk factors for developing severe disease [9–11]. Wide spectrum of manifestations is explained by SARS CoV-2 having target cells in multiple organs and systems like respiratory tract including lung parenchyma,

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ileum, bladder, esophagus, heart, kidney, hypothalamus, pituitary, adrenal glands etc. These target cells have angiotensin converting enzyme-2 (ACE2) receptors in their cell wall. ACE2 binding affinity of the SARS CoV-2 spike protein ectodomain is one of possible mechanisms by which entry to these cells is facilitated [1, 12]. Histopathological and immune histochemical studies have clearly demonstrated a direct assault on these organs/systems by the SARS CoV-2 virus [13].

Though main portal of entry is respiratory system and hence the dominantly affected one, SARS CoV-2 reaches other systems through mechanism yet unknown. Neuroinvasive potential of SARS CoV-2 has been discussed in recent literature in the context of plausible explanation for variety of neurological manifestations of COVID-19 [14–16]. Earlier studies have demonstrated that SARS CoV-1 and MERS CoV (Middle east respiratory syndrome CoV) have the potential to enter and invade central nervous system, dominantly in brainstem structures [12, 17, 18]. Owing to the similarities in viral structure and receptor binding domain, neurotropism of SARS CoV-2 can be speculated from the evidence found from SARS CoV-1. Though sparse, direct evidence of neurotropism of SARS CoV-2 is emerging in literature. A recent report by Paniz–Mondolfi et al. [19], demonstrated by electron microscopy, presence of viral particles in postmortem brain tissues of a COVID-19 patient. Another report by Moriguchi et al. [20], evidenced the presence of SARS CoV-2 in cerebrospinal fluid. It has been postulated that SARS CoV-2 can enter the central nervous system by several mechanisms such as invasion through olfactory epithelium [21], synapse-connected route from terminal nerve endings [22] and transmission across blood-brain barrier by endothelial damage [19]. Clinical implications of neuropropensity of SARS CoV-2 range from mild symptoms like headache, dizziness etc., to severe manifestations like seizures, encephalitis, Guillain-Barre syndrome and cerebrovascular events. Involvement of autonomic nervous system (brainstem and hypothalamus) can lead to dysfunction of vital organs including cardiovascular system, thereby significantly influencing the outcome in COVID-19 patients. Cardiovascular system is also affected by the virus leading to manifestations like acute myocardial infarction, myocarditis and arterial & venous thrombosis. Monitoring the cardiac autonomic function of patients with COVID-19 can help us identify the individuals who are at risk of developing adverse cardiovascular outcomes.

Autonomic dysfunction has been reported in viral infections like retrovirus (human immunodeficiency virus, human T-lymphotropic virus), herpes viruses, flavivirus, arbovirus, enterovirus 71 and lyssavirus [23]. Current literature on autonomic dysfunction in COVID-19 patients is very sparse, barring a few case reports [24, 25]. Though various other methods were employed in these clinical settings to elicit dysautonomia, heart rate variability (HRV) measurement was seldom used for this purpose. During last two decades, HRV is considered most objective, reproducible and validated tool for demonstration of autonomic dysfunction [26]. Frequency and time domain measures of HRV have emerged as the most promising markers of balance between parasympathetic and sympathetic activity of autonomic nervous system that controls homeostasis of all vital organs.

Eliciting an association between autonomic dysfunction and COVID-19 could clarify the genesis of many clinical manifestations and importantly the unpredictable natural course of the illness. In this study, we intended to evaluate for autonomic imbalance and the direction of its deviation in COVID-19 subjects by measuring HRV and comparing the differences in HRV metrics between SARS CoV-2 positive and negative individuals.

Methods

This was an analytical cross-sectional study involving adult COVID-19 patients admitted for treatment in PSG Institute of Medical Sciences, Coimbatore, a specialized COVID-19 treating center in South India during March to June 2020. Sample frame during this period was approximately 500 inpatients whose COVID-19 status was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) test of nasopharyngeal and oropharyngeal swab. Eligible inpatients (test group) of both genders, aged between 25 and 60 years were recruited from the sample frame by convenient sampling method. Patients with uncontrolled hypertension, coronary artery disease, cerebrovascular accident, family history of young myocardial infarction were excluded. Similarly, those patients on treatment with beta-blockers, inhaled or oral beta-mimetics, theophylline and other drugs with potential chronotropic effects were excluded. Patients with severe disease (those requiring oxygen support and/or intensive care) were also excluded. The comparison group included age and gender-matched healthy individuals from among the employees of medical school who tested negative for SARS CoV-2 by RT-PCR of their nasopharyngeal and oropharyngeal swab and fulfilled above mentioned selection criteria. This study was cleared by the institutional human ethics committee, and all study participants gave written informed consent. All such subjects recruited for the study underwent a detailed clinical examination.

Assessment of HRV

Study participants in both the groups were instructed to refrain from smoking, caffeine intake for 2 h and alcohol intake for 36 h. They should have had adequate rest, at

least 8 h of uninterrupted sleep on the night before the assessment of HRV and normal breakfast on the day of assessment. They were made to lie quietly in a couch in supine position for 5 min to alleviate the anxiety in a sound attenuated room with dim lighting and the temperature ranging from 20 to 25 °C.

After explaining the procedure to the subject, HRV, a quantitative marker of the autonomic activity was assessed using an ambulatory ECG system (INCO digital NIVIQURE, Bangalore, India) in lead II for 5 min. It is a multichannel digital data acquisition system which enables to acquire, analyze and store ECG data. ECG data was obtained at a sampling rate of 1,024 Hz in standard lead II configuration. The interface RS232C-compatible module was used to transfer data from the recording unit to the computer. The transferred data was analyzed using inbuilt software system. The series of RR intervals obtained were subjected to frequency and time domain analysis. Time domain indices of HRV indicate the amount of variability in measurements of interbeat interval (IBI), which represent time period between successive heartbeats. Time domain measures include standard deviation of NN intervals (SDNN), root mean square of successive RR interval differences (rMSSD) and percentage of successive RR intervals (pNN50). Frequency domain measurements estimate the distribution of absolute or relative power into frequency bands. Frequency domain measures include low frequency (LF) and high frequency (HF) [26]. HF component indicates the parasympathetic activity, whereas LF represents both sympathetic and vagal activity, and SDNN, rMSSD and pNN50 measure parasympathetic activity [26, 27].

Statistical analysis

The data were examined initially for normality of distribution and homogeneity of variance and expressed in terms of mean ± SD or percentage. Data with wide dispersion were expressed in log transformed values. Data from continuous variables were compared between study groups using Student's *t*-test whereas categorical variables were compared using Chi-Square test. Mann–Whitney *U* test was performed for variables that were not normally distributed. HRV variables that were significantly different between the groups were used as dependent variables for logistic regression analysis. For this purpose, HRV measures were dichotomized to categorical variable (artificial categorization using median split [28]) using an arbitrary cut-off value that was close to the median as reported by Nunan et al. [27] in their systematic review of studies involving normal subjects. Stepwise logistic regression analysis was employed to identify significant covariates and to evaluate association between HRV measures and SARS CoV-2 status after adjusting for important confounders like age, gender and comorbidity. Subgroup analysis was performed to characterize clinical and HRV profiles of SARS CoV-2 infected subjects based on symptom status. A p value of <0.05 was considered significant. All statistical analyses were carried out using SPSS version 17 software.

Results

In total, 106 participants were recruited for the study, which included 63 (59.4%) COVID-19 subjects and 43 (40.6%) healthy individuals who tested negative for SARS CoV-2. Among COVID-19 patients 33 (52.3%) had mild to moderate symptoms and 30 (47.6%) were asymptomatic. Mean age of the study population was 49.09 ± 14.21 years with 71 (67%) male subjects and 35 (33%) female subjects (Figure 1).

Comparing COVID-19 patients and healthy subjects, both groups were age and gender matched. Vital parameters like heart rate, SPO_2 at room air, systolic and diastolic blood pressure showed no difference between the study groups. Among frequency domain HRV parameters, HF and LF powers were significantly decreased among COVID-19 patients as compared to healthy individuals. HF/LF ratio and LF/HF ratio were not different between the groups (Table 1). Among time domain parameters, mean rMSSD was significantly higher in the COVID-19 group compared to healthy individuals (Figure 2). Mean SDNN value was higher among COVID-19 subjects with a trend toward statistical significance.

In the above graphical representation, the box spans the interquartile range, ends of the box represent upper and lower quartiles, bold horizontal line within the box represents the median value and the whiskers show the

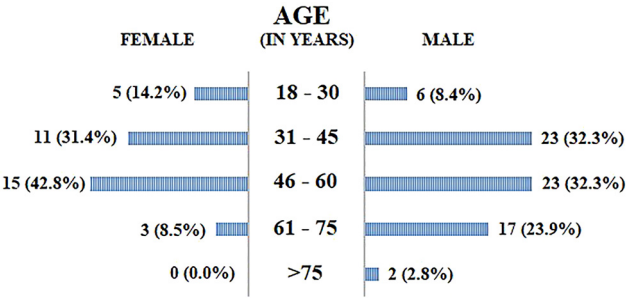


Figure 1: Age and gender distribution of study population.

Table 1: Comparison of clinical profile and heart rate variability parameters between COVID-19 subjects and healthy individuals. Data shown as mean ± SD and frequency {n (%)}.

Variables	COVID-19 subjects (n=63)	Healthy subjects (n=43)	p-Value
Age, years	48.39 ± 16.3	50.1 ± 10.5	0.517
Gender			0.449
Male	44 (69.8%)	27 (62.8%)	
Female	19 (30.2%)	16 (37.2%)	
SPO_2 in room air, %	95 ± 6	97 ± 1	0.678
Systolic BP, mmHg	124 ± 15	121 ± 6	0.386
Diastolic BP, mmHg	77 ± 7	77 ± 4	0.818
HR	84 ± 15	81 ± 15	0.151
HF[a]	1.79 ± 0.15	2.09 ± 0.39	0.0001*
LF	33.47 ± 20.31	77.46 ± 50.8	0.0001*
HF/LF ratio	4.16 ± 4.56	2.34 ± 1.89	0.248
LF/HF ratio	0.70 ± 0.71	0.64 ± 0.35	0.282
pNN50	4.45 ± 8.12	1.99 ± 3.23	0.561
rMSSD[a]	1.49 ± 0.58	1.15 ± 0.29	0.019*
SDNN[a]	1.89 ± 0.33	1.73 ± 0.10	0.10
rMSSD>40	19 (30.6%)	2 (4.7%)	0.001[b]
SDNN>60	27 (45.0%)	11(25.6%)	0.044[b]

SPO_2, oxygen saturation; BP, blood pressure; HR, heart rate; SDNN, standard deviation of NN intervals; rMSSD, root mean square of successive RR interval differences; pNN50, percentage of successive RR intervals; LF, low frequency power; HF, high frequency power. *Mann–Whitney *U* test, level of significance 0.05. [a]Log transformed values. [b]Chi square test, level of significance 0.05.

Table 2: Stepwise logistic regression analysis of association between COVID-19 infection and parasympathetic overtone as defined by rMSSD>40 or SDNN>60.

Parasympathetic overtone	Odds ratio (95% CI)	p-Value
rMSSD>40		
Unadjusted	9.058 (1.98–41.35)	0.004
Adjusted for age and gender	8.670 (1.86–40.30)	0.006
Adjusted for age, gender and diabetes mellitus type 2	8.174 (1.75–38.22)	0.008
Adjusted for age, gender and presence of comorbidities	7.609 (1.61–35.94)	0.01
SDNN>60		
Unadjusted	2.380 (1.014–5.58)	0.046
Adjusted for age and gender	2.427 (1.020–5.77)	0.045
Adjusted for age, gender and diabetes mellitus type 2	2.417 (1.002–5.83)	0.049
Adjusted for age, gender and presence of comorbidities	2.620 (1.070–6.44)	0.035

minimum and maximum values. The circles with numericals are outliers.

Based on the published data on range of observations and median value of normal subjects, we defined parasympathetic overtone when subjects had rMSSD more than 40 and SDNN more than 60 [27]. These dichotomous variables indicated that COVID-19 patients had higher parasympathetic overtone than the healthy subjects (Table 1). Stepwise logistic regression analysis revealed a significant association between SARS CoV-2 infection status and parasympathetic overtone after adjusting for age, gender, T2DM or the presence of one or more comorbidities (Table 2).

Subgroup analysis was performed comparing the characteristics between symptomatic and asymptomatic subjects with COVID-19 (Table 3). Symptomatic COVID-19 patients were elderly and predominantly males as compared to asymptomatic patients. Symptomatic patients more frequently had at least one co-morbidity. Common

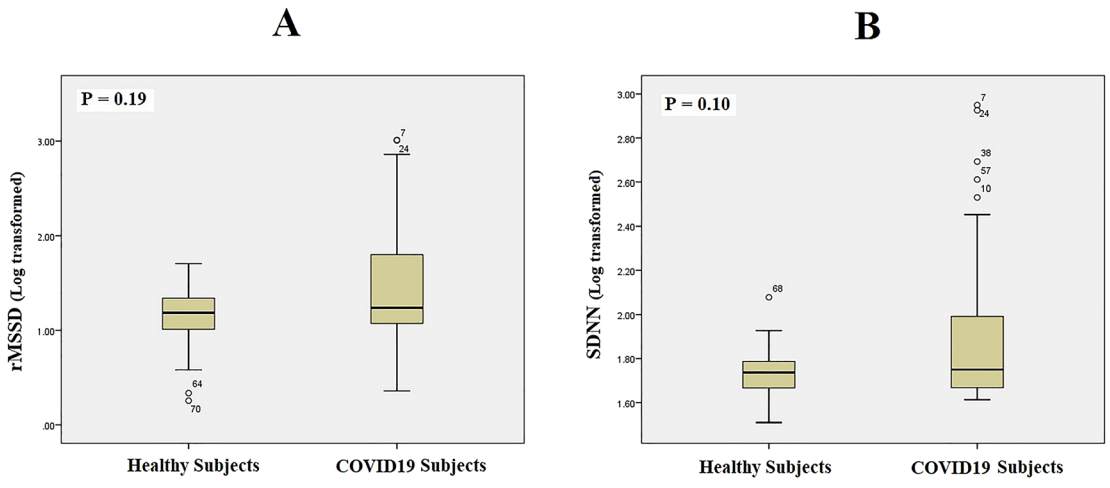


Figure 2: Boxplot graphical representation of rMSSD (A) and SDNN (B) among COVID-19 patients and healthy subjects.

Table 3: Subgroup analysis of clinical profile and heart rate variability parameters among symptomatic and asymptomatic COVID-19 subjects. Data shown as mean ± SD and frequency {n (%)}.

Variables	Symptomatic COVID-19 subjects (n=33)	Asymptomatic COVID-19 subjects (n=33)	p-Value
Age, years	57.59 ± 13.5	38.57 ± 13.1	0.0001[c]
Gender			0.006[b]
Males	28 (84.8%)	16 (53.3%)	
Females	5 (15.2%)	17 (46.7%)	
SPO_2 in room air, %	94 ± 9	97 ± 1	0.072
Systolic BP, mmHg	128 ± 16	121 ± 15	0.097
Diastolic BP, mmHg	77 ± 7	77 ± 8	0.763
Comorbidities			
DM type 2	11 (36.7%)	7 (23.3%)	0.260
Systemic hypertension	10 (33.3%)	2 (6.7%)	0.010[b]
Any one or more of above comorbidities	20 (66.7%)	8 (26.7%)	0.002[b]
HR	88.14 ± 17.11	80.55 ± 12.98	0.055
HF	64.61 ± 21.22	68.58 ± 19.33	0.442
LF	35.38 ± 21.22	31.37 ± 19.40	0.437
HF/LF ratio	3.82 ± 4.35	4.54 ± 4.83	0.518
LF/HF ratio	0.79 ± 0.83	0.61 ± 0.55	0.650
pNN50	4.58 ± 8.73	4.30 ± 7.53	0.358
rMSSD[a]	1.41 ± 0.62	1.56 ± 0.53	0.086*
SDNN[a]	1.87 ± 0.36	1.91 ± 0.3	0.135

SPO_2, oxygen saturation; BP, blood pressure; HR, heart rate; SDNN, standard deviation of NN intervals; rMSSD, root mean square of successive RR interval differences; pNN50, percentage of successive RR intervals; LF, low frequency power; HF, high frequency power. *Mann–Whitney *U* test, level of significance 0.05. [a]Log transformed values. [b]Chi square test, level of significance 0.05. [c]Student's *t*-test, level of significance 0.05.

co-morbidities among symptomatics were DM type 2 (36.7%), Systemic hypertension (33.3%), obesity (10%), hypothyroidism (13.3%) and psychomorbidity (10%). Both frequency and time domain HRV parameters showed no difference between the subgroups.

Discussion

Our study clearly demonstrated an increased parasympathetic activity in COVID-19 patients, independent of important confounders like age, gender and comorbidities including diabetes mellitus. Autonomic dysfunction in COVID-19 subjects in our study was best demonstrated by time domain measures of HRV namely rMSSD and SDNN with predefined cut-off values. HF and LF powers in frequency domain were significantly decreased in COVID-19 subjects, though their ratios were not significantly different when compared to the healthy uninfected subjects. In a subgroup analysis of COVID-19 subjects, HRV measures were not different between asymptomatics and mild to moderate symptomatic. To our knowledge, our study was the first to demonstrate higher rate of autonomic imbalance among COVID-19 patients as compared to uninfected healthy group.

Our search through literature yielded scanty reports on autonomic dysfunction among COVID-19 patients. Ritwik Ghosh et al. [24] reported in one case of COVID-19, clinical features of autonomic dysfunction in the form of sinus arrhythmia, postural hypotension, intermittent profuse sweating, constipation, erectile dysfunction and squeezing sensation in the chest. Cardiac dysautonomia was not studied in this report. In another case report, Reiner Buchhorn et al. [25], studied a 58-year-old COVID-19 patient with 24 h ECG monitoring and found a significant decrease in heart rate and a paradoxical decline in HRV. But for these two case reports suggesting a possible autonomic dysfunction, no studies have yet, evaluated the presence of this important physiological disharmony in COVID-19 patients.

Though scanty in the context of COVID-19, plethora of evidence available in the literature demonstrating autonomic dysfunction in other infectious diseases like human immunodeficiency virus [29–31] human T Cell lymphotropic virus [32], Epstein Barr virus [33], cytomegalovirus [34], West Nile virus [35], Chagas disease [36, 37], diphtheria [38], leprosy [39], dengue[40], tetanus [41] and botulism [42]. Methodological differences in evaluating autonomic function as well as variations in study design do not allow head-to-head comparison of these studies with our findings in COVID-19 patients**.** In our search through literature, we found only two reports using HRV to link autonomic dysfunction with infection. Jeevagan Vijayabala et al. [43] postulated sympathetic dysfunction as one of the pathogenetic mechanisms in Dengue shock syndrome . Robert Carter et al. [40] examined HRV of 27 children during defervescence in dengue viral infection and found that cardiac parasympathetic activity was the major cause for reduction in heart rate during this period of illness. Their observations were mainly based on frequency domain measures of HRV after correcting for baseline changes in heart rate. Contradictory to the above observations, La-Orkhun et al. [44] found no significant changes in various time and frequency domain metrics of HRV at least 24 h after defervescence, and follow-up conducted at least 14 days after defervescence. Our findings are consistent with Jeevagan Vijayabala et al. and Robert Carter et al., that we too found an increased parasympathetic activity in COVID-19 patients as demonstrated by the increase in time domain variables of HRV. This study further substantiates

two cases as cited above that reported autonomic dysfunction due to COVID-19. Proposed mechanisms of viral infection induced autonomic dysfunction include invasion of the central nervous system and the direct viral, toxin-mediated or immune-mediated involvement of the peripheral and autonomic nervous system [45].

Unlike the time domain variables, we found that frequency domains of HRV, specifically the LF/HF ratio were not different between the COVID-19 subjects and the healthy subjects. Traditionally LF/HF ratio was considered a marker of sympatho-vagal balance in cardiovascular system. However recent evidence this ratio merely indicates one part of complex non-linear interaction between sympathetic and parasympathetic nervous systems confounded by multiple physiological and mechanical factors [46]. Some of these confounders may have been missed in our study protocol. Also, smaller sample size may explain our inability to detect subtle difference in LF/HF ratio between the groups.

Our study involved a standardized measurement of heart rate variability using an ambulatory 5 min ECG recording in lead II. Precision of our evaluation was further augmented by stringent selection of subjects and optimal conditions for HRV measurement during conduct of the study. Our study is limited by participation of only asymptomatics and mild to moderate symptomatic patients with SARS CoV-2 infection. Severely ill COVID-19 patients were excluded from the study because we believed that factors like stress, cytokine storm, drugs like steroids, vasopressors etc., positive pressure ventilation, prolonged hospitalization and related psychomorbidity that typically characterize these patients may have a confounding effect on our observations. None of our study subjects had any of these factors that could have possibly altered our findings. Sample size of the study was not adequate to provide enough statistical power to our findings. But we believe that this preliminary report can serve a stimulus for future research in this direction. A larger sample size could have facilitated further evaluation of association between symptoms and autonomic function in our subjects.

In conclusion, this study has found that COVID-19 is associated with autonomic dysfunction whose mechanism and prognostic implications need to be evaluated further. HRV measurement, being a simple, noninvasive, inexpensive tool, its utility in clinical practice as a possible rapid diagnostic and prognostic marker needs to be assessed in future research.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This study was cleared by the local institutional human ethics committee and all study participants gave written informed consent.

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
2. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 2020;395:470–3.
3. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016;24:490–502.
4. Gorbalenya AE, Baker SC, Baric RS, Groot RJ, Drosten C, Gulyaeva AA, et al. Severe acute respiratory syndrome-related coronavirus: the species and its viruses: a statement of the Coronavirus Study Group. Nat Microbiol 2020;5:536–44.
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel corona virus-infected pneumonia in Wuhan, China. JAMA J Am Med Assoc 2020;323:1061–9.
6. Ellul MA, Benjamin L, Singh B, Lant S, Michael BD, Easton A, et al. Neurological associations of COVID-19. Lancet Neurol 2020;19: 767–83.
7. Vaira LA, Salzano G, Deiana G, De Riu G. Anosmia and Ageusia common findings in COVID-19 patients. Laryngoscope 2020;130: 1787.
8. Coronavirus disease 2019 (COVID-19) situation report. Available from: www.who.int›docs›20200306-sitrep-46-covid-19 [Accessed 11 Jan 2021].
9. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;3099:19–20.
10. Guan W, Ni Z, Hu Y. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708–20.
11. Zhang JJ, Dong X, Cao YY. Clinical characteristics of 140 patients infected by SARS-CoV-2 in Wuhan, China. Allergy 2020;75: 1730–41.
12. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med 2020;14:185–92.
13. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 2004;203:622–30.
14. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol 2020:1–4. https://doi.org/10.1002/jmv.25728.
15. Huang P, Tang L, Ren L, Liu L. Research progress in nervous system damage caused by SARS-CoV-2. Zhong Nan Da Xue Bao Yi Xue Ban 2020;28:1247–54.

16. Yashavantha Rao HC, Jayabaskaran C. The emergence of a novel coronavirus (SARS-CoV-2) disease and their neuroinvasive propensity may affect in COVID 19 patients. J Med Virol 2020;92: 786–90.
17. Li K, Wohlford Lenane C, Perlman S, Zhao J, Jewell AK, Reznikov LR, et al. Middle East respiratory syndrome coronavirus causes multiple organ damage and lethal disease in mice transgenic for human dipeptidyl peptidase 4. J Infect Dis 2016;213: 712–22.
18. McCray PB Jr, Pewe L, Wohlford Lenane C, Hickey M, Manzel L, Shi L, et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J Virol 2007;81: 813–21.
19. Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednicky J, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). J Med Virol 2020;92:699–702.
20. Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, Takamino J, et al. A first Case of Meningitis/Encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis 2020.
21. Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol 2008;82:7264–75.
22. Li YC, Bai WZ, Hirano N, Hayashida T, Taniguchi T, Sugita Y, et al. Neurotropic virus tracing suggests a membranous coating mediated mechanism for transsynaptic communication. J Comp Neurol 2013;521:203–12.
23. Carod-Artal FJ. Infectious diseases causing autonomic dysfunction. Clin Auton Res 2018;28:67–81.
24. Ghosh R, Roy D, Sengupta S, Benito-Leon J. Autonomic dysfunction heralding acute motor axonal neuropathy in COVID-19. J Neurovirol 2020;26:964–66.
25. Buchhorn R, Baumann C, Willaschek C. Heart rate variability in a patient with coronavirus disease 2019. Int Cardiovasc Forum J 2020. https://doi.org/10.17987/icfj.v20i0.685.
26. Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 1996;93:1043–65.
27. Nunan D, Sandercock GRH, Brodie DA. A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. Pacing Clin Electrophysiol 2010; 33:1407–17.
28. De Coster J, Gallucci M, Anne-Marie R. Iselin. Best practices for median splits, artificial categorization and their continuous alternatives. J Exp Psychopathol 2011;2:197–209.
29. DM FS, Umekawa S, Kocher M, Kallianpur KJ, Shikuma CM, Low P. Symptoms of autonomic dysfunction in human immunodeficiency virus. Open Forum Infect Dis 2015;2:ofv103.
30. Mittal CM, Wig N, Mishra S, Deepak KK. Heart rate variability in human immunodeficiency virus-positive individuals. Int J Cardiol 2004;94:1–6.
31. Becker K, Gorlach I, Frieling T, Haussinger D. Characterization and natural course of cardiac autonomic nervous dysfunction in HIV-infected patients. AIDS 1997;11:751–7.
32. Alamy AH, Menezes FB, Leite AC, Nascimento OM, Araujo AQ. Dysautonomia in human T-cell lymphotrophic virus type I-associated myelopathy/tropical spastic paraparesis. Ann Neurol 2001;50:681–5.
33. Bennett JL, Mahalingam R, Wellish MC, Gilden DH. Epstein-Barr virus-associated acute autonomic neuropathy. Ann Neurol 1996; 40:453–5.
34. Nakao K, Namekawa M, Kondo S, Ono S, Nakano I. Subacute autonomic and sensory neuropathy closely related to cytomegalovirus infection preceded by frequent syncopal attacks. Rinsho Shinkeigaku 2016;8:555–9.
35. Bode AV, Sejvar JJ, Pape WJ, Campbell GL, Marfin AA. West Nile virus disease: a descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003. Clin Infect Dis 2006;42: 1234–40.
36. Junqueira LF Jr. Insights into the clinical and functional significance of cardiac autonomic dysfunction in Chagas disease. Rev Soc Bras Med Trop 2012;45:243–52.
37. Vasconcelos DF, Junqueira LF Jr. Cardiac autonomic and ventricular mechanical functions in asymptomatic chronic chagasic cardiomyopathy. Arq Bras Cardiol 2012;98:111–9.
38. Illarramendi X, Buhrer-Sekula S, Sales AM, Bakker MI, Oliveira A, Nery JA, et al. High prevalence of vasomotor reflex impairment in newly diagnosed leprosy patients. Eur J Clin Invest 2005;35: 658–65.
39. Idiaquez J. Autonomic dysfunction in diphtheritic neuropathy. J Neurol Neurosurg Psychiatry 1992;55:159–61.
40. Carter R, III, Hinojosa-Laborde C, Convertino VA. Heart rate variability in patients being treated for dengue viral infection: new insights from mathematical correction of heart rate. Front Physiol 2014;5:46.
41. Lin TS, Chen LK, Lin TY, Wen SH, Chen MC, Jan RH, et al. Autonomic dysfunction because of severe tetanus in an unvaccinated child. PediatrNeonatol 2011;52:169–71.
42. Merz B, Bigalke H, Stoll G, Naumann M. Botulism type B presenting as pure autonomic dysfunction. Clin Auton Res 2003; 13:337–8.
43. Vijayabala J, Attapaththu M, Jayawardena P, De Silva SG, Constantine G. Sympathetic dysfunction as a cause for hypotension in dengue shock syndrome. Chin Med J 2012;125:3757–8.
44. La-Orkhun V, Supachokchaiwattana P, Lertsapcharoen P, Khongphatthanayothin A. Spectrum of cardiac rhythm abnormalities and heart rate variability during the convalescent stage of dengue virus infection: a Holter study. Ann Trop Paediatr 2011;31:123–8.
45. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol 2013;4:26.
46. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18–22.

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Association between consumption of chocolate and risk of type 2 diabetes mellitus

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Abstract

Background: There is a substantial interest in the potential role of chocolate consumption and its association with the risk of type 2 diabetes mellitus. The objective of this study was to examine the association of consumption of chocolate with the risk of type 2 diabetes mellitus.

Methods: A case control study was conducted at PSG Hospitals between 1st June 2017 and 1st July 2017. All those who had newly diagnosed type 2 diabetes and attended Department of Endocrinology for follow-up visit were the cases and the relatives accompanying them without the history of diabetes were the controls. Chocolate consumption and other dietary factors were elicited. The mediating factors studied were age, sex, Body Mass Index (BMI), educational levels, smoking status, alcohol consumption, family history of diabetes, potassium intake, magnesium intake and use of statins.

Results: After adjusting the potential confounders, chocolate consumption was inversely associated with risk of type 2 diabetes ($p<0.05$) with odds ratio of 0.564 (95% CI = 0.32 - 0.98).

Conclusions: Our findings suggest that moderate consumption of chocolates (preferably 1 to 2) per week has a decreased risk of type 2 diabetes mellitus. Potential applications of this knowledge include recommendations by health care professionals to encourage individuals to consume a wide range of phytochemical rich foods include chocolates in moderate amounts. The results from our study also suggest that adjusting for mediating factors did not alter the results and hence other unknown factors may explain inverse relation between chocolate consumption and type 2 diabetes mellitus.

Keywords: Chocolate consumption, Potassium intake, Type 2 diabetes mellitus, Tea consumption

Introduction

There is a substantial interest in the potential role of chocolate and one of its primary bioactive compounds, flaven-3-ols in prevention and management of cardio vascular disease (CVD).[1-5] Few randomized controlled clinical trials (RCT) have examined mechanisms by which chocolate in cocoa and flaven-3-ols potentially reduce CVD risk.[6-10] However, the predominant focus of these studies has been blood pressure effects and many of these studies examined its effects on systolic and diastolic blood pressure, LDL and HDL cholesterol. In addition,

some of these RCT's reported a reciprocal relation between insulin resistance and chocolate consumption.

There were few observational studies which supported the association between cocoa intakes and reduced diabetes risk. Chocolate contains caffeine, flavonoids and minerals such as magnesium that could influence the risk of diabetes mellitus. An inverse association between the consumption of caffeine, flavonoids and magnesium with the role of diabetes mellitus has been reported with inconsistent results.[11-12] In contrast, cocoa and chocolate have beneficial effects in insulin resistance, oxidative stress and inflammation which play important roles in pathogenesis of diabetes mellitus. However, it is still unclear, whether chocolate consumption is associated with risk of diabetes mellitus and whether this association differs according to the characteristics of the study population. The present study is aimed to find the association between chocolate consumption and risk of diabetes mellitus.

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Study population and design

A case-control study was conducted at PSG Hospitals. All those who have newly diagnosed in the past 1 year as type 2 diabetes and attending the Department of Endocrinology for follow up between 1stJune 2017 and 1st July 2017 were considered as cases. Controls were those who accompany with them and do not have history of diabetes. We then retrospectively evaluated their food practices and other demographic information.

General assessments

The general assessment survey was conducted at the outpatient Department of Endocrinology, PSG Hospitals, Coimbatore. The general questionnaire contained questions on demographic characteristics. Body weight (in kg) and height (in meter) were also measured. However, in few cases, we were not able to measure height. Food intake was assessed using a validated Food Frequency Questionnaire in this population and from this magnesium intake and potassium intake were estimated.[13] The questionnaire also asked about chocolate consumption, coffee intake, tea intake, smoking status, practice of alcohol intake, whether previously diagnosed as diabetes, other medical histories and medications taken.

Statistical analysis

To examine the association between chocolate consumption and incidence of type 2 diabetes, we used four logistic regression models. In the first model, odds ratio and 95% confidence intervals for type 2 diabetes mellitus were calculated against a reference group of low consumers of chocolate adjusted for age (continuous) and gender (male/female).

A second logistic regression model corrected the other known diabetes risk factors include smoking status (present/absent), BMI, education attainment (classes up to 12th standard/college and above), family history of diabetes (present/absent); alcohol intake (gm. per week). Body mass index was calculated by the formula, body weight (kg) divided by the square of the body height (meter). A third model corrected for coffee and tea consumption. Mediating factors were included in the final model. The mediating factors considered were daily intake of magnesium and potassium which were elicited from their food consumption practices and the effects of long term medications like the use of statins.

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The study included 150 cases and 150 controls. Demographic characteristics of the study participants among cases and controls were presented in Table 1.

Table 1: Distribution of the participant's demographic characteristics□□

□□r□□□□□□□	□□□□□□□ □□□□□ □□□r□M□□□□□□□□ □	□□□r□□□□□ □□□□□ □□□r□M□□□□□□□□ □	□□□ □□□□□
Age (years, continuous)	53.29 ± 11.52	41.75 ± 12.44	p < 0.05
BMI (kg/m^2, continuous)	26.12 ± 5.09	25.99 ± 5.21	0.824
Potassium (mg/day)	624.11 ± 151.14	661.14 ± 209.54	p < 0.001
Magnesium (mg/day)	134.27 ± 50.19	123.30 ± 44.96	0.082
□□□□			0.246
Male	72 (48%)	62 (41.3%)	
Female	78 (52.0%)	88 (58.7%)	
□d□□□□□□□□□□□□□□			p < 0.001
Up to 12th Standard	108 (72.0%)	85 (56.7%)	
College and above	42 (28.0%)	65 (43.3%)	
□□rr□□□□□ □□□□□□□□□□□			0.569
Yes	17 (11.3%)	14 (9.3%)	
No	133 (88.7%)	136 (90.7%)	
□□□□□□□□□□□□			0.427
rarely/moderately	144 (96.0%)	141 (94.0%)	

weekly/daily	6 (4.0%)	9 (6.0%)	
[illegible]			p < 0.001
Yes	91 (60.7%)	51 (34.0%)	
No	59 (39.3%)	99 (66.0%)	
[illegible]			0.317
Yes	1 (0.7%)	0 (0.0%)	
No	149 (99.3%)	150 (100%)	

[illegible]

[illegible]	[illegible]		[illegible]		
Do not take on a weekly basis	131	87.3%	102	68%	233
1-2 chocolates per week	17	11.3%	38	25.3%	55
3-4 chocolates per week	1	0.7%	5	3.3%	6
≥5 chocolates per week	1	0.7%	5	3.3%	6

[illegible]

	[illegible]	[illegible]	[illegible]	[illegible]
Odds ratio	0.559	0.601	0.540	0.564
95% CI for diabetes	0.336 - 0.932	0.352 - 1.026	0.312 - 0.936	0.324 - 0.980
p Value	0.026	0.062	0.028	0.042

Among the variables studied, all differences between case subjects and control subjects were comparable except for age, educational level and family history of diabetes. Cases were slightly older in age compared to controls. The mean and standard deviation of age (years) among cases were 53.29±11.52 and that of controls were 41.75±12.44. This difference was statistically significant ($p < 0.05$).

The mean and standard deviation of potassium intake among cases were 624.11±151.14 and that of controls were 661.14±209.54. This difference was statistically significant ($p < 0.001$). 72% of the participants among diabetic were having education up to 12th standard whereas the similar value for non-diabetic was only 56.7%. This difference was statistically significant ($p < 0.001$). Among diabetic, 60.7% of them had family history of diabetes whereas the similar value for non-diabetic was only 34.0%. This difference was statistically significant ($p < 0.001$).

87.3% of the cases do not take chocolate on a weekly basis whereas the similar value for controls was only 68%. 11.3% of the cases take 1 to 2 chocolates on a weekly basis whereas the similar value for controls was 25.3 % (Table 2). In the multivariate regression model (Table 3), it was observed that the participants who consume more chocolate had a significantly decreased risk of diabetes compared with those who do not take chocolate on a weekly basis (odds ratio = 0.564, 95% CI = 0.324 - 0.980).

[illegible]

In our study, self-reported chocolate intake was significantly inversely associated with diabetes mellitus. Many studies supported our findings that chocolate consumption may lower risk of diabetes mellitus. The recent Maine- Syracuse Longitudinal Study (MSLS) reported that persons who eat chocolate at least once a week have a lower prevalence of diabetes and are at lower risk for a diagnosis of diabetes for five years later.[14]

Beneficial effects of cocoa and chocolate on risk factors related to diabetes mellitus were reported in short term randomized clinical trials as well as long term experimental studies5,8. It was reported in same studies that cocoa or chocolate improves insulin resistance, oxidative stress and inflammation. However, whether these results are the possible mechanisms resulting in an inverse association between chocolate consumption and risk of diabetes is still unclear. It is also uncertain that which substance in chocolate is responsible for the inverse association with diabetes mellitus that we have observed.

However, it is important to differentiate between the natural product cocoa and the processed product chocolate which is an energy dense food. In addition, physical activities, diet and other life style factors must be carefully balanced to avoid differential weight gain over time.

Our study has several limitations. Since this was a case - control study, cause and effect relations between chocolate consumption and diabetes cannot be established. This has not been established in any study in the literature also. A bidirectional relationship could not be ruled out, modest amounts of chocolate protect against diabetes but some diabetic patients chose to eat modest amounts of chocolates.

It is also unclear if the benefits of the chocolates are limited only to dark chocolate. It is widely hypothesized that cocoa flavonols, found in larger amounts in dark chocolate, responsible for its health benefits and more large scale prospective studies are required to provide more information on whether only dark chocolate is beneficial or chocolate in general is beneficial. It is also possible that chocolate consumption may represent an overall marker for a cluster of favorable socio demographic profiles and thereby healthier life style behaviors and better health status. This could explain, at least in part, the observed associations with the insulin biomarkers.

[illegible]

Study findings suggest that moderate consumption of chocolates (preferably 1 to 2) per week has a decreased risk of type 2 diabetes. Potential approach of this knowledge includes recommendations by health care professionals to encourage individuals to consume a wide range of phytochemical rich foods include chocolates in moderate amounts.

The results from our study also suggest that adjusting for mediating factors did not alter the results and hence other unknown factors may explain inverse relation between chocolate consumption and type 2 diabetes mellitus. Continued studies are necessary to study the role of specific factors regarding the association of chocolate consumption and type 2 diabetes mellitus.

[illegible] [illegible]M[illegible]

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[illegible]

1. Corti R, Flammer AJ, Hollenberg NK, Luscher TF. Cocoa and cardiovascular health. Circulation. 2009;119:1433-41.
2. McCullough ML, Chevaux K, Jackson L, Preston M, Martinez G, Schmitz HH, et al. Hypertension, the Kuna, and the epidemiology of flavanols. J Cardiovasc Pharmacol. 2006;47:S103-9.
3. Schewe T, Steffen Y, Sies H. How do dietary flavanols improve vascular function? A position paper. Arch Biochem Biophys. 2008;476:102-6.
4. Buijsse B, Feskens EJM, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch Intern Med. 2006;166:411-7.
5. Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. Am J Clin Nutr. 2012;95:740-51.
6. Ding EL, Hutfless SM, Ding X, Girotra S. Chocolate and prevention of cardiovascular disease: a systematic review. Nutr Metab (Lond). 2006;3:2.
7. Ried K, Sullivan T, Fakler P, Frank OR, Stocks NP. Does chocolate reduce blood pressure? A meta-analysis. BMC Med. 2010;8:39.
8. Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, et al. Flavonoids, flavonoid-rich foods and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2008;88:38-50.
9. Taubert D, Roesen R, Schomig E. Effect of cocoa and tea intake on blood pressure: a meta-analysis. Arch Intern Med 2007;167:626-34.
10. Desch S, Schmidt J, Kobler D, Sonnabend M, Eitel I, Sareban M, et al. Effect of cocoa products on blood pressure: systematic review and meta-analysis. Am J Hypertens. 2010;23:97-103.
11. Matsumoto C, Petrone AB, Sesso HD, Gaziano JM, Djousse L. Chocolate consumption and risk of diabetes mellitus in the Physicians' Health Study. Am J Clin Nutr. 2015;101:362-7.
12. Dong JY, Xun P, He K, Qin LQ. Magnesium intake and risk of type 2 diabetes: meta-analysis of prospective cohort studies. Diabetes Care. 2011;34:2116-22.
13. Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K. Indian food composition tables 2017. National Institute of Nutrition. 2017;150-206.
14. Crichton GE, Elias MF, Dearborn P, Robbins M. Habitual chocolate intake and type 2 diabetes mellitus in the Maine- Syracuse Longitudinal Study: (1975-2010): Prospective observations, Appetite. 2017;108:263-8.

[illegible]r [illegible]Mathew AC, Kishan S, Joy M, Manoj DS, Amirthvarshan A, Kumar RS. Chocolate consumption and its relation to risk of type 2 diabetes mellitus. Int J Adv Med 2017;4:1473-6.

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Original Article

Comparison of Polymerase Chain Reaction (PCR), Microbiological and Histopathological Observations in the Diagnosis of Endometrial Tuberculosis

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Abstract

Background

Female genital tuberculosis often faces diagnostic challenges due to the asymptomatic nature of the disease. Our study aims at comparing the microbiological and histopathological results with PCR in diagnosing genital tuberculosis in endometrial curettage specimens.

Methods

Around 139 patients with diverse gynaecological complaints were recruited for the study, and endometrial curettage specimens were collected. The specimens were subjected to microbiological culture and staining, histopathological examination and PCR to look for the presence of *M. tuberculosis*. Statistical analyses of the PCR results include calculating sensitivity, specificity, positive and negative prediction values and positive and negative likelihood ratios.

Results

PCR yielded a detection rate of 41.7% (58/139) when compared to the microbiology (2.15%) and histopathology results (1.43%). PCR with *hsp65* and *cfp10*, in combination, detected 20% of the cases. Statistical analyses were suggestive that PCR with *hsp65* showed a higher sensitivity and specificity of 50% and 92.59% respectively.

Conclusion

The results obtained in this study suggest that for a definitive diagnosis, combinations of the results from various diagnostics techniques can only be considered.

Keywords: PCR, Female genital tuberculosis, Culture, Histopathology

Introduction

Female genital tuberculosis continues to be an important cause for infertility, especially in developing countries like India accounting for about 7–15% [1]. This is a form of secondary tuberculosis after a primary encounter of tuberculosis, either pulmonary or extrapulmonary [2]. *Mycobacterium tuberculosis* remains abeyant for a longer duration, reactivates and causes harmful effects on female genital tract which develops as female genital tuberculosis. Among the various sites, fallopian tubes and endometrium are affected the most, followed by ovary, cervix and vagina [3]. Tubal obstruction, impairment of implantation and ovulatory failure occur as a result of genital tuberculosis [4]. Therefore, infertility, chronic abdominal pain and menstrual dysfunctions are often found associated with this disease. On the other hand, 11% of women affected are asymptomatic [5] and so remain underdiagnosed.

Various diagnostic procedures like microbiological culture, smear microscopy, histopathology, hysterosalpingography (HSG) and ultrasonography are in use, though each of which had proven unhelpful in diagnosing certain cases. PCR is emerging as an important tool in view of its rapidness, sensitivity and specificity reported by many researchers [6, 7]. Since endometrial tuberculosis leaves only traces of bacilli, molecular techniques are potentially considered important for mycobacterial demonstration.

In our study, we have made an attempt to compare the results between PCR, microbiology and histopathology as well as correlate its results with the phenotype displayed by the patients. This investigation would help us in understanding the epidemiology of this disease, commonly appeared clinical evidences and superiority of techniques that can be employed in its diagnosis.

Materials and Methods

Patient Enrollment

Around 139 women belonging to the reproductive age group who attended the Obstetrics and Gynecology clinic in PSG Hospitals featuring symptoms suggestive of genital TB like chronic pelvic inflammatory disease, infertility, tubo-ovarian mass, secondary amenorrhoea and history of pulmonary tuberculosis were recruited for this study. Ethical committee approval was obtained for this study from Institutional Human Ethics Committee, PSG Institute of Medical Sciences & Research, Coimbatore (IHEC No: 10/214). Pregnant women and patients in whom dilatation and curettage (D&C) were contraindicated were excluded in this study. Informed consent was obtained from all individual participants included in the study and detailed history, and gynaecological examination were done on

the patients. Endometrial curettings collected from the patients were subjected to microbiological culture in Lowenstein–Jensen's (LJ) medium, acid fast staining, histopathological examination and PCR for detecting *M. tuberculosis.*

Microbiological Examinations

The endometrial curettings suspended in saline were vortexed in the presence of glass beads to obtain an even suspension, and the samples were subjected to Ziehl Neelson (acid fast) staining technique for smear positivity. The samples were inoculated onto Lowenstein–Jenson's medium and incubated for 4 weeks at 37 °C. To confirm the absence of growth, incubation was extended to 2 weeks more.

Histopathological Observations

The endometrial curettings were suspended in 10% formalin and processed. The samples were stained with haematoxylin and eosin to look for the presence of caseating granulomas with epitheloid cells, lymphocytes, plasma cells and giant cells which is suggestive of tuberculosis.

Polymerase Chain Reaction from Endometrial Curettings

The endometrial curettings were suspended in lysis buffer (50 mM KCl, 10 mM TrisHCl pH 8.3, 1.5 mM $MgCl_2$, 0.1% Nonidet P-40, 0.5% Tween-20) and homogenized. DNA was extracted using the standardized protocol [8]. PCR for amplifying three mycobacterial gene segments was carried out. The primer sequences and the amplicon length are mentioned in Table 1. 20 µl of PCR mix consisted of 50 ng of DNA mixed with 0.2 mM of each of the four dNTPs (dATP, dCTP, dGTP, dTTP), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 1.5 mM MgCl2, 0.05 µM of each forward and reverse primers, 1 U/µl *Taq* polymerase and nuclease free water. The reaction was supplemented with positive and negative controls. The PCR parameters followed were 35 cycles of denaturation (94 °C for 5 min), primer annealing (60 °C for 50 s) and extension (72 °C C for 1 min). The final extension was carried out at 72 °C C for 5 min, and the samples were put on hold at 4 °C C. The PCR products were run on 1.5% agarose gel, and the bands were visualized under UV light using a gel documentation system.

Table 1

Primer details of three mycobacterial gene segments

S. no	Gene segment	Primer sequences (FP – Forward primer, RP – Reverse primer)	Size of PCR product (bp)
1	*Hsp65*	FP-5′ ACCAACGATGGTGTGTCCAT 3′ RP-5′ CTTGTCGAACCGCATACCCT 3′	441
2	*Cfp10*	FP-5′ GGCAGAGATGAAGACCGATG 3′ RP-5′ GCTTATTGGCTGCTTCTTGG 3′	191
3	*Esat6*	FP-5′ CATGACAGAGCAGCAGTGG 3′ RP-5′ CCCTATGCGAACATCCC 3′	291

Statistical Parameters

Statistical parameters like sensitivity, specificity, positive and negative prediction values and positive and negative likelihood ratios were calculated.

Results

Clinical Presentations of the Patients

The common presenting symptoms found in the study population were primary infertility (60%) and irregular cycles (47.5%). Secondary infertility and abdominal pain account for about 16.5% and 10%, respectively. White discharges (7.2%), secondary amenorrhoea (6.5%), dysmenorrhoea (5.7%) and fibroid uterus (4.3%) were manifested by the patients too. Hypomenorrhoea (1.4%), oligomenorrhoea (3.6%), menorrhagia (2.9%), ectopic pregnancy (1.4%) and history of tuberculosis (1.4%) constituted to a meagre amount (Fig. 1).

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Fig. 1

Frequency of presenting symptoms in the study population. The percentage of different clinical presentations observed in the patients recruited for this study was depicted. Primary infertility and irregular cycles were presented more frequently, while ectopic pregnancy and oligomenorrhoea were presented the least

Correlation of Microbiological and Histopathological Results with PCR

For PCR detection, primers specific for three mycobacterial gene segments, namely *hsp65, cfp10* and *esat6*, were used. *Hsp65* is the mycobacterium genus-specific gene segment was employed for the diagnosis of genital TB [9]. *cfp10* and *esat6* were the gene segments present in *M. tuberculosis*, *M. africanum* and virulent *M. bovis,* thus comprising the *M. tuberculosis* complex [10, 11].

Overall, 58/139 samples were positive at least for one primer through PCR with a detection rate of 41.7%. On the contrary, only 2/139 samples were positive for histopathology with a detection rate of 1.43%. Microbiological examinations detected two cases of *M. tuberculosis* and a case of non-tuberculous mycobacteria with a positivity rate of 2.15%. Based on the PCR results, positive samples were grouped into four categories depending on the number of gene segments amplified for each sample (Table 2).

Table 2

Analysis of PCR-positive samples

Gene segments amplified	No of samples positive	Percentage
hsp65, cfp10 and *esat6*	4/139	2.9
hsp65 and *cfp10*	28/139	20
hsp65	12/139	8.6
cfp10	14/139	10

Out of 139 samples, four samples were positive for all the three gene segments. Both histopathological findings and microscopic techniques (acid fast staining & culture) yielded the negative results for these samples. *Hsp65* and *cfp10* gene segments were identified in 28/139 samples. Among 28 samples, only one sample detected tubercle bacilli through culture on LJ medium. Histopathological examinations yielded the negative results. In 12/139 samples, *hsp65* alone was detected. In 1/12 sample, mycobacterial culture and histopathology results were positive for *M. tuberculosis*. Non-tuberculous mycobacterium was detected in one sample. *cfp10* was detected in 14/139 samples which showed the negative results for microbiology and histopathology. There were 81/139 samples which gave the negative results for PCR. Out of this, one sample was positive for histopathology.

Overall, it is clear from the data that *hsp65* and *cfp10* either alone or in combination have been successful in detecting 58/139 samples. In combination, *hsp65* and *cfp10* have detected up to 20% of cases which microbiological and histopathological assays were unable to detect (Table 2).

Correlation of Clinical Symptoms with PCR Results

The varied clinical clues exhibited by the patients and their respective PCR results were compared (Table 3). The predominant clinical symptoms associated with the PCR-positive samples were irregular menstrual cycles and primary infertility accounting for 50–75% of the cases. Secondary infertility,

secondary amenorrhoea, hypomenorrhoea, dysmenorrhoea, oligomenorrhoea, chronic abdominal pain and white discharges were found in 10–25% of the cases. History of tuberculosis and fibroid uterus constitute a very meagre percentage of 3.5–7% (Table 3).

Table 3

Correlation of clinical symptoms with PCR results

Clinical features	PCR amplicons found			
	hsp65*, *cfp10* and *esat6	***hsp65* and *cfp10***	***hsp65***	***cfp10***
Primary infertility (%)	50	75	66	50
Secondary infertility (%)	–	21.4	8.3	20
Secondary amenorrhoea (%)	25	7.1	8.3	7.1
Hypomenorrhoea (%)	25	3.5	–	–
Oligomenorrhoea (%)	25	–	8.3	20
Dysmenorrhoea (%)	25	7.1	–	7.1
Menorrhagia (%)	–	3.5	–	–
White discharges (%)	25	10.7	–	21.4
Irregular cycles (%)	75	39	25	50
Abdominal pain/PID (%)	–	3.5	8.3	21.4
Ectopic pregnancy (%)	–	7.1	–	–
History of tuberculosis (%)	–	3.5	–	3.5
Fibroid uterus	–	7.1	8.3	7.1

The patients in whom all the three gene segments of mycobacteria were amplified belonged to the reproductive age group 27–31, marked with irregular menstrual cycles, primary infertility, hypomenorrhoea, dysmenorrhoea, oligomenorrhoea and white discharges.

The 28 patients in whom *hsp65* and *cfp10* gene segments were amplified belonged to the age group of 19–47. They indicated gynaecological irregularities like primary infertility (75%), secondary infertility (21.4%), secondary amenorrhoea (7.1%), white discharges (10.7%), fibroid uterus (7.1%) and ectopic pregnancy (7.1%). 39% of these 28 patients had irregular menstrual cycles. A previous history of abdominal tuberculosis was found in a patient. 3.5% of these patients had chronic pelvic inflammatory disease (PID). Other menstrual problems like menorrhagia, hypomenorrhoea and dysmenorrhoea were also associated in these patients.

Hsp65 alone was amplified in 12 samples. Primary infertility (66%), irregular cycles (25%), 8.3% each of secondary infertility, secondary amenorrhoea, abdominal pain, fibroid uterus and oligomenorrhoea were the clinical symptoms associated in these patients.

In 14 samples, only *cfp10* was amplified. A history of pelvic tuberculosis was also associated with a case. 50% of these patients had irregular cycles and primary infertility. White discharges, chronic abdominal pain and PID were observed in 21.4% of patients. 20% of patients had secondary infertility

and oligomenorrhoea. 7.1% of the patients had complaints like secondary amenorrhoea, fibroid uterus and dysmenorrhoea.

Sensitivity and Specificity of TB PCR

Microbiological culture was considered as the gold standard technique to diagnose endometrial TB. All culture-positive cases in our study were considered as positive cases. Based on this, the sensitivity, specificity and positive and negative predictive values for the TB PCR were calculated (Table 4). Using any one of the primers, the sensitivity and specificity of PCR were found to be 75% and 59.2%, respectively. The sensitivity and specificity were high for primer set *hsp65* when compared with other combinations of primers.

Table 4

Sensitivity, specificity and predictive values for the TB PCR

PCR primers	Statistical parameters					
	Sensitivity (%)	Specificity (%)	Positive predictive values (%)	Negative predictive values (%)	Positive likelihood ratio	Negative likelihood ratio
PCR Positive (for any one of the primer)	75	59.26	5.17	98.77	1.84	0.42
PCR positive for all the three primer sets (*hsp65*+ *esat6*+ *cfp10*+)	0.00	97.04	0.00	97.04	0.00	1.03
PCR positive for two primer sets (*hsp65* and *cfp10*)	20	82.91	3.57	97.04	1.17	0.96
PCR positive for one primer set (*hsp65* alone)	50	92.59	16.67	98.43	6.75	0.54
PCR positive for one primer set (*cfp10* alone)	0.00	89.63	0.00	96.80	0.00	1.12

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Discussion

Our results indicated lower detection rates for culture (2.15%) and histopathological analyses (1.43%) and higher identification rates for PCR. The similar results were obtained for histopathology (1.87%) and microbiology (2.05%) findings recorded earlier [12]. Other studies reported a higher detection rate of 65% for PCR with endometrial biopsies [13].

PCR-positive four samples (for all three gene segments) showing the negative results for microbiology and histopathology may be due to the lower sensitivity of culture and histopathological analyses. The possible reason for culture insensitivity may be poor recovery of bacilli as the sparse number of bacilli is a characteristic feature of genital TB [14]. The false-negative histopathology results may be due to the shedding of endometrium leaving insufficient time for granuloma formation or damaged endometrium [2, 15].

A PCR and culture negative sample displaying positive result may be due to the co-presence of other pathogens which might have precipitated the lesions. This also confirms the fact that the PCR results correlate with that of culture and have shown reliability.

Review of available data on microbiological diagnosis of genital TB indicates that BACTEC 460 TB culture had a sensitivity of 7.14–40% and a specificity of 90–100% [16–18] which improved upon usage of PCR as an additional tool in diagnosing genital TB. The histopathological method showed a sensitivity of 10.7% and specificity of 100% [16]. These figures reveal that there was a need for development of a better sensitive method for genital TB with subtle presentations and lower bacterial load. In our setting, around 20% of the positive cases were spotted positive by *hsp65* and *cfp10*. Statistical parameters indicate a higher sensitivity and specificity of PCR when any one of the primers was used. Among the primer combinations, *hsp65* primers demonstrated higher probabilities of finding mycobacteria with higher specificity of 92.59% as it is a mycobacterium genus-specific gene, but it showed a lower sensitivity rate of 50% possibly because of the presence of both the endometrial DNA and the mycobacterial DNA in the sample which might give rise to more inhibitory effects on PCR. Lower sensitivity range of 55% in detecting *M. tuberculosis* through PCR was also reported earlier [19] where the usage of low-cost PCR reagents was the reason for lower sensitivity, though it was developed for applications in low income settings [20]. Similarly, lower sensitivity of PCR (57%) in diagnosing genital TB was reported in another study where combination of many sets of primers was suggested to be used to improve the sensitivity.

Conclusion

The gold standard test to diagnose endometrial tuberculosis remains the culture technique: LJ medium and MGIT liquid culture. In the current scenario, all the clinical decisions are best made depending on the culture results although collaborative tests may be supportive. Therefore, PCR can only be used as an additional method along with histopathology, microbiology and imaging tools.

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Biography

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Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no competing interests.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Human Ethics Committee, PSG Institute of Medical Sciences and Research, Coimbatore (IHEC No: 10/214).

Informed Consent

Informed consent was obtained from all patients for being included in the study.

Footnotes

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References

1. Jindal UN, Bala Y, Sodhi S, et al. Female genital tuberculosis: early diagnosis by laparoscopy and endometrial polymerase chain reaction. *Int J Tuberc Lung Dis.* 2010;14(12):1629–1634. [PubMed] [Google Scholar]

2. Grace GA, Devaleenal DB, Natrajan M. Genital tuberculosis in females. *Indian J Med Res.* 2017;145(4):425–436. [PMC free article] [PubMed] [Google Scholar]

3. Sharma JB. Current diagnosis and management of female genital tuberculosis. *J Obstet Gynaecol India.* 2015;65(6):362–371. doi: 10.1007/s13224-015-0780-z. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

4. Ghosh K, Ghosh K, Chowdhury JR. Tuberculosis and female reproductive health. *J Postgrad Med.* 2011;57:307–313. doi: 10.4103/0022-3859.90082. [PubMed] [CrossRef] [Google Scholar]

5. Bhanothu V, Theophilus J, Rozati R. Detection of *Mycobacterium tuberculosis* among infertile patients suspected with female genital tuberculosis. *Am J Infect Dis Microbiol.* 2014;2(2):22–33. [Google Scholar]

6. Rana T, Singh UB, Kulshrestha V, et al. Utility of reverse transcriptase PCR and DNA-PCR in the diagnosis of female genital tuberculosis. *J Med Microbiol.* 2011;60:486–491. doi: 10.1099/jmm.0.025080-0. [PubMed] [CrossRef] [Google Scholar]

7. Kumar P, Benny P, Jain M, et al. Comparison of an in-house multiplex PCR with two commercial immuno-chromatographic tests for rapid identification and differentiation of MTB from NTM isolates. *Int J Mycobacteriol.* 2014;3:50–56. doi: 10.1016/j.ijmyco.2013.12.001. [PubMed] [CrossRef] [Google Scholar]

8. Chowdhury RG, Paine SK, Bhattacharjee B, et al. Infestation of endometrium by *Mycobacterium tuberculosis* bacilli—cause of reproductive failure. *Al Ameen J Med Sci.* 2010;3:322–331. [Google Scholar]

9. Prasad S, Singhal M, Negi SS, et al. Targeted detection of 65 kDa heat shock protein gene in endometrial biopsies for reliable diagnosis of genital tuberculosis. *Eur J Obstet Gynecol Reprod Biol.* 2012;160(2):215–218. doi: 10.1016/j.ejogrb.2011.11.015. [PubMed] [CrossRef] [Google Scholar]

10. Guo S, Xue R, Li Y, et al. The CFP10/ESAT6 complex of *Mycobacterium tuberculosis* may function as a regulator of macrophage cell death at different stages of tuberculosis infection. *Med Hypotheses.* 2012;78(3):389–392. doi: 10.1016/j.mehy.2011.11.022. [PubMed] [CrossRef] [Google Scholar]

11. Forrellad MA, Klepp LI, Gioffré A, et al. Virulence factors of the *Mycobacterium tuberculosis* complex. *Virulence.* 2013;4(1):3–66. doi: 10.4161/viru.22329. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

12. Abdissa S, Abebe T, Ameni G, et al. Endometrial tuberculosis among patients undergoing endometrial biopsy at Tikur Anbesa specialized hospital, Addis Ababa, Ethiopia. *BMC Infect Dis.* 2018;18(1):304. doi: 10.1186/s12879-018-3202-x. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

13. Chaubey L, Kumar D, Prakash V, et al. Menstrual blood versus endometrial biopsy in detection of genital tuberculosis by using nested polymerase chain reaction in an endemic region. *J Hum Reprod Sci.* 2019;12(1):35–39. doi: 10.4103/jhrs.JHRS_149_17. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

14. Srivastava I, Bhatambare GS, Deshmukh AB, et al. Genital tuberculosis: evaluating microscopy, culture, histopathology and PCR for diagnosis “All Play Their Role” *Int J Curr Microbiol Appl Sci.* 2014;3(4):439–445. [Google Scholar]

15. Sharma JB, Sharma E, Sharma S, et al. Female genital tuberculosis: revisited. *Indian J Med Res.* 2018;148:S71–S83. [PMC free article] [PubMed] [Google Scholar]

16. Thangappah RB, Paramasivan CN, Narayanan S. Evaluating PCR, culture & histopathology in the diagnosis of female genital tuberculosis. *Indian J Med Res.* 2011;134:40–46. [PMC free article] [PubMed] [Google Scholar]

17. Jindal UN, Verma S, Bala Y. Favorable infertility outcomes following anti-tubercular treatment prescribed on the sole basis of a positive polymerase chain reaction test for endometrial tuberculosis. *Hum Reprod.* 2012;27(5):1368–1374. doi: 10.1093/humrep/des076. [PubMed] [CrossRef]

[Google Scholar]

18. Radhika AG, Bhaskaran S, Saran N, et al. Comparison of diagnostic accuracy of PCR and BACTEC with Lowenstein–Jensen culture and histopathology in the diagnosis of female genital tuberculosis in three subsets of gynaecological conditions. *J Obstet Gynaecol.* 2016;36(7):940–945. doi: 10.1080/01443615.2016.1174829. [PubMed] [CrossRef] [Google Scholar]

19. Carroll K, Hobden J, Miller S, et al. *Jawetz, Melnick, & Adelbergs medical microbiology.* 2. Shenzen: McGraw-Hill; 2016. [Google Scholar]

20. Sethi S, Dhaliwal L, Dey P, et al. Loop-mediated isothermal amplification assay for detection of *Mycobacterium tuberculosis* complex in infertile women. *Indian J Med Microbiol.* 2016;34:322–327. doi: 10.4103/0255-0857.188323. [PubMed] [CrossRef] [Google Scholar]

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Correlation of the Expression of BRAF V600E Mutation With Various Phenotypic Expressions of Thyroid Neoplasms

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Abstract

Aims

We aimed to assess the incidence of the BRAF V600E mutation in thyroid neoplasms at a tertiary care center and its association with various phenotypic features.

Methods and material

We included all cases diagnosed as thyroid neoplasm in the past decade at the Department of Pathology of our institute and obtained their clinical details from the medical records department of the institute after obtaining permission from the authorities and due International Human Epigenome Consortium clearance. We included data on age, sex, clinical presentation, hormone status, and T and N status of the malignant neoplasms. Hematoxylin and eosin (H&E) slides of all cases were evaluated for the type

of neoplasm, nuclear features, invasion into the capsule and vascular spaces, extrathyroidal extension, lymph node metastases, mitoses, necrosis, and presence/absence of amyloid. Paraffin blocks of sections with high tumor density and less normal tissue were chosen for evaluation after H&E staining. The slides showing tumors with large areas of hemorrhage, cystic change, or necrosis were excluded. Two primers were used to amplify a 339-bp fragment containing the V600E mutation in exon 15 of BRAF. Tissues were prepared from formalin-fixed paraffin-embedded (FFPE) blocks, and DNA was isolated using a standard protocol BRAF NF and BRAF NR Primer Standardized Protocol For FFPE Tissue DNA. Percentages and tables have been used for data presentation.

Results

Among 47 identified cases, 14 were positive for the BRAF V600E mutation and had papillary carcinoma (n = 9) or follicular neoplasms (n = 5; follicular adenoma, n = 3; follicular carcinoma, n = 2). In the BRAF-positive papillary carcinomas, five cases were aged 20-30 years, eight were female, eight (88.88%) were euthyroid, and one was hypothyroid. Furthermore, 55.55% (5/9 cases) of BRAF-positive cases were stage I, 33.3% (3/9 cases) were stage II, and 0.02% (1/9 cases) were stage III.

Conclusions

In our cohort, 31% of cases of papillary thyroid carcinoma (PTC) and 18.72% of follicular neoplasms expressed the BRAF V600E mutation.

BRAF V600E mutation-positive papillary thyroid carcinomas consistently showed all characteristic nuclear features, such as nuclear crowding, overlapping, and grooves.

Considering the greater prevalence in the younger age group, the importance of mutation surveillance in PTCs for a total thyroidectomy may be warranted in mutation-positive patients.

Keywords: braf v600e mutation, papillary carcinoma, thyroid gland, thyroid neoplasms, thyroid malignancies

Introduction

Thyroid cancers account for approximately 1% of all malignancies and are one of the most common malignancies of the endocrine system [1,2]. The development of malignancy in the thyroid is influenced by both environmental factors as well as genetic factors. Specifically, the thyroid is vulnerable to genotoxic effects of radioactive iodine and nongenotoxic effects (thyroid-stimulating hormone stimulation) of iodine deficiency both of which can lead to neoplastic lesions in the thyroid [3].

Most thyroid tumors can be diagnosed based on morphological assessment and immunohistochemical findings [4,5] As altered gene expression can be discovered before morphological changes, knowledge regarding the genetic background helps in understanding thyroid carcinogenesis and facilitates the planning of appropriate therapeutic strategies and development of drugs that target specific molecules.

Thyroid tumors are classified based on the 2004 World Health Organization guidelines [3]. Recently, the RAS-RAF-MAP kinase signaling pathway has been implicated in many cancers, particularly more frequently in thyroid carcinomas, with the BRAF V600E mutation playing a vital role in papillary thyroid carcinoma. Furthermore, the BRAF mutation is specific for papillary carcinoma, is associated with poorer outcomes, and is frequently noted in high-grade tumors [6-8]

BRAF: an overview

BRAF is a serine-threonine kinase belonging to the family of RAF proteins. It acts upstream of MEK1/2 kinases in response to RAS signals. BRAF mutations are involved in early thyroid carcinogenesis and are somatic genetic alterations rather than germline mutations [9,10]. BRAF mutations occur early and play an important role in the pathogenesis of papillary thyroid carcinoma (PTC). Point mutations of BRAF are the most common types of mutations and occur in approximately 40%-45% of cases [11,12]. Other studies have reported [13,14] BRAF mutation rates of 35%-69% in PTC. The most common BRAF mutation is V600E, which is from missense thymine (T) to adenine (A) transversion at nucleotide 1799 in exon 15. This results in the substitution of valine by glutamate at residue 600. A less common mutation is the K601E mutation found in thyroid cancer [15,16]. Structurally, the RAF protein is divided into two functional domains, namely, the N-terminal and C-terminal regulatory domains, with three conserved regions (CR 1,2, and 3). CR1 and CR2 are present in the N-terminal domain and CR3 in the C-terminal domain [17].

Normally, RAS proteins are attached to the plasma membrane on the cytoplasmic side of the cell, endoplasmic reticulum, and Golgi membrane and are activated by growth factors binding to receptors at the plasma membrane. In an inactive state, RAS proteins bind to guanosine diphosphate (GDP), and upon activation, exchange GDP with guanosine triphosphate (GTP), leading to a conformational change that produces active RAS [2]. This activated RAS binds to the RAS-binding domain in CR1 of RAF, recruits RAF to the membrane, and activates a downstream signaling cascade wherein RAF phosphorylates mitogen-activated protein kinase (MAPK) [17]. Extracellular signal-regulated kinases 1 and 2 (MEK 1 and 2) become activated, which consecutively phosphorylate and activate extracellular signal-regulated kinases 1 and 2 (ERK 1 and 2). Activated ERK migrates to the nucleus where it activates various transcription factors that trigger cell cycle progression and result in cell proliferation and differentiation [8]. The RAF protein has three isoforms, ARAF, BRAF, and CRAF. Among these, BRAF is the most common isoform and most commonly mutated isoform because it has higher basal kinase activity [17] Additionally, BRAF protein expression is higher in hematopoietic cells, neurons, and testicles; is the predominant isoform [18,19] in thyroid follicular cells; and is the most potent activator of the MAPK pathway. Gain-of-function BRAF mutations function as an alternate pathway for aberrant activation of ERK signaling, resulting in constitutive activation of BRAF kinase, i.e., it can phosphorylate MEK as monomers in a RAS-independent manner. Such chronic stimulation of the MAPK pathway results in increased proliferation but reduced survival and differentiation of cells [11]. This signaling cascade is implicated in the etiology of several human cancers including malignant melanoma; thyroid, colorectal, and ovarian carcinomas; and carcinomas of the biliary tract, ovary, and cervix [9,20]

BRAF expression in thyroid carcinomas

Thyroid malignancies are predominantly diagnosed based on morphological assessment alone, and an immunohistochemical study is beneficial in confirming the diagnosis and establishing prognosis if tumor(s) exhibits unusual patterns. A significant improvement has been noted in the knowledge of molecular alterations over the past two decades in all tumors, including thyroid malignancies, and oncogenic BRAF activation represents one of the most prevalent molecular alterations. Reportedly, BRAF mutations are associated with poorer clinicopathological outcomes [21,22]. Among its various subtypes, BRAF most commonly occurred in the tall cell variant of PTC, followed by conventional PTC, and less commonly in the follicular variant of PTC. The tall cell variant is the most aggressive tumor, indicating that BRAF mutations are associated with poorer outcomes [9,23]. Other factors associated with the mutation and aggressive tumor phenotype include older age, extrathyroidal tumor invasion, lymph node, and distant metastasis, higher tumor stage, and poorly differentiated cancer [21,22,24-26].

Frasca et al [27] found that the presence of the BRAF V600E mutation in PTC is associated with aggressive tumor behavior and that tumor aggressiveness is independent of tumor size, suggesting that even small BRAF-positive tumors carry a higher risk of progression and invasiveness than BRAF-negative tumors.

BRAF mutations can be readily tested on thyroid tissue samples obtained by fine-needle aspiration with high preoperative clinicopathological prognostic capabilities in PTC. However, they have limited diagnostic value because [28-30] of low sensitivity in cytologically indeterminate specimens that are mostly non-PTC and therefore do not harbor the BRAF mutation. Nonetheless, controversies regarding BRAF mutations with poorer clinicopathological outcomes in papillary thyroid cancers have been reported in some studies [31,32]. In the thyroid, apart from papillary carcinomas, BRAF mutations are expressed in anaplastic carcinoma and poorly differentiated carcinoma (prevalence, 20%-30%, and 10%-15%, respectively).

BRAF-mutated, poorly differentiated, anaplastic carcinoma will have a papillary component, suggesting that these tumors progress from BRAF-positive papillary carcinoma [33-36]. The BRAF V600E mutation is not found in follicular thyroid cancers and benign thyroid nodules [23]. However, the other BRAF mutation, K601E, has been detected in follicular adenoma, carcinoma, and follicular variants of PTC. However, Kebebew et al reported a BRAF V600E mutation in one case of follicular carcinoma [22]. Newly developed mitogen extracellular kinase (MEK) inhibitors are particularly promising therapeutic agents for thyroid cancer. With these advances, it has become clearer that BRAF mutation will likely have a significant impact on the clinical management of PTC. Thus, this study aimed to assess the incidence of the BRAF V600E mutation in thyroid neoplasms at a tertiary care center and its association with various phenotypic features.

Materials and methods

We included all cases diagnosed as thyroid neoplasm in the past decade at the Department of Pathology of our institute and obtained their clinical details from the medical records department of the institute after obtaining permission from the authorities and due International Human Epigenome Consortium clearance (IRB number/Proposal number: 10/098). We included data on age, sex, clinical presentation, hormone status, and T and N status of the malignant neoplasms. Hematoxylin and eosin (H&E) slides of all cases were evaluated for the type of neoplasm, nuclear features, invasion into the capsule and vascular spaces, extrathyroidal extension, lymph node metastases, mitoses, necrosis, and presence/absence of amyloid. Paraffin blocks of sections with high tumor density and less normal tissue were chosen for evaluation after H&E staining. The slides showing tumors with large areas of hemorrhage, cystic change, or necrosis were excluded. Two primers were used to amplify a 339-bp fragment containing the V600E mutation in exon 15 of BRAF. Tissues were prepared from formalin-fixed paraffin-embedded (FFPE) blocks, and DNA was isolated using a standard protocol BRAF NF & BRAF NR Primer Standardized Protocol For FFPE Tissue DNA (Figures 1-4). Percentages and tables have been used for data presentation.

Figure 1

Open in a separate window

Steps in BRAF mutation analysis in various thyroid neoplasms.

FFPE: formalin-fixed paraffin-embedded; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism

Figure 2

Open in a separate window

Agarose gel electrophoresis: DNA extraction.

Figure 3

Open in a separate window

Agarose gel electrophoresis: PCR amplification.

PCR: polymerase chain reaction

Figure 4

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Polyacrylamide gel electrophoresis: restriction digestion.

Results

Of the 50 identified cases, DNA could only be extracted from 47 because extraction proved futile in the remaining three cases (Table 1).

Table 1

Master chart showing demographic variables, morphologic subtype of thyroid neoplasms, stage, and mutant status.

PTC: papillary thyroid carcinoma; FN-LMP: follicular neoplasm - low malignant potential

S.No	Age/ Sex	Hormone Status	Size	Other features	Diagnosis	Stage	BRAF
1	48/F	Euthyroid	0.4cm	-	Papillary microcarcinoma	I	Mutant
2	65/F	Hypothyroid	1.3cm	-	Papillary carcinoma	I	Mutant
3	30/F	Euthyroid	0.3cm	-	Encapsulated follicular variant of Papillary carcinoma	I	Mutant
4	28/F	Euthyroid	0.3, 1.4cm	Multifocal, cystic	Papillary carcinoma	I	Mutant
5	32/F	Euthyroid	1cm	Cystic	Papillary carcinoma	I	Mutant
6	48/M	NA	4.2cm	-	Follicular variant of PTC	I	Wild
7	38/M	Euthyroid	0.5cm	-	Papillary microcarcinoma	I	Wild
8	50/F	Euthyroid	0.3-1.0cm	Multifocal	Papillary carcinoma	I	Wild
9	30/F	Euthyroid	0.4cm	-	Papillary microcarcinoma	I	Wild
10	53/M	Euthyroid	2.0cm	-	Papillary carcinoma	I	Wild
11	27/F	Hyperthyroid	0.5cm	-	Papillary microcarcinoma	I	Wild
12	32/F	Euthyroid	1.5cm	-	Papillary carcinoma	I	Wild
13	41/F	Euthyroid	0.3-0.8cm	Multifocal, stromal bone formation, FA	Papillary carcinoma	I	Wild
14	52/F	Euthyroid	0.4-2.3cm	Multifocal	Papillary carcinoma	II	Mutant
15	29/M	Euthyroid	2.5cm	-	Papillary carcinoma	II	Mutant
16	27/F	Euthyroid	2.4cm	-	Papillary carcinoma	II	Mutant
17	49/F	Euthyroid	3.5cm	Multifocal, cystic	Papillary carcinoma	II	Wild
18	51/F	Euthyroid	3cm	Cystic	Papillary carcinoma	II	Wild
19	70/M	Euthyroid	2.0-4.0cm	Multifocal	Papillary carcinoma	II	Wild
20	27/F	Euthyroid	3.0cm	-	Papillary carcinoma	II	Wild
21	45/F	Euthyroid	3.5cm	-	Papillary carcinoma	II	Wild
22	59/M	Euthyroid	1.0-3.0cm	Multifocal	Papillary carcinoma	II	Wild

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Only 14 cases were positive for the BRAF mutation; of these, nine were of papillary carcinoma (Figure 5).

Figure 5

Open in a separate window

Papillary thyroid carcinoma H&E stain (A x10, B x20, C x20, D x20).

A, B, C: Papillary thyroid carcinoma - Showing characteristic papillary architecture, characteristic nuclear features with ground class chromatin, nuclear grooving, nuclear crowding, nuclear overlapping, and intranuclear cytoplasmic pseudo-inclusions.

D: Papillary thyroid carcinoma (follicular variant) - Showing prominent follicular architecture with characteristic nuclear features with ground class chromatin, nuclear grooving, nuclear crowding, nuclear overlapping, and intranuclear cytoplasmic pseudo-inclusions.

Out of the 14 BRAF-positive cases, five were follicular neoplasms, with the latter category comprising three cases of follicular adenomas and two of follicular carcinomas (Tables 1, 2) (Figure 6).

Table 2

Break up of BRAF V600E-positive cases (n = 47).

Types of neoplasms	BRAF V600E positive - mutant type (%)	Wild type (%)	Total
Papillary carcinoma: (Conventional papillary thyroid carcinoma - 8 cases) (Encapsulated follicular variant of papillary thyroid carcinoma - 1 case)	9 (31%)	20 (69%)	29
Follicular neoplasm: (Follicular adenoma - 3 cases) (Follicular carcinoma - 2 cases)	5 (31.25%)	22 (68.75%)	16
Medullary carcinoma	0	1 (100%)	1
Anaplastic carcinoma	0	1 (100%)	1
Total	14 (29.8%)	33 (70.2%)	47

Figure 6

Open in a separate window

Follicular adenoma H&E stain (A x20, B x40).

A: Follicular adenoma showing marked circumscription and encapsulation.

B: Follicular adenoma showing a prominent repetitive microfollicular pattern.

The nine cases of BRAF-positive papillary carcinomas predominantly comprised individuals aged 20-30 years (Tables 1, 3), were almost all female (8/9) (Tables 1, 4), and were euthyroid (8/9, 88.88%) (Tables 1, 5).

Table 3

Number of BRAF-positive papillary thyroid carcinoma and age distribution.

Age group	Number of cases
20–30 years	5
31–40 years	1
41–50 years	1
51–60 years	1
61–70 years	1

Table 4

BRAF-positive carcinomas and sex distribution.

Male	Female
One case	Eight cases

Table 5

BRAF-positive papillary thyroid carcinoma and hormonal status.

Number of BRAF-positive cases	Hormonal status
8	Euthyroid
1	Hypothyroid

Next, eight of the nine cases were euthyroid (88.88%), whereas one was hypothyroid (Tables 1, 5). Additionally, 55.55% of BRAF V600E-positive cases were found to be stage I (5/9 cases), 33.33% to be stage II (3/9 cases), and 11.11% to be stage III (1/9 cases) (Tables 1, 6).

Table 6

BRAF-positive papillary thyroid carcinoma stage at presentation.

BRAF-positive papillary thyroid carcinoma staging	Number of cases
Stage I	5
Stage II	3
Stage III	1
Stage IV	0

Scoring for nuclear and other microscopic features revealed characteristic features compared with other features observed for BRAF-positive papillary carcinomas (Tables 7, 8). The scoring was done on a relative qualitative double consensus scale based on mild (Score 1), moderate (Score 2), and severe (Score 3) appearance of the nuclear features.

Table 7

Relative qualitative double consensus scoring for nuclear features of papillary thyroid carcinoma.

Histologic nuclear feature	Mild	Moderate	Severe
Nuclear enlargement (NE)	1	2	3
Nuclear irregularity (NI)	1	2	3
Chromatin clearing (CC)	1	2	3
Nuclear crowding (NC)	1	2	3
Nuclear grooves (NG)	1	2	3
Nuclear pseudoinclusions (PI)	1	2	3
Fibrosis (Fib)	1	2	3
Psammoma bodies (PB)	1	2	3

Table 8

Scoring for nuclear features and other microscopic features.

NE: nuclear enlargement; NI: nuclear irregularity; CC: chromatin clearing; NC: nuclear crowding; NG: nuclear grooves; PI: nuclear pseudoinclusion; Fib: fibrosis; PB: psammoma bodies; HPF: high-power fields

S. No.	Id. No.	NE	NI	CC	NC	NG	PI/10HPF	Fib	PB/10HPF	Inflammatory cell aggregate/10HPF
1	458/06	1	1	2	3	3	0	1	2	1
2	2780/06	2	1	3	3	2	0	0	0	0
3	2874/07	2	1	3	3	2	1	1	0	0
4	1837/08	2	2	1	3	2	2	1	0	0
5	1865/08	2	1	3	2	2	1	1	0	0
6	2222/08	2	1	3	2	1	0	2	2	0
7	229/09	2	0	1	3	2	0	0	0	0
8	1218/09	2	0	1	2	3	0	0	0	0
9	2548/09	3	3	1	1	2	2	3	3	3
Average score		2	1.1	2	2.4	2.1	0.6	1	0.7	0.4

Discussion

The BRAF V600E mutation is most often expressed in stage III (30%). Importantly, its expression, particularly in the early stages, indicates an aggressive course and warrants a total thyroidectomy, whereas its expression in the later stages is associated with poor outcomes.

In our group of 30 papillary carcinoma cases, DNA could be extracted from 29 and only nine expressed the BRAF V600E mutation. Its prevalence is lower than that reported in the literature, i.e., 40%-45% of PTCs harbor the mutation.

We found that five of the nine cases of BRAF V600E-positive papillary carcinoma were in the 20-30-year age group. Rossella et al found that V600E was expressed more in an older age group [6]. Given the greater prevalence in the younger age group, the importance of mutation surveillance in PTCs for a total thyroidectomy may be warranted in mutation-positive patients.

We found five cases of stage I (55.55%) and three cases of stage II (33.33%) showing BRAF positivity, indicating a significantly higher expression in stages I and II lesions. Therefore, we reanalyzed the reports of PTC.

This high incidence of V600E mutation can be explained by the fact that at a tertiary care hospital, the radiologist and cytopathologist could probably detect PTC at an early stage. Furthermore, the strongest argument against using BRAF mutation as an independent prognostic and predictive factor in PTC is its high prevalence (30%-80%). As the V600E mutation is associated with poor outcomes, these patients require very close follow-up. We found no correlation between nuclear features and BRAF V600E mutations; however, the characteristic nuclear crowding, overlapping, and grooves were consistently observed (Tables 6, 7).

The incidence of the BRAF V600E mutation in follicular neoplasms was 31% (five out of 16 cases). In contrast, the literature shows that the V600E mutation is rarely seen in follicular neoplasms, with very few reports that have identified this mutation in follicular neoplasms with minimal incidence [37]. Therefore, a reanalysis of routine H&E slides identified two cases of focal papillary architecture with nuclear features, which might have been overlooked during the morphological assessment. Despite their exclusion, the percentage incidence of the V600E mutation in follicular neoplasm was 18.72% (three out of 16 cases). Nonetheless, papillary carcinoma, particularly the microfollicular type, was possibly missed on routine evaluation as specimens were not extensively sampled, rather were only drawn from the most representative areas of the lesion.

Overall, we show that a significant number of cases of papillary carcinoma (31%; 9/29) and follicular neoplasms (18.7%; 3/16) express the V600E mutation. A larger prospective study, including preoperative cytology specimen and surgically resected specimens of the same patients, is needed to establish the utility of the V600E mutation as a routine diagnostic tool. After development, the primers would be cost-effective to patients.

An increase in the incidence of papillary carcinoma in areas where there is high iodine intake has been reported [3,38,39] and there is high iodine supplementation to correct iodine deficiency, particularly in the population with an existing V600E mutation. This raises the question of establishing dietary iodine supplementation levels to prevent the incidence of non-neoplastic conditions including multinodular goiter. Furthermore, papillary carcinoma usually remains intra-thyroid and few cases may present with rapid onset of clinical manifestations with enlargement of multiple cervical lymph nodes [40]. Therefore, a larger, multicenter, prospective study that includes cytology specimens collected preoperatively and following surgical resection is essential to establish the relationship between the incidence of papillary carcinoma and V600E mutation in the background of iodine supplementation and deficiency and to prove the utility of BRAF in routine diagnosis. However, the most recent

American Thyroid Association (ATA) recommendations do not indicate a routine application of BRAF status for initial risk stratification in differentiated thyroid cancer due to a lack of evident confirmation of a direct influence of mutation on the increase in relapse risk.

Further, considering the increase in the prevalence of the BRAF V600E mutation in follicular neoplasms, a larger study of the V600E mutation in follicular neoplasms is vital to observe changes in the expression patterns of mutations in the Indian population.

Conclusions

In our cohort, 31% of cases of PTC and 18.72% of follicular neoplasms expressed the BRAF V600E mutation.

BRAF V600E mutation-positive PTCs consistently showed all characteristic nuclear features, such as nuclear crowding, overlapping, and grooves.

Considering the greater prevalence in the younger age group, the importance of mutation surveillance in PTCs for a total thyroidectomy may be warranted in mutation-positive patients.

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Notes

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The authors have declared that no competing interests exist.

Human Ethics

Consent was obtained or waived by all participants in this study. Institutional human ethics committee, PSG institute of medical sciences and research issued approval 10/098. Proposal number / IRB number : 10/098 Project title : A Study of Correlation of the Expression of BRAF V600E Mutation with Various Phenotypic Expressions of the Thyroid Neoplasms. Name of the investigators: Dr H Volga, Dr Subramaniam Ramkumar M.D Name of the guide: Dr Shanthakumari Name of the co-guide: Dr S Ramalingam, Dr Sudha Ramalingam, Dr S Thiagarjan Waiver of consent: Yes Review type: Exempt Date of the meeting: N/A Decision: Approved Approval date: 24.03.2011 Continuing panel review: Not needed

Animal Ethics

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

References

1. Carcangiu ML. *Histology for Pathologists, Third Edition.* Philadelphia: Lippincott Williams & Wilkins; 2006. Thyroid gland. [Google Scholar]

2. DeLellis R, Lloyd RV, Heitz PU, Eng C. *IARC Press.* Lyon: IARC Press; 2004. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Endocrine Organs. [Google Scholar]

3. Abul K, Abbas AM. *Robbins and Cotran Pathological Basis of Disease.* New York: Saunders; 2004. The endocrine system; pp. 1155–1183. [Google Scholar]

4. Immunohistochemical diagnosis of papillary thyroid carcinoma. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL. *Mod Pathol.* 2001;14:338–342. [PubMed] [Google Scholar]

5. CD56, CD57, HBME1, CK19, Galectin-3 and p63 immunohistochemical stains in differentiating diagnosis of thyroid benign/malign lesions and NIFTP. Tastekin E, Keskin E, Can N, et al. *Pol J Pathol.* 2019;70:286–294. [PubMed] [Google Scholar]

6. BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. Elisei R, Ugolini C, Viola D, et al. *J Clin Endocrinol Metab.* 2008;93:3943–3949. [PubMed] [Google Scholar]

7. BRAF and KRAS mutations in papillary thyroid carcinoma in the United Arab Emirates. Al-Salam S, Sharma C, Afandi B, Al Dahmani K, Al-Zahrani AS, Al Shamsi A, Al Kaabi J. *PLoS One.* 2020;15:0. [Google Scholar]

8. Papillary thyroid carcinoma: an overview. Al-Brahim N, Asa SL. *Arch Pathol Lab Med.* 2006;130:1057–1062. [PubMed] [Google Scholar]

9. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Xing M. *Endocr Rev.* 2007;28:742–762. [PubMed] [Google Scholar]

10. The genomic landscape of thyroid cancer tumourigenesis and implications for immunotherapy. Singh A, Ham J, Po JW, Niles N, Roberts T, Lee CS. *Cells.* 2021;10:1082. [PMC free article] [PubMed] [Google Scholar]

11. Molecular diagnostics of thyroid tumors. Nikiforov YE. *Arch Pathol Lab Med.* 2011;135:569–577. [PubMed] [Google Scholar]

12. Effect of BRAF V600E mutation detection of fine-needle aspiration biopsy on diagnosis and treatment guidance of papillary thyroid carcinoma. Qi W, Shi C, Zhang P, Feng L, Wang J, Chen D. *Pathol Res Pract.* 2020;216:153037. [PubMed] [Google Scholar]

13. Molecular markers in thyroid cancer diagnostics. Kato MA, Fahey TJ 3rd. *Surg Clin North Am.* 2009;89:1139–1155. [PubMed] [Google Scholar]

14. Molecular testing in the diagnosis of differentiated thyroid carcinomas. Ferrari SM, Fallahi P, Ruffilli I, et al. *Gland Surg.* 2018;7:0–29. [PMC free article] [PubMed] [Google Scholar]

15. Genetic considerations in thyroid cancer. Patel KN, Singh B. *Cancer Control.* 2006;13:111–118. [PubMed] [Google Scholar]

16. Genomic hallmarks of thyroid neoplasia. Giordano TJ. *Annu Rev Pathol.* 2018;13:141–162. [PubMed] [Google Scholar]

17. KRAS and BRAF: drug targets and predictive biomarkers. Vakiani E, Solit DB. *J Pathol.* 2011;223:219–229. [PubMed] [Google Scholar]

18. Genetic basis and gene therapy trials for thyroid cancer. Al-Humadi H, Zarros A, Al-Saigh R, Liapi C. https://pubmed.ncbi.nlm.nih.gov/20181629/ *Cancer Genomics Proteomics.* 2010;7:31–49. [PubMed] [Google Scholar]

19. Combinatorial therapies in thyroid cancer: an overview of preclinical and clinical progresses. Laetitia G, Sven S, Fabrice J. *Cells.* 2020;9:830. [PMC free article] [PubMed] [Google Scholar]

20. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Wan PTC, Garnett MJ, Roe SM, et al. https://pubmed.ncbi.nlm.nih.gov/15035987/ *Cell.* 2004;116:855–867. [PubMed] [Google Scholar]

21. The BRAFV600E mutation analysis and risk stratification in papillary thyroid carcinoma. Scheffel RS, de Cristo AP, Romitti M, et al. https://pubmed.ncbi.nlm.nih.gov/34033285/ *Arch Endocrinol Metab.* 2021;64:751–757. [PubMed] [Google Scholar]

22. The prevalence and prognostic value of BRAF mutation in thyroid cancer. Kebebew E, Weng J, Bauer J, et al. *Ann Surg.* 2007;246:466–470. [PMC free article] [PubMed] [Google Scholar]

23. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. Kondo T, Ezzat S, Asa SL. *Nat Rev Cancer.* 2006;6:292–306. [PubMed] [Google Scholar]

24. Clinical significance of BRAF (V600E) mutation and Ki-67 labeling index in papillary thyroid carcinomas. Nakayama H, Yoshida A, Nakamura Y, et al. https://pubmed.ncbi.nlm.nih.gov/17972530/ *Anticancer Res.* 2007;27:3645–3649. [PubMed] [Google Scholar]

25. BRAF V600E and lymph node metastases in papillary thyroid cancer. Chen P, Pan L, Huang W, et al. *Endocr Connect.* 2020;9:999–1008. [PMC free article] [PubMed] [Google Scholar]

26. Analysis of the association of BRAFV600E mutation and Ki-67 overexpression with clinical and pathological characteristics in papillary thyroid cancer. Espenbetova M, Krykpayeva A, Zamanbekova Z, Zhumanbaeva Z, Azizov B, Mukanova A, Glushkova N. *Radiat Environ Biophys.* 2021;60:233–241. [PubMed] [Google Scholar]

27. BRAF(V600E) mutation and the biology of papillary thyroid cancer. Frasca F, Nucera C, Pellegriti G, et al. *Endocr Relat Cancer.* 2008;15:191–205. [PubMed] [Google Scholar]

28. Utility of BRAF V600E mutation detection in cytologically indeterminate thyroid nodules. Rowe LR, Bentz BG, Bentz JS. https://pubmed.ncbi.nlm.nih.gov/16606457/ *Cytojournal.* 2006;3:10. [PMC free article] [PubMed] [Google Scholar]

29. BRAFV600E mutation analysis in fine-needle aspiration cytology specimens for diagnosis of thyroid nodules: the influence of false-positive and false-negative results. Zhao CK, Zheng JY, Sun LP, Xu RY, Wei Q, Xu HX. *Cancer Med.* 2019;8:5577–5589. [PMC free article] [PubMed] [Google Scholar]

30. False-negative BRAF V600E mutation results on fine-needle aspiration cytology of papillary thyroid carcinoma. Paek SH, Kim BS, Kang KH, Kim HS. *World J Surg Oncol.* 2017;15:202. [PMC free article] [PubMed] [Google Scholar]

31. Detection of BRAF mutation in thyroid papillary carcinomas by mutant allele-specific PCR amplification (MASA) Sapio MR, Posca D, Troncone G, et al. *Eur J Endocrinol.* 2006;154:341–348. [PubMed] [Google Scholar]

32. BRAF mutation associated with other genetic events identifies a subset of aggressive papillary thyroid carcinoma. Costa AM, Herrero A, Fresno MF, Heymann J, Alvarez JA, Cameselle-Teijeiro J, García-Rostán G. *Clin Endocrinol (Oxf)* 2008;68:618–634. [PubMed] [Google Scholar]

33. Pathology and genetics of thyroid carcinoma. DeLellis RA. *J Surg Oncol.* 2006;94:662–669. [PubMed] [Google Scholar]

34. Molecular pathology of poorly differentiated and anaplastic thyroid cancer: what do pathologists need to know? Volante M, Lam AK, Papotti M, Tallini G. *Endocr Pathol.* 2021;32:63–76. [PMC free article] [PubMed] [Google Scholar]

35. Progression of papillary thyroid carcinoma to anaplastic carcinoma in metastatic lymph nodes: solid/insular growth and hobnail cell change in lymph nodes are predictors of subsequent anaplastic transformation. Odate T, Oishi N, Kawai M, et al. *Endocr Pathol.* 2021 [PMC free article] [PubMed] [Google Scholar]

36. Evidence that one subset of anaplastic thyroid carcinomas are derived from papillary carcinomas due to BRAF and p53 mutations. Quiros RM, Ding HG, Gattuso P, Prinz RA, Xu X. *Cancer.* 2005;103:2261–2268. [PubMed] [Google Scholar]

37. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Soares P, Trovisco V, Rocha AS, et al. *Oncogene.* 2003;22:4578–4580. [PubMed] [Google Scholar]

38. Thyroid cancer and thyroiditis in Salta, Argentina: a 40-yr study in relation to iodine prophylaxis. Harach HR, Escalante DA, Day ES. *Endocr Pathol.* 2002;13:175–181. [PubMed] [Google Scholar]

39. Thyroid cancer, thyroiditis and dietary iodine: a review based on the Salta, Argentina model. Harach HR, Ceballos GA. *Endocr Pathol.* 2008;19:209–220. [PubMed] [Google Scholar]

40. Papillary carcinoma thyroid presented with extensive local lymph nodal metastasis. Jagtap SV, Patil D, Chetan Chetan, Gupta SO. https://www.achr.co.in/article-details/6887 *P Arch Cytol Histopathol Res.* 2018;3:113–115. [Google Scholar]

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Depression among elderly diabetics at Peripheral health centres attached to a private medical college in Coimbatore: a cross sectional study

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ABSTRACT

Introduction; Depression is an illness that may affect and be affected by diabetes mellitus. The clinical guidelines for older adults with diabetes mellitus have recognized this by recommending screening for and management of depression in diabetic elderly people. The aim of this study was to find out the prevalence of depression using PHQ -9 questionnaire among elderly diabetics in peripheral primary health care centres attached to a Private medical college. **Objectives:** 1. To find out the prevalence of depression among elderly diabetics 2. To find out the factors associated with depression among elderly diabetics **Methodology:** This cross sectional study was conducted among known diabetics above 60 years of age attending the NCD clinic of Peripheral health centres at Peelamedu, Vedapatti, Neelambur during December 2017 – April 2018. The socio demographic factors and morbidity factors such as fasting blood sugar, duration of diabetes, history of hypertension, past history of diabetes related complications, hours of sleep were included to find out the association with levels of depression. **Results:** Among the study population, 40.4 % were having the signs of depression in which the majority have only minimal symptoms of depression. The factors such as Female Gender, Fasting blood sugar >126 mg/dl and hours of sleep < 7 hours found to be statistically significant association with depression. **Conclusion:** The prevalence of depression using PHQ 9 is 40.4% and the factors such as female gender, fasting blood sugar more than 127 mg/dl and sleep at night less than 7 hours were found to have statistically significant association with depression.

Key word: Depression, PHQ-9, Screening, Prevalence, Elderly diabetics.

INTRODUCTION

Depression is an illness that may affect and be affected by diabetes mellitus. Depression is an independent risk factor for the onset of type 2 diabetes mellitus. It negatively affects the course of diabetes and is associated with increased risk of complications, hyperglycemia, and mortality. Depression may exert its negative effect through hormonal, neuronal, or immune system changes that directly affect the body's ability to produce or use insulin or, the effect of depression may be indirect, by resulting in poor self-care behaviours, such as overeating, drinking alcohol, not exercising, skipping medications, or failing to keep medical appointments. Thus, identifying and treating depression in diabetes is strongly recommended.[1]

Evidence suggests that depression may relate to poorer glycaemic control[2] and that poorer control predicts a poorer course of depression in adult samples.[3]There is some evidence that depression does not prospectively predict worsening of blood glucose control in the elderly, but more research needs to be done to understand this relationship in this group.

In interactions with the health care system, the presence of depression may influence decisions made by health care providers concerning medical outcomes (e.g., blood glucose and blood pressure). For example, providers may set goals that are easier to achieve, yet are less stringent, when they believe that depression will interfere with a patient's ability to adhere to a stricter regimen. Depression can also have an effect on patients' own goals and preferences for treatment, because depressed patients may feel too hopeless or helpless to embark on the lifestyle changes that are most beneficial for diabetes self-care.

Thus, in many different ways, diabetes may become more difficult to manage for elderly people who are also depressed, and depression may become more difficult to resolve in those who have diabetes, resulting in higher health care utilization and medical costs for those with this comorbidity.[4] The clinical guidelines for older adults with diabetes mellitus have recognized this by recommending screening for and management of depression in diabetic elderly people.

Tools such as the short form of the Geriatric Depression Scale, the Patient Health Questionnaire-9, and the newly

developed Depression and Suicide Screen are useful. [5] The present study used PHQ – 9 questionnaire to arrive at a provisional diagnosis of depression as patients with minimal symptoms (score 5-9), minor depression (score 10-14), moderate Major depression (score 15-19), severe major depression (score >20).

The objectives of this study were to find out the prevalence of depression using PHQ -9 questionnaire among elderly diabetics in peripheral primary health care centres attached to a Private medical college and to find out the factors associated with depression.

METHODOLOGY

The Department of community medicine of PSG Institute of Medical sciences and Research has one Urban (Peelamedu) and Two Rural health training centres (Vedapati and Neelambur) to deliver primary health care to people. These centres have basic laboratory services to manage Non communicable diseases (NCD). All these three centres run NCD clinic on a particular day of the week and maintain NCD register. This cross sectional study was conducted among known diabetics above 60 years of age attending the NCD clinic of Peripheral health centres at Peelamedu, Vedapatti, Neelambur during December 2017 – April 2018. By purposive sampling, 104 elderly diabetics who came for review during the study period at NCD clinic were selected. All registered diabetics above 60 years of age attending the NCD clinic were included and those who were on treatment for pre-existing major and minor mental illness were excluded.

After obtaining approval from Institutional Human Ethics Committee, informed consent obtained from the patients at NCD clinics of peripheral health centres. Study Questionnaire with PHQ-9 administered to the patients till the sample size of 100 reached. Questionnaire contains the following items i.e. Socio demographic variables, fasting blood sugar, presence of hypertension, complications related to diabetes and hours of sleep at past night to find out the association of above factors on depression among the study population.

Those patients found to be having depression will be followed up based on degree of depression at the peripheral health centres. Proportions, test of statistical significance (Chi square) and odds ratio with 95% confidence interval (CI) using SPSS version 24 were calculated.

RESULTS

Table 1: Distribution of study population at Peripheral Health Centres

Peripheral Health centres	Study population	Percent (%)
UHTC (Peelamedu)	46	44.2
VHTC (Vedapatti)	29	27.9
NHTC (Neelambur)	29	27.9
Total	104	100

The present study intended to find out the prevalence of depression among elderly diabetics and the factors associated with depression. There were 104 elderly

Table2: Prevalence of depression among the study population

Depression	Study population	Percent (%)
NO DEPRESSION	62	59.6
DEPRESSION	42	40.4
Total	104	100

Table 3: PHQ 9 Grading of Depression among the study population

PHQ 9 Grading	Study population	Percent (%)
NO DEPRESSION	62	59.6
MINIMAL SYMPTOMS	25	24
MINOR DEPRESSION	9	8.7
MODERATE MAJOR DEPRESSION	5	4.8
SEVERE MAJAOR DEPRESSION	3	2.9
Total	104	100

Table 4: Socio Demographic and co-morbidity variables of the study population

Variables	Category	Study population (n=104)	Percent (%)
Age in years	60-69	73	70.2
	70-79	27	26
	>80	4	3.8
Gender	Male	47	45.2
	Female	57	54.8
Educational status	Illiterate	32	30.8
	Primary	39	37.5
	Secondary	26	25
	Intermediate	4	3.8
	College	3	2.9
Marital status	Married	76	73.1
	Divorced	1	1
	Widow	27	26
Socio-economic status (modified Prasad scale August 2017)	CLASS I Rs>5910	22	21.2
	CLASS II Rs 2960 - 5909	34	32.7
	CLASS III Rs 1770-2959	16	15.4
	CLASS IV Rs 890-1769	22	21.2
	CLASS V Rs <890	10	9.6
Duration of Diabetes	<10 YEARS	81	77.9
	>11 YEARS	23	22.1
Fasting blood sugar level	FBS <126 mg/dl	31	29.8
	FBS >127 mg/dl	73	70.2
Hours of sleep	<7 hours	83	79.8
	>8 hours	21	20.2
Hypertension	Yes	53	51
	No	51	49
Complications	Yes	47	45.2
	No	57	54.8

Table 5: Factors associated with depression in the study population by univariate analysis

Study variables	Category	Depression	No depression	Unadjusted Odds Ratio 95% CI	P Value
Age	60-70 years	32(39.5%)	49(60.5%)	1.178	
	>71 years	10(43.5%)	13(56.5%)	(0.46-3.00)	0.732
Gender	Male	12(25.5%)	35(74.5%)	3.241	
	Female	30(52.6%)	27(47.4%)	(1.40-7.48)	**0.005***
Educational status	Up to Primary	30(42.3%)	41(57.7%)	0.781	
	Secondary and above	12(36.4%)	21(63.6%)	(0.33-1.83)	0.569
Marital status	Married	31(40.8%)	45(59.2%)	0.939	
	Divorced/widow	11(39.3%)	17(60.7%)	(0.38-2.27)	0.89
Socioeconomic status	Class I,II & III	27(37.5%)	45(62.5%)	1.471	
	Class IV& V	15(46.9%)	17(53.1%)	(0.63-3.41)	0.368
Duration of Diabetes	<10 YEARS	33(40.7%)	48(59.3%)	0.935	
	>11 YEARS	9(39.1%)	14(60.9%)	(0.36-2.41)	0.544
Fasting blood sugar level	FBS ≤126 mg/dl	8(25.8%)	23(74.2%)	2.506	
	FBS ≥127 mg/dl	34(46.6%)	39(53.4%)	(0.99-6.33)	**0.038***
Hours of sleep	<7 hours	38(45.8%)	45(54.2%)	0.279	
	>8 hours	4(19.0%)	17(81.0%)	(0.08-0.89)	**0.028***
Hypertension	Yes	18(34.0%)	35(66.0%)	0.579	
	No	24(47.1%)	27(52.9%)	(0.26-1.27)	0.174
Complications	Yes	16(34.0%)	31(66.0%)	0.615	
	No	26(45.6%)	31(54.4%)	(0.27-1.36)	0.231

* Statistically significant (p< 0.05)

diabetics (Table. 1) included in the study. Among the study population, 40.4 % were having the signs of depression (Table.2), of which 24% have only minimal symptoms of depression (Table.3).

The socio demographic factors i.e. age, gender, education, income per capita per month and morbidity factors such as fasting blood sugar, duration of diabetes, history of hypertension, past history of diabetes related complications, hours of sleep and PHQ -9 score are shown in Table.4.

Each of the above variables were categorized into two to find out the statistically significant (p< 0.05) association with depression among the study population using chi square test (Table. 5) in SPSS version 24. The factors such as Female Gender, fasting blood sugar >126 mg/dl and hours of sleep < 7 hours found to be statistically significant association with depression in the present study.

DISCUSSION

In the present study the prevalence of depression among elderly with diabetes mellitus was found to be 40.4%. The study conducted by SA Black et al showed that 31.1% of the older diabetic individuals reported high levels of depressive symptoms. [6] Prevalent major depressive episodes were present in 15.4% of the subjects with diabetes, which was significantly higher than in nondiabetic subjects in a study conducted among community-dwelling elderly persons with diabetes mellitus by P. de Jonge et. al. [7] In a study conducted by Manjiri D Pawaskar et al. among Medicare enrolled elderly population with type 2 diabetes mellitus, 17% had depressive symptoms. [8] In a study conducted by Kee-Lee Chou et al, on Prevalence of depression among elderly Chinese with diabetes, 26% of elderly diabetics reported elevated levels of depressive symptoms. [9] Elderly female diabetics are more among depressed (OR 3.2, 95%CI: 1.40-7.48) than men in this study which is statistically significant. Finkelstein EA et al reported diagnosed annual prevalence rates of major depression more among elderly female diabetics. In our study there is no significant association between the subjects with duration of diabetes less than 10 years and more than 10 years whereas in a study done by Yu-Ling Bai et al. a significant difference was found for depression scores between subjects with diabetes for <1 year and those with diabetes for >10 years.[10] Elderly with high Fasting blood sugar >127 mg/dl have higher risk of depression (OR 2.5, 95%CI: 0.99-6.33) than those with Fasting blood sugar <126 mg/dl in the present study. PJ Lustman et al reported in the meta analytic review of 24 studies that Depression is associated with hyperglycemia in patients with type 1 or type 2 diabetes[3]. However, association of depression with hyperglycemia in elderly diabetics is not documented. Elderly diabetics who slept more than 8 hours at night had lesser risk of depression (OR 0.279, 95%CI: 0.08-0.89) than those who slept less than 7 hours at night in this study. The factors such as age, education,

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income, marital status, past history of diabetes related complications and hypertension were not found to be significant in this study. This study has few limitations like very small sample size, FBS is taken as an indicator for glycemic control instead of Hb A1C and PHQ 9 administered by different doctors at three health centres.

Conclusion:

This cross sectional study among elderly diabetics in primary health centre setting of a private medical college revealed that prevalence of depression using PHQ 9 is 40.4% and the factors such as female gender, fasting blood sugar more than 127 mg/dl and sleep at night less than 7 hours were found to have statistically significant association with depression.

REFERENCES

1. Trief PM. Depression in elderly diabetes patients. Diabetes spectrum. 2007 Apr 1;20(2):71-5
2. Finkelstein EA, Bray JW, Chen H, Larson MJ, Miller K, Tompkins C, Keme A, Manderscheid R: Prevalence and costs of major depression among elderly claimants with diabetes. Diabetes Care 2003Feb 1:26:415–420
3. Lustman PJ, Anderson RJ, Freedland KE, DeGroot MK, Carney RM, Clouse RE: Depression and poor glycemic control: a meta-analytic review of the literature. Diabetes Care 2000 23:934 –942,
4. Finkelstein EA, Bray JW, Chen H, Larson MJ, Miller K, Tompkins C, Keme A, Manderscheid R: Prevalence and costs of major depression among elderly claimants with diabetes. Diabetes Care 2003Feb 1:26:415–420,
5. Hoyl MT, Alessi CA, Harker JO, Josephson KR, Piertruszka FM, Koelfgen M, Mervis JR, Fitten LJ, Rubenstein LZ: Development and testing of a five-item version of the Geriatric Depression Scale. J Am Geriatr Soc 1999 47 :873–878,
6. S.A.Black, Increased health burden associated with comorbid depression in older diabetic Mexican Americans. Results from the Hispanic Established Population for the Epidemiologic Study of the Elderly survey; Diabetes Care 1999 Jan;22(1):56-64.
7. De Jonge P, Roy JF, Saz P, Marcos G, Lobo A. Prevalent and incident depression in community-dwelling elderly persons with diabetes mellitus: results from the ZARADEMP project. Diabetologia. 2006 Nov 1;49(11):2627-33
8. Pawaskar MD, Anderson RT, Balkrishnan R. Self-reported predictors of depressive symptomatology in an elderly population with type 2 diabetes mellitus: a prospective cohort study. Health and quality of life outcomes. 2007 Dec;5(1):50
9. Chou KL, Chi I. Prevalence of depression among elderly Chinese with diabetes. International Journal of Geriatric Psychiatry. 2005 Jun;20(6):570-5
10. Bai YL, Chiou CP, Chang YY, Lam HC. Correlates of depression in type 2 diabetic elderly patients: A correlational study. International journal of nursing studies. 2008 Apr 1;45(4):571-9.

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Brief Communication

Needs Assessment of Ophthalmology Education for Undergraduate Medical Students – A Study from a Medical College in South India

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ABSTRACT

Background: Adequate ophthalmic diagnostic and clinical skills are essential for practitioners in primary care settings as well as specialty care physicians. The objectives of this study were to assess the adequacy of ophthalmology teaching in undergraduate medical education and to evaluate the comfort of medical students in diagnosing common eye problems and performing ophthalmic skills. **Methods:** A questionnaire based, cross-sectional survey was conducted among third-year undergraduate students from a medical college in South India at the end of ophthalmology training from February 2014 to December 2014. The main outcome measures were hours of classroom-based instruction and clinical exposure to ophthalmology received by the students and their comfort level in diagnosing common eye problems and performing ophthalmic skills. **Results:** 134 students participated in the study. They had received an average of 96.2 ± 5.9 and 112.5 ± 11.3 hours of classroom and clinic-based instruction, respectively. The participants' comfort in diagnosing eye problems was satisfactory for cataract and eyelid disorders but not for ophthalmic emergencies. Only 45.5% had satisfactory knowledge in community ophthalmology. Respondents were more proficient in visual acuity testing (93.3%) and assessment of pupillary reaction (80.6%) than direct ophthalmoscopy (41%). **Discussion:** Undergraduate medical students from India received a greater amount of ophthalmology instruction compared to the International Council of Ophthalmology task force recommendations. Gaps in community ophthalmology and knowledge-skills discrepancies were noted. Review of curriculum, appropriate training resources, and effective teaching methods tailored towards primary care may be useful to improve the training.

Keywords: Comfort, knowledge, ophthalmology, undergraduate medical education

Background

Ophthalmology makes up a significant proportion of primary care consultations.[1] A basic knowledge of ophthalmology is essential in the management of several neurologic, endocrine, rheumatic, and infectious conditions. Despite the relevance of ophthalmology to medical practice, little time is allocated to teaching of ophthalmology in undergraduate medical education and the majority of primary care physicians do not feel that they have received adequate undergraduate ophthalmic education.[2] Also several studies have noted that medical students receive too little ophthalmic training.[3-5]

In India, the five and one-half year undergraduate medical training consists of four and one-half years of teaching of subjects comprising the medical curriculum and one year of internship. Ophthalmology is being taught in the third year after preclinical and paraclinical subjects.

The state of undergraduate ophthalmology training from medical colleges in India has not been studied so far. Evaluation

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of ophthalmic knowledge and skills of undergraduates at the end of ophthalmology training would help in generating critical feedback on undergraduate ophthalmic curriculum planning. The objectives of this study were:

1. To assess the adequacy of ophthalmology teaching in undergraduate medical education
2. To assess the comfort level of medical students in diagnosing and treating common eye problems and performing ophthalmic skills.

Methods

After approval from the institutional review board, undergraduate medical students from a medical college in South India were included in the study at the end of their ophthalmology training (February 2014 to December 2014). The adequacy of ophthalmology education was quantified by determining the hours of classroom-based instruction and clinical exposure to ophthalmology received by the students and comparing these with the recommendations of the Medical Council of India (MCI) as well as the International Council of Ophthalmology (ICO).

A validated questionnaire [Appendix 1] was given to the students and their responses collected anonymously. The questionnaire consisted of three parts. Parts 1 and 3 assessed the students' comfort with respect to diagnosing specific ophthalmology conditions and performing ophthalmology-related skills on a Likert scale from 1 to 5 (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, and 5 = very comfortable). In terms of comfort level, we examined the ease with which study participants reported they were able to diagnose common eye problems and perform ophthalmic skills.

The second part assessed knowledge on certain topics on a Likert scale, also from 1 to 5 (1 = very poor, 2 = poor, 3 = average, 4 = good, and 5 = very good). The conditions and skills covered in the questionnaire were based on the topics covered in the undergraduate ophthalmology curriculum recommended by the MCI.[6]

Participants were also asked about the number of hours spent in self-directed learning and how comfortable they felt in managing ophthalmology-associated issues overall.

Likert scale-based responses were subsequently dichotomized and reduced to proportions by categorizing scores 1–2 as unsatisfactory or inadequate and 3–5 as satisfactory or adequate. Data were entered using the Statistical Package for the Social Sciences (version 19 (SPSS, Inc., Chicago, IL, USA) and examined using descriptive and analytical statistics to yield frequencies, percentages, and proportions.

Results

Of the 150 undergraduate medical students, 134 responded to the questionnaire (response rate = 89.6%).

The main modes of ophthalmology exposure were didactic lectures (35 hours), tutorials (37.5 ± 3.8 hours), seminars (23.7 ± 2.2 hours), and clinic-based learning. Our study participants had received an average of 96.2 ± 5.9 hours of classroom-based instruction. They also had received 112.5 ± 11.3 hours of clinic-based instruction in the eight weeks of clinical posting. 121 participants (90.3%) had spent up to 10 hours in self-directed learning while 13 participants (9.7%) had spent more than 10 hours in self-directed learning.

The results of the participants' comfort in diagnosing ophthalmic conditions are presented in Figure 1. Figure 2 shows the students' adequacy of knowledge in community and systemic ophthalmology as well as indications for ophthalmic referral. Figure 3 shows the study participants' comfort in performing MCI recommended clinical skills. Tables 1-3 show the numerical data for the corresponding figures. Finally, 101 participants (75.4%) felt comfortable in managing ophthalmology associated issues overall.

Discussion

This cross-sectional observational study attempted to quantify the adequacy of undergraduate ophthalmic education and students' comfort with regard to the management of ocular conditions.

In comparison with MCI recommendations, our study participants received a satisfactory number of hours of both classroom and clinic-based instruction. The MCI recommends clinical training of 8 weeks and a total of 100 teaching hours in ophthalmology, which includes 30 h of didactic lectures,

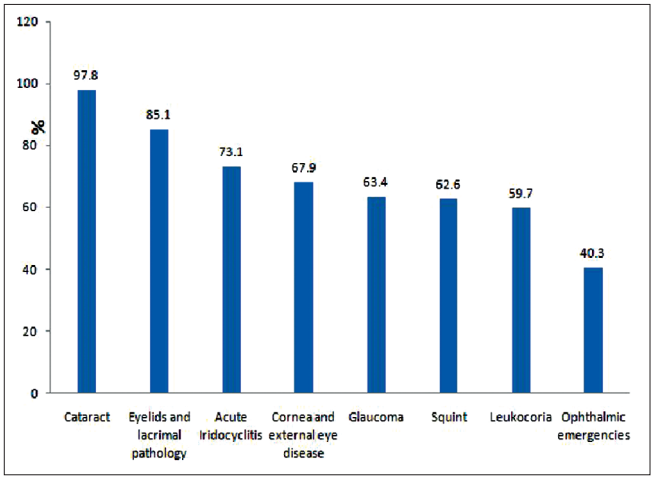


Figure 1: Medical students' comfort in diagnosing common ophthalmic conditions

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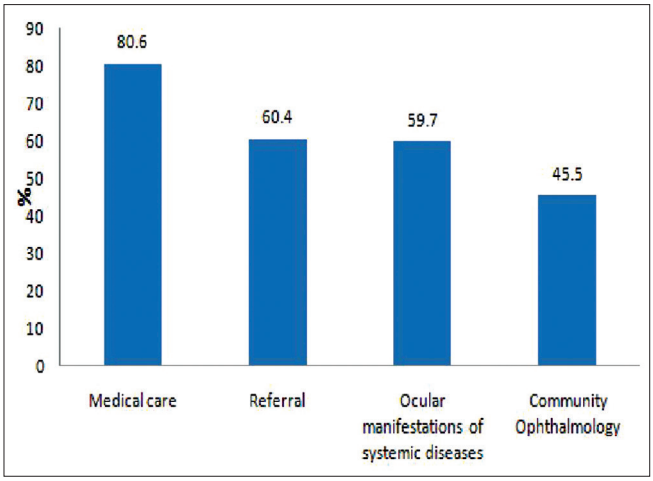


Figure 2: Students' adequacy of ophthalmic knowledge

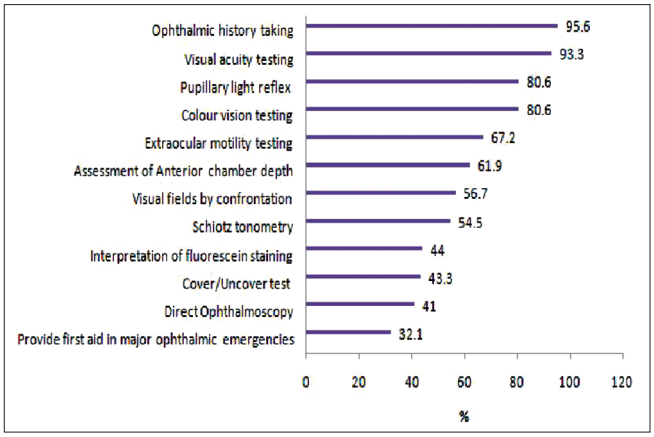


Figure 3: Students' self-reported proficiency in performance of ophthalmic skills

Table 1: Students' comfort in diagnosing common ophthalmic conditions

Ophthalmic conditions	Participants with adequate comfort in diagnosing common ophthalmic conditions (n=134), n (%)
Cataract	131 (97.8)
Eyelids and lacrimal pathology	114 (85.1)
Acute iridocyclitis	98 (73.1)
Cornea and external eye disease	91 (67.9)
Glaucoma	85 (63.4)
Squint	84 (62.6)
Leukocoria	80 (59.7)
Ophthalmic emergencies	54 (40.3)

60 h of tutorials and seminars (including integrated learning), and 10 h of self-directed learning.[7] These guidelines suggest a greater amount of ophthalmology exposure in undergraduate medical education in India when compared with ICO guidelines. The ICO recommends 40–60 h of ophthalmology exposure during undergraduate training.[8]

According to the curriculum recommended by the MCI, the goal of teaching ophthalmology to undergraduate medical students is to provide such knowledge and skills to the students that shall enable them to practice as a clinical and primary eye care physician.[6] The curriculum provides a list of competencies which need to be demonstrated by the student at the end of ophthalmology training. These include knowledge of common eye problems in the community; ability to manage common eye problems and identify indications for referral; recognize visual impairment and blindness in the community; and implement the National programme as applicable in the primary care setting.[7]

Most of the participants in the study reported a satisfactory level of comfort in diagnosing common ophthalmic problems. The fact that 97.8% reported a satisfactory level of comfort in diagnosing cataract is encouraging since cataract is the leading cause of preventable blindness in the developing world. Yet, despite this satisfactory level of comfort in managing common eye problems, most study participants' comfort in diagnosing ophthalmic emergencies such as endophthalmitis, rupture globe, and chemical injuries was found to be inadequate.

Nearly 60% reported to have adequate knowledge in ocular manifestations of systemic diseases. This could be because of better teaching resources as well as integration of ophthalmology topics into other subjects of the medical curriculum horizontally and longitudinally. The self-rated knowledge of more than one-half of the study participants was found to be unsatisfactory in community ophthalmology. With greater emphasis placed on teaching disorders of the eye, in theory lectures as well as in hospital settings, community ophthalmology is often neglected. A study on the status of ophthalmic medical education in the South-East Asia Region of World Health Organization has also highlighted this concern, where students have little exposure to community ophthalmology.[9]

The knowledge of most study participants on the indications of medical treatment and referral to eye clinics was adequate. With a significant shortage of ophthalmologists in developing countries, an adequate level of comfort of primary care physicians in providing medical care and accuracy in referral is essential. A high rate of referral places pressure on the limited resources of ophthalmic casualty departments and outpatient clinics.

More than 80% reported competency in ophthalmic history taking, visual acuity and color vision testing, and pupil examination. These are relevant not only in ophthalmology but also in general medicine, neurology, and trauma care. However, only 44% reported an adequate level of comfort in interpretation of fluorescein staining and 54.5% in Schiotz tonometry. This is an area of concern because corneal blindness

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Table 2: Students' adequacy of knowledge

Ophthalmic knowledge	Participants with adequate knowledge (*n*=134), *n* (%)
Medical care	108 (80.6)
Referral	81 (60.4)
Ocular manifestations of systemic diseases	80 (59.7)
Community ophthalmology	61 (45.5)

Table 3: Students' proficiency in performance of ophthalmic skills

Ophthalmic skills	Participants with adequate comfort in performing ophthalmic skills (*n*=134), *n* (%)
Ophthalmic history taking	128 (95.6)
Visual acuity testing	125 (93.3)
Pupillary light reflex	108 (80.6)
Color vision testing	108 (80.6)
Extraocular motility testing	90 (67.2)
Assessment of anterior chamber depth	83 (61.9)
Visual fields by confrontation	76 (56.7)
Schiotz tonometry	73 (54.5)
Interpretation of fluorescein staining	59 (44)
Cover/uncover test	58 (43.3)
Direct ophthalmoscopy	55 (41)
First aid in major ophthalmic emergencies	43 (32.1)

and glaucoma contribute to a major extent to the burden of blindness in India; adequate proficiency of the primary care physician in these skills will help in early diagnosis and treatment or referral, thereby reducing the risk of visual loss.

Only 41% of respondents indicated that they had adequate proficiency in performing direct ophthalmoscopy which is consistent with studies elsewhere.[2,10] This could be because of the limited time devoted to teaching ophthalmoscopy in the medical school curriculum as well as the technical challenges involved.

Although most students were comfortable in managing ophthalmology associated issues overall, gaps were noted in certain key areas. Review of the curriculum with greater emphasis on community eye care and blindness control strategies, adequate training resources, and effective teaching methods tailored toward primary care would help to increase the medical students' comfort in managing common eye problems.

Since our observations are from a single center, generalization of results is limited. Subjective bias due to the candidates varying ability to accurately self-evaluate is also a limitation. The choice of the study population was medical students at the end of ophthalmology training rather than interns because of the possibility of recollection bias. A similar study on interns or junior medical officers would provide more insight into the knowledge and gaps in training.

In spite of these limitations, this needs assessment study is the first of its kind in India to assess the adequacy of ophthalmic teaching and the knowledge and clinical skills of undergraduate medical students. Ophthalmology in India is being taught as part of the essential undergraduate medical curriculum and not exclusively as an elective. Students receive adequate exposure to ophthalmology, and the undergraduate curricular hours are greater than the guidelines published by the ICO. Since ophthalmology related topics and examination skills are included in most of the standardized medical licensing examinations and internal medicine boards, medical graduates from India are at a distinct advantage.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Dart JK. Eye disease at a community health centre. Br Med J (Clin Res Ed) 1986;293:1477-80.
2. Shuttleworth GN, Marsh GW. How effective is undergraduate and postgraduate teaching in ophthalmology? Eye (Lond) 1997;11(Pt 5):744-50.
3. Fan JC, Sherwin T, McGhee CN. Teaching of ophthalmology in undergraduate curricula: A survey of Australasian and Asian medical schools. Clin Exp Ophthalmol 2007;35:310-7.
4. Bellan L. Ophthalmology undergraduate education in Canada. Can J Ophthalmol 1998;33:3-7.
5. Noble J, Somal K, Gill HS, Lam WC. An analysis of undergraduate ophthalmology training in Canada. Can J Ophthalmol 2009;44:513-8.
6. Medical Council of India Regulations on Graduate Medical Education; 1997. Available from: http://www.mciindia.org/Rules-and-Regulation/GME_REGULATIONS.pdf. [Last accessed on 2016 Oct 11].
7. Medical Council of India Regulations on Graduate Medical Education; 2012. Available from: http://www.psgimsr.ac.in/wp-content/uploads/2012/pdf/others/mci-regulations-graduate-medical-education-12.pdf. [Last accessed on 2016 Oct 11].
8. International Task Force on Opthalmic Education of Medical Students, International Council of Opthalmology. Principles and guidelines of a curriculum for ophthalmic education of medical students. Klin Monbl Augenheilkd 2006;223 Suppl 5:S1-19.
9. World Health Organization. Study on the Status of Ophthalmic Medical Education in the South-East Asia Region - An Overview. Available from: http://www.v2020eresource.org/content/files/SEA_OPHTHAL_121.pdf. [Last accessed on 2017 Jun 03].
10. Gupta RR, Lam WC. Medical students' self-confidence in performing direct ophthalmoscopy in clinical training. Can J Ophthalmol 2006;41:169-74.

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Appendix

Appendix 1: Questionnaire

1. Using the table below, please indicate how comfortable You feel diagnosing the following issues based on your present knowledge:
   (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, 5 = very comfortable)
   Conjunctiva/cornea 1 2 3 4 5
   Viral conjunctivitis
   Allergic conjunctivitis
   Subconjunctival hemorrhage
   Pterygium
   Corneal abrasion/ulcer
   Keratomalacia
   Corneal blindness
   Eyelid/lacrimal sac 1 2 3 4 5
   Blepharitis
   Chalazion
   Stye
   Dacryocystitis
   Lens/Uvea 1 2 3 4 5
   Cataract
   Acute iridocyclitis
   Glaucoma 1 2 3 4 5
   Primary open-angle glaucoma
   Acute angle-closure glaucoma
   Ophthalmic emergencies 1 2 3 4 5
   Globe rupture
   Chemical injuries
   Endophthalmitis
   Pediatric ophthalmology 1 2 3 4 5
   Strabismus
   Leukocoria

2. Using the table below, please indicate your knowledge on the following issues:
   (1 = very poor, 2 = poor, 3 = Average, 4 = good, 5 = Excellent)
   Ocular manifestations of systemic diseases 12345
   Diabetic retinopathy
   Hypertensive retinopathy
   Tuberculosis
   Toxemia of pregnancy
   HIV
   Community ophthalmology 1 2 3 4 5
   Magnitude of blindness in India
   NPCB and implementation at various levels
   Functions of ophthalmic assistant
   Organization of eye camp
   Medical care/referral 1 2 3 4 5
   Indications of topical antibiotics
   Indications of topical steroids
   Indications of antiglaucoma drugs
   Indications of ophthalmology referral

3. Using the table below, please indicate how comfortable You feel performing the following ophthalmology-related skills:
   (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, 5 = very comfortable)
   Clinical assessment 1 2 3 4 5
   Ophthalmic history taking
   Visual acuity testing
   Test color vision
   Cover/uncover test
   Assessment of anterior chamber depth
   Pupil examination/swinging flashlight test
   Visual field testing by confrontation
   Extraocular movements
   Interpretation of fluorescein staining
   Schiotz tonometry
   Direct ophthalmoscopy
   Provide first aid in major ophthalmic emergencies

4. How many hours would you have spent in self-directed learning in ophthalmology?
   ("self-directed learning" describes a process by which individuals take the initiative, with our without the assistance of others, in diagnosing their learning needs, formulating learning goals, identify human and material resources for learning, choosing and implement appropriate learning strategies, and evaluating learning outcomes)
   1. 0–2 h 2. 2–5 h 3. 5–7 h
   4. 7–9 h 5. 0 > 10 h
5. How comfortable do you feel managing ophthalmology associated issues overall?
   1. Not comfortable at all 2. Somewhat comfortable
   3. Moderately comfortable 4. Comfortable 5. Very comfortable

THE END

Thank you for taking time for our survey.

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Practical Advice Paper

Academia–Industry Collaboration to Provide Interdisciplinary Experiential Learning Opportunities in Public Health Professions Education and Improve Health of Female Factory Workers

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ABSTRACT

Background: The community medicine department of our medical school, in addition to addressing its mandate to prepare undergraduate students for primary care and graduate students for careers in public health, administers several community-based interventions. An international organization involved with improving health of female factory workers globally invited us to partner in their efforts locally. We used the Precede-Proceed model to design an intervention to deliver the desired project outcomes. **Activities**: Recognizing that this partnership with industry would provide a learning opportunity to our Master's degree program students, we involved them in a needs assessment survey in order to make an educational diagnosis to identify the influencing and reinforcing factors. Our faculty and students interfaced with a multidisciplinary team including mid-level factory managers, health-care staff, and peer health educators within the textile industry. **Outcomes**: Through this industry–academia collaboration, our health professions training institution was able to provide supplementary experiential learning opportunities to students in our Master's degree in Community Medicine program by involving them in all the project stages from planning based on health needs assessment, to module design, implementation, and program evaluation along with interdisciplinary teams from the textile industry. Students then reflected on their learning experience using a modified Kolbe's experiential learning cycle to improve their performance when they replicated the intervention with the next factory under the same project. **Conclusion:** The use of PRECEDE–PROCEED model in the industry–academia collaboration and Kolb's framework provided supplementary experiential learning opportunities for deliberate practice, receiving feedback, and reflecting on their learning to our Master's in Community Medicine degree students.

Keywords: Educational diagnosis, female factory workers, peer health educator training, PRECEDE–PROCEED model, program evaluation, program planning, public health education

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Background

The Department of Community Medicine at our medical school in South India is involved in a number of community-based activities and programs that cater to the health needs of different population groups in urban and rural settings and also provide hands-on experiential learning opportunities to future public health specialists. One such program is directed toward promoting the health of women in the textile industry. Tirupur, a textile city in South India, is one of the leading exporters of garments. Recent years have seen a high inflow of migrant female workers, most of whom are between 18 and 25 years old.[1] These women workers often suffer from anemia, poor hygiene, inadequate pre- and post-natal care, sexual violence, and exposure to infections and illness.[2]

Business for social responsibility (BSR) is a network of more than 250 member companies all over the world that promotes various sustainable initiatives. Among the many strategies they use for ensuring sustainability is the "peer education model," which brings together international brands, academia, and top management of factories to educate women who work in these factories.[3] This model advocates for better conditions for workers with owners and is called "health enables returns." The project is also known as HERproject which serves as an acronym for "health enables returns" and also refers to the targeted beneficiaries who are the vulnerable women factory workers.

The Master's Degree program in Community Medicine (Public Health) administered by the department provides learning opportunities for acquiring core public health skills that enable graduates to function as public health consultants, educators, leader–managers, and researchers for improving the health of defined population groups.[4] The 1st year of the Master's Degree program introduces students to the foundation courses in epidemiology, nutrition, demography, biostatistics, and environment health. The 2nd and 3rd years provide them with opportunities to apply these skills and also to further develop leadership and management skills in the defined population groups as well as research competency (a community-based thesis is required). The Master's Degree students use structured experiential learning opportunities for deliberate practice, reflection on practice, and receiving feedback from faculty on the job as well as on their reflective log [Figure 1][5] to equip them with skills that they will be using for continuing professional development.

Context

Master's Degree students generally get opportunities to learn about health program planning and program evaluation in health systems or community settings serving defined population groups. Workers in factories are a defined

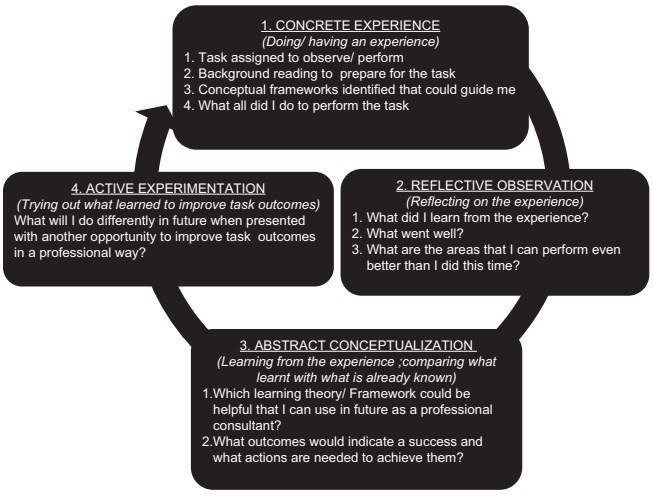


Figure 1: Reflective log using modified Kolb's cycle to optimize experiential learning

population group, so a collaborative partnership has been entered into between our medical school and BSR to address the health issues of the women workers and to promote good health. This academia–industry collaboration helped develop a need-based intervention, with academia contributing its expertise and industry, allowing its personnel and premises to function as resources to facilitate the implementation of the project. The community medicine department administering the Master's degree program involved the students in all the stages of the program planning, implementation, and evaluation.

The entire process of how the faculty and postgraduate students in the department of community medicine systematically designed and implemented the program within the industry is described in detail. We also describe how we built upon a well-known PRECEDE–PROCEED model by incorporating the concepts of community diagnosis and educational diagnosis to design a tailor-made intervention to improve the health of factory workers and thereby benefit the factory. It is hoped that sharing our experience will provide other health professions educational institutions with an alternate model of industry-based learning experience that they may be interested in replicating or adapting as an additional venue for community-based learning.

Activities

We chose to use the PRECEDE–PROCEED conceptual model to plan and apply the health behavioral theories for behavioral change.[6] We used the World Health Organization-recommended process of identifying the main health problems and their underlying social and other determinants known as "community diagnosis."[7,8] Based on the community diagnosis, we were able to identify the predisposing and enabling factors in the working environment. This means we could

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make an "ecological diagnosis" as well as identify behaviors that are contributing to ill health by making an "educational diagnosis" (PRECEDE phase 1 through 3). Once these two diagnoses were made, the tailor-made "treatment" – the health education program (Phase 4: Health promotion educational strategies, program implementation) was designed to correct the faulty behaviors and predisposing conditions in the working environment and thereby lead to improvement in health.

The "team-academia," comprising of faculty project advisor, faculty project coordinator, consultants, Master's Degree Public Health students, and social workers, was drawn from the department of community medicine of our medical school. Following an orientation meeting with the top and middle management of the industry, a team consisting of the top- and middle-level managers, the factory's basic health-care nursing team, and the peer leaders as peer educators was formed at the factory level. The female factory workers were the beneficiaries or target population of the initiative. A brief summary of the various steps involved in the program implementation and expected outcomes based on the PRECEDE–PROCEED model is shown in Figure 2.

Outcomes

Benefits and outcomes for the industry

The model as shown in Figure 2 in its phase 6–8 guides the user to plan and put in place a system for program monitoring and evaluation. We used Kirkpatrick's Training evaluation framework[9] to capture the effectiveness of the educational intervention program outcome. Table 1 summarizes the different outcomes of the program in the short term.

Table 1 shows how the peer educators found the program to be useful (Kirkpatrick Level 1). This influences program acceptance and its sustainability. It also captures their learning in terms of gain in knowledge (Kirkpatrick Level 2) and change in behavior (Kirkpatrick Level 3), thereby showing that capacity-building of peer educators was achieved. Successful transfer of learning to workplace (Kirkpatrick Level 4) was indicated by the peer educators being able to gain confidence and conduct several training sessions, which contributes significantly to program sustainability. Similarly, the program impact as demonstrated by bringing in return-on-investment (Kirkpatrick Level 5) in terms of health benefits to the workers translating to reduction in sickness absenteeism has convinced the factory management to institutionalize the program. Acceptance by another set of 25 factories in the region is a clear indicator of widespread recognition of the utility of the intervention and so ensures its sustainability.

Beneficial learning opportunities and outcomes for the academia

Table 2 shows the learning opportunities for the Master's Degree students in the industry that are unique to the project and are listed across the various phases of the model used.

Table 1: Effectiveness of the peer educators' training program

Kirkpatrick's level	Outcome indicators/measurement	Results from factories postintervention
Level 1: Reaction of the peer educators (satisfaction, usefulness, suggestions etc.)	Verbal and written feedback immediately after the peer educators' training session	"The program was very useful; earlier I used to be shy talking about HIV/AIDS. Not anymore
Level 2: Learning	Learners gain in knowledge was assessed by verbal questioning, conducting quiz sessions at the end of the training, etc. An end-line assessment done using the standard survey form among 10% of the workers	Most of the peer educators were able to reply quickly and correctly Awareness score on nutritious diet has improved from 40% to 75% Awareness score on necessity for doctors visit during pregnancy has increased from 85% to 33%
Level 3: Change in behavior Level 4: Transfer of learning to workplace	Number of outreach sessions conducted by peer educators Periodicity of interaction with workers on various modules Case studies from peer educators and workers Periodic meetings and interview with management	Confidence: " I am now confident in doing "Self Breast Examination" An average of 70 outreach sessions conducted by peer educators per factory varying from 60 to 80 based on workers strength Workers practicing regular exercise has increased from 30% to 55%
Level 5: Return on investment: Program impact	Monitoring certain indicators such as sickness-related absenteeism, trends in food wastage every month, worker turnover, and pattern of illness Willingness of the management to internalize the program and continue to provide resources for the sustainability of the program Program scaleup and expansion	Health benefits Menstrual problem-related absenteeism has decreased from 30% to 20% Complaints of musculoskeletal problems has decreased from 35% to 22% Management 's feedback regarding the program "Absenteeism due to health reasons is declining. It is helpful to reach our production target" "Posttraining we have seen visible changes among our workers. They are more energetic at and after work" Changes brought in the factories Change in the canteen menu, availability of nutritious snacks within the premises improved linkage with public health system for utilizing national programs for anemia prevention, deworming and other health services Started with three factories in 2012 the HERproject is scaled up to 25 factories in 7 years

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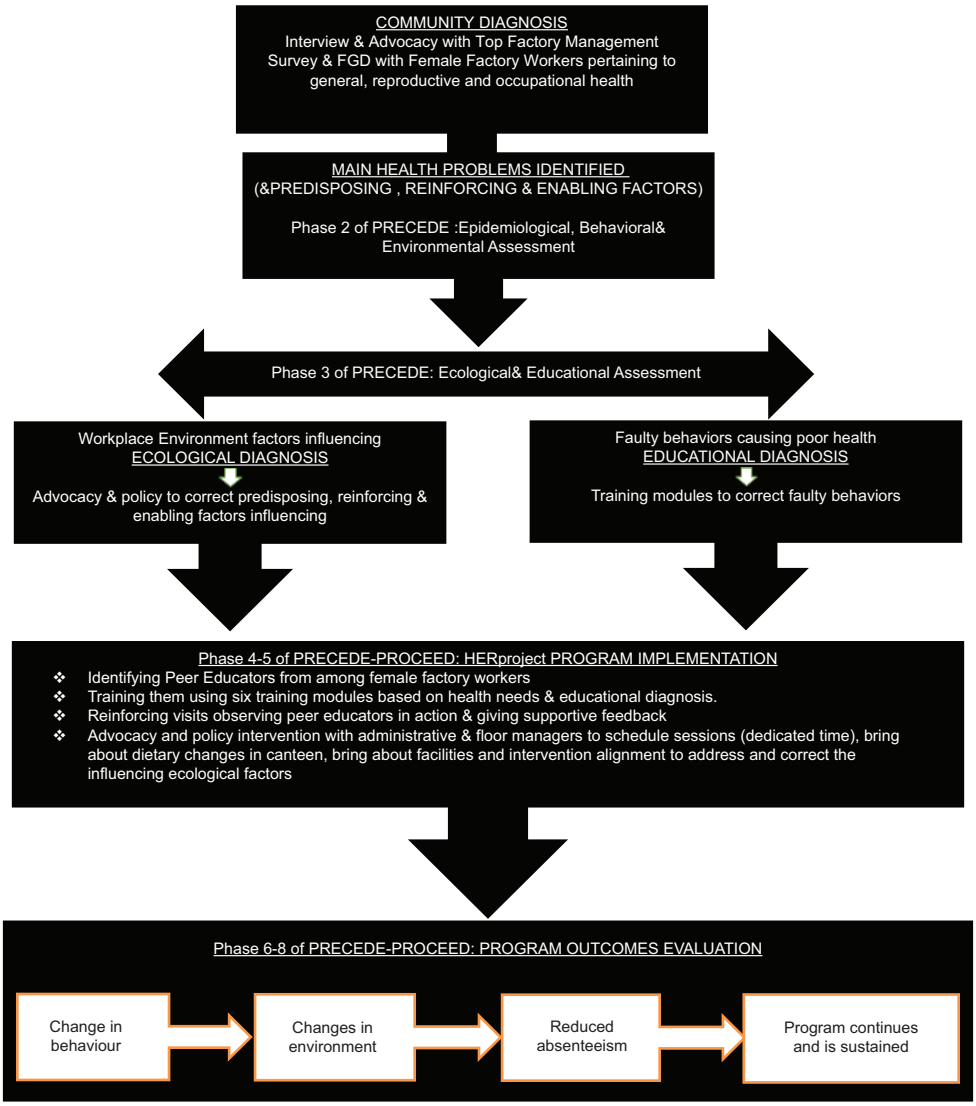


Figure 2: Flow diagram showing planning and implementation of the educational intervention

The concrete learning experiences in the second column are tagged with their resultant specific public health competencies and tools listed in the third column. They are effective communication, community diagnosis, educational diagnosis, training module design, working with interdisciplinary teams, use of adult learning principles, providing supportive feedback to peer educators, use of logic model for program planning, and identifying indicators to measure outcomes of any intervention.

Table 2 in its extreme right column captures the learning outcomes that emerged from the structured learning experiences stated in the column on its left and provides more insights about the benefits students reaped than any quantitative research would have elicited. These responses were captured from reflective logbooks students submitted after each learning experience they were involved in as part of their course. As shown in Figure 1, these reflective observations (step 2 in the Kolb's cycle) then provoke them to search for other conceptual frameworks that would illuminate and guide their next step in the step 3 namely "abstract conceptualization." This helps them to be better prepared for undertaking a similar professional activity by encouraging them to go through step 4 of "active experimentation" so that improved outcomes result.

Future Directions

The main factors that contributed to program sustainability are stated below and should find a place in project planning in future.

Contribution of the model for its sustainability

As indicated in Figure 2, it is clear that in addition to serving as a model to design an intervention, it facilitates, through the component of community diagnosis, in making an ecological

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Table 2: The PRECEDE-PROCEED model: Learning opportunities and learning outcomes for students in academia

PRECEDE-Proceed model for guiding and planning		Concrete experiences in industry	Concrete experiences in public health learning	Reflections by students on their learning experience (extracted from their Kolb's Reflective logbook) Student's reflections on learning
Phases of the model	Components of the model			
PRECEDE Phase 1	Social assessments, participatory planning, situational analysis	Advocacy with top level and middle management	"2-min elevator talk": Articulating the need for intervention, the intervention and benefits to industry	*"Got insight into ways for influencing policy, interagency collaboration, using research for developmental activities" "learnt the importance of getting all the stakeholders on board for the success of the program*
PRECEDE Phase 2	Epidemiological, behavioral, and environmental assessments	Site visits to industry for identification of priority health needs and behavioral and environmental determinants	Community diagnosis: HNA survey design, administration and analysis; prioritizing needs; design and do focus group discussion with stakeholders	*"learnt importance of HNA questionnaire - as an effective method to prepare tailor-made intervention" "Learned more about the needs and how to prioritize them Highlighted the areas of unmet need and provided a clear set of objectives to work towards to meet these needs*
PRECEDE phase 3	Educational and ecological assessments	FGD about predisposing, reinforcing and enabling factors	Educational diagnosis: Identifying faulty behaviors leading to ill health and their predisposing, reinforcing and enabling factors Health promotion theories for designing program strategies	*"Learnt to be a good listener, take down notes quickly while conducting FGDs" "I could motivate the silent non respondents in the group to respond and not allow a few to dominate the discussion" "Using the FGD helped in eliciting the predisposing and enabling factors" "I understood how the moderators skills play a major role in getting rich data from the FGDs"*
PRECEDE phase 4	Intervention alignment	Modules based on needs identified: Health behavior, ergonomics, diet in canteen	Use of ADDIE framework for module design (analyze need, design instruction, develop material, implementation of peer educator training and evaluation)	*"Before conducting any health activity in (the) field, structured (prior) planning helps achieve desired outcomes" "Use of standard frameworks helps achieve desired outcomes and we have the satisfaction of having used globally used instructional design frameworks." "how using a framework makes the intervention more structured and organized"*
PRECEDE-PROCEED phase 5	Implementing educational strategies and planning evaluation of process, impact and outcomes of program	Identification of peer trainers from among the factory workers, training of trainers, time scheduling for training sessions Used mid-level managers to identify and finalizing evaluation indicators	Working and leading interdisciplinary team within academia and the industry use of adult learning principles and interactive teaching, supportive ongoing feedback Use of logic model for identifying activities to be monitored for process evaluation	*"While interviewing the workers at the end of the study itself we became aware that the workers had become empowered with knowledge and sounded confident. Shows peer education as a strategy works" "Learnt importance of peer health education as a strategy to sustain the benefits of the intervention longer" "Got an opportunity to practice pedagogical skills and adult learning principles"*
PROCEED phase 6	Process evaluation	Measuring indicators of success of processes needed to carry out tasks and activities	Use of survey and focus groups to collect indicators of success Document study to corroborate evidence in support	*"Great opportunity to learn about (ways for) identifying indicators for evaluation" "helps to understand how important is to identify the right indicator that reflects the outcome expected from the program"*
PROCEED phase 7	Impact evaluation	Used top-level managers to identify indicators of success arising from carrying out project tasks and activities	Use of logic model for identifying short term indicators for impact evaluation	*"HERproject peer educators develop communication and leadership skills, and they use those skills to share their knowledge with their co-workers, with their communities, and with their families" "It helped me understand how targeted health education can impact the health of the workers in the long run"*

HNA=Health needs assessment, FGD=Focus group discussion, ADDIE=Analysis, Design, Development, Implementation and Evaluation

and educational diagnosis (Phase 3 of the PRECEDE–PROCEED model). It thus addresses "the need" for the program as reflected by the "diagnosis" and so the continuing need for the intervention ("treatment") contributes to its sustainability. From Figure 2, it also becomes clear that in the Phase 4–5 of the PRECEDE–PROCEED model as applied to the project, the strategy of using peer educators from among the factory workers, training them using the modules developed by academia based on the ecological and educational diagnosis described in the Phase 3 of the model, reinforcing visits for supportive supervision of the peer educators, together with advocacy and policy intervention by academia with administrative and floor managers are all program components that contribute to sustainability of the intervention.

Successful outcomes (return on investment) as a driver to ensure sustainability

Even after these phases of the program were completed, the outcomes were sufficiently compelling to convince the factory management in terms of "return on investment"

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to mainstream it along with other routine activities at the factory. Phases 6 through 8 of the model shown in Figure 2 highlight this result. Team academia, by consciously involving the factory administration to devise a feasible strategy of setting up the "peer education process" as well as having master-trainers at staff level within the factory, contributes significantly to the sustainability of the program.

Conclusion

Using a defined population group in an industrial setting provides a novel population group for students to apply theory to public health practice and helps them realize that irrespective of the target group they work with, the generic guiding principles are the same.

The PRECEDE–PROCEED model commonly used in health promotion program planning and evaluation was found to be helpful and demonstrated its usefulness to the students. Involving postgraduate students in the industry–academia collaboration provided supplementary experiential learning opportunities for deliberate practice, receiving feedback, and reflecting on their learning to our Master's in Community Medicine degree students. By involving them in all the stages from project planning based on health needs assessment, to implementation starting with advocacy with top-level management, training module design, training of peer educators, leadership and management of a multidisciplinary team in industry and program evaluation, they were able to apply their knowledge in a new context. The insights about their learning captured from their reflective portfolio logs using Kolb's experiential learning framework show that participation has enabled them to learn systematically prior to the intervention (PRECEDE phases), to implement the intervention tailored to the identified needs, and to devise program evaluation (PROCEED phases).

Use of Kolb's framework for their reflective log helped postgraduates to reinforce their learning and encouraged them to do background reading and plan for the tasks to be accomplished. It also helped them become reflective practitioners. These enhanced personal development practices help them to engage in continuing professional development to improve the quality of health-care delivery.

In addition, the industry provided another avenue to work collaboratively with multidisciplinary teams and thereby reinforced the value of collaborative learning and respecting team members and project stakeholders for the particular competencies and contributions that they bring together to deliver care and improve service effectiveness.

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Conflicts of interest

There are no conflicts of interest.

References

1. Suhasini Singh. India Cheap Labor Garment Export Industry. Fashionable and Famous at the Garment Worker's Cost. Global Research; 2009. Available from: https://www.globalresearch.ca/india-cheap-labor-garment-export-industry/13530. [Last accessed on 2018 Sep 10].
2. Saha TK, Dasgupta A, Butt A, Chattopadhyay O. Health status of workers engaged in the small-scale garment industry: How healthy are they? Indian J Community Med 2010;35:179-82.
3. Investing in Women for a Better World. BSR Report 2010. HERproject. Available from: https://herproject.org/downloads/BSR_HERproject_Investing_In_Women.pdf. [Last accessed on 2018 Feb 23].
4. Guidelines for Competency Based Postgraduate Training Programme for MD in Community Medicine. Available from: https://old.mciindia.org/PG-Curricula/MD-Community-Medicine.pdf. [Last accessed on 2019 Feb 14].
5. Elizabeth Fischer Turesky, Diane R Wood. Kolb's experiential learning as a critical frame for reflective practice. Academic leadership. Online J 2010;8(3), Article 25. Available from: https://scholars.fhsu.edu/alj/vol8/iss3/25. [Last accessed 2019 Feb 14].
6. Glanz K, Rimer BK, Lewis FM, editors. Health Behavior and Health Education: Theory, Research and Practice. 3rd ed. San Francisco: Jossey-Bass; 2002.
7. Andrea Carson Gielen CA, McDonald EM, Bone LR, Gary TL. Using the PRECEDE-PROCEED model to apply health behaviour theories. In: Glanz K, Rimer BK, Viswanath K, editors. Health Behaviour and Health Education: Theory Research and Practice. San Francisco: Jossey Bass; 2008. p. 407-34.
8. Vaughan JP, Morrow RH, editors. Manual of Epidemiology for District Health Management. Geneva: World Health Organization; 1989. Available from: http://apps.who.int/iris/bitstream/10665/37032/1/924154404X.pdf. [Last accessed on 2018 Sep 10].
9. Smidt A, Balandin S, Sigafoos J, Reed VA. The Kirkpatrick model: A useful tool for evaluating training outcomes. J Intellectual Develop Dis 2009;34:266-74.

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The Effect of Mode of Delivery on Breastfeeding Outcomes: A Hospital Based Study

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ABSTRACT

Background: Some studies suggested that a high rate of caesarean deliveries results in suboptimal breastfeeding outcomes. The relationship between mode of delivery and breastfeeding outcomes has been considered important given the rising prevalence of caesarean deliveries globally. The present study is to examine the relationship of mode of delivery with breastfeeding outcomes. **Materials and Methods:** This study was conducted at PSG Institute of Medical Science and Research, Coimbatore in the month of July 2018. Ethical clearance was obtained from the institution prior to the study. Chi square test was done to assess the association between socio-demographic variables, timely initiation of breastfeeding and exclusive breastfeeding with mode of delivery. The effect of caesarean on duration of breastfeeding was obtained by applying Kaplan-Meier method. **Result:** Prevalence of caesarean delivery was 36.2% and of vaginal delivery was 63.8%. A statistically significant association was obtained between caesarean deliveries and timely initiation of breastfeeding. However mode of delivery was not significantly associated with exclusive breastfeeding and duration of breastfeeding. **Conclusion:** The findings of our study indicates that caesarean deliveries have been associated with lower timely initiation n rates compared to vaginal deliveries.

Keywords: caesarean delivery, duration of breast feeding, exclusive breastfeeding, initial breastfeeding, vaginal delivery.

Introduction

Breastfeeding is a dynamic process comprising regulation between the mother's and infant's interconnected physiological, psychological, and behavioural systems [1]. Optimal infant and young child feeding is crucial for health and development.

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Women are recommended to breastfeed exclusively for 6 months and to continue breastfeeding until the child is 2 years. Breastfeeding is widely acknowledged as the best and safest form of infant feeding [2].

The global average caesarean delivery rate is 12.4% with an average annual rate of increase of 4.4%.The use of caesarean delivery has increased in the last few decades particularly in middle- and high-income countries [3]. According to a recent analysis of national health survey data, caesarean deliveries in India has gone up from 10% to 30 % raising the country's average caesarean section rates from 5% to 18%. An independent analysis of data from the National Family Health Survey (NFHS) (199293 to 2015-16) and Rapid Survey on Children 2013-14,India's caesarean-section rate was at 18 per cent which was higher than the

Lancet report's estimate of 10 per cent[4]. It is necessary to find whether the mode of delivery affect the breastfeeding outcomes.

The primary aim of the study was to examine the association between mode of delivery and subsequent breastfeeding outcomes – initiation of breastfeeding within one hour of birth, exclusive breastfeeding up to six months and duration of breastfeeding.

Materials and Methods

This study was conducted at PSG Institute of Medical Science and Research, Coimbatore in the month of July 2018. Ethical clearance was obtained from the institution prior to the study. Informed consent was obtained from all mothers interested in participating. Questions about mode of delivery and breastfeeding were collected using questionnaire. A total of 527 mothers were studied. Eligible mother include those who with the children age below four years attending paediatric outpatient department. Babies with congenital anomalies that interfere with breastfeeding were excluded from the study. Socio economic status was measured using Prasad classification. The classification is done based on the Consumer Price Index (CPI) value of 279 for Coimbatore [5]. Class 1 include people having per-capita income greater than or equal to Rs.6180. Class 2, class 3, class 4 include the people having range of pre-capita income Rs.3090-6179, Rs.1850-3089, Rs.930-1849 respectively and class 4 include people having per-capita income less than Rs.930.

WHO recommends that for those children less than six months of age, who fed only on mother's milk in the 24 hours preceding the survey were considered as exclusive breastfeeding.

Chi square test was done to assess the association between mode of delivery with socio-demographic variables, initiation of breastfeeding and exclusive breastfeeding. p value<0.05 was considered as statistically significant.

The effect of caesarean on duration of breastfeeding was obtained by using Kaplan-Meier method. The duration of termination of breastfeeding was the outcome event and those mothers who are continuing the breastfeeding during the survey were considered as censored. Total probability of termination of breastfeeding till that time interval was calculated by multiplying all the probabilities of termination of breastfeeding at all time intervals preceding that time. The log rank test was used to assess the equality of survival curves of vaginal and caesarean delivery. The data were analysed using SPSS[5].

Results

The sample included 527 mothers. 36.2% of mothers were delivered by caesarean and 63.8% of mothers gave birth by vaginal delivery.

Table 1 depicts the various socio-demographic and clinical variables according to mode of delivery. Increased number of antenatal visits (p<0.05) and term delivery (p<0.01) were negatively associated with caesarean delivery.

Table 2 describes the association of mode of delivery with timely initiation of breastfeeding, exclusive breastfeeding and duration of breastfeeding. Timely initiation of breastfeeding was lower among caesarean deliveries compared to vaginal deliveries and the difference was statistically significant (p<0.001).The prevalence of exclusive breastfeeding was 60.1% for vaginal delivery and 55.5% for caesarean. However the difference was not statistically significant (p=0.301).There was no difference between duration of breastfeeding and mode of delivery (p=0.851).

Table1: Distribution of socio-demographic variables by modes of delivery

Characteristics		**Vaginal delivery (%)**	**Caesarean (%)**	**p value**
Mother's age	15-24	76(22.6)	32(16.8)	0.190
	25-34	243(72.3)	145(75.9)	
	35-44	17(5.1)	14(7.3)	
Mother's education	Below 12^{th}	82(24.4)	56(29.3)	0.217
	Above 12^{th}	254(75.6)	135(70.7)	
Father's education	Below 12^{th}	79(23.5)	50(26.2)	0.494
	Above 12^{th}	257(76.5)	141(73.8)	

Number of antenatal visits	>8	253(75.3)	126(66.0)	p<0.05
	≤8	83(24.7)	65(34.0)	
Condition of mother after delivery	Well	319(94.9)	180(94.2)	0.731
	Sick	17(5.1)	11(5.8)	
Gestational age	Term	310(92.3)	159(83.2)	p<0.01
	Pre term	26(7.7)	32(16.8)	
Socio economic status	Class 1&2	273(81.2)	153(80.1)	0.748
	Class 3,4 &5	63(18.8)	38(19.9)	
parity	1	242(72)	123(64)	0.068
	≥2	94(28)	68(35.6)	

Table2: Breastfeeding outcomes by of mode of delivery

Breastfeeding pattern		**Vaginal (%)**	**Caesarean (%)**	**Chi-square**	**P value**
Timely initiation of breastfeeding	Yes	252(75.0)	46(24.1)	1.285	p<0.001
	No	84(25.0)	145(75.9)		
Exclusive breastfeeding	yes	202(60.1)	106(55.5)	1.071	0.301
	no	134(39.9)	85(44.5)		
Duration of breastfeeding	Mean Duration (Months)	18.357	18.680	0.035 *	0.851

*chi-square mentioned as log rank chi-square.

Discussion

We found that 56.5% of mothers surveyed reported initiating breastfeeding within one hour of birth and 58.4% reported exclusive breastfeeding for 6 months. The median duration of breastfeeding was 18 months. We observed a negative association between breastfeeding initiation within one hour and caesarean delivery. This contradicts the findings of Sara N K et al 2017[6] and supports the findings of Prior et al 2012 [7] and Hira Palla et al 2017 [8].

Despite the well-known benefits of nourishing infants exclusively with mother's milk, the rates of breastfeeding initiation, continuation and exclusive breastfeeding are still lower in India. Mothers and infants experiences during labour and delivery may affect lactation and breastfeeding outcomes. In this study we observed that caesarean deliveries have been associated with lower initiation rates compared to undergoing vaginal delivery. This may be due to the delay in mother-infant skin to skin contact, mother's post-surgery physical complications and effects of anaesthesia.

Research evidences showed that delayed onset of lactation is significantly higher among women with caesarean delivery compared to those with vaginal delivery. In addition, early contact with between mothers and infants during the first few hours after birth is important for forming a mother-infant bond that can lead to breastfeeding successful outcomes. Apart from it, operative care routines for post caesarean delivery can delay mothers from holding their infants and disrupt bonding between mother and infant. The mothers who undergo caesarean delivery stay longer in

hospital than mothers who experience vaginal delivery. Therefore a negative impact of surgical procedure delays breastfeeding initiation and babies were used to give formula feed from hospital.We did not find any association between mode of delivery and exclusive breast feeding. This supports the findings of Sara N K et al 2017[6] and contradicts the findings of Emily prior et al [7]. We did not find any association between mode of delivery and duration of breastfeeding which contradict the finding of Perez-Escamilla et al [9] and Amy J. Hobbs [10]. It has been suggested in these studies that lower duration of breastfeeding rates on women with caesarean delivery might be associated with physiologic mechanism that affect lactogens. However, in our study we could not establish a statistically significant association between type of delivery and duration of breastfeeding. The limitation of our study include response bias that contribute inaccurate information due to retrospective reporting. Another limitation is that the region of study has a generally higher socio-economic status and it was hospital based study. Further studies should address the obstacles based on delivery mode and other factors such as medical problems, women's knowledge around breastfeeding benefits, latching, and lifestyle changes on initiation of breastfeeding, exclusive breast feeding and duration of breastfeeding. In addition to mode of delivery, other factors that may influence breastfeeding initiation includes challenges encountered during nursing, availability of support systems, recommendation and attitudes of family, peers and health care professionals. Furthermore, large scale prospective cohort studies can provide more light in this area.

Conclusion

The findings of our study indicates that caesarean deliveries have been associated with lower timely breastfeeding initiation rates compared to mothers undergoing vaginal delivery.

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References

1. Kristin P. Tully, Helen L. Ball. Trade-offs underlying maternal breastfeeding decisions: A conceptual model. Matern Child Nutr. 2013. 9(1): 90–98.
2. Inger Aakre, Anne Marie Lilleengen, Marie LersethAarsand, Tor A.Strand, Ingrid Barikmo and SigrunHenjum. Infant feeding practices in the Saharawi refugee camps Algeria, a cross-sectional study among children from birth to six months of age. International Breastfeeding Journal. 2017.
3. Ana Pilar Betrán, Jianfeng Ye, Anne-Beth Moller, Jun Zhang, A. MetinGülmezoglu, Maria Regina Torloni. The Increasing Trend in Caesarean Section Rates: Global, Regional and National Estimates 1990-2014. 2016.
4. Caesarean section deliveries up from 10% to 30% percent in India. https://www.news18.com/news/lifestyle/health-andfitnesscaesareansectiondeliveries-up-from-10-to-30-percent-in-india-1347492.html. February 10, 2017, 2:05 PM IST
5. Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers. Available from: http://labourbureaunew.gov.in/UserContent/Linking_factor_CPI_IW.pdf?pr_id. Accessed August 10, 2018.
6. Sara N. Kiani, Katherine M. Rich, Darby Herkert, Cara Safon, Rafael Perez Escamilla. Delivery mode and breastfeeding outcomes among new mothers in Nicaragua. Matern Child Nutr. https://doi.org/10.1111/mcn.12. Wileyonlinelibrary.com/journal/mcn. 2017.
7. Emily Prior, Shalini Santhakumaran, Chris Gale, Lara H Philipps, Neena Modi, and Matthew J Hyde. Breastfeeding after cesarean delivery: a systematic review and meta-analysis of world literature. American Journal of Clinical Nutrition.https://doi.org/10.3945/ajcn.111.030254 .2012. 1113–1135.
8. Hira Palla and PanagiotaKitsantas. Mode of delivery and breastfeeding practices. International Journal of Pregnancy & Child Birth.2017.167-172.
9. RafaelPerez-Escamilla, Irene Maulen-Radovan, and Kathryn G. Dewey. The Association between Cesarean Delivery and Breast-Feeding Outcomes among Mexican Women. American Journal of Public Health.1996. 832-836.
10. Amy J. Hobbs, Cynthia A. Mannion, Sheila W. McDonald, Meredith Brockway and Suzanne C.

Tough. The impact of caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. BMC Pregnancy and Childbirth. 2016. 16-90.

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End-of-Life Practices in Rural South India: SocioCultural Determinants

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Abstract

Introduction:

End of life care of terminally ill is a sensitive topic in our socio cultural ethos. In a country where Euthanasia policies are widely debated, dignified death is a desired form of death. Euthanasia literally means “good death”. In India the debate still continues on practices related to euthanasia and its legalization until recently when the verdict on the passive euthanasia has been passed by the supreme court. In addition, lack of an effective palliative care system has led to complex situations towards the end of life. Globally, it is estimated that palliative care is needed in 40-60% of all deaths. However there is no training or facility to meet this demand.

Methodology:

A focus group discussion (FGD) was carried out among 22 residents in a rural area of Tamilnadu to identify the social and cultural determinants of end of life care practices. A FGD guide was prepared and after an informed consent the study was undertaken. An in depth interview was carried out among a sub group of participants.

Results:

The FGD and the IDI revealed several end of life practices in the rural areas such as Thalaikoothal, Feeding the ill with holy water and sand etc. The study also revealed the major determinants leading to such practices such as the social, and cultural beliefs in addition to economical and emotional factors.

Conclusion:

Several factors determine the end of life decisions in a family ranging from economical to social and cultural factors. While we are examining these factors, it is important to strengthen the palliative care provision in the country by building capacity and integrating it in primary care.

Keywords: End-of-life practices, euthanasia, palliative care

Introduction

End-of-life care of terminally ill is a sensitive topic in our sociocultural ethos. In a country where euthanasia policies are widely debated, dignified death is a desired form of death. Euthanasia literally means “good death.” It is also popularly known as “mercy death” as it is practised to lessen the pain of an individual and ensure a dignified death. In India, only recently a landmark verdict on the passive euthanasia has been passed by the Supreme Court.[1] In addition, lack of an effective palliative care system has led to complex situations toward the end of life. Globally, it is estimated that palliative care is needed in 40%–60% of all deaths.[2] Home-based palliative care models have been successfully implemented globally. The ultimate goal of these home-based care is to “promote, restore, and maintain a person's maximum level of comfort, function, and health, including care toward a dignified death”.[3] However, still it is an unmet need in the community leading to several practices intertwined with cultural and religious beliefs. These practices which are widespread do not come into the limelight for well-known reasons. Many individuals who are believed to be terminally ill are taken home to die a “peaceful” and “dignified” death surrounded by their loved ones. In addition, there are several social, religious, and cultural beliefs and factors embedded in this decision to take care of the terminally ill at home and end-of-life practices.[4] There are also traditional practices which are sparsely reported in several parts of the country which claim to liberate the elderly from a painful death and providing them relief, thereby making their death “dignified”.[4,5,6,7] Such practices have become firmly entrenched as social customs and may not be considered as euthanasia by the concerned people. However, there is a dearth in the literature which documents these practices and the several determinants which lead to these practices in rural Tamil Nadu. This study explores the several end-of-life care practices and the social, cultural, and religious dimensions associated with those practices in rural Tamil Nadu.

Methodology

The study was conducted in rural areas as defined by the Government of India in and around Coimbatore.[8] Two focus group discussions (FGDs) and four in-depth interviews (IDIs) were conducted. Ethical approval was obtained from the Institution Human Ethics Committee, and written informed consent was obtained from the study participants who are permanent residents of the area and aged >40 years and had experience in taking care of any elderly or terminally ill individuals in their household. They were selected by convenience sampling. The field guide was prepared before the FGD. The FGDs were led by a moderator and an observer for taking notes and observation. All FGDs were recorded with the participant's permission and transcribed.

Based on the FGD findings, IDIs were conducted among four women who were involved in end of life practices for people who are terminally ill.

Data collection and analysis

The FGDs and IDIs were conducted using a field guide to facilitate probing and sharing of the relevant information. Interviews, which lasted between 45 and 55 min, were audio recorded with participant's permission. The field guide included the following prompts to elicit participant views and experiences: (i) reasons for home care of the terminally ill (ii) the end-of-life rituals or practices (ii) reasons behind such rituals (their beliefs and customs) (iii) how do they feel about these practices, and (iv) what are their perceptions on dignified death. Using an framework approach, the data were managed, described, and explored. The interview transcripts were indexed and mapped on the basis of recurring themes. The transcripts were reviewed by both the authors, and a consensus was reached regarding the themes.

Results

The study revealed the various end-of-life practices in the rural area when a person is terminally ill and the common social, religious, and cultural beliefs associated with such practices. The FGD also brought out several practices and rituals that were prevalent in a community to end the life of an individual who is terminally ill to bring about a peaceful death and alleviate their suffering. Similar rituals are reported sporadically elsewhere.[5,6,9,10]

The rituals that are practised are as follows:

a. Oil bathing otherwise called as "*Thalaikoothal*" means giving a shower on the head in the regional language in which the terminally ill person is given an oil bath using gingelly oil and cold water several times in a week

b. Feeding with holy water or milk: Feeding water or milk to the bedridden, semi or unconscious person with an intention to end the suffering. Tender coconut water, "*Theertham*" – holy water, from different temples and Kasi temple and the holy water used for bathing the deity in a temple are all used for such rituals

c. Practices to address the attachment toward materialistic things: Water with soil or mud from the land owned by the terminally ill person is used with the notion that the terminally ill person is attached to his/her materialistic possession. This water mixed with the mud is fed to the ailing individual several times until the death occurs.

All these rituals hasten death by causing hypothermia, renal failure, and aspiration pneumonia, etc.

Transcription of the recordings revealed various reasons behind practising such rituals which fell under the following major themes [Figure 1]. (i) Socioeconomical, (ii) cultural, (iii) moral, and (iv) spiritual.

Figure 1

Major themes evolved during focus group discussion

Socioeconomical reasons

Most of them cited the huge costs involved in the hospitalization of terminally ill. Since death is almost inevitable they felt there is no reason to keep them in the hospital and pay huge bills.

"*I do not have enough money and facilities to take care of her, and she developed bed sores*."

"*Doctor said that she has to undergo treatment that will be expensive and cannot guarantee her good quality of life*"

Some also said that the hospitals do not accommodate very terminally ill patients for whom treatment options are limited.

"*The hospital asked us to take him home; they said they can't keep him in hospital*"

Some also expressed that it affected their social life they could not go anywhere for any social gatherings or attend other personal work since they always had to attend to the sick person. They could not be left alone, and there was no one else to take care of them.

"*I cannot go out leaving her and was not able to attend to my personal needs and work*"

Thus, socioeconomic condition, especially in the rural area came out as one of the major factor influencing the care of the elderly in their last stages of life resorting to rituals to end the life soon.

Customs and culture

Most participants believed in traditional customs and practices. They believed that the end-of-life practices/rituals as a custom done during the last stage of life to assure their loved ones a peaceful death.

"*Thalaikoothal is a tradition done to an elderly person in their last stages of life*"

The participant also said that it was a tradition done for old people who are bedridden for a long time. There were different customs followed to expedite and ensure a peaceful and dignified death to the person.

Customs such as giving milk and holy water are done, and it is believed that all family members and grandchildren of the ill have to perform that tradition to ensure peaceful death. It is also believed only when all the loved ones do so the deceased soul will be satisfied.

"*Milk and holy water should be given by all members of the family and close kin of the diseased, including grandchildren*"

The discussion also brought out that these traditions are carried out by the family members themselves or based on the suggestions of relatives and neighbors.

"*My relatives and neighbors said that by giving water mixed with mud from my land will give my mother peaceful death as she may be thinking about our l*and"

Moral duties

The study participants often cited about their moral duties toward parents and elders.

The discussion showed that people perceived these practices as moral duties toward their parents and elders and by doing such practices they help them attain peaceful death.

Spiritual

Some of the practices such as holy water and using water that is being used for bathing the idol of God are believed to help the dying person attain God. They also believe when all the members of the household give that holy water and pray the terminally ill person is relieved from suffering and will join their deceased ancestors after death.

"*When holy water is given God will call them to heaven*"

"*Kasi theertham (holy water) is kept at homes of the elderly to be given at the last stages of life*"

To reach God and to reach heaven after death were the most commonly cited reasons for giving holy water. These reasons are widely believed in the rural community (from both Hindu and Christian faiths).

Discussion

The article has explored the views and perception behind end-of-life practices and rituals. The study has highlighted various determinants and beliefs such as the socioeconomic, cultural, spiritual, and moral views behind the practices leading to the death of an individual who is terminally ill. In addition to end of life practices when a person is nearing death (e.g., feeding them holy water etc,) some practices are deliberately followed to hasten death to minimize further suffering and ensure a peaceful exit. It is important to know and understand such practices and the context behind them, to educate and design interventions to prevent those which cause deliberate death such as Thalaikoothal. Exploration of the various themes emerged shows that apart from the socioeconomic causes, customs, and cultural reasons among the closed communities encourage such practices. Moreover, the moral obligation of the son or daughter of a family to ensure that their parents or the elderly die a peaceful and dignified death is very evidently a strong-rooted belief. Therefore, they resort to such practices/rituals to alleviate the suffering of their dear ones. The discussion also brought out the community's perception that only good death can help people reach God. This was very evident when people who execute these rituals in the community said that they felt content in helping those who are suffering reach God. On another dimension, this study also undermines the need for an effective palliative care approach during the end of life. A community-based palliative care approach involving the multidisciplinary health team or the neighborhood network in palliative care model adopted in Kerala which is a community owned and driven by volunteers can be considered.[11]

Conclusion

The study has brought out the various end-of-life practices that are followed in rural areas to hasten death of their dear ones who are terminally ill. It also highlights the perception behind such practices. These insights can be targeted on giving awareness to the people and bring about changes in rural society. However, strong are these practices intertwined in our society under the cover of "dignified death," it has to be considered as an act of euthanasia and the sufferings of the elderly cannot be neglected. Hence, such practices have to be curtailed by bringing about awareness and penalizing such acts. Effective elderly care and whenever facilities are available, the Government should consider offering home-based care for the elderly. It should be incorporated into the primary health-care system.

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Conflicts of interest

There are no conflicts of interest.

References

1. *Landmark Ruling, Supreme Court Says Passive Euthanasia is Permissible.* [Last accessed on 2018 Dec 02]. Available from: https://www.indiatimes.com/news/politics-and-nation/landmark-ruling-supreme-court-says-passive-euthanasia-is-permissible-with-riders/articleshow/63228770.cms .

2. *World Hospice and Palliative Care Alliance. Global Atlas on Palliative Care at the End of Life.* London: World Hospice and Palliative Care Alliance; 2014. [Google Scholar]

3. Bhatnagar S, Gupta M. Future of palliative medicine. *Indian J Palliat Care.* 2015;21:95–104. [PMC free article] [PubMed] [Google Scholar]

4. Sharma H, Jagdish V, Anusha P, Bharti S. End-of-life care: Indian perspective. *Indian J Psychiatry.* 2013;55:S293–8. [PMC free article] [PubMed] [Google Scholar]

5. Shahina KK. *Mother, shall i kill you?* [Last accessed on 2018 Aug 20]. Available from: https://shahinanafeesa.wordpress.com/2010/11/20/mother-shall-i-put-you-tosleep/

6. Bhattacharya S, Bhattacharya S. What happens to the “hand that rocked the cradle”? A study of elderly abuse in India. *J Adult Prot.* 2014;16:166–79. [Google Scholar]

7. Chokkanathan S, Lee AE. Elder mistreatment in urban India: A community based study. *J Elder Abuse Negl.* 2005;17:45–61. [PubMed] [Google Scholar]

8. *Rural Indian: Definition of a Rural Area.* [Last accessed on 2018 Dec 03]. Available from: https://www.archive.india.gov.in/citizen/graminbharat/graminbharat.php .

9. Sellamuthu G. Social Determinants of Senicide, a Cultural Killing of Elderly People in South Tamilnadu: An Empirical Reflection. Third ISA Forum of Sociology. 2016 [Google Scholar]

10. [Last accessed on 2018 Aug 10]. Available from: http://www.who.int/violence_injury_prevention/violence/global_campaign/en/chap5.pdf .

11. Kumar SK. Kerala, India: A regional community-based palliative care model. *J Pain Symptom Manage.* 2007;33:623–7. [PubMed] [Google Scholar]

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Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019

Update From the GBD 2019 Study

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Abstract

Cardiovascular diseases (CVDs), principally ischemic heart disease (IHD) and stroke, are the leading cause of global mortality and a major contributor to disability. This paper reviews the magnitude of total CVD burden, including 13 underlying causes of cardiovascular death and 9 related risk factors, using estimates from the Global Burden of Disease (GBD) Study 2019. GBD, an ongoing multinational collaboration to provide comparable and consistent estimates of population health over time, used all available population-level data sources on incidence, prevalence, case fatality, mortality, and health risks to produce estimates for 204 countries and territories from 1990 to 2019.

Prevalent cases of total CVD nearly doubled from 271 million (95% uncertainty interval [UI]: 257 to 285 million) in 1990 to 523 million (95% UI: 497 to 550 million) in 2019, and the number of CVD deaths steadily increased from 12.1 million (95% UI:11.4 to 12.6 million) in 1990, reaching 18.6 million (95% UI: 17.1 to 19.7 million) in 2019. The global trends for disability-adjusted life years (DALYs) and years of life lost also increased significantly, and years lived with disability doubled from 17.7 million (95% UI: 12.9 to 22.5 million) to 34.4 million (95% UI:24.9 to 43.6 million) over that period. The total number of DALYs due to IHD has risen steadily since 1990, reaching 182 million (95% UI: 170 to 194 million) DALYs, 9.14 million (95% UI: 8.40 to 9.74 million) deaths in the year 2019, and 197 million (95% UI: 178 to 220 million) prevalent cases of IHD in 2019. The total number of DALYs due to stroke has risen steadily since 1990, reaching 143 million (95% UI: 133 to 153 million) DALYs, 6.55 million (95% UI: 6.00 to 7.02 million) deaths in the year 2019, and 101 million (95% UI: 93.2 to 111 million) prevalent cases of stroke in 2019.

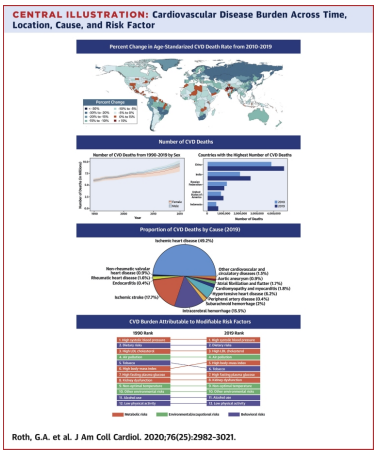
Cardiovascular diseases remain the leading cause of disease burden in the world. CVD burden continues its decades-long rise for almost all countries outside high-income countries, and alarmingly, the age-standardized rate of CVD has begun to rise in some locations where it was previously declining in high-income countries. There is an urgent need to focus on implementing existing cost-effective policies and interventions if the world is to meet the targets for Sustainable Development Goal 3 and achieve a 30% reduction in premature mortality due to noncommunicable diseases.

Key Words: cardiovascular diseases, global health, health policy, population health

Abbreviations and Acronyms: AC, alcoholic cardiomyopathy; AF, atrial fibrillation; AFL, atrial flutter; BMI, body mass index; CAVD, calcific aortic valve disease; CHA, congenital heart anomalies; CKD, chronic kidney disease; CVD, cardiovascular disease; DALYs, disability-adjusted life years; GBD, Global Burden of Diseases, Injuries, and Risk Factors Study; HAP, household air pollution; HHD, hypertensive heart disease; HICs, high-income countries; ICD, International Classification of Diseases; IHD, ischemic heart disease; IKF, impaired kidney function; IS, ischemic stroke; LDL, low-density lipoprotein; LMICs, low- and middle-income countries; LPA, low physical activity; MV, mitral

valve; PAD, peripheral artery disease; PM, particulate matter; RHD, rheumatic heart disease; SBP, systolic blood pressure; SDI, sociodemographic index; TMREL, theoretical minimum risk exposure level; UI, uncertainty interval; YLDs, years lived with disability; YLLs, years of life lost

Central Illustration



Cardiovascular disease (CVD) remains a major cause of premature mortality and rising health care costs (1,2). Cardiometabolic, behavioral, environmental, and social risk factors are major drivers of CVD. Consistent, comparable, and systematic analysis of long-term trends and patterns in global CVD are essential to guide public policy and provide benchmarks for decision makers. Beginning with ischemic heart disease (IHD) and stroke, this article provides information on the burden of CVD, including 13 underlying causes of cardiovascular death and 9 related risk factors at the global, regional, and national levels (Supplemental Figure 1).

This paper explores CVD trends from 1990 to 2019 and examines the extent to which population growth and aging explain the observed trends, sex differences, and regional patterns and how the epidemiology of the disease itself is changing. These trends show us where in the world CVD mortality and burden are increasing or declining and where progress has stalled (3). For each of the contributing causes of cardiovascular death and risk factors examined, we identify which regions and countries have the highest and lowest estimates of prevalent cases and number of deaths, as well as summary measures including number of years of life lost (YLLs), number of years lived with disability (YLDs), and the magnitude and temporal trends in disability-adjusted life years (DALYs) (1). For each section, the article also addresses how the summary measures of health and disease discussed inform investments in cardiovascular research, their implications for clinical and public health practice, and implications for health system development and national and regional policy.

To improve accessibility across a wide range of audiences, we have structured the review such that each section can be read independently for those most interested in a subset of causes or risks. Discussion pertinent to each topic is included within every section. This article is a collaborative effort involving the *Journal of the American College of Cardiology*, the National Heart, Lung, and Blood Institute, and the Institute for Health Metrics and Evaluation at the University of Washington designed to provide crucial population-level information that can guide action for CVD and risk factor prevention, treatment, and control (2).

Global Burden of Disease Study Methods

GBD 2019 is a multinational collaborative research study that estimates disease burden for every country in the world (1,4). The study is an ongoing effort, updated annually, and is designed to allow for consistent comparison over time from 1990 to 2019, by age and sex, and across locations. The study produces standard epidemiological measures such as incidence, prevalence, and death rates as well as summary measures of health, such as DALYs. DALYs represent the sum of years of life lost prematurely and years lived with disability; can be estimated from life tables, estimates of prevalence, and disability weights; and may be expressed as counts or rates. Annual updates to the study include new diseases, new data sources, and updates to methods. All results are available via the GBD Compare website (5), and all input data is identified via the Global Health Data Exchange website (6). The study is performed in compliance with Guidelines for Accurate and Transparent Health Estimates Reporting (GATHER) guidelines for reporting health estimates (1). Detailed methods of GBD 2019 are reported in the Supplemental Appendix and summarized here.

Each CVD cause and related health states were identified with standard case definitions. IHD represented acute myocardial infarction, chronic stable angina, chronic IHD, and heart failure due to IHD. Myocardial infarction was defined according to the Fourth Universal Definition of Myocardial Infarction and was adjusted to include out-of-hospital sudden cardiac death. Stable angina was defined according to the Rose Angina Questionnaire. Stroke was defined according to the World Health Organization definition and was estimated separately for 3 subcategories: 1) ischemic stroke (IS); 2) intracerebral hemorrhage; and 3) subarachnoid hemorrhage (7). Lower extremity peripheral artery disease (PAD) was defined by an ankle brachial index of <0.9. Symptomatic PAD was defined as self-report of symptoms of claudication among those with an ankle brachial index of <0.9. Atrial fibrillation (AF) and atrial flutter (AFL) were defined by electrocardiogram. Hypertensive heart disease (HHD) was defined as symptomatic heart failure due to the direct and long-term effects of hypertension. Cardiomyopathy was defined as symptomatic heart failure due to primary myocardial disease or toxin exposure to the myocardium, such as alcohol. Acute myocarditis was defined as an acute and time-limited condition due to myocardial inflammation using health system administrative data. Endocarditis and rheumatic heart disease (RHD) were defined by their clinical diagnosis. Estimates of RHD include cases identified by clinical history and physical examination, including auscultation or standard echocardiographic criteria for definite disease.

Mortality was estimated by using vital registration data coded to the International Classification of Disease (ICD) system or household mortality surveys known as verbal autopsy. Statistical methods were used to increase the comparability of mortality data sources, including the reclassification of codes that are nonspecific or unspecified, noise reduction algorithms, and Bayesian geospatial regression software (CODem, the cause of death ensemble model, Institute for Health Metrics and Evaluation, Seattle, Washington) that used location-specific covariates to create smoothed time trends for 204 countries and territories by borrowing strength over age, space, and time. GBD 2019 allowed for the production of estimates with uncertainty intervals (UIs) for all locations in every year, even when data were sparse or missing. Disease incidence and prevalence were produced by using a broad range of population-representative data sources identified by literature review and via study collaborations, including scientific reports of cohorts and registries, population surveys, microdata from registry and cohort studies, and health system administrative data. Consistent disease estimates were produced by using epidemiologic state-transition disease modeling software, DisMod-MR (Institute for Health Metrics and Evaluation), and Bayesian meta-regression software, MR-BRT (Institute for Health Metrics and Evaluation), that adjusted for study-level differences in measurement

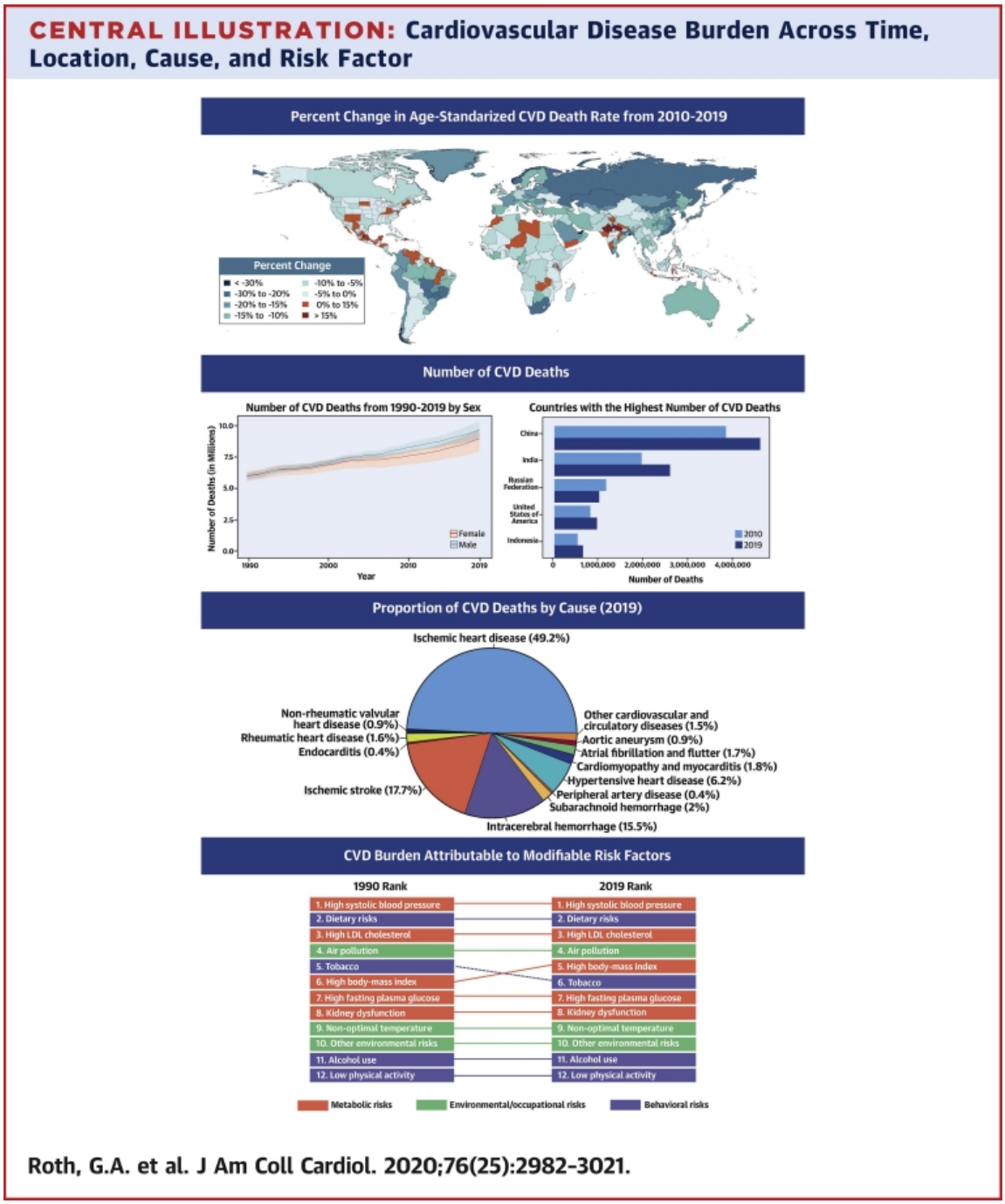
methods and case definitions. Risk factor exposures were estimated by using population-representative survey and surveillance data and geospatial Gaussian process regression models that borrowed strength across time and geography.

DALYs were calculated as the sum of YLLs, based on a reference maximum observed life expectancy, and YLDs based on standardized disability weights for each health state. Population-attributable fractions were calculated independently by risk factor by using risk exposures, estimates of relative risk based on meta-analyses, and theoretical minimum risk levels determined for each risk-outcome pair. Adjustment was made for comorbidity by simulating 40,000 individuals in each age-sex-country-year exposed to the independent probability of acquiring conditions based on their prevalence. The 95% UIs reported for each estimate used 1,000 draws from the posterior distribution of models, reported as the 2.5th and 97.5th values of the distribution. Age standardization was performed via the direct method, applying a global age structure from the year 2019. The University of Washington Institutional Review Board Committee approved the Global Burden of Diseases, Injuries, and Risk Factors Study (STUDY00009060).

Cardiovascular Diseases

Total CVDs

CVDs are common, have poor survival, and are increasing worldwide (Central Illustration). Prevalent cases of total CVD nearly doubled from 271 million (95% UI: 257 to 285 million) in 1990 to 523 million (95% UI: 497 to 550 million) in 2019, and the number of CVD deaths steadily increased from 12.1 million (95% UI: 11.4 to 12.6 million) in 1990, reaching 18.6 million (95% UI: 17.1 to 19.7 million) in 2019 (Figure 1A). The global trends for DALYs and YLLs also increased significantly, and YLDs doubled from 17.7 million (95% UI: 12.9 to 22.5 million) to 34.4 million (95% UI: 24.9 to 43.6 million) over that period.



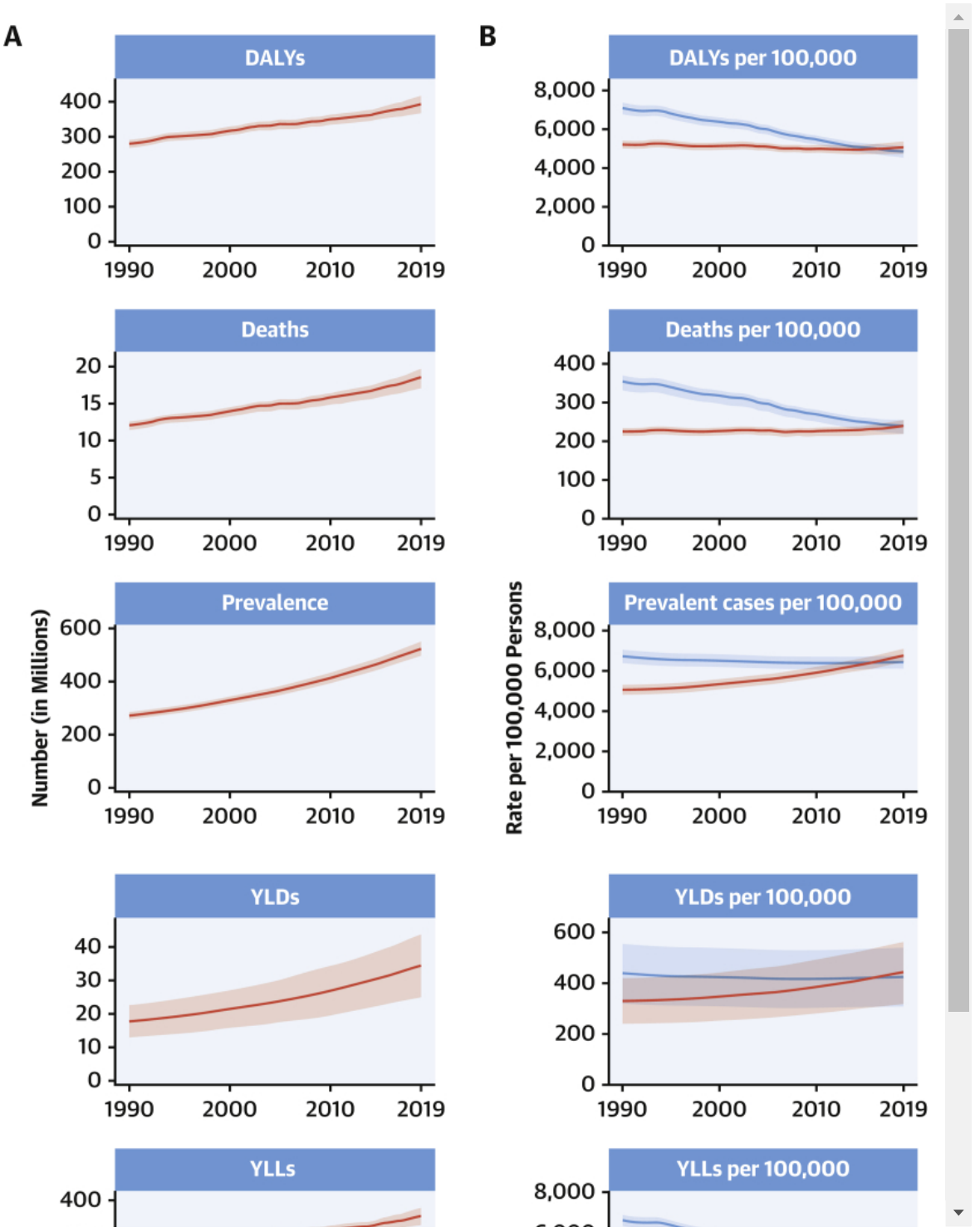
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Central Illustration

Cardiovascular Disease Burden Across Time, Location, Cause, and Risk Factor

Percent Change in Age-Standardized CVD Death Rate from 2010-2019. Map of the percent change in age-standardized CVD mortality rate from 2010 to 2019.**Number of CVD Deaths.** Total number of deaths due to CVD by sex, 1990 to 2019; total number of deaths due to CVD in 2010 and 2019 among the countries with the highest number of CVD deaths in 2019. **Proportion of CVD Deaths by Cause (2019)**. Proportion of total CVD deaths in 2019 by underlying causes. **CVD Burden Attributable to Modifiable**

Risk Factors. Comparison of the rankings of CVD DALYs attributable to modifiable risk factors in 1990 and 2019. CVD = cardiovascular disease; DALYs = disability-adjusted life years; LDL = low-density lipoprotein.



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Figure 1

Total Numbers and Rates of Cardiovascular Diseases

(A) Total number of DALYs, deaths, prevalent cases, YLDs, and YLLs due to cardiovascular diseases, 1990 to 2019. **Shaded region**s represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, prevalence, YLD, and YLL rates of cardiovascular diseases, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. DALYs = disability-adjusted life years; YLDs = years lived with disability; YLLs = years of life lost.

At the country level, age-standardized mortality rates for total CVD were highest in Uzbekistan, Solomon Islands, and Tajikistan and were lowest in France, Peru, and Japan, where rates were 6-fold lower in 2019. From 1990 to 2019, large declines in the age-standardized rates of death, DALYs, and YLLs, together with small gradual reductions in age-standardized rates for prevalent cases and YLDs, suggest that population growth and aging are major drivers of the increase in total CVD (Figure 1B).

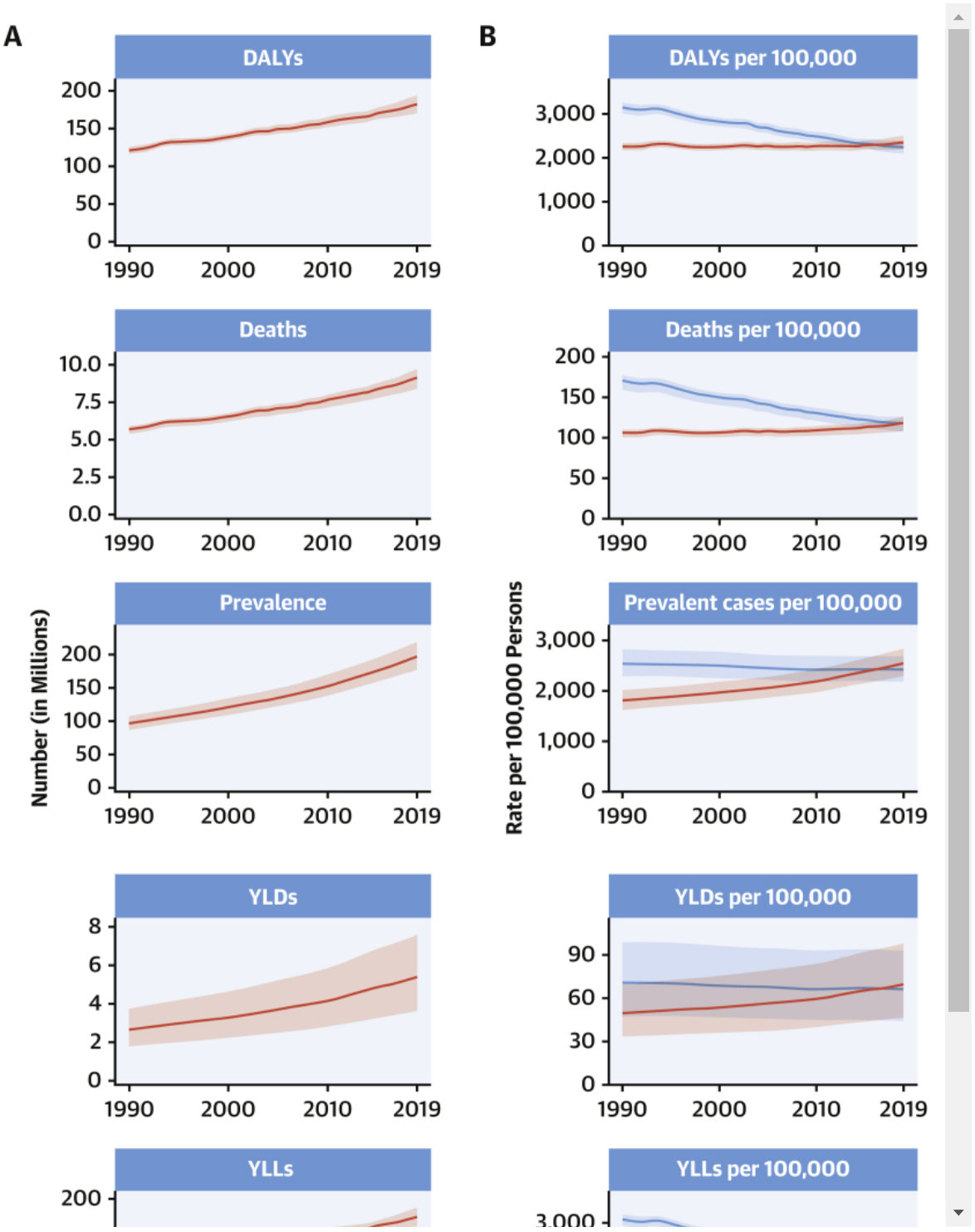
In 2019, total CVD DALYs were higher in men than women before age 80 to 84 years (Supplemental Figure 2). After this age, the pattern reverses. The sex differences in DALYs is most striking between ages 30 and 60 years (men greater) and age >80 years (women greater). The excess CVD deaths in women beginning at ages 80 to 84 years should focus attention to cause-specific mortality at older ages and have implications for secondary prevention strategies.

Among women, the age-standardized rates for DALYs were highest in Central Asia, Oceania, North Africa and the Middle East, and Eastern Europe; and lowest in High-Income Asia Pacific, Australasia, and Western Europe (Supplemental Figure 3). Among men, age-standardized rates for DALYs were highest in Central Asia, Eastern Europe, and Oceania; and lowest in High-Income Asia Pacific, Australasia, Western Europe, and Andean Latin America. At the country level, the highest age-standardized rates were estimated for many of the islands of Oceania, Uzbekistan, and Afghanistan, while the lowest rates for DALYs were seen in Japan, France, and Israel (Supplemental Figure 4). These regional and national differences in total CVD burden and mortality reflect differences in prevalence of CVD risk factors as well as access to health care (8). Differences in access to effective primary and secondary prevention strategies may also play a role in differences in total CVD burden, especially in low- and middle-income countries (LMICs) (9).

Global patterns of total CVD have significant implications for clinical practice and public health policy development (10). Prevalent cases of total CVD are likely to increase substantially as a result of population growth and aging, especially in Northern Africa and Western Asia, Central and Southern Asia, Latin America and the Caribbean, and Eastern and Southeastern Asia, where the share of older persons is projected to double between 2019 and 2050 (11,12). Increased attention to promoting ideal cardiovascular health and healthy aging across the lifespan is necessary (13). Equally importantly, the time has come to implement feasible and affordable strategies for the prevention and control of CVD and to monitor results (14).

Ischemic heart disease

The total number of DALYs due to IHD has risen steadily since 1990, reaching 182 million (95% UI: 170 to 194 million) DALYs and 9.14 million (95% UI: 8.40 to 9.74 million) deaths in the year 2019 (Figure 2A). GBD 2019 estimated 197 million (95% UI: 178 to 220 million) prevalent cases of IHD in 2019.



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Figure 2

Total Numbers and Rates of Ischemic Heart Disease

(A) Total number of DALYs, deaths, prevalent cases, YLDs, and YLLs due to ischemic heart disease, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, prevalence, YLD, and YLL rates of ischemic heart disease, 1990 to 2019**. Shaded regions** represent 95% uncertainty intervals. Abbreviations as in Figure 1.

Global age-standardized rates for DALYs, deaths, and prevalent cases declined over this time period, indicating that, on average, global increases in IHD have been due to population growth and aging, although even the age-standardized death rate is estimated to be increasing in many locations across South, East, and Southeastern Asia, including China (Figure 2B). Increasing absolute numbers of incident and prevalent IHD cases in most countries mean that national health systems will need to address increasing demand for IHD-related preventive and therapeutic services as these trends continue.

At the global level, substantially more total DALYs due to IHD were experienced by men than women. IHD DALYs rose rapidly for men beginning at age 30 years (Supplemental Figure 5). Men ages 45 to 49 years had almost as many DALYs due to IHD as women ages 65 to 69 years.

Age-standardized DALY rates due to IHD were highest in Eastern Europe, Central Asia, Oceania, and the Middle East/North Africa regions (Supplemental Figure 6). At the country level, extremely high rates were estimated for Uzbekistan, Ukraine, Tajikistan, and many of the islands of Oceania, with the lowest levels in Japan, Republic of Korea, and France (Supplemental Figure 7). Higher exposure to risks including tobacco and excessive alcohol use and restricted access to preventive health care may partly explain these patterns.

IHD remains a major threat to public health, and overall burden is increasing globally. In some locations over the past 5 years, including parts of the United States and United Kingdom, age-standardized IHD death rates are increasing, suggesting that long-term declines in IHD due to improved prevention and health care are no longer occurring in these locations. Health systems and countries need to focus on delivering effective interventions that will reverse these trends, including those that prevent and control diabetes, decrease obesity and high cholesterol, improve diet and physical activity, reduce tobacco and excessive alcohol use, integrate “polypills” for elevated blood pressure and related conditions, improve pre- and in-hospital care for acute coronary syndrome, and improve survival and quality of life for those living with the long-term sequelae of IHD, including heart failure. Social and economic factors remain fundamental drivers of IHD, suggesting that multisectoral interventions are needed to eradicate this disease (15).

Stroke

The total number of prevalent strokes, deaths, and DALYs due to stroke increased steadily from 1990, reaching 101 million (95% UI: 93.2 to 111 million) prevalent (85.3% [95% UI: 82.6% to 88.2%] increase) stroke survivors, 6.55 million (95% UI: 6.00 to 7.02 million) deaths from stroke (43.3% [95% UI: 31.0% to 55.4%] increase), and 143 million (95% UI: 133 to 153 million) DALYs due to stroke (32.4% [95% UI: 22.0% to 42.2%] increase) in 2019, with the bulk of the burden outside of the high-income world (Supplemental Figures 8A and 9A). Of 12.2 million (95% UI: 11.0 to 13.6 million) incident stroke cases, 7.63 million (95% UI: 6.57 to 8.96 million) (62.4%) were IS, 3.41 million (95% UI: 2.97 to 3.91 million) (27.9%) were intracerebral hemorrhages, and 1.18 million (95% UI: 1.01 to 1.39 million) (9.7%) were subarachnoid hemorrhages (Supplemental Figures 10A, 11A, and 12A).

Globally, age-standardized rates for deaths and DALYs due to stroke substantially declined over the same period of time (Supplemental Figures 8B, 10B, 11B, and 12B), suggesting that: 1) preventive measures are very effective at lowering risk of both ischemic and hemorrhagic stroke; and 2) on average, global increases in stroke burden have been largely due to population growth and aging.

Importantly, age-standardized rates for prevalence of stroke survivors have increased since 1990 in several locations including in China, Indonesia, and parts of the United States. Age-standardized death rates also increased in some locations, including Indonesia and the Philippines.

Age-standardized rates of DALYs and deaths due to stroke were substantially greater in men compared to women, but prevalence was greater in women, suggesting the possibility of greater risk of death and disability in men but better stroke survival in women. Similar patterns were observed in men and women with IS, intracerebral hemorrhage, and subarachnoid hemorrhage.

There is tremendous regional disparity in the burden of stroke. Age-standardized rates of deaths and DALYs due to stroke were highest in Oceania, Central Asia, East Asia, Southeast Asia, Eastern Europe, and sub-Saharan Africa (Supplemental Figure 13), and prevalence of stroke survivors was highest in Oceania, Southeast Asia, East Asia, and the Middle East/North Africa regions (Figure 3). These data suggest that applying preventive strategies, such as treatment of elevated blood pressure and cholesterol levels, can have major health benefits where stroke burden remains high, especially in those regions that did not show substantial decline over the past decades (i.e., Central Asia, Southern sub-Saharan Africa).

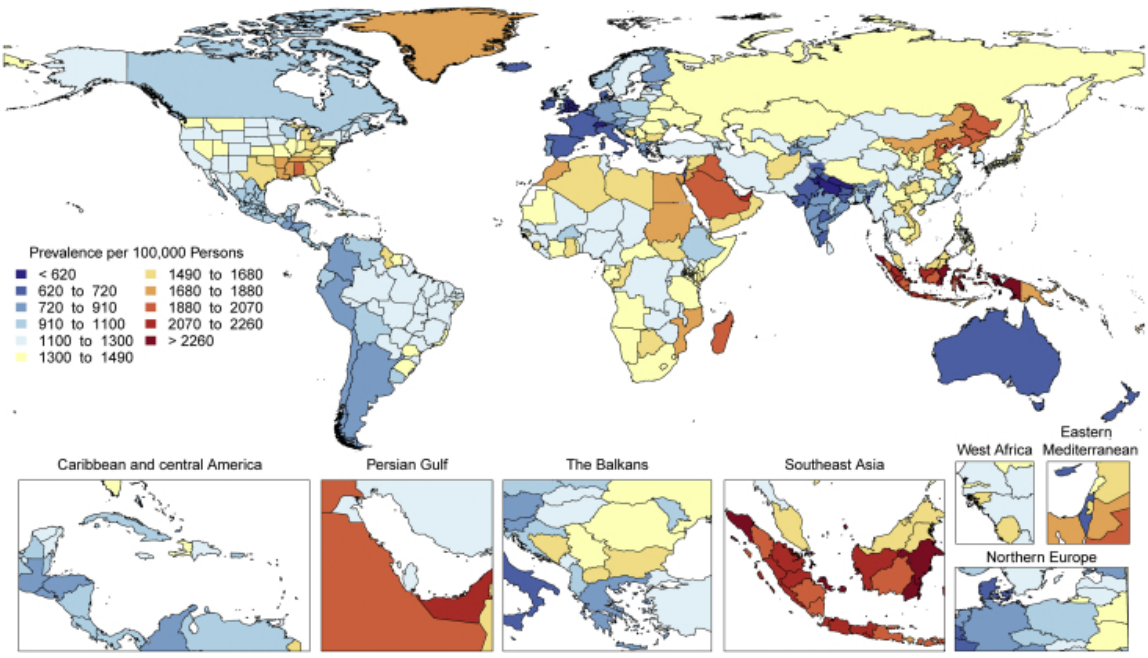


Figure 3

Map of Age-Standardized Prevalence of Stroke Survivors in 2019

Stroke remains the second-leading cause of death, and stroke burden in terms of DALYs is increasing. Primary stroke prevention strategies are not sufficiently effective as currently implemented in many countries. Large (15- to 20-fold) geographic variations in age-standardized stroke DALYs and mortality rates and moderate (4-fold) geographic variations in age-standardized prevalence may be related to the variations in stroke risk factors or the quality of and/or access to preventative care, acute stroke care, and stroke rehabilitation across the globe. Global and national health systems need to focus on new

approaches for delivering stroke prevention that can reverse these trends, including strategies to provide equal access to quality health care and prevention across all populations, and proven effective interventions that reduce mortality and improve outcomes post-stroke.

Hypertensive heart disease

The global prevalence of HHD has risen steadily over the last 3 decades, as have the total number of deaths, DALYs, YLLs, and YLDs due to this disease. In 2019, HHD was the main cause of 1.16 million (95% UI: 0.86 to 1.28 million) deaths and 21.5 million (95% UI: 16.4 to 23.9 million) DALYs annually, with a global prevalence of 18.6 million (95% UI: 13.5 to 24.9 million) cases (Supplemental Figure 14A).

The age-standardized prevalence and YLDs of HHD per 100,000 persons have been constant over time, and corresponding age-standardized rates of deaths, DALYs, and YLLs declined steadily until the mid-2000s and have leveled off since then (Supplemental Figure 14B). The trends for the absolute and age-standardized estimates closely resemble those for high blood pressure. Taken together, the increasing global prevalence and rates of HHD can be explained by population growth and aging, but the declining age-standardized rates of adverse outcomes are similar to those for IHD and stroke and are likely an effect of improved secondary prevention.

The age distributions of DALYs due to HHD are very similar in women and men until age 70 years (Supplemental Figure 15). This contrasts to the age distributions for high blood pressure, in which men are overrepresented at younger ages. The similarities by sex in DALYs calls for an equal attention to the clinical prevention and treatment of HHD in women and men.

The highest age-standardized rates of DALYs due to HHD are noted in Africa (Figure 4). DALYs due to HHD are particularly higher in Central sub-Saharan Africa, followed by most of Africa except Western sub-Saharan Africa, and Oceania. A similar geographic pattern exists for high blood pressure (with the exception of high rates of DALYs in men from high blood pressure in Central Asia and Eastern Europe). An overrepresentation of DALYs due to HHD in women can be noted in Central and Eastern sub-Saharan Africa (Supplemental Figure 16). The significance of this is uncertain because no such peaks are noted for high blood pressure or other contributing factors, and it can represent regional differences in disease coding, including that of peripartum cardiomyopathy. Inequalities in access to both primary and secondary prevention are a potential cause of these regional and sex differences.

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Figure 4

Map of Age-Standardized DALYs Due to Hypertensive Heart Disease in 2019

DALYs = disability-adjusted life years.

The global prevalence of HHD and absolute rates of adverse outcomes are expected to continue to rise due to population growth and aging. The age-standardized rates of adverse outcomes due to HHD are no longer decreasing, and with continuing global increases in obesity and diabetes, corresponding increases in left ventricular hypertrophy and other aspects of HHD are likely. Intensified global and

regional efforts to lower blood pressure and control other risk factors are needed, for example with population-wide reductions in dietary sodium intake. Continued surveillance of inequalities in adverse outcomes due to high blood pressure are necessary to inform such efforts.

Congenital heart anomalies

A total of 3.12 million (95% UI: 2.40 to 4.11 million) babies were born with congenital heart anomalies (CHA) in 2019, representing 2,305.2 per 100,000 live births (95% UI: 1,772.9 to 3,039.2 per 100,000 live births), a total of 13.3 million (95% UI: 11.5 to 15.4 million) people were living with CHA, and CHA was the underlying cause of 217,000 deaths (95% UI: 177,000 to 262,000 deaths), of which 150,000 deaths (95% UI: 120,000 to 184,000 deaths) were in infants <1 year (Figure 5A). The all-ages rates of DALYs, YLLs, and YLDs for CHA in 2019 were 241.6 per 100,000 (95% UI: 196.1 to 292.7 per 100,000), 234.0 per 100,000 (95% UI: 189.8 to 285.7 per 100,000), and 7.6 per 100,000 (95% UI: 3.7 to 12.7 per 100,000), respectively (Figure 5B).

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Figure 5

Total Numbers and Rates of Congenital Heart Anomalies

(A) Total number and number among children younger than 1 year of DALYs, deaths, prevalent cases, YLDs, and YLLs due to congenital heart anomalies, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, prevalence, YLD, and YLL rates of congenital heart anomalies, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. Abbreviations as in Figure 1.

Between 1990 and 2019, the global CHA birth rate was largely unchanged, but the all-ages death rate due to CHA declined 60.4% (95% UI: –71.9% to –41.4%) from 7.1 per 100,000 (95% UI: 5.0 to 10.5 per 100,000) in 1990 to 2.8 per 100,000 (95% UI: 2.3 to 3.4 per 100,000) in 2019. Age-standardized prevalence, death, and DALY rates remain lower than all-ages rates in low-income countries because of younger populations, higher birth rates of CHA, and much less access to the intervention that is needed to allow for the survival of children born with CHA.

The proportion of all infant deaths caused by CHA increased in all quintiles of the sociodemographic index (SDI) since 1990 except the highest because improvement in CHA mortality has lagged behind that of other causes, most notably infectious, respiratory, and diarrheal diseases (16). In low-SDI regions, CHA death rates have declined by 42.1% (95% UI: –61.9% to 12.1%) since 1990, compared with declines of 71.3% (95% UI: –74.7% to –64.6%) in high-SDI subgroups. Unlike other CVDs, prevention has limited ability to reduce the burden of CHA after birth; improvements in early diagnosis and access to cardiac surgery are the only solutions.

The total number of global CHA births remained steady, global CHA deaths decreased by 42.7% (95% UI: –59.3% to –15.2%), and the global number of individuals living with CHA increased by 28.2% (95% UI: 26.3% to 30.1%). There were increases in the 15- to 49-year and the 50- to 69-year age groups, in which the number of individuals living with CHA grew by 41.6% (95% UI: 40.0% to 43.2%) and 117.3% (95% UI: 114.7% to 120.0%) to 4.21 million (95% UI: 3.67 to 4.76 million) and

1.31 million (95% UI: 1.13 to 1.51 million), respectively, reflecting a cohort effect from the time of birth. Most of the prevalence increase occurred outside of high-income countries (HICs) and was due to improvements in survival and population growth. Health systems will be increasingly burdened with adolescents and adults needing care for their congenital heart conditions.

The very young age distribution in LMICs and large burden of YLLs due to CHA should drive a global mandate for investment in data collection and resources to expand access to infant heart surgery in regions in most need. In low-income countries with very young populations, the prevalence and mortality from RHD parallel lack of access to cardiac surgery services for CHA, and pooling resources to care for both of these diseases makes a stronger case for investment in surgical services (that includes both in-country surgical personnel and nongovernmental organizations) than for either disease alone.

Rheumatic heart disease

The prevalence of RHD has been rising steadily since 1990, reaching 40.5 million (95% UI: 32.1 to 50.1 million) currently affected in 2019 (Supplemental Figure 17A). Deaths decreased until 2012 but have stabilized since then and even started increasing since 2017 (306,000 [95% UI: 259,000 to 340,000] in 2019). DALYs and YLLs have slowly decreased to 10.7 million (95% UI: 9.21 to 12.1 million) and 8.68 million (95% UI: 7.43 to 9.77 million), respectively, in 2019, whereas YLDs have increased to 1.99 million (95% UI: 1.20 to 3.04 million).

Age-standardized rates for RHD prevalence have closely tracked with all-age rates, but age-standardized mortality has exceeded all-age mortality until the past few years, highlighting the differential mortality risk due to RHD observed in regions with differing age structures and levels of development (Supplemental Figure 17B). The narrowing of this differential tells a positive story about RHD care, although the dominance of China and India in the overall figures may mask persistent differentials in other high-prevalence countries and regions. The continuing increase in prevalence cannot be attributed to changes in age structure but likely reflects increased global awareness, the increasing availability of echocardiography for case definition, improved survival in some places, and the chronic nature of RHD.

Globally, the prevalence of RHD is estimated to peak between 20 and 29 years, remain relatively stable until 40 years, and then begin a steady decline, likely reflecting decreasing survival at older ages. Sex distribution is equal until the age of 15 years, after which women bear a higher burden in terms of prevalence across nearly all world regions. Higher rates of RHD in post-pubertal females are well described, but incompletely understood, and may reflect the interaction of biological, social, and environmental risk factors.

RHD burden continues to show substantial global heterogeneity (Supplemental Figure 18). The highest age-standardized DALY rates are seen in Oceania and South Asia (627.4 per 100,000 [95% UI: 404.1 to 918.0 per 100,000] and 348.5 per 100,000 [95% UI: 272.4 to 412.2 per 100,000], respectively) with the lowest in the highest-income regions (<25 per 100,000). Furthermore, although some regions have shown large declines between 1990 and 2019 (Eastern and Central Europe, East Asia, Central Latin America, high-income Asia Pacific, and North Africa and the Middle East), there are also regions with only modest improvements and regions where very little improvement has been seen (Oceania, South Asia, the Caribbean, and sub-Saharan Africa). There is also substantial within-region national and subnational variability in RHD DALYs, some examples of which include substantially higher RHD DALYs in Guyana and Haiti compared to the rest of Latin America and the Caribbean and the heterogeneity seen in the subnational data on RHD DALYs from India (Figure 6).

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Figure 6

Map of Age-Standardized DALYs Due to Rheumatic Heart Disease in 2019

DALYs = disability-adjusted life years.

RHD burden is highest among the world's most disadvantaged populations. The most marginalized and poorest populations regionally, nationally, and at a subnational level are not showing signs of improvement and continue to die early from RHD. Health systems in LMICs should support the recommendations of the 2018 World Health Assembly Global RHD Resolution with increased multisector investment in primary health care, improved sanitation and housing, infrastructure, secure medication supply chains, evidence-based RHD screening, prevention and management, and tertiary capacity to care for patients at the severe end of the RHD spectrum. RHD burden could be greatly decreased with an effective group A streptococcal vaccine, with several promising candidates currently in development (17). Future data collection is needed at subnational levels to identify and monitor populations with ongoing high RHD burdens.

Cardiomyopathy and myocarditis

DALYs due to cardiomyopathy and myocarditis have increased from 7.06 million (95% UI: 6.30 to 8.63 million) to 9.14 million (95% UI: 7.86 to 10.0 million) over the past 30 years, a pattern that is also seen in the rise of deaths from 238,000 (95% UI: 212,000 to 257,000) to 340,000 (95% UI: 285,000 to 371,000) (Supplemental Figure 19A). However, over the same period, the age-standardized rate of death has decreased from 8.0 per 100,000 (95% UI: 6.4 to 8.6 per 100,000) to 5.6 per 100,000 (95% UI: 4.5 to 6.3 per 100,000) in men and 5.8 per 100,000 (95% UI: 4.4 to 6.4 per 100,000) to 3.3 per 100,000 (95% UI: 2.7 to 3.6 per 100,000) in women. The age-standardized morbidity and mortality for men and women in 2019 remains different, at 6.5 per 100,000 YLDs (95% UI: 4.3 to 9.3 per 100,000 YLDs) and 4.2 per 100,000 YLDs (95% UI: 2.8 to 6.0 per 100,000 YLDs) and 148.9 per 100,000 YLLs (95% UI: 120.2 to 168.7 per 100,000 YLLs) and 71.4 per 100,000 YLLs (95% UI: 61.0 to 79.9 per 100,000 YLLs), respectively.

The prevalence and related mortality of cardiomyopathy and myocarditis increase throughout adulthood in both sexes, with a larger proportion of cases in men than in women. When myocarditis alone is considered, a similar trend was observed. Referring to 2019, in the age between 35 and 39 years, when myocarditis can commonly occur (18,19), the rate of myocarditis is 6.1 per 100,000 (95% UI: 4.2 to 8.7 per 100,000) in men and 4.4 per 100,000 (95% UI: 3.0 to 6.3 per 100,000) in women. Similar figures can be found in the range of ages between 20 and 44 years.

The increased prevalence associated with aging is more pronounced in cardiomyopathies than in myocarditis. Approximately a 6-fold higher age-related increase of other cardiomyopathy was observed in men between 35 and 39 years (10.8 per 100,000 [95% UI: 6.5 to 17.4 per 100,000]) versus those between 80 and 84 years (698.5 per 100,000 [95% UI: 429.6 to 1064.8 per 100,000]) compared with myocarditis in men of 35 to 39 years (6.1 per 100,000 [95% UI: 4.2 to 8.7 per 100,00]) versus those of 80 to 84 years (63.0 per 100,000 [95% UI: 43.6 to 87.9 per 100,000]). The relatively greater rise of other cardiomyopathy compared to myocarditis prevalence with age is constant, from 1990 to 2019.

Myocarditis-related mortality rate between 35 and 39 years was 0.2 per 100,000 (95% UI: 0.2 to 0.3 per 100,000) in men compared to 0.1 per 100,000 (95% UI: 0.1 to 0.2 per 100,000) in women in 2019. Alternatively, myocarditis resulted in death in 1 in 72 men (585 deaths per 42,200 incident cases) and 1 in 87 (324 deaths per 28,100 incident cases) women in this age bracket who were diagnosed in 2019. Between ages 80 and 84 years, the rate was higher, at 1 death for every 19 incident cases of myocarditis in men (1,800 deaths per 34,400 incident cases) and 1 death for every 15 incident cases of myocarditis in women (2,260 deaths per 34,900 incident cases).

Marked regional variations in other cardiomyopathy in age-standardized DALYs in both sexes were observed in 2019 (Supplemental Figure 20). These ranged from 5 to 46 DALYs per 100,000 in South Asia, East Asia, Andean Latin America, high-income Asia Pacific, and Central Latin America to 96 to 147 per 100,000 in Oceania, the Caribbean, high-income North America, and Western and Eastern sub-Saharan Africa to 230 to 271 per 100,000 in Central Asia, Eastern Europe, and Southern and Central sub-Saharan Africa. These regional variations may be explained by a higher prevalence of peripartum cardiomyopathy in Africa, Chagas disease in Central and South America, and alcohol-related cardiomyopathy in parts of Central Europe and Russia (20).

Although the morbidity and mortality rates from cardiomyopathies and myocarditis collectively present a substantial global disease burden in 2019, the regional differences in the burden (Figure 7) suggest that public health interventions should be tailored to the specific etiologies of cardiomyopathy to lower these rates in the future.

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Figure 7

Map of Age-Standardized DALYs Due to Cardiomyopathy and Myocarditis in 2019

DALYs = disability-adjusted life years.

Alcoholic cardiomyopathy

The global prevalence of alcoholic cardiomyopathy (AC) estimated by GBD 2019 was 708,000 cases (95% UI: 545,000 to 924,000 cases), approximately 9.1 cases per 100,000 (95% UI: 7.0 to 11.9 cases per 100,000) (Supplemental Figure 21A). Globally, AC was responsible for 71,700 deaths (95% UI: 60,200 to 82,000 deaths), 2.38 million YLLs (95% UI: 2.00 to 2.73 million YLLs), and 60,100 YLDs (95% UI: 38,500 to 88,300 YLDs). The total number of DALYs due to AC was 2.44 million (95% UI: 2.05 to 2.78 million). After a rapid increase from 1999, DALYs, deaths, and YLLs started decreasing from 2005 to 2019. The global prevalence and YLDs due to AC are increasing.

All-age and age-standardized rates of DALYs, deaths, prevalent cases, YLDs, and YLLs declined from 2005 to 2019 (Supplemental Figure 21B). This indicates that the global increase in AC prevalence is related, in part, to population growth and aging. Several countries in East Asia and the Caribbean regions showed opposite trends, with increasing age-standardized values for almost all indicators. Factors that might explain these regional differences remain incompletely understood.

At the global level, substantially more total DALYs due to AC were experienced by men than women. DALYs from AC rose rapidly among men beginning from age 25 years, being significantly higher in men than women across all ages (Supplemental Figure 22). Women are generally considered more susceptible to alcohol-induced damages than men, which may reflect sex-specific differences in alcohol consumption, type, blood level, distribution, or metabolism. However, the higher level of alcohol consumption and the higher frequency of alcohol problems among men could justify the observed higher rate of DALYs.

Age-standardized DALYs due to AC were higher in Central Europe as well as in the Caribbean (Cuba), followed by Australasia, North America, Central Asia, Western Europe, and tropical Latin America (Figure 8). Extremely high rates were seen in Eastern Europe, particularly in Russia and Kyrgyzstan. The lowest levels were observed in sub-Saharan Africa, Andean Latin America, and North Africa and the Middle East. Geographic differences reflect drinking patterns among regions, for example, rates of binge drinking, as well as social and cultural behaviors, such as abstaining from alcohol.

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Figure 8

Map of Age-Standardized DALYs Due to Alcoholic Cardiomyopathy in 2019

DALYs = disability-adjusted life years.

AC represents substantial morbidity and mortality among young and middle-age people, particularly men. The age-standardized DALYs are high in several countries, and the true burden of disease could be underestimated because alcohol consumption is often underreported (21). Additional policy support for public education and awareness is needed to reduce the harmful use of alcohol. Because alcohol negatively affects cardiovascular function, increased clinician emphasis on eliminating alcohol use among people with coexisting CVD is advisable. Research should be aimed at understanding the exact mechanisms of disease, susceptibility to alcohol damage, effective public health interventions, and treatments for AC.

AF and AFL

The total number of DALYs due to AF and AFL increased progressively from 3.79 million (95% UI: 2.96 to 4.83 million) in 1990 to 8.39 million (95% UI: 6.69 to 10.5 million) in 2019 (Supplemental Figure 23A). GBD 2019 estimated 59.7 million (95% UI: 45.7 to 75.3 million) prevalent cases of AF/AFL in 2019, about a doubling compared to the prevalent cases in 1990.

When standardized for age, prevalence of AF/AFL, DALYS, and death rates did not display marked changes between 1990 and 2019 (Supplemental Figure 23B). The age-standardized AF/AFL prevalence rate was 775.9 per 100,000 (95% UI: 592.4 to 990.8 per 100,000) in 1990 and 743.5 per 100,000 (95% UI: 571.2 to 938.3 per 100,000) in 2019. Age-standardized rates for DALYs did not change substantially between 1990, when they were 110.0 per 100,000 (95% UI: 87.7 to 139.2 per 100,000), to 2019, when they were 107.1 per 100,000 (95% UI: 86.2 to 133.7 per 100,000). Similarly, the age-standardized death rates per 100,000 were similar in 1990 at 4.3 per 100,000 (95% UI: 3.7 to 5.1 per 100,000) and 4.4 per 100,000 (95% UI: 3.7 to 5.0 per 100,000).

Comparing total numbers with age-standardized numbers suggests that global increases in AF/AFL are largely attributable to aging of the population and population growth.

Globally, under the age of 70 years, more total DALYs due to AF were experienced by men than women (Supplemental Figure 24). Between 70 and 74 years, men and women had similar DALYs. However, at age 75 years and older, this trend was reversed, with higher total DALYs experienced by women. These trends were observed in 1990 as well as 2019. These findings point toward sex-specific, age-related targets for the prevention and management of AF/AFL.

Age-standardized DALY rates due to AF/AFL were highest in high-income North America, Australasia, Central Asia, and Europe and were observed to be lowest in the high-income Asia Pacific region (Figure 9). Also, Andean and Central Latin America, the Caribbean, sub-Saharan Africa, and North Africa and the Middle East displayed low rates. However, because of a general lack of data from these regions, more detailed studies should be performed. The reasons for this significant regional variation may include increased ascertainment in high-income regions, differential control of risk factors, and genetic factors. A detailed comparison of potential differentiating features between highest DALY rate versus lowest DALY rate regions could yield valuable additional information.

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Figure 9

Map of Age-Standardized DALYs Due to Atrial Fibrillation and Flutter in 2019

DALYs = disability-adjusted life years.

AF/AFL are the most common arrhythmias and continue to make a progressive and substantial impact on public health at a global level. In most regions, prevalence rates are increasing, suggesting that more effort is needed to improve prevention and health care for AF/AFL at a global level. Health systems and countries will need to focus their efforts to reverse these trends by aggressive attention to the reduction of risk factors such as hypertension, diabetes, and obesity; better treatment of individuals with IHD and heart failure; and improved access to medications for thromboembolism prophylaxis.

Aortic aneurysm

The total number of YLLs due to aortic aneurysm, including both thoracic and abdominal types, has increased steadily since 1990, reaching 3.32 million YLLs (95% UI: 3.11 to 3.52 million YLLs) and 172,000 deaths (95% UI: 157,000 to 183,000 deaths) in 2019 (Figure 10A).

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Figure 10

Total Numbers and Rates of Aortic Aneurysm

(A) Total number of deaths and YLLs due to aortic aneurysm, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages death and YLL rates of aortic aneurysm, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. YLLs = years of life lost.

Age-standardized rates for YLLs and deaths declined over this period, indicating that, overall, global increases in aortic aneurysm have been due to population growth and aging (Figure 10B). This likely reflects improvement in diagnosis and treatment of risk factors.

At the global level, men bore a substantially higher burden of YLLs due to aortic aneurysm compared with women. YLLs from aortic aneurysm rose most steeply in the sixth decade of life for men, peaking by age 70 years (Supplemental Figure 25). Women younger than age 75 years accounted for less than one-half of YLLs due to aortic aneurysm. The gap between men and women narrowed with increasing age, with YLLs in women overtaking those of men at age 90 years. This could be explained by the generally longer lifespan of women compared with men. Despite the lower prevalence of abdominal aortic aneurysm than men, growth rate in women has been shown to be more rapid, rupture during surveillance 4 times more likely, and likelihood of fatal rupture 3 times as high, even after adjustment for age (22, 23, 24). For thoracic aortic aneurysms, women have a lower incidence but are more likely to have fatal consequences than men (25).

Age-standardized YLLs due to aortic aneurysm were highest in Eastern and Central Europe as well as Southern and Tropical Latin America, with extremely high levels noted in Montenegro and parts of Brazil (Supplemental Figure 26). The lowest levels were observed in Andean Latin America, North Africa and the Middle East, and East Asia, including Mexico, Iraq, and China. Risk factors, including tobacco use and hypertension, as well as access to screening and preventive health care, may partially explain these patterns.

Aortic aneurysm remains a major public health issue, with the overall burden in terms of number of YLLs and deaths increasing globally. Because age-standardized rates for YLLs and death due to aortic aneurysm declined from 1990 to 2019, the increase may be attributable to population growth and aging. However, because the risk factors for this disease remain common, most national health systems will need to address increasing demand for preventive care, including low-cost screening modalities for abdominal aneurysms in the high-burden regions mentioned earlier. The risk factors underlying the development of aortic aneurysm are common to other CVDs, such as IHD. As such, investing health system resources in these risk factors, including hypertension management and smoking cessation, could reduce the burden of other diseases in addition to aortic aneurysm. Combined with implementation of inexpensive screening technology, such as ultrasonography, where indicated, the morbidity and mortality due to aortic aneurysm can be significantly decreased globally.

Nonrheumatic valvular heart disease: calcific aortic valve disease

Calcific aortic valve disease (CAVD) occurs commonly among older adults with a normal trileaflet aortic valve and with a greater frequency among those with a congenital bicuspid aortic valve. CAVD is clinically important because severe obstruction causes symptoms and left ventricular dysfunction warranting surgical or transcatheter valve replacement (26). The prevalence of CAVD increases with age and is >1,000 per 100,000 beyond the age of 75 years.

Globally, both the prevalence and age-standardized prevalence of CAVD have increased steadily over the last 3 decades (Supplemental Figure 27B). In 2019, the age-standardized prevalence rose to approximately 116.3 cases per 100,000 (95% UI: 100.4 to 134.5 cases per 100,000) people, from about 45.5 cases per 100,000 (95% UI: 37.6 to 54.7 cases per 100,000) people in 1990. This increase may be attributable to increased prevalence of atherosclerotic risk factors associated with the development and progression of CAVD (27). Although age-standardized YLDs due to CAVD have increased in parallel with prevalence, there has been no significant change in age-standardized DALYs, most likely because most of the DALYs due to CAVD accrue from YLLs, whereas deaths due to CAVD (and consequently, age-standardized YLLs) have not increased over the years. The ready availability of aortic valve replacement in regions of the world where CAVD is most prevalent may be an important check on mortality.

In HICs, the age-standardized prevalence of CAVD is similar among both men and women, while the prevalence among men exceeds that among women to varying degrees in the other regions of the world. Population-based studies of the prevalence of nonrheumatic valvular heart disease remain rare, and further research is needed to understand if sex differences in prevalence are due to differences in disease burden, health-seeking behavior, or rates of diagnosis.

There is substantial heterogeneity among countries in age-standardized prevalence and DALYs due to CAVD (Figure 11). Prevalence is low (<20 per 100,000) in several regions of the world, notably, sub-Saharan Africa (excluding the south), most of Asia (including South and Southeast Asia), North Africa and the Middle East, and Oceania. In contrast, age-standardized prevalence is much higher (>200 per 100,000) in Australasia; high-income Asia Pacific and North America; and Central, Eastern, and Western Europe. The difference may reflect higher prevalence of other competing illnesses and lack of testing for CAVD in LMICs. Despite the low prevalence of CAVD in LMICs, DALYs due to CAVD are disproportionately high, possibly reflecting the limited access to timely aortic valve replacement (28).

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Figure 11

Age-Standardized DALYs Due to Nonrheumatic Calcific Aortic Valve Disease in 2019 by Region

Age-standardized DALY rate of nonrheumatic calcific aortic valve disease by region and sex with 95% uncertainty intervals, 2019. DALYs = disability-adjusted life years.

As populations age, CAVD becomes an increasingly important cause of morbidity and mortality. The increase in reported prevalence of CAVD may be a consequence of both an increase in the prevalence of risk factors for atherosclerosis and an increased awareness and investigation to detect disease. Although major advances have been made in the treatment of end-stage CAVD, research on ways to prevent the onset and progression of disease requires additional investments. In the populous regions of

the world such as South Asia and Africa, despite the low prevalence of CAVD, the absolute number of people with CAVD is large. Countries will need to invest in health care infrastructure and health workers to provide timely valve interventions for these patients to reduce related morbidity and mortality.

Nonrheumatic valvular heart disease: degenerative mitral valve disease

The major cause of nonrheumatic degenerative mitral valve (MV) disease is MV prolapse (29,30). Untreated, this can lead to chronic mitral regurgitation, AF, and heart failure (31). The total number of DALYs due to degenerative MV disease has increased since 1990 and is responsible for 883,000 DALYs (95% UI: 754,000 to 1,090,000 DALYs) and 34,200 deaths (95% UI: 28,300 to 43,300 deaths) in 2019 (Supplemental Figure 28A). GBD 2019 estimated 24.2 million (95% UI: 23.1 to 25.4 million) prevalent cases of degenerative MV disease in 2019.

Age-standardized rates for DALYs, deaths, and prevalent cases of degenerative MV disease declined over this time period, suggesting that the global increases in MV disease have been due to population growth and aging (Supplemental Figure 28B). The regions with the largest reductions in age-standardized DALYs between 1990 and 2019 are high-income Asia Pacific, North Africa and the Middle East, and Australasia. However, for some regions—for example, in Eastern Europe and Central Asia—age-standardized rates have not declined.

At the global level, substantially more total DALYs due to MV disease were experienced by women than men. For almost all age groups, MV disease DALYs were higher in women than men (Figure 12). Globally, women and men had similar DALYs in the 40- to 44-year age groups, but the levels diverged after age 65 years, when women had more than one-third more DALYs due to MV disease than men, peaking at age 75 to 79 years. Prior studies have found that women with severe regurgitation have higher mortality and lower surgery rates than men, which may account for some of the increased DALYs in older women (32).

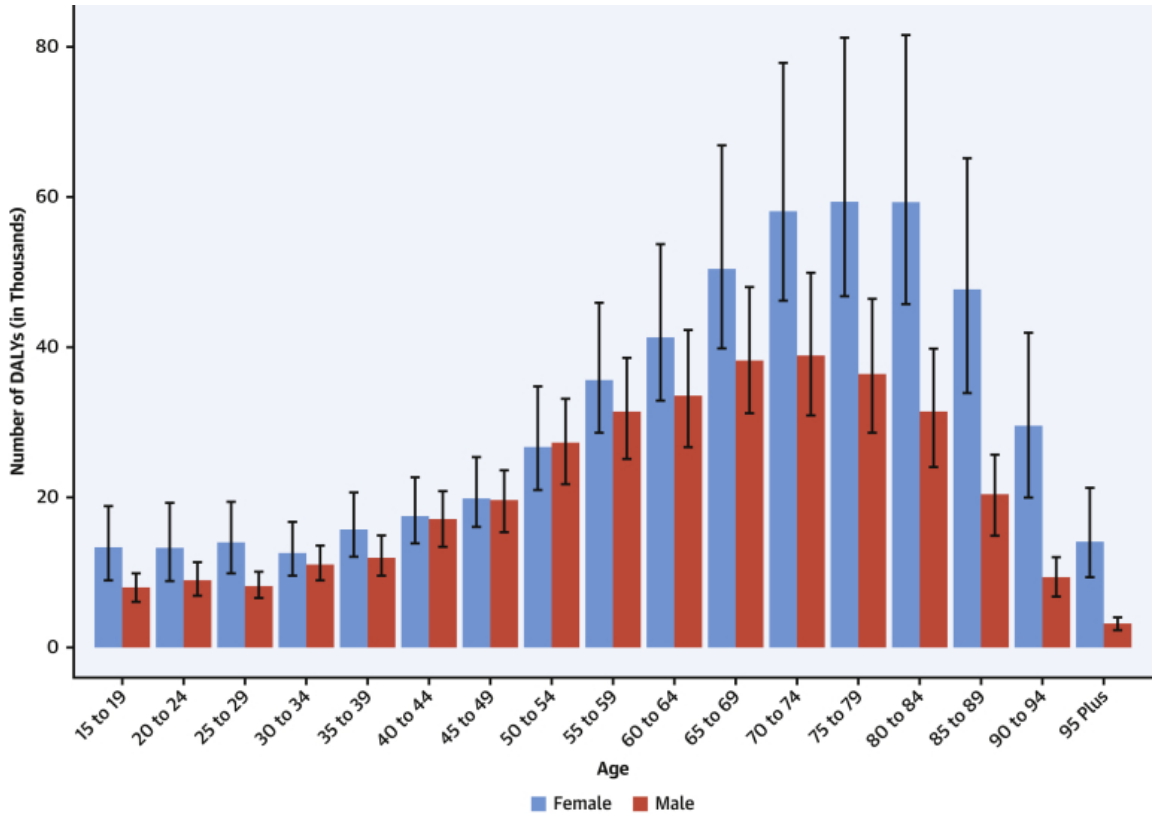


Figure 12

DALYs Due to Nonrheumatic Degenerative Mitral Valve Disease in 2019 by Age

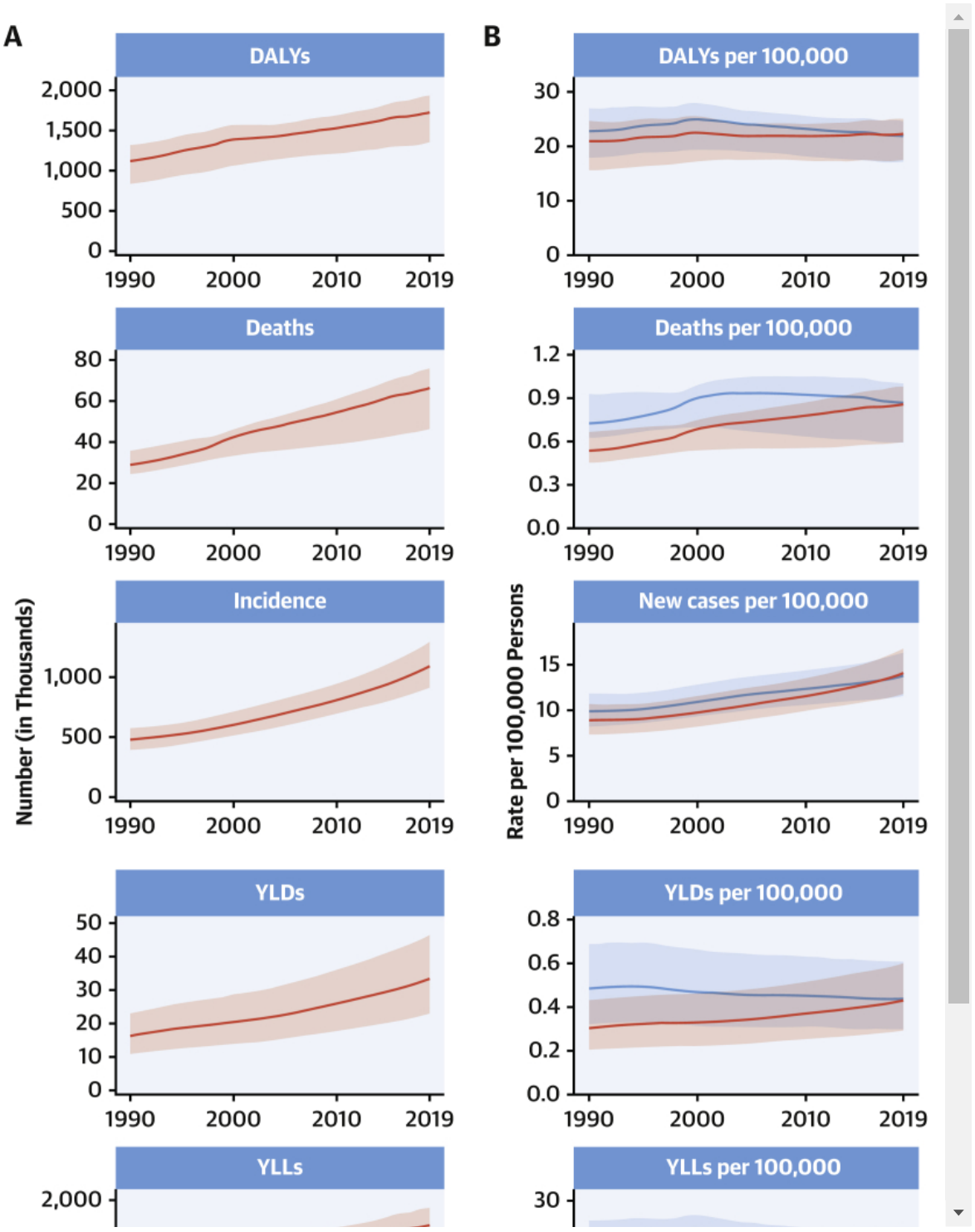
Number of DALYs due to nonrheumatic degenerative mitral valve disease by age and sex with 95% uncertainty intervals, 2019. Ages younger than 15 years were removed from the figure because they are not modeled for this cause. DALYs = disability-adjusted life years.

Age-standardized DALYs due to MV disease were highest for both men and women in Central Europe; specifically, high rates were seen in Serbia, Bosnia and Herzegovina, and Hungary, with the lowest levels seen in Southeast Asia, East Asia, and Andean Latin America (Supplemental Figure 29). Women had higher DALYs than men in most regions except Central Asia, Eastern Europe, Australasia, the Caribbean, and Western sub-Saharan Africa (Supplemental Figure 30).

Degenerative MV disease continues to be a major threat to public health, and the overall burden in terms of number of DALYs, deaths, and prevalent cases is increasing globally. Prevention of MV disease through reduction in risk factors remains a key public health issue. In addition, given an aging population in many regions, health systems should focus on improving access to diagnostic imaging (i.e., echocardiography) and subspecialty care for close follow-up. Improved access to surgical and percutaneous interventions is also needed to decrease mortality and long-term sequelae and improve quality of life for those with MV disease (33).

Endocarditis

The total number of DALYs due to endocarditis has risen steadily since 1990, reaching 1.72 million (95% UI: 1.36 to 1.94 million) DALYs and 66,300 deaths (95% UI: 46,200 to 75,900 deaths) in 2019 (Figure 13A). GBD 2019 estimated 1.09 million (95% UI: 0.913 to 1.30 million) incident cases of endocarditis in 2019.



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Figure 13

Total Numbers and Rates of Endocarditis

(A) Total number of DALYs, deaths, incident cases, YLDs, and YLLs due to endocarditis, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, incidence, YLD, and YLL rates of endocarditis, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. Abbreviations as in Figure 1.

Age-standardized rates show an increase in incidence, from 9.9 per 100,000 (95% UI: 8.2 to 11.8 per 100,000) to 13.8 per 100,000 (95% UI: 11.6 to 16.3 per 100,000), as well as deaths, from 0.7 per 100,000 (95% UI: 0.6 to 0.9 per 100,000) to 0.9 per 100,000 (95% UI: 0.6 to 1.0 per 100,000) (Figure 13B). Although there has been a slight increase in the DALYs, YLLs, and YLDs caused by endocarditis across all ages, once standardized for age structure and growth, these metrics are largely static between 1990 and 2019. The increase in death may be explained by an increasing proportion of endocarditis caused by virulent organisms such as staphylococci or complex infection in patients not fit for surgical intervention.

At the global level, more total DALYs due to endocarditis were experienced by men than women in 2019 (973,000 DALYs [95% UI: 736,000 to 1,120,000 DALYs] versus 751,000 DALYs [95% UI: 537,000 to 871,000 DALYs]). DALYs from endocarditis rose rapidly for both sexes from birth and reached a peak at age 55 to 59 years for men and age 65 to 69 years for women (Supplemental Figure 31). Women ages 75 years and older had more DALYs due to endocarditis than men in the same age group.

There is wide variation in the regional age-standardized DALY rate due to endocarditis (Supplemental Figures 32 and 33). In 2019, the highest rates were seen in Oceania, with 44.9 DALYs per 100,000 (95% UI: 30.7 to 60.7 DALYs per 100,000) (across men and women), followed by Southern Latin America (40.1 DALYs per 100,000 [95% UI: 32.7 to 49.6 DALYs per 100,000]) and Southeast Asia (38.6 DALYs per 100,000 [95% UI: 31.6 to 53.8 DALYs per 100,000]). There were also high rates in Eastern Europe (especially in men), the Caribbean, Tropical Latin America, and Africa. The lowest DALYs for endocarditis were seen in Central and East Asia. In large part, areas with high age-standardized DALY rates for endocarditis mirror those areas with a high prevalence of RHD, for example, in Oceania, Southeast Asia, and sub-Saharan Africa.

The epidemiology of endocarditis, including predisposing conditions, is heterogeneous. Therefore, it is not surprising that the endocarditis-associated YLDs and DALYs vary considerably by location. Both the endocarditis-associated YLDs and DALYs increased in 76.2% and 52.4% of locations, respectively. *Staphylococcus aureus* has become the predominant endocarditis pathogen in many areas of the world and is extremely virulent and a well-recognized marker of worse outcomes (34). As a result, mounting human and health care burdens mandate that an individualized approach be developed for each location to better understand the unique epidemiology of endocarditis so that efforts can be focused on management and prevention of this life-threatening condition.

Peripheral artery disease

The global numbers of prevalent cases and deaths due to PAD have risen consistently each year since 1990, resulting in a 2-fold increase to 113 million cases (95% UI: 99.2 to 128 million cases) and 74,100 deaths (95% UI: 41,200 to 128,000 deaths) in 2019 (Supplemental Figure 34A). Comparable trends in DALYs, YLLs, and YLDs have resulted in 1.54 million (95% UI: 1.01 to 2.37 million), 1.04 million (95% UI: 0.604 to 1.78 million), and 501,000 (95% UI: 235,000 to 898,000) respectively in 2019.

In keeping with the increase in numbers of prevalent cases, deaths, DALYS, YLLs, and YLDs, rates per 100,000 persons of all ages increased between 1990 and 2019 (Supplemental Figure 34B). The trends in age-standardized rates of deaths and YLLs were flat from 1990 to 2019, and prevalence, YLDs, and DALYs declined slightly. Overall, the PAD prevalence increase reflects population growth rather than a major change in age-specific incidence. In both 1990 and 2019, cross-sectional analyses show that the numbers of prevalent cases increased with age in both men and women up to 70 years of

age and prevalence rates increased throughout the whole age spectrum. Numbers and rates of cases were higher in women than men at all ages. Deaths and DALYs showed increases with age comparable to prevalence except that deaths and DALYs were higher in men than women up to very old age.

The numbers of prevalent cases increased in both men and women at all ages by up to 2-fold since 1990, but age-standardized prevalence rates were slightly lower in 2019 than in 1990. Numbers of deaths and DALYs were higher in 2019 compared to 1990 in both men and women at all ages, but over the same period, age-standardized death rates remained much the same, and DALY rates fell slightly.

Age-standardized DALY rates varied by world region in 2019 (Figure 14). Higher rates (>25 per 100,000 persons) were found in both men and women in Europe (western, central, and eastern), North America, Australasia, the Caribbean, and sub-Saharan Africa (central, eastern, and southern) (Supplemental Figure 35). Especially high rates occurred in men in Eastern Europe and areas of sub-Saharan Africa. Lowest DALY rates were found in Asia, Andean countries, West and North Africa and the Middle East. The pattern was similar in men and women, with most regions showing higher rates in men, but in regions with low overall rates, the sex differences were small. The burden of PAD is increasing not only in developed HICs but also in LMICs, where concomitant risk factors such as diabetes and obesity are increasing.



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Figure 14

Map of Age-Standardized DALYs Due to Peripheral Artery Disease in 2019

DALYs = disability-adjusted life years.

Despite decreases in PAD age-standardized prevalence, deaths, DALYs, YLDs, and YLLs, overall rates have increased because of worldwide increases in life expectancy. High DALY rates of PAD in Eastern Europe and parts of Africa, particularly in men, require investigation. PAD is highly prevalent and is both underrecognized and inadequately studied.

Modifiable Risk Factors

High systolic blood pressure

With aging of the population and population growth, the number of adults worldwide affected by high systolic blood pressure (SBP) increased from 2.18 billion (95% UI: 95% UI: 2.11 to 2.26 billion) to 4.06 billion (95% UI: 3.96 to 4.15 billion) from 1990 to 2019 (Supplemental Table 1). High SBP is defined by GBD 2019 according to a theoretical minimum risk exposure level (TMREL) of ≥110 to 115 mm Hg, which is the level of exposure that minimizes risk at the population level. In 2019, there were 828 million (95% UI: 768 to 888 million) adults with SBP >140 mm Hg, a common threshold for treatment with pharmacotherapies. The prevalence of adults worldwide with high SBP increased from 84,481.1 per 100,000 persons (95% UI: 81,641.5 to 87,579.2 per 100,000 persons) in 1990 to 88,971.1 per 100,000 persons (95% UI: 86,950.0 to 91,028.8 per 100,000 persons) in 2019.

From 1990 to 2019, the total number of DALYs due to high SBP increased from 154 million (95% UI: 139 to 169 million) to 235 million (95% UI: 211 to 261 million) (Supplemental Figure 36A). Additionally, between 1990 and 2019, the number of deaths (6.79 million [95% UI: 6.07 to 7.50 million] to 10.8 million [95% UI: 9.51 to 12.1 million]), YLDs (10.1 million [95% UI: 7.30 to 13.1 million] to 21.2 million [95% UI: 15.2 to 27.2 million]), and YLLs (144 million [95% UI: 129 to 158 million] to 214 million [95% UI: 191 to 237 million]) attributed to high SBP increased. The age-standardized rates of DALYs, deaths, and YLLs attributed to high SBP declined from 1990 to 2019, whereas the age-standardized rate of YLDs did not change substantially over this time period (Supplemental Figure 36B). These data indicate that the increases in total number of disease events can be attributed to population growth and aging. However, in some regions—for example, Oceania—the age-standardized rates of DALYs, deaths, YLDs, and YLLs increased between 1990 and 2019.

Globally, DALYs due to high SBP in 2019 were higher among men compared with women from ages 15 to 19 through 70 to 74 years, but higher among women compared to men in those 80 to 84 years or older (Figure 15). Age-standardized DALYs were higher among men compared with women in all regions of the world (Supplemental Figure 37).

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Figure 15

DALYs Due to High Systolic Blood Pressure in 2019 by Age

Number of DALYs due to high systolic blood pressure by age and sex with 95% uncertainty intervals, 2019. Ages younger than 15 years were removed from the figure because they are not modeled for this risk.

DALYs = disability-adjusted life years.

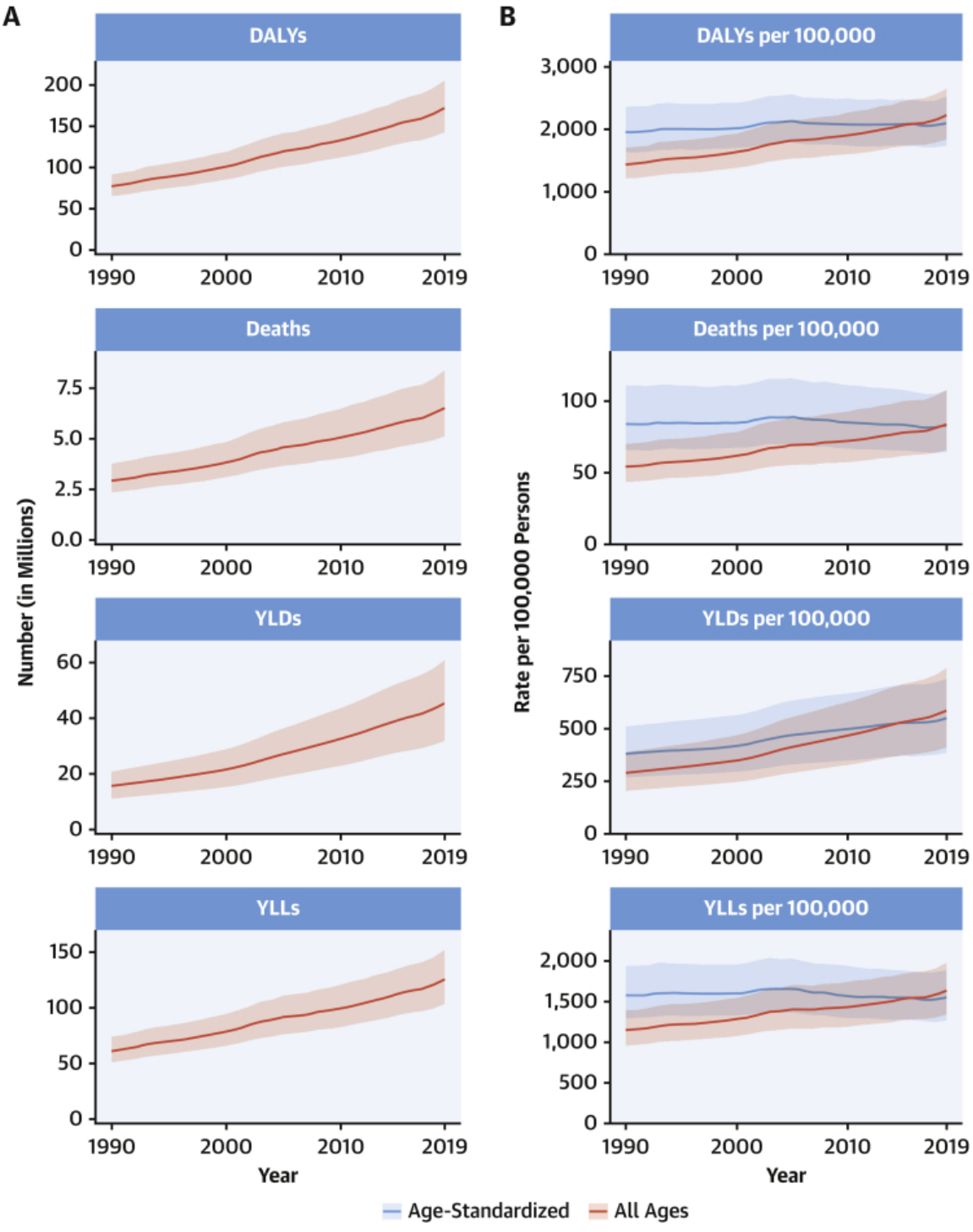
In 2019, there was an approximately 8-fold variation across regions in age-standardized DALYs attributed to high SBP (Supplemental Figure 38). Rates of DALYs were lowest for both women and men in the high-income Asia Pacific region and highest in Central Asia. Marked variation was also noted for rates of death, YLLs, and YLDs. The highest age-standardized rates of death and YLLs were noted in Central Asia and Eastern Europe among men and in Central Asia, North Africa and the Middle East, and Central sub-Saharan Africa among women. The highest rate of YLDs was noted in Central Asia, Central Europe, and Southeast Asia for men and in North Africa and the Middle East, East Asia, and Southeast Asia for women.

High SBP is a major public health challenge, affecting approximately 9 of 10 adults worldwide, and is associated with high rates of DALYs, death, YLDs, and YLLs. High SBP and its adverse health consequences can be prevented by eating a heart-healthy diet that includes <1 teaspoon of salt per day and adequate potassium from fruits and vegetables, maintaining a normal weight, increasing physical activity, and avoiding unhealthy alcohol intake (35).

High fasting plasma glucose

There are large differences between and within countries in the burden of high fasting plasma glucose, defined as above the TMREL of 4.8 to 5.4 mmol/l. The findings showed little improvement in age-standardized mortality rates from high fasting plasma glucose and significant increases in age-standardized YLDs between 1990 and 2019. Age-standardized mortality due to high fasting plasma glucose actually increased from 1990 to 2005, with a downward trend thereafter.

Between 1990 and 2019, a total of 134 million (95% UI: 107 to 171 million) deaths due to high fasting plasma glucose were recorded globally. The number of deaths increased from 2.91 million (95% UI: 2.34 to 3.75 million) in 1990 to 6.50 million (95% UI: 5.11 to 8.36 million) in 2019 (Figure 16A). The age-standardized mortality rate due to high fasting plasma glucose increased between 1990 and 2005 from 84.2 per 100,000 deaths (95% UI: 65.9 to 111.1 per 100,000 deaths) to 89.2 per 100,000 deaths (95% UI: 70.2 to 115.9 per 100,000 deaths) then declined to 83.0 per 100,000 deaths (95% UI: 64.5 to 107.1 per 100,000 deaths) in 2019 (Figure 16B). Age-standardized DALYs followed the same patterns with a peak in 2005 and then a decline until 2019. In contrast, age-standardized DALY rates were higher in 2019 at 2,104.3 per 100,000 (95% UI: 1,740.7 to 2,520.7 per 100,000) than in 1990 at 1,959.6 per 100,000 (95% UI: 1,638.7 to 2,362.4 per 100,000). Of course, this is the result of a sharp increase in age-standardized YLDs from 1990 to 2019. Men had higher DALY burden than women throughout the study period until the age of 80 years, after which women had a higher number of DALYs (Supplemental Figure 39). DALYs due to high fasting plasma glucose increased with age, peaked at 70 years, and then declined.



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Figure 16

Total Numbers and Rates of High Fasting Plasma Glucose

(A) Total number of DALYs, deaths, YLDs, and YLLs due to high fasting plasma glucose, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, YLD, and YLL rates of high fasting plasma glucose, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. Abbreviations as in Figure 1.

Age-standardized DALYs due to high fasting plasma glucose were higher in Oceania, followed by Central Asia (Supplemental Figure 40). There were large variations between countries in high fasting plasma glucose DALY burden, with notably high rates in Uzbekistan, Afghanistan, Papua New Guinea, Egypt, and Oman (Supplemental Figure 41). The highest age-standardized DALYs in 2019 were observed in Kiribati at 12,255.2 per 100,000 (95% UI: 9,799.7 to 15,073.0 per 100,000), and the highest age-standardized death rates were also observed in Kiribati at 435.4 per 100,000 (95% UI: 347.0 to 540.5 per 100,000). Large variations were also observed within countries. For example, the age-standardized DALY rate varied in Brazil from 1,571.8 per 100,000 (95% UI: 1,293.6 to 1,909.1 per 100,000) in Minas Gerais to 3,286.5 per 100,000 (95% UI: 2,735.5 to 3,907.3 per 100,000) in Alagoas, a 70.6% difference in the country.

These population-based estimates of plasma glucose level help track the global rise in diabetes. GBD 2019 showed low rates of physical activity (LPA) and poor diet in many countries. Both are major risk factors for high fasting plasma glucose and have led to caloric imbalance and higher rates of obesity, which has rapidly increased globally since 1990 and affects most geographic areas. There is a need to develop and implement both public health policies and clinical programs to reduce health disparities and disease burden due to diabetes.

High low-density lipoprotein cholesterol

The total number of DALYs due to high low-density lipoprotein (LDL) cholesterol, defined by a TMREL of 0.7 to 1.3 mmol/l, has risen steadily since 1990, reaching 98.6 million (95% UI: 80.3 to 119 million) DALYs and 4.40 million (95% UI: 3.30 to 5.65 million) deaths in 2019 (Supplemental Figure 42A). Over the same period, the YLDs and YLLs rose to 5.71 million (95% UI: 3.68 to 8.27 million) and 92.9 million (95% UI: 75.6 to 111 million), respectively. This indicates that the direction of the global trend for high LDL is increasing.

Over the study period, the global all-age rates for DALYs, deaths, and YLLs remained relatively static and increased for YLDs (Supplemental Figure 42B). During this time, the age-standardized rates for the same measures all declined. This difference between all-age rates and age-standardized rates suggests that although the global burden of LDL-related disease remains unacceptably high, on average, the observed increases in the burden of DALYs in locations like China and India have been driven primarily by population growth and aging. This trend is important for global health because it implies that there has been at least modest progress in reducing the burden of LDL-related disease globally. Age-standardized DALY rates attributable to elevated LDL cholesterol, however, are estimated to be increasing in some locations, including Pakistan and Saudi Arabia.

At the global level, significantly more total DALYs from elevated LDL were experienced by men than women. LDL-related DALYs increased rapidly for men beginning at age 30 years (Supplemental Figure 43). Men ages 40 to 44 years had as many DALYs due to LDL as women ages 60 to 64 and 80 to 84 years and more than women in all other age groups. In aging post-menopausal women, the LDL cholesterol levels are comparable to those of men of similar age. The difference in DALYs by sex and age suggests that the burden of premature LDL-related disease in men younger than 65 years may need focused public health attention.

Age-standardized DALYs due to high LDL cholesterol were highest in Eastern Europe, North Africa and the Middle East, Oceania, and Central Asia (Supplemental Figure 44). The lowest levels are present in high-income Asia Pacific, Australasia, Western Europe, and Andean Latin America and, by

country, in the Republic of Korea, Japan, Rwanda, France, Israel, and Spain (Figure 17). Potential explanations for these differences in regional patterns include the correspondingly LPA, increased high body mass index (BMI), dietary patterns, and increased tobacco use in the same regions and countries.

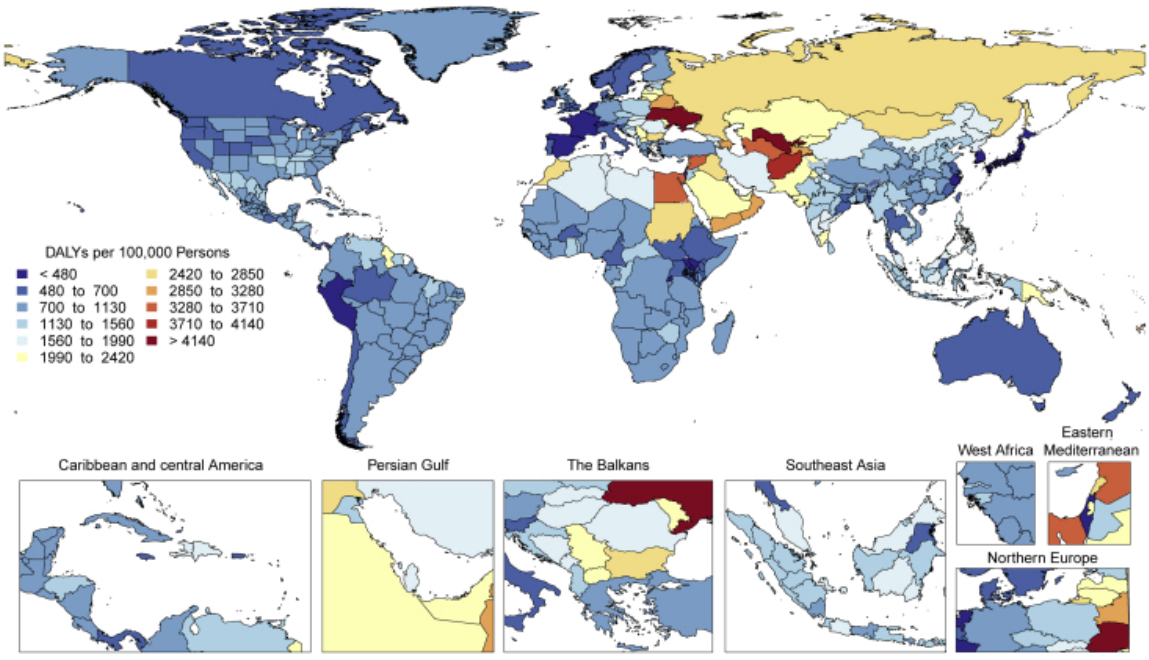


Figure 17

Map of Age-Standardized DALYs Due to High LDL Cholesterol in 2019

DALYs = disability-adjusted life years; LDL = low-density lipoprotein.

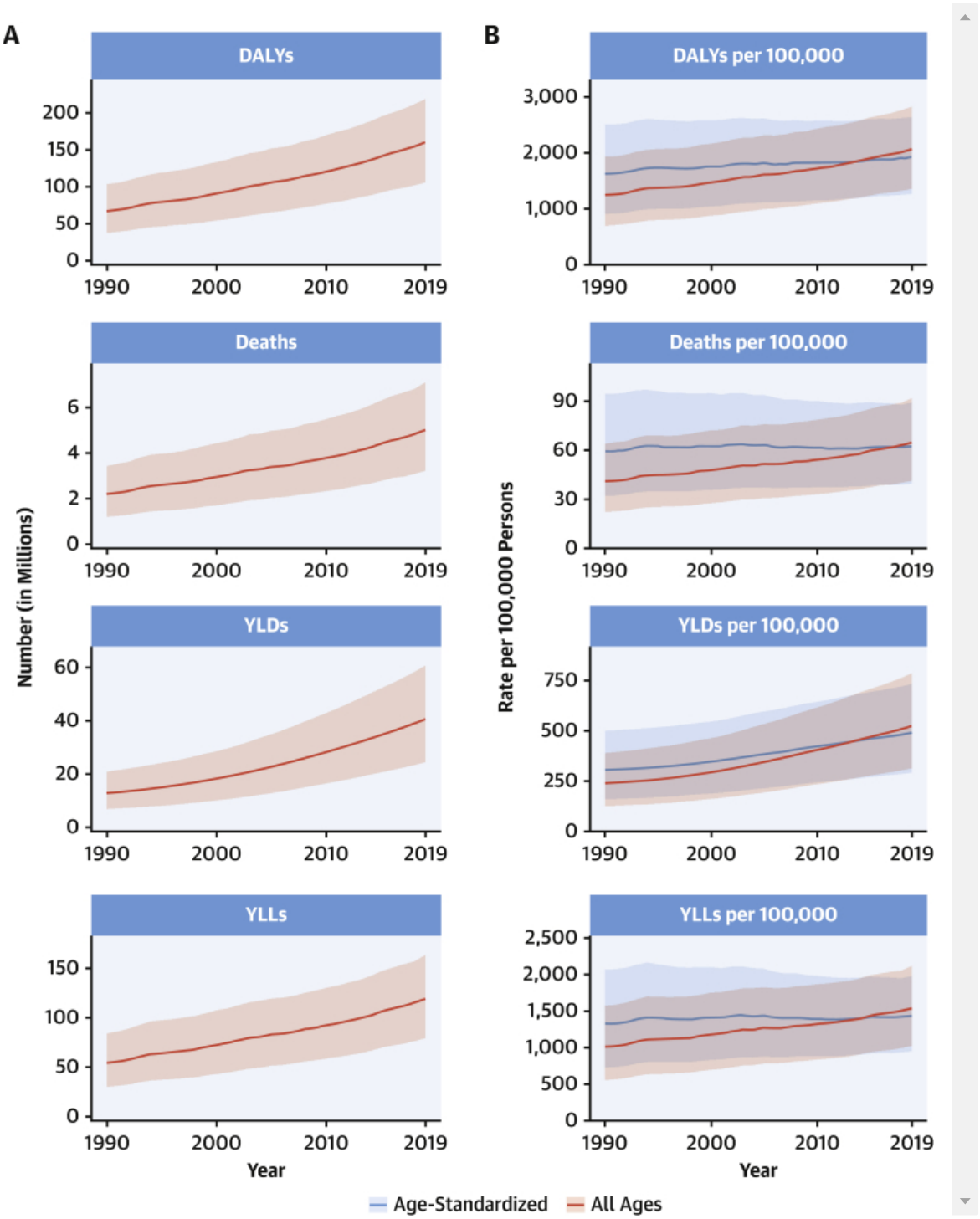
High LDL cholesterol remains a major threat to public health, and the overall burden in terms of number of DALYs, deaths, YLDs, and YLLs is increasing globally. In some locations, the risk associated with high LDL cholesterol is especially high and deserves immediate public health attention. Health systems and countries may need to focus on new approaches that can reverse these trends. These might include improved government policies on diet and tobacco, school physical activity programs, and, when needed, the use of lipid-lowering therapy in keeping with contemporary guidelines in which statins are the first choice. More global investment in research to address LDL cholesterol–related knowledge and therapeutic gaps is needed to address this persistent global health threat.

High BMI

Obesity, defined by elevated BMI (≥30 kg/m^2), has reached epidemic levels worldwide. Elevated BMI worsens most of the CVD risk factors, including adverse effects on blood pressure, blood sugar, lipids, and inflammation and has adverse effects on cardiac structure and function. Not surprisingly, hypertension, coronary heart disease, heart failure, and AF are increased with obesity (36).

Globally, 5.02 million (95% UI: 3.22 to 7.11 million) deaths and 160 million (95% UI: 106 to 219 million) DALYs were attributed to high BMI in 2019 (Figure 18A). High BMI, defined as above a TMREL of 20 to 25 kg/m^2 for adults and above normal weight for children, contributed to more YLLs

(119 million [95% UI: 79.6 to 164 million]) than YLDs (40.9 million [95% UI: 24.5 to 60.9 million]) in 2019. Globally, between 1990 and 2019, the absolute numbers of deaths attributable to high BMI increased (2.20 million [95% UI: 1.21 to 3.43 million] to 5.02 million [95% UI: 3.22 to 7.11 million]), as did DALYs (67.3 million [95% UI: 38.0 to 104 million] to 160 million [95% UI: 106 to 219 million]), YLDs (12.9 million [95% UI: 6.9 to 21.0 million] to 40.9 million [95% UI: 24.5 to 60.9 million]), and YLLs (54.4 million [95% UI: 30.2 to 84.4 million] to 119 million [95% UI: 79.6 to 164 million]).



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Figure 18

Total Numbers and Rates of High Body Mass Index

(A) Total number of DALYs, deaths, YLDs, and YLLs due to high body mass index, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, YLD, and YLL rates of high body mass index, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. Abbreviations as in Figure 1.

After standardization for population growth and aging, rates of deaths attributable to high BMI have increased only modestly from 1990 to 2019 (4.9% increase [95% UI: –7.3% to 24.6%]), DALYs (18.0% [95% UI: 2.2% to 42.3%]), and YLLs (8.3% [95% UI: –6.6% to 31.2%]), suggesting that population growth and aging of the population have contributed substantially to the global trends in obesity-associated burden of disease (Figure 18B). YLDs have increased more sharply (60.2% [95% UI: 41.3% to 90.2%]) from 1990 to 2019. Since population growth and aging from 1990 account for much of the absolute increases attributable to high BMI, the focus may need to be on strategies targeting prevention of weight gain at younger ages to decrease the burden of all measures, including YLDs.

Global DALYs attributable to high BMI are highest between ages 45 and 75 years and are slightly higher for men at younger and middle ages and for women at older ages (Supplemental Figure 45).

Age-standardized DALY rates due to high BMI are highest in Oceania, Central Asia, North Africa and the Middle East, Southern sub-Saharan Africa, Eastern Europe, Central Latin America, the Caribbean, and Central Europe (Supplemental Figure 46). Among countries, Japan has the lowest and Kiribati the highest age-standardized DALYs in the world (Supplemental Figure 47). The global findings are generally similar between men and women in most regions, but age-standardized DALYs are notably higher for men in Central Asia and Central and Eastern Europe.

Great efforts are needed to promote the prevention of obesity and its progression to more severe forms (36). A multifactorial approach is required to promote improving dietary quality, especially reductions in simple sugars, complex carbohydrates, and total calories. Community prevention programs, like the Diabetes Prevention Program, duplicated in many programs (37), are needed (36). Optimizing efforts to promote physical activity and exercise training are needed, as well as reductions in sedentary behavior and making neighborhoods, communities, and workplaces more suitable to allow for these healthier lifestyle behaviors. Potential governmental involvement, such as mandating caloric/nutrition menu labeling and regulation of food ingredients, as well as controlling advertisements and certain forms of beverage and food item taxation, etc., could be instituted. Governmental intervention was very successful decades ago in the North Karelia Project in Finland (36,38), and potentially, similar efforts could be successful for obesity intervention worldwide. Research is needed to identify effective methods of health promotion that will reduce obesity and align all allied health professions toward this important goal (36).

Impaired kidney function

GBD 2019 estimates use the term chronic kidney disease (CKD) to refer to the morbidity and mortality that can be directly attributed to all stages of CKD and the term impaired kidney function (IKF) to capture the additional risk of CKD from other associated conditions such as CVD and gout. The DALYs due to IKF nearly doubled from 1990 to 2019, reaching 76.5 million (95% UI: 67.8 to 86.3 million) DALYs and 3.16 million (95% UI: 2.72 to 3.62 million) deaths globally in 2019 (Supplemental Figure 48A). The estimated global prevalence of CKD is 9,011.9 per 100,000 (95% UI: 8,401.3 to 9,577.8 per 100,000) (697 million [95% UI: 650 to 741 million] people), 41.1% (95% UI: 38.9% to 43.4%) higher than in 1990 due to aging (Supplemental Figures 49A and 49B).

Age-standardized DALYs due to IKF declined slightly from 1,024.1 DALYs per 100,000 people (95% UI: 898.4 to 1,154.8 DALYs per 100,000 people) to 945.3 DALYs per 100,000 people (95% UI: 836.3 to 1,066.8 DALYs per 100,000 people) from 1990 to 2019 (Supplemental Figure 48B). This 7.7% (95% UI: –12.9% to –2.4%) decline in DALYs lags behind the 39.3% (95% UI: –43.5% to –34.9%) decline in DALYs for all risk factors combined. The age-standardized rate per 100,000 people for

YLDs from IKF increased from 115.9 (95% UI: 84.7 to 150.5) to 139.4 (95% UI: 101.8 to 181.8) from 1990 to 2019. Growth and aging of the global population resulted in an increase in all unadjusted disease burden measures for IKF.

CKD and its consequences generally rise markedly with age and are higher among men than women, but trends over time were similar across age and sex groups (Supplemental Figures 50 and 51).

Geographically, the highest age-standardized rates of DALYs due to IKF are now seen in Central Latin America, Central Asia, the Caribbean, North Africa and the Middle East, Oceania, and Southeast Asia (Figure 19). Men in Central Latin America have among the highest age-standardized DALYs at 2,091.2 per 100,000 people (95% UI: 1,791.6 to 2,424.8 per 100,000 people), double the global rate and 60% higher than in 1990 (Supplemental Figure 52), which represents an epidemic of CKD of unknown origin that appears to be common in coastal lowlands, with repeated heat stress, pesticide, and heavy metal exposure implicated as potential causes (39).

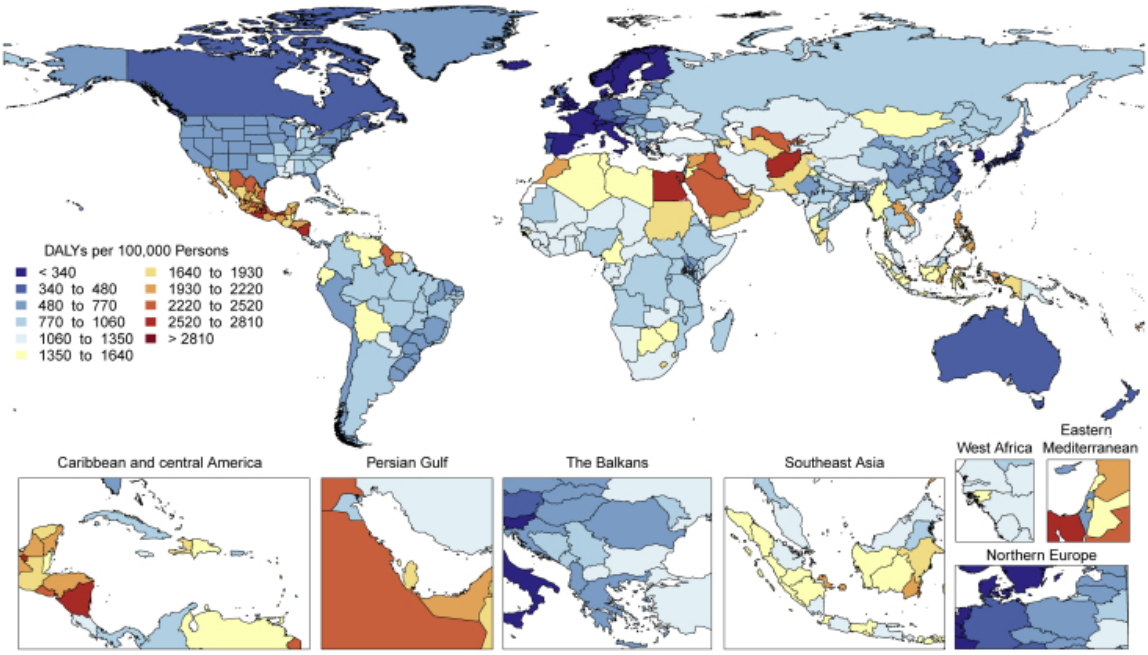


Figure 19

Map of Age-Standardized DALYs Due to Impaired Kidney Function in 2019

DALYs = disability-adjusted life years.

CKD is emerging as a growing global problem, with marked growth related to the aging population. Age-standardized DALYs for IKF have declined by only 7.7%, compared to 39.3% for all risk factors, and they increased in Central and Andean Latin America, the Caribbean, Central and Southeast Asia, Oceania, and Southern sub-Saharan Africa. Much of CKD remains undiagnosed and undertreated. Governments, health systems, physicians, and patient attention are needed to improve the diagnosis of CKD by measuring proteinuria and estimated glomerular filtration rate. There is also a need for earlier markers of kidney injury. Diabetes and hypertension are leading factors for CKD, its progression, and associated risk. Improving their prevention and control will reduce the burden of CKD. Therapeutic options for CKD are increasing with the proven benefits of renin-angiotensin system blockade in

proteinuria and possibly sodium-glucose cotransporter-2 inhibitors and mineralocorticoid receptor antagonists. Trials are underway to test prevention of kidney events in patients with CKD without diabetes. A combination of population-based public health measures for increased CKD recognition and awareness and improved therapies to reduce risk is needed to reduce the burden of CKD.

Ambient and household air pollution

Air pollution is the leading environmental risk factor for global health and the fourth largest risk factor for global mortality. Two main forms of air pollution quantified in GBD 2019 contribute substantially to the burden of CVD: ambient particulate matter with an aerodynamic diameter smaller than 2.5 μm ($PM_{2.5}$) and household air pollution (HAP) from the use of solid fuels for cooking. In 2019, CVD accounted for 51.5% and 30.5% of the total DALYS attributable to $PM_{2.5}$ and HAP, respectively.

Despite substantial reductions in ambient $PM_{2.5}$ concentrations in North America and Europe, levels remain high throughout most of the world (40). Global annual average population-weighted $PM_{2.5}$ levels increased slightly from 40.8 $\mu g/m^3$ in 1990 to 42.6 $\mu g/m^3$ in 2019. However, the global disease burden attributable to ambient $PM_{2.5}$ increased from 70.5 million (95% UI: 47.3 to 98.9 million) DALYs and 2.05 million (95% UI: 1.45 to 2.74 million) deaths in 1990 to 118 million (95% UI: 95.9 to 138 million) DALYs and 4.14 million (95% UI: 3.45 to 4.80 million) deaths, respectively, in 2019 (Supplemental Figure 53A). Age-standardized rates for DALYs and deaths attributable to $PM_{2.5}$ have remained relatively constant between 1990 and 2019, indicating that, on average, global increases in attributable disease have been due to population growth and aging, especially in locations with high exposures (Supplemental Figure 53B). Age-standardized DALYs attributable to $PM_{2.5}$ were higher than the global mean in East, Central, and South Asia; North Africa and the Middle East; and Southern and Western sub-Saharan Africa (Figure 20).

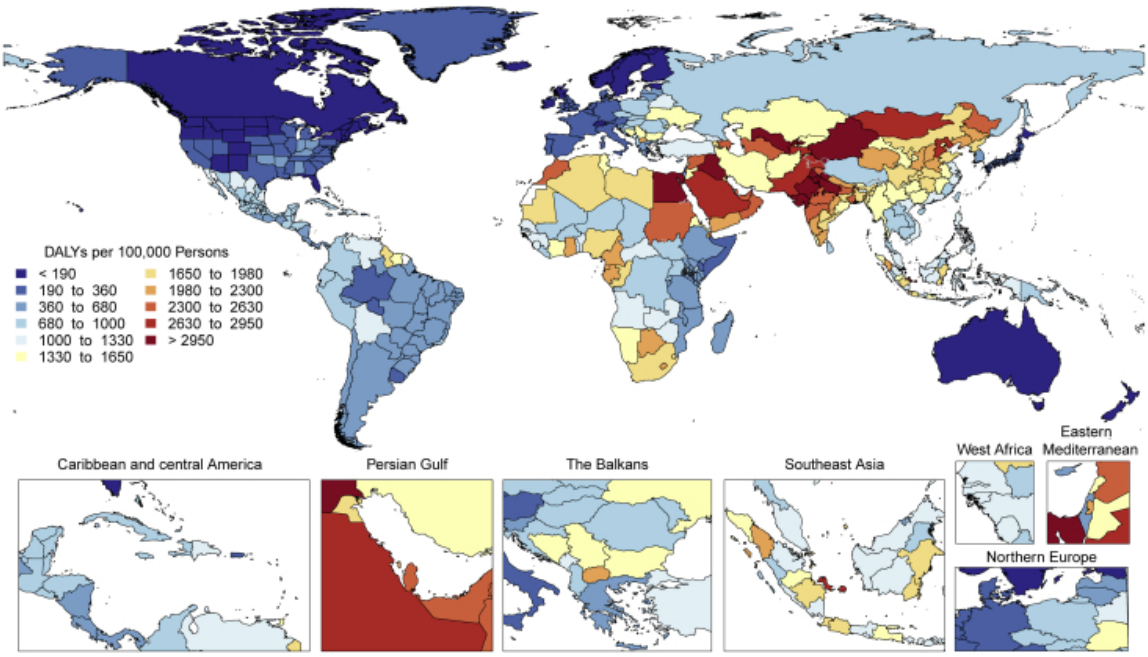


Figure 20

Map of Age-Standardized DALYs Due to Ambient Particulate Matter Pollution in 2019

DALYs = disability-adjusted life years.

In contrast to ambient $PM_{2.5}$, the burden attributable to HAP decreased sharply from 208 million (95% UI: 162 to 259 million) DALYs and 4.36 million (95% UI: 3.33 to 5.40 million) deaths in 1990 to 91.5 million (95% UI: 67.0 to 119 million) DALYs and 2.31 million (95% UI: 1.63 to 3.12 million) deaths in 2019 (Supplemental Figure 54A). Trends for both all-age and age-standardized DALYs and death rates attributable to HAP have also declined sharply, indicating the impact of exposure reduction (Supplemental Figure 54B). However, age-standardized rates of DALYs attributable to HAP remain high throughout much of sub-Saharan Africa, which has yet to experience the benefits of improved access to clean household energy sources seen in India, China, and much of Southeast Asia (Supplemental Figure 55). The highest rates of DALYs for HAP were in Oceania; Eastern, Western and Central sub-Saharan Africa; and South Asia, driven by differences in exposure.

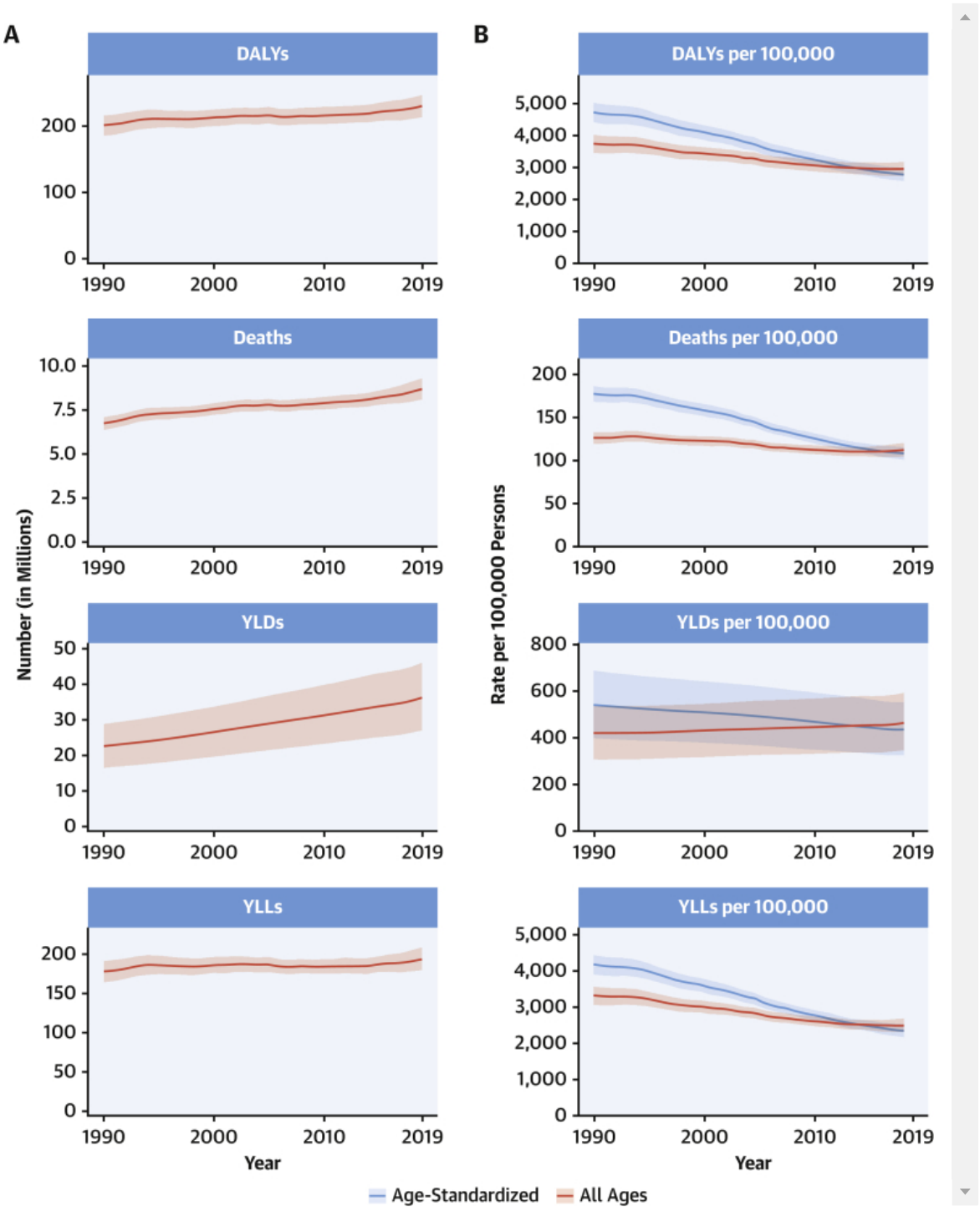
DALYs attributable to $PM_{2.5}$ and HAP were higher in men, driven by sex differences in rates of the diseases affected by air pollution (Supplemental Figures 56 and 57). These sex differences were less pronounced for HAP given that women tend to be more highly exposed. DALYs attributable to HAP had the largest impacts in infancy and early childhood, whereas for $PM_{2.5}$, it was between ages 30 and 90 years.

Increased attention to air pollution as a CVD risk factor is warranted and will require concerted action and collaboration between government health and environmental agencies. This is especially relevant given high levels of exposure in many parts of the world and links between $PM_{2.5}$ and health risks at levels substantially below those of current regulatory levels (41). As demonstrated in North America and Western Europe, air quality management, coupled with regulation and enforcement, is effective at reducing ambient air pollution and its disease burden (42). Similar approaches applied elsewhere, in combination with actions to reduce emissions of climate-forcing agents will be necessary. In the

interim, personal-level interventions, such as the use of portable air cleaners, may be effective (43). Continued reductions in HAP exposure through programs to improve access to clean energy sources will lead to both health and climate benefits (44).

Tobacco

Tobacco, including primary smoking, secondhand smoke, and use of chewing tobacco, accounted for 8.71 million (95% UI: 8.12 to 9.31 million) deaths and 230 million (95% UI: 213 to 246 million) DALYs in 2019 (Figure 21A). Of tobacco-attributable deaths, 36.7% were due to CVD. The number of YLLs attributable to tobacco (194 million [95% UI: 180 to 209 million]) far exceeded the number of attributable YLDs (36.1 million [95% UI: 27.0 to 46.0 million]), highlighting the outsized effect of tobacco on premature mortality.



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Figure 21

Total Numbers and Rates of Tobacco

(A) Total number of DALYs, deaths, YLDs, and YLLs due to tobacco, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, YLD, and YLL rates of tobacco, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. Abbreviations as in Figure 1.

Progress in reducing tobacco use and exposure has not kept pace with competing demographic forces, namely, population growth and population aging. Population growth, particularly in countries with a large population of tobacco users, has resulted in an increasing number of tobacco-attributable deaths and DALYs since 1990. When controlling for population growth, decreases in both death and DALY rates are observed. However, population aging has resulted in smaller decreases in all-age rates compared to age-standardized rates (Figure 21B). With approximately 80% of current smokers living in LMICs, these demographic forces will continue to counter progress in tobacco control for years to come.

In 2019, global smoking prevalence among men was 33.5% (95% UI: 33.1% to 33.9%), and smoking prevalence among women was 6.8% (95% UI: 6.6% to 7.0%). As a result of this difference, 75.4% of smoking attributable deaths occurred among men. The age pattern of tobacco use and exposure, combined with the age distribution of associated health outcomes, results in age-specific DALYs peaking among men ages 60 to 64 years and among women ages 65 to 69 years (Supplemental Figure 58).

The highest age-standardized tobacco-attributable DALY rates among men were observed in Eastern Europe, Central Asia, and Oceania (Supplemental Figure 59). Among women, the highest rates were observed in Oceania, high-income North America, and Central Europe. Thirty percent of all current smokers live in China, and nearly one-third of tobacco-attributable deaths occurred in China in 2019.

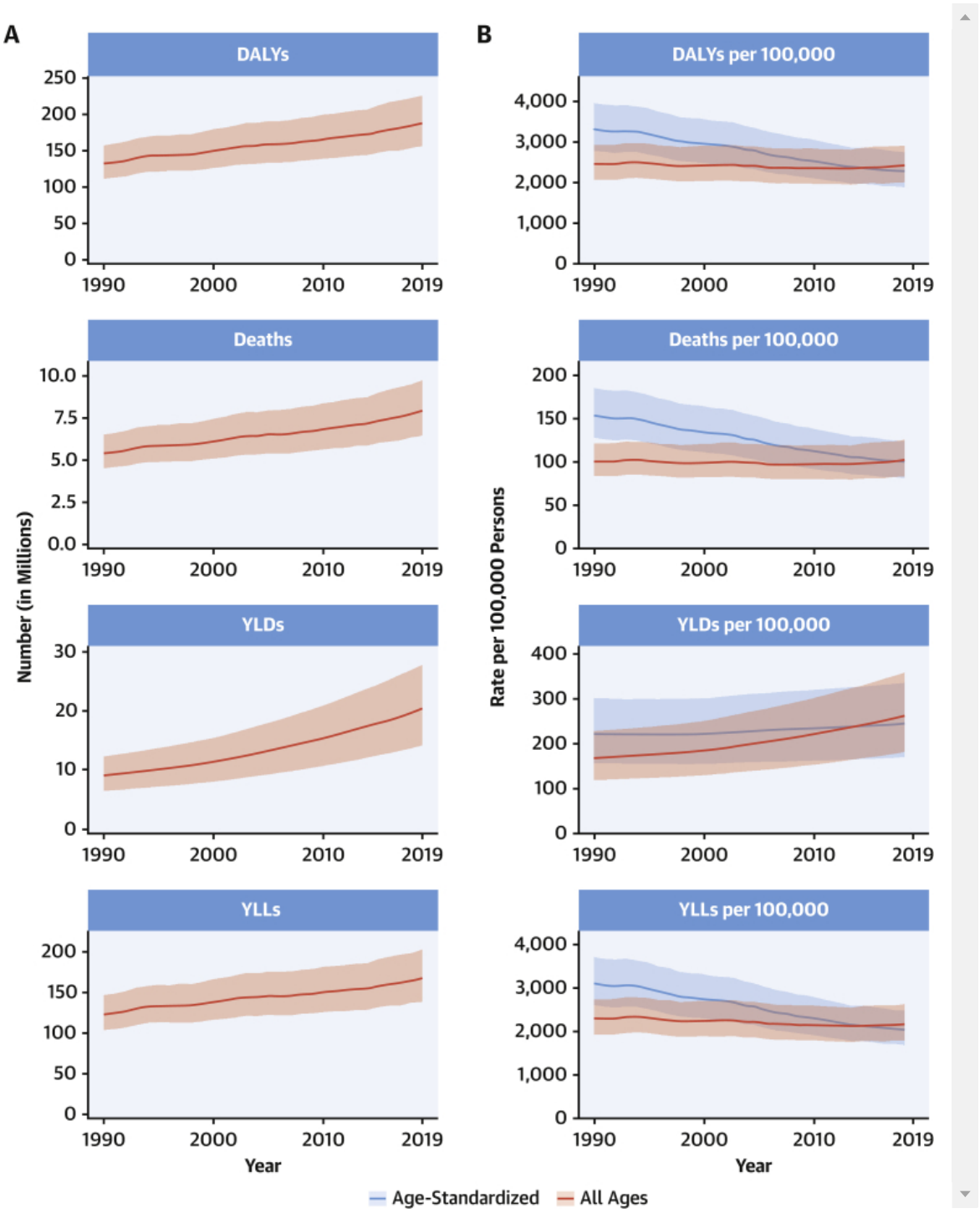
With more than 1 billion active tobacco users, the tobacco epidemic remains a central challenge to global health. The global disease burden of tobacco use continues to increase, attributable in large part to population growth and falling most heavily on LMICs, where the large majority of tobacco users live. Effective tobacco control measures available to governments and private sector institutions include policies that increase taxes on tobacco, fund mass media education campaigns, ban tobacco marketing and sponsorship, require prominent warning labels on tobacco products, require environments to be smoke-free, and ensure access to and delivery of tobacco cessation treatments in health care systems. The evidence-based measures are included in the World Health Organization's Framework Convention on Tobacco Control, a public health treaty that took effect in 2005 and has been ratified by 182 countries. Despite this global commitment, adoption of the Framework Convention on Tobacco Control measures has varied widely across countries. Increasing adoption and effective implementation of evidence-based tobacco control policies is a key worldwide public health priority. Looking ahead toward non-communicable disease targets set in the Sustainable Development Goals, accelerated progress in implementing proven policies and interventions is necessary to substantially reduce tobacco-attributable burden.

Dietary risks

In GBD 2019, dietary risks comprise the sum of adverse effects of diets in which 15 food types are either underconsumed (fruits, vegetables, legumes, whole grains, nuts and seeds, milk, fiber, calcium, omega-3 fatty acids from seafood, and polyunsaturated fatty acids) or overconsumed (red meat, processed meat, sugar-sweetened beverages, *trans*-fatty acids, and sodium).

CVDs are the primary consequence of these dietary risks, with 7.94 million (95% UI: 6.47 to 9.76 million) annual deaths and 188 million (95% UI: 156 to 225 million) annual DALYs attributed to dietary risks (Figure 22A). Worldwide, the absolute disease burden caused by dietary risks has risen for 30 years, regardless of how measured. Over the same period, age-standardized rates have fallen for

most measures, indicating the central role of population growth and population aging on the observed increases (Figure 22B). The exception is age-standardized rates of YLDs caused by dietary risks, which have grown in both absolute and proportional terms.



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Figure 22

Total Numbers and Rates of Dietary Risks

(A) Total number of DALYs, deaths, YLDs, and YLLs due to dietary risks, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. **(B)** Age-standardized and all-ages DALY, death, YLD, and YLL rates of dietary risks, 1990 to 2019. **Shaded regions** represent 95% uncertainty intervals. Abbreviations as in Figure 1.

Worldwide, the number of DALYs caused by dietary risks peaks between 55 and 70 years of age for both men and women, with a large excess of DALYs among men compared to women in every age group to 80 years old (Supplemental Figure 60). Age-standardized rates of DALYs caused by dietary risks are highest in Central Asia, Oceania, and Eastern Europe and lowest in Australasia, HICs of the Asia Pacific, and Western Europe (Supplemental Figures 61 and 62). Differences in age-standardized DALY rates among populations reflect a complex interplay between variations in diet quality, levels of exposure to different dietary risks, and access to interventions targeting the downstream clinical effects of CVDs.

The detailed effects of many dietary risks are poorly understood because assessments are hampered by the challenges of accurately quantifying exposures and separating the effects of each risk from those of important covariates. However, the effects of dietary risks on CVD remain large whether assessed as individual dietary factors or measures of overall diet quality, and there is little doubt about the importance of dietary risks as a cause of global disease burden.

Strong commercial interests driving the sales of unhealthy foods have made it challenging to persuade policy makers, clinicians, or community members to act decisively on diet quality. Easy access to healthy fruits, vegetables, and whole grains remains limited for much of the world. Many countries have adopted policies to limit consumption of added sugar, sodium, and harmful fats, but implementation has mostly been weak, and the impact on global health has been limited. Exceptions are regulatory and fiscal interventions such as soda taxes and beverage reformulation programs that have led to lower intakes of added sugars, proving the feasibility of determined intervention. Likewise, the removal of artificial *trans*-fat from the food supply in the United States has improved diet quality, although the amount of *trans*-fat in the global food supply remains high. Reliance on drug therapies targeting downstream metabolic risk factors caused by obesity may be inadequate. Ill health caused by dietary risks is anticipated to rise rapidly in LMICs over the coming years, and behavioral and policy interventions suited for implementation at scale in diverse settings around the world are urgently required.

Low Physical Activity

LPA is an important risk factor for IHD, stroke, diabetes, breast and colon cancers, and other noncommunicable diseases. The combination of increased risk for important causes of mortality and significant levels of LPA in most countries has led to physical inactivity being characterized as a pandemic. LPA is an important contributor of premature mortality, morbidity, and DALYs for adults in most of the world.

Globally, the total number of DALYs due to LPA, defined as <3,000 to 4,500 metabolic equivalent minutes per week, continuously increased from 8.61 million (95% UI: 4.28 to 15.9 million) in 1990 to 15.7 million (95% UI: 8.52 to 28.6 million) in 2019 (Supplemental Figure 63A). A linear trend was observed in the numbers of deaths due to LPA, reaching 832,000 premature deaths (95% UI: 427,000 to 1,470,000 premature deaths) in 2019. During the same time period, the estimated YLLs grew steadily from 7.51 million (95% UI: 3.62 to 14.4 million) in 1990 to 12.7 million (95% UI: 6.58 to 23.8 million) YLLs in 2019.

Over time, the all-ages rate of DALYs, deaths, and YLLs due to LPA has increased, and the age-standardized rate has decreased (Supplemental Figure 63B). This pattern reflects increased burden due to growth and aging of the population, an effect that is removed by age standardization.

Total global DALYs due to LPA were similar for men and women until the 65- to 69-year age group, with men consistently higher (Supplemental Figure 64). This pattern reversed in the 70- to 74-year age group as DALYs for women surpassed those for men. At all ages beyond 75 years, women had more DALYs than men, presumably driven by greater survival among women. LPA DALYs rose steadily for both men and women beginning at age 25 years, peaking for both sexes in the 80- to 84-year age group and declining thereafter.

Age-standardized DALYs due to LPA were highly variable by both region and sex. The highest rates were seen for both men and women in North Africa and the Middle East, Oceania, tropical Latin America, and the Caribbean (Figure 23), with high rates for men also observed in Central Asia (Supplemental Figure 65). The lowest age-standardized DALYs for LPA were seen for both men and women in high-income Asia Pacific, Southern Latin America, and Eastern sub−Saharan Africa; for men in high-income North America; and for women in Andean Latin America.

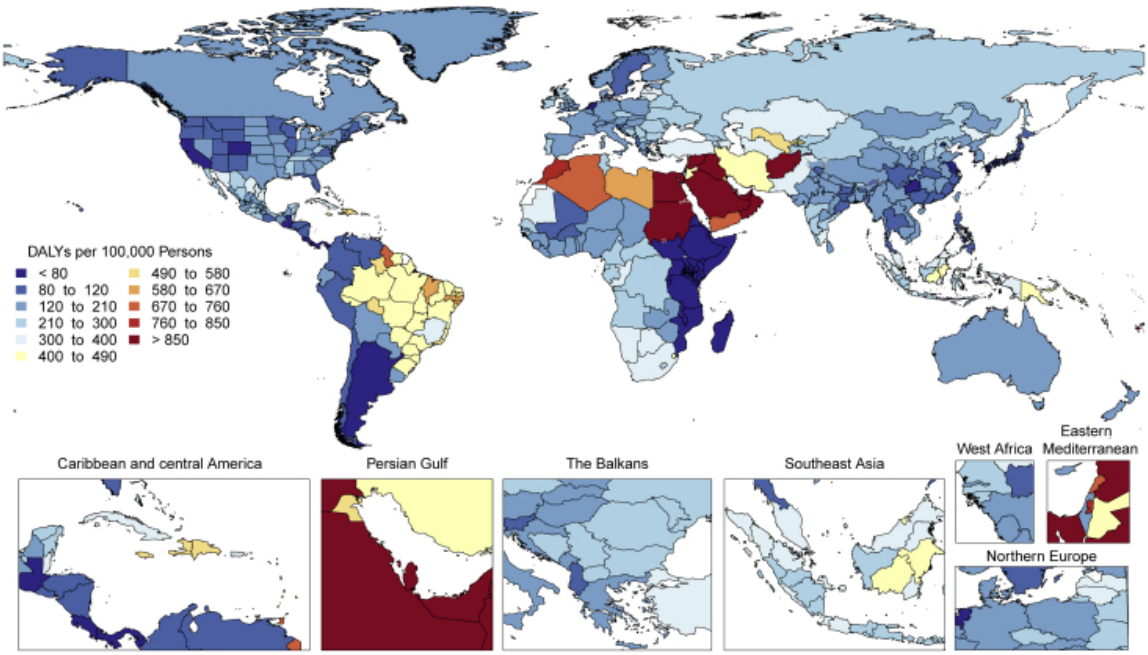


Figure 23

Map of Age-Standardized DALYs Due to Low Physical Activity in 2019

DALYs = disability-adjusted life years.

LPA remains a major threat to public health, and the overall burden is increasing globally even as age-standardized rates are declining. Although few health systems have historically focused on LPA, there is increased awareness and attention being paid to LPA as exemplified by the 2018 World Health Organization Global Action Plan for Physical Activity. However, the factors that influence population physical activity largely reside outside of the health sector. Increasing physical activity will depend on effective partnerships and collaboration between health and sectors such as transportation, education, urban planning, workplace health strategies, and environmental protection.

Subnational Disease Burden

GBD 2019 estimates disease burden within countries as well as between countries. Figure 24 shows the age-standardized rate of YLLs due to CVD for all countries, with Brazil, China, India, Indonesia, Japan, Kenya, Mexico, the United States, and the United Kingdom shown at the first subnational administrative level and Stockholm County shown separately from the rest of Sweden. Subnational disease estimation is made possible through collaboration with these governments. Subnational data reveal wide differences in DALYs within countries. For example, in Kenya in 2019, IHD DALYs were estimated to vary from 342.7 per 100,000 (95% UI: 212.4 to 531.7 per 100,000) in Turkana to 1,215.4 per 100,000 (95% UI: 856.8 to 1,612.4 per 100,000) in Nyeri, a 112.0% difference within Kenya. IHD DALYs in India ranged from 506.6 per 100,000 (95% UI: 379.6 to 819.0 per 100,000) in Mizoram to 5,413.2 per 100,000 (95% UI: 4,316.9 to 6,389.9 per 100,000) in Punjab, a 165.8% difference within the country. In Brazil, IHD DALYs varied from 771.2 per 100,000 (95% UI: 679.4 to 866.3 per 100,000) in Amazonas to 2,416.2 per 100,000 (95% UI: 2,176.7 to 2,686.2 per 100,000) in the Rio de Janeiro state, a 103.2% difference within the country. Within the United States, there was a 99.6% difference in IHD DALYs, ranging from 1,438.3 per 100,000 (95% UI: 1,210.7 to 1,699.0 per 100,000) in Utah to 4,293.1 per 100,000 (95% UI: 3,636.0 to 5,033.0 per 100,000) in West Virginia. In Mexico, they ranged from 890.5 per 100,000 (95% UI: 752.2 to 1,073.3 per 100,000) in Quintana Roo to 2,315.1 per 100,000 (95% UI: 1,932.1 to 2,716.3 per 100,000) in Chihuahua, an 88.9% difference within Mexico. Disease burden attributable to elevated levels of metabolic risk factors also varies significantly within countries.

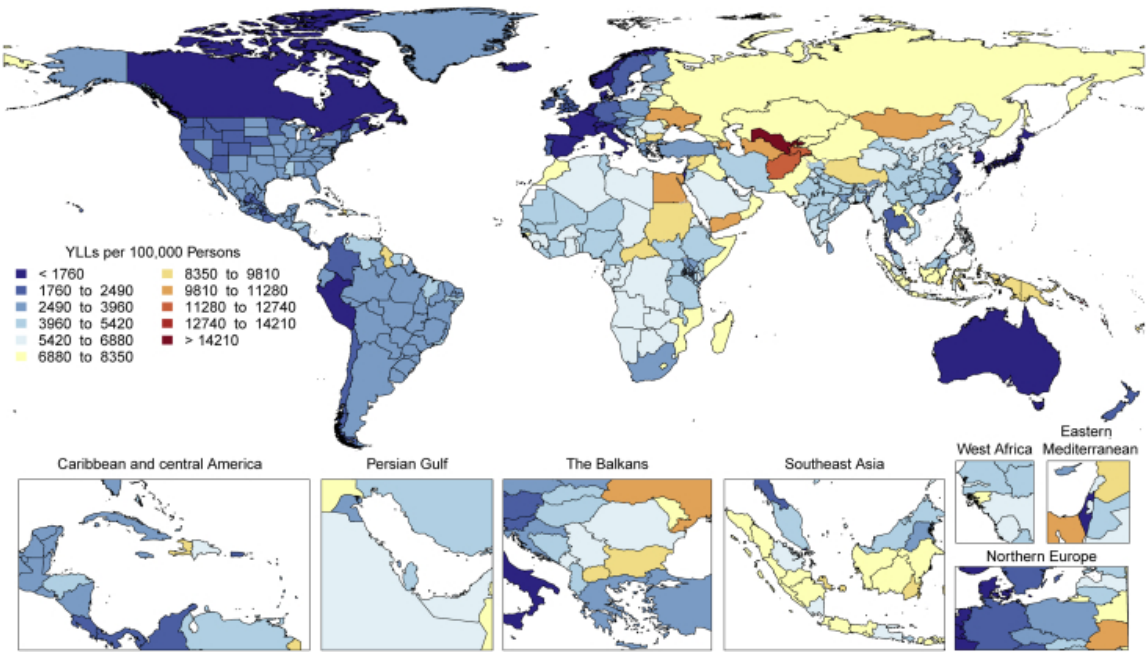


Figure 24

Map of Age-Standardized YLLs Due to Cardiovascular Diseases in 2019

YLLs = years of life lost.

Further analysis of the variation of CVD burden within countries will be vital to implementing health policies that reduce burden but also decrease disparities. The strategies to deal with these inequalities include: 1) targeting reduction in key modifiable risks including diet, tobacco, alcohol, physical

activity, and obesity; 2) improved access and quality of health care; and 3) a renewed focus on the social determinants of health. Addressing risk factors may prove to be an important initial opportunity to reduce disparities in parallel with broader efforts targeting social determinants, with potential indirect benefits for improving those social determinants, such as reduced financial hardship due to fewer acute cardiac events among working-age adults.

Several risks are increasing globally and warrant increased policy and health system attention, including high BMI, blood pressure, unhealthy cholesterol, and poor diet. Targeting subnational locations where metabolic risks are rising rapidly may be an important approach for testing new interventions and delivering resources where they will have the biggest impact. There is often a gap between the identification of a problem, the location of a solution, and the translation of that solution to an entire population. We currently lack a systematic, scalable process for determining which interventions are most likely to succeed in a given location, and research funding should be directed at addressing this gap.

Study limitations

Estimates from GBD 2019 should be interpreted in light of several limitations. Death certificates, although an essential source of data for public health, may be misclassified given the difficulty of identifying an underlying cause of death. GBD 2019 applies statistical methods to improve the comparability of health information coded using the ICD system and continuously evaluates, improves on, and reports the details of these methods. The use of different subnational population-based studies may lead to compositional bias of national estimates, although GBD 2019 adjusts variance and weighting to reflect this possibility. ICD system codes from administrative health facility data may represent a group of patients that differs from the standard case definition for a disease, although GBD 2019 applies adjustments to these data to account for differential access to health care and alternate case definitions. Access to CVD diagnostic technologies is likely to influence estimates of some conditions, with lower access resulting in lower observed prevalence. Some commonly used diagnostic tools—for example, electrocardiogram for AF—will underestimate true disease prevalence. Estimates are reported with an uncertainty interval that should always be carefully considered, given that it represents both data sparsity and differences in sample size across data sources and study locations. However, uncertainty may be underestimated in locations where no data on CVD of any kind are available. Limited data on CVD and resulting wider uncertainty intervals for parts of sub-Saharan Africa, Oceania, and Asia may restrict our ability to interpret trends in burden over time. An important use of GBD results is therefore to identify gaps in CVD health data and guide investments in future disease surveillance. In particular, better data are needed on the incidence of coronary events, cardiac arrest, and the severity and associated disability of chronic heart and vascular diseases. Comorbidity of CVD and the joint effects of multiple risk factors remains a topic where further investigation is also needed.

Conclusions and Recommendations

CVDs remain the leading cause of disease burden in the world. CVD continues its decades-long rise for almost all countries outside the high-income world and, alarmingly, age-standardized CVD rates have begun to rise in many locations where they were previously declining. There remains a large gap between identifying CVD as a problem, identifying the best package of solutions, and delivering them to the entire population.

GBD 2019 continues to be a platform that allows tracking and benchmarking of progress in the reduction of CVD burden. However, large investments are still needed to improve disease surveillance, including the expansion of robust vital registration systems, reliable electronic health records, and health surveys to all countries.

High rates of excess mortality are currently being observed because of the global severe acute respiratory syndrome coronavirus 2 pandemic, and much of this additional disease burden may be CVD due to the effects of both viral infection but also the unintended consequence of social distancing behaviors, such as changes in the delivery of health care (45,46). Further research in this area is urgently needed.

CVD was the cause of 6.2 million deaths occurring between the ages of 30 and 70 years in 2019. There is a pressing need to focus on implementing existing cost-effective interventions and health policies if the world is to meet the targets for Sustainable Development Goal 3 and achieve at least a 30% reduction in premature mortality due to noncommunicable disease by 2030. In the face of a global viral pandemic, we still must emphasize global commitments to reduce the suffering and premature death caused by CVD, which limits healthy and sustainable development for every country in the world.

Author Disclosures

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Footnotes

Listen to this manuscript's audio summary by Editor-in-Chief Dr. Valentin Fuster onJACC.org.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Appendix

For a complete list of the GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group, supplemental methods, and supplemental figures, please see the online version of this paper.

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Appendix

Supplemental Data 1:

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Supplemental Data 2:

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Supplemental Data 3:

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References

1. Vos T., Lim S.S., Abbafati C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* 2020;**396**:1204–1222. [PMC free article] [PubMed] [Google Scholar]

2. Mensah G.A., Roth G.A., Fuster V. The global burden of cardiovascular diseases and risk factors: 2020 and beyond. *J Am Coll Cardiol.* 2019;**74**:2529–2532. [PubMed] [Google Scholar]

3. Mensah G.A., Wei G.S., Sorlie P.D. Decline in cardiovascular mortality. *Circ Res.* 2017;**120**:366–380. [PMC free article] [PubMed] [Google Scholar]

4. Murray C.J.L., Aravkin A.Y., Zheng P. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* 2020;**396**:1223–1249. [PMC free article] [PubMed] [Google Scholar]

5. GBD Compare. https://vizhub.healthdata.org/gbd-compare/ Available at:

6. Global Health Data Exchange http://ghdx.healthdata.org/ Available at:

7. Tolonen Hanna, Mähönen Markku, Asplund Kjell. Do trends in population levels of blood pressure and other cardiovascular risk factors explain trends in stroke event rates? *Stroke.* 2002;**33**:2367–2375. [PubMed] [Google Scholar]

8. GBD 2017 Risk Factor Collaborators Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* 2018;**392**:1923–1994. [PMC free article] [PubMed] [Google Scholar]

9. Husain M.J., Datta B.K., Kostova D. Access to cardiovascular disease and hypertension medicines in developing countries: an analysis of essential medicine lists, price, availability, and affordability. *J Am Heart Assoc.* 2020;**9** [PMC free article] [PubMed] [Google Scholar]

10. Roth G.A., Forouzanfar M.H., Moran A.E. Demographic and epidemiologic drivers of global cardiovascular mortality. *N Engl J Med.* 2015;**372**:1333–1341. [PMC free article] [PubMed] [Google Scholar]

11. United Nations, Department of Economic and Social Affairs, Population Division World Population Ageing 2019: Highlights. ST/ESA/SER.A/430. https://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2019-Highlights.pdf Available at:

12. United Nations, Department of Economic and Social Affairs, Population Division World Population Prospects 2019: Highlights. ST/ESA/SER.A/423. https://population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf Available at: Available at: Accessed November 12, 2020.

13. Reynolds I., Page R.L., Boxer R.S. Cardiovascular health and healthy aging. In: Coll P.P., editor. *Healthy Aging: A Complete Guide to Clinical Management.* Springer International Publishing; Cham, Switzerland: 2019. pp. 31–51. [Google Scholar]

14. Fuster V. Global burden of cardiovascular disease: time to implement feasible strategies and to monitor results. *J Am Coll Cardiol.* 2014;**64**:520–522. [PubMed] [Google Scholar]

15. Gupta R., Wood D.A. Primary prevention of ischaemic heart disease: populations, individuals, and health professionals. *Lancet.* 2019;**394**:685–696. [PubMed] [Google Scholar]

16. Zimmerman M.S., Smith A.G.C., Sable C.A. Global, regional, and national burden of congenital heart disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Child Adolesc Health.* 2020;**4**:185–200. [PMC free article] [PubMed] [Google Scholar]

17. Vekemans J., Gouvea-Reis F., Kim J.H. The path to group A *Streptococcus* vaccines: World Health Organization research and development technology roadmap and preferred product characteristics. *Clin Infect Dis.* 2019;**69**:877–883. [PMC free article] [PubMed] [Google Scholar]

18. Ammirati E., Cipriani M., Moro C. Clinical presentation and outcome in a contemporary cohort of patients with acute myocarditis: multicenter Lombardy registry. *Circulation.* 2018;**138**:1088–1099. [PubMed] [Google Scholar]

19. Ammirati E., Veronese G., Brambatti M. Fulminant versus acute nonfulminant myocarditis in patients with left ventricular systolic dysfunction. *J Am Coll Cardiol.* 2019;**74**:299–311. [PubMed] [Google Scholar]

20. Sliwa K. Heart failure can affect everyone: the ESC Geoffrey Rose lecture. *Eur Heart J.* 2020;**41**:1298–1306. [PubMed] [Google Scholar]

21. Leon D.A., Shkolnikov V.M., McKee M., Kiryanov N., Andreev E. Alcohol increases circulatory disease mortality in Russia: acute and chronic effects or misattribution of cause? *Int J Epidemiol.* 2010;**39**:1279–1290. [PMC free article] [PubMed] [Google Scholar]

22. United Kingdom Small Aneurysm Trial Participants. Powell J.T., Brady A.R. Long-term outcomes of immediate repair compared with surveillance of small abdominal aortic aneurysms. *N Engl J Med.* 2002;**346**:1445–1452. [PubMed] [Google Scholar]

23. Mofidi R., Goldie V.J., Kelman J., Dawson A.R., Murie J.A., Chalmers R.T. Influence of sex on expansion rate of abdominal aortic aneurysms. *Br J Surg.* 2007;**94**:310–314. [PubMed] [Google Scholar]

24. Forbes T.L., Lawlor D.K., DeRose G., Harris K.A. Gender differences in relative dilatation of abdominal aortic aneurysms. *Ann Vasc Surg.* 2006;**20**:564–568. [PubMed] [Google Scholar]

25. Nienaber C.A., Fattori R., Mehta R.H. Gender-related differences in acute aortic dissection. *Circulation.* 2004;**109**:3014–3021. [PubMed] [Google Scholar]

26. Cheitlin M.D. Pathophysiology of valvular aortic stenois in the elderly. *Am J Geriatr Cardiol.* 2003;**12**:173–177. [PubMed] [Google Scholar]

27. Stritzke J., Linsel-Nitschke P., Markus M.R.P. Association between degenerative aortic valve disease and long-term exposure to cardiovascular risk factors: results of the longitudinal population-based KORA/MONICA survey. *Eur Heart J.* 2009;**30**:2044–2053. [PubMed] [Google Scholar]

28. Zilla P., Yacoub M., Zühlke L. Global unmet needs in cardiac surgery. *Glob Heart.* 2018;**13**:293–303. [PubMed] [Google Scholar]

29. Flack J.M., Kvasnicka J.H., Gardin J.M., Gidding S.S., Manolio T.A., Jacobs D.R. Anthropometric and physiologic correlates of mitral valve prolapse in a biethnic cohort of young adults: the CARDIA study. *Am Heart J.* 1999;**138**:486–492. [PubMed] [Google Scholar]

30. Freed L.A., Levy D., Levine R.A. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;**341**:1–7. [PubMed] [Google Scholar]

31. Avierinos J.-F., Gersh B.J., Melton L.J. Natural history of asymptomatic mitral valve prolapse in the community. *Circulation.* 2002;**106**:1355–1361. [PubMed] [Google Scholar]

32. Avierinos J.-F., Inamo J., Grigioni F., Gersh B., Shub C., Enriquez-Sarano M. Sex differences in morphology and outcomes of mitral valve prolapse. *Ann Intern Med.* 2008;**149**:787–794. [PMC free article] [PubMed] [Google Scholar]

33. Rosenhek R., Rader F., Klaar U. Outcome of watchful waiting in asymptomatic severe mitral regurgitation. *Circulation.* 2006;**113**:2238–2244. [PubMed] [Google Scholar]

34. Tleyjeh I.M., Abdel-Latif A., Rahbi H. A systematic review of population-based studies of infective endocarditis. *Chest.* 2007;**132**:1025–1035. [PubMed] [Google Scholar]

35. Whelton P.K., Carey R.M., Aronow W.S. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. *J Am Coll Cardiol.* 2018;**71**:e127–e248. [PubMed] [Google Scholar]

36. Lavie C.J., Laddu D., Arena R., Ortega F.B., Alpert M.A., Kushner R.F. Healthy weight and obesity prevention: *JACC* health promotion series. *J Am Coll Cardiol.* 2018;**72**:1506–1531. [PubMed] [Google Scholar]

37. Jiang L., Manson S.M., Beals J. Translating the diabetes prevention program into American Indian and Alaska Native communities: results from the Special Diabetes Program for Indians Diabetes Prevention demonstration project. *Diabetes Care.* 2013;**36**:2027–2034. [PMC free article] [PubMed] [Google Scholar]

38. Jousilahti P., Laatikainen T., Salomaa V., Pietilä A., Vartiainen E., Puska P. 40-year CHD mortality trends and the role of risk factors in mortality decline: the North Karelia project experience. *Glob Heart.* 2016;**11**:207–212. [PubMed] [Google Scholar]

39. Johnson R.J., Wesseling C., Newman L.S. Chronic kidney disease of unknown cause in agricultural communities. *N Engl J Med.* 2019;**380**:1843–1852. [PubMed] [Google Scholar]

40. Hammer M.S., van Donkelaar A., Li C. Global estimates and long-term trends of fine particulate matter concentrations (1998–2018) *Environ Sci Technol.* 2020;**54**:7879–7890. [PubMed] [Google Scholar]

41. Christidis T., Erickson A.C., Pappin A.J. Low concentrations of fine particle air pollution and mortality in the Canadian Community Health Survey cohort. *Environ Health.* 2019;**18**:84. [PMC free article] [PubMed] [Google Scholar]

42. Bennett J.E., Tamura-Wicks H., Parks R.M. Particulate matter air pollution and national and county life expectancy loss in the USA: a spatiotemporal analysis. *PLoS Med.* 2019;**16** [PMC free article] [PubMed] [Google Scholar]

43. Rajagopalan S., Brauer M., Bhatnagar A. Personal-level protective actions against particulate matter air pollution exposure. a scientific statement from the American Heart Association. *Circulation.* 2020 Nov 5 [E-pub ahead of print] [PubMed] [Google Scholar]

44. Shupler M., Hystad P., Gustafson P. Household, community, sub-national and country-level predictors of primary cooking fuel switching in nine countries from the PURE study. *Environ Res Lett.* 2019;**14** [Google Scholar]

45. Vestergaard L.S., Nielsen J., Richter L. Excess all-cause mortality during the COVID-19 pandemic in Europe—preliminary pooled estimates from the EuroMOMO network, March to April 2020. *Euro Surveill.* 2020;**25**:2001214. [PMC free article] [PubMed] [Google Scholar]

46. Rodríguez-Leor O., Cid-Álvarez B., Ojeda S. Impact of the COVID-19 pandemic on interventional cardiology activity in Spain. *REC Interv Cardiol.* 2020;**2**:82–89. [Google Scholar]

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Correlation between Type of Diet and Periodontal Parameters

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ABSTRACT

Introduction: Investigations about the Correlation between type of diet and periodontal parameters are very few and with less sample size. The main objective of this study was to establish the possible co-relation between type of diet and Wear patterns (erosion, abrasion, attrition) periodontal status (gingival index, periodontal pocket depth), plaque index and oral health parameters along with the help of questionnaire survey.

Materials and Method: Sample sizes of 260 healthy male and female subjects within the age group of 18-50 years were equally distributed into vegetarians and non-vegetarian groups. Clinical checkup will include examination of all the parameters. A questionnaire has been prepared for the participants for assessing the oral habits, dietary habits and the knowledge of oral health care. Statistical analysis of the data was conducted using pearson chi-square tests

Results: Periodontal findings comparing both the groups were not statistically significant. In the questionnaire survey, it was found that halitosis was greater in non-vegetarians when compared to vegetarians which were statistically significant. The remaining findings were not statistically significant.

Conclusion: Type of diet does not really affect the periodontal status of an individual. Both vegetarians and non-vegetarians show almost same level of gingival index, plaque index and periodontal pocket depths with dental education slightly lower in non-vegetarians than vegetarians with equal amount of regular dental visits. However, with proper oral hygiene procedures such as vertical brushing, brushing twice daily, using interdental aids and regular dental visits alone one can maintain good periodontal health regardless of diet being followed.

Keywords: *Vegetarian, Non-vegetarian, Periodontal parameters, full mouth examination, questionnaire, wasting diseases*

INTRODUCTION

Relation between diet and oral health is the most basic and often controversial subject and also there are increasing number of individuals complaining about oral health. Eating habits in developed and developing countries have changed in recent years. Various food stuffs have been introduced into diet that previously did not form a part of nutritional choices. This has resulted in an enrichment of daily diet, with the opportunity to vary foodstuffs more often and moreover this has led to the assumption, at least apparently, of wider spectrum of nutrients required for the wellbeing of the body.[1]Considering the relevance of effects of diet and food habits on oral health care, recent research reports have showed an actual co-relation between diet with that of parameters such as erosion ,attrition ,abrasion

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,gingivitis, periodontal status, amount of plaque etc. To understand this relation and promote better oral health ,better knowledge of diet is very important.

However, there is a lack of established facts and knowledge with regard to the concerned co-relation of diet and oral health parameters[2]. The purpose of this clinical study was mainly to understand and establish a better pathway to improve oral hygiene among the people in society with different food habits.

MATERIALS AND METHOD

Sample size of 260 healthy male and female subjects within the age group of 18-50 years, equally distributed in both the groups (vegetarians and non-vegetarians) respectively have participated in this study. Taking into consideration the possible risk factors affecting the parameters taken for the study, the exclusion criteria were: subjects with medical disorders, undergoing antibiotic or other anti-microbial therapy.

The inclusion criteria were subjects who were smokers and alcoholics. The subjects received verbal and written information about the study.

This study was a randomized controlled clinical study which included 260 participants, divided into two groups. Clinical checkup included examination of the following parameters: wearing of teeth, plaque index, periodontal status apart from which also the other parameters like color of gingiva, depth of periodontal pockets if present, gingival inflammation, bleeding on probing, number of decayed, missing teeth ; that is a full mouth assesment[2].A questionnaire was prepared for the participants for assessing the habits and the knowledge of oral health care among them. This was also done to sub categorize participants under the major study groups to narrow down the results obtained to a more accurate conclusion.

Baseline data on the medical history and previous hospital visits of the participants was recorded to minimize the factors affecting the results of the study and maintain the exclusion criteria. The participants were asked to answer the questionnaire.

Following this, clinical check-up was done on subjects for all the parameters included in the study using mouth mirror, an explorer and a periodontal probe[2]. The subjects were examined mainly for plaque to calculate plaque index, periodontal status, wearing of teeth which included abrasion, attrition and erosion and also for other parameters such as gingival inflammation, bleeding on probing and depth of gingival pocket.

Based on the clinical check-up the participants were asked to undergo dental treatment if needed or advised to take necessary oral prophylactic measures.

Clinical assessment of plaque index and gingival index was performed on four sites (buccal, lingual, mesial, distal) of the six key teeth (FDI tooth number 16,12,24,36,32,44) according to Loe H & Silness J. [3-6]. Each of the sites was given a score from 0-3 depending on the severity of the gingival condition.

Periodontal pocket depth and clinical attachment loss was recorded using William's graduated periodontal probe

STATISTICAL ANALYSIS

The calculation of the sample size was carried out using nQuery Advisor 6.0 (Statistical Solutions, Saugas, MA, USA). The analysis showed that a sample size of 160 in each group had a power of 80%. The recorded data were documented and analysed by the data procession program SPSS 17.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

In this study, 130 healthy male and female subjects aged 18-50 years for both the groups were included as participants. The prevalence of smoking and alcohol consumption was statistically similar in both the groups, with the non-vegetarian group being slightly greater.

Mean and range values of periodontal parameters are presented in table-1. There was greater probing depth in the vegetarian group compared to the non-vegetarian group, which was statistically not significant. While comparing the plaque index, it was higher in the vegetarian group and was statistically non-significant. But, even though statistically non-significant, the gingival index was seen to be a little greater in the non-vegetarian group when compared to the vegetarian group.

Table-1: Results of T-test for plaque index, gingival index and probing pocket depth among vegetarians and non-vegetarians

	1. DIET	N	Mean	Std. Deviation	t	df	P VALUE
PI	VEGETARIAN	129	1.22062	0.473661	1.299	258	0.195
	NON VEGETARIAN	131	1.14313	0.487665			
GI	VEGETARIAN	129	1.02744	0.560884	-0.173	258	0.863
	NON VEGETARIAN	131	1.03977	0.588518			
PPD	VEGETARIAN	129	2.7669	0.677827	0.506	238.97	0.614
	NON VEGETARIAN	131	2.71626	0.920669			

The wear pattern was not same among both the groups. Vegetarians had greater amount of attrition as compared to non-vegetarian group out of which occurrence of generalized attrition was found more in non-vegetarians. The cervical abrasion was more in non-vegetarian group as compared to vegetarians. This was also associated with the increased horizontal technique of brushing seen in non-vegetarian group.

Analysis of questionnaire showed that vegetarians and non-vegetarians had similar frequency of oral hygiene maintaining practices. It revealed that both the groups had similar number of dental visits. The brushing technique among the non-vegetarian group was more of horizontal technique when compared to the vertical technique used by vegetarian which was statistically significant. Also another statistically significant finding in the questionnaire was that halitosis was a more common complaint among the non-vegetarian group when compared to the vegetarian group. Level of dental education was about the same in both the groups apart from the type of brushing.

DISCUSSION

The effects of a vegetarian diet on systemic diseases like cancer, type 2 diabetes and coronary heart diseases have been studied by various authors and revealed predominantly less systemic diseases in vegetarians.[8,9]. Studies investigating correlations between vegetarianism and oral health are rare. Most authors assessed dental parameters or performed a saliva test.[10-14] In this study, emphasis on effect of diet on periodontal parameters is given. A very few studies have shown effect of diet on periodontal parameters as most of them compare oral health care among vegetarians and nonvegetarians.[15-18] Also, the studies done earlier have a comparatively smaller sample size (20-60), whereas this study has a significant sample size (n=130).

In addition, this study along with investigation of plaque score, gingival status, wear pattern have also gathered data regarding brushing technique, oral habits (smoking, alcoholism), oral hygiene practices, level of dental education and oral health problems via a multiple choice based questionnaire.

Our data indicates plaque index is slightly higher in vegetarian compared to non-vegetarian but it is not statistically significant. In our study the gingival index among non-vegetarians is slightly greater than vegetarians, yet statistically not significant. Since both the interrelated indices are inconsistent in both the groups and are almost similar, we believe that type of diet does not affect the periodontium in further causing gingivitis or periodontitis. In contrast a recent study by I Staufenbiel et al [2] in 2013 showed that gingival inflammation was significantly lesser in vegetarians. In addition, a study by Linkosalo et al.[16] revealed less bleeding on probing in vegetarians.

There are several pathways, which may lead to less inflammatory signs. Generally, vegetarians show more physical activity and a lower BMI. Hence, the prevalence of obesity, which may cause an increased local inflammatory response is lower. [19] Additionally, vegetarians consume a higher amount of antioxidants, which improves immune response, [20] and may lead to less inflammation.

Second, according to our study the average periodontal pocket depth in vegetarians was slightly higher than non-vegetarians. This is explained by the gingival score for vegetarians which was found slightly higher suggesting the presence of plaque causing slight loss of attachment, increasing the pocket depth. This may suggest chance of developing periodontal destruction irrespective of the type of diet and both vegetarians and non-vegetarians are equally susceptible to periodontal destruction. This is in contrast to the importance of an adequate nutrition for a healthy periodontium which was documented by Jenzschet al.[21]They demonstrated that solely a dietary change from non-vegetarian to vegetarian, according to the recommendations of Korber et al.[22]leads to better periodontal conditions. Jenzsch et al.[21] showed that 1 year after a dietary change without affecting dental hygiene and without dental therapy, patients had lower PPDs, less inflammatory signs and lower concentrations of interleukin-1β and interleukin-6 in the gingival crevicular fluid.

Third, the wear pattern observed among both the groups revealed that generalized attrition was more prevalent in non-vegetarians compared to vegetarians, but attrition of selected teeth was more common in vegetarian group. However, prevalence of cervical abrasion was notably greater in non-vegetarians than vegetarians. The potential etiological factors for such non-carious cavities are not very clear but it can be attributed to improper horizontal brushing technique. Interestingly, increased use of horizontal brushing technique in non-vegetarians was seen which was statistically significant.

This is in contrast to the recent study report given by Herman et al in 2011[1, 15], stating that vegetarians are more susceptible to non-carious cavity. He reported horizontal brushing technique is more in vegetarians than in non-vegetarians to his conclusion. In contrast, other authors could not find a different prevalence of erosion between vegetarians and non-vegetarians. Herman et al. [23] evaluated the prevalence of erosion and the consumption of acidic products in vegetarians and non-vegetarians. Regarding the prevalence of erosion, they could not show significant differences between the groups, although a significantly more frequent consumption of acidic products was observed among vegetarians.

Questionnaire analysis revealed equal level of dental education among both the groups and also similar frequency of regular dental visits. In contrary to this, various authors have showed higher level of dental education in vegetarians [24, 25] and also several studies have reported a lower use of prescription medications and health services by vegetarians.[25]. Statistically significant finding obtained from questionnaire reveals greater horizontal brushing in non-vegetarian group, this can be co-related to the increased amount of cervical abrasion seen in non-vegetarian group than in vegetarian group. This gives us quite a clear picture of how dental education is related to brushing technique which if faulty may cause cervical abrasion as seen in the non-vegetarian group.

Another statistically significant result from the questionnaire tells us halitosis was more common in non-vegetarians, which could probably arise because of the basic biochemical degradation of major component which constitutes an individual's diet. Meat being rich in protein undergoes putrefaction unlike carbohydrate that undergoes decomposition. Vegetarians usually tend to have high carbohydrate and low protein diet. When the results of smoking and alcoholism were matched in between vegetarians and non-vegetarians, both the group had similar distribution of smokers and alcoholics. This is in contrary to a study which states that Vegetarians consume less tobacco and alcohol, are physically more active and have a lower BMI compared with nonvegetarians.[21, 22]

CONCLUSION

Type of diet does not really affect the periodontal status of an individual. Both vegetarians and non-vegetarians show almost same level of gingival index, plaque index and periodontal pocket depths with dental education slightly lower in non-vegetarians than vegetarians with equal amount of regular dental visits. However, with proper oral hygiene procedures such as vertical brushing, brushing twice daily, using interdental aids and regular dental visits alone one can maintain good periodontal health regardless of diet being followed.

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REFERENCES

1. Al-Dlaigan YH, Shaw L, Smith AJ. Vegetarian children and dental erosion.International Journal

of paediatric dentistry 2001;11:184-92

2. I Staufenbiel, Knut Weinspach, G Forster, W Geurtsen, H Gunay. Periodontal condition in vegetarians: a clinical study ; European journal of clinical nutrition 2013
3. Rahmatulla M, Guile EE. Relationship between dental caries and vegetarian and nonvegetarian diets. Community Dent Oral Epidemol 1990; 18 : 227-8
4. Chaves ES, Wood RC, Jones AA, NEWbold DA, Manwell MA, Kornman KS.J Clin Periodontology 1993; 20:139-43
5. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. International Dental Journal 1975;25:229-35
6. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35
7. L.Laffranchi, F.Zotti, S.Bonetti, D.Dalessandri, P.Fontana. Oral implication of vegan diet: Obeservational study ; Minerva stomatol 2010;59
8. Key TJ, Appleby PN, Spencer EA, Travis RC, Allen NE, Thorogood M et al. Cancer incidence in British vegetarians. Br J Cancer 2009; 101: 192–197.
9. McEvoy CT, Temple N, Woodside JV. Vegetarian diets, low-meat diets and health: a review. Public Health Nutr 2012; 15: 2287–2294.
10. Linkosalo E, Markkanen H. Dental erosions in relation to lactovegetarian diet. Scand J Dent Res 1985; 93: 436–441.
11. Rahmatulla M, Guile EE. Relationship between dental caries and vegetarian and non-vegetarian diets. Community Dent Oral Epidemiol 1990; 18: 277–278. 12. Johansson G, Birkhed D. Effect of a long-term change from a mixed to a lactovegetarian diet on human saliva. Arch Oral Biol 1994; 39: 283–288. 13. Johansson G, Ravald N. Comparison of some salivary variables between vegetarians and omnivores. Eur J Oral Sci 1995; 103: 95–98.
14. Laffranchi L, Zotti F, Bonetti S, Dalessandri D, Fontana P. Oral implications of the vegan diet: observational study. Minerva Stomatol 2010; 59: 583–591.
15. Linkosalo E. Dietary habits and dental health in Finnish Seventh-Day Adventists. Proc Finn Dent Soc 1988; 84: 109–115.
16. Linkosalo E, Ohtonen S, Markkanen H, Karinpa¨a¨ A, Kumpusalo E. Caries, periodontal status and some salivary factors in lactovegetarians. Scand J Dent Res 1985; 93: 304–308.
17. Sedgley CM, Chu CS, Lo EC, Samaranayake LP. The oral prevalence of aerobic and facultatively anaerobic gram-negative rods and yeasts in semi-recluse human vegetarians. Arch Oral Biol 1996; 41: 307–309.
18. Sherfudhin H, Abdullah A, Shaik H, Johansson A. Some aspects of dental health in young adult Indian vegetarians. A pilot study. Acta Odontol Scand 1996; 54: 44–48.
19. Tonstad S, Butler T, Yan R, Fraser GE. Type of vegetarian diet, body weight, and prevalence of type 2 diabetes. Diabetes Care 2009; 32: 791–796.
20. Kuzmanova D, Jansen ID, Schoenmaker T, Nazmi K, Teeuw WJ, Bizzarro S et al. Vitamin C in plasma and leucocytes in relation to periodontitis. J Clin Periodontol 2012; 39: 905–912.
21. Jenzsch A, Eick S, Rassoul F, Purschwitz R, Jentsch H. Nutritional intervention in patients with periodontal disease: clinical, immunological and microbiological variables during 12 months. Br J Nutr 2009; 101: 879–885.
22. Ko¨rber KMTLC. Vollwerterna¨hrung. Konzeption einer zeitgema¨_en und nachhaltigen Erna¨hrung. Haug: Stuttgart, Germany, 2004.
23. Herman K, Czajczyn´ ska-Waszkiewicz A, Kowalczyk-Zaj ˛ac M, Dobrzyn´ ski M. Assessment of the influence of vegetarian diet on the occurrence of erosive and abrasive cavities in hard tooth tissues. Postepy Hig Med Dosw 2011; 65: 764–769.
24. Davey GK, Spencer EA, Appleby PN, Allen NE, Knox KH, Key TJ. EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. Public Health Nutr 2003; 6:259–269.
25. Baines S, Powers J, Brown WJ. How does the health and well-being of young Australian vegetarian and semi-vegetarian women compare with non-vegetarian? Public Health Nutr 2007; 10: 436–442.

The Effect of Kitchen Table Exercise Program on Strength, Balance and Functional Mobility in Elderly Population

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ABSTRACT

Background: The elderly population is the population of 60 years old and above and they are considered as a targeted population. This targeted population is more prone for risk of falls due to strength and balance dysfunction. So this study is conducted to improve this dysfunction with Kitchen table exercise program.

Objective: To find the effect of Kitchen Table Exercise Program on strength, balance and functional mobility in elderly population.

Materials and Method: 60 Subjects with 60 years of age and above included in the study. Subjects were divided into the two groups 30 in each group. Group A received the Kitchen table exercise program and Group B received conventional exercises. Both groups received 12 weeks of exercise program. In pre interventional assessment strength was taken by 1 RM; Balance was taken by Berg Balance scale and Functional mobility by Elderly mobility scale. Post interventional assessment was taken by the same outcome measures after 6th and 12th weeks of intervention.

Result: Both the groups showed improvement in the strength, balance and functional mobility. But the group A is improved in comparison with group B ($P<0.0001$)

Conclusion: The kitchen table exercise program can improve strength, balance and functional mobility in elder population.

Keywords: *Kitchen Table Exercise Program, Strength, Balance, Functional mobility, elderly population.*

INTRODUCTION

India has acquired label of an ageing nation with 7.7% of its population being more than 60 years old. The population of 60 years old and over are considered as elder people.[1-2] As the age advances the risk of fall increases[3-5]. Around 25%- 35% of older population are prone to fall i.e., one or more falls every year[4-7]. Strength of muscle decreases as the age advances and in older individual's activities become much harder (lifting a bag of groceries) or may not be able to perform activity without assistance. Impairment of muscle strength leads to falls (impairment in balance) and functional inability.[8] The muscle mass decrease by 50% between the age group of 20-90 years. This results in decline in strength which correlates with increased risk of fall[9]. Factors contributing to loss of balance are intrinsic and extrinsic factors. Intrinsic factor includes change in muscular strength.[10, 11]. Important parameter of balance performance includes range of motion, muscle strength, somatosensory function and the size and the quality of the base of support.[12-14] Rehabilitation of balance can work on static or dynamic balance. Dynamic balance is the ability to maintain balance while body is in motion and it keeps the centre of gravity over the base of support[15].

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Mobility is nothing but the physical ability to move. The first signs of decreasing mobility are mostly observed for more demanding mobility tasks, such as walking longer distances.[16] Environmental barriers and reduction in physical activities shows increased in level of mobility decline. Environmental barriers directly relates with fear of falling and moving outside which produces avoidance of outside activities[17]

Regular exercise has been shown to improve and maintain muscle strength and to improve mobility and balance in elderly individuals.[18] The rehabilitation of this targeted population should include a simple and understandable exercise program.

The current study is conducted with the same objectives to solve the above age related problems and make the older individuals independent in their functional activities. The kitchen table exercise program is designed to address the components of strength, balance and functional abilities. The Kitchen table exercise program is the simple exercise program.

But the effectiveness of this exercise program is still not studied in older population. So this study is conducted with an aim to find the effectiveness of KTE program on targeted population of community.

MATERIALS AND METHODOLOGY AND PROCEDURE

The study was conducted at Krishna Institute of Medical Sciences, Physiotherapy OPD, Karad. The Ethical clearance was taken from Institutional Ethical committee prior to the start of treatment. The subjects were taken as per the inclusion and exclusion criteria. The inclusion criteria was both male and female of 60 years of age and above having Berg balance scale score less than 45 and who were willing to participate was taken. Subjects with cardiac diseases, who have undergone recent surgeries and traumatic conditions, were excluded. An informed consent was taken from the subjects selected for the study.

The random sample of 60 subjects was studied in this experimental study. These subjects were divided into 2 groups with simple random sampling method. Group A was experimental group with the mean age of 69.86±6.42 years while the group B was an control group with a mean age of 71.9±6.68. Group A had a Kitchen table exercise program i.e., Sit to stand, Plantarflexion and Dorsiflexion, Weight bearing exercises, Standing with feet apart, Weight shifting exercises and Clock exercises.The group B was a control group.They were given conventional treatment of free exercises, Reach out exercises, Balance board exercises, Walking and Resisted exercises.

The pre treatment and post treatment assessment was done by outcome measures like 1 RM, Berg Balance Scale and Elderly mobility Scale.

Data analysis -The statistical analysis of 1 RM, Berg Balance Scale and Elderly mobility Scale was done by repeated measure anova test.

1) 1 Repetition Maximum:

Table 1: Comparison of pre and post 1 RM score within the group

	PRE	POST 6thWeek	**POST 12thWeek**	**P VALUE**	**F VALUE**	**INFERENCE**
GROUP A	28.73± 4.54	32.6±4.64	36.66±4.69	<0.0001	1011.7	Extremely significant
GROUP B	29.3 ±3.33	30.6±3.28	32.13±3.17	<0.0001	119.8	Extremely significant

In the Group A, the P value by repeated measure anova test was found to be <0.0001 which was extremely significant. In Group B, the P value by repeated measure anova test found to be <0.0001 which was extremely significant.

2) BERG BALANCE SCALE:

Table No.2: Comparison of pre and post Berg balance score within the group

	PRE	POST 6TH WEEK	POST 12THWEEK	P VALUE	F VALUE	INFERENCE
GROUP A	41.36±1.77	45.43±1.94	50.13±1.87	<0.0001	654.38	Extremely significant
GROUP B	41.7±2.20	43.56±1.97	45.2±2.09	<0.0001	296.85	Extremely significant

In the Group A The P value by repeated measure anova test was found to be <0.0001 which was extremely significant. In Group B, the P value by repeated measure anova test was found to be <0.0001 and which was extremely significant.

3) ELDERLY MOBILITY SCALE

Table No. 3:Comparison of Pre and Post ELDERLY MOBILITY SCALE score within the group

	PRE INTERVENTION	POST INTERVENTION 6TH WEEK	POST INTERVENTION 12TH WEEK	P VALUE	F VALUE	INFERENCE
GROUP A	10.56±1.16	13.76±1.07	17.03±0.92	P <0.0001	1046.8	Extremely significant
GROUP B	10.23±0.89	11.73±0.82	13.2±1.09	P < 0.0001	338.49	Extremely significant

In Group A, the P value by repeated measure anova test was found to be <0.0001 which was extremely significant. In Group B, the P value by repeated measure anova test found to be <0.0001 which is extremely significant.

RESULT

The Kitchen table exercise program i.e., Group A showed extremely significant improvement than the Group B which was controlled group.

DISCUSSION

This study was conducted in the elderly population of 60 years of age and above to determine the effect of kitchen table exercise program on strength, balance and function mobility. In this study, 60 subjects had participated who were screened as per the inclusion and exclusion criteria and analysed as per the pre test outcome measures out of which 35 were male subjects and 25 were female subjects. The mean age of subjects included in Group A was 69.86 and in Group B was 71.9 out of 60 subjects, this was statistically not significant. Subjects were analysed and were divided into two groups according to random sampling method. 30 subjects were included in Group A and were received Kitchen table exercise program. The treatment protocol was continued for 12 weeks. Similarly, 30 subjects were included in Group B and were received conventional exercises for strength, balance and functional mobility and the treatment protocol was continued for 12 weeks.

Pre and post treatment outcome measures for strength, balance and functional mobility was done with 1 Repetition maximum, Berg balance scale and elderly mobility scale. The specific treatment protocol was followed for 12 weeks.

Kitchen Table Exercise program is designed to be a self directed exercise program. The program consists of six levels and each level contains six exercises. The exercises are specifically designed to address components of strength, balance and functional mobility. The purpose of present study was to find out effect of Kitchen Table Exercise program on strength, balance and functional mobility in elderly people.These exercise program consists of various closed kinematic chain exercises, weight bearing exercises.

According to various studies it was analysed that closed kinematic exercises may help in improving quality of life of elder population. This study aims to improve quality of life of oldersubjects. In this study treatment protocol was given for twelve weeks. As this program consists of six levels each level of exercises were given for two weeks.

I ntra group comparison (within group) was analysed by repeated measure anova test for 1 Repetition Maximun, Berg Balance Scale and Elderly mobility Scale. This showed that there was extremely significant difference of Group A 1 RM score with (P<0.0001). Berg Balanve Scale scores was also extremely significant with (P<0.0001). Elderly Mobility Scale score shows extremely significant difference with (P<0.0001).

Similarly, In Group B, there was extremely significant difference of 1 RM with (P<0.0001) respectively. Berg Balance Scale score was also extremely significant with (P<0.0001). Elderly Mobility Scale score was also extremely significant with (P<0.0001).

In this study, an attempt was made to improve strength, balance and functional mobility. Improvement of subject on 12th week i.e. after the treatment program gets over.

The subject could perform exercise without support.

There could be improvement in balance in subject.

There was reduction in symptoms in both the groups but the reduction of symptoms was more in group A then group B.

The muscle strength increases consistently through gradual resistance exercises.[19,20]The body reacts to new positions based on the changes in the base of support.[12,13] Hence, various exercises such as open chain and closed kinematic exercises are used to improve balance.[21,22,23] Regular resistance exercise improve muscle function. Increases strength after damage and it prevents decline in muscle strength despite aging.[24,25]

In conclusion, the result of current study shows that Kitchen table exercise program ismore significant than routine exercise program for strength, balance and functional mobility in elderly people. Further studies can be done for longer duration of treatment protocol in order to determine the long term effect of this program.

CONCLUSION

Different approaches are used for improvement of the strength, balance and functional mobility in elderly people but this study concluded that the Kitchen Table Exercise program was more effective than the conventional routine exercises in improvement of the strength, balance and functional mobility and improving quality of life.

Hence, It is proved that Kitchen Table Exercise Program has significant effect in elderly population.

Ethical Clearance: Ethical clearance was taken from institutional ethical committee of KIMSDU, Karad

Source of Funding: Project is funded by KIMSDU, Karad

Conflict of Interest: No any conflict of interest

REFERENCES

1. Gopal K Ingale, Anita Nath, Geriatric health in India Concerns and Solution : India J Community Med. 2008 Oct; 33 (4) : 214-218.
2. Provisional Guidelines onStandard International Age Classification; Department of International Economical And Social Affairs; Series M No.74; United Nations, New York, 1982.
3. Tinetti ME, Bake DJ, McAway G, et.al. A multifactorial intervention to reduce risk of falling among elderly people living in community.NEng J Med. 1994; 331:821.827.
4. Hornbrook MC, stevens J. Wingfield DJ et. Al. Preventing falls among community dwelling oldr/er person: results from a randomized trial.
5. Blake: AJ. Morgan K, Bendaul MJ.et. al falls by wlderly people at home: prevalence and associated factors. Ageing. 1988;17:365-372.
6. Neitt MC, Cumming SR, Risk factors for recurrent nonsyncopalfalls : a prospective study. JAMA. 1989;261:2663-2668.
7. Tinetti ME, GInter SF. Identifying mobility dysfunction in elderly patients: standard neuromuscular examination or direct assessment. JAMA. 1988;259-1190-1193.
8. Buncher DM, deLateur BJ. The importance of skeletal muscle strength to physical function in older adults. Ann Behav med. 1991;13:91-98.

9. Tzankoff SP, Norris AH. Longitudinal changes in basal metabolic rate in man. J Appl Physiol.1978;33:536-539. Ol. 1999;86:188-194.
10. Whipple RH. Wolfson L2, Anerman PM. The relationship of knee and ankle weakness to falls in nursing home residents : an isokinetic studt .J. Am. Geriatrsoc. 1987;35:13-20.
11. Tinetti ME, speechley m, Ginter SF. Risk factoe of falls among elderly person living in community .nengl. J. Med 1988: 319 : 1701- 1707.
12. Shummway-Cook A, Woollacott MH: Motor Control: Theory and PracticeApplication. 2nd ed. Philadelphia, LW & W, 2001, 163–191.
13. Bennell KL, Hinman RS, Metcalf BR, et al.: Relationship of knee joint proprioception to pain and disability in individuals with knee osteoarthritis. JOrthop Res, 2003, 21: 792–797.
14. Fransson P, Johansson R, Hafstrom A, et al.: Methods for evaluation ofpostural control adaptation. Gait Posture, 2000, 12: 14–24.
15. Raymakers JA, Samson MM, Verhaar HJ: The assessment of body swayand the choice of stability parameters. Gait Posture, 2005, 21: 48–58.
16. Saino P, Koskinen S, Heliovaara M, Martelin T, Harkenen T, Hurri H, et al. Self-reported and test-based mobility limitation in a representative sample of Finns aged 30+ Scand J Public Health. 2006 ; 43 (4) : 378-386.
17. Rantakokko M, Manty M, Iwarsson S, Tormakangs T, Leinonnen R, Heikkinen E, et al. Fear of moving outdoors and development of outdoor walking difficulty in older people. J Am Geritr Soc.2009;57(4):634-640.
18. Keogh JWL, Morrid S, Barrett R. strength and co ordinationtraining are both effective in reducing the postural tremor amplitude of older adults. J Aging Phys Act, in press.
19. Prince WE: Rehabilitation technique in sports medicine. 3rd ed. USA:McGraw-Hill, 1999, pp170–187.
20. Iwasaki T, Shiba N, Matsuse H, et al.: Improvement in knee extensionstrength through training by means of combined electrical stimulationand voluntary muscle contraction. Tohoku J Exp Med, 2006, 209: 33–40.
21. Sauvage LR Jr, Myklebust BM, Crow-Pan J, et al.: A clinical trial ofstrengthening and aerobic exercise to improve gait and balance in elderlymale nursing home residents. Am J Phys Med Rehabil, 1992, 71: 333–342.
22. Heller BM, Pincivero DM: The effects of ACL injury on lower extremityactivation during closed kinetic chain exercise. J Sports Med Phys Fitness,2003, 43: 180–188.
23. Lin MR, Hwang HF, Wsang YW, et al.: Community-based tai chi and itseffect on injurious falls, balance, gait, and fear of falling in older people.PhysTher, 2006, 86: 1189–1201.
24. Lustosa LP, Silva JP, Coelho FM, et al.: Impact of resistance exercise program on functional capacity and muscularstrength of knee extensor in pre-frail community-dwelling older women: a randomized crossover trial. Rev Bras Fisioter,2011, 15: 318–324.
25. Cho SI, An DH: Effects of a fall prevention exercise program on muscle strength and balance of the old-old elderly.PhysTherSci, 2014, 26: 1771–1774.

Is Dietary Management is Essential for Gallbladder Diseases? A Review based on Available Literature

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ABSTRACT

BACKGROUND: In biliary system, Gall bladder diseases (GBD) are the most common disorder, and most of the cases are asymptomatic. It is also one of the most common diseases affecting 10-15 % of Caucasian adults (racial group having light-colored skin; white) in developed countries have GBD.

OBJECTIVE: To explore the evidence on patients' knowledge on common gallbladder diseases, as well as their practice regarding dietary management of GBD.

METHOD: Pub Med and EBSCO were searched for reviews on knowledge and practice regarding dietary management among patients affected with GBD.

RESULTS: The accessible literature refines to get 7 qualitative studies. In this narrative review, 6 research studies supported that dietary management is helpful in gallbladder diseases and may provide proper health, if it is followed. 1 research study shows that there is no remarkable result of low-fat diet in the prevention of gastrointestinal symptoms and specially gallstones.

CONCLUSION: Gallbladder diseases can not only be treated with medicines, but it actually requires dietary management so that it can be treated with diet.

KEYWORDS: *knowledge, practice, dietary management, patients, gallbladder diseases.*

INTRODUCTION

A gallbladder disease is one of the highest common gastrointestinal disorders, which result from a complex synergy of genetic and environmental factors. At present, it assumed as a big cause of abdominal morbidity, which greatly increase hospital admissions in the developing countries. In developed population of North India, its degree is 7.4%.where as in cadavers, it was only 6.3%. In some studies, the incidence of GBD in the Iranian men and women is quite low between the age group of 31-40 years. It increases sharply in men older than 60 years and women older than 50 years to more than ten-fold (12.5 and 24.6% in males and females 71-80 years respectively).

NEED FOR THE STUDY

GBD is a common medical condition, one among which is Cholelithiasis (formation of stone in the gallbladder), Cholecystitis (infection and inflammation of gallbladder) and Choledocholithiasis (formation of stone in the common bile duct). Regarding diet as an vital environmental agent, mounting cue in nutritional epidemiology commends that pattern assay is the most realistic approach to assess the associations between overall diet and health or disease. **Stinton and Shaffer, (2012)**, in their study showed that up to 80 % of cases are asymptomatic. **Amaral and Thompson, (1985); Erlinger,** state that diet is the most common risk factors for gallbladder disease specially high intake of energy leads to obesity where as high fat will increase the risk for GBD.

Gall bladder diseases are genuine and their vogue increases greatly with age; it is also decided by gender, body weight, and race. Gall bladder disease does not account serious notes such as colic and jaundice. Generally, gall bladder diseases are extensively cured by

cholecystectomy, and most of the cases are executed by laparoscopy.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1116086/

AIM OF THE STUDY

To identify the knowledge of the patients affected with gallbladder disease regarding the dietary management.

OBJECTIVE: To explore the evidence on patients' knowledge on common gallbladder diseases, as well as their practice regarding dietary management of GBD.

METHODOLOGY

Search strategy methods-

An electronic search of articles published in various journals has been done. Search was restricted to only English language.

The database search was PubMed and EBSCO.

Types of Studies- Cohort study, Case-control study, Phenomenological approach, Prospective analytical single center study, Randomized clinical trial, Descriptive study.

Types of Participants- Patients suffering from GBD.

Settings- Government and private hospitals.

For this narrative review, articles were searched on Pub Med and EBSCO, using keywords such as knowledge, practice, dietary management, patients, and gallbladder diseases. During initial search **13447** titles were retrieved andnumber of records screened after duplication were**12660** and rest **787** records were excluded. **35** records screened and records excludedwere **12625,** full text articles assessed for eligibility were**14.** Full text articles excluded due to irrelevant content and subscription were **21** and finally**7** studies included in qualitative synthesis.

Outcome: The outcomes from these studies clearly focus to the understanding about the knowledge and practice of gallbladder disease patients regarding their dietary management and up to which extent it is essential. Knowledge and practice of dietary management plays an important role in gallbladder disease patients.

DISCUSSION

According to **Gonzalez et.al, 2014,** the finding showed that abdominal adiposity**,** insulin resistance, obesity and nutritional factors such as higher intake of total energy, fats, sugar and long fasting periods are the cause of gallstone formation while satisfactory fiber intake in diet is a protective factor.[1]

According to **Menezes et.al, 2013**, it was observed that there was permanence, appearance or disappearance of symptoms postoperatively.[2]

As per the study of **Kim E and Lee Y, 2012**, it shows that there is a little association that dietary habits are related with cholesterol gallstone formation.[3]

According to **Johansson et.al, 2014**, the study suggested that Gall stones can be treated in the primary care, at home which are less serious for the patient as well as cheap for the society.[4]

As per the study conducted by **Jyi Tsai et.al, 2014**, the results suggested that high intake of vegetable protein can decrease the complication of cholecystectomy in women.[5]

According to **Bansal A et.al, 2014**, 43.56 years is the average of the clients with the ratio of 0.52:1 for male and female respectively In this study, fused type of regime, and multiple gallbladder calculi was observed.[6]

According to **Jessri and Rashidkhani, 2015**, dietary pattern system gives data related to the disease and dietary.[7]

Ethical Consideration:

This manuscript is ethically considered by the panel of Teerthanker Mahaveer College of Nursing, Teerthanker Mahaveer University after discussing with each and every point of this manuscript

Summary of Findings:

Out of 7 articles, 6 research studies supported that dietary management is helpful in gallbladder diseases and may provide proper health if it follow in appropriate manner.

Importance in Education:

Education is an important part for every individual not only to learn the facts about different aspects but

also to apply the facts in our life and more over that to follow them in daily routine in order to get a better life. Like the same way this review suggests that having a good dietary knowledge as well as practice during the gallbladder diseases is an essential part to follow in order to maintain a healthy lifestyle. Appropriate knowledge leads to better health which comes only with the education.

Future Significance:

It's always easier to prevent a problem rather than treat it, and this is especially true when it comes to the gallbladder problems.

Limitations:

- Computerized data bases were limited.
- Limited to only in pregnant women.

Strength:

- Article search was carried out on a significant problem
- Review could find out the gap in knowledge and practice on dietary management of patients affected with gallbladder diseases.

Weakness:

- Articles mainly focus on only knowledge and practice regarding dietary management.
- Only 7 qualitative articles were included for data synthesis due to limitations.

CONCLUSION

A healthy regime deals with a converse coalition with gallbladder disease. As a result, these data need to be acknowledged in future prospective studies for etiological purposes in both men and women to draw more deciding events. High intake of vegetable protein can reduce the effect of cholecystectomy in females.

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. Gonzalez-Hita M, Batis-Ruvalcaba C, Sanchez-Enriquez S. Diet and Nutritional Factors Related to Symptomatic Gallstone Disease in Women. J Clin Case Rep. 2014;4(458):2.
2. Menezes HL, Fireman PA, Wanderley VE, Menconça ÂM, Bispo RK, Reis MR. Randomized study for assessment of hypolipidic diet in digestive symptoms immediately following laparoscopic cholecystectomy. Revista do Colégio Brasileiro de Cirurgiões. 2013 Jun;40(3):203-7.
3. Kim E, Lee Y. A Descriptive Study of Gall Stone Patient's Dietary Habits and Nutritional Status. Korean Journal of Community Nutrition. 2007 Dec 1;12(6):826-37.
4. Johansson K, Sundström J, Marcus C, Hemmingsson E, Neovius M. Risk of symptomatic gallstones and cholecystectomy after a very-low-calorie diet or low-calorie diet in a commercial weight loss program: 1-year matched cohort study. International journal of obesity. 2014 Feb;38(2):279.
5. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Dietary protein and the risk of cholecystectomy in a cohort of US women: the Nurses' Health Study. American journal of epidemiology. 2004 Jul 1;160(1):11-8.
6. Bansal A, Akhtar M, Bansal AK. A clinical study: prevalence and management of cholelithiasis. International Surgery Journal. 2016 Dec 10;1(3):134-9.
7. Jessri M, Rashidkhani B. Dietary patterns and risk of gallbladder disease: a hospital-based case-control study in adult women. Journal of health, population, and nutrition. 2015 Mar;33(1):39.

Community Satisfaction Index as an Evaluation of Health Services Quality

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ABSTRACT

Quality control of service is the most important part in health service, so that the measurement of patient satisfaction level is absolute in effort to improve health service quality. Through these measurements, it can be known to what extent the dimensions of health services quality that have been held to meet patient expectations. This research aims to find out the result of community satisfaction index as an evaluation of health service quality in Brondong Primary Health Care of Lamongan Regency, using 9 elements taken from Permenpan Number 16 year 2014. The data were taken by observation with cross sectional study design using questionnaires analyzed descriptively. Samples were taken using Slovin formula, so that the respondent obtained 394. The measurement result shows that in general the quality of Brondong Primary Health Care of Lamongan Regency service is included in good category with CSI value of 3,050. However, when each element be viewed, there are still three elements with the lowest of community satisfaction index value indicating that there are still people who feel less satisfied with the service in Brondong Primary Health Care of Lamongan Regency. The three elements that have the lowest of community satisfaction index value are the element of service time, the service announcement, and the handling of complaints, suggestions, and inputs of the community. Therefore, this research suggests in Brondong Primary Health Care of Lamongan Regency needs to improve the quality, especially on the three elements with the lowest of community satisfaction index value.

Keywords: *community satisfaction index, health service quality.*

INTRODUCTION

The role and function of Primary Health Care in the implementation of Community Health Efforts and Individual Health Efforts should always pay attention to the needs of the community as a service user. In addition, it is necessary also the input and evaluation of the performance of Primary Health Care for use in efforts to improve and improve the service quality. Therefore, a system is required to get an analysis of community needs and expectations and feedback on Primary Health Care performance.

Patient satisfaction is a level of patient feeling that arises as a result of the health service performance it receives after the patient compares it to what they expect. The new patient will feel satisfied if the performance of health services obtained equal or exceeded the expectations and on the contrary, dissatisfaction will arise or the patient's disappointment will occur if the performance of health services obtained was not as expected[1].

To measure the level of patient satisfaction is not easy, because the effort to obtain the necessary information to measure the level of satisfaction of the patient will be faced with a cultural constraint, so that, there is a tendency of people who are reluctant or unwilling to express criticism, especially to government health service facilities. Service user satisfaction determines the next option, willing to keep using the service or move to another health facility.

Quality is a service that can meet customer expectations and understand customer needs in the future[2]. The quality of health services is the application

of medical science and technology to maximize health benefits without an increased risk[3]. Furthermore, the good quality of health services when patients are served with appropriate services, both in terms of competent way of serving, good communication, decision making together and cultural sensitivity[4]. Quality has an abstract concept[5]. The concept of abstract quality leads to the definition of quality depending on the perspective of each individual[4].

Previous research conducted by Analysis of the Effect of Patient Perception about Quality of Doctor Service on Patient Loyalty in Public Policlinic of Outpatient Care Installation of Panti Wilasa Citarum, Semarang Hospital in 2008, the result of the research shows there is a significant influence between medical technical skill, attitude, the delivery of information, the timeliness of service, and the availability of the doctor's consultation time on patient loyalty[6].

Brondong Primary Health Care as a public service provider is also responsible for carrying out the mandate from Permenpan and RB Number 16 Year 2014 on Guidelines for the Implementation of Public Satisfaction Survey of Public Service to obtain a Public Satisfaction Index nationally.

There are 9 elements or indicators to assess the satisfaction of society according as follows: The first element (E1) is a requirement to get service. In this element how society assess their easiness in fulfilling technical and administrative requirement to get service at government institution. Second element (E2) is the service procedures, in this element society assess the service unit of government institution based on society ease in comprehending service or procedure in the unit. The Third element (E3) is the service time, the duration in completing the service that has been determined by the service provider facilities and the accuracy of the schedule that has been determined. The fourth element (U4) is the cost/service tariff, here how the community assess the fairness and affordability of the service fee. The fifth element (E5) is a type of health service, where the community assesses the type of health services in health facilities is in accordance with the needs of the community. The sixth element (E6) is the competence / skill of the officer, where the aspect seen by the community is the skill and skill of the officer in providing the service. The seventh element (E7) is the behavior / attitude of the officers, hospitality and courteous of service providers in providing services to the community is an important point assessed by the community. The eighth element (E8) of the services ministry, which assessed is the ability of PHC officers to serve according to the standards. The ninth element (E9) is a tool for complaints of suggestions and input from the community as well as follow-up[7].

This study aims to determine the community satisfaction index as an evaluation of health services quality of Brondong Primary Health Care of Lamongan Regency in year 2017.

MATERIAL AND METHOD

The method of this research was an observational study with cross sectional study design used questionnaires analyzed descriptively. Data was collected in 2017 at Brondong Primary Health Center, while the population in this study was all patients who visited Brondong Primary Health Center for 1 year. Sampling in this study has been used Slovin formula with a fault tolerance of 5%, so that obtained 394 respondents.

Respondents were interviewed with questionnaires. The questionnaire contains questions about the level of patient satisfaction based on the elements of community satisfaction on Guidelines for the Implementation of Public Satisfaction Survey of Public Service.

The data that have been obtained, was processed in the way has been adopted from Kepmenpan Number Kep / 25 / M.PAN / 2 / 2004, on General Guidelines for Preparation of Public Satisfaction Index of Government Services Unit. Stages of data processing are as follows: First, Determine the value of weighted mean with the following formula:

$$\textbf{Weighted mean value} = \frac{The\ Sum\ of\ Weight}{The\ Sum\ of\ Elements} = \frac{1}{9} = 0{,}111$$

Second, determine the mean value per service element by:

The value per service element be summed up then divided by the number of respondents

Third, determine the weighted mean value of each element service by:

The mean value per service element is multiplied by the weighted mean value

Fourth, Calculation of Community Satisfaction Index of Service Unit, by adding 9 (nine) elements of weighted mean value

Table 1. Perception values based on The Calculation fo Community Satisfaction Index[8]

Perception Value	CSI Interval Value	CSI Conversion Interval Value	Quality of Services	Criteria of Performance
Dissatisfied (1)	1,00 – 1,75	25,00 – 43,75	D	Not Good
Less Satisfied (2)	1,76 – 2,50	43,76 – 62,50	C	Less Good
Satisfied (3)	2,51 – 3,25	62,51 – 81,25	B	Good
Very Satisfied (4)	3,26 – 4,00	81,26–100,00	A	Very Good

RESULTS AND DISCUSSION

Based on interviews with the help of questionnaires to 394 respondents who were patients who visited Brondong Primary Health Care, the result of Community Satisfaction Index based on the calculation that has been adopted from Kepmenpan Number Kep / 25 / M.PAN / 2 / 2004 can be seen in table 2 below.

Table 2. Calculationf of Community Satisfaction Index in Brondong Primary Health Care in 2017

Element	1	2	3	4	Amount	Weight per elements	Mean Value per elements	Weighted Mean per elements
E1	2	25	316	51	1204	0.111	3.056	0.340
E2	0	27	318	49	1204	0.111	3.056	0.340
E3	1	36	324	33	1177	0.111	2.987*	0.332*
E4	0	23	331	40	1199	0.111	3.043	0.338
E5	1	24	320	49	1205	0.111	3.058	0.340
E6	0	24	316	54	1212	0.111	3.076	0.342
E7	0	24	306	64	1222	0.111	3.102	0.345
E8	1	24	331	38	1194	0.111	3.030*	0.337*
E9	0	27	324	43	1198	0.111	3.041*	0.338*
CSI value								3.050

NOTE - * The lowest value element

The Table 2. above Indicts that the patient satisfaction index with the quality of health service in Brondong Primary Health Care in Lamongan Regency is 3.050. If that refers to the quality of service category from Kepmenpan Number Kep / 25 / M.PAN / 2/2004, so that the health service quality of Brondong Primary Health Care of Lamongan Regency is included in category B, which means that health service quality of Brondong Primary Health Care was good.

Elements that have the highest satisfaction value is the seventh element that is the behavior / attitude of the officer with the value of Community Satisfaction Indeks (CSI) of 3.102 are included in the good category. Friendliness and courteous service providers in providing services to the community is an important point assessed by the community.

Elements that have the second highest value is the sixth element that is the competence or skill of the officer with the value of CSI of 3,076 are included in the category elements above is a potential owned by

Brondong Primary Health Care, where qualified human resources are able to provide maximum performance in an organization.

Element that has the third highest value is the fourth element that is about the type of health services with the value of CSI of 3.058 are included in either category. It is seen that the clarity of the types of services available, listed on the announcement board, banner, brochures and leaflet in Brondong Primary Health Care, and the type of health services available is the result of the survey of the needs and expectations of the community about the types of services available at the Brondong Health Center[9].

The element that gets the first lowest value is the third element that is about the time of service with the value of CSI amounted to 2.987. Although it is still included in the good category but needs to get attention for improvement. It is seen that many patients are queuing up and the target of waiting time service has not been achieved due to less supportive systems such as slow internet access and search for old medical records. The long waited queue will cause a negative perception on the customer about the production, the quality of service and satisfaction[10].

Element that got the second lowest value is the eighth element of the service information with the value of CSI 3.030. It is still seen some employees who have not implemented commitments that have been agreed together as there are still employees who arrived late and not in accordance with the specified working hours or still seen some employees are less friendly so that it can provide a negative assessment by the community.

The third lowest element is the ninth element of complaint handling, suggestion and community input with the value of CSI of 3,041. This is still an obstacle for Brondong Primary Health Care if the complaints, suggestions and inputs of society in the form of facilities and infrastructure, such as lack of seating, lack of loudspeakers, and lack of air conditioning. Comfortable environment is one of expected service by customer, while a service can be said to be qualified if it can meet customer expectations[2]. In this context, the Primary Health Care is only able to make submission of the completeness of the higher level of infrastructure facilities, where the realization takes a long time. So that condition causes perception of dissatisfying society to health service at Brondong Primary Health Care of Lamongan Regency.

Previous research reveal on the Analysis of Influence of Patient Perception about Quality of Doctor Service on Patient Loyalty in Public Policlinic of Outpatient Care Installation of Panti Wilasa Citarum Semarang Hospital in 2008, the result of the research shows that there is a significant influence between medical skill, attitudes, delivery of information, timeliness of service, and availability of time doctor consultation on patient loyalty[6]. Another study, entitled "Analysis of the Effect of Patient Perception about Quality of Obstetric and Gynecology Specialist Doctor With Interest of Patient Re-visit in Outpatient Care Installation of RSI Sultan Agung Semarang in 2006" showed the result of perception about the accuracy of the officers and medical technical skills affect the interest of re-visit[11].

The above description shows that the need to improve the quality of Brondong Primary Health Care of Lamongan Regency service to be able to increase the satisfaction of the community of health service users.

Health care user satisfaction is often used as an indicator of the quality of health services. This is because health facilities have orientation to the user (customer orientated) in ordering their services, so health facilities need to evaluate the quality of service based on the perspective of the service user. Service user satisfaction is the user's perception and evaluation of the services they receive[12].

CONCLUSIONS

The quality of health services at Brondong Primary Health Care of Lamongan Regency based on the measurement of 9 elements or indicators adopted from Permenpan and RB Number 16 year 2014, is generally considered good by the community of Health-Care users. However, when every element have been viewed, there are still three elements with the lowest CSI value indicating that there are still people who feel less satisfied with the service Brondong Primary Health Care of Lamongan Regency. The three elements that have the lowest CSI value are the elements of service time, the service announcement, as well as the handling of complaints, suggestions and community input. Therefore, this study has been suggested that Brondong Primary Health Care of Lamongan Regency still needs to improve the quality of service, especially on the

three elements with the lowest CSI value based on the element or indicator from Permenpan Number 16 year 2014, because the satisfaction of users of health services is often used as an indicator of the quality of health services.

Ethical Approval: Related departments should be assured about the confidentiality of the results of questionnaires.

Conflict of Interest: The authors report no conflict of interest.

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REFERENCES

1. Pohan. (2007). Jaminan Mutu Pelayanan Kesehatan. Jakarta:EGC.
2. Supriyanto, S. & Wulandari, R.D. (2011). Manajemen Mutu Pelayanan Kesehatan. Surabaya Percatakan Pohon Cahaya
3. Donabedian, A. (1988). The Quality of Care How Can it Be Assessed ? JAMA, 260(12), pp.1743-48
4. Mosadeghrad, A.M. (2013). Healthcare service quality: towrds a broad definition. International Journal of Health Care Quality Assurance, 26(3), pp.203-19.
5. Antony, J. & Taner, T. (2006). Comparing public and private hospital care service quality in Turkey. Leadership in Health Service, 19(3), pp.i-x.
6. Asmita. (2008). Analisis Pengaruh Persepsi Pasien Tentang Mutu Pelayanan Dokter Terhadap Loyalitas Pasien di Poliklinik Umum Instalasi Rawat Jalan Rumah Sakit PantiWilasa Citarum Semarang Tahun 2008 Jurnal Kesehatan Masyarakat (FKM UNDIP JKM)
7. Peraturan Menteri Pendayagunaan Aparatur Negara Dan Reformasi Birokrasi Nomor 16 Tahun 2014 Tentang Pedoman Survei Kepuasan Masyarakat Terhadap Penyelenggaraan Pelayanan Publik.
8. Keputusan Menteri Pendayagunaan Aparatur Negara Nomor Kep/25/M.PAN/2/2004 Tentang Pedoman Umum Penyusunan Indeks Kepuasan Masyarakat Unit Pelayanan Instansi Pemerintah.
9. Permenkes Nomor 75 Tahun 2014 Tentang Puskesmas.
10. Pruyn, A. & Smidts, A. (1998). Effects of waiting on the satisfaction with the service: Beyond objective time measures. International Journal of Research in Marketing, 15,pp.321-34.
11. Karyati. (2006). Analisis pengaruh persepsi pasien Tentang Mutu Pelayanan Dokter Spesialis Obstetri dan Ginekologi Dengan minat Kunjungan Ulang pasien di Instalasi Rawat Jalan RSI SultanAgung Semarang: Program Studi Magister Manajemen Program Pascasarjana Universitas Diponegoro.
12. Moore, A.D., Hamilton, J.B., Knafl,G.J.,Godley,P.A.,Carpenter,W.R., Bensen, J.T., Mohler, J.L., Mishel,Merle. (2013). The Influence of Mistrust, Racism, Religious Participation and Access to Care on Patient Satisfaction for African American Men: The North Carolina-Louisiana Prostate Cancer Project. Hournal of The NationalMedical Association, 105(1), pp.59-68.

Examining Preconception Care Related to Knowledge among Reproductive Age Women: A Narrative Review

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ABSTRACT

Aims: Determine the realization of women realizing for importance of optimizing health prior to pregnancy. **Background :**The idea of preconception care (care before pregnancy), has came into in recent years, as a vital element , not only for improving young adult and women's health during pregnancy birth, but for future health. Preconception care is a positive new approach to upgrade the health of future children through primary intervention. Although most women have no positive attitude towards preconception care as they found that it would harm their health and will not acquire the healthy pregnancy due the certain domestic reasons or their cultural belief. **Methods:** Pub Med-Medline, EBSCO were searched for research articles in awareness on preconception care among women of reproductive age and found little awareness regarding preconception care. **Results:** Out of 9 articles, 6 research studies supported that awareness regarding preconception care revealed that variation in the level of awareness **Conclusion:** The review concludes that awareness on preconception care reveals very low level of awareness. **Clinical significance**: Preconception care is to reduce the risk of unfavorable health effect for the women and unborn baby by optimizing the women health awareness before planning and conceiving pregnancy.

Keywords: *Awareness, Preconception care, and Reproductive age women.*

INTRODUCTION

Preconception care (PCC) aims to promote the health of women of reproductive age before conception, while preventing pregnancy-related outcomes such as low birth weight, premature birth, and infant mortality.

The beginning of Preconception care was started to prevent low birth weight. Then later it came with slogan "Caring for Our Future," Preconception care first developed in developed countries like London, Britain etc. Preconception care is a set of planning that aim to analyze and reshape the biomedical, behavioral, and social risks to a woman's health or pregnancy outcome through prevention and management.

Preconception health care is a protection given to a woman before pregnancy to manage conditions and behaviors which could be a risk to women and baby. It targets those particular women who are never been pregnant as well as the women who could be pregnant in the near future. The key for encouraging the preconception health is to meet the effective medical care, healthy behavior, strong support and safe environment at home and at work.

It is targeting the existing risk before pregnancy where by using the beneficial resources for enhancing the improvement of reproductive health and optimizing the knowledge before conception and it provides the chance to intervene prior for to optimize health of the potential mothers and prevent from affecting the fetus.

Preconception care is a very strenuous task for

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engaging the public for the awareness as it is a form of patient care or an intervention provided to the women of an reproductive age, or the women who enters in child bearing age or having the desires of before pregnancy to improve the health outcomes and prevent from any deleterious effect over the health of the mother and fetus.

Worldwide in 2010, 287000 women died, 3.1 millions newborn babies died in their first month of life while 14.9 million were born prematurely and 2.7 million were stillborn. These statistics represents that due to the following risk factors are associated to change the health of the mother and child or severely it effected the entire process of healthy outcome behavior which results death. Hence need of preconception care is necessary for further reduction in the risk of fetal and maternal deaths and the planning of intervening services are more in range so as to reduce the problems.

In India very less number of Women received Preconception Care. National Family Health Survey (NFHS) found that 37 percent of all pregnant women not received the preconception care during their pregnancies where 13% of literate women received the preconception care as compared to half of illiterate who have not received care and this survey analyses the various additional services are engaging a women in preconception care so as to improve the healthy behavior and indented the most of pregnancies result in good maternal and fetal outcomes , some pregnancies may result in adverse health effect for the women and fetus and some of these outcome cannot be prevented.

Optimizing a women health so there is a need to identify the barrier to preconception care and introducing the risk reduction intervention and screening methods to a women of child bearing age and may contribute in the reduction of maternal and infant mortality rates.

METHODOLOGY

Search strategy methods-

An electronic search of articles published in various journals has been done. Search was restricted to only English language.

Types of studies: descriptive Cross -sectional studies, qualitative, mixed longitudinal descriptive and survey study

Type of participants: Community women, health care workers, health professional workers.

Setting: Maternal and health care centers and community areas.

The systematic search was conducted by framing the terms separately and in combination with the different variables with different search terms .In addition to this, a manual Pub Med search and EBSCO was undertaken by using the keywords and search synonyms and found the relating articles, in addition where 9 articles were found. Initial search retrieved 1382 articles over which 116 articles were selected manually. Duplicates were removed and 40 articles were reviewed for eligibility. 76 articles were excluded because they don't match with the study criteria. 21 more studies were excluded due to subscription and ten articles due to irrelevant content. Finally nine articles were selected where six were quantitative and two were qualitative and one was mixed longitudinal descriptive studies. These articles are supporting the used study.

DISCUSSION

The quantitative studies conducted on knowledge regarding preconception care among the women were reviewed. Most of the studies were conducted in foreign countries including North west Ethiopia , Malaysia, Nigeria, Australia, Jordan, England, London and concluded that women were having less knowledge on preconception care .

According to **Adeoye Tokunbo O et al, 2017**, it concluded that there is high level of awareness among health workers especially in doctors about 83.3%. Introduction of various in training services in professional education will ultimately improve the knowledge and awareness of preconception care services [1]

According to **Yitayal Ayalew et.al, 2016**, finding showed that overall 27.5% of women are aware of preconception care indicated very low level of awareness and suggesting that there is a need to give importance for delivery of health education about preconception care to a woman in order to increase their knowledge [2]

According to **Prashana Gautam et.al, 2016**, Overall 57.3% belongs to the highest level of knowledge in the area of reproductive health risk factors and the lowest in an area of health promotion and suggested that initiation of education programme on different aspects of preconception care will improve the health risk and

obtain the healthy future outcomes [3]

According **Roshanani Bkasim et.al 2016,** majority of participants had good knowledge, attitude and practice towards preconception care was about 98%, 45.2% and 51.9%. Pre-pregnancy care information can be integrated into educational programmes especially in secondary schools and this information should also be provided to all women who attend antenatal clinics [4]

According to **N.A.AL-Akour et.al, 2015** revealed that nearly 50% of the participants were awarded about preconception care associated factor. Majority of women and men are aware of the changes that should be made prior to conception [5]

According to **Judith Stephenson et.al, 2016,** revealed that 20 professionals expressed the low level of awareness regarding preconception health situations and ignorance about responsibility for delivery of preconception care and declares linkage between the health professionals input and healthy behavior before the pregnancy will improve the awareness and brings wider benefits for public health [6]

According to **S. Braspenningx et.al, 2013**, a systematic review on awareness, current situation and recommendation of future aspects of preconception care, Forty-six articles were identified for review and it shown that preconception care that might be result in excellence pregnancy outcomes, including e.g. a reduction of congenital abnormalities. Health care workers are in favor of the implementation of preconception care, but claim that they don't have enough knowledge to do so. In results the general population shows interest in receiving the preconception care and wanted to increases the awareness of preconception care and this should be implemented in terms of checklist and health education[7]

According to **Helena Toumainen et.al, 2013,** the results suggested that Women had modest or poor awareness of preconception health issues and the study concluded that raising preconception health will improves the pregnancy outcomes [8]

According to **Eric AP Steegers et.al, 2012,** Women expressed a positive attitude towards preconception care. Due to some aspects or subjective norms around the process of becoming pregnant may falling out with the current practice of preconception care. There is a Need for enhancing the awareness by adopting the following interventions and education programme among the women [9]

According to **Keith A. Frey et.al, 2006,** Majority of women understand the importance of optimizing their health prior to preconception and there is a need to forming the focused group so that entire women will be involved to improve the deficiencies over the health of the mother and fetus [10]

Ethical Consideration: This manuscript is ethically considered by the panel of Teerthanker Mahaveer College of Nursing, Teerthanker Mahaveer University after discussing with each and every point of this manuscript

SUMMARY FINDINGS

The available literature is refined to get six quantitative, two qualitative and one mixed approach.

Out of Nine articles, six research studies findings are showing the awareness on preconception care of a woman and categorized the level of awareness as low, poor, average and good. Under these categories the awareness was seen and found that majority of women have poor awareness regarding various aspects of preconception care.

Most of the studies were refined and concluded that recommendation is necessary to cover all reproductive age women under the boundaries of preconception programmes and services so as to reduce the harmful impact over the health of the mother and child. Five articles show the majority of women have the modest or poor awareness of preconception heath issues. They perceived that it can be reduced by addressing the preconception care in different areas of perspectives and as found that challenges faced by women is critical to achieve preconception care due to the following factors like unawareness of family planning, health lifestyle, smoking/alcohol, folic acid supplementation etc.

FUTURE SIGNIFICANCE

Based on this review the finding reveals that majority of women have lack of knowledge on preconception care which is greatly playing an undesirable effect over the health of mother and fetus. To overcome the problems there is a need to improve the preconception care health and pregnancy outcomes and this will protect the mother and fetus from various associated factors

affecting the health. It will lead to improve maternal nutrition, tackle obesity, related issues, under nutrition and micronutrients deficiencies. In order to decrease the mortality and morbidity rate. Various maternal services and programmes will be performed so that every individuals will be saved and acquire healthy pregnancy in a future

Nearly half of the pregnancies in United State are unplanned. Therefore the challenges of preconception care are more progressed. It is not only addressing the pregnancy planning but also address the women who are seeking for the medical consultation and care to secure their health but they failed to approach due to the cultural and educational barriers. The introduction of various educational programmes and screening programme for the women of child bearing age on an ongoing basis will analyse the prospective of maternal-fetal risk and hazard before and between the pregnancies.

Several national and international medical organization and advocacy group have focused on the promotion of health before conception, resulting in development of clinical recommendation and education materials and clinician will encourage the women to formulate the reproductive health plan so it will promote the healthy pregnancy outcomes.

CONCLUSION

Majority of studies highlights that education is needed to improve the level of knowledge on different aspects of preconception care among reproductive age women. Initiating the offer of preconception health intervention at this point is needed. First, and for most step is to give importance for those who belongs to deprived backgrounds, so that it will increases the potential to engage them in health educational programmes of preconception care and improves the knowledge regarding preconception care.

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Conflict of Interest: Nil

REFERENCES

1. Freda MC, Moos MK, Curtis M. The history of preconception care: Evolving guidelines and standards. Maternal and Child Health Journal. 2006 Sep 1;10(1):43-52.
2. Dubé B, Benton T, Cruess DG, Evans DL. Neuropsychiatric manifestations of HIV infection and AIDS. Journal of Psychiatry and Neuroscience. 2005 Jul;30(4):237.
3. Bhutta ZA, Dean SV, Imam AM, Lassi ZS. A systematic review of preconception risks and interventions. Karachi: The Aga Khan University. 2011.
4. Bhatnagar B, Williams AC, editors. Participatory development and the World Bank: potential directions for change. World Bank Publications; 1992.
5. Tokunbo OA, Abimbola OK, Polite IO, Gbemiga OA. Awareness and perception of preconception care among health workers in Ahmadu Bello University teaching university, Zaria. Tropical Journal of Obstetrics and Gynaecology. 2016 May 1;33(2):149.
6. Ayalew Y, Mulat A, Dile M, Simegn A. Women's knowledge and associated factors in preconception care in adet, west gojjam, northwest Ethiopia: a community based cross sectional study. Reproductive health. 2017 Dec;14(1):15.
7. Gautan P, Dhakal R. Knowledge on preconception care among reproductive age women. Saudi J Med Pharm Sci. 2016;2(1):6.
8. Kasim Rosnani, Draman nani et al 2012. *Knowledge, attitude & practice care among women attending maternal health clinics* .Education in medicine journal .volume 8, issue 4,Pp:57-58.
9. Al-Akour NA, Sou'Ub R, Mohammad K, Zayed F. Awareness of preconception care among women and men: a study from Jordan. Journal of Obstetrics and Gynaecology. 2015 Apr 3;35(3):246-50.
10. Stephenson J, Patel D, Barrett G, Howden B, Copas A, Ojukwu O, Pandya P, Shawe J. How do women prepare for pregnancy? Preconception experiences of women attending antenatal services and views of health professionals. PLoS One. 2014 Jul 24;9(7):e103085.
11. Tuomainen H, Cross-Bardell L, Bhoday M, Qureshi N, Kai J. Opportunities and challenges for enhancing preconception health in primary care: qualitative study with women from ethnically diverse communities. BMJ open. 2013 Jul 1;3(7):e002977.
12. Braspenningx S, Haagdorens M, Blaumeiser B, Jacquemyn Y, Mortier G. Preconceptional care:

a systematic review of the current situation and recommendations for the future. Facts, views & vision in ObGyn. 2013;5(1):13.

13. van der Zee B. Preconception care: concepts and perceptions: An ethical perspective. 2013 Feb
14. Frey KA, Files JA. Preconception healthcare: what women know and believe. Maternal and child health journal. 2006 Sep 1;10(1):73-7.

Impact of Parental Stress on Body Mass Index of Children

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ABSTRACT

Background: India is undergoing a rapid epidemiological and nutritional transition characterized by persistent nutritional deficiencies, as evidenced by the prevalence of children suffering from stunting, anemia, and iron and zinc deficiencies. Subsequently, there is a steep rise in the prevalence of childhood obesity, diabetes, cardiovascular disease etc. The impact of obesity or underweight upon a child's mental health, immediate and future social life is as important as the impact on physical health. So, the need of the hour is to address child's weight-related problems of being obese or underweight at an early stage of life in connection with their primary care and primary care giver/parents.

Aim: To assess the impact of parental stress on body mass index of children within Indian school setting.

Method: A sample of 250 students and their parents were contacted personally to obtain information on parental stress scale and body mass index of children. Students with the age range of 10-14 years (grade IV -IX) from three different schools of Jaipur city.

Result: The findings of the present study found to be statically significant that there is a difference in parental stress level associated with children's BMI.

Conclusion: Present study paves the way for future studies examine the relationships of parental stress with body mass index of children, as well as BMI on academic performance. The findings suggest that higher level of parental stress leads to higher level of BMI in children which could possibly lead to poor academic performances within a period of middle school aged students.

BACKGROUND

Childhood obesity is one of the major public health challenges of the 21st century. The problem is encompassing globally and is rapidly affecting many underdeveloped and developed countries, particularly in urban settings[1]. Globally in 2016, the number of overweight infants and children is estimated to be over 42 million and about 35 million of these are living in developing countries[1].

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India is undergoing a rapid epidemiological and nutritional transition characterized by persistent nutritional deficiencies, as evidenced by the prevalence of children suffering from stunting, anemia, and iron and zinc deficiencies[2,3]. Subsequently, there is a steep rise in the prevalence of childhood obesity, diabetes, cardiovascular disease etc. Studies from different regions of India within last decade are also evident of similar trend [2,3]. This trend has been challenging over recent years and can be presently considerd as different forms of the National malnutrition problem.

The most surprising aspect of all the attention is beign paid to the prevelance with numbers and figures and physical consequences of childhood obesity without going in depth of the problem and its global perspective. But, this alone can not slove the severity of the problem. The impact of obesity upon a child's mental health,

immediate and future social life is as important as the impact on physical health [4]. So, the need of the hour is to address the wider issues of child's weight-related problems of being obese or underweight at an early stage of life in connection with their early care and primary care givers/parents (for e.g. Can parental stress lead to abnormalities related to BMI (underweight/overweight) .The parent becomes a model for the their child. The child observes how the parents and other people around them respond to the child's needs. The child learns to adapt their behaviour to suit their environment therefore, children often repeat their parent's behaviours, which may then be carried into their adulthood [4,5]. Some previous studies found a relationship between stressful events or situations (stressors) resulting in negative physiologic or psychological responses (stress) in parents of obese and underweight children [5,6]. There are several mechanisms for how parent stressors can influence child obesity development [7]. Parents experiencing multiple stressors or who perceive that they are under stress may spend less time with their children, use less effective parenting approaches, or both [8]. This can yield less supervision for children, who may make unhealthy food and activity choices resulting in overweight or underweight. Similarly, stressed parents may be less likely to model and encourage their children to engage in physical activity as thi may be a sign of risk towards obesity [8,9].

Parent stressors that have been associated with childhood obesity and underweight include poor physical and mental health, financial strain, and single-family households [8-10]. Although, multiple stressors of parents can elicit a "stressor pile-up" causing adverse physical health consequences in children [10] and a number of studies have examined associations between dietary and psycho-social factors and prevalence of underweight, overweight, and obesity at the International level (i.e. American states,[11,12] Arab states[13]) little research has examined similar associations in India.

Therefore, the primary aim of the present study is to assess the impact of parental stress on body mass index of children within Indian school setting.

MATERIALS AND METHOD

Study design:

A school-based ex-post facto study with contrast group comparison was conducted in three private schools from different parts of Jaipur city of Rajasthan state after obtaining permission from the school administration, parents and children. The study was conducted over a short period of three months from August to October 2017 to avoid the examination related stress issues with children, parents and school administration to interfere with the results of the present study. This is not an intervention based research and no clinical trials on samples were conducted. The synopsis of this study has gone through stringent evaluation by Students Research Degree Committee with an external member in it.

Participants:

Present study included 250 students, with equal number of males and females in the age range of 10 to 14 years (enrolled in class V to IX) attending regular school and their parents. Studens School Principals, Class Teachers and Parental consents were obtained through a circular explaining the purpose and procedure of the study. Those present at the time of study and consented to participate were included in the study.

The socio-demographic characteristics of the sample included their religion (Hindu/non-Hindu), diet pattern (vegetarian/non-vegetarian), academic records, and number of siblings. The sample selected was from three different private schools of Jaipur city with comparable fee structure and parental education, justifying the similar socio-economic status of these children.

The justification for choosing the sample for this age group was:

1.There is a steep rise of obesity among school going children and the age of onset is declining [4]. To nib at bud this psychosomatic disorder, we need to identify its correlates at an early age to evolve preventive and curative measures.

2. The age group of 10-14 years fall in pre-adolescence where usually the body starts taking shape with pubertal changes [4,14].Adolescents of 10-14 years of age constitute a homogeneous group who have characteristics similar [14], have knowledge about health promoting and health hindering life style to some extent. They start taking independent decisions about themselves and their lifestyle and food choices. Younger children are more dependent on their parents in their lifestyle (selection, amount and frequency of food, screen time and sleep patterns). Moreover, relationship

with parents develop and impact health gradually and it seems the right time to study these health dynamics in relation to parental stress.

3. Students of grade V to IX are not hassled by board exams, competitions, and future career issues compounding to adaptive behaviors like over or under eating.

Exclusion criteria

Those students/ parents did not give the written consent to participate in the study.

Those who were taking treatment for certain alignments.

Procedure:

Parental stress scale developed by Berry and Jones (1995) based on 5 point scale with satisfactory levels of internal reliability (.83), and test-retest reliability (.81) and satisfactory convergent validity with various measures of emotions, role satisfaction, perceived stress, marital satisfaction, job satisfaction and social support.

Body mass index of children was measured by BMI machine (Model-Omeron HBF-121). Criteria for measuring BMI - Underweight - a percentile range of less than 5, Normal weight –a percentile range of 5 to less than 85, Overweight - a percentile range of 85 to less than 95 and Obese - a percentile range equal to or greater than 95 [15,16].

Statistical Package for Social Sciences, Version 22 for Windows (SPSS 22) was used to analyze the quantitative data. One way ANOVA was computed and .05 level of confidence was used to interpretate the results of the present study.

FINDINGS

Table. 1 Mean, standard deviation of parental stress on body mass index of children

Body Mass Index	Mean	Std. Deviation
Normal	37.95	4.379
Underweight	49.72	7.393
Obese	74.50	8.069
Total	54.92	17.869

Table.1.2. F value of parental stress and body mass index in children

Source	Df	F	Sig.
Corrected Model	**2**	**767.407**	**.000***
Intercept	1	14734.123	.000
BMI	2	767.407	.000

***Significant at (p=.01)**

Table 2. Chi square value, df and significance value of body mass index in children and their academic performance

	Value	df	P value
Pearson Chi-Square	**121.747[a]**	**4**	**.000***
Likelihood Ratio	137.282	4	.000
Linear-by-Linear Association	99.742	1	.000

***Significant at (p=.01)**

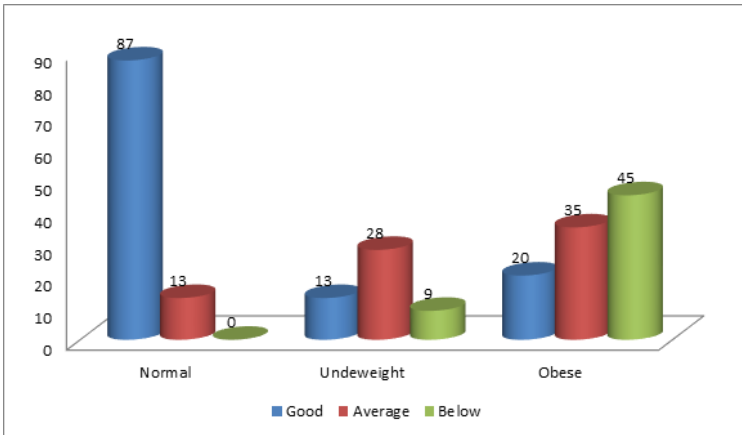


Figure 1. Body mass index and academic performance of children

DISCUSSION

The findings of the study are very promising and conclusive showing a clear trend of how parental stresss relates with BMI . The F ratio of parental stress and BMI of their children of the present study found to be statistically significant (*F = 767.407 , df=2 ,p=.00;table1 and 1.2)*. The results clearly show that the parental stress of children with body mass index labelling them as obese or overweight was significantly high. Moreover, the mean of parental stress of children with different BMI provides a clear difference (mean of obese/overweight children found to be *74.50* and mean of normal and underweight children *37.95, and 49.72* respectively). The findings support studies indicating positive relationship between parental stress with overweight and obesity in children [4-10]. The probable reasons could be that stress in parents leads to poor family functioning and poor communication which involves the disturbed emotional, physical and psychological activities between family members and has been found to be strongly associated with childhood obesity [17,18].Moreover, parental stress have also been associated with poor parenting style such children who experience authoritarian and neglectful parenting have higher risk of being overweight/obese [19]. Parental Stress also influences their attachment style with their children [19,20]. When children relate and interact with high stressed parents , they develop insecure attachment style (avoidant or anxious attachment style) which is associated with disturbed weight regulation in children [20]. House and professional demands (i.e. family environment, interpersonal relation, their education or socio-economic status combined with host of other variables) act as the major reason for stress among parents, which could be associated with higher/lower metabolic syndrome in children [21-22]. Many chronically stressed parents become chain smokers and consume excessive alcohol or drugs and become habitual drinkers/ addicts. Such pathological behaviours of parents can directly affected the child mental and physical health responsible for child obesity or underweight [23].The present evidences shows that parental stress can be a strong predictor of higher or lower than normal BMI levels in children.

Although the academic performance of children was unaffected by the stress of parents, but it is noteworthy that the body mass index of children was found to be stastically significant with academic performance of children *(x2=121.747,df=4,p=.00 ; table 2; figure 1)* in the present study. Significantly greater numbers of children having obese/overweight or underweight scored average or poor performance and children with normal BMI were found to be good in their academic performance. Some previous studies support these findings by reporting the association of cognitive abilities, poor nutrition and BMI, stating that cognitive ability is influenced by obesity and the likelihood of being obese is influenced by the quality of nutrition, so poor nutrition is associated with obesity leading to poor academic performance of child [24,25,26]. However, not all available studies have found a positive association between BMI and academic performance [27,28]. Such observations suggest that obesity is a complex problem there are so many factors which could be responsible for increase and decrease of body mass index of children.

FUTURE DIRECTION AND CONCLUSION

Since, this study was conducted only in three modern high schools of the Jaipur (Rajasthan) with a small sample of 250, a similar study of larger population from diverse locations and demographic specifications could be planned for greater generalizability of the study and results could be treated with more cross sectional and longitudinal researches. Separate stress responses of father and mother along with an account of stress level of children with different BMI could be taken and evaluated for better understanding of these complex demograpic and psycho social health issues.

In order to address the problem associated with BMI of school children we need to address the problem of stress of their parents. Interventions to reduce parental stress, household stress, environmental stress for the parents are required to help them to cope with stress might help to reduce obesity and underweight in children [22]. Interventions to alter and create healthy home environment has to be stressed. Educational support to children and counselling facilities for their stressed parents might help in providing healthy life style including diet and exercise behaviours in children to foster healthy habits and optimum growth in children.

Present study paves the way for future studies with more robust and comprehensive research designs and examine the relationships of parental stress mental health with body mass index of children as well as their academic performance.

Conflict of Interest: The author(s) declare that they

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REFERENCES

1. Bhadoria A, Sahoo K, Sahoo B, Choudhury A, Sufi N, Kumar R. Childhood obesity: Causes and consequences. Journal of Family Medicine and Primary Care. 2015;4(2):187.
2. Popkin B, Doak C. The Obesity Epidemic Is a Worldwide Phenomenon. Nutrition Reviews. 2009;56(4):106-114.
3. Gupta R. Nutrition and the Diseases of Lifestyle. 1st ed. New Delhi: epartment of community medicine AFMC, Pune in Collaboration with WHO India Office.; 2009.
4. Rao G. Child obesity. 1st ed. Amherst, N.Y.: Prometheus Books; 2006.
5. Gundersen C, Lohman B, Garasky S, Stewart S, Eisenmann J. Food Security, Maternal Stressors, and Overweight Among Low-Income US Children: Results From the National Health and Nutrition Examination Survey (1999-2002). PEDIATRICS. 2008;122(3):e529-e540.
6. Koch F, Sepa A, Ludvigsson J. Psychological Stress and Obesity. The Journal of Pediatrics. 2008;153(6):839-844.e3.
7. Francis D, Meaney M. Maternal care and the development of stress responses. Current Opinion in Neurobiology. 1999;9(1):128-134.
8. Lohman B, Stewart S, Gundersen C, Garasky S, Eisenmann J. Adolescent Overweight and Obesity: Links to Food Insecurity and Individual, Maternal, and Family Stressors. Journal of Adolescent Health. 2009;45(3):230-237.
9. Gundersen C, Mahatmya D, Garasky S, Lohman B. Linking psychosocial stressors and childhood obesity. Obesity Reviews. 2011;12(5):e54-e63.
10. Gibson L, Allen K, Byrne S, Davis L, Zubrick S. The role of family and maternal factors in the development and maintenance of childhood obesity. Obesity Research & Clinical Practice. 2013;7:e118-e119.
11. Parks E, Kumanyika S, Moore R, Stettler N, Wrotniak B, Kazak A. Influence of Stress in Parents on Child Obesity and Related Behaviors. PEDIATRICS. 2012;130(5):e1096-e1104.
12. Williams KJ, Taylor CA, Wolf KN, Lawson RF, Crespo R. Rural Remote Health. 2008 Apr-Jun; 8(2):932. Epub 2008 May 22.
13. Alnohair S. Obesity in Gulf Countries. International Journal of Health Sciences. 2014;8(1):79-83.
14. Devi R. Childhood Obesity. 1st ed. Hyderabad, India: Icfai University Press; 2010.
15. Jordan A. Children, Adolescents, Obesity, and the Media. PEDIATRICS. 2011;128(1):201-208.
16. Singh Chahar P. Physiological basis of Growth and Development among Children and Adolescent in Relation to Physical Activity. American Journal of Sports Science and Medicine. 2014;2(5A):17-22.
17. Chen J, Kennedy C. Family Functioning, Parenting Style, and Chinese Children's Weight Status. Journal of Family Nursing. 2004;10(2):262-279.
18. Carlson C, Cooper C, Spradling V. Developmental implications of shared versus distinct perceptions of the family in early adolescence. New Directions for Child and Adolescent Development. 1991;1991(51):13-32.
19. Arredondo E, Elder J, Ayala G, Campbell N, Baquero B, Duerksen S. Is parenting style related to children's healthy eating and physical activity in Latino families?. Health Education Research. 2006;21(6):862-871.
20. Pareek A, Joshi U. A Study Of The Relationship Between Attachment Style And Body Mass Index Of Children Using Ex-Post Facto Research Design. Journal of Indian Association for Child and Adolescent Mental Health. 2018;. Upublished.
21. Ge X, Conger R, Elder G. Coming of Age Too Early: Pubertal Influences on Girls' Vulnerability to Psychological Distress. Child Development. 1996;67(6):3386.
22. Puder J, Munsch S. Psychological correlates of childhood obesity. International Journal of Obesity. 2010;34(S2):S37-S43.
23. Kar S, Debroy A, Sarma R, Islam F. Substance abuse amongst the street-children in Guwahati City, Assam. Annals of Medical and Health Sciences Research. 2014;4(9):233.
24. Hollar D, Lopez-Mitnik G, Hollar T, Agatston A, Lombardo M, Messiah S. Elementary school-based obesity prevention intervention effect on waist circumference among multiethnic 6- to 13-year-olds. Canadian Journal of Diabetes. 2011;35(2):207.

25. Francis H, Stevenson R. The longer-term impacts of Western diet on human cognition and the brain. Appetite. 2013;63:119-128.
26. Taras H, Potts-Datema W. Chronic Health Conditions and Student Performance at School. Journal of School Health. 2005;75(7):255-266.
27. Franz D, Feresu S. The relationship between physical activity, body mass index, and academic performance and college-age students. Open Journal of Epidemiology. 2013;03(01):4-11.
28. Baxter S, Royer J, Hardin J, Guinn C, Devlin C. The Relationship of School Absenteeism With Body Mass Index, Academic Achievement, and Socioeconomic Status Among Fourth-Grade Children. Journal of School Health. 2011;81(7):417-423.

Utilisation of Janani Suraksha Yojana among Women in Urban and Rural Areas of Western Uttar Pradesh

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ABSTRACT

Background: In April 2005, under the National Rural Health Mission, the Government of India launched a scheme known as Janani Suraksha Yojana (JSY). It aims to promote institutional deliveries among pregnant women living below poverty line in all the states and Union Territories of the country with special focus on low performing states. **Aims & Objectives:** To find out the extent of utilisation of the JSY scheme by rural and urban women in the community. **Material & Method :** The present study was done in the catchment area of rural and urban health and training centre of Subharti Medical College. The sample size was 150 each in urban and rural area. **Findings:** Complete antenatal care was received by only 19.2% of the beneficiaries. 24% of the beneficiaries in urban area and 17% of the beneficiaries in rural area received complete antenatal care. Among the women who registered under JSY, 71% of the women received money after delivery in rural area and 82% of the women received money in urban area.

Keywords : *Janani Suraksha Yojana; Utilisation; Urban; Rural; Western Uttar Pradesh.*

INTRODUCTION

Mother and child constitute a priority group in a community. They comprise of approximately 57.5% of the total population and constitute a vulnerable group.[1]

In April 2005, under the umbrella of National Rural Health Mission (NRHM) in response to the slow and varied progress in improvement of maternal and neonatal health, the Government of India launched a scheme known as Janani Suraksha Yojana (JSY).[2]

The scheme aims to promote institutional deliveries among pregnant women living below poverty line in all the states and Union Territories (UTs) of the country with special focus on low performing states (LPS). In Uttar Pradesh, about 2.33 million beneficiaries have been benefited under JSY in 2014-15.

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METHOD

The present study was conducted in Khajoori area (RHTC) and Multan Nagar area (UHTC) of the department. The sample size was calculated using prevalence of institutional delivery taken from National Family Health 2005-06 Survey (NFHS-3) India-Uttar Pradesh which was 22%[3] and was rounded off to 300, 150 each from rural and urban area, respectively. Fourteen colonies are covered under UHTC, there were 3300 families residing there, out of which 488 families had married females above 19 years who had delivered in the past one year and seven villages are covered by RHTC, there were a total of 4436 families with 627 families having married females above 19 years who had delivered in the past one year. Number of families to be included in the study from each selected colony was calculated by proportion method using a simple proportion formula.[4]

$$n_i = nN_i/N$$

Where, n_i= sample size in i^{th} stratum.

n= Total sample size.

N_i= Total number of units in i^{th} stratum.

N= Total number of units in all the strata.

The calculated number of families in each colony were selected by Simple Random Sampling technique using online random number generator. Data was analysed using appropriate statistical tests by Statistical Package for Social Sciences (SPSS) software version 19.0 and Microsoft excel 2013. Ethical clearance was obtained from ethical committee of Swami Vivekanand Subharti University, Meerut. Informed written consent was obtained from each participant after explaining them about the purpose of the study.

FINDINGS

Table 1 shows that out of the total 182 beneficiaries who were aware about JSY, 164 (90%) beneficiaries got registered under JSY, 113 of which belonged to rural area and 51 were from urban area. 83.5% of the beneficiaries went for at least 3 antenatal checkups, 91.2% got the appropriate doses of Tetanus Toxoid and only 28.6% consumed 100 tablets of Iron and Folic Acid (IFA). Only 10.2% of the women in rural area and 7.3% of beneficiaries in urban area were given postnatal care under JSY. BCG and Zero polio were given to approximately 94% of the babies of the beneficiaries and all the beneficiaries were given advice on breastfeeding. The difference in the number of beneficiaries who had minimum three antenatal checkups in rural and urban areas was seen to be statistically significant.

Complete Antenatal care (ANC) includes beneficiaries who went for 3 or more ANC visits, 2 doses of TT or booster dose and received 100 IFA tablets. Table 2 shows that overall, Complete ANC was received by only 19.2% of the beneficiaries. 24% of the beneficiaries in urban area and 17% of the beneficiaries in rural area received complete antenatal care.

In the present study, 38.3% beneficiaries had to travel <5km to the place of delivery, 41.8% travelled 5-10km, 17.7% travelled 11-20km and 2.1% travelled >20km to their place of delivery. The distance of place of delivery from residency varied in rural and urban areas and this was seen to be statistically significant. It was observed that many of the beneficiaries went to district and tertiary care hospital at their own will or referral from peripheral hospital (PHCs/CHCs) due to lack of Basic Emergency obstetric care services. 61% of beneficiaries used hired vehicle to reach the place of delivery, 39% had personally owned vehicle. Amount incurred on transportation was reimbursed in just 1.4% cases.

70% of the deliveries in the rural area and 60% of the deliveries in the urban area were conducted in an institution. Government facilities were the most common institution for delivery in both rural and urban areas with 84% and 58% of the institutional deliveries, respectively. Accredited Private facility accounted for only 1% of the institutional deliveries in rural area and for about 14% institutional deliveries in urban area. 15% and 28% of the institutional deliveries in rural and urban areas, respectively were conducted in other private facilities. This distribution was seen to be statistically significant.

Table 3 shows that 100% of the beneficiaries in rural area and only 29% of the beneficiaries in urban area were contacted/ advised for institutional delivery during pregnancy and this variation was seen to be statistically significant. Grassroot level workers (65%) followed by Others (22%) were the most common people to advise for institutional delivery in rural area while Other sources (51%) followed by Doctor (36%) were the most common sources in urban areas and this distribution was statistically significant. Place of delivery was identified in advance in 69.8% of the beneficiaries who were aware about JSY. In rural area, Doctors conducted the delivery in 69% of the beneficiaries aware about JSY while in urban, 78% of the deliveries were conducted by doctors.

Table 4 shows that among the women who were registered under JSY, 71% of the women received money after delivery in rural area and 82% of the women received money in urban area. More than three fourth of the beneficiaries received money within a week after discharge in both rural and urban area. 90% of the rural beneficiaries and 93% of the urban beneficiaries received the appropriate amount of money for their respective areas.

Table 1: Antenatal, Natal and Postnatal Services Received by the JSY Beneficiaries (Type of maternal / child care services received)

VARIABLES FREQ		RURAL (n=127)		URBAN (n=55)		TOTAL (n=182)		χ^2	p value
		%	FREQ	%	FREQ	%			
Registration during pregnancy	Yes	113	89.0	51	92.7	164	90.1	0.61	0.436
	No	14	11.0	4	7.3	18	9.9		
At least three ante natal check-ups	Yes	101	79.5	51	92.7	152	83.5	4.86	**0.028**
	No	26	20.5	4	7.3	30	16.5		
Appropriate doses of TT	Yes	117	92.1	49	89.1	166	91.2	0.44	0.507
	No	10	7.9	6	10.9	16	8.8		
Consumed 100 tablets of IFA	Yes	35	27.6	17	30.9	52	28.6	0.21	0.646
	No	92	72.4	38	69.1	130	71.4		
Post natal care	Yes	13	10.2	4	7.3	17	9.3	0.40	0.528
	No	114	89.8	51	92.7	165	90.7		
BCG to the child	Yes	122	96.1	49	89.1	171	94.0	3.29	0.070
	No	5	3.9	6	10.9	11	6.0		
Zero polio to the child	Yes	119	93.7	53	96.4	172	94.5	0.52	0.469
	No	8	6.3	2	3.6	10	5.5		
Advice on breast feeding	Yes	127	100.0	55	100.0	182	100.0	NA	NA
	No	0	0.0	0	0.0	0	0.0		

Table 2: Distribution of beneficiaries according to the Complete Antenatal care Services received by them.

VARIABLES FREQ		RURAL (n=127)		URBAN (n=55)		TOTAL (n=182)	
		%	FREQ	%	FREQ	%	
Complete Antenatal care	Yes	22	17	13	24	35	19.2
	No	105	83	42	76	147	80.8

Table 3: Distribution of the beneficiaries with regard to details regarding preparation for institutional delivery

VARIABLES FREQ		RURAL (n=127)		URBAN (n=55)		TOTAL (n=182)		χ^2	p value
		%	FREQ	%	FREQ	%			
Whether anyone contacted/advised during pregnancy for institutional delivery	Yes	127	100	16	29	143	78.6	114.61	**<0.001**
	No	0	0	39	71	39	21.4		
Person who contacted/ advised for institutional delivery	Grassroot Worker	82	65	7	13	89	48.9	41.45	**<0.001**
	Doctor	17	13	20	36	37	20.3		
	Others	28	22	28	51	56	30.8		

***Cont...*Table 3: Distribution of the beneficiaries with regard to details regarding preparation for institutional delivery**

Identification of place of delivery in advance	Yes	89	70	38	69	127	69.8	0.02	0.894
	No	38	30	17	31	55	30.2		
Person who conducted the delivery	Doctor	87	69	43	78	130	71.4	1.77	0.414
	Nurse	7	6	2	4	9	4.9		
	Others	33	26	10	18	43	23.6		

Table 4: Details regarding Receipt of Cash Incentives under JSY

VARIABLES FREQ		**RURAL (n=80)**		**URBAN (n=42)**		**TOTAL (n=122)**		**χ^2**	**p value**
		%	**FREQ**	**%**	**FREQ**	**%**			
Time of receipt of money	At time of discharge	5	6	3	7	8	6.6	1.10	0.776
	Within a week after discharge	59	74	30	71	89	73.0		
	Within 2-4 weeks after discharge	12	15	5	12	17	13.9		
	More than 4 weeks after discharge	4	5	4	10	8	6.6		
Total amount received (in Rs.)	As per JSY guidelines	72	90	39	93	4	3.3	0.27	0.601
	Less than the JSY guidelines	8	10	3	7	44	36.1		
Type of problems faced in receiving the money	No problem	69	86	34	81	103	84.4	0.59	0.443
	Made several contacts to receive money	11	14	8	19	19	15.6		
Person who paid the money	By Health worker	30	38	12	29	42	34.4	0..97	0.324
	Direct to account	50	63	30	71	80	65.6		

DISCUSSION

Majority of women (90%) got registered during pregnancy. The results are consistent with the findings of Kumari V., et al (2009)[5] and Banerjee B., et al(2009).[6]

The study found that 83.5% had more than three antenatal checkups and it was higher (92.7%) in urban than in rural areas (79.5%). Similar findings were seen in studies conducted Dolma Y, et al (2015)[7] and Panja T.K., et al (2012)[8].

In our study, 91.2% beneficiaries got the appropriate doses of Tetanus Toxoid and only 28.6% consumed 100

tablets of IFA. In Annual Health Survey (AHS) 2012-2013,[9] it was found that mothers who received at least one Tetanus Toxoid (TT) injection were 83.2% in rural UP, 88% in urban UP and 84.1% in total and 82.9% in rural Meerut, 86.5% in urban Meerut and 84.3% in total. Mothers who consumed IFA for 100 days or more were 8.3% in rural UP, 16% in urban UP and 9.7% in total and 12.3% in rural Meerut, 16.4% in urban Meerut and 14% in total. NFHS IV (2015-16)[10] data shows that in Uttar Pradesh, 86% mothers in rural and 88.5% mothers in urban UP received protection against neonatal tetanus in their last birth. Only 10.9 % mothers in rural UP and 20.2% mothers in urban UP consumed IFA tablets for 100 days or more. The indicators in Meerut were in general found better than those of Uttar Pradesh. Mothers whose last births was protected against neonatal tetanus were 91.1% in rural Meerut and 86.1% in urban Meerut. Similar results have been shown as per the report of DLHS (2002-2004).[11]

Complete Antenatal care was received by 19.2% of the beneficiaries in our study with 17% and 24% beneficiaries in rural and urban areas, respectively, receiving complete antenatal care. Similar results were observed by Kumari V,[5] et al where Complete ANC was availed by 25.5% of the women.[12] According to NFHS IV[10] data, in rural India, only 16.7% mothers had full antenatal care while it was 31.1% in urban India. Complete antenatal care was received by 3.8% mothers and 13.5% mothers in rural and urban UP, respectively.

In the present study, 70% and 60% deliveries in rural and urban area, respectively were conducted in an institution. According to NFHS IV,[10] 78.9% deliveries in India occur in an institution and 75.1% and 88.7% in rural and urban areas, respectively. Similar results were also found in the studies done by Vora SK., et al(2009).[12]

Findings of the present study reflect the role of ASHA in motivating women for institutional delivery. Of all the women who were aware about JSY, 100% beneficiaries in rural area and only 29% beneficiaries in urban area were contacted/advised for institutional delivery during the course of pregnancy. Similar pattern was seen in the study by Dolma Y, et al[7] where ASHA were the source of motivation in 58.1% followed by doctor in 20% cases and ANM (16.3%) while 3.7% cases got motivated through other channels like TV, Radio, friends, relative and 2% of women were motivated by AWW.

CONCLUSION

Out of the total 182 beneficiaries who were aware about JSY, 164 (90%) beneficiaries got registered under JSY, 113 of which belonged to rural area and 51 were from urban area. Complete antenatal care was received by only 19.2% of the beneficiaries of which 24% of the beneficiaries belong to urban area and 17% of the beneficiaries were from rural area. 70% of the deliveries in the rural area and 60% of the deliveries in the urban area were conducted in an institution. Amount incurred on transportation was reimbursed in just 1.4% cases. Among the women who were registered under JSY, 71% of the women received money after delivery in rural area and 82% of the women received money in urban area.

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REFERENCES

1. Park K. Park's Textbook of Preventive & Social Medicine, 24th Edition. 1167, Prem Nagar, Jabalpur-482001, India. M/S Banarasidas Bhanot Publishers, 2017; p. 555.
2. Nandan D, Malini S, Tripathy RM. A Rapid Appraisal on Functioning of Janani Suraksha Yojana In South Orissa, National Institute of Health and Family Welfare. New Delhi. A Case Study.2007-08. Available from: file:///C:/Users/HP%20PC/Downloads/A_Rapid_Appraisal_on_Functioning_of_Janani_Suraksh.pdf [Accessed on 25th August 2017].
3. International Institute for Population Sciences (IIPS) and Macro International. 2008. National Family Health Survey (NFHS-3), India, 2005-06: Uttar Pradesh. Mumbai: IIPS.
4. Singh D, Choudhary F. Theory and Analysis of Sample Survey Designs. 3rd ed. New Delhi: New Age International Publishers; 1997; p132.
5. Kumari V, Dhawan D, Singh AR. Advantages as perceived by the beneficiaries of Janani Suraksha Yojana (JSY) in Bikaner district. J Dairying Foods Home Sci 2009; 28 (3 & 4): 247-9.
6. Banerjee B.A. Qualitative Analyses of Maternal and child health services of an urban health centre by asseessing client perception in terms

of awareness, satisfaction and service utilization. National Journal of Community Medicine; 2009:12(1). p27-36.

7. Dolma Y, Nazki S.G., Munshi I.H., Angmo R. Assessment of Janani Suraksha Yojana (JSY) Component Under NRHM in Selected Districts of Kashmir Valley: A Descriptive Study. Journal of Medical Science and Clinical Research. Vol 3 (3), Mar 2015. p17-22.
8. Panja TK, Mukhopadhyay DK, Sinha N, Saren, AB, Sinhababu A, Biswas AB. Are institutional deliveries promoted by Janani Suraksha Yojana in a district of West Bengal, India? Indian J Public Health. 2012;56 (1);69-72.
9. Registrar General & Census Commissioner, India, Ministry of Home Affairs, Government of India. ANNUAL HEALTH SURVEY 2012-13 FACT SHEET Uttar Pradesh.http://www.censusindia.gov.in/vital_statistics/AHSBulletins/AHS_Factsheets_2012-13/Factsheet-uttar_pradesh.pdf. [Accessed on 25th August 2017].
10. International Institute for Population Sciences, Mumbai. National Family Health Survey 4 India Fact Sheet. Ministry of Health and Family Welfare, Government of India. 2015-16. Available from:http://rchiips.org/NFHS/pdf/NFHS4/India.pdf [Accessed on 25th August 2017].
11. International Institute for Population Sciences (IIPS). 2010. District Level Household and Facility Survey (DLHS-3), 2007–08: India. Mumbai: IIPS.
12. Vora K .Maternal health situation in India: A case study. Journal of Health, Population and Nutrition. 2009; 27(2):184–201.

Comparison of Microleakage in Repairing Furcal Perforations in Biodentine with Cyanoacrylate and Biodenine Alone: An Invitro Study

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ABSTRACT

Perforations in the furcation region are one of the major complications leading to **failure** of **endodontic treatment** and causes significant impact on the long term prognosis of the tooth. It is estimated that furcation perforation is the **second major cause** of failure in endodontics.Biodentine is a calcium silicate-based bioactive material. It is a powder liquid system, powder composed of Tri-calcium silicate, Di-calcium silicate, Calcium carbonate and oxide, Iron oxide, Zirconium oxide. Liquid consist of Calcium chloride, Hydro soluble polymer.It is easy to handle owing to its **ease of manipulation** and a **short setting time** approximately 12 minutes, has **high alkaline pH** and is a **biocompatible material** makes it a favourable material for perforation repair,whereas cyanoacrylate has been shown to be an excellent **tissue adhesive** and **hemostatic agent** which is well tolerated by the tissues of the oral cavity, promotes healing of the tissues. Hence,this study would compare the microleakage of mineral trioxide aggregate and cyanoacrylate cement , in repairing furcal perforations using 2% methylene blue dye solution , conc. Nitric acid, ELISA plates for detecting the maximum dye leakage.

Keywords: *Perforations, bioactive material, short setting time, tissue adhesive, hemostatic agent,microleakage*

INTRODUCTION

In endodontic practice procedural accidents are encountered that will affect the prognosis of root canal space therapy. [1]

One of these accidents is **endodontic perforation**; which can often result in the loss of tooth. [1]

The root perforation can be repaired **surgically**, but a furcation perforation is surgically **inaccessible**.[1]

Therefore, furcal perforations have a more **unfavorable prognosis** than the perforations occurring in the middle and apical root thirds.[1]

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Repairing a perforation plays a vital role in preventing bacterial contamination of the furcal area. Hence a material which is used for sealing should provide an **adequate seal, be biocompatible, and should be bactericidal.**

Amalgam, Cavit, Super-EBA, glass ionomer cements and resins are materials used for sealing furcal perforations.[2]

Biodentine is a **high-purity calcium silicate**–based dental material composed of tricalcium silicate, calcium carbonate, zirconium oxide, and a water-based liquid containing calcium chloride as the setting accelerator and water-reducing agent.[1]

It is recommended for use as a **dentin substitute** under resin composite restorations and an endodontic repair material because of its **good sealing ability,** high compressive strengths, **short setting time (10minutes)**, biocompatibility, bioactivity, and biomineralization

properties. [1]

Tissue adhesives, such as **cyanoacrylates**, have been used in dentistry and in medicine because of their adhesive potential to the human tissue, even in the presence of **moisture**, their biological compatibility, surface isolation, hemostatic properties and bacteriostatic features.[3]

In endodontics, Cyanoacrylate has been used to **seal** the **remaining dentin** of endodontically treated teeth as it was found to control **micro-leakage** of oral fluid at the tooth / filling interface. [3]

No studies have been documented to evaluate the sealing ability of biodentine with cyanoacrylate for perforation repair.

Materials and methods

SOURCE OF THE DATA

The study was conducted in the Department of Conservative Dentistry, A.B.Shetty Memorial Institute of Dental sciences on 20 extracted human mandibular molars collected from the Department of Oral and Maxillofacial surgery, A.B.Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore.

Method of collection of data

Selection of Teeth:

Inclusion Criteria : Intact Mandibular molars with minimal caries and restoration.

Exclusion criteria: Teeth with extensive caries

Procedure

Twenty extracted mandibular molar teeth were used.

The molars were amputated **3 mm** below the furcation area by using a **diamond disk** followed by preparation of Endodontic access cavity .

A perforation was made **between the orifices** to the furcation area by using a high speed long shank carbide **round bur** (2 mm in diameter).

Every molar were then covered completely including cavity walls and pulpal floor with two successive layers of **varnish**.

Study design:

20 extracted mandibular molars with 5 molars in each group

Groups divided as follows

Group 1: Biodentine (bioactive dentin substitute)

Group 2 : Cyanoacrylate and biodentine (bioactive dentin substitute)

Group 3: Positive control (Unsealed molars)

Group 4: Negative control (Non perforated molars)

Experimental groups : Group 1 and Group 2

Furcation perforation sealed with biodentine (bioactive dentin substitute)

Furcation perforation sealed with biodentine, cyanoacrylate

Control groups: Group 3 and Group 4

EVALUATION OF EXPERIMENTAL GROUPS

To avoid dye penetration through other areas, all experimental teeth were coated homogeneously with two coats of **varnish** for 1 to 2mm around the perforation.

After 24 hours of drying, all teeth were immersed in at **2% methylene blue solution** for 48 hours.

After removal from the dye, the teeth were rinsed with water and **dried** at **room temperature** for 24 hours.

The teeth were placed in vials containing **1ml** of **concentrated** (65 wt%) **nitric acid** until complete dissolution.

Vials were centrifuged at **9000 rpm** for 7 minutes.

Two hundred microliters of the supernatant from each sample was transferred to a 96 well plate.

Sample absorbance were read by an **automatic spectrophotometer** at **550 nm** using concentrated nitric acid as a blank.

SAMPLE ANALYSIS

ANOVA test was used to compare the mean of the different groups. Post hoc Tukeys test was used in the procedure of pair-wise comparisons between the group when ANOVA test is significant. The level of significance were set at p value < 0.05.Statistical analysis was

performed with SPSS 16.0 software(Statistical Package for Scientific Studies).

FINDINGS

TABLE1: Sample absorbance values obtained from each sample measured using a spectrophotometer at 550nm.

Biodentine	Biodentine with cyanoacrylate	Positive control	Negative control
0.138	0.081	0.314	0.024
0.092	0.039	0.412	0.040
0.076	0.044	**0.98**	0.032
0.112	**0.033**	0.518	0.016
0.082	0.062	0.756	0.010

Table 2: Oneway ANOVA Test depicting the mean, standard deviation and ANOVA results of the various groups

Descriptives				
	No. of samples	**Mean**	**Standard Deviation**	**P value**
Bioactive dentine substitute	5	.10000	.025259	<0.001
Bioactive dentine substitute and cyanoacrylate	5	**.05180**	.019588	
Positive control	5	**.59600**	.270426	
Negative control	5	.02440	.012033	
Total	20	.19305	.270895	

- According to the results the positive control showed the highest mean absorbance (0.596)i.e. maximum dye leakage/ dye extraction.
- Bioactive dentine substitute(0.1) showed next mean dye absorbance value.
- Bioactive dentine substitute with Cyanoacrylate (0.051) showed lesser dye absorbance i.e. lesser dye leakage/ dye extraction than biodentine.
- The ANOVA test depicted overall significant differences between the groups in dye absorbance so a Post Hoc TUKEY test for pair wise comparison between the mean of the groups was performed.

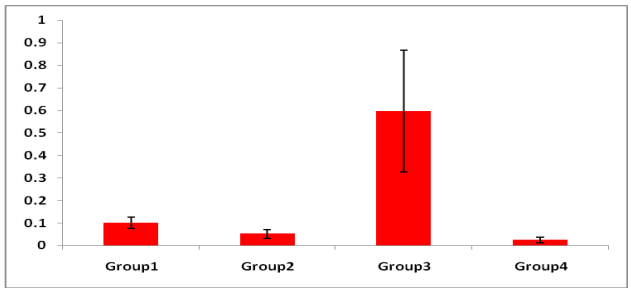
Table 3: Post Hoc Tests depicting pair wise comparison between the groups

(I) grp	(J) grp	Mean Difference (I-J)	P value
Bioactive dentine substitute	Bioactive dentine substitute and cyanoacrylate	.048200	.943
	Positive control	-.496000*	.001
	Negative control	.075600	.817
Bioactive dentine substitute and cyanoacrylate	Bioactive dentine substitute	-.048200	.943
	Positive group	-.544200*	.001
	Negative control	.027400	.988
Positive control	Bioactive dentine substitute	.496000*	.001
	Bioactive dentine substitute and cyanoacrylate	.544200*	.001
	Negative control	.571600*	.001
Negative control	Bioactive dentine substitute	-.075600	.817
	Bioactive dentine substitute and cyanoacrylate	-.027400	.988
	Positive group	-.571600*	.001

- Amongst all the groups Bioactive dentine substitute alone , Bioactive dentine substitute with cyanoacrylate had significantly lesser absorbance than positive control. ($p < 0.05$)
- Bioactive dentine substitute alone (0.1) and Bioactive dentine substitute with cyanoacrylate(0.051) showed a significant difference with positive control group when

compared with each other.

- Positive control group(0.596) showed highly significant difference with Bioactive dentine substitute and Bioactive dentine substitute with cyanoacrylate.



- The mean difference is significant at the 0.05 level.

Graph 1: BAR DIAGRAM REPRESENTING THE SAMPLE ABSORBANCE (DYE EXTRACTION) OF THE VARIOUS MATERIALS USED

- The important factors that are considered in sealing furcation perforations are sealing ability of the repair materials and their extrusion into furcation areas. In this study perforation sealing ability of Bioactive dentine substitute and Bioactive dentine substitute with CYANOACRYLATE was evaluated.
- This study uses the **dye extraction** method by Camp and Pashley (2003) to study the sealing ability of the materials. This method is a **more reliable technique** than dye penetration as it considers all the **dye absorbed** by the samples used. [4]
- A study comparing the efficacy of dye penetration to dye extraction method concluded that, dye extraction method may be preferred because of its **simplified procedure** and its ability to **simulate oral conditions**.[4]
- A spectrophotometer reader is used here to measure the **fraction of incident light** transmitted through a solution. [5]
- It can be used to determine concentration of compounds in solutions because concentration of the absorbing compound can be determined by the amount of light absorbed by the sample as it is directly proportional to it.[5]
- In this study **negative control**, showed **lowest dye absorbance** (0.024) and had absorbance values were near blank HN03 (0) and **positive controls** had the **highest dye absorbance** (0.596).
- In this study results showed that sample absorbance of the bioactive dentine substitute was more than that of bioactive dentine substitute with cyanoacrylate,which suggest **better sealing ability of bioactive dentine substitute with cyanoacrylate as a repair material in furcal perforations .**
- CYANOACRYLATE alone was not used in the study because of the chances of the **adhesive seepage due to the greater flow of the material** whereas is was used in combination with bioactive dentine substitute to enhance the **seal** of the furcal perforation region since its acts as a **water proof dressing**.
- Further studies are required before bioactive dentine substitute(Biodentine) with cyanoacrylate could be used a furcal repair material in clinical use.

CONCLUSION

- From this present study we could infer that the sample absorbance of BIODENTINE alone was more than that of BIODENTINE WITH CYANOACRYLATE.
- Statistically sealability of BIODENTINE and BIODENTINE WITH
- CYNOACRYLATE had a significant difference suggesting that the
- BIODENTINE WITH CYANOACRYLATE had a better sealing ability compared to BIODENTINE alone.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

REFERENCES

1. Yahya MM. Sealing ability of Biodentine as a root Perforation Treatment Material (An in Vitro Study).
2. Kakani AK, Veeramachaneni C, Majeti C, Tummala M, Khiyani L. A Review on Perforation Repair Materials. Journal of clinical and diagnostic research: JCDR. 2015 Sep;9(9):ZE09.
3. Lage-Marques JL, Conti R, Antoniazzi JH. The

use of Histoacryl in endodontics. Braz Dent J. 1992;3(2):95-8.

4. Camps J, Pashley D. Reliability of the dye penetration studies. J Endod. 2003;29:592–4.
5. Jeevani E, Jayaprakash T, Bolla N, Vemuri S, Sunil CR, Kalluru RS. Evaluation of sealing ability of MM-MTA, Endosequence, and biodentine as furcation repair materials: UV spectrophotometric analysis. Journal of conservative dentistry: JCD. 2014 Jul;17(4):340.
6. Silva LA, Pieroni KA, Nelson-Filho P, Silva RA, Hernandéz-Gatón P, Lucisano MP, Paula-Silva FW, de Queiroz AM. Furcation Perforation: Periradicular Tissue Response to Biodentine as a Repair Material by Histopathologic and Indirect Immunofluorescence Analyses. Journal of Endodontics. 2017 May 2.
7. Barkhordar RA, Javid B, Abbasi J, Watanabe LG. Cyanoacrylate as a retrofilling material. Oral Surgery, Oral Medicine, Oral Pathology. 1988 Apr 1;65(4):468-73.
8. Leggat PA, Kedjarune U, Smith DR. Toxicity of cyanoacrylate adhesives and their occupational impacts for dental staff. Industrial health. 2004;42(2):207-11.
9. Malkondu Ö, Kazandağ MK, Kazazoğlu E. A review on biodentine, a contemporary dentine replacement and repair material. BioMed research international. 2014;2014.
10. Hamad HA, Tordik PA, McClanahan SB. Furcation perforation repair comparing gray and white MTA: A dye extraction study. J Endod. 2006;32:337–40.
11. Kaya S, Ozer SY, Yavuz I, Aydin H. Comparison of dye extraction or dye penetration methods to quantitatively determine microleakage of three different root canal sealers. Dentistry. 2011;1:1–5.
12. Azevedo CL, Marques MM, Bombana AC. Cytotoxic effects of cyanoacrylates used as retrograde filling materials: an in vitro analysis. Pesquisa odontológica brasileira. 2003 Jun;17(2):113-8.
13. Torabinejad M, Ford TR. Root end filling materials: a review. Dental Traumatology. 1996 Aug 1;12(4):161-78.

Mental Health among High and Higher Secondary School Students

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ABSTRACT

The objective of the present study was to examine the relationship between mental health and academic achievement among high and higher secondary students. The sample consists of 511 (boys 288 and girls 233) high and higher secondary students were randomly selected from Chennai and Kancheepuram district schools in Tamil Nadu. Correlation and independent t-test, ANOVA were used for analyzing the data. The result shows that there is significant difference between mental health and sample sub groups. More over the result reveals that the positive correlation between mental health and academic achievement of high and higher secondary students. Early detection for indications of mental health problems and understanding factors contributing to stress among students would promote better understanding of mental health in future.

Keywords : *Mental health , Academic achievement*

INTRODUCTION

Mental Health of the learner is very important for efficient learning and proper development of personality. Mental Health which today is recognized as an important aspect of one's total health status is a basic factor that contributes to the maintenance of physical health as well as social effectiveness. Mental Health may be defined as the adjustment of the individuals to themselves and the world at large with a maximum of effectiveness, satisfaction, cheerfulness and socially considerate behavior and the ability of facing and accepting the realities of life, with minimum friction and tension. Hadfield (1952) defined "Mental Health is the full and harmonious functioning of the whole personality.[1] According to statistics from the World Health Organization (2003b), 12% of global diseases (121 million people suffer from depression, 70 million from alcoholism, 24 million from schizophrenia and 37 experiences dementia) were a result of mental health problems.[2] By 2020 as indicated by the World Health Organization (2003b) the burden will be increased by nearly 15%.[3] This will result in the loss of disability-adjusted life-years to illness and young adults in developing countries seem to be the most prone. Hill and Wigfield (1984) states that test anxiety is one of the most important aspects of negative motivation and has direct debilitating effects on school performance.[4] Students' Academic, Behavioral, Cognitive, Affective factors also influenced their mental health. Students poor academic performance and inconsistent of attendance are leads to their mental health problems.[5] (DeSocio & Hootman, 2004)

OBJECTIVES OF THE STUDY

To find out the impact of mental health of high and higher secondary school students.

To find out the influence of mental health on academic achievement of high and higher secondary school students.

HYPOTHESES

H.1) There is no significant difference between mental health of high and higher secondary students and the subgroup of the sample.

H.2.1	Sex
H.2.2	Parent education
H.2.3	Standard
H.2.4	Family income
H.2.5	Recreational activities
H.2.6	Study time

H.2). There is no significant relationship between mental health and academic achievement

H.1.1 Behavior problem and conduct

H.1.2 General positive affective

H.1.3 Hyper active

H.1.4 Emotional

H.1.5 Pro social

H.1.6 Depression

H.1.7 Anxiety

METHOD

The sample consists of 511 high and higher secondary school students, of whom 288 were boys and 223 are girls from chennai and kancheepuram district school in TamilNadu. Stratified random sampling technique used in the present study under this process, the entire universe or population is divided into a number of homogenous or types of classes. On the present study high and higher secondary students can be divided into number of sex (Boys, Girls) Parent Education (Non graduate, Graduate), Standard (High school level, Higher secondary level) Family Income (Above 12,000, Below 12,000), Recreational activities (Involved and Uninvolved), Study Times (Morning, Evening, Both).

Instruments and Techniques applied

Mental health scale

Mental health of individual was measured by mental health inventory which was developed by Veit & Ware (1983) in RAND Corporation. To measure the mental health among high and higher secondary students, the student form was prepared and which was consisted of 78 statements with four responses like always, often, sometimes and never. Mental health inventory (MHI) have are seven dimensions of mental health inventory are behavioral problem conduct , general positive affective , hyperactivity, emotional, pro social , depression , and anxiety are helpful to measure the student's mental health status.

Analysis

Descriptive analysis was done using the statistical package SPSS to stratify the subgroups of the sample by the method of finding our averages, deviation and t test. Correlation was dome in order to find out the relationship between Mental Health and Academic Achievement.

Purpose and nature of the test

This study intends to find out the level of mental health and the various psychological issues which the students are being faced and the influence of this issues may cause of their slow learning. Administration of the tool was conducted individually to ensure accuracy.

ANALYTICAL DISCUSSION

Table 1 and 2 represents the mean and standard deviation of the mental health scores based on the subgroups of the sample.

Table 1 : Mean and S.D of the Mental Health score based on the subgroups of the sample

	Sub group	*N*	Mean	*S.D*	*t*- value	Level of significance
Sex	Boys	288	187.57	35.88	3.893	<0.01**
	Girls	223	199.31	31.60		
Parent education	Non graduate	280	186.48	34.75	4.560	<0.01**
	Graduate	231	200.22	32.82		
standard	High school level	257	195.82	30.56	2.070	0.14*
	Higher secondary level	254	189.52	37.94		
Family income	Above 12,000	253	189.56	36.50	2.037	0.04*
	Below 12,000	258	195.76	32.82		
Recreational activities	Involved	441	196.30	31.47	6.137	<0.01**
	Uninvolved	70	169.96	43.52		

Note . *p<0.05, **p<0.01

Table 2: ANOVA for significant difference in Mental Health and its Factors among students with respect to Study Time

	Study Time			*F* value	*P* value
Mental Health	**Morning**	**Evening**	**Both**		
	184.71[a] (41.48)	189.49[a] (30.96)	198.51[b] (32.45)	7.18	< .001**

Note: The value within bracket refers to SD

Different alphabet among Educational Qualifications denotes significant at 5% Level using Duncan Multiple Range Test (DMRT)

***p*<0.05, ***p*<0.01**

Table 1 represents the mean and standard deviation of the mental health scores based on the subgroups of the sample. There was a significant effect of sex $t = 3.893$, parent education $t=4.560$, recreational activities $t=6.137$ and $p = 0.001$. Since the p value of mental health and its dimensions less than the 0.001. It reveals that there is significant difference between mental health and sample sub group. So hypothesis H.2 is rejected.

Moreover result indicated that in sex, girls mean score are high than boys. The above result with the respect to the gender is in concordance with the study done by Nanda, A.K.(2001), Female students had better mental health than male students[6] and Peterson et al (1991) found that adolescent girls generally experienced more challenging and stressful events than boys.[7] In parent education, graduate level parents' children are having good mental health. Students who are involved in recreational activities, are having good mental health than uninvolved students. It further shows that significant effect at $p = 0.005$ level in students standard $t = 2.070$ and family income $t= 2.034$. It revealed that those who are studying high school level having good mental health than higher secondary level students and if the family income below 12,000/- those students having good mental health than other.

Table 2 emphasized that in DHRT analysis of $F = 7.18$, $p = 0.001$. So students study time also significant effect of their mental health. It reveals that the students those who are preferred to study both morning as well as evening, they have good mental health. The students spending the amount of time is correlated with their academic achievement [8](Gettinger & Walter, 2012). It may be observed from table 1 and 2 that there exists significant difference with almost all the chosen sub groups of the sample and hypothesis H.1 is rejected.

Table 3: Correlation between Mental health and Academic Achievement

Variables	Sub – variables	*r*- value	Level of significance
Mental health	Behviour Problem and Conduct	0.401	**<0.01****
	General Positive Affective	0.473	<0.01**
	Hyper Active	0.459	<0.01**
	Emotional	0.434	<0.01**
	Pro social	0.458	<0.01**
	Depression	0.446	<0.01**
	Anxiety	0.476	<0.01**
	Overall Mental Health	0.549	<0.01**

Note . *p<0.05, **p<0.01

Table 3 shows that the correlation value of Behaviour problem and conduct r = 0.401, general positive affective $r = 0.473$, hyper active $r = 0.459$, emotional $r = 0.434$, pro social $r = 0.458$, depression $r = 0.446$, anxiety $r = 0.476$ and over all mental health $r = 0.549$. Since the p value of mental health and its dimensions less than the 0.001. It reveals that there is significant positive correlation between mental health and academic achievement. So the hypothesis H.2 is rejected at 1% level. If the students have good mental health, they can study well and their academic performance also good. This result corroborating with the study of Anita Chawla (2012) indicated that High academic achievement corresponds to high mental health[9] and similar findings like maintaining emotional

balance among students through a psychologist by using auto counselling increased their academic performance[10] Panchanatham (1999). To be precise, The result of the above table reveal that mental health plays a vital role in determining the academic achievement of the high and higher secondary students.

CONCLUSION AND SUGGESTIONS

The study also reveals that there are gender difference in the level of mental health, Therefore, it understands that both boys and girls are having similar in their innate capacities, girls seems to process higher level of mental health. In the area of gender-role conflicted men has suggested that these men are not only more likely to experience psychological distress, but also less likely to use available services.[11] Marks (2001) & Wood (1995)

It can perceived that the students those who are having the habit of studying at morning as well as evening , those students are higher mental health and involvement of recreational activities also helps to develop the good mental health

The study clearly reflects that there is a positive relationship between mental health, and academic achievement among high and higher secondary level students. Students, who are having good mental health, are reflecting better in academic achievement also. Whereas poor mental health result is, poor in academic achievement.

Ethical Clearance: Not required

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. Mangal, S, K., (2007). Essential of educational psychology, New Delhi: Prentice-hall of India Private Limited. Retrieved from https://books.google.co.in
2. World Health Organization. (2003). The Mental Health Context: Mental Health Policy and Service. Retrieved from http://www.who.int/mental_health/media/investing_mnh.pdf
3. Guidance Package. Geneva: WHO. Retrieved from http://www.who.int/ whr/2003 /en/ whr03_en.pdf
4. Cizek , J. G., & Burg, S. S. (2006). Addressing Test Anxiety In A High – Stakes Environment Strategies for Class Rooms and Schools, California: Crowing press.
5. Macklem, L, G. (2010). Evidence-Based School Mental Health Services: Affect Education, Emotion Regulation Training, and Cognitive Behavioral Therapy, Retrieved from https://books.google.co.in
6. Nanda, A.K. (2001). In mental health of high school students: A comparative study, Indian psychological review, 56(1). 2-7.
7. Krenke, S. I. (1995). Stress, coping and relationship in adolescence, London: psychology press.
8. Harrison, R, J., & Evans, W, S. (2017). School Mental Health Services for Adolescents, Retrieved from https://books.google.co.in
9. Chawla, A. (2012). Mental Health and Its Relation to Academic Achievement: A Brief Note On Auto-suggestion To Improve Mental Health, Indian Streams Research Journal, 2 (7). 1-7.
10. Shirazi, M., Khan, A. M., Ansari, & Mohd.F.A.(2012). Mental health in relation to personality characteristics among professional and non-professional students, Research world, 3(1). 8-10.
11. Yorgason, B. J., Linville, D., & Zitzman, B.(2008). Mental Health among College Students, Journal of American college health, vol. 57(2). 179-181.

Gender Gap in Literate Life Expectancy –A District-Wise Study in India

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ABSTRACT

Inadequate literacy describe as a strong barrier to access healthcare services .So, in the present study, the innovative indicator literate life expectancy (LLE) has been implemented to highlight the social differences existing in different parts of the country. Wolfgang Lutz (1995) was the first to develop LLE which gives an expected number of years a person lives in a literate state i.e., able to read and write under current mortality and literacy conditions. The obtained results showed that compared to men, women experienced less number of years in the literate state in almost all the districts of the selected states. It is believed that findings of the result in this study will help the policy makers and the government to focus more on literacy and programs relating to elementary education at the district level for the welfare and well being of the society.

Keywords: literate-life expectancy, education, gender-gap, India, district.

INTRODUCTION

Literacy is one of the important factors in the life of an individual. Lack of literacy directly affects the health of an individual, so the importance of education on physical health is fundamental. Inadequate literacy will also describe as a strong barrier to access healthcare services. So, in this perspective we tried to implement the literate life expectancy indicator to bring out the scenario of the people residing in different parts of the country .The concept of literate life expectancy (LLE) was first developed by Wolfgang Lutz in 1995 as an indicator of social development and quality of life. It is interpreted as an expected number of years a person lives in a literate state i.e., able to read and write under current mortality and literacy conditions [1]. The indicator is innovative and simple which accounts only two essential elements of social development namely literacy and life expectancy[2].It is based on age-specific mortality rates and age-specific literate proportions and computed by means of life table method [3]. The aim of this indicator is to look at person-years of life but in a literate state [2].

Lutz (1995)[4] mentioned that this indicator has numerous advantages over other indicators of social and educational development like literacy rate by sex and age-standardized literacy rate. It is not an abstract index on a relative scale and can be expressed in terms of individual years of life [1]. Moreover, LLE is the only social development indicator which can be projected into the future on the basis of other already accepted forecasts and thus illustrates both the path dependence and realistic prospects of development [5]. This indicator does not reflect any measure of economic income rather it is based purely on individual characteristics like literacy and mortality [6]. In addition to this, LLE can also be readily calculated for both the sexes separately that makes it very suitable for gender-specific analysis. It can also be evaluated for any subgroups of the population. The indicator not only shows the current level of social development but it also portrays the nation's possibility for future development [2]. LLE is an absolute number of social development which does not depend on any assumption.

Numerous studies in the literature showed that LLE has been widely used by various researchers for measuring the social development of a country which includes Khan and Asaduzzaman (2007) in Bangladesh[6], Medina (1996) in Mexico[2] and Huang and Nanjo(1998) in China[7], Chattopadhyay (2010) in India[5]. But the study on LLE at the district level is very limited. The LLE for females at the district level of India based on 2001 census data has been studied by Choudhury et.al (2013)[1] for the first time in the literature. Since then no study has been undertaken at the district level of India

with regards to LLE. The district is the basic territorial unit of administration in India and also the keystone of the whole administrative structure. It is believed that social development of any country is not possible if the equity conditions which are reflected at the micro level are not considered. So, the analysis of LLE at the micro level is essential to get a socio-development scenario in the administrative units. Out of 640 districts as per 2011 census of India, 190 districts from the selected states are included in the present analysis.

OBJECTIVE

With this backdrop, the objectives of the present study are:

1.To calculate the literate life expectancy (LLE) at the district level of some selected states representing various zones of India namely Assam from the North-East, Gujarat from the West, Kerala from the South, Rajasthan from the North, Uttar Pradesh from the Central and West Bengal from the East respectively for both the sexes based on 2011 census.

2. To assess the gender gap in terms of LLE at birth for the districts of the selected states mentioned above to capture the existing heterogeneity in the country.

DATA AND METHODOLOGY

The required data for the analysis are ordinary life tables and age-specific proportions of literate at the district level. The district-wise life tables for both the sexes have been constructed in a similar manner as the method developed by Choudhury and Sarma (2011)[8]. The one parameter model life tables for the major states and their corresponding districts were generated by Choudhury and Sarma (2011)[8] where the life expectancy at birth was the only input. As the life expectancy at birth at the district level is not readily available, so it has been estimated by the method of regression approach. Hence, the life tables at the sub-state level are constructed from the estimated expectation of life at birth. Secondly, information on literacy data was taken from the C8 table of the Census of India based on 2011 which is labelled by "*Educational Level By Age and Sex Population Age 7 and above"*. Data on literacy in the census table are structured in single, five and seven year age groups namely 0-6, 7, 8, ... 19, 20-24,25-29,...,70-74,75-79,80+ by sex and place of residence. The age group 0-6 were divided into single year ages by applying Karup-King Interpolation formula. Then all the single year ages are converted into following year age groups viz.,0-1, 1-4, 5-9, 10-14, 15-19,…,60-65,65-70,70+ respectively. Also, the corresponding population data were obtained to calculate the literacy rate.

The LLE estimation follows the ordinary life table method which is used to compute mortality based life expectancy, with the only addition being that the number of person-years at each age is weighted by the age-specific proportion literate. If P_x is the age-specific proportions in age x then the literate person-years lived in the age x (L_x) is given by the following equation:

$$L_x \times P_x = L_x$$

where L_x denotes the total number of person-years living in age x.

Then the LLE (e_x^0) is obtained by dividing the cumulative literate person-years (T_x) with the number of survivors (l_x) i.e.

$$e_x^0 = \frac{T_x}{l_x}$$

where $T_x = \sum L_x$

RESULTS AND DISCUSSION

The 2011 Census of India reports that the total literacy rate of the country stood at 74.04 %. Due to the limitation of the space, only the highest and lowest values of LLE at birth for the districts of the selected states are presented in the following table (Table 1). Table 2 displays the number and percentages of districts in each of the selected states having a literate life expectancy at birth below 20 years, 20-40 years, 40-60 years and above 60 years for both the sexes respectively. Table 3 presents the district-wise gender differential in LLE at birth.

Table 1: **Maximum and minimum values of LLE at birth at the district level of Assam and Some of its Selected States for the period 2011**.

States	Number of Districts	Districts with (male)		Districts with (female)	
		Maximum LLE at birth	Minimum LLE at birth	Maximum LLE at birth	Minimum LLE at birth
Assam	27	Kamrup Metropolitan (49.80)	Dhubri (29.79)	Kamrup Metropolitan (49.62)	Dhubri (22.68)
Gujarat	26	Ahmedabad (51.55)	Dohad (32.93)	Navsari (46.61)	Dohad (19.61)
Kerala	14	Pathanamthitta (66.02)	Wayanad (56.14)	Pathanamthitta (69.78)	Kasaragod (52.43)
Rajasthan	33	Jaipur (50.37)	Banswara (29.06)	Kota (37.16)	Jalor (15.83)
Uttar Pradesh	71	Gautam Buddha Nagar (47.40)	Shrawasti (26.58)	Kanpur Nagar (40.71)	Shrawasti (12.85)
West Bengal	19	Purba Medinipur (55.99)	Uttar Dinajpur (34.68)	Kolkata (47.55)	Uttar Dinajpur (23.75)
Total	190	Pathanamthitta (66.02)	Shrawasti (26.58)	Pathanamthitta (69.78)	Shrawasti (12.85)

The above table (Table 1) depicts that out of the total of 27 districts of Assam, the highest LLE at birth for males prevailed in the Kamrup Metropolitan district with 49.80 years. This means that a person in this district on an average will survive for 49.80 years in a literate state. Lowest male LLE at birth prevails in the Dhubri district with 29.79 years. Similar districts also performed the highest and lowest score in case of females. The corresponding figures for LLE at birth ranges from 22.68 years (lowest) to 49.62 years (highest).

In Gujarat amongst the 26 districts, Ahmadabad occupied the highest position while Dohad stood the lowest position in terms of male LLE at birth with 51.55 years and 32.93 years respectively while in case of females, Dohad remains the lowest position with 19.61 years and Navsari district is the highest with 46.61 years. Among the 14 districts of Kerala, the highest male LLE at birth is indicated in the Pathanamthitta district with 66.02 years and lowest is recorded in the Wayanad district with 56.14 years. For females, Pathanamthitta district remained the highest position in 69.78 years while in Kasaragod district, females had only 52.43 years as the lowest value.

In Rajasthan out of 33 districts, Jaipur district registered the highest LLE at birth (50.37 years) among the males while lowest male LLE at birth is recorded in Banswara district with 29.06 years. Kota recorded the highest position (37.16) years in terms of female LLE at birth and the lowest is found in Jalor district with 15.83 years.

Among 70 districts of Uttar Pradesh, a higher value of LLE at birth is noticed in Gautam Buddha Nagar district and lowest is found in the Shrawasti district for males. The corresponding figures stood at 47.40 years and 26.58 years respectively. Highest female LLE at birth is found in Kanpur Nagar with 40.71 years and lowest is seen again in Shrawasti district with 12.85 years respectively. The situation of females in the Shrawasti

district in terms of literacy performed poorly as males are spending more than the double number of years in a literate state as compared to the female counterparts. Also for the same state, the value of LLE score varies widely within the districts amongst the females as the highest figure for female LLE at birth is more than three times the lowest figure of female LLE at birth. Possibly due to economic backwardness and enlightenment towards education are the major causes of the very low level of female literacy in this district[9].

Finally, out of a total of 19 districts of West Bengal, the highest male LLE at birth is found in the Purba Medinipur district with 55.99 years and Uttar Dinajpur district stood the lowest position in terms of LLE at birth (34.68 years). In case of females, LLE at birth is highest in Kolkata district with 47.55 years and lowest is seen in Uttar Dinajpur with 23.75 years. The district Uttar Dinajpur is characterized by low-health care and livelihood access and widespread rural poverty. This district is a largely agricultural district, rapid population escalation restricts the absorption of the new rural workforce into farm-based employment, while the low level of urbanization restrains the growth of the non-farm sector[10] .These are the reasons that had made the level of literacy not up to the mark as compared to other districts. The table reveals that the people of the backward districts are having less years of education in comparison to the people of the advanced districts. Possibly people of the backward districts are unaware about the value of education and the government schemes that were related to literacy. It is also noticed from the Table 1 that the level of education among the districts of Uttar Pradesh is very poor for both the sexes. One interesting feature which is found that the female LLE at birth in the Pathanamthitta district has surpassed the male LLE by 3.76 points.

Table 2: Difference between male and female LLE at birth among the districts of the selected states.

States	Number of districts	Districts with	
		Highest Difference	Lowest Difference
Assam	27	Dhemaji (11.09)	Kamrup Metropolitan (0.18)
Gujarat	26	Banas Kantha (14.11)	Navsari (1.16)
Kerala	14	Kasaragod (5.41)	Ernakulam (0.05)
Rajasthan	33	Karauli (20.44)	Ganganagar (10.56)
Uttar Pradesh	71	Sant Ravidas Nagar (17.62)	Kanpur Nagar (3.89)
West Bengal	19	Puruliya (18.46)	Kolkata (3.69)
Total	**190**	**Karauli (20.34)**	**Ernakulam (0.05)**

The existence of gender gap is also reflected in the above table (Table 2). In Assam, the highest male-female difference in terms of LLE at birth is found in Dhemaji with 11.09 years while the lowest is seen in Kamrup Metropolitan with 0.18 years. Amongst the 26 districts of Gujarat, the highest gender differential in terms of LLE at birth occurred in Banas Kantha district with 14.11 years and the lowest is reflected in Navsari by 1.16 years. In Kerala, the highest male-female difference with regards to LLE at birth is found in Kasaragod with 5.41 years and the lowest is observed in Ernakulam with 0.05 years. In Rajasthan, the male-female gap in association to LLE at birth is noticed in Karauli (20.44 years) and the lowest prevailed in Ganganagar with 10.56 years. Similarly, out of 71 districts in Uttar Pradesh, the gender gap in terms of LLE at birth ranges from 3.89 years to 17.62 years. Finally, in West Bengal among the 19 districts, the highest gender difference in terms of LLE at birth is seen in Puriliya district with 18.46 years and the lowest revealed in Kolkata with 3.69 years. The possible reason for the huge gender discrimination might be due to the fact that school dropout rate among the girls is the highest since due to poverty in some families where parents cannot afford to send all the children to school, boys get the opportunity to attend the school as they give more priority to educate the boys.

Conclusion: The innovative indicator LLE has been implemented to highlight the social differences existing in different parts of the country. Compared to men, women experienced less number of years in the literate state in almost all the districts of the selected states. It is believed that findings of the result in the present paper will help the policy makers and the government to focus more on literacy and programs relating to elementary education at the district level for the welfare and well being of the society. Attention should be highly focused

on increasing the educational level, particularly among the females as literacy is the crucial parameter for the empowerment of women in the society.

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REFERENCES

1. Choudhury, L., Sarmah, G. R., & Sarma, R. (2013). Literate Life Expectancy for females of Assam and selected states of India: a district-level analysis of the 2001 census data. Journal of Population and Social Studies, 21(2 (Suppl.)).Health
2. Medina, S. (1996). Implementing a new indicator of social development in Mexico: literate life expectancy (LLE). International Institute for Applied Systems Analysis.
3. Lutz, W., & Goujon, A. (2004). Literate life expectancy: charting the progress in human development. The end of world population growth in the 21st century: new challenges for human capital formation and sustainable development, 159-86.
4. Lutz, W. (1995). Literate life expectancy, Popnet, 26 (winter), 1-5, International institute of applied system analysis, Luxemburg, Austria.
5. Chattopadhyay, A., & Sinha, K. C. (2010). Spatial and gender scenario of literate life expectancy at birth in India. Asia Pacific Journal of Public Health, 22(4), 477-491.
6. Khan, H.R. & Asaduzzaman (2007). Literate life expectancy in Bangladesh: A new approach of social indicator. Journal of Data Science, 5, 131-142
7. Huang, R. & Nanjo, Z. (1998). Measurement of social development in China using literate life expectancy. Jinkogaku Kenkyu, May (22), 25-30. Pmid: 12321693 [pub med-indexed for Medline].
8. Choudhury L, Sarma R. Generation of model life tables for the major states of India. International Journal of Statistics and Analysis. 2011; 1(4):419-41.
9. Singh, P., & Bhandari, M. (2017). Literacy Level of Muslim Girls in Uttar Pradesh.
10. Roy, C. (2011). Economic backwardness of Uttar Dinajpur: a block level analysis.

Evaluation of a Community Orientation Program for First Year Medical Undergraduate Students

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ABSTRACT

Background: Against the backdrop of the new changes effected in the curriculum by the affiliating university a short-term community orientation programme was designed for the medical undergraduate (MBBS) students. This study aims at evaluating the one-week community orientation program based on students' feedback, their performance in the assessment test and to understand the correlation between feedback evaluation scores and the student assessment scores.

Method: Feedback was obtained by an anonymized self administered questionnaire which assessed their improvement in understanding through likert scale and understanding the factors which facilitated or hindered learning through open ended questions. Student assessment was conducted in the form a 20-item MCQ test. Spearman's correlation was done to find out the association between program feedback scores and student assessment scores.

Results: 61.5% said that the program had greatly improved their understanding of the topics. Field visits (72.6%) and intra-session activities (71.6%) had helped in improving their understanding greatly and lecture mode of teaching (68%) was perceived by majority of students as a session that had slight or no change in the understanding of the subject. Students scored highest on topics that used activities in intra-session activities (95.3 %), field visits and group discussions (93.5% each) followed by role plays (91.6%).

Conclusion: Active teaching methods are greatly accepted by students and help them attain their learning objectives and feedback serves as an effective evaluation tool for assessing teaching programs.

***Keywords**: Community orientation, Curriculum, Evaluation, Medical education, Students*

INTRODUCTION

Medical education is the basis on which high quality health care is built. So it becomes imperative to train medical students to become competent doctors of tomorrow. The medical curriculum should train doctors to respond to the health needs of the community.[1] Medical Schools shares responsibility for a comprehensive set of health services to a defined population in a given geographical area, consistent with values of quality, equity, relevance, and efficiency for developing and assessing innovative models integrating population and individual health activities, for learning and for conducting health research, and so it is important to orient the students to work as community physicians. [2]

The focus of medical education has changed from individual hospital centred to promotion of health of the community. [3] The ROME program by WHO emphasised on community oriented medical education to produce doctors who are socially responsible and who possess the technical, scientific and management competence which

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will enable them to play a useful role in the development of comprehensive health systems based on primary health care.[4] A curriculum which gives consideration for and emphasises on community oriented teaching alone would help achieve the goal of developing primary care physicians. To keep up with the changing needs of the society the University to which this institute is affiliated has revised the Graduate medical curriculum.

Hence against the backdrop of the new changes effected in the curriculum by the affiliating university of our medical school, we designed a short-term community orientation programme for the medical undergraduate (MBBS) students under the aegis of the Department of Community Medicine of the medical school. It is also a well established fact that individuals have diverse learning styles and therefore for any teaching program to be successful different teaching strategies addressing the varying and diversified learning preferences of students have to be employed. [5] By encouraging active learning in students, their performances also improves in examinations.[6] Further, student feedback is an effective tool for evaluating the teaching-learning methods and contributing towards the success of the learning program. [7,8] It gives rich information which helps to improve the program and teaching methods.

Thus keeping in mind the diverse learning preferences of the students and the short term and long term goals of the graduate medical curriculum a learning program was designed. This study aims at evaluating the one-week community orientation program based on students' feedback, to assess the knowledge gained by students based on their performance in the assessment test and to understand the correlation between feedback evaluation scores and the student assessment scores

METHOD

This is a cross sectional study using retrospective data obtained from feedback evaluation and assessment scores of community orientation program for 1st year undergraduate medical students. The week long program in community medicine is the first exposure for the students to the subject. A total of 150 students underwent the orientation program.

Study setting:

The Tamil Nadu Dr. M.G.R. Medical University (to which our medical school is affiliated) introduced a curriculum in community medicine for I year medical undergraduate (MBBS) students, [9] according to which the students must have knowledge about:

- the evolution of community medicine
- definition of health and factors affecting it
- health care delivery system
- demographic pattern of the country
- the roles of the individual, family, community and socio-cultural milieu in health and disease
- social factors related to health
- disease and disability in the context of urban and rural societies
- the impact of urbanization on health and disease

Therefore a community orientation program was designed, which spread over 60 hours and focussed on the various objectives that the program had planned to attain. The program was also constructed in a way that it incorporated the various teaching styles addressing the diversified learning preferences of students. Table 1 shows the schedule planned for the one week orientation program comprising all the topics to be covered as per the new university curriculum.

Table 1: The schedule designed for the orientation program based on areas stressed by the university curriculum

Session / Areas / Topics / Concepts	Program Objectives	Teaching / Learning Method	Duration (minutes)
Introduction to the Department of Community Medicine	To understand as to why they are posted to community medicine: its importance vis-a-vis community health	Lecture	60
History of public health, changing concepts of health	To understand the evolution of community medicine To define health and factors affecting it	Lectures using story line	60

Cont... **Table 1: The schedule designed for the orientation program based on areas stressed by the university curriculum**

Principles of Primary Health Care	Enunciate the principles and elements of primary health care	Interactive lecture, Question & Answer sessions	60
Health Care Delivery System (Principles, Services available and utilization of health care services)	Describe the health care delivery system	Interactive lecture, Question & Answer sessions	60
Group work & Discussion: Health Care Delivery System: PHC Health Care Delivery System: Taluk Hospital Health Care Delivery System: Tertiary Hospital	Describe the organization and functions of the health care team at Primary Health Centre, Community Health Centre and District levels.	Lecture Group work & Discussion Pre-visit briefing Visit to health centres/ hospital	120 for group discussion
Demographic trends, cycle	Understand the demographic pattern of the country and fertility	Lecture	60
Fertility and factors affecting it		Interactive lectures	60
Family welfare and population control		Interactive lectures	60
Hospital sociology, doctor patient relationship, ethics	The principles of practice of medicine in hospital and community Setting including medical ethics	Role play	120
Social factors influencing health- Socio economic status, cultural factors	Social determinants of health, disease and disability in the context of urban and rural societies	Lecture, Question & Answer session, MCQs	60
Urbanization and its impact on health	Appreciate the impact of urbanization on health and disease	Lecture	60
Beyond hospital- reaching out to the community	Understand the principles of practice of medicine in hospital and community Setting including Medical ethics Develop rapport with the community using various participatory tools	Interactive lectures	60
Health communication	Describe the principles and practice of health education and to apply appropriate communication skills to bring about behavioural change in the community.	Intra session activities	90
Field visit and Presentations on visit to the Community	Observe and interpret the dynamics of community behaviour	Health education in the field by students	240

The orientation program incorporated various teaching methods to address the diverse needs of students. Some of the sessions had lecture classes. Story lines comprising the various historic changes in the public health field were used to teach the changing concepts of public health. Interactive lectures comprised of theory lectures and question and answer sessions in between and using probe questions to elicit answers of already known facts from students. Small group discussions were done before field visits on the various levels of health care to ensure that they had sufficient knowledge of the services available, thus enabling them to have a better understanding of the different health care systems during the visits. Through role play students were able to understand doctor patient relationship and medical ethics. Intra session activities

included giving students an information and asking them to communicate among their peers involving different methods of communication and encouraging them to come out with the challenges and barriers they had come across in doing the activity.

Students conducted supervised health education sessions in the community using various audio- visual aids like flip charts and posters.

Student's feedback and assessment was done at the end of the posting. Feedback was obtained using self-administered questionnaire which assessed their understanding of the subject using likert scale (greatly improved, slightly improved, no change) and understanding the factors which facilitated or hindered learning using open ended questions. Student assessment was conducted by administering a 20-item MCQ test which was aligned with the objectives.

The data are presented as frequencies and proportions. Spearman's correlation was done to find out the association between program feedback scores and student assessment scores.

106 students (includes males and females) who completed the feedback and the assessment test were included in the study. Sampling method was universe.

RESULTS

Of the total 150 students, 106 students completed program evaluation questionnaire.

Table 2 Shows the various teaching techniques used and the understanding of the sessions based on student's feedback

	Teaching-learning methods	Understanding about the topic/concept (n=106)		
		Improved greatly (%)	Improved Slightly (%)	No change (%)
1	Field Visits	79.3	19.5	1.2
2	Intra-session activities	73.7	24.4	1.9
3	Student Presentations	66.4	31.7	1.9
4	Used story line	61.9	32.4	5.7
5	Role plays	60.6	35.6	3.8
6	Group discussions	63.9	30.9	5.2
7	Interactive lectures	56.6	36.8	6.6
8	Lectures	32.1	50	17.9

Table 2 shows that 61.5% of students said that the program had greatly improved their understanding of the topics; 32.8% said that their understanding improved slightly and 5.7% said that the program made no change in their understanding of the subject.

Individual sessions feedback was analyzed based on the mode of teaching-learning used. The result showed that field visits (79.3%) and intra-session activities (73.7%) had helped in improving their understanding greatly and lecture mode of teaching (68%) was perceived by majority of students as a session that had slight or no change in the understanding of the subject.

Table 3 shows based on the assessment students scored highest on topics that used activities in intra-session activities (95.3 %), field visits and group discussions (93.5% each) followed by role plays (91.6%).

Table 3 Shows the comparison between student's feedback and the Assessment scores obtained (n=106)

	Teaching-learning methods	Improved greatly (% of students who responded that their knowledge improved greatly)	Assessment scores (% of students who gave correct answer)	Correlation between Program evaluation and Assessment Score (significance)
1	Field Visits	79.3	93.5	**0.778***
2	Intra-session activities	73.7	95.3	
3	Student Presentations	66.4	77.6	
4	Used story line	61.9	86.0	
5	Role plays	60.6	91.6	
6	Group discussions	63.9	93.5	
7	Interactive lectures	56.6	75.7	
8	Lectures	32.1	49.5	

* - significant (p<o.o5)

There was a significant correlation (r=0.778) between the student's feedback and assessment scores as shown in table.3

Table 4: Leading factors that were mentioned in feedback regarding facilitating and hindering factors of the orientation program

Facilitating	Hindering
Field visits Interaction with the community Teaching with videos	Lecture classes Lengthy duration of theory classes

DISCUSSION

Our study shows that based on student feedback, active forms of learning like field visits which provided opportunities for experiential learning, intra-session activities and student's presentation had greatly improved students understanding of the concepts. It also showed that lectures had played a minimal role in improving their understanding of the subject. This shows that students prefer to be active in their learning process. Active learning processes are desirable for many students as it helps them attain the learning outcomes. [10] Studies among undergraduate medical students have showed that interactive teaching styles are more popular than didactic lectures; and that the least preferred teaching method was didactic lectures.[11,12] Our study results are in agreement with the findings from these studies.

Our study also showed that teaching techniques rated as 'greatly improved' their understanding based on student's feedback had also scored well in the assessment test. But techniques which were rated as 'not greatly improved' their understanding based on student's feedback had actually scored high in the assessment test scores. Role play and group discussions which were rated by 40% of the students as 'not improved' their understanding greatly had actually resulted in more than 90% of them scoring well in those topics. A probable explanation would be that the task of preparing for class outside class hours and stage fear as mentioned by students in the feedback would have given a subjective feeling of rating them as not so good an option but had actually led to improvement in knowledge.

Similar studies have shown that techniques which involve greater effort from the student's side had led to attainment of learning objectives. However for these methods students lack the enthusiasm because of the comfort from lectures. [13] Also studies show that when student's presentation or participation is there, the anxiety is more among students.[14] So these would have been probable causes for students not giving a very positive feedback on methods like role-play but had actually made them attain their learning objectives.

Our study also showed that feedback on 'factors that facilitated their learning', most of the students have

quoted that field visits greatly improved their learning, and factors like lectures and long theory sessions have hindered their learning process.

The study also shows a significant positive correlation between student's feedback and the assessment scores. This brought out the fact that student's feedback is not only an effective evaluation tool to assess the teaching program; if properly designed it can be used effectively as an assessment test for evaluating student's understanding of the subject.

Overall, the findings of this study suggest that for community orientation program in undergraduates, active teaching methods or opportunities for active learning are greatly accepted by students and had helped them attain their learning objectives. Students learn best by field visits, and activities in between theory sessions have helped students be active in their learning process. It also reiterates the fact that anonymized student's feedback is an effective way of evaluating teaching sessions.

Though role plays and group discussions have not been perceived by students to improve their understanding it actually had resulted in attaining their learning objectives. Lack of enthusiasm and additional efforts needed from students could be a reason; further research is needed to understand why students haven't perceived those methods to improve their understanding though it actually had.

Our study used student's feedback which was subjective and the feedback though anonymous was collected by the department faculty which could have influenced the results.

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REFERENCES

1. Chastonay P, Brenner E, Peel S, Guilbert JJ. The need for more efficacy in medical education. Med Educ 1996;30:235-238.
2. The Consensus Document. Global Consensus for Social Accountability of Medical Schools. [cited 2017 Dec 2]. Available from: www.healthsocialaccountability.org
3. Jones R, Higgs R, de Angelis C, Prideaux D. Changing face of medical curricula. Lancet 2001;357:699-703.
4. World Health Organization. Re-Orientation of Medical Education. New Delhi: WHO SEARO; 1991. [cited 2017 August 5]. Available from: http://apps.searo.who.int/PDS_DOCS/B0085.pdf
5. Zhenhui R. Matching Teaching Styles with Learning Styles in East Asian Contexts. The Internet TESL Journal. 2001;7. [cited 2012 Apr 30]. Available from: http://iteslj.org/Techniques/Zhenhui-TeachingStyles.html
6. Yoder J, Hochevar C. Encouraging active learning can improve students? performance on examinations. Teach Psychol 2005;32:91-95.
7. Badyal, DK, Bala S, Kathuria P. Student evaluation of teaching and assessment methods in pharmacology. Ind Jour Pharmacol 2010;42: 87–89. http://doi.org/10.4103/0253-7613.64502
8. Husain M, Khan S. Students' feedback: An effective tool in teachers' evaluation system. Int J Appl Basic Med Res 2016;6:178–181. http://doi.org/10.4103/2229-516X.186969
9. The Tamil Nadu Dr. MGR Medical University. MBBS Syllabus and Curriculum. [cited 2017 August 4]. Available from: https://www.tnmgrmu.ac.in/images/2017/syllabus%20and%20curriculum/mbbs/mbbs-yr3-syllab-02032017.pdf
10. Carpenter JM. Effective teaching methods for large classes. Journal of Family and Consumer Sciences Education 2006;24:13-23.
11. Costa ML, van Rensburg L, and Rushton N. Does teaching style matter? A randomised trial of group discussion versus lectures in orthopaedic undergraduate teaching. Med Educ 2007;41:214-217. doi: 10.1016/j.cppeds.2014.01.002.
12. Papanna K, Kulkarni V, Tanvi D, Lakshmi V, Kriti L, Unnikrishnan B, Akash S, Tejesh S, Kumar SS. Perceptions and preferences of medical students

regarding teaching methods in a Medical College, Mangalore India. Afr Health Sci 2013;13:808–813. http://doi.org/10.4314/ahs.v13i3.41

13. Hunt D, Haidet P, Coverdale J, Richards B. The effect of using team learning in anevidence-based medicine course for medical students. Teach Learn Med 2003;15:131-139.

14. Adib-Hajbaghery M, Aghajani M. Traditional Lectures, Socratic Method and Student Lectures: Which One do the Students Prefer? WebmedCentral MEDICAL EDUCATION 2011;2:WMC001746. doi: 10.9754/journal.wmc.2011.001746

Effectiveness of an 'Abridged Course in Medical Education Technology' based on Students' Evaluation of the Teachers' Performance

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ABSTRACT

Background: To sustain the standards of quality medical education, the faculty needs to be well trained in the medical education technologies, both traditional and innovative. Students are the real beneficiaries of all training programmes conducted for faculty development and they appreciate good teaching. Their involvement in all possible aspects of teaching and learning will go a long way in achieving the best outcomes.

Objective: To assess the effectiveness of an 'Abridged course in Medical Education Technology', for entry level teachers, based on the students' evaluation of the (teaching) performance of the teachers.

Materials and Method: An experimental study in which an educational intervention was done and the effectiveness of the 'Abridged course' was assessed. Teaching by the teacher trained through the 'Abridged course' formed the 'intervention' in the study. 'Evaluation of the teaching performance' by the students was the outcome studied.

Results: The performance of teacher as assessed by the students had a higher mean score (61.28, SD 9.8) for residents who underwent training in medical education technology, as compared to other residents (56.81, SD 9.2) with a p-value of <0.001.

Conclusion: This study highlights that an abridged course in teaching methodology for entry level medical teachers improves the quality of their teaching.

Keywords: *Medical Education Technology, Abridged course, Effectiveness, Teachers' performance, Evaluation.*

INTRODUCTION

Faculty members of medical colleges play an important role in the training and instruction of future doctors and are expected to address a wide variety of educational goals. Due to the complexity of effective teaching, medical teachers need to be able to deploy many teaching skills. There is a growing consensus now on the fact that, to sustain the standards of quality medical education, the faculty needs to be well trained in the Medical Education Technologies.

Pre- service teachers' training routinely takes place in the general education sector in many countries. However this is not widely practiced in the field of medical education. World over, efforts are made to make teachers more efficient in their assignments by giving them various forms of training.[1] To improve the

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quality of teaching, faculty development programmes exist in most educational institutions. More emphasis is now given to faculty development programs which include planned activities to enhance faculty members' teaching and education- research skills. The Minimum Requirements laid down by the Medical Council of India (MCI) mandates every medical college in the country to have a Medical Education Unit (MEU) for faculty development and curriculum improvement.[2]

We have developed an eight hour 'Abridged course in Medical Education Technology' adapted from the curriculum of the Basic Course of the Medical Council of India, and customized it to meet our requirements, with an intention to impart to the entry level teachers the knowledge, attitudes and skills that they require to carry-out their routine teaching assignments. In an earlier study, we evaluated the programme in a faculty group and found that it was effective in improving their knowledge, motivation to teach and practice.[3] Another important aspect of any educational program design is assessing program effectiveness. There are only a few published researches that demonstrate the effectiveness of such educational interventions.[6-13] Most studies have relied on indirect measures such as learner- satisfaction surveys or self-assessment by participants. Actual effectiveness of the training can be assessed only through studies on implementation of the learned concepts by the faculty and the impact of the same as gauged by students' learning. It is to be appreciated that students are the real beneficiaries of all training programmes conducted for faculty development. The current study made an attempt to assess the effectiveness of our 'Abridged course in Medical education Technology', for entry level teachers, based on the students' evaluation of the (teaching) performance of their teachers.

OBJECTIVE

To assess the effectiveness of an Abridged course in Medical education Technology, for entry level teachers, based on the undergraduate students' evaluation of the (teaching) performance of the teachers.

MATERIALS AND METHOD

Study setting: A private Medical college in Kerala which has undergraduate, post graduate and super specialty training with an annual intake of 100 MBBS students, 95 junior residents [those who join for their post graduate training (MD, MS)] and 35 Senior residents [those who enroll for super specialty training (DM, MCH)].

Study design – an experimental study in which an educational intervention was done to assess the effectiveness of an abridged course. Teaching by the teacher trained through the abridged course formed the 'intervention' in the study. 'Evaluation of the teaching performance' by the students was the 'outcome' studied.

Sample size and sampling technique: Six junior residents of community medicine were stratified according to their year of joining and were randomized into two groups. One group received an educational intervention and the other group served as control.

Intervention:

Step-1 - A curriculum for an abridged course in Medical Education Technology was designed to impart to the residents the knowledge, attitudes and skills they require to carry-out their routine teaching assignments. The entire course duration was eight hours. The course content included Systems approach to medical education, Teaching-Learning process, Principles of Adult learning, Self- Regulated and Self-Directed learning, Taxonomy and domains of learning, writing Specific Learning Objectives, Microteaching, Curriculum, Interactive and Innovative teaching methods and teaching aids, Clinical teaching including bedside teaching etc. Three out of the six residents, received the training based on this curriculum.

Step-2 – The residents who were trained undertook 234 student hours and the residents who were not trained 213 student hours of teaching respectively. The teaching sessions were of similar topics from the usually prescribed schedule of classes for undergraduate students of the department of Community Medicine in our institute.

Thus the trained residents' classes constituted the intervention-limb of the experiment.

Evaluation: The impact of the course on teaching abilities was assessed using outcome levels defined by Kirkpatrick which included reaction, learning, behaviors and results.[14] To evaluate the first level (K1) participants' responses regarding the impact of the training on them was evaluated using a researcher-administered feedback which captured qualitative descriptions of their reactions

to the course. In the second level (K2), the learning of the participants' was evaluated. A pre- evaluation was done using a self –administered questionnaire including closed and open-ended questions to assess the cognitive, affective and psycho-motor components of the Teaching-Learning process in medical education technology. A post- evaluation was done using the same questionnaire after one week of the completion of the training. The questionnaire was concurrently administered to both groups of residents and the results of this evaluation were compared.

In the third level (K3), the participants' behavioral changes (with reference to teaching) were measured. This was the objective of the present study. For this the "trained" and the "untrained" residents were given teaching assignments on similar topics to teach the same batch of MBBS students. The topics were allotted to residents by lottery method. Thereafter, students, who were blind on the intervention, were asked to evaluate the teaching of the individual residents, using a structured evaluation format at the end of each session. This format drew ideas from the standardized format used in micro-teaching exercises used by the Medical Council of India's Regional centres and others. (This format was discussed with the faculty of our centre and accepted and consisted of 14 points which the student needed to rate on a Likert scale of 1-5).Thus the participants' teaching behaviour as evaluated by the under graduate students were compared with the control group residents.

The fourth level (K4) involves evaluation of the program's long-term impact on the learners' learning outcomes which will be the reflection of the results of the training. This evaluation was not one of our listed objectives (because data collection would be complex, time-consuming and costly). However a pre and post test (maximum 10 marks, multiple choice questions) to assess knowledge gain among students was conducted for each session, to study student's cognitive gain after the session. This took care of possible ethical issues also.

Analysis: Data were entered into Excel sheet and analysed using Statistical Package for Social Sciences software for Microsoft Windows version 20**.** Pre and post test scores of residents were described using mean and standard deviation. Scores were calculated for teaching merits of residents based on the responses from students and were compared using independent sample t-test. The students' pre and post-test scores were analysed to look for differences if any using paired sample t- test.

Ethical Consideration: Ethical clearance was obtained from the Institutional Ethics Committee.

RESULTS

The K1 level evaluation was based on the participant feedback. The feedback given by the trained residents was very encouraging and positive. Participants who underwent the course were "impressed with the workshop" and expressed their "willingness to introduce and implement these methodologies in their day to day teaching/learning exercises."

The comparison between the pre and post scores of the trained residents helped in assessing the K2 level. The mean knowledge score in teaching methods among junior residents prior to and after intervention was 2.33 (SD 0.57) and 8.67 (SD 4.16) in the intervention group while the figures were 2 (SD 1) and 1.67 (SD 0.57) for the control group respectively.

The evaluation of K3 level was the objective of the current study. The performance of teacher as assessed by the students had a higher mean score (61.28, SD 9.8) for residents who underwent training in medical education technology, as compared to other residents (56.81, SD 9.2) with a p-value of <0.001. (The result is shown in Table 1.)

The pre and post-test scores obtained by students were compared using paired t test. There was statistically significant improvement in knowledge among students taught by both 'trained' and 'untrained' residents. The mean score difference in the pre and post test scores being 4.1 in the trained residents' classes and 3.76 in the untrained residents' sessions with a p- value of <0.001 in both the groups. (Table 2)

TABLE 1: Performance of Teachers as Assessed by the Students

	Total number of responses from the students who attended the class	Mean scores	Standard Deviation	p value
Trained residents	234	61.28	9.8	<0.001
Untrained residents	213	56.81	9.2	

TABLE 2: Pre and Post Test Scores of Students

	Number of students attended the class	Mean scores	Standard Deviation	p value
Sessions by Trained residents Pre Post	234	5.13 9.03	1.4 1.1	<0.001
Sessions by untrained residents Pre Post	213	3.19 7.35	1.5 1.8	<0.001

DISCUSSION

Our study supports the evidence that abridged courses in Medical education technology for faculty development enhances their teaching skills. Many previous studies that were reviewed report similar outcomes as gains in knowledge, changes in attitudes, satisfaction with the program, and self-report of changes in behavior. However, many of these published studies lacked a control group.[6-13] Our findings are important because we have included a comparison group, and have evaluated the intervention from students' perspectives which will increase the validity and reliability of our findings.

The results of a systematic review of 53 faculty development programs found that such programs resulted in high levels of satisfaction and changes in attitudes, knowledge, skills, and teacher behaviours.[15] The review also highlighted the fact that participants consistently found such programs acceptable, useful and relevant to their objectives. Participants who underwent our course also were "impressed with the workshop" and expressed their "willingness to introduce and implement these methodologies in their day to day teaching/learning exercises."

In the K2 dimension, the pretest and posttest results showed that there were significant increases in participants' knowledge and noticeable differences between study and control group. These findings are also consistent with the findings of the systematic review.[15] The elevated post-test marks among the entry level teachers signify the immediate effectiveness or usefulness of the training with regard to their learning.

In the K3 dimension, we obtained students' evaluations of the trained teachers, and compared it with teachers who were not trained using the Abridged course in Medical Education Technology. The results were positive and showed statistically significant improvements in formers' teaching skills, but no significant improvements in the latter's, as judged by the students. This result is evidence of the positive impact of the program on teacher- effectiveness as a result of changes in their teaching behavior/ performance. Positive changes observed by students in the intervention group is a major impetus to have a pre-induction faculty training program in medical colleges/ schools. Teachers who receive some form of systematic training definitely perform better and this is appreciated by the students. This can also catalyze changes that make them better learners.

The improvement in teaching skills alone may not necessarily bring about immediate changes to student learning. While it is rational to believe that improving the pedagogic skills of (entry level and other) teachers promotes learning by medical students, there is only limited evidence suggesting that the effectiveness of teaching perceived by students can positively affect their performance and learning outcomes. Our study has shown an increase in knowledge gained by both group of students taught by 'trained' as well as the 'untrained' teachers. Many perceive medical students as persons with capacity to adapt and self-motivate, likely to possess the ability to achieve assessed learning outcomes, regardless of the teaching they receive**.** However we contend that organized training in medical education technology will make the teachers' roles more effective.

According to all the dimensions that we evaluated, the program had a positive impact on teaching abilities. Effect of randomization was uncertain due to the small

numbers. Potential confounding factors need to be considered which could be intrinsic to student participants or extrinsic related to the resident teachers. Despite these limitations, the study findings are highly relevant. This study highlights that an abridged course in teaching methodology for entry level medical teachers improves the quality of their teaching. The changes in teaching behaviour have been appreciated by the students also. This in turn should help the student/ learner in achieving short and long-term goals. Directions for future research should include an understanding of how to accurately and reliably evaluate the result of the training as regards improved student learning i.e., the desired final outcome. We conclude that faculty development programmes will promote high quality of teaching and must be an essential activity of every medical school. Therefore designing formal approaches to achieve the goals in this area is recommended.

Conflict of Interest: The authors have no conflict of interest to declare.

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Ethical Clearance: Ethical clearance was obtained from the Institutional Ethics Committee.

REFERENCES

1. Allen C. In - Service Training of Teachers. Review of Educational Research. 1940;10(3):210
2. Vision 2015[Internet]. 1st ed. Medical Coucil of India;2011 [cited 10 September 2016]. Available from : http://www.mciindia.org/tools/anouncement/MCI booklet.pdf
3. John A, Joy TM, Paul N, Javed R, Sasidharan A, Menon UK. Effectiveness of a 'Short training in Teaching Methodology' for entry level Medical Teachers. Indian Journal of Community Health 2016; 28 (3):300-4
4. Cox M. Cultural Responsiveness and Motivation in Preparing Teachers. Saarbrucken:LAP LAMBERT Academic Publishing;2011.
5. Bland CJ, Froberg DG. A systematic approach to faculty development for family practice faculty. J Fam Pract. 1982 Mar;14(3): 537-43.
6. Sheets K, Henry R. Evaluation of a faculty development program for Family Physicians. Med Teach. 1988;10(1):75-83.
7. Johnson D, Zammit-Montbello A. A new faculty training model for countries developing academic family medicine programs. Academic Medicine.1990;65(5):346-8.
8. DeWitt T. Developing Community Faculty. AM J Dis Child.1993;147(1):54.
9. Pololi L, Clay M, Lipkin M Jr, Hewson M, Kaplan C, Frankel R. Reflections on integrating theories of adult education into a medical school faculty development course. Med Teach. 2001;23(3):276-283.
10. Gordon G, Levinson W. Attitudes toward learner-centered learning at a faculty development course. Teaching and Learning in Medicine. 1990;2(2):106-109.
11. Skeff K. Improving clinical teaching. Evaluation of a national dissemination program. Archives of Internal Medicine. 1992;152(6):1156-1161.
12. Rawer H, Morton A, McCulloch R, Heyes L, Ryan J. Delivering training in teaching skills to hospital doctors. Med Teach. 1997;19(3):209-211
13. Nagdeo NV,Chari S.Basic Workshop for Medical Teachers: Effectiveness and Impact. NJIRM.(2014) [cited 10 September 2015];5(2):107-114.
14. Kirkpatrick D. Evaluating Training Programs. San Francisco: Berrett-Koehler;1994
15. Steinert Y, Mann K, Centeno A, Dolmas D, Spencer J, Gelula M et al. A systematic review of faculty development initiatives designed to improve teaching effectiveness in medical education: BEME Guide No.8 Med Teach. 2006;28(6):497-526.

An Economic Analysis of Small Scale Industries in Pudukkottai District (Tamil Nadu)

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ABSTRACT

The important role-played by Small Scale Enterprises in promoting the economic development of India in recent decades especially in providing more jobs for rural people and bringing about a more equitable distribution of national income has been well recognizedWhile the majority of small enterprises depend on retained profit and informal source of credit for expansion, with growth in size, the need increases rapidly for external finance for working and fixed capital. In recent years, following the successful example of Japan, the Republic of Koria and India, in mobilizing and channeling finance to SSI,many schemes are experimented with a wide array of new financial institutions, instruments and innovations. The significant role that this sector plays in the development strategy of India's economy is not only based on the fact of its being, labour-intensive and capital saving in character but also in 3 areas namely Production, Employment and Export.

Keyword: *Economic Development, Working Capital, Production, Employment and Export.*

INTRODUCTION

Generally a dry and hot climate prevails in the district(Pudukkottai District) which is not favorable for agricultural sector.So agricultural sector is inadequate to provide livelihood to the entire working force. On the other hand, the establishment of large- scale-industries cannot solve the problem of seasonable unemployment found in India,which is solved by SSI sector only. More-over the growth of capitalist enterprises and concentration of the means of production in few hands have led to the emergence of inequalities of income and wealth. So SSI units are ideal instruments for decentralization of industrial production is achieved especially in Pudukkottai District.

PudukkottaiDistrict, formed in January 14, 1974 out of certain pockets of the then Trichy and ThanjavurDistricts, has on area of 4663 Sqkm with a coastal line of 39 Kms. The District lies between 78.25 and 79.15 of the eastern longitude and between 9.50 and 10.40 of the northern latitude. It is bounded by Trichy District in the north and west, Sivagangai District in the south and Bay of Bengal in the east. Pudukkottai District is economically, industrially and educationally a backward district. Both the Central and State Government have declared it as an economically backward district. In order to promote the industrial scene in such backward areas both the Government of India and the Government of Tamil Nadu provide so many concessions and incentives to SSI units through District Industries Centre, which is located in Collector Office,TrichyRoad[1].

OBJECTIVES OF THE STUDY

The potential role of small-scale industries in Pudukkottai District during the period 1995-2000 is examined here. The present study is an analytical and descriptive one, based on a survey of One Hundred Small Scale Industrial Entrepreneurs.One Hundred Entrepreneurs were selected out of (5441) Five Thousand Four Hundred and Forty One units on the basis of random method of sampling. A preliminary sketch of the growth of Small-Scale Industries in India and Tamil Nadu in general and Pudukkottai District in particular is presented. The SSI units Pudukkottai District have classified into three categories.

1. **Agro-based processing units**-Food, Rice Mill,Wood,Oil and Rubber, Coir Units(Total 30 units).

2. Industry- based production units-Gem Cutting,

Paper, Aluminium,Metal Products, EngineeringGoods, ElectricalUnits (Total 40 Units)

3.Business based units – Beverages, Pan (Beeda), Xerox, PrintingPress (Total 30 units),Gross Total 100.

Among the sample units selected at random, Industries manufacturing Aluminium,Paper,Plastic,Rubber,Chemical,Coir,Metal Products are found in large numbers in this study area of the One Hundred sample units, Fifty Two units are run by sole proprietorship and Forty Eight units are run by Partnership concerns. Sole Proprietorship is preferred more in Pudukkottai District[2].

(A) MAJOR OBJECTIVES

1.To find out the Capital Labour ratio of small-scale units.

2.To know the degree of capital output ratio of SSI units in Pudukkottai District.

3.To access the extent of capacity utilization of SSI units in the study region.

4.To analyses the structure and performance of small-scale industries.

5.To study the problems faced by various small-scale industrial units.

6.To identify the factors retarding the growth of small-scale industrial units in the study area and

7.To understand the sickness of Small Scale Industries in the study area.

HYPOTHESIS

The following hypotheses were formed.

1.The Credit given for the operations on development purpose is more useful than loans given on a general basis.

2.There is a relationship between the traditional background of the owners and entrepreneurs and the growth of small-scale industrial units.

3.The borrowings from commercial banks have paved the way for the growth of SSI units

4.The financial assistance to SSI from Central and State Government is inadequate.

5.The growth of productivity is positively associated with growth in input.

6.The manufacturing sector is not experiencing technological change.

7.The factor productivity and capital intensity are inter-related.

METHODOLOGY

Researchers collected both secondary 1990-95 period and primary 1995-2000 periods of data's:

The primary data were collected through questionnaire from the entrepreneurspersonally, particulars required to fulfill the objective of the study were asked in the questionnaire.

The District Industry Centre and the Commercial Banks given the Secondary Data. For further informations,books,journals,magazines and other relevant material were referred too. The data collected from various sources were transcribed in to a master table to facilitate easy tabulation;percentages and ratios were worked out.Tools like the Granger test,Sims test and modified Sims test were also used to analyse the data.

The study covers the SSI Units registers with the District Industry Centre,Pudukkottai District during 1995-2000.Among the total units of 5441, 2290 SSI Units are sick and some are closed.The remaining 3150 units have been found to be healthy units and are efficiently operating during the period 1995-2000.Researchers had taken 100 units at random and have found that 42units are sick in Pudukkottai District.

The researchers had also used secondary data through the Reports of Finance, Government of India,National Planning Commission, Reports of Ministry of Finance, State Planning Commission of Tamil Nadu,IOB Annual Credit Plan Reports,Articles of journals,Research papers.The published data of SIDCO, TIIC have been used and have been referred to[3].

CHAPTERISATION OF THE STUDY

The study is presented in seven chapters.

First chapter deals with introduction.In the Second chapter early studies are reviewed many studies on this subject undertaken by researchers have brought

to light,the innumerable difficulties that the Small-Scale entrepreneurs have had to face in running their enterprises in India

The Third chapter deals with objectives, hypothesis,methodology and there is a discussion on the profile of the sample units in Pudukkottai District. Chapter Four discusses land factors for SSI.Chapter five deals with problems of SSI units and strategy for resolvingsuch problems. In Sixth chapter, there is a discussion and proof with statistical models.In chapter Seven findings,suggestions and conclusion are presented.

The study shows how much capital invested in every SSI units,working capital,value of production,reason for low production,estimates of cost of production,number of workers employed in each SSI units,mode of employment of workers. Marketing conditions,they too are experiencing many constraints,such as shortage of working capital and raw material,poor infrastructure facilities and irregular power supply.

LIMITATIONS OF THE STUDY

1. The study does not cover the institutional assistance for sick units of SSI.

2. Further this study does not cover the performance of some Central Government Institutions because of the scarcity of data.

3. Researchers could not receive Secondary Data sources from PudukkottaiTamiI Nadu Industry Investment Corporation Office directly;only data gathered through Annual Credit Plan Report published annually by IOB (Lead Bank)Pudukkottai District, is available.

4. The Researchers were not able to collect more as well as correct information from the respondents.Their ignorance and inexperience are stumbling blocks for the data collection.

5. The study is related to private SSI units only.No public sector units were considered in the process.

ANALYSIS OF THE CHAPTER.

The total population of the district as per 1991census is Thirteen Lakhs,Twenty Seven Thousand and One Hundred Forty Eight (13, 27,148) of which males and females are almost equally divided.The total population of the district as per 2001 census is 14, 52,269 of which male's population was 7, 20,847,Female population was 7,31,422. According to 2001 census, Female population was more than Male population in Pudukkottai District.

The study is confirmed to pudukkottai district,which is an industrially backward area in Tamil Nadu and it is now slowly developing into industrialized district of the state.This district has got 5441 registered SSI units (99-00), medium large-scale industries-34, Handicrafts units-2328 and Pudukkottal district is having 9 Taluks and 13 blocks. There is on SIPCOT industrial complex in the district 6km from head quarters with an area of 412acres and 76 plots are available here which is located Trichy road,the extension of Cauvery water for the industrial units in the estate is planned[4].

The Tamil Nadu forest plantation corporation had also drawn up a detailed scheme for replacing the old fielding cashew plantations in the district with high yielding varieties. The TNFPC has brought over 1000 hectares of land eucalyptus and many of land under and many private farmers were also keen on taking up eucalyptus plantations.So that related industries could come up.

Out of the 18 institutions 3 banks have shown excellent performance by achieving 100/- target PNB-174% achievements,Bank of India 136% achievement,ICICI – 125% achievements.

REVIEW OF BANK PERFORMANCE -1995-2000

Target-144.29 Crores93%

Achievements-134.83 Crores

Of the One hundred units selected for this study fifty three percent are located in Pudukkottai and seventeen percent in Mathur and MandiyurnearbyTrichy. Out of the one hundred SSI industries collected through random sampling method during the period of 1995-2000. Pudukottai proper town having 53 units of SSI units Alangudi 6 units, Aranthangi 6 units, Ponamarravathy 1 unit, Mathur, Mandiyur 17 SSI units, Keeranur 5 units,Viralimalai 2 units,Kottaipatinam 2 units, Athanakootai 1 unit, Tirumayam 2 units, Kandarvakottai 1 unit,Annavasal 4 units. Total 100 units were collected by researchers through proper questionnaire.

The location of the majority of the sample units in Pudukkottai town and Mathur block must be attributed to the pioneering role played by these blocks in the Industrial Development of the District and the availability of infrastructural facilities in these two centers.

RESULTSTHEORETICAL PROVED

1.The credit given for specific operations on development purpose is more useful than loans given on general basis.

One of the striking findings of the present study is the fact that the Credit for specific operations is 95 of the entrepreneurs have taken to SSI as a since of profitability, lessrisk, priorexperience, self employment,5 units have been started with the only aim of profitability earned from SSI units. Earning more profit is more useful and it is specific operation than other purposes that is less risk,prior experience,and self employment-as a general basis.

2. There is a relationship between the traditional backwardness and social background of the owners and the growth of SSI.

People in the age group between40-60 are continuing (i.e.) 56 units in Pudukkottai District) the work of their ancestors professions. This hypothesis is proved. Backward Caste people have some political influence through which they are dominating SSI units and Scheduled Caste people did not shine inSmall Scale Industrial units due to poverty,ignorance and the apathy shown by officials too. This hypothesis is also well proved[5].

3. The borrowing from commercial banks and the growth of SSI units.

In Pudukkottai District,commercial banks were established for improvement of SSI sectors particularly 72% of loans given to SSI units during the period of 1995-2000 by commercial banks,especially(IOB, SBI BANKS) had been well proved the financial strength of commercial banks than other sources of finances.This hypothesis is proved.

4. The State Government financial assistance to SSI units is very poor.

Only 8% of the loans were given to SSI units by the State Government.This hypothesis is also proved.

5. Statistical Proved

The growth of productivity is positively associatedwith growth in input:-Emerging input likecapital structure from this study according to SSI development in this district has also been worked out.It is presumed that greater the K/O ratio of output, the greater will be need for fixed capital and the sizes of output are highly correlated.For different industries in Pudukkottai District,it is that input that is investment and output are highly correlated.It is found that capital investment and output are highly correlated,as rank correlation is 0.70 where as capital investment and output at different levels are found 0.72.It means that at district level size of total capital employed plays important role for generation of income through value of output proceeds due to location choice of SSI units in Pudukkottai District[6].

6. The manufacturing sector is not experiencing technological change – it is proved.

Capital productivity in PDKT District - 1.082%

Capital Intensity - 3.296%

Raw Material Productivity- 1%

But Labour Productivity - 4.487% (per year)

7. Capital intensity and the factor productivity are inter-related

This hypothesis is disproved

Rs.26, 000/- will be required to employ one labourer annually in 1981.This ratio is higher than the other states. Rs.18, 418 will be required to one labourer in other states in the same year. Recently between 1995 and 2000 O/K,ratios shows productivity of capital. It shows how much output is produced by a unit of capital. Sample units of PDKT Districtshows O/K ratio is 0.84 i.e. Rs.84, 000/- will be required to a unit of output which is higher than All India level ratio of 0.15 i.e. Rs.15, 000/- will be required to a unit of output or registered manufacturing sector.

FINDINGS AND SUGGESTIONS

1.Government Liberalization, Globalization, Privatization policies are not favorable to SSI Units in PDKT District.

2. TNEB levied heavy power rate for SSI,which is very heavy to bear.

3. Most of the units in SIPCOT complex are very sick due to low power supply mismanagement,lack of working capital, power shortage, and marketing problems.

4. Lakshmi Shanmuga Spinning Mill,NamanaSamuthram which arelocated in Pudukkottai district is sick due to rise in raw material prices, heavy interest rates levied by banks according to Board of Industrial Financial Re-construction. Only remedy is government must take over all the important,SSI units with strict Government order.

5. Central exercise duty exemption should be granted to SSI and tiny sectors[7].

CONCLUSION

No doubt if the SSI meets challenges with determination,innovation and hard work they can even compete with foreign products also which are chapter even after levy of duties. The lowering of interest rates-on their (SSI Units) borrowings to 5 or 6%, by a sympathetic attitude on the part of nationalized banks to those sick units in particular and by greater co-ordination among Government banks and other financial institutions and the entrepreneurs and the researchers portrays many invaluable ideas to develop the economically backward district.

Ethical Clearance: Yes

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. Dhar, P.N "The Role of Small Scale Enterprises in Indian Economic Development",Delhi 1962, P.12
2. Lakshmi NarasaishM.DeevenaMurgarot, "Development of Small Scale Industry", Discovery Publishing House, Vyas, JN, SSI,Hand Book,Ahamadabad-1986 P.5.
3. Annual Credit Plan Reports,Lead Bank, Pudukkottai, 1993,1994,1996,1997,1998,1999,200 0. Kurien C.T, "Rethinking of Economics'', New Delhi, 1996.
4. Dandekar V.M. "The Indian Economy" population, poverty and employment,New Delhi, Vol.2, New Delhi, 1996.
5. Kuchhal SC, "The Industrial Economy of India" Chittanya.Hindu January 2, 1982, February 12, 13
6. Kurien C.T, "Small Sector in New Industrial Policy" in Uma Kapila(ed) op.ct.1996.

Role of Microfinance and Women Empowerment in Madurai District, Tamil Nadu

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ABSTRACT

The key elements in empowerment are enabling and providing power and they reinforce each other. Women's empowerment is a multifaceted concept that extends to the economic, social, cultural, political and psychological aspects of women's life. The major objectives of the study are to study the socio economic impact of women SHGs on group members and to know the role of SHGs in providing rural credit. Total 50 women were interviewed. 10 SHGs of were selected by simple random sampling method. As far as the selection of group members is concerned, five members were deliberately selected from each group. The study is purely descriptive. In order to achieve the objective of poverty eradication, every poor person should have easy access to credit for starting small economic activities based on local resources. However by 1970, the limitations and the critique of the welfare approach became obvious and resulted in the development of a number of alternative approaches based on equity, poverty alleviation, efficiency, and empowerment. Microfinance is not merely a financial business, but is a strategy or tool for empowerment of women particularly the poor. The microfinance concept when coordinated with the SHG lending system, those people who have been deprived of credit, could have their fair share of credit.

Keywords*: Self Help Groups (SHGs), Micro Finance, Women Empowerment, Credit, Poverty Eradication, Economic development.*

INTRODUCTION

The Concept of women empowerment is defined as the process by which women take control and ownership of their choices. The core elements of empowerment here have been defined as agency (the ability to define one's goals and act upon them), awareness of gendered power structures, self-esteem and self-confidence. Empowerment can take place at a hierarchy of different levels individual, household, community and societal and it is facilitative by providing encouraging factors (e.g. lack of resources and skills)[1]. In this connection micro finance with self-help groups play an effective role for promoting women empowerment. Indian women have to be made as an integral part of the socio economic structure of the society. The social values, norms, which influence social expectations regarding the behavior of women, will determine a role and her position in society to a great extent in the process of changing era. Changes have been taken place in the position of Indian women as a part of the process of transformation of the traditional society[3]. The situation of women actually has changed completely in the last thirty years. In the beginning of 1970s; women were behind in the field of development. There were no changes for the empowerment of women. In the Third World both rural and urban women were playing a significant role in the activities of managerial decision making. Self-help groups have been instrumental in the empowerment of women by enabling women to work together as a collective agency[5]. Women command few economics resources and frequently rely on family or male members. However, self-help groups, when combined with savings and credit, have enabled women to benefit economically by monetizing their contribution and in the process have empowered them to become agents of change. Another related aspect is that self-help groups have facilitated the formation of social capital where people learn to work together for a common purpose in a group or organization. Women learn to work for a common purpose in a group or organization. They learn to work for a common purpose in self-help groups leading to the formation of social capital. Although women form nearly half of the human capital, they are still the most deprived and neglected segments of the

society despite the constitutional guarantee, and for equal rights and privileges for men and women. Women constitute to be the victims of a process of economic, social, cultural and political marginalization[2]. While a large part of the world countries look at women's issues in terms of paternalism and well-being, the concept of women's empowerment in the social, political, and economic order as a pre-requisite of human development is hardly given the priority that it deserves.

THE CONCEPT OF MICROFINANCE AND SELF-HELP GROUPS

The concept of SHG which has been gaining significance especially in area of micro-finance is mainly the off-shoot of the success of group based approach to savings and credit for the rural poor followed by the Grameen Bank of Bangladesh. The group based approach not only enables the poor to accumulate capital by way of small savings but also, and more importantly, helps them to have easy access to formal credit facilities[7]. These groups by way of joint liability enable the poor to overcome the problem of collateral, which is a major hurdle in gaining access to institutional credit. The joint liability not only improves group member's accessibility to credit but also creates conditions or mechanisms like peer monitoring, leading to better loan recoveries. Formation of Self-Help Groups is the prime-mover as well as integral part of microfinance based credit delivery system and involves the following three basic steps:

- Group formation (formation, development and strengthening of the groups to evolve into self-managed people's organizations at grassroots level);
- Capital formation through the Revolving Fund, skill development (management skills for management of their organizations as well the activity); and
- Taking up economic activity (micro-enterprises) for income generation.

Microfinance refers to the supply of loans, savings and other basic financial services to the poor. It envisages mobilizing thrift and supplementing it with loan from the financial institutions. By providing access to financial services, microfinance plays an important role in the fight against the many aspects of poverty. Main objectives of microfinance are:

1. Poverty alleviation
2. Empowerment of women
3. Financial sustainability
4. Increased outreach and impact

OBJECTIVES OF THE STUDY

1. To study the socio economic impact of women SHGs on group members.
2. To know the role of SHGs in providing rural credit.
3. To study on status and impact of Micro finance in India.

METHODOLOGY

The present study focused on Madurai District and selected village in this District SHGs are functioning in the very success manner. The primary data were collected during the period January 2014 with the help of interview schedule. The schedule included related to general information about the SHGs members, socio demographic profile, income, expenditure, savings and loan schemes available to SHGs members.Total 50 women were interviewed.10 SHGs of was selected by simple random sampling method. As far as the selection of group members is concerned, five members were deliberately selected from each group. The study is purely descriptive. The researcher has undertaken every possible effort to fill up the gaps of information by conducting individual interviews.

SOCIO ECONOMIC IMPACT OF SHGS

Women self-help groups have been proving their significance in entrepreneurship development, marketing and active participation in social welfare campaigns, fighting against alcoholism, child marriages, dowry and superstitions. Saving is the principal activity of self-help groups. It was observed that after joining SHGs, the women members had become habitual of savings and depositing a certain amount regularly with their respective groups.

Table 1: Monthly Savings, Loan Purpose and Credit Facility availed by Responds

1) Monthly Savings	No. of the respondents	Percentage
Up to 25	13	26
26 – 50	24	48
51 – 100	7	14
101 – 250	4	8
151 and above	2	4
2) Loan Purpose	No. of the Respondents	Percentage
Business activity	12	24
Household needs	15	30
Festivals	4	8
House construction and Repair	7	14
Education	5	10
Repayment of loan	3	6
Other Purposes	4	8
3) Loan Amount (in Rs.)	No. of the Respondents	Percentage
Up to Rs. 500	2	4
501 – 1000	13	26
1001 - 2500	16	32
2501 – 5000	12	24
5001 – 10000	5	10
10001 and above	2	4
4) Loan Repayment	No. of the Respondents	Percentage
Regular	42	84
Irregular	8	16

Source: Field Survey, 2014.

As far as repayment of loan was concerned, 84 percent women members were regular at repayment of loan whereas 16 percent respondents were irregular. The major reasons of irregular repayment were use of loan amount for unproductive purpose, uncertain and inadequate income of the respondents.

The major aim of the SHGs is to promote savings and to credit for the productive purposes. This is true because many people in the study area join the SHGs for getting loan and promote their personal savings, in addition to get social status Table-1.The highest number of the respondents 30 percent had used loan amount for meeting household needs, such as, purchasing food grains, clothes etc. whereas repayment of loan was the main purpose in case of 6 percent women respondents.

RURAL CREDIT AND SHGS

One of the reasons for joining SHGs is to avail credit which is true in the present study area. The second objective of the present study is to know the rural credit by SHGs. The credit organizations like nationalized banks, Cooperative Societies and so on, follow many formalities to provide credit to the rural people. At the same time village money lenders change very high rate of interest. In this situation SHGs are the boon to the rural people, because instead of approaching banks individually, SHGs can easily approach the banks and other institutions to get loan. The SHGs get loan from credit institutions refinance (share) to the members in the SHGs. In the study the prevailing interest rate is 1 percent to 4 percent. Therefore members are repaid the loan in time.

Table 2: Types of Loans in the SHGs

Sl. No.	Types of the Loan	Maximum amount Rs.
1	Business Loan	20000 to 25000
2	Marriage Loan	Up to 20000
3	Repay the old Loan	10000 to 15000
4	Medical Loan	10000 to 15000
5	House repairing Loan	Up to 5000
6	Cattle Loan	5000 to 7500

Source: Field Survey, 2014.

Most of the respondents are received business loan and repay the old loan. Cattle loan are received by agricultural labour and famers. Marginalized people are received by house repairing loan in study area. All the SHGs are having a procedure to upper limit of loan. Table – 3 gives to maximum limit of loan amount in SHGs in study area.

Table 3: Amount of Loan Availed by the members through SHGs

Sl. No.	Amount of Loan	No. of respondents	Percentage
1	Less than 5000	8	16
2	5000 to 10000	10	20
3	10000 – 15000	12	24
4	15000 – 20000	13	26
5	Above 20000	7	14
	Total	**50**	**100**

Source: Field Survey, 2014.

Table 4: Repayment of Loan by SHGs members

Sl. No.	Amount of Loan	No. of respondents	Percentage
1	Repayment in time	31	62
2	Repayment in advance	11	22
3	Repayment not in time	8	16
	Total	**50**	**100**

Source: Field Survey.2014.

Moreover banks instruct the members to save minimum Rs.200 per month. So repayment is very easy to SHGs. The loans can be used by individual group members for their personal needs, sometime the group may invest on any economic activities. Nowadays many SHGs are starting small business, cottage industries, food processing units etc. The SHGs in the study are granting the loan to their member for various purposes. The maximum loan amount per members is decided by the personal by the general body meeting (Table 4). Almost the all members in the study areas are availing the loan facilitates SHGs.

STATUS OF MICROFINANCE IN INDIA

The microfinance initiatives of NABARD yielded remarkable success and the SHG bank linkage programme has emerged as the largest microfinance programme in the world. The pilot project started in 1992 has turned into a national movement leading to the socio-economic empowerment of women.

Table No. 5: SHG – Bank Linkage Programme 2013-14 : Physical

(As on 31 March 2010)

Total number of SHGs savings linked with banks	:	69.53 lakh
Out of total, [of which] exclusive Women SHGs	:	53.10 lakh
Out of total, [of which] -SGSY SHGs	:	16.94 lakh
Total number of SHGs credit linked during 2009-10	:	15.87 lakh
Out of total [of which] exclusive Women SHGs credit linked	:	12.94 lakh
Out of total ,[of which]- SGSY SHGs credit linked	:	2.67 lakh
Total number of SHGs having loans outstanding as on 31 March 2010	:	48.51 lakh
Of which exclusive Women SHGs	:	38.98 lakh
Of which-SGSY SHGs	:	12.45 lakh
Estimated number of families covered up to 31 March 2010	:	97 million

Source: NABARD, Annual Report 2013 - 2014

IMPACT OF MICROFINANCE

The micro-enterprises run by the SHG members through the microfinance provided improve their economic condition as follows;

- It enables the poor to take advantage of existing opportunities
- It builds up their assets
- It generates self-employment
- It develops micro enterprises
- It raises their income
- It builds up their self-confidence and self-esteem
- In enhances overall economic growth
- In enhances domestic savings and improves financial market
- It provides escape route from poverty

The benefits are not merely economic; they have also helped the women gain qualities of leadership; attain self-confidence, economic self-sufficiency, self-respect and concentrates on capacity building among women. The women undergo training programmes and discuss various issues that concern them and their village. They have become very articulate and enterprising. Microfinance will also give an access to support network thus enabling them to advance their individual and collective interests at local and macro level. Sustainable microfinance services lead to women's individual economic empowerment by stimulating women's micro-enterprise development and leading to increased income under women's control[10]. Women's control over these resources will then lead to increased well-being (health, nutrition, literacy, and housing) and reduced mortality rates for women and their children. Microfinance has the effect of increasing the political empowerment of individuals in a society. Microfinance can primarily strengthen political empowerment through two mechanisms. First, if microfinance improves the economic and the social conditions of the poor, the poor's political empowerment will consequently rise due to an increase in their self-efficacy. Second, in addition to increased self-efficacy, microfinance can also increase the social capital in a society, which then improves individual's access to political information and capacity to participate in politics.

RECOMMENDATIONS OF THIS STUDY

The following recommendations are made on a pragmatic basis and with a view to provide a new base-line of action.

1. There is a need for providing social security schemes like provident fund, medical benefits, pension etc., to the members of SHGs.
2. The government, bank and educational institutions should regularly arrange training programmes for the SHGs members for improvement of their managerial and behavioral competency.
3. The banks should relax their terms and conditions while giving loans to the SHGs. Bank should not impose any minimum limit for the savings.
4. There is a need for enacting separate act to provide legal framework for the functioning of SHGs in the country.
5. Encouraging the media with the help SHGs organizations to project positive images of women and girl child, change the mind-set of the people and thus promote balanced portrayals of women and men.
6. The government department should make budgetary allocation for training components for the Self Help Group members.

CONCLUSION

Microfinance is not merely a financial business, but is a strategy or tool for empowerment of women particularly the poor. The microfinance concept when coordinated with the SHG lending system, those people who have been deprived of credit, could have their fair share of credit. Considering the vast number of poor, discriminated and underprivileged women and the need of financial services, there is tremendous scope for micro financing through SHGs in India. The NBFCs, Banks and Voluntary Organizations play a crucial role in the micro finance market. In order to achieve the objective of poverty eradication, every poor person should have easy access to credit for starting small economic activities based on local resources. Women self-help groups can play a very positive role in socio economic empowerment of poor women. Micro finance can play

a crucial role in achieving Millennium Development Goals and reducing world's poverty by 50 percent by the end of 2015. Poverty reduction is possible by providing easy access to credit for small entrepreneurial activities. Empowerment of women can be promoted through socio economic activities at grass root level. Confidence build up by SHGs helps women members to fight against injustice and secure their rights.

Ethical Clearance- Completed

Sources of Funding - Self

Conflict of Interest - NIL

REFERENCES

1. Angapapillai (2012), "Performance of Self-help Groups: A Micro Study", Southern Economist, Vol. 50, No. 12.
2. Arjun Yallppa Pangannavar (2012), "Self-help Groups and Women Empowerment in India", New Century Publications, New Delhi.
3. Damodar Gujarathi (2011), "Econometrics by Example", Palgrave Macmillan Publication, London.
4. Heggade (2006), "Economic Thought in Ancient and Medieval India with Special Reference to Kautilya", Arjun Publishing House, Mysore.
5. India Rural Development Report 2012-2013 (2013), Orient Blackswan Private Ltd., New Delhi.
6. Jeyaseelan (2014), "Women in Work", APH Publishing Corporation, New Delhi.
7. Muniruddin Qureshi (2003), "Social Status of Indian Women", Anmol Publications, New Delhi.
8. Ram Krishna Mandal (2012), "Women in the 21st Century", Discovery Publishing House Pvt. Ltd., New Delhi.
9. Sarwani (2014), "Two Decades of CEDAW: Challenges and Responses – National and Internationals Perspectives",the Tamil Nadu Dr. Ambedkar Law University Publications, Chennai.
10. Tharabai et.al. (2004), "Micro Credit and Social Capitalism in Rural Tamil Nadu", Social Welfare, Vol. 50, No. 10.
11. Vijaya (2013), "Gender Relations and Government Politics", MJP Publications, Chennai.
12. Vir Dharam and Kamlesh Mahajan (2006), "Changing Status and Emerging Problems of Indian Women", New Academic Publications, New Delhi.

An Empirical Study on Retail Demand for Store Brand Pickles in Tirunelveli, Tamil Nadu

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ABSTRACT

Retail business hasexpanded quickly within a short period in India and has raised critical concerns such as management of service level while meeting consumer needs. That impact the performance of the retailers and their cost-effectiveness. The paper probesthe estimation of demand for a store brand household item, namely Pickle, and demonstrates the benefits of multinomial logistic regression as a useful tool for handling categorical demographic variablesvery frequently used as predictors. Retailers can benefit immensely, by the methodology for other similar products. Moreover, they can enhance space optimization and achieve greaterprofitability per unit retail space and improve customer satisfaction. The research finds that the probability of buying the store brand pickle increases with age of customer and evinces a clear gender bias in the inclination to buy store brand Pickle.

Keywords: *inventory management, assortment planning, multinomial logistic regression, R environment*

INTRODUCTION

Economic policies changes, growth of middle class population, and higher in per capita income hasspurred economic growth in India,leading to prosperi*ty* retail sectoras a resultnow the retail sector represents 10% of India's GDP and a 8% share of employment. Additionally, food inflation is exerting pressure on the retailers to reduceoperating cost to sustainbusiness and profitability. Food price inflation has strained consumer budgets leading them to reduce frequency of visits to the stores and also their purchase volumes. Though it is well known that inflation is due to dynamics of global economic environment, and uncertain rainfall, it has certainly broughtoperations efficiency to retailer's attention.

The competition in the retail in industry in India has been dynamicdue tothe emergence of retail chains and modern retail stores. The critical issues in retailing is the decision about the variety of items a retailer decides to carry for satisfying the consumer. While a wide product assortment meetsevery consumer's needs, it increases inventory. Additionally, it leads to shelf space allocations problems. The problems acquire intensity since retail space is expensive and limited. In view of such constraints, retailers need to use efficient assortment strategies. This means that the retailers would strive to find an optimal mix of products to minimize cost of operations without affecting service levels. This pivots on estimating demand for products. Regression, moving average, and exponential smoothing are frequently used to estimate aggregate demand. At SKU level, logistic regression has been suggested by literature for demand estimation.Logistic regression helps in establishes link between demographics and consumer choice of products. Demographics have been found to be good predictors for retail demand.Demographic variables are categorical in nature and simple regression is not suitable. Logistic regression and multinomial logistic regression are best options. This research uses multinomial logistics regression to determine the probability of purchase as a function of demographic variables age, gender.

Pickles are an integral part of food in Indian households and are traditionally prepared at home using several spices in various combinations leading to different tastes and health benefits. The combination of spices and methodology determines the shelf life of the pickles. It pertinent to point out that homemade pickles do not use preservatives but are prepared in such a manner that they remain good for long durations (2 to 3 years). More over pickles are known to be used only after a period of maturation, that is generally

not less than 6 months. In recent times due to lack of expertise and time families have started to purchase pickles made commercially, which are known to use preservatives and other artificial ingredients. The older generation still believes that pickles made at home are better tasting, hygienic, and good for health as they are devoid of artificial chemicals. While brand owners claim that their products are equivalent to homemade pickles, consumers' trust seems to be latent. Our study does not explicitly dwell into the hygiene aspect of the off the shelf pickles, but it seems that hygiene is an intrinsic part of the consumer choice and purchase intentions. Since we have not explicitly studied the hygienic aspects of the pickle as commercial products, the research does not make any comments on that aspect

Abundant literature on studies on various aspects of supply chain management for FMCG goods including food and grocery items is available. In this study how,inventory can be smartly managed in retail outlets is presented. The retail outlets are the first point contact for consumers and they relay the consumer requirements up the supply chain to the manufacturers. Variables such as floor space, shelf space, display methodology etc. have been identified as the measures that determine selling efficiency.

LITERATURE REVIEW

Retailer's assortment of products is dependent on the target customers, shelf space availability, brand perception, financial strength, and the competitive profile. Even though many retailers claim to offer a one stop shopping experience, providing the whole range of product a random customer would want to buy is next to impossible. Moreover,there is empirical evidencethat on an average customer visit at least three shops before fulfilling all their requirements. The number of consumers served by a retail outlet is so large that satisfying each customer in the target set would be difficult[1] and consumers first-choice preference changes from time to time [2,3]. Consumer preferences are directed by factors such as fulfilment of global and local utility, estimated search cost, and availability of substitutes etc. Such changes can occur due to satiation, need for a change due to changing objectives, social position[4], or to know[5,6] about other items. Such changes lead customers to seek variety. Findings of the above papers means that high levels of heterogeneity in consumer preferences are ubiquitous and a retailer may have to stock an extensive variety of products and SKUs. Stocking of inventory is limited by availability of resources. In addition, studies have evinced that offering too many choices negatively affects consumer perception about the store[7,8] because consumers are beset with the amount of choice and find it difficult arrive at a purchasing decision and the customers may not come back to the store[9]. Moreover, apparent variety has an influence on consumers' buying decision and a mismatch between actual variety and perceived variety can potentially negatively impact buying experience and may lead to lost sales or the consumer [10,11,12]. Perceived variety is dependent on method of display and the symmetry of the assortment [13]. Further retailers may lose sales (lost sales to extent of 4% occurs due to OOS[14] due to out of stock situations in which the consumer can either buy a substitute or buy the preferred product from a competing store. The following table shows reactions to stock out situations:

Multinomial Logit model (MNL)

Theory of utility is the basis for the MNL model. Each customer relates a utility for the purchase or no-purchase of a particular category/SKU. The no-purchase decision is coded in the model as product 0, i.e.when a customer chooses product 0 it is considered a no purchase decision. The consumer's utility related to choose of a product j from S U {0} the Union of set of products carried by the retailer and product 0 is represented as U_j. The utility U_j is considered to be sum of a deterministic part and a random part

$$U_j = u_j + \varepsilon_j$$

The random portion is modeled as a double exponential random variable with the following distribution:

$$\Pr \{X < \varepsilon\} = \text{Exp} (-\text{Exp} – (\varepsilon/\mu + \gamma))$$

Where γ is Euler's constant (0.57722). Its mean is zero, and variance is $\mu^2\pi^2/6$. As the degree of heterogeneity among the customers increases μ also increases. The ε_j are independent across consumers. Hence the product wise general utility for each consumer is same; the actual realized utility may be different based on the level of heterogeneity of the customer population. Additionally, unobservable factors determining the utility of the product to the individual may also be a cause. An individual maximizes utility when choosing a product from the available set. Hence, the probability

that an individual chooses product j from S U {0} can be represented as

$$p_j(s) = \Pr\{Uj = \max_{k \in sU\{0\}} (U_k)\}$$

The double exponential distribution being closed under maximization we can write the probability that a random customer chooses product j from S U {0} as (For proof refer Anderson et al (1992)19.

$$pj(s) = e^{\mu j/\mu} / \sum_{k \in sU\{0\}} e^{\mu k/\mu}$$

Guadagni and Little (1983)[15], show how MNL model can be used for estimating demand for a group of products.

The major criticism for the MNL model is due to Independence of Irrelevant Alternatives (IIA) property. This property is true when ratio of choice probabilities for any two alternatives is independent of ratios for other such alternatives. When we compare two brands within a category, choices within one brand will only lead to cannibalization under high brand loyalty and this property would not be true. Such situations are not rare. One way of overcoming this is to use a Nested Logit Model that is the customer first makes a choice of the brand and follows it up with choosing the SKU. Probability of choosing within a brand follows an exponential distribution and hence the choice probability between two brands also follows an exponential distribution and we can write the total probability as

In which the first term on the right hand side refers to the choice between brands and the second term refers to the choice within a brand. One difficulty in using this nested logit model is that we need know the product attributes the customer uses in the choice process and how the customer prioritizes them. The MNL is also deficient in capturing intricate issues with substitution behavior. The model cannot differentiate between products that have same penetration rate but different substitution rates.

METHODOLOGY

A retail store frequented many consumers located in a residential area was chosen for the study. The name of store and Picklebrand is being kept confidential for commercial reason at the request of the store owner. Mall encounter method of sampling was used to find out purchase intention of store the brand pickle.Ashort demographic profile consisting only of gender and age was also collected as only these two demographic variables are used in this analysis. In total details from 133 customers was collected and analyzed. The analysis was carried out using "nnet" package in the R environment. Chi square test function available in the generic R environment was used to perform chi square test prior to performing the multinomial regression.

RESULTS AND DISCUSSION

The sample profileis as in table 1

Table 1: Sample Profile

Total number of respondents	133
Gender ratio	62% male and 38 % female
Age group	
Group I 20-25 years	39%
Group II 26 -30 years	30%
Group III 31 -35 years	31%

As a firststep the data was put through a chi square test to see if there are significant differences between the groups based on gender, age and education. The Routput for chi square test is given in table 2:

Table 2: Pearson's Chi-squared test with Yates' continuity correction for gender Vs purchase intention

data: product purchase		
Chi Square	Df	P – Value
12.032	1	0.005228

The null hypothesis that there is no gender-based difference in purchase intentions is rejected at alpha of 5%. Which indicates that there is significant difference men and women about choice of Pickel brands.A similar chi square test on age and purchase preference yielded the following results:

Table 3: Pearson's Chi-squared test for Age group vS Purchase intension

data: purchase of product		
Chi Square	Df	P – Value
10.036	2	0.006619

Which clearly shows that the purchase preference of different age groups is different and highly significant at an alpha of 5%.

The output of the multinomial logistic regression using "multinom" function in nnet package is given below in table 4

Table 4:; Multinomial Regression: logit z associated with purchase intention and independent variables Age and Gender

Coefficients:	Values	Std. Err.	P values
Intercept	3.50769445	1.225891	0.0042185035
Age	-0.09448192	0.041458	0.0226683025
Gender	-1.46494357	0.397377	0.0002273296

Residual Deviance: 165.2999

AIC: 171.2999

From the table it is clear that all the coefficients are significant at an $\alpha = 0.05$. The following regression equation (* is used as multiplication sign) can be constructed from the above

(logit) Z = 3.5077 – 0.0945*Age – 1.465*Gender (1)

The probabilities can be calculated using equation 2

(2)

From the regression equation it can be inferred that the variables age and gender have negative effect on the probability of purchase, i.e. as we move from group I to Group II in the age category the probability of purchase diminishes by a factor of 0.0945 and between men and women the change in probability is to the extent of 1.46. This is clear from the predicted probabilities for the sample.

The predicted probabilities are given in table 5

Table 5: Predicted probabilities

Age	Gender	Logit(Z)	Exp(Z)	Predicted probabilities
34	1	-1.1696	0.31	0.24
30	1	-0.7917	0.453	0.31
33	1	-1.0752	0.341	0.25
31	0	0.57875	1.784	0.64
28	1	-0.6027	0.547	0.35
30	0	0.67324	1.961	0.66
30	1	-0.7917	0.453	0.31

Cont... **Table 5: Predicted probabilities**

22	0	1.42909	4.175	0.81
24	1	-0.2248	0.799	0.44
29	1	-0.6972	0.498	0.33
20	1	0.15311	1.165	0.54
30	0	0.67324	1.961	0.66
31	1	-0.8862	0.412	0.29
21	1	0.05863	1.06	0.51
30	1	-0.7917	0.453	0.31
32	0	0.48427	1.623	0.62
31	1	-0.8862	0.412	0.29
27	1	-0.5083	0.602	0.38
27	1	-0.5083	0.602	0.38
20	0	1.61806	5.043	0.83
22	1	-0.0359	0.965	0.49
20	0	1.61806	5.043	0.83
25	1	-0.3193	0.727	0.42
23	1	-0.1303	0.878	0.47
31	0	0.57875	1.784	0.64
24	1	-0.2248	0.799	0.44
33	1	-1.0752	0.341	0.25

Gender is coded as Male = 1 and Female = 0

Summary analysis of the predicted probabilities based on gender and age group is presented in table 6. From the table it can inferred that women of all age groups have greater preference for the store brand Pickle whereas younger men prefer the store brand Pickle than older men.

Table 6: Average Predicted Probabilities

Age group 20 - 25	
W	M
0.79	0.48
Age group 25-30	
W	M
0.68	0.35
Age group 31-35	
W	M
0.61	0.25

CONCLUSION

It has been established, how demographic data can be used to estimate demand for packaged food item such as Pickle. Additional demographic details could be used to get further clarity about the purchase intent of customers but the investigation becomes tedious and statistical significance may not be realized, thereby making the results not useful for realistic demand estimation. Additionally, the study provides direction for future research with more detailed demographic profile of customers to enable the retailers to manage their inventory and shelf space well and reduce cost of operations. Further simulation can be used to produce greater clarity into the buying behavior of consumer. Moreover, the analysis presented here can also be used by manufacturers to develop marketing plans for their merchandises. The preference for the store brand is gender as well as age dependent. Older age customers have greater preference for the store brand pickle when compared to younger customers. Women have greater preference for the store brand pickle across all age groups but men of younger age have greater preference for the store brand pickle than older age men.

Ethical Clearance- Not applicable

Source of Funding- Self

Conflict of Interest: Nil

REFERENCES

1. Green, Paul E. and Abba M. Krieger (1985), ‘‘Models and Heuristics for Product Line
2. Selection,’’ Marketing Science, 4 (1), 1–19.
3. McAlister, Leigh and Edger A. Pessemier(1982) “Variety seeking behavior and interdisciplinary Review”, Journal of Consumer Research. 9 (Decmber) 311-22
4. Kahn, Barbara E. (1998), “Dynamic Relationships with Customers: HighVariety Strategies,” Journal of Academy of Marketing Sciences, 26 (Winter), 45–53. (1999), “Introduction to the Special Issue: Assortment Retailing,” Journal of Retailing, 75 (3), 289–93.
5. Ariely, Dan and J. Levav (2000), “Sequential Choice in Group Settings: Taking the Road Less Traveled and Less Enjoyed,” Journal of Consumer Research, 27 (December), 279–90.
6. Brickman, P. and B. D’Amato (1975), “Exposure Effects in a Free-Choice Situation,” Journal of Personality and Social Psychology, 32 (September), 415–20.
7. McAlister, Leigh (1982), “A Dynamic Attribute Satiation Model of Variety-Seeking Behavior,” Journal of Consumer Research, 9 (September), 141–50.
8. Huffman, Cynthia and Barbara E. Kahn (1998), “Variety for Sale: Mass Customization or Mass Confusion,” Journal of Retailing, 74 (Winter), 491–513.
9. Iyengar, S. and M. Lepper (2000), “When Choice Is Demotivating: Can One Desire Too Much of a Good Thing?,” Journal of Personality and Social Psychology, 6, 995–1006.
10. Fitzsimons, Gavan J., Eric A. Greenleaf and Donald R. Lehmann (1997), “Consumer Satisfaction with both Product and Decision: Implications for the Supply Chain,” Working Paper, UCLA.
11. Broniarczyk, Susan M., Wayne D. Hoyer and Leigh McAlister (1998), “Consumers’ Perceptions of the Assortment Offered in a Grocery Category: The Impact of Item Reduction,” Journal of Marketing Research, 35 (May), 166–7.
12. Kahn, Barbara E. and Brian Wansink (2004), “Impact of Perceived Variety on Consumption Quantity,” Journal of Consumer Research, 30 (4), 519–34.
13. Godek, John, J. Frank Yates and Seigyoung Auh (2001), “Evaluation of Customized Products: The Effects of Assortment and Control,” Working paper, University of Michigan.
14. Young, Michael E. and Edward A. Wasserman (2001),

"Entropy and Variability Discrimination," Journal of Experimental Psychology: Learning, Memory and Cognition, 27 (1), 78–293.

15. Corsten, Daniel and Thomas W. Gruen (2004), "Stock-Outs Cause Walkouts," Harvard Business Review, 82 (5), 26–8.
16. Guadagni, P.M., J.D.C. Little. 1983. A logit model of brand choice calibrated on scanner data. Marketing Science. 2 203–238.

Nutritional and Sensory Evaluation of Moringa Oleifera Cookies

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ABSTRACT

Cookies are a favorite American sweet, made from dough of cereal flour and brown sugar that contains a relatively high ratio of fat, such as butter, vegetable shortening, or margarine. The increasing popularity of cookies especially among children is growing and has become commercialized. This study is an alternative approach to biscuit manufacturing, in order to develop healthy cookies, as it would supplement the essential nutrients needed for all age groups. The different blends of cereal flour (Millet, corn meal and rye flour) with wheat flour and Moringa oleifera leaf powder were processed into cookies in the following ratios 50:30:20 (wheat flour, millet flour, Moringa) (WMM), 40:30:30 (wheat flour, rye flour, Moringa) WRM, 30:30:40 (Wheat flour, corn meal flour, Moringa) (WCM). The sensory evaluation of the cookies samples from the blends was performed using a 9-point hedonic scale. The highest mean score of the cookie was analyzed further for its nutrient content. The results of the sensory evaluation are to show that there were significant differences in the diverse attributes that were determined such as in color, texture, taste, flavor and general acceptance. It was analyzed further for its nutrient content. Incorporating moringa leaf in cookies or any other baked foods may be claimed as a means of boosting nutrition and can be served as an alternative approach to the manufacture of healthy cookies.

Keywords: *Moringa oleifera, nutri cookies, Malnutrition herbal cookies, Healthy cookies.*

INTRODUCTION

Cookie making ingredients are flour, eggs, sugar, butter and flavoring agent. It may comprise other ingredients such as raisins, corn flakes, chocolates, coconut powder and various nuts as per the flavor required. Cookie dough is basically made with flour, fat, moistening agent, raising agent and flavoring agent in various ratios and methods of preparation. Moringa Oleifera is used as a source of Malnutrition relief. Moringa trees have been used to resist malnutrition, especially among infants and nursing mothers. Since Moringa flourishes in arid and semiarid environments, it may provide a versatile, nutritious food source all over the year. There are lots of nutritional benefits in the whole Moringa Oleifera tree[1]. I have researched cookies with the leaves of Moringa Oleifera (Drumstick leaves) with regular cookie ingredients. Drumstick tree is called the powerhouse of minerals and is the most common tree in India. Its leaves, fruits, flowers, bark and seeds are all edible. They all have medicinal values and are used as ingredients in most dishes. The leaves are especially beneficial in the treatment of many ailments due to their various medicinal properties as they are rich in iron content[2].

NEED FOR THE STUDY

Cookies are served at high tea or as snacks across the world. The increasing popularity of cookies especially among children is growing and has become commercialized. This study is an alternative approach to biscuit manufacturers in order to develop healthy cookies, as it would supplement the essential nutrients needed for all age groups. This study is mainly targeted to reduce the malnutrition rate, which can be achieved by the consumption of Moringa oleífera. The cookie, which is made by incorporating Moringa leaves, has all the vital nutrients, which will help in bringing down the malnutrition rate to a great extent[3]. Moringa Oleifera itself has various health benefits such as stabilizing the blood pressure, controlling glucose level in case

of diabetes, used in treating diarrhea and bronchitis. The main aim of the study is to develop and perform the sensory evaluation of Moringa oleifera cookies.

METHODOLOGY

The present study was undertaken to study the "sensory evaluation". Data collected, comprised of the following:

MATERIALS AND METHOD

DEFINING THE PROBLEM

Malnutrition describes a state of imbalance between the dietary needs of the body and the type of diet provided to the body. It impacts brain development, behavioral development, cognitive development and perhaps most visibly growth development. More specifically, the numbers of underweight (low weight-for-age), stunted (low height-for- age) and wasted (low weight-for height) children are common measures for the number of malnourished children in a particular country[4]. Children are more at risk for serious complications due to nutritional deficiencies than adults. Kids require different amounts of Iron at various ages and stages. Infants who are breastfed tend to get enough iron from their mothers only until 4-6 months of age. Iron is the major nutrient, which is used to treat malnutrition. After 12 months of age, toddlers are at risk for Iron deficiency because they are no longer entitled to breast-feeding. Thus, they are forced to Consume Iron rich food in some other forms. Iron is found in foods such as dark leafy greens, red meat, and egg yolks. Moringa Oleifera is rich in Iron Content[5]. A dosage of just two or three spoonsful of Moringa leaves Powder provides a substantial amount of Iron. Moringa Olefera when Consumed in raw form is bitter in taste which makes it very hard for kids to consume them. Thus, when it is incorporated in cookies, it becomes edible and much tastier.

SELECTION OF INGREDIENTS

Whole Wheat Flour

The three parts of the wheat kernels are the germ, bran and the endosperm. Refined wheat flour is made up almost entirely of the endosperm which is a compound of starches and proteins. These proteins and starches allow a baker to create a dough with a lot of strength that is able to trap the gas produced by the yeast and this in turn gives the dough volume[7]. Absence of endosperm in the dough would restrict rise or not possess a shape. This is a reason baking with a white flour is so considerable. As it has more percentage of flour protein than any other grains it can be used as an essential ingredient in cookie making. The demerits of baking with only the endosperm let's go off all the vitamins, minerals, fats and fiber that are contained in the germ and bran during the milling process[8]. These areas are where all the health benefits of wheat reside. It is essentially the entire kernel of wheat milled up into flour form which contains nutrition and flavor.

Shaping:

Due to the weakened state of dough prepared with whole-wheat flour it is significant to handle the dough gently. When shaping, the dough will have a tendency to tear much easier than when using only Refined wheat flour and it will not get the dough as stiff or Shape it firmly.

Selection of Secondary Flour:

The following flours were used as Secondary flours in the cookies:

Rye flour:

Whole grain rye flour contains, as a percentage of dry matter, 56—70% starch, 8—1 3% proteins, 2—3% lipids, 2% ash and 1 5—2 1 % total dietary fiber. In addition, it is rich in minerals, vitamins, especially B-vitamins, and phytochemicals.

Corn-Meal flour:

Corn meal is flour made by grinding dried corn. It can be ground into a variety of consistencies and is also known as corn flour when it is finely ground. Corn meal flour contains nutrients like vitamin B6, Carbohydrates, dietary fiber, Protein and Calories.

Millet flour:

Millet is a rich source of magnesium, a mineral that acts as a co-factor for more than 300 enzymes, including enzymes involved in the body's use of glucose and insulin secretion. Millet is gluten-free and non-allergenic. In Western countries, dried leaves are sold as dietary supplements, in either powder or capsule form.

STANDARDIZATION OF MORINGA OLEIFERA COOKIES

Standardized recipes are used every day in child nutrition operations as a guide to preparing the foods served to customers. A standardized recipe is a recipe that has been carefully adapted and tested to ensure that it will produce a consistent product every time it is used[9]. The recipes of three cookies with various flour the incorporation of moringa oleifera were developed with specific ingredients.

Table 1: Standardized Sample I, II & III — moringa oleifera and Rye, Corn-meal and Millet flour incorporated Cookies One Portion is Four Cookies (4Ogms)

Ingredients for Sample I	Ingredients for Sample II	Ingredients for Sample III	Methodology
Whole wheat flour (80gm)	Whole wheat flour (60gm)	Whole wheat flour (100gm)	Moringa oleifera was de- stalked, sundried and powdered.
Moringa oleifera powder (60gm)	Moringa oleifera powder (80gm)	Moringa oleifera powder (40gm)	Cream together all the ingredients till soft in texture.
Rye flour (60gm)	Corn-meal flour (60gm)	Millet flour (60gm)	The dough was refrigerated for 15mins.
Clarified Butter (90gm)	Clarified Butter (90gm)	Clarified Butter (90gm)	Rolled out the dough and shaped them with cookie cutter.
Honey (10ml)	Honey (10ml)	Honey (10ml)	baked at 170*c for 12-15 minutes.
Palm Sugar (30gm)	Palm Sugar (30gm)	Palm Sugar (30gm)	Cooled at room temperature
Milk powder (2gm)	Milk powder (2gm)	Milk powder (2gm)	Stored in air tight container

The recipe standardization process of the three-nutri cookies was done for recipe verification, product evaluation, and quantity adjustment. Reviewing the recipe in detail, preparing it, verifying its yield, performed recipe verification and recording changes. Product evaluation was focused in determining the acceptability of the product produced from the recipe[10]. Changing the recipe yield and ingredient amounts was done in the quantity adjustment phase. A recipe went through all these phases several times before becoming standardized at the necessary quantity for an operation. Faculty and students of School of Hotel and catering Management worked together on the recipe standardization process. Input from students and other panel members were critical during the evaluation phase.

SELECTION OF METHOD AND TOOL FOR THE STUDY

Sensory method is an experimental study for the newly developed products of three different cookies in order to test, evaluate and get feedback on Sample I, Sample II and Sample III. Thirty (30) panelists were selected to conduct sensory characteristics of cookies which were assessed for sensory acceptability level of the panel members[11]. The sensory profile was taken on appearance, odor, texture, taste, flavor and acceptance.

PREPAR&TION OF HEDONIC SCORECARD

The sensory acceptability tests for moringa oleifera cookies was determined by using 9 point hedonic scale ranging from 1 indicating Dislike Extremely to 9 suggesting Like Extremely with a neutral category of 5 indicating neither like nor dislike for parameters like appearance, aroma, texture, taste, over all acceptance using questionnaire as tool.

CONDUCT OF THE STUDY

Sensory tests were carried out in a sensory light and good ventilation, and away from the preparation room. evaluation room. All the samples were prepared with

utmost care. Texture was maintained and carried out for the same in all samples. The volume of all the samples were equal. Samples were labeled as Sample I (moringa oleifere and Rye) Sample II (moringa oleifere and Corn-meal) and Sample III (moringa Oleifere and millet) to avoid bias.

DETERMINATION OF PROXIMATE NUTRIENT ANALYSIS

The highest mean score of the product, Sample II was analyzed further for its nutrient content which includes energy, carbohydrate, fat, dietary fiber, calcium, iron and sugar[12]. The energy was calculated by FAO method. The proximate nutrients composition such as protein, dietary fiber, total sugar and iron of sample II was performed according to AOAC method Carbohydrates were determined by different method.

RESULTS AND DISCUSSION

The purpose of the study is to Eradicate Malnutrition. The analysis of the data results is presented in this chapter.

The results of three different samples have shown that the texture of the cookies is same in all samples and varied slightly only in Sample III. In Sample II the taste has a mean score of 8, which is greater than the other two samples. The mean scores of Aroma and Appearance in all the three samples didn't have much of an impact. The Acceptance mean score did not have a vast difference between Sample I and Sample III but in Sample II it is 8.32 which was liked, compared to the other samples of the cookies. The results indicate that functional and adding Moringa Oleifera to many bakery products could develop health products with improved sensory quality. To make the Moringa Oleifera cookies more palatable a higher proportion of fine Moringa leaves may be incorporated.

RESULTS

Table 2- Mean Scores of Sensory Evaluation of Moringa Oleifera cookies

SENSORY EVALUATION	SAMPLE I MEAN SCORES	SAMPLE II MEAN SCORES	SAMPLE III MEAN SCORES
APPEARANCE	7.28	7.68	7.04
TEXTURE	7.36	7.60	7.04
TASTE	6.96	8.08	7.28
ODOUR	7.44	7.88	7.52
ACCEPTANCE	7.16	8.32	7.44
OVERALL AVERAGE	**7.24**	**7.91**	**7.26**

Sensory Evaluation of Products

Prepared cookies were subjected to sensory analysis based on 9-point hedonic rating scale for taste, texture, appearance, aroma and after-taste Using a panel of 30 untrained members. Trained members were chefs and academicians, bakery and Culinary preparations. Panel members were advised to use sensory evaluation sheet, mark and convert them into scores. The scores were based on the following criteria: Like extremely: 9; Like very much: 8; Like moderately: 5. , 7; like slightly: 6; Neither like nor dislike extremely: 1.

NUTR1TIVE VALUE OF MORINGA OLEIFERA COOKIES:

(SAMPLE II MORINGA OLEIFERA AND CORN MEAL)

The proximate nutrient analysis of Sample [I tisane was determined. The results expressed on l00 grams of

weight basis, are presented in table 3.

Table 3: Proximate Nutrient Analyses **Per**: l00 grams

S.NO	Parameters	Methods	Result
1.	Energy (By Calculation)	FAO Method	498.48 kcal
2.	Carbohydrate (By Difference)	CTL/SOP/FOOD/262-2014	65.96gm
3.	Total Fat	AOAC 19th Edn.2012,920.39g	23.58gm
4.	Protein (N*6.25)	DGHS Manual 3,2005	5.61gm
5.	Dietary Fiber	AOAC 19th Edn.2012,985.29	5.79gm
6.	Total sugar	AOAC 19th Edn.2012, 925.05	16.15gm
7.	Iron as Fe	AOAC 19th Edn.2012, 99.11	2.40mg

DISCUSSION

The study is based on the implementation of Moringa oliefera in cookies using various combinations of flours. The goal of this study is mainly to reduce the rate of malnutrition by adding Moringa in the cookies as the core ingredient to make Moringa tasty. when Moringa is consumed barely it is bitter in taste. This Cookies helps in improving nutrition value. The medicinal values present in the Moringa oliefera is also present in the cookies therefore there are various health benefits in the cookies. Moringa has been advocated as a natural nutrition for the tropics and is being used to combat malnutrition due to its high protein and fiber content. The developed product of this cookies for this study may be served as an alternative approach to the manufacture of healthy cookies.

CONCLUSION

There are various types of cookies prepared by using different ingredients. A Moringa Oleifera cookie aims at increasing the nutritional value and eradicating malnutrition. Nowadays throughout the world people are consuming Herbal Cookies, Non-Processed foods, organic vegetables, etc. For a disease-Free and healthy life consuming the Moringa Oleifera in the form of cookies makes it more edible and tasty. Usually Herbal items though it has lots of medicinal, it cannot be eaten without processing. These leaves are incorporated in various medicines since olden days. In Ayurveda this is the most important ingredient, thus incorporating it in cookies makes it healthy and edible. Incorporating Moringa leaf in cookies or any other baked food may be claimed as a means of boosting nutrition in India where Malnutrition is prevalent. The developed product of Moringa Oleifera cookies for this study may be served as an alternative. In the manufacturing of cookies. It is high time that we kick-start our life with our undiscovered leaves. Limitations of the study are, Eliminating the raw smell from the cookies was a little tough job, the leaves are to be cleaned carefully as it has lots of stalk in it, Preserving the Moringa oleifera leaves were hard, The moisture in the leaves had to be shallow fried in clarified butter to enhance the flavor for cookies and it had to be dried properly before it was added as an ingredient.

Conflicts of Interest: Conflicts of Interest declared none.

Source of Funding – Self

Ethical Clearance - Nil

REFERANCE

1. Journal of Food Composition and Analysis. Schmidt, M. & Zizka, G. “Moringa olefera”. African plants — a Photo Guide. (2014). Akubor P. Functional

properties and performance of cowpeal/ plantain/ Wheat flour blends in biscuits. (20Ò3) Plant Foods for Human Nutrition.;58(3): 1 -8.

2. Hooda S, Jood S. Organoleptic and nutritional evaluation of herbal biscuit 34. Supplemented with untreated and treated fenugreek flour. (2005) Food Chemistry.;90(3):427-35.
3. Rory P.H. McBurney", Caroline Griffin", Alison A. Paule, David C. Greenberg "The nutritional composition of African wild food plants: from Compilation to utilization" (June—August 2004)
4. Alvarez-Leite, J. j. "Nutrient Deficiencies Secondary to Bariatric Surgery." Current Opinion in Clinical Nutrition and Metabolic Care 7 (September 2004)
5. Amelia, E. J. "Feeding and Hydration Issues for Older Adults with Dementia." Nursing Clinics of North America 39 (September 2004)
6. Shivani B, Asna U, Prabhasankar P. Effect of incorporation of mint in texture, colour and sensory parameter of biscuits. International Journal of Food properties. 37. 2006; 9(4):691-700.
7. Aust Prescr 2014; 37:120-3 Bryan, J., S. Osendorp, D. Hughes, et al. "Nutrients for Cognitive Development in School-Aged Children." Nutrition Reviews 62 (August
8. Nutritive Value of Indian Foods. National Institute of Nutrition, Catherjn Atkinson, Joanna farrow and Valerie barrett "The Cookie book" (2005) 10987654321 (28-41)
9. Christopher Duggan, jo B. Watkins, w. Allan. Nutrition in pediatrics: basic science, clinical application (2008) Hamilton: BC Decker. pp. 127—141. ISBN 978-1-550093612
10. Drisya CR, Swetha BG, Velu V, Indrani D, Singh RP. Effect of dried Murraya koenigii leaves on nutritional, textural and organoleptic characteristics of cookies. (2015)
11. Ghebremichael, K. A.; Gunaratna, K. R.; Henriksson, H; Brumer, H; Dalhanm-iar, G. "A simple purification and activity assay ofthe coagulant protein from Moringa oleifera seed". (2005) Water Res 39 (1 1): 2338—44. Doi: l0.1016 / j. watres.2005.04. Ol2. PMID 15921719.
12. Journal of Food Science and Technology.; 52(1): 500—506. Encyclopedia Britannica, in Food Research International. ;50(2):704—709.

Occupational Health Hazards among IT Sector Women Employees in Tamil Nadu

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ABSTRACT

In view of this healthcare importance, the real economic growth rate depends on the quality of human life especially in improving the women's standard of living around the world. At present, a large number of women are working in all sectors viz., primary sector, secondary sector and service sector in both the rural and urban areas. Further, they are actively participating in each and every job and express their knowledge and capacity to the society. However, most of the women are dominated by men and are affected psychologically and are under pressure in their work places. The term occupational hazards refer to a risk accepted as a consequence of a particular occupation. Regarding the occupational problems, the women employees in IT sector are facing health hazards like eye fatigue, headache, thyroid problem and others. It could be seen that over a past decade, a large number of women employees in IT sector suffer from heart related diseases. Moreover, the Poor health creates huge strain on physical and mental pressure in working places. At present, the large numbers of women employees also face many social, cultural and economic challenges. Hence, this study is an attempt to identify and analyse the reasons that affects the health of IT sector women employee respondents and give suitable measures to reduce the work related stress in IT sector among women employee respondents. Further, this study focused on primary data and the tools of analyses with using statistical techniques like percentages, t-test and rank value method. As a result found that, the urban area women respondents are highly affected as compared to that of rural area women respondents among the IT sector field. By and large, the study suggested that spiritual meditation is a way to help the reducing stress in psychologically and work related stress reduced through improving communication and technical skill.

Keywords: Women empowerment, health, IT sector, stress, economic development, communication skill, spiritual meditation

INTRODUCTION AND STATEMENT OF THE PROBLEM

In view of this healthcare importance, the real economic growth rate is based on to develop the quality of human life and especially to improve the women's standard of living around the world. The human development report (1995) stated that the basic capability for human development are living a long and healthy life, being educated and better standard of living in a society[1]. At present, a large number of women are working in all sectors like primary sector, secondary sector and service sector in which both rural and urban areas. Further, they are actively participating in each and every job and exposure their knowledge and capacity to the society. However, most of them women are conquered by men and they are affected in both psychology and mental pressure in working places[2]. The term occupational hazards refers to the stress related to the works require to be addressed without any delay. Regarding the occupational problems, the software women employees are facing continuously like eye fatigue, headache, thyroid problem and others. It could be seen that over a past decade, a huge number of software women employees are suffered due to heart diseases. Moreover, the Poor health creates huge strain on physical and mental pressure in working places[5]. At present, the large numbers of women employees are facing many problems from different directions like social problems, cultural problems, and economic problems. More specifically,

women are not only facing problems in working places and from her homes also. But some of them are actively participated in domestic work like cooking, washing, drawing water, preparing food and so on. Additionally, the physical health was majorly suffered due to that insufficient sleep as regularly[7]. Meantime, without yoga activity may be disturbed their psychological feelings also. The adverse impact of health is one of the major problems to makes gender equities in the society. In view of these all problems, maintaining health nutrition, and yoga and exercises, proper training and improving knowledge, good sleeping, and air conditioner facilities are the major solution to reduce their stress in software industries. Hence, the present study makes an attempt to analyze the health hazards among IT sector women employees in Tamil Nadu.

OBJECTIVES

To analyse the reasons for health affect in IT sector women employee respondents in selected region.

To suggest the suitable measures to prevent the work related stress in IT sector.

HYPOTHESIS

Rural area women employees are majorly affected as compared to urban area women employees in IT sector.

METHODOLOGY

Drawn from the present study has been focused on primary data and it involves a multi-stage sampling method. Further, the present study is covered four stages and the accurate information is derived from the field of inquiry in IT sector, Chennai city, Tamil Nadu: (i) Selection of Chennai district in Tamil Nadu, (ii) Selection of major towns' viz., Ambattur, Thiyagarayanagar, Kodambakkam and Nungambakkam in Chennai district (iii) Selection of few software industries in selected areas, (iv) Selection of rural area and urban area women employee respondents according to Tamil Nadu state and Other States in software sector. Among these four stages of investigation, 115 sample respondents are selected for the present study. In which, 63 respondents are collected from Tamil Nadu state and the remaining 52 respondents are collected from other states viz., Kerala, Bangalore, Sikkim, etc. Out of these 63 women respondents in Tamil Nadu, 27 respondents belonging to rural areas and the remaining 36 respondents are belonging to urban areas. On the other hand, out of 52 respondents in other states women respondents, 23 respondents are belonging to rural areas and the remaining 29 respondents are belonging to urban areas. In whole, 50 respondents are from rural area respondents and the remaining 65 respondents are from urban area respondents. Moreover, the present study is used by the convenient sampling method and the period covers from 2017 - 2018.

TOOLS FOR DATA ANALYSIS

To analyse the reasons for health affect in IT sector women employee respondents, rank analysis and t-test analysis have been applied.

To identify the major factors to prevent the work related stress in IT sector, the percentage analysis has been employed.

RESULTS AND DISCUSSIONS

TABLE 1: CAUSES OF WORK RELATED STRESS

Dimension on job stresses	Rural Area (Mean Value)	Rank	Urban Area (Mean Value)	Rank
Age Discrimination	7.23	7	6.80	10
Wage Discrimination	6.92	10	6.94	8
Heavy Workload	7.64	6	7.23	6
Gender Discrimination	7.70	5	6.89	9
Unable to achieve their Target	5.78	12	7.20	7
Family Problems	7.98	4	8.90	2

Cont... **TABLE 1: CAUSES OF WORK RELATED STRESS**

Health Issues	8.98	1	9.94	1
Problem with Co-workers	8.23	3	8.89	3
Could not provide maternity Leave	7.18	8	6.78	11
Poor working place	8.30	2	7.80	5
Sexual Harassment	6.99	9	6.21	12
Nightshift Work	6.78	11	8.01	4

Source: Computed

Table 1 analyzes the causes of work related stress with the help of utilizing the rank analysis method. Among the ranking method and estimating the mean value in rural area, most of the respondents are highly suffered by health issues under working in software sector which constitute the mean value at 8.98, followed by poor working places (8.30), problems with co-workers (8.23), family issues (7.98), gender discrimination (7.70), Heavy workload (7.64), age discrimination (7.23), could not provide maternity leave (7.18), sexual harassment (6.99), wage discrimination (6.92), working in night shift (6.78), and the remaining unable to achieve their target (5.78). In the case of urban area by using the mean value with the help of ranking method, the mean value is highly recorded in health issues (9.94), followed by the family issues (8.90), problem with co-workers (8.89), working in night shift (8.01), poor working places (7.80), heavy workload pressure (7.23), unable to achieve their target (7.20), wage discrimination (6.94), gender discrimination (6.89), age discrimination (6.80), could not provide maternity leave (6.78) and the remaining sexual harassment (6.21). As a result found that both rural and urban areas women respondents, health issues and health problems are mainly dominated the job related stress in the study area.

TABLE 2: CAUSES OF WORK RELATED STRESS WITH USING T- TEST METHOD

Dimension on job stresses	**Rural Area (Mean Value)**	**Urban Area (Mean Value)**	**t-value**	**p-value**
Age Discrimination	7.23	6.80	-2.019	.002*
Wage Discrimination	6.92	6.94	-1.090	.078(NS)
Heavy Workload	7.64	7.23	0.989	.049**
Gender Discrimination	7.70	6.89	-2.109	.067(NS)
Unable to achieve their Target	5.78	7.20	2.009	.093(NS)
Family Problems	7.98	8.90	-1.897	.003**
Health Issues	8.98	9.94	-3.478	.042**
Problem with Co-workers	8.23	8.89	-2.870	.021**
Could not provide maternity Leave	7.18	6.78	-3.092	.088(NS)
Poor working place	8.30	7.80	0.678	.097(NS)
Sexual Harassment	6.99	6.21	-0.110	.027**
Nightshift Work	6.78	8.01	1.201	.044**

Source: Computed, *1% level of significant, **5% level of significant, NS – Not significant

Table 2 examines the job related stress with testing of t-test analysis. The null hypothesis is based on "rural area women respondents are highly affected in work related stress with compared to urban area women respondents". The following results are mentioned the rural area respondents are highly suffered in job related stress with compared to urban area respondents. Age discrimination is found to statistically significant at 1% level, and the remaining factors are statistically significant at 5% level viz., heavy workload pressure, family issues, health problems, problem with co-worker, sexual harassment and working in night shift are found to be highly affected in rural area as compared to urban area respondents. Hence, accept the null hypothesis for the above mentioned factors. Rests of them, wage discrimination, gender discrimination, unable to achieve their target, could not provide maternity leave and the remaining poor working places are not significant. As a result found that, the urban area respondents are highly affected in these mentioned factors as compared to that of rural area women respondents. Hence, reject the null hypothesis for above the mentioned factors and accepted alternative hypothesis.

TABLE 3: IMPACT ON PERSONAL STRESS USING ANOVA

Factors	Groups Variable	Sum of Squares	df	Mean Square	F	Sig.
Child Care	Between Groups	.106	1	.106	.112	.739
	Within Groups	258.450	113	.954		
	Total	258.557	114			
Family Issues	Between Groups	.802	1	.802	1.014	.315
	Within Groups	214.260	113	.791		
	Total	215.062	114			
Personal Financial Burden	Between Groups	1.265	1	1.265	1.378	.241
	Within Groups	248.874	113	.918		
	Total	250.139	114			

Source: Computed

Table 3 interprets the impact on personal stress among IT sector women employees. It is seen that the rural area women respondents are highly affected due to the personal stress as compared to urban area respondents. The personal stress factors such as child care, family issues, and personal financial burden are statistically not significant at 5% or 1% level. Thus it leads to accept the alternative hypothesis i.e., urban area women respondents are highly affected in personal stress as compared to rural area respondents.

Table 4: Prevention of Work Related Stress

Parameters	YES	NO	Total
Group Discussion with Other Employees	96 (83.5)	19 (16.5)	**115 (100)**
Improve Communication and Technical Skill	82 (71.3)	33 (28.7)	**115 (100)**
Proper Training	78 (67.8)	37 (32.2)	**115 (100)**
Spiritual Meditation	99 (86.1)	16 (13.9)	**115 (100)**
Yoga and/or Exercises	83 (72.2)	32 (27.8)	**115 (100)**

Source: Computed; Figures in Parentheses denote percentages

Table 4 exhibits the factors attributing to prevent the work related stress among IT sector women employees in selected region. It is seen that more than three fourth of the respondents are stated that agree with spiritual meditation may reduce the work related stress which constitute 86.1 percent, followed by 83.5 percent of them where recorded group discussion with other employees, nearly three fourth of the respondents (72.2 %) are agree with yoga and/or exercises may reduce the work related stress, 71.3 percent of them recorded that improving communication and technical skills and the least recorded by the proper training method at 67.8 percent in selected region. It is noticed that on the table, spiritual meditation and group discussion may reduce the work related stress among IT sector women employees.

POLICY SUGGESTIONS

Protecting the women employees for the long hours of work in the software sector. Because the long hours of work due to increase the physical stresses and psychological stresses. Hence, all the software institutional should banned the long hours of work.

Improve the gender sensitive related and monitor progress about regulatory framework, software technologies and labour policies, and create infrastructural facilities are drawn it by the regulatory information as follow the ICT policies.

Improve the software mechanisms monitor the progress and to encourage gender development in IT field.

All the software institutions should strengthen their capacity through technical support, gender equality and ICT.

All the software institutions should provide the training facilities to the employees for improving the technical and communication skills and create more employment opportunities.

Government should promote software technologies, because it gives more taxation to the central and state Governments.

Government should collaborate with ICT sector. Because recently number of people depend on the software technologies. If the Government undertakes the ICT, it will helpful to increase the most employment opportunities.

Ethical Clearance : Completed

Source of Funding: Self

Conflict of Interest : NIL

REFERENCES

1. United Nations Human Development Report, Gender and Human Development: New York; 1995.
2. Bates J. A. model for the science of decision, Philosophy of Science, 1954; 21 p. 326-339.
3. Tor Busch. Gender differences in self-efficacy and attitudes toward computers, Journal of Educational Computing Research, 1955; 12, p. 147-158.
4. Kowalski, Gregory S, et al. Decision making in joint agricultural operations, Behavioral Sciences and Community Development, 1977; 11(2): 77-87.
5. Heskett J.L, Sasser, W.E, Schlesinger. The service profit chain, New York: The Free Press, 1997 p. 97-104.
6. Beukema L. Quality of Working Life and Workload in Home Help Services: A Review of the Literature and a Proposal for a Research Model, Scandinavian Journal of Caring Society, 1998; 15, p. 12-24.
7. Minai P, Lopamudra, R.P. "Role of farm women in decision making process related to IT activities", Research Highlights, JADU, 2000; 10(2): 64-68.
8. Daniels, Vinzant. Participation of rural women in decision making, Indian Journal of Extension Education, 2000; 29(3&4): 40-47.
9. Traut, C.A, Larsen, and S.H. Feimer. Hanging on or Fading Out? Job Satisfaction and the Long-Term Worker, Public Personnel Management, 2000; 29 p. 343-351.
10. Allen, T.D. Consequence Associated With Work-to-Family Conflict: A Review and Agenda for Future Research, Journal of Occupational Health Psychology, 2000; 5, p. 278-308.
11. Wendy Cukier. Constructing the IT Skills Shortage in Canada: The Implications of Institutional Discourse and Practices for the Participation of Women. SIGMS Conference, 2003; April, p. 1-12.
12. Jane Margolis. The Computer World Could use More IT girl, The Los Angeles Times, 2003; 21 May, p 8.
13. Andrea H. Tapia and Lynette Kvasny. Recruitment is never enough: Retention of Women and Minorities in the IT Workplace, SIGMS, 2004.

Gender preference and Awareness of PCPNDT Act among Rural Reproductive Age Group Wome

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ABSTRACT

Introduction: Sex ratio is defined as number of females per 1000 males. Decline in sex ratio leads to child marriage and poor status of women and it will affect us socially, economically and politically. The Government of India introduced the Preconception and Prenatal Diagnostic techniques act in 2003 to prevent the misuse of modern medical technologies for prenatal sex determination. This study was conducted among rural women of reproductive age group (20-35yrs) in a village of Trichy, southern India with the aim of studying gender preference and awareness about PCPNDT act.

Method: This cross sectional study was conducted using a questionnaire which comprised two sections including Socio demographic details and questions regarding gender preference, awareness about PCPNDT act.

Results: In our study, 120(60%) knew that prenatal sex determination was possible, 106(53%) participants had gender preference and Out of the participants preferring gender, 96(90.6%) of them preferred male babies. Mother-in-laws were the major persuaders. Relatives were the major source of information regarding prenatal sex determination tests. Only 29(14.5%) knew about the PCPNDT act.

Conclusion: Majority of the study population were aware of the possibility to do prenatal sex determination using Ultrasound techniques but not aware of the PCPNDT act and its provisions. Apart from legislative measures, people need to be educated about gender equality and PCPNDT Act.

Keywords: *PCPNDT act, prenatal sex determination, reproductive women, gender preference.*

INTRODUCTION

Sex ratio is defined as number of females per 1000 males. As per 2011 census, sex ratio in India is 940 females per 1000 males, which continues to be significantly adverse towards women[3]. Decline in sex ratio leads to child marriage and increasing number of child brides leads to poor status of women and it will affect us socially, economically and politically. There will be increased mother and child morbidity and mortality associated with early child birth. An associated phenomenon is the abduction and trafficking of girls. In this distorted social scenario marked by growing anti-social behaviour, crimes against women like rape, molestation, eve teasing, child marriages; forced polyandry etc. is also expected to rise. Adverse sex ratio also called female deficit syndrome female feticide, sex selective abortion is being the most common scenario in the present two child norm.

Child sex ratio indicates future sex ratio prediction. According to census 2011, child sex ratio for India is 919. Sex selective abortion has mainly two determinants- Gender preference and Sex determination. Former is influenced by beliefs in the Community and latter is influenced by Health Care providers and legislators.

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Sex selective abortion is almost reported across every part of our nation irrespective of the socioeconomic status, religion and rural or urban background. Usage of appropriate modern technological advancement like ultrasound paves the way for prenatal identification of the sex and thereby declining sex ratio at birth. The main reason for female feticide is that female child are vulnerable to harassment and burden of dowry for the parents at their later stages while a male child earns and supports the family.

The government of India introduced the prenatal diagnostic techniques act in 1994 which was later amended in 2003 to the Preconception and Prenatal Diagnostic Techniques Act[1].The violation of this act can lead to a fine of Rs.10000 and three years imprisonment for first offence and further increase in punishment and fine for subsequent offences[1].The significance of this law is that it was necessitated to prevent the misuse of modern technology. It does not involve only change in social behaviour and practices; it demands ethical medical practice and the regulation of medical technologies that have the potential to be misused. This thus puts onus on the medical professionals and the general public as well .It is the responsibility to educate the people about the PCPNDT act and its significance. This study was conducted among reproductive age group women (20-35yrs) to study gender preference pattern and awareness regarding provisions of PCPNDT act.

OBJECTIVES

To determine the gender preference and awareness about PCPNDT act among women of reproductive age group (20-35 years of age) in villages of Trichy district.

MATERIALS AND METHOD

This study was conducted in a rural area (Sangenthi Village) in Trichy district, south India. The study population included women of reproductive age group (20-35 years of age). A total of 200 women participated in the study. After obtaining informed written consent and explaining the purpose of the study, data was collected using a pre-structured questionnaire which comprised a set of questions which included demographic details, socio economic status, gender preference and awareness. Participant's identity and collected information were kept confidential.

STATISTICAL ANALYSIS

Data were entered in MS EXCEL and statistical analysis done using EPIINFO software.

RESULTS

1. The results of our study are discussed in the following headings:
2. Socio-demographic details.
3. Gender preference.
4. Who persuade for prenatal sex determination?
5. Knowledge about possibility of prenatal sex determination and opinion about prenatal sex determination
6. Source of information for prenatal sex determination
7. Awareness about the provisions of PCPNDT act

1. Socio Demographic details:

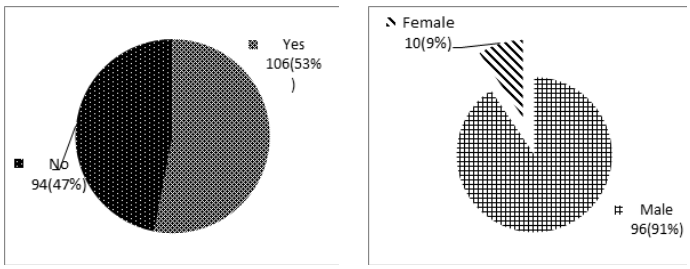
A total of 200 reproductive women of age between 20-35 years were assessed for their knowledge, awareness and attitude regarding prenatal sex determination and PCPNDT act using a questionnaire. Results can be represented here using descriptive statistics which includes socio demographic details and key findings as represented in pie, bar and table separately. Age of the participants ranged between 20-35 years with mean age of 27 years, median of 27 and mode of 28, Standard deviation 3.515.

Majority of the study participants were housewives 81, (40.5%).Most of the participants completed middle school 69, (34.5%). About 11 participants were illiterate (5.5%).Among them, most study participants belonged to nuclear family 145, (72.5%).Among our study population, most of them belonged to middle and upper middle socio economic scale (According to modified BG Prasad scale, 2014). These demographic details are illustrated in table 1.

Table 1: Socio demographic details of the study population

Socio Demographic Factor		Frequency	Percent
Occupation	Agricultural labourer	39	19.5
	Daily wage labourer	29	14.5
	Housewife	80	40
	Teacher	19	9.5
	Tailor	8	4
	Others	25	12.5
Education	Illiterate	11	5.5
	Primary	21	10.5
	Middle	80	40
	Higher secondary	52	26
	UG	33	16.5
	PG	3	1.5
Family	Joint	43	21.5
	Nuclear	145	72.5
	Extended	7	3.5
	Three generation	5	2.5
Socioeconomic class (BG Prasad's Classification)	Upper	3	1.5
	Upper middle	97	48.5
	Middle	94	47
	Lower middle	6	3
	Lower	0	0

2. Gender preference:



Male babies were the most preferred gender even nowadays. Among our study participants, 106(53%) had gender preference and out of them 96 (48%) preferred male over female babies. (Figure 1)

Figure 1 Gender preference among study population

Who persuade for prenatal sex determination? :

On analysis, it was found out that mothers-in-law were the major persuaders towards prenatal sex determination tests (102, 51%) followed by husbands (28, 14%). (Figure 2)

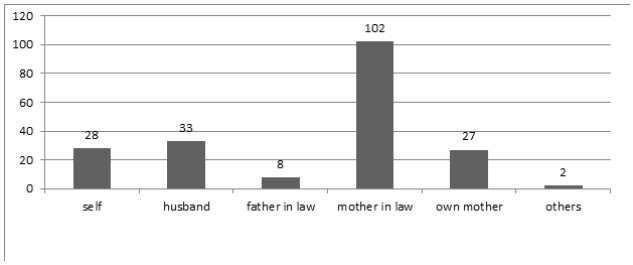


Figure 2 Who persuades for prenatal sex determination?

Knowledge about possibility of prenatal sex determination:

Among the study group, 120 (60%) of the study participants knew that prenatal sex determination was possible and 30 (15%) of them told it was not possible. (Figure 3)

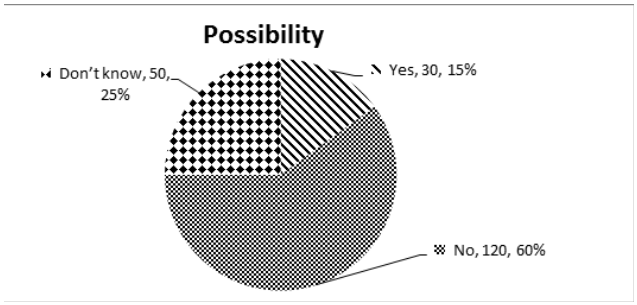


Figure 3 Knowledge about possibility of prenatal sex determination:

Source of information about prenatal sex determination:

According to our study, relatives were the major source of information regarding prenatal sex determination tests (106, 53%) followed by friends (58, 34%). (Figure 4)

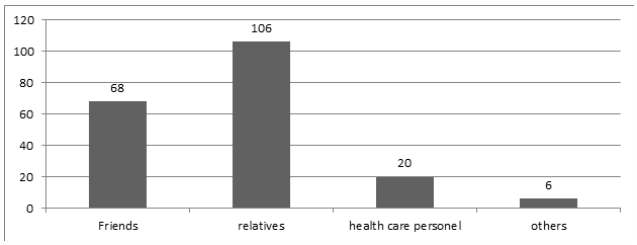


Figure 4 Source of information:

Awareness about PCPNDT act:

On observation of our study analysis, only 29 (14.5%) of the study participants knew the Government act against prenatal sex determination. (Figure 5)

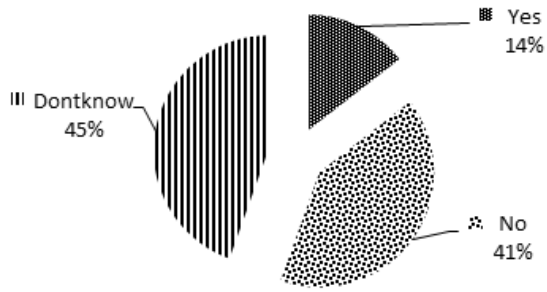


Figure 5 Awareness about PCPNDT act:

Irrespective of their knowledge on PCPNDT act, 40 (20%) of study participants knew that prenatal sex determination was punishable. Among the participants who knew prenatal sex determination is punishable, 26 (13%) knew that both doctors and people who seek for prenatal sex determination tests are punishable.

DISCUSSION

In India, first legal response to control the practice of sex determination and sex selective abortions came into effect in the year 1994 through the introduction of PNDT act which was amended later in 2004 as PCPNDT (Pre-Conception and Prenatal Diagnostic Techniques) act[6]. Though sex selective abortions have been reported all over India, registration and punishment rates have been less so far. Low registration and punishment rates may be due to improper implementation and lack of awareness of the act among public[6].In our study, 53% (106) of the participants had gender preference and among them, 96 of the participants need male babies. This indicates that gender preference is still prevalent.

In our study, mothers-in-law were the major persuaders towards prenatal sex determination. They can be educated regarding gender equality during antenatal visits of their daughters-in-law. About 60% (120) of the participants didn't know the possibility of prenatal sex determination, which is still an advantage for preventing sex selective abortion. Only 29 (14%) were aware of the PCPNDT act, which indicates that media, health care personnel, etc should take part in disseminating knowledge about gender equality and PCPNDT act. People should be educated that, those who seek for prenatal sex determination tests are also punishable by law, thus reducing the rate of prenatal sex determination.

In the present study, 60% of the participants knew

about possibility of sex determination which was lower than the study conducted at Puducherry (82.03%)[9], Mumbai[8], Maharashtra (73.5%) and Bareilly, Uttar Pradesh (80%)[7].In our study, only 21.5% were aware that USG is used for prenatal sex determination, which was lower than the study conducted in Puducherry (91.4%)[9], Hassan(73%)[10] and Mumbai (91.4%)[8].

Relatives (53%) were the main source of information in our study. Our observations were similar to the study conducted in Jammu[11] which was conducted in rural area among 400 people. In a study reported from Hassan (90%)[10], media was the main source of information.

In the present study, 14.5% of participants were aware of the government law against prenatal sex determination and 20% of the total participants knew that prenatal sex determination was punishable. These were lower than the study conducted in Puducherry (awareness of government law- 67.9%, punishable- 84.3%)[9], Hassan (punishable-53%)[10]and Mumbai (awareness of government law- 34.3%, punishable- 44.1%)[8] among which Puducherry(160 pregnant women)[9]and Mumbai(143 pregnant women)[8] were hospital based study while Hassan study included Teachers[10].

In our study, 1.5% said that only doctors should be punished , 4.5% said that only people who seek for sex determination should be punished and 13% were aware that both doctors and people who seek sex determination should be punished. This was lower than the study conducted in Puducherry (only doctors are punishable-3.9%, only people are punished-19.53%, both are punishable- 71.88%)[9].

CONCLUSION

More than half of our study population knew about the prenatal sex determination, still only one-tenth of them knew it was punishable. Nearly half of the study population preferred to know the sex of their unborn child and out of them 90% preferred male babies. Apart from legislative measures, people need to be educated about gender equality and PCPNDT Act.

Ethical Clearance: Obtained from Institutional Ethical committee, Chennai Medical College Hospital and Research Centre, Trichy, Tamil Nadu – 621105.

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Conflict of Interest: Nil

REFERENCES

1. Handbook on Preconception and Prenatal Diagnostic Techniques act and Rules with amendments published by the Ministry of Health and Family welfare Government of India 2006.
2. Government of India (GOI) The Pre-Natal Diagnostic Techniques (Regulation and Prevention of Misuse) Amendment Act. 2003. [Last accessed on 2012 Apr 14].www.xiss.ac.in/RCH/Others/PNDT_Act.pdf ;Available from: http://mohfw.nic.in/PNDTAmendments.htm
3. Office of Registrar General and Census Commissioner, India. Population in age group 0-6 years by sex and sex ratio (0-6).Census of India, Government of India. 2011. [Last accessed on 2011 Apr 09]. Provisional population totals. Available from: http://www.censusindia.gov.in/2011-prov-results/indiaatglance.html .
4. Pre –Conception and Prenatal Diagnostic Techniques (Prohibition of sex selection act, 1994). Annual Report 2010-11. (Cited December 2013) Available from http://mohfw.nic.in/WriteReadData/l892s/5772615131Annual%20Report%20part-6.pdf)
5. Census of India. Gender composition 2011. Available at http: //censusindia.gov.in /Census_And_You/gender_composition.aspx. Accessed on 13 March 2016.
6. Government of India (GOI). 2003. The Pre-Natal Diagnostic Techniques (Regulation and Prevention of Misuse) Amendment Act. www.xiss. ac.in/RCH/Others/PNDT_Act.pdf.Available at http://mohfw.nic.in/PNDTAmendments.htm.Accessed on 14 April 2012.
7. Srivastav S, Kariwal P, Kapilasrami MC. A community based study on awareness and perception on gender discrimination and sex preference among married women (in reproductive age group) in a rural population of district Bareilly, Uttar Pradesh. Nat J Commun Med. 2011 Jul;2:273-6.
8. Shidhaye PR, Giri PA, Nagaonkar SN, Shidhaye RR. Study of knowledge and attitude regarding prenatal diagnostic techniques act among the pregnant women at a tertiary care teaching hospital in Mumbai.Journal of education and health

promotion. 2012;1.

9. Sharmila V, Babu TA, Singh D. Knowledge, awareness and attitude about prenatal sex determination, pre-conception and pre-natal diagnostic techniques act among pregnant women in the South Indian union territory of Puducherry. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2016 Dec 15;5(10):3470-4.
10. Metri SS, Venktesh GM, Thejeshwari HL. Awareness regarding gender preference and female foeticide among teachers in the Hassan District, South India.Journal of clinical and diagnostic research. 2011;5:1430-2.
11. Dhingra R,Manhas S. Perception of rural Rajput community of Jammu regarding prenatal sex determination tests. Studies on Home and Community Science. 2011 Dec 1;5(3):161-4.

Breast Feeding Literacy and Belief - A Community based Cross Sectional Study among Reproductive Age Group Women in Tamil Nadu

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ABSTRACT

Background: Breast feeding have several benefits to both mother and infant; however despite strong evidences in support of breast feeding, its ignorance has remained high worldwide. **Objectives:** To assess the knowledge and attitude regarding breast feeding and its practices among reproductive age group women in rural area in Tamil Nadu. **Materials and Method:** A cross sectional community based study including 116 married reproductive age group women. A structured interview schedule consisting of questions about knowledge about breast feeding and Iowa Infant Feeding Attitudes Scale to assess attitude was administered to 116 reproductive age group women **Result:** Out of 116 subjects, 31.85% practiced exclusive breast feeding and only 61.2% of participants were aware of exclusive breast feeding, all the participants felt initial milk to be discarded, 8.6% are aware that breastfeeding protects against cancer; 23.2% felt that water is essential for baby along with breast milk; 25.8% were aware that lactation can be used as a contraceptive modality. Majority 103(88%) of mothers had neutral attitude towards breast feeding. **Conclusion:** The attitude and knowledge level towards breast feeding is still remaining low, which signifies the urgent need to address the issue. Like unmet needs for family planning "unmet adequacy of breast feeding" should be assessed and addressed.

Keywords: *Attitude, Belief, Breast feeding, Knowledge, Literacy, Reproductive age group women*

INTRODUCTION

Breastfeeding is one of the life saving nutrition for children and also improves future health for both mother and child .[1] More than 15% of 24 lakh death could be averted in India by optimal feeding practices.[2] Over the last couple of decades there has been an increase interest in the promotion of exclusive breast feeding as best feeding method for new borns.[3] Exclusive breast feeding is as infant feeding with human milk without addition of any other liquids or solids.[4] The WHO and UNICEF (1990) have recommended exclusive breast feeding for six months followed by introduction of complementary foods and continue breastfeeding for 24 months or more.[5]

For infants not being breast fed there is increased incidence of infectious morbidity like otitis media, gastroenteritis and pneumonia. There is also evidence of an increased incidence of breast cancer, ovarian cancer in mothers who neglect feeding. [6-9] The positive effects of breast feeding in the mortality pattern [10], intelligence [11] and long term consequences on their life[12] is well recorded.

For effective promotion of breast feeding active intervention should be promoted in the fields of health systems and services, home and family environment, community environment, work environment and policy environment or a combination of any of above.[13] In

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recent years breast feeding has declined as a result of urbanization and maternal employment outside the home which eventually lead to formula feeding. In this study we aimed to assess the knowledge and attributes regarding breastfeeding and its practices among the reproductive age group of women in Tamil Nadu.

METHODOLOGY

This was a community based cross sectional study conducted among married reproductive age group women (18-49 years) who were selected consecutively for a period of 2 months (Dec 2017-Jan 2018). The study was conducted in Sangethi village (the rural field practice area of the institution) in Trichy district, Tamil Nadu.

The sample size was calculated using an estimated 50% prevalence of good knowledge about breast feeding. For 95% confidence limits and an absolute precision of 10%, calculated sample size was 96. Adding 10% non response rate, the minimum sample size came up to 106.

The predesigned structured questionnaire which included three parts

Part A: Socio-demographic data including age, education, income, religion, marital status.

Part B: Knowledge regarding breast feeding and practices (15 questions). Correct answer was awarded one point and wrong zero.

Part C: The Iowa Infant Feeding Attitudes Scale (IIFAS) [14] was used to assess mothers' attitudes toward breastfeeding. The scale included 17 attitude items to determine level of agreement to each question. A 5-point Likert scale from strongly disagrees to strongly agree was applied to all questions. Approximately half of the questions were negatively worded (i.e. 1, 2, 4, 6, 8, 10, 11, 14, and 17). Total IIFAS score ranged from 17 to 85 with higher scores reflecting more positive attitudes on breastfeeding. Total scores were grouped into three, (1) positive to breastfeeding (70-85), (2) neutral (49-69), and (3) positive to formula feeding or negative to breast feeding (17-48).

Written informed consent was obtained from the mothers who were willing to participate in the study. The data were analyzed using Epi info software. The relationship between Knowledge scores, IIFAS scores and socio-demographic variables was tested using appropriate tests

RESULTS

Socio-demographic details

The sample of reproductive age group married women (n=116) were of the age range from 19-45 years. The mean age (± SD) of the population was 29.91(±6.04) years. Maximum 45(38.8%) of the population belonged to more than 30 years. Majority 74(66.4%) of them had school education. More than half 64(534.3%) were homemakers and 61(52.6%) belonged to joint family. Majority 88(75.9%) of them were Hindu religion and had a mean (±SD) age at marriage of 21.84 (±2.81) years. Mean (±SD) years of marital life were 8.27(±6.62) years. The mean (±SD) age at marriage of the group was 21.84 (±2.81) years. Mean (±SD) years of marital life were 8.27(±6.62) years.

Mother's Knowledge about the breast feeding

The mean (±SD) knowledge score of the women was 9.39 (±1.55) which shows more than 50th percentile and range of the score lies between 6 and 14. (Table 1)

Table 1: Knowledge about breast feeding

		True(%)
1	Protection for baby	115(99.1%
2	Breast milk is the ideal food for the baby	40(34.4%)
3	Water is essential for baby along with breast milk	27(23.2%)
4	Prelacteal feeds are good to the baby	30(25.8%)
5	Initial milk – colostrums to be discarded	116(100%)
6	Promotes mother-baby relation	114(98.2%)
7	Frequent sucking increases breast milk production	60(51.7%)
8	Lactation helps lose weight gained during pregnancy	68(58.6%)
9	Lactation prevents next pregnancy	30(25.8%)
10	Protection against cancer	10(8.6%)
11	Breast feeding should be stopped after 6 months	96(82.7%)
12	Drugs can be excreted in breast milk	114(98.2%)
13	Smoking can affect breastfeeding	115(99.1%)
14	Alcohol can affect breastfeeding	83(71.5%)
15	Duration of exclusive breastfeeding is 6 months	71(61.2%)

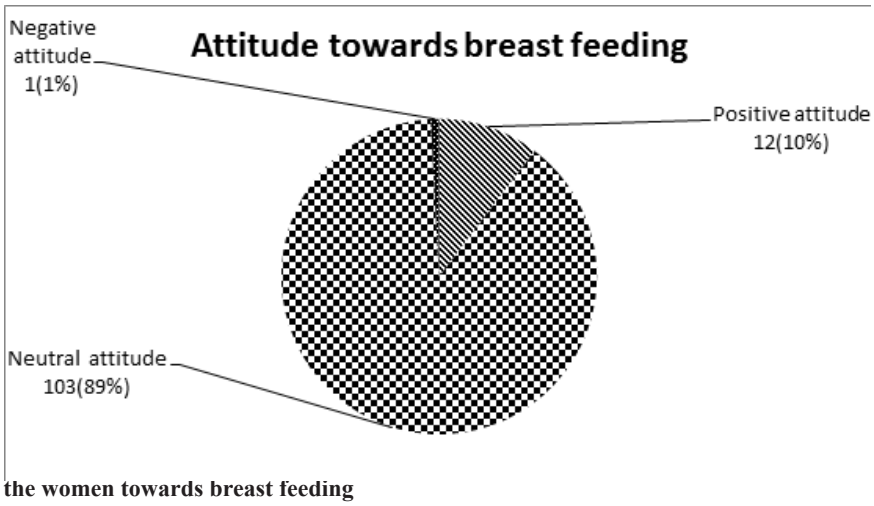


Figure 1: Attitude of the women towards breast feeding

In the study we found that majority of attitude questions scored a mean score of 3 or above indicating a neutral to positive attitude towards breast feeding. (Figure 2)

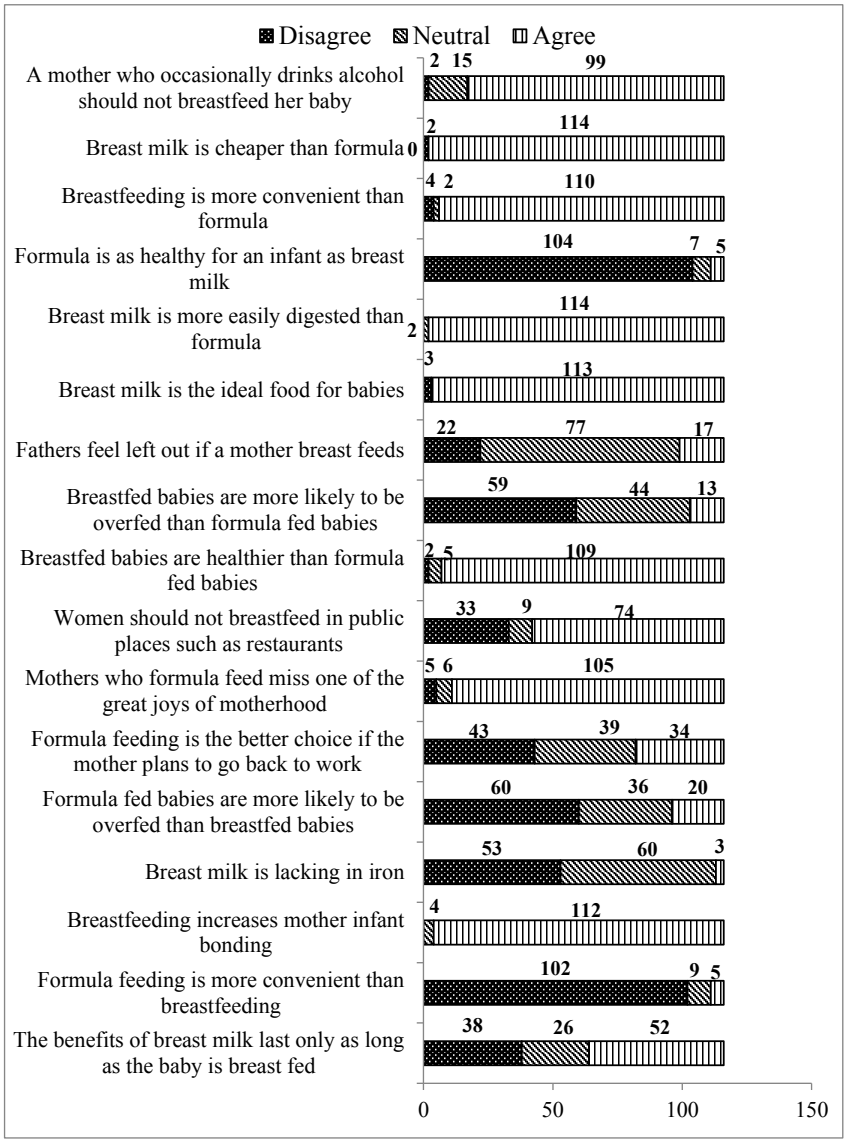


Figure 2: The Iowa Infant Feeding Attitudes Scale

In the study we did Kruskal Wallis test to find association between socio- demographic variables and knowledge score. Variables like family type, religion and act of giving prelacteal feeds were significantly associated with knowledge score. Other variables were not significantly associated with knowledge score.

Table 2: Multiple linear regression, factors associated with knowledge score

Variable	Regression coefficient	95% confidence interval	p value
Type of family	0.38	0.09 to 0.85	0.12
Religion	0.09	0.44 to 0.24	0.57
Prelacteal feeds given	0.83	0.24 to 1.42	0.006

*Significant p value <0.05

The table shows multiple linear regressions of knowledge score with the socio-demographic variables. Act of giving prelacteal feeds was found to be significant. Median knowledge score was 10 for those who didn't give prelacteal feeds compared to a score of 9 for those who gave. If one unit change happens in not giving prelacteal feeds knowledge score change by 0.83 and is statistically significant.

In the study we did one way Anova test to find association between socio- demographic variables and attitude score. Variables like family type, type of delivery, duration of exclusive breast feeding and act of giving prelacteal feeds were significantly associated with attitude score. Other variables were not significantly associated with attitude score.

Table 3: Multiple linear regression, factors associated with attitude score

Variable	Regression coefficient	95% confidence interval	p value
Type of family	0.73	-2.19 to 0.72	0.73
Type of delivery	1.53	-3.36 to 0.29	0.04*
Prelacteal feeds given	4.42	2.12 to 6.71	<0.001*
Exclusive breast feeding duration	0.81	-1.31 to -0.30	0.002*

*Significant p value <0.05

The table shows multiple linear regressions of attitude score with the socio-demographic variables. Type of delivery, duration of exclusive breast feeding and act of giving prelacteal feeds were significant. Mean attitude score for people who had normal delivery were more compared to those who had caesarean section. If one unit change happens in type of delivery attitude score change by 1.53 and is statistically significant.

Mean attitude score of people who didn't give prelacteal feed was 67.11 compare to score of 61.94 for those who gave. If one unit change happens in not giving prelacteal feeds attitude score change by 4.42 and is statistically significant.

Mean attitude score of people who exclusively breast feed for six months was less compare to those who did. If one unit change happens in decreased duration of exclusive breast feeding attitude score change by 0.8 and is statistically significant.

DISCUSSION

This study investigated knowledge and attitude of breast feeding among reproductive age group women. WHO recommends six months of exclusive breast feeding for infants, but in our study, 31.86% of participants practiced exclusive breast feeding. This finding is lower compared to that of NFHS 4 data of India (54.9%) and Tamil Nadu [15](48.3%).

In our study all the participants felt initial milk to be discarded, in contrary to other studies in India where the importance of colostrum was known to 56% of mothers[3] and 75-90% of mothers. [16] In our study, only 8.6% were aware that breastfeeding protects against cancer; 23.2% felt that water is essential for baby along with breast milk; 25.8% are aware that lactation can be used as a contraceptive modality..

Regarding the knowledge score mean score lies more than the 50th percentile denoting good knowledge about breast feeding which is consistent with other studies.[17,18] In our study women from joint and extended family, Muslim religion and who didn't give prelacteal feeds scored more in knowledge score. Women who live with multiple family members got influenced by family (particularly grandmother) in the decision and may result in increased knowledge. This finding is consistent with other studies.[19, 20]

Knowledge score was more among Muslim religion in this study can be due to decreased number of Muslim participants and also continued support from the part of the family for breast feeding which is similar to other studies.[21]

In the study we found that majority 103(88.8%) had neutral attitude towards breast feeding which is comparable to a study done in Bangalore.[18, 22, 23] Highest neutral attitude was in the questions "Fathers feel left out if a mother breast feeds" (66.38%), "Breast milk is lacking in iron" (51.7%) and" Breastfed babies are more likely to be overfed than formula fed babies" (37.93%). This result is similar to other studies.[21] In the present study 93.97% of the mothers believed that breastfeeding is healthier for babies. This result is higher than the results of other studies.[21, 24] Mothers' negative attitude toward breastfeeding could be due to lack of mothers' knowledge about breast milk contents and benefits and lack of facility to support mother's breastfeeding in workplace, feel uncomfortable to breastfeed in public places and negativity towards father's involvement in breastfeeding

This study showed attitude score was more for those from joint family, who had normal delivery, those who didn't give prelacteal feeds and those who exclusively breast fed less than 6 months. Again family influences the attitude as more support and supervision would be there in joint family with respect to a nuclear family. Also there will be a significant attitude change among working mothers and non working which is not significant in this study. Normal delivery may have made the women more comfortable in breast feeding due to the physical condition and immediate recovery than who had caesarean section. The finding that women who had exclusively breast fed less than 6 month having more favorable attitude than the other is in contrast to other studies[25, 26] which shows favorable attitude for those who exclusively breast fed for more than 6 months. The reason can be that women, who exclusively breast fed children for less than 6 months, may have suffered adverse events for infant or mother which was attributed to lack of breast feeding by health care practitioners.

The study lacked qualitative approach which prevented the researchers from delving deeper into other issues like cultural beliefs and deep rooted fads/myths regarding feeding practises. The study would have been more informative if more women were involved in different periods of their life like adolescent people, antenatal women, postnatal women and women in the late age groups. Also the knowledge and attitude of health care practitioners about breast feeding should have provided the present level of quality in health system.

CONCLUSION

This study reveals that knowledge and attitude of mothers towards breastfeeding is far from satisfactory. There is still a need for programmes which support and encourage breastfeeding focusing more on the benefits of breast milk. Training of health care deliverers in institutional setups on need for appropriate and timely intervention in certain breast feeding pattern like discarding initial milk should be executed. Also counseling of antenatal mothers on breastfeeding must be stressed.

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Conflict of Interest: None declared

Ethical Approval: Taken

REFERENCES

1. Victora, Cesar, G., et al., 'Breastfeeding in the 21st Century: Epidemiology, mechanism and lifelong effect', The Lancet, 2016, vol. 387, pp. 475-490.
2. Ekambaram M, Bhat V, Ahamed MA. Knowledge, attitiude and practice of breastfeeding among postnatal mothers. Current Pediatric Research. 2010;14(2).
3. Sokol, E., Aguayo, V. and Clark, D., 2007. Protecting breastfeeding in West and Central Africa: 25 years implementing the international code of marketing breast milk substitutes. Unicef Publication.
4. WHO. The WHO Global Data Bank on Infant and Young Child Feeding. WHO Nutrition for Health and Development; 2009. (Cited 2014 July 21). Available from: http://www.who.int.
5. WHO. 1990. Innocent declaration on the protection, promotion and support of breastfeeding. World Health Organization: Geneva
6. Ip S, Chung M, Raman G, et al. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess (Full Rep) 2007;153:1–186.

7. Nathavitharana KA, Catty D, McNeish AS. IgA antibodies in human milk: epidemiological markers of previous infections? Arch Dis Child Fetal Neonatal Ed. 1994;71:F192–F197.
8. Peterson JA, Patton S, Hamosh M. Glycoproteins of the human milk fat globule in the protection of the breast-fed infant against infections. Biol Neonate. 1998;74:143–162.
9. Horta BL, Bahl R, Martinés JC, et al. Evidence on the long-term effects of breastfeeding: systematic review and meta-analyses. Geneva: World Health Organization; 2007. pp. 1–57.
10. Sankar, M. J. et al (2015). Optimal breastfeeding practices and infant and child mortality: a systematic review and meta-analysis. Acta Paediatrica, Special Issue: Impact of Breastfeeding on Maternal and Child Health. Volume 104, Issue Supplement S467, pages 3–13
11. Horta, B. L. et al (2015). Breastfeeding and intelligence: a systematic review and meta-analysis. Acta Paediatrica, Special Issue: Impact of Breastfeeding on Maternal and Child Health. Volume 104, Issue Supplement S467, pages 14-19.
12. Horta, B.L. et al (2015). Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: a systematic review and meta-analysis. Acta Paediatrica, Special Issue: Impact of Breastfeeding on Maternal and Child Health. Volume 104, Issue Supplement S467, pages 30-37.
13. Sinha B, Chowdhury R, Sankar MJ, Martines J, Taneja S, Mazumder S, Rollins N, Bahl R, Bhandari N. Interventions to improve breastfeeding outcomes: a systematic review and meta-analysis. Acta Paediatrica. 2015 Dec 1;104(S467):114-34.
14. De la Mora A, Russell DW, Dungy CI, Losch M, Dusdieker L. The Iowa Infant Feeding Attitude Scale: analysis of reliability and validity. J Appl Psychol, 1999; 29:2362-80.
15. National Family Health Survey – 4; rchiips.org/nfhs/pdf/NFHS4/TN/India_FactSheet.pdf. (Accessed on October 2017).
16. Subbaiah N. A study to assess the knowledge, attitude, practice and ptoblems of postnatal mothers regarding breast feeding. Nursing J Ind 2003; 94 (8) ; 177-179.
17. Gupta A Arora V. Bhatt B. The state of world`s breastfeeding; India report card 2006. International baby food action network (IFBAN) Asia Pacific. In-Dia 2006.
18. Oche MO, Umar AS, Ahmed H.Knowledge and practice of exclusive breastfeeding in Kware, Nigeria. Afr HealthSci, 2011; 11:518-23.
19. Ekanem IA, Ekanem AP, Asuquo A, EyoVO. Attitude of working mothers to exclusive breastfeeding in Calabar municipality, cross river State, Nigeria. Journal of Food Research, 2012; 1:71-75.
20. Assessment of Breastfeeding Knowledge among Mothers in Erbil City. Zanco J. Med. Sci.2010; Special issue 2.
21. Dllak A, Al-Rabeei N, Aljahmi Y. Breastfeeding Knowledge, Attitude, and Practices among Mothers Attending Health Centers in Sana'a City. ARC Journal of Public Health and Community Medicine. 2016; 1(2):9-17.
22. Inoue M, Binns CW, Katsuki Y, Ouchi M. Japanese Mothers. Asia Pacific journal of clinical nutrition. 2013 Apr 1; 22(2):261-5.
23. Chen S, Binns CW, Liu Y, Maycock B, Zhao Y, Tang L. Attitudes towards breastfeeding-the Iowa infant feeding attitude scale in Chinese mothers living in China and Australia. Asia Pacific Journal of Clinical Nutrition. 2013 Apr 1; 22(2):266-9.
24. Hasanpour K. Exclusive Breastfeeding and Factors Affecting Knowledge, Attitude and Practice of Mothers in Rural and Urban Regions of East Azerbaijan, Iran. Life Science Journal. 2013;10(5s).
25. Sittlington J, Stewart-Knox B, Wright M, Bradbury I, Scott JA. Infant-feeding attitudes of expectant mothers in Northern Ireland. Health Educ Res. 2007; 22(4):561–70.
26. Inoue M, Binns C, Katsuki Y, Ouchi M. Japanese mothers' breastfeeding knowledge and attitudes assessed by the Iowa Infant Feeding Attitudes Scale. Asia Pac J Clin Nutr. 2013; 22(2):261–5.

A Study on Implementation of Powdered Cottonseed and Oil on Bread

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ABSTRACT

In general, bakers use refined flour for making various bread products. In this project we have made an attempt to add powdered cottonseed and oil as an adjunct. We have incorporated powdered cottonseed proportionate to whole wheat flour (i.e., 10: 90; 20:80; 30: 70) in making breads. During this attempt, it was found that these products tasted better than whole wheat breads. The tested products were found to have better taste, texture, aroma and overall appearance by the panelists using hedonic rating scale. As compared with bread from whole wheat, the tested products with powdered cottonseed have improved volume. The combination of whole wheat flour and powdered cottonseed has good hydration characteristics, creates high-fiber and/or reduced-calorie breads. Also, we believe the tested products using powdered cottonseed will help people to overcome certain nutritional deficiencies, specifically vitamin A, D and E.

Keywords: *Implementation, Powdered Cottonseed, Wheat, Cottonseed Bread*

INTRODUCTION

Cotton (*Gossypium hirsutum*) ranks next to corn and wheat in value in most of the western countries (Morrison, 1956), Cotton, the king of natural fibers is mainly cultivated for its lint which is the most sought-after textile fiber till date due to its inherent eco-friendly and comfort characteristics. It is also one of the important cash crops of many of the Afro-Asian countries like India, Iran, Egypt, Sudan, Uzbekistan, Tanzania, etc. and plays a major role in their economic development[1]. This research was aimed at the implementation of the powdered cottonseed and oil on bread products. We have used powdered cottonseed and cottonseed oil under various proportions in making bread products. We have made three samples of bread products with different proportions of powdered cottonseed and cottonseed oil in the recipes. We have incorporated powdered cottonseed proportionate to whole wheat flour (i.e., 10: 90; 20:80; 30: 70) in making breads. It contains a high amount of antioxidants which are essential for good health. It is also rich in Vitamin E, which like antioxidants, is important in fighting free-radicals. It is well known that as much as 60-70% of seed is available from seed cotton during ginning. Cottonseed oil is obtaining by extracted from the seeds of cotton plants of various species, mainly Gossypium hirsutum and Gossypium herbaceum, Cottonseed oil has traditionally been used in foods in ayurvedic cooking in Asian countries as shortening product. Cottonseed oil is a popular frying oil for the restaurant and snack-food manufacturing industries. In this research we have incorporated cottonseed oil as moistening agent for bread products. Cottonseed oil is low in cholesterol which makes it a great choice for those who want to lower their cholesterol levels[2]. It has a light, neutral taste that is accepted by people who do not like oils with concentrate flavors.

NEED FOR THE STUDY

The market for bread products has opened a gateway for enormous growth of Bakery. Anybody from any country loves bread products and has become a favorite one. It reflects the irony and latent humor that life often presents with and which we are often unable to appreciate. Certain bread products are good for health and at the same time satisfy taste buds has indulgent food option[3]. From the above factors it has become prominent the bread product has played a dominant role in the industry. This helps to generate awareness and also become a convenient food for all age group. This study is considered as an important one to analyze the pros and cons and health benefits of implementation of

powdered cottonseed in the bread products.

SCOPE OF THE STUDY

Use of same vegetable oil for repeated frying, common practice across the country in spite of health hazards Food safety and standards authority has noted that the repeated use of same oil making dough in bread products leads to changes in the physiochemical, nutritional and sensory properties of edible oil. Keeping this in view, the quality of the oil must be monitored to avoid the use of degraded oil for cooking purposes. Instance, Omega 3fatty acid is only found in mustard, rape seed, flax seed oil and soy-bean oil. In this study cotton oil was used as an alternate ingredient which provides supplementary benefits to the human body system[4]. Also, we believe the tested products using powdered cottonseed and their oil will help people to overcome certain nutritional deficiencies, specifically vitamin A, D and E.

OUTCOME OF THE STUDY

At the outset it has been found that the implemented cottonseed bread products are good in taste and nutritive values. It is also price reasonable so that volume of scale is also satisfactory. Marketing is facing a healthy competition in which Cottonseed has enormous fiber content[5]. The panelists prefer the most implementation of cottonseed product and its flavor. The product is generally having a warm welcome from the untrained panelists. This study analyses the implementation of cottonseed in the bread products and its nutritional benefits.

PILOT STUDY

The study which is done among the panelists helped in finding the overall quality of the cottonseed implemented bread products. The samples were given to the panelists and they were requested to evaluate the products using the hedonic scale method. The total sample of the target market segment is around 50 panelists.

MATERIALS AND METHODOLOGY

Collection of Materials:

Cottonseeds were procured from a local market. The main ingredients of the bread includes flour, yeast, sugar, salt, water cottonseed oil were also collected. The different quantities of ingredients taken for the sample A, B and C were given in the table below:

Table 1: Recipe formulation and standardization of three types of breads incorporated with cotton seed oil, cotton seed milk and cotton seed powder

S.No	Ingredients	Sample-A	Sample-B	Sample-C
1	Powdered Cottonseed	20 grams	40 grams	60 grams
2	Whole Wheat Flour	180 grams	160 grams	140 grams
3	Salt	5 grams	5 grams	5 grams
4	Sugar	30 grams	30 grams	30 grams
5	Yeast	10 grams	10 grams	10 grams
6	Cottonseed Milk	25 ml	50 ml	75 ml
7	Water	75 ml	50 ml	25 ml
8	Cottonseed Oil	2 tbsp	2 tbsp	2 tbsp

Bread Production incorporated with cottonseed powder:

The cottonseeds were allowed to dry in the sunlight and it was powdered without the cotton linter.

The cottonseeds were soaked overnight and the milk was extracted from the soaked cottonseeds. The cottonseed milk was ensured that it should doesn't has any impurities.

The dough were kneaded for 5-7 minutes

The cottonseed oil was applied on the dough and it was allowed for first fermentation upto 20 minutes.

After the dough is fermented, it was divided into 35-40 grams each.

The divided dough was then shaped and arranged in a greased tray.

Then it was allowed for second fermentation, upto 20 minutes

After the dough is fermented completely, it was baked in 220C for 15-20 minutes until it gave Golden Brown colour.

Once the dough was baked it was allowed to cool for some time. Then the breads were demounted from the tray[6].

STATISTICAL TOOLS USED

Analysis of data is the process by which data is converted into useful information. Raw data as collected from questionnaires cannot be used unless it is processed in some way to make it amenable to drawing conclusions. The various statistical tools used by the researcher for data analysis in this study are:

Percentage Analysis

$$\text{Percentage (\%)} = \frac{\text{Number of Respondents}}{\text{Total Respondents}} \times 100$$

t-Test

Graphs were used to represent the data for the better and accurate interpretation of results.

Percentage analysis:

Percentage method is used to make significant comparisons between various sets of data. Each items in the table is expressed as a percentage of the total in such way as to facilitate comparison of data and to describe the relationship between the variables under study[7]. The formula used here is given below.

t-Test

T-tests are used to compare two means to assess whether they are from the same population. T-tests presume that both groups are normally distributed and have relatively equal variances. The t-statistic is distributed on a curve that is based on the number of degrees of freedom (df). There are three kinds of t-tests: independent-samples, paired-samples, and one-sample.

Sensory Evaluation

These sensory evaluation methods are widely used both by the food industry and academics. Sensory analysis of food relies upon evaluation by our senses (odour, taste, colour, tactile, temperature, pain etc). Only by applying exact scientific testing methods can reproducible results be obtained and analyzed statistically[8]. In the present study the sensory evaluation was carried on between three various proportions of implemented cottonseed bread to find out the acceptance level of Sample A, B & C with 50 untrained panelists of School of Hotel and Catering Management Students and faculty from the Vels University, Tamil Nadu, India and completed the questionnaire. Sample infusions were coded A, B and C and served randomly to panelists. About 30 gm of each infusion was served in a full plate. Three samples were served at a time. The tests were carried out in ten minutes time. The sensory acceptance studied which includes taste, colour, aroma and temperature. Panel members scored their accepted level on a 9-point Hedonic scale. In this approach numbers from1 to 9 were assigned to the scale's and score 9 indicates 'like extremely', score 1 indicates 'dislike extremely' and score 5 indicates neither like or dislike.

Calculations of Nutritive Value

All three types of standardized bread incorporated with powdered cotton seed and cotton seed oil were calculated for its nutritional value using Calculation Nutritive values of Indian Foods by NIN[9].

Statistical Analysis

Sensory scores of three types of standardized bread incorporated with powdered cotton seed and cotton seed oil were dealt by usual statistical procedures. The mean and the difference between mean (t-test) was calculated. The mean scores obtained for code C was high and there was significant difference between the Code B and C.

RESULTS AND DISCUSSIONS

The present investigation was undertaken with the following major objectives.

To study about the implementation of powdered cottonseed in bread products.

To study about the use of cottonseed oil in bread products as a moisturizing agent

To study about the nutritional content of powdered cottonseed and cottonseed oil.

To study about the proportion in which the powdered cottonseed and its oil implemented in bread products.

To study about the drawback of implementation of powdered cottonseed in bread products.

This chapter furnishes the findings of the study entitled "A Study on Implementation of Powdered Cottonseed and Oil on Bread Products". The results are categorized and presented in this chapter under the following headings-

Sensory Analysis of Bread Samples incorporated with powdered cottonseed and cottonseed oil

Percentage was calculated for Sensory analysis of bread Samples incorporated with powdered cottonseed and cottonseed oil of three samples such as Sample A, Sample B and Sample C[10]. The Sample A implemented with 25% of powdered cottonseed and cottonseed oil, Sample B implemented with 50% of powdered cottonseed and cottonseed oil, Sample C were implemented with 75% powdered cottonseed and cottonseed oil.

Table 2: Percentage Distribution of Sensory Scores of Sample A

S. No	Test Scores	Percentage (%) of Taste Scores (N=50)	Percentage (%)of Texture Scores (N=50)	Percentage (%) of Appearance Scores (N=50)	Percentage (%)of Flavour Scores (N=50)	Percentage (%)of Overall Scores (N=50)
1	6	54%(27)	50%(25)	40%(20)	50%(25)	56%(28)
2	7	32%(16)	36%(18)	46%(23)	34%(17)	26%(13)
3	8	8%(4)	12%(18)	14%(7)	8%(4)	8%(4)
4	9	6%(3)	2%(1)	0(0)	8%(4)	10%(5)
	Total	**100%(50)**	**100%(50)**	**100%(50)**	**100%(50)**	**100%(50)**

Table 3: Percentage Distribution of Sensory Scores of Sample B

S.No	Test Scores	Percentage (%) of Taste Scores (N=50)	Percentage (%) of Texture Scores (N=50)	Percentage (%) of Appearance Scores (N=50)	Percentage (%)of Flavour Scores (N=50)	Percentage (%) of Overall Scores (N=50)
1	6	6%(3)	4%(2)	10%(5)	14%(7)	14%(7)
2	7	44%(22)	56%(28)	42%(21)	44%(22)	48%(24)
3	8	40%(20)	36%(18)	44%(22)	30%(15)	30%(15)
4	9	10%(5)	4%(2)	4%(2)	12%(6)	8%(4)
	Total	100%(50)	100%(50)	100%(50)	100%(50)	100%(50)

Table 4: Percentage Distribution of Sensory Scores of Sample C

S.No	Test Scores	Percentage (%) of Taste Scores (N=50)	Percentage (%) of Texture Scores (N=50)	Percentage (%)of Appearance Scores (N=50)	Percentage (%) of Flavor Scores (N=50)	Percentage (%) of Overall Scores (N=50)
1	6	8%(4)	10%(5)	2%(4)	10%(5)	6%(3)
2	7	26%(13)	24%(12)	44%(22)	30%(15)	36%(18)
3	8	24%(12)	48%(24)	20%(10)	52%(26)	44%(22)
4	9	42%(21)	18%(9)	32%(16)	8%(4)	14%(7)
	Total	100%(50)	100%(50)	100%(50)	100%(50)	100%(50)

Table 5: Significant score from the overall sensory evaluation for all the Sample -A, B, C

S. No	Sensory Attributes	Significant Score for Sample A	Significant Score for Sample B	Significant Score for Sample C
1	Taste	7(6.66)	8(7.54)	8
2	Texture	7(6.66)	7(7.40)	8(7.74)
3	Flavor	7(6.74)	7(7.40)	8(7.8)
4	Appearance	7(6.74)	7(7.42)	8(7.58)
5	Overall	7(6.72)	7(7.32)	8(7.66)

SUMMARY

The summary stated in this chapter gives a vital description about the procedure used for data collection and analysis undertaken for the study. Different aspects of rear in connection data is analyzed using various statistical tools and charts. The hedonic rating scale and assessed for its organoleptic qualities like taste, texture, flavor, appearance and overall acceptance of the bread products[11,12]. Bread products were served to untrained panelists. Panelists asked to access each coded sample, comparing it to the control (C), and to point the degree of difference using a 9-point scale where 0 = neither like nor dislike and 9 = extremely like. The significant sensory score of the Taste was 7(6.66), Texture was 7(6.66), Flavor was 7(6.74), Appearance was 7(6.74), Overall was 7(6.72) for the Sample-A. The significant score for the mean of the Taste was 8(7.54), texture was 7(7.40), Flavor was 7(7.40), Appearance was 7(7.42), Overall was 7(7.32) for the Sample-B. From the above findings of the sensory evaluation it is evident that the sample-C which has the highest proportion of powdered cottonseed and cottonseed milk has the highest significant score among the three samples.

CONCLUSION

Major consumers of bread are people from the lower middle class and economically weaker segments is blamed for wide spread poor health. Bread is the economical and basic instant food available for consumption. In India bread is still a secondary staple food when compared to chapati, puri or rice. It is important to realize that when bread ingredients especially refine flour is used vital nutrients are destroyed and also become unfit to consume. The tested bread products prepared with cottonseed were found to have better taste, texture, aroma and overall appearance by the panelists. As compared with bread from whole wheat, the tested products with powdered cottonseed have improved volume. The combination of whole wheat flour and powdered cottonseed has good hydration characteristics permits large percentage of the flour in bread to create high-fiber and/or reduced-calorie breads. In terms of bread when the nutritious part of the grain is removed it essentially becomes a form of sugar. The end result of excessive consumption of white bread and other processed form of grain products results in nutritional deficiencies in particular contribute to a wide range of illness. From this project it is suggested to shed excess fat and improve health is to moderate and

normalize the insulin response by limiting the intake of refined flour. Also, we believe the tested products using powdered cottonseed will help people to overcome certain nutritional deficiencies, specifically vitamin A, D and E. It also has enormous fiber content as the cotton by nature rich in fiber. It is recommended that the cottonseed bread can be a substitute for the whole wheat bread in the same economic price with reasonable nutritive content. This product can be successfully developed in the bakery industry in future.

Conflicts of Interest: The Authors Declare No Conflict Of Interest.

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REFERENCES

1. Dr. Poonam Khetrapal Singh. WHO Regional Director for South-East Asia. How to Beat the diabetes Epidemic. (updated; 2016 April 07; cited; 2016 April 15). Available Form: http://www.bangkokpost.com/opinion/opinion/924 161/how-to-beat-the-diabetes-epidemic
2. Chandan Prasad "Consumption of a Diet Rich in Cottonseed Oil (CSO) Lowers Total and LDL Cholesterol in Normo-Cholesterolemic Subjects" Pg. No 4 (June 2012)
3. N. P. Osti and S. B. Pandey "Use of Whole Cotton Seed and Cotton Seed Meal as a Protein Source in the Diet of Ruminant Animals: Prevailing situation and opportunity" (2011)
4. R. H. Balasubramanya & A. J. Shaikh "UTILISATION OF COTTONSEED BY-PRODUCTS" Pg. No. 12 (2014)
5. A.Mujahid, M.Abdullah, A.R Barque "Nutritional Value of Cottonseeds and its derived products" Pg No. 4 (2000)
6. Nacer Bellaloui, Salliana R. Stetina, and Rickie B. Turley "cottonseed protein, oil, and mineral status in near-isogenic Gossypium hirsutum cotton lines expressing fuzzy/lintel and fuzz less/lintel seed phenoty pes under field conditions" (2011)
7. Dan Gui Wenbin Liu, Xianping ShaoWeina Xu "Effects of different dietary levels of cottonseed meal" (2013)
8. Tashev &Todorov, (2009) CSM was incorporated in dairy animal diet
9. Thomas B. Osborne and Lafayette B. Mendel "The Use of Cottonseed as Food"
10. Charles E. Munroe, "The use of cottonseed oil as food, and for medicinal purposes"
11. John P. Cherry, Leah C. Berardi "Cottonseed Protein Derivatives as Nutritional and Functional Supplements in Food Formulations" (2006)
12. J.T.Lawhon, C.M. Hater, "A Whippable Extract From Glandless Cottonseed Flour"
13. Sajid Latif, Farooq Anwar "Characterization of Enzyme-Assisted Cold-Pressed Cottonseed Oil" (2011)
14. Nutrition Therapy Recommendations for the Management of Adults with Diabetes. BMJ Open Diabetes Research & Care. American Diabetes Association. 2014; 37; 120-143. Available from: http://care.diabetesjournals.org/
15. Dimple Singh-Ackbarali, Rohanie Maharaj.Sensory Evaluation as a Tool in Determining Acceptability of Innovative Products Developed by Undergraduate Students in Food Science and Technology at The University of Trinidad and Tobago. Journal of Curriculum and Teaching. 2014;3(1): 10-27.

Phacoemulsification in Patients with Fuchs Heterochromic Iridocyclitis

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ABSTRACT

Purpose: To compare the safety and efficacy of phacoemulsification to treat cataract in patients with Fuchs heterochromic iridocyclitis (FHI).

Method: A Retrospective, multicenter study was done. Patients with cataract after FHI who had phacoemulsification were evaluated retrospectively. Complications (intraoperatively and postoperatively), operative time, visual acuities, and surgically induced astigmatism were evaluated.

Results: The mean follow-up period was 10.46 ± 2.56 months. The types of cataract included posterior sub-capsular cataract in 45 (86.5 %) eyes and milky cataract white in 7 (13.4 %) eyes. The mean preoperative vision was 0.8 ± 0.24. The mean day 1 post-operative vision was 0.28 ± 0.26 (Figure1). The mean final vision was 0.24 ± 0.10 (P <0.001). The BCVA remained less than 6/60 due to recurrent uveitis, glaucoma, vitreous opacities, healed posterior KP's and CME. However, pre-operative vitreous opacities (P =0.001) and posterior KP's (P = 0.006) had a significant effect on the final visual outcome.

Conclusions: Patients with a history of FHI and cataract having phacoemulsification with PC IOL implantation had excellent visual results with a relatively low complication rate.

***Keywords**: Phacoemulsification, Fuchs Heterochromic iridocyclitis, Cataract.*

INTRODUCTION

Fuchs heterochromic iridocyclitis (FHI) is an inflammatory eye disease of unknown cause. It is still a matter of debate, what triggers inflammation in FHI; the rubella virus, Toxoplasma gondii, and cytomegalovirus have been some of the probable causes.[1]

The prevalence of cataract in FHI is about 15% to 75%. [2] Cataract surgery in patients with uveitis is often done to visually rehabilitate the patients and better visualize the fundus to manage posterior segment pathology.[3] However, he most important step in the management of complicated cataracts is adequate preoperative control of inflammation; most authors consider the absence of cells in the anterior chamber to be synonymous with inflammatory control, as mild cellular reaction in the vitreous may persist even in inactive stages of FHI.[4]

The management, visual outcomes, and postoperative course in patients with uveitis may vary according to the type of uveitis as well as the technique of cataract surgery. Conventional extracapsular cataract extraction (ECCE) was associated with complications like bleeding from fragile vessels, posterior capsular rent with vitreous loss, secondary glaucoma, and retinal detachment. However, with modern small incision surgical techniques, introduction of acrylic intraocular lenses (IOLs), and more in-depth management of complications, there has been a great improvement in visual outcomes in patients with FHI. Phacoemulsification is now the preferred surgical modality of treating cataract in patients with uveitis. [5-6]

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In the present study we retrospectively reviewed the outcomes of phacoemulsification in patients with FHI.

MATERIAL AND METHOD

Fifty-four eyes of 48 patients with FHI who had phacoemulsification with in-the-bag implantation of intraocular lens were evaluated retrospectively; and the primary and secondary outcome measures evaluated were the postoperative vision and complication rate.

The diagnosis was based on the criteria proposed by Kimura et al. [7]; the institutional review boards and the local ethics committees approved the trial. A written, informed consent for the study, based on Helsinki protocol was obtained from all the participating patients.

Exclusion criteria

Patients were excluded if their follow-up period was of less than six months and if they had posterior synechia, cystoid macular edema (CME) and a history of trauma and systemic diseases like diabetes mellitus. Patients with sulcus-to-sulcus and sulcus-to-bag implantation of the IOL were also excluded from the study.

The primary outcome measure was improvement in the post-operative visual acuity. The secondary outcome measure was the rate of postoperative complications.

The pre-operative protocol included routine investigations such as total and differential leucocyte counts, erythrocyte sedimentation rate and blood sugar levels, Mantoux test, chest X-ray, X-rays of the cervical spine and sacroiliac joints. Special investigations included rheumatoid factor, angiotensin converting enzyme assay, anti-nuclear factor, human leucocyte antigen typing and enzyme-linked immunosorbant assay (ELISA) for TORCH infections (Toxoplasmosis, Other agents, Rubella, Cytomegalovirus and Herpes), human immunodeficiency virus and tuberculosis.

B-scan ultrasonography was performed in cases where funduscopy was not possible due to a dense cataract. Gonioscopy was done in all patients with Sussman's four mirror, hand-held

gonioscope (Ocular Instruments, USA). Intraocular pressure was measured with applanation tonometry.

A minimum inflammation free period (defined as five or less than five cells per high power field in the anterior chamber) of a minimum of three months was a pre-requisite for eligibility for surgery. [8] Pre-operatively, topical antibiotic eye drops were prescribed to all patients. No patient received oral corticosteroids.

SURGICAL TECHNIQUE

Peribulbar anaesthesia was delivered. Asepsis was achieved; this included ciprofloxacin (0.3%) eye drops 6 to 8 times, 24 hours before the procedure; instillation of 5% povidone-iodine solution in the cul-de-sac before surgery; and ensuring patency of the nasolacrimal duct. In the preoperative holding area, periocular skin was cleansed with 10% povidone-iodine solution. On the operating table, periocular cleansing was repeated and a drop of 5% povidone-iodine solution instilled on the ocular surface.

Phacoemulsification was performed with peribulbar anaesthesia. Two side-port corneal incisions were created 180 degrees apart with a 20G microincision vitrectomy knife. Anterior chamber entry was fashioned with a 2.8-mm keratome. Anterior chamber was maintained with 2% hydroxypropyl methylcellulose. Trypan blue–assisted continuous curvilinear capsulorrhexis was done as described previously. Cortical cleavage hydrodissection was done just below the anterior capsule rim and the nucleus rotated in the bag. Phacoemulsification was performed with an Infinity vision system (Alcon, Inc) using the phaco-chop method. Bimanual irrigation/ aspiration technique removed cortical matter. IOL was implanted in the capsular bag. Paracentesis was hydrated. At the end of surgery, a subconjunctival injection of 20 mg gentamicin and 4 mg dexamethasone was given to all patients.

POSTOPERATIVE CARE

Patients were followed up on the first, third, and seventh postoperative days, then weekly for 2 weeks, monthly for 2 months, and every 3 months thereafter. The postoperative regimen included topical moxifloxacin every 4 hours for 7 days and topical betamethasone 0.1%, every 2 hours, tapered over 6 to 8 weeks depending on response. At each follow-up day, VA, AC reaction, and vitreous haze were evaluated. Goldman applanation tonometry was done on the first postoperative day, and if normal, repeated at monthly intervals for 3 months. Detailed fundus examination was done at 1 week, 1 month, and repeated at 3-month intervals.

STATISTICS

Vision was compared using the non-parametric Wilcoxon Test. The final vision and complication rate were compared with Mann Whitney test. The P value was calculated at 1 % and 5 % levels. A P value less than 0.001 at 1 % and less than 0.005 at 5% was considered statistically significant.

RESULTS

Two cases were excluded as their follow-up period was of less than six months. The number of valid cases was 52. The mean age was 36.41± 3.66 years. The types of cataract included posterior sub-capsular cataract in 45 (86.5 %) eyes and milky cataract white in 7 (13.4 %) eyes.

ELISA for TORCH infections was positive in one case. The mean follow-up period was 10.46 ± 2.56 months. Table 1 shows the baseline characteristics of the patients.

Snellen's visual acuity was converted to Log MAR units for comparison. The mean preoperative vision was 0.8 ± 0.24. The mean Day 1 post-operative vision was 0.28 ± 0.26 (Figure1). The mean final vision was 0.24 ± 0.10 (P <0.001) (Figure 2). Table 2 shows the percentage of patients at each level of corrected distance vision (CDVA). At the final follow-up, 88.6% of the patients had a vision of 0.5 or better (6/12).

The BCVA remained less than 6/60 due to recurrent uveitis, glaucoma, vitreous opacities, healed posterior KP's and CME (Table 4).

PREOPERATIVE SEQUELAE AND FINAL VISION

Pre-operative findings like heterochromia, iris atrophy or nodules, and vessels on the trabecular meshwork (TM) when present did not have a significant effect on the final visual acuity (Figure 1). However, pre-operative vitreous opacities (P =0.001) and posterior KP's (P = 0.006) had a significant effect on the final visual outcome (Table 2).

OCULAR INFLAMMATION

twenty eyes had a mild to moderate anterior chamber reaction on the first post-operative day.

At the end of the first post-operative month, six eyes had 2 + anterior chamber cells and four eyes had 1 + anterior chamber cells. Topical steroids were continued for eight weeks and resulted in the resolution of inflammation in most of these cases. However, five eyes had recurrent episodes of uveitis and developed PCO subsequently. Nd: YAG capsulotomy was offered after a quiet phase of three months. The eyes with recurrent uveitis had persistent vitreous haze at the final follow-up examination. (P = 0.001).

COMPLICATIONS

Intraoperative complications (Table 3) included hyphema in three eyes (5.7 %), which did not obscure visualization during surgery. Posterior capsule rent with a vitreous loss occurred in one case. A multi-piece PMMA IOL was implanted in the sulcus following anterior vitrectomy. The implanted IOLs were Acrylic in 17 eyes, PMMA in 34 eyes and silicone in 1 eyes. PMMA IOLs were implanted in those patients who could not afford foldable lenses.

The PCO was significantly more in the PMMA group (P = 0.001). There was no case of new onset postoperative glaucoma. However, the eyes of six patients had medically-controlled glaucoma prior to the surgery. At a mean postoperative duration of 1.86 ± 0.81 months, elevated IOP was seen in five eyes. A sustained rise of IOP, despite maximum topical therapy, developed in three eyes (P = 0.074). These patients were referred to the glaucoma clinic for further management. New-onset CME was clinically seen in sic (11.5 %) eyes. CME was detected within the second month in three eyes and within four months in the rest of the eyes.

Topical therapy with ketorolac four times a day for six weeks resulted in the resolution of the

CME in three eyes. However, two eyes with recurrent uveitis and one eye following Nd: YAG laser capsulotomy, had persistent CME on the final follow-up (P = 0.060). Posterior capsule opacification was a common cause (12/19 %) of postoperative reduction of vision (P < 0.001).

The mean duration of PCO after surgery was 3.42 ± 1.78 months. Pearl form of PCO was seen in eight (62.3 %) eyes and the fibrous form in four (33.3 %) eyes. All eyes with PCO except one achieved good vision after Nd: YAG laser capsulotomy; and CME was the cause of reduced vision in this one eye.

Table 1: Baseline Parameters

Age (years)	36.41±3.66
Sex n (%)	
Male	30 (57.7)
Female	22 (42.3)
Follow up (months)	10.46 ± 2.56
Preoperative VA (Log MAR)	0.8 ± 0.24
Postoperative VA (day 1)	0.28 ± 0.26
Postoperative VA(final)	0.24 ± 0.10

Table 2 Pre-operative finding and visual outcome

Pre-op finding	**N**	**%**	**P value (Mann Whitney test)**
Heterochromia	36	69.2	0.103
Iris atrophy	8	15.3	0.973
Iris nodules	0	0	NA
Vitritis	8	15.3	0.008
Raised IOP	5	9.6	0.079
Vessels on TM	4	7.6	0.074
Posterior KP's	10	19	0.006

Table 3 Complication and visual outcome

COMPLICATION	**n**	**%**	**P value**
Hyphema	3	5.7	0.079
IOL deposits	1	1.9	0.185
PCO	11	21.1	0.001
Vitreous opacities	4	7.6	0.002
Secondary glaucoma	3	5.7	0.597
Recurrent uveitis	4	7.6	0.002
Persistent CME	3	5.7	0.081



Figure1. Pigment Cells on Endothelium and Lens surface in Fuchs Heterochromic iridocyclitis.

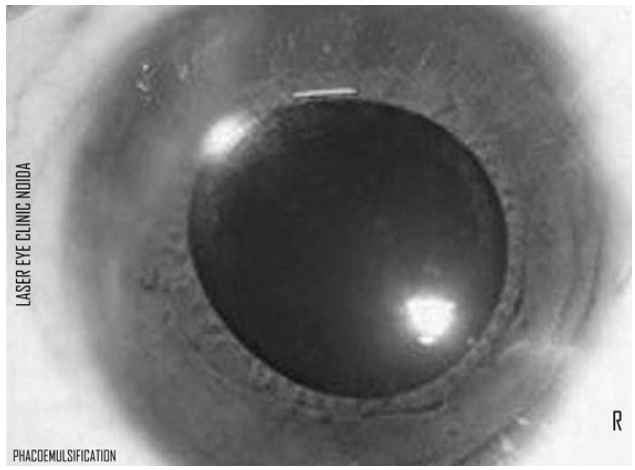


Figure 2. Phacoemulsification in Fuchs Heterochromic iridocyclitis

DISCUSSION

Cataract is a common complication of FHI. However, unlike age-related cataract, patients present at a relatively younger age (<40 years). In the present study, the mean age at presentation was 36.4 ± 3.66 years.

The treatment of cataract in patients with FHI has evolved from intracapsular cataract extraction and conventional ECCE to phacoemulsification and from implantation of AC IOLs to acrylic posterior chamber IOLs. Younger patient age and the posterior subcapsular location of most of these cataracts make phacoemulsification an easy procedure with fewer complications. In most countries and settings, phacoemulsification is now the preferred technique of cataract surgery. However, the outcomes of cataract surgery are sometimes difficult to assess as eyes with different types of uveitis respond differently to surgery.

In a RCT, Bhargava et al found that phacoemulsification is not always successful in fragmenting extremely dense nuclei with extensive posterior synechia; therefore, in general, authors routinely performed scleral tunnel incisions for phacoemulsification of uveitic cataracts, as it may be necessary to enlarge the incision to facilitate manual nuclear extraction. However, as synechia do not occur in FHI, scleral tunnel incisions were not done in the present study. 10

Intraocular lens implantation in an inflamed eye may cause concern because of exaggerated postoperative inflammation. However, FHI is not typically associated with severe uveitis in the postoperative period. In the present study, a mild to moderate reaction was seen in 15.3% of eyes. Recurrent uveitis with persistent vitreous haze at final follow-up examination was seen in 4.6% of eyes. Due to lack of posterior synechia in FHI and the fact that peripheral iridectomy was not performed in any case, postoperative inflammation was relatively mild. In a series of 103 eyes with complicated cataract after FHI and ECCE or phacoemulsification with posterior chamber IOL implantation, Tejwani et al did not find any case with severe postoperative inflammation, although 3 eyes had persistent vitreous haze at final follow-up.[6]

Most studies report excellent visual outcomes after cataract surgery in FHI. In the present study, 88.6% of patients achieved a CDVA of 20/63 or better at 6 months. Moreover, the mean SIA was significantly lower as compared to other studies in which small incision cataract surgery (SICS) was done in uveitic cataract ($P < 0.001$). Presumably, this was a result of the larger incision used by these authors for SICS. Our results were comparable to the studies by Budak et al and Gee and Tabbara. [11-12]

Posterior capsule opacification is a common complication in FHI, with an incidence of 20% to 40%. [13-14] The incidence of PCO in our study was 21%. The incidence of PCO is significantly higher with polymethyl metha acrylate IOLs as compared with acrylic IOLs, and PCO rates do not significantly differ between hydrophobic or hydrophilic acrylic IOLs at 6months' follow-up.[15-16] The higher incidence of PCO in the present study could be explained by the fact that a significant number of patients had PMMA implanted due to financial constraints.

Although there was no case of new onset postoperative glaucoma, the eyes of 8 patients had medically controlled glaucoma before surgery. Elevated IOP was seen in 3 eyes at a mean postoperative duration of 1.06 ± 0.4 months; 3 eyes developed a sustained rise in IOP despite maximum topical therapy with 2 drugs. These patients were referred to the glaucoma clinic for further management.

A shortcoming of the present study is that only 1 technique of phacoemulsification was studied. The results and complications like endothelial cell loss may vary slightly with the technique of nucleus delivery or chopping used.[17]

In conclusion, patients with a history of FHI and cataract having phacoemulsification with PC IOL implantation had excellent visual results with a relatively low complication rate.

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REFERENCES

1. Cerqueira PMG, Silva FTBGCD, Carricondo PC, Olivalves E, Hirata CE, Yamamoto JH. Outcomes of phacoemulsification in patients with uveitis at a tertiary center in São Paulo, Brazil: a review of cases from 2007 to 2012. Arq Bras Oftalmol.2017;80(2):104-107.
2. Ram J, Kaushik S, Brar GS, et al. Phacoemulsification in patients with Fuchs' heterochromic uveitis. J Cataract Refract Surg. 2002; 28:1372–1378.
3. Budak K, Akova YA, Yalvac I, Somer D, Aslan BS, Duman S. Cataract surgery in patients with Fuchs' heterochromic iridocyclitis. Jpn J Ophthalmol. 1999;43(4):308-11.
4. Bhargava R, Kumar P, Sharma SK, Arora Y. Phacoemulsification Versus Manual Small Incision Cataract Surgery in Patients with Fuchs Heterochromic Iridocyclitis. Asia Pac J Ophthalmol (Phila). 2016;5(5):330-4.
5. Cheng B, Liu Y. [Phacoemulsification in eyes with Fuchs' heterochromic iridocyclitis]. Yan Ke Xue Bao. 2000 Jun;16(2):97-8.
6. Tejwani S, Murthy S, Sangwan VS. Cataract extraction outcomes in patients with Fuchs'

heterochromic cyclitis. J Cataract Refract Surg. 2006;32(10):1678-82.

7. Kimura SJ, Hogan MJ, Thygeson P. Fuch's syndrome of heterochromic cyclitis. Arch Ophthalmol 1955; 54:179-86.
8. Hogan MJ, Kimura SJ, Thygeson P. Signs, and symptoms of uveitis. I. Anterior uveitis. Am J Ophthalmol 1959; 47:155-70.
9. Kosker M, Sungur G, Celik T, Unlu N, Simsek S. Phacoemulsification with intraocular lens implantation in patients with anterior uveitis. J Cataract Refract Surg. 2013 Jul;39(7):1002-7.
10. Bhargava R, Kumar P, Sharma SK, Kumar M, Kaur A. Phacoemulsification versus small incision cataract surgery in patients with uveitis. Int J Ophthalmol. 2015; 8:965-70.
11. Gee SS, Tabbara KF. Extracapsular cataract extraction in Fuchs 'heterochromic iridocyclitis. Am J Ophthalmol. 1989; 108:310–314.
12. Budak K, Akova YA, Yalvac I, et al. Cataract surgery in patients with Fuchs heterochromic iridocyclitis. Jpn J Ophthalmol. 1999; 43:308–311.
13. Javadi MA, Jafarinasab MR, Araghi AA, et al. Outcomes of phacoemulsification and in-the-bag intraocular lens implantation in Fuchs heterochromic iridocyclitis. J Cataract Refract Surg. 2005; 3:997–1001.
14. Kosker M, Sungur G, Celik T, et al. Phacoemulsification with intraocular lens implantation in patients with anterior uveitis. J Cataract Refract Surg.2013; 39:1002–1007.
15. Leung TG, Lindsley K, Kuo IC. Types of intraocular lenses for cataract surgery in eyes with uveitis. Cochrane Database Syst Rev. 2014;3:CD007284.
16. Mehta S, Linton MM, Kempen JH. Outcomes of cataract surgery in patients with uveitis: a systematic review and meta-analysis. Am J Ophthalmol. 2014; 158:676–692.e7.
17. Bhargava R, Sharma SK, Chandra M, Kumar P, Arora Y. Comparison of endothelial cell loss and complications between phacoemulsification and manual small incision cataract surgery (SICS) in uveitic cataract. Nepal J Ophthalmol. 2015;7(14):124-34.

Nutritional and Sensory Evaluation of Moringa Oleifera Cookies

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ABSTRACT

Cookies are a favorite American sweet, made from dough of cereal flour and brown sugar that contains a relatively high ratio of fat, such as butter, vegetable shortening, or margarine. The increasing popularity of cookies especially among children is growing and has become commercialized. This study is an alternative approach to biscuit manufacturing, in order to develop healthy cookies, as it would supplement the essential nutrients needed for all age groups. The different blends of cereal flour (Millet, corn meal and rye flour) with wheat flour and Moringa oleifera leaf powder were processed into cookies in the following ratios 50:30:20 (wheat flour, millet flour, Moringa) (WMM), 40:30:30 (wheat flour, rye flour, Moringa) WRM, 30:30:40 (Wheat flour, corn meal flour, Moringa) (WCM). The sensory evaluation of the cookies samples from the blends was performed using a 9-point hedonic scale. The highest mean score of the cookie was analyzed further for its nutrient content. The results of the sensory evaluation are to show that there were significant differences in the diverse attributes that were determined such as in color, texture, taste, flavor and general acceptance. It was analyzed further for its nutrient content. Incorporating moringa leaf in cookies or any other baked foods may be claimed as a means of boosting nutrition and can be served as an alternative approach to the manufacture of healthy cookies.

Keywords: *Moringa oleifera, nutri cookies, Malnutrition herbal cookies, Healthy cookies.*

INTRODUCTION

Cookie making ingredients are flour, eggs, sugar, butter and flavoring agent. It may comprise other ingredients such as raisins, corn flakes, chocolates, coconut powder and various nuts as per the flavor required. Cookie dough is basically made with flour, fat, moistening agent, raising agent and flavoring agent in various ratios and methods of preparation. Moringa Oleifera is used as a source of Malnutrition relief. Moringa trees have been used to resist malnutrition, especially among infants and nursing mothers. Since Moringa flourishes in arid and semiarid environments, it may provide a versatile, nutritious food source all over the year. There are lots of nutritional benefits in the whole Moringa Oleifera tree[1]. I have researched cookies with the leaves of Moringa Oleifera (Drumstick leaves) with regular cookie ingredients. Drumstick tree is called the powerhouse of minerals and is the most common tree in India. Its leaves, fruits, flowers, bark and seeds are all edible. They all have medicinal values and are used as ingredients in most dishes. The leaves are especially beneficial in the treatment of many ailments due to their various medicinal properties as they are rich in iron content[2].

NEED FOR THE STUDY

Cookies are served at high tea or as snacks across the world. The increasing popularity of cookies especially among children is growing and has become commercialized. This study is an alternative approach to biscuit manufacturers in order to develop healthy cookies, as it would supplement the essential nutrients needed for all age groups. This study is mainly targeted to reduce the malnutrition rate, which can be achieved by the consumption of Moringa oleífera. The cookie, which is made by incorporating Moringa leaves, has all the vital nutrients, which will help in bringing down the malnutrition rate to a great extent[3]. Moringa Oleifera itself has various health benefits such as stabilizing the blood pressure, controlling glucose level in case of diabetes, used in treating diarrhea and bronchitis. The main aim of the study is to develop and perform

the sensory evaluation of Moringa oleifera cookies.

METHODOLOGY

The present study was undertaken to study the "sensory evaluation". Data collected, comprised of the following:

MATERIALS AND METHODS:

DEFINING THE PROBLEM

Malnutrition describes a state of imbalance between the dietary needs of the body and the type of diet provided to the body. It impacts brain development, behavioral development, cognitive development and perhaps most visibly growth development. More specifically, the numbers of underweight (low weight-for-age), stunted (low height-for- age) and wasted (low weight-for height) children are common measures for the number of malnourished children in a particular country[4]. Children are more at risk for serious complications due to nutritional deficiencies than adults. Kids require different amounts of Iron at various ages and stages. Infants who are breastfed tend to get enough iron from their mothers only until 4-6 months of age. Iron is the major nutrient, which is used to treat malnutrition. After 12 months of age, toddlers are at risk for Iron deficiency because they are no longer entitled to breast-feeding. Thus, they are forced to Consume Iron rich food in some other forms. Iron is found in foods such as dark leafy greens, red meat, and egg yolks. Moringa Oleifera is rich in Iron Content[5]. A dosage of just two or three spoonsful of Moringa leaves Powder provides a substantial amount of Iron. Moringa Olefera when Consumed in raw form is bitter in taste which makes it very hard for kids to consume them. Thus, when it is incorporated in cookies, it becomes edible and much tastier.

SELECTION OF INGREDIENTS

Whole Wheat Flour

The three parts of the wheat kernels are the germ, bran and the endosperm. Refined wheat flour is made up almost entirely of the endosperm which is a compound of starches and proteins. These proteins and starches allow a baker to create a dough with a lot of strength that is able to trap the gas produced by the yeast and this in turn gives the dough volume[7]. Absence of endosperm in the dough would restrict rise or not possess a shape. This is a reason baking with a white flour is so considerable. As it has more percentage of flour protein than any other grains it can be used as an essential ingredient in cookie making. The demerits of baking with only the endosperm let's go off all the vitamins, minerals, fats and fiber that are contained in the germ and bran during the milling process[8]. These areas are where all the health benefits of wheat reside. It is essentially the entire kernel of wheat milled up into flour form which contains nutrition and flavor.

Shaping:

Due to the weakened state of dough prepared with whole-wheat flour it is significant to handle the dough gently. When shaping, the dough will have a tendency to tear much easier than when using only Refined wheat flour and it will not get the dough as stiff or Shape it firmly.

Selection of Secondary Flour:

The following flours were used as Secondary flours in the cookies:

Rye flour:

Whole grain rye flour contains, as a percentage of dry matter, 56—70% starch, 8—1 3% proteins, 2—3% lipids, 2% ash and 1 5—2 1 % total dietary fiber. In addition, it is rich in minerals, vitamins, especially B-vitamins, and phytochemicals.

Corn-Meal flour:

Corn meal is flour made by grinding dried corn. It can be ground into a variety of consistencies and is also known as corn flour when it is finely ground. Corn meal flour contains nutrients like vitamin B6, Carbohydrates, dietary fiber, Protein and Calories.

Millet flour:

Millet is a rich source of magnesium, a mineral that acts as a co-factor for more than 300 enzymes, including enzymes involved in the body's use of glucose and insulin secretion. Millet is gluten-free and non-allergenic. In Western countries, dried leaves are sold as dietary supplements, in either powder or capsule form.

STANDARDIZATION OF MORINGA OLEIFERA COOKIES

Standardized recipes are used every day in child nutrition operations as a guide to preparing the foods served to customers. A standardized recipe is a recipe that has been carefully adapted and tested to ensure that it will produce a consistent product every time it is used[9]. The recipes of three cookies with various flour the incorporation of moringa oleifera were developed with specific ingredients.

Table 1: Standardized Sample I, II & III — moringa oleifera and Rye, Corn-meal and Millet flour incorporated Cookies One Portion is Four Cookies (4Ogms)

Ingredients for Sample I	Ingredients for Sample II	Ingredients for Sample III	Methodology
Whole wheat flour (80gm)	Whole wheat flour (60gm)	Whole wheat flour (100gm)	Moringa oleifera was de-stalked, sundried and powdered.
Moringa oleifera powder (60gm)	Moringa oleifera powder (80gm)	Moringa oleifera powder (40gm)	Cream together all the ingredients till soft in texture.
Rye flour (60gm)	Corn-meal flour (60gm)	Millet flour (60gm)	The dough was refrigerated for 15mins.
Clarified Butter (90gm)	Clarified Butter (90gm)	Clarified Butter (90gm)	Rolled out the dough and shaped them with cookie cutter.
Honey (10ml)	Honey (10ml)	Honey (10ml)	baked at 170*c for 12-15 minutes.
Palm Sugar (30gm)	Palm Sugar (30gm)	Palm Sugar (30gm)	Cooled at room temperature
Milk powder (2gm)	Milk powder (2gm)	Milk powder (2gm)	Stored in air tight container

The recipe standardization process of the three-nutri cookies was done for recipe verification, product evaluation, and quantity adjustment. Reviewing the recipe in detail, preparing it, verifying its yield, performed recipe verification and recording changes. Product evaluation was focused in determining the acceptability of the product produced from the recipe[10]. Changing the recipe yield and ingredient amounts was done in the quantity adjustment phase. A recipe went through all these phases several times before becoming standardized at the necessary quantity for an operation. Faculty and students of School of Hotel and catering Management worked together on the recipe standardization process. Input from students and other panel members were critical during the evaluation phase.

SELECTION OF METHOD AND TOOL FOR THE STUDY

Sensory method is an experimental study for the newly developed products of three different cookies in order to test, evaluate and get feedback on Sample I, Sample II and Sample III. Thirty (30) panelists were selected to conduct sensory characteristics of cookies which were assessed for sensory acceptability level of the panel members[11]. The sensory profile was taken on appearance, odor, texture, taste, flavor and acceptance.

PREPAR&TION OF HEDONIC SCORECARD

The sensory acceptability tests for moringa oleifera cookies was determined by using 9 point hedonic scale ranging from 1 indicating Dislike Extremely to 9 suggesting Like Extremely with a neutral category of 5 indicating neither like nor dislike for parameters like appearance, aroma, texture, taste, over all acceptance using questionnaire as tool.

CONDUCT OF THE STUDY

Sensory tests were carried out in a sensory light and good ventilation, and away from the preparation room. evaluation room. All the samples were prepared with utmost care. Texture was maintained and carried out for the same in all samples. The volume of all the

samples were equal. Samples were labeled as Sample I (moringa oleifere and Rye) Sample II (moringa oleifere and Corn-meal) and Sample III (moringa Oleifere and millet) to avoid bias.

DETERMINATION OF PROXIMATE NUTRIENT ANALYSIS

The highest mean score of the product, Sample II was analyzed further for its nutrient content which includes energy, carbohydrate, fat, dietary fiber, calcium, iron and sugar[12]. The energy was calculated by FAO method. The proximate nutrients composition such as protein, dietary fiber, total sugar and iron of sample II was performed according to AOAC method Carbohydrates were determined by different method.

RESULTS AND DISCUSSION

The purpose of the study is to Eradicate Malnutrition. The analysis of the data results is presented in this chapter.

The results of three different samples have shown that the texture of the cookies is same in all samples and varied slightly only in Sample III. In Sample II the taste has a mean score of 8, which is greater than the other two samples. The mean scores of Aroma and Appearance in all the three samples didn't have much of an impact. The Acceptance mean score did not have a vast difference between Sample I and Sample III but in Sample II it is 8.32 which was liked, compared to the other samples of the cookies. The results indicate that functional and adding Moringa Oleifera to many bakery products could develop health products with improved sensory quality. To make the Moringa Oleifera cookies more palatable a higher proportion of fine Moringa leaves may be incorporated.

RESULTS

Table 2- Mean Scores of Sensory Evaluation of Moringa Oleifera cookies

SENSORY EVALUATION	SAMPLE I MEAN SCORES	SAMPLE II MEAN SCORES	SAMPLE III MEAN SCORES
APPEARANCE	7.28	7.68	7.04
TEXTURE	7.36	7.60	7.04
TASTE	6.96	8.08	7.28
ODOUR	7.44	7.88	7.52
ACCEPTANCE	7.16	8.32	7.44
OVERALL AVERAGE	**7.24**	**7.91**	**7.26**

Sensory Evaluation of Products

Prepared cookies were subjected to sensory analysis based on 9-point hedonic rating scale for taste, texture, appearance, aroma and after-taste Using a panel of 30 untrained members. Trained members were chefs and academicians, bakery and Culinary preparations. Panel members were advised to use sensory evaluation sheet, mark and convert them into scores. The scores were based on the following criteria: Like extremely: 9; Like very much: 8; Like moderately: 5. , 7; like slightly: 6; Neither like nor dislike extremely: 1.

NUTR1TIVE VALUE OF MORINGA OLEIFERA COOKIES:

(SAMPLE II MORINGA OLEIFERA AND CORN MEAL)

The proximate nutrient analysis of Sample [I tisane was determined. The results expressed on l00 grams of weight basis, are presented in table 3.

Table 3: Proximate Nutrient Analyses **Per**: l00 grams

S.NO	Parameters	Methods	Result
1.	Energy (By Calculation)	FAO Method	498.48 kcal
2.	Carbohydrate (By Difference)	CTL/SOP/FOOD/262-2014	65.96gm
3.	Total Fat	AOAC 19th Edn.2012,920.39g	23.58gm
4.	Protein (N*6.25)	DGHS Manual 3,2005	5.61gm
5.	Dietary Fiber	AOAC 19th Edn.2012,985.29	5.79gm
6.	Total sugar	AOAC 19th Edn.2012, 925.05	16.15gm
7.	Iron as Fe	AOAC 19th Edn.2012, 99.11	2.40mg

DISCUSSION

The study is based on the implementation of Moringa oliefera in cookies using various combinations of flours. The goal of this study is mainly to reduce the rate of malnutrition by adding Moringa in the cookies as the core ingredient to make Moringa tasty. when Moringa is consumed barely it is bitter in taste. This Cookies helps in improving nutrition value. The medicinal values present in the Moringa oliefera is also present in the cookies therefore there are various health benefits in the cookies. Moringa has been advocated as a natural nutrition for the tropics and is being used to combat malnutrition due to its high protein and fiber content. The developed product of this cookies for this study may be served as an alternative approach to the manufacture of healthy cookies.

CONCLUSION

There are various types of cookies prepared by using different ingredients. A Moringa Oleifera cookie aims at increasing the nutritional value and eradicating malnutrition. Nowadays throughout the world people are consuming Herbal Cookies, Non-Processed foods, organic vegetables, etc. For a disease-Free and healthy life consuming the Moringa Oleifera in the form of cookies makes it more edible and tasty. Usually Herbal items though it has lots of medicinal, it cannot be eaten without processing. These leaves are incorporated in various medicines since olden days. In Ayurveda this is the most important ingredient, thus incorporating it in cookies makes it healthy and edible. Incorporating Moringa leaf in cookies or any other baked food may be claimed as a means of boosting nutrition in India where Malnutrition is prevalent. The developed product of Moringa Oleifera cookies for this study may be served as an alternative. In the manufacturing of cookies. It is high time that we kick-start our life with our undiscovered leaves. Limitations of the study are, Eliminating the raw smell from the cookies was a little tough job, the leaves are to be cleaned carefully as it has lots of stalk in it, Preserving the Moringa oleifera leaves were hard, The moisture in the leaves had to be shallow fried in clarified butter to enhance the flavor for cookies and it had to be dried properly before it was added as an ingredient.

Conflicts of Interest: Conflicts of Interest declared none.

Source of Funding – Self

Ethical Clearance - Nil

REFERANCES

1. Journal of Food Composition and Analysis. Schmidt, M. & Zizka, G. “Moringa olefera”. African plants — a Photo Guide. (2014). Akubor P. Functional properties and performance of cowpeal/ plantain/ Wheat flour blends in biscuits. (20Ò3) Plant Foods for Human Nutrition.;58(3): 1 -8.
2. Hooda S, Jood S. Organoleptic and nutritional

evaluation of herbal biscuit 34. Supplemented with untreated and treated fenugreek flour. (2005) Food Chemistry.;90(3):427-35.

3. Rory P.H. McBurney", Caroline Griffin", Alison A. Paule, David C. Greenberg "The nutritional composition of African wild food plants: from Compilation to utilization" (June—August 2004)
4. Alvarez-Leite, J. j. "Nutrient Deficiencies Secondary to Bariatric Surgery." Current Opinion in Clinical Nutrition and Metabolic Care 7 (September 2004)
5. Amelia, E. J. "Feeding and Hydration Issues for Older Adults with Dementia." Nursing Clinics of North America 39 (September 2004)
6. Shivani B, Asna U, Prabhasankar P. Effect of incorporation of mint in texture, colour and sensory parameter of biscuits. International Journal of Food properties. 37. 2006; 9(4):691-700.
7. Aust Prescr 2014; 37:120-3 Bryan, J., S. Osendorp, D. Hughes, et al. "Nutrients for Cognitive Development in School-Aged Children." Nutrition Reviews 62 (August
8. Nutritive Value of Indian Foods. National Institute of Nutrition, Catherjn Atkinson, Joanna farrow and Valerie barrett "The Cookie book" (2005) 10987654321 (28-41)
9. Christopher Duggan, jo B. Watkins, w. Allan. Nutrition in pediatrics: basic science, clinical application (2008) Hamilton: BC Decker. pp. 127—141. ISBN 978-1-550093612
10. Drisya CR, Swetha BG, Velu V, Indrani D, Singh RP. Effect of dried Murraya koenigii leaves on nutritional, textural and organoleptic characteristics of cookies. (2015)
11. Ghebremichael, K. A.; Gunaratna, K. R.; Henriksson, H; Brumer, H; Dalhanm-iar, G. "A simple purification and activity assay ofthe coagulant protein from Moringa oleifera seed". (2005) Water Res 39 (1 1): 2338—44. Doi: l0.1016 / j. watres.2005.04. Ol2. PMID 15921719.
12. Journal of Food Science and Technology.; 52(1): 500—506. Encyclopedia Britannica, in Food Research International. ;50(2):704—709.

Emotional Impact of Diabetic Foot Ulcer

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ABSTRACT

A descriptive design was used to assess the emotional impact. The sample consisted of 20 patients with diabetic foot ulcer satisfying the sample selection criteria. The setting was surgical OPD of Sri Ramachandra Hospital. The instrument consisted of two parts Part I-demographic variables, Part II-emotional domain of diabetic foot ulcer scale developed by Johnson& Johnson (2002). Emotional domain that measures the level of depression consisted of 17 items in 5 point likert scale. Data were collected on the day of selection on a one to one basis through interview method**.** The study findings revealed that 10(50 %) patients belonged to the age group of 66-75yrs. Regarding wound classification, 14(70%) patients had ulcer that penetrated to capsule or bone. The emotional impact among diabetic foot ulcer patients were, 12(60%) patients had moderate depression and 6(20%) had mild depression and 2(10%) had severe depression. There is no statistically significant association noted between emotional impact and background variables.

Keywords: *emotional impact; depression, diabetic foot ulcer.*

INTRODUCTION

Diabetic foot ulcer is one of the long term complications of diabetes mellitus. Diabetic foot ulcers are the sores which develop over the feet. It is one of the common cause of amputation.85% of amputation are due to diabetic foot ulcer among patients with type 2 diabetes mellitus[1] and 20% of hospital admission in people with diabetes are due to foot ulcers[2] and average stay at hospital is 9-10 days[3].

Advancement in health care facilities and treatment modalities have reduced mortality and extended the life expectancy of people. The long term treatment and deformity related to diabetic foot ulcer puts the individual in reduced social activities, increased family tension, limited employment, financial hardship[4] ultimately makes the patient emotionally weak and finds it difficult to cope up with the situation. The emotional impact of diabetic foot ulcer is often overlooked when medical care is given importance. In the present study, the investigators assessed the emotional impact among patients with foot ulcer

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STATEMENT OF THE PROBLEM

A Study to assess the emotional impact of foot ulcer among patients with diabetic foot ulcer at Sri Ramachandra hospital, Chennai.

OBJECTIVES OF THE STUDY:

To identify the emotional impact of foot ulcer among patients with diabetic foot ulcer.

To associate the emotional impact of foot ulcer with selected background variables among patients with diabetic foot ulcer.

METHODOLOGY

A.Materials and Methods:

Research design : Descriptive design

Setting: Surgical Outpatient Department of SRH.

Population: Patients with Diabetic Foot Ulcer

Sample size: 20

Sampling technique: Convenience sampling technique

Sampling criteria:

1. Inclusion criteria

a. Patients with diabetic foot ulcer for 4weeks

b. Both male and female patients were included

2. Exclusion criteria

a. Patients not volunteering to participate

b. Patients with known depression

Description of the instrument

Part I - Background variables: Age, gender, residence, educational status, duration of diabetes mellitus, glycemic control and wound classification.

Part II - Emotional domain of diabetic foot ulcer scale developed by Johnson& Johnson (2002). Emotional domain that measures the level of depression consisted of 17 items in 5 point likert scale,1-not at all,2-slightly,3-moderately,4-quite a bit,5-extremely

Score interpretation is given as

<17 no depression

18-34 mild depression

35-51 moderate depression

52-68 severe depression

69-85 very severe depression

Data collection procedure

Ethical permission was obtained from Instituitional Ethics Committee Ref no.(IEC-NI/13/APR/33/24). All the subjects satisfied the criteria were selected. The researcher approached each selected patient and explained the purpose and nature of the study. The patient's consent was obtained individually and a one to one interview was conducted to obtain background information and identify emotional impact. 30min was spent for each patient to collect the data.

Statistical analysis

Descriptive (Frequency, Percentage distribution, mean and standard deviation) and inferential (chi square) statistics were used.

RESULTS

Table 1. Frequency and Percentage Distribution of Background Variables among patients with Diabetic foot ulcer (N=20).

SI. NO	DEMOGRAPHIC VARIABLES	FREQUENCY	PERCENTAGE
1.	**Age (in years)** (a) 36-45	-	-
	(b) 46-55	4	20
	(c) 56-65	6	30
	(d) 66-75	10	50
2.	**Gender** (a) Male	16	80
	(b) Female	4	20

Cont... **Table 1. Frequency and Percentage Distribution of Background Variables among patients with Diabetic foot ulcer (N=20).**

3.	**Residence** (a) Rural	8	40
	(b) Sub urban	6	30
	(c) Urban	6	30
4.	**Educational Status** (a) No formal education	3	15
	(b) Primary	8	40
	(c) High school	6	30
	(d) Higher secondary	3	15
	(e) Collegiate	-	-
5.	**Duration of diabetes mellitus** (a) <1 year	-	-
	(b) 1-5 years	-	-
	(c) 5-10 years	6	30
	(d) >10 years	14	70
6.	**Glycemic control** PPBS (mg/ dl) a) <250	4	20
	b) 251-350	12	60
	c) >351	4	20
7.	**Wound classification** (a) IA	3	15
	(b) IIA	3	15
	(c) IIIA	14	70

Table 1 represents the frequency and percentage distribution of background variables. Majority, 10(50%) of the patients were between 66-75 yrs of age. With respect to gender 16(80%) were male. Maximum 8(40%) are from rural area. Majority of the patients educational status was primary school 8(40%). Regarding duration of diabetes mellitus 14(70%) has diabetes for more than 10yrs

Table 2. Mean and standard deviation of Emotional Impact of diabetic foot ulcer among patients with diabetic foot ulcer (N=20).

Si.no	Variable	Mean	Standard Deviation
1.	Emotional impact	39.35	10.68

Table 2 shows the mean emotional impact score as 39.35 with the SD 10.68.

Table 3. Level of emotional impact of foot ulcer among patients with diabetic foot ulcer (N=20).

Si.no	Level of emotional impact	Frequency	Percentage
1.	Normal	-	-
2.	Mild depression	06	30
3.	Moderate depression	12	60
4.	Severe depression	02	10
5.	Very severe depression	-	-

Table 3 depicts the level of emotional impact, 12 (60%) patients have moderate depression and 2(10%) of patients have severe depression and 6 (30%) have mild depression.

DISCUSSION

Description of the study variables

Table1 represents the frequency and percentage distribution of background variables. Majority, 10(50%) of the patients were between 66-75 yrs of age. With respect to gender 16(80%) were male. Maximum 8(40%) are from rural area. Majority of the patients educational status was primary school 8(40%). Regarding duration of diabetes mellitus 14(70%) has diabetes for more than 10yrs. Pengzi Zhang (2016) in is study on Global prevalence of diabetic foot ulcer the ulcer was found to be more prevalent in males, Type 2 Diabetes mellitus, older people and longer duration of diabetes[5].

The first objective of the study was to assess the emotional impact of foot ulcer among patients with diabetic foot ulcer.

The emotional impact of diabetic foot ulcer was assessed by using the Diabetic Foot Scale (DFS) developed by Johnson& Johnson (2002). Emotional domain that measures the level of depression consisted of 17 items in 5 point likert scale.

(Table 3) shows analysis of level of emotional impact, 12 (60%) patients have moderate depression and 2(10%) of patients have severe depression. The study findings are consistent with the results of Maria Teresa de Jesus Pereira (2014) study were 50 patients with diabetic foot ulcer was studied for feelings of powerlessness using Powerlessness Assessment Tool for adults (PAT) with the mean total score 50.12. The results indicate that patients with diabetic foot ulcers had strong feelings of powerlessness[6].

(Table 2) shows the mean emotional impact score to be 39.35 with the SD 10.68.

The second objective of the study was to associate the level of emotional impact of foot ulcer with selected background variables among patients with diabetic foot ulcer.

There was no significant association found between the background variables.

CONCLUSION

Depression symptoms at various levels are present among most of the patients suffering from diabetic foot ulcer due to long term management, life style modification and recurrence of disease. Nurses need to play an important role in meeting the emotional needs of patients and help to lead a better quality of life.

Conflict of Interest: Conflict of interest declared none.

Source of Funding: Self

Ethical Clearance: Ethical permission was obtained from Institutional Ethics Committee (Sri Ramachandra institute of higher education and research, Deemed to be University).

REFERENCES

1. Brownrigg, J.R.W., Apelqvist, J., Bakker, K., Schaper, N.C., and Hinchliffe, R.J., Evidence-based managed of PAD & the Diabetic Foot. Euro J Vasc and Endovasular Surg, 2013. 45(6): p. 673-681.
2. Yazdanpanah, L., Nasiri, M., Adarvishi, S., Literature review on the management of diabetic foot ulcer. World J Diabetes, 2015, 6(1): p. 37-53.
3. Centers for Disease Control and Prevention. Average Length of Stay (LOS) in Days of Hospital Discharges for Nontraumatic Lower Extremity Amputation with Diabetes as a Listed Diagnosis, United States, 1988-2009.

4. Goodridge D, Trepman E, Embil JM. Health-related quality of life in diabetic patients with foot ulcers: literature review. Wound Ostomy Continence Nurs. 2005 Nov-Dec;32(6):368-77.

5. Pengzi Zhang, Jing Lu, Yali Jing, Sunyinyan Tang, Dalong Zhu & Yan Bi. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Annals of Medicine.2017. 49(2): p.106-116.

6. Maria Teresa de Jesus Pereira, Geraldo Magela Salomé, Diego Guimarães Openheimer, Vitória,Helena Cunha Espósito, Sergio Aguinaldo de Almeida, Lydia Masako Ferreira. Feelings of Powerlessness in Patients with Diabetic Foot Ulcers. Wounds a compendium of clinical research and practice.2014;26(6):172-177.

A Review of Epidemiology of Unintentional Injuries among Children in India

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ABSTRACT

Childhood injury is a major public health problem which requires immediate attention. According to the estimates of WHO, Injuries are of higher health concern in every country causing over 3.8 million deaths per year where Unintentional injuries account for almost 90% of these cases in Lower and middle income countries. Every year, approximately 875,000 children are killed and nonfatal injuries affect the lives of 10 to 30 million globally. In India, the mortality rate related to injuries among under-5 children is contributing to 5.9% of the total deaths. The objective of this study is to estimate the magnitude and prevalence of Unintentional injury distribution & pattern among children of 1-18 years of age in India. A thorough search was done using PUBMED, BMC PUBLIC HEALTH, IJPH, BMJ PAEDIATRICS and finally narrowing down to Pub Med where the articles published for the last ten years were extracted. The studies revealed that RTI, Burns, Falls, Drowning & Bites were the common causes and where meagre knowledge associated to the given injuries were significant influence to the pattern of Injury occurrence. Distribution & pattern of injury, including the health- seeking behaviour seemed to vary across both in Rural and Urban settings. Poor perception of injury was a common indicator of prevalence. Strategies on prevention and education still need improvisation. 90% of childhood injuries were both predictable and preventable. Unintentional injuries account for both the functional and financial healthcare burden on population under unintentional injury.

Keywords used: *Injury in India, Unintentional injuries in India, Childhood injury in India, Childhood and Injuries.*

INTRODUCTION

The perspective of some events never takes a better turn until there is more importance added to it by valuing that with something of historical origin. As we go on the same path we realize that Injury is not an unknown guest. This dates back more than a hundred years ago in England where a playful adventure of an 8-month-old turned to a tragic case of drowning.[1] Unintentional injuries are one of the leading causes of death and disability among children in most of the countries.[2] According to the baseline scenario of 2015, the deaths due to unintentional injuries are higher than the intentional injuries.[3] Indirect estimates of WHO says Injuries are of higher health concern in every country causing over 3.9 million deaths per year where Unintentional injuries account for almost 90% of these cases in Lower and middle income countries, which show an emerging trend towards the rise of unintentional childhood injuries especially among the 0-14 years of age children. The commonly seen unintentional injuries are Road traffic injuries, fall, Burn, Drowning, Poison related injuries which may be due to ingestion of medication and chemical fluids. Each has its own numbers on prevalence data. [4,5]

A growing child has little knowledge about its surrounding environment and this presents bigger chances of hazardous encounters. Each child is unique to every parent, whether they live in an undeveloped or the urban atmosphere. Child survival is important for the improvement of both national and international

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child health.[6] While presently available data exhibit increased frequency of cases in the rural population (north or the southern part of India), the number of hospital episodes continues to increase among the rural community.[7] Where perceptions and treatment seeking behaviour are still a matter of high concern.[8,9] This can be a major indicator of under-reported information, therefore, presenting a necessity for further investigation. Moreover, the psychosocial torment the family of the injured child goes through is unquantifiable. Another important part of this scenario is the financial burden and healthcare need that it inflicts.[10,11] Disability adjusted life years (DALY) associated with unintentional injuries had indicated that there has been an increase in which proves to project greater chances of lifelong functional impairments and Disability. Studies affirm the facts that though hundreds of deaths occur due to injuries and/or violence, many millions suffer from non-fatal injuries according to injury prevention report of WHO. Distribution of the previously mentioned injuries is most prominent among males over female children.[12]

Rationale: Though most of the injuries are preventable and predictable, there is a lack of social and political will to make a radical change in the incidence rate. While some of the studies provide evidence to the prevailing scenario in the northern part of the country the evolving epidemiological trend is still to improve in the southern side in better understanding the burden of the present issue that could provide a better picture which can help discover feasible interventions.[13, 14]

MATERIALS AND METHOD

The review initiated by a self-addressed guideline which helped the researcher set clearly defined rules as to the screening of studies.

Eligibility criteria: The inclusion criteria for selecting the studies was

1) Study was done in India,

2) Studies were only related to injuries, and

3) The participants were <18 years. The articles extracted were full-text from the last 10 years.

There was heterogeneity of literature (including study designs and sample size) that was recorded in the review, so there were meagre reasons to perform any synthesis of quantitative information.

Exclusion criteria: Whereas violence-related injuries, International studies, Birth injuries, Studies that were addressing any birth defects or congenital abnormalities were rejected.

Information sources: The review began with a web-based search on Google, to get a clearer picture of the available studies. Further exploration of evidence was carried out using PUBMED DATABASE, BMC PUBLIC HEALTH, AJPH, and BMJ PAEDIATRICS and was later restricted to the PUBMED DATABASE to maintain the integrity of the search and because of the availability of full-text papers. The given MESH terms were used to gather additional literature (Table 1).

TABLE I: Mesh terms that were used to generate a search of articles

MESH Terms
Wounds AND Unintentional Injuries (INDIA)
"Unintentional" AND (Childhood Injuries) NOT Intentional
"Childhood injuries" AND India
Unintentional Childhood Injuries AND India NOT Disability
Wounds AND Injuries (Unintentional injuries in INDIA) NOT Elderly

Risk of bias detection: Review articles for Unintentional injuries were also checked for any details on reliable evidence on estimates under Indian scenario. Further, the articles were screened using strobes checklist for observational studies as well as the Cochrane Collaboration's tool also used for assessing risk of bias. Information regarding the quality of the papers was assessed exhibiting clarity in Inclusion/ Exclusion criteria, study design, operational definitions (if any), sampling strategies and also the duration of each study.

Every study was also screened for ethical clearance by an Institutional review board or ethical committee, Informed consent (especially for community-based studies) and also whether the informant was a parent or caregiver.

RESULTS

Study selection: After the exhaustive search, the studies were narrowed down to only studies done in India, out of which 8 studies were selected. Thereon the Mesh terms (Table 1) were customized for an effective search strategy. The keywords were initially used to acquire the current literature with any unintentional injury study with several study designs. Studies using randomized controlled designs and case-control study design were excluded. The search result of each search strategy was recorded. The recurrences of some articles were observed to be more than once is any of the employed strategies and therefore those articles was included. This additionally determined the quality of the studies selected.

Synthesis of results: The overall prevalence of Unintentional injury in the selected studies among the age group of 1-18 years was observed around 7% to 23%. The common cause of injury was fall, followed by RTA and Burns. The minimum study duration was two months and the maximum duration is one year. A Study was done by **Parameswaran** had clearly mentioned sampling methodology including the derivation method that contrasts between both rural and urban-based study. These studies provided a better foundation which followed a discrete structure of information using analysis. Community-based studies had well defined operational definitions, sampling strategies, and sampling interval. Majority of the studies were done in the rural part of India.[10]Some literature carried a rural community-based study covering households in villages and studies had defined operational definitions that addressed better clarity among each level of data collection. **WHO TEACH-VIP 2** Guidelines were adopted by community- based studies to develop questionnaires for a survey on injuries.[15] Certain studies have provided details regarding tools which were translated and back-translated and most of the study articles were cross-sectional designs. There was a lack of clarity of the statistical method used amongst substantial studies. Most of the literature contains analysis done using statistical package SPSS or EPI Info followed by STATA. Studies included used Chi-square analysis followed by other studies with only descriptive and frequencies. Most of the unintentional injuries seemed to occur during daytime.

A Study done by **Bhanderi** extracted basic information like demographic details alongside other information on injury by activity, severity and equipment-related injury and observed the difference in distribution of frequency of cases among male gender than females except for study done by **Bhuvaneshwari** which shows that females getting injured over males was significantly higher, the common injury being Falls.[14,16]Another study done by **Inbaraj** showed that 89.3% of the children with primary caregivers had a higher rate of injury as compared to mothers as caregivers. Further adding that most of the Unintentional injuries fall between 0 to 14 years.[9]The home was the most common place of injury especially in the summer and **Sivamani** highlighted an effective method of validation (Lincoln Peterson capture-recapture) that ensured complete coverage of household survey data. A meagre amount of households refused to give consent. The studies included had One year as recall period of any unintentional injury.[17]

DISCUSSION

Literature shows that the trend of unintentional injuries needs a significant attention and research especially from the caregiver point of view. In many situation the unintentional injuries goes unnoticeably.[18] There is still demand high quality study, because quality study will to help provide a wider picture and the true incidence of injuries.[19] The health-related quality of life is very poor, especially among children.[20,21] It could be an iceberg phenomenon that health research could assist within gathering evidence for more interventions, and these measures can create a need for longitudinal studies.[22,23] Present status has proven that surveillance has effective strategy to prevent child injury, the Haddon's Matrix model will help to understand prevention of unintentional injuries.[17] The current community-based studies show that there is an increase in cases and a decrease of case reporting in community, where case reporting is also importance. Institutional based studies are sometimes lacking in essential information, even if it's a minor injury and still, it requiring extra attention. The quality of community-based studies proves to be an actual picture of a current situation. The integration of child health programmes in prevention strategies would provide a sustainable result on reducing injuries.[24] Overall there are certain individual risk factors and other social and physical factors which may changes over time, resulting in the distribution of prevention strategies and risk

factors in low and middle- income countries (LMIC) due to globalization. The use of geospatial methods, like mapping, to understand the epidemiology is also help to estimating what is the present magnitude and pattern of occurrence of unintentional injuries in identifying priority areas. [25,26] These types of approaches may help to produce the validated research findings.

CONCLUSION

A safer home environment for a child should be available at every household. Most of the unintentional Injuries are preventable rather than accidental. It is still predictable and therefore preventable rather than inevitable. The community needs more awareness of the effects of unintentional injuries among children so that could help to make a better policy. Most of the evidence is either missing or they are lack of manpower to document any such epidemiological data, leading to undermining the quality of research.

Conflict of Interest: None.

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Ethical Clearance: Not Applicable

REFERENCE

1. Towner E, Towner J. Developing the history of unintentional injury: the use of coroners' records in early modern England. Injury prevention. 2000 Jun 1;6(2):102-5.
2. Mickalide A, Carr K. Safe Kids Worldwide: preventing unintentional childhood injuries across the globe. Pediatric Clinics. 2012 Dec 1;59(6):1367-80.
3. World Health Organization. Projections of mortality and burden of disease, 2004-2030. Geneva, Switzerland. 2008.
4. Balan B, Lingam L. Unintentional injuries among children in resource poor settings: where do the fingers point?. Archives of Disease in Childhood. 2012 Jan 1;97(1):35-8.
5. Chandran A, Hyder AA, Peek-Asa C. The global burden of unintentional injuries and an agenda for progress. Epidemiologic reviews. 2010 Apr 1;32(1):110-20.
6. United Nations. Department of Public Information. Millennium Development Goals Report 2009. United Nations Publications; 2009.
7. Sheriff A, Rahim A, Lailabi MP, Gopi J. Unintentional injuries among children admitted in a tertiary care hospital in North Kerala. Indian journal of public health. 2011 Apr 1;55(2):125.
8. Alrimawi I, Watson MC, Hall C. Prevention of unintentional injuries at home in children younger than 5 years in Ramallah District: a case study approach. The Lancet. 2017 Aug 1;390:S3.
9. Inbaraj LR, Rose A, George K, Bose A. Incidence and impact of unintentional childhood injuries: a community based study in rural South India. The Indian Journal of Pediatrics. 2017 Mar 1;84(3):206-10.
10. Parmeswaran GG, Kalaivani M, Gupta SK, Goswami AK, Nongkynrih B. Unintentional childhood injuries in urban delhi: A community-based study. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2017 Jan;42(1):8.
11. Thanh NX, Hang HM, Chuc NT, Lindholm L. The economic burden of unintentional injuries: a community-based cost analysis in Bavi, Vietnam. Scandinavian journal of public health. Supplement. 2003;62:45.
12. Verma S, Lal N, Lodha R, Murmu L. Childhood trauma profile at a tertiary care hospital in India. Indian pediatrics. 2009 Feb 1;46(2).
13. Mohan D, Kumar A, Varghese M. Childhood injuries in rural north India. International journal of injury control and safety promotion. 2010 Mar 1;17(1):45-52.
14. Bhuvaneswari N, Prasuna JG, Goel MK, Rasania SK. An epidemiological study on home injuries among children of 0–14 years in South Delhi. Indian journal of public health. 2018 Jan 1;62(1):4.
15. Meddings D. MENTOR-VIP—a global mentoring program for violence and injury prevention. Injury Prevention. 2007 Feb 1;13(1):69-.
16. Bhanderi DJ, Choudhary S. A study of occurrence of domestic accidents in semi-urban community. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2008 Apr;33(2):104.

17. Sivamani M, Balraj V, Muliyil JP. Validity of a surveillance system for childhood injuries in a rural block of Tamilnadu. Indian journal of community medicine. 2009 Jan;34(1):43.
18. Kalaiselvana G, Dongre AR, Mahalakshmy T. Epidemiology of injury in rural Pondicherry, India. Journal of injury and violence research. 2011 Jul;3(2):62.
19. Smith GS, Barss P. Unintentional injuries in developing countries: the epidemiology of a neglected problem. Epidemiologic reviews. 1991 Jan 1;13(1):228-66.
20. Jagnoor J, Prinja S, Christou A, Baker J, Gabbe B, Ivers R. Health-related quality of life and function after paediatric injuries in India: a longitudinal study. International journal of environmental research and public health. 2017 Sep 28;14(10):1144.
21. Watson MC, Errington G. Preventing unintentional injuries in children: successful approaches. Paediatrics and Child Health. 2016 May 1;26(5):194-9.
22. Renu G, George A. Childhood injury an iceburg of phenomenon. IOSR Journal of Dental and Medical Sciences. 2014;13(8):18-23.
23. Alonge O, Hyder AA. Reducing the global burden of childhood unintentional injuries. Archives of disease in childhood. 2014 Jan 1;99(1):62-9.
24. Bhamkar R, Seth B, Setia MS. Profile and risk factor analysis of unintentional injuries in children. The Indian Journal of Pediatrics. 2016 Oct 1;83(10):1114-20.
25. Soori H, Khodakarim S. Child unintentional injury prevention in Eastern Mediterranean Region. International journal of critical illness and injury science. 2016 Jan;6(1):33.
26. Alonge O, Khan UR, Hyder AA. Our shrinking globe: implications for child unintentional injuries. Pediatric Clinics. 2016 Feb 1;63(1):167-81.

Study of Socio-Demographic Factors Affecting Infant Feeding Practices in an Urban Area of Punjab

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ABSRACT

Background: Optimal infant and young child feeding practices rank among the most effective interventions to improve child health.Breastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants; it is also an integral part of the reproductive process with important implications for the health of mothers.Many factors affect the feeding practices including education of mother, awareness about the benefits of breastfeeding. So this study was done to know the factors affecting the infant feeding practices.

Material and Method: A cross-sectional study was conducted in the urban field practice area of the Department of Community Medicine of SGRD Institute of Medical Sciences and Research, Amritsar. All the mothers of infants were included in the study. House to house survey was done to collect information about demographic profile, feeding practices and socio cultural factors affecting the feeding practices.

Results: It was observed that 53.7% mothers started breast feeding next day, 30.4% mothers started breast feeding the same day and 16% started breast feeding immediately.Pre lacteal feed was given by most of the mothers (89.5%). The common pre lacteal feed given was honey (67.3%). The colostrum was given by 59.1% of mothers. Weaning after 6 months was started by 53.3% mothers while 46.7% started after 4 months. Education had a significant association with age at weaning (p=0.018).

Conclusion: The present study revealed that various inappropriate feeding practices are prevalent in the community.Health and nutrition programmes should be concentrated on counselling of the mothers regarding breastfeeding especially exclusive breastfeeding.

Keywords*: Feeding, Infant, mother*

INTRODUCTION

Optimal infant and young child feeding practices rank among the most effective interventions to improve child health. In 2006 an estimated 9.5 million children died before their fifth birthday, and two thirds of these deaths occurred in the first year of life. Under-nutrition is associated with at least 35% of child deaths. It is also a major disabler preventing children who survive from reaching their full developmental potential.It is estimated that sub-optimal breastfeeding, especially non-exclusive breastfeeding in the first 6 months of life, results in 1.4 million deaths and 10% of the disease burden in children younger than 5 years.[1]

Adequate nutrition during infancy and early childhood is fundamental to the development ofeach child's full human potential. It is well recognized that the period from birth to two years ofage is a "critical window" for the promotion of optimal growth, health and behavioral development. Longitudinal studies have consistently shown that this is the peak age for growth faltering, deficiencies of certain micronutrients, and common childhood illnesses such as diarrhea.[2]

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Efforts to promote modest nutritional improvements such as changes in feeding behaviour will have a beneficial impact on mortality rates over time. Feeding practices adopted by mothers depends on the knowledge, attitude, socio-cultural tradition they are exposed to.[3]

Breastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants; it is also an integral part of the reproductive process with important implications for the health of mothers. Review of evidence has shown that, on a population basis, exclusive breastfeeding for 6 months is the optimal way of feeding infants. Thereafter infants should receive complementary foods with continued breastfeeding up to 2 years of age or beyond.[4]

Despite the many benefits of EBF, sound breastfeeding practices are not the norm in many countries, and large differences exist in the EBF rates between regions and among countries. The promotion, support and protection of optimum breastfeeding take a different type of engagement from the health system, because breastfeeding is a social behaviour and not a medical practice. It is not a typical intervention, because there is no pharmaceutical product to be purchased or distributed and the practice is not dependent on a facility or health provider. These factors pose particular challenges, which have been addressed with varying degrees of success by breastfeeding programmes.[5]

According to DLHS 4 in Punjab, 56.4% of Children age 0-5 months are exclusively breastfed and 74.4% of Children age 6-9 months are receiving solid/semi-solid food and breast milk.[6]

Many factors affect the feeding practices including education of mother, awareness about the benefits of breastfeeding. So this study was done to know the factors affecting the infant feeding practices.

MATERIAL AND METHOD

A cross-sectional study was conducted in theurban field practice area of the Department of Community Medicine of SGRD Institute of Medical Sciences and Research from April to June 2014. The mothers of infants were included in the study. A pre-designed andpre tested questionnaire was used to collect theinformation. Verbal consent was taken from the mothers after explaining the purpose of the study. Out of 263 mothers 257 were willing to participate in the study.House to house survey was done to collect information about demographic profile, feeding practices and socio cultural factors affecting the feeding practices. Statistical analysis was done by using SPSS 20.0.

RESULTS

It was a cross-sectional study conducted in the field practice area of Community Medicine, SRDIMSAR, Amritsar. There were total 263 infants in the area.The mothers of these infants were contacted. Out of 263 mothers, 257 gave consent to participate in the study. Most of the mothers were in the age group of 25-30 years. 19.8% of mothers were illiterate and rest were literate up to different levels a shown in table. 87.9% were housewives. Education profile of the fathers revealed that 55.6% were educated up to matric, 26.8% up to middle school, 13.6% above matric and only 10 (3.9%) were illiterate. Majority of the fathers (61.9%) were semiskilled workers, 30.7% were skilled and 7.4% were unskilled workers (Table I).

There were 138 (53.7%) male and 119 (46.3%) female children. Out of total children, 56.4% belonged to joint family and 43.6% children were from nuclear family. Majority of the children (87.2%) were Sikh by religion and 12.8% were Hindus. It was observed that 45.9% mothers had one, 33.1% had two, 14.8% had three and 6.2% had four living children respectively. Immunization status of 87.2% children was complete till date.

While all the mothers started breast feeding their child after birth, 80.2% mothers were still practicing breast feeding at the time of study. It was observed that 53.7% mothers started breast feeding next day, 30.4% mothers started breast feeding the same day and 16% started breast feeding immediately. The most common reason for delay in breast feeding was due to no milk secretion (58.8%), followed by caesarean section (26.1%), traditional practice (10.9%), baby asleep (2.3%) and mother tired (1.9%) respectively.

Pre lacteal feed was given by most of the mothers (89.5%). The common pre lacteal feed given was honey (67.3%). The colostrum was given by 59.1% of mothers while 40.9% discarded the colostrum. The most common reason for not giving colostrums was ceasarian section in 45.7% of mothers, while in 38.09% of mothers it was no milk secretion and 16.19% thought it is harmful for the baby. Feeding on demand was practised in 68.9%

children, while 10.1, 6.6, 13.2 and 1.2% were fed after every 4h, 3 h, 2 h and 1 h respectively.

There was significant association between educational status of mother and initiation of breast feeding (p<0.018). Only 62.6% mothers knew about exclusive breastfeeding. There was significant association between education status of mother and knowledge of exclusive breastfeeding (p= .047). Weaning after 6 months was started by 53.3% mothers while 46.7% started after 4 months. Education had a significant association with age at weaning (p=0.018). 101 (39.3%) mothers used bottle along with breastfeed to feed their children

DISCUSSION

The study was done among the infant mothers. Most of the mothers were in the age group of 25-30 years. Similarly in a study conducted in rural area of Karnataka most of the females (60%) belonged to age group of 21-25 years[7]. 19.8% of mothers were illiterate and rest were literate up to different levels a shown in table. 87.9% were housewives.

There were 138 (53.7%) male and 119 (46.3%) female children. Out of total children, 56.4% belonged to joint family and 43.6% children were from nuclear family. Majority of the children (87.2%) were Sikh by religion and 12.8% were Hindus. It was observed that 45.9% mothers had one, 33.1% had two, 14.8% had three and 6.2% had four living children respectively. Immunization status of 87.2% children was complete till date.

While all the mothers started breast feeding their child after birth, 80.2% mothers were still practicing breast feeding at the time of study. It was observed that 53.7% mothers started breast feeding next day, 30.4% mothers started breast feeding the same day and 16% started breast feeding immediately. This is in contrast to the findings of study done among postnatal women in Southern India where 61% of mothers started breastfeeding within 1 hour.[8]The most common reason for delay in breast feeding was due to no milk secretion (58.8%), followed by caesarean section (26.1%), traditional practice (10.9%), baby asleep (2.3%) and mother tired (1.9%) respectively.In a study done in Meerut, among those (79.5%) who initiated breast feeding late, the most common reason was social custom and belief (52.8%). Other reasons were mother and baby illness (41.2%), breast milk insufficiency

(4.4%) and difficulty of infant to attach to breast (1.6%).[9]

Pre lacteal feed was given by most of the mothers (89.5%). The common pre lacteal feed given was honey (67.3%).In a study done in Warangal, TalanganaPrelacteal feed was given in 57% out of which 51.1% were given honey with fingers.[10]The colostrum was given by 59.1% of mothers. The most common reason for not giving colostrums was ceasarian section in 45.7% of mothers, while in 38.09% of mothers it was no milk secretion and 16.19% thought it is harmful for the baby. Feeding on demand was practised in 68.9% children. In a study done in a rural area of Puducherry demand feeding was practiced by 80.1% of mothers.[11]

Only 62.6% mothers knew about exclusive breastfeeding. There was significant association between education status of mother and knowledge of exclusive breastfeeding (p= .047). Weaning after 6 months was started by 53.3% mothers while 46.7% started after 4 months. Preferred food for weaning was homemade in 89.5% of mothers while in 10.5% it was ready to cook food. Similarly in a study done in rural Delhi, breast milk plus semisolids were being given to 65.1% infants 6-9 months of age.Homemade semisolids were more popular (72.6%) than commercial ones (24.5%).[12]39.3% mothers used bottle along with breastfeed to feed their children.

CONCLUSION

The present study revealed that various inappropriate feeding practices are prevalent in the community. Delayed initiation of breastfeeding, deprivation from colostrumand improper complementary feeding are practiced and this lead to under nutrition in children. Knowledge of mother, traditions, socioeconomic level of the family affects the feeding practices. Health and nutrition programmes should be concentrated on counselling of the mothers regarding breastfeeding especially exclusive breastfeeding.

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Ethical approval: The study was approved by theInstitutional Ethics Committee.

Table No: 1Socio-Demographic Profile of Respondents

Parameters	**Frequency**	**Percentage**
Age of mothers		
20-25	67	26.1
25-30	133	51.8
30-35	52	20.2
>35	5	1.9
Religion		
Sikh	224	87.2
Hindu	33	12.8
Type of Family		
Nuclear	112	43.6
Joint	145	56.4
Literacy Status of mother		
Illiterate	51	19.8
Middle	98	38.1
Matric	54	21.0
Above matric	54	21.0
Literacy status of Father		
Illiterate	10	3.9
Middle	69	26.8
Matric	143	55.6
Above Matric	35	13.6
Occupation of mother		
Housewife	226	87.9
Working Outside	31	12.1
No. of Living Children		
1	118	45.9
2	85	33.1
3	38	14.8
≥4	16	6.2

Table No: 2 Relation of Education with Feeding Practices

Practices evaluated	Education			
	Illiterate	Upto Middle school	Up to matric	Above matric
Initiation of breast feeding				
within 1 hr	7	12	6	16
same day	12	37	13	16
next day	32	49	35	22
Chi-square = 15.35, df = 6, p = 0.018				

Cont... **Table No: 2 Relation of Education with Feeding Practices**

Bottle feeding Yes	33	33	14	21
No	18	65	40	33
Chi-square = 19.153, df = 3, p = 0.000				
Weaning After 4 months	25	30	27	38
After 6 months	26	68	27	16
Chi-square = 15.35, df = 6, p = 0.018				
Knowledge on exclusive breastfeeding Yes	29	72	30	30
No	22	26	24	24
Chi-square = 7.95, df = 3, p = 0.047				

REFERENCES

1. Infant and young child feeding. WHO. Available at http://www.wpro.who.int/nutrition_wpr/publications/infantchildfeeding.pdf.
2. Guiding principles for complementary feeding of breastfed child. Pan American Health Organization. WHO. Available athttp://www.who.int/nutrition/publications/guiding_principles_compfeeding_breastfed.pdf
3. Ezechukwu CC, Egbuonu I, Ugochukwu EF, ChukwukaJO.Maternal attitudes to breast feeding in the concept of the Baby Friendly Hospital Initiative. Journal of BiomedicalInvestigations. 2004;2(2):82–85.
4. Nutrition. Exclusive breastfeeding. WHO. Available at: http://www.who.int/nutrition/topics/exclusive_breastfeeding/en/
5. Improving Exclusive Breastfeeding Practices. UNICEF Web-based Orientation Series for Programme and Communication Specialists. Availableat:
6. http://nutritioncluster.net/wpcontent/uploads/sites/4/2013/12/C4D-in-EBF-manual-6-15-2010-final.pdf
7. District Level Household and Facility Survey -4. State Fact Sheet, Punjab.Ministry of Health and Family Welfare. Available at: http://rchiips.org/pdf/dlhs4/report/PJ.pdf
8. Madhu K, Chowdary S and Masthi R. Breast Feeding Practices and Newborn Care in Rural Areas: A Descriptive Cross-Sectional Study.Indian J Community Med. 2009 Jul; 34(3): 243–246.
9. Kamath SP, Garg D, KhanMK, JainA , BaligaBS. Perceptions and Practices regarding Breastfeeding among Postnatal Women at a District Tertiary Referral Government Hospital in Southern India. Scientifica Volume 2016 (2016), Article ID 5430164, 6 pages
10. Singhal P, Garg SK, Chopra H, Jain S,Bajpai SK, Kumar A.Status of Infant and Young Child Feeding Practices With Special Emphasis on Breast Feeding in an Urban Area of Meerut. Journal of Dental and Medical Science. 2013;7(4):7-11
11. Kumari SMV, Rani GS, Rao BB. A Study on Infant Feeding Practices in Rural Areas of Warangal District, State of Telangana, India. International Journal of Contemporary Medical Research 2017;4(4):789-791.
12. **VijayalakshmiS, Patil R, Datta SS, Narayan KA and Stephen F.** Feeding Practices and Morbidity Pattern of Infants in a Rural Area of Puducherry-A Follow Up Study. J Community Med Health Educ 2014, 4:4
13. Taneja DK, Saha R, Dabas P, Gautam VP, Tripathy Y, Mehra M. A study of infant feeding practices & underlying factors in a rural area of Delhi. Indian Journal of Community Medicine 2003;3(3):107-11

On the Structure of Infant Mortality using Accelerated Failure Time (AFT) Model: A Comparative Study based on National Family Health Survey (NFHS) Data in India

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ABSTRACT

This paper analyzes the effect of socioeconomic and demographic factors on infant mortality in India. The Paper also examines reasonable comparison of the three National Family Health Survey (NFHS), the largest sample survey of India from the 1993-1994, 1998-1999 and 2005-06 with respect to different determinants of infant mortality. Accelerated Failure Time (AFT) model is used to measure the direct effect of the above factors on the survival of infants. On the basis of Akaikes Information Criterion (AIC), the Weibull AFT model fits better to other selected models. In all the three NFHS, Weibull AFT model shows the significant effect of the factors on infant survival.

Keywords: *Infant mortality, determinants of infant mortality, NFHS, Cox proportional hazard model, AFT model*

INTRODUCTION

Study about infant mortality rate as a measure of infant death and its pattern recognition play a crucial role of health status scenario for any nation as infant mortality rate is widely accepted and longstanding indicator of well-being of children. In this perspective the infant mortality rate is an important indicator of a country's financial welfare too. The infant mortality rate is defined as the number of infant death among 1000 live births during the first year of life. The study about infant of a country is one of the main focus for the planners of the country. In 2015, 4.5 million deaths occurred in the infancy period (WHO, report). Infant mortality rate is high in developing countries like India, as compared to developed countries. India has experienced an impressive decline in infant death during the previous two decades but, it is still high as compared to other developed countries.

Thus, study of the overall picture of the survival of infant "Survival Analysis" provides the platform to move further in the same line. Survival analysis is defined as a collection of methods for analyzing the data where the response variable is time until the occurrence of death of infant. The field of survival analysis experienced tremendous growth during the latter half of the 20th century. The most profound methodologies so developed are Kaplan-Meier (non-parametric survival method) used to estimate the survival function (i.e. probability that an infant survive beyond a given time), and the Cox proportional hazard model (semi-parametric method) to examine the covariate effects on the hazard function (i.e. risk of death, in an interval after a specified time, condition on the subject having survived to that specified time).

Various studies on the basis of these models have been done, such as unsupplemented breastfeeding is more strongly associated with fewer infant deaths than supplemented breastfeeding[1]. In Egypt, the effect of household income on infant and early childhood mortality at household level is studied by incorporating the socioeconomic and demographic variables[2]. An attempt has been made to examine the impact of breastfeeding and maternal health care programs on infant mortality corresponding to other socio-demographic variables[10]. It is also essential to study the behavior of infant deaths in developing countries like India and determine the effect of demographic and socioeconomic factors on infant mortality.

Although the Cox-proportional hazard model finds widespread applicability in the survival analysis, it holds

some assumptions such as the regression coefficient is constant over time (proportional hazard assumption), combination of the covariates is linear and link function is exponential. Proportional hazard (PH) assumption received most attention in research and applications as well. Most research often ignored and not checked the assumption of proportional hazard that makes the result's accuracy questionable. Our study tests the assumption of proportional hazard on the basis of schoenfeld residuals and reaches to conclusion that the PH assumption is not fulfill. Therefore Cox-PH model could not be applied to test the effect of different covariates on the survival of infants. Thus, in this situation Accelerated Failure Time (AFT) model is a good alternative over these models, which relaxes the PH assumptions. AFT model is a parametric survival model used to see the pattern of the survival time of infants corresponding to different covariates. AFT models measure the direct effect of the explanatory variables on the survival time instead of hazard in Cox-PH models. This characteristic of AFT model makes easy interpretation of the results, because parameter measures the effect of corresponding variable on the mean of survival time.

DATA AND METHODOLOGY

The National Family Health Survey (NFHS) was carried out as the principal activity of a collaborative project to strengthen the research capabilities of the Population Research Centers in India, initiated by the Ministry of Health and Family Welfare, Government of India, and coordinated by the International Institute of Population Science (IIPS) Mumbai. There have been four rounds of the survey conducted, during 1992-93 (NFHS I), 1998-99 (NFHS II), 2005-06 (NFHS III) and in 2015-16 (NFHS IV) but, till now information on fourth round is not available. The main objective of the NFHS survey is to provide reliable and up-to-date information on various demographic events and their estimates for a comprehensive portrait of population, health, and nutrition in India as well as in each of its states. This paper utilizes three NFHS's (NFHS I, NFHS II and NFHS III) for the survival analysis.

AFT model is used to see the association of survival time of infants with covariates. For a random time to event T_i an AFT model proposes the following relationship between explanatory variables and $\mathrm{Y_i} = \log \mathrm{T_i}$.

$$\mathrm{Y_i} = \mu + \alpha_1 \mathrm{x_{1i}} + \alpha_2 \mathrm{x_{2i}} + \cdots + \alpha_\mathrm{j} \mathrm{x_{ji}} + \cdots + \alpha_\mathrm{k} \mathrm{x_{ki}} + \sigma \epsilon_\mathrm{i}$$

where μ is intercept term, x_{ji} is the value of the jth explanatory variable, $j = 1, 2, \ldots, k$, for the ith individual, $i = 1, 2, \ldots, n$, σ is the scale parameter and ϵ_i is a random variable assume to have particular distribution. On the basis of this relationship survival function at time t is defined as,

$$S_i(t) = P(T_i \geq t)$$

$$S_i(t) = P[\exp(\mu + \varphi x_i + \sigma \epsilon_\mathrm{i}) \geq \mathrm{t}],$$

where

$$\varphi x_i = \alpha_1 x_{1i} + \alpha_2 x_{2i} + \cdots + \alpha_k x_{ki}$$

Now $S_i(t)$, can be written in the form,

$$S_i(t) = P\left[\exp(\mu + \sigma \epsilon_\mathrm{i}) \geq \frac{t}{\exp(\varphi x_i)}\right]$$

$$S_i(t) = S_0\left[\frac{t}{\exp(\varphi x_i)}\right]$$

where $S_0(t)$ is the baseline survival function i.e. the survivor function of an individual at $x_i = 0$. $\exp(-\varphi x_i)$ is said to be the acceleration factor (Y) for the ith individual.

RESULTS

The survey included 11047, 7345 and 8301 live births in NFHS I, II and III respectively. Out of complete information, 2053 (18%), 975 (13%) and 1595 (19%) deaths have been occurred in the respective NFHS. Among the live births, 5672(5372), 3862 (3483), 4245 (4056) were male (female) and proportion of infants belonging to rural area was 76% in NFHS I and II while this proportion was 66% in NFHS III. 66%, 53% and 48% infants were those whose mothers were not educated in the three surveys. 15% of infants were not breastfed in NFHS I and III, while this proportion was 12% in NFHS II.

Table 1, shows the results of global Schoenfeld test regarding to NFHS I, II, & III which was used to test the independence between time and Schoenfeld residuals. An insignificant relationship between residuals and time supports the proportional hazard assumption. Breastfeeding, mother education, birth order and place of delivery have shown a significant relationship between residuals and time in all three NFHS. Global test was also significant which means that the proportional hazard assumption not met in whole the model. Results of Table 1, summarizes that the Cox-proportional hazard model could not be applied for infant mortality. Alternative approach of Cox-proportional hazard model i.e. AFT model was used which relaxed this assumption but required to determine the probability distribution of data set. Weibull versus exponential versus logistic were proposed distributions for the study. The selection of the distribution was done on the basis of Akaike's information criterion (AIC). Table 2, shows AIC value of different AFT models corresponding to each NFHS and it was minimum for the Weibull distribution. Therefore, this study included Weibull AFT models to study the effect of different covariates on infant survival.

Figure 1, which was based on NFHS I data, illustrates that the survival curve was highest for the infants who were breastfed, lower for the first and fourth order birth, higher when mother delivered their child in private hospital, lower for the infants whose mothers were not educated, higher for the infants who were born in first four years of marriage span of their parents, lower for the infants who came from small family size (i.e. 1-3 members in the household) as compared to other categories. Figure 2 and 3, show that the infant survival curves in NFHS II and III, have quite similar pattern as NFHS I.

In this study AFT model included nine covariates (breastfeeding, mother age, mother education, place of delivery, sex of infant, place of residence, marital status, birth order and family size) to see the effect of these covariates on the survival of infants regarding to each NFHS. Table 3, shows the results of AFT model on the basis of NFHS I, II and III data. Firstly, we will discuss the results of AFT Model related to NFHS I data. On the basis of these results, survival time for the infants who were breastfed was significantly increased by the factor 41.674 as compared to the infant who were not breastfed. Risk of death was high for first birth order and significantly decreased two times in second birth order as compared to the first. Survival of Infants having mother age 30-34 years was strong and significantly increased 1.519 times as compared to the infants of mother age 15-18 years. Survival of infant was high in the category where mother belonged to the secondary and higher level group of education and increased by 2.125 times as compared to the infant whose mother was not educated. Survival of infants who were born in a private hospital was significantly increased nearly three times as compared to the infant who were born at home. Survival time of infant where the duration of the parents marriage was 5-9 years and more than 9 years was significantly decreased by the factor 0.362 and 0.282 times respectively as compared to early married couple (0 – 4 years of parents marriage). Survival of the infant who belonged to family size of 4-6 members was significantly increased 1.5 times as compared to the infants who belonged to small family size of 1 – 3 members. Survival of the infant who belonged to the urban area was significantly better than the infant of rural area and also better for female child as compared to the male child. Behavior of AFT model related to NFHS II and III data was similar to NFHS I data except some significant results, which are discussed in the next section.

Discussion and Comparison among NFHS's

In all three NFHS, survival of infants was highly increased, where they were breastfed, even in the presence of control for several other demographic factors. Breast milk is nature's perfect food for the baby and it contains immunity boosting antibodies and healthy enzymes. Finding is consistent with the

finding from other authors[6,8]. In NFHS II, delivery in government hospital was not significantly associated with infant survival, while in NFHS I and III, survival of infants significantly increased when delivery took place in a government hospital as compared to home. In NFHS I and III, survival of female child was significantly increased, but in NFHS II it was not significant may be because of less careness, social taboos among the other reasons. Survival of infant belonged to small family size was decreased in each NFHS as compared to big family size. Small family does not have proper required care to infant may be one reason for less survival. Joint families have lower risk of infant mortality as compared to nuclear families[4].

Rural area has significantly poor survival of infants as compared to urban areas and this pattern was similar in all three NFHS. It means that till NFHS III there was no sufficient development of health improvement conditions as required in rural areas. Survival of infants significantly increased for those who were born from early married couples, may be due to the decrement of fertility in women after a fix period of time. Survival of infant of mother age 15 – 18 years was significantly decreased in NFHS I and II, but in NFHS III, result was not significant may be due to the medical awareness in the population. In all three NFHS, survival of infants significantly increased, when mothers were highly educated as compared to uneducated mothers. The educated mothers have better knowledge of health care facilities. They are expected to live in healthy environment and take more nutritious food. Maternal education results into rising the age at marriage, enhances socioeconomic status and emphasis on child quality[3] and hence reduces the risk of death in infancy.

CONCLUSION

It is observed from the study that the most relevant factor for the survival of the infant is breastfeeding, therefore it should be encouraged. For achieving the required reduction in infant mortality government should further make some policies to develop rural areas and to reduce the risk of survival of the first birth order, proper medical care should be implemented in addition to existing one. There is also an essential requirement to encourage female for the higher education level and discourage home delivery. On the basis of this study we may conclude that small family size has a negative impact and the couples who plan their child during the first four year of their marriage have a positive impact on the survival of infant.

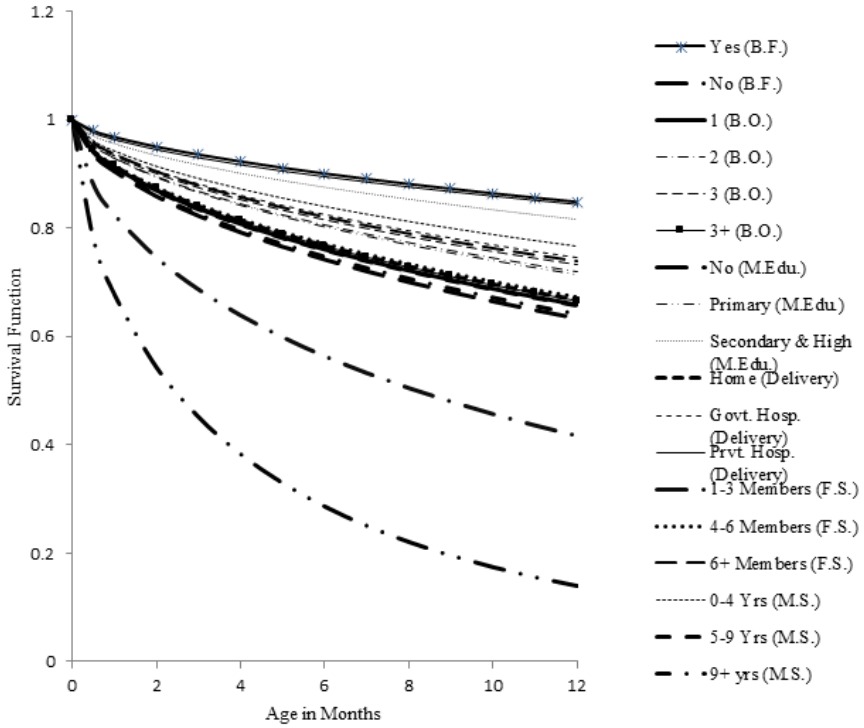


Figure 1: Parametric Survival Curves for NFHS I

Note: B.F.: Breastfeeding
B.O.: Birth Order
M.Edu.: Mother Education
F.S.: Family Size
Govt. Hosp.: Government Hospital
Prvt. Hosp.: Private Hospital
M.S.: Marital Status

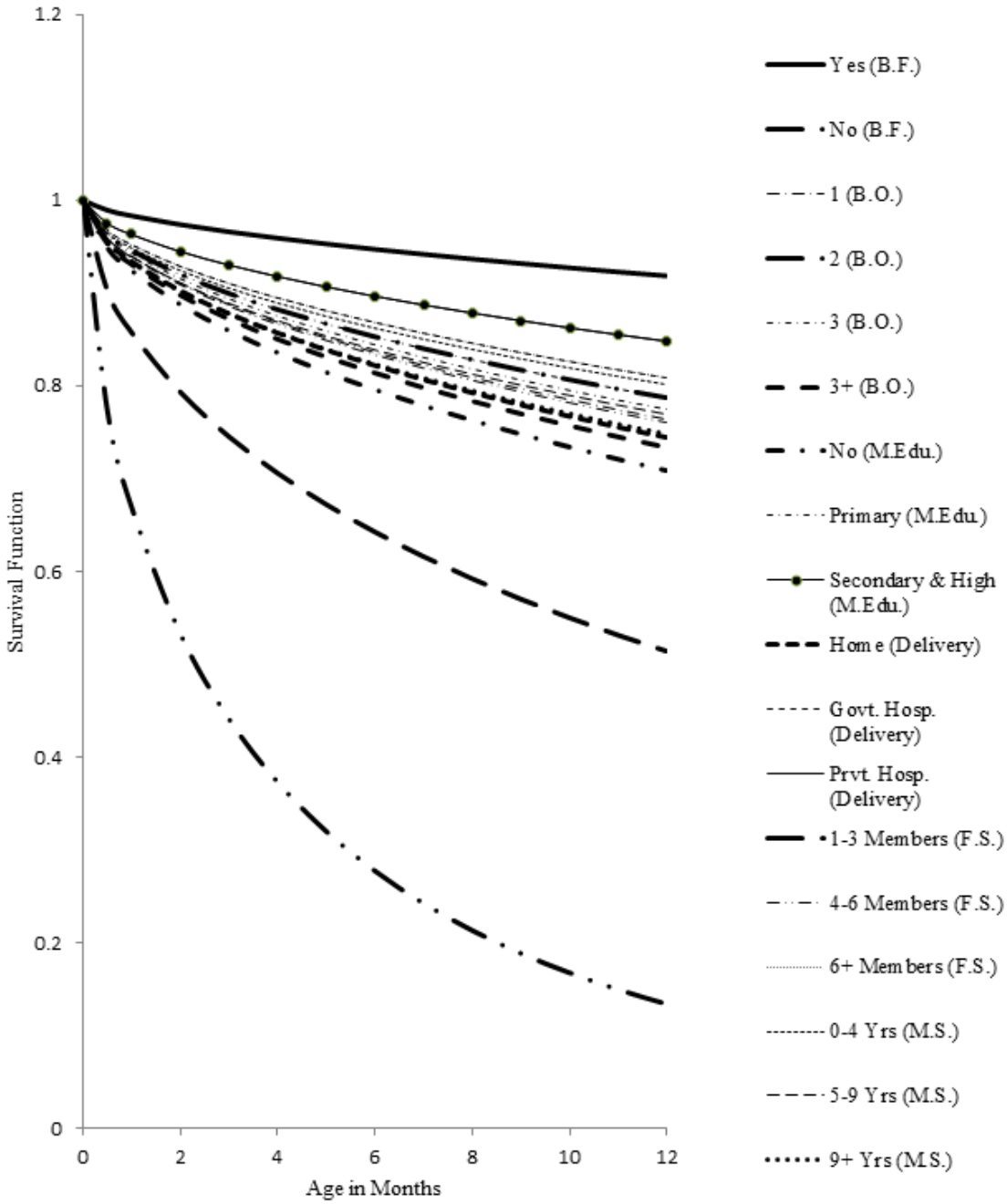


Figure 2: Parametric Survival Curves for NFHS II

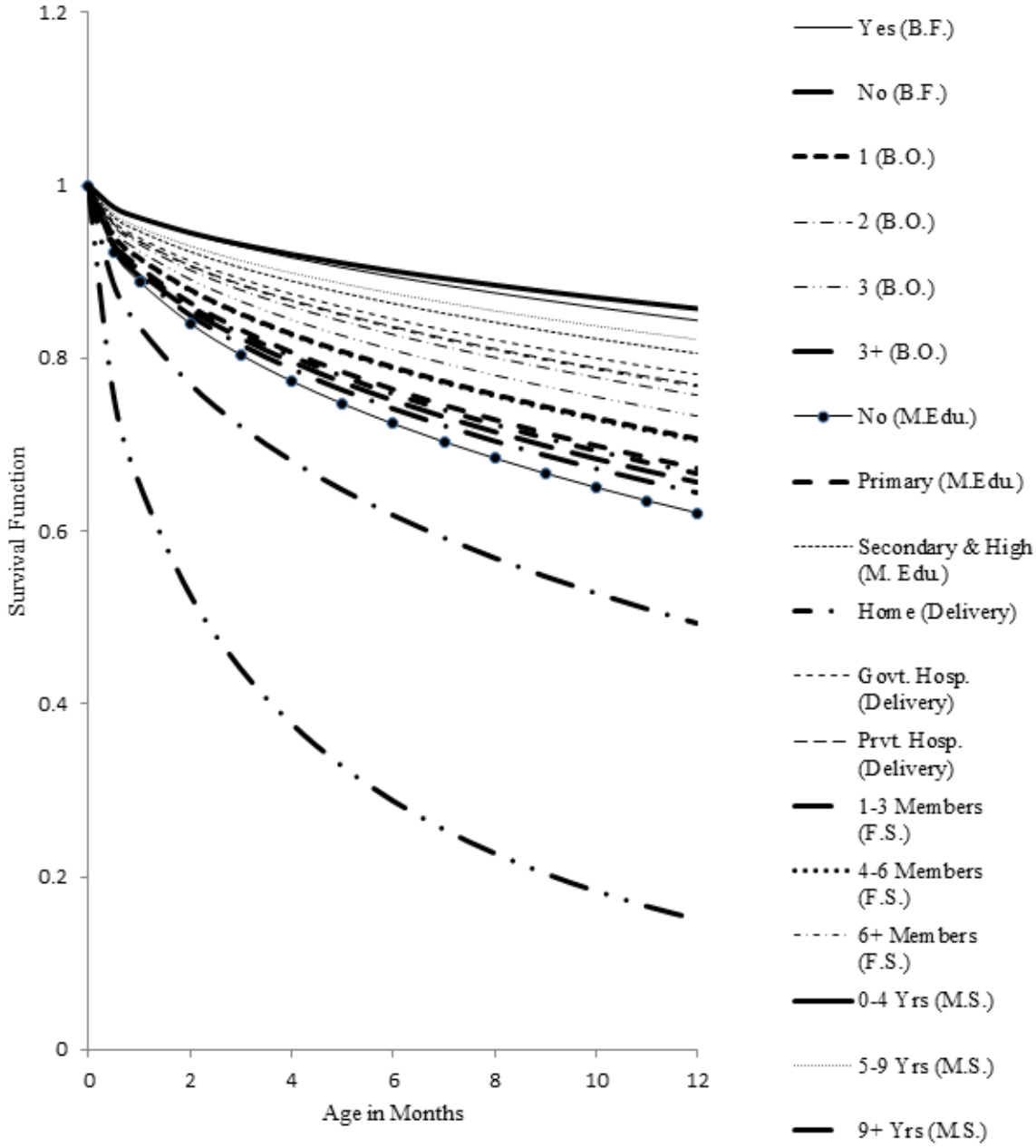


Figure 3: Parametric Survival Curves for NFHS III

Table 1: Global Schoenfeld test for NFHS I, II & III data

Covariates	χ^2 NFHS I	p-value NFHS I	χ^2 NFHS II	p-value NFHS II	χ^2 NFHS III	p-value NFHS III
Breastfeeding	9.091	0.003	5.300	0.021	9.753	0.002
Mother age	0.372	0.542	1.090	0.296	0.6412	0.423
Sex	2.868	0.090	3.460	0.063	3.3803	0.066
Mother education	10.239	0.001	5.870	0.015	8.572	0.003
Birth Order	14.072	0.000	3.170	0.075	6.0943	0.014
Place of delivery	22.305	0.000	19.000	0.000	31.621	0.000
Place of residence	0.28	0.597	2.210	0.137	1.9197	0.166
Marital Status	0.221	0.638	1.140	0.285	0.0263	0.871
Family size	1.188	0.276	0.000	0.989	3.3707	0.066
GLOBAL	116.169	0.000	94.000	0.000	130.1486	0.000

Table 2: AIC values for different Distribution

Models	AIC Values
NFHS I	**-**
Weibull	20089.14
Exponential	20900.53
Logistic	26273.03
NFHS II	**-**
Weibull	9997.88
Exponential	10487.33
Logistic	13272.76
NFHS III	**-**
Weibull	15998.04
Exponential	16906.59
Logistic	21621.53

Table 3: Summary Statistics of AFT Model for NFHS I, II & III

Covariates	p-value NFHS I	Y NFHS I	p-value NFHS II	Y NFHS II	p-value NFHS III	Y NFHS III
Intercept	0.000	1.594	0.000	1.599	0.000	2.404
Breastfeeding	0.000	41.674	0.000	132.687	0.000	41.720
Mother Age (years)						
15 – 18[a]	-	-	-	-	-	-
19 – 24	0.022	1.230	0.322	1.124	0.621	0.939
25 – 29	0.003	1.383	0.002	1.930	0.528	1.094
30 – 34	0.002	1.519	0.064	1.317	0.465	1.129
35 and more	0.017	1.459	0.014	3.724	0.979	1.005
Mother Education						
No Education[a]	-	-	-	-	-	-

Cont... **Table 3: Summary Statistics of AFT Model for NFHS I, II & III**

Primary	0.152	1.121	0.004	1.368	0.439	1.074
Secondary or Higher level	0.000	2.125	0.000	2.157	0.000	2.266
Birth Order						
1[a]	-	-	-	-	-	-
2	0.000	2.139	0.588	0.941	0.000	2.863
3	0.000	2.842	0.205	1.202	0.000	4.293
4 and more	0.000	2.702	0.032	1.416	0.000	3.551
Place of Delivery						
Home[a]	-	-	-	-	-	-
Govt. Hospital	0.006	1.266	0.149	1.175	0.004	1.308
Private Hospital	0.000	2.981	0.000	1.771	0.000	1.417
Other	0.054	0.404	0.763	1.192	0.397	0.704
Place of Residence						
Rural[a]	-	-	-	-	-	-
Urban	0.000	1.367	0.000	1.499	0.000	1.457
Sex	0.000	1.309	0.125	1.127	0.001	1.233
Marital status (years)						
0 – 4[a]	-	-	-	-	-	-
5 – 9	0.000	0.363	0.091	0.818	0.000	0.186
10 and more	0.000	0.282	0.003	0.592	0.000	0.157
Family Size						
0 – 3[a]	-	-	-	-	-	-
4 – 6	0.000	1.427	0.000	1.503	0.023	1.245
7 and more	0.000	1.725	0.000	1.484	0.000	1.645

Note: [a]Reference Category

Ethical Clearance- Research is based on the secondary data of National Family Health Survey, India.

Source of Funding- NIL

Conflict of Interest - NIL

REFERENCES

1. Butz WP, Habicht JP, Davanzo J. Environmental factors in the relationship between breastfeeding and infant mortality: The role of sanitation and water in Malaysia. American Journal of Epidemiology, 1984; 119 (4): 516-525.
2. Casterline JB, Cooksey EC, Ismail AF. Household income and child survival in Egypt. Demography, 1989; 26: 15-35.
3. Caldwell JC. Education as a factor in mortality decline: An examination of Nigerian data. Population Studies, 1979; 33(3): 395-413.
4. Hirve S, Ganatra B. A Prospective Cohort Study on the Survival Experience of under-five children in rural western India. Indian Pediatrics, 1997; 34: 995-1001.
5. Hobcraft JN, McDonald JW, Rutstein SO. Socio economic Factors in Infant and Child Mortality: Across-national Comparison. Population Studies, 1984; 38(2): 193-223.
6. Jelliffe DB, Jelliffe EFP. Human Milk in the Modern World. Oxford University Press, Oxford, 84 – 96.
7. Muluye S, Wencheko E. Determinants of infant mortality in Ethiopia: A study based on the 2005 EDHS data. Ethiop. J. Health Dev. 2012; 26 (2): 72-77.
8. Nath DC, Donna LL, Steel MS. Analysis of birth interval in a non contracepting Indian population. University of Washington, 1993.

9. National Family Health Survey: (NFHS) Reports for India and states (1992-93, 1998-99 and 2005-06).
10. Singh KK, Pandey N, Gautam A. Effect of Breastfeeding and Maternal Health Care Programme on Infant Mortality. Demography India, 2007; 36 (2): 253-266.
11. World Health Organization: Care of mother and baby at the health center: A practice guide, Geneva: Maternal Health and Safe Motherhood Programme, Division of Family Health. 1994.

Retinal Degeneration Using Iris Image through Machine Learning

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ABSTRACT

Retinal degeneration is one of the vital cause of vision loss. Retinal degeneration is induced through the declension of the retina middle part. The aim of our proposed paper is to detect retinal degeneration by using image processing. This involves segmentation, plotting of histogram, edge detection. The performance measures are calculated and compared with those of normal eye by machine learning singular value decomposition and correlation..

Keywords: *Retinal degeneration, machine learning, histogram, edge detection*

INTRODUCTION

Now a day the main challenge faced by developing countries is to prevent the eye diseases like retinal degeneration. Generally most affected are senior citizens. Reason for degeneration is eventually demise of cells in retina. Other cause of degeneration is blockage in vein and diabetic. The degeneration causes blindness, starting with tunnel vision, peripheral sight loss. The main cause of these types of degeneration is genetic disorder and ageing. It is necessary for new approaches involving machine learning to diagnose and detect these effects in time for better health adjunct system. It helps the society. Damage of macula and a little blemish approximately close to the midpoint of eye in the retina leads to retina degeneration and subsequently vision loss. The retina degeneration progress is quick and might lead to vision loss in one eye or both eyes. The general symptom of retina degeneration is blurred region close to the mid of vision.

In past years researchers much attention about the sight of the eye [2,11,13]. Several attempts have been deal to search for eyes degeneration[5,7]. Contrast and balance of gray is useful to identify feature extraction[9,17,14]. in [3], discuss about the correlation between iris. Analyses of iries and eyelids are using Hough Transform [10-12]. Filters are used to identify the detection of edges [6], Fractal features extraction are used for image segmentation[4,8,16]. In[9] used the images of facial through dimension of fraction. In [15], a method to identify the shape of the iris images. This is done through image segmentation and edge detection[18]

Data Base

The data base in this work is Retina Gallery. Fig.1 depicts the iris of a normal person.



Fig:1 Normal Iris

METHODOLOGY

The methodology involves, machine learning and singular value decomposition. The images are selected from the database and processed using MATLAB-R2015a. For each image are computed. Singular value decomposition is used for comparison of normal iris and degenerated iris.

If a real symmetric n x n matrix and orthogonal matrices U and V and a diagonal matrix S then .

Any non-square matrix is converted as a square matrix through singular value decomposition in order to calculate the correlation coefficient. Detection is mainly based on the comparison of correlation coefficient normal iris and degenerated iris.

RESULTS AND DISCUSSION

The performance measures namely sensitivity, specificity and accuracy of the input image are calculated in order to diagnose the iris for detection of macular degeneration. Through the diagnostic test the result shows that the output is true positive that means not affected from disease. It measures the degree of veracity of a diagnostic test on a condition.

These measures are based on the results of edge detection described in the following

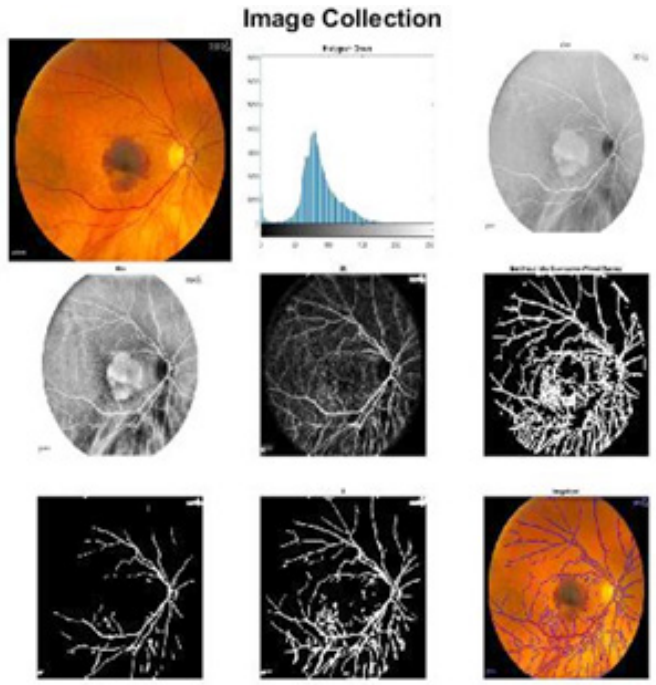


Fig:2 Stages of segmentation, edge detection

Fig :2 shows that the stages of segmentation, edge detection of degenerated eye through machine learning. It shows that it connects to the brain and the blood vessels are connect through the nerve. This ratio is a measure of the growth of macular degeneration. The histogram plot reveals the intensity of light distribution and peak of macular degeneration black spot.

The original image is converted from RGB to Green Channel. Complement structuring Element Morphological operation is done by Median Filter. Removal of background and image adjustment using intensity of the image is performed. It reveal that it show the segmentation of the retina blood vessels through morphological operation.

Below table presents to numerical measures for parameters A is accuracy, S is sensitivity, Sp is specification

Table1. Numerical measures of parameters

Image	A	S	Sp	Fpr 1-specificity	PPV
1	0.8118	0	1	0	0
2	0.7597	0	1	0	0
3	0.9348	0.7160	0.9919	0.0031	0.7377

Cont... **Table1. Numerical measures of parameters**

4	0.6624	0	1	0	0
5	0.8485	0	1	0	0
6	0.9355	0	1	0	0
7	0.8783	0	1	0	0
8	0.9614	0.0355	1	0	1
9	0.8449	0.0436	0.993	0.006	0.9250
10	0.9005	0	1	0	0
11	0.6973	1	1	0	0
12	0.9142	0.0152	0.9998	0.002	0.8750
13	0.9620	0	1	0	0
14	0.3625	0	1	0	0
15	0.8879	0	1	0	0
16	0.8690	0	1	0	0
17	0.8612	0.0082	0.9993	0.006	0.6667
18	0.8733	0	1	0	0
19	0.9449	0	1	0	0
20	0.8441	0	1	0	0

The above table 1 shows that the numerical measure of the parameter. The conclusion on the comparison of normal iris and degenerated iris is based on correlation coefficient values. In table 2, detection of macular degeneration is presented along with correlation coefficient. The value of the sensitivity and specification shows that iris information. Form the above results to compare the iris using correlation analysis.

Table2. Correlation coefficient & detection of retina degeneration

Image	Correlation coefficient	Detection
1	0.0053	Affected
2	0.0060	Affected
3	0.0046	Affected
4	0.0008	Affected
5	0.0077	Affected
6	0.0048	Affected
7	0.0072	Affected
8	0.0042	Affected
9	0.0023	Affected
10	0.0075	Affected
11	0.0031	Affected

Cont.. **Table2.**

12	0.0022	Affected
13	0.0064	Affected
14	0.0042	Affected
15	0.0033	Affected
16	0.0089	Affected
17	0.0106	Affected
18	0.0078	Affected
19	0.0044	Affected
20	0.0107	Affected

Histogram

Drusen identify through asymmetric distribution from histogram of an image .Compounding of like distribution in asymmetric kurtosis. The statement of the skewness is given below table3

Table: 3 Histogram distribution analysis

Skew is equal to zero	Playtkurtic	Output of an image is combining of more than two distribution
	Mesokurtic	Only one distribution is close to large drusen
	Leptokurtic	Incessant background
Positively skewed	Playtkurtic	Drusen in approximately equally distributed
	Mesokurtic	Difficult to differentiate
	Leptokurtic	Little area of drusen of eminent chroma
Negatively skewed	Playtkurtic	90 % of the area is in background
	Mesokurtic	90 % of the area is in background
	Leptokurtic	90 % of the area is in background

Normal image histogram

From the below Fig: 3 shows that according to shape of skewers is positive. So difficult to differentiate drusen from the images

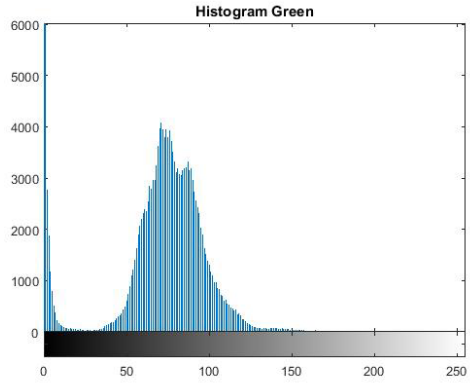


Fig:3 Normal Image Histogram

Abnormal image

From the below Fig: 4 shows that according to shape of skewers is negative and more 90 % of drusen is distributed in an image

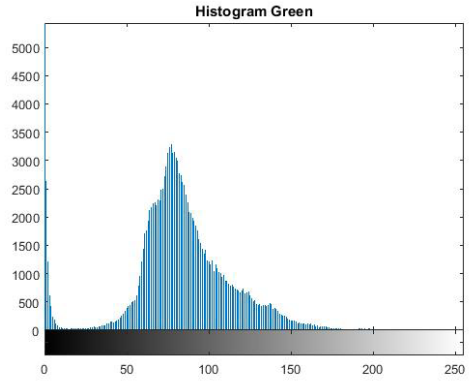


Fig :4 abnormal image histogram

CONCLUSION

The proposed robust methodology to detect retina degeneration using singular value decomposition and correlation coefficient is discussed. Singular value decomposition extraction is used to select the most significant segmentation method for identifying retina degeneration. From histogram analysis to identify the stage of degeneration . These results are very useful to finding the retina degeneration. .

Compliance with ethical Standards

Ethical approval: The article does not contain any studies with human participants or animal performed by any of the authors.

Source of Funding - Self

Conflict of Interest: The authors declare that they have no conflict of interest.

Informed consent: Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Alamelu Mangai, Jagadish Nayak and V. Santhosh Kumar A Novel Approach for Classifying Medical Images Using Data Mining Techniques, J., International Journal of Computer Science and Electronics Engineering, 2013, 1(2), 188-192.
2. Bianchini, M., Sarti, L., An eye detection system based on neural autoassociators, artificial neural networks in pattern recognition, In: Proceedings Second IAPR Workshop, ANNPR 2006, 244–252.

3. Dobes, M., Martineka, J., Skoupila D., Dobesovab, Z., Posps, J., Human eye localizationusing the modified Hough transform, Optik , 2006, 117, 468–473.

4. Du, G., Eye location method based on symmetry analysis and high-order fractal feature, IEE Proc. Vis. Image Signal Process. 2006, 153 (1),11–16.

5. Inbarathi.R, Karthikeyan.R Detection of Retinal Hemorrhage in Fundus Images by Classifying the Splat Features Using SVM, International Journal of Innovative Research in Science, Engineering and Technology, 2014, 3(3),1979-1986.

6. Kim, H., Lee, H.J., Kee, S.C. Seok, A fast eye localization method for face recognition, In: 13th IEEE International Workshop on Robot and Human Interactive Communication,2004, 241–245.

7. Koh, E.J., Rhee, P.K., Image context-driven eye location using the hybrid network of k-means and RBF, Lecture Notes in Computer Science, v 4222LNCS-II, in: Advances in Natural Computation—Second International Conference, ICNC 2006, Proceedings, 2006, 540–549.

8. Langade Umesh, Malkar Mrunalini, Swati Shinde, Review of Image Processing and Machine Learning Techniques for Eye Disease Detection and Classification, International Research Journal of Engineering and Technology, 2016, 3(3),547-551.

9. Lin, K.H., Lan, K.M., Siu, W.C., Locating the eye in human face images using fractaldimensions, IEE Proc. Vis. Image Signal Process. 2001, 148, 413–421.

10. Machala, L., Pospısil, J., Proposal and verification of two methods for evaluation of the human iris video- camera images, Optik , 2001, 112 (8), 335–340.

11. Mandelbrot, B.B., Fractal analysis and synthesis of fracture surface roughness and related forms of complexity and disorder, Int. J. Fract., 2006, 138 (1–4), 13–17.

12. Nagarajan.D (2017) Three dimensional visualization of brain using machine learning. International Journal of pure and applied mathematics, Vol:117 no.7,pp.459-466.

13. Parul , Neetu Sharma, A Study on Retinal Disease Classification and Filteration Approaches, International Journal of Computer Science and Mobile Computing, 2015, 4(5), 158-165.

14. Preeti, Jyotika Pruthi, Review of Image Processing Technique for Glaucoma Detection, International Journal of Computer Science and Mobile Computing, 2013, 2(11), 99-105.

15. Rizon, M., Kawaguchi, T., Automatic eye detection using intensity and edge information, Proc. IEEE TENCON 2, 2000, 415–420.

16. Yan, S.C.H., He, X.F., Hu, Y.X., Zhang, H.J., Bayesian shape localization for face recognition using global and local textures, IEEE Trans. Circuits Syst. Video Technol., 2004, 14(1),102–113.

17. Zhang, L., Lenders, P., Knowledge-based eye detection for human face recognition, in: Proceedings of the 4th Int. Conf. on Knowledge-based Intelligent Electronic System, KES, 1, 2000, 117–120.

18. Nagarajan.D, Lathamaheswari.M, Sujatha.R and Kavikumar.R "Edge Detection on DICOM Image using Triangular Norms in Type-2 Fuzzy" International Journal of Advanced Computer Science and Applications(IJACSA), 9(11), 2018. http://dx.doi.org/10.14569/IJACSA.2018.091165

To Study the Frequency of Cystic and Solid Lesions among Solitary Thyroid Nodules

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ABSTRACT:

BACKGROUND: In practical and numerical terms, the solitary nodule is the most important target of thyroid FNAC. Since FNAC can provide an unequivocal benign diagnosis in 60% of patients with benign nodules, its potential to reduce the number of unnecessary operations is significant. Of all the thyroid glands that on surgical resection prove to contain solitary nodules, 70- 80% are benign adenomas and about 10-30% is malignant growth. Over the past two decades, fine needle aspiration cytology (FNAC) has become an essential step in the evaluation of thyroid nodules, due to its superior diagnostic reliability and cost effectiveness. **AIMS & OBJECTIVES:** The present study aims to explore the frequency of cystic and solid lesions among solitary thyroid nodules (excluding multiple nodular lesion). **MATERIAL & METHOD:** 140 patients of more than or equal to 15 years of age presenting with solitary thyroid nodule were the subjects of the current study. Subjects were referred for fine needle aspiration by the treating clinicians. **OBSERVATIONS:** Male: Female ratio was (18/122) 1:6.8 and age of the patients ranged from 15 years to 62 years. Maximum number of cases was in 3rd and 4th decades. 4 patients were in 7th decade. Cytodiagnostic break up of all 122 cases included colloid goitre 31 cases (25.5%), hyperplastic nodule 3 cases (2.5%), lymphocytic thyroiditis 2 cases (1.6%), Hashimoto's thyroiditis 7 cases (5.7%) and subacute thyroiditis 5 cases (4.0%), from non-neoplastic category. **CONCLUSION:** It was concluded that FNAC of thyroid is a safe, simple, cost- effective, reliable and accurate investigation for cases of solitary thyroid nodule.

KEYWORDS: *FNAC, Solitary Thyroid nodules, cystic , Solid , Histological Correlation.*

INTRODUCTION

The thyroid gland is a bilobed organ situated in the anterior neck in front of the upper trachea. The normal thyroid gland in adult weighs 15-30 gm.[1] Normal function of the thyroid gland is to secrete thyroxine (T4) and triiodothyronine (T3). FNAC is highly successful in triaging patients with solitary thyroid nodule into operative and non-operative groups.[2] It may also be diagnostic for certain thyroid lesions, such as colloid goiter, Hashimoto's thyroiditis, papillary carcinoma, anaplastic carcinoma, large cell lymphoma, and metastatic carcinoma. In practical and numerical terms, the solitary nodule is the most important target of thyroid FNAC.[3]

Over the past two decades, fine needle aspiration cytology (FNAC) has become an essential step in the evaluation of thyroid nodules, due to its superior diagnostic reliability and cost effectiveness.[4, 5] Since FNAC can provide an unequivocal benign diagnosis in 60% of patients with benign nodules, its potential to reduce the number of unnecessary operations is significant.[6, 7, 8, 9]

Palpable thyroid nodules occur in 4-7% of the population (10 to 18 million persons) but nodules found incidentally on USG suggest a prevalence of 19-67%.10 Thyroid carcinoma occurs in roughly 5-10 % of palpable nodules.[10] Thyroid cancers constitute only about 1-2% of all malignant neoplasms in most populations and are three times more common in women than in men.[11]The prevalence of thyroid cancer in patients undergoing fine needle aspiration biopsy is estimated to be about 4%. Of all the thyroid glands that on surgical resection prove to

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contain solitary nodules, 70- 80% are benign adenomas and about 10-30% is malignant growth.[12]

AIMS& OBJECTIVES

The present study aims to explore the frequency of cystic and solid lesions among solitary thyroid nodules. (excluding multiple nodular lesion).

OBJECTIVES:

1. To study the distribution of thyroid lesions according to age & sex.

2. To study the frequency of cystic & solid lesions amongst solitary thyroid nodules.

MATERIAL & METHOD

This study was performed in the Department of Pathology, D.Y. Patil Medical College, Kolhapur during a study period of 2 year from June 2008 –to May 2010.140 patients of more than or equal to 15 years of age presenting with solitary thyroid nodule were the subjects of the current study. Subjects were referred for fine needle aspiration by the treating clinicians.

Observations

Table -1: DISTRIBUTION OF CASES ACCORDING TO SEX

S. NO.	SEX	NO.of cases	PERCENTAGE
1	Female	122	87.1
2	Male	18	12.9
	Total	140	100.0

Table -2 Distribution of cases according to Age

S.No.	Age (range)	No. Of cases	Percentage
1	10-19	6	4
2	20-29	49	35
3	30-39	41	29
4	40-49	28	20
5	50-59	12	9
6	≥60	4	3
	Total	140	100.0

Distribution of cases according to Age

Table – 3: FREQUENCY OF CYSTIC AND SOLID THYROID NODULES AND THEIR ADEQUACY

S. No.	Lesion	No. of cases	Percentage	Adequacy			
				Adequate		Inadequate	
				No.	%	No.	%
1	Solid	122	87.14	114	93.4	8	6.6
2	Cystic	17	12.14	7	41.2	1O	58.8
3	Solid /Cystic	1	0.71	1	100	0	0
	Total	140	100	122		18	

Cases were also divided into solid, cystic and solid/ cystic on the basis of the aspiration of fluid during procedure or on ultrasonographic findings.

Most of the nodules were solid accounting for 122cases out of 140 (87.14%) and of the remaining cases, 17 cases (12.14%) were cystic and only one case (0.71%) was partly solid cystic.

Majority of the aspirates from solid nodules were adequate i.e. 114 out of 122 (93.4%) and only 8 out of 122 (6.6%) were inadequate, where as most of the

aspirates from cystic nodules (10 out of 17 i.e. 58.8%) were considered inadequate and only 7 of the 17 (41.2%) were considered adequate. Aspirate from partly solid/cystic nodule was found to be adequate.

DISCUSSION

In the present study, a total of 140 cases of solitary thyroid nodule were subjected to fine needle aspiration cytology (without imaging guidance). Of these, 122 cases (87%) were found to be adequate by Goellner's criteria[13] and 18 cases (13%) were considered inadequate (Table-3).

Table - 4: **Comparision of Distribution of cases according to sex**

	Hussain Muhammad [13] (2001)	**Raza Sohail (2006)**	**Guhamallick M et al [8] (2008)**	**Uma Handa [9] (2008)**	**Foad Ali Moosa [7] (2010)**	**Present Study**
Female	78%	69.56%	66.66%	86.4%	69.4%	87%
Male	22%	30.43%	33.44%	13.6%	30.6%	13%
Female /Male Ratio	3.56:1	2.33:1	2.03:1	6.35:1	2.21:1	6.7:1

In all these studies no of females presenting as thyroid lesions is higher than males.

Table - 5: Comparision of no of cystic and solid thyroid nodules with other studies

	Silverberg[14] (1997)	**Bellantone R et al[15] (2004)**	**Raza sohail et al (2006)**	**Present study**
Solid	70%	-	70%	87.14%
Cystic	30%	15-37%	12.5%	12.14%
Solid/ cystic	-	-	17.5%	0.71%

In our study the percentage of solid lesion was obtained 87.14% which was higher to those quoted by Silverberg[14] (70%) & Raza sohail et al (70%).

In our study % of cystic lesion was 12.14% which was similar to those quoted by Raza sohail et al (12.5%) & lower than those quoted by Silverberg[14] (30%). In our study % of solid/cystic lesion was 0.71% which was lower than those quoted by Raza Sohail et al (17.5%). Bellantone R [15]et al (2004) mentioned the frequency of cystic thyroid nodules to be 15-37% of all surgically excised thyroid nodules.

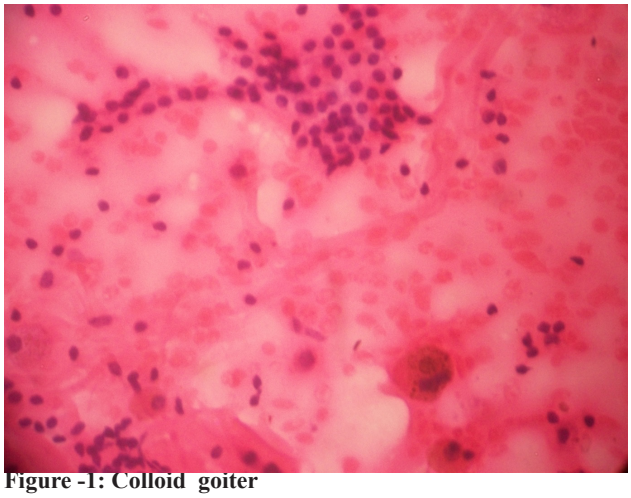


Figure -1: Colloid goiter

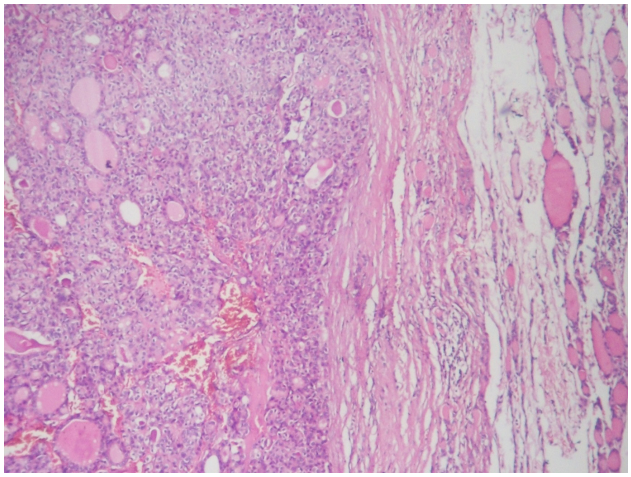


Fig- 2: Follicular adenoma

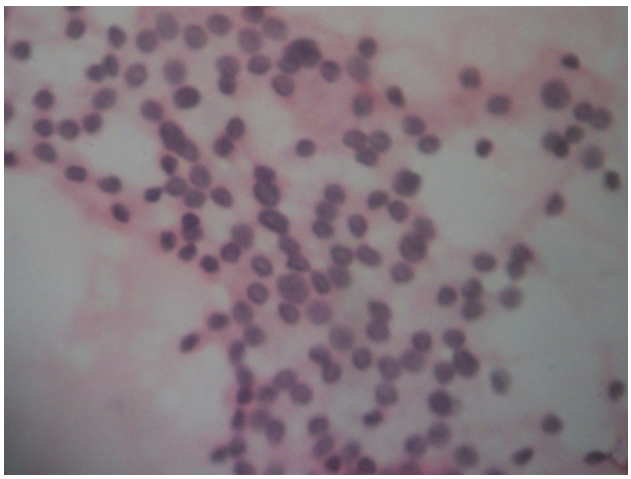


Fig – 3 Colloid goiter

CONCLUSION

Colloid goiter was the most common lesion amongst all non – neoplastic diagnoses. Great majority of these cases showed scant to moderate cellularity , good amount of colloid with or without histiocytes and monolayered honey comb sheets, thus enabling easy diagnosis Thus it was concluded that FNAC of thyroid is a safe, simple, cost- effective, reliable and accurate investigation for cases of solitary thyroid nodule as firm diagnosis can be made on cytology alone in vast majority of cases, thus obviating the need for unnecessary surgical procedures. Cystic lesions mandate careful clinical correlation.

Ethical Clearance – Taken from ethical committee

Source of Funding- Self

Conflict of Interest- None

REFERENCES

1. McNicol AM, Lewis PD. The endocrine system. Systemic pathology Churchill Livingstone, Edinburgh, 1996; 132-155.
2. Lngegowda JB, Muddegowda PH, Rajesh KN, Ramkumar KR. Application of pattern analysis in fine needle aspiration of solitary nodule of thyroid. J 2010 Jan; 27(1):1-7. doi: 10.4103/0970-9371.66688
3. Orell SR et al. Manual and Atlas of fine needle aspiration cytology. 4th Edn. Churchill Livingstone, an imprint of Elsevier, 2005: 125-164.
4. KantasuebSarunya, Sukpan Kornkanok. The study of thyroid lesions and the correlation between histopathological and cytological findings at Maharaj Nakorn Mai Hospital between 2003 & 2007. Chiang Mai Med J 2010; 49: 105-110.
5. BalochNaseem M, Ali Shazia. Contribution of fine needle aspiration cytology (FNAC) in the diagnosis of malignant thyroid nodules. Pakistan J. Of Surgery 2008; 24 :19-21
6. Gharib H, Goellner JR. Fine needle aspiration biopsy of the thyroid: an appraisal. Ann Intern Med. 1993; 118: 282-289.
7. Moosa Ali Foad. Up to what extent FNAC is accurate in detecting malignancy in solitary thyroid nodule. Medical Channel 2010; 16: 280-283.
8. Guhamallick M, Sengupta S. Cytodiagnosis of thyroid lesions – usefulness and pitfalls. A study of 288 cases. J of cytology 2008; 25:6-9.
9. Handa Uma, GargSukant. Role of fine needle aspiration cytology in diagnosis & management of thyroid lesions. J of cytology 2008; 25: 13-17.

10. Mazzaferri EL. Thyroid cancer in thyroid nodules : finding a needle in the haystack, Am. J. Med. 1992; 93: 359-62

11. Castro MR, Gharib H. Thyroid nodule and cancer: when to wait and watch when to refer. Postgrad Med 2000; 107 (1): 113-24.

12. Willems JS, Lowhagen T. The role of fine needle aspiration in the management of thyroid disease. Clinics in Endocrinology and Metabolism, 1981; 10: 267-273.

13. Goellner JR, Gharib H, Grant CS, Johnson DA. Fine Needle aspiration cytology of the thyroid, 1980 to 1986. Acta Cytol 1987;31:587-590.

14. HamburgerJi, Hussain M et Al. Increasing the accuracy of fine needle biopsy for thyroid nodules. Arch Pathol Lab Med.1989;113: 1035-1041.

15. Silverberg SG, De Lellis RA, Frable WJ : Principles and practice of surgical pathology and cytopathology : Churchill Livingstone, 1997; 2665-2708

16. Bellantone R, Lombardi CP et al. Management of cystic or predominantly cystic thyroid nodules. The role of ultrasound – guided fine – needle aspiration biopsy. Thyroid Vol. 14:1:2014

Prevalence of Pregnancy Associated Listerioisis in and Around Puducherry, India

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ABSTRACT

Background: Listeriosis ranks the most frequent source of death among the bacterial food-borne illnesses with high Case fatality rate of 20% to 30% and neonatal death rate 50%. Listeriosis incidence in pregnancy has been reported 18 to 20 times higher than in general population. Hence the present study was planned to evaluate the prevalence of pregnancy associated Listeriosis in and around Puducherry India.

Method: A total of 335 pregnancy associated cases which includes 129 pregnant women, 34 women with miscarriage/ spontaneous fetal loss and 172 neonates from whom 147 blood samples,138 amniotic fluid or HVS, 2 diarrheal stool samples,19 CSF, 14 placental bits or umbilical cord and 15 abortus material were aseptically collected and processed for isolation of *Listeria spp.*

Results: The overall prevalence of pregnancy associated Listeriosis was 6.3%. Pregnant women accounted for 11.62% positive cases, 8.82% of women with spontaneous abortion /miscarriage and 1.74% of neonates were found positive for Listerioisis in the present study.

Conclusion: Considering the increased incidence of pregnancy associated Listeriosis in our setting, this disease should be considered as an important differential diagnosis in clinical practice especially among pregnant women and women undergoing miscarriage or fetal loss.

Keywords : *pregnant women, neonates, Listeriosis, miscarriage.*

INTRODUCTION

Listeriosis ranks the most frequent cause of death among all the bacterial food-borne illnesses[1] with case fatality rate of about 20% to 30%, neonatal death rate 50% and hospitalization rate 91%[2,3] Listeriosis incidence in pregnancy has been reported 18 to 20 times higher than in general population and moreover Listeriosis infection in pregnancy shows poor prognosis for the fetuses[4]. Listeriosis in pregnancy results in intrauterine infection leading to complications like spontaneous abortion, still birth, preterm labor and neonatal infections like neonatal sepsis or meningitis resulting in morbidity and mortality[5] .In India reports on human Listeriosis are scanty with only few documented cases and less than 15 publications such as isolation of this pathogen from women with premature birth, spontaneous abortions or miscarriage or from women with bad obstetric history patients in Mumbai[6], in Delhi[7] in south India[8] and few other places[9,10]. A report on Listeriosis in 2nd trimester pregnant women from Chandigarh[11]. Isolation of *Listeria* in cases with neonatal meningitis or meningoencephalitis from North India[12], from Mumbai[13] and from South India[14]. Listeriosis has been considered as an emerging foodborne disease in India and *Listeria monocytogenes* a growing threat[15]. Besides all Listeriosis in India largely remains ignored mainly because the clinical diagnosis

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of Listeriosis is insufficient owing to its varied clinical forms and complex immunopathological aspects. Most often naturally occurring Listeriosis in humans has been unrecognized or unexposed owing to the lack of a rapid, simple, appropriate and reliable diagnostic tool[16]. Moreover the low incidence rate and failure to isolate the pathogen due to its rarity or lack of awareness or many a times it's been missed owing to lack of identification[17]. Till date the literature reviews suggest there is paucity of data pertaining to the *Listeria* infections among pregnant women and neonates in this subcontinent. The altering food habits, non-availability of vaccine for this disease, the capability of *Listeria spp.* to survive at refrigeration or low temperatures and its case fatality rate suggests that Listeriosis could be the single largest killer in humans and animals[18]. Moreover the increased food borne incidences of *Listeria spp.* has gained huge public health significance. Hence the present study was planned to evaluate the prevalence of pregnancy associated Listeriosis in and around Puducherry India.

METHOD

A cross-sectional study conducted over a period of 1 year from June 2015 to May2016 in the Department of Microbiology, Sri Lakshmi Narayana Medical College, Hospital and Government General Hospital, Puducherry, India. This study was approved by the Institutional Human Ethics Committee and Informed consent was obtained from all participants included in the study. In order to accomplish the objective a total of 335 cases which includes 129 pregnant women, 34 women with miscarriage/ spontaneous fetal loss, 172 neonates (infants aged≤ 4weeks) presenting with either fever/ flu-like illness, diarrhea , vomiting , with signs and symptoms of sepsis / meningitis / meningoencephalitis / encephalitis, who fulfilled the inclusion criteria from various hospitals in and around Puducherry such as Sri Lakshmi Narayana Institute of Medical Science, Rajiv Gandhi Government Women and children Hospital, India Gandhi Government General Hospital & Post Graduate Institute and Pondichery AIDS Control Society were accounted for this study. From the above mentioned cases 147 blood samples,138 amniotic fluid or HVS, 2 diarrheal stool samples,19 CSF, 14 placental bits or umbilical cord and 15 abortus material were aseptically collected, instantly transported to microbiology laboratory and processed immediately for the isolation of *Listeria spp.* following the USDA protocol proposed by Mc Clain and Lee in 1988 with certain modifications. Briefly the specimens were enriched by 2step enrichment method i.e, by inoculating in UVM broth -1 and incubating for 24 hrs at 30°C followed by enriching in UVM broth -2 with incubation at 30°C for 24 hours. Then a loopfull inoculum from enriched UVM broth- II was plated on to selective medium PALCAM agar and observed for *Listeria* colonies after incubation for 24 hrs at 37°C. Grayish glistening colonies surrounded with a diffusing black zone surrounding the colonies were presumptively taken as *Listeria* colonies[19]. The identified *Listeria* colonies were then subjected to Gram's staining and examined morphologically for Gram positive coccobacilli. Isolates showing tumbling motility at 20°C to 25°C was then confirmed for genus *Listeria* on the basis of Latex agglutination test using LK07- Hi *Listeria* Latex Test Kit -Hi-Media, India. The identified *Listeria* isolates were further subjected to biochemical characterization using KB012A-Hi *Listeria* identification kit to differentiate genus *Listeria* up to *spp.* level. The isolates exhibiting catalase, esculin, voges proskauer test and methyl red positive and nitrate test negative were considered as "presumptive" *Listeria* isolates. Isolates which showed α-methyl - D mannoside, glucose, rhamnose, lactose and sucrose positive reaction, xylose and mannitol negative reaction were considered as *Listeria monocytogenes* and the rest were categorized as other *Listeria spp.*

RESULT

The current study was conducted over a course of 1 year extending from June 2015 to May 2016 and was executed to determine the prevalence of pregnancy associated Listeriosis in and around Puducherry India. A total of 335 cases i.e., pregnant women, women with spontaneous abortion and neonates aged ≤ 4weeks presenting either with fever, flu –like illness, diarrhea, vomiting, with signs and symptoms of sepsis/ meningitis/ meningoencephalitis who fulfilled our inclusion criteria were enrolled for the study.

Table No.1 : Overall Prevalence of Pregnancy associated Listeriosis

No. of cases	No. of cases positive	Percentage (%)
335	21	6.3%

In this study out of 335 pregnancy associated cases screened for Listeriosis nearly 21 cases

were found positive for Listeriosis, with this the overall prevalence of pregnancy associated Listeriosis was 6.3% (Table No.1). The prevalence of Listerioisis among pregnancy associated cases included in the study is shown in Table No.2. Out of 129 pregnant women screened in the present study nearly 15 (11.62%) cases showed positive for Listeriosis. Of the 34 women with spontaneous abortion /miscarriage screened 3 (8.82%) cases turned positive for Listerioisis. 3/172 (1.74%) neonates screened were found positive for Listerioisis in the present study.

Table No.2 : Prevalence of Listerioisis among Pregnancy associated cases included in the study.

Pregnancy associated cases	No. of cases	No. of *cases positive*	Percentage (%)
Pregnant women	129	15	11.62%
Women with spontaneous abortion / miscarriage	34	3	8.82%
Neonates	172	3	1.74%

DISCUSSION

Listeriosis a dreaded disease of the west appears to have knocked the Indian subcontinent mainly due to rapid unplanned urbanization and globalization[20]. The disease in humans is chiefly through ingestion of food items contaminated with *Listeria* and the clinical spectrum of this disease varies from self-limiting febrile diarrhea to more serious invasive Listeriosis including bacteremia, sepsis, meningoencephalitis and abortion/ still birth[21]. The present study was proposed to determine the prevalence of pregnancy associated Listeriosis in and around Puducherry India. The overall prevalence of pregnancy associated Listerioisis in the present study was 6.3%. The results of the present study was found to be in agreement with the reports from most of the countries such as a report from Lombardy region Italy which reported nearly 15/134 were pregnancy-associated Listerioisis[22]. The number of Listeriosis cases recorded from 2004 to 2007 through the U S. *Listeria* Initiative has shown 16·9% of cases were pregnancy associated , reports from *Listeria* initiative during 2009 to 2011 reported through MMWR has showed pregnancy associated Listeriosis to be 14%[23]. Most reports from USA and Europe has shown about 15% and 6- 18% of Listeriosis cases respectively are pregnancy related[24,25]. Although the pregnancy associated Listeriosis is considered as a benign disease of the mother mainly because of its grave outcome in fetus, the pregnancy related Listeriosis is a major medical issue to be addressed that requires a deep clinical suspicion, highlighting the need for effective prevention in this category. Moreover the lack of awareness regarding the risk of certain food products in pregnancy would have contributed to pregnancy associated Listeriosis. Hence there is urgent requirement for preventive action among the pregnant women including the education on the avoidance of certain unsafe foods in pregnancy, as well as decontamination and food hygiene. Further steps addressing the decline in pregnancy related Listeriosis with improved industry and regulatory efforts to reduce *Listeria* contamination in foods is required. Hence a higher concern from the healthcare community or physician awareness towards pregnancy-associated Listeriosis is also mandated.

Pregnant women accounted for a considerable proportion of Listeriosis cases 11.62% followed by and women with spontaneous abortion / miscarriage accounted for 8.82% These results are concurrent with earlier published works where in the pregnant women accounted for a majority of all Listerial infection in humans , with the incidence of *Listeria* infection in pregnant women to be 12 per 100,000 in comparison to 0.7 per 100,000 in general public[26].The results are also in accordance with the recent published reports were an increasing incidence of pregnancy related Listeriosis has been furnished[26, 27]. Our report on isolation of *Listeria* from 8.82% of women with spontaneous abortion / miscarriage is well correlated with most studies conducted in India such as one reported by Krishna et al in 1966 with isolation of *Listeria* from 14% of cases with abortion or miscarriage or stillbirth in Mumbai[6] and isolation of *Listeria* from habitual abortions reported by several authors[28,29]. Whereas the results of the present study was quiet higher when compared to a report published by Bhujwala and Hingorani in 1975 who reported 1.34 % of cases with abortion or miscarriage screened were positive for *Listeria*[7]. The reasons for current rise in Listeriosis infection among pregnant women and women with spontaneous abortion or miscarriage could include the poor food hygiene practices, environmental and other host factors[4,27]. Hence

Listerioisis to be included in the differential diagnosis in clinical practices especially among pregnant women, women with spontaneous abortion / miscarriage.

Neonatal Listeriosis in our study was found to be 1.74% which is in par with few reports published earlier and the estimated prevalence of Neonatal Listeriosis is 8.6 per 100 thousand[30,31]. Thomas et al in 1981 from North India has shown 0.2% neonatal Listeriois in total live births[12]. Although, widely known that Listeriosis in pregnancy or maternal Listeriosis is extremely mild for pregnant women themselves, but it is of life- threatening with highly fatal outcomes for the neonates[26]. Moreover neonates and women during the antenatal period are highly susceptible to Listeriosis apparently due to their depressed or lack of CMI. Hence the present study emphasizes the need to screen for pregnancy associated Listerioisis in order to prevent further complications such as preterm labor, still birth, neonatal sepsis and meningitis which is extremely fatal as neonatal morbidity and mortality is possibly preventable with timely diagnosis and quick interventions. Hence care must be taken to report this pathogen in routine diagnosis.

Conclusion: Considering the increased incidence of pregnancy associated Listeriosis in our setting, this disease should be considered as an important differential diagnosis in clinical practice especially among pregnant women and women undergoing miscarriage or fetal loss. The pregnancy associated Listeriosis in our study was the highest that is currently described, signifying that effective or forceful preventative action against this group will have a great impact in lowering the abortion rates and fetal loss. This study concludes that a very high index of clinical suspicion and due diligence is mandatory in laboratory diagnosis of maternal or pregnancy associate Listeriosis.

Ethical Clearance- Institution Ethics committee (Human studies): No. IEc/C- P/62/2015

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REFERENCES

1. Esteban JI, Oporto B, Aduriz G, Juste RA, Hurtado A. Faecal shedding and strain diversity of Listeria monocytogenes in healthy ruminants and swine in Northern Spain. BMC Veterinary Research. 2009 Dec;5(1):2.
2. Low JC, Donachie W. A review of Listeria monocytogenes and listeriosis. The Veterinary Journal. 1997 Jan 1;153(1):9-29.
3. Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. Microbes and Infection. 2007 Aug 1;9(10):1236-43.
4. Lamont RF, Sobel J, Mazaki-Tovi S, Kusanovic JP, Vaisbuch E, Kim SK, Uldbjerg N, Romero R. Listeriosis in human pregnancy: a systematic review. Journal of perinatal medicine. 2011 May 1;39(3):227-36.
5. Vázquez-Boland JA, Domínguez-Bernal G, González-Zorn B, Kreft J, Goebel W. Pathogenicity islands and virulence evolution in Listeria. Microbes and infection. 2001 Jun 1;3(7):571-84.
6. Krishna U, Desai MW, Daftary VG. Listeriosis: a clinico-bacteriological study. Journal of Obstetrics and Gynecology of India. 1966;16:304-10.
7. Bhujwala RA, Hingorani V. Perinatal listeriosis: a bacteriological and serological study. The Indian journal of medical research. 1975 Oct;63(10):1503.
8. Stephen S, Indrani R, Achyutha Rao KN, Padma Rao A. Listeriosis in human abortions—including a brief review of literature. J. Obstet. Gynecol. India. 1978 Aug;28:497-501.
9. Malik SV, Barbuddhe SB, Chaudhari SP. Listeric infections in humans and animals in the Indian subcontinent: a review. Tropical animal health and production. 2002 Sep 1;34(5):359-81.
10. Rocourt, J.R. and WHO. Human listeriosis: 1989. Geneva: World Health Organization.1991; WHO/ HPP/FOS/91.3.
11. Gupta V, Gautam V, Mehta N, Kumari I, Joshi RM. Listeriosis in second trimester of pregnancy: case report from India. Japanese journal of infectious diseases. 2003 ;56(2):60-1.
12. Thomas S, Verma IC, Singh M, Bhujwala RA. Study of neonatal listeriosis in north India. Indian Journal of Medical Research. 1981;73:28-32.
13. Gogate AA, Deodhar LP. Meningitis due to Listeria monocytogenes:(a case report). Journal of postgraduate medicine. 1981 Oct 1;27(4):240.
14. Khan S, Sujath S, Harish BN, Praharaj I, Parija SC. Neonatal Menigitis due to Listeria monocytogenes: a case report from Southern India. J Clin Diagn Res. 2011;5(3):608-9.

15. Chugh TD. Emerging and re-emerging bacterial diseases in India. Journal of biosciences. 2008 Nov 1;33(4):549-55.
16. Barbuddhe SB, Malik SV, Kumar JA, Kalorey DR, Chakraborty T. Epidemiology and risk management of listeriosis in India. International journal of food microbiology. 2012 Mar 15;154(3):113-8.
17. Hernandez-Milian A, Payeras-Cifre A. What is new in listeriosis?. BioMed research international. 2014;2014.
18. Liu D. Identification, subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. Journal of medical microbiology. 2006 Jun 1;55(6):645-59.
19. McClain D, Lee WH. Development of USDA-FSIS method for isolation of Listeria monocytogenes from raw meat and poultry. Journal-Association of Official Analytical Chemists. 1988;71(3):660-4.
20. Tirumalai PS. Listeriosis and Listeria monocytogenes in India. Wudpecker J Food Technol. 2013;1(6):98-103.
21. Julián A, Jiménez Á, by Górgolas M, Fernández R, Fernández ML. Infections by Listeria monocytogenes in adults. Clinical and microbiological aspects of a changing disease. Infectious Diseases and Clinical Microbiology. 2001 Jan 1; 19 (7): 297-303.
22. Mammina C, Parisi A, Guaita A, Aleo A, Bonura C, Nastasi A, Pontello M. Enhanced surveillance of invasive listeriosis in the Lombardy region, Italy, in the years 2006-2010 reveals major clones and an increase in serotype 1/2a. BMC infectious diseases. 2013 Dec;13(1):152.
23. Silk BJ, Mahon BE, Griffin PM, Gould LH, Tauxe RV, Crim SM, Jackson KA, Gerner-Smidt P, Herman KM, Henao OL. Vital signs: Listeria illnesses, deaths, and outbreaks—United States, 2009–2011. MMWR. Morbidity and mortality weekly report. 2013 Jun 7;62(22):448.
24. Filipello V, Amato E, Gori M, Huedo P, Ciceri G, Lomonaco S, Pontello M. Epidemiology and Molecular Typing of Pregnancy-Associated Listeriosis Cases in Lombardy, Italy, over a 10-Year Period (2005–2014). Infectious diseases in obstetrics and gynecology. 2017;2017.
25. Charlier C, Perrodeau É, Leclercq A, Cazenave B, Pilmis B, Henry B, Lopes A, Maury MM, Moura A, Goffinet F, Dieye HB. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. The Lancet Infectious Diseases. 2017 May 1;17(5):510-9.
26. Janakiraman V. Listeriosis in pregnancy: diagnosis, treatment, and prevention. Reviews in Obstetrics and Gynecology. 2008;1(4):179.
27. Khatamzas E, Hughes H, Grant KA, McLauchlin J, Bowler IC. The increasing prevalence of listeriosis—what are we missing?. QJM: An International Journal of Medicine. 2009 Dec 30;103(7):519-22.
28. Aljičević M, Bešlagić E, Zvizdić Š, Hamzić S, Mahmutović S. Listeria monocytogenes as the possible cause of the spontanous abortion in female of the fertile age. Bosnian journal of basic medical sciences. 2005 Nov 20;5(4):89-92.
29. Tridente V, Cataldi UM, Mossa B, Morini F, Bonessio L, Ciardo A, Salvi M. Caso d'infezione MATERNA e neonatale provocata da Listeria monocytogenes. Clinica terapeutica. 1998;149(4):307-11.
30. Cherubin CE, Appleman MD, Heseltine PN, Khayr W, Stratton CW. Epidemiological spectrum and current treatment of listeriosis. Reviews of infectious diseases. 1991 Nov 1;13(6):1108-14.
31. Clinical practice guidelines on Listeriosis in pregnancy by royal college of physicians of Ireland 2015.

Title: Dengue cases Treated in Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar in Year 2016

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ABSTRACT

Introduction: Sri Guru Ram Das Institute of Medical Sciences & Research, Amritsar is a tertiary health care institution. Dengue is a viral infection mainly transmitted by female *Aedes aegypti* and *Ae. Albopictus* mosquitoes. It has spread globally in recent decades. Dengue virus (DENV) has four distinct and closely related serotypes i.e. DENV-1, DENV-2, DENV-3 and DENV-4. Severe dengue causes serious illness and deaths. Dengue has no treatment but can be prevented and controlled. **Materials and methods:** Data of confirmed dengue cases treated in SGRDIMSAR, Amritsar in year 2016 were collected; analyzed and valid conclusions were drawn. **Findings:** In year 2016, 198 dengue cases were reported. Maximum number of cases, 57 (28.8%) was found in age 21-30 years, majority, 118 (59.6%) were males and higher number of cases, 177 (89.4%) was found in urban area. All the cases were reported from August to November with the peak in September. Maximum cases 160 (80.8%) were found positive by NS-1 Ag. Mean gap between admission and testing of cases was 5 ± 2.1 days. Average length of stay of cases in hospital was 6.1 ± 2 days. **Conclusion:** Emphasize, to take all preventive measures, vector control, appropriate hospital care of cases and promote the use of NICD Coolers.

Keywords: *Dengue, Severe dengue, Aedes aegypti, Aedes albopictus.*

INTRODUCTION

Dengue is a human arbovirus disease transmitted by the female mosquito of the genus Aedes, mainly *Aedes aegypti* and *Ae. Albopictus*[1]. There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3, and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. Subsequent infections by other serotypes increase the risk of developing severe dengue. However, cross-immunity to other serotypes after recovery is only partial and temporary[2].

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Global burden of Dengue

The World Health Organization (WHO) considers dengue as a major global public health challenge in the tropic and sub tropic nations. Dengue has seen a 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel and lack of health care facilities [3,4,5]. One recent (2013) estimate indicates that 390 million dengue infections occur every year (95% credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically (with any severity of disease) [6]. Another (2012) study, of the prevalence of dengue, estimates that 3.9 billion people in 128 countries are at risk of infection with dengue viruses[7].

Situation in India

Dengue is endemic in all states and union territories (UTs) of India. Both Aedes aegypti and Aedes albopictus are the main competent vectors for dengue virus in India[3].

India receives 75% of its rainfall during the southwest monsoon period from June to September. Indian monsoon rainfall provides ample breeding habitats for *Ae. aegypti*, thus leading to high vector densities[8].

In the early 2000s, dengue was endemic in a few southern (Maharashtra, Karnataka, Tamil Nadu and Pondicherry) and northern states (Delhi, Rajasthan, Haryana, Punjab and Chandigarh). It has recently spread to many states, including the union territories. In addition to the increased number of cases and disease severity, there has also been a major shift in the geographical range of the disease. Dengue had been restricted to urban areas, but it has now spread to rural regions[9]. In year 2017, the spike in cases of dengue was the highest in the last one decade, according to the data from National Vector Borne Disease Control Programme (NVBDCP and National Health Profile 2018. From less than 60,000 cases in 2009, cases increased to 188,401 in 2017—more than a 300 per cent spike[10]. Cement water tanks, water coolers, plastic containers and tiers are the preferred breeding habitats of Aedes mosquitoes. Coconut shells and latex cups are important breeding sites in Kerala and Lakshadweep Island[11]. National Research Development Corporation of India found that in India about 60 to 70% of the Aedes mosquito breeding occurs in coolers in the urban areas and about 40% in rural areas[12]. National Centre for Disease Control (NCDC) has developed a modified cooler with a covered water tank which prevents breeding of mosquitoes thus very helpful in preventing vector breeding and contributes towards control of dengue as a public health problem particularly in urban areas. [13]

A dengue vaccine, Dengvaxia (R), has been registered in several countries[14]. But it has not yet been approved by the Ministry of Health and Family Welfare, Government of India, because more clinical trials are thought to be necessary in India[15].

Present study aims to describe the Epidemiological profile of confirmed cases of dengue admitted in year 2016 in medicine and pediatric ward at Sri Guru Ram Das Institute of Medical Sciences & Research, Amritsar, Punjab, India.

MATERIAL AND METHOD

A retrospective, descriptive study was conducted out in Sri Guru Ram Das Institute of Medical Sciences & Research (SGRDIMSAR), Amritsar. Epidemiological data of confirmed cases of dengue and deaths due to dengue in year 2016 were collected from dengue wards established in SGRDIMSAR, Amritsar. The study population included all the patients having their serum samples found confirmed for dengue by IgM Mac Elisa or NS-1 Ag test done in Government Medical College, Amritsar during this period.

Data Analysis: Data collected were analyzed by using SPSS software version 22 and the valid conclusions were drawn. $p < 0.05$ and $p < 0.01$ were considered significant and highly significant respectively.

FINDINGS

In year 2016, 198 confirmed cases of dengue were reported in SGRDIMSAR. No death was reported. Mean time gap in the date of admission of suspected dengue patients and testing of their blood samples for dengue was 5.01 ± 2.13 days. Average length of stay of dengue case in hospital was 6.12 ± 2.01.

Mean age of dengue cases was 33.21 ± 15.94 years. Maximum age was found to be 85 years and the minimum age was 2 months.

Table 1. Age wise distribution of confirmed cases of dengue

Age in years	No.	%
<1	1	0.5
1-5	1	0.5
6-10	8	4.0
11-20	35	17.7
21-30	57	28.8
31-40	38	19.2
41-50	35	17.7
> 50	23	11.6
Total	198	100.0

X^2 = 114.6 d. f. = 7 p = 0.000

Table 1 is showing the age wise distribution of confirmed cases of dengue. Maximum number of cases 57 (28.8%) were found in 21 to 30 years age groups and the minimum number, 1(0.5%) was found both in age groups < 1 and 5 years. The difference in the number of cases reported in different age groups was found highly significant statistically.

Table 2. Sex wise distribution of confirmed cases of dengue

Sex	No.	%
Male	118	59.6
Female	80	40.4
Total	198	100.0

$X^2 = 7.3$ d. f. =1

p = 0.007

Table 2 is showing the sex wise distribution of confirmed cases of dengue. It is evident from this table that higher number of males cases, 118 (59.6%) than the female cases, 80 (40.4%) was found. The difference in the number of cases reported in the male and female sexes was found highly significant statistically.

Table 3. Area wise distribution of confirmed cases of dengue

Area	No.	%
Urban	177	89.4
Rural	21	10.6
Total	198	100.0

$X^2 = 1.2$ d. f. = 1

p = 0.000

Table 3 is showing the area wise distribution of confirmed cases of dengue reported. Most of the cases 177 (89.4%) were reported from urban area and the difference in the number of cases reported from urban and rural areas was found highly significant statistically.

Table 4. Rural block wise distribution of confirmed cases of dengue

Block	No.	%
Baba Bakala	2	9.5
Lopoke	2	9.5
Manawala	5	23.8
Ramdas	2	9.5
Tarsika	1	4.8
Threawal	1	4.8
Verka	8	38.1
Total	21	100.0

$X^2 = 13.3$ d. f. =6

p = 0.03

Table 4 shows the block wise distribution of confirmed cases of dengue in rural area. Maximum number of cases, 8 (38.1%) was reported from block Verka followed by Manawala, 5 (23.8%) and the minimum 1 in each block Tarsika and Threawal. The difference in the number of cases reported from different rural blocks was found highly significant statistically.

Table 5. Month wise distribution of confirmed cases of dengue

Month	No.	%
August	20	10.1
September	138	69.7
October	39	19.7
November	1	0.5
Total	198	100.0

$X^2 = 225.6$ d. f. = 3

p = 0.000

Table 5 is showing the month wise distribution of confirmed cases of dengue. This table shows that all the cases of dengue were reported from August to November only. The peak of cases, 138 (69.7%) was found in the month of September followed by 39 (19.7%) cases in October. Only one case was reported in November. Rising trend of the reporting of dengue cases had been observed from August to September and there was a declining trend from September to November. The

month wise difference in the number of cases reported was found highly significant statistically.

Table 6. Test wise distribution of confirmed cases of dengue

Test	No.	%
NS-1Ag	160	80.8
IgM Mac ELISA	38	19.2
Total	198	100.0

$X^2 = 75.2$ d. f. = 1 $p = 0.000$

Table 6 shows the test wise distribution of confirmed cases of dengue. Maximum cases, 160 (80.8%) were found positive by NS-1 Ag while only 38 (19.2%) were found positive by IgM Mac ELISA. The test wise difference in the number of cases found positive has been found highly significant statistically.

DISCUSSION AND CONCLUSION

Mean time gap in the date of admission of confirmed dengue patients and testing of their blood samples for dengue was 5.01 ± 2.13 days. Average length of stay of dengue case in hospital was 6.12 ± 2.01. It is recommended that testing of the blood samples of dengue cases should be done on the same day of admission to reduce the dengue transmission to other non infected persons. This will also reduce the average length of stay of dengue cases in hospital. The impact of these effects will reduce the morbidity, severity and mortality of dengue and will give a useful economic effect by the reducing the expenditure of resources on the management of confirmed cases of dengue.

Age wise distribution in SGRDIMSAR in year 2016 shows the highest number of dengue confirmed cases, 57(28.8%) was found in age group 21-30 years which resembles the previous study conducted there in year 2015 and also a study conducted in Government Medical College, Amritsar in 2016 which also showed the highest number of cases in age group 21 to 30 years and most of the cases found in adult age [11, 19].

Sex wise distribution shows a male predominance (59.6%) in our study and this finding is in concordance with that of an earlier study done by Antony in a Medical College Hospital in Kerala, India[20].

Area wise distribution shows maximum number of cases, 177(89.4%) reported from urban area and 21(10.6%) cases reported from rural area in year 2016. Previous study done in SGRDIMSAR in year 2017, reported 13.1% cases from rural area, while in year 2008, district Amritsar, reported all the 196 suspected cases of dengue from the urban rural only[13]. These studies conducted in SGRDIMSAR have shown that the dengue is spreading from the urban to the rural areas. The findings pertaining to the present study also provide similar picture with other parts of the country and show the need to lay emphasis on prevention and control of dengue in rural areas also.

Our study shows that all the cases occurred from August to November with peak in September month. These findings are similar to the findings of studies done in Brazil in 2015 and Bengaluru in 2017 which show that dengue is highly seasonal and increases primarily during the rainy season when vector breeding rate is very high. Study done in Bengaluru showed maximum cases of dengue reported in September month which also resembles our study[16, 21].

CONCLUSION

Present study shows that dengue had affected the most economically productive age group, more males than females were being affected. It had largely confined to urban areas and all the cases were reported during monsoon and post monsoon season (August to November). Although there was no mortality but morbidity was fairly high. As vaccine is still not licensed to use in India so vector control and appropriate; and timely management of dengue cases is the only method of choice. The entire preventive and control measures should be started well before the onset of dengue season. Promote use of NICD Desert Coolers. Conduct campaigns for behavior change communication like use of personal protective measures, Insecticide treated bed nets and wearing of full sleeve clothes during the day, environmental sanitation, no water collections in small containers in the surroundings and vector control. Control measures like establishment of separate dengue wards in hospitals, availability of equipments, materials and drugs etc. for early diagnosis and management of dengue cases should be taken well before the onset of dengue season.

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REFERENCE

1. Lambrechts L, Scott TW, Gubler DJ: Consequences of the expanding global distribution of Aedes albopictus for dengue virus transmission. PLoS Negl Trop Dis. 2010, 4:1-9.
2. World Health Organization (WHO). Dengue and severe dengue. Available from: http://www.who.int//news-room/fact-sheets/detail/dengue-and-severe-dengue.
3. Gubler DJ. Dengue and dengue Hemorrhagic fever. Clin Microbiol Rev. 1998;11:480–96.[PMC free article] [PubMed]
4. New ed. Geneva, Switzerland: World Health Organization; 2009. World Health Organization (WHO). Dengue- Guidelines for Diagnosis, Treatment, Prevention and Control.
5. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: A continuing global threat. Nat Rev Microbiol. 2010;8(Suppl):S7–16. [PMC free article] [PubMed]
6. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL et.al. The global distribution and burden of dengue. Nature. 2013;496:504–7. doi:10.1038/nature12060.
7. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6:e1760. doi:10.1371/journal.pntd.0001760.
8. Guhathakurta P, Rajeevan M, Sikka DR et al. Observed changes in southwest monsoon rainfall over India during 1901–2011. Int J Climatol. 2015; 35: 1881–98.
9. Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. Trans R Soc Trop Med Hyg. 2012; 106: 273–82.
10. National Vector Borne Disease Control Programme, Ministry of Health and Family Welfare, New Delhi. Directorate General of Health Services. Dengue cases and death in the country since 2010. Available from: http://www.nvbdcp.gov.in/den-cd.html, accessed 7 September, 2018.
11. Mahajan SL, Devgun P, Brar APS, Sood A. Epidemiological Features of Dengue Cases Treated in SGRDIMSAR Amritsar. Indian Journal of Public Health Research & Development. 2017 July-September; 8(3):266-70.
12. National Research Development Corporation, A Government of India Enterprise. Brief review of mosquito proof cooler. New Delhi, India 2009.
13. Mahajan SL, Singh C, Devgun P. Trends of dengue in district Amritsar from 2009 to 2013. Indian Journal of Public Health Research & Development. 2015 October-December; 6(4):311-7.
14. World Health Organization. Dengue vaccine: WHO position. Wkly Epidemiol Rec 2016; 30: 349–364.
15. Mutheneni S. R., Morse A. P., Caminade C. and Upadhyayula S. M. Dengue burden in India: recent trends and importance of climatic parameters. Emerging Microbes & Infections. 2017:6: e70. doi:10.1038/emi.2017.57.
16. Aguiar M, Rocha F, Pessanha JEM et al. Carnival or football, is there a real risk for acquiring dengue fever in Brazil during holidays seasons? Sci Rep. 2015; 5: 8462.
17. Villar L, Dayan GH, Arredondo-García JL et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. N Engl J Med. 2015; 372: 113–23.
18. Nikam AP, Bhise PR, Deshmukh MM. Seroprevalence of Dengue infection in clinically suspected cases of dengue at tertiary care hospital in central India. Int J Med Res Rev. 2015;3:593-6.
19. Paul R, Oberoi L, Singh K, Devi P**.** A comprehensive study of dengue fever patients admitted to a tertiary care hospital in Amritsar. Asian Pacific Journal of Health Sciences. 2017; 4(4): 100-3.
20. Antony J, Celine TM. A descriptive study on dengue fever reported in a Medical College Hospital. Sahel Med J. 2014;17:83-6.
21. Dhanya P. R., Shilpa K., Hittinahalli V. A comparative study on dengue and malaria infections and analysis on the prevalence of co-infection from a rural tertiary care hospital**.** Int J Res Med Sci. 2018 Jun;6(6):2111-5.

Mediating Effect of Social Support on Stress among Parents of Children with Intellectual Disability

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ABSTRACT

Parents of children with intellectual disability put more care and effort in raising their children. The perceived stress faced by this segment of parents is more than that of the parents with normal child. It is therefore essential for the caregivers to release their burden to perform normal care giving functions. The objective of the study was to determine the effect of socio-economic demographics and social support on the perceived stress of parents of children with intellectual disability. The study was conducted in Chhattisgarh state among 100 parents of children with intellectual disability. Perceived stress scale and Social support scale were the toolsused in the study for data collection. Purposive sampling technique was the method adopted in the study for the selection of samples in the study. Multiple hierarchal regression analysis was used to test the proposed model. Structural equation modeling (SEM) was used to further confirm the mediating effect of social support on income and stress. Result indicated that socio-demographic variables income, education and gender significantly contributed to the variation of stress and showed negative association with stress. However age did not contribute significantly in the variation of stress. Result of mediating effect of social support showed significant association between income and stress.

Keywords: *Parents, Children with intellectual disability, Social support, Stress, Regression analysis.*

INTRODUCTION

Mental disability in children has been a curse for the parents and creates economic burden for the family.[1] Caregiver means an individual who provides direct care and support for chronically ill people.[2]Parents need to put extra effort and time in fulfilling the responsibilities towards their ward.The level of perceived burden is associated with higher risks of depression and lower quality of life for parents of children with intellectual disability.[3]Recently the American Association on intellectual and developmental disabilities (AAIDD) have approved a new nomenclature for mental retardation of children and have renamed it as intellectual disability[4]

Children with intellectual disabilities throw challenge on their parents, as they have more of adjustment problems.[5]It often upsets their life by increasing their perceived level of stress.[6]Parents of children with intellectually disabled account for more stress.[7]The behaviour and wellbeing of a child is found to have a greater impact on mother.[8] Intellectual disability of a children is remarked by ill communication and lack of academic and social skills.Parental stress is defined as "multidimensional response to physical, psychological, emotional social and financial stressors usually associated with the experience of caring". [9]

Emotional support is the one of the first type of support which is intangible in nature. Instrumental support is the second type of support and is regarded as tangible and direct ways of assisting others.[10]Its elements constitutes of financial boosting or materialistic gains.[11]Informational support is the third type of

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support which is based on providing useful advice, guidance and suggestions to individuals,[12]which act as a prospect to solve problems.[13] The fourth type of support is companion support which inculcates social belongingness and calls for shared social activities.[14]

Many studies reveal that there are significant association between parental stress and social support. Families having children with intellectual disability experience more stress and less social support.[14,15]Care giving burden for mothers are associated with higher education and less social support.[16]Gross income and social support was found to have indirect overall effects on psychological health outcome.[17] Low levels of social support were the most influential predictors of depression and anxiety in mothers.[18] There is strong correlation between family support and parenting stress.[19] In a study contradictory result was found and the relationship between the amount of informal support and level of parental stress was not confirmed.[20] Moderation effect of social support was confirmed in perceived stress and resilience.[21] Psychological stress was inversely related to perceived social support.[22] Few other studies explore the influence of various socio-demographic variables and its correlation with stress in parents of intellectually disabled children.[23] Income is found to be clinically significant with stress among parents of children's with intellectual disability.[24]Gender was significantly associated with risk of stress among parents.[25]

METHOD

*Design:*Parents of intellectual disabled child were the sampling unit whereas purposive sampling techniquewas used for selecting samples for the study.

Population: All the parents of the children with intellectual disability of Chhattisgarh were considered for the study. For locating such population at primary level, hospitals at district headquarter were contacted. Information's obtained from rehabilitation centers in Chhattisgarh was considered for the study.

Tools: The Perceived Stress Scale [26]is used in the study. To assess social support a Social Support Scale [27]was used in the study. Internal consistency Cronbach α coefficient of the scale was found to be 0.92 which confirmed the internal consistency of data.[28] Both of the scales were found to be reliable and valid.

The Demographic details such as age group, gender, income and educational qualification were also used in the survey form. A detailed description of the purpose of the study and the information requested for was provided in the covering letter.

Procedure

A survey based method was used to collect the data from the sample which lasted for about 13 weeks (i.e., from May to July 2017). 150 respondent parents participated in the study. Data were collected through personal means by the distribution of questionnaire in the beginning of May and was collected, as a when completed till July, 108 questionnaires were collected in the end, with a response rate of 72%. 100questionnaireswere found to be completed in all aspects, and hence were considered for inclusion for the final study.

RESULT

The demographic details of the respondents are presented in Table 1. The total sample size for the study constitute of 100 respondent parents, which are equally distributed in terms of male and female group.

Table 1 Demographic information of the participants (N = 100)

Variables	Frequency	Percentage	Mean	SD	CI [95%]
Gender					
Male	50	50%			
Female	50	50%			
Age (Years)			35.19	2.733	34.65 - 35.73
31-35	56	56%			

Cont... **Table 1 Demographic information of the participants (N = 100)**

36 – 40	44	44%			
Income (INR)			25459.95	9099.37	23654 - 27265
10000 - 25000	36	36%			
25001 – 40000	64	64%			
Education level					
UptoHigher Secondary	53	53%			
Graduate and above	47	47%			

Hierarchical multiple regression analysiswas used in the study to predict the affect of study variables on stress as shown in the Table 2. [29]

Table 2: Hierarchical Multiple Regression analysis forPredictorsonStress

Predictors	**Model 1**			**Model 2**			**Model 3**			**Model 4**		
	β	**t**	**VIF**	**B**	**t**	**VIF**	**β**	**t**	**VIF**	**β**	**t**	**VIF**
Income	-.544	- 6.422	1.000									
Education				-.213	-2.532	1.042						
Gender							-.362	- 4.561	1.651			
Social Support										-.476	- 6.917	1.030
R	.544			.583			.748			.799		
R^2	.296			.340			.559			.639		
ΔR^2	.296			.044			.079			.220		
ΔF	F(1,98) = 41.241**			ΔF (1,97) = 6.410*			ΔF (1,96) = 20.800**			ΔF (1,95) = 47.844**		

In model 1, factor income made significant contribution in variation of the stress F (1, 98) = 41.241, p<0.01) and explained 29.6 % of the variance in stress (R = 0.544, ΔR = 0.296). The standardised beta value (β = 0.-544, p<0.01) indicated significant negative association between predictor income and stress level of parents; it means higher the income lower will be parenting stress.

In model 2, factor education made significant contribution in variation of the stress (ΔF (1, 97) = 6.410, p<0.05). The introduction of factor education explained additional 4.4% variance in stress with overall 34% (R = 0.583, ΔR = 0.044). The predictor education was found to have significant negative association (β = 0.-213, p<0.01) with stress; that is indicate graduate and post graduate parents showed less stress.

In model 3, factor gender (1 = Male, 2 = Female) made significant contribution in variation of the stress (ΔF (1, 96) = 20.800, p<0.01) and explained overall 55.9% of variance in stress (R = 0.748, ΔR = 0.079); the model explained additional 7.9% of the variance in stress. The results indicated significant negative association between predictor gender of the participants and their stress (β = 0.-362, p<0.01); that means mothers showed higher stress in comparison to fathers of children with intellectual disability.

In the final model, main predicting factor in the study i.e. social support made significant contribution in variation of the stress (ΔF (1, 95) = 47.844, p<0.01)

and explained overall 63.9% of variance in stress (R = 0.799, ΔR = 0.220); the model explained additional 22% of the variance in stress. The results indicated (β = 0.-476, p<0.01) significant negative association between predictor social support and stress; that means those who perceived higher social support in the society, family or friends their stress level was decreased.

Findings clearly indicated that age did not contribute significantly in the variation of stress; factors income, education, gender and social support were found to be significantly negatively and directly correlated with stress of the participants. Result indicates the explaining percentage of all predictors was 63.9%; this total of the variance included 29.6% for income, 4.4% for education, 7.9% for gender, and 22% for challenge.

Variance inflation factor (VIF) found, ranged from 1.000 to 1.651, which was distant from the 1.0 to 4.0, criteria that may indicate multicollinearity concern.[30] It means that multicollinearity found significant correlation between all predicting variables.

All 100 cases were also included for confirm mediation effectworked out by structural equation model (SEM).[31] Significance of mediation was examined by bootstrap.[32] AMOS licensed version 24 was used for data calculation. Figure - 1 suggested that the total effect is .639, p<. 01; direct effect found (.296, p<.01). In addition, figure - 1 suggested that the indirect effect is (.369, p< .01) of income on stress through social support was statistically significant. The mediation model showed statistical good model fit of data(CMIN/DF= < 2.01, RMSEA = .079, GFI = .984, NFI = .929 and CFI =.957).[33]

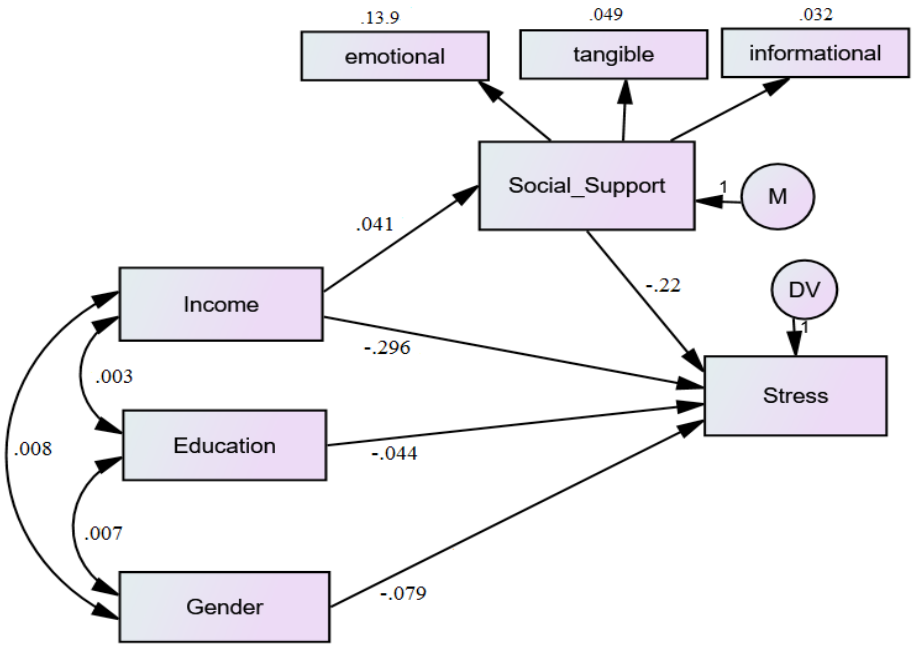


Figure 1 Mediating model of Social Support on the link between income and stress

DISCUSSION

The purpose of this study is to identify the effect of social support on stress level of parents of children with intellectual disability. It also studied the effect of various socio demographic variables like income, education and gender on the stress level of intellectually disabled children. The variable income is a significant predictor of stress and is negatively associated, which means that with the rise in income of the parents, there will be reduction in the stress or caregiving burden and is consistent with the previous findings of the related studies.[24] Similarly education was also significant predictor of stress and is negatively associated confirming that there is decrease in the stress level of the care givers with the rise in their education level.Researcher had similar findings in their studies.[17]Another demographic factor gender was significant predictor of stress is in keeping with the findings of other studies.[25]The impact of social support

on stress of the parents of children with intellectual disability was significant and is very much consistent with the past studies.[15,18]However the dimension age was insignificant on stress and was not found consistent with the earlier studies. The role of social support as a mediating variable between income and stress was found significant in the study.[22]

CONCLUSION

Stress has become an integral part of life. The condition of stress is developed within the environment. Parents of children with intellectual disability have an extra responsibility for caring their child and are subjected to more stressful conditions. As there is a relationship between social support and stress, the parents in order to reduce stress need more of emotional, tangible and informational support from the surroundings. Socio demographics of the parents also have significant relationship with the stress level. As income is a necessary condition of managing family, its increase leads to decrease in the stress level of the parents. Similarly with rise in the level of education, there is up gradation of knowledge component and thus decreases the stress level. Mother is subjected to more stress than the father as concluded in the study. Social support plays a satisfactory mediating role as a predictor between income and stress as concluded in the model fit. The model proposed in the study shows a good fit.

Conflict of Interest – No

Ethical Clearance – Data from the participants were obtained after taking their consent to participate in the research work. Ethical clearance was obtained from the departmental ethical committee.

Source of Funding – Self

REFERENCES

[1]. Busch SH, Barry CL. Mental health disorders in childhood: Assessing the burden on families. Health Affairs. 2007 Jul 1;26(4):1088-95.

[2]. Stucki BR, Mulvey J. Can Aging Baby Boomers Avoid the Nursing Home: Long-term Care Insurance for" aging in Place". American Council of Life Insurers; 2000.

[3]. Aronson KJ. Quality of life among persons with multiple sclerosis and their parents. Neurology. 1997 Jan 1;48(1):74-80.

[4]. American Association on Intellectual and Developmental Disabilities (AAIDD). 2007. http:/ aaidd.org.

[5]. Bharati R. Adjustment problems of educable mentally retarded. International Journal of Scientific and Research Publications. 2012;2(6):1-5.

[6]. Oshodi YO, Umeh CS, AfolabiLesi FE, Eigbike-Aideyan M, Adeyemi JD. Burden and psychological challenges in caregivers of children with intellectual disabilities in a child neurology clinic in Lagos, Nigeria. International Journal of Developmental Disabilities. 2014 Oct 1;60(4):226-34.

[7]. Dumas JE, Wolf LC, Fisman SN, Culligan A. Parenting stress, child behavior problems, and dysphoria in parents of children with autism, Down syndrome, behavior disorders, and normal development. Exceptionality: A Special Education Journal. 1991 Jan 1;2(2):97-110.

[8]. Heller T, Hsieh K, Rowitz L. Maternal and paternal caregiving of persons with mental retardation across the lifespan. Family relations. 1997 Oct 1:407-15.

[9]. Kasuya RT, Polgar-Bailey MP, MPH Robbyn Takeuchi MS. Caregiver burden and burnout a guide for primary care physicians. Postgraduate Medicine. 2000 Dec 20;108(7):119.

[10]. Slevin ML, Nichols SE, Downer SM, Wilson P, Lister TA, Arnott S, Maher J, Souhami RL, Tobias JS, Goldstone AH, Cody M. Emotional support for cancer patients: what do patients really want?. British Journal of Cancer. 1996 Oct 1;74(8):1275-9.

[11]. Heaney CA, Israel BA. Social networks and social support. Health behavior and health education: Theory, research, and practice. 2008 Jul 23;4:189-210.

[12]. Krause N. Social support, stress, and well-being among older adults. Journal of gerontology. 1986 Jul 1;41(4):512-9.

[13]. Tilden VP, Weinert C. Social support and the chronically ill individual. The Nursing Clinics of North America. 1987 Sep;22(3):613-20.

[14]. Uchino BN. Social support and physical health: Understanding the health consequences of

relationships. Yale University Press; 2004.

[15]. Friedrich WN, Friedrich WL. Psychosocial assets of parents of handicapped and nonhandicapped children. American journal of mental deficiency. 1981 Mar.

[16]. Oh H, Lee EK. Caregiver burden and social support among mothers raising children with developmental disabilities in South Korea. International Journal of Disability, Development and Education. 2009 Jun 1;56(2):149-67.

[17]. Raina P, O'Donnell M, Rosenbaum P, Brehaut J, Walter SD, Russell D, Swinton M, Zhu B, Wood E. The health and well-being of caregivers of children with cerebral palsy. Pediatrics. 2005 Jun 1;115(6):e626-36.

[18]. Boyd BA. Examining the relationship between stress and lack of social support in mothers of children with autism. Focus on autism and other developmental disabilities. 2002 Nov;17(4):208-15.

[19]. Hassall R, Rose J, McDonald J. Parenting stress in mothers of children with an intellectual disability: The effects of parental cognitions in relation to child characteristics and family support. Journal of intellectual disability research. 2005 Jun 1;49(6):405-18.

[20]. Duvdevany I, Abboud S. Stress, social support and well-being of Arab mothers of children with intellectual disability who are served by welfare services in northern Israel. Journal of Intellectual Disability Research. 2003 May 1;47(4-5):264-72.

[21]. Wilks SE, Croom B. Perceived stress and resilience in Alzheimer's disease caregivers: Testing moderation and mediation models of social support. Aging and Mental Health. 2008 May 1;12(3):357-65.

[22]. Lovell B, Moss M, Wetherell MA. With a little help from my friends: Psychological, endocrine and health corollaries of social support in parental caregivers of children with autism or ADHD. Research in developmental disabilities. 2012 Apr 30;33(2):682-7.

[23]. Dave D, Mittal S, Tiwari D, Parmar MC, Gedan S, Patel V. Study of anxiety and depression in caregivers of intellectually disabled children. Journal of Research in Medical and Dental Science. 2017 Mar 25;2(1):8-13.

[24]. Datta SS, Russell PS, Gopalakrishna SC. Burden among the caregivers of children with intellectual disability: associations and risk factors. Journal of Learning Disabilities. 2002 Dec;6(4):337-50.

[25]. Magaña S, Schwartz SJ, Rubert MP, Szapocznik J. Hispanic caregivers of adults with mental retardation: Importance of family functioning. American Journal on Mental Retardation. 2006 Jul;111(4):250-62.

[26]. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of health and social behavior. 1983 Dec 1:385-96.

[27]. Pandey D, Shrivastava P. Psychometric properties and confirmatory factor analysis of the social support scale. International Journal of Indian Psychology. 2016;3(4):191-8.

[28]. Gliem JA, Gliem RR. Calculating, interpreting, and reporting Cronbach's alpha reliability coefficient for Likert-type scales. Midwest Research-to-Practice Conference in Adult, Continuing, and Community Education. 2003 Oct 8-10: 82-88.

[29]. Cohen J, Cohen P, West SG, Aiken LS. Applied multiple correlation/regression analysis for the behavioral sciences. UK: Taylor & Francis. 2003.

[30]. Pandey D. Psychoneuroimmunology of Chronic Illness. RED'SHINE Publication. Inc; 2016 Oct 26.

[31]. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behavior research methods. 2004 Nov 1;36(4):717-31.

[32]. Cheung GW, Lau RS. Testing mediation and suppression effects of latent variables: Bootstrapping with structural equation models. Organizational Research Methods. 2008 Apr;11(2):296-325.

[33]. Randall ES, Richard GL. A Beginner's Guide to Structural Equation Modeling. 2010. 1996;79(2):286-7.

Risk of HIV Infection among Tuberculosis Patients of Jaintia Tribes, Meghalaya

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ABSTRACT

Tuberculosis (TB) and Human Immunodeficiency Virus (HIV) co-infection is associated with special diagnostic and therapeutic challenges and constitutes an immense burden on healthcare system of heavily infected countries like India. The association of TB with HIV is two-folded, and HIV infection being the most potent risk for a latent TB infection to convert to active disease. Aim of the study is to understand the risk factors associated with HIV infection among the TB patients of Jaintia Hills, Meghalaya. The data was collected from 317 TB patients belonging to the age group 15-50 years from different health centres of Jaintia Hills, Meghalaya. Out of all the TB patients, 39 (12.3%) were infected with HIV. The most important risk factors associated with HIV were found to be religion ($p<0.0$), marital status ($P<0.01$), education ($p<0.02$), occupation ($p<0.00$), income group ($p<0.05$). Early detection of TB and HIV co-infection is necessary to facilitate early treatment and ART initiation, thereby strengthening the control of TB and HIV.

Keywords: *Tuberculosis, HIV, Co-Infection, Jaintia Hills, Meghalaya.*

INTRODUCTION

India is the country with the highest burden of TB. It was estimated that globally around 9.6 million people were infected with TB, out of which 2.8 million cases were reported from India[1]. HIV is fuelling the global TB epidemic; the prevalence of this virus in TB patients is a sensitive indicator of the spread of the disease into the general population in many regions[2]. The effect of TB/HIV co-infection is bidirectional and synergistic/ aggravating[3]. Infected with both HIV and TB is one of the world's leading cause of death. The epidemic of AIDS has accelerated the rates of transmission and mortality due to TB. Although, the incidence of TB/HIV co-infection has decreased slowly with the implementation of antiretroviral therapy (ART), but unfortunately, in recent years globalization with economic and cultural exchanges has contributed to spread these diseases[4]. Both diseases are intricately linked to malnutrition, unemployment, poverty, alcohol consumption, drugs abuse[5]. Moreover, the prevalence of HIV among TB patients could be more in India, because 42% of these patients are unaware of their HIV status, the other reason being high rate of immigration from other states[6].

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METHOD

A cross-sectional study was conducted among 317 patients receiving treatment for TB at the various health centres (District Tuberculosis Hospital, Civil Hospital, PHC, CHC, UHC) in Jaintia Hills (East and West), Meghalaya. Since, the present study was a part of ICMR funded project entitled "Prevalence and Risk Factors leading to HIV Infection among the Two Tribes of Jaintia Hills, Meghalaya and Ethical clearance for ICMR funded project was sought from Amity University Research Ethics Committee. The patients involved in study were the individual suffering from TB and was registered in the hospital between 2014-2016, all subjects belonged to the age group 15-50 years and belonged to the tribal community of Meghalaya (pnar, biate, khasi, garo). Before data collection written permission was sought from District Medical and Health Officer (DM&HO) and Medical officers (MOs) of all

the health centres. Informed consent was obtained from each patient prior to conduct the interview and gathered information kept confidential. Patients who refused to give consent and who were unable to understand the questions were excluded from the study. Structured schedule was used to collect the demographic data such as age, sex, religion, community, education, occupation, marital status etc. from each patient. Information related to risk factors associated with TB and HIV was gathered. HIV screening was done for all the tuberculosis patients using WHO evaluated "Rapid Test Kit" following NACO guidelines. Statistical analysis was carried out using SPSS V22. Differences between frequencies were tested using Chi-square test (significance level $p<0.05$). Multivariate analysis was used to evaluate the associated risk factors of HIV infection among the TB patients. Odds ratios were obtained with corresponding 95% confidence intervals.

RESULTS

The present study was conducted among 317 patients diagnosed with tuberculosis in Jaintia Hills, Meghalaya and among them 39 (12.3%) respondents were found to be infected with HIV.

Correlation of socio-demographic parameters with TB/HIV co-infection

The number of HIV infected TB patients were found to be more in the age group 25-34 years (16.1%) as compared to other age groups and was found to be statistically significant at 5% probability level ($p<0.05$). Most of the Christian community patients (20.7%) were infected with both TB and HIV while only around 5% Niamtre and Hindu population were infected with TB/HIV co-infectious diseases. Statistical analysis shows a significant variation of marital and HIV status ($p<0.05$), with increased risk of HIV among unmarried patients (20.3%). Another important parameter for HIV prevention is education, it was observed that the cases of HIV positive were found to be illiterate (9.5%) and 15.2% were informally educated. Around, 10.9% infected cases had their education below or up to secondary (including primary and middle level of education); however, 41.7% patients pursued or pursuing further education after secondary (higher secondary, diploma courses, vocational studies and post graduation level of education). The distribution of educational level among HIV positive and negative was found to be statistically significant ($p<0.05$). Majority of the HIV infected studied populations were unemployed (36.4%) and belong to lower economic group (16.7%). Statistically significant differences were observed with respect to occupation and economic status between the HIV positives and negative patients ($p<0.05$).

Sexual Practices and Behaviour

In the present study, around 95% of the TB patients were found to sexually active and risk of HIV infection was higher among them (10.7%). Promiscuous sexual behavior (more than one sexual partner) (79.2%) was found in the study population, out of which 13.9% were infected with HIV co-infection. This factor emerged as very important risk factors among TB patients. Although, the association between sexual relationship, numbers of sex partners with respect to HIV status was found to be statistically insignificant ($p>0.05$).Out of 300 sexually active respondents 33% had history of sexually transmitted disease; among them 18 patients were infected with this viral disease. The association between history of sexually transmitted disease with respect to HIV status was found to statistically significant ($p<0.05$). The study revealed that almost 13% TB patients infected with HIV had not used condom during sexual practices. Moreover, the history of alcohol abuse injectable drug use was found to be 10.8% and 26.1% (respectively). The association between alcohol abuse, intravenous drug use and HIV infection was found to be statistically significant ($p<0.05$) (Table 1).

Table 1: Risk Factors associated with HIV infection among the TB patients:

Variables	Patients infected with Only TB		Patients infected with both TB/ HIV		Total	p value
	N	%	N	%	N	
History of other sexually transmitted diseases (n=300)						
Yes	81	81.8	18	18.2	99	0.000*
No	154	98.1	3	1.9	157	
Don't know	27	61.4	17	38.6	44	

Cont... **Table 1: Risk Factors associated with HIV infection among the TB patients:**

Alcohol abuse (n=317)						
Yes	257	89.2	31	10.8	288	0.009*
No	21	72.4	8	27.6	29	
Injecting drug use (n=317)						
Yes	17	73.9	6	26.1	23	0.03*
No	261	88.8	33	11.2	294	
Total	278	87.7	39	12.3	317	

DISCUSSION

The present study indicates the prevalence of HIV (12.3%) among the tribal tuberculosis patients in Jaintia Hills, which is similar to the findings in the states of Odisha, Jharkhand and some areas of West Bengal[2]. In India, the number of HIV cases among TB patients is found to be high in Mumbai (30%), Pune (28.75%) and Mangalore (21%) compared to low prevalence in New Delhi (0.68%), Jammu and Kashmir (1.6%), Aligarh (2.8%), Indore (4%) and Tamil Nadu (4.7%)[7-12]. The association between marital status, education and HIV infection was found to be significant ($p<0.05$) in accordance with other studies conducted in all over the world[13-14]. However, Occupation was also found to be a risk factor for HIV infection in tuberculosis patients, such significant association was observe in other studies[15-16]. In the present study, a strong statistical significance ($p<0.05$) was found with respect to sexually transmitted diseases, alcohol abuse and injecting drug use and HIV infection among the TB patients of Jaintia Hills, which is also reported from different parts of world[17-18].

The most important risk associated with HIV infection in the present study was religion, education, occupation (high in unemployed, coalmine workers and truck drivers), low-socio economic status, marital status, presence of sexually transmitted diseases, use of recreational substances use such as alcohol before sex and injecting drugs. Whereas, there were no significant association with age, gender, community, multiple sex partners and use of condom though these parameters described as high risk factors in some studies[19-20].

CONCLUSION

HIV infection is on the rise among TB patients in all over the world. Based on the findings of this study there is a high HIV prevalence in this TB patient population. Majority of the friends of the local tribal people were from Nepal, Bangladesh, Assam, Manipur etc. and some of them were using injecting drugs on daily basis and shared the needles with the others; they at times forced others to use it. This is a great concern especially as it might affect both patient management and public health prospective. In view of associated risk of TB/ HIV co-infection, early detection and treatment of TB and HIV co-infection is necessary along with promotion of condom use and not using substance need to be considered as HIV prevention measures for TB patients in various infection prevention programmes in Jaintia Hills, Meghalaya. It is alo very important to make sure the availability of anti-tuberculosis regimens in all Health Centres of Jaintia Hills.

Conflict-of-Interest: NA.

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Ethical Clearance: The present study was a part of Indian Council of Medical Research (ICMR) funded project, so, the Ethical clearance was sought for the project from Amity University Research Ethics Committee.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2017. http://www.who.int/tb/publications/global_report/MainText_13Nov2017.pdf
2. Manjareeka M and Nanda S. Prevalence of HIV infection among tuberculosis patients in Eastern India. Journal of Infection and Public Health 2013;

6: 358-362.

3. Sharma SK, Mohan A and Kadhiravan T. HIV-TB CoInfection: Epidemiology, Diagnosis and Management. Indian Journal of Medical Research 2005; 121: 550-567.
4. Wong ML, Chan R, Tan HH, Yong E, Lee L, Cutter J, et al. Sex work and risky sexual behaviors among foreign entertainment workers in urban Singapore: findings from Mystery Client Survey. Journal of Urban Health 2012; 89: 1031-1044.
5. ArunMohan MV, Tejaswi HJ and Ranganath TS. Socio-demographic profile of TB-HIV co-infected adults and it's association with tuberculosis treatment outcome, in a South Indian city. International Journal Of Community Medicine And Public Health 2016; 3: 3498-3503.
6. Soyam VC, Das J, Rajeeva TC, Boro P and Kohli C. Prevalence and socio-demographic correlates of HIV among Tuberculosis patients of DOTS centre in Delhi. Asian Journal of Medical Sciences 2015; 7: 53-58.
7. Jain SK, Aggarwal JK, Rajpal S, Baveja U. Prevalence of HIV infection among tuberculosis patients in Delhi - A sentinel surveillance study. Indian Journal of Tuberculosis 2000; 47: 21-26.
8. Ramachandran R, Datta M, Subramani R, Baskaran G, Paramasivan CN and Swaminathan S. Seroprevalence of human immunodeficiency virus (HIV) infection among tuberculosis patients in Tamil Nadu. Indian Journal of Medical Research 2003; 118: 147-151.
9. Swaminathan S, Ramachandran R, Baskaran G, Paramasivan CN, Ramanathan U, Venkatesan P, et al. Risk of development of tuberculosis in HIV-infected patients. The International Journal of Tuberculosis and Lung Disease 2000; 4: 839-844.
10. Tripathi S, Joshi DR, Mehendale SM, Menon P, Joshi AN, Ghorpade SV, et al. Sentinel surveillance for HIV infection in tuberculosis patients in India. Indian Journal of Tuberculosis 2002; 49: 17-20.
11. Bahl R, Singh B and Singh R. Prevalence of HIV infection among patients of pulmonary tuberculosis attending chest disease hospital, Jammu (Jammu and Kashmir). Indian Journal of Community Medicine 2007; 32 :288-289.
12. Pai SB. HIV and tuberculosis. Indian Journal of Tuberculosis 2006; 53: 43-46.
13. Mitku AA, Dessie ZG, Muluneh EK, and Workie DL. Prevalence and associated factors of TB/HIV co-infection among HIV Infected patients in Amhara region, Ethiopia. Afr Health Sci. 2016; 16: 588-595.
14. Méda ZC, Sombié I, Sanon OWC, Maré D, Morisky DE and Chen Y-MA. Risk Factors of Tuberculosis Infection Among HIV/AIDS Patients in Burkina Faso. AIDS Res Hum Retroviruses 2013; 29: 1045-1055.
15. MartieL CT. SusanE VWBM. William KD and Jason EGY. Occupational Risk Factors for Tuberculosis Among Healthcare Workers in KwaZulu-Natal, South Africa. Clinical Infectious Diseases 2016; 62 :S255–S261.
16. Field MJ. Occupational Risk of Tuberculosis, National Academies Press (US), Washington (DC) 2001. https://www.ncbi.nlm.nih.gov/books/NBK222463/
17. Miller AC, Nelson AK, Livchits V, Greenfield SF, Yanova G and Yanov S, et al. Understanding HIV Risk Behavior among Tuberculosis Patients with Alcohol Use Disorders in Tomsk, Russian Federation. PLoS One 2016; 11: e0148910.
18. Deiss RG, Rodwell TC and Garfein RS. Tuberculosis and Drug Use: Review and Update. Clin Infect Dis. 2009; 48: 10.
19. Kharsany ABM and Karim QA. HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. Open AIDS J. 2016; 10: 34-48.
20. Peltzer K. Longitudinal analysis of HIV risk behaviour patterns and their predictors among public primary care patients with tuberculosis in South Africa. SAHARA J. 2018; 15: 1-6.

Does Ict Influences Rural Government School Teachers Beliefs?-Exploring Teachers Opinion on Usage of Ict as Teaching and Learning Tool

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ABSTRACT

The world is experiencing quick changes due to outburst of new technologies. These transformations are visible throughout biosphere together with rising countries like India, China, and Indonesia etc. These changes need to embraced by the developing countries in sequence to get better value in education and as well as to strengthen the classroom teaching and learning process.

Objective: The main objective of the study is to identify educators' viewpoint about advance technology introduction integration in Education

Method: The present survey was conducted in Tirutanni taluk among primary, middle and higher secondary rural government school teachers in order to identify their beliefs on ICT. 200 Questionnaires was distributed and 152 teachers participated in the survey. 48 teachers did not participate in the survey. The response rate for the study was 76%.CFA was used to analyze the research model. The study contributes to the research and fills the research gap of limited studies done in this research area.

Results: The findings reveal that teachers believe that ICT influences teaching and it can use as a teaching and learning tool.

Conclusion: Usage of ICT by teachers leads to quality education**.**

Keywords: *Teachers beliefs, Technology devices, educator and learning tools.*

OVERVIEW

The domain is undergoing quick fluctuations due to outburst of new technology software. These transformations are visible throughout biosphere together with rising countries like India, China, and Indonesia etc. These changes need to embraced by the developing countries in sequence to get better value in in education and as well as to strengthen the classroom teaching and learning process. ICT has become a universal theme and information technology has started to revolutionize all countries, reaching every person by giving an end to traditional methods of teaching.[1].

The Indian education system has started to realize the major advantages of Information and communication technology as a valuable tool to be used in schooling and knowledge that provides larger entrance to greater communication; the operation activities of learning and pedagogical improvement[2]. Students have their everyday usage in ICT through smart phones, social networks like Instagram, Facebook etc.[3]

The frequent challenges seen by the rural teachers are the need of awareness and familiarity how to employ internet and explore data. It discourages teachers from usage of ICT in classroom teaching process[4]. On the other hand the noteworthy changes implemented by rural teachers are changing their traditional teaching methods to application methods in ICT mode. The usage of advance technology in coaching and education process involves advance technology abilities known as ICT education that involves knowledge on fundamental computer operations and practice. Usage of advance technology devices in daily lives has made life easier[5]. Teachers need to use ICT as it is not value addition but it

has started to become essential in daily process.

As per the Indian advanced technological devices usage trendy education and its integration in to classrooms was much stressed in the national policy on education in 2016[6]. But its actual implementation started around 2 decades ago. In 1992 Programme of Action which was formed, stressed on usage of ICT in schools. Various smart schemes were introduced to make educators improve their skills on ICT. Though this initiative did not take up well the usage of SMART resources were started in higher education.

The author[7] has stated that usage of ICT leads to professional development and such technology skills need to be developed by teaching professionals. The researchers [8] in their study communicates teachers attitudes proved to be significantly positive towards the usage of ICT. With these positive attitudes the required skill sets for ICT need to be acquired by the rural school teachers.

MATERIALS AND METHOD

India is one of the fast growing countries in usage of internet. The spread of internet usage among them have seen rapid growth in urban areas when compared to rural. Rural India is still lagging behind in terms of internet services and availability of ICT. As per the census of India 2018, The Indian youth population who are 50% below the age of 25 years are the main users of Information and Communication. The number of internet users in India by 2022 would be 511.89 million [9]. Among the population usage of advanced technology by teachers in the ground of instruction has increased. Both state and central government has also framed policies on usage of ICT. The Education policy 2017-18 of Tamilnadu has also emphasized usage of ICT everywhere. But rural area has been experiencing limited connectivity of internet to urban areas. Various researchers have stated that the degree of digital competence of its teaching staff needs to be increased The researchers [10] have found that teachers are expected to equip themselves with the 21st century skills on computers.

This study was designed to analyze the beliefs of rural government school teachers advanced technology usage as instruction and knowledge tool. The study also helps route and explore about the beliefs on ICT integration in Education.

LITERATURE REVIEW

Educators viewpoint about Advance Technology usage as a Instruction and Knowledge Tool

Teachers are termed as demonstrators and they are the primary factor for implementing development and innovation in education. Their perceptions do play a vital role in implementation of educational technology in improving the performance of the students Among educators (teachers) male members proved to have more confidence in use of ICT to female[11].

It is also stated that technology has more value when utilized with subjects like mathematics, solving word problems[12]. Some quote Education Technology is effective but its effective usage in classrooms is associated to instructors' outlooks and their near wisdom.

Educators Beliefs about Technology Integration in Education

[13]The authors in their study has revealed that advance technology view point by educators and its implementation in the area of education.

Aim of the research study

The prime demonstrable aim of the research was to pinpoint educators' beliefs about ICT as a teaching and learning tool and Integration of ICT in Education.

Resources and method

Tool

This is assessable research learning and data was collected using survey method.

Population

One rural government coeducation school was considered for the study. 200 teachers participated in the survey and structured questionnaire were distributed. 152 teachers participated in the study. 48 teachers did not return the filled in questionnaire.

Data Assessment

Data was assessed using CFA. AMOS software was used version 21.

Research process

Assessment method was engaged to gather the data.

Inquiry forms were distributed to 200 rural government school educators for the force of determining the soundness and consistency of the inquiry tool. The soundness values for individually are: educators' beliefs about ICT as a teaching and learning tool (0.687), educators' viewpoint about advance technology usage in education (0.624). People for the real data collection were rural government school educators. Data was analyzed by using CFA with Amos version 21.

RESULTS

Confirmatory factor analysis was done with AMOS 21.0 software version to understand the teachers' beliefs.

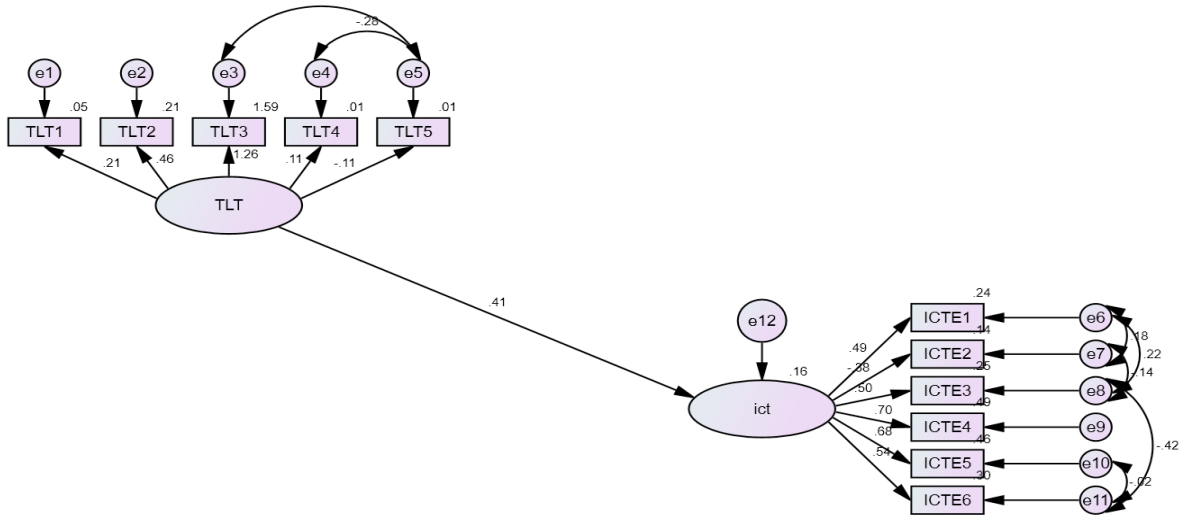


Diagram 1 showing Confirmatory Factor Analysis

Table 1: Showing significance of the model (Group number 1 - Default model)

			P
Ict	<---	TLT	.001
TLT1	<---	TLT	000
TLT2	<---	TLT	.001
TLT3	<---	TLT	.022
TLT4	<---	TLT	.099
TLT5	<---	TLT	.435
ICTE1	<---	Ict	.000
ICTE2	<---	Ict	.001
ICTE3	<---	Ict	***
ICTE4	<---	Ict	***
ICTE5	<---	Ict	***
ICTE6	<---	Ict	***

The results of the study reveals that the constructs on advance technology usage as a coaching and knowledge tool proved to be significant with alpha value less than $p<0.05$. The constructs on teachers beliefs on the implementation of ICT in education process is also proved to be significant with values less than $p<0.05$.

It can also be inferred from above table and diagram that teachers do strongly believe that ICT can be used as to tool of instruction in the subject matter in the curriculum. ICT helps to stimulate critical thinking of the students and it contributes substantially to students teaching and learning process. Hence teachers are strongly convinced is usage of **advance technology usage as a coaching and knowledge tool.**

The perceptions of educators on advance technology usage in their jobs in an effective manner are proved to be significant in the study. The study also provides insights in to usage of advance technology in the area of teaching will prove to be useful for the society in the future. At the same time teachers too believe that too much usage of ICT usage will isolate students and separate them from learning process. **Therefore ICT in usage of education process are believed to be effective but need to be used with caution.**

DISCUSSION

The results showed that educators believe that advance technology can be benefited as a teaching and learning tool. Teachers belief on using ICT for management and educators work preparation, ICT contribution as learning tool are significant as per the analysis.

Further in the study, teachers' beliefs on ICT integration in education are found to be significant. Teachers are interested to involving advance technology in instruction as it improves the teaching and learning process which is relevant to the study done by[14]. Teachers are ready and are always interested in learning new technologies which helps them to enhance their skills in technologies[15].

The study further reveals that rural government school teachers do have negative thoughts and they feel insecure in usage of ICT in education and they do face lot of challenges in using ICT for their respective subjects.

Hence it is important that government should take steps in solving the challenges faced by rural government school teachers by making regular investments in infrastructure facilities in government schools.

CONCLUSION

The study has shed light on two important things like advance technology as a coaching and knowledge tool and secondly ICT introduction in the field of education. The results discussed in the study also prove to be significant. However there are constraints found in ICT implementation practically due to lack of infrastructure facilities. ICT implementation in rural areas will lead to technological and skill enhancement of rural government school teachers and leads to quality education for rural children.

Author contributions: The author has collected the data on her own and she wants to specialize in the area of ICT.

Ethical Clearance: NA

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. A Sife, E Lwoga, C Sanga New technologies for teaching and learning: Challenges for higher learning institutions in developing countries.2007;3(2).
2. Yusefee et al The impact of information and communication technology on economic growth: evidence from developed and developing countries, Economics of Innovation and New Technology 2011;20(6).
3. Yunus&WekkeThe application of multicultural education and applying ICT on Perantren in south Sulaesi, 2009.
4. Wang The current practice of integration of information communication technology to English teaching and the emotions involved in blended learning, Turkish online Journal of Educational Technology 2014; 13(3),188.
5. Rumpa Das Integrating ICT in Teaching Learning Framework in India: Initiatives and Challenges, Bhatter College Journal of Multidisciplinary Studies 2012.
6.

7. Alcuin Mwalongo Teachers' perceptions about ICT for teaching, professional development, administration and personal use International Journal of Education and Development using Information and Communication Technology.2011;7 (3)p. 36-49.
8. Chang Zhu. Organizational culture and technology-enhanced innovation in higher education. Technology, Pedagogy and Education 2015:24:1,p 65-79.
9. Manuel Area Moreira, Olga Cepeda Romero, Luis felicianogarcía Primary and Secondary Education students' perspectives on the school use of ICT 2018;36.p. 229-276.
10. O'Neal, LaToya J.; Gibson, Philip; Cotten, Shelia R Elementary School Teachers' Beliefs about the Role of Technology in 21st-Century Teaching and Learning, Computers in the Schools, 2017;34(3) p192-206.
11. Almekhlafi, A. G., &Almeqdadi, F. A. .Teachers' Perceptions of Technology Integration in the United Arab Emirates School Classrooms. Educational Technology & Society, 2010; 13(1), pp 165–175.
12. Charles Buabeng-Andoh ICT usage in Ghanaian secondary schools: teachers' perspectives, The International Journal of Information and Learning Technology 2015;32(5) p. 300-312.
13. K. M. Cassim, S. D. EyonoObono On the Factors Affecting the Adoption of ICT for the Teaching of Word Problems. Proceedings of the World Congress on Engineering and Computer Science, 2011;1,p.19-21.
14. Steve Higgins & David Moseley Teachers' thinking about information and communications technology and learning: beliefs and outcomes, Teacher Development, 2001; 5(2),p. 191-210.
15. Sudha S Understanding the relationship between rural government school teachers perceptions, technological knowledge and ict awareness on technological pedagogical knowledge, Indian Journal of Public Health Research and Development, 2018;9(2): p.251-256.
16. Norton.P&Willburg K. Teaching with technology, Belmont, Wadsworth Publishing:2003.

Evaluation of Levels of Gunas in Indian Athletes Using Prakriti Concept

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ABSTRACT

Background: Our civilization has always recognized extraordinary individuals, whose performance in sports, arts, and science is vastly grander to that of the rest of the population. The present study was conducted to assess the levels of gunas of Indian athletes at three levels using the Prakriti concept.

Materials and method: The present study consisted of 107 school level athletes, 110 college level athletes and 76 Elite level athletes with age group of 15-18 years, 19- 24 years and 16- 45 years. Mathew IAS Rating Scale was applied. The instrument measures three broad behavioral tendencies (personality components, dimensions): Inertia (Tamas), Activation and Stability. Partial least squares structural equation modeling (PLS-SEM) approach was used to assess the coefficient regression values of the individual contributing factors to total personality values

Results: Factor loadings of all items presence of convergent validity. At school level stability was found to be negative with β= -0.494, activation was found to be positive with β= .352 and Inertia (Tamas) was found to be positive. Activation was found to be positive with β= .45 and Inertia (Tamas) was not significant in the elite athletes and negative.**Conclusion:** The performance of Indians at international level has been very mediocre and understanding the personality from school to elite level being predominantly result oriented (Rajas) can help in changing the approach at grass root level.

Keywords: *Athletes, Gunas, Prakriti*

INTRODUCTION

Personality has been defined as "psychological qualities that contribute to an individual's enduring and distinctive patterns of feeling, thinking and behaving"[1] whereas successful performance is defined as having both the development and mastery of knowledge, skills, and abilities and the capability to consistently and reliably deliver (i.e., perform) at the time of performance.[2]

Our civilization has always recognized extraordinary individuals, whose performance in sports, arts, and science is vastly grander to that of the rest of the population. Assumptions on the causes of these individuals' astonishing abilities and performance are as old as the first records of their achievements.[3]

Type based assessments in which personality has been assessed by categories an individual as one type or another. This fails to support the concept of "type" (e.g. introvert or extravert) as successful athletes have been both introverts and extroverts as shown in various researches. Thus type fails to support the concept of "type".[4]Samkhya theory of personality on the other hand provides the assessment of each individual of their present state and steps to proceed to next level in relation to traits to improve performance.[5]

Samkhya theory of personality shows the insights in accepting human nature as per Indian thoughts. Various Indian researchers have also deliberated on this concept

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of Triguna in their writings.[6]

The Triguna concept shows the great teacher of classical yoga, Patanjali, says (Yoga Sutras II.18), "The world of objects is composed of the three gunas—the principles of illumination (Sattva), Activation (Rajas)) and Inertia (Tamas).

The guna are of three kinds and are present in all individual at all the time and these gunas act together and never exist in isolation. Based on the above understanding, personalities are categorized into three, viz., Stability (Sattvic), Activation (Rajasic) and Tamasic types. Individuals differ in their cognitive, affective, and personality aspects according to interplay of Triguna.[7]

Guna has its own effect on the intellect and action. Each guna has a balanced and positive state and excess or negative state.

Stability (Sattva) Guna

From the Bhagavad Gita (14.6): "Of these three, sattva, untainted, luminous, free from sorrow, binds by means of attachment to knowledge and joy." As balanced state: Stability (Sattvic) quality has been described as being "free from attachment and vanity and absolutely unruffled in success and failure." In excess Stability (Sattvic) generates self-righteousness, complacency and self-satisfaction. That means happy in finding the solution but not acting on it when required.[8]

Activation (Rajas) Guna

From the Bhagavad Gita (14.7): "Activation (Rajas) is marked by passion born of craving and attachment; it binds the embodied Self to never-ending activity. "Activation (Rajas) is described as an intermediary between Inertia (Tamas) and Stability (Sattva).[9]

Inertia (Tamas) Guna

From the Bhagavad Gita (14.8): "Tamas, ignorance-born, deludes all embodied beings; it binds them by means of dullness, indolence and sleep." Tamas present in a balanced state represents qualities such as ease, loyalty, patience, stability and being grounded. Shutting down or coming to rest brings forgetfulness and possibility of renewal.[10]

The present study was done to assess the reasons for poor performance of Indian athletes at international level using the Triguna concept with objective to assess the levels of gunas of Indian athletes at three levels using the Prakriti concept.

MATERIAL AND METHOD

The present study includes purposive sample of school level athletes (n=107), college level athletes (n=110) and Elite level athletes (n=76). The study protocol was approved from institutional ethical committee. All participants were informed and written consent was obtained. The participants in interschool and intercollegiate meet belonging to various states. Elite athletes were representing India at international level.

Age group of school level was 15-18, college levels were from 19-24 and elite level age varied from 16 to 45. Mathew IAS Rating Scale was applied. The instrument measures three broad behavioral tendencies (personality components, dimensions): Inertia (Tamas), Activation and Stability. They cut across cognitive, temperament and motivational modalities. It contains 35 items and allows the subject to discriminate and assess type and strength of guna for each questionnaire. Reliability was established as standardization details for athletes' assessment was not found in the literature.

STATISTICAL ANALYSIS

The obtained data was statistically analyzed using IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. Partial least squares structural equation modeling (PLS-SEM) approach was used to assess the coefficient regression values of the individual contributing factors to total personality values.

RESULTS

Table 1 indicates Reliability and Validity. Here *Cronbach's Alpha* (CA) - Values of all the constructs exceeds or equivalent to the level of 0.75. *rho_A Reliability* – Measure of consistency all values greater than .75 *Composite Reliability* (CR) – All the values in the above table are greater than .75 . *Factorial Validity* - Factor loadings of all items in the present study are more than 0.5, thus indicating presence of convergent validity.

Figure 1 indicates Measurement Model Diagram showing the contribution of three broad behavioral tendencies personality components: Inertia (Tamas) (I), Activation (A) and Stability (S)

Table 2 indicates school level athletes displaying mean score of significant Regression (β) path coefficients, T statistics and p values obtained by estimating standard errors for the estimates-School athletes. The values are highly significant. At school level: Stability was found to be negative with β= -.494 (p = .000*), activation was found to be positive with β= .352 p*** highly significant and Inertia (Tamas) is found to be positive with β = 0.279 (p = .000*)

Tables 3 indicates for college level Athletes displaying mean significant Regression (β) path coefficients, T statistics and p values obtained by estimating standard errors for the estimates-College athletes. The values are statistically significant. At college level stability was found to be negative with β= -.46 (p = .000*), Activation is found to be positive with β= .19 (p = .000*) and Inertia (Tamas) is found to be positive with β = 0.45 (p = .000*)

Table 4 indicates Elite International Level Athletes which Displaying significant Regression (β) path coefficients, T statistics and p values obtained by estimating standard errors for the estimates-Elite athletes. At Elite level: Stability is found to be negative with β= -.46 (p = .000*), Activation is found to be positive with β= .45 (p = .000*) and Inertia (Tamas) is not significant in the elite athletes and negative with β = -0.09 (p = 0.31).

Tables 5 indicates Trend of Triguna at three levels of sports in India where Trend of the three personality aspects vis. Inertia (Tamas), Activation (Rajas) and Stability Sattvic) for the three distinct levels of sports (School, College & Elite) was compared. It indicates Stability is highly significant however perceived negatively at all three levels. Activation is highly significant and is perceived positively however activation is high at school level and highest at Elite level. Inertia (Tamas) is only significant at school and college level and not significant at elite level. Stability was found to be similar at all three levels. Activation was highest at the elite level and Inertia (Tamas) was found to be highest in college level.

Table 1 Reliability and Validity

Personality Components	Cronbach's Alpha	rho_A	Composite Reliability
School Level Athletes			
Activation	0.749	0.806	0.788
Inertia (Tamas)	0.742	0.779	0.775
Stability	0.823	0.85	0.851
College Level Athletes			
Activation	0.752	0.792	0.79
Inertia (Tamas)	0.913	0.919	0.923
Stability	0.894	0.902	0.907
Elite International Level Athletes			
Activation	0.968	0.973	0.971
Inertia (Tamas)	0.856	0.872	0.882
Stability	0.972	0.974	0.974

Cronbach's Alpha (CA) - Values of all the constructs exceeds or equivalent to the level of 0.75.

rho_A Reliability – Measure of consistency all values greater than .75

Composite Reliability (CR) – All the values in the above table are greater than .75 .

Factorial Validity - Factor loadings of all items in the present study are more than 0.5, thus indicating presence of convergent validity.

Table 2: **Displaying significant Regression (β) path coefficients, T statistics and p values obtained by estimating standard errors for the estimates-School athletes**

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	T Statistics (|O/ STDEV|)	P Values	
Activation -> IAS	0.35	0.35	0.03	11.88	0.000	
Inertia (Tamas) -> IAS	0.27	0.29	0.04	6.455	0.000	
Stability -> IAS	-0.49	-0.46	0.02	18.836	0.000	

Table 3: displaying significant Regression (β) path coefficients, T statistics and p values obtained by estimating standard errors for the estimates-College athletes

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	T Statistics (|O/ STDEV|)	P Values	
Activation -> IAS	0.193	0.214	0.044	4.359	0.000	
Inertia (Tamas) -> IAS	0.453	0.442	0.052	8.743	0.000	
Stability -> IAS	-0.469	-0.454	0.02	24.003	0.000	

Table 4: Displaying significant Regression (β) path coefficients, T statistics and p values obtained by estimating standard errors for the estimates-Elite athletes

	Original Sample (O)	Sample Mean (M)	Standard Deviation (STDEV)	T Statistics (|O/ STDEV|)	P Values	
Activation -> IAS	0.456	0.45	0.05	7.71	0.000	
Inertia (Tamas) -> IAS	-0.09	0.01	0.09	1.003	0.316	
Stability -> IAS	-0.51	-0.47	0.20	2.53	0.012	

Table 5: Trend of the three personality aspects vis. Inertia (Tamas), Activation (Rajas) and Stability Sattvic) for the three distinct levels of sports (School, College & Elite)

Personality Components	School Athletes	College Athletes	Elite Athletes
Inertia (Tamas) -> IAS	0.279	0.453	-0.096
Activation -> IAS	0.352	0.193	0.455
Stability -> IAS	-0.494	-0.469	-0.514

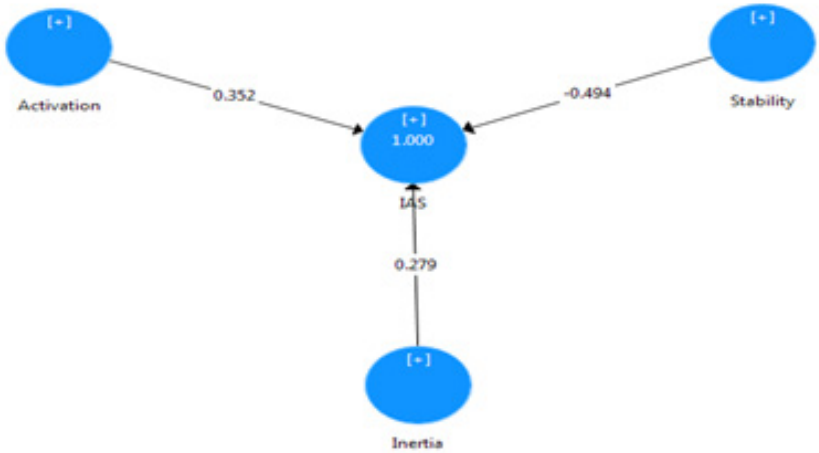


Figure 1: Measurement Model - Diagram showing the contribution of three broad behavioral tendencies personality components: Inertia (Tamas) (I), Activation (A) and Stability (S)

DISCUSSION

While assessing the result through the prism of the triguna concept one has to understand the fundamental in using this theory is that all the three gunas, namely Inertia (Tamas), Activation (Rajas), and Stability (Sattvic) are essential and necessary for the functioning of the human mind and body complex. An athlete has to use both his mental skill and physical skills equally and concurrently at a given time. Thus, in sports, the balance of each guna is a must to perform. The study highlights issues present in the Indian athletes based on the Triguna concept of personality.

We observed that the mean values of the gunas in the Indian athlete at all three levels suggest that stability is the most predominant guna, next dominant guna is activation followed by Inertia. However, Sattvic is highly significant at all three levels but have negative regression coefficients.

Stability denotes the quality of intelligence which creates harmony, staying calm, balanced, and control. It has also been shown that staying calm, being balanced and in control are vital traits for successful performance as it lower anxiety levels and improve performance. Higher levels of stability suggest that the Indian athletes are not in favor of approaching the sports in a detached, process-oriented and balanced manner.[11]

Activation, on the other hand, has a positive regression coefficient at all three levels suggests that they prefer approaching sport in an enthusiastic, excited, ambitious, alert, passionate, persevering and motivated way which are qualities of elite athletes as shown by various researches.[12] However, activation with positive coefficient and Stability with negative coefficient suggests that excessive activation which is characterized by being overactive, uncontrolled energy, impatient, hasty in planning things for the future, risk-taking, rash, go-getting, acquisitive, aggressive, greedy, competitive, , ego-involved assertiveness, dominance, inability to

be a follow a process, egoistic, which leads to result orientation , instant gratification and poor performance. It has been shown that too much of motivational level and result orientation narrows the cognitive skill and limits identifying the difference between result orientation and process orientation approaches which leads to failures in motor skills in sports.[13]

Nature of sports activities influence and cultivates different kind of abilities and thinking process among participants personality, group activities results in more social and democratically values, whereas individual activities make them to more rigid and narrow minded. But both nature of sports make sportsperson to have mastery over the emotion and learn how to regulate and control them according to situation. A study by Shankara Murthy[14] revealed that Sattva guna is positively related to organisational setup because Sattvic personality consists of passion, patience, control on behaviour, creative leadership, and intellectual ability.

Prasunavv et al[15] found that Sattvic personality performs better than Rajasic and Tamasic personalities. Authors concluded that trigunas and tridoshas directly control all the physiological processes, and individual motivation and cognitive levels are closely related with Prakriti.

It has been found that inertia (Tamas) is the quality of dullness, ignorance, and Inertia (Tamas) is highly significant however was not found significant in the elite group. The school and college level are still not entirely dedicated to sports as the elite is which justifies the presence of Inertia (Tamas) (tamas) guna in these two groups. Inertia (Tamas) also signifies a lack of knowledge and state of ignorance which will be prevalent at the school level due to lack of experience.[16]

This gunas represents Prakriti (personality) which suggest the Indian athlete believes in a more result-oriented approach than to a process orientated approach in sports. Activation which is result oriented approach and instant gratification are negating the Stability guna which is knowledge-based orientation and is the signification of delayed gratification. Sport is a lifelong process and not a onetime event and requires a patient and systematic approach which in the Prakriti concept denotes as Stability (Sattvic) guna.[17] The triguna scale can help each athlete to understand their gunas and that balancing the gunas is the key to consistent performance and any guna in excess leads to poor performance.

CONCLUSION

This study will serve to advance understanding and theoretically underpin the personality using Triguna concept to assess and provide intervention to improve sports performance in Indian athlete at individual level. Failures of Indian athletes at international level have not been assessed from individual athlete level rather has been assessed on a national level.

Conflict of Interest: NIL

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REFERENCES

1. Pervin LA, Cervone D, John OP. Personality Psychology: Theory And Research. Ninth Edition. Jakarta. Kencana Media Group. 2010.
2. Rao KR. Theism of Pre-Classical Sāṃkhya. Prasaranga, University Of Mysore; 1966.
3. Anuradha MV, Kumar YL. Trigunas In Organizations: Moving Toward An East-West Synthesis. International Journal Of Cross Cultural Management 2015 Aug; 15(2):195-214.
4. Aoyagi MW, Portenga ST. The Role of Positive Ethics And Virtues In The Context Of Sport And Performance Psychology Service Delivery. Professional Psychology: Research And Practice. 2010 Jun; 41(3):253.
5. Dey & Pahwa. Prakriti and its associations with metabolism, chronic diseases, and genotypes: Possibilities of new born screening and a lifetime of personalized prevention. J Ayurveda Integr Med. 2014 Jan; 5(1):15-24.
6. Rastogi S. "Development and validation of a Prototype Prakriti Analysis Tool (PPAT): Inferences from a pilot study". Ayu. 2014; 33(2):209-218.
7. Rudrani Mukherjee. Concept of Personality type in West and Ayurveda. Ayurveda Integr Med. 2014 Jan; 5(1):15-24.
8. Gucciardi DF. Mental Toughness: Progress And Prospects. Current Opinion In Psychology. 2017 Aug 1; 16:17-23.
9. Hata T, Ilundáin-Agurruza J. Eastern Philosophy. Inroutledge Handbook Of The Philosophy Of

Sport 2015 Mar 5 (Pp. 110-126). Routledge.

10. Northouse PG. Leadership: Theory And Practice. Sage Publications; 2018 Feb 9.
11. Shilpa and Murthy. Understanding Personality Dynamics from Ayurveda. Indian Journal of Health and Wellbeing 2014; 3 (2): 57-59.
12. Frawley D. Mantra Yoga And The Primal Sound: Secrets Of Seed (Bija) Mantras. Lotus Press; 2010.
13. Gucciardi D, Gordon S, Editors. Mental Toughness In Sport: Developments In Theory And Research. Routledge; 2011.
14. Shankara Murthy KM, Patil SS. Comparative study on personality traits and emotional intelligence between individual and team athletes. IJAR 2015; 1(8):503-5.
15. Prasunavv, Sharma Bk, Narayana A. Comparative study of personality with Ayurvedic Prakriti. International Journal of Ayurveda and Pharma Research 2015 Dec 17;2(1).
16. SUD. Application Of Ayurvedic Principles For Talent Identification In Sports--A Hypothetical Review. ICSSPE Bulletin (17285909). 2016 Oct 1(71).
17. Vealey RS. The Conceptualization And Measurement Of Sport-Confidence (Self-Confidence, Sport Psychology) (Doctoral Dissertation, University Of Illinois At Urbana-Champaign).

Effect of 8 Weeks Body-Weight Resistance Training on High-Normal Blood Pressure and Stage 1 Hypertension Subjects-Pilot Study to Validate the Protocol

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ABSTRACT

Background: Resistance training has shown to decrease the blood pressure (BP) in Indians, but assessing 1 repetition maximum (1-RM) and use of equipment is difficult for individuals who wants to do it in home. Body-weight exercises are simple yet provide resistance to exercising muscles. **Objective:** To see the effect of 8 weeks progressive body weight resistance training (RT) on BP in subjects with high-normal BP and stage I hypertension.

Materials &Method: A randomized controlled trial was conducted in Hisar, an urban area of Haryana and study was carried out during January to June 2017. 20 patients diagnosed with either high-normal BP or stages 1 hypertension were recruited after screening of inclusion &exclusion criteria. For resistance training (RT) group, participants were exercised 30-40 minutes/session and 3 Sessions/week for 8 weeks. Control group, there was no training for 8 weeks but offered exercise intervention afterwards. Outcome variable was BP which was measured at baseline as well as 2 week intervals thereafter up to 8 weeks. Data was analyzed using IBM SPSS v21.0 software. **Results:** There was a significant gradual SBP reduction in resistance training (RT) group up to 6 weeks (MD -11.36, p=0.001 at 6 week), but insignificant rise in BP at 8 week (MD -10.85, p=0.03). Resistance training (RT) reduced DBP significantly at 6th week (MD -6.36, p<0.001). **Conclusion:** In Resistance training group, current exercise protocol should be modified to reduce BP in this population. **Trial Registration:** CTRI/2017/03/008280. **Source of Fund**: None.

Keywords: *Resistance training; Hypertension; Exercise therapy; Body weight*

INTRODUCTION

Prevalence of cardiovascular disease (CVD) is increasing day by day in developing countries like India. High blood pressure accounts for the greatest proportion of deaths (about 30%) attributed to CVD risk factors in India.[1, 2] The percentage of risk increase in people with older age, gender, greater Body mass index (BMI $\geq$ 23 Kg/m^2), large waist circumference, sedentary occupation, illiterate envionment, diabetes mellitus and raised urine protein, serum creatinine. In order to abate the burden of hypertension and related health outcomes, it is important to understand and establish the relationships between major risk factors for elevated blood pressure. However, evidence regarding these relationships may not generalizable to developing countries, such as India because most of the evidence from developed countries.[3,4] Lack of awareness about hypertension, risk factors of hypertension (HTN), diet modification and treatment choice are important cause of increased prevalence. With increases in rate of literacy, rate of prevalence of hypertension decreased, thus education and community-based screening should be considered as having a potential role in controlling HTN in India.[3, 5]

Early screening of hypertension and development of affordable intervention program (pharmacological

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and nonpharmacological treatment) is the need of time. One important part of non pharmacological treatment is exercise training that improves blood pressure. There are different types of exercises which may effectively to lower blood pressure (BP). Some other researcher also found benefits of physical activity and aerobic training in lowering blood pressure more than regular walking. [6,7] The results of various meta-analytical studies showed that isometric & dynamic resistance exercise and aerobic exercise does lower BP however, the sample sizes of the trials to date are generally small. [8,9,10,11] Resistance training has shown to decrease the blood pressure (BP) in Indians, but assessing 1 repetition maximum (1-RM) and use of equipment is difficult for individuals who wants to do it in home. Body-weight exercises are simple yet provide resistance to exercising muscles. In western countries, Nascimento et al et al (2014) suggested that 14 weeks of resistance training has positive effects on functional capabilities and limitations of the elderly by increasing muscular strength, cardiovascular function, decreasing coronary artery disease (CAD) risk, and preventing hypertension but Juliano Casonatto et al (2016) found that a single bout of resistance exercise can have a BP-lowering effect that last for up to 24 hours.[12] But the effect of dynamic resistance protocol is yet to be noticed in India. There are some resistance protocols that differ in periodization, volume, intensity, and training frequency. These contributing factors vary the control of BP in during short and long-term training periods. Hence there is need of an effective resistance training (RT) protocol that can be used to prevent and treat high blood pressure. Therefore, the objective of the study was to develop an effective resistance exercise protocol for the Indian community.

METHODOLOGY

Study design

A randomized controlled trial was conducted in Guru Jambheshwar University of Science & Technology (GJUS&T), Hisar situated in an urban area of Haryana. Participants were recruited from local hospitals and department of Physiotherapy, GJUS&T. Patients were selected by stratified random sampling and randomized into two groups: a control group (n=10) and resistance group (n=10). Since it was pilot study so sample size was not calculated.

Inclusion and exclusion criteria

The inclusion criteria for the study was any males or females of middle aged adults of 30-45 year, who was apparently high normal BP and stage 1 hypertensive diagnosed by physician and passed physical activity ready questionnaire (PAR-Q) developed by American Cardiology Society of Medicine and were willing to participate in the study. The exclusion criteria for the study if were chronic alcoholic or were having any other pathological conditions such as Musculoskeletal conditions, Neurological conditions and Cardio-respiratory conditions that prevent the subject to perform exercise training.

Ethical consideration

Ethical approval for the study was obtained from the institutional ethical committee of GJUS&T, Hisar vide letter no PTY/2016/555 on 14/10/2016. We followed Helsinki guidelines (1964) because our study was on human subjects. Present study protocol was also registered under clinical registry trial (CTRI/2017/03/008280).

Randomization and allocation concealment: Urban area was divided into four blocks and each block has five wards. After that physiotherapist randomly select one ward from each block by chit method. Physiotherapist recruited 1 participant randomly by her convenience. Recruitment for the study was restricted by block allocation (3 males and 3 females each in high normal BP and stage II hypertension) through order selection means first come and first selection. Same sequence of selection of participants was followed in each block. All participants were blinded to the treatment and also assessor was also blind to the treatment. Hence present trial was a double blinded, randomized and active controlled trial.

Procedure

The participants were asked for their consent prior to participating in the study. The participants were assured about the confidentiality of the information provided. 20 patients diagnosed with high normal BP (n=10, 5 male & 5 female) and stage 1 hypertension (n=10, 5 male & 5 female) were recruited from Department of Physiotherapy, G.J.U.S. & T., Hisar, by referral from the hospitals. A trained hospital staff and trained physiotherapy lab technician measured baseline data for various outcome measures for all subjects before the start

of the treatment process. Participants followed their usual medical treatment during the course of this study. Blood pressure and heart rate of the subjects was measured by using a Dr Morepen Blood Pressure Automatic Monitor Model. According to WHO 2013, subjects were asked to remain seated, positioning their left arm level with their heart, taking three measurements with at least a one minute gap between each measurement. In this study, mean systolic and diastolic BP were calculated using the average of three measurements. The four stages are normal blood pressure Stage (SBP≥120-129 mmHg; DBP≥80-89 mmHg), high normal HTN (SBP≥130-139 mmHg; DBP≥80-89 mmHg), Stage 1 HTN (SBP≥140-159 mmHg; DBP≥90-99 mmHg) and Stage 2 HTN (SBP≥160 mmHg; DBP≥100 mmHg).

Protocol

For Resistance training group, an experienced physiotherapist taught different types of exercise to the participants and after that they have done exercise in home based setting. Participants exercised 3 times per week for 8 weeks. Each session started with a 5 min warm up followed by eight exercises for major muscle groups- Push Up, Squatting, Sit-Up, Hamstring Curls, Trunk Extension in Quadrepod Position, Shoulder Press, Heel Raises and Bridging using body weight in sequence and followed 5 min cool down. Participants had performed 3 sets of each exercise per session. The progressive resistance training (PRT) protocol was designed to provide progressive increase in volume (6-8 repetition in first 2 weeks, 8-10 repetitions in next 2 weeks and 12-15 repetitions in last 2 weeks) and change in positioning (showing in Table 1-Annexure A). Participants of control group did normal daily activities without any specific training. Post intervention data was collected after completion of eight weeks of intervention for all outcome measures.

Table 1: 8 weeks resistance training Protocol

Type of Exercise	1-2 weeks	3-4 weeks	5-6 weeks	7-8 weeks
Push up	Wall push up	Table push up	Ground push up	Push up with weight
Squatting	Wall squat	Free squat	Lunges	Lunges with Weight
Sit-up	Sit-up with hand on side	Sit-up with hands on chest	Sit-up with hand behind neck	Sit-up with weight
Trunk Extension in Quadrepod	Trunk Extension in Quadrepod with hand raise	Trunk Extension in Quadrepod with leg raise	Trunk Extension in Quadrepod with hand & leg raise	Trunk Extension in Quadrepod with weight
Pull Up	Partial 50% BW*	75% BW*	100% BW* (Full hanging)	100% BW* (Full hanging)
Heel Raises	B/L** Heel Raises	U/L*** Heel Raises	U/L*** Heel Raises with weight	U/L*** Heel Raises with weight
Bridging	B/L Bridging with 10 sec hold	B/L Bridging with 20 sec hold	U/L Bridging with 5 sec hold to each leg	U/L Bridging with 10 sec hold to each leg

*BW-body weight; **B/L-Bilateral; ***U/L-Unilateral

Data analysis

Data were analyzed using IBM SPSS v21.0 software. An independent-samples t-test was conducted to compare the two groups (resistance and control). For a repeated measures design ANOVA test had been used. Results were explored as mean±SD. Data was reported with mean difference (MD) along with 95% confidence interval. Significant level set at "$p \leq 0.05$".

RESULTS

All participants were middle aged with age in between 30-45 years. Demographic details of participants were shown in table 2 (Annexure-A).

Table 2: Descriptive statistics of demographic characteristics

Group	Variables	Age (years)	Height (cm)	Weight(kg)	BMI**	WC*
Resistance training(RT)	Mean±SD	42.30±5.31	164.77±5.81	69.80±6.28	25.76±2.59	35.10±2.02
	Range	17.00	15.22	18.00	6.00	9.30
Control group	Mean±SD	39.80±3.22	166.06±12.5	68.20±12.23	24.59±2.38	35.10±2.60
	Range	10.00	30.48	38.00	8.50	8.00

*WC-waist circumference; **BMI-Basal metabolic rate

There was a significant gradual SBP reduction in resistance training (RT) group up to 6 weeks (MD -11.36, p=0.001 at 6 week), but insignificant rise in BP at 8 week (MD -10.85, p=0.03). Resistance training (RT) reduced DBP significantly upto 6th week (MD -6.36, p<0.001). When the resistance training (RT) results were compared with control, there was no statistically significant difference in BP (p>0.05).

In repeated measure of resistance group, when we compared Baseline SBP to 2nd week SBP (MD .27, p≥0.01; 95% CI -3.80-3.26), 4th week SBP (MD 7.82*, p≤0.01; 95% CI 1.74-13.9), 6th week SBP (MD 11.36*, p≤0.01; 95% CI 5.70-17.02) and 8th week SBP (MD 10.85, p≥0.01; 95% CI 1.28-20.4) then result is highly significant in contrast to control group up to 6th week but rise in BP seen after 6th week to 8th week. When we compared baseline DBP to 2nd week DBP (MD 0.0, p≥0.01; 95% CI -1.72-1.72), 4th week DBP (MD 3.18, p≥0.01; 95% CI -2.69-9.05), 6th week DBP (MD 6.36*, p≤0.01; 95% CI 4.13-8.60), 8th week DBP (MD 2.24, p≥0.01; 95% CI -3.53-8.02) then result is highly significant in contrast to control group at 6th week only.

DISCUSSION

The aim of study was to develop an exercise protocol for adults. Initially during 2 weeks, SBP and DBP rise because of the exercise pressor reflex to the cardiovascular center in the medulla from proprioceptors (mechanoreceptors and metaboreceptors) in active muscles. Arterial pressure rises to overcome the resistance to muscle perfusion caused by elevated intramuscular pressure interrupting arterial blood flow.[13]

The present study evaluated the effects of resistance exercise programs without dietary restriction on cardiac fitness variables such as blood pressure and heart rate. The results suggest that there was gradual reduction in SBP and DBP in resistance training group up to 6 weeks and was significant but during 6-8 weeks result is inconsistent (some patient's showed decrease in BP but rest showed increase in BP and the result was nonsignificant). The possible reason for this may be due to progression in intensity of exercise and it is not optimal for some patient. The findings of this study about the response of SBP and DBP to this resistance exercise protocol corroborate the results of Wilborn et al demonstrated that the BP is significantly higher after 85% of 1 RM than 65% of that 1 RM. They found no significant differences in the response of SBP between exercising at 65 and 85% of 1 RM.[14] The results of this study suggest that reasons other than mechanical compression and Valsalva maneuver may be the cause of the increase in BP due to the fact that 1 RM caused minor responses in BP and they concluded that the longer duration of exercise at 65% of 1 RM is most likely to cause the largest increase in SBP and HR at this intensity. Our study findings also supported by Mota and colleagues found 16 weeks of moderate intensity dynamic resistance training reduced SBP/DBP about 14/ 4 mmHg among 32 older women with controlled hypertension.[15] Moraes et al. found 12 weeks of moderate intensity dynamic resistance training reduced SBP/DBP approximately 16/12 mmHg among 15 middle-aged men with hypertension.[16] These findings suggest that moderate intensity and duration of dynamic resistance training may be viable as stand-alone antihypertensive lifestyle therapy among adults with hypertension.[17]

Strength of our study was stratification of sample i.e we included male & female hypertensive and high normal BP and stage 1 hypertensive patients, so results can be generalized to middle aged populations. To the best of our knowledge, we are first to study home based intervention in community dwelling hypertensive so this intervention saved time and benefit patients.

Limitation of our study was that duration of our study was relatively short for home based training but it was not possible to do long duration study in community and training of patients was not monitored although we had demonstrated it properly before start of treatment. But as there was significant reduction in blood pressure in resistance training group, result confirmed that patients had done it properly.

CONCLUSION

The results of the present study indicate that resistance training protocol is significant up to 6 weeks in reducing resting blood pressure but during 6- 8 weeks increase in BP revealed that volume or repetition of body weight resistance training exercise overload the patients so repiptition and intensity of exercise should be remained same as in 4^{th} to 6^{th} week. Hence we used this protocol as a preventive measure in patients who are at risk of prehypertensive and used in stage 1 hypertensive.

Conflict of Interest: There is no conflict of interest.

Source(s) of support: Nil

Registration number in case of a clinical trial: CTRI/2017/03/008280

Conflicts of Interest: None

REFERENCES

1. Patel V, Chatterji S, Chisholm D, Ebrahim S, Gopalakrishna G, Mathers C,et al. Chronic diseases and injuries in India. Lancet. 2011; 377(9763):413–28.
2. Anchala R, Kannuri NK, Pant H, Khan H, Franco OH, Di Angelantonio E, et al. Hypertension in India: a systematic review and meta-analysis of prevalence, awareness, and control of hypertension. J Hypertens. 2014; 32 (6): 1170–7.
3. Enu A, Jayakant S. Hypertension Stages and Their Associated Risk Factors among Adult Women in India. Journal of Population and Social Studies. 2017; 25(1): 42-54.
4. Dorothy T. Morala-Dimaandal. Differences of functional status among elderly women in urban and rural settings – Self-report and performance-based measures, Advances in Physiotherapy 2009;11(1) :13-21. DOI: 10.1080/14038190701852317
5. Farag, Y. M., Mittal, B. V., Keithi-Reddy, S., Acharya, V. N., Almeida, A. F., C, A., Singh, A. K. Burden and predictors of hypertension in India: results of SEEK (Screening and Early Evaluation of Kidney Disease) study. BMC Nephrology. 2014; 15(1): 42.
6. A. Tollbäck, A. Ståhle. Effects of Aerobic Group Training on Exercise Capacity, Muscular Endurance and Recovery in Elderly Patients with Recent Coronary Events: A Randomized, Controlled Study, Advances in Physiotherapy 2001; 3(1):29-37. DOI: 10.1080/140381901300039305
7. Ilkka Vuori. Physical activity and health: Metabolic and cardiovascular issues, Advances in Physiotherapy 2007; 9 (2) :50-6. DOI: 10.1080/14038190701395887
8. Sonu P, Kulandaivelan S, Vandana P. Effect of Aerobic Training and Resistance Training on Blood Pressure in Indian: Systematic Review. International Journal of Science and Research (IJSR) 2016; 5(4):1373-1376.
9. Sonu P, S. Kulandaivelan, Vandana P. Effect of resistance exercise training on blood pressure in Indians: Systematic review. Saudi Journal of Sports Medicine. 2016.
10. Sonu P, Kulandaivelan S, Varun P, Vandana P. Effect of aerobic exercise training on blood pressure in Indians: Systematic review. International Journal of Chronic Diseases. 2016; (1370148).
11. Sonu P, Kulandaivelan S, Vandana P. Effect of Isometric Strength Training on Blood Pressure: Systematic Review of Literature with Specific Emphasis on Indians. Journal of Exercise Science & Physiotherapy JESP. 2016; 12(2):166- 174.
12. Casonatto **J** et al. The blood pressure-lowering effect of a single bout of resistance exercise: A systematic review and meta-analysis of randomized controlled trials. European Journal of Preventive Cardiology. 2016; 23(16).
13. Chulvi-MI, Sanchis-CJ, Tortosa-MJ, Cortell-TJM. Exercise for Hypertension. http://dx.doi.org/10.5772/65035
14. Wilborn C, Greenwood M, Wyatt F, et al. The effects of exercise intensity and body position on cardiovascular variables during resistance exercise. 2004; 7(4): 29–36.
15. Mota MR, Oliveira RJ, Terra DF, Pardono E, Dutra

MT, de Almeida JA, et al. Acute and chronic effects of resistance exercise on blood pressure in elderly women and the possible influence of ACE I/D polymorphism. Int J Gen Med. 2013; 6:581–7.

16. Moraes MR, Bacurau RF, Casarini DE, Jara ZP, Ronchi FA, Almeida SS, et al. Chronic conventional resistance exercise reduces blood pressure in stage 1 hypertensive men. J Strength Cond Res. 2012; 26:1122–9.
17. Shaw BS. Resting cardiovascular function improvements in adult men following resistance training. Afr J Phys Health Educ Recreat Dance. 2010; 16:402–10.

Management of Patient with Metabolic Encephalopathy – A Case Study

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ABSTRACT

The Metabolic Encephalopathy is a series of neurological disorders caused by systemic illness, such as diabetes, liver disease, renal failure and heart failure. Metabolic encephalopathy usually develops acutely or sub acutely. If left untreated, however, metabolic encephalopathy may result in secondary structural damage to the Brain. A 43 year-old patient was admitted to the ICU in a confused state with complaints of dyspnoea and weakness. On the day of admission, he complained of dry cough, fever, sore throat since 5 days accompanied by body ache, weakness and multiple episodes of vomiting since a day. On investigation, traces of ketones were detected in Urine and had increased PT-INR. Throat swab indicated Fungal stains. He had high fever and tachycardia. Tracheostomy was performed and was put on Ventilator to improve his respiratory functions. Nebulization was initiated BD. The case was reviewed for 5 days. His prognosis was very poor and deteriorating. His relatives were informed about his condition and the symptomatic treatment was continued.

Keywords: *Metabolic, encephalopathy, unconscious.*

INTRODUCTION

Encephalopathy is a difficult term because it can be used to denote either a disease or finding.[1]

The word metabolic encephalopathy was used for the first time in 1912 when Kinner Wilson tried to explain the state of global cerebral dysfunction. It is caused by systemic stress, that can vary in the clinical picture from a very mild disorder to a deep coma state with decerebrate rigidity.[2]

Metabolic encephalopathy is defined as a diffuse cerebral dysfunction, typically manifesting as changes in cortical functions and as disorders of consciousness, ranging from confusion to coma.[3] Others have suggested that metabolic encephalopathy is a temporary or permanent illness, more a symptom than a disease. It includes various forms of pathological conditions that are predominantly manifested by disorders of mental functioning. However, metabolic encephalopathy may also be defined as a systemic disorder with diffuse brain damage affecting the hemispheres, brain stem, and reticular activating system.[4]

Epidemiological and Demographic Data

Encephalopathy correlates with age. The number of patients with encephalopathy increase after the age of 65 years. People older than 75 years, who reside in nursing homes have 60% chance of developing encephalopathy, whereas in a population younger than 55 years, this proportion is 1.1%.[5,6] Encephalopathy occurs in 10–40% of hospitalized patients older than 65 years, whereas 8–70% of patients develop septic encephalopathy. According to a data from the United States, encephalopathy is being registered in 100–200,000 patients with anoxia and 12–16% of patients with thiamine deficiency, every year. In patients with cirrhosis, the development of hepatic encephalopathy is noted to be in 45–80% of cases, depending on the severity of liver damage.[1,5,6]

Etiology

Causes like Hypoxia, Ischemia, systemic disease, and toxic agents hepatic and renal insufficiency, pancreatitis, malnutrition, electrolyte imbalances sepsis, infection, vasculitis, and malignancy are common in majority of

the cases. Metabolic encephalopathy may also occur as a result of various toxic agents such as alcohol, sedatives (barbiturates, narcotics), psychiatric agents (tricyclic antidepressants, anticholinergics, phenothiazines), heavy metal poisoning, organic phosphates, and other drugs (anticonvulsants, corticosteroids, penicillin, etc.).[7,8]

Case Presentation of Metabolic Encephalopathy

A 43 year-old male patient was admitted to Intensive Care Unit in a confused state with complaints of dyspnoea and weakness. On the day of admission, he complained of dry cough, fever, sore throat since 5 days accompanied by body ache, weakness and multiple episodes of vomiting for 1 day. His medical history includes Diabetes, Hypertension and Hypothyroidism. Due to deteriorating condition manifested by irrelevant talk & breathing difficulty, he was shifted to Intensive Care Unit. The patient was thoroughly assessed; he was disoriented, had dyspnoea and high-grade fever. On investigation, traces of ketones were detected in Urine and had increased PT-INR. Throat swab indicated Fungal stains. A Rapid Sequence Intubation (RSI) was performed and patient was put on ventilator support to maintain respiratory function when he failed to maintain the saturation with CPAP. The patient was cautiously monitored and all the parameters were investigated as per the protocols.

Day 1

The patient presented with the complaints of dry cough, fever, sore throat since 5 days and accompanied by body ache, weakness and multiple episodes of vomiting since 1 day. He is a known case of Diabetes, HTN & Hypothyroidism. He had high fever (104.50F), Pulse – 140 beats/min and other vital parameters were normal. Patient was put on medications such as Tab Triolmesar 20 Mg OD, Tab Dimicron XR 60 OD, and Cap. Rifit CD OD. He was also put on IV fluids to correct the electrolyte imbalances.

Day 2

Patient shifted to Intensive Care Unit as his condition got worse with repeated dyspnoea & exhibited irrelevant talks. The patient was thoroughly assessed; he was conscious, irritable, confused, and had breathing difficulty. On examination there was a layer of pus formation on the buccal mucosa. His vitals were not stable with parameters like; temperature (1030F), Pulse (120 beats/min) and respiratory rate (24 breaths/min). His urinalysis reports depicted the positive for ketone bodies, elevated PT-INR and RFT values. His HBA1C level was 8.7. Pus drained and sent for investigation. He was put on CPAP to improve his respiratory functions and a RT was inserted for enteral feeding.

Day 3

Patient had altered levels of consciousness with dysarthria but was able to express in writing. ABG analysis & Blood sugar was monitored every 4 hours. There was no significant difference in hemodynamic parameters. Gradually his condition worsened and he became unconscious. The congestion in throat increased due to abscess formation and inflammation and later festering was seen. Due to this, RSI was performed and patient was put on ventilator. Nebulization was initiated BD. The prognosis was intimated to his relatives.

Medical Management

Treatment

Sr. No	Name of the Medicine
	Inj. Pan : 40mg OD
	Inj Paracetamol: 1gm TDS
	Inj Monocef: 2gm BD
	Inj Levoflox: 750mg OD
	Inj Optineuron : OD
	Inj Mucomix 1gm IV
	Inj Meropenem: 1gm TDS
	Inj Linid: 600mg BD
	Inj. Doxy: 100mg BD
	Inj Hydrocortizone : 30mg QID
	Inj. Metrogyl: 500mg TDS
	Tab Etilaam: 0.5mg HS
	Tab Allegra M: 130 mg BD
	Tab Azee: 500mg OD
	Tab Ivabrad: 5mg OD
	Tab Eltroxin: 100mg OD
	Tab Triolmesarto: 20mg OD
	Tab Amlong:2.5mg BD
	Tab Triol: 20mg OD
	Tab Towaptan: 15mg
	Inj Human Atrapid
	Sy Kesol 10ml
	Sy. Duphalac: 20ml HS
	Neb. Devolin 2.5ml TDS
	Neb. Budecort 0.5mg BD

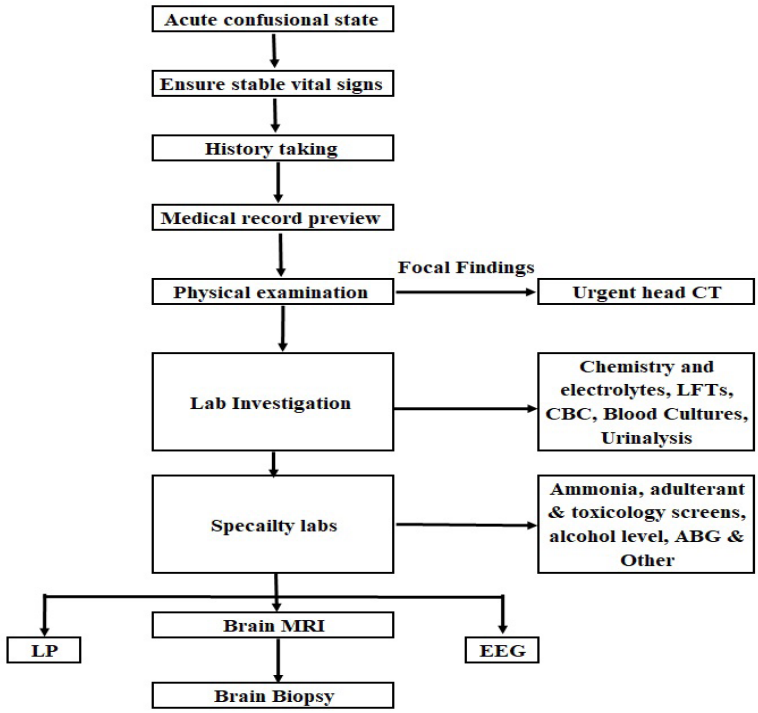


Fig: 01 - Approach to evaluation of acute confusional state of patient with metabolic encephalopathy

Diagnostic Findings

Table: 01 – ABG results of the patient

Sr. No.	Investigations	15 Jan 2018	16 Jan 2018	17 Jan 2018	18 Jan 2018
	Na+	128 mEq/L	128 mEq/L	122 mEq/L	124 mEq/L
	K+	3.9 mEq/L	3.9 mEq/L	3.9 mEq/L	3.8 mEq/L
	Cl	97 mEq / L	97 mEq / L	90 mEq / L	103 mEq / L
	pH	7.48	7.51	7.39	7.49
	pCO2	24 mmhg	23 mmhg	30 mmhg	32 mmhg
	pO2	143 mmhg	75 mmhg	63 mmhg	65 mmhg
	Hct	45%	50%	38%	41%
	Hco3	17.9 mmol/L	18.4 mmol/L	21 mmol/L	20.5 mmol/L
	Hco3 std	22.0 mmol/L	23.0 mmol/L	25 mmol/L	24 mmol/L
	SPO2	99%	96%	88%	90%

Table : 02 – Biochemical Test results

Sr. No.	Investigation	Values	Normal Values
	Liver Function Test		
	Total Protein	5.6 g/dL	6.0-7.8 g/dL
	Albumin	2.1 g/dL	3.5-5.5 g/dL
	Globulin	3.5 g/dL	2.5-3.5 g/dL

Cont... **Table : 02 – Biochemical Test results**

	AG (Albumin/Globulin) Ratio	0.6	0.8-2.0
	BD (blood sugar- Becton Dickinson)	0.69 mmol/L	4.0-6.0 mmol/L. (fasting).
	BI (Bismuth,Blood)	0.61 µg/L	0-5 µg/L
	SGOT	233 U/L	7 – 21 U/L
	SGPT	138 U/L	8 – 32 U/L
	Alkaline Phosphate	49 U/L	32 – 110 U/L
	Urea	49.3 mg/dL	48 8 – 25 mg/dL
	Sr. Creatinine	1 mg/dL	0.9 0.2 – 0.6 mg/dL
	Platelets	64000	140,000 – 450,000/ml
	Hb%	11.6 g/dL	11 – 16 g/dL
	WBC	11.9^3 / mm3	3.8 – 11.0 10^3 / mm3
	HBA1C	8.7	

Transthoracic Echo Report depicted the following findings:

Left Ventricular Hypertrophy

Chamber Dimensions are normal

No regional wall motion abnormality is seen

Normally LV Systolic function; LVEF - 60%

No Pulmonary HTN

Normal Valves

No clots/vegetation/effusion

Impression:

Poor echo window

Left ventricular hypertrophy

DISCUSSION

The 43 year-old male patient admitted in Intensive Care Unit in confused state with complaints of dyspnoea and weakness. On the day of admission, he complained of dry cough, fever, sore throat since 5 days accompanied by body ache, weakness and multiple episodes of vomiting since 1 day. On investigation, traces of ketone bodies was detected in Urine and had increased PT-INR. Throat swab indicated Fungal stains. Patient was put on medications like Tab Triolmesar 20 Mg OD, Tab Dimicron XR 60 OD, and Cap. Rifit CD OD. He was also put on IV fluids to correct the electrolyte imbalances. On 3rd day, Patient's was in a state of confusion. Gradually his condition worsened and he became unconscious. The congestion in throat increased due to abscess formation and inflammation. An emergency tracheostomy was performed and patient was put on ventilator.

Similar case was encountered with a 29-year old man who presented within the thrombolysis time window to the emergency department (ED) with sudden-onset dysarthria, ataxic and left-sided weakness while smoking cannabis. He was assessed in the ED and found to have a NIHSS of 3. A CT brain scan and extra cranial CTA revealed no significant findings, however, as he was clinically improving, he was not given intravenous thrombolysis. Subsequent MR brain imaging confirmed marked acute bilateral white matter changes typical of drug-induced encephalopathy.

His initial lab investigations were normal. His thyroid function responded well to treatment with Carbimazole, Propanolol and Hydrocortisone and improved rapidly and was discharged with counselling on cannabis use but re-presented twelve hours later with worsening dysphagia, dysphonia, breathlessness and bilaterally weak upper limbs and requiring intensive care support. His symptoms significantly improved and

he was followed up by the endocrinology team.[9]

Ethical Clearance- Taken from the Research Advisory Committee.

Source of Funding - Self

Conflict of Interest - Nil

REFERENCES

1. Papadopoulos MC. Davies DC. Moss RF, Tighe D, Tighe D, Bennett ED, Pathophysiology of septic encephalopathy: A review. Crit Care Med 2000:28(8):3019-24.
2. Angel MJ, Young GB. Metabolic encephalopathies. Handb Clin Neurol 2011;29:837-82
3. Angel MJ, Chein R, Young GB. Metabolic Encephalopathies. Handb Cil Neurol 2008;60:115-66.
4. Aminoff AJ. Anoxic, metabolic and toxic encephalopathy In Neurology Web Med Sci VII January 2003. Available from: http://edmedia.emory.edu/GStaton/Anoxic,%20Metabolic,%20and%20Toxic%20Encephalopathies
5. Bajaj JS, Wade JB, Sanyal AJ. Spectrum of neurocognitive impairment in cirrhosis: Implications for the assessment of hepatic encephalopathy. World J Gastroenterol 2008;14:3609-15
6. Poordad FF. Review article: The burden of hepatic encephalopathy. Alimet Pharmacol Ther 2006; 25:3-9
7. Upanc V, Vargek-Solter V, Demarin V. Metabolic encephalopathy. Acta Clin Croat 2003;42:351-7.
8. Sanap MN, Worthley LI. Neurologic complication of critical illness: Part I. Altered states of consciousness and metabolic encephalopathies. Crit Care Resusc 2002;4:119-32.

Multiple Massive Maxillary Exostoses – A Case Report with Complete Denture Rehabilitation

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ABSTRACT

Exostoses are benign, asymptomatic bony outgrowth arising from cortical bone and occasionally from spongy bone along the maxillary or mandibular arches. They tend to appear in early adolescence and may very slowly increase in size with age. Theyare not usually a concernto a patient with complete dentition. However, in an edentulous patient they may pose a major concern for denture placement over these areas, leading to pressure and ulceration, which demands their complete surgical excision. The following article presence a rare case ofbilateral massive buccal exostoses, which was incompatible with the patient ability of prosthetic rehabilitation and hence a resective osseous surgery was carried outfollowed by a complete denture rehabilitation.

Key words: *Bony outgrowth, complete denture, Exostoses*

INTRODUCTION

Natural tooth lost due to various reasons has to be restored by artificial means to rejuvenate the esthetic and functional profile of human face. However, in patients who cannot afford for implants, complete and partial dentures still remains the most popular modality of prosthetic replacement.[1] In situations where bony irregularities such as bony exostoses, tori occur the need for surgical correction becomes mandatory. These anatomical abnormalities which are more common in certain races such as Malaysians tend to remain insignificant in healthy dentition but become enigmatic in edentulism by interfering with denture fabrication.[2] Besides that, these bony prominences can compromise the oral health of the individual by traumatizing the overlying thin soft tissue. Hence the host bed (the alveolar bone and related soft tissues) has to be modified to an extent where the prosthesis can be accommodated without any compromise. Herewith a case of massive exostoses has been presented with complete denture rehabilitation.

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CASE PRESENTATION

A 59-year-old, healthy male patient presented for a prosthetic rehabilitation of his missing teeth. On examination, the patient was partially edentulous with few mobile teeth and root stumps, necessitating extraction of the remaining teeth and replacement with a complete denture. Besides, intraoral examination also revealed multiple overgrowths located on the buccal aspect of the maxilla in the premolar and molar areas on the left side and from the canine to molar region on the right side. All the mucosal surfaces over the lesion appeared to be healthy[Figure 1].On palpation the lesions were bony-hard, lobulated, measuring approximately 2.5 cm x 1.5 cm on either side. The patient had not noticed this mass before since it did not interfere with his speech,

chewing, phonetics or other oral functions. He had no significant past medical history or family history of similar lesions. However, when enquired regarding his dietary habits, being a fisherman, almost every meal included consumption of seafood in various forms.

Radiographic examination showed multiple well-defined radiopaque areas in the premolar and molar region bilaterally [Figure 2]. A maxillary occlusal radiograph also revealed that these masses were the bony thickening of the cortical plate on the buccal surface of the maxilla [Figure 3].These enlargements of the cortical plate of the buccal surface of the maxilla without any systemic abnormality confirmed the diagnosis of bilateral buccalexostoses.

In view of the complete prosthetic rehabilitation, aresective osseous surgery was planned to excise the bony masses following the extraction of the remaining teeth. After explaining the potential risks and benefits of surgery, an informed consent was obtained from the patient. In the first appointment all the remaining teeth were extracted under local anesthesia. During the second appointment preprosthetic surgery was planned on the right side and in the following appointment surgery was planned for the lesion on the left side. Three weeks elapsed between each appointment.

After giving appropriate local anesthesia, a full thickness mucoperiosteal flap was reflected for a complete access to the exostoses by giving crestal incision and vertical releasing incisions [Figure 4]. Segmental removal of bony exostoses was done with carbide bur under copious irrigation followed by smoothening and debridement of surgical bed (to clear out the osseous debris) with football diamond bur and saline irrigation [Figure 5,6].The flap was sutured with 3-0 black silk. Pressure was given on the operative site with wet gauze to prevent fluid accumulation and also to facilitate adhesion of periosteum to the underlying bone. Routine postoperative instructions were given. Medications including systemic antibiotics and analgesics were prescribed. The sutures were removed after 1-week. As healing was uneventful bilaterally, [Figure 7] patient was recalled for complete denture rehabilitation after 4 weeks [Figure 8].



Figure 1 – Pre-operative intraoral view with periodontally compromised teeth and severe exostoses.



Figure 2 – Orthopantomograph with exostosis evident in maxillary premolar and molar region bilaterally.



Figure 3 – Maxillary occlusal radiograph depicted adequate bone between the exostosis and maxillary sinus.

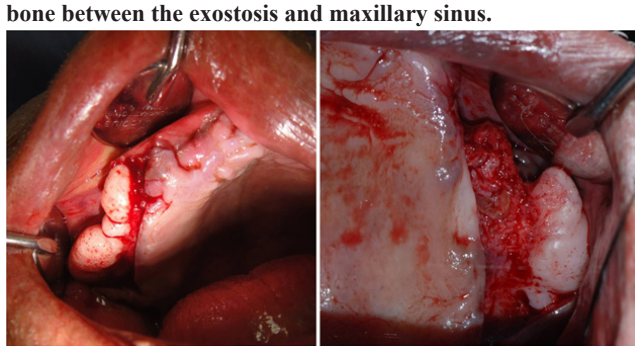


Figure 4 – Triangular flaps were designed and elevated and exostosis clearly visible.

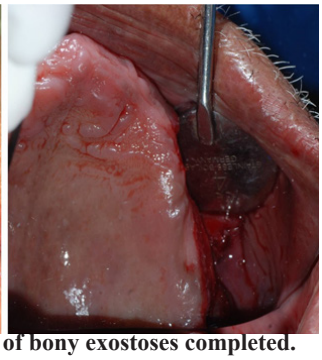



Figure 5 – Segmental removal of bony exostoses completed.

Figure 6 – Excised exostoses.




Figure 7 – Uneventful healing noted after 4 weeks of surgery.

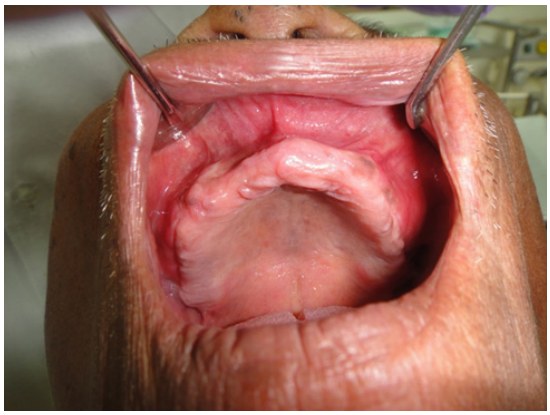


Figure 8 – Complete Denture rehabilitation.

DISCUSSION

Anexostosis is a benign, localized, peripheral overgrowth of bone which frequently occurs in long bones where tendons and muscles are inserted. In the jaw bones it may be a nodular, flat or pedunculated protuberance located on the alveolar surface. Maxilla is shown to exhibit the highest prevalence rate of 5.1:1 in comparison to mandible and a male predominance with an incidence of 1.66:1 among the genders. Prevalence of exostosis varies greatly depending on the population studied, with higher incidence in mongoloids than in Caucasoid. Depending on the anatomical locationthey are termed as torus palatinus (midline of the hard palate), torus mandibularis (lingual aspect of mandible), or buccal/palatal bone exostoses.[3]

Buccalexostoses occur along the buccal aspect of the maxilla or mandible, usually in the premolar and molar regions yet occasionally they may occur in other parts of the jaw. Palatal exostoses are found on the palatal aspect of maxilla, most commonly in the tuberosity region.[3] These exostoses may present either as a smooth bulging of the bone surface continuous with the adjacent area or as discrete, multilocular spherical projections with a broad base that forms a nodular cluster in the similar form noted in our patient.[4]

Glickman and Smulow further classified buccal alveolar bone enlargement in to exostosis and lipping. Though their etiology is unclear, the authors considered it as buttressing bone formation in response to trauma from occlusion and suggested that bone formation occurs to reinforce bony trabeculae for functional adaptation.[5]

Though the etiology of exostosishas been investigated by several authors, no consensus has been reached. Several authors have postulated the etiology to be multifactorial where there is an interaction of genetic and environmental factors, masticatory hyperfunction and continued growth.[3] Gorsky et al. postulated that the etiology of osseous outgrowth might be multifactorial, including environmental factors acting in a complicated and unclear interplay with genetic factors. The quasi-continuous genetic or threshold theory states that the environmental factors must first reach a threshold level before the genetic factors can express themselves in the individual; hence both genetic and environmental factors determine expressivity, making the etiology multifactorial.[3] Literature reveals that the prevalence of these bony enlargements increases with age, indicating a relationship between age and their occurrence.[6] It has also been theorized that because some of these outgrowth are found with some frequency during the middle phase of life, suggesting an environmental and functional factors, particularly those related to masticatory stress.[6] A correlation between the presence of these bony enlargement in patient with parafunctional habits has also been demonstrated. Other causative factors include dietary habits (taking supplements or food rich in calcium) and nutritional disturbances.[7] Eggen et al. suggested saltwater fish consumption to be related to these on outgrowths, probably due to higher levels of polyunsaturated fatty acids and Vitamin D that is involved in bone growth.[8] In our patient, it was difficult to distinguish the true origin of the exostoses since it was on an edentulous region, presence of few attrited teeth and consumption of seafood may suggest masticatory hyperfunction and saltwater fish consumption as

cofactors in origin of the exostoses.

Patients with exostoses seldom complain of unaesthetic appearance, speech difficulties due to limited tongue movement and food lodgment leading to malodor. Few patients may experience trauma or ulceration when masticating hard or sharp food as the mucosa covering the bony protuberances is reported to be thinner than the surrounding mucosa.[9] Hence, when a patient presence with exostoses the overlying mucosa might have a normal appearance, but may appear whitish or ulcerated if exposed to any trauma. Furthermore, recording oral impressions and seating of dentures are difficult in these patients.

The diagnosis of a buccalexostosis is generally done by simple clinical examination. However, radiographs might be required in situations where the bony protuberances are extremely large, or multiple, or vary in consistency to rule out underlying bone pathology. Radiographic appearance will show a normal bony pattern or a slightly radiodense image with a higher density than that of the surrounding bone.[9] In our patient, orthopantomograph and maxillary occlusal radiographs were taken which revealed multiple radiodense masses near premolar and molar region bilaterally. Though not mandatory, few authors have suggested taking a cone-beam computed tomography as a helpful diagnostic tool to identify anatomical variations in exostosis. [10] Additionally, it can also be helpful in assessing the relationship of the exostosisto maxillary sinus.

Distinction between exostoses and similar pathology arising from gingival enlargement and bone outgrowths remains important. Other differential diagnoses include osteomyelitis, osteoma, osteosarcoma, osteochondromas, organized subperiosteal hematoma, mature ossifying fibroma, periosteal osteosarcoma, chondrosarcoma, sclerosteosis, fibrous dysplasia, Paget's bone disease.[1]

Patient with multiple bony growths or lesions not in the classic torus or exostoses location should be evaluated for Gardner's syndrome. This autosomal dominant syndrome shows other features like multiple osteomas, sebaceous cysts and soft tissue tumours of skin, intestinal polyposis and multiple impacted supernumerary teeth.[1] Though our patient presented with multiple exostoses, absence of other associated features ruled out the diagnosis of Gardner's syndrome.

Due to their benign nature, most exostoses do not require treatment unless any esthetic problems, functional problems or discomfort in hygiene maintenance or phonetics are raised by the patient. Other reasons for surgical intervention include periodontal or prosthodontics complications.[11] In our case surgical extirpation was necessary for prosthetic rehabilitation and hence decided to surgical remove the exostoses.

When treatment is elected, the exostoses may be chiseled off or removed by bone-burr cutting. Conservative surgical removal with bony recontouring is the treatment of choice, with occasional recurrences expected.[1] Besides, these exostoses masses can be used as a source of autogenous bone graft for correction of intraoral bone defects.The possible complications after surgery include hematoma, edema, wound dehiscence, oro-antral communication and infection. However, our patient did not encounter any such complications. Biopsy for diagnostic support is usually not recommended. However, biopsy of the excised mass shall be performed if there is any dilemma regarding diagnosis.

CONCLUSION

Rehabilitation of edentulous mouth is an arduous task especially in the presence of substantial anatomical interferences. Since in our patient the radiograph depicted adequate bone between the exostosis and maxillary sinus, total removal was feasible. Treatment planning and management often involves multidisciplinary approach comprising specialties from prosthodontics and oral surgery. The present case demonstrates that complete excision of huge exostoses can be accomplished with predictable results and least postoperative sequela. Radiographs should be used as an aid wherever necessary and a segmental approach has been recommended during surgical removal in the presence of massive exostoses to avoid oro-antral communication.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Source of Funding: Self

Ethical Clearance: Ethical clearance has been taken from Institutional Ethical Committee

REFERENCES

1. Medsinge SV, Kohad R, Budhiraja H, Singh A, Gurha S, Sharma A. BuccalExostosis: A Rare

Entity. Journal of International Oral Health : JIOH. 2015;7(5):62-64.

2. Yaacob H, Tirmzi H, Ismail K. The prevalence of oral tori in Malaysians. J Oral Med 1983;38:40-2.
3. Smitha, K., Smitha, GP. Alveolar exostosis–revisited: a narrative review of the literature. Saudi J. Oral. Dent. Res. 2015;6 (1): 67–72.
4. Chandna S, Sachdeva S, Kochar D, Kapil H. Surgical management of the bilateral maxillary buccalexostosis. Journal of Indian Society of Periodontology. 2015;19(3):352-355.
5. Glickman I, Smulow JB. Buttressing bone formation in the periodontium. J Periodontol 1965;36:365–70.
6. Jainkittivong A, Langlais RP. Buccal and palatal exostoses: Prevalence and concurrence with tori. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90:48-53.
7. Antoniades DZ, Belazi M, Papanayaiotou P. Concurrence of torus palatinus with palatal and buccalexostoses. Case report and review of the literature. Oral Surg Oral Med Oral Pathol Oral RadiolEndod. 1998;85 :552–7.
8. Eggen S, Natvig B. Relationship between torus mandibularis and number of present teeth. Scand J Dent Res 1986;94:233–40.
9. García-García AS, Martínez-González JM, Gómez-Font R, Soto-Rivadeneira A, Oviedo-Roldán L. Current status of the torus palatinus and torus mandibularis. Med Oral Patol Oral Cir Bucal. 2010;15(2):e353–60.
10. Siddiqui H, Singh DK, Mishra S, Mandal A. Bilateral buccalexostosis evaluated by cone-beam computed tomography: A rare accidental finding. Indian J Dent Sci 2017;9:34-7.
11. Lee KH, Lee JH, Lee HJ. Concurrence of Torus Mandibularis with Multiple BuccalExostoses. Archives of Plastic Surgery. 2013;40(4):466-468.

A Hybrid Ensemble Classification Approach to Determine the Impact of Asthma in Association with Gastro Esophageal Reflux Symptoms

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ABSTRACT

Objectives: This implementation work focuses on the predicting severity of respiratory problems of asthmatic patients from the dataset of the PFT report with the significant parameters of Gastro Esophageal Reflux symptoms (GER).

Methodology: The pulmonary functionality test (PFT) report of the asthmatic patients is associated with the significant parameters of GER symptoms to determine the impact of GER symptoms on asthma using a proposed hybrid ensemble classification.

Methods/Statistical Analysis: Using R statistical tool a model has been developed for ensemble classification by stacking the SVM and Random Forest algorithms and boosting with the improved Gradient Boosting algorithm.

Findings: It has been identified that the asthmatic patients who have been reported as 'normal' or 'mild' in the PFT report also have the respiratory problems often and urge for frequent check – ups. This can be due to the implications of significant symptom parameters of GER.

Applications: The outcome of the developed model HMMC describes about the classification accuracy of the applied dataset of the asthmatic patients with GER symptoms and predicts the severity of asthma in asthmatic patients more accurately rather than the outcome of the existing classification techniques.

Keywords: *boosting, asthmatic Patients, PFT, GER, ensemble classification, HMMC.*

INTRODUCTION

Prediction and assortment of medical datasets serve in reducing the count of diagnostic issues to recognize the diseases there by affording economical solutions for healthcare systems and medical diagnosis software system. Data pre-processing operates an essential role in prime powerful and capable data for data mining. Feature selection aid in contributing essential characteristics for construction of extensive predictive models. The processing of medical data mining has more potential for exploring the hidden patterns in the knowledge sets of the medical domain. These patterns will be used for clinical diagnosing. However, the obtainable raw medical knowledge is heterogeneous and voluminous in nature. This collected information is often integrated to give a user orienting approach to novel and hidden patterns within the information. In my previous work the significant parameters of the GER symptoms have been identified [1] . And this work deals with the implications of the significant GER parameters of the asthmatic patients with their PFT report value. This leads to the way to find the accuracy of the classification prediction.

EXISTING METHODOLOGY

Chun-Rong Huang et al [2] proposed that "GER can be diagnosed by typical symptoms of acid regurgitation or heartburn sensation at the epigastric or mid chest regions".E.A.Boiler et al [3] proposed that "In western countries, 10–30% of the population experiences symptoms of gastro esophageal reflux (GER)". Sudha Pandit et al [4] propose that "The prevalence of GER

is primarily based on the acid reflux symptoms and these symptoms are not always present in patients with endoscopic evidence of esophagitis". D. Vincent et al [5] proposes that "Gastro-esophageal reflux (GER) has been suspected as a causal factor, but the relationship between GER and asthma remains controversial". The existing work focuses only upon the environmental factors such as living, working environment and parental history as the main causes of respiratory diseases [6] .But the fact is GER may cause, trigger or exacerbate pulmonary disease like bronchial asthma and many other diseases.

PROPOSED METHODOLOGY

A questionnaire can be scientifically accepted by following the standard criteria such as valid, responsive and reliable and it is well determined and established by psychometric methods elsewhere. Such a questionnaire is often administered directly by the patient, filled in, and is straightforward to know as these attributes guarantee prime quality information assessment. The GER symptoms are not always known in the endoscopy test and at the same time the asthmatic patient reported as 'normal' is not always having the normal respiration. This is due to the fact that GER has implications over asthma. This leads to conduct a prospective assessment of GER prevalence in a population of asthmatic patients and to find the prediction accuracy of severity of asthma using the proposed classification model called Hybrid Meta Model Classification(HMMC).

SELECTION OF ALGORITHMS

Random Forest is the flexible machine learning algorithm and produces accurate result with hyper parameter tuning. This supervised learning algorithm builds multiple decision trees, merges them together to get a more accurate and stable prediction [7]. It can also handle missing values and large dataset with higher dimensionality effectively [8].Support Vector Machine (SVM) is used in binary classification and also applied for pattern recognition and it is a promising classification approach for prediction purpose [9].

DATASET DESCRIPTION

- The PFT results for the asthmatic patients are got as raw data as .xps files from the Asthma and Allergy Resource Centre, Chennai, Tamil Nadu, India. Around 2000 raw data has been collected and entered in a spread sheet and stored as .csv file.
- The GER symptom parameters have been assessed to the above asthmatic patients through the questionnaire form and entered in a spread sheet and stored as .csv file.

The resultant significant GER symptom parameters from my previous research work and the PFT report data are taken under consideration for the purpose of classification.

The dataset comprises of

- PID(Patient ID)
- Age
- Gender
- Heartburn (GER symptom)
- Dysphagia (GER symptom)
- Nausea (GER symptom)
- PFT Report
- FreqCheckup

Heartburn	Dysphagia	Nausea	Report	Freq Checkup
voften often noften notAtAll	voften often noften notAtAll	voften often noften notAtAll	normal mild severe	yes no

Table 1: Possible values of attributes

EXPERIMENTAL RESULTS

CLASSIFICATION TREE

The Classification tree has the structure of a flowchart in which each node represents a condition test on an attribute and that each branch indicates the outcome of the condition test. Each terminal or leaf node indicates the class labels**.** Fig 1 shows the distribution of class labels of the PFT report with GER key attributes.

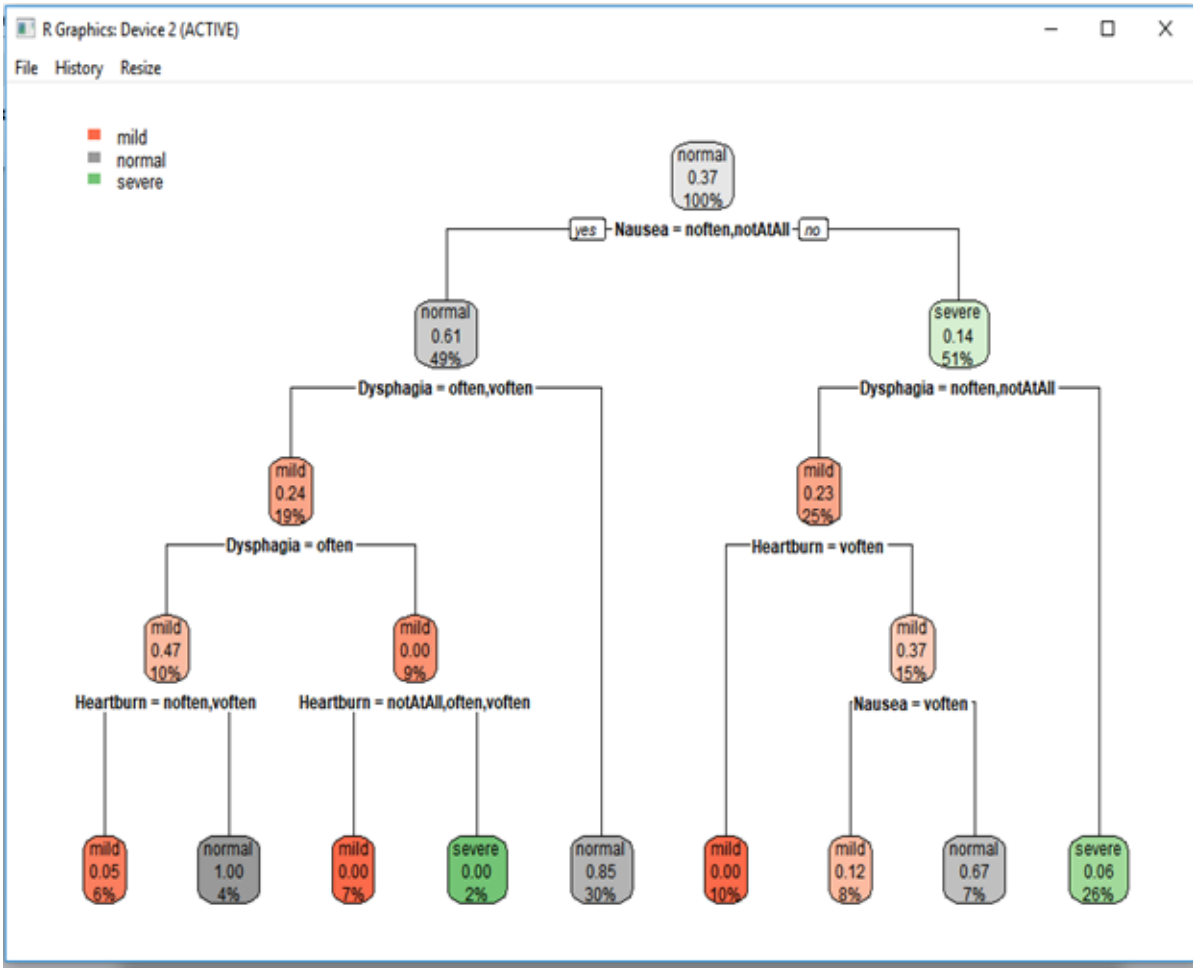


Fig 1: Classification Tree

CLASSIFICATION ALGORITHMS

In random forest algorithm the importance of a variable can be estimated by looking the rate of increase in prediction error when data for that variable is permuted while all the others are left unchanged. The calculations are carried out tree by tree as the random forest is developed [10].

"SVM converges fast and leads to high accuracy. When scores of multiple parameter datasets are combined, majority voting reduces noise and increases recognition accuracy" [11]. It also includes avoidance of over fitting effectively [12] .The built SVM model has the accuracy of 94.85% [Fig 2] and the accuracy of the Random Forest model has the greater accuracy of 95.15% [Fig 3].

BOOSTING ALGORITHMS

C5.0 is the extension of C4.5 algorithm. It gives a binary tree or multi branch tree and uses the gained information for splitting criteria. For estimating missing values it uses other attributes .It represents how the rules are generated with high accuracy and low memory usage [13] .Stochastic Gradient Boosting

is one of the powerful classification algorithms that attains maximum accuracy 96.97% whereas the accuracy of C5.0 is 93.64% which is lesser than SGB. SGB is powerful in terms of classifier and prediction at less execution time.

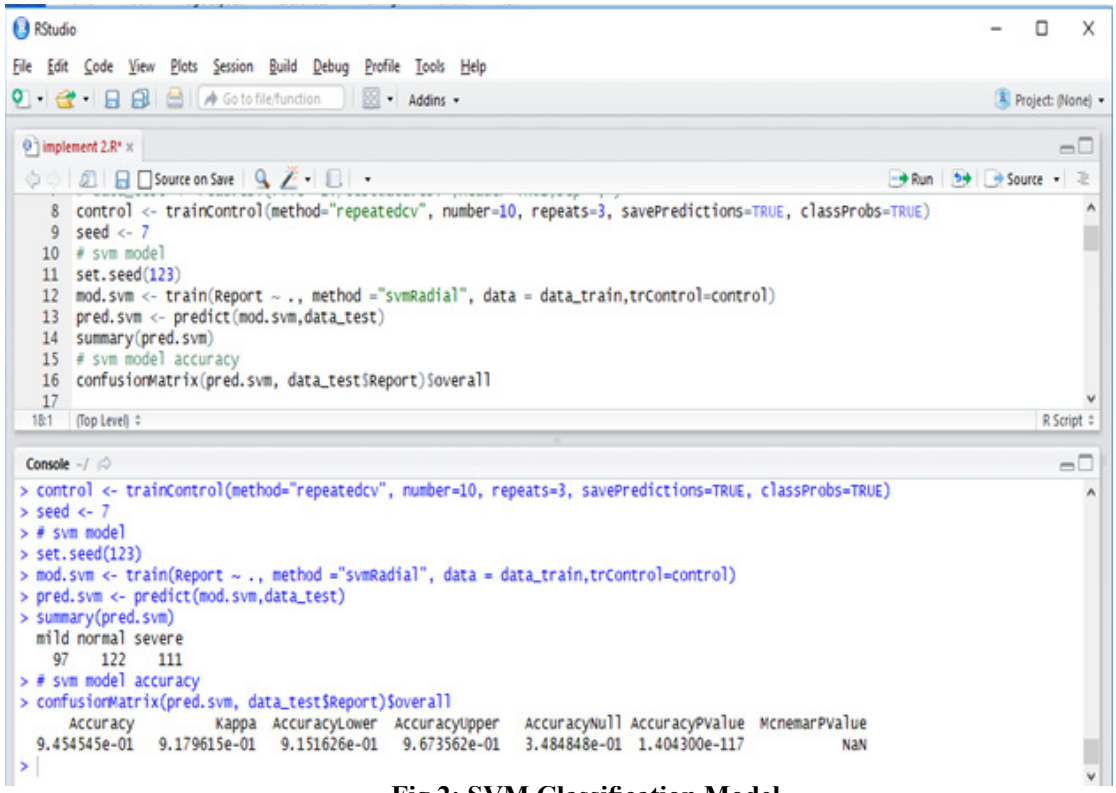


Fig 2: SVM Classification Model

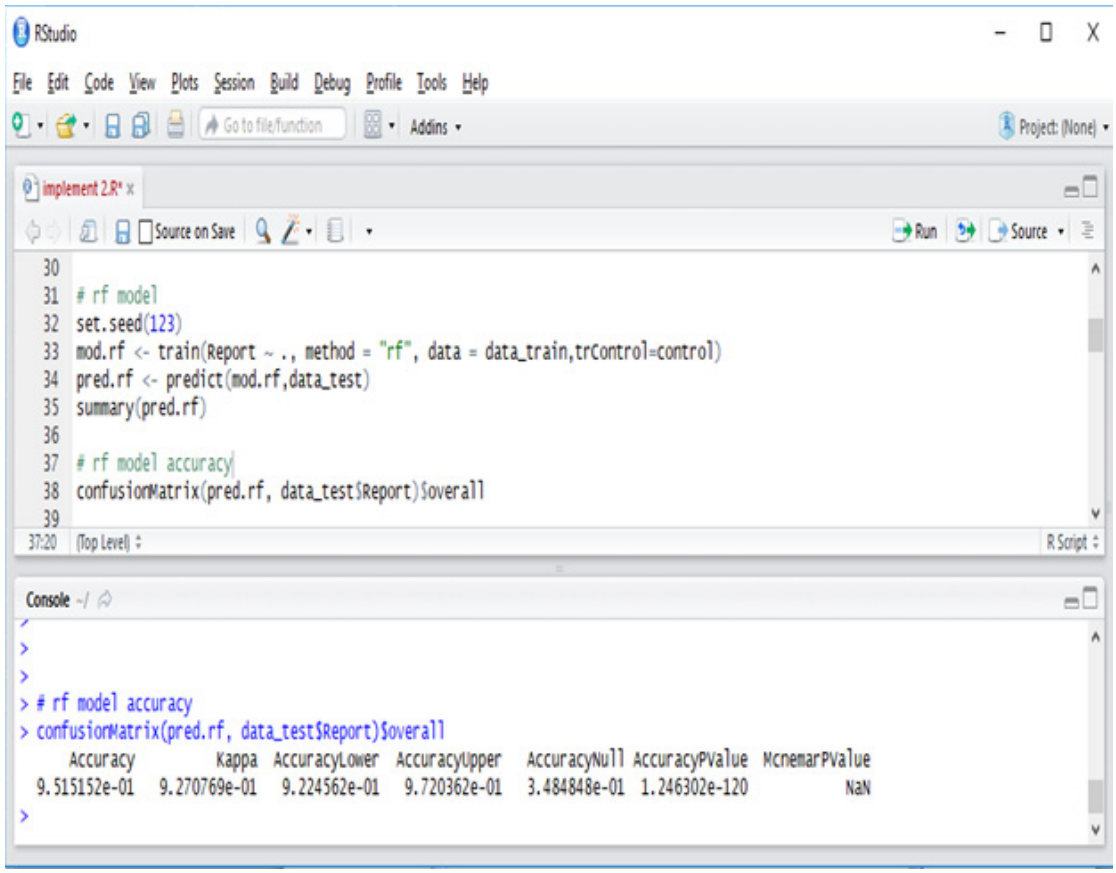


Fig 3: Random Forest Classification Model

PROPOSED MODEL

The hybrid function model can be generated for the predictive purpose with less error and high accuracy without affecting the data consistency [14]. Hybrid model reduces the diagnostic procedure to identify the diseases and to mine the hidden pattern of knowledge [15]. A Hybrid model is needed to diagnose the complexity of disorders and requires more empirical evidences before incorporated into clinical practise [16].

HYBRID META MODEL CLASSIFICATION (HMMC)

Step 1: Splitting the dataset into training and testing data.

Step 2: The developed Random Forest Classification Model and the SVM Classification Model is Combined by the ensemble classification technique called Stacking.

Step 3: Then the stacked outcome is fed into another ensemble classification technique called **Boosting.**

Step 4: The Boosting concept is implemented by the proposed **Improved Gradient Boosting algorithm** to get the maximum accuracy in less time.

PERFORMANCE ANALYSIS

Model Built Using	Accuracy
SVM	94.84.%
Random Forest	95.15%
HMMC	99.4%

Table 2: Classification Accuracy

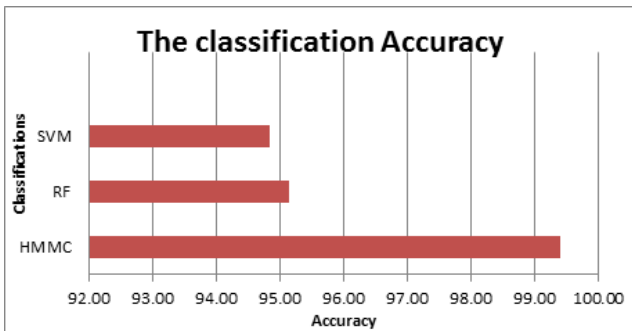


Fig 4: Classification Accuracy Graph

From the Fig 4 the classification accuracy depicts that the proposed model HMMC has the maximum accuracy rather than the existing classification techniques.

CONCLUSION

From the above implementation it has been interpreted that the results of the proposed ensemble model classification HMMC shows improved overall classification accuracy significantly rather than the existing model classification accuracy. This model confirms that the significant GER symptom parameters have high impact on asthmatic patients. Even though the asthmatic patient's PFT report says the condition of the patients as normal or mild, the existence of the significant GER symptom parameters makes the patient's respiration more worst and their by urging them for frequent check-ups.

Ethical Clearance-Not Required (only getting the test report from the hospital).

Source of Funding- Self.

Conflict of Interest – Nil.

REFERENCES

[1] Kasturi K, Prasanna S. Implications of Asthma over Gastro Esophageal Reflux Symptoms Using Regression Analysis. Journal of Advanced Research in Dynamical and Control Systems. 2018[Special Issue]; 11:1066-1072.

[2] Chun-Rong Huang , Yan-Ting Chen ,Wei-Ying Chen , Hsiu-Chi Cheng, Bor-Syang Sheu. Gastroesophageal Reflux Disease Diagnosis Using Hierarchical Heterogeneous Descriptor Fusion Support Vector Machine. IEEE.2016;63(3)

[3] Bolier, E., Kessing, B., Smout, A. and Bredenoord, A. (2013). Systematic review: questionnaires for assessment of gastroesophageal reflux disease. Diseases of the Esophagus, 28(2), pp.105-120.

[4] Pandit S, Boktor M, Alexander JS, Becker F, Morris J. Gastroesophageal reflux disease: A clinical overview for primary care physicians. Pathophysiology. 2018 Mar 1;25(1):1-1.

[5] Vincent D, Cohen-Jonathan AM, Leport J, Merrouche M, Geronimi A, Pradalier A, Soule JC. Gastro-oesophageal reflux prevalence and relationship with bronchial reactivity in asthma. European Respiratory Journal. 1997 Oct 1;10(10):2255-9..

[6] Rohini K, Suseendran G. Aggregated K Means Clustering and Decision Tree Algorithm for Spirometry Data. Indian Journal of Science and Technology. 2016 Nov 30;9(44)..

[7] Casanova R, Saldana S, Chew EY, Danis RP, Greven CM, Ambrosius WT. Application of random forests methods to diabetic retinopathy classification analyses. PLOS one. 2014 Jun 18;9(6):e98587.

[8] Pantanowitz A, Marwala T. Missing data imputation through the use of the Random Forest Algorithm. InAdvances in Computational Intelligence 2009 (pp. 53-62). Springer, Berlin, Heidelberg.

[9] "Hua S, Sun Z. A novel method of protein secondary structure prediction with high segment overlap measure: support vector machine approach1. Journal of molecular biology. 2001 Apr 27;308(2):397-407.

[10] Liaw A, Wiener M. Classification and regression by randomForest. R news. 2002 Dec 3;2(3):18-22.

[11] Ding CH, Dubchak I. Multi-class protein fold recognition using support vector machines and neural networks. Bioinformatics. 2001 Apr 1;17(4):349-58.

[12] Boser BE, Guyon IM, Vapnik VN. A training algorithm for optimal margin classifiers. InProceedings of the fifth annual workshop on Computational learning theory 1992 Jul 1 (pp. 144-152). ACM.

[13] Pandya R, Pandya J. C5. 0 algorithm to improved decision tree with feature selection and reduced error pruning. International Journal of Computer Applications. 2015 May;117(16):18-21.

[14] Banihashemi S, Ding G, Wang J. Developing a hybrid model of prediction and classification algorithms for building energy consumption. Energy Procedia. 2017 Mar 1;110:371-6.

[15] Raghavendra S, Indiramma M. Hybrid data mining model for the classification and prediction of medical datasets. International Journal of Knowledge Engineering and Soft Data Paradigms. 2016;5(3-4):262-84..

[16] Esbec E, Echeburúa E. The hybrid model for the classification of personality disorders in DSM-5: a critical analysis. Actas Esp Psiquiatr. 2015 Sep 1;43(5):177-86.

Prevalence of Dysmenorrhea among Female Medical Students and its Impact on their Day to Day Activities

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ABSTRACT

Background: Dysmenorrhea refers to painful menstruation with associated symptoms such as backache, headache etc. and is quite common among young women. In many, it hampers normal day to day activities resulting in lost work-hours.

Aim: This study was taken up to estimate the prevalence of dysmenorrhea among female medical students, its impact on sufferers' daily activities and their knowledge of treatment options.

Materials and Method: This was a cross-sectional study conducted among 150 medical students of ages 18 – 21 in Tagore Medical College, Chennai. All subjects were given a questionnaire with questions pertaining to pain during menstruation, its severity, other associated symptoms, effect on day-to-day activities as well as management options. Using information from the questionnaire, grading of subjects into mild, moderate and severe dysmenorrhea was done.

Results and Discussion: Overall prevalence of dysmenorrhea among the study population was found to be 72.67 % with commonest symptoms associated being headache (88%) and backache (64%). Of the dysmenorrheic population, 42% had mild, 39% had moderate and 19% had severe dysmenorrhea. Low class concentration (59%), class absenteeism (34%), college absenteeism (20%), limitation of social activity (52%) and curtailment of sporting activities (57%) of sufferers were found, all of these being more in the severe dysmenorrhea group. Regarding knowledge of medication, only 12% and 20% knew that paracetamol and mefenamic acid were treatment options. Dysmenorrhea was managed mainly by rest (52%) while 19% used analgesics. The majority considered dysmenorrhea to be a normal female experience and did not think it warranted any treatment.

Conclusion: Painful menstruation is considered to be a normal female experience by young female medicos. They need to be educated on effective management methods and given knowledge of appropriate medications so that dysmenorrhea will no longer be the cause of lost work hours or poor quality of life.

Keywords*: dysmenorrhea, class concentration, college absenteeism, medical students, menstruation*

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INTRODUCTION

In the world of today, where women have made significant strides in education and professional growth, it appears that dysmenorrhea is one burden many of them continue to carry. Dysmenorrhea is quite common among young women. Shaw's textbook of gynaecology

defines dysmenorrhea as "cramping pain accompanying menstruation".[1] It is associated with symptoms such as lower abdominal pain, backache, pain in the thighs, nausea / vomiting, fatigue, headache, lightheadedness and even diarrhea.[1,2] In many young women, dysmenorrhea is so severe that it hampers their normal activities one to two days every month and is a major reason for school and college absenteeism.[2,3]

Going by the findings of different studies, the prevalence of dysmenorrhea appears to vary greatly between 45 and 95%[4] depending on the population under study and method of data collection. Many women, however, consider dysmenorrhea to be a normal phenomenon and do not feel it merits medical attention. Untreated dysmenorrhea results in lost work hours in schools, colleges and offices. It affects the person's personal and family life, and has social and economic consequences as well.[3]

This study was taken up to determine the prevalence of dysmenorrhea among female medical students in Tagore Medical College, Chennai, its impact on sufferers' daily activities and knowledge of treatment options.

MATERIALS AND METHOD

The study was done among 150 female first and second year medical students at Tagore Medical College and Hospital, Chennai, Tamil Nadu, using a cross-sectional study design and simple random sampling. Ethical approval was taken from the institutional ethical committee. Healthy unmarried nulliparous female students of age ranging from 18-21 years were included in the study after obtaining written consent from them. Students having polycystic ovarian disease, dysfunctional uterine bleeding, primary amenorrhea, any pelvic pathology, secondary dysmenorrhea or any other gynecological disease were excluded from the study.

Data collection:

After obtaining written consent from the participants, the structured and validated study questionnaire was administered. It carried questions on details pertaining to the menstrual cycle, pain during menstruation, its severity and other associated symptoms, effect on day-to-day activities as well as management options.

The severity of menstrual pain was estimated by the visual analogue scale and scoring for severity of dysmenorrhea was done based on information from the questionnaire using the multidimensional scoring system of Andersch and Milsom.[5] This system grades dysmenorrhea into mild, moderate and severe based on the pain score, effect on daily activities and requirement for analgesia. In mild dysmenorrhea, there is mildly painful menstruation, but daily activities are hardly affected and analgesia rarely required. The moderate category has more painful menstruation so that daily activities are curtailed. However, analgesics help to manage the symptoms. In severe dysmenorrhea, the menstrual pain is severe with associated vegetative symptoms, daily activities are clearly affected and analgesics are not effective.

Statistical analysis:

Data was entered in Excel sheet and analysis was done using SPSS software. Statistical analysis was performed using appropriate tests such as student t test and chi square. P value of < 0.05 was taken as statistically significant.

RESULTS AND DISCUSSION

A total of 150 students took up the questionnaire and all of them completed it. Overall prevalence of dysmenorrhea among the study population was found to be 72.67 % (109/150), while 27.3 % (41/150) said they experience no menstrual pain.

Cycle characteristics of the dysmenorrheic women are shown in Table 1. Of the 109 dysmenorrheic students, 102 reported normal cycle lengths; 22% reported a longer than normal duration of period; 10% reported using five or more pads per day and an overwhelming 58% reported the passage of clots in menstrual blood.

Table 1: Cycle characteristics in dysmenorrheic women

Parameters		Women with dysmenorrhea (n = 109)	Percentage
Age at menarche	<= 11 years	8	7.3
	12 to 14 years	90	82.6
	>14 years	11	10.1
Cycle length	21 to 35 days	102	93.6
	>35 days	5	4.6
	Don't know	2	1.8
Duration of period	<=5 days	85	78.0
	>5 days	24	22.0
Pad usage per day during menstruation	< = 4	98	89.9
	5 to 7	11	10.1
Presence of clots	Yes	63	57.8

Table 2 lists associated symptoms present along with their frequency. The commonest symptoms were headache (88%), backache (64%), abdominal cramps (64%) and mood changes (39%). Fatigue occurred in 27.5%. In the study by Singh et al fatigue occurred in 70.9%, backache in 62% and headache in 26.6%.[6] A study done among Thai adolescents showed abdominal cramps at 78 %, backache at 59%, mood changes at 57% and fatigue at 43%.[7]

Table 2: Associated symptoms in dysmenorrheic women

Symptoms	Women with dysmenorrhea (n = 109)*	Percentage
Headache	96	88.1
Backache	70	64.2
Abdominal cramps	70	64.2
Mood Changes	42	38.5
Fatigue	30	27.5
Nausea	20	18.3
Loss of Appetite	19	17.4
Diarrhoea	09	8.3
Fainting	09	8.3
Dizziness	07	6.4
Vomiting	05	4.6

*Most women had more than one symptom

Of the dysmenorrheic population in our study, 42 % (46) had mild dysmenorrhea, while 39 % (42) belonged to the moderate category. Nineteen percent (21) of the students had severe dysmenorrhea (Table 3). The prevalence of dysmenorrhea among 18-21 year old nursing students in Mumbai was reported to be 45% with 18% having mild dysmenorrhea, 40% having moderate and 42% having severe dysmenorrhea[8] while another study done in three medical colleges in India reported 73% prevalence with 63.3% having mild, 30.4% having moderate and 6.3% having severe dysmenorrhea.[6] A study done among adolescents in Rajasthan reported a rural prevalence of 81.5% and an urban prevalence of 76 %.[9] Of the rural category, 52 % had mild, 26.5 % moderate and 3 % had severe dysmenorrhea.[8] Of the urban group, 56.5 % had mild, 12.5 % moderate and 7 % severe. A Gwalior study reported an incidence of 79.6 % dysmenorrhea among adolescents there.[10] Our findings

are comparable with these earlier estimates.

Table 3 shows the impact of dysmenorrhea on the day to day lives of affected young women. We found 59% being affected by low class concentration overall among the dysmenorrheics with 85.7% from the severe category, 78.6% from the moderate and 30.4% from the mild category. These differences were statistically significant (p=0.000). The Thai study found a total of 63.6% of dysmenorrheics suffering from poor class concentration with 51.4% from the mild group, 73.4 % from the moderate group and 87.1% from the severe group being affected.[7]

Class absenteeism stood at 90.4% among the severe, 35.7% among the moderate and 6.5% among the mild dysmenorrheics. 76.1% of severe dysmenorrheics and 9.5% of moderate dysmenorrheics had to miss a whole day of classes. These differences also were found to be statistically significant (p=0.000). The Thai study showed 80.6% of the severely dysmenorrheic, 27.7% of the moderate and 11.7% of the mild group missing classes.[7] Singh's study revealed 31.64% college absenteeeism among dysmenorrheics and 8.9% class absenteeism.[6] Our study also found that normal daily activity was curtailed in 53% of the dysmenorrheics with 95.2%, 76.2% and 13% of the severe, moderate and mild categories respectively being affected. Social activity also was limited in 52% of sufferers and sporting activities in 57%. Singh et al found an overall 67.1% of subjects reporting withdrawal from academic, social and sports activites.[6]

Table 3: Impact of dysmenorrhea on students' daily activities

	Grade of Dysmenorrhea				
	Severe n = 21	**Moderate n = 42**	**Mild n = 46**	**Total n = 109**	**P value**
Low class concentration*	18 (85.7 %)	33 (78.6 %)	14 (30.4 %)	65 (59.6 %)	.000
Class absenteeism*	19 (90.4 %)	15 (35.7 %)	3 (6.5 %)	37 (33.9 %)	.000
College absenteeism for the whole day*	16 (76.1 %)	4 (9.5 %)	0 (0 %)	20 (18.3 %)	.000
Limits normal daily activity*	20 (95.2 %)	32 (76.2 %)	6 (13.0 %)	58 (53.2 %)	.000
Limits social activity*	14 (66.7 %)	33 (78.6 %)	10 (21.7 %)	57 (52.3 %)	.000
Limits sport activity	13 (61.9 %)	28 (66.7 %)	21 (45.7 %)	62 (56.9 %)	.121

* statistically significant

For management of dysmenorrhea, resting appears to be the preferred method with 52% opting for the same. 21% did nothing, while 19% used analgesics (Table 4). Other home remedies like heating pads and herbal remedies were used by 7% of the dysmenorrheics. In a study at a university in Ankara, 69% of female students used analgesics, 56.5 % used heat application and 71.4 % took rest to manage menstrual pain.[11] The Thailand study found 92% of dysmenorrheics opting for rest, 34% using heating pads and 32% using analgesics.[7]

Of the dysmenorrheic population in our study, 54% had no knowledge of pharmacologic agents that could be used for pain management in dysmenorrhea (Table 4). Only 12% and 20% knew that paracetamol and mefenamic acid were treatment options. Around 12.8% were aware of naproxen and other NSAIDs. In the Thai study, awareness levels were much higher at 98.8% for paracetamol and 85% for aspirin, though only 6% knew about mefenamic acid.[7] Our population has very low awareness regarding analgesics and their use in dysmenorrhea.

Table 4: Management methods and knowledge regarding pharmacologic agents for dysmenorrhea

Management of Dysmenorrhea		
	Frequency	**%**
Nothing	23	21.1
Only Rest	57	52.3
Rest and Analgesics	21	19.3
Other Methods	8	7.3
Knowledge of Drugs		
None	59	54.1
Paracetamol	14	12.8
Mefenamic Acid	22	20.2
Others	14	12.8

Surprisingly, 96% of mild, 93% of moderate and 67% of severe dysmenorrheics considered dysmenorrhea to be a normal female experience and did not think it warranted any treatment (Table 5). Only 4%, 7% and 33% of mild, moderate and severe dysmenorrheics respectively felt that they need not suffer, but could seek medical help to alleviate their pain. These findings were comparable with those of the Thai study.[7] This shows that an overwhelming majority of young women look on dysmenorrhea as normal and only a very small percentage opt for treatment.

Table 5: Attitude towards dysmenorrhea

	Grade of Dysmenorrhea			
	Severe (n = 21)	**Moderate (n = 42)**	**Mild (n = 46)**	**Chi Square P value**
Normal female experience	14 (66.7%)	39 (92.9%)	44 (95.7%)	.001
I need not suffer	7 (33.3%)	3 (7.1%)	2 (4.3%)	

Appropriate health education will go a long way in letting young women know that dysmenorrhea is not a normal female experience and needs to be evaluated and treated. Getting dysmenorrhea treated assumes more importance in the light of recent research findings suggesting that it might predispose sufferers to other chronic pain conditions as they grow older.[12]

CONCLUSION AND IMPLICATIONS

This study serves to estimate the prevalence and severity of dysmenorrhea in young medical students and assess its impact on their daily activities.

Going by the findings of our study, painful menstruation appears to be a burden carried by a majority of young female medicos. Our finding of 19% students having severe dysmenorrhea shows the extreme suffering that many of them experience on a regular basis. They need to be educated that this is not necessarily a normal female experience, but needs to be managed effectively by using appropriate medication. Knowledge of these medications also needs to be given them as well as the assurance that these are not addictive as fear of addiction seems to deter many from using analgesics. The possible role of dysmenorrhea in predisposing individuals to other

chronic pain conditions also needs to be highlighted.

Creating awareness and giving health education regarding symptom management could help students manage the pain better and minimize the work-hours lost due to dysmenorrhea.

It will be beneficial if this study could be done in other population groups and also in the general community. It will give an idea of the public health burden of dysmenorrhea and its impact on the lives of young women both in the personal and socio-economic domains, so that appropriate remedial measures could be promoted to help them have a better quality of life.

Ethical Clearance: Taken from the Institutional Ethics Committee, Tagore Medical College and Hospital

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. Padubidri VG, Daftary SN. Howkins and Bourne Shaw's Textbook of Gynaecology. 16th ed. Elsevier 2015.
2. Coco AS. Primary dysmenorrhea. Am Fam Physician. 1999 Aug; 60(2):489-96. Epub 1999/08/28.
3. George A, Bhaduri A. Dysmenorrhea among adolescent girls – symptoms experienced during menstruation. Health Promotion Educ. 2002; 17:4
4. Kural MR, Noor NN, Pandit D, Joshi T, Patil A. Menstrual characteristics and prevalence of dysmenorrhea in college going girls. J Family Med Prim Care. 2015 Jul-Sep; 4(3): 426–431. doi: [10.4103/2249-4863.161345]
5. Andersch B, Milsom I. An epidemiological study of young women with dysmenorrhea. Am J Obstet Gynecol. 1982; 144(6):655-60.
6. Singh A, Kiran D Singh H, Nel B, Singh P, Tiwari P. Prevalence and severity of dysmenorrhea: a problem related to menstruation, among first and second year female medical students. Indian J Physiol Pharmacol. 2008; 52(4):389–397.
7. Tangchai K, Titapant V, Boriboonhirunsarn D. Dysmenorrhea in Thai adolescents; prevalence, impact and knowledge of treatment. J Med Assoc Thai. 2004 Oct; 87 Suppl 3:S69-73.
8. Shah M, Monga A, Patel S, Shah M, Bakshi H. A study of primary dysmenorrhea in young students-a cross sectional view. Healthline. 2013; 4(2):1-4
9. Chauhan M, Kala J. Relation Between Dysmenorrhea and Body Mass Index in Adolescents with Rural Versus Urban Variation. J Obstet Gynaecol India. 2012 Aug; 62(4): 442–445. Published online 2012 Jun 1. doi: [10.1007/s13224-012-0171-7]
10. Agarwal AK, Agarwal A. A Study of Dysmenorrhea During Menstruation in Adolescent Girls. Indian J Community Med. 2010 Jan; 35(1): 159–164. doi: [10.4103/0970-0218.62586]
11. Aktas D. Prevalence and Factors Affecting Dysmenorrhea in Female University Students: Effect on General Comfort Level. Pain Manag Nurs. 2015; 16(4):534-43. doi:10.1016/j.pmn.2014.10.004.
12. Bernardi M, Lazzeri L, Perelli F, Reis FM, Petraglia F. Dysmenorrhea and related disorders. F1000Res. 2017; 6:1645. doi: 10.12688/f1000research.11682.1. eCollection 2017.

Factors Enhancing Academic Performance in Management Courses among Post Graduate Pupils' of a Management Institution

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ABSTRACT

Purpose - The idea of the research is to look at the role of factors enhancing learners' presentation. The factors influence on academic performance such as teaching styles, communication method, assessment methods and ICT skills are considered. The Dependant variable academic performance is considered as univariate.

Research methodology- Purposive Sampling was used in the study to collect the data; structured Questionnaire was distributed to 110 respondents of students (male and female). 10 Questionnaires were found to be unfilled so total 100 respondents (n=100). CFA and SEM were the statistical tools applied for the analysis in AMOS-21 to check the hypothesis of learn.

Findings - This analysis shows a positive and significant relationship between the independent variables teaching styles and ICT skills and the dependant variable Academic performance.

The variable ICT Skills is a new variable used in review of literature in India. But it has started to gain momentum in all aspects. The study also reveals the significance of ICT usage through the study among management students.

Practical implication – The paper have definite confines. The study was pertaining to management students only. On the other hand the implications are the results of the study can be used for the purpose of developing new strategies in the field of academics in the management students. For researchers this study can be extended for future variables with more related variables. Secondly it can spread across to other educational institutions also.

Uniqueness – This study is an initial try to know, 'enhance of variables relating to academic performance of Post graduate Students in Indian context.

Keywords *– Teaching, pedagogy, academic performance, communication.*

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INTRODUCTION

Many countries have different approaches towards teaching and learning. Research studies have been carried out to investigate the best ways of teaching. One such facet of teaching in teachers' personal qualities are directly related to the subject matter of teaching. Hence

choice of the method and teachers' through the substance of the issue becomes important under learning context. There is a collaborative interaction among individual traits, the process by teacher utilize to communicate the substance of subject matter, and the method's display as learner[1]. The Methods through the teacher was teaching as exacting instance of requirements, idea, and behaviors that instructor exhibit in study hall. This study was used in conceptual framework.[2] the teaching method's as explain by Teacher through the Methods styles handling in class and study environment[3]. In 20th century different learning methods were followed. Special pedagogy was change in modern trend, since the pupils call for to learning process. What? , When? , How? To instruct lessons? The authority method is related to lectures styles or teachers' styles. Demonstrated method means coaching styles. The facilitator style means action method. The delegator style means peer feedback[4]. Hybrid method is blended learning. Theoretical discussion of teaching styles as a focus of presentation and study has become a part of the vernacular in. As a part of coaching technique (ICTs-awareness) get into class-rooms. Now a day's learning process was continued in (www.) web based learning[4]. Student's personality development (emotion, attitude) to reference[5]

Statement of the problem

Many researchers analyzed student's presentation (performance) by Grade point average (GPA) and cumulative grade point average (CGPA) was found. In this articles followed by Grade point value[6]. Some researcher to examining the quarterly, half yearly and annually to collect the pupil progress in required research. The aim of the present study was concentrate pupil's learning and outcome of the performance (exam results) was calculated by GPA. The pupil attitude, skills, interest on listening skill and extra activities (NSS, sports and NCC and yoga[7]. Private institution vision is to be a hub of quality in the midpoint of Tamil Nadu. Though the academic deans and the excellence and declaration of commission have noted that while some Pupils do highly and other do not complete healthy. Describe the pupil to check the progress in calendar year. Every calendar year to check institute quality and student enrolment, mark sheet and degree awarded, maintained by university Grants commission (UGC).

Theoretical Model

A model framework recommended by Lebcir model was taken as a base model for the study. They are teaching styles, ICT-skills and academic performance. The researcher has tested this frame work in the form of questionnaire, distributed to the students of management studies in a University - education institution.

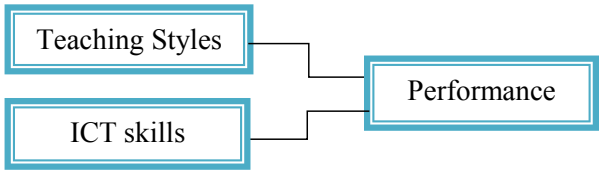


Figure 1. Theoretical Model

REVIEW OF LITERATURE

Review of literature explains the conceptual model for teaching styles[8]. For instance, had explained five various proceed towards learners. He had identified six categories which contain Task oriented common planner of the subject[9]. While institution of higher education pedagogical in management course current and alternative communicating approach, outcomes are weighted more[10]. He has shown examined Teaching with style in alliance with ICT as one of the most dynamic inventions which helps to tackle the challenges faced by world in 21st century and its application in academics has become a priority in most countries during the last decade. The usage of ICT tools in ME makes teaching and learning more effective. It also helps in wider dissemination of knowledge among students from all sections of the society. The literature review is divided into two parts independent as: Need for Teaching style in Management colleges, Benefits of using ICT in teaching and one Dependent as Academic performance.

Teaching styles:

Teaching method is a fundamental brick in teaching pedagogy, which has exposed to special details given by many research persons. Other research studies also highlight about teaching performance and the similarity between the instructing style and his or her thinking of the professor [11]. Furthermore, instructing style was specific and related with the different special sets of classroom instructing style' as explained[12]. 'Talk about the study submits to the person's enveloping instructional traits that continue to change the learning process'. On the other hand, the normal attribute get beginning these previous studies are the coaching behavior and wisdom

course of action.

ICT-Skills

The world of internet has shown tremendous growth and improvement over the past two decades[13]. The article focuses on the relativity and influence of ICT skills on academic performance of the students. A few previous studies on integration and usage of these skills in academic performance by students were found[14]. Hence this becomes one of the major research gaps of the present study. Study by Angeles highlights on usage of ICT skills has a major advantage[15]. According to the survey taken and highlights on the differences in the attitude in the usage of ICT skills in academic performance between boys and girls. ICT skills are fundamental for student and teacher inter related[16].

Academic performance:

There is a dearth in review of literature based on academic performance. Studies done on linguistic students' highlights the importance of academic performance among students. He has shown educative uses of ICT and its usage in academic performance and the results reveal there is a positive relationship between two variables[17].

Research question:

1. Does teaching styles and ICT impacts academic performance of students?

Objective of the study:

1. To analyze the association among teaching styles, ICT and academic performance.

Hypotheses of the study

H1. There is significant relationship between teaching styles, ICT and academic performance.

Materials and Methods

The research was carried out the association between teaching styles, ICT skills and academic performance measured among 110 post graduate students. The researcher administered a survey through questionnaire to discover the number of students learning skills and ICT skills in academic performance.

Sampling procedure

The respondents of this study consist of Management students' at the private university. Purposive sampling was used for the research study. The total number of students (male and female) of the management students' structured Questionnaire was distributed to 110 respondents of students (male and female). 10 Questionnaires was found to be unfilled so total 100 respondent filled quality questionnaire.

Data Analysis

Table I. Overall Reliability of the Data

Alpha(α) std. things	Number of things
.877	14

Variables	Cronbhach's Alpha	No of Item
Academic performance	.799	5
Teaching styles	.658	4
ICT-skills	.794	5

Overall reliability instrument study was 0.877.

The present paper to analyze reliability of the total Item is 0.875 is significant individual alpha (Teaching styles) is 0.70 and ICT-skills is 0.794 and dependent variable academic performance is 0.799 the on the whole 0.875.It is value note that 0.70 should be an good enough alpha value and coefficient still lower coefficients used in a few research Explain above the table.

Sample Adequacy Table II.(KMO test)

KMO test.	.836
Chi-Square(approx)	534.590
The (Bartlett's Test) Degrees of freedom(DF)	
Significance	91
	.000

The value received through KMO test was.836 which is above the threshold of .7. The Chi-square value was 534.590 and the significant p value is .000. Hence the sample taken for the study is adequate in nature.

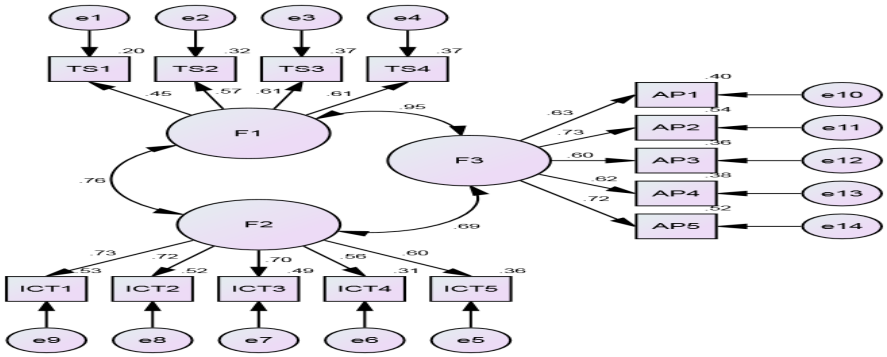


Diagram 1 showing CFA analysis

Table III showing testing of Hypothesis

Variables	The relation	Hypothesis
Teaching styles	Student's academic performance	Supported
ICT skills	Student's academic performance	Supported

The tested hypothesis as per regression analysis proves to be **significant.**

CONCLUSION

This study was carried to explain the role of teaching styles and ICT skills. Both the variables do play a vital role in the academic performance. ICT usage has been one of the weak areas in the field of Indian education, but it has started to gain momentum. Future studies with more mediation variables like gender and GPA can be used.

Conflict of Interest: Nil

Ethical Clearance: NA

Source of Funding: Self

REFERENCES

1. Grasha. A matter of style: The teacher as expert, formal authority, personal model, facilitator, and delegator. Online Heldref publications in collaborating with JSTOR to digitize, preserve and extend access to college Teaching vol.42 No.4 (Fall 1994).[accessed: 27/08/2010] available on URL https://jstor.org/stable/27558675.page 142-149.
2. Lebcir RM, Wells H, Bond A. Factors affecting academic performance of international students in project management courses: a case study from a British post 92 university, Online International Journal of Project Management, Vol. 26 No. 3, (2008). Available from URL https://doi.org/10.1016/ijproman http://www.sciencedirect.com [copy right @ 2008 ELSEVIER LTD and IPMA. all right reserved]. pp. 268-274.
3. Anthony J, Warner L, Kessler R. Comparative epidemiology of dependence on tobacco, Alcohol, controlled substances, and inhalants: basic finding from the national co morbidity survey. Online experimental and clinical psychopharmacology vol.2 No.3 1994. [Accepted April 23 1994] page 244-268.
4. Abdullah AL, Mutairi. Factors affecting business students' performance in Arab open university: the case of Kuwait. Online international journal of business and management volume.6 No.5 may 2011 [accepted December 7 2010] available from, URL: http://www.ccscnet.org/ijbm/ DOI:10.5539/ijbm.v6n5p146.
5. Arthur E poropat. Personality and academic performance. Online research gate January 2017. DOI: 10.1007/978-3-319-28099-8_989-1. Available from https://www.researchgate.net/publication/3138.

6. Feast V. The impact of IELTS scores on performance. Online international educational journal v olume.3 No.4 2002. Available online URL https://www.flindes.edu.au/education/iej.
7. Samuel Embiza Tadesse, Kibrom Gidey. Identification and analysis of factors that affects students learning among university students. Online research on humanities and social sciences volume 5 No.13 2015. Available from URL http://www.iiste.org/ ISSN (paper) 2224-5766 ISSN (online) 2225-0484.
8. Darwish Abdulrahamn Yousef. Factors influencing academic performance in quantitative courses among under graduate business students of a public higher education institution. Online journal of international education in business volume 10 No.1 2017 [accepted 13 January 2017] DOM: 101108JIEB-07-2016-0016. Available from URL http://www.emeraldinsight.com/2046-469x.htm PP12-30.
9. Virgil Frunza. Implications of teaching styles on learning efficacy. Online procedia social and behavioral sciences 127(2014) DOI: 10.1016/j.sbspro.2014.03.268. Available from URL http://www.sciencedirect.com 342-346.
10. Belal R. Students' perception of computer assisted learning: a empirical study. Online international journal of management in education volume 5 No.1 2011. DOI: 1504/ijmie 2011. 037755. Available from URL http://inderscience.com/offer.php?id=37755 pp6378.
11. Anne K, Ditcher. Effective teaching and learning in higher education with particular reference to the undergraduate education of professional engineers. Online int .j. engng ed. volume. 17 No.1 2001 [accepted 12 march 2000] printed in Great British pp 24-29.
12. Welborn, Timothy Tunde Oladokun. Factors influencing academic performance of real estate students in Nigeria. Online Property management Volume 34 No. 5 2016 [accepted 24 March 2016] DOM: 101108/pm.09.2015.0045. Available from URL http://www.emeraldinsight.com/0263-7472.htm PP 396-414
13. Cooper J. The digital divide: the special case of gender. Online Blackwell publishing ltd [accepted 4th may 2006] volume 22 pp320-334.
14. Angeles Iniesta Bonillo M, Raquel Sanchez Fernandez, Walesska Schlesinger. Investigating factors that influence on ICT usage in higher education: a descriptive analysis. Online int rev public nonprofit mark (2013)10:163-174. DOI: 10.1007/s12208-013-0095-7 [accepted 2 may 2013]. Available from Springer Verlag Berlin.
15. Raquel Sanchez Fernandez, Walesska Schlesinger. Investigating factors that influence on ICT usage in higher education: a descriptive analysis. Online int rev public nonprofit mark (2013)10:163-174. DOI: 10.1007/s12208-013-0095-7 [accepted 2 may 2013]. Available from Springer Verlag Berlin.
16. Raquel Sanchez Fernandez, Walesska Schlesinger. Investigating factors that influence on ICT usage in higher education: a descriptive analysis. Online int rev public nonprofit mark (2013)10:163-174. DOI: 10.1007/s12208-013-0095-7 [accepted 2 may 2013]. Available from Springer Verlag Berlin.
17. Muhdin Muhammed Hussen. Determinants of economic students academic performance: case study of Jimma university Ethiopia. Online international journal of scientific and research publications volume 6 issue 1 January 2016. Available from URL http://www.ijsrp.org/ ISSN 2250-3153.

Antibiotic Suscepyibility Pattern of *Staphylococcus aureus* and Methicillin – Resistant *Staphylococcus aureus* Isolated from Various Clinical Specimens in a Tertiary Care Teaching Hospital, Pondicherry

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ABSTRACT

Introduction: *Staphylococcus aureus* is one of the most common human pathogen capable of causing a wide range of infections. *Staphylococcus aureus* is a common cause of both community and hospital infections. It was the endemic microorganism in several reports of nosocomial infections which induced high mortality and morbidity. This microorganism is a virulent bacterium that can cause serious infections including skin and soft tissue infections, wound infection, bacteremia, pneumonia and endocarditis. It is estimated that *Staphylococcus aureus* has developed the ability to acquire resistance to all classes of antimicrobial agents and methicillin-resistant *S. aureus* (MRSA) has become a major problem in many hospitals worldwide. Hospital acquired infection (HAI) due to multidrug resistant bacteria like MRSA are a growing problem in many health care institutes. **Materials and Method:** A total of 568 clinical isolates of *Staphylococcus aureus* were isolated from various clinical specimens received in the Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry from April 2014 to December 2017, were included in the study. Isolation and identification of *Staphylococcus aureus* was done by standard conventional microbiological methods. Identification of MRSA was done as per standard guidelines of CLSI by using Oxacillin broth microdilution method. Antibiogram was determined by Kirby-Bauer disc diffusion method on Mueller-Hinton agar with zones of inhibition. **Results:** *Staphyloccus aureus* was susceptible to Vancomycin (94.7%), followed by Teicoplanin (91.7%), Linezolid (88.5%), Amikacin (85.7%). In case of MRSA all the isolates showed 100% susceptibility to vancomycin, followed by Teicoplanin (97.5%), Amikacin (84.7%) linezolid (80.3%) and Clindamycin (54.8%). In case of MRSA all the isolates were 100% susceptible to vancomycin followed by teicoplanin (97.5%), amikacin (84.7%) linezolid (80.3%) and clindamycin (54.8%).

Conclusion: MRSA infections are emerging as a serious health problem in health care set up. Joint efforts by clinicians, clinical microbiologists, hospital management and public health authorities are required to meet the challenge of MRSA on the forefront.

Keywords: *Staphylococcus aureus, MRSA, Oxacillin, Vancomycin.*

INTRODUCTION

Staphylococcus aureus has been renowned as an important cause of human disease for more than 100 years [1]. Alexander Ogston had first isolated *Staphylococcus aureus* from a surgical abscess in 1880 and described the role of *Staphylococcus aureus* in localized infection and septicemia, including the use of animal models for infection [2].

Staphylococcus aureus has emerged as one of the main important human pathogens, and has over

the past decades, been a leading cause of hospital and community-acquired infections. Staphylococcal infections give rise to a wide spectrum of symptoms and diseases in humans. The bacterium is well characterized and known to have a diverse arsenal of virulence factors that causes a prominent inflammatory response [3,4].This pathogen affects both immune competent and immuno compromised individuals, frequently resulting in high morbidity and with complications, which constitute problem to health care institutions. *S. aureus* has been reported by several studies as the causative agent of wide variety of diseases of supportive infections such as boil, wound infection, pustule, subcutaneous and sub-mucosa abscesses, osteomyelitis, mastitis, impetigo, septicemia, meningitis, bronchopneumonia, food poisoning, a common cause of vomiting, diarrhea, and urinary tract infections[5].

S.aureus was discovered in 1880, its effective treatment was started in1940, penicillin was the only drug used for treatment of the infections caused by this bacterium. After in late1940 and throughout 1950, *S.aureus* developed resistance mechanism to penicillin. Introduction of Methicillin was done in 1961 to treat these resistant strains nd within a year or later, clinicians had encountered methicillin - resistant *Staphylococcus aureus* which became a big threat. Now strains of MRSA are simultaneously resistant to a list of different groups of antibiotics, including vancomycin which is often considered our last line of antibacterial defence. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as one of the major pathogens in both hospitaland community settings.[6]

In 1961, British scientists discovered MRSA, the first case of this "superbug" in the United States occurred in 1968.[7] MRSA is due to the acquisition of *mecA* gene that carried on a large mobile genetic element, the staphylococcal cassette chromosome, and which encodes a low affinity penicillin-binding protein 2a (PBP2a) to β-lactam antibiotics. The *mecC* gene when present, may also mediate methicillin resistance. The *mecA* complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics.[8] Hospital acquired MRSA is frequently multidrug resistant. This limits the therapeutic options to a few antimicrobials, which are toxic, complicated to administer and expensive. As a consequence patients have to be hospitalized for a longer duration, treatment costs are increased and associated mortality also rises. This has a significant impact on individual patients and institutions. An additional concern of grave significance is the emergence of vancomycin intermediate *Staphylococcus aureus* (VISA) and more recently vancomycin resistant *Staphylococcus aureus* (VRSA).[9,10] Hence this study was conducted to evaluate Antibiotic susceptibility pattern of *Staphylococcus aureus* and to determine methicillin- resistant *Staphylococcus aureus* (MRSA) isolates from various clinical specimens.

MATERIALS AND METHOD

A total of 568 consecutive, clinically significant, non-repetitive clinical isolates of *Staphylococcus aureus* were isolated from various clinical specimens (pus, urine, wound swabs, sputum,ear swabs, body fluids, throat swabs, catheter tip, nasal swabs, blood) received in the Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry, during the period of April 2014 to December 2017 were included in this study.

Specimen collection:

The specimens were collected in sterile containers following aseptic measures and transported to the laboratory without delay and processed immediately. All the specimens were inoculated on 5% sheep blood agar and Mac Conkey's agar and plates were incubated aerobically at 37°C.

Identification of *Staphyloccus aureus* :

Colony morphology, Gram reaction, pigment production, catalase, and mannitol fermentation tests were performed and allocated to appropriate genera to the isolates. Golden yellow colored colonies on nutrient agar were noted and slide coagulase and tube coagulase was performed for differentiation of *S.aureus*.[11]

Identification of Methicillin-resistant *Staphylococcus aureus* by Cefoxitin disk diffusion method*:*

The MRSA strains were identified by using Cefoxitin (30µg) disc on Muller Hinton agar (Hi-Media Laboratories, Mumbai).Zone size was interpreted according to CLSI criteria, susceptible, ≥22 mm; resistant, ≤21 mm .[12]

Identification of MRSA by Oxacillin broth dilution method*:*

MICs of oxacillin for all isolates showing reduced zone of inhibition by cefoxitin didk diffusion were determined by broth microdilution method as described by Raghabendra Adhikari et al[13] and CLSI guidelines[14]. The concentrations of oxacillin used were 0.0125 μg/mL to 128 μg/mL.

Antibiotic susceptibility.

Antibiotic sensitivity was tested using the Kirby-Bauer disc diffusion method (1966) on Mueller-Hinton agar according to antibiotic Clinical and Laboratory Standards Insititute disc susceptibility testing guidelines (2013).[14] Bacterial suspension equivalent to 0.5 McFarland was prepared by mixing 3-5 well isolated colonies in 3-4 ml of sterile physiological saline. Each suspension was inoculated on Muller Hinton agar (Hi-Media Laboratories, Mumbai) using sterile cotton swab and antibiotic disks were applied and incubated aerobically at 37°C. Antibiogram was determined for the following antibiotics Teicoplanin (30µg), Vancomycin (30µg), Gentamicin (50µg), Amikacin (30µg), Linezolid (30µg), Ciprofloxacin (10µg), Cefotaxime (30µg), Clindamycin (2µg), Co-trimaxazole (25µg), Erythromycin (15µg), Penicillin (10units), Tetracycline (30µg) (Hi-Media Laboratories, Mumbai).

RESULTS

Out of 568 clinical isolates, 321 (56.5%) patients were from male and 247 (43.4%) were female. Majority of the patients were of age 41-50 (17.2%) as shown in **(Table- 1 and Table- 2).** Distributions of various clinical specimens were given in the (**Table- 3).**

Of the 568 *Staphyloccus aureus* recovered, 366(64.4%) exhibited resistance by Cefoxitin disk diffusion method and 361(63.5%) were found to be MRSA by broth microdilution method and the cutoff value is 4µg/ml. Among them 127 had high level resistance with MIC of >128µg/ml. The MIC of Oxacillin for ***S.*** *aureus* **isolates ranged from 0.064** µg/ml to 256 µg/ml.

Staphyloccus aureus was susceptible to vancomycin (94.7%), followed by teicoplanin (91.7%), linezolid (88.5%), amikacin(85.7%).

In case of MRSA all the isolates were 100% susceptible to vancomycin followed by teicoplanin (97.5%), amikacin (84.7%) linezolid (80.3%) and clindamycin (54.8%).

Table- 1: Distribution of *Staphyloccus aureus* according to the Age (n=568).

Characteristics	No.of isolates	Percentage
Age(in years):		
1 -10	37	6.5%
11-20	69	12.1%
21-30	81	14.2%
31-40	98	15.3%
41-50	102	17.2%
51-60	87	15.3%
61-70	72	12.6%
71-80	19	3.3%
81-90	3	0.52%

Table-2: Gender wise distribution of specimens: (n=568).

Gender	No. of isolates	Percentage
Male	**321**	**56.5%**
Female	**247**	**43.4%**

Table-3: Distribution of *Staphyloccus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) from various clinical specimens:

Clinical specimens	No. of *S aureus* (n=568).	No. of MRSA (n=361).	Percentage of MRSA (63.5%)
Sputum	174	113	64.9%
Urine	56	39	69.6%
Body fluids	19	11	57.8%
Wound swabs	150	92	61.3%
Nasal swabs	9	4	44.4%
Throat swabs	7	4	57.1%
Catheter tip	13	6	46.1%
Pus	94	68	72.3%
Ear swabs	5	3	60%
Blood	41	21	51.2%

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Table-4: Antibiotic suscepyibility pattern of *Staphylococcus aureus* and Methicillin - resistant *Staphylococcus aureus.*

	Staphylococcus aureus (n=568)		MRSA by Oxacillin broth microdilution. (n=361).	
Antibiotic	**suscepyibility**	**Percentage%**	**Suscepyibility**	**Percentage%**
Vancomycin (30µg)	538	94.7**%**	361	100**%**
Teicoplanin (30µg)	521	91.7**%**	352	97.5**%**
Gentamycin(50µg)	421	74.1**%**	152	42.1**%**
Amikacin(30µg)	487	85.7%	306	84.7**%**
Linezolid(30µg)	503	88.5**%**	290	80.3**%**
Ciprofloxacin(10µg)	326	57.3**%**	152	42.1**%**
Cefotaxime(30**µg)**	321	56.5**%**	142	39.3**%**
Clindamycin(2µg)	259	45.5**%**	198	54.8**%**
Co-trimaxazole(25µg)	301	52.9**%**	177	49**%**
Erythromycin(15µg)	261	45.9**%**	141	39**%**
Penicillin(10 units)	162	28.5**%**	0	0**%**
Tetracycline(30µg)	173	30.4**%**	48	13.2**%**

DISCUSSION

Staphylococcus aureus is one of the most infectious agents with high prevalence in various communities and healthcare institutions. MRSA is recognized as a major cause of nosocomial infections which result in significant morbidity and mortality rates. It is a very common cause of infection in hospitals and is most liable to infect new born babies, surgical patients, old and malnourished persons and patients with diabetes and other chronic diseases. The important reservoirs of MRSA in hospitals are infected or colonised patients and transient carriage and hands of health care workers is the predominant mode for patient to patient transmission.[15]

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In our study prevalence of MRSA was found to be (63.5%),similarly such percentages have previously reported in studies from verma etal (80.89%)[16],Suresh Jaiswal et al (72%),[17] Dr.S.Kulkarni et al (70.3%),[18] Tiwari et al (69.1%),[6] Joshi.S et al [19] and Ali et al (68%),[20] Yadhav et al (66.84%),[21] Shilp Arora et al(59.3%),[24] Chandrasekhar et al (56.7%),[23] Anupurba et al (54.8%),[22] Rijal.K et al(51.6%).[2]

MRSA strains were predominantly isolated from pus samples (72.3%) which correlates with Yadav et al (86%),[21] Dr.S.Kulakarni et al (64.67%),[18] Bandaru S Rao et al (64.38%),[23] in contrast Ankur kumar et al [27] and Bilal Ahmed Mir et al [28]reported (21.42%) and (27.5%).

Staphyloccus aureus was susceptible to Vancomycin (94.7%), followed by Teicoplanin (91.7%), Linezolid (88.5%), Amikacin (85.7%).All the MRSA isolates were 100% sensitive to Vancomycin in the present study. The sensitivity followed by Teicoplanin (97.5%), Amikacin (84.7%), Linezolid (80.3%) and Clindamycin (54.8%) correlates with Bhatt CP et al [29], Gitau et al [30], Ankur Kumar et al.[27] and Bandaru S Rao et al.[26]

CONCLUSION

MRSA is emerging as a potential threat to our hospitals with a predilection for critically ill patients. Regular surveillance of hospital acquired infections, promotion of infection control precautions and formulation of definite antibiotic policy can be helpful in preventing MRSA infections from acquiring an alarming proportion. Good basic hygiene measures are extremely important, not only for hospital staff but also for patients and visitors, and it has a positive impact in infection control.

Ethical Clearance: Taken from Sri Lakshminarayana Institute of Medical Sciences,Pondicherry. InstitutionalEthicscommittee(HumanStudies)Ref. No.IEC/C-P/50/2014.

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REFERENCES

1. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med .1998; 339: 520-532.
2. Ogston A .Micrococcus Poisoning. J Anat Physiol.1882;17: 24-58.
3. Nsofor CA, Ohale CU, Nnamchi CI. Distribution and Antibiotics Susceptibility Pattern of *Staphylococcus aureus* Isolates from Health Care Workers in Owerri, Nigeria. Scholarly Journal of Biological Science. 2015; 4(4):29–32.
4. Onanuga A, Awhowho GO. Antimicrobial resistance of *Staphylococcus aureus* strains from patients with urinary tract infections in Yenagoa, Nigeria. J Pharm Bio allied Sci. 2012; 4(3):226–230.
5. Akerele J, Akonkhai I, Isah A. Urinary pathogens and antimicrobial susceptibility: A retrospective study of private diagnostic laboratories in Benin City, Nigeria. J Med Lab Sci. 2000; 9:47–52.
6. Tiwari HK, et al., Methicillin resistant *Staphylococcus aureus*: prevalence and antibiogram in a tertiary care hospital in western Nepal. The Journal of Infection in Developing Countries. 2009; 3(9):681-684.
7. Priya Datta,et al., Evaluation of various methods for the detection of Methicillin-resistant *Staphylococcus aureus* strains and susceptibility patterns. Journal of Medical Microbiology (2011);60, 1613-1616.
8. Chambers HF.The changing epidemiology of *Staphylococcus aureus*?Emerg Infect Dis 2001;7:178-182.
9. Cosgrove. et al.,The impact of methicillin resistancein Staphylococcus aureusbacteremia on patient outcomes:mortality, length of stay and hospital charges. Infect Control Hosp Epidemiol 2005; 26: 166-174.
10. Apple baum PC. Reduced glycopeptide susceptibility in Methicillin- resistant *Staphylococcus aureus* (MRSA).Int J Antimicrob Agents 2007; 30: 398-408.
11. Bailey & Scott's, Diagnostic Microbiology, 20th edition, chapter 13, 222.
12. Bauer, A. W., Kirby, W.M. M. and Sherris, J. C. Antibiotic susceptibility testing by a single disc method. AM. J. Pathol.,1966; 45: 493-496.
13. Adhikari R, et al. Detection of Methicillin Resistant *Staphylococcus aureus* and Determination of Minimum Inhibitory Concentration of Vancomycin for *Staphylococcus aureus* Isolated from Pus/Wound Swab Samples of the Patients

Attending a Tertiary Care Hospital in Kathmandu, Nepal. Can J Infect Dis Med Microbiol. 2017;2017:2191532.

14. Clinical and Laboratory Standards Insititute, Performance Standards for Antimicrobial Susceptibility testing; 22 nd information supplement, M100-S22 Wayne, PA; 2013.

15 Tuo P, et al. Nosocomial *Staphylococci* in a neonatal and pediatric intensive care unit. Pediatr Med Chir. 1995;17:117-122.

16. Verma S et al,Growing problem of methicillin resistant *staphylococci*:Indian scenario. Indian Jornal of Medical Sciceces 2000;54:535-540.

17. Jaiswal, S. et al.,A Study of Methicillin Resistant Pattern on Clinical Isolates of *Staphylococcus aureus* in Tertiary Care Hospitals of Pokhara.BMR Microbiology,2016; 2(1);1-8

18. Dr.S.Kulkarni, Dr A. Khare, Ms Dardi CharanKaur. Prevalence of methicillin resistant *staphylococcus aureus*- A study in a tertiary care rural hospital Department of Microbiology, MIMER Medical College, Talegaon (Dabhade), Tal- Maval, Dist. Pune (MS), India- 410507. Indian Journal of Basic and Applied Medical Research; June 2014; 3(3):414-421.

19. Joshi S, Ray P, Manchanda V, Bajaj J, Gauatm V, Goswami P, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in India: prevalence and susceptibility pattern. Indian J Med Res 2013; 137(2):363–369.

20. Ali AM, Abbasi SA, Arif S, Mirza IA. Nosocomial infectionsdue to methicillin-resistant *Staphylococcus aureus* in hospitalized patients. Pak J Med Sci 2007; 23: 593-596.

21. yadhav et al .Prevalence and antibiogram of methicillin resistant *staphylococcus aureus* isolates at a tertiary care hospital in bangalore, south india. International Journal of Current Research and Review. 2014; 6(17): 37- 40.

22. Anupurba S etal, prevalence of methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital in eastern Uttar Pradesh.Ind J. Med. Micobiology.,2003;56:138-140.

23. Chandrashekar D K et al. Prevalence of Methicillin Resistant *Staphylococcus aureus* in a tertiary care hospital in Gulbarga, Karnataka, J. of Pharmaceutical and Biomedical Sciences. June 2012,19(19):1-3.

24. Shilpa Arora, Pushpa Devi, Usha Arora, Bimla Devi. Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital in Northern India. J Lab Physicians. Jul-dec 2010;2(2):78-81.

25. Rijal.k. et al.,Prevalence of Methicillin resistant *staphylococcus aureus* in school children of pokhara.Nepal Med col J,2008;10(3):192-195.

26. Bandaru N.rao et al,A preospective study of methicillin resistant *staphylococcus aureus*. Journal of pharmaceutical and scientific innovation.2012; 1(2):37-40.

27. AnkurKumar etal, Prevalence of Methicillin Resistant *Staphylococcus aureus* in patients admitted in a Tertiary Care Hospital of North India Department of Microbiology,Teerthanker Mahaveer Medical College and Research Centre, Moradabad, U.P., India International Journal of Advance Researchand Innovation. 2015;3(1):113-117.

28. Bilal ahmad mir, Srikanth. prevalence and antimicrobial susceptibility of methicillin resistant *staphylococcus aureus* and coagulase-negative staphylococci in a tertiary care hospital. Asian journal of clinical research 2013; 6(13):231-234.

29. Bhatt, CP. Antibiotic susceptibility pattern of staphylococcus aureus and methicillin-resistant *staphylococcus aureus* in a tertiary care hospital. Journal of Pathology of Nepal, 2014; 4(7): 548-551.

30. Gitau etal. Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates from clinical specimens at Kenyatta National Hospital Gitau et al.BMC ResNotes.2018; 11:226-231.

Effect of Retrowalking, a Non-Pharmacological Treatment on Pain, Disability, Balance and Gait in Knee Osteoarthritis: A Randomized Controlled Trial

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ABSTRACT

Knee osteoarthritis is a common condition in various orthopedic clinics and hospitals. Degenerative changes occur in the cartilage covering the articular surfaces of the joints leading to pain, crepitus, stiffness, limitation of movement and deformity. The purpose of the study was to examine the effect of treadmill retrowalking in patients suffering from knee osteoarthritis. Patients with age more than 40 years diagnosed with knee osteoarthritis by an orthopedician referred to or visiting the O.P.D, Department of Physiotherapy, Guru Jambheshwar University of Science & Technology, Hisar were assessed. Forty two eligible patients were randomly allocated to conventional group and retrowalking group. The conventional group received range of motion exercises, strengthening exercises, stretching and flexibility exercises. The patients in the retrowalking group underwent treadmill retrowalking in addition to conventional exercises. Both the interventions were given thrice weekly for six weeks. The data analysis was done using SPSS 16.0. Independent and dependent t-test was used to compare between and within group differences. Comparison of mean scores of conventional and retrowalking group showed statistically significant differences for all the variables. The study concludes that retrowalking when given along conventional exercise program alleviates pain, decreases disability, enhances balance and gait performance by improving step and stride length, increases cadence and walking speed in knee osteoarthritis patients.

Keywords – *retrowalking, knee osteoarthritis, conventional exercises, pain, disability, balance, gait.*

INTRODUCTION

Knee osteoarthritis (OA) is a progressive disease commonly encountered in the out-patient departments (O.P.D) of various orthopedic clinics and hospitals. The increasing prevalence of knee OA worldwide is suggestive of a physically crippled society [1]. Degenerative changes occur in the cartilage covering the articular surfaces of the joints leading to pain, crepitus, stiffness, limitation of movement and deformity which adds to the increase in knee replacements [2].The prevalence of knee osteoarthritis in India is 28.7% [3].

Patients with knee osteoarthritis modify their gait in response to pain. Such patients walk with shorter step and stride length, low cadence and have slower walking speed. These changes, which are possible unloading mechanisms, begin in early stages of medial knee osteoarthritis as identified by computerized gait analysis using spatiotemporal parameters [4-6].

Exercises are commonly employed in the management of knee osteoarthritis as they alleviate pain and physical disability in osteoarthritis patients without adverse effects produced by drugs and surgical procedures [7, 8].

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Retrowalking or backward walking is used nowadays in the management of many orthopedic, neurological and sports conditions [9-12]. It changes muscle activation pattern, lowers knee joint compressive forces and improves strength of quadriceps muscle. The effects of retrowalking on pain, physical disability, quadriceps strength and balance in knee osteoarthritis have been studied [13-17]. However, none of the studies have investigated the effectiveness of a retrowalking program on spatiotemporal gait parameters in knee osteoarthritis patients. The present study aims to know the effect of retrowalking in combination with a conventional exercise program on pain, disability, balance, step length, stride length, cadence and walking speed in patients suffering from knee osteoarthritis.

MATERIAL AND METHOD

Settings and Study participants

Patients with age more than 40 years diagnosed with knee osteoarthritis by an orthopedician who were referred to or attended the O.P.D, Department of Physiotherapy, Guru Jambheshwar University of Science &Technology, Hisar were assessed. Patients having a history of any inflammatory, infectious, traumatic condition of the knee joint, with any previous surgery or any invasive procedure of knee joint, history of cardiac disease, lower limb injury or pathology, fixed deformity of knee, any skin problems around the knee joint, lacking independent ambulation or requiring use of any walking aid, neurological disorders, patients with severe knee osteoarthritis (grade 4 or those referred for knee replacement surgery) and those unable to comply with study protocol were excluded from the study.

In case of bilateral osteoarthritis, more symptomatic knee joint was considered for the study. Forty two patients fulfilling the eligibility were included in the study. The patients were explained the procedure of the study and gave their written consent.

Intervention groups

The patients were then randomly divided into two groups i.e. conventional exercise group and retrowalking group by computer generated random number tables. The Conventional group received conventional exercise program which consisted of hot packs for 10 minutes followed by exercises [18]. These consisted of range of motion exercises, muscle strengthening exercises in form of isometric and isotonic exercises, muscle stretching exercises and flexibility exercises. The patients in the Retrowalking group followed retrowalking protocol on a treadmill in addition to the conventional exercise program [14]. Prior to the treatment, every patient was made familiar with retrowalking on the treadmill. The intervention began with a session of 3-5 minutes forward walking on the treadmill for warm up followed by 10 minutes of retrowalking on the treadmill at a comfortable speed at 0˚ inclination under the supervision of the therapist. The treadmill was placed within a specially designed metal framework with handrails support for safety purposes. Patients were instructed to report any problem faced during retrowalking. Both interventions were given for three sessions in a week for a total duration of six weeks.

Outcome measures

Pre and post intervention testing was done for all outcomes measures for both groups at two time points i.e. at 0 weeks and after 6 weeks of intervention. Visual Analogue Scale (VAS) was used to measure pain intensity [19], Western Ontario and McMaster Osteoarthritis Index (WOMAC) was used for disability [20] and Timed up and Go Test (TUG) was used to assess balance [21]. The measurement of spatio-temporal gait parameters like step length and stride length was done using foot print method [22]. A ten meter walkway was used for step and stride length measurements. The measurements were taken using a measuring tape. The step length was measured as the distance (in cm) between the heels of two consecutive steps of both feet while the stride length was measured as the distance (in cm) between the heels of two successive steps of the same foot. The walking speed (m/s) was measured as the total distance (10 m) of the walkway divided by the time taken to cover the distance. Cadence was evaluated by counting the number of steps per minute during which the patient was made to walk in a corridor at a normal speed.

STATISTICAL ANALYSIS AND RESULTS

The data was analyzed using SPSS 16.0. Dependent t-test was used for comparing between group differences for all variables such as pain, disability, balance, step length, stride length, cadence and walking speed between conventional group and retrowalking group (Table 2) while Independent-t test was used to compare between group differences (Table 3). The level of significance

was set at $p<0.05$. All the values are presented in mean and standard deviation. The mean age, height and weight of study patients was 52.5±8.75 years, 161.15±9.45 cm and 73.69±.65 kg respectively (Table 1). The conventional group consisted of 8 males and 13 females. The retrowalking group included 12 males and 9 females. Pre and post comparison of study variables show significant improvement in both groups after intervention (Table 2).Comparison of mean scores of conventional and retro-walking group show statistically significant differences for all the variables thus showing better results in case of retrowalking group (Table 3).

Table1. Basic demographic data of patients

Characteristics	**Conventional Group (n= 21)**	**Retrowalking Group (n=21)**
Age(years)	54.3±10.2	50.7±7.3
Height(cm)	159.6±8.9	162.7±10.0
Weight(kg)	71.5±8.9	75.7±10.4
BMI(Kg/m^2)	28.1±8.9	28.6±10.2
Sex(M/F)	8/13	12/9

Table 2. Comparison of study variables at pre and post intervention for conventional and retrowalking group.

Variables	**Group**	**0 week(pre)**	**6 week(post)**	**t value**	**p value**
Pain (VAS)	Conventional	7.92±0.98	5.20±1.17	13.866	≤0.001
	Retrowalking	7.82±1.08	2.85±0.88	30.040	≤0.001
Physical disability(WOMAC)	Conventional	51.69±7.32	31.79±6.10	26.599	≤0.001
	Retrowalking	46.84±6.44	20.79±4.20	20.934	≤0.001
Balance (TUG)	Conventional	10.78±0.72	10.36±0.79	8.800	≤0.001
	Retrowalking	10.24±1.36	9.22±1.06	6.353	≤0.001
Step Length(cm)	Conventional	54.86±5.71	55.09±5.96	-2.500	≤0.05
	Retrowalking	55.52± 4.38	56.90± 4.59	-7.319	≤0.001
Stride length(cm)	Conventional	105.52±11.9	105.9±12.1	-2.961	≤0.001
	Retrowalking	107.9± 8.23	110.4 ± 8.64	-8.971	≤0.001
Cadence (steps/min)	Conventional	101.10±4.41	103.0±4.32	-7.684	≤0.001
	Retrowalking	102.8±5.14	107.4±4.93	-17.593	≤0.001
Walking Speed(m/s)	Conventional	0.87±0.10	0.96±0.09	-4.590	≤0.001
	Retrowalking	0.93±0.12	1.08±0.14	-6.323	≤0.001

Table3. Comparison of mean scores of variables between conventional and retrowalking group

Variables	Conventional group (n=21)	Retrowalking group (n=21)	t value	p-value
Pain(VAS)	2.71± 0.89	4.97± 0.75	- 8.83	≤ 0.001
Physical disability (WOMAC)	19.89 ± 3.42	26.05 ± 5.70	- 4.24	≤ 0.001
Balance (TUG)	0.42 ± 0.22	1.03 ± 0.70	- 3.83	≤ 0.001
Step length(cm)	0.24 ± 0.44	1.38 ± 0.86	- 5.41	≤ 0.001
Stride length(cm)	0.38 ± 0.59	2.52 ± 1.29	- 6.93	≤ 0.001
Cadence(steps/min)	1.90 ± 1.14	4.62 ± 1.20	- 7.52	≤ 0.001
Walking speed(m/s)	0.09 ± 0.08	0.16 ± 0.09	- 2.57	≤ 0.05

DISCUSSION

Pain and disability were significantly reduced post intervention in both groups with a greater decline in the retrowalking group. The findings are in line with the former studies that have confirmed the benefits of retrowalking on pain and physical functions in knee osteoarthritis [13-14, 17]. Isometrics, range of motion and stretching exercises lead to an overall rise in the strength of the lower limb musculature thus reducing pain and disability [23-24]. Backward walking is a closed chain exercise capable of causing a decrease in the patella-femoral compression forces, reduced force absorption at knee, increase in quadriceps strength and reduced eccentric patellar tendon loading [25-29]. Thus, it possesses greater functional benefits which could be the possible reason for pain and disability reduction in the retrowalking group.

Balance was improved in both the groups after six weeks of intervention but a greater improvement was seen in retrowalking patients. The findings of the present study are consistent with conclusions of many previous researches. The integration of the sensory system, the motor system and the musculoskeletal system is related with improved balance after backward walking [10]. Retrowalking causes an increase in the knee extensor strength, alters the pattern of muscle activation, decreases compressive forces and thus pain in the knee joint, and improves motor control ability leading to an improvement in balance [10,12,16,30-32].

Greater improvement was evident in the retrowalking group for all gait parameters. This is the first study to evaluate the effects of retrowalking on gait parameters such as step length, stride length, cadence and walking speed in patients with knee osteoarthritis. The beneficial effects of backward walking on gait parameters have been investigated in stroke and cerebral palsy which have been attributed to reversal of temporal cycling of the muscle contractions and an improvement in balance as well as motor function of the lower limbs leading to improvised gait performance [11, 32].

A study concluded that backward treadmill walking was capable of achieving sufficient stimulus to augment hamstring flexibility [9].This reason may have increased the step length and stride length in knee osteoarthritis patients. Antero-posterior balance, step length and velocity in healthy adults were improved after backward walking in another study due to greater stimulation of lower limb muscles, greater activity of quadriceps muscle and high energy consumption [33]. A rectification in the abnormal gait after retrowalking in the present study enhanced the gait performance.

CONCLUSION

Retrowalking given along conventional exercise program for six weeks decreases pain, disability, improves balance, step length, stride length, cadence and walking speed in knee osteoarthritis. Although present investigation involved local patients who participated in study, a larger cohort study involving more number of patients of different races and diverse stages of osteoarthritis are required. Also, future studies could be done using gait laboratory for more accurate interpretation of results. The experimental outcomes of this study will be helpful for physicians and physiotherapists in deciding the exercise protocols

to achieve better results in patients suffering from knee osteoarthritis.

Conflict of Interest- None

Source of Funding- Self

Ethical Clearance- The ethical permission to conduct the study was acquired from the Institutional Ethical Committee (vide letter no. PTY/2015/800 dated 03/11/2015).

REFERENCES

1. Heidari B. Knee Osteoporosis prevalence, risk factors, pathogenesis and features: Part-I Caspian J Intern Med. 2011; 2(2):205-212.
2. Van Manen MD, Nace J, Mont MA. Management of Primary Knee Osteoarthritis and Indications for Total Knee Arthroplasty for General Practitioners. J Am Osteopath Assoc 2012; 112(11):709–715.
3. Pal CP, Singh P, Chaturvedi S, Pruthi KK, Vij A. Epidemiology of knee osteoarthritis in India and related factors. Indian Journal of Orthopaedics. 2016; 50(5):518-522. doi:10.4103/0019-5413.189608.
4. Haydor G.,Ergin S, Yavuzer G. Kinetic and kinematic characteristics of gait in patients with medial knee arthrosis. Acta Orthop Scand 2002; 73(6): 647-652.
5. Bejek Z, ParoczaiR, Illyes A, Kocsis L, Kiss RM. Gait parameters of patients with osteoarthritis of the knee joint. Physical Education and Sport 2006; 4:9-16.
6. Childs JD, Sparto PJ, Fitzgerald GK, Bizzini M, Irrgang JJ. Alterations in lower extremity movement and muscle activation patterns in individuals with knee osteoarthritis. Clin Biomech (Bristol, Avon) 2004; 19:44–9. [PubMed]
7. Page CJ, Hinman RS, Bennell KL. Physiotherapy management of knee osteoarthritis. International Journal of Rheumatic Diseases 2011; 14:145-151.
8. Fransen M, McConnell S, Harmer AR et al. Exercise for osteoarthritis of the knee: a Cochrane systematic review. Br J Sports Med. 2015; 49(24):1554-7.
9. Whitley CR and Dufek JS. Effects of backward walking on hamstring flexibility and
10. low back range of motion. Int. J. Exerc. Sci. 2011;4:192-198.
11. Hao WY and Chen Y. Backward training improves balance in school-aged boys. Sports
12. Med. Arthrosc. Rehabil. Ther. Technol. 2011; 3:24.
13. Yang YR, Yen JG, Wang RY et al. Gait outcomes after additional backward walking training in patients with stroke: a randomized controlled trial. Clin. Rehabil.2005; 19:264-273.
14. Shankar P, Bhandiwad RM, Pai H. Effectiveness of retrowalking in chronic osteoarthritis of knee joint. Innovative Journal of Medical and Health Science. 2013; 3(1):19-22.
15. Gondhalekar GA, Deo MV. Retrowalking as an adjunct to conventional treatment versus conventional treatment alone on pain and disability in patients with acute exacerbation of chronic knee osteoarthritis: A randomized clinical trial. N Am J Med Sci. 2013; 5(2):108-12.
16. Anadkat H, Ajith, Kumar D. Effectiveness of retrowalking treadmill training on pain and disability in knee osteoarthritis: a randomized controlled trail. Int J Pharm Bio Sci. 2015;6(4):43-50.
17. Joshi S, Vij JS, Singh SK. Retrowalking: A new concept in physiotherapy and rehabilitation. International Journal of Scientific Research. 2015; 4(10): 5-9.
18. Jagga V. Effect of retrowalking on dynamic balance and mobility in patients with knee osteoarthritis. International Educational Scientific Research Journal. 2016; 2(7): 10-11.
19. Rathi M, Palekar T, Varghese A. Effecacy of backward walking on patient with osteoarthritis of knee on quadriceps strength, pain and physical functions. Indian Journal of Physiotherapy and Occupational Therapy.2014; 8(4):192-196.
20. Durmus D, Alayli G, Bayrak IK, Canturk F. Assessment of the effect of glucosamine sulphate and exercise on knee cartilage using magnetic resonance imaging in patients with knee osteoarthritis: A randomized controlled trial. Journal of Back and Musculoskeletal Rehabilitation. 2012; 25:275-284.
21. Olaogun MO, Adedoyin RA, Anifaloba RO. Reliability and concurrent validity of visual analogue scale and modified verbal rating scale of pain assessment in adult patients with knee osteoarthritis in Nigeria. South African Journal of

Physiotherapy. 2003; 59(2):12-5.

22. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: A health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee.J Rheumatology.1998; 15:1833-1840.
23. Dobson F. Timed Up and Go test in musculoskeletal conditions. Journal of Physiotherapy. 2015; 61(1):47.
24. Kaur S, Singh V. Quantitative Gait Analysis of Healthy Adults Using Foot Print Method and Win Track. Journal of Nursing and Health Science. 2014; 3(3):16-21.
25. Diracoglu D, Aydin R, Baskent A, Celik A. Effects of kinesthesia and balance exercises in knee osteoarthritis. JCR: Journal of Clinical Rheumatology. 2005; 11(6):303-10.
26. Anwer S, Alghadir A. Effect of isometric quadriceps exercise on muscle strength, pain, and function in patients with knee osteoarthritis: a randomized controlled study. Journal of Physical Therapy Science. 2014; 26(5):745-8.
27. Flynn TW, Soutas- Little RW. Mechanical power and muscle action during forward and backward running. J Orthop Sports Phys Ther. 1993; 17: 108-112.
28. Chen LY, Su FC, Chiang PY. Kinematic and EMG analysis of backward walking on treadmill. Proceedings of the 22nd Annual EMBS International Conference, July 23-28, 2000, Chicago IL.
29. Cipriani DJ, Armstrong CW, Gaul S. Backward walking at three levels of treadmill inclination: an electromyographic and kinematic analysis. J. Orthop. Sports Phys. Ther. 1995; 22:95-102.
30. Flynn TW, Soutas- Little RW. Patellofemoral joint compressive forces in forward and backward running. J. Orthop. Sports PhysTher. 1995; 21:277-282.
31. Lee M, Kim J,Son J,Kim Y. Kinematic and kinetic analysis during forward and backward walking. Gait & Posture.2013; 38: 674-678.
32. Bal BS, Dureja G, Kaur P. An empirical study of backward walking treadmill training on static and dynamic balance in adolescent girls. Medicina Sportiva. 2012; VIII (1): 1743-1749.
33. Khalaj N, Osman NA, Mokhtar AH, Mehdikhani M, Abas WA. Balance and risk of fall in individuals with bilateral mild and moderate knee osteoarthritis. PloS One. 2014; 18;9(3):e92270.
34. Kim SG, Ryu YU, Je HD, Jeong JH, Kim HD. Backward walking treadmill therapy can improve walking ability in children with spastic cerebral palsy: a pilot study. International Journal of Rehabilitation Research. 2013;36:246-52.
35. Cha H-G, Kim T-H, Kim M-K. Therapeutic efficacy of walking backward and forward on a slope in normal adults. Journal of Physical Therapy Science. 2016; 28(6):1901-1903. doi:10.1589/jpts.28.1901.

To Live or to Leave? – The Ethical Factors Influencing the Parsi Community's Health

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ABSTRACT

A healthy living and a healthy community complement one another. A healthy living can undoubtedly ensure the health of a community. A community's health is strongly affected and influenced by its members, their individual health and habits, morals and principles, their perspectives towards life in general and particular, the social capital, the immediate environment and of course the religious and spiritual bent of mind of its people. The deterioration of the health of a community may result in significant changes in its population rate. It is thus very imperative that such an important issue needs to be addressed. The unique Parsi community though an essential part of India's multi-cultural fabric and diversity, is perhaps the only minority outside Europe to have the combination of a higher proportion of older inhabitants and a lower fertility rate. This holds the possibility of a total ethnic extinction of this minority despite having the potential to contribute substantially towards the overall growth of the country. But no matter how alienated as a minority it feels, it must always be protected at any cost. The present paper makes a humble attempt to bring to community health improvement of the Parsi people an approach that emphasizes on identifying various factors held responsible for the rapid decline of the Parsi population. Such an approach would facilitate in protecting and subsequently intensifying the growth of the community.

Keywords: *Parsi community, Minority, Health, Declining population, Survival.*

INTRODUCTION

A community's health is greatly affected and influenced by its members, their personal health and habits, their ethics, perspectives towards life, their social capital, the immediate environment and of course their religiosity and spirituality. The small yet unique Parsi community is no exception. The community which had fled to India from Iran some 1200 years ago to escape religious persecution by the fanatical Muslim invaders could stand independent on the Indian soil on the execution of some terms and conditions: to adopt the local Gujarati language as well as the local dress code; to hand-over their weapons and embrace peace; to venerate cows; to not to spread their religion outside their community; and to perform their marriages in the dark as the Hindus do. A community which is well known for its uphill struggle, an astonishingly high presentation among the upper echelons of our society, for its honesty, integrity and fidelity, the Parsi emigrants had proved to be immensely loyal towards keeping their oath intact in all situations. This had in many ways prevented their complete assimilation into the Indian society and ultimately handicapped the growth of the community in terms of demography, which has become the most alarming issue of the community.

However, the community becomes the victim of its own success. Despite an illustrious past and an enduring success, their numbers are declining so fast that their survival in the next century has become a question of debate. According to the 2001 census, their numbers have fallen off from 115,000 in 1941 to 69,601 in 2001, out of which around 40,000 are scattered across the globe. The numbers are believed to have declined further since then. It has been declining by 12% every census decade. The consistent sliding represents a tragic loss of cultural diversity for the Parsis. The community, though an essential part of India's cultural fabric and diversity, is perhaps the only community outside Europe to have experienced a population and fertility decline. *The four minorities constitute 5 per cent of the country's total population. Of this, the Christians account for 2.3 per cent, Sikhs for 1.9 per cent, Buddhists for 0.8 per cent,*

while the Parsis stand at .007 per cent.[1] *This has become o*ne of the most alarming issues for the community. The community with its dwindling population has already entered the final stage of demographic transition. Late marriage, no marriage, low birth rate with a large number of aging population, fecundity, foetal loss, neonatal and infant mortality, urban medical problems like cancer, neurological disorders, leukemia, Alzheimer's, the desire to keep the bloodline 'pure' because of the 'No Conversion' commitment and of course an accelerated migration to the west often contribute to the drastic reduction in the population of the community.

Surveys show that late marriages and voluntary or involuntary childlessness are some of the prominent factors for the decline of population in the community. Deaths have consistently outstripped population replacement rate since the 1950s, possibly due to medical and socio-cultural reasons. Further, the percentage of elderly population in the total population is as high as 31.0 per cent, as per the 2001 Census (Paul). For every 4 deaths in the community only one child is born, which is quite low as a birth rate. Deaths have exceeded births in every year since 1955. While the ageing was at 23 per cent compared to the national average of 7.7 per cent the age group of children below 15 years was 10 to 12.5 per cent as compared to 33 per cent the ageing rate was rapid even compared to most advanced countries where Canada has 9.5 per cent, USA 11.5 per cent and 15.1 per cent in United Kingdom. Moreover, the replacement level of ageing population with the younger persons was merely 1.1 per cent.[2]

Another vital factor that leads to an alarming deterioration of the Parsi population is its socio-cultural aspect. As the community gets highly urbanized and polished in course of time, women become more conscious of their future and prefer higher education and a successful career to marrying at an early age. This, however, results in rising problems over the availability of brides to marry. A large numbers of women, who never get married or choose late marriage, have been identified as potential issues contributing to the reduced fertility, thus impacting the growth of the population. The average age at marriage in 1962 was 26.5 years for women and 31.4 years for men, and has hovered at about this level ever since (Patel 2010). This is the community which has unfortunately the highest number of bachelors and spinsters in the world. According to a research conducted in the year 1973, it has been proved that women among aged 31-45 years, 27% had never married.[3]

Researches prove that the total fertility rate (TFR) of the Parsis has been waning off for over a century. It has dipped below the viable rates. This is not because the Parsis are biologically infertile but because of their individual choice. Only one out of nine Parsi families has a child under the age of 10 years. Even couples who can have babies do not want to have because of other priorities. When in 1881, the total fertility rate among the Parsis in Bombay was 4.41 (Visaria, 1974), it comes down to 0.94 in 1999 (Unisa et al. 2008). 1961-70 was the first period when the TFR was found to be below replacement level (Visaria 1974).[4] Between 2005 and 2014, only 43 births were recorded, though there were 176 deaths. The first 50 days of 2015 recorded two deaths and no birth.[5] This is an outcome of the fact that more than the average Parsi women either do not get married or choose to marry between 35-40 years at which stage, a woman generally loss her important fertility years and find it difficult to conceive. Couples, who decide upon a baby, go for a single child (0.8) throughout their life time to avoid family obligations in their professional front. Apart, more than average couples have the responsibility to look after five or more elderly persons in the family. That could also be listed as one of the important reasons of not planning for a second baby. Aditi Kapoor in her article 'The Parsis; Fire on Ice', writes in the following manner "unless something is done to augment their fast depleting numbers and to revive their religion, the Parsis after an illustrious past could well just fade out in oblivion".[6]

On one side, no marriage and late marriage have become trends among the community members, while on the other side marriage outside of the community is frowned upon. The community fears getting merged with other dominant cultures. They believe that if their ethnic identity is lost, that may raise questions against their value system eventually leading to the collapse of a brand image. A pure Parsi blood is the key to protect the Zoroastrian root is what the Parsis have believed right from the beginning and they remain stick to this in order to avoid community crisis. Therefore, children born out of mix marriages are not considered to be Parsi as they are not allowed to enter the fire temple, neither are they allowed to perform their *navjyot* ceremony. As a result the community does not get any chance to escalate its number. As a homogeneous community, intra-family

marriages between cousins, uncles and nieces have become the demand of the community. Based on the genealogical database of the Parsi population, it has been studied that the Zoroastrians' breeding among themselves has led to an increased genetic traits often resulting in genetic diseases and disorders, longevity and reduced fertility.

As Parsis are thought to be genetically homogenous, a number of researches have been conducted on the community members to identify genes involved in various diseases like cancer, multiple sclerosis (MS) and neurological movement disorder like Parkinson, to develop new treatments and diagnosis for the same. It has been identified by a study conducted of by the Mumbai Cancer Registry that the community is particularly venerable to endometrium, lymphomas and leukemia, thyroid, pancreas, bladder, corpuis uteri, and prostate cancer, which is twice as susceptible as compared to the non-Parsis. The research was conducted by distributing questionnaire among a large group of Parsis regarding their life styles. It found that factors like literacy rate, socio-economic condition, living habits and westernized dietary are particularly contributing to the problem. Apart, the role of an increasingly limited pool also occupies an important role in accelerating the process of extinction in the community.

According to the Deputy Director, Mumbai Cancer Registry, the Parsi women are more prone to breast cancer than the non-Parsis. The affliction is present 1.6 times higher than that of the other communities.[7] In fact, it is the second commonest disease seen among the female Parsis of Bombay. Infrequent breast feeding, fecundity which leads to inbreeding are sighted as some of the major causes of breast cancer among the Parsi women. Apart, cancers of the cervix, corpus uteri, ovarian cancer, cancer of the fallopian tube are also quite frequently diagnosed among the Parsi females.[8] A review of literature on cancer risk among the Parsis in Bombay presents a direct estimation of the true level of cancer risk in this community as compared to the total population of Bombay. Surveys show that the Parsis rank second in broad ligament, ovarian, and fallopian tube cancer (Bombay Cancer Registry). As far as the Parsi males are concerned, they are more prone to prostate cancer. Age- specific rates illustrate that the prostatic lesions start occurring between the ages of 40- 50. However, the highest occurrence is found between 65-69 (Bombay Cancer Registry).

As a highly prosperous, philanthropic and civilized community with an abundant contribution to the growth of modern India, we perceive that the community members have proved to be so diligent that they ignore their social and biological life. On one hand, they marry late or do not marry at all and restrict themselves to a single child even if they do. On the other hand, the members of the community are playing against these deadly diseases, which have made its survival almost impossible in the next century. Thus, to increase the number in order to save the dwindling community from total extinction in the near future is the need of the hour. In this regard, the government, officials and community leaders have agreed to increase its birth-rate by adopting various possible methods to halt a decline towards extinction.

The following measures have been taken which may add substantial value to the population growth of the community while at the same time they would also check the further decline in the number.

The UNESCO Parsi Zoroastrian project namely Parzor Project has been jointly initiated by the Bombay Panchayat and UNESCO in the year 1999 to prevent population crisis in the community and to promote and preserve the great Zoroastrian culture and legacy. The project also intends to establish a connection among the people of Afghanistan, Uzbekistan, Pakistan, and Iran as these countries are the rooms for many Parsis.[9]

To help the Parsis increase their population and become an influential community the Ministry of minority affairs launched a scheme called Jiyo Parsi in 2013. The scheme is funded by the Government with an objective of creating awareness among the community members for lineage inflation. The scheme is executed in two parts: advocacy and medical assistance for reproduction and financial help for women health. The former works with a purpose to act upon family planning, convincing young Paris to get married soon and have more children at the right age, whereas the later offers financial support to the married couples facing fertility problems.[10] Adopting scientific protocols and structured interventions are what the community members are recommended to do.

The second phase of Jiyo Parsi that came up towards the end of 2017, covers expenses of child care as well as of elderly dependents.

Besides, enlightenment through different magazines, articles, books, counseling, giving a broader perception of the present demographic figure of the community, helps its members to realize their dwindling number, would attract young Parsis to breed more.

Though very rare, Intercommunity marriage has also been encouraged among the community members to boost up the population at any cost.

Couples undergoing infertility treatment prefer artificial insemination and surrogacy to boost up the population.

DISCUSSION AND CONCLUSION OF THE STUDY

In an age of globalization, Community health has become a major area of concern. As healthy living can significantly promote a healthy community, factors affecting the health of a community should always be eluded. Values and ethical factors powerfully influence a community's mental, physical and psychological wellbeing. Being a community that is staring at its extinction, Parsis need to stress their diminishing population so as to realize their existence in the near future and to act upon that. As a unique minority, which has essentially kept its religion unsullied and its core beliefs apart despite powerful influences from other cultures, the community deserves to be protected and preserved at any cost. There is a dire need to identify, introspect and comprehend the existing issues first at the individual level and then it needs to be addressed at the community level in order to strengthen the population and sustain the identity whose demise would be a major loss to world culture.

Conflict of Interest: Nil

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REFERENCES

1. Paul C. Non-Muslim minorities high on socio-economic ladder. Indian Express. 2008 May 3.
2. High ageing rate, health problem worry Parsi community. The Times of India (Ahmadabad Ed.) [Internet]. [2018 Aug 2] 2001 Oct 22: 1. https://timesofindia.indiatimes.com/city/ahmedabad/High-ageing-rate-health-problems-worry-Parsi-community/articleshow/1440137447.cms.
3. C Shroff Z, C Castro M. The Potential impact of intermarriage on the population decline of the Parsis of Mumbai, India. Demographic Research [Internet]. 2011 [cited 2 Aug 2018]; 25; 545-64.
4. Available from: https://www.demographic-research.org/volumes/vol25/17/25-17.pdf
5. Visaria L. Demographic Transition among Parsis: 1881-1991: I: Size of Parsi Population. Economic and Political Weekly [Internet]. [Cited 2018 Aug 8] 1974: 9 (41):1735-41. Available from: https://www.jstor.org/stable/4364076.
6. Sharma A. The curious case of the vanishing Parsis. Hindustan Times [Internet]. [cited 15 July 2018] 2015. Available from: https://www.hindustantimes.com/brunch/the-curious-case-of-the-vanishing-parsis/story-60jjm3jRNXr0RZTqg57zzJ.html
7. Kapoor A. The Parsis: Fire on Ice. Times of India. 1989.
8. Bawa R. Parsis more prone to cancer: study. The Times of India [Mumbai Ed.]. [Cited 14 Apr 2018] 2000. Available from https://timesofindia.indiatimes.com/city/mumbai/Parsis-more-prone-to-cancer-study/articleshow/1458448068.cms
9. Jussawtalla D, Deshpande V, Haenszel W, Natekar M. Differences observed in the site incidence of cancer between the Parsi Community and the total population of Greater Bombay: A Critical Appraisal [Internet]. Bombay Cancer Registry of the Indian Cancer Society, Bombay, India and the Biometry Branch, National Cancer Institute, Bethesda, Maryland, U.S.A; 1970 Jan 2: 56-66. Available from: http://images.biomedsearch.com/5428617/brjcancer 00466-0072.pdf?AWSAccess KeyId=AKIAIBOKHYOL P4MBMRGQ& Expires=1533859200&Signature=y LhA7RpnUQ69td%2BeNVUvjFiEdbQ%3D
10. High Level Debate No 3 on Social networking [Internet]. 2010 May 11. Geneva, Switzerland: UNESCO and WSIS. Available from: http://www.unesco.org/new/http:/
11. Vikram R. The Parsi way of life. The Pioneer [Internet]. [Cited 12 July 2018] 2015. Available from: https://www. dailypioneer.com/sunday-edition/agenda/people/the-parsi-way-of-life.html

A Review on Plasma Glucose: Preventing Mongering of Madhumeha

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ABSTRACT

People believe that the new is better than the old, that the advanced is better than the simple, that more is better than little. How good is plasma glucose screening? Statistical medicine, sold all over as very scientific originates from scandal of poor research says Douglas G Altman, head of the medical statistics, London. Ayurvedic health care providers following International diagnostic criteria of Diabetes Mellitus for diagnosis of *Madhumeha* (Diabetes Mellitus). This was recently proved to be disease mongering and selling sickness. Many studies have consistently demonstrated that history taking and physical examinations are the most important factors in arriving at correct diagnosis, whereas lab tests play only minor role and too often palpably illogical laboratory findings are accepted without question. Ayurvedic physicians should strictly follow classical method of diagnosis of *Madhumeha*. The quotation from a Cardiologist Mimi Guarneri "Beyond the power of most sophisticated medical equipment is a physician's humanity – the listening ear, the healing touch, the devices of healers throughout time", holds good at this juncture

Key words: *Madhumeha; Diabetes Mellitus; plasma glucose; Screening; Disease mongering*

INTRODUCTION

Majority of Ayurvedic physicians, having a great influence of poor research[1] based western medicine gradually are going away from clinical symptoms of diseases in general and *Madhumeha* (Diabetes Mellitus) in particular based on laboratory results. If laboratory reports are abnormal, asymptomatic healthy person is medicalised and drugged or operated upon,[2] irrespective of truth whether he or she is suffering or not. It is unfortunate that such a great science Ayurveda which has existed for "times out of mind" in India is slowly deviating to follow the modern medical system of laboratory diagnosis even in the well segment of the population. Even in the western medicine treatment of asymptomatic hyperglycemia has been shown to be futile, if not dangerous. Even the newly invented disease in western medicine IGT (Impaired glucose Tolerance) has been shown to benefit more by life style changes rather than modern medicine like Rosiglitason in a large study.[3]

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Euboxic Ayurvedic doctors have been neglecting the definition of disease defined in different classics of Ayurveda, as a state in which both the body and mind are subjected to pain and misery.[4-6] Chambers Dictionary also defines disease which is similar to Ayurvedic definition as an unhealthy state of body or mind; a disorder, illness or ailment with distinctive symptoms.[7,8] The laboratory diagnostic criteria of Diabetes Mellitus of western medical science which is flourishing with unscientific practice[9] should not be followed for diagnosis of Madhumeha because, the medical profession is being bought by the pharmaceutical industry, not only in terms of the practice of medicine, but also in terms of teaching and research, says Arnold Relman, a Harvard professor and former editor of the New England Journal of Medicine.[3]

Madhumeha vis-à-vis Diabetes Mellitus

"Prakarshen Prabhutam Prachuram Varam Varam Va Mehati Mutratwagam Karoti iti Prameha" Prameha is characterized by increased quantity of urine associated with or with out the increased frequency of micturition.[10] Poly urea *(Prabhoot Mutratwam)* and turbidity of the urine *(Avila Mutratwam)* are the two cardinal features of this disease.[11] *Prameha* refers to repeated, excessive and turbid urination in terms of frequency, quantity and clarity. The term *"Prameha"* has two parts. *'Pra'* meaning abundant and *'Meha'* meaning 'passing of Urine'. Thus *Prameha* means passing of large quantity of urine. Ayurveda fixed the normal quantity as four *Anjalis* (1600 ml) and puts frequency of at six.[12]

The aggravated *Bahudrava Shleshma* (liquid property of *Kapha dosha*) by its specific etiology initiates the process of manifestation of *Prameha* (poly urea with turbity) because of the *Saithilyata* (lassitude) developed in the body. The *Bahudrva Shleshma* comes in contact with *Rasa* (plasma), *Rakta* (Blood), *Mamsa* (Muscle), *Meda* (Adipose tisuue), *Majja* (Bone marrow), *Shukra* (Semen / Ovum) , *Kleda* (moisture), *Lasika* (lymphatic fluid), *Vasa* (type of fat) and *Ojas* (immunity) and while spreading it first gets mixed with *Medas* (adipose tissue) because there is an increase in the quantity of *Medas* which is also *Asamhata* (uncondensed) and *Aghana* (more liquid) and also because *Kapha* and *Medas* share identical qualities. The specific nature of *Samprapti* (pathophysiology) of *Madhumeha* (Diabetes Mellitus) brings *Sara* (essence of tissues) and *Dravabhaga* (liquid portion) of involved *Dhatus* (tissues) to *Basti* (urinary bladder) leading to *Prabhoot*, *Avila* and *Madhura Mutratwam (sweet urine)*.[13,14] Ayurvedic diagnosis of *Madhumeha* should have this *Samprapti* not mere increase in the plasma glucose.

Diabetes term is derived from Greek word dia and bainein means through and to go respectively and diabetes literally means pass through. Mellitus means sweet. Thus Diabetes mellitus means passing of sweet urine which gives similar meaning of *Madhumeha* which refers to literary meaning of honey like urine. Sweet taste of diabetic urine is noticed in 1670 by Thomas Willis and later is showed that sweetness of urine is due to sugar by Mathew Dobson in 1776. Bouchardt and Peligot in 1838 proved that the sugar of diabetic urine is glucose. Qualitative test for urine sugar was perfected by Hermann Fehling in 1848 and semi-quantitative test by Francis Benedict in 1908.[15] The understanding of diabetes mellitus also started with passing of sweet urine not plasma glucose but present day health care providers diagnosing it based on only plasma glucose level.[16] Diabetes Mellitus is known disease from very ancient times. Charaka in his treatise gives a very elaborate clinical description of *Madhumeha,* which cannot be surpassed by even modern text books.

Ghost of Plasma Glucose and Screening

Random glucose more than 200 mg/dl and Plasma glucose of 126 mg/dl or higher after an overnight fast, documented more than one occasion with associated symptoms are the essentials of Type 1 diabetes mellitus diagnosis. The essentials of diagnosis of Type 2 diabetes are plasma glucose of 126 mg/dl or higher after an overnight fast on more than one occasion and after 75 gm oral glucose, diagnostic values are 200 mg/dl or more 2 hours after the oral glucose. Polyuria, polydipsia, ketonuria and weight loss generally are uncommon at time of diagnosis. Candida vaginitis in women and Belanophosthitis in men may be an associated initial manifestation. Many patients have few or no symptoms.[17] This international diagnostic criterion with no symptoms takes liberty of life and puts healthy individual into bottom less pit till last respiration.

Total body scan (TBS) including plasma glucose has become a routine among educated, economically rich people and national program in developed countries. Whole body scanning is currently marketed in the medical field to make healthy individual ill and who will rarely become healthy as Professor Isan burg says. A study reported in Journal of the National Cancer Institute says that routine screening for prostate cancer using the prostate specific antigen (PSA) leads to over diagnosis.[18] In Netherland, in 1982 around 1000 children were referred to pediatrician after a positive screening result which was shown to be false for congenital hypothyroidism.[19] More test, more false- positive result.[20,21] This type new born and children screening programs with false positive results create potential parental stress.[22]

The Pap smear screening program for cervical cancer in Bristol to prevent cancer, where in 13000 women needed to be screened over 20 years to prevent one death.[23] To prevent one cardiac event per year about 1000 patients needed to treat with newly introduced Polypill which is nothing but old wine in new bottle.[24] This is the use of screening which could damage public health and nation treasure.[25] The plasma glucose screening will show natural normalcy in asymptomatic person as abnormal and encourages physicians with linear thinking to drug healthy mind and body by creating ghost fear.

Epidemiologists to cause epidemics[26] predicted that Diabetes affects one in 20 adults world wide and 333 million cases are predicted world wide by 2025[27] and to sell drugs to increase mortality rate which has been proved by recent clinical trail.[28,29] Statistical science is one of the leading causes of death in United States of America.[30] To prevent and stop disease mongering The Pew Charitable trust has given US$ six million campaign by name 'prescription project' to reduce the influence of pharmaceutical industry marketing on US physicians and doctors-in –training.[31]

Linear versus Nonlinear Science

Professor B.M.Hegde in his classic "What Doctors Don't Get to Study in the Medical School" says The problems lie in medicine's difficulties in defining normality, the devil of false positives, and our limited understanding of the natural history of disease. The most common way of defining normal is that the measure lies within two standard deviations of the mean.[3] Defining normal and abnormal plasma glucose level in a dynamic non- linear human body which has the natural super power to manage altered internal environment to great extent is impossible by linear science. Euboxic Ayurvedic physicians should follow the time honored diagnostic methods of health and make use of *Trvidha* (Three types), *Chaturvidha* (Four types), *Shadvidha* (Six types), *Ashtavidha* (Eight types), *Dashavidha* (Ten types) *Pareeksha* (Examination) and *Nidan Panchaka* (Five methods of diagnosis) for diagnosis of diseases in general and *Madhumeha* in particular except in rare conditions as Sushruta says to make use of other sciences to come to a conclusion.[32]

Twentieth century great physician, Lord Platt a great teacher of medicine at the university, in 1949 wrote that, if you listen to your patient long enough, s/he will tell you what is wrong with her/him, which supports Ayurvedic *Nidana Panchaka* concept. Later his students did prove that with a very well executed prospective, double blind, randomized, hi-tech based (even PET scanner) study of the role of history taking, physical examination, and investigations in medical diagnosis. The study showed that 80% of the final accurate diagnosis and 100% of the future management strategies could be arrived at the end of listening to the patient and reading the GP's referral letter. This is refined slightly by examination and investigations.[33]

Diabetic Medicine 1999 published a revolutionary study report on the effect of insulin on symptomatic and asymptomatic hyperglycemic patients. Quality of life and plasma glucose in insulin treated diabetics clearly showed real relief only in those patients who were symptomatic before treatment. Asymptomatic hyperglycemic patients experienced more problems with social functioning and pain with treatment[16] is the proof for efficacy of drugs in symptomatic hyperglycemia and adverse effect of medicalization. Author's humble request to Ayurvedic practitioners is to stick to their own diagnostic criteria rather than slavishly follow the modern medical criteria. In other words of Sir William Osler, It is a safe rule to have no teaching without a patient for a text, and the best teaching is that taught by the patient himself.[34] We must understand that Ayurveda is a Holistic, non linear dynamic science unlike the Reductionist science of Western medicine.

CONCLUSION

Madhumehi (Diabetic patient*)* needs a *Vedasindu* (Ayurvedic scholar*)* to understand *Madhumeha* (Diabetes Mellitus*),* not a machine. So Ayurvedic physicians should follow classical method of diagnosis of *Madhumeha* not euboxic diagnosis which is very recently born for disease mongering and to sell sickness.

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REFERENCES

1. Douglas G Altman, The scandal of poor medical research. British Medical Journal. 29 January, 1994; 308; 283-284
2. Ray Moynihan, Iona Heath, David Henry, Selling sickness: the pharmaceutical industry and disease mongering. British Medical Journal. 13 April, 2002; 324; 886-890
3. B.M.Hegde. What Doctors Don't Get to Study in the Medical School? 1ed. Parasa Medical Publisher; Hyadarabad; 2006, 250p
4. Sushruta. Sushrata Samhitaa. 6ed. Varanasi; Chaukhambha Orintelia; 1997. 23p
5. Agnivesha. Charaka Samhitaa. 4ed. Varanasi; Chaukhambha Sanskrit Sansthan; 1994. 194p
6. C. Dwarakanata. Introduction to kayachikitsa. 2ed. Varanasi; Chaukhambha Orientalia; 1986.18p
7. Richard Smith, In search of "non-disease". British

Medical Journal. 13 April, 2002; 324; 883-885

8. Anne G, Cutler. Stedman's Medical Dictionary, Illustrated 23rd Indian Edition. S Chand &Company Ltd; New Delhi; 1986. 401p
9. Richard Smith, Unscientific practice flourishes in science. British Medical Journal. 4 April, 1998; 316; 1036
10. Madhavakar. Madhavanidanam.12ed. Varanasi; Chaukhambha Sanskrit Sansthan; 1992. 1p
11. Vaagbhata. Ashtanga Hradayam. 8ed.Varanasi; Chaukhambha Orientalia; 1998. 502p
12. A.R.V.Murthy, R.H.Singh, Concept of Prameha / Madhumeha (Contradictions and Compromises). Ancient Science of Life. October, 1989; 9(2);71-79
13. Agnivesha. Charaka Samhita. 4ed. Varanasi; Chaukhambha Sanskrit Sansthan; 1994. 212p
14. A.R.V.Murthy, R.H.Singha, Concept of Prameha / Madhumeha – Contraindications and Compromises. Ancient Science of Life. 1989; 9;80-85
15. DM Vasudevan, Sreekumari S, Kannan Vaidhyanathan. Text book of biochemistry for medical students. 7ed, New Delhi;Jaypee Brothers Medical Publishers (P) Ltd; 2013;323P
16. P. P. M. Goddijn, H. J. G. Bilo, E. J. M. Feskens, K. H. Groenier, K. I. van der Zee, B. Meyboom-de Jong, Longitudinal study on glycaemic control and quality of life in patients with Type 2 diabetes mellitus referred for intensified control. Diabetic Medicine. 1999; 16; 23–30
17. Stephen J. McPhee. Maxine A. Current Medical Diagnosis and Treatment. 46ed. United States of America; MacGraw-Hill; 2007.1219p.
18. Deborah Josefson Nebraska, PSA Screening leads to over diagnosis, study says. British Medical Journal. 13 July, 2002; 325;61
19. T. Tymstra, False positive results in screening tests: Experiences of parents of children screened for congenital hypothyroidism. Family Practice, 1986; 3;92-96
20. Beth A, Tarini, Dimitri A, Christakis and H, Gilbert Welch. Newborn Screening In the Tandem Mass Spectrometry Era: More Tests, more False-Positive Results. Pediatrics. 2006; 118;448-456
21. Susan E. Waisbren, Simone Albers, Steve Amato, Mary Ampola, et el, Effect of Expanded Newborn Screening for Biochemical Genetic Disorders on Child Outcomes and Parental Stress. Journal of American Medical Association. 2003; 290;2564-2572
22. Elizabeth A, Gurian, Danial D, Expanded Newborn screening for biochemical disorders: The effect of a False- Positive Result. Pediatrics. 2006; 117;1915-1921
23. A E Raffle, Balden, M M Quinn, P J Babb, M T Brett, Outcomes of screening to prevent cancer: analysis of cumulative incidence of cervical abnormality and modeling of cases and deaths prevented. British Medical Journal. 26 April, 2003; 326; 901-904
24. Costantino G, Ceriani E, Rusconi AM, Montano N, Prevention of Cardiovascular Disease with a polypill. British Medical Journal. 2007; 369;185
25. Stewart-Brown Sarah, Farmer Andrew, Screening could seriously damage your health. British Medical Journal. 22 February, 1997; 314;533
26. Editorials, Do epidemiologists cause epidemics? The Lancet. 17 April, 1993; 341(8851); 993-994
27. M Thompson, R Perera, Prevention of diabetes. British Medical Journal, 14 October, 2006; 333;764-765
28. Nesto RW, Bell D, Bono RO, et al, Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. Diabetes Care. January 2004; 27 (1); 256-63
29. James McCormack Trisha Greenhalgh, Seeing what you want to see in randomized controlled trials: versions and perversions of UKPDS data. British Medical Journal. 24 June, 2000; 320; 1720–1723
30. Barbara Starifield, Is US Health Really the Best in the World? Journal of American Medical Association. 26 July, 2000; 284(4);483
31. Michael McCarthy, US campaign tackles drug company influence over doctors. The Lancet. 3 March, 2007; 369 (9563);730
32. Sushruta. Sushruta Samhitaa.6ed. Varanasi; Chaukhambha Orientalia; 1997. 18p
33. B.M.Hegde, Leave the well alone. British Medical Journal. 20 April, 2007; 334,0
34. Christopher A. Feddock, The Lost art of Clinical Skills. The American Journal of Medicine. 2007; 120;374- 378.

Introduction of a Universal EMR Integrated Online Healthcare Management System Mobile App in Hospitals Throughout India and its Benefits to Patients, Hospitals and Governments

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ABSTRACT

Healthcare was,is and always will be a booming sector for business with a lot of scope for progress for the country.With the advent of mobile technology,it would be imprudent to not consider the integration of mobile technology into the healthcare system.This article looks to briefly explain the advantages of having a mobile app for the collection,processing & distribution of data to result in improved patient care on the patient's side,decrease in costs on the hospital's side and improvement in the overall condition of health on the country's side,through something as small as a mobile app.

Key words: *Universal EMR, Integrated online healthcare management system.*

INTRODUCTION

The penetration of smartphones into healthcare settings has increased due to the requirement for faster access to information,which could reduce morbidity,mortality and costs.Smartphone apps have transformed the way in which hospitals work by having an everlasting impact on the healthcare industry in various fields not only on the clinical side but also on the administrative and management sides of the hospital. Healthcare majors like Kaiser Permanente and Cigna have jumpstarted this part of the healthcare industry with innovative tech solutions to help doctors,hospitals and life science organizations become more efficient.[1] Previously,the healthcare sector was focused more on the manufacture of medical supplies and devices for various healthcare settings.But presently,trends have changed and they have transitioned to custom-tailored mobile applications that have higher functionality and ease of use to the point that it is contributing in bulk to the A.I. industry in the field of healthcare.The goal of these solutions is to slash healthcare expenditure and to cater to patients to bring about better quality and faster results in healthcare.

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Quality

Many studies have looked at whether or not EMRs actually enhance the quality of healthcare delivered. [2]A 2011 study in diabetes care showed evidence that healthcare settings having an EMR system provide improved quality of patient care.[3]The EMR system will ultimately enhance care coordination as well. Since authorized personnel using an EMR system can electronically(even with a smartphone) view the patient's complete medical history,it reduces the chances of guessing histories and frequent and numerous specialist visits.It allows appropriate urgent care in emergencies. EMRs in a way promote prevention by presenting doctors and patients with faster and more accurate retrieval of test results,spotting of missing patient data and making available evidence-based suggestions for preventive services.

Time

Implementation of an EMR system can effectively bring about a drop in the identification time of patients after hospital admission.A research study in the Annals of Internal Medicine shows that after the adoption of an EMR system,a significant drop in time of 65% had been observed(from 130 to 46 hours).[4]

Countries like Australia,Canada,United States,Sweden,Finland,Netherlands,Denmark,Estonia and even the UAE had started implementation of EMR(Electronic Medical Records) as early as the 1990's.[5]In China,they have been able to create a system where EMRs from all primary,secondary and tertiary hospitals have been compiled to form 'Big Data' which is later used for various government projects.

Big data and its applications.

Big data has been defined as "high-volume,high-velocity and/or high-variety information assets that demand cost-effective,innovative forms of information processing that enable enhanced insight,decision making and process automation."[6]China is already making use of big data with almost 90% of its hospitals using an EMR system.Such systems are currently being used for public health promotion(disease monitoring and population management),healthcare management(quality control and performance measurement),drug and medical device surveillance,routine clinical practice(risk prediction,diagnosis accuracy, and decision support) and research.As of 2016,numerous academic research projects have used many national datasets collected by means of electronic software or apps(using desktops and smartphones) to predict and foresee the present and future clinical and economic burden of chronic diseases like cardiovascular disease,diabetes,kidney disease and chronic obstructive pulmonary disease.Moreover,other national administrative databases,inclusive of most of the national standardized discharge summaries of in-patients and the national death registry along with hundreds of millions of patient records,have been used by medical and public health researchers.

Such kind of big data that can easily be used to survey trends and correlations among major diseases in turn provides substantial evidence for healthcare policy making.Newer implementations of data analytics,like machine learning to replace much of the work of radiologists and anatomical pathologists,may also be used and is a booming area of study in China currently. Such applications need in-depth,higher-quality clinical information along with long-term follow-ups and hence need to be studied more in China's case.

Why the need for Universal EMR Integrated Online Healthcare Management System ?

Hospital Information System (HIS) is an essential component of healthcare that is responsible primarily for the administrative needs of a hospital or a healthcare setting.In various settings a HIS is also a comprehensive integrated data collection system pioneered to deal with most requirements of a healthcare setting's operation,including but not limited to medical,managerial,financial and legal concerns and also the related handling and delivery of services.

AHISusuallyhandlesofficialdocumentation,financial situation reports,personal data,utilities and sometimes even stock amounts.Of utmost importance to HIS is the security of information like patient data,patient medical history,prescriptions,procedures,operations and lab test results.By doing so,they also provide a universal source of information about a patient's medical history that can be accessed by authorized personnel like physicians or nurses (or maybe even the Government in the future for specific surveys).Such a system can multiply many folds the capability of healthcare workers to coordinate and efficiently provide care,by providing immediate access to the patient's health history and records.A patient's lab test data contains visually interpreted results like X-ray,CT,MRI etc,which may be accessible by means of digital versions available on the personnel's smartphone.A HIS also acts as a means of communication among healthcare providers internally within the organization.

METHODOLOGY

Review of literature,Online search and Interview.

DISCUSSION

Interview with Dr.Basil Peechakara,who is an Indian educated prospective US residency applicant.He has passed his USMLE steps and applied for residency positions in the US after completing quite a few clerkships at various hospitals of one of the best hospital groups in the United States:Cleveland Clinic.This interview is on the American healthcare system and "paperless

hospitals"- specifically the use of smartphones to collect, gather and process data in hospitals by means of apps.

Q)As a future medical resident working in the United States already using an app for hospital administration,what would be your opinion on the use of such apps?How has it helped the clinical side?

A)It helps the clinical side by reducing the clutter of clerk work which exists if there is no electronic medical record.It also provides a standard for documentation and orders to nurses/pharmacies which otherwise is ill defined if written on paper.

Q)How many other hospitals in your knowledge use this technology?

A)I have seen this system only in the US.But I have heard from my friends that at least lab reports are uploaded in a software in some Indian hospitals for prompt delivery to the doctors.I have not worked in many Indian hospitals.

Q)How likely are you and your peers to recommend such an app to others?

A)I would highly recommend such technology because it is time we embrace it and stop relying on outdated protocols involving human labor.

Q)How do you think it can benefit the hospital?

A)It would be useful to have centralized medical records accessible throughout the hospital by medical professionals..

Q)Would you want to add anything else?

A)The main hurdle in its implementation is changing the outlook of veteran doctors who have relied on paper their entire careers.Change needs to be embraced to push Indian hospitals to become on par with hospitals abroad.

Advantages of having Hospital Administration apps.

OPD Management

Theappcouldbecometheperfectsolutiontooutpatient management problems and help streamline processes including patient registration(online appointment booking through the app while using one's smartphone) and also appointment rescheduling and cancellation.The streamlining nature of the app would help in establishing a better queue management system(at admission and registration counters,billing counters,pharmacies,labs etc.) and inventory management system.Clinical consultation recording including patient history/ allergies,problems,examinations done,diagnostic tests prescribed,diagnosis,treatment plans,prescriptions and lab test requests and results,billing etc can all be entered/ordered using a smartphone from anywhere by authorized personnel.Inpatient care management solutions including admission,triage,nursing care and doctor care can be administered.Drug administration and prescription can be done entirely within the app with the help of integration with hospital pharmacy and billing.An integrated inventory management system can be operationalised along with bed and ward management,problem/diagnosis recording,surgery requests and scheduling.Discharge summary with billing and other care record inclusion before final sign-off all can all be done using a single app on the phone. In bed and ward management,colour codes can be used to distinguish between free,occupied and in-preparation beds.

Laboratory/Diagnostic management:The app could enable immediate lab/diagnostic requests,store results and send back reports online,decreasing the need of manpower for logistic of the said results or personnel rushing to the nearest computer.

Integration of OPD,IPD and radiology departments through main server with subsequent linkage to billing.

Invoice/billing management

Invoicing could be seamlessly integrated with the inpatient,outpatient and inventory management systems with the help of strong back-end support (large databases for excess data storage and stable servers for the app to run).

One could see in real time,paid and unpaid patient invoices along with advance payment and part-payment options reducing the need to physically go to the billing department and print invoices (and rather save a huge sum on stationary not to mention a reduction in requirement for manpower).

Inventory management

Stock keeping of various commodities such as pharmaceutical products,stationary,disposables,laundry etc.

Strategic compartmentalization of various stock types for more efficient allocation of resources to respective departments.

Real time updates on stock levels for future references.

Pharmacy management

An appropriate pharmacy management within the APP could include inventory and stock-taking,low stock alerts with the option to dispense batch closest to expiry earlier.

Eradicating errors caused by manual intervention or workarounds.

Avoiding drug diversion and manipulation.

Executing compliance protocols and monitoring compliance violations.

Ability to trace delivery of medical supplies in real time within an entire healthcare setting.

Online Point of sale (POS) module to manage and control sales,generate reports based on daily,day-wise,monthly,month-wise or yearly sales made. POS could incorporated along with inventory management system as well.

Enhancing inventory control and decreasing costs along with prior purchase order generation notifications for low-stock & new stock items.

Data analytics

Data analytics within a hospital setting could help better understand patient trends and the hospital's performance by means of data intelligence.

The app could be used to view multi-dimensional interdepartmental data and gain deep access into more specific details that might be important in strategic decisions

Graphical display of data like for eg:- most commonly prescribed medicines,most demanding issues,patient visit frequencies,demographics of patients registered with hospital,tests most frequently conducted etc.

Administrative settings

Easy to use administrative settings to assign rights and privileges to various hospital users e.g. front staff, nurses,doctors,surgeons,support staff etc.

Customize and/or add hospital letters such as Sick Notes,Referral Letters and Transfer Notes etc.

Improves on responsiveness and patient satisfaction.

Enhances patient care by sending data to the (POC) Point Of Care as opposed to an administrative location.

Creates a system devoid of miscommunication and confusions.

SMS services could be made available in app to communicate effectively with patients.

Apply and alter various hospital service rates and discounts

Deriving more efficient workflow processes.

Integration of all administrative work within the hospital.

With this,the costs generated on repetitive creation of reports,maintenance of physical records and the resource allocations for the same can be drastically brought down. This ultimately would aid the hospital management to minimize a large section of their expenditure.

Proper feedback systems may also be introduced to patients in lieu of small discounts on their next visit.

Patients could be offered special tier level benefits looking at their history and out of pocket spending at the hospitals.

How do hospitals benefit from this?

All of the above salient features of the app used by professionals within the hospital can help in reducing the hospital's spending on manpower and stationary in addition to saving a lot of time.This will enable the hospital to treat more patients and reduce the opportunity costs incurred.In addition,by adhering to industry regulations such as HIPAA (Health Insurance Portability and Accountability Act),a secure environment could be created for the efficient interchange of clinical data in various healthcare settings.Three level security is present on all major smartphones today which involves pattern drawing,passwords and fingerprint recognition which guarantees safety of data entry by any personnel within the hospital as compared to the non- secure

traditional way of stamps and signatures.Patients would be happier as they would not have to physically carry their documents throughout the hospital as everything can be accessed online.

Why the need for a common centralized app for all hospitals in India?

The government would have access to big data (discussed earlier) through the databases based on data that are collected by single universal app in real time at any given point of time. The advantages in addition to applications would include:

Constant health surveillance of the population. The scope for statistical analysis of the country's health in this case is boundless.

No need for large investment on data collection means like desktops as it can be collected on a smartphone which almost every professional would have in today's tech-savvy world.

Lesser manpower and time required to do surveys which could be done in a fraction of the time required now without the app.

It could also contribute a great deal to GOIs 'Digital India' Scheme.

This initiative would also help the government keep a tight leash on the limitless nature of the private healthcare sector by having constant access to the data of private hospitals (the bounds would, of course, be negotiated by government and private entities) as well,resulting in less manipulation of records as everything is electronic and has to be updated in real time.

Regular and random data audits of any hospital may be conducted from any place in the country by the Government as all records are available online thereby increasing the minimum healthcare standard of the country and ultimately increasing India's global healthcare and medical tourism appeal (butterfly effect).

Obstacles to be faced

Servers have to be stable and up and running at all times.

Backend tech for the commissioned app has to be very stable and ready for collection of Big Data.A list of homegrown tech companies could be approached for such services.

Strong intranet and internet connections are required everywhere in the hospital.

The hospital or healthcare setting needs constant and good quality access to the intranet and to the internet.

Veteran doctors need to embrace this idea keeping in mind the bigger picture.

One of the biggest obstacles to be faced would be the acceptance of such an idea among the more experienced veteran doctors who are used to ink and wood and prefer to stay within that framework.To get them out of that safe-zone by helping them to understand that it would make things a lot easier not just for their patients but also for themselves through the implementation of such tech would be a challenge.Training them to use such a system would also be something to keep in mind.

Initial costs to create electronic records and how to view them on devices like Desktops or Tablets.

Conflict of Interest : None

Source of Funding : Self

Ethical Clearance : Taken from Institutional Ethics committee

REFERENCES

1. Iyer, S. How healthcare mobile apps help doctors and patients.[Internet]. 2016. [13th September] Available at: https://www.softwebsolutions.com/resources/healthcare-mobile-app-development-services.html

2. Himmelstein DU, Wright A, Woolhandler S. Hospital computing and the costs and quality of care: a national study. The American Journal of Medicine. January 2010. DOI:10.1016/j.amjmed.2009.09.004. PMID 19939343

3. Cebul RD, Love TE, Jain AK, Hebert CJ. Electronic health records and quality of diabetes care. The New England Journal of Medicine. September 2011. DOI:10.1056/NEJMsa1102519. PMID 21879900

4. Chaudhry B, Wang J, Wu S, Maglione M, Mojica W, Roth E, Morton SC, Shekelle PG. Systematic review: Impact of health information technology

on quality, efficiency, and costs of medical care. Annals of Internal Medicine. May 2006. DOI:10.7326/0003-4819-144-10-200605160-00125. PMID 16702590

5. Tera Tuten, 8 Countries Doing Electronic Health Records Right.[Internet].2012.[3rd April] Available from: https://blog.soliant.com/healthcare-news/8-countries-doing-electronic-health-records-right/

6. Luxia Zhang, Haibo Wang, Quanzheng Li, Ming-Hui Zhao, Qi-Min Zhan; Big data and medical research in China. 05 February 2018. BMJ 2018;360:j5910. Available at: https://www.bmj.com/content/360/bmj.j5910

Antibiotic Susceptibility Profile of Methicillin Resistant *Staphylococcus Aureus*- A Cross-Sectional Study

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ABSTRACT

Background: Antibiotic resistance is a worldwide phenomenon observed among clinical isolates of *Staphylococcus aureus*. The increasing prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) demands the availability of other essential antibiotics for the treatment. Our study is conducted to find out the various antibiotic susceptibility pattern observed among MRSA isolates. *Methods***:** Kirby Bauer disk diffusion method was performed on all the clinical isolates to determine the antibiotic susceptibility pattern and D test to identify the inducible clindamycin strains. *Results***:** of the 573 clinical isolates of Staphylococcus spp, 191 (33.3%) were MRSA which showed maximum antibiotic resistance to erythromycin (60.2%) followed by cotrimoxazole (19.4%) and ciprofloxacin (14.7%). Inducible clindamycin was found to be 24.6% among MRSA isolates. *Conclusion***:** Monitoring of the antibiotic resistance and sensitivity pattern is essential for the effective disease management and complete recovery from the disease.

Key words: *D test, Kirby Bauer disk diffusion method, Staphylococcus aureus*

INTRODUCTION

Staphylococcus is a commonly isolated pathogen from most of the skin and soft tissue infections. There has been a widespread antibiotic resistance observed among these clinically isolated pathogens, more commonly among nosocomial pathogens[1]. *Staphylococcus aureus* is one such pathogen which shows remarkable propensity for the development of various antibiotic resistance[2]. Inappropriate choice of antibiotic therapy results in a higher rate of increased clinical morbidity and mortality among patients[3].Methicillin resistant staphylococcus aureus (MRSA) strains are frequently isolated from hospital and community acquired infections showing multiple drug resistance pattern[4]. With multiple drug resistance MRSA strains the treatment options become limited. Rapid spread of such antibiotic resistant strains have been reported worldwide especially from the developing countries owing to the misguided use of antibiotics and its easy availability over the counter of pharmacy[5,6].

Increased incidence of MRSA cases have been reported from various studies in India as well[6]. Such increasing incidence of resistance strains in the recent times produces a significant impact on the disease management and treatment of infections[7]. Hence, control of MRSA emergence is essential in order to manage the introduction and spread of new infections which could be achieved by following universal precautions and updating on the changing patterns of resistance mechanisms by means of regular epidemiological studies[8]. MRSA shows wide variations in antibiotic resistance patterns by developing chromosome resistance to commonly used antibiotics like penicillin and cepahalosporins which are extensively used in the treatment of skin and soft tissue infections[7,9]. Also, the increased emergence of inducible clindamycin resistance ($iMLS_B$) strains among MRSA cases widens the gap of treatment and management of such infections[10-15].

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To manage the treatment of MRSA and inducible clindamycin resistant cases, the resistant pattern can be easily determined by Kirby-Bauer disk diffusion method and D-test method. The inclusion of D test in the regular clinical diagnostic procedure will further improve the management of the treatment progression with the commonly used antibiotics. Our study thus would help to assess as well as manage the resistant cases and also determine the rate of such cases in this part of the country on a routine basis.

MATERIALS AND METHOD

The present study was conducted in the Department of Microbiology, Sri Venkateshwara Medical College and Hospital, Ariyur, Pondicherry. The study was initiated after getting the approval from the Institute Ethics clearance committee. We analysed 376 strains of *Staphylococcus aureus* which mainly included samples like pus and wound exudates, coming for routine culture and sensitivity to the Microbiology laboratory. The clinical specimens were inoculated on blood agar and MacConkey's agar, and were examined for bacterial growth after 24 hours of incubation. Colony morphology, gram staining, catalase test, coagulase test, mannitol fermentation, methyl red test, voges proskauer test, nitrate reduction test and urease test were performed to confirm the pathogen. Antibiotic susceptibility was performed for *Staphylococcus aureus* as per the as per the CLSI guidelines[16].

Methicillin resistance was detected by using cefoxitin disk (30µg) by Kirby Bauer disk diffusion method. Other antibiotics included for observing the various antibiotic resistance included- penicillin (10 units), amikacin (30 µg), ciprofloxacin (5 µg), co-trimoxazole (1.25/23.75 µg), doxycycline (30 µg), erythromycin (15 µg), clindamycin (2µg), vancomycin (30 µg & E strip) and linezolid (30 µg). *Staphylococcus aureus* ATCC 25923 was used for quality control.

Inducible clindamycin resistance ($iMLS_B$) was detected by using double disk diffusion method (D-test)[17]. Lawn culture of 0.5 MacFarland standard of bacterial suspension was made on Mueller-Hinton agar plate. Erythromycin disk (15 µg) and clindamycin disk (2 µg) were placed at a distance of 15 mm apart (edge to edge). The plates were incubated overnight and the zones of inhibition were measured. Flattening of the shape of clindamycin zone facing erythromycin disk giving a D shaped appearance is considered to be positive for inducible clindamycin resistance ($iMLS_B$) by D- test. Strains resistant to both erythromycin and clindamycin were considered as constitutive MLS_B resistant whereas strains resistant to erythromycin and susceptible to clindamycin were taken as MLS_B phenotype[18].

RESULTS

Among the 376 strains of *Staphylococcus aureus* isolated from the various clinical samples in our study, 191 were MRSA strains. Most of the isolates were from the age group 31-45 years. On comparision, males (68.6%) were found to be more commonly affected when compared to the females (31.4%). Most of the samples were from pus (47.1%) and exudates (39.3%). The antibiotic resistance pattern of MRSA is shown in Table 1.

Of the 191 Strains, 47(24.6%) exhibited inducible resistant to clindamycin. Table 2 shows the different phenotypes of MRSA based on the susceptibility pattern to erythromycin and clindamycin.

Resistance rates of MRSA strains for more than one antibiotic were also observed among as shown in Table 3. Antibiotic susceptibility pattern for the commonly used antibiotics were compiled and found to be more resistant among MRSA isolates than in methicillin sensitive *S.aureus* (MSSA)(Table 4).

Table1. Antibiotic resistance pattern of MRSA (N=191) for other antibiotics

Antibiotic	Resistant strains N (%)
Penicillin (10 units)	191 (100)
Amikacin (30µg)	24 (12.6)
Ciprofloxacin (5µg)	28 (14.7)
Co-trimoxazole (1.25/23.75 µg)	37 (19.4)
Doxycycline (30µg)	15 (7.9)
Erythromycin (15µg)	115 (60.2)
Clindamycin (2µg)	8 (4.2)
Vancomycin- E strip	4 (2.1)
Linezolid (30µg)	4 (2.1)
Cefoxitin (30µg)	191 (100)

MRSA-Methicillin resistant *S.aureus*

Table 2: Susceptibility to ERY and CL among MRSA strains (N=191)

Susceptibility pattern (Phenotype)	MRSA N (%)
ERY-S, CL-S	76 (39.8)
ERY-R, CL-R	8 (4.2)
ERY-R, CL-S; D test positive	47 (24.6)
ERY-R, CL-S; D test negative	60 (31.4)
Total	**191 (100)**

ERY- Erythromycin, CL-Clindamycin, S-Sensitive, R-Resistant

Table 3. Antibiotic resistance of MRSA strains (N=191) for more than two antibiotics

MRSA strains	No of resistant isolates to erythromycin and cotrimoxazole	No of resistant isolates to ciprofloxacin and cotrimoxazole	No of resistant Isolates to cotrimoxazole and doxycycline
Sensitive	76 (39.8)	163 (85.34)	176 (92.15)
Resistant	115 (60.21)	28 (14.66)	15 (7.8)
Total	191 (100)	191 (100)	191(100)

MRSA- Methicillin resistant staphylococcus aureus

Table 4. Antibiotic susceptibility results of MRSA strains (N=191)

Antibiotics	Strain (N)	Sensitive (%)	Resistant (%)	*P* value
Penicillin	MRSA	0 (0.0)	191 (100)	0.000
	MSSA	25 (13.5)	160 (86.5)	
Amikacin	MRSA	167 (87.4)	24 (12.6)	0.000
	MSSA	179 (96.8)	6 (3.2)	
Ciprofloxacin	MRSA	0 (0.0)	191 (100)	0.000
	MSSA	25 (13.5)	160 (86.5)	

Cont... **Table 4. Antibiotic susceptibility results of MRSA strains (N=191)**

Cotrimoxazole	MRSA	154 (80.6)	37(19.4)	0.008
	MSSA	167 (90.3)	18 (9.7)	
Doxycycline	MRSA	0 (0.0)	191 (100)	0.000
	MSSA	25 (13.5)	160 (86.5)	
Erythromycin	MRSA	76 (39.8)	115 (60.2)	0.000
	MSSA	123 (66.5)	62 (33.5)	
Clindamycin	MRSA	183 (95.6)	8 (4.2)	0.061
	MSSA	183 (98.9)	2 (1.1)	
Vancomycin	MRSA	187 (97.9)	4 (2.1)	0.048
	MSSA	185 (100.0)	0 (0.0)	
Linezolid	MRSA	187 (97.9)	4 (2.1)	0.048
	MSSA	185 (100.0)	0 (0.0)	
Cefoxitin	MRSA	0 (0.0)	191 (100)	0.000
	MSSA	25 (13.5)	160 (86.5)	

MRSA-Methicillin resistant *S.aureus;* MSSA- Methicillin sensitive *S.aureus*
(P<0.005 is significant)

DISCUSSION

Our study highlights the increased incidence of MRSA strains (191) isolated form skin and soft tissue infections as reported in previous studies[19,20]. Out of 191 strains, 24.6% were inducible clindamycin resistant. Though the isolation rate of MRSA strains was high, these strains showed good susceptibility to other antibiotics commonly used for the treatment. Antibiotics like amikacin, doxycycline, clindamycin and cotrimoxazole are great choices for treatment of such antibiotic resistant strains. In-vitro determination of antibiotic resistance of *Staphylococcus aureus* with special reference for MRSA is essential in selection of effective drugs for the staphylococcal diseases and infections[21]. Also such strains are becoming multi-drug resistant and developing increased resistance to second line antibiotics.

MRSA strains in our study also showed multidrug resistance as observed in other studies[22,23]. There is also an increase in resistance for vancomycin and linezolid which are the most important antibiotics for the treatment of MRSA infections[24,25]. In our study, 2.1% of MRSA strains showed resistance towards vancomycin and linozolid. All the strains were resistant to penicillin (100%). Inducible clindamycin resistance was found to be 24.6% which was in accordance with the other reported studies[26,27]. Detection of inducible clindamycin is essential in order to avoid treatment failure with this potent drug which could be absorbed by the system very easily. Though, the emergence of such strains is rampant with the MRSA isolation, judicious choice of antibiotics can help in the outcome of patient cure.

Strength of the study

As reported in various studies, simultaneous detection of antibiotic resistance and inducible clindamycin resistance among the clinical isolates proves very beneficial in the care and management of the clinical outcome which was also highlighted in our study.

Limitations of the study

Being a cross sectional study we could not indicate cause-effect relationship. Also, small sample size is a limitation in our study. Resistant isolates has to be studied at molecular level via PCR methods.

CONCLUSION

Increased rate of MRSA strains isolated in our

study with multiple drug resistance and also inducible clindamycin resistance indicates the importance of rapid identification of such strains before the initiation of antibiotic treatment.

Source of Support: Nil

Conflict of Interest: None.

Ethical Clearance- The study was commenced after getting approval by the Research and Institute Ethics Committee of Sri Venkateshwara Medical College Hospital and Research Centre, Ariyur, Puducherry

REFERENCES

1. Nizami Duran, Burcin Ozer, Gulay Gulbol Duran, Yusuf Onlen, Cemil Demir. Antibiotic resistance genes and susceptibility pattern in staphylococci. Indian J Med Res 2012; 389-396.
2. Gupta M, Singh N P, Kumar A Kaur I R. *Cefoxitin disk diffusion test - Better predictor* of methicillin resistance in Staphylococcus aureus. Indian J Med Microbiol 2009; 27:379-80.
3. Luna CM, Rodríguez-Noriega E, Bavestrello L, Gotuzzo E. Treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in Latin America. Rev Chilena Infectol 2010; 27:94-103.
4. Shenoy MS, Bhat GK, Kishore A, Hassan MK. Significance of MRSA strains in community associated skin and soft tissue infections. Indian J Med Microbiol 2010; 28(2):152-4.
5. Sarma JB, Ahmed GU. Characterisation of methicillin resistant S. aureus strains and risk factors for acquisition in a teaching hospital in northeast India. Indian J Med Microbiol 2010, 28(2):127-9.
6. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistance Staphylococcus aureus (MRSA) In India: Prevalence and susceptibility pattern. Indian J Med Res 2013; 363-369.
7. Arunava Kali, Selvaraj Stephen, Sivaraman Umadevi, Shailesh Kumar, Noyal Mariya Joseph, Sreenivasan Srirangaraj. Changing trends in resistance pattern of methicillin resistant Staphylococcus aureus. J Clin Diagn Res 2013; 7(9): 1979-1982.
8. Vidhani S, Mehndiratta PL, Mathur MD. Study of methicillin resistant *S. aureus* (MRSA) isolates from high risk patients. Indian J Med Microbiol 2001; 19(2):13-16.
9. Neerja Jindal, Rubina Malhotra, Pragati Grover, Seema Singh, Renu Bansal & Satvir Kaur. Methicillin resistant *Staphylococcus aureus* (MRSA) in Malwa region of Punjab (North-West India). Indian J Med Res 2016; 371-372.
10. V Deotale, DK Mendiratta, U Raut, P Narang. Inducible clindamycin resistance in Staph*ylococcus aureus* isolated from clinical samples. Indian J Med Microbiol 2010; 38(2): 124-6.
11. Adhikari R P, Shrestha S, Barakoti A and Amatya R. Inducible clindamycin and methicillin resistant Staphylococcus aureus in a tertiary care hospital, Kathmandu, Nepal. BMC Infectious Diseases 2017; 17:483.
12. Tyagi S, Oberoi A. Prevalence of inducible clindamycin resistance among Staphylococcal isolates in a tertiary care hospital in North India. Indian J Med Microbiol 2015; 33(2): 327-28.
13. *Angel M R, Balaji V, Prakash* J, *Brahmadathan K N, Mathews* M S. *Prevalence* of *inducible clindamycin resistance* in *gram positive organisms* in a *tertiary care centre.* Indian J Med Microbiol 2008; 26:262-4.
14. Aleksandra AD, Misic MS, Mira ZV, Violeta NM, Dragana IT, Zoran BM et al. Prevalence of inducible clindamycin resistance among community associated staphylococcal isolates in central Serbia. Indian J Med Microbiol 2014; 32:49-52.
15. Ghosh S, Banerjee M. Methicillin resistance & inducible clindamycin resistance in Staphylococcus aureus. Indian J Med Res 2016; 143(3):362-364.
16. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement M100-S25 Wayne, PA: CLSI; 2015.
17. *Woods, Charles R. MD, MS. Macrolide-Inducible Resistance to Clindamycin and the D-Test.* The Pediatric Infectious Disease Journal 2009; 28 (12): 1115-1118.
18. Baragundi Mahesh C, Kulkarni Ramakant B,

Sataraddi Jagadeesh V. The Prevalence of Inducible and Constitutive Clindamycin Resistance Among the Nasal Isolates of *Staphylococci.* J Clin Diagn Res 2013; 7(8): 1620-1622.

19. Kale P, Dhawan B. The changing face of community-acquired methicillin-resistant *Staphylococcus aureus*. Indian J Med Microbiol 2016; 34(3): 275-285.
20. Shobha KL, Rao PS, Thomas J. Survey of *Staphylococcus* isolates among hospital personnel, Environment and their antibiogram with special emphasis on Methicillin resistance. Indian J Med Microbiol 2005; 23(3):186-188.
21. Dilnessa Tebelay and Bitew Adane. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. BMC Infect Dis 2016; 16:398.
22. Buzaid Najat, Elzouki Abdel Naser, Taher Ibrahim,Ghenghesh Khalifa Sifaw. Methicillin resistant Staphylococcus aureus (MRSA) in a tertiary surgical and trauma hospital in Benghazi, Libya. J Infect Dev Ctries 2011; 5(10):723-726.
23. Anupurba S, Sen M R, Nath G, Sharma B M, Gulati A K, Mohapatra T M. Prevalence of methicillin resistant staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol 2003; 21(1): 49-51.
24. KS Ashwin, NP Muralidharan. Vancomycin-resistant enterococcus (VRE) vs Methicillin-resistant *Staphylococcus aureus* (MRSA). Indian J Med Microbiol 2014; 29 (3):166-167.
25. Kalawat U, Sharma KK, Reddy S. Linezolid-resistant *Staphylococcus* spp. at a tertiary care hospital of Andhra Pradesh. Indian J Med Microbiol 2003; 29 (3):315-315.
26. Felipe S. Lupinacci, Daniel Bussius, and Marcelo J. Mimica. High prevalence of clindamycin resistance in Staphylococcus aureus blood culture isolates in São Paulo, Brazil. J Lab Physicians 2016; 9(4):314-316.
27. Dua'a Jarajreh, Amin Aqel, Hamed Alzoubi, Wael Al-Zereini. Prevalence of inducible clindamycin resistance in methicillin-resistant *Staphylococcus aureus*: the first study in Jordan. J Infect Dev Ctries 2017; 11(4):350-354.

The Multiple Ways of Constructing the Overmind

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ABSTRACT

The priorities and focus given by our current neural set-up might help us to win the short-run; but in the context of the overall evolution, the self-centric focus looks absolutely meaningless. As per science and a genuine introspective analysis, the human system is made of nothing other than fundamental particles and the fundamental sensations. In the long run, therefore, one can only identify either with the interconnected cosmic processes or with the conscious energy that appears to direct it towards more valuable and sustainable structures. To control and direct our current levels of self-talk in accordance with the long-term objectives, one needs to develop the over-mind, after the module of many other control systems that have already developed in nature.

Keywords: *Teleological Motive, Voluntary and involuntary Memory, Synapse, Plasticity of Brain*

BACKGROUND

Despite the fast-track changes in the standards of life, we still need to walk up to the food, extend our hand to push it up to the mouth or to drive away the fly that might come on the way. There's nothing uniquely different in the manner in which the nervous and the muscular systems function in both animals and human beings for executing such actions.

That which sets us apart is probably something else. It's just the fact that human beings engage in the construction of statements even as they carry out these utterly mundane activities. Simplistic though it might sound, this facility in itself is a huge development over the previous brain structures. Language function may be seen as the additional layer built over the unconscious mental control of the body such as the reflex movements and the involuntary muscular excitements and inhibitions[1].

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The conscious process was developed through attention and is regulated through the recruitment of dopamine in the pre-frontal cortex. However attention isn't entirely unique to the humans. The only tweak is that we have used the very same brain structure enabling us to pay attention as a convenient tool to build the semantic memory.

The art of language brought with it a whole lot of blessings and burdens as well. Undoubtedly, the type of self-talk we experience at present helps us to deal with much more complex situations than what the other animals can cope with. But it also fills us with endless anxiety and fear fueled by the prior expectations of the worst possible scenarios. Essentially, we are so absorbed in the meaningfulness made available through neural conditioning that we end up as puppets rather than thinkers.

By utilizing the same facility of attention modulated and supported by dopamine one can also elevate the current neural system to a still higher level of meaningfulness. The strategy may be seen as the reciprocal methodology. It is this human possibility and in some extra-ordinary individuals a skill already initiated that we would define as the over-mind.

Sri Aurobindo2 introduced the term, to mark the penultimate stage of the possible growth of mind, prior to the arrival of the supermind. Over-mind[3], for Aurobindo, is an intuitive way of finding the unity between the subject and the object. Laudable though it is, this way of description hardly delves into the fundamental elements that have built the macroscopic entities called the subject or the object, at least not in line with the findings of science, including cognitive neuroscience. And for this reason we wouldn't implicate the doctrine of Sri Aurobindo here. We would merely borrow the term for a different purpose.

Science has accomplished the immense task of finding the real connections in the stunning cosmic evolution even up to the point of the workings of the brain and has implicitly shown to each individual how they are nothing other than a speck of mechanistic and biological streak in the overall evolutionary forward march. It's high time, therefore, that we reassess the kind of inner talk spontaneously put forward by our neural conditioning. We are in need of re-working the human connectome, taking advantage of the fundamental plasticity of brain. One could call this the conscientious project of constructing the over-mind, which again implies nothing other than a possible new direction of attention, towards some specific objectives.

If attention is all that was required to build complex mental behavior and the language system, a renewed attention to reassess the entire module of self-talk is certainly possible. One can bring about an additional control system right in the midst of the outpouring of our mind similar to the growth of the master control genes and the DNA-binding proteins[4]. It is such deliberate attempt to build a new control system that we have defined as the over-mind.

MATERIALS AND METHOD

The Nobel laureate, Sir Charles Sherrington (1857-1952), would be our critical reference, for more than one reason. Sherrington was the first to show to the world that "muscle excitation was inversely proportional to the inhibition of an opposing group of muscles"[5]. The remarkable discovery was termed the 'reciprocal innervation'[6]. The objective of this article is also nearly similar, although on a purely conscious plane. As neuroscience has come up with all the evidence to show that our current level of self-talk is the excitation of a carefully selected and continually reinforced neural pathways, guided especially by the self-interests of the individual, it just calls for invoking a renewed scheme of attention paid in terms of reciprocal application to fit the self-talk in the context of the overall workings of the entire cosmic system. The moment I recognize that whatever I considered as myself has fallen into one of the many types of the cosmic formulae – in this case, minute sensations building up a huge mental construct as it similarly occurs in billions and billions of other individuals -- there's no ground on which the traditional 'self' can stand any longer, except, of course, the ground of the universe itself. It is this kind of reciprocal deflection of attention, turning an utterly limited concentration to its genuine extensions of all kinds, we would be dealing with here.

In addition, Sherrington has explored the concept of the teleological motive of Nature in The Gifford Lecture5 series. This is an exceptional work for no other Western scientist of the post-modern era has given us a bold and straightforward approach to the divine starting right from the system of Nature. However, the arguments given here might be still wanting, as eminently pointed out in the review given by William Elis[7].

The reason again is not far to seek. Sherrington couldn't move out of the dualistic conception of Descartes, expounded three centuries earlier. Rather than analytically splitting down reasoning and willing back to the minute sensations and the language system built on a specific group of sensations, Sherrington attributed such activities to the soul[8]. This would be unacceptable to any credible scientific methodology, for the current evidence clearly shows that the whole of nature including our mental judgments are made of atomic parts, such as electrons and protons in our neural system or the memory of sensations caused by them.

The argument of the Teleological Drive - traditionally known as the Divine -- can only arise as an analogical one after the module of the mental skills experienced by us, although ours is just an evolutionary set-up. We can also raise the irresolvable question of how the totally varying types such as electrons and sensations finally managed to instruct and influence each other. How the whole lot of minute sensations convert into meaningful summary statements about the entire universe. The Divine motive that has gone ahead and achieved all its works stands out loud and clear, not

only from the fundamental intentional set-ups of nature but more especially from the unnatural links such as electric conduction and the meaningful association of sensations. And it comes out equally impressive when we reflect on the highly inexplicable Big Bang in which all known laws are expected to collapse. Sherrington, for one, hadn't gone into such acid test arguments.

The crucial data preserved through the historical events of world religions powerfully suggesting the presence of something more fundamental to the universe is an extra boost to any positive conclusion we would make in this direction. The reciprocal control of meaning that we wish to develop as the over-mind stands only to be enriched by clarifying the concept and the material available through religions as well.

THE REASSESSMENT OF SELF-TALK

Nature has consistently built one beautiful structure over the other. The manner it does so is a spell-binding story, with each step getting even more intriguing than the previous one – far more brilliant than any human machine that we have managed to build so far. The habitual workings of our mind is just the top-most achievement of nature until now. And it's seriously doubted if anything more complex and brilliant than this could be produced through any other lineage of cosmic evolution. But that doesn't imply the human mind can't be developed further. The reason why we couldn't take it to a higher level of operation until now has to do with our failure to see the continuity of all developments. It isn't a bad idea therefore to list out the significant milestones of cosmic evolution before describing how mind itself can be oriented towards yet another jump-start.

The rapid developments of the very first second of the universe have been theoretically described. From utter fluidity and the highest energy state possible, things got progressively cooler and more expansive, leaving the very first configurations called nuclei and atoms9. It took 240,000 years for hydrogen and traces of lithium to be formed and about 240 million years for the first generation of stars to set fire. The medium sized third generation stars such as the sun came into picture 9 billion years later and it took another one and a half billion years for the early forms of cells to appear.

If the self-replicating cell is something unique in all of cosmic configurations, its existence and operation is just unthinkable without the essential prior stages through which the universe had expanded earlier. The planet earth that turned itself into the fitting abode for the arrival of the cell, is held in its rotational movement within the cosmic balance of galaxies and solar systems. It's obvious therefore that more complex realities have been continuously built within the bedrock of the previous arrangements. Nothing new can ever arise except in and through the laws prevailing earlier.

In the context of the evolution of life, the manner in which the central nervous system finally emerged provides an equally breath-taking story. From the nerve nets[10] and ganglion serving specific functions of the body in lower species, the complex system of cerebellum, the hemi-spheres of cortical lobes and the deep brain parts such as thalamus, hypothalamus, hippocampus and amygdala were gradually put in place. The frontal lobes that enable the executive actions are the latest addendums to this magnificent structure.

While the diffuse nervous system and even the non-human central nervous system were meant only for the co-ordination of the body movements, the control of visceral fluids, detection of food, partner and alarm signals, human beings managed to carve out specific regions in cortices and other brain-parts to produce a new type of memory. It is thanks to the extra-ordinary invention of language and the parallel support system that developed in the brain due to its fundamental plasticity, human beings could go ahead with acquiring knowledge about the whole of universe and converting all of it in verbal notation.

The conscious realm appears to have developed based on the pain and rewards that surrounds the individual. The way to get over this highly concentrated drama is simply by learning all the neural set-ups that favor this specific module and by observing the kind of thoughts and emotions that spontaneously spring up at different moments of life. The closer one captures through the power of attention, the programmed modules of self-talk put forward by the neural system, the more prepared we would be to challenge it and to take it forward to a new scale. The age-old practice of imagining an exclusive entity called 'self' or attributing the self-talk to something as mysterious as the soul would begin to thin out under the power of science combined with an introspective analysis.

The Reciprocal Control

This is not to imply that the over-mind is something entirely new to be developed. The extent of inhibitory processes already at work in our pre-frontal cortices has enabled us to choose the most appropriate action, word and behavior, suppressing a whole lot of other possibilities. The over-mind in this sense is already at work in us. What we are suggesting here is that this role could be further refined towards some specific objectives.

It's only after perfecting hundreds of involuntary muscular controls, one particular section of the sensual data relating either to the sound or the picture of alphabets has catapulted us to a world-wide-web of thoughts, emotions and values. To enjoy the chillness of a bath, one may have to shut down the automatic thought processes that side-track us from the immediate pleasure. Such wisdom was rightly advocated in Buddhist traditions more than two millennia earlier. Attending to just one split second is a brilliant way of avoiding the mega story spun by the mind.

But we also have the freedom to reflect over the beauty of inter-connectedness starting all the way from the early stages of cosmic evolution. The moment we know that the self we had relied on is a construct of nature taking no more than bits and pieces of particles making up the DNA of our cells and bits and pieces of sensations including the language-sensations, the question of the teleological motive in all of universe is bound to haunt our attention.

The meaningfulness given by the language system supported by neurotransmitters and neuromodulators is defining no more than an utterly limited circle at present. Reflecting on the long chain of evolution that has cleverly brought about the construct of the individual self, you would not only feel one with all physical and chemical designs, it would be impossible thereafter to separate yourself from the essential undercurrents of Nature.

The Indian tradition reached out to these truths in its own inimitable style. As per Mandukya Upanishad[11], the wakeful and the dream states are caused by the various organs and limbs exposed to the world, while Atman is identified with that which lies beyond deep-sleep. Something keeps you alive even when you deep-sleep. It brings back to you the right kind of memories the moment you wake up. That ultimate base is Brahman, as per the inspiring dialogue between Uddalaka and his son Svetaketu in Chandogya Upanishad[12].

The remarkable intuition of the pre-scientific era doesn't mean one can progress much by going to sleep and acting as if the identity with Brahman is already achieved. On the contrary, right in the wakeful state, if you start focusing on the essential fact that chemical designs are the base of each of your thought and that something else ought to have planned even the complex brain structures, there's no other go but to find yourself united with the cause of all causes. Again the Upanishadic wisdom is hard to come by without reflecting on the Buddhist ideal of looking at the utterly limited minute parts that build up the whole or without reflecting on the absolute certainty of mind over matter as demonstrated through Jesus of Nazareth or without wondering over the divine act of dismantling some of the misleading statements of Christianity through the Book of the Holy Quran.

CONCLUSION

The human system is dangling between two extremes. It can only split up into the basal elements from which it evolved or it can unite itself with the topmost conscious energy that appears to have guided from within one beautiful construct after the other. The priorities and focus given by our current neural set-up can only give us a façade of meaning and joy. It stands utterly ill-prepared for any of the long-term objectives or significance. To control and direct it as per the huge knowledge available through science, one needs to develop the over-mind, which is already at work in us through some of the religious teachings, social norms and behavior.

Ethical Clearance : COMPLETED

Source of Funding : Self

Conflict of Interest - Nil

REFERENCES

1. Kandel ER. In Search of Memory. New York: W.W. Norton & Company; 2006
2. Aurobindo. The Life Divine, New York: Greystone Press; 1949, American Edition
3. Sen I. Sri Aurobindo's Theory of Mind [Internet] (cited November 4, 2018) Philosophy East and

West; 1952: Vol1, No.4. Available from https://www.jstor.org/stable/1396996

4. Gene Regulation and Expression [Internet] (cited Novembar 4, 2018). Available from: https://www.internationalconnectionsacademy.com/content/media/527934-442012-85944-AM-1958216437.pdf
5. Wikipedia [Internet] (cited Novembar 4, 2018). Available from https://en.wikipedia.org/wiki/Charles_Scott_Sherrington
6. Sherrington C. Man: On His Nature, The Gifford Lecture, Edinburgh, Cambridge University Press, 1940.
7. Sherrington C. Man on His Nature [Internet] (cited November 5, 2018) The Gifford Lectures. Available from https://www.giffordlectures.org/lectures/man-his-nature
8. Elis W. Discussion: Man on his Nature by Sir Charles Sherrington, Philosophy, (1941), Vol.16, No.62, pp 198-207.
9. Mastin L. Timeline of the Big Bang [Internet] (cited October 28, 2018) The Physics of the Universe. Available from https://www.physicsoftheuniverse.com/topics_bigbang_timeline.html
10. Anatomy of Nervous System [Internet] (cited October 28, 2018) Brittanica Encyclopedia, Available from https://www.britannica.com/science/nervous-system/Diffuse-nervous-systems
11. Mandukya Upanishad [Internet] (cited November 5, 2018) Available from https://mast.queensu.ca/~murty/ind6.pdf
12. Chondokya Upanishad [Internet] (cited November 6, 2018) Available from swamij.com/upanishad-chandogya.htm

Are Soft Drinks Soft on Teeth? A Study on Dental Erosion Caused by Soft Drinks Marketed in India

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ABSTRACT

Introduction: Awareness of dental erosion by public is still not widespread, and there is paucity of information on how much erosion do various soft-drinks marketed in India cause.

Objectives: To quantify the amount of erosion that various soft-drinks may cause, in relation to their baseline pH, titrable acidity and calcium contents.

Method: Six types of drinks- Coca-cola, Sprite, Maaza, Lipton iced lemon tea, Tropicana orange juice and yoghurt were used, with water as control. Baseline pH was determined by pH electrode, baseline titrable acidity was determined by amount of sodium hydroxide (NaOH) required for making pH of 5.5 and calcium content by Atomic Absorption Spectrophotometry. Five teeth each of maxillary premolars were randomly allocated into each of 7 groups. After incubation in 100 ml for 2 hours, 12 hours and 24 hours, amounts of calcium released was determined.

Results: Mean calcium release after 24 hours was highest for Coca-cola with lowest pH, followed by Maaza, Sprite, Orange juice, Lemon tea, yoghurt and water, which released 1.38µg/ml/mm^2, 1.28µg/ml/mm^2, 1.04µg/ml/mm^2, 0.91µg/ml/mm^2, 0.49µg/ml/mm^2, 0.34µg/ml/mm^2 and 0µg/ml/mm^2 of calcium respectively. Statistically significant differences were found for calcium release in coca-cola, maaza, sprite and orange juice from baseline to 2, 12 and 24 hours (p-value < 0.00).

Conclusion: Beverages with lower baseline pH and lower baseline calcium concentration showed higher calcium release. Though, direct application of the results of this study to in-vivo conditions cannot be fully made due to other host and dietary factors.

Key words: *Dental erosion, Soft drinks, pH, calcium concentration.*

INTRODUCTION

One of the most important outcomes of modern lifestyle is the changed eating habits. To keep pace with an expeditious lifestyle, people are resorting to more and more processed foods and bottled beverages. This in turn, has resulted in an ever increasing prevalence of dental erosion.[19]

Dental erosion (DE) is defined as the tooth wear occurring by chemical dissolution caused by acids of any origin.[11] Being an inorganic tissue, the integrity of enamel is greatly influenced by the pH and presence of minerals in saliva. Whenever there is a drop in pH of saliva below the critical value of 5.5, tooth enamel is demineralized. The pH above critical pH is regarded as supersaturated and favors crystallization of mineral. Under normal conditions, a tooth does not lose its mineral

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since saliva is usually supersaturated with respect to its mineral concentration.[2] At this pH, the ionic product *(Ip)* of the mineral is said to be equal to its solubility product *(Ksp)*.[15] Whenever oral cavity is exposed to acidic foods and beverages, the pH of solution becomes less than critical pH *(Ip<Ksp)* and saliva becomes unsaturated with respect to mineral, which then precipitates out into the solution.[2] Frequent and prolonged exposure to low pH may result in a more rapid demineralization of the enamel surface.[2, 15]

Initially DE is manifested as loss of tooth shine, followed by flattening of convex structures. With the continuing acid exposure, concavities form on smooth surfaces and cupping occurs on incisal/occlusal surfaces.[1] DE can be caused due to external or internal factors. Extrinsic factors being frequently consumed acidic foodstuffs or beverages and acidic contaminants present in the working environment. Intrinsic factors are acids regurgitated due to gastro-esophageal refluxes.[1, 12] In the modern societies extrinsic factors are becoming an important cause for erosion due to the increased consumption of acid drinks as soft drinks, sport drinks, fruit juices and fruit teas.[5]

There are studies done in developed countries which measure the erosive potential of soft drinks marketed in their countries. [5, 10, 13-14] Hence, this study was conducted with an aim to quantify the amount of enamel surface demineralization that various beverages may cause, in relation to the baseline pH, titrable acidity and calcium contents. The objectives were: i. to measure baseline calcium content of various soft drinks by Atomic absorption Spectrophotometry. ii. to measure baseline pH by pH electrode. iii. to measure titrable acidity (neutralizable acidity) determined as the volumes of 0.1 M sodium hydroxide (NaOH) required to increase the pH of 50 milliliter (mL) of beverages to 5.5. iv. to measure and compare calcium concentrations in the drinks after 2 hours, 12 hours and 24 hours.

MATERIALS AND METHOD

This invitro study was conducted to quantify the amount of enamel surface demineralization that various soft-drinks and juices may cause, in relation to their baseline pH, titrable acidity and calcium contents.

Analysis of pH, Titrable Acidity and Calcium:

Six commonly available soft drinks and juices in the Indian market were chosen for study. The following drinks were used: Coca-cola™, Sprite™, Maaza™, Lipton Iced tea™, Tropicana orange juice™ and Danone yoghurt™. Water was used as a control.

Immediately after opening the bottles, their pH was determined at 25º C by pH electrode (Micropro – Gradmate). Three pH readings were measured for each beverage and the mean value calculated. The buffering effect of each drink was determined by monitoring pH after serial additions of 0.025 mL of 0.2 mol/L of sodium hydroxide (NaOH) to 5mL of each drink. Then, their Calcium concentrations were determined by Atomic Absorption Spectrophotometer (Thermo Jarrel Ash Atomic Absorption Spectrometer). Five readings were taken for each beverage and the mean value was calculated.

Preparation of Enamel Specimens:

A total of 35 freshly extracted human premolars were collected from young adults, who got their teeth extracted for orthodontic reasons. Immediately after tooth extraction, surrounding soft tissues were removed and were placed in a 0.9 % sodium chloride solution for up to 7 days. All teeth were then examined microscopically for any irregularities of mineralization, especially for genetic diseases of enamel mineralization, for cracks or for other defects.

All the teeth were covered with three coats of acid resistant nail varnish, except for a 4 × 4 mm window left for exposure. This was done to standardize all teeth, so that, calcium can leach out from the same amount of surface area from all teeth. Five teeth each of maxillary premolars were randomly allocated into each of the 7 groups.

Study design:

Each tooth was incubated in 100 mL beverage for upto 24 hours at 37º C in air tight containers. After incubation at 2 hours, 12 hours and 24 hours, the amounts of calcium released from each beverage was determined by selecting the median value of the five readings taken using Atomic Absorption Spectrophotometry. The increase in calcium in the drinks, as a result of erosive action was then calculated.

Statistical Analysis:

The collected data were computerized and processed

using MS Excel and Stata 10.0 version. Kruskal Wallis ANOVA was applied to test any significant differences in calcium release from baseline to 2 hours, 12 hours and 24 hours. Wilcoxon Matched Pairs test was applied to test if there is any significant difference in the percentage change in calcium levels from baseline to 2 hours, 12 hours and 24 hours. Pearson's correlation coefficient was applied to compare the DE caused by various drinks, in relation to their baseline pH, titrable acidity and calcium contents.

RESULTS

In this invitro study on determination of erosive potential of drinks, 6 drinks were analyzed for their baseline calcium concentration and pH value and titrable acidity. The pH values ranged from 2.32 to 6.45, with coca-cola having the lowest pH and water, the highest. Coca-cola had lowest titrable acidy (1.06 ± 0.75) and orange juice had the highest (3.02 ± 0.87). Baseline calcium contents ranged from 22.07µg/mL for coca-cola to 251.31µg/mL for yoghurt. (Table 1)

The increase in calcium as a result of erosive action was measured after 2 hours, 12 hours and 24 hours. It was seen that after 2 hours, Sprite showed highest calcium release of 7.09µg/mL followed by Coca-cola which released 6.2µg/mL and Yoghurt showed lowest calcium release of 1.49µg/mL and water, 0µg/ml. Statistically significant differences were seen in calcium release between baseline to 2 hours, in all the drinks, except in maaza and yoghurt (p-value< 0.05). And that, after 12 hours, coca-cola showed the highest calcium release of 12.81µg/mL followed by maaza and sprite. After 24 hours also coca-cola showed the highest calcium release of 22µg/ml. Again here, after 12 hours and 24 hours, statistically significant differences were seen in calcium release in all the drinks, except yoghurt (p-value< 0.05). (Table 2)

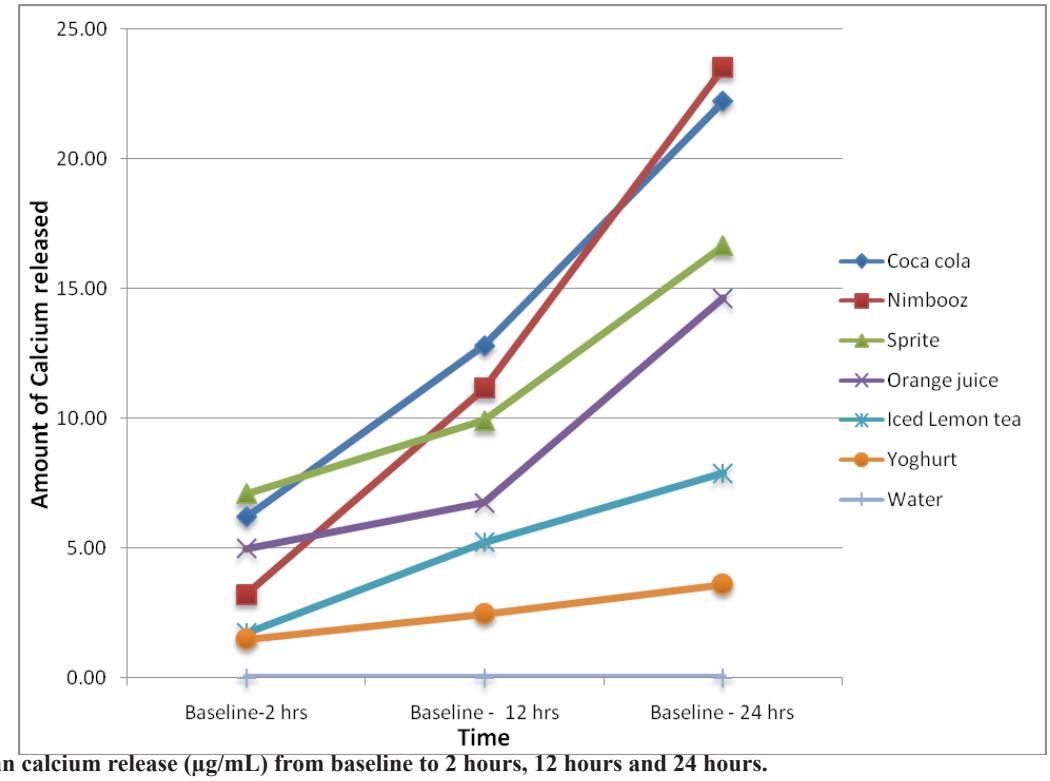
Table 3 gives the percent gain in Calcium in the drinks after 2, 12 and 24 hours. It was seen that, statistically significant differences between baseline to 2 hours, 12 hours and 24 hours were found in all the drinks, except in yoghurt at all times and maaza from baseline to 2 hours. Pearson's correlation coefficient showed no significant correlation in the amount of DE caused by various drinks, in relation to their baseline pH, titrable acidity and calcium contents.

Table 1: Mean baseline calcium concentration and baseline pH of the drinks.

Groups	Baseline Calcium (µg/mL)	Baseline pH	Baseline Titrable Acidity at 5.5 (ml)
Coca-Cola	22.07	2.32	1.06 ± 0.75
Maaza	89.79	3.08	1.93 ±0.17
Sprite	25.56	3.22	1.12 ± 0.33
Orange juice	62.13	3.67	3.02 ± 0.87
Iced lemon tea	35.63	3.35	1.87 ± 0.54
Yoghurt	251.312	4.34	2.71 ± 0.39
Water	0	6.45	_

Table 2: Percentage gain in Calcium after 2, 12 and 24 hours

Groups	% gain in 2hrs	% in 12 hrs	% in 24 hrs
Coca-Cola	28.118*+	58.05*+	100.64*+
Maaza	3.59	12.45*+	26.21*+
Sprite	27.76*+	38.83*+	65.14*+
Orange juice	8.04*+	10.89*+	23.57*+
Iced lemon tea	4.87*+	14.70*+	22.19*+
Yoghurt	0.59	0.99	2.23
Water	0.00	0.00	0.00
*p<0.05; + Applied Wilcoxon Matched Pairs test			



Graph 1: Mean calcium release (µg/mL) from baseline to 2 hours, 12 hours and 24 hours.



Fig. 1: Premolars coated with nail varnish, leaving a 4 × 4 mm window on the middle third of labial surface

DISCUSSION

The loss of dental hard tissue as a consequence of changed eating habits is increasingly gaining importance. Unusual drinking habits like that of holding acidic beverage in the mouth before swallowing increases the contact time and thus amplifies the risk of erosion. Bedtime consumption of acid beverages is considered as a risk factor among children. [19] Also, studies conducted previously suggest that the length of time the teeth are suffused in acidic environment is more decisive to erosion than the volume of beverage consumed. [5] It is known that carbonated drinks are frequently held in the mouth until all bubbles have dissipated. Hence the contact time of carbonated drinks with teeth can be much longer than that for non-carbonated beverages.[5, 19] On account of such shifts in eating habits in recent years, it is very important for dental professionals to alarm the public on harmful effects of these beverages on dental health. Hence, this study was conducted with an aim to quantify the amount of erosion caused by various beverages.

A large number of techniques have been developed in order to determine both the change in and loss of tooth surface structures induced by erosion. Some of the techniques used most frequently are: surface roughness measured with an Optical Profilometric Device, determination of Micro Hardness, Scanning Surface Microscopy, Microradiography, Confocal Laser Scanning Microscopy (CLSM) and Chemical Analysis of leached out minerals.[3-4, 7-8, 10, 13-14, 18, 20] The quantitative light-induced fluorescence (QLF) and the Nano Indentation techniques are some recent methods.

Enamel consists of 34 to 39 percent calcium by weight (dry weight) and 16 to18 percent phosphorus by weight. [20] Chemical analyses have an advantage over other methods that they are not only sensitive and precise, but also DE of natural enamel surfaces can be investigated as there is no need of polishing [4]. Therefore, a quantitative determination of calcium and phosphorus dissolved through erosive procedure is one of the most apposite methods in order to measure erosion. [6]

In this in-vitro study, pH of all the test beverages ranged from 2.32 to 4.34, which was below the critical pH of 5.5. Those beverages with lowest pH and baseline calcium concentrations showed the most pronounced erosive effect on the enamel. This is in accordance with the studies conducted by Sales-Peres et al (2007) [7] and Fatilah AR and Rahim ZHA. (2008).[6]

Titrable acidity is another important aspect that has a role in causing DE. Higher titrable acidity was observed for orange fruit juice and fruit based drinks, because of which they are expected to have higher erosive potential than carbonated beverages, but was not so in our study. This could be because of the higher pH of orange juice and iced lemon tea as compared to that of carbonated beverages which is in accordance with the study conducted by Tadakamadla J et al (2015) [17] and Jensdottir T et al (2006).[9] Yoghurt showed the lowest calcium release, which had the highest ph, titrable acidity and calcium concentration of all the test drinks. Thus, decrease in DE was seen with decreasing baseline calcium concentrations in the drinks, similar to the study done by Pereira HS et al (2013), where, soft drinks supplemented with metallic ions reduced dissolution of bovine enamel.[16]

Yet, the present study is not without any limitations. There are other factors that protect the oral environment from DE, like pellicle and salivary components preventing dissolution, frequency and time interval between beverage consumption, the simulation of which in invitro conditions is not feasible.

CONCLUSION

From the present study, it can be inferred that coca-cola, which had the lowest baseline calcium concentration and lowest pH showed the most pronounced erosive effect on dental enamel. Other than water, yoghurt showed the lowest calcium release, which had the highest ph and calcium concentration of all the test drinks. Baseline pH and baseline calcium concentration showed a significant correlation with the amount of calcium released.

Based on these analyses, personalized preventive programs may be suggested to the individuals who consume such beverages frequently. It may comprise dietary advice, optimized fluoride therapy, use of buffering medication and emphasis for nondestructive tooth brushing habits with low abrasive toothpastes.

Conflict of Interest - The authors do not disclose any conflicts of interest

Source of Funding - Self

Ethical Clearance - Ethical clearance was obtained by the Institutional Review Board

REFERENCES

1. Addy M, Edgar WM, Embery G. Tooth wear and sensitivity: Clinical advances in restorative dentistry. Thieme; 2000 Apr 17.
2. Almeida e Silva JS, Baratieri LN, Araujo E, Widmer N. Dental erosion: understanding this pervasive condition. Journal of Esthetic and Restorative Dentistry. 2011 Aug 1;23(4):205-16.
3. Attin T, Becker K, Hannig C, Buchalla W, Hilgers R. Method to detect minimal amounts of calcium dissolved in acidic solutions. Caries research. 2005 Aug 19;39(5):432-6.
4. Barbour ME, Rees JS. The laboratory assessment of enamel erosion: a review. Journal of dentistry. 2004 Nov 30;32(8):591-602.
5. Carvalho de Sales-Peres SH, Magalhães AC, Machado MA, Buzalaf MA. Evaluation of the erosive potential of soft drinks. European journal of dentistry. 2007 Jan;1(1):10.

6. Fathilah AR, Rahiml ZH. The effect of beverages on the release of calcium from the enamel surface. Ann Dent Univ Malaya. 2008 Mar;15:1-4.
7. Ganss C, Lussi A, Klimek J. Comparison of calcium/phosphorus analysis, longitudinal microradiography and profilometry for the quantitative assessment of erosive demineralisation. Caries research. 2005 May 13;39(3):178-84.
8. Jensdottir T, Bardow A, Holbrook P. Properties and modification of soft drinks in relation to their erosive potential in vitro. Journal of dentistry. 2005 Aug 31;33(7):569-75.
9. Jensdottir T, Holbrook P, Nauntofte B, Buchwald C, Bardow A. Immediate erosive potential of cola drinks and orange juices. Journal of Dental Research. 2006 Mar 1;85(3):226-30.
10. Larsen MJ, Nyvad B. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. Caries research. 1998 Nov 26;33(1):81-7.
11. Lussi A, editor. Dental erosion: from diagnosis to therapy. Karger Medical and Scientific Publishers; 2006.
12. Lussi A, Jaeggi T. Dental Erosion in Children. Monogr Oral Sci, 2006 20:140–65.
13. Lussi A, Kohler N, Zero D, Schaffner M, Megert B. A comparison of the erosive potential of different beverages in primary and permanent teeth using an in vitro model. European Journal of Oral Sciences. 2000 Apr 1;108(2):110-4.
14. Nogueira FN, Souza DN, Nicolau J. In vitro approach to evaluate potential harmful effects of beer on teeth. Journal of dentistry. 2000 May 31;28(4):271-6.
15. Nunn JH. Prevalence of dental erosion and the implications for oral health. European Journal of Oral Sciences. 1996 Apr 1;104(2):156-61.
16. Pereira HA, Leite AD, Italiani FD, Kato MT, Pessan JP, Buzalaf MA. Supplementation of soft drinks with metallic ions reduces dissolution of bovine enamel. Journal of Applied Oral Science. 2013 Aug;21(4):363-8.
17. Tadakamadla J, Kumar S, Ageeli A, Vani NV. Enamel solubility potential of commercially available soft drinks and fruit juices in Saudi Arabia. The Saudi Journal for Dental Research. 2015 Jul 31;6(2):106-9.
18. Willershausen B, Schulz-Dobrick B. In vitro study on dental erosion provoked by various beverages using electron probe microanalysis. European journal of medical research. 2004 Sep 29;9(9):432-8.
19. Willershausen B et al. Influence of apple juice on human enamel surfaces of the first and second dentition - an in vitro study. Eur J Med Res (2008) 13: 349-54.
20. Willis JB. Determination of calcium and magnesium in urine by atomic absorption spectroscopy. Analytical Chemistry. 1961 Apr;33(4):556-9.

Decentralized Internet of Things

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ABSTRACT

The Internet of things (IoT) is the network of physical devices, vehicles, home appliances, and other items embedded with electronics, software, sensors, actuators, and network connectivity which enable these objects to connect and exchange data. It is estimated that by 2020 IoT will consist of 30 billion devices with a global market value of $7.1 trillion. With large number of devices connected to the internet, security becomes a major factor to consider. Along with security micro- payments via the internet of things becomes a huge risk. Our target with this project is to rectify both problems with the use of Blockchain technology. We want to create a decentralized peer-to- peer network of IoT devices which share a common distributed database. This database is known as a blockchain. Blockchain ensures the ownership and integrity of the data solving the security problem faced by IoT. Also, cryptocurrency written over this blockchain would solve the micropayments problem of IoT. The cryptocurrency design methodology takes into account that most IoT devices would be embedded systems thereby optimizing cryptographic standards for embedded systems. With this project we aim to improve the security risks which relate from data sharing and to reduce data redundancy by above 80%.

Keywords—*Distributed Systems, Internet of Things, Decentral- ized, Blockchains.*

INTRODUCTION

A blockchain is a continuously growing list of records, called blocks, which are linked and secured using cryptogra- phy. Each block typically contains a cryptographic hash of the previous block, a timestamp and transaction data. By design, a blockchain is inherently resistant to modification of the data. It is an open, distributed ledger that can record transactions be- tween two parties efficiently and in a verifiable and permanent way. For use as a distributed ledger, a blockchain is typically managed by a peer-to-peer network collectively adhering to a protocol for validating new blocks. Once recorded, the data in any given block cannot be altered retroactively without the alteration of all subsequent blocks, which requires collusion of the network majority [1].

We aim to use this technology to implement it in Internet of Things. We do this to improve the security risks which relate from data sharing and to reduce data redundancy. We tend to create a decentralized peer-to-peer network of IoT devices which share a common distributed database [2]. Blockchain ensures the ownership and integrity of the data solving the security problem faced by IoT.

METHODOLOGY

To maintain a distributed ledger/database a mesh network is created. Each node need not be aware of every other node in the network. Although, every node should be connected to every other node directly or through nodes. Whenever a node creates a new firmware/ file it notifies the closest nodes which in-turn notifies the other nodes. Thereby, every node on the network receives the new file. Every node then checks the consistency of the file using proof-of-kernel algorithm. Once a node checks the file it adds the hash of the file to the blockchain. At least 50% of the nodes on the network must verify the file and add it to their blockchain to make it immutable. This phenomenon is known as consensus. Once consensus is achieved the file becomes immutable and available to everyone. Every node minimally consists

of a Node MCU, SD card reader and SD card, battery, battery charging circuit [3].

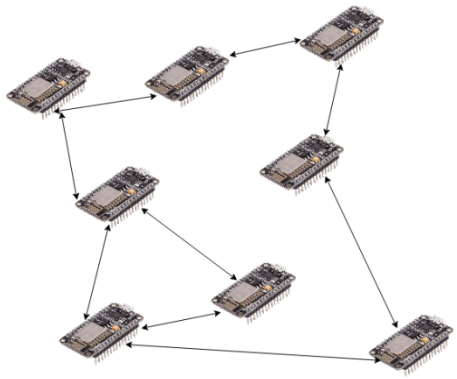


Fig. 1. Mesh network on NodeMCU nodes

Node MCU

Node MCU is an open source IoT platform. It includes firmware which runs on the ESP8266 Wi-Fi SoC from Espres- sif Systems, and hardware which is based on the ESP-12 module. The firmware uses the Lua scripting language. It is based on the eLua project, and built on the Espressif Non-OS SDK for ESP8266. It uses many open source projects, such as lua-cjson and spiffs[4].

SD card

This SD Card module can make your SD application easier and simple. It is easily interfaced as a peripheral to your arduino sensor shield module. Through programming, you can read and write to the SD card using your arduino

TP4056 - Li-ion battery charger

This module is made for charging rechargeable lithium batteries using the constant-current/constant-voltage (CC/CV) charging method. In addition to safely charging a lithium battery the module also provides necessary protection required by lithium batteries.

Li-ion battery

A lithium-ion battery or Li-ion battery (abbreviated as LIB) is a type of rechargeable battery in which lithium ions move from the negative electrode to the positive electrode during discharge and back when charging. Li-ion batteries use an intercalated lithium compound as one electrode material, compared to the metallic lithium used in a non-rechargeable lithium battery.

SYSTEM DESIGN

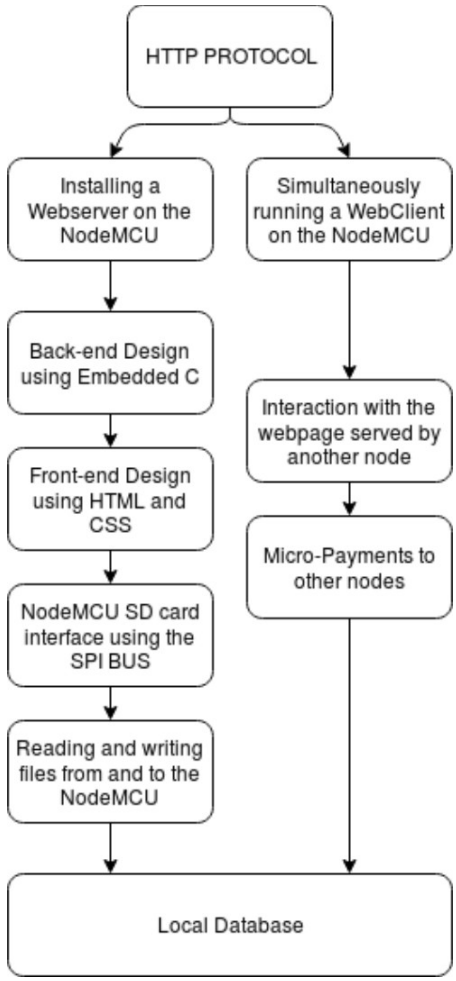


Fig. 2. Flowchart representing system design methodology

HTTP and File Storage

The HTTP Protocol runs both the web server and the web client on the on the NodeMCU. While normally we use a web server on the NodeMCU, we use a web client on the same NodeMCU. This is done so that the NodeMCU can send files and make payments in the same node[5].The web server has the complete back-end design in Embedded C. This is to interact with the various independent nodes created by the NodeMCU's. The Front-End is designed such that it can display the values received by the NodeMCU. We use HTML and CSS for the data retrieval and designing the user interface [6].

We will be using SD card in SPI mode, first we need to connect the SD card breakout board with NodeMCU. By de- fault FatFS module is disabled in nodemcu firmware, and we build the firmware with FatFS support using an online build tool. We load the new firmware and enter the following commands in the serial console;

it will list the files in root directory of the SD card [7]. We can open a file using file. open and can check out the file API for list of functions available.

We then store these values on the local database which is our SD module in the NodeMCU. The web client interacts with other nodes and is builds on the interface similar to the web server. Micro-Payments are done to the other nodes.

Blockchains

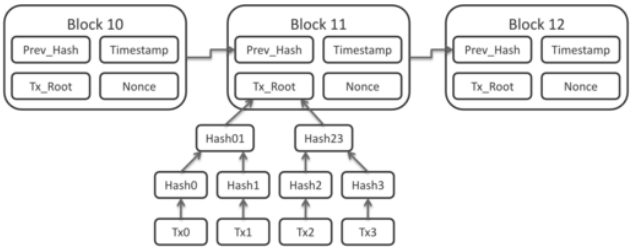


Fig. 3. Interlocking of blocks

A blockchain is a continuously growing list of records, called blocks, which are linked and secured using cryptogra- phy. Each block typically contains a cryptographic hash of the previous block, a timestamp and transaction data. By design, a blockchain is inherently resistant to modification of the data. It is ”an open, distributed ledger that can record transactions be- tween two parties efficiently and in a verifiable and permanent way”[8].

For use as a distributed ledger, a blockchain is typically managed by a peer-to-peer network collectively adhering to a protocol for validating new blocks. Once recorded, the data in any given block cannot be altered retroactively with- out the alteration of all subsequent blocks, which requires collusion of the network majority [9].Decentralized consensus has therefore been achieved with a blockchain. This makes blockchains potentially suitable for the recording of events, medical records, and other records management activities,such as identity management, transaction processing, documenting provenance, food traceability or voting [10].

RESULTS

We executed the code on two nodes to verify the working of the blockchains. Before we could upload the blockchain code we had to write in the genesis block into the NodeMCUs. If a node didn't have a genesis matching with the network it would not be able to buy or contribute to the network. For a node with the genesis block in existence successive blocks would be added to the text files (initial blocks from the balance.txt are added below)

be added to the text files (initial blocks from the balance.txt are added below)

```
{"from":"","to":"","amount":"50",
    "fileName":"blinkesp.bin",
    "description":"Helloworld"}
{"from":"chirag","to":"chirag",
    "amount":"50","fileName":"blinkesp.bin",
    "description":"Helloworld"}
{"from":"chirag","to":"swaroop",
    "amount":"50","fileName":"blinkesp.bin",
    "description":"Helloworld"}
{"from":"swaroop","to":"swaroop",
    "amount":"50","fileName":"blinkesp.bin",
    "description":"Helloworld"}
{"from":"swaroop","to":"chirag",
    "amount":"50","fileName":"blinkesp.bin",
    "description":"Helloworld"}
{"from":"chirag","to":"aditya",
    "amount":"50","fileName":"blinkesp.bin",
    "description":"Helloworld"}
```

As soon as new blocks are added to the balances.txt or the common ledger they would read by the front-end javascript and be presented on the browser page for the user.

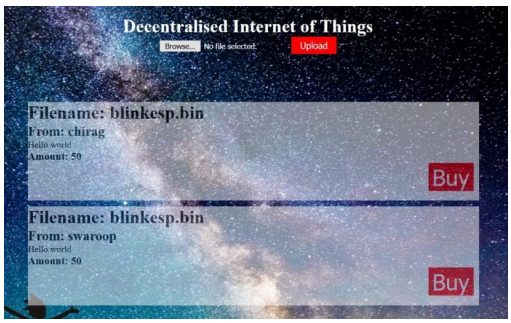


Fig. 4. **Front-end of the blockchain**

```
//putyoursetupcodehere,torunonce:
 SHA256 hasher;
 byte hash[SHA256_SIZE];

 String fileName ="";
 String from ="";
 String to ="";
 String amount ="";
 String description ="";
 String prevHash ="";
 String curHash;

 String compiled = from + to + fileName +
     amount + description + prevHash;
 char charBuf[200];
 compiled.toCharArray(charBuf,
     compiled.length());
 hasher.doUpdate(charBuf);
 hasher.doFinal(hash);

 for(int j=0;j<32;j++){
  curHash += String(hash[j], HEX);
 }

 StaticJsonBuffer<500> jsonBuffer;
  JsonObject& root =
      jsonBuffer.createObject();
  root["from"] = from;
  root["to"] = to;
  root["fileName"] = fileName;
  root["amount"] = amount;
  root["description"] = description;
  root["prevHash"] = prevHash;
  root["curHash"] = curHash;

  String groot;
  root.printTo(groot);
  File newFile = SD.open("balance.txt",
      FILE_WRITE);
  if(newFile){
   newFile.println(groot);
   newFile.close();
  }
  else
   Serial.println("Erroropeningfile..");
}
```

APPENDIX A
HARDCODING THE GENESIS BLOCK ONTO THE DEVICE

Genesis.ino

```
#include <ArduinoJson.h>

#include <ESP8266WiFi.h>
#include <WiFiClient.h>
#include <ESP8266WebServer.h>
#include <ESP8266HTTPClient.h>
#include <ESP8266httpUpdate.h>
#include <ESP8266mDNS.h>
#include <Crypto.h>
#include <SPI.h>
#include <SD.h>

voidsetup() {

  SD.begin(4);

voidloop() {
  //putyourmaincodehere,torun
      repeatedly:
}
```

CONCLUSION

With the proposed blockchain design we have proved that the security risks related to data sharing and reduction in data redundancy have been reduced by above 80%.In future more blockchain can be added and further reduction in redundancy rate can be achieved

ACKNOWLEDGMENT

We would like to express our deepest gratitude to our guide, Mr. M. Manigandan his valuable guidance, consistent encouragement, personal caring, timely help and providing me with an excellent atmosphere for doing research. All through the work, in spite of his busy schedule, he has extended cheerful and cordial support to us for completing this research work. J.Selvakumar has been of tremendous help for giving us a clear understanding of cryptography on embedded devices too.

Ethical Clearance: I testify on behalf of all co-authors that our article submitted to Indian Journal of Public Health Research & Development.

Title: Decentralized Internet of Things.

All authors: Jafar A. Alzubi, J.Selvakumar, Omar. A. Alzubi, R. Manikandan

1) This material has not been published in whole or in part elsewhere;

2) The manuscript is not currently being considered for publication in another journal;

3) All authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

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REFERENCES

[1] S. S. J. D. Tareq Ahram, Arman Sargolzaei and B. Amaba, "Blockchain technology innovations," IEEE Xplore Digital Library, 2016.

[2] J. MandritaBanerjee and K.-K. RaymondChoo, "A blockchain future to internet of things security: A position paper," IEEE Xplore Digital Library, 2016.

[3] M. Samaniego and R. Deters, "Blockchain as a service for iot," IEEE Xplore Digital Library, vol. 4, 2015.

[4] "Blockchain for the internet of things (white paper)," 2017.

[5] R. V. Pan Wang and Z. Li, "Introduction: Advances in iot research and applications," Springer, 2015.

[6] "IoT chain whitepaper," 2017.

[7] K. CHRISTIDI and M. DEVETSIKIOTIS, "Blockchains and smart contracts for the internet of things," IEEE ACCESS, 2016.

[8] M. M. Antonio. F.Skarmeta, Jose L.Hernandez-Ramos, "A decentralized approach for security and privacy challenges in the internet of things," IEEE World Forum on Internet of Things (WF-IoT), 2014.

[9] J. Z. Rodrigo Roman and J. Lopez, "On the features and challenges of security and privacy in distributed internet of things," Elsevier Computer Networks, 2013.

[10] A. V.Marco Conoscenti and J. C. D. Martin, "Peer to peer for privacy and decentralization in the internet of things," IEEE/ACM 39th International Conference on Software Engineering Companion (ICSE- C), 2017.

Self Reported Oral Pain and Dysfunctions Associated with Radiation Induced Oral Mucositis among Head and Neck Cancer Patients - A Prospective Observational Study

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ABSTRACT

Objectives: This study aimed at identifying and describing self reported oral pain and dysfunctions associated with oral mucositis among patients with head and neck cancer treated with radiation .

Method: A prospective observational study was conducted on 30 head and neck cancer patients who were undergoing radiation therapy and consented to participate. Data was collected by using a baseline proforma, WHO Oral Toxicity scale and Patient reported oral mucositis symptom scale .The patients were followed up during the entire course of radiation therapy and were monitored for the development of oral mucositis and were asked to rate their pain and associated dysfunctions on 100 mm scale.

Result: The present study findings revealed that all the subjects developed oral mucositis at the end of third week which was progressed to grade 3 or 4 mucositis by the end of therapy. The scores of oral pain and oral dysfunctions were progressively increased during the course of treatment with its peak at the end of treatment. Cumulative dose of radiation therapy and receipt of concurrent chemotherapy were found to have a significant association with oral dysfunction. The study also noted a significant positive correlation with severity of mucositis and oral dysfunctions.

Conclusion: Severe and painful oral mucositis is a dose limiting adverse effect of radiation therapy and its detrimental effect on oral functions is significant .Hence adequate preventive measures of mucositis to be identified to enhance the quality of life of head and neck cancer patients receiving radiation therapy.

Keywords: *oral pain, oral dysfunctions, head and neck cancer patients*

INTRODUCTION

Head and neck cancer treatment generally consists of a combination of radiotherapy, surgery, and chemotherapy. Majority of patients with head and neck cancer exhibit local or regionally advanced disease and hence the treatment option is usually a multimodal therapy including radiotherapy, surgery, and chemotherapy to achieve maximum tumour control. This multimodal and aggressive cancer therapy causes a plethora of short-term and long-term oral and oropharyngeal sequelae which adversely affects the oral functions and results in poor quality of life [1].

Oral Mucositis is the most common and debilitating reaction arising from the cancer treatment. Mucositis is described as an inflammation of the oral mucosa resulting from chemotherapy or ionizing radiation[1]. Earliest manifestation oral mucositis appear with a dosage of radiation equivalent to 1000 CGY, which generally occurs in the first week of treatment and clinically manifests as a rash in the oral mucosa which

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often progresses to skin loss and ulceration. Ulcers are typically covered by a white pseudo membrane[2, 3].

Mucositis is a clinical challenge with an incidence of 36-100% among head and neck cancer patients receiving radiation therapy[4] and is a major burden on head and neck cancer patients as it adversely affects the oral functions. Oral pain associated with radiation therapy induced mucositis is a significant contributor to emotional distress and often leads to lower food intake potentially resulting in undernourishment and weight loss[5] .In radiotherapy-induced mucositis, the pain intensity escalates at week 3 ,peeking at week 5 and persists for weeks. Pain is intense and interferes with oral functions[6] and results in dry mouth, dysgeusia , dysphagia , altered speech , and chewing or eating difficulties[7].

Oral mucositis, pain, dysphagia, and altered taste perception may lead to loss of appetite, malnutrition , reduced treatment compliance and radiation treatment breaks with a possible interference on tumour control. A systematic review conducted on literature on pain in chemo radiation therapy treated head and neck cancer patients reported that mucositis pain is frequent (80%), and interfere with daily activities (40%) and social activities (60%) [8]. These consequences greatly affect the patient's total quality of life[9] and may lead to severe disability which can increase the costs of care, tube feeding, extended hospitalization, or unanticipated re-hospitalization, and can cause modification to or interruption of the cancer therapy itself [10].

Hence an investigation of self reported oral pain and oral dysfunctions associated with radiation induced oral mucositis could help to reduce the patients anxiety by explaining them how the oral pain will affect their oral function .Addressing the patient reported clinical outcomes during cancer therapy helps the care givers to identify and institute possible novel protocols for reducing and managing the complications during head and neck irradiation .

METHOD

Population and sampling of study

The study employed a descriptive approach and 30 histopathologically confirmed patients of head and neck cancer scheduled to receive radiotherapy at Sri Shankara Cancer and research institute, Bangalore were selected randomly as per the inclusion criteria. Exclusion criteria included patients younger than 18 years, patients who had oral surgery within the previous 6 weeks, and patients with co morbid conditions. The study was approved by the hospital ethical committee. Informed written consent was obtained from the subjects and the subjects were followed up during the entire course of radiation therapy

DATA COLLECTION METHODS

Base line Performa was used to collect demographic data and the clinical data regarding the site of cancer, daily and cumulative dose of radiation therapy, type of chemotherapy medications were obtained from the subjects' medical records. The clinical manifestations of oral mucositis was evaluated by WHO toxicity criteria every week till the end of radiation therapy and was graded in to 4 categories ranging from 0-4 based on soreness, erythema, presence of ulcerations, ability to swallow solid food and the extent to which alimentation is not possible. At each clinical examination the participants completed a Patient reported oral mucositis symptom scale (PROMS) to appraise how oral mucositis affected common oral functions. The PROMS scale consists of 10 questions that are answered on a visual analogue scale (VAS), by setting a mark on each horizontal line measuring 100 mm. Two questions focused on mouth pain and change in taste and the other 8 questions focused on how much their mouth sores affected different oral functions on the day of the clinical examination.

DATA ANALYSIS

The data were typed into spreadsheet and SPSS (Statistical package for Social Science, IBM Corporation) version 23 was used for statistical analysis. Descriptive statistics using frequency, mean and standard deviation were employed to summarize the demographic and clinical characteristics of the participants and severity of oral pain and dysfunctions. Chi square test was computed to determine the association between oral dysfunctions and clinical characteristics .Spearman rank correlation was computed to identify the correlation between oral mucositis and oral dysfunctions.

RESULTS

Description of characteristics of the subjects

A total of 30 patients who fulfilled the eligibility criteria were included in the study and the study comprised of 77% males. The mean age was 60.9 years and 23% of them were educated up to higher secondary. The participants were diagnosed with carcinoma of oral cavity (63%), Laryngeal cavity (23%) and pharyngeal cavity (13%). A high proportion of tumours were classified as T2 (44%), followed byT3 (30%); T4 (23%) and T1 (3%) and 92% of the subjects had undergone surgical resection of the primary tumours site. Majority (47%) of the patients received a cumulative dose of 70 Gy and a fractionated dose of 2Gy per day (73%). 46.66% of the subjects received concurrent chemotherapy during radiation therapy and all of them received Cisplatin and 5 Fluorouracil.

Onset and severity of oral mucositis

The analysis revealed that the early manifestations of oral mucositis (grade 1 or grade 2) appeared in 43% of the patients by the end of first week and all the subjects developed Radiation induced oral Mucositis at the end of third week. Grade 3 or 4 mucositis was present in all the subjects by seven weeks and Ryles tube feeding was initiated for 4 subjects who developed grade 4 mucositis. (Fig1) The cheeks, lower lip and ventral and lateral tongue were the predominant sites of ulceration. Mean mucositis score was increased from 0 at baseline to 3.4 ± 0.31 at the end of the treatment. (Fig2)

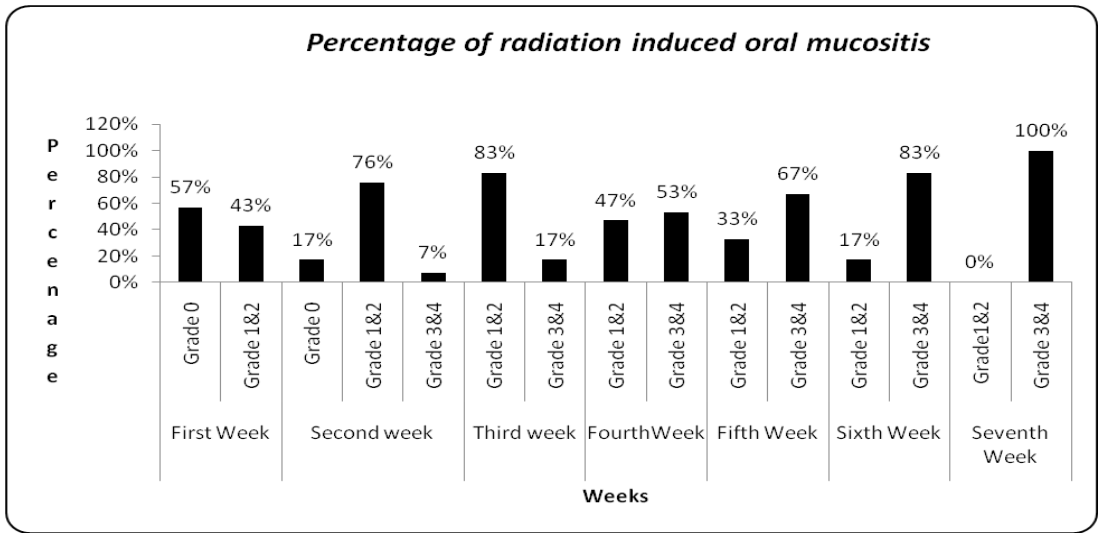


Fig1. Onset and severity of radiation induced mucositis

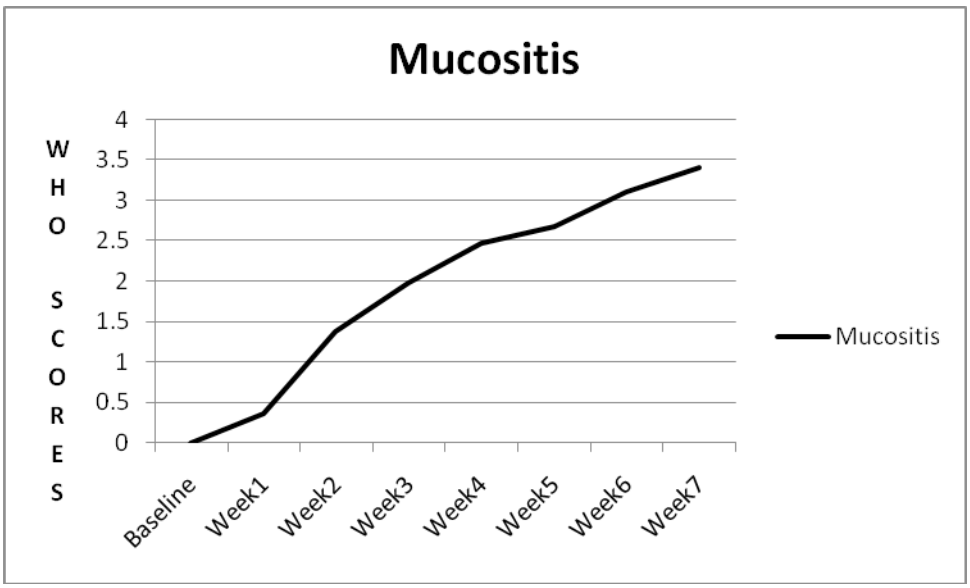


Fig 2: Mean mucositis scores during the course of radiation therapy

Oral pain and oral dysfunctions associated with radiation induced oral mucositis.

The scores of all the ten components of PROMS increased gradually during the cancer treatment period. Oral pain scores associated with mucositis were progressively increased from 17.3± 1.53 at the end of first week to 75.45 ± 1.95 at the end of seven weeks. Ninety percent of the subjects (n=27) were taking analgesic medication more or less constantly during the course of radiation therapy and despite this medication the participants reported consistently increasing pain score throughout the entire period of radiation therapy. Difficulty eating hard foods, Change of taste and difficulty in swallowing were considerably more affected by oral mucositis than the other components of the Patient reported oral mucositis symptoms scale (Table.1)

Table1: Comparison of mean scores of oral dysfunctions during the course of radiation

SL No	Items	First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Mean score
1	Oral pain	17.3	26.6	38.65	46.14	57	63.93	75.45	46.44
2	Difficulty speaking	16.13	22.3	32.73	40.7	49.7	57.15	63.25	40.28
3	Restriction in speaking	8.33	17.66	28.88	48.13	56.17	59.85	60.25	39.9
4	Difficulty eating hard food	23.16	35.46	53.3	64.1	78.8	85	88.9	67.59
5	Difficulty eating soft food	13.56	21.1	36.07	46.8	58.93	66.2	77.1	45.68
6	Restriction in eating	11.37	20.9	38.7	50	66.2	66.56	69.6	46.19
7	Difficulty in drinking	12.7	18.8	32.67	41.33	48.76	55.43	60.45	38.59
8	Restriction in drinking	9.2	17.16	29.06	37.56	48.4	54	56.25	35.95
9	Difficulty in swallowing	15.46	27.26	40.5	54.26	65.06	75.8	82.85	51.59
10	Loss of taste	17.4	27.76	50.56	63.7	76	85.03	91.47	58.84

Chi square analysis revealed a significant association between daily & cumulative radiation dose and receipt of concurrent chemotherapy with oral dysfunction among head and neck cancer patients (Table2). Spearman rank correlation was computed and revealed a significant correlation between mucositis and prom aggregate score at the end of therapy (r=0.79, p=0.02)

Table 2: Association between selected clinical variables and oral dysfunction among head and neck cancer patients N=30

Variables	Less than median	More than median	Chi value	P value
Age in years				
40-60	5	8	0.832	0.858
>60	6	11		
Site of cancer		10		
Oral	9	4		
Pharyngeal	0	5	3.45	0.178
Laryngeal	2			
Treatment				
RT	10	6	5.12	0.023
CT+CT	3	11		
Daily RT Dose				
1.8-2 Gy	9	5	5.67	0.017*
>2Gy	4	12		
Cumulative RT Dose				
60 Gy	5	2	10.28	0.006**
61-70	5	4		
>70Gy	1	13		

*significant at0.05, **significant at 0.01

DISCUSSION

Head and neck irradiation is often accompanied by significant and profound acute oral mucositis which adversely affects many of the oral functions. Clinical significance of oral mucositis is attributed to a complex interaction of the patient's perception of the altered oral function[11].The present study observed that all the subjects developed oral mucositis by the end of 3 rd week and intensified in severity by sixth or seventh week of radiation therapy. This finding supports the previous findings that oral mucositis is a distressing condition which is observed at the first week of head and neck irradiation and reaches its maximum intensity at the end of the treatment[12].

The suffering caused by oral mucositis is multifaceted. Pain due to oral mucositis is the most frequently reported patient related complaint affecting quality of life[9]. In the present study the subjects reported a consistently increasing pain score during the course of radiation therapy despite the intake of analgesics. These findings corroborates the observations made by a previous prospective observational study conducted on head and neck cancer patients which reported a poor pain control and high intake of analgesics during radiation therapy indicating neuropathic nature of pain which adversely affect activities of daily living and require a greater dose of analgesics[13].

Severe and painful oral mucositis is associated with a high level of oral dysfunctions and in general, the most common presentations of oral mucositis-related dysfunction are dysphagia, changes in food tastes, dry mouth, eating and drinking difficulties and inability to speak[12, 14]. It is observed in the current study that the oral functions eating, drinking, swallowing, speaking and sense of taste were affected and change of taste, difficulty eating hard foods and difficulty in swallowing were the most affected dysfunctions. Previous studies have reported similar findings that dysphagia (88%), change of taste (92%) [12], and difficulty in eating solids (90%) [13, 15] were the most prevalent oral dysfunctions among patients during head and neck irradiation. A cumulative dose of more than 30 Gy can cause damage to the taste buds and salivary glands and impairs the individual's ability to detect basic tastes (sweetness, sourness, saltiness, and bitterness)[16].

Dysphagia combined with loss of taste can interfere with the patients' food and fluid intake with significant weight loss and malnutrition[12].

Cumulative radiation dose and receipt of concurrent chemotherapy were found to have a significant association with the severity of oral dysfunctions (p =0.01)and the study also observed a significant positive correlation between mucositis and oral dysfunctions. Similar findings have been reported in a cross sectional study on eighty eight head and neck cancer patients with oral mucositis receiving cancer therapy in which intensity of oral mucositis were significantly correlated with distress score of oral dysfunction (r = .791, P < 001) ,dysphagia (r = .513, P < 001) and loss of taste (r = .578, P < 01)[12]. The findings indicate that oral mucositis adversely affects oral functions and an altered oral function is common sequelae of oral mucositis.

The current study is limited to a small sample size of 30 and to a single setting. The study was aimed to quantify the severity of oral dysfunctions perceived by head and neck cancer patients during radiation therapy and hence did not involve any objective measurement of these dysfunctions .

CONCLUSION

The findings have demonstrated that the intensity of oral dysfunction during head and neck irradiation were moderately high from the patients' point of view and hence increased attention and treatment of oral dysfunctions are essential to achieve the best outcomes of cancer therapies.

Conflicts of Interest: The authors declare no conflict of interest

Ethical Clearance: The study was approved by the hospital ethical committee. Patients and their caregivers indicated their willingness to participate in the study after the details of the study had been explained to them. The subjects were also informed that they had the right to withdraw from the study at any time during the course of the study and written informed consent was obtained from them.

Source of Support: Self

REFERENCES

1. Sciubba JJ, Goldenberg D. Oral complications of radiotherapy. Lancet Oncol 2006; 7:173-85.
2. Elting LS, Keefe DM, Sonis ST, Garden AS, et al. Patient-reported Measurements of Oral Mucositis in Head and Neck Cancer Patients Treated With Radiotherapy With or Without Chemotherapy and Impact on Quality of Life. 2008;(October):2704–13.
3. Volpato LER, Silva TC, Oliveira TM, Sakai VT, Machado MAAM. Radiation therapy and chemotherapy-induced oral mucositis. Braz J Otorhinolaryngol. 2007;73(4):562–8.
4. Arora H, Pai KM, Maiya A,Vidyasagar MS, Rajiv A. Efficacy of He-Ne Laser in the prevention and treatment of radiotherapy-induced oral mucositis in oral cancer. Oral Surg Oral Med Oral Pathol Oral Radio endode 2008;180–7.
5. Hébuterne X, Lemarié E, Michallet M, Montreuil CB De, Schneider SM. Prevalence of Malnutrition and Current Use of Nutrition Support in Patients With Cancer. 2014; Journal of Parenteral and Enteral Nutrition 2014 ;Volume 38 (2)Feb 196 –204
6. Nasiloski KS, Paula A, Gomes N. Oral complications in patients receiving head and neck radiation therapy: a literature review.Odontol. 2014 ; vol.62(4):395-400
7. Epstein JB, Frcd C, Robertson M, Emerton S, Phillips N, Stevenson-moore P, et al. Quality of life and oral function in patients treated with radiation therapy for head and neck cancer. Head Neck 2001;(May):389–98.
8. Mirabile A, Airoldi M, Ripamonti C, Bolner A, Murphy B, Russi E, et al. Critical Reviews in Oncology / Hematology Pain management in head and neck cancer patients undergoing chemo-radiotherapy : Clinical practical recommendations. Crit Rev Oncol / Hematol [Internet]. Elsevier Ireland Ltd; 2016;99:100–6. Available from: http://dx.doi.org/10.1016/j.critrevonc.2015.11.010
9. Epstein JB, Beaumont JL, Gwede CK, Murphy B, Garden AS, Le Q, et al. Longitudinal Evaluation of the Oral Mucositis Weekly Questionnaire-Head and Neck Cancer , a Patient-reported Outcomes Questionnaire. Cancer 2007; 109: 1914–22.
10. Elting LS, Cooksley CD, Chambers MS, Garden AS. Bonu s 1.6 C Issue Es. Int J Radiat Oncol [Internet]. 2007 Jul;68(4):1110–20. Available

from: http://linkinghub.elsevier.com/retrieve/pii/S0360301607002477

11. Kwong KK There A, Approaches N. Prevention and Treatment of Oropharyngeal Mucositis Following Cancer Therapy : Are there new approaches? Cancer Nurs. 2004;27(3). 183-205.
12. Cheng KK. Oral mucositis , dysfunction , and distress in patients undergoing cancer therapy. J Clin Nurs 2007; Nov;16(11):2114-21
13. Gussgard AM, Jokstad A, Wood R, Hope AJ , Tenenbaum H. Symptoms Reported by Head and Neck Cancer Patients during Radiotherapy and Association with Mucosal Ulceration Site and Size : An Observational Study. PLos ONE2015; 10(6): e0129001. https://doi.org/10.1371/journal.pone.0129001
14. Öhrn KEO, Dalarna H, Sciences C. Experiences of oral care in patients with haematological malignancies or head and neck cancer.European Journal of Cancer Care 2003; Vol 12(3): 274-82
15. Cartmill Beena, Ward Elizabeth, Macginley Olivia, Porceddu Sandro. Oral Health, Dysphagia, Distress, and Health Service Needs of Head and Neck Cancer. Survivors 5 Years Post-Chemo radiotherapy . Int J Oral Dent Health 2016;2(1):1–9.
16. Lesan S, Alirezaei S, Aminnia M, Kazemian A. The Prevalence of Oral Complications in Patients undergoing head and Neck Radiotherapy. Advances in Bioresearch 2016;7(March):86–92.

A Comparative Evaluation of Antibiotic Susceptibility Pattern of Methicillin Sensitive and Methicillin Resistant *Staphylococccus Aureus* Isolated from Clinical Specimens in a Tertiary Care Hospital

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ABSTRACT

Introduction: *Staphylococcus aureus* is a major human pathogen that causes a variety of suppurative (pus-forming) infections had evolved as a major cause of hospital acquired (nosocomial) infection humans. MRSA, a resistant variant of *Staphylococcus aureus* is resistant various classes of antibiotics such as penicillins, methicillin, cephalosporins and the fluoroquinolones, it is often reflected as a superbug. Methicillin-resistant *Staphylococcus aureus* (MRSA) posed to be an uncontrollable, nuisance in serious public concern. Vancomycin, a glycopeptides is the treatment of choice for MRSA infections. In recent times development of vancomycin resistant *Staphylococcus aureus* (VRSA), strains all around the world with highest being reported in health-care settings, the alternatives are Oxazolidons which includes Linezolid and polycyclic compounds such as Tetracycline and Tigecycline. **Materials and Method:** A total 620 *Staphylococcus aureus* isolates employed in this study was conducted at Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry All the isolates were collected between July 2014 to April 2017 in the study. All the isolates were identified as *S. aureus* by culture and biochemical tests which included test for clumping factor, free and bound coagulase and mannitol fermentation. **Results:** The average resistance seen in Ampicillin(93.8%), Cephelexin(88.6%), Cefotaxime(86.4%), Cloxacillin (17.9%), Erythromycin(82.4%), Gentamicin(10%), Ciprofloxacin(50.2%), Clindamycin(44.6%), Penicillin(90.5%), Tetracycline(84.8%). **Conclusion:** The present study shows health care institutions face constant and evermore problems because of *Staphylococcus aureus* . Active screening and proper infection control procedures need to be adopted to control *Staphylococcus aureus* infection.

Keywords: *Staphylococcus aureus, MRSA,Antibiotic resistance.*

INTRODUCTION

Staphylococcus aureus belongs to the family *Micrococcaceae* and is part of the genus *Staphylococcus*, which contains more than 30 species such as *S.epidermidis*, *S. saprophyticus* and *S. haemolyticus*. Among the *staphylococcal* species, *S. aureus* is by far the most virulent and pathogenic for humans.

S. aureus is a 1 μm, Gram-positive cell that in the laboratory may be observed as single cells, in pairs or as grape-like irregular clusters. It is characterized as coagulase and catalase positive, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow *staphylococci*.[1]

Alexander Ogston presented in 1880 at the Ninth Surgical Congress in Berlin his work establishing

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the causative role of bacteria in wound infection and subsequent septicemia. Building on the teachings of his senior contemporaries,Louis Pasteur and Joseph Lister,Ogston had observed pus from 88 human abscesses under his microscope and noted Gram-positive spherical "micrococci" [2 3] .Ogston named them staphyle, the Greek expression for a bunch of grapes. In 1884, Rosenbach succeeded in isolating yellow bacterial colonies from abscesses and named them *Staphylococcus aureus*, "aureus" from the Latin word for golden.[4]

S. aureus has the ability to adapt to different environments and it may colonize the human skin, nails, nares and mucus membranes and may thereby disseminate among recipient host populations via physical contact and aerosols[5]. Colonization with *S. aureus* is an important risk factor for subsequent *S. aureus* infection.[6] [7] *Staphylococcus aureus* has been recognized historically as a virulent and important humanpathogen.[8]*S. aureus* causes a variety of infections ranging from skin and soft tissue infections to invasive diseases such as bacteremia, endocarditis, pneumonia,visceral abscesses, osteoarthritis and septicaemia.[9]

MRSA, a resistant variant of *S. aureus* is resistant to various classes of antibiotics it is often referred to as a super-bug.During the last three decades, many articles have been published describing and debating the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA).[10]This work was stimulated by the ongoing impact of infections caused by MRSA and the difficulties in preventing or treating them.[11] [12] Nevertheless, uncertainties regarding the transmission and control of MRSA remain.[13] For instance, new epidemic strains of multi resistant *S. aureus* have continued to emerge and decline for unknown reasons in different places since 1961.[14] [15] [16] It remains unclear why some European countries such as the UK are affected by the rising occurrence of epidemic invasive strains of MRSA,[17] whereas countries like Denmark have nearly abolished their MRSA problem.[18] Moreover, some local MRSA outbreaks have disappeared without any particular control efforts or antibiotic restriction,[19] while others institutions report MRSA propagation or reappearance despite various attempts to contain it.[16]

Almost 40 years after the first report of MRSA in the UK, the study by Farrington and colleagues published in this issue of QJM, describes another astonishing MRSA experience, and presents new insights into the never-ending struggle to prevent MRSA infections. Several aspects of the retrospective study by Farrington and co-authors attract our attention. First, this paper describes in a very detailed and careful manner a 12-year experience with MRSA at Addenbrooke's Hospital in Cambridge.[20] The authors demonstrate that sporadic or epidemic strains of MRSA could be controlled over 10 years through the use of a stringent, but labour-intensive control policy including screening of high-risk patients, transfer of patients to isolation rooms, and ward closure to new admissions in case of MRSA transmission. Since 1993, however, an increase of patients admitted with MRSA halted the initial `search and destroy' strategy, resulting in a dramatic hospital-wide MRSA outbreak.[21]

The present study was designed to investigate the incidence of a comparative evaluation of Antibiotic susceptibility Pattern of Methicillin sensitive and Methicillin resistant *Staphylococccus aureus* isolated from clinical Specimens in a Tertiary Care hospital.

MATERIALS AND METHOD

This study was carried out to determine the antimicrobial susceptibility pattern of Methicillin sensitive and Methicillin resistant *Staphylococcus aureus isolated from different clinical specimens (Table: 1)* between April 2014 to January 2018 in the Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, pondicherry were included in the study.

Isolation of *Staphylococcus aureus* from clinical samples: A total of 620 consecutive isolates of *S.aureus* were isolated from clinical specimens. All the specimens were inoculated on nutrient agar, blood agar and incubated at 37°C overnight. The biochemical tests such as haemolysis, Gram's stain, catalase, coagulase, and sugar fermentation were done for further confirmation.[22]

Antibiotic Susceptibility test by disc diffusion assay:

The antimicrobial sensitivity testing was carried out by disc agar diffusion technique using Mueller-Hinton agar plates and the results interpreted according to clinical laboratory standards international (CLSI) guidelines.[23] [24] The clinical isolates of *S. aureus* were Gram-stained before the test. The *S. aureus* cultures were inoculated in peptone water and incubated at 37°C, the inoculums was adjusted to a turbidity equivalent of

0.5 McFarland standard. The entire agar surface was swabbed. The following antibiotic discs were applied Ampicillin (10mcg), Cephelexin (30mcg), Cefotaxime (30mcg), Vancomycin (5mcg), Erythromycin(30 mcg), Gentamicin (10mcg), Ciprofloxacin(10 mcg), Clindamycin (2mcg), Penicillin(10 units), Tetracycline (30mcg) and the plates were incubated at 37°C and examined after 18–24 h.

Identification of MRSA

All the isolates of *Staphylococcus aureus* isolates were subjected to Cefoxitin disc diffusion testing using a 30µg Cefoxitin disc.[24] The results were interpreted according to CLSI guidelines 2014. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and ≥ 22 mm was reported as methicillin sensitive.Since Kirby Bauer disc diffusion method is not recommended for susceptibility testing of *Staphylococcus aureus* to vancomycin, determination of minimum inhibitory concentration (MIC) has to be done either by broth dilution method.

RESULTS

Out of 620 clinical isolates 363(58.3%) patient were from male and 257(41.4%) were female patients. Majority of the patients were age of 61-70 years. The distribution of clinical specimens were given in the below table (Table- 1). Of the 620 isolates of *Staphylococcus aureus*, 268 isolates were found to be methicillin-resistant *Staphylococcus aureus* (MRSA) and 352 were MSSA. The prevalence of MRSA and MSSA were 43.2% and 56.7% respectively.

Table- 1: Characteristics of specimen (n=620)

S.No	Characters	No	Percentage
1.	**Age(yrs):**		
	1-10	24	3.8 %
	11-20	32	5.1 %
	21-30	86	13.8 %
	31-40	72	11.6%%
	41-50	104	16.7%
	51-60	78	12.5%
	61-70	126	20.3%
	71-80	98	15.8%
2.	**Sex:**		
	Male	363	58.5%
	Female	257	41.4%
3.	**Distribution of samples:**		
	Urine	192	30.9%
	Pus	167	26.9%
	Wound	33	5.3%
	Nasal swab	19	3%
	Throat swab	21	2.5%
	Sputum	96	15.4%
	Blood	37	5.9%
	Cathertip	09	1.4%
	Vaginal swab	04	0.6%
	Bodyfluids	42	6.7%

Table 2:Antibiotic Susceptibility profile of *Staphylococcus aureus* from clinical specimens (n=620)

S.No	Antibiotic	Sensitivity of MSSA No% (n=352)	Resistant of MSSA No % (n=352)	Sensitivity of MRSA No% (n= 268)	Resistant of MRSA No % (n= 268)
1	Ampicillin(10mcg)	28(7.9%)	324 (92%)	256(95.5%)	12(4.4%)
2	Cephelexin (30 mcg)	58(16.4%)	294(83.5%)	64(23.8%)	204(76.1%)
3	Cefotaxime(30mcg)	84(23.8%)	268(76.1%)	146(54.4%)	122(45.5%)
4	Vancomycin(30mcg)	352(100%)	0	259(96.6%)	9(3.3%)
5	Erythromycin(30 mcg)	38(10.7%)	314(89.2%)	253(94.4%)	15(5.5%)
6	Gentamicin (10mcg)	40(11.3%)	312(88.6%)	58(21.6%)	210(78.3%)
7	Ciprofloxacin(10 mcg)	204(57.9%)	148(42%)	112(41.7%)	156(58.2%)
8	Clindamycin (2mcg)	308(87.5%)	44(12.5%)	198(73.8)	70(26.1%)
9	Penicillin(10 units)	0	352(100%)	0	268(100%)
10	Tetracycline (30mcg)	194(55.1%)	158(44.8%)	68(25.3%)	200(74.6%)

DISCUSSION

MRSA is a major nosocomial isolate in hospitals which is responsible for higher morbidity and mortality. (Costantini). MRSA strains are resistant to several antibiotics like macrolides, lincosides, aminoglycosides and beta-lactum penicillin and cephalosporins. The MRSA isolates subjected to antibiotic sensitivity pattern in the present study reveals that the isolates have also developed resistance to other antibiotics tested.

The present study showed that males had a higher isolation rate of *S. aureus* than females. Different studies have depicted variations in the prevalence rates of MRSA in different countries. The overall relative proportion of MRSA was 43.2%. The overall relative proportion of MSSA was 56.8% similar findings were seen by Arora.S et al [25] from North India, reported 46% MRSA.

The isolates of MRSA and MSSA exhibited 100 percent resistance to Penicillin similar results were reported by Chandrashekar et al.,[26] in contrast a study by Jayatilleke K et al reported 17% sensitivity.[28] The lowest drug resistant was observed for Vancomycin (3.3%), where as a study from North India by Ankur Kumar et al [27]reported 100 percent sensitivity.

CONCLUSION

Regular surveillance of antibiogram of both community acquired and hospital-acquired infections of *staphylococcus aureus* is required in formulating and monitoring the antibiotic policy. As these pathogens acquire resistance to new classes of antimicrobial agents controlling the usage of glycopeptides and linezolid is necessary in avoiding the emerging resistance trends.

only in MRSA cases should be encouraged

Preservation of glycopeptides and linezolid for use

only in MRSA cases should be encouraged

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REFERENCES

1. Washington C Winn,Elmer W Koneman,et al.Koneman's Color Atlas and Textbook of Diagnostic Microbiology LippincottWilliams & Wilkins, Philadelphia.2006.
2. Ogston A. On Abscesses. Rev Infect Dis. 1984; 6:122–128.
3. Ian A. Myles,Sandip K. Datta, *Staphylococcus*

aureus: an introduction.Semin Immunopathol. 2012 March ; 34(2): 181–184.

4. Ogston A .*Micrococcus* Poisoning. J Anat Physiol.1882;17:24-58.
5. Lowy, F.D., *Staphylococcus aureus* infections. N Engl J Med 1998; 339:520-532.
6. Wertheim, H.F., Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 2000;364:703-705.
7. Williams, R.E.,Healthy carriage of *Staphylococcus aureus*: its prevalence and importance.Bacteriol Rev 1963; 27:56-71.
8. Von Eiff, C.,et al,Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med, 2001 ;344:11-16.
9. Baron, E J. The detection, significance andrationale for control of methicillin –resistant *Staphylococcus aureus*. Clinical Microbiology News letter.1992; 14:129-135.
10. Latha JP, Rajendran S, Uma maheswari G, Sumathi.Characterization of Methicillin-Resistant *Staphylococcus aureus* in a Tertiary Care Teaching Hospital, South India. J Med Microb Diagn 2014:3(6):169. doi:10.4172/2161-0703.1000169.
11. Michel M, Gutmann L. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *enterococci*:therapeutic realities and possibilities. Lancet 1997; 349:1901-1906.
12. Harbarth S, Rutschmann O, Sudre P, Pittet D. Impact of methicillin resistance on the outcome of patients with bacteremia caused by *Staphylococcus aureus*. Arch Intern Med 1998; 158:182-189.
13. Pittet D, Waldvogel FA. To control or not to control colonization with MRSA...that's the question! Q J Med 1997; 90:239-241.
14. Ridley M, Lynn R, Barrie D, Stead KC. Antibiotic-resistant *Staphylococcus aureus* and hospital antibiotic policies. Lancet 1970;1:230-233.
15. Cafferkey MT, Hone R, Coleman D, et al. Methicillin-resistant *Staphylococcus aureus* in Dublin 1971-1984. Lancet 1985;2:705-708.
16. Ayliffe GA. The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 1997; 24(1):74 -79.
17. Speller DC et al. Resistance to methicillin and other antibiotics in isolates of *Staphylococcus aureus* from blood and cerebrospinal fluid, England and Wales, 1989-1995. Lancet 1997; 350:323-325.
18. Rosdahl VT, Knudsen AM. The decline of methicillin resistance among Danish *Staphylococcus aureus* strains.Infect Control Hosp Epidemiol 1991; 12:83-88.
19. Kayser FH. Methicillin-resistant *staphylococci* 1965-1975. Lancet 1975; 2:650-653.
20. Jevons MP. Celbenin-resistant *staphylococci*. Br Med J 1961; 1:124-125.
21. Farrington M, Redpath C, Trundle C, Coomber S, Brown NM. Winning the battle but losing the war: 12 years of methicillin-resistant *Staphylococcus aureus* infection at a teaching hospital. Q J Med 1998; 91:539-548.
22. Bailey & Scott's, Diagnostic Microbiology, 20th edition, chapter 13, 222.
23. Bauer, A. W., Kirby, W.M. M. and Sherris, J. C. Antibiotic susceptibility testing by a single disc method. AM. J. Pathol., 1966; 45: 493-496.
24. Clinical and Laboratory Standards Insititute, Performance Standards for Antimicrobial Susceptibility testing; 22 nd information supplement, M100-S22 Wayne, PA; 2014.
25. Arora S, et al.Prevalence of Methicillin – resistant *Staphylococcus aureus* in a Tertiary Care Hospital in Northern india.Journal of Physician.2010;2(2):78-81.
26. Chandrashekhar DK, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital in Gulbarga, Karnataka. Journal of Pharm Biomed Sci. 2012;19(6):1-3.
27. Ankur Kumar etal, Prevalence of Methicillin Resistant *Staphylococcus aureus* in patients admitted in a Tertiary Care Hospital of North India. International Journal of Advance Research and Innovation.2015:3(1):113-117.
28. Jayatilleke K, Bandara P. Antibiotic sensitivity pattern of *Staphylococcus aureus* in a tertiary care hospital of Sri Lanka.Sri Lanka Journal of Infectious Diseases 2012; 2: 13-17.

Efficient approaches forPrediction of Brain Tumor using Machine Learning Techniques

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ABSTRACT

Tumor is one of the most prevalentdiseases in the brain.It is the reason why the diagnosis and treatment of the brain tumor have critical importance. MRI (Magnetic resonance imaging) is a scientific maneuver used to produce a computerized image of internal body tissue. MRI image is a diagnostic approach is used for detection ofa brain tumor and classifies it as atype malignant and benign. There aredifferent processes to detect the brain tumor.Image processing, feature extraction, and many algorithms havebeen implemented for the detection of brain tumor, but a convincing and accurate technique to detect thetumor'sprecise position and to diagnose in minimal time is in great need. The execution and complexity involved in the medical image segmentation process are enhanced by applying feature extraction techniques and mathematical models. Furthermore, to improve the accuracy and performance of detection of brain tumor using existing Artificial Neural Network and Naïve Bayes Classifier, In this article we propose a novel technique using mathematical analysis to predict and enhance the better models. The proposed method has been implementedfor detecting the type of brain tumor and its specific location in the brain. The proposed system will be used to diagnose the patient's brain tumor with goodaccuracy success rate.

Keywords—*Magnetic Resonance Image, Braintumor, Malignant, Benign, Feature extraction, Preprocessing, Artificial Neural Network, Naïve Bayes Algorithm, Canny Edge Detection.*

INTRODUCTION

Brain tumor appears when an abnormal cell is formedin the brain,known as intracranial neoplasm. There is a dual category of brain tumor cancerous tumor or malignant and benign tumor.All category of brain Tumor may produce a syndrome that varies lean on the portion of the braininvolved. The origin of most of the brain tumor is unknown. The tumor iscategorized by the tumor's origin and if its malignancy as a benign and malignant.

Malignant or cancerous tumor consists of a cancerous cell that cannot be eliminatedquickly, eventually leading to death. Hence malignant are more harmful then benign. MRI is a widely used technique in neurosurgery& neuroscience. It gives a computerized view of tissue, thus helps to detect a human brain tumor. The MRI scanning is more appropriate than CT scanning for diagnosis; it does not affect the human body because it does not use any radiation.The Treatment of tumor starts with the doctor obtaining the family and personal medical history and performing a complete physical examination.The brainis dividedinto four parts - frontal lobe, parietal lobe, Temporal lobe, Occipital lobe,and the Cerebellum, pituitary gland and Brainstem..

The typical Existence rate for malignant brain tumor patients is only 34.7%. Nearly 16,620 people will expire from a malignant brain tumor in the year 2018 in the United States.In this paper,we useFuzzy C means, and K means for segmentation of the MRI Images.The artificial neural network is a proficiency classifier used to categorize the tumor. This paper is organized as follows: covers Literature Review, Analysis on recognition of tumor following the Proposed methodology, with expected output and finally the conclusion with acknowledgment.

LITERATURE REVIEW

Dr. Babuanto et al [1] the MRI brain image using anisotropic diffusion filter discrete wavelength transform based feature is extracted and the extracted feature are given as a input to the segmentation stage SVM(Support vector Machine) was used for tumor segmentation and classification. The result came as a showing output that brain tumor is either critical or normal stage further on basis of that treatment will be executed or the accuracy of proposed method is 86%.

Prof. Akshta, et al [2] they detect a brain tumor by thresholding method application of morphological operation and extraction of tumor region for further analysis and shrinking the image and calculation of the area is efficient parameter in detecting the stage of brain tumor patient. Histogram used to summarize the data, the lapsed time for entire program is 9.14 sec but not able to normalize the category of tumor and not applicable for color images.Warintorn phusomsai, et al [3] the investigation possibilities to improve the recognition and its exactitude performance is established against SVM and a traditional ELM that is 90% against 64% and 70%. Later to improve in H-ELM in aspect of computational complexity with high dimension and large data sets.. ELM with sigmoid accomplishes the finest performance the highest precision is around 70% comparable to hard limit, radial basis sine and triangular basis.

R Manikandan, et al [4] usingof clustering method of grouping a set of pattern into a number ofcluster. To intention anautomatic tool for brain tumor recognition using MRI image data sets. Classification region using their multi parameter value make the study of region of physiological and pathological interest and definable for future enhancement.Sanjeevkumar, et al [5] including hybrid approach includes discrete wavelet transform (DWT) to be usage for abstraction of feature. The suggested hybrid approach is functional to brain MRI images to classify that tumor is either malignant or benignant and the linear accuracy vary from 80% to 90%.B. Hema Kumar, et al [6] have refined a Non-invasive grading and prognosis prediction methodology using CT and MRI images that preclude the necessity of biopsy to certify the survival of tumor and there by helps the Diagnostic in accurate diagnosis. The result generated from the FETGS System. The FETGS proposed method as 92.6% sensitive and 82.38% specific with the entire image set in case of benign and malignant. Pawar,Ganbote et al[7] used feature extraction to obtain most relevant data from the unique data set KNN Algorithm and C means algorithm has been used for showing the space of interest. They overcome with the restriction of the existing system and enhancing the detecting the tumor and providing the size and space of the tumor. B Devkota, et al [8] they used mathematical morphological reconstruction. The image is being preprocessed to disregardthe noise and then segmented to find an interest point of region with probable tumor. The limitation in the separation method such as deprived accuracy and high computational rate, asubstitute method for segmentation was being proposed. NarkhadeSachin, et al [9] their objective is to perceive the location and boundary of tumor automatically. Based on symmetric and unsymmetrical region it able to define that whether the tumor is detected like if symmetric then brain tumor is weekend and when unsymmetrical then it is recognized as a brain tumor. MRI of healthy brain has an almost bilateral symmetry. Nilesh B, et al [10] they optimized extracted feature only the relevant feature are held for advance analysis thus lessen the numerical unpredictability of arrangement of cerebrum tumor.

Anupurba Nandi et al [11] uses K-means clustering where the perceived tumor display certain abnormality which is then modified by the use of morphological operator along with basic image processing technique to achieve the objective of splitting the normal cell with the tumor cell. EmanMaksoud et al [12] they introduced an well-organized image segmentation methodology using k-means clustering technology assimilated with Fuzzy c means algorithm. Following by thresholding and label set dissection stages to deliveraccurate brain tumor detection.Limitationisn't able to diminish the time strategy so it will propose to demonstrate in several experiments. Garima Singh, et al [13] Efficient MRI is done with the help of SVM and naïve Bayes Classifier algorithm to provide accurate prediction and classification of the brain tumor. Many algorithm has been implemented and the further research work is going on to get a better result to detect and recognize the tumor's place and to diagnosis it with help of MRI images and CT scan.

ANALYSIS ON RECOGNITION OF TUMOR

The segmented images are then subjected to feature extraction. The feature for the classified images is analyzed through the algorithm and [2] CT scan using

ionizing radiation while MRI using a strong magnetic field to arrange the nuclear magnetization followed by a change in the calibration of the magnetization by radio frequencies that can be detected by the scanner.

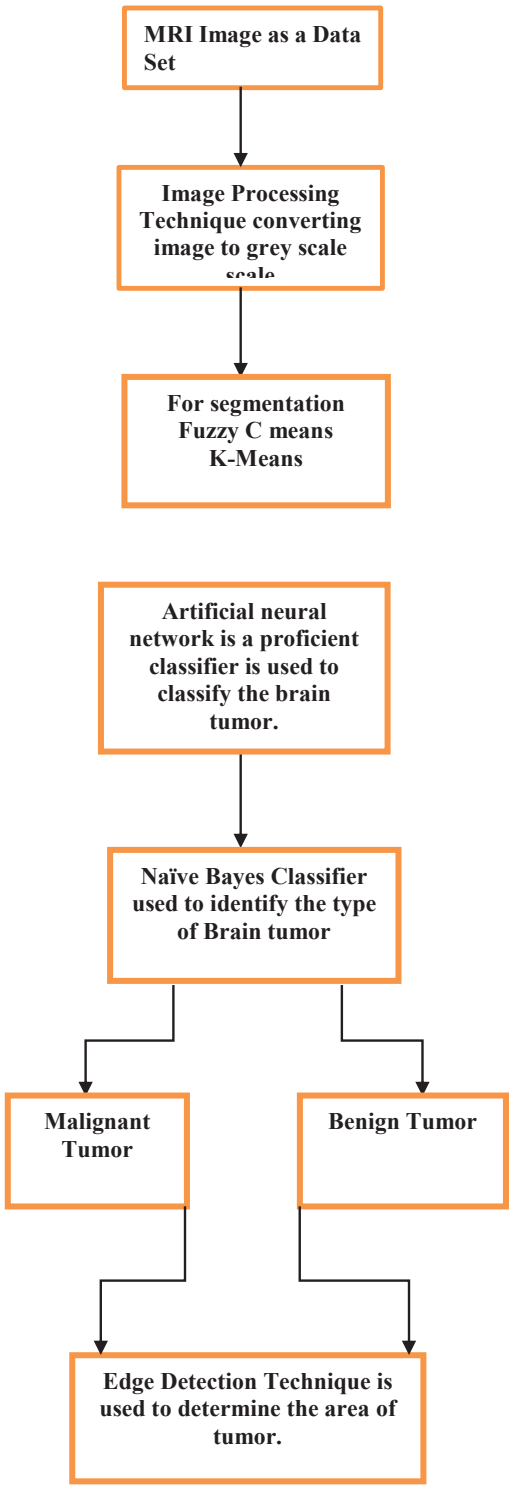


Figure 1. Architectural Diagram of the Proposed System

PROPOSED METHODOLOGY

The proposed methodology of brain tumor detection is implemented. The process to detect the tumor and classify it with the stage and forms of tumor. It is essential to know the category of tumor to diagnosed it, and to retrieved the stage then it is easy to rectify or to diagnose the tumor it need further process and as shown in figure 1 will standard to deliberate it in descriptive way.

MRI Image Data Set: The MRI image can usually be obtained from the patient's database on the computer when the person undergoes the MRI scanning.MRI images are usually black and white, single-channel images. Description of gray shading in a black and white image is done by reading the image as a grid of black spots on a white background vice-versa.

Image Preprocessing Technique: MRI image cannot be used directly as an input for the proposed system. The captured image be passed through the noise removal filter. With the assistance of a median filter, the image is being filtered, thus improving the image quality and increasing the accuracy.The median filter is less delicate than other outliers as the rate of every pixel is gained by the moderate of the near-by pixel.

Feature Extraction: [7] Feature Extraction is used to obtain the mostappropriate information from original data by using different techniques. This technique is used when the image size is large, and feature illustration is needed to complete the tasks quickly. [4]Segmentation it is to partition the filtered image into its constituent region or objects. It should end if point of interest has been isolated.

Analysis of Algorithm:An enormous number of algorithms has been executed to recognize the brain tumor and to classify the tumor and the stage.Artificial neural network and KNN algorithm is usedfor detecting the tumor and further,Bayes Classifier and edge detection techniques are used to classify and give a better accuracy in the detection of tumor. Genetic algorithms also arrange the solutions to search and optimization of the problems. These operators are crossover, mutation, and regeneration etc. The genetic algorithm originates with the initialization of the population which is an iterative system. The iteration is referred to as a generation and in each such generation; the capability is evaluated corresponding to each. In the optimization problem, the

fitness reflects the rate of the objective function in the optimization problem. K-NN Algorithm is also feasible because it combines k nearest points based on their distances and joins them in a cluster and these clusters are then evaluated. The fuzzy c means is also used topredict the tumor accurately.Mathematical models such as least square method, Entropy, Correlation and Cramer's rule to prophesyare also used to enhance a better system to detect the brain tumor.

Statistical features are used, except the features are applied as an input of Artificial neural network. The features are discussed below

Contrast: It is described as the separation of the darkest and brightness area of the image.

Contrast $=\sum_{i,j=0}^{n=1} P_{ij}(i-j)^2 \sum_{i,j=0}^{n=1} P_{ij}(i-j)^2$ ---------- (1)

Where P_{ij} = Probability i=training data j= testing data.

Correlation: It is computed into what is known as a correlation coefficient, which ranges under the value -1 and +1.

Correlation$=\sum_{i,j=0}^{n-1} P_{ij} \frac{(i-\mu)(j-\mu)}{\partial^2} \sum_{i,j=0}^{n-1} P_{ij} \frac{(i-\mu)(j-\mu)}{\partial^2}$ ---------- (2)

Homogeneity: Homogeneity iscalculated from gray scalelevel of image to simplify the classification.

Homogeneity$= \sum_{i,j=0}^{n-1} \frac{P_{ij}}{1+(i+j)^2} \sum_{i,j=0}^{n-1} \frac{P_{ij}}{1+(i+j)^2}$ ---------- (3)

Entropy: The entropy is extreme when everyentry in P_{ij} is equal, such as a matrix resembles to an image, when there is no preferred gray-level pair of the image.

Entropy $=\sum_{i,j=0}^{N-1} -\ln(P_{ij}) P_{ij} \sum_{i,j=0}^{N-1} -\ln(P_{ij}) P_{ij}$ ---------- (4)

Variance: Variance is regularly used to identify how much pixel varies from the neighboring pixel and used to classify in a different region.

Variance $= \sum_{i,j=0}^{N-1} P_{ij}(i-\mu)^2 \sum_{i,j=0}^{N-1} P_{ij}(i-\mu)^2$ ---------- (5)

Where,

Mean $\mu = \sum_{i=0}^{N-1} i, P(i) \sum_{i=0}^{N-1} i, P(i)$ ---------- (6)

Classification of tumor: SVM is one of the most prevailing classification algorithm which is competent of giving greater enactment in terms of certainty when compared with the other classification algorithms.. SVM classifier identifies the trouble by discovering out ahyperplane with biggest limit, i.e.the maximal limit of hyper-plane SVM has the certain property of concurrently minimizing the classification fault and higher range of the geometric limits. By reconstructing it into high dimensional area, it searches for a linear optimal unscrambling hyper-plane. With the support of Naïve Bayes Classifier, the brain tumor is classified as benign, malignant. Bayesian Classificationevaluates the class of unidentified data items using probabilistic statistical model. The challenge in Bayesian classification is to regulate the class of data sample which have some count of attributes. It evaluates the class of this undefined data item on the base of recognized data item which areproviding with the class label for training purpose [10].

Let consider D be the dataset of n objects such that $X_1, X_2, X_3 \ldots\ldots\ldots X_n$. Each objects has t attribute such that $A_1, A_2, A_3 \ldots\ldots\ldots A_t$. There are m classes $C_1, C_2, C_3 \ldots\ldots\ldots C_m$. Naïve Bayes Classifier predicted the unidentified data sample X which is without the label to the class C_i. if an only if

$P(C_i | X) > P(C_j | X)$ for $1 \leq j \leq m; j \neq i$. ---------- (7)

Then X is assigned to C_i. This is called Bayes Decision Rule.

Edge detection means finding the object boundary in an image. Canny Edge Detection technique is a conventional edge detection technique. It is improved techniques which doesn't trouble the feature of edges in an image. It is then applied to the propensity to find the edge and serious value of threshold. Canny Edge detection algorithm produced the same edge map. Scaling technique is used to detect the outer limit boundary of the tumor part to define its size by which it isable to know that the stage of tumor. As all the block is being discussedto detect the brain tumor and to resolve it,the next following section deal with the expected outcome of the proposed system to give a good accuracy result to recognize a brain tumor.

CONCLUSION & FUTURE WORK

Existing method provides the recognition of tumor with the assistance of MRI image, it's number of pixels and the space of tumor. The proposed system recognizes the exact size of tumor and space of tumor in brain using artificial neural network algorithm.Classification methods as a naïve Bayes classifier, canny edge detection technique for scaling purpose and mathematical model such as entropy contrast homogeneity and correlationis also used to improve the accuracy of the system. The proposed technique is useful to the physician for treatment of patients.The development of better techniques comes under future work. the existing systems for detection of brain tumor & the related algorithms which is used to detect brain tumour is viewed upon. Many algorithmsare proposed, but there is recognition of accurate size and stage of the tumor for curing it so that the abnormal cell never developis still a challenge.The proposed system used in the genetic algorithm and classification based technique to detect the tumor which gives an idea to physicians to cure the tumor. The future work will focus the analysis of the stored data with some mathematical formula which helpful to define the exact position of the tumor in brain.

Ethical Clearance: I testify on behalf of all co-authors that our article submitted to Indian Journal of Public Health Research & Development.

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REFERENCES

1. Mathew, A. R., &Anto, P. B. y), Tumor detection and classification of MRI brain image using wavelet transform and SVM. International Conference on Signal Processing and Communication (ICSPC), 2017: 75-78
2. Hunnur, S. S., Raut, A., &Kulkarni, S. Implementation of image processing for detection of brain tumors International Conference on Intelligent Computing and Control Systems (ICICCS), 2017: 278-283.
3. Phusomsai, W., So-In, C., Phaudphut, C., Thammasakorn, C., &Punjaruk, W. Brain tumor cell recognition schemes using image processing with parallel ELM classifications on GPU. International Joint Conference on Computer Science and Software Engineering (JCSSE), 2016: 1-6.
4. Manikandan, R., Monolisa, G. S., &Saranya, K. A Cluster Based Segmentation of Magnetic Resonance Images for Brain Tumor Detection. Middle-East Journal of Scientific Research, 2013; 14(5): 669-672.
5. Kumar, S., Dabas, C., &Godara, S. Classification of Brain MRI Tumor Images: A Hybrid Approach. Procedia Computer Science, 2017:*122*: 510-517..
6. Kumar, B. H., Malathi, R., &Natarajan, R. A. Non-invasive grading and prognosis of Brain Tumours using fuzzy inferencing for rural India. Materials Today: Proceedings,2018:5(1):1921-1928.
7. Pawar, B., Ganbote, S., Shitole, S., Sarode, M.,

&Pandharpatte, R. Optimizing Problem of Brain Tumor Detection Using Image Processing.2017.

8. Devkota, B., Alsadoon, A., Prasad, P. W. C., Singh, A. K., &Elchouemi, A. Image Segmentation for Early Stage Brain Tumor Detection using Mathematical Morphological Reconstruction. *Procedia Computer Science*, 2018;*125*: 115-123.
9. Khairnar, V., &Kadu, S. Brain Tumor Detection Based On Mathematical Analysis and Symmetry Information. arXiv preprint arXiv:1403.6002,2014.
10. Bahadure, N. B., Ray, A. K., &Thethi, H. P. Feature extraction and selection with optimization technique for brain tumor detection from MR images. International Conference on Computational Intelligence in Data Science 2017:1-7.
11. Nandi A Detection of human brain tumour using MRI image segmentation and morphological operators. International Conference on Computer Graphics, Vision and Information Security.2015:55-60.
12. Abdel-Maksoud, E., Elmogy, M., & Al-Awadi, R. Brain tumor segmentation based on a hybrid clustering technique. Egyptian Informatics Journal, 2015;16(1): 71-81.
13. Singh, G., & Ansari, M. A. Efficient detection of brain tumor from MRIs using K-means segmentation and normalized histogram. 1st India International Conference on Information Processing 2016:1-6.

Association between Socio-Demographic Variables and Alcohol Dependency among Alcoholics attending Alcohol Deaddiction Camp Held at Gundlupet, Karnataka, India

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ABSTRACT

Introduction: Alcohol drinking is common practice in many parts of the world including India. It leads to serious social, physical and mental consequences as per the survey of WHO 2011. Many studies have shown that alcohol use exposes the individual to acute health conditions like road traffic accidents and risk of acquiring chronic diseases. Hence the present study was conducted to determine the association between socio-demographic variables with severity of alcohol dependence among the chronic alcoholics. **Methodology:** It is a cross sectional study conducted at Gundlupet, rural part of Mysuru District. Severity of Alcohol Dependence Questionnaire (SADQ) was used to measure the severity of alcohol dependency among the chronic alcoholics. Information on socio-demographic variables was obtained by a self-administered questionnaire. The obtained data was entered in Microsoft excel and analyzed using SPSS version 23. Descriptive statistics and Chi-Square analysis was used to find the association between socio-demographic variables and severity of alcohol dependency. **Results:** 78% of alcoholics had Severe Alcohol dependency, 8% had Moderate and 4% had Mild alcohol dependency. Residence and Education were found to be significantly associated with severity of alcohol dependency. **Conclusion:** Morethan ¾ of the alcoholics are having severe alcohol dependency while remaining are having mild and moderate alcohol dependency. Place of Residence and Education were found to be significantly associated with severity of Alcohol dependency.

Key words: *Alcohol dependency, Socio-demographic variables, Rural India*

INTRODUCTION

Even today consuming alcohol is a common practice in the history of mankind. It affects the individual physical, social and psychological health status. It is a progressive disease where an alcoholic finds a lot of difficulties to quit drinking and falls in to its effects knowing its bad effects. Alcoholic faces various problems due to his addiction to alcohol. If he stops drinking, he will face withdrawal symptoms.[1] Systematic reviews of various longitudinal studies show that different socio demographic variables have differential effects on the alcoholics and they are very influential in motivating them to an alcohol dependency status.[2] Smith study indicates that alcohol users are increasing in the world wide and there is a urgent need to curb this progressive disease. According to their study conducted among alcoholics who have visited the OPD of Savitha medical college Chennai found that 65% of alcoholics are having substantial dependency on alcohol and 3% of alcoholics are severely dependent on alcohol.[3] Hence in this present study we try to understand if the socio-demographic variables influence the dependency on alcohol.

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OBJECTIVE

To determine the association of the socio-demographic variables with severity of Alcohol dependency among chronic alcoholics attending the Deaddiction camp at Gundlupet Taluk

METHODOLOGY

The present community based cross sectional study was conducted among 50 alcoholic participants who participated in a community based Alcohol Deaddiction camp held at Gundlupet, Mysuru district from 07-07-2017 to 14-7-2017. Purposive sampling was adopted. Informed consent was received before conducting the interview. Confidentiality and voluntariness were the guiding principles of the study. The study was approved by the institutional ethical committee. Severity of Alcohol Dependence Questionnaire (SADQ) was used for collecting the details of dependency. The SADQ is a short, easy-to-complete, self-administered, 20-item questionnaire designed to measure severity of dependency on alcohol. There are five subscales with four items in each. They are physical withdrawal, affective withdrawal, withdrawal Relief Drinking, alcohol consumption, and rapidity of reinstatement. Each item is scored on a 4-point scale, ranging from Almost Never to Nearly Always, resulting in a corresponding score of 0 to 3. Thus the total maximum score possible is 60 and the minimum is 0. A score of 30+ indicates severe alcohol dependence and scores between 16 -30 indicates moderate dependence and score below 16 indicates a mild physical dependency.[4]Interview was conducted on 13th and 14th of July 2017 to ensure that the respondent was psychologically fit to respond to the questionnaire.

STATISTICAL ANALYSIS

The obtained Data was entered in Microsoft excel and analyzed using SPSS V.22. Descriptive statistics like mean, proportion and standard deviation was used and inferential statistics like Fisher Exact Test was used to determine the association between socio-demographic variables and severity of alcohol dependency. The difference in the proportions was considered statistically significant at $p \leq 0.05$.

RESULTS

The mean age of the study participants was 37.8 ± 9.6 yrs, the range being 22-71yrs with 64% less than 40 yrs. All the study participants were males. 50% belonged to rural area and 76% of them had completed Pre-University education. There was equal proportion of skilled and semi-skilled professionals and majority had evening and night shifts. The mean income was 1, 04,308 ± 69,306 Rs. 68% were married and all the married participants had children. 74% belonged to nuclear family and the rest to joint family and three generation families.

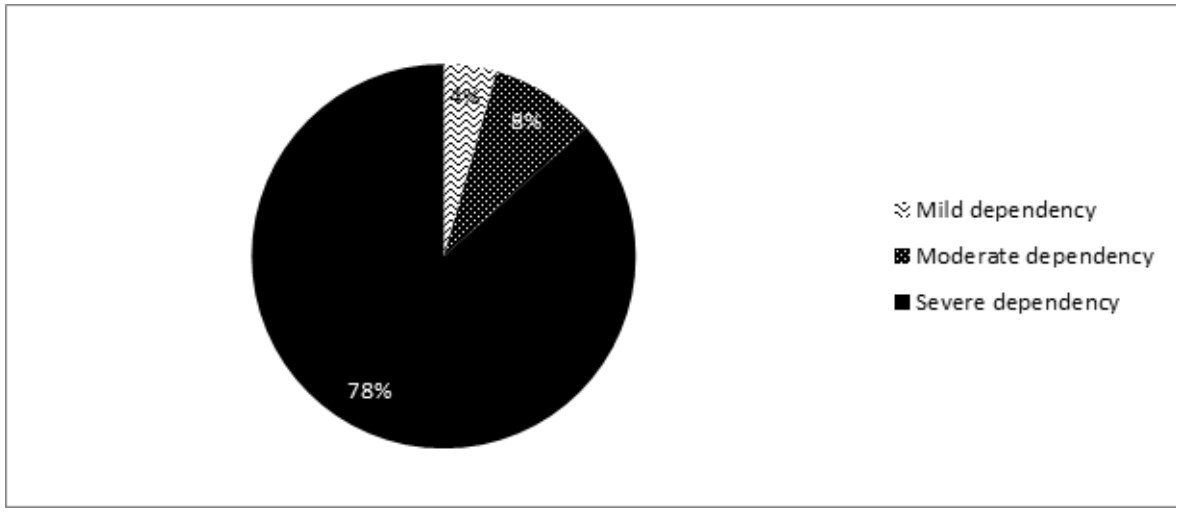


Fig- 1 Showing Alcohol Dependency among study subjects according to SAD Questionnaire

Among the study participants 78% had Severe Alcohol dependency as determined by Severity of Alcohol dependency Questionnaire (SAD Questionnaire), 8% had Moderate and 4% had Mild alcohol dependency

Table 1: Association of Alcohol dependence with Socio-demographic variables

Variable	Mild and Moderate (Score <16)	Severe (Score ≥16 -30)	p-value (Fisher Exact test)
Age			
<40yrs	8	24	0.495
≥40 yrs	3	15	
Residence			
Urban	2	23	0.017*
Rural	9	16	
Education			
<Pre University	8	30	0.013*
≥Pre University	3	9	
Occupation			
Skilled	6	19	0.733
Unskilled	5	20	
Shift in work			
Morning	3	19	0.206
otherwise	8	20	
Annual income			
<1,00,000	8	28	0.951
≥1,00,000	3	11	
Marital status			
Married	7	25	0.977
Single	4	14	
Type of family			
Nuclear	8	29	
Three Generation	1	0	0.154
Joint	2	10	
Children			
Present	6	23	0.793
Absent	5	16	

**significant at p-value <0.05*

Residence (p=0.017) and Education (p=0.013) were found to have significant association with severity of alcohol dependency

DISCUSSION

WHO Statistics reported that there is significant association between SADQ score and annual income (p= 0.005), marital status (p=0.04), education (p< 0.0001), occupation (p=0.008) and work timings (p=0.002). WHO also reported that severe dependency of alcohol among urban inhabitants is due to lack of family support, increased peer pressure and easy availability of alcohol. Residence (urban or rural) and education are found to be significantly associated with severity of alcohol dependency.[5] Pillai, et al., showed that significant associations between ADS score and annual income (p=0.001), education (p=0.001)and work timings (p< 0.0001). Further he proves that alcohol dependent men take more sick leave than other employees and men with

lower education and lower standard of living would opt for a risky usual quantity of alcohol (c60 g/ drinking day).[6]In our study Education and Residence were the two socio-demographic variables found to be statistically associated with severity of Alcohol dependency. With respect to Education it was seen that lesser the education more severe is the dependency. This could be attributed to educated individuals being better informed about the ill effects of alcohol compared to the counterpart; it was also found that individuals belonging to urban area had a more severe dependency. This may be due to better availability of alcohol in urban areas, peer pressure and lack of monitoring due to nuclear family which is mostly the type of families at the urban setting. Froms the above studies and our study we conclude that socio-demographic variables influence the individual to addiction. The strength of our study is that it tries to find the association of various socio-demographic variables with severity of alcohol dependency in rural area which is a less explored research question .The limitation would be the small sample size and since all the study participants were males the influence of socio-demographic variables in female alcoholics has not been explored.

CONCLUSION

Among the study participants it was estimated that 78% had severe dependency, 8% had Moderate dependency and 4% had mild dependency of alcohol. Place of residence and education of an individual were found to be significantly determine the severity of alcohol dependency.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional Ethical Committee

REFERENCES

1. Alcohol use disorder - Symptoms and causes [Internet]. Mayo Clinic. 2018 [cited 20 August 2018]. Available from: http://www.mayoclinic.com/health/alcoholism/DS00340
2. Hoffman P, Tabakoff B. Alcohol Dependence: A Commentary on Mechanisms. Alcohol and Alcoholism. 1996;31(4):333-340.
3. Smith R. [Internet]. 2018 [cited 20 August 2018]. Available from: http://www.telegraph.co.uk/health/3333411/Alcohol-consumption-doubles-since-the-1960s.html
4. Stockwell T, Murphy D, Hodgson R. The Severity of Alcohol Dependence Questionnaire: Its Use, Reliability and Validity. British Journal of Addiction. 2010;78(2):145-155.
5. Scientific Facts on Alcohol [Internet]. Greenfacts.org. 2018 [cited 20 August 2018]. Available from: http://www.greenfacts.org/en/alcohol/index.htm
6. Pillai A, Nayak M, Greenfield T, Bond J, Nadkarni A, Patel V. Patterns of alcohol use, their correlates, and impact in male drinkers: a population-based survey from Goa, India. Social Psychiatry and Psychiatric Epidemiology. 2012;48(2):275-282.

Prescription Pattern of Antibiotics for Upper Respiratory Tract Infection in Shah Alam, Malaysia

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ABSTRACT

Background: Upper respiratory infection (URTI) is a contagious infection of the upper respiratory tract which includes the nose, pharynx and larynx. Most of these infections are viral in origin yet treated often with antibiotics. Overuse of antibiotics is a worldwide problem. The aim of this study is to evaluate the prescribing pattern of antibiotics in management of URTI among doctors in Shah Alam and to determine the antibiotics most often prescribed for URTI.

Material & Method: A cross sectional study was conducted at two private hospitals and seven clinics in Shah Alam. A total number of 128 doctors participated in this study. The data was collected through well–structured questionnaires. Analysis of data was done by using descriptive statistics.

Result: This study showed few prescriptions for antibiotics in treatment of URTI as most of doctors (89%) did not recommended it. Although the number of cases of URTI diagnosed weekly was high, yet most of them were non-bacterial and did not require any drugs. However, most of the doctors interviewed had not attended clinical antibiotic training though they were well aware of the latest Malaysian Antibiotics Guidelines. Amoxycillin was the most prescribed antibiotic in certain cases of URTI which needed antibiotic prescription.

Conclusion: Most of URTI cases do not require antibiotics for treatment. The prescription pattern of antibiotics in Upper Respiratory Tract Infection showed no difference between the doctors involved in hospitals and clinics.

Keywords: *Antibiotics, Upper respiratory tract infection, doctors*

INTRODUCTION

Upper Respiratory Tract Infection (URTI) is a contagious infection of our upper respiratory tract which includes the nose, pharynx, larynx, and bronchi. It may manifest as common cold, pharyngitis, laryngitis, epiglottitis, laryngotracheobronchitis (Croup), otitis media and sinusitis. Most of these cases are caused by viruses like rhinovirus, adenovirus, parainfluenza virus, herpes simplex virus, respiratory syncytial virus, Epstein-Barr (EB) virus, influenza virus, Coxsackie A virus, coronavirus and cytomegalovirus. Only 10% of these cases are caused by bacteria and warrant antibiotics. The common bacteria causing URTI are Beta-hemolytic streptococci, Pneumococci, Staphylococci, H. influenza, Corynebacterium diphtheria, and Chlamydia trachomatis[1]. Overuse of antibiotics is a worldwide problem[2]. Hence, WHO has used antibiotic prescription for URTI as an indicator for assessing the optimum

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or overuse of antibiotics[3].According to WHO report on prescription pattern of antibiotics in developing countries, over the period of 1982 - 2006, had shown increase in the rate of antibiotic prescription by 71%[4,5]. Antibiotic use in URTI was 85-90% in developing countries like China, India, Thailand and South Africa[6,7]. This can cause adverse effects, increase healthcare cost and may result in increased resistance to antibiotics. This study was conducted with the aim to evaluate the current trend in prescribing of antibiotics for URTI in Shah Alam, Malaysia.

MATERIAL AND METHOD

This is a cross sectional study, conducted on 128 doctors working in two private hospitals and seven clinics in Shah Alam evaluating the prescription pattern concerned with the use of antibiotics in the management of URTI. The convenient sampling technique was used. Medical officers in general medicine, otorhinolaryngology, pediatrics and emergency departments were included in this study. Houseman officer and other hospital staff who are not involved in drug prescription were excluded. Permission was taken from the heads of department of all the hospitals and clinics to conduct thestudy. Ethical clearance was obtained from the institutional ethical board.

Study tool: A questionnaire was developed after extensive literature search. It was pilot tested on a small group of 10 doctors and revised for ambiguity. The questionnaire included questions on their educational status, experience, status of their training on their antibiotic use, the frequency of treating patients with URTI and attitude towards prescribing antibiotics. A written informed consent was attached to each survey form that was answered by the respondents. A random selection of prescriptions from URTI patients treated in these healthcare institutions were collected to verify the actual prescribing behaviors and compared with the answered questionnaire.

Statistical Analysis: All the data collected was statistically analyzed by using SPSS version.

RESULT & ANALYSIS

In this study, a total of 128 doctors participated from 2 hospitals and 7 clinics in and around Shah Alam, Malaysia. 62.5% had >5 years of experience in clinical practice while 37.5% had 3-5 years of experience. Most of them (82%) had no special training on use of antibiotics .114 of the 128 doctors were aware of the latest Malaysian antibiotic guidelines (Table1).

Table 1 shows the number of doctors with their experience, clinical training on antibiotic use and familiarity with latest Malaysian Antibiotic Guidelines.

		Number of doctors
1	Working experience	3–5 years 48 (37.5%)
		>5 years 80 (62.5%)
2	Have clinical antibiotic training	Yes 23 (18%)
		No 105 (82%)
3	Familiar with latest Malaysian antibiotics guidelines	Yes 114(89%)
		No 14(11%)

URTI was encountered frequently by our study population. The number of cases of URTI treated by the doctors per day is shown in Figure 1. Fifty-five doctors claimed to see more than 10 cases of URTI per day, while forty said they saw about >5-10 cases per day. Eight doctors dealt with 1-5 cases while twenty-three treated less than 1 patient of URTI per day.

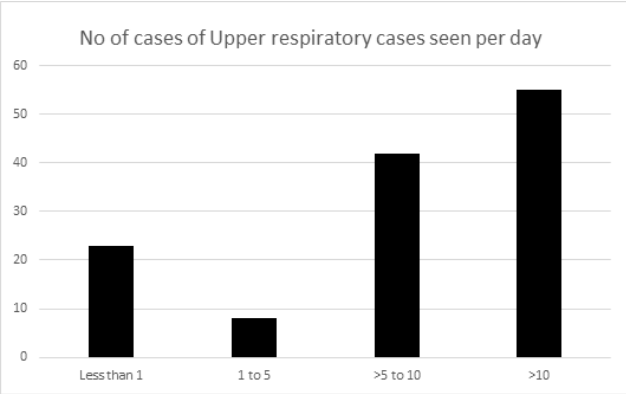


Figure1: No. of URTI cases seen by the study population per day.

Recommendation for antibiotic use:

In our study, only 14 out of 128 doctors (11%) recommended antibiotics for URTI treatment while the vast majority 114(89%) did not prescribe it. The diagnosis for which antibiotics prescribed were acute tonsillitis, acute sinusitis and otitis media. Among the antibiotics prescribed, the most common drug was of penicillin group (amoxicillin), followed by cephalosporin and macrolides (Figure2).

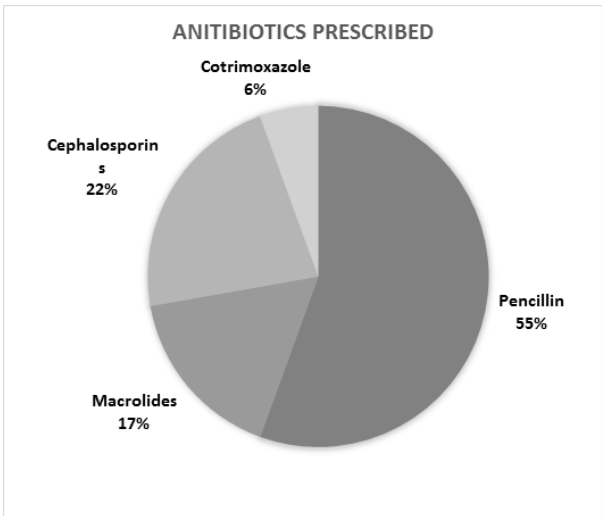


Figure 2: Antibiotics prescribed by the study group.

Alternative treatment methods used in the treatment of URTI:

The doctors in our study group used alternative options to provide symptomatic relief to patients with URTI. Seventeen recommended high dose of vitamin C, forty-three prescribed antihistamines, twenty-five suggested hydration therapy, fifteen traditional remedies while twenty-five recommended rest and allowing self-recovery (Figure3).

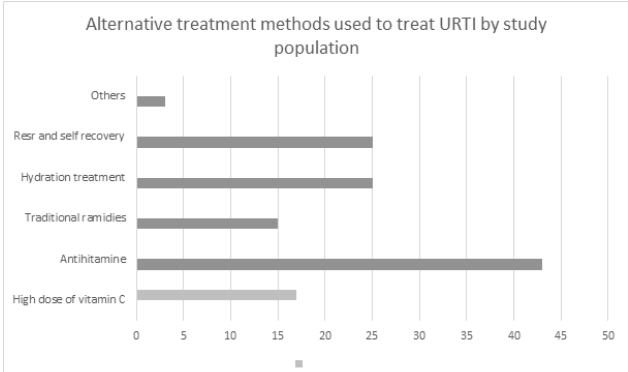


Figure 3: Alternative treatment methods used to treat URTI by study population.

DISCUSSION

In this study, a total of 128 doctors participated. Most of them were expert in the treatment of URTI with 80 having 5 years or more experience, while 48 had about 3-5 years of clinical experience. Due to their vast experience, they were aware of the latest Malaysian National Antibiotics Guidelines (NAG). However, majority of them had never attended any workshop / training on antibiotic use. It is a challenge for clinicians to identify between viral and bacterial infection. Most of the drugs given are aimed to minimize the symptoms and promote effective recovery. In our study, most of the doctors (89%) did not recommend antibiotics for URTI cases with only 11% preferring to use antibiotics in treatment of URTI. An earlier study in primary care settings in Malaysia by Teng et .al reported use of antibiotics in 33.8% of URTI cases [1]. In a similar study in India, Kotwani and Hollaway reported use of antibiotic in 45% and 57% of uncomplicated URTI cases in public and private hospitals respectively[8].Similar data was presented in a systematic review from China where 83.7 % of patients with URTI were prescribed antibiotics. An alarming 18.4% were prescribed 2 antibiotics while 1.1% got 3 antibiotics. The authors however mentioned about the downward trend in antibiotic prescription in recent years[2]. Mohitosh et.al in Bangladesh too reported URTI to be a common cause for antibiotic prescription[9].Even in a developed country like France a study on antibiotic use in paediatric age group between 2008-2012 reported an unacceptable higher use of antibiotics [10].

We have observed a decrease in the prescriptions for antibiotics in our study. This may be attributed to the study being conducted in an urban area with availability of specialist care and the doctors being aware of the antibiotic guidelines. The reasons cited by the respondents for not prescribing antibiotics were to

prevent antibiotic resistance among patients, to prevent side effects especially to infants and young children, the type of causative microorganism and the level of severity of the case. Instead of antibiotics, some of the doctors in our study preferred to use alternative methods to treat URTI and provide symptomatic relief. Prescription of anti-histamines was the commonest among them. Others included giving high doses of Vitamin C to help boost the immune system, hydration treatment or fluid replacement, anti-inflammatory drugs or suggesting rest as they believed in self-recovery. Similar observation was made by Reshmi et al who also reported the use of symptomatic treatment for patients with URTI[11].

In our study, there were 14 cases of URTIs where antibiotics were used. In some cases of tonsillitis, laryngitis and sinusitis antibiotic coverage was needed to prevent complications in immunocompromised individuals or when virulent organism was suspected.

The most prescribed drug was of Penicillin group (amoxicillin), followed by cephalosporin and macrolides. Similar prescription pattern has been described by Teng et .al [1], The choice of antibiotics given maybe due to the commonest causative organisms being gram-positive type of bacteria such as *Streptococcus*. In Bangladesh, Mohitosh[9] reported cephalosporins to be the commonest antibiotic prescribed. In a similar study in Kerala by Reshmi et .al[11], azithromycin was the commonest antibiotic prescribe

This is in contrast to the observations of Shamsuddin et.al [12] who in 2013 reported inappropriate use of antibiotics in 18% for URTI.

CONCLUSION

This study reveals that most doctors in Shah Alam are familiar with Malaysian Antibiotic Guidelines, which is reflected in fewer prescriptions for antibiotics in the treatment of URTI cases. When dealing with URTI, alternative symptomatic treatment like vitamin C, antihistamines, good hydration were advocated by our study group. However, a similar study in future on a larger population including both urban and rural healthcare settings as well as both private and public hospitals would be able to substantiate the changing trend in the prescription pattern of antibiotics among doctors for URTI in Malaysia.

Limitation of the study:

This study was conducted on a small population of doctors. All the doctors were working in urban, private health care settings. Hence the findings of this study do not reflect on the rural and public healthcare settings.

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Conflict of Interest: Nil.

Source of Funding: Self

REFERENCES

1. Teng CL, Tong SF, Khoo EM, Lee V, Zailinawati AH, Mimi O, Chen WS, Nordin S. Antibiotics for URTI and UTI Prescribing in Malaysian primary care settings. Australian family physician. 2011;40(5):325-9.
2. Li J, Song X, Yang T, Chen Y, Gong Y, Yin X, Lu Z. A systematic review of antibiotic prescription associated with upper respiratory tract infections in China. Medicine. 2016 May;95(19).
3. WHO The World Medicines Situation 2011. http://apps.who.int/medicinedocs/documents/s18064en/s18064en.pdfhttp://apps.who.int/medicinedocs/documents/s18064en/s18064en.pdf.
4. WHO The World Drug Situation 1988. http://apps.who.int/medicinedocs/documents/s16222e/s16222e.pdfhttp://apps.who.int/medicinedocs/documents/s16222e/s16222e.pdf.
5. WHO. Medicines Use in Primary Care in Developing and Transitional Countries. 2009; http://www.who.int/entity/medicines/publications/primary_care_8April09.pdfhttp://www.who.int/entity/medicines/publications/primary_care_8April09.pdf.
6. Zhang W, Shen X, Wang Y, Chen Y, Huang M, Zeng Q, Fan M, Bergman U, Yang Y. Outpatient antibiotic use and assessment of antibiotic guidelines in Chinese children's hospitals. European journal of clinical pharmacology. 2008 Aug 1;64(8):821-8.
7. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA,. Laxminarayan R. Global antibiotic consumption 2000 to 2010: an analysis

of national pharmaceutical sales data. The Lancet Infectious Diseases. 2014 Aug 1;14(8):742-50.

8. Kotwani A, Holloway K. Antibiotic prescribing practice for acute, uncomplicated respiratory tract infections in primary care settings in N ew D elhi, I ndia. Tropical Medicine & International Health. 2014 Jul;19(7):761-8.
9. Biswas M, Roy DN, Tajmim A, Rajib SS, Hossain M, Farzana F, Yasmen N. Prescription antibiotics for outpatients in Bangladesh: a cross-sectional health survey conducted in three cities. Annals of clinical microbiology and antimicrobials. 2014 Dec;13(1):15.
10. Touboul-Lundgren P, Bruno P, Bailly L, Dunais B, Pradier C. Paediatric antibiotic prescriptions in primary care in the Alpes-Maritimes area of southeastern France between 2008 and 2013. European Journal of Clinical Microbiology & Infectious Diseases. 2017 Mar 1;36(3):509-16.
11. Reshmi T. M., C.I. Sajeeth., Thangamani .'A Study on Drug Prescribing Pattern in Upper Respiratory Tract Infections among Pediatrics in Tertiary Care Hospital', International Journal of Pharmacy & Pharmaceutical Research, (2016) ;6(3), pp. 78–86.
12. Shamsuddin S, Akkawi ME, Zaidi ST, Ming LC, Manan MM. Antimicrobial drug use in primary healthcare clinics: a retrospective evaluation. International Journal of Infectious Diseases. 2016 Nov 1; 52:16-22.

Re-Standardization Makassar Healthy City based on Local Needs

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ABSTRACT

This study aimed to develop Makassar Healthy City standards and indicators based on local needs, including key indicators, general indicators and specific indicators according to the settings choices. Through in-depth interviews and focus group discussions, this study found that general indicators only change indicators regarding the level of awards, from national and international awards to provincial, national and international awards. Furthermore, the main indicators still refer to national policies. Seven of nine settings were identified in Makassar City based on local needs. There are changes or additions to indicators in almost all settings. Re-standardization of healthy districts/cities should be carried out in all districts/cities based on the local conditions.

Keywords: *Re-standardization, Healthy Cities, Local Needs*

INTRODUCTION

The Healthy Cities movement in Indonesia has long been carried out, even though it was officially effective since the publication of the Joint Regulation between the Ministry of Home Affairs and Health [1,2]. Adipura and Healthy Cities are different but the goal of Adipura can strengthen the Healthy Cities goal [3]. Indonesia has a Healthy Cities concept that is different from other countries [4,5]. Although Healthy Cities in Indonesia has been running for more than a decade [4,6], the environmental and social health problems are the still main problem in urban areas, but the districts/cities receive the Healthy Cities award from the central government, how is the Healthy Cities really in Indonesia? How does the central government assess and determine the indicators used? The central government can set national indicators and standards, but it can be different from local governments because they have different needs, resources, capacities and even values and cultures so that different solutions can occur [7].

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Several previous studies relating to Healthy Cities in Indonesia have been conducted [1,3,6], but specific research especially studying the Healthy Cities indicator used is rarely done. This study aimed to develop Makassar Healthy City standards and indicators based on local needs, including key, general and specific indicators according to the settings choices.

MATERIALS AND METHOD

This study was carried out in Makassar City, namely at Regional Planning and Development Board (Bappeda), Health Office, Tourism Office, Social Service, Food Security Service, Sanitation Office, City Planning Office, Healthy City Trustees and Healthy City Forum. Data to be collected consists of primary data and secondary data. Primary data was collected through depth interviews, observations and Focus Group Discussion (FGD). In-depth interviews were conducted with heads of agencies related to healthy cities structures such as Bappeda, Health Office, Tourism Office, Social Service, Food Security Service, Cleanliness Office, Healthy City Trustees and Healthy City Forum. This study used a qualitative research method with a case study approach where qualitative researchers conducted face to face interactions throughout the study [8]. The variables or concepts in this study refered to the main indicators,

general indicators and specific indicators of Makassar Healthy City. Special Healthy City indicators refer to the settings of healthy districts/cities in Indonesia and can be developed based on local needs. Data analysis was conducted qualitatively with thematic analysis.

RESULTS

Seven of nine healthy city settings have been identified for Makassar City, namely:

a. Healthy settlements, public facilities and infrastructure

1. A clean air program

2. Appeal for the use of low-sulfur fuel and energy efficiency, through circulars, decrees, billboards, or leaflets.

3. Regulations governing non-smoking area

4. Incidence pneumonia

5. Frequency carry out an ambient air quality test for a moment

6. Implementation of clean air programs through 5 movements: 1 billion trees, car free days, areas without smoking, cycling and walking facilities

7. Use of environmentally friendly alternative energy: solar cells, methane

8. The air quality index category

9. The existence of monitoring activities for chimney emissions in industrial activities

10. The existence of motor vehicle emission testing activities

11. River management policy

12. River cleanliness conditions

13. Riverbank conditions

14. Carry out river monitoring

15. Water Quality Index Value

16. Community involvement in river management

17. Carry out conservation activities around the river banks

18. Clean water service coverage

19. Coverage of drinking water quality

20. Drinking Water Access Coverage

21. Percentage of population using healthy latrines 64%

22. Stop Defecation Village

23. The efforts of the Regional Government to encourage people not to open defecation

24. Drainage Planning Noting the Eco-Drain Concept

25. Local Government Program in encouraging Community Participation in the construction of drainage

26. The active role of the community reports the existence of inundation

27. Public & Private Engagement in Maintenance of Drainage Systems

28. The functioning of the drainage canal

29. Community involvement in the process of maintaining drainage

30. Services and public access to waste water infrastructure and facilities

31. Domestic Waste Water Treatment Plant

32. Quality Test of Communal IPAL Waste Water Effluent

33. Availability of Fecal Treatment Plant

34. Stool trucks operate into the Fecal Treatment Plant

35. General environmental conditions in residential environments

36. Waste handling includes activities

37. Implementation Waste management with the 3R principles

38. Final processing sites Waste does not pollute the environment

39. Management of methane gas in landfill

40. Length of garbage collection at TPS

41. Aides larva numbers in settlements

42. PSN and Routine Work

43. Incidence) of filariasis cases

44. Housing / settlement Free of floods

45. Number of healthy houses

46. Incidence / case of pulmonary tuberculosis

47. Outbreaks of diarrheal diseases

48. Outbreaks of DHF

49. Outbreaks of Malaria

50. Number of villages that have health service facilities

51. Percentage of posts in the regency in accordance

with the number of health centre

52. The number of active alert villages

53. Health centre with Sanitation Clinic services

54. Tree planting program for the community

55. Available parks and urban forests

56. Percentage of schools implementing healthy schools (UKS)

57. Percentage of schools that take part in the "Adiwiyata program"

58. Availability of Healthy Canteen

59. Availability of adequate toilets (separate between men and women)

60. Availability of places of worship in schools

61. "Saptapesona School "security, order, cleanliness, beauty, and hospitality

62. Availability of segregated bins

63. Waste Management in schools

64. Available public facilities (sports and play)

65. Market management policy program

66. Supervision regarding market management

67. Market community involvement in participating in market sanitation

68. Clean urban market environment

69. Enough toilets and meet the requirements on the market

70. Parking lots available in urban markets

71. Avalilable play facilities for children

72. The arrangement of informal traders

73. Regulations on handling street vendors

74. Availability of Healthy Canteen

75. Availability of adequate toilets (separate men and women)

76. Availability of disaggregated bins

77. Waste management in traditional markets

78. Availability of places of worship

b. Traffic Order Zone and Transportation services

1. Car free day area

2. Clean, orderly and neat terminal conditions

3. Public facilities in the terminal

4. Availability of traffic signs

5. Special facilities for nursing mothers at the terminal

6. Crime in the terminal decreases

7. Availability of smoking ban in the terminal area

8. Availability of smoking ban on the vehicle

9. Examination of the feasibility of public transport vehicles

10. The number of traffic accidents decreases

11. The existence of suitable pedestrian facilities

12. Non-smoking public transport vehicles

13. A routine health inspection program for public transport drivers

14. Availability of bus stops that meet the requirements

15. Availability of an orderly traffic area

16. Availability of a workshop to monitor exhaust emissions

17. Availability of a smart driving training program for the driver

18. The awarding of exemplary public vehicle crew training awards

19. Giving an award of obedience to the driver

c. Healthy industries and offices

1. Locations specifically for industrial zones

2. A smoking ban in the office environment

3. Places of worship in offices

4. Special arrangements for the informal sector

5. Health insurance for workers in all industries

6. The presence of industrial waste pollutes the environment

7. Community complaints about cases of industrial pollution

8. Decreasing work accident rates

9. Decreased unemployment

10. Availability of training activities in the vocational training center

11. Implementation of Environmental Management

12. Special room for nursing mothers

13. Enough Toilets (separate Men and Women)

14. Availability of Parking Lots

15. Availability of Healthy Canteen

16. Maintain cleanliness

17. Availability of first aid

18. Availability of Green Open Space

19. Availability of disaggregated bins

d. Healthy Tourism

1. Availability of tourist information in public places

2. Information on health facilities for tourists at the location

3. The entire hotel is worthy of health

4. Availability of healthy restaurants

5. Increasing number of tourists per year

6. Tourists have been insured

7. Food poisoning in tourists for the past year

8. Decreased cases of accidents in tourism objects

9. Transportation available in tourist areas

10. Emergency response/safety hall in tourist area

11. Public facilities available

12. Waste disposal facilities available

13. Clinic/first aid facilities available

14. Telecommunication facilities available

15. Souvenir facilities available

16. Worship facilities available

17. The presence of tourism police

18. Smoke-free zone

19. Availability of Parking Lots

e. Food Security and Nutrition

1. Increased food crop production

2. Food Production Supervision

3. Food Diversity

4. Food Processing Monitoring

5. Cases of malnutrition

6. Availability of food reserves and storage

7. Food availability

8. Cases of pesticide poisoning on farmers

9. An extension of integrated pest control and use of pesticides

10. The functioning of food distribution institutions in the community

11. Food Distribution Monitoring

12. The existence of organic farming programs by the government and the community

f. Healthy-Independent Community Life

1. The existence of a regular sports movement in the community/office

2. Decreased cases of drug use

3. The existence of community groups for drug and HIV/AIDS prevention

4. Availability of community compliance not to smoke in public places

5. Availability of an anti-smoking movement

6. Coverage of clean water services

7. Availability of a routine inspection of the quality of clean water

8. Increased quality of clean water

9. Healthy food, restaurant Food Management Place services

10. Government program on healthy home improvement

11. Increased coverage of the use of health services

12. Availability of youth counseling service facilities

13. Availability of a routine health check on school children

14. Childbirth assistance by trained personnel

15. Availability of mental health services at health centers

16. A functioning sanitation clinic service

17. Availability of an anti-alcohol movement by the community

18. The existence of an anti-drug movement by the community

19. Availability of is a special drug prevention service

20. Decreased drug use cases

21. Increased immunization coverage

22. The functioning of an active Posyandu

23. Coverage of visits to health centre

24. PSN movement in schools, households, public spaces

25. Free aides larvae at school, household and public spaces

26. The existence of community movement in preventing degenerative diseases

27. Information on risk and efforts to prevent degenerative diseases

28. The existence of community groups in overcoming nutritional problems

29. Increased Protein Energy Deficiency for pregnant women

30. Cases of pregnant women who are anemic and lack iodine

31. Decreased people lack of vitamins

32. Decreased weight of low birth baby

33. Increased awareness of nutrition families

34. Number of health centre that provide basic services for the poor

35. The existence of health insurance for the community

g. Healthy Social Life

1. Decreasing poverty rate (4% -6%)

2. The existence of beggars and homeless people in public facilities

3. Decreasing number of drug users

4. The decline in the number of prostitutes

5. Availability of a halfway house for street children

6. The existence of informal workers

7. The existence of children's creativity development and elderly productivity

8. The existence of an education program dealing with disability

9. Availability of facilities for people with disabilities in public places

10. Regional government policy on handling communities in remote areas

11. Public transportation facilities to remote areas

12. Health facilities in remote areas

13. Adequate Child Care Facilities

14. Special programs for abandoned, elderly

15. Disaster preparedness program for the community

16. Incidents of riots/anarchists

17. Having a contingency plan for a disaster problem

18. Availability of facilities for disabled people

19. Availability of street children control program

DISCUSSION

Environmental infrastructure facilities are places used by the general public to function properly. Specific indicators of the settlement area arrangement of healthy facilities and infrastructure nationally are 59 assessments while based on the results of FGD 78 special indicators are obtained. This environmental aspect is very important because it is a determinant of public health status[9-11].

Additional indicators of this setting include monitoring activities for chimney emissions in industrial activities, motor vehicle emission testing activities, access to drinking water. Other indicators include the availability of healthy canteens in schools, the availability of places of worship in schools, waste management in schools, availability of play facilities for children in traditional markets, availability of healthy canteens in traditional markets.

Indicators of the setting area of traffic and transportation services, nationally, there are 16 indicators of assessment while based on local needs there are additions to 19 indicators. These additional indicators are the availability of traffic signs, the existence of smoking bans on vehicles and the existence of a rider compliance award. The problem of transportation and traffic congestion is a big issue, especially in large cities [12,13]. The impact is quite complex on health both from the social aspect and the incidence of disease [14] . Furthermore, there are 11 indicators for industrial estate and office buildings, while based on in-depth interviews there are changes to 19 assessment indicators. The additional indicators include the availability of separate toilets between men and women, and availability of parking spaces, availability of healthy canteens.

Specific indicators of the setting of healthy tourism, nationally, there are 13 assessments, while based on focus group discussion there are 19 indicators. The indicators are available telecommunications facilities, available souvenir facilities, available facilities for places of worship and the availability of parking spaces. Healthy tourism is one indicator of healthy cities set globally [15,16].

Specific indicators of the setting of food and nutrition security, nationally, there are 8 indicators while based on in-depth studies identified 12 indicators, namely: the supervision of food production and processing, and the existence of a variety of foods.

Specific indicators, the setting of healthy-independent communities independent, nationally, there are 38 indicators of assessment, while based on the study there are only 35 indicators. Some indicators are not valid in the Makassar City, such as the presence of new cretin patients; this is because cases of cretin patients have not been found in Makassar City. Finally, the indicators for a healthy social life, nationally, there are 17 indicators of assessment while based on local needs there are 19 indicators. The indicators include the existence of facilities for disabled people, and the existence of a street children control program. One of the biggest problems in implementing healthy cities is social problems [17,18].

CONCLUSION AND RECOMMENDATION

This study found that the main indicators still refer to national policies. Seven of nine settings have been identified for Makassar Healthy Cities. There are changes or additions to indicators in almost all settings. This study recommends that the standardization of healthy districts/cities should be carried out in all districts/cities based on the local needs.

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Ethical Clearance: Taken

REFERENCES

1. Palutturi S, Chu C, Moon JY, Nam EW. A Comparative Study on Healthy City Capacity Mapping: Indonesia and Korea. The Social Sciences. 2015;10(6):848-854.
2. MOHA & MOH. The guideline of healthy districts/ cities implementation (Joint Regulation Between the Ministry of Home Affairs and the Ministry of Health) Number: 34/2005 and Number: 1138/ MOH/PB/VIII/2005. Jakarta, Indonesia: Healthy Cities Advisory Team; 2005.
3. Palutturi S, Rutherford S, Davey P, Chu C. Comparison Between Healthy Cities and Adipura in Indonesia. Malaysian Journal of Medicine and Health Sciences. 2013;9(1):35-43.
4. MOH. The Guide of Healthy District/City Implementation (Joint Regulation between The Ministry of Home Affairs and the Ministry of Health) In: The Ministry of Home Affairs and the Ministry of Health, ed. Jakarta: Advisory Team of the Healthy District/City at Central Level,; 2005.
5. Palutturi S. Healthy Cities: Global Concepts, Local Implementation for Indonesia. Yogyakarta: Pustaka Pelajar; 2018.
6. Palutturi S, Zulkifli A, Syam A, et al. The Key Challenges and Recommendations for Healthy Cities Implementation of North Kolaka, Indonesia. Indian Journal of Public Health Research & Development. 2017;8(2).
7. Werna E, Harpham T. The implementation of the healthy cities project in developing countries: lessons from Chittagong. Habitat International. 1996;20(2):221-228.
8. Creswell JW. Research Design: Qualitative, Quantitative and Mixed Approach. Yogyakarta: Pustaka Pelajar; 2013.
9. Mirowsky J. Education, social status, and health: Routledge; 2017.
10. Bard D. Health and environment, prevention or precaution? Soins; la revue de reference infirmiere. 2018;63(823):24-27.
11. Roychoudhury N, Dasgupta A, Roy K, Bandyopadhyay L, Mandal S, Paul B. HEALTH STATUS OF FIELD LEVEL FEMALE HEALTH PERSONNEL: STUDY IN A RURAL AREA OF WEST BENGAL. INDIAN JOURNAL OF APPLIED RESEARCH. 2018;8(6).
12. Kimura N, Yanagisawa K. Traffic jam hypothesis: Relationship between endocytic dysfunction and Alzheimer's disease. Neurochemistry international. 2017.
13. Sylla FK, Faye A, Fall M, Anta T-D. Air Pollution Related to Traffic and Chronic Respiratory Diseases (Asthma and COPD) in Africa. Health. 2017;9(10):1378.
14. Sørensen M, Pershagen G. Transportation noise linked to cardiovascular disease independent from air pollution. European Heart Journal. 2018.
15. Hancock T, Norris T, Lacombe R, Perkins F.

Healthy Cities and Communities: The North American Experience. Healthy Cities: Springer; 2017:215-240.

16. Tsouros AD. Healthy cities: A political project designed to change how cities understand and deal with health. Healthy Cities: Springer; 2017:489-504.
17. De Leeuw E, Simos J. Healthy Cities: The theory, policy, and practice of value-based urban planning: Springer; 2017.
18. Spector M, Kitsuse JI. Constructing social problems: Routledge; 2017.

The Effect of Progressive Muscle Relaxation in Reducing Fatigue among Nurses in Mental Hospital

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ABSTRACT

Nursing, an occupation dominated by women, is typically high in job demands, work stress levels, and fatigue. Fatigue on nurses affect the neurocognitive function and also the performance of the nurses.

Progressive Muscle Relaxation, a technique involving sequential tensing and relaxation of major skeletal muscle groups, aims to reduce feelings of tension, to lower perceived stress, and to induce relaxation.

This research aimed to find out the effect of progressive muscle relaxation in reducing fatigue on nurses at Mental Hospital with indicators of pulse rate, oxygen saturation, and subjective feelings of fatigue. This quasi-experimental study was performed on 42 nurses within-subject design with a pre-post test, which was conducted in two treatment i.e. routine activity (without progressive muscle relaxation) and progressive muscle relaxation that performed in one group of the same subjects at different times.

The progressive muscle relaxation consists of two basic parts to progressive relaxation: 1) the tension in muscles, and 2) the relaxation of each muscle group. The results showed that the progressive muscle relaxation may reduce fatigue. It can be observed that the progressive muscle relaxation had influence in the decreasing of pulse rate, increasing of oxygen saturation, and decreasing of the subjective feeling of fatigue score.

Keywords: *Nurse Fatigue, Progressive Muscle Relaxation, Pulse Rate, Oxygen Saturation, Subjective Feeling Fatigue*

INTRODUCTION

Fatigue implies in negative impact in short and long term. Short-term impact, for example, the emergence of discomfort, decreased strength and decreased motor control. The impact of short-term leads to reduced performance, productivity, quality of work, and an increase in the incidence of accidents and human error. In the long term, prolonged fatigue can lead to absenteeism, skeletal muscle disorders, and disability.

Fatigue is commonly observed in nurses. Workload, work hours, work structures, and many other factors can indirectly or directly cause fatigue in multiple industries and affect safety [(1)]. Fatigue on nurses can lead to medical errors, performance setbacks, decreased mental acuity, and social issues [(2,3)]. That becomes an issue that stimulate efforts to examine and deal with the problems of fatigue on nurses to improve the quality of service to patients and improve patient satisfaction as well as to find management approaches a comprehensive fatigue to create a supportive work environment for nurses to prevent fatigue and improving performance [(4)].

Based on a previous study conducted in September 2017 about fatigue of nurses in Mental Hospital, the result which has been obtained showed that 52% of nurses experienced moderate to heavy with fatigue, while the rest experienced mild fatigue. The fatigue experienced by nurses include a physical or psychic fatigue-related workload, lack of personnel and the working environment with the smells of the less savory originating from the patient's body that sometimes cause nausea and dizziness, as well as internal factors, be age, gender, educational level and marital status.

Progressive muscle relaxation techniques (Progressive Muscle Relaxation) is one of the non-

pharmacological intervention and is a systematic technique that can be used to reduce stress and achieve a state of relaxation [5]. Some of the results of the study mentioned that the progressive muscle relaxation therapy is effective for losing muscle tension, reduces stress levels and lower blood pressure [6] and provide immediate and significant effects against fatigue marked with a decrease in pulse rate [7]. The purpose of this research is to implement and to find out the effect of progressive muscle relaxation on nurses at Mental Hospital. The results then could be a recommendation in the preparation of the program of health promotion in the workplace.

METHODS

The study design was quasi-experimental with a pre-post trial within the subject, which was conducted in two treatment i.e. routine activity (without progressive muscle relaxation) and progressive muscle relaxation that performed in one group of the same subjects at different times. This study aimed to know the direct effect of progressive muscle relaxation on the nurse's fatigue objectively with the indicator of pulse rate, oxygen saturation, and subjectively with the subjective feeling of fatigue.

This research was conducted on 42 nurses at Mental Hospital. The criteria of a sample is a nurse managing staff; the sample does not have a history of infectious diseases or degenerative; minimum 1-year working period and at the time of research, not just finished running on leave (± 1 month before).

Measurement of fatigue was done after the respondents have the activity or work approximately 2 hours (pre-test) and after the treatment (post-test) (Figure 1). The instrument used to measure the fatigue objectively is the Pulse Oximeter with the aim to measure oxygen saturation and pulse rate. Meanwhile, the subjective measurement was carried out using the Subjective Self-questionnaire Rating Test (SSRT) from the Industrial Fatigue Research Committee which consists of 30 items of questions. The questionnaire consists of 10 questions that described the decreased activity (decrease in the level of activity), 10 the question illustrated the decline in the motivation of work (symptoms of weakening motivation) and 10 questions described the physical fatigue symptoms (symptoms of physical fatigue).

The analysis was carried out with the application of SPSS statistics version 22.0 to find out the difference in the average of the measurements before and after treatment as well as the measurement of the average difference between the treatment routine activity and progressive muscle relaxation treatment.

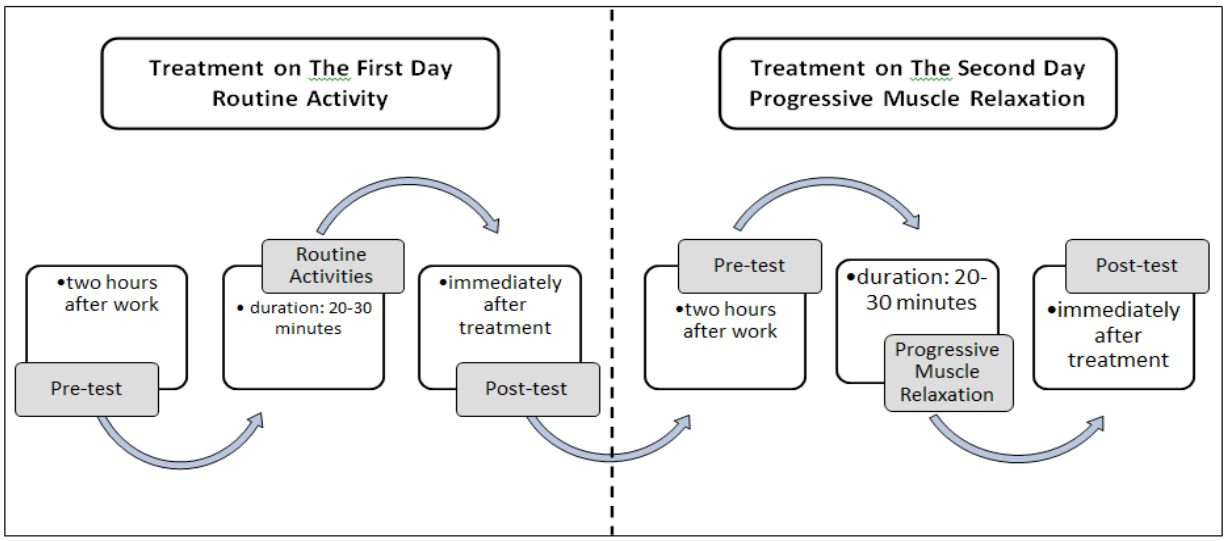


Figure 1 Quasi-Experiments Design with Pre-Test and Post Test within The Subject.

Progressive muscle relaxation techniques are sequential contractions and relaxation movements consisting of 14 movements and involve 5 muscle groups, namely arm/hand muscles, facial muscles, upper chest-abdomen, hips and thighs, and legs and feet. Progressive muscle relaxation sessions lasted for 20 to 30 minutes. The relaxation process started by tensing the muscle group; hold that tension for 5-7 seconds, then relaxing the muscles slowly for 20-30 seconds. Each movement is done 2-3 times. The fourteen of progressive muscle relaxation movements were: 1) exercising the hand muscles; 2) training rear hand muscle; 3) training the large biceps/muscles on the upper part of the arm; 4) training the shoulder muscles to relax; 5 and 6) relaxing facial muscles, such as the forehead, eyes, jaw, and mouth; 7) relaxing the tension experienced by the jaw muscles; 8) relaxing the muscles around the mouth; 9) relaxing the front and back neck muscles; 10) training the front neck muscles; 11) training back muscles; 12) relaxing the chest muscles; 13) training the abdominal muscles; and 14) training the leg muscles such as thighs and calves.

RESULTS

Characteristics of Respondents

Table 1 Frequency Distribution Based On The Characteristics of Respondents

Variable	Percentage	SD	Mean	(Min-Max)
Age				
25-35 years	78,6 %	5,33	32,48	25 – 46
36-45 years	16,7 %			
≥ 45 years	4,7 %			
Sex				
Male	31%	-	-	-
Female	69%			
Nutrition Status (by Body Mass Index)				
Underweight	0 %	3,65	25,11	18,90 – 34,29
Normal Range	57,14 %			
Overweight	28,57 %			
Obesity	14,29 %			
Length of Work				
0-5 years	28,57 %	5,55	8,48	2 – 25
6-10 years	50 %			
≥ 10 years	21,43 %			

The Objective and Subjective Measurements

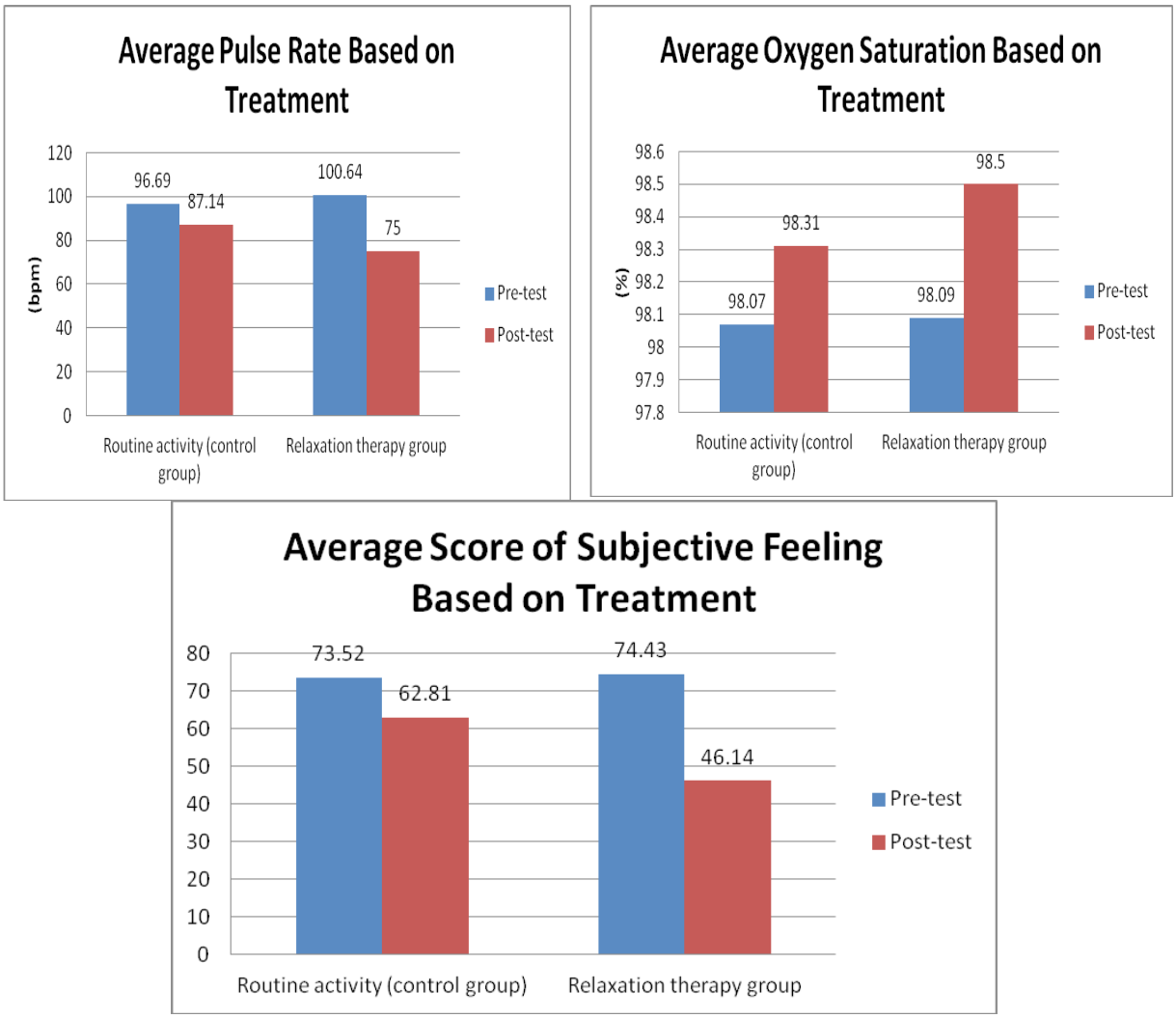


Figure 2. Above right: Average Pulse Rate Based on Treatment, above left: Average Oxygen Saturation Based on Treatment, below: Average of Score of Subjective Feeling Based on Treatment

From the Figure 1 above shows that there is an increasing exhaustion of the respondents on measuring pre-test on the first day of treatment (routine activity) and the second day of treatment (progressive muscle relaxation) compared with the initial measurement (baseline). It can be observed from the increase in the average frequency of the pulse, a decrease in average oxygen saturation as well as an increase in the average score of subjective feelings of fatigue. Instead, on the measurement of post-test showed a decrease in fatigue for each treatment (frequency of the pulse oxygen saturation is reduced, increasing and decreasing the subjective feeling of fatigue score) compared the results of the pre-test.

Table 2 Measurement According to The Treatment

Treatment	Pulse Rate (times per minute)		Oxygen Saturation (%)		Subjective Feelings of Fatigue (Score)	
	Mean Difference Pre-Post	SD	Mean Difference Pre-Post	SD	Mean Difference Pre-Post	SD
Routine activity (n= 42)	9,548	4,660	0,238	0,692	10,714	12,208
Progressive Muscle Relaxation (n=42)	25,643	5,318	0,405	0,798	28,286	9,759

Table 3 Decreased Fatigue Level Based on The Treatment

Variable	Routine Activity		Progressive Muscle Relaxation		Mean Difference	R^2
	Mean	SD	Mean	SD		
Pulse Rate	87,14	2,58	75,00	4,55	12,14	0,778
Oxygen Saturation	98,31	0,84	98,50	0,99	0,19	0,434
Subjective Feelings of Fatigue	62,81	13,28	46,14	11,42	16,67	0,623

Based on treatment, measurement results on indicator pulse and the subjective feeling of fatigue showed a significant difference between routine activities with progressive muscle relaxation (see Table 2). The magnitude of the influence of progressive muscle relaxation in reducing fatigue can also be observed from the coefficient of determination (R^2) shown for the respective indicators (see Table 3).

DISCUSSION

Characteristics of Respondents

The range of age of respondents in this study was between 25 to 46 years. A person's age is very influential factors towards the pulse which is an indicator of the work of the heart. This is similar to research conducted by Houghton, et al. which explained the function of the vascular decreased significantly with aging and contribute to decreased heart rate [8]. Age also influence on physical strength and mental muscles, that showed that there was a certain age at which a worker would experience changes work achievement. At an older age generally occurs a decrease in muscle strength, but it was balanced by a better emotional stability than a young workforce that can be positive in doing work [9]. This is in accordance with the research done by Chang, et al. which mentioned that work fatigue related to age and physiological symptoms [8], a lot of health problems [10]. This was in accordance with the research conducted by Hunter mentioned that gender contributes to muscle strength [11]. In addition, other studies also mentioned that at a particular age and sex, children, adults, and men are more resistant to fatigue the muscle than older people and women [12].

The calculation based on nutritional status, 42 of those respondents, 18 people were categorized as respondents with excess body weight and obesity (42.86%) according to Indonesian Ministry of Health criteria year 2013. Consequently, it might affect the ability and endurance of the respondents in their work performance which closely related to the status of nutrition.

The influential working period significantly related to fatigue. The longer working period effect on work fatigue levels caused the level of monotonous work that has accumulated over the years [13]. Workers with a longer working period has more experience and more familiarity with the work which prevent the occurrence fatigue [9]. The previous study also mentioned that nurses working with less than 2 years have a fatigue score higher than the nurses who work for more than 6 years [14]. Another research showed a statistical relationship between nurse fatigue with age and work experience. The results show that the longer work experience and older age related to the lower the perceived fatigue. This is because with increasing experience in the work, the less pressure of the job [15].

The Effect of Progressive Muscle Relaxation on Pulse Rate

Pulse rate was affected by the presence of stress or at the rest or break time. The factors that affect the pulse of which are gender, age, physical activity, fitness level, ambient temperature, body posture, emotion, body size, as well as the consumed drugs [16].

This result was in line with the results of previous research which showed a decrease in the frequency of the pulse on nurses after making the progressive muscle

relaxation [17]. The other studies have also proved that the progressive muscle relaxation therapy give immediate and significant effects against fatigue is characterized by a decrease in pulse rate [7]. Furthermore, research shows that the progressive muscle relaxation can heart rate, blood pressure, oxygen consumption and regulate the activity of sweat glands, changing patterns of brain waves, and finally, it lowers the activity of physical motor [18].

The Effect of Progressive Muscle Relaxation on Oxygen Saturation

The results of this research were not in accordance with the previous which mentioned that at the time of relaxation happens, there was an increase in oxygen levels [19]. Other studies also mentioned that at the time of relaxation of muscle fibers, the extension occurs with declining conductance of nerve impulses to the brain, decreasing brain activity, and other body functions as the response of relaxation. The other responses to relaxation were the declining number of pulse, breathing, decreased blood pressure, and increased consumption of oxygen [20].

The absence of any significant differences between the average oxygen saturation (post-test) when just doing routine activity when done with progressive muscle relaxation may occur due to oxygen saturation values in each person is different and many factors influence, among others 1) internal factors, i.e., age, gender, diseases of pulmonary function disturbance, acidity (pH), and the partial pressure of carbon dioxide (CO2); and 2) external factors, namely food intake to increase the levels of Hb, early mobilization and gymnastics parturition [21]. However, these results may differ when progressive muscle relaxation did more often and regularly.

The Effect of Progressive Muscle Relaxation on Subjective Feelings of Fatigue

The results of this research are consistent with research done Ivancevich, et al [22]. which mentioned that progressive muscle relaxation psychologically could produce a feeling of a healthy, quiet and peaceful, a feeling that everything was in control as well as a decrease in the tension and anxiety. The study mentioned that progressive muscle relaxation technique to effectively cope with tension, anxiety, insomnia, depression, fatigue, irritable bowel syndrome, muscle spasms, neck and back pain, high blood pressure and mild phobia; the study mentioned that progressive muscle relaxation was a systematic therapy effective for overcoming the stress on nurses to reach a state of deep relaxation [17]. Another study revealed that Progressive Muscle Relaxation was one of the non-pharmacological intervention and a systematic technique that can be used to reduce stress and achieve a state of relaxation [5].

CONCLUSION

The results showed that the progressive muscle relaxation technique was more effective for reducing fatigue on nurses, compared with just doing routine activities without relaxation. It was observed from the decline of its influence on the frequency of the pulse and lowers the subjective feeling of fatigue score respondents indicating a more significant result than routine activity.

Conflict of Interest: Hereby the authors declared that there is no conflict of interest in this research with any other parties.

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Ethical Clearance: This research has been approved by Ethical Board Committee, Faculty of Public Health University Indonesia and has been approved for ethical clearance by Ethical Board Committee, Faculty of Public Health University Indonesia.

REFERENCES

1. Dorrian J et al. The Ability to Self-Monitor Performance when Fatigued. PubMed-Journal Sleep Res US Natl Libr Med Natl Institutes Heal 9(2)137-44. 2000;
2. Kunert K, King ML, Kolkhorst FW. Fatigue and Sleep Quality in Nurses. J Psychosoc Nurs Ment Health Serv. 2007;45(8):30–7.
3. Han K, Trinkoff AM, Geiger-brown J. Factors Associated With Work-Related Fatigue and Recovery in Hospital Nurses Working 12-Hour Shifts. 2015;62(10):409–14.
4. Martin DM. Nurse fatigue and shift length: a pilot study. Nurs Econ. 2015;33(2):81–8.
5. Essa RM et al. Effect of Progressive Muscle Relaxation Technique on Stres, Anxiety and

Depression After Hysterectomy. J Nurs Educ Pract Int Peer-reviewed Open Access J Nurs Spec. 2017;Vol 7.

6. Kumutha V, Aruna S, Poongodi R. Effectiveness of Progressive Muscle Relaxation Technique on Stress and Blood Pressure among Elderly with Hypertension. IOSR J Nurs Heal Sci. 2014;3(4):1–6.
7. Trisnowiyanto B. Pengaruh Immediet Instrumental Music Hearing Therapy Dengan Progresive Muscle Relaxation Exercise Terhadap Rest Heart Reat. J Phederal Penjas. 2015;11(2).
8. Houghton D, Jones TW, Cassidy S, Siervo M, MacGowan GA, Trenell MI, et al. The effect of age on the relationship between cardiac and vascular function. Mech Ageing Dev [Internet]. Elsevier Ireland Ltd; 2016;153:1–6. Available from: http://dx.doi.org/10.1016/j.mad.2015.11.001
9. Setyawati L. Selintas tentang kelelahan kerja. Yogyakarta: Amara Books; 2010.
10. Suma'mur P. Higiene Perusahaan dan Kesehatan Kerja. Jakarta: Toko Gunung Agung; 2010.
11. Hunter SK. Sex Differences and Mechanisms of Task-Specific Muscle Fatigue. Exerc Sport Sci Rev. 2009;37(3):113–22.
12. Wan JJ, Qin Z, Wang PY, Sun Y, Liu X. Muscle fatigue: General understanding and treatment. Exp Mol Med [Internet]. Nature Publishing Group; 2017;49(10):e384-11. Available from: http://dx.doi.org/10.1038/emm.2017.194
13. Gempur S. Ergonomi Manusia, Peralatan, dan Lingkungan. Jakarta: Prestasi Pustaka; 2004.
14. Sung K, Seo Y, Kim JH. Relationships between Compassion Fatigue, Burnout, and Turnover Intention in Korean Hospital Nurses. J Korean Acad Nurs [Internet]. 2012;42(7):1087. Available from: http://synapse.koreamed.org/DOIx.php?id=10.4040/jkan.2012.42.7.1087
15. Abbaszadeh et al. The Relationship Between "Compassion Fatigue" And "Burnout" Among Nurses. Ann Trop Med Public Heal 10869-73. 2017;
16. Acharya A, VP Reddaiah NB. Nutritional Status and Menarche in Adolescent Girls in an Urban Resettlement Colony of South Delhi. Indian J Community Med. 2006;31(1):7–8.
17. Patel P. A study to assess the effectiveness of progressive muscle relaxation therapy on stress among staff nurses working in selected hospitals at Vadodara city. IOSR J Nurs Heal Sci. 2014;3(3):34–59.
18. Tsitsi T, Charalambous A, Papastavrou E, Raftopoulos V. Effectiveness of a relaxation intervention (progressive muscle relaxation and guided imagery techniques) to reduce anxiety and improve mood of parents of hospitalized children with malignancies: A randomized controlled trial in Republic of Cyprus and Greece. Eur J Oncol Nurs [Internet]. Elsevier Ltd; 2017;26:9–18. Available from: http://dx.doi.org/10.1016/j.ejon.2016.10.007
19. Smeltzer S, Bare B. Keperawatan Medikal-Bedah. Jakarta: ECG; 2012.
20. Potter P, Perry A. Fundamental of Nursing : Fundamental Keperawatan. 7th ed. Jakarta: ECG; 2010.
21. Brunner, Suddarth. Keperawatan Medikal Bedah. 8th ed. Jakarta: ECG; 2001.
22. Ivancevich JM, Konopaske R, Matteson MT. Perilaku dan manajemen organisasi. 7th ed. Jakarta: Erlangga; 2007.

Situational Analysis of Career Choices among Indonesian Nurses Returnees

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ABSTRACT

Background: There are few studies about the Indonesian nurse returnees' career after working and living overseas. Therefore, the present study addressed the current status of their employment. **Purpose:** This paper explores the situation of return migration among Indonesian nurses who return to the country of origin. **Methods:** Descriptive study using the latest survey of Indonesian nurse returnees. **Results:** The career situation of Indonesian nurse returnees varies on the post-migration stage. Half of the returnees were working out of nursing areas (57.2%) at the time of the survey. Returnees predominantly work as own account worker when they get back into the labor market. While the rest were working as private employees, Japanese translators, Indonesian state own enterprises employee, hotel staff, insurance agent, and teacher. **Conclusion:** The result of this research inquiry adds new understanding and knowledge in an area of return migration of nurses that has been relatively unexplored. Returnee career situations were relatively similar number between nursing and non-nursing areas.

Keywords: *return migration, career choices, Indonesian nurses.*

INTRODUCTION

In regard to the international nursing migration, Indonesia has become an exporter of nurses to economically developed countries[1-3]. The United Arab Emirates, Netherlands, Kuwait, Great Britain, and Saudi Arabia have been among the receiving countries since 1996[4]. The active recruitment of Indonesian nurses abroad is dominated by private recruiters followed by government facilitation[5]. Regulation of international nurse migration under bilateral agreement has grown through national regulatory bodies which started with Japan followed by East Timor [6, 7].

The generalizability of much-published research on the return migration issue is problematic, not only in the Indonesian context but also in the global landscape. Return migration is often discussed as a new strategy to get a brain gain. However, early evidence indicates this could also lead to brain waste[8]. Following the current review, it was apparent that published studies failed to gain insight on return to practice for nurses in the post-migration stage. This indicates a need to understand various circumstances among nurse returnees under workforce migration flows. This present study seeks to remedy these problems by analyzing Indonesian returnees on their current occupational choice.

METHODS

A descriptive study design was conducted to describe the current occupational of Indonesian nurse returnees. A survey involving Indonesian nurse returnees were conducted to track the latest returnees' occupation.

RESULTS

The career situation of Indonesian nurse returnees varies on the post-migration stage (Table 1). As can be seen, half of the returnees were working out of nursing areas (57.2%) at the time of the survey. Returnees predominantly work as own account worker when they get back into the labor market. While the rest were working as private employees, Japanese translators,

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Indonesian state own enterprises employee, hotel staff, insurance agent, and teacher. Respondents who chose not to return to nursing cited bad working conditions (86%), poor salary (74%), lack of confidence with their nursing skills (62%), unclear career paths or stalled career progressions (55%), difficulty in finding nursing jobs (58%), shift work (44%), unrecognized working experience in Japan (40%), feeling neglected by officials (40%), lack of respect (38%), and difficulty dealing with the certificate of registration (*Surat Tanda Registrasi)* process (22%). These reasons were repeated among returnees who opted out altogether from the nursing field. Deskilling was also cited by returnees who worked in Japan by stated they had lost their technical skills such as the ability to administer Intra Venous (IV) therapy. An emphasis on basic nursing care was voiced by returnees as an example of deskilling, as this activity was associated with lower grade work in Indonesia.

On the other hand, for those who stayed in nursing areas based on their employment setting the majority now work in private hospitals (27.2.6%). In contrast with previous respondents, those who stay in nursing jobs said they are passionate about nursing (92%), have a desire to help others (90%), expect to transfer skills and knowledge from Japan (88%), are aligned with their educational background (80%), have opportunities to open private nursing practices (67%), have opportunities to develop nursing specialties (particularly in geriatric nursing) (77%), have opportunities to develop long-term care services (54%), and have pride and noble works (41%).

Table 1 Career choices of participants (N=250)

Variables	n	%
Non-nursing area	143	57.2
Own account workers	66	26.4
Private employee	30	12
Japanese translator	24	9.6
Indonesian state own enterprises	4	1.6
Hotel	5	2
Insurance agent	7	2.8
Teacher	7	2.8
Nursing area	107	42.8
Nurse in private hospital	68	27.2
Nurse in district hospital	28	11.2
Nurse in Japanese company	9	8.4
Community health center	2	1.9

As Table 2 illustrates, the nursing specialties of returnees are widely spread out into different specialties. These include medical and surgical nursing (10.4%), general nursing (10.4%), mother and child nursing (4.8%), emergency nursing (4.8%), and community health nursing (0.8%).

Table 2 Nursing area based on specialties (N=107)

Nursing specialties	n	%
General nursing	26	10.4
ICU/Pediatric care nursing	7	2.8
Emergency nursing	12	4.8
Operating theater	9	3.6
Medical & surgical nursing	26	10.4
Mother and child nursing	12	4.8
Management nursing	8	3.2
Geriatric nursing	5	2
Community health nursing	2	0.8

DISCUSSION

This study provides a profile of the career choices of Indonesian nurses after their return from overseas. The choice of nursing and non-nursing areas is quite similar with regard to numbers. One study in Jamaica showed a slightly higher proportion (76%) among returnees who come back into bedside nursing [9]. While other studies on the return migration of nurses did not clearly mention how many returnees came back into nursing[10-12]. Findings from this present study show that the majority of returnees were working in various settings, including hospitals, clinics and communities. The highest numbers were working in private hospitals, followed by district hospitals, clinics in Japanese companies and community health centers. This data is similar in vein to the labor market for the Indonesian health workforce, which is dominated by the private sector[13]. The introduction of a zero growth policy on civil service numbers, liberalization of hospital ownership, and the passing of decentralization law in 1999 has significantly contributed to the dramatic growth of private health providers in Indonesia [13]. The presence of few returnees in district hospitals might reflect the low capacity of local government to employ nurses. On the other hand, nurses who work in Japanese clinic companies might feel interested in utilizing their skills and acquisition of the Japanese language. Returnees might possibly gain benefits by receiving a higher salary in Japanese company rather than a local business. With regard to returnee specialties in nursing, the dominant numbers were in medical and surgical nursing, and the fewest were in community health nursing. Further analysis on the competency of returnees and availability of job market are deserved to be done.

Returnees who work in non-nursing areas were dominated by the self-employed or those who were their own account worker. Piracha and Vadean (14) viewed that returning migrants have a higher propensity to become self-employed. Those who opted out from nursing areas, also found work as private employees, Japanese translators, insurance representatives and teachers. A number of the most highly qualified nurses who work outside of nursing were evident in this study, especially those who were titled in *Kangoshi* and *Kaigofukushishi* positions. These returnees had excellent nursing competencies and a great opportunity to contribute to the development of nursing. They cited some reasons such as low salaries, unrecognized working experience, and lack of appreciation from the government as reasons for choosing to leave nursing jobs. This feeling of being uncared for and neglected among returnees might lead to demotivation toward the nursing profession itself. From the point of view of the nursing profession, brain waste was observed within this group. They could not transfer the skills and knowledge they had acquired abroad. Most health professional migration studies have highlighted that brain waste occurs during the immigration stage [15-17]. In contrast to earlier ideas, this study observes that brain waste exists during the post-migration stage. This finding supports previous research which found that return migrants who were qualified could not find opportunities in their field of specialization [18]. In order to derive the maximum benefits of bilateral labor migration, Indonesia should manage migration of professional nurses by improving skill matching and recognition of skills. At the same time, in order to maximize the potential positive outcome of return migration this issue must be addressed with the Japanese counterparts through bilateral meetings.

CONCLUSION

The result of this research inquiry adds new understanding and knowledge in an area of return migration of nurses that has been relatively unexplored. Returnee career situations were relatively similar number between nursing and non-nursing areas. However, the brain waste phenomenon was evident among nurses as many were working outside nursing areas.

Ethical Clearance: This study obtained the Certificate of Ethical Clearance from the Research Ethics Committee of Faculty of Nursing, Universitas Airlangga, number 696-KEPK.

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Conflict of Interest: None.

REFERENCES

1. Kemenkokesra. Decree of the Coordinating Minister for People's Welfare of the Republic of Indonesia Number 54 Year 2013 About the Health Manpower Development Plan 2011-2025. Jakarta: Kemenkokesra; 2013.
2. Kurniati, Efendi F. Human Resources for Health Country Profile of Indonesia. New Delhi: WHO

South-East Asia Region; 2017.

3. Efendi F, Nursalam N, Kurniati A, Gunawan J. Nursing qualification and workforce for the Association of Southeast Asian Nations Economic Community. Nurs Forum. 2018;53(2):197-203.
4. Agus Suwandono M, Anhari Achadi, Ketut Aryastami Human Resources On Health (HRH) For Foreign Countries: A Case Of Nurse 'Surplus' In Indonesia. Asia Pacific Action Alliance of Human Resources for Health; Srilanka: AAAH; 2005.
5. BNP2TKI. The Role of BNP2TKI on Mapping and Harmonization Migrant Workers, Jakarta: BNP2TK; 2012.
6. MOFA. Technical arrangement between the National Agency for the Placement and Protection of Indonesian Workers With the Ministry of Health of the Democratic Republic of Timor-Leste About Manpower Placement and Protection of Indonesian Midwives to Timor-Leste by Bilateral Agreement. Jakarta: Ministry of Foreign Affairs; 2011.
7. MOFA. Indonesia-Japan Economic Partnership Agreement (Join Press Statement) Japan: Ministry of forein Affairs of Japan (MOFA); 2006 [Available from: http://www.mofa.go.jp/region/asia-paci/indonesia/joint0611-2.html.
8. Wahba J. Who benefits from return migration to developing countries? Germany: IZA World; 2015 [Available from: http://wol.iza.org/articles/who-benefits-from-return-migration-to-developing-countries/long.
9. Brown. Workforce Losses and Return Migration to the Caribbean: A Case Study of Jamaican Nurses. Center for Migration Studies special issues. 1996;13(4):197-223.
10. Adzei F, Sakyi E. Drivers of return migration of Ghanaian health professionals: perspectives from doctors and nurses in urban Ghana. International Journal of Migration, Health and Social Care. 2014;10(2):102-20.
11. Blouin C, Debnath P. CARICOM return migration and brain circulation: case study of Caribbean-born nurses. Canadian Foreign Policy Journal. 2011;17(2):101-14.
12. World Bank. The Nurse Labor and Education Markets in the English-Speaking CARICOM: Issues and Options for Reform Caribbean: World Bank; 2009 [Available from: http://goo.gl/2C73aG.
13. Anderson I, Meliala A, Marzoeki P, Pambudi E. The production, distribution, and performance of physicians, nurses, and midwives in Indonesia : an update. Washington: World Bank Group; 2014. Contract No.: 25 December.
14. Piracha M, Vadean F. Return Migration and Occupational Choice: Evidence from Albania. World Development. 2010;38(8):1141-55.
15. Alam N, Merry LA, Islam MM, Cortijo CZ. International Health Professional Migration and Brain Waste: A Situation of Double-Jeopardy. Open Journal of Preventive Medicine. 2015;5(03):128.
16. Blythe J, Baumann A, Rheaume A, McIntosh K. Nurse Migration to Canada Pathways and Pitfalls of Workforce Integration. Journal of Transcultural Nursing. 2009;20(2):202-10.
17. ICNM. Nursing Workforce Sustainability: The International Connection. Philadelphia: ICNM; 2015.
18. Wickramasekara P. Policy responses to skilled migration: Retention, return and circulation: International Labour Organization; 2003.

Analysis of Factors Affecting Malnutrition among Elderly in Panti Werdha Mojopahit Mojokerto

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ABSTRACT

Background: Old age as the final stage of the cycle in life is the natural development stage that will be experienced by every individual who reaches old age and is a reality that cannot avoid. In 2020, the number of elderly humans is predicted to be 28.8 million (11.34%) with a life expectancy of 71.1 years. The increase in the number of elderly populations is due to the rise in the socio-economic level of the community, progress in the field of health services, and an increased level of public knowledge. In 2020-2025 it is estimated that Indonesia's elderly population is ranked fourth after China, India and the United States.

Material and Method: The research method uses factor analysis. The technique of sampling used in research is purposive sampling, with the elderly population at the Nursing Home. The purpose of this study is specifically to find out; Appetite; change in body weight; old cars; stress; neuropsychological disorders, BMI. The Collecting data using instruments, the questionnaire was used. Analysis results using SPSS version 25.0.

Findings: The research can be said that the variables and samples used to allow for further analysis. This evidence by the KMO value of 0.504 which means higher than 0.5. after that, we can see Rotated Component Matrix[a]

Conclusion: Malnutrition of Risk factors in the elderly people at Mojopahit Mojokerto nursing home can be described as follows, Informal factors for the elderly: mobility, neuropsychological disorders; External factors: control nurses about elderly body weight.

Keywords: *Elderly, Nursing, Nursing Home, Malnutrition.*

INTRODUCTION

Malnutrition is a problem that always arises and becomes a serious thing for parents, elderly as the final stage of the cycle in life is the natural development stage that will be experienced by every individual who reaches old age and is a reality that it cannot avoid.[1] Regarding population in the elderly, malnutrition appears today as the primary basis for public health. Mal-nutrition is caused by insufficient or inadequate nutritional intake which makes various damaging effects such as muscle wasting and body defenses. The same means that mal-nutrition in the elderly causes and worsens the state of weakness or dependence and contributes to the development of self-morbidity,[2-5] a large number of older adults in Indonesia in the future brings positive and negative impacts. The positive effect, if the elderly population is in a healthy, active and productive condition. Seen from the other side, the large number of elderly people is a burden. if the elderly has health problems that result in an increase in the cost of health services, a decrease in income/ income, an increase in disability, the absence of social support and an environment that is not friendly to the elderly population.[6]

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Aging is a process of gradual or continuous change, personal, physical and social experiments and a unique perception of life, which is happy for some people and others, it is the initial value of a dark and sad stage of life.[7] The aging process associated with changes in body composition, precisely due to a decrease in lean body mass. Malnutrition is a multifactorial disease, and in the elderly, many risk factors can worsen physiological performance that affects muscle mass and fat (FM), which globally seen for weight loss and body mass index (BMI). There are other things to note, the existence of chronic diseases, many drugs, cognitive impairment, depression, loss of role, and social isolation can act systematically with a decrease in digestive function, smell, and saliva, as well as hormonal profiles, which affect the nutritional status of the elderly.[8] For this purpose, it seems essential to identify malnutrition factors experienced by the elderly in high risk; In this case, a necessary step to promoting elderly health is gaining knowledge of their experiences and concerns during the aging process. The first step to identifying malnutrition at this age is to use reliable instruments and methods.[9]

The world population is rapidly aging with estimates that this year until 2050 the proportion of the world's population above 60 years will increase from 11% to 22%. The expected increase in the absolute number of older adults will triple from 605 million to 2 billion during this period.[10] Based on population projection data, it estimated that in 2017 there would be 23.66 million older adults in Indonesia (9.03%). It predicted that the elderly population in 2020 (27.08 million), in 2025 (33.69 million), in 2030 (40.95 million) and 2035 (48.19 million).[6] Because the number of older adults continues to increase, the provision of a better healthcare process for the elderly at both the hospital and the community is an obligation. Often, the focus of nutrition in the elderly is a healthy diet and exercise to minimize the risk of developing breast cancer from a lifestyle (such as cardiovascular disease, type 2 diabetes mellitus). Thus, there is ample evidence to suggest that energy-protein malnutrition (PEM) is a common and frequent problem in this age group, including in hospitals, nursing homes, and community environments.[11]

The primary challenge along this line of research is to find out the most influential factors in malnutrition in the elderly in nursing homes using the mini nutritional assessment. There are 18 the Mini Nutritional Assessment (MNA) items grouped in four parts: anthropometric assessment (weight, height, and weight loss); general assessment (lifestyle, drug use, and mobility); evaluation of diet (amount of food, food and fluid intake, and feeding autonomy); and subjective assessment (self-perception of health and nutritional status).[12]

Malnutrition in old age will become a more complex problem in the physical and psychological advanced age. However, researchers only want to see what factors impact on elderly seen from questionnaire MNA in nursing homes.

As a result, due to several malnutrition tests in the elderly, the current study was conducted to analyze the malnutrition factors with MNA. As a basis for state policy and the effectiveness of future interventions. Therefore, the present study aims to develop and evaluate MNA from instruments used to determine the most dominant factors in cases of elderly malnutrition

MATERIAL AND METHOD

This research is a Factor Analysis study to determine the factors that have the most impact on the case. Factor analysis (FA) is a multivariate method and can use as an extension of the primary component analysis. The primary purpose of the FA is to describe the relationship between a set of variables that observed with a small number of unobservable variables called factors.[13] The research community, both in the quantitative phase, included those in the Mojopahit nursing home in Mojokerto, East Java, Indonesia 2018. The research sample was older adults in nursing homes. The participants gave their informed verbal approval. Inclusion criteria are elderly, not those who experience total communication disruption and bed rest.

The exclusion criteria were refusing to continue the interview. To find out assessing nutritional status as part of an evaluation of the standard of elderly patients in clinics, nursing homes, hospitals, or among those who were evaluated for nutritional status, weak and fast, MNA was validated and developed jointly by the Internal Medicine Center and Clinical Gerontology of Toulouse (France), Clinical Nutrition Program at the University of New Mexico (United States), and the Nestle Research Center in Lausanne (Switzerland).[14-17]

The data collection process, researchers, used MNA which is complete in less than 15 minutes. Each response point has a numerical value and contributes to the score, which has a maximum value of 30. In people with advanced age with cognitive impairment, the test requires help from families from old age or the health nursing team. Older adults interviewed are classified into three nutritional levels based on a score between 0 and 30. A score of 24 or higher means satisfying nutritional status; a score of 17 to 23.5 means the risk of malnutrition; scores below 17 conclude protein-energy malnutrition. Guidelines for determining height according to knee height and body mass index (BMI) for all patients are included to facilitate using the tool. MNA is easier to manage, patient-friendly, inexpensive (no laboratory tests required), susceptible (96%) and specific (98%) and reproduced.[17-20]

Interviews are written carefully according to the questionnaire and then analyzed in the shortest time possible or approximately 15 minutes. Interview analysis is carried out by 2 participants (member checking) and 3 teaching staff members or researchers (external verification). In the third step, each questionnaire point is determined and entered by guiding the results of the interview and literature review. In this study, the first inductive and deductive method used. Data were analyzed using SPSS 25.0 software. Data from questionnaires that have completed and then tabulated, 27 elderly are asked to comment on this instrument, and then an examination is carried out by the MNA questionnaire examined. The classified data will proceed to the data analysis process (factor analysis). The factors that have known the results will be adjusted in value with other factors so that the dominant factor obtained.

To determine that 2 research members can carry out an assessment correctly, the principal researcher conducts a common perception in data retrieval, so that there will be no errors during the field assessment. The time used in this perception equation is 1 day in the campus class. The stages that are carried out are pre and post-test understanding of MNA instruments. in the event of any misunderstanding, it carried out until the member can carry out field assessments.

MNA only shows malnutrition in humans and here in parents. If the total score is> 23.5, 17-23.5, <17, it means that there is no malnutrition, the risk of malnutrition, or malnutrition, respectively.[21] MNA was validated to determine mal-nutrition for Turkish geriatric patients in 2015.[22] The calculate body mass index, height measured to centimeters (cm), and body weight weighed to half a kilogram (kg) with the same measuring instrument. MNA is performed in all respondents or the elderly even if their MNA-Short Form (SF) scores ≥ 12.

The structure evaluation of the questionnaire factor, exploratory factor analysis was applied. To answer this question "whether the measurement scale for malnutrition in the elderly with several factors or not," we use factor analysis that directly uses SPSS 25.0.

The final step includes determining the reliability of the questionnaire with internal consistency. KMO and Bartlett's Test calculated for the sig level. Research. The current article with the Code of Ethics STIKes.PPNI. LPPM.1316.13 approved at the Community Service Research Institute of Institute of Health Science Bina Sehat PPNI.

FINDINGS

Panti Werdha Mojopahit is a technical implementation unit of the Health and Social Welfare Office of the Mojokerto Regional Government that provides services to all people, especially those who are disadvantaged. Socially, this institution provides services to the elderly. In this place the elderly get love, physical and spiritual care, so they can enjoy their old days and get inner and inner peace. This Nursing Home located on Jl Raya Brangkal No. 862 (Front of Sooko Police Station), District Sooko Kab. Mojokerto Postal Code: 61361 Tlfn: (0321) 328894. The northern part of the nursing home located not far from the Kedungmaling market. The south of this institution adjacent to the Masjid Raya Desa Gemekan, Sooko District, Hospital and DIAN HUSADA, Sooko sub-district health center which has the advantage for 47 health services, and dealing with the Sooko District Police Station and the Sooko sub-district office in Mojokerto Regency and the location of this nursing home is right on the side of the inter-provincial highway, with the busy streets of both private and public vehicles.

Table 1: KMO and Bartlett's Test

KMO and Bartlett's Test		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.504
Bartlett's Test of Sphericity	Approx. Chi-Square	14.053
	df	15
	Sig.	.522

The above results, it can be said that the variables and samples used to allow for further analysis. This evidence by the KMO value of 0.504 which means higher than 0.5.

After the sig level. It is known and can be continued to determine what factors I influence that can be seen in the table as follows, determining variables to certain factors follows the magnitude of the correlation between variables with coefficients, namely to the significant relationship. It saw table 2.

Table 2. Rotated Component Matrix[a]

Rotated Component Matrix[a]			
	Component		
	1	2	3
Penurunan Asupan makanan	.116	-.822	.282
Penurunan berat badan	.338	.746	.214
Mobilitas	.674	.287	-.030
Stress	.246	.212	-.672
Neuropsiko	.170	.103	.828
IMT	.889	-.111	-.006
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.[a]			
a. Rotation converged in 5 iterations.			

Determination of variables to certain factors follows the magnitude of the correlation between variables with coefficients, namely to the significant association. It was the conclusion of the table. 2 then the member factors and variables are:

1. First factor:

a. Mobility

b. Body Period Index

2. Second Factor:

a. Weight loss

3. Third Factor:

a. Neuropsychological

DISCUSSION

The first factor consists of activities that must be done by the elderly and body mass index; this means that the elderly and controlling the elderly interpreted as more elderly. The second factor is almost the same as the monitoring of body mass index, and the third factor is the aging process itself which is experiencing neuropsychological disorders. In conclusion, the factors that affect malnutrition among elderly people at Mojopahit Mojokerto nursing home can be described as follows:

Informal factors for the elderly: mobility, neuropsychological disorders

External factors: control nurses about elderly body weight

CONCLUSION

Nutritional status assessment with Mini Nutritional Assessment (MNA) is a multiparameter in screening as well as field assessments of malnutrition in the elderly. This MNA questionnaire consists of 18 questions divided into 4 domains: evaluation for anthropometry, evaluation for food intake, general evaluation of old lifestyle and subjective assessment of advanced age. MNA scores are reliable and can be relied upon to detect the risk of malnutrition in the elderly. In conclusion, using this MNA to look at the factors that cause starvation is better than malnutrition in the elderly. there are 2 malnutrition factors in the Mojopahit Mojokerto nursing home, namely informal elements of the elderly: mobility, neuropsychological disorders, and external factors: controlling nurses about elderly body weight

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REFERENCES

1. Chen CCH, Schilling LS and Lyder CHJJoan. A concept analysis of malnutrition in the elderly. 2001; 36: 131-142.
2. Lacau St Guily J, Bouvard É, Raynard B, et al. NutriCancer: A French observational multicentre cross-sectional study of malnutrition in elderly patients with cancer. *Journal of Geriatric Oncology* 2018; 9: 74-80. DOI: https://doi.org/10.1016/j.jgo.2017.08.003.
3. Leij-Halfwerk S, Verwijs M, van Houdt S, et al. Best estimates of malnutrition risk in European elderly. *Clinical Nutrition* 2018; 37: S253-S254. DOI: https://doi.org/10.1016/j.clnu.2018.06.1895.
4. Maitre I, Van Wymelbeke V, Amand M, et al. Food pickiness in the elderly: Relationship with dependency and malnutrition. *Food Quality and Preference* 2014; 32: 145-151. DOI: https://doi.org/10.1016/j.foodqual.2013.04.003.
5. Siddique N, O'Donoghue M, Casey MC, et al. Malnutrition in the elderly and its effects on bone health – A review. *Clinical Nutrition ESPEN* 2017; 21: 31-39. DOI: https://doi.org/10.1016/j.clnesp.2017.06.001.
6. Health IMo. Elderly Analysis in Indonesia 2017, https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwih3-yruJTeAhWItY8KHXqyA5gQFjAAegQICRAC&url=http%3A%2F%2Fwww.depkes.go.id%2Fdownload.php%3Ffile%3Ddownload%2Fpusdatin%2Flain-lain%2FAnalisis%2520Lansia%2520Indonesia%25202017.pdf&usg=AOvVaw1pZHfpZoN-XHD-uoHyy3HF (2017, accessed 20 October 2018).
7. Farajzadeh M, Gheshlagh RG, Sayehmiri KJIjocbn, et al. Health related quality of life in Iranian elderly citizens: a systematic review and meta-analysis. 2017; 5: 100.
8. Donini LM, Poggiogalle E, Pinto A, et al. Chapter 20 - Malnutrition in the Elderly. In: Martin CR and Preedy VR (eds) *Diet and Nutrition in Dementia and Cognitive Decline*. San Diego: Academic Press, 2015, pp.211-222.
9. Damayanthi H, Moy FM, Abdullah KL, et al. Prevalence of malnutrition and associated factors among community-dwelling older persons in Sri Lanka: a cross-sectional study. *BMC geriatrics* 2018; 18: 199. 2018/09/01. DOI: 10.1186/s12877-018-0892-2.
10. WHO. Facts about ageing, http://www.who.int/ageing/about/facts/en/ (2014, accessed 201 October 2018).
11. Agarwal E, Miller M, Yaxley A, et al. Malnutrition in the elderly: A narrative review. *Maturitas* 2013; 76: 296-302. DOI: https://doi.org/10.1016/j.maturitas.2013.07.013.
12. Guigoz Y, Lauque S and Vellas BJJCigm. Identifying the elderly at risk for malnutrition. The Mini Nutritional Assessment. 2002; 18: 737-757.
13. Alkarkhi AFM and Alqaraghuli WAA. Chapter 9 - Factor Analysis. In: Alkarkhi AFM and Alqaraghuli WAA (eds) *Easy Statistics for Food Science with R*. Academic Press, 2019, pp.143-159.
14. Wang X, Gao J and Li X. Effect of the nutritional support in elderly patients with malnutrition. *Clinical Nutrition* 2018; 37: S306. DOI: https://doi.org/10.1016/j.clnu.2018.06.2069.
15. Ngo J and Serra-Majem L. Hunger and Malnutrition. *Reference Module in Food Science*. Elsevier, 2018.
16. Jeejeebhoy KN and Duerksen DR. Malnutrition in Gastrointestinal Disorders: Detection and Nutritional Assessment. *Gastroenterology Clinics of North America* 2018; 47: 1-22. DOI: https://doi.org/10.1016/j.gtc.2017.09.002.
17. Cereda EJCOiCN and Care M. Mini nutritional assessment. 2012; 15: 29-41.
18. Dent E, Chapman I, Piantadosi C, et al. Screening for malnutrition in hospitalised older people: Comparison of the Mini Nutritional Assessment with its short-form versions. 2017; 36: E8-E13.
19. Gupta A and Rehman A. *Measurement scales used in elderly care*. CRC Press, 2017.
20. Helminen H, Luukkaala T, Saarnio J, et al. Comparison of the Mini-Nutritional Assessment short and long form and serum albumin as

prognostic indicators of hip fracture outcomes. 2017; 48: 903-908.

21. Soysal P, Isik AT, Arik F, et al. Validity of the Mini-Nutritional Assessment Scale for Evaluating Frailty Status in Older Adults. *Journal of the American Medical Directors Association* 2018. DOI: https://doi.org/10.1016/j.jamda.2018.07.016.
22. Sarikaya D, Halil M, Kuyumcu ME, et al. Mini nutritional assessment test long and short form are valid screening tools in Turkish older adults. 2015; 61: 56-60.

The Development of the Vestibular Stability in Children Who are Engaged in Football Taking into Account their Nervous System

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ABSTRACT

In this study, we investigated the effect of typological peculiarities of manifestations of properties of the nervous system of young football players the ability to accurately and consistently perform motor actions in conditions of vestibular irritation. 32 young football players of 10-11 years participated in the pedagogical experiment. 16 athletes were engaged in experimental methodic which is based on using the same exercise and methods development of the ability to react quickly, but different components of the load, for players with a strong nervous system (8 people), the load was intensive, but for players with a weak nervous system (8 persons) - volumetric load. The other 16 football players made up the control group. For 8 months of the experiment there was a significant increase in the ability to react quickly football players 10-11 years old in the experimental group, which was engaged in by a special methodic. The football players with a strong nervous system indicator has improved from 10.1±0.6 time to 12.0±0.4 time ($P<0.05$), and football players with a weak nervous system – from 9.1±0.3 time to 10.1±0.4 time ($P<0.05$). In the control group also occurred the positive, but not significant changes ($P> 0.05$). The study proved the effectiveness of the use of the typological properties of the nervous system as a differentiated method of developing the ability to ability to accurately and consistently perform motor actions in conditions of vestibular irritation of young football players. This approach allows to improve the quality of the training process of young football players.

Keywords: *Typology, Vestibular stability, Football players, Differentiated approach.*

INTRODUCTION

Football is the most popular sport in all the world. Football is played at different levels, amateur and professional. Football is played by men, women and children. In order to become a great football player must be persistently engaged from childhood. The general level of training of a football player consists of such types of training as his technical training, physical training, theoretical knowledge, tactical thinking and some other types of training.

One of the most important types of training for a football player, especially at a young age, is his technical level of preparation[10,20]. Technical training of an athlete, a football player is a level of coordination abilities[4,7,26].

Each sport has its own characteristics. However, it is the specific coordination abilities that make up the technical training of the athlete. While General coordination abilities are the Foundation for the development of specific abilities.

In the modern literature there are a number of classifications of specific coordination abilities, one of the most popular classifications is the classification proposed by Hirtz and Lyakh. One of the most important abilities the authors distinguish is vestibular stability[8,17].

Age 10-11 years is the most favorable for the development of children's ability to One of the most important abilities the authors distinguish is accurately and consistently perform motor actions in conditions of vestibular irritation[5,11,23].

The game of the football players is carried out in the constantly emerging unexpected situations, which require the manifestation of the speed of reaction, the ability to concentrate and switch attention, space-time accuracy. It is necessary to have the ability to accurately and consistently perform motor actions in conditions of vestibular irritations[22].

In order to fully reveal and realize the athlete's ability to use an individual approach to training activities. In children's and youth sports, as a rule, this approach is called differentiated. That is, during the training session, children are divided into groups, taking into account some criterion, for example, in terms of technical training, physical development, growth, physical training and some others[1,3,13].

Criteria for dividing players into groups can be very diverse. However, in our opinion, it is necessary to rely on the typological features of the manifestation of the properties of the nervous system, that is, the strength-weakness indicators of the nervous system in the process of excitation. This is a new and poorly understood approach in the training activity of athletes. The use of typology in the training process was studied on athletes - in sprint track and field athletes[12], basketball[16,19], gymnastics[14], table tennis[25].

In all the studies listed above, the effectiveness of the typological criterion was proved. At the same time, we were unable to find studies on football players, the preparation of which is carried out based on the difference in the properties of the nervous system (strength and weakness of the excitation process).

The aim of the study is to improve the technical training of football players, using a differentiated approach based on typological features of the manifestation of the properties of the nervous system due to the development of the ability to accurately and consistently perform motor actions in conditions of vestibular irritation.

MATERIAL AND METHOD

The study used empirical and theoretical methods.

1. Theoretical methods - the study of materials on the problem being studied, the study of the training system for young athletes involved in football, analysis of literary sources.

2. Empirical methods:

a) Basic pedagogical experiment - determination of the strength of the nervous system[24], determination of the level of development of the ability to vestibular stability[8];

b) Mathematical and statistical methods[21,27].

Criteria for evaluating the effectiveness of the methodic

The nervous system of players was determined by the method of "Tapping-test".

Sheet A4 is divided into six arranged three in a row of equal squares, according to the signal, the players begin to put dots in each rectangle. 5 seconds of work in each squares. The work is the rapid putting points in the square. You can move from one rectangle to another by command only. After the sixth rectangle the exercise ends. Result - count the number of points in each rectangle, build a work schedule. The type of nervous system is determined according to the schedule[24].

The ability to balance was evaluated in "turns on the gymnastic bench".

The subject must perform on the narrow surface of the gymnastic bench and not fall. The turn is complete when the subject returns to the starting position. The bench is located narrow side up, and the wide side lies on the floor. Result: the best of two attempts is taken into account, the number of turns performed in 20 with an accuracy of 0.5 turns[8].

Description of the experiment

In total, 32 football players of 10-11 years took part in the experiment. Prior to the experiment, the players were divided into KG and EG by random sampling[2, 28]. Each group had the same number of players with a strong and weak nervous system of the process of excitation.

During the training year, the CG players were engaged in the standard program 6, and the players in the EG were engaged in an experimental methodic. In football training in EG, a differentiated approach based on the typology of the nervous system was used. During the experiment, 130 training sessions were conducted for 90 minutes each training session.

Mathematical and statistical processing of the

experimental results was carried out using the parametric criterion (t-student)[21,27], used by Microsoft Excel 2007. Correlation analysis was performed using the Bio Stat 2009 program. The result was significant at a value of P> 0.05.

Feature of training in EG:

1. After a short warm-up, the football players of the EG performed exercises for 20 minutes on the development of vestibular stability in different subgroups strong and weak nervous system:

1) a Series of 6-10 jumps on the site with turns left and right, performed by different schemes: a) left 90 degrees + 90 degrees (the amount of turns can be varied within 45-360 degrees); b) the same right; C) 90 degrees left + 90 degrees right (such as "turn-turn").

2) Jumping on the spot and moving forward or backward with a turn of 540-720 degrees.

3) Run with turn (signal) for 180-360 degrees.

4) Running with rotation left and right and other exercises[18, 29].

2. The main methods of developing the reaction were repeated, varied and playing methods[9,15].

3. The main methodical admission were - the introduction of new exercises or the complication of old exercises, changing the speed of the exercise and the introduction of new targets[9,15].

4. The main difference in the training of football players with a strong and weak nervous system were the load components. The load for players with a strong nervous system was intense, and for players with a weak nervous system - a voluminous load.

The intensity of the load increased by increasing the number of exercises and reducing rest, and the volume increased due to the increase in the number of repetitions and rest intervals.

The intensity of the exercise is 150-170 beats per minute. The duration of the exercise for football players with a strong nervous system 20-30 seconds, for weak 30-40 seconds. The rest-time - until complete recovery; the character of the rest-time passive.

RESULTS

At the beginning of the study groups and subgroups were formed. Then, between EG and KG was played a friendly match. This match showed an objective level of possession of the technical skills of young football players. The meeting ended with a score of 1-1. Before the beginning of the pedagogical experiment, all football players passed a control test "turns on the gymnastic bench", which shows the level of development of the vestibular stability. There were no significant and significant differences between the indices of soccer players of CG and EG and within the groups (P> 0.05).

Table 1. Indicators of the ability to vestibular stability of football players 10-11 years old (M±m)

Indicators	The strength of the nervous system	KG			EG			P (2-5)
		Before	After	P	Before	After	P	
		1	2	3	4	5	6	
turns on the gymnastic bench (number of times)	Strong	10,1±0,6	12,0±0,4	**t=2,85 P<0,01**	9,9±0,4	10,2±0,4	t=0,58 P>0,05	**t=3,1 P<0,05**
	Weak	9,1±0,3	10,1±0,4	**t=2,20 P<0,05**	9,4±0,6	9,6±0,6	t=0,35 P>0,05	t=0,7 P>0,05
		t=1,55 P>0,05	**t=3,36 P<0,05**	–	t=0,7 P>0,05	t=0,78 P>0,05	–	–

The results in the test "Stop rolling the ball with a foot" (from 9.1 to 10.1 time) correspond to the average level of development of the ability to accurately and consistently perform motor actions in conditions of vestibular irritation[18].

Indicators of the ability to vestibular stability to football players 10-11 are presented in Table 1.

After the experiment was over, positive changes occurred in the test "turns on the gymnastic bench".

In KG in football players with a weak and weak nervous system, the indicators improved, but not significantly (P> 0.05).

In football players of the EG with a strong nervous system, the indices improved from 10.1±0.6 to 12.0±0.4 time (P <0.01), and for players with weak nervous system - from 9.1±0.3 up to 10.1±0.4 time (P <0.05). Such indicators correspond to a high level of development of the ability to accurately and consistently perform motor actions in conditions of vestibular irritation for the age of 10-11 years[18].

After the end of the pedagogical experiment of the boys' team, the EG and KG again played a friendly match among themselves. The game ended with a confident victory of football players EG with a score of 5-0. In the game, the guys from EG looked much faster, agile and more technically than the football players from the KG. Such a result allows us to judge the effectiveness of the methodic.

DISCUSSION

In different sports, General and specific coordination abilities are very important, especially in team sports such as football. For football players, one of the most important specific coordination abilities is the ability to vestibular stability[4,7,10]. Vestibular stability-the ability to accurately and consistently perform motor actions in conditions of vestibular irritation (somersaults, throws, turns)[11,20,26].

In training work with athletes, especially with children, an individual or differentiated approach is very important. It allows the effective use of hidden functionality and physical reserves of the body[1,3,13].

When using a differentiated approach, there may be different criteria, such as the growth of a player, his technical training and some other criteria. One of the effective criteria for dividing athletes into groups is the type of nervous system in the process of excitation[12,14,16,19,25].

To date, there are methodic in which tools and methods, the components of the load that are aimed at developing the abilities of athletes with different types nervous system are briefly described. In our methodic, for the first time in detail, we specified all the parameters of the means, methods and components of the load when performing physical exercises for athletes 10-11 years, involved in football.

It is important to understand that athletes with a strong nervous system better tolerate the load - intensive. Athletes with a weak nervous system work better with a volume load[12,14,16,19,25]. In our study, this theory is fully confirmed.

In the course of the experiment, new results were obtained and specific recommendations were given on working with young football players 10-11 years old, who have different types of the nervous system.

CONCLUSION

The experimental methodic of differentiated development of the ability to vestibular stability in football players aged 10-11 with different types of nervous system had a positive and progressive impact on soccer players EG (P <0.01).

In terms of football players in the KG, who were engaged in the standard methodic, the results of the ability to accurately and consistently perform motor actions in conditions of vestibular irritation also improved, but not significantly (P> 0.01).

The victory of the EG over the KG with a score of 5-0 indicates that the football players of the EG surpassed the players of the CG in all respects, including technical training.

Conflicts of Interest - The author declare that hi have no conflict of interest.

Ethical approval - All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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REFERENCES

1. Abramov EE (2006) A differentiated approach to the construction of training loads of players training groups. Dissertation, Moscow State Academy of Physical Culture.
2. Afanasiev VV (2007) Mathematical statistics in team sports. Academy, Moscow.

3. Battalov IM (2006) Differentiated approach to training basketball-students. Dissertation, All-Russian Scientific Research Institute of Physical Culture and Sports.
4. Boichenko SD, Karseko EN, Leonov VV, Smotritskiy AL (2003) On some aspects of the concept of coordination and coordination abilities in physical education and sports training. Theory and Practice of Physical Culture, pp 15-18.
5. Feoktistov MF. Periods of sensitivity of development of physical abilities among various contingents of pupils at average school age. Uchenye zapiski universiteta imeni P.F. Lesgafta 2010; 62:118-120. http://doi.org/10.5930/1994-4683.2010.04.62.p118-120
6. Godik MA (2011) Football: a model of training program of sports training for youth sports schools, specialized youth school of Olympic reserve. Soviet sport, Moscow.
7. Grigoryan MR (2009) Technical training of young football players based on the differentiation of specialized loads with increased coordination complexity. Dissertation, Kuban State University of physical education, sport and tourism.
8. Hirtz P (1985) und andere. Koordinative Fähigkeiten im Schulsport. Volkseigener Verlag, Berlin.
9. Holodov ZhK, Khuznetsov VS (2009) Theory and methods of physical training and sports. Akademia, Moscow.
10. Jaakkola J, Watt A, Kalaja S. Differences in the Motor Coordination Abilities Among Adolescent Gymnasts, Swimmers, and Ice Hockey Players. Human Movement 2017; 1:44-50. https://doi.org/10.1515/2017-0006
11. Karpeev AG. Long-term strategy of sports and technical preparation with due regard for age peculiarities of motor coordination development. Uchenye zapiski universiteta imeni P.F. Lesgafta 2008; 38:35-39. https://doi.org/10.5930/1994-4683.2008.04.38.p35-39
12. Kostyunina LI, Kiryanova LA, AnisimovaYA. Special features of the manifestation of nervous system typological properties among sprint track and field athletes. Uchenye zapiski universiteta imeni P.F. Lesgafta 2010; 62:38-42. https://doi.org/10.5930/1994-4683.2010.05.63.p38-42
13. Kozhevnikov VS (2011) Differentiated planning of the main training facilities for players of 15-16 years old in the preparatory period on the basis of their bioenergetic types. Dissertation, Kama State Academy of Physical Culture of Sport and Tourism.
14. Krivoshchekova ON (2005) The development of speed and strength abilities of young gymnasts with different individual psychological characteristics. Dissertation, Siberian State University of Physical Culture and Sports.
15. Khuramshin YuF (2007) Theory and methods of physical training. Soviet sport, Moscow.
16. Lubkin YuV (2004) Efficiency of actions of basketball players of different game roles in connection with typological features of the properties of the nervous system. Dissertation, Herzen State Pedagogical University of Russia, St.Petersburg.
17. Lyakh VI, Witkowski Z, Zhmuda V (2002) Specific coordination abilities as a criterion for predicting sport achievements of football players. Theory and Practice of Physical Culture, pp 21-25.
18. Lyakh VI, Witkovski Z (2010) Coordination training in football. Soviet sport, Moscow.
19. Makarov YM, Hussain A-T. Typological profile of person properties of the basketball players aged 16-18 years old depending on the style of game activity. Uchenye zapiski universiteta imeni P.F. Lesgafta 2011; 73:122-124. https://doi.org/10.5930/1994-4683.2011.03.73.p122-124
20. Oskolkov VA, Kshinin II. Coordination abilities in structure of motor possibilities of the young boxers with various tactical manners of the duel conducting. Uchenye zapiski universiteta imeni P.F. Lesgafta 2011; 77:121-124. https://doi.org/10.5930/1994-4683.2011.07.77.p121-124
21. Paramonov VV. Development of abilities to rational activity in the changed conditions of young football players' training. Uchenye zapiski universiteta imeni P.F. Lesgafta 2011; 82:124-127. https://doi.org/10.5930/1994-4683.2011.11.81.p124-127
22. Parmuzina YuV (2006) Methods of practicing aerobics with young players. Dissertation, Volgograd State Academy of Physical Culture.
23. Petukhov AV (2007) Formation of the foundations of individual technical and tactical skills of young football players. Soviet sport, Moscow.

24. Raigorodskiy DJ (2001) Practical psychodiagnostics. Methods and tests. Method of express-diagnostics of the properties of the nervous system by E. P. Ilyin's psychomotor measures (Tapping-test). Publishing house Bakhrakh-M, Samara.
25. Voronin EV (2007) The conditionality of a choice of style of play in table tennis typological features of the basic properties of the nervous system and psychomotor performance. Theory and Practice of Physical Culture, pp 5-10.
26. Witkowski Z (2003) Coordination abilities of young players: diagnostics, structure and ontogeny. Dissertation, Academy of Physical Education in Katowice.
27. Zheleznyak YuD, Petrov PK (2002) Basis of methodological activity in physical culture and sport. Academy, Moscow.
28. David FN, Cramer H. Mathematical methods of statistics. Biometrika 1947; 3/4 (34):374 https://doi.org/10.2307/2332454.
29. Nikitin AV. Coordination abilities of young football players-goalkeepers in their structure of motor abilities. Uchenye zapiski universiteta imeni P.F. Lesgafta 2011; 81:108-112 https://doi.org/10.5930/1994-4683.2011.11.81.p108-112.

Patterns of Mortality Caused by Natural Disasters and Human Development Level: A South Asian Analysis

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ABSTRACT

This study aimed at exploring the correlation between the patterns of mortality and affected people caused by natural disasters and human development level at country level in South East Asia (SEA). The study utilized quantitative data on deaths and affected people caused by natural disasters from CRED and other databases. Huge deaths and affected people concentrated on lower HDI countries in comparison with middle ones in SEA. Desegregated disaster subtypes, overlapping data, no universal scale about severity and magnitude measurement demonstrated lead barriers for conducting statistical test between impact variables and development level with value. With the combination of all parameters of natural disaster scales, a universal and accepted scale might be suggested to avoid misleading in disaster data collection and thereby develop scientific techniques for vulnerability assessment.

Keywords: *Natural disaster; Mortality; Affected people; South East Asia; Human development level*

INTRODUCTION

Large scale natural disasters have demonstrated unprecedented deaths and affected people based on severity and magnitude for the past decades around the world. Though natural hazards are connected with geographical fault lines, climatic shifts and meteorological extreme events, decrease in widespread deaths and affected people largely depends on country specific health system, existing logistics, coping strategies and human development level. Globally, 4000 massive natural disasters had been accounted for estimating 1.5 million deaths during 2005-2015. Several studies on natural disasters related mass causality and affected people have often turned into hazard and event specific disasters in the global settings.[1] Existing disaster documents reveal that there is a great gap of adequate information on country specific mortality and affected people caused by hydro-meteorological and geophysical disasters and its relation with human development value.[2] Disaster management logistics and preparedness initiatives have carried conviction for few people in low resource settings. In high income settings, people's exposure to deaths and affected people accounts for estimating lower rates due to strong disaster policy, existing coping resources. SEA is one of the most disaster-prone regions in the world. Geologically, the subcontinent is situated in the active tectonic plate movements in the India Ocean, which have been the source of major earthquakes and tsunamis. Climatic variability, tropical monsoons, and proximity to the coastlines are the direct risk factors for generating tropical cyclones and storms in the Indian Ocean.[3]

Natural disasters pose for occurring sudden or slow onset furious impacts with atmospheric, hydrologic and geologic roots.[4] Around five million deaths and millions more injuries worldwide are the direct consequences of disasters since 1960. Most of the deaths have concentrated in developing countries. As a single disaster, drought has killed over 2.5 million people

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while earthquakes caused 1 million more fatalities. Furthermore, storms and cyclone claimed over 750, 000 deaths during the last decades.[5] Over the past twenty years, more than 50% of all fatalities were reported in low human development countries though they shared only a tenth of those exposed to natural disasters. Subsequently, around 85% of the people exposed to natural disasters are the inhabitants of medium and low human development countries.[6] Deaths are the direct result of sudden onset natural catastrophe while affected is an aggregated figure of all injured, homeless and affected during the period of disaster.[7] The HDI refers to the geometric mean of normalized indices for three dimensions separately.[8] As a dimension of health for HDI, life expectancy has observed a disproportionate increase over the past quarter century. Education and standard of living, based on mean of years of schooling and per capita income[8] respectively are attributed to promise improved socio-economic status through increasing livelihood capacities, housing in low risk areas, expensive disaster insurance and adequate access to warning and evacuation information system. The overall objective of the study is to explore the patterns of mortality and affected people caused by natural disasters and their relationship to the level of human development at country level in SEA.

MATERIALS AND METHOD

The study approached a content analysis of academic research articles, conference papers and scientific documents based on mortality and affected people caused by natural disasters in the duration of 2014-2015. This study employed quantitative method of data collection. Deaths and affected data had been primarily collected from CRED database (EM-DAT). For measuring the severity and magnitude of each disaster, the study also used quantitative data from Relief web, OCHA, UNDP, Flood list, Wikipedia and different media sources. The researcher checked and rechecked data repeatedly from multiple sources for verifying the validity.

Concentrating on multiple scales for measuring magnitude level, a general scale valued 1 to 5 had been adopted for combining original disaster magnitude levels into a standardized benchmark. This piece of work utilized human development value from UNDP country reports and correlated impact variables caused by disasters for the duration of 2014-2015. The duration of data collection took two months, May to June, 2016. The collected data had been analyzed by using descriptive statistics in SPSS version 20. Multi variate analysis was administered for correlating impact variables caused by natural disasters with the value of country specific human development index.

RESULTS

Table 1: Profile of deaths caused by natural disasters 2014-2015

Disasters	Frequency	Deaths	Percent (%)
Earthquake	13	9457	52.84
Extreme temperature	6	3796	21.21
Flood	40	3160	17.66
Landslide	15	997	5.57
Storm	17	483	2.70
Total	**92**	**17, 897**	**100.00**

The table 1 shows that there was no relation between number of disaster episodes and number of deaths occurred in SEA. Out of 92 natural disasters recorded, approximately 53% deaths occurred due to earthquakes while 21.21% fatalities occurred due to extreme temperature in the past two years. More frequency of disaster episodes does not demonstrate incidence of deaths higher or lower in the current study. In the study, 17.66%, 5.57% and 2.70% deaths were reported from flood, landslide and storm respectively.

Patterns of Mortality and Affected People and HDI

Mortality and HDI

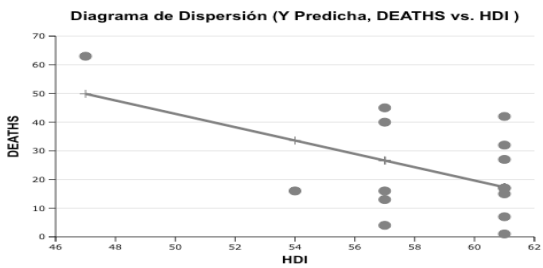


Figure 1: Number of deaths and HDI

The figure 1 shows that over 60 deaths were reported for HDI 0.47 in Afghanistan while around 40-0

deaths were noted for HDI 0.61 in India and Bhutan respectively. The number of deaths lowered when the value of HDI showed an increase in the line graph data. It was observed that deaths caused by natural disasters had a positive correlation with the level of human development for each country. It was an inverse or indirect correlation between deaths and HDI variables. This relationship was considered statistically significant in the study. So, it was clearly found that deaths caused by natural disasters followed a positive correlation with the value of human development for each specific country.

Flood occurred mortality and HDI level

In the case of floods, there was also an inverse correlation between the value of human development index and the number of deaths and this correlation was shown as statistically significant.

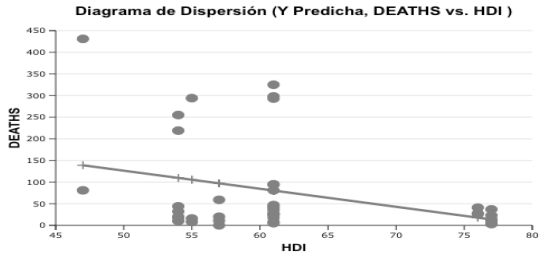


Figure 2: Deaths caused by flood and HDI

With regard to explaining deaths caused by flood during 2014-2015, it was clearly found that nearly 450 deaths were identified for lowest HDI 0.47 while less than 50 deaths were reported for the highest HDI 0.77 in the study line graph 2. The study data on flood demonstrated that deaths followed an inverse statistical relationship with the level of HDI in quantitative study.

Storm induced deaths and HDI value

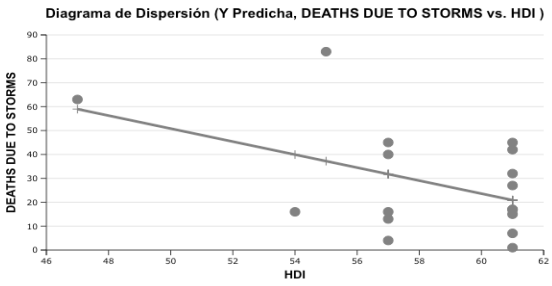


Figure 3: Correlation between deaths and HDI

In the graph 3, it was clearly noted that over 80 deaths caused by storms were identified for HDI score 0.55 of Nepal while around 60 deaths caused by storms were reported for HDI value 0.47 of Afghanistan in the study. Most of the reported deaths caused by storms followed a slight declination for higher HDI countries in the figure. In addition, more deaths caused by storms demonstrated for lower HDI values in comparison with higher ones in the study. The relationship between variables was negative or indirect but reported as statistically significant.

Correlation of Mortality based on Standardized Magnitude (SM) and HDI

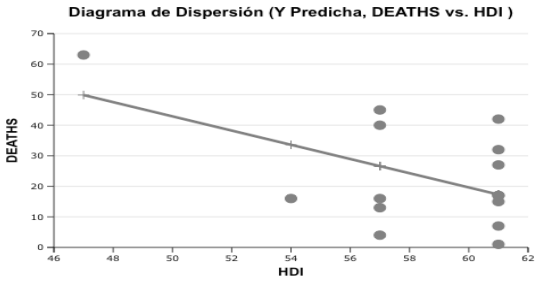


Figure 4: Deaths of SM 1 and HDI

Overall, it was found that over 60 deaths caused by disasters of magnitude one were reported for low HDI score 0.47 in Afghanistan focused in the graph 4. The number of deaths of magnitude one declined sharply following higher HDI value in the study. It was an indirect or negative correlation between deaths caused by disasters and HDI level. It was considered as statistically significant in the study.

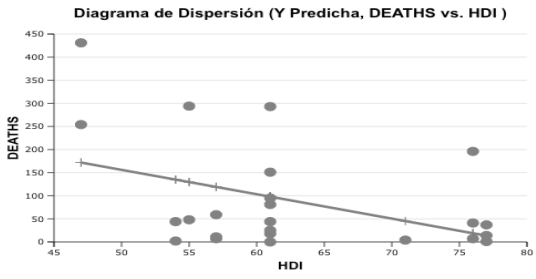


Figure 5: Deaths of SM 3 and HDI

In case of disaster magnitude 3, approximately 450 deaths were noted for lowest HDI value 0.47 in Afghanistan while around 5 deaths were reported for highest HDI values in Iran and Sri Lanka respectively (Figure 5).

DISCUSSION

Mortality Trends and Human Development Level

The SEA has experienced large scale disasters and widespread deaths and affected in the past couple of years. The frequency and destructiveness of natural hazards have found a considerable increase during the last 30 years.[9] Likewise, in comparison with disaster impacts in 2014, a higher proportion in deaths and affected people caused by natural disasters was found in 2015. Overlapping in data records, misleading in database records and multiple characters of similar natural disasters usually limit higher statistical analysis to connect disaster impact factors with development variables. In World Health Organization's estimation, statistics of death and affected people vary widely due to technical challenges. Limited resources for collecting and compiling disaster related data, inadequate vital registration system, political biases and misleading in reporting were noted as the challenging barriers for reporting country wise natural disaster data.[10]

The number of deaths caused by flood, earthquake and storm were found to be statistically correlated with country specific HDI value. The relationship was indirect or negative and found statistically significant. Exposure to disasters and deaths caused by natural disasters concentrated a lot on low human development countries while more impact factors concentrate in middle human development countries in comparison with that from high human development countries. Bangladesh and the United States experienced tropical cyclone 3.4 times and 12.1 times respectively, resulting consecutive deaths of 7,468 and 223 people on average per year during the twenty years 1980-2000.[10] Landslide and extreme temperature did not find any correlation with the value of human development.

Flooding showed a significant correlation with the level of human development for country specific profile. India, a middle HDI country has experienced a fairly proportion of flood related impact factors in the past years. With the highest frequency and magnitude, deaths and affected people caused by flooding undoubtedly concentrated on the level of development. But, the correlation between level of development and impact factors were focused as inverse or indirect statistically. In case of earthquake, the findings reveal that the correlation between deaths and affected people caused by earthquake as well as structural factors becomes apparent more rather than the level of human development value. Most of earthquakes concentrated on low middle human development countries like Nepal, Afghanistan, Pakistan and India and caused large scale deaths and injuries across the region. However, the yearly approximate 250 deaths were noted from storm, in the past couple of years. Another regional study finding from SEA reveals the different results. The yearly average deaths from storm were reported approximately 2000 during the periods 2010-2014 [3].

Human Development Level and Affected from Disasters

The correlation between human development level and affected people from flood, earthquake and landslide respectively was considered indirect or inverse. It was not statistically significant relationship. In addition, no correlation was found between HDI level and affected people from storm. The most affected people from natural disasters were found for middle HDI in India during the past two years 2014-2015. Flood, the frequently occurred event in India made a significant difference with all other disaster affected people for SEA countries. It was a bit confusing to correlate all the data from affected people with development level. Limitation of data on affected people from different natural disasters had been the great barrier for correlating with development level for country specific explanation. The most obvious finding showed that affected people from natural disasters appear mostly in middle HDI countries in SEA context.

Correlation between Deaths and Affected People

Deaths caused by natural disasters demonstrated a direct or positive correlation with affected population in the past couple of years. When deaths from natural disasters find an increase, the number of affected people also finds a positive increase in the same direction. These findings are quite similar with the statistical results of yearbook 2011. Between 1991-2000 and 2001-2010, around 10 and 60 deaths per million people respectively were reported in comparison with 21 and 22 affected people per thousand people respectively from natural disasters in SEA.[11] Overall, the higher the number of deaths reported in natural disaster situations, the largest the number of affected people appeared in the database.

HDI Value and Mortality Analysis

In SEA, countries with lower HDI value experiences more deaths caused by natural disasters of lower magnitude in comparison with countries with higher HDI value. The number of deaths caused by natural disasters demonstrated comparatively higher in Afghanistan, Pakistan, Nepal, and Bangladesh in comparison with other countries in SEA in the periods 2014-2015. The correlation of deaths caused by disaster magnitude 3 with HDI value was approached negatively in the study. However, majority of deaths of disaster magnitude 3 occurred in lower HDI countries like Afghanistan, Pakistan, Nepal and Bangladesh for the past two years. Conversely, higher HDI countries Iran, Sri Lanka, Maldives, Bhutan and India experienced lower deaths for disaster magnitude 3 in the same periods. The inverse relationship was indirect and statistically significant. On the basis of standardized magnitude 2 and 4, it was noted that there was correlation between the variables. It was an indirect or negative correlation. It was not statistically significant. Fatalities from earthquake 2015 in Nepal made a considerable difference for showing more significant relationship between human development value and deaths.

CONCLUSION

SEA countries continue to experience disproportionate impacts of natural disasters. Most of the deaths from natural disasters concentrated on low human development countries like Afghanistan, Nepal, Pakistan and Bangladesh in comparison with fatalities from other middle human development countries in SEA. Disaster affected people dominated in middle HDI countries in comparison with that from low HDI backgrounds. Limitation of data on affected people, overlapped disaster data among disaster subtypes, misleading in reporting in database, and misreporting in newspapers and journals demonstrated perceived barriers for considering statistical significant and positive correlation between impact variables and HDI level. Considering different disaster indicators for measuring severity and magnitude, a universal and well accepted scale for aggregating large scale disaster data and framing benchmark might be suggested for avoiding misleading in disaster data collection and developing scientific tools for vulnerability estimation.

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Ethical Clearance : The study was dependent on recorded data from EM-DAT database as per institutional rules. So, the study did not look for any individual consent. But, it took officially recognized institutional permission for using their reserved dataset.

REFERENCES

[1] Songer T, The Epidemiology of Natural Hazards, PhD Dissertation, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pennsylvania, USA

[2] The Rise of Natural Disasters in Asia and the Pacific (2013) Learning from ADB's Experience, Independent Evaluation, Asian Development Bank, Manila, Philippines

[3] ESCAP (2015) Overview of Natural Disasters and their Impacts in Asia and the Pacific, 1970 – 2014, ESCAP Technical Paper, Information and Communications Technology and Disaster Risk Reduction Division, March 2015

[4] Watson J T, Gayer M and Connolly M A (2007) Epidemics after Natural Disasters, Emerg infect Dis, 2007 Jan, 13 (1), 1-5, doi: 10.3201/eid1301.060779

[5] Cropper M L and Sahin S (2009) Valuing Mortality and Morbidity in the Context of Disaster Risks, Policy Research Working Paper, The World Bank

[6] United Nations Development Program (UNDP), Human Development Reports 2004

[7] Guha-Sapir D, Hargitt D, Hoyois, P (2004) Thirty years of Natural Disasters 1974-2003: the numbers. Presses universitaires de Louvain, Louvain-la-Neuve. Brussels, Belgium

[8] United Nations Development Program (UNDP), Human Development Reports 2015

[9] Seck P (2007) Links between Natural Disasters, Humanitarian Assistance and Disaster Risk Reduction: A Critical Perspective, Occasional Paper, Human Development Report Office

[10] World Health Organization (WHO), Natural Disaster Report 2015

[11] Statistical Yearbook for Asia and the Pacific 2011

Isolation and Identification of the Fungi Associated with Mosquitos and Utilizing their Filtrates in the Biocontrol of *Culex molestus* Forskal

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ABSTRACT

The following study included the isolation and identification of the fungi associated with the adult mosquito spread out throughout the Kerbala Governorate \ Iraq. A total of 94 samples were collected from different areas . Of these samples 69 fungi were isolated and laboratory identified to belong to 6 genera . *Aspergillus spp.* scored the highest rate reaching 56.5%, whereas *Trichoderma viride* scored the least *rate* of 2.8%. Three species of the *Aspergillus* were Isolated and identified, where the *A.niger* was the more frequent as its rate was 44.9% .The two neighbourhoods of Towayreg and Alhussainiya recorded the highest rate up to24.6%,and 20.2% *respectively*. As regards the effect of the isolated fungal filtrates on the percent of mortality of the adult of *Culex molestus*, the results also explained the superiority of *A.flavus* filtrates by spray and feeding the adults with food containing that filtrates where the mortality percent reached72.97% and70.83 %respectively. The concentration 100 % of the fungal filtrates resulted in the highest mortality rate up to 100 % after 96 hours of the treatment .

Key words : *A.flavus , A.niger , Culex molestus Forskal.*

INTRODUCTION

Mosquitos are considered blood-sucking arthropods which belong to the order Diptera [1] of the family Culicidae which is of great importance both in the medical and veterinary fields because they are a bio vector for several dangerous pathogenic pests ,and *are* the most prevalent among human environment [2] . Due to the medical importance of mosquitos, scientists were interested in combating both them and the damages resulting from utilizing chemical pesticides suffered by the environment, humans and the non targeted organisms [3] , thus the need arose to use bio control [4] where the fungi were utilized in insect control as they have the ability to infect nearly 200 types of insects which belong to order of Coeloptera andLepidoptera, wherebyfungi act as insect pathogens through contact, producing spores that attach to the insect's body and dissolve the proteins,chitin and fat incorporated in the insects body [5] .

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In view of the lack of previous studies concerning the isolation and identification of the fungi accompanying the mosquitos spread out in the Kerbala Governorate, this study aims at:Isolation and identification of the fungi associated with the mosquitos spread in different areas in Kerbala Governorate,Testing the efficiency of various concentrations of filtrates in affecting adult *Cx. molestus* by utilizing different methods.and Determination of the relation between the effect of the concentration and the period of exposure and their effect on the percent of mortality.

MATERIAL AND METHOD

Collection of samples

A total of 94 samples were collected from adult mosquitos from different areas of the Kerbala Governorate which are Towayreg, Alhussaineya, districts of Al- Zahraa, Albobyat, qantart Alsalam,Ramadan and the old city center using a specialized insect collection trap. brought to the laboratory and treatment in accordance with [6] then each 5 insects were put in a Petri dish containing Potato dextrose agar added to the

antibiotic Chloramphenicol at a concentration of 250 mg/lt, and incubated at 27 ℃ for an interval of 5-7 days, after which they were examined, the growing fungi isolated and purified on a new growth medium .

Identification of fungi

The isolated fungi were identified according to the standardized method [7] ,utilizing the categorization key [8,9] .The percentile was calculated according to the following equation [10]

Percentile of encounters =(number of times the fungus is encountered / the total number of samples) X 100

Preparation of fungal filtrates

A broth potato dextrose agar was prepared according to the standard doctrine [11] , it was distributed in 250 ml beakers in the amount 150 ml in each beaker, and the medium was inoculated with discs of the isolated fungal cultures at the age of 7 days, each alone, at a rate of three discs for each beaker. The beakers were incubated at 27 ℃ and shaken every three days to distribute the fungal growth. After two weeks filtering the inoculations using the Whatman No.1 filter paper using a bechner cone with the assistance of a vacuum pump, the filtration was repeated utilizing the fine filter containing a fine filter paper and then the concentrations were prepared: 25 %, 50% ,75 % ,and 100 % [12].

Collection and identification of mosquito samples

Egg rafts were collected from the*Cx. molestus* from one of the heavy sewage discharge locations in the district of Ramadan ,Kerbala Governorate. The rafts were placed in strict laboratory conditions in order to hatch and were breeded until reaching the adult.To confirm the identification process, a biological phenomenon was utilized, namely the ability of this type of females to lay its first raft of eggs without needing a blood meal [13] .

Effect of the fungal filtrates on the mortality of the *Cx. molestus* adult by spraying

The effect of the **filtrates** on the adult mosquitos was examined at the age of 24 hours after spraying them with the **filtrates** , where 10 insects were placed in a glass bottle of 250 ㎝³. The refined items were sprayed at a rate of 5 ml **filtrates** / refined, at an amount of three refined items for each concentration using a small sterilized atomizer from an approximate height of 15 cm. As to the control treatment, they were sprayed only with sterilized distilled water.The percent of mortality scored after 48 and 96 hours from treatment were recorded, and the values corrected according to [14] .

Effect of the fungal filtrates of some fungi introduced through nutrition on the percent of adult mortality of *Cx. molestus*

Filtrates of two isolated fungi introduced through nutrition were used and their effect on adult *Cx.molestus* was tested,where 5㎝³ of the fungal filtrates of each concentration prepared was mixed with 5 ㎝³ of a sugar solution at 10% concentration The mixture was distributed in sterilized plastic Petri dishes of a diameter of 9 cm, then the dishes were placed in breeding boxes containing 10 adult with three refined items for each fungal filtrates. The percent of mortality were calculated after 48 and 96 hours of treatment [14] .

All results were analysed according to the complete randomized factorial design, and calculated by using the Least significant difference at a probability level of 0.05[15]

RESULTS AND DISCUSSION

In the current study 69 isolates of the fungi were isolated and they were found to belong to 6 genera, the most prevalent percent belonged to the imperfect fungi followed by the cystic ones(table1).The reason for this is that the imperfect fungi produce vast numbers of small sized reproductive units in addition to their ability to spread over extended distances and their ability to form certain structures to resist environments hostile to their growth [16] .

Table(1) shows as well that the most frequently encountered fungus was the *Aspergillus*, where the percent of its encounters was 56.5 % while the least fungi to appear were both *Fusarium solani* and *Trichoderma viride*, as the percent of their encounters was 5.7 % and 2.8 %, respectively.

These results were consistent with other studies that indicated the isolation of several fungi from different insects , one of those is [10, 17, 18, 19] .As for the types, the same table shows that *Aspergillus spp.* was diagnosed through three types which are *A.niger*, the most frequent encountered at 44.9 % , followed by *A.flavus* at 8.6 %

, then *A.candidas* at 2.8 % . This result contradicts the findings of [18] and [20] , [21] stated that the reason of the frequent encounters of *Aspergillus spp.* which exceed the other species is due to the fact that they are one of the widespread fungi abundant in the warm places, in addition to them producing great numbers of spores.

Table 1.The percent of isolated fungi from adult mosquitos

Percent of encounters %	**Number of encounters**	**The fungus**
56.5	39	Aspergillus sp.
44.9	31	A.niger
8.6	6	A.flvus
2.8	2	A.candidus
13.0	9	Penicillium sp
11.5	8	Candida sp.
10.1	7	A .alternaria
5.7	4	Fusarium.solani
2.8	2	T.viride

The results of the study also showed significant differences in the percents of encounters of fungi in the samples examined according to the locations(table 2),. The highest rate of encounters was also recorded for the areas Altowayreg, Alhussainiya and Albobyat with a significant gap separating them from other areas at rates of 24.6 % ,20.2 % and18,8 % respectively. The reason for the high rate of them being rural areas with an abundance of gardens and fields , as well as the relatively high humidity rates there due to the presence of rivers and small streams in them,

Table 2. The effect of the districts studied on the percent of the encounters of the isolated fungi

	fungi %	**Districts**							**Fungi**
		Old city center	**Kantarat Alsalam**	**Ramadan district**	**Alzahra-a district**	**Albobyat**	**AlHuss-ainiya**	**Towayreg**	
44.9		1.4	4.3	2.9	**10.1**	**7.2**	**7.2**	**11.6**	***A.niger***
8.6		0	0	0	1.4	**2.9**	**2.9**	**1.4**	***A.flavus***
2.8		0	1.4	0	0	0	1.4	0	***A.candidus***
13.0		1.4	0	0	2.9	2.9	**2.9**	**2.9**	***Penicillium sp.***
11.5		1.4	0	2.9	0	2.9	1.4	2.9	***Candida sp.***
10.1		0	0	2.9	0	2.9	**2.9**	**1.4**	***A. alternaria***
5.7		0	1.4	0	0	0	**1.4**	**2.9**	***F.solani***
2.8		1.4	0	0	0	0	0	1.4	***T.viride***
		5.6	7.1	8.6	14.5	18.8	20.2	24.6	Rate of district effect
	For interference = 0.5712			For fungi = 0.2019			For district = 0.2019		L.S.D. 0.05

Table 3 demonstrated the superiority of the fungal filtrate of *A.flavus* with significant differences separating them from the rest of the treatments with a mortality rate reaching72.97 % ,and that the concentration 100 % showed a perceptible superiority in the mortality rates than the rest of concentrations used, while the fungal filtrate *Candida sp*. produced the least mortality rate at 23.76 % in comparison with the rest of the concentrations. The same table showed as well that the interval of 96 hours was the most effective compared to less intervals as the mortality rate in that interval was88.88 % which is significantly different from the rest of the time intervals as confirmed by the results shown in the table itself indicating the significant overlap between the effect of the fungal filtrate and the interval elapsed after the treatment where the greater the increase in the interval of the treatment and the concentration of the filtrates, the more the increase in the mortality rate whatever the different types of fungi. The reason of this disparity between these fungi could be the ability of these fungi to secrete analysed enzymes and fungal toxins which affect the biological activities of the living organisms leading to their demise [22].

Our current studies confirm with [23] who confirmed that the fungus *B.bassiana* is effective against the adult of *Haematobia irritans* which produced mortality rates of 98.4 % after 4 days from treatment and 100 % after 7 days from treatment and [24] determined the superiority of the concentration 100 % for the fungal filtrate *B. bassiana* to the rest of the other dilutions where the highest mortality rate for the adult of *Cx. pipienspipiens* was 100 % during 2 and 4 days, respectively and the least mortality rate was 16.33 % and 50.00 % during 2 and 4 days respectively.

Table 3. The effect of the fungal filtrates using the spray method in the percent of mortality for the adult mosquito *Cx . molestus*

Concentration	**Percent of adult mortality after**								**Average**
	48 hour				**hour 96**				
Filtrates of fungi	25	50	75	100	25	50	75	100	
A.niger	19.9	41.25	41.42	60.0	35.0	55.5	80.0	100	56.63
A.flavus	28.75	65.0	80.0	90.0	40.0	90.0	90.o	100	72.97
A.candidus	16.11	16.11	41.25	67.5	21.24	48.01	61.5	100	38.16
Penicillium sp	33.33	33.33	45.0	55.5	33.33	45.00	71.43	100	52.12
Candida sp	16.25	16.25	30.0	36.6	24.25	24.25	37.5	100	23.76
A. alternaria	22.5	45.0	50.0	65.0	29.25	66.66	80.0	100	57.30
F.solani	21.66	33.33	35.0	67.5	33.33	43.57	87.5	100	52.74
T.viride	10.0	16.25	50.0	90.0	23.33	50.01	68.75	100	51.04
Control	0	0	0	0	0	0	0	0	0
Average intervals	19.00	29.61	40.85	61.29	26.65	47.01	64.07	88.88	
L.S.D. 0.05	For filtrates = 0.1997			For intervals = 0.1584			For concentrations = 0.1395		

Results of table (4)showed that the highest mortality rates increase by the increase of the concentration of the fungal filtrate and the increase of the interval after the treatment ,which were 31.67 % for the concentration 25 % and increased to 47.78 % and 95.00 % and 100 % for the concentrations 50, 75 and 100 %, respectively after 96 hours from treatment compared to the mortality rates in the lesser intervals. The effect of these fungi may be due to their ability to produce toxins which affect the insect pests and the mortality rate increases with the increase of its concentration as well as the interval of exposure [22].

Table 4. The effect of some fungal filtrates using the nutrition method on the percent of mortality for the adult mosquito *Cx . molestus*

Concentration	Percent of adultl mortality after								Average
	48 hour				hour 96				
	25	50	75	100	25	50	75	100	
A.flavus	33.33	45.0	80.0	80.0	45.o	83.33	100	100	70.83
A. alternaria	16.33	23.33	50.0	80.0	50.0	60.0	90.0	100	58.70
Control	0	0	0	0	0	0	0	0	0
Average interval	16,55	22.78	43.33	53.33	31.67	47.78	95.0	100	
L.S.D. 0.05	For filtrate= 0.2566		For interval= 0.5491		For concentration= 0.4222		For interference = 0.7202		

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REFERENCES

1. 1-IIango,S.;Athirstalaxmi ,D.; Manomani ,P.and atarvizhi M. Repellency of aromatic plants based ingredients against mosquitoes .Int .J .Appl.Biol .Pharm.tech. 2016.7(2):55-61.
2. 2-AlHasnawy, N. K. F .,.The bioeffect of two strains of bacteria *Bacillus thurigensis* and the growth regulator Applaud and the pesticide Abte in control of mosquito *Culex molestus* (Diptera : Culicidae) in the Karbala Governorate. 2014. Master thesis-Faculty of Agriculture- Karbala University.
3. 3- AlJanaby, A.A. and AlEisa, ,The effect of the water and alcohol extract of the *Nerium Oleander* L. plant in the control of the *Meloidogyneineognita* larvae and some untargeted living organisms, The scientific university of Karbala journal. .2013 :11(3)
4. 4-Rajesh ,K.;Dhanase Karan, D. and Tyagi,KMosquito survey and larvicidal activity of action bacterial isolates against Culex larvae (Diptera: Culicidae) , journal of the Saudi society of Agricultural sciences .2014.2(6):233-239.
5. 5- Hanonik,S. B.; Mohamed ,Al. A., Mansour, I. .,Said ,A., A., Salah ,A. and Said, Al .The use of the fungus *B.bassiana* (Bals.) Vuill, as an important element in the integrated insect management. .2000.
6. 6-Pereira,E.;Sarquis,M.;Keepler,R.;Hamada,N. and Alencar,YFiLamentous fungi associated with mosquito larvae(Diptera: Culicidae)in municipalities of the Brazilian Amazon .Neotropical Entomology .2009.38(3):352-359.
7. 7-. Forbes , B.A. ;Sohm,D.F. and Weissfeld , A.S. Diagnostic Microbiology. 10 th edn .,Mosby Inc .1998.
8. 8- Ellis , D.; Davis ,S.; Alexiou, H.; Hondke, R. and Bartley,RDescriptions medical fungi . second edition. University of Adelaide. .2007. AUSTALIA:204pp.
9. 9-Pitt,J.IAlaboratory guide to common *Penicillium species* .Commonwealth Scientific and Industrial Research Organization .1988.187pp.
10. 10-AlJaboury, A. N.HIsolation and identification of the fungi accompanying some types of Aphid insects

and suppressing their parasitic and secretory abilities against the *Boyernirii* (Homoptera : Aphidiade) insect. The Technical College / Musayyib, Master thesis, 2007. page 63.

11. 11-Sarhan, A. T. The practical fungal science. First part.The University science college. Baghdad. 2012
12. 12-Singh,G.and Parkash,S.Fungi Beauveria brassiana(Balsama) metabolites for controlling malaria and filarial in tropical countries .Advan.In Biomed.Resh. .2010.9:238-242.
13. 13-Hayes,J.A.study of culex pipiens Linnaeus In the Ohio Mississippi river basin in relation to St. Louis encephalitis (SLE) Mosq .News. 1973. 33(2): 447-454.
14. 14-Saleh, M. A.,. Medical and veterinary insects. Faculty of Agriculture - University of Alexandria. Maaref Institute , Alexandria, Egypt. Pages .2004.182-184.
15. 15-SAS. Statistical analysis system .Users Guide. Statistical Version 9. 1th Ed. SAS.Institute Incorporated Cary .N.C.USA. 2012.
16. 16-Samson , A.R. ; Evans , H.C. and Latage , J. P. Altlas of the Entomopathogenic fungi , spring – Verlgar . Berlin . 1988.187pp.
17. 17-AlYoussef , A.A.. The efficiency of some fungi in the biocontrol of the *Aphis Fabsaescopolli* (Homoptera: Aphididae) on the *Vicia Fabae* plant. Missan Journal for Academic studies. 2008.13;69-77 .
18. 18-Saleh, T. A.; Tarek, M. A. and Abd Ali, T. The cumulative effects and the non- cumulative effects of the water extracts and some of the organic solvents extracts of the plants Eucalyptus and Datura on the larvae of the mosquito *Culex quinquefasciatus*. The Anbar journal of Agricultural sciences. 2010.8(4): 321-333.
19. 19- AlGhanemy , A.A.R. Comparison of the efficiency of some fungi and the products of their metabolism in the control of the mosquito *Culexquinquefasciatus*. Master thesis/ Faculty of Science/ university of Kadesiah. 2016. Page 123.
20. 20- AlJameily, S. N. A. Evaluation of the efficiency of some fungi which accompany the mosquito *Culexquinquefaciatus* in its biocontrol.Master thesis/Kadesiah university
21. 21-AlRady, Z. F. A ..The effect of the alcohol and water extracts of the roots of the *Costus specious* on some types of *Aspergillus* spp. Fungus in the rats experimentally infected with the bronchopulmonary aspergillosis. Faculty of science- Kadesiah University: 2013.126 pages.
22. 22-Ibrahim, I.K. and Karkaz, M. T. Al The fungal toxins and their effects and hazards. The Abbaa center for agricultural research. 1998.. 243 pages..
23. 23- Mahdi, Estabrek Mahmoud; Ahmed Ali Eissa and Hesham Nagi Hamid.. Study of the effect of different dilutions of the fungal filtrate of the fungus *Beauveri abassian* on the mosquito *Culex pipiens pipiens* L. (Diptera: Culicidae). 2015
24. 24-Lohmeyer, K.H.and Miller,J.A.Pathgenicity of three formulation of entompathogenic fungi for control of adult Haematobia irritians(Diptera:Muscidae). .2006.

Clinical and Molecular Profile of Patients with Breast Cancer in Tikrit Province

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ABSTRACT

Background: breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women, a good description of the patients' clinical and molecular characteristics is essential to better understanding of the disease.

Patients and method: A retrospective study that included 79 patients diagnosed with breast cancer, the study carried out during the period of 1st January 2014 to 1st December 2015.

The aim of the study: evaluate the patients' clinical profile and molecular profile for females with breast cancer.

Results: Mean age of the patients was 52.9 ± 10.1 years and the BMI was 28.8 ± 4.7 kg/m^2, 73.4% ER positive, 70.9% with PR positive, and 24.1% Her2 positive. T-staging was 13.9%, 57.0%, 25.3% and 3.8% (T1 to T4 respectively) while N-staging was 32.9%, 39.2%, 10.1% and 17.7% (N0 to N3 respectively).

Conclusion: The clinical and molecular profile of Iraqi women patients with breast cancer was similar to reported in the literature, with mean age at diagnosis in the six decade, 73.4% ER positive, 70.9% with PR positive, and 24.1% Her2 positive.

Key words: *age, estrogen receptor, progesterone receptor, her2, Iraq*

INTRODUCTION

Globally, breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women. In the United States, breast cancer is the most commonly diagnosed cancer and the second most common cause of cancer death in women. In addition, breast cancer is the leading cause of death in women ages 40 to 49 years. Breast cancer is treated with a multidisciplinary approach involving surgical oncology, radiation oncology, and medical oncology, which has been associated with a reduction in breast cancer mortality [1].

Breast cancer is the commonest cancer in women worldwide[2]. Its incidence has increased over several decades, while the death rate has fallen because of improved survival [3]. Survival has probably improved because of a mixture of earlier detection of the disease (both through screening and earlier symptomatic presentation), biological changes that have made the disease more susceptible to hormonal therapy, and improved treatment [4, 5].

Incidence rates vary from 19.3 per 100,000 women in Eastern Africa to 89.7 per 100,000 women in Western Europe and are high (greater than 80 per 100,000) in developed regions of the world (except Japan) and low (less than 40 per 100,000) in most of the developing regions[6]. According to the International Agency for Cancer Research and GLOBOCAN 2008, the Age-Standardized Incidence Rates (ASR) in Iraq was (31.1/100,000), similar as compared to the countries surrounding Iraq, Kuwait (47.7), Saudi Arabia (22.4), Jordan (47.0), Syria (23.0), Iran (18.4), Turkey (28.3) [6]. The current work aimed to evaluate the patients' clinical profile and molecular profile for females with breast cancer.

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METHOD

A retrospective study that included 79 patients diagnosed with breast cancer, the study carried out in Tikrit Teaching Hospital during the period of 1st January 2014 to 1st December 2015.

The patients enrolled in this study were already diagnosed with breast cancer either on adjuvant chemotherapy, hormonal therapy or on regular follow up, all of them were females. The data of all the patients were obtained included the histopathological reports that confirm their diagnosis regarding breast subtypes, grading and staging. Also, immunohistochemical (IHC) reports that confirm their hormonal status (ER, PR HER2). The patients in this study underwent surgical intervention either by biopsy or mastectomy with axillary clearance, and then the formalin-fixed paraffin-embedded tissue blocks were sent to the hospital laboratory for H&E and IHC. Male patients with breast cancer were exclude from this study.

RESULTS

Mean age of the patients was 52.9 ± 10.1 years and the BMI was 28.8 ± 4.7 kg/m^2, both the molecular and clinical profile and staging are illustrated in table 1 and 2.

Table 1: molecular profile of the patients

Parameters	Value
ER Status	
Negative	21 (26.6%)
+1	20 (25.3%)
+2	24 (30.4%)
+3	14 (17.7%)
ER Positive	58 (73.4%)
PR status	
Negative	23 (29.1%)
+1	22 (27.8%)
+2	15 (19.0%)
+3	19 (24.1%)
PR positive	56 (70.9%)
Her2 status	
Negative	37 (46.8%)
+1	11 (13.9%)
+2	14 (17.7%)
+3	17 (21.5%)
Her2 positive	19 (24.1%)

ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor 2
Data presented as number (%)

Table 2: clinical profile and tagging of the patients

Parameters	Value
T staging	
1	11 (13.9%)
2	45 (57.0%)
3	20 (25.3%)
4	3 (3.8%)
N staging	
0	26 (32.9%)
1	31 (39.2%)
2	8 (10.1%)
3	14 (17.7%)
Metastasis	17 (21.5%)
Bone	9 (11.4%)
Liver	7 (8.9%)
Lung	6 (7.6%)
Data presented as number (%)	

DISCUSSION

In the current study mean age of the patients was 52.9 ± 10.1 years, which is in agreement with Kensler et al with mean age 57.1 ± 10.1 years [7], and with Hu et al (50 years) [8], but younger that Elebro et al with median 65 years at diagnosis [9], in meta-analysis of 19 studies median age were between 49 – 61 years [10], these studies agree with findings of the current study about age at presentation of breast cancer.

In the current ER positive reported in 73.4% and PR positive reported in 70.9%, Kensler et al. reported higher ER+ that current study with 89.2% of the cases [7], ER+ was higher in Hu et al study with 88.7% but PR+ expression was similar to the current study with 72.2% of the cases [8], Elebro et al reported similar findings to Hu et al [9], In Honma et al PR+ expressed in 208/403 (51.6%) of the cases [11], in Park et al study 78.4% had ER+ status and 69.8% had PR+ status [12], in Castellano et al 680/938 (72.5%) had PR+ [13], these variability in the distribution

of ER and PR in different studies can be attributed to different criteria in these studies and specific population that the study examined, the result of the current study fall within the range of the reported studies.

In the current study T-staging was 13.9%, 57.0%, 25.3% and 3.8% (T1 to T4 respectively) while N-staging was 32.9%, 39.2%, 10.1% and 17.7% (N0 to N3 respectively), the following studies reported similar findings in Carreño et al T-stage of the tumor (T1, T2, T3, and T4) was 45.5%, 42.3%, 6.6% and 6.6% respectively, while node positive disease reported in 34.9% [14], In Honma et al node positive presented in 186/403 (46.2%) of the cases [11, 15], Park et al T-staging was 15.9%, 62.1%, 21.9% (Tis, T1, and T2-3) while N – staging (0 through 3) was 15.6%, 49.2%, 29.9% and 5.3% [12], Castellano et al T – staging was 57.7%, 28.3%, 2.7%, and 2.9% [13].

In the current study her2+ expressed in 24.1% of the cases, in Honma et al study Her2+ expressed in (5.0%) of the cases [11], Park et al 21.9% of the cases expressed Her2+ [12], Castellano et al Her2+ in 10.8% [13], Hu et al Her2+ in 8.6% [8], Kensler et al Her2+ presented in 20.4%[7].

CONCLUSION

The clinical and molecular profile of Iraqi women patients with breast cancer was similar to reported in the literature, with mean age at diagnosis in the six decade, 73.4% ER positive, 70.9% with PR positive, and 24.1% Her2 positive.

Conflict of Interest : None

Ethical Clearance: Informed written consent was obtained from all the participants in the study, and the study and all its procedure were done in accordance with the Helsinki Declaration of 1975, as revised in 2000. The study was approved by Tikrit Teaching Hospital, department of medicine.

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REFERENCES

1. Kesson EM, Allardice GM, George WD, Burns HJ, Morrison DS. Effects of multidisciplinary team working on breast cancer survival: retrospective, comparative, interventional cohort study of 13 722 women. BMJ (Clinical research ed). 2012;344:e2718.10.1136/bmj.e2718
2. Althuis MD, Dozier JM, Anderson WF, Devesa SS, Brinton LA. Global trends in breast cancer incidence and mortality 1973-1997. Int J Epidemiol. 2005;34(2):405-12.10.1093/ije/dyh414
3. Botha JL, Bray F, Sankila R, Parkin DM. Breast cancer incidence and mortality trends in 16 European countries. European journal of cancer (Oxford, England : 1990). 2003;39(12):1718-29, https://www.ejcancer.com/article/S0959-8049(03)00118-7/fulltext
4. Brown SB, Mallon EA, Edwards J, Campbell FM, McGlynn LM, Elsberger B, et al. Is the biology of breast cancer changing? A study of hormone receptor status 1984-1986 and 1996-1997. British journal of cancer. 2009;100(5):807-10.10.1038/sj.bjc.6604934
5. Hashimi SJMA, Hyder A, Abdulla KN, Fawzi HA. Female Cancers: Incidence, Characteristics and Death Rate in Misan Province, Iraq International Journal of Science and Research 2018;7(6):1843-45.10.21275/ART20182746
6. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer. 2010;127(12):2893-917.10.1002/ijc.25516
7. Kensler KH, Poole EM, Heng YJ, Collins LC, Glass B, Beck AH, et al. Androgen Receptor Expression and Breast Cancer Survival: Results From the Nurses' Health Studies. J Natl Cancer Inst. 2018.10.1093/jnci/djy173
8. Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, et al. Androgen receptor expression and breast cancer survival in postmenopausal women. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011;17(7):1867-74.10.1158/1078-0432.ccr-10-2021
9. Elebro K, Bendahl PO, Jernstrom H, Borgquist S. Androgen receptor expression and breast cancer mortality in a population-based prospective cohort. Breast Cancer Res Treat. 2017;165(3):645-57.10.1007/s10549-017-4343-0
10. Vera-Badillo FE, Templeton AJ, de Gouveia

P, Diaz-Padilla I, Bedard PL, Al-Mubarak M, et al. Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis. J Natl Cancer Inst. 2014;106(1):djt319.10.1093/jnci/djt319

11. Honma N, Horii R, Iwase T, Saji S, Younes M, Ito Y, et al. Clinical importance of androgen receptor in breast cancer patients treated with adjuvant tamoxifen monotherapy. Breast cancer (Tokyo, Japan). 2012.10.1007/s12282-012-0337-2
12. Park S, Koo J, Park HS, Kim JH, Choi SY, Lee JH, et al. Expression of androgen receptors in primary breast cancer. Annals of oncology : official journal of the European Society for Medical Oncology. 2010;21(3):488-92.10.1093/annonc/mdp510
13. Castellano I, Allia E, Accortanzo V, Vandone AM, Chiusa L, Arisio R, et al. Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers. Breast Cancer Res Treat. 2010;124(3):607-17.10.1007/s10549-010-0761-y
14. Carreno G, Del Casar JM, Corte MD, Gonzalez LO, Bongera M, Merino AM, et al. Local recurrence after mastectomy for breast cancer: analysis of clinicopathological, biological and prognostic characteristics. Breast Cancer Res Treat. 2007;102(1):61-73.10.1007/s10549-006-9310-0
15. Jumaah Jebur N, J. Kadhim D, M. Firhan N, Adnan Fawzi H. Assessment of health-related quality of life of Iraqi patients with inflammatory bowel disease. International Journal of Research in Pharmaceutical Sciences. 2018;9(3):829-35.10.26452/ijrps.v9i3.1577

Evaluation of "Sublay" and "Onlay" Mesh Hernioplasty Techniques of Ventral Hernial Repair

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ABSTRACT

Ventral abdominal hernias are common surgical conditions that can be presented as emergency and elective cases. Repairing ventral hernias have always been a huge challenge to the surgeons. Mesh hernioplasty was considered as a gold standard to prevent or minimize the incidence of recurrence, but a question arises where should the the mesh sublay or onlay be placed? This research compares the procedures and outcomes of both onlay and sublay techniques in ventral hernia repair. A prospective study of was conducted at the surgical unit of Al-Imam Al-Hussein Medical City on 120 patients submitted for ventral hernias repair. Cases were registered in a year starting from 1st January 2015 to 1st January 2016) and followed up till January 2017. Study population was divided into two groups: 60 patients under group A that included patients who had undergone onlay mesh repair and another 60 patients as group B who were treated with sublay mesh repair. Data collected from both the groups included operation time, placement, and duration needed for drain removal, wound infection, and recurrence rate. Quarterly follow-up for 2 years was performed. Data were analyzed using SPSS 18.0 software; Fisher's exact test was considered as appropriate for analyzing statistical significance; $p < 0.05$ was considered to be statistically significant. The research outcomes indicated postoperative complications such as seroma formation in two patients (3.33%) from the sublay group, whereas 12 (20%) from the onlay group. Wound infection was found in one patient (1.66%) in the sublay group whereas in 6 (10%) patients in the onlay group. No septic mesh was removed in the sublay treated group, whereas one mesh was removed in the onlay type. In the onlay group recurrence was found in 4 patients (6.66%), whereas there was no recurrence in the sublay group. To conclude the sublay mesh hernioplasty is a better alternative to onlay mesh hernioplasty for all forms of ventral hernia cases.

Key words: *sublay; onlay; mesh repair; ventral hernia; umbilical hernia.*

INTRODUCTION

Protrusion of the abdominal contents through the fascia of the anterior abdominal wall is called a ventral hernia. Such defects are categorized as congenital, spontaneous, or acquired or based on their abdominal wall location. For example, hernias occurring from the xiphoid process to the umbilicus are the epigastric; those at the umbilicus are termed as umbilical; hernias that are highly uncommon and spontaneous hernias that occur in the midline below the umbilicus are paraumbilical and hypogastria hernias. Acquired hernias typically occur after surgical incisions, and are therefore termed incisional hernias. Although not a true hernia, diastasis recti can present as a midline bulge. In such a condition, the linea alba is stretched that leads to bulging at the medial margins of the rectus muscles. Abdominal wall diastasis may occur at other sites other than the midline. There is no facial ring or hernial sac, and surgical correction is avoided, unless it is significantly symptomatic.

According to the national operative statistics, 15–20% of all abdominal wall hernias were incisional hernias. Epigastric and umbilical hernias constitute 10% of all hernias. In women, incisional hernias are twice more common than in men. Clear evidence demonstrating that the suture type at the primary operation affects the formation of hernia is lacking [1]. The formation of ventral hernia is linked with many factors including gender,

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age, obesity, sleep apnea, emphysema, and prostatism. Other factors associated with collagen destruction in the lung increased hernia formation. The use of a suture to wound length ratio of 4: 1 has been shown to reduce the formation of incisional hernia significantly as compared with the 1 cm bites and 1 cm advanced suturing technique typically used by most surgeons [2].

Whether the incisional hernia rate is influenced by the type of initial abdominal incision remains controversial. As noted, the incidence of ventral herniation post-midline laparotomy ranges from 3% to 20% and doubles in case of a surgical site infection. A meta-analysis of many cases shows that the incidence of ventral hernia formation post different types of abdominal incisions are 2.5% for paramedian incisions, 7.5% in transverse incisions, and 10.5% in case of midline incisions. [3].

AIM OF THE STUDY

To compare onlay and sublay techniques of ventral hernia repair in terms of procedures and outcomes.

Patients and methods

This prospective comparative study was carried out on 120 patients of abdominal hernias admitted in the general surgical unit of Al-Imam Al-Hussein Medical City. Cases were registered in one year starting from 1st January 2015 till the 1st of January 2016) and followed up continued till January 2017. The majority of the cases were registered at the beginning of the year 2015, wherein the average follow-up was 1–2 years with quarterly visits, followed by clinical examination and confirmation by way of ultrasound.

In this study, 60 cases of ventral hernias were managed by onlay (group A) mesh repair and 60 others by sublay (group B) mesh repair. A similar type of mesh (polypropylene) in both the groups was used, and whether onlay or sublay technique was to be followed was left to the surgeon's preference and experience. Follow up of patients of both the groups was performed by checking the following parameters: operative time; drain placement time, which is one drain placed subcutaneously; wound complications; and recurrence rate.

Inclusion criteria

All patients of both genders with the following criteria were included in the study:

1. Post-laparotomy midline incisional hernias and recurrent hernias.
2. Primary hernias (umbilical, paraumbilical, or epigastric), diagnosed on clinical examination and confirmed by ultrasound.

Exclusion criteria

The following groups of patients who had different comorbidities with different degrees of influence on the outcome of the surgery, based on the degree of severity of these comorbidities were excluded from this study to make the procedure the most probable cause of complications:

1. Morbidly obese patients with BMI>40 kg\m^2.
2. Patients with diabetes mellitus.
3. Patients with abdominal malignancy and cirrhosis with end-stage liver disease.
4. All patients with chronic obstructive pulmonary disease (COPD) such as asthma.
5. Patients with obstructive uropathy such as benign prostatic hypertrophy (BPH).
6. Patients presented as emergency cases such as strangulated hernia with signs of obstruction (abdominal distention, vomiting, and absolute constipation) and those who lacked follow-up.
7. Preexisting skin infection at the site of hernia with local signs of inflammation (redness, hotness, and tenderness).

Operative Technique

A/Sublay mesh repair

The principles of the retrorectus or sublay mesh repair included two main steps: mesh placement deep into the recti muscles and mesh extension well beyond the hernial defect. The medial edge of each rectus muscle was identified by palpation, and the extreme medial edge of each rectus sheath was incised along its length to reach the submuscular space. This relatively bloodless plane could be created to the lateral edges of the rectus muscle on each side. Primary "peritoneal" closure was obtained using posterior rectus sheath above the arcuate line, the peritoneum itself, or excess sac below the arcuate line. The posterior rectus sheath along with the peritoneum

is closed with zero proline sutures. Then, the mesh is fastened around and well beyond the defect (about at least 5 cm). The center point of the mesh was assigned by a stitch to avoid malpositioning of the mesh and edges of the mesh were fixed to the posterior rectus sheath by way of multiple stitches. Organs within the abdomen are isolated from injury using the mesh along with a layer of posterior rectus sheath and peritoneum. Adhesions are thereby prevented due to the high viscosity of the mesh. The edges of the muscular sheath were sutured over the mesh by nonabsorbable nylon sutures [4-5].

B/Onlay Mesh Repair

Onlay repair was performed with skin incision over the bulge or the defect. Both the rectus sheath and the defect containing the hernia contents were identified using sharp and blunt dissection. The hernial sac was clearly dissected and the contents were dealt with while the margins of the defect were held by Kocher forceps. The sac's was treated by reducing its contents into the abdominal cavity. With nonabsorbable sutures, the defect in the linea alba was closed and a proline mesh of adequate size was placed on the rectus sheath and fixed with stiches. Redivac suction drains were used for all cases of the two groups with mean time periods of 5 and 7 days for sublay and onlay techniques, respectively, and removed when drainage was observed to less than 20 ml in 24 hours. All surgeries were performed under general anesthesia, and all patients were administered 1 gm 3rd-generation cephalosporin antibiotic preoperatively at the time of induction and continued till the 2nd postoperative day (1 gm) daily. Data were analyzed using SPSS 18.0 software; Fisher's exact test was considered as appropriate for analyzing statistical significance; $p < 0.05$ was considered to be statistically significant.

RESULTS

A total of 120 patients of ventral hernias were managed by both sublay mesh and onlay mesh repair techniques. The youngest patient was 22 year old and the oldest 77 year old; the mean age of the patients was 48 ±5 years. The majority of the patients were female, that is, 90 patients, which represented 75%, and 30 male patients represented 25% of the sample. The majority of the patients were between 51 and 60 years totaling to 40 patients who represented 33.33% of all patients studied. The second age group was 41–50 years, which constituted 25% of the patients (i.e., about 58% or more than half of patients from age 40–60 years as shown in Table 1.

Table 1. Age and gender distribution.

Age in years	Male (30)	Female (90)	Total	Percent (%)
20-30	4	5	9	7.5
31-40	8	12	20	16.66
41-50	6	24	30	25
51-60	8	32	40	33.33
61-70	3	12	15	12.5
71-80	1	5	6	5
Total	30	90	120	100

In this study, most hernias repaired were of spontaneous type (60%), whereas those of incisional type were less and represented 40% of the sample

Seroma formation was observed in 2 patients (3.33%) in the sublay group, whereas in 12 patients (20%) of the onlay group. Wound infection was seen in one patient (1.66%) of the sublay technique group, whereas in in 6 (10%) patients of the onlay group. One patient (1.66%) of the second group suffered from mesh infection and needed its removal, whereas none was observed in the sublay group. Wound edge necrosis occurred in one of the patients (1.66%) of the onlay repair, which was managed by excision of the necrotic edge and primary suturing, and no such case of flap edge necrosis occurred in the sublay group. Paralytic ileus as a complication was observed equally in both studied groups, with one such case (1.66%) in each group. Regarding recurrence rate in the duration of 2 years, follow-up in the sublay group exhibited in no recurrence (0%), whereas that in the onlay group it was observed in 4 patients (6.66%).

The tests of significance were assessed by *p* value, and it was discovered that it was statistically significant in three postoperative complications that are still the main problems in the surgical management of hernias which are as follows:

1. Seroma Formation
2. Wound Infections
3. Recurrence rate

Otherwise, there were no significant differences (statistically) in other variables in the study, as shown in Table 2.

Table 2. Postoperative complications

Postoperative complications	Sublay group n = 60 (%)	Onlay group n = 60 (%) (%)	*p* value
Seroma	2 (3.33)	12 (20)	<0.05
Wound infection	1 (1.66)	6 (10)	<0.05
Mesh removal	0 (0)	1 (1.66)	>0.05
Recurrence	0 (0)	4 (6.66)	<0.05
Flap necrosis	0 (0)	1 (1.66)	>0.05
Paralytic ileus	1(1.66)	1(1.66)	1.000

DISCUSSION

Ventral abdominal wall hernias are a common surgical problem encountered in clinical practice. The outcome of the surgery is based not only on the technique used but also on the experience of the operator, meticulous dissection, tension-free repair, and so on [6]. Many methods are available to deal with these hernias. Commonly practiced techniques for hernial repair use mesh, which is placed either in a sublay or onlay position [7]. The refinement of the sublay technique decreased the recurrence rates and resulted in an overall better outcome that renders it suitable to be declared the gold standard care of ventral hernias. Primary tissue repair is associated with higher unacceptable recurrence rate. Nowadays tension-free mesh repair is an ideal hernia repair technique [8].

However, the optimal technique for mesh placement has not been established and remains controversial. The prosthetic mesh can be placed either between the subcutaneous tissues of the abdominal wall and the anterior rectus sheath (onlay mesh repair) on in the preperitoneal or retromuscular (sublay mesh repair). The latter technique has several advantages, one of them being not transmitting the infection from subcutaneous tissues down to the mesh as it lies quite deep inside the subcutaneous tissues [9]. Increased intraabdominal pressure that acts anteriorly on the margins of the mesh tends to push it toward the abdominal wall rather than distracting it away from it. Some studies suggest that the use of the sublay technique as a treatment option for ventral hernias appears to be less complicated than the onlay technique [10, 11, 12].

Ethical Clearance- taken from the scientific committee in Department of Maxillofacial Surgery, College of Dentistry, Kerbala University, Iraq. In their reference no.89 at 2nd. October 2017.

Source of Funding- Self-funding research.

Conflict of Interest – None

CONCLUSIONS

The sublay mesh hernioplasty is a better alternative to onlay mesh hernioplasty for all forms of ventral hernia cases.

Minimal complications and better outcome with sublay mesh hernioplasty in comparison with the onlay technique.

RECOMMENDATIONS

Because of less complications of infections, seroma formation and low recurrence rate in sublay mesh repair during 2 years follow up in which most of the recurrence happens, the use of this method for the repair of ventral hernias is encouraged.

We recommend increasing the number of the patients in the study groups and prolongation of follow-up time to obtain more reliable results.

REFERENCES

1. Rucinski, James, Mark Margolis, Georgia Panagopoulos, and Leslie Wise. "Closure of the abdominal midline fascia: meta-analysis delineates the optimal technique." *The American surgeon* 67, no. 5 (2001): 421.
2. Muysoms, F. E., S. A. Antoniou, K. Bury, G. Campanelli, J. Conze, D. Cuccurullo, A. C. de Beaux et al. "European Hernia S (2015) European Hernia Society guidelines on the closure of abdominal wall incisions." *Hernia* 19, no. 1: 1-24.
3. Carlson, Mark A., Kirk A. Ludwig, and Robert E. Condon. "Ventral hernia and other complications of 1,000 midline incisions." SOUTHERN MEDICAL JOURNAL-BIRMINGHAM ALABAMA- 88 (1995): 450-450.

4. Duce, A. M., J. M. Mugüerza, R. Villeta, J. Martin, A. Gutierrez, M. Diez, F. Noguerales, and J. Graneil. "The Rives operation for the repair of incisional hernias." *Hernia* 1, no. 4 (1997): 175-177.
5. Klinge, Uwe, Joachim Conze, Carsten J. Krones, and Volker Schumpelick. "Incisional hernia: open techniques." *World journal of surgery* 29, no. 8 (2005): 1066-1072.
6. Chien, J., P. Tsai, K. Liu, S. E. Wang, Y. M. Shyr, C. H. Su, and T. H. Chen. "Open suture repair and open onlay technique for incisional hernia in elderly patients with multiple comorbidities." *Int J Appl Sci Technol* 1 (2011): 34-40.
7. Stey, Anne M., Marcia M. Russell, Catherine A. Sugar, Bruce L. Hall, David S. Zingmond, Elise H. Lawson, and Clifford Y. Ko. "Extending the value of the National Surgical Quality Improvement Program claims dataset to study long-term outcomes: rate of repeat ventral hernia repair." *Surgery* 157, no. 6 (2015): 1157-1165.
8. Korenkov, M., S. Sauerland, M. Arndt, L. Bograd, E. A. M. Neugebauer, and H. Troidl. "Randomized clinical trial of suture repair, polypropylene mesh or autodermal hernioplasty for incisional hernia." *British Journal of Surgery* 89, no. 1 (2002): 50-56.
9. Aoda, Furat Shani, and Alaa Sharif Ibrahim. "Sublay versus onlay mesh repair of ventral hernia." *AL-QADISIYAH MEDICAL JOURNAL* 9, no. 16 (2013): 208-216
10. Aoda, Furat Shani, and Alaa Sharif Ibrahim. "Sublay versus onlay mesh repair of ventral hernia." *AL-QADISIYAH MEDICAL JOURNAL* 9, no. 16 (2013): 208-216.
11. Saber, Aly, and K. Bayumi Emad. "Onlay versus sublay mesh repair for ventral hernia." *J Surg* 4, no. 1-1 (2015): 1-4.
12. Kharde, Kundan, Bharat Dogra, Sunil Panchabhai, Karan Rana, Srihari Sridharan, and Sai Kalyan. "A comparative study of onlay and retrorectus mesh placement in incisional hernia repair." *Medical Journal of Dr. DY Patil University* 6, no. 3 (2013): 258-258.

Estimated Analysis on Environmental Health Risk of 2.5 Micron Particulate Matter to Urban Communities in South Jakarta

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ABSTRACT

Background : Urban areas with their numerous activities including transportation, industrial and building construction activities are places that may potentially become the sources of pollution for human health, especially in areas with high population density. $PM_{2.5}$ potentially causes health problems to humans because inhaled pollution remains in alveoli. This may trigger respiratory diseases to cardiovascular diseases.

Objective : The study was to determine the risk level of $PM_{2.5}$ exposure in Jakarta on people living in areas around the *ready-mix* industry

Material & Method : The analysis carried out in this study is an environmental Health Risk Analysis (HRA) conducted to associated with pattern of activities, anthropometric data, and PM 2.5 concentration. This study involved 92 respondents with 10 study area points.

Result: The result of study reveals that there is 1 point having the risk of experiencing health problems with the Hazard Quotient HQ> 1. Although, overall, the people who reside in the study area are on average still relatively safe up to the present, when the health risk in the environment has exceeded the safe limit as at point 1 there must be a risk estimation at 9 other points to identify the safe limit in future.

Keywords *: Air Pollutant, Particulate Matter, Health Risk Analysis, Urban Area.*

INTRODUCTION

Particulate matter of less than 2.5 micron in size is in the form of gas and condensation from the activity of construction and demolition works, and mining operations. $PM_{2.5}$ particle matter is formed from gas and condensation of high temperature vapors during combustion and industrial activities [1]. $PM_{2.5}$ can remain in the air for a long time and may fly to areas hundreds of miles in distance [2] and can easily penetrate through buildings [3]. Consequently, air pollution can affect human health.

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Quoted from *Greenpeace* based on the air quality monitoring conducted by the American Embassy in South Jakarta and Central Jakarta, the air quality of $PM_{2.5}$ parameter is very hazardous for the health. The results of monitoring on $PM_{2.5}$ air quality by *Greenpeace* also showed results which are not different from the US Embassy's monitoring with the air pollution index value of AQI 153 in Central Jakarta, and 169 in South Jakarta. The air quality monitoring carried out since January 2017 in 21 locations in the Jakarta Metropolitan Area indicates that the last six months shows an unhealthy air level for humans and will cause more serious health impacts to sensitive groups, such as children, pregnant women, and elderly people [4].

The $PM_{2.5}$ mass concentration in Ahmedabad, India ranged from 0.032 to 0.106 mg/m^3, and Yokohama, Japan 0.363 – 0.04778 mg/m^3, while the PM 2.5 concentration in Beijing ranged between 0.07 and 0.1 mg/m^3 [5]. The

particulate air pollution mostly comprises $PM_{2.5}$ which size is smaller than 1/30rd part of human hair with a diameter of 70 μm consisting of sulfate, nitrate, organic compounds, ammonium compounds, metals, acidic material [6]. Five specific sources of $PM_{2.5}$ namely sulfate, residual oil, traffic, metal industry and ground steel [7].

METHOD

Sampling Site

Jakarta, based on Indonesian Statistics in 2017, is one of the largest cities in Indonesia with the population density of 15,663 people / km^2 where a number of medium and large-scale industries operate reaching 1,410. South Jakarta hosted 68 medium and large-scale industries in 2012. Analysis on the health risk of $PM_{2.5}$ was performed in two districts in South Jakarta, namely in Jagakarsa District with a population density of 15,148 people/km^2 and Pasar Minggu District which has a population density of 14,029 people/km^2.

Sampling Methods

High Volume Air Sampler (HVAS) is a device that is used to collect particles through filtration that can collect particles up to 0.1 μm in size with an efficiency of 99.95% for a particle size 0.3 μm. The total number of particles accumulated in the filter for 1 hour is analyzed gravimetrically during which the flow rate is monitored. The results were displayed in the form of particulate mass units collected per volume unit of air samples taken as μg/m^3. This method is adjusted to the Indonesian National Standard (SNI) method 19-7119.3-2005 with normative references following the United States Environmental Protection Agency (USEPA) method IO-2.1 1999. Sampling of ambient air for Total Suspended Particulate Matter (SPM) and PM_{10} use the method reference from the Third Edition 501 of high volume measurement of size classified as Particulate Matter and Government Regulation of the Republic of Indonesia Number 41 of 1999 concerning air pollution control. Measurement of $PM_{2.5}$ concentration, temperature, humidity, and wind direction were carried out at 10 points.

Health Risk Analysis Methods

Health Risk Assessment (HRA) can be observed by knowing the characteristics of effects that potentially harm human health by pollutant exposure [8]. HRA is the process to estimate the nature and possible adverse health effects to humans who may be exposed to hazardous substances. Its four basic steps are hazard identification, exposure analysis, dose-response assessment, and risk characterization. Hazard identification aims to recognize various hazards that adversely affect the health, while exposure analysis has the purpose to identify exposure pathways of risk agent so that the total intakes received by individuals in the risky population can be determined. Dose-response assessment is to estimate the amount of substances needed to cause varying degrees of adverse health effects. Health risk level is expressed as Risk Quotient (RQ) or Hazard Quotient (HQ). HQ is calculated by comparing the intake value and the Reference Concentration (RfC) [9].

The risk level for non-carcinogenic contaminants is determined by calculating the HQ. If the HQ equals to or exceeds one, there is a risk that may endanger the health.

$$HQ = Intake/RfC$$

Where intake indicates the average daily intake of non-carcinogenic substances during the exposure period. RfC indicates reference concentration [10] The intake of risk agent must be calculated for all exposure pathways in accordance with the anthropometric characteristics and the activity pattern of the population at risk with the following equation,

$$Intake = \frac{CA \times IR \times ET \times EF \times ED}{BW \times AT}$$

Where CA indicates the concentration of $PM_{2.5}$ (mg/m^3) in the air, IR indicates the inhalation rate (m^3/hour), ET indicates the exposure time (hours/day), EF indicates the exposure frequency (days/year), ED indicates the exposure duration, BW indicates the body weight (Kg), and AT indicates the average time (days), 30 (years) x 365 (days/year).

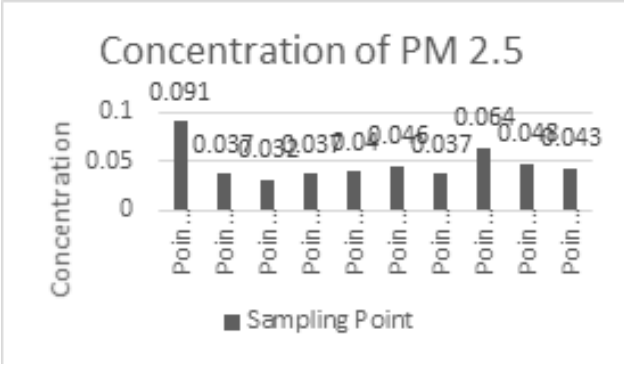
RESULTS AND DISCUSSION

$PM_{2.5}$ Concentration

Table 1. Climatological Factor of Exposure

Parameter	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9	Point 10
C(mg/m3)	0.091	0.037	0.032	0.037	0.040	0.046	0.037	0.064	0.048	0.043
T (℃)	28.6	30.8	30.8	31.1	28.8	29.5	30.3	32.6	31.8	32
RH (%)	74	68	68	64	64	59	56	50	53	52
Wind	North	North	North	North	West	North	West	East	South	East

The highest PM2.5 concentration is at point 1 (Table 1), namely 0.091 mg/m3. This graphic (Graphic 1) exceeds the threshold value set by the National Air Ambient Quality Standard (NAAQS) namely 0.035 mg/m^3. Air humidity at point 1 is also at the highest value among other points which is 74%, which is contrary to the temperature. The temperature at point 1 is at the lowest 28.6%. Low temperature air will pose more risk to the health because the cold air will be trapped and is difficult to exit the area [11].



Graphic 1. $PM_{2,5}$ Concentration

Risky Assignment

The reference concentration (RfC) is the reference dose obtained from the US EPA. As RfC has not been determined for $PM_{2.5}$, the RfC value in this study refers the safe limit that was proposed by the US-EPA National Ambient Air Quality Standard (NAAQS) in 2006 for $PM_{2.5}$ namely 0.035 mg/m^3. To equalize the intake with the RfC, the concentration of risk agent is reduced using the EPA default value, namely IR = 0.83 m^3/hour, ET = 24 hours/day, EF = 350 days/year, BW = 70 kg, AT = 365 days/year, ED = 30 years, then obtained:

RfC = 0.035 mg/m^3 x 0.83 m^3/h x 24 hours/day x 350 hours/year x 30 years

70 kg x 30 x 365 hours/year

RfC = 0.009 mg/kg/day

Hence, the safe limit based on the reference concentration is 0.009 mg per kilogram of body weight per day. This value is used to determine the risk of $PM_{2.5}$ exposure in calculating the risk level (Table 2).

Table 2. Risk level of $PM_{2.5}$ concentration at the study points

Site point	$PM_{2.5}$Concentration (mg/m^3)	LADD (mg/kg/day)	HQ Value	HQ
1	0.091	0.01576	1.7511	HQ ≥ 1
2	0.037	0.00535	0.5948	HQ < 1
3	0.032	0.00653	0.7254	HQ < 1
4	0.037	0.00798	0.8861	HQ < 1
5	0.04	0.00567	0.6296	HQ < 1
6	0.046	0.00403	0.4473	HQ < 1
7	0.037	0.00610	0.6775	HQ < 1
8	0.064	0.00199	0.2212	HQ < 1
9	0.048	0.00697	0.7749	HQ < 1
10	0.043	0.00703	0.7807	HQ < 1
Average	0.041	0.00512	0.5690	HQ < 1

Estimating Risk

Each point has different time spans to experience risks. The range of risky time for each point is determined based on the community condition and environment condition.

Table 3. Average of anthropometry and $PM_{2.5}$ concentration

Point	Concentration (mg/Nm^3)	Weight (Kg)	Exposure Time (hours/day)	Exposure Frequency (days/year)	Duration (years)
Point 1	0.091	52.1	23	358	20.5
Point 2	0.037	64.7	23	350.67	23.22
Point 3	0.032	55.4	23	358	21.7
Point 4	0.037	63	24	363.97	27.13
Point 5	0.04	64.2	24	359	18.3
Point 6	0.046	55.7	23	358	10.8
Point 7	0.037	58.7	24	362.5	19.9
Point 8	0.064	65.4	24	365	4
Point 9	0.048	60	24	365	17.67
Point 10	0.043	55.4	24	365	18.89
Average	0.041	58.5	24	362	15

Table 4. Estimating Health Risk Analysis Several Years Later

Point	HQ								
	Now	2022	2024	2028	2029	2030	2031	2032	2033
1	1.751	2.093	2.264	2.605	2.691	2.776	2.861	2.947	3.032
2	0.595	0.697	0.865	0.984	1.014	1.043	1.073	1.102	1.132
3	0.725	0.859	0.801	0.916	0.945	0.974	1.003	1.032	1.061
4	0.886	1.017	1.082	1.213	1.245	1.278	1.311	1.343	1.376
5	0.630	0.767	0.836	0.974	1.008	1.042	1.077	1.111	1.146
6	0.447	0.613	0.696	0.861	0.903	0.944	0.986	1.027	1.069
7	0.678	0.814	0.882	1.018	1.052	1.086	1.120	1.154	1.188
8	0.221	0.442	0.553	0.774	0.829	0.885	0.940	0.995	1.051
9	0.775	0.950	1.038	1.213	1.257	1.301	1.345	1.389	1.433
10	0.781	0.946	1.029	1.194	1.235	1.277	1.318	1.359	1.401
Average	0.569	0.721	0.797	0.948	0.986	1.024	1.062	1.100	1.138

In year 2018 (at present) only point 1 has risk. Point 1 is located 200 meters from a readymix industry, to the northwest direction the same direction as the dominant wind direction in May 2018. This causes the PM2.5 concentration to be at the highest point 1 compared to other points. Risk estimation which also involves anthropometric data and patterns of community activity (Table 3) indicates that currently the people who reside at point 1 may be at the risk of experiencing health problems. 4 years later in 2022, the HQ value at point 4 will already have risks exceeding 1 (HQ≤. 2 years afterward points 9 and 10 will be in risky areas, and 4 years later point 7 will be in a risky area, then each point will have risk with an interval of 1 year. Up to 15 years thereafter, in 2033, 10 points in the study area will be at risk of experiencing health problems if the concentration value, anthropometric data and activity patterns do not change (Table 4).

Safe Limit

Inhaled $PM_{2.5}$ can deposit deep in alveoli and may cause inflammation and oxidative stress and alter the cardiac work system. Earlier studies on primarily respiratory health effects, mentioned that $PM_{2.5}$ is one of factors that cause cardiovascular diseases (CVD) including hypertension, increased visits to hospitals, and cardiovascular mortality [12]. High plaque deposit in arteries causes sudden cardiac arrest and other cardiovascular diseases [13].

Estimating safe concentration as the highest limit of PM 2.5 concentration value in an area is calculated using the formula to find safe concentration,

$$CA = \frac{RfC \times BW \times AT}{IR \times ET \times EF \times ED}$$

Of the 10 research points (Table 4), point 1 is the location currently at risk based on the value of HQ. Determination of safe concentration at point 1 with PM2.5 concentration is 0.091 mg/m^3 with the exposure duration of 20.5 years. In calculating safe concentration, the highest concentration that can be tolerated with the activity pattern and anthropometric data at point 1 is 0.052 mg/m^3. If the concentration under the same condition exceeds 0.052 mg/m^3 there will be a health impact on the community particularly in the respiratory system.

The researchers observed that $PM_{2.5}$ exposure is related to dementia [12]. Weuve et al showed that older US women (70 years or older) living in areas with higher levels of $PM_{2.5}$ have more rapid cognitive decline over a 2 year period [14]. They concluded that long-term exposure (7-14 years) for particulate matters at the level typically experienced by many individuals in the United States is associated with significantly worse cognitive decline in older women.

Increased $PM_{2.5}$ concentration also increases risk of inpatient treatment for myocardial infarction, dysrhythmias, heart failure and cardiac arrest [15]. With regard to non-fatal effects, the risk of myocardial infarction has been estimated to be 1.48 times greater in increased PM2.5 concentration (0.025 mg/m^3 in two hours) [16]. Increased $PM_{2.5}$ in humans corresponds with the incidence of atherosclerosis and carotid inima-media thickening [17]. Some studies suggest that each decrease by 0.01 mg/m^3 of long-term exposure to $PM_{2.5}$ can increase by up to 22 months of life expectancy for persons 30 years of age or older. Based on a research in 2004, an increase of $PM_{2.5}$ by 0.01 mg/m^{3}s is associated with an increased risk of mortality by 8% to 18% [13].

CONCLUSION

Health risks can be seen from the risk level of $PM_{2.5}$ exposure in areas around a readymix industry. The level of health risk is determined not only by exposure to pollutant concentration, but involves anthropometric data and patterns of the community's activities that affect the high rate of public health risk in certain areas. Health risks can be viewed from the risk level of $PM_{2.5}$ exposure in the environment around a readymix industry.

Ethical Clearance – Taken from Universitas Indonesia Ethics Commision

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Competing Interest

The authors report no competing interest

REFERENCES

1. Block ML & Calderon-Garciduens L. Air pollution: mechanisms of neuroinflammation and CNS

disease. Trends Neurousci. 2009;32(9):506

2. Chang T, Gross T, Neidel M. Particulate pollution and the productivity of pear packers. American Economic Journal: Economic Polic*y*. 2016;8(3):141-169
3. Vette AF, Rea AW, lawless PA, et al. Characterization of indoor-outdoor aerosol concentration relationship during the Fresno PM Exposure Studies. Aerosol Sci Technol.2001;34(1):118-126
4. Greenpeace. Pembunuh Senyap di Jakarta. (Quiet Killers in Jakarta. Jakarta : Greenpeace South East Asia. 2017.
5. Hongbing Ji, Briki Meryem, Cai Li. 2016. Potential Threat of Heavy Metals and PAHs in PM2.5 in Different Urban Functional Areas of Beijing. 2016;178-179: 6-16
6. EPA. Final rule: fine particulate matter in the national ambient air quality standards: state implementation plan requirements. http://www.regulations.gov accessed on 23/06/2018 [internet]. 2016
7. Rosalia O, Wispriyono Bambang, Kusnoputranto H, Karakteristik Risiko Kesehatan Non Karsinogen pada Remaja akibat pajanan inhalasi debu particulate matter <1,5(PM 2,5). (Characteristics of Non-Carcinogen Health Risk to Teenagers due to Exposure and Inhaled Particulate Matter <1.5(PM 2.5)) Jurnal MKMI. 2018;14 (1) : 26 - 35
8. Aldrich, Tim E, and Jack Griffith. Environmental Epidemiology and Risk Assessment. New York: Van Nostrand Reinhold. 1993
9. USEPA. Guideliness for Exposure Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington DC. 1992
10. EPA. Protocol Outline Risk Assessment Guidance for Superfund. 1989
11. Chandra, B. Pengantar Kesehatan Lingkungan. (Introductory to Environmental Health) EGC, Jakarta. 2006
12. Cipriani G, Danti S, Carlesi C, & Borin G. Danger in the Air : Air Pollution and Cognitive Dysfunction, 2018;33(6), 333–341.
13. Shilton V, Giess P, Mitchell D., & Williams C. Relationships between Indoor and Outdoor Respirable Particulate Matter. 1902
14. Weuve J, Puett RC, Schwartz J, Yanoskly JD, laden F, Grodstein F. Exposure to particulate air pollution and cognitive decline in older women, Arch Intern Med. 2012;172(3):210-227
15. Wellenius GA, Schwartz J, Mittleman MA. Air Pollution and hospital admissions for ischemic and hemorrhagic stroke among Medicare beneficiaries. Stroke. 2005;36: 2549-2553
16. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and triggering of myocardial infarction. Circulation. 2001;103: 2810-2815
17. Hoffmann B, Moebus S, Möhlenkamp S, Stang A, Lehmann N, Dragano N. et al. Residential exposure to traffic is associated with coronary atherosclerosis. Circulation 2007;116: 489–496.

Effects of Multimicronutrient and IFA Supplementation in Preconception Period Against Birth Length and Birth Weight: A Randomized, Double Blind Controlled Trial in Banggai Regency, Central Sulawesi

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ABSTRACT

Background: The beneficial effects of MMN and Iron Folic Acid (IFA) supplementation on pregnancy outcomes is still debatable. The objective of this study is to know the effect of IFA and MMN supplementation since preconception period on pregnancy outcomes. Multimicronutrient deficiencies may contribute to low birth weight and birth length which is associated with increased risk of infant morbidity and mortality in developing countries.

Method: The research was conducted in three sub-districts of Banggai district, namely, Luwuk, North Luwuk, and South Luwuk. This was a double blind study, randomized controlled trial, providing multimicronutrient for women from preconception period using prospective design with saturated sampling technique. A total of 19 preconception women followed until pregnancy and look at the pregnancy outcomes were enrolled from September 2016 to Januari 2018.

Results: The mean birth weight of infants born to pregnant women in the MMN group was heavier at 3142.5 g with a value (p= 0.001). LBW percentage of pregnant women MMN group was smaller, equal to 8.3% with value (p=0,863). The average length of infant born to pregnant women in MMN group was longer, 49,5 cm with value (p=0,001). Short birth length (<48 cm) of pregnant mother MMN group smaller, equal to 41,7% with value (p=0,515).

Conclusions: Pregnant women who get MMN intervention produce better pregnancy outcomes.The nutritional status of women before pregnancy is very important to achieve mother and fetal welfare and also considered as the most important thing for governing fetal growth.

Keywords: *Preconception period, multimicronutrient, birth weight, birth length*

INTRODUCTION

Low birth weight (LBW) is associated with the increasing risk of infant morbidity and mortality in developing countries[1]. It has been estimated that infants weighing 2000–2500 and 2500–3000 g at birth are four- and two-times higher risk of post neonatal mortality than those weighing 3000–3500 g, respectively. Through its impact on fetal growth and micronutrient stores, nutrition of the pregnant woman contributes to infant morbidity and mortality[2,3,4].

Epidemiological studies suggest that the interaction between maternal nutritional intake, hormonal disorders, and placental development is a determinant of stunting. Stunting events begin in the womb and continue until at least 24 months of age; so that the preconception period

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up to 2 years is a critical period for the occurrence of stunting and a priority for intervention. Babies born less than 2500 grams are classified as premature and small during pregnancy (small for gestational age) a high risk for stunting.

Maternal Mortality Rate in Banggai district in 2014 is relatively high, (279/100.000) compare to the national target 2015 (102/100.000). To overcome these problems, innovative efforts as a breakthrough to accelerate improvement of the nutritional and health status of Preconception Women (PcW) has been started in Integrated Health Post (Posyandu) for PcW. The services to pregnant women or antenatal care have been hampered by the delay of first contact (K1) of pregnant women, and the big number of cases of anemia in pre-pregnant women (preconception). Therefore, preconception women services supposed to be a very important action, which is part of Maternal and Child Health Service (MCH) in Banggai District. Preconception health services are health services for women of reproductive age, before the first pregnancy which is a public health strategy to improve women's health and reduce maternal and child mortality. By following the preconception health service, the mother to-be are able to get early identification of pregnancy risk factors such as anemia before pregnancy, identifying and managing maternal conditions and behaviors during pregnancy which may pose a risk to both mother and newborn.

UNICEF/WHO/UNU proposed a multimicronutrient (MMN) prenatal supplement to replace the existing iron folic acid (IFA) supplement, which has been recommended for decades as a means of preventing maternal anaemia[5,6]. Multimicronutrient supplementation since preconcepcetion period is considered a feasible public health strategy in areas with micronutrient deficiencies and can potentially benefit both mothers and their infants[7,8]. Still, the evidence for benefits of MMN supplementation on pregnancy outcome is sparse[1]. We conducted a randomised, double blind controlled trial in which the effects on birth weight and birth length multimicronutrient supplementation with RDA of 15 multimicronutrients were compared with the conventional iron folic acid (IFA) supplement containing ferro sulfate / ferro fumarate or ferro gluconate and 0.25 mg folic acid.

Multimicronutrient (MMN) supplementation

Maternal micronutrients deficiency during pregnancy is an outstanding public health issue worldwide. Due to the increased nutritional requirement, pregnant women are vulnerable to micronutrients deficiency. However, the beneficial effect of MMN supplementation on preconception women and during pregnancy and on postnatal growth of children are still unclear. There has been a scarse studied on the prevention of MMN deficiencies as a strategy to reduce the risk of prenatal anaemia and attention has tended to focus instead on iron supplementation during pregnancy, with conflicting results.

MMN supplementation should be considered as a strategy for improving the micronutrient and haematological status of preconception women of reproductive age. MMN supplementation using the United Nations International Multiple Micronutrient Preparation (UNIMMAP) is a balanced preparation of 15 vitamins and minerals formulated by an Expert Committee of the United Nations Children's Fund, primarily for using by pregnant and lactating women. The MMN tablet consist of vitamin A (retinyl acetat) 800 RE, vitamin E 10 mg, selenium 65 microgram, vitamin D3 (Colecalciferol) 200 IU, vitamin B1 (Thiamin) 1.4 mg, vitamin B2 (Riboflavin) 1.4 mg, vitamin B3 (Niacin) 18 mg, vitamin B6 1.9 mg, vitamin B12 (cyanocobalamin) 2.6 microgram, asam folat 400 microgram, vitamin C 70 mg, iron 30 mg *(iron sulphate),* zinc 15 mg *(zinc sulphate)*, iodium 150 microgram and cuprum *(cooper sulfat)* 2 mg.

MATERIAL AND METHOD

A randomized, double blind controlled Trial

To fulfill a double-blind requirement, MMN from UNICEF in the form of tablets was processed into capsules. The MMN tablet and IFA tablet is crushed and then put into a combination of pink and white capsules. MMN capsules and control capsules are both made with similar weight and color. Subjects, data collectors and researchers did not know whether the capsules contained MMN or iron folate, so the parties involved in the field did not know whether the subjects were in the MMN group or iron folate group.

Randomisation and intervention

The women enrolled in the study were individually randomized with iron folic acid and multimicronutrient supplements. MMN supplementation using the United

Nations International Multiple Micronutrient Preparation (UNIMMAP) is a balanced preparation of 15 vitamins and minerals formulated by an Expert Committee of the United Nations Children's Fund, primarily for using by pregnant and lactating women. One group received a daily multimicronutrient tablet containing of 15 micronutrients as recommended in the future prenatal supplement. The control group received the conventional prenatal iron folic acid (IFA) supplement. Preconception women were instructed to take one tablet weekly if not in menstruating period, and to take one tablet daily if in menstruating periode and during pregnancy preferably together with breakfast.

The manufacturer provided the tablets in containers with a colour code for each intervention group. This code was kept secret from study participants, study personnel, and data analysts until data cleaning and preliminary data analysis had been carried out. For managing of administration in the field, randomization by excel randomization six block program AABB, ABAB, ABBA, BBAA, BABA, and BAAB was done. However, the health workers who collected outcome data after delivery did not have any knowledge of intervention group of the women.

Android gate way

There has been conducted a 24-hour consultation and monitoring of capsules consumption through android. The team of the first 1000 days of life provides 24-hour consultation services through android to ensure the consumption of capsule and question – answer of daily emerging issues during preconception period–positive pregnancy test up to the delivery.

Study area and population

The study was carried out from September 2016 to Januari 2018 in three sub-districts of Banggai district, namely, Luwuk, North Luwuk, and South Luwuk Central Sulawesi.

Infant Measurements

Newborn weight were measured to the nearest 1 mm with a TANITA digital scale. To measure the infant's body length using a length board. All measurements were made in the health centers. To ensure reliability, all anthropometric variables were measured twice, by regional public hospital or clinic staff who have been trained and under the supervision of researcher. The average of the 2 measures was used for analysis. The weighing scales were calibrated monthly.

Statistical analyses

The type of statistical test used is adjusted to the type of variable being tested, if there is a test of the relationship between categorical data and categorical data then using the chi-square test, the average difference test depends on the normality of the data (Anova or Kruskal Wallis) when testing the relationship of categorical data and numerical data. Meanwhile the confidence interval used was 95% with p-values < 0.05 were considered significant. All statistical analyses were performed with SPSS 23.0 (SPSS® Inc.)

RESULTS

Homogeneity Test

Homogeneity between groups before treatment is very important in an experimental study, to ensure that differences in conditions after treatment between groups is indeed an effect of treatment (Meneinert, 1986). In table 1 it can be seen that in both IFA and MMN supplementation groups all variables were homogeneous with p value> 0.005 by testing the homogeneity of variance with Anova test.

Table 1. Homogeneity Test for Preconception Women in IFA Supplementation Group and MMN Supplementation Group

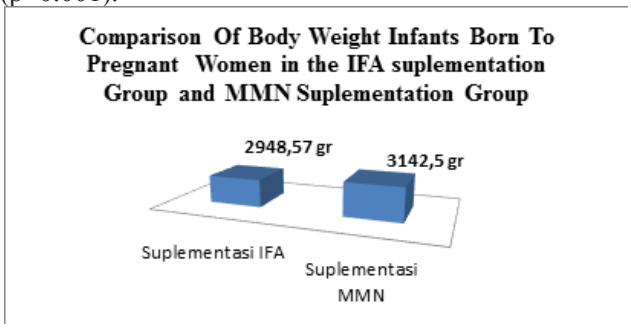
No	Variable	P value*
1	Residential area	0,633
2	Mother's age	0,989
3	Education mother	0,321
4	Father's education	0,648
5	Mother's work	0,499
6	Father's work	0,102
7	Systolic blood pressure at preconception periode	0,079
8	Diastolic blood pressure at preconception periode	0,189
9	Maternal weight at preconception periode	0,086
10	Maternal height at preconception periode	0,848
11	Body mass index at preconception periode	0,818

Cont... **Table 1. Homogeneity Test for Preconception Women in IFA Supplementation Group and MMN Supplementation Group**

12	Mid Upper Arm Circumference at preconception periode	0,601
13	Maternal Haemoglobin level at preconception periode	0,467
14	Maternal Sflt-1 value at preconception periode	0,661
15	Maternal VEGF value at preconception periode	0,766

**Anova test (test homogenity of variance)*

From graph 1, it can be seen that the comparison of the average body weight of infants born in the IFA Suplementation group was lower (2948 g ± SD 344.40) compared to the average body weight of infants born in the MMN supplementation group (3142.5 g±SD 464.48) and the results of statistical tests showed that there were significant differences in the average of body weight infants born to pregnant women in both groups (p=0.001).



Graph 1. Comparison Of Body Weight Infants Born To Pregnant Women in the IFA Supplementation Group and MMN Supplementation Group

Table 2. Comparison of Body length Infants Born To Pregnant Women in the IFA and MMN Suplementation Group

Body length of Infant Born	**IFA Suplementation Group (n=7)**			**MMN Suplementation Group (n=12)**		
	Mean ± SD	**Median**	**Min± Max**	**Mean ± SD**	**Median**	**Min± Max**
	47,86 cm ± 2,41	**48 cm**	**50 cm ± 43 cm**	**49,5 cm ± 2,54**	**49 cm**	**55 cm ± 46 cm**
Mean difference body length of Infant Born To Pregnant Women in the IFA and MMN supplementation group						
Difference	1,64 cm					
95% CI	47,66 – 50,13					
P value	0,001					

From table 2 shows that in the IFA supplementation group the average body length of infants born to pregnant women was 47.86 cm±2.41 median value of 48 cm and the average body length of infants born to pregnant women in MMN supplementation group was 49.5 cm±2.54 and median value was 49 cm. The difference in body length of the IFA and MMN supplementation groups was 1.64 cm. The mean birth weight of infants born to pregnant women in the MMN group was heavier at 3142.5 g with a value (p = 0.001). The average length of infant born to pregnant women in MMN group was longer, 49,5 cm with value (p = 0,001).

Table 3. Category Body Length of Infant Born to Pregnant Women in the IFA and MMN Supplementation Group

Supplementation Group	Short birth length (<48 cm)		Normal birth length (≥48 cm)		Total		P value
	n	%	n	%	n	%	
IFA	4	57,1	3	42,9	7	100	0,515
MMN	5	41,7	7	58,3	12	100	
Total	**2**	**47,4**	**17**	**52,6**	**19**	**100**	

**Chi Square Test*

From table 3 shows that short birth length (<48 cm) of pregnant mother MMN group smaller, equal to 41,7% with value (p = 0,515).

DISCUSSION

The effect of MMN supplementation on infants' birth weight

Birth weight is a simultaneous effect of energy and protein intake factors that play a dominant role, as well as the role of MMN in determining nutritional status since the preconception period. The meta-analysis of MMN administration and pregnancy outcomes conducted[9] explains that the exact mechanism of how MMN supplementation can affect pregnancy outcomes is not yet understood, but MMN can improve immune function thereby reducing the risk of infection in the mother, improving maternal energy metabolism, improve response to stress, and increase the volume of plasma and erythrocytes circulating in the body.

The effect of MMN supplementation on infants' birth length

Between the metaphysics and epiphyses lies the growth plate, where bone growth occurs, so that the bones can become long. The increase in height occurs because of the increasing number of cells in the modified growth plate by MMN supplementation, wherein the content of vitamin D which is a steroid prohormon plays an important role in calcium absorption by regulating calcium absorption in the small intestine. Vitamin D stimulates the synthesis of calcium binding proteins and phosphorus binding proteins in the small intestinal mucosa.

Bone mineralization is positively associated with bone mineral density (BMD). Vitamin D deficiency causes a decrease in calcium absorption which causes the release of calcium from the bone to maintain circulating calcium concentration[10]. Vitamin D also plays a role in stimulating the differentiation and proliferation of chondrocytes so that the growth of the bone growth plate is better, resulting in more body length addition compared to the IFA supplementation group. The more nutrient content, the better absorption of vitamins so that its utilization is more effective for bone growth.

There are a number of biological pathways that explain the role of vitamin D that can affect maternal health, placental and fetal growth during pregnancy. In vitro research shows that vitamin D plays an important role in the metabolism of glucose and insulin which can affect the availability of energy for the fetus.

Vitamin D modulates the immune system that can help shape the right maternal immune response to the placenta which also regulates the key target genes associated with proper placental implantation. Vitamin D plays a direct role in the production of antimicrobial peptides such as katelicidin, which are produced after activation regulated by vitamin D receptors where the production requires 25 (OH)-D which plays an important role in preventing infection during pregnancy. Vitamin D also affects bone and muscle growth[11].

Recent studies have suggested a direct role of vitamin D in the regulation of Hoxa-10 in human endometrial stromal cells. Overall, the study shows that endometrial expression of Hoxa-10 plays an important role in controlling uterine reception, implantation, and desidualization[12] and will further influence fetoplacental

blood flow.

CONCLUSION

Pregnant women who get multimicronutrien (MMN) intervention since preconception period produce better pregnancy outcomes. Posyandu for PcW in Banggai District has became an innovative intervention to increase the health status of PcW starting from preconception period. Preconception period is the best time for women to modify their dietary habits and select healthy nutritional patterns that associated with better birth outcomes.

Ethical Clarance: The study was approved by the Hasanuddin University with the number 1578/ H4.8.4.5.31/31/PP.36-Kometik/2016. All women received an anti-helminthes prophylaxis with albendazole (400 mg base) before receiving the supplementation.

Sources of Funding: Self-funded

Conflict of Interest: Nil

REFERENCES

1. Kaestel P, Michaelsen KF, AAby P, and Friis H. Effects of prenatal multimicronutrient supplements on birth weight and perinatal mortality: a randomised, controlled trial in Guinea-Bissau. European Journal of Clinical Nutrition 2005; 59:1081–1089.
2. Ceesay SM, Prentice AM, Cole TJ, Foord F, Weaver LT, Poskitt EM & Whitehead RG. Effects on birth weight and perinatal mortality of maternal dietary supplements in rural Gambia: 5 year randomised controlled trial. BMJ 1997; 315: 786–790.
3. Tomkins A. Nutrition and maternal morbidity and mortality. Br. J. Nutr. 2001; 85 (Suppl 2): S93–S99.
4. Christian P. Maternal nutrition, health, and survival. Nutr. Rev. 2002; 60: S59–S63.
5. Huffmann SL, Baker J, Schumann J & Zehner ER. The case for promoting multiple vitamin/mineral supplements for women of reproductive age in developing countries. The LINKAGES Project, Washington; 1998.
6. UNICEF, WHO & UNU. Composition of a multi-micronutrient supplement to be used in pilot programmes among pregnant women in developing countries. New York: UNICEF; 1999.
7. de Onis M, Villar J & Gulmezoglu M. Nutritional interventions to prevent intrauterine growth retardation: evidence from randomized controlled trials. Eur. J. Clin. Nutr. 1998; 52 (Suppl 1): S83–S93.
8. Shrimpton R & Schultink W. Can supplements help meet the micronutrient needs of the developing world? Proc. Nutr. Soc. 2002; 61: 223–229.
9. Shankar AH, Prasad AS. Zinc and immune function: The biological basis of altered resistency to infection. Am J Clin Nut 1998; 68: 447-63.
10. Eamon Laird, Mary Ward, Emeir McSorley, J.J. Strain and Julia Wallace. Vitamin D and Bone Health; Potential Mechanisms. Nutrients 2010; 2(7): 693-724.
11. Haugen J, Ulak M, Chandyo RK, Henjum S, Thorne-Lyman AL, Ueland PM, Midtun O, Shresta PS, and Strand TA. Low Prevalence of Vitamin D Insufficiency among Nepalese Infants Despite High Prevalence of Vitamin D Insufficiency among Their Mothers. Nutrients 2016; 8(12): 825.
12. Lim Roach. Metabolism and Nutrition. Mosby Elsevier; 2017.

Ineffective Regulation of Narcotics Crime Prevention (Criminology Perspective)

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ABSTRACT

The reality is that it should be a reason for us to worry because it threatens the future of the young generation who are the holders and successors of the nation's relay. That said because the impact caused by drugs is so tragic. The impact of drugs includes physical, psychological, social and economic impacts. This study aims to find out the causes of the increasing number of crime narcotics in the perspective of criminology. This type of research is an empirical research. Police officials, advocates, and narcotics abusers were taken as samples in this study. Data collection is done through interviews and documentation. The results showed that the regulation of narcotics crime prevention has not been effective in practice. Narcotics crime should have been reduced to the lowest in the presence of Act Number 35 the Year 2009 on Narcotics, but in reality, tend to increase every year. In the perspective of criminology with the observation of the age, sex, education, and work of narcotics perpetrators, it is found that multiple factors are interrelated, including psychological factors, sociological factors, and economic factors as criminal etiologies that trigger an increase in narcotics crime.

Keywords: *Narcotics crime, Criminology, Crime etiology.*

INTRODUCTION

The danger of drugs is no doubt. However, drug abuse is increasingly prevalent in various countries around the world, including Indonesia. When someone starts taking drugs, there is a high chance of experiencing addiction. The longer, the user will need a higher dose in order to feel the same effect. When the effects of drugs begin to disappear, users will feel uncomfortable due to the appearance of withdrawal symptoms and will want to use them again [1].

Drugs that dissolve in the body will be channeled through blood throughout the body, including the brain. The effects of drugs depend on the type consumed, dosage, duration of use, and body size of the person who consumed them. In addition to influencing the body, the danger of drugs can also cause things that interfere with a person's quality of life. An addict is also more susceptible to sexually transmitted infections, accidents, and suicide attempts due to being under the influence of drugs[2]

According to data quoted from the Provincial National Narcotics Agency (BNNP) of South Sulawesi, the impact of drugs includes physical, psychological, social and economic impacts. Physical effects such as nervous system disorders (neurological): convulsions, hallucinations, and disturbances of consciousness. The psychological impact is not the normal ability to think, feeling anxious. Dependence/always need medicine. Socio-economic impacts, for example, always harm the community, both economic, social, health and legal.[3]

Drug distribution and use at this time are very worrying. In a sign that Indonesia is a transit place for drug trafficking by the international kingpin. We should be concerned and alert to the dangers of drugs that threaten the young generation of this nation. Drugs have a very broad impact and can damage the joints of the nation's life. The danger of drugs is actually very massive, even less destructive than the dangers of corruption itself.[4.] The crime data cited above is the result of the national scale collection of the Republic of Indonesia case. In the local scale, it is also found that crime statistics tend to increase every year. In Makassar City in the period of 2012 till. In 2015 the crime rate was: in 2012 there were 288 people, in 2013 there were

255 people, in 2014 there were 323 people, and in 2015 there were 429 people data from Polrestabes Makassar, 2016. Only in 2013 did the number of Narcotics crimes experience a decline, which is 33 people decreased, and in 2015 again increased to 106 people.

Subjective approach is to look at individual factors as determinants of the occurrence of crime, wrong because of your own fault (SS). In essence, this approach is influenced by indeterministic theory [5]. While the objective approach is to look at factors outside individual conditions as the cause of crime, wrong because the Environment is Wrong (LS). This approach is influenced by deterministic theory.

MATERIAL AND METHOD

Research sites

This research was carried out in Makassar City, namely at the Makassar Polrestabes. The researcher took this location, considering that at that location, it was the first time that narcotics crime cases were examined by police law enforcement officers.

Type of Research

The type of research in this study is empirical research. The goal is to reveal the etiology of narcotics crime with the aim of providing legal solutions to the increase in crimes from a criminological perspective.

Data Collection Techniques

In this study interview and documentation, techniques were used. The interview technique was carried out on police law enforcement officers, advocates, narcotics abusers including users, dealers and narcotics dealers in the city of Makassar. While documentation is carried out through the collection of statistical data on crimes found in the Makassar Police

Data analysis

The data analysis method used is qualitative analysis, by analyzing and describing the data obtained both from primary data and secondary data, the results of research through a criminological approach to the etiology of narcotics crime and its countermeasures.

RESULTS AND DISCUSSION

Criminology as the science of various knowledge, the body of knowledge, is dominantly defined as the science that seeks to investigate the causes of the symptoms of evil in ways that exist in it. By conducting an investigation into the causes of these crimes, this is then the scope of the study of criminal etiology which is none other than one of the main branches of criminology [6]. Related to the increasing number of narcotics crimes with a view to revealing their criminal etiology, four variables will be used which consist of the age of the perpetrator, the sex of the perpetrator, the education of the perpetrator and the work of the perpetrator.

As stated above, that because criminology uses various sciences, then to measure all four variables, it cannot be separated from anthropological approaches, psychological approaches, and sociological approaches as well as in the applied field of criminology: criminal anthropology, criminal psychology, and sociology criminal.

Age of Narcotics Crime Actors

Formulation of different criminal responsibilities between children (12 to 18 years) with adults is a further consequence of a person's cognitive phase to be able to think fully or not be able to think fully

In a psychology perspective, especially developmental psychology, adulthood should be one of the physical variables that can prevent a person from committing a crime. Ansem of developmental psychology if connected with statistics of narcotics criminals based on one's adult age, it does not support the reduction in the number of narcotics criminals. This can be noted through the following table 1:

Table 1: Number of Narcotics Crimes in Makassar City Based on the Age of the Perpetrators (2012 to 2015)

Years 10-17	Age (year)				
	18-20	21-25	26-30	> 31	
2012	15	36	59	70	122
2013	18	27	63	42	105
2014	20	39	61	75	131
2015	19	44	103	85	178
Total	72	146	286	272	536

The interesting thing from the presentation in Table 1 above is that the increase in the age of narcotics criminals is directly proportional to the increase in narcotics criminals. Physical and biological development of the stage of perfection, but perfectly cognitive is unable to hold the offender acting based on the norms of goodness contained in him as a reflection of his social environment. According to National Police Headquarters data contained in the book Population Perspective of Islam written by M Cholil Nafis, from 2004 to March 2009, there were 98,614 cases (97% more) teenage children were drug users[1].

Narcotics perpetrators from 10 to 17 years of age and 18 to 21 years of age are essentially unable to live independently. He has not been able to make a living on his own, has not been able to earn money independently, even if there is money in his hand it is usually obtained from his close relatives. He was most likely to get close to giving his mother's father. Therefore if you want to use or consume narcotics that are expensive, you will certainly be constrained from the financial aspect to buying narcotics ingredients.

Crime is learned through interaction with other people by learning techniques of crime, distinctive motivation, encouragement, rationalization, and attitude6. While narcotics crimes committed by adults are not influenced by totality alone. The country of Indonesia as a developing country with a poverty rate is still high, and at the same time more and more demands to live in luxury 7 cause social consequences of irregularities. The deviation is by becoming a narcotics dealer so that the finances are sufficient to meet all needs that can increase its prestige.

Gender of Narcotics Crimes

Comparing the quantity of crime based on sex, the perpetrator is still a series of individual factors as their physical and psychological state. Although in examining narcotics crime by looking at the sex of the perpetrator, it cannot be separated from environmental factors. The combination of these individual factors and environmental factors is then referred to as the bio-sociological approach[3]. The quantity of narcotics criminals who are always dominated by male-sex offenders rather than a female can be considered in the following Table 2:

Table 2: Number of Narcotics Crimes in the City of Makassar Based on Gender Actors

(2012 to 2015)

YEAR	Gender	
	Male	Female
2012	259	17
2013	243	21
2014	269	39
2015	369	45
TOTAL	1.140	122

Based on Table 2 above, there are two things that need to be explained further, namely: (1) Why are the number of narcotics perpetrators dominated by male sex offenders? (2) Why do narcotics perpetrators, both male and female, tend to increase every year? Although in 2013, male sex offenders experienced a decline. The opportunity is that men who are more demanding work to be outside the home, than women are more at home taking care of their children, family, and husband's eating needs, of course, the opportunity for men to become narcotics abusers is more open than with women [8]

It is different from narcotics abusers who are female, the cause they commit crimes is not because of economic problems which are the main reason. They become more abused by their association with the workplace. In some cases the average woman is also a narcotics abuser because the family conditions are broken (broken home), so by becoming a drug user, her family's problems for a moment do not burden her mind again [9].

Education Status of Narcotics Crime Actors

The purpose of education for everyone is essential to form their personality with integrity, responsibility, and agility in differentiating a good and right decision. The analogy is that the higher the level of education a person means the more his experience goes through the process of internalization of a shared value so that the output is easier to avoid all actions that have been criticized by the community in their environment.

One research in the United States also shows that prison inmates at the "Richland Parish Detention Center," Louisiana, generally only have education in

grade 2 elementary school to grade 1 junior high school (second grade to seven grade). Those with low education are always wrong in having perceptions about life and law[10]. Some of the results of the above research, in fact, contradict the results of research found on the number of narcotics crimes in the city of Makassar, when viewed from the education status of each perpetrator. It is precisely at the level of high school education, the perpetrators are more compared to narcotics criminals who are in junior and elementary education levels. More details about the conflict can be noted in the following table 3:

Table 3: Number of Narcotics Crimes in Makassar City Based on the Level of Education Actors (2012 to 2015)

Years	Education			
Elementary School	Junior High School	Senior High School	College	
2012	15	36	195	42
2013	41	55	152	7
2014	66	90	147	20
2015	68	127	216	18
Total	190	308	755	87

Based on Table 3 above shows that the highest number of narcotics abusers occurs at the level of high school education compared to the level of elementary and junior high school education. Only the narcotics abusers with higher education status then the number of perpetrators, suddenly shows a decrease.

Apart from that which is a striking difference, so that narcotics criminals with higher education status suddenly decline, none other than because the population in Makassar city is far more high school educated than college graduates.

Job Status of Narcotics Crime Actors

A person's work will certainly be a reflection if the economic situation becomes sufficient to meet his life's needs. If many people crave a profession as a civil servant, actually they are craving for a more established economy. A person's job status that is directly proportional to the economic situation, associated with the potential or not to become a narcotics abuser is very difficult to measure. Someone who has financial supplies, in the sense of an established economy, has the potential to become a narcotics abuser. For those in the group, even though the price of narcotics is expensive, it is definitely affordable, at least as a user.

The criminologist's conclusions about the close relationship between work and crime will be supported by the results of this study. The highest number of narcotics perpetrators is in fact played by those who are unemployed. This can be noted through the following table,

Table 4: Number of Narcotics Crimes Based on the Workers' Acts (2012 to 2015)

Occupation	Year (person)			
	2012	2013	2014	2015
Student	12	5	13	6
Collage Student	20	23	23	13
Government Employee	5	10	3	5
Private Employee	92	54	68	79
Police	5	-	1	-
Entrepreneur	59	58	67	108
Farmer/ Fisherman	-	2	1	2
Daily Labour	40	46	63	95
Unemployment	71	57	84	121
Total	304	225	323	429

Based on Table 4 above shows that the highest number of narcotics abusers occupy the first position in each year (other than 2013), namely those who are only unemployed. Then followed by those who work as entrepreneurs, day laborers, and private employees. It was found in this study that narcotics criminals with unemployment status, the main factor causing them to be involved in these crimes, are mostly caused by economic motives compared to other reasons, such as severe

stress so that narcotics are considered as life escape. This grouping is more dominant, the perpetrators act as dealers or bookies.

For narcotics abusers who come from the daily labor profession, the same situation applies to the entrepreneurial profession, which is only as a user, but not used during breaks. Daily laborers actually use narcotics with the aim to boost adrenaline work while eliminating fatigue after a full day's work. While narcotics offenders who come from the entrepreneurial profession and private employees are caused by their orientation in a social class. One experiences a shift from one choice to another because it is faced with different social activities[11,12]. Then the narcotics offenders who come from the profession of daily labor, are mostly caused by ecological and physical factors, each of these factors intertwines, narcotics cannot be separated from their parts of life and work.

CONCLUSIONS AND SUGGESTIONS

The results of this study indicate that the regulation of narcotics crime prevention has not been effective in practice. The narcotics crime rate should have been reduced as low as possible, but in reality, it tends to increase every year. In a criminology perspective with observations of age, sex, education, and occupations of narcotics criminals, it was found that multiple factors are interrelated, including psychological factors, sociological factors, and economic factors as criminal etiologies that trigger an increase in narcotics crime rates.

Reaction to the breaking law against these crimes is by eliminating the criminogenic factor which is the cause of the crime (abolitionist system). Priority should be given to increasing employment opportunities, then counseling and education of narcotics hazards in all lines regardless of the age and profession of each person. Police and BNN must prioritize capturing narcotics dealers because these are the main causes of the emergence of dealers in the narcotics business with very promising benefits.

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REFERENCES

1. Lampung Province National Narcotics Agency. Influence of Narcotics in Youth . Rakyatku News Online, http://news.rakyatku.com/read/62133/2017/08/22/ pengaruh-narkoba-di-kalangan-remaja, accessed on July 3, 2018. 2017.
2. Rahmadona E, Agustin H. Factors Relating to Drug Abuse in Prof. RSJ HB. Sa'anin. J Health Andalas Health [Internet]. 2014; 8 (2): 60–6. Available from: http://jurnal.fkm.unand.ac.id/index.php/jkma/
3. Judge PP, Criminal BH, Law F, Hasanuddin U. Criminological review of narcotics abuse by individual students. 2014;
4. Sujatmiko A. Danger of Drugs [Internet]. D3 Student Scientific Work. 2011. Available from: http://research.amikom.ac.id/index.php/DMI/article/view/5071
5. Bishop RC. Determinism and Indeterminism. 1998;
6. Weitzman ML. Principles of Criminology. Q J Econ. 1998; 113 (2): 331–60.
7. Merton RK. Social Structure and Anomie. Am Sociol Rev [Internet]. 1938; 3 (5): 672. Available from: http://www.jstor.org/stable/2084686?origin=crossref
8. Brantingham PJ, Faust FL. Crime & Delinquency. 1976;
9. Davies CH and RB. Forensic Psychology, by G. Davies, C. Hollin and R. Bull (eds). Psychiatry, Psychol Law [Internet]. 2008; 15 (3): 500–1. Available from: http://www.tandfonline.com/doi/abs/10.1080/13218710802492533
10. Buonanno P, Montolio D, Vanin P. Does Social Capital Reduce Crime? J Law Econ [Internet]. 2009; 52 (1): 145–70. Available from: http://www.journals.uchicago.edu/doi/10.1086/595698
11. Palutturi, S., Chu, C., Moon, J.Y., Nam, E.W. A comparative study on healthy city capacity mapping: Indonesia and Korea, Social Sciences (Pakistan) 2015.
12. Pulubuhu, D.A.T., Evans, K., Arsyad, M., Mallongi, A. Understanding the perspectives of village leaders and institutions in transforming social conflict into peace and health. Indian Journal of Public Health Research & Development, March 2018, Vol. 9, No. 3

The Effect of $PM_{2.5}$ Exposure on Workers' Enzymatic Superoxide Dismutase (SOD) Concentration at a Ready-Mix Concrete Factory in 2018

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ABSTRACT

The lungs are exposed to $PM_{2.5}$ via inhalation; $PM_{2.5}$ then settles in the alveoli and circulates via the blood system, causing chronic obstructive pulmonary disease and cardiovascular issues. $PM_{2.5}$ triggers the formation of superoxide anions and provide signals for the formation of superoxide dismutase . Workers at ready-mix concrete factories had long-term exposure to high doses of $PM_{2.5}$. This study investigates the effects of $PM_{2.5}$ on the blood SOD concentration of workers.

This cross-sectional study involved 53 subjects chosen with random sampling and interviewed to obtain individual characteristic data. $PM_{2.5}$ value was measured by gravimetric analysis using High Volume Air Sampler and EPAM-5000. The SOD value was tested with the RanSOD-Kit and read using RX-Monza. Data were analyzed with chi-square and multiple logistic regression.

It was determined that the workers exposed to $PM_{2.5}$ ^{3}65 mg/m^3 had a 10x higher risk of obtaining a SOD concentration of <84.40 U/mL than the workers exposed to $PM_{2.5}$ <65 mg/m^3.

There is a significant relationship between $PM_{2.5}$ exposure and SOD concentration. Working status and length of employment were identified as contributing variables (p-value 0.029; OR 10.165; CI 1.276–90.994). This study suggests that $PM_{2.5}$ exposure leads to a decrease in SOD concentration. It is necessary to take preventive measures for workers.

Keywords: *superoxide dismutase, antioxidant, oxidative stress, $PM_{2.5}$*

INTRODUCTION

The total population of Indonesia in 2010 was 237.641.326, including the urban population of 118.3 million (49.7%) and the rural population of 119.3 million (50.2%) [1]. Such a large population increases the need for building construction and transportation facilities; thus, ready-mix concrete plays an important role in the building material industry. Batching plants in the ready-mix concrete industry increase the concentration of particulate matter ($PM_{2.5}$) with a diameter of 2.5 mm, impacting the health of workers. The real-time risk calculation shows that 21.4% of batching plant workers are in the at risk category [2]. The safest area that can be inhabited by members of a nearby community is more than 2.5 km from the center of the cement industry and has a $PM_{2.5}$ concentration of about 28 mg/m^3 [3]. In the presence of fine particles ($PM_{2.5}$), neutrophils from asthmatic patients showed an increasing tendency to generate reactive oxygen species (ROS) [4].

Silica and iron comprise the majority of PM_{10} and $PM_{2.5}$ in dwellings between a 500 m and 3000 m radius

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from the cement factory [5]. $PM_{2.5}$ is the external factor that triggers the formation of ROS in the body [6]. $PM_{2.5}$ with an aerodynamic diameter of <10 μm has a greater impact on human health [7].

The $PM_{2.5}$ surface is rich in transition elements that can increase free radicals. $PM_{2.5}$ triggers chronic obstructive pulmonary disease (COPD); causes inflammation, impaired lung function, and immunity dysfunction; and can change the structure of respiratory epithelium [8]. ROS will damage molecules, such as proteins, carbohydrates, and even DNA[9], through the lipid peroxidation reaction [10]. ROS plays several physiological roles, such as cell signaling. ROS are normally generated as by-products of oxygen metabolism, environmental stressors (i.e., ultraviolet, ionizing radiation, pollutants, heavy metals), and xenobiotics [11].

At certain levels, free radicals give signals to the body to establish the superoxide dismutase (SOD) enzyme. The human body provides antioxidants to convert the oxidants into less harmful substances [12]. This SOD enzyme converts superoxide anion radicals into oxygen molecules and hydrogen peroxide which are less reactive. SOD is the first line of defense affected by free radicals. The decrease of total antioxidant capacity is significantly related to the increase of lipid peroxidation[13]. In the blood assays of patients with COPD, the oxidative markers are higher, while antioxidants (catalase, copper, and SOD) are significantly reduced [14].

The purpose of this research is to study the effect of $PM_{2.5}$ exposure on workers' SOD enzymatic blood concentration at the ready-mix concrete factory in Jakarta.

MATERIALS AND METHOD

a. Study Design

This research is a cross-sectional design study using simple random sampling from 125 workers. Blood samples were taken from 53 subjects and assayed at the Health Laboratory of Jakarta. The subjects were the workers of a concrete ready mix factory in batching plants; all subjects signed informed consent and were neither ill nor taking special medication.

b. $PM_{2.5}$ measurement

Fine particles (<2.5 mm $PM_{2.5}$) were collected from ambient by Tisch high volume air sampler in temperature 28.29±2.15°C and 74.34±8.13%RH. The concentration of indoor $PM_{2.5}$ was measured by 2.5mm-impactor gravimetric HazDust EPAM-5000.

c. Superoxide dismutase (SOD) measurement

Blood SOD enzyme concentration was assayed by RanSOD^{O} Kit and read by RX-Monza. The role of this measurement is to get the dismutation of the toxic superoxide anion to hydrogen peroxide and oxygen. This method employs xanthine and xanthine oxidase to generate superoxide anion radicals. It will react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to create a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction. One unit of the SOD is the one which causes a 50% inhibition of the rate of reduction of INT. The INT reduction rate is linearly associated with the inhibitory activity by SOD [15]. The INT method is easy and inexpensive, which can be used in routine erythrocyte and serum analyses for SOD [16].

d. Body Mass Index

Characteristics of subjects were obtained by interviewing them using questionnaires. The body weight obtained by calibrated scale and the body height obtained by microtoise.

e. Statistical Analysis

We analyzed these data by chi-square, while the final model was analyzed by multiple regression.

f. Ethics

This study has passed the ethical clearance by the Research Ethics and Community Service Commission, Public Health Faculty, Universitas Indonesia (282/UN2/F10/ PPM/00.02/2018 dated 17 April 2018).

RESULTS

This research was performed in a batching plant. The subjects were mostly outdoor workers, such as mixer truck drivers, technicians, central mixer unit operators, and waste operators; while only 12 people were administrative employees. The production process is determined as a wet batching plant type where materials are mixed with water in a central mixer and directly poured into the mixer truck [17]. The determination of sample points referred to the National Standard SNI 19-7719.6-2005.

Table 1. Distribution of $PM_{2.5}$ Concentration Based on Sampling Points

Sampling point	Sampling location	$PM_{2.5}$ (μg/m³)
1	Stockpile	129.05
2	Central mixer & silo	117.45
3	Laboratory, workshop & waste treatment	113.35
4	Outdoor gate & parking lot	120.30
5	Office	46.75

The distribution of $PM_{2.5}$ concentration can be seen in Table 1. The highest amount of $PM_{2.5}$ concentration was found in the stockpile area (129.05 mg/m³) where material loading and unloading activities are carried out using trucks. The lowest amount of $PM_{2.5}$ exposure was found in the office (46.75 mg/m³), the distance of this workspace is relatively far from the center of $PM_{2.5}$ exposure.

Table 2. Statistic Overview of $PM_{2.5}$ SOD Concentration and Characteristics of Subjects

Variables	Mean	Median	Standard deviation	Min-max
$PM_{2.5}$ (μg/m³)	101.65	113.35	30.47	46.75–129.05
SOD concentration (U/mL)	99.46	84.40	58.03	30.60–398.40
Age (year)	34.40	34.00	8.58	20–53
Length of employment (year)	4.64	3.00	4.53	1–22
Vegetables &fruits consumption (portion/week)	33.88	28.00	17.08	2–70
Body mass index (kg/m²)	24.16	23.51	6.01	15.72–33.35

As shown in Table 2, the average $PM_{2.5}$ concentration is 101.65 μm/m³. This value exceeds the maximum limit of 65 μm/m³ as regulated in the Government Regulation No. 41 of 1999. According to this value, we classified subjects into two groups: those who were exposed to $PM_{2.5}$ above 65 μm/m³ and below 65 μm/m³.

Table 3. Subject Characteristics

Variables	n	%
Number of subjects exposed to $PM_{2.5}$		
<65 mg/m³	12	22.60%
≥65 mg/m³	41	77.40%
SOD enzymatic concentration		
≥84.40 U/mL	26	49.10%
<84.40 U/mL	27	50.90%
Age		
< 28 years	15	28.22%
≥ 28 years	38	71.78%
Working status		
Group 1 (not in contact with the mixer truck)	23	43.40%
Group 2 (in contact with the mixer truck)	30	56.60%
Length of employment		
<3 years	19	35.80%
≥3 years	34	64.20%
Smoking		
No	11	20.80%

Cont... **Table 3. Subject Characteristics**

Yes	42	79.20%
Vegetables and fruit consumption		
enough (≥35 portion/week)	24	45.30%
less (<35 portion/week)	29	54.70%
BMI		
Normal (18.5 ≤ BMI < 25)	26	49.10%
Not normal (BMI<18.5 or BMI≥25)	27	50.90%

Based on Table 3, the number of subjects that received $PM_{2.5}$ exposure ³65 mg/m³ is 77.40%. Furthermore, the percentage of subjects who had SOD value below 84.4 U/mL is about 49.10%. There were 38% of the subjects who were 28 years of age or older. The SOD level in human blood starts to decrease with age at 28 years old [18]. The percentage of subjects who consumed approximately 35 portions of vegetables and fruit in a week was 54.70%.

Table 4. Correlation of SOD Concentration with $PM_{2.5}$ Exposure and Workers' Characteristic

Variables	**SOD (U/mL)**				**Total**		**OR (95%CI)**	**p-Value**
	≥84.4		<84.4					
	N	%	n	%	n	%		
$PM_{2,5}$								
<65 mg/m³	10	83.3	2	16.7	12	100	7.06 (1.37–36.40)	0.03
≥65 mg/m³	17	41.5	24	61.0	41	100		
Age								
<28 years	6	40.2	9	60.0	15	100	0.54 (0.16–1.82)	0.49
≥28 years	21	55.3	17	47.4	38	100		
Working status								
Group 1	13	59.1	9	39.1	23	100	1.75 (0.58–5.30)	0.47
Group 2	14	45.2	17	60.0	30	100		
Length of employment								
<3 years	6	31.6	13	68.4	19	100	0.29 (0.09-0.94)	0.07
≥3 years	21	61.8	13	41.2	34	100		
Smoking								
No	7	63.6	4	36.4	11	100	1.93 (0.49–7.57)	0.54
Yes	20	45.2	22	54.8	42	100		
Vegetable and fruit consumption								
Sufficient	12	45.8	12	54.2	24	100	0,93 (0.32–2.75)	0.90
Not sufficient	15	51.7	14	48.3	29	100		
BMI ratio								
Normal	14	50.0	12	50.0	26	100	1.26 (0.43–3.70)	0.89
Not normal	13	48.1	14	51.9	27	100		

By chi-square test, as shown in Table 4, we obtained the p-value of 0.03 for the correlation between $PM_{2.5}$ exposure and SOD concentration. Statistically, there is a significant difference in SOD concentration in workers exposed to $PM_{2.5}$ ³65 mg/m³ and <65 mg/m³.

Table 5. Model for the Correlation between SOD Concentration and $PM_{2.5}$ Exposure

Variables	Coeff.B	p-value	Odd ratio	95%CI	
				Lower	Upper
$PM_{2.5}$	2.319	0.029	10.165	1.276	80.994
Working status	-0.714	0.373	0.490	0.102	2.359
Length of employment	-1.074	0.095	0.342	0.097	1.204
Constant	-0.769	0.401	0.463		

Multivariate regression was conducted as shown in Table 5, it can be concluded that workers who received $PM_{2.5}$ exposure of more than 65 mg/m³ in the batching plant were at ten times higher risk of having the SOD concentration below 84.4 U/mL than those who received $PM_{2.5}$ exposure below 65 mg/m³.

DISCUSSION

The deposition of particulate matter in human respiratory tract depends on the size of the particles: larger particles are deposited in the upper respiratory tract, while smaller particles penetrate deep into the alveolar spaces [19]. $PM_{2.5}$ as an external factor produces free radicals in the body and brings about the oxidative stress. They trigger the forming of free radicals, such as superoxide anion. The metal content of $PM_{2.5}$ acts as an electron donor. Superoxide anion creates a signal to the body to produce a large amount of SOD enzymatic antioxidants. If there is an imbalance between prooxidants and antioxidants due to increased ROS (from chronic exposure), pathological reactions appear and cause various diseases, such as chronic obstructive pulmonary disease [20].

Daily workplaces are related to the decrease of SOD concentration. The percentage of workers in Group 2 who had SOD concentration below 84.4 U/mL was 60%, and these people were technicians, waste operators, and drivers. Stockpile area was the highest concentration place that may be caused by some ready-mix concrete activities, such as material unloading, material reversal, and mobilization of back-haw vehicles.

The workers need to consume a sufficient amount of vegetables and fruits to provide themselves with exogenous nutritional sources which can improve health by controlling free radicals. Some research found that changes in moisture content during storage, cooking, and processing stages can misrepresent changes in nutrient content [21]. Steamed vegetables have better texture quality than boiled ones, whereas boiled vegetables show limited discoloration [22].

Increased physical activity is believed to increase energy expenditure beyond dietary intake that results in weight loss. In view of this, workers can be directed to adjust their BMI ratio to the normal category by performing a healthier lifestyle, such as doing physical activity, consuming more vegetables and fruits, and checking their health regularly [23].

A long-term exposure of $PM_{2.5}$ forms a ROS accumulation that triggers the DNA signal to initiate the production of SOD enzymes, which leads to a lower level of SOD concentration than that in humans who are slightly exposed to $PM_{2.5}$. Long-term exposure of $PM_{2.5}$ with a ten times higher dose leads to a decrease in SOD and glutathione peroxide concentration and an increase in malondialdehyde concentration in blood [24].

Good technologies and management systems are the best ways to resolve the dust problem in stockpile, central mixer, conveyor, and workshop areas [25]. Relevant organizations should establish their hazard identification procedures and prepare a work plan by applying the hierarchy of controls.

CONCLUSION

There is a significant correlation between $PM_{2.5}$ exposure and SOD concentration in blood, so it is

necessary to take preventive actions in a ready-mix concrete plant to prevent diseases in workers. This research specifically deals with the issue of $PM_{2.5}$ pollution and SOD enzymes antioxidant in workers at a ready-mix concrete plant that can serve as an early detection signal for the workers.

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REFERENCES

1. BPS. (2010). Population census year 2010. Available from: http://sp2010.bps.go.id/
2. Azni IN. Wispriyono B. Sari M. Health risk analysis of PM_{10} exposure in ready-mix industrial workers PT. X, Plant Kebon Nanas, East Jakarta. J MKMI. 2015;203-209.
3. Novirsa R, Ahmadi UF. Risk analysis of $PM_{2.5}$ exposure in ambien air at noon towards community in Cement Industrial Estate. J Kesehatan Masyarakat Nasional. 2012;7(4):173-9.
4. Sierra-Vargas MP. Guzman-Grenfell AM. Blanco-Jimenez S. Sepulveda-Sanchez JD *et al*. Airborne particulate matter $PM_{2.5}$ from Mexico City affects the generation of Reactive Oxygen Species by blood neutrophils from asthmatics: an in vitro approach. Journal of Occupational Medicine and Toxicology. 2009;4-17:doi:10.1186/1745-6673-4-17
5. Gatot S. Content of elements in PM_{10} and $PM_{2,5}$ dust in cement factory, Bogor and in residence area by using X-Ray Fluorescence (XRF). Proceeding of the 6th National Seminar on Neutron and X-Ray Scattering. 2005;ISSN 1410-7686.
6. Halliwell B. Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br.J.Pharmacol.2004;142:231-255
7. Xing Y. Xu Y. Shi M. Lian Y. The impact of $PM_{2,5}$ on the human respiratory system. Journal of Thoracic Disease. 2015;8(1):E69-E74. doi:10.3978/j. issn.2072-1439.2016. 01.19
8. Ni L. Chuang C. Zuo L. Fine particulate matter in acute exacerbation of COPD. Frontiers in Physiology. 2015; 6: 294: doi:10.3389/fphys.2015.00294.
9. Birandra SK. Roles of free radicals in the toxicity of environmental pollutants and toxicants. Journal of Clinic Toxicology. 2013; 8:13. DOI: 10.4172/2161-0495.S13-e001
10. Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology. 2006;141:312–322.
11. Pizzino G, Irrera N, Cucinotta M *et al*. Oxidative stress: harms and benefits for human health. Oxidatice Medicine and Cellular Longevity. 2017; https://doi.org/10.1155/2017/8416763
12. Biswas SK. Rahman I. Environmental toxicity, redox signaling and lung inflammation : The role of glutathione. Mol Aspects Med. 2009;60-76. Colagar AH. Karimi F. Jorsaraei SGA. Correlation of sperm parameters with semen lipid peroxidation and total antioxidants levels in astheno- and oligoasheno-teratospermic men. Iranian Red Crescent Medical Journal. 2013;15(9):780-785.
13. Bajpai J. Prakash V. Kant S *et al*. Study of oxidative stress biomarkers in chronic obstructive pulmonary disease and their correlation with disease severity in North Indian Population Cohort. Indian Chest Society. 2018; doi:10.4103/lungindia. lungindia_205_16.
14. Podczasy JJ *et al.* Reduction of iodonitrotetrazolium Violet by Superoxide Radicals. Biochem Biophys Res Commun. 1988; PMID:2829896.
15. Haghighi AZ. Wei R. Measurement of superoxide dismutase in erythrocytes and whole blood using Iodinitrotetrazolium Violet. Journal Analytical Letters. 1998; 31(6): 981-990.
16. Cazacliu B. Ventura A. Technical and environmental effects of concrete production: dry batch versus central mixed plant. Journal of Cleaner Production. 2010;18(13):1320-1327.
17. Bolzana AD. Bianchia MS. Bianchia NO. Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: Influence of sex, age and cigarette smoking, Clinical Biochemistry. 1997;30(6):449-454.
18. Ling SH. Eeden SF. Particulate matter air pollution exposure: Role in the development and exercabation of Chronic Obstructive Pulmonary Disease. International Journal of Chronic Obstructive

Pulmonary Disease. 2009;4:233-243.

19. Domej W. Oetti K. Renner W. Oxidative stress and free radicals in COPD – implications and relevance for treatment. International Journal of COPD. 2014;9:1207-1224.
20. Rickman JC. Barrett DM. Bruhn CM. Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds. Journal of the Science of Food and Agriculture. 2007;87:930-944.
21. Miglio C. Chiavaro E. Visconti A. Fogliano V. Pellegrini N. Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. Journal Agricultural and Food Chemistry. 2008;56:139-147.
22. Ministry of Health. Buku Panduan Germas (Handbook of the Healthy Living Movement). 2017.
23. Lu X. Ye Z. Zheng S. Ren H. Zeng J *et al*. Long-term exposure of fine particulate matter causes hypertension by impaired renal D1 Receptor–Mediated Sodium excretion via upregulation of G-Protein-Coupled Receptor Kinase Type 4 Expression in Sprague-Dawley rats. Journal of the American Heart Association. 2018;7:e007185.
24. EPA. Environmental guidelines for the concrete batching industry. EPA Publication. 1998.

Association between $PM_{2.5}$ and Oxidative Stress Using Malondialdehyde Biomarker among Workers in a Concrete Batching Plant in 2018

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ABSTRACT

Background: Severe air pollution has affected the health of individuals both nationally and globally. The dangerous pollutant $PM_{2.5}$ could trigger oxidative damage, which has been widely studied in relation to health problems, such as lung cancer, chronic obstructive pulmonary, and cardiovascular diseases. Malondialdehyde (MDA), which is a secondary product of the lipid peroxidation of polyunsaturated fatty acids, is a marker of oxidative stress.

Objective: This study aims to analyze the association between $PM_{2.5}$ and MDA concentration in concrete batching plant workers as a population at risk of dust pollution.

Material & Method: This research was conducted in April–May 2018 with a cross-sectional method involving 53 samples of workers chosen by simple random sampling. Beside $PM_{2.5}$ and MDA concentration, other variables including smoking status, supplement consumption, age, physical activity, body mass index, years of work, and rotational shift working were also studied.

Results: The results indicated a significant correlation of $PM_{2.5}$ and age with MDA concentrations in concrete batching plant workers. P value between $PM_{2.5}$ and MDA concentration was 0.025 with OR 15,791 CI (1,410-176,833). These findings suggest that exposure to $PM_{2.5}$ leads to oxidative stress as evidenced by MDA concentration. These findings suggest that exposure to $PM_{2.5}$ leads to oxidative stress as evidenced by MDA concentration.

Keywords: *$PM_{2.5}$, oxidative stress, malondialdehyde (MDA), concrete batching plant, industry, age.*

INTRODUCTION

Ambient air quality is one of the major factors affecting human health at both the global and the national level. An estimated 72 percent of premature deaths are caused by ischemic heart disease, 14 percent by chronic obstructive pulmonary disease or lower respiratory tract infections, and 14 percent by lung cancer related to air pollution.[1] Compared to other pollutants, particulate meters (PM) are the most dangerous for human health. The toxicity characteristics of $PM_{2.5}$ are related to metal and organic components that are absorbed into the particles or constitute the $PM_{2.5}$ formers themselves as well as the biological components, sulfates, nitrates, acidity, and reactive gases absorbed by the surface of particles such as O_3.[2] Research has shown associations between health effects and $PM_{2.5}$-forming components, such as iron, nickel, zinc, ammonium nitrate, carbon elements, organic carbon, nitrates, and sulfates.[3]

Air pollution due to high $PM_{2.5}$ levels has been associated with increasing incidences of

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respiratory diseases, such as lung cancer, chronic obstructive pulmonary disease (COPD), and cardiovascular disease.[1, 4 – 7] In animal experiments, air pollution has been shown to induce oxidative stress. Epidemiological studies have also indicated an association between exposure to air pollution in urban areas and oxidative stress in bus drivers.[8] Malondialdehyde (MDA) is a secondary product of polyunsaturated fatty acid (PUFA) oxidation, which determines the degree of lipid peroxidation used as an oxidative stress biomarker.[6, 8, 9] Research showed an association between particulate dust levels and the incidence of oxidative stress in school students in China, as evidenced by MDA levels in urine. Another study also proved that MDA levels in children living in industrialized areas were higher than those of children living in non-industrial areas. Weiwei Lin et al. in Beijing identified associations between pollutant concentrations of CO_2, $PM_{2.5}$, SO_2, NO_2, and CH with MDA concentrations in the urine of schoolchildren.[4, 8, 10]

Research has shown that workers are the population at risk of COPD or cardiovascular diseases due to exposure to $PM_{2.5}$ in their working environment.[11–14] Concrete batching plants and ready mix plants produce concrete for large-scale construction. Concrete batching plants with poor settings and controls can pollute the surrounding environment with wastewater, dust, and noise.[15] This study aims to analyze the association between $PM_{2.5}$ exposure and oxidative stress using the MDA biomarker among workers in a concrete batching plant. In addition, this study also investigates other factors of MDA concentration variables including smoking status, supplement consumption, age, physical activity, body mass index, years of work, and rotational shift working.

MATERIALS AND METHOD

a. Study Design

This research was carried out in a concrete or ready mix batching plant in Jakarta, Indonesia with cross-sectional study on April 2018. A total of 53 workers were selected using simple random sampling and calculated based on the average difference hypothesis test formula.[16] The inclusion criteria were that each participant must be 18–55 years old, one who worked in the position as his or her main job, and a willing research respondent who signed an informed consent.

b. Sample Collection and Analysis

Blood samples were collected by certified phlebotomy nurses from a certified laboratory, using anti-coagulant EDTA in the batching plant before being carried to laboratory for analysis. The plasmas were then separated from other parts of blood and reacted using *thiobarbituric acid* (TBA) as a catalyst which brought in pink-color substance that could be read by spectrophotometry 532 nm wavelength.[17, 18]

c. $PM_{2.5}$ Exposure and Other Variables

The concentration of $PM_{2.5}$ was measured in the ambient air of the working environment. There are five air sampling points consisting of point 1 (stockpile), point 2 (central mixer and silo), point 3 (lab, workshop, and waste treatment), point 4 (gate and parking lot), and point 5 (the office). *High Volume Air Sampler* (HVAS) was used for the measurement of outdoor-based air samples and Haz Dust EPAM 5000 for indoor-based air samples. Calculation of $PM_{2.5}$ concentration was performed using the gravimetry method for HVAS and direct reading for Haz Dust EPAM 5000. Air sampling was carried out twice at every point for one hour on the day and one hour at night. Other factors of MDA concentration consisting of smoking status, supplement consumption, age, physical activity, body mass index, years of work, and rotational shift working were assessed using questionnaires.

d. **Statistical Analysis**

We analyzed the concentration of $PM_{2.5}$ in μg/m^3. The comparison of MDA concentration between workers who worked in the high and low levels of $PM_{2.5}$ was shown. The final model of analysis involving multivariate logistic regression and using SPSS 22nd version was conducted to understand the association between $PM_{2.5}$, MDA concentration and other factors consisting of smoking status, supplement consumption, age, physical activity, body mass index, years of work, and rotational shift.

RESULTS

Based on the respondents' distribution, the concentration of $PM_{2.5}$ was not normally distributed with the median value of 113.35 μg/m^3 and the standard

deviation of 30.27. The minimum value of $PM_{2.5}$ inhaled by respondents was 46.75 μg/m^3, while the maximum was 129.05 or 95%. 95% CI assumed that the concentration of $PM_{2.5}$ dust in the batching plant of PT X was 93.01–109.70 μg/m^3. The average concentration of MDA was 0.264 nmol/mL with the variation of 0.048 nmol/mL. The lowest concentration of MDA was 0.176 nmol/mL, while the highest was 0.351 nmol/mL. These results showed that 95% of respondents had MDA concentrations in blood of 0.251 to 0.277 nmol/mL (Table 1).

Table 1 Distribution of Respondents by $PM_{2.5}$ and MDA Concentration in Concrete Batching Plant

Variable	Average	Standard Deviation	Min-Max	95% CI
$PM_{2.5}$ Concentration	113.35 μg/m^3	30.27	46.75–129.05	93.01–109.70
MDA Concentration	0.264 nmol/mL	.048	0.176–0.351	0.251–0.277

For further analysis, $PM_{2.5}$ concentrations were categorized based on the Indonesian regulation concerning air pollution which sets the maximum concentration of $PM_{2.5}$ at 65 μg/m^3. MDA concentrations were to be categorized according to the mean into two groups consisting of below average and above average. MDA was below average if the concentration is 0–0.263 and above average if the concentration is 0.264 or more (Table 2).

Chi-square test was performed to see the relationship between MDA variables and various independent variables including $PM_{2.5}$ concentrations, smoking status, supplement consumption, age, physical activity, body mass index, years of work, and rotational shift. This analysis shows that only $PM_{2.5}$ concentration and age were significantly related to MDA concentration with p-value of under 0.05.

Table 2 MDA Concentration in Respondents by $PM_{2.5}$ Concentration and Age

Variables		MDA Concentration						OR	95% CI
		<0.264 nmol/ML		≥0.264 nmol/ML		Total			
		N	%	N	%	N	%		
$PM_{2.5}$ concentration									
	> 65 μg/m3	24	58.5	17	41.5	41	100	7.06	**1.37-36.40***
	≤ 65 μg/m^3	2	16.7	10	83.3	12	100		
Age									
	≥ 28 years old	22	57.9	16	42.1	38	100	3.78	**1.02-14.06***
	< 28 years old	4	26.7	11	73.3	15	100		

The last analysis was done in multivariate logistic regression. This modeling aims to validate the relationship of one major variable with the dependent variable by controlling some confounding variables.[19] It can be explained that workers who breathe $PM_{2.5}$ air at work points that do not meet the quality standard are 15.791 times more likely to have MDA concentrations above average after being controlled by age, body mass index, and work shift (Table 3).

Table 3 Multivariate Analysis

Variables	β	p-value	OR	95% CI	
				Lower	*Upper*
$PM_{2.5}$ concentration	2.759	0.025	15.791	1.410	176.833
Age	1.629	0.032	5.096	1.149	22.603
Shift rotation	-0.399	0,706	0.671	0.084	5.333
Body mass index	0.541	0,407	1.771	0.458	6.839
Constant	-3.355				

DISCUSSION

This study, whose design involves twofold measurement of $PM_{2.5}$, found a high level of $PM_{2.5}$. Referring to the Indonesian regulation concerning air pollution, the maximum concentration of $PM_{2.5}$ was set at 65 μg/m^3 for outdoor areas. There was a significant concentration difference between indoor-based and outdoor-based areas. Indoor-based concentration of $PM_{2.5}$ was below the maximum value. $PM_{2.5}$ concentration becomes the most determining factor in MDA concentration. However, the relationship between $PM_{2.5}$ and MDA concentration is also controlled by age.

Age is one of the factors associated with oxidative stress. Some studies with biomarkers like MDA, isoPGF2α, GPx, GSH-Px showed a significant association between age and concentration of biomarkers in either the blood or urine. [20-22] In line with the research by Bolzani et al., this study showed there is a significant relationship between MDA and respondents' age. The presence of higher MDA concentrations in individuals aged 28 years or more may be associated with a decline in SOD that is used as an antioxidant. Biologically, age is a complex concept that basically refers to the physiological capacity and physiological reserves of organs and systems within an individual. This physiological capacity decreases with age.[22] According to the Environment Protection Authority of Australia, several potential sources of air pollution from the concrete batching plant are the delivery of raw materials in trucks, trailers and tankers, storage of raw materials in bunkers and stockpiles, raw material transfer at the front end of loaders, conveyors, wagons, and agitators as well as leakage or spillage of raw materials from silos, inspection cover and channel wor.[23] Concrete raw material consisting of aggregate, cement, and fly ashes is the main cause of the high-level $PM_{2.5}$. A study by Ayse Orman et al. conducted at a cement plant showed that field workers exposed to cement dust directly had higher MDA concentrations than office workers.[24] Other studies also showed that $PM_{2.5}$ concentrations are related to MDA concentrations in the urine or in the blood both in children and in adults. [4, 8, 25, 26]

Higher MDA concentration in workers exposed to $PM_{2.5}$ may be due to the presence of metals which are the components of the $PM_{2.5}$ themselves in the batching plant. The metal compositions present in the particulates Cr, Co, Ni, Mn, Zn, V, Cu, and especially Fe and Aromatic Poly Hydrocarbons (PAHs) which are the main compositions of particulates and may lead to additive or synergetic interactions with the particle surface area. Either stand-alone or together, the chemical and physical characteristics of PAH and metals can produce reactive oxygen species (ROS) and oxidative stress.[27-29] Research suggests that silica particles derived from cement or fly ash in the batching plant can induce ROS that controls antioxidant defenses in the lungs and causes lipid peroxidation and cell damage. Among the different aldehydes that can be formed as a secondary product of fat destruction, Malondialdehyde (MDA) is one of the most mutagenic products of toxic fat peroxidation.[9] The higher the MDA in a person, the worse the oxidative stress is found in the person. This is related to the adverse health consequences for that person.[29]

CONCLUSION

This study proves that the concentration of $PM_{2.5}$ is the biggest risk factor associated with MDA concentration of workers in the concrete batching plant.

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REFERENCES

1. WHO. Ambient (Outdoor) Air Quality and Health [Internet]. World Health Organization: Fact Sheet. 2016 [cited 2018 Jan 1]. Available from: http://www.who.int/mediacentre/factsheets/fs313/en/.
2. Mazzoli-Rocha F, Fernandes S, Einicker-Lamas M, Zin WA. Roles of oxidative stress in signaling and inflammation induced by particulate matter. Cell Biol Toxicol. 2010;26(5):481–98.
3. Bell ML, Dominici F, Ebisu K, Zeger SL, Samet JM. Spatial and temporal variation in PM2.5 chemical composition in the United States for health effects studies. Environ Health Perspect. 2007;115(7):989–95.
4. Lin W, Zhu T, Xue T, Peng W, Brunekreef B, Gehring U, et al. Association between changes in exposure to air pollution and biomarkers of oxidative stress in children before and during the Beijing Olympics. Am J Epidemiol. 2015;181(8):575–83.
5. Tovalin H, Valverde M, Morandi MT, Blanco S, Whitehead L, Rojas E. DNA damage in outdoor workers occupationally exposed to environmental air pollutants. Occup Environ Med. 2006;63(4):230–6.
6. Valavanidis A, Vlachogianni T, Fiotakis K, Loridas S. Pulmonary oxidative stress, inflammation and cancer: Respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health. 2013;10(9):3886–907.
7. Wiebe S, England TN. Journal Medicine. English J. 2001;345(5):311–8.
8. Bae S, Pan XC, Kim SY, Park K, Kim YH, Kim H, et al. Exposures to particulate matter and polycyclic aromatic hydrocarbons and oxidative stress in schoolchildren. Environ Health Perspect. 2010;118(4):579–83.
9. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014.
10. Peluso M, Munnia A, Ceppi M, Giese RW, Catelan D, Rusconi F, et al. Malondialdehyde-deoxyguanosine and bulky DNA adducts in schoolchildren resident in the proximity of the Sarroch industrial estate on Sardinia Island, Italy. Mutagenesis. 2013;28(3):315–21.
11. Costello S, Brown DM, Noth EM, Cantley L, Slade MD, Tessier-Sherman B, et al. Incident ischemic heart disease and recent occupational exposure to particulate matter in an aluminum cohort. J Expo Sci Environ Epidemiol [Internet]. 2014;24(1):82–8. Available from: http://dx.doi.org/10.1038/jes.2013.47.
12. Han BC, Liu IJ, Chuang HC, Pan CH, Chuang KJ. Effect of welding fume on heart rate variability among workers with respirators in a shipyard. Sci Rep [Internet]. 2016;6:1–7. Available from: http://dx.doi.org/10.1038/srep34158.
13. Lai CY, Lai CH, Chuang HC, Pan CH, Yen CC, Lin WY, et al. Physicochemistry and cardiovascular toxicity of metal fume PM2.5: A study of human coronary artery endothelial cells and welding workers. Sci Rep [Internet]. 2016;6(August):1–12. Available from: http://dx.doi.org/10.1038/srep33515
14. Pan CH, Chan CC, Huang YL, Wu KY. Urinary 1-hydroxypyrene and malondialdehyde in male workers in Chinese restaurants. Occup Environ Med. 2008;65(11):732–5.
15. Mishra V. Indoor air pollution from biomass combustion and acute respiratory illness in preschool age children in Zimbabwe. Int J Epidemiol 2003;32(5):847–53.
16. Lemeshow S, Hosmer Jr DW, Klar J, Lwanga SK. Part 1: Statistical Methods for Sample Size Determination. Adequacy Sample Size Heal Stud [Internet]. 1990;247. Available from: http://apps.who.int/iris/bitstream/10665/41607/1/0471925179_eng.pdf?ua=1.

17. Wills ED. Mechanisms of lipid peroxide formation in animal tissues. 1966;667–76.

18. Grotto D, Santa Maria L, Valentini J, Paniz C, Schmitt G, Garcia SC, et al. Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. Quim Nova. 2009;32(1):169–74.

19. Hastono SP. Analisis Data pada Bidang Kesehatan. 1st ed. Jakarta: RAJAGRAFINDO PERSADA; 2016.

20. Babizhayev MA, Savel'yeva EL, Moskvina SN, Yegorov YE. Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. Am J Ther. 2011;18(6):209–26.

21. Kozakiewicz M, Kornelia K, Józef K. Markers of oxidative stress and erythrocyte antioxidant enzyme activity in older men and women with differing physical activity. 2013;48:1141–6.

22. Bolzán AD, Bianchi MS, Bianchi NO. Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: Influence of sex, age and cigarette smoking. Clin Biochem [Internet]. 1997;30(6):449–54. Available from: http://www.sciencedirect.com/science/article/pii/S0009912097000477.

23. Environmental Protection Authority, State Government of Victoria. Environmental Guidelines for the Concrete Batching Industry. Australia; 1998.

24. Orman A, Kahraman A, Çakar H, Ellidokuz H, Serteser M. Plasma malondialdehyde and erythrocyte glutathione levels in workers with cement dust-exposure silicosis. Toxicology. 2005;207(1):15–20.

25. Kim K, Park E-Y, Lee K-H, Park J-D, Kim Y-D, Hong Y-C. Differential oxidative stress response in young children and the elderly following exposure to PM2.5. Environ Health Prev Med [Internet]. 2009;14(1):60–6. Available from: http://link.springer.com/10.1007/s12199-008-0060-y.

26. Peluso M, Srivatanakul P, Munnia A, Jedpiyawongse A, Ceppi M, Sangrajrang S, et al. Malondialdehyde-deoxyguanosine adducts among workers of a Thai industrial estate and nearby residents. Environ Health Perspect. 2010;118(1):55–9.

27. Bernard SM, Samet JM, Grambsch A, Ebi KL, Romieu I. The potential impacts of climate variability and change on air pollution-related health effects in the United States. Environ Health Perspect. 2001;109 Suppl(May):199–209.

28. Kim KH, Kabir E, Kabir S. A review on the human health impact of airborne particulate matter. Environ Int [Internet]. 2015;74:136–43. Available from: http://dx.doi.org/10.1016/j.envint.2014.10.005.

29. Romieu I, Castro-Giner F, Kunzli N, Sunyer J. Air pollution, oxidative stress and dietary supplementation: A review. Eur Respir J. 2008;31(1):179–96.

Teachers as Promoters of Sex Education Among Adolescents with Mental Retardation

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ABSTRACT

Adolescents with mental retardation have the right to receive health information including sex education. This study aims to obtain information about the sex education practices implemented by teachers at Special School-C Dharma Asih in Depok 2018. The study used a qualitative approach with Rapid Assessment Procedure (RAP) design. The research participant consisted of eight teachers, the headmaster, parents of student, a health promotion officer at the Depok Jaya Primary Health Care, and staff at Regional 2 Education Branch Office of West Java Province. The results showed that sex education was provided classically and incidentally at Special School-C Dharma Asih. The teachers not only had adequate knowledge of sex education but also believed that sex education should be given to adolescent with mental retardation as early as possible. The constraints faced by the teacher included the lack of a sex education module and educational tools that support sex education in schools. Schools do not have specific policies related to sex education of adolescents with mental retardation. Cooperation between teachers, parents, primary health care workers, and education offices has not been optimal for encouraging sex education. This study suggests that the involvement of all stakeholders in the development of special policies related to sex education for adolescents with mental retardation should be encouraged.

Keywords : *Sexuality education, adolescents, mental retardation, teachers.*

INTRODUCTION

The prevalence of mild, moderate and severe mental retardation world wise is estimated to be 2%, 1.5% and 0.3%, respectively. In Indonesia, the prevalence of mental retardation is 13.68%, amounting to 290,837 people.[1]

Adolescents with mental retardation have a below-average intelligence level (IQ <70) that causes impairments in cognitive, language, motoric and social functions.[2,3] However, they have same sexual drive as adolescents with normal development.[4]

Teachers can be promoters of sex education for adolescents with mental retardation. However, a lack of self-confidence in teaching about sex and the perceptions of sex education as a taboo can hamper the provision of comprehensive sex education.[5,6]

No special curriculum for sex education exists in Indonesia. The provision of sex education in school is still integrated with several subjects, such as natural sciences, religion, citizenship, and physical, sports and health.[7,8] Sex education is also incidentally delivered because teachers do not have sex education-related modules or special guidelines.[9]

Training for teachers on sex education is still very limited, whereas training on pre-education can improve a teacher's initiative in providing sex education.[10,11]

Based on this background, this study aims to obtain extensive information on the sex education practices that have been carried out by teachers at the school for the disabled, Special School-C Dharma Asih, in Depok.

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MATERIALS AND METHOD

This qualitative study applied a Rapid Assessment Procedure (RAP) design at Special School-C Dharma Asih in Depok, in May-June 2018. Data collected through in-depth interviews and observation. The analysis method used was thematic analysis. Data validity was assessed by triangulation of the source, method, and data.

RESULTS

Knowledge

Results of study indicated that almost all teachers had adequate knowledge of sex education. Only one teacher considered sex education to be related to sexual intercourse.

Several aims of sex education were conveyed by teachers, such as protecting children from sexual abuse, guiding children based on norms, and enhancing children's skills of taking care of and maintaining their reproductive health.

The teachers were also aware that children with mental retardation enter the adolescent period at the age of 13-15 years. In female adolescents, some of the physical changes that occur are menstruation, breast growth and hip enlargement. Male adolescents, on the other hand, experience physical changes, such as wet dreams, voice change, moustache growth, and the emergence of Adam's apple. Children with mental retardation who begin to enter puberty often show aggressive behaviour at school, such as demonstrating emotions that are difficult to control, gazing at someone for a prolonged period, getting physically close to someone, poking, hugging Someone suddenly, and masturbating in public.

Perception

Almost all teachers thought that adolescents with mental retardation need sex education because parents do not necessarily provide sex education at home. Almost all teachers felt confident when delivering sex education.

Nevertheless, one teacher thought that sex education does not need to be delivered comprehensively because it is considered taboo and would encourage the adolescents to exhibit deviant behaviours.

Supporting Facilities

Supporting facilities are important for enhancing the effective provision of sex education for adolescents with mental retardation. The tools often used by teachers are pictures, both printed and digital, displayed through InFocus or accessed directly through mobile phones. In this study, one teacher taught certain sex education materials through direct demonstrations. This approach is considered to be more casual, allowing the children to feel more open and less shy.

The teachers did not have sex education modules available for use. All teachers thought that module provision is important for facilitating the systemic provision of sex education.

This study observed that Special School-C Dharma Asih had no supporting facilities like banner, posters or leaflets related to sex education. The walls in the school's hallway were only used to display announcements, documents concerning the school's activities, and some of the students' crafts.

Sex Education-Related Program and Policy

Sex education-related program and policy are used to guarantee the continuity of sex education practices at schools. Unfortunately, the Education Office, schools, and Depok Jaya Primary Health Care have not developed any sex education-related programs and policies. The Education Office only deals with general curriculum-related programs, while sex education is under the responsibility of Social Affairs Office. Schools do not provide special programs because sex education is already integrated in the instruction of several related subjects, such as religion, citizenship, the natural sciences, and physical, sports and health. The Depok Jaya Primary Health Care's reason for not implementing sex education programs is due to limited human resources, time, and funds.

Seminar and Training

Seminar and training are useful to improving the knowledge and skills of teachers in sex education. Some of the teachers said they had participated at a seminar on sex education in 2006 held by one of universities in Bandung. Other teachers said that they had not yet participated in sex education-related seminars and trainings. The teachers thought that the regular provision of trainings and seminars on sex education is required

to increase their insight into sex related issues among adolescents with mental retardation.

According to the information given by the Primary Health Care in Depok, there was in fact a routine training held by the Education Office for the teachers responsible for teaching the health-related curriculum. Unfortunately, Special School-C Dharma Asih has not yet received the curriculum materials. As the Primary Health Care Centre has not yet proposed that special school for the disabled should participate in the training.

Sex Education Practice

Sex education was classically and incidentally delivered by the teachers based on the needs. Classically delivered sex education is integrated in the teaching several subjects. By contrast, incidentally delivered sex education was conducted on a case-by-case basis when adolescents behaved in sexually deviant ways, such as suddenly hugging someone, watching porn or masturbating at school.

The contents often taught in sex education involved an introduction to sexual organs and functions, sexual abuse prevention, sex hygiene, reproductive rights and the ways to making friends with the same and opposite sex. One exemplary aim was to ensure that the adolescents understood that they should not be engaged in a romantic relationship during their education and that they may get married later after graduation and get a job.

The methods often applied by the teachers while providing sex education were lectures and personal briefing given to the children. The use of these methods was adjusted according to the children's needs. The lecture method was applied during class teaching, while the personal approach was applied when certain children exhibited deviant sexual behaviours.

Evaluation of the delivered materials was conducted by asking the adolescents about the information delivered and directly observing the changes in the adolescents' attitudes and behaviours. In addition, several of the teachers performed the evaluation by asking parents about the adolescents' behaviours at home.

DISCUSSION

Almost all teachers had adequate knowledge of sex education and the sexual development processes of adolescents with mental retardation. Sufficient knowledge will encourage a comprehensive sex education practice. Travers, Tincani, Whitby and Booutot stated that before delivering sex education, the caregiver should have a good knowledge of the definitions and objectives of sex education, the concept of human development, the child's personal ability, and sexual behaviours, and culture in the greater society.[12]

Nevertheless, there are still teachers who have misleading perceptions that sex education means the teaching of sexual intercourse. Sex education-related training for teachers at special schools for the disabled is still minimal. The results of this study showed that none of the teachers had participated in a sex education-related seminar or training within the last five years. This result was further corroborated by the statement of the officer at the Education Office that no sex education-related seminars or trainings for teachers at special schools for the disabled have been held by the office.

The lack of education and training for teachers at special schools for the disabled shows the low level of attention given by the government to the sex-related issues among adolescents with mental retardation. According to Nurse, training will improve the knowledge and ability to identify sexual problems, such as sexual abuse.[11] Byrne, Rietdijk and Pickett mentioned that training on pre-education for teachers can elevate the teachers' knowledge of sexual concepts, improve their pedagogical skills and enhance their comprehension of the methods of delivery.[10]

Despite the fact that the teachers consider sex education to be important for adolescents with mental retardation, no specific policy related to sex education in schools exists. Byrne, Rietdijk, and Pickett stated in their study that school policy is important for influencing the response and behaviour of teachers toward the practice of sex education.[10]

According to the United Nations Educational, Scientific and Cultural Organization, a teacher may experience some conflicts while delivering sex education. Some teachers may not be confident and may feel confused when handling sex-related issues. Therefore, teachers need guidelines that give a clear picture of what to teach and how to teach it.[13]

Sex education can be carried out classically, namely by integrating sex education materials into the teaching of several subjects. A study by Pakasi and Reni

showed that sex education-related materials in schools are provided especially in natural sciences, religious and physical, sports, and health classes.[5] Tjasmini discussed an integrated sex education model, in which self-development programs teach several skills, such as bathing, teeth brushing, vulva hygiene during menstruation, toilet use, getting dressed, communication, and makeup application.[14] A special curriculum for sex education is needed to make sex education more systematic.

Sex education can also be applied incidentally when adolescents show deviant sexual behaviors, such as suddenly hugging someone, watching porn videos or masturbating in class. According to Aziz, the teaching of sex education can be performed directly when the child is exhibiting a certain condition.[15]

The contents taught in sex education at the school under study included an introduction to sexual functions and organs, the prevention of sexual abuse, sex hygiene, reproductive rights and ways to establish relationships with friends of the same and opposite sex. These materials were quite comprehensive. However, according to Aziz, some topics have not been taught by teachers, such as risky sexual behaviours, sexually transmitted infections (STIs) and human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS), abortion and contraception,[15] even though they are also important for adolescents with mental retardation. Rosdami, Djaswadi and Sumarni asserted that the adolescents with less knowledge of reproductive health, STIs and HIV/AIDS, abortion, and contraception are susceptible to risky sexual behaviours.[16]

Sex education materials are delivered gradually based on the needs and the ability of adolescents in receiving information. Tutar Güven and İşler thought that sex education must be adjusted according to the ability level of the adolescents with mental retardation.[17] As it difficult for them to receive information, the sex education provided must be in an understandable language.

In practicing sex education, the teachers use instructional media in the form of high-quality printed images or digital images displayed through InFocus or directly accessed via tablets or mobile phones. The use of media is considered effective for enhancing the children's comprehension of the information provided. Psychologically, children with mental retardation prefer learning through visual media rather than written media.[15] In addition, Travers, Tincani, Whitby and Boutot pointed out that the use of media such as videos, images and diagrams, is effective for conveying sex-related information to children with special needs.[12] A study by Palupi revealed children's positive responses toward the development of animated videos for sex education, suggesting that children are more interested in watching animated videos of sex education than listening to lectures.[18]

To determine whether the sex-related information has been relayed successfully, teachers conduct an evaluation by directly asking the adolescents with mental retardation about the materials delivered via several questions. Teachers also observe any changes in the adolescents' attitudes and behaviours. Teachers even ask the parents about the adolescent's behaviour at home as part of evaluation.

Teachers consider the parents' involvement in sex education to be important, as the adolescents spend more time at home than at school. According to the International Planned Parenthood Federation, several of key players in the provision of sex education to adolescents with mental retardation include teachers, parents, peer educators, and service providers.[19]

The absence of collaboration between stakeholders is one of the barriers that schools face in the practice of sex education. Collaboration is needed to ensure the delivery of comprehensive sex education. The groups that can effectively encourage the practice of sex education are teachers, parents, health workers, and acedemicians.[20]

CONCLUSION

The teachers at Special School-C Dharma Asih had adequate knowledge of sex education. The teacher considered it important to provide sex education to adolescents with mental retardation as early as possible. The constraints faced by the teachers involved the lack of a sex education-related module and supporting facilities in the form of visual learning media to improve the practice of sex education at school. Schools need specific policies to encourage the sustainable practice of sex education. In addition, collaboration between teachers, the students' parents, the Primary Health Care Centre and the Education Office is needed to establish

the provision of comprehensive sex education.

Conflict of Interest: There was no conflict of interest of this study.

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Ethical Clearance: Ethical review was conducted in accordance with the procedures at the Faculty of Public Health of the Universitas Indonesia and was approved by the Health Research Ethics Committee of the Faculty of Public Health of the Universitas Indonesia

REFERENCES

1. Social Ministry of the Republic Indonesia. The social ministry in numbers: social welfare development. Jakarta: Social Ministry of the Republic Indonesia; 2012.
2. World Health Organization (WHO). The world health report 2001, mental health: new understanding, new hope. France: World Health Organization; 2001.
3. Ministry of Health of the Republic of Indonesia. Guidelines for child health services in special schools for health workers. Jakarta: Ministry of Health of the Republic of Indonesia; 2011.
4. Sarwono, S. Adolescent psychology. Jakarta: Rajawali Perss; 2013.
5. Pakasi, Reni. Between needs and taboos: sexuality and reproductive health education for adolescents in high school. Makara Health Series. 2013;17(2):79-87.
6. Rushbrooke E, Murray CD, Townsend S. What difficulties are experienced by caregivers in relation to the sexuality of people with intellectual disabilities? A qualitative meta-synthesis. Res Dev Disabil. 2014 Apr;35(4):871-86.
7. Ministry of Education. Guide to the implementation of special education curricula, special programs for self-education in junior high schools, the usual range of mild mental retardation. Jakarta: Directorate of Special School Development of the Ministry of National Education; 2016.
8. Ministry of Education and Culture. At a glance the implementation of national education in 2013/2014. Jakarta: Center for Education and Culture Data and Statistics; 2015.
9. Jannah, SN. Teachers' perceptions of the development and sexual education of autistic children of adolescents in exceptionally noble, autonomous schools in Yogyakarta. Orthodactic Widia Journal. 2016;5(8):796-805.
10. Byrne J, Rietdijk W, Pickett K. Teachers as health promoters: factors that influence early career teachers to engage with health and wellbeing education. Teaching and Teacher Education. 2018 Jan;69;289-99.
11. Nurse AM. Coaches and child sexual abuse prevention training: impact on knowledge, confidence, and behavior. Children and Youth Services Review. 2018 May;88:395-400.
12. Travers J, Tincani M, Whitby PS, Boutot EA. Aligment of sexuality education with self determination for people with significant disabilities: a review of research and future directions. Education and Training in Autism and Developmental Disabilities. 2014 Jun;49(2):232-47.
13. United Nations Educational, Scientific and Cultural Organization (UNESCO). Revised edition: international technical guidance on sexuality education, an evidence-informed approach. Paris: United Nations Educational, Scientific and Cultural Organization; 2018.
14. Tjasmini M. Self-development learning in helping to understand reproductive health in mentally retarded girls. Edutech. 2014;1(2).
15. Aziz S. Sex education for children with special needs. Yogyakarta: Gava Media Publisher; 2015.
16. Rosdami, Djaswadi, Sumarni. Effect of personal factors on premarital sexual behavior in adolescents. National Public Health Journal. 2015;9(3):215-21.
17. Tutar Güven S, İşler A. Sex education and its importance in children with intellectual disabilities. Journal of Psychiatric Nursing. 2015 Jan;6(3):143-8.
18. Palupi P. Development of an animated video sex education media for early childhood to prevent sexual violence in children in play group budas rimba purwokerto. E-journal of Education Technology

Study Program. 2017;6(7):712-22.

19. International Planned Parenthood Federation (IPPF). IPPF framework for comprehensive sexuality education (CSE). London: International Planned Parenthood Federation Adolescents Team; 2010.

20. Ivankovich MB, Fenton KA, Douglas JM Jr. Considerations for national public health leadership in advancing sexual health. Public Health Rep. 2013 Mar-Apr;128 Suppl 1:102-10.

Improper Use of Inhaler Technique in the Control of Asthma in Adult

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ABSTRACT

Background: Asthma is a chronic inflammatory disease of the airways in which they are involved a lot of cells and elements cellular and that in susceptible individuals causes recurrent episodes of wheezing, shortness of breath when breathing, feeling tightness of chest and cough. **Aim of the study:** To assess the asthmatic patient's inhaler use technique, any error if present. **Patients and method:** A cross sectional study conducted in consulting respiratory unit in Baghdad teaching hospital from the 1st of July 2017 to the end of February 2018. **Results:** Device preparing errors was present in 47.2% of asthmatic patients, failure to exhale before inhalation was present in 80.2% of patients, exhalation during inhalation was present in 10% of patients, short inhalation time was present in 51.7% of patients, not rinsing mouth with water after inhalation was present in 85.5% of patients and repetition more than recommended was present in 22.8%. **Conclusions:** There is error in using the asthmatic inhalers treatment technique.

Keyword: *Asthma, inhaler, device*

INTRODUCTION

The concept of asthma has been evolutionary. Until 1975 it is considered that asthma is produced by a contraction of the bronchi; With the advent of fibrobronchoscopy and bronchoalveolar lavage, it was discovered that there is also chronic inflammation of the airway, which generates the symptoms; later, the progress in the techniques of bronchial biopsy with which it will be recognized and created in the 1990s, in the sense that the disease is reversible. Remodeling of the airway secondary to chronic repetitive inflammation, which explains that over time, the uncontrolled asthmatic patient has persistent obstruction.(1)

Due to its multifactorial etiology, respiratory functional alterations and its diverse clinical expressions, according to the age of presentation, there is no definition that completely describes this entity. Therefore, defining asthma is complex, since it is a multifactorial disease (etiology, morphological characteristics, functional changes and clinical manifestations), in which there is an interaction between the immune system and the autonomic nervous system. However, the most complete definition is that which takes into account both the functional and the cellular point of view, that is why the definition that best adapts is that proposed by the Global Initiative for Asthma (GINA 2006), (2) that proposes asthma as a chronic inflammatory disease of the airways, in which many cells and cell products play an important role. Chronic inflammation produces an increase in airway reactivity and recurrent episodes of wheezing, respiratory distress, intercostal retractions, coughing and chest tightness, especially at night and in the morning.(3) These episodes are associated with airflow obstruction, reversible spontaneously or with treatment. Inflammation also causes an increase in the bronchial response to a wide variety of stimuli. Thus, in all cases it is emphasized that asthma is a chronic inflammatory alteration of the airways, which can be associated in an acute or chronic way. with airflow limitation as a result of bronchoconstriction, edema, mucous secretion, inflammation and, in some patients, remodeling of the airways. The remodeling of the airway corresponds to those structural changes characterized by thickening of the reticular lamina with deposits of subepithelial and perivascular fibrin, hyperplasia of the mucous glands, smooth muscle and vascular. This determines the thickening of the wall of

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the bronchi of greater conduction (accessible to their study by mucosal biopsy) and also in the small airway (accessible by bronchoalveolar lavage). [4]

Epidemiology

The prevalence of asthma varies according to the geographical area, climate, lifestyle and economic development of each region. [5] The World Health Organization (WHO) estimates that it affects 300 million people and even today It is the cause of death worldwide, estimating 250 000 asthma deaths per year. [2] In the last four decades, the prevalence increased significantly, mainly in industrialized cities. [6] However, in the last 10 years prevalence has been established. plateau, where despite remaining high, no increases have been reported.

Epidemiological studies are being carried out throughout the world, predominantly cross-sectional, in order to determine the prevalence of asthma and its tendency. Thus, in 1991, the International Study of Asthma and Allergies in Childhood (ISAAC = International Study of Asthma and Allergies in Children) was created, together with the European Community Respiratory Health Survey (ECR-HS = European Community of Respiratory Health), Studying young adult population, have determined that countries such as United States of America, Canada, United Kingdom, New Zealand and Australia have higher prevalences, while the lowest are in China, India, Ethiopia, Indonesia and Eastern Europe. The ISAAC and the ECRHS show a great variability in the spatial distribution of asthma. Epidemiological studies in Latin America have revealed differences in the prevalence of asthma, with figures from 5.7 to 16.5% in the pediatric population. [7]

Industrialized countries and urban areas are among risk factors of the disease. [8] The current reported prevalence in the Middle East region is somewhat lower, varying between 5.6% in Saudi Arabia and 8.5% in Kuwait. In Iraq, approximately 200,000 patients per year with asthma are either hospitalized or treated in an Emergency Room. [8]

Etiology and classification

Attempts have been made to classify asthma according to its causes, intensity and obstruction of the airway valued by the maximum expiratory volume at the end of the first second (FEV1) or peak expiratory peak (PEF) and its response to management. This is not appropriate, since asthma is multifactorial and both genetic and environmental factors contribute.Previously, asthma was classified as extrinsic, which had an allergic component, and in intrinsic asthma, which represented a group of patients where there was no justified cause and with negative allergy laboratory tests. Asthma can be classified by etiological factors, severity or type of airflow limitation. Given that it is a heterogeneous disease, there are multiple causal factors both for its induction and for its exacerbation, from viral infections in childhood to occupational exposure in adults. Severity is estimated by a combined evaluation of symptoms, amount of beta two bronchodilator agonists (β2) to control symptoms and lung function. [9]

Classification based on severity

In this type a series of variables are grouped which include:

a) The frequency of symptoms during the day and night,

b) Its repercussion in the daily activities carried out during the day and night,

c) The use, number of times and the dose in which β2 agonist is used to treat the symptoms and

d) Lung function.

This classification is useful, since it relates the inflammation of the airway with the clinic and with the indexes of severity of symptoms according to the intensity of the asthma. In addition, it links the clinical data with the degree of obstruction of the airway and the variability of the Para clinical parameters that measure this obstruction (FEV 1 or PEF). In this way, chronic asthma has been subdivided into: mild intermittent, mild persistent, moderate persistent and severe persistent. The advantage of this type of classification is that in a practical way it allows to start treatment based on the severity and establish, later, a treatment plan according to the control and stabilization of symptoms. It has been proposed by the National Program of Education for Asthma (NAEPP). Thus, the severity of patients with asthma can be classified in one of these four steps before treatment and during the monitoring of it. If the asthma control is maintained, the degree of asthma is reclassified and another management is installed according to the intensity. [10]

METHOD

Study design and study setting:

A cross sectional study conducted in consulting respiratory unit in Baghdad teaching hospital from the 1st of July 2017 to the end of February 2018.

Patients:

A total of 400 adults with persistent asthma for at least 6 months duration, and agreeing to the Global Initiative for Asthma criteria, the patients within the age ≥18 years old and who had received at least one dose of inhaled corticosteroids and long-acting beta-2 agonists, either in separate or fixed combinations, irrespective of pharmacologic agent(s).

Data Collection

The data were collected by using a specially designed questionnaire form. At first socio-characteristics (age, on age, sex, education, occupation, current therapies, outcome measures of asthmatic patient's treatment and inhaler technique) of the patients were collected

Statistical Analysis

The analyses were conducted with statistical package for social science IBM-software (SPSS 22). Data are expressed as mean ± SD or number and percent.

Results:

A total of 400 adult asthmatic patients with mean age of 42.7±13.3 years.The inhaler types constituted MDI with spacer (2.5%), MDI (79%), Diskus (0.8%), Turbuhaler (5.2%), MDI & Turbuhaler (11.5%) and MDI & Diskus (1%). (Figure 1)

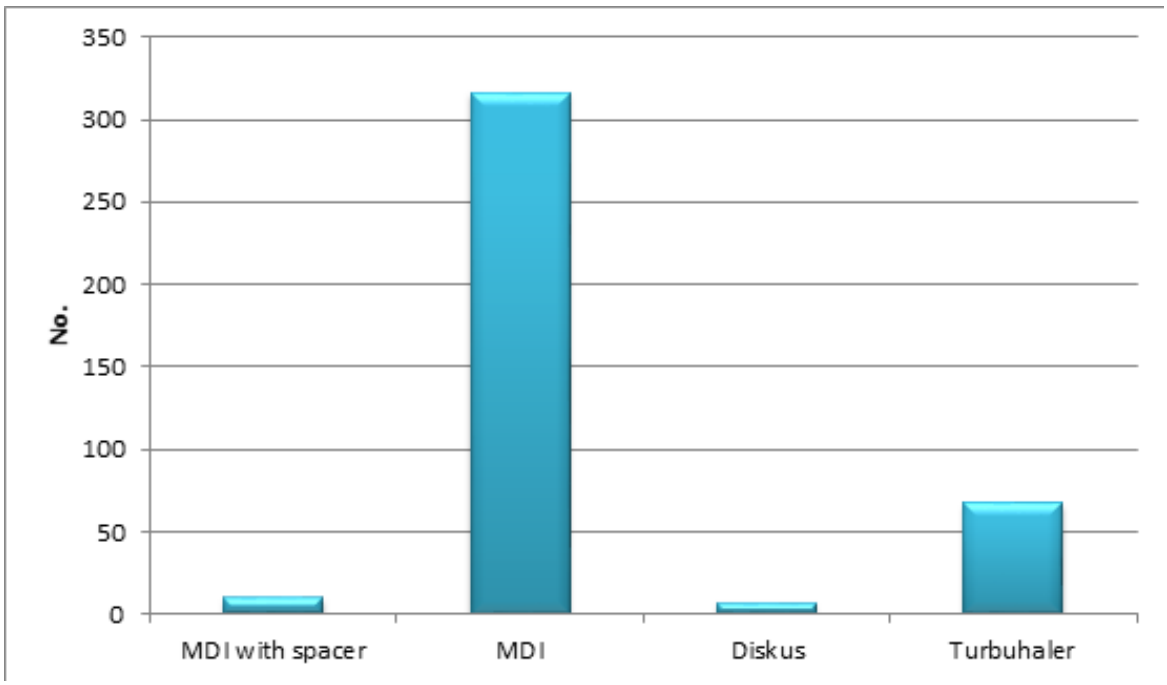


Figure 1: General types of inhalers.

Device preparing errors was present in 47.2% of asthmatic patients, failure to exhale before inhalation was present in 80.2% of patients, exhalation during inhalation was present in 10% of patients, short inhalation time was present in 51.7% of patients, not rinsing mouth with water after inhalation was present in 85.5% of patients and repetition more than recommended was present in 22.8% of asthmatic patients. (Table 1)

There was a highly significant association between daytime symptoms > 2/weeks and Diskus inhaler type ($p<0.001$). A highly significant association was observed between bronchodilator need and MDI with spacer type ($p<0.001$). There was a highly significant association between night waking and Diskus inhaler type ($p<0.001$). A highly significant association was observed between activity limitation and MDI type ($p<0.001$). There was a highly significant association between well controlled

onset and MDI with spacer type (p<0.001). (Table 2)

Table 1: Main errors when patients used inhaler.

Variable	No.	%
Device preparing errors		
Yes	189	47.2
No	211	52.8
Total	400	100.0
Failure to exhale before inhalation		
Yes	321	80.2
No	79	19.8
Total	400	100.0
Exhalation during inhalation		
Yes	40	10.0
No	360	90.0
Total	400	100.0
Short inhalation time		
Yes	207	51.7
No	193	48.3
Total	400	100.0
Failure of the patients to hold breath for 5-10 seconds		
Yes	272	68.0
No	128	32.0
Total	400	100.0
Do not rinse mouth with water after inhalation		
Yes	342	85.5
No	58	14.5
Total	400	100.0
Repetition more than recommended		
Yes	91	22.8
No	309	77.2
Total	400	100.0

Table 2: Distribution of clinical characteristics according to inhaler types.

Variable	MDI & spacer		MDI		Diskus		Turbuhaler		P
	No.	%	No.	%	No.	%	No.	%	
Daytime symptoms more than twice a week									
Yes	0	-	313	99.1	7	100.0	49	73.1	<0.001* S
No	10	100.0	3	0.9	0	-	18	26.9	
Bronchodilator need more than twice a week									
Yes	10	100.0	306	96.8	7	100.0	53	79.1	<0.001* S
No	0	-	10	3.2	0	-	14	20.9	
Night waking due to asthma									
Yes	0	-	175	55.4	7	100.0	10	14.9	<0.001* S
No	10	100.0	141	44.6	0	-	57	85.1	
Activity limitation due to asthma symptoms									
Yes	0	-	194	61.4	4	57.1	20	29.9	<0.001* S
No	10	100.0	122	38.6	3	42.9	47	70.1	
Score									
Well controlled	10	100.0	3	.9	0	-	19	28.4	<0.001* S
Partially controlled	0	-	85	26.9	0	-	36	53.7	
Non controlled	0	-	228	72.2	7	100.0	12	17.9	

**Fishers exact test, S=Significant.*

Highly significant association between device preparing errors and MDI inhaler type (p<0.001), between short inhalation time and Diskus inhaler type (p<0.001), Failure to hold breath for 5-10 seconds among patients used MDI inhaler type (p<0.001), Failure to exhale before inhalation was significantly higher among patients used MDI inhaler type (p<0.001). Exhalation during inhalation was significantly higher among patients used MDI inhaler type (p=0.008). (Table 3)

Table 3: Distribution of outcome measures according to inhaler types.

Variable	MDI & spacer		MDI		Diskus		Turbuhaler		P
	No.	%	No.	%	No.	%	No.	%	
Devise preparing errors									
Yes	0	-	170	53.8	0	-	19	28.4	<0.001* S
No	10	100.0	146	46.2	7	100.0	48	71.6	
Failure to exhale before inhalation									
Yes	0	-	287	90.8	3	42.9	31	46.3	<0.001* S
No	10	100.0	29	9.2	4	57.1	36	53.7	

Cont... **Table 3: Distribution of outcome measures according to inhaler types.**

Exhalation during inhalation									
Yes	0	-	40	12.7	0	-	0	-	**0.008*** S
No	10	100.0	276	87.3	7	100.0	67	100.0	
Short inhalation time									
Yes	0	-	178	56.3	4	57.1	25	37.3	**<0.001*** S
No	10	100.0	138	43.7	3	42.9	42	62.7	
Failure to hold breath for 5-10 seconds									
Yes	0	-	247	78.2	3	42.9	22	32.8	**<0.001*** S
No	10	100.0	69	21.8	4	57.1	45	67.2	
Do not rinse mouth with water after inhalation									
Yes	10	100.0	271	85.8	7	100.0	54	80.6	0.2* NS
No	0	-	45	14.2	0	-	13	19.4	
Repetition more than recommended									
Yes	0	-	84	26.6	0	-	7	10.4	**0.008*** S
No	10	100.0	232	73.4	7	100.0	60	89.6	

**Fishers exact test, S=Significant, NS=Not significant.*

DISCUSSION

In the current study the main inhaler used was MDI with mainly used error than other types, and this type lead to critical error which is mentioned by Price D et al, [11] as fallowing (Failure to remove the cap, not holding the inhaler upright, actuation not corresponding to inhalation; actuation before inhalation, actuation not corresponding to inhalation; actuation is too late (Puff 1), failure to actuate, failure to inhale, inhale too fast, inhalation through the nose). Westerik JA et al, [12] found that errors in Diskus inhaler technique are common among patients with asthma, with over half of patients in this study making ≥1 serious errors. [13, 14]. Before inhalation, do not rinse mouth with water after inhalation and Failure to hold breath after inhalation)

Which is in agreement with Westerik JA et al,[12] when the two errors most frequently reported in our study (failure to exhale before inhalation and insufficient [or absent] breath-hold at the end of inhalation) are in agreement with previously published reports of the most frequently observed errors with the Diskus being ‘‘no exhalation before inhalation’’ and ‘‘no breath holding after inhalation’’. But the third most common error in former study (inhalation that was not forceful from the start), which in agreement with that also been reported by other previous studies [15-17].

CONCLUSION

There is error in using the asthmatic inhalers treatment technique and the most common error were do not rinse mouth with water after inhalation then failure to exhale before inhalation.

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REFERENCES

1. Jover Lopez E . Asma bronquial I: Epidemiología, Patogenia, Definición. Medwave 2007;7 (1):e1901
2. The Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention; 2006.
3. Global Initiative for asthma. Pocket guide for asthma management and prevention (for adults and

children older than 5 years). uptodate; 2015.

4. Palacios VM, Zavalu VM. Algunas expectaciones acerca del asma. Dominio de las Ciencias. 2018;4(1):17-27.
5. Liu A, Szefler S. Advances in childhood asthma: Hygiene hypothesis, natural history and management. J Allergy Clin Immunol. 2003; 111: 787-92.
6. Braman SS. The global burden of asthma. Chest. 2006; 30 Suppl 1: 45-125.
7. Mallol J, Solé D, Asher I, et al. Prevalence of asthma symptoms in Latin America: the International Study of Asthma and Allergies in Childhood (ISAAC). Pediatric pulmonology. 2000 Dec;30(6):439-44.
8. MOH-Iraq/ National guidelines for health care physicians. Guidelines for the Diagnosis and Management of Asthma, Directorate of Public Health Non-Communicable Disease Section Iraq, July 2012.
9. Lemanske RF Jr, Busse WW. Asthma. J Allergy Clin Immunol. 2003; 111: S502-S19
10. Busse WW, Lemanske RF Jr. Asthma. N Engl J Med. 2001; 344: 350-62.
11. Price D, Bosnic-Anticevich S, Briggs A, et al. Inhaler Error Steering Committee. Inhaler competence in asthma: common errors, barriers to use and recommended solutions. Respiratory medicine. 2013 Jan 1;107(1):37-46.
12. Westerik JA, Carter V, Chrystyn H, et al. Characteristics of patients making serious inhaler errors with a dry powder inhaler and association with asthma-related events in a primary care setting. Journal of Asthma. 2016 Mar 15;53(3):321-9.
13. Haughney J, Price D, Barnes NC, et al. Choosing inhaler devices for people with asthma: current knowledge and outstanding research needs. Respir Med 2010;104: 1237–1245.
14. Papi A, Haughney J, Virchow JC, et al. Inhaler devices for asthma: a call for action in a neglected field. Eur Respir J 2011;37:982–85.
15. Melani AS, Zanchetta D, Barbato N, et al. Inhalation technique and variables associated with misuse of conventional metered-dose inhalers and newer dry powder inhalers in experienced adults. Ann Allergy Asthma Immunol 2004;93:439–446.
16. Lavorini F, Magnan A, Dubus JC, et al. Effect of incorrect use of dry powder inhalers on management of patients with asthma and COPD. Respir Med 2008;102:593–604.
17. Lee SM, Chang YS, Kim CW, et al. Skills in handling turbuhaler, diskus, and pressurized metered-dose inhaler in korean asthmatic patients. Allergy Asthma Immunol Res 2011;3:46–52.

Ecological Studies of Certain Aphid Species and their Associated Predators on Wheat Plants at Qadisiyah Distract, Iraq

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ABSTRACT

Background: Studies were carried out at Qadisiyah district, Iraq to study certain aphid species infestation and their associated predators on wheat plants during season 2016-2017. Methods: Survey and population density of piercing sucking pests and their predators on wheat plants were investigated. Results: The dominant aphids were wheat, *Schizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius), *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* (Linnaeus) and *Diuraphis noxia* (Mordvilko). Whereas, the prevailing associated predators were *Coccinella undecimpunctata*(L.), *Coccinella septempunctata*(L.), *Chrysoperla carnea* (Steph.) *Metasyrphus corollae* F., and a few number of true spiders. Obtained results showed that The infestation with *S. graminum* was much higher wheat plantations than other aphids in 2016-2017 season . *Schizaphis graminum* had three peaks of activity were recorded on the last week of January, March and April (455.1,3250.7 and 907.6 individuals/100 tillers, resp.) in 2016-2017 season. Meanwhile, *S. avenae* had two peaks of activity were recorded on the last week of, March and April (2010.9 and 570.2 individuals/100 tillers, resp.).Also, *C. septempunctata* had two peaks of activity were recorded on the last week of, March and Second week of May (35.7 and 25.0 individuals/100 tillers, resp.) on the same period of study.

Keywords: *Cereal aphid, Predators, wheat.*

INTRODUCTION

Aphid are one of the insect groups whose economic importance increases with the development of agriculture[1,2]. Cereal aphid are the serious pests attacking cereal crops, particularly wheat , barley and corn not only in Iraq[3,4] but also in many other countries ; i.e. Egypt , Souther Russia, Iran, Afghanistan, and countries bordering the Mediterranean Sea[2,5]. The species that are capable of causing substantial yield reductions are *Schizaphis graminum* (Rondan), bird cherry oat aphid , *Rhopalosiphum Padi* L., *R. maidis.* F., *Sitibion avenae* F. and *Diuraphis noxia*[2,6]. The use of insecticides in controlling aphids generally, leads to many problems, not only increasing resistant strains of aphids to these chemical substances, but also in induction of pollution to man and beneficial insects such as bees and other pollinators, insect parasitoids and predators[7,8]. Numerous publications documented the supperession of cereal aphid population by natural enemies[2,9,10]. Therefore , this investigation aims to study the role of aphids phagous insects in reducing aphid population on wheat crop in Qadisiyah district, Iraq. Study the effect temperature and relative humidity on the population density of wheat aphid and associated predators on field.

MATERIALS AND METHOD

The present investigation was carried out at fields of Qadisiyah district. Iraq during 2016- 2017 season.

Survey and Population Density of Piercing Sucking Pests and Their Predators on wheat Plants: Surveying of major piercing sucking pests and their predators took place during successive growing season 2016-2017 on wheat plant. The cultivated area was one donum(2500sq.m) ,the sowing date was in the 1st November in 2016-2017 season. Sapling started after about three weeks from planting and continued to the harvesting time. The sample size was 100 wheat tillers and chosed randomly from plants. Direct count of adults

of injurious insects and predators on wheat plant was undertaken. Since the predators under investigation differed in their living habits, activities, and distributions on the host plants infested with different prey and host insects the it was necessary to use one sampling method for all groups such as leaves sampling plant method in order to explain the relationship between pests and their associated predators. Sample of 100 tillers of wheat plants were biweekly in some few cases, picked up randomly and placed in paper bags to be examined carefully under microscope. The number of insect pestes and predators (immature stages and adults) in most cases were directly counted. The collected specimens were kept in vials containing 75% ethyl alcohol with some drops of glycerin to keep their tissues soft, and labeled for date. Identification according to key of Aphididae, described by (Helmi, 2011) and department of classification in plant protection Research Institute, Agriculure Research Center, Egypt.

Study the Effect of Certain weather factors of the Sucking Pests (Aphid species) and Their Predator on Wheat: Daily recorded of minimum temperature, maximum temperature and relative humidity were obtained from Agrometerological station at AL Qadisiyah region during the period of study. The relationship between the biweekly mean number of aphids and predators individuals and the corresponding biweekly means of the previously mentioned weather factors were statistically analyzed. The simple correlation coefficient between weather parameters and the number of pests and their associated predators were studied according to[11,12].

RESULTS AND DISCUSSION

Survey of certain aphid species and their associated predators in wheat plant: The seasonal abundance of the dominant aphid species infesting wheat plants were Green cereal- bug aphid *Schizaphis graminum* (Rondani), English grain aphid *Sitobion avenae* (Fabricius), Coron leaf aphid *Rhopalosiphum maidis*(Fitch), Bird cherry oat aphid *Rhopalosiphum padi* (Linnaeus) and Russian wheat aphid *Diuraphis noxia* (Mordvilko). As seen from Table 1. the percentage in fertation of *R. padi*, *R. maidis*, *S. avenae*, *S. graminum* ,and *D. noxia* were 14.77%, 12.48%, 26.91%, 41.63 and 4.21%, respectively during season 2016- 2017. Similar result were obtained by Alhag *et al* ., 1996 and Abd el – Aziz 2014, who mentioned that five species of cereal aphid found infesting wheat (*R. padi, R. maidis, S. graminum, S. avenae and D. noxia*). Meanwhile, the dominant aphid species were *Coccinella undecimpunctata* (L.), *Coccinella septempunctata* (L.), *Chrysoperla carnea* (Stephens) and *Metasyrphus corollae* (Fab.). The percentage in fertation of *C. undecimpunctata* , *C. septempunctata* , *C. carnea* and *M. corallae* were 25.98%, 38.93%, 20.85%, and 14.24%, respectively during season 2016-2017 (Table 2) . These result agree with Abd El- Aziz 2014, who surveyed that *C. undecimpunctata* , *C. carnea* and *M. corollae* on cereal aphid in wheat plant.

Table 1. Number of aphid insect species and their percentages to total catch related to their orders at wheat plants at Qadisiyah district in the growing season 2016 - 2017.

Aphid species	**2016- 2017**	
Order Homoptera	**Total individuals**	**%**
R. padi	4252.95	14.77
R. maidis	3592.2	12.48
S. avenae	7746.6	26.91
S. graminum	11985.76	41.63
D. noxia	1211.6	4.21
total	28789.11	100

Table 2. Number of beneficial insect species and their percntages to total catch related to their orders in wheat fields at Qadisiyah district in the growing season 2016 - 2017.

Beneficial insect species	**2016- 2017**	
Order Coleoptera	**Total individuals**	**%**
C. undecimpunctata	119.5	25.98
C. septempunctata	179.1	38.93
Order: Neuroptera *C. carnea*	95.9	20.85
Order : Diptera *M. corolla*	65.5	14.24
Total	460	100

Population density of certain aphid species infested wheat plants during the period of study: Five species of aphids were recorded during 2016-2017 season these aphid species were *S. graminum*, *S. avenae*

R. padi, R. maidis, and *D. noxia*.

A.1.*Schizaphis graminum:* Data in (figer1) showed initial number of *S. graminum* occurred in last week of November with a total number of 15.6 nymph/ 100 tiller. Three peaks of activity were recorded at last week of January, March and April (455.1 , 3250.7 and 907.6 individuals).

A.2.*Sitobion avenae:* *S. avenae* began to appear on wheat in 4th week of November. Two peaks of activity were recorded at 4th week of March and April (2010.9 and 570.2 individuals). (Fig 1)

A.3. *Rhopalosiphum maidis* and *R. padi*: Data fig 1 showed initial number of *R. maidis* and *R. padi* occurred in 4th week of November with a total number of (3.7 and 5.3 individuals). One peaks of activity was recorded at last week of February (960.6 and 1050.7 individuals) for *R. maidis* and *R. padi* respectively.

A.4. *Diuraphis noxia*: Numbers of *D. noxia* associated with wheat plant during 2016-2017 season are tabulated in (Fig 1) initial number were stated on last week of December(10.2 individuals / 100 tillers) and gradually increased to reach the maximum (310.2 individuals / 100 tillers) .On the other hand Darwish and Ali ., 1991 found the aphids *S. graminum* and *R. maidis* reached their maximum abundance (406 and 518 / plant) in upper Egypt. These results in agreement with those of Slman., 2006 and Sabbour., 2007 in Egypt, they found that the wheat plants were infested by four cereal aphid, *R. padi, R. maidis, S. graminum*, and *S. avenae.* They reported also that, the population of aphid peaked on fourth week of March. and coincided with the highest number of *C. undecimpunctata.* Tabasum *et al.,* 2012 observed a maximum aphid population on wheat during February 24 and then a decline observed up to march 8. again on march 8 up to march 17.

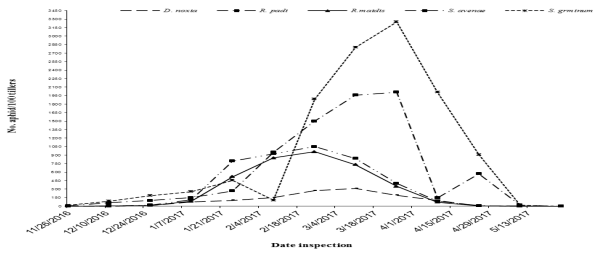


Fig. 1. Seasonal abundances of certain aphid species on wheat plants in 2016 /2017 season at Qadisiyah district.

Seasonal Abundance of Predators Associated with Certain Aphid Species infesting wheat at Qadisiyah District: Catches of predatory species revealed the following species *Coccinella undecimpunctata*, *Coccinella septempunctata*, *Chrysoperla carnea* and *Metasyrphus corollae* and few number of true spiders.

***B.1 .C. septempunctata*:** The *C. septempunctata* began to appear on wheat plants at low density during second week of December (1.2 individuals). Two peaks of activity were attained on 4th of March and 2nd of May (35.7 and 25.0 individuals).

***B.2.C. undecimpunctata*:** The number of *C. undecimpunctata*, took place in the 2nd week of December (1.5 individuals). Its increased to reach their maximum in 4th week of March (31.5 individuals) .

***B.3.C. carnea* :** The *C. carnea*, began to appear on wheat plants at low density during 2nd of December(1.3 individuals). It's increased to reach their maximum in 4th week of March (20.2 individuals) .

B.4. *M. corolla:* Initial number of *M. corollae* , were stated on last week of December(1.5 individuals / 100 tillers) and gradually increased to reach their maximum (15.3 individuals). However , El- Heneidy and Rizk,2004, Slman and Ahmed, 2005 and Yigit *et al* , 2007 , recorded that *C. undecimpunctata*, and *C. carnea* the most specific aphid predator of cereal aphid in wheat fields. Also, Samad, 2004 and El- Heneidy and Rizk, 2004 , recoded that *Metasyrphus corollae* in the third predators position after *C. undecimpunctata*, and *C. carnea.*

Fig. 2. Seasonal abundances of certain predators species on wheat plants in season 2016 /2017 at Qadisiyah district.

Effect of wheather factros on the population density of certain aphid species and their predators on wheat plants

1- aphid

On *R. padi:* The obtain results indicated that the (minimum, maximum and mean) temperatures cleared a negative significant correlation with population of *R. padi* on wheat during 2016- 2017 (-0.5088, - 0.4574 and -0.4833). Meanwhile the mean relative humidity showed a negative slight correlation in the same season.

On *R. maidis:* The (minimum and mean) temperature cleared a negative significant correlation with population of *R. maidis* (-0.4870 and -0.4611) on wheat . However, max., temperature showed slight negative correlation (-0.4349) and the relative humidity showed a slight positive correlation with population *R. maidis* on wheat during season 2016-2017(table 3).

On *S. avenae* and *D. noxia:* The temperature (minimum, maximum and mean) and relative humidity cleared a slight a negative correlation with population of *S .avenae* (-0.1646, -0.1458, -0. 1552 and -0.1402) while, were (-0.335, -0.3036, -0. 3198 and -0.0016) during 2016-2017 season (table 3).

On *S. grminum:* The temperature (minimum, maximum and mean) showed a slight positive correlation with population of *S. grminum* while, the relative humidity cleared a slight negative correlation during season 2016-2017(table 3). These agree with Abd El-Aziz, A.A. A. 2014 who reported that the temperature had a great effect on biology of *S. grminum.* Powell 1986 in United Kingdom mentioned that the low abundance of cereal aphid population infesting wheat may due to the combined action of natural enemies as well as the a variability of climatic conditions from year to another.

Table (3)Simple correlation between certain aphid species and climatic factors on wheat during 2016-2017 season at Qadisiyah district .

Variable		Insect Pests			
Source	*R.padi*	*R. maidis*	*S. avenae*	*S.grminum*	*D. noxia*
Min.Temp.	-0.5088*	-0.4870*	-0.1646	0.0330	-0.3355
Max. Temp.	-0.4574*	-0.4349	-0.1458	0.0258	-0.3036
Mean Temp.	-0.4833*	-0.4611*	-0.1552	0.2932	-0.3198
R.H	-0.0535	-0.0535	-0.1428	-.01857	-0.0016

CONCLUSION

Obtained results showed that the infestation with *S. graminum* was much higher wheat plantations than other aphids in 2016-2017 season. *C. undecimpunctata,* and *C. carnea* the most specific aphid predator of cereal aphid in wheat fields. Statistical analysis showed that temperature and relative humidity were significant with some insects and insignificant with the others.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required.

REFERENCE

1. Stary, P. Aphid parasite (Hymenoptera : Aphidiidae) of the Mediterranean area. Transactions of the Czechoslova Academy of Siences, Series of Mathematica and Natural Siences1976; 86: 1- 95.
2. El- Heneidy, AH, Gonzalez D , Ahmed MA, Ibraheem MM , Megahed HE , Abdel- Awal WM , Adly D .Performance of certain Exotic aphid parasitoid species towards cereal aphids under laboratory, field cage and open wheat field conditions in Egypt. Egyptian Journal of Biological Pest control 2006; 16 (2);67-72.
3. El- Heneidy, AH. Efficacy of aphidophagous insects against aphids at wheat field in Egypt. Egypt. J. Biol. Pest Control, 1994 ;4(2):113-123.
4. USAID. Improving grain production in Iraq. The Agriculture Reconstruction and Development Program for Iraq; 2006.
5. Kindler, SD, Breen JP. Reproduction and damage by Russian wheat aphid (Homoptera: Aphididae) as influenced by fungal endophytes and cool season turf grasses .J. Econ. Entomol., 1991; 84(2):685-692.
6. Alhag, EA, Al- Rokaiboh, AA , Zaitoon, AA. Natural enemies of cereal aphids in sprinkler irrigated wheat in central Saudi Arabia. Bull. Fac.

Agric. Univ. Cairo 1996, 47:649-663.

7. Abdul- Satar, S Al – Saadi, NA Quda, A Al - Haidari , HS Al- Azzawi, A. Insect predators of Aphis nerii in Baghdad area , their predatory efficiency and feeding perference. J. of Biol. Sci. Res. 1998 ;19;31-40.
8. Saleh, AAA , El Sharkawy, HM El- Santel FS , Abd El - Salam RA .Seasonal abundance of certain piercing Sucking pests on cucumber plants in Egypt. Egypt. Acad. J. Biolo. Sci., 2017; 10 (7):65-79.
9. Hopper,W. Bio control of cereal aphids in the United Kingdom by a range of natural enemies. Uni. Pertantian Malysia1995; 4:159-162.
10. Tabsum, S Noorka, I R Fzal MA , Ali A. Screening best adopted wheat lines against aphid (*Schizaphis graminum* Rondani) population. Pak. Entomol., 2012; 3(1)51-53.
11. Helmi A and Khafaga AF. Molecular Fingerprinting of Certain Cereal Aphids in Egypt (Hemiptera: Sternorrhyncha: Aphididae) Using RAPD and ISSRs Markers. Journal of Entomology, 2011: 8: 327-340.
12. Snedecor, GW, Cochrran WG. Statistical Methods 6th Ed., Iowa state Univ.Press, Ames Iowa, USA;1967 .
13. SAS. Institute Inc.SAS/ STAT users, guide. 6-12 edition cary, Nc, SAS. Institute Inc.; 1999.
14. Abd el Aziz, AA. Ecological and biological studies on some insects infesting wheat crop and their associated predators. M. Sc. Thesis. Fac. of Agric., Mansoura University, Egypt 2014 ;120pp.
15. Darwish, YA ,Ali, AM. Field population trend of cereal aphids and their natural enemies on corn plants in Upper Egypt. Assiut J. Agric. Sci. 1991; 22(4): 33- 42.
16. Slman, FAA .Incidence of cereal aphids and seasonal abundance of their parasitoids in wheat fields in Sohag(Upper Egypt) Assiut. Jour. of Agric. Sci., 2006 ; 37 (2): 211-220.
17. Sabbour, MM. Effect of some natural bio agent and natural enemies against aphid in wheat fields Egyptian J. Bio. Pest. Cont., 2007 ;17(1/2):139-145.
18. El- Heneidy, AH , Rizk ,G N. Comparative study of cereal aphids species and their associated predators and parasitoids in two different wheat regions in Egypt. Egyptian J. Bio. Pest cont. 2004; 14 (1): 217-224.
19. Slman, FAA, Ahmed, MA. Seasonal abundance of ceral aphids and ladybrid beetle, *Coccinella undecimpunctata* (L) on four cereal crops in South Egypt. Assiut Jour. of Agric. Sci, 2005;36 (4) : 205-215.
20. Yigit, A Sertkaya , E Tiryakioglu, M. Population fluctuations of cereal aphids, *Rhopalosiphum padi* (L) and *Sitobion avenae* (F.) (Homoptera : Ahididae) and their impact on crop loss parameters of wheat. Turkey, Turk. Entomol. Dreg, 2007; 31 (1): 21-34.
21. Samad, SSM. Comparative study of cereal aphid species and their associated predalors and parasitoids in two different wheat regions in Egypt. Egyptian J. Biol. Pest Control. 2004; 14(1): 183-191.

Influence bacterial inoculant of local isolates of azotobacter vinelandii and irrigation water quality on growth and yield of wheat *(Triticum aestivum* L.)

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ABSTRACT

The present study deals with the isolation and characterization of *Azotobacter vinelandii* and A pots experiment was conducted at the canopy wood of Agriculture - University of Al-Muthanna (station 2) during winter season of 2016-2017 to study the effect of inoculant bacterial and irrigation water quality and their interactions on yield and growth of wheat. Treatments included four levels of inoculant bacterial (without inoculation A_0 and inoculation with three locally *Azotobacter vinelandii* coded A_1,A_2A_3 and three water qualities river water (0.752 ds.m^{-1} W_0), mixed water (3.6 ds.m^{-1} W_1) and drainage water (6.5 ds.m^{-1} W_2). The results showed, generally bacterial inoculant treatments was superior to the un-inoculant treatment A0 in most character studied and A_2 treatment achieved best result compared with other isolation locally, in height plant, dry weight plant, weight of 1000 seeds, yield and nitrogen uptake) was recorded (99.10 cm, 15.50 g plant^{-1}, 29.29 g, 32.95 g plant^{-1}, 73.56 mg plant^{-1}) sequentially. Generally increasing salinity in irrigation water significantly decreased growth characters, yield, and yield components of plant. Interaction between biofertilizer treatments and salinity had a significant effect on plant growth and yield and yield components of plant and the best result were had been gave by A2W0 in all traits studied (height plant, dry weight plant, weight of 1000 seeds, yield and nitrogen uptake) which was recorded (106.88cm, 17.97g plant^{-1}, 30.34g, 39.34g plant^{-1}, 97.43mg plant^{-1}) respectively.

Key words: *Azotobacter vinelandii, water salinity, inoculant.*

INTRODUCTION

Bio-fertilizers are cheap sources for plants nutrient and alternative mineral fertilizers using. They also reduce soil and water pollution as well as Bio-fertilizers different benefits forms by reducing the of chemical fertilizers quantities[1]. Agricultural development Sustainable is requires optimal use of microorganisms effectiveness and their biological activity in agricultural soils, which is an environmentally safe alternative to the availability of plant nutrients compared to chemical fertilizers[2]. Azotobacter most important for plant growth due to their role of nitrogen supply for plants and it is have been high efficiency of nitrogen fixation and hormones growth production[3]. Many studies refereed that Iraq's soils have been an overabundance of bacteria but there is many factors are inhibit growth and its existence such as drought and salinity of soil[4].Water Quality of irrigation are one of most important of basic natural resources for many centauries of the world particularly that located in semi-dry and dry area which use water irrigation in agricultural production so that are limit factor to development of agriculture in many countries. quality of water irrigation consider when using of water have a salt, the water irrigation quality can be using to expended area which use for agriculture production by mixing it with fresh water and use bacterial inoculants, to reduce harmful of water salt. The study aim is to investigate three isolates, which use inoculants of local bacteria and three levels of water irrigation salinity to determine best level can be suitable for inoculant bacterial and plant growth of wheat plant.

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MATERIALS AND METHOD

Isolation and bacteria diagnosis

Three soil rhizosphere samples were collected from geographical local of Al Muthanna province shown in table 1.. Sucrose mineral-salts broth media was used to soil inoculation by *Azotobacter vinelandii* isolates. Soil dilution prepared Take 1 ml to test tubes inoculation by above medium, which was making and sterilized by autoclave for 20 min at 121 °C and 15 lb.by. Tubes incubated at 30 ° C for 7 days and were testing a thin white membrane observation on the surface, which is an indicator of *Azotobacter* spp. Growth taking 0.1 ml of the tubes, which gave a growth indication and spread on a Petri dish surface containing the medium Sucrose mineral-salts agar. Dishes Incubate at 28 ° C for 2-3 days were re streak to obtain pure colonies of bacteria, White colonies were then extracted, a brownish dye was given to the medium.

Table 1. Numbers of soil samples and names of areas and fields collected from them.

Sample number	Geographical	Plant in soil
1	Rumaitha istrict	Wheat
2	Warka district	Alfalfa
3	Samawah district	Barley

Field experience A field experiment was designed to study the effect three *A. vinelandii* isolates and three levels of water irrigation quality. CRD was designed using two factors, as shown below:-

The first factor:-

Bacterial inoculants by four levels symbols taken : -

A0 = without inoculant, A1 = local isolate of Rumaitha district -Muthanna province, A2 = local isolate of Warka district -Muthanna province and A3 = local isolate of Samawah district -Muthanna province

The second factor:-

Water irrigation quality by three levels the symbols taken : -

W0 = 0.750 ds. m^{-1} (river water), W1 = 3.6 ds.m^{-1} (mixed water) and W2= 6.5 ds.m^{-1} (well water). Experiment was carried out by three replicates: Experimental units number = 4× 3 × 3 = 36 units.

Soil preparation

The experiment was conducted in lath-house at the College of Agric., University of AL-Muthanna season 2016-2017. The wheat seeds were cultivated Aba 99 variety in 15/11/2017. Nitrogen fertilizer was added in the form of urea (46% N) at 20 kg N 1 ha^{-1}. a stimulant for the action of the bacteria. The phosphate fertilizer was added as a superphosphate fertilizer (48% P_2O_5) by 60 kg. ha^{-1} potassium was added by 120 kg ha^{-1} in the form of potassium sulfate K_2SO_4 (43% K)., when the wheat arrived to maturity at the end of the winter season of 2017. the wheat crop was harvested by cutting the plants from the area near the surface of the soil, also recorded the following measurements:Plant height, plant dry weight, grain yield and nitrogen uptake of plant.

Table 2. The following table shows chemical, physical and biological characteristics.

pH 1:1	Ece ds.m^{-1}	OM%	Available% N	Available% P	Availabe% K	Sand gm kg^{-1}	Silt gm kg^{-1}	Clay gm kg^{-1}	Total bacteria
7.75	4.25	0.65	24.3	12.5	275	232	388	379	$2.8x10^7$
						Silty clay			

RESULTS AND DISCUSSION

The colonies of the isolated were smooth, convex, glistening and opaque on media. The isolate was Gram-negative, motile rods, positive for catalase and oxidase test. The biochemical characterization (QTS-24 miniaturized identification tests) based on carbon/ nitrogen source utilization pattern revealed that the isolate can utilize more carbon sources than that of nitrogen and indicated highest (98%) similarity to the genus *Azotobacter* when compared with Bergey's Manual of Determinative Bacteriology[5]. The isolate was positive

for tryptophan deaminase, sodium citrate, sodium melonate, ornithine decarboxylase, H2S production, urea hydrolysis, indole acid from glucose, acid from maltose, acid from sucrose, acid from rhamnose, acid from sorbitol and acid from raffinose. The comparison of this biochemistry characteristic depicted 98% similarity *A. vinelandii.* Height Plant cm. The results showed in table (3) the inoculant bacterial effected in the height plant. A treatment A2 was best which recorded a mean 99.10cm with an increase reached 28.83% compared control relative, we can explain to inoculation with *Azotobacter vinelandii* is an ideal tool to supply the soil with a high density of beneficial microorganisms[6]. An increase of the height plant average may be due to the inoculant's characteristics. A researchers Abd El-Gawad et al, 2009 and Zaied, 2009 referred that the secretions of bacteria conduce an important role in the elongation of plant cells, and increased division of plant cells, as well as their activity in the nitrogen fixation, which provided some of the nitrogen in Different stages of growth. The impacts positive are may be due to the effect of environmental factors in the isolates of bacteria isolated from salt environmental zones whereas it is did not adaptation to the new environment[8].

Table 3. Effect of inoculant bacterial and different level of water quality in plant height (cm)

Mean A	W2	W1	W0	
76.58	72.88	77.22	79.66	A0
87.55	83.55	89.88	89.22	A1
99.10	87.88	102.55	106.88	A2
89.33	84.22	89.55	94.22	A3
	83.13	89.80	91.74	Mean W
	A*W	W	A	L.S.D
	6.08	2.31	4.51	

A data of analysis statics revealed a water quality factor influenced on plant height so W_0 treatment was superior which recorded 91.74 cm compared with other treatment $W_1$and W_2 which that reached 89.80, 83.13 cm respectively. A decreases of height plant due to water quality are bad because salinity of water particular in$W_1$and W_2 treatment. The positive response of yield as a result of inoculation may be due to the high ability of these microbes in N2-fixation and the secretion of several compounds that increase soil fertility and decomposition of organic materials that increase the plant's ability to grow and increase productivity.

Dry weight

The results showed in table (4) the inoculant bacterial effect in the dry weight of vegetable plant. for *Azotobacter vinelandii* locally isolated treatment A2 was superior recorded a mean increase of 15.50 gm plant^{-1} with an increase of 29.81% compared control relative, An increase explain to inoculation bacterial with *Azotobacter vinelandii* which is have many characteristics such is provide macro elements as N,P,K and micro elements and secretion some hormones which is promoting plant growth. The results agreed with many researchers[2,7].

Table 4. Effect of inoculant bacterial and different level of water quality in dry weight of the plant (g plant^{-1}).

	W2	W1	W0	
11.94	9.50	11.58	14.75	A0
14.44	12.45	14.69	16.19	A1
15.50	13.41	15.11	17.97	A2
14.74	12.95	14.41	16.87	A3
	12.08	13.95	16.45	Mean A
	A*W	W	A	L.S.D
	0.53	0.33	0.38	

The results in table 4 referred that using water irrigation quality are possibility influence on dry weight of plant. The river water (W0) was used recorded high values reached 16.45 gm plant^{-1} than compared with W1,W2 treatments which gave (13.95, 12.08) gm plant^{-1} respectively. The interaction dual of bio-fertilizer and water irrigation quality in the dry weight of the plant A2W0 was recorded 17.97 g plant^{-1} . A percentage increase was 33.33% maybe that cause of Water irrigation quality effect and inoculant which has the ability to release nitrogen-fixing bacteria through growing season and It has already been shown that the use of nitrogen fixing bacteria with high effective ability in solubilizing organic and inorganic phosphates (Rahim et al, 2016) .

Grain yield (g plant$^{-1)}$

The results of the statistical analysis shown in table

5, which refer effect of inoculant bacterial type and water quality in the grain yield. The locally bacteria A2 were superior to the other treatments regardless the water quality type it recorded 32.95 g plant^{-1}. This is refer to bacteria ability to produce a growth stimulated such as Indol Acitic Acid.This is confirmed by Samurai et al, (2006) as well as it Sidrophorses secretions, which is a sticking to micro elements such as iron and zinc and availability increase its as well as its enzymatic activity and nitrogen fixation[7]. This can supply sufficient N2 and assist the hydrolysis of a wide range of P compounds leading to increased crop production[11]. These results confirmed by the works were carried out by[12,13,14].

Table 5. Effect of inoculant bacterial and different level of water quality in grain yield (g plant^{-1}).

	W2	W1	W0	
26.93	23.59	26.80	30.39	A0
29.57	24.52	28.26	35.92	A1
32.95	28.99	30.53	39.34	A2
28.64	24.68	27.59	33.66	A3
	25.45	28.30	34.83	Mean A
	A*W	W	A	L.S.D
	2.11	1.007	1.162	

The results showed that water irrigation W0 was superior in yield trait which recording 34.83 g plant^{-1} compared with mixed water (W1) and drainage water (W2). This refer to water irrigation efficiency decreased when salinity of water had been increased, which is osmotic pressure was increased, also there is many harmful effective of water saline of inoculant and yield plant. These results are in agreement with those obtained by[15]. The statically analysis showed a treatment had coded A2W0 was superior to the rest of the treatments which recorded 39.34 g plant^{-1}, In general it can be said that the use of biofertilizers with wheat plant at all levels of salinity of irrigation water gave positive results as compared to the control. This is due to the high ability of these microbes in N2-fixating atmosphere and the secretion of several compounds that increase soil fertility and decomposition of organic materials that increase the plant's ability to grow and increase productivity under those levels of salinity of irrigation water[16,17].

Nitrogen uptake (mg plant^{-1})

Data presented in table 6. indicated that inoculant bacterial treatments significantly increased the Nitrogen uptake in dry weight of vegetable plant of Wheat compared with inoculated plants. The treatment (A2) was superior to the other treatments it recorded 73.56 mg plant^{-1}. This result is compatible with the finding of Mahmoud *et al*. (2012) and Paritosh *et al.* (2013).

Table 6. Effect of inoculant bacterial and different level of water quality in nitrogen uptake (mg plant^{-1})

	W2	W1	W0	
55.31	39.37	53.46	73.11	A0
63.88	50.40	59.20	82.04	A1
73.56	56.96	66.28	97.43	A2
68.33	53.65	61.48	89.87	A3
	50.10	60.11	85.61	Mean A
	A*W	W	A	L.S.D
	3.11	2.007	3.162	

The results showed increasing salinity irrigation water caused decreasing nitrogen uptake, compared with River water. W0 treatment had gave the highest values of nitrogen uptake in vegetable plant, It's had been reached 85.61 mg plant^{-1}. The gradual depression occurred in all the growth characters of plant due to the irrigation with saline water. Thus, as salinity is a condition of excess salts in soil solution, it affects plant by increasing the osmotic pressure of the soil solution. These results are in agreement with those obtained by Ebrahim *et al.* (2010). They found that increasing salinity in irrigation water decreased growth characters of plant. The interaction between Biofertilizer and irrigation water salinity had a significant effect on nitrogen uptake of wheat plants. The highest value of nitrogen uptake by plants were recorded 97.43 mg plant^{-1}when inoculated with *Azotobacter* and, irrigated by river water (A2W0).

Ethical Clearance : Taken from ethical committee of institution.

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REFERENCE

1. Al-Sefat L. Biological fertilization and its role in reducing the problems of water pollution with chemical fertilizers. Faculty of Natural Resources

and Environmental Sciences - Omar Al - Mukhtar University - Al - Bayda - Libya. 2012.

2. Zaied KA, Kosba ZA, Nassef MA and El-Sanossy AS. Enhancement nitrogen fixation via inducing recombinants in *Azotobacter* SPP. Australian Journal of Basic and Applied Sciences, 2009; 3(2):1369-1385.
3. Michavila G, Ricciardi ML, de Souza Borges C, Pomares MF, Saccol de Sa´ EL. Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. 2017; 50;521-577.
4. Davey CB and Rovira AD. Biology of the rhizosphere. In: Carson,E.W. (Ed.). The Plant Root and Its Environment. Univ. Press. Of Virginia, Charlottesville, 1974 : 153-204.
5. Holt JG, Kreig NR, Sneath PHA, Staley JT, Williams ST.Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, 1994.
6. De Gregorio A, Pedrotti E, Russo L, Morselli M. Minimally invasive combined glaucoma and cataract surgery: clinical results of the smallest Ab interno gel stent. Int Ophtalmol. 2017 May 29; Epub.
7. Abd El-Gawad AM, Hendawey MH and Farag HIA. Interaction between biofertilization and canola genotypes in relation to some constituent under Siwa Oasis conditions.Research Journal of Agriculture and Biological Sciences, . 2009; 5(1):82:96.
8. Bashan Y, Holguin G, de-Bashan LE. Azospirillum-plant relationships: physiological, 2012.
9. Rahim N, Parviz O, Horieh S, Iraj R, Mohammad AM. Phosphate solubilization characteristics of efficient nitrogen fixing soil Azotobacter strains. IRAN. J. MICROBIOL. 2016;Vol. (6)N(4), 285-295 Number 4 285-295
10. Samurai IK and Hamdallah SR. Effect of inoculation with azotobacter and isospermal bacteria in absorption of certain nutrients and concentration Plant Hormones and Growth of Tomato Crops. Iraqi Agricultural Science Series. 2006; Vol(37) Issue (3).
11. Garg SK, Bhatnagar A, Kalla A, Narula N. In vitro nitrogen fixation, phosphate solubilization, survival and nutrient release by Azotobacter strains in an aquatic system. Bioresource Technology 2001; 80:101-109.
12. Omid M and Jalilian J. Response of safflower seed quality characteristics to different soil fertility systems and irrigation disruption. International Research Journal of Applied and Basic Sciences, 2012; 3 (5): 968-976.
13. Mina P, Ayenehband A and Modhej A. The effect of biological and chemical fertilizers and plant density onquality and quantity yield of Safflower (*Carthamus tinctorius*.L) underAhvaz condition. International journal of Agronomy and Plant Production. 2013; 4(3): 524-529.
14. Paritosh P, Pati BK, Ghosh GK, Mura SS and Saha A. Effect of biofertilizers and sulphur on growth, yield, and oil content of hybrid sunflower (*Helianthus annuus*. L) In a typical lateritic soil. Open Access Scientific Reports, 2013;2 (1):1-5.
15. Siddiqee AH. Influence of salt stress on some physiological and biochemical attributes, and oil composition of a potential oilseed crop safflower (*Carthamus tinctorius* L.). Ph.D. Thesis, University of Agriculture, Faisalabad. 2010: 29 p.
16. Abou-Aly HE, Zaghloul RA, Ehsan A, Hanfy A, Rahal GH and EL-Meihy RM. Colonization of pepper roots with salt-tolerant PGPR as inducer for saline stress. The eleventh conference of agricultural development researches, 27-30 March, 2012. Fac. Agric, Ain Shams Univ., Egypt.
17 Aymen EM, Zhani K, Meriem BF and Hannachi C. Seed priming for better growth and yield of safflower (*Carthamus tinctorius*) under saline condition. Journal of Stress Physiology & Biochemistry, 2012; 8 (3): p125.
18. Mahmoud, G, Moghaddasi MS and Omidib AH. The Effects of biological and chemical nitrogen fertilizers on agronomical traits of winter safflower cultivars in Saveh region of Iran. Annals of Biological Research. 2012; 3 (11):5141-5144
19. Ebrahim N, Mohammadi-Nejad G, Yousefi K and Farahbakhsh H. Evaluation salinity tolerance of safflower (*Carthamus tinctorius* L.) Genotypes at different vegetative growth stages. Intl. J. Agron. Plant. Prod. 2010; 1 (4): 105-111.

Effect of Zinc Methionine on Some of the Productive Traits of Broilers

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ABSTRACT

The trial was designed to evaluate the addition of zinc methionine to the diet on some productive and carcasses performance of broiler, the study was conducted in the poultry field, Animal Production Department, Agriculture Faculty, Al-Muthanna University, from 1/4/2018 to 15/5/2018. A total of 400 broiler, one day, unsexed Ross308 strain were randomly distributed to four treatments (100 chick / treatment), each treatment included four replicates (25 chick per replicate), The chickens were reared in four-floor batteries with a measuring 2 x 1.5 meters per floor. The treatments of the experiment were as follows: First treatment (T1): a control treatment, zinc methionine was added at levels 400, 500 and 600 g per ton of feed for T2, T3 and T4 treatments respectively. The results showed that the T4 resulted in a significant improvement ($p \leq 0.05$) in body weight, body gain, feed consumption, feed conversion and mortality compare with other treatments.

Keywords: *zinc methionine, broilers, productive.*

INTRODUCTION

Rare metals are important in feeding poultry, as it affects the speed of growth and metabolic rate, which is reflected in their production performance such as eggs or meat, because it has an important role in vital processes[1.] As well as necessary for the permanence of life, it is an enzyme-stimulating agent involved in the construction of its systems[2]. Zinc is a rare mineral and contributes to the synthesis of more than 300 enzymes[3], formation of blood cells red increased thyroid hormone secretion[4]. Many researchers point to the benefits of zinc as it has the role of manufacturing RNA and DNA, restoring tissue growth and contributing to bone mineral deposition, increases the production of eggs, increases the shell thickness and improves the conversion coefficient of broilers[5,6,7]. The lack of zinc in the diet will affect the growth rate as well as the irregularity of bone formation, decrease in feathering and the decrease of the body's immunity against many diseases[8]. There are many studies on the use of zinc in poultry diets that its addition affects the productive qualities, the addition of high-grade zinc to the diet was given the highest increase in weight gain[9], the same results followed with addition of zinc as well as improved food conversion coefficient[10]. The addition of zinc to broiler broilers led to increased feed consumption and improved food conversion coefficient, and a significant decrease in the mortality, this improvement in body weight, weight gain and feed conversion was positively reflected on the measure of the production index[6,11], this was found by Abbas (2018), observed a significant improvement in body weight, weight gain, feed conversion, production index, significant decrease in mortality and significant improvement of thigh, wing and neck when adding organic zinc to 80 mg / kg feed[13]. Conducted a study on the addition of zinc at levels of 120,90,60,30,0 mg / kg stock feed in improving growth rate as well as good consumption of daily feed. Ezzat et al. (2013) noted that the addition of zinc at different levels of chicken broilers contributed to a higher than normal high body weight. Kwiecień et al. (2016) fed meat broilers on zinc-containing diets that led to a positive increase in chest and femoral weight. Jahanian et al. (2008) reported that the addition of zinc Methionine (Zn Met) to the diets improved the proportion of reflux and the proportion of breast meat and increased liver weight. Al-Daraji et al. (2012) noted that the intake of zinc in

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large quantities more than the need of the body will prevent the absorption of copper from the bowel, leading to the emergence of anemia. Zinc is poorly absorbed by the intestines so we increase its concentration more than the required requirement of 40 mg / kg feed, as indicated by NRC (1994) to absorb what is needed by the bird and the rest is thrown out of the body and this leads to obstruction absorption of copper from the intestine, The idea of adding zinc methionine (Zn Met) to the diet to know its effect on some of the productive and carcasses qualities of broilers.

MATERIALS AND METHOD

A TOTAL OF 400 CHICKS ONE DAY, ROSS BROILER, were randomized disterbuted to four treatments (100 chick/ treatment), Each treatment consisted of four replicates (25 chick per replicate), experiment treatments were as follows: The first treatment (T1): a control treatment in which the birds fed on a diet without addition. The second treatment (T2): In which the birds were fed on a diet, 400 g / t of zinc methionine was added. The third treatment (T3): in which the birds were fed on a diet, 500 g / t of zinc methionine was added. The fourth treatment (T4): In which the birds were fed on a diet, 600 g / t of zinc methionine was added. The chicks were fed by starter for 1-21 days and Grower for 22-35 days. The ratios were calculated according to table 1. The water and diet were provide ad libitum. Studied traits were weekly body weight, weight gain, feed consumption, feed conversion, mortality and production index[17].

Table 1. Basal diet

Contents	**Starter(1-21 days)**	**Finisher(22-35 days)**
Corn	60	66
Soya bean meal[1]	35	28
Primex[2]	2.5	2.5
Limestone	0.5	0.5
Dicalcium phosphate	0.7	0.5
Oil	1.2	1.7
Anti-toxin	0.05	0.05
Antifungal	0.05	0.05
Total	100	100
Calculated chemical composition		
Total protein (%)	22	20
Metabolism energy (kilocalorie/ kg)	2950	3100
Methionine (%)	0.51	0.48
Methionine + cystine (%)	0.90	0.80
Lysine (%)	1.15	1.00
Calcium (%)	1.00	0.90
Available phosphorus (%)	0.43	0.40
Fiber (%)	3.00	3.50
Fats (%)	2.80	3.00

1. The soybeans used by an Argentine source contained 48% crude protein and energy representing 2,400 kcal / kg.

2.Premix Max Care contains 16% protein, 900 kilocalorie energy, 10% lysine, 9.5% methionine and 9.5% sodium, 2% fat, 1% fiber, 12% calcium, phosphoric 13% Sodium, 5%. Calcium phosphate was (21.8% calcium and 18% phosphorus).

3. Chemical composition of the components of diet was calculate according to NRC (1994).

Statistical analysis

The data were analyzed in a one-way analysis using the statistical program[20], The averages were compared using the Duncan Multiple Test[21].

RESULTS AND DISCUSSIONS

BODY WEIGHT

Significant increase (P≤0.05) for the treatment (T4) in the body weight at the 5 weeks age, the body was 1995 g for T4 and 1968, 2939 and 1873 g, the other treatments T3, T2 and T1 respectively, These results were consistent with Kucuk et al. (2003): Ezzati et al. (2013): Abbas (2018) who observed a significant superiority of the final body weight when adding organic zinc to broilers, this improvement in body weight may be due to the zinc as an enzyme, important for metabolism, thus improved body weight[2]. The modern broiler breeds were characterized by rapid growth and therefore need zinc to become larger. This increases the body weight by increasing the level of zinc in the diets, but does not reach the excessive limit that allows it to bind with copper and impede its absorption and the incidence of anemia.

Table 2. Effect of Addition of Different Levels of Zinc Methionine (Zn Met) on body weight (g) of broiler (± S.E.).

Treatments	Age (week)				
	1	2	3	4	5
T1	111. 66±0.49	298.46 b±0.94	577.09 b±5.06	1162.42 d±7.42	1873.31 d±11.48
T2	110.82±0.82	301.16 ab±1.05	589.35 b±4.32	1194.76 c±8.13	1939.74 c±10.67
T3	112.18±0.77	304.62 a±2.01	600.11 ab±4.19	1212.34 b±8.44	1968.82 b±10.82
T4	111.42±0.29	308.02 a±1.14	611.60 a±5.04	1231.09 a±7.36	1995.36 a±9.98
Sig.	N.S	*	*	*	*

T1: A control treatment in which birds are fed on a diet without additives. T2: fed the birds on a diet supplemented with 400 g / t of zinc methionine. T3: fed the birds on a diet supplemented with 500 g / t of zinc methionine. T4: fed birds on a diet supplemented with 600 g / t of zinc methionine. (Mean ±S.E) means ± standard error. (N.S) No significant differences between mean values. * There were significant differences between the mean values at the probability level (P≤0.05).

Body gain

Table 3. shows a significant increased (P≤0.05) in for the cumulative body gain weight. The fourth treatment (T4) recorded the highest value of this trait at 1955 g on the rest of the experiment, which recorded the lowest values significantly and reached 1833 g. These results were consistent with the findings of Innoconti et al. (2004): Ao et al., (2006): Abbas, (2018), which showed that the addition of zinc to the broiler chicks resulted in a significant improvement in body gain.

Table 3. Effect of Addition of Different Levels of Zinc Methionine (Zn Met) on body gain (g) of broiler (± S.E.) .

Treatments	Age (week)					Total
	1	2	3	4	5	
T1	71. 66±0.27	186.80 c±0.97	278.63 c±1.74	585.33 c±3.56	710.89 d±4.86	1833.31 d±9.73
T2	70.82±0.33	190.18 bc±1.06	288.18 b±1.55	605.41 b±4.01	744.98 c±5.06	1899.74 c±10.55
T3	72.18±0.09	192.49 ab±0.52	295.49 a±0.94	612.23 ab±3.64	756.48 b±3.95	1928.82 b±10.49
T4	71.42±0.13	196.60 a±0.79	303.58a±1.47	619.49 a±2.97	764.27 a±4.44	1955.36 a±9.30
Sig.	N.S	*	*	*	*	*

T1: A control treatment in which birds are fed on a diet without additives. T2: fed the birds on a diet supplemented with 400 g / t of zinc methionine. T3: fed the birds on a diet supplemented with 500 g / t of zinc methionine. T4: fed birds on a diet supplemented with 600 g / t of zinc methionine. (Mean ±S.E) means ± standard error. (N.S) No significant differences between mean values. * There were significant differences between the mean values at the probability level (P≤0.05).

Feed Consumption

Table 4. shows that there is a significant superiority (P≤0.05) for birds of the fourth treatment (T4) for the consumption of the cumulative feed, recorded a value of 3400 g / bird at the age of 5 week for the other of the experimental birds, which recorded values less 3381, 3358 and 3308 g / bird for treatments T1, T2 and T3 respectively. The reason may be that nutrition at high levels of zinc leads to an increase in microscopic microbes that enter the zinc in their diet, which have a major role in the digestion of fiber, especially the secum, which works to increase the speed of food passage in the intestines, Which increases the need for birds to consume large amounts of feed[23].

Table 4. Effect of Addition of Different Levels of Zinc Methionine (Zn Met) on feed consumption (g) of broiler (± S.E.)

Treatments	Age (week)					Total
	1	2	3	4	5	
T1	113.93±0.64	308.22 b±0.92	479.24 c±2.49	1041.89 c±6.94	1364.91 b±9.26	3308.19 c±16.76
T2	113.31±0.34	311.90 ab±0.70	487.02 bc±1.95	1053.41 b±4.33	1393.11 a±8.44	3358.75 b±20.12
T3	115.49±0.21	313.76 ab±0.57	493.47 b±2.11	1059.16 ab±7.46	1399.49 a±9.14	3381.37 ab±18.37
T4	113.56 ±0.29	318.49 a±0.32	503.94a±1.50	1065.52 a±5.22	1398.61 a±8.86	3400.12 a±17.77
Sig.	N.S	*	*	*	*	*

T1: A control treatment in which birds are fed on a diet without additives. T2: fed the birds on a diet supplemented with 400 g / t of zinc methionine. T3: fed the birds on a diet supplemented with 500 g / t of zinc methionine. T4: fed birds on a diet supplemented with 600 g / t of zinc methionine. (Mean ±S.E) means ± standard error. (N.S) No significant differences between mean values. * There were significant differences between the mean values at the probability level (P≤0.05).

Feed conversion

Table 5. indicates a significant improvement (P≤0.05) for the feed conversion of T3 and T4 and recorded the best improvement of the feed conversion of 1.75 and 1.74 respectively, compared to T1 (control), which recorded the worst feed conversion was reached 1.80. while T2 did not differ significantly with the treatment of T1 (control), and T3 and T4 were not different. The improvement in the feed conversion may be due to the introduction of zinc in the synthesis of fatty acids, which contribute significantly to the improvement of the general health of birds and thus improve the conversion coefficient of food[24].

Table 5. Effect of Addition of Different Levels of Zinc Methionine (Zn Met) on feed conversion (g diet/ g weight gain) of broiler (± S.E.).

Treatments	Age (week)					Total
	1	2	3	4	5	
T1	1.59±0.002	1.65 b±0.002	1.72 b±0.002	1.78 b±0.002	1.92 c±0.003	1.80 b±0.002
T2	1.60±0.001	1.64 ab±0.002	1.69 ab±0.002	1.74 a±0.002	1.87 b±0.001	1.77 ab±0.002
T3	1.60±0.001	1.63 ab±0.002	1.67 a±0.002	1.73 a±0.002	1.85 ab±0.002	1.75 a±0.002
T4	1.59±0.001	1.62 a±0.002	1.66 a±0.001	1.72 a±0.002	1.83 a±0.002	1.74 a±0.002
Sig.	N.S	*	*	*	*	*

T1: A control treatment in which birds are fed on a diet without additives. T2: fed the birds on a diet supplemented with 400 g / t of zinc methionine. T3: fed the birds on a diet supplemented with 500 g / t of zinc methionine. T4: fed birds on a diet supplemented with 600 g / t of zinc methionine. (Mean ±S.E) means ± standard error. (N.S) No significant differences between mean values. * There were significant differences between the mean values at the probability level (P≤0.05).

CONCLUSION

The high levels of zinc methionine (600 mg per ton of feed) resulted in improved all productive traits of broilers compared with other treatments.

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Ethical Clearance: Not required.

REFERENCES

1. Yatoo MI, Saxena A, Deepa PM, Habeab BP, Devi S, Jatav RS and Dimri U. Role of Trace elements in animals: A review, Vete World. 2013: 6(12):963-967.
2. Swiatkiewicz S, Arczewska –Wlosek A and Jozefiak D. The efficacy of organic minerals in poultry nutrition review and implications of recent studies. Worlds Poultry Sci. J. 2014: 70:475-486.
3. Plum L, Rink L, Haase B and Hajo A. The Essential Toxin: Impact of Zinc on Human Health. Int J Environ Res Public Health. 2010: 7 (4): Pol. J. Food Nutr. Sci. 57(Suppl. 4C):577–581.
4. Park YM, Kang SG, Lee BH and Lee HJ. Decreased thyroid function in Korean women with bipolar disorder receiving valproic acid. J. General Hospital Psychiatry. 2011: 33(2), 200-13.
5. Sahin N, Onderci M and Sahin K. Effects of dietary chromium and zinc on egg production egg quality ,and some blood metabolites of laying hens reared under low ambient temperature. Biol. Trace Elem. Res. 2002: 85:47-58.
6. Salim HM, Jo C and Lee BD. Zinc in broiler feeding and nutrition. Avian Biology Research. 2008: 1(1), 5-18.
7. Rouhalamini SM, Salarmoini M and Asadikaram GH. Effect of zinc sulfate and organic chromium supplementation. 2014: p. 32.
8. Suttle, NF. The Mineral Nutrition of Livestock, 4th ed. CABI Publishing, Oxfordshire, UK. 2010: 43 p.
9. Innoconti AS, Zimmerman JG, Ferry A, Scozzafava S and Supuran CT. Carbonic anhydrase inhibitors. Inhibition of the zinc and cobalt gammaclass enzyme from the archaeon Methanosarcina thermophila with anions. Bio org. Med. Chem. Lett. 2004: 14: 3327-3331.
10. Ao, T, Pierce JL, Power R, Dawson KA, Pescatore AJ, Cantor AH and Ford MJ. Evaluation of Bioplex Zn as an organic zinc source for chicks. International Journal of Poultry Science. 2006: 5(9), 808-811.
11. Salim, HM, Lee HR, Jo CS, Lee K and Lee BD. Supplementation of graded levels of organic zinc in the diets of female broilers;effects on performance and carcass quality. British Poultry Science. 2011: 52(5):606–612.
12. Abbas S. The effect of diet supplementation with Organic Zinc on productive performance, carcass characteristics, and immunological responses of broiler chicks. Master theses. Animal Production. College of Agriculture. University of Baghdad. 2018: 122 p.
13. Feng J, Ma WQ, Niu HH, Wu XM, Wang Y and Feng J. Effects of zinc glycine chelate on growth, hematological, and immunological characteristics in broilers. Biol. Trace Elem. Res. 2010: 133:203–211.
14. Ezzati MS, Bozorgmehrifard MH, Bijanzad P, Rasoulinezhad S, Moomivand H, Faramarzi S and Stabraghi E. Effects of different levels of zinc supplementation on broilers performance and immunity response to Newcastle disease vaccine. Eur. J. Exp. Biol. 2013: 3(5), 497-501.
15. Kwiecień M, Winiarska-Mieczan A, Milczarek A, Tomaszewska E and Matras J. Effects of zinc glycine chelate on growth performance,88 carcass characteristics, bone quality, and mineral content in bone of broiler chicken. Livestock Science. 2016: 191, pp.43-50.
16. Jahanian R, Moghaddam HN, Rezaei A, Haghparast AR. The influence of dietary zinc-methionine substitution for zinc sulfate on broiler chick performance. Journal of Biological Science. 2008: 8(2):321-327.
17. Al-Daraji HJ, Al-Hayani WKhA, Al-Husseini AH.. The blood physiology of birds. Ministry of Higher Education and Scientific Research, University of Baghdad. Agriculture college. 2012: 325 p.
18. NRC; National Research Council. Nutrient Requirements of Poultry . 9th ed., National

Academic press , Washington , DCV USA . 1994: p. 120.

19. Al-Fayyad HAA, and Naji SAH. Poultry Products Technology. Ministry of Higher Education and Scientific Research, University of Baghdad. Agriculture college. 1989: 457 p.
20. SAS. SAS / STAT Users Guide for personal Computer; Release 6-12. SAS Institute Inc . Cary , Nc . USA. 2001:117 p.
21. Steel RGD and Torrie JH. Principles and procedure of statistics. 2nd Edition. New York, Mcgraw Hill book company. 1980: 97 p.
22. Kucuk O, Sahin N and Sahin K. Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. Biological trace element research. 2003: 94(3), 225-235.
23. Hudson BP, Dozier WA, Wilson JL, Sander JE and Ward TL. Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic sources of zinc from hatching to 65 wk of age. Journal of Applied Poultry Research. 2004: 13(2), pp.349-359.
24. Batal AB, Parr TM and Baker DH. Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poultry Science. 2001: 80(1), pp.87-90.

Topical Combination of Nifedipine with Lidocaine for Anal Fissure Treatment

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ABSTRACT

Background: A fissure consist of crack or tear in the vertical axis of the anal canal between the anal verge and the dentate line. Anal fissure is treated by two ways either medical or surgical treatment or both.

Objective: evaluate the healing response of topical application of nifedipine with lidocaine in acute and chronic anal fissure

Material and method: A prospective study of one hundred patients presented with anal fissure to outpatient surgical clinic in Al-Diwaniyah teaching hospital, the patients divided into two groups, 40 patients (15 males and 25 females) with acute anal fissure, and 60 patients (25 males and 35 females) with chronic anal fissure.

Results: There was no statistically significant difference in the healing rate between acute and chronic lesion, however the rate of healed lesion in acute setting is higher than that of chronic setting (80% vs. 56%)

Conclusion: Topical application nifedipine and lidocaine effective in treating acute anal fissures with the healing response of 85%, and prevent evolution of acute anal fissures to chronic anal fissure.

Keywords: *acute, chronic, anal fissure, Medical treatment.*

INTRODUCTION

Anal fissure is the most common cause of severe anal pain and bleeding [1]. Anal fissure is an elongated ulcer in anoderm below dentate line, more common posteriorly due to more adherent of anoderm to underlying tissue in posterior midline so the blood supply is significantly low as shown by the Doppler flow-metry study [2]. It is estimated that 25% of a women and 8% of a men have anterior fissure [3]. Constipation and passing of hard faces lead to tearing of the mucosa causing pain and bleeding[4].

The pain of anal fissure is severe and always disproportionate to the severity of physical lesion, it may be so severe that patients may avoid defecation for days until it becomes inevitable, this lead to harden of a stools, which farther tear the anoderm during defecation [5]. This ulcer is ischemic ulcer and due to hypertonicity of internal anal sphincter due to pain and spasm of sphincter, this causing a vicious cycle and this can lead to chronic anal fissure [6].

Chronic anal fissure have traditionally managed with lateral internal sphincterotomy or anal dilatation sphincterotomy, however it has been associated with incontinence in up to 35% of patients. Furthermore, this does not take into account normal weakening of the sphincter with age as well as the possibility of future anorectal surgery or obstetrical trauma. Dilatation of the anal canal has also been associated with sphincteric tears and subsequent incontinence [7]. Controlled pneumatic dilatation, however is a variation to the technique that may reduce the risk of sphincter in jury [7].

More recently, less invasive strategies have been adopted to induce sphincter relaxation topical agents including nifedipine reduce internal sphincter pressure

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and increase blood supply to ischemic ulcer by decreasing the internal anal sphincter tone, and topical agents nifedipine also have anti-inflammatory action. As lidocaine, is the most common topical anaesthetic for anal fissure, which break the vicious cycle of pain [8, 9].

During pregnancy and following childbirth the fissures occur as a superficial split in anterior anoderm may progress to a chronic anal fissure [10]. A typical fissure may be multiple, off the midline, large and irregular, these may be caused by inflammatory bowel disease, local or systemic malignant, venereal infection (syphilis, HIV) trauma, tuberculosis, and chemotherapy [11]. The aim of the current study to evaluate the healing response of the anal fissure to topical application of nifedipine with lidocaine gel, and prevent evolution of acute anal fissure to chronic anal fissure.

MATERIAL AND METHOD

A prospective study carried out in the surgical outpatient clinic of AL-Diwaniyah teaching hospital from January 2017 to January 2018. The study included one hundred patients, each patients were followed up during the admission period, examination of the anal fissure and its signs, like puckered or spatulated anus, anal sphincter tone by doing per rectum examination (subjective feeling) for the chronic fissure to decided wither its tight or relax, examination for the skin tag (present or absent), and also examination for pressure which indicate the chronicity of anal fissures.

After that the patients were given the formula (cream Nifedipine (0.3%) – lignocaine (1.5%) Combination) [12] and explain to them who to use the formula, the frequency of visit every two weeks to evaluate the response to the formula.

According to the signs and symptoms, the patients were divided into two groups, the first group with acute anal fissure (15 males and 25 females) and second group with chronic anal fissure (25 males and 35 females). In Both groups patients were instructed to apply cream locally into the anal canal twice daily for 3 weeks after defecation [12].

Anal fissure was defined as acute when its symptoms (moderate to severe pain during defecation, bleeding per rectum) is within 3 to 6 weeks of onset, the patients were assessed with history and physical examination [13]. Chronic anal fissure defined by duration of symptoms longer than 3 months, the presence of induration at fissure edges, sentinel pile, hypertrophied anal papillae, and circular muscle fibres at the base of the cutaneous defect [14]. The response was shown as stopping or decreasing the bleeding, decreasing the pain, the relaxation of internal anal sphincter and disappearance the induration.

STATISTICAL ANALYSIS

Analysis of our data done using the software program: SPSS 21 (Statistical Package for Social Sciences). Numeric data were represented by the mean ± standard error, while the categorical data represented by numbers and percentages. Independent t-test was used to study the difference between two. For the study of the association between categorical data, the Chi-Square test used. The significant level considered when the P value < 0.05 [9, 15].

RESULTS

There was no statistically significant difference in the healing rate between acute and chronic lesion, however the rate of healed lesion in acute setting is higher than that of chronic setting (80% vs. 56%)

Table 1: healing of lesions in male after 6 weeks

	Healed	Not healed	P value
Acute (15)	12 (80%)	3 (20%)	0.123
Chronic (25)	14 (56%)	11 (44%)	

There was no statistically significant difference in the healing rate between acute and chronic lesion.

Table 2: healing of lesions in male after 8 weeks

	Healed	Not healed	P value
Acute (15)	13 (86.7%)	2 (13.3%)	0.591
Chronic (25)	20 (80%)	5 (20%)	

There was no statistically significant difference in the healing rate between acute and chronic lesion, however the rate of healed lesion in acute setting is higher than that of chronic setting (80% vs. 57.1%)

Table 3: healing of lesions in female after 6 weeks

	Healed	Not healed	P value
Acute (25)	20 (80%)	5 (20%)	0.064
Chronic (35)	20 (57.1%)	15(42.9%)	

There was no statistically significant difference in the healing rate between acute and chronic lesion.

Table 4: healing of lesions in female after 8 weeks

	Healed	Not healed	P value
Acute (25)	23 (92%)	2 (8%)	0.455
Chronic (35)	30 (85.7%)	5 (14.3%)	

DISCUSSION

Today, the treatment of chronic anal fissure has become challenging because of irreversible damage and deformation of the internal anal sphincter. Additionally, given that most patients with anal fissure are young and worried about non-voluntary faeces excretion and the consequences after surgery, the use of alternative medicines for treating patients with anal fissure has been of recent concern [16].

In the current study the healing response of acute anal fissure (AF) after (6 weeks) for both females were 80%, and after extended therapy (8 weeks) the healing response of acute anal fissure for females was 92% and for males were 86.7%, our findings were in agreement with Antropoli et al in which 141 patients treated with nifedipine topically (0.2% nifedipine gel) every 12 hours for three weeks they reported a total remission from acute anal fissure after 21 days of therapy to be 95% [17]. Our findings were in agreement with Digennaro et al in which 171 patients with acute AF treated with Diltiazem gel applied every 12 h for 40 days the rate of efficacy was 89.4 % (complete healing: 64.3 %, improvement: 25.1 %) [18], also in agreement with Katsinelos et al The effect of topical nifedipine in treatment of acute anal fissure showed that a course of (8 weeks) nifedipine achieved 85.2% remission indicated by resolution of symptoms and healing of fissure and the unhealed 14.8% that 50% undergo lateral internal sphincterotomy and 50% of them continue therapy for four additional weeks that resulting in healing the fissure [19].

In the current study chronic anal fissure the healing response after (6 weeks) for females were 57.1% (20 patients) and for males were 80% (20 patients), but after extended therapy (8 weeks) the healing response of chronic anal fissure for females were 85.7%% (30 patients) and for males were 80% (20 patients). Our findings were in agreement with Digennaro et al in which 171 patients with acute AF treated with Diltiazem gel applied every 12 h for 40 days with chronic fissure the rate of efficacy was 62.8 % (complete healing: 23 %, improvement: 39.8 %) [18]. In a study conducted by Golfam et al, 110 individuals were studied with chronic anal fissure, out of whom 60 were treated with nifedipine and 50 were controls (conventional treatment). In the nifedipine group, 70% of patients achieved recovery (which is in agreement with our findings), and in controls, 12% recovery was observed after four weeks. In the Golfam et al. study, recovery and healing of the pain in the nifedipine group were significantly different from that of the control group [20].

Oral and topical calcium channel blockers (CCBs) have recently been shown to lower the anal resting pressure by relaxing the internal anal sphincter [21-23]. The transport of calcium through the L-type calcium channels is important for the maintenance of internal anal sphincter tone [24]. As opposed to glyceryl trinitrate, which reduces resting anal tone by releasing nitric oxide, nifedipine (a calcium channel blocker) reduces the tone and spontaneous activity of the sphincter by decreasing the intracellular availability of calcium [24].

CONCLUSION

Topical application nifedipine and lidocaine effective in treating acute anal fissures with the healing response of 85%, and prevent evolution of acute anal fissures to chronic anal fissure.

Conflict of Interest None

Ethical Clearance: Informed written consent was obtained from all the participants in the study, and the study and all its procedure were done in accordance with the Helsinki Declaration of 1975, as revised in 2000. Approved by Al-Diwaniyah teaching hospital, department of surgery.

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REFERENCES

1. Nelson R. Non surgical therapy for anal fissure. The Cochrane database of systematic reviews [Internet]. 2006 2006; (4):[CD003431 p.]. Available from: http://europepmc.org/abstract/MED/17054170 https://doi.org/10.1002/14651858.CD003431.pub2.
2. Knight JS, Birks M, Farouk R. Topical diltiazem ointment in the treatment of chronic anal fissure. The British journal of surgery. 2001;88(4):553-6.10.1046/j.1365-2168.2001.01736.x
3. Madoff RD, Fleshman JW. AGA technical review on the diagnosis and care of patients with anal fissure. Gastroenterology. 2003;124(1):235-45, https://www.gastrojournal.org/article/S0016-5085(03)50035-X/pdf
4. Mancini I, Bruera E. Constipation in advanced cancer patients. Supportive Care in Cancer. 1998;6(4):356-64, https://link.springer.com/article/10.1007%2Fs005200050177
5. Lund JN, Scholefield JH. Glyceryl trinitrate is an effective treatment for anal fissure. Diseases of the colon and rectum. 1997;40(4):468-70,
6. McCallion K, Gardiner K. Progress in the understanding and treatment of chronic anal fissure. Postgraduate medical journal. 2001;77(914):753-8, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1742193/pdf/v077p00753.pdf
7. Tranqui P, Trottier DC, Victor JC, Freeman JB. Nonsurgical treatment of chronic anal fissure: nitroglycerin and dilatation versus nifedipine and botulinum toxin. Canadian Journal of Surgery. 2006;49(1):41-5, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3207506/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3207506/pdf/20060200s00009p41.pdf
8. Hang MTH, Smith BE, Keck C, Keshavarzian A, Sedghi S. Increasing efficacy and reducing side effects in treatment of chronic anal fissures: A study of topical diazepam therapy. Medicine. 2017;96(20):e6853.10.1097/MD.0000000000006853
9. Mohammed MA, Kadhim KA, Jasim GA, Fawzi HA. Metformin compared to insulin for the management of gestational diabetic. Int J Res Pharm Sci. 2018;9(3):1063-7.https://doi.org/10.26452/ijrps.v9i3.1630
10. Patel JR, Dudhamal TS. A comparative clinical study of Yashtimadhu Ghrita and lignocaine-nifedipine ointment in the management of Parikartika (acute fissure-in-ano). Ayu. 2017;38(1-2):46-51.10.4103/ayu.AYU_93_17
11. Sigle GW, Kim R. Sexually Transmitted Proctitis. Clinics in Colon and Rectal Surgery. 2015;28(2):70-8.10.1055/s-0035-1547334
12. Singh B, Khichy S, Kumar A, Singh S, Neki N. COMPARATIVE STUDY TO OBSERVE EFFECTS OF TOPICAL NIFEDIPINE WITH LIGNOCAINE AND TOPICAL SUCRALFATE WITH LIGNOCAINE IN ACUTE ANAL FISSURE. Journal of Advanced Medical and Dental Sciences Research. 2016;4(6):81,
13. Hafeez U, Choudhry M, Khan S. Comparison of Healing Rate between Surgical and Nonsurgical Management of Acute Anal Fissure. PAKISTAN JOURNAL OF MEDICAL & HEALTH SCIENCES. 2016;10(4):1414-6,
14. Sonarkar RK, Deshmukh SD, Akhtar MA, Bindlish R. Segmental lateral internal Sphincterotomy: a new technique for treatment of chronic anal fissure-clinical outcome and review of literature. IJSS. 2016;2(3):30,
15. Yahya RN, Kasim AA, AlGawwam G, A. Fawzi H. Treatment satisfaction with disease-modifying therapy in relapsing-remitting multiple sclerosis in Iraq. International Journal of Research in Pharmaceutical Sciences. 2018;9(3):836-43. https://doi.org/10.26452/ijrps.v9i3.1578
16. Khaledifar B, Mahmoudi MYA, Mobasheri M. A Double-Blind Randomized Trial Comparing the Effectiveness and Safety of Nifedipine and Isosorbide Dinitrate in Chronic Anal Fissure. The Malaysian Journal of Medical Sciences : MJMS. 2015;22(5):42-9, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5295740/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5295740/pdf/mjms-22-5-042.pdf
17. Antropoli C, Perrotti P, Rubino M, Martino A, De Stefano G, Migliore G, et al. Nifedipine for local

use in conservative treatment of anal fissures: preliminary results of a multicenter study. Diseases of the colon and rectum. 1999;42(8):1011-5,

18. Digennaro R, Pecorella G, La Manna S, Alderisio A, Alderisio A, Jr., De Pascalis B, et al. Prospective multicenter observational trial on the safety and efficacy of LEVORAG(R) Emulgel in the treatment of acute and chronic anal fissure. Techniques in coloproctology. 2015;19(5):287-92.10.1007/s10151-015-1289-2
19. Katsinelos P, Kountouras J, Paroutoglou G, Beltsis A, Chatzimavroudis G, Zavos C, et al. Aggressive treatment of acute anal fissure with 0.5% nifedipine ointment prevents its evolution to chronicity. World Journal of Gastroenterology : WJG. 2006;12(38):6203-6.10.3748/wjg.v12.i38.6203
20. Golfam F, Golfam P, Khalaj A, Sayed Mortaz SS. The effect of topical nifedipine in treatment of chronic anal fissure. Acta medica Iranica. 2010;48(5):295-9, http://acta.tums.ac.ir/index.php/acta/article/download/4051/4026
21. Agaoglu N, Cengiz S, Arslan MK, Turkyilmaz S. Oral nifedipine in the treatment of chronic anal fissure. Digestive surgery. 2003;20(5):452-6.10.1159/000072715
22. Perrotti P, Bove A, Antropoli C, Molino D, Antropoli M, Balzano A, et al. Topical nifedipine with lidocaine ointment vs. active control for treatment of chronic anal fissure: results of a prospective, randomized, double-blind study. Diseases of the colon and rectum. 2002;45(11):1468-75.10.1097/01.dcr.0000029705.79796.53
23. Ho KS, Ho YH. Randomized clinical trial comparing oral nifedipine with lateral anal sphincterotomy and tailored sphincterotomy in the treatment of chronic anal fissure. The British journal of surgery. 2005;92(4):403-8.10.1002/bjs.4882
24. Cook TA, Brading AF, Mortensen NJ. Differences in contractile properties of anorectal smooth muscle and the effects of calcium channel blockade. The British journal of surgery. 1999;86(1):70-5.10.1046/j.1365-2168.1999.00998.x

Relationship between Diabetes Mellitus and Tuberculosis in Indonesia

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ABSTRACT

Backgrounds: Tuberculosis was one of the 10 caused of death in the world. Tuberculosis was caused by *mycobacterium tuberculosis* which was transmitted through droplets. One risk factor for tuberculosis was diabetes mellitus. The risk of death due to tuberculosis in people who had diabetes would be higher than whom did not have tuberculosis. This study was aimed to determine the relationship between diabetes mellitus and tuberculosis.

Method: This study used a cross sectional study design. The data analyzed were 34,257 people who participated in the Indonesia Family Life Survey 5 in 2013-2014. Prevalence Odds Ratio crude, Prevalence of adjusted odds ratios and 95% CI were calculated using the chi-square and the multivariate binary logistic.

Result: The prevalence of tuberculosis in this study was 6.6%. From the chi-square results there was a significant relationship between diabetes mellitus and tuberculosis with POR 3.88 (2.49-6.05). Multivariate binary logistic result was a significant relationship between diabetes mellitus and tuberculosis with POR 4.93 (3.01-8.1) after being adjusted by confounder variables.

Conclusions: There was a significant relationship between diabetes mellitus and tuberculosis.

Keywords: *Diabetes Mellitus, Tuberculosis, Indonesia*

INTRODUCTION

Tuberculosis was one of the top 10 caused of death in the world[(1).] Tuberculosis caused by *mycobacterium tuberculosis* which was transmitted through droplets. Every one positive smear would transmit 10-15 people or 17% probability of infected each contact[(2)]. About a quarter of the people in the world had latent tuberculosis where the person had been infected with *mycobacterium tuberculosis* but they were not sick and could not transmit it. In 2017, 10 million people suffered from tuberculosis and 1.6 million people died. Two-thirds tuberculosis cases in the world were India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh and South Africa. The prevalence of Tuberculosis at Indonesia in 2016 was 361,893 residents[(1)].

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WHO had set a target for ending TB endemic by 2030 by reducing the mortality rate by 95% and the incidence rate by 90% by 2035[(1)]. The success rate of treatment for all tuberculosis cases in 2017 was 85%, this number had increased from the previous year. To achieve it, we must take preventive actions besides success rate of treatment targets. People of productive age, malnutrition or diabetes, HIV, and smoker had high risk of tuberculosis. People with weak immunity due to chronic diseases such as diabetes mellitus had a high risk of getting tuberculosis. 15% of cases of tuberculosis in the world was probably related to diabetes mellitus. The risk of death due to tuberculosis in people who had diabetes would be higher than those who did not have tuberculosis. 7.9% of cases of tuberculosis in the world were caused by smoking[(1)]. Based on research in China by Zhang et al., smoking could increase the risk of tuberculosis by 1.28 times[(3)]. The largest proportion of tuberculosis cases in Indonesia in 2016 at the age of 25-34 years was 18.07% followed by age 45-54 at 17.25%[(4)].

Based on a retrospective cohort study in Taiwan in 2011-2012 by Yen et al., underweight could increase the risk of death from tuberculosis by 2,14[5]. Poor ventilation could facilitate the spread of *mycobacterium tuberculosis* by air. Based on research conducted by Halim, Naning and Satrio poor ventilation could increase the risk of tuberculosis by 2.21 times[6].

Based on the explanation above, the researcher wanted to know the relationship between diabetes mellitus and tuberculosis.

METHOD

This research used cross sectional study design. This study used Indonesian Family Life Survey (IFLS) data 5. The survey was conducted by the University of Southern California and RAND in 13 provinces in 2014 to 2015[7]. The survey was carried out by observation, interviews, and measurements on weight and height. The sample used in this study amounted to 34,256 people. In this study using all respondents aged ≥15 years who took the IFLS survey 5. In this study the exposure was diabetes mellitus and its outcome was tuberculosis. Determination of tuberculosis status by interviewing whether the respondent had or had not been diagnosed with tuberculosis by a doctor. Determination of DM status by interviewing whether the respondent had or had not been diagnosed with DM by a doctor.

Measurements of body weight and height were carried out by trained interviewers. Variable BMI (Body Mass Index) uses the category by Ministry of Health there were[8]:

Skinny category : BMI <18.5

Normal category : BMI ≥18,5 - <24,9

Fat category : BMI ≥25.0 - <27.0

Category of obesity : BMI ≥27.0

For smoking behavior variables, education was also obtained by interview method. Whereas the variable of house ventilation and the type of house floor were obtained by observation by the interviewer. Age variables used the age category by statistics Canada there were[9]:

Children : 0-14

Youth : 15-24 years

Adult : 25-64 years

Elderly : >65 years old

Data processing was started by merge data so it became a data set that matches the selected variable. Then re-coding and categorizing the variables of age, BMI, education, smoking behavior, number of cigarettes consumed, type of floor, and cooking fuel. Data analyzed were descriptive analysis, bivariate analysis, and cox regression analysis.

RESULTS

This study with a total sample of 34,257 after the data cleaning process contained missing data were on sex variables (6 respondents), age variables (7 respondents), education variables (93 respondents), BMI variables (2,035 respondents), cooking fuel variables (50 respondents), ventilation variables and type of floor (1 respondent). Table 1 was the characteristics of the respondents there were gender, age, education, smoking behavior, cigarette consumption per day, body mass index, ventilation conditions, type of house floor and cooking fuel for tuberculosis. Table 1 also provided potential confounders. Variables that had the potential as confounders were gender, age, education, smoking behavior, body mass index, type of house floor and cooking fuel.

Table 1. The Characteristic Respondents of Tuberculosis

Characteristic	Tuberculosis		Total	P-Value
	Yes	No		
	n (%)	n (%)	n (%)	
Sex				
Man	874 (5,4)	15.459 (94,6)	16.333 (47,7)	0,000
Woman	1.385 (7,7)	16.534 (92,3)	17.919 (52,3)	
Missing			6 (0,0)	
Age				

Cont... **Table 1. The Characteristic Respondents of Tuberculosis**

15-24 years	337 (4,7)	6.910 (95,3)	7.247 (21,2)	0,000
25-64 years	1.600 (6,6)	22.799 (93,4)	24.399 (71,2)	
>65 years	322 (12,4)	2.283 (87,6)	2.605 (7,6)	
Missing			7 (0,0)	
Education				
Education Low	610 (5,1)	11.385 (94,9)	11.995 (35,0)	0,000
Education Moderate	1.016 (5,8)	16.534 (94,2)	17.550 (51,2)	
Education High	626 (13,5)	3.994 (86,5)	4.620 (13,5)	
Missing			93 (0,3)	
BMI				
Skinny	220 (5,3)	3.950 (94,7)	4.170 (12,2)	0,000
Normal	1.051 (5,8)	16.939 (94,2)	17.990 (52,5)	
Fat	833 (8,3)	9.208 (91,7)	10.041 (29,3)	
Missing			2.057 (6,0)	
Smoking				
Yes	486 (4,5)	10.426 (95,5)	10.912 (31,9)	0,000
No	1.578 (7,3)	20.093 (92,7)	21.671 (63,3)	
Ever Smoking	196 (11,7)	1.479 (88,3)	1.675 (4,9)	
Cigarette Consumption per Day				
≤18 cigarette	2.110 (6,5)	30.113 (93,5)	32.223 (94,1)	0,147
>18 cigarette	150 (7,4)	1.885 (92,6)	2.035 (5,9)	
Ventilation				
Good	1.971 (6,7)	27.434 (93,3)	29.405 (85,8)	0,052
Poor	289 (6,0)	4.563 (94,0)	4.852 (14,2)	
Missing			1 (0,0)	
Type of Floor				
Soil	39 (3,0)	1.278 (97,0)	1.317 (3,8)	0,000
Non Soil	2.221 (6,7)	30.719 (93,3)	32.940 (96,2)	
Missing			1 (0,0)	
Cooking Fuel				
High Risk	238 (3,4)	6.864 (96,6)	7.102 (20,7)	0,000
Non Risk	2.018 (7,4)	25.088 (92,6)	27.106 (79,1)	
Missing			50 (0,1)	

In this study physicians who had been diagnosed with tuberculosis by 2,260 (6.6%) people, and those who had never been diagnosed with tuberculosis were 31,998 (93.4%) people. In sex variable, the highest was woman with 17,919 (52.3%) people, while the largest age group was in the 25-64 year age group with 24,399 (71.2%) people. The most moderate education variable was 17,550 (51.2%) people, while the most body mass index (BMI) with the normal category was 17,990 (52.5%) people. In the smoking variable the largest proportion of non-smoking was 21,671 (63.3%) people, and cigarette consumption was ≤18 cigarettes per day amounting to 32,223 (94.1%) people. A total of 29,405 (85.8%) respondent had good house ventilation, while the most variable type of house floor was a type of non-soil floor of 32,940 people (96.2%). Variable non-risk cooking fuels was 27,106 (79.1%) people. In table 1, it can be seen that there were a lot of tuberculosis in women of 1,385 (7.7%), and in age group of >65 years was 322 (12.4%). High education amounted to 626 (13.5%) people with normal category BMI of 833 (8.3%) people and had a smoking behavior of 196 (11.7%) people with consumption of cigarettes > 18 cigarettes per day of 150 (7.4%) people were have higher prevalence of

tuberculosis than others. Subjects with high prevalence of tuberculosis had good house ventilation amounted to 1,971 (6.7%) people, and non-soil type of floor house were 2,221 (6.7%) people. Subjects with high prevalence of tuberculosis using non risk cooking fuels were 2,018 (7.4%) people.

Table 2. Analysis of bivariate and multivariate mellitus diabetes and tuberculosis.

Variable	Tuberculosis (N=34.257)				POR crude	95% CI	Pvalue	POR adj	95% CI	Pvalue
	Yes		No							
	N	%	N	%						
DM										
Yes	25	1,1	92	0,3	3,88	2,49-6,05	0,000	4,93	3,01-8,1	0,000
No	2.235	98,9	31.906	99,7						

In table 2 it can be seen that there was a relationship between diabetes mellitus and tuberculosis. People with diabetes mellitus had a risk of 3.88 (95%CI 2.49-6.05) times for tuberculosis rather than whom did not have diabetes mellitus. Multivariate analysis is done by entering all variables suspected of being confounders. The POR after adjusted variables of sex, age, education, smoking behavior, body mass index, type of house floor and cooking fuel was 4.93 (95%CI 3.01-8,1).

DISCUSSION

The prevalence of tuberculosis in this study was 6.6% (2,260) of 34,257 respondents. Tuberculosis was common in women, age groups> 65 years, higher education, fat BMI, ever smoking behavior, type of floor of the house that was not soil, and non-risk cooking fuel. There was a significant relationship between diabetes mellitus and tuberculosis (POR 3.88 95%CI 2.49-6.05). After being controlled by the confounder variable there was a significant relationship between diabetes mellitus and tuberculosis (POR 4.93 95%CI 3.01-8,1).

In case-control research conducted by Alisjahbana et al., diabetes mellitus was increase the risk of OR 4.7 (95%CI 2.7–8.1) tuberculosis. After adjusted variable confounders (such as socioeconomics, environmental factors, contact with patients, and history of tuberculosis) the result did not reduce the strength of the association between diabetes mellitus and tuberculosis[10]. This was consistent with a systematic review of 13 observational studies conducted by Jeon and Murray that diabetes mellitus increases the risk of RR 3.11 (95%CI 2.27-4.26) in the occurrence of tuberculosis[11]. This was consistent with the systematic review conducted by Al-Rifai et al., that diabetes mellitus patients had 3.59 times (95%CI 2.25–5.73), 1.55 times (95%CI 1.39–1.72), and 2.09 times (95%CI 1.71–2.55) increased risk of active prospective in four TB, 16 retrospective, and 17 case-control studies. Diabetes mellitus with uncontrolled blood sugar had a higher risk of developing tuberculosis compared to diabetes mellitus with controlled blood sugar[12]. In a cohort study in Australia conducted by Dobler, Flack and Marks diabetes mellitus increases the risk of tuberculosis 1.48 (95%CI 1.04-2.10) in people with diabetes mellitus and 2.27 (95%CI 1.41 -3.66) in people with diabetes mellitus who use insulin. In this study also states that the attributable risk was small, so that if the incidence of diabetes mellitus in the population was high, screening and treatment of diabetes mellitus patients was necessary[13]. According to Harries et al., and Dooley and Chaisson it was more reliable to screening tuberculosis patients when patients undergo treatment compared to the start of treatment. Tuberculosis was a chronic disease that is likely to increase blood sugar due to cytokine stimulus, causing false positive diabetes mellitus if screening is too early[14][15].

In a study conducted by Workneh, Bjune and Yimer in ethiophia, stated that the risk of tuberculosis with diabetes mellitus dying increased 3.96 (1.76–8.89) times compared with no diabetes mellitus. This may be due to poor glycemic control and impaired immune cells in patients with diabetes mellitus[16]. This is according to what was done in a study conducted by Martens et al., in mice, that mice have acute diabetes mellitus will be exposed to tuberculosis after 4 weeks of infection[17]. According to Dooley and Chaisson, diabetes mellitus

increased sensitivity to *mycobacterium tuberculosis* through multiple mechanisms. The mechanisms directly related were hyperglycemia and cellular insulinopenia, and indirect effects on the macrophage and lymphocyte function. Most important cells in prevented tuberculosis infection were phagocytes (alveolar macrophages and their precursor monocytes) and lymphocytes[15].

The limitations of this study was the use of interview methods for smoking behavior variables so that the results are likely to be underestimated. The use of interviews with tuberculosis and diabetes mellitus status can result in underestimated results. While the variable of home ventilation was only done by observation by the interviewer without any measurement. The results of this study had controlled confounder variables, but there were potential confounder who had not been controlled such as social economy and contact with tuberculosis patients.

CONCLUSION

The results of this study there was a relation between diabetes mellitus and tuberculosis. This study was present information for tuberculosis prevention and further research.

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Conflict of Interest: None declared

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Ethical Clearance: The IFLS surveys and their procedures were properly reviewed and approved by IRBs (Institutional Review Boards) in the United States (at RAND) and in Indonesia at the University of Gadjah Mada (UGM) for IFLS5. Ethical clearance taken from IRB (institutional Review Boards) committee Thus all requirements for consent for adults and children were met and approved by those IRBs before fieldwork could begin.

REFERENCES

1. WHO. Tuberculosis [Internet]. 2018 [cited 2018 Jan 19]. Available from: http://www.who.int/mediacentre/factsheets/fs104/en/
2. Widoyono. Tropical diseases: Epidemiology, Transmission, Prevention & Eradication. Jakarta: Erlangga; 2005.
3. Zhang H, Li X, Xin H, Li H, Li M, Lu W, et al. Association of Body Mass Index with the Tuberculosis Infection: A Population-based Study among 17796 Adults in Rural China. Sci Rep [Internet]. 2017;7:41933. Available from: http://dx.doi.org/10.1038/srep41933
4. Ministry of Health. Indonesia Health Profile 2016. Jakarta; 2017.
5. Yen YF, Chuang PH, Yen MY, Lin SY, Chuang P, Yuan MJ, et al. Association of body mass index with tuberculosis mortality: A population-based follow-up study. Med (United States). 2016;95(1):1–8.
6. Halim, Naning R, Satrio DB. Risk Factors of Pulmonary Tuberculosis in Children 1 - 5 Years Old in Kebumen Regency. Jambi Univ Res J Sci Ser. 2015;17(2):26–39.
7. Strauss J, Witoelar F, Sikoki B. The Fifth Wave of the Indonesia Family Life Survey : Overview and Field Report. 2016.
8. Ministry of Health. Basic Health Research. Jakarta; 2013.
9. Statistics canada. Age Categories, Life Cycle Groupings. 2017.
10. Alisjahbana B, Crevel R van, Sahiratmadja E, Heijer M den, Maya A, Istriana E, et al. Diabetes mellitus is strongly associated with tuberculosis in Indonesia. NT J TUBERC LUNG DIS. 2006;10(6):696–700.
11. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: A systematic review of 13 observational studies. PLoS Med. 2008;5(7):e152.
12. Al-Rifai RH, Pearson F, Critchley JA, Abu-Raddad LJ. Association between diabetes mellitus and active tuberculosis: A systematic review and meta-analysis. PLoS One. 2017;12(11):e0187967.
13. Dobler CC, Flack JR, Marks GB. Tuberculosis

and diabetes mellitus: Time for an integrated approach. BMJ Open. 2012;2(e000666).

14. Harries AD, Murray MB, Jeon CY, Ottmani SE, Lonnroth K, Barreto ML, et al. Defining the research agenda to reduce the joint burden of disease from diabetes mellitus and tuberculosis: Viewpoint. Trop Med Int Heal. 2010;15(6):659–63.
15. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. Lancet Infect Dis [Internet]. 2009;9(12):737–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19926034%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2945809
16. Workneh MH, Bjune GA, Yimer SA. Diabetes mellitus is associated with increased mortality during tuberculosis treatment: A prospective cohort study among tuberculosis patients in South-Eastern Amahra Region, Ethiopia. Infect Dis Poverty [Internet]. 2016;5(22). Available from: http://dx.doi.org/10.1186/s40249-016-0115-z
17. Martens GW, Arikan MC, Lee J, Ren F, Greiner D, Kornfeld H. Tuberculosis susceptibility of diabetic mice. Am J Respir Cell Mol Biol. 2007;37:518–24.

A Cross-secsional Study: Analysis Risk Factors Against Pulmonary TB AFB Positive in Indonesia

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ABSTRACT

Background: Indonesia is a third rank in TB cases worldwide. TB is a disease caused by multifactors where the environment interacts with host-related factors. So, the study was conducted to identify the multifactors and to get the main risk factor of pulmonary TB AFB positive in Indonesia.

Methods: A cross-sectional study, Subjects were the age group ≥15 years who were followed by the interview and had complete data. Pvalue <0,025 was considered statistically significant and included to final model by screening the factors which able to predict the prevalence of pulmonary TB.

Results: The prevalence of pulmonary TB AFB positive was 1.72%. Chi square test showed statistically significant association against pulmonary TB were gender OR=1.42(1.26-1.60), Diabetes Mellitus history OR=3(2.35-3.82), Contact with active TB patients OR=3.49(2.90-4.20). Multivariate analysis showed, Active smoker, gender, age category, contact with active TB patients, and diabetes mellitus history had role against pulomonary TB AFB positive in Indonesia. The main risk factor was active smoker OR=3.71.

Conclusion: Age, gender, diabetes mellitus, active smoker, and contact with active TB patients were significantly risk factors to predict pulmonary TB. Active smoker had 3.71 greater risk of having pulmonary TB compared with non pulmonary TB.

Keywords: *Risk factors, pulmonary TB AFB positive, Indonesia*

INTRODUCTION

Globally, it was estimated 10.0 million people (9.0–11.1 million) developed TB disease in 2017. The highest cases countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%)[(1)]. Earlier in the 20th century, the discipline of pulmonology had been driven by epidemic levels of TB in the USA (the rate of PTB in New York City was 247 per 100 000) [(2)]. In 2017, the global incidence for TB was 48.9 per 100 000 males and 39.7 per 100 000 females [(3)]. In 2014, the World Health Assembly embraced an ambitious resolution to reduce deaths from TB by 95% by 2035 [(4)].

Indonesia is the third rank in TB worldwide. The management of *M. tuberculosis* infection was a new priority action for "End Tuberculosis Strategy". Hence, identifying the significant risk factors of TB in Indonesia is essential effort to do. The SDG and End TB Strategy targets set for 2030 cannot be achieved without intensified research/study and development. The main reason, we use this survey to identify risk factors to predict pulmonary TB AFB positive and to get the main risk factor against pulmonary TB in Indonesia.

Study Design and Data Collection

Design was a cross-sectional using Prevalence Survey TB 2013-2014 data. It collected information on individual, household and community level data using multistage cluster stratified random sampling on≥15 years old[(5)]. Selected cluster was 156 district from 33 provinces in Indonesia.Field supervision during data collection was carried out regularly by the Balitbangkes research team and partners (WHO, KOMLI Tuberculosis, Directorate of Tuberculosis and TORG). Supervised activities included pre-survey, data collection, panel

team activities, and data analysis.

Measures

The quality of surveys was guaranteed at all stages by recruiting qualified teams, compliance with standard operating procedures for all activities, trials, team training and regular supervision. Socio-Demographic Characteristics and Risk Factors; age , gender, level of education, DM history, tuberculosis history, active smoker, passive smoker, and contact with active tuberculosis patients. Pulmonary TB was difined by the result of microscopic test from respondents' sputum.

Statistical Analysis

Logistic regression was performed to calculate the risk factors and to get the final model of pulmonary TB AFB postive.

RESULTS

Table 1. Assosiation of pulmonary TB AFB positive

Characteristics	Tuberculosis		Non Tuberculosis		Total	OR	95%CI	p
	n =1,091	%	n = 62,190	%				
Age Category (years)								
15-24	104	0.7	14,188	99.3	14,292	1		
25-34	236	1.6	14,509	98.4	14,745	0.27	0.21-0.34	0.0001
35-44	246	1.8	13,453	98.2	13,699	0.59	0.49-0.71	0.0001
45-54	236	2.3	10,185	97.7	10,421	0.67	0.56-0.79	0.0001
≥ 55	269	2.7	9,855	97.3	10,124	0.85	0.71-1.01	0.070
Gender								
Female	532	1.5	35,750	98.5	36,282	1		
Male	559	2.1	26,440	97.9	26,999	1.42	(1.26-1.60)	0.0001
Education Level								
High	362	1.7	21,532	98.3	21,894	1		
Low	729	1.8	40,658	98.2	41,387	1.06	(0.94-1.21)	0.321
DM History								
No	1018	1.6	60,739	98.4	61,757	1		
Yes	73	4.8	1,451	95.2	1,524	3	(2.35-3.82)	0.0001
Tuberculosis History								
No	0	0	61,346	100	61,346	1		
Yes	1091	56.4	844	43.6	1,935	-	-	-
Active Smoker								
No	805	1.8	42,784	98.3	43,589	1		
Yes	286	1.5	19,406	98.5	19,692	0.78	(0.68-0.90)	0.0001
Passive Smoker								
No	410	2.1	18,711	97.9	19,121	1		
Yes	681	1.5	43,479	98.5	44,160	0.71	(0.63-0.81)	0.0001
Contact with active tuberculosis patients								
No	957	1.6	59,794	98.3	60,751	1		
Yes	134	5.3	2,396	94.7	2,530	3.49	(2.90-4.20)	0.0001

The prevalence of pulmonary TB AFB positive in Indonesia was 1.72%. The highest proportion of TB was ≥ 55 years group 2.7%. The proportion of TB in male was 2.1% higher but non TB female was 98.5% higher.

Table 2. Final Model Prediction

Risk Factors	B	df	p	OR	95%CI
Gender	0.837	1	0.000	2.31	1.99-2.67
Education	0.162	1	0.014	1.17	1.03-1.34
DM history	1.027	1	0.000	2.79	2.18-3.57
Active smoker	-0.773	1	0.000	0.46	0.39-0.54
Contact with active tuberculosis patients	1.277	1	0.000	3.58	2.97-4.32
Constanta	-4.454	1	0.000	0.012	

The final model to predict of pulmonary TB BFA positive in Indonesia;

Pulmonary TB = -4.454+0.837(Gender)+0.162(Education)+1.027(DM History)-0.773(Active Smoker)+1.277(Contact with Active Tuberculosis Patients)

Probability to suffer TB:

$$\frac{1}{1 + e^{\text{Pulmonary TB}}}$$

No difference of education level between TB and non TB. Respondents had DM history and suffered TB 4.8% higher than non TB only 1.6 %. Almost non TB had no tuberculosis history (100%). The proportion of TB and no TB between active smoker and passive smoker were the same. In respondents with contact active TB patients, the proportion of TB was 5.3% and non TB was 94.7% while the proportion of no contact with active TB, TB was 1.6% and non TB was 98.3%.

Male have risk 1.42 greater of pulmonary TB. Education level has no significant risk of TB. DM has risk 3 greater of pulmonary. Active smoker and passive smoker were a protective to pulmonary TB. Respondents contact with active TB patients has risk 3.49 greater than those who have no contact.

DISCUSSION

Risk of pulmonary TB AFB positive could be accurately predicted by gender, education, DM, active smoker, and contact with active tuberculosis patients. Study in Ambo Hospital, the risk of one or more family member with history of TB (OR=4.4;95%CI1.5–12.9) [6]. Study in Vietnam found RR of smear-positive disease among household contacts was 6.4 (95%CI,4.5-9.0;P<0.001)[7]. Quoted from Ambo Hospital study,45% of TB cases had a family history of TB compared with 11% of the controls (OR=7.55; P< 0.001). The risk appears to increase when the contact is with a close family member[5]. Study in China identified the cumulative risk of active TB among close contacts 1.48% (95%CI 1.15%–1.88%)[6].

Study of Ojha Institute, living with TB AFB positive patients are at high risk[8].Although TB control programmes have averted millions of deaths, their effects on transmission and incidence rates are not yet widely detectable[36].The second risk factor is DM. It risks 2.79 times to pulmonary TB. WHO collaborative framework for TB-DM recommends bidirectional screening[9]. The global diabetes epidemic is steadily increasing in every country[10,11]. High prevalence, severe clinical characteristics and poor outcomes of pulmonary TB patients with DM highlight the necessity of early bidirectional screening and comanagement in Shanghai, China[12].

TB-DM comorbidity is characterized by increase inflammation with elevated circulating levels of inflammatory cytokines and other factors[37]. Cohort study by Kim found risk of DM RR=3.57 (95% CI 3.07-5.16) [38]. Prevalence of DM progressively increased 27.9%. Patients age 55-64 years had the highest association of DM (OR=3.53) compared with those under 45 years [13]. Adults with DM had significantly higher odds of TB (aOR 1.90; 95%CI 1.15–3.14) compared to adults

without DM after adjusting for age, sex, smoking status, history of active TB, and foreign born status[14].

DM were seven times represented in TB patient population (OR=6.6;95%CI=3.788–11.60) in comparison to the general population[15]. In Maryland, severity of DM affects the risk of developing TB adjusting for HIV status, age, weight, and foreign birth (OR=6.5 95%CI 1.1–38,P=0.039)[16]. TB infection prevalence among patients with DM type 2 was 51.3%[17]. In Asian population, DM is an independent risk factor for all cause mortality with HRs 1.17-1.27[13].The peak prevalence of DM observed in countries of Asia, North America, and Oceania[18].DM increased risk of developing and dying from an infectious disease[19].TB is the leading killer among global infectious diseases[35].

The third risk factor is gender.Male have risk 2.31 greater suffer pulmonary TB. Epidemiological information shows the differences between men and women in prevalence of infection, rate of progression from infection to disease, incidence of clinical disease, and mortality due to TB[20].Study from Iran, The incidence of TB in males and females was 62% and 38%[21]. Study populations in China, Male was significantly associated with increased risk of TB HR 2.36 (95% CI 1.30–4.30) [22].

South Nigeria; 2625 TB patient, 61.4% were males while 38.6% were females with ratio of 1.59[23].Male predominance in TB incidence which has been previously explained[24]. Investigation of TB Prevalence in Nigera Delta, male 63% of the prevalence while females 37%[25]. Kaplan–Meier survival analysis demonstrated significantly higher mortality in the men (p=0.005). Multivariate analysis, male was an independent risk factor (OR=1.96; 95% CI, 1.12–3.41)[26].

The fifth, education was the risk factor to TB. Study in US shown under High school graduate had risk 3.23 (95%CI 2.16–4.83) greater to latent tuberculosis infection[27]. Cross-sectional study in Semarang, Indonesia in 2012 found low education was 56.7% and high education was 43.3%[28].

Finding from this study active smoker is a protective factor (OR=0.46) to prevent pulmonary TB. It is needed continued study to examine the relationship of active smoker on patophisiology of pulmonary TB to determine the consistency of the effects of active smoker of pulmonary TB AFB positive. Using another design of study which is able to evaluate association between active smoker and tuberculosis correctly. Finding of active smoker in this study shows opposite result from another study. Smoking is causally associated with active tuberculosis[29]. Smoking is significantly associated with pulmonary TB in India (OR for mild, moderate and heavy were 2.28, 2.51 and 2.74)[30].

CONCLUSION

Ministry of health launched “find and treat until healing” program in 2016. One of indicator is case detection rate by investigation contact by health worker with population around index cases. A guideline on investigation among close contacts of infectious cases exist in regulation “Minister of health Number 67 year 2016” mentioned about investigation contact strategy[31]. WHO also has recommended contact investigation. Household contact investigation strategies can bring case detection in community. This strategies are particularly effective in high TB burden regions[32].

TB is a major cause illness and death worldwide especially in Asia and Africa. DOTS has lead to significant increase in the treat ment success (82.7%) (WHO target 85%) and decrease in the default and failure rates [40]. Continued program collaboration between TB and DM programs will help to integrate public health programs. DM is risk factor for the development of pulmonary TB and both disease present endocrine alterations likely to play in certain immune-endocrine-metabolic associated disorders[39].

Screening for active tuberculosis in DM should lead to earlier detection of TB[33]. Monitoring, recording and reporting on case numbers and outcomes are responsibility of collaborative framework between TB and DM programs.TB control in low and middle income countries are isoniazid preventive therapy for preventing TB among HIV and household contacts of infectious TB patients, clinical algorithms for diagnosing smear-negative TB disease in patients seeking care, screening algorithms for excluding TB-HIV-infected individuals eligible for preventive therapy, and programmatic provision of second-line treatment for multidrug-resistant TB[34].

Ethical Considerations: Ethics testing conducted by the Balitbangkes ethics committee. Written informed consent was obtained from all respondents before data collection was carried out.

Limitations

This study is a secondary data analysis, not all covariate variables were examined.

Conflict of Interest: Both author declared that no competing interest exist.

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REFERENCES

1. WHO. Global TB Report. France: WHO. 2018.
2. Davies PDO, et al. Smoking and tuberculosis :The epidemiological association and immunopathogenesis. 2006.
3. Metrics GH. Measuring progress from 1990 to 2017 and projecting attainment to 2030 of the health-related Sustainable Development Goals for 195 countries and territories.2018;2091–138.
4. Engla NEW, Journal ND.Tuberculosis Elimination in the United States-The Need for Renewed Action. 2017;2017–9.
5. Kementerian Kesehatan.SPTB 2013-2014. Jakarta;2015.
6. Tenna Ephrem, Bezatu Mengiste, Frehiwot Mesfin WG.Determinants of active pulmonary tuberculosis in. 2012;1–8.
7. Fox GJ, et al.Household-Contact Investigation for Detection of Tuberculosis in Vietnam Greg. 2018;221–9.
8. Jiang Q, et al.Assessment of tuberculosis contact investigation in Shanghai , China : An 8- year cohort study. 2018;108(1052):10–5.
9. Khan TR, Ahmed Z. Active case finding of sputum positive pulmonary tuberculosis in household contacts of tuberculosis patients in Karachi , Pakistan. 2014;2(1).
10. Silva DR, Muñoz-torrico M, Duarte R, Galvão T, Bonini EH, Arbex FF.Risk factors for tuberculosis : diabetes , smoking , alcohol use , and the use of other drugs. 2018;44(2):145–52.
11. Alotaibi A, Perry L, Gholizadeh L, Al-ganmi A.Incidence and prevalence rates of diabetes mellitus in Saudi Arabia: An overview. 2017;7(4):211–8.
12. Wu Z, Guo J, Huang Y, Cai E, Zhang X, Pan Q, et al. Diabetes mellitus in patients with pulmonary tuberculosis in an aging population in Shanghai, China: Prevalence, clinical characteristics and outcomes. J Diabetes Complications. 2016;30(2):237–41.
13. Ko P, Lin S, Tu S, Hsieh M. High diabetes mellitus prevalence with increasing trend among newly-diagnosed tuberculosis patients in an Asian population :A nationwide population-based study. 2015;0(375):148–55.
14. Barron MM, Shaw KM, Mckeever K, Ali MK, Magee MJ. Diabetes is associated with increased prevalence of latent tuberculosis infection : Findings from the National Health and Nutrition Examination Survey , 2011 – 2012. 2018;139:366–79.
15. Bridson T, Matthiesson A, Owens L, Govan B, Norton R, Ketheesan N. Diabetes : A Contributor to Tuberculosis in Tropical Australia. 2015;93(3):547–8.
16. Dooley KE, Tang T, Golub JE, Dorman SE, Cronin W.Impact of Diabetes Mellitus on Treatment Outcomes of Patients with Active Tuberculosis. 2009;80(4):634–9.
17. Mart G, Serrano CJ, Casta E, Enciso-moreno L, Garc Y, Lira D, et al. Associated Risk Factors for Latent Tuberculosis Infection in Subjects with Diabetes. 2015;46:221–7.
18. Nasir A, Hussain S, Siddiqui N, Umer K, Tabrez S, Sharma M. Diabetes & Metabolic Syndrome : Clinical Research & Reviews Detrimental association between diabetes and tuberculosis : An unresolved double trouble. 2018;12(6):1101–7.
19. Hah BARS. Quantifying the Risk of Infectious Diseases for People With Diabetes BAIJU. 2003;26(2).
20. Diwan VK, Thorson A. Sex , gender , and tuberculosis. 1999;353:1000–1.
21. Babamahmoodi F, Alikhani A, Charati JY, Ghovvati A, Ahangarkani F, Delavarian L, et al. Clinical Epidemiology and Paraclinical Findings in Tuberculosis Patients in North of Iran. 2015;2015.
22. Gao L, Li X, Liu J, Wang X, Lu W, Bai L, et al. Incidence of active tuberculosis in individuals with

latent tuberculosis infection in rural China : follow-up results of a population-based , multicentre , prospective cohort study. 2017;1053–61.

23. Obaseki DO, Jumbo J, Obaseki DO, Ikuabe PO. Tuberculosis and gender parity in a TB Referral Centre ,. 2012;2012.
24. Alkhawaja S, Romaihi E Al, Aljowder W, Fawzy N, El A, Azam M, et al. Epidemiology of Tuberculosis in Bahrain and Pre-employment Screening. 2018;3(1):1–4.
25. Owned G, Harcourt P, Azuonwu O, Ngozika WG. Investigation of Prevalence of Tuberculosis Infection Outcome in Two Government Owned Hospitals in Port Harcourt, Nigeria Delta. 2018;3(1):1–4.
26. Feng J, Huang S, et al. Gender differences in treatment outcomes of tuberculosis patients in Taiwan:a prospective observational study.2012.
27. Lindsay RP, Shin SS, Garfein RS, Rusch MLA, Novotny TE. The Association between Active and Passive Smoking and Latent Tuberculosis Infection in Adults and Children in the United States : Results from NHANES. 2014;9(3):1–8.
28. Dian Saraswati Lintang. Jurnal Kesehatan Masyarakat. J Kesehat Masy. 2014;8(2):113–20.
29. Chiang C, Slama K, Enarson DA. EDUCATIONAL SERIES : TOBACCO AND TUBERCULOSIS Associations between tobacco and tuberculosis SMOKE INCREASE THE RISK OF. 2007;11(3):258–62.
30. Rao VG, Bhat J, Yadav R, Muniyandi M, Bhondeley MK, Sharada MA, et al.Tobacco smoking : a major risk factor for pulmonary tuberculosis–evidence from a cross-sectional study in central India. 2014;(June):474–81.
31. Indonesia Ministry of Health. Republik Indonesia.2016.
32. Shelby T, Meyer AJ, Ochom E, Turimumahoro P, Babirye D, Katamba A, et al. Social determinants of tuberculosis evaluation among household contacts: a secondary analysis. Public Heal Action VOL 8 no 3 Publ 21 Sept 2018 Int. 2018;I(3):118–23.
33. Liang Li, Yan Lin, Fengling Mi, Shouyong Tan BL. Screening of patients with tuberculosis for diabetes mellitus in China. 2012;17(pp1294-1301).
34. Cobelens F, Kampen S Van, Ochodo E, Atun R, Lienhardt C. Research on Implementation of Interventions in Tuberculosis Control in Low- and Middle-Income Countries:A Systematic Review. 2012;9(12).
35. Hospital MG, Conroy J. Tuberculosis Control in New York City-A Changing Landscape. 2014;2362–5.
36. Dye C, Lönnroth K, Jaramillo E, Raviglione M. Trends in tuberculosis incidence and their determinants in 134 countries. 2009:683–91.
37. Pavan N, Moideen K, Sivakumar S, Menon PA, Viswanathan V, Kornfeld H, et al. Tuberculosis-diabetes co-morbidity is characterized by heightened systemic levels of circulating angiogenic factors. 2017;74(1):10–21.
38. Jeon CY, Murray MB. Diabetes Mellitus Increases the Risk of Active Tuberculosis. 2008;5(7).
39. Díaz A, Attilio LD, Bongiovanni B, Santucci N, Bertola D, Fern R, et al. An adverse immune-endocrine pro fi le in patients with tuberculosis and type 2 diabetes. 2016;101.
40. Abu SM, Omar MM, Al GF, Mohammad OE, Sh R. Tuberculosis situation in Port Said governorate before and after Direct Observed Therapy Short Course Strategy. 2015;64(2):441–7.

The Achievement of Ministry of Health Polytechnic Semarang as an Excellent Service University Upon Implementing A Decade of Internal Quality Assurance System

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ABSTRACT

Background: Since 2007 the head of the Semarang Health Ministry Polytechnic has established and implemented an Internal Quality Assurance System to guarantee the quality of services and graduates as well as to oversee the implementation of higher education regulations as a form of public accountability. The research objective was to examine trends in the level of student satisfaction with lecturer performance and education administration services since the quality assurance was implemented for a decade.

Method: The number of samples ranged from 10-20% with a random sampling technique from the population of Semarang Health Ministry Polytechnic students ranging from 5000 to 7,000 people spread across 7 campuses in 5 different cities in Central Java. Student satisfaction with lecturer performance and educational administration services is measured by giving questionnaires to students and then the data are analyzed by statistical correlation test of Pearson Product Moment.

Results: The results showed that for a decade the level of student satisfaction with lecturer performance and educational administration services continued to increase. There is a significant relationship between student satisfaction with education administration services.

Conclusion: Further research is qualitatively needed to assess the level of student satisfaction in a holistic manner related to matters that are considered important by students on lecturer performance and educational administration services.

***Keywords**: : Excellent Service, Quality Assurance, Health Polytechnic, A Decade.*

INTRODUCTION

Ministry of Health Polytechnic Semarang (MHPS) as one of the government-owned educational institutions that provide health education services in Indonesia is a community service institution that is an integral part of the national education system. Students are users of educational services, and administrative services are the essential resources for educational institutions [(1)]. Research on the level of student satisfaction with MHPS services shows that the level of student satisfaction is highest in aspects of lecturer performance, administrative and laboratory services [(2)]. Measuring the level of student satisfaction with lecturers' performance and administrative services in 1 decade (consecutive time) is very necessary for educational institutions as the primary capital in developing strategies to improve the quality of teaching, learning, various supporting services, and infrastructure. Improper strategy formulation can have a severe impact on institutions regarding improving the quality of education, design and curriculum development and institutional reputation [(3)]. On the other hand, if the strategy is formulated appropriately can have a significant impact on these matters above.

To improve and guarantee the quality of services and graduates, since 2007 PKS has implemented an ISO 9001-based Internal Quality Assurance System (SPMI) and obtained an ISO 9001 certificate in 2009 and obtained recertification every 3 years until June 2018. Based on the Decree of the National Accreditation Board of Universities High No. 0365 / SK / BAN-PT / Accredited / PT / I / 2017, MCC institutions have been

accredited with Good status. The accreditation of study programs is carried out by the Independent Accreditation Institution of Health Higher Education resulting in almost 50% of the 14 study programs with the A status accreditation (Excellent).

The level of student satisfaction with educational administration services is essential for higher education providers. The service is categorized as quality if the service received is following the expected service. The quality of higher education services is divided into 3 (three) dimensions, namely service to students, lecturers' abilities/performance and the physical environment of universities. The mission of MHPS is to organize a professional learning and teaching process. To support the implementation of the mission, it is necessary to analyze the extent to which the institution has provided services to its customers. Besides that, this is also an effort to support MHPS to maintain the certificate of ISO 9001 (*Quality Management System*) and improve the results of study program accreditation.

Since 2009 MHPS has been measuring and evaluating customer satisfaction every year, especially in the academic process which is the primary process of the education services business provided, based on the things mentioned above, the author wishes to examine the trend of the level of student satisfaction on the performance of lecturers and educational administration services from 2009 to 2017.

METHODOLOGY

This study included descriptive analytic research. The research data used were primary data from 2009 - 2017. Data collection techniques were used through observation, questionnaires, documents, and literature. The population is students spread across 7 campuses in 5 different cities in Central Java (Semarang, Magelang, Blora, Pekalongan, and Purwokerto) with an average of 5000-7000 students per year. Sampling is done by random sampling technique. The number of samples is calculated according to the formula [4] as below:

$$n = \frac{Z^2_{1-\alpha/2}\, P\,(1-P)\, N}{d^2\,(N-1) + Z^2_{1-\alpha/2}\, P\,(1-P)}$$

P = proportion of students not satisfied

d = precision = 5%

N = the number of students PKS

Z = 1.96

Based on this formula, each year the number of samples is obtained as follows:

Table 1. Sample Distribution

Year	Number of Samples (People)
2009	556
2010	624
2011	601
2012	512
2013	860
2014	850
2015	850
2016	850
2017	1150

Measurement of perceptions held students on lecturer performance and institutional administration services by giving questionnaires answered with Likert scale [5] ranging from Excellent (5), Good (4), Satisfactory (3), Poor (2), and Awful (1).

Items used to measure lecturer performance include: timeliness in the teaching and learning process, material mastery, the ability to arouse student attention to the courses given, ability to create student activities in thinking and acting, the ability to connect the lessons provided with the students' knowledge or experiences, the ability to choose the right learning media, providing refreshing on the material taught, giving opportunities for students to play an active role in group work, ability to understand and treat students according to their characteristics, and providing evaluations describing student progress, achievements, as well as shortcomings must be corrected.

The items used to measure educational administration services include the availability of learning physical administration facilities, accessibility and dissemination of learning administration service procedures, availability of building physical facilities, availability of physical media for learning, availability of administrative officers provide services, willingness of administrative officers to help and deliver services quickly, the friendly and trustworthy administrative officers and the attentive administrative officers.

The relationship between lecturer performance and educational administration services with student satisfaction was analyzed by using *Pearson Product Moment* correlation test with $\alpha = 0.05$.

RESULTS

The results of the validity test on lecturer performance variables between 0.508 to 0.698 and educational administration service variables are between 0.545 to 0.75. This shows that item statements to measure the variables of the performance of lecturers and educational administration services are valid. Further, the values of Cronbach's alpha ranged from 0.881 to 0.889. Thus the value of Cronbach's alpha on lecturer performance variables and education administration service variables is above 0.8 indicating that item statements to measure the variable performance of lecturers and educational administration services are reliable [6].

Age of respondents ranged from 18 to 22 years where most of the respondents were female (80%). The average level of student perceptions of the performance of lecturers showed that the highest student perceptions scored > 4 on the item "mastery of material" (4.15) and "giving students the opportunity to play an active role in group work "(4.04) while the lowest on the item "timeliness in the teaching and learning process" (3.64).

The level of student satisfaction with the performance of lecturers fluctuated with the lowest in 2010 (score 3.3) and the highest in 2017 with a score of 3.82. Most students (47.9%) are seen as "satisfied," and 37.6% with the category "dissatisfied" to the performance of lecturers and a small proportion of students felt very dissatisfied (7.8%) and very satisfied (6.8%). The highest score of student perceptions was > 4 on the item "availability of learning administration physical facilities in terms of attendance document, college journal, semester learning plan well "(4.04) and the lowest on the item of " availability of building physical facilities like lecture room, discussion and presentation room"(3.75). The score of student perceptions about the education administration service from 2009 to 2017 increased by 20% from 3.07 to 3.77. Most students (51.6%) were satisfied, and 34% of students felt dissatisfied with the educational administration services that had been done, while 7.5% of students felt very dissatisfied and 6.9% of students felt very satisfied. The results of the correlation analysis between lecturer performance and educational administration services with student satisfaction are shown in Table 2.

Table 2. Correlation between Lecturer Performance and Educational Administration Services to Student Satisfaction

No	Variable	Pearson Correlation	Coefficient of Determination (%)	p- Value
1	Lecturer Performance	0.579	33.50	0.102
2	Educational Administration Services	0.954	90.90	0,000

Table 2 shows a significant correlation (p-value <0.001) between educational administration services and student satisfaction levels while lecturer performance is not correlated with the level of student satisfaction.

DISCUSSIONS

The score of the level of student satisfaction towards the education administration service of the Ministry of Health Polytechnic Semarang (MHPS) from 2009 - 2017 has increased from year to year ranging from 20% from 3.07 to 3.77, approaching score 4 with satisfying categories. Continuous improvement is in line with the principle of the Internal Quality Assurance System which has been implemented by the Semarang Ministry of Health Polytechnic since 2007 to ensure the quality of services and graduates, oversee the ongoing implementation of higher education and as a form of public accountability, and education administration services begin 2 years later (2019). Improving the quality of education administration services continuously plays an essential role in maintaining and enhancing

the quality of education management [7]. In 2017, the Ministry of Health Polytechnic Semarang received an award certificate from the Ministry of Research and Higher Education , the Directorate General of Learning and Student Affairs for their earnest efforts in realizing a quality culture through the application of the Internal Quality Assurance System or in Indonesia is known as *Satuan Pengawas Mutu Internal* (SPMI). The use of internal quality assurance is an effort to improve the internal performance of educational institutions, especially teaching and learning methods and processes [8]. Since 2007 the MHPS has implemented SPMI based on ISO 9001, over time SPMI has been determined to follow government regulations and refer to the National Standards for Higher Education. The results of the application of SPMI have a significant impact on the results of accreditation, especially the accreditation of study programs, nearly 50% of the total 14 study programs have resulted in A (Excellent) accreditation status, the highest level in Indonesian educational accreditation system.

The results of this study indicate that better education administration services the higher the level of student satisfaction. The highest student perception scores > 4 are found on the item "availability of learning physical administration facilities (attendance document, college journal, semester learning plan) and functioning well." This result is consistent with the research conducted at the Nusa Dua Tourism College of Bali where academic service quality has a positive and significant effect on student satisfaction by 89.5% [9]. Student satisfaction can be increased by improving the quality of educational services through physical evidence variables, reliability, responsiveness, assurance, and empathy [10].

Educational administration services are positively correlated with student satisfaction. The quality of service dramatically influences customer satisfaction — services provided by service providers in proving something that is not really so that consumers can feel the services provided to consumers. Customer satisfaction is determined by various types of services customers get as long as they use several stages of the service. Satisfaction obtained in the early stages of service can lead to perceptions of excellent service quality for the next step so that customers feel satisfied with the overall service. The service situation is related to the internal conditions of the customer so that it affects service performance. Service performance can be determined by function, service process and the physical environment where service is provided. Customer perception of a service is determined by the three things above. Therefore, service providers must provide services beyond what is expected by consumers so that these service providers become the best from other service providers [11].

CONCLUSION

Although students of the Semarang Ministry of Health Health Polytechnic have been satisfied with the performance of lecturers and educational administration services, these institutions must continue to improve and improve SPMI continuously in line with the needs of stakeholders including students. To maintain lecturer performance and educational administration services, institutions need to allocate monetary resources to focus on developing human resources, especially discipline and expanding learning facilities on campus. Further research is qualitatively are necessary to assess the level of student satisfaction in a holistic manner related to matters that are considered important by students on lecturer performance and educational administration services.

Ethical Clearance: Ethical clearance was obtained from The Ministry of Health Polytechnic Semarang, Indonesia. We also wish to thank all the participants who contributed to this study.

Conflict of Interest: Nil.

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REFERENCES

1. Dahlstrom E, Brooks DC, Bichsel J. The current ecosystem of learning management systems in higher education: Student, faculty, and IT perspectives. Research report. Louisville, CO: ECAR, September 2014.
2. Nugraheni, H., Sudiarto, Lestari, S. & Kartikasari, Y. Applying cartesian gap analysis in determining internal and external service quality of educational provisions. International Journal of Multidisciplinary Education and Research, 2018: 3 (1): 21-25.
3. Grebennikov L, Shah M. Student voice: using qualitative feedback from students to enhance their university experience. Teaching in Higher Education. 2013 Aug 1;18(6):606-18.

4. Levy PS, Lemeshow S. Sampling of populations: methods and applications. John Wiley & Sons; 2013 Jun 7.
5. Teeroovengadum V, Kamalanabhan TJ, Seebaluck AK. Measuring service quality in higher education: Development of a hierarchical model (HESQUAL). Quality Assurance in Education. 2016 Apr 4;24(2):244-58.
6. Malhotra N. K.(2010). Marketing Research: An Applied Orientation Global Edition, 6th Edition, Upper Saddle River: New Jersey.
7. Sallis E. Total quality management in education. Routledge; 2014 Aug 7.
8. Chiaha GT, Nane-Ejeh SO. Quality assurance indicators for school transformation: A paradigm shift. International Letters of Social and Humanistic Sciences. 2014 Oct 8;42:72-81.
9. Rinala IN, Yudana IM, Natajaya IN. The effect of academic service quality to students' satisfaction and loyalty in the college of tourism Nusa Dua Bali. Journal of Indonesian Administrative Education. 2013 Sep 16;4(1).
10. Ali F, Zhou Y, Hussain K, Nair PK, Ragavan NA. Does higher education service quality effect student satisfaction, image and loyalty? A study of international students in Malaysian public universities. Quality Assurance in Education. 2016 Feb 1;24(1):70-94.
11. Yeo RK, Li J. Beyond SERVQUAL: The competitive forces of higher education in Singapore. Total Quality Management & Business Excellence. 2014 Feb 1;25(1-2):95-123.

Snakehead Fish (*Chana striata*) Powder Formulation for Increasing Calorie and Protein Intake in Malnourished Children

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ABSTRACT

Background: The cases of malnutrition in South Kalimantan are still high, and two districts which are included in the prevalence of undernourishment are South Hulu Sungai District and Batola District. This study aims to increase protein intake in children with malnutrition by giving additional input in the form of snakehead fish powder.

Method: The method of this study was quasi-experimental. This study used two groups with one treatment. The study was conducted at Berangas Health Center, Alalak Sub-District, Barito Kuala District, South Kalimantan. The research subjects were 50 people, with criteria for malnourished children under five who did not increase their weight scales in rows twice. The analysis used the t-test with the significance level used was $p < 0.05$.

Results: There was no difference in calorie intake between the group who received conventional toddler formula and the group who received the fish formula, and there was a difference in protein intake between the group who received conventional toddler formula and the group that received the recommended fish formula.

Conclusion:. The formula of snakehead fish is perfect for sufferers of malnutrition because the high protein in snakehead fish contains high levels of albumin that can improve cells and body tissues and help to boost growth in toddlers.

Keywords: *Malnutrition, toddlers, snakehead fish, South Kalimantan*

INTRODUCTION

Indonesia as a developing country still faces considerable nutritional problems. Malnutrition in children under five occurs because at that age more nutritional needs and toddlers are stages of maturity that are prone to nutrition. Lack of energy and protein is malnutrition due to low consumption of energy and protein in daily food, so it does not meet the nutrition adequacy rate.

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Protein deficiency is one of the leading nutritional problems that is often found in toddlers in Indonesia and other developing countries. Protein deficiency has an impact on growth, intellectual development, and productivity between 20 - 30%, while also having a direct effect on morbidity and mortality [(1)].

Based on Research data in 2013 cases of under nutrition in South Kalimantan were 8.2%, malnutrition 19.2% and tends to continue in recent years. This figure shows that cases of malnutrition in South Kalimantan are still high mainly in two districts: South Hulu Sungai District and Batola District. Malnutrition data in the Berangas Health Center in Alalak Subdistrict, Barito Kuala Regency up to June 2016 were: 1 malnutrition (0.045%), toddler (under the red line) 73 toddlers (3.3%)

and those treated in TFC (Therapeutic Feeding Center) as many as six people. This case increased compared to data in 2015, cases of malnutrition were one person (0.045%), toddlers 53 people (2.31%) and none were treated at TFC. In 2017, malnutrition continued to increase in Alalak District as many as 374 children under five (30.6%) with the highest nutritional status, namely in Beringin Village as many as 57 toddlers (16%) [2].

Nutritional therapy in malnourished children has a significant role in accelerating the healing of diseases. Errors in feeding will slow the healing of the disease. Excess or lack of nutrition can worsen the condition of the child, and can even cause death. Therefore, parents should be informed about the patient's rights in the care and feeding of malnourished children. Nutritional therapy for sufferers of malnutrition is applied in several phases, namely: the stabilization phase, the transition phase and the rehabilitation phase [3].

Various studies to improve nutritional status in sufferers of malnutrition including by providing snakehead fish extract which has a high albumin content can help increase albumin levels in patients with poor nutrition with low albumin levels below 2.5 mg%. Other studies also reported that the use of snakehead fish as a source of protein turned out to maintain the albumin value of patients hospitalized in the hospital so that it can help speed up the healing process of patients [4]. This study aims to determine the formula for fragrant fish to increase calorie intake and protein in children with malnutrition.

RESEARCH METHOD

This study was a quasi-experimental study, and this study used two groups with one treatment.. The research location was carried out at Berangas Health Center, Alalak District, Barito Kuala District, South Kalimantan. The subjects of the study were 25 control groups and 25 treatment groups with the criteria of malnourished children who did not increase their weight scales twice — respondent data consisting of name, age, gender. The data was collected by interviewing and filling out the questionnaire. Food intake data (formula of potatoes with snakehead fish) were obtained by talking the subject directly by using a 2x24 hour food recall form for two times a day. 2x24 hour food recall results were analyzed using *Nutri2008* — a different test using t-test. Toddler formula is a modification of the formula F 100 plus a special biscuit for toddlers. Snakehead Fish Formula is a modified formula for sufferers of malnutrition consisting of food ingredients: snakehead fish, potatoes, carrots, milk, vegetable oil, sugar, and salt.

RESULTS

The difference in calorie intake in the group of malnourished children under five who received formula toddler compared to the group of malnourished children under five who received the formula for snakehead fish was p = 0.079, which means there was no difference in calorie intake in the two groups. Differences in protein intake in the group of malnourished children under five who received formula toddler compared to the group of malnourished children under five who received snakehead fish formula was p = 0, 016 which means there is a difference in calorie intake in both groups.

Table 1. Calorie Intake Rate of Malnutrition Patients

Calorie Intake Rate Category	Toddler Formula Group		Snakehead Formula Fish Group		p-value
	n	%	n	%	
a. Over	17	68	7	28	
b. Good	5	20	5	20	
c. Mild Deficit	1	2	4	16	0.079
d. Moderate Deficit	0	0	2	10	
e. Severe Deficit	2	10	7	26	
Total	25	100	25	100	

Table 1 shows most levels of caloric intake in the group that received toddler formula were in over categories, namely 68% while in the group that received the formula for snakehead fish the biggest category was also over (28%) with p-value = 0.079.

Table 2. Protein Intake Under Nutrition Patients

Calorie Intake Rate Category	Toddler Formula Group		Snakehead Formula Fish Group		p-value
	n	%	n	%	
a. Over	11	44	14	64	
b. Good	12	48	6	16	
c. Mild Deficit	1	4	1	4	0.016
d. Moderate Deficit	0	0	2	8	
e. Severe Deficit	1	4	2	8	
Total	25	100	25	100	

Table 2 shows the level of protein intake in the group that received formula toddlers is mostly in the over category, which is 44%, while the group that gets the formula for snakehead the biggest category is also over (64%) with p-value = 0.016.

DISCUSSION

Optimizing the handling of nutritional problems in children under five can be done through diversifying the development of additional food formulas by considering the nutritional aspects, health benefits, acceptability, endurance and local food resource advantages —local food material, among others, in the form of snakehead fish (*Chana striat*) [5].

The advantages of snakehead fish include the womb protein is higher than other food ingredients such as eggs, chicken, and beef. Besides, collagen protein of snakehead fish is also lower than livestock meat, which ranges from 3-5% of total protein. Low collagen causes snakehead fish meat becomes easier to be digested by babies, elderly groups, and also people who just recovered from illness. [8]. Besides the snakehead fish is known as a fish that can heal wounds, reduce pain, and postoperative discomfort [9]. Many snakehead fish are caught in the area the waters of Kalimantan that are not far away from the community living area , and the processing is still limited to being dried fish or sold freshly. Potential of snakehead fish development for other purpose is big but if consumed directly there are still people who don't feel willing to consume because of the original head shape snakehead fish resembles a snake. If consumed fresh, it requires more seasoning because snakehead fish tastes plain. Processing of fish snakehead into flour can be an alternative in decreasing fishy aroma and enhancing acceptance and consumption of snakehead fish [10].

The level of caloric intake in toddlers who get formulas in the form of biscuits and milk, is more in the over intake category (68%), because the calorie content of cookies every 100 grams is 458 kcal, and in milk powder skim every 100 grams is 362 kcal. The form of food in the form of biscuits which are packaged in dry form becomes small portions, but the calorific value is high. The level of caloric intake in the group that received the formula for snakehead fish was much the same in the category of more consumption (28%) and the level of heavy deficit intake (28%). For comparison, 100 grams of potato contain 83 kcal, skim milk every 100 grams is 362 kcal and fragrant fish every 100 gram is 74 Kcal.

The level of protein intake in group I which received formula for toddlers was at most good intake categories (48%), and protein intake in group II who received the formula for the most type was 64%. The calorific value of fresh fish in 100 grams is 25.2 grams. Herring protein is also high in albumin. The high albumin content in snakehead fish causes this fish is used to overcome hypoalbumin [6].

Based on statistical tests (p = 0.079), there was no difference between group I calorie intake (toddler formula) and group II calorie intake (snakehead fish formula), and there was a difference between group I protein intake (formula toddler) with group II (fish formula snakehead) with a value of p = 0.016.

Calories in toddler formulas are given according to the condition and condition of toddlers' weight of malnutrition, formula F 100 containing 1000 kcal, protein 29 grams plus 30-gram biscuits at 137.4 kcal, protein 6.9 grams so that it amounts to 1137.4 kcal and 35, 9 grams of protein. This can increase calorie and protein intake for all malnourished toddlers. The formula, F 100 consists of 50 grams of sugar, 60 grams of vegetable oil, 80 grams of skim milk, added mineral mix and water could be in the form of a gel, so that it is diluted plus water and stirred until homogeneous, making it easy to consume. The number of Calories in a fragrant fish formula with a composition of 200 grams of potatoes, 10 grams of sugar, 20 grams of skim milk, 50 grams of harp fish, 30 grams of carrots, and 10 grams of vegetable oil for two recipes are 1142 Kcal and 36.6 grams of protein.

The value of calories and protein that are not different in the two formulas can be a reference for giving formulas to better sufferers of malnutrition. The difference in protein intake is more significant in the group that gets the formula for snakehead fish can be provided more often for sufferers of malnutrition. Snakehead fish that have high protein values also contain high albumin, and its function is to maintain fluid balance in the body that can prevent edema [7]. Another feature of albumin is to help the formation and repair of cell tissue in the body for the development and growth of thermal children [8].

Snakehead fish has a very high protein content of albumin, the results of the study revealed that in the nutritional content of snakehead fish there was 6.2% albumin. Albumin is a type of protein that is found in 60% of human blood plasma whose function is to maintain and improve tissue and improve health in patients with malnutrition and poor nutrition [4].

CONCLUSION

There is no difference in calorie intake in the group that received formula toddlers with a group of toddlers who received formula of fragrant fish. There was a difference in protein intake in the group that received formula toddlers with a group of toddlers who received formula of fragrant fish. The formula of snakehead fish is perfect for sufferers of malnutrition because high protein snakehead fish also contains high levels of albumin that can improve cells and body tissues and help to enhance the growth of toddlers.

Ethical Clearance: This study has been conducted an ethical review by the Banjarmasin Ministry of Health Polytechnic Research Ethics Commission with No. 257 / KEPK-PKB / 2018. We also wish to thank all the participants who contributed to this study.

Conflict of Interest: Nil.

Source of Funding: Nil.

REFERENCES

1. Stevens GA, Bennett JE, Hennocq Q, Lu Y, De-Regil LM, Rogers L, Danaei G, Li G, White RA, Flaxman SR, Oehrle SP. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. The Lancet Global Health. 2015 Sep 1;3(9):e528-36.

2. Rahman A, Lahdimawan A, Arifin S, Indriasari R. The Analysis of Risk Factors Associated with Nutritional Status of Toddler in Posyandu of Beringin Village, Alalak Sub-District, Barito Kuala District. Indian Journal of Public Health Research & Development. 2018 Oct 1;9(10).

3. Bhandari N, Mohan SB, Bose A, Iyengar SD, Taneja S, Mazumder S, Pricilla RA, Iyengar K, Sachdev HS, Mohan VR, Suhalka V. Efficacy of three feeding regimens for home-based management of children with uncomplicated severe acute malnutrition: a randomised trial in India. BMJ global health. 2016 Dec 1;1(4):e000144.

4. Romadhoni AR, Afrianto E, Pratama RI, Grandiosa R. Extraction of Snakehead Fish [Ophiocephalus Striatus (Bloch, 1793)] into Fish Protein Concentrate as Albumin Source Using Various Solvent. Aquatic Procedia. 2016 Aug 1;7:4-11.

5. Liu A, Zhao L, Yu D, Yu W. Study on malnutrition status and changing trend of children under five years old in China. Wei sheng yan jiu= Journal of

hygiene research. 2008 May;37(3):324-6.

6. Asikin AN, Kusumaningrum I. Albumin profile of snakehead fish (Channastriata) from East Kalimantan, Indonesia. In IOP Conference Series: Earth and Environmental Science 2018 Apr (Vol. 144, No. 1, p. 012035). IOP Publishing.

7. Herumuryawan M, Hardaningsih G. Effect of Striped Snakehead fish (ophiocephalusstriatus) extract supplement pills compared to human albumin infusion on Albumin Serum, Lipid Profile, Malondialdehyde and IL-8 serum level on Nephrotic Syndrome. Pakistan journal of medical & health sciences. 2017 Oct 1;11(4):1601-6.

8. Fallahi GH, Latifi S, Mahmoudi M, Kushki D, Ashtiani MT, Morteza A, Rezaei N. Growth Status and Its Relationship with Serum Lipids and Albumin in Children with Cystic Fibrosis. Acta Medica Iranica. 2016 May 10;54(4):276-9.

9. Sahid NA, Hayati F, Rao CV, Ramely R, Sani I, Dzulkarnaen A, Zakaria Z, Hassan S, Zahari A, Ali AA. Snakehead Consumption Enhances Wound Healing? From Tradition to Modern Clinical Practice: A Prospective Randomized Controlled Trial. Evidence-Based Complementary and Alternative Medicine. 2018.

10. Hidayati D, Faizah A, Prasetyo EN, Jadid N, Abdulgani N. Antioxidant Capacity of Snakehead Fish Extract (Channa striata) at Different Shelf Life and Temperatures. In Journal of Physics: Conference Series 2018 Jun (Vol. 1028, No. 1, p. 012021). IOP Publishing. 9.

Spatial Analysis of Hypertension Risk Factors Incidence in South Kalimantan Province

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ABSTRACT

Background: This study aims to identify spatial effects on risk variables that influence the incidence of hypertension in South Kalimantan Province because the region has specific geographical variations.

Method: The research method used is cross-sectional with a population of all households in the province, city districts in Indonesia. The data to be analyzed in this study is the incidence of hypertension data in 13 regencies of South Kalimantan Province.

Results: It is identified 5 groups of districts/cities in South Kalimantan Province that have similarities regarding risk factors affecting the incidence of hypertension.

Conclusion: The results obtained can be used by the relevant leaders as a consideration in determining policies to suppress and control the incidence of hypertension in the province of South Kalimantan by considering factors related to specific hypertension disease in the regency/city area of each group.

Keywords: *Spatial, Hypertension, South Kalimantan, Indonesia*

INTRODUCTION

Hypertension is one of the non-communicable diseases that become public health problem [(1)]. Uncontrolled hypertension can lead to degenerative diseases, such as congestive heart failure, kidney failure and vascular disease [(2)]. Hypertension is called the silent killer because it is asymptomatic and can cause fatal strokes. Although it cannot be treated, prevention and management can reduce the incidence of hypertension and accompanying diseases. Hypertension is the number 3 cause of death after stroke and tuberculosis, reaching 6.7% of the death population in all ages in Indonesia [(3)].

According to the guidelines of the World Health Organization (WHO) in 2011, one billion people in the world suffer from hypertension, two-thirds of whom are in developing countries that are low-middle income [(4)]. The prevalence of hypertension will continue to increase sharply, predicted in 2025 later, around 29% of adults worldwide suffer from hypertension. Hypertension has resulted in the deaths of approximately 8 million people each year, 1.5 million deaths occur in Southeast Asia, of which a third of the population suffers from hypertension [(5)].

Based on basic health research in 2013, the prevalence of hypertension in Indonesia obtained through measurements at ≥ 18 years of age was 25.8% revealed the highest in Bangka Belitung 30.9%, followed by South Kalimantan 30.8%, East Kalimantan 29.6% and West Java 29.4% [(6)]. The prevalence of hypertension in Indonesia obtained through a questionnaire diagnosed by health personnel was 9.4%, diagnosed by health personnel or taking medication at 9.5%. So there are 0.1% who make their own medicine — respondents who have normal blood but are taking hypertension medication at 0.7%. Thus the prevalence of hypertension in Indonesia is 26.5%.

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The aim to be achieved in this study is to identify spatial effects on risk variables that influence the incidence of hypertension in the province of South Kalimantan.

METHOD

This research is a secondary data-based documentation study. To complete the information contained in the research objectives required primary data collection through observational research with a survey approach. The data used were data from the 2013 Basic Health Research (Riskesdas) and BPS. The data is the result of a descriptive cross-sectional survey with a population of all households in the province, city districts in Indonesia. Primary data was also taken through a questionnaire on the respondents include knowledge, attitudes, self-concept, and family support — the location of this research in Banjarmasin Kalimantan Selatan. The research period was 2015 to 2016.

The data to be analyzed in this study is the incidence of hypertension in 13 regencies of South Kalimantan Province.

Table 1: Description of Research Variables

Variables	**Description of Variables**	**Types of Variables**
Y	Percentage of residents who have been diagnosed with hypertension / high blood pressure by health personnel	Response
Variables	Description of Variables	
X_1	Percentage of male population	Predictor
X_2	Percentage of population with primary education	Predictor
X_3	Percentage of population with smoking habits every day	Predictor
X_4	Percentage of people who have moderate physical activity habits.	Predictor
X_5	Percentage of population who have fruit consumption habits seven times a week	Predictor
X_6	Percentage of people who have a habit of consuming vegetables seven times a week	Predictor
X_7	Percentage of people who have a habit of consuming salty food more than one time per day	predictor
X_8	Percentage of people who have the habit of eating food fat/cholesterol / fried more than one time per day	predictors
X_9	Percentage of population with medical assurance / veteran / retired	predictors

RESULTS

The full Geographical Weighted Regression (GWR) model for each location is presented in the Appendix. Districts in South Kalimantan Province have different models of the prevalence of hypertension. Based on the variables that are significant for each area, a grouping of districts/cities is formed that has similar variables which significantly influence the prevalence of hypertension. The cluster of districts/cities that have related variables that have a significant effect on the incidence of hypertension is presented in Table 4

Table 2: District / City Grouping Based on Significance

Spatial Groups	Variables significance	Variables Description of Variables
Tanah Laut	X3, X4, X6	Percentage of hypertensive patients diagnosed (Y) Percentage of the population of male sex (X_1) Percentage of population with basic education (X_2) Percentage of population with daily smoking habits (X_3) Percentage of people with physical activity (X_4) Percentage of the population consuming fruit - ingredients seven times in 1 week (X_5) Percentage of people who consume vegetables seven times in 1 week (X_6) Percentage of people who drink salty food more than 1 time per day (X_7) Percentage of the population consuming fatty food consumption/cholesterol / fried food more than one time per day (X_8) Percentage of population with health insurance ownership (X_9)
Tanah Bumbu	X3, X4, X6	
Kota Baru	X4, X8	
Banjar	X4, X5, X6, X7	
Kota Banjarmasin	X4, X5, X6, X7	
Banjar Baru	X4, X5, X6, X7	
Barito Kuala	X3, X4, X9	
Tapin	X1, X5	
Hulu Sungai Selatan	X1, X5	
Hulu Sungai Tengah	X1, X5	
Hulu Utara River	X1, X5	
Tabalong	X1, X5	
Balangan	X1, X5	

Grouping based on significant variables is divided into five groups. There are districts/cities that have similar variables that have a significant effect on the surrounding areas, but there are districts/cities that have their uniqueness because the variables that are having a significant impact are not the same.

Based on the results of spatial regression, there are five groups. Districts/cities that have similar variables that have a significant effect on the surrounding districts/cities. The results of the above study indicate that group 1 consists of Tanah Laut and Tanah Bumbu Districts with characteristics Percentage of population with daily smoking habits (X_3), Percentage of people with physical activity (X_4), Percentage of the population consuming vegetables seven times in 1 week (X_6). Group 2, Kota Baru Regency with the characteristics of the percentage of the population having physical activity (X_4), Percentage of the population consuming consumption of fatty/cholesterol / fried foods more than once per day (X_8). Group 3 consists of Banjar Regency, Banjarbaru City, Banjarmasin City, with characteristics Percentage of population who have physical activity (X_4), Percentage of people who consume fruits 7 times in 1 week (X_5), Percentage of population consuming vegetables 7 times in 1 week (X_6), the percentage of people who drink salty food more than 1 time per day (X_7). Group 4 in Barito Kuala Regency with characteristics Percentage of population with smoking habits every day (X_3), Percentage of people with physical activity (X_4), Percentage of population with Askes / JPK PNS / Veteran / Pension ownership (X_9). Group 5 consists of Tapin Regency, Hulu Sungai Selatan, Hulu Sungai Tengah, Hulu Sungai Utara, Tabalong, Balangan with characteristics Percentage of the population of male sex (X_1), Percentage of the population consuming fruits seven times in 1 week (X_5).

DISCUSSION

Spatial analysis is a set of methods for finding and describing the level/pattern of a spatial phenomenon so that it can be better understood. By conducting spatial analysis, new information is expected to emerge which can be used as a basis for decision making in the area under study. The methods used are very varied, from visual observation to the use of mathematics / applied statistics [7].

As a method, spatial analysis seeks to assist planners in analyzing the condition of the problem based on data from the target area. And the concepts that underlie a spatial analysis are distance, direction, and relationships. The combination of the three regarding a region will vary to form a significant difference that distinguishes one location from another. Thus distance, direction, and the relationship between the position of an object in an area with objects in another region will have a clear difference. And these three things are things that are always present in spatial analysis with certain stages depending on the planner's perspective in looking at a problem of spatial analysis [8].

The analysis using the Geographic Weighted Regression (GWR) method aims to determine the variables that influence the prevalence of hypertension at each observation location, namely the sub-district in the province of South Kalimantan. The result described is the prevalence of hypertension modeling using the Geographic Weighted Regression (GWR) method.

CONCLUSION

The results obtained can be used by the relevant leaders as a material consideration in determining policies to suppress and control the incidence of hypertension in the province of South Kalimantan. By considering factors related to specific hypertension disease in the regency/city area of each group.

Ethical Clearance: Ethical clearance was obtained from The Ministry of Health Polytechnic Banjarmasin, Indonesia. We also wish to thank all the participants who contributed to this study.

Conflict of Interest: Nil.

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REFERENCES

1. Peltzer K, Pengpid S. The prevalence and social determinants of hypertension among adults in Indonesia: A cross-sectional population-based national survey. International journal of hypertension. 2018;2018.
2. Liang X, Chen J, Liu Y, He C, Li T. The effect of hypertension and diabetes management in Southwest China: A before-and after-intervention study. PloS one. 2014 Mar 14;9(3):e9180).
3. Marczak L, Williams J, Loeffler M. Global Deaths Attributable to High Systolic Blood Pressure, 1990-2016. Jama. 2018 Jun 5;319(21):2163.
4. Hatano S, Strasser T. Primary Pulmonary Hypertension: Report on a WHO Meeting, Geneva, 15–17 October 1973.1975. Geneva: World Health Organization. 2018.
5. Castillo R. SSA 03-1 Prevalence and management of hypertension in Southeast Asia. Journal of hypertension. 2016 Sep 1;34:e4.
6. O'Sullivan D. Geographically Weighted Regression: The Analysis of Spatially Varying Relationships, by AS Fotheringham, C. Brunsdon, and M. Charlton. Geographical Analysis. 2003 Jul;35(3):272-5.
7. Leung Y, Mei CL, Zhang WX. Statistical tests for spatial nonstationarity based on the geographically weighted regression model. Environment and Planning A. 2000 Jan;32(1):9-32
8. Fotheringham AS, Charlton ME, Brunsdon C. Geographically weighted regression: a natural evolution of the expansion method for spatial data analysis. Environment and planning A. 1998 Nov;30(11):1905-27.

Health Improvement after Childbirth with Traditional Snack Consumption

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ABSTRACT

Background:. After giving birth, most mothers complain of pain, so they are not fit. Traditional ingredients is usually consumed by postpartum mothers to restore health, but no research has been carried out to find the effectiveness. The study aimed to determine the effect of consuming traditional food called *kekerit* on fitness by using rats before applied further to human beings.

Method: The study was conducted using *randomized pre-post test control group design* on female, white rats that were made pregnant and waited until delivery. The sample was 18 rats consisting of 9 treatment groups and nine control groups. The study was conducted at the Laboratory of the Faculty of Veterinary Medicine, Bogor Agricultural University of Indonesia by assessing fitness through swimming three times on days 1, 5 and 11, and then the data were analyzed by employing *t-test.*

Results: The mean swimming pool of intervention rats 101.00 ± 6.65 seconds compared to controls 68.65 ± 6.58 seconds on the first day and there was a significant difference in the fitness of rats with p= 0.039 .

Conclusion: Upon successful completion test on rats, the health of postpartum mothers may be accelerated to recover after consuming *kekeri*t, but further research is needed to examine the feasibility.

Keywords*: Fitness, Strength, Post-Partum, traditional snack*

INTRODUCTION

After giving birth as many as 76% of women experience at least one health problem within eight weeks after giving birth. Feeling discomfort in the form of pain after childbirth, excessive sweating, breast swelling, constipation, hemorrhoids and perineal pain [(1)]. Overcoming this problem some people believe in doing taboos [(2)]. Perform rituals or drink concoctions so that they can improve health and strengthen the body [(3)].

The culture of drinking potions, usually herbs, especially Javanese people in Indonesia, is believed to be efficacious as a traditional treatment that is safer and cheaper compared to modern medicine. Mothers do this during pregnancy, childbirth, childbirth, and breastfeeding by 80.5% every day to drink it as an effort to maintain health [(4)]. This habit occurs in postpartum mothers, especially the Sundanese in the Permu Immigration Village, Kepahiang District, Bengkulu Province, in the form of consuming *kekerit* as a snack that is believed to be able to restore health during the postpartum.

Kekerits snack is a traditional powdered food consumed during childbirth consisting of grains, discoveries, cooking spices and leaves, roots or stems. Ingredients consist of the keratin grain the form of: *Oryza sativa, Arachis hypogaea L, Phaseolus radiatus L, Glycine max*, and a little *Zea mays* ssp. Mays. The herbs and spices: *Zingher officinale rocs*, *Kaempferia galangal L*, *Curcuma domestica Val,* as well as Various spices: *Myristica fragrans Hout, Ammonium cardamom*

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and other leaves such as medicinal plants [5].

Kekerit Snacks have been prepared and made by parents or family since the age of pregnancy over seven months. The way to make it is still simple using all the dried ingredients (the results of drying) are roasted and after being cold pounded until smooth and stored in a jar to be ready to be served. This habit has been passed down from generation to generation, originally from the Sundanese but because of its efficacy, other tribes such as Java, and the Serawai or Rejang tribes from the Bengkulu province also followed, thus becoming the local wisdom of this village [6].

The paradigm that develops in today's society, consuming *kekerit* as a traditional herb is felt to be beneficial which is harmless and has no side effects. This assumption is not entirely correct, for that there needs to be scientific proof through clinical trials using experimental animals such as a rat. The existence of these tests is expected to be able to find out the benefits of creaking in improving health, especially fitness. The purpose of this study was to determine the effect of consumption of snacks *kekerit* by a rat on physical fitness after childbirth.

METHOD

This research was conducted by *randomized pre-post test control group design,* which is to perform fitness tests in the treatment group by giving extra food *kekerit* while the control consumed only food standard ration from the Food and Drug Supervisory Board of the Republic of Indonesia (BPOM RI). The research was carried out at the Laboratory of the Faculty of Veterinary Medicine, Bogor Agricultural University, from April to September 2016. Samples were used by rat *Striped Sprague Dawley* female who had given birth to 18 children, aged 2.5-3 months, weighing 150-300grams.

Each experimental group was divided into 3 for observation of fitness on days 1, 5 and 11 in the control group or treatment through swimming test. The fitness test is carried out using rat swimming in a rectangular tub filled with water. Each mouse swims three times and counts in seconds from starting to swim to being unable to swim.

Before the treatment, rats were acclimatized for two weeks, and then rats were synchronized lust by using prostaglandin preparations with the double injection method. After that, the rats have been mated together using a 1: 3 comparison stud. Then the pregnant rat has waited until they give birth within 22-25 days.

Each rat was given a food ration based on the Indonesian Supervisory Food and Drink Board standard and placed in groups with the same environment and treatment, and the room temperature was set with a range of 20-28ºC, 50 + 10% humidity and a 12-hour dark-light cycle (RSPCA, 2011). Shortly after giving birth, the rats were weighed and then the treated rats were fed snacks *kekerit* with through the stomach every day with a dose of 0.370 gr/kg body weight as much as one administration at 9-10 is.

RESULTS

The content of substances Nutritional *Kekerit* Snacks

Kekerit snacks laboratory tests have been conducted at Bogor Agricultural Biochemistry at the content of nutrients contained in these foods are as follows:

Table 1. Substance Nutrient Content

No.	Component	%
1.	Macro Nutrient	
	a. Substances.Water	4.3% gr
	b. Ash	0.5% gr
	c. Protein	18.7% gr
	d. Fat	38.4% gr
	e. Rough Fiber	0.5% gr
	f. Carbohydrate g. Energy	5.6% gr 443 Kcal
2	Vitamins	
	Vitamin A	2.5 mcg / g
3	Analysis of minerals	
	a. Ferum	3.94 mg / g
	b.Zinc	0.22 mg / g
	c. Magnesium	0.02mg / g

Source: Biochemical Laboratory, 2016

Examination of bioactive components is analyzed to see whether there is nothing (qualitative) does not assess how much. Qualitative analysis results detected were flavonoids, triterpenoids, phenol and tannin (Table 2).

Table 2. Qualitative Analysis of Bioactive Components

	Qualitative Analysis	Indicators
	a.Alkaloid	Negative
	b.Flavonoids	Positive
	c.Triterpenoid	Positive
	d. Phenol	Positive
	e.Hydroquinone	Negative
	f.Saponin	Negative
	g. Tannins	Positive

Kekerit Snacks Effect Against Rat Physical Activity

Physical healthiness assessment of rats can be seen from the ability to swim and judged by the length of time that can be carried by rats to swim.

Table 3. Effect of Giving *Kekerit* Snacks Based on Swimming Time

Day	Parameters	Control	Treatment	*p-value*
1	Second	68.65 ± 6.58 101.00	± 6.65	0.039 *
5	Seconds	87.15 ± 18.17	129.50 ± 52, 04	0.391
11	Seconds	67.30 ± 9.90	173.15 ± 36.98	0.06

*Note: test paired sample t-test * <0.05 = significant*

DISCUSSION

Health for every human being is something important for activities. Fitness, especially from the first day after giving birth can support health, primarily due to pain and fatigue after childbirth [7]. Previous research has shown that postpartum mothers are physically active characterized by higher health awareness and can reduce the risk of postpartum depression [8].

The results showed that, the swimming ability of rat given snacks *kekerit* was longer. On the first day of, the control rats were only able to survive swimming for 68.65 seconds while the rats that received *kekerit* snacks were able to survive for 101 seconds. This situation shows that these rats are more fit than control rat, because swimming means being able to do activities and of course in a fit condition [9].

The ability to engage in energy needs, *kekerit* snacks contain 443 calories of energy derived from food in the form of 70% grain. The food is from black sticky rice, peanuts, soybeans, corn which is an energy source. High energy content in this substance can meet the additional food requirements during childbirth and breastfeeding. In the first six months it takes 330 calories of energy, 20 g of protein, 11 g of fat, 45 g of carbohydrate [10]. Based on Table.1 *Kekerit* snacks can meet these needs in 100 grams containing 18.7 grams of protein, 38.4 grams of fat, 5.6 grams of carbohydrates and 443 calories. The content of this nutrient can be an additional source of daily nutrients for nursing mothers. Nursing mothers generally feel hungry faster because at the same time the mother must fulfill her nutritional needs and produce milk. *Postpart*um mothers often consume *kekerit* snacks until 40 days postpartum. This high enough energy is good for supporting breastfeeding for babies and preventing supplementary feeding which is usually the reason for mothers not to breastfeed exclusively [11].

The presence of protein in *kekerit* snacks is beneficial in the postpartum period which functions as improving aerobic performance (endurance), increasing anaerobic capacity to increase strength and energy [12]. Also, protein is an essential substance for the structure and function of the body and the recovery of reproductive organs during postpartum [13].

The results of previous studies reported that, herbal medicine *Galohgor* contained Fe 0.221 mg, and Zinc 0.34 mg. While this study obtained a higher Fe content,

ie 3.94 mg and Zinc lower than 0.22 mg (Table.1). The need for Fe is 26 mg for breastfeeding mothers with an addition of 6 mg. Iron in food is in the form of iron-hem as in hemoglobin and myoglobin animal foods, and iron-them in plant foods. Iron can be absorbed by the body up to 25%, while ironnonhem can be absorbed by the body only 5%. 100% *kekerit* snack in plant foods, then 0.197 mg is absorbed and if the mother consumes 50 gram *kekerit* snacks per day, about 31% can be fulfilled from *kekerit* and can prevent the occurrence of anemia due to Fe deficiency and such cases often occur in postpartum mothers as much as 50-60% [14].

Flavonoids are beneficial for health as an anti-inflammatory, anti-oxidant and antiproliferative and anticancer activity [15]. Flavonoid food sources in kekerit snacks come from nuts which are as anti-oxidant [14]. In *kekerit* snacks contain spices such as kencur, ginger, turmeric. Ginger and turmeric contain flavonoids and *triterpenoids* (Table 2) can be used as a source of antioxidants and treatment of chronic diseases and mineral deficiencies [10]. *Triterpenoid* is a component found in plants that have an odor and can be isolated from plant material which functions to capture free radicals in the human body. One of the ingredients of *kekerit* is *Centella Asiatica* which contains *triterpenoids* which can improve the body's immune system, which contributes to the fitness of post-partum mothers [16].

CONCLUSION

Kekerit snacks tested at the rat can be beneficial to the health of postpartum mothers, supported from existing content in the form of macro-nutrients, vitamins and minerals, and antioxidants. Further testing needs to be done to humans or postpartum mothers so that the immediate effects and benefits are known that *kekerit* is a healthy traditional snack.

Ethical Clearance: This research has been carried out and obtained ethical approval from the Health Ministry Polytechnic Research Ethics Commission in Bengkulu (Approval Number: DM.01.04 / 007/3 / V / 2016). The authors would like to thank the laboratory of the Faculty of Veterinary Medicine and the laboratory IPB Biochemistry that has helped in the research process. The assistance provided is meaningful for the completion of this study.

Conflict of Interest: The author states that in this study there were no conflicts of interest.

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Conflict of Interest: Nil.

REFERENCES

1. Barennes H, Simmala C, Odermatt P, Thaybouavone T, Vallee J, Martinez-Ussel B, Newton PN, Strobel M. Postpartum traditions and nutrition practices among urban Lao women and their infants in Vientiane, Lao PDR. European journal of clinical nutrition. 2009 Mar;63(3):323.
2. Fikawati S, Syafiq A. Maternal Calorie Intake is a Significant Factor Associated with 6 Months of Exclusive Breastfeeding among Lactating Mothers in Depok City, Indonesia. Malaysian Journal of Nutrition. 2017 Jan 1;23(1).
3. Lo Bue MC, Priebe J. Revisiting the socioeconomic determinants of exclusive breastfeeding practices: evidence from Eastern Indonesia. Oxford Development Studies. 2018 Jul 3;46(3):398-410.
4. Bardosono S, Prasmusinto D, Hadiati DR, Purwaka BT, Morin C, Pohan R, Sunardi D, Chandra DN, Guelinckx I. Fluid intake of pregnant and breastfeeding women in Indonesia: a cross-sectional survey with a seven-day fluid specific record. Nutrients. 2016 Nov 22;8(11):651..
5. Smith JL, Brooker S. Impact of hookworm infection and deworming on anemia in non-pregnant populations: a systematic review. Tropical medicine & international health. 2010 Jul;15(7):776-95.
6. Koren G, Mills E, Dugoua JJ, Perri D. Herbal medicines in pregnancy and lactation: an evidence-based approach. CRC Press; 2006 Jan 17.
7. Kalisch BJ, Lee S, Dabney BW. Outcomes of inpatient mobilization: a literature review. Journal of clinical nursing. 2014 Jun;23(11-12):1486-501.
8. Jennings M, Smith JA. A resource book for lay members of ethical review and similar bodies worldwide. RSPCA Research Animals Department; 2015 Jan.

9. Yordi EG, Pérez EM, Matos MJ, Villares EU. Antioxidant and pro-oxidant effects of polyphenolic compounds and structure-activity relationship evidence. InNutrition, well-being and health 2012. InTech.
10. Xiao ZP, Peng ZY, Peng MJ, Yan WB, Ouyang YZ, Zhu HL. Flavonoids health benefits and their molecular mechanism. Mini reviews in medicinal chemistry. 2011 Feb 1;11(2):169-77.
11. Lamxay V, de Boer HJ, Björk L. Traditions and plant use during pregnancy, childbirth and postpartum recovery by the Kry ethnic group in Lao PDR. Journal of Ethnobiology and Ethnomedicine. 2011 Dec;7(1):14.
12. Jagim AR, Jones MT, Wright GA, Antoine CS, Kovacs A, Oliver JM. The acute effects of multi-ingredient pre-workout ingestion on strength performance, lower body power, and anaerobic capacity. Journal of the International Society of Sports Nutrition. 2016 Dec;13(1):11.
13. Dos-Santos JW, de Mello MA. Endurance swimming periodized training in rats. Journal of Exercise Physiology Online. 2010 Oct 1;13(5):29-44.
14. Koren G, Mills E, Dugoua JJ, Perri D. Herbal medicines in pregnancy and lactation: an evidence-based approach. CRC Press; 2006 Jan 17.
15. Xiao ZP, Peng ZY, Peng MJ, Yan WB, Ouyang YZ, Zhu HL. Flavonoids health benefits and their molecular mechanism. Mini reviews in medicinal chemistry. 2011 Feb 1;11(2):169-77.
16. Trinidad PT, Rosario SS, Marco PD, Aida CM, Melissa PB. Zingiber officinale and Curcuma longa as potential functional foods/ingredients. Food and Public Health. 2012;2(2):1-4.

Evaluation of Apelin, Periostin and Tartrate-resistant Acid Phosphatase-5B in Ankylosing Spondylitis Male Patients According to their Disease Activity

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ABSTRACT

Introduction: Apelin is a proinflammatory adipokine that can be induced by other inflammatory mediators. Periostin is produced by different types of tissue under the influence of inflammation and mechanical stress.

Aim: The aim of this study was to evaluate serum (Apelin, Periostin (POSTN), and Tartrate-resistant acid phosphates (TRACP)-5b among ankylosing spondylitis (AS) male patients with active and inactive disease and find the correlation between disease activity and the studied parameters.

Material and Methods: Fifty-eight male Ankylosing spondylitis (AS) patients were included in this study. Serum Apelin, Periostin (POSTN), and tartrate-resistant acid phosphates (TRACP)-5b were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits. Disease activity was determined by using a BASDAI score.

Results: Ankylosing spondylitis (AS) male patients with active disease had a significantly high serum level of serum TRACP-5b (P-value <0.001) while serum of (Apelin and Periostin) levels shows no significant differences (P-value > 0.05) when to AS males with inactive disease.

Conclusion: The results of this study suggest that serum Apelin and periostin levels show no differences among AS male patients with active when compared to AS male patients with the inactive disease, but serum TRACP-5b was elevated in AS male patients with active disease.Correlations between disease activity (BASDAI score), and serum POSTN and with serum TRACP-5b have been found.

Keywords: *Ankylosing spondylitis, Apelin, Periostin, TRACP-5b, and BASDAI.*

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic, systemic rheumatic disease with long-term inflammation that affects mostly the joints of axial skeleton (sacroiliac and other joints in the spine (vertebra) and less commonly peripheral joints[1]. AS influence the quality of life, the newly formed bones (at vertebral sides) is the main factor to determine the disease's outcome[2]. Morningstiffness of more than one hour, fatigue and the pain in the lower back at the night and in the morning arethe mainsymptoms of AS[3]. The pathogenesis of AS consist of a number of different inter-related processes, each of which is controlled by different pathways and factors[4]. There is a combination of genetic and nongenetic factors leads to clinical disease[5]. Ankylosing spondylitis is mostly affecting young people[6]. AS is seen in male more than female, the ratio of 2:1, and the familial occurrence is common[3,7]. The diagnosis of AS before the occurrence of permanent damage is not easy, the diagnosis usually delays between 5 to 10 years from the onset of disease symptoms due to missed diagnose of young men with

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inflammatory back pain (IBP) and the radiographic changes in the sacroiliac joints (a late feature of the disease) needed many years to appear by conventional radiology[1,8,9]. The diagnosis of AS is made by a mixture of radiological changes, clinical features, and laboratory results, however, classification criteria are available for patient categorization[3]. Apelin is a one of adipokines, is an endogenous peptide that has the ability to bind to the apelin receptor (APJ or AR) which is also known as an orphan G protein-coupled receptor (GPCR)[10]. Apelin can act as pro-inflammatory adipokines, in addition to that other inflammatory mediators such as (TNF-α and IL6) can induce it is an expression[11]. It has been found that apelin increases in obesity, cardiovascular disease, cancer[12-15].

Periostin (POSTN) is a 90 KD, secretory matricellular protein consisting of 836 amino acids in human [16]. POSTN is a multi-functional proteinsince it can express by a wide range of human tissue due to mechanical stress or injury[17]. It is involved in the bone anbolism via regulation of Wnt-β-catenin signaling. It also plays a major role in the pathogenic mechanisms of syndesmophyte formation in ankylosing spondylitis[36]. TRACP-5b is specifically from osteoclast thus TRACP-5b considered as a new, sensitive important biomarker of bone resorption, and data show that it is the only marker reflecting osteoclasts number and metabolic activity of osteoclast[18]. Various techniques are available for the measurement of AS. However, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is a self-administrating tool that composed of six formulated questions, each of which has ten scores that assess fatigue, spinal pain, joint pain, and the duration and the severity of morning stiffness use to determine the activity of the disease[19]. With this background, the aim of the present study is to compare serum apelin, periostin, and TRACP-5b levels among AS male patients according to their disease activity (BASDAI Score). Also to find the correlation between disease activity and the studied parameter in the ankylosing spondylitis.

MATERIALS AND METHOD

Patient enrollment

Fifty -eight male patients with ankylosing spondylitis under the age of 50 were involved in this study. Oral consent was taken before enrolled the patients in the study.

Blood biochemistry

The blood samples were taken from both healthy males and AS male patients in fasting condition to determine serum (Apelin, Periostin and TRACP-5b) levels using commercial ELISA Kits and according to the manufacturer's instructions (the kits were manufactured by My BioSource Inc., USA). Patients with diseases other than AS have been excluded from the study. All patients were subjected to Bath ankylosing spondylitis disease activity index (BASDAI) questionnaire to determine disease activity. Patient with Scores of 4 or greater suggest active disease, and patients with scores below 4 refer to the inactive disease[20].

Statistical analysis

Statistical analysis was done using SPSS version 23 computer software.

RESULTS

Table 1 represents the variation of apelin, periostin and tartrate-resistant acid phosphatase in active and inactive BASDAI condisions. Statistically non-significant (P=0.51) difference in serum apelin level (Mean±SD) were found in the inactive AS disease (2556.9±439.2pg/ml) as compared to the active AS disease (2488.0±351.6pg/ml). Similar results were obtained with the serum periostin levels. While, a significant increase (P<0.001) in serum TRACP-5b level (Mean±SD) was found in with active disease (7.4±1.9mlU/ml) when compared to the inactive disease (5.5±1.8mlU/ml). Scatter diagram with the fitted regression line showing the linear correlation between disease activity (BASDAI score) and serum periostin among patients with ankylosing spondylitis (AS) is depicted in the Figure 1.

Table 1: The comparison of the studied parameter according to disease activity in ankylosing spondylitis (AS) male patients.

	Disease activity		
Parameter	**Inactive (BASDAI score <4) No= 28**	**Active (BASDAI >= 4) No=30**	**P-value**
Apelin (pg/ml)	2556.9 ±439.2	2488 ±351.6	0.51 [NS]
Periostin (ng/ml)	3.3 ±0.7	3.0 ±0.6	0.06 [NS]
Tartrate-resistant acid phosphatase (mlU/ml)	5.5 ±1.8	7.4 ±1.9	<0.001 [S]

[S]= significant differences between two independent means using student-t-test at 0.05 level. Mean±SD

The Correlation coefficient (r) and significance between the studied parameters in ankylosing spondylitis (AS) male patients and disease activity were depicted in Table 2. There was a significant weak positive correlation between disease activity and serum TRACP-5b (r=0.371, P=0.004) and a significant weak negative correlation between disease activity and serum POSTN (r=-0.34, P=0.009), while no correlation has been found between disease activity and serum Apelin.

Table 2: The Correlation coefficient (r) and the significance between the studied parameters in ankylosing spondylitis (AS) male patients and disease activity.

	Serum Apelin (pg/ml)	**Serum Periostin (ng/ml)**	**Serum Tartrate resistant acid phosphatase (mlU/ml)**
Disease activity (BASDAI score)	r= -0.03 P=0.82[NS]	r= -0.34** P=0.009	r= 0.371** P=0.004

[NS] =no significant difference between two independent means using students-t-test at

0.05 level.

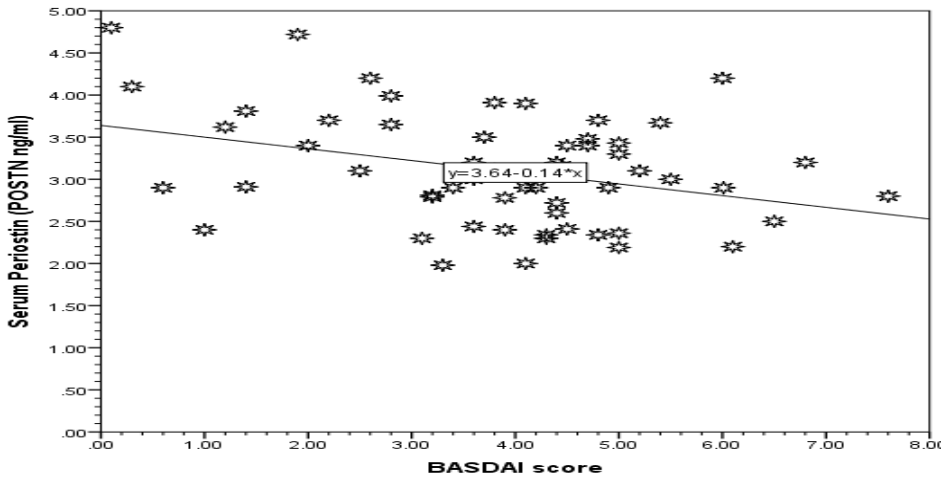


Figure 1: Scatter diagram with the fitted regression line showing the linear correlation between disease activity (BASDAI score) and serum periostin (ng/ml) among patients with ankylosing spondylitis (AS).

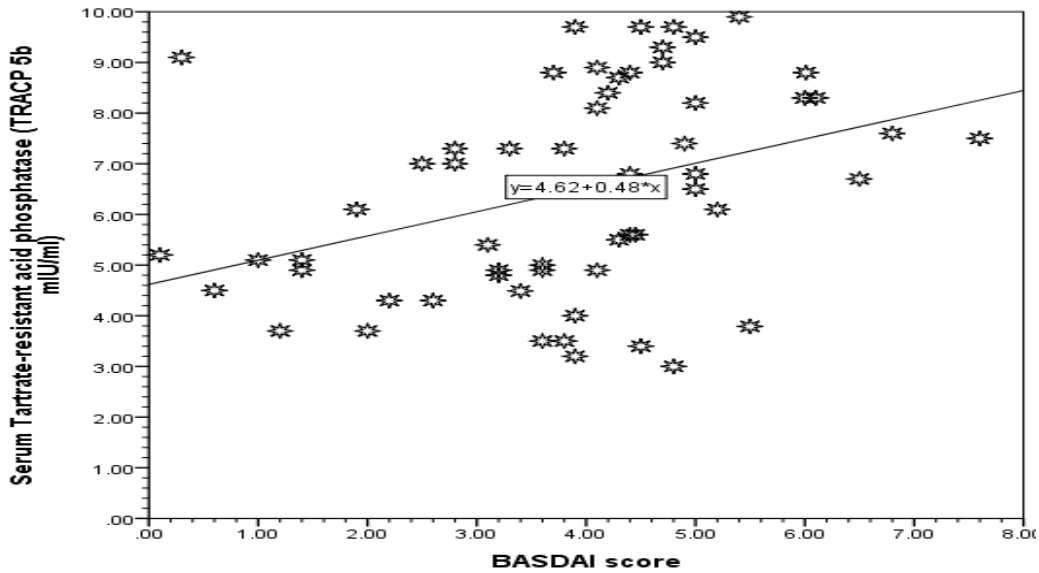


Figure 2: Scatter diagram with the fitted regression line showing the linear correlation between disease activity (BASDAI score) and serum Tartrate-resistant acid phosphatase (mlU/ml) among male with ankylosing spondylitis (AS).

DISCUSSION

Ankylosing spondylitis (AS) is a long-term inflammation of the spine in addition to other joints; it affects men more than women usually in young age and associated with significant alterations in the bone metabolism[21,22]. Hence, in the present study only male individuals are considered. Apelin, adipokines can promote inflammation in obese person through playing the key role in the communication between the adipose tissue and the immune system but the mechanism is not totally known[23,24]. The ample amount of literature reported the correlation of apelin with obesity[25-30]. Periostin as a molecule is expressed in a wide variety of tissues; it has the ability to act as a structural and signaling molecule[16].

This study shows no significant change in serum apelin, and periostin levels but there is a significant increase in TRACP-5b serum level of AS male patients with active disease (Table 1). In addition to that, it shows a positive correlation between disease activity and serum TRACP-5b (r = 0.371, p=0.004) (Table 2, Figure 1) which agrees with the previous studies[2,31]. A positively correlated between serum TRACP-5b level and disease activity were reported in previous studies[2,31] which inturn indicated the reduction in bone formation and the elevation in bone loss are the manifestations of bone metabolism of AS patients as a result of the inflammatory process[27,28]**. High disease activity associated with an increase and related to bone resorption process in patients with AS[32,33,38]. While the finding of this study disagrees with Éric** et al[34] that found no correlation between disease activity and TRACP-5b possibly due to differences in sample size.

This study also shows a negative correlation between disease activity and serum POSTN level (r=-0.340, p=0.009) (Table 2 and Figure 2). The results are agree with the previous studies[35]. Solmaz et al[35] that suggested the suppression of periostin expression in AS patients occurred with high disease activity and related to the progression of radiological damage. While, another study[36] reported lower activity in initial stages which elevated in advanced disease activity, systemic inflammation simultaneously less extensive radiographic damage due to the role of IL-13.

In the present study, serum Apelin level shows no correlation to disease activity (Table 2). Our results are in accordance with previous reports[32,34]. The accumulation of adipocytes there by increased in apelin occurring under obesity condition can be closely linked with functional ability and structural integraty of lymphatic vessels but no bone structure and function. Apelin signaling leads to enhanced lymphatic and blood vessel integrity[26,35-37]. However, Hartavi et al[39] showed a negative correlation between serum apelin and disease activity. Is worth to mention that the association between the activity of the disease and bone turnover markers has not been fully studied[35].

CONCLUSION

In the present study, no correlation has been found between serum apelin and disease activity. A weak negative correlation was found between disease activity and serum POSTN. A weak positive correlation was found between disease activity and serum TRACP-5b.

Ethical Clearance: The ethical clearance was taken from College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq. Oral consent was taken before enrolled the patients in the study.

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. Ghasemi-Rad M, Attaya H, Lesha E, Vegh A, Maleki-Miandoab T, Nosair E, Sepehrvand N, Davarian A, Rajebi H, Pakniat A, Fazeli SA, Mohammadi A. Ankylosing spondylitis: A state of the art factual backbone. World J Radiol. 2015;7(9):236–252.
2. Park JH, Lee SG, Jeon YK, Park EK, Suh YS, Kim HO. Relationship between serum adipokine levels and radiographic progression in patients with ankylosing spondylitis A preliminary 2-year longitudinal study. Medicine (Baltimore). 2017; 96:33-7854.
3. Heijde DVD. Ankylosing Spondylitis. In: Klippel JH, Stone JH, Crofford LJ, H. P, editors. Primer on Rheumatic Diseases.Thirteenth. 2015. p. 193–199.
4. Longo DL. The spondyloarthritides. In: Anthony S. Fauci, editor. Harrison Textbook of Rheumatology.3rd ed. Mcgraw hill company education; 2013. p. 136–40.
5. Khan MA. Update on spondyloarthropathies. Ann Intern Med. 2002;136(12):896–907.
6. Sieper J. Ankylosing spondylitis. In: Watts RA, Conaghan PG, Denton C, Foster H, Isaacs J, Müller-Ladner U, editors. Oxford testbook of rheumatology. fourth. United kingdom: oxford university press; 2013. p. 879–89.
7. Ranjith MP, Divya R. A case of ankylosing spondylitis and discussion of the literature. J Clin Diagnostic Res. 2013;7(6):1180–2.
8. Sieper J, Braun J, Rudwaleit M, BoonenA, Zink A. Ankylosing spondylitis : an overview. Vol. 61, Ann Rheum Dis. 2002.
9. Shaikh S A. Ankylosing spondylitis: recent breakthroughs in diagnosis and treatment. J Can Chiropr Assoc. 2007;51(4):249–60.
10. Hu PF, Tang JL, Chen WP, Bao JP, Wu LD. Increased apelin serum levels and expression in human chondrocytes in osteoarthritic patients. Int Orthop. 2011;35(9):1421–6.
11. Wattanachanya L, Lu WD, Kundu RK, Wang L, Abbott MJ, O'Carroll D, Quertermous T, Nissenson RA. Increased bone mass in mice lacking the adipokine apelin. Endocrinology. 2013;154(6):2069–89.
12. Zhang L, Takara K, Yamakawa D, Kidoya H, Takakura N. Apelin as a marker for monitoring the tumor vessel normalization window during antiangiogenic therapy. Cancer Sci. 2016; 107(1):36-44.
13. Sonmez A, Celebi G, Erdem G, Tapan S, Genc H, Tasci I, Ercin CN, Dogru T, Kilic S, Uckaya G, Yilmaz MI, Erbil MK, Kutlu M. Plasma apelin and ADMA Levels in patients with essential hypertension. Clin Exp Hypertens. 2010; 32(3):179-183.
14. Bircan B, Cakır M, Kırbag S, Gul HF. Effect of apelin hormone on renal ischemia/reperfusion induced oxidative damage in rats. Ren Fail. 2016; 38(7):1122-1128.
15. Wysocka MB, Pietraszek-Gremplewicz K, Nowak D. The role of apelin in cardiovascular diseases, obesity and cancer. Front Physiol. 2018;9:557.
16. Bonnet N, Garnero P, Ferrari S. Periostin action in bone. Mol Cell Endocrinol. 2016;432(1):75–82.
17. Idolazzi L, Ridolo E, Fassio A, Gatti D, Montagni M, Caminati M, Martignago I, Incorvaia C, Senna G. Periostin: The bone and beyond. Eur J Intern Med. 2017;38(3):12–16.
18. Szulc P, Bauer DC. Biochemical Markers of Bone Turnover in Osteoporosis. Fourth Edi. Osteoporosis: Fourth Edition ; 2013:1573-1610.
19. Bobek D. Scoring of disease activity using BASDAI and ASDAS method. reumatizam. 2012;59(1):5–10.
20. Zochling J. Measures of symptoms and disease status in ankylosing spondylitis. Arthritis care Res. 2011;63:S47–S58.

21. Chen HH, Chen TJ, Chen YM, Ying-Ming C, Chen DY. Gender differences in ankylosing spondylitis-associated cumulative healthcare utilization: a population-based cohort study. Clinics. 2011;66(2):251–154.

22. Grisar J, Aringer, M K Redlich, PM Bernecker, W Wolozcszuk, JS Smolen PP. Ankylosing Spondylitis, Psoriatic Arthritis, and Reactive Arthritis Show Increased Bone Resorption, But differ with Regard to Bone Formation. Ann rheumdis. 2001;60(suppl 1):387.

23. Caruso C, Balistreri CR, Candore G. The role of adipose tissue and adipokines in obesity-related inflammatory diseases. Mediators Inflamm. 2010;2010:19.

24. Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: Not a passive bystander. Autoimmun Rev. 2014;13(9):981–1000.

25. Tapan S, Tascilar E, Abaci A, Sonmez A, Kilic S, Erbil MK, Ozcan O. Decreased plasma apelin levels in pubertal obese children. J Pediatr Endocrinol Metab. 2010; 23(10):1039-46.

26. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci. 2013; 9(2):191-200.

27. Kotanidou EP, Kalinderi K, Kyrgios I, Efraimidou S, Fidani L, Papadopoulou-Alataki E, Eboriadou-Petikopoulou M, Galli-Tsinopoulou A. Apelin and G212A apelin receptor gene polymorphism in obese and diabese youth. Pediatr Obes. 2015,10(3):213-9.

28. Ba HJ, Chen HS, Su Z, Du ML, Chen QL, Li YH, Ma HM. Associations between serum apelin-12 levels and obesity-related markers in Chinese children. PLoS One. 2014; 9(1):e86577.

29. Krist J, Wieder K, Kloting N, Oberbach A, Kralisch S, Wiesner T, Schon MR, Gärtner D, Dietrich A, Shang E, Lohmann T, Drebler M, Fasshauer M, Stumvoll M, Bluher M. Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. Obes Facts. 2013; 6(1):57-69.

30. Wang S, Liu J, Cao Y, Zhang J. Changes of serum bone gla protein and tartrate-resistant acid phosphatase and their influencing factors in patients with ankylosing spondylitis: A Report of 60. J Anhui Tradit Chinese Med Coll. 2012;2012:1.

31. Park MC, Chung SJ, Park YB, Lee SK. Bone and cartilage turnover markers, bone mineral density, and radiographic damage in men with ankylosing spondylitis. Yonsei Med J. 2008;49(2):288

32. Lange U, Jung O, Teichmann J, Neeck G. International original article relationship between disease activity and serum levels of vitamin D metabolites and parathyroid hormone in ankylosing spondylitis. Osteoporos Int. 2001;12(12):1031–5.

33. Ghozlani I, Ghazi M, Nouijai A, Mounach A, Rezqi A, Achemlal L, Bezza A, El Maghraoui A. Prevalence and risk factors of osteoporosis and vertebral fractures in patients with ankylosing spondylitis. Bone. 2009;44(5):772–6.

34. Éric T, Dumoulin G, Saas P, Nguyen NU, Hue G Le, Wendling D. Increased tartrate-resistant acid phosphatase serum levels in ankylosing spondylitis and relationship with the inflammatory process. Ann Rheum Dis. 2008;67(3):430–2.

35. Solmaz D, Uslu S, Kozacı D, Karaca N, Bulbul H, Tarhan EF, Ozmen M, Can G, Akar S. Evaluation of periostin and factors associated with new bone formation in ankylosing spondylitis: Periostin may be associated with the Wnt pathway. Int J Rheum Dis. 2018, 21(2):502-509.

36. Sakellariou GT, Anastasilakis AD, Bisbinas I, Oikonomou D, Gerou S, Polyzos SA, Sayegh FE. Circulating periostin levels in patients with AS: Association with clinical and radiographic variables, inflammatory markers and molecules involved in bone formation. Rheumatol. 2014;54(5):908–14.

37. Genre F, Miranda-Filloy JA, Lopez-Mejias R, Carnero-Lopez B, Ochoa R, Rueda J, González-Juanatey C, Blanco R, Llorca J, González-Gay MA. Apelin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-alpha antagonist therapy. Clin Exp Rheumatol. 2013;31(4):532–7.

38. Sawane M, Kajiya K, Kidoya H, Takagi M, Muramatsu F, Takakura N. Apelin inhibits diet-induced obesity by enhancing lymphatic and blood vessel integrity. Diabetes. 2013; 62(6):1970-80.

39. Hartavi A, Alayli G, Alacam H, Bilgici A, Durmus D, Bedir A, Kuru O. Apelin level in ankylosing spondylitis: association with disease activity, clinical parameters and radiographic changes. Ann Rheum Dis. 2014, 73. 1039-1040.

Sleep Disturbances and Self-Management among Adolescents with Nocturnal Asthma in Al- Najaf City

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ABSTRACT

Self-management improves quality of life among adolescents with nocturnal asthma. This study aimed to identify sleep disturbances and self-managements regarding asthmatic attack among adolescents with nocturnal asthma. Therefore, a cross sectional study was conducted at the Emergency Department in Al-Najaf, during the period October 15th 2017 to September 18th 2018. The study included 100 adolescents with nocturnal asthma who met the inclusion criteria, a pre-structured validated questionnaire was used, including demographic information, sleep disturbances variables and self-managements questionnaire. Data were managed and analyzed using the statistical package for social sciences software version 25 and appropriate statistical tests were applied accordingly. The findings revealed that (66.6%) of patients had moderate and (30%) had severe sleep disturbances. Also, (77.7%) of patients had a poor and (22.2%) had fair self-management. It had been significantly found that females were less aware for self-management, ($P<0.05$), other characteristics showed no significant association, ($P>0.05$). In conclusion, an evidence of sleep disturbances among the adolescents that result from asthmatic attack during the night. Deficit knowledge regarding self-management of asthmatic attack and other female gender can contributed to such attacks.

Keywords: *Nocturnal asthma, Sleep disturbances, Self-management .*

INTRODUCTION

Asthma is a chronic inflammatory disease complex that occurs in the airways(bronchi) in the lungs. Being widespread globally, asthma kills one person in every 250 deaths in the world [1]. According to the World Health Organization(WHO) estimate in 2005 it affects around 300 million people worldwide. Asthma is among the highest twenty chronic conditions for global ranking of disability-adjusted life years in adolescents. Death rates from asthma in children globally range from 1% to 7% of 100 000. The prevalence of asthma has increased in developing countries over the last 30 years but now appears to have stabilized, with about 10–12% of adults, 12-14% of adolescents and 15% of children affected by the disease. In developing countries the prevalence of asthma had been much lower [2].

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Asthma is characterized by recurrent attacks of breathlessness and obstruction of airflow causes frequent occurrence of wheezing, shortness of breathing, chest tightness, and cough especially nighttime, or early morning which vary in severity and frequency from person to person. These episodes are usually associated with variable airflow obstruction that is often reversible, either spontaneously or with treatment [3]. Nocturnal asthma usually worsens at night, the systems representing this nocturnal worsening of symptoms are not totally seen but rather might be driven by circadian rhythms of circulating hormones (epinephrine, cortisol, and melatonin and neural instruments). An increase in airway inflammation at night has been reported. This finding may reflect a reduction in endogenous anti inflammatory mechanism [4,5] . It has also been shown that adolescents are more prone to shorter sleep duration and poor sleep habits. Focusing on adolescent youth is critical as many health-related behavioral patterns that are formed during adolescence continue in adulthood and affect adult health outcomes[6] .

Adolescent sleep is important because it might be both a cause and the result of health problems. However, it still remains a neglected topic in adolescent health research, partly because there are widespread misunderstandings about adolescents' needs for sleep. In fact, the sleep needs of teenagers are not very dissimilar to those of primary school aged children. They remain higher than adult needs across the second decade of life; probably because sleep plays a big part in creating the environment for healthy brain development, adolescent brains are still changing and refining across this age period [7].

Patients and Method

This was a cross-sectional study carried out in Al-Najaf city-Iraq at the Center for Allergy and Asthma in Al-Sadr teaching hospital, and the emergency department of Al-Hakim and Al-Zahra teaching hospitals during the period 15th October 2017 to 18th September 2018. Included 100 adolescents with nocturnal asthma

Study Instrument included the following parts:

Part I: Patients' Demographic Data and Clinical Data

Part II: Patients' Sleep disturbances in asthmatic adolescent scale

Part III: Asthma self-management questionnaire .

Data were collected through a structured interview technique with the subjects and the statistical analysis performed using the statistical package for social sciences and appropriate statistical tests and procedures applied accordingly with level of significance of < 0.05.

FINDINGS

Sleep disturbances were mild in 4%, moderate in 66% and severe in 30% of the studied group, (Table 1). Responses of the study participants towards Self-management items were poor in most of these items giving poor overall self-management evaluation , (Table 2). No statistically significant association had been found between self- management and sleep disturbances from one side and demographic characteristics of the studied group from the other side except one correlation was significant which was between female gender and poor Self-Management, (P<0.05), (Table 3), furthermore, females had significantly lower mean score of self-management, 3.91 ± 1.97 compared to males (mean score: 4.94 ± 2.41), (P<0.05), (Table 4)

Table 1. Distribution of sleep disturbances among the studied group

Sleep disturbance	No.	%
Mild	4	4.00
Moderate	66	66.0
Sever	30	30.0
Total	100	100.0

Table 2: Responses and evaluation of the study participants toward the self-management questionnaire items (N = 100).

Item	Response				Mean score	Evaluation
	Preferred		Unpreferred			
	No.	%	No.	%		
Methods of prevent asthma	0	0.0	100	100.0	0.00	Poor
Taking a prescribed two puffs	39	39.0	61	61.0	0.39	Fair
Not having asthma symptoms	63	63.0	37	37.0	0.63	Fair
Maintenance medicines	19	19.0	81	81.0	0.19	Poor
Correct way to use peak flow	29	29.0	71	71.0	0.29	Poor
Rescue medicines	8	8.0	92	92.0	0.08	Poor
What using your inhaler	4	4.0	96	96.0	0.04	Poor
After using inhaler	56	56.0	44	44.0	0.56	Fair

Cont... **Table 2: Responses and evaluation of the study participants toward the self-management questionnaire items (N = 100).**

Having a symptoms	27	27.0	73	73.0	0.27	Poor
Taking more medicine than prescribed	23	23.0	77	77.0	0.23	Poor
Benefits of using peak flow meter	2	2.0	98	98.0	0.02	Poor
Exercises	21	21.0	79	79.0	0.21	Poor
Asthma can be cured by	13	13.0	87	87.0	0.13	Poor
Asthma flare-ups	34	34.0	66	66.0	0.34	Fair
Course of steroids	64	64.0	36	36.0	0.64	Fair
Control asthma	44	44.0	56	56.0	0.44	Fair
Overall score					0.28	Poor

Table 3. Correlation coefficient and P. value for self- management and sleep disturbances.

Variable	Self-Management		Sleep disturbances	
	Correlation coefficient	P. value	Correlation coefficient	P. value
AGE	0.02	0.88	-0.06	0.52
BMI	-0.08	0.41	0.08	0.46
Gender (female)	-0.228	0.023	0.00	0.99
Residence	-0.19	0.06	-0.01	0.92
Order in the family	0.08	0.42	-0.04	0.67
Education	-0.19	0.06	-0.05	0.63
Mother job	0.02	0.83	0.09	0.35
Father job	-0.07	0.48	-0.12	0.24
Alone room	0.02	0.88	0.12	0.24
Income	0.11	0.28	0.00	0.98
Smoking	-0.15	0.15	0.10	0.30
Medications	-0.15	0.12	0.13	0.20
Allergy	-0.11	0.28	0.14	0.17
Type of Allergy	-0.03	0.75	0.01	0.93

Table 4. Comparison of mean scores of self- management of both genders.

Gender	N	Total score for self-management	
		Mean	Std. Deviation
Male	53	4.94	2.41
Female	47	3.91	1.97
t-test=2.318, P. value = 0.023			

DISCUSSION

Adolescents have suffer from moderate sleep disturbances of nocturnal asthma. Adolescents have difficulty sleeping on a daily almost, the highest rate (66%), this is due to asthmatic attack lead to sleep disturbances during night, this study agrees with the study conducted by Levin et al [8]. Overall, patients responses to the items of asthma self-management questionnaire

ASMQ revealed wide gaps in knowledge of asthmatic patients about the disease, the role of different medication and method of prevention of asthma attack and 77% of adolescents have poor self-management about asthmatic attack during night time such as the recruited adolescents had poor knowledge of the technique of using the inhaler with regard to the steps taken during the use of the device. Where linked the improper use of inhaler among asthmatic patients with poor control of the disease with a lack of health education and irregularity of follow up visits as significant predictors of improper use [9,10] .

In this study asthmatic patients were correlation sleep disturbance and self-management in gender that the male more knowledge than female (53%), the low level of knowledge about the most important items that help the patient to self-manage asthma can be attributed to the absence of formal health education [11]

CONCLUSION

Based on the study results the study, there was an evidence of sleep disturbances among the adolescents due to asthmatic attack during the night. Deficit knowledge regarding self-management of asthmatic attack , gender, residency, and levels of education could play a role in these attacks. The intensity of sleep disturbance at night could contributed to difficult sleep, drowsiness during the day, and early morning awakening. There were very wide gaps in the adolescent's knowledge of the most essential items about the disease, medications and preventive measures and how to use the inhaler that decreases asthma exacerbations. Therefore, it is recommended to create voluntary courses to raise awareness among adolescents and educate parents in cooperation with specialized medical staff. An educational programs targeting asthmatic adolescents and parents could improve self-management and commitment to self-care as an important part of reducing the risk of asthma and morbidity, using standard health care guidelines, and get benefit of the social media, posters educational publications to educate about self-management and risk factors that increase attacks of asthma.

Ethical Permission : The study protocol was approved by the council of the college of nursing , university of Kufa and the ethical committee of researches . Verbal informed consents were obtained from all participants and their parents, Data were collected according to the World Medical Association Declaration of Helsinki 2013

Conflict of Interest : None.

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REFERENCES

1. AI E, Alharthi BJ, Alharthi SA. Self-management and control of asthma among adult patients in King Faisal medical complex Taif, KSA , 2017; 7: 24-29.
2. Global Asthma Network. The global asthma report 2014. Auckland, New Zealand. 2014;769.The Global Asthma Report 2014; 5: 28-36
3. Gater A, Nelsen L, Fleming S, Lundy JJ, Bonner N, Hall R, Marshall C, Staunton H, Krishnan JA, Stoloff S, Schatz M. Assessing asthma symptoms in adolescents and adults: Qualitative research supporting development of the asthma daily symptom diary. Value in Health. 2016 Jun 1;19(4):440-50.
4. de Oliveira Francisco C, Bhatawadekar SA, Babineau J, Reid WD, Yadollahi A. Effects of Physical Exercise Training on Nocturnal Symptoms and Sleep Quality in Adults with Asthma: A Systematic Review. In: Pulmonary Rehabilitation in Non-Copd And Lung Transplantation American Thoracic Society , 2018; 2180-8.
5. Carr TF, Kraft M. Management of severe asthma before referral to the severe asthma specialist. The Journal of Allergy and Clinical Immunology: In Practice. 2017 Jul 1;5(4):877-86.
6. Koinis Mitchell D, Kopel SJ, Williams B, Cespedes A, Bruzzese JM. The association between asthma and sleep in urban adolescents with undiagnosed asthma. Journal of School Health. 2015 Aug;85(8):519-26.
7. D Erik, E. Adolescent sleep: review of characteristics, consequences, and intervention. Journal of Sleep Disorders: Treatment & Care 2012; 1 (2): 1–8
8. Levin AM, Wang Y, Wells KE, Padhukasahasram B, Yang JJ, Burchard EG, Williams LK. Nocturnal asthma and the importance of race/ethnicity and genetic ancestry. American journal of respiratory and critical care medicine. 2014 Aug 1;190(3):266-73.
9. Hamdan AJ, Ahmed A, Abdullah AH, Khan M, Baharoon S, Salih SB, Halwani R, Al-Muhsen S. Improper inhaler technique is associated with poor

asthma control and frequent emergency department visits. Allergy, Asthma & Clinical Immunology. 2013 Dec;9 (1): 8-12.

10. Prentice B, Homaira N, Jaffé A. Increased doses of inhaled corticosteroids during home management of asthma flare-ups do not reduce the need for systemic steroids. Journal of paediatrics and child health. 2017 Sep;53(9):915-7.
11. Wiecha JM, Adams WG, Rybin D, Rizzodepaoli M, Keller J, Clay JM. Evaluation of a web-based asthma self-management system: a randomised controlled pilot trial. BMC pulmonary medicine. 2015 Dec;15(1):17-25

Factors Associated with the Occurrence of Anemia in Pregnant Women in the Work Area of Baraka Public Health Center, Enrekang District, South Sulawesi Indonesia

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ABSTRACT

Study of factors related to anemia in pregnant women in the work areas of Baraka Public Health Facility of Enrekang District aimed to know the parity, mother's age, and inter-pregnancy interval with anemia in pregnant women was conducted. The method used in this study is a laboratory observation method with a descriptive approach, where each respondent was examined for hemoglobin (Hb) levels as a dependent variable and data about independent variables. The study was conducted from June to October 2017. The results showed that the parity factor and age of pregnant women had a relationship with the incidence of anemia, where the p-value <ά (0.05), the distance of pregnancy did not have a significant relationship with the incidence of anemia, where the value of p> ά (0.05). From the results of the study concluded that the age of pregnant women is the most influential factor in the incidence of anemia in pregnant women in Baraka Public Health Center, Enrekang District.

Keywords: *age, anemia, Baraka, inter-pregnancy interval, parity, pregnant women*

INTRODUCTION

Public health development in Indonesia is mainly aiming to increase life expectancy, improve the quality of life of human resources, and improve the quality of life in order to improve family welfare so that it can deliver optimal levels of public health. Health is one of the basic human needs, so it needs to endeavor so that every citizen is increasingly aware of the importance of health for himself and his environment, and can increasingly behave good healthy life.

Undermutrition is one of the main problems of public health in Indonesia. Especially in poor pupulation[9]. Nutrition problem, especially in pregnant women, is a big problem because it is a supporting factor for anemia in pregnant women. Anemia during pregnancy is a significant health problem in order to improve public health. Regarding maternal and child health, anemia is a major nutritional problem in developing countries including Indonesia.

Anemia is one of the factors that become an indicator of the success of a nation's health development that describes socio-economic capabilities in meeting the needs of quality and quantity of people's nutrition [1].

Anemia during pregnancy is a significant health problem to improve the degree of public health related to public health. Regarding maternal and child health, anemia is a major nutritional problem in developing countries, including Indonesia.

Data from the World Health Organization (WHO) in 2010 showed that anemia in pregnant women ranged from 20-85% with hemoglobin levels (Hb ≤ 11 g/dL). In developing countries including Indonesia, the infant mortality rate and the percentage of anemia during pregnancy are the most sensitive indicators to describe health status, especially maternal health in infants. The Indonesian Health Demographic Survey (SKDI) in 2012 showed that maternal mortality (MMR) in Indonesia was 228/100,000 live births. The causes of maternal

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deaths due to bleeding were 29.67%, infections 5.51%, abortion 1.77%, prolonged labor 1.65%, and others 34.35%. Maternal mortality is one indicator of the success of health services in a country. The direct cause of maternal death is bleeding, eclampsia and indirect causes are anemia [4].

Pregnancy is a natural process and not a pathological process. Pregnancy usually develops and results in the birth of a term healthy baby through the birth canal, but this is not always as expected, normal conditions can be pathological/abnormal. From the medical record data of Baraka Health Center in 2015 showed the number of pregnant women who had their pregnancies examined in 2015 as many as 196 people with some cases of anemia as many as 72 pregnant women. Nowadays, researchers interested to do research to determine factors related to the incidence of anemia in pregnant women in the working area of Baraka District Enrekang Health Center. The factors studied included: parity, age, and inter-pregnancy interval [2].

Anemia is a decrease in the number of red blood cells or the concentration of hemoglobin in the blood circulation. Causes of anemia include malnutrition, lack of iron, malabsorption, massive blood loss such as labor, abnormal menstruation, chronic infectious diseases such as pulmonary tuberculosis, intestinal worms, malaria, and others. According to World Health Organization, anemia in pregnant women is classified based on Hb levels of pregnant women into three categories as follows: normal (Hb ≥ 11 g/dL), mild anemia (Hb 8-10 g/dL), and severe anemia severe (Hb <8.0 g/dL) [10].

MATERIAL AND METHOD

The type of research used in this study was observational with a descriptive cross-sectional study approach. Pregnant women who examined themselves at the Baraka Public Health Center in Enrekang District included in this study were examined their hemoglobin (Hb) levels. Data on dependent and independent variables are collected simultaneously, to obtain information about the relationship of several factors to the incidence of anemia in pregnant women in the working area of the Baraka Public Health Center in Enrekang District.

This study was carried out from June to October 2017. The populations in this study were pregnant women who were in the working area of the Baraka Health Center in Enrekang Regency who came to have their pregnancies examined during the study period. The samples in this study were pregnant women who had their pregnancies examined at Baraka Health Center in Enrekang Regency. The sample size in this study was 65 people. The sampling technique in this study is the total sampling technique.

The independent variables in this study were factors related to the incidence of anemia in pregnant women: parity, the age of pregnant women, and inter-pregnancy interval. The dependent variable in this study was anemia in pregnant women.

The data obtained in this study include primary data, namely hemoglobin levels examined directly in pregnant women with the Sahli method. Also, other primary data obtained in this research is filling in questionnaires distributed to pregnant women to identify data relating to knowledge, education, age, parity, and inter-pregnancy interval. Other than that, secondary data also obtained including a profile of the Department of Health of Enrekang District and the medical record of the patients from Baraka Public Health Center in Enrekang District.

Materials and tools used in this study are 3 mL syringe, tourniquet, cotton, lancet, a reaction tube, 20 µl micropipette, Sahli hemometer, alcohol 70%, and HCl 0.1 N solution.

DATA ANALYSIS

The analysis used in this study is regression analysis which aims to determine the relationship of each independent variable to the dependent variable. Regression analysis also used to know which variable, from the three independent variables, is the most dominant relationship with the incidence of anemia in pregnant women.

FINDING

Hemoglobin level of pregnant women in the working area of Baraka Public Health Center displayed in Table 1. Twenty-six of respondents (40%) suffered from anemia based on their hemoglobin level while the other 39 pregnant women (60%) not. Table 2 displayed that as much as 84.6 % (55 pregnant women) are low-risk parity while 15.4 others are high-risk parity.

Table 1 Distribution of Respondents Based on Occurrence of Anemia in Pregnant Women in Baraka Health Center in Enrekang District in 2017

Hemoglobin level	**(n)**	**(%)**
Anemia	26	40
Normal	39	60
Total	65	100

Source: Primary Data 2017

Table 2 Distribution of pregnant women who examined their pregnancies according to parity at the Baraka Public Health Center in Enrekang District in 2017

Parity	(n)	(%)
High Risk	10	15.4
Low Risk	55	84.6
Total	65	100

Source: Primary Data 2017

Table 3 Distribution of pregnant women who check their pregnancies according to the age of pregnant women at the Baraka Health Center in Enrekang Regency in 2017

Age	**(n)**	**(%)**
High Risk	11	16,9
Low Risk	54	83,1
Total	65	100

Source: Primary Data 2017

Based on the age, it is shown in Table 3 that 11 of respondents (16.9%) are high risk, while the majority of them (83.1% or 54 people) are low risk. It is displayed in Table 4 that all (100%) of women included in this study were at low risk based on the inter-pregnancy interval.

Table 4 Distribution of pregnant women who check their pregnancies according to the inter-pregnancy interval at the Baraka Public Health Center, Enrekang District in 2017

Inter-pregnancy interval	**(n)**	**(%)**
High Risk	0	0
Low Risk	65	100
Total	65	100

Source: Primary Data 2017

DISCUSSION

Anemia in pregnancy is a condition where the hemoglobin (Hb) level is lower than the standard value which is less than 11.0 g/dL. Pregnant women whose hemoglobin levels 8.1-10 g/dL, 6.5-8 g/dL, and < 6.5 g/dL were classified as suffering mild, moderate, and severe anemia, respectively (Tabrizi and Barjasteh

2015). Anemia in Indonesia is still considered a public health problem since its high prevalence, especially for pregnant women. Anemia in adults can cause fatigue and weakness which resulting in lowering productivity and the declining of work capacity[3].

Anemia can be caused by malnutrition, lack of iron, malabsorption, massive blood loss (such as labor and abnormal menstruation), and chronic infectious diseases (including pulmonary tuberculosis, intestinal worms, malaria, and others)[6][7].

Parity is the number of deliveries performed or experienced by a woman with live births or stillbirths. The number of births can affect the body's metabolic process. The present data of prevalence of anemia in pregnant women (40%) was higher compared to national health survey conducted by Ministry of Health [5] .

The analysis using chi-squared with ά 0.05 showed that that there is strong association between parity and the incidence of anemia among pregnant women in Baraka Public Health Center of Enrekang District (p-*value* = 0.000). The result means that high risk parity (or pregnancy more than 3 times) has a higher chance of anemia compared to low risk parity. High risk parity in pregnant women usually relates to the level of knowledge that the mother lacks in the impact of the number of pregnancies. There is still lack of dissemination of information through counseling about the normal number of pregnancies by every woman.

Age is one factor that cannot be changed. Epidemiological attention is made to certain age groups because it has its own important problems. Age grouping is usually based on the presence of a problem and is specifically associated with other health factors. The age referred to in this study is the age of pregnant women with high risk i.e. less than 20 years and more than 35 years. Mother's age is very influential on the development of reproductive organs. This relates to the physiological state of the mother's body in accepting presence and supporting the development of the fetus. A woman entering the age of marriage will experience a certain phase in her life where healthy reproductive age is between 20-35 years. There was a significant relationship (p *value*= 0.000) between the age of pregnant women with the incidence of anemia among pregnant women in Baraka Public Health Center of Enrekang District based on chi-squared test.

Inter-pregnancy interval is the interval between two consecutive births of a woman. Setting the interval between pregnancies has an impact on the health of the mother and her baby, the inter-pregnancy interval that is too tight (<2 years) is more risky than the longer inter-pregnancy interval (> 2 years). This is because the anatomy of a woman needs time to restore her health and adequate nutritional intake. Based on this research, there is no association between interpregnancy interval and the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District based on chi-square test (p-*value* = 0.40).

CONCLUSION

there is a significant association between parity and the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District

there is significant association between the age of pregnant women and the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District

there is no association between the inter-pregnancy interval with the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District

the age of pregnant women is the most factor that associated with anemia in pregnant women in Baraka Public Health Center of Enrekang District.

Conflict-Of-Interest Statement : In this study between researchers and research, subjects did not have a conflict of interest, because subjects did not have personal or informal relationships with researchers.

Source of Funding : The source of funds in this study came from the Research of Health Workforce Development of the Ministry of Health of the Republic of Indonesia.

Ethical Clearance : The ethics of this study were obtained from the Ethics Commission for Health Research, Poltekkes Kemenkes Makassar.

REFERENCES

1. Arisman. Nutrition and Life Cyle. Nutrition Research and Project Ministry of Education. Faculty of Medicine Sriwijaya Univerisity, Palembang 2010 (2). Available from: https://www.belbuk.com/buku-

ajar-ilmu-gizi-gizi-dalam-daur-kehidupan-edisi-2-p-3860.html.

2. Baraka Health Centre. Baraka Health Report: Enrekang. South Sulawesi. 2015.
3. Barkley JS, Kendrick KL, Codling K, Muslimatun S, Pachon H. Anemia prevalence over time in Indonesia: estimates from the 1997, 2000, and 2008 Indonesia family life surveys. Asia Pac J Clin Nut. 2015. 24(3): 452-455.
4. BKKBN. The National Population and Family Planning Committe report. Jakarta. 2013.
5. [MoH] Ministry of Health. 2013. Basic Health Research. Research and Development Department, Ministry of Health.
6. Sabina S, Iftequar S, Zaheer Z, Khan MM, Khan S. An overview of anemia in pregnancy. Journal of Innovations in Pharmaceuticals and Biological Sciences 2015. (2): 144-151. Available From: www.jipbs.com/VolumeArticles/FullTextPDF/78_JIPBSV2I208.pdf
7. Sifakis S, Pharmakides. Anemia in pregnancy. Annals of the New York Academy of Sciences. 2000. Available from: https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.2000.tb06223.x
8. Tabrizi FM, Barjasteh S. Maternal hemoglobin levels during pregnancy and their association with birth weight of neonates. Iran J Ped Hematol Oncol. 2015. 5(4): 211-217. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26985354.
9. Usfar AA, Lebhthal E, Atmarita, Achadi E, Soekirman and Hadi H. Obesity as a poverty-related emergin nutrition problems: the case of Indonesia. National prevalence of obesity. Obesity reviews 2010. 11:924-928. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20977602
10. WHO U; UNU. Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. Geneva: World Health Organization; 2001. WHO/NHD/01.3, 2013. Available from: https://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/.

Effect Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Honey Bee *Trigona spp* on *Streptococcus pyogenes*

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ABSTRACT

Streptococcus pyogenes one cause of respiratory tract infections including pharyngitis. Seventy-three percent of doctors prescribe antibiotics for pharyngitis. In developing countries, antibiotics are prescribed for 44-97% of hospitalized patients, sometimes with inappropriate doses. Improper antibiotic use can cause side effects such as allergies or diarrhea, increase health care costs, and increase the possibility of selection for antibiotic resistance. Research needs to be done on natural antibiotics found in natural ingredients. One of the natural ingredients empirically has many properties and is relatively safe is honey from bees. Honey has substances that are bactericidal and bacteriostatic like antibiotics. This study aimed Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) honey bee *Trigona spp* against *Streptococcus pyogenes* in vitro. In concentration of 2.5%, 5%, 7.5%, 10%, and 12 , 5% and knowing the effect of honey bee *Trigona spp* with a concentration of 2.5%, 5%, 7.5%, 10%, and 12.5% against *Streptococcus pyogenes* in vitro. Type of research is the experiment with the Posttest-Only Control Group Design, the research material of honey from *Trigona spp* independent variable is the concentration of honeybees *Trigona spp*. The dependent variable is the level of turbidity produced in a tube to measure MIC and the number of colonies *Streptococcus pyogenes* on Blood Agar Plate media to measure MBC. The results of the MIC determination showed that all concentrations showed clear, MBC results the largest number of colonies occurred at a concentration of 2.5% of the total colony colonies and colonies growth the smallest occurred at a concentration of 10% of the number of colonies 0 colonies, Conclusions of MIC cone honey bee *Trigona spp* against bacteria *Streptococcus pyogenes* occurred at a concentration of 2.5%., MBC moth honey *Trigona spp* against bacteria *Streptococcus pyogenes* occurred at a concentration of 10% . There is an influence of the honey *Trigona spp* on the bacteria *Streptococcus pyogenes*, which is indicated by the value of p <0.05, which is 0,000 using the Linear Regression test.

Keywords: *MIC, MBC, Streptococcus pyogenes.*

INTRODUCTION

Streptococcus pyogenes (Streptococcus group A) is one of the causes of respiratory tract infections including pharyngitis. In Indonesia every year ± 40 million people visit health care centers because of pharyngitis[1]. In the United States, 15−30% of cases of pharyngitis are in school-age children and 5-15% of cases of pharyngitis in adults. Usually occurs in the winter, which is a result of infection with Streptococcus, hemolytic, group pharyngitis is rare in children less than three years[2].

Seventy-three percent of doctors prescribe antibiotics for pharyngitis, most of which are caused by bacteria. In developing countries, antibiotics are prescribed for 44-97% of hospitalized patients, sometimes with inappropriate doses. Incorrect antibiotic use (indications, selection, length of administration, and improper doses) can cause side effects such as allergies or diarrhea, increase health care costs, and increase the likelihood of selection for antibiotic resistance[3]. Antibiotic resistance in Indonesia has been found, such as staphylococcus

aureus which is resistant to methicilin[4] and vancomicin[5].

Indonesia is rich in natural ingredients that can be used as an antibacterial, anti-fungal or anti-larvae. As Anredera cordifolia[6], lime[7] for antibacterial, Cananga odorata (Lamk). Hook[8], Carica papaya Linn[9], Syzygium polyanthum[10] for anti-larvae and Kaempferia galanga L.[11], Jatropha curcas[12] as antifungal. Another natural material that is widely used is honey. Bee honey empirically has many properties and is relatively safe, Research Wineri et al. (2013) proved that honey has an active effect against and inhibits pathogenic bacteria that cause infection, such as *Streptococcus pyogenes* which can cause pharyngitis[13]. Honey has substances that are bactericidal and bacteriostatic like antibiotics. Bacteria cannot live and develop in honey because honey has antibiotic substances such as anti-viral interferon, and inhibin which can inhibit bacterial growth[14].

MATERIALS AND METHOD

This type of research is the experiment the form of a Posttest-Only Control Group Design.

The research was carried out at the Bacteriology Laboratory of the Health Analyst Department of the Health Ministry of Health Banjarmasin January 2018. The research material was honey from the honeycomb (*Trigona spp*).

The independent variable was the concentration of honeybees *Trigona spp*. The dependent variable is the level of turbidity produced in the tube to see MIC and the number of colonies *Streptococcus pyogenes* on BAP (Blood Agar Plate) media to see MIC.

Research steps include bee determination at the Entomology Laboratory of the Faculty of Biology, Gadjah Mada University, Yogyakarta. The sampling of honey bee honey and sterility testing. Making suspense of bacteria. *Streptococcus pyogenes* Making a series of bee honey concentration in each sterile test tube as in the following table. :

Table 1.Honey Dilution

SterileTube	Concentration of	Honey (ml)	Aquades Solution (ml)
1	5%	0.25 ml	4.75 ml
2	10%	0.50 ml	4.50 ml
3	15%	0.75 ml	4, 25 ml
4	20%	1.00 ml	4.00 ml
5	25%	1.25 ml	3.75 ml

Treatment of Research: Determination of MIC :: Preparing a sterile test tube and labeled according to variations in the concentration made of 2.5%, 5%, 7.5%, 10%, 12.5%, PC (positive control), NC (negative control) and HC (honey control). From each concentration, 1ml was taken and added 1 ml of bacterial suspense. The positive control tube filled with 1 ml of 5 µg of Levofloxacin antibiotics and 1 ml of bacterial suspension. The negative control tube was filled with 1 ml of sterile TSB and added with 1 ml of bacterial suspension. The honey control tube was filled with 1 ml of bee honey plus 1 ml of sterile TSB. Mix well and incubated for 24 hours at 37ºC in the incubator. The results were read by finding a tube containing the lowest honey content but still being able to inhibit bacteria (clear solution) which was declared as MIC. After obtaining the results of Minimum inhibitory concentration, the examination continued with the determination of MIC[11].

Determination of MIC: The tube that was determined MIC was taken as much as 50 µl then leveled on the surface of Nutrient Agar (NA) media using a spreading rod. Incubated for 24 hours at 35ºC in an incubator[15]. Read the results by counting the number of colonies growing on the surface of the media using the colony counter and then looking for concentrations that do not show bacterial growth at all. Five repetitions.

RESULTS AND DISCUSSION

Table 2: Results of Minimum Inhibitory Concentration (MIC)

Level of clarity of repetition of	Concentration of honey beetle					Control	
	2.5%	5%	7.5%	10%	12.5%	Negative (KN)	Positive (KP)
1	Clear	Clear	Clear	Clear	Clear	Cloudy	Clear
2	Clear	Clear	Clear	Clear	Clear	Cloudy	Clear
3	Clear	Clear	Clear	Clear	Clear	Cloudy	Clear
4	Clear	Clear	Clear	Clear	Clear	Cloudy	Clear
5	Clear	Clear	Clear	Clear	Clear	Cloudy	Clear
average	Clear	Clear	Clear	Clear	Clear	Cloudy	Clear

Table .3: Results of Minimum Bactericidal Concentration (MBC)

Clarity level of repetition of	Concentration of honey beetle					Control	
	2.5%	5%	7.5%	10%	12.5%	Negative (KN)	Positive (KP)
1	40	8	1	0	0	4416	0
2	35	25	5	0	0	3888	0
3	31	8	2	0	0	4304	0
4	31	14	1	0	0	4112	0
5	38	20	0	0	0	3984	0
Average	35	15	2	0	0	4141	0

The average number of colonies *Streptococcus pyogenes for* each concentration in determining the minimum Bactericidal concentration is presented as in Figure 1.1.

Fig. 1 Average Number of colonies *Streptococcus pyogenes* on Determination of Minimum Bactericidal Concentration (MBC)

Based on the results of normality data testing using Shapiro-Wilk, it is known the asymp value. Sig = 0.365; 0,408; 0,223; 0.782. Asymp value. Sig> 0.05, it can be stated that MIC data is normally distributed.

Table. 4 Normality Test Results for the Number of colonies *Streptococcus pyogenes* on Determination of Minimum Bactericidal Concentration (MBC)

	Minimum Bactericidal Concentration	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistics	Df	Sig.	Statistics	Df	Sig.
Number of Colonies *Streptococcus Pyogenes*	2.5%	.238	5	.200*	.892	5	.365
	5%	.225	5	.200*	.900	5	.408
	10%	.261	5	.200*	.859	5	.223
	KN.	172	5	. 200*	.956	5	.782 The

The test continued with the ANOVA test to assess the quality of the regression equation obtained. Based on the ANOVA test results obtained a significant value of 0,000 so that the equation obtained is not used.

Regression test results obtained a significance value of 0,000. The value of significance <α = 0.05 can be stated treatment of different concentrations of honey bees effect on the growth of *Streptococcus pyogenes* in vitro.

The results of the determination coefficient test, the R square value is 0.420. This value is to conclude that the effect of bee honey on the growth of *Streptococcus pyogenes* is 42% (obtained from 0.420 x 100%), which means that honey bee has a weak influence on the growth of *Streptococcus pyogenes*.

The results showed that the higher the level of concentration of honey bee honey added, the greater the ability of inhibitory power and killing power on the growth of *Streptococcus pyogenes*. This is characterized by the occurrence of clarity in TSB media tube dilution test (table 2) and a decrease in the number of colonies *Streptococcus pyogenes* that grow on the surface of the nutrient agar media (table 3). Based on the results of the regression test obtained a significance value <α = 0.05, it can be stated that the treatment of different concentrations of bee honey affects the growth of *Streptococcus pyogenes*.

Based on Erywiyatno's (2014) research on the minimum concentration of honey which can inhibit the growth of Group A beta-hemolytic Streptococcus by diluting honey at concentrations of 10%, 20%, 40%, 60%, 80%, and 100%, it shows that the minimum inhibitory concentration (MIC) on packaged honey against the growth of Group A beta Streptococcus hemolyticus is positive (+) at a concentration of 90% and Minimum Bactericidal Concentration (MBC) is at a concentration of 95%[16].

According to Wineri (2013) based on the origin of manufacture, honey is divided into natural honey and packaged honey. Physically packaged honey has similarities with natural honey, but there are differences in nutritional content. Natural honey has high sugar content in the form of fructose 38.19%, glucose 31%, and sucrose 1.31%. The sugar content found in natural honey causes the viscosity of natural honey to be thicker compared to packaged honey, this is due to the process of making packaged honey there is a stage of giving water and other mixtures so that the volume of honey packs becomes more. Also, packaged honey does not contain enzymes, vitamins, and minerals as found in natural honey. Therefore, the results of the study of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in the honey bee honey (*Trigona spp*) occur at low concentrations compared to the results of Erywiyatno's research using packaged honey.

Honey has substances that are bactericidal and

bacteriostatic like antibiotics. Bacteria cannot live and develop in honey because honey contains an element of potassium which is an element that prevents moisture so that it can inhibit bacterial growth. Based on the results of research it has been known that honey has the broad spectrum antibiotic activity to fight pathogenic bacteria. Honey also contains phenol, peroxide and non-peroxide components, has a thick viscosity, and a low pH that can inhibit bacterial growth. The hygroscopic properties of honey can attract water from the living environment of bacteria which results in dehydrated bacteria. Honey is also an immunomodulator by triggering macrophages to produce cytokines involved in killing bacteria and repairing tissues. These antibacterial properties are effective in inhibiting the growth of *Salmonella typhi, Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus* and *Pseudomonas aeruginosa*[13].

Non-sting honey bee *Trigona spp* has been shown to contain high phenolic levels. Phenolic benefits for health include functioning as antioxidants, antibacterial and anti-inflammatory. The research examined the phenolic content in honey produced by Apismellifera, A. dorsata, A. cerana, and two types of Trigona sp. The results showed that the phenolic content of honey was Trigona superior to honey from the genus Apis bee. The values of the phenolic content of honey studied were 602.4 mg EAG (Galatea Acid Equivalent) per kg of honey; 589.2 mg; 510.4 mg; 791.5 mg; and 1,058.8 mg[17].

The mechanism of action of phenol compounds in killing bacterial cells is three ways, namely denaturing bacterial cell proteins, inhibiting cell wall synthesis, and damaging bacterial cell membranes. Phenol compounds denature bacterial cell proteins by forming hydrogen bonds with bacterial proteins. Results in the structure of bacterial proteins damaged and enzymes were becoming inactive. In addition, the mechanism of phenol in inhibiting bacterial cell wall synthesis by poisoning the protoplasm and breaking peptidoglycan bonds[18].

Flavonoids contained in honey bee which also functions as antibacterial Flavonoids can damage cell membranes by inhibiting the synthesis of macromolecules, depolarizing cell membranes and inhibiting DNA, RNA and protein synthesis. inhibits the function of the cytoplasmic membrane and inhibits energy metabolism in bacteria[19].

The antibacterial activity of honey is also related to its osmotic condition. Honey is a supersaturation solution of sugar that has an osmotic ability. Water molecules react strongly with sugar in honey; there is only a small amount of free water left for the growth of microorganisms. The enzyme activity of glucose oxidase will produce hydrogen peroxidase. Decomposition of hydrogen peroxidase will increase reactive free radicals. These free radicals will react to organisms resulting in death. Osmotic pressure can cause microorganisms to dehydrate and die[20].

CONCLUSION

MIC honey *Trigona spp* against bacteria *Streptococcus pyogenes* occurs at a concentration of 2.5%. MBC bee honey *Trigona spp* against bacteria *Streptococcus pyogenes* occurs at a concentration of 10%. There are an influence of honey *Trigona spp* bacteria *Streptococcus pyogenes*,

Ethical Clearance: Taken From *Health Research Ethics Committee* Politeknik Kesehatan Banjarmasin

Conflict of Interest: Nil

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REFERENCES

1. Ministry of Health of the Republic of Indonesia. Clinical Practice Guide for Doctors at Primary Health Care Facilities Revised Edition. Ministry of Health of the Republic of Indonesia. Jakarta; 2014
2. Acerra, JR. Pharyngitis. Department of Emergency Medicine. Hofstra North Shore, 2017
3. Malino IY, Utama DL, Soenarto Y, 2013. McIac criteria for diagnosis of acute group-A-hemolytic streptococcal pharyngitis. Paediatrica Indonesiana. 2013; 53, 258-263.
4. Lutpiatina L, NN Agistni Eriana. Staphylococcus aureus and Methicillin resistant Staphylococcus aureus From The Diabetic Ulcer. Medical Laboratory Technology Journal. 2018; 4(1), 26-29
5. Dwiyanti RD, A Muhlisin, A Muntaha. MRSA and VRSA in Paramedics at Ratu Zalecha Hospital Martapura (MRSA dan VRSA pada Paramedis RSUD Ratu Zalecha Martapura). Medical Laboratory Technology Journal. 2015; 1(1), 27-33
6. Dwiyanti RD, Nurlailah, I.K. Widiningsih. Water Effectiveness of Binahong Leaf Decoction (Anredera

cordifolia) Against the Growth of Salmonella typhi (Efektivitas Air Rebusan Daun Binahong (Anredera cordifolia) Terhadap Pertumbuhan Salmonella typhi). Medical Laboratory Technology Journal. 2015; 1(1), 1-6

7. Dwiyanti RD, L. Lutpiatina, Lime Soap as Bacteria Reducer, Medical Laboratory Technology Journal. 2018; 4(1), 6-20
8. Nuryati A, Siti nuryani, AD wibowo. Effect of Simplicia of Kenanga Flowers (Cananga odorata (Lamk). Hook.) As Electric Mat Against Knock-Down of Aedes Aegypti Mosquitoes (Pengaruh Simplisia Bunga Kenanga (Cananga odorata (Lamk). Hook.) sebagai Mat Elektrik Terhadap Knock-Down Nyamuk Aedes Aegypti). Medical Laboratory Technology Journal. 2015; 1(1), 34-37
9. Swastika D, Lenie Marlinae, Laily Khairiyati. Effect of Simplicia of Kenanga Flowers (Cananga odorata (Lamk). Hook.) As Electric Mat Against Knock-Down of Aedes Aegypti Mosquitoes (Peran Ekstrak Daun Pepaya (Carica papaya Linn) terhadap Kematian Larva Nyamuk Aedes aegypti). Medical Laboratory Technology Journal. 2016; 2(2), 66-69
10. Dwiyanti RD, Rion Dediq, A Thuraidah. Effect of Simplicia of Kenanga Flowers (Cananga odorata (Lamk). Hook.) As Electric Mat Against Knock-Down of Aedes Aegypti Mosquitoes (Daya Bunuh Ekstrak Air Daun Salam (Syzygium polyanthum) terhadap Larva Aedes sp). Medical Laboratory Technology Journal. 2017; 3(1), 17-21
11. Annisa R, Erfan R, Lutpiatina L. Potential of Kencur Rimpang Extract (Kaempferia galanga L.) Inhibits the Growth of Candida albicans (Potensi Ekstrak Rimpang Kencur (Kaempferia galanga L.) Menghambat Pertumbuhan Candida albicans). Medical Laboratory Technology Journal. 2016; 2(2), 70-76
12. Ni Made Niagita Wiratni, I Nyoman Jirna, IGA. Sri Dhyanaputri. Potential Antifungal of Jatropha Stem Leaves Against the Growth of Candida albicans (Potensi Antifungi Tangkai Daun Jarak Pagar Terhadap Pertumbuhan Candida albicans). Medical Laboratory Technology Journal. 2017; 3(2), 63-67
13. Wineri E, Rasyid R, Alioes Y. Comparison of natural honey inhibition with packaged honey in vitro against group A beta hemolytic Streptococcus as a cause of pharyngitis. Andalas Health Journal. 2013; 3 (3), 376-380.
14. Rio YBP, Djamal A, Estherina. Comparison of the antibacterial effects of native Sikabu honey with Lubuk Minturun honey on Escherichia coli and Staphylococcus aureus in vitro. Jurnal Kesehatan Andalas. 2012; 1 (2), 59-62.
15. Lutpiatina L. Widiyawati, Muntaha A. Potential of Noni (Morinda citrifolia L.) on Growth of Salmonella sp. JCPS Journal of Current Pharmaceutical Sciences. 2018; 1(2), 48-53
16. Erywiyatno, L., Djoko, SSBU., & Kriharyani, D. Pengaruh Madu Terhadap Pertumbuhan Bakteri Streptococcus pyogenes, Analis Kesehatan Sains. 2012; 1(1), 2302-3635.
17. Kek SP, Chin NL, Yusof YA, Tan SW, Chua LS. Total phenolic contents and color intensity of Malaysian honeys from the Apis spp. and Trigona spp. bees. Agriculture and Agricultural Science Procedia. 2014; 2, 150-155.
18. Naidu, AS and RA Clemens. Natural Food Antimicrobial Systems. LCC: CRC Press; 2000
19. T.P. Tim Cushnie, Andrew J. Lamb. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 2005; 26, 343–356
20. S. Hamad, Honey Therapy: A Practical Guide to 84 Diseases, Plus for Mental Stamina. Depok, Pustaka Iman; 2007

Study of Humeral Immune Response and Some of The Blood Variables in Mice Balb /c Teated with LPS of *Klebsiella pneumoniae* Antigen and Glycyrrhiza glabra Extract

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ABSTRACT

In this work, lipopolysaccharides of *Klebsiella pneumonia* and extract of Glycyrrhiza glabra were used to investigate the humoral immunity in mice Balb/c (in vivo). In contrast, little is known about humeral immunity induced by LPS and Glycyrrhiza glabra. Some parameters were used to achieve this study, are WBC and differential count, Concentration of antibodies IgA, IgM, IgG and Concentration of complement proteins type C3 and C4 / mg / 100. The results showed there are a significant change in a parameters values in IgA, IgM, IgG and Concentration of complement proteins type C3 and C4 ,also in CBC and differentiated WBC.

Keywords: *LPS, immunology, extract of Glycyrrhiza glabra, in vivo, Klebsiella pneumoniae.*

INTRODUCTION

The Glycyrrhiza glabra (licorice) plant has a long and storied his Tory of utilization in both Eastern and Western societies pre-dating the Babylonian and Egyptian realms, The antiquated Greeks and Romans are known to have cultivated the plants in the third century, Licorice was a prescriptive specialist of Hippocrates in the treatment of asthma dry hack and other "pectoral diseases[1,2,4]. The licorice (Radix Glycyrrhizae or Liquiritiae radix), which is comprehensively utilized as a part of prescription and business, is gotten from the sweet foundation of different types of Glycyrrhiza. Licorice is local to southern Europe and parts of Asia; it is a standout amongst the most usually utilized homegrown [3,5]. *Klebsiella pneumoniae* is a Gram-negative bacterium that colonizes the skin, mouth, gut mucosa, and oropharynx, is likewise a crafty pathogen causing an assortment of contaminations, including bacteremia, urinary tract diseases also, liver abscesses [6,7]. The Glycyrrhiza glabra extricates were additionally found to Fortify humoral resistance in chickens tentatively tainted with the Newcastle infection (NDV), The talked about plant conveys various medical advantages what's more, applies mitigating, antibacterial, antifungal what's more, hostile to cancer-causing impacts because of the nearness of an- Anthraquinones, saccharides and cell reinforcement vitamins (A, C and E). [8,9,10,11,12]

METHODOLOGY

Identification of K.pneumoniae

Microscopical examination (Gram staining film)

Smears were prepared by taking a small amount of isolated colony from a bacterial culture on a glass slide by mixing a colony of the testing bacterium with a drop of distilled water, stained with Gram stain and examined using an oil immersion objective lens under 100X power.

Cultural characteristics

The morphological characteristics of isolated colonies were carefully studied with the aid of a lens 10X lens. Depending on the above two criteria the cultures were primarily classified as pure or mixed cultures.

The growth of the bacterial isolates

The bacterium *K.pneumoniae* was obtained from the College of Science, University of Iraq. This isolate

was a subculture in 10 ml Nutrient broth and incubated for 48 hr at 37 ºC. Then the insulation of bacteria was achieved by taking 4 ml of the culture broth in 1000 ml of sterilized nutrient broth and incubated in the same conditions in a shaker incubator at 100 cycles/ min for 48 hours, then Centrifugation of culture broth at 3000 cycles/min for 15 min at 4 ºC and the sediment was suspended in Phosphate buffer saline (pH 7.2) and centrifugated by same condition three times until getting the pure sediment and stored in 4 ºC.

Extraction and purification of LPS

LPS of *K.pneumoniae* was extracted using a reference [13,14], and purified using the modified Vogel's method [15,16].

Extraction of licorice

Licorice was extracted according to a papers [17,18,19.20,21.22].

Laboratory animals

Males of Swiss white mice Balb/c with old 6-7 weeks in this study which obtained from Medicative Control Center, Ministry of Health, Baghdad, Iraq

RESULTS



Figure 1 The licorice plant



Figure 2 The licorice plant after extraction

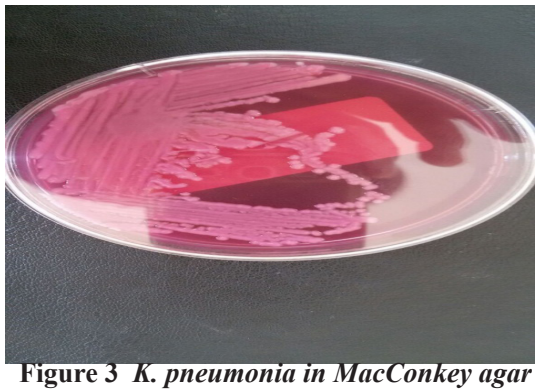


Figure 3 *K. pneumonia in MacConkey agar*

Table 1 Effect of immunization of mice using LPS antigen of *K. Pneumonia* and *Glycyrrhiza glabra extract* on antibodies IgA, IgM, IgG mg / 100

Treatments	IgA	IgM	IgG
LPS of *K. Pneumonia*	226.18±5.44 b	120.9±2.66 ab	1170.2±21.33b
Glycyrrhiza glabra extract	142.82±5.11 c	95.5±2.87 c	720.24±21.45 c
LPS & Glycyrrhiza glabra extract	187.34±3.33 a	121.88±2.33 a	1115.34±31.8 a
Phosphate buffer (control)	141.82±5.11 c	91.5±2.87 c	7`22.24±21.45 c

Legend: The different letters in the same column refer to the significant differences (p<0.05).

Table 2 Effect of immunization of mice using LPS antigen of *K. Pneumonia* and *Glycyrrhiza glabra* extract in a concentration of the complement proteins type C3 and C4 / mg / 100.

Treatments	C3	C4
LPS of *K. Pneumonia*	122.56±1.82 b	44.7±1.23 ab
Glycyrrhiza glabra extract	80.4±2.33 d	22.78±1.4 d
LPS & Glycyrrhiza glabra extract	132.68±2.35 a	48.23±1.04 a
Phosphate buffer (control)	82.4±2.33 d	25.78±1.4 d

Table 3 Effect of immunization of mice using LPS antigen of *K. Pneumonia* and *Glycyrrhiza glabra* extract in In the total and differential count of white blood cells.

Treatments	WBC mm3	Neutrophile %	Lymphocyte %	Acinophile %	Basophie %
LPS of *K. Pneumonia*	12040±646.9 [b]	52.81±0.67 b	45.58±1.36 b	2.4±0.48 a	0
Glycyrrhiza glabra extract	5651±107.7 [c]	51. ±0.29 c	47. ±0.42 a	2.0±0.05 a	0
LPS & Glycyrrhiza glabra extract	16130±508.9 a	54.07±1.67 a	44.86±1.55 c	2. ±0.23 a	0
Phosphate buffer (control)	5667±107.7 c	50.12±0.29 c	48. ±0.42 a	2. ±0.05 a	0

Legend: The different letters in the same column refer to the significant differences (p<0.05).

Legend: The different letters in the same column refer to the significant differences (p<0.05)

DISCUSSIONS

Firstly ,the effect of the immunization by using lipopolysaccharides (LPS) of *K. Pneumonia* and *Glycyrrhiza glabra extract* on IgG, IgM, IgA antibody level is reported in table 1. The results of the effect of LPS antigen injections of *K. Pneumonia* at IgG, IgM, IgA antibody level. The results showed significantly higher levels of antibodies in the treatment using LPS antigen, *Glycyrrhiza glabra extract* and LPS antigen as compared with the control groups and the treatment in which the extract was used For the unit. The antigen (LPS) with *Glycyrrhiza glabra extract* showed the highest difference of antibodies IgA, IgM, IgG, 187.3, 121.8 and 1115.3, respectively, compared with the Control group and the treatment of the *Glycyrrhiza glabra extract*, which did not register any significant difference. The results of the present study agree with [23,24,25,26.27]. that *K. Pneumonia* infection leads to an increase in antibody production. Elevation in antibody levels in treating mice may be due to the ability of the *K. Pneumonia* antigen to stimulate lymphocyte reproduction. The results of the current study were also agreed with [27,28]. There was a significant increase in antibody levels of IgG, IgM, and IgA since the increase in the concentration of these antibodies is indicative of the development of humoral immunity.

Secondly , the effect of the immunization by using lipopolysaccharides (LPS) of *K. Pneumonia* and Glycyrrhiza glabra extract in a concentration of the complement proteins type C3 and C4 are reported in table 2. The results showed an increase in the concentration of complement proteins type C3 and C4 which the treat by LPS antigen, Glycyrrhiza glabra extract compared with the control groups. The antigen (LPS) with *Glycyrrhiza glabra* extract showed the highest difference in the concentration of C3 and C4 complemental proteins at 132.68, 48.23 respectively, compared with the control group and the treatment with the Glycyrrhiza glabra extract, which showed no significant difference. Łukawska, Polcyn-Adamczak, and Niemir, (2018) were found that the concentration of C3 and C4 complemental proteins was significantly higher in mice which the immunization by using lipopolysaccharides (LPS) compared with the control group.

Finally, The effect of the immunization by using lipopolysaccharides (LPS) of *K. Pneumonia* and Glycyrrhiza glabra extract in a WBC and differential WBC count are reported in table 3. The results of the present study showed significant differences towards increasing the total number of white blood cells in the treatment in which the LPS antigen with the Glycyrrhiza glabra extract where it gave the highest significant difference. In the differential count of white blood cells, the results showed significant differences in the number of Neutrophils compared to the control groups and the treatment in which the extract was used alone. The results also showed that there were significant

differences in the reduction of the number of white blood cells lymphocytes compared to control groups. The highest value of total white blood cells in the group was treated with LPS antibody with the Glycyrrhiza glabra extract 16130 compare of control group 5667. The differential count was the highest number of treated cells with LPS with Glycyrrhiza glabra extract, where it was 54.07 % compared to the control. The results of the total number of white blood cells in our study are consistent with Khaertynov *et al*.,(2018) showing that the injection of Swedish white mice with *K. Pneumonia* leads to an increase in the total number of white blood cells compared with the control group.

CONCLUSION

The results showed there are a significant change in a parameters values in IgA, IgM, IgG and Concentration of complement proteins type C3 and C4 ,also in CBC and differentiated WBC comparative with others parameters.

Ethical Clearance : Taken from Medicative Control Center, Ministry of Health, Baghdad, Iraq.

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Conflict of Interest : Nil

REFERENCES

1- Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp .), its extract and powder as a food ingredient , with emphasis on the pharmacology and toxicology of glycyrrhizin. 2006;46:167–92.

2 Slomka MJ, Pavlidis T, Banks J, Shell W, McNally A, Essen S, Brown IH. Validated H5 Eurasian real-time reverse transcriptase–polymerase chain reaction and its application in H5N1 outbreaks in 2005–2006. Avian diseases. 2007 Mar;51(s1):373-7.

3- Zhao Y, Kong C, Cui X, Cui H, Shi X, Zhang X, Hu S, Hao L, Wang Y. Detection of infectious laryngotracheitis virus by real-time PCR in naturally and experimentally infected chickens. PloS one. 2013 Jun 28;8(6):e67598.

4- Santander Parra SH, Nunez LF, Buim MR, Astolfi-Ferreira CS, Piantino Ferreira AJ. Development of a qPCR for the detection of infectious laryngotracheitis virus (ILTV) based on the gE gene. British Poultry Science. 2018 May 26(just-accepted).

5- Bagchi D, editor. Sustained Energy for Enhanced Human Functions and Activity. Academic Press; 2017 Aug 7.

6- Clarke BR, Ovchinnikova OG, Kelly SD, Williamson ML, Butler JE, Liu B, Wang L, Guo X, Follador R, Lowary TL, Whitfield C. Molecular basis for structural diversity in serogroup O2-antigen polysaccharides in Klebsiella pneumoniae. Journal of Biological Chemistry. 2018 Feb 12:jbc-RA117.

7 Abdul-Cader MS, Amarasinghe A, Palomino-Tapia V, Ahmed-Hassan H, Bakhtawar K, Nagy E, Sharif S, Gomis S, Abdul-Careem MF. In ovo CpG DNA delivery increases innate and adaptive immune cells in respiratory, gastrointestinal and immune systems post-hatch correlating with lower infectious laryngotracheitis virus infection. PloS one. 2018 Mar 7;13(3):e0193964.

8- Łukawska E, Polcyn-Adamczak M, Niemir ZI. The role of the alternative pathway of complement activation in glomerular diseases. Clinical and experimental medicine. 2018 Feb 15:1-22.

9- Zhang T, Chen J, Wang C, Zhai X, Zhang S. Establishment and application of real-time fluorescence-based quantitative PCR for detection of infectious laryngotracheitis virus using SYBR Green I. Poultry science. 2018 Jul 20;97(11):3854-9.

10- Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyenova DR, Lubin SA, Skvortsova NN. Virulence Factors and Antibiotic Resistance of Klebsiella pneumoniae Strains Isolated From Neonates With Sepsis. Frontiers in medicine. 2018;5.

11- Trautmann M, Ruhnke M, Rukavina T, Held TK, Cross AS, Marre R, Whitfield C. O-antigen seroepidemiology of Klebsiella clinical isolates and implications for immunoprophylaxis of Klebsiella infections. Clinical and diagnostic laboratory immunology. 1997 Sep 1;4(5):550-5.

12- Rollenske T, Szijarto V, Lukasiewicz J, Guachalla LM, Stojkovic K, Hartl K, Stulik L, Kocher S, Lasitschka F, Al-Saeedi M, Schröder-Braunstein J. Cross-specificity of protective human antibodies against Klebsiella pneumoniae LPS O-antigen. Nature immunology. 2018 May 14:1.

13- Westphal O. Extraction with phenolwater and further applications of the procedure; Methods in carbohydrate chem, Vol. 5.

14- Lüderitz OT, Westphal OT, Staub AM, Nikaido HI. Isolation and chemical and immunological characterization of bacterial lipopolysaccharides. Microbial toxins. 1971;4:145-233.

15- Staub AM. Bacterial Lipido-protinopolysaccharides ('O'Somatic Antigens) Extraction with Trichloroacetic Acid. Methods in Carbohydrate Chem. 1965;5:92-3.

16 Delahooke DM, Barclay GR, Poxton IR. A re-appraisal of the biological activity of bacteroides LPS. Journal of medical microbiology. 1995 Feb 1;42(2):102-12.

17- Kitagawa I. Licorice root. A natural sweetener and an important ingredient in Chinese medicine. Pure and applied chemistry. 2002 Jan 1;74(7):1189-98.

18- Pan X, Liu H, Jia G, Shu YY. Microwave-assisted extraction of glycyrrhizic acid from licorice root. Biochemical Engineering Journal. 2000 Jul 1;5(3):173-7.

19- Kim HS, Lee SY, Kim BY, Lee EK, Ryu JH, Lim GB. Effects of modifiers on the supercritical CO 2 extraction of glycyrrhizin from licorice and the morphology of licorice tissue after extraction. Biotechnology and Bioprocess Engineering. 2004 Nov 1;9(6):447-53.

20- Wu J, Lin L, Chau FT. Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. Ultrasonics sonochemistry. 2001 Oct 1;8(4):347-52.

21 Alissandrakis E, Daferera D, Tarantilis PA, Polissiou M, Harizanis PC. Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey. Food chemistry. 2003 Sep 1;82(4):575-82.

22- Khanahmadi M, Ghaffarzadegan R, Khalighi-Sigaroodi F, Naghdi Badi H, Mehrafarin A, Hajiaghaee R. Optimization of the Glycyrrhizic Acid Extraction from Licorice by Response Surface Methodology. Iranian Journal of Chemistry and Chemical Engineering (IJCCE). 2018 Feb 1;37(1):121-9.

23- Julianelle LA. Immunological relationships of encapsulated and capsule-free strains of Encapsulatus pneumoniae (Friedländer's bacillus). Journal of Experimental Medicine. 1926 Nov 1;44(5):683-96.

24- Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. Journal of experimental medicine. 1933 Apr 1;57(4):571-95.

25. Shattock PF. The use of serology in the classification of micro-organisms. Microbiology. 1955 Apr 1;12(2):367-74.

26- Avery OT, Goebel WF. Chemo-immunological studies on conjugated carbohydrate-proteins: II. Immunological specificity of synthetic sugar-protein antigens. The Journal of experimental medicine. 1929 Sep 30;50(4):533.

27- Vinogradov E, Frirdich E, MacLean LL, Perry MB, Petersen BO, Duus JØ, Whitfield C. Structures of lipopolysaccharides from Klebsiella pneumoniae. Elucidation of the structure of the linkage region between core and polysaccharide O chain and identification of the residues at the non-reducing termini of the O chains. Journal of Biological Chemistry. 2002 May 1.

28- Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyenova DR, Lubin SA, Skvortsova NN. Virulence Factors and Antibiotic Resistance of Klebsiella pneumoniae Strains Isolated From Neonates With Sepsis. Frontiers in medicine. 2018;5.

Spore-Forming Bacteria in Honey

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ABSTRACT

Honey can be contaminated with microorganisms, especially spore-forming bacteria. Bacteria that can survive in honey are *Bacillus sp* and *Clostridium sp*. This study aimed to determine the presence of *Bacillus sp.* and *Clostridium sp.* on Trigona and Apis bee honey and honey packs after 1 year of storage. *Trigona sp.* honey, *Apis sp.* honey was taken directly from the honeycomb located in Hulu Sungai Tengah and Tanah Laut, South Kalimantan. Honey packs were 2 types of honey supplements for children taken randomly as much as 6 packs, at a drugstore in the Banjarbaru market. Honey packs were examined in laboratory before and after storage for 1 year at room temperature. Laboratory tests were performed on samples with parameters of TPC (Total Plate Count), *Bacillus sp.*, *Clostridium sp*., pH and moisture content. Results of research on Trigona honey found 50% of *Bacillus sp*., 10% of *Clostridium sp.*. Apis honey contained bacillus 50%, clostridium 5%. Honey packs before storage contained 83% bacillus, 33% clostridium, after 1 year storage bacillus 50%, clostridium 50%. It is recommended to research the germination inhibition factor of bacterial spores in honey.

Keywords: *Spore-forming bacteria, honey, Trigona sp., Apis sp.*

INTRODUCTION

Microorganisms contained in honey can affect the quality and safety of honey. Microbes found in honey are bacteria and fungi. These microbes can come from nectar, bees, humans and dust. The bee intestine contains 70% Gram negative bacteria such as *Achromobacter*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia coli*, *Flavobacterium*, *Klebsiella*, *Proteus*, and *Pseudomonas*. Gram negative bacteria such as coliform have commonly contaminated food ingredients. They can contaminate sauces[1], dumplings[2], even water sources such as wells[3]. It also contain 27% of Gram-positive bacteria such as *Bacillus*, *Bacteridium*, *Streptococcus* and *Clostridium spp* and 1% fungi[4].

Most bacteria could not grow and multiply in honey, but some bacteria are found in honey[5]. Spore-forming bacteria such as *Bacillus sp*., *Clostridium perfringens*, and *Clostridium botulinum* can survive in honey at 25°C.

Clostridium botulinum is presented in studies of honey samples in Argentina and Brazil. Honey containing *Clostridium botulinum* even becomes the cause of botulism in infants in California[6]. *Clostridium botulinum* spores contaminated honey is one source of botulism[7].

Bacillus sp is a bacterium that is widely found in the environment. can contaminate water, soil, air and even the air of the laboratory space[8]. *Bacillus cereus* is a bacteria caused food poisoning that can occur at infective dose of at least 10^3 bacteria per gram of food. These bacteria contaminate many foods including infant formula[9], baby rice cereal[10], cooked rice[11]. The heat resistance character of Bacillus spores cause them to persist in food. This diarrhea is caused by the enterotoxins produced during the growth of bacteria in the small intestine[12].

Methods and materials

This type of research was descriptive observation. Research samples are *Trigona sp*. honey, *Apis sp*. honey, and honey packs in the form of children supplement honey. *Trigona sp*. honey and *Apis sp.* honey were taken

directly from the honeycomb located in Hulu Sungai Tengah and Tanah Laut, South Kalimantan. Honey packs were 2 types of honey supplements for children taken randomly as much as 6 packs, at a drugstore in the Banjarbaru market. Honey packs were examined in laboratory before and after storage for 1 year at room temperature. Laboratory tests were performed on samples with parameters of TPC (Total Plate Count), *Bacillus sp.*, *Clostridium sp*., pH and moisture content.

Bacterial isolation used thioglycholate media (Merck) and blood agar plate (Merck). Incubation of blood agar plate for aerobic and anaerobic used Oxoid Anaerobic Gas Generating Kit (Anaerobene TM 2.5L AN0025A) and Oxoid Anaerobic Indicator (BR0055B). Suspect colonies of *Bacillus sp*. on the blood agar plate have characteristics of 4 - 5 mm colony diameter, pieces, rough, grey, uneven edges, slightly wavy, an-hemolytic. Suspect colonies of *Clostridium sp*. on blood agar plate have characteristics of 5 - 10 mm colony diameter, clear, smooth, shiny, creeping, darker colored in the middle of the colony, keeping, hemolytic. Colonies that grew on media were colored with Gram stain and produced images of Gram-positive bacteria with spored-bacil form. Media for biochemical tests were Simon citrate (Merck), SIM (Merck), TSIA (Merck), glucose, lactose, mannose, maltose, saccharose. Biochemical test results for *Bacillus sp.* were glucose +, lactose -, mannose +/-, maltose +/-, sakarosa +, indol -, motility +, citrate -, TSIA: slope -, base +, H2S -, gas -. Biochemical test results for *Clostridium sp.* were glucose +, lactose - / +, mannose -, maltose +, sakarosa +, indol -, motility +, citrate -, TSIA: slope -, base +, H2S -, gas -.

Measurement of honey pH used pH meter (Lutron PH-209), moisture content was calculated by gravimetric method.

RESULT AND DISCUSSION

Table 1. Spore Forming Bacteria and *Trigona sp.* Honey Characteristics

Number	Sample Code	Spore-Forming Bacteria		pH	Color
1	MKA	-	-	3,88	Brownish Yellow
2	MKB	-	-	3,54	Brownish Yellow
3	MKC	-	-	3,19	Brownish Yellow
4	MKD	-	-	3,60	Brown
5	MKE	-	-	3,15	Brownish Yellow
6	MKF	*Bacillus sp*	-	3,01	Brownish Yellow
7	MKG	*Bacillus sp*	-	3,31	Brownish Yellow
8	MKH	-	-	3,74	Brownish Yellow
9	MKI	-	-	3,18	Brownish Yellow
10	MKJ	*Bacillus sp*	-	3,20	Brownish Yellow
11	MKK	*Bacillus sp*	-	3,27	Brownish Yellow
12	MKL	*Bacillus sp*	-	3,14	Brownish Yellow
13	MKM	*Bacillus sp*	-	3,16	Brownish Yellow
14	MKN	*Bacillus sp*	-	3,11	Brown
15	MKO	*Bacillus sp*	-	3,19	Brownish Yellow
16	MKP	-	*Clostridium sp*	3,48	Brownish Yellow
17	MKQ	*Bacillus sp*	-	3,01	Brownish Yellow
18	MKR	-	-	3,57	Brown
19	MKS	*Bacillus sp*	-	3,23	Brownish Yellow
20	MKT	-	*Clostridium sp*	3,09	Brownish Yellow
Total (%)		10(50)	2(10)		

Table 2. Spore Forming Bacteria and *Apis sp.* Honey Characteristics

Number	Sample Code	**Spore-Forming Bacteria**		**pH**	**Color**
1	LMA	-	*Clostridium sp*	6,4	Bright Yellow
2	LMB	-	-	5,1	Bright Yellow
3	LMC	-	-	4,3	Brownish Yellow
4	LMD	-	-	5,9	Bright Yellow
5	LME	*Bacillus sp*	-	4,6	Brownish Yellow
6	LMF	*Bacillus sp*	-	4,0	Bright Yellow
7	LMG	*Bacillus sp*	-	4,7	Bright Yellow
8	LMH	*Bacillus sp*	-	4,2	Bright Yellow
9	LMI	*Bacillus sp*	-	5,9	Bright Yellow
10	LMJ	-	-	5,7	Bright Yellow
11	LMK	*Bacillus sp*	-	6,0	Brownish Yellow
12	LML	*Bacillus sp*	-	6,2	Bright Yellow
13	LMM	-	-	3,4	Bright Yellow
14	LMN	-	-	5,3	Bright Yellow
15	LMO	*Bacillus sp*	-	4,7	Brownish Yellow
16	LMP	-	-	4,9	Bright Yellow
17	LMQ	*Bacillus sp*	-	4,1	Bright Yellow
18	LMR	-	-	4,2	Bright Yellow
19	LMS	-	-	4,2	Bright Yellow
20	LMT	*Bacillus sp*	-	4,4	Bright Yellow
Total (%)		10(50)	1(5)		

Tabel 3. Bacteria in Honey Packs

	Sample Code	Before Storage			After Storage		
	Code	Average Number of Bacteria (Colony/g)	Spore-Forming Bacteria		Average Number of Bacteria (Colony/g)	Spore-Forming Bacteria	
1	A1	$2{,}31 \times 10^2$	*Bacillus sp*	-	$5{,}1 \times 10^2$	-	-
3	B1	$1{,}40 \times 10^2$	*Bacillus sp*	-	$4{,}12 \times 10^3$	*Bacillus sp*	-
5	C1	$9{,}52 \times 10^2$	*Bacillus sp*	*Clostridium sp*	$1{,}71 \times 10^3$	-	*Clostridium sp*
2	A2	$1{,}61 \times 10^2$	*Bacillus sp*	-	$2{,}5 \times 10^2$	-	*Clostridium sp*
4	B2	$1{,}631 \times 10^3$	Bacillus sp	Clostridium sp	$3{,}2 \times 10^3$	*Bacillus sp*	-
6	C2	$1{,}75 \times 10^2$	-	-	$2{,}23 \times 10^3$	*Bacillus sp*	*Clostridium sp*
Total (%)			5(83)	2(33)		3(50)	3(50)

Table 4. pH, Water Content in Honey Packs

Sample Code	pH	% water content	Expired Time	Suplement honey composition on packs	
A1	6,0	14,63	Nov 2016	Honey Curcuma Date Extract Propolis	
B1	5,82	13,90	Mei 2017		
C1	5,75	15,28	April 2017		
A2	5,57	21,73	Maret 2019		Honey Habbatusauda Date Extract Propolis
B2	5,55	23,46	Feb 2019		
C2	5,52	22,97	Mei 2019		

Bacterial contamination in honey bee might also come from soil containing bacterial spores. Bacteria could live on moist and humid soils and environments where many livestock lived[13]. The spore-forming bacteria found in honey in this study were *Clostridium sp* and *Bacillus sp*. This study was in line with research on honey samples in Argentina found *Clostridium botulinum* as much as 1.1% and in brazil as much as 7.06%[6]. *Bacillus cereus* was found as much as 42% in baby food in Isfahan, Iran[14]. *Clostridium sp*. and *Bacillus sp*. form the metabolically inactive endospores but are resistant to adverse environmental conditions including heat. Spores could germinate under favorable conditions and could cause food spoilage and food poisoning after consumption[15].

The genera *Clostridium* and *Bacillus* are commonly found in soil, water, and gastrointestinal tracts of insects and animals, as well as humans. Both genera grow in low oxygen environments. Clostridia require more anaerobic atmosphere, but some species is aero-tolerance. *Clostridium* and *Bacillus* pathogen species can survive in and out of various host species. The disease is often associated with toxins and protein spores that include gangrene gas, food poisoning, diarrhea, pseudomembranous colitis, and enterotoxemia[16]. The pH values of all honey samples in this study were acidic (pH 3.01-6.4). The lowest acidity was detected in *Trigona sp*. (3.30 ± 0.246), followed by *Apis sp*. honey (4.91 ± 0.856), and honey packs (5.70 ± 0.19). There was a significant difference of honey pH between *Trigona sp.* with *Apis sp.* honey ($P < 0.05$). The pH of honey in this study was close to the pH value of honey in the previous study, ie Yemen, Egypt, Saudi, Kashmir (pH 4,114-4,637)[17]. Other studies have also shown that the pH of honey is close to similar values, ie in Indian, Algerian, Brazilian, Spanish and Turkish honey (pH 3.49-4.70)[18]. Honey with acidic pH is not a good medium for microbial growth. The pH 7.4 media supports better spore growth than pH 5.5[19]. Spores were inactivated at lower pH values of the media[20].

The degree of honey acidity correlates with the fermentation of sugars in honey into organic acids. The acidity of honey causes honey to stabilize against microbial decay Bogdanov et al. (2008). The acidity of honey may also indicate that the honey sample has a high mineral content[21].

The results showed that after 1 year storage at room temperature honey packs tend to show increased number of germs, such as *Bacillus sp.* and *Clostridium sp*. The food composition and storage conditions determine germination and development of living spores[22]. The composition of honey that contained more than 180 substances, including amino acids, enzymes, proteins, vitamins, minerals, ash, organic acids and phenol compounds[18] can be a good growth medium for microbes.

The color of honey varies like dark yellow, blackish brown or black. The most important aspect that affect the marketing value of honey is the color of honey. Color

change may be associated with mineral content, heavy metal contamination, and high temperature or light exposure[23]. The results showed the honey color were bright yellow to brown. *Trigona sp*. honey tend to have more brown color, a more acidic pH and a more watery texture. The color of honey is affected by the mineral content in it[24]. This is because *Trigona sp* bees consume more minerals. The amount of *Trigona sp.* Honey production is much less because these bees produce more propolis[25].

CONCLUSION

Trigona honey contained bacillus sp 50%, clostridium 10%. Apis honey contains bacillus 50%, clostridium 5%. Honey packs before storage contains 83% bacillus and 33% clostridium. After 1 year storage honey packs contains bacillus 50% and clostridium 50%.

Ethical Clearance: Taken From *Health Research Ethics Committee* Politeknik Kesehatan Banjarmasin

Conflict of Interest: Nil

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REFERENCES

1. Dwiyanti RD, L Lutpiatina. Bacteriological Quality of Pentol Tomato Sauce in Banjarbaru (Mutu Bakteriologis Saus Tomat Pentol di Banjarbaru). Medical Laboratory Technology Journal. 2016; 2(1), 1-5
2. Yunus R, Ruth Mongan, Rosnani. Gram Negative Bacterial Contamination on Siomay Snacks in Kendari City (Cemaran Bakteri Gram Negatif pada Jajanan Siomay di Kota Kendari). Medical Laboratory Technology Journal. 2017; 3(1), 11-16
3. Roebiakto E, G Setiadi, YJ Supriyadi. Overview of Bacteriological Quality and Risk Level of Dug Well Water Pollution in Sungai Ulin Village, Banjarbaru City (Tinjauan Kualitas Bakteriologis dan Tingkat Risiko Pencemaran Air Sumur Gali di Kelurahan Sungai Ulin Kota Banjarbaru). Medical Laboratory Technology Journal. 2017; 3(1), 32-36
4. M. Gilliam and D. Prest, Microbiology of feces of the larval honey bee, Apis mellifera, J. Invertebr. Pathol. 1987; 47(1), 70–75
5. Snowdon JA, The microbiology of honey—meeting your buyers' specifications, Am. Bee J. 1999; 1999(139), 51–60
6. Schocken-Iturrino RP, Carneiro MC, K. E, Sorbara JOB, Rossi OD, and Gerbasi LER, Study of the presence of the spores of Clostridium botulinum in honey in Brazil, FEMS Immunol. Med. Microbiol. 1999; 24(3), 379–382
7. Peck M.W., Biology and genomic analysis of Clostridium botulinum, Adv Microb Physiol. 2009; 2009(55), 183–265
8. L. Lutpiatina, Uricase Products from *Bacillus sp*. Laboratory Contaminants, Med. Lab. Tecnol. J. 2015; 1(2), 96-101
9. Jun H, Kim J, Bang J, Kim H, Beuchat L.R, and Ryu J.H., Combined effects of plant extracts in inhibiting the growth of Bacillus cereus in reconstituted infant rice cereal, Int. J. Food Microbiol. 2013; 160, 260–266
10. Wang J, Ding T, and Oh D.H, Effect of temperatures on the growth, toxin production, and heat resistance of Bacillus cereus in cooked rice, Foodborne Pathog. Dis. 2014; 2014(11), 133–137
11. Becker H, Schaller G, von Wiese W, and Terplan G, Bacillus cereus in infant foods and dried milk products, Int. J. Food Microbiol.1994; 1994(23), 1–15
12. Madigan M.T, Martinko J.M, and Parker J, Enterotoxins, in Brock Biology of Microorganisms., T. C, Ed. USA: Prentice Hall, 2003; 746–748.
13. Ebrahim Rahimi, Fahimeh Abdos, H. Momtaz, Zienab Torki Baghbadorani, and Mohammad Jalali, Bacillus cereus in Infant Foods: Prevalence Study and Distribution of Enterotoxigenic Virulence Factors in Isfahan Province, Iran, Scientific World Journal, 2013.
14. M. L. Stecchini, M. Del Torre, and P. P. M. Stecchini, Survival Strategies of Bacillus Spores in Food, Indian J Exp Biol 51. (11), 905–909
15. McKillip J.L, Prevalence and expression of enterotoxins in Bacillus cereus and other Bacillus spp., Antonie Van Leeuwenhoek. 2000; 77, 393–399
16. S.A.El Sohaimy, S.H.D.Masry, and M.G.Shehata, Physicochemical characteristics of honey from different origins, Ann. Agric. Sci. 2015; 60, 279–287
17. S. Ouchemoukh, H. Louaileche, and P. Schweitzer, Physicochemical characteristics and pollen spectrum of some Algerian honeys, Food Control. 2007; 2007(18), 52–58

18. A.K. Warda, H. M. W. den Besten, Na Sha, T. Abee, and M. N. N. Groot, Influence of Food Matrix on Outgrowth Heterogeneity of Heat Damaged Bacillus Cereus Spores, Int J Food Microbiol. 2015; 201, 27–34
19. S. Bogdanov, T. Jurendic, R.Sieber, and P. Gallmann., Honey for nutrition and health: a review, J. Am. Coll. Nutr. 2008; 27, 677–689
20. S.A. Mohammed and E.E. Babiker, Protein structure, physicochemical properties and mineral composition of Apis mellifera Honey samples of different floral origin, Aust. J. Basic Appl. Sci. 2009; 3(3), 2477–2483
21. M. H. J. Wells-Bennik et al., Bacterial Spores in Food: Survival, Emergence, and Outgrowth, Annu Rev Food Sci Technol. 2016; 7, 457–482
22. S. Bogdanov, Physical Properties of Honey. Bee Product Science; 2010
23. M. Moniruzzaman, M.I. Khalil, and S. H. G. Sulaiman, Physicochemical and antioxidant properties of malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera, BMC Complement. Altern. Med. 2013; 13, 1–1251
24. Kuntadi, Honey: Its composition, properties and properties, November. Jakarta: Sylva Tropika Popular Information on Science and Technology; 2002.
25. M. A. Wijayanti, E. Herdiana, and Sugeng Yuwono, Effect of Bee Propolis on Plasmodium berghei infection in Swiss Mice, Berkala Ilmu Kedokteran. 2003; 35(2), 81–89

Comparison of CT, MRI, and Diffusion–Weight MRI in Differentiation Cystic Brain Tumors (Prospective Study)

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ABSTRACT

Objective: To determine the ability of MRI, diffusion–weight MRI images and ADC (apparent diffusion coefficient) in comparison to CT scan to differentiation cystic brain tumors.

Method: From January 2017 to October 2018, this prospective study included 60 patients (age range 20 to 85 years, mean age 55 years) diagnosed clinically as having cystic brain tumor. A conventional MRI was done on Philips MRI a 1.5T using T2, T1 and fat-suppressed technique and echo-planar spin-echo sequence diffusion weight and ADC is carried for all patients with CT scan.

Results: All CT scan and MR imaging features were categorized brain lesion as cystic brain tumor according to the features of imaging which were compared the result with histopathological findings. Sixty patients, 34 male and 26 female the diagnosis was confirmed with pathological findings which included Primary malignant cystic brain tumors 16 (20%) Primary benign cystic brain tumor 24 (26%) and Metastatic tumor 20 (32%). The sensitivity for diagnosis primary malignant cystic tumor 60%, Primary benign cystic brain tumor 100% and the sensitivity of DWI for diagnosis metastasis tumor 81%. Diffusion–weight MRI and ADC is good modality for differentiation cystic necrotic tumor.

Conclusion: According to our results we concluded that MRI has more accuracy than CT scan for diagnosis brain tumors and biopsy correlation. Both CT scan and MRI reports for diagnosis of benign or malignant tumor compared with pathologic findings and they were not significant.

Keywords: *Brain Neoplasms, Computerized Tomography, Magnetic Resonance Imaging, Diffusion–Weight, ADC (apparent diffusion coefficient) .*

INTRODUCTION

There is MRI protocol for preoperative cystic brain tumor assessing include T1 weighted with gadolinium enhanced, T2 weighted and diffusion weighted to evaluate the lesion, vascularity and blood brain barrier integrity these sequence result in correct diagnosis in majority of cases[1].

all primary benign tumor can be diagnosis by DWI. The sensitivity of DWI for diagnosis of primary benign cystic brain tumor is 100% [2,3].

computed tomography CT) and magnetic resonance image (MRI) are being routinely used to screen the neonate for plausible intracranial problems [4-7].

Diffusion –weighted MR has an establish role in the differential diagnosis between brain abscess, cystic tumor and between epidremoid cyst and arachnoid cyst[8].

CT scan remains a good choice in diagnosis of some conditions like bone or vascular involvement and metastases to the skull base [9].

There is protocol for typical MRI in preoperative assessing tumor of brain include T2 and T1 weighted, diffusion weighted to evaluate the lesion T1 weighted with gadolinium enhanced, blood brain barrier integrity, irregularity and vascularity these sequence result in correct diagnosis in majority of cases [10,11].

The aim of this study was to investigate whether the different cystic brain tumors can be differentiated

on CT scan, MRI and diffusion –weight (MRI) and apparent diffusion coefficient (ADC) on the basis of their appearance and characterization of signal intensity.

PATIENTS AND METHOD

In this cross-sectional study, 60 patients aged 20–85 years (mean age 50 years) with clinically suspected brain tumor were included. The patients were having different clinical presentations; headache 42 (70%), Dizziness 12 (20%), and Confusion 6 (10%).

For all patients, CT scan, conventional MRI preoperative and diffusion–weighted MRI were done to show characterization of brain tumor by CT appearance whether cystic or solid. All solid tumors were excluded from this study, then MRI signal intensity was done.

This study was carried out in department of surgery, Hilla teaching hospital, Iraq, in the period extended from January 2017 to October 2018. Chief complains of all patients documented from their medical record. Pathologists evaluated the lesions as benign or malignant, without any information about CT scan or MRI reports. CT scan reported cystic or solid lesion, with or without calcification and edema, shifting and enhancement. CT scan or MRI reports were concluded lesions as benign or malignant. The MRI pictures had been seen and confirmed by two radiologists to differentiate the brain tumor whether followed by operative exploration in the same hospital, the median time between initial MRI and surgery 31 days (range 5 days to 76 days).

CT scan by Philips 64 slice then Magnetic resonance imaging (MRI) was carried out on a Philips 1.5T system using multiplanar sequence T2, T1 and fat-saturation and sequence diffusion weight is carried for all subjects. All MR imaging features were categorized according to the features of imaging as malignant or benign without clinical details.

MRI Protocol

In this study Philips MRI 1.5 T units was used, with different planes (coronal, sagittal and axial) see the brain lesion whether cystic or solid, site, texture, extension, and intensity. Used multiplanar sequence diffusion weight with ADC do for all patients. In this study, the thickness of slice was 5 mm and the slices numbers averaged 14, which decrease the effects of eventual motion and volume partial between diffusion-weighted imaging and conventional MR.

RESULTS

Sixty subjects admitted in teaching hospital in Babylon in Iraq have different symptom and signs. In this study, age of patients included ranged from 25-85 years mean age (55 year), 34 male and 26 female, then routine CT scan and conventional MRI examination was done, in conventional MRI examination show the patient normal or abnormal, if there is mass in brain or not, the abnormal patient who have cystic mass in brain do DW MRI in department of radiology in the same hospital to determine the mass, appearance of mass, nature and side then do ADC (apparent diffusion coefficient), the appearance of MRI are read by two radiologist without known history of patient to determine the diagnosis and mention the report, then all patient do surgery in in the same hospital. In all 60 patients the diagnosis was confirmed with histological evaluation which include primary benign cystic tumor 24 (40%), have primary malignant brain tumor (cystic glioma) 16 (26%) and patients have metastatic tumors 20 (34 %).

All primary benign cystic brain tumors (arachnoid cyst and epidermoid cyst) can diagnosed by CT scan show hypodense area fluid density (Figure 1), DWI and ADC show high signal on DW MRI and low signal on ADC, so the sensitivity for diagnosis arachnoid cyst and epidermoid cyst on DW MRI are 100%. (Figures 2). The pattern of enhancement on CT scan and MRI can be shown in (Table 2).

Sixty patients by using CT scan, which 13 (54%) were diagnosed benign from 24 benign tumor, 10 (62.5%) malignant from 16 malignant tumor and 9 (45%) were diagnosed metastasis from 20 metastasis tumor. Sensitivity, specificity, positive predictive value and negative predictive value of CT scan were 83%, 10%, 93% and 3% respectively. The accuracy of this method was 78%.

While by MRI Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of MRI were 94%, 27%, 93%, 3% and 88% respectively.

The sensitivity for diagnosis primary cystic malignant brain tumor only 6 from 16 primary malignant cystic brain tumor (Figure 3) show hyper intense on DWI and hypo intense on ADC while the remain primary cystic tumor 6 show hypo intense on DWI and hyper intense on ADC Table 1) make the sensitivity of DWI foe diagnosis primary malignant cystic tumor 60%, in

metastatic tumors 4 out of 20 metastatic tumors showed hyper intense on DWI and hypo intense on ADC while the remain showed hypo intense on DWI and hyper intense on ADC make the sensitivity of DWI for diagnosis metastasis tumor 81%. as shown in (Figure 4).

Table 1: Signal intensity of cystic cerebral tumor on DWI and ADC

Type of cerebral lesions	**Number**	**DWI**	**ADC**
Primary malignant cystic brain tumors	N=16 N=0	High signal Low signal	Low signal High signal
Metastatic tumors	N=15 N=5	Low signal High signal	High signal Low signal
Arachnoid cyst	N=14 N=0	Low signal	High signal
Epidermoid	N=10 N=0	High signal	Low signal
Total	60		

Table 2: Pattern of enhancement on CT scan and MRI

D/D	**CT scan**	**MRI**						**PET**
		T1	**T2**	**Gad enhanced**	**DWI**	**ADC**	**MRS**	
Primary cystic neoplasm	Hypodense with rim enhancement	Hypointense	Hyperintense	Thick rim or nodular	variable	high	Suppressed NAA and Creatinine. Elevated choline and lactate in high grade glioma	Increased Metabolic Uptake
Metastasis	Hypodense lesions with rim enhancement	Hypointense	Hyperintense	Thick rim or nodular	Dark	high	Suppressed NAA and Creatinine. Elevated choline and lacate variable.	Increased Metabolic Uptake
Primary benign cystic tumor	Ring enhancing lesions with surrounding edema. multilobulated lesions may be seen s/o daughter abscesses	Core hyperintense, surrounding hypointense edema	Cavity hypointense, surrounding hyperintense edema	Ring enhancement	Bright	low	Lactate, lipids, amino acids, acetate and succinate peaks	Increased Metabolic Uptake

MRI=Magnetic resonance imaging, DWI=Diffusion weighted imaging, ADC=Apparent diffusion coefficient, MRS= Magnetic resonance spectroscopy, PET=positron emission tomography, CT= computerized tomography, NAA=N acetyl aspartate.

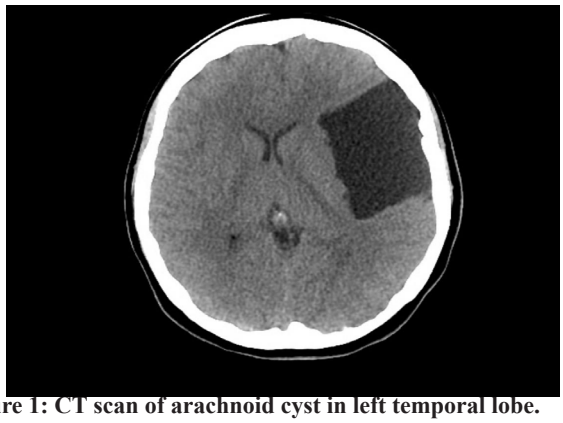


Figure 1: CT scan of arachnoid cyst in left temporal lobe.

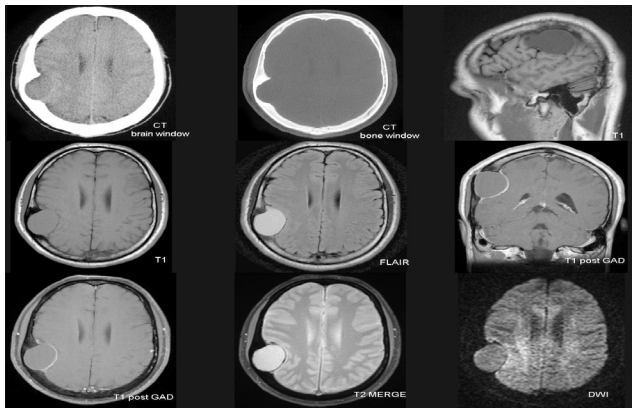


Figure 2: Arachnoid cyst in right parietal lobe

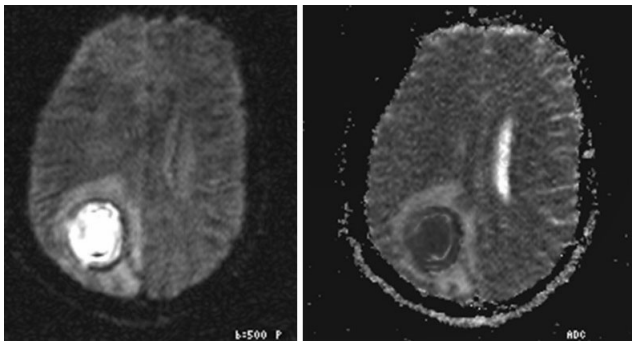


Figure 3: Cystic glioma in right occipital lobe.



Figure 4: Metastatic tumor from lung cancer in left occipital lobe on DWI shows low signal surrounding by high signal edema

DISCUSSION

According to our result, CT scan and MRI were sensitive imaging in intra cranial tumors but they are not specific. Their high positive predictive value and low negative predictive value also made them reliable diagnostic procedures when it is difficult to access mass directly.

In this study relation between CT scan findings and pathologic findings surveyed and concluded that some findings like cystic or solid lesions, calcification, shifting, edema and enhancement did not predispose biopsy reports, but hydrocephaly found as a predictive finding in CT scan reports for determining malignant tumors.

MRI is a good module using multiple relaxation properties seen in tissue types to provide information on content of masses of soft tissue, the result of this information show the character of masses of soft tissue [12,13]. Diffusion-weighted MR imaging is found to be highly specific and sensitive for the diagnosis of cerebral infarction in acute stage [14].

In this study, the sensitivity of DWI 90% for diagnosis of primary brain tumor. This result goes with finding of Bukte [15]. DWI (diffusion–weight MR imaging) has shown promise in differentiating malignant neoplasm and brain abscess the ability of DWI to diagnosed brain abscess enables a neurosurgeon to change planning of management, all brain abscess restricted water diffusion as indicated by hyper intense on DWI and diminished on ADC this finding similar to finding by Leuthardt [16].

In this study we focused on ability of DWI and ADC to characterize necrotic tissue from water content of brain mass that DAI restricted all water in abscess causing the abscess high signal on it and low signal on ADC this finding similar to finding by several reports[4,11].

In this study relation between CT scan findings and pathologic findings surveyed and concluded that some findings like cystic or solid lesions, calcification, shifting, edema and enhancement did not predispose biopsy reports, but hydrocephaly found as a predictive finding in CT scan reports for determining malignant tumors.

In this study the sensitivity of DWI for diagnosis abscess are 100 %, for diagnosis primary benign cystic brain tumor (arachnoid and epidermoid cyst) are 100 %

this result goes with finding of several reports [13].

In tumor lesion presence large areas of necrosis can be well detected on the elevated ADC [17]. One of the early reported applications of diffusion imaging in brain tumors diagnosis was the differentiation between primary benign epidermoid and extra-axial cysts[18]. In our study found Diffusion-weighted imaging is good modality for distinguishing brain abscess from cystic or necrotic tumor lesions, is more best than performing conventional MRI alone the finding similar to finding by Chang [19].

In this study, patients were classified into three main groups; first group: the patients with cystic or necrotic malignant brain tumors of high grade necrotic glioma (16 cases); second group: is benign cystic masses, arachnoid and epidermiod cysts (24 cases); and third group: metastatic cystic tumor (20 cases).

On visual assessment, all brain abscesses showed ring enhancement at post contrast T1 weighted images and brain abscesses are hyper intense on DWI and hypo intense on ADC maps (restricted diffusion) make the sensitivity of diffusion weighted for diagnosis brain abscess 100% this result similar to finding by Leuthardt[20].

CONCLUSION

According to our results we concluded that MRI has more accuracy than CT scan for diagnosis brain tumors and biopsy correlation. Both CT scan and MRI reports for diagnosis of benign or malignant tumor compared with pathologic findings and they were not significant. There is widely applied of Diffusion-weighted MRI imaging for diagnosis of various diseases including the detection of cystic brain tumors from abscess, and distinguishing arachnoid from epidermoid cysts.

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Ethical Clearance : The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

REFERENCES

1- Taj-Aldean KA. The Validity of Magnetic Resonance Imaging (MRI) in Characterizing Benign and Malignant Uterine Endometrial Cavity Masses. Res J Pharm Biol Che Sci. 2016; 7(6):750-754.

2- Taj-Aldean KA. Role of MRI In Comparison With DWI-MRI In Diagnosis Of Intracranial Meningioma . A prospective study. Indian J Public Health Research & Development., 2018:9(10): 336-341.

3- Taj-Aldean KA. Role of diffusion weight in differential diagnosis of cerebral cystic lesion. A prospective study. Int J Pharm Clin Res., 2017:9(1):1-5.

4- Taj-Aldean KA. Cranial CT scan. and sonographic finding in term and preterm newborn. A prospective study. Indian J Public Health Research & Development., 2018:9(10): (387-391).

5- Cole L, Dewey D, Letourneau N, Kaplan BJ, Chaput K, Gallagher C, et al. Clinical Characteristics, Risk Factors, and Outcomes Associated With Neonatal Hemorrhagic Stroke: A Population-Based Case-Control Study. JAMA Pediatr 2017; 171(3):230-238.

6- Golomb MR, MacGregor DL, Domi T, Armstrong DC, McCrindle BW, Mayank S, et al. Presumed pre- or perinatal arterial ischemic stroke: risk factors and outcomes. Ann Neurol. 2001; 50:163–8.

7- Armstrong-Wells J, Johnston SC, Wu YW, Sidney S, Fullerton HJ. Prevalence and predictors ofperinatal hemorrhagic stroke: results from the kaiser pediatric stroke study. Pediatrics.2009; 123:823–8.

8- Mascalchi M, Filippi M, Floris R, Fonda C, Gasparotti R, Villari N. Diffusion –weighted MR of the brain: methodology and clinical application. Radiol Med. 2005;109(3):155-97.

9- Poussaint TY, Gudas T, Barnes PD. Imaging of neuroendocrine disorders of childhood. Neuroimaging Clin N Am. 1999;9(1):157-75.

10- Wang S, Kim S, Chawla S, et al. Differentiation between glioblastomas and solitary brain metastasis using diffusion tensor imaging. Neuroimage. 2009; 44(3):653-660.

11- Taj-Aldean KA. The Validity of Magnetic Resonance Imaging (MRI) in Characterizing Benign and Malignant Uterine Endometrial Cavity Masses. Res J Pharm Biol Che Sci. 2016; 7(6):750-754.

12- Huber S, Medl M, Baumann L, Gzembirek H, Value of ultrasound and magnetic resonance in the preoperative evaluation of suspected ovarian masses, Anticancer Res, 2002; 22: 2501-2507.

13- Taj-Aldean K.A. Validity of Magnetic Resonance Imaging (MRI) in characterizing adnexal masses: a prospective study. J Chem Pharmaceut Sci. 2017; 10(1): 142-146.

14- Lutsep HL, Albers GW, DeCrespigny A, Kamat GN, Marks MP, Moseley ME. Clinical utility of diffusion-weighted magnetic resonance imaging in the assessment of ischemic stroke. Ann Neurol 1997;41:574–580.

15- Bukte Y, Paksoy Y, Genc E, Uca AU. Role of diffusion-weighted MR in differential diagnosis of intracranial cystic lesions. Clin Radiol. 2005; 60(3):375-383.

16- Leuthatdt EC, Wippoid FJ, Oswood MC, Rich KM. Diffusion –weighted MR imaging in the preoperative assessment of brain abscesses. Surg Neurol. 2002; 58(6):395-402.

17- Lyng H, Haraldseth O, Rofstad E. Measurement of cell density and necrotic fraction in human melanoma xenografts by diffusion weighted magnetic resonance imaging. Magn Reson Med. 2000; 43(6):828–836.

18- Holdsworth SJ, Skare S, Newbould RD, Bammer R. Robust GRAPPA-accelerated diffusion-weighted readout-segmented (RS)-EPI. Magn Reson Med. 2009; 62 (6): 1629–1640.

19- Chang SC, Lai PH, Chen WL, Weng HH, Ho JT, Wang JS, et al. Diffusion-weighted MRI features of brain abscess and cystic or necrotic brain tumors: Comparison with conventional MRI. Clin Imaging. 2002; 26(4):227–236.

20- Leuthatdt EC, Wippoid FJ, Oswood MC, Rich KM. Diffusion –weighted MR imaging in the preoperative assessment of brain abscesses. Surg Neurol. 2002; 58(6):395-402.

Health Literacy as a Risk Predictor of Cardiovascular Diseases among Informal Sector Worker in Makassar City

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ABSTRACT

Background: Health literacy is a concept that can influence a person's health status. The purpose of this study was to determine the relationship between health literacy and the risk of cardiovascular disease in informal sector cooks in Makassar City. **Method**: The quantitative study method with cross sectional design was carried out in the working area of the Tamalanrea Health Center in 80 informal sector cooks determined by purposive sampling. Data collection uses HLS-EU prevention of cardiovascular disease, and the form of the Jakarta Cardiovascular Score. Processing data with SPSS version 20 for Windows, analyzing data with Chi Square test on 95% CI and $\rho<0.05$. The results showed that more than half of the informal sector cooks had a low literacy level (63.7%) and were in the high risk category of cardiovascular disease (52.5%). **Result:**There is a significant relationship between low health literacy and the risk of cardiovascular disease ($p = 0,006$). **Conclusion:** low health literacy is very influential on the risk of cardiovascular disease. It is recommended to use a preventive health based literacy model for informal sector workers to be able to be independent in seeking their health.

Keywords: *Health literacy, Risk of cardiovascular disease*

INTRODUCTION

According to WHO (2016), deaths from cardiovascular disease have affected more than 75% of the population in low and middle income countries.[1] This condition is caused due to the fact that integrated primary health care programs for early detection and treatment for people who have risk factors are often not utilized properly, so the disease is slowly detected and eventually experiences death.[2]

Indonesia is one country that has a high risk of premature death. According to WHO (2016), non-communicable diseases are estimated at 75% as a cause of death from all deaths, as much as 35% of the proportion of deaths caused by cardiovascular disease and predicted to continue to increase.[3]

The international world continues to try to reduce the incidence of non-communicable diseases. World Heart Federation (WHF) has initiated a cardiovascular disease prevention and control program called 25 by 25 Global Target. This program was created with the aim of reducing the premature mortality rate due to CVD by 25% in 2025.[4]

On national scale, Indonesia has taken several control measures against the risk of cardiovascular disease, such as making guidelines for controlling heart and blood vessel disease[5], GERMAS programs (Healthy Life Society Movement), and CERDIK appeal (regular health checks, eliminate cigarette smoke, diligently exercise physically, healthy and balanced diet, adequate rest, and manage stress), but the results are not maximal. The results of the Basic Health Research (Riskesda)

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prove the increase in cases of cardiovascular disease in 2018 compared to 2013. [6,7]

Research conducted by Kurniawidjaja (2007) in several companies in Indonesia, found that 40% - 58.3% were caused by cardiovascular disease.[8] Informal sector cooks are workers with a high prevalence rate of the incidence of hypertension. Research conducted by Bosu (2015) found the highest prevalence of hypertension in cooks in the West African region at 68.9% compared to other types of work.[9] Factors causing cardiovascular disease in workers, can be caused by the work environment such as noise,[10-13] work stress,[14-16] chemicals in the form of pollutants,[17,18] workloads,[19,20] and healthy lifestyle.[8,14] Kurniawidjaja reported thatcase identification results of cardiovascular disease in workers at an automotive factory and found 23% of workers at high risk, 50% at risk of developing cardiovascular disease, where the dominant risk factors for health problems were factors that could be intervened with healthy life behaviors.[8]

The results of a study conducted by Won et al. (2013) found that knowledge is a significant predictor of cardiovascular events in workers.[21] According to Setyawaty, the lack of knowledge and skills regarding occupational health and safety in the workplace has resulted in workers being at risk of getting accidents due to work and work-related illnesses and diseases due to work relations.[22]

Health literacy is what underlies good health knowledge and is very influential on the healthy behavior of individuals.[23] Research conducted by Andrade et al (2017), to characterize specific knowledge of cardiovascular disease, especially stroke and myocardial infarction (MI), and its relationship with sociodemography factors, health knowledge and clinical history, in 1624 Portuguese residents, found important gaps in knowledge related to cardiovascular health in Portuguese society. Andrade et al (2017), suggested the need for a strategy and practice of health education to improve knowledge of cardiovascular health among the Portuguese population.[24]

METHOD

This study used a cross sectional study from January to February 2018 on 80 informal sector cooks determined by purposive sampling technique. Data collection used the HLS-EU cardiovascular disease questionnaire, consisting of 15 questions for disease prevention for workers.[23] Health literacy questionnaire is a standard questionnaire but researchers modified the questionnaire so that it was easier to understand by respondents without changing the purpose of each question.

The results of the validity test by comparing the r value of the table with r count obtained by the value of r count (0.635 - 0.853)> r table, so the questionnaire was declared valid. The instrumental reliability test results are using the Cronbach's Alpha formula, obtained by the reliable coefficient value of 0.943> 0.60 then the statement is said to be reliable. Determination of the risk of cardiovascular disease using the form of the Jakarta Cardiovascular Score. Jakarta Cardiovascular Score is a standard questionnaire so that validity and reliability tests are not carried out. Obtained from the risk value for gender, age, blood pressure measured using a Sphygmomanometer, body mass index (height measured by microtoise which has a precision of 0.1 cm; body weight was measured by tread scales), smoking, diabetes based on the statement of respondent has ever been diagnosed by a health worker and physical activity based on the statement of respondents who refer to the criteria in the Jakarta Cardiovascular Score, with a sensitivity level of 77.9% and high specificity of 90%, against the Framingham study scoring.[25] Processing data with SPSS version 20 for Windows, analyzing data with Chi Square test on 95% CI and $\rho < 0.05$.

RESULT

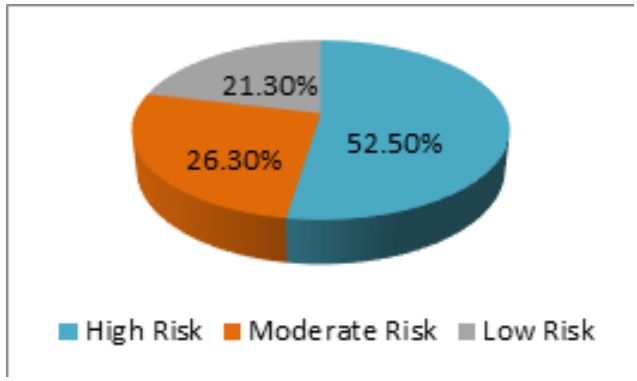


Figure 1. Distribution of Risk of Cardiovascular Disease to Respondents

In Figure 1, the data collection results show that the highest percentage of cardiovascular disease risk in respondents is in the high risk category (52.5%).

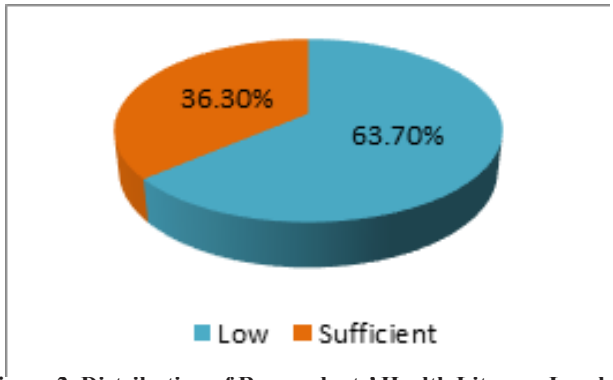


Figure 2. Distribution of Respondents' Health Literacy Levels

Figure 2 shows the identification of respondents' Health Literacy using the HLS-EU questionnaire, where the largest percentage of respondents was in the category of low Health Literacy (63.8%).

Table 1: Relationship between Health Literacy and the Risk of Cardiovascular Disease in Respondents

Health Literacy	Risk of cardiovascular disease			Total	ρ - Value
	High	Moderate	Low		
Low	33(64,7%)	12(23,5%)	6(11,8%)	51 (100%)	0,006
Sufficient	9(31,0%)	9(31,0%)	29(100%)	29(100%)	
Total	42(52,5%)	21(26,3%)	17(21,3%)	80 (100%)	

p value < 0,05

Table 1 shows a high percentage of respondents with low literacy, had a high risk of cardiovascular disease (64.7%). There is a trend of an increased risk of cardiovascular disease in respondents who have low health literacy. Table 1 also shows a significant correlation between the level of health literacy and the risk of cardiovascular disease.

DISCUSSION

Health literacy is a process carried out by a person related to his health condition in terms of making appropriate decisions so that he is always in a healthy condition.[26-28] The results of this study indicate the level of health literacy of respondents in the less category (63.7%).

The level of health literacy that is lacking is a condition that must receive attention from the government because it involves a basic understanding of the risk of an illness. The level of health literacy can be influenced by several determinants, the first is a personal determinant that includes age, gender, race, socio-economic status, education, employment and income, and the second is the determinants of society and the environment including demographic conditions, culture, language and community systems) and social determinants (family support and relations).[28,29]

In addition, access to health information also affects a person's ability to obtain health information and education from health care providers.[30] Especially for informal sector workers, inherent characteristics such as low education levels, lack of access to health information and health services, will ultimately lead to poor workers' health. The results of a survey conducted by the ILO in collaboration with Jamsostek (Workers' Social Security), found that 80% of informal sector workers do not have health insurance other than expecting guarantees from their families.[31]

Regarding the risk of cardiovascular disease, the results of this study prove a significant relationship between the level of health literacy and the risk of cardiovascular disease. There is aincreasing trend risk of cardiovascular disease in workers with low health literacy than workers with sufficient health literacy.

Research conducted by Ko et al (2013), corroborates the results of this study. Ko et al. conducted a study of

the relationship between health literacy and knowledge and control of disease in patients with hypertension in Singapore, found a significant relationship between health literacy of patients with hypertension and knowledge of hypertension.[32]

Relationship between health literacy with different cases of disease is carried out by several researchers, namely Kalichman and Rompa (2000) and Giselle et al (2006). This study provides evidence that health literacy is a factor that has a significant relationship with the health and treatment of patients with HIV AIDS.[26,33] According to Nutbeam, health literacy is the goal of the concept of behavioral-based health and communication education that aims not only at lifestyle changes but also to achieve awareness of the effects of health and encourage individuals and communities to act in dealing with health problems.[27]

This study proves that informal sector workers with a low level of health literacy have the potential to experience the risk of cardiovascular disease, so a model of preventive action based on health literacy needs to be made to

CONCLUSIONS

this study shown that health literacy greatly influences cardiovascular disease. informal sector workers with a low level of health literacy have the potential to experience the risk of cardiovascular disease.

RECOMMENDATIONS

Health literacy model are a recommended to prevent cardiovascular disease in informal sector workers in order to be independent in seeking their health.

Conflict of Interest: There is no conflict of interest.

Ethical Clearence: Taken from Hasanuddin University Ethics Committee with number: 768/H4.8.4.5.31/PP36-KOMETIK/2017.

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REFERENCES

1. WHO. Cardiovascular diseases (CVDs). http://wwwwhoint accessed at 6 November 2018.
2. WHO. Cardiovascular Disease. http://wwwwhoint/cardiovascular_diseases accessed at 6 November 2018.
3. WHO. Indonesia, Risk of Premature Death Due to NCDS (%). http://wwwwhoint Accessed at 6 November 2018. 2016.
4. Rilantono.I.L&Rahajoe.A.U. Cardiovasculare in woman: Challenge in 21 Century. Faculty od Medicine Publisher, Indonesia University; 2014 P
5. Ministry of Health RI. Guideline for Controlling Cardiovascular. Ministry of Health RI; 2007.
6. Ministry of Health RI. Heart Disease Causes The Highest Mortality. www.depkes.go.id2017 accessed at 6 November 2018.
7. Ministry of Health RI. Indonesian Healthy Portrait of Riskesdas. www.depkes.go.id2018 accessed at 20 November 2018.
8. Kurniawidjaja M. Occupational Health Theory And Aplication. Indonesia University Press. 2010.
9. Bosu WK. The prevalence, awareness, and control of hypertension among workers in West Africa: a systematic review. Global health action. 2015;8:26227.
10. Assunta C, Ilaria S, Simone de S, Gianfranco T, Teodorico C, Carmina S, et al. Noise and cardiovascular effects in workers of the sanitary fixtures industry. International journal of hygiene and environmental health. 2015;218(1):163-8.
11. Ismaila SO, Odusote A. Noise exposure as a factor in the increase of blood pressure of workers in a sack manufacturing industry. Beni-Suef University Journal of Basic and Applied Sciences. 2014;3(2):116-21.
12. Gan WG, et.al. Exposure to loud noise, bilateral high-frequency hearing loss and coronary heart disease. Occupation and Environmental Medicine. 2016;73(1).
13. Girard SA, Leroux T, Verreault R, Courteau M, Picard M, Turcotte F, et al. Cardiovascular disease mortality among retired workers chronically exposed to intense occupational noise. International archives of occupational and environmental health. 2015;88(1):123-30.
14. Hegg-Deloye S, Brassard P, Prairie J, Larouche D, Jauvin N, Poirier P, et al. Prevalence of risk factors for cardiovascular disease in paramedics. International archives of occupational and environmental health. 2015;88(7):973-80.
15. Van Droogenbroeck F, Spruyt B. Do teachers

have worse mental health? Review of the existing comparative research and results from the Belgian Health Interview Survey. Teaching and Teacher Education. 2015;51:88-100.

16. Backe EM, Seidler A, Latza U, Rossnagel K, Schumann B. The role of psychosocial stress at work for the development of cardiovascular diseases: a systematic review. International archives of occupational and environmental health. 2012;85(1):67-79.
17. Burroughs Pena MS, Rollins A. Environmental Exposures and Cardiovascular Disease: A Challenge for Health and Development in Low- and Middle-Income Countries. Cardiology clinics. 2017;35(1):71-86.
18. Miller *ea*. Long-Term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women. The new england journalof medicine. 2007;356(5).
19. Black JK, Balanos GM, Whittaker Previously Phillips AC. Resilience, work engagement and stress reactivity in a middle-aged manual worker population. International journal of psychophysiology : official journal of the International Organization of Psychophysiology. 2017;116:9-15.
20. Mulyanto. Number of Employees Killeddue to Fatigue Workin Japan is Increases.https:// internasionalkompascom accessed at 7 November 2018.
21. Won.JU HO, Hwang.WJ. Actual Cardiovascular Disease Risk and Related Factors A Cross-sectional Study of Korean Blue Collar Workers Employed by Small Businesses. Workplace Health & Safety. 2013;61(4).
22. Setyawati L. A Glimpse of Work Fatigue. Yogyakarta: Amara Books; 2010.
23. Doyle.G CK, Fullam.J. The European Health Literacy Survey :Results From Ireland. Dublin Ireland University. 2012.
24. Andrade N, Alves E, Costa AR, Moura-Ferreira P, Azevedo A, Lunet N. Knowledge about cardiovascular disease in Portugal. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2018;37(8):669-77.
25. Kusmana D. Effect of Stop Smoking Accompanied by Regular Exercise on Population Survival Power in Jakarta. Indonesia University; 2002.p.129-131
26. Kalichman.SC RD. Functional health literacy is associated with health status and health-related knowledge in people living with HIV-AIDS. Journal of Acquired Immune Deficiency Syndromes 2000;25(4):337-44.
27. Nutbeam D. Health literacy as a public health goal: a challenge for contemporary health education and communication strategies into the 21st century. Health Promotion International. 2006;15(3).
28. Sorensen KVdB, S.Fullam, J.Doyle, G.Pelikan, J.Slonska, Z.Brand, H.Consortium Health Literacy Project, European. Health literacy and public health: a systematic review and integration of definitions and models. BMC public health. 2012;12:80.
29. Sun *ea*. Determinants of health literacy and health behavior regarding infectious respiratory diseases: a pathway model. BMC public health. 2013;13(261).
30. McNaughton CDJ, T. A.Kripalani, S. Low literacy is associated with uncontrolled blood pressure in primary care patients with hypertension and heart disease. Patient education and counseling2014. p. 165-70.
31. ILO. Social Security for Workers in the Informal Economic Sector in Indonesia: Look for flexible programs that are targeted. International Labor Organization. 2010.
32. Ko YB, T, et al. Health literacy and its association with disease knowledge and control in patients with hypertension in Singapore. International journal of cardiology. 2013;168(4):e116-7.
33. Giselle ea. Health Literacy Is a Predictor of HIV/ AIDS Knowledge. Family Medicine. 2006;38(10).

Anxiety Level of Dental Care among Adolescents in Kepulauan Selayar District

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ABSTRACT

Introduction: Dental health has not seen any tangible results that needs an attention and require cooperation from various parties. One of the contributing factors is the lack of data on the level of community dental and oral health care needs that owned by the local government so that efforts to actualize healthy communities including dental and oral health are less than optimal. The objective of the study is to identify the level of anxiety of dental services among adolescents in Kecamatan Benteng, Kepulauan Selayar Distict. **Subjects and Methods:** The present study is a purposive-sampling study, adolescents between the age of 17 to 21 years old in state senior high school. This study obtained 511 students from three different schools and processed with SPSS Version 16. **Discussion:** A total of 511 students showed four level of anxiety— Not Anxious, Less Anxious, Anxious and Very Anxious. Most student experienced a not anxious level (56,8%), while only 2,3% students showed a very anxious level. In addition, the respondent was divided into two groups, based on visiting dentist in the last two years. Respondents who have had last visit to the dentist more than 2 years witnessed more anxiety than they who are visiting dentist in the last two years. **Conclusions:** The cause of anxiety to dental treatment is influenced by many factors, which relate closely to the pain history on dental treatment both in the adult and children.

Keywords: *Anxiety, Dentist Care, Adolescents, Kepulauan Selayar District.*

INTRODUCTION

Kepulauan Selayar is a district in South Sulawesi Province , Indonesia, which Benteng city is the capital city. This district has an area of 903.35 km² and a population of ± 100,000 inhabitants. This area is the only regency in South Sulawesi that its entire territory separated from the mainland of South Sulawesi and more than that region Kepulauan Selayar District consists of a group of islands so that is the archipelago region. This place consists of 123 islands, both large islands and small islands extending from North to South which has a stable number of schools each year. Those are elementary school 139 units (16,207 students), junior high school 52 units (5,232 students), senior high school 8 units (2,343 students) , and vocational school 7 units (1,481 students).

In addition, it has variety of health facilities. There are one general hospital, 13 public health centre and 61 units supporting community health centre. On the other hand, oral and dental health problems are found in Kepulauan Selayar which need to get attention and require cooperation from various parties. Moreover, efforts to overcome have not seen any tangible results when

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measured with public dental health indicators. One of the contributing factors is the lack of data on the level of community dental and oral health care needs that owned by the local government so that efforts to actualize healthy communities including dental and oral health are less than optimal. If this continues, obviously dental and oral health especially in the community will continue to decline. And ultimately affect their quality of life[1,2].

Anxiety is a vague feeling of discomfort, with a shadow that something undesirable will happen (Kargan & Haveman, 1976). Surveys generally showed that most of the general population avoid regular visits to dentists because they are afraid to do so. In recent studies, anxiety about dental care is associated with the length of time since the last visit to dental care and the greatest frequency of cancellation visits. Anxiety about dental care restricts partial, or prevents all oral health care services. An anxious person shows more damage or missing teeth and less restored teeth [4.5]. The level of anxiety is closely related to the method of oral and dental treatment that will be given to the patient. Patients with high levels of anxiety will find it more difficult to receive dental and oral care as well[3, 13].

SUBJECTS AND METHOD

The present study was a purposive-sampling study among adolescents between the ages of 17 to 21 years old in state senior high school. This study obtained 511 students from three different schools. Those schools were Benteng Senior High school, Muhammadiyah Senior High school and Benteng Vocational High school. Examination of survey was based on questionnaires/survey forms. Researchers attend all senior state high school and vocational schools in each location then explained the purpose of visits, followed by the distribution of a questionnaire /survey form and filling the questionnaire/survey form. Eventually, the data collected was processed with SPSS Version 16.

DISCUSSION

Results showed that the characteristics of respondents consists of age, sex, class grade, parental education level , last visit to dentist and anxiety of dental services. Majority respondents was in 16-17 years old (75.5%) and the least age was in 20-21 years old (0.8%). In addition, most of respondents were women (58.1%), while another was only 41.9%. The respondents was generally second grade (57,9%) and most of them had parents with bachelor education level (51,1%). In the last two years, it had 52,4% who had visited the dentist. Furthermore, most of the respondents do not feel anxious about dental services (56.8%). The data result is shown in Table 1 below:

Table 1. Characteristics of respondents

Respondents Characteristics	n	%
Age Group (year)		
16 - 17	386	75 , 5
18 - 19	121	23 , 7
20 - 21	4	0, 8
Gender		
Man	214	41 , 9
Women	297	58 , 1
Grade		
1	12	2 , 3
2	296	57 , 9
3	203	39 , 7
Parents' Education		
High school	250	48 , 9
Bachelor	261	51 , 1
Last Visit To Dentist > 2th		
Yes	268	52 , 4
No	243	47 , 6
Anxiety Level of dental services		
Not Anxious	290	56 , 8
Less Anxious	149	29 , 2
Anxious	60	11 , 7
Very Anxious	12	2 , 3

Source: Primary Data

Table 1 showed that most of adolescences were not anxious of dental services. Even thought it showed a not anxious level, there were other anxiety levels which almost a half of the results. This sums that anxiety of dental services was still an issue in Kepualaun Selayar District. Moreover, the correlation of anxiety level and last dentist visiting is shown in table 2.

Table 2. Correlation of anxiety level to dental services and last dentist visit.

Anxiety	Last visit to the dentist > 2 years				Amount	
	Yes		No			
	n	%	n	%	n	%
Not Anxious	145	54,1	145	59,7	290	56 , 8
Less Anxious	82	30,6	67	27,6	149	29 ,2
Anxious	33	12,3	27	11, 1	60	11 , 7
Very Anxious	8	3,0	4	1 , 6	12	2 , 3
Amount	268	100	243	100	511	100

Source: Primary Data

Table 2 shows that in the last two years visiting the dentist had almost the same number in all level of anxiety. Respondents who've had last visit to the dentist more than two years mostly felt not anxious (54,1%). This number has slightly lower than them who were not visiting the dentist, which were 59,4%. In contrast, other anxiety levels on respondents who visited the dentist experienced a little more percentage compare to them who had not visited.

Different types of anxiety components are interconnected with one another, but it is important to repeat here that there is no one-on-one relation between the components. Some people are actually very anxious about dental treatment but regularly and fairly cooperatively examined while others may actually avoid visiting dentists.[6,14]

Approach through education alone is not enough, because it only gives partial impact on behaviour change. Although humans need information and skills, they have no motivation to implement the newly-given information[15]. The idea that research alone is not enough, supported by research conducted by Todd et al (1982). Although 91% of the sample size is large enough to agree that regular visits to dentists are important to maintain dental health, 43% say they go to the dentist only when experiencing symptoms. The imbalance between knowledge and the actual behaviour may related to the motivation factor, especially the consequences seen from its actions.[6]

A review of the literature estimates that 9% of the world population suffer from a sense of fear/anxiety to dental treatment, with a reduction in prevalence as age increase. The cause of anxiety to dental treatment is influenced by many factors, which relate closely to the pain history on dental treatment both in the adult and children. The prevalence of fear to dental care is considered high (24.3 %), but lowers than fear of snakes, heights or injured physically.

Surprisingly, among all the phobias, the most common is dental phobia (3.7 %). These findings should be on alert by both researchers and dental care practitioners as a very real problem with the aim to finding ways to improve the conditions. [12] Fear to dental care usually begins in childhood with a negative experience, commonly expressed as a painful experience and/or being treated by a rough dentist.

Although it tends to decrease with age increasing, anxiety of dental care can last until adolescence and continue into adulthood. This is important. Therefore, the dentist should be able to identify these patients, with the aim to plan dental interventions that can reduce an individual's anxiety level. [12]

CONCLUSIONS

The cause of anxiety to dental treatment is influenced by many factors, which relate closely to the pain history on dental treatment both in the adult and children.

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REFERENCES

1. Al Omari WM, Al-Omiri MK. Dental anxiety among university students and ts correlation with their field of study. Journal of Applied Oral Science. 2009; 17(3):199-203.
2. Madfa AA. Prevalenceof dental anxiety and fear among medical students at University of Thamar. American Journal of Health Research. 2015; 3(1-2):5-9.
3. Aartman IH, Jongh A, Meulen MJ. Psychological charachteristics of patients applying for treatment in a dental fear clinic. European Journal of Oral Sciences. 1997; 105(5P1):384-388.
4. Humphris GM, Dyer TA, Robinson PG. The modified dental anxiety scale: Uk general public population norms in 2008 with further psychometrics and effects of age BMC oral health. 2009; 9(1):1.
5. Nicholas Emmanuel, Collado Valerie, Faulks Denise, Bullier Brigitte, Hennequin Martine. A National Cross-sectional Survey of Dental Anxiety in The French AdultPopulation. BMC Oral Health. 2007; vol.7.No.12.p.2. Accessed on Mei,20th2010
6. Kent GG, Blinkhorn AS. Pengelolaan Tingkah Laku Pasien pada Praktik Dokter Gigi. In: Budiman JA,editor. Kecemasan dan Penyebabnya. Ahli bahasa: Lilian Yuwono. Jakarta: EGC; 2005: 63-137.
7. D. Locker, A Liddell, and D Burman. Dental fear and anxiety in an older adult population. Community Dentistry and Oral Epidemiology. 1991; 19 (2): 120-124.
8. A. Al-Namankany, P. Ashley, and A. Petrie. The development of a dental anxiety scale with cognitive component for children and adolescents. Pediatric Dentistry. 2012; 34 (7): 219-224.
9. Berggeren U, Meynert G.Dental fear and avoidance: cause, symptoms. And consequences. I am dent assoc. 1984; 109 (2).
10. Akeel Riyadh F, abduljabarnadel,. Dental anxiety among patient attending king saud university. [internet]. Saudi dental journal 2000,vol.12. No.3.p.124.available from :http://www.sclsjournal.org. accessed on Mei 20th2010.
11. Udoye C.I.,oginni F.O,.dental anxiety among patients undergoing various dental treatment in nigerian teaching hospital. The journal of contemporary dental practice.2005. Vol.6 No.2., available from : http://www.thejcdp.com accessed on Mei 17th2010
12. Soraya Coelho LEAL, Danielle Matos de Menezes, Jo E. Frecken. Assosiated of ART with pain in dental treatment. J Appl Oral Sci 2009; 17(sp. issue):84-8.
13. Armfield JM, Pohjola V, Joukamaa M, Mattila AK, Souminen AL, Lahti SM. Exploring the association between somatization and dental fear and dental visiting. Europian Journal of Oral Sciences, 2011; 119(4):288-293.
14. M. Frazer and S. Hampson. Some personality factors related to dental anxiety and fear of pain. British Dental Journal. 1988; 165 (12): 436-439.
15. UW Bowman, A Wennstrom, U Stenman, and M Hakeberg. Oral health-related quality of life, sense and coherence and dental anxiety. BMC Oral Health. 2012; 12 (14).

Evaluation and Strategic Planning of Playground for Kids to Reduce the Accident Risk (Case Study in Immanuel Kindergarten Batu City)

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ABSTRACT

Essentially, kids world are identic with playing activities, mainly in early age kids. Through games, children are experiencing the learning process. This study aims to evaluate and provide recommendations to reduce the risk of accidents at Kindergarten. The result shows that there were several accidents experienced by children when playing in a playground such as bumping into a friend, crashing into a game device, stumbling, and falling from the playground. The playground in Immanuel Kindergarten generally has a relatively narrow and hollow place but has a clear guardrail so that children remain within reach. Some students play not according to how to play the tool, which increases the risk of falling. In general, there are differences between playgrounds at Immanuel Kindergarten and playground standards (p value 0.03<0.05), which is caused by several factors that influence human, method, equipment and environment. The improvements suggestions are referred to the strategy that is most likely to be implemented, namely the SO strategy to provide prior knowledge to children before playing and manage to play facilities.

Keywords: *playground, evaluation, t-test, fishbone, SWOT*

INTRODUCTION

Children are unique individuals and have all the potential that can be developed. Children naturally have the ability to develop their potential. This ability is obtained by children through the experience of associating with people in their environment, whether parents, siblings, peers, or other adults. Early experiences of hanging out with their environment can be obtained by children from an early age. Early childhood is a child who is in the age range of 0 years from birth to the age of 6 years.[1,2]

The early period (0-6 years) becomes a sensitive period that is the period of physical and psychological maturation functions that are ready to respond to the stimulation provided by the environment. This period is a time to study physical, cognitive, language, social-emotional, self-concept, discipline, independence, art, morals, and religious values[8]. Basically, the world of children is identical with playing, especially at an early time. Through games, children experience the learning processes.[3]

One place for children's education or kindergarten (TK) which has a children's play area as a means of learning is Immanuel Kindergarten Batu City. This kindergarten has an outdoor children's play area with various playground equipment such as swings, slides, and globe climbing. During breaks, the children are very active in trying various games that require physical ability in the play area. However, children's playground equipment and the playing environment can pose a great risk if not carefully designed and maintained. Based on preliminary studies conducted at Immanuel Kindergarten Batu City, there were several accidents experienced by children while playing in the playground. The following is data about accidents during play experienced by children recorded within 1 month based on the results of interviews with TK Immanuel teachers, namely 40%

crashing a friend, 33% stumble, 20% crashing into a game tool, 7% falling from a game tool.

Management of children's play area needs to be considered so that it can provide benefits to playing with children. In addition, good management of various aspects such as game tools, playing methods, supervision, and the playing environment can also reduce accidents while playing. Therefore, the design and construction of children's playgrounds in schools, especially kindergartens require emphasis on security and comfort aspects to ensure their level of safety.[4,5]

METHOD

Time and Place

Study about the evaluation of playground in kindergarten takes a place in Immanuel Kindergarten. This study was conducted during June-August 2018.

Data Collection

Data collection was done by collecting primary data and secondary data. Primary data in the study were collected through observation of play activities and children's behavior. Then do an evaluation by filling in the checklist of a good playground. Data related to accidents while playing and safety culture while playing for children were also conducted by interviewing several accompanying teachers who accompanied the children while playing. Secondary data that used in this research is the standard data on a good playground.

Data Processing Method

Based on the results that have been obtained, data processing is carried out through comparative studies with dependent sample t-test by comparing the standards of good playgrounds with observations obtained directly from observations in the field, as well as secondary data obtained through supporting studies. In addition, an analysis was carried out using a causal diagram and formulation of strategies to correct problems with the SWOT method.

RESULT AND DISCUSSION

Physical Conditions of Playground

One of the things that need to be considered in the management of the playground is the condition of the environment where children play. This can be in the form of the physical condition of the land and building play, or the condition of outdoor playground equipment that is in the playground. The playground at Immanuel Kindergarten has an area of approximately 40 m^2 with a variety of game tools namely climbing the globe, swings, seesaw, as well as a number of agility rides where students will face obstacles such as climbing, bowing through holes, and so on.

The children's playground in Immanuel Kindergarten generally has a relatively narrow place so that children who play and run are very likely to collide with other children. In addition, there are several holes on the surface of the playground that cause the possibility of a child falling because of the hole. However, the children's playground has a guardrail made of iron fences and hedgerows so that the children keep playing in the place provided and affordable by the teacher's supervision.

Safety Culture

The culture of child safety when playing is an important thing that must be introduced to children so that children can use the facility to play properly and not to engage themselves or others. During playing, all students of Immanuel Kindergarten were always accompanied by several teachers, so that the children were still under surveillance. Teachers of Immanuel Kindergarten carry out controlling functions so that children keep playing safely and preventing accidents. However, there are still children who have a tendency to endanger themselves while playing. Here are some examples of cases of misuse of play facilities performed by children:

One of the students climbed the stairs not through the steps, but through the ladder barrier which should be used to hold hands so as not to fall. This can endanger the child because with the position of the body as shown below, the child easily loses balance and allows an accident to fall from the playground.

Some students are seen standing in the swings and handrails, so that when a swing moves it can cause children to lose balance.

Based on the results of interviews with the teachers of Immanuel Kindergarten, children are always reminded when using the game but not in accordance with the actual way of use. There is no specific overview for children about the available play facilities and how to

play, so children are free to explore the usefulness of the tool. But every resting hour and the child is free to play in the outdoor area, each teacher has informed students to be careful in playing.

Comparative Study

The comparative study was conducted to evaluate the place of play of observation objects with a good standard of the playground, and analyze the causes of various aspects so that further steps can be identified.

Comparison of Playgrounds Immanuel Kindergarten with the Standards

This comparative study was carried out using the student-t test to compare whether there was a difference between the object's playground and the predetermined standard. The actual situation in Immanuel Kindergarten is perceived value, which is an assessment of the observations perceived by observers. While the standard playground is the expected value. This assessment uses an interval scale of 1-3 with successive provisions that arenot appropriate, appropriate, and very appropriate. After getting perceived and expected values, the gap or difference between the two is calculated by subtracting the expected value from the perceived value.

Table 1. Comparative Value of Playground in Immanuel Kindergarten with Standards

No.	Aspects	Perceived	Expected (Standard)	Gap
1.	Equipment and play areas do not have the following: sharp points or angles; splinters or protrusions that might catch children's clothing; peeling paint; loose or rusty parts; small parts that can be released or present the danger of suffocation, aspiration, or consumption; danger of strangulation; components that can tear the skin, pinch, thin, or destroy body tissues.	1	3	2
2.	To prevent the trap of the head or limbs of children, all openings while playing or other equipment are too large for the child's head or limbs to be trapped or too small to be inserted into the child's body.	2	3	1
3.	To prevent finger traps, openings are smaller than 3/8 inches or larger than 1 inch.	3	3	0
4	Other toys and equipment used in and around the water play area are made of solid plastic or metal. No one uses glass material.	2	3	1
5	Children do not play in areas where there are water bodies including tubs, buckets, washbasins, toilets, swimming pools, pools, irrigation canals, or installed wading pools, unless adults watching are within arms that provide a touch of supervision.	2	3	1
6	There is no danger of tripping	0	3	3
7	There is no danger of being hit by goods	3	3	0
8	Hazardous equipment are not available or cannot be accessed by children using barriers such as fences.	2	3	1
9	Children wear hats or hats in full to protect their faces from the sun if they are not in a shady place.	0	3	3
10	Physical activity / outdoor time is not taken as a punishment.	3	3	0
11	Caregiver / Teacher uses simple rules for behavior that children can understand. They use clear, direct and simple commands, and descriptive praise.	2	3	1
12	Caregivers / Teachers encourage positive behavior, promote self-regulation, and model desired behavior.	3	3	0
13	Caregivers / Teachers support children in learning appropriate social skills and emotional responses as well as providing predictable daily routines and schedules.	3	3	0

In determining whether there is a difference between the object of observation and the standard, the t-test is carried out by setting the significance level or alpha of 5%. H_0 shows that there is no difference between the object of observation and the standard, while H_1 shows the difference between the object of observation and the standard used.

Based on these results, it is known that the sig value is 0.003 <0.05, so H_0 is rejected. This means that overall, there are differences between the object of observation and the standard. This difference can occur, among others, because there are some things that are not in accordance with the standard of the playground.

Improvements can be made by improving aspects that have a large gap between perceived, and expected. The biggest gap is worth 3, namely in the aspect of no tripping danger and aspects of the use of hats in outdoor activities. Here are examples that are suitable for amusement park improvements:[6,7]

The use of pedestal playgrounds that are safe and flat like carpets or fine sand, so there are no holes that can make a child trip.

Use a hat or head protection when children play in an open area so that children are protected.

Cause-Effect Analysis

Generally, there are several factors that cause children's playgrounds, especially those in kindergartens, to still cause potential accidents in children. The following is a causal diagram to explain the root causes of potential accidents when playing:

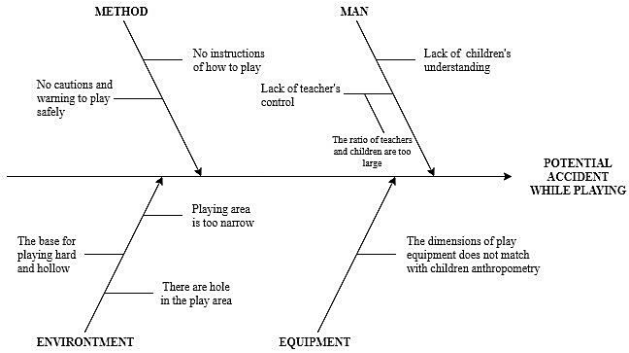


Figure 1. Cause-Effect Diagram

Strategic Planning

Based on the SWOT analysis it can be seen that in developing playgrounds in Immanuel Kindergarten Batu City there are very influential factors, which is internal factors including strengths and weaknesses and external factors including opportunities and threats. The following is an internal factor evaluation (IFE) and external factor evaluation (EFE) table from the playground in Immanuel Kindergarten:

Table 2. Table Matrix IFE and EFE

	Strength			
No.	Information	Weight	Rating	Score
1.	The game facilities that are owned are quite varied, among others, a vehicle for agility, swings, globe climbing and slide.	0,2	4	0,8
2.	There are teachers who accompany children every play.	0,2	3	0,6
	Subtotal	0,4		1,4
	Weakness			
No.	Information	Weight	Rating	Score
1.	Lack of information about how to play	0,2	2	0,4
2.	Lack of debriefing on children regarding safety in playing	0,1	3	0,3
3.	The playing environment is too narrow	0,1	2	0,2
4.	The base for playing hard and hollow	0,2	2	0,4
	Subtotal	0,4		1,3
	TOTAL	1		2,7
	Oportunity			
No.	Information	Weight	Rating	Score
1.	Game facilities that are owned can increase children's agility and motor power	0,5	4	2
	Subtotal	0,5		2

Cont... **Table 2. Table Matrix IFE and EFE**

	Threat			
No.	Information	Weight	Rating	Score
1.	Land degradation	0,2	3	0,6
2.	Facilities that are damaged due to high playing frequency	0,3	3	0,9
	Subtotal	0,5		1,5

Based on the analysis in the IFE matrix, the strength factor has a total score of 1.4, and the weakness factor has a total score of 1.3. The results of the EFE matrix indicate the opportunity factor has a score of 2, while the threat factor has a value of 1.5. Then it is known that the strength value above the weakness value, with the difference (+) 0.1 and the opportunity value above the threat value with the difference (+) 0.5. From the results of the identification of these factors it can be described in the SWOT Cartesian Diagram

From the SWOT diagram, the playground at Immanuel Kindergarten is in quadrant 1, which is aggressive. Being in quadrant I is a very favorable situation, because the Immanuel Kindergarten playground has the opportunity and strength so that it can take advantage of the opportunities that exist. The strategy that must be applied in this condition is to support aggressive growth policies (Growth Oriented Strategy).[6,7,8]

The explanation of strategic planning can be described below:

Strength: The game facilities that are owned are quite varied, among others, a vehicle for agility, swing, globe climbing and slide, there are teachers who accompany children every play.

Weakness: Lack of information about how to play, lack of debriefing on children regarding safety in playing, the playing environment is too narrow, the base for playing hard and hollow.

Opportunity: Game facilities that are owned can increase children's agility and motor power.

SO Strategy: Using strength to utilize opportunity=3.4, manage game facilities to increase children's motor power, the teacher gives play instructions and helps students optimize their physical condition to improve motor skills

WO Strategy: Minimize weakness to utilize opportunity=3.3, provide information about how to play, provide information related to safety in playing, expand the playground so that it can add games that increase child agility, improve the playground so that it doesn't cause accidents.

Threat: Land degradation, facilities that are damaged due to high playing frequency

ST Strategy: Using strength to overcome threats=2.9, maintain the feasibility of playing equipment, give instructions on how to play so as not to damage the game

WT Strategy: Minimize weakness to avoidthreats=2.8, optimize available land and choose a safe place to play.

The strategy that is applied in the development of the playground in Immanuel Kindergarten is to support aggressive growth policies (Growth Oriented Strategy) that is to manage game facilities in order to increase children's motor power, and provide play instructions and help students optimize their physical condition to improve motor skills.[7,8]

CONCLUSIONS

The playing environment tends to be narrow and hollow, causing several accidents, including colliding and tripping. Children do not fully understand the safety of playing so that there are still children who have the potential to cause accidents. In general, there are differences between playgrounds at Immanuel kindergarten and playground standards (sig. 0.03 <0.05). The biggest difference lies in the aspect of no tripping danger and aspects of the use of hats on outdoor activities caused by several factors including human, method, equipment, and environment. Proposed suggested improvements refer to the strategy that is most likely to be implemented, namely the SO strategy to provide prior knowledge to children before playing, and manage play facilities.

Ethical Clearance: Not required

Source of Funding: Self funding

Conflict of Interest: There is no conflict of interest exist.

REFERENCES

1. Alamo MR. Design for Fun: Playgrounds. Barcelona: LINKS Internasional. 2002.
2. Ministry of Education RI. Republic of Indonesia Law No.20 of 2003 concerning the National Education System. 2003.
3. Francis and Marcus, CC. People Places: Design Guidelines for Urban Open Space. John & Sons; 1998.
4. Mustofa, MH. Work Productivity Planning from Productivity Evaluation Results with Fishbone Method in Packaging Printing Company PT. X. HEURISTIC Journal of Industrial Engineering. 2014; (11) 1.
5. Pearce and Robinson. *Strategic Management*. Jakarta: Binarupa Aksara; 1997.
6. Sutojo, S and Kleinsteuber, F. Marketing Management Strategy. Jakarta: Damar Mulia Pustaka; 2002.
7. Tamara, A. Implementation of Swot Analysis in the Mandiri Business Savings Product Marketing Strategy. Journal of Business Research and Management. 2016; 4 (3): 395-406.
8. Yamin, M. and Sanan, JS. Early Childhood Education Guide. Ciputan: Gaung Persada Press Group; 2013.

Correlation Tuberculosis Drugs Treatment Phase with SGPT/ Bilirubin Total Level and Correlatioan both of the Enzymes from TB Patients

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ABSTRACT

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis that can infect some organs of the body such as lungs, kidneys, and bones. Multidrug therapy method using Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, and Streptomycin. Anti Tuberculosis Drugs (ATD) have side effects on the liver, skin, and nerves. Unwanted one is hepatotoxic. This research aims to determine correlation long of ATD administration with total Bilirubin and SGPT level from pulmonary TB patients at Sungai Tabuk 1, and Martapura Barat PHC also determines a correlation between the Total Bilirubin with the SGPT . 25 people as the sample with accidental sampling technique. This research is the analytic survey with cross-sectional study design. The results is 1.36 mg / dL for 0-2 months, 3-4 months were 1.19 mg / dL, and 5-6 months1.13 mg / dL for mean of Total Bilirubin and 30,58 u/l, 19,83 u/l dan 15,16 u/l for mean SGPT level. Spearman test is p 0,001 for Bilirubin ; 0,002 for SGPT with duration ATD administration and 0,000 between those enzymes. A conclusion is there is the significant correlation between duration of administration ATD with total Bilirubin (-0,632) and SGPT (-0,597) While the decrease of level value from both enzymes had the strong correlation (0,972). Recommended for further research to the same respondents with another ATD.

Keywords: *ATD; Treatment Phase; SGPT; Total Bilirubin*

INTRODUCTION

Pulmonary tuberculosis (TB) is a disease caused by the bacteria Mycobacterium tuberculosis which transmitted through the air, usually attacks the lungs but can also attack other organs of the body[1]. Pulmonary tuberculosis is a chronic disease that is still a health problem in the world, including in Indonesia. Six countries accounted for 60% of new tuberculosis cases, namely India, Indonesia, China, Nigeria, Pakistan, and South Africa

According to Indonesian health profile data and information in 2016, the number of new tuberculosis cases was 156,723 cases whereas South Kalimantan ranked 22nd at 2,811 cases consisting of 1,733 men and 1,078 women. The number of tuberculosis cases is possible because of problems in the health center, including patient noncompliance for treatment and low quality of sputum collected by patients for examination[2]. Annual report on Sungai Tabuk 1 and Martapura Barat Health Center in 2016 pulmonary tuberculosis (TB) cases ranked first for infectious disease cases. Since 1995, Indonesia has adopted a WHO-recommended treatment strategy, namely the Directly Observed Treatment Shortcourse (DOTS) strategy. The implementation of the DOTS strategy is proven to reduce tuberculosis mortality[3].

The minimum treatment for tuberculosis for six months with 2 phases namely intensive phase is the initial treatment phase is detected until the second month, and the continuous phase is the treatment phase after the intense period of the 3rd month until the 6th month completed. General therapy is given Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, and Streptomycin. Anti-tuberculosis drugs (ATD) have side effects on the liver, skin, nerves and can cause gastrointestinal. The severe impact that is currently in focus is the effect of anti-tuberculosis drugs on the liver, which causes hepatotoxic, known as Antituberculosis Drug-induced Hepatotoxicity (ATDH)[5].

This is because of the liver functions as the center of a metabolic disposition of all drugs and foreign substances in the body. In the liver, the medication is changed to be more hydrophilic, so it can dissolve in water and can excrete in urine or bile. The resulting liver traces have a direct effect, namely by producing enzyme-drug complexes. This complex will then cause cell dysfunction, membrane dysfunction, and T cell cytotoxic response[6].

Tests that can be done to assess hepatotoxic liver function include measurement of serum bilirubin levels, aminotransferase or transaminase, alkaline phosphatase, Gamma GT, and albumin. The progressive increase in SGPT and Bilirubin levels is very dangerous. Continuous improvement will result in drug-induced hepatitis. When drug-induced hepatitis occurs, the treatment must be stopped, if it occurs in the intensive phase, after the normal SGPT level returns, the treatment is continued with the same drug unless pyrazinamide is replaced with streptomycin. But if it occurs in the advanced phase, after the normal SGPT level returns, the treatment is continued with the same drug, Isoniazid, and Rifampisin[7]. Pyrazinamide should not be given to patients with the abnormal liver function if the patient in the intensive phase has the abnormal liver function.

In the previous study by Pramastuti1 out of 27 patients, there was an increase in SGPT of 22 people[4]. Another supportive study was a study conducted by Khadka[8], out of 114 patients 92 patients had elevated SGPT within normal limits, and 22 patients had elevated SGPT above the normal limit. While the increase in bilirubin levels is used as a specific indicator of hepatic impairment, which can be fatal if not treated early[9]. Besides that, there is an association between the increase in SGPT with Bilirubin in patients with Hepatitis C[10]. From the description of the previous background to find out the hepatotoxic effect of Anti Tuberculosis (ATD) drugs, a study was conducted to determine the relationship of the duration of Anti Tuberculosis (ATD) treatment to SGPT levels and Bilirubin and to determine the relationship of decreased SGPT levels with Bilirubin pulmonary tuberculosis patients.

MATERIALS AND METHOD

The study is an Analytical Survey with a Cross-Sectional design. The study sample was 25 patients with pulmonary tuberculosis category I on intensive phase treatment and advanced phase at Sungai Tabuk 1 Community Health Center and West Martapura Community Health Center. Sampling technique Accidental sampling with criteria for pulmonary TB patients over 18 years with regular treatment. The equipment used by Pharma 08 photometer, Randox RX Monza photometer. Reagents used from the biosystem.

RESULTS AND DISCUSSION

Table 1. Results of measurement of SGPT levels and total bilirubin

Respondents	Giving medicine 0-2 months				Giving medicine 3-4 months				Giving medicine 5-6 months			
	SGPT (u/l)		Bilirubin (mg/dl)		SGPT (u/l)		Bilirubin (mg/dl)		SGPT (u/l)		Bilirubin (mg/dl)	
1	49,5	A	1,77	A	23	N	1,27	A	13	N	1,08	N
2	23,5	N	1,28	A	26	N	1,3	A	39,25	A	1,49	A
3	19,5	N	1,21	A	19	N	1,12	A	19,5	N	1,09	N
4	36,5	A	1,40	A	21,5	N	1,22	A	18,5	N	1,16	A
5	45,5	A	1,37	A	15,25	N	1,10	N	12	N	1,05	N
6	22	N	1,23	A	14,25	N	1,12	A	23	N	1,24	A
7	34,5	N	1,36	A					12,5	N	1,05	N
8	17	N	1,2	A					11,25	N	1,04	N
9	34,5	N	1,52	A					11,5	N	1,03	N
10	23,25	N	1,28	A								
Average	30,58		1,35		19,83		1,19		15,16		1,13	

Description: N = Normal, A = abnormal Bilirubin Total (N: 1.0 mg / dL, A:> 1.0 mg / dL)

SGPT (N: 0-29 U / L, A ≥ 29 U / L)

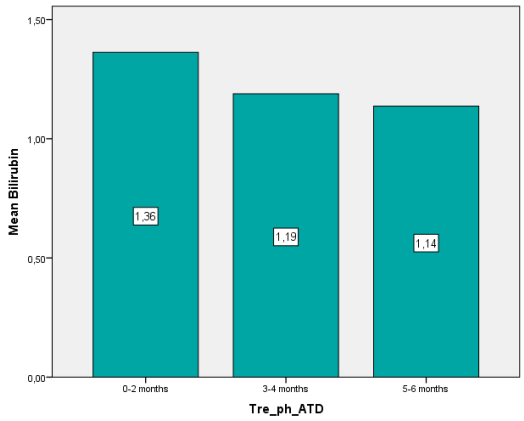


Figure 1. Duration of Use with Average Total Bilirubin

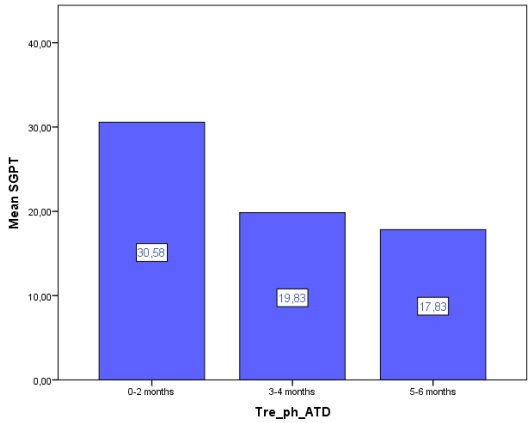


Figure 2. Duration of Use with Average SGPT

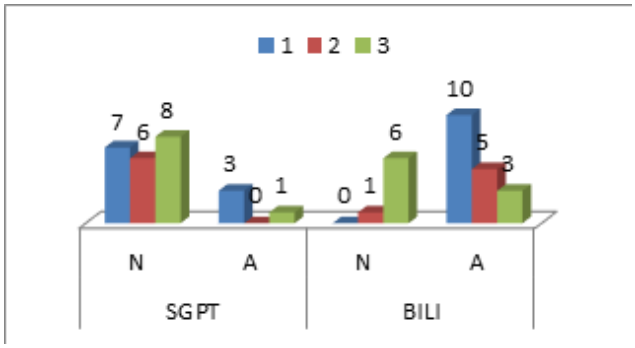


Figure 3. Normal and abnormal levels of SGPT and Bilirubin

Table 2. Respondents' length of work

Treatmen Phase	Long of work		
	< 8 hr	**8 hr**	**>8 hr**
0-2 months	60,0	30,0	10,0
3-4 months	30,0	50,0	20,0
5-6 months	-	100,0	-

Table 3. Respondents' sleep duration

Treatmen Phase	Long of Sleep		
	< 6 hr	**6-8 hr**	**>8 hr**
0-2 months	10,0	70,0	20,0
3-4 months	-	83,3	16,7
5-6 months	22,2	77,7	-

Table 4. Respondents' smoking habits

Treatmen phase	Smoking	
	Yes	No
0-2 months	30,0	70,0
3-4 months	33,3	66,7
5-6 months	44,4	55,6

DATA ANALYSIS RESULTS

Table 5. Results of statistical analysis

Test statistics	Long ATD with SGPT		Long ATD with Bilirubin Total		SGPT with Bilirubin Total	
	p	**Koef korelasi**	**p**	**Koef korelasi**	**p**	**Koef Korelasi**
Normality	0,010	-	0,022	-	-	-
Spearman	0,002	-0,597	0,001	-0,632	0,000	0,972

Single-dose Rifampicin can interfere with excretion of bilirubin so that it can increase unconjugated bilirubin which causes real or subclinical jaundice, without accompanied hepatocellular damage. This can be temporary and will occur early in patients with previous liver abnormalities[11]. Rifampicin also increases INH metabolism into isonicotinic acid and hydrazine, both of which are hepatotoxic. The half-life of plasma AcHz (Acetyl Hydrazine) which is a metabolite of INH shortened by Rifampicin and AcHz rapidly converted into its active metabolite by increasing the rate of oxidative elimination from AcHz, which is related to the high incidence of liver necrosis caused by INH and Rifampicin in combination[12].

On examination of SGPT levels in intensive phase treatment, 5 samples with SGPT levels exceeded normal values because pulmonary tuberculosis patients experienced the highest increase in transaminase enzymes in the fourth week to the sixth week after administration of ATD[13]. This was due to intensive treatment with four types of anti-tuberculosis drugs, namely Isoniazid, Rifampicin, Pyrazinamide and Ethambutol are consumed every day for two months of treatment[14]. And the incidence of hepatotoxicity was higher in patients with latent tuberculosis infection who received combination therapy with Rifampicin and Pyrazinamide compared with patients who only given Isoniazid[15].

Isoniazid inhibits CYP1A2 gene function which functions to detoxify hydrazine, where hydrazine is an isoniazid metabolite which, if further metabolized by cytochrome P450 will produce a product that causes hepatotoxic. While the pyrazinamide toxicity mechanism is unknown, according to case studies in mice, pyrazinamide inhibits the activity of several CYP450.12 And the metabolism of Rifampin starts from acetylated rifampicin to acetylation, then hydrolyzed to 3-formyl rifampicin[11].

In addition to factors from consumption of ATD, SGPT levels exceeding normal values can also be caused by various factors. Based on the results of the questionnaire submitted, all patients on intensive phase treatment who had SGPT levels exceeding normal values were in the age range of 40 to 65 years. This is consistent with Clarasanti's study, with samples with high levels of transaminase enzymes after ATD therapy at most in the 41-50 year age group of 16 people[16].

Also, 2 out of 5 patients who have SGPT levels exceeding normal values are active smokers. Smoking causes lipid peroxidation which causes damage to normal cell membranes from the liver. If liver cell damage occurs, there will be an increase in SGPT and SGOT in smokers compared to nonsmokers[17]. Smoking and shortness of breath also affect the increase in bilirubin levels in line with Lavigne's research results Smoking worsens the condition of pulmonary TB patients. Shortness of breath causes reduced oxygen intake which affects the decrease in oxygen in the body. Lack of oxygen is harmful to the body because it can damage organs such as the liver which plays a very important role in removing toxic substances[24].

Based on the results of examination of SGPT levels in the intensive phase it can be seen that SGPT levels were only 1.5 times higher than normal. Treatment does not need to be stopped if the increase in SGPT levels is less than three times, only to strictly monitor the liver function of the patient while undergoing treatment.7 ATD hepatotoxicity does not occur in every patient but can cause extensive and permanent liver injury and can cause death if not detected at the initial stage[18].

All results in the advanced phase of 3-4 months and eight patients in the advanced phase 5-6 months had normal SGPT levels. Because the type of ATD given in that phase only consists of Isoniazid and Rifampicin and is only consumed three times a week for four months of treatment[14]. And how to administer ATD 3 times per week compared to each day has little impact on the occurrence of hepatotoxicity[15].

In the advanced phase of 5-6 months, there is 1 sample that has SGPT levels exceeding normal values. Hepatic reactions usually appear during the first two months of treatment but can appear at any time during the treatment period[16]. And this can occur due to hypersensitivity reactions in susceptible individuals.

Increased bilirubin levels only occur in the first two and three months of ATD therapy. This is because in the first and second months an intensive phase of ATD administration, giving four treatment regimens at

once for two months. This is due to the inhibition of bile synthesis and excretion of bile acids and bilirubin and cholesterol in bile[20].

Based on the questionnaire submitted the patient worked more than 8 hours and had less than 8 hours of sleep, which caused a lack of rest time. Poor sleep is also the main cause of liver cirrhosis because the body's system requires enough hours for the detoxification process. Like Pramudiantoro's statement that there are several factors that can increase SGPT levels are insufficient rest, too tired and consumption of drugs[24].

Bilirubin is the final breakdown product of heme (hemoglobin), increased in liver cell damage and cholestasis[10]. Increased destruction of erythrocytes that are not matched by the speed of conjugation and secretary of bile flow resulting in increased bilirubin levels, and conjugated bilirubin cannot escape the bile to the intestine so will re-enter and be absorbed into the bloodstream, elimination is less due to the presence of bile duct obstruction or problems in processing bilirubin.[21,22]. Bilirubin levels that are above normal values are also influenced by several factors including hemolytic anemia which is a toxic effect from isoniazid and rifampicin[20,12]. Thuraidah A, et al 2017 states that there is a long relationship between consumption of anti-tuberculosis drugs and anemia[25].

CONCLUSION

The conclusion of this study is that there is a significant relationship between the duration of use of anti-tuberculosis drugs with total bilirubin levels ($p = 0.001 < \alpha$) and SGPT ($p = 0.002 < \alpha$) with a correlation value of -0.632 for Bilirubin Total and -0.597 for SGPT, which states the relationship is moderately correlated and the longer the use of the drug levels are getting lower. While the significant relationship between the decrease in total bilirubin levels and SGPT has a strong correlation (0.972).

EthicalClearance: Taken From *Health Research Ethics Committee* Politeknik Kesehatan Banjarmasin

Conflict of Interest: Nil

Source of Funding: Self

REFERENCES

1. Zubaidah T. Factors Affecting Decreasing TB Cure Rates in Banjar district in 2013. Jurnal Buski. 2013; 4(4),192-199.
2. Lutpiatina L, Wahidah, Nurhilaliah, D Rakhmina, Rifqoh. Sputum Quality Of The Anytime And Outset For Examination Acid-Resistance Bacilli, Medical Laboratory Technology Journal. 2018; 4 (1), 26-29
3. WHO, WHO Global Tuberculosis Report 2016, World Health Organization; 2016
4. Pramastuti, I. The Relationship Between Giving Anti-Tuberculosis Medication and Transaminase Enzyme Levels in Tuberculosis Patients in New Cases at Temanggung Hospital, Faculty of Medicine, Sebelas Maret University, Surakarta; 2011
5. Tostmann, A, Boeree, M.J., Aarnoutse, Rob, E., Lange, Wiel, C.M.de., Ven, A. J A M van der., dan Dekhuijzen, R. Antituberculosis drug-inducedhepatotoxicity: Concise up-to-date review, Journal of Gastroenterology and Hepatology. 2008; 23,192-202.
6. Bayupurnama, P. Drug-inducing Hepatotoxicity, in Sudoyo AW, Setiyohadi B, Alwi I, Simadibrata M, Setiati S (eds), Textbook on Internal Medicine Volume I, Edition IV, FK-UI; Jakarta; 2006
7. PDPI. Guidelines for Diagnosis and Management of Tuberculosis in Indonesia 2011; Jakarta; 2011
8. Khadka J, Malla P, 2009, The Study of Drug Induced Hepatotoxicity in ATT patients attending in National Tuberculosis Centre in Bhaktapur, SAARC Journal of Tuberculosis, Lung Disease and HIV/ AIDS. 2009; 6(2),17-21.
9. Sharma SK, Singla R, Sarda P, Mohan A, Makharia G, Jayaswal A, et al. Safety of different reintroduction regimens of antituberculosis drugs after development of antituberculosis treatment-induced hepatotoxicity. Clin Infect Dis. 2010;50(6):833-9.
10. Aleya dan KN Berawi. Correlation of SGOT/SGPT with Bilirubin levels in Hepatitis C patients in Dr.H.Abdul Moeloek Hospital, Majority. 2014;4(9)
11. Istiantoro, Y,H, Setiabudy,R. Tuberculostatic and Leprostatic, in Gunawan SG, Nafrialdi RS, Elysabeth, Pharmacology and therapy, Edition ke 5, FKUI, Jakarta; 2012
12. Pandit, A, Saehdeva, T, Bafna, P, 2012, Drug Induced Hepatotoxicity : A review, Journal of Applied Pharmaceutical Science. 2012; 2(5),233-243.

13. Praditya EP. Clinical Profile of Hepatitis Patients Affected by Anti-tuberculosis Drugs at Siti Hajar Hospital Medan tahun 2012: Medan; 2015
14. Kementerian Kesehatan RI. National Tuberculosis Control Handbook, Jakarta: Kemenkes; 2014
15. Luthariana Lies, Karjadi H Teguh, Hasan Irsan, Rumende Martin, 2017, Risk Factors for the Occurrence of Anti-Tuberculosis Drug Hepatotoxicity in HIV/AIDS Patients, Jurnal Penyakit Dalam. 2017; 4(1), 23-28.
16. Clarasanti I, Wongkar MCP, Waleleng BJ. Description of Transaminase Enzymes in Tuberculosis Patients who are treated with Anti-Tuberculosis Drugs at Prof. Hospital. Dr. R. D. Kandou Manado, Jurnal e-Clinic. 2016; 4(1), 1-6.
17. Alsalhen KS, Abdalsalam RD, 2014, Effect of cigarette smoking on liver functions, International current pharmaceutical. 2014; 3(7), 291
18. Govindan, N. The incidence of Hepatotoxicity in pulmonary tuberculosis patients using first-line anti-tuberculosis drugs at Adam Malik Haji Hospital in 2010, University of Northern Sumatra; Medan; 2011
19. Julita I. Clinical Pharmacokinetic Aspects of Potential Hepatotoxic Drugs in Hospitalized Patients in the Lung Ward DR. M. Djamil Padang period October 2011 – Januari 2012; Padang; 2012
20. Amirudin R. Physiology and Biochemistry of the Heart In: A Textbook on Internal Medicine. Volume I, Edition V. Jakarta: InternaPublishing; 2009
21. American Association for Clinical Chemistry. Hepatic Function Panel. England: Trustworthy Health Information; 2014
22. Kee, Joyce Fever. Laboratory & diagnostic examination guidelines, Ed.6 –Jakarta: EGC; 2007
23. Lavigne M, Rocher I, Steensma C, Brassard P. The impact of smoking on adherence to treatment for latent tuberculosis infection. BMC Public Health; 2006
24. Pramudiantoro, E. Relationship of Sleep Rest with Levels of SGOT / SGPT in Hepatitis Patients in the Inpatient Room of Kraton Hospital in Pekalongan Regency, Faculty of Nursing and Health Sciences University of Muhammadiyah Semarang: Semarang; 2013
25. Thuraidah A, RA Widya Astuti, D Rakhmina. Anemia and duration of consumption of anti-tuberculosis drugs (Anemia dan Lama Konsumsi Obat Anti Tuberculosis). Medical Laboratory Technology Journal. 2017; 3(2), 42-46

An Environmental Health Risk Assessment of Workers' Ambient Exposure to Particulate Matter of 2.5 Microns or Less at a Concrete Batching Plant

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ABSTRACT

Background. Workers in the concrete industry can be negatively affected by exposure to particulate matter. Particulate matter that is 2.5 microns in width or less ($PM_{2.5}$) is one of the most dangerous air pollutants because it can be inhaled into the lungs and enter the bloodstream. **Objective.** This research analyzed workers' health risks due to inhalation exposure to $PM_{2.5}$ in at a concrete batching plant. **Materials & Method.** The risk was calculated using an environmental health risk assessment method to determine the value of the risk quotient (RQ). The RQ was obtained by dividing the body exposure intake by the reference concentration. If the value of RQ>1, then risk management is necessary. This study calculated the risk of $PM_{2.5}$ exposure for 59 workers. Samples were collected at 4 points in the plant for 1 hour using a high-volume air sampler; each point was sampled 2 times, once during the day and once at night. **Results.** The average concentration of $PM_{2.5}$ was 120 mg/m^3 that means the concentration was above the quality standard. The calculation shows that $PM_{2.5}$ is risky to workers in life time duration (25 years) with the average yield at least for the next 9 years because reach RQ=1,096. Thus, risk management is required for the next 25 years by reducing $PM_{2.5}$ concentration.

KEYWORDS: *$PM_{2.5}$, Concrete Batching Plant, Environmental Health Risk Assessment*

BACKGROUND

Fine particle or $PM_{2.5}$ is one indicator of air pollution. Particulate matter consists of a complex mixture of solid and liquid particles of organic and inorganic substances suspended in the air.[20, 23] Tracheobronchial penetration of PM is related to particle size and efficiency of the lung defense mechanism[12, 21] Particles smaller than 10 micron cause larger problem, because these particles can enter the lungs and then enter the alveoli into the bloodstream[7] Exposure of $PM_{2.5}$ can cause acute and chronic effects[11, 18] WHO estimates that around 7 million people die every year from exposure to fine particles in polluted air that penetrate deep into the lungs and cardiovascular system, causing diseases including stroke, heart disease, lung cancer, chronic obstructive pulmonary diseases and respiratory infections, including pneumonia[13, 26] These fine particles can penetrate deep into the lungs and then irritate and damage alveolar wall, it will interfere lung function which clinically causes coughing, shortness of breath, and increased risk of Chronic obstructive pulmonary disease (COPD) associated with impaired lung function[9, 16]

The source of $PM_{2.5}$ pollution from industrial activities are usually comes from mining activities, factory chimneys, burning products and the cement industries[4] Batching Plant is a factory that ready mix concrete in accordance with the mix design that is ordered by the contractor who is doing construction[19] Ready mix concrete basically consists of water, cement, fine aggregate and coarse aggregate. Ready mix concrete components include calcium, silica, aluminum,

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magnesium, iron oxide and sulfur dioxide compounds along with fly ash, fine aggregates, coarse aggregates and admixture. These materials are source of $PM_{2.5}$ pollution[5, 15]

The objective of this study was to assess the level of non-carcinogenic health risks caused by workers' inhalation exposure to $PM_{2.5}$ in ambient air at a concrete batching plant. The ultimate goal was to formulate a risk management recommendation. Previous environmental health risk assessments (EHRAs) have been conducted to pinpoint where $PM_{2.5}$ exposure affects people working in the cement industry, and they have said that the most considerable risk is in areas closest to pollutant sources[3]

MATERIALS AND METHOD

The method used in this study is the EHRA, which consists of several steps: (1) hazard identification, (2) exposure assessment, (3) dose-response analysis, and (4) risk characterization[14, 17]. Two kinds of samples were used as variables in the risk assessment. The first sampling technique is interviewed workers to know the activity and antrhopometric chracteristics. The second technique was the use of a high-volume air sampler to measure the $PM_{2.5}$ concentrations in the ambient air at the batching plant. The short-term sampling method was used in which samples are collected for 1 hour. The research sample consisted of 59 workers as respondents while the criteria for including each respondent were as follows: (1) a permanent worker in the plant, (2) 18-55 years old when the samples were taken, and (3) a worker in an outdoor area. $PM_{2.5}$ samples were taken at 4 points in outdoor areas and were collected 2 times at each location, once during the day and once at night. The chronic daily intake (CDI) of $PM_{2.5}$ was calculated using the following equation[14] :

Where:

CDI = Chronic Daily Intake (mg/kg/day),

C = Concentration of risk agent (mg/L for drinking water and mg/kg for food),

R = Rate of intake or consumption (L/day for drinking water and kg/day for food),

t_E = Time of exposure (hours/day),

f_E = Frequency of annual exposure (day/year),

Dt = Duration of exposure, year (real time or projection, 30 years for residential default value),

Wb = Weight (kg),

tavg = Average time period (Dt x 365 days per year for non-carcinogenic substances, 70 years x 365 days per year for carcinogenic substances).

Estimation of the level of health risks derived from calculations using Risk Quotient (RQ) is calculated through the equation[14] :

CDI = Intake (mg/kg/day)

RfC = Reference Concentration (mg/kg/day)

RESULT

Hazard Identification. The risk agent in this study is the exposure of $PM_{2.5}$ toward respiration of human body. The highest concentration location for $PM_{2.5}$ exposure is located in the stockpile area during the morning shift which is 137.2µg/m^3, and the lowest is in the silo and parking lot during the morning shift which is 107.5µg/m^3. The average concentration per day of $PM_{2.5}$ is 120µg/m^3 (Table 1).

Table 1. Concentration of $PM_{2.5}$ on 4 points in Batching Plant

No.	Location	Measurement Time	Concentration (µg/m^3)	Average concentration perday (µg/m^3)
1	*Stockpile* Area	09.15-10.15	137,2	129,05
		21.10-22.10	120.9	
2	Central Mix & Conveyor	14.40-15.40	133,5	117,45
		20.00-21.00	101,4	
3	Lab, Workshop, & Waste Treatment	11.10-12.10	114,4	113,35
		21.10-22.10	112,3	
4	Silo & Parking Lot	15.00-16.00	107,5	120,3
		20.00-21.00	133,1	
Total				**120**

Exposure Assessment. Weight measurement and worker activity patterns aim to determine $PM_{2.5}$ exposure to workers in the Batching Plant. Variables that need to be known from the worker activity to calculate the intake are in Table 3. The Frequency of Exposure (f_E) aims to find out the number of working days of workers for one year. Every worker has a 12-day leave per year. The working day of workers in outdoor is 9 per 10 days, so the working days per year multiplied by 365 days is 329 days/year, then minus the days of leave, so total frequency of exposure is **317 days/year.**

The duration of Real Time Exposure is based on the actual time conditions of all respondents, but workers can work in the Batching Plant until their retirement and are constantly exposed to $PM_{2.5}$ exposure. Therefore, it is necessary to calculate the Duration of Life Time Exposure ($Dt_{lifetime}$) with an EPA default value for industry which is **25 year**[8].

The average time period (t_{avg}) is the average time of year the workers working at the Batching Plant. To find out the value by calculating the duration of exposure multiplied by the number of days per year which is 365 days/year for non-carcinogenic exposure (Dt × 365 days/ years for non-carcinogenic substances). The calculation is:

$$T_{avg} = D_t \times 365 \text{ days/year}$$

$$T_{avg} = 25 \text{ year x } 365 \text{ days/year}$$

$$T_{avg} = \mathbf{9.125 \text{ days/year}}$$

Based on the results of the data retrieval, it was found that these Batching Plants have a concentration of $PM_{2.5}$ of 0.12mg/m^3 with the average body weight of the worker 66.83kg and the inhalation rate 0.64m^3/hour, based on the activity pattern of working for 12 hours per day for 317 days per year with the average worker working for 2 years (Table 2), based on the calculation of the formula for the intake value which is **0.0016 mg/ kg/day**. The calculation is an actual duration of work ($Dt_{real\ time}$) of the workers. Because the company will continue to operate and the workers should work until their retirement, it is also necessary to calculate the life time intake ($Dt_{life\ time}$) which is 25 years for the industry[6] The calculation of lifetime intake produces a value of **0.0124 mg/kg/day**.

Table 2. Distribution of Anthropometry and Activity Patter of Respondent

Variables	Mean	Median	Min	Max	Normality Test
Age (year)	33,83	33	20	53	Normal
	66,83	65,00	41	101	Normal
Weight (Kg)	0,6361	0,63	0,53	0,73	Normal
Inhalation Rate (m^3/day)					
Time of Exposure (hour/day)	12,2669	12,5	9	15	Normal
Duration of Exposure *Real time* (year)	3,3153	2	3 month	16	Abnormal

Dose-Response Analysis. The purpose of the Dose-Response Analysis in this study is to calculate the Reference Concentration (R_fC) with the intake calculation and the value in safe limits that do not cause detrimental effects on workers. The calculation value of R_fC is **.**

Risk Quotient. The Risk characterization is calculated to determine the level of risk for workers exposed to $PM_{2.5}$. If the value is RQ <1 then exposure is not risky, whereas if the value is RQ> 1 it is considered risky exposure. The calculation of the total risk of Batching Plant with real time duration using the same equation produces **RQ = 0.412** meaning that $PM_{2.5}$ exposure is not at risk in the Batching Plant with real time exposure duration. Based on the calculation of the life time duration, the risk has begun since the worker has worked for 9 years because the value of RQ> 1 is equal to 1.096. (Table 3). The result of RQ value shows risk, then the exposure with the life time duration requires risk management.

Table 3. Risk Projection

2 years	5 years	9 years	15 years	20 years	25 years
0,412	0,609	1,096	1,826	2,435	3,044

DISCUSSION

The calculation of environmental health risk assessment of $PM_{2.5}$ exposure to Batching Plant workers shows that $PM_{2.5}$ is not a risk to workers for the duration of real time exposure, but it is risky to workers in the duration of lifetime exposure. Data regarding worker characteristics, anthropometric factors, and activity patterns are required to determine the amount of risk that individuals are exposed to at certain $PM_{2.5}$ concentrations. The results of the univariate analysis regarding the characteristics of respondents show that the average age of workers at the Batching Plant is 34 years old. Based on the research about the association between $PM_{2.5}$ exposure and impaired lung function, the human respiratory system stalls to 80% in late adolescence, which is 18 years old for women and 20 years old for men. During its development, the lungs can experience modification and damage that are at risk for experiencing respiratory diseases during adulthood. After achieving optimal lung function, the advancing age will affect the decline in lung function to reach 28 ml per year. Thus, the advancing age will increase the risk of respiratory health[10]

The average concentration (C) of $PM_{2.5}$ at the Batching Plant in 4 sampling points during days and nights is 120 $\mu g/m^3$. The measurement results show that in every point of sampling the concentration of $PM_{2.5}$ passes the limit value. The permissible concentration of $PM_{2.5}$ within 24 hours according to several regulations such as the Indonesian Government Regulation number 41 of 1999 is 65 $\mu g/m^3$; on the other hand, according to the National Ambient Air Quality Standards - US EPA[8], it is 35 $\mu g/m^3$, and according to WHO air quality guidelines[25], it is 25 $\mu g/m^3$. The measured concentration exceeds the quality standard, meaning that the exposure is dangerous for humans and the environment. The highest concentration is in the stockpile area with the measurements during morning shift of 137.2 $\mu g/m^3$. Stockpile is a place to store coarse aggregate (gravel) and fine aggregate (sand). Much particulate dust spreads when the aggregate is blown away by the wind because it is placed in an open space. The aggregate transfer to the Load-In Hopper uses Wheel Loaders, the movement of Wheel Loader tires and the smoke of the vehicle can also spread particulate dust. These things allow high concentration of $PM_{2.5}$ in the air[2, 22]

This study uses calculation done individually and on the average of RQ value at Batching Plant. The calculation of the Batching Plant area with real time exposure duration does not indicate any risk, but the duration of life time exposure shows that health risk will start from the duration of work for 9 years. Therefore, risk management is necessary. Risk management is carried out on the duration of life time exposure due to RQ > 1. Risk management planning can be done in arithmetic calculation of intake with various modifications. What can be done is to do risk management to reduce $PM_{2.5}$ concentration.

CONCLUSION

The conclusion of this study is that the average concentration of $PM_{2.5}$ at Batching Plant pass the limit value based on The Indonesian Government Regulation number 41 of 1999, The National Ambient Air Quality Standards, and WHO air quality guidelines. The average calculation of risk with real time duration is not treacherous because RQ=0,412. But, the calculation shows that $PM_{2.5}$ is risky to workers in life time duration (25 years) with the average yield at least for the next 9 years because reach RQ=1,096. Therefore, risk management can be done by reducing the $PM_{2.5}$ concentration.

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Ethical Approval: This research number of the ethical approval from the Ethical Research Committee is 280/UN2.F10/PPM.00.02/2018 dated April 6th 2018.

Conflict of Interest: There is no conflict of interest for this research.

REFERENCES

1. Ambient Air Pollution: A Global Assessment for Exposure and Burden of Disease. Geneva: World Health Organization (WHO); 2016.
2. Araújo IPS, Costa DB, Moraes RJB De. Identification and Characterization of Particulate Matter Concentrations at Construction Jobsites. Sustainability [Internet]. 2014;6:7666–88. Available from: www.mdpi.com/journal/sustainability
3. Bio FY, Sadhra S, Jackson C, Burge PS. RESPIRATORY SYMPTOMS AND LUNG FUNCTION IMPAIRMENT IN UNDERGROUND GOLD MINERS IN GHANA. GHANA Med J. 2007;41(2):38–47.
4. Chellam S, Kulkarni P, Fraser MP, Fraser MP. Emissions of Organic Compounds and Trace Metals in Fine Particulate Matter from Motor Vehicles : A Tunnel Study in Houston , Texas Emissions of Organic Compounds and Trace Metals in Fine Particulate Matter from Motor Vehicles : A Tunnel Study in Houston ,. J Air Waste Manage Assoc [Internet]. 2011;2247. Available from: http://www.tandfonline.com/loi/uawm20
5. Environment Protection Authority (EPA). ENVIRONMENTAL GUIDELINES FOR THE CONCRETE BATCHING INDUSTRY. Melbourne Victoria: 1998.
6. Environmental Protection Agency (EPA). Exposure Factors Handbook. 2011.
7. Environment Protection Authority (EPA). Health and Environmental Effects of Particulate Matter (PM). [Internet]. 2016 [cited 2017 Dec 29]. Available from: https://www.epa.gov/pm-pollution/health-and-environmental-effects-particulate-matter-pm
8. Environmental Protection Agency (EPA). National Ambient Air Quality Standards for Particulate Matter; Final Rule. 2006.
9. Haikerwal A, Akram M, Smith K, Sim MR, Meyer M, Tonkin AM, et al. Impact of Fine Particulate Matter (PM2.5) Exposure During Wildfires on Cardiovascular Health Outcomes. J Am Hear Assoc [Internet]. 2007;1–11.
10. Hastiti LR. PAJANAN PM 2 , 5 DAN GANGGUAN FUNGSI PARU SERTA KADAR PROFIL LIPID DARAH (HDL , LDL , KOLESTEROL TOTAL , TRIGLISERIDA) PADA KARYAWAN PT X , KALIMANTAN SELATAN TAHUN 2012. J Kesehat Masy Univ Indones. 2013;
11. Hu G, Zhong N, Ran P. Air pollution and COPD in China. J Thorac Dis. 2015;7(1):59–66.
12. Jang A. Particulate Air Pollutants and Respiratory Diseases. In: intech. 2012.
13. Krewski D, Burnett RT, Goldberg MS, Hoover BK, Consultants H, Siemiatycki J, Jerrett M, et al. OVERVIEW OF THE REANALYSIS OF THE HARVARD SIX CITIES STUDY AND AMERICAN CANCER SOCIETY STUDY OF PARTICULATE AIR POLLUTION AND MORTALITY Daniel Krewski Michal Abrahamowicz The reanalysis of the Harvard Six Cities Study (Dockery et al ., 1993) and the Ame. J Toxicol Environ Health. 2003;Part A(66):1507–51.
14. Louvar JF, Louvar DB. Health and Environmental Risk Analysis: Fundamental with Applications. 2nd ed. USA: Prentice hall PTR; 1998.
15. Murthy NRD, Ramaseshu D, Rao MVS, Engineering DC. STUDIES ON FLY ASH CONCRETE UNDER SULPHATE ATTACK IN ORDINARY , STANDARD AND HIGHER GRADES AT EARLIER AGES. ASIAN J Civ Eng (BUILDING HOUSING). 2007;8(2):203–14.
16. Paulin L, Hansel N. Particulate air pollution and impaired lung function [version 1 ; referees : 3 approved] Referee Status : In: F1000Research. 2016. p. 1–9.
17. Rahman A. Prinsip Dasar, Metode, dan Aplikasi Analisis Risiko Kesehatan Lingkungan. Depok: Universitas Indonesia; 2017.
18. Shi L, Zanobetti A, Kloog I, Coull BA, Koutrakis P, Melly SJ, et al. Low-Concentration PM2.5 and Mortality: Estimating Acute and Chronic Effects in a Population-Based Study. Environ Health Perspect. 2016;(1):46–52.
19. Titan America. MATERIAL SAFETY DATA SHEET for Ready-Mix Concrete. 2001. p. 1–4.
20. Upadhyay S, Ganguly K, Stoeger T. INHALED AMBIENT PARTICULATE MATTER AND. Eur Med J. 2014;(October):88–95.
21. Wang C, Tu Y, Yu Z, Lu R. PM 2 . 5 and Cardiovascular

Diseases in the Elderly : An Overview. Int J Environ Res Public Health [Internet]. 2015;12:8187–97. Available from: www.mdpi.com/journal/ijerph

22. Wang J, Ogawa S. Effects of Meteorological Conditions on PM 2 . 5 Concentrations in. Int J Environ Res Public Health [Internet]. 2015;9089–101. Available from: www.mdpi.com/journal/ijerph
23. World Health Organization (WHO). Ambient Air Pollution: A Global Assessment for Exposure and Burden of Disease. Geneva. 2016.
24. World Health Organization (WHO). CLIMATE AND HEALTH COUNTRY PROFILE – 2015 INDONESIA.; 2015. 1-8 p.
25. World Health Organization (WHO). WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Geneva; 2005.

RAPD Identification of Bacteria Isolated from Arm Third Degree Burn Wound

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ABSTRACT

This study aims to isolate different types of bacteria from the wounds of the third degree burns in the forearm area and to define those types at the morphological level and using the RAPD-PCR technique. And study the sensitivity of isolated species towards certain types of antibiotics traded. 35 swabs of third-degree burns were collected in the forearm area of 35 patients. 12 species of bacteria were isolated and purified from those swabs. The necessary tariff tests were carried out.

The isolated bacteria were identified as morphology and the result was the following: Bacillus sp, Enterobacter sp, E.Coli sp, Klebseilla sp , Pseudomonas aeruginosa and MRStaphylococcus aureus. Seven antibiotics; amoxicillin , Cefaclor, ampicillin , amoxicillin/clavulonic acid , Ciprofloxacin , Imepenim and Vancomycin were tested to identify the isolates of the isolated bacteria. DNA was extracted from isolates. The RAPD-PCR method was applied to DNA extracts according to a standard method using 3 primers. This technique resulted in repeated trip patterns used for genetic differentiation. The phylogeny tree in Figure 2 obtained from statistical analysis of the RAPD-PCR band pattern of 12 bacterial isolates using three RAPD primer shows that there are only two main groups branched from one ancestor at linkage distance 16%. One of these groups contains isolate number 10, while the other group contains isolate no.12 and branched at linkage distance 15% to other two sub groups one of them contains isolates 1, 11 while the other group contains the other remaining 8 isolates. The study showed different types of infectious bacteria according to the age of the injured and the causes of injury, which is worth studying on a larger scale.

Keywords: *third degree burn; infection; bacteria; identification; RAPD-PCR; sensitivity*

INTRODUCTION

The skin is the first line of the immune system of the body as well as containing nerve cells to do the sensory function.[1, 2] Burns are one of the most common home injuries, especially in children. Burns are not limited to the pain and biting sensation, but can cause severe damage to skin and skin, causing skin cells to die in the affected area.[3] Although severe burns may require emergency measures to prevent complications, burns are often recovered without complications, taking into account the degree of incineration and the cause[4, 5]. Burns are divided into three main types depending on the degree of damage to the skin and skin; first-degree burns, mildest damage, second-degree burns and third-degree burns, the most severe type of burns [5] .

The immune response changes after the injury burns, especially the third degree, where the first defense wall of the body drops and inhibits immunity and less efficiency. The burns of the third degree on the largest proportion of the enemy and whenever the destruction of the skin on a large scale, the greater the cases of infection [4, 6] .

RAPD reactions are PCR reactions, using a small-size primer that can be randomly attached to multiple DNA sequences in the bacterial genome and the creation of repeated autopsy patterns that are used in genetic differentiation[7]. If there is a mutation in the DNA template at the site that was previously a supplement to

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the paint, a PCR product will not be produced, resulting in a different pattern of the DNA parts that are inflated on the gel. The other difference between this method and ordinary PCR is the use of two different temperatures. Because bacteria are the main cause of nosocomial infection, especially in patients with burns[6].

The aim of this study was to determine the genetic pattern of RAPD from strains of isolated bacteria from burn patients to characterize their genetic diversity.

MATERIALS AND METHOD

The 35 sterile swabs samples were collected from burned arms at ------- hospital after having a signed agreement form of the patient's family.

Samples were properly labeled with patients Name, Age, and Gender. The samples then were processed at the laboratory.

We used 3 different media to cultivate the isolated bacteria; Nutrient agar, Blood agar, and MacConkey's agar with the addition of antifungal agent like azole .The media was autoclaved for 15 minutes at 121°C.

Streaking of the samples on the plate were done in three plates each with one of the previously mentioned media and incubated for 48 hours on 37° C.

The colonies that appeared on the plates were sub cultured and purified. The pure colonies obtained from the sub culturing and purification process were numbered and labeled.

Morphological and biochemical identification of the purified isolates were performed[4]. Table 1 summurize the biochemical tests used to identify the bacterial isolates. Table 2 shows the list of antibodies used in identification of the bacterial shambles.

Table 1 the biochemical tests used to identify the bacterial isolates.

	Bacillus sp	**Enterobacter sp**	**E.Coli sp**	**Klebseilla sp**	**Pseudomonas aeruginosa**	**MRStaphylo-coccus aureus**
Catalase test	+	+	-	+	+	+
Oxidase test	+	+	-	+	+	+
Eg coagulase	-	-	+	-	-	+
Litmus milk decolorization test	-	+	-	+	-	-
IMVIC	+	+	-	+	+	+

Table 2 the list of antibodies used in identification of the bacterial shambles.

	amoxicillin	**Cefaclor**	**ampicillin**	**Amoxicillin clavulonic acid**	**Ciprofloxacin**	**Imepenim**	**Vancomycin**
Bacillus sp	S	R	S	R	S	S	S
Enterobacter sp	R	S	R	S	S	R	S
E.Coli sp	S	R	S	S	R	R	S
Klebseilla sp	R	S	R	R	S	S	S
Pseudomonas aeruginosa	S	S	R	R	S	S	R
MRStaphylo-coccus aureus	R	R	S	S	R	R	R

DNA extraction

. From a bacterial suspension 200 ml was used in genomic DNA extraction using DNA extraction kit (Bio-Rad , USA).

RAPD-PCR reaction

DNA amplification was performed on a thermo cycler (Bio Rad, USA) in a final volume of 25 µL containing 21µL of master mix (thermo, usa), 2.5 µL of 50 mM primer and1.5 µL genomic DNA .list of primers used in this study as following : primer 1 (3 - AGCGGGCCAA-5), primer 2(3-GCGCGATCTGGTTCACTCG-5), primer 3 (3- CAGTGAAAATTCACTGGCAAC-5). [8].

The cycling conditions were as follows: initial denaturation at 96 8C for 2 min followed by 3 cycles of denaturation at 94 8C for 1 min, annealing at 36 8C for 2 min, extension at 72 8C for 2 min and 29 more cycles of denaturation at 94 8C for 1 min, annealing at 58 8C for 1 min, extension at 72 8C for 1 min and a final extension at 72 8C for 5 min[8].

The RAPD-PCR products were loaded on a 1.5% (w/vol) agarose gel with 0.5 mg/ml of Ethidium bromide, and were analyzed by gel electrophoresis and banding patterns were observed in Gel-Documentation system (Uvitec, UK). A 1 kilobase DNA ladder (Fermentas, Canada) was used as a molecular size standard. The RAPD fingerprints were analyzed and genotypes were assigned on the basis of number and weight of band differences. Each reaction was repeated at least three times for reproducibility. The minor non reproducible reactions were excluded from the study.

RESULTS AND DISCUSSION

After infection with third degree burns is a critical condition to affect the immune system. Topical antibiotics are used intravenously to prevent and treat infection. The collected samples were divided into groups according to type and by age and then by isolated bacterial species after their morphological and biochemical identification. This result was recorded in Table 4. The males and females were eliminated 51.5% and 48.5%, respectively. The majority of the studied samples were of the age of 25-45 years by 60.1%. The most common cause of the eye injury was the incidence of burns as a result of edible oil by 42.9%.

Table3. statistical analysis of the patients data

	No	percentage	Types of bacteria isolated
Sex			
male	18	51.5%	Enterobacter, E.Coli, Klebseilla, Bacillus Pseudomonas aeruginosa
Female	17	48.5%	Enterobacter, E.Coli, Klebseilla, Bacillus Pseudomonas aeruginosa
Age			
1-10	5	14.2%	E.Coli, Bacillus, Enterobacter
10-25	9	25.7%	Klebseilla, Enterobacter, Bacillus
25-45	21	60.1%	Staphylococcus, E.Coli, Enterobacter , Pseudomonas aeruginosa Pseudomonas aeruginosa
Couse of burn			
Direct fire	8	22.8%	Klebseilla, Enterobacter, Bacillus
Hot water	10	28.5%	E.Coli, Bacillus, Pseudomonas aeruginosa
Cooking oil	15	42.9%	Staphylococcus, E.Coli, Enterobacter
Electricity	2	5.8%	E.coli , Klebsella

The results of the current study showed the presence of infection in burns of the third degree in the forearm. These results are consistent with the study conducted by[6], which investigated the association of microbes with burn patients . In 2004, isolated a number of different bacterial isolates from post-burn infection, including S. aureus, epidermis and klebsiella [2, 9]. These results are concise with our study, which isolated us by eight different types of bacteria including Pseudomonas aeruginosa, Staphylococcus aureus, klebseilla, E.coli, epidermis, Enterobacter, and bacillus. Adeyemiet al; in 2006, it was reported that most severe burns were due to the association of pseudo-auregenousa, klebseilla, staphylococcus aureus, E. coli and Staphylococcus epidermis. [3, 10]The present study agrees with the previous researcher. It is also in line with research conducted by [10-12]. The results of our study are consistent with the results[7] . The causes of this high frequency of Pseudomonas aeruginosa, Staphylococcus aureus and Klebsella may be present in abundance in the air, and can remain within the waste range of environmental conditions and show resistance against a number of antibiotics. The current research work is also consistent with reports[13, 14]. It is well documented that children 1-15 years of age are more likely to have burns[14]. The results of our study showed that the highest percentage of burn injuries in the age group 25-45 years were recorded 60% followed by age group. These results are consistent with the research work of [13, 15].

The 12 bacterial isolates were tested on molecular levels by DNA fingerprinting with random primers (RAPD) (figure 1). The RAPD analysis data of these bacterial isolates can be used for further molecular identification using 18srRNA sequence. The phylogeny tree in Figure 2 obtained from statistical analysis of the RAPD-PCR band pattern of 12 bacterial isolates using three RAPD primer (Figure 2) shows that there are only two main groups branched from one ancestor at linkage distance 16%. One of these groups contains isolate number 10, while the other group contains isolate no.12 and branched at linkage distance 15% to other two sub groups one of them contains isolates 1, 11 while the other group contains the other remaining 8 isolates.

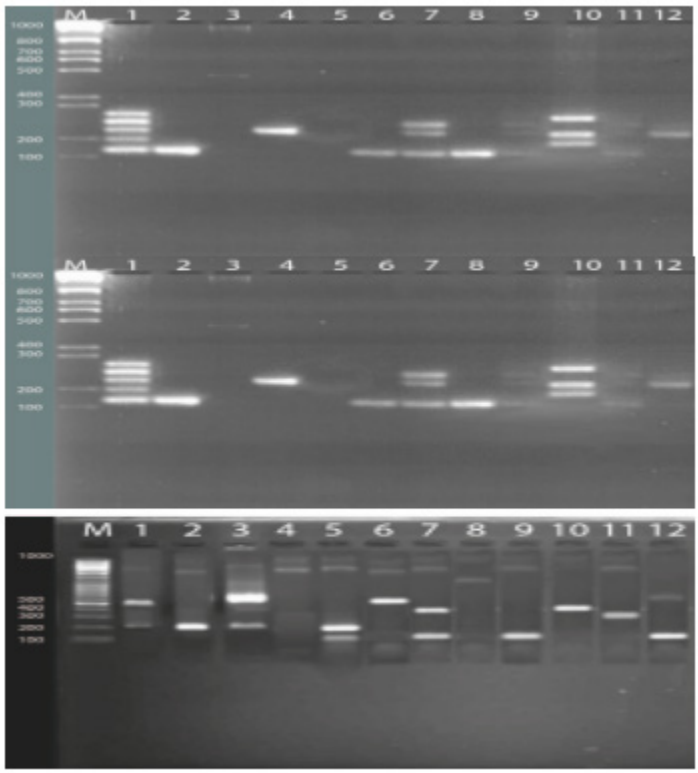


Figure 1 – RAPD-PCR fingerprints of 12 isolates from burn patients A: using primer1 B: using primer2 C: using primer3. M: DNA molecular weight marker (1 kilo base), 1–12: Samples

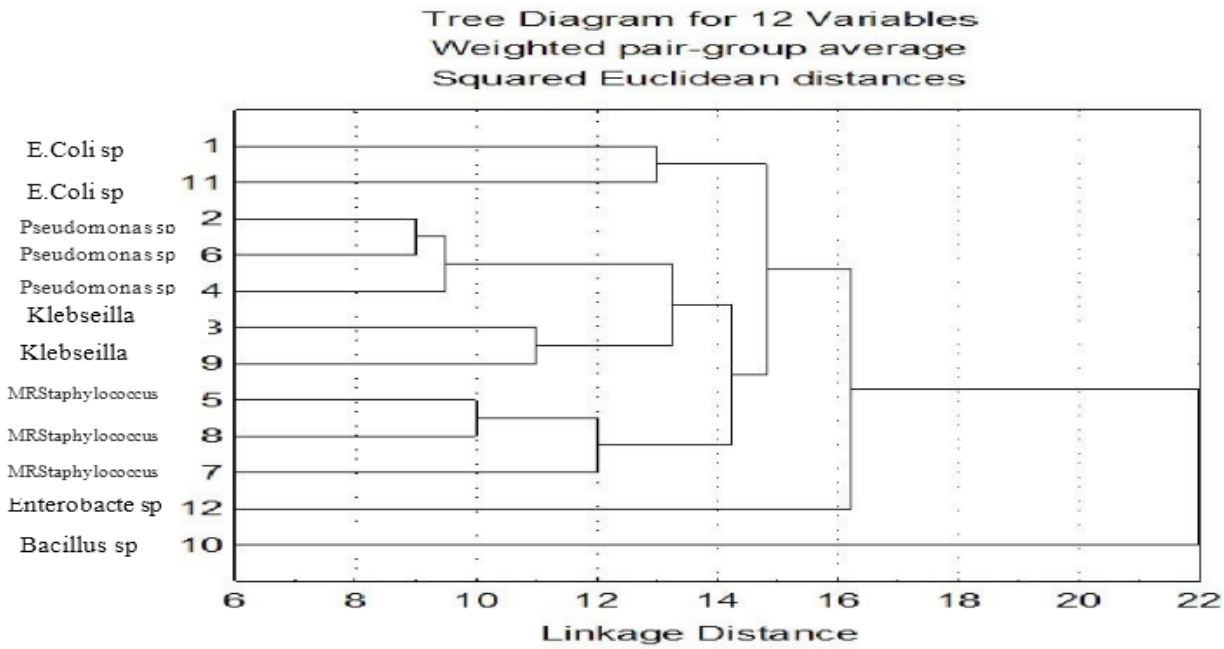


Figure 2. The phylogenetic tree

The gelectrophorasis gel showes some common bands of some bacterial species indicating that those samples are related on moleculer levels. The data of the RAPD analysis with those specific primers previously mentioned in table 4 was enlisted to relate the data obtained from the morphological identification , antibiotic sensitivity test and moleculer analysis of the tested isolates.

The data revealed that isolates 10, 12 were have the most far genetic distance among the twelve bacteria isolates, while isolates 1, 11 were laid in the same group indicating genetic similarity between these two isolates which in turn differ from the 8 remaining bacterial isolates. This results indicates that there were only one species of Enterobacter (sample 12) and Bacillus (sample 10). Isolates 1and 11 represents 2 species of E.Coli while Pseudomonas sps showed to be in 3 different species (samples 2,6,4) . MRStaphylococcuson the other hand represented by 3 subspecies 5,8,7. There were 2 isolates from the morphological identification appeared to by Klebseilla sp while genetically they were not in the Staphylococcus branch on the phylogenetic tree analysis.

The difference in genetics observed in this study is not expected because the result is different depending on the cause of the injury and the difference in the age of the patient. Some researchers in their experiments also reported a wide genetic variation and the appearance of some unexpected bacterial species in cases of burns in different degrees.[16]

CONCLUSION

The study showed genetic differences among the types of bacteria isolated from wounds from burns of the third degree. It also showed the presence of some unknown organisms that show morphology that they will be developed and therefore we recommend completing research on those species to define them molecularly. The study showed different types of infectious bacteria according to the age of the injured and the causes of injury, which is worth studying on a larger scale.

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Source of Funding: Self.

Ethical Clearance: Not required.

REFERENCES

1. Neyestanaki, D. K., Mirsalehian, A., Rezagholizadeh, F., Jabalameli, F., Taherikalani,

M., & Emaneini, M. Determination of extended spectrum beta-lactamases, metallo-beta-lactamases and AmpC-beta-lactamases among carbapenem resistant Pseudomonas aeruginosa isolated from burn patients. Burns, 2014.40(8), 1556-1561.

2. Bertrand, J. Y., Giroux, S., & Golub, R. Bibliography Current World Literature,2018. (12)3, emergence, 10, 03.
3. Salimi, H., Owlia, P., Yakhchali, B., & Lari, A. R. Characterization of Pseudomonas aeruginosa in Burn Patients Using PCR-Restriction Frag-ment Length Polymorphism and Random Amplified Polymorphic DNA Analysis. Iranian Journal of Medical Sciences, 2015. 35(3), 236-241.
4. Faraji, F., Mahzounieh, M., Ebrahimi, A., Fallah, F., Teymournejad, O., & Lajevardi, B. Molecular detection of virulence genes in Pseudomonas aeruginosa isolated from children with Cystic Fibrosis and burn wounds in Iran. Microbial pathogenesis,2016. 99, 1-4.
5. Schaal, J. V., Leclerc, T., Soler, C., Donat, N., Cirrode, A., Jault, P., & Bargues, L. Epidemiology of filamentous fungal infections in burned patients: A French retrospective study. Burns,2015. 41(4), 853-863.
6. Neyestanaki, D. K., Mirsalehian, A., Rezagholizadeh, F., Jabalameli, F., Taherikalani, M., & Emaneini, M. Determination of extended spectrum beta-lactamases, metallo-beta-lactamases and AmpC-beta-lactamases among carbapenem resistant Pseudomonas aeruginosa isolated from burn patients. Burns,2014. 40(8), 1556-1561.
7. Vaněrková, M., Mališová, B., Kotásková, I., Holá, V., Růžička, F., & Freiberger, T. Biofilm formation, antibiotic susceptibility and RAPD genotypes in Pseudomonas aeruginosa clinical strains isolated from single centre intensive care unit patients. Folia microbiologica,2017. 62(6), 531-538.
8. Mahmmudi, Z., Gorzin, A. A., & Emami, A. Comparison of genetic and drug resistance patterns in Pseudomonas aeruginosa isolated from the burn wound patients using primers 272 and 277. BIOSCIENCE BIOTECHNOLOGY RESEARCH COMMUNICATIONS, 2016.9(1), 32-38.
9. Saadatian Farivar, A., Nowroozi, J., Eslami, G., & Sabokbar, A. RAPD PCR Profile, Antibiotic Resistance, Prevalence of armA Gene, and Detection of KPC Enzyme in Klebsiella pneumoniae Isolates. Canadian Journal of Infectious Diseases and Medical Microbiology, 2018.
10. Latteef, M.N.R.N.S., A.B. Jassim, and M.I. Nader, Rapid Identification of Pseudomonas aeruginosa by Using Real Time PCR. 2017.
11. Zarei, O., Shokoohizadeh, L., Hossainpour, H., & Alikhani, M. Y. Molecular analysis of Pseudomonas aeruginosa isolated from clinical, environmental and cockroach sources by ERIC-PCR. BMC research notes,2018. 11(1), 668.
12. Finnerty, C. C., Jeschke, M. G., Branski, L. K., Barret, J. P., Dziewulski, P., & Herndon, D. N. Hypertrophic scarring: the greatest unmet challenge after burn injury. The Lancet,2016. 388(10052), 1427-1436.
13. Asadpour, L. Antimicrobial resistance, biofilm-forming ability and virulence potential of Pseudomonas aeruginosa isolated from burn patients in northern Iran. Journal of global antimicrobial resistance,2018. 13, 214-220.
14. Abdel-Sayed, P., Kaeppeli, A., Siriwardena, T., Darbre, T., Perron, K., Jafari, P., ... & Applegate, L. A. Anti-microbial dendrimers against multidrug-resistant P. aeruginosa enhance the angiogenic effect of biological burn-wound bandages. Scientific reports, 2016.6, 22020.
15. Latifi, N. A., & Karimi, H. Correlation of occurrence of infection in burn patients. Annals of burns and fire disasters,2017. 30(3), 172.
16. El-Kased, R. F., Amer, R. I., Attia, D., & Elmazar, M. M. Honey-based hydrogel: In vitro and comparative In vivo evaluation for burn wound healing. Scientific reports,2017. 7(1), 9692.

The Impact of Social Media in Improving Patient's Mental Image Towards Healthcare Provided by Private Hospitals' in Amman/Jordan

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ABSTRACT

The main objective of this study is to identify the impact of social media tools on improving patients' mental image of the healthcare provided by private hospitals (an applied study in Amman city).

An analytical descriptive style was used to achieve this goal because this method was appropriate for the nature of the study. A questionnaire was designed as a tool to collect data. The questionnaire was first presented to a number of experts in the marketing field. The study population consisted patients treated by private hospitals in Amman, Jordan. The study sample was a proper sample of this population: (550) patients received copy of the questionnaire, and (513 copies were returned and deemed suitable analysis.

The study shown that social media had a statistical significant impact on patients' mental image of private hospital, and Facebook shown the greatest effect cognitive and behavioural dimension, whereas Twitter showed the greatest effect on the influence dimension.

Keywords*: Social Media; Mental Image; Healthcare Service; Private Hospital; Amman.*

INTRODUCTION

All animal species have perfected a system of communication, but humans are the only species capable of spoken language [1, 2]. Communications are important for many reasons. For humans in particular, communication a sense of social cohesion. As mankind has evolved over the centuries, our means of communication have followed suit. Communication has existed in different forms since man appeared on Earth [2]. Communication methods have evolved over time, from direct contact to letters to landline phones and now to communication through social media [1].

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The internet plays an essential and important role in facilitating communication between people and organizations locally on a global and international scale [3]. Business organizations have adopted electronic means to promote their products and take advantage of the benefits offered by these new forms of communication [4].

The information revolution ushered in by the internet leads organizations to seek to adapt these methods of communication to reach their targeted markets and strengthen their relationships with segments of these markets. This is done by circulating and, publishing information about their products [4,5] these promotions may be communicated by the organization itself or through electronic communication methods, especially social media, the goal of building a positive mental image of organization's products or services. Organizations do this to gain popularity, name recognition and good reputation [6].

Like all other organizations, hospitals particularly private hospitals work hard to build a positive mental image of the services they offer to patients. In building this image, they are aware of the importance of communicating and sharing information with patients [7].

This need, requires them to adapt the modern communication methods, including social media and to use these methods to circulate and publish information about their services [8]. These services vary in quality which influences patient thoughts, feelings and behaviours towards the services of these private hospitals [9].

Building a positive mental image is not an easy matter. The adopted strategies must affect the three dimensions of mental image (the cognitive dimension, Influence dimension and behavioural dimension) [10]. This can be done only by providing accurate information, establishing credibility and maintaining continuous communication with patients in a way that creates the best possible mental image and influences patient's future behaviour choosing and trusting a particular hospital [11.8].

Previous studies have indicated that there is, at least, one social media account for every household in the world .This finding clearly demonstrates how far these communication methods spread and how quickly society has accepted them, regardless of the cultural, social, religious, racial and ethnic differences among people [12,13].Social media gathers people from different location and countries under one umbrella called the internet [6].

In the last few years the healthcare economy has further developed as a result of technological developments and the implementation of ideas and principles borrowed from business economic analysis [8,14].

Because of this hospitals and healthcare centers have transitioned from location for patients lodging hygienic system that provide many types of integrated healthcare services, meets patients' their health needs and improve patients' opinions of healthcare [15]. Based on business principles, economic analysis and the concept of the healthcare economy, healthcare marketing has become an essential way for healthcare system to address heavy and increasing market competition [6,16]. Healthcare marketing maintains relationships with exiting patients and attract new patients. Also increases patient satisfaction and improves mental the image of the healthcare services provided by these systems [11].

From this perspective, this study examines the role of social media in proving patients 'mental image of the healthcare services provided by private hospitals.

Study questions

Administrators of hospitals and healthcare centers have increasing focused on their presence on social media and its effects on the organization's target audiences. They believe these methods will reach the largest number of patients while incurring low operational costs (i.e. an internet connection ,electronic devices such as computers and mobiles and the salaries of the employees who manage these accounts) It is worthwhile to mention that information circulated by social media is uncontrolled , which causes problems for private hospitals in Jordan.

The study assumes that many private hospitals in Jordan have account or on at least one social media. A field survey of private hospitals found that the most commonly used social media methods are websites, Facebook and Twitter.

Based on this information, the research questions were formulated as follow:

What is the impact of social media (e.g., websites, Facebook and Twitter) on patients' mental image of the healthcare services provided by private hospitals?

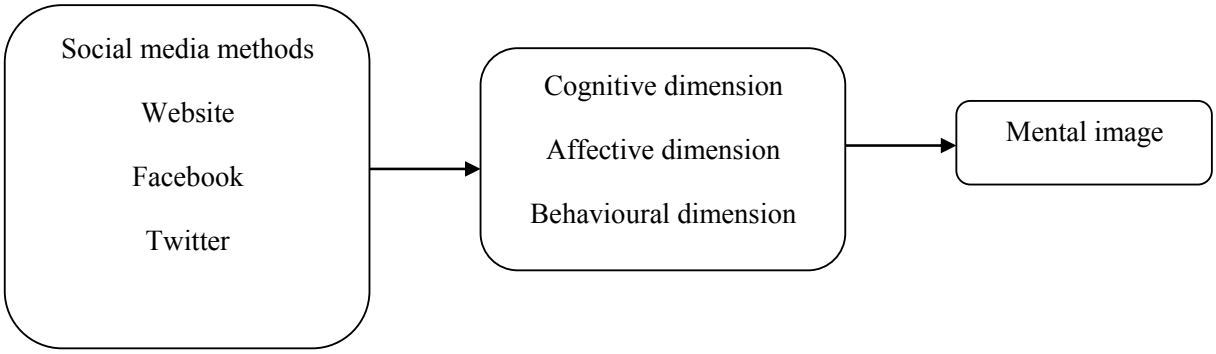
Study objectives

The current study aims to understand the impact of social media (websites, Facebook and Twitter) on the mental image of healthcare services provided by private hospitals.

Study model

The following diagram (Figure-1) represents the study model based on the study problem, objectives and hypotheses, with reference to [4,17-24].

Summary of Study Model Components



Study tools

Figure 1. Study model.

Study methodology

Study tools

The study tool was (the questionnaire) and the data it provided to the researcher from the research sample. The opinions of many specialists in the field, such as researchers, scholars, the authors and university professors have been considered in finalizing the questionnaire and explaining it is related to the subject.

The questionnaire is divided into two part:, the first part is concerned with the demographic data of people respondents, and , the second part includes the paragraphs that measure the questionnaire domains (fields).

The uses five –point Likert scale as follows questionnaire answers, Likert Scale, of five scales, was relied on:

((5) strongly approve, (4) approve, (3) neutral, (2) do not approve, (1) strongly do not approve).

Study sample

The sample is derived from the population of patients who have received healthcare services at private hospitals in Amman, the capital of Jordan. The number of those hospitals 2015 was (41) covering different fields of medicine. A proper sample of (550) patient received, the questionnaire. They returned were (513) valid copies of the questionnaire, for a response rate of 93.27%.

stability of the study tool

The Cronbach's alpha test was used to measure the stability of the measurement tool. T the value of (α) reached (0.988) for the questionnaire as a whole. This is an excellent value since it is higher than the accepted value (0.60).

The following table (Table-1: Stability Test) presents the value of (α) for each variable.

Table 1. Stability test.

Variable	**(α) value**
Website	0.951
Facebook	0.919
Twitter	0.940
Cognitive Dimension	0.945
Affective Dimension	0.943
Behavioural Dimension	0.959
Social Media Methods	0.972
Mental Image	0.961

Overlapping (Correlated) relation test

The VIF test and the tolerance test were used to test the extent of the relationship between the independent variables. and of VIF was less than (0.10) and the value of tolerance is greater than (0.10) which is evidence that there is no multicollinearity

The statistical treatment used in the study

The researcher (scholar) relied on a number of

statistical tests to support the study objectives by using the Statistical Package for the Social Sciences (SPSS) program.

RESULTS

Many statistical methods were used within SPSS to reach the results of this study.

There were (66) questions in the questionnaire, which was designed especially for this study. They were distributed on six pivots (axes) of social media methods as explained in the following table (Table-3):

Table 2. Study questions distributed on pivots (Axes) of social media methods.

Serial	Pivot (Axis) of Social Media Methods	No. of Questions for Each Pivot
01	Website	18
02	Facebook	18
03	Twitter	18
	Mental Image Dimensions:	
04	# Cognitive Dimension	4
05	# Affective Dimension	4
06	# Behavioural Dimension	4
	Total	66

The Impact of Social Media Methods on Mental Image Dimensions Table-4 explains the impact of each method on mental image in a combined form, using (one-simple t-test) to test the branch hypotheses.

It is notable that the value for the t-test for the three methods is greater than the tabulated value (1.96); based on this, these hypotheses are accepted. In the following (Table-5), we present the regression coefficients (B, Beta, t, Sig.) for social media methods.

We notice that the value of F is (179.981) and is of statistical of significance at the level of (0.05; this indicates is a statistically significant impact of social media on patients 'mental image of healthcare services. The strength of the relationship R= 0.842 and the independent variables explain 61.2 % of the variation of the following variable.

In addition, the drawn value for each independent variable statistically significant at the level (0.05). This indicates that there is an impact for each variable on the following variable. Facebook has the highest impact because the value of Beta= 0.406 is the greatest value.

Discussion and conclusion

DISCUSSION

This study found the following results:

Social media has a statistically significant impact on the mental image of healthcare services, Facebook has the greatest effect. This result agrees with what is presented in the literature where social media methods play an important role in the marketing field. Furthermore, this result coincides with the results of previous studies, such as Hashem [19] Kaite and Piligrimiene [18], and Nikolova [17]. This confirms the importance of using social media in marketing campaigns, for hospitals, the scope of this study.

Statistical treatment finds the presence of a statistical significant impact of websites on mental image dimensions for healthcare services provided by private hospitals, at the level ($\alpha \leq 0.05$). Additionally, there is an impact of Facebook on mental image dimensions for healthcare services provided by private hospitals, at the level ($\alpha \leq 0.05$).In addition, there is an impact of Twitter

on mental image dimensions for healthcare services provided by private hospitals, at the level ($\alpha \leq 0.05$).

Social media has a statistically significant impact on the influence dimension for healthcare services provided by private hospitals. Twitter has the highest effect. This can be explained because Twitter is in interactive news site with users inside and outside Jordan, whose opinions may play an important role in creating the mental image of healthcare services. This result coincides with the results of previous studies, such as Hashem [19] Kaite and Piligrimiene [18], and Nikolova [17].

Moreover, social media has a statistically significant impact, on the cognitive dimension of healthcare services provided by private hospitals. Facebook has the highest effect. This can be explained because Facebook is an interactive social site connecting family and friends inside and outside of Jordan; this interaction increases users' cognitive about the healthcare services provided by private hospitals, the scope of the study. This result matches the results of previous studies, such as Hashem [19] Kaite and Piligrimiene [18], and Nikolova [17].

Social media also has statistically significant impact, on the behavioural dimension for healthcare services provided by private hospitals. Facebook has the highest effect. This can be explained because Facebook is an interactive social site connectin family and, friends inside and outside of Jordan; this interaction contributes to the development of behaviour plans for healthcare services provided by private hospitals, the scope of this study. This result matches the results of previous studies, such as Hashem [19] Kaite and Piligrimiene [18], and Nikolova [17].

The mental image dimensions, of g healthcare services provided by private hospitals, contribute to improving patients' mental image; at the level ($\alpha \leq 0.05$). This shows that the general average of (4.101) reflects a high level of approval towards cognitive dimension. Thus, there is a high level of positive cognition for the provided healthcare services. It also shows that the general average of (3.977) reflects a higher approval level towards the influence dimension. This means that there is a high level of positive influence for the provided healthcare services. It also shows, that the general average of (3.931) reflects a high level of approval towards behavioural dimension. This means that there is a high level of positive approval for the provided healthcare services.

CONCLUSION

Although this study is very limited, t it provides an important contribution to studying and investigating mental image and its three dimensions and the impact of social media on each dimension of mental image. It also explores how best to improve mental image in the healthcare sector, an important and vital field in Amman, Jordan. This issue concerns e society in a significant and vital way because everyone needs healthcare services regardless of ages.

Future studies must explore other aspects of the subject of healthcare services and social media , including how to create a positive mental image for these services.

Ethical Clearance: Not required

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. 1. ODLYZKO, Andrew. The history of communications and its implications for the Internet. 2000.
2. 2. Rogers, E.M. A History of Communication Study: A Biographical Approach. New York: The Free Press. 1994.
3. 3. Grujanac, M.P. "How Does Internet Facilitated Communication Impact Teacher and Parent partnerships?"Doctoral dissertation, Loyola University Chicago. 2011.
4. 4. AlSamydai, M., D. Dajani, and S. Yaseen. "Factors Influence the Use of E-Promotion Means in Jordanian Business Organization." International Journal of Management Science. 2014; 3 (1):433–445.
5. 5. Fill, C., and S. Turnbull. Marketing Communications: Brands, Experiences and Participation. London: Pearson Education Limited.175, 2016.
6. 6. Alsamydai, M.J., and R.O. Yousif. Service Marketing. Amman, Jordan: Al Massira for Publishing and Distribution, 98. 2010.
7. 7. Mayberry, R. M., D. A. Nicewander, H. Qin, and D. J. Ballard. "Improving Quality and Reducing

Inequities: A Challenge in Achieving Best Care." Proceedings (Baylor University. Medical Center. 2006; 19 (2):103–118.

8. 8. Ventola, C. L. "Social Media and Health Care Professionals: Benefits, Risks, and Best Practices." Pharmacy and Therapeutics. 2014; 39 (7):491–520.
9. 9. Mosadeghrad, A. M. "Factors Influencing Healthcare Service Quality." International Journal of Health Policy and Management. 2014; 3 (2):77–89.
10. 10. Di Marino, E. "The Strategic Dimension of Destination Image: An Analysis of the French Riviera Image from the Italian Tourists' Perceptions." Paper presented at 17th International Tourism and Leisure Symposium, Barcelona, 17–18 May. 2008.
11. 11. Shepherd, A., C. Sanders, M. Doyle, and J. Shaw. "Using Social Media for Support and Feedback by Mental Health Service Users: Thematic Analysis of a Twitter Conversation." BMC Psychiatry. 2015:15 (1):29.
12. 12. Alsamydai, M.J., and R. O. Yousif. Customer Behavior Quantitative and Analytic Entrance. Amman, Jordan: Al Massira for Publishing and Distribution, 181–202. 2005.
13. 13. Cooley-Strickland, M., T. J. Quille, R. S. Griffin, E. A. Stuart, C. P. Bradshaw, and D. Furr-Holden. "Community Violence and Youth: Affect, Behavior, Substance Use, and Academics." Clinical Child and Family Psychology Review. 2009; 12 (2):127–156.
14. 14. Park, J., and F. Gunn. "The Impact of Image Dimensions toward Online Consumers' Perceptions of Product Aesthetics." Human Factors and Ergonomics in Manufacturing & Service Industries. 2016; 26 (5): 595–607.
15. 15. Hawn, C. "Take Two Aspirin and Tweet Me in the Morning: How Twitter, Facebook, and Other Social Media Are Reshaping Health Care." Health Affairs 2009; 28 (2): 361–368.
16. 16. Foray, D., and B-Å. Lundvall. "The Knowledge-Based Economy: From the Economics of Knowledge to the Learning Economy."In The Economic Impact of Knowledge,115-121. Routledge. 1998.
17. 17. Nikolova, S.N. "The Effectiveness of Social Media in the Formation of Positive Brand Attitude for the Different Users."Doctoral dissertation, Master Thesis, University of Amsterdam, Feb. 2012, Accessed 24 November 2015 http://dare.uva.nl/cgi/arno/show. Cgi.
18. 18. Kuvykaitė, R., and Ž. Piligrimienė. "Communication in Social Mediafor Company's Image Formation." Economics and Management. 2013; 18 (2):305–317.
19. 19. Hashem, T. "The Impact of Social Media on Customers' Image for Mobiles." Journal of Advances in Humanities and Social Sciences. 2016; 2 (5): 269–277.
20. 20. Ali Alsamydai, Ghaida' Baqa'in, Ibrahim Al-Helli, Hala Aladwan, Karim Salman, Rola Herimi, and Prof. Talal Aburjai ―The Role of Information Resources on Building Health Awareness -Study Conduct in Amman Jordan, International Research Journal of Pharmacy and Medical Sciences (IRJPMS), Volume 1, Issue 6, pp. 44-49, 2018.
21. 21. Ali AL-Samydai , Lena A. Al-Elaime , Sundos H. Abbadi, Lubna Al-Khareisha, Measuring Awareness of Over the Counter Analgesics Use and Risks Associated With It Among Jordanian Population In Amman,Asian Journal of Pharmaceutical research and Development. 2018; 6 (5):9-15 DOI: http://dx.doi.org/10.22270/ajprd.v6i5.429.
22. 22. Alsamydai, A. M. J. the opinions of pharmacists about the possibility of using electronic means of communication by pharmaceutical companies to facilitate the process of trade exchange. International Journal of Business Management & Research. Vol. 4, Issue 6, Dec 2014, 63-72.
23. 24. Alsamydai, A. M. J., & Baqer, A. B. M. Measuring patient satisfaction regarding the quality of healthcare service provided by pharmacists. International Journal of Medicine and Pharmaceutical Science Vol. 5, Issue 6, Dec 2015, 71-84.

Sequencing of the Exon 17 C/T, Intron 3 and Intron 8 in INSR (Insulin Receptor Gene) to Identification New SNPs in Iraqi Women with Polycystic Ovarian Syndrome (PCOS)

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ABSTRACT

In this case-control design study , 46 clinically diagnosed Polycystic Ovarian Syndrome (PCOS) in Iraqi women and 20 healthy Iraqi female were enrolled for analysis of the Exon 17 C/T Single Nucleotide Polymorphisms in Insulin Receptor Gene *(INSR*) gene as predisposing molecular marker for PCOS and its relationship with diabetes. Genomic DNA was extracted from whole blood of each subject using wizard genomic DNA purification kit. Specific primers for Single Nucleotide Polymorphism (SNPs) analysis of *INSR* Exon 17, Intron 8 and 3 were used Single Nucleotide Polymorphism-Polymerase Chain Reaction (SNPs-PCR) amplification reaction. Post PCR Restriction Fragment Length Polymorphism (PCR-RFLP) was done for PCR-Products using the Restriction Enzyme *PMLI*. In conclusion, the Exon 17 C/T Single Nucleotide Polymorphism in *INSR* gene can consider as predisposing molecular marker for PCOS that can transmitted offspring, colorectal cancer (CRC) and Breast Cancer in Iraqi women and the Intron 8 Single Nucleotide Polymorphism can consider as predisposing molecular marker for PCOS and Diabetes that can be transmitted offspring in Iraqi women. The hormonal values were takes as detected markers for PCOS such as Luteinizing Hormone (LH) values that appears in high level in women with PCOS either they were married or unmarried and the Follicle Stimulating Hormone (FSH) that appear in low levels in women with PCOS.

Keywords: *Exon 17 C/T, Intron 3, Intron 8, Insulin Receptor Gene, SNPs, Polycystic Ovarian Syndrome.*

INTRODUCTION

Polycystic ovarian syndrome, which used to be called Stein and Leventhal Syndrome, is a common condition affecting 5-10% of women of childbearing age. This disorder is probably the most common hormonal abnormality in women of reproductive age. In 95% of women with PCOS, an ultrasound of the ovaries will reveal cysts that can be seen on the surface of the ovary. These ovarian cysts are often lined-up to form the appearance of a "pearl necklace" [1] .

The susceptibility genes for PCOS are unknown; several candidate genes have been evaluated. Most of researchers found the evidence that *INSR* gene show consistent; linkage and association with PCOS. The *INSR* receptor gene comprises 22 exons spanning 120kb on chromosome 19. Mutations in exon 17 and intron 13, 8 that encode the tyrosine kinase domain of the insulin receptor, have been shown severe insulin resistance and hyperinsulinemia. Two possible approaches are used to identify a genetic locus for PCOS genes: (i) association studies where a predisposing allele is expected to be found more frequently in the affected population than the normal individuals and (ii) linkage studies where the probands and their families are investigated to determined if particular genomics landmarks are distributed independently or in linkage with the phenotype. While the mode of inheritance is not required for the association studies, it requires a relatively large set of individual for a clear conclusion [2].

Many genes presented expression suggesting thus that the genetic abnormality in PCOS affects signal transduction ruling insulin action and their secretion.

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Although the cause of PCOS is not well understood, insulin resistance may be a key factor. Insulin is vital for the transportation and storage of glucose at the cellular level; it's helps to regulate blood glucose level and has a role in carbohydrate and lipid metabolism. These conditions put those with PCOS at a higher risk of developing type 2 diabetes (T2D) and cardiovascular disease [3,4].

The aims of this study is to examine whether the insulin receptor *INSR* gene contributes to genetic susceptibility to the PCOS and to analyze the exon 17 C/T single nucleotide polymorphism in INSR a predisposing molecular marker for PCOS.

MATERIAL AND METHOD

Blood Samples.

The samples were collected from AL-Yarmok Hospital in Baghdad, from 17 December 2013 to 17 June 2014 using EDTA tubes and sterile syringe which consist of 70 unrelated females (20 controls, 50 patients). The samples were transferred to the laboratory for DNA extraction. Sixty-eight human blood samples consisting of 46 samples infected with PCOS that have different ages ranged between 11-67 years old and their weight ranged between 30-120 kg. According to these data there will be 4 groups that classified due to their ages and weight, these are the properties of each groups.

DNA extraction that used in this study.

DNA extraction that used in this study according to the Genomic® Wizard Purification Kit .

Estimation of the DNA concentration by the Nanodrop equipment.

The DNA concentration was determined by using the Nanodrop according to [5].

Estimation of DNA quality

Estimation of DNA quality also can assess by simple analyzing the DNA by Agarose gel electrophoresis [5].

Amplification of DNA by PCR technique.

PCR reaction performed using the following:

Specific primers and their preparation.

There are variant SNPs in the *INSR* gene were selected based on way of Xu *et al*. [6].

Go Taq@ Green Master Mix

The master mix reactions were used to achieve the homogeneity of reagents and reduced the risks of contamination. All amplifications were performed on ice in aseptic condition using a laminar air flow hood [6].

Table (1) the sequences and genes locations of specific primers [6].

Primers	Sequence	genes Locations
*Rs8108622 / rs10500204 (forward) Rs8108622 / rs10500204 (reverse)	5`GTCCCAGATACCAAGGATGTGC3` 5`GAGAATTAGCCAAGCGAGAGTGT3`	INTRON 3
** RS2059807 (forward) Rs2059807(reverse)	5`GACCCAGTATGCCATCTTTGTG3` 5`TGCTGAGCCCAGGAGTTTG3`	INTRON 8
*** RS1799817 (forward) Rs1799817 (reverse)	5`TCCAGAAAGTGATGAGAACGTGAT3` 5`GGTCAACGAGTCAGCCAGTCT3`	EXON 17

*primer 1, **primer 2, ***primer 3

Protocol of specific PCR

This protocol consists of: PCR primers, PCR Mix , Amplification reaction, PCR programe according to [1,6].

Detection single nucleotide polymorphisms (SNPs) by RFLPs

Some samples were surveyed for characterizing single nucleotide polymorphisms (SNPs) on Insulin receptor gene **(INSR gene)** by RFLPs technique (PCR amplification, followed by restriction enzyme digestion [6].

Restriction Enzyme *PMlI*

PMLI* is** a restriction enzyme that isolates from product source ***Pseudomonas maltophilia . There are several key factors to consider when setting up a restriction endonucleases digest using the proper amount of DNA, (incubation time, temperature and buffer) provided by (Biolabs). enzyme and buffer components in the correct reaction volume that lead to having optimal digestion.

Detection of single nucleotide polymorphism (SNPs) by automated sequencing.

Several DNA sample from women that have PCOS were taken to have PCR reaction with different molecular markers, according to NICEM Company / USA to have an automated sequencing by ABI3730XL APPLIED BIOSYSTEM machine , which gave the identity of the genes comparison with the original genes in Gene Bank in NCBI.

Results and discussion

Table (2): Unmarried and Married Women with PCOS .

Unmarried Women with PCOS (G1).			**Married Women with PCOS (G2).**		
Samples ID	**Ages (Years)**	**Weight (Kg)**	**Samples ID**	**Ages (Years)**	**Weight (Kg)**
N12	19	60	**N2**	23	95
N15	18	60	**N3**	28	82
N16	13	75	**N6**	32	95
N18	11	38	**N8**	29	99
N19	27	60	**N9**	30	75
N23	22	55	**N11**	21	75
N26	25	57	**N13**	31	75
N30	15	53	**N14**	24	79
N32	15	80	**N21**	35	65
N35	28	60	**N28**	28	80
N39	12	80	**N33**	30	88
N42	30	50	**N38**	24	85
N45	21	65	**N40**	24	88
			N41	30	67

Table (3) Unmarried and married Women with PCOS .

Unmarried Women with PCOS (G3).			Married Women with PCOS. (G4).		
Samples ID	**Ages (Years)**	**Weight (Kg)**	**Samples ID**	**Ages (Years)**	**Weight (Kg)**
N1	40	85	**N10**	64	85
N4	37	60	**N31**	67	114
N7	42	75	**N44**	63	73
N17	47	68	**N46**	58	83
N20	45	65			
N22	40	62			
N27	45	75			
N29	39	75			
N34	40	90			
N36	50	80			
N37	47	74			
N43	41	75			

Concerning molecular genetic studies, PCOS is one of the most extensively studied endocrinopathys in women and attention has been given to insulin resistance with special focus on the ***INSR*** gene. Therefore in this study we used family- based analysis in order to investigates the relationship between the INSR gene SNPs and the genetic component of PCOS [2,6,7].

Results of RFLP

After PCR technique for all the samples , several PCR product were selected such as (46 , 11 , 31 , 5 , 2 and 14) with different primers to incubate them with restriction enzyme PMLI at 37°C for 1 hour and then these samples were migrated in 0.5 X TBE buffer with 2.5% Agarose gel for (1 hour , 90 V). The result was three restriction fragments they are 317 bp , 264 bp and 43 bp.

In figure (1) that refer to have three restriction fragments on line 13 which belongs to sample N31 for the primer (rs17799817) which has 317bp, 264 bp but the last fragment which is 43 bp did not appear in the gel due to its small size.

While in figure (2) illustrate the line 1 which belongs to sample N5 for the primer (rs8108622 and rs10500204) has three restriction fragments they are 317 bp, 264bp, 43bp.

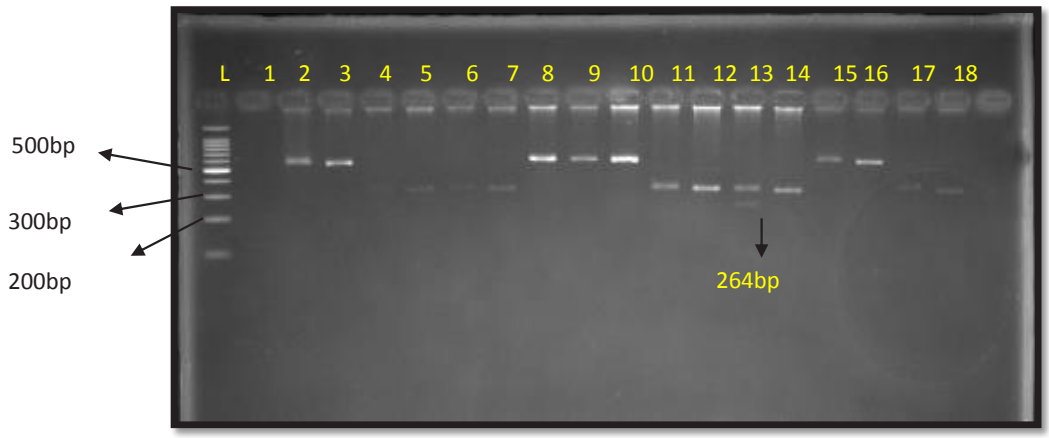


Figure (1): The PCR- RFLP analysis of intron 8 of the INSR gene. The line 13 that refer to sample N31 that has 317bp , 264bp only.

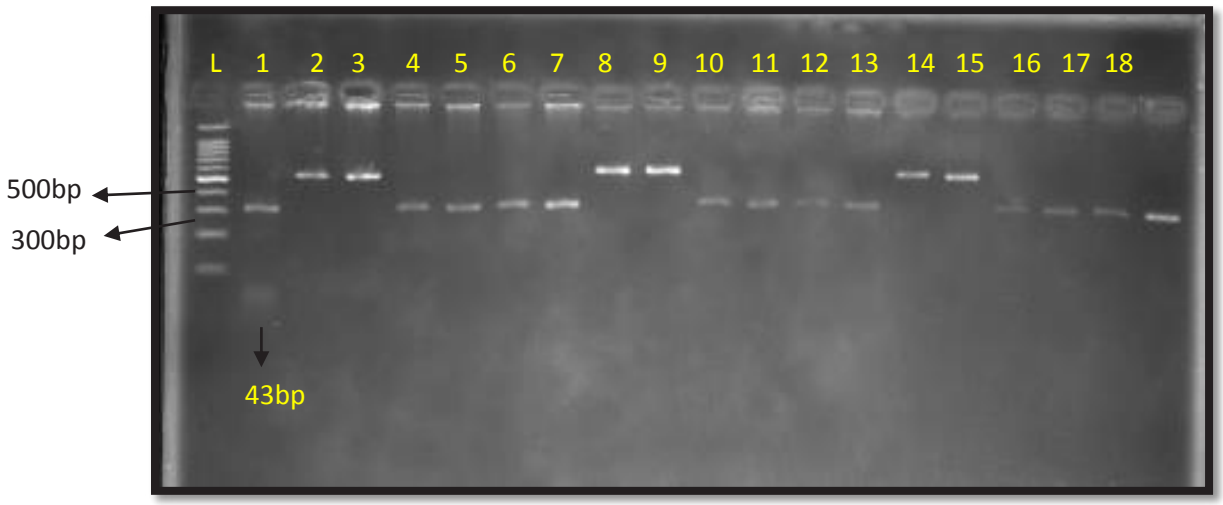


Figure (2): The PCR – RFLP analysis of exon 17 of the INSR gene. The line 1 that refer to the sample N5 that has 317bp, 264bp and 43bp.

That's mean that the sample N5 for primer (rs8108622 and rs10500204) and the sample N31 for the primer (rs17799817) have the same sequence as same as *PML1* sequence. The other samples that did not have any restriction fragments because of one of these reasons:

A. changing in their sequence.

B. has a mutation.

C. changing in their original replication

The SNPs in exon 17 of the INSR gene in the tyrosine kinase domain of the insulin receptor have paid more attention [2,8]. Found an association between rs1799819 and PCOS.

They argued for the first time that the INSR gene itself took part in the development of PCOS, that's was not similar to the data of this study whose found that the rs8108622 & rs10500204 and rs2059807 have an association with PCOS while the rs1799819 does not have an association with PCOS in Iraqi women.

In India, the researchers found an association of rs1799817 with indices of insulin resistance and hyperandrogenimia in the same subgroup [1,7]. According to the results of this study that found the rs1799817 have not significantly over transmitted to PCOS off spring from their parents by using a genetic approach of association analysis, while the rs2059807 and rs8108622 & rs10500204 were significantly over transmitted to PCOS off spring from their parents whose has an agreement with [9] and [1].

The insulin regulates cell growth and apoptosis by binding to its receptor (INSR) gene. Many studies show the variation in insulin pathway play a plausible role in the development of Colorectal Cancer (CRC) and breast cancer. The goal of their study was to evaluate the incidence of insulin receptor (*INSR* rs1799817) in people of Taleghani Hospital to investigate the role of this polymorphism in increased of CRC and breast cancer [10] ; also there are many research articles have been published in this site which give support to the hypothesis that patients with insulin resistance syndrome such as obesity and T2D , might be at higher risk for developing cancer than the general population [11] ,which is similar to the result of this study that found the rs1799819 have an association in increased of CRC and breast cancer in Iraqi women.

However, the precise mechanism that link insulin resistance and cancer have not yet been fully understood there were more details of molecular mechanistic understanding is required to interpret the existing data together with preclinical and clinical studies.

In addition, environmental factors are also involved in PCOS. The interaction with genes may be a key point of the pathogenesis in PCOS, not only the gene itself. When we use the family trios analyzing the subject's genotypes, the interaction would be exorcized. Therefore, there may be one reason for finding little association between two SNPs and PCOS instead of four

SNPs [1,6].

This study covered a large series of PCOS trios and represent the first family that association with providing data about INSR gene and PCOS in Iraqi women. Finally, we used to mention that PCOS in a multi factorial disease.

RESULTS OF SEQUENCING

These PCR product samples were sent outside the country to have an automated DNA sequencing at NICEM company/ USA and worked by ABI3730XL APPLIED BIOSYSTEM machine, the INSR gene sequence was investigated because this method is more discriminating [11]. From comparison of the sequence with that in the database in Gene Bank by BLAST program, the gene was identified (98%) with no gaps (0%), there were five circles that illustrates these comparisons and their means, the first and second circle means at the subject 173674 there is 2 SNPs linked to INSR gene they are: (Addition in sequence and Replace C instead of T [12] .

While the third circle mean at the subject 173914 there was 1 SNP linked to the same gene it`s.. (Replace G instead of T). The fourth and fifth circles means at the subject 173914 there were 2 SNPs linked to same gene and they are (Replace G instead of T and Replace C instead of A) as shown in figure(3).

This sample was belonging to sample no.5 of primer 3. This sequence was approved to have PCOS in patient that transmitted off spring from the parents and in future can have CRC or breast cancer, this result was decomentated for the first time in Iraq.



Figure (3): The automated sequencing of INSR (P1) from PCOS patient that have (98%) indentify with (0%) of gaps . This result was approved to have PCOS that transmitted off spring from the parent with CRC or breast cancer.

While the sample no. 14 of primer 2 that refer to was sent to the same company in USA to have an automated DNA sequencing, from comparison of the sequence with that in the database in Gene Bank by BLAST programs, the gene was identified as (97%) with (1%) of gaps. There were three circles that illustrates these comparisons and their means**.** The first circle mean at the subject 132782 there is 1 SNP that linked to INSR gene which is addition of nucleotide in the sequence, While the second circle mean at the subject 132842 there was 1 SNP which is replace G instead of T and the third circle mean there was a semi conservative sequence that refer to the of the gene. This result was approved to have PCOS in patient that transmitted off spring from the parents with diabetes disease.

CONCLUSION

The exon 17 C/T single nucleotide polymorphisms in INSR can`t consider as predisposing molecular marker for PCOS in Iraqi women , while the SNPs in intron 3 and intron 8 can be consider as predisposing molecular marker for PCOS in Iraqi women. **Found that INSR (P3)** can consider as a molecular marker that approved to have CRC or Breast cancer transmitted offspring

from the parent. **Found that the INSR (rs2059807)** can consider as a molecular marker that approved to have PCOS with diabetes disease which transmitted off spring from the parent.

Conflict of Interest: The author has no disclosures to report.

Source of Funding: Self.

Ethical Clearance: Not required.

REFERENCES

1. Maysaa, A.R. Molecular Screening for Single Nucleotide Polymorphism in Insulin Receptor Gene in Iraqi Women with Polycystic Ovarian Syndrome. *Int. J. Mod. Biol. Med*.;2013. 3(1): 17-26.
2. Moran, L.J.; Ko, H.; Misso, M.; Nonkes,M.; Talbot, M.; Freson, M.; Thendon, M.; Stepto, N. and Tedee, H.J."Diary Composition in the Treatment of Polycystic Ovary Syndrome: A Systematic Review to Inform Evidence Based Guidelines ". Hum. Reprod.;2013. 19(5):432.
3. Hoeger, K.M. and Oberfield, S.H.E. Do Women with PCOS have a Unique Predisposition to Obesity? Fertil. Steril;2012.97:13-17.
4. Ewens, K.; Stewart, D.R. ; Ankenar, W.; Urbanek,M.; Mcalliste, J.M.; Chen, C.; Baing, K.M.; Parker, S.C.J.; Franks, S. and Berga, S.L. Does PCOS have development Origins? Fertil. Steril;2012. 97:2-6.
5. Maniatis, T.; Fritsch, E.F. and Sambrook, J.Molecular Cloning: A Labrotary Manual, Cold Spring Harbor laboratory, N.Y.;1982.
6. Xu, X.; Zhao, H.; Shi, Y.; You, L.; Bian, Y.; Zhao, Y. and Chen, Z.J. Family Association Study between INSR gene Polymorphisms and PCOS in Han Chinese. Reprod. Biol. Endorinol. ;2011. 9: 76.
7. Mukherjee, S.; Shaikh, N.; Khavale, S.; Shinde, G.; Meherji, P.; Shah, N.; and Maitra, A. Genetic Variation in Exon 17 of INSR is associated with insulin resistance and hyperandrogenemia among lean Indian women with Polycystic Ovary Syndrome. Eur. J. Endocrinol. ;2009. 160: 855 – 862.
8. Siegel, S.; Futterweit, W,; Davies, T.F.; Conceppcion, E.S.; Greenberg, D.A.; Villanueva, R. and Tomer, Y. A C/T single nucleotide polymorphism at the tyrosine kinase domain of the insulin receptor gene is associated with polycystic ovary syndrome. Fertil Steril. ;2002. 78: 1240-1243.
9. Alnakash, H.A. and Al-tae`e, N.K. Polycystic Ovarian Syndrome: The Correlation between the LH / FSH Ratios and disease manifestations. *Mid. East Fertil Societ. J. ;*2010. 12:35-40.
10. Karimi, K.; Arkani, M.; Safaei, A.; Vahedi, M.; Mohebi, S.R.; Fatemi, S.R. and Zali, M.R. Relationship between Insulin Receptor (INSR rs1799817) and Colorectal Cancer (CRC). Shahid Beheshti University of Medical Science;2012. 22(92): 30-35.
11. Arcidiacono, B.; Iiritano, S.; Nocero, A.; Passedente, K.; Maria, T.; Ventura, V.; Foti, D.; Cheifari, E.; and Brunetti, A. Insulin Resistance and Cancer Risk: An Overview of Pathogenic Mechanisms. *J.Diab.Res ;*2012. 789174:1-12.
12. National Center for Biotechnology Information, (NCBI) United States National Library of Medicine, NCBI dbSNP build 138 for human;2013.

Factors Influencing Woman Behavior to Visit Dental Clinic to Improve their Smile

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ABSTRACT

Women, nowadays, become more interested or more oriented to plastic surgery including smile improvement. Although smile improvement popularity is increasing among Iraqi women, but published researches are rare in this field. Consequently, the objective of this study is to determine the factors that affect woman behaviour and direct her to improve the smile.

The theory of planned behavior, developed by Ajzen in 1985 (1), and the variables were adopted and formed according to study objectives, i.e. attitude, social, perceive, cost and dentist. The study approach and methodology depended on collecting data from 367 women in Baghdad city, data was analyzed by using the proper statistical methods (SPSS) and AMOS analysis.

There are positive correlations between attitude, social, perceived, cost, dentist and intention; and the results show that the dentist has mostsignificant effect on intention with correlation215**and Sig 0.000.There is positive relationship between Intention and behavior with correlation 0.298** and Sig 0.000

This paper provides information about factors that impact behaviour of Iraqi woman to improve the smile, and as far as we know, it is considered to be the first study, in this field, in Iraq.

Keywords: *Dental Clinic, Smile, Attitude, Social, Perceived, Cost, Intention, Woman Behavior*

INTRODUCTION

Dentistry has developed, as other medical fields, especially in the last fifty years. This development does not focus only on dental treatment, but also ensures providing beauty services requested by the patient, smile improvement upon patient request (2,3). The patient need or request for plastic surgeries may be due to social pressures or perhaps due to change in beauty standards evaluations which represents the motive behind beauty revolution. It is known that media, concerned with beauty, feed this beauty revolution. As Morley, 1999 and Priest and Priest, 2004 mentioned(4,5).

In today's society, most people, including women, have the desire to be better than before concerning enhancement of the external look. The physical appearance has an important and efficient role in self-respect and achieving success in social, functional and work. The human nature makes individuals who own positive characteristics an attractive people; those are usually received proper behavior and treatment, in many different situations, and gain from it many benefits, due to Sarwer et al 2004 (6).

Chetan et al in 2013(7) , indicates that patients have to think before taking any decision towards any cosmetic dentistry. They must consider the nature of the process, benefits, costs, results. For this, this study will concentrate on smile improvement, costs and dentist capability. The woman approach towards improving her smile is a planned behavior that depends on intention which is affected by social factors and situations, awareness of benefits, costs and the dentist. For that, this study will be based on adaptation of planned behavior theory which was developed by Ajzen in 1985 (1).

The theory of planned behavior, developed by Ajzenin 1985 (1), is an explanatory model that has been widely applied in diverse studies on behavioral intention

[8,9]. The theory states that attitude toward behavior, subjective norms, and perceived behavioral control, together shape an individual's behavioral intentions and behaviors.

Previous studies and hypotheses development

Schmidt et al in 2003 [10], indicated in his study, concerning spontaneous face expressions that the smile considered to be one of the most important human face expressions which supports reward value for the attractive face. Goldstein in 1969[11]finds, in his study concentrated on the needs for beauties in dentistry, that smile comes in the second degree after eyes and considered to be the basic advantage that affects face attraction. Attractive smile helps to win elections and sell products for companies.

Cheng and Cheng in 2017 [12], indicate that smile is one of the most important expressions among faceexpressions in human that supports reward value for attractive face. Face attraction plays a major role in social interaction, character evaluation, performance and employment horizon [13]

The smile plays an important role in face expression and the look and smile represents face expressions that indicate to cordiality, agreement and appreciation. Distinctive and attractive smile leaves an impression in others. Those who have an attractive smile excite or inflame interest or the desire to smile. Teeth appearance proved that it affects other judgment for attraction of a person face beside the personal characters like quite, joy and relaxation [14,15]

Shavi et al in 2013[16], explains in his study, smile analysis in Haryanavi females, that people who own attractive smile got a bigger chance to success and social acceptance, because the un-harmonic smile reduces face beauty and may cause un comfortability in social and professional life while the happy smile represents a harmonic relation between teeth and the lips.

The women will do their best to hide any defects in the teeth. In fact this is the main motive for women to look for dentist, that is, to gain attractive smile. They will look for information from different resources like relatives, friends, social media, celebrities' photos, actresses and others whom they publish their photos on social media like Facebookand the comments of other users [17].

The study ofAhrari et al in 2015[18], concluded that face attractiveness, including teeth and smile beauty, influences on social reactions and on self-concept, psychological welfare and social behavior. Persons, who are more attractive, are more successful in school, employment interviews and even in selection of their partners [19].

No doubt, smile provides face with great beauty attraction which plays a major role in influencing the social interactions of a person, belonging to the group, acceptance from others, beside its effect on "being" or "self" concept and social behavior [20]. The objective from dental treatment, like dental implants or orthodontics appliances, is smile improvement [21].

Worldwide, smiles are known and evaluated as friendly. The real smile, that express person internal feeling, considered an indicator to acceptance and warm. Women, who have shining and friendly smile, will have less level of despair and sadness, say after 30 years, than their study colleagues [22] .

Al Taki et al in 2017 [23] mentions that smile is a remarkable express in the face distinguished and it used as efficient tool for the social interaction.

Piete et al in 2007[13], mentions that he underpins the psychosocial importance and thedental significance of an attractive smile.When a woman realizes that she is facing a problem by un-satisfaction of her smile, and the feeling of social and psychosocial pressures, then she starts to collect data about smile types by consulting the reference groups andthe media, then according to this information she shall take the proper decision to improve the smile [24].

Attitude

an attitude is a disposition to respond a positive or negative toward the object[25].

H1. Attitude has a negative effect on the attention

Subjective norm is defined as any social influence that may determine if theindividual performs or does not perform the behavior [26,27].

H2. Social norm has a negative effect on the attention

Perceived behavioral control is defined as the level of confidence an individual has about their ability to

perform the behavior based on how easy or difficult they perceive its performance as it relates to hindrances or facilitators [26, 27].

H3. Perceives has a negative effect on the attention

Cost

In spite of many women are seeking to improve the smile, aiming to gain an attractive look and achieve the benefits that will achieve satisfaction to them,psychological stability and social interaction, but costs are the decisive and final factor in making this decision.

Dental care request is the most health care types that are effected by prices, because patients, usually, sensitive towards prices [28, 29].

H4: Cost has a negative effect on the attention

Dentist

Dentist is the basic motive and the actual provider for the service of dental health care due to his distinguished knowledge, capabilities and skills that fit and match his job nature.

Dentist capability and services quality that he provides play a major role in attracting patients to request dental health careand provide services of very high quality [30,31,32].

H5. Dentist has a negative effect on the attention

The intention

The intention is the immediate antecedent of actual behavior and the relative degree of influence that attitude, subjective norm, and perceived behavior control have on intention may vary in significance by population and behavior studied [33,34,35,36].

H6. Intention has a negative effect on the behavior

STUDY METHODOLOGY

We tested our hypotheses by using the collected data returned fromapplying the questionnaire on sample of 420 women who have smile improvement, where (420) copies of the questionnaire were distributed for women, and (367) were returned and valid for analysis, which is 87%.

Data analyses

Test of Reliability

A reliability coefficient of (Cronbach's Alpha) 70% or higher is considered "acceptable" in most social science research situations. The result of this test in the current study is 72%

The structural model and the hypothesized relationships were tested by using Amos analysis

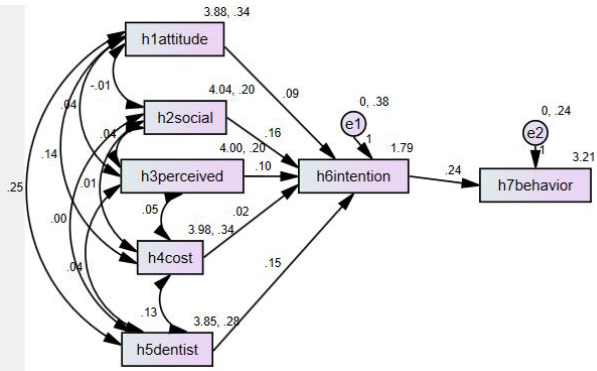


Fig1: Results of the structural model

Table (1) Person correlation coefficient

		h1attitud	h2socia	h3perceived	h4cost	h5dentist	h6intention
h6 intention	Pearson Correlation	.199**	.123*	.131*	.127*	.215**	1
	Sig. (2-tailed)	.000	.018	.012	.015	.000	
	N	367	367	367	367	367	367

RESULTS

The Person correlation coefficient

There are positive correlations between Attitude, Social, Perceived, Cost, Dentist and Intention and the results show that the dentist has most significant effect on intention withCorrelation.215** and Sig 0.000

Table (2)The Correlations between the intention and thebehavior

		h6intention	h7behavior
h6 intention	Pearson Correlation	1	.298**
	Sig. (2-tailed)		.000
	N	367	367
h7 behavior	Pearson Correlation	.298*	1
	Sig. (2-tailed)	.000	
	N	367	367

**. Correlation is significant at the 0.01 level (2-tailed).
There is positive correlations between intention and behavior

Table (3)Model Summary[b] -ANOVA[a]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	df	F	Sig.
1	.263[a]	.069	.056	.62495	5 361 366	5.355	.000[b]

a. Predictors: (Constant), h5dentist, h2social, h3perceived, h4cost, h1attitude

The results of the table confirm the validity of the multiple linear regression model, where the calculated F value is 5.355, which is greater than the table value of 2.26, and the value of Sig is 0.000, indicating that there is an effect of the independent factors on the intention of the Woman to improve the smile The value of R Square indicates that the independent variables (position, cognition, social factor, cost, and dentist) explain what (.069) of the changes that affect the intention of the mirror to improve the smile

Table (4) Model Summary[b] -ANOVA[a]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	df	F	B	t	sig	
1	.298[a]	.089	.086	.49478	1 365 366	35.495	.240	5.958	.000	
a. Predictors: (Constant), h6intention										
Dependent Variable: h7behavior										

The results of the table confirm the validity of the multiple linear regression model, where the calculated F value is greater than the table value 3.89, and the Sig value is 0.000, indicating that there is an effect of the intention on the behavior of the mirror to improve the smile, Square suggests that the intention is to explain what it is (.089) of the changes that affect the behavior of the woman to improve the smile

CONCLUSION AND DISCUSSION

The current study applied the theory of planned behavior to examine the factors influencing Woman behavior to improve the smile. In the absence of previous studies on the subject. Since existing studies examining this issue are limited, the present study can contribute in providing a better understanding of the factors influence the woman behavior to improve the smile in Iraq.

The main findings of this study indicate that all independent factors have a positive relationship with the intention to improve the smile and that the strongest relationship is between the dentist and the behavioral intention and the relationship between the behavior and intention positive relationship also.

The results indicate that the value of F is 5.355 and the sig is 0.000. This indicates that there is an effect of the independent factors on the behavioral intention of the woman to improve the smile. The F value is 35.495 and the sig is 0.000 which indicates the effect of the intention on the behavior of the woman to improve the smile

The results revealed significant relationship of five factors behavioral intentions, and significant relationship between the behavioral intentions and behavior of woman to improve the smile. This is consistent with the theory of planned behavior.

Ethical Clearance: Not required

Source of Funding: Self

Conflict of Interest: Nil

REFERENCES

1. Ajzen, I. From intention to actions: A theory of planned behavior. In Kuhl,J.&Bechmann,J.Action Control: From Cognitions to Behavior; New York: 1985.
2. Mandel, I.The image of dentistry in contemporary culture, 129: Journal of the American Dental Association; 1998.
3. Christensen, G.J. Are prosthodontics a vital part of dentistry, 133: Journal of the American Dental Association; 2002.
4. Morley, J.The role of cosmetic dentistry in restoring a youthful appearance, 130: Journal of the American Dental Association; 1999.
5. Priest, G. and Priest, J.Promoting esthetic procedures in theprosthodontic practice, 13:Journal of Prosthodontics; 2004.
6. Sarwer, D.B.; Magee, L. and Clark, V. Physical appearanceand cosmetic medical treatments: physiological and socio-cultural influences, 2: Journal of Cosmetic Dermatology; 2004.
7. Chetan,P.; Tandon, P.;Singh,G. K.;Nagar,A.;Prasad,V. and Chugh,V. K. Dynamics of a smile in different age groups, 83(1): The Angle Orthodontist; 2013.
8. Lee, J.;Cerreto, F. A. and Lee, J. Theory of planned behavior and teachers' decisions regarding use of educational technology, 13(1): Educational Technology & Society;2010.
9. Ajzen, I. The theory of planned behavior, 1: In P. A. M. Lange, A.W. Kruglanski& E. T. Higgins (Eds.), Handbook of theories of social psychology. London, UK: 2012.
10. Schmidt, K.L.; Cohn, J.F.&Tian, Y.Signal characteristics of spontaneous facial expressions: automatic movement in solitary and social smiles, 65(1):BiolPsychol; 2003.
11. Goldstein, R.E. Study of need for esthetics in dentistry, 21: J Prosthet Dent;1969.
12. Cheng, H.C. and Cheng, P.C., Factors affecting smile esthetics in adults with different types of anterior overjet malocclusion, 47(1): The Korean Journal of Orthodontics: 2017.
13. Pieter, A.A.; Paul, O. andAnne Marie, K.Smile attractiveness. Self-perception and influence on personality, 77(5):The Angle Orthodontist; 2007.
14. Thompson L, Malmberg J, Goodell N, Boring R . The distribution of attention across a talker's face. Discourse Processes. 2004 .38: 145–168
15. Rehman, K.; Khan, F.R. and Rahman, M.Asseing the perception of smile attractiveness in young

duilts. Apilot study, 20(4): JPDA; 2011.

16. Shavi, G. and Kanta, R.S. Smile analysis in Haryanavi Females, 2(2): IJMDS; 2013.
17. Meier, E.& Gray, J.Facebook photo activity associated with body image disturbance in adolescent girls, 17(4):Cyberpsychology, Behavior, and Social Networking; 2014.
18. Ahrari, F.;Heravi, F.; Rashed, R.; Zarrabi, M.J. and Setayesh, Y. Which Factors Affect Dental Esthetics and Smile Attractiveness in Orthodontically Treated Patients?, 12(7): Journal Dent (Tehran); 2015.
19. Nevin, J.B. andKein, R. Social psychology of facial appearance. In: Nanda R, editor. , editor. Biomechanics and esthetic strategies in clinical orthodontics. 1st ed., St Louis. 2005.
20. Sarver, D.M. Esthetic orthodontics and orthognathic surgery, St. Louis, Mosby; 1998.
21. Kelages, U.; Bruckner, A. andZentner, A.Dental Aesthetics, self-awareness, and oral health-related quality of life in young adults, 26:Eur J Orthod; 2004.
22. Goman, C.K.ASmile Can Change Your Brain. Can An Emoticon Do The Same?//www.forbes.com/sites/carolkinseygoman/2015/07/10/a-smile-can-change-your-brain-can-an-emoticon-do-the-same/#4769ea1a1afa; 2015.
23. Al Taki, A.; Mohammed, T.H. and Hamdan, A.M.Influence of the Smile Line on Smile Attractiveness in Short and Long Face Individuals, 2017:Int J Dent; 2017.
24. Solomon, E.S. The past and future evolution of the dental workforce team, 76(8):Journal dentist education; 2012.
25. Fishbein,M.,&Ajzen,I. Belief,attitude,intention,and behavior: An Introduction to theory and research. Reading,MA: Addison-Wesley; 1975.
26. Ajzen, I. The theory of planned behavior, 50: Organizational Behavior and Human Decision Processes; 1991.
27. Ajzen, I.; Brown, T. C.;&Carvajal, F. Explaining the discrepancy between intentions and actions: The case of hypothetical bias in contingent valuation,30(9): Personality and Social Psychology Bulletin;2004.
28. Phelps, C.E. Health economics .Addison Wesley Educational Publications,New York,London, Bookmark: Sixth edition; 1997.
29. Wall, T. andGuay, A. The Per-Patient Cost of Dental Care, A Look Under the Hood. American Dental Association; 2016.
30. Holden, L.C. and Moore, R.S. The development of a model and implementation process for clinical governance in primary dental care, 196: Br Dent J.; 2004.
31. Yamalik N. Quality systems in dentistry. Part 1. The increasing pressure for quality and implementation of quality assurance and improvement (QA/I) models in health care. Int Dent J. 2007;57:338–46
32. Alaghemandan H, Yarmohammadian MH, Khorasani E, Rezaee S. Efficiency improvement of dentistry clinics: introducing an intervening package for dentistry clinics, isfahan, iran. International journal of preventive medicine. 2014 Feb;5(2):176.
33. Ajzen, I., &Fishbein, M. The influence of attitudes on behavior. In D. Albarracin, B. T. Johnson, & M. P. Zanna (Eds.), The Handbook of Attitudes. Mahwah, NJ: Erlbaum; 2005.
34. Ali Alsamydai, Ghaida' Baqa'in, Ibrahim Al-Helli, Hala Aladwan, Karim Salman, Rola Herimi, and Prof. Talal Aburjai ―The Role of Information Resources on Building Health Awareness -Study Conduct in Amman Jordan, International Research Journal of Pharmacy and Medical Sciences (IRJPMS), 2018; Volume 1, Issue 6, pp. 44-49.
35. Ali AS, Al-Elaime LA, Abbadi SH, Al-Khareisha L. Measuring Awareness of Over the Counter Analgesics Use and Risks Associated With it Among Jordanian Population in Amman. Asian Journal of Pharmaceutical Research and Development. 2018 Oct 18;6(5):9-15. DOI: http://dx.doi.org/10.22270/ajprd.v6i5.429.
36. Alsamydai, A. M. J. the opinions of pharmacists about the possibility of using electronic means of communication by pharmaceutical companies to facilitate the process of trade exchange. International Journal of Business Management & Research. 2014 Dec; 4 (6):63-72.

CD4+ Cell Impacts of Orally Red Fruit (*Pandanus conoideus*) Oil Extract in HIV Patients with Antiretroviral Therapy

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ABSTRACT

Background and Objective: The efficacy of red fruit (*Pandanus conoideus*) oil extract on CD4+ cells was evaluated in HIV infected patients with taking antiretroviral therapy. **Materials and Method:** Seventy patients with an age of 17-45 years, with confirmed HIV infection with a CD4 count of 200-349 cells/μL, were divided into the antiretroviral (ARV) control (n = 35) and the antiretroviral + red fruit capsule (ARV+RFC)-treated (n = 35) groups. In ARV+RFC group, they were given oral RFC (1 g daily for two months). Monitoring evaluations of the laboratory were performed at baseline (0 months) and two months during the study. **Results:** The result showed that the CD4+ cells count was increased in the patient who took ARV+RFC compared with ARV alone ($P<0.05$). **Conclusion:** By these result, it can be concluded that the red fruit oil extract may have an excellent immunomodulatory impact and has potential as an adjuvant in the management of HIV patients.

Keywords: *Antiretroviral therapy, CD4+ cell, HIV/AIDS, Immune, Pandanus conoideus*

INTRODUCTION

The acquired immune deficiency syndrome (AIDS) is an infectious disease caused by a virus namely human immunodeficiency virus type 1 (HIV-1) [1, 2]. This disease remains a global public health problem with an estimated 690,000 people living with HIV in Indonesia area in 2015 [3]. The prevalence of HIV infection and AIDS of Papua Province was higher than another province of Indonesia [4]. In the Merauke District of Papua Province of Indonesia, the prevalence of HIV/AIDS has become a severe problem. In 1992-2017, the total number was 1,063 people living with HIV in Merauke Regency, 902 people had AIDS, and 537 died due to AIDS-related causes [4]. The populations of people HIV/AIDS include direct and indirect high-risk men, transgender people, female sex workers, people who inject the drug, and men who have sex with men [5].

Nowadays, antiretroviral therapy (ARV) was successful alternative treatment in people infected with human immunodeficiency virus (HIV) 6, 7. However, there were limited to ARV treatment in patients HIV/AIDS about by some factors, which include drug resistance 8-10, toxicity 11, drug-drug interaction 12 and drug-food interaction 13. Other than that ARV treatment required lifelong use, failed treatment response, the optimal time to start treatment and switching regimens 14. Therefore, management of patients HIV/AIDS are required to reduce the negative impact and improved impact the efficacy of highly active antiretroviral treatments

Numerous herbal medicines are being used in combination with ARV treatment to improve the efficacy of HIV/AIDS therapy. The metabolite compounds of

medicinal plants have been the most successful source of potential traditional medicine [15-21] and also can be used as an alternative for an interaction with antiretroviral therapy in patients HIV/AIDS [22]. Larson *et al.* [23] reported that the combination medicinal plant extracts from Papua New Guinea with ARV exhibited alter the concentration of antiretroviral drugs in the body and their efficacy. Another literature showed that medicinal plant extracts were considered as an excellent source for management of HIV/AID patients [24].

The red fruits (*Pandanus conoideus*), namely Buah Merah in Indonesia, is a plant in the family Pandanaceae [25]. It is an endemic plant that easily found in Papua regions of Indonesia and has been used as a traditional herb Papua community to treat cancer, rheumatoid arthritis, stroke, and HIV/AIDS [26]. Some other pharmacological activities that have been reported in recent years include its anti-inflammation and anticancer [27, 28].

In the current work was investigated the clinical efficacy of red fruits (*Pandanus conoideus*) oil extract in HIV infected patients with taking antiretroviral therapy. This working point to the potential of developing red fruits oil extracts for management HIV infected patients.

MATERIALS AND METHOD

Study Location and Design

Recruitment and drug administration took place in Merauke Hospital, Merauke Regency of Papua Province, Indonesia. Laboratory tests were performed at a Molecular Biology and Immunology Laboratory for Infectious Diseases, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

In this work, ethical clearance was obtained from Hasanuddin University of Medicine Faculty Research Ethics Committee (532/H4.8.4.5.31/PP36-KOMETIK/2017) before data collection commenced. The study was conducted in randomized controlled groups. Patients qualified for inclusion in the study were ambulatory, with the base CD4 line was between 200-349 cells/μL, the body weight of 45-70 kg and treated with ARV. Excluded were pregnant women and kidney failure, body weight under than 45 kg and no treated ARV in three months.

Study Medication and Monitoring

Seventy HIV-infected patients of either gender aged between 17 and 45 years were selected in this study. The patients were divided into the ARV (antiretroviral) control (n = 35) and the antiretroviral + red fruit capsule (ARV+RFC)-treated (n = 35) groups. RFC gelatin (1000 mg red fruit extract per capsule) was administered to the patients. The dosage was 1000 mg (one capsule) daily for two months. Monitoring evaluations of the laboratory were performed at baseline (0 months) and two months during the study.

$CD4^+$ Cells Count

The efficacy determinations were the change from baseline (0 months) to month 2 in the $CD4^+$ cell count. $CD4^+$ cell counts were determined using Human CD4 ELISA Kit (Catalog No. LS-F11086, LSBio, LifeSpan BioSciences, Inc.).

Statistical Analysis

All data of results were analyzed by SPSS statistical software 16.0 (SPSS Inc., Chicago II, USA) and comparison ANOVA was used with considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

A total of seventy HIV-infected patients participated in the research and were completed $CD4^+$ cell counts at baseline and final time points. There was a gradual increase in the mean $CD4^+$ cell counts from 71.78 (11.74-164.12) cells/μL at baseline to 123.62 (28.49-217.61) cells/μL after two months treatment of RFC (Figure 1). In ARV group showed that a lower increase in the mean $CD4^+$ cell counts from 83.90 (16.43-153.87) cells/μL at baseline to 96.15 (18.24-222.45) cells/μL after two months therapy. A significant increase ($P<0.05$) in the mean $CD4^+$ cell counts was observed in patients treated with ARV+RFC for two months (52.16%) when compared to control ARV (12.24%). These results indicated that the application of ARV+RFC could be an enhancement of the immune status of the patients compared with ARV only. Thus, red fruit has potential as an adjuvant in the management of HIV patients. This study can be completed information on previous research in the application of red fruit oils extract for improved immune systems [28]. This research evaluated the efficacy of red fruit oils extracts in HIV-infected patients. The efficacy effect of red fruit oils extract was probably derived from its bioactive compounds, one of which is carotenoid[25]. Carotenoid has been widely studied and

has been shown to possess anti-cancer [29], antioxidant [30], as well as immunomodulatory capacity [31]. As an immunomodulator, carotenoid has been shown enhance lymphocyte blastogenesis, increase the population of specific lymphocyte subsets, increase lymphocyte cytotoxic activity, and stimulate the production of various cytokines [32].

Results showed that $CD4^+$ cell counts in ARV+RCV treatment were significantly increased in HIV-infected patients as compared to ARV controls (123.62 cells/µL vs. 83.90 cells/µL, respectively). Results indicated that red fruits extract oils functional as immunostimulants; because the $CD4^+$ cell counts reflect the immunologic status of HIV-infected patients [33, 34]. Thus it is possible that the functional interaction of red fruit extract oils with antiretroviral therapy as an immunostimulant in HIV-infected patients occur at the level metabolism through enzyme induction or inhibition [23]. Another possible mechanism that metabolite constituents of red fruits extract oils remedies may affect ARV metabolism as a result of their efflux drug transporter systems [35, 36].

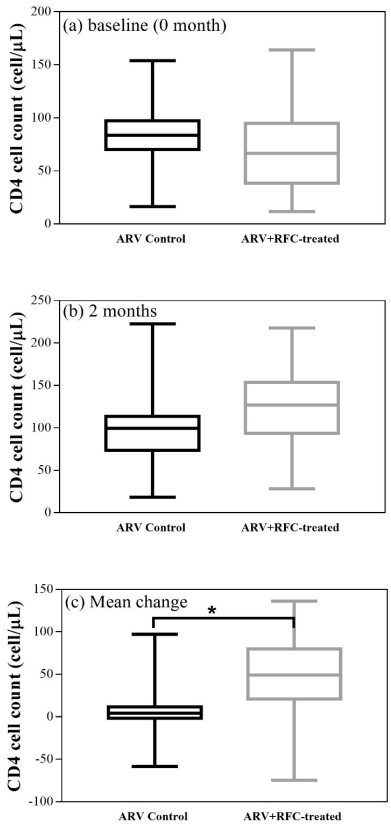


Figure 1 CD4 cell count response of ARV (antiretroviral) control and ARV+RFC (red fruit capsule)- treated in the baseline (a), two months (b), and mean change (c). *p<0.05 vs. ARV control.

CONCLUSION

In conclusion, our study shows that the increased level of $CD4^+$ cells count by ARV+RFC was provide positive benefits in HIV/AIDS therapy.

Conflict of Interest - We declare that we have no conflict of interest.

Ethical Clearance – Taken from Hasanuddin University of Medicine Faculty Research Ethics Committee (532/H4.8.4.5.31/PP36-KOMETIK/2017).

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REFERENCES

1. Kretova OV, Fedoseeva DM, Gorbacheva MA, Gashnikova NM, Gashnikova MP, Melnikova NV, et al. Six Highly Conserved Targets of RNAi Revealed in HIV-1-Infected Patients from Russia Are Also Present in Many HIV-1 Strains Worldwide. Molecular Therapy - Nucleic Acids. 2017;8:330-44.
2. Zaidane I, Wakrim L, Oulad Lahsen A, Bensghir R, Chihab H, Jadid FZ, et al. Interleukin 28B rs12979860 genotype and Human Immunodeficiency Virus type 1: Susceptibility, AIDS development and therapeutic outcome. Hum Immunol. 2018;79(1):70-5.
3. Pendse R, Gupta S, Yu D, Sarkar S. HIV/AIDS in the South-East Asia region: progress and challenges. J Virus Erad. 2016;2(Supplement 4):1-6.
4. Tambaip T, Karo MB, Hatta M, Natzir R, Islam AA. Trends in HIV/AIDS epidemics in Merauke-Papua, Indonesia, from 1992-2017. Asian Journal of Epidemiology. 2017;10(2):76-82.
5. Wijayanti F, Tarmizi SN, Tobing V, Nisa T, Akhtar M, Trihandini I, et al. From the Millennium Development Goals to Sustainable Development Goals.: The response to the HIV epidemic in Indonesia: challenges and opportunities. J Virus Erad. 2016;2(Suppl 4):27.
6. Roncero C, Fuster D, Palma-Álvarez RF, Rodriguez-Cintas L, Martinez-Luna N, Álvarez FJ. HIV And HCV infection among opiate-

dependent patients and methadone doses: the PROTEUS study. AIDS Care. 2017;29(12):1551-6.

7. Van Epps P, Kalayjian RC. Human Immunodeficiency Virus and Aging in the Era of Effective Antiretroviral Therapy. Infect Dis Clin North Am. 2017;31(4):791-810.
8. Crowell CS, Maiga AI, Sylla M, Taiwo B, Kone N, Oron AP, et al. High Rates of Baseline Drug Resistance and Virologic Failure Among ART-naive HIV-infected Children in Mali. Pediatr Infect Dis J. 2017;36(11):e258-e63.
9. Meloni ST, Chaplin B, Idoko J, Agbaji O, Akanmu S, Imade G, et al. Drug resistance patterns following pharmacy stock shortage in Nigerian Antiretroviral Treatment Program. AIDS Res Ther. 2017;14(1):58.
10. Tsai H-C, Chen I-T, Wu K-S, Tseng Y-T, Sy C-L, Chen J-K, et al. High rate of HIV-1 drug resistance in treatment failure patients in Taiwan, 2009–2014. Infect Drug Resist. 2017;10:343-52.
11. Hardy H, Esch LD, Morse GD. Glucose disorders associated with HIV and its drug therapy. Ann Pharmacother. 2001;35(3):343-51.
12. Chastain DB, Franco-Paredes C, Stover KR. Addressing Antiretroviral Therapy-Associated Drug-Drug Interactions in Patients Requiring Treatment for Opportunistic Infections in Low-Income and Resource-Limited Settings. J Clin Pharmacol. 2017;57(11):1387-99.
13. Dresser GK, Spence JD, Bailey DG. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clin Pharmacokinet. 2000;38(1):41-57.
14. Misgena DK. The pattern of immunologic and virologic responses to highly active antiretroviral treatment (HAART): Does success bring further challenges? Ethiop J Health Dev. 2011;25(1):61-70.
15. Karo MB, Hatta M, Patellongi I, Natzir R, Tambaip T. IgM antibody and colony fungal load impacts of orally administered ethanol extract of plectranthus scutellarioides on mice with systemic candidiasis. J Pharm Pharmacogn Res. 2018;6(1):27-34.
16. Made Artika I, Khasanah U, Bintang M, Nurcholis W. Extraction of total flavonoid contents and antibacterial activities from curcuma aeruginosa RoxB. rhizome using two level half factorial design. Der Pharma Chemica. 2016;8(21):35-9.
17. Nurcholis W, Ambarsari L, Purwakusumah ED. Curcumin analysis and cytotoxic activities of some *Curcuma xanthorrhiza* Roxb. accessions. Int J PharmTech Res. 2016;9:175-80.
18. Nurcholis W, Khumaida N, Syukur M, Bintang M. Variability of total phenolic and flavonoid content and antioxidant activity among 20 *Curcuma aeruginosa* Roxb. accessions of Indonesia. Asian J Biochem. 2016;11:142-8.
19. Nurcholis W, Khumaida N, Syukur M, Bintang M. Variability of curcuminoid content and lack of correlation with cytotoxicity in ethanolic extracts from 20 accessions of *Curcuma aeruginosa* RoxB. Asian Pac J Trop Dis. 2016;6(11):887-91.
20. Nurcholis W, Khumaida N, Syukur M, Bintang M, Ardyani IDAAC. Phytochemical screening, antioxidant and cytotoxic activities in extracts of different rhizome parts from *Curcuma aeruginosa* Roxb. Int J Res Ayurveda Pharm. 2015;6(5):634-7.
21. Nurcholis W, Munshif AA, Ambarsari L. Xanthorrhizol contents, α-glucosidase inhibition, and cytotoxic activities in ethyl acetate fraction of Curcuma zanthorrhiza accessions from Indonesia. Rev Bras Farmacogn. 2018;28(1):44-9.
22. Hernández MV, Gómez MFH, Blanco JR. The influence of alternative medicine in highly active antiretroviral treatment. Farmacia Hospitalaria. 2009;33(1):31-6.
23. Larson EC, Hathaway LB, Lamb JG, Pond CD, Rai PP, Matainaho TK, et al. Interactions of Papua New Guinea medicinal plant extracts with antiretroviral therapy. J Ethnopharmacol. 2014;155(3):1433-40.
24. Gambari R, Lampronti I. Inhibition of immunodeficiency type-1 virus (HIV-1) life cycle by medicinal plant extracts and plant-derived compounds. In: Khan MTH, Ather A, editors. Advances in Phytomedicine. 2: Elsevier; 2006. p. 299-311.
25. Sarungallo ZL, Hariyadi P, Andarwulan N, Purnomo EH, Wada M. Analysis of

α-Cryptoxanthin, β-Cryptoxanthin, α -Carotene, and β-Carotene of Pandanus Conoideus Oil by High-performance Liquid Chromatography (HPLC). Procedia Food Science. 2015;3:231-43.

26. Wismandanu O, Maulidya I, Indariani S, Batubara I. Acute toxicity of red fruits (Pandanus conoideus Lamk) oil and the hepatic enzyme level in rat. J Phytopharmcol. 2016;5(5):176-8.
27. Achadiani, Sastramihardja H, Akbar IB, Hernowo BS, Faried A, Kuwano H. Buah Merah (Pandanus conoideus Lam.) from Indonesian herbal medicine induced apoptosis on human cervical cancer cell lines. Obes Res Clin Pract. 2013;7:31-2.
28. Khiong K, Adhika OA, Chakravitha M. Inhibition of NF-κB pathway as the therapeutic potential of red fruit (*Pandanus conoideus* Lam.) in the treatment of inflammatory bowel disease. Jurnal Kedokteran Maranatha. 2010;9(1):69-75.
29. Linnewiel-Hermoni K, Khanin M, Danilenko M, Zango G, Amosi Y, Levy J, et al. The anti-cancer effects of carotenoids and other phytonutrients resides in their combined activity. Arch Biochem Biophys. 2015;572:28-35.
30. Beta T, Hwang T. Influence of heat and moisture treatment on carotenoids, phenolic content, and antioxidant capacity of orange maize flour. Food Chem. 2018;246:58-64.
31. Sepp T, Karu U, Sild E, Männiste M, Hõrak P. Effects of carotenoids, immune activation and immune suppression on the intensity of chronic coccidiosis in greenfinches. Exp Parasitol. 2011;127(3):651-7.
32. Chew BP. Role of Carotenoids in the Immune Response1. J Dairy Sci. 1993;76(9):2804-11.
33. Kim MJ, Chang H-H, Kim SI, Kim YJ, Park DW, Kang C, et al. Trend of CD4+ Cell Counts at Diagnosis and Initiation of Highly Active Antiretroviral Therapy (HAART): Korea HIV/AIDS Cohort Study, 1992-2015. Infection & Chemotherapy. 2017;49(2):101-8.
34. Hatta M. Evaluation of anti-phenolic glycolipid-I IGM and CD4/CD8 t cell subsets value as high risk determination indicator for individuals residing in a leprosy endemic area in Indonesia. Med J Indonesia. 1999;8(3):160-5.
35. Mills E, Cooper C, Seely D, Kanfer I. African herbal medicines in the treatment of HIV: Hypoxis and Sutherlandia. An overview of evidence and pharmacology. Nutrition Journal. 2005;4:19.
36. Tamuno I. Traditional medicine for HIV infected patients in antiretroviral therapy in a tertiary hospital in Kano, Northwest Nigeria. Asian Pac J Trop Med. 2011;4(2):152-5.

Effect of Iron Overload on Some Physiological and Biochemical Variables in Immature Female Rats

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ABSTRACT

The current study was conducted to investigate the effect of (100 mg / kg b.w) on the weight of some organs (liver, heart, spleen, kidneys, ovaries, and uterus) and some physiological and biochemical parameters in immature female rats at the weaning age (22) days, the experimental animals were divided into two groups, the first group included (10) females were gave the solution of iron sulfate daily by mouth for 14 days, and the second group included (10) females were treated naturally and considered as a control group. The results of the iron treatment showed a significant increase ($P \leq 0.05$) in weight of the liver and spleen, and a significant decrease in body weight, while no significant difference was observed in the weight of the heart, kidney, ovaries and uterus. While in the physiological and biochemical parameters studied, the results showed a significant increase in the level of malondialdehyde, the concentration of hemoglobin (Hb), packed cell volume (PCV), and iron level, with a significant decrease in the level of glutathione compared to control group. It is concluded that iron has harmful effects on studied parameters and organs, indicating that iron is stressful and has a negative effect if it increases over the normal level.

Keywords: *iron overload, physiological criteria, immature female rats, oxidative stress, body weight.*

INTRODUCTION

Pollution is a common problem at present, and the world around us is saturated with polluting substances, such as those used in the environment by humans as well as many naturally occurring substances such as vapors rising from active volcanoes, similar to pollution from raw materials, factories, human and sewage products. [1] The evolution of the world and the geological changes in the earth crust have led to changes in the distribution of essential soil elements such as iron, zinc, copper, selenium, molybdenum, manganese, chromium, cobalt and iodine. [2] These elements are widely dispersed in the environment either through their natural presence or as industrial wastes. Human and animal species are exposed to these minerals from various sources, including air, soil, water and contaminated food. [3] Exposure to these minerals causes many functional disorders, including loss of fertility for many reasons; one of it is the increase of iron ratio in the body [iron overload) or iron deficiency. [4] Transitional metals (such as iron) are catalysts in the oxidation reactions in living organisms. Therefore; the damage caused by these minerals appears in the tissues as oxidative damage. The most affected organs are the liver, kidneys ,and testicles, these effects include dehydration, low body weight, loss of appetite, renal tubules necrosis and pulmonary hemorrhage. [5]

Excessive iron administration increases the amount of free iron that collected in the body and then participates in the Finton reaction, leading to the generation of active oxygen species, causing damage to the cells and tissues of the body as well as attacking the embryos in the of pregnancy and causing severe oxidative damage. The oxidative damage caused by free radicals due to excessive iron inside the body, is a clear indication of the body's access to the state of poisoning that has a negative impact on human health. [6]

The current study aims to

1- Investigation the effect of iron on some blood variables by measuring the: level of hemoglobin (Hb), the packed cell volume of blood (PCV), the level of iron blood serum.

2 - Detection of the negative effects caused by

the free radicals stimulated the iron formation in the immature female rats.

MATERIALS AND METHOD

In this study, 20 immature female albino rats were used at the age of 22 days. Their weights ranged from (30 - 45 g). They were raised in the animal house in the college of the Veterinary Medicine / University of Tikrit. The animals were subjected to laboratory conditions represented by temperature (5 ± 25 ° C), with light cycle (10) hours and (14) dark hours. The animals were kept in plastic hutch with a diameter (20 x 25 x 20 cm) and then divided into two groups, each group included (10) animals. The first group was treated with (100 mg / kg b.w) of ferrous sulphate solution in a daily dose for 15 days orally through tubular feeding, while the other group considered as a control group, and then the animals were killed at the age of 37 days.

The Weight of internal viscera and genitals

Animals were anesthetized using a special glass vessel containing ether-saturated cotton. The weight of the animals was recorded prior to the killing. The abdominal cavity was opened with forceps and scissors, the opening continued until the thoracic cavity reached the internal organs quickly. The internal organs, which included the liver, heart, spleen and kidneys, ovaries (right and left), and uterine, were extracted. After cleaning these organs from the surrounding tissue and by using a sensitive electrical balance, the weight of these organs was weighed.

The variable studied:

Determination the level of glutathione in the blood serum

The level of glutathione was estimated using the modified method used by **Rifai (2018)**. [7]

Determination the level of malondialdehyde in the blood serum

The serum lipid peroxidation level was assessed by measuring the level of malondialdehyde (MDA) as a final product of over-oxidation fat. [8]

Determination packed cell volume of the blood (PCV)

PCV was estimated according to **Guyton, (2006)** [9] , where the glass tube containing heparin was used by performing a minor vein wound in the internal eye organization and before filling the capillary tube with blood, blocked from the other end by the artificial clay, and then placed directly in the microhematocrit centrifuge for 5 minutes at 12000 xg. The results were then read on the microhematocrit reader.

Determination the concentration of hemoglobin (Hb)

Hemoglobin concentration was estimated by using the cyanomethemoglobin method, where the special kit was used to estimate Hb, from SYRBIO company, (French).

Determination of iron level in the blood serum

The iron level in blood serum was estimated by using the ready measurement kit from SYRBIO (French), and then the light absorption of the complex color produced was reading using the spectrophotometer at the wavelength 535 nm.

STATISTICAL ANALYSIS

The data were analyzed statistically by Sigma Stat program. One way analysis of variance was used, and the differences between variables were determined using: Duncan multiple range test.

THE RESULTS

Table (1) shows the effect of oral iron sulfate on body weight, heart, kidney, spleen, liver, ovaries, and uterus (mg)in female immature rats.

variables	Mean ± standard error	
	Control group	Iron treatment group
Body weight (gm)	97.64 ± 3.37 B	67.43 ± 2.71 a
Liver weight (mg)	347.67 ± 142.81 B	4632.17 ± 174.42 a
Spleen weight (mg)	698.83 ± 15.64 B	867.33 ± 12.78 a
Heart weight (mg)	496.83 ± 23.84 A	511.33 ± 22.91 a
Kidney weight (mg)	413.33 ± 9.82 A	423.50 ± 8.38 a
Ovary weight (mg)	12.16 ± 0.61 A	10.17 ± 0.61 a
Uterine weight (mg)	44.17 ± 1.92 A	45.83 ± 1.51 a

Horizontal different letters mean that there is a significant difference between groups at a significant level (P <0.05).

Number of animals in each group = 10

Table (2) shows the effect of oral iron therapy on the level of malondialdehyde, hemoglobin concentration, packed cell volume (PCV), level of iron and glutathione in immature adult rats.

variables	Mean ± standard error	
	Control group	Iron treatment group
Serum glutathione level (µmol/l)	5.12 ± 0.08 a	3.14 ± 0.13 b
MDA level (µmol/l)	0.32 ± 0.01 B	0.97 ± 0.02 a
Hb (gm/100ml)	11.12 ± 0.15 B	14.23 ± 0.17 a
PCV (%)	39.72 ± 0.54 B	44.22 ± 1.49 a
serum iron level (µgm/ml)	175.01 ± 2.24 B	242.84 ± 3.38 a

Horizontal different letters mean that there is a significant difference between groups at a significant level (P <0.05).

Number of animals in each group = 10

DISCUSSION

The current study showed that given the iron at dose (100 mg/ kg of body weight) caused a significant decrease in body weight, and this result came correspondent with the findings of **Han (2011)** [10] who pointed out that rats exposed to iron overload are susceptible to anorexia, which may be due to the irregular physiological functions of the body as well as the effect of iron overload on the absorption of food from the intestines. The result was identical to that indicated by **Garibay (2011)** [11] who observed a significant decrease in body weight in iron-treated animals excessively compared to control group, this was attributed to the fact that giving high doses of iron causes loss of appetite and then to the weight loss.

The results of this study showed a significant increase in the weight of the liver and spleen, this result was agreed to that of **Sharaf,.(2002)** [12] which indicated a direct relationship between the weight of the liver and spleen on the one hand and dietary iron content on the other hand; as the liver and spleen are the most organs affected by the quantity of Iron in the body and this may explain the moral increase in the weight of these two organs due to the iron overload in experimental animals. However, this result was contrary to the results of by **Eybl, (2011)**. [13] which did not record a relationship between the weight of the liver and spleen and the body iron level on the other hand, this result was consistent with that indicated by Stal, (1999) [14] that the rats treated with heavy metals suffered from a rise in the weight of the liver and spleen and he attributed that to the decrease of liver glutathione level; due to the lack of antioxidant enzymes in it and then the occurrence of oxidative damage, and functional disorder of the liver, and may be due to what pointed out by **Stohs, (1999)** [15] that the site of the main collection of iron in the body is the liver and the spleen, and the iron is responsible for cases of oxidative damage in each, this is confirmed by studies that patients exposed to iron overload suffering from hepatic cell damage. [16]

The significant increase in the weight of liver and spleen in the iron treatment group was corresponding with the findings of **Karaca. (2010)** [17], which indicated that this increase may be due to increased inflammatory processes in those organs as iron is an oxidative component and catalyst of this inflammatory processes at the same time, and that the liver and spleen are one of the main centers of the presence of iron in the body so the cause of iron overload doubling inflammatory processes, which ends with an increase in weight in these two organs. The small age of animals in this group may be helpful in increasing the inflammatory processes that occur within the body due to the high level of free radicals and the consequent increase in the rates of oxidative damage; due to the low efficiency of anti-

oxidant systems.

Treatment with iron showed no significant difference in heart, kidney, ovary, and uterine weight compared with the control group. This result was agreed with **Chandra, (2011)**. [18], which indicated that the exposure of immature rats to heavy metals did not cause a significant increase in heart, kidneys, testis weight, and subsequent sexual glands and growth in pre-puberty age are affected by the secretion of sex hormones, which begin to secrete at puberty. Sex hormones work on the retention of nitrogen, which is involved in the manufacture of amino acids and proteins, so no significant difference in weight at these ages. It may also be due to the shortening of the period of the dosage which is (14) days so that the iron cannot show the negative effects during this period to those values [17] [5]. The treatment with iron showed a significant decrease in the level of glutathione in the blood serum. The result was consistent with 14-**Stal, (1999)** [14], which observed" a decrease in the level of glutathione in rats treated with heavy metal"; as a result of the lack of antioxidant enzymes. The synthesis of active oxygen species by non-enzymatic reactions of iron with the thiol groups in plasma leads to a reduction in the ability of total antioxidants (catalase and superoxide dismutase) in plasma, as well as the treatment with iron significantly increased the level of malondialdehyde, and this result was consistent with **Girotti, (1994).** [19], which indicated "that the treatment of rats with heavy metals led to an increase in lipid peroxidation and a decrease in antioxidant enzymes". As the cellular membranes are rich in unsaturated fats, which is a major target for effective oxygen varieties, in addition, effective oxygen varieties are responsible for breaking down most of the main proteins involved in cell formation as well as reducing the levels of antioxidants.

Iron ion has the potential to facilitate the modification of hydrogen peroxide (H_2O_2) to the hydroxyl root through Fenton reaction, which is believed to be an important initiator for lipid peroxidation [20], the increase in the level of malondialdehyde is an important indicator of the increase in free radical which causes increase in the lipid peroxidation, which is the final product of it is the malondialdehyde. [21]

The results obtained showed a significant increase in the level of hemoglobin (Hb), the packed cell of blood (PCV), and the level of iron blood serum. This result was agreed with the findings of **Rincker, (2012)**. [22]," which confirmed a significant increase in hemoglobin in the case of continuous exposure to nutritional iron, which stimulates the synthesis of Hb, also he indicated" that the concentration of hemoglobin was directly proportional to the PCV", also he indicated "that the increase in iron leads to a decrease in the concentration of serum transferrin, which coincides with the high levels of hemoglobin synthesis" this decrease in the transferrin concentration; is due to the reduction of iron transport from its storage sites to the sites of the hem synthesis, and this is why the rise of iron liver and spleen in the iron overload.

CONCLUSIONS

It is concluded that iron overload has harmful effects on studied parameters and organs, indicating that iron is stressful and has a negative effect if it increases over the normal level which produce oxidative condition .

Conflict of Interest: Nil.

Source of Funding: Self.

Ethical Clearance: We have not collected any samples from humans.

REFERENCES

1. Bahuguna, A. Testicular protein profile (SDS-PAGE) study of zinc deficient Wister albino rat. 2007, Ind J Exp Bio,46:27-34
2. Marta Z. Jagoda T. and Halina M. The role of metallothionein interactions with other proteins. 2014, Vol.(14) , pp: Pages 1343-1356.
3. Monisha J., Tenzin T., Naresh A., et al Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology. 2018, Vol. 7(2): 60–72
4. Valko, M.. Free radicals and antioxidants in normal physiological functions and human disease. 2007, Int. J. Biochem. Cell. Biol.,39:44–84.
5. Sreedhar, B. A protective role for zinc on intestinal peroxidative damage during oral iron repletion. (2004), Biochemical and Biophysical Research Communications, 318 : 992–997.
6. Jiancheng Yang, Gejing Zhang, Dandan Dong and, Peng Shang: Effects of Iron Overload and Oxidative Damage on the Musculoskeletal System in the Space Environment: Data from Spaceflights and Ground-Based Simulation Models. 2018, Int. J. Mol. Sci., 19, 2608

7. Nader Rifai Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2018. 6th Edition. ELSIVER. USA..
8. Wysocka, R. Metabolic control quality and free radical activity in diabetic patients. 1995. Diab. Res. Clin. Prac., 27: 193-197.
9. Guyton, A.. Textbook of Medical Physiology. 2006. 11th edi . Elsevier Inc- Philadelphya, Pennsylvania. 425-426.
10. Han, J.. H and L Ferritin subunit mRNA expression differs in brain of control and iron deficient rats. 2011,Am. J. Nutr. 132:2769-2774.
11. Garibay, E.. Iron absorption during recovery from malnutrition. 2011 J. AM. Colle. Nutr. 20(4):286-292.
12. Sharaf, K. Iron bioavailability of green leafy vegetables for anemic and healthy rats mesopotamia . 2002 J. Agric. 31(4): 155-165.
13. Stal, P. Iron increases ethanol toxicity in rat liver. 1999. J. Hepatol. 17:108-115.
14. Eybl, V. Comparative study of natural antioxidants curcumin, resveratrol and melatonin in cadmium-induced oxidative damage in mice. 2011. Toxicology. 225:150-156.
15. Stohs, S. Oxidative mechanisms in the toxicity of metal ions. 1999. Free Radical Biol Med, 18(2): 321-336.
16. Bodiga, S. Concurrent repletion of iron and zinc reduces intestinal oxidative damage in iron- and zinc-deficient rats. 2012,World J.Gastroentero .13(43): 5707-5717
17. Karaca, K. . Pubertal arrest due to Zn deficiency, The effect of zinc supplementation Hormones. 2010,6(1):71-74
18. Chandra, A. Effect of curcumin on chromium-induced oxidative damage in male reproductive system. 2011, Environ. Toxicol. And Pharmacol. 24:160-166.
19. Girotti, A. Damaging effects of oxygen radicals on released erythrocyte ghosts. 1994, J. Biol.Chem., 259: 1744-1752.
20. Nielson, F. Plasma Malondialdehyde as biomarker for oxidative stress : refrence interval and effects of life-stylefactors. 2007, Clin. Chem., 43(7): 1209-1214.
21. Amirkhizi, F. The relationship of body iron stores and oxidative stress markers related to atherosclerosis in women of reproductive age. 2010, Atherosclerosis Journal, 4(4): 159-165.
22. Rincker, M. effect of dietary iron supplementation on growth performance, hematological status and whole –body mineral concentration of nursery pigs. 2012, J. Anim. Sci., 82:3189-3197.

Factors Associated with the Occurrence of Anemia in Pregnant Women in the Work Area of Baraka Public Health Center, Enrekang District, South Sulawesi Indonesia

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ABSTRACT

Study of factors related to anemia in pregnant women in the work areas of Baraka Public Health Facility of Enrekang District aimed to know the parity, mother's age, and inter-pregnancy interval with anemia in pregnant women was conducted. The method used in this study is a laboratory observation method with a descriptive approach, where each respondent was examined for hemoglobin (Hb) levels as a dependent variable and data about independent variables. The study was conducted from June to October 2017. The results showed that the parity factor and age of pregnant women had a relationship with the incidence of anemia, where the p-value <ά (0.05), the distance of pregnancy did not have a significant relationship with the incidence of anemia, where the value of p> ά (0.05). From the results of the study concluded that the age of pregnant women is the most influential factor in the incidence of anemia in pregnant women in Baraka Public Health Center, Enrekang District.

Keywords: *age, anemia, Baraka, inter-pregnancy interval, parity, pregnant women*

INTRODUCTION

Public health development in Indonesia is mainly aiming to increase life expectancy, improve the quality of life of human resources, and improve the quality of life in order to improve family welfare so that it can deliver optimal levels of public health. Health is one of the basic human needs, so it needs to endeavor so that every citizen is increasingly aware of the importance of health for himself and his environment, and can increasingly behave good healthy life.

Undermutrition is one of the main problems of public health in Indonesia. Especially in poor pupulation[9]. Nutrition problem, especially in pregnant women, is a big problem because it is a supporting factor for anemia in pregnant women. Anemia during pregnancy is a significant health problem in order to improve public health. Regarding maternal and child health, anemia is a major nutritional problem in developing countries including Indonesia.

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Anemia is one of the factors that become an indicator of the success of a nation's health development that describes socio-economic capabilities in meeting the needs of quality and quantity of people's nutrition [1].

Anemia during pregnancy is a significant health problem to improve the degree of public health related to public health. Regarding maternal and child health, anemia is a major nutritional problem in developing countries, including Indonesia.

Data from the World Health Organization (WHO) in 2010 showed that anemia in pregnant women ranged from 20-85% with hemoglobin levels (Hb ≤ 11 g/dL). In developing countries including Indonesia, the infant mortality rate and the percentage of anemia during pregnancy are the most sensitive indicators to describe health status, especially maternal health in infants. The Indonesian Health Demographic Survey (SKDI) in 2012 showed that maternal mortality (MMR) in Indonesia was 228/100,000 live births. The causes of maternal deaths due to bleeding were 29.67%, infections 5.51%,

abortion 1.77%, prolonged labor 1.65%, and others 34.35%. Maternal mortality is one indicator of the success of health services in a country. The direct cause of maternal death is bleeding, eclampsia and indirect causes are anemia [4].

Pregnancy is a natural process and not a pathological process. Pregnancy usually develops and results in the birth of a term healthy baby through the birth canal, but this is not always as expected, normal conditions can be pathological/abnormal. From the medical record data of Baraka Health Center in 2015 showed the number of pregnant women who had their pregnancies examined in 2015 as many as 196 people with some cases of anemia as many as 72 pregnant women. Nowadays, researchers interested to do research to determine factors related to the incidence of anemia in pregnant women in the working area of Baraka District Enrekang Health Center. The factors studied included: parity, age, and inter-pregnancy interval [2].

Anemia is a decrease in the number of red blood cells or the concentration of hemoglobin in the blood circulation. Causes of anemia include malnutrition, lack of iron, malabsorption, massive blood loss such as labor, abnormal menstruation, chronic infectious diseases such as pulmonary tuberculosis, intestinal worms, malaria, and others. According to World Health Organization, anemia in pregnant women is classified based on Hb levels of pregnant women into three categories as follows: normal (Hb ≥ 11 g/dL), mild anemia (Hb 8-10 g/dL), and severe anemia severe (Hb <8.0 g/dL) [10].

MATERIAL AND METHOD

The type of research used in this study was observational with a descriptive cross-sectional study approach. Pregnant women who examined themselves at the Baraka Public Health Center in Enrekang District included in this study were examined their hemoglobin (Hb) levels. Data on dependent and independent variables are collected simultaneously, to obtain information about the relationship of several factors to the incidence of anemia in pregnant women in the working area of the Baraka Public Health Center in Enrekang District.

This study was carried out from June to October 2017. The populations in this study were pregnant women who were in the working area of the Baraka Health Center in Enrekang Regency who came to have their pregnancies examined during the study period. The samples in this study were pregnant women who had their pregnancies examined at Baraka Health Center in Enrekang Regency. The sample size in this study was 65 people. The sampling technique in this study is the total sampling technique.

The independent variables in this study were factors related to the incidence of anemia in pregnant women: parity, the age of pregnant women, and inter-pregnancy interval. The dependent variable in this study was anemia in pregnant women.

The data obtained in this study include primary data, namely hemoglobin levels examined directly in pregnant women with the Sahli method. Also, other primary data obtained in this research is filling in questionnaires distributed to pregnant women to identify data relating to knowledge, education, age, parity, and inter-pregnancy interval. Other than that, secondary data also obtained including a profile of the Department of Health of Enrekang District and the medical record of the patients from Baraka Public Health Center in Enrekang District.

Materials and tools used in this study are 3 mL syringe, tourniquet, cotton, lancet, a reaction tube, 20 µl micropipette, Sahli hemometer, alcohol 70%, and HCl 0.1 N solution.

DATA ANALYSIS

The analysis used in this study is regression analysis which aims to determine the relationship of each independent variable to the dependent variable. Regression analysis also used to know which variable, from the three independent variables, is the most dominant relationship with the incidence of anemia in pregnant women.

FINDING

Hemoglobin level of pregnant women in the working area of Baraka Public Health Center displayed in Table 1. Twenty-six of respondents (40%) suffered from anemia based on their hemoglobin level while the other 39 pregnant women (60%) not. Table 2 displayed that as much as 84.6 % (55 pregnant women) are low-risk parity while 15.4 others are high-risk parity.

Table 1 Distribution of Respondents Based on Occurrence of Anemia in Pregnant Women in Baraka Health Center in Enrekang District in 2017

Hemoglobin level	(n)	(%)
Anemia	26	40
Normal	39	60
Total	65	100

Source: Primary Data 2017

Table 2 Distribution of pregnant women who examined their pregnancies according to parity at the Baraka Public Health Center in Enrekang District in 2017

Parity	(n)	(%)
High Risk	10	15.4
Low Risk	55	84.6
Total	65	100

Source: Primary Data 2017

Table 3 Distribution of pregnant women who check their pregnancies according to the age of pregnant women at the Baraka Health Center in Enrekang Regency in 2017

Age	(n)	(%)
High Risk	11	16,9
Low Risk	54	83,1
Total	65	100

Source: Primary Data 2017

Based on the age, it is shown in Table 3 that 11 of respondents (16.9%) are high risk, while the majority of them (83.1% or 54 people) are low risk. It is displayed in Table 4 that all (100%) of women included in this study were at low risk based on the inter-pregnancy interval.

Table 4 Distribution of pregnant women who check their pregnancies according to the inter-pregnancy interval at the Baraka Public Health Center, Enrekang District in 2017

Inter-pregnancy interval	(n)	(%)
High Risk	0	0
Low Risk	65	100
Total	65	100

Source: Primary Data 2017

DISCUSSION

Anemia in pregnancy is a condition where the hemoglobin (Hb) level is lower than the standard value which is less than 11.0 g/dL. Pregnant women whose hemoglobin levels 8.1-10 g/dL, 6.5-8 g/dL, and < 6.5 g/dL were classified as suffering mild, moderate, and severe anemia, respectively (Tabrizi and Barjasteh 2015). Anemia in Indonesia is still considered a public health problem since its high prevalence, especially for pregnant women. Anemia in adults can cause fatigue and weakness which resulting in lowering productivity and the declining of work capacity[3].

Anemia can be caused by malnutrition, lack of iron, malabsorption, massive blood loss (such as labor and abnormal menstruation), and chronic infectious diseases (including pulmonary tuberculosis, intestinal worms, malaria, and others)[6][7].

Parity is the number of deliveries performed or experienced by a woman with live births or stillbirths. The number of births can affect the body's metabolic process. The present data of prevalence of anemia in pregnant women (40%) was higher compared to national health survey conducted by Ministry of Health [5] .

The analysis using chi-squared with ά 0.05 showed that that there is strong association between parity and the incidence of anemia among pregnant women in Baraka Public Health Center of Enrekang District (p-*value* = 0.000). The result means that high risk parity (or pregnancy more than 3 times) has a higher chance of anemia compared to low risk parity. High risk parity in pregnant women usually relates to the

level of knowledge that the mother lacks in the impact of the number of pregnancies. There is still lack of dissemination of information through counseling about the normal number of pregnancies by every woman.

Age is one factor that cannot be changed. Epidemiological attention is made to certain age groups because it has its own important problems. Age grouping is usually based on the presence of a problem and is specifically associated with other health factors. The age referred to in this study is the age of pregnant women with high risk i.e. less than 20 years and more than 35 years. Mother's age is very influential on the development of reproductive organs. This relates to the physiological state of the mother's body in accepting presence and supporting the development of the fetus. A woman entering the age of marriage will experience a certain phase in her life where healthy reproductive age is between 20-35 years. There was a significant relationship (p *value*= 0.000) between the age of pregnant women with the incidence of anemia among pregnant women in Baraka Public Health Center of Enrekang District based on chi-squared test.

Inter-pregnancy interval is the interval between two consecutive births of a woman. Setting the interval between pregnancies has an impact on the health of the mother and her baby, the inter-pregnancy interval that is too tight (<2 years) is more risky than the longer inter-pregnancy interval (> 2 years). This is because the anatomy of a woman needs time to restore her health and adequate nutritional intake. Based on this research, there is no association between interpregnancy interval and the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District based on chi-square test (p-*value* = 0.40).

CONCLUSION

there is a significant association between parity and the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District

there is significant association between the age of pregnant women and the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District

there is no association between the inter-pregnancy interval with the incidence of anemia in pregnant women in Baraka Public Health Center of Enrekang District

the age of pregnant women is the most factor that associated with anemia in pregnant women in Baraka Public Health Center of Enrekang District.

Conflict-Of-Interest Statement: In this study between researchers and research, subjects did not have a conflict of interest, because subjects did not have personal or informal relationships with researchers.

Source of Funding : The source of funds in this study came from the Research of Health Workforce Development of the Ministry of Health of the Republic of Indonesia.

Ethical Clearance : The ethics of this study were obtained from the Ethics Commission for Health Research, Poltekkes Kemenkes Makassar.

REFERENCES

1. Arisman. Nutrition and Life Cyle. Nutrition Research and Project Ministry of Education. Faculty of Medicine Sriwijaya Univerisity, Palembang 2010 (2). Available from: https://www.belbuk.com/buku-ajar-ilmu-gizi-gizi-dalam-daur-kehidupan-edisi-2-p-3860.html.

2. Baraka Health Centre. Baraka Health Report: Enrekang. South Sulawesi. 2015.

3. Barkley JS, Kendrick KL, Codling K, Muslimatun S, Pachon H. Anemia prevalence over time in Indonesia: estimates from the 1997, 2000, and 2008 Indonesia family life surveys. Asia Pac J Clin Nut. 2015. 24(3): 452-455.

4. BKKBN. The National Population and Family Planning Committe report. Jakarta. 2013.

5. [MoH] Ministry of Health. 2013. Basic Health Research. Research and Development Department, Ministry of Health.

6. Sabina S, Iftequar S, Zaheer Z, Khan MM, Khan S. An overview of anemia in pregnancy. Journal of Innovations in Pharmaceuticals and Biological Sciences 2015. (2): 144-151. Available From: www.jipbs.com/VolumeArticles/FullTextPDF/78_JIPBSV2I208.pdf

7. Sifakis S, Pharmakides. Anemia in pregnancy. Annals of the New York Academy of Sciences. 2000. Available from: https://nyaspubs.onlinelibrary.

wiley.com/doi/abs/10.1111/j.1749-6632.2000.tb06223.x

8. Tabrizi FM, Barjasteh S. Maternal hemoglobin levels during pregnancy and their association with birth weight of neonates. Iran J Ped Hematol Oncol. 2015. 5(4): 211-217. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26985354.

9. Usfar AA, Lebhthal E, Atmarita, Achadi E, Soekirman and Hadi H. Obesity as a poverty-related emergin nutrition problems: the case of Indonesia. National prevalence of obesity. Obesity reviews 2010. 11:924-928. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20977602

10. WHO U; UNU. Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. Geneva: World Health Organization; 2001. WHO/NHD/01.3, 2013. Available from: https://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/.

Assessment of Learning Disabilities among Deaf Adolescent

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ABSTRACT

Objective: To assess learning disabilities among deaf adolescent. To find association between sociodemographic and learning disorder of t deaf adolescent. **Methodology**: A cross sectional descriptive design was carried out through the present study in order to achieve the early stated objectives for period from February 2016 to March 2017.**Conclusion** the majority of the study sample was male. The study subjects have 12-14 for age was exceedingly and result study was residence mostly rural. Level of education for father and mother was generally primary school .The economic status was good during study, behavioral characteristics that most affect. Overall evidence of research on the prevalence of additional disabilities with deafness is very low. **Recommendation** needs to be made here is that a comprehensive research program focusing on deaf adolescent with multiple disabilities is very much needed. Data provide the basis for recommendation of a battery of tests for use by school, professional, and general psychologists in evaluating deaf adolescent

Key wards: *Assessment, Learning, Disabilities, Deaf, Adolescent.*

INTRODUCTION

Deaf and dump the term deaf is frequently applied to those who are deficient in hearing power in any degree, however slight, as well as to people who are unable to detect the loudest sounds by means of the auditory organs. It is impossible to draw a hard and fast line between the deaf and the hearing at any particular point[1].

Mentioned National Deaf Children's Society November (2012) known the incidence rates of lasting childhood hearing weakening for children in the Trent area. Study is identify 38.7% of the children were institute to have another clinical or developmental problem and half of these children had at least two additional problems. Since then there have been major developments in the technology used to assess deafness, with objective measures in place allowing for hearing testing from birth. In England, the newborn hearing screening program offers 99.9% of babies born a hearing screening test within the first few weeks of birth[2].

Deafness is either congenital or acquired. Congenital deafness arises from some natural cause which deprives the child of hearing from its birth. Acquired deafness arises from disease, accident, or other causes. The deaf are divided into two classes the totally deaf, and the partially deaf the latter being subdivided into five classes[3].

Those who perceive the human voice when it is used close to the ear, without being able, however, to distinguish the separate sounds, by those who can distinguish the vowels when they are loudly pronounced in the ear; those who understand (but with difficulty) some words known t0 them when these are clearly pronounced in their ear[4]. Without effort, understand all that is clearly pronounced in their ear; and finally those who can hear the raised voice, but not sufficiently well to follow general conversation, or to attend to what is going on in a class of hearing children. All those coming within this division are fit subjects for schools for the deaf and dumb[5].

Statement of problem:

Assessment of Learning Disabilities among Deaf Adolescent in AL-Amal Center of Al-Najaf Al- Ashraf Governorate

The study aims:

1- To assess Learning Disabilities among Deaf Adolescent.

2- To find association between sociodemographic and learning disorder of Deaf adolescent.

Methodology: A cross sectional descriptive design was carried out through the present study in order to achieve the early stated objectives for period from February 2016 to March 2017.

Design of the study: A cross sectional study descriptive design was conducted. Each individual in the sample was interviewed using a specific questionnaire form.

Setting of the study: The study was conducted at AL-Amal Center of Al- Najaf Al- Ashraf Governorate.

The sample of the study: The Simple purposeful sample of (60) Assessment of Learning Disabilities among Deaf Adolescent in AL -Amal Center.

RESULTS

Table (1): The Statistical distribution of study group by their Socio-Demographic Data

Items	Sub-groups	Study group Total = 60	
		Frequency	Percentage
Age / Years	12-14	41	**68.3**
	15-17	19	**31.7**
Gender	Male	34	**56.7**
	Female	26	43.3
Residency	Urban	22	**36.7**
	Rural	38	**63.3**
Level Education of father	Illiterate	17	**27.7**
	read and write	14	**23.3**
	Primary school	17	**28.9**
	Secondary school	7	**11.7**
	Institute or College	4	**6.7**
	Higher studies	1	**1.7**
Level Education of mother	Illiterate	11	**18.3**
	read and write	10	**16.7**
	Primary school	26	**43.3**
	Secondary school	8	**13.3**
	Institute or College	5	**8.3**
	Higher studies	0	**0.0**
Economic Status	Good	35	**58.3**
	Not good	25	**41.7**

Table (1) shows statistical distribution of patients group by their socio-demographic data, it explains that the majority of the patients subgroup are: patients with ages between (12-14) years old (68.3%), male patients (56.7%), those who live rural residents (63.3%), and finally those with good economic status (58.3%) the level of education father was Primary school (28.9) % and level of education mother was primary school was (43.3%).

Table (2) The mean of scores and assessment of behavioral characteristics for study group

Behavioral characteristics	mean of scores	Stander division	Assessment.
attention easily distracted	2.87	1.07	**Failure**
Impulsive	2.63	1.20	**Failure**
Famous to the point of folly	3.17	1.38	**Pass**
Behavior difficult to predict	3.35	1.33	Pass
cannot control himself (speaking without ear, jumping from a chair, etc.)	2.80	1.45	Failure
Intensive	2.57	1.26	**Failure**
Always rude with others	3.62	0.98	**Pass**
movement cannot stabilize	2.78	1.11	**Failure**
Very easily consulted other children	3.17	1.11	Pass
behavior often does not suit the attitude	3.38	1.02	**Pass**
Furious and irritable	2.72	1.13	**Failure**
Moody	2.88	1.20	**Failure**
Global Mean of Score	2.99	1.19	**Failure**

This table shows the mean of scores and assessment of behavioral characteristics for study group, the statistical assessment considers any item (pass) when the mean of score is higher than the cut-off point for the mean of score (cut-off point = 3) .

Table (3)The mean of scores and assessment of cognitive difficulties for study group

Cognitive difficulties	M.S.	S.D.	Assessment
Unable to remember the printed word	2.95	1.27	**Failure**
It is difficult for him to recognize letters and numbers	3.10	1.31	**Pass**
It lacks the ability to distinguish sizes	3.57	1.26	**Pass**
The ability to distinguish (right, left, up, below)	4.12	1.21	**Pass**
His ability to balance is very weak	3.82	1.01	**Pass**
Has a weak audio memory	1.85	1.30	**Failure**

Cont... **Table (3)The mean of scores and assessment of cognitive difficulties for study group**

He finds it difficult to distinguish sound stimuli	1.48	1.09	**Failure**
Its kinetic coherence is generally very weak	3.58	1.16	**Pass**
Has a weak visual memory	3.48	1.55	**Pass**
His ability to use his hands is very weak	3.87	1.13	**Pass**
Lack of skill in the performance of motor skills in general	4.03	0.87	**Pass**
He is able to listen but he does not understand what he hears	3.67	1.55	**Pass**
It lacks the ability to retrieve simple geometric shapes	3.65	1.18	**Pass**
Global Mean of Score	3.32	1.22	**Pass**

Table shows the mean of scores and assessment of cognitive difficulties for study group, the statistical assessment considers any item (pass) when the mean of score is higher than the cut-off point for the mean of score (cut-off point = 3) .

Table (4): the correlation between demographic data and behavioral characteristics for study group

Demographic items	Chi Square	Coefficient of Contingency	P value
Age	0.00	0.01	0.94
Gender	0.46	0.09	0.50
Residence	0.03	0.02	0.86
Economic Status	3.77	0.24	0.58
Level of education of father	2.19	0.19	0.70
Level of education of mother	0.00	0.01	0.94

This table shows the correlation between demographic data and behavioral characteristics for study group, it is clear from the table that there is no significant correlation; because the p-value is higher than 0.05, except for the residence which shows a significant correlation cognitive difficulties.

DISCUSSION

Discussion of study results will be presented in with assessment of learning disabilities among deaf adolescent.

In the current study show higher incidence in the age (12-14). Concerning the gender, the study results reveal that the majority of the subject are male, this study subject reveal the most of participant is male[6].

Their results indicate that the majority of subjects are rural residents rather than the

big cities. Regarding fathers and mothers education level most of participant are

Primary school. Their results indicate that the majority of study subjects are low monthly income[7].

The study result shows that the final assessment of academic difficulties and determination of cognitive patients domains are pass except the assessment of knowledge patient regarding behavioral domain is poor. While the overall assessment for the patients knowledge regarding learning difficulties is good[8].

The study results show that there is a no significant relationship between academic behavioral and cognitive difficulties and their demographical data that mean the age, gender, residency, economic status and fathers and mothers level of education do not effect on their learning difficulties[9].

Assessments should also be go together with by observations of the integrity of the implementation of the intervention, including the amount of time paid on supplemental instruction, especially if the child does not appear to be making progress. School psychologists are often well organized in this area of assessment. Although

a psychologist operating outside of a school may not be in a position to do curriculum-based assessments or to in my opinion evaluate the intervention, such assessments should be expected, especially if the referral is to a private academic therapist[10].

Impost of written expression is especially important because it is the most common type of LD in clinical children and because writing problems are more common in general population studies in school students than are reading problems[11].

CONCLUSIONS

According to the present study findings, the researcher can make the following conclusions: Most of the sample male was majority; the study subjects have 12-14 for age was exceedingly. The result study was residence mostly rural, the level of education for father and mother was generally primary school. Economic status was good during study. The behavioral characteristics that most affect. Cognitive difficulties assessment was no affect. The correlation between demographic data and behavioral no significant correlation. The overall evidence of research on the prevalence of additional disabilities with deafness is very low.

Recommendations:

1-The recommendation that needs to be made here is that a comprehensive research program focusing on deaf adolescent with multiple disabilities is very much needed.

2-These data provide the basis for recommendation of a battery of tests for use by school, professional, and general psychologists in evaluating deaf adolescent.

Declaration of Interest: Nil.

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REFERENCES

1. Zwerling C, Whitten PS, Davis CS, Sprince NL. Occupational injuries among workers with disabilities: the National Health Interview Survey, 1985-1994. Jama. 1997;278(24):2163-6.
2. Wiley S, Arjmand E, Jareenmeinzen D, Dixon M. Findings from multidisciplinary evaluation of children with permanent hearing loss. International journal of pediatric otorhinolaryngology. 2011;75(8):1040-4.
3. Cömert M, Güneş AE, Sahin F, Saydam G. Quality of life and supportive care in multiple myeloma. Turkish journal of haematology : official journal of Turkish Society of Haematology. 2013;30(3):234-46.
4. Crowe T, Jani S, Jani S, Jani N, Jani R. A pilot program in rural telepsychiatry for deaf and hard of hearing populations. Heliyon. 2016;2(3):e00077-e.
5. He P, Luo Y, Hu X, Gong R, Wen X, Zheng X. Association of socioeconomic status with hearing loss in Chinese working-aged adults: A population-based study. 2018;13(3):e0195227.
6. Mekonnen M, Hannu S, Elina L, Matti K. The Self-Concept of Deaf/Hard-of-Hearing and Hearing Students. The Journal of Deaf Studies and Deaf Education. 2016;21(4):345-51.
7. Keller BK, Morton JL, Thomas VS, Potter JF. The effect of visual and hearing impairments on functional status. Journal of the American Geriatrics Society. 1999;47(11):1319-25.
8. Grattagliano I, Ubaldi E, Bonfrate L, Portincasa P. Management of liver cirrhosis between primary care and specialists. World journal of gastroenterology. 2011;17(18):2273-82.
9. Fletcher JM, Francis DJ, Morris RD, Lyon GR. Evidence-based assessment of learning disabilities in children and adolescents. Journal of clinical child and adolescent psychology : the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53. 2005;34(3):506-22.
10. Mayes SD, Calhoun SL, Lane SE. Diagnosing children's writing disabilities: different tests give different results. Perceptual and motor skills. 2005;101(1):72-8.
11. Mayes SD, Calhoun SL, Bixler EO, Vgontzas AN, Mahr F, Hillwig-Garcia J, et al. ADHD subtypes and comorbid anxiety, depression, and oppositional-defiant disorder: differences in sleep problems. Journal of pediatric psychology. 2009;34(3): 328-37.

Evaluation of Drug Management Information System to Improve Quality and Users Satisfaction: Case Study at a Primary Health Center

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ABSTRACT

Drug and supplies management plays an important role in health systems. A web-based drug management information system, *Sistem Informasi Pengelolaan Obat* (SIPO) was developed to support interaction and communication among the internal entities of a district health office (DHO), including primary health centers (PHC). Using a qualitative approach, the objective of this descriptive study is to assess SIPO's performance in terms of success in addressing problems related to drug and medical supplies management. The results indicate that SIPO has a positive impact for both PHCs and the DHO. The result found that the users are satisfied with the system. One element that needs to be improved is integration ability; currently, SIPO cannot connect to other health-related information systems. Generally, SIPO is considered to have a positive impact, both individually and on the organization.

Key words: *health information systems, supply chain management, medicine*

INTRODUCTION

Information technology has a positive impact on healthcare[1]. Health information technology (HIT) can potentially improve healthcare quality and outcomes by innovating healthcare delivery processes, including (a) providing clinicians with timely and appropriate patient information; (b) enhancing care coordination; (c) increasing physician compliance with care guidelines; (d) facilitating clinical monitoring by means of large-scale screening and aggregation of data; (e) improving clinical workflows; (f) improving communication among clinicians, and between clinicians and patients; and (g) decreasing medication.

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One of the uses of HIT in the health sector is on supply chain management (SCM) for drug and medical supplies[2]. SCM can be defined as a system of suppliers, manufacturers, distributors, retailers, and customers, in which material typically flows downstream from suppliers to customers (except for reverse logistics), while information flows in both directions. SCM can improve efficiency and effectiveness in terms of planning, coordinating, and controlling the movement of materials[3]. In Indonesia, medicines and medical supplies are assigned particular importance in health sector management because, as valued assets with a monetary dimension, drugs and medical devices are audited periodically.

Effective and transparent tracking systems allow pharmacies to accurately record inventory components, such as medication expiration dates and physical quantities[4]. Accordingly, there has been significant research emphasis on the role of information sharing and information integration as a way of achieving better supply chain performance[5].

Drugs and medical supplies are an important component of modern healthcare systems, reducing the burden of diseases across the globe[6]. The application of SCM practices in the healthcare sector relates not only to physical goods, like drugs, pharmaceuticals, medical devices, and health aids, but also to the flow of patients [7]. In managing medicines and medical supplies, one of the key objectives is to ensure the availability and quality of drugs at every level, both in healthcare institutions and in pharmaceutical installations in regions, provinces, and centrally. In accordance with the Indonesia Presidential Regulation concerning National Health Systems, SCM must be properly designed to maintain an adequate supply of drugs; maintain the availability of drugs according to buffer stock; maintain drugs in good condition during the distribution process to ensure their quality, safety, and efficacy; minimize drug wastage due to damage or expiration; ensure the accuracy of documents and logistics information systems; provide information for estimating drug needs; and ensure that each district's drug needs are met through appropriate planning.

One entity that plays an important role in Indonesia's national health system is the Puskesmas, or primary health center (PHC), which is responsible for local delivery of public health services. In carrying out its functions, which include prevention, promotion, and basic treatment, the PHCs depend on the supply of medicines and medical supplies to ensure delivery of health services to the community. PHCs receive drugs and medical supplies from the pharmaceutical installation at the district or city level; to maintain accountability, each PHC must send monthly reports on stock and usage of these items to the pharmaceutical installation at the district health office (DHO). These reports are then used as the basis for supplies allocation for the following month.

One common difficulty in SCM is controlling the movement of drugs and medical supplies at the healthcare facility level; the inefficient and irrational use of medicines has also become a problem at all levels of the health service[4]. In the city of Depok, the health office has complained about the difficulty of promptly obtaining accurate information about stock, expenditure, and usage of drugs and other medical supplies, which has adversely impacted planning, budgeting, procurement, and distribution.

In 2014, in collaboration with the Faculty of Public Health at Universitas Indonesia, the Depok Health Office developed an information system called *Sistem Informasi Pengelolaan Obat* (Drugs and Medical Supplies Management Information System; SIPO) for managing medicines and medical supplies. Depok City's PHCs and DHO had previously used a paper-based system to record and report drug and medical supplies, which was tabulated on spreadsheets and saved as separate files. SIPO is an internet-based system that connects PHCs with the DHO. The system was developed to assist distribution of drugs and medical supplies and to automate report preparation, meaning that PHCs and health offices are no longer charged with manual reporting to the DHO. In this study, we evaluate and assess the utilization of SIPO in terms of its success as an information system.

METHOD

This is a descriptive study, which employs qualitative methods to assess the success of SIPO. We apply DeLone and McLean's model for measuring the success of an information system[8], using six aspects, each of which has its own variables. These are **system quality** (flexibility, integration, response time, error recovery, system convenience, and language), **information quality** (completeness, precision, reliability, currency, and output format), **service quality** (assurance, empathy, and responsiveness), **user satisfaction** (repeat purchases and repeat visits), **individual benefit** (usefulness, ease of use, speed of use, productivity, and effectiveness), and **organizational benefit** (organizational effectiveness, service effectiveness, contributing to achieving goals, and productivity).

There are seven informants involved in this assessment, six users from the PHCs and one from the DHO. The informants were selected purposefully for their familiarity with the system. Informants were asked to give a mark or score using ten point scale to measure users satisfaction for each variable[9]. Zero (0) is represent for very dissatisfied, five is represent for neutral, and ten (10) is represent for very satisfied. In this study, we only conducted a descriptive analysis to know how much user satisfaction regarding to the SIPO.

RESULTS AND DISCUSSION

The SIPO system was developed to aid monitoring and management of drugs across health centers

and pharmaceutical installations, and to facilitate communication relating to drug management. At the DHO level, SIPO is used for recording receipts, distribution, and monitoring of stock at health centers and in warehouses (pharmaceutical installations), and for making required reports. At PHC level, SIPO is also used to record recipe-based drug expenditures. All required reports are created automatically.

Table 1. Score Results

Aspect	Scores (n=7)
System quality	
Flexibility	7.9
Response time	6.8
Integration	0.0
Error recovery	7.8
System convenience	8.0
Language	8.3
Average	6.5
Information quality	
Completeness	7.3
Precision	7.9
Reliability	8.0
Currency	7.9
Output format	8.8
Average	8.0
Service quality	
Assurance	8.3
Empathy	7.4
Responsiveness	8.1
Average	7.9
User satisfaction	
Repeat purchases	8.0
Repeat visits	8.0
Average	8.0
Individual benefit	
Usefulness	8.3
Ease of use	8.5
Speed of use	8.4
Productivity	7.9
Effectiveness	8.5
Average	8.3
Organizational benefit	
Organizational effectiveness	8.5
Service effectiveness	8.8
Contributing to goals	8.5
Productivity	8.5
Average	8.5
Overall average	7.9

Based on these results, users feel that the quality of the SIPO system is slightly satisfy (average 6.5), except for integration. The integration indicator has the lowest score because SIPO cannot yet connect to other systems to facilitate the users' work.

On information quality, the lowest score from users is for completeness (average 8.0) SIPO does not yet facilitate the input of all information regarding drugs that expire, which is very important because drugs that expire earlier must be issued before drugs that expire later (first expired, first out) and drugs that arrive first must be removed before drugs that arrive later (first in, first out). In other words, there are several indicators that need to be added to SIPO, both for input and output.

In terms of user satisfaction, informant stated that they are satisfied with SIPO (average 8.0). Users say that they will continue to use SIPO even if it is not required, because the data and information produced helps simplify work, especially in terms of reporting.

For individual benefits, the informants consider that SIPO has a significant individual impact and supports their daily work, including improving productivity, and particularly report generation. Overall, informants at both the PHC and DHO feel that SIPO has a positive effect on their organizations, particularly on drug and medical supplies management. The DHO can control every transaction at every PHC, with manual reporting no longer required.

SIPO was developed as an electronic recording and reporting system to replace the previous paper-based approach. As changing methods to improve a system always present major challenges, it is important to assess the level of user acceptance. An information system must be useful if it is to improve performance[10].

To ensure that drugs and medical supplies are made available in a timely way at health facilities, a functioning country-wide drug supply system should be in place. Each player in the system should also be responsible and accountable for the system's operation[11]. SCM integrates all aspects of the supply chain from first supplier to end user. The main objectives of SCM are (a) overall cost reduction, (b) lead-time reduction, (c) inventory and shortfall reduction, (d) transportation cost reduction, and (e) purchase cost reduction[12].

Effective management and distribution of drugs

helps to ensure availability and assessment of future need[4]. There is extensive evidence that the use of information technology to manage work provides many advantages, including cost effectiveness, efficiency, and quality of care[13]. To achieve improved performance, the healthcare supply chain must be efficient and integrated[3]. To meet these needs, SIPO integrates the recording and reporting of drug and medical supplies at PHC and DHO levels by incorporating information technology into the business process. Both directly and indirectly, SIPO has changed the flow of the business process and bureaucratic management of drugs and medical supplies in the city of Depok.

To ensure a system's sustainability, several factors must be considered, including user motivation, leadership, and user satisfaction. Having the right user attitude and skills base—together with good leadership, an IT-friendly environment, and good communication—can have a positive influence on system adoption[14]. The Depok City Health Office stipulates that each health center must use SIPO to record and report its drugs or be denied access to drug distribution. This is an important motivator for the use of SIPO in health centers.

Leaders play an important role in information technology implementation[15]. The implementation of SIPO was strongly encouraged by leaders at both PHC and DHO levels. One important role of leadership in this context was the approval function built into the system; at the DHO, the head of the pharmaceutical installation must give online approval for any item to be distributed to health centers, and the item can only be processed if it has been approved. To address the problem of bureaucracy, the process can continue even when the leader is not present in the office, the leader still can approve through their smartphone. Approval function also can be giving to another person who responsible to this. Similarly, the head of each PHC plays a leadership role in system sustainability by means of a monthly approval action, and monthly reports are then sent automatically to the DHO.

The role of leadership in driving HIT use and innovation is informed by the technology-enabled environment principle[16]. User satisfaction is influenced by several factors, including perceived usefulness, ease of use, user expectations, user experience, user skills, user involvement in system development, organizational support, perceived attitude of top management, and user attitude to information systems[17].

Our observations indicate that SIPO can improve users' performance in drug recording and reporting. Using SIPO, reports are automatically produced each month, meaning that users no longer need to print and send hard copies to the DHO. Users at the DHO receive information directly about the number and types of drugs needed by each PHC in Depok City, making the process of distributing drugs faster and timelier. Other studies have identified a close relationship between SCM practices and organizational improvement[18,19]. Successful use of HIT can assist such organizations in leveraging resources[20].

CONCLUSIONS

Success of a system can be assessed in six main aspects: system quality, information quality, service quality, user satisfaction, impact on individuals, and impact on the organization. Generally, SIPO is considered to have had a positive impact, both individually and on the organization.

Although the SIPO system can be said to be a success, the issue of system integration remains a problem, because SIPO is still a stand-alone system. From the viewpoint of the quality of information produced by SIPO, the completeness of the indicators is also a goal for future system improvements, because there are several reports that have not yet been implemented in SIPO. Another required improvement is in the input systems.

Users report satisfaction with SIPO at both the PHC and DHO levels; they feel helped by SIPO and do not feel forced to use it, even though it is actually mandatory. Users at the PHC and DHO also assess SIPO as having a positive individual impact; however, productivity is scored lower because, even if users feel more productive using SIPO, further improvements are needed to be able to monitor drug and medical supplies.

Users at the PHC and DHO also assess SIPO as having a positive organizational impact. Drug management at the PHC and DHO has become more effective and efficient and contributes to achieving organizational goals in terms of drug supervision and monitoring.

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related to this study.

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REFERENCES

1. Buntin MMB, Burke MMF, Hoaglin MCM, Blumenthal D. The benefits of health information technology: A review of the recent literature shows predominantly positive results. Health Aff [Internet]. 2011;30(3):464–71. Available from: http://content.healthaffairs.org/content/30/3/464.short
2. Rubenfeld GD. Using computerized medical databases to measure and to improve the quality of intensive care. J Crit Care. 2004;19(4):248–56.
3. Chandra C, Kachhal S. Managing the healthcare supply chain: trends, issues and solutions from a logistics perspective. Sixt Annu Soc Heal Syst Manag Eng forum. 2004;1–9.
4. Iqbal MJ, Geer MI, Dar PA. Medicines Management in Hospitals: A Supply Chain Perspective. Syst Rev Pharm [Internet]. 2017;8(1):80–5. Available from: http://www.sysrevpharm.org/article/109
5. Goswami S, Engel T, Krcmar H. A comparative analysis of information visibility in two supply chain management information systems. J Enterp Inf Manag. 2013;26(3):276–94.
6. Kokilam MB, Joshi HG, Kamath VG. Strengthening the Pharmaceutical Supply Chain Management with Information Communication Technology Intervention. J Health Manag [Internet]. 2016;18(2):274–89. Available from: http://journals.sagepub.com/doi/10.1177/0972063416637719
7. de Vries J, Huijsman R. Supply chain management in health services: An overview. Supply Chain Manag An Int J. 2011;16(3):159–65.
8. DeLone WH, McLean E. The DeLone and McLean Model of Information Systems Success. J Manag Inf Syst. 2003;19(9):9–30.
9. Coelho PS, Esteves SP. The choice between a 5-point and a 10-point scale in the framework of customer satisfaction measurement. Int J Mark Res. 2007;49(3):313–339.
10. McDaniel L. A comparison of the impart of acceptance and support on the motivation to use information systems. Bus Renaiss Q. 2011;50–76.
11. Mkoka DA, Goicolea I, Kiwara A, Mwangu M, Hurtig AK. Availability of drugs and medical supplies for emergency obstetric care: Experience of health facility managers in a rural District of Tanzania. BMC Pregnancy Childbirth [Internet]. 2014;14(1):1–10. Available from: BMC Pregnancy and Childbirth
12. Buxmann P, Ahsen A von, Díaz LM, Wolf K. Usage and evaluation of Supply Chain Management Software – results of an empirical study in the European automotive industry. Inf Syst J [Internet]. 2004;14:295–309. Available from: http://doi.wiley.com/10.1111/j.1365-2575.2004.00172.x
13. Frimpong JA, Jackson BE, Stewart LM, Singh KP, Rivers PA, Bae S. Health information technology capacity at federally qualified health centers: a mechanism for improving quality of care. BMC Health Serv Res [Internet]. 2013;13(1):35. Available from: http://bmchealthservres.biomedcentral.com/articles/10.1186/1472-6963-13-35
14. Ahmadi M, Damanabi S, Sadoughi F. A comparative study of the proposed models for the components of the national health information system. Acta Inform Medica. 2014;22(2):115–9.
15. Goldzweig CL, Towfigh A, Maglione M, Shekelle PG. Costs and benefits of health information technology: New trends from the literature. Health Aff. 2009;28(2).
16. Brammer C, Kirtane J, Beasley C. Improving Health Through Health IT. Healthc Exec. 2012;27(5):70.
17. Adam Mahmood M, Burn JM, Gemoets LA, Jacquez C. Variables affecting information technology end-user satisfaction: a meta-analysis of the empirical literature. Int J Human-Computer Stud [Internet]. 2000;52:751–71. Available from: http://www.idealibrary.com
18. Harrison JP, McDowell GM. The role of laboratory information systems in healthcare quality improvement. Int J Health Care Qual

Assur [Internet]. 2008;21(7):679–91. Available from: http://www.emeraldinsight.com/doi/10.1108/09526860810910159

19. Mathur B, Gupta S, Meena ML, Dangayach GS. Healthcare supply chain management: literature review and some issues. J Adv Manag Res [Internet]. 2018;15(3):265–87. Available from: https://www.emeraldinsight.com/doi/10.1108/JAMR-09-2017-0090

20. Kennedy BL. Exploring the Sustainment of Health Information Technology: Successful Practices for Addressing Human Factors [Internet]. Northcentral University; 2011. Available from: http://eserv.uum.edu.my/docview/863217305?accountid=42599.

Analysis of Risk Factors for Changing Conversion of Pulmonary Tuberculosis AFB Positive Patients in the Intensive Phase, Makassar City, Indonesia

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ABSTRACT

Objective. The association of risk factors of tuberculosis has been known but still rarely performed risk factors to result of convertion among patient tuberculosis in 2 month treatment of DOTS. The study aims to know the risk factors of convertion against pulmonary tuberculosis BTA positive in Makassar.

Method. This was a case control study. Subjects of this study were pulmonary tuberculosis AFB positive patients in 2 month treatment of DOTS (intensive phase) in Labuang Baji hospital and Lung Health Center, 111 patients from December 2013 until February 2014. Cases were positive smear pulmonary tuberculosis patient who experiences no conversion at the end of the intensive phase of treatment and control were patients with positive smear pulmonary tuberculosis who did not experience conversion at the end of the intensive phase.

Result. The proportion of pulmonary tuberculosis AFB positive patients were convertion 33,3% and no convertion 66,7%. Chi square test showed statistically significant association against convertion ($p<0,05$) were education.

Conclusion. Education was important factor for changing convertion of pulmonary tuberculosis AFB patients. Health promotion program about tuberculosis should be the best solution to improve the proportion convertion among patients.

***Keywords**: Risk Factors, Convertion, pulmonary tuberculosis AFB Positive patients.*

INTRODUCTION

Tuberculosis is still a public health problem that is a global challenge. Tuberculosis is a direct infectious disease caused by *Mycobacterium tuberculosis* and can be transmitted by air. *Mycobacterium tuberculosis* when, sneezing, talking or laughing. it attacks the lungs, but can also attack other organs. This causes health problems in millions of people every year and the second rank as a cause of death in infectious diseases worldwide after HIV[1].

WHO has global target in the context of eradicating pulmonary tuberculosis in 2030. It is monitoring the progress of the pulmonary tuberculosis eradication program to ensure failure of treatment for pulmonary tuberculosis up to 85%. Based on 2009 data. Globally, the incidence of pulmonary tuberculosis reached 137 cases per 100,000 population. While the highest percentage of achievement of treatment success occurred in 2008 at 86%[5]. And in 2013 the success rate of TB treatment increased to 90.5%. In this case, eventhough the National tuberculosis control program has succeeded in achieving global targets but in 2013, Indonesia will still got the fifth rank in the country with the highest tuberculosis burden in the world, an estimated number of new cases of 410,000-520,000 tuberculosis prevalence rates of 272 per 100,000 residents while the estimated incidence is 183 per 100,000 population[2].

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A study conducted by Tierney DB, On MDR TB patients who started treatment between 1999-2002 were 87.7% experienced sputum conversion with a median conversion time of 59 days (IQR:31-92). Normally, tuberculosis patient would experience convertion after 2 month treatment (intensive phase) except for patients were not regularly to do treatment DOTS[10].

Based on the target proportion of conversions in the South Sulawesi was 85% while in the main health care services proportion of conversion were low. Labuang Baji hospital and Lung Health Center in Makassar had proportion of convertion were 12% and 15% in 2013. Due to the above data, it can be seen that the level of achievement of targets at both health services very low. So, it was needed a study to determine the risk factors for changing conversion of pulmonary tuberculosis patients.

METHOD

Study Sample

Ethical Consideration

Written informed consent was obtained from all respondents before data collection was carried out.

Study Design

This study uses a case control design. Data collection was conducted in December 2013 to February 2014.

Population and Sampel

The study population was new positive smear pulmonary tuberculosis patients who underwent an intensive treatment period in the DOTS strategy, Makassar city, Indonesia. Samples were a part of the population who had completed intensive phase treatment at Labuang Baji General Hospital and Community Lung Health Center, there were 111 patients. Cases were positive smear pulmonary tuberculosis patient who experiences no conversion at the end of the intensive phase of treatment and control were patients with positive smear pulmonary tuberculosis who did not experience conversion at the end of the intensive phase. The sampling technique used exhaustive sampling techniques to select cases while controls were selected by accidental sampling technique[3].

Socio-Demographic Characteristics and Risk Factors

We include demographic information and individual characteristics (age, gender, education, occupation, socio-economic, tuberculosis, knowledge measured using the *guttman* scale), DM, regular treatment DOTS, role of PMO, and side effects of tuberculosis drugs as risk factors analyzed in this study.

Measures

Measurement of Sputum Convertion

Measurement of sputum conversion was seen based on AFB microscopic examination in positive smear pulmonary TB patients at the end of the 2-month intensive phase of treatment.

Tuberculosis Measurement

The diagnosis of tuberculosis was based on the results of microscopic smear positive smear in patients before receiving intensive phase treatment for 2 months.

Statistical Analysis

Analysis was carried out after getting information about risk factors using a questionnaire that had been tested for validity and reliability. Data processing was done electrically using computerized SPSS 20 for Windows programs. Data analysis was done by univariate by looking at the description of the frequency distribution of each independent variable, bivariate by *Chi-square* test to determine the relationship of the frequency of the dependent variable with the independent variable to see the risk factors against the results of conversion in patients pulmonary tuberculosis AFB positive.

RESULTS

Analysis and description of each study variable as a risk factor against result convertion of 111 patients, the proportion of convertion (33,3%) and no convertion (66,7%).

Tabel 1. Analysis of Risk Factors for Changing Conversion of Pulmonary Tuberculosis AFB Positive Patients in The Intensive Phase.

Variable (Factor Internal)	**Convertion Case**		**Non Convertion Control**		**Total**	**OR**	**95% CI**	***P* Value**
	n =37	**%**	**n =74**	**%**				
Age (years)								
<48	26	70,3	55	74,3	81	0,817	0,340-1,963	0,650
≥48	11	29,7	19	25,7	30			
Gender								
Male	29	78,4	55	74,3	84	**1,252**	0,489-3,208	0,639
Female	8	21,6	19	25,7	27			
Education Level								
Low	13	35,1	37	50	61	0,542	0,240-1,223	**0,138**
High	24	64,9	37	50	50			
Knowledge								
Low	16	43,2	32	43,2	58	**1,723**	0,777-3,821	**0,179**
High	21	56,8	42	56,8	53			
Job								
Work	28	75,7	43	58,1	71	0,446	0,185-1,077	**0,069**
No Work	9	24,3	31	41,9	40			

Tabel 2. Analysis of Risk Factors for Changing Conversion of Pulmonary Tuberculosis AFB Positive Patients in The Intensive Phase.

Variable (Factor Eksternal)	**Convertion Case**		**Non Convertion Control**		**Total**	**OR**	**95% CI**	***P* Value**
	n =37	**%**	n =74	%				
Social Economy								
Low	23	62,2	51	68,9	74	**1,350**	0,590-3,086	0,477
High	14	37,8	23	31,1	37			
Diabetes Melitus								
Yes	3	8,1	1	1,4	4	0,155	0,016-1,548	**0,072**
No	34	91,9	73	98,6	107			
Regular Treatment DOTS								
Low	21	56,8	38	51,4	59	**1,243**	0,562-2,751	0,591
High	16	43,2	36	48,6	52			
Role DOTS								
Low	21	56,8	48	64,9	69	0,711	0,317-1,593	0,406
High	16	43,2	26	35,1	42			
Role of Health Worker								

Low	22	59,5	47	63,5	69	0,843	0,375-1,892	0,678
High	15	40,5	27	36,5	42			
Effect of Drugs								
Yes	24	64,9	55	74,3	79	**1,568**	0,668-3,679	0,300
No	13	35,1	19	25,7	32			

Based on risk factor variables, the largest proportion were in no control group (table 1 and table 2). It can also be seen that the highest risk factor internal was of gender (OR=1.252), knowledge (OR=1.723) and the highest risk factor eksternal was of social economy (OR=1.350), regular treatment DOTS (OR=1.243), effect of drugs (OR=1,568).

DISCUSSION

Based on the results of the analysis, it is known that gender, Kowledge, sosial economy, regular treatment DOTS and effect of drugs, are risk factors for conversion among smear pulmonary tuberculosis patients in Makassar. Cases of smear-positive TB, especially men and those with risk factors identified for non-conversion, must be monitored closely during their treatment period. In patient compliance counseling and education to reduce TB non-conversion risk factors, and to increase TB conversion and cure rates[4].

In a study, which stated that TB knowledge was poor among respondents and many misconceptions about TB[6]. stated that the lack of education and distance from home to health center were significantly related to the delay in seeking care from the service provider. This was further demonstrated by a prospective cohort study conducted in South Africa, which found that illiterate patients were more likely to interfere with treatment[7]. Patients in this study demonstrated a good understanding of transmission and risk factors for TB. However, some patients are not aware the causes of TB and the transmission. Health education efforts must take advantage of these positive findings of learning[8]. Explained the knowledge of patients who are very low can determine the patient's disobedience to take medication because of the lack of information provided by health workers about pulmonary tuberculosis disease, treatment and its prevention[12]. Knowledge of patients about TB does not directly affect the increase motivation. Social support directly affects the increase in knowledge TB sufferers. The most powerful aspects of knowledge are treatment methods and side effects[15].

The results of this study are in not accordance with the research conducted by Maulidya states there is no relationship between knowledge and compliance with treatment for TB patients.This means, the higher or the lower of patient's knowledge does not affect his recovery[14]. TB knowledge is low and many misconceptions about TB these should be targeted through health education programs. Both TB knowledge gaps could be better addressed through anintegrated health education program[16]. These findings highlight the need for health education efforts to strengthen accurate information dissemination to promote sound TB knowledge and attitudes among patients[17]**.** Lower education was an important factor in TB[20].

while social support for TB patients reduced default. Further prospective randomised studies are necessary to evaluate the impact and to determine the most cost-effective social support for improving treatment outcomes of TB in patients, especially among populations at risk of default[25]. Interventions to reduce default should therefore concentrate on socially disadvantaged patients[23]. Our findings measure the risks associated with a variety of social factors and tuberculosis. Poverty, diabetes were independently associated with an increased risk of tuberculosis[21].

The consistent study in evaluating and comparing time and predictors of culture conversion, in Latvia got results a median sputum conversion rate of 60 days (range 4-462 days) of 77% of patients who experienced sputum conversion19. Shortening treatment from 6 to 4 months in adults with noncavitary disease and culture conversion after 2 months using current drugs resulted in a greater relapse rate. The combination of noncavitary diseaseand 2-month culture conversion was insufficient to identify patients with decreased risk for relapse18. TB patients remained smear-positive after 2 months of treatment. Patients with cavitation, higher smear grading and those who had not used DOTS continu ouslyin the initial treatment

phasehadalonger time to sputum smear conversion22

The recommended tuberculosis treatment is at least 6 months. It must be completed but if the patient feels healed, patients stopped the treatment. This is due to the lack of knowledge of the informantion about the importance of treatment. Therefore, there should be counseling about the importance of TB treatment and the consequences of uncomplete treatment[11]. Relationship between age and type of treatment is found 36 people (100%) productive age respondents get OAT category 1. Based on the results of statistical tests, p value is 0.023 where ρ <0.05 so it can be concluded that there is a significant relationship between age with type of treatment. It is possible that immunological systems at unproductive age (>50 years) are very susceptible to various diseases, including pulmonary tubeculosis. The older the age will be a functional change in physiological, pathological and decreased body defense systems. It will affect the body's ability of tuberculosis treatment[13].

Duration of Tuberculosis treatment more than 2 months can result dropping out. After the intensive treatment, Patients must take antibiotics for at least six months to nine months. Uncomplete treatment will cause drug resistance. Therefore, it is needed to educate TB patients about the importance of completing each stage of TB treatment, so that patients can complete the treatment[10].

In 1107 eligible patients, the treatment success rate was 81.5% and the default rate 9.4% (respectively 60.4% and 17.0%).The main risk factors for treatment default can be infl uenced. Interventions to reduce default should therefore concentrate on socially disadvantaged patients[23]. 2–3 drugs, HR 7.2, 95%CI 3.3–16.0, P<0.001) was significantly associated with an increased risk of default for MDR- TB treatment[24].

LIMITATION

The results obtained in this study may still be influenced by selection bias when choosing controls from accident sampling. The use of the control case design has a weakness because it has valid controls to compare with the case and the sample in this study is unadequat while this study has several variables, it makes the actual risk factors in the theory do not match with the results of this study.

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REFERENCES

1. WHO. Global Tuberculosis Report 2012. Genewa: WHO Press; 2012.
2. Ministry of Health RI, Directorate General of Disease Control and Environmental Health. Indonesian health profile, Ministry of Health of the Republic of Indonesia. Jakarta; 2013.
3. Budiarto. Biostatistic. Jakarta; 2002.
4. Mlotshwa M, Abraham N, Beery M, Williams S, Smit S, Uys M, et al. Risk factors for tuberculosis smear non-conversion in Eden district, Western Cape, South Africa, 2007-2013: A retrospective cohort study. BMC Infect Dis [Internet]. 2016;16(1):1–12. http://dx.doi.org/10.1186/s12879-016-1712-y.
5. WHO. Global tuberculosis control:a short up date to the 2010 report. 2010.
6. Wynne A, Jhangri GS, Richter S, Alibhai A, Rubaale T, Kipp W. Tuberculosis in the era of infection with the human immunodeficiency virus: Assessment and comparison of community knowledge of both infections in rural Uganda. BMC Int Health Hum Rights [Internet]. 2012;12(1):1. Available from: BMC International Health and Human Rights.
7. Z. NDWANDWE ZSI. NON- Health system related factors affecting tuberculosis treatment outcomes : A cas-control study. UMGUNDUNDLOVU Healty District. Vol. 39, Animal Genetics. 2008.
8. Kigozi, N. G. *et al.* (2017) 'Tuberculosis knowledge, attitudes and practices of patients at primary health care facilities in a South African metropolitan: Research towards improved health education', *BMC Public Health*, 17(1), pp. 1–9. doi: 10.1186/s12889-017-4825-3.

9. Tierney DB, Franke MF, Becerra MC, Virú FAA, Bonilla CA, Sánchez E, et al. Time to culture conversion and regimen composition in multidrug-resistant tuberculosis treatment. PLoS One. 2014;9(9):1–6.
10. Karim SSA, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray A, et al. NIH Public Access. English J. 2011;362(February 2009):697–706.
11. Nugroho RA. Qualitative Study of the Factors Underlying the Drop Out Treatment of Lung Tuberculosis. Kemas. 2011;7(1):83–90. Available from: http://journal.unnes.ac.id/index.php/kemas.
12. Nitari R, Irvan M, Ifdelia S. Relation of Compliance Level of Patients with Lung Tuberculosis with Health Behavior, OAT Side Effects and Role of PMO in Intensive Phase Treatment at Seberang Padang Health Center September 2012 - January 2013. Health Journal Andalas [Internet]. 2017;6(2):345–50. Available from: https://journal.fk.unand.ac.id/index.php/jka/article/view/702.
13. Kurniawan N, Rahmalia S, Indriatia G. Factors Affecting Success in Treatment of Lung Tuberculosis. Jom. 2015;2(1):729–241.
14. Maulidya YN, Redjeki ES, Fanani E. Factors Affecting the Success of Pulmonary Tuberculosis (TB) Treatment in Post-Treatment Patients at Dinoyo Health Center. 2016
15. Rachmawati T, Turniani L. The effect of social support and knowledge about TB on motivation for recovery of pulmonary tuberculosis patients treated at the health center. Researcher of the Buletan Health System. 2006;(1):134–41.
16. Wynne A, Jhangri GS, Richter S, Alibhai A, Rubaale T, Kipp W. Tuberculosis in the era of infection with the human immunodeficiency virus : assessment and comparison of community knowledge of both infections in rural Uganda. 2012.
17. Kigozi NG, Heunis JC, Engelbrecht MC, Rensburg APJ Van, Rensburg HCJD Van. Tuberculosis knowledge , attitudes and practices of patients at primary health care facilities in a South African metropolitan : research towards improved health education. 2017;1–9.
18. Johnson JL, Hadad DJ, Dietze R, Maciel ELN, Sewali B, Gitta P, et al. Shortening treatment in adults with noncavitary tuberculosis and 2-month culture conversion. Am J Respir Crit Care Med. 2009;180(6):558–63.
19. Holtz TH, Sternberg M, Kammerer S, Laserson KF, Riekstina V, Zarovska E. Annals of Internal Medicine Article Time to Sputum Culture Conversion in Multidrug-Resistant Tuberculosis : Predictors and Relationship to Treatment Outcome. Ann Intern Med. 2000;144(3):650–9.
20. Miranda MD, PhD A, Golub J, Lucena F, Gurgel F, Dietze R. Tuberculosis and AIDS co-morbidity: linkage of tuberculosis and AIDS databases. XVII Int AIDS Conf 3-8 August 2008, Mex City, Mex. 2008;13(June 2006):137–41.
21. Coker R, McKee M, Atun R, Dimitrova B, Dodonova E, Kuznetsov S, et al. Risk factors for pulmonary tuberculosis in Russia: Case-control study. Br Med J. 2006;332(7533):85–7.
22. Wang JY, Lee LN, Yu CJ, Chien YJ, Yang PC. Factors influencing time to smear conversion in patients with smear-positive pulmonary tuberculosis. Respirology. 2009;14(7):1012–9.
23. Kliiman K, Altraja A. Predictors and mortality associated with treatment default in pulmonary tuberculosis. 2010;14(October 2009):454–63.
24. M. T. Gler, L. J. Podewils, N. Munez, M. Galipot, M. I. D. Quelapio TET. Impact of patient and program factors on default during treatment of multidrug-resistant tuberculosis. Int J Tuberc lung Dis. 2012;1064(May 2012):955–60.
25. Jakubowiak WM, Bogorodskaya EM, Borisov ES, Danilova DI, Kourbatova EK. Risk factors associated with default among new pulmonary TB patients and social support in six Russian regions. Int J Tuberc Lung Dis. 2007;11(1):46–53.

Role Therapy of Camel Milk to Treatment Hepatitis Mice Which Induce *Via Listeria Monocytogenes*

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ABSTRACT

The goal of the current study was to administered the role of camel milk on hepatic histopathological induced via experimental infection with *L.monocytogenes* in Swiss albino mice. The mice were divided into four groups (A, B, C and D). The control group received water and fed *ad libitum*. The other groups received orally *L. monocytogenes*, Group B sacrificed after fourteen days. Group C received 1ml of camel milk, Group D received *ad libitum* of camel milk. Mice of both groups sacrificed after two weeks. Livers of all mice groups were taken to histological study. Histological sections of the liver of group B detected more sever changes including the acquisition of granular appearance for most tissue of liver lobes, hepatic cells necrosis with multiply micro abscesses compared with control groups. While liver of groups C and D showed normal structure of liver in most sections were represented via reduce the acquisition of granular appearance and increases number of Kupffer cells and in liver of group C while in group D showed several Hepatic cells to be ready for division, hyperplasia and hypertrophy of kupffer cells.

Keywords*: Role therapy, Camel milk, hepatitis, L. monocytogenes, Adult mice*

INTRODUCTION

Each year in developed countries, food-borne pathogens are responsible for causing millions of gastrointestinal disorders [1] . *L . monocytogenes* is an important food–borne opportunistic human and animal pathogen [2]. It was responsible for numerous food-borne outbreaks with high hospitalization and mortality average worldwide, specially affecting patient immune compromised adult, elderly and pregnant women [3]. These due to the *L. monocytogenes* is widely spread in natural and has been isolated form diverse of environmental source [4] like soil, plants, water, food chain, food processing [3] dairy product, raw milk, fruit and vegetables [5].As found on nonfood contact surface [6]. This wide spread of bacteria is due to resistant to hard environmental conditions, including psychotropic, high NaCl concentration and low pH. In numerous ways, *L. monocytogenes* can enter into food, processing plants, its ability to survive for long period in the environment, on food processing plants and its ability to grow at very low temperature (2C° -4C°), also to survive in or on food for prolonged period under bad conditions [7, 8].

In many parts of the world, Camel milk considered as one of the key ingredients of several traditional medicines as well as for human diet. It has a high bioactive value as result of higher composed of antimicrobial factors like Immunoglobulins, Lactoferrin and Lysozyme [9]. Recent studies confirmed that the composition of camel milk is unique in terms of anti-oxidative factors, antibacterial, antiviral, antifungal, antitumor activity, hypoglycemic and anti-cancer, to prevent aging for autoimmune diseases effect [10, 11].

The present research was proposed with an objective to study pathogenicity of *L. monocytogenes in* adult mice and role of camel milk to reduce pathological changes which induced by this bacteria.

MATERIAL AND METHOD

Bacteria strains and preparation of inoculums

The standard culture of *L. monocytogenes* (ATCC) was obtained from Medya Diagnostic center/ Erbil city/ Iraq. It was assessed for it purity carried out some recommended biochemical tests to ensure that the diagnosis was correct.

Camel's milk source

The milk sample was collected from Hawija which is 45 Km far from Kirkuk city/ Iraq. Camel was in the first month of lactation period fed mainly on pasture, which grows in the natural grassland in addition to the dates, bran and water. It was obtained milk samples by a manual way milking (Hand milking). Milk was transported to the laboratory in cooled conditions, then keeping it in the refrigerator under temperature (4-2) º C until use.

Pathogenicity of bacteria

L. monocytogenes were grown overnight in Brain Heart infusion liquid medium plus 6% glucose and yeast extra in an incubator at 37Cº. Bacterial cells were harvested by centrifugation 3000 rpm for 10 minutes, washed three times in phosphate-buffered saline. The bacterial suspensions were diluted to achieve the final suspension 4.5×10^6 CFU/ml and numbers of viable *L. monocytogenes* was done according to [12].

Animals used in the experiment

A total of 40 Swiss albino mice 27±2 in weigh, aged 4-6 weeks were used in this study. They were purchased from college of Veterinary Medicine/ University of Tikrit/ Iraq. Mice were kept in the animal house in wire-meshed stainless steel cages (five/ cags). The environment in the animal house was controlled in which the temperature was maintained at (20 - 24C°) with 12 h light/ dark cycle with good ventilation. They were fed by commercial pellet diet. The animals were kept in this environment for 7 days before starting the experiment for acclimatization.

Experimental Groups and Histology:

Mice divided randomly into four groups comprising 10 mice in each group and fed the same diet throughout the experimental period. Group A: given water and fed *ad libitum*. Group B: given dose of bacteria suspension 4.5×10^6 CFU orally one time for one a day. Group C: given 1ml of Camel milk orally two times a day. Group D: given *ad libitum* of Camel milk .

All animals in group A and B were scarified and dissected after 14 days. As for the rest of group C and D the same procedure was used for bringing about a bacterial infection. Then animals were left for 14 days and treatment will be started in the morning of fourteen days by camel milk for the groups B and C. finally scarified the animals of two groups after two weeks of the treatment. Liver of all groups were fixed in 10% neutral buffer formalin. After 48 hours, livers were trimmed and processed routinely in paraffin, sectioned at 5µm ,and stained with hematoxylin and Eosin stain [13]

RESULT & DISCUSSION

Histological examination of liver controlling mice group (group A) showed that hepatic cell, portal area and vasculature appear normal .The liver of group mice B showed hepatitis which acquisition of granular appearance for most tissue of liver lobes (Fig 1) as compared to the normal architecture of hepatic cells. In other sections observed focal necrosis, vacuolated and karyolysis most of hepatic cell's nucleus also infiltration of inflammatory cells in central vein and hyperplasia of their endothelium (Fig 2). Additionally, results showed multiple micro abscesses sprinkled in parenchyma in liver sections for all mice of group B characterized by necrosis of hepatic cells, dense infiltration of lymphocytes and few neutrophils (Fig 3). Also, there was macrophages in some sections (Fig 4). The granular appearance of liver tissue attributed to infiltration of inflammatory cells between hepatic cells where [14] mentioned that *L. monocytogenes* have a cell wall component that is mitogenic for lymphocytes and lipid components, which stimulate the monocytosis for which it is named. On the other hand, these bacteria have listeriolysin O which have highly lytic role for nucleated cells and it can prompting abroad range of various cells death kinds. Perhaps this explains the presence of nuclei at different stages of lysis [15]. Additionally, *L. monocytogenes* carried via blood to liver after crossing the intestinal barrier, this is the majority of the invading bacteria be trapped in the liver [16]. Infected hepatic cells react to *listeria* infection via liberation neutrophil chemoattractions and display boost in adhesion to neutrophils, leading to micro abscess [17]. Monocytes are other early responding cell type that infiltrates infectious foci within 24 -48 hours and this occurs following both oral and iv infection [18].

While the microscope examination of mice group C showed normal structure of liver in most sections with reducing granular appearance and demonstrated normal central vein. Also, normal arrangement of hepatocytes and sinusoids Liver with few numbers of inflammatory cells around central vein in some sections (10).

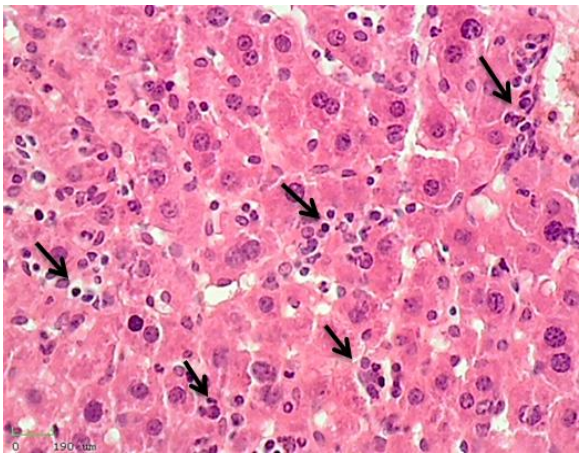


Figure 1. Liver section showing acquisition of granular appearance (H & E)X400

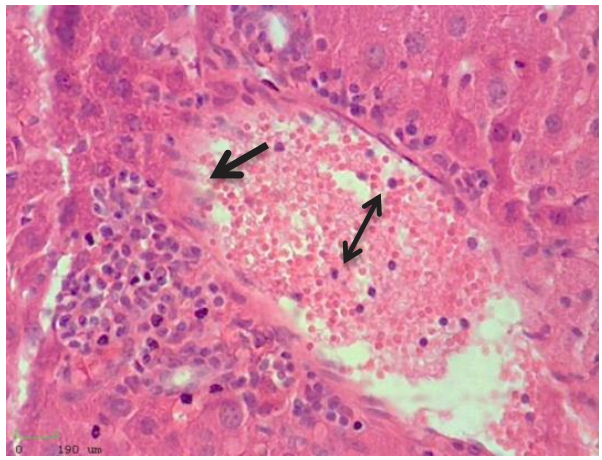


Figure2. Liver section showing infiltration of inflammatory cells in central vein, hyperplasia their endothelium (H & E) X400

In parenchyma , hyperplasia of kupffer cells was noticed in some sections of this group with (11) in other hand there was showed absent of the inflammatory cells inside central and portal vein, and normal endothelial lining them. While the histological architecture of the liver of mice group D was similar to mice group A. Absent granular appearance, micro abscesses (12). Several hepatic cells also were showed to be ready for division (13), hyperplasia and hypertrophy of kupffer cells (14).

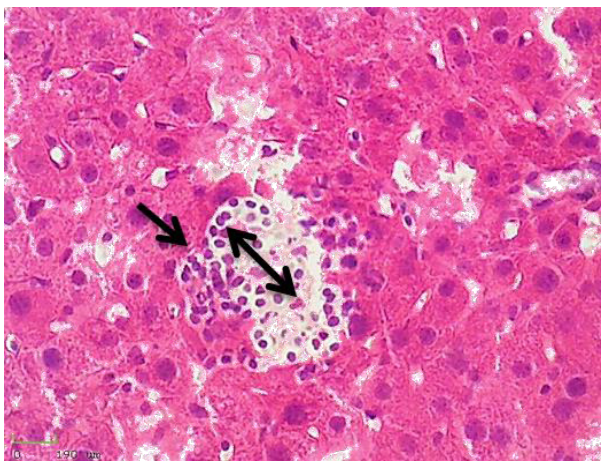


Figure3. Liver section showing i micro abscesses in parenchyma of liver , necrosis of hepatic cells (H & E)X400

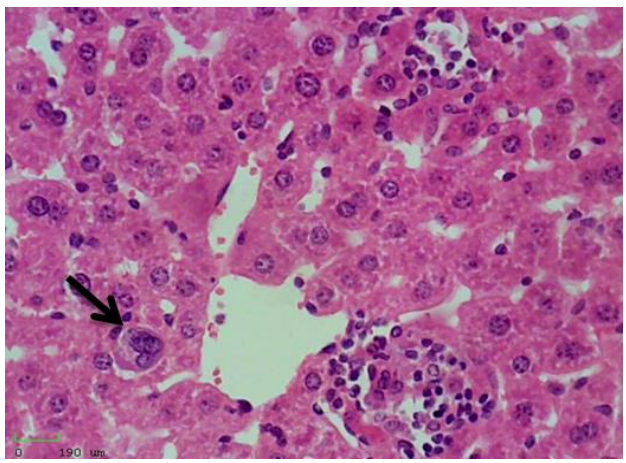


Figure 4. Liver section showing macrophages (H&E)X400

Many studies in vivo and vitro have proofed a significant backing for proving camel milk protective effects on liver destruction. It acts as antibacterial, antiviral, anti-fungal and anti-parasitic activity [19, 20] because it has antibacterial enzyme like Lactoferrin, lysozyme, immunoglobulins, N-acetyl –S– glucosaminidase, protective protein like casein, stronger immune system and smaller immunoglobulins than other ruminates [21]. The reduction of granular appearance of liver tissue of mice group C and its absent in mice group D may be due to the role of anti-inflammatory ingredients of camel milk it is Lactoferrin which has been distinguished avoid TNF-α, IL-1 and IL-6 in mononuclear cell in vitro and vivo response to LPS stimulation [22]. The anti-inflammatory role of Lactoferrin can be attributed as it has positive charge in its surface, which interacts with negatively charged moieties like proteoglycan on the surface of immune cells.

This combination can trigger signaling pathways that produce physiological anti- inflammatory [23]. Not only have these actions also Lactoferrin acts as antibacterial which prevents the reach of iron at the site of infection which is nutrient for the bacteria. In this case it plays bacteriostatic effect [24]. Lack of iron in the environment obliges bacteria to move because it cannot adhere to the host surfaces [25]. Additionally [25, 26] reported that Lactoferrin blocks pathogens adhesion to target cells. This characteristic was suggested for the first time gainst enteroinvasive *Escherichia coli* (HB. 101) and later against *Staphylococcus aureus, Yersinia enterocolitica, Shigella dysenteriae, L. monocytogenes, Helicobacter pylori, Neisseria gonorrhoeae, Pemphigus neonatorum, Bordetella pertussis, Streptococcus pyogenes and Y. pseudotuberculosis*. The repair of liver to normal architecture can be attributed to the role of synerging of Lactoferrin and lysozyme. Where they mentioned [27, 28, 29] that the gram positive bacteria have anionic molecules like lipoteichoic acid of the surface, Lactoferrin which binds with this molecules and this electrostatic binding decreases the overall negative charge of cell wall making easy effectiveness of antibacterial ingredients like lysozyme and antibiotics. This improvement due to the effective effect of the ingredient of this milk and its unique medicinal properties in terms of its high concentration of vitamins and protective proteins and immunological. This feature made it distinctive different from other animals in terms of containing high concentration of antioxidants of vitamins A, B2, C and E [30, 31]. These anti-oxidant vitamins play an important role in preventing tissue damages by removing free radicals [32]. It also works to protect the cells from the effect on internal toxins, which act on the oxidation process that is accompanied different tissue changes [33]. Also, it contains high concentration of Magnesium, Potassium, Sodium, Copper and Zinc [34]. While, [35] suggested that the Zinc in camel milk plays an important role in stimulating hepatic cell to mitotic division and differentiation the cells of the immune system. It may elaborate the increase of numbers of Kupffer and increase the volume of hepatic cells prepared for division.

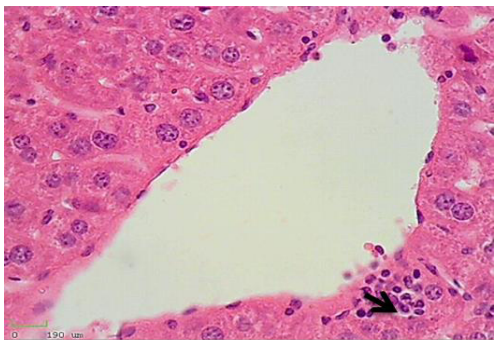


Figure 5. showed normal structure of liver, normal central vein, normal arrangement of hepatocytes ,few number of inflammatory cells around central vein ↘ (H&E)X400

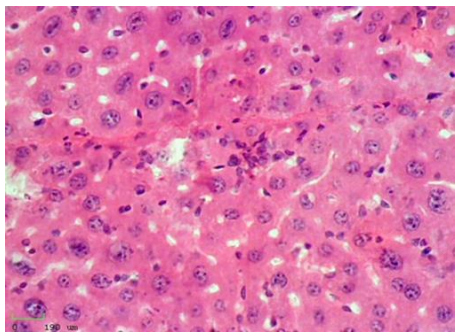


Figure 6. Liver section showing few number of inflammatory cells , hyperplasia of Kupffer cells (H&E)X400

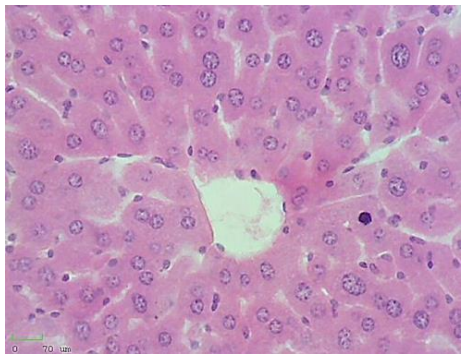


Figure 7.showed normal hepatic cells arrangement around central vein and lining with normal endothelium,sinusoids.(H&E)X400

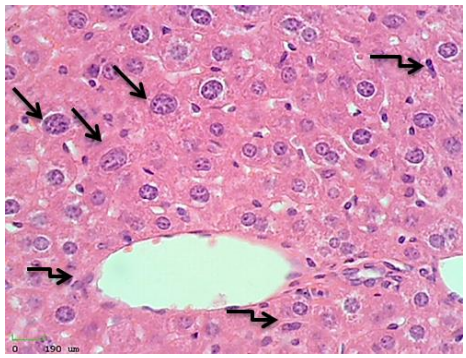


Figure 8. showed several hepatic cells to be ready for division hypertrophy of Kupffer cells (H&E)X400

Conflict of Interest: Nile

Source of Funding: Self-Funded

The Ethical Committee of the concerned institute approved the research protocol. The purpose and procedures of the study were to be explained to all the study subjects,and informed consent was to be obtained from them.

REFERENCES

1. Negi M, Rawool DB, Vergis J, Dhaka P, Vijay D, et al. Isolation and identification of pathogen Listeria monocytogenes from diarrhocal cases in human infants and young animals. Adv. Anim. Vet. Sci. 2014; 2 (5s): 5-10.
2. Holch A, Ingmer H, Lich T. & Gram, L. *Listeria monocytogenes* strain encoding premature stop codons in *lA* invade mice and guinea pig fetuses in orally dosed dams. 2013;2 (12): 1799-806.
3. López PR, Herrera JJ R, Vázquez-Sánchez D. & Cabo M L. Current knowledge on *Listeria monocytogenes* biofilms in food-related environments: Incidence, resistance to biocides. Ecol. Biocontrol. J. Foods. 2018; (7): 85.
4. Abu Owda S M . Bacteriological quality of fresh vegetables salad in schools canteens and Restaurants in Gaza strip. The Islamic university Gaza. Biological science. Master Degree of Science in Biological Sciences. The Islamic University-Gaza. 2018.
5. Laksanalamai P, Joseph L, Silk B, Burall L,Tarr, et al. Genomic characterization of *Listeria monocytogenes* strains involved in a multistate listeriosis outbreak associated with Cantaloupe in US. PLoS One. 2012; 7 (7): Pe42448.
6. Lakicevic B, & Nastasijevic I. *Listeria monocytogenes* in retail establishments: Contamination routes and control strategies. Food Rev. Int., . 2017; 33: 247–269.
7. Vinothkumar R, Arunagiri K, & Sivakumar T. Studies on pathogenic *Listeria monocytogenes* from marine food resources. Int. J. Curr. Microbiol. App. Sci.,2013; 1 (1): 86-93.
8. Bala S. Food microbiology and frontier. 2nd ed. 383-409 pp;2001.
9. El-Fakharany E M, Tabil A, Abd El-Wahab A, et al. Potential activity of camel milk-amylase and lactoferrin against hepatitis C virus infectivity in HepG2 and lymphocytes. Hepatitis Monthly.2008; 8: 101-109.
10. Sharma Ch, and Chandan S. Therapeutic value of camel milk review. Adv. J. Pharm. Life Sci. Res.2014; 2: 13-37.
11. Al-Juboori A T, Mohammed M, Rashid J, Kurian J, & El-Refaey S. Nutritional and medicinal value of camel (*Camelus dromedrius*) milk: In second international conference on food and environment: The quest for a sustainable future, Budapest, Hungary2013; 22-24: 221-232.
12. Miles AA, Misra SS, & Irwin J O. The estimation of the bactericidal power of blood. J. Hyg.1938; 38: 732-749.
13. Luna L . Manual of histological staining methods of the armed forces institute of pathology, New York. 3rd .Mc Graw- ill.pp:258; 1968
14. Czuprnski Ch S, and Balish E. Pathogensis of *Listeria monocytogenes* for Gnotobiotic rat.1981; 32 (1): 323-331.
15. Hernández-Flores K G,& Vivanco-Cid H. Biological effects of listeriolysin O: implications for vaccination. Biomed. Res. Inter., 2015; 9.
16. Hoelzer K, Pouillot R, and Dennis S. Animal models of listeriosis: a comparative review of the current state of the art and lessons learned. Vet. Res.,2012; 43 (18): 1-27.
17. Rogers H W, Callery M P, and Unanue E R. *Listeria monocytogenes* induces apoptosis of infected hepatocytes. J. Immunol.,1996; 156: 679-684.
18. Jones G S, Smith VC. & D'Orazio SE. *Listeria monocytogenes* replicate in bone marrow-derived cd11c+ cells but not in dendritic cells isolated from the murine gastrointestinal tract. J. Immunol., Pub. Med.2017.
19. Mona E, Ragia O, Abeer A,& Mosa T. Biochemical effect of fermented camel milk on diarrhea in rats.

N.Y. Sci. J.2010; 3 (5): 106-111.

20. Gizachew A, Teha J, & Birhanu T. Review on medicinal and nutritional values of camel milk. Natu. Sci.2014; 12 (12).

21. Kula J. Medicinal values of camel milk. Int. J. Vet. Sci. Res.2016; 2 (1): 18-25.

22. Arab HH , Salama A, Samir SS, Abdelghany TM, Omare HA, et al. Camel ilk attenuates rheumatoid arthritis via inhibition of mitogen activated protein kinase pathway. Cell Physiol. Biochem.2017; 43: 540-552.

23. Legrand D. verview of lactoferrin as a natural immune modulator. J. Pediatrics,2016; 173: 10-15.

24. Keykanlu HR I, Zibaei S, Ardjmand M, and Safekordi, A.A. (2016). Fluorocarbon nanostructures (PFOB-NEP) as camel milk lactoferrin and its anti-cancer effects on human breast cancer cell line MCF7. Bulgarian Chem. Comm., 48 (2): 323– 331.

25. Jahani, S.; Shakiba, A. & Jahani L. The antimicrobial effect of lactoferrin on Gram-negative and Gram-positive bacteria. Int. J .Infect.2015; 2(3): e27954.

26. Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, &Tomita M.Identification of the bactericidal domain of lactoferrin. Biochim. Biophysiol. Acta,1992; 1121 (1-2):130-136.

27. Barbiroli A, Bonomi F, Capretti G, Iametti S, Manzoni M, Piergiovanni L, & Rollini M. Antimicrobial activity of lysozyme and lactoferrin incorporated in cellulose-based food packaging. Food Control, 2012; 26 (2): 387-392.

28. Leitch E C, and Willcox MDP. Elucidation of the anti-staphylococcal action of lactoferrin and lysozyme. J. Med. Microbiol.1999; 48 (9): 867-871.

29. Wang B, Timilsena Y, Blanch EW, and Adhikari B. Lactoferrin: structure, function, denaturation and digestion.2017.

30. AL-Hashem F. Camel's milk protects against Aluminum Chloride induced toxicity in the liver and kidney of White Albino Rats. Am. J. Biochem. Biotechnol.2009; 5 (3): 98-109.

31. Afifi MEM. Effect of camel's milk on cispatin induced nephrotoxicity in Swiss Albino Mice. Am. J. Biochem. Biotechnol.2010; 6 (2): 141-147.

32. Yousef MI. Aluminum induced changes in hematobiochemical parameters, lipid per oxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicology.2004; 215: 97-107.

33. Lomnitski L, Nyska A, Ben-Shaul V, Maronpot R, Haseman J, Bergman HM, & Grossman S. Toxical. Pathol.,2000; 28 (4): 580-587.

34. Gader AG, Abdel M, and Abdulqader AA. The unique medicinal properties of camel products: A review of the scientific evidence. J. Taibah Uni. Med. Sci.2016; 11 (2): 98-103.

35. Faye B, Konuspayeva M, Narmuratova and Loiseau G. Compa fatty gross composition of milk in bactrian camel and dromedary. J. Camel Sci.2008; 1: 48-53.

Physical Activity: Mine Workers' Behavior Related With Metabolic Syndrome

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ABSTRACT

Metabolic syndrome is a term for risk factors for heart disease and diabetes mellitus. Workers have different lifestyle behaviors and work patterns that can cause metabolic syndrome. Based on medical check-up data in 2016, there were found several cases of dyslipidemia (97.5%), visual impairment (47.8%), BMI> 25 (45.2%), abnormal audiometry (17%), liver enzyme disorder (1.7%), and hypertension (10 %). Among those cases, there are 3 problems which are identified as the components of the metabolic syndrome. This study was conducted to explain the factors related to metabolic syndrome in mine workers to make control and prevention programs. A cross-sectional design was used to obtain workers lifestyle data by analyzing lifestyle questionnaire results and medical check-up data which includes central obesity, triglycerides, HDL, blood pressure and fasting blood sugar. There were significant relationships between physical activities with metabolic syndrome. No significant relationship was found between diet pattern, sleep duration, and knowledge of metabolic syndrome. Promotion and preventive controls are needed to prevent the metabolic syndrome in population, and screening of metabolic syndrome in all workers need to perform by the company to find out the magnitude of the problems.

Keywords: *Metabolic syndrome, occupational health, coal mining*

INTRODUCTION

The metabolic syndrome (METS) consists of five factors for Atherosclerotic Cardiovascular Disease (ASCVD), that is atherogenic dyslipidemia, high blood pressure, dysglycemia, pro-thrombotic state, and inflammatory state, which occurs most often in obese people. The contribution of each component to cardiovascular risk necessarily varies between individuals but, in combination, both multiply the risk of ASCVD (1).

According to the American Heart Association / National Heart, Lung, Blood Institute update ATP III MS and IDF report, the main purpose for diagnosing METS is to identify patients at high risk of heart disease and type 2 diabetes mellitus who require lifestyle therapy to reduce this risk [(2)]. Most people with METS are obese and do not settle. Urbanization and access to cheap food create a pandemic of METS. Thus, primary interventions should be lifestyle changes, ie calorie restriction, improved food quality, and increased physical activity [(1)].

Some research on METS of International Diabetes Federation (IDF) decided to use the definition of ATP III 2001 as a starting point and modify and update it to reflect the current goals [(3)]. According to a new definition, a person suffering from METS, they should have central obesity plus two of the other four additional factors. These four factors were elevated triglyceride levels ≥ 1.7 mmol / l (150 mg/dl), decreased HDL cholesterol: <1.03 mmol / l (40 mg/dl) in men and <1.29 mmol / l (50 mg/dl), in women (or specific treatment for these lipid abnormalities), elevated blood pressure (systolic ≥ 130 or diastolic 85 mmHg) or treatment of previously diagnosed hypertension, fasting blood glucose ≥ 5.6 mmol / l (100 mg/dl) or previously diagnosed type 2 diabetes.

METS was found in 22.8% and 22.6% of US men and women. METS was found in 4.6%, 22.4%, and 59.6% of men of normal weight, overweight, and obese,

respectively, and a similar distribution was observed in women. Older age, postmenopausal status, Mexican American ethnicity, higher body mass index, smoking, low household income, high carbohydrate intake, and physical inactivity were associated with increased likelihood of METS [4]

A METS-related study conducted on executive workers in Jakarta in 2011 showed that in general, the prevalence of METS was high (21.6%). The prevalence of METS in male executives (24.7%) was higher than for female executives (11.8%). Demographic, lifestyle, sex, and physical activity characteristics are associated with METS [5].

XYZ is a coal mining company, where the mining processing environment takes place has a health impact. Based on preliminary research by reviewing HIRADC (Hazard Identification Risk Assessment and Determining Control), medical check-up result and clinic visit found that disease that occurs among workers not only related to work environment but related to work behavior and healthy lifestyle.

MATERIAL AND METHOD

This cross-sectional study used questionnaire and medical check-up in 2017. This study aimed to describe the prevalence and factors related to METS in coal mining company XYZ. Hypothesis test using Chi-Square test and T-test to see the relation between dietary pattern, physical activity, sleep duration and knowledge with METS. Research conducted at XYZ, a coal mining company operating in South Kalimantan. The study was conducted in January - May 2018.the population study is the workers of XYZ coal mining site of South Kalimantan. From the total of 196 workers, 105 workers selected after adjusting with inclusion criteria. This research was using FFQ to get dietary pattern information, GPAQ for physical activity and questionnaire that develop by the researcher for sleep duration and knowledge.

FINDINGS

Table 1 Prevalence of METS From 2012-2017

Year	METS		Total
	Yes	No	
2012	6	74	80
	7,5%	92,5%	100.0%
2013	6	92	98
	6,1%	93,9%	100.0%
2014	17	81	98
	17,3%	82,7%	100.0%
2015	16	83	99
	16,2%	83,8%	100.0%
2016	20	77	97
	20.6%	79,4%	100.0%
2017	44 47,8%	48 52,2%	92 100%

The prevalence of METS obtained by analyzing medical check-up result. It was found that METS in miners increase from 2012-2017. In 2017, employees with METS were 47.8%, while those without METS were 52.2%.

Table 2 Distribution of Component METS in 2017

Component	Normal		Abnormal	
	N	%	N	%
Central Obesity	58	63	34	37
Triglycerides	33	35,9	59	64,1
HDL	50	54,3	42	45,7
Blood Pressure	11	12	81	88
Fasting Blood Sugar	84	91,3	8	8,7

The METS component which many respondents had is a high blood pressure of (88%), followed by triglycerides, HDL, central obesity, and fasting blood sugar respectively.

Table 3 Distribution of METS Based on Numeric Independent Variable

METS	Min	Max	Mean	SD	SE	P value
Knowledge Score						
No METS	33,30	100,00	68,76	15,616	2,254	
METS			69,89	16,49	2,485	0,736

It was found that there was no difference in the mean score of knowledge in the group who had METS and did not have METS (p-value = 0,736). In the group with METS, the mean score was 68,76, whereas in the group with no METS the mean score was 69,89.

Table 4 Distribution of METS Based on Categoric Independent Variable

		METS		P-value	OR (95% CI)
Variable		**Yes**	**No**		
		%	%		
Sleep duration	Short sleep duration (<6 hours)	57,1	42,9	0,364	1,600 (0,580-4,412)
	Long sleep duration (>8 hours)	43,8	56,3	0,904	0,933 (0,304-2,864)
	Normal sleep duration (6-8 hours)	45,5	54,5		
Diet Pattern	Often	2 (33,3)	4 (66,7)	0,469	0,524 (0,091-3,012)
	Rare	42 (48,8)	44 (51,2%)		
Physical Activity	Inactive (<600 METs-min per week)	15 (68,2)	7 (31,8)	0,032	3,030 (1,097-8,363)
	Active (≥600 METs-min per week	29 (41,4)	41 (58,6)		

Bivariate analysis showed that there was no difference in the proportion of metabolic syndrome in the type of food consumed rarely and often consumed. In the diet often groups as much as 48,8% had METS and on rare diet groups as much as 33,3% had METS. The odds -ratio (OR) value obtained at 0,524 (95% CI 0,091-3,012); it means that diet often has a risk for METS of 0,524 times compared with rare diet. The results showed that there was no significant relationship between frequent food intake with METS (p-value = 0,469).

In variable sleep duration, the group that had the highest percentage of METS was the short sleep duration group, which was 57,1% with an OR value of 1,600 (95% CI 0,580-4,412). The OR value for the long sleep duration group was obtained at 0.933 (95% CI 0,304-2,864). However, it can be seen from the p-value, both short sleep duration and long sleep duration are not related to the METS (p-value = 0,364; p-value = 0,904).

The results of data analysis demonstrated that there is a difference in the proportion of METS in both groups of physical activity. The OR value was obtained at 3,030 (95% CI 1.097-8,363); it mean that less physical activity had a risk of METS of 3,030 times compared to active physical activity. P-value showed that there was a significant relationship between physical activity with METS (p-value = 0,032).

DISCUSSION

Based on the results of research on 92 mining workers XYZ coal mining company located in South Kalimantan found the prevalence of METS in 2017 amounted to 47,8%. When compared with previous research on METS in Indonesia, this result higher than result from Soewondo's study 28,4%. [6]

According to the consensus of the definition of METS, there was the fact that METS was a progressive disease and increases the risks of ASCVD, for example, some components tend to worsen over time. Some things that usually increase annually are weight gain, gradual loss of muscle mass, stiffness in the arteries, decreased capacity of pancreatic beta cell secretion, mitochondrial dysfunction, and increased inflammatory changes in adipose tissue, and age-related [1].

The largest component of the METS contributing to this study was high blood pressure obtained in 88% of

respondents.

Dietary Pattern

Food intake is a determinant factor in the diet, which is described in the frequency of eating, skips breakfast and eating habits outside the home associated with obesity. It has been agreed that a high-fat diet will increase total energy intake and increase the likelihood of obesity. [7]

Assessment of the frequency of food consumption was done using the Food Frequency Questionnaire (FFQ). Food consumption was divided into carbohydrates, animal protein, vegetable protein, vegetables, fruit, milk and its products, fast food, snacks and soft drinks. After obtaining the overall food frequency data, then the food ingredients are determined which are at risk of causing METS. In this study, diet pattern was not related with METS. This result was not in line with several studies that suggest that diet was related with METS. Dietary patterns characterized by high consumption of fish and foods with whole grains and low consumption of processed products, sugar and sweets and preserved meat are associated with a lower risk of METS with a reduced risk of lower HDL cholesterol concentrations and increased glucose concentration [8]. This might be happening because there was no difference in the variety of food consumed by each worker because the company has provided canteen facilities and meal menu settings.

Physical Activity

Physical inactivity increases the risk of CVD and types 2 diabetes mellitus and other can worsen other risk factors. Increased physical activity promotes weight loss and maintenance in obese people and modifies obesity-related risk factors, including increasing visceral adipose tissue loss, improving insulin sensitivity, increasing HDL cholesterol, and decreasing triglyceride levels [4]: "

The results of this study showed that workers who were an inactive category (<600 METs-minutes per week) 3 times (OR = 3,030) were at risk of having METS compared with workers who were an inactive category and statistical tests showed that physical activity significantly related with the incidence of METS. These findings are in line with studies showing that the percentage of adults who do most of the sedentary activity was more at risk of developing METS [9] 21.4% of the participants studied had 3 or more risk factors for METS with low HDL levels as the most risk factor, Moderate-Vigorous Physical Activity (MVPA) with at least 10 minutes per session, Low Physical Activity (LPA) and body mass index explained 16% HDL variation. [10]

Sleep Duration

Sleep duration was not significantly related with METS. Sleep was recognized as an important mediator in an individual's health status. Given that the number of short and long sleep gradually increases [11]. The results of this study are not in line with some studies that have reported that short sleep hours (generally less than 6 hours per day) or long (generally more than 8-9 hours per day) sleep duration was associated with an increased risk of chronic diseases such as obesity, hypertension, diabetes, and METS [12]

Several studies have reported that short sleep duration was associated with an increased risk of METS, but conversely, long sleep duration was not associated with METS [13–15].

In this study, there was no association between short sleep hours and long sleep hours with METS. This was due to the working hours arranged by the company so that very few workers work overtime or leave early. Workers who live in the company accommodation can go straight back home to rest, and workers living outside the company accomodation are provided with shuttle buses so that they return home on time. With a working system like this, it was very possible for a person to return to his place of residence and immediately rest.

Knowledge

Worker's knowledge was measured using a questionnaire that contained several questions about METS. None of this research results are suitable for groups that are not with METS. The results show that no difference in the scores of knowledge in the group who had METS and did not have METS. This was in line with Kamso et al (2011) research on the groups in Jakarta who see the level of education as the uniqueness of the METS. In the study, there was no significant relationship between education and METS [5]. This was in contrast to several studies that have shown that the level of education has a significant relationship with METS [6].

Previous studies show that women have a lack of understanding of diet and exercise in reducing CVD. One way to reduce CVD incidence was to improve adherence to healthy lifestyles by educating, filtering, detecting and treating the modifiable risk factors because women do not practice heart-healthy behavior on a regular basis. This attributed to their limited knowledge and lack of attention and knowledge to CVD which can lead to poor motivation to change behaviour [16,17].

CONCLUSION

Dietary pattern, sleep duration, and knowledge were not related to METS. Factor contributed to METS in miners based on the results of was physical activity. Promotion and preventive controls are needed to prevent the METS in population. The researcher suggests performing screening of METS in all workers to find out the magnitude of the problems that occur in the company. It is necessary to improve physical activity and exercise programs for workers who had METS and all workers.

Conflict of Interest: Hereby the authors declared that there is no conflict of interest in this research with any other parties.

Ethical Clearance: This research has been approved by Ethical Board Committee, Faculty of Public Health University Indonesia and has been approved for ethical clearance by Ethical Board Committee, Faculty of Public Health University Indonesia.

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REFERENCES

1. Grundy SM. Metabolic syndrome: A multiplex cardiovascular risk factor. J Clin Endocrinol Metab. 2007;92(2):399–404.
2. Assmann G, Guerra R, Fox G, Cullen P, Schulte H, Willett DW, et al. Harmonizing the Definition of the Metabolic Syndrome: Comparison of the Criteria of the Adult Treatment Panel III and the International Diabetes Federation in United States American and European Populations. Am J Cardiol. 2007;99(4):541–8.
3. Alberti KGMMG, Zimmet P, Shaw J. Metabolic syndrome - a new world-wide definition. A consensus statement from the international diabetes federation. Diabet Med [Internet]. 2006;23(5):469–80. Available from: isi:000237012700003
4. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2016;102(8):353–76.
5. Kamso S. Prevalensi dan Determinan Sindrom Metabolik pada Kelompok Eksekutif di Jakarta dan Sekitarnya. J Kesehat Masy. 2011;6(2):85–90.
6. Soewondo P, Purnamasari D, Oemardi M, Waspadji S. Prevalence of Metabolic Syndrome Using NCEP / ATP III Criteria in Jakarta, Indonesia : The Jakarta Primary Non-communicable Disease Risk Factors Surveillance 2006. Acta Med Indones-Indones J Intern Med. 2006;42(4):199–203.
7. Hill JO, Melanson EL, Wyatt HT. Symposium: Dietary Composition and Obesity: Do We Need to Look beyond Dietary Fat? J Nutr. 2000;130(April):284–8.
8. Suliga E, Kozieł D, Cieśla E, Głuszek S. Association between dietary patterns and metabolic syndrome in individuals with normal weight: A cross-sectional study. Nutr J [Internet]. 2015;14(1):1–10. Available from: http://dx.doi.org/10.1186/s12937-015-0045-9
9. Saleh D, Arnardottir ES, Bjornsdottir E, Olafsdottir KA, Benediktsdottir B, Gislason T. Interrelationships among sedentary behaviour, short sleep, and the metabolic syndrome in adults. Eur Respir J. 2013;47(1):1–9.
10. Alkahtani S, Elkilany A, Alhariri M. Association between sedentary and physical activity patterns and risk factors of metabolic syndrome in Saudi men: A cross-sectional study. BMC Public Health [Internet]. 2015;15(1):4–10. Available from: http://dx.doi.org/10.1186/s12889-015-2578-4
11. Bin YS, Marshall NS, Glozier N. Sleeping at the limits: The changing prevalence of short and long sleep durations in 10 countries. Am J Epidemiol. 2013;177(8):826–33.
12. Yoon HS, Yang JJ, Song M, Lee HW, Han S, Lee SA, et al. Correlates of self-reported sleep duration in middle-aged and elderly Koreans: From the Health Examinees Study. PLoS One. 2015;10(5):1–22.
13. Chaput JP, McNeil J, Després JP, Bouchard C, Tremblay A. Short sleep duration as a risk factor for the development of the metabolic

syndrome in adults. Prev Med (Baltim) [Internet]. 2013;57(6):872–7. Available from: http://dx.doi.org/10.1016/j.ypmed.2013.09.022

14. Itani O, Kaneita Y, Tokiya M, Jike M, Murata A, Nakagome S, et al. Short sleep duration, shift work, and actual days taken off work are predictive life-style risk factors for new-onset metabolic syndrome: a seven-year cohort study of 40,000 male workers. Sleep Med [Internet]. 2017;39:87–94. Available from: https://doi.org/10.1016/j.sleep.2017.07.027
15. Xi B, He D, Zhang M, Xue J, Zhou D. Short sleep duration predicts risk of metabolic syndrome: Asystematic review and meta-analysis. Sleep Med Rev. 2014;18(4):293–7.
16. Amarasekara P, De Silva A, Swarnamali H, Senarath UPUL, Katulanda P. Knowledge, Attitudes, and Practices on Lifestyle and Cardiovascular Risk Factors among Metabolic Syndrome Patients in an Urban Tertiary Care Institute in Sri Lanka. Asia-Pacific J Public Heal. 2016;28:32S–40S.
17. Mirza A-S, Aslam S, Perrin K, Curtis T, Stenback J, Gipson J, et al. Knowledge, attitudes and practices among patients with coronary artery disease in Dhaka, Bangladesh. Int J Community Med Public Heal [Internet]. 2016;3(10):2740–8. Available from: http://ijcmph.com/index.php/ijcmph/article/view/211

Correlation Analysis of Sleep Duration, Dietary Habits, Physical Activity and Knowledge with Blood Pressure on Engineering Workers, Procurement, and Construction (EPC)

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ABSTRACT

Background: There have been five cases of hypertension to EPC workers in the last five years (2012-2017). To minimize the risk of hypertension in workers and to prevent greater losses in terms of productivity and medical costs arised from the disease, it is necessary to acknowledge the distribution of blood pressure in EPC workers and analyze any factors related to blood pressure, as a screening of hypertension occurred related to worker health promotion program.

Objective: The aim of this study is to analyze the risk factors associated with blood pressure (systolic, diastolic) on workers of EPC.

Method: A quantitative approach in a cross-sectional design which comprised the subjects of the study as samples are 97 workers of EPC

Result: The systolic blood pressure (SBP) and diastolic blood pressure (DBP) is higher among workers with abnormal sleep duration (SBP: 125,15±8,57, DBP: 84,71±6,02), frequently dietary habits in fast food consumption (SBP: 140,00±0,00, DBP: 90,00±0,00), lack of physical activity (SBP: 122,75±9,20) and lack of knowledge about hypertension. There were significant differences in the mean systolic (R^2 = 0,049) and diastolic blood pressure (R^2 = 0,061) in workers in the form of sleep duration.

Conclusion: The majority of worker's blood pressure is categorized prehypertension based on blood pressure category by JNC-7. Results indicate that abnormal sleep duration, the frequency of protein consumption, physical activity that does not fit with the recommendation and lack of knowledge about hypertension contribute to higher of worker's blood pressure.

***Keywords**: blood pressure (systolic, diastolic), risk factors, EPC workers*

INTRODUCTION

Hypertension is regarded the main factors of heart disease and stroke which lead to premature death worldwide. At least, 970 million people experiencing hypertension. In high-income countries, about 330 million people experienced hypertension compared to about 640 million people in developing countries. By 2025 it is estimated that there will be 1.56 billion adults living with hypertension [(1)].

Several factors related to hypertension comprised of modifiable risk factors (lack of physical activity, unhealthy diet, obesity, alcoholism, sleep apnea, high cholesterol, diabetes, smoking, and stress) and unmodified risk factors (family history, age, gender, and chronic kidney disease[(4)]. Many studies linked genetic factors, such as age, sex, ethnicity and family history and lifestyle factors, such as alcohol consumption, smoking, poor diet, lack of physical activity, stress with hypertension [(2)]. Furthermore, some research mentions that hypertension is related to the level of knowledge [(3)].

EPC company, whose biggest asset is workers, is engaged in providing engineering, procurement and construction services for development and maintenance

of oil and gas companies. It is expected that workers is able to maintain their health, resulting more productiveness to perform. Yet, there occurred five cases of hypertension in EPC company over the past five years (2012-2017). Nevertheless, a health promotion program is not available in the company which causing them hardly to prevent and control risks of hypertension. In order to minimize the risk of hypertension in workers and to prevent greater harm in terms of productivity and medical costs due to hypertension in EPC company, it is crucial to measure the frequency distribution of blood pressure among the workers and analyze the factors which contributed to the elevation of the blood pressure.

MATERIAL AND METHOD

This cross-sectional study aimed to determine the correlation between the independents and dependent variable. This research was conducted in an EPC company from January to June 2018 where gathering 97 workers. The study conducted BP measurement using sphygmomanometer by a competent paramedic. An interview-administered questionnaire was used to collect data, which included the respondent's identity, knowledge, sleep duration, physical activities, and food frequency. Workers knowledge was recorded by the questionnaire adopted from American Heart Association (AHA). Data on self-reported physical activity were collected using the Global Physical Activity Questionnaire (GPAQ) by WHO, whereas the food frequency was adopted from the FFQ (Food Frequency Questionnaire). The data were analyzed with the SPSS program, univariate and bivariate data analysis using comparative hypothesis testing which is the Mann-Whitney test.

FINDINGS

The bivariate analysis of two variables, BP and modified risk factors, showed that the mean of SBP and DBP are higher among workers with abnormal sleep duration (SBP: 125,15±8,57, DBP: 84,71±6,02), frequently dietary habits in fast food consumption (SBP: 140,00±0,00, DBP: 90,00±0,00), lack of physical activity (SBP: 122,75±9,20) and lack of knowledge about hypertension (SBP: 122,59±9,88, DBP: 82,21±9,52), this finding may contribute to high BP among workers. Whereas, in the dietary habit, frequently protein consumption has the highest average SBP (124,09±10,21) while the frequency of rarely protein consumption has the highest DBP average. (83,64±8,09) as well as with snack consumption (SBP: 123,06±8,59, DBP: 82,39±8,81). A rare frequency beverage consumption (SBP: 123,32±9,33, DBP: 82,87±8,52) have higher blood pressure compared to other categories (see Table 1).

Table 2 Comparison of Blood Pressure (Systolic, Diastolic) Based on Modifiable Factors in EPC Workers

Variable	SBP			DBP		
	Mean (Min-Max)	SD	R^2	Mean (Min-Max)	SD	R^2
Sleep duration						
Abnormal (<6 hours or >8 hours)	125,15(110,00-140,00)	8,57	0,049*	84,71 (75,00-95,00)	6,02	0,061
Normal (6-8 hours)	120,71(100,00-140,00)	9,79		80,32 (60,00-100,00)	9,28	
Dietary habit - Protein (red meat)						
Frequently	124,09 (100,00-140,00)	10,21		81,63(60,00-100,00)	8,59	
Rarely	122,03 (110,00-140,00)	9,53	0,065	83,64 (70,00-100,00)	8,09	0,066
Never	0	0		0	0	
Dietary habit - Fast Food						
Frequently	140,00(105,00-140,00)	0,00		90,00 (60,00-100,00)	0,00	
Rarely	121,76 (100,00-140,00)	9,38	0,070	81,41(60,00-100,00)	8,07	0,072
Never	124,55(140,00-140,00)	10,11		84,54(90,00-90,00)	11,51	
Dietary habit - Snacks						
Frequently	121,49 (105,00-140,00)	10,52		82,39 (60,00-95,00)	8,81	
Rarely	123,06 (100,00-140,00)	8,59	0,058	81,33 (60,00-100,00)	8,27	0,075
Never	0	0		0	0	
Dietary habit - Beverages (coffee, Soda, Alcohol)						
Frequently	117,28 (100,00-140,00)	10,09		78,18(60,00-90,00)	8,15	
Rarely	123,32 (100,00-140,00)	9,33	0,069	82,87(60,00-100,00)	8,52	0,076
Never	121,39 (110,00-140,00)	9,67		80,28 (70,00-90,00)	8,31	
Physical activity						
Not fit the recommendation (<600)	122,75(110,00-140,00)	9,20	0,070	81,88 (70,00-100,00)	8,14	0,076
Fit the recommendation (≥600)	121,93 (100,00-140,00)	9,90		81,84 (60,00-100,00)	8,85	
Knowledge						
Lack (<70)	122,59 (100,00-140,00)	9,88	0,070	82,21(60,00-100,00)	9,52	0,076
Good (≥70)	121,89 (100,00-140,00)	9,31		81,44 (60,00-95,00)	7,27	

Table 2 presents results from statistic test against differences in mean BP on modifiable risk factor variables. The only statistically significant variables were sleep duration against SBP (R^2 = 0,049) and DBP (R^2 = 0,061). Whereas, other modified risk factors such as dietary habits-protein (SBP R^2 = 0,065; DBP R^2 = 0,066), fast-food (SBP R^2 = 0,070; DBP R^2 = 0,072), snacks (SBP R^2 = 0,058; DBP R^2 = 0,075), beverages (SBP R^2 = 0,069; DBP R^2 = 0,076), physical activity (SBP R^2 = 0,070; DBP R^2 = 0,076) and knowledge (SBP R^2 = 0,070; DBP R^2 = 0,076) did not show significant results, meaning there was no difference in mean BP between categories in these variables against SBP and DBP.

Table 2 Comparison of Blood Pressure (Systolic, Diastolic) Based on Modifiable Factors in EPC Workers

Variable	SBP			DBP		
	Mean (Min-Max)	SD	R^2	Mean (Min-Max)	SD	R^2
Sleep duration						
Abnormal (<6 hours or >8 hours)	125,15(110,00-140,00)	8,57	0,049*	84,71 (75,00-95,00)	6,02	0,061
Normal (6-8 hours)	120,71(100,00-140,00)	9,79		80,32 (60,00-100,00)	9,28	
Dietary habit - Protein (red meat)						
Frequently	124,09 (100,00-140,00)	10,21		81,63(60,00-100,00)	8,59	
Rarely	122,03 (110,00-140,00)	9,53	0,065	83,64 (70,00-100,00)	8,09	0,066
Never	0	0		0	0	
Dietary habit - Fast Food						
Frequently	140,00(105,00-140,00)	0,00		90,00 (60,00-100,00)	0,00	
Rarely	121,76 (100,00-140,00)	9,38	0,070	81,41(60,00-100,00)	8,07	0,072
Never	124,55(140,00-140,00)	10,11		84,54(90,00-90,00)	11,51	
Dietary habit - Snacks						
Frequently	121,49 (105,00-140,00)	10,52		82,39 (60,00-95,00)	8,81	
Rarely	123,06 (100,00-140,00)	8,59	0,058	81,33 (60,00-100,00)	8,27	0,075
Never	0	0		0	0	
Dietary habit - Beverages (coffee, Soda, Alcohol)						
Frequently	117,28 (100,00-140,00)	10,09		78,18(60,00-90,00)	8,15	
Rarely	123,32 (100,00-140,00)	9,33	0,069	82,87(60,00-100,00)	8,52	0,076
Never	121,39 (110,00-140,00)	9,67		80,28 (70,00-90,00)	8,31	
Physical activity						
Not fit the recommendation (<600)	122,75(110,00-140,00)	9,20	0,070	81,88 (70,00-100,00)	8,14	0,076
Fit the recommendation (≥600)	121,93 (100,00-140,00)	9,90		81,84 (60,00-100,00)	8,85	
Knowledge						
Lack (<70)	122,59 (100,00-140,00)	9,88	0,070	82,21(60,00-100,00)	9,52	0,076
Good (≥70)	121,89 (100,00-140,00)	9,31		81,44 (60,00-95,00)	7,27	

DISCUSSION

Our findings showed that dietary habit, mean of BP among workers is higher in frequently consumption of fast food and beverages such as coffee, alcohol, and soda. Supporting to our findings, Ugorji [4] stated that the implementation of the Dietary Approach to Stop Hypertension (DASH) intervention program for African-American women with hypertension doing a healthy diet by consuming fruits, vegetables, low-fat dairy products and increasing potassium intake can reduce SBP by 8-14 mmHg and reduce sodium intake by consuming no more than 100 mmol (2.6 grams of sodium or 6 grams of sodium chloride) per day can reduce SBP by 2-8 mmHg [4,5].

In our study, similar with a study conducted by Bosu [6], workers with physical activity which unmeet with WHO recommendations (MET < 600) have a greater average blood pressure compared to the group with WHO recommendations of physical activity (MET ≥600). Bosu [6] suggested that the prevalence of hypertension was significantly higher in people with low physical activity compared with people who performed moderate or intense physical activity. This applies to workers with high activity demands such as car workers, plant operators and planter who have a lower prevalence of hypertension compared to workers in individually task such as traders, executives, and civil service [6,7]. In sleep duration, the mean difference of SBP and DBP in respondents who had abnormal sleep duration was higher

than respondents who had normal sleep duration, similar to Gangswisch study [8]with widespread consequences for health and safety.(1. Sleep duration of ≤5 hours per night was significantly associated with increased risk of hypertension (hazard ratio, 2.10; 95%, CI, 1.58-2.79) with the age range of the study subjects as 32-59 years.

This study underlines that workers with lack of knowledge about hypertension have higher SBP and DBP compared to workers with good knowledge. The earlier study showed that knowledge about hypertension is better for participants who have control than participants who did not, then the results of the logistic regression model analysis showed that men participants (OR = 1.31) with better knowledge of hypertension (OR = 1.19), awareness of suffering from hypertension (OR = 2) and adhering to regular treatment (OR = 1.48,) are more likely to control hypertension.

Our findings highlight several modifiable factors which are sleep duration, dietary habit, physical activity, and knowledge, only the sleep duration variable, have a correlation with systolic blood pressure with $R^2 = 0{,}049$. The correlation between sleep duration and high BP had been examined in Song [9]. This study revealed that respondents who had sleep duration <6 hours had higher mean SBP (111.9 ± 0.9) compared with respondents who had a duration of sleep 6 - 8 hours (108.9 ± 0.3) and >8 hours (106.7 ± 0.7) with $R^2 = 0{,}049$, while the mean DBP was also related to sleep duration, respondents with sleep duration <6 hours had the highest mean diastolic blood pressure (73.8 ± 0.6) with $R^2 = 0{,}061$. It occurs due to excess sympathetic nerve activity after a short period of sleep, if short duration of sleep continues, structural adaptations such as left arterial and ventricular hypertrophy can occur due to an increase in hemodynamic load, so that the cardiovascular system is adjusted to maintain a higher blood pressure balance. Several other studies have shown that this finding usually occur in women, the mechanism has not been clearly identified, but this may be due to hormonal and psychosocial changes associated with menopausal [9] . Moreover, it is suggested that an increase in systolic blood pressure due to insufficient sleep can be related to the risk of isolated systolic hypertension in the future, besides the possibility of decreasing sleep duration can be related to hardening of the material which will cause arteriosclerosis and isolated systolic hypertension [10].

CONCLUSION

The findings show that the majority of workers' BP is determined prehypertension based on BP category by JNC-7. Evidence of this study indicates that modified risk factors (abnormal sleep duration, the frequency of protein consumption, proper physical activity and lack of knowledge about hypertension) contribute to higher of worker's blood pressure. Data analysis signify that there were significant differences in the mean SBP in workers in the form of sleep duration.

Conflict of Interest: Hereby the authors declared that there is no conflict of interest in this research with any other parties.

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Ethical Clearance: This research has been approved by Ethical Board Committee, Faculty of Public Health University Indonesia and has been approved for ethical clearance by Ethical Board Committee, Faculty of Public Health University Indonesia.

REFERENCES

1. Chockalingam A, Campbell NR, Fodor JG. Worldwide epidemic of hypertension. Can J Cardiol. 2006;22(7):553–5.
2. Kim I-G, So W-Y, Sung DJ. The relationships between lifestyle factors and hypertension in community-dwelling Korean adults. J Phys Ther Sci [Internet]. 2015;27(12):3689–92. Available from: https://www.jstage.jst.go.jp/article/jpts/27/12/27_jpts-2015-595/_article
3. Legido-Quigley H, Lopez PAC, Balabanova D, Perel P, Lopez-Jaramillo P, Nieuwlaat R, et al. Patients' knowledge, attitudes, behaviour and health care experiences on the prevention, detection, management and control of hypertension in Colombia: A qualitative study. PLoS One. 2015;10(4):1–16.
4. Ugorji J. Developing a Lifestyle Modification Toolkit to Prevent and Manage Hypertension Among African American Women. Walden University; 2014.
5. Ghezelbash S, Ghorbani A. Lifestyle Modification and Hypertension Prevention. ARYA Atheroscler

J. 2012;8(Special Issue in National Hypertension Treatment):202–7.

6. Bosu WK. Determinants of Mean Blood Pressure and Hypertension among Workers in West Africa. J Hypertens. Hindawi Publishing Corporation; 2016;2016:1–19.
7. Tenkorang EY, Kuuire V, Luginaah I, Banchani E. Examining Risk Factors for Hypertension in Ghana: Evidence from the Study on Global Ageing and Adult Health. Glob Health Promot. 2017;24(1):14–26.
8. Ramos AR, Gangwisch JE. Is sleep duration a risk factor for stroke? Neurology. 2015;84(11):1066–7.
9. Song, M. Y., Sung, E., Jung, S. P., Lee, K. M., Keum, S. H., & Ryu SD. The association between sleep duration and hypertension in non-obese premenopausal women in Korea. Korean J Fam Med. 2016;37(2):130–4.
10. Angles J, Kenarsary A, Paddock S, Schuck Z. The Physiological Relationship of Sleep Duration with Blood Pressure , Heart Rate , and Reaction Time.

Phenotypic and Molecular Study of *mecA* Gene in MRSA Isolated from Clinical Cases in Misan Province /Iraq

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ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a common strain of *S. aureus* caused infections lead to increased of risk in clinical outcomes and death, and compared with infections caused by non-resistant strains of the same bacteria patients consume more resources of health-care, where their hospitalization was prolonged because most preferred drugs such as β-lactam antibiotics not have the ability to produce bactericidal effect on this bacteria. Therefore this study aimed to: Isolation and identification of MRSA by using conventional and confirmatory techniques, detection of phenotypic traits related to their antibiotic resistance and estimation the occurrence of *mecA* gene in identified bacteria. A total of 274 samples, were collected from local hospitals in Misan Province during the period from December 2017 to April 2018. Diagnostic results using microscopic, cultural, biochemical tests and vitek 2 system proved that 106 bacterial isolates belong to *Staphylococcus aureus.* The susceptibility test of *S. aureus* isolates was performed toward many types of antibiotics, from all isolates 99(93.4%) were determined as MRSA by oxacillin antibiotic and 93(87.7%) were determined as MRSA by cefoxitin antibiotic. The results of molecular estimation of *mecA* gene showed that 89(89.9%) and 91(97.8%) from phenotypically positive MRSA isolates had this gene for oxacillin and cefoxitin antibiotics respectively.

Keywords: *Staphylococcus aureus, MRSA, Antibiotic resistance, mecA gene.*

INTRODUCTION

Methicillin-Resistance *Staphylococcus aureus* (MRSA) is a pathogenic bacteria causing diseases extended from surface skin and tissue infections to severe and lethal infections.[1,2]It has been generally considered as a public health problem, thus it was classified into two different groups, community-acquired (CA-MRSA) and healthcare-acquired (HA-MRSA).[3,4]

MRSA has the capacity to rapidly develop drug resistance.[5] Because it can develop several mutations conferring it high resistance for wide range of antibiotics. [6] Penicillin binding proteins (PBPs) involved in the collecting of the peptidoglycan in bacterial cell wall. These proteins have a high affinity to β-lactams, by bind these antibiotics the active site of PBPs thus cell wall synthesis inhibited.

PBP2a alternative proteins with low tropism to these antibiotics.[7] So PBP2 continues in cell wall synthesis even in the presence of high concentrations of β-lactam antibiotics.[8] These proteins encoded by *mecA* gene, which carrying on a mobile genetic element called Staphylococcal chromosomal cassette *mec* (SCC*mec*).[9] The transcription of this gene regulated by two regulatory systems that mecI-mecR1 and the penicillinase blaR1-blaI-encoded regulatory elements.[10] The majority of researches in this field suggested that *mecA* gene presented in all MRSA strains.[11] Thus it considered the corner stone responsible for MRSA phenomenon.[11] Also MRSA produce a β-lactamase enzyme, which damages the functional perfection of the β-lactam antibiotics by split the β-lactam ring of penicillin molecules.[12]

MATERIALS AND METHOD

Collection of samples

A total of 274 samples, were collected from different clinical cases (Burns, Wound, Nasal, Oral, Ear, Throat, Vaginal, Bedsore and Urinary tract infections) from local hospitals in Misan Province during the period from December 2017 to April 2018. Collected swabs were

streaked on mannitol salt agar then incubated at 37C° for 24 hours, the suspected colonies depending on the morphological bases were selected for the identification by conventional methods and Vitek2 system.

Antimicrobial susceptibility test

Susceptibility testing was determined by the agar disk diffusion method,[13] and national committee for Clinical and Laboratory Standards Institute guidelines,[14] using antibiotics utilizes as conventional therapy for MRSA infections.

Extraction of bacterial chromosomal DNA

DNA extraction was done toward *S. aureus* isolates according to Presto™MinigDNA Bacteria Kit protocol. The Chromosomal DNA then subjected to monoplex PCR. The integrity of extracted DNA was tested using Agarose Gel Electrophoresis.

Molecular estimation of *mecA*gene using PCR technique

The protocol used depending on Bioneer manufacturer's instructions. All PCR components were assembled in PCR tube. The specific primer for *mecA* gene have been choose according to [15] as in table (1), was prepared by the company of Bioneer, South Korea.

Table (1): Primers used in this study

Gene	Primer sequences (5' → 3')	Product length (bp)
mecA	F: CGGTAACATTGATCGCAACG R: TTTGCCAACCTTTACCATCG	985

Then template DNA and primers were added into the AccuPower®. Taq premix tubes for get 20ml reaction volume.Eppendorf PCR tubes were placed in the thermocycler (Eppendorf, Germany) and the right PCR cycling program parameter conditions were conducted as in table (2), then seven microliters of amplified PCR product were loaded to the agarose gel wells with standard molecular weight of DNA ladder was loaded in one well and running using TBE 1X buffer was added to the electrophoresis tank, tray with agarose was immersed in electrophoresis tank. Electrophoreses run at 65 volt for 120 min, the gel stained with 0.5 µg/ml of ethidium bromide was visualized by Gel Documentation.[16]

Table (2): PCR cycling program parameter conditions

Genes	Temperature °C/ time					Cycles number
	Initial denaturation	Cycling conditions			Final extension	
		Denaturation	Annealing	Extension		
mecA	95/5 min	95/45 sec	55/1 min	72/2 min	72/7 min	35

RESULTS AND DISCUSSION

Susceptibility test

The results of susceptibility testing showed that all *S. aureus* isolates were multi-drug resistant (MDR) when done toward 18 antibiotics from different groups, therefore the percentage of resistance of these isolates is that; ß-lactam group: Methicillin, Ampicillin, Oxacillin, Cefoxitin, Cefotaxime which showed percentage of resistance were 100, 98.1, 93.4, 87.7, 84% respectively, to Erythromycin was 80.2%, to Tetracycline and Doxycycline were 79.2, 56.6% respectively, and to Trimethoprim-sulfamethoxazole the resistance was 71.7%.

The resistance to Gentamicin and Amikacin appeared in 63.2, 49.1% of *S. aureus* respectively and the rate of resistance was 61.3% to Rifampin. *S. aureus* isolates also were resistant to Levofloxacin and Ciprofloxacin in rate 59.4, 49.1% respectively, to Clindamycin was 51% and to Vancomycin was 50%. The rate of resistance to Imipenem was 47.2%, while to Nitrofurantoin was 38.7%.

The bacterial isolates show a contrast in antibiotics resistance, due to the diversity in the mechanisms of resistance of *S. aureus* isolates such as the production of PBP2a and ß-lactamase enzymes that inhibit these antibiotics.[17] The reason for the other depends on the virulence factors of bacteria, where some isolates show amount of virulence more than others,[18] as well as the difference in the sources of sampling, tests circumstances and the type of techniques that used in the study, all these combined may lead to a difference in resistance and sensitivity levels.[19] the point of high resistance is noticeable, because it may lead to failure in therapy, extended disease, increased health care expenses and death.[20]

Out of 106 *S. aureus* isolates 99(93.4%) and 93(87.7%) were identified as MRSA determined by oxacillin and cefoxitin antibiotics respectively, because for susceptibility testing now typically uses oxacillin and/or cefoxitin.[21] Oxacillin can be considered a representative antibiotic for susceptibility test to all β-lactams.[4] Cefoxitin also used for MRSA detection, because it is a powerful influence of *mecA* gene that shows to be less affected than oxacillin by isolates have the ability to produce penicillinase excessively.[21]

Molecular estimation of *mecA*

Polymerase chain reaction technique (PCR) utilize in the amplification of *mecA* gene. This gene is one of the most common and important virulence genes in *S. aureus* where it considered the primarily gene responsible for the widely antibiotic resistance, therefore was selected in this study. Also the molecular amplification of this gene is recognized as a hallmark to diagnose MRSA because these genes are highly conserved among these bacteria.[11]

The results for *mecA* gene detection illustrate that 89(89.9%) and 91(97.8%) from phenotypically positive MRSA isolates had this gene for oxacillin and cefoxitin respectively, figure (1-A,B).In present study the occurrence of *mecA* gene was higher than that obtained by other researchers such as[22-26] which were (18.8, 29, 39.2, 45.1, 54.5%) respectively. While other studies revealed that the percentage of incidence of *mecA* gene has been more than this study such as[11, 27-30] which were (90.2, 95, 96, 98.1, 98.3%) respectively.

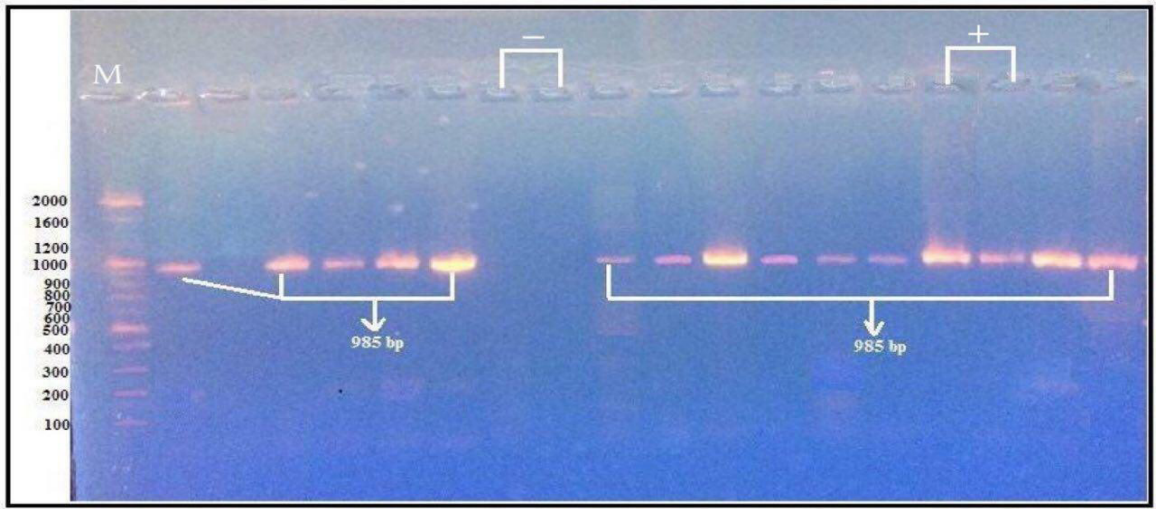


Figure 1-A: Ethidium bromide-stained Agarose Gel Electrophoresis of *mecA* gene of MRSA isolates, Lane M DNA molecular size Ladder (100-2000)bp, Lane with positive marker refer to the isolates that have *mecA* gene, Lane with negative marker refer to the isolates that not have this gene.

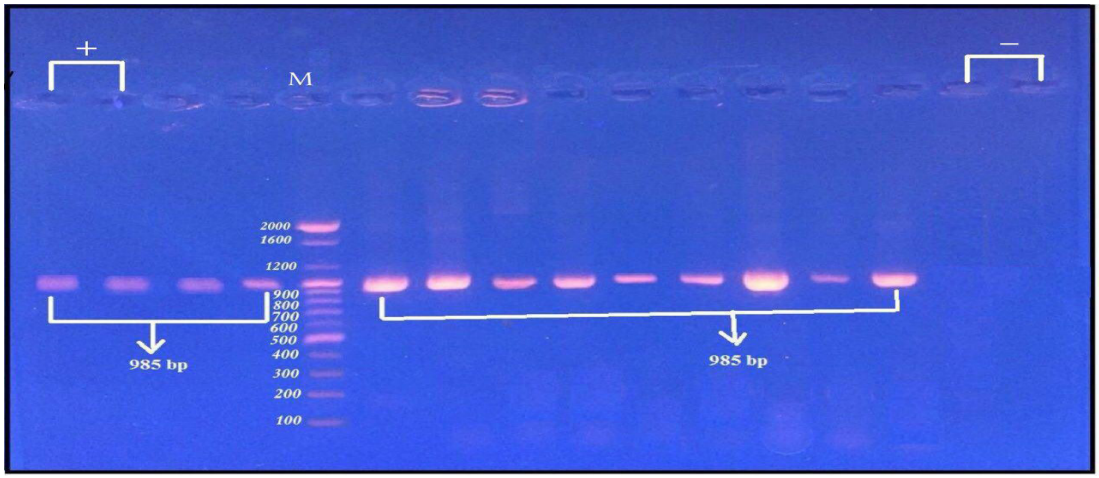


Figure 1-B: Ethidium bromide-stained Agarose Gel Electrophoresis of *mecA* gene of MRSA isolates, Lane M DNA molecular size Ladder (100-2000)bp, Lane with positive marker refer to the isolates that have*mecA*gene, Lane with negative marker refer to the isolates that not have this gene.

According to the results in present study we can concluded that cefoxitin is more reliable than oxacillin in MRSA identification, and this confirmed by study conducted by.[31] Therefore it currently recommended by CLSI (2017) for MRSA detection.

S. aureus which phenotypically positive MRSA while gave a negative results to *mecA* gene, this could be referred they have an alternative mechanism instead of this gene for antibiotic resistance including the alteration of the penicillin binding proteins (PBPs) leading to the hyper-production of methicillinase or β-lactamase,[14] subsequently this can explain why not all isolates that resistant to β-lactams not have this gene.

The studies has been showed that 90% of *S. aureus* resist penicillins due to β-lactamase (penicillinase) production,[33] also[34] confirmed that resistance to penicillins occur also by the production of trans-membrane proteins associated with the cell wall. These proteins represent enzymatic activity such as Transpeptidases and carboxypeptidases a target of penicillins and cephalosporins, where it works to change the target of ß-lactams.[35] The study of [36] affirmed the role of plasmids in the production of β-lactamase enzymes and their responsibility on the encoding of some PBP2a, and study by[33] has been linked between the resistance to antibiotics and sources responsible for this resistance, where they were found that most of the resistance shown by the strains of *S. aureus* has plasmid origin.

CONCLUSIONS

MRSA strains have a high resistance to ß-lactam group and most other antibiotics,the greatest antibiotic resistance in this study is returned to the isolates that taken from burns and wounds,cefoxitin antibiotic is more reliable than oxacillin antibiotic in MRSA detection and the *mecA* gene was high prevalence within MRSA strains.

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Ethical Clearance: It is no behalf of authors certify that the research conducted after being got official ethics clearance.

REFERENCES

1. Yang Y, Hu Z, Shang W, Hu Q, Zhu J, Yang J, Peng H, Zhang X, Liu H, Cong Y, Li S, Hu X, Zhou R, Rao X. Molecular and phenotypic characterization revealed high prevalence of multidrug-resistant methicillin-susceptible *Staphylococcus aureus* in Chongqing, Southwestern China. Microb Drug Resist 2017; 23(2): 241–246.
2. Ezeamagu C, Imanatue I, Dosunmu M, Odeseye A, Baysah G, Ania D, Odutayo F, Agyei G. Detection of methicillin resistant and toxin-associated genes in *Staphylococcus aureus*. J Basic and ApplSci 2018; 7(1): 92-97.

3. Grumann D, Nupübel U, Bröker BM. *Staphylococcus aureus* toxins-their functions and genetics. Infect Genet Evol 2014; 21: 583-592.

4. Loftus, RW, Dexter F, Robinson, ADM. Methicillin-resistant *Staphylococcus aureus* has greater risk of transmission in the operating room than Methicillin-sensitive S. aureus. Am J Infect Control 2017; 46(5): 520-525.

5. Zhanel GG, Love R, Adam H, Golden A, Zelenitsky S, Schweizer F, Gorityala B, Wiens PRS, Rubinstein E, Walkty A, Gin AS, Gilmour M, Hoban DJ, Lynch JP, Karlowsky JA. Tedizolid: a novel oxazolidinone with potent activity against multidrug-resistant gram-positive pathogens. Drugs 2015; 75(3): 253-270.

6. Bradley P, Gordon C, Walker TM, Dunn L, Heys S, Huang B, Earle S, Pankhurst LJ, Anson L, Cesare M, Piazza P, Votintseva AA, Golubchik T, Wilson DJ, Wyllie DH, Feuerriegel S, Kohl TA, Ismail N, Omar SV, Smith EG, Buck D, McVean G, Walker AS, Peto TE, Crook DW, Iqbal Z. Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*. Nat Commun 2015; 6:10063.

7. Müller S, Wolf AJ, Lliev LD, Berg BL, Underhill DM, Liu GY (2015). Poorly cross-linked peptidoglycan in MRSA due to *mecA* induction activates the inflammasome and exacerbates immunopathology. Cell Host Microbe 2015; 18(5): 604-612.

8. Fishovitz J, Hermoso JA, Chang M, Mobashery S. Penicillin-Binding Protein 2a of Methicillin-Resistant *Staphylococcus aureus*. IUBMB Life 2014; 66: 572-577.

9. Liu J, Chen D, Peters BM, Li B, Xu Z, Shirliff ME. Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant *Staphylococcus aureus*. MicrobPathog 2016; 101: 56-67.

10. Chovanová R, Mikuláová M, Vaverková S. Modulation of mecA Gene Expression by Essential Oil from Salvia sclarea and Synergism with Oxacillin in Methicillin Resistant *Staphylococcus epidermidis* Carrying Different Types of Staphylococcal Chromosomal Cassette mec. Int J Microbiol 2016; 10.

11. Elhassan MM, Ozbak HA, Hemeg HA, Elmekki MA, Ahmed LM. Absence of the *mecA* Gene in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Specimens in Shendi City, Sudan. Biomed Res Int 2015; 5.

12. Waters EM, Rudkin JK, Coughlan S, Clair GC, Adkins JN, Gore S, Xia G, Black NS, Downing T, Neill EO, Kadioglu A, O'Gara JP. Redeploying β-lactam antibiotics as a novel antivirulence strategy for the treatment of Methicillin-resistant *Staphylococcus aureus* infections. J Infect Dis 2016; 215(1): 80-87.

13. Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Path 1966; 45: 493-496.

14. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M100-27th ed. Wayne, PA, USA; 2017.

15. Stotts SN, Nigro OD, Fowler TL, Fujioka RS, Steward GF. Virulence and antibiotic resistance gene combinations among *Staphylococcus aureus* isolated from coastal waters of Oahu, Hawaii. J Young Invest 2005; 12: 1-8.

16. Mishra AK, Sharma K, Misra RS. Isozyme and PCR-based genotyping of epidemic *Phytophthoracolocasiae* associated with taro leaf blight. Archives of Phytophathology and Plant Protection 2010; 43(14): 1367-1380.

17. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJV. Understanding the mechanisms and drives of antimicrobial resistance. Lancet 2016; 387(10014): 176-187.

18. Kumar N, David MZ, Boyle-Vavra S, Sieth J, Daum RS. High *Staphylococcus aureus* Colonization Prevalence among Patients with Skin and Soft Tissue Infections and Controls in an Urban Emergency Department. J ClinMicrobiol 2015; 53(3): 810–815.

19. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. PerspectMedicinChem 2014; 6: 25-64.

20. Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: An emerging crisis. InterdiscipPerspect Infect Dis 2014; 7.

21. Wu MT, Burnham CA, Westblade LF, Dien BJ, Lawhon SD, Wallace MA, Stanley T, Burd E, Hindler J, Humphries RM (2016). Evaluation of

Oxacillin and Cefoxitin Disk and MIC Breakpoints for Prediction of Methicillin Resistance in Human and Veterinary Isolates of *Staphylococcus intermedius* Group. J ClinMicrobiol 2016; 54(3): 535-542.

22. Martini R, Hörner R, Graichen DÂS. Antimicrobial susceptibility profile and research of *mecA*and *erm* genes in coagulase-negative staphylococci isolated from platelet concentrates bags. Braz J Pharm Sci 2017; 53(1): 15195.
23. Abbas A, Srivastava P, Nirwan PS. Prevalence of MLSB Resistance and Observation of erm A & erm C Genes At A Tertiary Care Hospital. J ClinDiagn Res 2015; 9: 8-10.
24. Rajabiani A, Kamrani F, Boroumand MA, Saffar H. mec-A-mediated Resistance in *Staphylococcus aureus* in a Referral Hospital, Tehran, Iran. Jundishapur J Microbiol 2014; 7: 9181.
25. Pournajaf A, Ardebili A, Goudarzi L, Khodabandeh M, Narimani T, Abbaszadeh H. PCR- based identification of methicillin resistant *Staphylococcus aureus* strains and their antibiotic profiles. Asian Pac J Trop Biomed 2014; 4(1): 293-297.
26. Elkenany RM. Genetic Characterization of Enterotoxigenic Strains of Methicillin-Resistant and Susceptible *Staphylococcus aureus* Recovered from Bovine Mastitis. Sci Alert 2018; 11: 1-8.
27. Kuar DC, Chate SS. Study of Antibiotic Resistance Pattern in Methicillin Resistant *Staphylococcus aureus* with Special Reference to Newer Antibiotic. J Glob Infect Dis 2015; 7(2): 78-84.
28. Eshetie S, Tarekegn F, Moges F, Amsalu A, Birhan W, Huruy K. Methicillin resistant *Staphylococcus aureus* in Ethiopia: a meta-analysis. BMC Infect Dis 2016; 16: 689.
29. Becker K, Denis O, Roisin S, Mellmann A, Idelevich EA, Knaack D, Alen S, Kriegeskorte A, Köck R, Schaumburg F, Peters G, Ballhausen B. Detection of mecA- and mecC-Positive Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates by the New Xpert MRSA Gen 3 PCR Assay. J ClinMicrobiol 2016; 54(1): 180-184.
30. Ito H, Sato K, Koganezawa M, Ote M, Matsumoto K, Hama C, Yamamoto D. Fruitless Recruits Two Antagonistic Chromatin Factors to Establish Single-Neuron Sexual Dimorphism. Cell 2012; 149(6): 1327-1338.
31. Sharma S, Srivastava P, Kulshrestha A, Abbas A. Evaluation of different phenotypic methods for the detection of methicillin resistant *Staphylococcus aureus* and antimicrobial susceptibility pattern of MRSA. Int J Community Med Public Health2017; 4(9): 3297-3301.
32. Xu L, Liang W, Wen Y, Wang L, Yang X, Ren S, Jia N, Zuo X, Liu G. An ultrasensitive electrochemical biosensor for the detection of mecA gene in methicillin-resistant *Staphylococcus aureus*. BiosensBioelectron 2017; 99: 424-430.
33. Yu F, Liu Y, Qi X, Lu C, Ding Y, Li D, Liu H, Wang L. Antimicrobial susceptibility, virulence determinant carriage and molecular characteristics of *Staphylococcus aureus* isolates associated with skin and soft tissue infections 2015; 19(6): 614- 622.
34. Akindolire MA. Detection and Molecular Characterization of Virulence Genes in Antibiotic Resistant *Staphylococcus aureus*from Milk in the North West Province. Ph.D. Thesis, North West University, Mafikeng, North West Province, South Africa; 2013.
35. Munita JM, Arias CA (2016). Mechanisms of Antibiotic Resistance. MicrobiolSpectr 2016

A Solution for Nosocomial Infection in Healthcare Facilities

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ABSTRACT

Cross-infections in healthcare facilities, collectively known as Nosocomial Infection (NI), remain a global issue. Numerous people in Healthcare facilities (HFs) die all the time due to the spread of such diseases, specially NIs. Governmental HFs are burdened with increased expenses as a result of the unchecked transmission of cross-infections across their departments. This conceptual paper, firstly, attempts to present the severity of NI prevalence and propose a hypothesis in discussions of the role of HF layouts in controlling such cross-infections. Lastly, it focuses on the comprehension for controlling of the risks of cross-infections transiting between HF departments and facilities.

Keywords: *Nosocomial infections, cross infection, healthcare facility, Risky departments.*

INTRODUCTION

A healthy societal culture is one that is educated, work-oriented, and makes contributions to the arts while providing services that are usually inaccessible to less healthy societies [1, 2]. Therefore, societies have developed obligations for provisioning better health services to every patient [1, 3]. Among such measures would be the establishment of more Healthcare Facilities (HF) units within various regions throughout the country, which could lead to increased prosperity that brings improvements to people's quality of life [4].

For every healthcare facility, there are certain problems that can affect institutional service quality. Increasing healthcare-related errors, injuries among workers, and decreasing patient recovery can all lead to increased nursing and other staff turnover, loss of work time, and further disabilities. Increasing costs as well as infection rates are among the problems which every HF experiences as a result [5]. Of these, Nosocomial Infections (NIs) are among the main hurdles facing HFs and the medical establishment. This article intends to scrutinise the risk of NIs.

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MATERIAL AND METHOD

The current study can be classified as a qualitative research. The valuation of research literature made through reading of published manuscripts from scholarly database. Near 50 manuscripts was studied which comprises articles from the national and global studies with considerable effect on the topic.

First, the literature review tries to present fundamentals of cross-infection especially in HFs. Then, the review of the literature focused on the role of HF layout in controlling of NI risk. Lastly, based on the existing literature, the knowledge gap in this area is highlighted.

FINDING AND DISCUSSION

'Nosocomial' as a term derives from the Greek wording of '*Nosos*' in reference to 'disease', along with '*Komeion*', which refers to care [6]. NI, also termed as HF cross-infection, comprises a type infectious process whereby patients acquire infections during hospitalisations [6-10]. 'Nosocomial' describes a disease that is inflicted on patients subject to treatments for other ailments or else undergoing general healthcare [7, 11]. Berket [7] noted that NIs are clinically observed in hospitalised patients or else in subjects who have been discharged for a few days.

A fuller understanding regarding cross-infections followed the early breakthroughs of Pasteur, Koch, and Lister, during the advent of the 'Bacteriological Era' of modern science. Microorganisms were found to transfer between objects and persons across sites [12]. With late 19th-century medical progress came great Western advances in disinfection and treatment via asepsis. These appeared to signal a final triumph over cross-infections, an achievement that was short-lived however [13]. Moreover, as the initial model for this study, the existence of cross-infections in healthcare facilities was first reported by Cruickshank [14] (as mentioned by Ayliffe and Lilly [15]) in his description of *Streptococcus pyogenes* infections among patients admitted to hospitals. The report by Cruickshank [14] remains the foundational basis for studies of NIs, which this paper also covers. Modern primary care units continue to host dense populations of very ill subjects that are exposed to far greater risks regarding cross-infection [16]. NI prevalence is considered to be a primary socio-economic and medical issue that affects all developing as well as developed countries [17, 18].

By 1941, memoranda regarding the prevention of NIs in wounds were advising that each hospital appoint '. . . full-time specialised officers to supervise infectious disease control . . .' [19]. By 1944, it was being suggested that all hospitals establish representative committees for doctor, nursing, and laboratory staff as well as administrators, for investigating and designing measures to manage cross-infections [19]. The NI pandemic of *Staphylococcus aureus* from the 1940s through to the 1950s resulted in the mandating of further guidelines [19]. Jacoby [20] noted that cross-infections remain a critical problem in HF management. Fisher [21] noted that medical decision-making should regard a germicide's value (capacity for killing pathogens) on its ability to fight cross-infections and not in terms of price alone.

With several hospitals for communicable diseases being operational in the last century, soon it was realised that contagions occurred not only in surgical and obstetric patients (which was emphasised in the later part of the 19th century) but also in the medical patients. Soon it was realised that several bacterial, as well as viral, contagions spread in HFs and brought about these contagions [13]. Also in the recent years, Hertzberg [22] highlights that the threat of spreading of the infections in certain HF units, for instance, SARS (Severe Acute Respiratory Syndrome) epidemic that occurred in 2003, wherein there were 128 instances of patients getting infected from direct or indirect exposure to a patient of SARS [23]. Recently, the admission of a patient infected with Ebola virus to HFs in Dallas, Texas, gave rise to a need to test and supervise more than 180 other individuals, among whom several were hospital staff, who had been in close contact with the Ebola virus patient or with 2 other nurses who got infected after being exposed to that patient [22].

Studies have mentioned that several people are infected with NIs across the globe [24, 25]. The infection from dispersal of microorganisms in NIs, HFs, cause an unwanted and a grave threat to the healthcare system quality in the nation [17, 18]. NIs extend the stay of the HF, causing long-term ailment for the patient, increasing the resistance of the patients to the antimicrobials, can result in death, raises the cost of the HF to be borne by the patients and their families and bring about a tremendous monetary burden on the nation's present healthcare system [18, 26]. On a yearly basis about 400,000 - 600,000 NIs impact the German patients, causing 10,000 – 15,000 deaths [27, 28]. NIs are to blame for 37,000 demises in Europe annually [26]. Also, 722,000 severe NI cases were reported in the US HFs in the year 2011 [29, 30]. Based on a 2016 report, it was stated that, in Pennsylvania, around 247 HFs had provided data (about NIs) to the National Healthcare Safety Network for the duration of a year [31]. Also, on any specified day, 1 patient out of every 25 in the HFs would be having at least 1 NI [29, 30]. NIs also causes huge economic losses each year. In Europe, these total about €7 billion, which involves direct expenses and causes an additional 16 million-day stay at the HF; also the United States of America registered a loss of $6.5 billion due to NIs [26]. Riu, Chiarello[32] calculated the expenditure of 12 hospitals in Spain and noticed that just NIs had been responsible for 64.2 percent of the total expenses of opposing occurrences.

Nowadays, the growth of antimicrobials use and improvements in the medical procedures have caused several invasive processes being utilised on the patients, which in turn increases the risk of new NIs [28]. These diseases are still regarded as a hazard to this day in the age of modern antibiotics [7, 33]. Congestion in the hospitals, lags in implementing extra precautions, crowding and combining patients having contagious diseases, shifting of patients from one part of the hospital to another, defects in the building structure, insufficient staff preparation and scarcity of personal safety devices

also play an important role towards the threat of cross-infections [34].

The HF environment is contaminated by several types of infectious organisms and moreover it is a store of the nosocomial microbes that could contaminate the patients at the time of their HF stay [35]. Khan [36] mentioned that, while the rates of infection in the various HFs cannot be determined exactly, one can get a rough approximation of these rates based on several dependent parameters like the sort of HFs (i.e., private or public), service offered by these HFs, etc. [36].

Hospital is an instance of a multi-product establishment which provides several facilities like patient care, fitness promotion, training of health personnel, and even assist research regarding health-related issues [3, 37]. Every HF structure (such as the hospital) accommodates many divisions which offer their specific services [38]. For having a valid HF, several other factors like the quality of design of the HF blueprint must also be taken into account. A good and accommodating HF environment avoids further injuries and also offers help and emotional support to the sick persons during the recuperation process [5].

The physical structure of the HF contributes significantly in regulating NIs and reducing the risk of infection transmission [39]. Nonetheless, there are several variations in the layout of the HF around the globe, and regulation of infections by using specific engineering structures is still a matter of debate [40]. The technical research is concentrated on resolving the way in which an enhanced design can minimise the threats in the HFs [41]. The essential safety risk considerations must be made at the time of the planning phase and must be a recurrent process at the time of reviewing the design [38]. Now, it has been understood that the measures for regulating infections must be combined with the design and development of HF structures together with its operations [42]. These days, a lot of focus is given to the HF environment and its correlation with health of the patient, which has brought about the development of efficient blueprints [43]. Risk of NIs due to shifting of the patients to another hospital or area or to another division in the same facility has been recorded [44]. As per Ong [45], cross-infections can take place via transmission of microbes from personnel-to-personnel, patient-to-patient and department-to-department. With regards to the NIs, certain departments are impacted more than the others, and few can be the causes because of the existence of cross-infection. The cause and effect department are termed as risky. Therefore, risky departments are separated from one HF to another; it can be determined from Ducel [46] who substantiate the impact of extent of prevention, policies, type, size, technology and other parameters related to HFs. Although there is considerable amount of evidence which connects the HF environment to the transmission of infections, it is dispersed among several domains and is yet not systematically evaluated [47].

CONCLUSION

Therefore, it can be summarised that for every government of countries and HF, the control of NIs is a significant issue. The theory described in this article implies that NIs has a long history, but till now, no decisive way has been found to minimise the severity of risks for them. In relation to this field, some advancements have been made in medicine and other science, which the scientists believe require more research. The major challenge is that these infections can get transmitted across HFs and various departments. There is a considerable gap in the endeavour to find a solution for the prevention of this problem. In addition, as mentioned in this article, the HFs layout has a critical role in controlling of NIs. The discovery of a solution in this field, specifically for the departments at high risk, will prove to be more beneficial and appropriate.

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REFERENCES

1. Wenzel R, Bearman G, Brewer T, Butzler B. A guide to infection control in the hospital Fourth ed. Boston: International Society for Infectious Diseases; 2008.
2. Parsia Y, Tamyez PF. ROLE OF HEALTHCARE-FACILITIES LAYOUT DESIGN, HEALING ARCHITECTURE, ON QUALITY OF SERVICES. International Journal of Civil Engineering and Technology (IJCIET) 2018;6(7):80-92.
3. Phiri M. Health Building Note 00-01 general design guidance for healthcare buildings. Department of health.; 2014.
4. Ahmadi-Javid A, Seyedi P, Syam SS. A survey

of healthcare facility location. Computers & Operations Research. 2017;79:223-63.

5. Reis P, Chambers B. Planning, design, and construction of health care facilities. Second ed. U.S.A: Joint Commission Resources Mission; 2009.
6. Zerganipour F, Ajami S, Ketabi S, Samimi A. Critical success factors to control nosocomial infection by "wireless sensor network" in intensive care unit. Journal of information technology & software engineering. 2016;6(173):2.
7. Berket W, Hemalatha K, Getenet B, Wondwossen T. Update on bacterial nosocomial infections. European review for medical and pharmacological sciences 2012;16:1039-44.
8. Mohammed M, Mohammed AH, Mirza MABa, Ghori A. Nosocomial infections: an overview. International research journal of pharmacy. 2014;5(1):7-12.
9. Lax S, Gilbert JA. Hospital-associated microbiota and implications for nosocomial infections. Trends in molecular medicine. 2015;21(7):427-32.
10. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance. Asian Pacific Journal of Tropical Biomedicine. 2017;7(5):478-82.
11. Parsia Y, Puteri FMT, Sorooshian S. Microbial Troubles Of Hospitalization. International Journal of Pharmacy & Technology 2017;9(1):28447-50.
12. Krapp, K. The gale encyclopedia of nursing & allied health (Vol. 1). UK: GALE GROUP; 2006.
13. Forder AA. A brief history of infection control - past and present. South African Medical Journal. 2007;97(11):1161-4.
14. Cruickshank R. The bacterial infection of burns. Journal of Pathology and Bacteriology. 1935;41:367-9.
15. Ayliffe GAJ, Lilly HA. Cross-infection and its prevention. Journal of Hospital Infection. 1985;6:47-57.
16. Krapp K. The GALE ENCYCLOPEDIA of Nursing & Allied Health. UK: GALE GROUP; 2006.
17. Adamus W, editor Quality management in hospitals and its role in infections provision. In Proceedings of the International Symposium on the Analytic Hierarchy Process; 2011.
18. Farzianpour F, Bakhtiari A, Mohammadi M, Khosravizadeh O. Analysis of nosocomial infections in selected teaching hospitals, Qazvin, Iran. Health. 2014;6(18):2425-32.
19. Meers PD. The organization of infection control in hospitals Journal of Hospital Infection. 1980;1:187-91.
20. Jacoby NM. A SCHEME FOR THE PREVENTION OF CROSS INFECTION IN CHILDREN'S WARDS. Arch Dis child. 1944;1(1):26-8.
21. Fisher HJ. Concept, Cross-Infection and Concern Hospital Topics. 1977;55(4):3.
22. Hertzberg VS. The Risk of Cross Infection in the Emergency Department: A Simulation Study. infection control & hospital epidemiology 2018;36(6):687-93.
23. Varia M, Wilson S, Sarval S, McGeer A. nvestigation of a nosocomial outbreak of severe acute respiratory syndrome (SARS) in Toronto, Canada. CMAG. 2003;169(14):285–92.
24. Shalini S, Vidyasree MD, Abiselvi A, and, Gopalakrishnan S. Impact and effect of nosocomial infections: a review. . Research journal of pharmaceutical, biological and chemical sciences 2015;6(1):947-51.
25. Khazaei S, Khazaei S, Ayubi E. Importance of Prevention and Control of Nosocomial Infections in Iran. Iranian Journal of Public Health. 2018;47(2):307-8.
26. WHO. Health care-associated infections fact sheet. 2014.
27. Ott E, Saathoff S, Graf K, Schwab F, and, Chaberny IF. The prevalence of nosocomial and community acquired infections in a university hospital. Dtsch arztebl int. 2013;110(31-32):533-40.
28. Behnke M, Hansen S, Leistner R, Diaz L, Alberto Peña, Gropmann A, Sohr D, et al. Nosocomial infection and antibiotic use: a second national prevalence study in Germany. Deutsches Ärzteblatt International. 2013;110(38):627.
29. Adamski C, Daly ER, Dreisig J. State of new hampshire healthcare□associated infections 2014 hospital report. Concord:New Hampshire Department Of Health And Human Services. 2015.

30. Ward RL. Healthcare-associated infections in Utah, 2014 annual report. Salt lake city: Utah department of health. 2015.

31. Centers-for-disease-control-and-prevention. National and state healthcare associated infections-progress report Penmsylvania department of health. 2016.

32. Riu M, Chiarello P, Terradas R, Sala M. Incremental cost of nosocomial bacteremia according to the focus of infection and antibiotic sensitivity of the causative microorganism in a university hospital. Medicine. 2017;96(17).

33. Kouchak Fa, Askarian M. Nosocomial infections: the definition criteria Iran journal medical science. 2012;37(2):72-3.

34. Québec CIN. Infection Prevention and Control Measures in the Emergency Department. BIBLIOTHÈQUE ET ARCHIVES NATIONALES DU QUÉBEC LIBRARY AND ARCHIVES CANADA QUEBE; 2013.

35. Nyamogoba H, and , Obala AA. Nosocomial infections in developing countries: cost effective control and prevention. East African medical journal. 2002;79(8):435-41.

36. Khan HA, Mehboob R, Ahmad A. Nosocomial infections and their control strategies. Asian pacific journal of tropical biomedicine. 2015;5(7):509-14.

37. Padgaonkar AS. Modeling and analysis of the hospital facility layout problem: Faculty of New Jersey Institute of Technology; 2004.

38. Abel LK, Reese A. Planning, Design, and Construction of Health Care Facilities. Third ed. U.S.A: Joint Commission Resources Mission; 2015.

39. Rao LCS. Designing hospital for better infection control : An experience. Med journal armed forces India. 2004;60(1):63-6.

40. Dettenkofer M, Seegers S, Antes G, Motschall E, Schumacher M, Daschner F. Does the architecture of hospital facilities influence nosocomial infection rates? A systematic review. Infection Control & Hospital Epidemiology. 2004;25(1):21-5.

41. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, et al. Architectural design influences the diversity and structure of the built environment microbiome. The ISME journal. 2012;6(8):1469.

42. Stockley, J., Constantine, C., Orr, K.(). Building new hospitals: a UK infection control perspective. Journal of Hospital Infection. 2006; 62(3), 285-299.

43. Elf M, Fröst P, Lindahl G, Wijk H. Shared decision making in designing new healthcare environments—time to begin improving quality. BMC health services research. 2015;15(1):114.

44. Kulshrestha A, Singh J. Inter-hospital and intra-hospital patient transfer: Recent concepts. Indian Journal Anaesthesia. 2016;60(7):451–7.

45. Ong MS, Magrabi F, Post J. communication intervention to improve adherence to infection control precautiona: A randomised crossover trial. BMC Infectious Diseases 2013;13(72):1-9.

46. Ducel, G., Fabry, J., & Nicolle, L. Prevention of hospital-acquired infections: a practical guide (2 ed.): World Health Organization; 2002.

47. Zimring C, Jacob JT, Denham ME, Kamerow DB, Hall KK, Cowan DZ, et al. The role of facility design in preventing the transmission of healthcare-associated infections: Background and conceptual framework. HERD: Health Environments Research & Design Journal. 2013;7(1_suppl):18-30.

Quality of Life Determination among Hemophiliac Children

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ABSTRACT

Objective: To identify the quality of life determination among hemophilic children at al -hakeem general hospital. To find out the relationship between hemophiliac children quality of life and their socio-demographic characteristic of age, gender, parents educational levels, family type and socio-economic status

Methodology: A descriptive study designed to find the quality of life among hemophilic children. The study was carried out the period from March 6th to April 4th 2016.

Conclusion: The result of this study indicated that the quality of live sample of male children with hemophilia was impaired, and that impairment according to the total Hemophilia –Quality of live score was greater among adolescents than children aged 8-12 years. Moreover, number of bleeding event, number of school absences, mother s' education level, severity of hemophilia, insight to health status and insight to suffering from hemophilia were associated with hemophilia –Quality of live. In addition, family income and parent- administration of the product were the factors which predict the quality of live in children with hemophilia. Therefore, in order to improve Hemophilia –Quality of live adolescents, patients who were given drug infusion by third party care givers excluding parents and also patients with lower familial income are suggested to receive more effective interventions by authorities, managers and health care providers. For evidence based practice, further studies on Hemophilia –Quality of live in children are recommended.

Keywards: *Quality of life, Determination, among, Hemophiliac, Children.*

INTRODUCTION

Hemophilia is a group of genetic diseases characterized by reduced to obviously altered blood clotting resulting from a deficiency of one of the factors essential for blood coagulation, Hemophilia is an X-linked congenital bleeding disorder which occurs at a frequency of approximately 1 in every 10,000 births; it is caused by a deficiency in coagulation factor VIII hemophilia A, representing 80–85 % of the total number of diagnosed cases or factor IX hemophilia B, both of which are related to a mutation of the clotting factor gene. The number of affected persons worldwide is estimated to be about 400,000 [1].

The life expectancy among people born with hemophilia who have access to currently available, adequate forms of treatment should approach normal. The rising life expectancy of men living with hemophilia. is related to advances in bleeding prevention and hemostatic control, and the general consensus of experts in the field is that it is becoming increasingly practicable for hemophilia patients to lead more ,for example, to participate in sports activities, have a professional career, and build a family [2].

Hemophilia is a rare, chronic, inherited disease primarily affecting males [1]. It is an X-linked bleeding disorder caused by a defective Factor VIII or Factor IX allele and leading to impaired clotting characterized by spontaneous bleeding and excessive bleeding after surgery or trauma. Many genetic illnesses exhibit a range of expressivity. Expressivity refers to the severity of the disease. Some children with hemophilia will exhibit mild expressivity. Among them, there are 3957 (61%) Factor VIII deficiency hemophilia A frequency of severe, moderate and mild hemophilia A was 47, 33 and 20%, respectively [3].

Children with hemophilia may lose opportunities to achieve their potentialities during school and later in life.4 Individuals with severe hemophilia A Factor VIII level (≤1%) experience frequent bleedings, often into soft tissue and joints, leading to joint damage[4].

According to individuals with hemophilia experience the impairment in quality of live and among children aged 8-16 years old the highest impairment was in the "physical health" subscale It was also shown that the dimension of "physical health" was lowest in the subscale of quality of live in children and adolescents with hemophilia A[5].

Statement of problem:

Quality of Life among Hemophiliac Children:

1. To identify the Quality of Life among Hemophilic Children At Al Hakeem General Hospital.

2. To find out the relationship between hemophiliac children quality of life and their socio-demographic characteristic of age, gender, parents educational levels, family type and socio-economic status.

Design of the study: A descriptive study designed to find the Quality of Life among Hemophilic Children. The study was carried out the period from March 6th to April 4th 2016.

Setting of the study: The study was carried out in At Al Hakeem General Hospital in Al-najaf Al-Ashraf governorate.

The sample of the study: Collected sample of (40) patient of the Quality of Life among Hemophilic Children.

RESULTS

Table (1) The observed frequencies and percentages of patients' groups according to socio-demographic data and clinical characterizations

Groups		Frequency (total 40)	Percentage (%)
Age (years)	12 ≤	22	55
	≥ 12	18	45
Gender	Male	36	90
	Female	4	10
Residence	Urban	31	77.5
	Rural	9	22.5
Educational Level	Illiterate	0	0
	Read & Write	3	7.5
	Primary School	24	60
	Secondary School	13	32.5
	Graduate	0	0
	Postgraduate	0	0
Father Educational Level	Illiterate	4	10
	Read & Write	11	27.5
	Primary School	6	15
	Secondary School	6	15
	Graduate	13	32.5
	Postgraduate	0	0
Mother Educational Level	Illiterate	4	10
	Read & Write	11	27.5
	Primary School	10	25
	Secondary School	9	22.5
	Graduate	6	15
	Postgraduate	0	0

Cont... **Table (1) The observed frequencies and percentages of patients' groups according to socio-demographic data and clinical characterizations**

Socio-economic Status	High	13	32.5
	Middle	19	47.5
	Low	8	20
Type of Hemophilia	A	27	67.5
	B	13	32.5
	C	0	0
Intensity of the disease	Mild	14	35
	Moderate	17	42.5
	Severe	9	22.5
Suffering from bleeding	Mild	9	22.5
	Moderate	23	57.5
	Very severe	8	20

This table shows that most of the study sample (55%) are leass than 12 years old, (90%) are males, (77.5%) are urban residents, (60%) are primary school graduated, (32.5%) of the study sample fathers levels of education is graduate, (27.5%) of the study sample mothers levels of education is able to read and write, and (47.5%) of the study sample present with a middle socio-economic status.

Above table shows that (67.5%) are patients suffering from type A of hemophilia, (42.5%) are present with moderate intensity of the disease, and, (57.5%) are suffering from moderate bleeding.

Table (2): Descriptive statistics of initial assessment for determination of quality of life for hemophilic children patients towards physical domain

Physical domain	Groups	Freq.	Percentage (%)	M	SD	Assessment
Difficulty in Walking	Always	5	12.5	2.13	0.53	Failure
	Sometimes	**25**	**62.5**			
	Never	10	25			
Difficulty in Running	Always	11	27.5	1.93	0.48	Failure
	Sometimes	**21**	**52.5**			
	Never	8	20			
Difficulty in daily activity	Always	9	22.5	2.18	0.54	Failure
	Sometimes	15	37.5			
	Never	**16**	**40**			
Difficulty in Lifting something	Always	**16**	**40**	1.88	0.47	Failure
	Sometimes	13	32.5			
	Never	11	27.5			
Difficulty in Taking a bath	Always	7	17.5	2.48	0.62	pass
	Sometimes	7	17.5			
	Never	**26**	**65**			
Difficulty in Doing chores	Always	10	25	2.05	0.51	Failure
	Sometimes	**18**	**45**			
	Never	12	30			
Body pain	Always	10	25	1.98	0.49	Failure
	Sometimes	**21**	**52.5**			
	Never	9	22.5			

Cont... **Table (2): Descriptive statistics of initial assessment for determination of quality of life for hemophilic children patients towards physical domain**

Muscle spasm	Always	7	17.5	2.25	0.56	Failure
	Sometimes	16	40			
	Never	**17**	**42.5**			
Joints pain	Always	**19**	**47.5**	1.68	0.42	Failure
	Sometimes	15	37.5			
	Never	6	15			
Bleeding after injury	Always	**23**	**57.5**	1.55	0.39	Failure
	Sometimes	12	30			
	Never	5	12.5			

This table shows assessment for determination of quality of life for hemophilic children patients towards physical domain. In relation to all of variable have failure assessment accept Difficulty in Taking a bath was pass assessment for determination of quality of life.

Table (3): Descriptive Statistics of Initial Assessment For Determination of Quality of Life For Hemophilic Children Patients Towards Emotional Domain

Emotional domain	Groups	Freq.	Percentage (%)	M	SD	Assessment
Being afraid	Always	14	35	1.88	0.47	Failure
	Sometimes	17	42.5			
	Never	9	22.5			
Being sad	Always	7	17.5	2.15	0.54	Failure
	Sometimes	20	50			
	Never	13	32.5			
Being angry	Always	12	30	2.05	0.51	Failure
	Sometimes	14	35			
	Never	14	35			
sleeping trouble	Always	6	15	2.35	0.59	pass
	Sometimes	14	35			
	Never	20	50			
Being worried	Always	4	10	2.48	0.62	pass
	Sometimes	13	32.5			
	Never	23	57.5			
Feeling depressed	Always	7	17.5	2.48	0.62	pass
	Sometimes	7	17.5			
	Never	26	65			
Feeling despair	Always	7	17.5	2.50	0.63	pass
	Sometimes	6	15			
	Never	27	67.5			
Loss of Confidence	Always	4	10	2.10	0.53	Failure
	Sometimes	25	62.5			
	Never	10	25			

This table shows assessment for determination of Quality of Life For Hemophilic Children Patients Towards Emotional Domain. In relation to all of variable have failure assessment accept sleeping trouble, Being worried, Feeling depressed and Feeling despair was pass assessment.

Table (4): Descriptive Statistics of Initial Assessment for Determination of Quality of Life for Hemophilic Children Patients towards Social Domain

Social domain	Groups	Freq. (40)	Percentage (%)	M	SD	Assessment
Other kids don't support him	Always	13	85.7	2.13	0.53	Failure
	Sometimes	9	8.6			
	Never	18	5.7			
Other kids refuse him	Always	2	84.3	2.50	0.63	Failure
	Sometimes	16	11.4			
	Never	22	4.3			
Teasing from other kids	Always	2	62.9	2.53	0.63	Failure
	Sometimes	15	24.3			
	Never	23	12.9			
Can't do things as others	Always	8	42.9	2.03	0.51	Failure
	Sometimes	23	35.7			
	Never	9	21.4			
Keeping up when play	Always	12	65.7	1.90	0.48	Failure
	Sometimes	20	22.9			
	Never	8	11.4			
Don't going to the touristic journeys	Always	9	91.4	2.28	0.57	Failure
	Sometimes	11	4.3			
	Never	20	4.3			
Do not going to the gardens	Always	15	85.7	2.00	0.50	Failure
	Sometimes	10	8.6			
	Never	15	5.7			
Do not like the visitors	Always	7	80	2.45	0.61	pass
	Sometimes	8	11.4			
	Never	25	8.6			
Don't like to visit the relatives	Always	23	87.1	1.68	0.42	Failure
	Sometimes	7	8.6			
	Never	10	4.3			
Do not Sharing conversations with my family members	Always	14	78.6	2.13	0.53	Failure
	Sometimes	5	17.1			
	Never	21	4.3			

This table shows Assessment For Determination of Quality of Life For Hemophilic Children Patients Towards Social Domain. In relation to all of variable have failure assessment accept Do not like the visitors was pass assessment.

Table (5): Descriptive Statistics of Initial Assessment For Determination of Quality of Life For Hemophilic Children Patients Towards School Domain

School domain	Groups	Freq.	Percentage (%)	M±SD		Assessment
Pay attention in class	Always	5	12.5	2.30	0.58	Failure
	Sometimes	18	45			
	Never	17	42.5			
Forgetting things	Always	1	2.5	2.48	0.62	pass
	Sometimes	19	47.5			
	Never	20	50			
School work	Always	5	12.5	2.18	0.54	Failure
	Sometimes	23	57.5			
	Never	12	30			
Missing school due to illness	Always	17	42.5	1.83	0.46	Failure
	Sometimes	13	32.5			
	Never	10	25			
Missing school to go to hospital	Always	19	47.5	1.70	0.43	Failure
	Sometimes	14	35			
	Never	7	17.5			
Decrease in activity due to sudden bleeding	Always	11	27.5	1.85	0.46	Failure
	Sometimes	24	60			
	Never	5	12.5			
Missing school due to joint pain	Always	18	45	1.58	0.39	Failure
	Sometimes	21	52.5			
	Never	1	2.5			
Difficulty in sitting on sharp-edged seats	Always	22	55	1.48	0.37	Failure
	Sometimes	17	42.5			
	Never	1	2.5			
Prevented from sport to avoid bleeding	Always	30	75	1.38	0.34	Failure
	Sometimes	5	12.5			
	Never	5	12.5			

This table show Assessment For Determination of Quality of Life For Hemophilic Children Patients Towards School Domain. In relation to all of variable have failure assessment accept Forgetting things was pass assessment.

Table(6): Association Between Demographical Characteristics and Overall Assessments Due To Quality of Life Towards Hemophilic Children Patients (Under/Upper) Cutoff Point

Demographical Characteristics & Overall assessments due to quality of life	Contingency Coefficient	P-value	C.S.
Age Groups	0.233	0.413	NS
Gender	0.087	0.683	NS
Residence	0.045	0.740	NS
Father education Level	0.019	0.872	NS
Mother education Level	**0.034**	**0.044**	S
Socio-Economic Status	0.209	0.074	NS

Non-Significant at P<0.05

This table show significant correlation between Quality of Life Towards Hemophilic Children and Mother education Level at p-value (0.044). Other variables have no significant correlation between Demographical Characteristics and Assessments Due To Quality of Life Towards Hemophilic Children Patients. In relation to Age Groups, Gender, Residence, Father education Level and Socio-Economic Status at p-value (0.413, 0.683, 0.740, 0.872,and 0.074) respectively.

This chapter present a systemically organized interpretation and reasonably derived Discussion of results the support of available literatures and related studies.

DISCUSSION

Determination of the quality of life of children and adolescents with hemophilia is an important part in the holistic management of this chronic disease, and this is attainable through the use of disease - related Quality of live questionnaires. Hemophilia is an X-linked congenital bleeding disorder caused by a deficiency of coagulation factor VIII (FVIII) (in hemophilia A) or factor IX (FIX) (in hemophilia B). The deficiency is the result of mutations of the respective clotting factor genes. Hemophilia has an estimated frequency of approximately one in 10,000 births [6].

Throughout the course of the present study, as shown in table (1) which refers to the statistically distribution of the observed frequencies, percentages of some related demographical characteristics variables for all the studied sample. Regarding to the gender, the findings indicate that gender (90%) are males. Regarding to the Residence (77.5%) are urban residents. Regarding to the father and mother education level the study findings show that almost one third of the study sample their father Graduated and mother are Read & Write (32.5%), (27.5%) respectively. Regarding to the socioeconomic status, it was found that most of the study sample are from middle level of SES[7].

Table (2) Shows that the study findings represent that (67.5%) are patients suffering from type A of hemophilia, (42.5%) are present with moderate intensity of the disease, and, (57.5%) are suffering from moderate bleeding. The association between the severity of hemophilia and quality of live in our study was consistent with other studies. Poon et al. was demonstrated that individuals with more severe hemophilia and higher self-reported joint pain and motion limitation had poorer HR quality of live scores, particularly in the physical aspects of HR quality of live[8].

It was also revealed that the patients with various severities of hemophilia showed different impairments in their HR Quality of live. Severely affected patients were more impaired in their 'physical functioning' than moderate or mild patients. The severity of the disease may influence joint movement, physical activity, treatment and relationships with others, and these factors might negatively impact the quality of live[9].

CONCLUSION

The result of this study indicated that the quality of live sample of male children with hemophilia was impaired, and that impairment according to the total Hemophilia –Quality of live score was greater among adolescents than children aged 8-12 years. Moreover, number of bleeding event, number of school absences, mother s' education level, severity of hemophilia, insight to health status and insight to suffering from hemophilia were associated with hemophilia –Quality of live. In addition, family income and parent- administration of the product were the factors which predict the quality of live in children with hemophilia.

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REFERENCES

1. Lentz SR, Rangarajan S, Karim FA, Andersen PD, Arkhammar P, Rosu G, et al. The potential correlation between patient-reported symptoms and the use of additional haemostatic medication for joint bleeding in haemophilia patients with inhibitors: a post hoc exploratory analysis of recombinant activated factor VII data from the ADEPT2 trial. Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis. 2017;28(3):224-9.
2. Cömert M, Güneş AE, Sahin F, Saydam G. Quality

of life and supportive care in multiple myeloma. Turkish journal of haematology : official journal of Turkish Society of Haematology. 2013;30(3):234-46.

3. Shapiro AD, Donfield SM, Lynn HS, Cool VA, Stehbens JA, Hunsberger SL, et al. Defining the impact of hemophilia: the Academic Achievement in Children with Hemophilia Study. Pediatrics. 2001;108(6):E105.
4. Dekoven M, Wisniewski T, Petrilla A, Holot N, Lee WC, Cooper DL, et al. Health-related quality of life in haemophilia patients with inhibitors and their caregivers. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(2):287-93.
5. Lindvall K, Von Mackensen S, Berntorp E. Quality of life in adult patients with haemophilia--a single centre experience from Sweden. Haemophilia : the official journal of the World Federation of Hemophilia. 2012;18(4):527-31.
6. Stonebraker JS, Bolton-Maggs PH, Soucie JM, Walker I, Brooker M. A study of variations in the reported haemophilia A prevalence around the world. Haemophilia : the official journal of the World Federation of Hemophilia. 2010;16(1):20-32.
7. de Wee EM, Mauser-Bunschoten EP, Van Der Bom JG, Degenaar-Dujardin ME, Eikenboom HC, Fijnvandraat K, et al. Health-related quality of life among adult patients with moderate and severe von Willebrand disease. Journal of thrombosis and haemostasis : JTH. 2010;8(7):1492-9.
8. Skevington SM, Lotfy M, O'Connell KA. The World Health Organization's WHOQOL-BREF quality of life assessment: psychometric properties and results of the international field trial. A report from the WHOQOL group. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2004;13(2):299-310.
9. Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, et al. Guidelines for the management of hemophilia. Haemophilia : the official journal of the World Federation of Hemophilia. 2013;19(1):e1-47.

Sensitivity and Specificity of Linear Gingival Erythema as Immune Suppression Marker in Pediatric HIV-infected at UPIPI Soetomo General Hospital Surabaya, Indonesia

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ABSTRACT

Introduction: Strikingly, more than 95% pediatric HIV infections worldwide are in developing countries. Although ART can reduce significantly the risk of HIV transmission to children born from HIV-infected mothers, this can be effective only if the women know their HIV status. In many developing countries, such as Indonesia, the lack of access by pregnant mothers to laboratory facilities may deprive them of knowing their HIV status, thus the opportunity to transmit the disease to their children is high, knowing no preventive intervention is attempted. To date, it is estimated that the number of HIV infected children in Indonesia who are left undiagnosed is still high, therefore a study on clinical markers of HIV that can aid in the diagnosis and predict the severity of the disease among children is critical. In this study, Linear Gingival Erythema is assessed on its correlation to degree of immune suppression to be used as HIV clinical marker in laboratory-poor settings in Indonesia. **Method**: HIV-infected children admitted to Soetomo General Hospital between June – October 2017 were screened and examined intraorally for the presence of LGE. Other relevant data were obtained from questionnaire-guided interview to parents/guardians and from patient's medical record. **Results**: LGE was found in 21.43% children and statistical analysis shows significant correlation of LGE to degree of immune suppression. **Conclusion**: Further study with larger study population size is required to analyze the sensitivity and specificity of LGE to be used as HIV marker in predicting a particular degree of immune suppression, specific to Indonesian pediatric population.

Keywords: *Oral Manifestation, Children with HIV/AIDS, Linear Gingival Erythema, CD4+*

INTRODUCTION

Human Immunodeficiency Virus (HIV) continues to be a major global health issue. United Nations Program on HIV/AIDS (UNAIDS) estimated 36.7 million people worldwide were living with the infection in 2016, with an extremely high proportion (95%) resides in developing countries. Despite the discovery of ART decades ago, the growth rate of HIV-infected population is still unacceptably high; 5.000 new infections per day, which 400 among them are children below 15 years.[1,2]

Most (90%) children were infected with HIV through mother to-child transmission (MTCT).[3] Although the administration of ART during pregnancy and other preventive interventions can significantly lower the risk of MTCT from 45% to less than 2%, this is not the case for many developing countries, such as Indonesia.[4]

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Indonesia does not screen for HIV infection as part of its routine antenatal care due to laboratory limitations in many areas.[5] This concerning condition causes many children born from unaware women of their HIV status do not get the necessary preventive interventions from the outset, thus they are very vulnerable to get vertically infected. This is proven by the drastic increase of reported new HIV infections among Indonesian children below 4 years between 2010-2015 by >230%.[6]

To date, it is estimated that the number of HIV infected children who are left undiagnosed in Indonesia is still high.[1] When no laboratory facilities are available, clinical indicators becomes the solely most important marker in making presumptive diagnosis of HIV infection and predict the severity of the disease.[7,8] Oral manifestations have longed been studied to be used as HIV clinical indicators, however no studies to date have analyzed the correlation of these oral lesions to predict certain degree of immune suppression among Indonesian pediatric population. One of the most commonly found oral lesions in HIV pediatric infection is Linear Gingival Erythema (LGE).[9] The aim of this study is therefore to analyze the correlation of LGE to degree of immune suppression among pediatric patients admitted to Intermediate Care and Infectious Disease Centre (UPIPI), Soetomo General Hospital, Surabaya, Indonesia.

MATERIAL AND METHOD

A cross-sectional study was carried between June – October 2017 at the outpatient clinic UPIPI, Soetomo General Hospital. The study protocol was approved by Ethics Committee of Dr. Soetomo Hospital (No:326/Panke.KKE/V/2017) and Health Research Ethical Clearance Commission Universitas Airlangga (No:137/HRECC.FODM/VIII/2017). Pediatric patients under 18 years with confirmed HIV diagnosis according to the Indonesian Ministry of Health guidelines and attended the center between the study period were screened. The age limit set for pediatric patients in this study follows the regulations applied by the Indonesian legal system.[10]

Prior to the study, attending parents/guardians of the eligible patients were explained about the study objectives. Approval from parents/guardians regarding the participation of their children in the study was obtained by written consents. Oral mucosal status of each patient was then assessed by a trained oral medicine specialist using disposable plastic mouth mirrors and sterile gauze pads under artificial lighting. Identification of LGE was based on its clinical features according to EC-Clearinghouse diagnostic criteria as a distinct fiery red band along the margin of the free gingiva, with the amount of erythema is disproportionately intense for the amount of plaque seen.[9]

Other relevant data of the patients were obtained from their respective parents/guardians through questionnaire-guided interview and from their medical records. Data collected include age, gender, use of ART, and latest laboratory result on CD4 values (CD4% or CD4 counts). Viral load counts were not accessible to most patients; thus, this parameter was excluded from the present study. To determine patients' immune status closest to the condition at oral examination, CD4 values were restricted only up to 6 months interval from the date of examination. This time range follows the Indonesian Ministry of Health guidelines on pediatric CD4 monitoring[11]. CD4 value of each patient served as a baseline to determine patient's degree of immune suppression according to classification system of Centers for Disease Control (CDC) (Table 1).[12,13]

Correlation between LGE and degree of immune suppression was analyzed using chi-square test. Statistical Package of Social Sciences (SPSS) 17.0 for windows was used and p values <0.05 were considered significant.

RESULTS

The study was conducted among HIV-positive children admitted to the outpatient clinic at UPIPI, Soetomo General Hospital, Indonesia. Between June – October 2017, a total of 47 HIV-positive children who attended the center were screened. This number is much lower than the total number of children registered at the center. Although all HIV-positive children are required to pay periodic visit to the center for monitoring, the reality did not seem so. Many parents/guardians did not attend the center with their children, unless their children show any symptoms. Far distance between the center and their homes and the fact that their children need to attend classes at schools were the most-heard reasons from parents/guardians who came to the center to get the prescribed ART for free on behalf of their children.

Out of the 47 children screened, a relatively high proportion (19 children) had to be further excluded. Reasons for exclusion were parental refusal in providing consents, children being not cooperative during examination, and no CD4 values found within 6 months interval from the date of oral examination on patients' medical record. Not all children were routinely get their CD4 tested due to the high laboratory cost. Consequently, the total number of children included in the study is 28 children.

Summary of age range, ART status, and degree of immune suppression of these children are presented in Table 2. Oral examinations on 28 Indonesian children found LGE in 6 (21.43%) children (Fig 1 & 2). Three (10.71%) children with LGE were on ART while the other 3 (10.71%) were ART naïve (Table 2).

Indonesian children were categorized into several degrees of immune suppression. Two (7.14%) children with LGE were in degree of no immune suppression, while the other 4 (14.29%) children with LGE were in degree of severe immune suppression (Table 2). Statistical analysis to correlate the occurrence of OHL according to child's immune suppression was found to be statistically significant (p value = 0.027) (Table 3).

Table 1. **Immunologic categories based on age-specific CD4 % and CD4 counts.**

Immunologic category	**Age of child**				
	< 12 months		**1-5 years**		**> 5 years**
	cells/mm^3	**%**	**cells/mm^3**	**%**	**cells/mm^3**
No immune suppression	≥ 1.500	≥ 25	≥ 1.000	≥ 25	≥ 500
Moderate immune suppression	750 - 1499	15 - 24	500 - 999	15 – 24	200 – 499
Severe immune suppression	< 750	< 15	< 500	< 15	< 200

Table 2. Summary of age, ART status, and degree of immune suppression in the study population

	No. of Patients	
	OHL present	**OHL absent**
Total	6 (21.43%)	22 (78.57%)
Age		
Age range (years)	1 - 15	
Mean age	6.8 ± 3.1	
HAART		
Yes	3 (10.71%)	21 (75%)
No	3 (10.71%)	1 (3.57%)
Degree of immune suppression		
No	2 (7.14%)	16 (57.14%)
Moderate	-	3 (10.71%)
Severe	4 (14.29%)	3 (10.71%)

Table 3. Statistical relationship between LGE and immune suppression degree in the study population

	No. of Patients		**Chi-square**	**P value**
Degree of Immune Suppression	**LGE present**	**LGE absent**		
No	2 (7.14%)	16 (57.14%)	7.2590	0.027*
Moderate	-	3 (10.71%)		
Severe	4 (14.29%)	3 (10.71%)		

*statistically significant

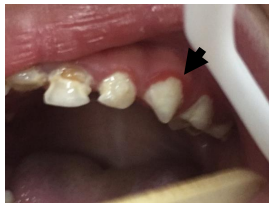


Figure 1. Four years old, ART naïve female patient

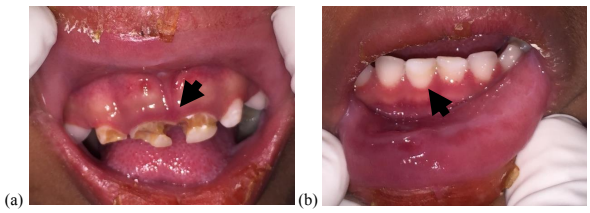


Figure 2. Five years old, ART naïve male patient (a) Maxillary LGE (b) Mandible LGE

DISCUSSION

Oral lesions can not only indicate HIV infection, they are also among the earliest clinical features of the infection that can aid in predicting the course of the disease. EC-Clearinghouse clinical diagnostic criteria, the term of reference for most studies in HIV-associated oral diseases, classified LGE into lesions commonly associated with pediatric HIV infection. LGE was formerly known as HIV-gingivitis and it is the most common form of HIV-associated periodontal disease in infected children.[14]

LGE has been correlated to fungal infection of the Candida species, yet the nature etiology of this lesion is still a controversy, as other studies reported different findings.[15] Umadevi et al reported the possibility of certain viruses, such as CMV, EBV, and papillomavirus in initiating and causing LGE progression.[16] On the contrary, Aas et al. found *Saccharomyces cerevisiae*, *Gamella spp*., *Dialister spp*., *Streptococcus spp*., and *Veillonella spp*. as the causative agents of LGE [17]. Despite the discrepancies between studies, it is now widely accepted that Candida contributes to the occurrence of LGE as many clinical cases were effectively treated with antifungal treatment.[15,18]

In this study, the prevalence of LGE was found to be 21.43%. This prevalence is higher than other studies in developing countries in Uganda (0.84%) [19] and India (2.27% [20] and 9.05% [21]) yet lower than a study conducted in Venezuela (27.03%) [22]. The varying prevalence of LGE between countries may be due to the distinct characteristics among the populations (proportion of the population on ART, their adherence to ART, immune status of the population, nutritional status, etc.), as well as the influence of cultural and geographical factors of each country.

Furthermore, EC-Clearinghouse classification has also associated LGE to be more prevalent in children HIV infection than in adults.[18] Comparing this study to another study conducted in 2014 among adult patients at the same centre (UPIPI, Soetomo General Hospital) supports this classification as LGE was found to be much lower in adult patients (5.83%) than among pediatric children in this study.[23]

ART has been widely accepted to be highly effective at inhibiting HIV replication, although it is not curative. Combination of ART can dramatically suppress the number of virus circulating in the plasma, even to a level of undetectable. With the successful viral suppression, immunologic recovery is expected and the number of opportunistic infections to manifest should be lower.[24] In this study, although LGE were also found in children undergoing ART, LGE were found more predominantly in children who had not received treatment as seen in Table 2.

Although LGE in this study were found in children with severe immune suppression yet also in children with no immune suppression, the occurrence of LGE is more notable in children with severe immune suppression (4 children with LGE among 7 children with severe immune suppression) than in children with no immune suppression (2 children with LGE among 18 children with no immune suppression). This trend indicates that LGE will be more likely to manifest when immune status of the host is continuously declining. Statistical analysis between the presence of LGE with degree of immune suppression is also proven to have a solid relationship (p value = 0.027) (Table 3).

CONCLUSION

Immune status was significantly correlated with the presence of LGE in this study. However, its sensitivities and specificities to predict a specific degree of immune suppression is yet still need to be assessed. Further study with larger Indonesian pediatric HIV/AIDS population size is thus recommended.

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REFERENCES

1. The Joint United Nations Program on HIV/AIDS. UNAIDS data book 2017. Available from http://www.unaids.org/sites/default/files/media_asset/20170720_Data_book_2017_en.pdf. Accessed on 10 January 2018.
2. World Health Organization. Immunization, Vaccines and Biologicals: HIV/AIDS. Available from http://www.who.int/immunization/topics/hiv/en/index1.html. Accessed on 18 January 2018.
3. Chukwuemeka IK, Fatima MI, Ovavi ZK, Olukayode O. The impact of a HIV prevention of mother to child transmission program in a nigerian early infant diagnosis centre. Nigerian Medical Journal: Journal of the Nigeria Medical Association. 2014;55(3):204-208.
4. Prestes-Carneiro LE. Antiretroviral therapy, pregnancy, and birth defects: a discussion on the updated data. HIV/AIDS (Auckland, NZ). 2013;5:181-189
5. Rahmalia A, Wisaksana R, Meijerink H, Indrati AR, Alisjahbana B, et al. Women with HIV in Indonesia: are they bridging a concentrated epidemic to the wider community? BMC Research Notes. 2015;8:757.
6. Directorate General CDC & EH - Ministry of Health of Indonesia. Laporan situasi perkembangan HIV-AIDS di Indonesia Jan-Maret 2017. Available from http://www.aidsindonesia.or.id/ck_uploads/files/Laporan%20HIV%20 AIDS%20TW%201%20 2017.pdf. Accessed on 10 January 2018
7. Pakfetrat A, Falaki F, Delavarian Z, Dalirsani Z, Sanatkhani M, et al. Oral Manifestations of Human Immunodeficiency Virus-Infected Patients . Iranian Journal of Otorhinolaryngology. 2015;27(78):43-54
8. dos Santos Pinheiro R, França T, Ribeiro C, Leão J, de Souza I, et al. Oral manifestations in human immunodeficiency virus infected children in highly active antiretroviral therapy era. Journal of Oral Pathology & Medicine. 2009;38(8):613-622.
9. European Community Clearinghouse. Classification and diagnostic criteria for oral lesions in HIV infection. Journal of Oral Pathology and Medicine. 1993;22:289-91
10. Indonesian Law and Regulations. Undang-undang Republik Indonesia nomor 23 tahun 2002 tentang perlindungan anak. Available from http://www.kinerja.or.id/pdf/86547a83-b8d6-461f-afae-f16f968fae8e.pdf. Accessed on 10 April 2017.
11. Ministry of Health Republic of Indonesia. Pedoman penerapan terapi HIV anak 2014. Available from http://www.spiritia.or.id/dokumen/pedoman-hivanak2014.pdf. Accessed on 10 April 2017.
12. Centers for Disease Control. Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. MMWR Morbidity and Mortality Weekly Report 1994;43:1-10
13. Centers for Disease Control. Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged < 18 months and for HIV infection and AIDS among children aged 18 months to < 13 years. MMWR Morbidity and Mortality Weekly Report 2008;57:1-10
14. Prabhu RV, Prabhu V, Chatra L, Shenai P. Oral manifestations of HIV. Journal of Tropical Diseases and Public Health. 2013; 1:111
15. Portela M, Cerqueira D, Soares R, Castro G. Candida spp. in Linear Gingival Erythema lesions in HIV- infected children: reports of six cases. International Journal of Science Dentistry. 2012;1(57): 51–55.
16. Umadevi M, Adeyemi O, Patel M, Reichart PA, Robinson PG. Periodontal diseases and other bacterial infections. Adv Dent Res. 2006;19(1):139–145
17. Aas JA, Barbuto SM, Alpagot T, Olsen I, Dewhirst FE, et al. Subgingival plaque microbiota in HIV positive patients. Journal of Clinal Periodontology. 2007;34(3):189–195
18. Seneviratne CJ and Jayasinghe RD. Association of Candida with Linear Gingival Erythema in HIV-infected subjects. In: Oral Candidosis, Berlin: Springer; 2015. pp. 83-94
19. Rwenyonyi CM, Kutesa A, Muwazi L, Okullo I, Kasangaki A, et al. Oral manifestations in HIV/AIDS-infected children. European Journal of Dentistry. 2011;5(3):291-298.
20. Manikandan S, Kumar VS, Ramesh K. Oral manifestations of HIV infected children undergoing highly active antiretroviral therapy. Journal of Pierre Fauchard Academy. 2013:27(2):49-52
21. Subramaniam P, Kumar K. Oral mucosal lesions

and immune status in HIV-infected Indian children. Journal of Oral Pathology and Medicine. 2015;44(4):296-9

22. Davila ME, Gil M. Oral manifestations and dental caries in children exposed to Human Immunodeficiency Virus. Rev Salud Publica. 2011;13(5):833-43
23. Nugraha AP, Ernawati DS, Parmadiati AE, Soebadi B, Triyono EA, et al. Correlation Linear Gingival Erythema, candida infection and CD4+ counts in HIV/AIDS patients at UPIPI RSUD Dr. Soetomo Surabaya, East Java, Indonesia. Journal of International Dental and Medical Research. 2017;10(2):322-326
24. B-Lajoie M-R, Drouin O, Bartlett G, Nguyen Q, Low A, et al. Incidence and prevalence of opportunistic and other infections and the impact of antiretroviral therapy among HIV-infected children in low- and middle-income countries: a systematic review and meta-analysis. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. 2016;62(12):1586-1594.

Maternal Mortality with Panel Regression Approach Model based on Maternal and Child Health Revolution Program or etc Performance Indicators at Nusa Tenggara Timur Province Indonesia

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ABSTRACT

Nusa Tenggara Timur (NTT) government had launched a local specific program, namely Maternal and Child Health Revolution Program (MCHRP) or *Revolusi KIA* at 2009. However, maternal mortality still becomes one of the biggest public health problems in NTT Province in Indonesia.

The slow decreasing of Maternal Mortality Ratio (MMR) need a study in order to determine MCHRP performance indicators that have the biggest influence in decreasing maternal mortality ratio at each district of NTT Province at a specific range of time in order to develop a more effective, efficient and directed plan or policy for maternal and child health intervention program.

The aim of this research is to identify and analysis factors that influence the maternal mortality ratio of 2011 – 2015 based on MCHRP performance indicators at every district and municipality of NTT Province.

This applied research used data from MCHRP indicators program reports from 2011 to 2015 at Public Health Division of NTT Health Office with 22 districts and municipalities as unit analysis. The data analyzed using panel data regression.

Result from this analysis shows that the trend of performance indicator achievements tends to fluctuate and imbalanced for every district and municipality. The best Panel Data Regression Model that can be used for evaluating the achievement of MCHRP performance indicators toward MMR at NTT is the Random Effect Model that takes into account the place and time effect error. Indicators that have significantly influenced in decreasing MMR at NTT are the complete Ante Natal Care (ANC) visit, childbirth assisted by a skilled health professional with midwifery competence, complete postpartum care (PNC) visit and complication care with determination coefficient (R^2) 90%.

Keywords: *Maternal Mortality Ratio (MMR), Maternal and Child Health Revolution Program, Panel Data Regression*

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INTRODUCTION

Indonesia is one of developing countries that did not reach MDG's target in decreasing MMR to 102/100,000 live birth in 2015[(1)] Based on data from Demographic and Health Survey (DHS) there was a significant increasing of MMR in Indonesia from 228/100,000 live births in

2007 to 359/100,000 live births in 2012. Furthermore, based on Beetwen Sensus Population Survey in 2015 the MMR in Indonesia is 305/100,000 live births, which is still far from the MDG's target.

Since 2009, NTT as one of the provinces in Indonesia that has a highest MMR has been trying to accelerate the decreasing of maternal and child morbidities and mortalities with a tremendous program called Maternal and Child Health Revolution Program (MCHRP) or *Revolusi KIA*.

This program runs through a regulation that all childbirth must be assisted by skilled health professional with midwifery competence at adequate health care facility for 24 hours[3]. This regulation has an impact on the increased numbers of health care facilities and health care facilities with basic obstetric and neonatal emergency care at NTT from 292 sub district health center or *puskesmas* with 61 *puskesmas* equipped by basic obstetric and neonatal emergency care (20.89%) in 2009 to 374 *puskesmas* with 374 *puskesmas* equipped by basic obstetric and neonatal emergency care (62.57%).

However, this MCHRP seems to be not yet effective in reducing the MMR at NTT. The annual reduction of MMR tends to slowly decrease which is 220/100,000 live birth in 2011, 200/100,000 live birth in 2012, 185,6/100,000 live birth in 2013, 169/100,000 live birth in 2014 and 133/100,000 live birth in 2015.The low achievements of MCHRP performance indicators which are complete ANC visit coverage is 61.63%, childbirth assisted by skilled health professional with midwifery competence coverage is 69.97% and childbirth at health care facilities coverage is 69.95%[4]. These facts showed that there are still 34% childbirth delivered at home and around 30% childbirth still assisted by traditional birth attendance that put the maternal at risk. When obstetric complication on childbirth happened it can be lead to maternal and child dead if handling late that can be caused by late referee caused by geographic, social cultural or economic factors[5][6].

There are some challenges and obstacles in the process of reducing MMR at NTT such as the insufficient knowledge and awareness from the community toward the importance of maintaining health and safety of pregnant women; insufficient nutrition status and health of pregnant women; limited access of pregnant women to qualified health care facilities and comprehensive obstetric care caused by geographic and topographic factors (archipelago, highland and hill) and the patriarchy culture; insufficient numbers of health care facilities and insufficient of health professionals (quantity, quality and distribution)[7][8].

Thus to make the initiatives in reducing MMR program at NTT succeed, it is important to define the achievement indicators of MCHRP that have the biggest effect in reducing MMR at every district and municipality at NTT at a specific period of time in order to develop a more effective, efficient and directed plan or policy for maternal and child health intervention program.

The aim of this research is to identify and analysis factors that influence the maternal mortality ratio of 2011 – 2015 based on performance indicators of MCHRP at each district and municipality of NTT Province.

MATERIAL AND METHOD

This applied research used data from MCHRP indicators program reports from 2011 to 2015 at Public Health Division of NTT Health Office with 22 districts and municipalities as unit analysis. The data analyzed using panel data regression because it takes into account the weighting of individual effect (district/municipality) and time period (2011 – 2015).

Analyzed variables in this research are the response variables, i.e. MMR, predictor variables, i.e. ANC 1st coverage, 4 times ANC coverage, childbirth assisted by skilled health professionals with midwifery competent coverage, 3 times PNC visits, complication care coverage and the number of puskesmas equipped by basic obstetric neonatal emergency care at every district and municipality.

Panel Regression Model analyzed by eviews7 software consists of three models which are the common effect model, the fixed effect model and the random effect model.

The best regression model was selected by using Chow test and Hausman test. Chow test is used to choose between the common effect model and the fixed effect model. If the null hypothesis accepted, then the common effect model chose, whereas Hausman test used to choose between the fixed effect model and the random effect model. If the null hypothesis accepted, then random effect model chose. The best regression

model will use to estimate the panel data.

FINDINGS

Table 1 shows that MMR average and the achievements of MCHRP performance indicators tends to fluctuate and imbalance at every district and municipality in NTT Province, which are shown from standard deviation and the big difference of minimum and maximum at every research variable. MMR average from 2011 to 2015 is 181.5/100.000 live birth.

There is a significant difference between every district and municipality with range between 107.40/100.000 live births to 219.8/100.000 live birth. PNC visit indicator and complication care indicator coverage also showed a big significant difference between every district and municipality in the 5 years time period.

Table 1: Description of research variables of NTT Province 2011 – 2015

Indicator/Research Variables	Mean	SD	Min	Max
MMR	181.50	32.96	107.40	219.8
ANC (1th visit) coverage	86,00	11.82	38.9	127.5
Complete ANC (minimum 4 times visit) coverage	61.80	14.41	30.7	92.1
Skilled health professional with midwifery competence birth delivery assisted coverage	74.00	5.30	69.10	76.60
PNC (minimum 3 times visit)	77.10	18.4	11.3	102.6
Complication care coverage	52.00	26.00	6.9	117.4
Puskesmas equipped by obstetric and neonatal emergency care coverage at every district/municipality	5.00	3.00	2.00	16.00

The data panel regression analysis benefit to look for MMR difference at every district and municipality in the time period. Every indicator very depended by the difference of time (year) and place (district/ municipality).

The test result of the best panel data regression can be seen in table 2. The result from Chow test shown p value = 0.000 meaning that the common effect model rejected. Whereas the result from Hausman test show p value = 0.138 meaning that the random effect model accepted that can be used further to estimate performance indicator of MCHRP toward MMR at every district and municipality NTT in 2011 – 2015 time period. Random effect model resulting from the analysis for MMR estimation at NTT province has taken into account the heterogeneity of unobservable place and time variables and reduce the colinearity between predictor that resulted a more efficient estimation[9]

Table 2: The best panel regression model test result

Test	Statistic value	*p*-value
Chow Test	F = 5.1631	0.000
Hausman Test	X^2 = 2199	0.138

Based on the result on table 3 performance indicators of MCHRP that significantly influence the MMR in NTT are ANC visit, childbirth assisted by skilled health professionals with midwifery competence, PNC visit and complication care. The indicator that has the biggest influence in reducing MMR in NTT Province is the complete ANC visit.

Every regression coefficient in the Random Effect Model that has negative value means that if we increase the four indicators mention above then the MMR will decrease, vice versa.

The R^2 *adjusted* values of this model is 0.9008 which is included in the very good category that mean about 90.08% change in MMR can be explained by these four predictor variables in the model.

Regression coefficient of the complete ANC visit i.e. -0.4814 mean that if the complete ANC visit increase 10%, then the MMR will have a chance to decrease at about of 4/100,000 live births annually both for the district and municipal level estimation and province level estimation. The interpretation of coefficient regression of other variables in the model can be done similarly.

Table 3 Results from Random Effect Model Analyze

Specifications of the indicators				
Variable	**Coefficient**	**Standard Error**	**t-statistic**	**P value**
Constanta (C)	6.6354	2.2724	2.9199	0.0043
Complete ANC (minimum 4 times visits) coverage	-0.4814	0.1808	-2.6626	0.0205
Childbirth delivery assisted by a skilled health professional coverage	-0.0952	0.0436	-2.2319	0.0267
PNC (minimum 3 times visits) coverage	-0.0950	0.0418	-2.3167	0.0258
Complication care coverage	-0.0942	0.0367	-2.4810	0.0238
The best model specification				
R^2	0.9276	F statistic	123.6292	
R^2*Adjusted*	0.9008	p value	0.0000	

DISCUSSION

The result of this research shows that the MCHRP performance indicator that has the biggest contribution in decreasing MMR at every district and municipality in NTT on 2011 – 2015 time period is the complete ANC visit or a minimum 4 times ANC visits indicator. Increasing 10% complete ANC visit coverage will have a chance to decrease MMR in the amount of 4/100.000 live birth.

Hence this indicator needs to get more attention in order to prevent complication and maternal and child mortality. ANC visit related with maternal behavior in health caring and access to health care facilities [(10)].

In the reality, many community members at NTT still consider that the pregnancy is a usual and natural stage of life that lead pregnant women not regularly visit health professional.

These beliefs have an implication of the high numbers of un-detection of maternal with high risk factors. These un-threaten maternal high risk factors lead to the delay on getting care that can cause a serious health consequences such as hospitalization or even death both for the mother or the baby[(11)]. Even though around 80% of maternal mortality are preventable if they regularly visit health professional for ANC. ANC visits is important in order to early detection and on time care for threaten the complication at pregnancy, labor and post partum which can help in reducing MMR [(12).]

This research also found that the average of ANC visit at NTT Province at 2001 – 2015 period was 61.80%.

The utility of the ANC at health facilities influenced by the low of women's autonomy in economic (freedom to regulate their family budget allocation), low freedom on mobility and low authority in decision making which can be caused by maternal status in their family and community. The other factors that can influence ANC visits are the attitude of pregnant women themselves of the importance of their health care especially antenatal care. Supports from family members and community leaders and also information and assistance from heath professionals toward ANC visits can increase maternal intention[(13)(14)] but also in reproductive health since ICPD 1994. However, very little empirical evidence is available about women's role on maternal health service utilization in Nepal. This paper explores dimensions of women's autonomy and their relationship to utilization of maternal health services. METHODS The analysis uses data from the Nepal Demographic and Health Survey, 2011. The analysis is confined to women who had given birth in the 5 years preceding the survey (n = 4,148. Therefore, in order to increase the ANC visits coverage at NTT as the most influenced factor in reducing MMR, factors that need more attention are women's autonomy.

The Prenatal class or *Kelas Ibu Hamil* can become a medium for increasing women's autonomy with target and material modification. The Prenatal class that used to target the pregnant women can also make the husband and childbearing age couples as target to prepare them for their pregnancies later. The materials that can be added in the Prenatal class, such as the rule of women in fulfill the reproduction's health right and the need for reproductive health at every cycle. Hopefully with the increasing knowledge of the pregnant mother and the husband about the reproduction's health will benefit from increasing the complete ANC visit coverage, childbirth assisted by skilled health professionals with midwifery competence coverage and PNC visit coverage.

Besides that, the network that already exists between health professionals with the traditional birth attendance must be more developed in order to make a safe pregnancy, clean and safe childbirth and postpartum by reducing negative myths regarding pregnancy, childbirth and postpartum but still keep culture values that exist in the community in order to reduce MMR and Child Mortality Ratio. This is also important because many pregnant women still choose the traditional birth attendant assist in childbirth, reasoned by their personal reasons such as more familiar, understand and can help with traditional childbirth ritual and also because the traditional birth attendant can assist the maternal and the child up to 40 days after delivery.

CONCLUSIONS

In order to decrease MMR at NTT through the MCHRP, the performance indicators that have the biggest influence are increase the complete ANC visit coverage, the complete PNC visit coverage and childbirth assisted by skilled health professionals with midwifery competence coverage and PNC care visit coverage, respectively, which can increase by target and material modification in Prenatal Class and also by strengthening the network between health professional and traditional birth attendance.

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Ethical Clearance: There are no human participants involved in this research. However the procedure of this research had already gotten ethical approval of Health Research Ethics Committee, Faculty of Public Health Airlangga University.

Informed Consent: This research used secondary data. No informed consent needed.

REFERENCES

1. WHO Group. Trends in Maternal Mortality: 1990 to 2015. Geneva, Switzerland; 2015.
2. Ministry of Health of Republic of Indonesia (Kementerian Kesehatan RI). Indonesia Health Profile (Profil Kesehatan Indonesia) 2015. Jakarta; 2016.
3. Nusa Tenggara Timur Province Health Office (Dinas Kesehatan Prov. NTT). Maternal And Child Health Revolution Program (MCHRP) Guidelines (Pedoman Revolusi KIA di Provinsi NTT), Kupang, NTT: Dinas Kesehatan Prov NTT; 2009.
4. East Nusa Tenggara Province Health Office (Dinas Kesehatan Prov. NTT).. East Nusa Tenggara Province Healh Profile 2015 (Profil Kesehatan Provinsi Nusa Tenggara Timur Tahun 2015. Kupang), NTT; 2016.
5. Thaddeus S, Maine D. Too Far to Walk: Maternal Mortality in Context. Soc Sci Med. 1994;38(8):1091–110.
6. Gabrysch S, Campbell OM. Still too far to walk: Literature review of the determinants of delivery service use. BMC Pregnancy Childbirth. 2009;9(34):1–18.
7. Ratu-Ludji ID. (Development Approach: Social Ecological Model of Health Behavior in reducing MMR of Kupang District (Pengembangan Pendekatan "Social Ecological Model of Health Behavior untuk Penurunan Angka Kematian Ibu (AKI) di Kabupaten Kupang). Universitas Airlangga; 2013.
8. Rogaleli YC, Sadukh JP. Maternal Mortality Model of Maternal And Child Health Revolution Program (MCHRP) using Geographically Weighted Poisson Regression (Pemodelan Maternal Mortality pada target Revolusi KIA di Provinsi NTT menggunakan Geographically Weighted Poisson Regression), Kupang, NTT; 2015.

9. Baltagi BH. Econometric Analysis of Panel Data. 3th ed. Chichester: John Wiley & Sons Ltd; 2005.
10. Sibiya MN, Ngxongo TSP, Bhengu TJ. Access and utilization of antenatal care services in a rural community of Thekwini district in KwaZulu-Natal. Int J Africa Nurs Sci. Elsevier; 2018;8(December 2017):1–7.
11. Oyerinde K. Can Antenatal Care Result in Significant Maternal Mortality Reduction in Developing Countries? J Community Med Health Educ. 2013;3(2):1–2.
12. Kilpatrick SJ, Crabtree KE, Kemp A.Preventability of Maternal Deaths: Comparison Between Zambian and American Referral Hospitals. Am Coll Obs Gynaecol. 2002;100(2):321–6.
13. Yumni H, Suhariadi F, Sudirham O. Women's Autonomy to Improve Quality Of Maternal Health. Innov J Med Heal Sci. 2016;6(6):171–5.
14. Adhikari R. Effect of Women's autonomy on maternal health service utilization in Nepal: A cross sectional study. BMC Womens Health. BMC Women's Health; 2016;16(26):1–7.

Role of microRNA-423 Gene Variation in Women at Higher Risk of Breast Cancer in Tabuk of Saudi Arabia

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ABSTRACT

Aim: Background- MicroRNA has attracted increasing attention due to their possible involvement in the development of various types of cancer. Many studies have been conducted to investigate the association between miR-423 rs6505162C/T and Breast cancer risk; however, the results are not consistent. Therefore, we investigated the association of microRNA-423 rs6505162C>A gene variations with Breast cancer cases, sex match healthy controls and *Women* at higher *risk* of *breast cancer* susceptibility in Saudi women.

Methodology: This study was conducted on 90 subjects among whom 30 were *Women* at higher *risk* of *breast cancer* (WHR), 30 were Breast cancer cases and 30 were gender matched healthy controls. The microRNA-423 rs6505162C>A gene variations was performed using Amplification refractory mutation system PCR method.

Results: A significant difference was observed in genotype distribution between w*omen* at higher *risk* of *breast cancer* and Breast cancer cases (P=0.04) and between sex match healthy controls and *Women* at higher *risk* of *breast cancer* (P=0.009). The frequencies of all three genotypes CC,CT,TT reported in the w*omen* at higher *risk* of *breast cancer* were 20%, 43% and 37% , Breast cancer patients 53%,37% and 10% and in sex matched healthy controls 57%, 30% and 13% respectively. Our results indicated that the miR-423-rs6505162C>T gene variant is associated with an increased risk of *Women* at higher *risk* of *breast cancer* in Codominant inheritance model (OR=0.20, 95% CI=(0.05-0.80) p=0.020, TT vs CC; Dominant model (OR=0.26, 95% CI= (0.86-0.81), p=0.020, CT+TT vs CC) and no significance was reported in Recessive model (OR=0.38, 95%CI= (0.12-1.21), P=0.07, TT vs CC+CT) inheritance models tested. While, the T allele significantly increased the risk in *Women* at higher *risk* of *breast cancer* (OR= 0.38; 95% CI= (0.18-0.78); p=0.009 compared to C allele.

Conclusion: Our findings indicated that microRNA-423 TT genotype and T allele are associated with an increased susceptibility to Breast cancer in high risk w*omen* . Furthers studies with larger sample sizes are necessary to confirm our findings.

Keywords : *Women at higher risk of breast cancer, MicroRNA,SNP-single nucleotide polymorphism,*

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INTRODUCTION

Breast cancer is the second most commonly diagnosed cancer worldwide [(1)]. Breast cancer (BC) has a major impact on health of women worldwide and the Kingdom of Saudi Arabia (KSA) is no exception. It is considered the most common malignancy and embodies the second leading cause of cancer deaths after lung

cancer. The malignancy carries tremendous socio-economic, emotional, and public health implications. It is estimated that more than one million new cases of BC are diagnosed annually. Breast cancer incidence rates in Arab women have increased during the last 24 years, but women are still being diagnosed with BC at more advanced stages of the disease [2,3] .An estimated 5-10% of all breast cancer cases are thought to be hereditary or caused by known genetic factors. A mutation in the BRCA1 or BRCA2 gene is one of the main known causes of hereditary breast cancer.MicroRNAs are a class of small, endogenous, non-coding, single-stranded, highly conserved and tissue-specific RNA molecules which take part in the regulation of target mRNAs expression at the post-transcriptional level [4] . The discovery of miRNAs has been followed by findings highlighting their important and diverse roles in many molecular pathways and biological processes, including development, apoptosis, differentiation, and cell proliferation [5] , as well as their implication in various human diseases including cancer. Growing evidence indicates that miRNAs can work as oncogenes or tumor suppressors, depending on which gene(s) they modulate [6] .Recent evidence suggests a role for miR-SNPs in breast cancer susceptibility including work by Hu et al. where the presence of mutant alleles of MIR196A2 rs11614913 and MIR499A rs3746444 significantly increased breast cancer risk in Chinese women [7]. However in genetic association analysis of Caucasian populations and functional studies in breast cancer cell lines performed by Hoffman et al. the presence of SNP in MIR196A2 rs11614913 was significantly associated with reduced risk of breast cancer, as well as less efficient processing of MIR196A2 and reduced capacity to regulate target genes, indicating additional factors may be at work in this SNP's effect on breast cancer [8]. Additionally, Kontorovich et al. found 2 SNPs, rs6505162 and rs895819 located in MIR423 and MIR27A precursors respectively, to be significantly associated with decreased risk of breast cancer in BRCA2 mutation carriers from a Jewish population [9].However the SNP rs895819 was also significantly associated with reduced risk of developing breast cancer in families with a history of non-BRCA related breast cancer in a later study performed by Yang et al [10]. Finally rs2910164, a miR-SNP located in the 3p strand of MIR146A, was found to be associated with a younger age of diagnosis in familial breast cancer for BRCA1 mutation carriers [11].Many epidemiological studies have examined the association of miRNA SNPs with cancer susceptibility [12]. In BC, several case–control studies and meta-analyses have evaluated associations between miRNA gene polymorphisms and BC risk in European,[13] Asian [14], Arab[15], and Jewish[9],] populations. Many studies have been conducted to investigate the association between miR-423 rs6505162C/T and Breast cancer risk; however, the results were not consistent.

MATERIAL AND METHOD

This study was conducted on 90 subjects among whom 30 were Breast cancer cases, 30 were *Women* at higher *risk* of *breast cancer* (WHR) and 30 were sex matched healthy controls. The Breast cancer cases were histologically confirmed .The sex-matched healthy women with no history of any types of cancer and not related to the patients. The first degree relatives of Breast cancer patients were selected as Women at higher *risk* of *breast cancer*. Patients with any previous history of cancer were excluded from this study.

Sample collection

After assessing the clinicopathological findings of Breast cancer patients, a 4ml sample of peripheral blood was collected by venipuncture in EDTA tubes from each patient and healthy control.

DNA extraction

DNA extraction was done by using DNeasy Blood Kit (cat 69506) from Qiagen (Germany) as per the manufactures instructions. The extracted DNA was dissolved in nuclease-free water and stored at 4°C until use. Quality and integrity of DNA were checked by NanoDrop™ (Thermo Scientific, USA).

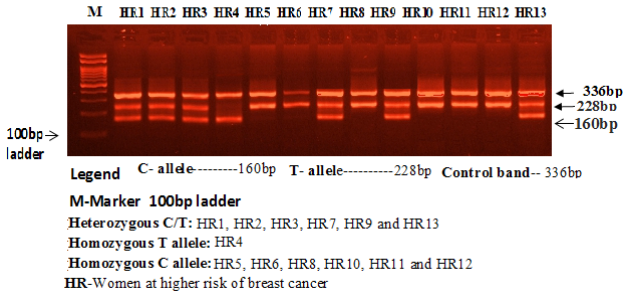
Genotyping for miR-423 rs6505162C>T) by using amplification-refractory mutation system PCR

The miR-423-rs6505162 C>T genotyping was done by using amplification-refractory mutation system PCR .The ARMS primers were designed by using Primer3 software as depicted in Table 1.

Table 1. Primer sequence of miR-423 genotyping (rs6505162 C>T)		
Direction	Sequence	Product size
miR-423 FO:	5′-TTTTCCCGGATGGAAGCCCGAAGTTTGA-3′	336 bp
miR-423 RO:	5′-TTTTGCGGCAACGTATACCCCAATTTCC-3′	
miR-423FI (T allele):	5′-TGAGGCCCCTCAGTCTTGCTTCCCAA-3′	228bp
miR-423 RI (C allele):	5′-CAAGCGGGGAGAAACTCAAGCGCGAGG-3′	160bp

The ARMS-PCR was performed in a reaction volume of 25uL containing template DNA (50ng), FO -0.30uL , RO -0.30uL , FI-0.20uL , RI -0.20uL of 25pmol of each primers and 10uL from GoTaq® Green Master Mix (cat no M7122) (Promega, USA). The final volume of 25 uL was adjusted by adding nuclease free ddH2O .Finally 2ul of DNA was added from each patient. The amplification conditions used were at 94 ^{0}C for 12 minutes followed by 35 cycles of 94^{0}C for 35sec, 62^{0}C for 40 sec, 72^{0}C for 40 sec followed by the final extension at 72^{0}C for 10 minutes. The amplification products were separated by electrophoresis through 2% agarose gel stained with 0.5µg/mL ethidium bromide and visualized on a UV transilluminator. Primers FO and RO flank the exon of the miR-423-rs6505162 C/T gene, resulting a band of 336bp to act as a control for DNA quality and quantity. Primers Fwt and RO amplify a wild-type allele (C allele), generating a band of 160bp, and primers FO and Rmt generate a band of 228bp from the mutant allele (T allele) as depicted in **Figure 1**).

Figure 1 Genotyping for miR-423 rs6505162C>T) by using amplification-refractory mutation system PCR



STATISTICAL ANALYSIS

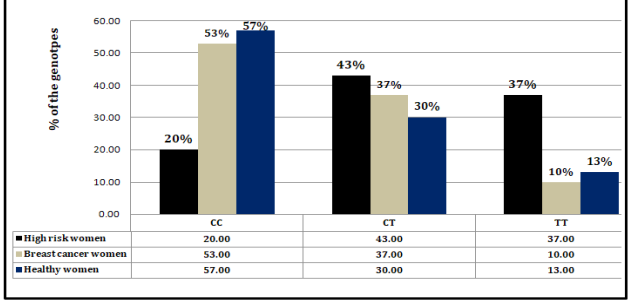
The differences in the miR-423 gene allele and genotype frequencies between the three groups were evaluated using Chi-square test. The associations between miR-423 (rs6505162 C>T) genotypes and w*omen* at higher *risk* of *breast cancer,* breast cancer cases and healthy controls were estimated by computing the odds ratios (ORs), risk ratios (RRs) and risk differences (RDs) with 95 % confidence intervals (CIs A p-value < 0.05 was considered significant. All statistical analyses were performed using SPSS 16.0.

RESULTS

This case controls study was performed on ninety subjects .Thirty of ninety (30/90) were Breast cancer cases, Thirty of ninety (30/90) were w*omen* at higher *risk* of *breast cancer* (WHR) and Thirty of ninety (30/90) were sex matched healthy controls .All the samples were screened for miR-423 rs6505162 C>T genotyping by using amplification-refractory mutation system PCR assay. The frequencies of all three genotypes of miR-423 rs6505162 gene polymorphism CC,CT and TT in three groups was analyzed as depicted in the **Figure 2.**

The frequencies of all three genotypes CC,CT,TT reported in the w*omen* at higher *risk* of *breast cancer* was CC 20%, CT 43% and TT 37% , Breast cancer patients was CC 53%, CT 37% and TT 10% and in sex matched healthy controls was CC 57%, CT 30% and TT 13%.The significant difference was observed in genotype distribution between w*omen* at higher *risk* of *breast cancer* and Breast cancer cases (P=0.04) and between sex match healthy controls and *Women* at higher *risk* of *breast cancer* (P=0.009).

Figure 2 Genotype distribution of miR-423 rs6505162 C>T gene polymorphism in three groups - , women at higher risk of breast cancer ,Breast cancer cases and healthy controls



miR-423 rs6505162 C>T gene variation between women at higher risk of breast cancer (WHR) and Breast cancer Case

The genotype distribution of miR-423 rs6505162C>T gene variation between w*omen* at higher *risk* of *breast cancer* (WHR) and Breast cancer Case is summarized in Table 3. A statistically significant difference was observed in the frequencies of miR-423CC, CT and TT genotypes among w*omen* at higher *risk* of *breast cancer* (WHR) and Breast cancer Case (p=0.040). The frequency of T allele (fT) was found to be higher among w*omen* at higher *risk* of *breast cancer* (0.58) than the breast cancer cases (0.29) whereas, the higher frequency of C allele (fC) was observed among Breast cancer cases (0.71) than the w*omen* at higher *risk* of *breast cancer* (WHR) (0.42). The T allele was found to be a risk factor Breast cancer as depicted in table 2.

Table 2: miR-423 rs6505162 C>T gene variation between w*omen* at higher *risk* of *breast cancer* and Breast cancer Case

Subjects	N =	CC	CT	TT	C	T	X2	Df	P value
High risk women	30	6(20%)	13(43%)	11(37%)	0.42	0.58	6.02	2	0.04
Breast cancer women	30	16(53%)	11(37%)	03(10%)	0.71	0.29			

miR-423 rs6505162 C>T gene variation between women at higher risk of breast cancer (WHR) and sex matched healthy controls. The genotype distribution of miR-423 rs6505162C>T gene variation between w*omen* at higher *risk* of *breast cancer* (WHR) and sex matched healthy controls is summarized in Table 3. A statistically significant difference was observed in the frequencies of miR-423CC, CT and TT genotypes among w*omen* at higher *risk* of *breast cancer* (WHR) and sex matched healthy controls (p=0.009). The frequency of T allele (fT) was found to be higher among w*omen* at higher *risk* of *breast cancer* (WHR) (0.58) than the sex matched healthy controls (0.28) whereas, the higher frequency of C allele (fC) was observed among sex matched healthy controls (0.72) than the w*omen* at higher *risk* of *breast cancer* (WHR) (0.42). The T allele was found to be risk factor women at higher *risk* of *breast cancer* (WHR) as depicted in **table 3.**

Table 3: **miR-423 rs6505162 C>T gene variation between w*omen* at higher *risk* of *breast cancer* (WHR) and sex matched healthy controls**

Subjects	N =	CC	CT	TT	C	T	X2	Df	P value
High risk women	**30**	6(20%)	13(43%)	11(37%)	0.42	0.58	9.25	2	0.009
Healthy women	30	17(57%)	9(30%)	04(13%)	0.72	0.28			

Risk of Women at higher risk of breast cancer, Breast Cancer with miR-423 (rs6505162C/T) gene polymorphism in BC patients:

A multivariate analysis based on logistic regression like odds ratio, risk ratio and risk difference with 95% confidence intervals were calculated for each group to estimate the association between the miR423-rs6505162C>T genotypes and risk of *Women* at higher *risk* of *breast cancer* in Saudi patients as depicted in **table 4.**

Our findings showed that the miR-423-rs6505162C>T gene variant was associated with an increased risk of *Women* at higher *risk* of *breast cancer* in Codominant inheritance model (OR=0.20, 95% CI=(0.05-0.80) p=0.020, TT vs CC; Dominant model (OR=0.26, 95% CI= (0.86-0.81), p=0.020, CT+TT vs CC) and no significance was reported in Recessive model (OR=0.38, 95%CI= (0.12-1.21), P=0.07, TT vs CC+CT) inheritance models tested. While, the T allele significantly increased the risk in *Women* at higher *risk* of *breast cancer* (OR= 0.38; 95% CI= (0.18-0.78); p=0.009 compared to C allele.

T able 4: Association of miR-423 rs6505162 C>T gene variation with breast cancer					
Genotypes	**High risk women**	**Breast cancer patients**	**OR (95% CI)**	**Risk Ratio(RR)**	**P-Val**
	(N=30)	(N=30)			
Codominant					
miR-423 -*CC*	06	16	1(ref.)	1(ref.)	
miR-423 -*CT*	13	11	0.31 (0.019-1.09)	0.50 (0.23-1.09)	p=0.060
miR-423 -TT	11	03	0.20(0.05-0.80)	0.42(0.19-0.90)	p=0.020
Dominant					
miR-423 -*CC*	06	16	1(ref.)	1(ref.)	
miR-423 *(CT+ TT*)	24	14	0.26(0.86-0.81)	0.46(0.22-0.96)	p=0.020
Recessive					
miR-423 *(CC+ CT*)	19	27	1(ref.)	1(ref.)	
miR-423 (TT)	11	06	0.38(0.12-1.21)	0.63(0.39-1.04)	p=0.07
Allele					
miR-423 -C	25	43	1(ref.)	1(ref.)	
miR-423-T	35	17	0.38(0.18-0.78)	0.60(0.41-0.88)	p=0.009

DISCUSSION

In the present study, we analyzed the correlation between genetic polymorphisms in *miR-423*- rs6505162 and susceptibility to w*omen* at higher *risk* of *breast cancer* (WHR) in Saudi Arabian population. The mi*R-423* - rs6505162 polymorphism was revealed to be associated with an overall increased risk in w*omen* at higher *risk* of *breast cancer* (WHR). The higher prevalence of miR-423-TT genotypes were reported in w*omen* at higher *risk* of *breast cancer* 37% vs *breast cancer* 10% & gender matched controls 13% similarly the higher prevalence of miR-423-CT genotypes were reported in w*omen* at higher *risk* of *breast cancer 43*% vs *breast cancer* 37% & gender matched controls 30% . The significant difference was observed in genotype distribution between w*omen* at higher *risk* of *breast cancer* and Breast cancer cases (P=0.04) and between sex match healthy controls and *Women* at higher *risk* of *breast cancer* (P=0.009).A confirmed carrier of a genetic mutation that increases the risk for breast cancer, (BRCA1or BRCA2). Confirmed" means the person has had genetic testing and a genetic mutation was found. They may not have breast cancer but they "carry" the genetic mutation and are at a higher risk for developing the disease .Most inherited cases of breast cancer are associated with one of two abnormal genes: BRCA1 or BRCA2 gene. Women with an abnormal BRCA1 or BRCA2 gene have up to an 85% lifetime risk of developing breast cancer besides have a much higher-than-average lifetime risk of ovarian cancer; estimates range from 15% to 60% .

CONCLUSION

Our findings indicated that microRNA-423 TT genotype and T allele are associated with an increased susceptibility to Breast cancer in high risk w*omen* . Furthers studies with larger sample sizes are necessary to confirm our findings.

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Ethical Clearance : This Research study was approved by the Research ethics committee, University of Tabuk .

Conflict of Interest : Authors do not have conflict of interest.

REFERENCES

1. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer .http://globocan.iarc.fr].
2. Brown R, Kerr K, Haoudi A, Darzi A .Tackling cancer burden in the Middle East: Qatar as an example.Lancet Oncol. 2012 Nov; 13(11):e501-8.
3. Miller AB .Screening for breast cancer in the Eastern Mediterranean Region. East Mediterr Health J. 2010 Oct; 16(10):1022-4.
4. Shivdasani RA .MicroRNAs: regulators of gene expression and cell differentiation. Blood. 2006 Dec 1; 108(12):3646-53.
5. Cheng AM, Byrom MW, Shelton J, Ford LP.Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res. 2005; 33(4):1290-7.
6. Zhang B, Pan X, Cobb GP, Anderson TA.microRNAs as oncogenes and tumor suppressors.Dev Biol. 2007 Feb 1; 302(1):1-12.
7. 29. Hu Z, Liang J, Wang Z, Tian T, Zhou X et al. Shen H. Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. Hum Mutat. 2009;30(1):79–84.
8. 30. Hoffman AE, Zheng T, Yi C, Leaderer et al. microRNA miR-196a-2 and Breast Cancer: A Genetic and Epigenetic Association Study and Functional Analysis. Cancer Res. 2009;69(14):5970–7.
9. Kontorovich T, Levy A, Korostishevsky M, Nir U, Friedman E. Single nucleotide polymorphisms in miRNA binding sites and miRNA genes as breast/ ovarian cancer risk modifiers in Jewish high-risk women. Int J Cancer. 2010;127(3):589–97.
10. Yang R, Schlehe B, Hemminki K, Sutter C et al. A genetic variant in the pre-miR-27a oncogene is associated with a reduced familial breast cancer risk. Breast Cancer Res Treat. 2010;121(3):693–702.
11. Shen J, Ambrosone CB, DiCioccio RA, Odunsi K, Lele SB, Zhao H. A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. Carcinogenesis. 2008;29(10):1963–6.
12. O'Day E, Lal A .MicroRNAs and their target gene networks in breast cancer.Breast Cancer Res. 2010; 12(2):201.
13. Catucci I, Yang R, Verderio P, Pizzamiglio S, Heesen et al .Evaluation of SNPs in miR-146a, miR196a2 and miR-499 as low-penetrance alleles in German and Italian familial breast cancer cases.Hum Mutat. 2010 Jan; 31(1):E1052-7.
14. Hu Z, Liang J, Wang Z, Tian T, Zhou X et al .Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. Hum Mutation. 2009 Jan; 30(1):79-84.
15. Alshatwi AA, Shafi G, Hasan TN, Syed NA et al. Differential expression profile and genetic variants of microRNAs sequences in breast cancer patients. PLoS One. 2012; 7(2):e30049.

The Susceptibility of Aedes Aegypti to Cypermethrin Used in Vector Control Programs of Dengue Hemorrhagic Fever

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ABSTRACT

Background: Cypermethrin is an insecticide commonly used in dengue vector control program. Reasonably different levels of susceptibility in mosquitoes of Aedes aegypti in the two districts of Central Java Indonesia are suspected to occur as a result of using cypermethrin continuously.

Method: The study was conducted using a survey method to the community to take samples of Aedes aegypti eggs after becoming an adult female mosquito toward the susceptibility of 0.05% cypermethrin.

Results: The results showed that the mosquito Aedes aegypti in Kebumen are resistant to the insecticide 0.05% cypermethrin. Similarly, the mosquito Aedes aegypti in Banyumas also is immune to the same insecticide.

Conclusion: Mosquitoes in the district of Kebumen and Banyumas regency are resistant to the insecticide of 0.05% cypermethrin. It is suggested for health agencies to conduct periodic evaluations (3-5 years) on the effectiveness of pesticides used, especially in areas with high endemicity status so that control can be applied efficiently.

Keywords: *vulnerability, Aedes aegypti, cypermethrin*

INTRODUCTION

The number of cases of DHF (Dengue) always fluctuate annually [(1)]. Various efforts to control dengue vector has been implemented either physically, chemically or biologically. Physical controls in Indonesia are commonly known as the practice of 3M, (*Menguras, Menutup, Mengubur*- to drain, to shut, to burry namely mosquito nest elimination using 3 M (drain, close and bury or destroy) that remove the tub or toilet, shut the water reservoirs (buckets, drums, jars) and bury or destroy the used goods such as cans, tires, plastic, and others. This method, however, has not worked satisfactorily proved by the number of dengue cases are still common [(2)].

Some vector control method has been widely known and used by the dengue control program at the central level in the areas of environmental management, biological control, chemical control, community participation, protection of individuals and the rule of law. Control of Dengue is primarily intended to break the chain of transmission, i.e., the vector control. The dengue vector control in endemic areas which are not targeted will not be sustainable and has not been able to break the chain of transmission. This may due to the method adopted not referring to the data/information vectors as well as still rely on the use of insecticides.

One of the efforts to control the vector is through cut transmission by killing adult mosquitoes quickly with insecticide application or known by the term fogging. It is still often found fogging is performed not in appropriate dose, not to the right target and not based on a scientific basis. This triggeres the occurrence of resistance in addition to the fact that some places use the same insecticide group in a long time. Insect resistance

to insecticides commonly occurs after 2-20 years of use. Cypermethrin is insecticides often used in dengue vector control programs in each district in Central Java in recent years and ironically this substance is found no longer useful as the mosquito has been resistant [3] besides to 0.8% malathion and 0.25% permethrin [4].

Kebumen and Banyumas are two districts in the southern part of Central Java province which includes using cypermethrin in the last three years. Kebumen is a county located in the lowlands and by the Indonesian Ocean, while Banyumas is situated in the highlands and bordered by the beach. Their geographical and topographical differences that allow growth, the joy of life and adaptability of mosquito Aedes aegypti can be different as well. Cypermethrin associated with the use of insecticides may have different levels of vulnerability in the mosquito Aedes aegypti in the two districts as a result of using cypermetrin continuously.

METHODOLOGY

This type of research is observational research with a cross sectional approach by conducting surveys to endemic areas of dengue fever in the district of Kebumen and Banyumas in Central Java province, Indonesia.

Samples were the Aedes aegypti obtained from the highest dengue cases in Kebumen (districts of Sempor) and Banyumas (the District of South Purwokerto). Adult mosquitoes collected by hatch (rearing) obtained through the installation of ovitrap across the two districts. The number of mosquitoes used in research is 125 each region bringing the total number of mosquitoes = 125 head x 2 areas = 250 Aedes aegypti adult female mosquitoes. Tests were carried out by using WHO standard kit equipment such as susceptibility test set consisting of three pairs of test tubes and three pairs of control tubes. Test tube comprises of a tube collector mosquito (risela oil-coated paper) and the tube contact insecticides (coated paper impregnated).

The calculation result is determined by the provision if the results obtained mosquito mortality in the control group. If it is <5%, then it is ignored. If the death of larvae in the control group is > 20%, the research is considered failed, and the test should be repeated. The percentage protection (feeding inhibition, mortality or knockdown effect) was estimated by Abbot formula as PE = (NC - NT)/NT × 100%, where NC and NT are the number of the mosquito on control and treatment, respectively [5]. The mortality rate of Aedes *aegypti* is interpreted as follows:

vulnerable: if the death of the mosquito larvae is 98-100% tolerant: if the death of larvae mosquito is 80-97% resistant: when the mosquito larvae mortality is <80%

RESULTS

The insecticides used for fogging on the location of the study are described below.

Table 1. The Insecticides Used for Fogging

No.	Location	Insecticides Group	Frequency	Duration of use
1.	Kebumen Regency	Synthetic pyrethroid	1-2 times/year	> 5 years
2.	Banyumas Regency	Synthetic pyrethroid	1-2 times/year	> 5 years

Temperature measurements are carried out every day, i.e., air temperature at the test and 24 hours after testing. Temperature measurements obtained can be seen in the table below.

Table 2. Measurements of Air Temperature

Sampel		Data Testing	Initial temperature (°C)	Temperature after 24 hours (°C)
1	Jatinegara Village	1	25	26
		2	25	26
		3	25	26
2	Karangklesem Sub-district	1	25.5	26
		2	25.5	26
		3	25.5	26

Humidity measurements were performed everyday, i.e., air humidity during testing and observation 24 hours after trial. The humidity measurements obtained can be seen in the table below.

Table 3. Humidity Measurement Results

Sample		Data Testing	Initial humidity(%)	Humidity after 24 hours (%)
1	Jatinegara Village	1	77	78
		2	77	78
		3	77	78
2	Karangklesem Sub-district	1	78	78
		2	78	78
		3	78	78

The susceptibility status of Aedes aegypti resistance to 0.05% Cypermethrin is obtained through test or susceptibility test. The resistance test data was collected from the results of Aedes aegypti mosquito count which died in the 0.05% Cypermethrin, exposure group after being observed for 1x24 hours. Aedes aegypti mosquito resistance test result in exposure group of 0.05% Cypermethrin insecticide in Jatinegara village can be seen in the following table.

Table 4. Aedes aegypti Mosquito Resistance Test Results in Jatinegara Village

No.	Samples	Mosquitos	Death	Alive	Mortality Rate (%)	Note
1	1	20	3	17	15	Resistant
2	2	20	7	13	35	Resistant
3	3	20	7	13	35	Resistant
Average		20	5.7	14.3	28.3	Resistant

From table 4 it can be seen that the population of Aedes aegypti mosquitoes in Jatinegara village, Kebumen regency with 3 times test have Aedes aegypti mosquito death rate respectively: 1st test as much as 15%, 2nd test as much as 35%, and 3rd test as much as 35% with the average percentage of deaths is 28.3%. Thus it can be said that Aedes aegypti mosquitoes in Jatinegara Village, Kebumen Regency have been resistant to 0.05% Cypermethrin insecticide.

In the control group, the test was performed using three sets of test tubes. The experimental procedure is similar to the resistance test in the exposed group. What distinguishes is in the control group it applies a non-insecticide paper. The death of Aedes aegypti mosquito in the control group can be seen in the following table.

Table 5. Resistance Test Results Aedesaegypti mosquito in the Control Group

No.	Samples	Mosquitos	Death	Alive	Mortality Rate (%)
1	1	20	0	20	0
2	2	20	0	20	0
3	3	20	1	19	5
Average		20	0.3	19.7	1.7

Aedes aegypti mosquito resistance test result in exposure group of 0.05% Cypermethrin insecticide in Karangklesem Urban Village, South Purwokerto District, Banyumas Regency can be seen in the following table.

Table 6. Resistance Test Result of Aedes aegypti Mosquito on Exposure Group

No.	Samples	Mosquitos	Death	Alive	Mortality Rate (%)	Note
1	1	20	7	13	35	Resistant
2	2	20	5	15	25	Resistant
3	3	20	2	18	10	Resistant
Average		20	4.7	15.3	23.3	Resistant

Based on Table 6, it can be seen that the population of Aedes aegypti mosquito in Karangklesem village, South Purwokerto subdistrict, Banyumas regency with 3 times test have Aedes aegypti mosquito death rate: 35% in experiment 1, 25% in test 2, and check 3 as much as 10% with the average percentage of death 23.3%. Thus, it can be said that Aedes aegypti mosquito in Karangklesem Village, District of South Purwokerto, Banyumas Regency has been resistant to 0. 05% Cypermethrin insecticide

In the control group, the test was performed using three sets of test tubes. The experimental procedure is similar to the resistance test in the exposed group. What distinguishes is in the control group it apllies a non-insecticide paper. The death of Aedes aegypti mosquitoes in the control group can be seen in the following table.

Table 7. Aedes aegypti Mosquito Resistance Test Result in Control Group in Banyumas Regency

No.	Samples	Mosquitos	Death	Alive	Mortality Rate (%)
1	1	20	0	20	0
2	2	20	1	19	5
3	3	20	1	19	5
Average		20	0.7	19.3	3.3

Based on Table 7 it can be seen that the control group mortality from Aedes aegypti mosquitoes on the 0.05% Cypermethrin insecticide resistance test in Karangklesem Subdistrict of Purwokerto Selatan District of Banyumas Regency with three times test, the control group mortality rate is 3.3%.

DISCUSSION

The result of temperature measurement shows that the temperature in the study room is 25-26 ° C. In general, mosquitoes will lay eggs at temperatures around 20-30 ° C, so it can potentially breed the Aedes aegypti mosquito [6].

The optimum temperature of mosquito breeding is between 25 ° C-27 ° C and will stop entirely at <10 ° C or> 40 ° C [7]. Thus the temperature of the room in the laboratory is still in optimum condition for the heat of Aedes aegypti mosquito development. The result of initial humidity test measurement and after observed 1x24 hour gets the result between 77-78%. The need for high humidity affects mosquitoes to find moist and wet places as a place to rest or rest. At moisture less than 60%, the age of the mosquito is short [8]. Humidity in the laboratory room is adjusted with moisture in place of mosquitoes to rest or to rest area, so that at the time of mosquito breeding and at the time of the testing process of mosquito in excellent condition and stable.

The test data of resistance with susceptibility test method was obtained from Aedes aegypti mosquito count which died on Cypermethrin 0.05% exposure group after observed for 1x24 hours. Aedes aegypti mosquito resistance test in 0.02% Cypermethrin insecticide group in Jatinegara village, Sempor sub-district, Kebumen regency with three times analysis has Aedes aegypti mosquito average percentage of death = 28.3%. Thus it can be said that Aedes aegypti mosquitoes in Jatinegara Village, Sempor Sub-district, Kebumen Regency have been resistant to 0.05% Cypermethrin insecticide.

In the control group, the test was performed using three sets of test tubes. The death of Aedes aegypti mosquitoes in the mean control group was 1.7%. Due to the control mortality <5%, it does not need to be corrected with the Abbot formula. Aedes aegypti susceptibility or resistance to Cypermethrin 0,05% in Karangklesem Village, District of Purwokerto Selatan, Banyumas Regency known that Aedes aegypti mosquito average percentage of death is 23.3%. Thus it can be said that Aedes aegypti mosquito in Karangklesem Village, District of South Purwokerto, Banyumas Regency has been resistant to Cypermethrin insecticide 0,05%.

Tests on the control group of Aedes aegypti mosquito in Karangklesem Village, District of Purwokerto Selatan, Banyumas Regency were done using three sets of the test tube. The death of Aedes aegypti mosquitoes in the control group was 3.3%. Due to the control mortality <5%, it does not need to be corrected with the Abbot formula.

This way, communities should not always use insecticides in mosquito control but advisable to control by methods of repair or to clean the environment such as eradication of mosquito nest.

Other researchers may conduct susceptibility studies of Aedes aegypti mosquitoes against other types of insecticides or similar research with different research situation to enlarge the generality.

CONCLUSION

The application of insecticides in the control of dengue vectors performed at the study sites (Kebumen and Banyumas districts) by the health authorities has found the use of synthetic pyrethroid insecticides for more than five years. This makes Aedes aegypti mosquitoes in Jatinegara village, Sempor sub-district, Kebumen regency has been resistant to 0.05% cypermethrin insecticide (average percentage of mosquito deaths test of 28.3%). Likewise, the results of mosquito susceptibility test using susceptibility test method showed that Aedes aegypti mosquito in Karangklesem village, South Purwokerto subdistrict, Banyumas district had been resistant to 0.05% cypermethrin insecticide (average of mosquito death rate percentage was 23.3%). This makes the government necessary to find an alternative to combat the mosquito as in ineffectiveness of the insecticide may be one cause affecting the transmission of dengue fever is hard to eliminate.

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REFERENCES

1. Departemen Kesehatan R.I. Direktorat Jenderal Pengendalian Penyakit Dan Penyehatan Lingkungan (DIT.JEN. PP & PL), 2007, Ekologi dan Aspek Perilaku Vektor , Jakarta : DIT.JEN. PP & PL.
2. Direktorat Jendaral P2PL, 2012, Pedoman Penggunaan Insektisida (Pestisida) Dalam Pengendalian Vektor, Jakarta : Kementerian RI.
3. Bina Ekawati, Dyah Widiastuti, Rahmawati dan Sunaryo, 2015, Peta Status Kerentanan Aedes aegypti (Linn) Terhadapa Insektisida Cypermetrin dan Malathion di Jawa Tengah, Ciamis : Loka Litbang P2B2.
4. Sayono, dkk, 2012, Distribusi Resistensi Nyamuk Aedes aegypti Terhadap Insektisida Sipermetrin Di Semarang, http://dinus.ac.id/repository/docs/ajar/rsistensi_aedes.pdf
5. Waka M, Hopkins RJ, Glinwood R, Curtis CF: The effect of repellents Ocimum forskolei and DEET on the response of Anopheles stephensi to host odours. J Med Vet Entomol. 2006, 20: 373-376. 10.1111/j.1365-2915.2006.00645.x.
6. Soegeng Soegijanto, 2006, Demam Berdarah Dengue, Edisi 2, Surabaya : Airlangga University Press.
7. World Health Organization / Organisasi Kesehatan Dunia (WHO), Demam Berdarah Dengue : Diagnosis, Pengobatan, Pencegahan dan Pengendalian Edisi 2, Jakarta : Penerbit Buku Kedokteran EGC.
8. World Health Organization (WHO), 2016, Test Procedures For Insecticide Resistance Monitoring In Malaria Vector Mosquitoes, 2d Edition.

Effectiveness of Hand Hygiene Training by Kirkpatrick Model

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ABSTRACT

The aim of this organisational development was to improve Hand Hygiene education and training compliance in Hospital by extension, improve hand hygiene compliance. Hand Hygiene is identified as the single most important intervention in reducing the transmission of Healthcare Associated Infections (HAIs). In 2009, The World Health Organization published comprehensive evidence-based guidelines on Hand Hygiene in healthcare, which introduced a standardized approach to Hand Hygiene practice; "The Five Moments for hand Hygiene". These guidelines have been adopted by the hospital and core to our education and training programme. The model was used to guide this research. The Kirkpatrick model was employed to evaluate the hand hygiene education and training, effectiveness of training at the different levels was being evaluated through construct on the basis of literature review. For level the respondents are who had completed training 7 months earlier. The results indicated that reaction of the participants were positive for training, secondly they had applied skills and knowledge which they had learnt from training. However, advanced research is needed to asses the impact of applying hand hygiene on spread of infection in hospital patient through measuring HAIs rate in this hospital.

Keywords: *Training evaluation, Kirkpatrick model, hand hygiene compliance, HAIs*

INTRODUCTION

Nosocomial infections are a major problem faced by hospitals and they are mostly caused by hand contact. Hand hygiene is the simplest and most effective way to prevent nosocomial infections [(1)]. In accordance with Sung Chin Pan, [(19)] hand washing compliance rate is still low on health workers. An education and training program on hand hygiene is needed to provide knowledge, understanding, and improve skills for hand hygiene according to WHO's Hand Hygiene five-point guidance.

Hand Hygiene Training helps health workers to understand the knowledge and skills needed to perform their daily work by prioritizing patient safety [(14)]. By evaluating Hand Hygiene Training of Kirkpatrick's four levels model it is expected to be measured the training effectiveness, the improvement efforts can be done for the implementation of further training or as a proposal to management and organization to improve the implementation of hand hygiene so as to achieve organizational goals in accordance with key indicators that have been set.

Training and Development

Education and training is the process of adding, encouraging and updating the knowledge, skills and abilities of human resources to move towards better performance in accordance with organizational goals [(6)]. It also encourages organizational commitment, improves employee's performance to address internal and external challenges, encourages increased knowledge and improves skills, work process efficiency, problem solving development and also builds self confidence that will has impact on performance improvement [(7)].

Education and training programs should be evaluated to determine how efforts to improve the next program, to determine whether the program needs to be continued or not and to determine the performance of the education and training units and the determination of the budget [(7)].

Training Evaluation Model

Kirkpatrick Model

An easy-to-apply training evaluation model is Kirkpatrick's four levels [(10)]

a. Reaction; The measured aspect is the level of satisfaction of the training participants on the training implementation, including elements of materials, implementation, means, and instructor's ability. The material elements are divided into systematics and presentation of the material, the ease of the material to be understood, the contribution of the material in the enhancement of knowledge and insight and it can be applied to the work, and the conformity of the material with the training objectives. The implementation elements are divided into several sub-elements of conformity with the stipulated schedule, the timeliness of the training, the willingness of the committee to assist the trainees. The elements of facility are divided to be sub-elements of audio-visual or visual aid quality, consumption, seminar kits, room and room facilities (light, wide, acoustics and ventilation). Elements of instructor's ability are divided into sub-elements of material mastery, delivery techniques, how to answer questions, language and gestures. The evaluation is done by using a questionnaire that should be filled by the trainees at the end of the training session.

b. Learning; The measured aspect is the addition of knowledge and understanding level of the participants after the training. The measured result is the learning result rather than the impact on the trainees' performance. Evaluation is done by providing pre-post test at the beginning and end of the training session.

c. Behaviour; How the behaviour change occurs in each individual trainees, this means how the knowledge acquired during training can be developed into skills improvement and applied in daily work.

d. Result; It is the process of measuring how the training program has impacts on the achievement of organizational goals, as a result of improving the trainees' performance. In this case the evaluation is done by looking at the hospital key indicator that is HAIs rate.

Hand Hygiene and Healthcare Associated Infections (HAIs)

The presence of close relationship between hand hygiene compliance and reduced risk of HAIs infection [2,3]. Hand Hygiene is the foundation of the patient's safety which will always be applied with the prescribed procedures in each process of patient care. But the low compliance rates are a common problem commonly found in health care [25]. This is what causes the transmission of microorganisms that causes HAIs can not be avoided. The high compliance rates of hand hygiene and the existence of organizational policies on this matter have an impact on the decrease in hospital HAIs [3]. The key are not lies only in health workers, but also the policies and commitments of leadership.

The effectiveness of hand hygiene compliance measurements requires the same perception of hand hygiene process terminology, which includes indications, opportunities, and actions [4].

Indication is a rational principle that becomes the basis to conduct hand hygiene. The hospital should have written policy on technical and indications to conduct hand hygiene. In accordance with WHO's Manual for Observer, the definition of hand hygiene indication "is the reason why hand hygiene is necessary at a given moment". It is based on the risk of transmitting microbe from one surface to another, using terminology before and after contact. Some examples of indications for hand hygiene based on both WHO and CDC are as follows:

- Before contact with patient
- Before performing aseptic action
- After contact with blood or blood products and body fluids
- After contact with the patient
- After touching the environment around the patient

When measuring the compliance rates, it should be clear about these indications, in accordance with WHO, it was referred as "Five Moment Hand Hygiene" [1]. Opportunities represent an opportunity to perform hand hygiene in a period of time when health workers perform patient care in accordance with prescribed indications. Action shows the performance of health workers while performing hand hygiene. Every opportunity will always be followed by the implementation of hand hygiene conducted by health workers.

HAIs (Healthcare Associated Infections) are an infection that occurs during the patient receiving care at the hospital or other health facility, the infection is not found or not being in an incubation period at the time of patient's arrive. Included in this definition are infections acquired in hospital but only show symptoms

after the patient was departed from the hospital. WHO [1], Global data on HAIs is currently very limited, but it is generally mentioned that the prevalence of HAIs in developing countries is higher than developed countries (10.1% - 7.6%). The most commonly found infections are those related to the use of invasive instruments or procedures i.e. catheter associated urinary tract infection (CAUTI), central-line associated bloodstream infection (CLABSI), ventilator associated infection (VAP), and Surgical Site Infection (SSI), some include peripheral IV line Infection or Phlebitis. HAIs can be prevented by doing hand hygiene that is the key to patient-based care, because hand hygiene is the easiest, cheapest and effective procedure to prevent HAIs [2]. The research objectives are to evaluate the effectiveness of hand hygiene training with Kirkpatrick' framework including 4 levels: reaction, learning, behaviour, and results.

MATERIALS AND METHOD

Hand hygiene training evaluation was conducted in January 2018 while the Training of Infection Prevention and Control which contained Hand Hygiene material was held in May 2017. As the priority participants were the workers of Surabaya Islamic Hospital who served in high risk units such as Operation Room, Maternity Room, Haemodialysis Unit, Laboratory Unit, Radiology Unit, ICU, *Zam-zam* Room (Neonatus), Emergency Unit, Nutrition Unit, and Sanitation and Environmental Health Unit. Training location is at meeting room of Surabaya Islamic Hospital. Training was conducted for two days starting at 08.00 up to 16.00 o'clock. The numbers of trainees in the initial stage are 30 participants. The training instructor is the team of Infection Prevention and Control that has been certified in basic level of IPC training.

This study focuses on evaluation of Hand Hygiene training based on Kirkpatrick's four level model, including reaction from trainees, learning after training, behaviour change after training, and training contribution to the achievement of hospital key indicator. For level 1, evaluation was performed by filling out the questionnaire of trainees' satisfaction. Level 2 was assessed from the pre-post test of trainees based on the WHO's Hand Hygiene Knowledge Test questionnaire. For level 3, the evaluation was performed by measuring the hand hygiene compliance rate, which was assessed after 8 months post training. The training compliance rate would be compared to the unattended-training health worker. Hand hygiene compliance measurements were performed with the WHO tool, i.e. HHOT (Hand Hygiene Observational Tools), and calculated using the *Epiinfo* method. Level 4 was measured from the hospital's key indicator that is hospital's HAIs rate.

FINDINGS AND DISCUSSIONS

Effectiveness of Level 1 Reaction

In overall the training process was well organized, the weakness is only on facilities and infrastructure factors that is limited space, and training time is too short compared with the given material density.

Measurement of trainees' perception of the implementation of each element are: it is said not satisfied with the range below 50 to 60, it is said less satisfied if the range between 61 to 70, it is said satisfied if the range of values between 71 to 80, and it is said very satisfied if the range above 81 to 100.

Table 1 Level of Trainee's Satisfaction

Evaluation Elements	Score	Category
Training material		
Systematics	87	Very satisfied
Clarity	80	Satisfied
Contribution	80	Satisfied
Advantages	90	Very satisfied
Conformity	85	Very satisfied
Training implementation		
Schedule conformity	84	Very satisfied
Timeliness	78	Satisfied
Committee assists	80	Satisfied
Committee willingness	82	Very satisfied
Infrastructure facility		
Audio and visual aid	75	Satisfied
Conformity of visual aid	81	Very satisfied
Consumption	76	Satisfied
Seminar kits	75	Satisfied
Facilities	75	Satisfied
Room	78	Satisfied
Instructor ability		
Material mastery	88	Very satisfied
Delivery techniques	75	Satisfied
Ability to answer questions	75	Satisfied
Language	82	Very satisfied
Gesture	88	Very satisfied

Source: Education and Training Unit of Surabaya

Islamic Hospital, 2017

Effectiveness of Level 2 Learning

The measured aspect is the trainees' understanding of the training materials. Prior to the training, a questionnaire was handed out based on WHO's tool "Hand Hygiene Knowledge Test" and at the end of the training session was done post test with the same questionnaire.

From the result of pre-post test given during Hand Hygiene training, it is seen that there is an increase of knowledge from the training participants, where the knowledge is expected to increase their awareness to do hand hygiene in accordance with the established moment. With increasing knowledge it will encourage self confidence of workers to apply the learning culture gained during the training.

Table 2 The increasing result of Pre and Post test

Pre Test	**Post Test**	**Increasing**
40.2	81.73	41.57

Source: Education and Training Unit of Surabaya Islamic Hospital, 2018

Effectiveness of Level 3 Behaviour

The impact of hand hygiene training that has been done shows the difference in compliance rate between health workers who received training and who did not. With increased knowledge it is easier for health workers to recognize risk factors for the transmission of microbes through the hands of health workers, and disconnect the transmission chain by applying hand hygiene according to established guidelines. Increased knowledge gained during training will also encourage the self confidence of health workers to apply hand hygiene skills to their daily work according to the steps in the guidelines.

Table 3: The comparison of compliance rate of hand hygiene

Moment	**Training**	**Non Training**
ICU room		
Moment 1	48	45
Moment 2	85	49
Moment 3	40	80
Moment 4	75	45
Moment 5	46	30
Emergency room		
Moment 1	42	22
Moment 2	46	46
Moment 3	88	68
Moment 4	40	42
Moment 5	32	20
Inpatient room		
Moment 1	72	38
Moment 2	80	72
Moment 3	92	72
Moment 4	68	37
Moment 5	72	42

Effectiveness Measurement of Level 4 Result

Assessment of effectiveness at the previous level should be illustrated at the level 4. With the development of knowledge and skills of health workers, they have the provision to make changes in behaviour in their daily work. The HAIs rate as a key indicator of the hospital will describe the level of workers' compliance in hand hygiene, because the largest medium of microbe transmission is through the hands of health workers.

Limitations of the study are the duration of research between training programs implemented and the time of research that are only about 7 months, it can not describe the trend of HAIs rate. With sufficient knowledge of the basics of prevention of infections acquired during the training program it is expected the cases of HAIs such as SSI, CAUTI, VAP, CLABSI and Phlebitis can be found through the implementation of hospital surveillance. From the results of research, the HAIs rate is still below the standard set by the hospital that is 2% for SSI and 15% for Phlebitis.

Table 4 HAIs rate of Hospital

Months	Phlebitis	SSI	VAP/HAP	CAUTI	CLABSI
May	3%0	0	0	0	0
June	8%0	0	0	6%0	0
July	8%0	0	0	0	0
August	6%0	0.8%	0	0	0
September	2%0	0.4%	0	0	0
October	4%0	0	0	0	0
November	4%0	0	0	0	0
December	4%0	0.5%	0	0	0

Source: IPC Committee of Surabaya Islamic Hospital, 2017

CONCLUSION

The research shows that hospital workers show increased knowledge and skills after following the hand hygiene training program. Therefore, hand hygiene training program must be done continuously. It is need further research to see the impact of HAIs rate in hospitals that are closely related to the transmission of microbes that are distributed through the hands of health workers.

Conflict of Interest: The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any fi nancial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-fi nancial interest (such as personal or professional relationships, affi liations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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REFERENCES

1. WHO. WHO Guidelines for hand hygiene in healthcare (advanced draft), 2006 (http://www.whoint/patientsafety/challenge/en) Geneve. World Health Organization.
2. Center for Disease Control and Prevention . Guidelines for hand hygiene in healthcare settings; recommendations of the healthcare Infection Control Practices Advisory Committee and the HIPAC/SHEA/APIC/IDSA (http;//www.cdc.gov/mmwr/pdf/RRS5116.pdf)
3. Pittet D, Mouruga P, Perneger TV; Compliance with handwashing in teaching hospital. Infection Control Program. Ann Intern Med 1999
4. Measuring Hand Hygiene Adherence. Overcoming the challenge (http;//www.jointcommision.org)
5. Mehwish Rafiq (2015),Training Evaluation in Organization using Kirkpatrick Model; A Case Study of PIAMesh, Entrepreneurship & Organization Management Journal.
6. Vemic, J,(2007) Employee Training and Development and Learning Organization, Facta Universitatis Series. Economic and Organization 4 209- 216.
7. Edens PS, Bell ST (2003) Effectiveness of Training in Organizations; A Meta Analysis of Design and Evaluation Feature. American Psychological Association
8. Maha Mousa Mohammed Mosaa, Eman (2015)

Evaluation of educational Program on nurses knowledge and practice regarding Standart precaution of infection control measures in outpatient clinics. International Journal of Advances Research.

9. Tahereh Changiz, Moh Fakhari, Athar Omid (2014) Kirkpatrick Model a Framework for Evaluating the Effectiveness of Short-term and Inservice Training Program, Iranian Journal of Medical Education
10. Kirkpatatrick, Donald, (1994). Evaluating Training Programs the four levels. San Fransisco, CA;Berret-Kohler Publisher.
11. Roger C Jeensen (2005),Safety Training; Flowchart Model Facilitates Development of Effective Course, Safety Health and Industrial Hygiene13, http;//digital.commons.mtech.edu/shih/13
12. Ann-Margaret Dunn-Navarra (2011), Relationship between system -Level Factors and Hand Hygiene Adherence, J Nurs Care 2011, National Institute of Health Colombia edu.
13. Sylvester Mulli Maingi (2015), Factor Influencing Compliance with Hand Hygiene Guidelines Among Healthcare Providers in Kenya; A Case of Level Five, Embu County,
14. Noreen Hynes(2015) A hand Hygiene Education and Training Improvement Strategy in Acute Hospital Setting, Royal College of Surgeon in Ireland e-publication @RCSI
15. George Osoro Momanyi(2016). Value of Training on motivation among healthcare worker in Norok County, Kenya, Pan African Medical Journal-ISSN
16. Ludmilla Praslova,(2010). Adaptation of Kirkpatrick's four level models of training criteria to assessment of learning outcomes and program evaluation in higher education, Springer Science+Business Media,LLC,2019
17. Roger, Kaufmann(1994). Levels of evaluation: Beyond Kirkpatrick, Human Resource Development Quarter Vol 3, No 4 Willey Online Library.
18. Mark R Chassin,Md,(2015) Sustaining and Spreading Improvement in Hand Hygiene Compliance..Vo, The Joint Commission Journal. Journal on Quality and Patient Safety
19. Mark R Chassin,MD(2015) Improving Hand Hygiene at Eight Hospital in the United States by Targeting Specific Causes of Noncompliance. The Joint Commission Journal, Journal on Quality and Patient Safety.
20. Sung-Ching Pan (2013) Compliance of Health Care Workers with Hand Hygiene Practices; Independen advantages of overt and covert observer. PloS ONE 8(1) Journal

Quantitative Risk Assesment of Crystalline Silica Exposure in Ceramics Industry

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ABSTRACT

Crystalline silica can be found in Ceramics Industry. Ceramics are basically made of clay, feldspar and silica sand. Ceramics manufacturing process starts from the processing of materials, forming, drying, burning and gazing. Crystalline silica consists of three types of quartz, tridymite and cristobalite which are distinguished by their forming temperature. Crystaline silica dust is very dangerous for health workers are exposed continuously it can cause a silicosis. Prevention and control efforts can be done one of them by conducting a risk analysis study on crystalline silica exposure. In this research will be discussed about the analysis of crystalline silica exposure into production workers in the ceramics industry.

The purpose of this study was to assess the risk level of crystalline silica due to silicosis. Subjects in this study were 47 production workers with the exposure to crystalline silica between 0,007 – 0,4 mg / m^3. From the result of risk analysis, it was found that 42,6% of workers had unsafe risk which was confirmed by radiology examination was 6,4% of workers with abnormal condition. Complaints experienced by workers include shortness of breath, cough and phlegm. Long term exposure to 5-20 years to the next tends to increase the risk of the effects caused by exposure to crystalline silica. Based on the results of this study it is recommended that decision makers take action to implement effective risk management strategies to prevent the long-term effects of crystalline silica exposure to the workplace.

Keywords: *risk analysis, crystalline silica, ceramic industry*

INTRODUCTION

Exposure to the existing hazards in the work environment in the ceramics industry most widely encountered is silica dust, total dust and heat pressure. Silica dust is the main pollutant in the ceramics industry because it is one part in the raw material. Crystalline silica is one of the most widely encountered minerals of the earth, with wide exposure in working environments and ambient environments[1]. silica belongs to Group 1 substances that are carcinogenic in humans[2]. In China, the association with silica dust exposure and mortality from all causes of respiratory illness, respiratory tuberculosis, and cardiovascular disease is significant[3]. Worldwide estimated at least two to three million workers work roughly exposed to silica each year[1]. Silica crystal dust is inhaled from the work environment can cause pulmonary fibrosis (silicosis), decreased lung function, pulmonary inflammation, and lung cancer have been associated with glomerulonephritis and disorders of the liver, spleen, and immune system[1]. The prevalence of silicosis data varies from country to country. Studies conducted in the United States show that there is 3600-7300 new cases of silicosis per year in 1987-1996[3]. Studies conducted at a cement plant found radiological silicosis suspicion of 0.5%[4] . A study conducted at one of the cement plants in West Java showed that the incidence of silicosis was 2.06% in 1990-2003[5]. A previous study showed that 32% of ceramic material samples contains free silica throughout the Taiwan[6].

Several recent epidemiological studies have shown that the present value of standard silica dust are insufficient for guidelines to protect and prevent chronic silicosis[6]. The threshold value of crystalline silica dust

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in Indonesia is based on the Ministry of Manpower and Transmigration No. PER.13 / MEN / X / 2011 on the threshold value of physics and chemical factors in the workplace set at 0.05 mg / m^3 [7]. Cases of pneumoconiosis ranked first Occupational Diseases (OD) in Japan and China[8]. Based on the silicosis surveillance program in Ontario it was found that the incidence of silicosis was a significant increase in more than 8% of respondents after exposure to silica dust for 35 years [9]. The risk analysis approach is one way to determine the level of risk due to crystalline silica dust present or some future time making it possible for early prevention efforts.

METHOD AND MATERIAL

This type of research is descriptive research using quantitative analysis method. The study design used was a risk analysis method taken from risk analysis steps, risk management, and risk communication used to assess and predict the occurrence of health effects as a result the presence of a hazardous exposure to this case is exposure to crystalline silica. The design of this research is cross sectional where the environmental data collection (silica dust measurement) and taking of rontgen photo taken at same time period. The population of this study are all workers that work in the production section from the processing of raw materials to packing which amounted to 50 people. The sample of this study is the total population with the exclusion factor of workers not suffering and never exposed from tuberculosis, so the sample in this study amounted to 47 people. The measurement of crystalline silica dust in the working environment is based on NIOSH 7500 method of XRD[10].

The risk is calculated based on the amount of intake of agent entering the worker's body so that it can be known how much the risk of health effects on workers. The data and information required to calculate the silica dust intake of the worker's body is all variables in the following [11].

$$I_{nk} = \frac{C \times R \times t_E \times f_E \times D_t}{W_b \times t_{avg}}$$

Level of risk to be expressed in Risk Quotien notation (RQ). To do risk characterization is done by comparing the intake of Rfc from the agent.

$$RQ = \frac{I_{nk}}{RfD \text{ atau } RfC}$$

The Rfc value was obtained using the data onto Chen et al of 6 µg / m3 3. The Rfc unit is converted first into units of m^3 / kg / day, by multiplying the Rfc value with the adult inhalation default value of 20 m^3 / day and dividing by the default value of the adult 70 kg[14]. Risk levels are expressed in numbers or decimal numbers without units. The risk level is said to be safe whenever its intake ≤ Rfc is represented by RQ ≤ 1. The risk level is said to be unsafe when the intake values ≥ Rfc is expressed by RQ ≥ 1[15].

FINDING

Table 1. The pattern of crystal silica dust exposure in production workers

	Concentration of c-silica dust	Respiratory rate (m^3 / h)	Intake of c-silica dust (mg / m3 / day)	Risk Quostion
Mean	0,0686	0,6572	0,00192	2,2343
Median	0,0540	0,6600	0,00129	1,5017
Mode	0,0540	0,66	0,00024	0,0459
Std. Deviation	0,0704	0,0218	0,00260	3,0251
Range	0,4387	0,10	0,01668	19,391
Minimum	0,0078	0,59	0,00004	0,0456
Maximum	0,4466	0,69	0,01672	19,4365

From table 1 the research results can be seen that the concentration of crytaline silica dust at work ranged from 0.0078 - 0.4466 mg / m^3 with an average of 0.0686 mg / m^3. The average respiration rate of workers is 0.6572 m^3 / hour. Intake of silica dust based on exposure pattern and anthropometric data of worker ranged from 0,00004 - 0,01672 mg / m^3 / day with average intake 0,00192. Risk level (RQ) ranged from 0,0456 - 19,4365 with value RQ ≥ 1 equal to 42,6% from total respondent. Age of respondents in this study between 23 - 56 years with an average age of 42.38 years. The working period ranges from 2 - 25 years with an average of 18.06 years. Workers work for 8 hours per day with 1 hour break time to exit the workroom so that the effective hours of labor are in the workroom for 7 hours.

Tabel 2. Estimate the value of the risk level of chronic exposure to production workers

Duration	Risk *Quotient* (RQ)	Persentase (%)
5 year	RQ ≤ 1	51,1
	RQ ≥ 1	48,9
10 year	RQ ≤ 1	42,6
	RQ ≥ 1	57,4
I5 year	RQ ≤ 1	34,0
	RQ ≥ 1	66,0
20 year	RQ ≤ 1	29,8
	RQ ≥ 1	70,2

Tabel 2. shows that in the projection of exposure over the next 20 years an increase in the value of RQ ≥ 1 can be interpreted that there is an increased risk due to exposure to c-silica dust up to 20 years in the future.

DISCUSSION

Continuous exposure to c-silica dust can have both long-term and short-term effects[16]. The source of c-silica dust part production comes from raw materials in the form of clay and silica sand containing free silica that make ceramic to be harder and stronger[12]. The heating process in the production process to a temperature of about 1200 ^oC will also form another fraction of the more reactive c-silica dust. The level of c-silica dust has a uniform distribution throughout the workplace with an average grade of 0.068 mg / m^3. The temperature used in the production process will also affect the convection and radiation to the temperature rise of the work environment. The working environment temperature ranges from 31.3 - 36.6 ^OC which makes the c-silica dust become drier and lighter so it is easily carried by the airflow present in the work environment and can expand the spread of the dust. Silicosis is still present in workers who die affected by exposure to silica dust between 0.05 - 0.1 mg / m^3 [3]. From the result of the measurement of c-silica dust in the working environment by using personal sampler on each respondent got result that exceeds limit value based on Indonesia regulation (Ministry of Manpower and Transmigration No. PER.13 / MEN / X / 2011) amounted to 55.3%. The value of concentration is very influential on the value of intake in workers the higher the concentration of dust c-silica in the work environment the higher the value of the intake so that the value of risk will also increase[11].

Chest radiography can be used to assess the exposure of silica dust. The interpretation of the results of a thorax examination should be done using the radiographs of pneumoconiosis guidance from the ILO[13]. Based on the calculation of risk values over the next 20 years based on table 2 there is an increased risk. This happens because exposure to c-silica dust is prolonged even further if not effectively controlled[16].

CONCLUSION

From this study it can be concluded that 42.6% of respondents mempuyai unsafe risk level of exposure to c-silica dust so potentially health problems due to exposure to c-silica dust. Based on the estimated calculation of chronic exposure risk, the risk level tends to increase to the next 20 years.

Conflict of Interest: None

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REFERENCE

1. Chen, W. et al. (2012) "Long-term exposure to silica dust and risk of total and cause-specific mortality in Chinese workers: A cohort study," PLoS Medicine, 9(4). doi: 10.1371/journal.pmed.1001206.
2. "IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: Silica, Some Silicates, Coal Dust and Para-Aramid Fibrils. Lyon, 15-22 October 1996." (1997) IARC monographs

on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer, 68, hal. 1–475.

3. Office of Environmental Health Hazard Assessment (2005) "Chronic Toxicity Summary."
4. Salawati, L. (2017) "Silikosis," JURNAL KEDOKTERAN SYIAH KUALA Volume, 17(April), hal. 20–26.
5. Rinawati, P. (2015) "Coal Worker ' S Pneumoconiosis," 4, hal. 49–56.
6. Department Of Health And Human Services, Centers for Disease Control and Prevention National Institute for Occupational (2002) "Health effects of occupational exposure to respirable crystalline silica," Washington, DC: National Institute for Occupational Safety and Health, 129.
7. PER.13/MEN/X/2011 (2011) "Peraturan Menteri Tenaga Kerja Dan Transmigrasi," Peraturan Menteri Tenaga Kerja Dan Transmigrasi.
8. Services, H. (1998) "Toxicological Profile," (205). doi: http://dx.doi.org/10.1155/2013/286524.
9. Suhariyono, G., Bunawas dan Makhsun (2006) "ANALYSIS OF FREE SILICA (SiO2) IN RESPIRABLE DUST WITH METHOD OF NIOSH 7500," Prosiding Seminar Nasional Keselamatan, Kesehatan, dan Lingkungan II, Pusat Tenaga, (December 2006), hal. 0–15.
10. National Institute for Occupational Health and Safety (2003) "SILICA , CRYSTALLINE , by XRD (filter redeposition)," NIOSH Manual of Analytical Methods, 7.
11. Louvar FL and Louvar BD (1998).Eenvironment Risk Analysis Volume 2. New Jersey: Prentice Hall PTR.
12. Bakri, R., Utari, T. dan Sari, P. (2008) "Koalin Sebagai Sumber SiO2 Untuk Pembuatan Katalis Ni/ SiO2: Karakterisasi Dan Uji Katalis Pada Hidrogenasi Benzena Menjadi Sikloheksana," Makara, Sains, 12(1), hal. 37–43.
13. Siswanto (1996) Penyakit Akibat Debu Silika. Surabaya: Balai Hiperkes dan Keselamatan Kerja Jawa Timur Departemen Tenaga Kerja.
14. U.S. Environmental Protection Agency (1994) "Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry,". doi: EPA/600/8-90/066F.
15. P2PL KEMENKES (2011) Pedoman Analisis Resiko Kesehatan Lingkungan. Jakarta: KEMENKES RI.
16. Suma'mur (2014) Higene Perusahaan dan Kesehatan Kerja (HIPERKES). 2 ed. Jakarta: CVSagung Seto.

Central Obesity as a Risk Factor for Hypertension in Women : in Bogor District

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ABSTRACT

Hypertension in women is increased by 30.9% and 26.6% of central obesity in Indonesia. The purpose of this study is to study the relationship of central obesity with hypertension in women aged 18 years and over in Bogor District year 2016. This study used cross sectional design with *cox regression analysis*. The analysis is used to see how much impact of Central Obesity with hypertension incidence after backward elimination procedure test. Total sample in this study was 525 samples. Results from this study showed an influence of age > 45 years on the association of central obesity with hypertension in women with a PR of 1:56 (95% CI 1084-2120) and the discovery of potentially confounding variables which is age. The risk of central obesity with hypertension increased after controlled by age as much as PR 1:52 (95% CI 1:07 to 2:16). Central obesity affects the incidence of hypertension in women. It is suggested that all women to increase physical activity, adjusting the diet and conduct regular health checks to maintain the weight to avoid central obesity.

Keywords: *Central Obesity, Age, Hypertension, Women, Health*

INTRODUCTION

Hypertension is a global public health problem. Hypertension is often called the silent killer, because there was no physical signs of high blood pressure and there is no record of complaints in patients with hypertension.[1] According to WHO (World Health Organiation) in 2011, 1 billion people worldwide suffer from Hypertension and two thirds of them are from low and middle-income developing countries. Hypertension in women has increased around the world in this decade. Especially in Indonesia, Hypertension in women is higher than in men. [2]

Hypertension is the latest issue to be considered in the Sustainable Develompment Goals (SDGs) program. Therefore, there is Presidential Instruction No. 1 of 2017 on Healthy Living Community Movement. The purpose of this movement is to improve prevention and early detection of diseases, especially on Non Contagious Diseases in Indonesia. One way of this GERMAS program is to check blood pressure once every month at POSBINDU in every area of the first health facility in Indonesia.[3]

Central obesity mechanism for hypertension could lead to unbalanced glucose tolerance. High levels of insulin would circulate and reduce the sensitivity of insulin metabolic activity. These conditions could lead to hyperinsulinemia that affects the rise in blood pressure.[4]

Measurement of abdominal circumference can be useful for identifying population-based risk that can cause hypertension in adult women. A study revealed that an increase in the occurrence of hypertension is influenced by the increase of abdominal circumference and age in women.[5] Another study has also revealed that waist circumference may increase the risk of hypertension. Measurement of waist circumference is one step to prevent the occurrence of hypertension in Asians.[6]

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There are many risk factors which caused hypertension, such as age, gender, smoking habit, physical activity, obesity and central obesity, fruit and vegetable consumption, genetic factors, and stress. Hypertension in women can lead to more serious complications than in men. Therefore there should be hypertension prevention for women, especially in women based on risk factors. In addition, hypertension in menopausal women can contribute to an increase in incidence of hypertension. This incidence has relation to the loss of estrogen and pituitary hormones because they are produced in excess, their weight gain or a combination of other influences. Moreover, in infertile women, there are some who use oral contraceptives. Oral contraceptives could cause sodium buildup, increase fluid volume / plasma, and could increase weight. The use of oral contraceptives with more than (> 50μgr) could cause hypercholesterolemia in women.[7]

Several studies conducted in Indonesia shows that central obesity has a significant relationship with hypertension in women.[8] Other studies also explain that central obesity may increase the risk of hypertension by 1.6 times compared with non-obese central ones [9]. Based on the results of the study of Obesity Association of Indonesia in 2004, the prevalence of obesity in women is higher (11:02%) than in men (9:16%).[10] Another study conducted in Bogor District in 2007 explained that abdominal circumference is one of the risk factors associated with the incidence of hypertension.[11]

Bogor District is an area that has a high prevalence of hypertension and central obesity. Prevalence of hypertension in Bogor district is 27.6% while central obesity is 24% in 2013. This study was conducted in Bogor District. However, prevalence of hypertension and central obesity in women aged 18 years and above is not known. Therefore it is necessary to conduct research related to central obesity and hypertension in women in Bogor District.

MATERIALS AND METHOD

This research is a quantitative research with cross sectional study design using secondary data of GERMAS 2016. The data is a data of Bogor District. This design observes and measures the main risk factors (central obesity) and other risk factors (age, physical activity, smoking, total cholesterol, also fruit and vegetable consumption) and the measurement of dependent variables (hypertension) at the same time. The variable in this study is limited to the variables which available in the data GERMAS 2016. Estimated minimum sample in this study is 260 samples which determined by using two sample populations / two tail with a power of 80%. The analysis uses Cox Regression analysis because the prevalence of outcome is more than 10%. Partisipant rate in this study is 100%. Samples taken were 525 samples.

It is determined as not Central Obesity when the measurement of abdominal circumference in women is < 80 cm and it is Central Obesity if the result > 80 cm.[11] The age of respondents is grouped using ROC cut off point value, if Not Risk (<45 years cut off point ROC) and Risk (> 45 years cut off point ROC). The measurement in hypertension variabel is conducted when someone has a result of systolic blood pressure ≥140mmHg and or diastolic blood pressure ≥ 90mmHg. Not hypertension if the systolic blood pressure is < 140mmHg and or tekanan diastolic blood pressure < 90mHg.[12] Physical activity is determined as Enough if the frequency is 3-5 times a week with duration of at least 150 minutes / week. However it is determined as Less activity, if it is less than 3-5 times a week with duration of less than 150 minutes.[13] A person who is exposed to secondhand smoke either as an active smokers or as a passive smokers,[14] Cholesterol levels in the blood, if the total cholesterol level > 200 mg / dL it is high whereas if the total cholesterol level <200mg / dL it is low [15] and the average consumption of fruit and vegetable intake > 5 servings per day.[16]

RESULTS

In this study a multivariate cox regression analysis was used to examine the relationship between central obesity and hypertension occurrence in women aged 18 years and over after controlled with age, total cholesterol, physical activity, fruit and vegetable consumption and smoking. In **Table 1** from 525 respondents, 49.71% women aged 18 years and over suffered from hypertension while 50.29% had normal blood pressure. In addition, women who are central obesity is recorded at 77.33% whereas there are 22.67% of those women who is not central obesity. Percentage of female respondents by age group 46-55 years at most is 29.71%. The percentage of women whose physical activity is less is 66.67% compared with the physical activity which is enough has 33.33%, the percentage of women who are active smokers or passive smokers exposed by

cigarette smoke is lower at 48.57% than unexposed or non-smokers at 51.43%, the percentage of women who consumed less fruits and vegetables is lower which is 48.57% compared with women who consumed enough fruits and vegetables has 51.43%, and the percentage of women which has a high cholesterol levels is lower at 30.67% compared with the percentage of women who have low cholesterol levels which is 69.33%.

Table 1. Frequency Distribution of Hypertension In Women

Variabel		n	%
Hypertension	Hipertensi	261	49,71
	Normal	264	50,29
Central Obesity	Central Obesity	406	77,33
	Normal	119	22,67
Age	18-25	26	4,95
	26-35	84	16
	36-45	145	27,62
	46 – 55	156	29,71
	56-65	82	15,62
	>65	32	6,10
Physical activity	Less	350	66,67
	Enough	175	33,33
Smoke	Yes	255	48,57
	No	270	51,43
Fruit Vegetable Consumption	Less	255	48,57
	Enough	270	51,43
Total cholesterol	High	161	30,67
	Low	364	69,33

Based on **Table 2**, the stratified analysis results shows that age > 45 years is at risk for the occurrence of central obesity with hypertension 1.516 times higher compared with age < 45 years that not has a strong enough risk for the occurance of central obesity with hypertension. From these results we could say that the age affect central obesity with hypertension. The prevalence of central obese women aged 45 years and over suffer from hypertension is higher at 64.44% compared with the women who is not central obese at 42.86%. Furthermore, in central obese women aged under 45 years suffering from hypertension is higher at 37.58% compared with non-central obesity at 24.29%.

Table 2. StratificationAnalysis Central Obesity with Hypertension Adjusted by Age In Women

Variabel		**Hypertension**		**PR (95%CI)**	**p-*value* Test homogenity**
		Hypertension (%)	**Normal (%)**		
Age ≥45	Central Obesity	167(64,44)	90(35,02)	1,51 (1,084–2,12)	0,98
	Normal	21(42,86)	28(57,14)		
Age < 45	Central Obesity	56(37,58)	93(62,42)	1,54 (0,97-2,45)	
	Normal	17(24,29)	53(75,71)		

Based on **Table 3** it could be seen the relationship of central obesity with the incidence of hypertension in women after being adjusted with other variables. The risk of central obesity is 1.55 (95% CI 1.09-2.20) times occurrence of hypertension in women after adjusted by age, total cholesterol, smoking, consumption of fruits and vegetables and physical activity. The result shows that age is a confounding variable against the relationship of central obesity with the incidence of hypertension. Where the risk of hypertension is higher in age compared with central obesity, age may increase the risk of occurrence of hypertension in women by 1.69 times.

Table 3. Association of Central Obesity with Hypertension in Women

Variable	Coeff	P	PR	95% CI	
				Lower	Upper
Central Obesity*	0,439	0,014	1,55	1,09	2,20
Age*	0,524	0,000	1,69	1,27	2,23
Total Cholesterol	0,113	0,399	1,12	0,86	1,45
Smoking	0,215	0,088	1,24	0,96	1,58
Consumption of fruit and vegetable	- 0,136	0,349	0,87	0,65	1,16
Physical Activity	- 0,082	0,607	0,92	0,67	1,26

PR; Prevalens Ratio, CI; Confidence Interval * P <0.0,5 test using multivariate Cox Reggresion (adjusted by covariate variable: age, total cholesterol, smoking, fruit and vegetable consumption and physical activity).

Based on **Table 4**, the relationship of central obesity with hypertension at risk of 1.52 (95% CI 1.07-2.16) times greater after controlling it with the age variable. It could be argued that women with central obsessions will develop hypertension as much as 1.52 times greater than those who are not central obesity after being controlled by age. The results of this analysis indicate that there is still a significant relationship between central obesity and hypertension in women after controlled by age.

Table 4. Final Model of Cox Regression Association Central Obesity with Hypertension In Women

Variable	p-value	PR	95% CI	
			Lower	Upper
Central Obesity*	0,017	1,52	1,07	2,16
Age*	0,000	1,73	1,32	2,28

PR; Prevalens Ratio, CI; Confidence Interval * P <0.0,5 test using multivariate Cox Reggresion (adjusted by confounding variable: age).

DISCUSSION

Analysis has been done to prove the relationship of central obesity with hypertension after controlled with covariate variables. It was revealed that the relationship of central obesity with hypertension has a significant relationship after controlled by confounding variable which is age. The effect of central obesity with hypertension after controlled by risky age was 1.52 (95% CI 1.07-2,16), while the effect of age on the relationship of obesity with hypertension is 1.73 (95% CI 1.32-2 , 28). The effect of age on the relationship of central obesity with hypertension is higher, therefore age variable is a confounding variable to the relationship of central obesity with hypertension. However, central obesity has a significant relationship with hypertension even before or after controlled by other variables, especially by the age variable. A study conducted by Jenssen explains that an increase in the age of a person, increases the risk when entering the age of 40 years to 59 years, after age of 60 years and over, the incidence of hypertension with central obesity is lower than when entering the age of 40 to 59 years. But these results explain that the increasing size of a person's abdominal circumference increases the risk of sickness in an aged person.[17]

According to Poirier measurement of abdominal circumference can prevent the risk of increased systolic blood pressure and diastolic blood pressure after adjusted by age, sex, BMI and insulin resistance intake. Previous studies have found that abdominal circumference is a major determinant of hypertension. Research conducted in a cohort study by American and Japanese people found that a person whose stomach circumference is at risk can suffer from hypertension with more than 40%. This number is higher when a person with central obesity is exposed to hypertension is women rather than men after being adjusted by age.[18] The abdominal circumference increases with age and higher at older age than younger ones. Increased abdominal circumference is higher in women which is 4.3cm per 15 years[19]

Research conducted by Snijder explains that age is one of the effect on the relationship of central obesity with the incidence of hypertension, especially in women. Women aged> 35 years with central obesity are associated with increased diastolic blood pressure and systolic blood pressure.[20] Excessive abdominal circumference and increasing age is a risk for the occurrence of hypertension. This study proved, that women who are central obesity and at age 45 years has greater risk of hypertension. The risk of central obesity in this study on hypertension after adjusted by age is still quite high.

CONCLUSION

Central obesity affects the incidence of hypertension in women. The older a person is, the greater the risk of hypertension when central obesity. It is suggested that all women to increase physical activity, adjusting their diet and conduct regular health checks to maintain the body weight, so they can avoid central obesity.

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Competing Interest: This research is part of final task of University of Indonesia students, thus, there is no competition in conducting this research.

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REFFERENCE

1. Kemenkes RI. Non-Communicable Diseases. Jakarta: Ministry of Health; 2016
2. Agency for Health Research and Development. Basic Health Research (RISKESDAS) 2013. Lap Nas 2013. 2013;1-384.
3. Sardjoko S. Mainstreaming Health in Sustainable Development Goals (SDGs). Konf Nas-7 Promotion of Health:2017
4. Kotsis, Vasilios, Stabouli S, Papakatsika S, Rizos Z, Parati G. "Mechanisms of Obesity-Induced Hypertension." Hypertension Research . Nature Publishing Group. 2010;386–93
5. Olinto M, Nacul L, Gigante D, Costa J, Menezes A, Macedo S. Waist circumference as a determinant of hypertension and diabetes in Brazilian women: a population-based study. Public Health Nutr. 2004;7(5):629–35.
6. Yap S, Yang Z, Wang J, Bacon SL, Campbell TS. Waist circumference, not body mass index, is associated with blood pressure in a sample of young Chinese adults. J Hum Hypertens. 2006;20(11):904–6.
7. Rosenthal T, Oparil S. Hypertension in women. J Hum Hypertens. 2000;14(10–11):691–704.
8. Triwinarto A, Muljati S, Jahari AB. Cut-Off Point Body Mass Index (BMI) and Round Stomach as Indicators of Diabetes Risk and Hypertension in Adults In Indonesia. Penel Nutrition Eating 2012. 2012;35(2):119-35.
9. Julianty P. Factors Affecting the Occurrence of Hypertension in Urban Areas (Data Analysis Riskesdas 2007). Nutrition Indonesia. 2010; 33 (1): 59-66.
10. Trisna, SH. Factors Related to Central Obesity in Adult Women (30-50 Years) in Kecamatan Lubuk Sikaping Year 2008. J Public Health. 2009; 3 (2): 68-71.
11. Pradono J, Indrawati L, Murnawan T. Problems And Risk Factors Associated With The Occurrence Of Hypertension In Bogor Regency Prov. West Java. Health Research Bulletin. 2013; 41 (2): 61-71.
12. Mancia G, Fagard R, Narkiewicz K, Redán J,

Zanchetti A, Böhm M. Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC). J Hypertens. 2013;31(10):1925–38.

13. Cho NH, Whiting D, Guariguata L, et al. International Diabetes Federation. IDF Diabetes Atlas Sixth edition. IdfOrg. 2014;1–2.
14. Ministry of Health RI. General Guidelines for Obesity Control. In Jakarta; 2016.
15. Ministry of Health RI. Technical Guidelines Skrining/ Health Specific Examination in The Framework of The Healthy People Movement Of 2016. 2016:1-6.
16. National Cholesterol Education Program (NCEP) Expert Panel. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Arch Intern Med. 2002;(6):284.
17. Janssen I. Influence of age on the relation between waist circumference and cardiometabolic risk markers. Nutr Metab Cardiovasc Dis. 2009;19(3):163–9.
18. Poirier P, Lemieux I, Mauriège P, Dewailly E, Blanchet C, Bergeron J, et al. Impact of waist circumference on the relationship between blood pressure and insulin: The Quebec health survey. Hypertension. 2005;45(3):363–7.
19. WHO. Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation. World Heal Organ. 2008;8–11.
20. Snijder MB, Zimmet PZ, Visser M, Dekker JM, Seidell JC, Shaw JE. Independent and opposite associations of waist and hip circumferences with diabetes, hypertension and dyslipidemia: The AusDiab Study. Int J Obes. 2004;28(3):40–9.

Health Promoting Lifestyle and its Associated Factors among Private University Students in Shah Alam, Selangor

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ABSTRACT

Healthy lifestyle is a major strategy to promote current and subsequent health status. Lifestyle is the way of living of individuals, families, and societies which can be healthy or unhealthy. The objective of the present study was to find out healthy promoting lifestyle level and its associated factors among the private university students in Shah Alam, Malaysia. A cross- sectional study was conducted among 500 students using convenience sampling. Self-administrated questionnaires with 52 items of health promoting lifestyle habits with 6 subscales was used. The results showed that mean score of health promoting lifestyle scale was 136.18 ± 22.48. Half of the respondents found to have normal weight (50.2%) followed by (28.4%) overweight and (12.4%) obese. The association between gender and health promoting lifestyle was significant (P<0.001). While, Body Mass Index was not significantly associated with health promoting lifestyle (P=0.837). As a conclusion, the mean score of healthy lifestyle practice was 136, prevalence of overweight and obese was (40.8%) among university students. More promotion is needed in promoting healthy lifestyle.

Keywords: *Healthy promoting lifestyle, obesity, university students.*

INTRODUCTION

Healthy lifestyle is a major strategy to promote current and subsequent health status. The aim of this study is to assess the status of health promoting the lifestyle and its determinants among university students Health promotion is a process for empowerment of individuals in order to improve their health status. The definition of an individual from health is placed in the center of individual viewpoint toward health promoting behaviour. At this level, health is defined as using some positive qualities recommended by World Health Organization. Health is the fulfillment of human potential, maintenance of balance and goal orientation in the environment [1]. Considering the changes happened in the study modes and living in campus dormitories, there is an assumption that many students may experience a wide range of unhealthy habits such as high level of fast food intake, low level of physical activity and irregular sleep, as well. Moreover, the young age of students may lead them to believe that they are in good health, and this would be a possible reason why they are not very conscious of their health behaviours [2].

Healthy life depends on many factors, including genetics, but certain habits can have a major effect. Health lifestyles are important for health and social identity, yet little is known about their development in early life [3].

The health habits of university students are a special concern, since they represent a major segment of the young population and they are at a stage of their lives during which important lifestyle modifications take place [4]. high prevalence of risk factors for chronic diseases and poor health-seeking behavior, despite accessibility and affordability to all levels of health care [5].

Based on Pakseresht et al. 2017 [6] study which measured six domains of health promoting lifestyle by using the 52-item Health Promoting Lifestyle Profile (HPLP II). Convenient sample of 343 students from the University was selected in 2013-14. While girls scored higher in eating habits and health accountability, boys did better in physical activity. However, the scores of all

these three dimensions were undesirable in both genders. Conclusion of Health Promoting lifestyle scores were undesirable in university students and significantly related to eating and physical activities. Therefore, there is an urgent need of developing program on healthy lifestyle for them.

The aim of the present study was to find out healthy promoting lifestyle level and its associated factors among the private university students in Shah Alam, Malaysia.

METHODOLOGY

A descriptive, cross-sectional study design is used in this study which was carried out from September 2016 until February 2017 in private university in Shah Alam. The sample size required for this study was 500 respondents. This calculation using the single population mean formula.

A validated self-administrated questionnaire with 52 items [7] was distributed to 500 students. The questionnaire compromised of five sections which are:

Part A: Socio-demography. Composed of five questions.

Part B: Subscales include Health Responsibility with 9 items, Physical activity with 8 items, Nutrition with 9 items, Spiritual Growth with 9 items, Interpersonal Support with 9 items, and Stress Management with 8 items. Composed of 52 questions in total.

The raw data were checked for any exclusion, legibility and consistency before being coded for analysis. For the purpose of data analysis and hypothesis testing, several statistical tests were applied using the Statistical Package for Social Science (SPSS) version 20.0. Score for overall health promoting lifestyle is calculated by the mean of the 52 items by scoring 1 (never), sometime (2), often (3), routinely (4). The maximum total score for each subscale ranged from 32 to 36 and minimum total score for each subscales ranged 0-18. Respondents independent t-tests, chi square test, one-way anova test were used to identify any statistically significant associations between explanatory independent and outcome variables.

This study has been approved by the International Medical School of the Management and Science University (MSU) Shah Alam. Respondents are entitled to choose freely to participate in the study without any coercion. The respondents were assured that participation in the survey was voluntary and all the information will remain confidential. All participants will be given written informed consent. The participants confidentially details in the questionnaire will be kept secured. Questionnaire will then be collected upon completion.

RESULTS

A total of 500 respondents who participated in this study fulfilled the criteria of inclusions and had answered the given questions with the answer of their choice.

Table 1 shows the frequency of respondents according to the gender of 500 respondents. It show the percentage of male and female was same (50%). The lowest race is India with 148 respondents (29.6%). Body Mass Index (BMI) show half of the respondents is a normal weight (50.2%). Body Mass Index (BMI) show half of the respondents is a normal weight (50.2%).

It shows physical activity is the lowest mean among six subscales with 18.64 ± 4.99. The highest mean of subscales is spiritual growth with 26.88 ± 5.91. The total score is 136.18 ± 22.48 with the maximum of total mean is 191.

Table 3 show the male are overweight and obese with 110 students (44%) compared to the female with 94 students (37.6%). The p value is not significant (p=0.145).

It shows the mean of total score health promoting lifestyle for male is higher than a female with 138.49 ± 23.45 and 133.87 ± 21.25. Moreover, there is association between gender and health promoting lifestyle with p value is significant with (p=0.021). Alternative hypothesis is accepted.

Table 5 show India has the highest mean of health promoting lifestyle among Chinese and Malay with 137.28 ± 21.07. The p value is not significant (p=0.051). There is no association between health promoting lifestyle and race. Null hypothesis is accepted

DISCUSSION

The main finding of our current study that, the mean score of healthy lifestyle practice was 136 out of 208 , prevalence of overweight and obese was (40.8%) among university students.

Our results about healthy lifestyle was better than previous study done in Guangzhou city, mainland China. The mean score for each of the six dimensions was lower than 70. Gender and grade were the factors primarily associated with health-promoting lifestyles among undergraduates [8] .

Based on (Motlagh et al. 2011) [9] , the overall health-promoting lifestyle profile had a mean of 130.31 ±19). The highest mean in the subscales was 26.03±5.04) for spiritual growth and the lowest was 16.24±4.28) for physical activity. Based on this previous study results, there are the same results according to my current research study which is female students have a low mean of total score of health promoting lifestyle of female students (136.18 ± 22.48). Also lowest subscales for physical activity (18.64 ± 4.99) with mean of female (17.43) than male (19.86) and the highest subscales also same which is spiritual growth.

Body Mass Index (BMI) in our study showed that half of the respondents is a normal weight (50.2%). Its followed by overweight with 142 respondents (28.4%), and obese with 62 respondents (12.4%). Its show the student of the not have the a good health lifestyle according to a total of the overweight and obese of students is considered high with almost half of the respondents (40.8%). Poor diet and its consequences like obesity is the common healthy problem in urban societies. Unhealthy lifestyle can be measured by BMI. Urban lifestyle leads to the nutrition problems like using fast foods and poor foods, increasing problems like cardiovascular. More promotion and campaign about awareness of obesity is needed to prevent further complication of chronic disease for example diabetes, heart disease, coronary heart disease, heart attack, high blood pressure.

According the previous study by (Najat Yahia, et al. 2008) [10], they conclude that majority of the students (64.7%) were of normal weight in Lebanese university students. It shows that the normal weight was higher than the obesity and overweight. Similar findings of prevalence of obesity among male university students were reported in recent studies. High prevalence rate of overweight and obesity was also reported in a study conducted in Kuwait University among 842 students, at 32% and 8.9%, respectively [11].

Regarding the race, Indian has the highest mean of health promoting lifestyle among Chinese and Malay with 137.28 ± 21.07. The second highest mean is Chinese with 137.21 ± 24.28. Malay has the lowest mean among the race with 134.29 ± 21.72. It is possible because of the culture and ethnics based on each race and religions. There are no comparison with previous study between health promoting lifestyles and race. But there is a previous study of Religiosity, dietary habit, intake of fruit and vegetable, and vegetarian status among Seventh-Day Adventists in West Malaysia (Tan et al. 2016) [12], Religion has been shown to be salutary on health, and a possible link between religion and positive health outcomes is diet.

Table 1: Socio demographic characteristic of the respondents

Variable	Frequency (N)	Percentage (%)
Gender Male Female	 250 250	 50.0 50.0
Age 17 – 21 years old 22 – 26 years old >27 years old	 291 204 5	 58.2 40.8 1.0
Race Malay Chinese India	 175 177 148	 35.0 35.4 29.6
Study field Science Non science	 277 233	 55.4 44.6
Body Mass Index Underweight Normal Overweight Obese	 45 251 142 62	 9.0 50.2 28.4 12.4

Table 2: Subscales of health promoting lifestyle.

Variable	Minimum	Maximum	Mean
Health responsibility	10.0	36.0	20.96
Physical activity	8.0	32.0	18.64
Nutritional	10.0	36.0	22.45
Spiritual growth	9.0	36.0	26.88
Interpersonal relation	12.0	36.0	26.43
Stress management	9.0	32.0	20.82
Total score	83.0	191.0	136.18

Table 3: Association between gender and BMI status of the respondents.

	Body Mass Index	**(BMI)**	**POR**	**P value**	**X2**	**95% CI**
	Normal	**Overweight and obese**				
	N (%)	**N (%)**				
Gender						
Male	140 (56.0)	110 (44.0)	0.767	0.145	2.120	0.536 – 1. 097
Female	156 (62.4)	94 (37.6)				

*Chi Sqaure test was performed, level of significant at p <0.05

Table 4: Association between gender and health promoting lifestyle.

	Gender			
Variable	**Male**	**Female**	**P value**	**95% CI**
	Mean ± SD	**Mean ± SD**		
Total score health promoting lifestyle	138.49 ± 23.45	133.87 ± 21.25	**0.021***	0.687 – 8.553

*Independent t test was performed, level of significant at p <0.05

Table 5: Association race and between health promoting lifestyle.

Race	N	Mean race (SD)	P value
Malay	175	134.29 (21.72)	
Chinese	177	137.21 (24.28)	0.051
India	148	137.28 (21.07)	

*Independent t test was performed, level of significant at p <0.05

CONCLUSION & RECOMMENDATIONS

As a conclusion, the mean score of healthy lifestyle practice was 136, prevalence of overweight and obese was (40.8%) among university students.

In view of the results, the respondents show low of health promoting lifestyle profile especially in physical activity level. On top of that, there was a significant association between gender and health promoting lifestyle. Therefore, more the project recommends plans to elevate the poor healthy lifestyles into control into high awareness and practice of health promoting lifestyle. Aggressive health promotional campaigns and social mobilization by relevant agencies are needed to increase knowledge lifestyle diseases. Emphasis should be placed on the limitations, such as seeking early step to control the lifestyles disease to be serious such as control the fast food intake and obesity. Information, education and communication materials maybe provided in areas like schools or colleges making it more accessible for the residents to obtain. Campaign and promotion of healthy lifestyle is needed. And more the physical activity event among university students recommended. In conclusion, in spite of the study and education and communication programs to identify barriers to action and to control the lifestyle and chronic diseases in future among the university student.

Conflicts of Interest : The authors declare no conflicts of interest

Eethical Clearance: Taken from Management and Science University Ethical Committee.

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REFERENCES

1. Tol A, Tavassoli E, Shariferad GR, Shojaezadeh D. Health-promoting lifestyle and quality of life among undergraduate students at school of health, Isfahan University of Medical Sciences. J Health Sys Res 2012;7(4):442-8.
2. Mehri A, Solhi M, Garmaroudi G, Nadrian H, Sighaldeh SS. Health promoting lifestyle and its determinants among university students in Sabzevar, Iran. Int J Prev Med. 2016;7:65.
3. Stefanie Mollborn, Elizabeth Lawrence. Family, Peer, and School Influences on Children's Developing Health Lifestyles . Journal of Health and Social Behavior 2018.
4. Petroski EL, Oliveira MM. Atividade f.sica de lazer e est.gios de mudan.a de comportamento em professores universit.rios. Rev Port Cien Desp 2008; 8: 209-218.
5. Laskar AR, Sharma N, Bhagat N. Lifestyle disease risk factors: a study of awareness and prevalence in North Indian community in Delhi. IJCM 2010;35(3):426-28.
6. Pakseresht S, Rezaei K, Pasha A, KazemNejad Leili E, Hasandoost F. Health Promoting Lifestyle among Students at Guilan University of Medical Sciences. J Holist Nurs Midwifery. 2017; 27 (1) :19-26
7. Walker, S. N., Sechrist, K. R., Pender, N. J. (1987), The Health-Promoting Lifestyle Profile: Development and Psychometric Characteristics. Nursing Research 36, 76-81.
8. D. Wang, C. Q. Ou, M. Y. Chen, and NI. Duan, "Healthpromoting lifestyles of university students in Mainland China," BMC Public Health, vol. 9,

article no. 379, 2009.

9. Motlagh Z, Mazloomy-Mahmoodabad S, Momayyezi M. (2011), Study of Health-promotion behaviors among university of medical science students . ZJRMS.; 13 (4) :29-34.
10. Najat Yahia, Alice Achkar, Abbass Abdallah and Sandra. Rizk eating habits and obesity among Lebanese university students. Nutrition Journal 2008; 7:32 doi:10.1186/1475-2891-7-32
11. Al-Isa AN: Obesity among Kuwait University students: an explorative study. The Journal of the Royal Society for the Promotion of Health. 1999, 119 (4): 223-227. 10.1177/146642409911900404.
12. Tan MM, Chan CK, Reidpath DD. Religiosity, dietary habit, intake of fruit and vegetable, and vegetarian status among Seventh-Day Adventists in West Malaysia. J Behav Med 2016:1–12.

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Are Body Image Issues Affecting Our Adolescents? A Cross-sectional Study among College Going Adolescent Girls

Subhashini Ganesan, S. L. Ravishankar, Sudha Ramalingam
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Abstract

Background: Body image relates to how people think and feel about their own body. In today's society, with the growing sense of ideal body image, adolescents try to lose or gain body weight to attain that perfect body. Body image perception is still naive, and this research will try to understand these unexplored areas, where there is paucity of body image-related studies. **Objectives:** The objective of the study is to find out the proportion of girls dissatisfied about body image, and the association of various factors with body image dissatisfaction and to ascertain the weight control behaviors adopted by adolescent college girls. **Methodology:** A cross-sectional study was done among 1200 college girls in Coimbatore. A semi-structured questionnaire was used to collect data on various factors associated with body image dissatisfaction. Body mass index (BMI) of the participants was calculated. **Results:** Body image dissatisfaction was there among 77.6% of the girls. It was found that factors such as higher BMI, sociocultural pressure to be thin and depression were all significantly associated with body image dissatisfaction. The most commonly followed weight control behaviors were eating small meals and skipping meals. Improving the appearance and body shape were the main reasons for weight control behaviors. **Conclusion:** This study establishes the fact that body image dissatisfaction is no longer a western concept and affects Indian adolescent girls to a great extent. Hence, effective interventions have to be planned to increase awareness on ideal body weight and protect our young generation from pressures of negative body image.

Keywords: Adolescent, body image dissatisfaction, body weight, college girls, weight control

Introduction

Body image relates to how people think and feel about their own body. It relates to a person's perceptions, feelings, and thoughts about his or her body and is usually conceptualized as incorporating body size estimation, evaluation of body attractiveness, and emotions associated with body shape and size.[1]

Adolescents are particularly vulnerable to body dissatisfaction due to the physiological, social, and psychological changes they are going through.[2] For adolescent girls, a range of body and social changes take place during and following puberty that can strongly influence their body image and become more pronounced by late adolescence.[3]

Perception of overweight and dissatisfaction with body size, rather than actual weight appears to be a potent force behind girls' dieting and weight control behaviors. Such behavior is related to negative physical and psychological changes and can lead to life-threatening conditions including eating disorders.[4]

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With rapid economic development and affluence, there is a progressive shift toward thinness as the ideal body shape and increasing concerns about body weight leading to dissatisfaction.[5]

Factors contributing to body image problems have been studied, and biopsychosocial model of risk factors was developed by Paxton.[6] This model [Figure 1] suggests that biological factors, such as body size, together with individual psychological characteristics such as low self-esteem, depression, and perfectionism, which refers to individuals desire to attain idealistic goals without failing, social influences, and interpersonal interactions, all contributes to the development of body image.

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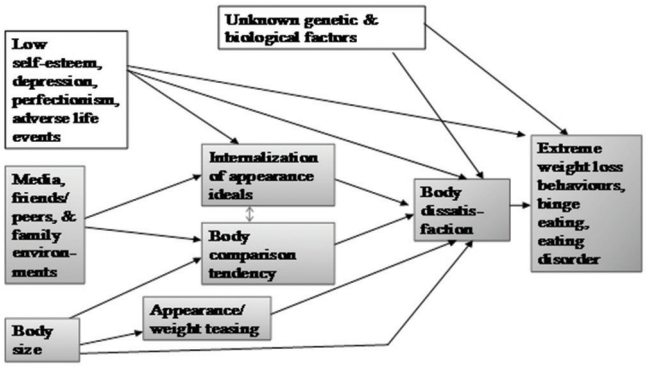


Figure 1: Biopsychosocial model of risk factors for the development of body dissatisfaction by Susan J Paxton

There is a paucity of studies in India and gaps remain in our understanding of the factors responsible for the development of attitudes and behavior related to body weight and appearance among adolescent girls.[7]

Hence, this study aimed to find out the proportion of girls dissatisfied about body image, and the association of various factors with body image dissatisfaction and to ascertain the various weight control behaviors adopted by adolescent college girls.

Methodology

This study was conducted in a college located in an urban area of Coimbatore. With an estimated prevalence of body image dissatisfaction of 33.3% from a study in Mangalore,[8] sample size was calculated to be 978, with the precision of 3.3%, and nonresponse 20%. After approval by the Institutional Human Ethics Committee, 1220 college going adolescent girls in the age group of 18–19 years, were enrolled in the study by convenience sampling. After obtaining informed consent, a semi-structured questionnaire was used to collect the data on body image dissatisfaction and the associated factors namely demographic factors, socioeconomic and media influence, sociocultural pressure, self-esteem, perfectionism, and depression. Furthermore, data regarding weight control measures adopted were collected.

Measuring body image dissatisfaction

Body image dissatisfaction of the adolescents was measured using the Stunkard silhouettes Figure Rating Scale (FRS). It includes drawing of nine silhouettes ranging from very thin (1) to very heavy (9). Students were presented with female versions of the FRS and were asked to identify the figure they currently perceived themselves with, and the figure they would wish to have. Discrepancy scores were calculated based on the difference between the actual and ideal figures chosen. Positive scores indicate a desire to be smaller, while negative scores suggest a desire to be larger. The discrepancies between the current and desired figures were interpreted as an indication of body image dissatisfaction.[9,10] Hence, participants who had discrepancies in the figures chosen were identified as having body image dissatisfaction.

Measuring factors associated with body image dissatisfaction

The factors associated with body image dissatisfaction such as self-esteem, depression, sociocultural pressure, media influence, and perfectionism were measured using previously validated questionnaires and using Likert scale ratings, and score was given.[11-15] Rosenberg Self-Esteem Scale was used to measure Self-esteem with the Likert scales ranging from strongly agree (score 1) to strongly disagree (score 4). Higher scores indicate higher self-esteem.[11] Depression was measured using Center of Epidemiological studies (CES) Depression questionnaire with Likert scale which graded from rarely to mostly with the scoring of 0–3, respectively. Score on the CES-Depression questionnaire range from 0 to 60, with higher scores indicating more severe levels of depressive symptoms.[12]

Socio-cultural pressure was measured with the pressure grading from none (score 1) to a lot of pressure (score 5). The scoring ranges from 10 to 50.[13] Media influence was measured using SATAQ 3 questionnaire with Likert scale grading of completely disagree to completely agree with a scoring of 1–5. The scoring ranges from 14 to 70.[14] Perfectionism was measured using the EDI questionnaire with Likert scale grading from always to never with score from 1 to 6, respectively. The scoring ranges from 6 to 36.[15]

The height and weight of the participants were measured and body mass index (BMI) was calculated. According to the WHO standards in our study, BMI <18.5 kg/m^2 was classified as underweight, a BMI of 18.5–24.9 kg/m^2 was considered normal, overweight is 25–29.9 kg/m^2, and obesity is ≥30 kg/m^2.[16]

The association between the possible risk factors and body image dissatisfaction was assessed by univariate analysis using Chi-square test, *t*-test, and odds ratio was estimated. Those factors found significant by univariate analysis were further subjected to multivariate logistic regression analysis to identify factors that were significantly associated with body image dissatisfaction.

Results

Majority of the study participants (60.6%) were from an urban background. About 67.5% of them belonged to Class I and Class II and 32.5% of them belong to Class III and Class IV socioeconomic status according to modified Prasad classification.[17]

Based on the BMI, about half (53.6%) of them were having normal BMI while 26.7% of them were undernourished, 15.7% were overweight, and 3.9% were obese.

The study showed that 947 (77.6%) girls were dissatisfied with their body image (95% CI: 75.2–79.9). Among the 947 participants who were dissatisfied the mean score of the perceived silhouette figure was 4.0 ± 1.5 (four corresponds to the normal range of BMI), and the mean of the chosen ideal

silhouette was 3.3 ± 0.8 (three corresponds to the lower end of the normal BMI toward the underweight range).

Table 1 shows the distribution of body image dissatisfaction based on the calculated BMI of the participants. It shows that 58.3% of the participants of normal BMI also were dissatisfied with their appearance and wanted to reduce weight. In the underweight category, 23.3% were satisfied, and 7.4% wanted to reduce their weight further showing the tendency of the college students to have a thin appearance. Tables 2 and 3 show the association of body image dissatisfaction with its risk factors by univariate analysis. Higher BMI, higher socioeconomic status, depression, increased sociocultural pressure to be thin, media influence and low self-esteem were found to be significantly associated with body image dissatisfaction.

There was no significant association with the place of residence and perfectionism.

Table 4 shows multivariate logistic regression exploring the association of body image dissatisfaction with those risk factors having statistically significant association with body image dissatisfaction by univariate analysis.

Factors such as higher BMI, higher socioeconomic status, depression, and increased sociocultural pressure to be thin were found to be significantly associated with body image dissatisfaction by multivariate logistic regression analysis.

Among the 1220 students who participated in the current study, 791 (64.8%) of them had undertaken at least one weight control measure in the past 1 year. Of which 157 (19.8%) were satisfied and 634 (80.2%) were dissatisfied with their body image.

Among the multiple options of weight control measures adopted, the most common method was taking small meals (53.2%) followed by skipping meals (42.7%). Extreme measures such as taking laxatives (5.3%) and vomiting after meals (9.7%) were also seen.

Among participants who followed weight control measures, multiple reasons were given by them, of which 61.7% gave improving the appearance and body shape as the main reason for going in for weight control measures and 33.5% said to look better in clothes.

Our study also showed that 67% of participants who were dissatisfied with their body image were adopting one or more weight control measures while in the satisfied group 57.5% have adopted weight control measures. The difference was found to be statistically significant ($P < 0.01$) with an odds ratio of 1.49 (1.13–1.97).

Table 1: Distribution of body image dissatisfaction of study participants based on calculated body mass index (*n*=1220)

BMI status (BMI in kg/m²)	Total number	Frequency of dissatisfaction	
		Increase weight	Reduce weight
Underweight (<18.50)	326 (26.7)	226 (69.3)	24 (7.4)
Normal (18.50-24.99)	654 (53.6)	84 (12.8)	381 (58.3)
Overweight (25.00-29.99)	192 (15.7)	0	185 (96.4)
Obese (≥30.00)	48 (3.9)	0	47 (97.9)
Total	1220 (100)	310 (25.4)	637 (52.2)

BMI: Body mass index

Table 2: Association of body image dissatisfaction with the risk factors (continuous variables) by univariate analysis

Factor	Mean±SD		OR (95% CI)	*P*
	Satisfied	Dissatisfied		
Media influence	37.60±8.96	40.35±8.19	1.04 (1.02-1.05)	<0.001
Depression	15.7±7.38	19.2±9.19	1.05 (1.03-1.06)	<0.001
Sociocultural pressure	15.49±5.57	19.08±7.70	1.08 (1.05-1.10)	<0.001
Perfectionism	19.09±5.53	19.21±6.09	1.00 (0.98-1.02)	0.77
Self esteem	18.65±3.20	17.92±3.30	0.937 (0.90-0.97)	<0.01
BMI	19.92±2.27	21.99±4.40	1.15 (1.111.20)	<0.001

SD: Standard deviation, BMI: Body mass index, CI: Confidence interval, OR: Odds ratio

Table 3: Association of body image dissatisfaction with the risk factors (categorical variables) by univariate analysis

Factors	Category	Total	Body image dissatisfaction (%)	*P*
Socioeconomic class	Class I	53	43 (81.1)	<0.001
	Class II	643	510 (79.3)	
	Class III	380	302 (79.5)	
	Class IV	144	92 (63.9)	
Place of residence	Urban	739	569 (77)	0.52
	Rural	481	378 (78.6)	

Table 4: Association of body image dissatisfaction with the risk factors by multivariate logistic regression

Factor	AOR	95% CI	*P*
Media influence	1.02	1.00-1.03	<0.05
Depression	1.04	1.01-1.05	<0.001
High self esteem	0.98	0.94-1.03	0.411
Increased sociocultural pressure	1.05	1.02-1.07	<0.001
Higher BMI	1.13	1.08-1.18	<0.001
Higher socioeconomic status	1.28	1.06-1.54	<0.001

BMI: Body mass index, AOR: Adjusted odds ratio, CI: Confidence interval

Discussion

Our study showed that body image dissatisfaction was there in 77.6% of the adolescents. This was similar to a study done by Sasi and Maran in Chennai among adolescents above 12 years of age which showed a prevalence of 81%.[18]

Our study also showed that 23% of the underweight people were satisfied with their BMI, and among those who were dissatisfied 7.4% wanted to reduce their weight further. Similarly, 71% of the normal BMI category was dissatisfied

with their appearance of which 58.3% wanted to reduce their weight. This clearly shows the tendency for liking toward thin body shapes.

Socioeconomic status of an individual impacts their body image perceptions and satisfaction/dissatisfaction with the body. Studies have shown that body image dissatisfaction is seen more among higher socioeconomic status.[19] Our study also showed that the proportion of girls having body image dissatisfaction, who belonged to class I socio economic status are slightly higher than other classes and it was statistically significant.

Studies have also shown that urban adolescents have more body image dissatisfaction compared to rural,[20] but our study did not show any significant association.

Our study also showed that increased socio-cultural pressure, depression and higher BMI had significant association with body image dissatisfaction. Literature also shows that these factors are associated with body image dissatisfaction.[21-23]

Our study failed to show any significant association between body image dissatisfaction and media influence, self-esteem, and perfectionism which were also established in other studies.[24-26]

This study also showed that 64.8% of the college students had undertaken at least one weight control measure in the past 1 year. It also showed that girls who were dissatisfied with their appearance tend to do more weight control measures and the most common weight control measures were eating small meals, skipping meals, and avoiding certain foods. It also showed that the majority of students have cited improving the appearance and to look better in clothes as main reasons for going in for weight control measures. This was also found similar to other studies which showed that appearance was a major motivation for dieting practices.[27,28]

Our study was done in a large sample and measured the different factors which were associated with body image dissatisfaction. The study of these factors is crucial for improving our understanding of body image dissatisfaction in adolescent girls which is still naive in our cultural setup.

Limitations

Although the study area was in a college which had both self-financing and government aided divisions which brought together adolescents from different socioeconomic and cultural backgrounds, it was done in only one college - this restriction may limit the generalizability of the findings.

Conclusion

This study establishes the harsh fact that body image perceptions and dissatisfaction is no longer a western concept and affects our adolescent girls to a great extent. There is also a tendency favoring toward thin body figures which is evident in the study.

Recommendations

Effective interventions to improve awareness on ideal body weight, media literacy, and harmful effects of extreme weight control measures have to be planned, and young people both maintain a healthy body weight and are protected from the pressure of negative body image and weight control practices.

Further studies are recommended to establish the cause-effect relationship of the identified factors.

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Conflicts of interest

There are no conflicts of interest.

References

1. Grogan S. Body Image: Understanding Body Dissatisfaction in Men, Women and Children. London: Routledge; 1999. Available from: http://www.art-therapy.gr/images/stories/book_library/new/%CE%92%CE%99%CE%92%CE%9B%CE%99%CE%91/art%20therapy/Body-Image-Understanding-Body-Dissatisfaction-in-Men-Women-a.pdf. [Last accessed on 2016 Oct 15].
2. Clay D, Vignoles VL, Dittmar H. Body image and self-esteem among adolescent girls: Testing the influence of sociocultural factors. J Res Adolesc 2005;15:451-77.
3. Littleton HL, Ollendick T. Negative body image and disordered eating behavior in children and adolescents: What places youth at risk and how can these problems be prevented? Clin Child Fam Psychol Rev 2003;6:51-66.
4. Mann AH, Wakeling A, Wood K, Monck E, Dobbs R, Szmukler G, *et al.* Screening for abnormal eating attitudes and psychiatric morbidity in an unselected population of 15-year-old schoolgirls. Psychol Med 1983;13:573-80.
5. Cash TF, Morrow JA, Hrabosky JI, Perry AA. How has body image changed? A cross-sectional investigation of college women and men from 1983 to 2001. J Consult Clin Psychol 2004;72:1081-9.
6. Paxton SJ. Evidence, Understanding and Policy: A Perspective from Psychology – Prevention, Early Intervention and Treatment of Body Image Problems. Body Image: Evidence, Policy, Action. Report of a Multidisciplinary Academic Seminar on Behalf of the Government Equalities Offi ce. United Kingdom; 2003. Available from: https://www.gov.uk/.../uploads/.../Susan_Paxton_-_Body_Image_Seminar__3_.docx. [Last accessed on 2017 Dec 16].
7. Chugh R, Puri S. Affluent adolescent girls of Delhi: Eating and weight concerns. Br J Nutr 2001;86:535-42.
8. Priya D, Prasanna KS, Sucharitha S, Vaz NC. Body image perception and attempts to change weight among female medical students at Mangalore. Indian J Community Med 2010;35:316-20.
9. Lo WS, Ho SY, Mak KK, Lam TH. The use of Stunkard's figure rating scale to identify underweight and overweight in Chinese adolescents. PLoS One 2012;7:e50017.
10. Bhuiyan AR, Gustat J, Srinivasan SR, Berenson GS. Differences in body shape representations among young adults from a biracial (Black-white), semirural community: The Bogalusa heart study. Am J Epidemiol 2003;158:792-7.
11. Gray-Little B, Williams VS, Hancock TD. An item response theory analysis of the rosenberg Self-esteem Scale. Pers Soc Psychol Bull 1997;23:443-51.
12. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Appl Psychol Meas 1977;1:385-401.

13. Reina SA, Shomaker LB, Mooreville M, Courville AB, Brady SM, Olsen C, *et al.* Sociocultural pressures and adolescent eating in the absence of hunger. Body Image 2013;10:182-90.
14. Thompson JK, van den Berg P, Roehrig M, Guarda AS, Heinberg LJ. The sociocultural attitudes towards appearance scale-3 (SATAQ-3): Development and validation. Int J Eat Disord 2004;35:293-304.
15. Garner DM, Olmstead MP, Polivy J. Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia. Int J Eat Disord 1983;2:15-34.
16. Eveleth PB. Physical status: The use and interpretation of anthropometry. Report of a WHO Expert Committee. Am J Hum Biol 1996;8:786-7.
17. Mangal A, Kumar V, Panesar S, Talwar R, Raut D, Singh S, *et al.* Updated BG Prasad socioeconomic classification, 2014: A commentary. Indian J Public Health 2015;59:42-4.
18. Sasi RV, Maran K. Advertisement pressure and its impact on body dissatisfaction and body image perception of women in India. Glob Med J Indian Ed 2012;3:1-9.
19. Felden EP, Teixeira CS, Gattiboni BD, Bevilacqua LA, Confortin SC, da Silva TR. Body image perception and socioeconomic status in adolescents: Systematic review. Rev Paul Pediatr 2011;29:423-9.
20. Mishra SK, Mukhopadhyay S. Eating and weight concerns among sikkimese adolescent girls and their biocultural correlates: An exploratory study. Public Health Nutr 2011;14:853-9.
21. Calzo JP, Sonneville KR, Haines J, Blood EA, Field AE, Austin SB, *et al.* The development of associations among body mass index, body dissatisfaction, and weight and shape concern in adolescent boys and girls. J Adolesc Health 2012;51:517-23.
22. Stice E, Whitenton K. Risk factors for body dissatisfaction in adolescent girls: A longitudinal investigation. Dev Psychol 2002;38:669-78.
23. Marsella AJ, Shizuru L, Brennan J, Kameoka V. Depression and body image satisfaction. J Cross Cult Psychol 1981;12:360-71.
24. Grabe S, Ward LM, Hyde JS. The role of the media in body image concerns among women: A meta-analysis of experimental and correlational studies. Psychol Bull 2008;134:460-76.
25. Fortes Lde S, Cipriani FM, Coelho FD, Paes ST, Ferreira ME. Does self-esteem affect body dissatisfaction levels in female adolescents? Rev Paul Pediatr 2014;32:236-40.
26. Boone L, Soenens B, Braet C. Perfectionism, body dissatisfaction, and bulimic symptoms: The intervening role of perceived pressure to be thin and thin ideal internalization. J Soc Clin Psychol 2011;30:1043-68.
27. Kalarchian MA, Levine MD, Klem ML, Burke LE, Soulakova JN, Marcus MD. Impact of addressing reasons for weight loss on behavioral weight-control outcome. Am J Prev Med 2011;40:18-24.
28. Brink PJ, Ferguson K. The decision to lose weight. West J Nurs Res 1998;20:84-102.

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Original Article

Peer Educators as Change Leaders – Effectiveness of Peer Education Process in Creating Awareness on Reproductive Health among Women Workers in Textile Industry

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Abstract

Context: India is one of the leading manufacturers of garments with roughly 80% of the workforce dominated by female migrant workers. Lack of education and access to resources in these women contribute to unsafe sexual behaviors, sometimes leading to unwanted pregnancies or sexually transmitted infections (STIs). Awareness raising, and peer education in particular, is extremely effective in addressing reproductive health issues. **Aims:** This study aims to study the effectiveness of peer health education in improving the awareness on reproductive health among women workers in textile industries. **Settings and Design:** A health educational interventional study for women in garment and spinning mills using peer education process carried out over a period of 18 months. **Subjects and Methods:** The process includes orientation to factory management, health needs' assessment, identifying health topics, peer educators' selection, peer educator trainings followed by outreach sessions, and impact measurement. The impact of peer education on reproductive health has been assessed among 597 participants from 9 factories. **Statistical Analysis Used:** Data were entered in SPSS version 24 and analyzed. Results were expressed as percentages. Chi square test was used where appropriate to find association between categorical variables. **Results:** The mean age of the workers in both the groups is 25.4 (±9.6) years and 25.1 (±8.3) years. In both the groups, most of the workers were single and more than half of them reside within the factory premises. Significant change in menstrual hygiene practices, knowledge in family planning methods, STIs, and cervical cancer has been observed following peer health education. **Conclusions:** A well-designed health educational interventional program using peer educators could be a successful approach to create awareness on sensitive topics.

Keywords: Peer health education, reproductive health, textile industry, women workers

Introduction

India has made a name for itself as a garment manufacturing center of global renown. The textiles and garments industry contributes 16.63% of India's export earnings. However, India's niche in the global garments market has been carved out at the cost of lakhs of workers in this industry's predominantly female and migrant labor force. Roughly 80% of garment workers are women between the ages of 21 and 25. Most are semi-skilled migrant workers and the sole earning members in their families.

These women workers often suffer from anemia, poor hygiene, inadequate pre- and post-natal care, sexual violence, and exposure to infections and illness. Lack of education and access to resources contribute to unsafe sexual behaviors, sometimes leading to unwanted pregnancies or sexually transmitted infections (STIs). They also contribute to high rates of malnutrition, maternal and infant mortality, and the spread of sexually transmitted and other preventable diseases such as HIV/AIDS and tuberculosis.[1]

Facility-based studies done in Chennai[2] and other parts of India[3] have shown that unwanted pregnancies among young

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unmarried women are high and emphasize the need for educating them about contraceptives. Data from subnational study conducted in 2006 have shown that around 80% of young women perceive the need for family life education. The same study has shown that peers are the major sources of information regarding contraception.[4]

Awareness raising, and peer education in particular, is extremely effective in addressing reproductive health issues. Women are grateful for such education focusing on personal and family health, with information they have seldom received before. An environment focused on education rather than behavior change empowers the women to make better health choices for themselves.

Peer education typically involves training and supporting members of a given group to affect change among members of the same group. The peer education model initiated by business for social responsibility for improving health of women at factories has proven to be an effective model that encourages women to share their knowledge with other family and community members extending the benefits well beyond the factory floor.[5,6] A study conducted among women workers in garment industries has shown that peer education approach is effective in raising the awareness on STIs. The study also concludes that it is likely that the health education program may help to reduce the burden of healthcare costs to the community in the long run.[7]

This study looks into the effectiveness of peer health education in improving the awareness on reproductive health among women workers in textile industries.

Subjects and Methods

This study is part of an ongoing health education program done by the Department of Community Medicine for women workers in garment and spinning mills located within 100 kilometer radius from our institution. Pooled baseline and endline assessment data of 597 workers from nine industries which include six spinning mills and three garment factories have been analyzed for the study purpose. The study has been approved by the institution's human ethics committee.

Data were entered and analyzed using statistical package for social sciences (SPSS) version 19 (IBM SPSS statistics Company, IBM corporation). Results are presented as percentages. Chi-square test is done for statistical association wherever appropriate.

Salient steps of the health education program

As part of the program, baseline assessment is conducted by survey method using a prevalidated questionnaire among 10% of women workers chosen by simple random sampling. Health education was then conducted by peer education process. Peer educators were selected by the factory representatives(supervisory level) using the following guidelines: workers who have ability to read and write in local language, working in the factory for at least one year and having exhibited leadership skills. As a measure to ensure sustainability, nurse, female human resource (HR) representatives, and volunteers from staff level were also included. Peer educator strength in each factory ranged from 10 to 20 proportionate to the total strength of the women workforce.

Peer education process

Health education in reproductive health issues focused on menstrual hygiene and reproductive tract infections, STIs including HIV and hepatitis B, family planning and cancers. Health education sessions for the peer educators were conducted once in 2 months. Each session was conducted for over 2 h by interactive lecture aided by flip charts and videos supplemented with demonstrations where appropriate. Participatory methods such as body mapping exercise were used to teach anatomy of reproductive system. Energizers were conducted to break monotony and avoid boredom.

Following the session, peer educators conduct outreach sessions during weekends, lunch breaks, etc., for their coworkers in small groups ranging between 10 and 15. These sessions were facilitated by the HR department in coordination with the nurse. Informal discussions also took place in leisure times in the dormitories. A refresher session was conducted after a month following the main session to reemphasize the important points and clarify any doubts the peer educators might have. An endline assessment at the end of the program was done among 10% of randomly chosen workers.

Results

The pooled data of 597 female workers interviewed during baseline (316) and endline (281) assessment were included for analysis. The mean age of the workers in both the groups is 25.4 (±9.6) years and 25.1 (±8.3) years.

Table 1 shows the distribution of the female workers based on certain sociodemographic variables.

Table 1: Distribution of workers based on certain sociodemographic variables

Sociodemographic variables	Baseline (%) (n=316)	Endline (%) (n=281)
Mean age (years)	25.4±9.6	25.1±8.3
Marital status		
Single	69.3	71.8
Married	29.8	26.4
Divorced/widowed/separated	0.9	1.8
Educational status		
None	7.7	6.8
Primary	27.5	30.0
Secondary	53.8	52.5
Postsecondary	11.0	10.7
Residence		
Within the factory premises	52.2	67.3
Home outside factory	47.8	32.7

In both the groups, most of the workers were single and more than half of them reside within the factory premises.

Table 2 shows the comparison of practices during menstruation before and after intervention. A statistically significant ($P < 0.01$) improvement in frequency of changing pads has been observed before and after intervention. The results also reveal a significant ($P < 0.01$) decrease in menstrual problem-related absenteeism.

A significant increase in knowledge on awareness about family planning and its methods has been observed following the intervention [Table 3].

Knowledge regarding both HIV and hepatitis B has improved significantly among the workers, but very few women (17.1%) had awareness about hepatitis B before intervention compared to HIV where the preintervention knowledge was 74.4% [Table 4].

During baseline assessment, it was observed that only 40% women have heard of cervical cancer, and among them, only 24.6% were aware about the symptoms [Table 5]. Following health education, significant increase in knowledge has been observed.

Discussion

The mean age of the female workers in the textile industry is around 25 (±9 years). Similar age profile was observed in studies conducted among women workers in textile industries in Bangladesh and China.[8,9] Around 30% of the workers in our factories have no or limited literacy. This finding is similar to studies done in other parts of India,[10] but low literacy rate in Chinese workers is only around 2%.[9] More than half of the workers in our study population reside the dormitories within the factories. This is in contrast to situation in others places[8] where most workers come from their residence. Women of younger age together with limited literacy and being away from home warrant need for creating awareness and providing enabling environment for reproductive health issues in industries.

Peer education process is a successful method in bringing about awareness, especially in sensitive health issues. Peer educators could be powerful change agents as evidenced by interventional studies in HIV/AIDS using peer educators.[11,12] Our study has also proven that peer education process has led to significant change in knowledge and practice in reproductive health issues. A significant improvement in menstrual hygiene practices (59.7% vs. 84.2%, $P < 0.05$) and decrease in absenteeism (36.6% vs. 27.2%, $P < 0.05$) during menstruation has been observed. There is slight increase in the proportion of workers using sanitary pads, but this difference is not statistically significant. This could be due to a relatively higher proportion of workers already using sanitary pads.

Although 75% of women have heard about family planning methods, the baseline assessment findings showed that only around a quarter of them could mention few contraceptive methods. Peer educators were able to bring a significant improvement in knowledge on the different contraceptive methods (8.2% vs. 55.6%, $P < 0.05$). This is important considering the age profile of the workers and most of them being away from home. Our study has shown that peer education has been effective in bringing about a significant awareness about various contraceptive methods.

Educational intervention on STI focusing on HIV and hepatitis B has brought a significant increase in awareness, especially in hepatitis B (17% vs. 89%, $P < 0.05$). Many of the workplace based health educational interventions focus mainly on HIV[13] even though the awareness on hepatitis B is very low[14] conducted among women in reproductive age. Our study also showed that the preintervention knowledge was only 17%.

Despite being the leading cause of all cancers among women in India, the awareness of cervical cancer and its symptoms was

Table 2: Comparison of certain practices during menstruation before and after intervention

Parameters	Baseline ($n=303$) (%)	Endline ($n=279$) (%)	*P*
Frequency of changing pads more than two times a day	181 (59.7)	235 (84.2)	0.000
Absenteeism during menstruation	111 (36.6)	78 (27.9)	0.026
Use of sanitary pads during menstruation	277 (91.4)	261 (93.5)	0.748

P<0.05 is considered statistically significant

Table 3: Knowledge regarding family planning and its methods

Parameters	Baseline ($n=316$) (%)	Endline ($n=281$) (%)	*P*
Heard of family planning	231 (74.3)	257 (94.5)	0.000
Can mention at least two methods of family planning	26 (8.2)	176 (55.6)	0.000

P<0.05 is considered statistically significant

Table 4: Knowledge regarding HIV and hepatitis B

Parameters	Baseline ($n=316$) (%)	Endline ($n=281$) (%)	*P*
Heard of HIV	235 (74.4)	260 (92.5)	0.000
Heard of Hepatitis B	54 (17.1)	250 (89.0)	0.000

P<0.05 is considered statistically significant

Table 5: Knowledge regarding cervical cancer and its symptoms

	Baseline (%)	Endline (%)	*P*
Heard of cervical cancer	(n=316) 126 (39.9)	(n=281) 247 (87.9%)	0.000
Heard of cervical cancer symptoms	(n=126) 31 (24.6)	(n=247) 193 (78.1)	0.000

P<0.05 is considered statistically significant

less among our study population. Community-based studies in other parts of the country have shown even higher level of lack of awareness.[15] Our study showed a significant improvement in knowledge of cervical cancer (39.9% vs. 87.9%, $P < 0.05$), and its symptoms were brought about following health education (24.6% vs. 78.1%, $P < 0.05$).

Conclusions

A significant increase in knowledge and practice has been brought about in menstrual hygiene, family planning methods, STIs, and cervical cancer. Our study has shown evidence that peer education approach is effective in bringing about significant change in knowledge in reproductive health issues. A well-designed health educational interventional program using peer educators could be a successful approach to create awareness on sensitive topics.

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Conflicts of interest

There are no conflicts of interest.

References

1. Singh S. India Cheap Labor Garment Export Industry. Fashionable and Famous – At the Garment Worker's Cost. Global Research; 2009. Available from: https://www.globalresearch.ca/india-cheap-labor-garment-export-industry/13530. [Last accessed on 2018 Feb 23].
2. Kalyanwala S, Zavier AJ, Jejeebhoy S, Kumar R. Abortion experiences of unmarried young women in India: Evidence from a facility-based study in Bihar and Jharkhand. Int Perspect Sex Reprod Health 2010;36:62-71.
3. International Institute of Population Sciences and the Population Council. Youth in India: Situation and Needs 2006–2007. Mumbai: International Institute of Population Sciences. Available from: http://www.popcouncil.org/uploads/pdfs/2010PGY_YouthInIndiaReport.pdf. [Last accessed on 2018 Feb 23].
4. Vaidya D, Hussainy D. AIDS Awareness programme in industry. Indian J Occup Environ Med 2004;8:11-5.
5. Investing in Women for a Better World. BSR Report. Investing in Women for a Better World; 2010. HERproject. Available from: https://herproject.org/downloads/BSR_HERproject_Investing_In_Women.pdf. [Last accessed on 2018 Feb 23].
6. Hossain MI, Al Mahmud A, Bajracharya A, Rob U, Reichenbach L. Evaluation of the Effectiveness of the HERhealth Model for Improving Sexual and Reproductive Health and Rights Knowledge and Access of Female Garment Factory Workers in Bangladesh. The Evidence Based project. Population council. 2017. Available from: http://evidenceproject.popcouncil.org/. [last accessed on 2018 Feb 03].
7. Rianon N, Selwyn B, Shahidullah SM, Swint JM, Franzini L, Rasu R. Cost of health education to increase STD awareness in female garment workers in Bangladesh. Int Electron J Health Educ 2009;12:135-49.
8. Barkat A, Majid M, Ara R, Mahiyuddin G, Rahman M, Poddar A, *et al*. Study on Change in Attitudes and Behavior of Garment Owners, Managers and Workers Towards Gender and Reproductive Health Issues. Prepared for UNFPA Bangladesh: December, 2008. Accessed from: http://www.hdrcbd.com/admin_panel/images/notice/1386584302.07.%20change%20in%20attitude%20and%20behaviour%20of%20garment%20owners,%20managers%20and%20workers%20towards%20gender%20and%20rh%20issues.pdf. [Last accessed on 2018 Feb 23].
9. Lu C, Xu L, Wu J, Wang Z, Decat P, Zhang WH, *et al*. Sexual and reproductive health status and related knowledge among female migrant workers in Guangzhou, China: A cross-sectional survey. Eur J Obstet Gynecol Reprod Biol 2012;160:60-5.
10. Saha TK, Dasgupta A, Butt A, Chattopadhyay O. Health status of workers engaged in the small-scale garment industry: How healthy are they? Indian J Community Med 2010;35:179-82.
11. Cornish F, Campbell C. The social conditions for successful peer education: A comparison of two HIV prevention programs run by sex workers in India and South Africa. Am J Community Psychol 2009;44:123-35.
12. Van Rompay KK, Madhivanan P, Rafiq M, Krupp K, Chakrapani V, Selvam D. Empowering the people: Development of an HIV peer education model for low literacy rural communities in India. Hum Resour Health 2008;6:6.
13. Prabhu TR. Legal abortions in the unmarried women: Social issues revisited. J Obstet Gynaecol India 2014;64:184-8.
14. Jha S, Devaliya D, Bergson S, Desai S. Hepatitis B knowledge among women of childbearing age in three slums in Mumbai: A cross-sectional survey. Hepatol Med Policy 2016;1:5.
15. Harsha Kumar H, Tanya S. A study on knowledge and screening for cervical cancer among women in Mangalore city. Ann Med Health Sci Res 2014;4:751-6.

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Original Article

A Toolkit for Strengthening Health Care Policies and Infrastructure of Industries in Developing Countries

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Abstract

Background: The health risks faced by textile workers calls for a workplace health system that is comprehensive and accessible. To enhance the capacity of workplaces to strengthen their health system, a toolkit was developed by the Business for Social Responsibility (BSR), a non-profit global business network and sustainability consultancy. **Methods:** The Health System Strengthening (HSS) toolkit was designed to provide a set of tools, resources, and concrete steps for the factory management and health staff to work toward continuous improvement of their on-site health systems. It was then implemented with academic collaboration simultaneously In three factories/ in three manufacturing units/ in three workplaces in South India over 6 months to find out its usefulness as a self-reference tool for HSS. Monitoring and evaluation tools and indicators were developed based on the logic framework. **Results:** The main outcomes of the HSS pilot program include the formation of a health committee which was able to utilize the modules, perform a self-assessment of the health system, and come out with short- and long-term action plans for HSS under expert supervision and guidance. **Conclusions:** Overall, the toolkit was found to be an effective solution for HSS in industries which require expert guidance for implementation.

Keywords: Capacity building, health system strengthening, industries, toolkit

Introduction

The Indian subcontinent has seen tremendous industrial growth with the textile and garment industries contributing to 13% of the export earnings of the country.[1,2] This huge contribution from the textile industry has been the outcome of a large number of laborers involved in long hours of repetitive and monotonous work which could put them at risk of general, and occupational health problems unique to this industry.[3]

The health risks faced by the textile workers call for a workplace health system that is comprehensive and accessible. Health services that are currently offered within the workplace are underutilized as they are most often poorly equipped, lack trained staff, and have weak linkages with external healthcare providers. The existing external systems of healthcare in the private sector are either expensive or inaccessible, and on the other hand, the health services offered by the state are poorly utilized due to unfriendly timings, lack of adequate services, and facilities.[3,4]

The current health systems and services in the industries are modeled and regulated by the Indian Factories Act. The industries restrict themselves to fulfilling the requirements of the act and focus only on the basic parameters.[5] This is also partially fulfilled in most industries due to the lack of trained workforce available for providing occupational health services. Sharma *et al*.[6] identified a 50% deficiency of trained workforce for providing occupational health services in industries across India.

Saha, in his extensive review of occupational health status in India, has raised an urgent need for the development of

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institutions and infrastructure of occupational health, need for spreading awareness of occupational health-related issues among all stakeholders, need to increase awareness about the concept of occupational health nursing, and to have in policy for safety, health, and environment in the workplaces.[7] Growing industrialization, changing profile of health needs of workers together with the lack of a system for providing occupational health services, calls for an urgent need to set up comprehensive and sustainable workplace health programs (WHP).[6,8,9]

To enhance the capacity of the workplaces to strengthen their health system, a toolkit was developed by the Business for Social Responsibility (BSR), a non-profit global business network and sustainability consultancy. The overall goal of the toolkit is to provide a set of tools, resources, and concrete steps for factory management and health staff to work toward continuous improvement of on-site health services. The toolkit was pilot-tested by a collaborative effort of BSR and its academic partner based in South India. They teamed previously for a health educational interventional program called 'HERproject' targeted to improve the health of female workers in the textile and garment industry.[10]

This paper explains in detail the various phases of the development and implementation of the toolkit, monitoring, and evaluation framework used, the outcomes of the pilot initiative, and the perceptions of the industries about the usefulness and challenges in using the Health System Strengthening (HSS) toolkit.

Methods

The pilot program was implemented by the academic team simultaneously in three workplaces in South India over 6 months between May and October 2017. A detailed description of the two phases of the study is given below.

Description of the phases of the program

A framework of the various components of the two phases of the study is depicted in Figure 1 followed by a detailed description of the two phases.

Phase. 1a: Development of Health System Strengthening toolkit

The HSS toolkit comprises two main components: (1) Tools for self-assessment; (2) Capacity-building resources.

Tools for self-assessment

The self-assessment component of the toolkit consists of a clinic scorecard [Figure 2] and a mapping tool. The self-assessment tool known as the scorecard was used to benchmark the workplace health system's performance. The scorecard was divided into two sections (a) on-site services and (b) workplace systems and policies.

Each indicator under these benchmarks was demarcated as basic (B), middle (M), or high (H) based on the priority level. A workplace could self-score its performance by assigning a color code based on the presence and functionality of a particular indicator, and if any improvements are necessary. A mapping tool was developed along with the clinic scorecard to help the management teams map out the external health service provisions, assess existing partnerships, and identify potential partnerships to meet the workers' needs and implementation resources.

Capacity-building resources

The capacity-building component of the toolkit consisted of complementary resources for the workplace team and resources for the academic team. For the workplace team, the resources included self-reference modules and videos; for the academic team, facilitation resources were provided.

Phase 1b: Capacity building of the implementing partners

Phase 1b of the program included capacity building of the implementing (academic) team, selection of the intervention site, and pre-implementation preparations.

Phase 2: Pilot testing of the health system strengthening toolkit

The second phase of the program was initiated with a kick-off meeting with the senior management. This was followed by the baseline assessment of the workplaces using qualitative methods like interviews, focus on group discussions, and observation of clinics to understand the existing health system

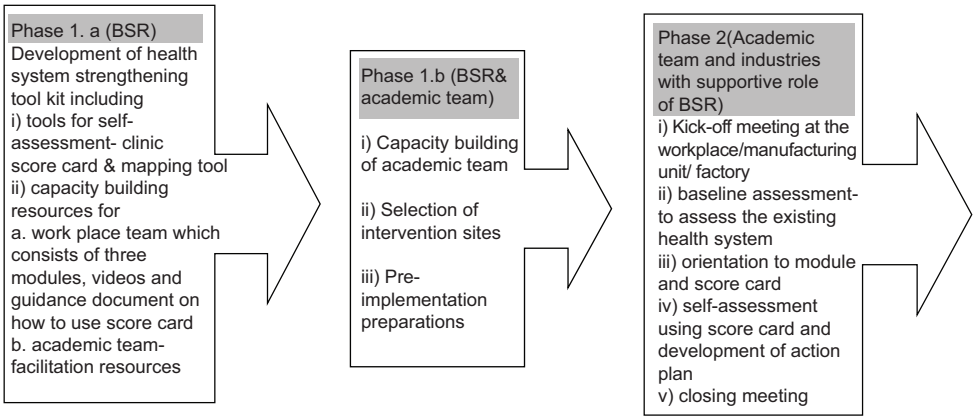
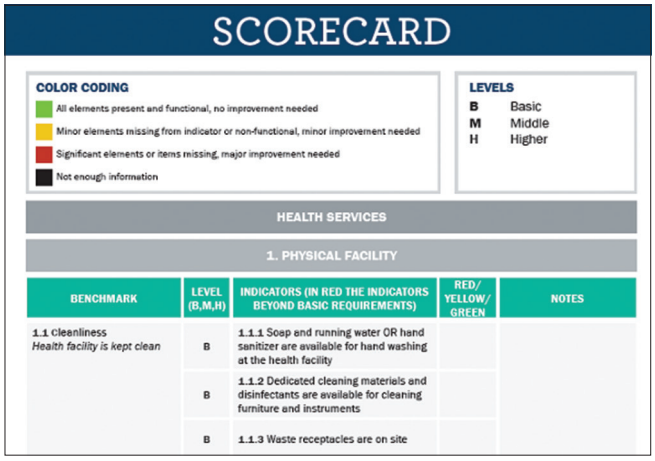


Figure 1: Framework of the phases of implementation of the program

of the factories. Following baseline assessment, a HSS team was formed at the factories and they were oriented to the modules and scorecard. The HSS team then conducted a self-assessment of the health system. The results of which were calibrated with the findings of the academic team. Short- and long-term action plans were developed by the committee based on the gaps identified. The final and crucial step was the closing meeting was attended by all stakeholders. The HSS team presented their action plans and the way forward.

Monitoring and evaluation framework

The logical model for program planning and evaluation which has been an effective tool for monitoring and evaluation of programs and interventions was used throughout the pilot of the HSS toolkit to monitor progress.[11] The summary of the various phases of the pilot program along with relevant indicators are given in Table 1.


SCORECARD

COLOR CODING
All elements present and functional, no improvement needed
Minor elements missing from indicator or non-functional, minor improvement needed
Significant elements or items missing, major improvement needed
Not enough information

LEVELS
B Basic
M Middle
H Higher

HEALTH SERVICES

1. PHYSICAL FACILITY

BENCHMARK	LEVEL (B,M,H)	INDICATORS (IN RED THE INDICATORS BEYOND BASIC REQUIREMENTS)	RED/ YELLOW/ GREEN	NOTES
1.1 Cleanliness *Health facility is kept clean*	B	1.1.1 Soap and running water OR hand sanitizer are available for hand washing at the health facility		
	B	1.1.2 Dedicated cleaning materials and disinfectants are available for cleaning furniture and instruments		
	B	1.1.3 Waste receptacles are on site		

Figure 2: Outline of the scorecard

Results

Section 1: Profile of the factories

A total of three factories (one garment and two spinning mills) were included for the pilot study with a worker strength ranging from 600 to 2000. A majority of the workers in the garment industry were women residing within the factory premises. All the factories have a functioning clinic with a staff nurse. The spinning mill had a full-time physician and nurses working in shifts. From the "mapping exercise," it was understood that all the factories had established linkages with external service providers, both public and private. Social protection benefits covering health and accident insurance are available through the Employees State Insurance (ESI) for all permanent workers.

Section 2: Outcome of intervention of the program

Table 2 provides a summary of a few areas of improvement identified in different assessment parameters like physical infrastructure and strengthening health committee. The areas of concordance and discrepancy between the factory and academic team can also be observed from the results.

Then, a concordant list of areas for improvement was developed by the teams in all three factories, which was one of the main expected outcomes of the program. Short-term action plans focused on improving clinic facilities, strengthening biomedical waste management, and ensuring availability and displaying health education materials. Long-term plans focused on improving the physical infrastructure of the clinics,

Table 1: Summary of monitoring and evaluation framework of the Health System Strengthening toolkit pilot-based on the logic model

Phases of the model	Activities in the program	Indicators
Input (resources)	Academia team to facilitate the health system strengthening at the factories	Number and composition of members of the academic team
	Toolkit with self-assessment component and capacity-building resources	Availability of the capacity-building resources
	Implementation package	Number and expertise of the members of the health team at the factory
	Health System Strengthening Committee identified and formed at the factory level	
Process	Baseline assessment by the academia team at the factory	Report on baseline assessment
	Periodic visits by the academic team for facilitation of the toolkit, building capacity of the factory HSS committee, and overall program monitoring	Number of visits conducted by the academic team
		Strength of members from the health team attending the meetings with the academic team
Output	Utilization of capacity-building resources by the HSS Committee, especially the clinic staff at the factory	Self-assessment results on the clinic scorecard
	Self-assessment of the factory health systems using the clinic scorecard	Presentation of the short- and long-term action plans
	Identification of areas for improvement and develop short-term, medium-, and long-term action plans for the health system strengthening	Approved action plans and completion of the short-term action items before pilot closing
Expected outcome	Implement the health system strengthening measures from the action plan	Periodic self-assessment using the "scorecard'
	Functioning of a comprehensive health system at the workplace	Improved clinic attendance
	Improved access to healthcare products and services	Improved satisfaction of the workers

Table 2: Areas for improvement identified by the health team

Assessment parameters	Factory 1		Factory 2		Factory 3	
	Factory health team	External evaluation	Factory health team	External evaluation	Factory health team	External evaluation
Physical facilities products equipment materials	Need for the improvement of the clinic facilities, increase the number of health personnel, and to provide patient-waiting area		Nil		Need to strengthen clinic facilities	
Facility policies and procedures	Not identified	Need to improve disinfection protocols, develop policies for ensuring confidentiality and safety during patient care	Not identified	Infection prevention protocols not in place	Need for more insurance facilities for workers as private facilities are more utilized.	
				Need for the development of policies for regular pre-placement screening for all workers and to develop policies to address grievances of the workers' concerns for health care		
Referral and provision of care	Need to organize non-communicable disease screening program		Nil		Nil	
Education and counseling	Not identified	Need for providing health education and counseling in nutrition, hygiene, contraception, and non-communicable disease risk factors, occupational diseases	Not identified	Need for providing health education and counseling in nutrition, hygiene, contraception, and non-communicable disease risk factors and occupational health risks	Increase scope for the nurses to be involved in health management like taking an active role in committees, providing counseling services, etc.	
Management system and corporate leadership	Need to initiate and involve health committee in health planning and budgeting		Need for a health committee, written policy on health management: the hiring of workers, appraisals, promotions, etc., and streamlining of health budgeting		Need for strengthening of health committee; their roles and responsibilities, develop written policy on health management-hiring of workers, appraisal, promotions, etc., and streamline health budgeting	

*"Nil" denotes that from the assessment no area for improvement identified by the factory and academic team. "Not identified" denotes the academic team has identified gaps but the health team missed the gaps

budget reallocation, and strengthening the healthcare team, and bringing policy-level changes.

Section 3: Reaction and perception of the factory team regarding the HSS program

Table 3 is a summary of the perceptions and experiences of the factory team regarding the program in all three factories. The HSS program was a new experience for all three factories.

Discussion

The present study was piloted to develop and implement a HSS package consisting of self-assessment tools to improve the health facility and resources for capacity building of the staff in the factories and to understand the usefulness of the package for the industries. This intervention is very much needed in workplaces as only 5–10% of the industries in the developing countries have employee health services, that too, mostly restricted to the manufacturing industries.[12]

The baseline assessment using a multipronged approach helped to understand the existing health systems at the factory level and the gaps and strengths in running their health systems parallelly with the business operations. All the factories had established a good linkage with the public service providers but utilization was less due to issues in timings and having to wait for long hours. Lack of awareness regarding the benefits of ESI services and low trust levels in the government health institutions were added reasons for poor utilization of the services. Rashidha, in her study in Kerala, observed similar reasons for the average or low utilization of ESI services among the respondents.[13]

The HSS toolkit was designed as a self-reference tool for workplaces. During its pilot run, valuable insights were gained from different stages of its development and piloting. While the toolkit was well-received by all the three pilot factories, it was realized that the toolkit in itself could not be used by the factories without proper orientation and facilitation on the different components and resources through a systematic approach.

The clinic scorecard was well appreciated as an internal audit tool by the factories to track their health systems periodically.

Table 3: Perceptions and experiences of the program from the pilot industries

Components	Perceptions of team members about the program
Capacity-building resources	*"The module on budgeting was a very useful resource for us as we had no idea earlier as to what are the components of a health budget. Now, we are able to do line listing of items."* **HR Manager**
	"The toolkit will be a good resource to improve our clinics as it gives us a guideline of how a clinic should function, why and how a health budget should be planned." **General manager**
Self-assessment using scorecard	*"Scorecard was a handy tool for us to self-assess our clinic and come up with short- and long-term plans to strengthen our clinic."* **HR manager**
	"Rating Methodology-Level (B, M, H) and Red, Yellow, Green very innovative. Just by seeing the color coding, we will be able to have a clear idea of which areas to improve. " **Nurse**
	"By using this scorecard-almost everything related to dispensary like what records to maintain, what information to display in the clinic, what cleanliness measures to be followed, was covered." **Nurse**
Overall impression of health system strengthening program	*"Apart from the module, you have given such valuable health- and hygiene-related guidelines to our staff."* **Labour Welfare Officer**
	"This strengthening process will be useful for us as we are applying for OSHA certification where health system strengthening is the main component." **Senior Manager**
Change in attitude and behavior following the program	*"More involvement from the nursing staff into the HR system. She now helps us in budgeting."* **HR Manager**
	"Increased proactiveness of the nurse." **HR Manager**
	"We feel the need to strengthen ourselves in managing communicable diseases, prevent outbreaks." **HR Manager**
Outcome of the program	*"We have modified the job description of our nurse widening her scope of work."* **Senior Manager**
	"We have improved the physical infrastructure of our health center." **Nurse**
	"We have started making our budget and we already notice that there is a decrease in the cost of medicines as we used to buy unnecessary medicines earlier." **HR manager**
	"We have made a policy to send our health staff for continuous professional development programs at least twice a year" **HR manager**

The role of the standardized assessment criteria using checklists or any validated tools for the improved quality of healthcare has been emphasized by many authors.[14,15] In the first attempt, workplaces tend to score themselves higher on the scorecard, assuming they have all things in place. However, an external non-biased assessment of the workplace health systems using the same scorecard can bring out completely different results. In our pilot study, we found discordance between the factories and external agencies in identifying gaps in counseling and education and policies and procedures. This finding is not surprising considering that workplaces generally look beyond occupational safety as observed by Pescud *et al*.[16] The WHO has emphasized the role of senior management in establishing workplace health policies and the participation of employees for the proper implementation of such health policies.[17]

An important outcome from the program was a better role clarification of the occupational health nurse, commitment of the management to establish policies for ensuring the continuous professional development of the nurse. The nurses who participated in the program have expressed that the scope of their job has been widened as an outcome of the pilot and they feel that they can play a proactive role in the industry. The multidisciplinary role of the occupational health nurses and the need for strengthening their capacity has been emphasized by the WHO.[17]

Another key outcome from this pilot was the realization that workplaces are primarily preoccupied with business operations, and despite the best intentions, may lack the knowledge and skills required to strengthen healthcare support for their workforce. While the HSS toolkit is well-designed for self-reference use for factories to fill this gap, it is not a complete solution in itself. For best results, it is preferable that an expert agency facilitates this toolkit and builds the capacity of the factory management toward strengthening their health systems.

Conclusions

Conducting a self-assessment using a validated tool is an effective method for workplaces to identify the gaps in their existing health systems, and further propels them toward their next steps in making action plans for short- and long-term duration. This exercise once facilitated by an expert organization can pave the way toward a self-sustainable practice that the workplaces can periodically continue to review and strengthen their health systems. A tool that was tested in the textile industries can as well be used in any industrial setup with expert guidance.

Ethics approval

The study was cleared by the Institutional Human Ethics committee of PSG Institute of Medical Sciences and Research, Coimbatore, India.

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Conflicts of interest

There are no conflicts of interest.

References

1. D'Ambrogio E. Workers' conditions in the textile and clothing sector: Just an Asian affair? Issues at stake after the Rana Plaza tragedy. Member Research Service. [posted 2014 August 15]. [Last accessed on 2017 May 05]. Available from: http://www.europarl.europa.eu/thinktank.
2. Annual Report. Ministry of textiles, Government of India. 2019. [Last accessed on 2020 Aug 21]. Available from: http://texmin.nic.in/sites/default/files/Textiles-AnnualReport2018-2019%28English%29.pdf.
3. Mehra R, Branscum P, Sharma MA. Needs assessment of factory workers in India for health promotion programs. J Community Med Health Edu 2012;2:128.
4. Saha TK, Dasgupta A, Butt A, Chattopadhyay O. Health status of workers engaged in the small-scale garment industry: How healthy are they? Indian J Community Med 2010;35:179-82.
5. Babu AS, Madan K, Veluswamy SK, Mehra R, Maiya AG. Worksite health and wellness programs in India. Prog Cardiovasc Dis 2014;56:501-7.
6. Sharma K, Zodpey SP, Tiwari RR. Need and supply gap in occupational health manpower in India. Toxicol Ind Health 2013;29:483-9.
7. Saha RK. Occupational health in India. Ann Glob Health 2018;84:330-3.
8. Suri S, Das R. Occupational health profile of workers employed in the manufacturing sector of India. Natl Med J India 2016;29:277-81.
9. Ahmed S, Raihan M. Health status of the female workers in the garment sector of Bangladesh. J Fac Econ Adm Sci 2014;4:43-58.
10. Business for Social Responsibility. Investing in women for a better world. HER project. 2010. [Last accessed on 2018 Feb 23]. Available from: https://herproject.org/downloads/BSR_HERproject_Investing_In_Women.pdf.
11. McCawley P. The logic model for program planning and evaluation. University of Idaho, Extension. 2002. [Last accessed on 2018 Sep 11]. Available from: http://www.uiweb.uidaho.edu/extension/LogicModel.pdf.
12. Rantanen J, Fedotov IA. Standards, principles, and approaches in occupational health services. Encyclopedia of Occupational health and safety. International Labour organization. [Last accessed on 2012 Mar 30]. Available from: http://www.ilocis.org/documents/chpt16e.htm.
13. Rashida KN. Awareness and use of employees' state insurance services in Dharmadam Panchayat, Kerala. J Inf Manag 2015;2:67-76.
14. Hughes RG. Tools and strategies for quality improvement and patient safety. In: Hughes RG, editor. Patient Safety and Quality: An Evidence-Based Handbook for Nurses. Rockville (MD): Agency for Healthcare Research and Quality (US); 2008.
15. Hales R, Terblanche M, Fowler R, Sibbald W. Development of medical checklists for improved quality of patient care. Int J Qual Health Care 2008;20:22-30.
16. Pescud M, Teal R, Shilton T, Slevin T, Ledger M, Waterworth P, *et al*. Employers' views on the promotion of workplace health and wellbeing: A qualitative study. BMC Public Health 2015;15:642.
17. World Health Organization. The role of the occupational health nurse in workplace health management. Regional Office for Europe & WHO European Centre for Environment and Health. Copenhagen: WHO Regional Office for Europe; 2001.

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Original Article

Enhancing identification and counseling skills of dental undergraduate students using a customized Tobacco Counseling Training Module (TCTM) – A piloting of the process using ADDIE framework

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Abstract

Background: Tobacco use has emerged as a major public health problem. But, most graduates in medical and dental schools receive limited systematic training. The objective of this education innovation project was to enhance dental undergraduate student's ability to identify tobacco users through oral manifestations and improve their counseling skills using a customized Tobacco Counseling Training Module (TCTM).

Methods: A TCTM for students of dentistry was developed using ADDIE framework as a guide. Content and construct validation of the module was done by six subject experts using Delphi technique for obtaining consensus. Pilot testing was done on 20 students of third year BDS. Pre- and post-intervention assessment of knowledge, attitude, self-confidence was done using learning outcomes questionnaire. Ability to correctly identify oral manifestations was assessed using extended item MCQs and tobacco counseling skills using a modified KEECC. The difference in mean scores were computed and subjected to further statistical analysis using SPSS version 22.

Results: There was a significant improvement in post intervention scores for mean knowledge (5.5 ± 1.4 to 13.2 ± 1.1), attitude (5.6 ± 0.9 and 8.5 ± 0.5), self-confidence (1.5 ± 0.5 and 3.1 ± 0.2), ability to correctly identify oral manifestations (5.2 ± 1.4 and 9.4 ± 0.8) and tobacco counseling skills.

Conclusion: It is possible to introduce the module in the existing curriculum and its effectiveness evaluation shows benefit in terms of Kirkpatrick's Level 1, 2, 3 (improvement in knowledge, attitude, self-confidence, ability to identify oral manifestations, and tobacco counseling skills) of training effectiveness.

Keywords:
ADDIE model, Calgary-Cambridge counselling framework, dental students, process piloting, tobacco counseling, oral manifestations

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Introduction

Tobacco use has emerged a major public health issue worldwide that has resulted in massive disability, disease, and death acquiring the dimension of an epidemic.[1] Smoking is a leading cause of preventable deaths across the nations. It is estimated that five million preventable deaths occur every year globally, attributable to tobacco use which is expected to double by 2020. Approximately one person dies every 6 seconds due to tobacco, accounting for one in 10 adult deaths. Nearly 80% of the more than one billion smokers worldwide live in low- and middle-income countries where the burden of tobacco-related illness and death is heaviest. Besides damaging personal health, tobacco use contributes to severe societal costs like reduced productivity, environmental damage, and poverty of the families.[2] India is the second largest producer and consumer of tobacco after China. More than one-third of adults use tobacco in India accounting for 275 million. Out of these 275 million users, 163.7 million consume smokeless tobacco, 69 million are smokers, and 42.3 million consume tobacco in both the forms.[3]

Medical and dental students upon graduation are often tasked with assisting to promote health and welfare of the population as part of the health system as well as for their individual patients. Literature suggests that most graduates in medical and dental schools worldwide receive limited training and are ill-equipped to treat tobacco dependence.[4] Although, Dental Council of India expects a dentist to identify a tobacco addict using oral manifestations and offer some basic counseling, there is lack of a structured training module in existing curriculum. Such a focused training to combat a common public health problem will also improve generic communication and health behavior counseling skill sub-components such as establishing rapport, focusing on affective behavioral issues, and cross-cultural communication. In this background, this education innovation intervention was undertaken to explore and test whether a systematic structured approach using a customized Tobacco Counseling Training Module (TCTM) will enhance dental undergraduate student's ability to identify tobacco users through oral manifestations of tobacco use and improve their skills in tobacco cessation counseling. The specific objectives of the study were:

- To do learning needs assessment by assessing the learner's pre-intervention status of knowledge, attitude, ability of undergraduate dental students to correctly identify oral manifestations and skills in tobacco counseling, and identify the gaps in knowledge and practice against recommended Public Health Service's Clinical practice guidelines by US Department of Health and Human Services for tobacco cessation counseling[5]
- To develop and validate a customized TCTM based on identified gaps in knowledge and practice and implement this intervention on a group of third year BDS students
- To determine the effectiveness of TCTM on knowledge, attitude, ability of undergraduate dental students to correctly identify oral manifestations, and their skills in tobacco counseling following its implementation.

Methods

This education innovation project was carried out over a period of one year from June 2017 to May 2018 at JSS Dental College and Hospital, Mysore. Ethical clearance was obtained from the Institutional ethics committee on 23rd February 2017. A mixed method research design was adopted. A customized tobacco cessation counseling training module for students of dentistry was developed using ADDIE framework (Analyse, Design, Development, Implement, Evaluate), which is a useful tool for building effective training and performance support.[6,7]

ADDIE step 1: Analyze (Learning needs assessment)

This was done using a combination of qualitative and quantitative research techniques. Training needs for a customized tobacco counseling module was identified using a pre-designed structured questionnaire on 10 undergraduate students, one focus group discussion (FGD) involving six interns, in-depth interview among two teaching faculty in Public Health Dentistry and four office bearers of Indian Association of Public Health Dentistry [Table 1]. FGD guide and in-depth interview guide were prepared. These were used by the principal investigator to elicit information on current practices of training students in tobacco counseling, perceived gaps in existing curriculum which needed to be addressed, the contents to be included while developing any such customized training module, method of teaching and evaluation to be adopted. The FGD and in-depth interview were audio recorded. An abridged transcript was prepared by listening to the tape of focus group and in-depth interview. Classical content analysis[8] method was used wherein we created smaller chunks of data and then placed a code with each chunk. These codes were then placed into similar groupings and counted. The frequency of each code (quantitative information) was supplemented with rich description of each code (qualitative information). Besides, five faculties in the department of public health dentistry were requested to participate in a free listing exercise where they enlisted all the important topics that in

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their opinion were to be included in training module. Summary of outcomes from needs assessment using FGD and in-depth interview is captured in Table 2. Key contents suggested by faculty during free listing were identified by card pile sorting. The needs analysis revealed that tobacco related topics were taught in bits and pieces in different specialty departments in dentistry and students found it difficult to integrate and apply what is being taught during real patient encounters. It was felt that there was a need for designing a structured training module to be taught by a multispecialty team. Teaching in such module should focus on tobacco trends and patterns, role of dentists in tobacco cessation counseling, scales for assessing tobacco dependence, behavioral theories that could be adopted for counseling, clinical tips for identifying and differentiating various oral manifestations in a tobacco user and a tobacco cessation counseling framework that could be used in dental clinical setup.

ADDIE Step 2: Design (Module designing)

A framework for TCTM was designed by synthesizing inputs from learning needs assessment, free listing among faculty who suggested the contents for such module and through literature review. The module was designed to be spread over five sessions of 90 minutes each, and broad contents, structure, delivery methods, duration, assessment and feedback for each of these sessions were incorporated. The initial five modules focused on (1) global pattern, trends and impact of tobacco consumption, (2) role of health and oral health care providers in reducing the tobacco burden, (3) alarming oral manifestations suggestive of tobacco use and differential diagnosis of these manifestations, (4) introduction to behavioral theories and motivational interview, (5) tobacco counseling using "STAR with

Table 1: Training Needs Assessment: Background of participants in focus group discussion, in-depth interview and free listing

	Gender		Designation				
	Male	Female	Interns	Postgraduate students	Lecturers/Assistant professors	Associate professors	Professors and above
Focus group discussion (*n*=6)	2	4	6	-	-	-	-
In-depth interview (*n*=6)	-	-	-	-	-	2	4*
Free listing (*n*=5)	-	-	-	-	3	2	-

*Office bearers of Indian Association of Public Health Dentistry

Table 2: Training needs assessment using focus group discussion and in-depth interview: Key findings

Major theme	Responses of participants with group consensus
Current practices	"Tobacco-related topics are taught by four different specialties in dentistry They are taught in different contexts without stressing on how to assess level of tobacco dependence and counseling"
Concerns on current practices	"Although tobacco topics are taught by four specialties, we find it difficult to apply in real life (clinical practice) situations." "We find it difficult to identify a tobacco addict using oral manifestations and also to differentially diagnose the lesion in a tobacco user." "The current practice of teaching has never made us think tobacco counseling is so important and we should do this in every possible patient encounter."
Need for a customized training program	"We need some dedicated hours of training exclusively for tobacco counseling in one department by a team of specialists from these four departments." "Focus in such training program should be on most common oral manifestations in a tobacco user and tips for clinically differentiating them from other similar lesions."
Topics (contents) suggested	"We need to know all health and oral health risks of tobacco use, its burden and impact on society." "We need to know about simple chair side tools for determining tobacco addiction". "We need to understand the fundamentals in tobacco counseling; Some demonstrations on how to counsel a tobacco addict".
Recommendations for mode of teaching and student assessment	"Preferably teaching on tobacco counseling should be done in Public Health Dentistry department by a multispecialty team during clinical postings." "Interactive teaching methods should be adopted with demonstrations of counseling" "Pictures, videos and real-life examples should be used for training." "Evaluation may be undertaken using quiz, match the following, MCQs etc., for each session." "Effectiveness of training may be elicited by using a customized questionnaire on knowledge, attitude and self confidence."

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5A and 5R approach" incorporated into Calgary—Cambridge communication skill framework.[9,10] This module was prepared for third year BDS students and can be implemented during clinical posting of students in Public Health Dentistry.

Addie Step 3: Development (Module development)

Development Part 1: Development and validation of content and structure of modules 1 to 5

At this stage, a trainer's (facilitator's) guide and a guide for student on each session that contained the resource material with specific learning objectives, well-defined learning outcomes, the teaching methods to be adopted, a worksheet with small group activities incorporated, mode of assessment, and session feedback were prepared. The focus areas (contents) in five sessions of TCTM have been listed in step 2 above. The fifth session focused on tobacco counseling using "STAR with 5 A and 5 R approach".[9,10] The content knowledge base for the tobacco cessation was developed from "STAR with 5 A and 5 R approach" used by US Public Health Service which is popular among public health dentists.[5] Content and context validation was done by six subject experts locally. Delphi technique was used to obtain consensus on the contents from six subject experts.[11] Summary of output from Delphi technique is shown in Table 3. TCTM was shared with six subject experts and each subject expert was requested to provide their inputs on relevance, appropriateness and applicability of contents in each session using a predesigned content validation form. The experts were requested to suggest their recommendations for modifications, deletions and addition of contents of the module with justifications. After receiving replies from each expert, a summary was prepared based on their inputs for each session and this was shared with all the experts again for them to refine it further. Based on recommendations of experts following second round of communication, a summary with explanations for inclusion of contents in each session and their relevance, appropriateness and usefulness in enhancing the knowledge, attitude, and skills related to identification and counseling was prepared. This was shared with the subject experts and they were requested to express their views and

Table 3: Summary of output from the Delphi technique

Purpose: Content and context validation of TCTM			
Number of subject experts participated: 06			
	Round 1		
Session	**Number of experts expressing their agreement with content and context of sessions**	**Number of modifications suggested for each session**	**Number of suggestions incorporated following this round**
Session 1: Global pattern, trends and impact of tobacco consumption	4	2	2
Session 2: Role of health and oral health care providers in reducing the tobacco burden	3	3	2
Session 3: Alarming oral manifestations in a tobacco user	3	3	3
Session 4: Introduction to behavioral theories and motivational interview	4	4	2
Session 5: Tobacco counseling using "STAR with 5 A and 5 R approach	5	3	2
	Round 2		
Session 1: Global pattern, trends and impact of tobacco consumption	6	None	None
Session 2: Role of health and oral health care providers in reducing the tobacco burden	5	2	1
Session 3: Alarming oral manifestations in a tobacco user	5	2	2
Session 4: Introduction to behavioral theories and motivational interview	4	2	1
Session 5: Tobacco counseling using "STAR with 5 A and 5 R approach"	5	1	1
	Round 3		
All the six subject experts expressed their consensus and agreement for the learning objectives, lesson plan, method of teaching adopted, activities planned, method of eliciting feedback for each session.			

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refine it further to modify the same, if needed. All the six subject experts expressed their consensus on the output content at this stage.

Development Part 2: Development of module 6 (Tobacco counseling incorporated into Calgary—Cambridge communication skill framework)

When the above mentioned training modules were shared with faculty mentor who was an expert in medical education, it was suggested to incorporate contents of "STAR with 5 A and 5 R approach" into the Calgary—Cambridge communication framework[12] to enable the students to engage in deliberate practice of tobacco counseling and receive feedback to improve performance. This necessitated the inclusion of sixth session of 120 minutes into the module. Addition of sixth session into the module necessitated revalidation of contents to make subject experts understand the importance of incorporating Calgary—Cambridge communication framework. But, three out of six subject experts who validated TCTM, initially, were out of country at that time. TCTM was supposed to be implemented in the institution within a week's time according to calendar of events in the department. Conventional process of reaching consensus on inclusion of sixth session using Delphi would have taken more time with no real time interaction. So, we tried a combination of WhatsApp and mobile conference call to get all these subject experts in different time zones to discuss the project synchronously. A WhatsApp group of all subject experts and moderator was created. An article that described the process of fitting Calgary-Cambridge framework to tobacco counseling was shared in group.[1] Subject experts were requested to post their comments in the WhatsApp group after referring the article. Some experts expressed diverging opinions on inclusion of sixth session to an already validated module. To explain the rationale and to reach consensus on revised module, mobile conference call was thought about. Mobile conference call following initial discussion using WhatsApp provided quick and timely interaction on diverging opinions. Queries raised by some experts were replied by either moderator or other subject experts. We were able to arrive at consensus in 25 minutes on revised contents, teaching plan for each session and evaluation tools for all the six sessions. As a result of all these processes, "The modified CALGARY – CAMBRIDGE communication framework for tobacco counseling" was developed and is shown as Annexure 1.

ADDIE Step 4: Implementation

The module containing five sessions of 90 minutes each and an additional session of 120 minutes for deliberate practice and evaluation of tobacco counseling pilot tested on twenty students of third year BDS in May 2018 on six consecutive days during clinical postings in Public Health Dentistry. Third year BDS students were divided by institution authorities into 10 clinical batches. Each batch had approximately 10 students. Two batches were selected out of 10 batches using lottery method for this pilot study and this constituted 20% of students in the batch. The flow chart of these sessions in TCTM is attached as Annexure 2 which provides an overview of sequencing, contents and lesson plan for each session. TCTM was implemented by a multidisciplinary team comprising of faculty from Public Health Dentistry, Community Medicine and Oral Medicine. Faculty selection for the multidisciplinary team was made by HOD of Public Health Dentistry based on interest of faculty in tobacco intervention and their availability to offer training in a sustained manner for subsequent years as well. Moreover, these faculties were also suggested by interns to be included in the multidisciplinary team for training on tobacco counseling during FGD. Students and faculty orientation was conducted before initiating the training module. These sessions incorporated a combination of various teaching methods like initial brainstorming, lectures using PowerPoint, videos, experience sharing, quiz, small group activities, role playing, etc.

ADDIE Step 5: Evaluation

To evaluate the effectiveness of the intervention (TCTM), a questionnaire was developed for assessing knowledge, attitude and self-confidence in tobacco counseling. MCQs with extended items were used for assessing the ability to correctly identify oral manifestations. An initial Learning Outcomes Questionnaire (LOQ) for assessing knowledge, attitude, and self-confidence in tobacco counseling was prepared using expert inputs and literature review. This LOQ had 30 items for assessing knowledge, 15 items for assessing attitude and 10 items for assessing self-confidence. The LOQ was communicated to six subject experts along with a content validation form. Each expert was requested to rate the relevance, appropriateness and clarity of each item on a five-point Likert scale with 1 being least favorable response to 5 being highly favorable response. Fourteen items from knowledge, 5 items from attitude and 4 items from self-confidence were deleted from the original questionnaire due to redundancy, lack of relevance and appropriateness. The final LOQ had 16 items for assessing knowledge, 10 items for attitude and 6 items for self-confidence. The initial tool for assessing ability to correctly identify oral manifestations had 17 items. During

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validation from six subject experts, three items were deleted due to redundancy, two for not being relevant and two for not being appropriate. Final tool had 10 extended item MCQs. Cognitive interview was conducted to assess the response process validity of these tools among ten final year BDS students. Known group validity of LOQ was assessed by using this questionnaire on three final year BDS students, three interns and three postgraduate students from Public Health Dentistry. Each correct response was given a score of one while incorrect response was scored zero. The total score for knowledge (score range 0–16), attitude (score range 0–10), and self-confidence (score range 0–6) in tobacco counseling was computed for each participant. Extended item MCQs was administered to three final year BDS students, three interns and three postgraduate students from Oral Medicine to assess known group validity. Tobacco counseling skills was assessed during role play by students in the sixth session using a modified Kalamazoo Essential Elements Communication Checklist (KEECC).[13] Here, students were divided into triads where one student acted as a tobacco user while others acted as doctor and recorder. This (formative assessment) was repeated till each student completed all three roles. However, score given by the observing trained faculty (summative assessment) was considered for statistical analysis on effectiveness of the TCTM. Reliability assessment of the tools for knowledge, attitude, self-confidence and ability to identify oral manifestations was done on 10 students using test-retest method.

Pre-intervention knowledge, attitude, self-confidence, ability to correctly identify oral manifestations and their skills in tobacco counseling was assessed before implementation of module on twenty students of third year BDS and post-intervention assessment was done following its implementation. The difference in mean scores were computed and subjected to statistical analysis using SPSS version 22 (IBM, Chicago, USA). Student and faculty satisfaction was collected using a pre-designed satisfaction survey questionnaire on 5 faculty and 20 students. Paired sample *t*-test was used for comparison between pre and post-intervention values while calculating the effect size. McNemar's Chi square test was used for comparing frequencies and percentages before and after implementation of module.

Results

Validity testing of the tool used. The scale level content validity index for evaluation tools assessing knowledge, attitude, self-confidence and ability to identify oral manifestations was 1.0. Cronbach's alpha values indicating the intra examiner reliability score for the tools evaluating knowledge, attitude and self-confidence, and ability to identify oral manifestations were 0.98, 0.96 and 0.97 respectively. In known group validity assessment, knowledge,

Table 4: Summary of validity and reliability of Learning Outcomes Questionnaire (LOQ) and extended item MCQs used for assessment of knowledge, attitude, self-confidence and identification skills

Assessment tool	Scale level content validity index/ Universal agreement (S - CVI/UA)	Intra rater reliability (Cronbach's alpha) *n*=10 participants
Learning Outcomes Questionnaire		
Knowledge (16 items)	1.0	0.98
attitude and self-confidence (10 items+6 items)	1.0	0.96
Extended item MCQs	1.0	0.97
Details of known group validity assessment for Learning Outcomes Questionnaire. (*n*=3 each from three groups)		
Outcome assessed	**Knowledge (16 items), (Mean±SD)**	**Attitude and self-confidence (16 items) (Mean±SD)**
Final year BDS students (*n*=3)	5.7±0.6	5.3±0.6
Interns (*n*=3)	7.7±0.6	6.7±0.6
Postgraduate students from Public Health Dentistry (*n*=3)	11.7±0.6	9.3±0.6
Statistical inference	*F*: 84.0, *P*: <0.001	*F*: 37.3, *P*: <0.001
Details of known group validity assessment for extended item MCQs (*n*=3 each from three groups)		
Outcome assessed	**Identification skill (10 items), (Mean±SD)**	
Final year BDS students (*n*=3)	5.3±0.6	
Interns (*n*=3)	7. 0±0.0	
Postgraduate students from Oral Medicine & Radiology (*n*=3)	8.7±0.6	
Statistical inference	*F*: 8.3, *P*: <0.001	

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attitude and self-confidence, ability to identify oral manifestations scores were higher for postgraduate students followed by interns while being least for the fourth year BDS students [Table 4].

Effectiveness of the Intervention (TCTM) in terms of Student learning i.e., Kirkpatrick level 2 of Training program evaluation: This is shown by the mean knowledge score increasing from 5.5 ± 1.4 to 13.2 ± 1.1 suggesting a significant improvement (t = 16. 9, P < 0.001, Effect size: 1.33). The mean attitude and self-confidence scores at pre-intervention were 5.6 ± 0.9 and 1.5 ± 0.5 respectively and they changed to 8.5 ± 0.5 and 3.1 ± 0.2 post-intervention. The difference was statistically significant for attitude (t = 16.5, P < 0.001, Effect size: 0.52) and self-confidence (t = 13.5, P < 0.001, Effect size: 0.34). Mean scores for identification of oral manifestations at pre and post-intervention were 5.2 ± 1.4 and 9.4 ± 0.8 with the difference being statistically significant (t = 12.8, P < 0.001, Effect size: 0.98) [Table 5]. With regard to tobacco counseling skills, none of the students did well during pre-intervention assessment while it increased to six (30%) after intervention. The positive change in their communication skills was statistically significant (X^2 Value: 11.7, P value: 0.001) [Table 6].

Effectiveness of the intervention (Student and Faculty reaction i.e., Kirkpatrick level 1 of training program evaluation): All five faculty (100%) and 15 students (95%) felt that the program was very much relevant and highly appropriate [Table 7]. The faculty also offered some specific suggestions for implementation of the training module to make the program even better.

Discussion

Tobacco use has become a common contributor to premature death and disability.[14] Learning counseling skills that facilitate behavior modification is one of the essential skill expected of a competent health care professional. These behavior interventions are beneficial in changing the life course of a tobacco consumer/addict that will have several social, economic and health related benefits to the individual, family and society as a whole.[14,15]

Literature indicates that health care professionals can learn tobacco cessation counseling skills to improve patient outcomes.[16-18] Literature also suggests that there is lack of structured training program in tobacco counseling for medical and dental undergraduates in the existing curriculum.[19] Dental undergraduates are in a strategic position to identify a tobacco user through oral manifestations and offer tobacco counseling. Dental Council of India also expects a graduating dental student to undertake tobacco counseling among patients with tobacco habits. It was evident in needs assessment undertaken in the present study that the existing curriculum does not have structured training program for training dental undergraduate students in tobacco counseling. It was realized during focus group discussion among interns

Table 5: Effectiveness of the TCTM intervention (knowledge & attitude)

Outcome variable (maximum score)	Pre-intervention score mean±SD	Post-intervention score mean±SD	Tests of significance
Knowledge (16)	5.5±1.4	13.2±1.1	t: 16.9 Effect size: 1.33 P: <0.001
Attitude (10)	5.6±0.9	8.5±0.5	t: 16.5 Effect size: 0.52 P: 0.001
Self - confidence (6)	1.5±0.5	3.1±0.2	t: 13.5 Effect size: 0.34 P: 0.001
Ability to correctly identify oral manifestations (10)	5.2±1.4	9.4±0.8	t: 12.8 Effect size: 0.98 P: 0.001

Table 6: Effectiveness of the TCTM intervention (tobacco counseling skills)

Tobacco counseling skills (using role play)	Done well n (%)	Needs improvement n (%)	Not done n (%)	Total n (%)
Pre - intervention (n=20)	0 (0)	4 (20)	16 (80)	20 (100)
Post - intervention (n=20)	6 (30)	14 (70)	0 (0)	20 (100)
Statistical inference		χ^2: 11.7, P: 0.001		

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Table 7: Effectiveness of the TCTM (student and faculty reaction)

Participants	Relevant	Very much relevant	Appropriate	Highly appropriate
Faculty (*n*=5)	-	5 (100)	-	5 (100)
Students (*n*=20)	1 (5)	19 (95)	1 (5)	19 (95)

that some topics related to tobacco habits, pattern of tobacco use, oral manifestations in a tobacco user and brief counseling was taught by different specialty departments. But, this compartmentalized training was not useful as many of them could not connect between what is being taught and its application in their routine practice. It was apparent after needs assessment that there was a need for a customized tobacco counseling training module for dental undergraduate students to enhance their skills in the identification of oral manifestations in a tobacco user followed by counseling. Hence, in response to this need, the TCTM was developed and tested on a group of third year BDS students. In BDS curriculum specified by Dental Council of India, a dental undergraduate student enters clinical postings in third year of BDS program. We introduced TCTM of six sessions as an introductory module on tobacco counseling for students entering the clinical settings. It was evident in the literature that early training on promotion of behavior change among medical students facilitated better acquisition and application of tobacco intervention skills.[19] The early exposure resulted in promoting a sense of belonging and provided usefulness in clinical environments. Moreover, early exposure of students to TCTM was intended to create a sense of responsibility among students as soon as they enter clinicals to make them realize the importance of identifying tobacco addicts using oral manifestations and also to highlight the importance of early intervention to make these addicts quit the habit. This was somehow not given due importance while eliciting case histories of patients by dental undergraduate students as noted during needs assessment. However, the training module is designed in such a way that this foundation course of six sessions in third year will be followed by three session's module to be undertaken among final year BDS students and one hour refresher session for interns. The modules for final year BDS students and interns have been developed and validated, but, are yet to be implemented in a sequential manner by a multispecialty team. This pilot study evaluated the effectiveness of TCTM on Kirkpatrick level 1 (reaction) and level 2 (learning). Here, the evaluation of knowledge, attitude and self-confidence was done using LOQ, extended item MCQs with clinical vignettes were used to assess ability to correctly identify the images of oral manifestations projected on a screen on a random basis. Here, the counseling skills were assessed while the students practiced it through role plays. However, in final year BDS and during internship, the evaluation of counseling is proposed to be undertaken using standardized patients and real patients, respectively. The sequencing of training module initially from third year BDS and extending into their final year and internship was thought to provide reinforcement at regular intervals throughout their curriculum to achieve optimum results. The first session on global pattern, trends and impact of tobacco consumption was incorporated to provide an overview on the tobacco consumption pattern and recent statistics on tobacco usage around the world and in India. It was realized through feedback after the session that many students were not even aware about facts on tobacco presented in the session. This session was included to enhance their knowledge on tobacco burden while motivating them to consider tobacco counseling as an important aspect in their clinical practice. Second session focused on role of health and oral health care providers in reducing tobacco burden and also provided some practice on assessing tobacco dependence using Fagerstorm scale among smokers and smokeless tobacco users. The intension of this session was to make participants realize the fact that dentists are in a key position to motivate patients to quit tobacco. Third session focused on alarming oral manifestations in a tobacco user and their diagnosis. It was included to provide clinical tips for identifying oral manifestations in tobacco user and their differential diagnosis. This was also one of the skills expected to be enhanced through TCTM. Fourth session focused on introduction to behavioral theories and motivational interview. It was expressed by the students during learning needs assessment that they are not aware of any behavioral theories which they could apply for lifestyle and behavior management. This, in fact was the rationale for inclusion of this session. The session was included to provide brief overview on most commonly used theories for behavior modification in medical profession. It was felt necessary to provide some knowledge to the students on various stages of change a person goes through during behavior modification which could be applied for other clinical purposes as well. Fifth session focused on tobacco counseling using expanded Calgary - Cambridge communication skill framework. Inclusion of communication skills framework in the module was done to make students to understand the importance of communication

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in motivating a person to adopt healthy lifestyle. Students expressed in their feedback that this session specifically helped them to understand the key components of communication in medical case history taking which was not known to them previously. Students expressed their willingness to adopt these key components in their routine clinical encounters as well besides tobacco counseling. The additional session of 120 minutes provided an opportunity for deliberate practice of tobacco counseling through role plays and also to evaluate student's performance in tobacco counseling in a simulated setting. Students expressed in their feedback that they enjoyed this session and also learnt their mistakes when their own batchmates identified the aspects that were done well, not done well or those that required improvement using a checklist. Besides, the feedback by faculty on individual student's performance also was appreciated by the students.

There was a significant increase in the knowledge, attitude and self-confidence of students towards tobacco counseling following implementation of TCTM. This could be attributed to focused and relevant topics being covered in one stretch by a multispecialty team. Some students have expressed in their feedback that the mode of training which included initial brainstorming, lectures, video demonstrations, experience sharing, interactive group discussion and small group activities were interesting and kept them engaged throughout the sessions. These results were similar to the findings of a study by Brown RL *et al*.[19] who in their study assessed whether a curriculum on tobacco intervention could garner student acceptance; improve relevant knowledge, attitudes, and self-confidence; and be applied in students' early clinical experience. They found significant improvement in all these attributes following implementation of Tobacco Intervention Basic Skills curriculum (TIBS). Ahmadian M *et al.* in their study compared the effectiveness of two educational methods in teaching tobacco cessation counseling (TCC) in dental practice for senior dental students.[20] They found both role play (RP) and problem-based learning (PBL) methods to be effective in enhancing the knowledge, attitude, and skill of the participants. Ability to correctly identify oral manifestations also improved significantly following implementation of training module. The session that specifically focused on clinical presentation of most common oral manifestations in a tobacco user and pathophysiology of these lesions which was taught by an expert in oral medicine was appreciated by the students. It was their perception through feedback that this session helped them in enhancing their ability to correctly identify these lesions while differentially diagnosing them. Students practiced counseling using a modified Calgary—Cambridge communication skill framework that incorporated STAR with 5A and 5R approach content for reference during deliberate practice using role plays. The counseling in practice sessions facilitated self-improvement through deliberate practice using role play in triads of students where one student acted as patient, one student as a doctor and other as an observer followed by feedback by observing faculty. This session of deliberate practice gave each student an opportunity to undertake counseling in a simulated setting. Students perceived that role play enabled them in understanding the sequence of communication contents to be followed during counseling process while they got exposed to the checklist used for evaluation during such counseling sessions. During this deliberate practice, one student who acted as evaluator was given the modified Kalamazoo Essential Elements Communications Checklist to evaluate the performance of the student who was acting as doctor. This gave an opportunity for students to understand how communication during a clinical encounter will be assessed using standard checklist. This modified evaluation checklist is attached as Annexure 3**.** It was found that their counseling skills improved with 30% of the students performing well following implementation of TCTM compared to none of the students before the intervention. Fernandes K *et al.* in their study to enhance communication skills of MBBS (Bachelor of Medicine and Bachelor of Surgery) students in counseling of tobacco users by using interactive teaching methods found role play, interactive session, anecdotes to play a vital role in improving their counseling skills.[21] Gordon JS *et al.* in their study found an innovative and interactive CD-ROM tobacco cessation training program to be effective in enhancing the knowledge level and attitudes of 47 third-year dental students and 30 dental hygiene students.[22] Faculty and students in our study also expressed complete satisfaction on the course content and method of teaching adopted. Faculty expressed their desire to incorporate this into the existing dental curriculum at the institution level. Davis JM *et al.* in their study to evaluate the effectiveness of a peer-reviewed tobacco curriculum (Tobacco Free! Curriculum) found an average increase in the amount to time spent by faculty on tobacco education. There was an increase of 85 minutes on tobacco education in dental hygiene curriculum. Besides, there was a large positive increase in the percentage of faculty members who formally assessed the use of 5As and 5Rs (21%–88%), and a dramatic increase (+100) in the percentage of faculty members who taught or included most of the 13 tobacco use cessation (TUC)

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interventions content areas following the introduction of the curriculum and training program.[23] Our findings were consistent with the results of these studies and others.[24,25]

Conclusions

Using a systematic process guided by the ADDIE Framework, it is possible to design a structured learning experience (curricular module) for equipping students with skills to manage a common public health problem. TCTM was found to improve knowledge, attitude, self-confidence, ability to identify oral manifestations and tobacco counseling skills among study participants (Kirkpatrick's Level 1, 2, 3). Students and faculty expressed their satisfaction about the TCTM with some specific suggestions.

Novelty: TCTM was developed through synthesis of contents from literature review and inputs obtained from students, faculty and experts during needs assessment. TCTM was implemented during clinical training of students in Public Health Dentistry by a multispecialty team. It required six days during their clinical training which was found to be practically feasible and well accepted by students and faculty. This study also describes the process of learning needs assessment, training module design, content and process validation, and training program evaluation using the ADDIE model of instructional design. The content matter (knowledge needed in tobacco use and cessation) was incorporated into the Calgary-Cambridge Counselling framework and validated by dental experts using Delphi technique. This can be used by fellow teachers in dentistry and other health care educators as it helps students engage in deliberate practice and receive feedback on performance to sharpen and improve their tobacco cessation communication skills.

Limitation: Time constraint was a limitation as intensive training was required on six consecutive days. It may be practically difficult to apply this for each small group in an academic year. Hence, it was suggested by faculty to consider three knowledge-oriented sessions in large-group mode for all students together to be then followed up by three skill-oriented sessions during clinical postings in small groups. This will reduce the time constraint (if any) and make it more practical for its replication in other institutions for common and important public health problems. It was mentioned by interns who participated in focus group discussion during needs assessment that no customized training was undertaken with regard to tobacco counseling. Hence, we thought of comparing pre and post-intervention scores among participants who underwent training as part of evaluation. However, comparison of performance of students who underwent training against those who were not offered such training would have given us chance to compare effectiveness of the current module against traditional method. This could not be undertaken owing to the time constraints. The long-term impact will be evaluated in the future when subsequent follow up sessions are undertaken during the fourth year BDS and internship.

Way forward: This customized tobacco counseling training module for dental undergraduate students would be evaluated by incorporating this as a value addition into the existing curriculum at the level of institution before recommending this as a curriculum reform to Dental Council of India.

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Declaration of participant consent

The authors certify that they have obtained all appropriate participant consent forms. In the form, the participant(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity, but, anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Reddy KS, Gupta PC. Report on tobacco control in India. Ministry of Health and Family Welfare. Government of India. 2004.
2. World Health Organization. Tobacco: Leading cause of death, illness and impoverishment. WHO 2013. Available from: https://www.who.int/news-room/fact-sheets/detail/tobacco. [Last accessed on 2019 Aug 17].
3. Report of the working group on disease burden for 12th five year plan. Working group on disease burden: Non communicable diseases. Directorate general of health services. Ministry of Health and Family Welfare. Government of India. 2011.
4. Llambi L, Barros PM, Parodi C, Cora M, Garces G. Teaching tobacco cessation to large student cohorts through the train the trainers and problem based learning strategies. Educ Health 2016;29:89-94.
5. Fiore MC, Jaen CR, Baker TB, Bailey WC, Benowitz NL, Curry SJ, *et al.*

[Downloaded free from http://www.indianjcancer.com on Wednesday, July 22, 2020, IP: 172.20.65.147]

Clinical practice guideline. Treating tobacco use and dependence: 2008 update. Rockville, MD: US Department of Health and Human Services, Public Health Service; 2008. Available from: http://www.ahrq. gov/clinic/tobacco/treating_tobacco_use08.pdf. [Last accessed on 2019 Sep 03].
6. ADDIE Model: Instructional Design. Available from: https://educationaltechnology.net/the-addie-model-instructional-design/. [Last cited 2018 Jul 10].
7. Guyer M. Training from A to E: Analysis to evaluation white paper. U.S. Department of Justice National Institute of Corrections. Available from link: https://info.nicic.gov/nicrp/system/files/032740.pdf. [Last cited 2018 Jul 10].
8. Dongre AR, Deshmukh PR, Kalaiselvan G, Upadhyaya S. Application of qualitative methods in health research: An overview. Online J Health Allied Scs 2009;8:3.
9. Boutwell L, Cook L, Norman K, Lindsey WT. A Pharmacist's guide for smoking cessation. Available from: https://cdn.ymaws.com/www.aparx.org/resource/resmgr/CEs/CE_Winter_Smoking_Cessation.pdf. [Last cited 2018 Jul 10].
10. Sandhu HS. Tobacco cessation in dental offices. J Can Dent Assoc 2001;67:153-7.
11. Cantrill JA, Sibbald B, Buetow S. The Delphi and nominal group techniques in health services research. Int J Pharm Pract 1996;4:67-74.
12. Kurtz S, Silverman J, Benson J, Draper J. Marrying content and process in clinical method teaching: Enhancing the Calgary–Cambridge guides. Academic Medicine 2003;78:802-9.
13. Joyce BL, Steenbergh T, Scher E. Use of the Kalamazoo essential elements communication checklist (Adapted) in an institutional interpersonal communication skills curriculum. J Grad Med Educ 2010;165-9.
14. McGinnis JM, Foege WH. Actual causes of death in the United States. J Am Med Assoc 1993;270:2207-12.
15. Carr A, Ebbert J. Interventions for tobacco cessation in the dental setting (Review). Available from: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD005084.pub2/pdf. [Last cited 2018 Jun 14].
16. Hughes JR. Motivating and helping smokers to stop smoking. J Gen Intern Med 2003;18:1053-7.
17. Humair JP, Cornuz J. A new curriculum using active learning methods and standardized patients to train residents in smoking cessation. J Gen Intern Med 2003;18:1023-7.
18. Eyler AE, Dicken LL, Fitzgerald JT, Oh MS, Wolf FM, Zweifler AJ. Teaching smoking-cessation counseling to medical students using simulated patients. Am J Prev Med 1997;13:153-8.
19. Brown RL, Pfeifer JM, Gjerde CL, Seibert CS, Haq CL. Teaching patient centered tobacco intervention to first year medical students. J Gen Intern Med 2014;19:534-9.
20. Ahmadian M, Khami MR, Ahamdi AE, Razeghi S, Yazdani R. Effectiveness of two interactive educational methods to teach tobacco cessation counseling for senior dental students. Eur J Dent 2017;11:287-92.
21. Fernandez K, Pandve HT, Debnath DJ. Use of interactive teaching methods in tobacco cessation program and examine it by using objective structured clinical exam. J Educ Health Promot 2013;2:28.
22. Gordon JS, Severson HH, Seeley JR. Development and evaluation of an interactive tobacco cessation CD-ROM educational program for dental students. J Dent Educ 2004;68:361-9.
23. Davis JM, Stockdale MS, Cropper M. Evaluation of a comprehensive tobacco cessation curriculum for dental hygiene programs. J Dent Educ 2010;74:471-9.
24. Hanioka T, Ojima M, Kawaguchi Y, Hirata Y, Ogawa H, Hinode D, *et al.* Education on tobacco use interventions for undergraduate dental students. Japanese Dent Sci Rev 2015;51:65-74.
25. Walsh SE, Singleton JA, Worth CT, Krugler J, Moore R, Wesley GC, *et al.* Tobacco cessation counseling training with standardized patient. J Dent Educ 2007;71:1171-8.

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Annexure 1

MODIFIED CALGARY – CAMBRIDGE COMMUNICATION FRAMEWORK FOR TOBACCO COUNSELING

I. ESTABLISHES INITIAL RAPPORT AND BUILDS POSITIVE RELATIONSHIP

- Greets patient,
- Introduces self,
- Demonstrates respect to the patient (uses words that show care and concern, uses tone, pace, eye contact, and posture that show care and concern)

II. OPENS THE DISCUSSION ON TOBACCO COUNSELING BY ASKING RELEVANT OPEN ENDED QUESTIONS ON TOBACCO HABITS

- **Explores (Asks about)** the patient's tobacco related problems with an appropriate **opening question** (e.g., "tell me about how you got into this habit?" and what happened after that till date" or "what questions related to this habit did you hope to get answered today?")
- **Encourages patient to tell the story** of the tobacco related problem(s) from when first started to the present in own words (clarifying reason for presenting now)
- **Listens** attentively to the patient's opening statement, without interrupting or directing patient's response
- **Facilitates** patient's responses verbally and non–verbally e.g., use of encouragement, silence, repetition, paraphrasing, interpretation
- **Picks up** verbal and non–verbal cues (body language, speech, facial expression, affect); checks out and acknowledges as appropriate
- **Makes necessary notes and confirms** tobacco related information elicited and looks for further information (e.g. "Tell me about any previous attempts to quit the habit and what happened then?")

III. GATHER ADDITIONAL RELEVANT INFORMATION ON FOUR "R"S (RISKS, RELEVANCE, REWARDS AND ROADBLOCKS)

- **Asks about frequency and duration of current habit,** current habit, risks involved, relevance of quitting, rewards of quitting, and anticipated roadblocks in such quit attempts,
- **Clarifies** details as necessary with more specific questions
- **Summarizes the information** gathered and provides opportunity for patient to correct or add information

IV. ASSESSES PATIENT'S LEVEL OF ADDICTION BY USING FAGERSTORM TEST FOR NICOTINE DEPENDENCE FOR SMOKING/USE OF SMOKELESS TOBACCO

- Informs the patient about the need for filling the small questionnaire (which may take approximately two minutes).
- Administers the questionnaire after obtaining informed consent

V. ADVICES PATIENT TO QUIT THE HABIT

- Tries to communicate the necessity to quit the habit by connecting the patient's current habit and adverse impact (social, economic and health related) it can have on the entire family.
- Uses the information gathered earlier by patient on risks, relevance, rewards and roadblocks to enhance his/her existing knowledge by giving additional relevant information.
- Adopts motivational interviewing technique while giving such advices

VI. ASSESSES PATIENT'S WILLINGNESS TO QUIT

- Elicits and documents the patient's willingness to quit by a question such as "what is your opinion on trying to quit the habit considering the risks and benefits of quitting and specially in the interest of your family"
- Gives opportunity to the patient to express freely without imposing

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If willing to quit,

VII. ASSIST THE PATIENT IN MAKING A PLAN TO QUIT

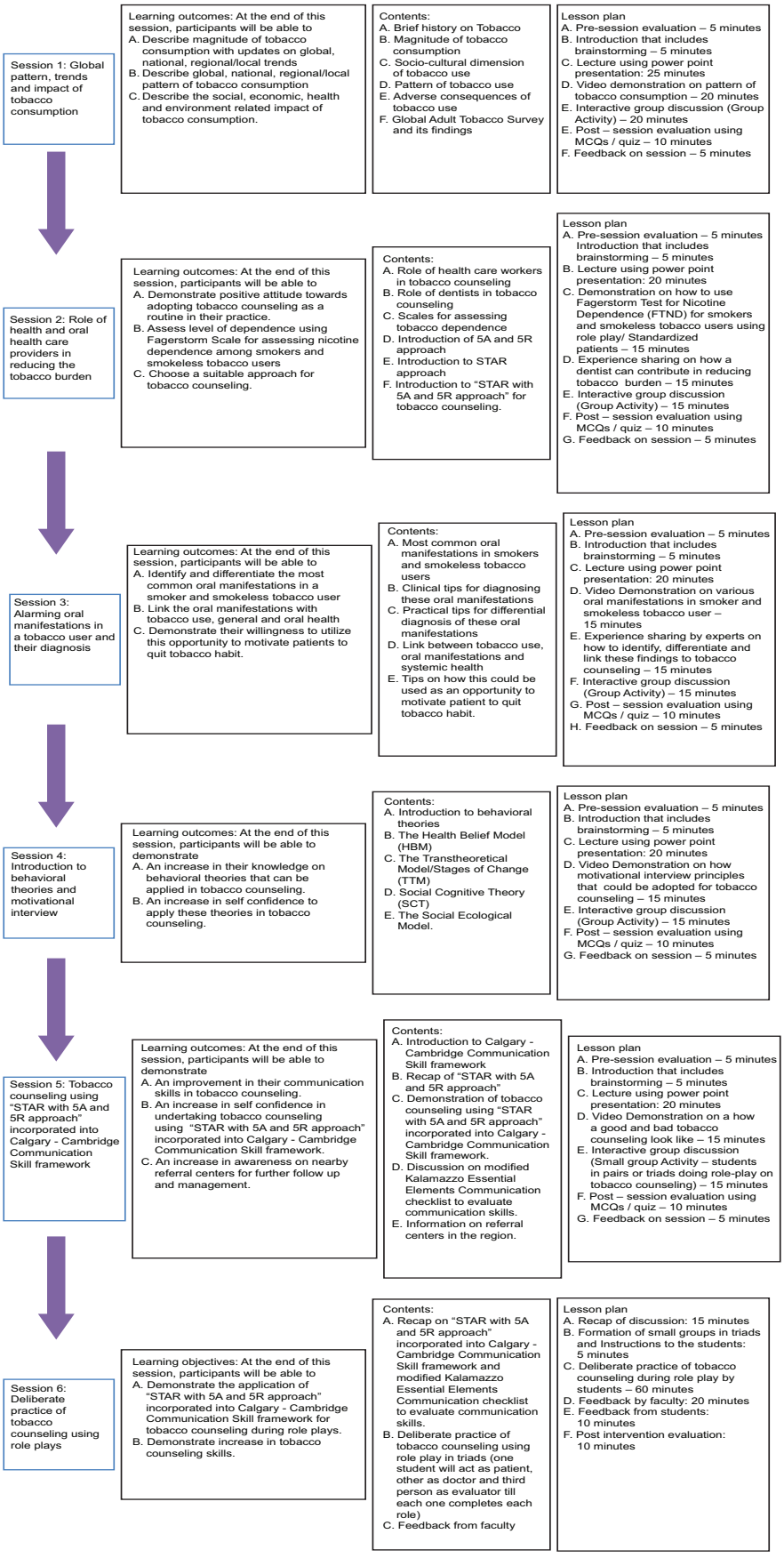
- **Explains STAR approach to the patient**
- S- Set a quit date: Gives flexibility to the patient to decide a date preferably within the next one month
- T- Tell your friends and family: Informs the patient to tell his/her decision to quit to all family members and friends who may encourage him to do so.
- A – Anticipate adherence challenge: Tells the patient that some withdrawal symptoms like head ache, irritability, lack of attention, etc may be experienced. Motivates the patient to be mentally prepared to face these short term challenges with determination. Encourages the patient to do so in the best interest of dependent family members.
- R – Remove all tobacco products from vicinity: Informs the patient about the necessity to keep tobacco products away so that they are not easily available in times of craving.

VIII. CLOSES THE DISCUSSION WITH AN ARRANGEMENT FOR FOLLOW UP

- Tries to set a date for follow up examination even if the patient is not willing
- Tries to set a date for follow up even when patient agrees to quit by adopting STAR Approach
- Asks whether the patient has questions, concerns, or other issues
- Acknowledges patient and closes the interview by greeting the patient

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Annexure 2: Flow chart of six sessions in TCTM



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Annexure 3

Modified Kalamazoo Essential Elements Communication Checklist for tobacco counseling

	Done well	Needs improvement	Not done
Build a Relationship			
Greets and shows interest in patient as a person			
Uses words that show care and concern throughout the interview			
Uses tone, pace, eye contact, and posture that show care and concern			
Open the Discussion			
Allows patient to complete opening statement without interruption			
Asks "Is there anything else?" to elicit a full set of concerns			
Explain and/or negotiates an agenda for the visit			
Gather Information			
Begins with patient's story using open-ended questions ("Tell me about…")			
Clarifies details as necessary with more specific "yes/no" questions			
Summarizes and gives patient opportunity to correct or add information			
Uses an appropriate scale to elicit the level of addiction to tobacco			
Advices patient to quit the habit and assess readiness to quit			
Discuss four “R”s effectively to reinforce and enhance existing knowledge			
Motivates patient by connecting the health, social and economic impacts			
Elicits patient's willingness to quit by clarifying the doubts			
Assist the patient in making a quit plan by explaining STAR approach			
Explains using words that are easy for patient to understand			
Provide closure by arranging a follow up			
Asks whether the patient has questions, concerns, or other issues			
Acknowledges patient and closes the interview			

Reference: The Bayer-Fetzer Conference on Physician-Patient Communication in Medical Education. Essential Elements of Communication in Medical Encounters: The Kalamazoo Consensus Statement. Academic Medicine. 76(4):390-393, April 2001

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Original Article

Effectiveness of Multi-Strategic Health Screening Cum Educational Intervention Model in Promoting Health of School Children in Rural Coimbatore

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Abstract

Background: Schools are the best setting for health promotion activities, and in India, for many, the schools are in fact the only nurturing and supportive place where they learn health information and have positive behavior consistently reinforced. Therefore, health promotion addressing the nutrition and personal hygiene habits among school children would improve health of school children and mold them into healthy productive citizens of tomorrow. **Objectives:** The objective of the study is to find the effectiveness of multi-strategic health screening cum educational intervention model in promoting the health of school children. **Methods:** A school-based intervention was designed using multi-strategic approach to promote the health of 2500 school children in 13 schools in rural Coimbatore. Logic model was used to plan the intervention, and the approach included health screening, nutrition and personal hygiene assessment and educational intervention in three phases over a period of 1 year. The multi-strategic approach comprised of approach through doctors, teachers, and through peer educators. The effectiveness of the intervention was assessed with improved nutrition and personal hygiene habits, improved body mass index status, and reduction in sickness absenteeism. **Results:** There was a significant improvement in nutrition and personal hygiene habits among school children. There was also significant reduction in the proportion of underweight category. Furthermore, a slight increase in sickness absenteeism was observed. **Conclusion:** Our study shows evidently that a multi-strategic health screening cum educational intervention model can be implemented and can be effective in bringing changes in the nutrition and personal hygiene habits thereby promoting the health of school children.

Key words: Educational intervention, health promotion, school children

Introduction

Schools have been a popular setting for health promotion activities for many years and served as the major institution in providing education and experiences that mold the young children into responsible and productive citizens.[1] School health programs and interventions are one of the most effective ways in reaching the children of that age. Well-designed educational interventions in schools can bring about awareness leading to change in attitudes and behaviors and thus promotion of healthy practices.

In India, the major health problems of school-going children are undernutrition and added to that, micronutrient deficiency, and overweight/obesity are increasing.[2] In addition, another major reason for poor health and infections among school children is the lack of awareness of the health benefits of personal hygiene. Diarrheal diseases, skin diseases, worm infestations, and dental diseases are most commonly associated with poor personal hygiene. It is also an established fact that infection and malnutrition form a vicious circle and retard children's physical development. Studies also show that repeated attacks of infections often compound the existing poor health of children, compromising children's attendance, and performance at school.[3]

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Hence, from a school health perspective, the major components to be addressed to improve the health of school children are improving their nutrition and personal hygiene. By doing this, we can target the major health problems of school children namely malnutrition and infections which are the major contributors to poor health and poor academic school performance, particularly in rural areas. Literature also shows that healthy eating habits in young children may prevent various chronic health disorders in childhood and adult life, including obesity, diabetes, hypertension, cardiovascular disease, cancer, and dental caries.[4,5] School environment also provides an easily accessible setting for interventions targeting the children to promote healthy lifestyles.[6,7]

There are many studies on interventions to promote healthy behaviors among school children, and systematic reviews have shown that interventions to promote healthy eating and physical activity were the most likely to be effective.[8,9] A lot of studies have evaluated school-based interventions on a diet of which a few are from low- and middle-income countries. Review shows that the interventions that were effective are high-intensity school-based interventions which were comprehensive and multicomponent.[10]

A study done in India has shown that school-based child-to-child personal hygiene education resulted in behavior change among children.[11] Another study done among adolescent school students in North India included intervention components like nutrition education lectures, promotion of physical activity, individual counselling by a trained nutritionist, increased availability of healthier food choices in the school canteen and involvement of teachers and parents, showed effectiveness in improving the diet of school children.[12]

Studies have also shown that while it appears that parental involvement is crucial in school interventions, challenges exist for recruiting and sustaining parent involvement in school-based programs.[13]

Studies have revealed that reaching the children through peer leaders were more effective than when done through adults,[14] especially when targeting behavioral changes and draws on the credibility that young people have a rapport with their peers and provides flexibility in meeting the needs of their peers.[15,16]

Similarly, the WHO also recommends that a variety of activities promoting a healthy diet and a strong educational component are likely to be more successful among school students.[17]

Hence, this study was conducted to find out the effectiveness of a multi-strategic health screening cum educational intervention model in promoting the health of school children.

Materials and Methods

Based on the extensive review of various interventions and recommendations of the WHO as well as keeping in mind, the cultural settings and resources available, a custom-made intervention was designed to promote the health of school children, and this included the following components:

Morbidity screening and dietary and hygiene gap identification

The school children were screened for general morbidities including eye and dental examination. The morbidities were documented in the health card. The body mass index (BMI) was measured, and the WHO charts were used to grade the BMI status. Then, they were also assessed for personal hygiene habits and nutritional habits using a checklist developed based on standard recommendations. The gaps in the recommended diet and personal hygiene habits were identified. Those who needed referral were also given a referral slip.

Individual counseling of students based on individual gaps identified

The students at the end of the screening were given individual counseling by doctors on their morbidities, about the gaps in their diet and personal hygiene practices and how they can be rectified.

Modification of teaching curriculum

The heads of the schools, along with the teachers were approached, and efforts were taken to include the nutrition and personal hygiene education as a part of their routine classes in which definite hours were allotted for educating students on nutrition and personal hygiene using the tailor-made module designed using standard recommendations of the National Health Agencies.

Designing modules for dietary and personal hygiene education

The teaching module on good nutritive diet and personal hygiene was prepared based on standard recommendations and the one regarding diet-based on the Indian Council of Medical Research/National Institute of Nutrition (ICMR/NIN) recommendations for a balanced diet. We designed the module in such a way that the student's interest, i.e., their future aspirations and career choices were linked to nutrition and personal hygiene practices. How a proper nutrition and personal hygiene practices could bring them close to their aspirations. Further, the module developed was a flip chart with pictures for easy understanding and the key points to be told during the session were printed on the back side of the flip for ease of using it. Studies showed that flipchart was an effective tool in poor resource setting for health education.[18] This could capture the student's interest and be a motivation for the positive behavior changes expected in them. Based on the evidence of the impact of using posters on imparting knowledge, a poster was also given for each school that had the key points of the module.[19]

Training of teachers for communicating the health promotion messages

The teachers were given training on how effectively the tailor-made modules could be used to educate the children on nutrition and personal hygiene habits.

After the training, their sessions were observed by doctors and feedback on the same was given to the teachers to increase their effectiveness in the communication of key messages.

Selection and supervision of peer educators

The peer educators were chosen from each class with the help of teachers. The selected peer educators were motivated to teach their peers and use of the modules that would aid the diet and hygienic behavior change. The peer education sessions were supervised and feedback was given to the peer educators to improve their communication skills.

Reinforcing, cascading, and buttressing approach

The students were approached using three different approaches – through doctors, teachers, and peers.

- First was through teachers, who have good face value and rapport with the students. We involved the teachers by training them to take sessions using our module designed
- Second was through doctors and health team, the doctors did the nutrition and personal hygiene assessment and counseling. Doctors had good credibility among the students and were always looked on by the students, in this way, it motivated the students to a positive change in behavior
- Third, we also used peer approach by selecting peer leaders in school in their same class and trained peers in using the module among their peers. It gave them recognition and motivation to deliver the modules to their peers.

Assessment tools and program evaluation

The assessment tools were designed based on standard recommendations (ICMR/NIN) and were developed as a checklist in quickly identifying the gaps in the recommendations. The same was piloted, and face validation was done among the experts of community medicine. The checklist developed assessed the nutrition and personal hygiene habits of children and based on this counseling was given. The tool designed was kept simple, user-friendly and effective in quickly identifying gaps and faulty habits in nutrition and personal hygiene. We also incorporated an evaluation component in the tool by giving a scoring for the assessment done.

The intervention was designed to promote the health of school children in 13 schools in rural Coimbatore. The study was done over a period of 1 year from 2014 to 2015. The preparatory work of planning the intervention, getting approval from authorities, acquiring funding, and designing the tailor-made modules were done during March–May 2015. When the schools reopened baseline assessments were done in all 13 schools and Phase 1 extended from June to August 2014, Phase 2 visits were done during September–November 2014 and final assessments were done during Phase 3 which extended from December 2014 to February 2015. The study population included all 2500 students from the age of 6–18 years of both sexes studying in these schools. The health team consisted of doctors, postgraduate students, interns, nurses, social worker, and health assistant who participated in the intervention.

Logic model was used to plan the intervention process leading to a desired result. It also helped to set out the evaluation priorities right from the beginning of the process.

Figure 1 shows the inputs needed for the project, the various activities planned, the output expected from these activities, and finally, the impact of the project, a desired outcome of the planned intervention.

The health education intervention was done in three phases. There was an interval of 3 months in-between these three visits. The final visit and assessment were done at an interval of almost 6 months from the baseline assessment. The Institution Human Ethics Committee approval was obtained for using the data for the study.

Phase 1

During the first visit, health screening was done, and the student was also assessed for the nutrition and personal hygiene habits and based on the assessment were given individual counseling. During the same visit, the teachers were given training by the health team member on how to use the tailor-made module on nutrition and personal hygiene to deliver the health education to students.

Phase 2

During the second visit, the students were again subjected to health screening and assessment of the nutrition and personal hygiene using the checklist. Based on which individual counseling and reinforcement of corrective measures were done. In this visit, the teachers were asked to deliver the health education session to the students with the help of the module, which was supervised and feedback was given. Child peer educators were selected from their own peers in each class.

Phase 3

During the third visit, again the students were screened and assessed for nutrition and personal hygiene and counseled individually. In this visit, peer educators delivered the health education which was supervised.

The module on nutrition and personal hygiene was given to each school, and a poster of the same was also placed on prominent areas of the school premises.

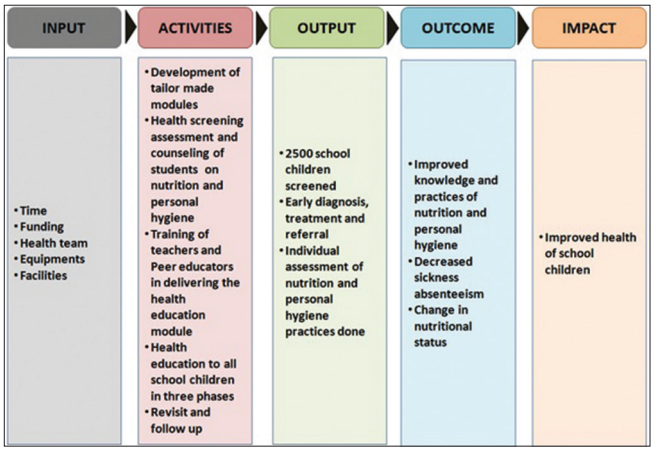


Figure 1: Logic model used in planning the intervention.

Measuring the effectiveness of the intervention

The outcome assessment indicators were chosen such that they would reflect the changes that we aimed doing this intervention. As our aim was to improve the health of school children by improving nutrition and personal hygiene practices, we chose the following indicators.

Change in the dietary and personal hygiene assessment score

The students were assessed for good nutritional intake and personal hygiene on the first visit following which a score was assigned against a maximum score of 10 each. The same was assessed at the end of the year; an improvement in score was considered improved practices of nutrition and personal hygiene.

Change in body mass index status

The BMI of school children was computed as a part of health screening so that the baseline BMI distribution could be compared with the change in the BMI distribution of the school population at the end of the intervention. Reduction in the underweight category was considered as a positive change indicating the effectiveness of the intervention.

Decreased absenteeism

This was another indicator that we chose. The days of absenteeism were noted down for each quarter; this was a proxy indicator indicating the health of school students. The mean days of absenteeism were compared using baseline and the mean days at the last quarter of the intervention. This indicator was chosen with the assumption that improved practices of good nutrition and personal hygiene would prevent most of the common communicable diseases thereby reducing sickness absenteeism.

Results

The total number of students was 2500 of which 2204 were assessed at the baseline assessment and 1836 were assessed during the final visit. The baseline (preintervention assessment) score and the postintervention score for a total score of 10, at the end of 1 year were documented.

Table 1 shows the basic characteristics of the study population; the participants belonged to the age group of 6–18 years.

Table 2 shows the pre- and post-intervention mean scores. Scoring was given on a total score of 10 each for both the nutrition and personal hygiene assessment. The difference between pre- and post-mean scores was found to be statistically significant. However, the increased figures for absenteeism shows we need to further ascertain whether the absenteeism is because of sickness or due to other extraneous reasons.

Table 3 shows the mean BMI distribution of the students, pre- and post-intervention. The BMI was calculated using the WHO standards,[20] and it was found that the difference in the distribution of BMI pre- and post-intervention was statistically significant. There was a significant reduction in the underweight category and improvement in the normal BMI category, though in the overweight and obese category there was a slight increase.

Table 1: Gender distribution of the study population at baseline and postintervention

Participants	At baseline assessment, *n* (%)	At postintervention assessment, *n* (%)
Boys	1128 (51.2)	937 (51.0)
Girls	1076 (48.8)	899 (49.0)
Total	2204 (100)	1836 (100)

Table 2: Indicators showing the effectiveness of the intervention

Indicators	Mean±SD		P
	Preintervention (*n*=2204)	Postintervention (*n*=1836)	
Diet assessment score	4.6±2.5	7.2±2.7	0.0001
Personal hygiene score	4.3±2.5	6.5±3.5	0.0001
Mean days of absenteeism	4.6±5.6	5.4±7.1	0.0001

SD: Standard deviation

Table 3: Body mass index distribution before and after intervention

BMI category	Preintervention (*n*=2204), *n* (%)	Postintervention (*n*=1836), *n* (%)	χ^2 and *P*
Underweight	505 (22.9)	356 (19.4)	77 and 0.024
Normal	1558 (70.7)	1351 (73.6)	
Overweight/obese	141 (6.4)	129 (7.0)	

BMI: Body mass index

Discussion

Our study aimed to show and share multipronged model of promoting the health of school children. The intervention was school-based in which we targeted the school children by individual counseling by doctors, health education by teachers, and also peer-led health education. The intervention had led to the improvement in the total mean score of diet and personal hygiene assessment among the students at the end of 6 months.

Our study highlights that students when individually screened for gaps in diet and hygiene and counseled for the same, the acceptance and understanding are better and when doctors themselves give the counseling, it increases the credibility of the information given. Second, we involved the teachers also who already had the confidence of the students, and so information from them would reinforce the key messages to the students even more. Third, we also chose peer educators among the same class since they would be easily accessible to their classmates and they had greater influence on their peers through peer pressure.

We also used tailor-made health education material which linked the academic performance and the career aspiration of students to their dietary and personal hygiene habits which was a motivation for the students to adapt better dietary and personal hygiene practices.

Our intervention showed that by involving the stakeholders during the planning and implementation stages, even a multipronged intervention can be implemented and can be effective in bringing changes in the diet and personal hygiene habits of school children, which was evident from the increase in postintervention behavior change scores as well as its positive health outcome reflected by the change in BMI distribution and particularly so reduction in underweight category at the end of 1 year.

Similar studies done in other places have shown that school based and peer-led programs have been successful in bringing out changes in regard to nutrition among school children. A study done in Canada also shows that peer educators models have been successful in bringing changes in elementary children also.[21] A study done in China among school adolescents have also proven to be effective in reducing sedentary behaviors in school children.[22] Systematic reviews have been done which discusses the success and failures of these models and need for further studies.[9]

Although BMI status and mean days of sickness absenteeism was taken as proxy measures, BMI status change reflected the status change of the whole study population and not necessarily the change in the individual status. In addition, mean days of absenteeism observed does not necessarily represent actual sickness since we did not insist on production of the sickness certificate. Furthermore, the increased absenteeism seen as a finding during the final impact assessment may be a confounder arising due to it being done in the festive season.

Our study also found out the practical difficulties in bringing changes in the habits of school children. Nutrition and personal hygiene habits cannot be inculcated in school children unless the resources and opportunities are provided. Parents and schools should be able to provide them with the proper nutrition, and environmental facilities and resources have to be provided for personal hygiene practices. The major drawback identified was the lack of parental involvement in the intervention, effectiveness of this intervention cannot be completely assessed unless the parents are involved.

Limitations

Our study focused on finding the effectiveness of an innovative intervention in which change in the nutrition and personal hygiene scores were taken as a main indicator. Since we could not find any existing tool that was user-friendly in assessing a large number of children in a short duration of time, we developed our own tool in the form of a checklist to assess nutrition and personal hygiene habits. The reliability and validity of this tool need to be tested. The other limitation was though our tool assessed the habits of recommended nutrition intake and personal hygiene practices the scores were self-reported, so increase in score had definitely shown knowledge increase but how far it was transformed into change in practices could not be evaluated. Another limitation of our study was due to resource constraint the individual-level data could not be compared preintervention and postintervention, we had to use aggregate data to compare scores.

Conclusion

Our study has shown evidently that this innovative model of health education where school children are intervened by individual counseling by doctors, teachers, and peer educators based health education sessions is effective in increasing the knowledge and practices of school children regarding nutrition and personal hygiene. Our evidence also showed a positive influence on the BMI of school children. Thus, this model of individual counseling involving health team, teachers, peer educators, and custom-made modules on relating nutrition and hygiene to their academic performances, aided by flipcharts and posters had created a positive impact on the ethos of the schools. Further studies with the involvement of parents and providing proper resources for aiding nutrition and personal hygiene practices are needed to study the real impact of the intervention.

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Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. Expert Committee on School Health Services & World Health Organization. Expert Committee on School Health Services: Report on the first Session. Geneva: World Health Organization; 7-12 August, 1950. Available from: http://www.who.int/iris/handle/10665/40137. [Last accessed on 2017 Nov 14].
2. Sunitha S, Gururaj G. Health behaviours & problems among young people in India: Cause for concern & call for action. Indian J Med Res 2014;140:185-208.
3. Department of Drinking Water Supply, Ministry of Rural Development, Government of India. Technical Note Series. School Water Supply, Sanitation & Hygiene Education: India. New Delhi: Department of Drinking Water Supply; 2004. Available from: http://www.ddws.gov.in/sites/upload_files/ddws/files/pdf/pdf/TechNoteSSHE_0.pdf. [Last accessed on 2017 Sep 11].
4. World Health Organization. Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation. Geneva: WHO Technical Report Series. 2003. p. 916.
5. Janssen I, Katzmarzyk PT, Boyce WF, Vereecken C, Mulvihill C, Roberts C, *et al*. Health Behaviour in School-Aged Children Obesity Working Group. Comparison of overweight and obesity prevalence in school-aged youth from 34 countries and their relationships with physical activity and dietary patterns. Obesity reviews 2005 May; 6 (2):123-32.
6. Lobstein T, Baur L, Uauy R; IASO International Obesity Task Force. Obesity in children and young people: A crisis in public health. Obes Rev 2004;5 Suppl 1:4-104.

7. Franks A, Kelder S, Dino GA, Horn KA, Gortmaker SL, Wiecha JL. School-based programs: Lessons learned from CATCH, Planet Health, and Not-On-Tobacco. InSchool Nutrition and Activity: Impacts on Well-Being. Apple Academic Press, Oakville, ON, Canada. 2015:147-62.
8. Shepherd J, Garcia J, Oliver S, Harden A, Rees R, Brunton G, *et al*. Barriers to, and Facilitators of, the Health of Young People: A Systematic Review of Evidence on Young People's Views and on Interventions in Mental Health, Physical Activity and Healthy Eating. Vol. 1, Overview. Vol. 2. Complete Report. London: Evidence for Policy and Practice Information and Co-Ordinating Centre, 2002.
9. Harden A., Oakley A, Oliver S. Peer-delivered health promotion for young people: A systematic review of different study designs Health Educ J 2001;60:339-53.
10. World Health Organization. Interventions on Diet and Physical Activity: What Works-Summary Report. World Health Organization; 2009. Available From: http://www.who.int/dietphysicalactivity/summary-report-09.pdf. [Last accessed on 2017 Dec 16].
11. Dongre AR, Deshmukh PR, Boratne AV, Thaware P, Garg BS. An approach to hygiene education among rural Indian school going children. Online J Health Allied Sci 2008;6:1-6.
12. Singhal N, Misra A, Shah P, Gulati S. Effects of controlled school-based multi-component model of nutrition and lifestyle interventions on behavior modification, anthropometry and metabolic risk profile of urban Asian Indian adolescents in North India. Eur J Clin Nutr 2010;64:364-73.
13. Story M. School-based approaches for preventing and treating obesity. Int J Obes Relat Metab Disord 1999;23 Suppl 2:S43-51.
14. Mellanby AR, Rees JB, Tripp JH. Peer-led and adult-led school health education: A critical review of available comparative research. Health Educ Res 2000;15:533-45.
15. Sloane BC, Zimmer CG. The power of peer health education. J Am Coll Health 1993;41:241-5.
16. McCrystal P, McAleavy G. Addressing health care in Northern Ireland through collaborative peer education. Int J Health Promot Educ 2000;38:76-85.
17. World Health Organization. Population-Based Approaches to Childhood Obesity Prevention. World Health Organization; 2012. Available from: http://www.who.int/dietphysicalactivity/childhood/WHO_new_childhoodobesity_PREVENTION_2027nov_HR_PRINT_OK.pdf. [Last accessed on 2017 Dec 16].
18. Caniza MA, Maron G, Moore EJ, Quintana Y, Liu T. Effective hand hygiene education with the use of flipcharts in a hospital in el Salvador. J Hosp Infect 2007;65:58-64.
19. Ilic D, Rowe N. What is the evidence that poster presentations are effective in promoting knowledge transfer? A state of the art review. Health Info Libr J 2013;30:4-12.
20. World Health Organization. Growth Reference 5-19 Years. Available from: http://www.who.int/growthref/who2007_bmi_for_age/en/. [Last accessed on 2015 Aug 18].
21. Stock S, Miranda C, Evans S, Plessis S, Ridley J, Yeh S, *et al.* Healthy buddies: A novel, peer-led health promotion program for the prevention of obesity and eating disorders in children in elementary school. Pediatrics 2007;120:e1059-68.
22. Cui Z, Shah S, Yan L, Pan Y, Gao A, Shi X, *et al.* Effect of a school-based peer education intervention on physical activity and sedentary behaviour in Chinese adolescents: A pilot study. BMJ Open 2012;2. pii: e000721.

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Original Article

Are Our Rural Adolescents Eating Healthy?: Implications for Redesigning School Health Interventions – A Cross Sectional Study in Rural Coimbatore

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Abstract

Background: Adolescence is a period of transition where independence in thinking and behavior is established and food choices that are made are followed for several years, and this can influence their health in adulthood. Hence, understanding the eating habits are necessary to plan effective nutritional interventions in adolescents. **Objectives:** The main objective of the study is to find out the extent of malnutrition among rural adolescents as well as evaluate their eating habits against recommended dietary food groups and to compare eating habits across gender and age groups. **Methods:** A cross-sectional study was done among 1425 adolescents from 13 rural schools from 2014 to 2015, and the variables considered in this study were age, sex, body mass index (BMI), and the eating habits of the adolescents. **Results:** Undernutrition was seen among 23% and overweight/obesity among 8% of adolescents. The habit of taking milk and milk products, fruits, and green leafy vegetables were very poor among the adolescents. Significant association was found between eating habits and BMI. Furthermore, late adolescents had better eating habits, and significant gender difference was seen in certain eating habits. **Conclusions:** The study shows that it is possible to understand the gaps in eating habits of adolescents, and this can be used to plan tailor-made nutritional interventions to adolescent groups as their eating habits are different and have long-term nutritional and health implications.

Key words: Adolescent, dietary gap assessment, faulty diet, nutritional diagnosis, school health

Introduction

Adolescence is a period of transition. The World Health Organization (WHO) defines the period of adolescents as 10–19 years of age.[1] It is a period of rapid growth and maturation in human development. It is a period where independence is established and adolescent makes choices of their own. The choices that are made are followed for several years and food habits are greatly established during this period, and this can influence their health in the adulthood.[2]

As global foods are widely marketed in low- and middle-income countries, it becomes important to understand the diet patterns and preferences.[3] In India, among adolescents, there is an increased consumption of fast foods contributing to overweight and obesity.[4] Furthermore, studies have shown that the food consumption pattern is poor among adolescents and have observed frequent consumption of energy-dense, nutrient-poor foods, and sugar-sweetened beverages, and the omission of a variety of healthy foods from their daily diet.[5]

In addition, cultural factors such as dietary practices and attitude toward food are evolving. These factors eventually influence the eating habits and today's adolescents lack proper knowledge on good eating habits and how those habits contribute toward their healthy nutritional status.

Hence, healthy eating behavior should be established in childhood and maintained during adolescence.[6] Adequate knowledge of healthy food choices and eating habits should be taught early to adolescents, as it can predispose them

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to lifestyle diseases in adulthood. Adolescents should be constantly motivated, and the need of the hour is bridging the knowledge gap on healthy eating.[7]

In India, there is a paucity of information about eating habits of adolescents, and limited information is available regarding various categories of food consumed by adolescents in relation to the food pyramid, which defines and identifies the balanced diet and corresponding nutritional status of an adolescent. Moreover, this type of nutritional data is even more difficult to find among rural population in India. Also, adolescents are the most important and vulnerable segment of population who have been largely ignored, and exclusive data pertaining to adolescent health are not widely available in Indian scenario.[8]

Hence, this study aimed to measure the extent of malnutrition among rural adolescents as well as evaluate their eating habits against recommended dietary food groups and to compare eating habits across gender and age groups.

Materials and Methods

A cross-sectional study was done among adolescents from 13 schools in rural area within the Madukkarai block, Coimbatore District, in 2014–2015. Prior permission was obtained from the concerned school health authorities, and this study protocol was approved by the Institutional Human Ethics Committee. Data used in this study were collected as a part of the school health project. The variables considered in this study were age, sex, body mass index (BMI), and the various elements in the recommended food portions that constitute the eating habits of the adolescents.

This school health program was funded by Larsen and Toubro, under their corporate social responsibility scheme. The funders' interest was only on the services to the rural school children and did not have a role in the research component. The research on the data obtained was done after obtaining ethics committee clearance from our institution. All adolescents from 10 to 18 years were included in the study and students who were absent from school on the day of visit were excluded from the study.

Anthropometric measurements such as height and weight of all students were recorded. Instruments were calibrated, and the BMI was calculated based on the measured height and weight. The nutritional status of the adolescent was determined based on the WHO BMI charts available, separately for boys and girls, from 5 to 19 years.[9] The WHO chart classifies them as severe thinness, thinness, and normal, overweight, and obese based on their BMI for their particular age. A questionnaire/checklist was designed to record the daily eating habits of school children based on the recommended portions of various categories listed in the food pyramid, as recommended by the ICMR and National Institute of Nutrition (NIN).[10] Based on the above balanced diet recommendations, questionnaire was developed which assessed the daily eating habits of the adolescents.[11] The questionnaire was a simplified tool with ten items, to assess gaps in the recommended portions of food category intake, by directly asking question that checks in Yes/No terms the compliance with daily recommended intake of cereals in the three main meals, daily intake of vegetables and green leafy vegetable, daily intake of milk and milk products, habit of taking a fruit daily, and intake of protein-rich foods in daily diet. In the assessment of protein-rich food/pulses recommended, portions can also be replaced by egg or other nonvegetarian food as per recommendations.[10] Hence, this assessment includes the assessment of egg and nonvegetarian food also. Additional questions were added to assess other eating habits such as the habit of taking mid-day snacks and questions that captured certain faulty eating habits of students like habit of skipping meals, taking junk food, and eating from street shops. The scoring was also given for each student based on the Yes/No terms in the questionnaire. Each positive response had a score of 1. Total score was 10 and a score of <10 indicates the gap from the recommended portion of food, or they have a faulty eating habit. Lesser the score, more is the gap in the recommended dietary portion of food category. Each student was individually enquired about the eating habits based on the questionnaire, and individual nutrition counseling was given to students who had a faulty eating habit or not complying with the recommended dietary food group intake standards.

All quantitative values were expressed in proportions. Normality test (Shapiro–Wilk test) was done and the nutritional score followed a nonnormal distribution. The association between eating habits and gender, age, and the nutritional status was tested using Chi-square test and Kruskal–Wallis test. All statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp (24.0), $P = 0.05$ or less, were considered as statistically significant.

Results

A total of 1425 adolescents between the ages, 10 and 18 years were included for this study, of which 53.1% belonged to early adolescents (10–13 years), 39.9% belonged to middle adolescents (14–16 years), and 6.9% belonged to late adolescents (17–18 years). The mean age was 13.38 ± 2.03 years. The study participants were 753 (47.2%) males and 672 (52.8) females.

Table 1 shows that a majority (68.8%) of adolescents had a normal BMI. However, more than a quarter of them (23.2% of

Table 1: Nutritional status of adolescents based on the WHO body mass index charts

Nutritional status	Male (*n*=672), *n* (%)	Female (*n*=753), *n* (%)	Total (*n*=1425), *n* (%)
Severe thin	53 (7.9)	23 (3.1)	76 (5.4)
Thin	145 (21.6)	109 (14.5)	254 (17.8)
Normal	429 (63.8)	551 (73.2)	980 (68.8)
Overweight	28 (4.2)	38 (5.0)	66 (4.6)
Obese	17 (2.5)	32 (4.2)	49 (3.4)

students who were undernourished including thin and severely thin and 8% were either overweight or obese) need dietary and other interventions.

The nutritional status of boys revealed 29.5% were either severely thin or thin, 4.2% were overweight, and 2.5% had obesity. Among girls, 17.6% were in severely thin or thinness category, 5.0% overweight, and 4.2% had obesity. This difference between boys and girls was statistically significant ($P < 0.001$).

Figure 1 shows the percentage of adolescents taking the recommended food groups daily as per the ICMR/NIN recommendations and also certain faulty eating habits such as eating junk food, eating from street shops, and skipping meals. It shows that none of the adolescents had the habit of taking green leafy vegetables daily. Furthermore, the habit of taking fruits and milk was very poor among the adolescents, thereby putting them at risk of micronutrient and vitamin deficiency which are not picked up if we do anthropometry alone as done in most school health programs.

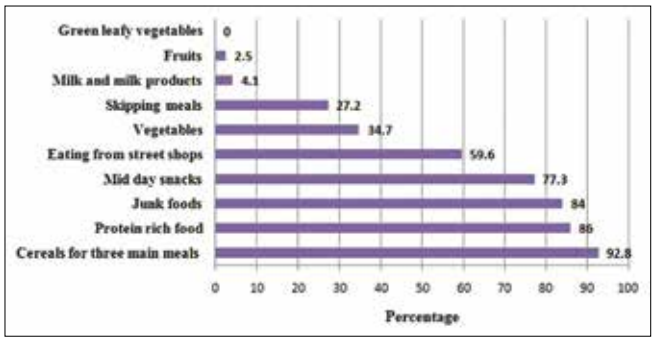


Figure 1: Pattern of eating habits among adolescents.

Table 2 shows that there is an association between eating habits and nutritional status of the adolescents. Chi-square test was used to find whether there was any significant difference in the eating habits among the adolescents of various nutritional statuses (based on BMI). Except for the habit of eating junk foods, others habits have significant association with BMI ($P < 0.001$).

Table 3 shows the difference in eating habits based on gender. Chi-square test was used to find whether there was any significant difference in the eating habits among the adolescent boys and girls. Odds ratio was also calculated. Habits of taking cereals, mid-day snacks, skipping meals, and eating from street shops showed significant difference between boys and girls.

The mean score obtained by the students on the diet assessment was compared between the age groups. Kruskal–Wallis test was used to find the statistical significance among the various age

Table 2: Association of eating habits and nutritional status of schoolchildren ($n=1425$)

Eating habits (recommended ICMR/NIN food category portion frequency)	Nutritional status (BMI categories)			χ^2	*P*
	Thin ($n=330$), *n* (%)	Normal ($n=980$), *n* (%)	Overweight/obese ($n=115$), *n* (%)		
Taking three main meals (cereals)	266 (80.6)	949 (96.8)	107 (93)	96.99	<0.001
Taking mid-day snacks	48 (14.5)	960 (98)	94 (81.7)	981.30	<0.001
Taking protein-rich food	193 (58.5)	921 (94.0)	112 (97.4)	272.29	<0.001
Taking milk and milk products	5 (1.5)	16 (1.6)	38 (33.0)	263.31	<0.001
Taking green leafy vegetables	0	0	0	0	0
Taking other vegetables	23 (7.0)	375 (38.3)	97 (84.3)	242.46	<0.001
Taking fruits	10 (3.0)	12 (1.2)	13 (11.3)	44.23	<0.001
Skipping meals	262 (79.4)	79 (8.1)	47 (40.9)	645.71	<0.001
Eating junk foods	276 (83.6)	816 (83.3)	105 (91.3)	4.99	0.08
Eating from street shops	113 (34.2)	655 (66.8)	82 (71.3)	116.02	<0.001

NIN: National Institute of Nutrition, BMI: Body mass index

Table 3: Association of eating habits and gender of the adolescents ($n=1425$)

Eating habits of adolescents	Male ($n=672$), *n* (%)	Female ($n=753$), *n* (%)	OR	χ^2	*P*
Taking three main meals (cereals)	609 (90.6)	713 (94.7)	1.84 (1.22-2.78)	8.74	0.004
Taking mid-day snacks	491 (73.1)	611 (81.1)	1.58 (1.23-2.03)	13.21	<0.001
Taking protein-rich food	576 (85.7)	650 (86.3)	1.05 (0.77-1.41)	0.10	0.760
Taking milk and milk products	29 (4.3)	30 (4.0)	0.92 (0.54-1.55)	0.09	0.428
Taking green leafy vegetables	0	0	0	0	
Taking other vegetables	243 (36.2)	252 (33.5)	0.88 (0.71-1.10)	1.13	0.290
Taking fruits	21 (3.1)	14 (1.9)	0.58 (0.29-1.16)	2.37	0.127
Skipping meals	208 (31.0)	180 (23.9)	1.42 (1.12-1.80)	8.90	0.003
Eating junk foods	551 (82.0)	646 (85.8)	0.75 (0.56-1.50)	3.80	0.060
Eating from street shops	368 (54.8)	482 (64.0)	0.68 (0.55-0.84)	12.62	<0.001

OR: Odds ratio

groups. The mean scores of early, middle, and late adolescents were 4.3 ± 1.27, 4.2 ± 1.23, and 4.6 ± 1.02, respectively. Late adolescents had a better nutritional score compared to early and middle adolescents. The difference was found to be statistically significant ($P = 0.006$).

Discussion

Our study shows that undernutrition or thinness among the rural school-going adolescents was a common problem (23.2%). Furthermore, 8% were overweight or obese and hence over a quarter of them need dietary and other interventions. The percentage of undernutrition and overweight was less in our study compared to a study done in Karnataka by Benazeera., where among school adolescents, 34% were belonging to the category of underweight, 21.33% were obese, and 10.67% were overweight.[12]

We also found that underweight was more common among boys and overweight and obesity were more among girls and hence targeted interventions for girls and boys are needed that address factors that could be different. This means further studies are needed to find out the reasons for the difference observed. A study done in Bangalore by Abraham and Anand showed similar findings among adolescent schoolchildren.[13] The study by Venkaiah *et al*. among rural adolescents in nine states also showed that underweight was more among boys than girls.[14]

Regarding eating habits, our study showed that the habit of taking green leafy vegetables daily was not there in any of the adolescents. Furthermore, the habit of taking fruits (2.5%) and milk and milk products (4.1%) was very poor. These habits were poor even in the normal BMI category. Thus, traditional school health programs that use anthropometry alone would not help identify vitamin and micronutrient deficiency unless anthropometry is combined with clinical screening for vitamin and micronutrient deficiency diseases and their contributing factors by doing a diet survey. A study done by Rathi *et al*. among adolescents in Kolkata also showed that 45% did not consume any fruit, 30% did not take vegetables, and 36% did not take milk and milk products. Our study showed that consumption was very less percentage maybe because it assessed daily habits, while the study in Kolkata showed the consumption based on the last 2 days.[5]

In our study, the eating habits of adolescents were found to have significant association with their nutritional status, except for habit of eating junk foods. Our study showed that habits like not consuming cereals for three main meals, not taking mid-day snacks, protein-rich food, and skipping meals were significantly associated with the underweight. In the overweight/obese category, factors such as milk and milk products, eating from street shops, and junk food were high, though junk food did not show any statistical significance. Similar findings were seen in a study by Amin *et al*. which showed that frequent consumption of sweets/candies and milk and milk products are associated with obesity.[15] However, even habits of eating vegetables and fruits were also high in the overweight/obese category. Since the study did not take into consideration the quantity of consumption and exact fruits and vegetables consumed, we could not comment on this. A study by Bhattacharjee *et al*. among adolescents showed that BMI correlated significantly with the dietary habits.[16] Another study by *et al*. showed that eating habits were a better predictor of BMI, especially among boys.[17]

In our study, association of gender with eating habits showed that habits such as consumption of cereals, having mid-day snacks, skipping meals, and eating from street shops showed significant differences. Habit of consuming cereals and having mid-day snacks were better among girls. A study by Rathi *et al*. also showed that females had better consumption of cereals.[5] However, higher percentage of girls had the habit of eating from street shops. This was again similar to a study which showed that significantly more mid-adolescent girls purchased from tuck shops than boys.[18] The habit of skipping meals was more common among boys than girls. Similarly, a study among Turkish Adolescents shows that one-third of the boys skip meals, while only one-sixth of the girls skip meals.[19] Furthermore, a systematic review done among young adults showed that men tend to skip breakfast more often than females, while skipping lunch and dinner was more common among females.[20]

Our study also showed that late adolescents had better eating habits as demonstrated by their higher nutritional scores compared to early and middle adolescents. The nutrition scores are least in the middle adolescents group. This might be because in early adolescents still parental influence is more as shown in a study which shows parental influences can influence the diet of the child.[21] In the late adolescence, the better scores might be because the adolescents become more aware about healthy eating, as shown in certain studies that late adolescents have better knowledge than the younger ones.[22] However, further studies are needed to establish the factors influencing their diet.

Limitations and strengths

Our study has several strengths; we have studied a large number of children in the study sample. A simple and effective questionnaire was used for identifying the gaps in the eating habits based on the standard ICMR/NIN recommendations for a balanced diet. In addition, standard methods like the WHO-developed BMI age charts were used to assess the nutritional status.

However, there were certain limitations, eating habits were assessed by self-reported data and quantitative measuring of the intake of the recommended portions of food groups was not done. We could not exclude the possibility of confounding effects by unmeasured variables such as physical activity and other comorbidities that influence the BMI. Furthermore, data did not include social factors known to affect BMI such as socioeconomic status, number of family members, and education of the mother. Although our study identified low

intake of green leafy vegetables and fruits, we had not planned to look for clinical manifestations of vitamin and micronutrient deficiencies and then look for correlation between the two. Also, since we have found that underweight was more common among boys and overweight and obesity were more among girls and hence further studies are needed to find out the reasons for the difference observed.

Notwithstanding the above limitations, our study findings show that there is scope for doing further in-depth studies to prove the preliminary findings of this study.

Conclusion

The study shows that it is possible to make a Nutritional Education Diagnosis by identifying the gaps in eating habits compared to the recommended one for school-going adolescents. This can then be used to plan tailor-made educational and nutritional programs for improving their eating habits. Using the rapid screening method used in the study, it would be relatively easy to conduct this exercise on a sample of schools in a district to capture gaps in diet at district level for conducting health education and awareness programs on nutrition for adolescents in schools at the district and using similar methods to scale it up for state-level as possibly a national-level initiative to extend school health nutritional interventions to adolescent groups, as their eating habits are different and have long-term nutritional and health implications for the population beyond adolescence.

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Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. WHO Health topics on Maternal, Child and Adolescent. Geneva: World Health Organization. Available from: http://www.who.int/maternal_child_adolescent/topics/adolescence/dev/en/. [Last accessed on 2016 Dec 09].
2. Wennberg M, Gustafsson PE, Wennberg P, Hammarström A. Poor breakfast habits in adolescence predict the metabolic syndrome in adulthood. Public Health Nutr 2015;18:122-9.
3. Shaikh NI, Patil SS, Halli S, Ramakrishnan U, Cunningham SA. Going global: Indian adolescents' eating patterns. Public Health Nutr 2016;19:2799-807.
4. Joseph N, Nelliyanil M, Rai S, Raghavendra Babu YP, Kotian SM, Ghosh T, *et al*. Fast food consumption pattern and its association with overweight among high school boys in Mangalore city of Southern India. J Clin Diagn Res 2015;9:LC13-7.
5. Rathi N, Riddell L, Worsley A. Food consumption patterns of adolescents aged 14-16 years in Kolkata, India. Nutr J 2017;16:50.
6. World Health Organization. WHO Regional office for Europe. Fact Sheet, 15 March, 2016. Available from: http://www.euro.who.int/__data/assets/pdf_file/0006/303477/HBSC-No.7_factsheet_Diet.pdf?ua=1. [Last accessed on 2016 Dec 09].
7. Kumar S, Ray S, Roy D, Ganguly K, Dutta S, Mahapatra T, *et al.* Exercise and eating habits among urban adolescents: A cross-sectional study in Kolkata, India. BMC Public Health 2017;17:468.
8. Sivagurunathan C, Umadevi R, Rama R, Gopalakrishnan S. Adolescent health: Present status and its related programmes in India. Are we in the right direction? J Clin Diagn Res 2015;9:LE01-6.
9. World Health Organization. WHO health topics on growth reference. Geneva: World Health Organization; 2007. Available from: http://www.who.int/growthref/who2007_bmi_for_age/ en/. [Last accessed on 2016 Dec 09].
10. National Institute of Nutrition. Dietary guidelines for Indians – A Manual. Hyderabad. National Institute of Nutrition. Available from: http://ninindia.org/DietaryguidelinesforIndians-Finaldraft.pdf. [Last accessed on 2014 Aug 12].
11. Chacko TV, Ganesan S. A tool for quickly identifying gaps in diet of school children for nutritional educational interventions. Indian J Public Health 2018;62:146-9.
12. Benazeera UJ. Association between eating habits and body mass index (BMI) of adolescents. Int J Med Sci Public Health 2014;3:9403.
13. Abraham RR, Anand AK. A study on nutritional status and its determinants in adolescents aged 11 to 17 years, from selected schools in Bangalore, India. Natl J Res Community Med 2017;6:2136.
14. Venkaiah K, Damayanti K, Nayak MU, Vijayaraghavan K. Diet and nutritional status of rural adolescents in India. Eur J Clin Nutr 2002;56:1119-25.
15. Amin TT, Al-Sultan AI, Ali A. Overweight and obesity and their association with dietary habits, and sociodemographic characteristics among male primary school children in alhassa, kingdom of Saudi Arabia. Indian J Community Med 2008;33:172-81.
16. Bhattacharjee P, Mukhopadhyay S, Joshi P, Singh S. Food habits and obesity: Astudy in adolescents. Int J Contemp Pediatrics 2017;4:336-40.
17. Djordjevic-Nikic M, Dopsaj M. Characteristics of eating habits and physical activity in relation to body mass index among adolescents. J Am Coll Nutr 2013;32:224-33.
18. Sedibe MH, Pisa PT, Feeley AB, Pedro TM, Kahn K, Norris SA. Dietary habits and eating practices and their association with overweight and obesity in rural and urban black South African adolescents. Nutrients 2018;10. pii: E145.
19. Soyer MT, Ergin I, Gursoy ST. Effects of social determinants on food choice and skipping meals among Turkish adolescents. Asia Pac J Clin Nutr 2008;17:208-15.
20. Pendergast FJ, Livingstone KM, Worsley A, McNaughton SA. Correlates of meal skipping in young adults: A systematic review. Int J Behav Nutr Phys Act 2016;13:125.
21. Reicks M, Banna J, Cluskey M, Gunther C, Hongu N, Richards R, *et al*. Influence of parenting practices on eating behaviors of early adolescents during independent eating occasions: Implications for obesity prevention. Nutrients 2015;7:8783-801.
22. Naeeni MM, Jafari S, Fouladgar M, Heidari K, Farajzadegan Z, Fakhri M, *et al*. Nutritional knowledge, practice, and dietary habits among school children and adolescents. Int J Prev Med 2014;5:S171-8.

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Potential Pitfalls in Wastewater Phosphorus Analysis and How to Avoid Them

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Abstract

Due to the increasing adoption of nutrient discharge regulations, many research groups are stepping into new territory with phosphorus (P) measurements. Accurate reporting of P concentrations in effluent from novel wastewater treatment technologies is critical for protecting both environmental and human health. Analysis of P in wastewater is prone to pitfalls because of the (1) variety of chemical forms of P in wastewater (orthophosphate, condensed P, and organic P), (2) availability of different chemical assays for measuring different P forms, and (3) different conventions in the units for reporting P. Here, we present a case study highlighting how these pitfalls affect analysis and interpretation of P measurements. We show that, when used appropriately, commercially-available kits are indeed accurate tools for evaluating reactive P and total P concentrations. For both standard solutions and real wastewater, we systematically remove steps from the total P protocol to show how protocol deviations affect the results. While standard solutions are important for validating analytical methods, commercially-available wastewater standard solutions only contain P as orthophosphate (reactive P).

We therefore demonstrate options for making a mixed-P standard solution containing acid-hydrolyzable and/or organic P compounds that can be used to validate both reactive P and total P assays.

Keywords: Wastewater, nutrient pollution, standard solutions, onsite treatment, phosphorus, phosphate, non-sewered sanitation

Introduction

An intense area of research is non-sewered sanitation solutions for rural areas and low- and middle-income countries[1-14] to address the urgent need for safely-managed sanitation to improve human health and the environment.[15,16] In addition to removing pathogens and organic compounds, a key requirement of wastewater treatment is removal of nutrients including nitrogen and phosphorus (N and P) due to their potential toxic effects on aquatic environments.[17,18] The concern with P is that phosphates are limiting nutrients in freshwater aquatic environments; an overabundance can lead to decreased dissolved oxygen, accelerated eutrophication, and harmful algal blooms.[19] These algal blooms can also pose a human health risk through the production of cyanotoxins.[20] Additionally, P is a limited, valuable natural resource that should be recovered and reused.[21,22]

Nutrient discharges from inadequate onsite wastewater treatment systems are a growing concern globally and likely underreported.[16,23] Affordable and sustainable methods for effective N and P removal in decentralized treatment systems remain technologically challenging.[24,25] Recently, an international standard ISO 30500 was specifically developed to provide performance requirements for non-sewered sanitation systems[26] and sets a minimum threshold reduction of 80% for total P. As P regulations are increasingly enforced, wastewater P content will need to be regularly measured and reported as new technologies are pilot-tested.[2,14,27,28] Standardized P reporting is required to compare performance across different technologies and wastewater streams to accelerate technology adoption, yet a critical gap exists due to lack of a common set of metrics.[29] This knowledge gap in reporting metrics for P and its different chemical species is also highlighted in a recent systematic review of nutrient removal and recovery technologies by Kogler et al.[30] In reviewing 292 articles across 46 sources, Kogler et al.[30] found that removal efficiency and achievable effluent concentrations for P removal and recovery technologies are generally underreported, with only 16% of the articles reviewed giving adequate quantitative information for meaningful interpretation of technology performance.

As we embarked on the journey of adding total P to our routine testing regimen, we identified several specific points of confusion. We determined that measurement and reporting of P requires particular attention because of the (1) variety of chemical forms of P in wastewater, (2) availability of several different chemical assays for measuring different forms of P, and (3) different conventions in the units used for reporting P. Due to the interrelated nature of these topics, there is potential for inconsistencies in reporting results for P. Here, we present a case study highlighting the importance of accurate and clear reporting of P units and strict adherence to chemical assay protocols for measuring P in wastewater samples. The objective of this paper is to share our experiences in identifying and overcoming these P-related pitfalls so that others may avoid these issues in their future work. The aims of this paper include (1) determining the root cause(s) of discrepancies in P results and (2) developing new standard solution mixtures to ensure accurate analytical method implementation and data reporting.

We begin with a brief review of the forms of P in wastewater and typical units used for reporting P. We then describe our recent experiences where we identified discrepancies in P concentrations we measured using a commercially-available test kit and those measured by a third-party lab for the same

samples. Due to the transparent and productive relationship with the third party lab, we were able to identify two distinct sources of confusion as the likely reason for these discrepancies: (1) the factor of 3.066 difference in results reported as mg L^{-1} "PO_4-P" versus mg L^{-1} "PO_4^{3-}"; and (2) the omission of the digestion heating step in the total P protocol. By systematically omitting steps from the total P chemical assay procedure, we show that skipping the digestion heating step results in a significant undercounting of total P, both for standard solutions and for real wastewater samples.

To avoid these issues in future studies, regularly testing known P standard solutions to validate analytical methods and reporting methods is needed. However, we observed that the common commercially-available wastewater standard solutions only contain P as orthophosphate (reactive P). This is perhaps due to the fact that soluble orthophosphate is the majority P form in effluents from traditional wastewater treatment systems and therefore also the P form of primary concern for anthropogenic nutrient pollution.[31] However, our analysis reveals that the assumption that orthophosphate = total P does not necessarily hold for wastewater influent of novel onsite wastewater treatment technologies. We therefore believe that including acid-hydrolyzable P and/or organic P components in known standard solutions is especially important to ensure accurate reporting of novel wastewater treatment technology performance. To this end, we have prepared and validated our own standard mixtures of orthophosphate, acid-hydrolyzable P, and organic P using readily available and inexpensive chemical reagents.

Methods

Unless otherwise indicated, all glass wares used in this study were cleaned by soaking in 1:1 HCl:water (v:v), rinsing thoroughly in distilled or ultrapure water, then filling with fresh distilled or ultrapure water and storing until needed. Mean and standard deviation values for replicate measurements were calculated using Excel. Data were plotted using OriginPro 2018.

Standard solutions

A mixed-parameter quality control standard solution for wastewater was purchased (Hach, product LCA720) that features 10.0 mg L^{-1} PO_4-P in addition to ammonia, nitrate, COD, and TOC. A wastewater influent inorganics quality control standard solution was also purchased (Hach, product #2833149) that contains 10 mg L^{-1} PO_4^{3-} in addition to ammonia, nitrate, COD, sulfate, and TOC. (See Supplemental Material for details.)

The other standard solutions used to validate assay performance were prepared in-house. Phosphate stock standard solutions (PO_4^{3-} = 10 and 25 mg L^{-1}, labelled P 10 and P 25, respectively) were prepared by dissolving potassium dihydrogen phosphate (KH_2PO_4) in distilled water. Mixed standard solutions were prepared using KH_2PO_4, sodium trimetaphosphate ($Na_3P_3O_9$), and adenosine 5'-triphosphate, disodium salt (ATP), all purchased from Sigma-Aldrich (see Supplemental Material for additional reagent information).

Real wastewater samples

Effluent from a toilet was collected to serve as wastewater samples for evaluation. A women's shared toilet facility at a privately-owned textile mill in Coimbatore, India (described in a previous study) was the sample collection site.[13] The facility is used by 20 to 50 employees and features five toilet stalls equipped with Indian squat plates, each connected to cistern flush. The effluent of one of the toilets can be easily tapped into from the pipe and was diverted from its septic tank to a 200 L collection tank. After collecting all toilet effluent (containing flush water, urine, and feces) from 8 am to 5 pm local

time, the wastewater was left to settle overnight. The supernatant was collected the morning after and then the contents of the tank were homogenized by with a macerator pump (Jabsco Model: 18950, 45 lpm). Both supernatant and macerated wastewater samples were analyzed with the commercially-available test kit on the same day.

Commercially-available test kits (ascorbic acid method)

Total P (method 8190) and reactive P (method 8048) test kits were purchased from Hach and used with a Hach DR 900 colorimeter. Unless otherwise indicated, the test kits were used exactly as per the manufacturer's instructions (see Supplemental Material for more detail). Both test kits use the ascorbic acid method, are USEPA accepted for reporting wastewater analyses, and the procedures follow USEPA and Standard Method 4500-P.[32] Distilled (in India) or ultrapure 18.2 MΩ cm (in the US) water was used for dilution as necessary to bring the samples within the assay detection range. The total P test kit includes two powder pillows (potassium persulfate, PhosVer 3), a bottle of sodium hydroxide 1.54 N, and glass reaction vials containing a pre-measured sulfuric acid solution. A Hach DRB 200 digester heating block was used for the digestion step in the total P protocol. The reactive P test kit includes one powder pillow (PhosVer3) and either glass reaction vials containing a pre-measured volume of deionized water or a reusable glass sample cell (see Supplemental Material).

Third-party laboratory measurement (stannous chloride method)

Samples sent to a NABL certified laboratory (T.STANES Coimbatore) were analyzed using the Indian Standard IS:3025 part 31[33] stannous chloride method for the measurement of total P, a method essentially similar to Standard Method 4500-P.[32] According to the IS:3025 part 31 stannous chloride method, samples are decolorized by shaking with activated carbon for 5 minutes, then filtered. A phenolphthalein indicator is added to the filtered sample, and if a pink color develops, the pink color is discharged through the addition of up to 5 drops of a strong acid solution. Finally, ammonium molybdate and stannous chloride reagents are added and allowed to react for 10 minutes. The sample is then quantified spectrophotometrically at 690 nm. Fresh standard solutions were prepared by the third-party lab to calibrate their instrument upon receipt of each set of samples. Distilled water was used for sample dilution when necessary to bring the sample within the assay detection range. Analytical results from the third-party lab were reported to us in units of "Phosphorus (mg/L)."

Results and Discussion

Forms of P relevant for wastewater analysis

In aqueous solutions relevant for wastewater treatment technologies, P exists in three main forms, commonly referred to as orthophosphate, condensed phosphate, and organic phosphate (Table 1).[32] Phosphate anions can be detected colorimetrically, and chemical assays are commercially available for measuring only orthophosphate (sometimes called "reactive P"), "acid-hydrolyzable P" (orthophosphate + condensed phosphate), or "total P" (orthophosphate + condensed phosphate + organic phosphate). The specific chemical reactions responsible for the colorimetric responses have been described in detail elsewhere[32] and should be included with each commercially-available assay. Briefly, orthophosphate anions form a colored complex with a transition metal under the chemical assay conditions.

Table 1.

Common assays, with naming and unit conventions, for forms of P in wastewater analysis.[32]

Commercially-available chemical assays	"Orthophosphate" or "reactive P"	"Acid-hydrolyzable P"	"Total P" or "total phosphate"
Forms of P detected	Orthophosphate	Orthophosphate + condensed phosphate	Orthophosphate + condensed phosphate + organic phosphate
Digestion required?	No	Yes	Yes
Strong oxidant required?	No	No	Yes
Typical reported units (mg L^{-1})	PO_4^{3-} or PO_4-P	PO_4^{3-} or PO_4-P	PO_4-P or P

The color response for all the different assays is due to detection of orthophosphate. Other forms of P (condensed phosphate and organic phosphate) must first be converted to orthophosphate before they can be detected. Condensed phosphates require a preliminary acid hydrolysis step at elevated temperatures before the colorimetric response can be observed. Some organic phosphates also require addition of a strong oxidant to the acid hydrolysis step. Orthophosphate and acid-hydrolyzable P are typically reported using units of either mg L^{-1} "PO_4^{3-}" (orthophosphate) or "PO_4-P" (orthophosphate as P). Total P is typically reported as mg L^{-1} "P."

Potential Pitfall #1: Distinguishing and converting between the different units used for P reporting

Note that the "typical" reporting units in Table 1 are just a guideline. In our experience, these do not appear to be hard rules, and all three units are used for reporting all types of phosphorus data. *The units alone will not tell you which forms of phosphorus have been measured.* It is therefore important to understand what the different units mean and how they are interrelated. Some standards, such as the ISO 30500, are written as percent reduction requirements; in this case, the units in which P quantities are reported is not particularly important as long as it is internally consistent within any given study. However, P units must still be accurately and clearly reported in order to compare values across different studies and to nominal values for other discharge standards. Briefly, units of "PO_4-P" and "P" are equivalent and therefore interchangeable. "PO_4^{3-}" units are calculated differently and a mathematical conversion must be performed to compare to the other units.

Converting between values reported in the three different units requires accounting for the mass of atoms which are counted in each unit. Units of PO_4-P and P only count the 1 P atom (30.97 amu). Therefore, values reported in mg L^{-1} PO_4-P will be identical to values reported in mg L^{-1} P and no conversion is required. In contrast, units reported in PO_4^{3-} count all atoms in the phosphate anion—1 P atom (30.97 amu) and 4 O atoms (each 16.00 amu). Values reported in mg L^{-1} PO_4^{3-} can be converted to P or PO_4-P by dividing by 3.066, which is the stoichiometric ratio of the mass of the phosphate anion (1P + 4O = 30.97 + 4(16.00) = 94.97 amu) to that of a single P atom (30.97 amu). Similarly, values reported in units of P or PO_4-P can be converted to units of PO_4^{3-} by multiplying by 3.066:

$$1.000\ \text{mg L}^{-1}\text{P} = 1.000\ \text{mg L}^{-1}\ \text{PO}_4\text{–}P = 3.066\ \text{mg L}^{-1}\ \text{PO}_4^{3-} \quad \text{Eq. (1)}$$

As an example, consider the two wastewater (WW) quality control standard solutions, both purchased from Hach for use in this study. The first WW standard solution (1) is listed by the supplier as containing "10.0 mg L^{-1} PO_4-P," while the other (2) is listed as containing "10 mg L^{-1} PO_4^{3-}." It is an easy mistake at first glance to think that the phosphate concentrations of these solutions are equivalent. However, when we convert WW standard solution (1) to PO_4^{3-} units, we see that it actually contains 3 times as much phosphate: (10.0 × 3.066) = 30.7 mg L^{-1} PO_4^{3-}. To directly compare these wastewater standard solutions, we will hereafter label them as (1) WW 30 and (2) WW 10 to reflect the expected phosphate concentration in PO_4^{3-} units.

We use a commercial test kit for total P (Hach 8190) for our in-house field testing, because this field-friendly method utilizes only two small, easily-transportable pieces of equipment (the DRB 200 digester and the DR 900 colorimeter) and pre-portioned reagents in pre-measured vials and sealed "powder pillow" packets. The results for total P are displayed by the DR 900 colorimeter in units of "mg L^{-1} PO_4^{3-}." We used the WW 30 standard solution described above to validate the total P test kit, as recommended by the manufacturer. Results were obtained for samples collected in glass bottles cleaned with or without a HCl acid wash. (The acid wash is used to eliminate possible contamination from the detergent used to clean bottles, which may contain phosphate.) Figure 1 shows that all results obtained were within 15% of the expected value (n = 4; mean = 29.6 ± 4.2 mg L^{-1} PO_4^{3-}), however the variability in the acid-washed results was lower, within 5% of the expected value (n = 3; mean = 30.6 ± 1.5 mg L^{-1} PO_4^{3-}). We therefore used acid-washed containers for all subsequent measurements, including with samples sent for analysis to the third-party lab.

Open in a separate window

Figure 1.

(A) Total P test results (measured by Hach kit) for the commercially-available standard solution WW 30 and in-house prepared phosphate solutions (P 25 and P 10). Each standard solution was tested multiple times; the results of individual tests are shown as open symbols, with the mean for each column shown as a short dashed line and the expected values shown as solid horizontal reference lines. The variability in test results is highest for WW 30 measured without acid washing of glassware (diamonds, n = 4, 29.6 ± 4.2 mg L^{-1} PO_4^{3-}). The variability is similar for all three standard solutions when glassware is acid washed prior to contact with the samples (circles; WW 30, n = 3, 30.6 ± 1.5; P 25, n = 6, 25.3 ± 1.0; P 10, n = 6, 10.8 ± 1.1). Position along the x-axis is not meaningful—data points within each column are offset from one another for clarity. (B) Total P measured for identical samples tested in-house with the Hach test kit and by the third-party lab. Values for units of PO_4^{3-} are shown on the left y-axis and values for units of PO_4-P are shown on the right y-axis. Expected values are shown as solid horizontal reference lines. Values reported by the third-party lab (in PO_4-P units) are similar to those measured in-house (in PO_4^{3-} units) once the correct unit conversion is applied.

In addition to the WW 30 standard solution, we evaluated phosphate solutions prepared in-house of known concentrations (PO_4^{3-} = 10, 25 mg L^{-1}, labelled P 10 and P 25, respectively) obtained by dissolving KH_2PO_4 in distilled water. Again, these solutions gave results close to the expected values (P 10, n = 6, mean = 10.8 ± 1.1 mg L^{-1} PO_4^{3-}; P 25, n = 6, mean = 25.3 ± 1.0 mg L^{-1} PO_4^{3-}). These results confirm that solutions of KH_2PO_4 can effectively serve as standards for P in orthophosphate form, and that acid-washing of sample glassware reduces test-to-test variability in the results.

Initial reports showed different units for P analysis performed by a third-party lab, leading to a discrepancy which required further validation. Our third-party collaborator, a certified laboratory located in the same city, uses the stannous chloride method for total P measurement. This third-party lab requires a large volume (>150 mL) of sample to run their assay, thus we diluted by 5× the WW 30 standard solution prior to delivering it. We also sent for analysis a sample of the P 25 solution.

The third-party lab reported values of 10.25 mg L^{-1} for the WW 30 sample and 7.84 mg L^{-1} for the P 25 solution, both reported with units of "Phosphorus (mg/L)." Initially, we were surprised to find that the third-party lab results appeared much lower than the values we expected (30.7 and 25.1 mg L^{-1}, respectively) for the WW 30 and P 25 samples. After clarifying discussions with the third-party lab, we determined that their results were reported in "PO_4-P" units. Thus, the expected values would be 10.0 and 8.15 mg L^{-1} for units of PO_4-P, respectively, for these two samples. In Figure 1B, the results are shown for both units of PO_4^{3-} (on the left axis) and PO_4-P (on the right axis). It is clearly seen that by resolving the ambiguity of the reported units, the total P values were measured by the third-party lab within a few percent from the expected values.

Potential Pitfall #2: Omission of steps from the total P test protocols

Despite reconciling the difference in reported units between our in-house total P test results and those from the third-party lab, we noticed that there was still a discrepancy between results from the two labs when real wastewater samples were analyzed. Total P results from the third-party lab were always lower than those measured in-house, although not always lower by a consistent nominal amount or percentage. Importantly, we learned through fruitful discussions with the third-party lab that while a digestion (heating) pre-treatment step is listed in the IS:3025 protocol, digestion was not actually performed. This indicates that while the third-party lab results were accurate in determining total P content from the standard solutions (where only orthophosphate was present), their procedure will under-report the total P content for samples where condensed and organic phosphates are present (e.g., real wastewater samples from field testing evaluation of onsite treatment technologies).

The full protocol for Hach total P measurement includes a digestion step performed by heating the sample in acid at 105 °C for 30 minutes with a persulfate ($S_2O_8^{2-}$) reagent added to the solution. Heating in acid converts any acid-hydrolyzable P to orthophosphate. The addition of the strong oxidant $S_2O_8^{2-}$ ensures that organic phosphates are fully converted to orthophosphate. Figure 2 shows in-house results for two raw wastewater samples where steps from the total P protocol were systematically and intentionally omitted. Samples A and B were collected on different days from the same shared toilet effluent, and the liquid supernatant as well as a macerated sample containing higher fecal load were analyzed. The primary soluble form of P in wastewater is orthophosphate, therefore orthophosphate is expected to dominate in the supernatant fraction, while the macerated fraction is expected to contain a higher concentration of organic and condensed P forms.

Figure 2.

Comparison of total P results obtained during systematic omission of testing steps for two raw wastewater samples A and B collected from the Coimbatore field testing site. The photograph shows the typical appearance of the supernatant and macerated sample fractions. For each sample, both the macerated fraction (closed circles) and the supernatant fraction (open circles) were measured. The macerated fraction contains more fecal matter and thus a higher total P concentration. Omitting the heating step ("-heat") from the testing protocol has the largest effect on the measured total P concentration, while omitting the persulfate reagent ("-$S_2O_8^{2-}$") has a smaller effect that is most visible for the macerated fraction. Samples were diluted with distilled water by 10× to 25× to bring them within the range of the total P Hach test kit.

Figure 2 shows that omitting the heating step has the largest effect on the total P results, both for the macerated and supernatant sample fractions. Omitting the $S_2O_8^{2-}$ reagent has a measureable, but smaller, effect. When the protocol is followed fully, the total P measured for Sample A was 45.8 mg L^{-1} PO_4^{3-} for the macerated fraction and 42.2 mg L^{-1} PO_4^{3-} for the supernatant fraction. These values drop to 35.4 and 35.6 mg L^{-1} PO_4^{3-}, respectively, when the heating step is omitted. This results in an under-reporting of total P by 23% for the macerated fraction and 16% for the supernatant. For Sample B, omitting the heating step results in under-reporting of total P by 35% for the macerated fraction and 21% for the supernatant. Omitting only the $S_2O_8^{2-}$ reagent under-reported the total P in the supernatant samples by ~5%, which is similar to the test-to-test variability for standard solutions as shown in Figure 1. In contrast, omitting only the $S_2O_8^{2-}$ reagent from the macerated fraction under-reports the total P by 8% for Sample A and 18% for Sample B.

In summary, total P content in raw wastewater samples can be substantially under-reported if any step in the digestion protocol is omitted. The largest differences in total P values were found with heating and/or persulfate omission for the macerated sample fractions. This shows that the risk of P under-reporting increases as the fecal content (and thus the amount of condensed and/or organic P) increases. This is important when testing new treatment technologies, particularly those which involve novel solid/liquid separation processes, solids-settling techniques, or other methods of solids treatment where solid fecal matter is broken down and mixed with liquid effluent.

Impact of the pitfalls

While the major contribution of water soluble P in human excreta is inorganic orthophosphate, orthophosphate may not always be the sole contributor to total P in effluent from onsite wastewater treatment technologies. Raw influent of onsite wastewater treatment technologies is highly variable and impacted by many factors such as number of toilet uses, flush volume, and diet.[34,35] We suspect that concentrations of condensed and/or organic P may be significant in influent and in effluent from some technologies with novel solid/liquid separation or solids processing methods.

Our group has previously evaluated one such novel system. We reported in a previous publication total P values of system effluent of 8.3 and 21.5 "mg P L^{-1}"[5] that we now suspect were measured omitting the heating step; thereby, those values represent only the orthophosphate as opposed to the total P. In a different publication,[13] we reported values of "P," without specifying the form (which was orthophosphate) nor clarifying the reporting units (mg L^{-1} P). In another recent paper,[14] we did specify

in the methods section that both reactive P and total P were reported in units of "mg L^{-1} PO_4^{3-}." However, table columns and figure axes in that work were labelled only as "mg L^{-1}" for both reactive P and total P. For added clarity in the future, we will explicitly include the full unit "mg L^{-1} PO_4^{3-}" or "mg L^{-1} P" in all tables and figure axes, as demonstrated in the present work.

From our experiences described here, particularly those during collaboration with a third-party laboratory, we now fully appreciate how critical it is for groups working on wastewater treatment technologies to report the correct P form in clear, specific units. Our concern that inconsistencies in P reporting are widespread is further supported by a recent systematic review of the literature on nutrient removal and recovery technologies by Kogler et al.[30] In sorting reports for P removal efficiencies and effluent concentrations, three separate categories were required for reports of "TP = total phosphorus" and "PO_4 = phosphate," and "P = general phosphorus species if species was not identified clearly."[30] This review shows that there is a prevalent need to standardize quantitative performance metrics of nutrient removal and reuse technologies.

The ambiguity in reporting units introduces lingering questions of whether variable P concentrations in wastewaters across different studies is due to variability in the wastewater itself or variability in reporting and analysis methods. For example, Cid et al.[2] reported 0.64 mmol L^{-1} "PO_4^{3-} + HPO_4^{2-}" in average macerated toilet wastewater from one of their self-contained bathroom + wastewater treatment and recycling prototypes. The specific analytical method used in that work is not reported, but assuming based on the units that this represents a measurement of orthophosphate (reactive P), this would be approximately 60.8 mg L^{-1} PO_4^{3-}. This value is similar to what we report here for total P concentrations in macerated toilet wastewater, but also aligns well with our previous report of reactive P in toilet effluent.[14] Another recent study by Reynaert et al.[27] reported 36.2 mg L^{-1} average soluble phosphate "PO_4" concentration in toilet wastewater influent from a urine-diverting toilet with solids separated from the flush water. While the use of the "PO_4" unit is ambiguous, the analytical test used (Spectroquant) appears to give results in units of PO_4-P. This would indicate that the average influent concentration in this study was 36.2 mg L^{-1} PO_4-P or 111 mg L^{-1} PO_4^{3-}, and the nominal average concentration in the treated clean water tank is 25.4 mg L^{-1} PO_4-P or 77.9 mg L^{-1} PO_4^{3-},[27] which would be unable to meet water reuse or discharge standards with nominal effluent P concentration limits (eg, <1 mg L^{-1} P in India).[36]

Pitfall prevention: Preparation of mixed-P standard solutions for analytical test validation

For pitfall #1, we determined that validating test protocols and reporting units for reactive P, both in-house and with the third-party lab, can be accomplished by testing commercially-available standard solutions of orthophosphate. But what about validating methods when samples contain condensed and/or organic forms of P? Researchers should clarify the analytical methods used by explicitly stating whether samples were digested prior to analysis. For example, Chapter 4500-P in the 22nd edition of Standard Methods for the Examination of Water and Wastewater contains ten subsections detailing sample filtration pretreatments, three digestion methods, and three colorimetric methods.[32] Therefore, simply referencing "standard method 4500-P" is insufficient for determining the fraction or form of P that has been analyzed. For samples containing condensed and/or organic P, definitive confirmation that digestion has been performed requires an analytical validation step testing a standard solution of known composition. However, the wastewater standard solutions readily available for purchase only contain orthophosphate. Testing these standard solutions will not clarify whether sample digestion is performed as required to convert all P forms to orthophosphate.

We therefore set out to prepare our own in-house, mixed standard solution for validating total P testing protocols. In addition to KH_2PO_4 to provide orthophosphate, we identified two non-hazardous compounds which are readily-available and relatively inexpensive to represent condensed and organic P forms: trisodium trimetaphosphate ($Na_3P_3O_9$) and adenosine 5'-triphosphate, disodium salt (ATP). Figure 3 shows the structures of these compounds and relevant properties, such as number of PO_4^{3-} equivalents (i.e., number of PO_4^{3-} anions detected in total P test per molecule of the compound). While KH_2PO_4 will be detected in a reactive P test, $Na_3P_3O_9$ and ATP will only be detected in a total P test, as digestion is required to hydrolyze P-O and O-C bonds to release free orthophosphate into the solution.

Open in a separate window

Figure 3.

Chemical compounds used in this study to make in-house mixed-P standard solutions. KH_2PO_4 dissociates readily to give phosphate anions that are detected in reactive P tests. $Na_3P_3O_9$ and ATP each contribute 3 phosphate equivalents, but these molecules must undergo acid digestion to release phosphate anions. $Na_3P_3O_9$ and ATP are thus detected in total P tests but are not detected in reactive P tests.

Solutions were first prepared containing only KH_2PO_4, $Na_3P_3O_9$, or ATP and analyzed using total P and reactive P Hach kits (see Supplemental Material for a complete table of all solution recipes). Figure 4 shows these results by comparing samples that contain no reactive P (top; ATP only and $Na_3P_2O_9$ only) to samples with only reactive P (bottom; P 25, P 10, WW 10). For samples with no reactive P ($Na_3P_3O_9$ and ATP), the total P results match the expected values only when the heating step was included in the protocol. When heating was omitted from the protocol, both samples showed <1.5 mg L^{-1} PO_4^{3-} for the total P measurement. As expected, the reactive P test for both $Na_3P_3O_9$ and ATP measured almost zero ($Na_3P_3O_9$ = 0.34 mg L^{-1} PO_4^{3-}; ATP = 0.08 mg L^{-1} PO_4^{3-}). Standard solutions purchased (WW 10) and prepared in-house (P 25, P 10) which only contained reactive P showed no difference in the values for the total P test when steps were omitted from the protocol. As expected, the results of the reactive P test and total P test are identical for samples containing only reactive P.

Figure 4.

Demonstration of the different results obtained from total P and reactive P tests for identical samples, including tests where steps were systematically omitted from the total P protocol. Expected values are shown as horizontal reference lines (dashed lines = ATP). As expected, samples containing condensed and organic P (top, triangles) show no response to the reactive P test (shaded column). Samples containing ATP (closed symbols) and $Na_3P_3O_9$ (open symbols) are accurately measured only when the total P test has been performed according to the protocol. Eliminating the heating step ("-heat" and "-heat, -$S_2O_8^{2-}$") results in a complete failure of the total P test. Eliminating only the persulfate pouch ("-$S_2O_8^{-2}$") shows only a small effect on the total P result for ATP. In contrast, samples that only contain reactive P (bottom, circles; WW 10, shaded symbols; P 25, open symbols; P 10, closed symbols) show nearly identical results for total P and reactive P (shaded column), and omitting steps from the total P protocol imparts no effect.

We also prepared two standard solutions with mixed-P forms that could be used to validate results from reactive P or total P tests using the same solution. Figure 5 shows total P and reactive P results for a 2-part standard solution (containing KH_2PO_4 and $Na_2P_3O_9$) and a 3-part standard solution (containing KH_2PO_4, $Na_3P_3O_9$, and ATP). Both the 2-part and 3-part standard solutions give measured total P concentrations close to their expected values. When the heating step is omitted, the total P results are nearly identical to the reactive P results.

Figure 5.

Comparison of total P and reactive P (shaded column) results for two mixed standard solutions prepared in-house. The 2-part mixed standard solution (open symbols) is a mixture of KH_2PO_4 and $Na_3P_3O_9$. The 3-part mixed standard solution (closed symbols) contains KH_2PO_4, $Na_3P_3O_9$, and ATP. Expected values are shown as horizontal lines (solid lines for 2-part standard solution; dashed lines for 3-part standard solution). Measured results for both mixed standard solutions were close to the expected values. When the heating step is omitted from the total P protocol, the measured value is the same as that for the reactive P test.

The results in Figure 5 show that a 2-part standard solution of KH_2PO_4 and $Na_3P_3O_9$ is sufficient to conclude whether the digestion heating step proceeds as necessary. These chemicals are affordable and do not require any special handling or storage considerations, so a solution of these two compounds could serve as a simple, yet robust mixed-P standard solution. The ATP salt has some disadvantages compared to $Na_3P_3O_9$; it is more expensive, requires storage under refrigeration, and can have a variable water content bottle-to-bottle, making the calculation of the expected concentration slightly more complicated (see Supplemental Material).

However, there are two advantages for why some researchers may choose to include ATP in their in-house standard-solution formulation. First, as persulfate is expected to affect hydrolysis of organic P (ATP) but is not necessary for hydrolysis of condensed P ($Na_3P_3O_9$), we hypothesized that including

ATP in the mixture could validate that the persulfate reagent is included in the total P protocol. When the $S_2O_8^{2-}$ is omitted from the solution of only $Na_3P_3O_9$ or from the WW 10 standard solution (see Figure 4), the total P result does not decrease. In fact, there is a slight increase in the measured total P values (55.6 to 56.6 mg L^{-1} PO_4^{3-} for $Na_3P_3O_9$ from 9.8 to 10.0 mg L^{-1} PO_4^{3-} for WW 10), although this change is well within the test-to-test variability demonstrated in Figure 1. On the other hand, when $S_2O_8^{2-}$ is omitted from the solution of only ATP, the total P value measured decreases from 46.4 to 43.8 mg L^{-1}. Similarly, for the 3-part mixture, omitting $S_2O_8^{2-}$ gives a small decrease in total P from 50.4 to 49.4 mg L^{-1}, however, this is on the border of the test-to-test variability of 1.0 to 1.5 mg L^{-1} shown in Figure 1. If validating inclusion of persulfate is desired, increasing the ratio of ATP in the 3-part mixed standard solution may be prudent. Alternatively, a 2-part standard solution containing only KH_2PO_4 and ATP would also be suitable. The second advantage of including ATP is that it also contains N atoms (5 N equivalents per ATP molecule) and can therefore be used to validate total nitrogen digestion protocols as well. Using the Hach low range total N kit (method 10071), we measured total N concentrations of 8.9 mg L^{-1} N in the ATP only solution and 2.0 mg L^{-1} N in the 3-part standard solution, both of which are in close agreement with the expected values of 11.4 and 2.3 mg L^{-1} N, respectively.

Finally, we evaluated the stability of the in-house mixed-P standard solutions over time, with and without refrigeration of the solutions. If either the $Na_3P_3O_9$ or ATP is susceptible to degradation over time (e.g., slow hydrolysis), then the reactive P concentration of the standard solutions should increase as the samples age. Figure 6 shows reactive P values measured for $Na_3P_3O_9$, ATP, 2-part, and 3-part mixtures after ageing for several days in sealed glass bottles. Only the 3-part mixture was stored under refrigeration; the other solutions were left in the laboratory ambient (21 °C). Small increases in the reactive P concentration were observed for all three solutions stored at room temperature (0.91, 1.98, and 1.40 mg L^{-1} PO_4^{3-} for ATP, $Na_3P_3O_9$, and the 2-part mixture, respectively) for 6 to 11 days. The 3-part mixture stored for 13 days at 4 °C increased by only 0.2 mg L^{-1} PO_4^{3-}. All of these values are within the test-to-test variability of 1.0 to 1.5 mg L^{-1} PO_4^{3-}, except for the $Na_3P_3O_9$ solution, which is only slightly higher.

Figure 6.

Comparison of reactive P results for freshly prepared samples (open symbols) and the same samples after ageing for several days (closed symbols) under laboratory conditions. Samples containing ATP and/or $Na_3P_3O_9$ show very small increases in reactive P when stored at 20 °C for 6 to 11 days. The 3-part standard solution showed no increase in reactive P after 13 days when stored at 4 °C.

In summary, solutions prepared in-house with $Na_3P_3O_9$ and/or ATP are sufficiently stable for several days without refrigeration to serve as standards for validation of total P protocols. These compounds show minimal degradation once dissolved in water and stored at room temperature. The mixed standard solutions can be used in-house or sent to third-party laboratories to confirm that the total P sample digestion protocol is followed correctly.

Conclusions

Accurate and clear reporting of P in effluent from novel wastewater treatment technologies is critical for preventing eutrophication of receiving water bodies and in protecting human health. New research into methods to mitigate nutrient discharge in effluent from non-sewered sanitation systems and the adoption of nutrient discharge standards has many research groups stepping into new territory with phosphorus measurements. We have shown that commercially-available kits are accurate tools for evaluating reactive P and total P concentrations in standard solutions, and results were consistent across the two testing methods used in-house and at third-party lab once the correct unit conversion was applied. We also described options for making a standard solution containing condensed and/or organic P compounds that can be used to validate the digestion step in total P assays. We hope that the mixed-P standard solution recipes will be useful for other research groups, as commercially-available wastewater standard solutions only contain orthophosphate.

The work described here leads us to give recommendations for best practices going forward:

1) Regularly include P standard solutions of known concentration in P analyses. We advise that both an orthophosphate and organic phosphate standard solution (or a mixture) be used to validate which P assay is being used, determine the presence of a digestion step, and confirm the reported units.

2) Clearly state the fraction of phosphorus measured—reactive P, acid-hydrolyzable P, or total P—when reporting phosphorus results. Neither the units for reporting P nor a reference to "standard method 4500-P" are sufficient to convey this important information.

3) Discontinue use of the unit "PO_4-P" in reporting results, and instead only use either PO_4^{3-} or P. We think this may be the root of much confusion in the field, as (1) "PO_4-P" and "P" units are interchangeable and therefore redundant, (2) "PO_4-P" and "PO_4^{3-}" are *not* interchangeable, yet look quite similar, and (3) "PO_4-P" is not a chemically meaningful notation (see Supplemental Material for further discussion).

We hope that these recommendations will be useful and widely adopted by groups (including ours) who are adding or continuing with total P analysis as part of their routine testing.

Supplemental Material

sj-pdf-1-ehi-10.1177_11786302211019218 – Supplemental material for Potential Pitfalls in Wastewater Phosphorus Analysis and How to Avoid Them:

Click here for additional data file. (573K, pdf)

Supplemental material, sj-pdf-1-ehi-10.1177_11786302211019218 for Potential Pitfalls in Wastewater Phosphorus Analysis and How to Avoid Them by Praveen Rosario, Ramya Viswash, Thamayanthi Seenivasan, Sudha Ramalingam, Katelyn L Sellgren, Sonia Grego and Lena Trotochaud in Environmental Health Insights

Footnotes

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Supplemental Material: Supplemental material for this article is available online. Supplemental tables, details of Hach kit test procedures, example soitchiometric calculations, discussion of chemically meaningful unit notations, and underlying data.

References

1. Cho K, Qu Y, Kwon D, et al.. Effects of anodic potential and chloride ion on overall reactivity in electrochemical reactors designed for solar-powered wastewater treatment. *Environ Sci Technol*. 2014;48:2377-2384. [PubMed] [Google Scholar]

2. Cid CA, Qu Y, Hoffmann MR. Design and preliminary implementation of onsite electrochemical wastewater treatment and recycling toilets for the developing world. *Environ Sci*. Water Res. Technol. 2018;4:1439-1450. [PMC free article] [PubMed] [Google Scholar]

3. Cid CA, Stinchcombe A, Ieropoulos I, Hoffmann MR. Urine microbial fuel cells in a semi-controlled environment for onsite urine pre-treatment and electricity production. *J Power Sources*. 2018;400:441-448. [PMC free article] [PubMed] [Google Scholar]

4. Huang X, Qu Y, Cid CA, et al.. Electrochemical disinfection of toilet wastewater using wastewater electrolysis cell. *Water Res*. 2016;92:164-172. [PMC free article] [PubMed] [Google Scholar]

5. Varigala SK, Hegarty-Craver M, Krishnaswamy S, et al.. Field testing of an onsite sanitation system on apartment building blackwater using biological treatment and electrochemical disinfection. *Environ Sci*. Water Res. Technol. 2020;6:1400-1411. [Google Scholar]

6. Künzle R, Pronk W, Morgenroth E, Larsen TA. An energy-efficient membrane bioreactor for on-site treatment and recovery of wastewater. *J Water Sanit Hyg Dev*. 2015;5:448-455. [Google Scholar]

7. Hanak DP, Kolios AJ, Onabanjo T, et al.. Conceptual energy and water recovery system for self-sustained nano membrane toilet. *Energy Convers Manag*. 2016;126:352-361. [PMC free article] [PubMed] [Google Scholar]

8. Tobias R, O'Keefe M, Künzle R, et al.. Early testing of new sanitation technology for urban slums: The case of the Blue Diversion Toilet. *Sci Total Environ*. 2017;576:264-272. [PMC free article] [PubMed] [Google Scholar]

9. Kamranvand F, Davey CJ, Sakar H, et al.. Impact of fouling, cleaning and faecal contamination on the separation of water from urine using thermally driven membrane separation. *Sep Sci Technol*. 2018;53:1372-1382. [PMC free article] [PubMed] [Google Scholar]

10. Rogers TW, Rogers TS, Stoner MH, et al.. A granular activated carbon/electrochemical hybrid system for onsite treatment and reuse of blackwater. *Water Res*. 2018;144:553-560. [PMC free article] [PubMed] [Google Scholar]

11. Ziemba C, Larive O, Reynaert E, Huisman T, Morgenroth E. Linking transformations of organic carbon to post-treatment performance in a biological water recycling system. *Sci Total Environ*. 2020;721:137489. [PMC free article] [PubMed] [Google Scholar]

12. Sahondo T, Hennessy S, Sindall RC, et al.. Field testing of a household-scale onsite blackwater treatment system in South Africa. *Sci Total Environ*. 2020;703:135469. [PMC free article] [PubMed] [Google Scholar]

13. Welling CM, Sasidaran S, Kachoria P, et al.. Field testing of a household-scale onsite blackwater treatment system in Coimbatore, India. *Sci Total Environ*. 2020;713:136706. [PMC free article] [PubMed] [Google Scholar]

14. Trotochaud L, Andrus RM, Tyson KJ, et al.. Laboratory demonstration and preliminary techno-economic analysis of an onsite wastewater treatment system. *Environ Sci Technol*. 2020;54:16147-16155. [PMC free article] [PubMed] [Google Scholar]

15. United National Children's Fund (UNICEF), World Health Organization. *Progress on Household Drinking Water, Sanitation and Hygiene 2000-2017: Special Focus on Inequalities*. World Health Organization; 2019. [Google Scholar]

16. WWAP (UNESCO World Water Assessment Programme). *The United Nations World Water Development Report 2019: Leaving No One Behind*. UNESCO; 2019. [Google Scholar]

17. Silbiger NJ, Nelson CE, Remple K, et al.. Nutrient pollution disrupts key ecosystem functions on coral reefs. *Proc Biol Sci*. 2018;285:20172718. [PMC free article] [PubMed] [Google Scholar]

18. Amin MN, Kroeze C, Strokal M. Human waste: an underestimated source of nutrient pollution in coastal seas of Bangladesh, India and Pakistan. *Mar Pollut Bull*. 2017;118:131-140. [PubMed] [Google Scholar]

19. Correll DL. The role of phosphorus in the eutrophication of receiving waters: A review. *J Environ Qual*. 1998;27:261-266. [Google Scholar]

20. Paerl HW, Otten TG. Harmful cyanobacterial blooms: causes, consequences, and controls. *Microb Ecol*. 2013;65:995-1010. [PubMed] [Google Scholar]

21. Desmidt E, Ghyselbrecht K, Zhang Y, et al.. Global phosphorus scarcity and full-scale P-recovery techniques: A review. *Crit Rev Environ Sci Technol*. 2015;45:336-384. [Google Scholar]

22. Sena M, Rodriguez Morris M, Seib M, Hicks A. An exploration of economic valuation of phosphorus in the environment and its implications in decision making for resource recovery. *Water Res*. 2020;172:115449. [PubMed] [Google Scholar]

23. Maxcy-Brown J, Elliott MA, Krometis LA, Brown J, White KD, Lall U. Making waves: Right in our backyard-surface discharge of untreated wastewater from homes in the United States. *Water Res*. 2021;190:116647. [PubMed] [Google Scholar]

24. Trotochaud L, Hawkins BT, Stoner BR. Non-biological methods for phosphorus and nitrogen removal from wastewater: A gap analysis of reinvented-toilet technologies with respect to ISO 30500. *Gates Open Res*. 2019;3:559. [PMC free article] [PubMed] [Google Scholar]

25. Bunce JT, Ndam E, Ofiteru ID, Moore A, Graham DW. A review of phosphorus removal technologies and their applicability to small-scale domestic wastewater treatment systems. *Front Environ Sci*. 2018;6:8. [Google Scholar]

26. ISO 030500:2018. *Non-Sewered Sanitation Systems - Prefabricated Integrated Treatment Units - General Safety And Performance Requirements For Design And Testing*. International Organization for Standardization; 2018. [Google Scholar]

27. Reynaert E, Greenwood EE, Ndwandwe B, et al.. Practical implementation of true on-site water recycling systems for hand washing and toilet flushing. *Water Res X*. 2020;7:100051. [PMC free article] [PubMed] [Google Scholar]

28. Ziemba C, Larivé O, Reynaert E, Morgenroth E. Chemical composition, nutrient-balancing and biological treatment of hand washing greywater. *Water Res*. 2018;144:752-762. [PMC free article] [PubMed] [Google Scholar]

29. Cornejo PK, Becker J, Pagilla K, et al.. Sustainability metrics for assessing water resource recovery facilities of the future. *Water Environ Res*. 2019;91:45-53. [Google Scholar]

30. Kogler A, Farmer M, Simon JA, Tilmans S, Wells GF, Tarpeh WA. Systematic evaluation of emerging wastewater nutrient removal and recovery technologies to inform practice and advance resource efficiency. *ACS EST Eng*. 2021;1:662-684. [Google Scholar]

31. Warwick C, Guerreiro A, Soares A. Sensing and analysis of soluble phosphates in environmental samples: A review. *Biosens Bioelectron*. 2013;41:1-11. [PubMed] [Google Scholar]

32. 4500-P. In: Rice EW, Baird RB, Eaton AD, Clesceri LS, eds. *Standard Methods for the Examination of Water and Wastewater*. 22nd ed. American Public Health Association; 2012. [Google Scholar]

33. Methods of sampling and test (physical and chemical) for water and wastewater, IS:3025 Part 31 - Phosphorus. *Indian Standard*. 1988. (reaffirm-ed 2003). https://law.resource.org/pub/in/bis/S02/is.3025.31.1988.pdf (accessed 18 May 2021).

34. Welling CM, Varigala S, Krishnaswamy S, et al.. Resolving the relative contributions of cistern and pour flushing to toilet water usage: Measurements from urban test sites in India. *Sci Total Environ*. 2020;730:138957. [PMC free article] [PubMed] [Google Scholar]

35. Rose C, Parker A, Jefferson B, Cartmell E. The characterization of feces and urine: A review of the literature to inform advanced treatment technology. *Crit Rev Environ Sci Technol*. 2015;45:1827-1879. [PMC free article] [PubMed] [Google Scholar]

36. Schellenberg T, Subramanian V, Ganeshan G, Tompkins D, Pradeep R. Wastewater discharge standards in the evolving context of urban sustainability - The case of India. *Front Environ Sci*. 2020;8:30. [Google Scholar]

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ORIGINAL ARTICLE

CR-Possum—Can It Be Used to Predict Morbidity? A Single-Centre Retrospective Study

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Abstract

Preoperative prediction of morbidity in colorectal cancer (CRC) surgery helps to optimize the surgical outcome. In this study, we aim to develop a dedicated equation for predicting operative morbidity using colorectal possum scoring system and also to validate the predictive accuracy of CR-POSSUM scoring system in prognosticating actual complications. We did a retrospective analysis of 322 patients undergoing colorectal cancer surgery from a single centre in South India from 2004 to 2016. Mortality and morbidity risk factors as defined by CR POSSUM were collected from 322 patients who underwent CRC surgery and were used to derive equations to predict morbidity, and the results were compared with the observed morbidity. Logistic regression analysis was used to derive the equation. The model fit and model discrimination were analysed using the Hosmer-Lemeshow statistical test for goodness of fit, the Nagelkerke R^2 and area under the receiver operating characteristic (ROC) curve respectively. Out of 322 patients, 103 (32%) patients developed complications and 10 (3%) died due to complications. The regression equation we derived has an overall correct classification of about 70% ($P < 0.01$) with positive and negative predictive value of 60% and 73% respectively. The Hosmer-Lemeshow goodness of fit was 3.147 ($P = 0.829$), and the Nagelkerke R^2 was 17% and area under ROC as model discrimination was 71.6%. Hence, CR-POSSUM scoring which was originally used for predicting mortality risk can also be extrapolated to predict morbidity.

Keywords Colorectal POSSUM · POSSUM · Colorectal surgery · SSI · Morbidity and mortality · Prediction model

Introduction

Colorectal malignancy is the third most common malignancy among men and the second most common among women around the world [1]. Fortunately, in India, the incidence is not too high. However, the incidence has been found to be rising, with the AAR (adjusted annual incidence rate) for colon cancer in men and women being 4.4 and 3.9 per 100,000, respectively [2]. Being in a country, where people living with poor access to quality healthcare, extreme presentation like advanced stage and perforation are common in tertiary care centres [3, 4]. In such scenarios, the mortality and morbidity for the patients undergoing surgery are relatively high [5]. Complications following major surgeries will proportionately increase the hospital cost and duration of hospital stay [6]. Apart from such hardships, complications also had an adverse impact on long-term survival [7]. So in a country like India, where the spending on health by the government is low and health insurance is under-penetrated, morbidity following a major surgical procedure will further drain the already constrained resources [8]. The complication rates are not uniform and cannot be generalised, as there are certain factors, such as age, patients' co-morbidity and operative severity, influencing the outcome of surgery [9]. So, putting together these factors and assessing the postoperative outcome preoperatively will help in optimizing the available resources. In such cases, the predictive scoring has to be more accurate, and its predictive ability should

Key message: CR-Possum can be used to predict morbidity in patient undergoing colorectal cancer surgery.

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be proven in multiple centres. In our study, we devised a morbidity scoring system with already established parameters in CR-Possum which influence the outcome of colorectal surgery in terms of mortality and assessed the model calibration and discrimination with statistical analysis tools.

Patient and Methods

Data of 506 patients undergoing colorectal surgery for colorectal malignancy from January 2004 to December 2017 was collected from the medical records department of a single tertiary care centre. The in-patient files were analysed and the required variables as per CR-POSSUM scoring [physiologic severity score (PSS) (age, cardiac failure if any, systolic blood pressure, pulse, urea, haemoglobin) and operative severity score (OSS) (operative severity, peritoneal soiling, operative urgency, cancer staging in Dukes)] were collected and entered in an excel data sheet. Patient files, with single missing variable, were excluded from the study. Therefore, only 322 patients with complete details were included. The dichotomous outcome of each patient, either dead/alive, with/without complication, was entered. Presence or absence of complications, such as haemorrhage, wound infection, wound dehiscence, anastomotic leak, deep vein thrombosis, pulmonary embolus, cerebrovascular accident, myocardial infarction, cardiac failure, impaired renal function and respiratory failure were also entered. Complications were also graded based on Clavien-Dindo scale of surgical morbidity, and all grades were considered. Duration of hospital stay in terms of number of days for each patient was also recorded. The study was approved by institutional ethics committee.

Statistical Analysis

The data was analysed using SPSS version 24. Mean and standard deviation were calculated for continuous variables. Chi-square test was done to check association between categorical variables. The equation to predict morbidity was derived using multiple logistic regression analysis, and the model discrimination was measured by calculating the area under ROC (receiver operating characteristic) curve. The Nagelkerke R^2 and the Hosmer-Lemeshow statistics were used for model calibration (goodness of fit). Positive and negative predictive values were also calculated. $P < 0.05$ was considered as statistically significant.

Results

Out of a total of 506 patients undergoing colorectal surgery for malignancy, 322 (63.2%) satisfied the criteria to be included in the study. Among the 322 patients, 103 (31.9%) developed complications and 10 (3%) patients died of complications. The average duration of hospital stay for all patients who underwent surgery was 15.5 days. The mean duration of in-hospital stay for patients with and without complications was 18 and 14.5 days respectively ($P = 0.01$) which was statistically significant. The proportion of patients with and without complications and in-hospital stay days are shown in Fig. 1. The summary of procedures and their associated complications were shown in Table 1. The complications rates were more in tumours located in sigmoid and rectum compared to other sites ($P < 0.05$). The complications were graded as per Clavin-Dindo classification. Wound-related complication tops the list (37%) followed by respiratory complications (18%). These are shown in Table 2. The patient's demographic characteristics and the in-hospital morbidity rates for the factors taken into consideration for CR-POSSUM scoring are shown in Table 3. Majority of the patients are in the age group less than 60 years (51%) and there is a male preponderance (61%). Table 4 summarizes the CR-POSSUM scoring system which we used for the analysis. The equation we derived using above data was $In\ (R/(1-R)) = -4.83 + 0.09(PSS) + 0.35(OSS)$($P < 0.001$). The positive and negative predictive value of the equation was 60.87% and 73.09% respectively. The overall percentage of correct classification was 70%. The goodness of fit was looked at to check whether the predicted probability was assigned accordingly to individual patient's outcome. The Hosmer-Lemeshow test statistics was 3.147 ($P = 0.829$) which was reasonably a good fit for higher the P value, more fit the model is. The Nagelkerke R^2 which was another test to check goodness of fit was fairly good (17%). Model discrimination calculated by area under ROC curve was 71.6% (Fig. 2), which was reasonably a fair discrimination. The observed and expected ratio was almost close to 1 among the different range of predictions.

Discussion

POSSUM AND P-POSSUM were original scoring systems which enumerated the mortality and morbidity in a patient undergoing major surgeries [10, 11]. These have been validated in numerous studies outside the UK, and their value in surgical audit has been proven beyond doubt [12–14]. A study conducted by the American College of Surgeons (ACS-NSQIP) in 2005 to 2006 showed that ten procedure groups among various general surgical operations accounted for 62% of complications, and colectomy was topping in the list [15]. It accounts for 24% of adverse events. CR-POSSUM, developed by Tekkis et al., a modification in the original scoring system, was designed for enumeration of mortality in patients undergoing colorectal procedures [16]. Since the mortality following colorectal surgery was brought down with

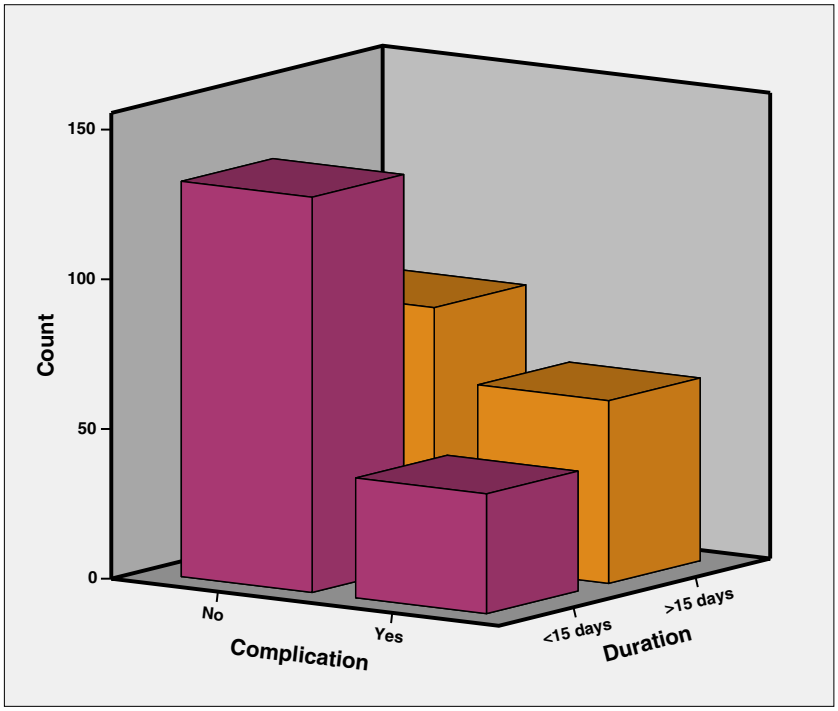


Fig. 1 Duration of hospital stay in patients with and without complications

improvement in postoperative critical care but the morbidity following such procedures especially in patients undergoing emergency surgery for perforation and obstruction was considerably high [17]. Complication rates in surgery for colorectal malignancy elective and emergency were 26 and 38% respectively. In our study, the overall complication rate was 31.9%. The original CR-POSSUM was devised by identifying odds of risk factor causing mortality. However, it was a well-known fact that mortality was the end point of severe morbidity [18, 19]. Therefore, we used the same scoring system to analyse the outcome in terms of complications in our study. The equation, though, using the same odds as for mortality did not overpredict morbidity in our study. But in the physiological severity scoring, except for preoperative cardiac status and urea level, other variables have no significant correlation

Table 1 Tumour location and procedures with in-hospital complication rates

Location of the tumour	Patients	Morbidity (%)	X^2 (P value)
Rectum	99	33(32)	14.40 ($P = 0.04$)
Sigmoid colon	77	31(30.1)	
Ascending colon	43	9(8.7)	
Transverse colon	39	9(8.7)	
Caecum	24	6(5.8)	
Descending colon	17	3(2.9)	
Rectosigmoid junction	11	7(6.8)	
Others	12	4(3.8)	
Type of procedure	Patients	Morbidity (%)	
Right hemicolectomy	99	25(24.2)	11.34 ($P = 0.18$)
Anterior resection	74	18(17.4)	
APR	46	19(18.4)	
Left hemicolectomy	34	9(8.7)	
Sigmoidectomy	31	14(13.5)	
Diversion colostomy	24	9(8.7)	
Total colectomy	5	3(2.9)	
Subtotal colectomy	2	1(0.9)	
Others	9	3(2.9)	

Table 2 Severity of complications graded as per Clavien-Dindo classification

Grade of complication	Type of complication	No. of patients (%) ($n = 103$)
I	Wound infection requiring drainage	16(15.5)
	Post op ventilation for > 1 day	6(5.8)
II	Wound infection needs resuturing	22(21.3)
	Respiratory infection	17(16.5)
	PTE/DVT	4(3.8)
III	Re-exploration due anastamotic leak	15(14.5)
	Burst abdomen/resuturing	3(2.9)
	UTI/sepsis	2(1.9)
	SMV thrombosis	1(0.9)
IV	Myocardial infarction	5(4.8)
	Bowel gangrene	1(0.9)
	Seizure	2(1.9)
	Sepsis and hypotension	1(0.9)
V	Severe sepsis	7(6.7)
	ARDS	2(1.9)
	Multivisceral metastasis	1(0.9)

Table 3 Patient demographic and in-hospital complication rates

Variables in consideration	No. of patients $n = 322$	No. of complication (n = 103)
Age		
< 60	165 (51.2%)	50 (48.5%)
61–70	104 (32.3%)	36 (35.0%)
71–80	38 (11.8%)	11 (10.7%)
> 80	15 (4.7%)	5 (4.9%)
Sex		
Male	197 (61.2%)	65 (63.1%)
Female	125 (38.8%)	37 (35.9%)
PSS		
6 to 10	273 (84.8%)	81 (78.6%)
11 to 15	49 (15.2%)	22 (21.4%)
Operative severity scoring		
Intermediate	27 (8.4%)	8 (7.8%)
Major	228 (70.8%)	61 (59.2%)
Complex major	67 (20.8%)	34 (33.0%)
Peritoneal soiling		
None	275 (85.4%)	80 (77.7%)
Local pus	6 (1.9%)	3 (2.9%)
Faceal contamination	41 (12.7%)	20 (19.4%)
Operative urgency		
Elective	280 (87%)	81 (78.6%)
Urgent	34 (10.6%)	18 (17.5%)
Emergency	8 (2.5%)	4 (3.9%)
Cancer staging		
Dukes A, B	162 (50.3%)	43 (41.7%)
Dukes C	120 (37.3%)	42 (40.8%)
Dukes D	40 (12.4%)	18 (17.5%)

with outcome. Since wound infection and its sequelae occupy a considerable share (37%) of complications in our study, the role of physiological factors influencing the result may be less significant compared to operative severity score where later affects the outcome in terms of surgical site soft tissue infection [20]. Next common complications were respiratory (16.5%) and anastamotic leak (14.5%). Regarding mortality, CR-POSSUM observed and expected mortality ratio was 1

Table 4 Colorectal POSSUM scoring system

Score	1	2	3	4	8
Physiologic score					
Age (yr)	≤ 60		61–70	71–80	≥ 81
Cardiac failure	None or mild	Moderate	Severe		
Pulse (beats/min)	40–100	101–120	> 120 or < 40		
Systolic BP (mmHg)	100–170	> 170 or 90–99	< 90		
Urea (mmol/l)	≤ 10	10.1–15	> 15		
Haemoglobin (g/dl)	13–16	10–12.9 or 16.1–18	< 10 or > 18		
Operative severity score					
Operative severity	Minor		Intermediate	Major	Complex major
Peritoneal soiling	None or serous	Local pus	Free pus/feces		
Operative urgency	Elective		Urgent		Emergency
Cancer staging (dukes)	None or dukes A, B	Dukes C	Dukes D		

and area under ROC curve was 80% showing good model discrimination. But with low mortality rate [$n = 10$ (9.7%)] in our study, the validity of it may not be reliable.

Among the various risk factors, age and cancer staging are considered non-modifiable risk factors. The rest are modifiable risk factors and give the extent of scope to which the complications can be prevented by addressing such factors. But once patients present to tertiary centres in emergency or semi-emergency conditions, most of the risk factors will be present. Tackling such elements will be time-consuming and lead to a further chance of increasing complication [21]. Surgical site-related complications top in our study (37%) and elsewhere, as well [22]. They have an adverse impact in terms of long-term survival [23]. Since operative factors play a significant role in outcome in terms of wound infection as per our study, measures that reduce the chance of acquiring surgical site infections with high level of evidence are appropriate and timely administration of antibiotic, discontinuation of prophylactic antibiotic within 24 h of surgery, optimization of blood glucose levels and avoiding spillage of bowel contents [21]. Similar to SSIs, respiratory complication and anastomotic leak leading to deep-seated infection also affect the long-term survival [24]. PSS and OSS both play a significant role in predicting the latter two complications here. Hence tackling both physiological and operative variable assumes an important role prevention of these two problems.

CR-POSSUM, which was originally developed to predict mortality, can also be used to predict morbidity. But, there are some limitations to our study. Both CR-POSSUM and POSSUM models for mortality and POSSUM model for predicting morbidity left many variables like preoperative nutrition level, operative duration, BMI and length of preoperative stay, all of which influence the eventual outcome. These were not considered while developing the equation [21]. But these scoring systems were developed 10 and 25 years ago respectively, and many new influencing variables were found in subsequent research.

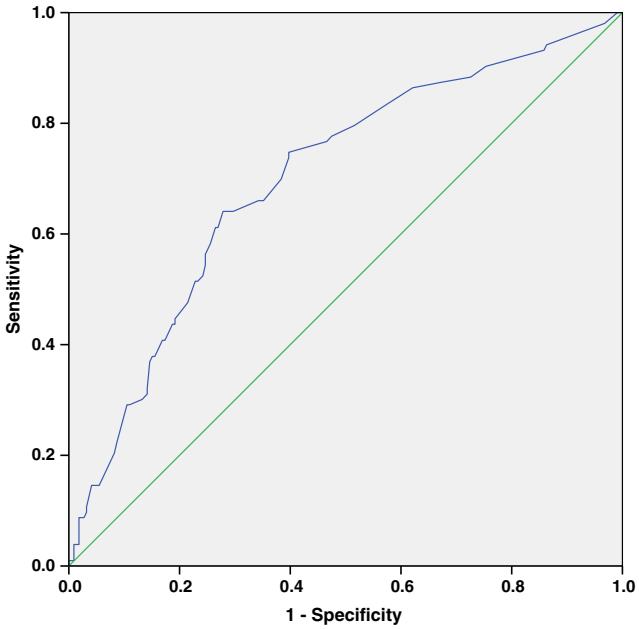


Fig. 2 Receiver operator characteristic curve (ROC) for the derived equation (Area under the curve is 71.6%)

Our results may be biased since there is a high proportion of SSI-related complication. We, therefore, validated the equation after excluding this group of patients. We excluded surgical site infection (SSI) in further analysis, to check whether physiological factors had a role in predicting non-SSI-related complications. We found that there was, indeed, a significant association ($P = 0.005$) between PSS and other non-SSI complications. The results were similar with few minor variations in predictive values. So, if a new model is developed considering the various new variables, then the predictive accuracy of such an equation can be close to reality.

To conclude, these predictive models once validated, across various centres in India, can be used as a measure of quality control in different institutions. Secondly, they can also be used to allocate resources and adequately trained professionals to deal with those patients whose expected morbidity and mortality are high.

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Compliance with Ethical Standards

Conflicting Interest The authors declare that they have no conflict of interest.

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References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008. Int J Cancer 127:2893–2917
2. Comparison of cancer incidence and patterns of all population-based cancer registries. Available from: http://www.ncrpindia.org/all_ncrp_reports/pbcr_report_2012_2014/all_content/pdf_Printed_Version/Chapter7_Printed.pdf. Assessed 26th Jan 2018
3. Sudarshan V, Hussain N, Gahine R, Mourya J (2013) Colorectal cancer in young adults in a tertiary care hospital in Chhattisgarh, Raipur. Indian J Cancer 50:337–340
4. Patil PS, Saklani A, Gambhire P, Mehta S, Engineer R, De'Souza A et al (2017) Colorectal cancer in India: an audit from a tertiary center in a low prevalence area. Indian J SurgOncol 8:484

5. MohdSuan MA, Tan WL, Soelar SA, Ismail I, Abu Hassan MR (2015) Intestinal obstruction: predictor of poor prognosis in colorectal carcinoma? Epidemiol Health 37:e2015017
6. Itani KM, Hawn MT (2013) Prevention of surgical site infection in colorectal surgery. Colorectal Cancer 2:467–475
7. Khuri SF, Henderson WG, DePalma RG, Mosca C, Healey NA, Kumbhani DJ (2005) Determinants of long-term survival after major surgery and the adverse effect of postoperative complications. Ann Surg 242:326–341
8. World Health Organisation global health expenditure database. Available from: https://data.worldbank.org/indicator/SH.XPD.TOTL.ZS. Accessed 7th Jan 2018
9. Longo WE, Virgo KS, Johnson FE, Oprian CA, Vernava AM, Wade TP, Phelan MA, Henderson WG, Daley J, Khuri SF (2000) Risk factors for morbidity and mortality after colectomy for colon cancer. Dis Colon Rectum 43:83–91
10. Prytherch DR, Whiteley MS, Higgins B, Weaver PC, Prout WG, Powell SJ (1998) POSSUM and portsmouth POSSUM for predicting mortality. Physiological and operative severity score for the enumeration of mortality and morbidity. Br J Surg 85:1217–1220
11. Copeland GP, Jones D, Walters M (1991) POSSUM: a scoring system for surgical audit. Br J Surg 78:355–360
12. Teeuwen PHE, Bremers AJA, Groenewoud JMM (2011) Predictive value of POSSUM and ACPGBI scoring in mortality and morbidity of colorectal resection: a case-control study. J Gastrointest Surg 15:294–303
13. Cengiz F, Kamer E, Zengel B, Uyar B, Tavusbay C, Unalp HR (2014) Comparison of different scoring systems in patients undergoing colorectal cancer surgery for predicting mortality and morbidity. Indian J Cancer 51:543–548
14. Baré M, Alcantara M, Gil M, Collera P, Pont M, Escobar A et al (2018) Validity of the CR-POSSUM model in surgery for colorectal cancer in Spain (CCR-CARESS study) and comparison with other models to predict operative mortality. BMC Health Serv Res 18:49
15. Schilling PL, Dimick JB, Birkmeyer JD (2008) Prioritizing quality improvement in general surgery. J Am CollSurg 207:698–704
16. Tekkis PP, Prytherch DR, Kocher HM, Senapati A, Poloniecki JD, Stamatakis JD, Windsor ACJ (2004) Development of a dedicated risk-adjustment scoring system for colorectal surgery (colorectal POSSUM). Br J Surg 91:1174–1182
17. Gallardo-Valverde JM, Calanas-Continente A, Baena-Delgado E, Zurera-Tendero L, Vazquez-Martinez C, Membrives-Obrero A, Muntane J, Arevalo-Jimenez E (2005) Obstruction in patients with colorectal cancer increases morbidity and mortality in association with altered nutritional status. Nutr Cancer 53:169–176
18. Dindo D, Demartines N, Clavien P-A (2004) Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 240:205–213
19. Petros AJ, Marshall JC, van Saene HK (1995) Should morbidity replace mortality as an endpoint for clinical trials in intensive care. Lancet 345:369–371
20. Mahajna A, Krausz M, Rosin D, Shabtai M, Hershko D, Ayalon A, Zmora O (2005) Bowel preparation is associated with spillage of bowel contents in colorectal surgery. Dis Colon Rectum 48:1626–1631
21. Pedroso-Fernandez Y, Aguirre-Jaime A, Ramos MJ, Hernández M, Cuervo M, Bravo A, Carrillo A (2016) Prediction of surgical site infection after colorectal surgery. Am J Infect Control 44:450–454
22. Young PY, Khadaroo RG (2014) Surgical site infections. Surg Clin North Am 94:1245–1264
23. Artinyan A, Orcutt ST, Anaya DA, Richardson P, Chen GJ, Berger DH (2015) Infectious postoperative complications decrease long-term survival in patients undergoing curative surgery for colorectal cancer: a study of 12,075 patients. Ann Surg 261:497–505
24. Tu R-H, Lin J-X, Li P, Xie J-W, Wang J-B, Lu J, Chen QY, Cao LL, Lin M, Zheng CH, Huang CM (2017) Prognostic significance of postoperative pneumonia after curative resection for patients with gastric cancer. Cancer Med 6:2757–2765

Predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates

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ABSTRACT

Background: Inadequate breastfeeding results in hypernatremia in otherwise healthy neonates. **Objective:** This study finds out the predisposing factors and clinical outcome of hypernatremia in breastfed late preterm and term neonates. **Materials and Methods:** A descriptive study was conducted in a tertiary hospital with retrospective data from July 2013 to June 2016. Babies with serum sodium ≥ 150 mEq/L were identified. Breastfed babies with little or no formula feeds, gestational age ≥ 34 weeks, and birth weight ≥ 2000 g were included. Babies with congenital anomalies, sepsis, prior parenteral fluid therapy and renal or endocrine diseases causing fluid and electrolyte disturbances were excluded. Total weight loss > 10% or daily weight loss > 5% from the birth weight were considered as excess weight loss. **Results:** A total of 201 babies were studied. Hypernatremia was significantly more common in babies of primiparous mothers ($p < 0.01$). There was no difference in incidence between vaginal born and cesarean-born babies. Positive correlation was seen between age at presentation, percentage of weight loss ($p = 0.001$) and serum sodium ($p = 0.01$). Significantly more cases were seen in summer season ($p < 0.01$). Excess weight loss, jaundice, fever, and dehydration were the common symptoms. Complications observed were acute kidney injury, seizures, intracranial bleed, apnea, and bradycardia. Babies with complications mostly presented after 7 days of age, had > 25% weight loss and had serum sodium ≥ 170 mEq/L. There was no death. Three babies had neurodevelopmental problems on follow-up. **Conclusion:** Breastfed babies of primiparous mothers and born in summer are predisposed to hypernatremia. Babies presenting at a later age had severe weight loss, severe hypernatremia, and complications.

Key words: *Breastfeeding, Hypernatremia, Primiparity, Summer season, Weight loss*

Exclusive breastfeeding for the first 6 months of life is recommended by the World Health Organization to optimize the growth and development of the infants [1]. Breast milk in addition to nutrients provides various immunological factors that help to reduce the diarrheal and respiratory illness in children [2]. Breastfeeding is an instinct behavior and many mother-baby dyads accomplish it successfully [3]. However, some babies may not receive enough milk from their mothers due to various reasons. If breastfeeding is inadequate, then malnutrition and hypernatremic dehydration can occur in the baby [4]. Moritz *et al*. have termed this condition as "breastfeeding associated hypernatremia" (BFAH) [5].

An increase in the incidence of BFAH in otherwise healthy neonates has been observed in the past few years [5-8]. The clinical features and complications seen in these babies have been reported in various studies [9]. It has been found that daily weighing of the babies helps in the early recognition of this illness [10,11]. Thus, the discrepancy in clinical features and complications reported in various studies were probably related to weight monitoring practices and timing of presentation of the cases [10,11]. This study was planned to find out the predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates in a hospital with regular weight monitoring practice.

MATERIALS AND METHODS

This descriptive study was done using retrospective data from July 2013 to June 2016. The study was conducted in an urban tertiary care medical college hospital with an average delivery rate of 2500 per year. Exclusive breastfeeding was promoted for all babies in the hospital. The mother-baby dyads were discharged at around 72 h after normal delivery and 120 h after cesarean delivery. As per hospital protocol, all babies were weighed daily. Excess weight loss was defined as total weight loss more than 10% from birth weight or weight loss per day more than 5% of birth weight [12]. Serum sodium level was checked for babies with excess weight loss. Hypernatremia was defined as serum sodium more than or equal to 150 mEq/L. Babies with hypernatremia were treated with liberal oral feeds, intravenous (IV) fluids, and other supportive therapies. Serum sodium <150 mEq/L was considered an end point of treatment.

The Institutional Ethics Committee approved the study. Babies admitted during the study period with hypernatremia were identified from the hospital database. Their case records were scrutinized and babies who fulfilled the following criteria were included in the study: (1) Breastfed babies with little or no formula supplementation, (2) gestational age more than or equal to 34 weeks, and (3) birth weight more than or equal to 2000 g. The babies who had major congenital anomalies, blood culture positive sepsis, received IV fluids within 48 h before diagnosis of hypernatremia and renal or endocrine disorders which may affect the fluid and electrolyte balance were excluded from the study. The following data were collected: Gestational age as completed weeks, age at diagnosis as completed days, birth weight, weight at diagnosis, maternal age, obstetric details, details about baby feeding, clinical features, treatment, complications, condition at discharge, and neurodevelopment at follow-up.

The total weight loss and weight loss per day were calculated as a percentage of the birth weight. Axillary temperature more than 38°C was considered as fever. The rate of decline in serum sodium was calculated using the sodium before and after treatment and the time taken to achieve it. Acute kidney injury (AKI) was diagnosed if serum creatinine increased by >0.3 mg/dl in 24 h or if it was >1.5 mg/dl with normal maternal renal functions [13]. Jaundice treated with phototherapy was considered as significant. At the time of discharge, babies were said to have improved if their serum sodium level was <150 mEq/L and was taking feeds well. At follow-up, their neurodevelopment was said to be normal if gross motor, fine motor, language, and social milestones were appropriate for age and had no other neurological problem.

SPSS software version 24 was used for analysis. Numerical data were presented as mean±standard deviation or median±interquartile range as appropriate. Categorical data were presented as percentages and analyzed using Chi-square test. Ordinal data were analyzed with Mann–Whitney U-test or Student's t-test. Correlation was done with Spearman rank correlation coefficient where appropriate. $p<0.05$ was considered as significant.

RESULTS

There were 213 babies with hypernatremia satisfying the inclusion criteria during the study period. 12 babies were excluded due to various reasons. The remaining 201 babies were considered for analysis (Fig. 1). During the study period, there were 7613 live births, with 4570 vaginal births, and 3043 cesarean births. There were 4463 primiparous and 3150 multiparous mothers. Hypernatremia was seen in 2.4% of vaginally born babies and 3.1% of cesarean-born babies. This difference was not statistically significant. 3.1% babies of primiparous mothers and 1.6% babies of multiparous mothers had hypernatremia, and this different was statistically significant (Chi-square 16.7, $P<0.01$). All babies were born of singleton pregnancy. Most of the (80%) mothers had no problem in breastfeeding their babies (Table 1).

Serum sodium had a negative correlation with mother's age ($r=-0.23$, $p=0.01$) and baby's gestational age ($r=-0.2$, $p=0.004$). No correlation was seen between serum sodium level and birth weight. A positive correlation was seen between age at presentation and total weight loss ($r=0.65$, $P=0.001$). Serum sodium at admission had a positive correlation with age at presentation ($r=0.704$, $p=0.01$) and percentage of total weight loss ($r=0.652$, $p=0.001$). More than 50% of the cases were seen during the summer season in this study. This seasonal variation in the incidence of cases was statistically significant (Chi-square-26.9, $p<0.01$) (Table 1).

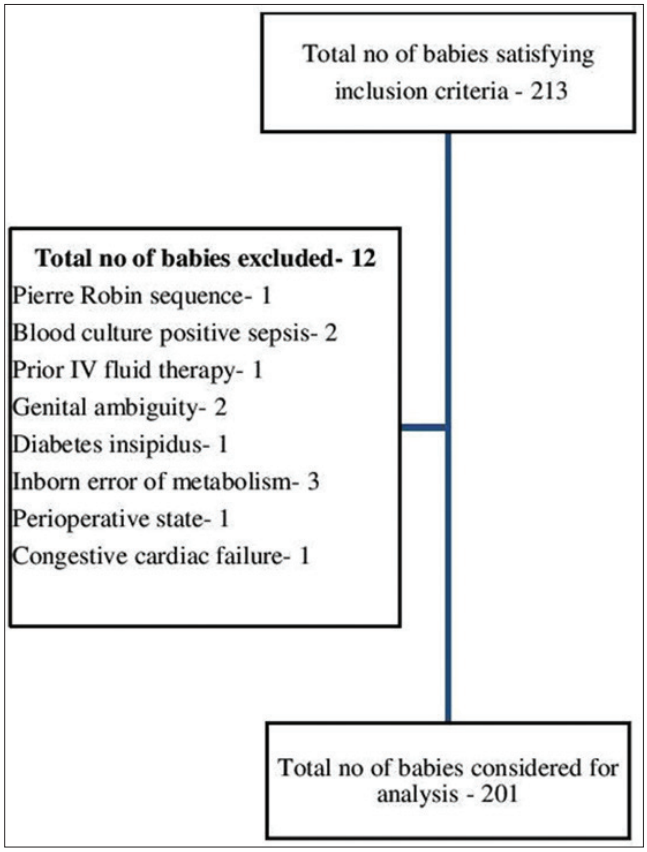


Figure 1: Flow of cases in the study

Significant jaundice was seen in 57.2% babies, but none required exchange transfusion. Phototherapy (median duration 24 h) before the onset of hypernatremia was given to 26 babies. Other common clinical findings observed were fever (33.8%) and dehydration (17.4%). No symptom other than excessive weight loss was seen in 21.4% of babies. Severe hypernatremia with serum sodium ≥170 mEq/L was seen in only five babies, and all other babies (98%) had moderate hypernatremia (serum sodium 150–169 mEq/L). About 89% of babies improved after treatment and got discharged. There was no death in the study population (Table 2). The babies who improved had a median birth weight of 3000 g (2800–3300), median admission weight of 2700 g (2460–2930), and median discharge weight of 2800 g (2540–3070).

Five babies (2.5%) had complications during the course of hypernatremia. One male baby with serum sodium of 151 mEq/L had apnea after vomiting of feeds and developed aspiration pneumonia. He improved with mechanical ventilation and antibiotics. All other four babies presented after 7 days of life

Table 1: Baseline characteristics of hypernatremic babies

Characteristics	Observation
Median maternal age (years) (n=183)*	26.9 (23-29)
Number of babies born to primiparous mothers	138/188 (73.4%)
Number of babies born to cesarean mothers	108 (53.7%)
Number of mothers reported problem in breastfeeding	42 (20.1%)
Male to female ratio	1.34:1
Median gestational age (weeks) (n=189)*	38 (37-39)
Median age at diagnosis (days)*	3 (2–18)
Number of babies presented on 2–3 days age	161 (80.1%)
Median birth weight (g)*	3080 (2810-3410)
Median weight at diagnosis (g)*	2710 (2470-3020)
Median daily weight loss (%)*	3.9 (3.3-4.7)
Median total weight loss from birth (%)*	11.3 (10-12.8)
Number of babies with 10–15% weight loss	139 (69.2%)
Season and number of babies admitted	
a. March to June (Summer)	103 (51.2%)
b. July to October	40 (19.9%)
c. November to February (Winter)	58 (28.9%)
Number of babies on exclusive breastfeeding	177 (88.1%)

*Median (IQR). IQR: Interquartile range

Table 2: Clinical features and outcome of hypernatremic babies

Characteristics	Observations
Median serum sodium at diagnosis (mEq/L)*	153 (151–155)
Serum sodium range and number of babies	
a. 150–159 mEq/L	184 (91.5%)
b. 160–169 mEq/L	12 (6%)
c. ≥170 mEq/L	5 (2.5%)
Mean time to normalize serum sodium	
a. 150–159 mEq/L (n=161)	17.8 h
b. 160–169 mEq/L (n=12)	26.6 h
c. 170–179 mEq/L (n=3)	48.8 h
d. ≥180 mEq/L (n=2)	71.3 h
Outcome	
a. Improved	178 (88.6%)
b. Left against medical advice	23 (11.4%)
Neurodevelopment problem at follow-up	3/154 (1.9%)

*Median (IQR). IQR: Interquartile range

with weight loss more than 25% of birth weight and severe hypernatremia (serum sodium ≥170 mEq/L). Complications observed were AKI (4), seizures (2), apnea (2), intracranial hemorrhage (1), and bradycardia (1). Peritoneal dialysis was done for one baby with AKI. No baby had peripheral or cerebral vessel thrombosis. Neurosonogram done for one baby with seizures showed increased white matter echogenicity. Another baby with seizures had normal neurosonogram. The treatment and outcome details of babies with severe hypernatremia are given in Table 3.

Follow-up details were available in 154 cases (76.6%) for a median duration of 1.5 months (0.5–11 months). Three babies had abnormal neurodevelopment. One female baby with serum sodium 150 mEq/L had a prolonged period of jaundice requiring phototherapy for 9 days. At follow-up, she had a global developmental delay with a seizure disorder. One male baby with serum sodium 150 mEq/L was found to have hyperactivity and aggressive behavior at 3 years. Neuroimaging was not available for these two babies. Another female baby had severe hypernatremia with AKI, seizures and normal neurosonogram in the neonatal period; and on follow-up at 1 year was found to have left hemiparesis and magnetic resonance imaging of the brain showed chronic infarct in right front parietal region and white matter changes in subcortical and deep cerebral white matter.

DISCUSSION

In this study, we have found BFAH to be more common among babies of primiparous mothers. Although breastfeeding is an instinct behavior, mothers have to learn the technique and need social support to successfully breastfeed their babies [3]. Systematic review on BFAH has found cesarean delivery, primiparity and absent previous breastfeeding experience as risk factors for BFAH [9]. However, there was no difference between cesarean babies and vaginally born babies in this study. Similarly, two studies had found no significant difference in mode of delivery among hypernatremic babies compared to control group [5,14].

It is stated that the parents of hypernatremic babies have poor recognition of their baby's illness [7,15]. About 80% of our baby's mothers have not felt any problem with their baby's feeding behavior. Excess weight loss gave a clue to identify these cases early. Thus, majority of our cases were identified at an early age, had less severe weight loss and less severe hypernatremia. Many studies report that babies presenting at a later age have higher weight loss and more severe hypernatremia [16,17]. Hence, daily weighing of babies along with appropriate lactation support during the first 4–5 days of life has been suggested for early detection of BFAH [10,11,18]. The seasonal variation in the incidence of hypernatremia was reported in some studies [12,19-21]. This was attributed to excess insensible water loss due to the hot climate in summer and overuse of room heaters in winter [9,21]. In this study, we have found significantly more number of cases during the summer season. This seasonal variation may be related to hot environmental temperature during summer seen in Tamil Nadu state of India where the study was done.

At admission, jaundice, fever, and dehydration were common symptoms in our study population. Lavagno *et al*. through systematic review have found jaundice, poor feeding, and dehydration each seen in about 45% of all reported cases [9]. Yang *et al*. have showed that percentage of weight loss within first 3 days of life has a positive correlation with jaundice at 72 h after birth in healthy term breastfed babies [22]. The hypernatremia and the resultant hyperosmolar state may damage the blood–brain barrier and facilitates bilirubin entry into nervous tissues at

Table 3: Clinical features and outcome of babies with severe hypernatremia

Gestational age (weeks)	Birth weight (g)	Total weight loss (%)	Day of diagnosis	Maximum Sr. sodium (mEq/L)	Complications	Treatment	Time to correct Sr. sodium (h)	Outcome at follow up
40	2700	25.9	12	182	Seizures, AKI, apnea, bradycardia	½ DNS+oral feeds; peritoneal dialysis; anticonvulsant	57	Normal
40	3630	14.3	4	173	Nil	½ DNS	43	Normal
37	2510	37.5	18	174	AKI, cerebral hemorrhage	NS bolus, ½ DNS	69.75	Normal
36	3060	27.5	8	173	AKI	½ DNS+oral feeds	33.5	Normal
Term	2700	24.1	17	186	AKI, seizures	NS bolus, DNS, ½ DNS; anticonvulsants	85.5	Left hemiparesis

AKI: Acute kidney injury

lower serum bilirubin level [5,23]. This has to be considered in one baby with neurodevelopmental impairment in our study who had hypernatremia and prolonged jaundice. About 20% of our babies had no symptoms at admission except for excess weight loss. Similarly Uras *et al*. and Korgali *et al*. have found that 14% and 2.4% of their hypernatremic babies were asymptomatic at admission, respectively [24,25].

Four out of five babies with severe hypernatremia had neurological and/or renal complications. Similarly, Ahmed *et al*. have found that babies with AKI presented at a later age, had higher weight loss and higher serum sodium [26]. However, less number of babies in this study had complications compared other studies [5,8,9,20,25-27]. This may be due to earlier age at presentation and a lesser degree of hypernatremia seen in the large proportion of babies in our study. There was no death in our study population and on follow-up only three babies had a neurodevelopmental problem. Mortality rate of 1-5% has been reported by various authors [8,26-28]. Koklu *et al*. have reported neurodevelopmental impairment in 47.8% cases [27]. Similarly, a high rate of neurodevelopmental impairment is also reported by Bolat *et al*. and Ergenekon *et al*. [29,30]. This emphasizes the need for formal neurodevelopmental follow-up of hypernatremic babies.

In this study, we have reported the correlation of serum sodium level with various other clinical parameters in babies with BFAH. Our study has the following limitations due to retrospective nature of the study: Some data were incomplete and long-term formal neurodevelopmental assessment was not done. Our study was hospital based, and many babies had a less severe degree of hypernatremia with fewer complications. A larger scale prospective study with neurodevelopmental follow-up is needed to have a clear understanding of the risk factors, complications and long-term effects of BFAH.

CONCLUSION

Breastfed late preterm and term babies born to primiparous mothers and born in summer season have predisposition to develop hypernatremia. These babies commonly presented with excess weight loss, jaundice, fever, and dehydration. Babies presenting beyond 7 days of age and with more than 25% weight loss are likely to have severe hypernatremia. Neurological complications and AKI were common complications observed with severe hypernatremia. These babies are at risk of neurodevelopmental impairment. Prospective study with long-term follow-up is needed.

REFERENCES

1. Kramer MS, Kakuma R. The Optimal Duration of Exclusive Breastfeeding-A Systematic Review. Geneva: World Health Organization; 2002.
2. Eidelman AI, Schanler RJ. Breastfeeding and the use of human milk. AAP policy statement. Pediatrics 2012;129:e827-41.
3. Volk AA. Human breastfeeding is not automatic: Why that's so and what it means for human evolution. J Soc Evol Cult Psychol 2009;3:305-14.
4. Livingstone VH, Willis CE, Abdel-Wareth LO, Thiessen P, Lockitch G. Neonatal hypernatremic dehydration associated with breast-feeding malnutrition: A retrospective survey. Can Med Assoc J 2000;162:647-52.
5. Moritz ML, Manole MD, Bogen DL, Ayus JC. Breastfeeding-associated hypernatremia: Are we missing the diagnosis? Pediatrics 2005;116:e343-47.
6. Laing IA, Wong CM. Hypernatraemia in the first few days: is the incidence rising? Arch Dis Child Fetal Neonatal Ed 2002;87:F158-62.
7. Cooper WO, Atherton HD, Kahana M, Kotagal UR. Increased incidence of severe breastfeeding malnutrition and hypernatremia in a metropolitan area. Pediatrics 1995;96:957-60.
8. Trotman H, Lord C, Barton M, Antoine M. Hypernatremic dehydration in Jamaican breastfed neonates: A 12-year review in a baby-friendly hospital. Ann Trop Paediatr 2004;24:295-300.
9. Lavagno C, Camozzi P, Renzi S, Lava SA, Simonetti GD, Bianchetti MG, *et al.* Breastfeeding-associated hypernatremia: A systematic review of the literature. J Hum Lact 2016;32:67-74.
10. Iyer NP, Srinivasan R, Evans K, Ward L, Cheung WY, Matthes JW. Impact of an early weighing policy on neonatal hypernatraemic dehydration and breast feeding. Arch Dis Child 2008;93:297-9.
11. Konetzny G, Bucher HU, Arlettaz R. Prevention of hypernatraemic dehydration in breastfed newborn infants by daily weighing. Eur J Pediatr 2009;168:815-8.
12. Bhat SR, Lewis P, David A, Liza SM. Dehydration and hypernatremia in breast-fed term healthy neonates. Indian J Pediatr 2006;73:39-41.
13. Subramanian S, Agarwal R, Deorari A, Paul V, Bagga A. Acute renal failure in neonates. Indian J Pediatr 2008;75:385-91.
14. Boskabadi H, Maamouri G, Ebrahimi M, Ghayour-Mobarhan M, Esmaeily H, Sahebkar A, *et al.* Neonatal hypernatremia and dehydration in infants receiving inadequate breastfeeding. Asia Pac J Clin Nutr 2010;19:301-7.

15. Oddie S, Richmond S, Coulthard M. Hypernatraemic dehydration and breast feeding: a population study. Arch Dis Child 2001;85:318-20.
16. Oddie SJ, Craven V, Deakin K, Westman J, Scally A. Severe neonatal hypernatremia: A population based study. Arch Dis Child Fetal Neonatal Ed 2013;98:F384-7.
17. Dommelen PV, Boer S, Unal S, Wouwe JP. Charts for weight loss to detect hypernatremic dehydration and prevent formula supplementation. Birth 2014;41:153-9.
18. Neifert MR. Prevention of breastfeeding tragedies. Pediatr Clin North Am 2001;48:273-97.
19. Jain S, Basu S. Hypernatremic dehydration in term and near-term neonates. Indian J Pediatr 2010;77:461.
20. Gomes SM, Fernandes A, Ramos H, Fernandes E, Santos M, Nascimento O, *et al*. Breastfeeding-associated hypernatremic dehydration. Einstein 2009;7:206-10.
21. Basiratnia M, Pishva N, Dehghani A. Prevalence of breastfeeding associated hypernatremia among hospitalized hyperbilirubinemia neonates. Iran J Neonatol 2014;5:12-6.
22. Yang WC, Zhao LL, Li YC, Chen CH, Chang YJ, Fu YC, *et al.* Bodyweight loss in predicting neonatal hyperbilirubinemia 72 hours after birth in term newborn infants. BMC Pediatr 2013;13:145.
23. Wennberg RP, Johansson BB, Folbergrová J, Siesjö BK. Bilirubin-induced changes in brain energy metabolism after osmotic opening of the blood-brain barrier. Pediatr Res 1991;30:473-8.
24. Uras N, Karadag A, Dogan G, Tonbul A, Tatli MM. Moderate hypernatremic dehydration in newborn infants: retrospective evaluation of 64 cases. J Matern Fetal Neonatal Med 2007;20:449-52.
25. Korgali EU, Cihan MK, Oguzalp T, Sahinbas A, Ekici M. Hypernatremic dehydration in breastfed term infants: Retrospective evaluation of 159 cases. Breastfeed Med 2017;12:5-11.
26. Ahmed A, Iqbal J, Ahmad I, Charoo BA, Ahmad QI, Ahmad SM. Complications due to breast-feeding associated hypernatremic dehydration. J Clin Neonatol 2014;3:153-7.
27. Koklu E, Gunes T, Ozturk MA, Kose M, Kurtoglu S, Yuksel F. A review of 116 cases of breastfeeding-associated hypernatremia in rural area of central Turkey. J Trop Pediatr 2007;53:347-50.
28. Unal S, Arhan E, Kara N, Uncu N, Alienfendioglu D. Breast-feeding-associated hypernatremia: Retrospective analysis of 169 term newborns. Pediatr Int 2008;50:29-34.
29. Bolat F, Oflaz MB, Güven AS, Özdemir G, Alaygut D, Dogan MT, *et al.* What is the safe approach for neonatal hypernatremic dehydration? A retrospective study from a neonatal intensive care unit. Pediatr Emerg Care 2013;29:808-13.
30. Ergenekon E, Unal S, Gücüyener K, Soysal SE, Koç E, Okumus N, *et al.* Hypernatremic dehydration in the newborn period and long-term follow up. Pediatr Int 2007;49:19-23.

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Prevalence and the factors influencing soil-transmitted helminths among school age children (5–14 years age) in a rural area of Coimbatore district

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Abstract

Context: Highest intensity of soil-transmitted helminthiasis (STH) is seen among school age children. **Aims**: The aim of this study is to find out the prevalence and factors associated with soil-transmitted helminthic infection among school age children (5–14 years) in a rural area of Coimbatore district. **Settings and Design:** The study was conducted in the field practice area of the Rural Health Training Centre (RHTC) Vedapatti, Coimbatore. RHTC caters to a total population of 23,841 distributed in 14 villages. After getting ethical clearance, five of the 14 villages of Vedapatti were selected by the cluster sampling method. Totally, 819 participated in the survey conducted between November 2015 and July 2016 in the field practice area. **Subjects and Methods:** Structured questionnaire was used to collect the information. Consent from parents and assent from child were obtained. Totally, 610 gave one adequate stool sample. Early morning samples were collected and transported to the laboratory within four hours. Formal ether concentration method was performed, and examination was done. **Statistical Analysis Used:** Data analysis was performed with the SPSS version 19 software. The prevalence is expressed in percentage with 95% confidence interval (CI). Univariate and multivariate analyses were performed. Strength of association was expressed in terms of odds ratio (OR) and adjusted OR with 95% CI. $P < 0.05$ was considered as statistically significant. **Results:** The prevalence of STH was 7.70% (95% CI: 5.58–9.82). *Ascaris lumbricoides* was highly prevalent 6.9% (4.89%–8.91%) followed by Hook worm 0.7% (0.04%–1.36%), and *Trichuris trichiura* 0.2% (0.15%–0.55%). Mulitivariate logistic regression analysis showed that pucca houses offered protection against STH. **Conclusions:** The prevalence of STH in a rural area of Coimbatore is 7.7% (95% CI: 5.58–9.82), and is continuing as a public health problem.

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Full Text

Introduction

The soil-transmitted helminthes causing major illness among human beings include Ascaris lumbricoides, Trichuris trichiura, Necator americanus, and Ancylostoma duodenale.[1] STH are considered neglected tropical diseases.[2] STH affects more than one fourth of the world's population, and it is reported more in tropical and subtropical areas.[3] The greatest number of cases are occurring in sub-Saharan Africa, the Americas, China, and East Asia.[4] In India, the reported prevalence of STH infection can be striked out varied from 7.8% to 92.6%.[5],[6],[7],[8],[9] The reported prevalence varies according to the place and time of the study conducted.

The highest intensity of soil-transmitted helminthes occur in school age children.[10] Children do not fully understand the connections between illness and behavior.[11] It has been demonstrated that children often than adults tend to go barefoot, resulting in more contact with soil, and fail to use sanitary facilities even when they are present.[12] STH infections are considered an important cause of sickness absenteeism. They are accounting for loss of 12.3% and 11.4% of disability adjusted life years in girls and boys, respectively.[13] Over 270 million preschool-age children, and over 600 million school-age children live in areas where sanitation is poor and these parasites are intensively transmitted.[14] In India, more than 241 million children are at the risk of parasitic worm infections. Ministry of Health and Family Welfare, Government of India launched the National Deworming Day (NDD)[15] on Feb 10, 2015. NDD aims to de-worm all children between the ages of 1–19 years. The tablets were supplied to children through government, government-aided and private schools, and Anganwadi centers.

When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent re-infection, a long-term impact can be achieved. Epidemiological studies are needed to identify the risk factors which may represent critical control points. There is no recently published article on the prevalence of soil-transmitted helminthes in Coimbatore, where open-air defecation is a common practice. As per the 2011 census, 46% of the households in Coimbatore district lack toilets. Hence, this cross-sectional study was proposed and conducted with the objective of finding the prevalence and risk factors associated with soil-transmitted helminth infections in school age children (5–14 years) in a rural area of Coimbatore district.

Subjects and Methods

The study was conducted in the field practice area of the Rural Health Training Center (RHTC) Vedapatti, Coimbatore. RHTC caters to a total population of 23,841 distributed in 14 villages.

of Thondamuthur and Madukarai block of Coimbatore district. The total number of school-aged children (aged 5–14 years) is 3266.

Ethical clearance was obtained from Institutional Human Ethics Committee, in 2014. The sample size was calculated to be 819 assuming 34.5% as prevalence, relative precision of 15%, design effect of 2, and nonresponse rate of 20%.[16] Five out of 14 villages were selected by the cluster sampling method. House-to-house survey was conducted in the five villages during November 2015–July 2016. All school-age children (aged 5–14 years) who were permanent residents of Vedapatti field practice area of RHTC were included in the study. Children who were not available on three visits within a week time were excluded from the study.

At the home setting, after getting consent from the parent and assent from the child any one of the parents of the child was interviewed in the evening hours of the day. A structured questionnaire was used to collect the information on demographic, socioeconomic, and environmental information such as availability of type of drinking water and availability of functioning sanitary toilet. Few questions were asked regarding the knowledge and attitude of the parents on STH. Respondent was asked to give comment on the personal hygiene practices of child such as washing hands with soap before taking food and after defecation, eating fallen foods on the floor, wearing foot wear outside the house and intake of de-worming tablets in the past 3 months. Observations were also made regarding the personal hygiene of the children related to STH such as clipped nails. Knowledge and attitude score was generated from the correct responses for the knowledge and attitude questions. The correct responses were given a score of one, and maximum score one could get was nine. Score five and above was considered as adequate knowledge and attitude score.

At the end of survey, a screw-capped plastic container with the identification of the child was given to collect the stool sample of the child on the next morning. Instructions regarding stool collection were given. The stool samples were collected and transported to microbiology laboratory within 4 h of sample collection. Stool samples were examined by saline wet mount and iodine wet mount.[17] Iodine preparation was done for the better visualization of morphological details of ova. All samples were re-examined after concentrating with WHO recommended.[18] Formalin-ether sedimentation method.[19] To reduce the human error, duplicate slides (both direct and after concentration method) were prepared from each sample and the slides were read by two different microbiologists and the opinion of chief microbiologist was considered as final.

Analysis

Data entry was made in the Microsoft Excel software in codes and analysis was done with SPSS-19 (IBM, Armonk, New York ,United States) computer package. The presence of ova of A. lumbricoides, Trichuris trichiura, N. americanus, and A. duodenale was considered as the dependent variable. The prevalence is expressed in percentage with 95% confidence interval (CI). Univariate analysis and logistic regression were performed to find the associations between the independent variables and dependent variables. Strength of association was expressed in terms of odds ratio (OR) and adjusted OR with 95% CI and $P < 0.05$ was considered as statistically significant.

Results

The results of this study are based on 610 children, who gave one adequate stool sample. Totally, 819 children participated in the survey; hence, the nonresponse rate was calculated as 25.52.

The distribution of the study population and their risk factors is shown in [Table 1] and [Table 2]. The mean age of the study population was 8.92 ± 2.68. Among them, children below 10 years (58.5%), female participants (52.1%), belonged to families with members four and above (54.92%), children living in kutcha and semi-pucca houses(80%), and children belonged to low socioeconomic status (52.3%) Class IV and V according to the modified Prasad classification were found in majority. Illiteracy was found among 20.2% of mothers. Open air defecation was found among 33.4% of the children. Regarding the toilet usage, only 50.7% of the children were using the toilets in the home. Around 66% did not wash their hands with soap before taking food. Moreover, 44.6% did not wash their hands with soap after defecation. Around 63% children regularly used foot wear and around 70% of the children had trimmed nail during the visit. Nearly half of the parents of children had adequate knowledge on STH. Only 59% reported intake of de-worming tablets in the past 3 months.{Table 1}{Table 2}

The prevalence of STH among 610 children who had given one adequate stool sample and whose parents participated in survey was 7.70% (95% CI: 5.58–9.82). Among all STH, A. lumbricoides was highly prevalent 6.9% (95% CI: 4.89%–8.91%) followed by Hook worm 0.7% (0.04%–1.36%), and Trichuris trichura 0.2% (0.15%–0.55%).

There was no significant association found between STH and factors influencing STH by univariate analysis. However, when all independent variables were subjected to multivariate logistic regression analysis, only pucca houses offered protection OR: 0.34(95% CI: [0.10–0.91]) against STH.

Discussion

Relatively high nonresponse rate (25.52%) was noted in this study. It is also evident from a similar study by Shubha and Fatima[20] that surveys involving bio samples shows high nonresponse rate (30.9%) when compared to simple questionnaire-based surveys.

The prevalence of STH in the study population is 7.7% (95% CI 5.58–9.82%). The results of the current study were less than other studies conducted in the nearby areas.

The explanations for the low prevalence are more number of risk group (29.13%) were found among the nonresponders compared to younger children (22.72%, P = 0.02). The study results were based on single stool sample, modified formol-ether concentration (FEC) method with low sensitivity (63.1%)[21] which could have estimated the low prevalence. The other probable explanations are Mass Drug Administration (MDA) under NDD[15] might have changed the scenario.[5],[6],[7],[8],[9] However, there are no sufficient data from to compare prevalence before de-worming in this area to support this statement. Coimbatore first round of NDD was just completed and second round was on-going at the time of the survey (2016-Feb). Kattula et al.[8] also explains the reason for the low prevalence of STH (7.8%) in Vellore area is due to Albendazole supplied in MDA along with diethylcarbamazine since 2004 Deworming using albendazole rapidly declines the overall prevalence of STH, and this is evident from Mani et al.[22] study on efficacy of albendazole in Tamil Nadu. However, there are other studies reported with a higher prevalence in the nearby areas. Kaliappan et al.[23] explains the reason for the increased prevalence (39%) in Thiruvanamalai District in the year 2013 could be due to five stool samples collected among tribal population where open-air defecation and bare foot walking were common practice. Ragunathan et al.[16] explains the reason for higher prevalence (34.56%) reported in Pudhucherry, a coastal area using FEC method in year 2006 is due to poor hygienic practices of the participants.

This study showed the most common soil-transmitted helminth as A. lumbricoides with the prevalence of 6.9% (95% CI: 4.89%–8.91%) followed by Hook worm 0.7% (95% CI: 0.04%–1.36%) and T. trichura 0.2% (95% CI: 0.15%–0.55%). In general studies showed that A. lumbricoides and T. trichura were more common among urban children and hookworm infestation among rural children.[24]

Regarding the factors influencing STH pucca houses offered protection OR: 0.34(95% CI: [0.10–0.91]) against STH, by multivariate analysis. A cross-sectional study by Kattula et al [8].showed that children living in Hut/Kutcha houses are at higher risk for STH.

Kattula et al.[8] also showed that children of more than 10 years of age, living in households without toilet, open air defecation, unhygienic practices like keeping untrimmed nails are important risk factors for STH. Kattula et al.[8] showed that the consumption of deworming tablets offered protection. However, there was no significant association noted in this study. This is a cross-sectional study; hence, the cause-effect relationship cannot be determined and is the major limitation of this study. Moreover, the other limitations are as follows: the study results were based on single stool sample which could have estimated the low prevalence. The newer techniques with higher sensitivity would have given higher prevalence. Sample size was not adequate to comment on factors influencing STH.

Conclusion

The prevalence of STH in a rural area of Coimbatore is 7.7% (95% CI: 5.58–9.82), and is continuing as a public health problem. Open-air defecation is a biggest concern (33.4%), hence, more private, public toilets should be built in this area. The children should be trained to use toilet facilities either private or public. It is very important to raise the awareness about these infections; hence, parents of children with inadequate knowledge should be given health education. When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent reinfection, a long-term impact can be achieved.

The prevalence of STH in a rural area of Coimbatore is 7.7% (95% CI: 5.58–9.82), and is continuing as a public health problem. Open-air defecation is a biggest concern (33.4%), hence, more private, public toilets should be built in this area. The children should be trained to use toilet facilities either private or public. It is very important to raise the awareness about these infections; hence, parents of children with inadequate knowledge should be given health education. When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent reinfection, a long-term impact can be achieved.

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Conflicts of interest

There are no conflicts of interest.

References

1 World Health Organization. Focusing on STH: Towards an Integrated Approach for STH Control. Available from: http://www.who.int/intestinal_worms/en/http://whqlibdoc.who.int/publications/2012/9789241503129_eng.pdf?ua=1. [Last accessed on 2019 Feb 24].
2 Statement Regarding Soil Transmitted Helminths, by CDC. Available from: http://www.cdc.gov/globalhealth/ntd/. [Last accessed on 2019 Feb 24].
3 Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 2014;7:37.
4 WHO. Eliminating Soil-Transmitted Helminthiasis as a Public Health Problem in Children Soil-Transmitted Helminthiasis Progress Report 2001-2010. WHO; 2012.
5 Ramesh GN, Malla N, Raju GS, Sehgal R, Ganguly NK, Mahajan RC, *et al*. Epidemiological study of parasitic infestations in lower socio-economic group in Chandigarh (North India). Indian J Med Res 1991;93:47-50.
6 Singh P, Gupta ML, Thakur TS, Vaidya NK. Intestinal parasitism in Himachal Pradesh. Indian J Med Sci 1991;45:201-4, 200.
7 Singh S, Raju GV, Samantaray JC. Parasitic gut flora in a north Indian population with gastrointestinal symptoms. Trop Gastroenterol 1993;14:104-8.
8 Kattula D, Sarkar R, Ajjampur SS, Minz S, Levecke B, Kang G. Prevalence & risk factors for soil transmitted helminth infection among school children in south India. Indian J Med Res 2014;139:76-82.
9 Naish S, McCarthy J, Williams GM. Prevalence, intensity and risk factors for soil-transmitted helminth infection in a South Indian fishing village. Acta Trop 2004;91:177-87.
10 World Bank. The global burden of disease. Appendix B. In: World Development Report: Investing in Health-World Development Indicators. New York: World Bank and Oxford University Press; 1993. p. 213-25.
11 Richmond JB, Kotelchuck M. Personal health maintenance for children. West J Med 1984;141:816-23.
12 Abera B, Alem G, Yimer M, Herrador Z. Epidemiology of soil transmitted helminths, Schistosomamansoni and haematocrit values among schoolchildren in Ethiopia. J Infect Dev Ctries 2013;7:253-60.
13 Curtale F, Pezzotti P, Sharbini AL, Al Maadat H, Ingrosso P, Saad YS, *et al*. Knowledge, perceptions and behaviour of mothers toward intestinal helminths in Upper Egypt: Implications for control. Health Policy Plan 1998;13:423-32.
14 World Health Organisation. Control of Tropical Disease. Geneva: World Health Organisation; 1998. Available from: http://www.who.int/neglected_diseases/en/. [Last accessed on 2019 Feb 24].
15 Information Regarding National Deworming Day in India from NHM Website. Available from: http://nrhm.gov.in/nrhm-components/rmnch-a/child-health-immunization/national-deworming-day-august-2016.html. [Last accessed on 2019 Feb 24].
16 Ragunathan L, Kalivaradhan SK, Ramadass S, Nagaraj M, Ramesh K. Helminthic infections in school children in Puducherry, South India. J Microbiol Immunol Infect 2010;43:228-32.
17 World Health Organization. Manual of Basic Techniques for a Health Laboratory. 2nd ed.. Geneva: World Health Organization; 2003.
18 World Health Organization. Bench Aids for the Diagnosis of Intestinal Parasites. Geneva: World Health Organization; 1994. Available from: http://apps.who.int/iris/handle/10665/37323. [Last accessed on 2019 Feb 24].
19 Chatterjee KD. Text Book of Parasitology. 13th ed. Darya Ganj - New Delhi, India: CBS Publishers & Distributors Pvt Ltd; 2015.
20 Shubha D, Fatima F. A coprological survey for assessing intensity of parasitic infection in school children: Cross-sectional study. Trop Parasitol 2011;1:88-93.
21 Yimer M, Hailu T, Mulu W, Abera B. Evaluation performance of diagnostic methods of intestinal parasitosis in school age children in Ethiopia. BMC Res Notes 2015;8:820.
22 Mani TR, Rajendran R, Munirathinam A, Sunish IP, Abdullah S, Augustin DJ. Efficacy of co-administration of albendazole and diethylcarbamazine against geohelminthiases: A study from South India. Trop Med Int Health 2002;7:541-8.
23 Kaliappan SP, George S, Francis MR, Kattula D, Sarkar R, Minz S, *et al*. Prevalence and clustering of soil-transmitted helminth infections in a tribal area in southern India. Trop Med Int Health 2013;18:1452-62.
24 Edirisuriya C, Beard FH, Hendry AJ, Dey A, Gidding HF, Hueston L, *et al*. Australian rubella serosurvey 2012-2013: On track for elimination? Vaccine 2018;36:2794-8.

Table 2 Association of soil transmitted helminthiasis with risk factors by univariate and multivariate analysis

Table 1. Association of soil transmitted helminthiasis with socioeconomic and demographic factors by univariate and multivariate analysis

			No.	Unadjusted	Adjusted	
[illegible]	<5	24	229	1.52 (0.80-2.90)	1.45 (0.76-2.77)	0.24
	>5	22	284	1	1	
[illegible]	Male	24	268	1.15 (0.60-2.18)	1.07 (0.58-2.00)	0.82
	Female	23	245	1	1	
[illegible]	Less than 5	22	253	1.18 (0.56-2.05)	1.21 (0.64-2.27)	0.55
	[illegible]	25	313	1	1	
[illegible]	Rural	1	116	0.36 (0.04-1.03)	0.34 (0.13-0.91)	0.05
	Urban Semi-permanent Mud-to-Brick	23	448	1	1	
[illegible]	Illiterate	16	116	1.08 (0.46-2.29)	1.03 (0.43-2.30)	0.94
	Literate	32	450	1	1	
[illegible]	Low SES	27	74	1.14 (0.60-2.19)	1.10 (0.56-2.20)	0.78
	High SES	21	274	1	1	

[illegible]

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Psychological well- being of women workers in a garment factory of South India-A cross sectional study

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ABSTRACT

Background: Working women are constantly facing the problems of role conflict or dual role. Due to role conflict, there are reports of psychological distress, mental tension, anxiety, etc, which affect the psychological well being of working women. Due to outside work, they have psychological problems such as anxiety, stress, burn out, fatigue, etc. This way, the mental health status of working women is neglected and hence, the present study **aims** to assess the psychological wellbeing of women in an industrial setup. **Methods:** A cross sectional study was done among women workers of 18 years and above. A standardized questionnaire was used to collect data. The data was analyzed using SPSS version 24. A total of 136 participants completed the study. **Results:** Present study showed that majority of the workers (67%) had low level of psychological well-being. **Conclusion:** Women workers have low level of psychological well-being and further studies are needed to understand factors influencing it.

Key Words: Psychological well-being, Women workers, Garment industry.

INTRODUCTION

"The mind is everything. What you think you become." – Buddha

According to the World Health Organization (WHO), mental health includes "subjective well-being, perceived self-efficacy, autonomy, competence, inter-generational dependence, and self-actualization of one's intellectual and emotional potential, among others". WHO further states that the well-being of an individual is encompassed in the realization of their abilities, coping with normal stresses of life, productive work and contribution to their community. [1]

Psychological well-being is a very subjective term meaning contentment, satisfaction with all elements of life, self-actualization (a feeling of having achieved something with one's life), peace and happiness.[2]Psychological well-being is defined as the presence of psychological and social skills and abilities that contribute to optimal functioning in daily life. [1]

The concept of mental health is inextricably linked to that of psychological well-being. A comprehensive model developed by C. Ryff provides an integration of different viewpoints. It encompasses six inter-related psychological dimensions: positive evaluation of one's self (Self-Acceptance), a sense of continued growth and development (Personal Growth), the belief that life is purposeful and meaningful (Purpose in Life), the possession of quality relations with others (Positive Relations with Others), the capacity to manage effectively one's life (Environmental Mastery), and a sense of self-determination (Autonomy). Lack of psychological well-being has been found to be a risk factor for depression. [3]

Garment workers around the world, especially those involved in tailoring, are predominantly women. The Indian garment industry is dominated by women workers and 80% of them are young women between 21-25 years. [4]

The workplace is one of the key environments that affect our mental well-being and health. It is difficult to quantify the impact of work alone on personal identity, self-esteem and social recognition; most mental health professionals agree that the workplace environment can have a significant impact on an individual's mental well-being. [5]

Working women have many problems such as career related problems, social problems such as domestic violence, health problems such as psychological distress due to role conflict, mental stress, tension, anxiety, physical health problems such as Anemia, arthritis, etc.

As discussed above, due to outside work, they have psychological problems such as anxiety, stress, burn out, fatigue, etc. This could lead to neglect of mental health. Hence this study is proposed with the objectives of

assessing the psychological well-being of women workers in garment industries and to find out its association with socio demographic factors.

MATERIALS & METHODS

A cross sectional study was conducted in a selected garment industry in Tirupur district of Tamilnadu state. This factory is mainly involved in manufacture of garments for export. Majority of the workers are unmarried women.

Sample size: Based on 63.5% prevalence of moderate level of psychological well-being study done by K.S.Velmurugan, Dr.K.Maheswari, in Tiruchirappalli [6] sample size was calculated as 136.

Inclusion criteria: There were 200 women workers above 18 years in our study factory. Prestuctured and pre validated questionnaire was administered to 136 women workers who were selected by simple random sampling.

Study tool: Carl Ryff's 18 item psychological well-being questionnaire translated into local language was used capturing various dimensions: autonomy, environmental mastery, personal growth, positive relations and self-acceptance, purpose in life. These items were measured using Likert scales of 1 to 6, with 1 indicating strong disagree and 6 indicating strong agree. The maximum score for psychological well-being that could be obtained under each dimension is 18.The maximum score that could be obtained for overall psychological well-being is 128.

Data collection: Data collection was done using the above tool as self administered questionnaire. Data entry and analysis was done using Statistical Package for Social Sciences (SPSS) version 24. The data were analyzed by using mean, standard deviation and two-way analysis of variance (ANOVA) and p <0.05 was considered statistically significant.

Ethical Issues: Approval for the study was obtained from the Institutional Human Ethics Committee of PSG Institute of Medical Sciences and Research.

RESULTS

A total of 136 participants completed the survey. The study participants were in the age group of 18 to 25 years. The participants were representative of different sections of the factory with majority (45%) being tailors. The demographical details of the participants are shown in Table1.

Table 2 shows the mean scores of the participants under each dimension of psychological well-being. For all dimensions the mean score was 12 and below which indicates low psychological well-being. The findings indicated that workers scored high in personal growth (M= 12.02, sd= 4.05) and least in environmental mastery (M=10.10, sd=4.05).

Table 1: Demographic details of the participants

Demographic factors	Percentage
Age	
Below 20 years	58%
Above 20 years	42%
Education Qualification	
High school	48%
Hr.sec.school	40%
Diploma	4%
Graduate	8%
Work experience	
Below 1 year	57%
Above 1 year	43%
Type of family	
Nuclear family	73%
Joint family	27%
Residence	
Hostel	97%
Day scholar	3%

Table 2: Overall level of workers Psychological well-being

Indicators of Psychological well-being	Mean±SD
Autonomy	11.50±3.39
Positive relations	11.92±2.37
Self-acceptance	10.13±3.52
Environmental mastery	10.10±4.05
Purpose in life	11.66±2.24
Personal growth	12.02±4.05
Total score	67.36±13.20

Table 3: Association of various factors with psychological well-being

Socio economic Factors	Total score of Psychological well-being (Mean±SD)	p value
Age		
Below 20 years	67.69±12.74	0.728
Above 20 years	66.89±13.91	
Education Qualification		
High school	68.39±13.26	
Hr.sec.school	67.59±13.14	
Diploma	68.20±14.06	0.241
Graduate	59.63±11.84	
Work experience		
Below 1 year	67.02±12.26	0.733
Above 1 year	67.81±14.46	
Type of family		
Nuclear family	67.02±13.00	0.625
Joint family	68.27±13.84	

Table 3 shows the association of various demographic variables with psychological well-being. Age was categorized less than 20 and more than 20, also between various educational level and work experience of at least a

year and whether they belonged to nuclear or joint family. The findings indicated that there is no significant difference between demographic factors like age, education and years of education with psychological well-being.

DISCUSSION

The present study showed that overall psychological well-being of women garment workers was low (mean value 67.36). In garment industry women workers work for longer hours (10 hours). Also due to high production pressure, hourly targets are set for workers. Staying away from family and having long working hours could be reasons which need to be explored.

Our study findings revealed relatively higher score for personal growth compared to other dimensions. It indicates that women workers had more positive feelings towards their continued development and ready to experience new challenges in life. Similarly, a study done on 2015 showed that a high score in personal growth and positive relations subscales. [7]

The result of present study concurs with a similar study done in Perambalur which showed that women in higher age have low psychological well-being. In our study there is no significant difference between workers age & their psychological well-being. But a woman with higher age shows low psychological well-being. In the same study it was found that women with more work experience had higher psychological wellbeing. In our study though not significant the mean score was comparatively higher among women with more work experience. [6]

In our study we couldn't find out any reasons for poor psychological well-being. Instead, we say above mentioned reasons could be the reason for poor psychological well-being. The present study concurs with a similar study, done in Mysore where the mean score for psychological well-being was 193. [8]

Conclusion and way forward

From the study we conclude that psychological well-being is low among women workers. Management should develop policies for addressing mental health issues and creating access to counseling services.
Though quantitative analysis did not reveal statistical association between psychological well-being and demographic profile, further qualitative studies would help in better understanding the factors influencing psychological well-being.

REFERENCES

1. Mental health [internet].[Updated 2019 March 7]. Available from: https://en.wikipedia.org/wiki/Mental_health
2. Psychological well-being: Staff and organization support program [internet]. 2010 Dec. Available from:https://www.sosp.org.mt/2010/12/22/psychological-well-being/
3. Ryff CD, KeyesCLM. The structure of psychological well-being revisited. Journal of personality and social psychology 1995; 69:719.
4. Suhasini Singh. Fashionable and famous - at the garment worker's cost.Global Research Publisher 2009;Available from:https://www.globalresearch.ca/india-cheap-labor-garment-export-industry/13530
5. Mental health and work: Impact, issue and practice. Nations for mental health 2000; Available from: https://www.who.int/mental_health/media/en/712.pdf
6. Velmurugan KS, Dr.Maheswari K. A Study on Psychological Well-Being of Women Working in Southern Railways Tiruchirappalli. International Journal of Scientific Research and Management 2017; 5:7370-75.
7. Melissa Ludban, Priscilla N, Gitimu. Psychological well-being of college students. Undergraduate Research Journal for the Human Sciences 2015; 14.
8. Srimathi NL, Kiran Kumar SK. Psychological Well being of Employed Women across Different Organisations. Journal of the Indian Academy of Applied Psychology 2010; 36:89-95.
9. Appendix: Items on Well-Being Measures 2016; available from http://online.fliphtml5.com/ffch/avfx/
10. Madhuchandra MK, Srimathi NL.Psychological Well-being among Doctors and Nurses: A Comparative Study. The International Journal of Indian Psychology 2016;3:203-10
11. Alex M. Wood, Stephen Joseph. The absence of positive psychological (eudemonic) well-being as a risk factor for depression: A ten year cohort study. Journal of Affective Disorders 2010; 213–17
12. Deepthi Shanbhag, Bobby Joseph. Mental Health Status of Female Workers in Private Apparel Manufacturing Industry in Bangalore City, Karnataka, India. International Journal of Collaborative Research on Internal Medicine & Public Health 2012; 4:1893- 1900
13. Richa Aryan,Deepika Kathuria. Psychological Wellbeing at Workplace:-An Analytical Study on It Sector. International Journals of Advanced Research in Computer Science and Software Engineering 2017; 7:223-28

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Special Report

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Quick Response Code:

Research Priorities in Maternal, Newborn, & Child Health & Nutrition for India: An Indian Council of Medical Research-INCLEN Initiative

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In India, research prioritization in Maternal, Newborn, and Child Health and Nutrition (MNCHN) themes has traditionally involved only a handful of experts mostly from major cities. The Indian Council of Medical Research (ICMR)-INCLEN collaboration undertook a nationwide exercise engaging faculty from 256 institutions to identify top research priorities in the MNCHN themes for 2016-2025. The Child Health and Nutrition Research Initiative method of priority setting was adapted. The context of the exercise was defined by a National Steering Group (NSG) and guided by four Thematic Research Subcommittees. Research ideas were pooled from 498 experts located in different parts of India, iteratively consolidated into research options, scored by 893 experts against five pre-defined criteria (answerability, relevance, equity, investment and innovation) and weighed by a larger reference group. Ranked lists of priorities were generated for each of the four themes at national and three subnational (regional) levels [Empowered Action Group & North-Eastern States, Southern and Western States, & Northern States (including West Bengal)]. Research priorities differed between regions and from overall

Supplementary material available from *http://www.ijmr.org.in/article.asp?issn=0971-5916;year=2017;volume=145;issue=5;spage=611;epage=622;aulast=Arora*

national priorities. Delivery domain of research which included implementation research constituted about 70 per cent of the top ten research options under all four themes. The results were endorsed in the NSG meeting. There was unanimity that the research priorities should be considered by different governmental and non-governmental agencies for investment with prioritization on implementation research and issues cutting across themes.

Key words Child health - Child Health & Nutrition Research Initiative - Indian Council of Medical Research - INCLEN - maternal health - Maternal, Newborn, and Child Health and Nutrition - newborn health - nutrition - research priority setting

National Steering Group[18]

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Nation-wide Network[18]

Names of participants in the Nation-wide Network are provided in Supplementary Table I (available on *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm6.pdf*) and also on *www.inclentrust.org*

Prioritization of research options in health is essential to plan for achieving efficient and impactful investment of limited resources against a large number of competing research options[1]. There is an increasing need and effort to set research priorities in health in a systematic way using a sound and transparent methodology and through engagement of the various key stakeholder constituencies[2,3]. Research priority setting (RPS) in India has traditionally been guided by a small group of experts, mostly identified from major metropolitan areas of the country. Subsequent to India's impressive yet inadequate improvement towards achieving Millennium Development Goals (MDGs) 1, 4 and 5, an expert group had convened on May 23, 2011 at the Indian Council of Medical Research (ICMR) Headquarters in New Delhi to discuss RPS. The participants observed that evidentiary gaps in the country impeded informed action in Maternal, Newborn, and Child Health and Nutrition (MNCHN) themes, and that an inclusive and transparent method should be adopted to decide the national research agenda. The agenda so developed should also identify areas for innovation and strategies to improve deliverability, efficiency, scalability and sustainability of existing interventions, avoid common blind spots in MNCHN research (*e.g*., still birth & neonatal health) and engage the multiple stakeholder constituencies including end users of any funded health research activity. This led to the formalization of the ICMR-INCLEN collaboration for undertaking the national RPS exercise for MNCHN using the Child Health and Nutrition Research Initiative (CHNRI) methodology. Since 2006-2007, the CHNRI priority setting methodology has been increasingly acknowledged as a flexible yet systematic priority-setting method for ranking competing research options using an objective, quantified and inclusive approach[2-7]. The method is opined to be effective at the national level where the results derived from inputs from national stakeholders can have a direct and prominent impact on the research investment policy[8]. As a major conceptual advancement, this method ranks the broadly defined competing research options that not only generate new knowledge but also synthesize evidence for efficient implementation of what is already known[9]. The ranking of these research options employs 'crowdsourcing' through the engagement of a wide range of stakeholder constituencies with due cognizance of their core expertise. The various dimensions of the research priorities are carefully examined by the experts using a predefined context and set of strictly defined scoring criteria. The collective optimism of the stakeholders, which can be considered as the 'wisdom of the crowd', helps in identifying research priorities through a democratic method of scoring, weighting and ranking of competing research options[1,5].

National Research Priority Setting (RPS) exercise for Maternal, Newborn, and Child Health and Nutrition (MNCHN)

The protocol was reviewed and approved by the Independent Institutional Ethics Committee of INCLEN, and the exercise was undertaken between 2012 and 2016. The context, in which the research priorities were identified in this exercise, are provided in Box 1.

The exercise was woven around four key structures, namely, the National Steering Group (NSG), the Thematic Research Subcommittees (RSCs) constituted for each of the four MNCHN themes (112 experts in India were identified based on active contribution to contemporary research), the Nation-wide Network for Crowdsourcing (experts of various disciplines related to MNCHN with due attention to regional representation

Box 1. Context of the Indian Council of Medical Research-International Clinical Epidemiology Network (ICMR-INCLEN) National Research Priority Setting Exercise for maternal, newborn, child health and nutrition

Purpose: Priority setting in maternal, newborn, and child health and nutrition for efficient and rewarding investment in research using a systematic, transparent, inclusive and consultative method.

Geography: India (National) and three regional levels *viz*., Empowered Action Group (EAG) & North-Eastern States, Southern & Western States, and Northern States & West Bengal.

Target population: Women of reproductive age (15-49 yr); pregnant women, newborns (0-28 days), under-five children (0-59 months) and children (up to the age of 18 yr).

Major areas of concern for research: Conditions that together contributed to at least 75% of the mortality and morbidity burden in Maternal, Newborn, Child Health and Nutrition in India during 2012-2013 as per the available evidence and expert opinion.

Time frame: 2016-2025.

Stakeholder constituencies (public and private sectors, health and non-health sectors): Researchers, professionals, public health functionaries, policy makers, communities and their leadership, civil society and donor agencies.

Translation and implementation context: Public and private health systems of India and their existing as well as future policies and programmes.

Source: Ref. 11, reproduced with permission with minor modifications

from 256 institutions) and the Larger Reference Group (LRG) (84 members group) constituted with Central and State policy decision makers, politicians and bureaucrats from key ministries, MNCHN programme managers, eminent researchers and representatives from research funding organizations. The list of participants in the Nationwide Network is provided in Supplementary Table I (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm6.pdf*). [The list of all participants in the exercise with respective institutional affiliation is available on *www.inclentrust.org*].

The NSG for the exercise, co-chaired by the Secretary, Department of Health Research (DHR) and Director General (DG)-ICMR and the Executive Director of The INCLEN Trust International, was formed with key officials from concerned Ministries, namely, Ministry of Health & Family Welfare (MoHFW) (National Health Mission, Child Health, Maternal Health and Nutrition Divisions, Directorate General of Health Services and DHR-ICMR), Ministry of Women and Child Development [Integrated Child Development Services (ICDS), Food and Nutrition Board] and Ministry of Science and Technology (Department of Biotechnology, Department of Science & Technology). There was a wide range of invited subject experts, Central and State programme managers, national and international donors and multilateral agencies, who were members. Two NSG meetings were organized, one at the initiation of the exercise to ratify the context and protocol (April 18, 2013) and second, at the conclusion for reviewing and endorsing the findings (February 4, 2016).

The Thematic RSCs contributed to research ideas in the first round of crowdsourcing, helped in refining and consolidating the ideas into research options, finalizing the scoring criteria, participated in second round of crowdsourcing for scoring the research options and presented the results to the NSG for ratification.

The Nationwide Network of experts participated in the first and second round of crowdsourcing for pooling of research ideas and scoring the research options, respectively.

The LRG assigned relative weights to the five scoring criteria, namely answerability, relevance, equity, innovation and out-of-the-box thinking and investment on research.

The exercise was centrally coordinated by the RPS Management Team at the Executive Office of The INCLEN Trust International, New Delhi.

The RPS Management Team established a national network of 1066 experts from institutions across India during 2012-2013. During the same period, it completed an extensive review of literature (research, academic and policy documents) on burden of diseases of MNCHN in India and presented the summary to the NSG at its first meeting. The NSG decided that topmost causes collectively accounting for at least 75 per cent of mortality and morbidity burden (Areas of Concern) for each of the RPS themes so identified shall guide pooling of research ideas and subsequent framing of research options for priority setting. Between September and December 2013, 3498 research ideas were pooled from the nationwide network using custom-designed online software (1st round of crowdsourcing). The participation rate was 42.3 per cent [of the 1178 experts contacted (1066-national members and 112-RSC members), 498 experts contributed research ideas]. Several of these research ideas were narrations comprising more than one research idea spanning across themes, areas of concern and domains. With the help of RSC members (January 2014-February 2015), these were split and refined into 4003 research ideas that were further consolidated into 373 research options (Maternal: 122, Newborn: 56, Child: 101 and Nutrition: 94) (*http://inclentrust.org/inclen/?page_id=8666*). Five criteria (answerability, relevance, equity, innovation and out-of-the-box thinking and investment on research) were identified and defined for scoring the research options (March-June 2015) (Box 2). This was done through a comprehensive review of criteria that had been used in previous exercises at national and global levels and iterative discussion with RSC members and national and international CHNRI experts (University of Edinburgh, Johns Hopkins Bloomberg School of Public Health and the WHO).

Scoring of the research options was done independently by 893 experts (of the 1536 experts approached; Participation rate: 58.1%) using a customized online (*SurveyMonkey.com*) platform (July-October, 2015) (2nd round of crowdsourcing). The scores obtained by the research options were adjusted with criteria weights as assigned by the LRG (November, 2015-January, 2016) as per standard CHNRI data analysis practices[10]. The Average Expert Agreement (AEA) was also calculated for each research option score. The detailed methodology and process adopted for this priority setting has been published

Box 2. Criteria used for scoring the research options

Answerability: Can the research be done through ethical, transparent, well-designed, 'do-able' studies with the existing local and national capacities and or by strengthening the existing capacities through regional or global collaboration?

Relevance: Is it likely that the research would address a high burden condition and critical gap in knowledge?

Innovation and out-of-box thinking to resolve complex and refractory challenges: Does the new research have the potential for transformative change in the health system/ health care?

Equity: Is it likely that the research product will address the differences in health and nutrition that are systematically associated with social, cultural and economic hierarchies, ethnicity, gender, environment and geographic disadvantages, thereby reducing inequities?

Investment on research: Is it likely that the potential impact and benefits of the new knowledge on health/ nutrition will outweigh the considerations of investments on research?

Source: Ref. 11, reproduced with permission

elsewhere[11]. The research options were categorized into four domains; description, discovery, delivery and development.

In addition to national priorities, the States and Union Territories in the country were grouped into three subnational regions to obtain regional priorities [Empowered Action Group (EAG) and North-Eastern States, States in Western and Southern India and States in Northern India (including West Bengal)] (Fig. 1).

The number of scorers ranged between 60 and 96 scorers across each region and theme. Therefore, four priority lists were obtained for each theme: one national and three regional priority lists. The NSG reviewed and discussed the ranked list of priorities and made focused observations on the patterns of research that had evolved as priorities and the possible interlinks between the priorities identified. It also discussed other potential priority research options that might not have made it to the topmost priority list. This helped enrich the structuring of the national research agenda and identify the way forward.

Outcome of the research priority setting (RPS) exercise in the four themes

About 43 per cent of all the research options scored pertained to the delivery domain and required implementation research methodology (Fig. 2). It was observed that in each of the four themes, a large



Fig. 1. Geographical context of the ICMR-INCLEN National Research Priority Setting Exercise for Maternal, Newborn, and Child Health and Nutrition (2016-2025) (Map courtesy: *www.mapsofindia.com* modified with permission).

proportion (70%) of the top ten prioritized issues were dealing with delivery domain of research and implementation of programmes. Amongst the top ten priorities identified at national level, delivery domain research options accounted for 80 per cent in maternal health, 70 per cent in newborn health, 60 per cent in child health and 70 per cent in nutrition themes. Significant differences were also observed between national and regional research priorities for all the four themes. The AEA was very high for the top ten priorities of research in the four themes both nationally and regionally. There were research options in the top ten regional priorities under various themes that did not figure in the national priority list. The Table enlists the top ten priorities identified under each theme by the exercise at the national level. Top ten priorities and their scores along with the national and region-specific ranks are provided in Supplementary Table II (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm7.pdf*). Key priority research options under each MNCHN theme are summarized below.

Maternal health

The network indicated the need for developing and evaluating screening checklists and management algorithms for severe acute maternal morbidities (SAMMs), near-miss events, high-risk pregnancies,

Table. Top ten priority research options in maternal, newborn, and child health and nutrition themes at national level

Theme	Rank	Research option
Maternal Health	1	Development and validation of algorithms for prevention, early detection and management of severe acute maternal morbidities and near-miss events in resource-constrained settings
	2	Strategies to improve quality of care during childbirth in the public health system, for example, medical practices, LSCS, active management of third stage of labour, EmOC, biophysical profiling for foetal assessment, application of epidural anaesthesia during delivery; beneficiary counselling and communication inside the labour room
	3	Early identification, referral and management of high-risk pregnancies (having maternofoetal morbidities including IUGR, stillbirths and preterms) at all levels of health care
	4	Introduction of a validated and cost-effective cancer screening programme of reproductive system for women in the community and health facilities
	5	Improving EmOC services [*e.g*., risk prediction, identification and communication; prompt referral; service availability (safe transportation, skilled personnel, capacity, logistic, blood storage); accountability; innovations]
	6	Assess blood transfusion needs, current availability and delivery mechanisms of blood for pregnant women based on PHC and CHC catchment areas in the context of prevailing burden of severe anaemia and post-partum haemorrhage
	7	Implementation research for effective delivery of evidence-based care protocols/algorithms for prevention and management of post-partum haemorrhage at different levels of care
	8	Improving maternal death audits, protocols and practices in the public health care system (body handling, support to family, communication, autopsy, death audit and causality ascertainment)
	9	Process and impact evaluation of public health programmes targeted for adolescents (ARSH, AFHS, RMNCH+A, RTI/STI screening services) in urban and rural areas
	10	Develop and validate a self-assessment check list for pregnant women to identify warning signs and need for care seeking
Newborn Health	1	Designing and evaluating curriculums for skill building and their retention for health personnel involved in newborn care in the community and at various levels of health care system (*e.g*., training in identification of warning signs, safe injection practices, administration of oxygen therapy, *etc*.)
	2	Engaging and empowering family members and community in the care of newborn (including family centred care): barriers, strategies to overcome, impact, cost-effectiveness
	3	Identifying appropriate and effective strategies (messages and channels of communication) to promote community awareness on newborn care practices and social mobilization for early healthcare seeking (including utilization of existing nutrition and health services) to prevent adverse outcomes
	4	Low cost, feasible, portable technological innovations in equipment to improve capacity (diagnosis, identification and management) and outreach for foetal & neonatal care (especially, LBW, preterm: CPAP, surfactant therapy, *etc*.,) at various levels of the health system and their impact evaluation
	5	Improving the implementation (service availability, quality, programme management and referral chain robustness) of neonate centric programmes and services (RMNCH+A, JSSK, NSSK, IMNCI & F-IMNCI, SNCUs, *etc*.)
	6	Implementation of an integrated and comprehensive maternal and newborn healthcare package for delivering continuum of care: barriers, strategies to overcome, need for governance modification, maternal and newborn outcomes
	7	Strategies to scale up home-based newborn care: Role assignment and rationalization for frontline workers, barrier identification and mitigation, cost-effectiveness, impact

Contd...

Theme	Rank	Research option
	8	Strategies for social, economic, skill and knowledge empowerment of women and its impact on newborn, child and women's health
	9	Establishing an innovative framework of monitoring and supervision with in-built mechanism of accountability to improve performance of frontline workers and health personnel involved in neonatal care (*e.g*., physical supervision; engaging PRIs and clients; use of ICT, telemedicine, maternal health)
	10	Development and validation of protocols for the management of pregnant women at risk of pre-term delivery, in the healthcare system [*e.g*., nutritional, pharmaceutical (steroids, betamimetics, progesterone, nitroglycerine patches, prophylactic antibiotics, *etc*.), surgical, exercise and lifestyle counselling]
Child Health	1	Develop locally relevant cost-effective strategies to expand the coverage of UIP by reaching segments of populations that are traditionally left out (address system 1 and community-Level 2 challenges) (*i*) VPD epidemiology, system capacity, cold chain, safety surveillance; (*ii*) Hesitancy, dropout, outreach strategies, KAP of care provider, community and clients
	2	Improving administrative data quality and strengthening data-driven child health programme monitoring, action and accountability at PHC and district levels (*e.g*., line listing of households with children with NDD, use of ICT, develop novel indicators)
	3	Development and validation of low-cost technologies for screening, referral and management of childhood pneumonia and ARI in the community and at various levels of health care (*e.g*., maternal health, point-of-care diagnostics & therapeutics, management protocols, *etc*.)
	4	Strategies to promote WASH practices in the community to improve child health and nutrition
	5	Development of cost-effective, feasible, validated point-of-care diagnostics for malaria in children for use at community and different levels of healthcare
	6	Development of evidence-based guidelines for rational use of antibiotics for childhood morbidities in India: choice of antibiotic; route and delivery systems (*e.g*., nebulizers); duration of therapy; monitoring criteria; adjunct therapies
	7	Development of an integrated child health programme for improving quality of life of children: challenges and barriers; strategies to overcome; feasibility across the country; effectiveness, cost-effectiveness
	8	Establishing an effective and sustainable vaccine preventable disease surveillance programme (especially, measles and rubella, pneumonia and diarrhoea) in India [*e.g*., defining syndromes (fever and rash) and programme thresholds, forging PPPs, building upon polio infrastructure, using technology (maternal health, GIS, *etc*.)]
	9	Identifying cost-effective strategies for supplementation of micronutrients and probiotics to prevent and control childhood diarrhoea, pneumonia and other infections
	10	To establish nationwide multicentric antimicrobial surveillance and antibiotic stewardship programme for infectious morbidities during childhood
Nutrition (Maternal and Child)	1	Identify and evaluate strategies to promote healthful lifestyle (physical activity and diet behaviour) in children through school and home-based interventions
	2	Determine characteristics of mother friendly work place policies and governance framework that enable optimal care and nutrition of pregnant and lactating women and their children: identify barriers and challenges to implement (*e.g*., financial security and compensation for loss of pay; crèches at workplaces; provision for breast milk expression and storage for working mothers)
	3	Process, impact and economic evaluation of NRCs for management of severely malnourished children (*e.g*., quality of care and client satisfaction; implementation gaps and challenges, reasons for underutilization and relapse; IEC to mothers during stay and at discharge; impact assessment, effectiveness of the RUTF used in NRCs and plausibility of indigenous preparation with the help of SHGs)

Contd...

Theme	Rank	Research option
	4	Identifying strategies for engaging the male partners, families and communities to improve the nutrition of women of reproductive age group and under-five children
	5	Process, impact, and economic evaluation of community-based management of childhood malnutrition (including SAM): role, effectiveness and accountability of various stakeholders (including frontline workers)
	6	Impact and economic evaluation of WASH practices in the community on the nutrition of women and children
	7	Cost-effective strategies to improve the quality, quantity and coverage of food supplements provided under the Mid-Day Meal Programme to improve the nutritional status of school-going children
	8	Determining optimal growth trajectory of LBW (preterm, SGA) babies: nutrient and calorie requirements; strategies to minimize, mitigate development of chronic diseases
	9	Development and popularisation of improved varieties of traditional food items rich in micronutrients (*e.g.*, iron rich millets): adoption of viable business models and modifying value and supply chains
	10	Strategies to overcome barriers and improve implementation of WASH practices in the community with particular focus on poor, socially disadvantaged groups

AFHS, Adolescent Friendly Health Services; ARI, Acute respiratory infections; ARSH, Adolescent reproductive and sexual health; CHC, Community Health Centre; CPAP, Continuous positive airway pressure; EmOC, Emergency obstetric care; F-IMNCI, Facility-based Integrated Management of Newborn and Childhood Illnesses; GIS, Geographic information system; ICT, Information and communication technology; IEC, Information, education, communication; IMNCI, Integrated Management of Newborn and Childhood Illnesses; IUGR, Intra uterine growth restriction; JSSK, *Janani Shishu Suraksha Karyakram*; KAP, Knowledge, attitude and practice; LBW, Low birth weight; LSCS, Lower segment cesarean section; NDD, Neuro-developmental disorders; NRC, Nutrition rehabilitation centres; NSSK, *Navjaat Shishu Suraksha Karyakram*; PHC, Primary Health Centre; PPP, Public private partnership; PRI, *Panchayati Raj* Institutions; RMNCH+A, Reproductive maternal newborn child and adolescent health; RTI, Reproductive tract infections; RUTF, Ready-to-use therapeutic food; SAM, Severe acute malnutrition; SGA, Small-for-gestational age; SHG, Self help group; SNCU, Special Newborn Care Units; STI, Sexually transmitted infections; UIP, Universal Immunization Programme; VPD, Vaccine preventable diseases; WASH, Water, sanitation and hygiene

post-partum haemorrhage, stillbirth, eclampsia and cancers. Development of point of care diagnostics and technological solutions for SAMMs, perinatal hypoxia and foetal distress were ranked as high priorities. Research on strategies to empower families and women for better self-care and timely care seeking, and skill enhancement (including role rationalization, task shifting and sharing) along with accountability of health providers at different levels were scored as important priorities. Process and impact evaluation of existing maternal health programmes to improve outcomes, developing implementation strategies to improve the quality of different aspects of maternal care in health system and expanding their coverage were considered important research areas. Development of pharmaceutical protocols for prevention and clinical management and novel technological solutions for identifying SAMMs, epidemiology of stillbirths and expanding coverage of reproductive tract infection (RTI)/sexually transmitted infection programmes and specific studies on symptomatic and asymptomatic RTI and its impact on stillbirth, low birth weight (LBW), intrauterine growth restriction (IUGR) and abortions were scored as important regional priorities.

Newborn health

Newborn health priorities included strategies to improve delivery and quality of care of newborn health-oriented programmes; skill and capacity enhancement of service providers including pre-service changes in the curriculum with in-built mechanisms of accountability; empowering mothers, families and communities to improve care seeking and quality of care in home environments; multicentric antimicrobial surveillance and antibiotic stewardship programme; development of point-of-care diagnostics/biomarkers for improving neonatal outcome, particularly for LBW neonates (preterm and IUGR) and neonatal sepsis; use of information and communication technology and utilization of m-Health to improve access to newborn care.

Child health

Six areas emerged prominently within child health: (*i*) issues related to coverage of Universal

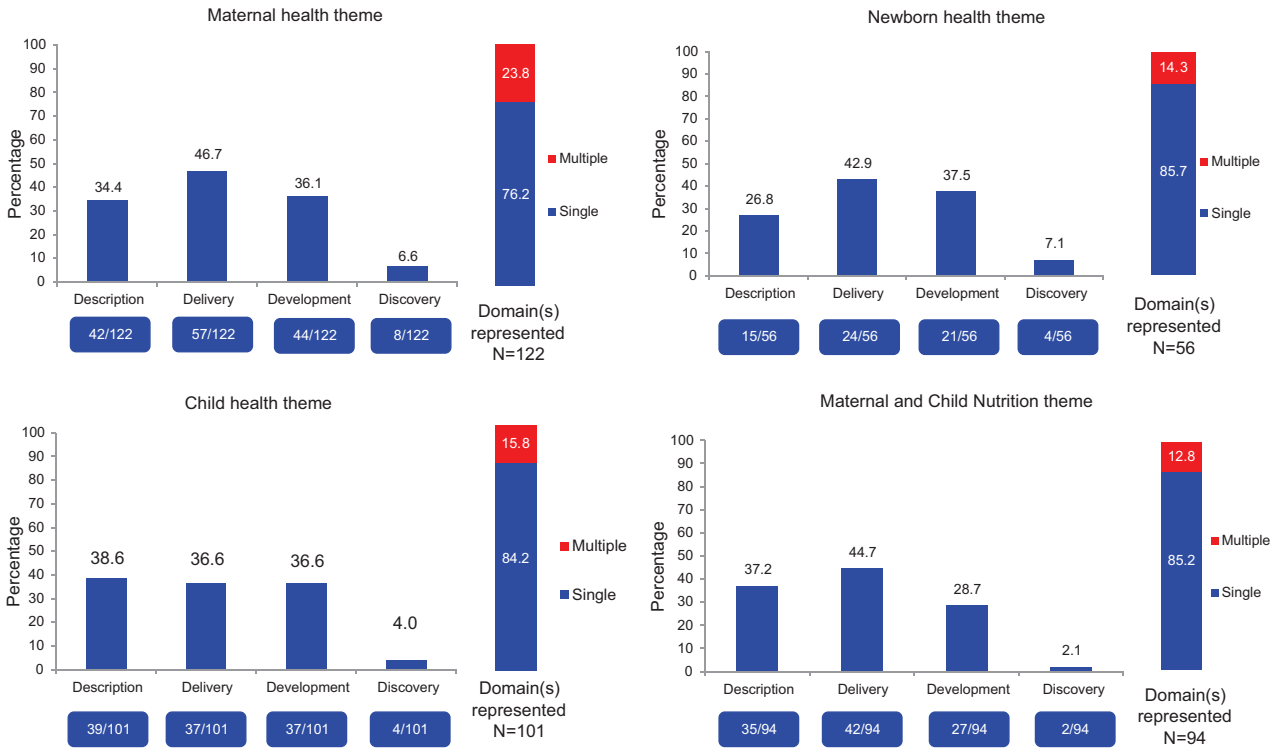


Fig. 2. Distribution of the research options in maternal, newborn, and child health and nutrition themes across the four domains of research.

Immunisation Programme (UIP) and surveillance for vaccine preventable diseases; (*ii*) point-of-care diagnostics; (*iii*) use of technology and development of biomarkers for the screening, referral and management of common childhood illnesses, namely, diarrhoea and acute respiratory infections (ARIs) along with improved control and management of malaria; (*iv*) rational use of antibiotics; (*v*) early childhood risk factors of adverse metabolic outcomes in later life, namely, obesity and metabolic syndrome; and (*vi*) water and sanitation hygiene (WASH). Operational and implementation research to effectively deliver different child health interventions in an integrated manner, including improving administrative data quality for decision-making, achieving Indian Public Health Standards at facilities, engaging community and its resources for better care seeking and care at home, strengthening curriculums for skill building and retention of personnel, was another area that scored high at both national and regional levels.

Maternal & child nutrition

Creation of mother friendly environments was an interesting and important priority to address issues of maternal and child nutrition. Research options that addressed the issues related to chronic diseases (*i.e.,* lifestyle behaviour modification interventions, point-of-care diagnostics and biomarkers for metabolic syndrome and nutrition of women around conception to prevent foetal programming and consequent risk for foetal origin of adult diseases) also scored high. Research options for enhancing skill of care providers and empowerment of families and communities including behavioural change communication were prioritized as was observed for other thematic areas. Research options around WASH (environmental and water hygiene) were scored high by experts across the country. Importantly, these research options were seen across all four themes. Research options to explore the performance and impact of various nutrition and food supplementation programmes (including community-based management of childhood malnutrition, midday meal, ICDS and food items provided under National Food Security Programme), improvement of administrative data quality for decision-making and the multisectoral governance of agriculture-nutrition-health nexus were prioritized at both national and regional levels. Anaemia (including issues related to absorption of nutrients), growth trajectory of LBW and breastfeeding were important cross-cutting areas for both mother and child perspective and were scored high.

The LRG had assigned the highest weight to the scoring criterion of 'relevance' and the lowest weight to 'investment in research'. The relative weights assigned to the five scoring criteria by different categories of members within the LRG did not vary significantly.

Discussion

The NSG strongly endorsed the research priorities identified by the exercise as appropriate for investment by different science and research departments of the Government of India and other national and international donor agencies and academic institutions over the next decade. It felt that the research agenda established with this exercise was in alignment with the national programme for achieving the recently enunciated health-related Sustainable Development Goals (SDGs) and will facilitate in accomplishing the unfinished agenda of the MDGs.

The exercise engaged experts who were well dispersed across the country. Thus, for the first time, regional MNCHN research priorities could be identified. In all the four thematic areas, there were differences between the top ten national and regional priorities. This observation made it imperative to take cognizance of the unique research needs of the regions. Although not part of the current project, the NSG suggested that a systematic effort must be made to determine factors that could explain differences in national and subnational (regional) priorities. The States falling under the three regions vary in terms of their health infrastructure, programme implementation and governance, development indicators and availability of baseline information on various health indicators. This may to some extent, explain the differences in the emerging research priorities. Better understanding of these factors will be important to design need-based research programmes and policies for different parts of the country. There are significant rural and urban differences for the health challenges and these have assumed greater significance in the light of rapid urbanization in certain parts of the country. The rural-urban divide will also have to be kept in mind when decisions on research investments are made.

In addition to the top priorities identified by the exercise, the NSG highlighted some more areas of research in MNCHN themes that deserve investment. These included role of environmental exposures in the occurrence of neurodevelopmental disorders such as autism; early childhood care and stimulation for improving cognitive outcomes; better description of childhood cancers and their risk factors; unsafe abortions; innovations to solve the 'nutrition enigma'; factors interfering with iron absorption in both women and children; impact of fluoride on health and nutrition of women and children; micronutrient deficiencies and occurrence of congenital birth defects (*e.g*., neural tube defects with folic acid deficiency); research into processes and strategies to make multisectoral governance for human nutrition workable and effective and technology innovation for developing better tools of nutrition assessment.

As part of Infant and Young Child Feeding and Complementary Feeding research agenda, NSG has also suggested that it is important to innovate strategies to promote breastfeeding; innovation is also needed in the development of low cost nutritious and healthy processed foods that require minimal cooking, are prepared from indigenous raw material and are appropriately fortified. This is important in the context of giving relief to severely time-constrained mothers who have now entered the workforce in a considerable way. Legislations affecting women and child health and nutrition are an important aspect to consider when research programmes and investment decisions are made. NSG suggested that researchers and funding agencies should work with Food Safety and Standards Authority of India in MoHFW to better understand regulatory issues related to food processing and related nutritional impacts.

Adolescents constitute almost 25 per cent of the Indian population. They suffer from several health problems that have been hitherto neglected. The foundation of several chronic diseases in the form of exposure to risk factors is laid during the adolescent years. NSG advised that adolescent-related research options were culled from the four themes and put forward separately with a caveat that scorers did not have an opportunity to see all the options in this section together. These have been put together in Supplementary Table III (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm8.pdf*). However, as adolescent health was not stated as a theme on its own for prioritization, the research options that were chosen from within the other sections may not reflect the true priorities in adolescent health. An independent exercise may be undertaken where adolescent health is given a focus (One example where a gap can easily be seen is interventions to target adolescent boys or interventions looking at accidental

injury or drowning which carry a huge burden in this age group).

The NSG acknowledged implementation research as an important area for consideration. This necessitates bridging the 'disconnect' between the research community, the policy and programme makers, implementers and the communities to achieve the desirable impact. Implementation research involves behavioural changes of health providers, and this cannot be overlooked. Some of the prioritized implementation research options can also help the national and State administrators to either commission research studies or decide to reset the way, in which the health system is working. This will also provide an opportunity for re-engineering health systems and programmes to improve their efficiencies: by dropping redundant programmes and/or making way for newer, refined and 'need of the hour' programmes. As a cross-cutting research priority, the NSG strongly pitched the imperatives of improving the quality of administrative data for better use in decision-making at ground level.

The NSG members observed that several of the priority research issues were cross-cutting and common across the four themes; these should be brought together as common research agenda for MNCHN. This will make the research investment efficient and effective by addressing more than one theme at a time and also align the research projects with the national goal to achieve universal healthcare in India. As a follow up on this suggestion, the investigator team has prepared a separate list of cross-cutting research options [Supplementary Table IV (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm9.pdf*)]. Similarly, as technology has cross-cutting usage, another list of research options related to research on the use of technology for health has been prepared for consideration by agencies with mandate to exclusively fund such research [Supplementary Table V (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm10.pdf*)].

The NSG strongly advised that the DHR and other science ministries along with MoHFW should make efforts to pool resources for addressing priority research issues and also encourage national and international agencies to come together to invest strategically for greater and faster impact. The NSG has suggested that a number of research issues have the ability to be 'game changers' for the health systems, and for the health and nutrition of mothers and children, in general. These can be systematically identified and taken up in a mission mode by science ministries and donor agencies as per their own prioritization and investment policies. Recognizing the challenges of programme delivery in the context of weak infrastructure, national policymakers and programme planners have already identified north-eastern States and EAG States for the focus of several developmental schemes including health and nutrition (*http://nhm.gov.in/nhm/nrhm.html*). To further accelerate the developmental process, region-specific research agenda should become natural corollary. Health is a State subject in India and implementation of research issues can be integrated with the programme monitoring and evaluation frameworks existing therein. The research agenda could be accomplished in an accelerated manner and effectively with multistakeholder and multi-agency engagement through a broader convergent innovation coalition that will add to existing sectoral and cross-sectoral capacity for science, social sciences, economics, technology, innovation, strategy and policy to achieve better health and nutrition for mothers and their children in India.

Conclusion

It was concluded that the logical way forward for this exercise would be to take up these research priorities with different governmental and non-governmental agencies for investment. Various government and non-governmental funding agencies were requested to now re-align their investment with these priorities to achieve better health and nutrition for women and children of India effectively and efficiently. Further, bridging the gap between researchers and programme implementers at the grass root level would be essential along with decentralization of strategies to have State and possibly, district centric solutions for the betterment of health and nutrition scenario in India. In view of the predominance of delivery and implementation research identified as priority in all thematic areas, it was suggested to explore the possibility of supporting such research projects from the programme funds.

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References

1. Rudan I, Sridhar D. Structure, function and five basic needs of the global health research system. *J Glob Health* 2016; *6* : 010505.
2. McGregor S, Henderson KJ, Kaldor JM. How are health research priorities set in low and middle income countries? A systematic review of published reports. *PLoS One* 2014; *9* : e108787.
3. Yoshida S, Wazny K, Cousens S, Chan KY. Setting health research priorities using the CHNRI method: III. Involving stakeholders. *J Glob Health* 2016; *6* : 010303.
4. Dua T, Tomlinson M, Tablante E, Britto P, Yousfzai A, Daelmans B, *et al.* Global research priorities to accelerate early child development in the sustainable development era. *Lancet Glob Health* 2016; *4* : e887-9.
5. Yoshida S. Approaches, tools and methods used for setting priorities in health research in the 21st century. *J Glob Health* 2016; *6* : 010507.
6. Wazny K, Sadruddin S, Zipursky A, Hamer DH, Jacobs T, Kallander K, *et al.* Setting global research priorities for integrated community case management (ICCM): Results from a CHNRI (Child Health and Nutrition Research Initiative) exercise. *J Glob Health* 2014; *4* : 020413.
7. Souza JP, Widmer M, Gülmezoglu AM, Lawrie TA, Adejuyigbe EA, Carroli G, *et al.* Maternal and perinatal health research priorities beyond 2015: an international survey and prioritization exercise. *Reprod Health* 2014; *11* : 61.
8. Sharma R, Buccioni M, Gaffey MF, Mansoor O, Scott H, Bhutta ZA; Canadian Expert Group on Maternal, Newborn, Child and Adolescent Health. Setting an implementation research agenda for Canadian investments in global maternal, newborn, child and adolescent health: a research prioritization exercise. *CMAJ Open* 2017; *5* : E82-9.
9. Rudan I. Setting health research priorities using the CHNRI method: IV. Key conceptual advances. *J Glob Health* 2016; *6* : 010501.
10. Rudan I, Gibson JL, Ameratunga S, El Arifeen S, Bhutta ZA, Black M, *et al.* Setting priorities in global child health research investments: Guidelines for implementation of CHNRI method. *Croat Med J* 2008; *49* : 720-33.
11. Arora NK, Mohapatra A, Gopalan HS, Wazny K, Thavaraj V, Rasaily R, *et al*. Setting research priorities for maternal, newborn, child health and nutrition in India by engaging experts from 256 indigenous institutions contributing over 4000 research ideas: a CHNRI exercise by ICMR and INCLEN. *J Glob Health* 2017; *7* : 011003.

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PREVALENCE OF DEPRESSION AND ITS ASSOCIATED FACTORS IN ADULTS WITH DIABETES MELLITUS: A CROSS-SECTIONAL STUDY IN A TERTIARY CARE CENTRE

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ABSTRACT

The prevalence of Diabetes Mellitus has been rising steadily owing to several factors such as sedentary lifestyle, obesity and an aging population. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India with up to 79.4 million individuals in India. Depression occurs frequently with diabetes but there are not many studies in India to estimate its prevalence and associated factors. This study was done with the aim of estimating the prevalence of depression among diabetes patients using the validated Patient Health Questionnaire-9 and also its associated factors. A cross sectional study was done on 100 consecutive patients with Diabetes Mellitus (both type 1&2) attending the Outpatient Endocrinology clinic from September 2015 to November 2015. The Patient Health Questionnaire diagnostic algorithm was used to identify major depression. The covariates studied were age, gender, duration of diabetes, presence of hypertension and glycaemic control. Chi-square test was done to find the association of demographic and clinical characteristics of depression. Multiple logistic regressional analysis was done to identify the statistically significant variables. The prevalence of major depression was found to be 35%. People with hypertension were found to have a significantly higher prevalence of depression (adjusted odds ratio=3.02, 95% CI: 1.17 to 7.7, P=0.022). Also, employed people were at a lower risk for depression when compared to the unemployed /retired population (adjusted odds ratio=0.319, 95% CI: 0.11-0.85, P=0.023).Interestingly age, duration of diabetes and glycaemic control were not significantly associated with depression. Over one-third of the patients with diabetes mellitus had depressive symptoms. The prevalence varied by demographic and clinical characteristics.

KEYWORDS

Diabetes, Depression, Patient Health Questionnaire- 9, Hypertension.

1. INTRODUCTION

The prevalence of Diabetes Mellitus has been rising steadily owing to several factors such as sedentary lifestyle, obesity and an aging population. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India with up to 79.4 million individuals in India.[1]

Depression occurs frequently with diabetes and has been found to be associated bidirectionally with diabetes mellitus.[2,3] However, it was observed that the reported prevalence in the studies varied tremendously in terms of definition, study design, source of subjects, time frame and measurement methods.[4-6] Moreover, with the predicted rise of diabetes in India, and risk factors

for depression such as low socioeconomic status, financial constraints being rampant here it is quintessential to establish regional studies as a guideline to aid in a holistic patient approach. [7]

Apart from this, most of these studies used self-administered questionnaires which cannot directly establish a diagnosis of depression. [4,5,6,8] Standard interviews, such as the Structured Clinical Interview for DSM Disorders (SCID)[9] and the Composite International Diagnostic Interview (CIDI) [10] can yield a clinical diagnosis according to the Statistical Manual of Psychiatric Disorders, 4th edition (DSM-IV) [11] ; however, lengthy assessments and high costs preclude the extensive use of such structured diagnostic interviews in large population-based surveys.

The Patient Health Questionnaire (PHQ) can establish provisional diagnoses of major and minor depression as well as evaluate the severity of depressive symptoms. [12] ; The PHQ-9 diagnosis of major depression yielded acceptable sensitivity and specificity. The present study aims to estimate the prevalence of depression among diabetes patients using the validated Patient Health Questionnaire-9 and its associated factors.

The variables taken into consideration for association with depression in this region include age, gender, duration of diabetes, glycaemic index and presence of hypertension . This stems from previous studies which show significant correlations between the above variables and depression[6,13,14]

2. MATERIALS AND METHODS:

This study is a cross-sectional study on 100 consecutive patients presenting at a tertiary care centre, aged >18yrs diagnosed with Type 2 diabetes mellitus by a certified Endocrinologist. The study was carried out after obtaining institutional ethics committee clearance. Written informed consent was obtained from the study participants and the PHQ-9 questionnaires were distributed. Those subjects that couldn't read were assisted in filling the questionnaires by an interpreter (Figure 1). During multivariate analysis we have combined the scores 0-9 (none, minimal and mild depression categories) as "no major depression" and scores of 10-27 (moderate, moderately severe and severe categories) as " major depression".[15,16]

Patients with complications from diabetes (retinopathy, neuropathy, nephropathy), previous history of mental illness, previous illnesses known to increase a risk for depression (CAD,CVA), and patients already on antidepressants for other symptoms were excluded from the study.

The demographic variables considered were age, gender, duration of diabetes, glycaemic index and presence of hypertension. Age was measured in completed years. Duration of diabetes and glycaemic control were taken from the patients' case records. Hypertension was defined in the study as systolic BP level of ≥140mmHg and diastolic BP of ≥90mmHg, or current treatment for hypertension with prescription medication. [17,18]

Data were analysed using SPSS(19). Chi-square test was done to find the association of demographic and clinical characteristics of depression. Further, multiple logistic regressional analysis was done to identify the statistically significant variables. *P* value of <0.05 was considered as statistically significant

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

NAME:_____ DATE:_____

Over the last *2 weeks*, how often have you been bothered by any of the following problems?
(use "✓" to indicate your answer)

	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself—or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or the opposite — being so figety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead, or of hurting yourself	0	1	2	3
	add columns		+	+
(Healthcare professional: For interpretation of TOTAL, please refer to accompanying scoring card).	TOTAL:			

10. If you checked off *any problems*, how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?	Not difficult at all _____ Somewhat difficult _____ Very difficult _____ Extremely difficult _____

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Figure 1: The Patient Health Questionnaire -9

3. RESULTS:

Among the 100 patients studied, 35 were found to have major depression (Figure 2). In the univariate analysis, those who were having hypertension and those who were unemployed were significantly associated with depression (P<0.05). In the multiple regression analysis(Table I), people with hypertension were found to have a significantly higher prevalence of depression (adjusted odds ratio=3.02,95% Confidence Interval :1.17 to 7.7,P=0.022) (Figure 3).Also, employed people were at a lower risk for depression when compared to the unemployed /retired population(adjusted odds ratio=0.319,95% Confidence Interval :0.11-0.85,P=0.023) (Figure 4) that bar graph has nothing written on x axis. Interestingly age, duration of diabetes and glycaemic control were not significantly associated with depression.

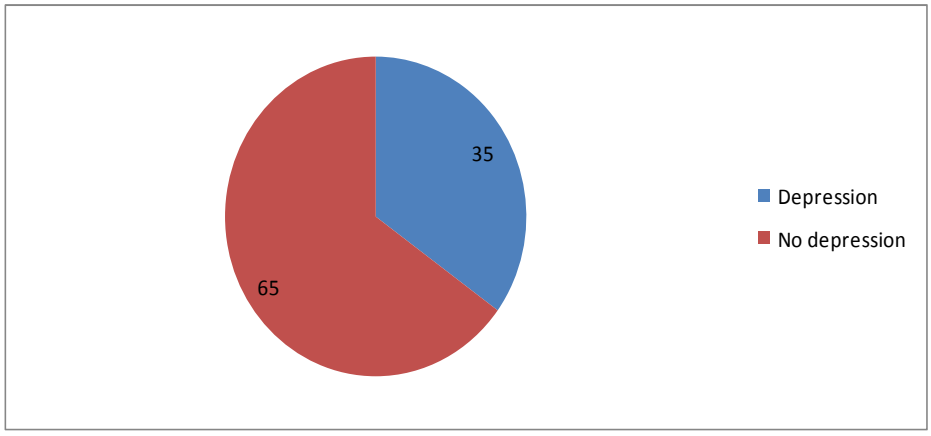


Figure 2. Percentage of patients with and without depression among the study group.

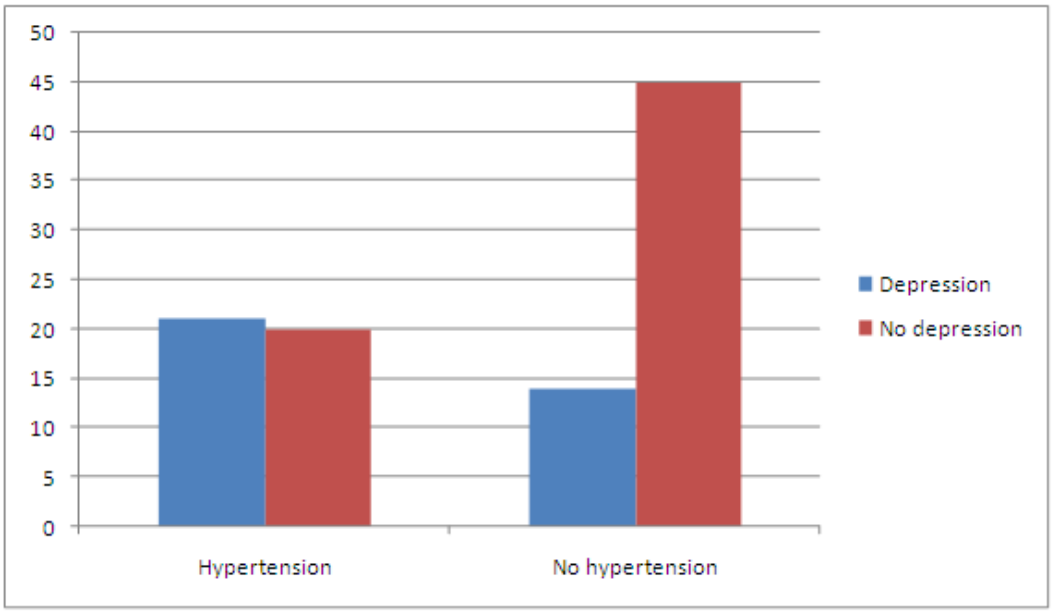


Figure 3. Comparison of the prevalence of Depression in patients with and without Hypertension

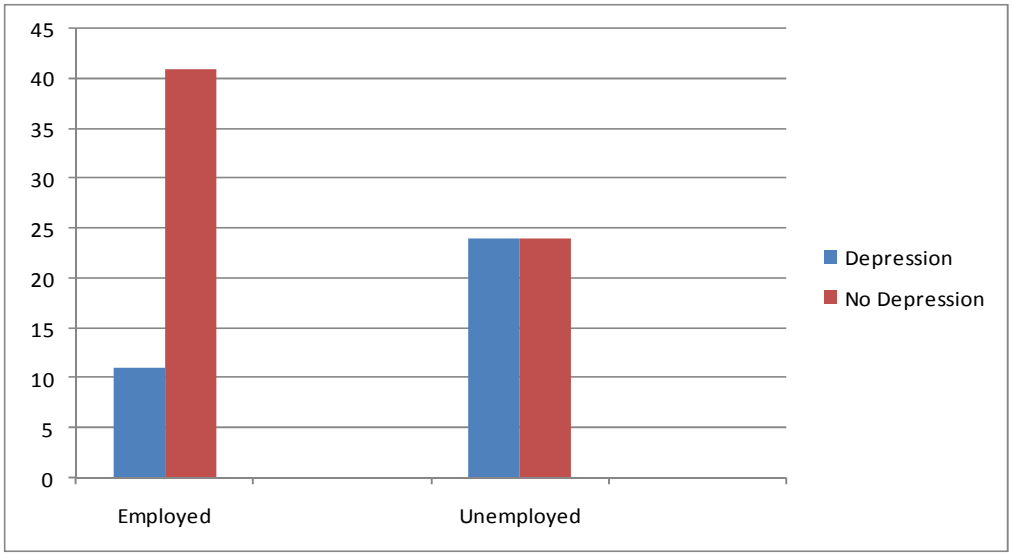


Figure 4. Prevalence of depression among employed and unemployed study participants

Table 1. Regression analysis of the associated factors of depression

Variable	Number studied	Percentages with depression	Adjusted odds ratio	*P* value*
Age				
<40 years	11	36.4	1	
More than 40 years	89	34.8	0.40(0.08-1.91)	0.251
Gender				
Male	51	27.5	1	
Female	49	42.9	1.32 (0.51-3.41)	0.513
Duration				
Less than 5 years	31	32.3	1	
More than 5 years	69	36.2	1.03(0.36-2.96)	0.946
Glycemic control				
<7	45	26.7	1	
>7	55	41.8	2.34(0.91-6.04)	0.077
Employment				
Unemployed/Retired	48	50.0	1	
Employed	52	21.2	0.31(0.11-0.85)	0.023*
Hypertension				
No	59	23.7	1	
Yes	41	51.2	3.02(1.17-7.77)	0.022*

4. DISCUSSION:

In the present study the prevalence of depression among patients with diabetes mellitus was found to be 35% which was slightly smaller than the prevalence in a study conducted by Joseph N et al., in an adjoining area that reported a prevalence of 45.3%.[13] It was also found to be more than twice as common as depression in the general population of our region.[7,19]

This leads us to ask the more imposing question, “Why is depression more prevalent in people with diabetes mellitus?”. The study by Talbot F et al., suggested that the extent to which diabetes intrudes in life, rather than diabetic complications per se or personal control, is a key factor in

relation to depressive symptomatology in individuals with diabetes. [20]In an animal model, Wang and colleagues demonstrated that experimentally induced type 1 diabetes impairs hippocampal neurogenesis, which may contribute to diabetes-related depression.[21] Another study demonstrated decreased plasma brain-derived neurotrophic factor (BDNF) in patients with type 2 diabetes compared to controls, which has been hypothesized to play a role in learning and memory and has also been implicated in the pathogenesis of depression.[22]

Among the risk factors, depression was found to be significantly associated with hypertension. This is in line with the findings of Saeed AK et al., where the prevalence of depression in hypertensive people with diabetes mellitus was 57% whereas in people with diabetes alone it was 40%. (P is less than 0.05). [23] There are also other studies which showed that depression is common in patients with uncontrolled hypertension irrespective of presence of diabetes and may interfere with blood pressure control. [24, 25] Studies have shown that plasma noradrenaline, as an indirect marker of sympathetic tone, was elevated in patients with essential hypertension.[26] There is also reported evidence showing abnormal autonomic nervous system function in depression, specifically regarding increased sympathetic activity and poor vagal control. Several studies have examined cerebrospinal fluid (CSF), plasma or urinary levels of norepinephrine (NE) and its major central nervous system metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG). Plasma NE and/or MHPG levels have been reported to be elevated in depressive patients.[27,28] Thus, the existing relationship between hypertension and depression by itself could have contributed to the statistically significant presence of depression among hypertensive people with diabetes mellitus.

Also, we found that depression among diabetes was significantly associated with employment status with a higher prevalence in the unemployed population. This is in line with other studies which showed a higher prevalence of depression in the unemployed population. [13,29,30] The unemployed individuals lack sociological functions such as time structure, status and identity, social contacts, participation in collective purposes and regular activity which could predispose them to depression.[30]

A methodological innovation of our study was the application of the PHQ-9[12] which was developed for clinical use, scoring each of the DSM IV criteria on grades from 0-3 based on the frequency of the symptoms[31] Scores of 20-27,15-19,10-14,5-9 and 1-4 represent severe, moderately severe, moderate, mild and minimal depression respectively. [11]

Our results indicate that a substantial number of people with diabetes are at an increased risk of having major depression, and those who care for patients with diabetes should routinely screen them for major depression using the PHQ-9 or other such instruments. Among the umpteen methods to screen for depression, the PHQ-9 is a promising tool with its ease of administration, convenient scoring system, brief and simple questions. Most importantly, the use of PHQ-9 can serve in comparisons of depressive symptomatology on an international basis given its worldwide validation. [32,33]

This study has some limitations. The study was conducted in a tertiary care hospital, so a possible selection bias cannot be excluded. This could be because the patients coming to the tertiary care hospital belong to the relatively higher socioeconomic status of the community. A large sample community based study could throw more light on this relationship. Since this study is a cross-sectional study, temporal relationship between depression and diabetes cannot be determined.

5. CONCLUSIONS

Over one-third (35%) of the patients with diabetes mellitus had depressive symptoms. It is widely associated with unemployment and co-existent hypertension in this population.

This emphasizes that the mind/body dualism is a false dichotomy and there is an increasing need to integrate mental health services in these patients. We recommend routine screening of diabetic patients with PHQ-9 to identify those at risk as it is a quick and easy tool to identify depression and refer patients appropriately for further counselling and support.

In addition, the issue of depression in association with socioeconomic factors such as unemployment is to be addressed by the health policy to aim for an overall betterment of the welfare of the people in this region.

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REFERENCES

[1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes-estimates for the year 2000 and projections for 2030. Diabetes Care2004;27:1047–53.

[2] Katon WJ.The Comorbidity of Diabetes Mellitus and Depression. Am J Med 2008;121(11, Suppl 2):S8–15.

[3] Golden SH, Lazo M, Carnethon M, Bertoni AG, Schreiner PJ, Diez Roux AV, et al. Examining a bidirectional association between depressive symptoms and diabetes. JAMA 2008;299:2751-9.

[4] Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes:a meta-analysis. Diabetes Care 2001;24:1069-78.

[5] Ali S, Stone MA, Peters JL, Davies MJ, Khunti K. The prevalence of comorbid depression in adults with type 2 diabetes: a systematic review and meta-analysis. Diabet Med 2006;23:1165-73.

[6] Barnard KD, Skinner TC, Peveler R. The prevalence of co-morbid depression in adults with type 1 diabetes: systematic literature review. Diabet Med 2006;23:445-8.

[7] Poongothai S, Pradeepa R, Ganesan A, Mohan V. Prevalence of Depression in a Large Urban South Indian Population — The Chennai Urban Rural Epidemiology Study (Cures – 70). PLoS One 2009;4:e7185.

[8] Fisher L, Skaff MM, Mullan JT, Arean P, Mohr D, Masharani U,et al. Clinical depression versus distress among patients with type 2 diabetes: not just a question of semantics. Diabetes Care 2007:30:542-8

[9] Spitzer RL, Williams JB, Gibbon M, First MB. The Structured Clinical Interview for DSM-III-R (SCID). I. History, rationale, and description. Arch Gen Psychiatry 1992;49:624–9.

[10] Wittchen HU. Reliability and validity studies of the WHO–Composite International Diagnostic Interview (CIDI): a critical review. J Psychiatr Res 1994;28:57-84.

[11] American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV [Internet]. 4th ed. Washington (DC): American Psychiatric Association; 1994 [cited 2010 Mar 8]. 866 p. Available from: http://www.psychiatryonline.com/DSMPDF/dsm-iv.pdf

[12] Kroenke K, Spitzer RL. The PHQ-9: a new depression diagnostic and severity measure. Psychiatric Annals 2002;32:509–21.

[13] Joseph N, Unnikrishnan B, RaghavendraBabu YP, Kotian MS, Nelliyanil M. Proportion of depression and its determinants among type 2 diabetes mellitus patients in various tertiary care hospitals in Mangalore city of South India. Indian Journal of Endocrinology and Metabolism 2013;17:681–88.

[14] Roy T, Lloyd CE. Epidemiology of depression and diabetes: a systematic review. J Affect Disord 2012:142 Suppl:S8-21

[15] Arroll B, Goodyear-Smith F, Crengle S, Gunn J, Kerse N, Fishman T, et al. Validation of PHQ-2 and PHQ-9 to Screen for Major Depression in the Primary Care Population. Ann Fam Med 2010;8:348–53.

[16] Sawaya H, Atoui M, Hamadeh A, Zeinoun P, Nahas Z. Adaptation and initial validation of the Patient Health Questionnaire - 9 (PHQ-9) and the Generalized AnxietyDisorder - 7 Questionnaire (GAD-7) in an Arabic speaking Lebanese psychiatric outpatient sample. Psychiatry Res 2016;239:245-52.

[17] The fifth report of the Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med 1993;153:154-83.

[18] Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee: Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.

[19] Egede LE, Zheng D, Simpson K. Comorbid depression is associated with increased health care use and expenditures in individuals with diabetes. Diabetes Care 2002;25:464–70.

[20] Talbot F, Nouwen A, Gingras J, Bélanger A, Audet J. Relations of diabetes intrusiveness and personal control to symptoms of depression among adults with diabetes. Health Psychol 1999;18:537-42.

[21] Wang S, Sun Z, Guo Y, Yuan Y, Yang B. Diabetes impairs hippocampal function via advanced glycation end product mediated new neuron generation in animals with diabetes-related depression. ToxicolSci 2009;111:72-9.

[22] Krabbe, K.S., Nielsen, A.R., Krogh-Madsen, R., Plomgard, P.,et al., Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. Diabetologia 2007;50:431-8.

[23] Saeed AK, Al-Dabbagh TQ. Type 2 diabetes and its association with hypertension and depression in an Iraqi population. Ann Saudi Med 2003;23:254-9.

[24] Rubio-Guerra AF, Rodriguez-Lopez L, Vargas-Ayala G, Huerta-Ramirez S, Serna DC, Lozano-Nuevo JJ.Depression increases the risk for uncontrolled hypertension. Experimental and Clinical Cardiology 2013;18:10-2.

[25] Nabi H, Chastang JF, Lefèvre T, DugravotA, Melchior M, Marmot MG, et al. Trajectories of depressive episodes and hypertension over 24 years:The Whitehall II prospective cohort study. Hypertension 2011;57:710–6.

[26] Goldstein D, Lake C. Plasma norepinephrine and epinephrine levels in essential hypertension. Federation Proceedings 1984;43:57-61.

[27] Lake CR, Pickar D, Zeigler MG, Lipper S, Slater S, Murphy DL. High plasma norepinephrine levels in patients with major affective disorder. Am J Psychiatry. 1982;139:1315-8.

[28] Roy A, Pickar D, Linnoila M, Potter WZ. Plasma norepinephrine levels in affective disorders. Relationship to melancholia. Arch Gen Psychiatry 1985;42:1181-5.

[29] Robert Friis , G. Nanjundappa. Diabetes, depression and employment status. SocSci Med 1986;23:471-75.

[30] Strandh M, Hammarström A, Nilsson K, Nordenmark M, Russel H. Unemployment, gender and mental health: the role of the gender regime. Sociol Health Illn.2013;35:649-65.

[31] Kroenke K, Spitzer RL, Williams JBW. The PHQ-Validity of a Brief Depression Severity Measure. J Gen Intern Med 2001;16:606–13.

[32] Burdzovic Andreas J, Brunborg GS. Depressive Symptomatology among Norwegian Adolescent Boys and Girls: The Patient Health Questionnaire-9 (PHQ-9) Psychometric Properties and Correlates. Front Psychol. 2017:8: 887.

[33] Titov N, Dear BF, McMillan D, Anderson T, Zou J, Sunderland M. Psychometric comparison of the PHQ-9 and BDI-II for measuring response during treatment of depression. Cogn Behav Ther. 2011:40:126-36

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Consumption of Tea and its Relation to Risk of Type 2 Diabetes mellitus

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ABSTRACT

Aim: The aim of the study was to examine the association of consumption of tea with the risk of type 2 diabetes mellitus. **Methods:** A case control study was conducted at PSG Hospitals between 1st June 2017 and 1st July 2017. All those who had newly diagnosed type 2 diabetes mellitus and attended Department of Endocrinology for follow-up visit were the cases and the relatives accompanying them without the history of diabetes mellitus were the controls. Tea consumption and other dietary factors were elicited. The mediating factors studied were age, sex, Body Mass Index (BMI), educational levels, smoking status, alcohol consumption, family history of diabetes mellitus, potassium intake, magnesium intake and use of statins. **Results:** After adjusting the potential confounders, tea consumption was inversely associated with risk of type 2 diabetes mellitus with odds ratio of 0.652 (95% CI = 0.43 – 0.98). The association was also observed with participants who consumed tea 3 or 4 cups per day compared to those who did not drink tea on a daily basis (odds ratio = 0.368, 95% CI = 0.14 – 0.96). However, the association was not observed among those who take 5 or more cups of tea per day (odds ratio = 0.328, 95% CI = 0.06 – 1.60).**Conclusion:** Our findings suggest that consumption of 3 to 4 cups of tea per day has a decreased risk of type 2 diabetes mellitus. The results from our study also suggest that adjusting for mediating factors did not alter the results and hence other unknown factors may explain inverse relation between tea consumption and type 2 diabetes mellitus. Further studies are warranted.

Keywords: Tea consumption, type 2 diabetes mellitus, chocolate consumption, potassium intake

Introduction

The prevalence of type 2 diabetes mellitus has increased dramatically in the past decades [1, 2].

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The majority of cases of diabetes mellitus could be prevented by changes in life style. However, the association of tea consumption with the risk of type 2 diabetes mellitus is not entirely clear. Few studies found an inverse association between tea intake and type 2 diabetes mellitus, while few other studies did not observe an association between tea consumption and risk of type 2 diabetes mellitus [3-6].The underlying mechanism regarding the relation between tea consumption and type 2 diabetes mellitus is also

unclear. Several components of tea have been suggested as possible causal factors [7].Magnesium plays a role in regulating insulin action and is inversely associated with insulin sensitivity and type 2 diabetes mellitus. Potassium intake has also been associated with reduced risk of diabetes mellitus [8].So far; there is no conclusive evidence about whether these factors explain the association of tea intake and type 2 diabetes mellitus. Besides these, earlier studies have not accounted for the effect of long term medication such as statin use which may confound the results. Therefore the present study is aimed to investigate the association of tea consumption with the risk of type 2 diabetes mellitus adjusting for multiple covariates.

Materials and Methods

Study population and design

A case-control study was conducted at PSG Hospitals. All those who have newly diagnosed in the past 1 year as type 2 diabetes mellitus and attending the Department of Endocrinology for follow up between 1stJune 2017 – 1st July 2017 were considered as cases. Controls were those who accompany them and do not have history of diabetes mellitus. We then retrospectively evaluated their food practices and other demographic information.

General assessments

The general assessment survey was conducted at the outpatient Department of Endocrinology, PSG Hospitals, Coimbatore. The general questionnaire contained questions on demographic characteristics. Body weight (in kg) and height (in meter) were also measured. However in few cases, we were not able to measure height. Food intake was assessed using a validated Food Frequency Questionnaire in this population and from this magnesium intake and potassium intake were estimated. The questionnaire also asked about smoking status, practice of alcohol intake, whether previously diagnosed as diabetes mellitus, other medical histories and medications taken. The information on baseline consumption of coffee, tea and other beverages were also collected. We also collected the data about consumption of chocolates, since chocolates are also a source of caffeine.

Statistical analysis

To examine the association between tea consumption and incidence of type 2 diabetes mellitus, we used four logistic regression models. In the first model, odds ratio and 95% confidence intervals for type 2 diabetes mellitus were calculated against a reference group of low consumers adjusted for age (continuous) and gender (male/female). A second logistic regression model corrected the other known diabetes mellitus risk factors which include smoking status (present/absent), BMI, education attainment (classes up to 12th standard/college and above), family history of diabetes mellitus (present/absent); alcohol intake (gm. per week). Body mass index was calculated by the formula, body weight (kg) divided by the square of the body height in meter. A third model corrected for coffee (considering the fact that those who drank a lot of tea tend to drink less coffee and vice versa) and chocolate consumption. Mediating factors were included in the final model. The mediating factors considered were daily intake of magnesium and potassium, which were elicited from their food consumption practices and the effects of long term medications particularly the use of statins[9].

Results

The study included 150 cases and 150 controls. Baseline characteristics of the study participants among cases and controls were presented in Table 1. Most of the demographic factors were comparable between cases and controls. However cases were having slightly older age compared to controls. Family history of diabetes mellitus and level of education was higher among cases compared to controls. In the multivariate regression model, odds ratio shows that the participants who drank more tea had a significantly decreased risk of diabetes mellitus compared with those who do not drink tea on a daily basis (odds ratio = 0.652, 95% CI = 0.43 – 0.98). On further analysis, it was observed that the participants who consumed 3 or 4 cups of tea per day had a decreased risk of type 2 diabetes mellitus compared to those who do not drink tea on a daily basis (odds ratio = 0.368, 95% CI = 0.14 – 0.96) (Table 3) with no significance in analysis of trend (Figure 1). The reduced risk was not observed among those who take 5 cups or more tea per day (odds ratio = 0.328, 95% CI = 0.06 – 1.60)

Table 1: Distribution of the participant's demographic characteristics

Variables	Cases No (%) or Mean ± SD	Controls No (%) or Mean ± SD	p value
Age(years, continuous)	53.29 ± 11.52	41.75 ± 12.44	p < 0.05
BMI(kg/m^2, continuous)	26.12 ± 5.09	25.99 ± 5.21	0.824
Potassium (mg/day)	624.11 ± 151.14	661.14 ± 209.54	p < 0.001
Magnesium (mg/day)	134.27 ± 50.19	123.30 ± 44.96	0.082
Sex			0.246
Male	72 (48%)	62 (41.3%)	
Female	78 (52.0%)	88 (58.7%)	
Educational levels			p < 0.001
Up to 12th Standard	108 (72.0%)	85 (56.7%)	
College & above	42 (28.0%)	65 (43.3%)	
Current smoking status			0.569
Yes	17 (11.3%)	14 (9.3%)	
No	133 (88.7%)	136 (90.7%)	
Alcohol Intake			0.427
rarely/moderately	144 (96.0%)	141 (94.0%)	
weekly/daily	6 (4.0%)	9 (6.0%)	
Family history of diabetes mellitus			p < 0.001
Yes	91 (60.7%)	51 (34.0%)	
No	59 (39.3%)	99 (66.0%)	
Use of statins			0.317
Yes	1 (0.7%)	0 (0.0%)	
No	149 (99.3%)	150 (100%)	

Table 2: Tea consumption and odds ratio for the risk of type 2 diabetes mellitus in the multivariate analyses

	Model 1 Adjusted for age and sex	Model 2 Adjusted for BMI, education, smoking, alcohol and family history of diabetes mellitus in addition to variables in Model 1	Model 3 Adjusted for coffee and chocolate consumption in addition to variables in Model 2	Model 4 Adjusted for potassium, magnesium and statins in addition to variables in Model 3
Odds ratio	0.753	0.648	0.613	0.652
95% CI for diabetes mellitus	0.533 – 1.066	0.441 – 0.953	0.410 – 0.915	0.433 – 0.982
p value	0.109	0.027	0.017	0.041

Table 3: Adjusted odds ratio* according to the consumption of tea cups per day

Category	Cases (N = 150)	Controls (N = 150)	Odds ratio	95% CI for EXP (B)	p value
Do not drink on a daily basis	34	27	1		
1-2 cups per day	81	84	0.522	0.236 – 1.157	0.109
3-4 cups per day	31	32	0.368	0.141 – 0.964	0.042
>5 cups per day	4	7	0.328	0.067 – 1.605	0.169

*adjusted for age, sex BMI, education, smoking, alcohol, family history of diabetes mellitus, coffee consumption, chocolate consumption, potassium intake, magnesium intake and use of statins

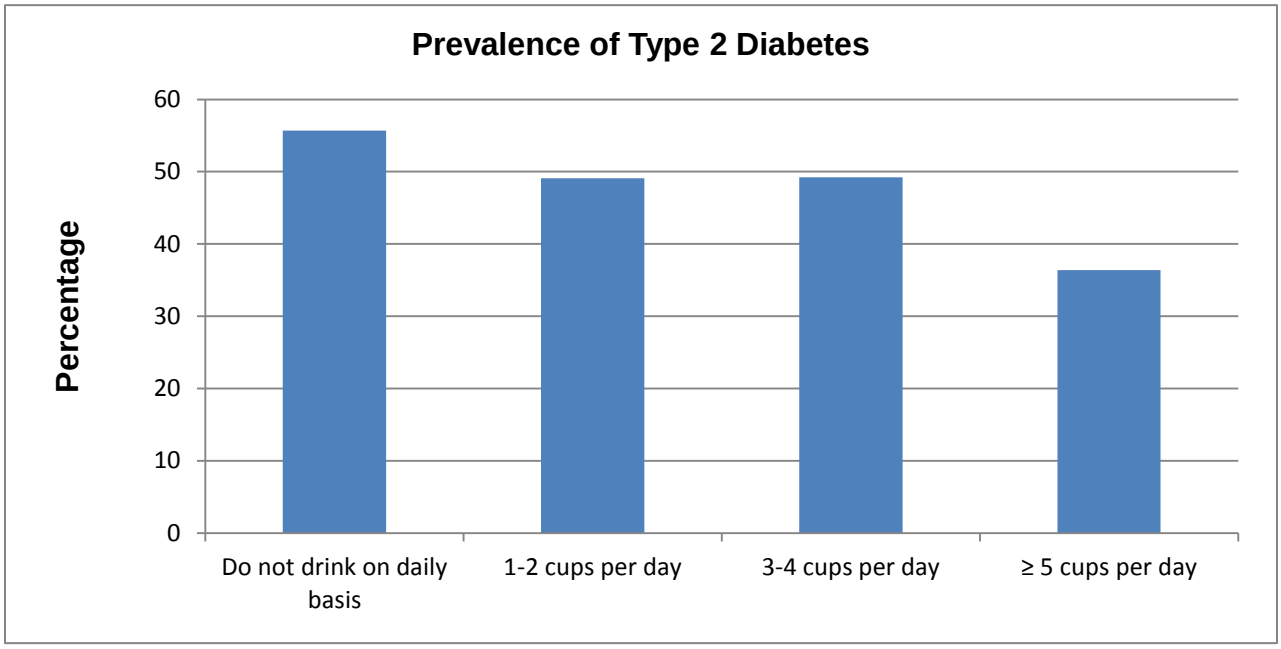


Fig1: Prevalence of type 2 diabetes mellitus according to the consumption of tea cups per day

Discussion

Several studies have investigated the association between tea consumption and type 2 diabetes mellitus[10-14]. Some of these studies did not find any association, while other studies observed an inverse association between tea consumption and type 2 diabetes mellitus. Another study found an association only for green tea and not for black tea. However, our study strongly support the findings of the recent meta-analysis[11] which concluded that consumption of 4 cups of tea per day may lower the risk of type 2 diabetes mellitus, but a reduced risk was not observed for 1 to 2 cups per day. In addition, we also observed that the reduced risk was not observed for those who take more than five cups of tea per day. This is perhaps due to the reduced sample size in this group.In univariate analysis, we observed an inverse association between potassium intake and the risk of type 2 diabetes mellitus. In our study, intake of magnesium and potassium were included in the final model to assess whether these factors mediated the observed association between tea intake and type 2 diabetes mellitus, but none affected the association. Hence, results from our study suggest that other factors may be more important to explain inverse relation between tea consumption and type 2 diabetes mellitus. Tea contains flavonols and flavones are antioxidants. Antioxidants may reduce the amount of reactive oxygen species, which activate stress-sensitive pathways. These pathways can lead to insulin resistance, impaired insulin secretion and beta cell dysfunction. Our study has several limitations, first we relied on self-reported tea consumption and therefore misclassification may have occurred. Second, we relied on self-reported information on non-diabetic status among controls. Third, we could not assess the influence of physical activities due to some practical reasons. The strengths of this study are a large number of covariates studied and extensive information on diet of the participants.

In conclusion, the results from our study suggest that consumption of 3 to 4 cups of tea per day has a decreased risk of type 2 diabetes mellitus. Further large scale prospective studies can provide more information in this field.

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intake using the dietary history of the participants. We do not have any conflict of interest and not received any research grants for this study.

References

1. Wild S, Roglic G, Green A, Sicree R, King H, "Global prevalence of diabetes mellitus: estimates for the year 2000 and projections for 2030". Diabetes mellitus Care.2004;27:1047–1053.
2. Hu FB, Manson JE, Stampfer MJ, et al, "Diet, lifestyle and the risk of type 2 diabetes mellitus mellitus in women" N Engl J Med 2001; 345:790–797.
3. Hamer M, Witte DR, Mosdol A, Marmot MG, Brunner EJ, "Prospective study of coffee and tea consumption in relation to risk of type 2 diabetes mellitus mellitus among men and women": The Whitehall II study. Br J Nutr 2008:1046–1053.
4. Iso H, Date C, Wakai K, et al, "The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes mellitus among Japanese adults" Ann Intern Med.2006; 144: 554–562.
5. Oba S, Nagata C, Nakamura K, Fujii K, Kawachi T, et al, "Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes mellitus in Japanese men and women" British Journal of Nutrition.2010;103:453–459.
6. Greenberg JA, Axen KV, Schnoll R, et al, "Coffee, tea and diabetes mellitus: the role of weight loss and caffeine" Int J Obes.2005;29: 1121–1129.
7. Waki K, Noda M, Sasaki S, et al, "Alcohol consumption and other risk factors for self-reported diabetes mellitus among middle aged Japanese": a population-based prospective study in the JPHC study cohort I, Diabet Med 2005; 22: 323–331.
8. S. van Dieren, C. S. P. M. Uiterwaal, Y. T. van der Schouw , D. L. van der A, J. M. A. Boer, et al, "Coffee and tea consumption and risk of type 2 diabetes mellitus" Diabetologia 2009; 52:2561–2569.
9. Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K, "Indian food composition tables" National Institute of Nutrition 2017: 150-206.
10. Odegaard AO, Pereira MA, Koh WP, Arakawa K, Lee HP, Yu MC, "Coffee, tea and incident type 2 diabetes mellitus": the Singapore Chinese Health Study, Am J Clin Nutr 2008; 88: 979–985.
11. Jing Y, Han G, Hu Y, Bi Y, Li L, Zhu D, "Tea consumption and risk of type 2 diabetes mellitus": a meta-analysis of cohort studies, J Gen Intern Med 2009;24: 557–562.
12. Polychronopoulos E, Zeimbekis A, Kastorini CM, et al, "Effects of black and green tea consumption on blood glucose levels in non-obese elderly men and women from Mediterranean Islands" (MEDIS epidemiological study), Eur J Nutr 2008; 47: 10–16.
13. Greenberg JA, Axen KV, Schnoll R, Boozer CN, "Coffee, tea and diabetes mellitus: the role of weight loss and caffeine" Int J Obes (Lond) 2005;29: 1121–1129.
14. Van Dam RM, Hu FB, Rosenberg L, Krishnan S, Palmer JR.Dietary calcium and magnesium, major food sources, and risk of type 2 diabetes mellitus in U.S. black women. Diabetes mellitus Care.2006; 29:2238–2243.

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The genetics of cardiac failure: Role of a G protein-coupled receptor polymorphism in therapeutic response in an Indian population

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Abstract

Background and Aim:

The incidence of heart failure (HF) is rising to epidemic proportions in developing countries like India. A lack of adequate Indian studies underscores the importance of pursuing research into HF in an Indian population. G protein-coupled receptor kinase 5 (*GRK5)* Gln41>Leu (rs2230345) polymorphism was reported as a genetic modifier associated with survival in HF patients. A prospective study was conducted to investigate the association of *GRK5* Gln41>Leu polymorphism with response to β-blocker therapy in Indian HF patients.

Methods:

HF patients (*n*=584) were recruited for the study. The patients were genotyped by tetra-primer based allele specific polymerase chain reaction and confirmed with Sanger sequencing. The HF patients were evaluated for *GRK5* gene expression and followed up for ~3 years. Drug dosages, cardiac output and hospitalization-free survival were evaluated as study outcomes. HF subgroups (i.e. systolic or diastolic dysfunction, biventricular dysfunction and pulmonary artery hypertension) were also analyzed in association with hospital-free survival.

Results:

HF patients showed genotype frequencies of AT (15%) and TT (1%). AT/TT genotype carriers showed downregulated *GRK5* gene expression and significant reduction in carvedilol drug dosage (p=0.0001). Moreover, AT/TT genotype carriers on β-blockers showed improved ejection fraction from 27% to 36% (p=0.0007) and increased hospitalization-free survival in comparison to other HF patients. HF patients with AA genotype showed an increased rate of hospital admission in comparison with patients with the AT/TT genotype. HF subgroups with the AT/TT genotype showed an increased hospitalization-free survival versus subgroups with the AA genotype.

Conclusions:

GRK5 Gln41>Leu polymorphism in response to β-blocker therapy improved cardiac function in HF patients.

Relevance for Patients:

This study presents a comprehensive clinicofunctional pharmacogenetic characterization of *GRK5* Gln41>Leu polymorphism in a cohort of Indian HF patients. *GRK5* Gln41>Leu polymorphism can confer improved cardiac function and reduce hospitalization, thus improving the quality of life in HF patients.

Keywords: β-blocker, genotype, GRK5, heart failure, India, polymorphism

1. Introduction

Heart failure (HF) is a major, multifactorial global health disease affecting approx. 6.5 million adults/year in the United States [1] and 491,600 – 1.8 million individuals/year in India [2]. The common etiologies of HF are coronary artery disease, hypertension, valvular heart disease, arrhythmia, dilated cardiomyopathy, infection, and inflammation [3]. Other risk factors include hypertension, ischemic heart disease, obesity, Type II diabetes mellitus, and rheumatic heart disease [2]. Growing evidence show that mortality rates as a result of HF are higher in Indian patients than in western countries with death records of 0.1-0.16 million individuals/year in India [4].

The use of beta (β)-adrenergic receptor (β-AR) antagonists continues to be the standard treatment for HF [5] and has effectively reduced mortality and morbidity rates over the past decade. β-AR, a G protein-coupled receptor expressed by cardiomyocytes has been documented as important for effective cardiac function [6]. Many studies have shown that elevated activation of β-AR results in abnormal β-AR signaling which leads to the pathogenesis of HF [7,8]. Therefore, much of the therapeutic drugs like β-blockers that target the β-AR, antagonize catecholamine-stimulated β-AR signaling [6] and have resulted in increased survival of HF patients.

As a regulator of β-AR signaling, G protein-coupled receptor kinases (GRKs) act by phosphorylating the activated β-AR, leading to receptor desensitization and diminished β-AR signaling [9]. Overexpression of GRK5 and GRK2 contribute to dysregulation of β-AR by excess desensitization and

is also considered a hallmark of HF pathology [10]. Therefore, genetic polymorphisms in β-AR regulator genes may influence HF outcome. One such functional polymorphism being *GRK5* Gln41>Leu (rs2230345, c.122A>T, p.Q41L) in the *GRK5* gene. This nonsynonymous single nucleotide polymorphism (SNP) in the noncatalytic regulatory domain is prevalent in African Americans and confers a gain-of-desensitization function akin to the effects of β-blockade in cells. This polymorphism, however, did not alter the risk of HF but facilitated β-blocker response and reduced death [11,12].

We conducted a prospective study in a cohort of Indian HF patients to investigate the association of *GRK5*Gln41>Leu polymorphism in relation to β-blocker therapeutic response. The hypothesis behind this study was that as GRKs impose β-AR blockade by desensitization, the presence of genetic GRK variants might alter the outcome in HF patients. Therefore, the main aim of this study was to identify *GRK5* variants and to study their response to β-blocker treatment which might allow curtailing β-AR blocker usage in these selected patients to avoid potential harmful effects.

2. Materials and Methods

2.1. Study population

The study was conducted in multispecialty, tertiary care hospital. This prospective study recruited HF patients (n=584) with systolic or diastolic dysfunction, dilated cardiomyopathy or biventricular dysfunction (BVD) that attended the in- and outpatient cardiology clinic in our hospital. The study was initiated after obtaining the institutional ethics committee approval and getting informed consent from the patients. The HF patients considered for this study were diagnosed following the Framingham criteria [13]. The Framingham criteria for HF diagnosis should include either two major criteria or one major and two minor criteria. The major criteria included acute pulmonary edema, cardiomegaly, hepatojugular reflex, neck vein distention, paroxysmal nocturnal dyspnea or orthopnea, pulmonary rales, and third heart sound (S3 gallop rhythm). The minor criteria included ankle edema, dyspnea on exertion, hepatomegaly, nocturnal cough, pleural effusion, and tachycardia (heart rate >120 beats/min). Healthy controls (n=60) without any comorbidity were recruited for gene expression studies.

Stage-appropriate medications given to HF patients included diuretics, angiotensin-converting enzyme (ACE) inhibitors or β-blockers or angiotensin receptor blockers (ARBs) alone or in combination. If needed, mineralocorticoid receptor antagonists and hydralazine were also included. β-blockers were determined by the treating physicians (60% carvedilol, 17% metoprolol, 12.5% nebivolol, and 10.5% bisoprolol) and defined as continuous therapy for at least 6 months. β-blocker dosages were monitored during the follow-up period. Initial dosage refers to the dosage administered at the start of the β-blocker therapy after the diagnosis of HF. The final dosage refers to the dosage followed at the last follow-up time. The number of hospitalizations was monitored for each patient and the follow-up data were collected for 3-7 years. The mean follow-up duration was 3.7 years and 3.5 years for the β-blocker group and non-β-blocker group.

2.2. Clinical and echocardiography assessment

A complete resting two-dimensional and M-mode echocardiography color flow and spectral Doppler as well as annular tissue Doppler imaging data were obtained from the subjects using a Philips IE33™ ultrasound system (Philips Medical Systems, Eindhoven, The Netherlands). Standard two-dimensional parasternal short-axis and apical 2- and 4-chamber views were recorded. Left ventricular (LV) ejection fraction (EF) was calculated using the Teichholz formula. Mitral Doppler signals were recorded in apical 4-chamber view and early diastolic mitral inflow peak velocity (E), late diastolic mitral inflow

peak velocity (A), and E/A ratio were calculated. Thus, confirmation of HF in patients was determined on clinical and echocardiography assessment. Echocardiographic parameters were measured according to current recommendations [14].

2.3. Genotyping

Blood samples from the patients were collected by venipuncture in EDTA coated vials and immediately aliquoted and frozen at −80°C. Genomic DNA was isolated using EZ-10 spin column blood genomic DNA purification kit (Bio Basic Inc., Markham, Ontario, Canada). *GRK5* (c.122A>T, p.Q41L) polymorphism was screened and confirmed through in-house developed tetra-primer based allele specific polymerase chain reaction (PCR) and confirmed with restriction fragment length polymorphism (RFLP) method and Sanger sequencing. PCR reaction mixture containing 100 ng of DNA, Taq polymerase mix (Ampliqon, Odense, Denmark), primers for tetra primer PCR (35nM GRK5F primer: 5'- GAAGGCTGTGGTCTAGTCTAGGGGAACAG - 3'and GRK5R primer: 5'- TTGATTAGCAAAAAGGTCAGCCTGGAGC - 3', 45nM SNPTF primer: 5' - TGGCTAATGTGAGGGAAC - 3'and SNPTR primer: 5' -TGTGTGAAGACCTCCGA - 3') or primers for RFLP method (50 nM BSRF primer: 5'- CTACAGCCCGTCCCTCTGT - 3'and BSRR primer: 5' - GCAAAAAGGTCAGCCTGGAGC - 3') were subjected to thermal cycling (Eppendorf, Hamburg, Germany). For RFLP, PCR product was digested using 1.5 units of *Bsr*I (New England Biolabs® Inc., Ipswich, Massachusetts, USA) as per manufacture instructions. The genotype was identified for tetra-primer PCR (AA: 580 bp and 225 bp, AT:580 bp, 380 bp and 225 bp, and TT:580 bp and 380 bp) and RFLP method (AA:202 bp and 179 bp, AT:381 bp, 202 bp and 179 bp, and undigested TT:381 bp) with these banding patterns after resolving on the gel.

2.4. Quantitative PCR (qPCR)

RNA was isolated from the HF patients and unaffected controls using the Aurum™ total RNA mini kit (Bio-Rad, Hercules, California, USA). The RNA was qualitatively verified on denaturing formaldehyde 1.2% agarose gel electrophoresis. Using 1 μg of RNA, respective cDNAs was synthesized using iScript™first strand cDNA synthesis kit (Bio-Rad, Hercules, California, USA). qPCR was carried out to compare *GRK5* gene expression in HF cases and healthy controls using relative quantification with β-actin as reference gene. PCR mixture of 20μL contained 2X SYBR green (Sso Advanced universal SYBR® Green supermix, Bio-Rad, Hercules, California, USA), 50 nM of forward and the reverse primer of the respective gene, cDNA template corresponding to 1 μg RNA and nuclease free water. The primers used were GRK5 FP (5' - ACCTGAGGGGAGAACCATTC - 3'), GRK5 RP (5' -TGGACTCCCCTTTCCTCTTT - 3'), ACTIN FP (5' - TCCCTGGAGAAGAGCTACG - 3'), and ACTIN RP (5' - GTAGTTTCGTGGATGCCACA - 3'). Reaction included an initial denaturation of 95°C for 3 min followed by 45 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 45 s, and a melt curve analysis (65 – 95°C) was performed at the end of 45 cycles. Normalized fold expression was calculated using $2^{-\Delta\Delta Ct}$ method.

2.5. Variables

In this study, the independent variables were age, sex, and socio-demographic data. Covariates were (EF%), comorbidities, use of β-blockers, and use of other medications. The covariates adjusted for hospital-free survival analysis were age, sex, EF%, and comorbidities.

2.6. Statistical analysis

Student *t*-tests (paired and unpaired) and Chi-square tests were used to assess significant differences in variables between the groups, drug dosages, genotype classes, gene expression, and EF%. Continuous variables are expressed as mean ± standard deviation (SD). Fischer's exact test was carried out to compare cardiac output between groups. Hardy-Weinburg equilibrium was assessed to compare predictions with that of the obtained results. Kaplan–Meier curves were also constructed to plot the percentage of survival of patients. Comparisons of overall survival were performed using a log-rank (Mantel-Cox) test. All the statistical analyses were carried out using GraphPad Prism software version 5.0 Windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Clinical and demographic characteristics of the HF patient cohort

The HF patient cohort (n=584) was considered for the study after clinical and echocardiographic assessment. Table 1 shows the clinical and demographic characteristics of the HF patient cohort. The HF patients were categorized into β-blocker group and non-β-blocker group. There was no statistically significant difference in variables such as age, sex, body mass index, and hemodynamics between the two groups. BVD (24%; p=0.002) and LV diastolic dysfunction (32.8%; p=0.002) were observed as statistically significant HF characteristics in the β-blocker group. Dilated cardiomyopathy (54%; p=0.0002) was observed as statistically significant HF etiology in the β-blocker group versus the non-β-blocker group whereas rheumatic heart disease (8%; p=0.0001) and coronary artery disease (44%; p=0.0001) were significantly higher in the non-β-blocker group. Type II diabetes mellitus (60%; p=0.0001) and its associated disorders were significantly frequent in the non-β-blocker group whereas systemic hypertension (42%; p=0.002) and dyslipidemia (25%; p=0.002) were higher in the β-blocker group. Approximately 52% of the patients were on β-blocker therapy. Among the medications administered, 38% of the HF patients received ACE inhibitors, 20% ARBs, 70% diuretics, 35% digitalis glycosides, 22% statins, and 19% anticoagulants.

Table 1

Clinical and demographic characteristics of the HF patient cohort

Variables	HF patient cohort (*n*=584)	
	β-blocker group (*n*=304)	Non-β-blocker group (*n*=280)
Mean Age±SD (years) (range)	60±10 (24 – 89)	59±11 (25 – 90)
Males (%)	73	73
Mean body mass index (kg/m^2) (range)	22 (19.5 – 24.5)	23.5 (21.1 – 26.5)
Mean follow up time (years) (range)	3.7 (3 – 7)	3.5 (3 – 7)
Hemodynamics		
Heart rate (beats/min)	78	80
Mean systolic blood pressure (mmHg)	128 (81 – 171)	123 (80 – 170)
Mean diastolic blood pressure (mmHg)	84 (50 – 139)	72 (55 – 130)
HF Etiology, n (%)		
Coronary artery disease	89 (26)	131 (47)*
Dilated cardiomyopathy	164 (54)	109 (36)*
Rheumatic heart disease	0	24 (8)*
HF Characteristics, n (%)		
LV systolic dysfunction	106 (35)	112 (40)
LV diastolic dysfunction	100 (32.8)	50 (18)*
Biventricular dysfunction	74 (24)	40 (14)*
Pulmonary artery hypertension	52 (17)	50 (18)
Comorbidities, n (%)		
Type II diabetes mellitus and its associated disorders	106 (35)	168 (60)*
Systemic hypertension	128 (42)	84 (30)*
Dyslipidemia	76 (25)	42 (15)*
Hypothyroidism	49 (16)	56 (20)
Cerebrovascular events	18 (6)	12 (4)
Renal insufficiency	9 (3)	14 (5)
Anemia	6 (2)	6 (2)

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*$p \leq 0.05$ versus β-blocker group.

3.2. GRK5 Gln41>Leu polymorphism in HF patient cohort

GRK5 Gln41>Leu polymorphism was screened by in-house developed tetra-primer based allele specific PCR and reconfirmed with RFLP and the Sanger sequencing method (Figure 1). Genotype distribution was in agreement with the Hardy-Weinberg equilibrium (p=0.595). The genotype (84% for AA, 15% for AT, and 1% for TT) and allele frequencies (0.916 for A allele and 0.084 for T allele) of *GRK5* Gln41>Leu polymorphism in the HF patient cohort is presented in Table 2. On comparing the genotype frequencies, AT genotype was more prevalent in the β-blocker group (19%) compared to the non-β-blocker group (10%) (p=0.011). The unaffected healthy controls were also screened for this polymorphism. The genotype and the allele frequencies of the healthy controls (Supplementary Table 1) did not differ significantly from the HF cases (p=0.94 for genotype frequency, p=0.82 for allele frequency) ensuring a lack of association of this polymorphism with development of HF.

Figure 1

Genotyping of GRK5 Gln41>Leu polymorphism. Tetra-primer based allele specific polymerase chain reaction (A) results on showing banding pattern for Gln/Leu heterozygous AT genotype (lane 2: 580 bp, 380 bp and 225 bp), Leu/Leu homozygous TT genotype (lane 3: 580 bp and 380 bp), Gln/Gln homozygous AA genotype (lanes 4, 5: 580 bp and 225 bp). RFLP (B) results showing banding pattern for Gln/Leu heterozygous AT genotype (lanes 2, 3: 381 bp, 202 bp and 179 bp), Leu/Leu homozygous TT genotype (lane 4: 381 bp) and Gln/Gln homozygous AA genotype (lanes 5, 6, 7: 202 bp and 179 bp). Lanes 1a, 1 b: 100 bp DNA ladder (Biobasic, Canada). Sanger sequencing results of Gln/Gln homozygous AA genotype C), Gln/Leu heterozygous AT genotype (D) and Leu/Leu homozygous TT genotype (E).

Table 2

GRK5 genotype and allele frequencies in the HF patient cohort

Genotype	HF patients (n=584), n (%)	HF patients (β-blockers) (n=304), n (%)	HF patients (non-β-blockers) (n=280), n (%)	p-value
AA	493 (84)	244(80)	249(89)	0.011
AT	85 (15)	57 (19)	28 (10)	
TT	6 (1)	3 (1)	3 (1)	
Allele				0.07
A	535 (91.6)	273 (90)	263 (94)	
T	49 (8.4)	31 (10)	17 (6)	

Results were expressed as n (%) for genotype and allele distribution. $\chi 2$ test was significant for genotype frequency and not significant for allele frequency between β-blocker group and the non-β-blocker group.

As the frequency of homozygous TT genotype was low, we intended to divide the HF patients into two group, those having AA genotype alone (AA genotype group) and those with AT and TT genotype (AT/TT genotype group), to study the influence of *GRK5* Gln41Leu (rs17098707) polymorphism on response to β-blocker therapy in the HF patients.

3.3. Downregulated GRK5 expression among the GRK5 Leu41 variants (AT/TT genotype)

The *GRK5* gene expression was evaluated among different groups of HF patients. The gene expression among The AT/TT genotype carriers showed a downregulated *GRK5* expression than AA genotype carriers among β-blockers and non-β-blockers. For gene expression analysis (Figure 2), healthy controls (n=60) were included for comparison. The β-blocker AT/TT group (n=60) showed a 0.5135±0.1593-fold decrease in gene expression and the β-blocker AA group (n=60) showed a slightly but significant upregulated pattern of 1.752±0.0.2775 gene expression (p<0.0001). On comparing the non-β-blockers, there was a significant difference in the gene expression (p<0.0001) between the AT/TT group (n=31, 0.5507±0.1322) and the AA group (n=60, 1.681±0.2688).

Figure 2

GRK5 expression among different groups of HF patients. β-blocker AT/TT group versus β-blocker AA group (p<0.0001), non-β-blocker AT/TT group versus non-β-blocker AA group (p<0.0001) along with healthy controls (HC) were analyzed for _GRK5_ normalized gene expression. Student's _t_-test was used to compare the groups.

3.4. Improvement in the EF of the GRK5 Leu41 variants (AT/TT genotype)

The EF% is the measure of LV systolic function and cardiac output. Among the β-blocker group (29±4.8%) and non-β-blocker group (41.5±2.3%), the mean EF% remained significantly varied (p=0.0286). The EF% of the patients before and after pharmacological treatment was recorded and improvement in their cardiac output was compared among the groups (Figure 3). The increment in the EF% after treatment was considered as an improvement in cardiac output. About 56% of HF patients on β-blockers and 41% on non-β-blockers showed improvement in their EF% (p=0.0004; Figure 3A). As expected, 71% of the *GRK5* Leu41 variants (AT/TT genotype) showed remarkably higher cardiac output (p=0.0001; Figure 3A) versus those with *GRK5*Gln41 (AA genotype, 45%). Since the non-β-blockers had HF preserved EF, only the β-blocker group having HF reduced EF was considered for further analysis. When the HF patients with different genotypes in the β-blocker group were compared it was evident that those with the AT/TT genotype (83%) improved significantly (p=0.0001; Figure 3B) compared to those with the AA genotype (49%).

Figure 3

Percentage improvement in cardiac output among the cohort of HF patients. The percentage of HF patients improved among β-blocker and non-β-blocker groups (p=0.0004) and different genotype groups of *GRK5* (p=0.0001) (A). The percentage of HF patients improved among β-blocker group with different genotypes (p=0.0001) (B). Fischer's exact test was carried out for analysis.

The improvement in cardiac output was compared among the HF patients in β-blocker group with different genotypes (Figure 4). EF% has improved from 25.2±1.041% to 28.2±0.7638% among the AA group (p=0.0091) and 27.4±1.021% to 36±1% in the AT/TT group (p=0.0007).

Figure 4

Improvement in ejection fraction in HF patient cohort. The improvement in EF% in HF patients with different genotypes of β-blocker group was measured. AT/TT group (p=0.0091) improved significantly in comparison with the AA group (p=0.0091). Paired t-test was carried out for computing p-values.

3.5. GRK5 Leu41 variants (AT/TT genotype) show decrement in β-blocker drug dosage

Among the β-blocker group (n=304), 60% of the HF patients were treated with carvedilol, 17% with metoprolol, 12.5% and 10% with nebivolol and Bisoprolol, respectively. The initial and the final drug dosages over the follow-up period were noted and analyzed. Initial dosages administered at the start of the β-blocker therapy and final dosages followed at the last follow-up were noted. There was a statistically significant reduction in carvedilol drug dosage in the overall β-blocker group when the initial (11.2±7.7 mg) and final drug dosages (10.2±7.1 mg) were compared (p=0.0114; Figure 5A). Furthermore, the drug dosages remained unchanged in the AA genotype group (p=0.8211; Figure 5B) when the initial (11.06±7.3 mg) and final (11.1±7.4 mg) drug dosages were compared. Interestingly, there was a statistically significant reduction in the drug dosage in the AT/TT genotype group (p=0.0001; Figure 5B) when the initial (11.6±9.2 mg) and the final (6.4±4 mg) drug doses were compared. Thus, the *GRK5* Leu41 variants in the homozygous and heterozygous forms were associated with a lower dose of β-blockers.

Figure 5

GRK5 Gln41>Leu polymorphism influences β-blocker (carvedilol) drug dosage. Carvedilol drug doses were reduced in the overall β-blocker group (p=0.0114) (A). The drug doses remained unchanged in the AA genotype group (p=0.8211), whereas in the AT/TT genotype group there was a significant reduction in the drug dosage (p=0.0001) (B). Paired t-test was used to compare the drug doses between groups.

3.5. Reduced event of hospitalization among the GRK5 Leu41 variants (AT/TT genotype)

The HF patients were followed-up for ~3 years (40 months) after the initiation of pharmacological treatment. The influence of β-blocker therapy and *GRK5* genotype on hospitalization-free survival of HF patients was studied through Kaplan–Meier survival curves (time-to-event analysis). When the overall HF patients administered with β-blockers and non-β-blockers were compared for hospital-free survival, the patients on β-blockers showed higher survival than the non-β-blockers (hazard ratio [HR]: 1.167, 95% confidence interval [CI]: 0.325 – 2.008, log-rank $p<0.0001$; Figure 6A). The same was found when the overall HF patients with AA and AT/TT genotypes were compared; the presence of AT/TT genotype reduced the risk for hospitalization (HR: 0.8387, 95% CI: 0.07042 – 1.607, log-rank $p<0.0001$; Figure 6B). On further analysis of the HF subgroups, β-blocker AT/TT group versus β-blocker AA group, it was found that the presence of AA genotype increased the risk for hospitalization (HR: 1.143, 95% CI: 0.4337 – 1.852, log-rank $p<0.0001$; Figure 6C) even under the influence of β-blocker therapy. When non-β-blocker AT/TT group versus non-β-blocker AA group was analyzed, AA genotype increased the risk for hospitalization (HR: 1.364, 95% CI: 0.7020 – 2.025, log-rank $p=0.0003$; Figure 6C). In the β-blocker AT/TT group versus non-β-blocker AT/TT group analysis, the use of non-β-blockers increased the risk for hospitalization (HR: 1.067, 95% CI: 0.463 – 1.670, log-rank $p=0.0231$; Figure 6C). As expected, when β-blocker AA group versus non-β-blocker AA group was analyzed, the presence of AA genotype and use of non-β-blocker therapy increased the risk of hospitalization (HR: 1.273, 95% CI: 0.441 – 2.104, log-rank $p<0.0001$; Figure 6C).

Figure 6

Influence of β-blocker therapy and *GRK5* genotype on hospital-free survival of HF patients. HF patients were stratified by β-blockers usage (β-blockers ($n=304$); non-β-blockers ($n=280$); $p<0.0001$) (A). HF patients were stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (GRK5 AA group ($n=493$); *GRK5* AT/TT genotype ($n=91$); $p<0.0001$) (B). β-blocker treated subjects and non-β-blocker subjects stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (β-blocker AA group ($n=244$); β-blocker AT/TT group($n=60$); non-β-blocker AA group($n=249$); non-β-blocker AT/TT group ($n=31$) (C). Log-rank test (Mantel-Cox) test was utilized for comparisons of survival curves.

The overall HF patients were subdivided based on HF characteristics such as systolic dysfunction ($n=218$), diastolic dysfunction ($n=150$), BVD ($n=114$), and pulmonary artery hypertension (PAH) ($n=102$). The groups were compared for hospitalization-free survival. The patients with PAH showed higher survival and patients with systolic dysfunction showed the lower survival than the other groups (log-rank $p=0.02$; Figure 7A). When the subgroups with systolic dysfunction and diastolic dysfunction were compared with *GRK5* polymorphism genotypes (AA and AT/TT), it was evident that the HF patients with AA genotype under both the subgroups indicated the lower hospitalization-free survival than the HF patients with AT/TT genotype under both subgroups (log-rank $p=0.0002$; Figure 7B). When the subgroups with BVD and PAH were compared with *GRK5* polymorphism genotypes (AA and AT/TT), it was evident that the HF patients with AA genotype under both the subgroups indicated lower hospitalization-free survival than those with the AT/TT genotype under both subgroups (log-rank $p=0.0051$; Figure 7C).

Figure 7

Influence of *GRK5* genotype on hospital-free survival of HF patient subgroups. HF patients were stratified by HF characteristics systolic dysfunction (*n*=218); diastolic dysfunction (*n*=150); BVD (*n*=114); PAH (*n*=102); *p*=0.02) (A). HF patients with systolic and diastolic dysfunction were stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (systolic dysfunction AA group (*n*=193); diastolic dysfunction AA group (*n*=128); systolic dysfunction AT/TT group (*n*=25); diastolic dysfunction AT/TT group (*n*=22); *p*=0.0002) (B). HF patients with BVD and PAH were stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (BVD AA group (*n*=90); PAH AA group (*n*=82); BVD AT/TT group (*n*=24); PAH AT/TT group (*n*=20); *p*=0.0051) (C). Log-rank test (Mantel-Cox) test was utilized for comparisons of survival curves.

To compare the frequency of hospital admissions between the genotype groups within a month, the rate of hospital admission/month was compared (Figure 8). From the results, it was evident that the HF patients in the AA group (0.0769±0.003; *n*=493) were significantly more frequently ($p<0.0001$) admitted in hospital than the AT/TT group (0.0409±0.004; *n*=91; Figure 8A). Among the β-blocker group, HF patients with the AA genotype (0.0790±0.005; *n*=244) were significantly more frequently admitted in the hospital than the AT/TT variants (0.0374±0.005; *n*=60; *p*=0.0002). Among the non-β-blocker group, HF patients with the AA genotype (0.0749±0.005; *n*=249) had more hospital admissions compared to the AT/TT variants (0.0477±0.008; *n*=31) although not statistically significant (*p*=0.0674; Figure 8B).

Figure 8

Comparison of frequency of hospital admission between the genotype groups. The AA group of HF patients (*n*=493) had more hospital admissions than the AT/TT HF group (*n*=91; $p<0.0001$) (A). Among the β-locker group, HF patients with the AA genotype had more frequent hospital admission than the AT/TT variants (*p*=0.0002) (B). A similar trend was observed among the non-β-blocker group but the results were no statistically insignificant (ns). β-blocker AA group (*n*=244); β-blocker AT/TT group (*n*=60); non-β-blocker AA group(*n*=249); and non-β-blocker AT/TT group (*n*=31). Student's *t*-test was used to compare the gr.

4. Discussion

Genetic polymorphisms impact HF outcomes. One such polymorphism is *GRK5*Gln41>Leu (A>T), which was proven to have a role in promoting β-AR desensitization [11]. Our results show that HF patients with *GRK5* Leu41 variant in its homozygous and heterozygous forms show decreased events in hospitalization and specifically respond to β-blockers and require reduced β-blocker drug dosage in comparison to *GRK5* Gln41 carriers. Moreover, *GRK5* Leu41 variants in combination with β-blocker therapy showed improved cardiac output.

The *GRK5* genotype frequencies in this study (AT/TT15% and TT1%) were comparatively lower than that reported in African Americans (AT/TT39% and TT6%) [15]. The T allele frequency in our study (0.084) was lower than African Americans (0.23) [15] but, higher than that found in the Chinese population (0.009) [16] and Caucasians (0.017) [17]. As GRK5 is highly expressed in myocardium [18], and given its established role in cardiac failure pathology [10], this prompted our group to examine and evaluate *GRK5* expression in a cohort of Indian HF patients. GRK5 is reportedly overexpressed in HF conditions and could attenuate cardiac burden in response to adrenergic surge [19,20]. In view of this, a slight upregulation observed in *GRK5* expression in a subpopulation of HF patients could be expected. However, gene expression was downregulated in the AT/TT subset of HF patients in our study. A possible reason could be that the role of overexpressed GRK5 in β-AR desensitization is compensated by the allelic variation, bringing about an augmented desensitization, thus attempting to regulate *GRK5* expression for better cardiac performance. Interestingly though inhibiting GRK5 expression restores the cardiac muscle damage incurred during HF [21]. These observations support the fact that *GRK5* Leu41 variant confers protection from congestive HF [22].

Human and rodent data have proven that *GRK5*Leu41 protected against death or cardiac transplantation [11]. Coinciding with these data, our study reaffirms that the *GRK5*Leu41 allele improved quality of life, promoted hospitalization-free survival of South Indian HF patients. Independently, both the administration of β-blocker therapy and the presence of Leu41 allele yielded a positive outcome on hospitalization-free survival. The Leu41 allele carriers among the different HF subgroups showed increased hospitalization-free survival in comparison to Gln41 carriers. The previous reports support the fact that Indians are highly sensitive to β-blockers [23] and also speculated that polymorphic variations in β-AR gene in Indians [24] might be the reason for higher sensitivity [25]. Our data indicate that the prevalence of *GRK5* gene polymorphisms in Indian HF patients and its role in cardiac failure might be the reason for responders to β-blocker treatment. In response to β-blocker treatment, *GRK5*Leu41 allele carriers (AT/TT genotype) showed better hospital-free survival than *GRK5* Gln41 allele carriers (AA genotype) and β-blocker naïve *GRK5*Leu41 and Gln41 allele carriers. This observation was also reported in a study where HF patients with the Gln41Leu genotype had reduced hospitalization rates (18.6%) [26]. These results also justify the continued use of β-blockers as standard therapy for cardiac failure and their benefits over other pharmacological interventions [27-29]. Interestingly, it was found that although Leu41 carriers among African Americans survived better than Gln41 carriers, they exhibited a reduced response to β-blockers [11,17] such as atenolol [30]. Another study among African race demonstrated that Leu41 impacted negatively on survival of HF patients, but the effect seemed to be stabilized upon β-blocker usage. In other words, Gln41 carriers survived better in the presence and absence of β-blockers [15].

The Leu41 allele seemed to act as a genetic β-blockade [11], promoting reduced dependency on β-blockers for improvement in HF patients. Although β-blocker has been validated as a lifesaving drug in HF, in view of its adverse effects such as HF deterioration, hypotension, and impaired left atrial function [31,32], minimizing its usage is recommended. In our study, the presence of Leu41 allele has helped in curtailing the dosage of carvedilol during the course of treatment and reduced the frequency of hospital admission compared to the Gln41 allele. Carvedilol, a nonselective β-blocker with better efficacy than other β-blockers [10,33] was used in 60% of the HF patients in this study. The usage of β-blockers has substantially improved the LV systolic function by increasing the EF in 56% of HF patients in this study. Literature also supports the usage of β-blockers in recovering from LV systolic dysfunction [34]. The improvement in EF was pronounced in *GRK5* Leu41 variants compared to Gln41 carriers and this observation coincided with another study reporting *GRK5* polymorphism having a

positive effect on systolic HF after β-blocker therapy [16]. Taken together, these data present convincing evidence that the Leu41 allele and β-blocker therapy synergistically work to stabilize cardiac function in Indian HF subjects.

Ethical Approval

Human samples to carry out the study were obtained following informed consent of patients according to the Declaration of Helsinki and its later amendments or comparable ethical standards. Research involving humans was authorized by the Institutional Ethics committee from PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.

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Appendix

Supplementary Table 1

***GRK5* genotype and allele frequencies in the HF patients and healthy controls**

Genotype	HF patients (*n*=584), *n* (%)	Healthy controls (*n*=400), *n* (%)	*p* value
AA	492 (84)	340 (85)	0.94
AT	86 (15)	56 (14)	
TT	6 (1)	4 (1)	
Allele			
A	535 (91.6)	368 (92)	0.82
T	49 (8.4)	32 (8)	

Results were expressed as *n* (%) for genotype and allele distribution. χ^2 test was non significant for genotype and allele frequencies between HF patient cohort and healthy controls group.

Conflict of Interest

The authors declare no conflict of interest.

The genetics of cardiac failure: Role of a G protein-coupled receptor polymorphism in therapeutic response in an Indian population

References

[1] Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart Disease and Stroke Statistics-2019 Update:A Report From the American Heart Association. *Circulation.* 2019;139:e56–528. [PubMed] [Google Scholar]

[2] Huffman MD, Prabhakaran D. Heart Failure:Epidemiology and Prevention in India. *Natl Med J India.* 2010;23:283–8. [PMC free article] [PubMed] [Google Scholar]

[3] Guo M, Guo G, Ji X. Genetic Polymorphisms Associated with Heart Failure:A Literature Review. *J Int Med Res.* 2016;44:15–29. [PMC free article] [PubMed] [Google Scholar]

[4] Seth S, Ramakrishnan S, Parekh N, Karthikeyan G, Singh S, Sharma G. Heart Failure Guidelines for India:Update 2017. *J Pract Cardiovasc Sci.* 2017;3:133–8. [Google Scholar]

[5] Bristow MR. Beta-Adrenergic Receptor Blockade in Chronic Heart Failure. *Circulation.* 2000;101:558–69. [PubMed] [Google Scholar]

[6] Steinberg SF. The Molecular Basis for Distinct Beta-Adrenergic Receptor Subtype Actions in Cardiomyocytes. *Circ Res.* 1999;85:1101–11. [PubMed] [Google Scholar]

[7] Madamanchi A. Beta-adrenergic Receptor Signaling in Cardiac Function and Heart Failure. *Mcgill J Med.* 2007;10:99–104. [PMC free article] [PubMed] [Google Scholar]

[8] de Lucia C, Eguchi A, Koch WJ. New Insights in Cardiac β-Adrenergic Signaling during Heart Failure and Aging. *Front Pharmacol.* 2018;9:904. [PMC free article] [PubMed] [Google Scholar]

[9] Zhang Y, Matkovich SJ, Duan X, Gold JI, Koch WJ, Dorn GW., 2nd Nuclear Effects of G-Protein Receptor Kinase 5 on Histone Deacetylase 5-Regulated Gene Transcription in Heart Failure. *Circ Heart Fail.* 2011;4:659–68. [PMC free article] [PubMed] [Google Scholar]

[10] Mangmool S, Parichatikanond W, Kurose H. Therapeutic Targets for Treatment of Heart Failure:Focus on GRKs and β-Arrestins Affecting β-AR Signaling. *Front Pharmacol.* 2018;9:1336. [PMC free article] [PubMed] [Google Scholar]

[11] Liggett SB, Cresci S, Kelly RJ, Syed FM, Matkovich SJ, Hahn HS, et al. A GRK5 Polymorphism that Inhibits Beta-Adrenergic Receptor Signaling is Protective in Heart Failure. *Nat Med.* 2008;14:510–7. [PMC free article] [PubMed] [Google Scholar]

[12] Dorn GW, 2nd, Liggett SB. Pharmacogenomics of Beta-Adrenergic Receptors and their Accessory Signaling Proteins in Heart Failure. *Clin Transl Sci.* 2008;1:255–62. [PMC free article] [PubMed] [Google Scholar]

[13] King M, Kingery J, Casey B. Diagnosis and Evaluation of Heart Failure. *Am Fam Physician.* 2012;85:1161–8. [PubMed] [Google Scholar]

[14] Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults:An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;28:1–39.e14. [PubMed] [Google Scholar]

[15] Johnson AE, Hanley-Yanez K, Yancy CW, Taylor AL, Feldman AM, McNamara DM. Adrenergic Polymorphisms and Survival in African Americans With Heart Failure:Results From A-HeFT. *J Card Fail.* 2019;25:553–60. [PMC free article] [PubMed] [Google Scholar]

[16] Kang S, Hong X, Ruan CW, Yu P, Yu SS, Chen M, et al. Effects of GRK5 and ADRB1 Polymorphisms Influence on Systolic Heart Failure. *J Transl Med.* 2015;13:44. [PMC free article] [PubMed] [Google Scholar]

[17] Cresci S, Kelly RJ, Cappola TP, Diwan A, Dries D, Kardia SL, Dorn GW., 2nd Clinical and Genetic Modifiers of Long-Term Survival in Heart Failure. *J Am Coll Cardiol.* 2009;54:432–44. [PMC free article] [PubMed] [Google Scholar]

[18] Vinge LE, Øie E, Andersson Y, Grøgaard HK, Andersen G, Attramadal H. Myocardial Distribution and Regulation of GRK and Beta-Arrestin Isoforms in Congestive Heart Failure in Rats. *Am J Physiol Heart Circ Physiol.* 2001;281:H2490–9. [PubMed] [Google Scholar]

[19] Chen EP, Bittner HB, Akhter SA, Koch WJ, Davis RD. Myocardial Function in Hearts with Transgenic Overexpression of the G Protein-Coupled Receptor Kinase 5. *Ann Thorac Surg.* 2001;71:1320–4. [PubMed] [Google Scholar]

[20] Ishizaka N, Alexander RW, Laursen JB, Kai H, Fukui T, Oppermann M, et al. G Protein-Coupled Receptor Kinase 5 in Cultured Vascular Smooth Muscle Cells and Rat Aorta. Regulation by Angiotensin II and Hypertension. *J Biol Chem.* 1997;272:32482–8. [PubMed] [Google Scholar]

[21] Sorriento D, Santulli G, Fusco A, Anastasio A, Trimarco B, Iaccarino G. Intracardiac Injection of AdGRK5-NT Reduces Left Ventricular Hypertrophy by Inhibiting NF-kappaB-Dependent Hypertrophic Gene Expression. *Hypertension.* 2010;56:696–704. [PubMed] [Google Scholar]

[22] Hendrickx JO, van Gastel J, Leysen H, Santos-Otte P, Premont RT, Martin B, et al. GRK5-a Functional Bridge between Cardiovascular and Neurodegenerative Disorders. *Front Pharmacol.* 2018;9:1484. [PMC free article] [PubMed] [Google Scholar]

[23] Rasool AH, Rahman AR, Ismail R, Hatim S, Abdullah AR, Singh R, et al. Ethnic Differences in Response to Non-Selective Beta-Blockade among Racial Groups in Malaysia. *Int J Clin Pharmacol Ther.* 2000;38:260–9. [PubMed] [Google Scholar]

[24] Ramu P, Kumar KN, Shewade DG, Swaminathan RP, Dutta TK, Balachander J, et al. Polymorphic Variants of Beta1 Adrenergic Receptor Gene (Ser49Gly and Arg389Gly) in Healthy Tamilian Volunteers. *Indian J Med Res.* 2010;132:62–6. [PubMed] [Google Scholar]

[25] Kumar KN, Ramu P, Rajan S, Shewade DG, Balachander J, Adithan C. Genetic Polymorphisms of Beta1 Adrenergic Receptor and their Influence on the Cardiovascular Responses to Metoprolol in a South Indian Population. *J Cardiovasc Pharmacol.* 2008;52:459–66. [PubMed] [Google Scholar]

[26] Huang J, Li C, Song Y, Fan X, You L, Tan L, et al. ADRB2 Polymorphism Arg16Gly Modifies the Natural Outcome of Heart Failure and Dictates Therapeutic Response to β-Blockers in Patients with Heart Failure. *Cell Discov.* 2018;4:57. [PMC free article] [PubMed] [Google Scholar]

[27] Chavey WE., 2nd The Importance of Beta Blockers in the Treatment of Heart Failure. *Am Fam Physician.* 2000;62:2453–62. [PubMed] [Google Scholar]

[28] Cesario DA, Fonarow GC. Beta-Blocker Therapy for Heart Failure:The Standard of Care. *Rev Cardiovasc Med.* 2002;3:14–21. [PubMed] [Google Scholar]

[29] Dézsi CA, Szentes V. The Real Role of β-Blockers in Daily Cardiovascular Therapy. *Am J Cardiovasc Drugs.* 2017;17:361–73. [PMC free article] [PubMed] [Google Scholar]

[30] Kurnik D, Cunningham AJ, Sofowora GG, Kohli U. Li is not Associated with Sensitivity to Beta(1)-Adrenergic Blockade in Humans. *Pharmacogenomics.* 2009;10:1581–7. [PMC free article] [PubMed] [Google Scholar]

[31] Sardana M, Syed AA, Hashmath Z, Phan TS, Koppula MR, Kewan U, et al. Beta-Blocker use is Associated with Impaired Left Atrial Function in Hypertension. *J Am Heart Assoc.* 2017;6:e005163. [PMC free article] [PubMed] [Google Scholar]

[32] Ko DT, Hebert PR, Coffey CS, Curtis JP, Foody JM, Sedrakyan A, et al. Adverse effects of Beta-Blocker Therapy for Patients with Heart Failure:A Quantitative Overview of Randomized Trials. *Arch Intern Med.* 2004;164:1389–94. [PubMed] [Google Scholar]

[33] DiNicolantonio JJ, Lavie CJ, Fares H, Menezes AR, O'Keefe JH. Meta-Analysis of Carvedilol Versus Beta 1 Selective Beta-Blockers (Atenolol, Bisoprolol, Metoprolol, and Nebivolol) *Am J Cardiol.* 2013;111:765–9. [PubMed] [Google Scholar]

[34] Bellenger NG, Rajappan K, Rahman SL, Lahiri A, Raval U, Webster J, et al. Effects of Carvedilol on Left Ventricular Remodelling in Chronic Stable Heart Failure:A Cardiovascular Magnetic Resonance Study. *Heart.* 2004;90:760–4. [PMC free article] [PubMed] [Google Scholar]

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Pediatric Research and Child Health

RESEARCH ARTICLE

The Emergence of Deafness, Blindness, Cerebral Palsy, and Mental Retardation among very low birth weight (VLBW) Infants, aged 21-24 Months

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Abstract

Purpose: The study aims to assess the emergence of deafness, blindness, cerebral palsy, and mental retardation among very low birth weight (VLBW) infants, aged 21-24 months.

Methods: A study was conducted with a total of 123 premature Saudi infants, who were recruited from King Khalid University Hospital. The association among demographic factors and neurodevelopmental morbidities were identified with the help of regression analysis.

Results: A significant relationship between deafness and cerebral palsy was found. However, there was no significant relationship between retinopathy of prematurity and intraventricular hemorrhage. Furthermore, maternal education plays a positive role in the neurodevelopment of preterm newborns.

Conclusion: This study has concluded that there has been significant relationship between cerebral palsy and deafness in preterm infants with very low birth weight. On the contrary, there was no significant relationship found between intraventricular hemorrhage and retinopathy of prematurity.

Keywords: Blindness, Cerebral Palsy, Deafness, Neonatal Care, Neonatal Morbidity

Abbrevations: **BPD**: Bronchopulmonary Dysplasia, **AAP:** American Academy of Pediatricians, **ROP:** Retinopathy of Prematurity, **HIE:** Hypoxic Ischemic Encephalopathy, **PPHN**: Persistent Pulmonary Hypertension, **PDA**: Patent Ductus Arteriosus

Introduction

The primary cause of neonatal morbidity and mortality is the preterm birth of an infant [1]. Developments in perinatal care have enhanced the chances of survival of preterm low birth weight infants of gestational age of less than 25 weeks. Although, the survival rate has increased, the chances of neonatal morbidity and neurodevelopment impairment have also augmented and increased. On the other hand, the lower the gestational age and the birth weight of the preterm infant; the higher the infants' likelihood of suffering from major neonatal morbidity [2].

The disabilities occur due to the improper development of the infant or poor functioning of nervous system. The limitations that are manifested during infancy or early stage of childhood include cognition, vision, behavior, and hearing. There are the modalities that these disabilities are traced in the early stage of neurodevelopment. However, the cognitive disabilities among infants and children includes mental retardation as well as it further leads to the learning disabilities. Moreover, distress and chronic stress usually leads to psychological changes, which further result in causing physical distress, psychiatric disorder, and psychological distress [3].

Early years of childhood are important for language development and communication because during this time the brain is at its highest capacity to go about structural changes in regard to external stimulation and is termed as neural plasticity [4]. According to American Academy of Pediatricians (AAP), there are 2% to 3% of infants with developmental disabilities. In most of the cases, no definite cause is ever found. Intellectual disabilities can be further classified as mild, moderate, severe, and profound; and this is done on the basis of various intellectual disabilities. Mental disability is usually result of genetic disorders; for instance, Down syndrome. In case of infants, the genetic disorders are usually detected at an

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early stage. It is due to certain physical or facial characteristics that alert the parents or the clinician.

Experts, who are always associated with disabled children, believed that in such cases early intervention pays off immensely [5]. Not much work has been done in order to investigate the outcome of early intervention for children, who are deaf and blind. However, various studies illustrated that, the early identification and intervention for hearing loss, proved out to be beneficial for infants as it enhances their language skills [6]. Continued ill-health of the mother can lead to further morbidities or disabilities and can negatively impact baby's health. Whereas, these complications and consequences are not much focused upon in various countries in low-income countries. Families situated in Middle Eastern regions complaint about isolation and lack of community resources to help them in improving their child's abilities. One of the main reasons behind the lack of awareness or medical care is that there are no health professionals or researchers available in such regions. In most cases, these disabilities also come forward due to the mother's depressive state [7].The aim of the present study is to assess the relationship between Retinopathy of Prematurity and Intraventricular hemorrhage as well as the relationship between deafness and cerebral palsy.

Methods

A total of 123 premature Saudi infants, with birth weight less than or equal to 1500 grams & gestation less than equal to 32 weeks were recruited. These infants that were chosen for the study were from the King Khalid University Hospital in which these premature infants were delivered. The study was approved from the ethics committee. The analysis was done on the basis of neurological and developmental assessment, along with medical and social history. It was done in order to determine functional and neurodevelopmental outcomes among infants. The neurologic examinations were performed by certified physicians, who had been well trained in this department. The neurological assessment included the evaluation of strength reflex, angles as well as posture. The demographics include age, gender, and gestation age and body weight. The factors examined included were maternal education, birth weight, early-onset sepsis, late-onset sepsis, grades 3 to 4 IVH/PVL, Bronchopulmonary Dysplasia (BPD), Retinopathy of Prematurity (ROP), Hypoxic Ischemic Encephalopathy (HIE), Persistent pulmonary hypertension (PPHN), Patent ductus arteriosus (PDA).

During the first four weeks of life, Intraventricular hemorrhage (IVH) is usually detected by head ultrasound during first four weeks of life. Moreover, it is graded in accordance with Papile's classification. Periventricular echogenicity, which is noticed on the head ultrasound and is performed at any time during the NICU stay, is known as Preventricular Leucomalacia (PVL). Retinopathy of Prematurity (ROP) is detected during routine retinal examination. Furthermore, Patent Ductus Arteriosus (PDA) is established through an echocardiography with signs of left-to-right shunting. Bronchopulmonary Dysplasia (BPD) refers to the requirement of oxygen at 36 weeks, corrected age.

Table 1: Gestational Age of the study participants

Gestational age	Number	Percent
26	3	2.4
27	6	4.9
28	25	20.3
29	23	18.7
30	36	29.3
31	14	11.4
32	11	8.9
34	3	2.4
35	1	0.8
38	1	0.8
Total	123	100

The data were analyzed to evaluate the impact of morbidities on various neurodevelopmental disabilities like blindness, deafness, cerebral palsy (CP) and mental retardation (MR). Severe mental retardation syndrome includes optic atrophy, microcephaly, that further results in impaired vision, defect in hearing, seizures, restricted movements of large joints and spasticity. The study examined the influence of maternal education and early intervention on BINS at 21 to 24 months infants. Maternal education is considered as one of the strongest determinants that ensures infant survival in developing countries. The statistical analysis was done to assess maternal education and its association with disabilities. BINS was classified into two different groups. First group was low and the other group was taken as moderate (Mod) and high. However, 'Low' illustrated that no further follow-up is required; whereas, 'high' suggested that further developmental testing is necessary. Descriptive statistics were calculated using mean and standard deviation. Spearman rank correlation analysis was done to find the correlation between Retinopathy of Prematurity and Intraventricular hemorrhage. Chi square test was done to find the association deafness and cerebral palsy. $P<0.05$ was considered as statistically significant (Table 2).

Table 2: Association between Deafness and CP

	CP (No)	CP(Yes)	Total
Deafness (No)	120(98.4%)	2(1.6%)	122
Deafness(Yes)	0 (0.0%)	1 (100%)	1
Total	120	3	123

Results

The total number of neonates who were examined in the study were 123. Out of the total, newborn females totaled 53 (43.1%). The mean (SD) of the gestation age in weeks was 29.65 (1.82). Amongst the total number of neonates, the lowest gestational age of preterm infants was 26 weeks and the highest gestational age was 38 weeks. Apart from this, the premature neonates that had a gestational age of 28 weeks were 25 (20.3%) infants. Similarly, the preterm neonates that had a gestational age of 30 weeks were 36 (20.3%) infants and were the highest number of infants in that particular classification group (Table 1). Among the 123 mothers, 85 (69.1%) were educated. The

correlation between ROP and IVH was examined and found that spearman, s rho r=0.145 p=0.109 which is not statistically significant.). The association between Deafness and Cerebral Palsy was found to be statistically significant

(Chi-square=40.38, P<0.001)

Discussion

The results revealed that there is significant relationship between Deafness and Cerebral Palsy. However, there was no significant relationship between Retinopathy of Prematurity (ROP) and Intraventricular hemorrhage (IVH). The results are consistent with the study conducted by Neubauer et al. (2008), in which it was discovered that the proportion of disabilities has increased with decreasing gestational age of the newborn. Infants having high grade intraventricular hemorrhage, neonatal seizures, necrotizing enterocolitis and bowel perforation were at a significant risk for developing and enduring major impairment [8].

In a study conducted by Wilson-Costello et al. (2007), modern therapies that were incorporated with regard to low birth weight infants were compared to traditional therapies that were provided to low birth weight infants in the previous two decades. The comparison features were the rate of survival and death with and without impairment and the therapies that were provided. The population comprised of 496 low birth weight infants born (2000-2002), 1749 neonates that were born (1982-1989) and 233 newborns born (1990-1999). The findings revealed that between 1990 and 1999, survival rate has increased from 49% to 68%, but so did neonatal morbidity. Therefore, it leads to survival without impairment as well as survival with impairment [9].

The therapy modification between 2000-2002 included a reduction of postnatal steroid use. Apart from this, antenatal steroid use was increased. It was observed that there were no changes in the rate of lung disease. In addition, there was found to be a decrease in morbidities; such as, severe intraventricular hemorrhage (IVH) and early-onset sepsis (EOS) and late-onset sepsis (LOS). With therapeutic care, the rate of neurodevelopmental impairment has reduced from 35% to 23%. More specifically, the rate of cerebral palsy reduced from 13% to 5%. It was concluded that there was significant reduction in the neurodevelopment impairment in neonates with very low birth weight between 2000-2002 as compared to a decade early. The improved outcomes and the increase in the survival rate of newborns with low birth weight were due to modern therapies that were being provided. These included both perinatal and neonatal factors. However, despite the use of postnatal steroids, the lung condition of the infants showed no improvement [10]. Many studies revealed that maternal education has a positive impact on neurodevelopmental outcome among very low birth infants. The results conducted by Voss et al. (2012) revealed that the maternal educational background was directly associated with the intellectual growth of pre-term infants. Low maternal educational background was connected to reduced composite intelligent quotient of pre-term kids, who were between the ages of 10-13 years old during the study [11-14].

Conclusion

The preterm birth of an infant can be the cause of neonatal morbidity and mortality. Preterm neonates with a very low birth weight have an increased risk of suffering from neurological, sensory, development, and functional morbidity. The neurodevelopment of all very low birth weight newborns must be monitored and the relevant therapies must be incorporated to ensure and optimize their survival. Some morbidities are directly associated with long-term outcomes of the development of preterm infants. This study has concluded that there is a significant relationship between Deafness and Cerebral Palsy in infants. On the contrary, there was no significant relationship found between Intraventricular hemorrhage (IVH) and Retinopathy of Prematurity (ROP). Additionally, maternal education played a significant role in the development of preterm newborns. However, more studies must be conducted in relation to preterm babies and neonatal morbidity and mortality to get further insights into the causes of morbidities, suffered by preterm babies with very low birth weight.

References

1. Alhaidari SI, Al Houssien AO, Alteraiqi MA, Al Arafah AM, Al Houssien RO, et al. (2016) Children's growth pattern and mothers' education and socio-economic status in Riyadh, Saudi Arabia. *Journal of Health Specialties* 4: 276. [View Article]
2. Ashford L (2002) Hidden suffering: disabilities from pregnancy and childbirth in less developed countries. Policy brief Washington, DC: Population Reference Bureau *Measure Communication* 6. [View Article]
3. Azar M & Badr LK (2006) The adaptation of mothers of children with intellectual disability in Lebanon. *Journal of Transcultural Nursing* 17: 375-380. [View Article]
4. Cole EB & Flexer C (2007) Children with hearing loss: Developing listening and talking. *San Diego: Plural Publishing Inc* [View Article]
5. Estes A, Zwaigenbaum L, Gu H, John TS, Paterson S, et al. (2015) Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life. *Journal of neurodevelopmental disorders* 7: 24. [View Article]
6. Guralnick MJ (Edn) (1997) The effectiveness of early intervention. *Baltimore: Paul H Brookes Publishing Company* [View Article]
7. Hack M, & Fanaroff A A (1999) Outcomes of children of extremely low birthweight and gestational age in the 1990's. *Early human development* 53: 193-218. [View Article]
8. Kennedy CR, McCann DC, Campbell MJ, Law CM, Mullee M, et al. (2006) Language ability after early detection of permanent childhood hearing impairment. *New England Journal of Medicine* 354: 2131-2141. [View Article]
9. Neubauer AP, Voss W & Kattner E (2008) Outcome of extremely low birth weight survivors at school age: the influence of perinatal parameters on neurodevelopment. *European journal of pediatrics* 167: 87-95. [View Article]
10. Sapolsky RM (2007) Stress, stress-related disease, and emotional regulation.[View Article]

11. United Nations Children's Fund (1997) Guidelines for monitoring the availability and use of obstetric services. New York, NY: United Nations Children's Fund 103.[View Article]

12. Voss W, Jungmann T, Wachtendorf M, & Neubauer A P (2012) Long-term cognitive outcomes of extremely low-birth-weight infants: The influence of the maternal educational background. *Acta Paediatrica* 10:569-573. [View Article]

13. Wilson Costello D, Friedman H, Minich N, Siner B, Taylor G, et al. (2007) Improved neurodevelopmental outcomes for extremely low birth weight infants in 2000-2002. *Pediatrics* 119: 37-45. [View Article]

14. Xiong T, Gonzalez F, & Mu DZ (2012) An overview of risk factors for poor neurodevelopmental outcome associated with prematurity. *World Journal of Pediatrics* 8: 293-300. [View Article]

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Research Paper

Physical properties of menstrual hygiene waste as feedstock for onsite disposal technologies

Sarani Sasidaran, Prateek Kachoria, Antony Raj, Sudha Ramalingam, Brian R. Stoner, Katelyn L. Sellgren and Sonia Grego

ABSTRACT

Onsite incinerators offer a promising solution to the challenges of menstrual waste disposal, particularly in institutional settings. However, thermochemical processes are well known to depend on the nature of the feedstocks and their moisture content (MC). An analysis is presented of the physical properties of menstrual waste as potential feedstock for onsite incinerators. Waste was generated in three institutional settings – from women of wide age range and from people with different socioeconomic backgrounds such as workers and students. Over 10 separate collections, 613 discarded menstrual pads were obtained with over 93% of the waste representing commercially disposable pads and less than 7% of the waste appearing to be homemade cloth pads. A surprising finding of this study was that a significant fraction of the commercially disposed pads (23–37%) had a large wet weight (over 20 g) which was associated with MC exceeding 50 wt%. This high MC was attributed to the practice of washing the pads prior to disposal, and it was much higher than what is standard for thermal processing. These data suggest that testing protocols for menstrual waste incinerator technology performance should include an evaluation of the effects of higher MC, unfolded pads, and occasional cloth pads.

Key words | incineration, menstrual health and hygiene (MHH), moisture content, pads, sanitation

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HIGHLIGHTS

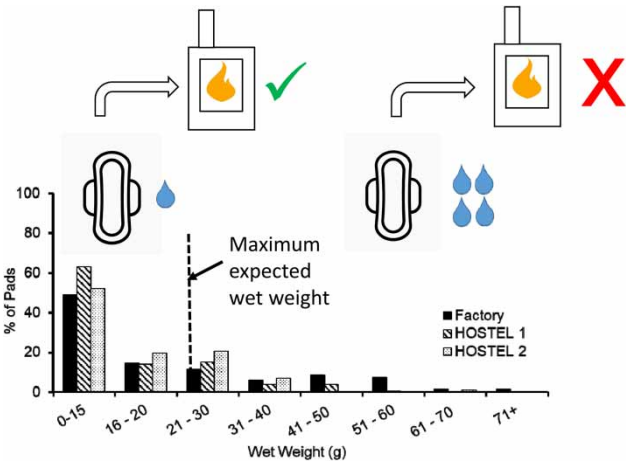
- Menstrual waste consists mainly of commercially disposable pads (93% or more) and of homemade cloth pads (6–7%).
- This study found that nearly a third of commercial pad waste had a wet weight of over 20 g, which was associated with moisture content (MC) exceeding 50 wt%.
- High MC waste is attributed to the practice of washing pads prior to disposal.
- Moist waste may result in pollutant emission and incomplete processing by onsite incinerators.

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GRAPHICAL ABSTRACT



INTRODUCTION

Menstrual hygiene is an essential aspect of sanitation for women and girls, which is often neglected in sanitation program implementation in low- and middle-income countries, yet is critical for women's health and dignity as well as environmental safety (Sommer *et al.* 2013; Tilley *et al.* 2013).

A body of research has shown that the lack of access to proper menstrual hygiene management has a negative impact on women's health (Balamurugan & Bendigeri 2012; Das *et al.* 2015) and women's participation in education and the workforce (Bodat *et al.* 2013; Sommer *et al.* 2016).

Challenges related to menstrual health and hygiene (MHH) in low- and middle-income countries are related to both access to safe sanitary products and safe disposal options at the household and community levels (Elledge *et al.* 2018).

The most used menstrual products are absorbents such as sanitary pads, tissue paper (disposable, one-time use) and cloth, typically homemade (Elledge *et al.* 2018). Cloth pads are the traditional and cheaper option, while disposable sanitary pads are increasingly available, especially in urban environments, and considered as aspirational products (van Eijk *et al.* 2016). Statistical associations have been reported between the use of disposable sanitary pads and younger age, higher social class, and urban residence (Elledge *et al.* 2018); however, affordability represents a major constraint (Crofts & Fisher 2012).

Disposal of used sanitary pads is a critical element of the MHH value chain. Cloth pads are cheaper and environmentally less polluting, but cleaning is challenging since it requires access to water, privacy and drying in the sun for its disinfecting effect, as opposed to a hidden drying area utilized for a sense of shame (van Eijk *et al.* 2016). Disposable pads offer convenience of use and are considered as aspirational products for women and girls; however, they present a solid waste challenge. A woman will have an average of 459 periods over her lifetime and dispose of 125–150 kg of sanitary waste (Bharadwaj & Patkar 2004).

The lack of disposal solutions leads to negative health outcomes, e.g. infrequent pad changes during school (Singh & Bhardwaj 2017) as well as environmental issues. Where solid waste management is not available, disposal practices for sanitary pads include throwing them in the open where they can contaminate water bodies, burning (in an open pit), burying them (whether waste is compostable or not), or flushing in the toilet, where they cause clogging and impair the biological degradation of human waste in sanitation systems (Path 2017).

In addition to the lack of sanitation infrastructure, menstruation is associated with stigma and taboos such as the perception of impure or shameful processes leading to restrictions of women's activities during menstruation

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and secrecy around menstrual fluid management (Sommer *et al.* 2013; Garg & Anand 2015). Importantly, cultural beliefs and taboos, in addition to convenience and information, contribute to the selection of disposal method (e.g. bury or burn) and to practices such as washing pads before disposal (Ali & Rizvi 2010; Sommer *et al.* 2013).

Onsite incineration is an option for managing the disposal of menstrual waste to achieve both pathogen and volume reduction, thus alleviating the environmental problems associated with improper disposal. Small-scale incinerators placed within a toilet facility may provide an effective and discrete way of disposing menstrual absorbents, align with decentralized waste treatment practices, and are well suited for institutional settings such as schools and worksites (Elledge *et al.* 2018).

Concerns in regard to the adoption of incineration are related to negative health and environmental impacts associated with the release of pollutants and toxic emissions as well as incomplete waste processing (Sommer *et al.* 2013); in the past decade, poor design and insufficient operation and maintenance have resulted in negative experiences for people (Tilley *et al.* 2013).

The Government of India has promoted the adoption of incineration for menstrual waste as part of its Swatch Bharat (Clean India) mission (2018). In India, menstrual waste volume is significantly high, with an estimated 121 million women disposing of 113,000 tons of menstrual hygiene waste (12.3 billion pads) annually (Path 2017). A number of programs implemented to increase access to sanitary pads have been successful in achieving widespread adoption recently, with an estimated 98% urban women using them (Dixit *et al.* 2016) and 64% of women in low-income areas gaining access to such pads (Garikipati & Boudot 2017). It is estimated that over 50 suppliers on the Indian market sell sanitary pad incinerators in institutional settings, with the majority being electric incinerators processing batch sizes as small as 10 pads (Bill & Melinda Gates Foundation 2021). The Government of India has classified sanitary pads as solid waste (SWM 2016), with associated emission thresholds for incineration, and since 2016 has stipulated the manufacturers of sanitary napkins to, among other things, provide wrappers for used sanitary napkins. However, lack of oversight and enforcement of regulations remains a concern for menstrual waste incineration (Elledge *et al.* 2018).

To our knowledge, there are no published reports on the performance of menstrual waste incinerators on the Indian market nor is there any information that includes the details of operation with actual menstrual waste.

Thermochemical processes such as incineration are well known to depend on the nature of the feedstock and its moisture content (MC). A very low MC is advantageous to incineration, and, to give some examples, the maximum MC allowed for thermal processes including incineration has been reported to be <15% (Burguete *et al.* 2016; Eke *et al.* 2019), while municipal solid waste thermal processes operate at no more than 20–25% moisture (Dong *et al.* 2016). In general, a higher MC can result in higher pollution and incomplete treatment (Youcai *et al.* 2004).

In order to enable the evaluation of menstrual pad incinerators based on realistic feedstock characteristics, this paper characterizes the physical properties of menstrual waste in the context of feedstocks for incineration. We collected and characterized menstrual waste from women's bathrooms from three institutional sites in India, and this paper reports our findings on the nature of the waste and its physical parameters: wet weight and MC.

MATERIAL AND METHODS

Site characteristics and study population

Menstrual waste was collected from three sites in the city of Coimbatore, India, between September 2019 and March 2020. All sites were communal women's toilet facilities: a private textile factory and two student residence halls in an educational institute, Hostel 1 and Hostel 2. The collection of pads was carried out by the caretakers (custodian or janitor) of each facility. The caretakers received an orientation about the purpose of the project prior to the beginning of the study and wore personal protective equipment (masks and gloves).

Site 1: The private textile factory site is a shared toilet for live-in and day workers. According to the warden, the number of users ranges between 20 and 50 women

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between 18 and 50 years of age. The facility has five toilet stalls, with a dedicated lidded bin for menstrual waste disposal. After an accumulation period of 2–3 weeks, the five bins were collected by the caretaker by placing the waste in one larger disposal bag and delivered to the investigators for analysis. Five separate collections were used for this study, yielding a total of 304 pads.

Site 2: Hostel 1 is a multistorey building housing approximately 900 female students, estimated to be between 23 and 30 years of age. Each floor contains a toilet facility with 25 individual toilet stalls. Each stall has a dedicated lidded bin for menstrual waste (Figure 1(a)). Caretakers empty the waste bins daily. Standard disposal operations involve the caretaker collecting the contents of all individual bins from one floor into one large disposal bag. In order to preserve the contents of each bin and prevent clumping of the pads, black garbage liner bags were provided to the caretakers the day before collection and placed in each bin. Black liners containing at least one pad were delivered to the investigators (Figure 1(b)). Four separate collections were carried out at this site for one or more floors for a total of 47 bags.

Site 3: Hostel 2 is a multistorey building housing approximately 350 female students, estimated to be between 23 and 30 years of age. Each floor contains a toilet facility with 10 individual toilet stalls. One waste bin is shared between two toilet stalls. The janitors empty the individual waste bins daily into one large disposal bag per floor, similar to hostel 1. Black garbage liners for the bins were provided to the caretakers the day before collection. After 1 day, liners containing at least one pad were delivered to the investigators. One collection was carried out at this site for a total of 24 bags.

Menstrual waste physical analysis

Analysis began the same day menstrual waste was received from the caretakers. Metal tongs were used to sort the waste into individual pads. For each pad, its type was recorded as either commercially disposable cellulose or homemade cloth (Figure 1(c)). Commercial pads were further analyzed for disposal shape and assigned one of the following categories: unfolded, rolled up/crumbled, or stuck together. Pads that were stuck together due to exposed adhesive liners were recorded as such and not analyzed further. Additional information collected from commercial pads included the presence of wings and additional coatings, as determined by a different color of the substrate material. Cloth pads were homemade and nonuniform in size; therefore, we recorded the length, width, and layers of cloth of the pad.

In a fume hood, an analytical balance was used to record the wet weight for all individual pads received. A subset of approximately 15–18 pads from each collection was further evaluated for MC analysis. Pads were assigned to three weight categories based on wet weight: low (5–20 g), medium (21–40 g), and heavy (>41 g). The pads for MC measurements were randomly selected in quantities proportional to their number in each of the three weight categories.

MC was calculated using the conventional formula $\%\ \mathrm{MC} = (W_{\mathrm{wet}} - W_{\mathrm{dry}}/W_{\mathrm{wet}})*100$, where W_{wet} is the wet



Figure 1 | (a) Photograph of a bathroom stall with individual waste bins. (b) Waste bin bag liner as received. (c) Disposed of homemade cloth pad. (d) Individually labeled commercial pads in a dry oven for moisture measurement.

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weight and W_{dry} is the dry weight. Pads were placed inside a vented hot air oven whose temperature did not exceed 100 °C (typically 55 – 70 °C) (Figure 1(d)). The drying temperature is lower than the standard value of 105 °C because of the limited temperature control capability of the oven. By drying at 55–70 °C, we ensured that no temperature swings exceeded 105 °C, which would cause mass loss to volatilization, and thus, we obtained an accurate dry weight value at the cost of longer drying times. The weight of the pads was recorded over multiple days until it did not change more than 4% from prior measurement, thereby providing the dry weight value. After the completion of analytical characterization, all specimens were discarded as biohazardous waste.

Ethics

PSG IMSR's Institute Human Ethics Committee (IHEC) reviewed and approved the study as exempted (2018/Appr/Exe/017). Duke University IRB determined the study to be non-human subject research.

RESULTS AND DISCUSSION

A total of 613 discarded menstrual pads were collected over multiple collections from three institutional sites, a factory and two residence halls from an institution of higher education (Hostels 1 and 2). Two types of menstrual waste were observed: commercially disposable cellulose pads and homemade cloth pads. The overwhelming majority (≥93%) of the pads collected from the three sites were commercially disposable pads, and of these, a large majority had wings or some type of cellulose coating as detected by different colored pad material. Homemade cloth pads were found in waste in two of the three sites, namely, the factory and Hostel 1. Results over multiple collections at the same sites were consistent over time. Table 1 summarizes the

Table 1 | Descriptive statistics of waste collection and its weight and moisture properties

	Collection sites			
Parameter	**Factory**	**Hostel 1**	**Hostel 2**	**Total number**
Collected pads	304	207	102	613
Collections	5	4	1	10
Commercial pads	284	194	102	580
Cloth pads	20	13	0	33
Commercial pads (%)	93	94	100	95
Featuring wings, coatings	244	192	99	535
Commercial pads in waste				
Unfolded	220	127	7	354
Rolled up/crumpled	8	73	95	176
Stuck together	22	7	0	29
Individually weighed pads	268	187	102	557
Wet weight median (g)	13.9	14.1	15.8	15.2
Wet weight minimum/maximum (g)	6.8/89.2	5.7/52.8	6.8/63.8	5.7/89.2
Pads with wet weight >20 g (%)	37	23	28	31
Moisture analysis of commercial pads				
Pads analyzed for moisture	44	64	17	125
Dry weight median (g)	8.5	7.6	8.9	7.8
Dry weight minimum/maximum (g)	4.8/11.7	4.7/13.7	5.4/14.1	4.7/14.1

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results of the physical analysis of the disposable commercial pads for all collections.

Properties of commercial pads

There was a difference in the manner of disposal across sites, with the pads at the factory site being largely disposed of as unfolded, while at the hostels, we observed a mix of unfolded and rolled up, often wrapped by a piece of newspaper. We note that India being a country with a washing culture, bathrooms do not feature toilet paper, which is commonly used as a wrapper prior to disposal in other geographies. Unfolded pads are likely to stick to each other and form clumps that may be problematic for incineration, cause them to stick to surfaces, or be improperly positioned inside the incinerator.

The number of clustered pads was higher at the factory site than at the hostels due, in part, to both the manner of collection and the adoption of liner bags for the menstrual waste bins at the hostels.

The wet weight of the pads had a similar median value consistent across sites (median wet weight between 13.9 and 15.8 g); however, the range was very broad, from 5.7 to 89 g. The dry weight of the pads had median values 7.6–8.9 g and ranged between 4.7 and 14.1 g, consistent with typical ranges for different commercial pad sizes.

Commercial pads are designed to absorb typically 5 ml and, in some models, up to 10 ml of liquid (Fraser *et al.* 2001). Given that blood density is slightly more than water (1.06 g/ml), we estimate that blood adds 5–10 g to the dry weight of pads. Using the largest dry weight measured, for pad, of 14 g, we estimate 14 g (pad) +5 g (blood) ~ 20 g as the maximum weight of used pads (dashed line in Figure 2), being aware that in some cases we may observe 14 + 10 = 24 g.

Figure 2 shows the wet weight distribution of the pads, indicating that approximately half of the collected waste was below 15 g, with the majority below the expected maximum weight of 20 g. However, a long distribution tail of wet weights higher than 20 g was recorded for all three sites, particularly at the factory site, where as many as 37% of the pads were heavier than 20 g. Weights are plotted against measured MC in Figure 3. The data show that, consistently across the three sites, heavier wet pads feature very high MCs reaching 80–90%. The dotted line at 50% MC

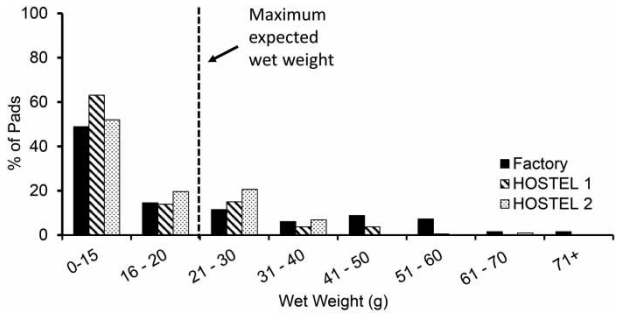


Figure 2 | Wet weight distribution of commercial pads shown as a percentage of the total number of pads from each site.

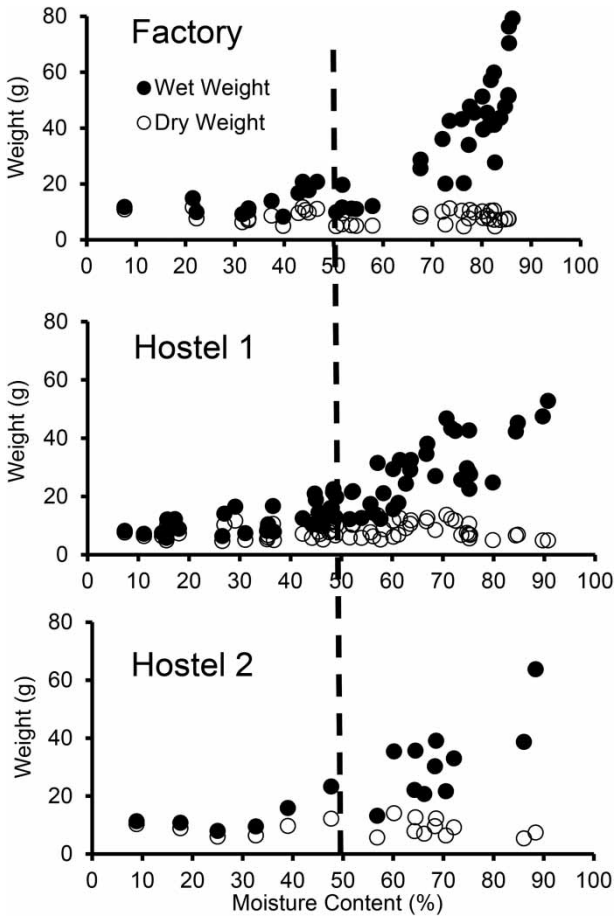


Figure 3 | MC results: wet and dry weights are reported by site. The dashed line represents 50% moisture, the expected upper limit for moisture for used menstrual pads.

corresponds to an 8 g dry pad receiving 8 ml of blood. This is expected to be an upper limit for MC for two reasons: (1) the typical volume of blood for pad rarely exceeds 5 ml and (2) in this study, drying of the pad can occur during

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the time between disposal and collection (time lag = 1 day for hostels, 1–2 weeks for the factory site). Yet, the measured moisture values are too high to be consistent with moisture from menstrual blood alone. While we did not conduct chemical analysis on the liquid, we note that since India has a washing culture, a tap with water per personal wash is available in each of the toilets included in this study. Thus, we infer that wet heavy pads derive from the practice of washing them prior to disposal (Ali & Rizvi 2010; Sommer *et al.* 2013). This practice is associated with the concept that menstrual blood is impure and the belief that others should not see it, and also possibly with a lack of awareness on proper disposal practices (Garg & Anand 2015).

While MC was not measured for all pads (wet weight was measured), all pads in Figure 3 whose wet weight exceeded 20 g had a MC exceeding 50% (dotted line). Thereby, a significant portion of disposed pads (23, 28 and 37%) with wet weight above 20 g were estimated to have a MC ranging from 50 to 90%. Such high moisture levels could be problematic for thermal processing because they significantly exceed the optimal 15–25% feedstock moisture (Dong *et al.* 2016; Eke *et al.* 2019).

Properties of cloth pads

Out of the 613 pads collected in this study, 33 were homemade cloth pads. We note that since the cloth pads are reusable, the low fraction of disposed cloth pads does not represent an indication of how prevalent their use is. It is, however, an indication that the menstrual pad incinerator should be evaluated for its ability to process feedstock that contains a percentage of cloth material that has been reused multiple times.

Figure 4(a) illustrates the distribution of wet weight of cloth pads. Higher weights were observed particularly at the factory site, where cloth as long as 88 cm and as wide as 38 cm was folded in layers to serve as a pad (for reference, the length and width of a typical commercial pad product is 25 cm length × 7.2 cm width). The MC of the homemade cloth was, however, narrower than that observed for the commercial pads and ranged between 16 and 40%. Also, the MC was not associated with wet weight, with some of the heavy pads consisting of a larger quantity of material. A possible explanation for the difference in MC between cloth and commercial pads is the fact that commercial cellulose pads are designed to absorb liquid and, therefore, when washed, retain a large amount of liquid, while cloth pads can dry more easily after disposal.

Comparison with prior studies

Prior studies of menstrual waste are based on surveys, and only a few of them address specific questions on disposal practices that are relevant to our physical assessment results.

A key finding of this study is that a significant fraction of the commercial pads (23–37%) was associated with a high MC consistent with washing prior to disposal.

Prior literature that investigated reproductive tract health included questions about the pad material type and the frequency of personal washing. Within the studies that included details on menstrual hygiene material, the topic of pad washing emerged in the context of cloth pads and their reuse (Omidvar & Begum 2010; Das *et al.* 2015), while separate studies addressed the topic of pad disposal, with no mention made on washing (Wagh *et al.* 2018). Combining

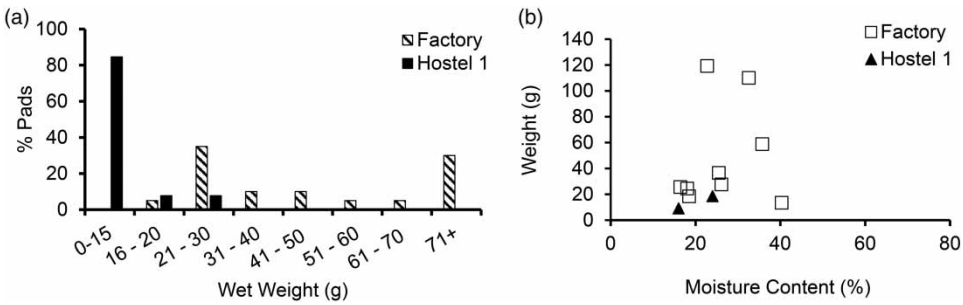


Figure 4 | Analysis of homemade pad properties. (a) Distribution of wet weight (factory: 20 pads; Hostel 1: 13 pads). No cloth pads were collected from Hostel 2. (b) MC was measured for a range of wet weights.

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questions on both washing and disposing, a study in Pakistan reported that as many as 60% of the respondents washed and discarded cloth pads; however, there was no explicit question about the washing of disposable pads (Ali & Rizvi 2010). We identified one publication (Kumar & Srivastava 2011) that, while reporting on social restrictions and taboos around menstruation, found that the majority of girls from residential areas in a city in India used disposable pads and that 25% of them washed such sanitary napkins after using them and then disposed them. This observation of washing sanitary napkins prior to disposal is consistent with the high MC in waste that we measured.

Another finding of our work is that a large portion of the pads are disposed of unfolded and, therefore, not wrapped in any paper or plastic. This result is not reflected in prior literature, because the question whether pads are wrapped prior to disposal was not directly asked. In Nayak *et al.* (2017), 98% of the respondents described the material selected to wrap pads (either paper or plastic), and only 1% of the surveyed women did not respond to the question. In Dipali *et al.* (2009), the questionnaire asked about the material the pad was disposed in, not whether it was wrapped; thus, no information on unfolded pads was captured. This difference between our findings and prior reports on this matter highlights the complementarity of physical assessments and surveys in the study of behavior and practices.

CONCLUSIONS

We investigated the physical properties of menstrual waste as feedstock for incinerators that represent an attractive solution to the challenges of menstrual waste disposal, particularly in institutional settings.

Menstrual waste was generated by women of different ages (18–50 years) and different education/income (workers vs. college students), yet consistent outcomes were observed across the two groups. The use of commercial cellulose pads was prevalent at all sites (>93%), in agreement with recent reports (Dixit *et al.* 2016; Garikipati & Boudot 2017). In two out of three sites, 6–7% of the waste consisted of homemade cloth pads, suggesting that a menstrual pad incinerator technology will encounter multiple types of feedstock. The commercial pads were disposed both unfolded or folded, indicating that the incinerator configuration should be robust with respect pad insertion and positioning inside the unit; folded pads were not wrapped in manufacturer-provided wrappers, despite country regulations and standard use instructions.

A major finding of this study was that a significant fraction of the commercial disposed pads (23–37%) had a large wet weight (over 20 g), which was associated with MCs exceeding 50%. This MC is much higher than ideal for thermal processing (<25%) and can be quite problematic for typical incinerators, leading to incomplete combustion and increased hazardous air emissions. We infer that the MC is due to the practice of washing pads before disposal, a practice that has been reported in washing cultures. While this study on menstrual waste in India is limited to three sites, hundreds of pads were collected in repeated collections over periods of months, and the results were consistent across collections. These findings suggest that further research is needed to fill the knowledge gap on practices in menstrual disposal and that testing protocols of disposal technologies should include an evaluation of the effects of high MC, unfolded pads, and occasional cloth pads on waste processing performance.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Ali, T. S. & Rizvi, S. N. 2010 Menstrual knowledge and practices of female adolescents in urban Karachi, Pakistan. *Journal of Adolescence* **33** (4), 531–541.

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Balamurugan, S. S. & Bendigeri, N. 2012 Community-based study of reproductive tract infections among women of the reproductive age group in the urban health training centre area in Hubli, Karnataka. *Indian Journal of Community Medicine: Official Publication of Indian Association of Preventive & Social Medicine* **37** (1), 34.

Bharadwaj, S. & Patkar, A. 2004 Menstrual hygiene and management in developing countries: taking stock. *Junction Social* **3** (6), 1–20.

Bill & Melinda Gates Foundation 2021 Menstrual hygiene management (MHM) incinerator market landscape: analysis of India and South Africa [version 1; not peer reviewed]. *Gates Open Research* **5**, 9. https://doi.org/10.21955/gatesopenres.1116712.1.

Bodat, S., Ghate, M. M. & Majumdar, J. R. 2013 School absenteeism during menstruation among rural adolescent girls in Pune. *National Journal of Community Medicine* **4** (2), 212–216.

Burguete, P., Corma, A., Hitzl, M., Modrego, R., Ponce, E. & Renz, M. 2016 Fuel and chemicals from wet lignocellulosic biomass waste streams by hydrothermal carbonization. *Green Chemistry* **18** (4), 1051–1060.

Crofts, T. & Fisher, J. 2012 Menstrual hygiene in Ugandan schools: an investigation of low-cost sanitary pads. *Journal of Water, Sanitation and Hygiene for Development* **2** (1), 50–58.

Das, P., Baker, K. K., Dutta, A., Swain, T., Sahoo, S., Das, B. S., Panda, B., Nayak, A., Bara, M. & Bilung, B. 2015 Menstrual hygiene practices, WASH access and the risk of urogenital infection in women from Odisha, India. *PLoS One* **10** (6), e0130777.

Dipali, N., Seema, A. & Rupali, G. 2009 Impact of health education on knowledge and practices about menstruation among adolescent school girls of Kalamboli, Navi-Mumbai. *Health and Population-Perspectives and Issues* **32** (4), 167–175.

Dixit, S., Raghunath, D., Rokade, R., Nawaz, S. A., Nagdeve, T. & Goyal, I. 2016 Awareness about menstruation and menstrual hygiene practices among adolescent girls in central India. *National Journal of Community Medicine* **7** (6), 468–473.

Dong, J., Chi, Y., Tang, Y., Ni, M., Nzihou, A., Weiss-Hortala, E. & Huang, Q. 2016 Effect of operating parameters and moisture content on municipal solid waste pyrolysis and gasification. *Energy & Fuels* **30** (5), 3994–4001.

Eke, J., Onwudili, J. A. & Bridgwater, A. V. 2019 Influence of moisture contents on the fast pyrolysis of Trommel fines in a bubbling fluidized bed reactor. *Waste and Biomass Valorization* **11**, 3711–3722.

Elledge, M. F., Muralidharan, A., Parker, A., Ravndal, K. T., Siddiqui, M., Toolaram, A. P. & Woodward, K. P. 2018 Menstrual hygiene management and waste disposal in low and middle income countries – a review of the literature. *International Journal of Environmental Research and Public Health* **15** (11), 2562.

Fraser, I., Warner, P. & Marantos, P. 2001 Estimating menstrual blood loss in women with normal and excessive menstrual fluid volume. *Obstetrics & Gynecology* **98** (5), 806–814.

Garg, S. & Anand, T. 2015 Menstruation related myths in India: strategies for combating it. *Journal of Family Medicine and Primary Care* **4** (2), 184.

Garikipati, S. & Boudot, C. 2017 To pad or not to pad: towards better sanitary care for women in Indian slums. *Journal of International Development* **29** (1), 32–51.

Kumar, A. & Srivastava, K. 2011 Cultural and social practices regarding menstruation among adolescent girls. *Social Work in Public Health* **26** (6), 594–604.

Nayak, P., Shukla, D., Tiwari, N., Mishra, O. & Mishra, R. 2017 Assessment of menstrual pattern and use of sanitary pads in adolescent girls of Varanasi slum: preliminary analysis. *Indian Journal of Preventive & Social Medicine* **48** (1–2), 9–9.

Omidvar, S. & Begum, K. 2010 Factors influencing hygienic practices during menses among girls from south India – A cross sectional study. *International Journal of Collaborative Research on Internal Medicine & Public Health* **2** (12), 0–0.

Path 2017 Pushing the Boundaries on the MHM Dialogue in India Management of Menstrual waste. Available at: https://www.path.org/resources/pushing-the-boundaries-on-the-menstrual-health-management-dialogue/.

Singh, P. & Bhardwaj, S. 2017 Study on hygiene and sanitary practices during menstruation among adolescent girls of Udham Singh Nagar district of Uttarakhand. *International Journal of Home Science* **3**, 21–23.

Sommer, M., Kjellén, M. & Pensulo, C. 2013 Girls' and women's unmet needs for menstrual hygiene management (MHM): the interactions between MHM and sanitation systems in low-income countries. *Journal of Water, Sanitation and Hygiene for Development* **3** (3), 283–297.

Sommer, M., Chandraratna, S., Cavill, S., Mahon, T. & Phillips-Howard, P. 2016 Managing menstruation in the workplace: an overlooked issue in low-and middle-income countries. *International Journal for Equity in Health* **15** (1), 1–5.

SWM Solid Waste Management Rules 2016 Central Pollution Control Board, Government of India. https://cpcb.nic.in/rules-2/ Accessed on 1 March 2021.

Tilley, E., Bieri, S. & Kohler, P. 2013 Sanitation in developing countries: a review through a gender lens. *Journal of Water, Sanitation and Hygiene for Development* **3** (3), 298–314.

van Eijk, A. M., Sivakami, M., Thakkar, M. B., Bauman, A., Laserson, K. F., Coates, S. & Phillips-Howard, P. A. 2016 Menstrual hygiene management among adolescent girls in India: a systematic review and meta-analysis. *BMJ Open* **6** (3), e010290.

Wagh, R. V., Upadhye, A. J. & Upadhye, J. J. 2018 Menstrual hygiene practices in young girls of urban India. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology* **7**, 1897–1902.

Youcai, Z., Stucki, S., Ludwig, C. & Wochele, J. 2004 Impact of moisture on volatility of heavy metals in municipal solid waste incinerated in a laboratory scale simulated incinerator. *Waste Management* **24** (6), 581–587.

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Original Article

Are Our Rural Adolescents Eating Healthy?: Implications for Redesigning School Health Interventions – A Cross Sectional Study in Rural Coimbatore

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Abstract

Background: Adolescence is a period of transition where independence in thinking and behavior is established and food choices that are made are followed for several years, and this can influence their health in adulthood. Hence, understanding the eating habits are necessary to plan effective nutritional interventions in adolescents. **Objectives:** The main objective of the study is to find out the extent of malnutrition among rural adolescents as well as evaluate their eating habits against recommended dietary food groups and to compare eating habits across gender and age groups. **Methods:** A cross-sectional study was done among 1425 adolescents from 13 rural schools from 2014 to 2015, and the variables considered in this study were age, sex, body mass index (BMI), and the eating habits of the adolescents. **Results:** Undernutrition was seen among 23% and overweight/obesity among 8% of adolescents. The habit of taking milk and milk products, fruits, and green leafy vegetables were very poor among the adolescents. Significant association was found between eating habits and BMI. Furthermore, late adolescents had better eating habits, and significant gender difference was seen in certain eating habits. **Conclusions:** The study shows that it is possible to understand the gaps in eating habits of adolescents, and this can be used to plan tailor-made nutritional interventions to adolescent groups as their eating habits are different and have long-term nutritional and health implications.

Key words: Adolescent, dietary gap assessment, faulty diet, nutritional diagnosis, school health

Introduction

Adolescence is a period of transition. The World Health Organization (WHO) defines the period of adolescents as 10–19 years of age.[1] It is a period of rapid growth and maturation in human development. It is a period where independence is established and adolescent makes choices of their own. The choices that are made are followed for several years and food habits are greatly established during this period, and this can influence their health in the adulthood.[2]

As global foods are widely marketed in low- and middle-income countries, it becomes important to understand the diet patterns and preferences.[3] In India, among adolescents, there is an increased consumption of fast foods contributing to overweight and obesity.[4] Furthermore, studies have shown that the food consumption pattern is poor among adolescents and have observed frequent consumption of energy-dense, nutrient-poor foods, and sugar-sweetened beverages, and the omission of a variety of healthy foods from their daily diet.[5]

In addition, cultural factors such as dietary practices and attitude toward food are evolving. These factors eventually influence the eating habits and today's adolescents lack proper knowledge on good eating habits and how those habits contribute toward their healthy nutritional status.

Hence, healthy eating behavior should be established in childhood and maintained during adolescence.[6] Adequate knowledge of healthy food choices and eating habits should be taught early to adolescents, as it can predispose them

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to lifestyle diseases in adulthood. Adolescents should be constantly motivated, and the need of the hour is bridging the knowledge gap on healthy eating.[7]

In India, there is a paucity of information about eating habits of adolescents, and limited information is available regarding various categories of food consumed by adolescents in relation to the food pyramid, which defines and identifies the balanced diet and corresponding nutritional status of an adolescent. Moreover, this type of nutritional data is even more difficult to find among rural population in India. Also, adolescents are the most important and vulnerable segment of population who have been largely ignored, and exclusive data pertaining to adolescent health are not widely available in Indian scenario.[8]

Hence, this study aimed to measure the extent of malnutrition among rural adolescents as well as evaluate their eating habits against recommended dietary food groups and to compare eating habits across gender and age groups.

Materials and Methods

A cross-sectional study was done among adolescents from 13 schools in rural area within the Madukkarai block, Coimbatore District, in 2014–2015. Prior permission was obtained from the concerned school health authorities, and this study protocol was approved by the Institutional Human Ethics Committee. Data used in this study were collected as a part of the school health project. The variables considered in this study were age, sex, body mass index (BMI), and the various elements in the recommended food portions that constitute the eating habits of the adolescents.

This school health program was funded by Larsen and Toubro, under their corporate social responsibility scheme. The funders' interest was only on the services to the rural school children and did not have a role in the research component. The research on the data obtained was done after obtaining ethics committee clearance from our institution. All adolescents from 10 to 18 years were included in the study and students who were absent from school on the day of visit were excluded from the study.

Anthropometric measurements such as height and weight of all students were recorded. Instruments were calibrated, and the BMI was calculated based on the measured height and weight. The nutritional status of the adolescent was determined based on the WHO BMI charts available, separately for boys and girls, from 5 to 19 years.[9] The WHO chart classifies them as severe thinness, thinness, and normal, overweight, and obese based on their BMI for their particular age. A questionnaire/checklist was designed to record the daily eating habits of school children based on the recommended portions of various categories listed in the food pyramid, as recommended by the ICMR and National Institute of Nutrition (NIN).[10] Based on the above balanced diet recommendations, questionnaire was developed which assessed the daily eating habits of the adolescents.[11] The questionnaire was a simplified tool with ten items, to assess gaps in the recommended portions of food category intake, by directly asking question that checks in Yes/No terms the compliance with daily recommended intake of cereals in the three main meals, daily intake of vegetables and green leafy vegetable, daily intake of milk and milk products, habit of taking a fruit daily, and intake of protein-rich foods in daily diet. In the assessment of protein-rich food/pulses recommended, portions can also be replaced by egg or other nonvegetarian food as per recommendations.[10] Hence, this assessment includes the assessment of egg and nonvegetarian food also. Additional questions were added to assess other eating habits such as the habit of taking mid-day snacks and questions that captured certain faulty eating habits of students like habit of skipping meals, taking junk food, and eating from street shops. The scoring was also given for each student based on the Yes/No terms in the questionnaire. Each positive response had a score of 1. Total score was 10 and a score of <10 indicates the gap from the recommended portion of food, or they have a faulty eating habit. Lesser the score, more is the gap in the recommended dietary portion of food category. Each student was individually enquired about the eating habits based on the questionnaire, and individual nutrition counseling was given to students who had a faulty eating habit or not complying with the recommended dietary food group intake standards.

All quantitative values were expressed in proportions. Normality test (Shapiro–Wilk test) was done and the nutritional score followed a nonnormal distribution. The association between eating habits and gender, age, and the nutritional status was tested using Chi-square test and Kruskal–Wallis test. All statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp (24.0), $P = 0.05$ or less, were considered as statistically significant.

Results

A total of 1425 adolescents between the ages, 10 and 18 years were included for this study, of which 53.1% belonged to early adolescents (10–13 years), 39.9% belonged to middle adolescents (14–16 years), and 6.9% belonged to late adolescents (17–18 years). The mean age was 13.38 ± 2.03 years. The study participants were 753 (47.2%) males and 672 (52.8) females.

Table 1 shows that a majority (68.8%) of adolescents had a normal BMI. However, more than a quarter of them (23.2% of

Table 1: Nutritional status of adolescents based on the WHO body mass index charts

Nutritional status	Male (*n*=672), *n* (%)	Female (*n*=753), *n* (%)	Total (*n*=1425), *n* (%)
Severe thin	53 (7.9)	23 (3.1)	76 (5.4)
Thin	145 (21.6)	109 (14.5)	254 (17.8)
Normal	429 (63.8)	551 (73.2)	980 (68.8)
Overweight	28 (4.2)	38 (5.0)	66 (4.6)
Obese	17 (2.5)	32 (4.2)	49 (3.4)

students who were undernourished including thin and severely thin and 8% were either overweight or obese) need dietary and other interventions.

The nutritional status of boys revealed 29.5% were either severely thin or thin, 4.2% were overweight, and 2.5% had obesity. Among girls, 17.6% were in severely thin or thinness category, 5.0% overweight, and 4.2% had obesity. This difference between boys and girls was statistically significant ($P < 0.001$).

Figure 1 shows the percentage of adolescents taking the recommended food groups daily as per the ICMR/NIN recommendations and also certain faulty eating habits such as eating junk food, eating from street shops, and skipping meals. It shows that none of the adolescents had the habit of taking green leafy vegetables daily. Furthermore, the habit of taking fruits and milk was very poor among the adolescents, thereby putting them at risk of micronutrient and vitamin deficiency which are not picked up if we do anthropometry alone as done in most school health programs.

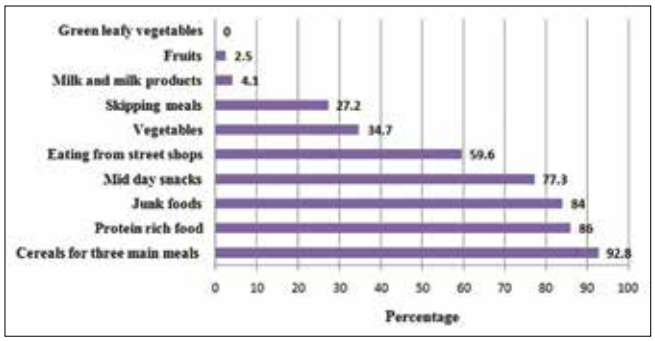


Figure 1: Pattern of eating habits among adolescents.

Table 2 shows that there is an association between eating habits and nutritional status of the adolescents. Chi-square test was used to find whether there was any significant difference in the eating habits among the adolescents of various nutritional statuses (based on BMI). Except for the habit of eating junk foods, others habits have significant association with BMI ($P < 0.001$).

Table 3 shows the difference in eating habits based on gender. Chi-square test was used to find whether there was any significant difference in the eating habits among the adolescent boys and girls. Odds ratio was also calculated. Habits of taking cereals, mid-day snacks, skipping meals, and eating from street shops showed significant difference between boys and girls.

The mean score obtained by the students on the diet assessment was compared between the age groups. Kruskal–Wallis test was used to find the statistical significance among the various age

Table 2: Association of eating habits and nutritional status of schoolchildren ($n=1425$)

Eating habits (recommended ICMR/NIN food category portion frequency)	Nutritional status (BMI categories)			χ^2	*P*
	Thin ($n=330$), *n* (%)	Normal ($n=980$), *n* (%)	Overweight/obese ($n=115$), *n* (%)		
Taking three main meals (cereals)	266 (80.6)	949 (96.8)	107 (93)	96.99	<0.001
Taking mid-day snacks	48 (14.5)	960 (98)	94 (81.7)	981.30	<0.001
Taking protein-rich food	193 (58.5)	921 (94.0)	112 (97.4)	272.29	<0.001
Taking milk and milk products	5 (1.5)	16 (1.6)	38 (33.0)	263.31	<0.001
Taking green leafy vegetables	0	0	0	0	0
Taking other vegetables	23 (7.0)	375 (38.3)	97 (84.3)	242.46	<0.001
Taking fruits	10 (3.0)	12 (1.2)	13 (11.3)	44.23	<0.001
Skipping meals	262 (79.4)	79 (8.1)	47 (40.9)	645.71	<0.001
Eating junk foods	276 (83.6)	816 (83.3)	105 (91.3)	4.99	0.08
Eating from street shops	113 (34.2)	655 (66.8)	82 (71.3)	116.02	<0.001

NIN: National Institute of Nutrition, BMI: Body mass index

Table 3: Association of eating habits and gender of the adolescents ($n=1425$)

Eating habits of adolescents	Male ($n=672$), *n* (%)	Female ($n=753$), *n* (%)	OR	χ^2	*P*
Taking three main meals (cereals)	609 (90.6)	713 (94.7)	1.84 (1.22-2.78)	8.74	0.004
Taking mid-day snacks	491 (73.1)	611 (81.1)	1.58 (1.23-2.03)	13.21	<0.001
Taking protein-rich food	576 (85.7)	650 (86.3)	1.05 (0.77-1.41)	0.10	0.760
Taking milk and milk products	29 (4.3)	30 (4.0)	0.92 (0.54-1.55)	0.09	0.428
Taking green leafy vegetables	0	0	0	0	
Taking other vegetables	243 (36.2)	252 (33.5)	0.88 (0.71-1.10)	1.13	0.290
Taking fruits	21 (3.1)	14 (1.9)	0.58 (0.29-1.16)	2.37	0.127
Skipping meals	208 (31.0)	180 (23.9)	1.42 (1.12-1.80)	8.90	0.003
Eating junk foods	551 (82.0)	646 (85.8)	0.75 (0.56-1.50)	3.80	0.060
Eating from street shops	368 (54.8)	482 (64.0)	0.68 (0.55-0.84)	12.62	<0.001

OR: Odds ratio

groups. The mean scores of early, middle, and late adolescents were 4.3 ± 1.27, 4.2 ± 1.23, and 4.6 ± 1.02, respectively. Late adolescents had a better nutritional score compared to early and middle adolescents. The difference was found to be statistically significant ($P = 0.006$).

Discussion

Our study shows that undernutrition or thinness among the rural school-going adolescents was a common problem (23.2%). Furthermore, 8% were overweight or obese and hence over a quarter of them need dietary and other interventions. The percentage of undernutrition and overweight was less in our study compared to a study done in Karnataka by Benazeera., where among school adolescents, 34% were belonging to the category of underweight, 21.33% were obese, and 10.67% were overweight.[12]

We also found that underweight was more common among boys and overweight and obesity were more among girls and hence targeted interventions for girls and boys are needed that address factors that could be different. This means further studies are needed to find out the reasons for the difference observed. A study done in Bangalore by Abraham and Anand showed similar findings among adolescent schoolchildren.[13] The study by Venkaiah *et al*. among rural adolescents in nine states also showed that underweight was more among boys than girls.[14]

Regarding eating habits, our study showed that the habit of taking green leafy vegetables daily was not there in any of the adolescents. Furthermore, the habit of taking fruits (2.5%) and milk and milk products (4.1%) was very poor. These habits were poor even in the normal BMI category. Thus, traditional school health programs that use anthropometry alone would not help identify vitamin and micronutrient deficiency unless anthropometry is combined with clinical screening for vitamin and micronutrient deficiency diseases and their contributing factors by doing a diet survey. A study done by Rathi *et al*. among adolescents in Kolkata also showed that 45% did not consume any fruit, 30% did not take vegetables, and 36% did not take milk and milk products. Our study showed that consumption was very less percentage maybe because it assessed daily habits, while the study in Kolkata showed the consumption based on the last 2 days.[5]

In our study, the eating habits of adolescents were found to have significant association with their nutritional status, except for habit of eating junk foods. Our study showed that habits like not consuming cereals for three main meals, not taking mid-day snacks, protein-rich food, and skipping meals were significantly associated with the underweight. In the overweight/obese category, factors such as milk and milk products, eating from street shops, and junk food were high, though junk food did not show any statistical significance. Similar findings were seen in a study by Amin *et al*. which showed that frequent consumption of sweets/candies and milk and milk products are associated with obesity.[15] However, even habits of eating vegetables and fruits were also high in the overweight/obese category. Since the study did not take into consideration the quantity of consumption and exact fruits and vegetables consumed, we could not comment on this. A study by Bhattacharjee *et al*. among adolescents showed that BMI correlated significantly with the dietary habits.[16] Another study by *et al*. showed that eating habits were a better predictor of BMI, especially among boys.[17]

In our study, association of gender with eating habits showed that habits such as consumption of cereals, having mid-day snacks, skipping meals, and eating from street shops showed significant differences. Habit of consuming cereals and having mid-day snacks were better among girls. A study by Rathi *et al*. also showed that females had better consumption of cereals.[5] However, higher percentage of girls had the habit of eating from street shops. This was again similar to a study which showed that significantly more mid-adolescent girls purchased from tuck shops than boys.[18] The habit of skipping meals was more common among boys than girls. Similarly, a study among Turkish Adolescents shows that one-third of the boys skip meals, while only one-sixth of the girls skip meals.[19] Furthermore, a systematic review done among young adults showed that men tend to skip breakfast more often than females, while skipping lunch and dinner was more common among females.[20]

Our study also showed that late adolescents had better eating habits as demonstrated by their higher nutritional scores compared to early and middle adolescents. The nutrition scores are least in the middle adolescents group. This might be because in early adolescents still parental influence is more as shown in a study which shows parental influences can influence the diet of the child.[21] In the late adolescence, the better scores might be because the adolescents become more aware about healthy eating, as shown in certain studies that late adolescents have better knowledge than the younger ones.[22] However, further studies are needed to establish the factors influencing their diet.

Limitations and strengths

Our study has several strengths; we have studied a large number of children in the study sample. A simple and effective questionnaire was used for identifying the gaps in the eating habits based on the standard ICMR/NIN recommendations for a balanced diet. In addition, standard methods like the WHO-developed BMI age charts were used to assess the nutritional status.

However, there were certain limitations, eating habits were assessed by self-reported data and quantitative measuring of the intake of the recommended portions of food groups was not done. We could not exclude the possibility of confounding effects by unmeasured variables such as physical activity and other comorbidities that influence the BMI. Furthermore, data did not include social factors known to affect BMI such as socioeconomic status, number of family members, and education of the mother. Although our study identified low

intake of green leafy vegetables and fruits, we had not planned to look for clinical manifestations of vitamin and micronutrient deficiencies and then look for correlation between the two. Also, since we have found that underweight was more common among boys and overweight and obesity were more among girls and hence further studies are needed to find out the reasons for the difference observed.

Notwithstanding the above limitations, our study findings show that there is scope for doing further in-depth studies to prove the preliminary findings of this study.

Conclusion

The study shows that it is possible to make a Nutritional Education Diagnosis by identifying the gaps in eating habits compared to the recommended one for school-going adolescents. This can then be used to plan tailor-made educational and nutritional programs for improving their eating habits. Using the rapid screening method used in the study, it would be relatively easy to conduct this exercise on a sample of schools in a district to capture gaps in diet at district level for conducting health education and awareness programs on nutrition for adolescents in schools at the district and using similar methods to scale it up for state-level as possibly a national-level initiative to extend school health nutritional interventions to adolescent groups, as their eating habits are different and have long-term nutritional and health implications for the population beyond adolescence.

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Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. WHO Health topics on Maternal, Child and Adolescent. Geneva: World Health Organization. Available from: http://www.who.int/maternal_child_adolescent/topics/adolescence/dev/en/. [Last accessed on 2016 Dec 09].
2. Wennberg M, Gustafsson PE, Wennberg P, Hammarström A. Poor breakfast habits in adolescence predict the metabolic syndrome in adulthood. Public Health Nutr 2015;18:122-9.
3. Shaikh NI, Patil SS, Halli S, Ramakrishnan U, Cunningham SA. Going global: Indian adolescents' eating patterns. Public Health Nutr 2016;19:2799-807.
4. Joseph N, Nelliyanil M, Rai S, Raghavendra Babu YP, Kotian SM, Ghosh T, *et al*. Fast food consumption pattern and its association with overweight among high school boys in Mangalore city of Southern India. J Clin Diagn Res 2015;9:LC13-7.
5. Rathi N, Riddell L, Worsley A. Food consumption patterns of adolescents aged 14-16 years in Kolkata, India. Nutr J 2017;16:50.
6. World Health Organization. WHO Regional office for Europe. Fact Sheet, 15 March, 2016. Available from: http://www.euro.who.int/__data/assets/pdf_file/0006/303477/HBSC-No.7_factsheet_Diet.pdf?ua=1. [Last accessed on 2016 Dec 09].
7. Kumar S, Ray S, Roy D, Ganguly K, Dutta S, Mahapatra T, *et al.* Exercise and eating habits among urban adolescents: A cross-sectional study in Kolkata, India. BMC Public Health 2017;17:468.
8. Sivagurunathan C, Umadevi R, Rama R, Gopalakrishnan S. Adolescent health: Present status and its related programmes in India. Are we in the right direction? J Clin Diagn Res 2015;9:LE01-6.
9. World Health Organization. WHO health topics on growth reference. Geneva: World Health Organization; 2007. Available from: http://www.who.int/growthref/who2007_bmi_for_age/ en/. [Last accessed on 2016 Dec 09].
10. National Institute of Nutrition. Dietary guidelines for Indians – A Manual. Hyderabad. National Institute of Nutrition. Available from: http://ninindia.org/DietaryguidelinesforIndians-Finaldraft.pdf. [Last accessed on 2014 Aug 12].
11. Chacko TV, Ganesan S. A tool for quickly identifying gaps in diet of school children for nutritional educational interventions. Indian J Public Health 2018;62:146-9.
12. Benazeera UJ. Association between eating habits and body mass index (BMI) of adolescents. Int J Med Sci Public Health 2014;3:9403.
13. Abraham RR, Anand AK. A study on nutritional status and its determinants in adolescents aged 11 to 17 years, from selected schools in Bangalore, India. Natl J Res Community Med 2017;6:2136.
14. Venkaiah K, Damayanti K, Nayak MU, Vijayaraghavan K. Diet and nutritional status of rural adolescents in India. Eur J Clin Nutr 2002;56:1119-25.
15. Amin TT, Al-Sultan AI, Ali A. Overweight and obesity and their association with dietary habits, and sociodemographic characteristics among male primary school children in alhassa, kingdom of Saudi Arabia. Indian J Community Med 2008;33:172-81.
16. Bhattacharjee P, Mukhopadhyay S, Joshi P, Singh S. Food habits and obesity: Astudy in adolescents. Int J Contemp Pediatrics 2017;4:336-40.
17. Djordjevic-Nikic M, Dopsaj M. Characteristics of eating habits and physical activity in relation to body mass index among adolescents. J Am Coll Nutr 2013;32:224-33.
18. Sedibe MH, Pisa PT, Feeley AB, Pedro TM, Kahn K, Norris SA. Dietary habits and eating practices and their association with overweight and obesity in rural and urban black South African adolescents. Nutrients 2018;10. pii: E145.
19. Soyer MT, Ergin I, Gursoy ST. Effects of social determinants on food choice and skipping meals among Turkish adolescents. Asia Pac J Clin Nutr 2008;17:208-15.
20. Pendergast FJ, Livingstone KM, Worsley A, McNaughton SA. Correlates of meal skipping in young adults: A systematic review. Int J Behav Nutr Phys Act 2016;13:125.
21. Reicks M, Banna J, Cluskey M, Gunther C, Hongu N, Richards R, *et al*. Influence of parenting practices on eating behaviors of early adolescents during independent eating occasions: Implications for obesity prevention. Nutrients 2015;7:8783-801.
22. Naeeni MM, Jafari S, Fouladgar M, Heidari K, Farajzadegan Z, Fakhri M, *et al*. Nutritional knowledge, practice, and dietary habits among school children and adolescents. Int J Prev Med 2014;5:S171-8.

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In-silico analysis of Covid-19 genome sequences of Indian origin: Impact of mutations in identification of SARS-Co-V2

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Abstract

Covid-19 disease caused by SARS-CoV-2 is still being transmitted in developed and developing countries irrespective of healthcare setups. India with 1.3 billion people in the world is severely affected by Covid-19 with 11.3 million cases and 157 000 deaths so far. We have assessed the

mismatches in WHO recommended rRT-PCR assays primer and probe binding regions against SARS-CoV-2 Indian genome sequences through in-silico bioinformatics analysis approach. Primers and probe sequences belonging to CN-CDC-ORF1ab from China and HKU-ORF1b from Hong Kong targeting ORF1ab gene while NIH-TH-N from Thailand, HKU-N from Hong Kong and US-CDCN-2 from USA targeting N genes displayed accurate matches (>98.3%) with the 2019 novel corona virus sequences from India. On the other hand, none of the genomic sequences displayed exact match with the primer/probe sequences belonging to Charité-ORF1b from Germany targeting ORF1ab gene. We think it will be worthwhile to release this information to the clinical and medical communities working in Indian Covid-19 frontline taskforce to tackle the recently emerging Covid-19 outbreaks as of March-2021.

Keywords: Covid-19, Primer and probe mismatches, rRT-PCR, Indian SARS-CoV-2 genome sequences

1. Introduction

Covid-19 pandemic disease is caused by novel Severe Acute Respiratory Syndrome corona virus 2 (SARS-CoV-2) worldwide. The virus has transmitted to both developed and developing countries irrespective of the healthcare setups [1]. Diagnosis of Covid-19 disease is vital to detect the infected individuals in community regarding quarantine and treatment, where real-time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) is employed to detect the presence of viral pathogen in swab samples collected from Covid-19 suspected patients [2]. The virus has a strong transmission potential and caused Covid-19 outbreak in several countries throughout the world. Prompt and early detection of SARS-COV-2 through rRT-PCR is essential for the disease control strategies planned by national healthcare agencies and Government programmes. To date, there are several rRT-PCR protocols developed by different nations and approved by WHO. SARS-CoV-2 is a RNA virus having the potential to evolve rapidly through high mutation rates [3]. Since its first outbreak in December 2019 in Wuhan province of China it rapidly circulated throughout the world with accumulation of nucleotide polymorphisms in the genomes of SARS-CoV-2 strains [[4], [5], [6]].

Mismatches in the primer and probe regions of SARS-CoV-2 strains may lead to their diminished ability binding to corresponding template DNA molecules during the initial steps of PCR reactions. Mutations in the primer and probe regions have been shown to affect the sensitivity of PCR reactions in case of other viral infections such as human immunodeficiency virus (HIV), Hepatitis B virus (HBV), H1N1 virus and dengue [[7], [8], [9], [10], [11], [12], [13]].

India being the second most populous country in the world severely affected by Covid-19 and as of 10-March-2021, 11 244 786 cases and 157 930 deaths has been reported in India since its first Covid-19 case reported on 30-January-2020 [14]. With 1.3 billion people living in diverse states of different culture and health care inequalities, early detection of SARS-CoV-2 infection with robust diagnostic tests are in great demand to control the disease transmission. Previous studies reported the mismatches in primer probe regions of Covid-19 PCR primers used the sequences representing SARS-CoV-2 strains from different countries, nevertheless there are no studies focusing on the SARS-CoV-2 genomes from Indian Sub-Continent. In this study, we evaluated mismatches in PCR primers against SARS-CoV-2 genomes from India through in-silico bioinformatics analysis approach [15,16].

2. Materials and methods

Briefly, a total of 3765 whole genome sequences of SARS-CoV-2 strains isolated from India were retrieved in FASTA format from GISAID (Global Initiative on Sharing All Influenza Data) database submitted as on January 30, 2021 (Supplementary Table 1) [17]. Further search criteria employed for collecting the sequences from the database is as follows: (i) “hCoV-19” was selected under virus name (ii) “Human” was selected under host name (iii) location was selected “Asia/India” (iv) complete genomes alone were included (>29000 bp length) and (v) high coverage genomes alone were considered (low coverage entries i.e. >5% Ns were excluded). The collected individual FASTA sequences were then merged as a single FASTA file along with the inclusion of the complete genome of Wuhan-Hu-1 as reference genome in 29903 bp length (NCBI Reference Sequence: NC_045512.2). Primers and probe sequences were collected and shortlisted from the recommended protocols in the literature and from WHO website (Table 1) [18]. A detailed protocol is presented as in supplementary file 1.

Table 1

Mismatch in the primer and probe sequences of different RT-qPCR assays recommended by WHO used to detect Covid-19.

Gene Target	Assay Name/Country	F/P/R	Sequence (5' - 3')	Position	sequ exhi ex ma n
N	US-CDC-N-1/USA	F	GACCCCAAAATCAGCGAAAT	28287–>28306	3449
		P	ACCCCGCATTACGTTTGGTGGACC	28 309–>28332	
		R	TCTGGTTACTGCCAGTTGAATCTG	28358<–28335	
	US-CDC-N-2/USA	F	TTACAAACATTGGCCGCAAA	29164–>29183	3701
		P	ACAATTTGCCCCCAGCGCTTCAG	29188–>29 210	
		R	GCGCGACATTCCGAAGAA	29230<–29213	
	CN-CDC-N/China	F	GGGGAACTTCTCCTGCTAGAAT	28881–>28902	2043
		P	TTGCTGCTGCTTGACAGATT	28934–>28953	
		R	CAGACATTTTGCTCTCAAGCTG	28979<–28958	
	NIH-TH-N/Thailand	F	CGTTTGGTGGACCCTCAGAT	28320–>28339	3703
		P	CAACTGGCAGTAACCA	28341–>28356	
		R	CCCCACTGCGTTCTCCATT	28376<–28358	
	NIID-JP-N/Japan	F	AAATTTTGGGGACCAGGAAC	29125–>29144	3691
		P	ATGTCGCGCATTGGCATGGA	29222–>29241	
		R	TGGCACCTGTGTAGGTCAAC	29282<–29263	
	HKU-N/Hong Kong	F	TAATCAGACAAGGAACTGATTA	29145–>29166	3701
		P	GCAAATTGTGCAATTTGCGG	29177<–29196	

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Table represents the Forward primer (F), Reverse primer (R) and Probe (P) sequences, their corresponding position in relation to Wuhan SARS-CoV-2 reference Genome, followed by the number of sequences exhibiting exact match. Sequences with variance of at least 0.5% might decrease the efficiency of primers and probes

binding to the corresponding template DNA sequences. About 324 sequences submitted between November 25 2020 to November 30 2020 displayed misalignment with the reverse primer and therefore they have been omitted from the study. * - Range of melting temperature is provided in case of degenerate nucleotide

A large-scale multiple sequence alignment (LS-MSA) was carried out for the given FASTA files using MAFFT (Multiple Alignment using Fast Fourier Transform) v.7 tool available online (https://mafft.cbrc.jp/alignment/server/) [25]. MSA was carried out with the following options, (i) "Allow unusual symbols", (ii) "same as input" for nucleotide sequence direction, (iii) "Aligned" output order and (iv) "Auto" strategy. The aligned sequences were then downloaded and visualized using AliView program [26]. Sequences representing from forward primer initial nucleotide to reverse primer end nucleotide regions were trimmed from rest of the whole genome alignments and saved separately as FASTA files. Variability within primer-probe sequences were estimated as described elsewhere [16]. Briefly, positional nucleotide numerical summary (PNNS) and entropy was calculated using PNNS (http://entropy.szu.cz:8080/EntropyCalcWeb/pnns) and entropy calculator (http://entropy.szu.cz:8080/EntropyCalcWeb/) available in Alignment Explorer respectively [27]. Further, sequence stratification of primer-probe alignments was carried out using SequenceTracer module in Alignment Explorer with the segregation of sequences into discrete groups of identical sequence variants along with their frequency for each primer/probe. During the execution of the program, sequences with stretches of NNNs and ambiguous sequences in the alignments were excluded from the study. For the removal of extremely low prevalent variants and sequencing errors in the data, a threshold of at least 0.5% incidence of variation was considered as meaningful and those sequences alone were further considered for analysis [16]. Finally, the melting temperature (Tm) of the original primer/probe sequence against the variant sequences was calculated after mapping the whole genome sequences against the same. Since the length of primers included in this analysis is > 13 nucleotides, the following formula was applied for calculating the melting temperature $Tm = 64.9 + 41*(yG + zC – 16.4)/(wA + xT + yG + zC)$ where w, x, y, and z are the number of A, T, G, and C nucleotides, respectively [28].

3. Results

In this study, whole-genome sequences of SARS-CoV-2 strains isolated in India were obtained from GISAID database and their sequence similarity against various primers/probe employed in detection assays recommended by WHO were investigated (Supplementary Table 1). Variations in sequences were identified after performing multiple sequence alignment with MAFFT and visualized using Entropy Calculator and Sequence Tracer. The obtained Results were segregated into distinct groups based on the nucleotide variations. As described earlier [16], to avoid considering mismatches raised due to sequencing errors, a threshold value of ≥0.5% was set, where only sequences showing variation frequency above this limit were considered further. Non-informative sequences were segregated separately as outgroups. Outgroup 1 included sequences having "*n*" in them while incomplete/short sequences were categorized as 'Outgroup1/2'. Targets with missing or no sequences were excluded from the data processing. The remaining sequences alone called as 'informative sequences' were considered for further analysis. These informative sequences were divided into two groups namely 'perfect matches' and 'mismatches'.

Among the assays targeted against N region of SARS-CoV-2 genome, NIH-TH-N from Thailand displayed highest accuracy with 98.35% of genome sequence matching exactly with corresponding primers and probe (Table 1). This was followed by both US-CDC-N-2 from USA and HKU-N from

Hong Kong with 98.3% sequences displaying exact matches. Primers and probe sequences employed in NIID-JP-N assay from Japan displayed exact matches with 3691 (98.03%) SARS-CoV-2 whole genome sequences. The assay US-CDC-N-1 from USA had exact match with 3449 (91.6%) sequences. It had two significant single nucleotide mutations at 28312th position showing mismatch (C28312T) with 194 (5.15%) sequences at probe binding region and 28299th position showing mismatch (C28299A) with 20 (0.53%) sequences in the forward primer binding region. It also showed two insignificant consecutive nucleotide change CA to TT (C28315T and A28316T) of 4 (0.1%) sequences at the probe binding region. Among the assays targeting N gene, CN-CDC-N were found to be least specific. About 2043 (54.26%) sequences displayed exact matches with sequences and probe involved in CN-CDC-N assay while 1471 sequences (39.07%) displayed significant trinucleotide variation (GGG→AAC) in the forward primer region starting at 28881st position and significant single nucleotide mismatch with 45 (1.19%) sequences at forward primer region (C28887T) (Supplementary Fig. 1).

Other assays targeting the ORF1ab and E genes were also analyzed for their exact match accuracies between genomic sequences and primer/probe region. Among the assays targeting ORF1ab genes, HKU-ORF1b assay primers and probe sequences displayed exact matches with 3707 (98.46%) sequences. It is to be noted that the primers and probe used in HKU-ORF1b assay from Hong Kong were found to have degenerative nucleotides (Y=C/T, W = A/T and R = A/G). Further, primers and probe employed in CN-CDC-ORF1abassay had exact matches with 3710 (98.53%) SARS-CoV-2 genome sequences (Table 1). None of the sequences exhibited exact match with the primers and probe involved in Charité-ORF1b assay from Germany. Among 3620 (96.15%) sequences, a significant single nucleotide mutation (C/G15516T) was detected. Both the forward and reverse primers of Charité-ORF1b assay contained degenerative bases (R = A/G in forward primer, S = G/C, Y=C/T in reverse primer) like HKU-ORF1b assay. These bases are included to increase the binding specificity of primers and probes. A single assay targeting the E region, Charité-E, was also evaluated for the alignment accuracy between primer/probe and genomic DNA sequences. About 3667 (97.39%) of sequences exhibited exact matches with SARS-CoV-2 whole genome sequences while 139 (3.691%) of sequences were omitted from analysis as ‘outgroups’ (Supplementary Fig. 1). It is generally recommended that the range of hybridization temperature of the primer/probe sets should be less than 10 °C. In our study we found that 4 primer/probe sets namely, US-CDC-N1/USA, US-CDC-N2/USA, CN-CDC-ORF1ab/China and Charité-ORF1b/Germany were deviating from the prescribed threshold hybridization temperature. Mutations in the primer/probe binding regions in US-CDC-N1/USA and Charité-ORF1b/Germany assays further increased the hybridization temperature range (Table 1).

Overall, the primers and probe sequences belonging to CN-CDC-ORF1ab from China and HKU-ORF1b from Hong Kong targeting ORF1ab gene while NIH-TH-N from Thailand, HKU-N from Hong Kong and US-CDC-N-2 from USA targeting N genes displayed accurate matches (>98.3%) with the 2019 novel corona virus sequences from India. On the other hand, US-CDC-N-1/USA and CN-CDC-N/China primers and probes targeting N gene of SARS-Co-V2 Indian genome sequences displayed only 91.6% and 54.26% respectively. Interestingly, none of the genomic sequences displayed exact match with the primer/probe sequences belonging to Charité-ORF1b from Germany targeting ORF1ab gene.

4. Discussion

Reliability in detection of diagnostically important viruses has been a major concern prevailing for decades in the field of virology. Specific binding of primers and the probes to the gene targets must be achieved to obtain the PCR gene products. Single or multiple nucleotide changes in primer and/or

probe binding sites inside the specific gene of interest would result in poor specificity of the primers and/or probes for the test being conducted. This study was conducted to re-evaluate the specificity of primers and probes for reported Indian sequences of 2019 novel Corona virus (SARS-CoV-2). Although more than 25 primer-probe sets are available in the literature, out of them only seven assays showed significant variations at the defined threshold [16]. Hence, specificity was evaluated only for the primers and probes described in different CDC (Center for Disease Control and Prevention) protocols published in the WHO website [18]. Though similar studies have been carried out previously, a data bias has been observed with respect to either geographical locations or fewer samples. In general, most whole genome SARS-CoV-2 sequences that are deposited in the databases are from locations that have an easy access to genome sequencing facilities or from the place of outbreak. Similarly, another study reported the sequencing and depositing the first set of whole genome sequences of SARS-CoV-2 from India, after the isolation and culturing of viral particles from two positive cases with a travel history to Wuhan, China [29]. Since then more than 1000 sequences from India have been deposited to GISAID. Given the fact that corona viruses undergo mutation at moderate rate compared to other RNA viruses, a timely reassessment of the primer/probe sequences employed in the detection of SARS-CoV-2 becomes necessary. The work design and tools employed in this work requires minimal *insilico* infrastructure and bioinformatics skills which except for sequence alignment skills. The same strategy has been successfully applied in the assessment of primer/probes involved in the detection of Influenza A and SARS-CoV-2 viral genomes.

Through stringent data selection parameters and assigning a threshold value of 0.5% as significant nucleotide variation detection parameter, none of the assays displayed exact accuracy with the SARS-CoV-2 sequences obtained from India. Major of the Results obtained were in contrast with the results obtained from studies involving genomic sequences from other countries. A study carried out by Khan and Cheung (2020) demonstrated that primer/probe sequences belonging toUS-CDC-N2, NIH-TH-N, NIID-JP-N, HKU-N, CN-CDC-ORF1ab, HKU-ORF1b and Charité-E displayed 100% accuracy with around 17000 SARS-CoV-2 whole genome sequences collected around the world [16]. Another similar study carried out by Vogels et al., 2020, indicated that the primer/probe sequences belonging to US-CDC-N1, US-CDC-N2 and Charité-E displayed 100% accuracy with around 992 whole genome sequences deposited in GISAID [30]. Previous studies on the genome wide analysis of SARS-CoV-2 strains at a global scale indicated that the single nucleotide transitions act as the major mutational type and on that basis the strains have been classified into three major clades. Further, amino acid changes were also observed in the proteomes of SARS-CoV-2 at a varying rate across six continents. Based on the rates of observed mutations per sample analysis, it has been shown that genomic sequences from India possess relative higher value at 8.40 against Germany (6.09) and Italy (5.92) [31]. Recently, a detailed phylogenetic analysis of SARS-CoV-2 genome sequences Indian origin carried out by Banu et al., 2020 indicated the presence of a distinct clade unique to India, named as clade I/A3i [32]. This cluster is characterised by a core set of 4 genetic variants and at a nucleotide substitution rate of 1.1×10^{-3} variants per site per year. The variations in the clade I/A3i were predominantly observed in the E and N genes compared to S gene (prevalent in the A2a cluster). All these observations validate the result that was obtained in this study. The SARS-CoV-2 sequences obtained from India represent a geographically unique cluster with a differential mutation patterns occurring across the genome compared to other clades across the globe. Therefore, the mutations occurring in the target regions where primer/probe sequences from the assays would bind eventually hamper the successful amplification of the genes during RT-qPCR resulting in possible false-negatives.

Accuracy of some of the assays observed in this study is in accordance with the previously published Results. For instance, CN-CDC-N assay from China targeting N gene displayed an accuracy of 54.26% which was comparable with 81.2% and 87.3% obtained by Khan & Cheung (2020) and Vogels et al.

(2020) respectively [16,30]. Similarly, none of the genomic sequences displayed exact match with the primer/probe sequences belonging to Charité-ORF1b from Germany targeting ORF1ab gene. The presence of a degenerative nucleotide (S) was found to be detrimental because of the presence of T in the corresponding position. This could be due to be fact that primers were designed earlier in the outbreak based on the sequences from SARS-CoV and bat-SARS-related CoV genomes. Therefore, removing this degeneracy would help improve the detection limit of this assay. On the contrary, the presence of degenerate bases in both primers and probe sequences involved HKU-ORF1b assay has proved beneficial, turned out to be one of the most accurate assays investigated in the study. We would like to point out that a mere single nucleotide mutation in the genomes would not have serious ramification in the successful amplification of the targets. Mismatches located within 5 bp from 3′ end of the primers and in the probe regions are known to have serious implications in the amplification of targets while mismatches at any other position are well tolerated [33]. In our study, we could identify a single assay US-CDC-N1 containing mismatch in the 3′ end probe region. In the light we recommend using this primer in RT-qPCR assays with caution.

5. Conclusion

In this study, we have analyzed the binding accuracy of various primers and probes involved in commonly employed RT-qPCR assays for the detection of SARS-CoV-2. We could find that US-CDC-N-1from USA, CN-CDC-N from China, and Charité-ORF1b from Germany contained one or another mismatch, either in primer or probe sequences. The Results obtained in this study indicate that separate strategies, unique to specific geographical locations should be developed in the containment of pandemic. Given the number of available SARS-CoV-2 whole genome sequences in the database, novel primers with a higher accuracy could be developed. Further, this study provides an *in silico* template for re-assessing the diagnosis of COVID-19 through RT-qPCR.

Sankarganesh Jeyaraj: Conceptualization, Methodology, Formal analysis, Writing, Reviewing and Editing; Kabilan Mani.: Methodology, Formal analysis, Interpretation, Writing and Editing; Divya Sri Kathiresan: Formal analysis, Summary report; Kannan Thirumalmuthu: Formal analysis; Sudha Ramalingam: Methodology, Reviewing and Editing; Ramalingam Sankaran: Methodology, Reviewing and Editing; All authors revised and approved the final manuscript

Declarations of competing interest

None.

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Footnotes

Appendix A Supplementary data to this article can be found online at https://doi.org/10.1016/j.mcp.2021.101748.

Appendix A. Supplementary data

The following are the Supplementary data to this article:

Multimedia component 1:

Click here to view.(420K, pptx)Multimedia component 1

Multimedia component 2:

Click here to view.(189K, pdf)Multimedia component 2

Multimedia component 3:

Click here to view.(107K, docx)Multimedia component 3

References

1. World Health Organization . 2021. Corona Virus Disease (COVID-19) Weekly Epidemiological Update and Weekly Operational Update.https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports [Google Scholar]

2. Sethuraman N., Jeremiah S.S., Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *J. Am. Med. Assoc.* 2020;323:2249. doi: 10.1001/jama.2020.8259. [PubMed] [CrossRef] [Google Scholar]

3. Wang C., Liu Z., Chen Z., Huang X., Xu M., He T., Zhang Z. The establishment of reference sequence for SARS-CoV-2 and variation analysis. *J. Med. Virol.* 2020;92(6):667–674. doi: 10.1002/jmv.25762. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

4. Forster P., Forster L., Renfrew C., Forster M. Phylogenetic network analysis of SARS-CoV-2 genomes. *Proc. Natl. Acad. Sci. U. S. A.* 2020;117:9241–9243. doi: 10.1073/pnas.2004999117. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

5. Phan T. Genetic diversity and evolution of SARS-CoV-2. *Infect. Genet. Evol.* 2020;81 doi: 10.1016/j.meegid.2020.104260. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

6. Wang C., Liu Z., Chen Z., Huang X., Xu M., He T., Zhang Z. The establishment of reference sequence for SARS-CoV-2 and variation analysis. *J. Med. Virol.* 2020;92:667–674. doi: 10.1002/jmv.25762. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

7. Christopherson C., Sninsky J., Kwok S. The effects of internal primer-template mismatches on RT-PCR: HIV-1 model studies. *Nucleic Acids Res.* 1997;25:654–658. doi: 10.1093/nar/25.3.654. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

8. Liu C., Chang L., Jia T., Guo F., Zhang L., Ji H., Zhao J., Wang L. Real-time PCR assays for hepatitis B virus DNA quantification may require two different targets. *Virol. J.* 2017;14:94. doi: 10.1186/s12985-017-0759-8. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

9. Chow C.K., Qin K., Lau L.T., Cheung-Hoi Yu A. Significance of a single-nucleotide primer mismatch in hepatitis B virus real-time PCR diagnostic assays. *J. Clin. Microbiol.* 2011;49:4418–4419. https://dio.org/10.1128/JCM.05224-11 [PMC free article] [PubMed] [Google Scholar]

10. Klungthong C., Chinnawirotpisan P., Hussem K., Phonpakobsin T., Manasatienkij W., Ajariyakhajorn C., Rungrojcharoenkit K., Gibbons R.V., Jarman R.G. The impact of primer and probe-template mismatches on the sensitivity of pandemic influenza A/H1N1/2009 virus detection by real-time RT-PCR. *J. Clin. Virol.* 2010;48:91–95. doi: 10.1016/j.jcv.2010.03.012. [PubMed] [CrossRef] [Google Scholar]

11. Yang J.R., Kuo C.Y., Huang H.Y., Wu F.T., Huang Y.L., Cheng C.Y., Su Y.T., Chang F.Y., Wu H.S., Liu M.T. Newly emerging mutations in the matrix genes of the human influenza A (H1N1) pdm09 and A (H3N2) viruses reduce the detection sensitivity of real-time reverse transcription-PCR. *J. Clin. Microbiol.* 2014;52:76–82. doi: 10.1128/JCM.02467-13. 1. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

12. Lee H.K., Lee C.K., Loh T.P., Chiang D., Koay E.S., Tang J.W. Missed diagnosis of influenza B virus due to nucleoprotein sequence mutations. *Euro Surveill.* 2011;16 PMID: 21871229. [PubMed] [Google Scholar]

13. Koo C., Kaur S., Teh Z.Y., Xu H., Nasir A., Lai Y.L., Khan E., Ng L.C., Hapuarachchi H.C. Genetic variability in probe binding regions explains false negative Results of a molecular assay for the detection of dengue virus. *Vector Borne Zoonotic Dis.* 2016;16:489–495. doi: 10.1089/vbz.2015.1899. [PubMed] [CrossRef] [Google Scholar]

14. World Health Organization . 2021. WHO health Emergency Dashboard.https://covid19.who.int/region/searo/country/ [Google Scholar]

15. Rana D.R., Pokhrel N. Sequence mismatch in PCR probes may mask the COVID-19 detection in Nepal. *Mol. Cell. Probes.* 2020;53 doi: 10.1016/j.mcp.2020.101599. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

16. Khan K.A., Cheung P. Presence of mismatches between diagnostic PCR assays and coronavirus SARS-CoV-2 genome. *R. Soc. Open. Sci.* 2020;7:6. doi: 10.1098/rsos.200636. 200636. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

17. Shu Y., McCauley J. GISAID: global initiative on sharing all influenza data–from vision to reality. *Euro Surveill.* 2017;22:13. doi: 10.2807/1560-7917.ES.2017.22.13.30494. 30494. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

18. Protocols SARS. nCoV-2 PCR. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf

19. Lu X., Wang L., Sakthivel S.K., Whitaker B., Murray J., Kamili S., Lynch B., Malapati L., Burke S.A., Harcourt J., Tamin A. US CDC real-time reverse transcription PCR panel for detection of severe acute respiratory syndrome coronavirus 2. *Emerg. Infect. Dis.* 2020;26:1654. [PMC free article] [PubMed] [Google Scholar]

20. National Institute for Viral Disease Control and Prevention China . National Institute for Viral Disease Control and Prevention; Beijing: 2020. Specific Primers and Probes for Detection 2019 Novel Coronavirus.http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html (China); 21 Jan 2020.Chinese. Available from: [Google Scholar]

21. Okada P., Buathong R., Phuygun S., Thanadachakul T., Parnmen S., Wongboot W., Waicharoen S., Wacharapluesadee S., Uttayamakul S., Vachiraphan A., Chittaganpitch M. Early transmission patterns of coronavirus disease 2019 (COVID-19) in travellers from Wuhan to Thailand, January 2020. *Euro Surveill.* 2020;25 [PMC free article] [PubMed] [Google Scholar]

22. Shirato K., Nao N., Katano H., Takayama I., Saito S., Kato F., Katoh H., Sakata M., Nakatsu Y., Mori Y., Kageyama T. Development of genetic diagnostic methods for novel coronavirus 2019 (nCoV-2019) in Japan. *Jpn. J. Infect. Dis.* 2020;73(4):304–307. [PubMed] [Google Scholar]

23. Chu D.K., Pan Y., Cheng S.M., Hui K.P., Krishnan P., Liu Y., Ng D.Y., Wan C.K., Yang P., Wang Q., Peiris M. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clin. Chem.* 2020;66(4):549–555. [PMC free article] [PubMed] [Google Scholar]

24. Corman V.M., Landt O., Kaiser M., Molenkamp R., Meijer A., Chu D.K., Bleicker T., Brünink S., Schneider J., Schmidt M.L., Mulders D.G. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020;25(3) [PMC free article] [PubMed] [Google Scholar]

25. Katoh K., Rozewicki J., Yamada K.D. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings Bioinf.* 2019;20(4):1160–1166. [PMC free article] [PubMed] [Google Scholar]

26. Larsson A. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics.* 2014;30:3276–3278. doi: 10.1093/bioinformatics/btu531. 22. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

27. Nagy A., Jiřinec T., Černíková L., Jiřincová H., Havlíčková M. Large-scale nucleotide sequence alignment and sequence variability assessment to identify the evolutionarily highly conserved regions for universal screening PCR assay design: an example of influenza A virus. In: Basu C., editor. *PCR Primer Design.* Humana Press; New York: 2015. pp. 57–72. [PubMed] [Google Scholar]

28. Wallace R.B., Shaffer J., Murphy R.F., Bonner J., Hirose T., Itakura K. Hybridization of synthetic oligodeoxyribonucleotides to phi chi 174 DNA: the effect of single base pair mismatch. *Nucleic Acids Res.* 1979;6:3543–3557. doi: 10.1093/nar/6.11.3543. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

29. Yadav P.D., Potdar V.A., Choudhary M.L., Nyayanit D.A., Agarwal M., Jadhav S.M., Majumdar T.D., Shete-Aich A., Basu A., Abraham P., Cherian S.S. Full-genome sequences of the first two SARS-CoV-2 viruses from India. *Indian J. Med. Res.* 2020;151:200–209. doi: 10.4103/ijmr.IJMR_663_20. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

30. Vogels C.B.F., Brito A.F., Wyllie A.L., Fauver J.R., Ott I.M., Kalinich C.C., Petrone M.E., Casanovas-Massana A., Catherine Muenker M., Moore A.J., Klein J., Lu P., Lu-Culligan A., Jiang X., Kim D.J., Kudo E., Mao T., Moriyama M., Oh J.E., Park A., Silva J., Song E., Takahashi T., Taura M., Tokuyama M., Venkataraman A., Weizman O.E., Wong P., Yang Y., Cheemarla N.R., White E.B., Lapidus S., Earnest R., Geng B., Vijayakumar P., Odio C., Fournier J., Bermejo S., Farhadia S., Dela Cruz C.S., Iwasaki A., Ko A.I., Landry M.L., Foxman E.F., Grubaugh N.D. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets. *Nat. Microbiol.* 2020;5(10):1299–1305. doi: 10.1038/s41564-020-0761-6. Epub 2020 Jul 10. PMID: 32651556. [PubMed] [CrossRef] [Google Scholar]

31. Mercatelli D., Giorgi F.M. Geographic and genomic distribution of SARS-CoV-2 mutations. *Front. Microbiol.* 2020;11:1800. doi: 10.3389/fmicb.2020.01800. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

32. Banu S., Jolly B., Mukherjee P., Singh P., Khan S., Zaveri L., Shambhavi S., Gaur N., Mishra R.K., Scaria V., Sowpati D.T. A distinct phylogenetic cluster of Indian SARS-CoV-2 isolates. *Open Forum Infect. Dis.* 2020 doi: 10.1093/ofid/ofaa434. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

33. Lefever S., Pattyn F., Hellemans J., Vandesompele J. Single-nucleotide polymorphisms and other mismatches reduce performance of quantitative PCR assays. *Clin. Chem.* 2013 doi: 10.1373/clinchem.2013.203653. [PubMed] [CrossRef] [Google Scholar]

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Original Research Article

A community based study to assess the prevalence and sociodemographic factors influencing undernutrition among children aged 6 to 14 years in a rural area of Coimbatore

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ABSTRACT

Background: Globally, malnutrition among school age children is a major public health concern. The objectives of the study were to determine the prevalence of under nutrition (thinness and stunting) among children aged 6 to 14 years in a rural area of Coimbatore district; to determine the socio-demographic factors influencing under nutrition (thinness and stunting) among these children.
Methods: A community based- cross sectional study was conducted from December 2015 to July 2016 in the field practice area of PSG Rural Health Training Center Vedapatti. Out of the 14 villages in this area, five villages (clusters) were selected by cluster sampling. Data was collected from 702 children aged 6 to 14 years in these five villages using a structured questionnaire. Z score was used to calculate nutritional status. Thinness is defined as the BMI for age (BAZ) <-2SD.Stunting is defined as the Height-for-age (HAZ) <-2SD.The association of Thinness with socio-demographic factors were studied by univariate analysis. The association of Stunting with socio-demographic factors were studied by univariate analysis and logistic regression analysis.
Results: The prevalence of Thinness was 15.5% (95%CI: 12.82% to 18.18%) and that of stunting was 18.8% (95%CI: 15.91% to 21.69%). In univariate analysis there was no statistically significant association between thinness and socio-demographic factors. Stunting showed statistically significant association with educational status (illiteracy) of mothers and working mothers in logistic regression analysis.
Conclusions: The current study revealed that undernutrition as indicated by thinness and stunting was prevalent to some extent among these children. Stunting was significantly higher among those children whose mothers were illiterate and working. Health education and encouraging working mothers will help to solve this.

Keywords: Thinness, Stunting, BAZ, HAZ, Coimbatore

INTRODUCTION

Globally, malnutrition among school age children is a major public health concern. More than 200 million school age children are stunted and underweight and if no action is taken and at this rate, about one billion school children will be growing up by 2020 with impaired physical and mental development.[1-5]

Malnutrition is the underlying cause of one third of the 7.6 million child deaths each year before their fifth birthday. Meeting this challenge is doubly urgent because among children who survive, chronic malnutrition causes devastating and irreversible damage.[6] Lack of nutritious food, coupled with infection and illness, means their bodies and brains don't develop properly and at least 170 million children are affected by stunting.[7,8] Conditions of

early life (from conception to two years) provide the foundations for adult life. Vicious interactions between malnutrition, poor health, and impaired cognitive development set children on lower development paths and lead to irreversible changes.[9]

The prevalence of malnutrition among children in India is highest in the world. Malnutrition in school going children is responsible for 22% of the country's burden of disease. Recent reports showed that Tamil Nadu has shown gains in terms of reduction of the problem of malnutrition, but it still has a high percentage of malnourished children.[11] However, there are only a few studies regarding under nutrition among children aged 6 to 14 years reported from Coimbatore. Hence this study was undertaken to estimate the prevalence of under-nutrition in the rural field practice area of the Department of Community Medicine of PSG Institute of Medical Sciences and Research, which will help in devising suitable strategies to reduce this problem in this area.

Objectives

The objectives of this study are to estimate the prevalence of under nutrition (Thinness and Stunting) among children aged 6 to 14 years in a rural area of Coimbatore district and to determine the socio-demographic factors influencing under nutrition (Thinness and Stunting) among children aged 6 to 14 years in a rural area of Coimbatore district.

METHODS

Field practice area of PSG Rural Health Training Center, Vedapatti containing 14 villages was selected for this study. Totally 2985 children of 6 to 14 years (School age children) were living in these 14 villages. The sample size calculated was 702, assuming prevalence of undernutrition as 29%, absolute precision of 5%,design effect of 2, and non response rate of 10%.[12,13] Five out of the 14 villages (clusters) were selected by cluster sampling. A community based- cross sectional study was conducted from December 2015 to July 2016 in these five villages, and data was collected using a structured questionnaire.

All the school-age children (aged 6–14 years) who were permanent residents in these five villages were included in this study. School- age children (aged 6–14 years) who were absent for 3 visits within a week were excluded. The number of children included in this study was 702. Ethical clearance was obtained from Institutional Human Ethics Committee, PSGIMSR. Informed consent from parent and assent from the child were obtained.

Age of the child, sex of the child, total number of family members, type of house, educational status of the mother, occupation of the mother, educational status of the father, occupation of the father, socio economic status (modified Prasad's classification), were the socio- demographic factors were considered as independent variables. Thinness and stunting were considered as dependent variables.

Standing height was measured (to 0.5 cms) using stadiometer, without shoes, the child standing erect and looking straight so that the inferior orbital margin and the tragus of the ear fall in a horizontal plane parallel to the ground. An electronic weighing scale was used to measure the weight in kilograms. The scale was calibrated. Zero error was checked, children were without shoes, in shirt and trousers or skirt. The weighing machine was placed on a firm and flat ground. Weight was recorded to the nearest 100 grams. The same balance was used throughout the study. Body Mass Index, and Height for age were expressed in standard deviation units (Z scores) from reference median as recommended by 2007 WHO growth reference for (5-19 years). The calculation for Modified Prasad's classification was done using the multiplication factor as 57.07 and Consumer Price Index for Industrial Workers (November 2015) in Coimbatore area as 250.[14] Age of the child was calculated from the date of birth to number of completed years of the child.

Thinness is defined as the BMI for age (BAZ) <-2SD. Stunting is defined as the Height-for-age (HAZ) <-2SD.

The various Interpretation of cut-offs of (BAZ)[15] are:

Overweight: >+1SD (equivalent to BMI 25 kg/m^2),
Obesity: >+2SD (equivalent to BMI 30kg/m^2),
Thinness: <-2SD, Severe thinness: <-3SD
Interpretation of cut-offs for (HAZ) are:
Stunting: HAZ <–2 Z score, or <3rd percentile
Severe stunting : <-3 Z score

All the data collected was entered with MS Excel and imported to WHO Anthro software. Z score was arrived by Anthro+ package using WHO standards for nutritional status classification. The output of Anthro was analyzed with SPSS version 19. Prevalence was expressed in percentage with 95% confidence intervals (CI). Distribution of Socio-demographic factors of the study participants were expressed in percentage. The association of thinness with socio-demographic factors was studied by univariate analysis. The association of stunting with socio-demographic factors was first studied by univariate analysis and those factors found significant were subjected to logistic regression analysis.

RESULTS

A total of 702 children aged 6 to 14 years residing in the five villages were studied. Majority of the participants were female children, belonged to nuclear families and low socio economic status. Regarding educational status, 19.5% of mothers and 17.7% of fathers were illiterate. We found 36.8% mothers were home makers.

Table 1: Association of thinness with socio-demographic factors by univariate analysis.

Factors	Category	Thinness		Unadjusted odds ratio (95% confidence interval)	P value
		Yes	No		
		N (%)	N (%)		
Age in years	Age less than 10	54 (15.6)	292 (86.4)	1.01 (0.67-1.52)	0.52
	Age 10 and above	55 (15.4)	301 (84.6)	1	
Sex	Female	50 (13.5)	321 (86.5)	0.72 (0.48-1.08)	0.07
	Male	59 (17.8)	272 (82.2)	1	
Total number of family members	>4	47 (14.8)	270 (85.2)	0.91 (0.60-1.37)	0.36
	4 or less	62 (16.1)	323 (83.9)	1	
Type of house	Kutcha	23 (15.9)	122 (84.1)	1.033 (0.63-1.71)	0.49
	Others	86 (15.4)	471 (84.6)	1	
Mother's educational status	Illiterate	23 (16.8)	114 (83.2)	1.12 (0.68-1.86)	0.37
	Literate	86 (15.2)	479 (84.8)	1	
Mother's occupation	Unemployed	39 (15.1)	219 (84.9)	0.95 (0.62-1.46)	0.45
	Employed	70 (15.8)	374 (84.2)	1	
Father's education	Illiterate	18 (14.5)	106 (85.5)	0.91 (0.53-1.57)	0.43
	Literate	91 (15.7)	487 (84.3)	1	
Father's occupation	Not working	0 (0)	6 (100)	1.19 (1.15-)1.22	0.36
	Working	109 (15.7)	587 (84.3)	1	
Socio economic status (modified Prasad Scale)	Lower SES (Class IV,V)	65 (17.8)	301 (82.2)	1.43 (0.95-2.17)	0.05
	High SES (Class I,II,III)	44 (13.1)	292 (86.9)	1	

(*p<0.05 statistically significant).

Table 2: Association of stunting with socio-demographic factors by univariate analysis.

Factors	Category	Stunting		Unadjusted odds ratio (95% Confidence Interval)	P value
		Yes	No		
		N (%)	N (%)		
Age	Age less than 10	62(17.9)	284(82.1)	0.89(0.61-1.30)	0.31
	Age 10 and above	70(19.7)	286(80.3)	1	
Sex	Female	66(17.8)	305(82.2)	0.87(0.60-1.27)	0.26
	Male	66(19.9)	265(80.1)	1	
Total number of family members	>4	59(18.6)	258(81.4)	0.98(0.67-1.43)	0.49
	4 or Less	73(19.0)	312(81.0)	1	
Type of house	Kutcha	24(16.6)	121(83.4)	0.83(0.51-1.34)	0.26
	Others	108(19.4)	449(80.6)	1	
Mother's educational status	Illiterate	37(27.0)	100(73.0)	1.83(1.18-2.83)	0.01*
	Literate	95(16.8)	470(83.2)	1	
Mother's occupation	Not working	38(14.7)	220(85.3)	0.64(0.43-0.97)	0.02*
	Working	94(21.2)	350(78.8)	1	
Father's education	Illiterate	28(22.6)	96(77.4)	1.33(0.83-2.13)	0.15
	Literate	104(18.0)	474(82.0)	1	
Father's occupation	Unemployed	1(16.7)	5(83.3)	0.86(0.10-7.446)	0.69
	Employed	131(18.8)	565(81.2)	1	
Socio economic status (modified Prasad scale)	Lower SES (Class IV,V)	81(22.1)	285(77.9)	1.59(1.08-2.34)	0.01*
	High SES (Class I,II,III)	51(15.2)	285(84.8)	1	

(*p<0.05 statistically significant).

Table 3: Association of stunting with socio-demographic factors by logistic regression analysis.

Factors	Category	Adjusted Odds Ratio (95% CI)	P value
	Illiterate	1.609(1.02-2.55)	0.04*
	Literate	1	
Mother's occupation	Not working	0.624(0.41-0.95)	0.03*
	Working	1	
Socio economic status (modified Prasad Scale)	Lower SES (Class IV,V)	1.460(0.97-2.20)	0.07
	High SES (Class I,II,III)	1	

(*p<0.05 statistically significant).

The overall prevalence of Thinness (including severe thinness) and stunting (including severe Stunting) as 15.5% (95%CI: 12.82% to 18.18%), 18.8% (95%CI: 15.91% to 21.69%) respectively.

The nutritional status based on BAZ Score shows that thinness was noted among 11.4% of the children and 4.1% were severely thin. Over weight was noted among 7.3% of the children and obesity was noted among 3.8%. The nutritional status based on HAZ Score showed that. Stunting was noted among 13.8% children and 5% children were severely stunted

There was no statistically significant association between the socio-demographic factors and thinness by univariate analysis as shown in Table 1. Table 2 shows the association of stunting with socio-demographic factors by univariate analysis. Educational status of mother (illiterate), working status of mother (non working) and low socioeconomic status were significantly associated with stunting by univariate analysis. However other demographic factors showed no statistical significance**.** The factors which were significantly associated with stunting by univariate analysis were subjected to logistic regression analysis and the results in Table 3 showed that educational status and working status of mothers are significantly associated with stunting. However, low socioeconomic status showed no statistically significant association with stunting in logistic regression analysis.

DISCUSSION

The overall prevalence of thinness in the current study was 15.5%. This finding is similar to that found in a cross sectional study conducted by Vinoth et al during 2013 in Southern part of India among school children of age (9-17 years), based on WHO-BAZ scores in which the prevalence of thinness was reported to be 13%. However, a higher prevalence was reported in few other studies.[16] Bose et al did a cross sectional study in 2007 among Bengalee Hindu children aged 6-14 years and showed the prevalence of thinness as 23.1%.[17] Anjum et al conducted a cross sectional study in Kashmir in 2008 among 5-14 year old children and reported 29% prevalence for thinness.[18] Chakraborty et al conducted a cross sectional study among 5-10 year old school children of Nandigram in 2003 and showed the prevalence of thinness was 62.9%.[19] Sameena et al conducted a cross sectional study in Aligarh in 2010 among 6-12 year old children and found a very high prevalence of thinness of 79.4%.[20]

The overall prevalence of stunting in our study was 18.8%. This finding is similar to that found in certain other studies conducted in India. The cross sectional study conducted by Vinod et al reported the prevalence of stunting as 19.8%.[16] Bose et al showed the prevalence of stunting as 17.2%.[17] Ruchika et al conducted a cross sectional study from Allahabad and reported a prevalence of 17.3% for stunting among children of 7-10 years age group.[21] Chowdhary et al from West Bengal conducted a cross sectional study among the 442 Santal children of Puruliya district of West Bengal aged 5-12 years and reported the prevalence of stunting as 17.0%.[22] Bandopadyay et al conducted a study in Navinagar Mumbai among school children (aged 5 to 17 years), and reported the prevalence of stunting as 16.8%.[23] A higher prevalence (40.4%) of stunting was found in a cross sectional study conducted by Hasan et al in Bangalore in 2010 among 6-14 year old children.[24] However Anjum et al found a lower prevalence (9.25%) for stunting.[18]

Regarding the various factors influencing thinness, none of the socio-demographic factors included in our study was found to have a statistically significant association with thinness. However, Vinod et al found that factors like literacy level of the mothers, occupational status of the mothers and socio economic status had a statistically significant association with thinness.[16]

Regarding the various factors influencing stunting, we found a significantly higher prevalence among those children whose mothers were illiterate. This may be due to lack of awareness of mother regarding nutritional status of her children. Srivastava et al showed that mother's education was found to be a strong predictor of child nutritional status.[1] Those children whose mothers are working were also found to have more stunting and the probable explanation is that working mother often finds less time to care for her children. Srivastava et al showed that children of nonworking mothers have better nutritional status than children of working mothers.[1] However, Sameena et al reported higher prevalence of stunting among children whose mothers were not working.[20] Mukherjee et al reported no significant relationship between the working status of mothers and prevalence of malnutrition in their study children.[25]

Our study has certain limitations. This study being a cross sectional study, the existence of a cause-effect relationship cannot be inferred. Regarding the factors influencing thinness and stunting, only socio-demographic factors were looked into in our study.

CONCLUSION

The current study revealed that under-nutrition as indicated by thinness and stunting was prevalent to some extent among the children aged 6 to 14 years in this rural community. It was observed that stunting was significantly higher among those children whose mothers were illiterate and working. Health education is to be given to mothers to create awareness about the problem of under-nutrition including its reduction and working mothers to be encouraged to pay more attention towards the health of their children.

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REFERENCES

1. Srivastava A, Mahmood SE, Srivastava PM, Shrotriya VP, Kumar B. Nutritional status of school-age children :A scenario of urban slums in India. Archives of Public Health. 2012;70(1):8.
2. Ara R, Huque SR, Adhikary M, Uddi MN, Mahmood AR, et al. Nutritional status among primary school children in a selected rural community. J Dhaka Med Coll 20: 2011:97-101.
3. UNICEF (2011). Levels and Trends in Child Mortality: Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation. Available at: https://www.unicef.org/media/files/Child_Mortality_Report_2011_Final.pdf. Accessed 1 February 2019.
4. Save the Children: A Life Free From Hunger: Tackling Child Malnutrition, 2012. Available at: http://www.savethechildren.org.uk/sites/default/files/images/A_Life_Free_From_Hunger.pdf. Accessed on 01 February 2019.
5. Global Monitoring Report 2012: Food Prices, Nutrition, and the Millennium Development Goals. Available at: http://www.imf.org/external/pubs/ft/gmr/2012/eng/gmr.pdf. Accessed 01 February 2019.
6. Chesire EJ, Orago AS, Oteba LP, Echoka E.Determinants of undernutrition among school age children in a Nairobi peri-urban slum. East Afr Med J. 2008;85:471-9.
7. Amuta E, Olusi T, Houmsou R. Relationship of intestinal parasitic infections and malnutrition among school children in Makurdi, Benue State – Nigeria. Internet J Epidemiol. 2009;7:1540-2614.
8. Garba CM, Mbofung CM. Relationship between malnutrition and parasitic infection among school children in the Adamawa region of Cameroon. Pakistan J Nutr. 2010;9:1094-9.
9. Reji P, Belay G, Erko B, Mulugeta M, Belay M. Intestinal parasiticinfections and malnutrition amongst first cycle primary school in Adama, Ethiopia. African J Primary Health Care Family Med. 2011: 3:1-5.
10. National nutrition monitoring bureau report on diet and nutrition status of adolescence, NIN, India 2002. Available at: http://nnmbindia.org/nnmbreport2001-web.pdf Accessed 01 February 2019.
11. Navaneethan P, Kalaivani T, Rajasekaran C, Sunil N. Nutritional status of children in rural India: a case study from Tamil Nadu, first in the world to initiate the Mid Day Meal scheme. Health. 2011;3:647-55.
12. Sundar RJ, Richard J. An Introduction to Biostatistics –A Manual for Students in Health Science, 3rd ed, 2004.
13. Shivaprakash NC, Joseph RB. Nutritional Status of Rural School-Going Children (6-12 Years) of Mandya District, Karnataka. Int J Sci Stud. 2014;2(2):39-43.
14. Mangal A, Kumar V, Panesar S, Talwar R, Raut D, Singh S. Updated BG Prasad socioeconomic classification, 2014: A commentary. IJPH. 2015;59:42-4
15. WHO Working group-Use and interpretation of anthropometric indicators of nutitional status Bulletin of WHO (1986) 64:924-41.
16. Selvaraj V, Sangareddi S, Velmurugan L, Muniyappan U, Anitha FS. Nutritional status of adolescent school children in a semi-urban area based on anthropometry. Int J Contemp Pediatr. 2016;3:468-72.
17. Bose K, Bisai S, Mukherjee S. Anthropometric characteristics and nutritional status of rural school children. Internet J Biological Anthropol. 2007;2:1-6.
18. Fazili A, Mir A, Pandit IM, Bhat IA, Rohul J, Shamila H. Nutritional Status of School Age Children (5 14 years) in a Rural Health Block of North India (Kashmir) Using WHO Z Score System. J Health Allied Sci. 2012;11:1-3.
19. Chakraborty R, Bose K. Very high prevalence of thinness using new international body mass index cut off points among 5-10 year old school children of Nandigram, west Bengal, India. Journal of Research in Medical Sciences: The Official J Isfahan Univ of Med Sci. 2009;14(2):129-33
20. Sultan S. Prevalence of stunting and thinness among school-age children of working and non-working mothers in rural areas of Aligarh District. Indian J Basic Applied Med Res. 2014;3(3):51-7
21. Ruchika H, Faizan A, Kesari K, Prasad R. Assessment of Nutritional Status of 7 10 Years School Going Children of Allahabad District: A Review. Middle East J Sci Res. 2008;3(3):109-15.
22. Chowdhary SD, Chakraboraty T, Ghosh T. Prevalence of under nutrition in Santal Children of

Puriliya district West Bengal. Indian Pediatrics. 2008;45 (1):43 46.
23. Bandopadhyay D. A Nutrition Survey of school children, Navi Nagar Mumbai. Med J Forum India. 1988;44 (1):31-4.
24. Hasan I, Zulkifle M, Haseeb A. An assessment of nutritional status of the children of government urdu higher primary schools of Azad Nagar and its surrounding areas of Bangalore. Arch Applied Sci Res. 2011;3(3):167-76.
25. Mukherjee M, Chaturvedi LC, Bhalwar C. Determinants of Nutritional Status of School Children. Med J Armed Forces India. 2008;64:227-231.

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Socio-Demographic and Lifestyle Factors Associated With Internet Addiction among Medical and Health Sciences Students

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ABSTRACT

Background: New innovations in technology produce large opportunities in communication, information and social interaction. The excess in unwanted use of internet by people led to internet addiction. Psychological, environmental and socio demographic factors in the lives of college students may leave them disproportionately vulnerable to internet addiction. **Aims and objectives:** This study was conducted with objectives to determine the prevalence of internet addiction among medical and health science students and its socio demographic correlates. **Material and methods:** This cross sectional study was conducted on medical and health science students at PSG Institute of Medical Sciences and Research and PSG College of Physiotherapy, Coimbatore. A total of 490 students were selected by simple random sampling. Validated internet addiction scale was used to assess the internet addiction. **Results:** Of the 490 students studied, 140(28.5%) students were having internet addiction. The factors associated with internet addiction were gender(males) ($p<0.01$) and those take frequent junk food ($p<0.001$).**Conclusion:** We had observed a high prevalence of internet addiction in medical and physiotherapy students. So strategies for controlling internet addiction is mandatory for healthy and safe use of internet, particularly students studying at professional colleges.

Key Words: Internet addiction, Medical students, Internet addiction score, Young's internet addiction scale, Likert scale

INTRODUCTION

The prevalence of internet addiction has increased dramatically in these decades. The majority cases of internet addiction could be prevented by changes in lifestyle[1]. Many studies have reported that internet addiction is more common in men than women[2]. Globally, about half of the population are using internet through smartphones. In developing countries like India, population using internet increases rapidly from 5 million in 2000 to 137 million in 2012[2]. A research conducted by Indian Market Research Bureau in June 2013, indicates that the internet usage in India has gone up with more and more internet users using the internet on a regular basis[2]. Nearly 71%of the world internet users are constituted by young people aged 15-24[3].

The rapid expansion and proliferation of the internet has provided better opportunities for communication, information and social interaction[2]. Internet technology was originally designed to facilitate communication and research activities, however the dramatic increase in the use of internet in recent years has led to internet addiction which is classified into a disorder by WHO[4-6]. After effects of internet addiction includes the impairment of academic performance, psychological wellbeing and interaction with family members, depression, anxiety, sleep disorders and dissociative experience. Studies were also shown that younger internet users were more at risk of becoming internet addicts than older users[5]. Psychological and environmental factors in the lives of college students may leave them disproportionately vulnerable to internet addiction[6]. Although there were studies reported on the association of socio demographic and clinical factors associated with internet addiction among college students, the relationship between dietary behaviour and lifestyle factors such as regular exercise, place of origin, education of parents on internet addiction among college students is unclear and more studies are needed.

The aim of the study is to assess the prevalence of internet addiction and its association with socio-demographic and lifestyle factors among medical and health sciences students.

MATERIAL AND METHODS

Study location and design: This cross sectional study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore and PSG College of physiotherapy, Coimbatore during April to July 2019. The study got

approval from Institute Human Ethics Committee and the consent of college and students were procured before the start of the study.With an expected prevalence of internet addiction of 50% and with 10% allowable error of the prevalence, 95% confidence limits and 20% non-response,the required sample size is 500. To meet the required sample size, 50% of the students from each batch (first year, second year, third year and forth year) were selected by Simple Random Sampling Technique. Informed consent was obtained from each study participant and administered a semi structured questionnaire as the study tool. Thus a total of 490 study participants were recruited from both the colleges.

The study tool has two parts. Part A consisted the socio demographic and lifestyle factors include year of the study, gender (male, female),regular exercise(yes, no),frequent junk food consumption (yes, no), residence(home, hostel, others), origin(city, town, village, others), father's education(below undergraduate, under graduate, postgraduate, others), mother's education(below undergraduate, under graduate, post graduate, others) and gadget(desktop, laptop, mobile, others).

Part B is the validated internet addiction questionnaire to measure internet addiction. The scale comprises of 20 questions, each questions had score ranging from 0-5(0=never, 1=rarely, 2=occasionally, 3=frequently, 4=often, 5=always)[7]. Based on these total scores, participants were classified as: normal user (score as: <50) and addicted user (score as:>=50).

The data were analyzed using SPSS version 24. Socio demographic variables and patterns of internet use have been described by frequency tables. The prevalence of internet addiction was described in terms of percentage. The descriptive statistics were calculated based on mean, standard deviation for quantitative variables and percentages for categorical variables. The association between internet addiction and socio demographic variables was assessed using Chi-square test and then performed a Multiple Logistic Regression analysis with internet addiction as dependent variable and independent variables including several socio demographic and life style factors.In all calculations, p value less than 0.05 was considered as statistically significant.

RESULTS

A total of 490 students were studied and there were 197(40.2%) males and 293(59.7%) females. 120 students were from 1st year, 134 students were from second year, 132 students were from third year and 104 students were from final year. Out of 490 students studied, 140 (28.5%) students were having internet addiction(Table 1). It was observed that males were reported more internet addiction than females (p<0.001).Students consuming junk food frequently were also more addicted than who were not frequently consuming (p<0.001)(Table 2). In the Multivariate analysis gender(males)(p<0.001) and those frequently consuming junk foods (p<0.001) were significantly associated with internet addiction(Table 3).

Table 1:Distribution of the students studied

Year of study	College of physiotherapy			College of medicine			Total
	Male	Female	Total	Male	Female	Total	
First year	13	32	45	38	37	75	120
Second year	17	33	50	29	55	84	134
Third year	20	30	50	36	46	82	132
Fourth year	14	31	45	30	29	59	104
Total	64	126	190	133	167	300	490

Table 2: Association of internet addiction with socio demographic and life style factors in univariate analysis

Variables		Number	Percentage	Chi-square	P-value
Class*	MBBS	98	32.7	6.358	0.012
	Physiotherapy	42	22.1		
Year	First year	33	27.5	7.76	0.051
	Second year	36	26.9		
	Third year	49	37.1		
	Fourth year	22	21.2		
Gender*	Male	74	37.6	13.053	0
	Female	66	22.5		
Junk food*	Yes	87	42.6	33.931	0
	No	53	18.5		
Exercise	Yes	42	31.6	0.809	0.368
	No	98	27.5		
Residence	Home	44	27.2	0.605	0.739
	Hostel	88	28.9		
	Others	8	34.8		
Origin	City	72	30	0.496	0.92
	Town	39	27.1		
	Village	27	27.6		
	Others	2	25		
Father's education	Below UG	28	20.6	6.102	0.107
	UG	46	32.45		
	PG	60	30.8		
	Others	6	35.3		
Mother's education	Below UG	37	24.2	2.102	0.35
	UG	49	30.4		
	PG	54	30.7		
Gadget	Desktop	5	45.5	2.243	0.523
	Laptop	11	28.2		
	Mobile	119	28.6		
	Others	5	20.8		

*=Significant with p value<0.05

Table3:Association of internet addiction with socio demographic and life style factors in multivariate analysis

Variables		Adjusted odds ratio	95% CI	
			Lower	Upper
Class	MBBS	1	-	
p=0.276	Physiotherapy	1.337	0.793	2.256
Year	First year	1	-	
P=0.116	Second year	1.389	0.71	2.716
	Third year	1.385	0.713	2.688
	Fourth year	2.117	1.123	3.991
Gender*	Male	1.964	1.251	3.083
p=0.003	Female	1	-	
Junk food*	Yes	2.961	1.928	4.546
p=0.000	No	1	-	
Exercise	Yes	1.017	0.628	1.645
p=0.946	No	1	-	
Residence	Home	1	-	
p=0.526	Hostel	1.045	0.644	1.698
	Others	1.823	0.64	5.191
Origin	City	1	-	
p=0.970	Town	0.912	0.542	1.534
	Village	1.045	0.578	1.892
	Others	0.811	0.129	5.095
Father's education	Below UG	1	-	
p=0.431	UG	1.684	0.84	3.375
	PG	1.398	0.638	3.066
	Others	2.081	0.597	7.258
Mother's education	Below UG	1	-	
p=0.971	UG	0.926	0.478	1.795
	PG	0.921	0.446	1.903
Gadget	Desktop	1	-	
p=0.510	Laptop	0.669	0.153	2.927
	Mobile	0.691	0.187	2.561
	Others	0.318	0.06	1.695

*=Significant with p value<0.05

DISCUSSION

Internet addiction has been found important as a new and increasing global problem. The consequences of Internet addiction has a huge impact on students, physically and mentally,including changing the lifestyle in order to spend more time on the internet, neglecting their own health, avoiding of major life activities, reducing the social relations, ignoring the family and friends[8-10]. The prevalence of internet addiction varies from 2% to 18% worldwide and from 0.3% to 11.8% inIndia[3,11-17].The world has witnessed an explosive growth of internet users; globally more than half of the population is using the internet[18-20]. Internet user population has increased from 360 million in December 2000 to 2.4 billion in June 2012[2]. In Asia it has grown from 114 million internet users on December 2000 to 1.07 billion in June 2012[2].

Gender has a statistically significant association with internet addiction in this study. The finding has been corroborated by many previous researches explained this gender difference with a reason that males involve more in online activities such as gaming, gambling, pornography etc.[2,10-19]. In general, men are more likely to enjoy searching for information of personal interest other than the requirements of work [20-23]. Men seemed to enjoy interactive online games more than women,which draw upon power and dominance. Men seem to enjoy the aspects of violence and dominance in such interactive games[24-27].Cyber sex is another area men seemed more attracted to than women. In general, men were more openly drawn to the sexually explicit material accessible through the internet[28-30].

One of the more important finding that is revealed through our study is that junk food addicts are more likely to be internet addicts than normal people. Previous studies do not give us any effective results on the relation of junk food consumption and internet addiction. These junk food are nutritionally poor and this is universally recognized as they have very little or no vitamins, minerals and proteins and rather have a high content of salt sugar and calories that can lead to numerous health conditions when ingested in large quantities. Though it is universally accepted that these "junk food" cause numerous health issues both on a short term and long term basis, a large population of people have been found addicted to it[31-34]. The brain has perceives the food we eat in an unique way. Evolution has been a major contributor in the fact that the entire human race as a whole are more likely to ingest carbohydrates and fats when compared to vitamins, minerals and proteins as it provides an immediate energy supply and contributes to increased blood sugar levels but due to the increasing sedentary lifestyle of people, this is leading to be a major risk factor. It has been demonstrated that when some food is ingested and it reaches the GIT, impulses are immediately sent to the brain. A vast segment of nerve supply is found connecting the brain and CNS to the gut[31]. In the brain exists a pathway called the reward pathway, the reward pathway involves several parts of the brain, the ventral tegmental area (VTA), the nucleus accumbens, and the prefrontal cortex. When activated by a rewarding stimulus (e.g., food, water), information travels from the VTA to the nucleus accumbens and then up to the prefrontal cortex. It has been found that the gastrointestinal tract sends signals to the brain's reward circuits every time junk food is consumed. In the presence of calories in the gastrointestinal tract triggers the release of dopamine, the major chemical transmitter acting on the reward circuitry.A particular lipid messenger in the gut, known as oleoylethanolamine (OEA), controls the brain's perception of reward value when it comes to food.[31] A recent study byTellez, et al.found that the brain's reward circuitry is highly sensitive to the energy content of foods, independent of sensory properties such as flavour[31]. It means that calorie sensing in the gastrointestinal tract is an important

activator of this brain's reward circuitry, and thus can cause alterations in gastrointestinal physiology. Thus the consumption of junk food leads is presumed to trigger the reward circuitry of the brain and found to have same effect on the reward circuitry just like addictive drugs like cocaine and heroin. Brain's reward circuitry was active in people those frequently consuming junk food, which make them vulnerable to internetaddiction[31-36].For susceptible people, eating junk foods can lead to full-blown addiction, which shares the same biological basis as addiction to abusive drugs.

Our study has some limitations. First, the prevalence of internet addiction is difficult to establish accurately because of limitations related to the population screened, the unwillingness of subjects to participate and unreliability on the history of internet usage revealed by study subject and hence its accuracy is unknown. Second, we have not collected the information from the students studying at Government colleges. Third, since the students were asked to report details of exposure and use to internet, recall bias cannot be ruled out. Another limitation of the study is that since there was self-reporting of data and hence social desirability bias can also be present.

Despite these limitations, our study has several strengths.The main strength of the study includes scientific selection of variables.We have selected students from various classes and that also selected both from medical and allied health sciences students. Thus we studied a heterogeneous representation of college students.Sampling bias has always been a major drawback of many of the earlier studies.This study has tried to minimize this bias by not sending the questionnaire by email or group networks instead the investigators went to the class and collected the required information. We have used a validated questionnaire and questionnaires were answered anonymously and the investigators were kept away from the classrooms where information was being collected.

Conclusion

In conclusion results from our study suggest that internet addiction was observed in one third of thesubjects. The explosive growth of the internet in the last decade had a huge impact on communication and inter-personal behavior. The present study unfolds that internet addiction in the majority of medical students is a dark reality that recovers timely remedial action of detection of internet addiction therefore assumes greater importance in professional institutions such as medical colleges. It is essential to develop strategies for the prevention of internet addiction. As an emerging global issue, awareness should be created among the students to reduce the occurrence of internet addiction behaviour and for promoting their healthy growth[33-37].Further analysis of internet addiction with usage patterns will be reported in a subsequent paper.

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REFERENCES

1. Anusha Prabhakaran, V. Rajvee Patel, D. JaishreeGanjiwale, et al. Factors associated with internet addiction among school going adolescents in Vadodra. Journal of Family Medicine and Primary Care. 2016; 5(4): 765-769.

2. Arvind Sharma, RupeshSahu, Pradeep Kumar Kasar, et al. Internet addiction among professional courses students; a study from Central India. International Journal of Medical Science and public Health.2014;3(9):1069-1073.

3. N R Ramesh, S Pruthvi and M S Phaneendra. A comparative study on social media usage and health status among students studying in pre-university colleges of urban Bangalore. Indian J Community Med. 2018; 43(3): 180–184.

4. Young KS and Case CJ. Internet abuse in the workplace: New trends in risk management. CyberpsycholBehav.2004;7:105-11.

5.Takeshi Sato. Internet addiction among students: Prevalence and psychological problems in Japan: Japan Medical Association Journal.2006; 49(7):8: 279-282.

6. Young KS and Rogers RC. The relationship between depression and internet addiction.CyberpsycholBehav.1998;1:25-8.

7. Young KS. Caught in the Net: How to recognize the signs of Internet addiction and a winning strategy for recovery. New York, NY: John Wiley & Sons,Inc:1998; P:196.

8.Ge Y, Se J and Zhang J. Research on relationship among internet-addiction, personality traits and mental health of urban left-behind children. Glob J Health Sci.2014; 7:60-9.

9. Bahrainian A and Khazaee A. Internet addiction among students: The relation of self-esteem and depression. Bull Environ Pharmacol Life Sci.2014;3:1-6.

10. Kormas G, Critselis E, Janikian M, et al. Risk factors and psychosocial characteristics of potential problematic and problematic internet use among adolescents: A cross-sectional study. BMC Public Health.2011;11:595.

11. Srijampana VV, Endreddy AR, Prabhath K, et al. Prevalence and patterns of internet addiction among medical students. Med J Dr DY PatilUniv.2014;7: 709.

12. Goel D, Subramanyam A and Kamath R. A study on the prevalence of internet addiction and its association with psychopathology in Indian adolescents. Indian J Psychiatry.2013;55:140-3.

13. Yadav P, Banwari G, Parmar C, et al. Internet addiction and its correlates among high school students: A preliminary study from Ahmedabad, India. Asian J Psychiatr.2013;6:500-5.

14. Bargh J A and McKenna K Y A. The Internet and social life. Annual Review of Psychology.2004;55:573–590.

15. Beranuy M, Oberst U, Carbonell X, et al. Problematic Internet and mobile phone use and clinical symptoms in college students: The role of emotional intelligence. Computers in Human Behavior.2009; 25: 1182–1187.
16. Morahan-Martin JM, Schumacker P. Incidence and correlates of pathological internet use. Comput Human Behav.2000; 16:13-29.
17. Chakraborty K, Basu D, Vijaya Kumar KG. Internet addiction: Consensus, controversies, and the way ahead. East Asian Arch Psychiatry.2010;20: 123-32.
18. Sharma A, Sahu R, Kasar PK, et al. Internet addiction among professional courses students: A study from Central India. Int J Med Sci Public Health.2014;3: 1069-73.
19. Li W, O'Brien JE, Snyder SM, et al. Characteristics of internet addiction/pathological internet use in U.S. university students: A qualitative-method investigation. PLOS One.2015;10: e0117372
20. Ho RC, Zhang MW, Tsang TY, et al. The association between internet addiction and psychiatric co-morbidity: A meta-analysis. BMC Psychiatry.2014;14: 183.
21. Krishnamurthy S and Chetlapalli SK. Internet addiction: Prevalence and risk factors: A cross-sectional study among college students in Bengaluru, the Silicon Valley of India. Indian J Public Health.2015;59: 115-21.
22. Canan F, Ataoglu A, Ozcetin A, et al. The association between internet addiction and dissociation among Turkish college students.Compr Psychiatry.2012;53: 422-6.
23. Ghamari F, Mohammadbeigi A, Mohammadsalehi N, et al. Internet addiction and modeling its risk factors in medical students, Iran. Indian J Psychol Med.2011;33: 158-62.
24. Salehi M, NoroziKhalili M, Hojjat SK, et al. Prevalence of internet addiction and associated factors among medical students from Mashhad, Iran in 2013. Iran Red Crescent Med J.2014;16: e17256.
25. Young KS, de Abreu CN. Internet Addiction: A Handbook and Guide to Evaluation and Treatment. New York: Wiley.2010. p. 239.
26. Guan SS and Subrahmanyam K. Youth internet use: Risks and opportunities. CurrOpin Psychiatry.2009;22: 351-6.
27. Yen JY, Ko CH, Yen CF, et al. The comorbid psychiatric symptoms of internet addiction: Attention deficit and hyperactivity disorder (ADHD), depression, social phobia, and hostility. J Adolesc Health.2007;41: 93-8.
28. Kuss DJ and Lopez-Fernandez O. Internet addiction and problematic internet use: A systematic review of clinical research. World J Psychiatry .2016;6: 143-76.
29. Durkee T, Carli V, Floderus B, et al. Pathological internet use and risk-behaviors among European adolescents. Int J Environ Res Public Health.2016;13.pii:E294.
30. Grover S, Chakraborty K and Basu D. Pattern of internet use among professionals in India: Critical look at a surprising survey result. Ind Psychiatry J.2010;19: 94-100.
31. L.A. Tellez, S. Medina, W. Ha, et al. A Gut Lipid Messenger Links Excess Dietary Fat to Dopamine Deficiency.Science.2013;34: pp.800-802
32. Recupero PR. Forensic evaluation of problematic internet use. J Am Acad Psychiatry Law.2008;36: 505-14.
33. Siomos KE, Dafouli ED, Braimiotis DA, etal.Internet addiction among Greek adolescent students. Cyberpsychol Behav.2008;11:653-7.
34. Lam LT, Peng ZW, Mai JC, et al. Factors associated with Internet addiction among adolescents. Cyberpsychol Behav.2009;12: 5515.
35. Niemz K, Griffiths M and Banyard P. Prevalence of pathological Internet use among university students and correlations with selfesteem, the General Health Questionnaire (GHQ) and disinhibition. Cyberpsychol Behav.2005;8: 562-70
36. Seo M, Kang HS and Yom YH. Internet addiction and interpersonal problems in Korean adolescents. Computers, informatics, and nursing: CIN.2009;27: 226-33.
37. Morrison CM and Gore H. The relationship between excessive internet use and depression: A questionnaire-based study of 1,319 young people and adults. Psychopathology.2010;43:121-6.

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Prevalence and correlates of anemia among women in the reproductive age (15–49 years) in a rural area of Tamil Nadu: An exploratory study

A. Jenit Osborn, G. M. Muhammad, S. L. Ravishankar, Anil C. Mathew

Abstract:

BACKGROUND: Anemia is a major public health problem among reproductive-aged women in India. Despite many programs implemented for decades to fight anemia, still the prevalence of anemia is high because its associated factors vary among different regions. This exploratory study was carried out to find the prevalence of anemia and factors influencing it among nonpregnant reproductive-aged women (15–49 years) in a rural area of Coimbatore.

MATERIALS AND METHODS: The study was conducted in the field practice area of Rural Health Training Centre, PSG IMSR, Coimbatore, during March 2016 to August 2016. Participants were recruited by two-staged random sampling method and 426 participants were enrolled. A predesigned semi-structured questionnaire was used to collect the data pertaining to the factors influencing the prevalence of anemia, and their hemoglobin level was measured using a hemoglobin photometer. The data were analyzed using SPSS-24. (IBM Corporation, SPSS Inc., Chicago, IL, USA) Univariate analysis was done using Chi-square test and the strength of association was estimated by odds ratio. Those factors which were found to be significant by univariate analysis were further subjected to multivariate logistic regression analysis. $P < 0.05$ was considered statistically significant.

RESULTS: The prevalence of anemia was estimated as 64.8% (95% confidence interval: 60%–69%). Significant predictors for anemia among the study participants were less frequent intake of green leafy vegetables (adjusted odds ratio [AOR] = 3.65, confidence interval [CI]: 2.17–6.12), low socioeconomic status (AOR = 3.36, CI: 1.93–5.84), illiteracy (AOR = 3.09, CI: 1.09–5.24), birth spacing <2 years (AOR = 2.49, CI: 1.19–5.25), excessive menstrual bleeding (AOR = 2.27, CI: 1.09–4.76), and inadequate knowledge regarding anemia (AOR = 2.03, CI: 1.19–3.44).

CONCLUSION: Anemia is still prevalent in high magnitude in reproductive-aged women, and measures to prevent and control it are not optimum. Hence, its multifactorial determinants have to be addressed to alleviate its burden.

Keywords:

Anemia, nonpregnant reproductive-aged women, risk factors for anemia

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Introduction

Anemia is a major public health problem, affecting 24.8% of the global population. The prevalence of anemia is disproportionately high in developing countries compared to developed countries due to poverty, illiteracy, inadequate dietary intake, certain infectious diseases, frequent pregnancy, and poor access to health services. The most vulnerable groups affected by anemia are children, adolescent girls, and women in the reproductive age, especially pregnant women.[1] Anemia can reduce the quality of life, leading to a substantial economic

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burden on the affected individuals and their families, communities, and societies.[2] According to the Fourth National Family Health Survey (NFHS-4), 54.4% and 56.9% of women of reproductive age are found to be anemic in rural areas of India and in Tamil Nadu, respectively.[3] Anemia is found to be more prevalent among reproductive-aged women in India, and it poses a significant morbidity risk, particularly among reproductive-aged women. Despite the fact that anemia is largely preventable and easily treatable if detected in time, it still continues to be a common cause of morbidity and mortality and is considered a silent killer.[4] This nutritional disability in the life of women will have adverse consequences on the developmental stages of the next generation and can have long-term impact and thus leave a perpetual effect.[5] The WHO recommends local studies for each region to provide baseline information for designing prophylactic and therapeutic strategies to control this problem.[4] Most of the studies carried out in different parts of the country are either hospital-based studies done among pregnant and lactating mothers or college/school-based studies done among adolescent girls, there is a scarcity of community-based studies among nonpregnant reproductive-aged women. Social, cultural, economic, and dietary features vary widely across India and hence the factors influencing anemia also vary based on the geographical location. Though the accurate data on the prevalence of anemia is essential to determine public health measures, in reality, accurate data are scarce in the study area, which impedes anemia preventive strategies. Identifying the local determinants and risk factors of anemia will help to plan for appropriate package of services to reduce the risks of morbidity and mortality associated with anemia and is one of the priorities for safe motherhood initiative.

Materials and Methods

Study design and setting

A community-based cross-sectional study was done in the rural field practice area of PSG Institute of Medical Science and Research, Coimbatore, during March 2016 to August 2016. The conceptual framework adopted in this study explains the possible causes of anemia and the interconnectedness of these factors in predisposing a woman to become anemic [Figure 1].[6]

Study participants and sampling

With an estimated prevalence of 55.4% from NFHS-4[3] study and 5% allowable error, and 20% nonresponse rate, the sample size was estimated as 480. The study participants were selected by two-staged random sampling method [Figure 2]. In the first stage, three villages were selected randomly from 14 villages. In the second stage, the required sample numbers were selected from three villages by probability proportional to the size of the villages. Individual participants from each village were chosen by SPSS-generated random numbers. Women in reproductive age group (15–49 years) who are permanent residents for at least 1 year were included, whereas pregnant women, those not willing to participate in the study, and absence at home on three visits were excluded from the study.

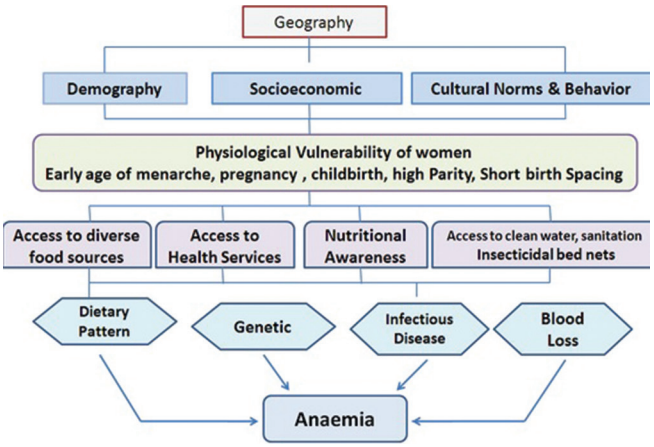


Figure 1: Conceptual framework for anemia among reproductive age women

Data collection tool and technique

After obtaining informed consent, a predesigned semi-structured questionnaire was used to collect the data pertaining to the factors influencing the prevalence of anemia namely demographic factors, socioeconomic, environmental factors, reproductive, and menstrual factors. Socio-economic status was assessed using Modified Prasad Classification. Knowledge was assessed by a series of twelve questions on causes, symptoms, complication, and prevention methods, and a scoring system was given and those who scored more than 50% were considered having adequate knowledge on anemia. Sixteen iron-rich food items were considered in our study based on the iron content given by the National Institute of Nutrition.[7] Height and weight were measured according to the WHO standard procedure,[8] and confidentiality of the study participants was ensured. Hemoglobin level was measured from capillary blood obtained by finger prick using a hemoglobin photometer, which is a reliable method in field surveys.[9] The sensitivity and specificity for the hemoglobin estimation by this photometer is 94.1% and 95.2%, respectively.[10] The Haemoglobin photometer was checked each day morning prior to data collection with the control microcuvette given along with the instrument by the manufacturer for the purpose of internal validation of the instrument. After every seventy samples examined by the photometer, the instrument was tested for reliability using same blood samples,

and it was also externally validated in the Pathology Laboratory of PSGIMSR using the Beckman Coulter LH 450 method. Difference in the value of hemoglobin was found to be in the range of 0.3–0.6 g/dL.

Ethical consideration

The study was carried out in accordance with the ethical standards of PSGIMSR Institutional Human Ethics Committee (IHEC – Proposal Code – 14/075), and the Ethical Principles for Medical Research was followed.

Operational definition

The WHO criteria for diagnosis of anemia in nonpregnant women with hemoglobin level <12 g/dL were used in this study. Grading of anemia was done on the basis of cutoff values: mild anemic (hemoglobin [Hb]: 11–11.9 g/dL), moderate anemic (Hb: 8–10.9 g/dL), and severe anemic (Hb: <8 g/dL).

Statistical analysis

Data were analyzed using SPSS-24. The prevalence of anemia was estimated with 95% confidence interval (CI). The associations between independent variables and hemoglobin level were tested using Chi-square test and the strength of associations was obtained using odds ratio. The variables which were found to be statistically significant in univariate analysis were further subjected to multivariate logistic regression analysis. $P < 0.05$ was considered statistically significant.

Results

Of the selected 480 women of reproductive age, 37 of them did not participate due to fear of finger prick, 7 of them were pregnant, and 12 of them were not present in their house during three visits and hence excluded from the study. A total of 426 nonpregnant women in the reproductive age group were enrolled in the study.

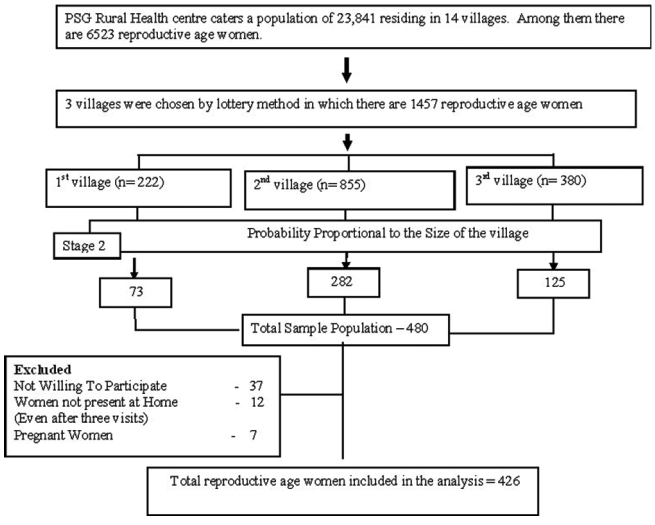


Figure 2: Flow diagram showing the sampling procedure

The demographic profile of the study participants is shown in Table 1. 18.8% of the study participants were in the age group of 25-29 years, 26.7% had education up to high School, higher proportion (63.8%) of the study participants were not working and majority of them (92.7%) belonged to Hindu religion. 71.6% of the participants were residing in a nuclear family and 37% of them belonged to low-socio-economic class (Class IV and V).

Table 2 shows the reproductive characteristics of the study participants. Nearly 75.6% of them have attained menarche before the age of 14 years, and 79.6% of the participants were married. Among those who were married, 25.7% of them got married before the age of 18 years, 9.6 % of them have more than 2 children, 28.6% have a history of abortion, and 8.3% of the individuals are currently using IUD. Among 218 people having two children and more, 45.4% had birth spacing <2 years. 10 participants had hysterectomy done, and 19 of them have attained menopause. Of the remaining 397 participants,

Table 1: Demographic profile of the study participants (*n*=426)

Variable	Frequency, *n* (%)
Age (years)	
15-19	51 (12.0)
20-24	75 (17.6)
25-29	80 (18.8)
30-34	66 (15.5)
35-39	59 (13.8)
40-44	48 (11.3)
45-49	47 (11.0)
Education	
Illiterate	64 (16.2)
Primary	29 (6.8)
Middle	63 (14.8)
High school	114 (26.7)
Higher secondary	68 (16.0)
Graduate	83 (19.5)
Occupation	
Working	154 (36.2)
Not working	272 (63.8)
Religion	
Hinduism	395 (92.7)
Christianity	27 (6.3)
Islam	4 (1.0)
Type of family	
Nuclear	305 (71.6)
Extended	121 (28.4)
Socioeconomic status	
Class I	22 (5.2)
Class II	102 (23.9)
Class III	144 (33.8)
Class IV	111 (26.1)
Class V	47 (11.0)

Table 2: Reproductive and menstrual characteristics of the study participants

Variable	Frequency, *n* (%)
Age at the time of menarche (years) (*n*=426)	
<14	322 (75.6)
≥14	104 (24.4)
Marital status (*n*=426)	
Unmarried	70 (16.4)
Married	339 (79.6)
Widowed	15 (3.5)
Divorced	2 (0.5)
Age at the time of marriage (years) (*n*=339)	
<18	87 (25.7)
>18	252 (74.3)
Number of children (*n*=339)	
0	22 (6.4)
1	99 (29.2)
2	185 (54.6)
3	24 (7.1)
>3	9 (2.7)
Age at the time of first child birth (years) (*n*=317)	
<19	35 (11)
≥19	282 (89)
Birth spacing (*n*=218)	
<2	99 (45.4)
≥2	119 (54.6)
History of abortion (*n*=339)	
Yes	97 (28.6)
No	242 (71.4)
Current IUD usage (*n*=339)	
Yes	28 (8.3)
No	311 (91.7)
Menstrual cycle (*n*=397)	
Regular	313 (78.8)
Irregular	84 (21.2)
Excessive menstrual bleeding (*n*=397)	
Yes	83 (20.9)
No	314 (79.1)

IUD=Intrauterine device

21.2% of them had irregular menstrual cycle and 19.5% of the individuals had excessive menstrual bleeding.

Regarding the environmental factors, only 64.3% of them were having toilet facility in their homes, 20.4% of them were having the practice of open-air defecation, and the rest were using community toilets. Almost 5.9% of the participants had the habit of barefoot walking, 8.2% of them had a history of passing worms in the stool, and 20% of them did not have the habit of washing hands with soap after defecation.

Regarding knowledge, 49.1% of them had adequate knowledge regarding anemia – its cause, symptoms, methods to prevent anemia – and knowledge regarding iron-rich foods. Almost 87.3% of the participants were on mixed diet and 40.1 of them have the habit of taking coffee along with food. Nearly half (48.6%) of them were having normal body mass index (BMI), while 19% of them were undernourished and 32.4% were overweight/obese. The mean BMI of the study participants was 23.03 ± 4.71 kg/m^2.

Prevalence of anemia

The prevalence of anemia among the study population was 64.8% (95% CI: 60–69). The mean hemoglobin of the study participants was 11.34 ± 1.63 g/dL. Out of the 276 anemic women, 55.07% of them were mildly anemic, 35.15% were moderately anemic, and 9.78% of them were severely anemic.

Factors influencing prevalence of anemia

The contributing factors associated with anemia in descending order as revealed by multivariate logistic regression [Table 3] were intake of green leafy vegetables (GLVs) (<2 times weekly), socioeconomic status (class IV and V), illiteracy, birth spacing <2 years between last two pregnancies, excessive menstrual bleeding, and inadequate knowledge regarding anemia. Factors such as open air defecation habit, history of passing worms in stool, not washing hands with soap after defecation, attained menarche <14 years of age, irregular menstrual cycle, following vegetarian diet, those having the habit of drinking coffee along with food, those having low BMI, intake of whole grains and mutton less frequently, which were found to have a significant association on univariate analysis did not show a significant association with anemia in the multivariate logistic regression analysis. There was no significant association between anemia and type of family, habit of barefoot walking, marital status, age at marriage <18 years, age at first child birth <19 years, having more than two children, current IUD usage, and other iron-rich foods.

Discussion

Anemia is largely preventable and easily treatable if the local determinants are identified. The WHO and UNICEF therefore reemphasize the urgent need to combat anemia and stress the importance of recognizing its multifactorial etiology for developing effective control programs.[11] Our study showed the prevalence of anemia to be 65% (95% CI: 60%–69%), which is 10% higher compared to NFHS-4[3] survey. According to the WHO, if the prevalence of anemia at community levels is more than 40%,[1] it is considered a problem of high magnitude.

Out of the 276 anemic women, majority of them were mild to moderately anemic and only few of them were severely anemic, which was consistent with that of other studies.[12,13] Hence, its prevention is essential for child

Table 3: Multivariate logistic regression analysis of factors influencing anemia

Variable	AOR	CI	*P*
Education			
Illiterate	3.09	1.09-8.81	<0.05
Literate	1		
Socioeconomic status			
Classes IV, V	1.38	1.93-5.84	<0.001
Classes I, II, III	1		
Practice open-air defecation			
Present	1.84	0.97-5.57	0.09
Absent	1		
History of passing worms in stools			
Present	2.83	0.88-9.04	0.08
Absent	1		
Washing hands with soap after defecation			
Present	0.89	0.44-1.21	0.75
Absent	1		
Menarche attained (years)			
<14	2.35	0.78-4.22	0.09
≥14	1		
Birth spacing (<2 years)			
Present	2.49	1.19-5.25	<0.05
Absent	1		
Regularity of menstrual cycle			
Regular	0.78	0.42-1.47	0.44
Irregular	1		
Excessive menstrual bleeding			
Present	2.27	1.09-4.76	<0.05
Absent	1		
Knowledge regarding anemia			
Inadequate	2.03	1.19-3.44	<0.05
adequate	1		
Nature of diet			
Vegetarian	2.57	0.89-6.29	0.10
Mixed	1		
Taking coffee along with food			
Yes	1.60	0.92-2.78	0.09
No	1		
BMI (Kg/m^2)			
<25	2.46	0.94-5.01	0.57
≥25	1		
Intake of whole grains (times/week)			
<2	2.36	0.89-8.44	0.102
≥2	1		
Intake of GLVs (times/week)			
<2	3.65	2.17-6.12	<0.001
≥2	1		
Intake of heme iron (time/week) (meat, poultry, and fish)			
<1	2.79	0.88-8.91	0.08
≥1	1		

AOR=Adjusted odds ratio, CI=Confidence interval, BMI=Body mass index, GLVs=Green leafy vegetables

survival and safe motherhood.[14] The identified anemic women were referred to the Rural Health Training Centre for further management. Data from the National Nutritional Monitoring Bureau Surveys[5] show that Indian women have a sustained low intake of iron, which may be due to various reasons such as economic, social, and lack of empowerment.

In our study, we identified six factors which had a significant association with anemia. The mean

hemoglobin level of significant predictors for anemia in the current study is shown in Figure 3. The first risk factor which was found to have statistical significant association with higher risk of anaemia was the intake of GLV. The study participants who took GLV less than twice a week had three times higher risk of becoming anaemic and this finding was also observed in other studies.[15,16] GLV is a good source of iron, and inadequate intake of GLV is one of the important causes of anemia. It is followed by socioeconomic status which had an inverse relationship with anemia. This finding is consistent with that of other studies.[17-19] Poor socioeconomic status is known to be associated with a number of factors such as high parity; short birth interval; poor diet, both in quantity and quality; lack of awareness regarding health and nutrition; a high rate of infectious diseases and parasitic infestations; as well as limited access to medical attention.[12]

The current study reveals the fact that illiterates have about three times higher risk of becoming anemic. Similar findings have been observed in other studies.[20,21] Better educated women enjoy the privilege of higher paying jobs and live in better environment, which will have a positive influence on their nutritional status. In our study, another factor associated with anemia is birth spacing, which is a similar observation in other studies also.[22,23] Repeated pregnancies at short interval deplete the iron stores, thereby leading to anemia, which depict the need for contraception education.[24]

Excessive menstrual bleeding is said to be present when a woman has long duration of menstruation (>5 days) or if there is passage of clots during the menstrual period. In the present study, those with excessive menstrual bleeding had two times higher risk of anemia. Similar findings have been observed in other studies.[19,23,25] We also observed that there was a significant association between inadequate knowledge regarding anemia and risk of anemia, which was similar to the findings of other studies.[26,27] Due to lack of adequate knowledge on anemic causes, ill effects, and preventive measures, incorrect choice of food can lead to nutritional deficiency,

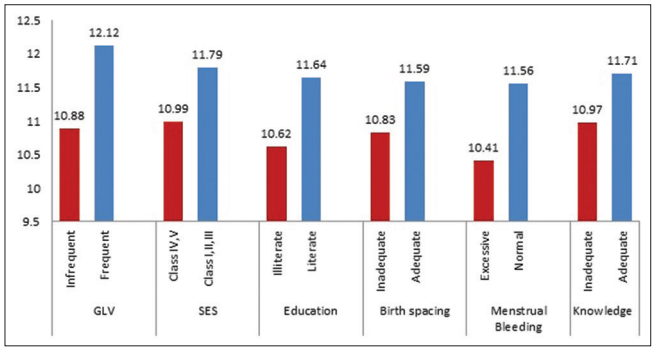


Figure 3: Mean hemoglobin levels of significant predictors for anemia in the current study

making them more prone to become anemic. No significant association was found between hemoglobin concentrations and anthropometric measurements, which was similar to that of another study.[28] Other demographic, reproductive, and socioenvironmental factors did not show a significant association with anemia.

India was the first developing country to commence a National Anaemia Prophylaxis Programme during the 4th five-year plan in 1970, providing iron and folic acid supplementation to all pregnant women and children with an aim to reduce the prevalence of anemia to below 25%.[29] The current study highlights that anemia still remains a public health issue of great magnitude among nonpregnant reproductive-aged women, even after nearly 50 years into the program. Women of childbearing age are at risk of anemia not only throughout their reproductive years but also during pregnancy. Measures to prevent and control anemia so far have largely been ineffective, hence strategies to combat anemia, therefore, should address all the causal factors and focusing our thrust on iron supplementation alone may not be able to provide the much-desired effect because the pathogenesis of anemia is multifactorial. First, the system of periodic screening for anemia among women of childbearing age should be developed and it should be considered one of the important primary healthcare activities at the center. Second, an IEC program to change knowledge, attitudes, household dietary practices, and kitchen garden activities is required to effect positive changes in consumption patterns, thereby improving the nutritional status. Economic empowerment of women can be attempted by organizing the women to form a self help group in order to carry out small scale business and thereby paving a way for small income. Optimal birth spacing (Minimum two years) should be enforced and the reproductive aged women should be made assertive to it.

Further studies are needed to determine the specific types of anemia and the quantum of dietary intake of iron, thereby giving a better insight into the situation.

Limitation

The study has few limitations. Because it is a cross-sectional study, cause–effect relationship cannot be determined. Dietary history was limited to self-reported food frequency, hence the quantum of dietary intake of iron could not be ascertained and confirmation of worm infestation by stool examination was not possible due to operational difficulty.

Conclusion

Anemia has been recognized as a public health problem for many years, but only little progress has been made in its control. The best predictors of anemia in descending order are intake of GLVs (<2 times weekly),

socioeconomic status (class IV and V by the Modified Prasad classification), illiteracy, birth spacing <2 years between last two pregnancies, excessive menstrual bleeding, and inadequate knowledge regarding anemia. Hence, to alleviate anemia burden, measures such as economic empowerment, improvement in awareness regarding anemia, dietary practice of taking frequent GLVs and making it easily available, and contraceptive education should be provided by an integrated multifactorial and multi-sectorial approach.

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Conflicts of interest

There are no conflicts of interest.

References

1. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr 2009;12:444-54.
2. Kaur K. Anaemia 'A Silent Killer' among women in India: Present scenario. Eur J Zoolog Res 2014;3:32-6.
3. International Institute for Population Sciences (IIPS) and ICF National Family Health Survey (NFHS-4), 2015–16: India. Available from: http://mohfwnicin/nfhsfactsheethtm. [Last accessed on 2020 Sep 02].
4. DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the World. World Health Stat Q 1985;38:302-16.
5. National Nutrition Monitoring Bureau. Hyderabad NNMB. Diet & nutritional status of population and prevalence of hypertension among adults in rural areas. National Institute of Nutrition, 2006. Available from: https://www.nin.res.in/downloads /NNMBReport06Nov20.pdf. [Last accessed on 2021 Mar 26].
6. USAID. United States Agency International Development. Geneva, Switzerland: Health Report, USAID; 2013.
7. Gopalan C, Rama S, Balasubramanian S. Nutritive Value of Indian. Foods-Revised ed. Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research; 1989. p. 50.
8. World Health Organization. Physical Status: The Use of and Interpretation of Anthropometry, Report of a WHO Expert Committee. Geneva, Switzerland: World Health Organization; 1995.
9. Johns W, Lewis SM. Primary health screening by haemoglobinometry in a tropical community. Bull World Health Organ 1989;67:627.
10. Akhtar K, Sherwani RK, Rahman K, Hasan J, Shahid M. HemoCue photometer: A better alternative of hemoglobin estimation in blood donors? Indian J Hematol Blood Transfus 2008;24:170-2.
11. World Health Organization. United Nations Children's Fund (2004) Focusing on Anaemia: Towards an Integrated Approach for Effective Anaemia Control. Joint Statement by the World Health Organization and the United Nations Children's Fund Geneva: World Health Organization; 2011.
12. Yip R. Forging effective strategies to combat iron deficiency-plenary papers-prevention and control of Iron deficiency: Policy and Strategy Issues. J Nutr 2002;132:802S.
13. Thankachan P, Muthayya S, Walczyk T, Kurpad AV, Hurrell RF. An analysis of the etiology of anemia and iron deficiency in young women of low socioeconomic status in Bangalore, India. Food Nutr Bull 2007;28:328-36.
14. Ramachandran P. Food fortification: A public health approach to bridge the gap between requirement and intake of micronutrients. Proceed Indian Natl Sci Acad 2018;84:913-22.
15. Rao S, Joshi S, Bhide P, Puranik B, Kanade A. Social dimensions related to anaemia among women of childbearing age from rural India. Public Health Nutr 2011;14:365-72.
16. Ramachandran P. Nutrition in Pregnancy. In: Gopalan C, Kaur S, editors. Women and nutrition in India, Special Publication No. 5. New Delhi: Nutrition Foundation of India; 1989. p. 153-93. 19.
17. Balarajan YS, Fawzi WW, Subramanian SV. Changing patterns of social inequalities in anaemia among women in India: Cross-sectional study using nationally representative data. BMJ Open 2013;3:e002233.
18. Rajaratnam J, Abel R, Ganesan C, Jayaseelan SA. Maternal anaemia: A persistent problem in rural Tamil Nadu. Natl Med J India 2000;13:242-5.
19. Venkatachalam J, Muthu R, Singh Z, Madhanraj K, Sathya G. Determinants of anemia among women in the age group of 11-49-A case control study in a rural area of Kancheepuram district, Tamil Nadu. EXCEL Int J Multidisci Manage Stud 2014;4:218-23.
20. Gebremedhin S, Enquselassie F. Correlates of anemia among women of reproductive age in Ethiopia: Evidence from Ethiopian DHS 2005. Ethiop J Health Dev 2011;25:22-30.
21. Ngnie-Teta I, Kuate-Defo B, Receveur O. Multilevel modelling of sociodemographic predictors of various levels of anaemia among women in Mali. Public Health Nutr 2009;12:1462-9.
22. Swarnlatha N. Prevalence of anaemia and its socio demographic determinants among pregnant women attending government maternity hospital, Tirupati, AP. Sudan J Public Health 2013;8:104-6.
23. Alquaiz AM, Gad Mohamed A, Khoja TA, Alsharif A, Shaikh SA, Al Mane H, *et al*. Prevalence of anemia and associated factors in child bearing age women in Riyadh, Saudi Arabia. J Nutr Metab 2013;1-7. https://doi.org/10.1155/2013/636585.
24. Asghari S, Mohammadzadegan-Tabrizi R, Rafraf M, Sarbakhsh P, Babaie J. Prevalence and predictors of iron-deficiency anemia: Women's health perspective at reproductive age in the suburb of dried Urmia Lake, Northwest of Iran. J Educ Health Promot 2020;9:332.
25. Kaur S, Deshmukh P, Garg B. Epidemiological correlates of nutritional anemia in adolescent girls of rural Wardha. Indian J Commun Med 2006;31:255-8.
26. Ahmad N, Kalakoti P, Bano R, Syed MMA. The prevalence of anaemia and associated factors in pregnant women in a rural Indian community. AMJ 2010; 3:276-280. Doi 10.4066/ AMJ.2010.286.
27. Mamta K, Vibha B. Anaemia: Impact assessment of dietary intervention on iron status of college going girls. Food Sci Res J 2011;2:76-9.
28. Adib Rad H, Sefidgar SA, Tamadoni A, Sedaghat S, Bakouei F, Bijani A, *et al*. Obesity and iron-deficiency anemia in women of reproductive age in northern Iran. J Educ Health Promot 2019;8:115.
29. Agarwal DK, Agarwal KN, Roychoudhry S. Targets in National Anemia Prophylaxis Programme for pregnant women. Indian Pediatr 1988;25:319-22.

Original Article

A study on contraceptive prevalence rate and factors influencing it in a rural area of Coimbatore, South India

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Abstract

Introduction: India is the first country to implement National Family Program in 1952. Acceptance of contraceptive methods is influenced by various factors at the individual, family, and community level with their roots in the socioeconomic and cultural milieu of Indian society. This study was carried to find the contraceptive prevalence rate among eligible couples as well as to find the factors influencing it in a rural area of Coimbatore. **Methodology:** The study was carried out using the data extracted from the family health survey conducted by trained field staff for the purpose of furnishing details regarding people residing in the field practice area of Rural Health Training Centre (RHTC), Vedapatti located in Coimbatore district after obtaining Institutional Human Ethical clearance. All eligible couples were included for the study. Contraceptive prevalence is expressed in percentage with 95% Confidence interval. Univariate analysis (Chi-square test) was done to find the association between contraceptive acceptance and independent variables. Strength of association is determined by odds ratio with 95% Confidence Interval (CI). $P < 0.05$ was considered statistically significant. **Results:** Contraceptive Prevalence Rate among eligible couples was found to be 75% (95% CI: 73.6–76.4). Most commonly used method among the study participants was tubectomy (81.6%), followed by condoms (11.4%), intrauterine devices (6.3%), and oral contraceptive pills (0.7%). Higher age of the women, religion, educational status of the women, socioeconomic status, working status of the women, number of living children, age at the time of marriage, and age at the time of first child birth were found to have statistical significant association with higher usage of contraception. **Conclusion:** The contraceptive prevalence rate was found to be higher in this study population and multiple factors were influencing the contraception usage among the eligible couples.

Keywords: Contraceptive prevalence, eligible couples, rural population

Introduction

Population explosion has been the major problem of India since Independence.[1] Considering the consequences, population stabilization has been identified as an essential prerequisite for the economic development of the nation resulting in improved quality of life.[2] India became the first country in the world to formulate National Family Planning Program in 1952, with the objective of "reducing birth rate to the extent necessary to stabilize the population at a level consistent with requirement of national economy."[2,3] As per the projections by United Nations, India will become the most populous country by the year 2045. One of the important components of family planning program management is to assess the potential demand for contraceptive services so as to mitigate the adverse impact of population growth.[4] Family planning can reduce maternal mortality by reducing the number of pregnancies, abortions, and the proportion of births at high risk.[5]

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Many empirical studies have shown the acceptance of contraceptive methods is influenced by various factors operating at the individual, family, and community level with their roots in the socioeconomic and cultural milieu of Indian society.[6] The contraceptive prevalence rate is "the percentage of women of reproductive age (15–49 years) who are married or in a union: Who are currently using, or whose sexual partner is currently using, at least one contraceptive method, regardless of the method used".[7,8] Contraceptive prevalence rate serves as a proxy measure of access to reproductive health services and is an indicator of health, population, development, and women's empowerment[7,8] that are essential for meeting many of the Sustainable Development Goals (SDG), especially the child mortality and maternal health related goals.[8]

If the population has ready access to good quality services at affordable cost, it will be possible for them to meet all their needs, achieve the desired family size, and enable the country to achieve population stabilization rapidly.[1,9] Majority of India's population (about 70%) live in the rural communities[9] but majority of the health facilities are concentrated in the urban localities leading to a disparity in access to health care. Contraceptive use is influenced by a wide range of factors including demographic, social, and economic characteristics.

There is paucity of community-based rural studies with regard to contraceptive usage. Identifying the prevalence of contraceptive usage and the local determinants could help to plan strategy and make necessary recommendations that would help improve utilization of family planning services. Hence, this study was carried out to determine the contraceptive prevalence rate among eligible couples as well as to study the association of selected social, economic and demographic factors with contraceptive usage in a rural area of Coimbatore.

Methodology

The study was carried in accordance with the ethical standards of PSGIMSR Institutional Human Ethics Committee and Ethical Principles for Medical Research was followed. The Ethics committee approval was obtained prior to carrying out the study. Date of approval : 18/12/2019. The Institute permission was obtained prior to carrying out the study. In the current study, the data were extracted from the family health survey conducted by trained field staff for the purpose of furnishing details regarding people residing in the field practice area of Rural Health Training Centre (RHTC), Vedapatti, a rural area of Tamil Nadu located in Coimbatore district. The RHTC caters to 14 villages with a population of 25,886.

Sample size was calculated based on NFHS IV report- Contraceptive Prevalence Rate of rural Tamil Nadu was 52.3%.[10] Using the formula, $n = 4\,pq/d^2$ where P is 52, q is 100-52, that is, q = 48 and d is the relative precision (5% of p), the sample size required was estimated to be 1,477. Though the required sample size was only 1,477, all 4,438 eligible couples in the field practice area were included for the current study and their data was analyzed.

Various demographic, social, and economic factors like age of women, number of children, age at marriage, age at first child birth, religion, caste, family type, educational status of husband and wife, working status of women, socioeconomic status classification (based on Modified Prasad classification - Class I – ≥Rs. 6,414; Class II – Rs. 3,207–6,413; Class III – Rs. 1,924–3,206; Class IV – Rs. 962–1,923, and Class V - ≤Rs. 961) were included in the study.

Statistical analysis

Data was entered in Microsoft excel and analyzed in SPSS 20.0 version Univariate analysis (Chi-square test) was used to find out the association between the independent variables and the dependent variable. Odds ratio was calculated with 95% Confidence Interval (CI) to find the strength of association. $P < 0.05$ was considered as statistically significant.

Results

Total number of eligible couples enrolled in the study was 4,438.

The demographic profile of the participants is shown in the Table 1. Majority of the participants (41.3%) were in the age group of 35–49 years, 96.3% of women were Hindus. Majority of married women (70.4%) were living in nuclear families, 64% were

Table 1: Demographic profile of the study population

Variable	Number (*n*=4438)	Percentage
Age of Women (completed years)		
15-24	1196	27.0
25-34	1408	31.7
35-49	1834	41.3
Religion		
Hindu	4276	96.3
Christian	133	3
Muslim	29	0.7
Caste		
General	1389	31.3
OBC	2926	65.9
SC/ST	123	2.8
Family Type		
Nuclear	3125	70.4
Extended	1313	29.6
Number of Living Children		
No child	443	10
One child	777	17.5
Two child	2841	64
≥3 children	377	8.5
Age at the Time of Marriage		
<18 years	1152	26
≥18 years	3286	74
Age at the Time of First Child		
<18 years	957	21.6
≥18 years	3481	78.4

having two children, 26% of the women in the study population got married before completion of 18 years and 21.6% women delivered a baby before completion of 18 years.

Table 2 shows that most of the eligible couples were educated up to middle school. Female literacy rate was 84.8%, whereas male literacy rate was 87.3%. Higher proportion (37.4%) belonged to Class IV socioeconomic class and 31.4% women were employed with salary.

Out of 4,438 eligible couples studied, 3,328 were current users of contraception at the time of the survey. Hence, the Contraceptive Prevalence Rate was 75% (95% CI: 73.6–76.4%) in this study population. Table 3 shows the various family planning methods adopted by the eligible couples. Most common preferred method was tubectomy (81.6%), followed by condoms (11.4%), intrauterine devices (IUDs) (6.3%), and oral contraceptive pills (OCPs) (0.7%).

Table 2: Education status and socio-economic class of the study participants

Variable	Number (*n*=4438)	Percentage
Educational status of Women		
Illiterate	675	15.2
Primary	698	15.7
Middle	1237	27.9
High school	688	15.5
Higher secondary	543	12.2
Graduate	597	13.5
Educational status of Husband		
Illiterate	565	12.7
Primary	682	15.4
Middle	1644	37.0
High school	566	12.8
Higher secondary	311	7.0
Graduate	670	15.1
Socio Economic Status (Modified Prasad Classification)		
Class I	309	7.0
Class II	798	18.0
Class III	1333	30.0
Class IV	1660	37.4
Class V	338	7.6
Working status of women		
Working	1394	31.4
Not working	3044	68.6

Table 3: Various family planning methods adopted by eligible couples

Family Planning Method Adopted	Number (Percentage)
Tubectomy	2717 (81.64)
Condom	378 (11.36)
Intra Uterine Device (IUD)	210 (6.31)
Oral Contraceptive Pill (OCP)	22 (0.66)
Vasectomy	1 (0.03)
Total	3328 (100)

The demographic factors influencing the contraception acceptance is shown in Table 4. The significant predictors for contraceptive acceptance are higher age of women, those who are having two living children, women who are married after 19 years of age, and those who had given birth to first child after 19 years of age.

The social and economic factors influencing the contraception acceptance is shown in Table 5. Factors like higher educational level of women, working women, and higher socioeconomic class are significantly associated with adoption of any of the contraceptive method.

Discussion

Contraceptive use and unmet need for family planning are keys to understand profound changes in fertility pattern and also helps to improve reproductive health. By understanding the concept of unmet needs, the gap between women reproductive intentions and contraceptive behavior can be understood. In this study, Contraceptive Prevalence Rate among eligible couples was found to be 75%, which is higher than studies reported by NFHS IV (52.3%),[10] Patel AA (55.3%),[11] Singh *et al.* (68.4%),[1] Makade *et al.* (68.4%),[12] and Gupta *et al.* (71.1%).[13] The contraceptive acceptance is quite high among the study population.

Most common method followed by the eligible couples was permanent method, that is, tubectomy (81.6%) which is higher than other studies by Shree *et al.* (70.9%),[14] Patel AA, *et al.* (33.7%),[11] and Nanda *et al.* (30.5%).[15] In our study, 11.4% couples use condoms which is similar to study done by Chandra *et al.* (11.7%)[3] and higher than Nanda *et al.* (5.1%).[15] Usage of IUDs was 6.3% which is lower than study done by Nanda *et al.* (45.8%)[15] and Shree *et al.* (11.3%).[14] Another important finding as that only one male individual has undergone vasectomy. This clearly portrays that even though permanent sterilization method in male (i.e., vasectomy) is an easier procedure and has lesser postoperative symptoms the responsibility for birth control still traditionally rests with the woman since she has to undergo the physical and psychological burden of unplanned pregnancy.

In our study, contraception usage showed significant association with higher age of the women, religion, education of the women, higher socioeconomic class, working status of the women, presence of two living children, age at the time of marriage as well as first child birth more than 18 years of age. There was no significant association with type of family, educational status of husband and caste.

In this study, the usage of contraception was significantly higher among those who were aged 35–49 years. The contraceptive acceptance was three times higher than those of aged 15–24 years, probably these couples would have completed their family. Similar findings were reported by Patel AA,[11] Valekar SS, *et al.*,[16] and Prateek *et al.*[2] wherein women of age group 35–49 years are using better contraception. The

Table 4: Association of Demographic variables with adoption of family planning methods

Variables	Adoption of Family Planning method		OR	95% CI	*P*
	Yes (*n*=3328) Total (%)	No (*n*=1110) Total (%)			
Age of women completed years					
15-24	742 (62)	454 (38)	1		
25-34	1048 (74.4)	360 (25.6)	1.78	1.5-2.11	<0.001
35-49	1538 (83.9)	296 (16.1)	3.18	2.67-3.78	<0.001
Religion					
Hindu	3220 (75.3)	1056 (24.7)	1		
Muslim	10 (34.5)	19 (65.5)	0.17	0.07-0.39	<0.001
Christian	98 (73.7)	35 (26.3)	0.92	0.61-1.39	0.67
Caste					
SC/ST	90 (73.2)	33 (26.8)	1		
OBC	2192 (74.9)	734 (25.1)	1.10	0.71-1.67	0.66
General	1046 (75.3)	343 (24.7)	1.12	0.72-1.73	0.59
Family Type					
Nuclear	2349 (75.2)	776 (24.8)	1		
Extended	979 (74.6)	334 (25.4)	0.97	0.83-1.13	0.67
Number of Living Children					
≥3 child	343 (91)	34 (9)	1		
2 child	2707 (95.3)	134 (4.7)	2.0	1.33-3.01	<0.001
1 child	275 (35.4)	502 (64.6)	0.05	0.04-0.08	<0.001
No child	3 (0.7)	440 (99.3)	0.0007	0.0002-0.0022	<0.001
Age at the Time of Marriage (Women)					
<18 years	2559 (77.9)	727 (22.1)	1		
≥18 years	769 (66.8)	383 (33.2)	0.57	0.49-0.66	<0.001
Age at the Time of First Child Birth (Women)					
<18 years	2854 (82)	627 (18)	1		
≥18 years	474 (49.5)	483 (50.5)	0.22	0.18-0.25	<0.001

contraceptive usage was significantly low (34.5%) among Muslims than Hindus (75.3%) but the proportion of Muslims was low in this study. This result was consistent with other studies like Singh AK, *et al.*[1] and Chandra *et al.*[3] The reason for lower usage of contraception among the Muslim couples could be because of religious and cultural beliefs on contraception. Hence, religion still plays a predictor role on deciding the choice of contraception.

Education of women leads to a greater awareness and a significant effect on family planning behavior and use of contraception. Our study revealed significantly higher usage of contraception among literates when compared with the illiterates. The literates will be able to understand the pros of contraceptive usage which could have brought the behavioral change. This result was consistent with studies done by Singh AK, *et al.*[1] Patel AA,[11] Gupta *et al.*,[13] Kumar N, *et al.*,[17] and Oppong FB, *et al.*[18] Improving education status can pave way to delay a women's marriage age, she will be able to seek a job and can help to change the attitude toward birth spacing and family size and empowers women to make a better decision.

In our study, contraceptive use was significantly higher among working women as they are more likely to be educated and their socioeconomic status is supposed to be better than the non-working women. Working women are likely to be more knowledgeable; would have wide exposure to media and social contacts which can influence their contraceptive behavior. This finding was similar to study done by Mukherjee *et al.*[19]

In the present study, it was found that as the socioeconomic status decreases, the usage of contraception also decreases. Contraception usage is significantly higher among class I when compared to Class V. This may be due to the fact that the higher socioeconomic status individuals have better accessibility and availability of contraception and they have better awareness regarding contraception. Similar finding was reported by Prateek *et al.*[2]

Number of living children had a significant impact on contraceptive usage. Those women who had completed their family by getting at least two children (small family norm) are using contraception higher than others. This may be due to the fact that many couples decided to complete their family with two children and also female sterilization (after institutional deliveries) was emphasized for women after they bear two children. Government policies and advisory of two child norm and benefits announced could have favored contraception acceptance by these couples. This finding was consistent with study done by Singh *et al.*[1] Shree *et al.*[14] Valekar SS, *et al.*,[16] and Gothwal M, *et al.*[20] Contrastingly, it was also found contraceptive usage was higher among those having two children compared with couples having three or more

Table 5: Association of Social and Economic variables with adoption of family planning methods

Variables	Adoption of Family Planning method		OR	95% CI	*P*
	Yes (*n*=3328) Total (%)	No (*n*=1110) Total (%)			
Educational status of Women					
Illiterate	445 (65.9)	230 (34.1)	1		
Primary	529 (75.8)	169 (24.2)	1.62	1.27-2.06	<0.01
Middle	966 (78.1)	271 (21.9)	1.84	1.49-2.28	<0.01
High school	569 (82.7)	119 (17.3)	2.47	1.9-3.21	<0.01
Higher secondary	405 (74.6)	138 (25.4)	1.52	1.17-1.96	<0.01
Graduate	414 (69.3)	183 (30.7)	1.17	0.92-1.49	0.19
Educational status of Men					
Illiterate	434 (76.8)	131 (23.2)	1		
Primary	501 (73.5)	181 (26.5)	0.84	0.64-1.09	0.17
Middle	1241 (75.5)	403 (24.5)	0.93	0.74-1.17	0.5
High school	431 (76.1)	135 (23.9)	0.96	0.73-1.28	0.79
Higher secondary	225 (72.3)	86 (27.7)	0.79	0.57-1.10	0.14
Graduate	496 (74)	174 (26)	0.86	0.66-1.13	0.26
Socio Economic Status					
Class I	247 (79.9)	62 (20.1)	1		
Class II	608 (76.2)	190 (23.8)	0.80	0.57-1.12	0.18
Class III	998 (74.9)	335 (25.1)	0.75	0.54-1.02	0.06
Class IV	1261 (76)	399 (24)	0.79	0.58-1.08	0.13
Class V	214 (63.3)	124 (36.7)	0.43	0.30-0.63	<0.001
Working status of women					
Not Working	2190 (71.9)	854 (20.1)	1		
Working	1138 (81.6)	256 (18.4)	1.73	1.48-2.03	<0.01

children. This could be due to the fact the need for contraception is not well appreciated by these couples.

Age at the time of marriage and age at time of first child birth also showed significant association with contraceptive usage. Those women who got married or gave birth before the age of 18 years used less contraception when compared with those who got married or gave birth to child after the age of 18 years. This may be because the former individuals are less aware of the contraceptive methods and their maturity levels are not favorable. Hence delaying the age of marriage of women as well as delaying the age of first child birth more than 18 years could have a positive impact on contraceptive acceptance. But studies by Prateek *et al.*[2] and Valekar SS, *et al.*[16] did not show significant association between contraception usage and age at marriage or age at first child birth.

Our study did not reveal association of contraception use and educational status of husband. This result is consistent with study done by Valekar SS, *et al.*[16] But studies by Prateek *et al.*[2] and Shree *et al.*[14] has shown association between educational status of husband and contraception usage. The current study did not show significant association of contraception use with caste and type of family, but a study by Nanda *et al.*[15] has shown significant association of contraception with caste and studies conducted by Singh *et al.*[1] and Valekar SS, *et al.*[16] has shown individuals in nuclear family are accepting the family planning methods higher than those in extended family.

This study has few limitations because other confounders which can influence the contraceptive prevalence like knowledge about contraception, sex preference, female autonomy, and unmet needs of contraception were not studied. Despite the data being retrieved from the routine family health survey, the reliability of the data is not a major concern as it was collected by trained field workers for purpose of family health survey.

Conclusion and Recommendation

From our study, it is obvious that the contraceptive prevalence was high among the study population and epidemiological factors like higher age of the women, religion, education of the women, higher socioeconomic class, working status of the women, presence of two living children, age at the time of marriage, as well as first child birth more than 18 years of age are influencing the contraception acceptance. Measures to sustain the gains of the family planning program should be given priority to sustain population stabilization and to achieve Net Reproductive Rate of one. Hence, on the first-hand targeted health awareness among those who are not using contraception could help to bring about a behavioral change so contraception usage can be maximized. Secondly, outreach contraceptive services should be intensified and expanded for easy access without fear of stigma by the society. Thirdly, reproductive health programs and policies should adequately involve men, by encouraging and motivation to adopt vasectomy as an easy method of family planning.

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Conflicts of interest

There are no conflicts of interest.

References

1. Arvind K, Alok C. Contraceptive Use among Rural Women: A Micro-Regional Study. Asia-Pac. J. Soc. Sci 2012;4:151-73.
2. Prateek S, Saurabh R. Contraceptive practices adopted by women attending an urban health centre. Afr Health Sci 2012;12:416-21.
3. Chandra R, Kandpal S, Negi K. Epidemiological correlates of contraceptive prevalence in rural population of Dehradun district. Indian J Community Med 2005;30:60-2.
4. Evaluation study on family programme [Last accessed 2014 Jul 11]. Available from: http://planningcommission.nic.in/reports/peoreport/cmpdmpeo/volume1/135.pdf.
5. Elizabeth L, Susheela S, Chowdhury, Sadia A. Fertility Regulation Behaviors and Their Costs: Contraception and Unintended Pregnancies in Africa and Eastern Europe & Central Asia. HNP discussion paper series. World Bank, Washington, DC. 2007; © World Bank. https://openknowledge.worldbank.org/handle/10986/13783 License: CC BY 3.0 IGO.
6. Rao A, Somayajulu U. Factors responsible for family planning acceptance with single child: Findings from a study in Karnataka. Demography India. 1999;28:65 73.
7. Organization WH. Sexual and Reproductive Health: Contraceptive Prevalence. Geneva: WHO; 2013.
8. UNDP. Indicators for monitoring the millennium development goals definitions rationale concepts and sources. United Nations; New York, 2003. Available From: https://docplayer.net/21179513-Indicators-for-monitoringthe-millennium-development-goals-definitions-rationale-conceptsand-sources-united-nations.html. [Last accessed 2020 Sep 02].
9. Barman S. Socio-economic and demographic differentials of contraceptive usage in Indian states: A study based on NFHS data. J Hum Ecol 2013;42:53-68.
10. International Institute for Population Sciences (IIPS) and ICF National Family Health Survey (NFHS-4), 2015–16: India Available from: http://mohfwnicin/nfhsfactsheethtm. [Last accessed 2020 Sep 2].
11. Anil A P. Knowledge and practices of contraception among married females of rural Tamil Nadu. Asian J Biomed Pharm Sci. 2015;5:1-4.
12. Makade KG, Padhyegurjar M, Padhyegurjar SB, Kulkarni R. Study of contraceptive use among married women in a slum in Mumbai. Natl J Community Med 2012;3:40-3.
13. Gupta RK, Singh P, Gupta R, Akhtar N, Gupta C, Sharma P. Contraceptive use: Its prevalence, awareness, practices and determinants in a rural population of Northern India. Int J Med Sci Public Health 2017;6:1543-8.
14. Shree V, Prasad R, Kumar S, Sinha S, Choudhary SK. Factors for non-acceptance of contraceptive methods amongst married women of reproductive age group in rural Patna. Int J Community Med Public Health 2017;4:1882-7.
15. Nanda J, Adak DK, Bharati P. Contraceptive practices among adolescent married women in Tamil Nadu, India. Asian Pac J Trop Dis 2011;1:137-41.
16. Valekar S, Chawla P, Tukaram H, Fernandez K, Kalra K. The socio-demographic determinants of contraceptive use among rural women in reproductive age group. J Women's Health Care 2017;6:1.
17. Kumar N, Das V, Agarwal A, Pandey A, Agrawal S, Care P. Pregnancy in women with previous two healthy children, associated factors, and acceptability of contraception among these women: A questionnaire-based, cross-sectional KAP study in world's second most populated country advocating two-child norm. J Family Med Prim Care 2020;9:2899-2906.
18. Oppong FB, Agbedra SY, Adomah AA, Amenyaglo S, Arhin-Wiredu K, Afari-Asiedu S, *et al.* Determinants of contraceptive use among sexually active unmarried adolescent girls and young women aged 15–24 years in Ghana: A nationally representative cross-sectional study. BMJ Open 2021;11:e043890.
19. Mukherjee BN. A comparison of the results of family planning KAP surveys in Haryana and Tamil Nadu, India. Stud Fam Plann 1974;5:224-31.
20. Gothwal M, Tak A, Aggarwal L, Rathore AS, Singh P, Yadav G, *et al.* A study of knowledge, attitude, and practice of contraception among nursing staff in All India Institute of Medical Sciences, Jodhpur, Rajasthan. J Family Med Prim Care 2020;9:706-10.

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Original Article

A Primary Care Approach for Oral Health Promotion among HIV Patients: An Interventional Study

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Abstract

Background: HIV-positive patients are at a greater risk for developing oral health problems, making them potentially susceptible to nutritional deficiencies, decreased adherence to treatment, and failure of highly active antiretroviral therapy. Their oral health needs are unmet due to a lack of awareness, social stigma, and other factors. The primary care workers (PCWs) are the first point of contact for health issues of these patients. **Aim:** The aim of the study was to assess the effectiveness of the educational intervention for the PCWs on oral health. **Materials and Methods:** This interventional pilot study was conducted at two residential centers for HIV patients in Mangalore. The participants included 15 PCWs working at these centers. An educational module was prepared as the intervention that included dental conditions that could compromise oral health, oral manifestations of HIV, and oral health care. A structured questionnaire was administered to the PCWs before the intervention, immediately after the intervention, and at 3 months. The PCWs were required to routinely examine the HIV patients for any dental or oral health problems and refer them to the dental hospital for treatment. The Statistical Package for the Social Sciences 20.0 software (Chicago. IL, USA) was used for analysis. $p < 0.05$ was considered statistically significant. Repeated measures analysis of variance and Chi-squared test were used for data analysis. **Results:** 73% of the caretakers had no training in oral health care. Analysis of the mean scores revealed a statistically significant improvement ($p < 0.05$) in their knowledge and skills from 2.53 ± 1.3 to 7.57 ± 1.2. There was also a statistically significant increase in the number of referrals to the dental hospital. **Conclusion:** Educating the PCWs showed positive outcomes enabling patients' access to regular professional oral health care, thus improving their quality of life.

Keywords: HIV, oral health, primary care workers

Introduction

HIV-related oral lesions are found in 30%–80% of HIV-infected individuals, and most often are the first clinical sign of the infection. While the presence of certain oral lesions might suggest an HIV infection, a few others might even signal the progression of the disease.[1] The oral manifestations of HIV are xerostomia, salivary gland disorders, oropharyngeal candidiasis, hairy leukoplakia, Kaposi's sarcoma, warts, recurrent aphthous ulcers, neutropenic ulcers, etc.[2] The dental conditions include dental caries, periodontal diseases, and tooth loss.[3,4] With the introduction of highly active antiretroviral therapy (HAART), there has been a significant decrease in the occurrence of the more severe types of oral lesions, but there is also an increase in frequency of the more common lesions such as candidiasis and periodontitis as compared to the noninfected individuals. Studies have shown that HIV patients are more susceptible to oral lesions and harmful dental conditions than otherwise healthy individuals.[5] These conditions are a risk factor for nutritional deficiencies as they affect mastication, swallowing, and other oral functions. They are also a potential barrier to adherence to treatment and have been shown to be associated with an increased risk of progression of HIV disease, severe immune deterioration that could lead to increased mortality.[6] These conditions have a physical, social, and psychological impact on the patients which is detrimental to their well-being and impairs their quality of life.[7] The oral

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and dental needs are unmet in HIV patients due to a lack of awareness on oral health and available services, late diagnosis, social stigma, and poverty.[8] Oral health is most often neglected as it is not given utmost priority. It is therefore vital that the oral health status of these patients is regularly monitored and treatment guaranteed.

Collaborations with various professions are the need of the hour in the current health care scenario to improve health outcomes. With regard to people living with HIV, an interprofessional teamwork has the potential to bring about a significant change in their overall health and well–being, leading to a better quality of life. The dental professionals primarily work to prevent and treat oral and dental diseases. However, the HIV patients might not be able to avail of their services due to their perceived barriers. The primary care workers (PCWs) play a crucial role by complementing the health-care system. They generally are at ideal positions and the first point of contact for health issues of patients and therefore can play a pivotal role in oral health promotion. The PCWs comprise doctors, nurses, pharmacists, counselors, and care takers who regularly tend to the patients. However, in order to facilitate oral health promotion, they themselves need to have adequate knowledge about the oral manifestations of HIV and its management. The primary objective of this study was to develop an oral health-care module and evaluate its effectiveness among the PCWs in promoting oral health among HIV patients. The secondary objective was to explore if this training model led to an increase in the number of patients seeking oral health care. Althrough this project, we strived to enable HIV patients overcome their barriers and ensure accessibility to oral health care by educating the PCWs.

Materials and Methods

This educational interventional study was carried out as a pilot initiative among PCWs of two HIV residential centers of Mangalore, housing 75 HIV-positive individuals. The PCW team included nurses, counselors, pharmacist, caretakers, and volunteers. The study was approved by the Institutional Human Ethics Committee (DCGI Reg. No. ECR/348/Inst/KA/2013). The study was explained to the participants, and written informed consent was obtained from them.

Module development and validation

A team comprising dental professionals (prosthodontists and dental public health Specialists) and physicians was formed to prepare the module for the intervention. The team together finalized the topics to be included in the module. Key areas included normal anatomy of oral cavity, general oral health, oral manifestations of HIV, early recognition of the lesions, harmful dental conditions, their management, documentation of cases, and diet. The module was tailor-made for ease of use for the PCWs that included illustrations using simple language. This was also translated to Kannada, which is the local language. The topics and content of the module were reviewed by five subject experts and a consensus was arrived at.

Health education of primary care workers and HIV patients

A training session was conducted for the PCWs by interactive lectures aided by video projector and demonstrations. A PowerPoint presentation was prepared to facilitate the intervention. It contained simplified text and images of lesions such as oropharyngeal candidiasis, linear gingival erythema, salivary gland enlargement, aphthous stomatits, oral hairy leukoplakia, and dental conditions such as dental caries, missing teeth, periodontitis, and dental fluorosis. They were also taught oral hygiene procedures and diet recommendations. The interventions were carried out both in English and in Kannada.

Following the training, the PCWs were expected to periodically conduct oral examinations of the patients, refer them for treatment, ensure that they are under the care of dental professionals, and also encourage them to follow dentist's recommendations. Referral cards were provided to the PCWs for referrals to the dentist.

A health education session was also conducted for the HIV-positive patients to enable them to perform self-examinations and identify the lesions in their oral cavity so as to report them to the PCWs.

Data collection and analysis

A preintervention baseline assessment was done for the PCWs, using a structured questionnaire. The questionnaire included sociodemographic information of the PCWs (age, gender, educational qualifications and employment status, years of experience, and past training in oral health), as well as their knowledge on oral health such as symptoms and clinical appearance of the lesions, preventive measures, and diet. There were eight questions on sociodemographic characteristics, nine questions on knowledge, and four questions on beliefs. The questionnaire was administered in English as well as Kannada to facilitate the workers who were not fluent in English. The PCWs were given an opportunity to clarify any doubts with the questionnaire. The participants completed the questionnaire in 30 min, following which the educational intervention was performed.

Posttest questionnaire was administered immediately after the intervention and after 3 months. Referrals were assessed by the number of patients visiting the dental hospital, confirmed by clinical data mentioned on the referral cards. Data were analyzed using the SPSS software, Version 20.0 (Chicago. IL, USA). The changes in the mean score pre- and post-intervention were assessed using the repeated measures analysis of variance.

Results

Fifteen PCWs completed the questionnaire. All of them were females between the age group of 20–65 years. A majority of them (80%) were educated beyond the 12th grade. Nearly 73% of the workers had not received any previous training on oral health care. All the PCWs believed that oral health

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is important for HIV patients. They also agreed that regular screening is necessary, and it is important to seek dental treatment for dental problems. However, 20% did not believe that poor oral health could compromise nutrition. The responses showed that they had poor baseline knowledge. The pretest showed a mean knowledge score of 2.5 ± 1.3. The mean score immediately after intervention was 5.8 ± 1.2 and after 3 months was 7.2 ± 1.2. Repeated measures analysis of variance showed a statistically significant difference between the scores ($p < 0.05$), [Table 1].

There was a 36% increase in the number of patients diagnosed and referred to the dental hospital pre- and post-intervention at 3 months. This increase was statistically significant as shown by the Chi-squared test [Table 2].

Discussion

Oral diseases cause pain, discomfort, altered taste, and a burning sensation. Dental conditions such as decay, periodontitis, and loss of teeth can affect mastication, swallowing, and speech.[9] Although the overall incidence of oral lesions has significantly reduced due to HAART, the prevalence is still high in patients in developing countries and vulnerable age groups due to the various risk factors involved.[6]

The current study aimed to improve the oral health-related outcomes of HIV-positive patients through an educational intervention targeted at improving the knowledge and competencies of the PCWs. Before the study, discussions with the PCWs and also with a few patients had revealed that patients suffered from stigma associated with their illness, which along with a lack of awareness and low socioeconomic status, prevented them from seeking dental treatment. They also disclosed the problems they faced regarding accessibility to oral health care.

This study helped to empower the PCWs with knowledge related to oral health and also improve their skills in educating patients to ensure that their oral health care needs are met. Kolisa and Ayo-Yusuf[7] in their study have stated that the nontrained care givers are less likely to provide advice on oral hygiene. Educational interventions do improve the caregivers' knowledge of oral lesions and their beliefs about oral care. Therefore, they can efficiently complement the health-care system.

This study was conducted at two residential centers for HIV patients. Most of the patients were school-going children below the age of 18 years. A majority of the children were orphans and completely dependent on the PCWs for all their needs. It was seen that there was not much of an emphasis on oral health due to ignorance as well as inaccessibility to oral health-care facilities.

The PCWs who took part in the study were co-operative and willing to conduct periodic oral examinations. Regular visits and telephone reminders by the dentists also motivated them to perform their duties. The model proved to be an effective method for oral health-care promotion. It improved the knowledge of the PCWs significantly. Ever since this model has been introduced at the HIV centers, there has been a regular stream of HIV patients visiting the dental hospital to avail dental treatment. This study shows results similar to a study that had been conducted by Koyio *et al*.[10] on the community and professional health-care workers in Nairobi. They too showed that the training programs significantly increased the primary and community health workers' knowledge, recognition, and management of oral lesions in HIV patients.

This is a pilot study, and the sample is limited to 15 PCWs. However, the positive outcome with this study shows that this model can be implemented on a larger scale so as to benefit a wider population of the HIV patients.

Conclusion

Despite the limitations of the study, this model shows that this model has the potential to benefit a larger number of HIV patients through its implementation to a larger group of PCWs in other centers. The PCWs have shown improved competencies with regard to diagnosing harmful oral and dental conditions and timely referrals to the dental hospital, therefore leading to an increased awareness among HIV patients about their oral health status. The patients can overcome the stigma in seeking treatment and improve their oral health-related quality of life. The study also suggests that an effective interprofessional collaboration can be developed between the dentists and the PCWs for the benefit of the patients.

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Table 1: Pre- and post- intervention mean scores

	Mean±SD	*p*
Pre	2.53±1.30	<0.001
Post	5.801±1.26	
Post 3 months	7.26±1.27	

Statistically significant at $p<0.05$. SD: Standard deviation

Table 2: Number of referrals made by the primary care workers-pre- and post- intervention (3 months)

	Number of patients	Number of referrals	Percentage	Difference (%)	χ^2	DF	*p*
Pre	75	18	24	36	19.818	1	<0.001*
Post 3 months	75	45	60				

*Statistically significant. DF: Degree of freedom

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Conflicts of interest

There are no conflicts of interest.

References

1. Grando LJ, Yurgel LS, Machado DC, Nachman S, Ferguson F, Berentsen B, *et al*. The association between oral manifestations and the socioeconomic and cultural characteristics of HIV-infected children in Brazil and in the United States of America. Rev Panam Salud Publica 2003;14:112-8.
2. Reznik DA. Oral manifestations of HIV disease. Top HIV Med 2005;13:143-8.
3. Leao JC, Ribeiro CM, Carvalho AA, Frezzini C, Porter S. Oral complications of HIV disease. Clinics (Sao Paulo) 2009;64:459-70.
4. Navazesh M, Mulligan R, Komaroff E, Redford M, Greenspan D, Phelan J. The prevalence of xerostomia and salivary gland hypofunction in a cohort of HIV-positive and at-risk women. J Dent Res 2000;79:1502-7.
5. Cavasin Filho JC, Giovani EM. Xerostomy, dental caries and periodontal disease in HIV+ patients. Braz J Infect Dis 2009;13:13-7.
6. Ottria L, Lauritano D, Oberti L, Candotto V, Cura F, Tagliabue A, *et al.* Prevalence of HIV-related oral manifestations and their association with HAART and CD4+ T cell count: A review. J Biol Regul Homeost Agents 2018;32:51-9.
7. Kolisa Y, Ayo-Yusuf O. Evaluation of caregivers' knowledge, beliefs and practices regarding oral lesions in HIV-patients: A pilot study. Health SA Gesondheid 2018;18:1-8.
8. Saini R. Oral lesions: A true clinical indicator in human immunodeficiency virus. J Nat Sci Biol Med 2011;2:145-50.
9. Mohamed N, Saddki N, Yusoff A, Mat Jelani A. Association among oral symptoms, oral health-related quality of life, and health-related quality of life in a sample of adults living with HIV/AIDS in Malaysia. BMC Oral Health 2017;17:119.
10. Koyio LN, Sanden WJ, Dimba E, Mulder J, Creugers NH, Merkx MA, *et al*. Oral health training programs for community and professional health care workers in Nairobi East District increases identification of HIV-infected patients. PLoS One 2014;9:e90927.

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Questionnaire

Age:

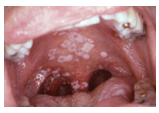
Sex:

Employment:

Educational qualification:

<12th Std
>12th std
Graduate
Post graduate

1. Have you received any training in oral health care
   Yes
   No
2. Do you know the oral screening/examination procedures for children
   a. No knowledge
   b. Partial knowledge
   c. Complete knowledge
3. Oral health care is important for HIV patients
   d. Strongly agree
   e. Agree
   f. Neither
   g. Disagree
   h. Strongly disagree
4. HIV patients are at a high risk for
   a. Dental caries
   b. Gum disease
   c. Dry mouth
   d. All of the above
5. Do you think poor oral health can compromise nutrition
   a. Strongly agree
   b. Agree
   c. Disagree
   d. Strongly disagree
6. Which of the following is true about oral lesions:
   a. Predict HIV progression
   b. Signal failure of antiretroviral treatment
   c. Causes pain, discomfort
   d. All of the above
7. The most common oral lesion in HIV patients is
   a. Hairy leukoplakia
   b. Candidiasis
   c. Acute necrotizing periodontitis
   d. Oral cancer
8. What does oral thrush/candidiasis look like

a.  b 

c  d 

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9. Identify healthy gingiva (gums)

a  b 

c  d 

10. Most important cause of dental caries in HIV patients is
    a. Tooth grinding
    b. Avoiding dentist
    c. Dry mouth
    d. Genetics
11. Signs of periodontal disease are
    a. Red, swollen gums
    b. Bleeding while brushing
    c. Teeth looking longer than before
    d. All of the above
12. Do you think it is important to seek dental treatment for dental problems (dental caries, gingival inflammation)
    a. Strongly agree
    b. Agree
    c. Disagree
    d. Strongly disagree
13. Regular oral screening for HIV patients is necessary
    a. Strongly agree
    b. Agree
    c. Disagree
    d. Strongly disagree
14. Home care for periodontal disease includes
    a. Antibacterial mouthwash
    b. Proper nutrition
    c. Pain killers
    d. All of the above
15. Which of the following is not true regarding nutrition
    a. HIV patients must eat diet high in vegetables, fruits, and whole grains
    b. Eat small amount of fats and sugars
    c. Do not need higher daily calorie intake
    d. Eat smaller meals more often
16. Do you know the referral procedures for the patients
    a. No knowledge
    b. Partial knowledge
    c. Complete knowledge
17. Have you previously referred patients for dental/oral care to the doctor
    a. Yes
    b. No
18. If yes
    a. How many
    b. Reasons

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Original Article

Parental Satisfaction with Pediatric Day-Care Surgery and its Determinants in a Tertiary Care Hospital

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ABSTRACT

Objective: The objective is to assess the level of parental satisfaction of pediatric day-care surgery and its different determinants.

Materials and Methods: This is a descriptive study performed in a tertiary care hospital in India among parents of pediatric day-care surgery patients from June 2013 to March 2015. The core questionnaire for the assessment of patient satisfaction for general day care (COPS-D) was used. Variables related to surgery, overall satisfaction, one open-ended question, and socio-demographic data were also collected. Calculated sample size was 121.

Results: The mean and standard deviation of parental satisfaction were estimated in eight domains of day care (COPS-D) using Likert scale 1–5. Preadmission visit had a mean of 4.63 (0.52), day of surgery 4.65 (0.58), operating room 4.76 (0.51), nursing care 4.46 (0.79), medical care 4.89 (0.48), information 4.51 (0.68), autonomy 4.64 (0.56), and discharge 4.50 (0.72). In elder children, there was less satisfaction on the information and discharge domains. Overall satisfaction was good in 88% of patients and was less than satisfactory when they had significant pain.

Conclusion: Perception of quality of pediatric day-care surgery was assessed with a questionnaire and was found to be good. Variables related to surgery such as pain may be included in the questionnaire for assessing satisfaction in the day-care surgery.

Keywords: *Day-care surgery, parental satisfaction, pediatric day-care surgery, quality in day-care surgery, quality of care, variables related to day-care surgery*

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Introduction

Day-care surgery is being practiced worldwide. For a child, it gives quicker return to a familiar home environment without compromising the safety of the child. This is an efficient way of reducing the costs and waiting time, maximizing the use of resources, and avoiding an unnecessary stay in the hospital.[1] Day-care surgery ranges from 20% to 68% of surgery being performed in various pediatric surgical units.[2] This is possible due to the advanced medical technology and a better understanding of what can be performed safely in a day.[3] Effective preoperative assessment and preparation with protocol-driven discharge are fundamental for safe day-care surgery.

Quality and safety in health care are of paramount importance.[4] The WHO has emphasized the importance of the patient's perspective in the evaluation of health-care delivery. After providing infrastructure and proper personnel to assure quality and safety, it is important to assess the services using a structured patient's satisfaction questionnaire. Measuring satisfaction is problematic as the evaluations are personal and subjective.[5] Expressed satisfaction reflects the patient's knowledge and expectations than the quality of the service received.[6] However, monitoring user satisfaction is important for quality care assessment and benefits patient care if their feedback influences subsequent service delivery.

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Two groups of determinants of satisfaction were distinguished in reviewing literature: those relating to the characteristics of respondents and those relating to health service delivery features. The patient's expectation is an important determinant among individual factors.[7] Care process is an important determinant regarding health delivery system.[8] Other determinants specific to day-care surgery are postoperative complications, postoperative pain, waiting time before surgery, readmission, and the clinical outcome.[3]

The different approaches to measure the patient satisfaction are based on health service attributes, holistic approach, expectation-based surveys, and those evolved from economic theory.[5,7] Among these, holistic approach includes all important determinants of patient satisfaction.[7] The patient satisfaction surveys using validated instrument should be able to measure the structure, process, and outcome of a health-care system.[9,10] The patient satisfaction score is indispensable to the assessment of quality.[8,11]

In different countries, systems are established to monitor the performance of health-care institutions using a framework of structure, process, and outcome indicators.[12] The aim of our study was to assess the level of parental satisfaction with pediatric day-care surgery and to find out the different determinants that influence the parental satisfaction.

Materials and Methods

This is a descriptive study conducted in PSG Institute of Medical Sciences and Research, a tertiary care private medical college hospital, in South India. The study period was from June 2013 to March 2015 among parents of pediatric day-care surgery patients. The Institutional Ethical Board approval was obtained (No: 13 / 036).

Inclusion criteria were children aged 1–15 years undergoing day-care surgery with the American Society of Anesthesiologists I and II and who can reach the hospital within 2 h in case of emergency. Exclusion criteria were children under 1 year and those who needed emergency surgery. Children under 1 year are generally admitted. The operations performed in day care are procedures as herniotomy, circumcision, and orchidopexy.

These children had a pre-operative assessment few days before the planned surgery. Parents were explained about day-care procedure, expected problems, anesthesia, and cost of surgery by the doctor and clinical nurse assistant. On the day of surgery, they were seen in the day-care area; surgery was performed under general anesthesia with or without caudal analgesia. They were monitored for 4 h and more if necessary. If the child had no problems, he was sent home after being seen by the treating surgeon on oral paracetamol for next 3 days. If necessary, they were admitted for observation. A follow-up visit was scheduled after 1 week.

Study tool

The core questionnaire for the assessment of patient satisfaction for general day care (COPS-D),[13] was chosen as it is a validated questionnaire designed for day care including day-care surgery. It assesses eight dimensions/domains: as preadmission visit (4 items), admission at the day-care center (3 items), operation room (3 items), nursing care (2 items), medical care (2 items), information (4 items), autonomy (3 items), and discharge and after care (3 items). The response for each item is assessed by 1–5 Likert scale; 1 being unsatisfied and 5 as very satisfied. Average of the item score constituted the domain score. Together all these categories aim to achieve a total picture of the quality of care. The reported internal consistency of the eight dimensions was good (α =0.82–0.90).

In addition, questions about variables related to surgery such as waiting time for surgery, postoperative complications such as vomiting, postoperative pain, readmission, and outcome of surgery were also included in the study. Postoperative pain was assessed on a numeric rating scale of 0–10. There was one item about the overall satisfaction of the quality of care which was marked on a scale of 1–10. One open-ended question was added for parent to comment regarding the services they have received on the day of surgery to counter the drawback of quantitative surveys. The responses were classified as compliments, complaints, and suggestions. Socio-demographic data were also collected.

The questionnaire was translated into the regional language and it was verified with back translation. The questionnaire was given to the parents when they came for their child's first postoperative visit, which was usually a week after surgery by the outpatient department staff. Informed consent was obtained from the respondent. Questionnaire response was kept anonymous. Incomplete questionnaires were excluded from the study. Privacy of the participants was ensured by excluding identification details from the study questionnaire. All questions were directed to the parents of the patients.

Sample size

We considered 75% as the anticipated satisfaction level of day-care surgery patients. At a confidence level of 95% and with 12% of relative precision, with the

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nonresponse rate of 30%, an overall sample size of 121 was derived. A total of 121 consecutive parents of day-care surgery patients were included in this study. Responses were entered in Epi Info™ 7.1.2.0 (Centre for Disease Control and Prevention, Atlanta, USA) and results were analyzed using R software (R version 3.0.1. The R foundation for Statistical Computing. Vienna, Austria). Descriptive statistics such as means and standard deviation, percentage, Chi-square test, and ANOVA test were used in the analysis. A P value < 0.05 was considered statistically significant.

Results

Respondents

All 121 respondents returned the questionnaire. Twelve questionnaires were excluded from the study for many missing values making the sample size to 109. The characteristics of the study population are presented in Table 1. Male children predominated in our study (85.3%). Children under 4 years were 62.38%. Graduates were 53.21% and illiterates were <3% in our study group. Income was classified according to four quartiles. Circumcision (n = 55), hydrocele (n = 19), hernia (n = 12), tongue tie release (n = 11), orchidopexy (n = 5), node biopsy (n = 5), mass excision (n = 7), and meatoplasty (n = 1) were the surgical procedures performed as day care during the study. Six patients underwent two procedures.

Satisfaction scores

Analysis of rating of satisfaction of eight domains of the day care in Likert scale of COPS-D [Figure 1] showed that parents were satisfied in most of the domains. However, by comparing satisfaction of various domains with different age groups by ANOVA test [Table 2], there was relatively less satisfaction in domains of information and discharge in the older age group (11–15 years) and this was statistically significant. Sex of the child, education status of the parent, and income did not make any difference in the satisfaction score of various domains.

Overall satisfaction score and other variables related to surgery

Overall satisfaction score of patients was fair (score 4–7) in 10 (9.17%) and good (score 8–10) in 96 (88%) patients. Three had not recorded this score, but none had written unsatisfactory. One-fourth of children who underwent day care had more than mild pain. There were moderate pain in 17 patients (15.59%) and severe pain in 11 patients (10.09%). Overall satisfaction score of patients who had more than mild pain was less than those who had mild pain and it was statistically significant [Table 3].

Table 1: Sociodemographic variables of the study participants (n=109)

Patient characteristic	n (%)
Gender	
Male	93 (85.3)
Female	16 (14.7)
Age (years)	
1-4	68 (62.38)
5-10	30 (27.52)
11-15	11 (10.09)
Education standard (parents)	
Illiterate	3 (2.75)
Primary school	10 (9.17)
Middle/high school	17 (15.59)
Higher secondary	21 (19.26)
Graduate	58 (53.21)
Monthly income in INR (parents)	
Not recorded	11 (10.09)
<8000	23 (21.1)
8001-12,000	26 (23.85)
12,001-20,000	23 (21.1)
20,001-100,000	26 (23.85)

INR: Indian rupee

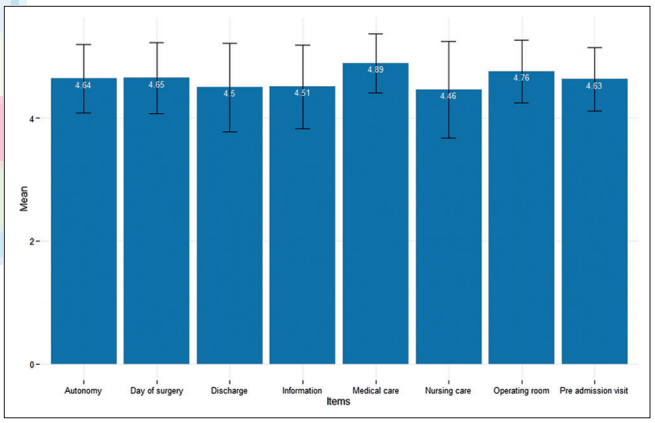


Figure 1: Comparison of rating of satisfaction in Likert scale (1–5) for the 8 dimensions/domains of core questionnaire for the assessment of patient satisfaction for general day care

Postoperative complications were present in 17 patients (15.60%) and it was temporary and mostly related to anesthesia. The problems encountered were nausea/vomiting (n = 5), urinary retention (n = 3), drowsiness (n = 2), headache (n = 2), sore throat (n = 1), bleeding (n = 2), and others (n = 2) as postoperative fever and wound infection. There was a delay in the operation theater (OT) in 13 patients (11.92%) of our study group. Both postoperative complications and delay in OT have not made any statistically significant difference in overall satisfaction score. Children who needed readmission were 2 (1.83%) out of 109. The outcome of surgery was good in all patients except one whose status was

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Table 2: Comparison of core questionnaire for the assessment of patient satisfaction for general day care and overall satisfaction score with different age group, sex, education level, and income

	Mean (SD)								
	Preadmission visit	Day of surgery	Operation room	Nursing care	Medical care	Information	Autonomy	Discharge	Overall s. score
Age group (years)									
1-4	4.65 (0.55)	4.62 (0.64)	4.80 (0.54)	4.56 (0.80)	4.88 (0.58)	4.58 (0.63)	4.64 (0.60)	4.64 (0.57)	9.27 (1.25)
5-10	4.60 (0.48)	4.65 (0.52)	4.68 (0.49)	4.25 (0.80)	4.93 (0.25)	4.55 (0.47)	4.66 (0.53)	4.31 (0.90)	9 (1.80)
11-15	4.59 (0.49)	4.81 (0.22)	4.75 (0.36)	4.45 (0.52)	4.86 (0.32)	4.00 (1.18)	4.57 (0.47)	4.18 (0.81)	9.1 (1.28)
F (df=2,106)	0.165	0.5	0.519	1.68	0.137	3.67	0.102	3.624	0.39 (df=2,103)
P	0.848	0.608	0.597	0.191	0.872	0.0287*	0.903	0.03*	0.678
Sex									
Male	4.62 (0.55)	4.64 (0.60)	4.78 (0.52)	4.45 (0.83)	4.88 (0.57)	4.50 (0.72)	4.66 (0.55)	4.51 (0.73)	9.23 (1.29)
Female	4.71 (0.38)	4.68 (0.47)	4.66 (0.43)	4.56 (0.47)	4.93 (0.25)	4.62 (0.38)	4.54 (0.66)	4.46 (0.64)	8.86 (2.09)
t	−0.8719	−0.287	0.9653	−0.7509	−0.6123	−1.0191	0.6902	0.2657	0.651
df	26.835	24.004	23.109	33.289	41.498	36.45	18.732	22.281	15.79
P	0.391	0.7765	0.3444	0.458	0.5437	0.3149	0.4985	0.7929	0.5241
Education									
Graduate	4.59 (0.43)	4.68 (0.47)	4.76 (0.38)	4.44 (0.67)	4.91 (0.37)	4.55 (0.53)	4.66 (0.43)	4.52 (0.55)	9.35 (0.95)
Less than graduate	4.67 (0.61)	4.61 (0.69)	4.77 (0.38)	4.49 (0.90)	4.87 (0.59)	4.48 (0.83)	4.62 (0.69)	4.49 (0.87)	8.97 (1.82)
t	−0.74278	0.65355	−0.0673	−0.26979	0.42831	0.52477	0.29666	0.24999	1.2844
df	88.78	86.26	81.22	91.48	82.833	83.154	81.843	82.829	70.014
P	0.4596	0.5151	0.9465	0.7879	0.6695	0.6011	0.7675	0.8032	0.2032
Income group									
<20,000	4.66 (0.56)	4.66 (0.58)	4.76 (0.55)	4.54 (0.72)	4.86 (0.57)	4.517 (0.74)	4.63 (0.63)	4.48 (0.80)	9.02 (1.61)
20,000-1,000,00	4.58 (0.45)	4.63 (0.59)	4.77 (0.42)	4.31 (0.89)	4.94 (0.22)	4.519 (0.58)	4.66 (0.43)	4.55 (0.54)	9.45 (0.93)
t	0.76289	0.29456	−0.069	1.3834	−1.0417	−0.0165	−0.3201	−0.51472	1.7381
df	90.154	73.631	94.367	63.208	100.21	92.264	100.69	101.05	103.42
P	0.4475	0.7692	0.9446	0.1714	0.3001	0.9869	0.7496	0.6079	0.08

*Statistically significant. SD: Standard deviation

Table 3: Pain, complications, and delay in operation theatre versus overall satisfaction score

	n (%)	Satisfaction score, mean (SD)	*t*-test	df	*P*
Pain					
Mild	65 (59.63)	9.5 (1.032)	2.6374	35.384	0.01233*
Others	28 (25.68)	8.62 (1.59)			
Not recorded	16 (14.67)				
Postop complications					
No	92 (84.40)	9.25 (1.3)	1.0005	18.803	0.3298
Yes	17 (15.60)	8.76 (1.95)			
Delay					
No	94 (86.23)	9.18 (1.39)	0.057	14.265	0.9546
Yes	13 (11.92)	9.15 (1.72)			
Not recorded	2 (1.83)				

*Statistically significant. SD: Standard deviation

not recorded and hence, its relationship to satisfaction could not be established.

Suggestions for improvement

Open-ended question was answered by 50 parents (45.87%). Compliments were given by 33 (30.27%), 6 (5.50%) gave a suggestion, and complaints were received from ten parents (9.17%). The complaints were about the delay at the pharmacy or dietary services. They also suggested improvement in waiting area, services provided by the staff nurses in the recovery area.

Discussion

Most of the study patients were male, the reason being the day-care procedures such as circumcision itself are more common in male children. Many of the study patients were under 4 years for the same reason and

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this is similar in pediatric day-care services in other hospitals.[2] Level of satisfaction was almost equal among the various income groups, but this may not be representative as this is a private hospital. There were no significant differences in satisfaction score among parents of varying educational level in the current study. However, in other studies, it has been noted that higher the education, lesser was the satisfaction. It has been argued that the parents with higher education come the awareness and their expectation from the health providers goes up.[14]

Parental satisfaction score of various domains of care in day-care surgery patients in the current study had ranged from 4.46 to 4.89 out of 5 (i.e., 89.2%–97.8%). However, areas such as information about day care and discharge need some improvement, especially when catering for children above 10 years. There was no similar study (using domains) to compare among pediatric age group. A study conducted in a private sector hospital in North India among inpatients reported satisfaction in various domains ranging from 64.8% to 98.2%.[15]

The secondary outcome of this study showed that although the overall outcomes of the day-care surgery in children were good, there were some areas of care which need to be improved. They were delay in performing surgery which needs better planning, postoperative pain relief, and postoperative complications. Mandhan *et al*., have reported postoperative pain in 40% of their day-care surgery children while in our study 26% had more than mild pain. However, the nature of pain was not graded in their study.[16] Myles *et al*. noted that patients who had postoperative pain were less satisfied like the current study and hence, this factor has to be taken into consideration to improve the quality of day-care surgery.[17]

The occurrence of postoperative complications in the current study was 15.60%. Mandhan *et al*., reported complication of 44% (n = 368) out of which surgical complications were only 4%. In addition to complications we have noted, the study reports about feeding disturbance in first 24 h.[16] It had not affected the overall satisfaction scores. It is probably because parents were primed for it in their preoperative visit and the effects were temporary. Delay in OT to start the procedure (11.92%) had not affected satisfaction in the current study; although, it had been a significant factor affecting patient's satisfaction in other day-care surgery studies.[2]

Only 2 (1.83%) out of 17 who had postoperative complications, needed readmission. Readmission rate of 1%–2% is suggested as acceptable in day-care surgery. Day-care surgery performed in government sector in India showed readmission rate of 5.7%.[18] Other studies showed readmission rate varying from 0.2% to 6.2%.[16] In the current study, the overall outcome could be explained by proper selection of cases. However, parents need to be informed about waiting time in OT, postoperative pain, and immediate postoperative complications as they seem to be an integral part of day-care surgery.

Overall satisfaction score (in the scale of 1–10) was 88% in the current study population. Other pediatric day-care surgery centers from developed countries showed 93%–97% satisfaction level.[16,19] The patient satisfaction level reported among inpatients in Indian studies range from 23.4% in the government sector to as high as 84% in a private tertiary care.[14,20]

Open-ended question had helped us to identify what patients appreciate and the specific area which needed improvements such as recovery area, dietary services, and pharmacy services. This information was passed on to hospital quality control department for making necessary changes.

Limitation of the study

Gratitude bias is possible in the study as it was conducted by the same department. However, we had tried to reduce this effect by giving a self-response questionnaire and questionnaire response was kept anonymous.

Conclusion

The patients look at a facility every time the service is delivered and form distinct opinions about its effectiveness. Health-care organizations that aspire to excellence must constantly assess the quality of their service experience through their patients' eyes. The patient feedback may be routinely practiced to improve the health-care system. Variables related to surgery as pain may be included in a questionnaire assessing satisfaction in day-care surgery as it affects overall satisfaction score.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Black N, Sanderson C. Day surgery; development of a questionnaire for eliciting patients' experiences. Qual Health Care 1993;2:157-61.

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2. Abdur-Rahman LO, Kolawole IK, Adeniran JO, Nasir AA, Taiwo JO, Odi T. Pediatric day case surgery: Experience from a tertiary health institution in Nigeria. Ann Afr Med 2009;8:163-7.
3. Bain J, Kelly H, Snadden D, Staines H. Day surgery in Scotland: Patient satisfaction and outcomes. Qual Health Care 1999;8:86-91.
4. Lengnick-Hall CA. The patient as the pivot point for quality in health care delivery. Hosp Health Serv Adm 1995;40:25-39.
5. Crow R, Gage H, Hampson S, Hart J, Kimber A, Storey L, *et al.* The measurement of satisfaction with healthcare: Implications for practice from a systematic review of the literature. Health Technol Assess 2002;6:1-244.
6. Taylor SA, Cronin JJ Jr. Modeling patient satisfaction and service quality. J Health Care Mark 1994;14:34-44.
7. Bjertnaes OA, Sjetne IS, Iversen HH. Overall patient satisfaction with hospitals: Effects of patient-reported experiences and fulfilment of expectations. BMJ Qual Saf 2012;21:39-46.
8. Ygge BM, Arnetz JE. Quality of paediatric care: Application and validation of an instrument for measuring parent satisfaction with hospital care. Int J Qual Health Care 2001;13:33-43.
9. Fitzpatrick R. Capturing what matters to patients when they evaluate their hospital care. Qual Saf Health Care 2002;11:306.
10. Lew VK, Lalwani K, Palermo TM. Factors affecting parental satisfaction following pediatric procedural sedation. J Clin Anesth 2010;22:29-34.
11. Donabedian A. The quality of care. How can it be assessed? JAMA 1988;260:1743-8.
12. van Dishoeck AM, Lingsma HF, Mackenbach JP, Steyerberg EW. Random variation and rankability of hospitals using outcome indicators. BMJ Qual Saf 2011;20:869-74.
13. Kleefstra S, Kool R, Zandbelt L, de Haes J. An instrument assessing patient satisfaction with day care in hospitals. BMC Health Serv Res 2012;12:125.
14. Akoijam BS, Konjengbam S, Bishwalata R, Singh TA. Patients' satisfaction with hospital care in a referral institute in Manipur. Indian J Public Health 2007;51:240-3.
15. Kumar S, Haque A, Tehrani HY. High satisfaction rating by users of private-for-profit healthcare providers-evidence from a cross-sectional survey among inpatients of a private tertiary level hospital of North India. N Am J Med Sci 2012;4:405-10.
16. Mandhan P, Shah A, Khan AW, Muniruddin, Hasan N. Outpatient pediatric surgery in a developing country. J Pak Med Assoc 2000;50:220-4.
17. Myles PS, Williams DL, Hendrata M, Anderson H, Weeks AM. Patient satisfaction after anaesthesia and surgery: Results of a prospective survey of 10,811 patients. Br J Anaesth 2000;84:6-10.
18. Ramanujam TM, Uma G, Usha V, Ramanathan S, Sujaritha R. Advantages and limitations of day surgery in children in a developing country. Pediatr Surg Int 1998;13:512-4.
19. Nurliza I, Norzi G, Azlina A, Hashimah I, Sabzah MH. Daycare tonsillectomy: A safe outpatient procedure. Hospital Sultanah Bahiyah, Alor Setar Malaysia experience. Med J Malaysia 2011;66:474-8.
20. Rao KD, Peters DH, Bandeen-Roche K. Towards patient-centered health services in India – A scale to measure patient perceptions of quality. Int J Qual Health Care 2006;18:414-21.

SHORT ARTICLE

Knowledge, Attitude and Practice of COVID 19 and the factors influencing them among a Rural Population in South India

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Abstract | Introduction | Methodology | Results | Conclusion | References | Citation | Tables / Figures

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Abstract

Background: Mitigation measures to control the novel coronavirus disease 2019 (COVID-19) depends on the public adherence to preventive measures which is influenced by their knowledge and attitude. **Aim**: To find the knowledge, attitudes and practices (KAP) of the rural population toward COVID-19 and to establish its socio-demographic influencing factors. **Material and methods**: KAP study regarding Covid-19 was done among the rural population during August 2020 using a semi-structured questionnaire. **Results:** Among 500 participants, the mean knowledge, attitude and practice score was 21.55± 2.9 (Range 0- 30), 30.0± 2.88 (Range 12- 36) 9.4± 1.25 (Range 0-13) respectively. Higher knowledge was associated with higher education and employed individuals. Favourable attitude was among those aged <35 years and having higher education. Optimistic practices were exhibited by employed individuals and when children <12 years are present in their home. **Conclusions:** Study participants preceded good knowledge, positive attitude towards COVID-19 but practice is not sufficient enough to win the battle against the dreadful disease due to considerable knowledge- practice gap.

Keywords

COVID-19; Knowledge; Attitude; Practice; Rural Population

Introduction

COVID-19 virus which emerged in Wuhan has spread over 200 countries and has become a pandemic claiming several lives. World Health Organization (WHO) announced a public health emergency of international concern to prevent its rapid spread.(1)In India, a confirmed case of COVID-19 was reported on 30thJanuary 2020, who was a student travelled from Wuhan, China.(2) More than eight million cases have been reported so far in India.[3] Tamilnadu, a state in India has the third largest number of cases approximately 7,30,000 cases till date.(3) While the government has issued advisory through various channels and media on the prevention and precautionary measures, people's observance is essential for controlling the spread of

COVID-19, which is affected by their knowledge, attitudes and practices (KAP) towards COVID-19. From compliance with lockdowns to following personal safety measures, the onus is on each individual to break the chain and flatten the curve.(4) While the urban community has the advantage and access of health care facilities and other ways of becoming aware of these measures such as social media and print media, the rural population may be limited in terms of access and awareness of these updates.(5) In addition, the recent trends indicate that the COVID crisis is moving towards the rural areas.(5) Considering the above facts it is more important and imminent to reach out to the community in a rural area to assess their knowledge, attitude and practice towards COVID 19 and recommend tailor made awareness programs to these population so that the disease control measures are effective and on-going prevention efforts can be strengthened. Therefore, we conducted a survey to investigate the knowledge, attitude and practice (KAP study) towards COVID-19 among the rural population as well as to find the socio-demographic determinants associated with it.

Aims & Objectives

1. To study the knowledge, attitude and practice (KAP) among the rural population towards COVID-19
2. To find the socio-demographic determinants influencing KAP among the study participants

Material & Methods

Study population and location: This cross-sectional study was conducted among those who attended the outpatient clinic at the PSG Rural Heath Training Centre (RHTC) attached to Department of Community Medicine in Coimbatore.

Questionnaire: A self-designed questionnaire was developed from WHO training material for emerging respiratory viruses including COVID-19(6) and Training Resources for COVID 19 Management in India. (7) Demographic details had questions on age, gender, occupation, education, socioeconomic class and presence of children less than 12 years. Knowledge section had 30 questions which dealt with clinical symptoms, transmission, high risk groups, prevention and control of COVID-19. Participants were asked to respond to knowledge items as either true or false, with an additional "don't know" option. The correct answer was assigned a score of one and false/I don't know answers were given a score of zero. The total score for knowledge ranged from zero to 30 with high score representing better knowledge. Those who scored more than 70 % of the score were considered as having adequate knowledge based on previous study.(8)

In the section on attitude, scores were calculated based on the respondents' answers to each of 12 attitudinal statements in likert scale as agree, neutral and disagree. Agree or Disagree was awarded 3 points based on positive or negative statement and the reverse one was given one point, neutral answer was given a score of 2. The total attitude score ranged from 12 to 36 with high scores indicating positive attitudes. In the section on practices, respondents were asked to respond "yes" or "no" to the 13 items. A score of one was given to answers that reflected good practice and a score of zero was given for wrong practice. Total practice score ranged from 0 to 13 higher averages represents a desirable practice.

Data Collection: IHEC clearance was obtained for the study. Convenient sampling method was used to enroll the participants attending the RHTC owing to the current pandemic situation and to conduct the physical interview with safety precautionary measures until a sample size of 500 was reached during the period of August 10 to August 30. Data was collected among permanent residents in the catchment area above 18 years after obtaining a detailed written informed consent.

Statistical analysis: Data was collected using epicollect app and was analyzed using SPSS (v20) software. Normality of the score was assessed using a test of normality - Kolmogorov-Smirnov and Shapiro-Wilk test. Comparison of KAP scores with demographic factors was done using independent samples t-test. Multivariate linear regression analysis was used to establish the relationship between independent variables and KAP outcome variable. P value < 0.05 was considered as statistically significant.

Results

Demographic information: Demographic profile of the enrolled 500 participants is shown in table 1. The mean age of the study participants was 37.49 ± 13.16. Among the participants who responded 52.6% were in the age group ≤ 35 years, 60.8% were females, 59% were employed with salary, 62.8% were educated above high school, 18.4 % belonged

to socio-economic class IV & V and 39.4% had children less than 12 years in their home.

Knowledge, Attitude and Practice Score related to COVID-19: The mean knowledge score was 21.55± 2.9 (Range 0- 30). 65.8 % of the participants scored more than 70 % and were considered as having adequate knowledge. 89% of them knew wearing mask help to reduce the transmission of the disease. 87% of the individuals said washing hands with water and soap can eliminate the disease causes. Nearly everyone (97.2%) knew that COVID-19 can be prevented by avoiding crowded places. The mean attitude score was 30.0 ± 2.88 (Range 12- 36). Majority of population (96.4 %) believed restriction of travel to contaminated area along with social distancing, wearing mask regularly and practising hand hygiene helps to prevent disease spread. 94.8% felt mental health of COVID-19 infected patients should be taken care. The mean practice score was 8.2 ± 1.25 (Range 0-13). Majority (99.6 %) of the participants use facial mask whenever they go out of home but 39.2 % have the habit of keeping the mask in the pocket and use it when required, 50.4% have the habit of pulling down the mask when talking to their friends / relatives .82 % avoid going out of home unnecessarily. Higher proportion (96.8%) of the participants practice washing hands with soap/ use sanitizer but only 36.6% wash hands with soap for at least 20 seconds.

Predictors for KAP regarding COVID_19: In multivariate regression analysis (as shown in Table 2) employed individuals (β=0.73: p=0.016), Higher education (β=1.35: p=0.002) have significant association with higher knowledge score. With regard to attitude those aged ≤ 35 years (β=0.69: p=0.016), Higher education (β=0.75: p=0.012) have good attitude score and is found to be statistically significant. Employed individuals (β=0.53: p=0.002) those who have children less than 12 years in their home (β=--0.31: p=0.010) have significant good practice.

Discussion

The battle against the novel COVID-19 is still in progress in India and evidence shows that public knowledge is important in tackling pandemics. Adequate knowledge was present among 65.8 % of the study participants. The knowledge acquired can be due to familiarity of the disease since this pandemic is in existence for more than six months. Significant predictors of participant knowledge were those who have education more than 10th standard and employed with salary. This supports the claim that educated and employed individuals have exposure to different information-gathering networks hence they are much aware which is consistent with other study. (9)

A positive and optimistic attitude among the study participants reflects the belief that COVID-19 was controllable and containable. Attitude was strongly associated with participants aged ≤ 35 years and those having higher education consistent with another study. (10) The younger generation use social media more often than the elderly individuals in the rural area which could have influenced their attitude.

Good practice indicates the translation of knowledge and attitudes into practices (behavioural change) which is a crucial determinant to control the disease. Despite government mitigation measures like restriction on public transport, gatherings, closures of theatres and malls etc. to enforce social distancing still practice measures are not satisfactory. Though 89 % of them are aware that wearing mask can reduce the transmission unfortunately nearly one-third of the participants has the habit of keeping the mask in their pocket and uses it when necessary and 50% have the habit of pulling down the mask while talking to their friends/relatives which hinder the purpose of using face mask. Similarly, though 87% of the individuals knew washing hands with water and soap can eliminate the disease only 36.6% wash hands with soap for at least 20 seconds. The predictors for optimistic practices in our study are employed individuals and presence of children less than 12 years in their home which was similar to another study. (9)

The current study has few limitations since community based sample surveys could reflect better KAP assessment but in the current situation it is not advisable. This study is one of the first among rural population assessing peoples' knowledge, attitudes, and practices toward COVID-19 and thus provides a useful baseline for future research. Though knowledge score is better in this study, good practice is inadequate which poses a major threat in the spread of the disease. There is a huge gap between knowledge and practice which emphasize an immediate need to address this gap for better disease control measures. Owing to relaxation of lockdown - colleges, schools, theatres and malls have started to function, so the potential barriers has to

be curbed and harness better practice in order to prevent the second wave of transmission.

Conclusion

There is a huge gap between knowledge and practice which emphasize an immediate need to address this gap for better disease control measures. Owing to relaxation of lockdown - colleges, schools, theatres and malls have started to function, so the potential barriers has to be curbed and harness better practice in order to prevent the second wave of transmission.

Recommendation

This study is one of the first among rural population assessing peoples' knowledge, attitudes, and practices toward COVID-19 and thus provides a useful baseline for future research. Encouragement of public to adopt precautionary behaviours for containment strategies as knowledge attitude and practice is foreground for public cooperation and backbone for implementing any health policy. Good practice indicates the translation of knowledge and attitudes into practices (behavioural change) which is a crucial determinant to control the disease.

Limitation of the study

Community based sample surveys could reflect better KAP assessment among rural population but in the current situation it is not advisable.

Relevance of the study

COVID-19 is an emerging infectious disease that poses a significant threat to public health. Preventive measures play an essential role in reducing infection rates and controlling the spread of the disease. This indicates the necessity of public adherence to preventive and control measures, which is affected by their knowledge, attitudes, and practices (KAP). Though knowledge score is better in this study, good practice is inadequate which poses a major threat in the spread of the disease.

Authors Contribution

All the authors have contributed at various stages of composition of the final manuscript.

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References

1. World Health Organization. Coronavirus disease 2019 (COVID-19): situation report, 82. 2020.
2. COVID-19 India. Tracker, 2020. [Online]. Available from: https://www.covid19india.org/ . [accessed on 02 Feb 2021].
3. COVID-19 Statewise Status. Available from: https://www.mygov.in/covid-19 . [accessed on 02 Feb 2021].
4. Marston C, Renedo A, Miles S. Community participation is crucial in a pandemic. Lancet. 2020;395(10238):1676-1678. doi: 10.1016/S0140-6736(20)31054-0. Epub 2020 May 4. PMID: 32380042; PMCID: PMC7198202.[PubMed].
5. The COVID-19 Crisis Is Moving to India's Rural Areas. Available from: https://science.thewire.in/health/covid-19-india-urban-rural/ [accessed on 02 Feb 2021].
6. World Health Organization. Emerging respiratory viruses, including COVID-19: methods for detection, prevention, response and control. Available from: https://openwho.org/courses/introduction-to-ncov . [accessed on 02 Feb 2021].
7. Ministry of Health and Family Welfare. Training Resources for COVID 19 Management. Available from: https://www.mohfw.gov.in/pdf/TrainingresourcesforCOVID1930MARCH.pdf . [accessed on 02 Feb 2021].
8. . Tamang N, Rai P, Dhungana S, Sherchan B, Shah B, Pyakurel P, Rai S. COVID-19: a National Survey on perceived level of knowledge, attitude and practice among frontline healthcare Workers in Nepal. BMC Public Health. 2020 Dec 14;20(1):1905. doi: 10.1186/s12889-020-10025-8. PMID: 33317486; PMCID: PMC7734608.[PubMed]
9. Yousaf MA, Noreen M, Saleem T, Yousaf I. A Cross-Sectional Survey of Knowledge, Attitude, and Practices (KAP) Toward Pandemic COVID-19 Among the General Population of Jammu and Kashmir, India. Soc Work Public Health. 2020;35(7):569-578. doi: 10.1080/19371918.2020.1806983. PMID: 32970548.[PubMed].
10. Olum R, Chekwech G, Wekha G, Nassozi DR, Bongomin F. Coronavirus Disease-2019: Knowledge, Attitude, and Practices of Health Care Workers at Makerere University Teaching Hospitals, Uganda. Front Public Health. 2020;8:181. doi: 10.3389/fpubh.2020.00181. PMID: 32426320; PMCID: PMC7204940.[PubMed]

Tables

TABLE 1 DEMOGRAPHIC PROFILE AND COMPARISON OF KNOWLEDGE, ATTITUDE AND PRACTICE SCORES AMONG DIFFERENT DEMOGRAPHIC VARIABLES BY UNIVARIATE ANALYSIS

Factors	Category	Total	Knowledge		P value	Attitude		P	Practice		P
			Mean	SD		Mean	SD	value	Mean	SD	value
Age	>35 years	237	21.16	3.38		29.60	2.98		8.16	1.23	0.443
	≤ 35 years	263	21.90	2.52	**0.005***	30.35	2.74	**0.003***	8.24	1.27	
Gender	Female	304	21.40	3.18	0.145	29.94	2.81		8.19	1.31	
	Male	196	21.80	2.61		30.08	3.01	0.616	8.20	1.21	0.89
Occupation	Unemployed	205	21.15	3.39		29.92	2.85		8.35	1.26	
	Employed	295	21.84	2.62	**0.011***	30.05	2.91	0.632	7.98	1.20	**0.001***
Education	Upto10th standard	186	20.56	3.63		29.42	2.79	**0.001***	8.17	1.35	0.67
	More than 10th standard	314	22.14	2.33	**<0.001***	30.33	2.88		8.22	1.19	
SES	Class IV,V	92	20.71	3.74		29.80	3.01		7.95	1.31	0.034
	Class I,II,III	408	21.75	2.74	**0.002***	30.04	2.85	0.480	8.25	1.23	
Children < 12 years in the home	Yes	197	21.80	2.63		30.04	2.85		8.32	1.26	**0.008***
	No	303	21.40	3.18	0.142	29.93	2.92	0.675	8.01	1.21	

TABLE 2 MULTIVARIATE LINEAR REGRESSION OF ASSOCIATION BETWEEN DEMOGRAPHIC CHARACTERISTICS WITH KNOWLEDGE, ATTITUDE AND PRACTICE SCORES OF COVID-19

	Factor	Knowledge		Attitude		Practice	
		Unstandardized coefficient B (95%CI)	P value	Unstandardized coefficient *B* (95%CI)	P value	Unstandardized coefficient *B* (95%CI)	**P value**
Age	>35 years ≤ 35 years	0.33 (-0.22 to 0.84)	.230	0.69 (0.12 to 1.24)	**.016***	0.07 (-0.16 to 0.31)	.571
Gender	Female Male	0.03 (-0.61 to -0.64)	.928	0.01 (-0.61 to 0.57)	.954	0.34 (- 0.05 to 0 .61)	0.09
Occupation	Unemployed Employed	0.73 (0.12 to 1.28)	**.016***	0.21 (-0.34 to 0..76)	.469	0.53 (0.27 to 0.78)	**.002***
Education	More than 10th standard Upto10th standard	1.35 (0.73 to 1.95)	**.002***	0.75 (0.18 to 1.34)	**.012***	-0.01 (-0.24 to 0.25)	.936
SES	Class IV,V Class I,II,III	0.45 (-0.29 to 1.22)	.273	0.14 (-0.85 to 0.51)	.675	0.25 (-0.02 to 0.56)	.100
Children < 12 years in the home	Yes No	0.25 (-0.19 to -0.74)	.299	0.38 (-0.84 to 0.17)	.160	0.31 (0.07 to 0.57)	**.010***

SHORT ARTICLE

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There is a huge gap between knowledge and practice which emphasize an immediate need to address this gap for better disease control measures. Owing to relaxation of lockdown - colleges, schools, theatres and malls have started to function, so the potential barriers has to be curbed and harness better practice in order to prevent the second wave of transmission.

Recommendation

This study is one of the first among rural population assessing peoples' knowledge, attitudes, and practices toward COVID-19 and thus provides a useful baseline for future research. Encouragement of public to adopt precautionary behaviours for containment strategies as knowledge attitude and practice is foreground for public cooperation and backbone for implementing any health policy. Good practice indicates the translation of knowledge and attitudes into practices (behavioural change) which is a crucial determinant to control the disease.

Limitation of the study

Community based sample surveys could reflect better KAP assessment among rural population but in the current situation it is not advisable.

Relevance of the study

COVID-19 is an emerging infectious disease that poses a significant threat to public health. Preventive measures play an essential role in reducing infection rates and controlling the spread of the disease. This indicates the necessity of public adherence to preventive and control measures, which is affected by their knowledge, attitudes, and practices (KAP). Though knowledge score is better in this study, good practice is inadequate which poses a major threat in the spread of the disease.

Authors Contribution

All the authors have contributed at various stages of composition of the final manuscript.

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References

1. World Health Organization. Coronavirus disease 2019 (COVID-19): situation report, 82. 2020.
2. COVID-19 India. Tracker, 2020. [Online]. Available from: https://www.covid19india.org/ . [accessed on 02 Feb 2021].
3. COVID-19 Statewise Status. Available from: https://www.mygov.in/covid-19 . [accessed on 02 Feb 2021].
4. Marston C, Renedo A, Miles S. Community participation is crucial in a pandemic. Lancet. 2020;395(10238):1676-1678. doi: 10.1016/S0140-6736(20)31054-0. Epub 2020 May 4. PMID: 32380042; PMCID: PMC7198202.[PubMed].
5. The COVID-19 Crisis Is Moving to India's Rural Areas. Available from: https://science.thewire.in/health/covid-19-india-urban-rural/ [accessed on 02 Feb 2021].
6. World Health Organization. Emerging respiratory viruses, including COVID-19: methods for detection, prevention, response and control. Available from: https://openwho.org/courses/introduction-to-ncov . [accessed on 02 Feb 2021].
7. Ministry of Health and Family Welfare. Training Resources for COVID 19 Management. Available from: https://www.mohfw.gov.in/pdf/TrainingresourcesforCOVID1930MARCH.pdf . [accessed on 02 Feb 2021].
8. . Tamang N, Rai P, Dhungana S, Sherchan B, Shah B, Pyakurel P, Rai S. COVID-19: a National Survey on perceived level of knowledge, attitude and practice among frontline healthcare Workers in Nepal. BMC Public Health. 2020 Dec 14;20(1):1905. doi: 10.1186/s12889-020-10025-8. PMID: 33317486; PMCID: PMC7734608.[PubMed]
9. Yousaf MA, Noreen M, Saleem T, Yousaf I. A Cross-Sectional Survey of Knowledge, Attitude, and Practices (KAP) Toward Pandemic COVID-19 Among the General Population of Jammu and Kashmir, India. Soc Work Public Health. 2020;35(7):569-578. doi: 10.1080/19371918.2020.1806983. PMID: 32970548.[PubMed].
10. Olum R, Chekwech G, Wekha G, Nassozi DR, Bongomin F. Coronavirus Disease-2019: Knowledge, Attitude, and Practices of Health Care Workers at Makerere University Teaching Hospitals, Uganda. Front Public Health. 2020;8:181. doi: 10.3389/fpubh.2020.00181. PMID: 32426320; PMCID: PMC7204940.[PubMed]

Tables

TABLE 1 DEMOGRAPHIC PROFILE AND COMPARISON OF KNOWLEDGE, ATTITUDE AND PRACTICE SCORES AMONG DIFFERENT DEMOGRAPHIC VARIABLES BY UNIVARIATE ANALYSIS

Factors	Category	Total	Knowledge		P value	Attitude		P	Practice		P
			Mean	SD		Mean	SD	value	Mean	SD	value
Age	>35 years	237	21.16	3.38		29.60	2.98		8.16	1.23	0.443
	≤ 35 years	263	21.90	2.52	**0.005***	30.35	2.74	**0.003***	8.24	1.27	
Gender	Female	304	21.40	3.18	0.145	29.94	2.81		8.19	1.31	
	Male	196	21.80	2.61		30.08	3.01	0.616	8.20	1.21	0.89
Occupation	Unemployed	205	21.15	3.39		29.92	2.85		8.35	1.26	
	Employed	295	21.84	2.62	**0.011***	30.05	2.91	0.632	7.98	1.20	**0.001***
Education	Upto10th standard	186	20.56	3.63		29.42	2.79	**0.001***	8.17	1.35	0.67
	More than 10th standard	314	22.14	2.33	**<0.001***	30.33	2.88		8.22	1.19	
SES	Class IV,V	92	20.71	3.74		29.80	3.01		7.95	1.31	0.034
	Class I,II,III	408	21.75	2.74	**0.002***	30.04	2.85	0.480	8.25	1.23	
Children < 12 years in the home	Yes	197	21.80	2.63		30.04	2.85		8.32	1.26	**0.008***
	No	303	21.40	3.18	0.142	29.93	2.92	0.675	8.01	1.21	

TABLE 2 MULTIVARIATE LINEAR REGRESSION OF ASSOCIATION BETWEEN DEMOGRAPHIC CHARACTERISTICS WITH KNOWLEDGE, ATTITUDE AND PRACTICE SCORES OF COVID-19

	Factor	Knowledge		Attitude		Practice	
		Unstandardized coefficient B (95%CI)	P value	Unstandardized coefficient *B* (95%CI)	P value	Unstandardized coefficient *B* (95%CI)	**P value**
Age	>35 years ≤ 35 years	0.33 (-0.22 to 0.84)	.230	0.69 (0.12 to 1.24)	**.016***	0.07 (-0.16 to 0.31)	.571
Gender	Female Male	0.03 (-0.61 to -0.64)	.928	0.01 (-0.61 to 0.57)	.954	0.34 (- 0.05 to 0 .61)	0.09
Occupation	Unemployed Employed	0.73 (0.12 to 1.28)	**.016***	0.21 (-0.34 to 0..76)	.469	0.53 (0.27 to 0.78)	**.002***
Education	More than 10th standard Upto10th standard	1.35 (0.73 to 1.95)	**.002***	0.75 (0.18 to 1.34)	**.012***	-0.01 (-0.24 to 0.25)	.936
SES	Class IV,V Class I,II,III	0.45 (-0.29 to 1.22)	.273	0.14 (-0.85 to 0.51)	.675	0.25 (-0.02 to 0.56)	.100
Children < 12 years in the home	Yes No	0.25 (-0.19 to -0.74)	.299	0.38 (-0.84 to 0.17)	.160	0.31 (0.07 to 0.57)	**.010***

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Original Research Article

Morbidity profile of truck drivers: a South Indian cross-sectional study

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ABSTRACT

Background: Recent studies have shown that workers on the transport industry are more predisposed to cardiovascular morbidity and other health morbidities due to their improper diet and sedentary lifestyle. This study was aimed at assessing the common morbidity profile among the truck drivers and associated demographic and clinical parameters considered relevant to the development of cardiovascular disease.
Methods: This was a cross sectional study conducted among 175 truck drivers aged 18-60 years at the Walayar check post, Coimbatore, southern India. After getting informed consent, a detailed physical examination was conducted. Socio demographic information was also elicited.
Results: It was observed that 50% of the truck drivers were overweight and obese. Hypertensives were 41.1% and diabetics were 12%. About 27.4% had raised systolic blood pressure (BP) and 29.1% had raised diastolic BP. Low back pain was observed in 34.3%, hip pain in 24.6%, knee pain in 9.7%, shoulder pain in 5.4%, diminished vision in 4.6%, giddiness in 2.3% and hearing defects in 1.1% of the truck drivers.56.6% were current smokers and 66.3% were taking alcohol consumption more than 4 times a month. Older age was found to be a significant independent predictor [odds ratio OR 1.04 (95% CI 1.03-1.05)] for the total number of cardio vascular disease morbidities.
Conclusions: We observed a higher morbidity among the truck drivers and observed that periodic health appraisals are needed.

Keywords: Truck driver, Morbidity, Hypertension

INTRODUCTION

Non communicable diseases are considered as one of the major health and development challenges of 21st century. The social and economic effect of increased morbidity and mortality associated with cardiovascular disease is a great challenge that has to be encountered in these decades.[1,2] Recent studies done in other countries have shown that workers in the transport industry are more predisposed to cardiovascular morbidity due to their improper diet and sedentary behavior and unhealthy life styles. The commonly seen morbidities reported among truck drivers were diabetes mellitus, obesity, hypertension and heart disease.[3-6] However, most of the research done in India among the truck drivers, have focused only on their sexual health. To the extent of our knowledge, very few studies were done in India to assess other co morbidities like cardiovascular morbidity profile. Many studies done in other parts of the country have reported that professional truck drivers and other road transport professionals have a higher risk of ischemic heart disease.[7-9]

Objectives

Considering the importance of truck drivers to our country's economy, more studies are needed to assess their morbidity profile in general and associated risk factors. Considering the inherent risks associated with the profession of truck driving and scarcity of studies aimed at evaluating the cardiovascular risk of morbidity profile in their profession, this study was aimed to assess the morbidity profile among truck drivers in general and to assess the independent predictors of the total number of cardiovascular risk factors identified among them. Thus, this study focused on truck drivers, whose working conditions favor the development of a set of cardiovascular risk factors and simultaneously impose difficulties in accessing health care and adhering to life style changes that enhance quality of life. The results would provide important information to be used at personal and community level to develop strategies and actions targeted at the prevention of cardio vascular disease and the promotion of general health of these workers.

METHODS

Study design, setting and participants

A cross sectional study was conducted on 25th August 2016 among 175 truck drivers aged 18-70 years at the Walayar check post, Coimbatore, Southern India. Ethical clearance was obtained from Institute Human ethics committee, PSG IMS&R. We also got permission from highway transport authorities. The study was conducted at Walayar check post which is located on a national highway of South India on 23rd August 2016 between 10am to 4pm. Drivers who came with valid driving license for submission of documents at the check post and those who gave informed consent were included. A detailed physical examination was conducted. Socio demographic questions were also included. Truck drivers with known congenital heart disease and genetic disorders were excluded. The truck drivers who exhibited risk factors were informed about their condition and advised to attend health care services.

Variables and measurement

Demographic details were collected first followed by measurement of systolic and diastolic blood pressures using mercury sphygmomanometer, two times over a period of 10 minutes and the mean value was calculated. Digital weighing machine was used to measure weight nearest to 0.1 kg. Height was measured with bare foot nearest to 0.1 cm using calibrated stadiometer. Random blood sugar was also measured. The following criteria and definitions were used: family history of CVD: when the individual mentions the occurrence of heart disease in a first degree relative, history of chronic diseases: truck drivers were asked whether they had been previously been diagnosed with any chronic diseases and what medicines they were taking, subjects were considered physically active if they are performing aerobic (walking, biking, dancing and racing) or anaerobic activities (body building) at least 150 minutes in a week and moderately active if they do 10 to 150 minutes in a week and insufficient if they do less than 10 minutes in a week, subjects were considered active smokers if they use tobacco daily in any quantity or at quit smoking in the previous 12 months. Subjects were considered non-smokers if they had not used tobacco for the past 12 months. For determining the body mass index (BMI) the truck drivers were weighed (in kg) without shoes on a 150kg mechanical anthropometric scale and height in meter. The BMI levels were classified according to the WHO guidelines; subjects were normal if their BMI was 18.5-24.5 kg/m^2, overweight if their BMI was 25.0-29.9 kg/m^2.[11] Grade 1 obese if the BMI was 30.0-34.9kg/m^2, grade 2 obese if the BMI was 35.0-39.9 kg/m^2 and grade 3 obese if the BMI was ≥40.0 kg/m^2. Hypertension was defined as systolic blood pressure greater than or equal to 140 mm Hg or diastolic blood pressure greater than or equal to 90 mmHg. Self-reported history of previously known disease was also included.

Sample size

With an expected prevalence of hypertension as 45% and 20% allowable error of prevalence, the minimum sample size required for the study was 122.[1] With a non-response rate of 20%, the total sample required for this study was 153. A total 175 truck drivers were studied.

Statistical methods

Data were analyzed using SPSS 19.Initially data were analyzed descriptively. The categorical variables were represented by absolute frequencies. The numerical variables were summarized with averages, minima, maxima and standard deviations. The existence of association between two categorical variables was verified using Chi square test. Student t test was used to compare the averages of the numerical variables between the groups of two independent samples. Mean (SD) were calculated for continuous variables and percentages for categorical variables. 95% confidence interval was calculated for prevalence assuming normal approximation. As the total number of morbidity is expected to follow Poisson regression, we have initially checked this assumption by analyzing whether mean of the total number of CVD risk factors is approximately equal to variance using Kolmogrov Smirnov Z test. After observing the goodness of fit, Poisson regression model was performed to determine the independent predictors of the total number of cardiovascular risk factors of the truck drivers. This includes diabetes mellitus, hypertension and overweight including obesity. The independent predictors considered were age, smoking, alcohol consumption, family history of CVD and duration of physical activity. A significant level of 5% used for all statistical tests.

RESULTS

Descriptive data

Table 1 shows some characteristics among the 175 truck drivers studied. The population consist young adults with mean age of 39.38 years with a range of 22 years- 67 years. 50% of the subjects were aged 40 years and above, 137 (78.3%) were ever married, 112 (64%) had education only up to 10th standard, 56% were current smokers, 66.3% consume alcohol more than 4 times a month and 42% had family history of CVD.

Table 1: Baseline demographic characteristics (n=175).

Variable	%	Minimum	Maximum	Mean	Median
Age (years)		22	67	39.38	39.00
Physical activity (in minutes per week)		0	460	93.25	0.00
Weight (kg)		46	100	73.23	73.00
Height (cm)		144	185.50	169.55	170.00
Body mass index (kg/m^2)		16.90	36.13	25.47	24.96
Systolic blood pressure (mmHg)		70	190	127.80	130.00
Diastolic blood pressure (mmHg)		55	120	80.29	80.00
Family history of CVD	41.7				
Smoking	56.0				
Alcohol consumption	66.3				

Table 2: Morbidity profile observed.

	Number (n=175)	%
Hypertension	72	41.1
Diabetes	21	12
Overweight/ obesity	86	49.1
Low back pain	60	34.3
Hip pain	43	24.6
Knee pain	17	9.7
Shoulder pain	10	5.4
Diminished vision	8	4.6
Giddiness	4	2.3
Hearing defects	2	1.1

Table 3: Distribution of hypertension according to body mass index.

Hypertension	BMI≤ 25 kg/m^2 (n=89)		BMI 25-29.99 kg/m^2 (n=72)		BMI≥ 30 kg/m^2 (n=14)	
	N	%	N	%	N	%
Systolic only	18	20.2	25	34.7	5	35.7
Diastolic only	19	21.3	24	33.3	8	57.1
Systolic + diastolic	27	30.3	35	48.6	8	57.1
General hypertension	28	31.5	36	50.0	8	57.1

Table 4: Distribution of Study subjects based on systolic and diastolic blood pressure.

Classification	Cut point	Number	%
Optimal	SBP<120	28	16
Normal	SBP 120-129	40	22.9
High normal	SBP 130-139	59	33.7
Hypertension	SBP ≥140	48	27.4
Optimal	DBP<80	49	28
Normal	DBP 80-84	73	41.7
High normal	DBP 85-89	2	1.1
Hypertension	DBP ≥90	51	29.1

Outcome data

The analysis of the parameters obtained during the clinical evaluation shows that (Table 2), 41.1% were having hypertension, 34.3% were having low back pain, 24.6% were having hip pain and 12 5% were diabetes. It was observed that 41.1% were overweight (Table 3) and 8.05 were obese. It was also observed that 1 subject was underweight. It was also observed that the mean BMI (Table 1) of 25.47 kg/m^2 was found to be in the overweight range (25 kg/m^2-29.99 kg/m^2). We are observing an increasing trend in the blood pressure according to the increase in BMI (Table 4).

Table 5: Factors associated with the total number of morbidity using Poisson regression model.

Variable	Odds ratio	95% CI	P value
Higher age	1.03	1.02 -1.05	p<0.001
Positive family history	1.04	0.83 -1.30	0.69
Smoking	1.04	0.83 -1.34	1.32
Alcohol consumption	0.82	0.65	1.04
Physical activity	1.00	0.99 -1.00	1.00

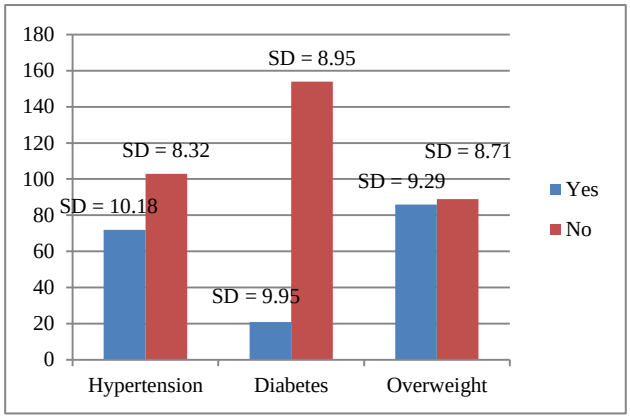


Figure 1: Mean (SD) of age (years) among cardio vascular disease morbidity.

In our study, 59 truck drivers (33.7%) were considered high normal and 48 truck drivers (27.4%) were hypertensive considering systolic blood pressure. Based on diastolic blood pressure, 2 drivers (1.1%) were considered as high normal and 51 drivers (29.1%) were hypertensive (Table 5). The prevalence of overweight and diabetes were higher in this study population compared to the recent study at Hyderabad in the year 2014. The prevalence of hypertension was similar to the reported prevalence of Hyderabad.[10]

Main results

It was observed that the mean is approximately equal to variance. From the results from Kolmogov Smirnov test, it is concluded that the total number of morbidity follows Poisson distribution (z=0.665, p=0.768) and hence decided to Poisson regression analysis. On using Poisson regression model, Age was found as a significant independent predictor [OR 1.03 (95% CI 1.02-1.05)] for the total number of cardio vascular disease morbidity. It was also observed that mean age of truck drivers with risk factors were between 42.45 years to 46.95 years which is the most productive years putting them at risk of CVD morbidity.

Other analyses

On further analysis it was observed that, a significant difference in the mean (SD) age of truck drivers between those who have hypertension 42.48 (10.18) compared with those who do not have hypertension 37.21 (8.32) (p<0.001). A similar difference in the age was observed for diabetes 46.95 (9.95) versus non diabetes 38.35 (8.95) (p<0.001) and for overweight and obesity 42.45 (9.29) versus others 36.41(8.71) (p<0.001).

DISCUSSION

The aim of the present study was to characterize a population of truck drivers with regard to demographic and clinical variables and the presence of cardiovascular risk factors. Our study highlights the need to retrospectively evaluate and systematize the presence of various risk factors in the population of long distance truck drivers. The main finding of our study is that truck drivers have a high prevalence of multiple factors that put them at high risk of cardiovascular events. This is an alarming prospect given the morbidity and mortality rates in the south Indian population and the risks these events pose to truck drivers and others who use roads.

Our study results showed that there is a high prevalence of behavioral risk factors such as overweight, obesity, hypertension, diabetes among the truck drivers and these risk factors were higher at older ages. A number of studies done on other parts other country have demonstrated that high prevalence of obesity among workers in the transport industry. According to Moreno et al., this category of workers in Brazil has a higher incidence of obesity, physical inactivity, inadequate diet, smoking habits, high levels of cholesterol and glycemia, hypertension, and obstructive sleep apnea in comparison to the general population in Brazil.[11,12] A study carried out in the United States involving more than 600 thousand workers found the highest prevalence of obesity to be among male employees who work as highway truck drivers.[13] A representative sample of the Australian in productive age compared ten different functional categories with regards to the risk of obesity and found that male employees of the transportation industry had a higher risk of overweight and obesity.[14] In our study, it was observed that nearly one third of the truck drivers were having low back pain (LBP). It has been pointed out by several studies that the factors leading occupational

LBP among truck drivers include exposure to vibration, repeatedly lifting heavy objects, strained postures, and sitting for work for many hours.[15] In a recent study conducted at Japan it was observed that 52.9% of the truck drivers were having LBP. Most drivers pointed out that vibration and road shock account for the LBP. Troup et al. noted that muscle fatigue due to mechanical stress such as road shock transmitted to the body and a restricted sitting posture contribute to truck drivers' LBP. From the viewpoint of prophylaxis, an improvement in the working condition is needed to reduce the incidence of drivers' LBP to some extent.

In this study we observed higher prevalence of hypertension among truck drivers. According to World Health Organization, systolic blood pressure greater than 115 mm Hg accounts 62% of cases of cardiovascular disease and 49% of cases of ischemic heart disease.[16] Moreover, arterial hypertension is one of the major risk factors of cerebro vascular accident. Many studies have also demonstrated an increase in the prevalence of systemic hypertension among professional drivers.[17,18]

In our study older age was found to be an independent predictor of the total cardiovascular morbidity profile life span. With aging, there is an increased acquisition of several CVD risk factors in an individual's life span. Several studies have shown that when age and other risk factors are used jointly to examine an individual's risk of CVD, it has been found that the contribution of age in the multivariate models may be a reflection of the intensity and duration of exposure to the traditional CVD risk factors.[19-23] Avoidance of these traditional risk factors at younger ages can reduce CVD risk associated with age per se.

People employed in the transport sector form a considerable workforce of our nation.[24-28] Intersectorial consideration between transport department and health department is needed to protect and provide health to transport employees. Thorough periodical medical examinations are to be conducted to these truck drivers and necessary steps are to be taken. In addition strict guidelines about diet are to be formulated to the truck drivers. In addition it is observed that the truck drivers must be educated at their younger age preferably early thirties to prevent overweight and obesity and thereby to reduce hypertension. Regular health education programs are to be conducted regarding various non-communicable diseases and attendance to such programs should be made compulsory. Strict guidelines about diet are to be formulated and implemented in all the eateries catering to the employees. Various recreational facilities, counseling centers with psychologists are to be established in all depots to help the employees cope with their stress.

Limitations

Our study has some limitations. First, this study does not allow the determination of causal associations since we used cross sectional study design. Another limitation of our study is that, we have not quantified the smoking and alcohol intake. Fasting blood sugar values were not studied. Because we were looking for a cluster of cardio vascular risk factors, another limitation of this study was the lack of measurements on lipid profiles or inflammatory markers.

CONCLUSION

In conclusion, we observed a higher morbidity among the truck drivers' ages. The data in the present study allow us to draw conclusion that truck drivers are highly vulnerable to developing CVD because of the high prevalence of variety of risk factors. Furthermore we must consider that the truck drivers we studied were adult men and that cardiovascular risk factors could decrease their maximum age and it affects their economy, the lives of the truck drivers and individuals who use the roads.

Recommendations and generalizability

More national studies are needed to look into what percentages of road traffic accidents involving human error and what percentages involve medical conditions involving cardiovascular conditions. Accordingly guidelines for the minimum standards required for fitness to drive those drivers with CVD. However, the driving restrictions may have disadvantage in the form of psychosocial effects on recipients and their family members. Instead, regular health education programs and appropriate healthcare action based on comprehensive public policies that focus on the truck drivers work environment and monitoring of these factors must be established at the national level. Accordingly various recreational facilities and counseling centers are to be established in highways for the truck drivers. Finally, intersectorial coordination between transport department and health department is needed to protect and promote health of transport employees.

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REFERENCES

1. Udayar SE, Sampath S, Arun D, Sravan S. Epidemiological study of cardiovascular risk factors among public transport drivers in rural area of Chittoor district of Andhra Pradesh. Int J Community Med Public Health. 2017;2(4):415-20.
2. Sharvanan EU, Konduru RK, Praveen BA Pawan K, sivachandran V, Srinivas T. Study of cardiovascular risk factors among transport drivers in rural area of Andhra Pradesh. Int J Res Health Sci. 2014;2(1):420-6.
3. Hirata RP, Sampaio LM, Leitão Filho FS, Braghiroli A, Balbi B, Romano S, et al. General characteristics and risk factors of cardiovascular disease among interstate bus drivers. Scientific World J. 2012;2012.
4. Tűchsen F, Andersen O, Costa G, Filakti H, Marmot MG. Occupation and ischemic heart disease in the European community: a comparative study of occupations at potential high risk. Am J Industrial Med. 1996;30:407-14.
5. Shin SY, Lee CG, Song HS, Kim SH, Lee HS, Jung MS, et al. Cardiovascular Disease Risk of Bus Drivers in a City of Korea. Ann Occupational Environ Med.1996;30:407-14.
6. Bigert C, Klerdal K, Hammer N, Hallqvist J, Gustavsson P. Time trends in the incidence of myocardial infarction among professional drivers in Stockholm 1977-96. Occupational Environ Med. 2004;61(12):987-91.
7. Malinauskiene V. Truck driving and risk of myocardial infarction. Przeglad lekarski. 2003;60:89-90.
8. Albright CL, Winkleby MA, Ragland DR, Fisher J, Syme SL. Job strain and prevalence of hypertension in a biracial population of urban bus drivers. Am J Public Health. 1992;82(7):984-9.
9. Ragland DR, Winkleby MA, Schwalbe J, Holman BL, Morse L, Syme SL, Fisher JM. Prevalence of hypertension in bus drivers. AAOHN J. 1989;37(2):71-9.
10. Sharma PK, Ganguly E. Morbidity profile of long distance truck drivers in Hyderabad city, India. J Dr. NTR Univ Health Sci. 2014;10;3(4):234.
11. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363:157-63.
12. Moreno CR, Louzada FM, Teixeira LR, Borges F, Lorenzi-Filho G. Short sleep is associated with obesity among truck drivers. Chronobiol Int. 2006;23(6):1295-303.
13. Caban AJ, Lee DJ, Fleming LE, Gómez-Marín O, LeBlanc W, Pitman T. Obesity in US workers: The national health interview survey, 1986 to 2002. Am J Public Health. 2005;95(9):1614-22.
14. Allman-Farinelli MA, Chey T, Merom D, Bauman AE. Occupational risk of overweight and obesity: an analysis of the Australian Health Survey. J Occupational Med Toxicol. 2010;5(1):14.
15. Troup JDG. Drivers bavk pain its prevention: A review of the postural, vibratory and muscular factors, together with the problem of transmitted road-shock. Applied Ergonomics. 1978: 207-214.
16. Kelsey JL, Hardy RJ: Driving of motor vehicles as a risk factor for acute herniated lumbar intervertebral disc. Am J Epidemiol. 1975;102:63-73.
17. Kelsey JL: An epidemiological study of acute herniated lumbar intervertebral disc. Rheumatol Rehab. 1975;14:144-59.
18. Frymoyer JW, Pope MH, Clements JH. Risk factors in LBP: An epidemiological survey. J Bone Joint Surg. 1983;65:213-8.
19. Bovenzi M, Zadini A. Self-reported Low Back Symptoms in Urban Bus Drivers Exposed to Whole-Body Vibration. Spine. 1992;17:1048-59.
20. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors and impact of urbanization. Circulation. 2001;104:2746-53.
21. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet. 2006;367(9524):1747-57.
22. Bigert C, Gustavasson P, Hallqvis J, Hogstedt C, Lewne M, Plato N, et al. Myocardial infarction among professional drivers. Epidemiology. 2003;14(3):333-9.
23. Mathew Anil C, Veyilmuthu R, Dhanush M, PremAnand ES, Xavier ChristuRajan V. Factors associated with hypertension among truck drivers: a cross sectional study at a check post on a national highway in South India. Int J Med Res Health Sci. 2017;6(5):126-9.
24. Allman-Farinelli MA, Chey T, Merom D, Bauman AE. Occupational risk of overweight and obesity: an analysis of the Australian Health Survey. J Occupational Med Toxicol. 2010;5(1):14.
25. Miyamoto M, Shirai Y, Nakayama Y, Gemebun Y, Kaneda K. An epidemiologic study of occupational low back pain in truck drivers. J Nippon Med School. 2000;67(3):186-90.
26. Ragland DR, Winkleby MA, Schwalbe J, Holman BL, Morse L, Syme SL, et al. Prevalence of hypertension in bus drivers. Int J Epidemiol. 1987;16(2):208-14.
27. Winkleby MA, Ragland DR, Fisher JM, Syme SL. Excess risk of sickness and disease in bus drivers: a review and synthesis of epidemiological studies. Int J Epidemiol. 1988;17(2):255-62.
28. Gersh BJ, Sliwa K, Mayosi BM, Yusuf S. Novel therapeutic concepts the epidemic of cardiovascular disease in the developing world: global implications. Eur Heart J. 2010;31(6):642-8.

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RESEARCH PAPER

Pediatric Appropriate Evaluation Protocol for India (PAEP-India): Tool for Assessing Appropriateness of Pediatric Hospitalization

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Objectives: To develop and assess Pediatric Appropriateness Evaluation Protocol for India (PAEP-India) for inter-rater reliability and appropriateness of hospitalization.

Design: Cross-sectional study.

Setting: The available PAEP tools were reviewed and adapted for Indian context by ten experienced pediatricians following semi-Delphi process. Two PAEP-India tools; newborn (≤28 days) and children (>28 days-18 years) were developed. These PAEP-India tools were applied to cases to assess appropriateness of admission and inter-rater reliability between assessors.

Participants: Two sets of case records were used: (*i*) 274 cases from five medical colleges in Delhi-NCR [≤28 days (*n*=51); >28 days to 18 years (*n*=223)]; (*ii*) 622 infants who were hospitalized in 146 health facilities and were part of a cohort (*n*= 30688) from two southern Indian states.

Interventions: Each case-record was evaluated by two pediatricians in a blinded manner using the appropriate PAEP-India tools, and 'admission criteria' were categorized as appropriate, inappropriate or indeterminate.

Main outcome measures: The proportion of appropriate hospitalizations and inter-rater reliability between assessors (using kappa statistic) were estimated for the cases.

Results: 97.8% hospitalized cases from medical colleges were labelled as appropriate by both reviewers with inter-rater agreement of 98.9% (k=0.66). In the southerm Indian set of infants, both reviewers labelled 80.5% admissions as appropriate with inter-rater agreement of 96.1% (*k*= 0.89).

Conclusions: PAEP-India (newborn and child) tools are simple, objective and applicable in diverse settings and highly reliable. These tools can potentially be used for deciding admission appropriateness and hospital stay and may be evaluated later for usefulness for cost reimbursements for insurance proposes.

Keywords: *Bed use, Cost, Hospital stay, In-patient, Utilization*.

Efficient and rationale allocation of health resources in India and other developing countries is essential. The recent National Health Policy of India and Ayushman Bharat Yojana aim at healthcare universalization and improving both out-patient and in-patient accessibility [1,2]. Hospitalizations consume a major proportion of healthcare resources in India and 50.9% of the resources are utilized for secondary (34.8%) and tertiary (16.1%) level services [3]. There is severe shortage of in-patient beds in India (0.7 beds/1000 population vs. world average of 3.96) with long waiting period for hospitalization particularly at government hospitals [4]. Standardizing admission and discharge processes has improved utilization, patient-flow and waiting time in several countries, and appropriateness evaluation protocols (AEP) are in wide use [5-7]. At present no Pediatric

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Appropriateness Evaluation Protocol (PAEP) tool is available for use in India.

We describe the development and pilot-testing of the PAEP-India tool to determine the inter-rater reliability for appropriateness of two sets of hospital admissions: (*i*) 274 in-patients from five medical colleges from Delhi and surrounding states and (*ii*) 622 hospitalized infants from a southern Indian cohort.

METHODS

Development of PAEP Tool

The tool development process used semi-Delphi technique [8]. The PAEP tool adaptation for India (PAEP-India) process followed the steps: (*i*) an expert group of ten experienced pediatricians from ten medical colleges, each with >10 years of clinical experience was constituted considering the differences in symptomology, diseases and threshold for hospitalization, the experts felt need for developing separate tools for newborns (≤28 days) and children (>28 days-18 years); (*ii*) review of the available literature was done and seven PAEPs (from 6 high- and 3 middle-income countries) were sourced [9-19]; (*iii*) two rounds of review and pilot testing of the tools was done by the experts; (*iv*) a face-to-face meeting was held for finalization of tools.

Each tool has two sections; 'admission criteria' to assess the appropriateness at admission and 'day of care' criteria for the appropriateness of hospitalization duration. For the PAEP- India (child) tool, the group made some amendments in both the admission criteria and day of care criteria for children (***Box* 1**).

The PAEP-India (newborn) tool was drafted with reference to the available management protocols for newborns [21-23]. The group did not suggest any change in the draft and finalized it. Finally, 'admission criteria' section comprised of 44 items in both the tools, and the 'day of care' for newborn and child tools comprised of 27 and 29 items, respectively (***Web Annexure* I** and **II**).

The group of ten pediatricians who participated in the development of the PAEP-India tools were invited as raters. Five pairs of raters were made and each pair was assigned the same set of case sheets in a blinded manner; raters in a pair were not from the same institution and did not know the other member of their pair. All the raters underwent orientation to have common understanding of using the PAEP reviewers' manual.

We had two sets of case-records drawn from different settings for inter-rater reliability assessment: 274 pediatric cases records drawn from five medical colleges located in Delhi and surrounding states (admitted during July-September 2015), and 622 cases-records from Kollam and Coimbatore, who were part of a cohort of 30688 infants recruited and followed-up in another study [24]. The case-records were anonymized and assigned unique study numbers.

The Ethics Committees of participating Institutes reviewed and approved the study. Waiver of informed consent was granted for data collection from the case records from five medical colleges. Informed consent was obtained for recruiting the infant cohort from Kollam and Coimbatore.

Statistical analysis: Inter-rater agreement was evaluated by the Cohen's kappa (*k*) statistic [25]. Landis and Koch guidelines were adopted as benchmark scales of κ coefficients (moderate: 0.41-0.60, substantial: 0.61-0.80, and almost perfect: 0.81-1.0) [26]. Statistical analyses was performed using STATA version 15.0 (StataCorp LLC, Texas, USA). Overall agreement was the proportion of judgements in which two raters agreed on categorizing as appropriate, inappropriate and indeterminate. We assessed the inter-rater reliability only for the 'admission criteria'; the 'day of care criteria' section could not be evaluated as complete clinical, nursing and laboratory assessment records were not available for most of the days in most of the case sheets in both datasets.

Box 1 ADMISSION CRITERIA AND DAY OF CARE CRITERIA FOR DEVELOPMENT OF PAEP-INDIA*(CHILD) TOOL

- "severity of illness" section, "any fever for >48 hours when a diagnosis has not been reached" was revised to ">72 hours".
- Considering accidents, "burns/inhalational injury" and "exposure to poison and snake/ scorpion bite" were added.
- Under the "severe electrolyte/acid base/ hematological abnormality" section, "hypocalcaemia", "raised creatinine", "thrombocytopenia", "increased respiratory rate" were added, modified "total leukocyte count cut-off to <5000/ mm^3" and added "raised diastolic blood pressure" to hypertension.
- Under the "intensity of services" section, revised "nebulisation use at least every 4 hours".
- Day of care criteria: added "lack of suitable care taker availability (for abandoned child)/protected place" under "patient condition" section; and clarified "unstable vitals in last 48 hours" under the "within 48 hours of the day reviewed" section.

* *PAEP-India: Pediatric Appropriateness Evaluation Protocol*

TABLE I CLINICAL DIAGNOSES IN THE TWO DATA SETS USED FOR INTER-RATER AGREEMENT FOR APPROPRIATENESS OF ADMISSION USING PAEP-INDIA TOOLS

Characteristic	*Delhi and Surroundings (n=274)*	*Kollam and Coimbatore (n=622)*
Children >28 d -18 y		
Infections	122	524
Acute respiratory infections	23	427
Acute gastroenteritis	26	33
CNS infections	20	
Urinary tract infections	3	19
Acute febrile illness[@]	28	25
Other infections[#]	22	20
Congenital diseases[€]	4	26
Other systemic diseases	43	68
Seizure/CNS disorders	15	25
Other medical disorders*	28	43
Surgical conditions	54	4
Gastrointestinal	19	4[^]
Urological	12	0
Other surgical conditions	23	0
Neonates (<28 d)		_
Medical problems	42	_
Neonatal sepsis	24	_
Other medical disorders[$]	18	_
Surgical conditions	9	_

*[@]Acute febrile illness including malaria, dengue, enteric fever, urinary tract infection, and other for evaluation; [#]Multiple system infection involves infection of more than one organ, may be with features of sepsis; [€]Congenital diseases including cardiac and central nervous system (CNS) malformations and other parts; *Other medical conditions including malignancy, coagulopathy, severe acute malnutrition, drug reaction, poisoning, constipation, nephrotic syndrome and hematemesis; [^]All 4 children had intussusception; [$]Other medical conditions in neonates included preterm care, respiratory distress syndrome, hyperbilirubinemia, hypo/hyper-glycemia, and birth asphyxia.*

RESULTS

The age strata of 274 case-records from Delhi and surrounding states were: ≤28 days (*n*=51), >28 days-12 months (*n*=48), 13-59 months (*n*=67), and >5-18 years (*n*=108). There were 54 surgical cases (20.8%). Out of 622 cases from southern India cohort, 471 (75.7%) were from Kollam and 151 (24.3%) were from Coimbatore. The median age at entry and exit were 48 days (range 32-175 days) and 153 days (range: 67-562 days), respectively. There were equal proportion of boys (50.3%) and girls (49.7%) in the cohort (***Table* I**). Out of 622 cases hospitalized to 146 hospitals, 50% (*n*=311) were admitted to tertiary (level 3), 49% (*n*=304) to secondary (level 2) and 1% (*n*=7) were admitted to primary care (level 1) care facilities respectively.

TABLE II INTER-RATER AGREEMENT FOR APPROPRIATENESS OF HOSPITALIZATION USING PAEP-INDIA TOOLS

Reviewer 1	*Reviewer 2*		
	Appropriate	*Inappropriate*	*Indeterminate*
Medical colleges in Delhi and surrounding areas (n=274)			
Appropriate	268	0	1
Inappropriate	2	1	0
Indeterminate	0	0	2
Hospitals in Kollam and Coimbatore (infants) (n=622)			
Appropriate	480	13	0
Inappropriate	9	68	2
Indeterminate	0	0	50

Kappa (*k*) coefficient was 0.66 (95% CI 0.30, 1.0) for the overall dataset for hospitalized cases in medical college. Both raters categorized 97.8% admissions as appropriate. The observed inter-rater agreements were >98% for aggregated and the disaggregated data according to age and gender. The agreement for PAEP appropriate cases and inappropriate cases were >98% with values of 0.66 and 0.49, respectively (***Table* II**).

The *k* for overall dataset was 0.89 (95% CI 0.84, 0.93) for the Kollam and Coimbatore infants. Overall both raters categorized 80.5% admissions as appropriate. The appropriate admissions in public and private hospitals were 84.5% and 78.5%, respectively. The observed agreement was >90% in most categories except for the level 1 hospitals (85.7%) which had just seven admissions.

DISCUSSION

This is the first effort to develop a tool for assessing the appropriateness of pediatric admission and hospitalization duration in India. The PAEP-India tools performed well for admission appropriateness assessment, both for newborns and children and across different levels of hospitals.

In the absence of a gold standard and true valid measure of appropriateness, the consensual validity is reflected through inter-rater agreement. Studies on admission appropriateness using the PAEP tools in different countries have reported variable levels of both observed agreement and kappa statistic (0.29-0.89) [9-12,14,16,19]. With PAEP-India tools, observed agreements were uniformly high with both the datasets.

WHAT IS ALREADY KNOWN?

- Country- and context-specific Pediatric Appropriateness Evaluation Protocol (PAEP) tools are in use for rational use of in-patient facilities.

WHAT THIS STUDY ADDS?

- India-specific PAEP tools for the assessment of appropriateness of pediatric and newborn hospitalizations were developed and tested for reliability with two sets of hospitalized children.

Despite a high observed agreement, lower kappa values may be observed, when the marginal values are imbalanced. On the contrary, higher kappa value may be observed for asymmetrical imbalanced marginal totals. Kappa is affected by prevalence and may not be reliable for rare observations. Thus very low kappa values may not necessarily reflect low overall agreement. Decision on performance of a tool should also consider the observed *versus* expected agreement, consistency across contexts, and suitability of the criteria for specific settings besides kappa statistics [27,28].

The reported proportion of appropriate pediatric admissions reported in literature range between 68%-89.5% and 59.3%-98% in high- and middle-income countries, respectively [9-12,14-16,19]. The proportion of appropriate admissions in the present study was high, particularly in medical colleges, in view of the higher demand and pressure for admission. A previous study reported that one-third of the adult patients overstay in hospitals [29]; which was triangulated by the perceptions of 83% of resident doctors and 43% of nurses in another study [30].

Awareness of the raters about the source of cases in first dataset might have influenced the high expected and observed agreements (>90%). For the second dataset (southern Indian infant cohort), the raters were neither aware about the hospitals nor involved in the study implementation or patient care. We could not assess the appropriateness of the duration of hospitalization due to lack of necessary information at the time of discharge. In the first dataset, the experts involved in development of PAEP-India tools also applied the tools in cases drawn from some medical colleges where they worked. These factors might have increased observed agreement and categorization of cases as appropriate. The findings for the level one health facilities from southern India may not be generalized as the number of hospitalizations were too small.

In conclusion, the PAEP-India tools performed consistently in two different settings demonstrating consensual validity. The advantages of the PAEP-India tools is their simplicity, objectivity and applicability in different hospitals. Further application and evaluation of these tools is required in diverse settings across India including health facilities of all levels for triangulating the evidence of its utility. Meanwhile the PAEP-India tools have potential application in insurance systems, quality assessment processes, and resource-allocation.

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REFERENCES

1. Ministry of Health and Family Welfare, Government of India. Ayushman Bharat Yojana (2018-2022). Government of India; 2018. Available from: *https://www.pradhanmantriyojana.in/ayushman-bharat-yojana/*. Accessed March 26, 2018.
2. Ministry of Health and Family Welfare, Government of India. National Health Policy, 2017. Government of India; 2017. Available from: *https://mohfw.gov.in/sites/default/files/9147562941489753121.pdf.* Accessed July 19, 2017.
3. National Health Systems Resource Centre. National Health Accounts Estimates for India (2013-14). Ministry of Health and Family Welfare Government of India; 2016. Available from:*https://mohfw.gov.in/sites/default/files/89498311221471416058.pdf.* Accessed July 19, 2017.
4. Planning Commission (Government of India). Report of the Working Group on Tertiary Care Institutions for 12th Five Year Plan 2012-2017. Government of India; 2011. Available from: *http://planningcommission.nic.in/aboutus/committee/wrkgrp12/health/WG_2tertiary.pdf.* Accessed July 19, 2017.
5. Ortiga B, Salazar A, Jovell A, Escarrabill J, Marca G, Corbella X. Standardizing admission and discharge processes to improve patient flow: A cross sectional study. BMC Health Serv Res. 2012;12:180.
6. Strumwasser I, Paranjpe NV, Ronis DL, Share D, Sell LJ. Reliability and validity of utilization review criteria. Appropriateness evaluation protocol, standardized

medreview instrument, and intensity-severity-discharge criteria. Med Care. 1990;28:95-111.
7. Kemper KJ, Fink HD, McCarthy PL. The reliability and validity of the pediatric appropriateness evaluation protocol. QRB Qual Rev Bull. 1989;15:77-80.
8. McMillan SS, King M, Tully MP. How to use the nominal group and Delphi techniques. Int J Clin Pharmacy. 2016;38:655-62.
9. Werneke U, Smith H, Smith IJ, Taylor J, MacFaul R. Validation of the paediatric appropriateness evaluation protocol in British practice. Arch Dis Child. 1997;77:294-8.
10. Esmail A. Development of the paediatric appropriateness evaluation protocol for use in the United Kingdom. J Public Health Med. 2000;22:224-30.
11. Esmaili A, Seyedin H, Faraji O, Arabloo J, Qahraman Bamdady Y, Shojaee S, *et al*. A pediatric appropriateness evaluation protocol for Iran children hospitals. Iran Red Crescent Med J. 2014;16:e16602.
12. Smith HE, Sheps S, Matheson DS. Assessing the utilization of in-patient facilities in a Canadian pediatric hospital. Pediatrics. 1993;92:587-93.
13. Gloor JE, Kissoon N, Joubert GI. Appropriateness of hospitalization in a Canadian pediatric hospital. Pediatrics. 1993;91:70-4.
14. Henley L, Smit M, Roux P, Zwarenstein M. Bed use in the medical wards of Red Cross War Memorial Children's Hospital, Cape Town. S Afr Med J. 1991;80:487-90.
15. Shafik MH, Seoudi TMM, Raway TS, Al Harbash NZ, Ahmad MMA, Al Mutairi HF. Appropriateness of pediatric hospitalization in a general hospital in Kuwait. Med Princ Pract. 2012;21:516-21.
16. Kreger BE, Restuccia JD. Assessing the need to hospitalize children: Pediatric appropriateness evaluation protocol. Pediatrics. 1989;84:242-7.
17. Werneke U, MacFaul R. Evaluation of appropriateness of paediatric admission. Arch Dis Child. 1996;74:268-73.
18. Bianco A, Pileggi C, Trani F, Angelillo IF. Appropriateness of admissions and days of stay in pediatric wards of Italy. Pediatrics. 2003;112:124-8.
19. Formby DJ, McMullin ND, Danagher K, Oldham DR. The appropriateness evaluation protocol: application in an Australian children's hospital. Aust Clin Rev. 1991;11:123-31.
20. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? Lancet. 2003;361: 2226-34.
21. Agarwal R, Deorari A, Paul VK. AIIMS Protocols in Neonatology. 1st ed. New Delhi: CBS Publishers; 2015.
22. National Neonatology Forum, India. Evidence based clinical practice guidelines. National Neonatology Forum; 2010. Available from: *http://www.nnfi.org/*. Accessed July 19, 2017.
23. Ministry of Health and Family Welfare, Government of India. Facility based new born care training packages. Government of India; 2014. Available from: *http://nhm.gov.in/nrhm-components/rmnch-a/child-health-immunization/child-health/guidelines.html*. Accessed April 16, 2016.
24. World Health Organization. Global Advisory Committee on Vaccine Safety, 15-16 June 2016. Wkly Epidemiol Rec. 2016;91:341-8.
25. Cohen J. A Coefficient of agreement for nominal scales. Educ Psychol Meas. 1960;20:37-46.
26. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159-74.
27. Feinstein AR, Cicchetti DV. High agreement but low kappa: I. The problems of two paradoxes. J Clin Epidemiol. 1990;43:543-9.
28. Cicchetti DV, Feinstein AR. High agreement but low kappa: II. Resolving the paradoxes. J Clin Epidemiol. 1990;43:551-8.
29. Chakravarty A, Parmar N, Bhalwar R. Inappropriate use of hospital beds in a tertiary care service hospital. Med J Armed Forces India. 2005;61:121-4.
30. Kanwar V, Salaria N, Khanduja P. How do nurses perceive hospital bed utilization: A prerequisite for quality improvement in nursing care. Int J Health Sci Res. 2014;4135-41.

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SHORT COMMUNICATION

Papillon–Lefèvre syndrome (PLS) with novel compound heterozygous mutation in the exclusion and Peptidase C1A domains of Cathepsin C gene

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Abstract

Papillon Lefevre syndrome (PLS) manifests with palmoplantar keratoderma, combined with a rapidly progressive periodontitis associated with mutations in Cathepsin C (*CTSC*) gene. This article reports a 15-year old male proband with typical PLS traits having a novel compound heterozygote with p.Q49X mutation in exon 1 and p.Y259C missense mutation in exon 6 of *CTSC* gene respectively. The exon 1 mutation, p.Q49X, (found in proband's mother) was located in exclusion domain and exon 6 mutation, p.Y259C (found in proband's father), was present in peptidase C1A, papain C-terminal domain. Interestingly, missense mutation p.Y259C identified in this study was found to be not reported so far. Upon computational analysis, this missense mutation was found to be lethal. Moreover, our protein modelling approach using mutant protein revealed the presence of monomeric structure on contrary to the tetrameric structure of the wild type protein. In addition, in vitro functional characterization of mutant p.Y259C expressed in HEK293 cells showed a significant reduction in CTSC activity (0.015 ± 0.009 mU/ml) when compared with wild type protein (0.21 ± 0.008 mU/ml). Thus, in this study, we have demonstrated that the pathogenic missense mutant p.Y259C might cause PLS by impaired CTSC function.

Keywords Papillon Lefevre syndrome · Cathepsin C · Compound heterozygote · Missense mutation · Palmoplantar keratoderma.

Abbreviations

PLS	Papillon lefevre syndrome
CTSC	Cathepsin C

Introduction

Papillon–Lefèvre syndrome (PLS) is an autosomal recessive disorder characterized by palmoplantar keratoderma in association with aggressively progressing periodontitis. Keratosis can occur in the knees and elbows [1, 2]. Mutations in *CTSC* gene predisposes to PLS [3] with an increased risk of medical manifestations in consanguineous marriages. Haim munk syndrome and PLS share common features but with supplementing components like arachnodactyly, acroosteolysis and onychogryphosis is found only in the former.

CTSC gene finds its locus on chromosome 11q14-q21 [4, 5] and encodes a lysosomal cysteine protease involved in activation of many serine proteinases within immune cells. It is involved in cell-mediated cytotoxicity, phagocytic destruction of bacteria, local activation or deactivation of cytokines. Many reports on *CTSC* mutation leading to loss of function

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of CTSC protein and development of PLS has been reported [6, 7].

In our study, the proband suspected to be a PLS case was screened for *CTSC* gene mutation and found to have a compound heterozygous mutation, p.Q49X in exon 1 and p.Y259C in exon 6 of *CTSC* respectively. p.Y259C is a novel missense mutation not reported so far in association with PLS. It is a pathogenic mutation with a monomeric structure and had reduced enzyme activity.

Materials and methods

Patient and family unit

A 15-year old boy who attended the Dermatology outpatient clinic of PSG Hospitals, Coimbatore, India was clinically examined for dermatological, dental and radiological findings. The patient and his parents consented for genetic analysis and therefore, blood samples were drawn from them. Third degree parental consanguinity was documented. The phenotypic characteristics were then compared with the genotype results.

Mutation analysis of *CTSC*

Genomic DNA was isolated using EZ-10 Spin Column Blood Genomic DNA MiniPreps Kit (Bio Basic Canada Inc). The seven exons of *CTSC* gene were amplified using the primer sets reported [6]. The PCR reaction mixture of 20 µl containing 50 ng DNA as the template, 50 nM each of the forward and the reverse primer, 1 mM each of dNTPs, in a 1X PCR buffer (with1.5 mM $MgCl_2$) with 1U of Taq Polymerase (Ampliquon, Germany) was incubated in a thermal cycler (Eppendorf, USA). The cycling parameters included 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 60 °C annealing for 40 s and extension of 72 °C for 1 min. The amplified exons were sequenced by Sanger's sequencing and analysed.

Computational analysis

The CTSC protein was analysed using InterProscan and PROSITE database tools to identify significant domains and functional active sites in the protein respectively. In order to predict the potential pathogenecity of *CTSC* mutations, two bioinformatics tools were used, PolyPhen-2 (Polymorphism Phenotyping v2) in a scale from 0.00 (for benign) to 1.00 (for deleterious) (http://genetics.bwh.harvard.edu/pph2) and Mutation Taster (http://www.mutationtaster.org/). Multiple protein sequence alignments were performed using COBALT (Constraint-based Multiple Alignment Tool). Moreover, the wild type and the mutant proteins were modelled using SWISS MODEL (https://swissmodel.expasy.org) tool to obtain the comparative 3D structure of both the proteins.

Site directed mutagenesis

The Q5 Site-Directed Mutagenesis Kit (NEB, USA) was used to construct the missense mutant, Y259C. The kit manufacturer's instructions were followed to obtain the mutant clones and were confirmed through sequencing.

Cell culture and transfection

HEK293T cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma Aldrich, USA) at 37 °C in a 5% CO_2 atmosphere. The cells were transiently transfected with the Lipofectamine 3000 reagent (Invitrogen, USA) and Opti-MEM Reduced Serum Medium in 24 well plates. After 48 h, the medium was replaced with complete DMEM with 100 µg Hygromycin to obtain the cells bearing the plasmid (pCMV3) with Hygromycin resistance.

Mutant and wild type CTSC protein expression and purification

The transfected cells were disrupted in 100 µl lysis buffer (10 mM Na_3PO_4, 0.1% Triton X-100) and centrifuged at 2500 g for 5 min at 4 °C. The supernatant (5 µg per lane) was separated on 10% SDS PAGE. Proteins were quantified using the Bradford assay. The His—CTSC protein fractions were selectively purified using Ni-NTA column (Qiagen, Germany). The protein mixture was incubated in the binding buffer (20 mM Tris/HCl, 200 mM NaCl; pH 7.5), washed (20 mM Tris/HCl, 200 mM NaCl, 5 mM imidazole; pH 7.5) and eluted (20 mM Tris/HCl, 200 mM NaCl, 200 mM imidazole; pH 7.5) to obtain pure protein fractions. The protein fractions were allowed to undergo activation by with 20 µg/ml papain at 37 °C, pH 6.5 for 6 h to generate active peptides of CTSC. To inhibit the activity of the mature enzyme, 1 mM iodoacetamide (Sigma Aldrich, USA) was used.

Recombinant CTSC enzyme activity assay

Enzymatic activity was assayed by mixing 20 µl of the purified protein with 30 µl reaction buffer (100 mM Na_3PO_4, 2 mM NaCl, 2 mM DTT), 30 µl dilution buffer (10 mM Na_3PO_4, 0.1% Triton -X-100), and 100 µl of chromogenic CTSC substrate (1.5 mM gly-phe-para-nitroanilide). Absorbencies were measured at 405 nm, samples were incubated at 37 °C for 1 h, and read again. Cathepsin C activity of samples, expressed as para-nitroanilide released (nmol/mg of protein * hour), was determined interpolating

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the difference between first and second reads in a standard curve (0.0351–2.24 nmoles) of pure 4 para-nitroaniline (4 pNA) (Sigma-Aldrich, USA) [8].

Statistical analyses

Data were expressed as mean ± standard error of the mean (SEM) from three independent experiments. The data were evaluated for statistical significance with unpaired *t* test. $p < 0.05$ was considered statistically significant. All the statistical analyses were carried out using GraphPad Prism software version 5.0 Windows (GraphPad Software, USA).

Results

Clinical manifestations of the patient with PLS

A 15 year old boy, born out of third degree consanguineous marriage, presented to the dermatology OPD with complaints of thickened skin involving the palms and soles since infancy. He had erythematous lesions involving elbows, knees and few areas of trunk. The patient gave history of failure of eruption of lower incisors following the fall of primary teeth. The patient had developed multiple furuncles in the past. On examination, the patient had diffuse palmoplantar keratoderma with multiple erythematous papules and plaques seen involving the dorsum of hands, feet, elbows, knees and few areas of the trunk. On examining the teeth, the lower central incisors were absent (Fig. 1a). The nails showed multiple transverse ridging. Biospy done from the cutaneous lesions showed evidence of psoriasiform hyperplasia of epidermis with dermis being unremarkable. Routine blood investigations and X ray of the hands were normal.

CTSC mutation analysis

The seven exons of the *CTSC* gene from the proband and parents were sequenced using Sanger sequencing method. Further, *CTSC* gene analysis (NCBI reference sequences for mRNA: NM_001814.6, Concensus CDS: CCDS44693.1 and Protein: NP_001805.4) of the proband showed a compound heterozygous mutation, Q49X in exon1 and Y259C in exon 6 (Fig. 1b, c). The parents were heterozygous carriers of the mutations and were unimpacted. The nonsense mutation *c.145C > T* (p.Q49X) in exon 1 was inherited from mother while missense mutation *c.776A > G* (p.Y259C) in exon 6 was from father (ESM_1).

Evolutionary conservation of *CTSC* and in silico analysis

The mutations, p.Q49X and p.Y259C belonged to the exclusion domain (25-142 aminoacids) and Peptidase C1A, papain C-terminal domain (231-458 aminoacids)

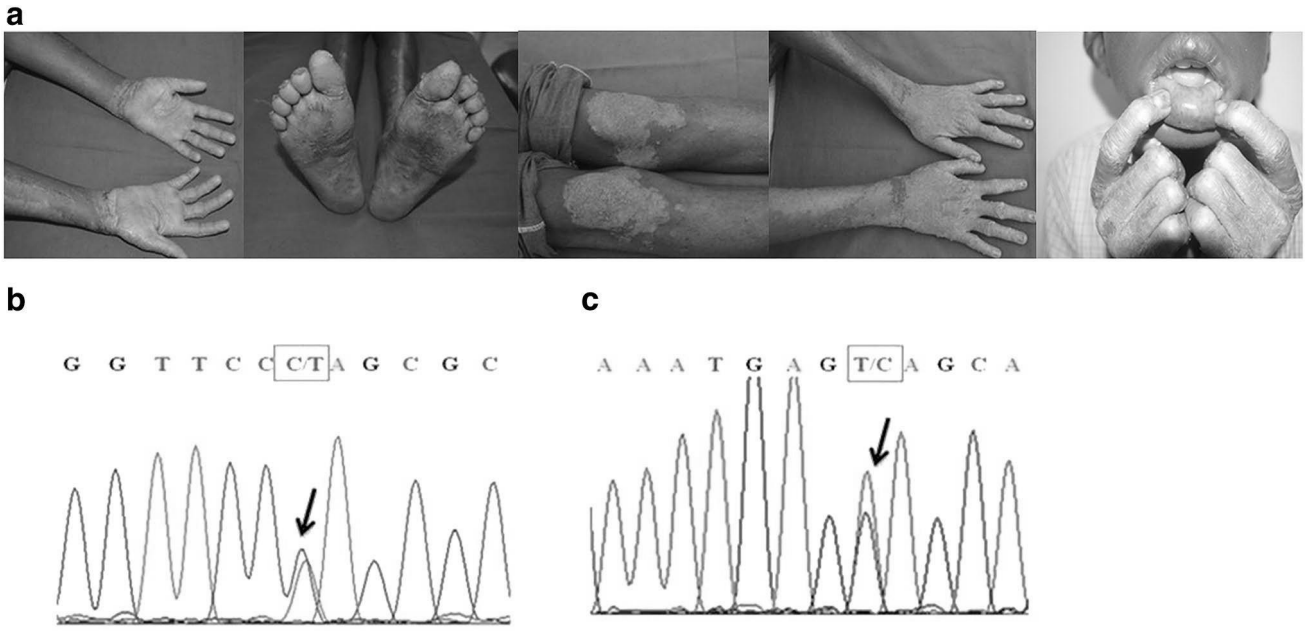


Fig. 1 Clinical observations and sequence results of *CTSC* in the patient. **a** Clinical examinations in the patient revealed multiple erythematous papules and plaques seen involving the dorsum of hands, feet, elbows, knees and few areas of the trunk. The nails showed multiple transverse ridging. The lower central incisor teeth were absent. Compound heterozygous mutation in the *CTSC* gene of the proband revealing **b** heterozygous mutation (*c.145C > T*) in exon 1 and **c** heterozygous mutation (*c.776A > G*) in exon 6

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respectively as identified from InterProscan analyses. The PROSITE database search revealed that cathepsin C protein has a thiol (cysteine) protease enzyme whose active sites are composed of Cys258, His405 and Asn427. PolyPhen2 predictions of the functional effect of the missense mutation proved that mutation was predicted to be damaging with a score of 1.00. Another tool, Mutation Taster also predicted this mutation to be disease causing. Moreover, the aminoacid Y (tyrosine) at 259th position was found to be highly conserved among different species like house mouse, rat, zebra fish, dog, cattle, chimpanzee, monkey, chicken and frog (Fig. 2a).

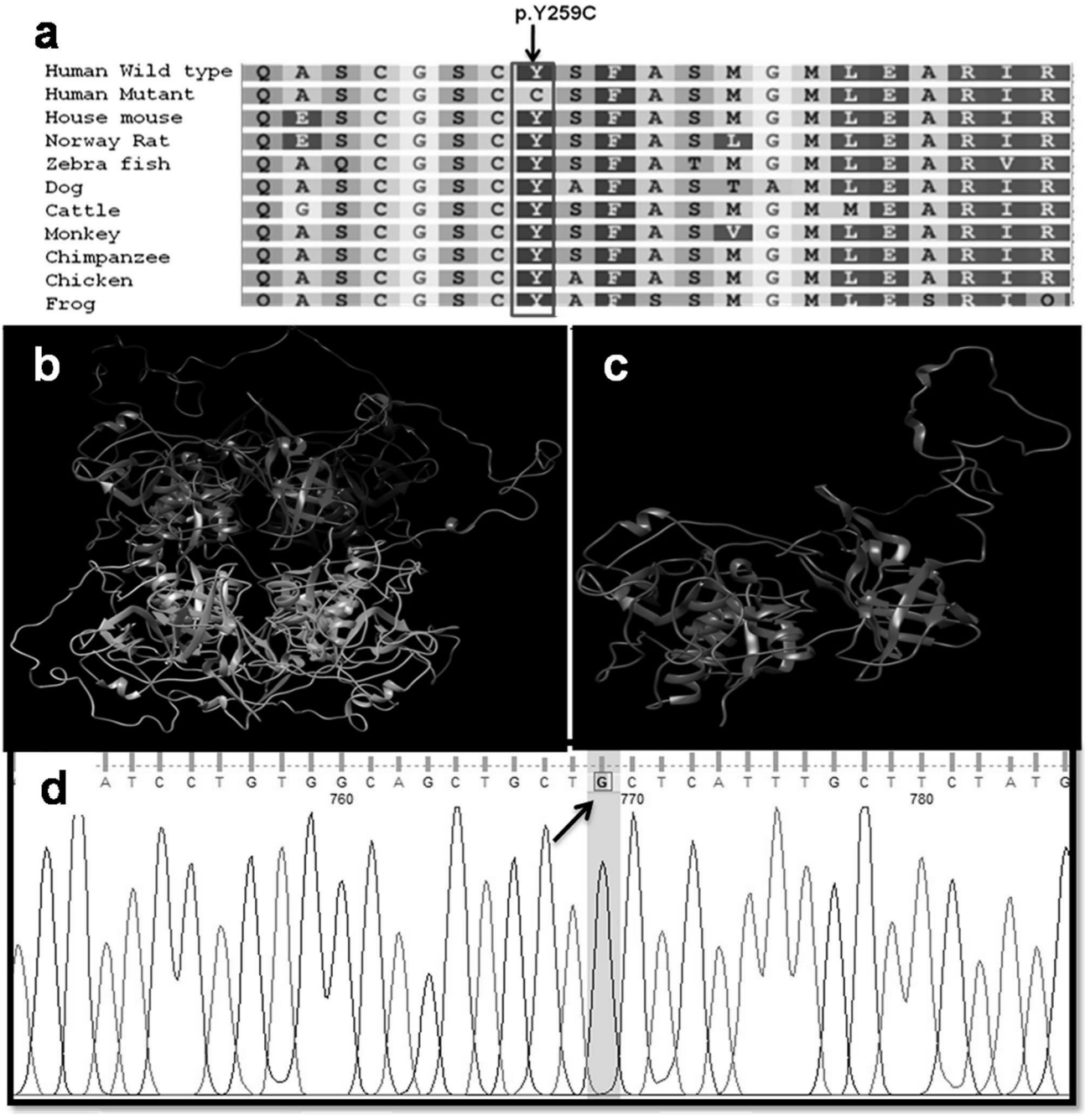


Fig. 2 Confirmation of pathogenecity of p.Y259C mutation of *CTSC* gene. **a** Cross-species multiple sequence alignment showing the high conservation of the aminoacid Y at 259th position. 3D structure of the proteins modelled using SWISS MODEL software showing the **b** tetrameric structure of the wild type protein-A chain (red), B chain (blue), C chain (green), D chain (pink) and **c** monomeric structure of the mutant protein (p.Y259C)-A chain (red) as predicted by the software. **d** Electropherogram of the mutant clone confirming *c.776A > G* mutation in CTSC gene to construct pCMV3-CTSC Y259C vector

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The protein sequence derived from the wild type and mutant protein (Y259C) were subjected to sequence based structure prediction using SWISS MODEL protein modelling software. The wild type protein was predicted by the SWISS MODEL as a tetramer having A, B, C and D chains (Fig. 2b). On the other hand, the mutant protein carrying the novel mutation p.Y259C was predicted to be only a monomer having the A chain (Fig. 2c).

In vitro expression of mutant p.Y259C CTSC protein

The pCMV3-CTSC vector was subjected to site directed mutagenesis to create a p.Y259C mutation. After mutagenesis, the clones were confirmed by Sanger's sequencing (Fig. 2d). To obtain the rCTSC protein, the following vectors were transfected into HEK 293T cell lines; pCMV3-CTSC, pCMV3-CTSC Y259C and pCMV3 negative control vector. After transfection, the cells bearing hygromycin resistant phenotypes were selectively harvested. DNA was isolated from the harvested cells and *CTSC* gene *was* amplified (Fig. 3a). The cells were lysed to obtain the protein fractions which were purified. The purified protein (inactive) was of size 55 kDa which is a propeptide. It was activated by papain treatment which yielded bands of sizes 25 kDa and 6 kDa (Fig. 3b).

Mutant p.Y259C CTSC shows reduced activity

The mutant p.Y259C CTSC protein showed a statistically significant reduction in its activity (0.015 ± 0.009 mU/ml) when compared to wild type CTSC protein (0.21 ± 0.008 mU/ml). Upon treatment with 1 mM iodoacetamide, a cysteine protease inhibitor, the activity of both mutant and wild type protein was reduced by 10 folds (Fig. 3c).

Discussion

PLS is caused by mutation in cathepsin C (*CTSC*) gene which encodes a lysosomal enzyme expressed predominantly in palms, soles, elbow, knee and gingiva. This explains the disordered keratinization leading to cutaneous lesions in these areas. Cathepsin C also plays an important role in the function of polymorphonuclear cells and macrophages, thus explaining the reason for recurrent pyoderma and gingivitis in PLS [9].

Among the PLS cases, missense mutations predominates nonsense and frameshift mutations in *CTSC* gene. The first compound heterozygous individual with PLS was reported in 2000 [10]. In the current study, a nonsense mutation p.Q49X (*c.145C > T*) and a missense mutation p.Y259C (*c.776A > G*) was present in a compound heterozygous state in the proband. This nonsense mutation truncated the protein to a 49 amino acid polypeptide as a nonfunctional enzyme resulting in PLS phenotype. p.Q49X has been reported in an Indian family coupled with PLS [11] and Haim Munk syndrome [12].

A missense mutation *c.776A > G* (p.Y259C) observed in the affected patient was located in Peptidase C1A, papain C-terminal domain which is important for the intracellular protein degradation activity of the enzyme as this domain corresponds to the 233 amino acids mature enzyme unit [13] of cathepsin C. Moreover, this mutation Y259C lies next to the active site Cys258, thus, raising a suspicion of any possible Cys-Cys disulfide bond formation between Cys258 and Cys259 in the mutant protein and abolishing the active site availability. The 3D structure prediction results show that the p.Y259C mutant protein lacked the tetrameric structure unlike the wild type protein and

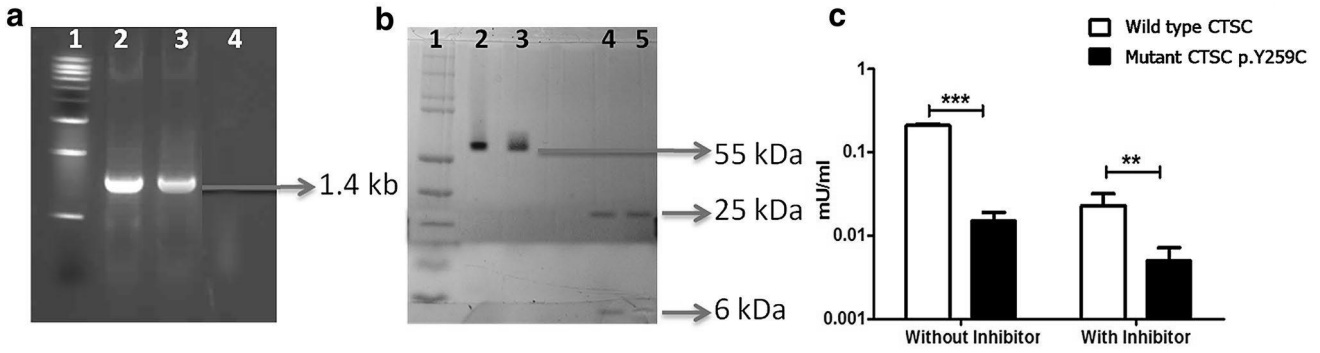


Fig. 3 Cloning, expression and enzyme activity of wild type and p.Y259C mutant CTSC protein. **a** *CTSC* amplified from cells transfected with wild type (lane 2), mutant plasmid (lane 3) and negative control vector (lane 4). **b** The image shows propeptide form of His-CTSC (lane 2) and His-CTSC Y259C (lane 3) purified from the cell lysate of wild type and mutant plasmid transfected HEK 293T cell lines. The active form of His-CTSC (lane 4) and His-CTSC Y259C (lane 5) after papain treatment is also shown. 5 µg of the protein was loaded onto 10% SDS PAGE and followed by Coomassie blue staining. Lane 1: Prestained protein ladder (Biorad, USA). **c** Mutant CTSC (p.Y259C) protein showed a statistically significant 14 fold reduction in the enzyme activity when compared to wild type CTSC protein. 1 mM iodoacetamide inhibited the CTSC enzyme activity proportionally in mutant CTSC (p.Y259C) and wild type CTSC by tenfolds

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had a monomeric structure (A chain). It is reported that tetramerization is crucial for the enzymatic activity [14]. Therefore, the structure modelled by SWISS MODEL substantiates the fact that this missense mutation has resulted in the development of PLS phenotype by abolishing the formation of tetrameric structure. Similarly, structural effects leading to functional abnormalities were also reported as a result of P35delL and R272P mutations in CTSC gene [15]. The inactive form of CTSC protein was of 55 kDa which after maturation yielded bands of molecular weights of 25 kDa and 6 kDa observed in earlier studies [16].

This missense mutation identified in our study, p.Y259C, was found as a novel pathogenic mutation associated with PLS syndrome In addition, there was a statistically significant 14 fold reduction in CTSC enzyme activity in the mutant protein when compared to the wild type protein. The presence of cysteine protease inhibitor, iodoacetamide further reduced the enzyme activity in both the wild type and the mutant proteins by 10 folds as observed in other studies [17]. Similarly, 85% reduction in the enzyme activity was observed in Mexican patients with PLS in a study [18]. Recently, many reports on requirement of early identification of PLS and proper dental management was emphasized [19–22]. Therefore, our study has helped to broaden the list of reported mutations associated with PLS.

Author contributions SM and TS: Conceptualization, design of study and interpretation; SM: Laboratory experimentation and Writing—original draft preparation; BP and RR: Clinical data collection interpretations; SR, TS and RS: Writing—review and editing; SR and RS: Funding acquisition and supervision; All the authors reviewed and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors did not declare any conflict of interest.

Ethical approval This study was approved by the Institutional Human Ethics Committee (IHEC), PSG Instituteof Medical Sciences and Research, Coimbatore, India and the study procedures were in accordance with the1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The patient and his parents gave written informed consent for geneticanalysis and for a future possible publication.

References

1. Papillon MN, Lefevre B (1924) Two cases of familial symmetric palmoplanter keratosis (Maleda's disease) in a brother and his sister. Alterations in both cases (in French). Bull Soc Francaise Dermatologie Syphiligraphie 31:81–84
2. Fageeh HN (2018) Papillon-Lefèvre syndrome: a rare case report of two brothers and review of the literature. Int J Clin Pediatr Dent 1:352–355. https://doi.org/10.5005/jp-journals-10005-1523
3. Korkmaz B, Caughey GH, Chapple I et al (2018) Therapeutic targeting of cathepsin C: from pathophysiology to treatment. Pharmacol Ther 190:202–236. https://doi.org/10.1016/j.pharmthera.2018.05.011
4. Hart TC, Bowden DW, Ghaffar KA, Wang W, Cutler CW, Cebeci I et al (1998) Sublocalization of the Papillon-Lefevre syndrome on chromosome 11q14-q21. Am J Med Genet 79(19980901):134–139
5. Lingeswaran A, Gopal SD (2018) A rare presentation of psychotic depression with suicidality in a case of papillon–lefèvre syndrome. Indian J Psychol Med 40:372–374
6. Toomes C, James J, Wood AJ, McCormick D, Lench N, Hewitt CH et al (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. Nat Genet 23:421–424. https://doi.org/10.1038/70525
7. Chedid JA, Salameh M, El-Outa A, Noujeim ZEF (2019) Papillon-Lefèvre Syndrome: diagnosis, dental management, and a case report. Case Rep Dent. https://doi.org/10.1155/2019/4210347
8. Bullón P, Castejón-Vega B, Román-Malo L et al (2018) Autophagic dysfunction in patients with Papillon-Lefèvre syndrome is restored by recombinant cathepsin C treatment. J Allergy Clin Immunol 142:1131–1143
9. Hattab FN (2019) Papillon-Lefèvre syndrome: from then until now. Stomatol Dis Sci 3:1. https://doi.org/10.20517/2573-0002.2018.22
10. Allende LM, García-Pérez MA, Moreno A, Corell A, Carasol M, Martínez-Canut P et al (2001) Cathepsin C gene: first compound heterozygous patient with Papillon-Lefèvre syndrome and a novel symptomless mutation. Hum Mutat 17:152–153
11. Selvaraju V, Markandaya M, Prasad PV, Sathyan P, Sethuraman G, Srivastava SC (2003) Mutation analysis of the cathepsin C gene in Indian families with Papillon-Lefèvre syndrome. BMC Med Genet 4:5. https://doi.org/10.1186/1471-2350-4-5
12. Rai R, Thiagarajan S, Mohandas S, Natarajan K, Shanmugasekar C, Ramalingam S (2010) Haim Munk syndrome and Papillon Lefevre syndrome–allelic mutations in cathepsin C with variation in phenotype. Int J Dermatol 49:541–543. https://doi.org/10.1111/j.1365-4632.2010.04300.x
13. Nakano A, Nomura K, Nakano H, Ono Y, LaForgia S, Pulkkinen L, Hashimoto I, Uitto J (2001) Papillon-Lefèvre syndrome: mutations and polymorphisms in the cathepsin C gene. J Invest Dermatol 116:339–343. https://doi.org/10.1186/1471-2350-4-5
14. Nagy N, Vályi P, Csoma Z, Sulák A, Tripolszki K, Farkas K et al (2014) CTSC and Papillon-Lefèvre syndrome: detection of recurrent mutations in Hungarian patients, a review of published variants and database update. Mol Genet Genomic Med 2:217–228. https://doi.org/10.1002/mgg3.61
15. Moghaddasian M, Arab H, Dadkhah E, Boostani H, Babak AR, Abbaszadegan MR (2014) Protein modeling of cathepsin C mutations found in Papillon-Lefèvre syndrome. Gene 538:182–187. https://doi.org/10.1016/j.gene.2013.11.079
16. Yang W, Xia W, Mao J, Xu D, Chen J, Feng S et al (2011) High level expression, purification and activation of human dipeptidyl peptidase I from mammalian cells. Protein Expr Purif 76:59–64. https://doi.org/10.1016/j.pep.2010.09.001
17. Dağlioğlu C (2017) Cloning, expression, and activity analysis of human cathepsin C in the yeast Pichia pastoris. Turk J Biol 41:746–753. https://doi.org/10.3906/biy-1704-4
18. Romero-Quintana JG, Frías-Castro LO, Arámbula-Meraz E, Aguilar-Medina M, Dueñas-Arias JE, Melchor-Soto JD et al (2013) Identification of novel mutation in cathepsin C gene causing

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Papillon-Lefèvre Syndrome in Mexican patients. BMC Med Genet 14:7. https://doi.org/10.1186/1471-2350-14-7
19. Yousry YM, El-Latif AEA, El-Gawad RYA (2018) Case report: clinical manifestation and dental management of Papillon-Lefèvre syndrome. F1000Res 7:1420. https://doi.org/10.12688/f1000research.16042.1
20. Tyagi S, Thomas AM, Balla V, Kundra R (2019) Cu-sil dentures: a novel approach of Papillon-Lefèvre syndrome management. J Indian Soc Pedod Prev Dent 37:303–307. https://doi.org/10.4103/JISPPD.JISPPD_256_18
21. Millet C, Virard F, Lienhart G, Ducret M (2020) Digital prosthodontic management of a young patient with Papillon-Lefèvre syndrome: a clinical report. J Prosthet Dent 123:548–552. https://doi.org/10.1016/j.prosdent.2019.03.013
22. Giannetti L, Apponi R, Dello Diago AM, Jafferany M, Goldust M, Sadoughifar R (2020) Papillon-Lefèvre syndrome: oral aspects and treatment. Dermatol Ther. https://doi.org/10.1111/dth.13336

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PREVALENCE OF DEPRESSION AND ITS ASSOCIATED FACTORS IN ADULTS WITH DIABETES MELLITUS: A CROSS-SECTIONAL STUDY IN A TERTIARY CARE CENTRE

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ABSTRACT

The prevalence of Diabetes Mellitus has been rising steadily owing to several factors such as sedentary lifestyle, obesity and an aging population. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India with up to 79.4 million individuals in India. Depression occurs frequently with diabetes but there are not many studies in India to estimate its prevalence and associated factors. This study was done with the aim of estimating the prevalence of depression among diabetes patients using the validated Patient Health Questionnaire-9 and also its associated factors. A cross sectional study was done on 100 consecutive patients with Diabetes Mellitus (both type 1&2) attending the Outpatient Endocrinology clinic from September 2015 to November 2015. The Patient Health Questionnaire diagnostic algorithm was used to identify major depression. The covariates studied were age, gender, duration of diabetes, presence of hypertension and glycaemic control. Chi-square test was done to find the association of demographic and clinical characteristics of depression. Multiple logistic regressional analysis was done to identify the statistically significant variables. The prevalence of major depression was found to be 35%. People with hypertension were found to have a significantly higher prevalence of depression (adjusted odds ratio=3.02, 95% CI: 1.17 to 7.7, P=0.022). Also, employed people were at a lower risk for depression when compared to the unemployed /retired population (adjusted odds ratio=0.319, 95% CI: 0.11-0.85, P=0.023).Interestingly age, duration of diabetes and glycaemic control were not significantly associated with depression. Over one-third of the patients with diabetes mellitus had depressive symptoms. The prevalence varied by demographic and clinical characteristics.

KEYWORDS

Diabetes, Depression, Patient Health Questionnaire- 9, Hypertension.

1. INTRODUCTION

The prevalence of Diabetes Mellitus has been rising steadily owing to several factors such as sedentary lifestyle, obesity and an aging population. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India with up to 79.4 million individuals in India.[1]

Depression occurs frequently with diabetes and has been found to be associated bidirectionally with diabetes mellitus.[2,3] However, it was observed that the reported prevalence in the studies varied tremendously in terms of definition, study design, source of subjects, time frame and measurement methods.[4-6] Moreover, with the predicted rise of diabetes in India, and risk factors

for depression such as low socioeconomic status, financial constraints being rampant here it is quintessential to establish regional studies as a guideline to aid in a holistic patient approach. [7]

Apart from this, most of these studies used self-administered questionnaires which cannot directly establish a diagnosis of depression. [4,5,6,8] Standard interviews, such as the Structured Clinical Interview for DSM Disorders (SCID)[9] and the Composite International Diagnostic Interview (CIDI) [10] can yield a clinical diagnosis according to the Statistical Manual of Psychiatric Disorders, 4th edition (DSM-IV) [11] ; however, lengthy assessments and high costs preclude the extensive use of such structured diagnostic interviews in large population-based surveys.

The Patient Health Questionnaire (PHQ) can establish provisional diagnoses of major and minor depression as well as evaluate the severity of depressive symptoms. [12] ; The PHQ-9 diagnosis of major depression yielded acceptable sensitivity and specificity. The present study aims to estimate the prevalence of depression among diabetes patients using the validated Patient Health Questionnaire-9 and its associated factors.

The variables taken into consideration for association with depression in this region include age, gender, duration of diabetes, glycaemic index and presence of hypertension . This stems from previous studies which show significant correlations between the above variables and depression[6,13,14]

2. MATERIALS AND METHODS:

This study is a cross-sectional study on 100 consecutive patients presenting at a tertiary care centre, aged >18yrs diagnosed with Type 2 diabetes mellitus by a certified Endocrinologist. The study was carried out after obtaining institutional ethics committee clearance. Written informed consent was obtained from the study participants and the PHQ-9 questionnaires were distributed. Those subjects that couldn't read were assisted in filling the questionnaires by an interpreter (Figure 1). During multivariate analysis we have combined the scores 0-9 (none, minimal and mild depression categories) as "no major depression" and scores of 10-27 (moderate, moderately severe and severe categories) as " major depression".[15,16]

Patients with complications from diabetes (retinopathy, neuropathy, nephropathy), previous history of mental illness, previous illnesses known to increase a risk for depression (CAD,CVA), and patients already on antidepressants for other symptoms were excluded from the study.

The demographic variables considered were age, gender, duration of diabetes, glycaemic index and presence of hypertension. Age was measured in completed years. Duration of diabetes and glycaemic control were taken from the patients' case records. Hypertension was defined in the study as systolic BP level of ≥140mmHg and diastolic BP of ≥90mmHg, or current treatment for hypertension with prescription medication. [17,18]

Data were analysed using SPSS(19). Chi-square test was done to find the association of demographic and clinical characteristics of depression. Further, multiple logistic regressional analysis was done to identify the statistically significant variables. *P* value of <0.05 was considered as statistically significant

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

NAME:_____ DATE:_____

Over the last *2 weeks*, how often have you been bothered by any of the following problems?
(use "✓" to indicate your answer)

	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself—or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or the opposite — being so figety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead, or of hurting yourself	0	1	2	3
	add columns		+	+
(Healthcare professional: For interpretation of TOTAL, please refer to accompanying scoring card).	TOTAL:			

10. If you checked off *any problems*, how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?	Not difficult at all _____ Somewhat difficult _____ Very difficult _____ Extremely difficult _____

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Figure 1: The Patient Health Questionnaire -9

3. RESULTS:

Among the 100 patients studied, 35 were found to have major depression (Figure 2). In the univariate analysis, those who were having hypertension and those who were unemployed were significantly associated with depression (P<0.05). In the multiple regression analysis(Table I), people with hypertension were found to have a significantly higher prevalence of depression (adjusted odds ratio=3.02,95% Confidence Interval :1.17 to 7.7,P=0.022) (Figure 3).Also, employed people were at a lower risk for depression when compared to the unemployed /retired population(adjusted odds ratio=0.319,95% Confidence Interval :0.11-0.85,P=0.023) (Figure 4) that bar graph has nothing written on x axis. Interestingly age, duration of diabetes and glycaemic control were not significantly associated with depression.

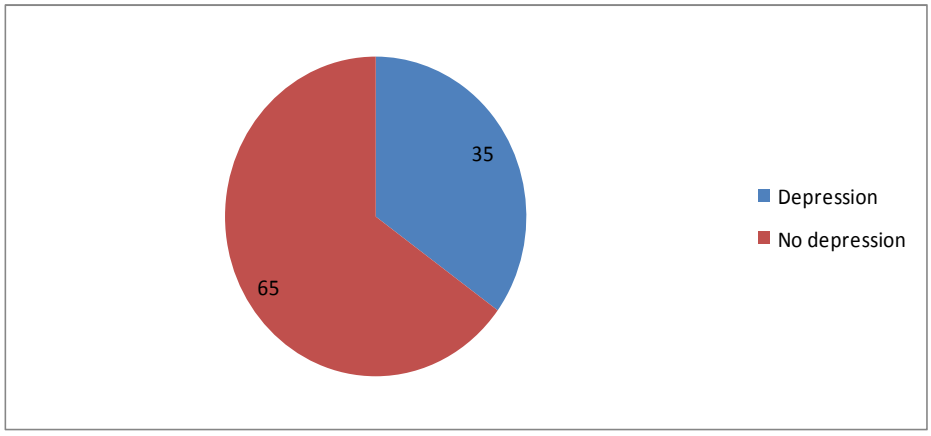


Figure 2. Percentage of patients with and without depression among the study group.

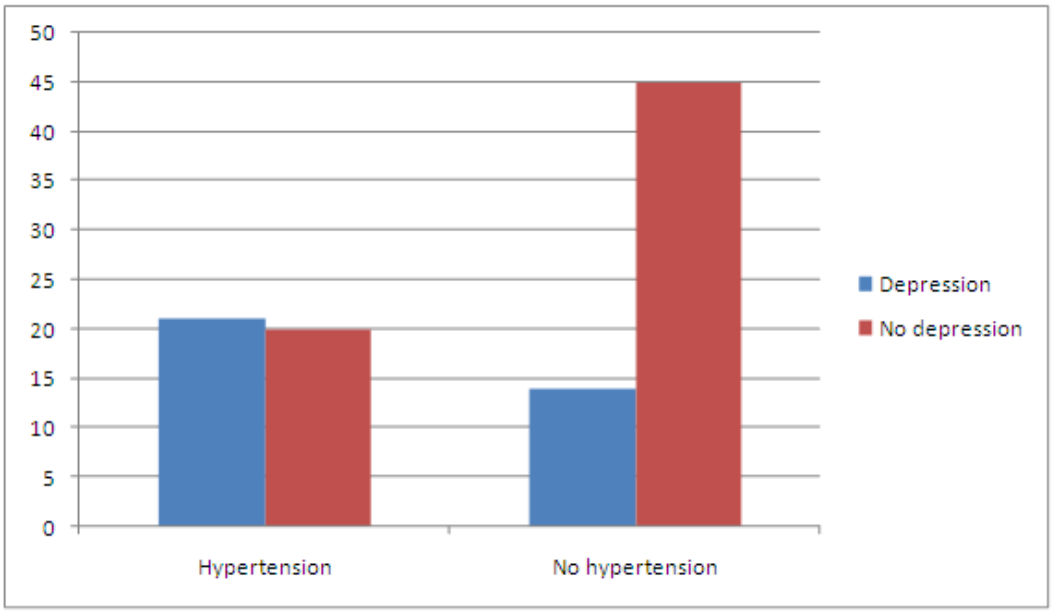


Figure 3. Comparison of the prevalence of Depression in patients with and without Hypertension

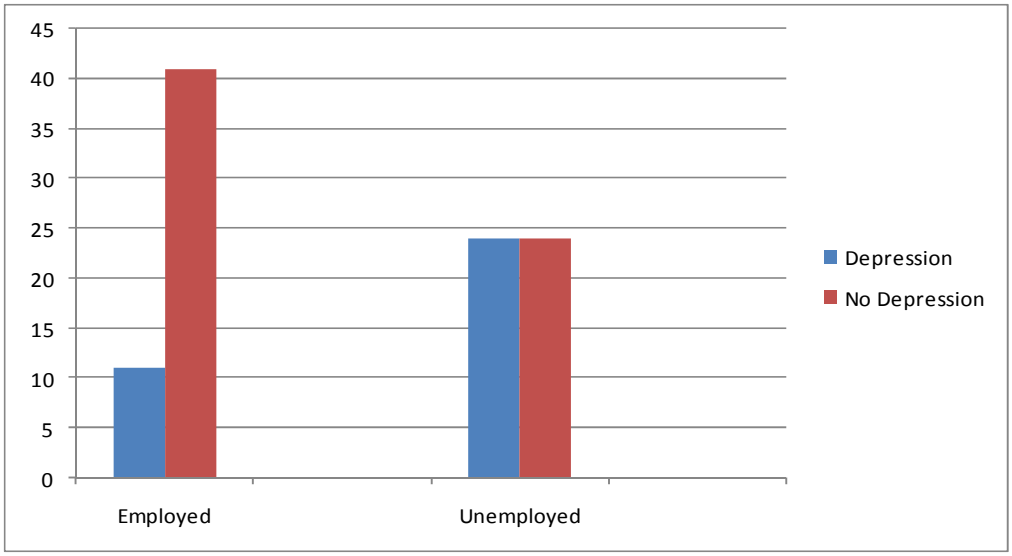


Figure 4. Prevalence of depression among employed and unemployed study participants

Table 1. Regression analysis of the associated factors of depression

Variable	Number studied	Percentages with depression	Adjusted odds ratio	*P* value*
Age				
<40 years	11	36.4	1	
More than 40 years	89	34.8	0.40(0.08-1.91)	0.251
Gender				
Male	51	27.5	1	
Female	49	42.9	1.32 (0.51-3.41)	0.513
Duration				
Less than 5 years	31	32.3	1	
More than 5 years	69	36.2	1.03(0.36-2.96)	0.946
Glycemic control				
<7	45	26.7	1	
>7	55	41.8	2.34(0.91-6.04)	0.077
Employment				
Unemployed/Retired	48	50.0	1	
Employed	52	21.2	0.31(0.11-0.85)	0.023*
Hypertension				
No	59	23.7	1	
Yes	41	51.2	3.02(1.17-7.77)	0.022*

4. DISCUSSION:

In the present study the prevalence of depression among patients with diabetes mellitus was found to be 35% which was slightly smaller than the prevalence in a study conducted by Joseph N et al., in an adjoining area that reported a prevalence of 45.3%.[13] It was also found to be more than twice as common as depression in the general population of our region.[7,19]

This leads us to ask the more imposing question, “Why is depression more prevalent in people with diabetes mellitus?”. The study by Talbot F et al., suggested that the extent to which diabetes intrudes in life, rather than diabetic complications per se or personal control, is a key factor in

relation to depressive symptomatology in individuals with diabetes. [20]In an animal model, Wang and colleagues demonstrated that experimentally induced type 1 diabetes impairs hippocampal neurogenesis, which may contribute to diabetes-related depression.[21] Another study demonstrated decreased plasma brain-derived neurotrophic factor (BDNF) in patients with type 2 diabetes compared to controls, which has been hypothesized to play a role in learning and memory and has also been implicated in the pathogenesis of depression.[22]

Among the risk factors, depression was found to be significantly associated with hypertension. This is in line with the findings of Saeed AK et al., where the prevalence of depression in hypertensive people with diabetes mellitus was 57% whereas in people with diabetes alone it was 40%. (P is less than 0.05). [23] There are also other studies which showed that depression is common in patients with uncontrolled hypertension irrespective of presence of diabetes and may interfere with blood pressure control. [24, 25] Studies have shown that plasma noradrenaline, as an indirect marker of sympathetic tone, was elevated in patients with essential hypertension.[26] There is also reported evidence showing abnormal autonomic nervous system function in depression, specifically regarding increased sympathetic activity and poor vagal control. Several studies have examined cerebrospinal fluid (CSF), plasma or urinary levels of norepinephrine (NE) and its major central nervous system metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG). Plasma NE and/or MHPG levels have been reported to be elevated in depressive patients.[27,28] Thus, the existing relationship between hypertension and depression by itself could have contributed to the statistically significant presence of depression among hypertensive people with diabetes mellitus.

Also, we found that depression among diabetes was significantly associated with employment status with a higher prevalence in the unemployed population. This is in line with other studies which showed a higher prevalence of depression in the unemployed population. [13,29,30] The unemployed individuals lack sociological functions such as time structure, status and identity, social contacts, participation in collective purposes and regular activity which could predispose them to depression.[30]

A methodological innovation of our study was the application of the PHQ-9[12] which was developed for clinical use, scoring each of the DSM IV criteria on grades from 0-3 based on the frequency of the symptoms[31] Scores of 20-27,15-19,10-14,5-9 and 1-4 represent severe, moderately severe, moderate, mild and minimal depression respectively. [11]

Our results indicate that a substantial number of people with diabetes are at an increased risk of having major depression, and those who care for patients with diabetes should routinely screen them for major depression using the PHQ-9 or other such instruments. Among the umpteen methods to screen for depression, the PHQ-9 is a promising tool with its ease of administration, convenient scoring system, brief and simple questions. Most importantly, the use of PHQ-9 can serve in comparisons of depressive symptomatology on an international basis given its worldwide validation. [32,33]

This study has some limitations. The study was conducted in a tertiary care hospital, so a possible selection bias cannot be excluded. This could be because the patients coming to the tertiary care hospital belong to the relatively higher socioeconomic status of the community. A large sample community based study could throw more light on this relationship. Since this study is a cross-sectional study, temporal relationship between depression and diabetes cannot be determined.

5. CONCLUSIONS

Over one-third (35%) of the patients with diabetes mellitus had depressive symptoms. It is widely associated with unemployment and co-existent hypertension in this population.

This emphasizes that the mind/body dualism is a false dichotomy and there is an increasing need to integrate mental health services in these patients. We recommend routine screening of diabetic patients with PHQ-9 to identify those at risk as it is a quick and easy tool to identify depression and refer patients appropriately for further counselling and support.

In addition, the issue of depression in association with socioeconomic factors such as unemployment is to be addressed by the health policy to aim for an overall betterment of the welfare of the people in this region.

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REFERENCES

[1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes-estimates for the year 2000 and projections for 2030. Diabetes Care2004;27:1047–53.

[2] Katon WJ.The Comorbidity of Diabetes Mellitus and Depression. Am J Med 2008;121(11, Suppl 2):S8–15.

[3] Golden SH, Lazo M, Carnethon M, Bertoni AG, Schreiner PJ, Diez Roux AV, et al. Examining a bidirectional association between depressive symptoms and diabetes. JAMA 2008;299:2751-9.

[4] Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes:a meta-analysis. Diabetes Care 2001;24:1069-78.

[5] Ali S, Stone MA, Peters JL, Davies MJ, Khunti K. The prevalence of comorbid depression in adults with type 2 diabetes: a systematic review and meta-analysis. Diabet Med 2006;23:1165-73.

[6] Barnard KD, Skinner TC, Peveler R. The prevalence of co-morbid depression in adults with type 1 diabetes: systematic literature review. Diabet Med 2006;23:445-8.

[7] Poongothai S, Pradeepa R, Ganesan A, Mohan V. Prevalence of Depression in a Large Urban South Indian Population — The Chennai Urban Rural Epidemiology Study (Cures – 70). PLoS One 2009;4:e7185.

[8] Fisher L, Skaff MM, Mullan JT, Arean P, Mohr D, Masharani U,et al. Clinical depression versus distress among patients with type 2 diabetes: not just a question of semantics. Diabetes Care 2007:30:542-8

[9] Spitzer RL, Williams JB, Gibbon M, First MB. The Structured Clinical Interview for DSM-III-R (SCID). I. History, rationale, and description. Arch Gen Psychiatry 1992;49:624–9.

[10] Wittchen HU. Reliability and validity studies of the WHO–Composite International Diagnostic Interview (CIDI): a critical review. J Psychiatr Res 1994;28:57-84.

[11] American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV [Internet]. 4th ed. Washington (DC): American Psychiatric Association; 1994 [cited 2010 Mar 8]. 866 p. Available from: http://www.psychiatryonline.com/DSMPDF/dsm-iv.pdf

[12] Kroenke K, Spitzer RL. The PHQ-9: a new depression diagnostic and severity measure. Psychiatric Annals 2002;32:509–21.

[13] Joseph N, Unnikrishnan B, RaghavendraBabu YP, Kotian MS, Nelliyanil M. Proportion of depression and its determinants among type 2 diabetes mellitus patients in various tertiary care hospitals in Mangalore city of South India. Indian Journal of Endocrinology and Metabolism 2013;17:681–88.

[14] Roy T, Lloyd CE. Epidemiology of depression and diabetes: a systematic review. J Affect Disord 2012:142 Suppl:S8-21

[15] Arroll B, Goodyear-Smith F, Crengle S, Gunn J, Kerse N, Fishman T, et al. Validation of PHQ-2 and PHQ-9 to Screen for Major Depression in the Primary Care Population. Ann Fam Med 2010;8:348–53.

[16] Sawaya H, Atoui M, Hamadeh A, Zeinoun P, Nahas Z. Adaptation and initial validation of the Patient Health Questionnaire - 9 (PHQ-9) and the Generalized AnxietyDisorder - 7 Questionnaire (GAD-7) in an Arabic speaking Lebanese psychiatric outpatient sample. Psychiatry Res 2016;239:245-52.

[17] The fifth report of the Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med 1993;153:154-83.

[18] Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee: Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.

[19] Egede LE, Zheng D, Simpson K. Comorbid depression is associated with increased health care use and expenditures in individuals with diabetes. Diabetes Care 2002;25:464–70.

[20] Talbot F, Nouwen A, Gingras J, Bélanger A, Audet J. Relations of diabetes intrusiveness and personal control to symptoms of depression among adults with diabetes. Health Psychol 1999;18:537-42.

[21] Wang S, Sun Z, Guo Y, Yuan Y, Yang B. Diabetes impairs hippocampal function via advanced glycation end product mediated new neuron generation in animals with diabetes-related depression. ToxicolSci 2009;111:72-9.

[22] Krabbe, K.S., Nielsen, A.R., Krogh-Madsen, R., Plomgard, P.,et al., Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. Diabetologia 2007;50:431-8.

[23] Saeed AK, Al-Dabbagh TQ. Type 2 diabetes and its association with hypertension and depression in an Iraqi population. Ann Saudi Med 2003;23:254-9.

[24] Rubio-Guerra AF, Rodriguez-Lopez L, Vargas-Ayala G, Huerta-Ramirez S, Serna DC, Lozano-Nuevo JJ.Depression increases the risk for uncontrolled hypertension. Experimental and Clinical Cardiology 2013;18:10-2.

[25] Nabi H, Chastang JF, Lefèvre T, DugravotA, Melchior M, Marmot MG, et al. Trajectories of depressive episodes and hypertension over 24 years:The Whitehall II prospective cohort study. Hypertension 2011;57:710–6.

[26] Goldstein D, Lake C. Plasma norepinephrine and epinephrine levels in essential hypertension. Federation Proceedings 1984;43:57-61.

[27] Lake CR, Pickar D, Zeigler MG, Lipper S, Slater S, Murphy DL. High plasma norepinephrine levels in patients with major affective disorder. Am J Psychiatry. 1982;139:1315-8.

[28] Roy A, Pickar D, Linnoila M, Potter WZ. Plasma norepinephrine levels in affective disorders. Relationship to melancholia. Arch Gen Psychiatry 1985;42:1181-5.

[29] Robert Friis , G. Nanjundappa. Diabetes, depression and employment status. SocSci Med 1986;23:471-75.

[30] Strandh M, Hammarström A, Nilsson K, Nordenmark M, Russel H. Unemployment, gender and mental health: the role of the gender regime. Sociol Health Illn.2013;35:649-65.

[31] Kroenke K, Spitzer RL, Williams JBW. The PHQ-Validity of a Brief Depression Severity Measure. J Gen Intern Med 2001;16:606–13.

[32] Burdzovic Andreas J, Brunborg GS. Depressive Symptomatology among Norwegian Adolescent Boys and Girls: The Patient Health Questionnaire-9 (PHQ-9) Psychometric Properties and Correlates. Front Psychol. 2017:8: 887.

[33] Titov N, Dear BF, McMillan D, Anderson T, Zou J, Sunderland M. Psychometric comparison of the PHQ-9 and BDI-II for measuring response during treatment of depression. Cogn Behav Ther. 2011:40:126-36

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Consumption of Tea and its Relation to Risk of Type 2 Diabetes mellitus

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ABSTRACT

Aim: The aim of the study was to examine the association of consumption of tea with the risk of type 2 diabetes mellitus. **Methods:** A case control study was conducted at PSG Hospitals between 1st June 2017 and 1st July 2017. All those who had newly diagnosed type 2 diabetes mellitus and attended Department of Endocrinology for follow-up visit were the cases and the relatives accompanying them without the history of diabetes mellitus were the controls. Tea consumption and other dietary factors were elicited. The mediating factors studied were age, sex, Body Mass Index (BMI), educational levels, smoking status, alcohol consumption, family history of diabetes mellitus, potassium intake, magnesium intake and use of statins. **Results:** After adjusting the potential confounders, tea consumption was inversely associated with risk of type 2 diabetes mellitus with odds ratio of 0.652 (95% CI = 0.43 – 0.98). The association was also observed with participants who consumed tea 3 or 4 cups per day compared to those who did not drink tea on a daily basis (odds ratio = 0.368, 95% CI = 0.14 – 0.96). However, the association was not observed among those who take 5 or more cups of tea per day (odds ratio = 0.328, 95% CI = 0.06 – 1.60).**Conclusion:** Our findings suggest that consumption of 3 to 4 cups of tea per day has a decreased risk of type 2 diabetes mellitus. The results from our study also suggest that adjusting for mediating factors did not alter the results and hence other unknown factors may explain inverse relation between tea consumption and type 2 diabetes mellitus. Further studies are warranted.

Keywords: Tea consumption, type 2 diabetes mellitus, chocolate consumption, potassium intake

Introduction

The prevalence of type 2 diabetes mellitus has increased dramatically in the past decades [1, 2].

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The majority of cases of diabetes mellitus could be prevented by changes in life style. However, the association of tea consumption with the risk of type 2 diabetes mellitus is not entirely clear. Few studies found an inverse association between tea intake and type 2 diabetes mellitus, while few other studies did not observe an association between tea consumption and risk of type 2 diabetes mellitus [3-6].The underlying mechanism regarding the relation between tea consumption and type 2 diabetes mellitus is also

unclear. Several components of tea have been suggested as possible causal factors [7].Magnesium plays a role in regulating insulin action and is inversely associated with insulin sensitivity and type 2 diabetes mellitus. Potassium intake has also been associated with reduced risk of diabetes mellitus [8].So far; there is no conclusive evidence about whether these factors explain the association of tea intake and type 2 diabetes mellitus. Besides these, earlier studies have not accounted for the effect of long term medication such as statin use which may confound the results. Therefore the present study is aimed to investigate the association of tea consumption with the risk of type 2 diabetes mellitus adjusting for multiple covariates.

Materials and Methods

Study population and design

A case-control study was conducted at PSG Hospitals. All those who have newly diagnosed in the past 1 year as type 2 diabetes mellitus and attending the Department of Endocrinology for follow up between 1stJune 2017 – 1st July 2017 were considered as cases. Controls were those who accompany them and do not have history of diabetes mellitus. We then retrospectively evaluated their food practices and other demographic information.

General assessments

The general assessment survey was conducted at the outpatient Department of Endocrinology, PSG Hospitals, Coimbatore. The general questionnaire contained questions on demographic characteristics. Body weight (in kg) and height (in meter) were also measured. However in few cases, we were not able to measure height. Food intake was assessed using a validated Food Frequency Questionnaire in this population and from this magnesium intake and potassium intake were estimated. The questionnaire also asked about smoking status, practice of alcohol intake, whether previously diagnosed as diabetes mellitus, other medical histories and medications taken. The information on baseline consumption of coffee, tea and other beverages were also collected. We also collected the data about consumption of chocolates, since chocolates are also a source of caffeine.

Statistical analysis

To examine the association between tea consumption and incidence of type 2 diabetes mellitus, we used four logistic regression models. In the first model, odds ratio and 95% confidence intervals for type 2 diabetes mellitus were calculated against a reference group of low consumers adjusted for age (continuous) and gender (male/female). A second logistic regression model corrected the other known diabetes mellitus risk factors which include smoking status (present/absent), BMI, education attainment (classes up to 12th standard/college and above), family history of diabetes mellitus (present/absent); alcohol intake (gm. per week). Body mass index was calculated by the formula, body weight (kg) divided by the square of the body height in meter. A third model corrected for coffee (considering the fact that those who drank a lot of tea tend to drink less coffee and vice versa) and chocolate consumption. Mediating factors were included in the final model. The mediating factors considered were daily intake of magnesium and potassium, which were elicited from their food consumption practices and the effects of long term medications particularly the use of statins[9].

Results

The study included 150 cases and 150 controls. Baseline characteristics of the study participants among cases and controls were presented in Table 1. Most of the demographic factors were comparable between cases and controls. However cases were having slightly older age compared to controls. Family history of diabetes mellitus and level of education was higher among cases compared to controls. In the multivariate regression model, odds ratio shows that the participants who drank more tea had a significantly decreased risk of diabetes mellitus compared with those who do not drink tea on a daily basis (odds ratio = 0.652, 95% CI = 0.43 – 0.98). On further analysis, it was observed that the participants who consumed 3 or 4 cups of tea per day had a decreased risk of type 2 diabetes mellitus compared to those who do not drink tea on a daily basis (odds ratio = 0.368, 95% CI = 0.14 – 0.96) (Table 3) with no significance in analysis of trend (Figure 1). The reduced risk was not observed among those who take 5 cups or more tea per day (odds ratio = 0.328, 95% CI = 0.06 – 1.60)

Table 1: Distribution of the participant's demographic characteristics

Variables	Cases No (%) or Mean ± SD	Controls No (%) or Mean ± SD	p value
Age(years, continuous)	53.29 ± 11.52	41.75 ± 12.44	p < 0.05
BMI(kg/m^2, continuous)	26.12 ± 5.09	25.99 ± 5.21	0.824
Potassium (mg/day)	624.11 ± 151.14	661.14 ± 209.54	p < 0.001
Magnesium (mg/day)	134.27 ± 50.19	123.30 ± 44.96	0.082
Sex			0.246
Male	72 (48%)	62 (41.3%)	
Female	78 (52.0%)	88 (58.7%)	
Educational levels			p < 0.001
Up to 12th Standard	108 (72.0%)	85 (56.7%)	
College & above	42 (28.0%)	65 (43.3%)	
Current smoking status			0.569
Yes	17 (11.3%)	14 (9.3%)	
No	133 (88.7%)	136 (90.7%)	
Alcohol Intake			0.427
rarely/moderately	144 (96.0%)	141 (94.0%)	
weekly/daily	6 (4.0%)	9 (6.0%)	
Family history of diabetes mellitus			p < 0.001
Yes	91 (60.7%)	51 (34.0%)	
No	59 (39.3%)	99 (66.0%)	
Use of statins			0.317
Yes	1 (0.7%)	0 (0.0%)	
No	149 (99.3%)	150 (100%)	

Table 2: Tea consumption and odds ratio for the risk of type 2 diabetes mellitus in the multivariate analyses

	Model 1 Adjusted for age and sex	Model 2 Adjusted for BMI, education, smoking, alcohol and family history of diabetes mellitus in addition to variables in Model 1	Model 3 Adjusted for coffee and chocolate consumption in addition to variables in Model 2	Model 4 Adjusted for potassium, magnesium and statins in addition to variables in Model 3
Odds ratio	0.753	0.648	0.613	0.652
95% CI for diabetes mellitus	0.533 – 1.066	0.441 – 0.953	0.410 – 0.915	0.433 – 0.982
p value	0.109	0.027	0.017	0.041

Table 3: Adjusted odds ratio* according to the consumption of tea cups per day

Category	Cases (N = 150)	Controls (N = 150)	Odds ratio	95% CI for EXP (B)	p value
Do not drink on a daily basis	34	27	1		
1-2 cups per day	81	84	0.522	0.236 – 1.157	0.109
3-4 cups per day	31	32	0.368	0.141 – 0.964	0.042
>5 cups per day	4	7	0.328	0.067 – 1.605	0.169

*adjusted for age, sex BMI, education, smoking, alcohol, family history of diabetes mellitus, coffee consumption, chocolate consumption, potassium intake, magnesium intake and use of statins

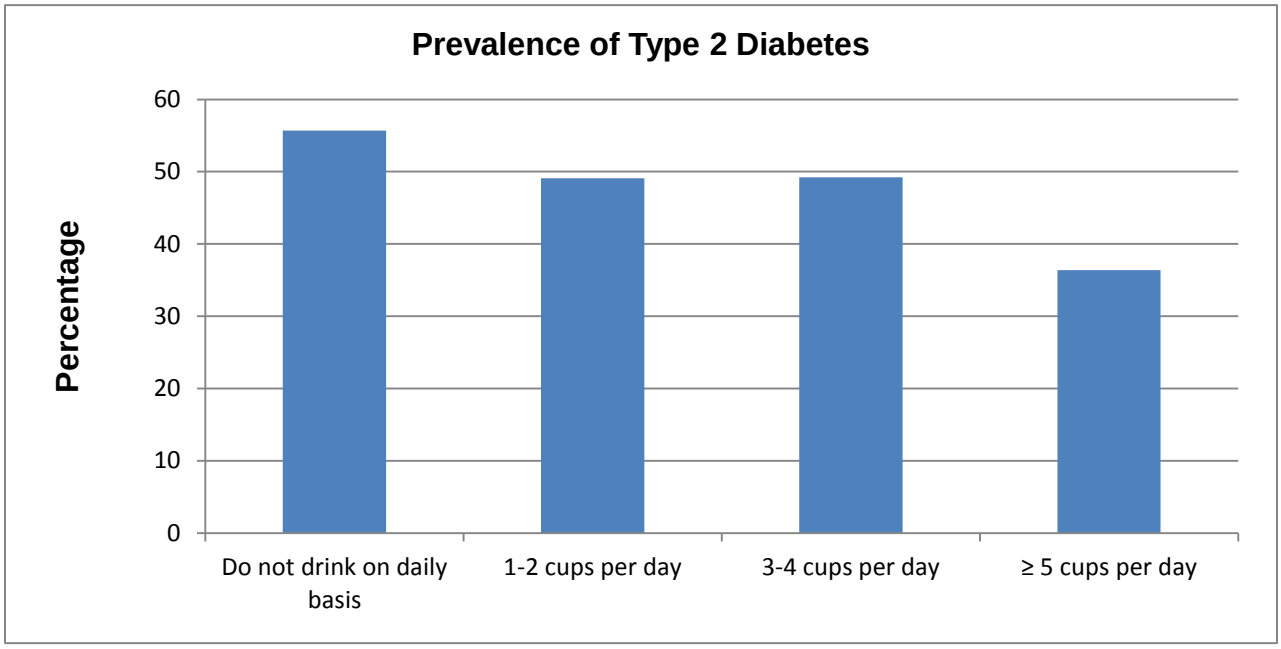


Fig1: Prevalence of type 2 diabetes mellitus according to the consumption of tea cups per day

Discussion

Several studies have investigated the association between tea consumption and type 2 diabetes mellitus[10-14]. Some of these studies did not find any association, while other studies observed an inverse association between tea consumption and type 2 diabetes mellitus. Another study found an association only for green tea and not for black tea. However, our study strongly support the findings of the recent meta-analysis[11] which concluded that consumption of 4 cups of tea per day may lower the risk of type 2 diabetes mellitus, but a reduced risk was not observed for 1 to 2 cups per day. In addition, we also observed that the reduced risk was not observed for those who take more than five cups of tea per day. This is perhaps due to the reduced sample size in this group.In univariate analysis, we observed an inverse association between potassium intake and the risk of type 2 diabetes mellitus. In our study, intake of magnesium and potassium were included in the final model to assess whether these factors mediated the observed association between tea intake and type 2 diabetes mellitus, but none affected the association. Hence, results from our study suggest that other factors may be more important to explain inverse relation between tea consumption and type 2 diabetes mellitus. Tea contains flavonols and flavones are antioxidants. Antioxidants may reduce the amount of reactive oxygen species, which activate stress-sensitive pathways. These pathways can lead to insulin resistance, impaired insulin secretion and beta cell dysfunction. Our study has several limitations, first we relied on self-reported tea consumption and therefore misclassification may have occurred. Second, we relied on self-reported information on non-diabetic status among controls. Third, we could not assess the influence of physical activities due to some practical reasons. The strengths of this study are a large number of covariates studied and extensive information on diet of the participants.

In conclusion, the results from our study suggest that consumption of 3 to 4 cups of tea per day has a decreased risk of type 2 diabetes mellitus. Further large scale prospective studies can provide more information in this field.

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intake using the dietary history of the participants. We do not have any conflict of interest and not received any research grants for this study.

References

1. Wild S, Roglic G, Green A, Sicree R, King H, "Global prevalence of diabetes mellitus: estimates for the year 2000 and projections for 2030". Diabetes mellitus Care.2004;27:1047–1053.
2. Hu FB, Manson JE, Stampfer MJ, et al, "Diet, lifestyle and the risk of type 2 diabetes mellitus mellitus in women" N Engl J Med 2001; 345:790–797.
3. Hamer M, Witte DR, Mosdol A, Marmot MG, Brunner EJ, "Prospective study of coffee and tea consumption in relation to risk of type 2 diabetes mellitus mellitus among men and women": The Whitehall II study. Br J Nutr 2008:1046–1053.
4. Iso H, Date C, Wakai K, et al, "The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes mellitus among Japanese adults" Ann Intern Med.2006; 144: 554–562.
5. Oba S, Nagata C, Nakamura K, Fujii K, Kawachi T, et al, "Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes mellitus in Japanese men and women" British Journal of Nutrition.2010;103:453–459.
6. Greenberg JA, Axen KV, Schnoll R, et al, "Coffee, tea and diabetes mellitus: the role of weight loss and caffeine" Int J Obes.2005;29: 1121–1129.
7. Waki K, Noda M, Sasaki S, et al, "Alcohol consumption and other risk factors for self-reported diabetes mellitus among middle aged Japanese": a population-based prospective study in the JPHC study cohort I, Diabet Med 2005; 22: 323–331.
8. S. van Dieren, C. S. P. M. Uiterwaal, Y. T. van der Schouw , D. L. van der A, J. M. A. Boer, et al, "Coffee and tea consumption and risk of type 2 diabetes mellitus" Diabetologia 2009; 52:2561–2569.
9. Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K, "Indian food composition tables" National Institute of Nutrition 2017: 150-206.
10. Odegaard AO, Pereira MA, Koh WP, Arakawa K, Lee HP, Yu MC, "Coffee, tea and incident type 2 diabetes mellitus": the Singapore Chinese Health Study, Am J Clin Nutr 2008; 88: 979–985.
11. Jing Y, Han G, Hu Y, Bi Y, Li L, Zhu D, "Tea consumption and risk of type 2 diabetes mellitus": a meta-analysis of cohort studies, J Gen Intern Med 2009;24: 557–562.
12. Polychronopoulos E, Zeimbekis A, Kastorini CM, et al, "Effects of black and green tea consumption on blood glucose levels in non-obese elderly men and women from Mediterranean Islands" (MEDIS epidemiological study), Eur J Nutr 2008; 47: 10–16.
13. Greenberg JA, Axen KV, Schnoll R, Boozer CN, "Coffee, tea and diabetes mellitus: the role of weight loss and caffeine" Int J Obes (Lond) 2005;29: 1121–1129.
14. Van Dam RM, Hu FB, Rosenberg L, Krishnan S, Palmer JR.Dietary calcium and magnesium, major food sources, and risk of type 2 diabetes mellitus in U.S. black women. Diabetes mellitus Care.2006; 29:2238–2243.

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The genetics of cardiac failure: Role of a G protein-coupled receptor polymorphism in therapeutic response in an Indian population

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Abstract

Background and Aim:

The incidence of heart failure (HF) is rising to epidemic proportions in developing countries like India. A lack of adequate Indian studies underscores the importance of pursuing research into HF in an Indian population. G protein-coupled receptor kinase 5 (*GRK5)* Gln41>Leu (rs2230345) polymorphism was reported as a genetic modifier associated with survival in HF patients. A prospective study was conducted to investigate the association of *GRK5* Gln41>Leu polymorphism with response to β-blocker therapy in Indian HF patients.

Methods:

HF patients (*n*=584) were recruited for the study. The patients were genotyped by tetra-primer based allele specific polymerase chain reaction and confirmed with Sanger sequencing. The HF patients were evaluated for *GRK5* gene expression and followed up for ~3 years. Drug dosages, cardiac output and hospitalization-free survival were evaluated as study outcomes. HF subgroups (i.e. systolic or diastolic dysfunction, biventricular dysfunction and pulmonary artery hypertension) were also analyzed in association with hospital-free survival.

Results:

HF patients showed genotype frequencies of AT (15%) and TT (1%). AT/TT genotype carriers showed downregulated *GRK5* gene expression and significant reduction in carvedilol drug dosage (p=0.0001). Moreover, AT/TT genotype carriers on β-blockers showed improved ejection fraction from 27% to 36% (p=0.0007) and increased hospitalization-free survival in comparison to other HF patients. HF patients with AA genotype showed an increased rate of hospital admission in comparison with patients with the AT/TT genotype. HF subgroups with the AT/TT genotype showed an increased hospitalization-free survival versus subgroups with the AA genotype.

Conclusions:

GRK5 Gln41>Leu polymorphism in response to β-blocker therapy improved cardiac function in HF patients.

Relevance for Patients:

This study presents a comprehensive clinicofunctional pharmacogenetic characterization of *GRK5* Gln41>Leu polymorphism in a cohort of Indian HF patients. *GRK5* Gln41>Leu polymorphism can confer improved cardiac function and reduce hospitalization, thus improving the quality of life in HF patients.

Keywords: β-blocker, genotype, GRK5, heart failure, India, polymorphism

1. Introduction

Heart failure (HF) is a major, multifactorial global health disease affecting approx. 6.5 million adults/year in the United States [1] and 491,600 – 1.8 million individuals/year in India [2]. The common etiologies of HF are coronary artery disease, hypertension, valvular heart disease, arrhythmia, dilated cardiomyopathy, infection, and inflammation [3]. Other risk factors include hypertension, ischemic heart disease, obesity, Type II diabetes mellitus, and rheumatic heart disease [2]. Growing evidence show that mortality rates as a result of HF are higher in Indian patients than in western countries with death records of 0.1-0.16 million individuals/year in India [4].

The use of beta (β)-adrenergic receptor (β-AR) antagonists continues to be the standard treatment for HF [5] and has effectively reduced mortality and morbidity rates over the past decade. β-AR, a G protein-coupled receptor expressed by cardiomyocytes has been documented as important for effective cardiac function [6]. Many studies have shown that elevated activation of β-AR results in abnormal β-AR signaling which leads to the pathogenesis of HF [7,8]. Therefore, much of the therapeutic drugs like β-blockers that target the β-AR, antagonize catecholamine-stimulated β-AR signaling [6] and have resulted in increased survival of HF patients.

As a regulator of β-AR signaling, G protein-coupled receptor kinases (GRKs) act by phosphorylating the activated β-AR, leading to receptor desensitization and diminished β-AR signaling [9]. Overexpression of GRK5 and GRK2 contribute to dysregulation of β-AR by excess desensitization and

is also considered a hallmark of HF pathology [10]. Therefore, genetic polymorphisms in β-AR regulator genes may influence HF outcome. One such functional polymorphism being *GRK5* Gln41>Leu (rs2230345, c.122A>T, p.Q41L) in the *GRK5* gene. This nonsynonymous single nucleotide polymorphism (SNP) in the noncatalytic regulatory domain is prevalent in African Americans and confers a gain-of-desensitization function akin to the effects of β-blockade in cells. This polymorphism, however, did not alter the risk of HF but facilitated β-blocker response and reduced death [11,12].

We conducted a prospective study in a cohort of Indian HF patients to investigate the association of *GRK5*Gln41>Leu polymorphism in relation to β-blocker therapeutic response. The hypothesis behind this study was that as GRKs impose β-AR blockade by desensitization, the presence of genetic GRK variants might alter the outcome in HF patients. Therefore, the main aim of this study was to identify *GRK5* variants and to study their response to β-blocker treatment which might allow curtailing β-AR blocker usage in these selected patients to avoid potential harmful effects.

2. Materials and Methods

2.1. Study population

The study was conducted in multispecialty, tertiary care hospital. This prospective study recruited HF patients (n=584) with systolic or diastolic dysfunction, dilated cardiomyopathy or biventricular dysfunction (BVD) that attended the in- and outpatient cardiology clinic in our hospital. The study was initiated after obtaining the institutional ethics committee approval and getting informed consent from the patients. The HF patients considered for this study were diagnosed following the Framingham criteria [13]. The Framingham criteria for HF diagnosis should include either two major criteria or one major and two minor criteria. The major criteria included acute pulmonary edema, cardiomegaly, hepatojugular reflex, neck vein distention, paroxysmal nocturnal dyspnea or orthopnea, pulmonary rales, and third heart sound (S3 gallop rhythm). The minor criteria included ankle edema, dyspnea on exertion, hepatomegaly, nocturnal cough, pleural effusion, and tachycardia (heart rate >120 beats/min). Healthy controls (n=60) without any comorbidity were recruited for gene expression studies.

Stage-appropriate medications given to HF patients included diuretics, angiotensin-converting enzyme (ACE) inhibitors or β-blockers or angiotensin receptor blockers (ARBs) alone or in combination. If needed, mineralocorticoid receptor antagonists and hydralazine were also included. β-blockers were determined by the treating physicians (60% carvedilol, 17% metoprolol, 12.5% nebivolol, and 10.5% bisoprolol) and defined as continuous therapy for at least 6 months. β-blocker dosages were monitored during the follow-up period. Initial dosage refers to the dosage administered at the start of the β-blocker therapy after the diagnosis of HF. The final dosage refers to the dosage followed at the last follow-up time. The number of hospitalizations was monitored for each patient and the follow-up data were collected for 3-7 years. The mean follow-up duration was 3.7 years and 3.5 years for the β-blocker group and non-β-blocker group.

2.2. Clinical and echocardiography assessment

A complete resting two-dimensional and M-mode echocardiography color flow and spectral Doppler as well as annular tissue Doppler imaging data were obtained from the subjects using a Philips IE33™ ultrasound system (Philips Medical Systems, Eindhoven, The Netherlands). Standard two-dimensional parasternal short-axis and apical 2- and 4-chamber views were recorded. Left ventricular (LV) ejection fraction (EF) was calculated using the Teichholz formula. Mitral Doppler signals were recorded in apical 4-chamber view and early diastolic mitral inflow peak velocity (E), late diastolic mitral inflow

peak velocity (A), and E/A ratio were calculated. Thus, confirmation of HF in patients was determined on clinical and echocardiography assessment. Echocardiographic parameters were measured according to current recommendations [14].

2.3. Genotyping

Blood samples from the patients were collected by venipuncture in EDTA coated vials and immediately aliquoted and frozen at −80°C. Genomic DNA was isolated using EZ-10 spin column blood genomic DNA purification kit (Bio Basic Inc., Markham, Ontario, Canada). *GRK5* (c.122A>T, p.Q41L) polymorphism was screened and confirmed through in-house developed tetra-primer based allele specific polymerase chain reaction (PCR) and confirmed with restriction fragment length polymorphism (RFLP) method and Sanger sequencing. PCR reaction mixture containing 100 ng of DNA, Taq polymerase mix (Ampliqon, Odense, Denmark), primers for tetra primer PCR (35nM GRK5F primer: 5'- GAAGGCTGTGGTCTAGTCTAGGGGAACAG - 3'and GRK5R primer: 5'- TTGATTAGCAAAAAGGTCAGCCTGGAGC - 3', 45nM SNPTF primer: 5' - TGGCTAATGTGAGGGAAC - 3'and SNPTR primer: 5' -TGTGTGAAGACCTCCGA - 3') or primers for RFLP method (50 nM BSRF primer: 5'- CTACAGCCCGTCCCTCTGT - 3'and BSRR primer: 5' - GCAAAAAGGTCAGCCTGGAGC - 3') were subjected to thermal cycling (Eppendorf, Hamburg, Germany). For RFLP, PCR product was digested using 1.5 units of *Bsr*I (New England Biolabs® Inc., Ipswich, Massachusetts, USA) as per manufacture instructions. The genotype was identified for tetra-primer PCR (AA: 580 bp and 225 bp, AT:580 bp, 380 bp and 225 bp, and TT:580 bp and 380 bp) and RFLP method (AA:202 bp and 179 bp, AT:381 bp, 202 bp and 179 bp, and undigested TT:381 bp) with these banding patterns after resolving on the gel.

2.4. Quantitative PCR (qPCR)

RNA was isolated from the HF patients and unaffected controls using the Aurum™ total RNA mini kit (Bio-Rad, Hercules, California, USA). The RNA was qualitatively verified on denaturing formaldehyde 1.2% agarose gel electrophoresis. Using 1 μg of RNA, respective cDNAs was synthesized using iScript™first strand cDNA synthesis kit (Bio-Rad, Hercules, California, USA). qPCR was carried out to compare *GRK5* gene expression in HF cases and healthy controls using relative quantification with β-actin as reference gene. PCR mixture of 20μL contained 2X SYBR green (Sso Advanced universal SYBR® Green supermix, Bio-Rad, Hercules, California, USA), 50 nM of forward and the reverse primer of the respective gene, cDNA template corresponding to 1 μg RNA and nuclease free water. The primers used were GRK5 FP (5' - ACCTGAGGGGAGAACCATTC - 3'), GRK5 RP (5' -TGGACTCCCCTTTCCTCTTT - 3'), ACTIN FP (5' - TCCCTGGAGAAGAGCTACG - 3'), and ACTIN RP (5' - GTAGTTTCGTGGATGCCACA - 3'). Reaction included an initial denaturation of 95°C for 3 min followed by 45 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 45 s, and a melt curve analysis (65 – 95°C) was performed at the end of 45 cycles. Normalized fold expression was calculated using $2^{-\Delta\Delta Ct}$ method.

2.5. Variables

In this study, the independent variables were age, sex, and socio-demographic data. Covariates were (EF%), comorbidities, use of β-blockers, and use of other medications. The covariates adjusted for hospital-free survival analysis were age, sex, EF%, and comorbidities.

2.6. Statistical analysis

Student *t*-tests (paired and unpaired) and Chi-square tests were used to assess significant differences in variables between the groups, drug dosages, genotype classes, gene expression, and EF%. Continuous variables are expressed as mean ± standard deviation (SD). Fischer's exact test was carried out to compare cardiac output between groups. Hardy-Weinburg equilibrium was assessed to compare predictions with that of the obtained results. Kaplan–Meier curves were also constructed to plot the percentage of survival of patients. Comparisons of overall survival were performed using a log-rank (Mantel-Cox) test. All the statistical analyses were carried out using GraphPad Prism software version 5.0 Windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Clinical and demographic characteristics of the HF patient cohort

The HF patient cohort (n=584) was considered for the study after clinical and echocardiographic assessment. Table 1 shows the clinical and demographic characteristics of the HF patient cohort. The HF patients were categorized into β-blocker group and non-β-blocker group. There was no statistically significant difference in variables such as age, sex, body mass index, and hemodynamics between the two groups. BVD (24%; p=0.002) and LV diastolic dysfunction (32.8%; p=0.002) were observed as statistically significant HF characteristics in the β-blocker group. Dilated cardiomyopathy (54%; p=0.0002) was observed as statistically significant HF etiology in the β-blocker group versus the non-β-blocker group whereas rheumatic heart disease (8%; p=0.0001) and coronary artery disease (44%; p=0.0001) were significantly higher in the non-β-blocker group. Type II diabetes mellitus (60%; p=0.0001) and its associated disorders were significantly frequent in the non-β-blocker group whereas systemic hypertension (42%; p=0.002) and dyslipidemia (25%; p=0.002) were higher in the β-blocker group. Approximately 52% of the patients were on β-blocker therapy. Among the medications administered, 38% of the HF patients received ACE inhibitors, 20% ARBs, 70% diuretics, 35% digitalis glycosides, 22% statins, and 19% anticoagulants.

Table 1

Clinical and demographic characteristics of the HF patient cohort

Variables	HF patient cohort (*n*=584)	
	β-blocker group (*n*=304)	Non-β-blocker group (*n*=280)
Mean Age±SD (years) (range)	60±10 (24 – 89)	59±11 (25 – 90)
Males (%)	73	73
Mean body mass index (kg/m^2) (range)	22 (19.5 – 24.5)	23.5 (21.1 – 26.5)
Mean follow up time (years) (range)	3.7 (3 – 7)	3.5 (3 – 7)
Hemodynamics		
Heart rate (beats/min)	78	80
Mean systolic blood pressure (mmHg)	128 (81 – 171)	123 (80 – 170)
Mean diastolic blood pressure (mmHg)	84 (50 – 139)	72 (55 – 130)
HF Etiology, n (%)		
Coronary artery disease	89 (26)	131 (47)*
Dilated cardiomyopathy	164 (54)	109 (36)*
Rheumatic heart disease	0	24 (8)*
HF Characteristics, n (%)		
LV systolic dysfunction	106 (35)	112 (40)
LV diastolic dysfunction	100 (32.8)	50 (18)*
Biventricular dysfunction	74 (24)	40 (14)*
Pulmonary artery hypertension	52 (17)	50 (18)
Comorbidities, n (%)		
Type II diabetes mellitus and its associated disorders	106 (35)	168 (60)*
Systemic hypertension	128 (42)	84 (30)*
Dyslipidemia	76 (25)	42 (15)*
Hypothyroidism	49 (16)	56 (20)
Cerebrovascular events	18 (6)	12 (4)
Renal insufficiency	9 (3)	14 (5)
Anemia	6 (2)	6 (2)

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*$p \leq 0.05$ versus β-blocker group.

3.2. GRK5 Gln41>Leu polymorphism in HF patient cohort

GRK5 Gln41>Leu polymorphism was screened by in-house developed tetra-primer based allele specific PCR and reconfirmed with RFLP and the Sanger sequencing method (Figure 1). Genotype distribution was in agreement with the Hardy-Weinberg equilibrium (p=0.595). The genotype (84% for AA, 15% for AT, and 1% for TT) and allele frequencies (0.916 for A allele and 0.084 for T allele) of *GRK5* Gln41>Leu polymorphism in the HF patient cohort is presented in Table 2. On comparing the genotype frequencies, AT genotype was more prevalent in the β-blocker group (19%) compared to the non-β-blocker group (10%) (p=0.011). The unaffected healthy controls were also screened for this polymorphism. The genotype and the allele frequencies of the healthy controls (Supplementary Table 1) did not differ significantly from the HF cases (p=0.94 for genotype frequency, p=0.82 for allele frequency) ensuring a lack of association of this polymorphism with development of HF.

Figure 1

Genotyping of GRK5 Gln41>Leu polymorphism. Tetra-primer based allele specific polymerase chain reaction (A) results on showing banding pattern for Gln/Leu heterozygous AT genotype (lane 2: 580 bp, 380 bp and 225 bp), Leu/Leu homozygous TT genotype (lane 3: 580 bp and 380 bp), Gln/Gln homozygous AA genotype (lanes 4, 5: 580 bp and 225 bp). RFLP (B) results showing banding pattern for Gln/Leu heterozygous AT genotype (lanes 2, 3: 381 bp, 202 bp and 179 bp), Leu/Leu homozygous TT genotype (lane 4: 381 bp) and Gln/Gln homozygous AA genotype (lanes 5, 6, 7: 202 bp and 179 bp). Lanes 1a, 1 b: 100 bp DNA ladder (Biobasic, Canada). Sanger sequencing results of Gln/Gln homozygous AA genotype C), Gln/Leu heterozygous AT genotype (D) and Leu/Leu homozygous TT genotype (E).

Table 2

GRK5 genotype and allele frequencies in the HF patient cohort

Genotype	HF patients (n=584), n (%)	HF patients (β-blockers) (n=304), n (%)	HF patients (non-β-blockers) (n=280), n (%)	p-value
AA	493 (84)	244(80)	249(89)	0.011
AT	85 (15)	57 (19)	28 (10)	
TT	6 (1)	3 (1)	3 (1)	
Allele				0.07
A	535 (91.6)	273 (90)	263 (94)	
T	49 (8.4)	31 (10)	17 (6)	

Results were expressed as n (%) for genotype and allele distribution. $\chi 2$ test was significant for genotype frequency and not significant for allele frequency between β-blocker group and the non-β-blocker group.

As the frequency of homozygous TT genotype was low, we intended to divide the HF patients into two group, those having AA genotype alone (AA genotype group) and those with AT and TT genotype (AT/TT genotype group), to study the influence of *GRK5* Gln41Leu (rs17098707) polymorphism on response to β-blocker therapy in the HF patients.

3.3. Downregulated GRK5 expression among the GRK5 Leu41 variants (AT/TT genotype)

The *GRK5* gene expression was evaluated among different groups of HF patients. The gene expression among The AT/TT genotype carriers showed a downregulated *GRK5* expression than AA genotype carriers among β-blockers and non-β-blockers. For gene expression analysis (Figure 2), healthy controls (n=60) were included for comparison. The β-blocker AT/TT group (n=60) showed a 0.5135±0.1593-fold decrease in gene expression and the β-blocker AA group (n=60) showed a slightly but significant upregulated pattern of 1.752±0.0.2775 gene expression (p<0.0001). On comparing the non-β-blockers, there was a significant difference in the gene expression (p<0.0001) between the AT/TT group (n=31, 0.5507±0.1322) and the AA group (n=60, 1.681±0.2688).

Figure 2

GRK5 expression among different groups of HF patients. β-blocker AT/TT group versus β-blocker AA group (p<0.0001), non-β-blocker AT/TT group versus non-β-blocker AA group (p<0.0001) along with healthy controls (HC) were analyzed for _GRK5_ normalized gene expression. Student's _t_-test was used to compare the groups.

3.4. Improvement in the EF of the GRK5 Leu41 variants (AT/TT genotype)

The EF% is the measure of LV systolic function and cardiac output. Among the β-blocker group (29±4.8%) and non-β-blocker group (41.5±2.3%), the mean EF% remained significantly varied (p=0.0286). The EF% of the patients before and after pharmacological treatment was recorded and improvement in their cardiac output was compared among the groups (Figure 3). The increment in the EF% after treatment was considered as an improvement in cardiac output. About 56% of HF patients on β-blockers and 41% on non-β-blockers showed improvement in their EF% (p=0.0004; Figure 3A). As expected, 71% of the *GRK5* Leu41 variants (AT/TT genotype) showed remarkably higher cardiac output (p=0.0001; Figure 3A) versus those with *GRK5*Gln41 (AA genotype, 45%). Since the non-β-blockers had HF preserved EF, only the β-blocker group having HF reduced EF was considered for further analysis. When the HF patients with different genotypes in the β-blocker group were compared it was evident that those with the AT/TT genotype (83%) improved significantly (p=0.0001; Figure 3B) compared to those with the AA genotype (49%).

Figure 3

Percentage improvement in cardiac output among the cohort of HF patients. The percentage of HF patients improved among β-blocker and non-β-blocker groups (p=0.0004) and different genotype groups of *GRK5* (p=0.0001) (A). The percentage of HF patients improved among β-blocker group with different genotypes (p=0.0001) (B). Fischer's exact test was carried out for analysis.

The improvement in cardiac output was compared among the HF patients in β-blocker group with different genotypes (Figure 4). EF% has improved from 25.2±1.041% to 28.2±0.7638% among the AA group (p=0.0091) and 27.4±1.021% to 36±1% in the AT/TT group (p=0.0007).

Figure 4

Improvement in ejection fraction in HF patient cohort. The improvement in EF% in HF patients with different genotypes of β-blocker group was measured. AT/TT group (p=0.0091) improved significantly in comparison with the AA group (p=0.0091). Paired t-test was carried out for computing p-values.

3.5. GRK5 Leu41 variants (AT/TT genotype) show decrement in β-blocker drug dosage

Among the β-blocker group (n=304), 60% of the HF patients were treated with carvedilol, 17% with metoprolol, 12.5% and 10% with nebivolol and Bisoprolol, respectively. The initial and the final drug dosages over the follow-up period were noted and analyzed. Initial dosages administered at the start of the β-blocker therapy and final dosages followed at the last follow-up were noted. There was a statistically significant reduction in carvedilol drug dosage in the overall β-blocker group when the initial (11.2±7.7 mg) and final drug dosages (10.2±7.1 mg) were compared (p=0.0114; Figure 5A). Furthermore, the drug dosages remained unchanged in the AA genotype group (p=0.8211; Figure 5B) when the initial (11.06±7.3 mg) and final (11.1±7.4 mg) drug dosages were compared. Interestingly, there was a statistically significant reduction in the drug dosage in the AT/TT genotype group (p=0.0001; Figure 5B) when the initial (11.6±9.2 mg) and the final (6.4±4 mg) drug doses were compared. Thus, the *GRK5* Leu41 variants in the homozygous and heterozygous forms were associated with a lower dose of β-blockers.

Figure 5

GRK5 Gln41>Leu polymorphism influences β-blocker (carvedilol) drug dosage. Carvedilol drug doses were reduced in the overall β-blocker group (p=0.0114) (A). The drug doses remained unchanged in the AA genotype group (p=0.8211), whereas in the AT/TT genotype group there was a significant reduction in the drug dosage (p=0.0001) (B). Paired t-test was used to compare the drug doses between groups.

3.5. Reduced event of hospitalization among the GRK5 Leu41 variants (AT/TT genotype)

The HF patients were followed-up for ~3 years (40 months) after the initiation of pharmacological treatment. The influence of β-blocker therapy and *GRK5* genotype on hospitalization-free survival of HF patients was studied through Kaplan–Meier survival curves (time-to-event analysis). When the overall HF patients administered with β-blockers and non-β-blockers were compared for hospital-free survival, the patients on β-blockers showed higher survival than the non-β-blockers (hazard ratio [HR]: 1.167, 95% confidence interval [CI]: 0.325 – 2.008, log-rank $p<0.0001$; Figure 6A). The same was found when the overall HF patients with AA and AT/TT genotypes were compared; the presence of AT/TT genotype reduced the risk for hospitalization (HR: 0.8387, 95% CI: 0.07042 – 1.607, log-rank $p<0.0001$; Figure 6B). On further analysis of the HF subgroups, β-blocker AT/TT group versus β-blocker AA group, it was found that the presence of AA genotype increased the risk for hospitalization (HR: 1.143, 95% CI: 0.4337 – 1.852, log-rank $p<0.0001$; Figure 6C) even under the influence of β-blocker therapy. When non-β-blocker AT/TT group versus non-β-blocker AA group was analyzed, AA genotype increased the risk for hospitalization (HR: 1.364, 95% CI: 0.7020 – 2.025, log-rank $p=0.0003$; Figure 6C). In the β-blocker AT/TT group versus non-β-blocker AT/TT group analysis, the use of non-β-blockers increased the risk for hospitalization (HR: 1.067, 95% CI: 0.463 – 1.670, log-rank $p=0.0231$; Figure 6C). As expected, when β-blocker AA group versus non-β-blocker AA group was analyzed, the presence of AA genotype and use of non-β-blocker therapy increased the risk of hospitalization (HR: 1.273, 95% CI: 0.441 – 2.104, log-rank $p<0.0001$; Figure 6C).

Figure 6

Influence of β-blocker therapy and *GRK5* genotype on hospital-free survival of HF patients. HF patients were stratified by β-blockers usage (β-blockers ($n=304$); non-β-blockers ($n=280$); $p<0.0001$) (A). HF patients were stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (GRK5 AA group ($n=493$); *GRK5* AT/TT genotype ($n=91$); $p<0.0001$) (B). β-blocker treated subjects and non-β-blocker subjects stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (β-blocker AA group ($n=244$); β-blocker AT/TT group($n=60$); non-β-blocker AA group($n=249$); non-β-blocker AT/TT group ($n=31$) (C). Log-rank test (Mantel-Cox) test was utilized for comparisons of survival curves.

The overall HF patients were subdivided based on HF characteristics such as systolic dysfunction ($n=218$), diastolic dysfunction ($n=150$), BVD ($n=114$), and pulmonary artery hypertension (PAH) ($n=102$). The groups were compared for hospitalization-free survival. The patients with PAH showed higher survival and patients with systolic dysfunction showed the lower survival than the other groups (log-rank $p=0.02$; Figure 7A). When the subgroups with systolic dysfunction and diastolic dysfunction were compared with *GRK5* polymorphism genotypes (AA and AT/TT), it was evident that the HF patients with AA genotype under both the subgroups indicated the lower hospitalization-free survival than the HF patients with AT/TT genotype under both subgroups (log-rank $p=0.0002$; Figure 7B). When the subgroups with BVD and PAH were compared with *GRK5* polymorphism genotypes (AA and AT/TT), it was evident that the HF patients with AA genotype under both the subgroups indicated lower hospitalization-free survival than those with the AT/TT genotype under both subgroups (log-rank $p=0.0051$; Figure 7C).

Figure 7

Influence of *GRK5* genotype on hospital-free survival of HF patient subgroups. HF patients were stratified by HF characteristics systolic dysfunction (*n*=218); diastolic dysfunction (*n*=150); BVD (*n*=114); PAH (*n*=102); *p*=0.02) (A). HF patients with systolic and diastolic dysfunction were stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (systolic dysfunction AA group (*n*=193); diastolic dysfunction AA group (*n*=128); systolic dysfunction AT/TT group (*n*=25); diastolic dysfunction AT/TT group (*n*=22); *p*=0.0002) (B). HF patients with BVD and PAH were stratified by *GRK5* Leu41 carrier status (AT/TT genotype) (BVD AA group (*n*=90); PAH AA group (*n*=82); BVD AT/TT group (*n*=24); PAH AT/TT group (*n*=20); *p*=0.0051) (C). Log-rank test (Mantel-Cox) test was utilized for comparisons of survival curves.

To compare the frequency of hospital admissions between the genotype groups within a month, the rate of hospital admission/month was compared (Figure 8). From the results, it was evident that the HF patients in the AA group (0.0769±0.003; *n*=493) were significantly more frequently ($p<0.0001$) admitted in hospital than the AT/TT group (0.0409±0.004; *n*=91; Figure 8A). Among the β-blocker group, HF patients with the AA genotype (0.0790±0.005; *n*=244) were significantly more frequently admitted in the hospital than the AT/TT variants (0.0374±0.005; *n*=60; *p*=0.0002). Among the non-β-blocker group, HF patients with the AA genotype (0.0749±0.005; *n*=249) had more hospital admissions compared to the AT/TT variants (0.0477±0.008; *n*=31) although not statistically significant (*p*=0.0674; Figure 8B).

Figure 8

Comparison of frequency of hospital admission between the genotype groups. The AA group of HF patients (*n*=493) had more hospital admissions than the AT/TT HF group (*n*=91; $p<0.0001$) (A). Among the β-locker group, HF patients with the AA genotype had more frequent hospital admission than the AT/TT variants (*p*=0.0002) (B). A similar trend was observed among the non-β-blocker group but the results were no statistically insignificant (ns). β-blocker AA group (*n*=244); β-blocker AT/TT group (*n*=60); non-β-blocker AA group(*n*=249); and non-β-blocker AT/TT group (*n*=31). Student's *t*-test was used to compare the gr.

4. Discussion

Genetic polymorphisms impact HF outcomes. One such polymorphism is *GRK5*Gln41>Leu (A>T), which was proven to have a role in promoting β-AR desensitization [11]. Our results show that HF patients with *GRK5* Leu41 variant in its homozygous and heterozygous forms show decreased events in hospitalization and specifically respond to β-blockers and require reduced β-blocker drug dosage in comparison to *GRK5* Gln41 carriers. Moreover, *GRK5* Leu41 variants in combination with β-blocker therapy showed improved cardiac output.

The *GRK5* genotype frequencies in this study (AT/TT15% and TT1%) were comparatively lower than that reported in African Americans (AT/TT39% and TT6%) [15]. The T allele frequency in our study (0.084) was lower than African Americans (0.23) [15] but, higher than that found in the Chinese population (0.009) [16] and Caucasians (0.017) [17]. As GRK5 is highly expressed in myocardium [18], and given its established role in cardiac failure pathology [10], this prompted our group to examine and evaluate *GRK5* expression in a cohort of Indian HF patients. GRK5 is reportedly overexpressed in HF conditions and could attenuate cardiac burden in response to adrenergic surge [19,20]. In view of this, a slight upregulation observed in *GRK5* expression in a subpopulation of HF patients could be expected. However, gene expression was downregulated in the AT/TT subset of HF patients in our study. A possible reason could be that the role of overexpressed GRK5 in β-AR desensitization is compensated by the allelic variation, bringing about an augmented desensitization, thus attempting to regulate *GRK5* expression for better cardiac performance. Interestingly though inhibiting GRK5 expression restores the cardiac muscle damage incurred during HF [21]. These observations support the fact that *GRK5* Leu41 variant confers protection from congestive HF [22].

Human and rodent data have proven that *GRK5*Leu41 protected against death or cardiac transplantation [11]. Coinciding with these data, our study reaffirms that the *GRK5*Leu41 allele improved quality of life, promoted hospitalization-free survival of South Indian HF patients. Independently, both the administration of β-blocker therapy and the presence of Leu41 allele yielded a positive outcome on hospitalization-free survival. The Leu41 allele carriers among the different HF subgroups showed increased hospitalization-free survival in comparison to Gln41 carriers. The previous reports support the fact that Indians are highly sensitive to β-blockers [23] and also speculated that polymorphic variations in β-AR gene in Indians [24] might be the reason for higher sensitivity [25]. Our data indicate that the prevalence of *GRK5* gene polymorphisms in Indian HF patients and its role in cardiac failure might be the reason for responders to β-blocker treatment. In response to β-blocker treatment, *GRK5*Leu41 allele carriers (AT/TT genotype) showed better hospital-free survival than *GRK5* Gln41 allele carriers (AA genotype) and β-blocker naïve *GRK5*Leu41 and Gln41 allele carriers. This observation was also reported in a study where HF patients with the Gln41Leu genotype had reduced hospitalization rates (18.6%) [26]. These results also justify the continued use of β-blockers as standard therapy for cardiac failure and their benefits over other pharmacological interventions [27-29]. Interestingly, it was found that although Leu41 carriers among African Americans survived better than Gln41 carriers, they exhibited a reduced response to β-blockers [11,17] such as atenolol [30]. Another study among African race demonstrated that Leu41 impacted negatively on survival of HF patients, but the effect seemed to be stabilized upon β-blocker usage. In other words, Gln41 carriers survived better in the presence and absence of β-blockers [15].

The Leu41 allele seemed to act as a genetic β-blockade [11], promoting reduced dependency on β-blockers for improvement in HF patients. Although β-blocker has been validated as a lifesaving drug in HF, in view of its adverse effects such as HF deterioration, hypotension, and impaired left atrial function [31,32], minimizing its usage is recommended. In our study, the presence of Leu41 allele has helped in curtailing the dosage of carvedilol during the course of treatment and reduced the frequency of hospital admission compared to the Gln41 allele. Carvedilol, a nonselective β-blocker with better efficacy than other β-blockers [10,33] was used in 60% of the HF patients in this study. The usage of β-blockers has substantially improved the LV systolic function by increasing the EF in 56% of HF patients in this study. Literature also supports the usage of β-blockers in recovering from LV systolic dysfunction [34]. The improvement in EF was pronounced in *GRK5* Leu41 variants compared to Gln41 carriers and this observation coincided with another study reporting *GRK5* polymorphism having a

positive effect on systolic HF after β-blocker therapy [16]. Taken together, these data present convincing evidence that the Leu41 allele and β-blocker therapy synergistically work to stabilize cardiac function in Indian HF subjects.

Ethical Approval

Human samples to carry out the study were obtained following informed consent of patients according to the Declaration of Helsinki and its later amendments or comparable ethical standards. Research involving humans was authorized by the Institutional Ethics committee from PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.

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Appendix

Supplementary Table 1

***GRK5* genotype and allele frequencies in the HF patients and healthy controls**

Genotype	HF patients (*n*=584), *n* (%)	Healthy controls (*n*=400), *n* (%)	*p* value
AA	492 (84)	340 (85)	0.94
AT	86 (15)	56 (14)	
TT	6 (1)	4 (1)	
Allele			
A	535 (91.6)	368 (92)	0.82
T	49 (8.4)	32 (8)	

Results were expressed as *n* (%) for genotype and allele distribution. χ^2 test was non significant for genotype and allele frequencies between HF patient cohort and healthy controls group.

Conflict of Interest

The authors declare no conflict of interest.

The genetics of cardiac failure: Role of a G protein-coupled receptor polymorphism in therapeutic response in an Indian population

References

[1] Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart Disease and Stroke Statistics-2019 Update:A Report From the American Heart Association. *Circulation.* 2019;139:e56–528. [PubMed] [Google Scholar]

[2] Huffman MD, Prabhakaran D. Heart Failure:Epidemiology and Prevention in India. *Natl Med J India.* 2010;23:283–8. [PMC free article] [PubMed] [Google Scholar]

[3] Guo M, Guo G, Ji X. Genetic Polymorphisms Associated with Heart Failure:A Literature Review. *J Int Med Res.* 2016;44:15–29. [PMC free article] [PubMed] [Google Scholar]

[4] Seth S, Ramakrishnan S, Parekh N, Karthikeyan G, Singh S, Sharma G. Heart Failure Guidelines for India:Update 2017. *J Pract Cardiovasc Sci.* 2017;3:133–8. [Google Scholar]

[5] Bristow MR. Beta-Adrenergic Receptor Blockade in Chronic Heart Failure. *Circulation.* 2000;101:558–69. [PubMed] [Google Scholar]

[6] Steinberg SF. The Molecular Basis for Distinct Beta-Adrenergic Receptor Subtype Actions in Cardiomyocytes. *Circ Res.* 1999;85:1101–11. [PubMed] [Google Scholar]

[7] Madamanchi A. Beta-adrenergic Receptor Signaling in Cardiac Function and Heart Failure. *Mcgill J Med.* 2007;10:99–104. [PMC free article] [PubMed] [Google Scholar]

[8] de Lucia C, Eguchi A, Koch WJ. New Insights in Cardiac β-Adrenergic Signaling during Heart Failure and Aging. *Front Pharmacol.* 2018;9:904. [PMC free article] [PubMed] [Google Scholar]

[9] Zhang Y, Matkovich SJ, Duan X, Gold JI, Koch WJ, Dorn GW., 2nd Nuclear Effects of G-Protein Receptor Kinase 5 on Histone Deacetylase 5-Regulated Gene Transcription in Heart Failure. *Circ Heart Fail.* 2011;4:659–68. [PMC free article] [PubMed] [Google Scholar]

[10] Mangmool S, Parichatikanond W, Kurose H. Therapeutic Targets for Treatment of Heart Failure:Focus on GRKs and β-Arrestins Affecting β-AR Signaling. *Front Pharmacol.* 2018;9:1336. [PMC free article] [PubMed] [Google Scholar]

[11] Liggett SB, Cresci S, Kelly RJ, Syed FM, Matkovich SJ, Hahn HS, et al. A GRK5 Polymorphism that Inhibits Beta-Adrenergic Receptor Signaling is Protective in Heart Failure. *Nat Med.* 2008;14:510–7. [PMC free article] [PubMed] [Google Scholar]

[12] Dorn GW, 2nd, Liggett SB. Pharmacogenomics of Beta-Adrenergic Receptors and their Accessory Signaling Proteins in Heart Failure. *Clin Transl Sci.* 2008;1:255–62. [PMC free article] [PubMed] [Google Scholar]

[13] King M, Kingery J, Casey B. Diagnosis and Evaluation of Heart Failure. *Am Fam Physician.* 2012;85:1161–8. [PubMed] [Google Scholar]

[14] Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults:An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;28:1–39.e14. [PubMed] [Google Scholar]

[15] Johnson AE, Hanley-Yanez K, Yancy CW, Taylor AL, Feldman AM, McNamara DM. Adrenergic Polymorphisms and Survival in African Americans With Heart Failure:Results From A-HeFT. *J Card Fail.* 2019;25:553–60. [PMC free article] [PubMed] [Google Scholar]

[16] Kang S, Hong X, Ruan CW, Yu P, Yu SS, Chen M, et al. Effects of GRK5 and ADRB1 Polymorphisms Influence on Systolic Heart Failure. *J Transl Med.* 2015;13:44. [PMC free article] [PubMed] [Google Scholar]

[17] Cresci S, Kelly RJ, Cappola TP, Diwan A, Dries D, Kardia SL, Dorn GW., 2nd Clinical and Genetic Modifiers of Long-Term Survival in Heart Failure. *J Am Coll Cardiol.* 2009;54:432–44. [PMC free article] [PubMed] [Google Scholar]

[18] Vinge LE, Øie E, Andersson Y, Grøgaard HK, Andersen G, Attramadal H. Myocardial Distribution and Regulation of GRK and Beta-Arrestin Isoforms in Congestive Heart Failure in Rats. *Am J Physiol Heart Circ Physiol.* 2001;281:H2490–9. [PubMed] [Google Scholar]

[19] Chen EP, Bittner HB, Akhter SA, Koch WJ, Davis RD. Myocardial Function in Hearts with Transgenic Overexpression of the G Protein-Coupled Receptor Kinase 5. *Ann Thorac Surg.* 2001;71:1320–4. [PubMed] [Google Scholar]

[20] Ishizaka N, Alexander RW, Laursen JB, Kai H, Fukui T, Oppermann M, et al. G Protein-Coupled Receptor Kinase 5 in Cultured Vascular Smooth Muscle Cells and Rat Aorta. Regulation by Angiotensin II and Hypertension. *J Biol Chem.* 1997;272:32482–8. [PubMed] [Google Scholar]

[21] Sorriento D, Santulli G, Fusco A, Anastasio A, Trimarco B, Iaccarino G. Intracardiac Injection of AdGRK5-NT Reduces Left Ventricular Hypertrophy by Inhibiting NF-kappaB-Dependent Hypertrophic Gene Expression. *Hypertension.* 2010;56:696–704. [PubMed] [Google Scholar]

[22] Hendrickx JO, van Gastel J, Leysen H, Santos-Otte P, Premont RT, Martin B, et al. GRK5-a Functional Bridge between Cardiovascular and Neurodegenerative Disorders. *Front Pharmacol.* 2018;9:1484. [PMC free article] [PubMed] [Google Scholar]

[23] Rasool AH, Rahman AR, Ismail R, Hatim S, Abdullah AR, Singh R, et al. Ethnic Differences in Response to Non-Selective Beta-Blockade among Racial Groups in Malaysia. *Int J Clin Pharmacol Ther.* 2000;38:260–9. [PubMed] [Google Scholar]

[24] Ramu P, Kumar KN, Shewade DG, Swaminathan RP, Dutta TK, Balachander J, et al. Polymorphic Variants of Beta1 Adrenergic Receptor Gene (Ser49Gly and Arg389Gly) in Healthy Tamilian Volunteers. *Indian J Med Res.* 2010;132:62–6. [PubMed] [Google Scholar]

[25] Kumar KN, Ramu P, Rajan S, Shewade DG, Balachander J, Adithan C. Genetic Polymorphisms of Beta1 Adrenergic Receptor and their Influence on the Cardiovascular Responses to Metoprolol in a South Indian Population. *J Cardiovasc Pharmacol.* 2008;52:459–66. [PubMed] [Google Scholar]

[26] Huang J, Li C, Song Y, Fan X, You L, Tan L, et al. ADRB2 Polymorphism Arg16Gly Modifies the Natural Outcome of Heart Failure and Dictates Therapeutic Response to β-Blockers in Patients with Heart Failure. *Cell Discov.* 2018;4:57. [PMC free article] [PubMed] [Google Scholar]

[27] Chavey WE., 2nd The Importance of Beta Blockers in the Treatment of Heart Failure. *Am Fam Physician.* 2000;62:2453–62. [PubMed] [Google Scholar]

[28] Cesario DA, Fonarow GC. Beta-Blocker Therapy for Heart Failure:The Standard of Care. *Rev Cardiovasc Med.* 2002;3:14–21. [PubMed] [Google Scholar]

[29] Dézsi CA, Szentes V. The Real Role of β-Blockers in Daily Cardiovascular Therapy. *Am J Cardiovasc Drugs.* 2017;17:361–73. [PMC free article] [PubMed] [Google Scholar]

[30] Kurnik D, Cunningham AJ, Sofowora GG, Kohli U. Li is not Associated with Sensitivity to Beta(1)-Adrenergic Blockade in Humans. *Pharmacogenomics.* 2009;10:1581–7. [PMC free article] [PubMed] [Google Scholar]

[31] Sardana M, Syed AA, Hashmath Z, Phan TS, Koppula MR, Kewan U, et al. Beta-Blocker use is Associated with Impaired Left Atrial Function in Hypertension. *J Am Heart Assoc.* 2017;6:e005163. [PMC free article] [PubMed] [Google Scholar]

[32] Ko DT, Hebert PR, Coffey CS, Curtis JP, Foody JM, Sedrakyan A, et al. Adverse effects of Beta-Blocker Therapy for Patients with Heart Failure:A Quantitative Overview of Randomized Trials. *Arch Intern Med.* 2004;164:1389–94. [PubMed] [Google Scholar]

[33] DiNicolantonio JJ, Lavie CJ, Fares H, Menezes AR, O'Keefe JH. Meta-Analysis of Carvedilol Versus Beta 1 Selective Beta-Blockers (Atenolol, Bisoprolol, Metoprolol, and Nebivolol) *Am J Cardiol.* 2013;111:765–9. [PubMed] [Google Scholar]

[34] Bellenger NG, Rajappan K, Rahman SL, Lahiri A, Raval U, Webster J, et al. Effects of Carvedilol on Left Ventricular Remodelling in Chronic Stable Heart Failure:A Cardiovascular Magnetic Resonance Study. *Heart.* 2004;90:760–4. [PMC free article] [PubMed] [Google Scholar]

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Pediatric Research and Child Health

RESEARCH ARTICLE

The Emergence of Deafness, Blindness, Cerebral Palsy, and Mental Retardation among very low birth weight (VLBW) Infants, aged 21-24 Months

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Abstract

Purpose: The study aims to assess the emergence of deafness, blindness, cerebral palsy, and mental retardation among very low birth weight (VLBW) infants, aged 21-24 months.

Methods: A study was conducted with a total of 123 premature Saudi infants, who were recruited from King Khalid University Hospital. The association among demographic factors and neurodevelopmental morbidities were identified with the help of regression analysis.

Results: A significant relationship between deafness and cerebral palsy was found. However, there was no significant relationship between retinopathy of prematurity and intraventricular hemorrhage. Furthermore, maternal education plays a positive role in the neurodevelopment of preterm newborns.

Conclusion: This study has concluded that there has been significant relationship between cerebral palsy and deafness in preterm infants with very low birth weight. On the contrary, there was no significant relationship found between intraventricular hemorrhage and retinopathy of prematurity.

Keywords: Blindness, Cerebral Palsy, Deafness, Neonatal Care, Neonatal Morbidity

Abbrevations: **BPD**: Bronchopulmonary Dysplasia, **AAP:** American Academy of Pediatricians, **ROP:** Retinopathy of Prematurity, **HIE:** Hypoxic Ischemic Encephalopathy, **PPHN**: Persistent Pulmonary Hypertension, **PDA**: Patent Ductus Arteriosus

Introduction

The primary cause of neonatal morbidity and mortality is the preterm birth of an infant [1]. Developments in perinatal care have enhanced the chances of survival of preterm low birth weight infants of gestational age of less than 25 weeks. Although, the survival rate has increased, the chances of neonatal morbidity and neurodevelopment impairment have also augmented and increased. On the other hand, the lower the gestational age and the birth weight of the preterm infant; the higher the infants' likelihood of suffering from major neonatal morbidity [2].

The disabilities occur due to the improper development of the infant or poor functioning of nervous system. The limitations that are manifested during infancy or early stage of childhood include cognition, vision, behavior, and hearing. There are the modalities that these disabilities are traced in the early stage of neurodevelopment. However, the cognitive disabilities among infants and children includes mental retardation as well as it further leads to the learning disabilities. Moreover, distress and chronic stress usually leads to psychological changes, which further result in causing physical distress, psychiatric disorder, and psychological distress [3].

Early years of childhood are important for language development and communication because during this time the brain is at its highest capacity to go about structural changes in regard to external stimulation and is termed as neural plasticity [4]. According to American Academy of Pediatricians (AAP), there are 2% to 3% of infants with developmental disabilities. In most of the cases, no definite cause is ever found. Intellectual disabilities can be further classified as mild, moderate, severe, and profound; and this is done on the basis of various intellectual disabilities. Mental disability is usually result of genetic disorders; for instance, Down syndrome. In case of infants, the genetic disorders are usually detected at an

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early stage. It is due to certain physical or facial characteristics that alert the parents or the clinician.

Experts, who are always associated with disabled children, believed that in such cases early intervention pays off immensely [5]. Not much work has been done in order to investigate the outcome of early intervention for children, who are deaf and blind. However, various studies illustrated that, the early identification and intervention for hearing loss, proved out to be beneficial for infants as it enhances their language skills [6]. Continued ill-health of the mother can lead to further morbidities or disabilities and can negatively impact baby's health. Whereas, these complications and consequences are not much focused upon in various countries in low-income countries. Families situated in Middle Eastern regions complaint about isolation and lack of community resources to help them in improving their child's abilities. One of the main reasons behind the lack of awareness or medical care is that there are no health professionals or researchers available in such regions. In most cases, these disabilities also come forward due to the mother's depressive state [7].The aim of the present study is to assess the relationship between Retinopathy of Prematurity and Intraventricular hemorrhage as well as the relationship between deafness and cerebral palsy.

Methods

A total of 123 premature Saudi infants, with birth weight less than or equal to 1500 grams & gestation less than equal to 32 weeks were recruited. These infants that were chosen for the study were from the King Khalid University Hospital in which these premature infants were delivered. The study was approved from the ethics committee. The analysis was done on the basis of neurological and developmental assessment, along with medical and social history. It was done in order to determine functional and neurodevelopmental outcomes among infants. The neurologic examinations were performed by certified physicians, who had been well trained in this department. The neurological assessment included the evaluation of strength reflex, angles as well as posture. The demographics include age, gender, and gestation age and body weight. The factors examined included were maternal education, birth weight, early-onset sepsis, late-onset sepsis, grades 3 to 4 IVH/PVL, Bronchopulmonary Dysplasia (BPD), Retinopathy of Prematurity (ROP), Hypoxic Ischemic Encephalopathy (HIE), Persistent pulmonary hypertension (PPHN), Patent ductus arteriosus (PDA).

During the first four weeks of life, Intraventricular hemorrhage (IVH) is usually detected by head ultrasound during first four weeks of life. Moreover, it is graded in accordance with Papile's classification. Periventricular echogenicity, which is noticed on the head ultrasound and is performed at any time during the NICU stay, is known as Preventricular Leucomalacia (PVL). Retinopathy of Prematurity (ROP) is detected during routine retinal examination. Furthermore, Patent Ductus Arteriosus (PDA) is established through an echocardiography with signs of left-to-right shunting. Bronchopulmonary Dysplasia (BPD) refers to the requirement of oxygen at 36 weeks, corrected age.

Table 1: Gestational Age of the study participants

Gestational age	Number	Percent
26	3	2.4
27	6	4.9
28	25	20.3
29	23	18.7
30	36	29.3
31	14	11.4
32	11	8.9
34	3	2.4
35	1	0.8
38	1	0.8
Total	123	100

The data were analyzed to evaluate the impact of morbidities on various neurodevelopmental disabilities like blindness, deafness, cerebral palsy (CP) and mental retardation (MR). Severe mental retardation syndrome includes optic atrophy, microcephaly, that further results in impaired vision, defect in hearing, seizures, restricted movements of large joints and spasticity. The study examined the influence of maternal education and early intervention on BINS at 21 to 24 months infants. Maternal education is considered as one of the strongest determinants that ensures infant survival in developing countries. The statistical analysis was done to assess maternal education and its association with disabilities. BINS was classified into two different groups. First group was low and the other group was taken as moderate (Mod) and high. However, 'Low' illustrated that no further follow-up is required; whereas, 'high' suggested that further developmental testing is necessary. Descriptive statistics were calculated using mean and standard deviation. Spearman rank correlation analysis was done to find the correlation between Retinopathy of Prematurity and Intraventricular hemorrhage. Chi square test was done to find the association deafness and cerebral palsy. $P<0.05$ was considered as statistically significant (Table 2).

Table 2: Association between Deafness and CP

	CP (No)	CP(Yes)	Total
Deafness (No)	120(98.4%)	2(1.6%)	122
Deafness(Yes)	0 (0.0%)	1 (100%)	1
Total	120	3	123

Results

The total number of neonates who were examined in the study were 123. Out of the total, newborn females totaled 53 (43.1%). The mean (SD) of the gestation age in weeks was 29.65 (1.82). Amongst the total number of neonates, the lowest gestational age of preterm infants was 26 weeks and the highest gestational age was 38 weeks. Apart from this, the premature neonates that had a gestational age of 28 weeks were 25 (20.3%) infants. Similarly, the preterm neonates that had a gestational age of 30 weeks were 36 (20.3%) infants and were the highest number of infants in that particular classification group (Table 1). Among the 123 mothers, 85 (69.1%) were educated. The

correlation between ROP and IVH was examined and found that spearman, s rho r=0.145 p=0.109 which is not statistically significant.). The association between Deafness and Cerebral Palsy was found to be statistically significant

(Chi-square=40.38, P<0.001)

Discussion

The results revealed that there is significant relationship between Deafness and Cerebral Palsy. However, there was no significant relationship between Retinopathy of Prematurity (ROP) and Intraventricular hemorrhage (IVH). The results are consistent with the study conducted by Neubauer et al. (2008), in which it was discovered that the proportion of disabilities has increased with decreasing gestational age of the newborn. Infants having high grade intraventricular hemorrhage, neonatal seizures, necrotizing enterocolitis and bowel perforation were at a significant risk for developing and enduring major impairment [8].

In a study conducted by Wilson-Costello et al. (2007), modern therapies that were incorporated with regard to low birth weight infants were compared to traditional therapies that were provided to low birth weight infants in the previous two decades. The comparison features were the rate of survival and death with and without impairment and the therapies that were provided. The population comprised of 496 low birth weight infants born (2000-2002), 1749 neonates that were born (1982-1989) and 233 newborns born (1990-1999). The findings revealed that between 1990 and 1999, survival rate has increased from 49% to 68%, but so did neonatal morbidity. Therefore, it leads to survival without impairment as well as survival with impairment [9].

The therapy modification between 2000-2002 included a reduction of postnatal steroid use. Apart from this, antenatal steroid use was increased. It was observed that there were no changes in the rate of lung disease. In addition, there was found to be a decrease in morbidities; such as, severe intraventricular hemorrhage (IVH) and early-onset sepsis (EOS) and late-onset sepsis (LOS). With therapeutic care, the rate of neurodevelopmental impairment has reduced from 35% to 23%. More specifically, the rate of cerebral palsy reduced from 13% to 5%. It was concluded that there was significant reduction in the neurodevelopment impairment in neonates with very low birth weight between 2000-2002 as compared to a decade early. The improved outcomes and the increase in the survival rate of newborns with low birth weight were due to modern therapies that were being provided. These included both perinatal and neonatal factors. However, despite the use of postnatal steroids, the lung condition of the infants showed no improvement [10]. Many studies revealed that maternal education has a positive impact on neurodevelopmental outcome among very low birth infants. The results conducted by Voss et al. (2012) revealed that the maternal educational background was directly associated with the intellectual growth of pre-term infants. Low maternal educational background was connected to reduced composite intelligent quotient of pre-term kids, who were between the ages of 10-13 years old during the study [11-14].

Conclusion

The preterm birth of an infant can be the cause of neonatal morbidity and mortality. Preterm neonates with a very low birth weight have an increased risk of suffering from neurological, sensory, development, and functional morbidity. The neurodevelopment of all very low birth weight newborns must be monitored and the relevant therapies must be incorporated to ensure and optimize their survival. Some morbidities are directly associated with long-term outcomes of the development of preterm infants. This study has concluded that there is a significant relationship between Deafness and Cerebral Palsy in infants. On the contrary, there was no significant relationship found between Intraventricular hemorrhage (IVH) and Retinopathy of Prematurity (ROP). Additionally, maternal education played a significant role in the development of preterm newborns. However, more studies must be conducted in relation to preterm babies and neonatal morbidity and mortality to get further insights into the causes of morbidities, suffered by preterm babies with very low birth weight.

References

1. Alhaidari SI, Al Houssien AO, Alteraiqi MA, Al Arafah AM, Al Houssien RO, et al. (2016) Children's growth pattern and mothers' education and socio-economic status in Riyadh, Saudi Arabia. *Journal of Health Specialties* 4: 276. [View Article]
2. Ashford L (2002) Hidden suffering: disabilities from pregnancy and childbirth in less developed countries. Policy brief Washington, DC: Population Reference Bureau *Measure Communication* 6. [View Article]
3. Azar M & Badr LK (2006) The adaptation of mothers of children with intellectual disability in Lebanon. *Journal of Transcultural Nursing* 17: 375-380. [View Article]
4. Cole EB & Flexer C (2007) Children with hearing loss: Developing listening and talking. *San Diego: Plural Publishing Inc* [View Article]
5. Estes A, Zwaigenbaum L, Gu H, John TS, Paterson S, et al. (2015) Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life. *Journal of neurodevelopmental disorders* 7: 24. [View Article]
6. Guralnick MJ (Edn) (1997) The effectiveness of early intervention. *Baltimore: Paul H Brookes Publishing Company* [View Article]
7. Hack M, & Fanaroff A A (1999) Outcomes of children of extremely low birthweight and gestational age in the 1990's. *Early human development* 53: 193-218. [View Article]
8. Kennedy CR, McCann DC, Campbell MJ, Law CM, Mullee M, et al. (2006) Language ability after early detection of permanent childhood hearing impairment. *New England Journal of Medicine* 354: 2131-2141. [View Article]
9. Neubauer AP, Voss W & Kattner E (2008) Outcome of extremely low birth weight survivors at school age: the influence of perinatal parameters on neurodevelopment. *European journal of pediatrics* 167: 87-95. [View Article]
10. Sapolsky RM (2007) Stress, stress-related disease, and emotional regulation.[View Article]

11. United Nations Children's Fund (1997) Guidelines for monitoring the availability and use of obstetric services. New York, NY: United Nations Children's Fund 103.[View Article]

12. Voss W, Jungmann T, Wachtendorf M, & Neubauer A P (2012) Long-term cognitive outcomes of extremely low-birth-weight infants: The influence of the maternal educational background. *Acta Paediatrica* 10:569-573. [View Article]

13. Wilson Costello D, Friedman H, Minich N, Siner B, Taylor G, et al. (2007) Improved neurodevelopmental outcomes for extremely low birth weight infants in 2000-2002. *Pediatrics* 119: 37-45. [View Article]

14. Xiong T, Gonzalez F, & Mu DZ (2012) An overview of risk factors for poor neurodevelopmental outcome associated with prematurity. *World Journal of Pediatrics* 8: 293-300. [View Article]

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Check for updates

Research Paper

Physical properties of menstrual hygiene waste as feedstock for onsite disposal technologies

Sarani Sasidaran, Prateek Kachoria, Antony Raj, Sudha Ramalingam, Brian R. Stoner, Katelyn L. Sellgren and Sonia Grego

ABSTRACT

Onsite incinerators offer a promising solution to the challenges of menstrual waste disposal, particularly in institutional settings. However, thermochemical processes are well known to depend on the nature of the feedstocks and their moisture content (MC). An analysis is presented of the physical properties of menstrual waste as potential feedstock for onsite incinerators. Waste was generated in three institutional settings – from women of wide age range and from people with different socioeconomic backgrounds such as workers and students. Over 10 separate collections, 613 discarded menstrual pads were obtained with over 93% of the waste representing commercially disposable pads and less than 7% of the waste appearing to be homemade cloth pads. A surprising finding of this study was that a significant fraction of the commercially disposed pads (23–37%) had a large wet weight (over 20 g) which was associated with MC exceeding 50 wt%. This high MC was attributed to the practice of washing the pads prior to disposal, and it was much higher than what is standard for thermal processing. These data suggest that testing protocols for menstrual waste incinerator technology performance should include an evaluation of the effects of higher MC, unfolded pads, and occasional cloth pads.

Key words | incineration, menstrual health and hygiene (MHH), moisture content, pads, sanitation

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HIGHLIGHTS

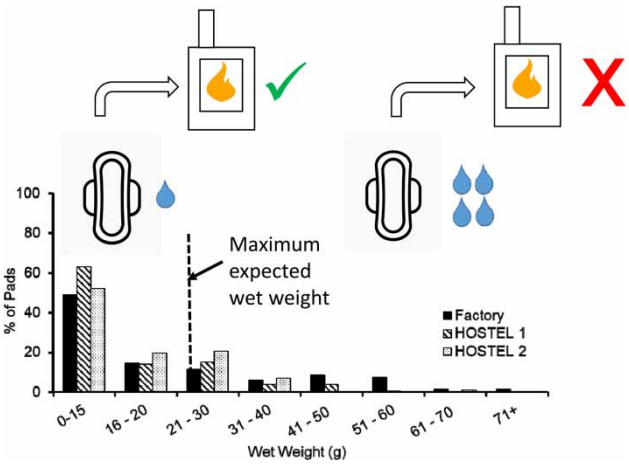
- Menstrual waste consists mainly of commercially disposable pads (93% or more) and of homemade cloth pads (6–7%).
- This study found that nearly a third of commercial pad waste had a wet weight of over 20 g, which was associated with moisture content (MC) exceeding 50 wt%.
- High MC waste is attributed to the practice of washing pads prior to disposal.
- Moist waste may result in pollutant emission and incomplete processing by onsite incinerators.

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GRAPHICAL ABSTRACT



INTRODUCTION

Menstrual hygiene is an essential aspect of sanitation for women and girls, which is often neglected in sanitation program implementation in low- and middle-income countries, yet is critical for women's health and dignity as well as environmental safety (Sommer *et al.* 2013; Tilley *et al.* 2013).

A body of research has shown that the lack of access to proper menstrual hygiene management has a negative impact on women's health (Balamurugan & Bendigeri 2012; Das *et al.* 2015) and women's participation in education and the workforce (Bodat *et al.* 2013; Sommer *et al.* 2016).

Challenges related to menstrual health and hygiene (MHH) in low- and middle-income countries are related to both access to safe sanitary products and safe disposal options at the household and community levels (Elledge *et al.* 2018).

The most used menstrual products are absorbents such as sanitary pads, tissue paper (disposable, one-time use) and cloth, typically homemade (Elledge *et al.* 2018). Cloth pads are the traditional and cheaper option, while disposable sanitary pads are increasingly available, especially in urban environments, and considered as aspirational products (van Eijk *et al.* 2016). Statistical associations have been reported between the use of disposable sanitary pads and younger age, higher social class, and urban residence (Elledge *et al.* 2018); however, affordability represents a major constraint (Crofts & Fisher 2012).

Disposal of used sanitary pads is a critical element of the MHH value chain. Cloth pads are cheaper and environmentally less polluting, but cleaning is challenging since it requires access to water, privacy and drying in the sun for its disinfecting effect, as opposed to a hidden drying area utilized for a sense of shame (van Eijk *et al.* 2016). Disposable pads offer convenience of use and are considered as aspirational products for women and girls; however, they present a solid waste challenge. A woman will have an average of 459 periods over her lifetime and dispose of 125–150 kg of sanitary waste (Bharadwaj & Patkar 2004).

The lack of disposal solutions leads to negative health outcomes, e.g. infrequent pad changes during school (Singh & Bhardwaj 2017) as well as environmental issues. Where solid waste management is not available, disposal practices for sanitary pads include throwing them in the open where they can contaminate water bodies, burning (in an open pit), burying them (whether waste is compostable or not), or flushing in the toilet, where they cause clogging and impair the biological degradation of human waste in sanitation systems (Path 2017).

In addition to the lack of sanitation infrastructure, menstruation is associated with stigma and taboos such as the perception of impure or shameful processes leading to restrictions of women's activities during menstruation

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and secrecy around menstrual fluid management (Sommer *et al.* 2013; Garg & Anand 2015). Importantly, cultural beliefs and taboos, in addition to convenience and information, contribute to the selection of disposal method (e.g. bury or burn) and to practices such as washing pads before disposal (Ali & Rizvi 2010; Sommer *et al.* 2013).

Onsite incineration is an option for managing the disposal of menstrual waste to achieve both pathogen and volume reduction, thus alleviating the environmental problems associated with improper disposal. Small-scale incinerators placed within a toilet facility may provide an effective and discrete way of disposing menstrual absorbents, align with decentralized waste treatment practices, and are well suited for institutional settings such as schools and worksites (Elledge *et al.* 2018).

Concerns in regard to the adoption of incineration are related to negative health and environmental impacts associated with the release of pollutants and toxic emissions as well as incomplete waste processing (Sommer *et al.* 2013); in the past decade, poor design and insufficient operation and maintenance have resulted in negative experiences for people (Tilley *et al.* 2013).

The Government of India has promoted the adoption of incineration for menstrual waste as part of its Swatch Bharat (Clean India) mission (2018). In India, menstrual waste volume is significantly high, with an estimated 121 million women disposing of 113,000 tons of menstrual hygiene waste (12.3 billion pads) annually (Path 2017). A number of programs implemented to increase access to sanitary pads have been successful in achieving widespread adoption recently, with an estimated 98% urban women using them (Dixit *et al.* 2016) and 64% of women in low-income areas gaining access to such pads (Garikipati & Boudot 2017). It is estimated that over 50 suppliers on the Indian market sell sanitary pad incinerators in institutional settings, with the majority being electric incinerators processing batch sizes as small as 10 pads (Bill & Melinda Gates Foundation 2021). The Government of India has classified sanitary pads as solid waste (SWM 2016), with associated emission thresholds for incineration, and since 2016 has stipulated the manufacturers of sanitary napkins to, among other things, provide wrappers for used sanitary napkins. However, lack of oversight and enforcement of regulations remains a concern for menstrual waste incineration (Elledge *et al.* 2018).

To our knowledge, there are no published reports on the performance of menstrual waste incinerators on the Indian market nor is there any information that includes the details of operation with actual menstrual waste.

Thermochemical processes such as incineration are well known to depend on the nature of the feedstock and its moisture content (MC). A very low MC is advantageous to incineration, and, to give some examples, the maximum MC allowed for thermal processes including incineration has been reported to be <15% (Burguete *et al.* 2016; Eke *et al.* 2019), while municipal solid waste thermal processes operate at no more than 20–25% moisture (Dong *et al.* 2016). In general, a higher MC can result in higher pollution and incomplete treatment (Youcai *et al.* 2004).

In order to enable the evaluation of menstrual pad incinerators based on realistic feedstock characteristics, this paper characterizes the physical properties of menstrual waste in the context of feedstocks for incineration. We collected and characterized menstrual waste from women's bathrooms from three institutional sites in India, and this paper reports our findings on the nature of the waste and its physical parameters: wet weight and MC.

MATERIAL AND METHODS

Site characteristics and study population

Menstrual waste was collected from three sites in the city of Coimbatore, India, between September 2019 and March 2020. All sites were communal women's toilet facilities: a private textile factory and two student residence halls in an educational institute, Hostel 1 and Hostel 2. The collection of pads was carried out by the caretakers (custodian or janitor) of each facility. The caretakers received an orientation about the purpose of the project prior to the beginning of the study and wore personal protective equipment (masks and gloves).

Site 1: The private textile factory site is a shared toilet for live-in and day workers. According to the warden, the number of users ranges between 20 and 50 women

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between 18 and 50 years of age. The facility has five toilet stalls, with a dedicated lidded bin for menstrual waste disposal. After an accumulation period of 2–3 weeks, the five bins were collected by the caretaker by placing the waste in one larger disposal bag and delivered to the investigators for analysis. Five separate collections were used for this study, yielding a total of 304 pads.

Site 2: Hostel 1 is a multistorey building housing approximately 900 female students, estimated to be between 23 and 30 years of age. Each floor contains a toilet facility with 25 individual toilet stalls. Each stall has a dedicated lidded bin for menstrual waste (Figure 1(a)). Caretakers empty the waste bins daily. Standard disposal operations involve the caretaker collecting the contents of all individual bins from one floor into one large disposal bag. In order to preserve the contents of each bin and prevent clumping of the pads, black garbage liner bags were provided to the caretakers the day before collection and placed in each bin. Black liners containing at least one pad were delivered to the investigators (Figure 1(b)). Four separate collections were carried out at this site for one or more floors for a total of 47 bags.

Site 3: Hostel 2 is a multistorey building housing approximately 350 female students, estimated to be between 23 and 30 years of age. Each floor contains a toilet facility with 10 individual toilet stalls. One waste bin is shared between two toilet stalls. The janitors empty the individual waste bins daily into one large disposal bag per floor, similar to hostel 1. Black garbage liners for the bins were provided to the caretakers the day before collection. After 1 day, liners containing at least one pad were delivered to the investigators. One collection was carried out at this site for a total of 24 bags.

Menstrual waste physical analysis

Analysis began the same day menstrual waste was received from the caretakers. Metal tongs were used to sort the waste into individual pads. For each pad, its type was recorded as either commercially disposable cellulose or homemade cloth (Figure 1(c)). Commercial pads were further analyzed for disposal shape and assigned one of the following categories: unfolded, rolled up/crumbled, or stuck together. Pads that were stuck together due to exposed adhesive liners were recorded as such and not analyzed further. Additional information collected from commercial pads included the presence of wings and additional coatings, as determined by a different color of the substrate material. Cloth pads were homemade and nonuniform in size; therefore, we recorded the length, width, and layers of cloth of the pad.

In a fume hood, an analytical balance was used to record the wet weight for all individual pads received. A subset of approximately 15–18 pads from each collection was further evaluated for MC analysis. Pads were assigned to three weight categories based on wet weight: low (5–20 g), medium (21–40 g), and heavy (>41 g). The pads for MC measurements were randomly selected in quantities proportional to their number in each of the three weight categories.

MC was calculated using the conventional formula $\%\ \mathrm{MC} = (W_{\mathrm{wet}} - W_{\mathrm{dry}}/W_{\mathrm{wet}})*100$, where W_{wet} is the wet



Figure 1 | (a) Photograph of a bathroom stall with individual waste bins. (b) Waste bin bag liner as received. (c) Disposed of homemade cloth pad. (d) Individually labeled commercial pads in a dry oven for moisture measurement.

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weight and W_{dry} is the dry weight. Pads were placed inside a vented hot air oven whose temperature did not exceed 100 °C (typically 55 – 70 °C) (Figure 1(d)). The drying temperature is lower than the standard value of 105 °C because of the limited temperature control capability of the oven. By drying at 55–70 °C, we ensured that no temperature swings exceeded 105 °C, which would cause mass loss to volatilization, and thus, we obtained an accurate dry weight value at the cost of longer drying times. The weight of the pads was recorded over multiple days until it did not change more than 4% from prior measurement, thereby providing the dry weight value. After the completion of analytical characterization, all specimens were discarded as biohazardous waste.

Ethics

PSG IMSR's Institute Human Ethics Committee (IHEC) reviewed and approved the study as exempted (2018/Appr/Exe/017). Duke University IRB determined the study to be non-human subject research.

RESULTS AND DISCUSSION

A total of 613 discarded menstrual pads were collected over multiple collections from three institutional sites, a factory and two residence halls from an institution of higher education (Hostels 1 and 2). Two types of menstrual waste were observed: commercially disposable cellulose pads and homemade cloth pads. The overwhelming majority (≥93%) of the pads collected from the three sites were commercially disposable pads, and of these, a large majority had wings or some type of cellulose coating as detected by different colored pad material. Homemade cloth pads were found in waste in two of the three sites, namely, the factory and Hostel 1. Results over multiple collections at the same sites were consistent over time. Table 1 summarizes the

Table 1 | Descriptive statistics of waste collection and its weight and moisture properties

	Collection sites			
Parameter	**Factory**	**Hostel 1**	**Hostel 2**	**Total number**
Collected pads	304	207	102	613
Collections	5	4	1	10
Commercial pads	284	194	102	580
Cloth pads	20	13	0	33
Commercial pads (%)	93	94	100	95
Featuring wings, coatings	244	192	99	535
Commercial pads in waste				
Unfolded	220	127	7	354
Rolled up/crumpled	8	73	95	176
Stuck together	22	7	0	29
Individually weighed pads	268	187	102	557
Wet weight median (g)	13.9	14.1	15.8	15.2
Wet weight minimum/maximum (g)	6.8/89.2	5.7/52.8	6.8/63.8	5.7/89.2
Pads with wet weight >20 g (%)	37	23	28	31
Moisture analysis of commercial pads				
Pads analyzed for moisture	44	64	17	125
Dry weight median (g)	8.5	7.6	8.9	7.8
Dry weight minimum/maximum (g)	4.8/11.7	4.7/13.7	5.4/14.1	4.7/14.1

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results of the physical analysis of the disposable commercial pads for all collections.

Properties of commercial pads

There was a difference in the manner of disposal across sites, with the pads at the factory site being largely disposed of as unfolded, while at the hostels, we observed a mix of unfolded and rolled up, often wrapped by a piece of newspaper. We note that India being a country with a washing culture, bathrooms do not feature toilet paper, which is commonly used as a wrapper prior to disposal in other geographies. Unfolded pads are likely to stick to each other and form clumps that may be problematic for incineration, cause them to stick to surfaces, or be improperly positioned inside the incinerator.

The number of clustered pads was higher at the factory site than at the hostels due, in part, to both the manner of collection and the adoption of liner bags for the menstrual waste bins at the hostels.

The wet weight of the pads had a similar median value consistent across sites (median wet weight between 13.9 and 15.8 g); however, the range was very broad, from 5.7 to 89 g. The dry weight of the pads had median values 7.6–8.9 g and ranged between 4.7 and 14.1 g, consistent with typical ranges for different commercial pad sizes.

Commercial pads are designed to absorb typically 5 ml and, in some models, up to 10 ml of liquid (Fraser *et al.* 2001). Given that blood density is slightly more than water (1.06 g/ml), we estimate that blood adds 5–10 g to the dry weight of pads. Using the largest dry weight measured, for pad, of 14 g, we estimate 14 g (pad) +5 g (blood) ~ 20 g as the maximum weight of used pads (dashed line in Figure 2), being aware that in some cases we may observe 14 + 10 = 24 g.

Figure 2 shows the wet weight distribution of the pads, indicating that approximately half of the collected waste was below 15 g, with the majority below the expected maximum weight of 20 g. However, a long distribution tail of wet weights higher than 20 g was recorded for all three sites, particularly at the factory site, where as many as 37% of the pads were heavier than 20 g. Weights are plotted against measured MC in Figure 3. The data show that, consistently across the three sites, heavier wet pads feature very high MCs reaching 80–90%. The dotted line at 50% MC

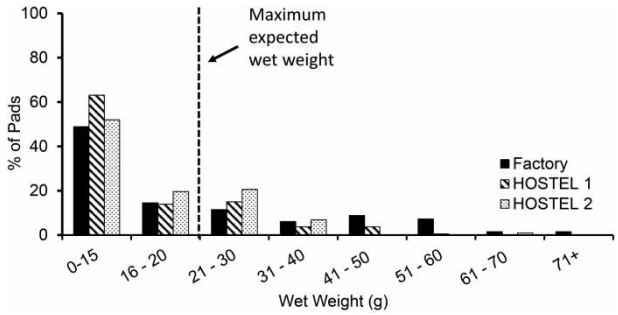


Figure 2 | Wet weight distribution of commercial pads shown as a percentage of the total number of pads from each site.

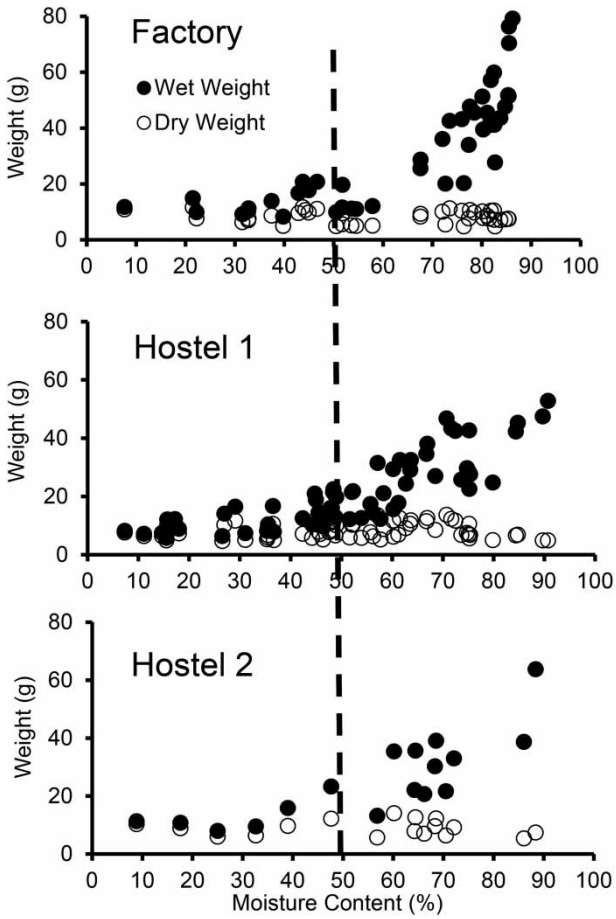


Figure 3 | MC results: wet and dry weights are reported by site. The dashed line represents 50% moisture, the expected upper limit for moisture for used menstrual pads.

corresponds to an 8 g dry pad receiving 8 ml of blood. This is expected to be an upper limit for MC for two reasons: (1) the typical volume of blood for pad rarely exceeds 5 ml and (2) in this study, drying of the pad can occur during

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the time between disposal and collection (time lag = 1 day for hostels, 1–2 weeks for the factory site). Yet, the measured moisture values are too high to be consistent with moisture from menstrual blood alone. While we did not conduct chemical analysis on the liquid, we note that since India has a washing culture, a tap with water per personal wash is available in each of the toilets included in this study. Thus, we infer that wet heavy pads derive from the practice of washing them prior to disposal (Ali & Rizvi 2010; Sommer *et al.* 2013). This practice is associated with the concept that menstrual blood is impure and the belief that others should not see it, and also possibly with a lack of awareness on proper disposal practices (Garg & Anand 2015).

While MC was not measured for all pads (wet weight was measured), all pads in Figure 3 whose wet weight exceeded 20 g had a MC exceeding 50% (dotted line). Thereby, a significant portion of disposed pads (23, 28 and 37%) with wet weight above 20 g were estimated to have a MC ranging from 50 to 90%. Such high moisture levels could be problematic for thermal processing because they significantly exceed the optimal 15–25% feedstock moisture (Dong *et al.* 2016; Eke *et al.* 2019).

Properties of cloth pads

Out of the 613 pads collected in this study, 33 were homemade cloth pads. We note that since the cloth pads are reusable, the low fraction of disposed cloth pads does not represent an indication of how prevalent their use is. It is, however, an indication that the menstrual pad incinerator should be evaluated for its ability to process feedstock that contains a percentage of cloth material that has been reused multiple times.

Figure 4(a) illustrates the distribution of wet weight of cloth pads. Higher weights were observed particularly at the factory site, where cloth as long as 88 cm and as wide as 38 cm was folded in layers to serve as a pad (for reference, the length and width of a typical commercial pad product is 25 cm length × 7.2 cm width). The MC of the homemade cloth was, however, narrower than that observed for the commercial pads and ranged between 16 and 40%. Also, the MC was not associated with wet weight, with some of the heavy pads consisting of a larger quantity of material. A possible explanation for the difference in MC between cloth and commercial pads is the fact that commercial cellulose pads are designed to absorb liquid and, therefore, when washed, retain a large amount of liquid, while cloth pads can dry more easily after disposal.

Comparison with prior studies

Prior studies of menstrual waste are based on surveys, and only a few of them address specific questions on disposal practices that are relevant to our physical assessment results.

A key finding of this study is that a significant fraction of the commercial pads (23–37%) was associated with a high MC consistent with washing prior to disposal.

Prior literature that investigated reproductive tract health included questions about the pad material type and the frequency of personal washing. Within the studies that included details on menstrual hygiene material, the topic of pad washing emerged in the context of cloth pads and their reuse (Omidvar & Begum 2010; Das *et al.* 2015), while separate studies addressed the topic of pad disposal, with no mention made on washing (Wagh *et al.* 2018). Combining

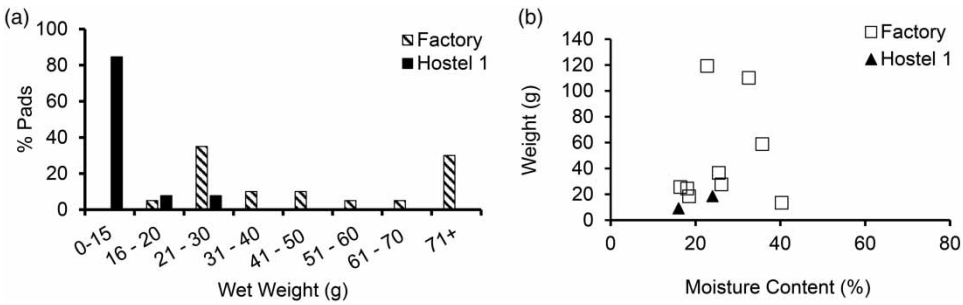


Figure 4 | Analysis of homemade pad properties. (a) Distribution of wet weight (factory: 20 pads; Hostel 1: 13 pads). No cloth pads were collected from Hostel 2. (b) MC was measured for a range of wet weights.

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questions on both washing and disposing, a study in Pakistan reported that as many as 60% of the respondents washed and discarded cloth pads; however, there was no explicit question about the washing of disposable pads (Ali & Rizvi 2010). We identified one publication (Kumar & Srivastava 2011) that, while reporting on social restrictions and taboos around menstruation, found that the majority of girls from residential areas in a city in India used disposable pads and that 25% of them washed such sanitary napkins after using them and then disposed them. This observation of washing sanitary napkins prior to disposal is consistent with the high MC in waste that we measured.

Another finding of our work is that a large portion of the pads are disposed of unfolded and, therefore, not wrapped in any paper or plastic. This result is not reflected in prior literature, because the question whether pads are wrapped prior to disposal was not directly asked. In Nayak *et al.* (2017), 98% of the respondents described the material selected to wrap pads (either paper or plastic), and only 1% of the surveyed women did not respond to the question. In Dipali *et al.* (2009), the questionnaire asked about the material the pad was disposed in, not whether it was wrapped; thus, no information on unfolded pads was captured. This difference between our findings and prior reports on this matter highlights the complementarity of physical assessments and surveys in the study of behavior and practices.

CONCLUSIONS

We investigated the physical properties of menstrual waste as feedstock for incinerators that represent an attractive solution to the challenges of menstrual waste disposal, particularly in institutional settings.

Menstrual waste was generated by women of different ages (18–50 years) and different education/income (workers vs. college students), yet consistent outcomes were observed across the two groups. The use of commercial cellulose pads was prevalent at all sites (>93%), in agreement with recent reports (Dixit *et al.* 2016; Garikipati & Boudot 2017). In two out of three sites, 6–7% of the waste consisted of homemade cloth pads, suggesting that a menstrual pad incinerator technology will encounter multiple types of feedstock. The commercial pads were disposed both unfolded or folded, indicating that the incinerator configuration should be robust with respect pad insertion and positioning inside the unit; folded pads were not wrapped in manufacturer-provided wrappers, despite country regulations and standard use instructions.

A major finding of this study was that a significant fraction of the commercial disposed pads (23–37%) had a large wet weight (over 20 g), which was associated with MCs exceeding 50%. This MC is much higher than ideal for thermal processing (<25%) and can be quite problematic for typical incinerators, leading to incomplete combustion and increased hazardous air emissions. We infer that the MC is due to the practice of washing pads before disposal, a practice that has been reported in washing cultures. While this study on menstrual waste in India is limited to three sites, hundreds of pads were collected in repeated collections over periods of months, and the results were consistent across collections. These findings suggest that further research is needed to fill the knowledge gap on practices in menstrual disposal and that testing protocols of disposal technologies should include an evaluation of the effects of high MC, unfolded pads, and occasional cloth pads on waste processing performance.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Ali, T. S. & Rizvi, S. N. 2010 Menstrual knowledge and practices of female adolescents in urban Karachi, Pakistan. *Journal of Adolescence* **33** (4), 531–541.

Downloaded from http://iwaponline.com/washdev/article-pdf/11/3/474/889950/washdev0110474.pdf
by guest

Balamurugan, S. S. & Bendigeri, N. 2012 Community-based study of reproductive tract infections among women of the reproductive age group in the urban health training centre area in Hubli, Karnataka. *Indian Journal of Community Medicine: Official Publication of Indian Association of Preventive & Social Medicine* **37** (1), 34.

Bharadwaj, S. & Patkar, A. 2004 Menstrual hygiene and management in developing countries: taking stock. *Junction Social* **3** (6), 1–20.

Bill & Melinda Gates Foundation 2021 Menstrual hygiene management (MHM) incinerator market landscape: analysis of India and South Africa [version 1; not peer reviewed]. *Gates Open Research* **5**, 9. https://doi.org/10.21955/gatesopenres.1116712.1.

Bodat, S., Ghate, M. M. & Majumdar, J. R. 2013 School absenteeism during menstruation among rural adolescent girls in Pune. *National Journal of Community Medicine* **4** (2), 212–216.

Burguete, P., Corma, A., Hitzl, M., Modrego, R., Ponce, E. & Renz, M. 2016 Fuel and chemicals from wet lignocellulosic biomass waste streams by hydrothermal carbonization. *Green Chemistry* **18** (4), 1051–1060.

Crofts, T. & Fisher, J. 2012 Menstrual hygiene in Ugandan schools: an investigation of low-cost sanitary pads. *Journal of Water, Sanitation and Hygiene for Development* **2** (1), 50–58.

Das, P., Baker, K. K., Dutta, A., Swain, T., Sahoo, S., Das, B. S., Panda, B., Nayak, A., Bara, M. & Bilung, B. 2015 Menstrual hygiene practices, WASH access and the risk of urogenital infection in women from Odisha, India. *PLoS One* **10** (6), e0130777.

Dipali, N., Seema, A. & Rupali, G. 2009 Impact of health education on knowledge and practices about menstruation among adolescent school girls of Kalamboli, Navi-Mumbai. *Health and Population-Perspectives and Issues* **32** (4), 167–175.

Dixit, S., Raghunath, D., Rokade, R., Nawaz, S. A., Nagdeve, T. & Goyal, I. 2016 Awareness about menstruation and menstrual hygiene practices among adolescent girls in central India. *National Journal of Community Medicine* **7** (6), 468–473.

Dong, J., Chi, Y., Tang, Y., Ni, M., Nzihou, A., Weiss-Hortala, E. & Huang, Q. 2016 Effect of operating parameters and moisture content on municipal solid waste pyrolysis and gasification. *Energy & Fuels* **30** (5), 3994–4001.

Eke, J., Onwudili, J. A. & Bridgwater, A. V. 2019 Influence of moisture contents on the fast pyrolysis of Trommel fines in a bubbling fluidized bed reactor. *Waste and Biomass Valorization* **11**, 3711–3722.

Elledge, M. F., Muralidharan, A., Parker, A., Ravndal, K. T., Siddiqui, M., Toolaram, A. P. & Woodward, K. P. 2018 Menstrual hygiene management and waste disposal in low and middle income countries – a review of the literature. *International Journal of Environmental Research and Public Health* **15** (11), 2562.

Fraser, I., Warner, P. & Marantos, P. 2001 Estimating menstrual blood loss in women with normal and excessive menstrual fluid volume. *Obstetrics & Gynecology* **98** (5), 806–814.

Garg, S. & Anand, T. 2015 Menstruation related myths in India: strategies for combating it. *Journal of Family Medicine and Primary Care* **4** (2), 184.

Garikipati, S. & Boudot, C. 2017 To pad or not to pad: towards better sanitary care for women in Indian slums. *Journal of International Development* **29** (1), 32–51.

Kumar, A. & Srivastava, K. 2011 Cultural and social practices regarding menstruation among adolescent girls. *Social Work in Public Health* **26** (6), 594–604.

Nayak, P., Shukla, D., Tiwari, N., Mishra, O. & Mishra, R. 2017 Assessment of menstrual pattern and use of sanitary pads in adolescent girls of Varanasi slum: preliminary analysis. *Indian Journal of Preventive & Social Medicine* **48** (1–2), 9–9.

Omidvar, S. & Begum, K. 2010 Factors influencing hygienic practices during menses among girls from south India – A cross sectional study. *International Journal of Collaborative Research on Internal Medicine & Public Health* **2** (12), 0–0.

Path 2017 Pushing the Boundaries on the MHM Dialogue in India Management of Menstrual waste. Available at: https://www.path.org/resources/pushing-the-boundaries-on-the-menstrual-health-management-dialogue/.

Singh, P. & Bhardwaj, S. 2017 Study on hygiene and sanitary practices during menstruation among adolescent girls of Udham Singh Nagar district of Uttarakhand. *International Journal of Home Science* **3**, 21–23.

Sommer, M., Kjellén, M. & Pensulo, C. 2013 Girls' and women's unmet needs for menstrual hygiene management (MHM): the interactions between MHM and sanitation systems in low-income countries. *Journal of Water, Sanitation and Hygiene for Development* **3** (3), 283–297.

Sommer, M., Chandraratna, S., Cavill, S., Mahon, T. & Phillips-Howard, P. 2016 Managing menstruation in the workplace: an overlooked issue in low-and middle-income countries. *International Journal for Equity in Health* **15** (1), 1–5.

SWM Solid Waste Management Rules 2016 Central Pollution Control Board, Government of India. https://cpcb.nic.in/rules-2/ Accessed on 1 March 2021.

Tilley, E., Bieri, S. & Kohler, P. 2013 Sanitation in developing countries: a review through a gender lens. *Journal of Water, Sanitation and Hygiene for Development* **3** (3), 298–314.

van Eijk, A. M., Sivakami, M., Thakkar, M. B., Bauman, A., Laserson, K. F., Coates, S. & Phillips-Howard, P. A. 2016 Menstrual hygiene management among adolescent girls in India: a systematic review and meta-analysis. *BMJ Open* **6** (3), e010290.

Wagh, R. V., Upadhye, A. J. & Upadhye, J. J. 2018 Menstrual hygiene practices in young girls of urban India. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology* **7**, 1897–1902.

Youcai, Z., Stucki, S., Ludwig, C. & Wochele, J. 2004 Impact of moisture on volatility of heavy metals in municipal solid waste incinerated in a laboratory scale simulated incinerator. *Waste Management* **24** (6), 581–587.

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Original Research Article

Pattern and prevalence of metabolic syndrome in obese adolescents aged 10-18 years: a community based study

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ABSTRACT

Background: The prevalence and magnitude of childhood obesity is increasing in pediatric age group. Incidence of metabolic syndrome is high among obese children and adolescents leading to increased risk of cardiovascular morbidity in long term. For this reason, recognition of metabolic syndrome in obese children is of great importance.
Methods: This study was a cross-sectional study carried out among children aged 10-18 years from both urban and rural schools in Coimbatore. Children were screened, sample for metabolic studies collected after consent and analyzed.
Results: Total 1582 children in the age group of 10-18 years were screened. 300 children were recruited. Among the studied group, 19.3% were overweight, 30.7% were obese. Metabolic syndrome was present in 55.1% of obese and overweight children. Hyperinsulinemia was present in 30% of obese children.
Conclusions: This study done in adolescent school children showed a strong association between obesity and early onset of metabolic syndrome. Early intervention with lifestyle modifications is strongly recommended to prevent long term cardiovascular morbidity.

Keywords: Adolescent children, Metabolic syndrome, Obesity

INTRODUCTION

The prevalence and magnitude of childhood obesity is alarmingly increasing in pediatric population. Incidence of metabolic syndrome is high among obese children and adolescents, and it proportionately increases with worsening obesity. There is significantly increased risk of coronary morbidity and mortality in early adulthood among obese adolescents.[1] For this reason, the recognition of the metabolic syndrome in obese children is of great importance from a clinical and public health perspective. There are only few studies regarding the prevalence of the metabolic syndrome in children and adolescents in India.[2-4] This cross-sectional study was done to evaluate the pattern and prevalence of metabolic syndrome among adolescents aged between 10-18 years in Coimbatore, India. Metabolic syndrome was defined by using WHO and modified National Cholesterol Education Program Adult Treatment Panel III [NCEP ATP III] criteria.

In 1988, Reaven and colleagues coined the term Metabolic Syndrome (MetS) that refers to a combination of hypertension, abdominal obesity, insulin resistance, and hyperlipidemia and it was closely associated with obesity and increased risk for atherosclerotic cardiovascular disease.[5,6] What was first considered as adult condition has now been recognized in children.[7] With increasing prevalence of childhood obesity, it is very likely that pediatric metabolic syndrome will also be more prevalent recently, with implications for future increase in cardiovascular morbidity and mortality.

The International Association for the Study of Obesity (IASO) and International Obesity Task Force (IOTF) estimate that 200 million school children are either overweight or obese.[8]

A recent study among 38,296 children aged 8-18 y from five cities of India reported the prevalence of overweight and obesity (based on IOTF reference) as 14.4% and 2.8% respectively.[9] This may have major implications towards increasing prevalence of non-communicable diseases like diabetes, hypertension and cardiovascular disease in early adulthood.[10] Worldwide estimates of the prevalence of MetS range from 1.2% to 22.6% for youth and 9.0% to 35.0% for adults, depending on the definition of MetS used, the region, the study design, the years of the study, and the age group and study population.[11-15] Only very few data are available in India in this context.

METHODS

This study was a cross-sectional study and was carried out among children aged 10-18 years from schools in Coimbatore, including urban and rural areas. Children were screened for overweight and obesity based on BMI and waist circumference.

Weight was measured while the subjects were minimally clothed without shoes in upright position using digital scales and recorded to the nearest 0.1 kg. Height was measured in a standing position, without shoes, using a stadiometer and inch tape to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in centimeters to the nearest 0.1 cm using a non-stretchable measuring tape, without any pressure to body surface at the narrowest level between the lower border of ribcage and iliac crest. Those adolescents classified under overweight and obesity are further evaluated for the presence of metabolic syndrome. Age and sex matched normal children were taken as control. They were selected by simple random sampling.

Informed consent was obtained from parent/guardians and assent from the children. A qualified physician measured blood pressure two times with the subject in a seated position during physical examinations after one initial measurement for determining peak inflation level using a standard mercury sphygmomanometer. The mean of two measurements was considered to be the participant's blood pressure. The systolic blood pressure was defined as the appearance of the first sound (Korotkoff phase 1) and diastolic blood pressure was defined as the disappearance of the sound (Korotkoff phase 5) during deflation of the cuff at a 2 - 3 mm/s decrement rate of the mercury column.

Fasting blood samples for the serum glucose and lipid profile was drawn after the subjects had fasted overnight. Samples were collected for rural children from rural health centre and urban children at PSG Hospital respectively. Fasting plasma glucose was measured on the day of blood collection by the glucose oxidase peroxide method, and lipid profile was measured by enzymatic assay on a Hitchi 704 system auto-analyzer. Lipid profiles [(triglyceride (TG), total cholesterol (C), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and very-low-density lipoprotein-cholesterol (VLDL-C)] were determined in the fasting blood samples.

Fasting insulin was measured. Pubertal stage was determined in each patient using Tanner's criteria and hyperinsulinism (H1) was defined according to the norms for fasting insulin according to pubertal stage: prepubertal ≥ 15 mIU/L, mid-puberty (breast, genital stages 2-4)≥30 mIU/L post-pubertal≥20 mIU/L. Insulin resistance (IR) was analyzed using the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR was calculated by the following formula: fasting insulin* fasting glucose/22.5. An HOMA-IR value greater than 3.16 was used to determine IR in pubertal students.

Definition of metabolic syndrome

For the diagnosis of MetS, the presence of at least three of the following criteria (in accordance with the WHO and National Cholesterol Education Program Adult Treatment Panel III [NCEP ATP III] recommendations) was considered. These criteria included:

- Obesity: BMI≥95th percentile for age and gender (6-18 years, WHO growth charts)
- Abnormal glucose homeostasis: the presence of one of the following:
  - Hyperinsulinemia and insulin resistance (using fasting blood insulin appropriate for pubertal stage or sum of insulin levels in the first 120 min of oral glucose tolerance test)
  - Elevated fasting glucose (EPG): FG≥110 mg/dL
  - Impaired glucose tolerance (IGT), glucose at 120 min: 140-200 mg/dL
- Hypertension: systolic/diastolic blood pressure ≥95th percentile for age and sex.
- Dyslipidemia: presence of one of the following: (AAP Clinical Report on Lipid Screening in children).
  - High TG (≥95th percentile for age and sex)
  - Low HDL-C levels (<5th percentile for age and sex).
  - High total or LDL-C (≥95th percentile for age and sex).

Inclusion criteria

Children and adolescents aged between 10-18 years of age studying in urban and rural schools in Coimbatore, India. Study was done over a period of one year.

Sample size

Previous studies have estimated the prevalence of metabolic syndrome to be 4%. With this prevalence, the power of 80% and excluding the underweight children (prevalence of 30%), the screening sample size calculated as 1582. Considering the prevalence of 8% for overweight and obese children, sample size for second stage was calculated to be 150 with equal number of normal children. The samples were distributed evenly through urban and rural areas.

The data were entered in individual proforma. The collected data were then converted to excel format.

Data analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 19. Data expressed as percentage. Chi square test was used to find out association between categorical variables.

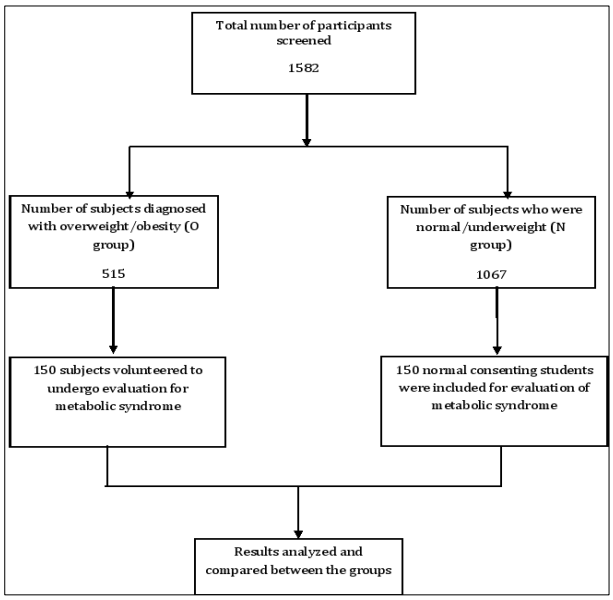


Figure 1: Flow diagram of subject recruitment.

RESULTS

Total number of school children recruited for this study in the age group of 10-18 years were 300, among whom 149 (49.7%) were boys and 151 (50.3%) were girls; in our study, there was almost equal distribution of boys and girls. Among the studied group 58 (19.3%), 92 (30.7%), 124 (41.3%), 26 (8.7%) children were overweight, obese and normal weight and underweight children respectively. Abnormal FBS (>110mg/dL) was seen in 5% of obese children and 2.1% in normal weight group, which was statistically significant. Abnormal total cholesterol level was found in 6% of obese and overweight group and 3.3% in normal group. (p value = 0.27) Triglyceride level was abnormal in 40.6% of O group children and 12.6% in N group children which was statistically significant (p=0.0001%). HDL was abnormal in 24.6% of O group children and 9.3% in N group children also had statistically significant difference (p=0.0001%). Hyperinsulinemia was present in 30% of O group children and 9.3% of N group children. HOMA - IR elevated in 60.6% of O group children and 22% in N group children. Both are statistically significant. Elevated BP was observed in 4.6% of N group children and 7.3% of O group children. Stage 1 hypertension seen in 3.3% of N group children and 14% of O group children which was statistically significant (p=0.002%). Among studied group MS was present in 55.1% in obese, overweight children. MS was more evidenced in the age group of 10-12 years (38.3%) as compared to the other age groups.

Table 1: Baseline characteristics of the study population.

Parameters		Number	Percentage
Age in years	10-12	122	40.7
	13-15	121	40.3
	16-17	57	19
Sex	Males	149	49.7
	Females	151	50.3
Overweight		58	19.3
Obese		92	30.7
Normal		124	41.3
Underweight		26	8.7

Table 2: Lipid profile, HOMA-IR, insulin levels, blood pressure between normal and overweight/obese children.

Variables		Overweight and obese(%)	Normal and underweight(%)	Chi square and p-value
TC		6	3.3	1.19,0.27
TGL		40.6	12.6	30.06,0.0001*
HDL		24.6	9.3	12.49,0.0001*
LDL		11.3	8.6	0.793,0.441
HOMA-IR		60.6	22	46,2,0.0001*
Insulin		30	9.3	20.27,0.0001*
BP	Normal	78.6	92	12.29, p- 0.002
	Elevated	7.3	4.6	
	Stage1	14	3.3	

Table 1 indicating baseline characteristics of the study population including 1. Age group, majority of the children studied were between 10-15 years of age (81%). 2. Equally distributed between both sexes. 3. 30.7% children were obese and 19.3% were overweight.

Table 2 depicting the variables of lipid profile, insulin levels and blood pressure between normal and overweight, obese group.

Table 3 depicts that 50% of children with obesity in the study population had criteria to fulfill metabolic syndrome. 5.1% of children in the overweight group and 3.3% in the normal weight group had metabolic syndrome.

Table 3: Metabolic syndrome in the study population.

Category	Number	Percentage
Normal and under weight	5	3.3
Overweight	3	5.1
Obese	46	50

Table 4 shows the distribution of metabolic syndrome, age wise amongst overweight and obese children. 38.3%between 10-12 years, 28.5% between 13-15 years and 21.4 percent 16 and 17 years fulfilled criteria for metabolic syndrome. This indicates that screening and interventions should be planned early.

Table 4: Distribution of metabolic syndrome age-wise among overweight and obese children.

Age	Number	Percentage
10-12	28	38.3
13-15	18	28.5
16-17	3	21.4

DISCUSSION

Metabolic syndrome is a group of risk factors which has been increasingly prevalent concurrent with obesity.[16] Consistently high prevalence of MS has been recorded in obese and overweight adolescents across the globe varying from 24% to 38.8% of obese children (Turkey). These figures have varied in accordance with the criteria used. Using a combination of WHO and ATP-III criteria in the present study, we found that 50% of obese and 5.1% of overweight adolescent had MS respectively. Such figures in children is a potential harbinger of adult onset cardiovascular morbidity and mortality thereon. In this study, we found that there was equal preponderance of male and female adolescents for MS.

The children between 10-12 years had higher percentage of MS as compared to older adolescents.[17] This fact highlights early screening and dietary and lifestyle modifications for younger adolescents.

Authors observed that there was no statistically significant difference in total cholesterol and LDL levels between the 2 groups. However, the circulatory triglyceride levels were statistically more in the overweight and obese children with MS. High TG has been reported in studies.[2,17,18] The significance of hypertriglyceridemia in adolescents as a harbinger of adult onset of cardiovascular disease is gradually being appreciated. (Another factor noted in this study was that a small subsect of normal weight adolescents had triglyceride values above the age specific cutoff with other parameters remaining normal.) Levels of HDL were statistically significantly lower in the overweight and obese groups as quoted by other studies.[17,19,2] Hyperinsulinemia was present in 30% of obese and overweight adolescents qualifying for MS as observed by Mehmet Atabek.[20] Acanthosis nigricans as an external marker for hyperinsulinemia was seen in 3% of obese adolescents. High FBS (>110mg/dL) was found in 5% of obese and overweight children in the study. HOMA - IR was found to be elevated in 60.6% of the overweight and obese group which was statistically significant (p value 0.0001).11 adolescents (7.3%) in the overweight and obese group had elevated BP and 14% of children in the group and high BP qualifying for stage I hypertension.

Limitation of the study was that detailed dietary assessment could not be done as it was a school based study. Exact duration of physical activity was not included in the proforma.

CONCLUSION

This study done in children between 10-18 years has shown a strong association between obesity and incidence of metabolic syndrome. Early intervention with lifestyle modifications is strongly recommended to prevent long-term cardiovascular morbidity.

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REFERENCES

1. Misra A, Shah P, Goel K, Hazra DK, Gupta R, Seth P, et al. The high burden of obesity and abdominal obesity in urban Indian schoolchildren: a

multicentric study of 38,296 children. Ann Nutr Metab. 2011;58(3):203-11.
2. Andrabi SM, Bhat MH, Andrabi SR, Kamili MM, Imran A, Nisar I, et al. Prevalence of metabolic syndrome in 8–18-year-old school-going children of Srinagar city of Kashmir India. Indian J Endocrinol Metab. 2013 Jan;17(1):95.
3. Singh N, Parihar RK, Saini G, Mohan SK, Sharma N, Razaq M. Prevalence of metabolic syndrome in adolescents aged 10-18 years in Jammu, J and K. Indian J Endocrinol Metab. 2013 Jan;17(1):133.
4. Bhalavi V. International Journal of Biomedical Research. Age (years).;15(15):159.
5. Reaven GM. Banting Lecture 1988: role of insulin resistance in human disease. Diab. 1988;37:1595-607.
6. Levitt NS, Lambert EV. The foetal origins of the metabolic syndrome -a South African perspective. Cardiovasc J S Afr. 2002;13:179-80.
7. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. Jama. 2002 Dec 4;288(21):2709-16.
8. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004 Jun 3;350(23):2362-74.
9. International Obesity Task Force. Available at: http://www. iaso.org/iotf/obesity/obesitytheglobalepidemic/, for Saudi, Canada, South Africa, Australia and NZ estimates. Accessed 23 December 2019.
10. Chakraborty P, Dey S, Pal R, Kar S, Zaman FA, Pal S. Obesity in Kolkata children: Magnitude in relationship to hypertension. J Natural Sci Bio Med. 2012;2:101-6.
11. Shah B, Anand K, Joshi P, Mahanta J, Mohan V, Thankappan K, et al. Report of the Surveillance of Risk Factors of Non-communicable Diseases (STEPS 1 and 2) From Five Centers in India – WHO India – ICMR initiative. New Delhi.
12. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;364(9468):1415-28.
13. Goodman E, Daniels SR, Morrison JA, Huang B, Dolan LM. Contrasting prevalence of and demographic disparities in the world health organization and national cholesterol education program adult treatment panel III definitions of metabolic syndrome among adolescents. J Pediatr. 2004;4(59): 445-51.
14. Tailor AM, Peeters PH, Norat T, Vineis P, Romaquera D. An update on the prevalence of the metabolic syndrome in children and adolescents. Int J Pediatr Obes. 2010;5(3):202-13.
15. Cameron AJ, Shaw JE, Zimmet PZ. The metabolic syndrome: prevalence in worldwide populations. Endocrinol Metab Clin North Am. 2004;33(2):351-75.
16. Sangun Ö, Dündar B, Köşker M, Pirgon Ö, Dündar N. Prevalence of metabolic syndrome in obese children and adolescents using three different criteria and evaluation of risk factors. J Clini Res Pediatr Endocrinol. 2011 Jun;3(2):70.
17. Invitti C, Maffeis C, Gilardini L, Pontiggia B, Mazzilli G, Girola A, et al. Metabolic syndrome in obese Caucasian children: prevalence using WHO-derived criteria and association with nontraditional cardiovascular risk factors. Inter J Obesity. 2006 Apr;30(4):627-33.
18. Jacob AS, Reetha G. Prevalence of metabolic comorbidities in obese children. Int J Contemp Pediatr. 2017;4(4):1450-5.
19. Singh R, Bhansali A, Sialy R, Aggarwal A. Prevalence of metabolic syndrome in adolescents from a north Indian population. Diab Med. 2007;24:2.
20. Atabek ME, Pirgon O, Kurtoglu S. Prevalence of metabolic syndrome in obese Turkish children and adolescents. Diab Res Clini Pract. 2006 Jun 1;72(3):315-21.

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Persistent organic pollutants-environmental risk factors for diabetes mellitus? – A population-based study

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Abstract

Background:

Globally, type-2 diabetes mellitus is increasing in epidemic proportions. A major cause of concern in India is the increasing incidence of cases, especially troubling is the observed increase in younger age groups with no risk factors. New evidence suggests that many environmental factors, such as air pollution, persistent organic pollutants (POPs), and environmental estrogens are implicated as risk factors for type-2 diabetes mellitus. Animal and human epidemiological studies have shown ubiquitous lipophilic substances, including POPs, are frequently associated with type-2 diabetes mellitus. Such studies have not been undertaken in Indian youth.

Methods:

This is a cross-sectional study that explored the association between POPs and type-2 diabetes mellitus in Indian urban and rural population. About 7 ml of venous blood was collected from all consenting patients and serum was separated immediately and was transported to the lab for further analysis. Serum levels of POPs, including organochlorine (OC) compounds and organophosphorus pesticides, were estimated using sample gas chromatography-mass spectrometry (GC-MS). The fasting blood sugar values and the serum levels of POPS were tested using Pearson correlation coefficient. The magnitude of increase in blood sugar corresponding to increase in POPs was analyzed using linear regression analysis. The odds ratios (ORs) were expressed at 95% confidence intervals (CIs).

Results:

Three OC pesticides and one organophosphate pesticide were strongly associated with increasing blood sugar levels after adjusting for age, sex, and body mass index – lindane (OR 4.95, 95% CI 1.03–23.73), DDT o, p' (OR 3.50, 95% CI 1.04–11.73), dimethoate (OR 19.31, 95% CI 4.22–88.37), and dichlorvas (OR 6.33, 95% CI 1.28–31.18).

Keywords: DDT, dimethoate, health effects, persistent organic pollutants, pesticides, type- 2 diabetes mellitus.

Introduction

Incidence of diabetes is rapidly increasing worldwide.[1] The dominant risk factors include obesity, sedentary lifestyle, poor diet, old age, ethnicity, family history of diabetes, and dyslipidemia. Prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and expected to rise to 4.4% by 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.[1] Type-2 diabetes is an epidemic in Asia, characterized by a rapid increase in onset at relatively young ages and low body mass index (BMI),[2] and environmental estrogens implicated in the rise of the same.[3] Persistent organic pollutants are a class of compounds characterized by their ability to persist in the environment, due to their low water, high lipid solubility and bio-magnification in the food chain.[4] POPs include polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls (PCB), hexachlorobenzene, p, p'-dichlorodiphenyldichloroethylene (DDE), and several organochlorine (OC) mixtures. These have been studied by international organizations for their impact on environment and humans alike and have been implicated in several adverse effects on human health especially with impaired glucose metabolism and prevalence of diabetes mellitus.[4,5] India, a highly populated middle-income country, is subjected to environmental contamination of POPs from several activities and sources like production units, illegal imports as well as stockpiles of obsolete pesticide.[6] Except for DDT seven other pesticide POPs listed in the Stockholm Convention are banned for manufacture but are in wide use because large stockpiles are still available. In a nationwide food survey in 2001, 75% of the samples had detectable levels of DDT with about 10–15% of the samples having levels of DDT that exceeded the levels prescribed by the Food and Agricultural organization and World Health Organization. Aldrin and dieldrin were also frequently detected pesticides in foodstuffs.[7,8] Racial and ethnic differences in responses to POPs are well known.[9] POPs may be associated with T2DM with a higher magnitude in Asians, contributing to the epidemic. Hence, prevention and control of diabetes is a top public health priority in Asian populations.[10]

Many studies based on self-reported diabetes and mortality rate analysis indicate an association between pollutants and diabetes/prediabetes.[11,12] Many POPs act as endocrine disrupting agents in animals and humans.[13] Concentrations of most of these pollutants have been diminishing in the

environment, food chain, and human body over recent decades in Europe and the United States. However, there are subgroups within these populations that still show an elevated body burden because of dietary habits and current or past exposures.[11] Diabetes has been associated with dioxin-like chemicals, nondioxin like PCBs, DDE, and/or other OCs in several cross-sectional investigations.[4,11,14,15] Altered glucose transport, lipid metabolism, and modifications in the insulin signaling pathways are potential mechanisms that might be involved in the association between POPs and diabetes.[11] The Jørgensen group showed that POPs may affect insulin secretion rather than insulin resistance.[16]

POPs metabolism, particularly low-level exposures, warrants investigations using a cohort study design.[17] There have been few studies that have used fasting blood glucose levels (≥126 mg/dL or 7.0 mmol/L), HbA1c, or random glucose level (≥200 mg/dL or 11.1 mmol/L) to diagnose diabetes.[4,14] The Morgan lab at University of Iowa College of Medicine, Iowa observed that workers with incident diabetes had significantly elevated levels of DDE+DDT in their serum compared to workers without diabetes.[18] Vasiliu *et al*.[12] found that PCBs were associated with incident diabetes in women but not in men and remained associated when cases encountered during the first 15 years of follow-up were excluded, suggesting that reverse causality was an unlikely explanation for the relationship. Investigations on Operation Ranch Hand Veterans, who were exposed to 2,3,7,8- tetra chlorodibenzo-p-dioxin (TCDD) through application of Agent Orange in Vietnam, were found to have incident diabetes associated with TCDD exposure.[19] Also, diabetes was associated with DDE exposure but not with mono-ortho PCB-118, total PCBs, or years of sport fish consumption.[5] In another case-control study it was confirmed that p, p'- DDE exposure could be a potential risk factor for T2DM.[11] A cross-sectional survey conducted in a heavily polluted area of eastern Slovakia demonstrated increasing serum concentrations of individual POPs considerably increased prevalence of prediabetes and diabetes in a dose-dependent manner.[20] Based on the current literature, there is a strong implied linkage between POPs exposure in humans and type-2 diabetes. There have been many studies analyzing the presence of POPs in Indian population, but none connecting the link and establishing association between POPs and diabetes.[21] Hence in this study, we investigated the association of blood sugar with the serum concentrations of POPs in south Indian population.

Methods

This is a cross-sectional study conducted at the rural and urban outreach centers affiliated to the PSG Institute of Medical Sciences and Research, Coimbatore, India. The study was conducted with approval from Institutional Human Ethics Committee at PSGIMS&R. A written informed consent was obtained from all eligible participants. All participants who consented were recruited in the study. Details about the study were informed by health workers from the center by house to house visit in addition to distribution of pamphlets which included details of the study and requirement of 8 h fasting for the collection of samples. Both male and female of age above 18 were included based on convenience sampling. Diabetics were confirmed as evidenced by fasting blood sugar above 126 mg/dL or HbA1c above 6.4% or use of hypoglycemic medications. Participants with type-1 diabetes and terminally ill conditions were excluded from the analysis. Basic demographic details, socioeconomic status, family history of diabetes, hypertension, general health, and medical history were elicited using a questionnaire. Anthropometric measurements were recorded and blood pressure was recorded in the sitting posture in the right arm. Age, gender, body weight, and height were recorded for each patient. Body mass index was calculated as weight in kilograms divided by height in meter squared. Blood was drawn after at least 8 h overnight fasting. Fasting blood sugar (FBS) was determined by hexokinase method which is a standard laboratory procedure. Serum POPs were determined by method described by Koc and Karakus.[22] Briefly, OC pesticide residues were determined using florisil packed-columns

and GC-MS. Linear response between FBS and POPs were measured by the Pearson correlation method. The association between increasing sugar level and POPs was calculated by linear regression after adjusting for age, sex and BMI. All statistical analyses were conducted using R version 3.5.1 (R Development Core Team, http://www.r-project.org).

Results

The total numbers of participants in our study was 191, out of which FBS was not available for five participants. The demographic characteristics for the remaining 186 participants are given in Table 1. Box plots for age and BMI stratified as per gender are given in Figure 1a and 1b. Of the 44 compounds, 11 of them contributed to 93% of total POPs load [Figure 2]. The serum levels of the following four compounds – lindane, dichlorvas, DDT o p', and dimethoate were significantly correlated with increasing blood sugar levels [Table 2]. Among the remaining compounds 31 were not comparable and seven were below limit of detection. Correlation statistics among selected compounds is illustrated in Figure 3, nonsignificant correlation indicated by cross mark, positive correlation by blue solid circles, and negative correlation by red solid circles. Size the circle indicates the magnitude of correlation. Figure 4a–d illustrates the trend analysis by Pearson method showing increasing linear relationship as sugar level increases. Since POPs are obesogenic, we checked its correlation between different strata of BMI [Table 3] and with age [Table 4]. The participants were categorized as normal, overweight, and obese based on the height and weight. The odds ratio (OR) and 95% confidence intervals (CIs) of POPs showed significant risks for compounds like lindane (OR 4.95, 95% CI 1.03–23.73), DDT o, p' (OR 3.50, 95% CI 1.04–11.73), dimethoate (OR 19.31, 95% CI 4.22–88.37), dichlorvas (OR 6.33, 95% CI 1.28–31.18). Similar compounds are found to be significantly associated with blood sugar after adjusting for age, sex, and BMI [Table 5].

Table 1

Demographics of participants enrolled in the study

Demographic characteristics of the participants (*n*=186)						
Gender (*n*)	**Male**	**56**	**Female**	**130**		
	Min	Mean	25%	Median	75%	Max
Age (year)	32	58.46	50.25	60	67	86
Height (cm)	137	154.3	149.2	152.5	158	184
Weight (kg)	31	61.75	54	61	68	126
BMI (kg/m^2)	16.44	25.86	22.35	25.43	28.95	46.28

n: number of participants

Table 2

Correlation between POPs (ng/mL) and serum blood sugar levels

Compound	Min	Mean	Median	Max	SD	r^2
Lindane	1.02	10.92	4.50	38.57	9.26	0.22 *
Dichlorvas	1.05	17.98	18.40	47.92	8.54	0.19 *
DDT o, p’	1.05	17.89	20.13	42.76	10.25	0.16 *
Dimethoate	15.98	27.17	25.35	75.68	9.44	0.36

P<0.05; SD – Standard deviation; r^2 – Pearson correlation coefficien

Table 3

Correlation between POPs (ng/mL) and BMI

Compound	Mean	Median	SD	r^2		
				Normal	Overweight	Obese
Dichrotophos	7.50	7.22	7.30	0.70 *	NA	0.42
Monochrotophos	71.22	52.68	78.63	0.07	-0.07	0.19 *
1,1 Biphenyl, 3,3’,4 Trichloro	1.34	1.13	0.41	-0.67 *	NA	-0.34
1,1 Biphenyl, 2,2’,4,4’,5,5’ Hexachloro	1.47	1.37	0.29	NA	0.9 *	NA

*P<0.05; SD – Standard deviation; r^2 – Pearson correlation coefficient

Table 4

Correlation between POPs (ng/mL) and age (years)

Compound	Min	Mean	Median	Max	SD	r^2
BHC alpha isomer	1.02	9.5	11.78	24.5	5.89	- 0.4*
Parathion methyl	3.32	142.7	28.51	2324.91	314.7	- 0.1*
DDE, p, p'	1.50	36.59	10.99	484.98	71.15	- 0.1*
DDD, o, p'	1.11	43.92	16.77	1160.8	124.67	- 0.2*
1,1 Biphenyl, 2,4,4' Trichloro	1.06	6.60	5.10	21.35	5.29	0.1*

*P<0.05; SD – Standard deviation; r^2 – Pearson correlation coefficient

Table 5

Risks of increasing sugar levels and POPs

Compound	Parameters	Odds ratio	95% CI		*P*
			Lower	Upper	
Lindane	POPs	4.9585	1.0357	23.7392	0.0491 *
	Age	1.5382	0.4401	5.3757	0.5023
	BMI	0.8169	0.028	23.804	0.9068
	Gender	NA	NA	NA	NA
DDT, o, p'-	POPs	3.5041	1.046	11.7382	0.044 *
	Age	2.3531	0.8923	6.2056	0.086
	BMI	0.3416	0.0236	4.9413	0.4321
	Gender	NA	NA	NA	NA
Dimethoate	POPs	19.3124	4.2205	88.3709	2.00E -04*
	Age	2.1495	0.6246	7.3971	0.228
	BMI	0.5344	0.0211	13.5535	0.7049
	Gender	NA	NA	NA	NA
Dichlorvas	POPs	6.3379	1.2882	31.1826	0.025 *
	Age	3.2142	1.0804	9.562	0.0381 *
	BMI	1.0741	0.0507	22.7355	0.9635
	Gender	NA	NA	NA	NA

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*P<0.05; CI – confidence interval

Figure 1

Box plots for (a) Age and (b) BMI categorized as per gender

Figure 2

Concentrations of major POPs compounds in the serum of the participants. End.Ketone - Endrin Ketone; Par.Methyl - Parathion methyl; Mono - Monocrotophos; Hept.Epoxide – Heptachlor Epoxide

Figure 3

Correlogram for POPs. Non-significant correlation indicated by cross mark, positive correlation by blue solid circles and negative correlation by red solid circles

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Figure 4

Trend plots for compounds showing significant correlation (a) Lindane (b) Dichlorvas (c) DDT (d) Dimethoate. (FBS – Fasting Blood Sugar)

Discussion

Our study demonstrated that environmental exposure to various OC and organophosphate pesticides are associated with prevalent type-2 diabetes in Indian population. Our findings are supported with published literature, which implicate OC pesticides in risk of diabetes among general population.[14,15] Benzene Hexachloride (BHC) beta isomer was shown to be a strongly correlated with age in our study. One possible mechanistic pathway for BHC isomers is via pancreatic and extra-pancreatic actions, i.e. caspase 3 activation due to reactive oxygen species generation causing membrane destabilization and mitochondrial dysfunction of the beta cell.[15,20,23,24] Other OCs like DDD and DDT were also moderately associated with sugar levels, their ORs a little above 1.00. Epidemiological evidence also substantiates the positive risk DDT and its metabolites can have on diabetes.[20,24] DDT and its metabolites are inducers of cytochrome P450 (CYP 2BA and CYP 4A1), enzymes involved in lipid and steroid metabolism.[25,26] Moreover, these compounds can inhibit aromatase, an enzyme that metabolizes steroids like cortisol.[27] As a result of aromatase inhibition, steroidal compounds tend to accumulate leading to anti-estrogenic effects, although at a receptor level, they are pro-estrogenic in action.[28] These effects coupled with cross talk with catecholamines in the adrenal gland lead to endocrine disruption. These compounds are also known to inhibit expression of Dio-2, low-density lipoprotein (LDL), Protein kinase B (AKT), extracellular-signal-regulated kinase (ERK), Patatin-like phospholipase domain-containing protein (PNPLA), and Glut-4 genes in target tissues.[29] Although we could not find epidemiological evidence for dimethoate even after PubMed search with the following terms “Dimethoate” [MESH] AND “Diabetes” [MESH], we were able to find animal studies

implicating omethoate (a break-down product of dimethoate) with insulin resistance.[30] The epidemiological evidences are summarized in Table 6. Major limitation of the study is the cross-sectional nature, which might not have captured the cause-effect relationship.[31,32]

Table 6

Epidemiological and animal studies substantiating our study

Compounds	Odds ratio (CI)	Exposure contrast	Reference
p, p'- DDT	2.52 (1.26-5.02)	20.8-26.6 vs. ≤20.7 (ng/g lipid adj.)	Everett and Matheson[24]
	1.96 (1.29-2.98)	≥20.7 vs. ≤20.7 (ng/g lipid adj.)	Everett and Matheson[24]
	1.84 (1.03-2.27)	QU3 vs. QU1 (ng/g lipid adj.)	Ukropec *et al.*[20]
p, p'- DDD	3.6 (0.8-16.3)	T3 vs. T1 (ng/g lipid std.)	Son *et al.*[31]
o, p'- DDT	12.3 (1.3-113.2)	T3 vs. T1 (ng/g lipid std.)	Son *et al.*[31]
β- HCH	2.3 (1-4.3)	≥ 1 vs.<1 ng/g ww	Cox *et al.*[15]
	2.67 (1.59-4.49)	≥ 9.35-< 9.35 ng/g lipid adj.	Everett and Matheson[24]
	1.08 (0.59-1.97)	QU3 vs. QU1 (ng/g lipid adj.)	Ukropec *et al.*[20]
	0.8 (0.3-2.2)	Q4 vs. Q1 pg/g	Lee *et al.*[4]
	8.2 (1.3-53.4)	T3 vs. T1 ng/g lipid adj.	Son *et al.*[31]
Lindane	2.02 (0.88-4.65)	Not available	Zhang *et al.*[30]
Dichlorvas	1.21 (0.98-1.49)	Not available	Montgomery *et al.*[32]
Dimethoate	Study suggested Omethoate could potentially cause insulin resistance.		Zhang *et al.*[30]

Conclusion

This exploratory study found that serum levels of several OCs and organophosphate pesticides were associated with type-2 diabetes in a south Indian population. This is the first study in the Indian population to report the association of POPs and type-2 diabetes mellitus. Our finding complement other studies on Asian groups providing evidence for adverse effects of POPs, which explains the current epidemic of type-2 diabetes in Asia.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care.* 2004;27:1047–53. [PubMed] [Google Scholar]

2. Magliano DJ, Sacre JW, Harding JL, Gregg EW, Zimmet PZ, Shaw JE. Young-onset type 2 diabetes mellitus-implications for morbidity and mortality. *Nat Rev Endocrinol.* 2020;16:321–31. [PubMed] [Google Scholar]

3. Bonini M, Sargis R. Environmental toxicant exposures and type 2 diabetes mellitus: Two interrelated public health problems on the rise. *Curr Opin Toxicol.* 2017;7:52–9. doi: 10.1016/j.cotox. 2017.09.003. [PMC free article] [PubMed] [Google Scholar]

4. Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, et al. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: Results from the national health and examination survey 1999-2002. *Diabetes Care.* 2006;29:1638–44. [PubMed] [Google Scholar]

5. Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of great lakes sport fish consumers. *Environ Health Perspect.* 2009;117:1076–82. [PMC free article] [PubMed] [Google Scholar]

6. Syed J, Malik R, Liu D, Xu Y, Wang Y, Li J, et al. Organochlorine pesticides in air and soil and estimated air–soil exchange in Punjab, Pakistan. *Sci Total Environ.* 2013;444:491–7. [PubMed] [Google Scholar]

7. Adeleye A, Sosan M, Oyekunle J. Dietary exposure assessment of organochlorine pesticides in two commonly grown leafy vegetables in South-western Nigeria. *Heliyon.* 2019;5:e01895. [PMC free article] [PubMed] [Google Scholar]

8. Olutona G, Aderemi M. Organochlorine pesticide residue and heavy metals in leguminous food crops from selected markets in Ibadan, Nigeria. *Legume Sci.* 2019;1:e3. [Google Scholar]

9. Nebert DW, Zhang G, Vesell ES. Genertic risk prediction: Individualized variability in susceptibility to toxicants. *Ann Rev Pharmacol Toxicol.* 2013;53:355–75. [PubMed] [Google Scholar]

10. Chan JC, Schymura MJ, Negoita S, Rej R, Carpenter DO. Diabetes in asia: Epidemiology, risk factors, and pathophysiology. *J Am Med Assoc.* 2009;301:2129–40. [Google Scholar]

11. Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type-2 diabetes mellitus. *Hum Exp Toxicol.* 2007;26:447–52. [PubMed] [Google Scholar]

12. Vasiliu O, Cameron L, Gardiner J, Dequire P, Karmaus W. Polybrominated biphenyls, polychlorinated biphenyls, body weight, and incidence of adult-onset diabetes mellitus. *Epidemiology.* 2006;17:352–9. [PubMed] [Google Scholar]

13. McLachlan JA. Environmental signaling: What embryos and evolution teach us about endocrine disrupting chemicals. *Endocrinol Revs.* 2001;22:319–41. [PubMed] [Google Scholar]

14. Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO. The Akwesasne Task Force on the Environment. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult native americans. *Environ Health Perspect.* 2007;115:1442–7. [PMC free article] [PubMed] [Google Scholar]

15. Cox S, Niskar AS, Venkat Narayan KM, Marcus M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among mexican americans: Hispanic health and nutrition examination survey, 1982-1984. *Environ Health Perspect.* 2007;115:1747–52. [PMC free article] [PubMed] [Google Scholar]

16. Jørgensøn ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance mong greenland inuit. *Diabetologia.* 2008;51:1416–22. [PubMed] [Google Scholar]

17. Longnecker MP. Pharmacokinetic variability and the miracle of modern analytical chemistry. *Epidemiology.* 2006;17:350–1. [PubMed] [Google Scholar]

18. Morgan DP, Lin LI, Saikaly HH. Morbidity and mortality in workers occupationally exposed to pesticides. *Arch Environ Contamin Toxicol.* 1980;9:349–82. [PubMed] [Google Scholar]

19. Michalek JE, Ketchum NS, Tripathi RC. Diabetes mellitus and 2,3,7,8-tetrachlorodibenzo-p-dioxin elimination in veterans of operation ranch hand. *Toxicol Environ Health Part A.* 2003;66:211–21. [PubMed] [Google Scholar]

20. Ukropec J, Radikova Z, Huckova M, Koska J, Kocan A, Sebokova E, et al. 2010.High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. *Diabetologia.* 2010;53:899–906. [PubMed] [Google Scholar]

21. Tomar LR, Agarwal MP, Avasthi R, Tyagi V, Mustafa M, Bannerjee BD. Serum organochlorine pesticide levels in patients with metabolic syndrome. *Indian J Endocrinol Metab.* 2013;17(Suppl 1):S342–4. [PMC free article] [PubMed] [Google Scholar]

22. Koc F, Karakus E. Determination of organochlorinated pesticide residues by gas chromatography-mass spectrometry after elutio in a florisil column. *Kafkas Univ Vet Fak Derg.* 2011;17:65–70. [Google Scholar]

23. Michalowicz J, Mokra K, Rosiak K, Sicińska P, Bukowska B. Chlorobenzenes, lindane and dieldrin induce apoptotic alterations in human peripheral blood lymphocytes (*in vitro* study) *Environ Toxicol Pharmacol.* 2013;36:979–88. [PubMed] [Google Scholar]

24. Everett CJ, Matheson EM. Biomarkers of pesticide exposure and diabetes in the 1999-2004 national health and nutrition examination study. *Environ Int.* 2010;36:398–401. [PubMed] [Google Scholar]

25. Kitamura S, Shimizu Y, Shiraga Y, Yoshida M, Sugihara K, Ohta S. Reductive metabolism of p, p'-ddt and o, p'-ddt by rat liver cytochrome p450. *Drug Metabol Dispos.* 2002;30:113–8. [PubMed] [Google Scholar]

26. Sierra-Santoyo A, Hernandez M, Albores A, Cebrian ME. Sex-dependent regulation of hepatic cytochrome p450 by ddt. *Toxicol Sci.* 2000;54:81–7. [PubMed] [Google Scholar]

27. Wójtowicz AK, Milewicz T, Gregoraszczuk EŁ. Ddt and its metabolite dde alter steroid hormone secretion in human term placental xplants by regulation of aromatase activity. *Toxicol Lett.* 2007;173:24–30. [PubMed] [Google Scholar]

28. Ndebele K, Graham B, Tchounwou PB. Estrogenic activity of coumestrol, ddt, and tcdd in human cervical cancer cells. *Int J Environ Res Public Health.* 2010;7:2045–56. [PMC free article] [PubMed] [Google Scholar]

29. La Merrill M, Karey E, Moshier E, Lindtner C, La Franco MR, Newman JW, et al. Perinatal exposure of mice to the pesticide ddt impares energy expenditure and metabolism in adult female offspring. *PLoS One.* 2014;9:e103337. [PMC free article] [PubMed] [Google Scholar]

30. Zhang Y, Ren M, Li J, Wei Q, Ren Z, Lv J, et al. Does omethoate have the potential to cause insulin resistance? *Environ Toxicol Pharmacol.* 2014;37:284–90. [PubMed] [Google Scholar]

31. Son HK, Kim SA, Kang JH, Chang YS, Park SK, Lee SK, et al. Strong associations between low-dose organochlorine pesticides and type-2 diabetes in korea. *Environ Int.* 2010;36:410–4. [PubMed] [Google Scholar]

32. Montgomery M, Kamel F, Saldana T, Alavanja M, Sandler D. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural health study, 1993-2003. *Am J Epidemiol.* 2008;167:1235–46. [PMC free article] [PubMed] [Google Scholar]

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Potential Pitfalls in Wastewater Phosphorus Analysis and How to Avoid Them

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Abstract

Due to the increasing adoption of nutrient discharge regulations, many research groups are stepping into new territory with phosphorus (P) measurements. Accurate reporting of P concentrations in effluent from novel wastewater treatment technologies is critical for protecting both environmental and human health. Analysis of P in wastewater is prone to pitfalls because of the (1) variety of chemical forms of P in wastewater (orthophosphate, condensed P, and organic P), (2) availability of different chemical assays for measuring different P forms, and (3) different conventions in the units for reporting P. Here, we present a case study highlighting how these pitfalls affect analysis and interpretation of P measurements. We show that, when used appropriately, commercially-available kits are indeed accurate tools for evaluating reactive P and total P concentrations. For both standard solutions and real wastewater, we systematically remove steps from the total P protocol to show how protocol deviations affect the results. While standard solutions are important for validating analytical methods, commercially-available wastewater standard solutions only contain P as orthophosphate (reactive P).

We therefore demonstrate options for making a mixed-P standard solution containing acid-hydrolyzable and/or organic P compounds that can be used to validate both reactive P and total P assays.

Keywords: Wastewater, nutrient pollution, standard solutions, onsite treatment, phosphorus, phosphate, non-sewered sanitation

Introduction

An intense area of research is non-sewered sanitation solutions for rural areas and low- and middle-income countries[1-14] to address the urgent need for safely-managed sanitation to improve human health and the environment.[15,16] In addition to removing pathogens and organic compounds, a key requirement of wastewater treatment is removal of nutrients including nitrogen and phosphorus (N and P) due to their potential toxic effects on aquatic environments.[17,18] The concern with P is that phosphates are limiting nutrients in freshwater aquatic environments; an overabundance can lead to decreased dissolved oxygen, accelerated eutrophication, and harmful algal blooms.[19] These algal blooms can also pose a human health risk through the production of cyanotoxins.[20] Additionally, P is a limited, valuable natural resource that should be recovered and reused.[21,22]

Nutrient discharges from inadequate onsite wastewater treatment systems are a growing concern globally and likely underreported.[16,23] Affordable and sustainable methods for effective N and P removal in decentralized treatment systems remain technologically challenging.[24,25] Recently, an international standard ISO 30500 was specifically developed to provide performance requirements for non-sewered sanitation systems[26] and sets a minimum threshold reduction of 80% for total P. As P regulations are increasingly enforced, wastewater P content will need to be regularly measured and reported as new technologies are pilot-tested.[2,14,27,28] Standardized P reporting is required to compare performance across different technologies and wastewater streams to accelerate technology adoption, yet a critical gap exists due to lack of a common set of metrics.[29] This knowledge gap in reporting metrics for P and its different chemical species is also highlighted in a recent systematic review of nutrient removal and recovery technologies by Kogler et al.[30] In reviewing 292 articles across 46 sources, Kogler et al.[30] found that removal efficiency and achievable effluent concentrations for P removal and recovery technologies are generally underreported, with only 16% of the articles reviewed giving adequate quantitative information for meaningful interpretation of technology performance.

As we embarked on the journey of adding total P to our routine testing regimen, we identified several specific points of confusion. We determined that measurement and reporting of P requires particular attention because of the (1) variety of chemical forms of P in wastewater, (2) availability of several different chemical assays for measuring different forms of P, and (3) different conventions in the units used for reporting P. Due to the interrelated nature of these topics, there is potential for inconsistencies in reporting results for P. Here, we present a case study highlighting the importance of accurate and clear reporting of P units and strict adherence to chemical assay protocols for measuring P in wastewater samples. The objective of this paper is to share our experiences in identifying and overcoming these P-related pitfalls so that others may avoid these issues in their future work. The aims of this paper include (1) determining the root cause(s) of discrepancies in P results and (2) developing new standard solution mixtures to ensure accurate analytical method implementation and data reporting.

We begin with a brief review of the forms of P in wastewater and typical units used for reporting P. We then describe our recent experiences where we identified discrepancies in P concentrations we measured using a commercially-available test kit and those measured by a third-party lab for the same

samples. Due to the transparent and productive relationship with the third party lab, we were able to identify two distinct sources of confusion as the likely reason for these discrepancies: (1) the factor of 3.066 difference in results reported as mg L^{-1} "PO_4-P" versus mg L^{-1} "PO_4^{3-}"; and (2) the omission of the digestion heating step in the total P protocol. By systematically omitting steps from the total P chemical assay procedure, we show that skipping the digestion heating step results in a significant undercounting of total P, both for standard solutions and for real wastewater samples.

To avoid these issues in future studies, regularly testing known P standard solutions to validate analytical methods and reporting methods is needed. However, we observed that the common commercially-available wastewater standard solutions only contain P as orthophosphate (reactive P). This is perhaps due to the fact that soluble orthophosphate is the majority P form in effluents from traditional wastewater treatment systems and therefore also the P form of primary concern for anthropogenic nutrient pollution.[31] However, our analysis reveals that the assumption that orthophosphate = total P does not necessarily hold for wastewater influent of novel onsite wastewater treatment technologies. We therefore believe that including acid-hydrolyzable P and/or organic P components in known standard solutions is especially important to ensure accurate reporting of novel wastewater treatment technology performance. To this end, we have prepared and validated our own standard mixtures of orthophosphate, acid-hydrolyzable P, and organic P using readily available and inexpensive chemical reagents.

Methods

Unless otherwise indicated, all glass wares used in this study were cleaned by soaking in 1:1 HCl:water (v:v), rinsing thoroughly in distilled or ultrapure water, then filling with fresh distilled or ultrapure water and storing until needed. Mean and standard deviation values for replicate measurements were calculated using Excel. Data were plotted using OriginPro 2018.

Standard solutions

A mixed-parameter quality control standard solution for wastewater was purchased (Hach, product LCA720) that features 10.0 mg L^{-1} PO_4-P in addition to ammonia, nitrate, COD, and TOC. A wastewater influent inorganics quality control standard solution was also purchased (Hach, product #2833149) that contains 10 mg L^{-1} PO_4^{3-} in addition to ammonia, nitrate, COD, sulfate, and TOC. (See Supplemental Material for details.)

The other standard solutions used to validate assay performance were prepared in-house. Phosphate stock standard solutions (PO_4^{3-} = 10 and 25 mg L^{-1}, labelled P 10 and P 25, respectively) were prepared by dissolving potassium dihydrogen phosphate (KH_2PO_4) in distilled water. Mixed standard solutions were prepared using KH_2PO_4, sodium trimetaphosphate ($Na_3P_3O_9$), and adenosine 5'-triphosphate, disodium salt (ATP), all purchased from Sigma-Aldrich (see Supplemental Material for additional reagent information).

Real wastewater samples

Effluent from a toilet was collected to serve as wastewater samples for evaluation. A women's shared toilet facility at a privately-owned textile mill in Coimbatore, India (described in a previous study) was the sample collection site.[13] The facility is used by 20 to 50 employees and features five toilet stalls equipped with Indian squat plates, each connected to cistern flush. The effluent of one of the toilets can be easily tapped into from the pipe and was diverted from its septic tank to a 200 L collection tank. After collecting all toilet effluent (containing flush water, urine, and feces) from 8 am to 5 pm local

time, the wastewater was left to settle overnight. The supernatant was collected the morning after and then the contents of the tank were homogenized by with a macerator pump (Jabsco Model: 18950, 45 lpm). Both supernatant and macerated wastewater samples were analyzed with the commercially-available test kit on the same day.

Commercially-available test kits (ascorbic acid method)

Total P (method 8190) and reactive P (method 8048) test kits were purchased from Hach and used with a Hach DR 900 colorimeter. Unless otherwise indicated, the test kits were used exactly as per the manufacturer's instructions (see Supplemental Material for more detail). Both test kits use the ascorbic acid method, are USEPA accepted for reporting wastewater analyses, and the procedures follow USEPA and Standard Method 4500-P.[32] Distilled (in India) or ultrapure 18.2 MΩ cm (in the US) water was used for dilution as necessary to bring the samples within the assay detection range. The total P test kit includes two powder pillows (potassium persulfate, PhosVer 3), a bottle of sodium hydroxide 1.54 N, and glass reaction vials containing a pre-measured sulfuric acid solution. A Hach DRB 200 digester heating block was used for the digestion step in the total P protocol. The reactive P test kit includes one powder pillow (PhosVer3) and either glass reaction vials containing a pre-measured volume of deionized water or a reusable glass sample cell (see Supplemental Material).

Third-party laboratory measurement (stannous chloride method)

Samples sent to a NABL certified laboratory (T.STANES Coimbatore) were analyzed using the Indian Standard IS:3025 part 31[33] stannous chloride method for the measurement of total P, a method essentially similar to Standard Method 4500-P.[32] According to the IS:3025 part 31 stannous chloride method, samples are decolorized by shaking with activated carbon for 5 minutes, then filtered. A phenolphthalein indicator is added to the filtered sample, and if a pink color develops, the pink color is discharged through the addition of up to 5 drops of a strong acid solution. Finally, ammonium molybdate and stannous chloride reagents are added and allowed to react for 10 minutes. The sample is then quantified spectrophotometrically at 690 nm. Fresh standard solutions were prepared by the third-party lab to calibrate their instrument upon receipt of each set of samples. Distilled water was used for sample dilution when necessary to bring the sample within the assay detection range. Analytical results from the third-party lab were reported to us in units of "Phosphorus (mg/L)."

Results and Discussion

Forms of P relevant for wastewater analysis

In aqueous solutions relevant for wastewater treatment technologies, P exists in three main forms, commonly referred to as orthophosphate, condensed phosphate, and organic phosphate (Table 1).[32] Phosphate anions can be detected colorimetrically, and chemical assays are commercially available for measuring only orthophosphate (sometimes called "reactive P"), "acid-hydrolyzable P" (orthophosphate + condensed phosphate), or "total P" (orthophosphate + condensed phosphate + organic phosphate). The specific chemical reactions responsible for the colorimetric responses have been described in detail elsewhere[32] and should be included with each commercially-available assay. Briefly, orthophosphate anions form a colored complex with a transition metal under the chemical assay conditions.

Table 1.

Common assays, with naming and unit conventions, for forms of P in wastewater analysis.[32]

Commercially-available chemical assays	"Orthophosphate" or "reactive P"	"Acid-hydrolyzable P"	"Total P" or "total phosphate"
Forms of P detected	Orthophosphate	Orthophosphate + condensed phosphate	Orthophosphate + condensed phosphate + organic phosphate
Digestion required?	No	Yes	Yes
Strong oxidant required?	No	No	Yes
Typical reported units (mg L^{-1})	PO_4^{3-} or PO_4-P	PO_4^{3-} or PO_4-P	PO_4-P or P

The color response for all the different assays is due to detection of orthophosphate. Other forms of P (condensed phosphate and organic phosphate) must first be converted to orthophosphate before they can be detected. Condensed phosphates require a preliminary acid hydrolysis step at elevated temperatures before the colorimetric response can be observed. Some organic phosphates also require addition of a strong oxidant to the acid hydrolysis step. Orthophosphate and acid-hydrolyzable P are typically reported using units of either mg L^{-1} "PO_4^{3-}" (orthophosphate) or "PO_4-P" (orthophosphate as P). Total P is typically reported as mg L^{-1} "P."

Potential Pitfall #1: Distinguishing and converting between the different units used for P reporting

Note that the "typical" reporting units in Table 1 are just a guideline. In our experience, these do not appear to be hard rules, and all three units are used for reporting all types of phosphorus data. *The units alone will not tell you which forms of phosphorus have been measured.* It is therefore important to understand what the different units mean and how they are interrelated. Some standards, such as the ISO 30500, are written as percent reduction requirements; in this case, the units in which P quantities are reported is not particularly important as long as it is internally consistent within any given study. However, P units must still be accurately and clearly reported in order to compare values across different studies and to nominal values for other discharge standards. Briefly, units of "PO_4-P" and "P" are equivalent and therefore interchangeable. "PO_4^{3-}" units are calculated differently and a mathematical conversion must be performed to compare to the other units.

Converting between values reported in the three different units requires accounting for the mass of atoms which are counted in each unit. Units of PO_4-P and P only count the 1 P atom (30.97 amu). Therefore, values reported in mg L^{-1} PO_4-P will be identical to values reported in mg L^{-1} P and no conversion is required. In contrast, units reported in PO_4^{3-} count all atoms in the phosphate anion—1 P atom (30.97 amu) and 4 O atoms (each 16.00 amu). Values reported in mg L^{-1} PO_4^{3-} can be converted to P or PO_4-P by dividing by 3.066, which is the stoichiometric ratio of the mass of the phosphate anion (1P + 4O = 30.97 + 4(16.00) = 94.97 amu) to that of a single P atom (30.97 amu). Similarly, values reported in units of P or PO_4-P can be converted to units of PO_4^{3-} by multiplying by 3.066:

$$1.000\ \text{mg L}^{-1}\text{P} = 1.000\ \text{mg L}^{-1}\ \text{PO}_4\text{–}P = 3.066\ \text{mg L}^{-1}\ \text{PO}_4^{3-} \quad \text{Eq. (1)}$$

As an example, consider the two wastewater (WW) quality control standard solutions, both purchased from Hach for use in this study. The first WW standard solution (1) is listed by the supplier as containing "10.0 mg L^{-1} PO_4-P," while the other (2) is listed as containing "10 mg L^{-1} PO_4^{3-}." It is an easy mistake at first glance to think that the phosphate concentrations of these solutions are equivalent. However, when we convert WW standard solution (1) to PO_4^{3-} units, we see that it actually contains 3 times as much phosphate: (10.0 × 3.066) = 30.7 mg L^{-1} PO_4^{3-}. To directly compare these wastewater standard solutions, we will hereafter label them as (1) WW 30 and (2) WW 10 to reflect the expected phosphate concentration in PO_4^{3-} units.

We use a commercial test kit for total P (Hach 8190) for our in-house field testing, because this field-friendly method utilizes only two small, easily-transportable pieces of equipment (the DRB 200 digester and the DR 900 colorimeter) and pre-portioned reagents in pre-measured vials and sealed "powder pillow" packets. The results for total P are displayed by the DR 900 colorimeter in units of "mg L^{-1} PO_4^{3-}." We used the WW 30 standard solution described above to validate the total P test kit, as recommended by the manufacturer. Results were obtained for samples collected in glass bottles cleaned with or without a HCl acid wash. (The acid wash is used to eliminate possible contamination from the detergent used to clean bottles, which may contain phosphate.) Figure 1 shows that all results obtained were within 15% of the expected value (n = 4; mean = 29.6 ± 4.2 mg L^{-1} PO_4^{3-}), however the variability in the acid-washed results was lower, within 5% of the expected value (n = 3; mean = 30.6 ± 1.5 mg L^{-1} PO_4^{3-}). We therefore used acid-washed containers for all subsequent measurements, including with samples sent for analysis to the third-party lab.

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Figure 1.

(A) Total P test results (measured by Hach kit) for the commercially-available standard solution WW 30 and in-house prepared phosphate solutions (P 25 and P 10). Each standard solution was tested multiple times; the results of individual tests are shown as open symbols, with the mean for each column shown as a short dashed line and the expected values shown as solid horizontal reference lines. The variability in test results is highest for WW 30 measured without acid washing of glassware (diamonds, n = 4, 29.6 ± 4.2 mg L^{-1} PO_4^{3-}). The variability is similar for all three standard solutions when glassware is acid washed prior to contact with the samples (circles; WW 30, n = 3, 30.6 ± 1.5; P 25, n = 6, 25.3 ± 1.0; P 10, n = 6, 10.8 ± 1.1). Position along the x-axis is not meaningful—data points within each column are offset from one another for clarity. (B) Total P measured for identical samples tested in-house with the Hach test kit and by the third-party lab. Values for units of PO_4^{3-} are shown on the left y-axis and values for units of PO_4-P are shown on the right y-axis. Expected values are shown as solid horizontal reference lines. Values reported by the third-party lab (in PO_4-P units) are similar to those measured in-house (in PO_4^{3-} units) once the correct unit conversion is applied.

In addition to the WW 30 standard solution, we evaluated phosphate solutions prepared in-house of known concentrations (PO_4^{3-} = 10, 25 mg L^{-1}, labelled P 10 and P 25, respectively) obtained by dissolving KH_2PO_4 in distilled water. Again, these solutions gave results close to the expected values (P 10, n = 6, mean = 10.8 ± 1.1 mg L^{-1} PO_4^{3-}; P 25, n = 6, mean = 25.3 ± 1.0 mg L^{-1} PO_4^{3-}). These results confirm that solutions of KH_2PO_4 can effectively serve as standards for P in orthophosphate form, and that acid-washing of sample glassware reduces test-to-test variability in the results.

Initial reports showed different units for P analysis performed by a third-party lab, leading to a discrepancy which required further validation. Our third-party collaborator, a certified laboratory located in the same city, uses the stannous chloride method for total P measurement. This third-party lab requires a large volume (>150 mL) of sample to run their assay, thus we diluted by 5× the WW 30 standard solution prior to delivering it. We also sent for analysis a sample of the P 25 solution.

The third-party lab reported values of 10.25 mg L^{-1} for the WW 30 sample and 7.84 mg L^{-1} for the P 25 solution, both reported with units of "Phosphorus (mg/L)." Initially, we were surprised to find that the third-party lab results appeared much lower than the values we expected (30.7 and 25.1 mg L^{-1}, respectively) for the WW 30 and P 25 samples. After clarifying discussions with the third-party lab, we determined that their results were reported in "PO_4-P" units. Thus, the expected values would be 10.0 and 8.15 mg L^{-1} for units of PO_4-P, respectively, for these two samples. In Figure 1B, the results are shown for both units of PO_4^{3-} (on the left axis) and PO_4-P (on the right axis). It is clearly seen that by resolving the ambiguity of the reported units, the total P values were measured by the third-party lab within a few percent from the expected values.

Potential Pitfall #2: Omission of steps from the total P test protocols

Despite reconciling the difference in reported units between our in-house total P test results and those from the third-party lab, we noticed that there was still a discrepancy between results from the two labs when real wastewater samples were analyzed. Total P results from the third-party lab were always lower than those measured in-house, although not always lower by a consistent nominal amount or percentage. Importantly, we learned through fruitful discussions with the third-party lab that while a digestion (heating) pre-treatment step is listed in the IS:3025 protocol, digestion was not actually performed. This indicates that while the third-party lab results were accurate in determining total P content from the standard solutions (where only orthophosphate was present), their procedure will under-report the total P content for samples where condensed and organic phosphates are present (e.g., real wastewater samples from field testing evaluation of onsite treatment technologies).

The full protocol for Hach total P measurement includes a digestion step performed by heating the sample in acid at 105 °C for 30 minutes with a persulfate ($S_2O_8^{2-}$) reagent added to the solution. Heating in acid converts any acid-hydrolyzable P to orthophosphate. The addition of the strong oxidant $S_2O_8^{2-}$ ensures that organic phosphates are fully converted to orthophosphate. Figure 2 shows in-house results for two raw wastewater samples where steps from the total P protocol were systematically and intentionally omitted. Samples A and B were collected on different days from the same shared toilet effluent, and the liquid supernatant as well as a macerated sample containing higher fecal load were analyzed. The primary soluble form of P in wastewater is orthophosphate, therefore orthophosphate is expected to dominate in the supernatant fraction, while the macerated fraction is expected to contain a higher concentration of organic and condensed P forms.

Figure 2.

Comparison of total P results obtained during systematic omission of testing steps for two raw wastewater samples A and B collected from the Coimbatore field testing site. The photograph shows the typical appearance of the supernatant and macerated sample fractions. For each sample, both the macerated fraction (closed circles) and the supernatant fraction (open circles) were measured. The macerated fraction contains more fecal matter and thus a higher total P concentration. Omitting the heating step ("-heat") from the testing protocol has the largest effect on the measured total P concentration, while omitting the persulfate reagent ("-$S_2O_8^{2-}$") has a smaller effect that is most visible for the macerated fraction. Samples were diluted with distilled water by 10× to 25× to bring them within the range of the total P Hach test kit.

Figure 2 shows that omitting the heating step has the largest effect on the total P results, both for the macerated and supernatant sample fractions. Omitting the $S_2O_8^{2-}$ reagent has a measureable, but smaller, effect. When the protocol is followed fully, the total P measured for Sample A was 45.8 mg L^{-1} PO_4^{3-} for the macerated fraction and 42.2 mg L^{-1} PO_4^{3-} for the supernatant fraction. These values drop to 35.4 and 35.6 mg L^{-1} PO_4^{3-}, respectively, when the heating step is omitted. This results in an under-reporting of total P by 23% for the macerated fraction and 16% for the supernatant. For Sample B, omitting the heating step results in under-reporting of total P by 35% for the macerated fraction and 21% for the supernatant. Omitting only the $S_2O_8^{2-}$ reagent under-reported the total P in the supernatant samples by ~5%, which is similar to the test-to-test variability for standard solutions as shown in Figure 1. In contrast, omitting only the $S_2O_8^{2-}$ reagent from the macerated fraction under-reports the total P by 8% for Sample A and 18% for Sample B.

In summary, total P content in raw wastewater samples can be substantially under-reported if any step in the digestion protocol is omitted. The largest differences in total P values were found with heating and/or persulfate omission for the macerated sample fractions. This shows that the risk of P under-reporting increases as the fecal content (and thus the amount of condensed and/or organic P) increases. This is important when testing new treatment technologies, particularly those which involve novel solid/liquid separation processes, solids-settling techniques, or other methods of solids treatment where solid fecal matter is broken down and mixed with liquid effluent.

Impact of the pitfalls

While the major contribution of water soluble P in human excreta is inorganic orthophosphate, orthophosphate may not always be the sole contributor to total P in effluent from onsite wastewater treatment technologies. Raw influent of onsite wastewater treatment technologies is highly variable and impacted by many factors such as number of toilet uses, flush volume, and diet.[34,35] We suspect that concentrations of condensed and/or organic P may be significant in influent and in effluent from some technologies with novel solid/liquid separation or solids processing methods.

Our group has previously evaluated one such novel system. We reported in a previous publication total P values of system effluent of 8.3 and 21.5 "mg P L^{-1}"[5] that we now suspect were measured omitting the heating step; thereby, those values represent only the orthophosphate as opposed to the total P. In a different publication,[13] we reported values of "P," without specifying the form (which was orthophosphate) nor clarifying the reporting units (mg L^{-1} P). In another recent paper,[14] we did specify

in the methods section that both reactive P and total P were reported in units of "mg L^{-1} PO_4^{3-}." However, table columns and figure axes in that work were labelled only as "mg L^{-1}" for both reactive P and total P. For added clarity in the future, we will explicitly include the full unit "mg L^{-1} PO_4^{3-}" or "mg L^{-1} P" in all tables and figure axes, as demonstrated in the present work.

From our experiences described here, particularly those during collaboration with a third-party laboratory, we now fully appreciate how critical it is for groups working on wastewater treatment technologies to report the correct P form in clear, specific units. Our concern that inconsistencies in P reporting are widespread is further supported by a recent systematic review of the literature on nutrient removal and recovery technologies by Kogler et al.[30] In sorting reports for P removal efficiencies and effluent concentrations, three separate categories were required for reports of "TP = total phosphorus" and "PO_4 = phosphate," and "P = general phosphorus species if species was not identified clearly."[30] This review shows that there is a prevalent need to standardize quantitative performance metrics of nutrient removal and reuse technologies.

The ambiguity in reporting units introduces lingering questions of whether variable P concentrations in wastewaters across different studies is due to variability in the wastewater itself or variability in reporting and analysis methods. For example, Cid et al.[2] reported 0.64 mmol L^{-1} "PO_4^{3-} + HPO_4^{2-}" in average macerated toilet wastewater from one of their self-contained bathroom + wastewater treatment and recycling prototypes. The specific analytical method used in that work is not reported, but assuming based on the units that this represents a measurement of orthophosphate (reactive P), this would be approximately 60.8 mg L^{-1} PO_4^{3-}. This value is similar to what we report here for total P concentrations in macerated toilet wastewater, but also aligns well with our previous report of reactive P in toilet effluent.[14] Another recent study by Reynaert et al.[27] reported 36.2 mg L^{-1} average soluble phosphate "PO_4" concentration in toilet wastewater influent from a urine-diverting toilet with solids separated from the flush water. While the use of the "PO_4" unit is ambiguous, the analytical test used (Spectroquant) appears to give results in units of PO_4-P. This would indicate that the average influent concentration in this study was 36.2 mg L^{-1} PO_4-P or 111 mg L^{-1} PO_4^{3-}, and the nominal average concentration in the treated clean water tank is 25.4 mg L^{-1} PO_4-P or 77.9 mg L^{-1} PO_4^{3-},[27] which would be unable to meet water reuse or discharge standards with nominal effluent P concentration limits (eg, <1 mg L^{-1} P in India).[36]

Pitfall prevention: Preparation of mixed-P standard solutions for analytical test validation

For pitfall #1, we determined that validating test protocols and reporting units for reactive P, both in-house and with the third-party lab, can be accomplished by testing commercially-available standard solutions of orthophosphate. But what about validating methods when samples contain condensed and/or organic forms of P? Researchers should clarify the analytical methods used by explicitly stating whether samples were digested prior to analysis. For example, Chapter 4500-P in the 22nd edition of Standard Methods for the Examination of Water and Wastewater contains ten subsections detailing sample filtration pretreatments, three digestion methods, and three colorimetric methods.[32] Therefore, simply referencing "standard method 4500-P" is insufficient for determining the fraction or form of P that has been analyzed. For samples containing condensed and/or organic P, definitive confirmation that digestion has been performed requires an analytical validation step testing a standard solution of known composition. However, the wastewater standard solutions readily available for purchase only contain orthophosphate. Testing these standard solutions will not clarify whether sample digestion is performed as required to convert all P forms to orthophosphate.

We therefore set out to prepare our own in-house, mixed standard solution for validating total P testing protocols. In addition to KH_2PO_4 to provide orthophosphate, we identified two non-hazardous compounds which are readily-available and relatively inexpensive to represent condensed and organic P forms: trisodium trimetaphosphate ($Na_3P_3O_9$) and adenosine 5'-triphosphate, disodium salt (ATP). Figure 3 shows the structures of these compounds and relevant properties, such as number of PO_4^{3-} equivalents (i.e., number of PO_4^{3-} anions detected in total P test per molecule of the compound). While KH_2PO_4 will be detected in a reactive P test, $Na_3P_3O_9$ and ATP will only be detected in a total P test, as digestion is required to hydrolyze P-O and O-C bonds to release free orthophosphate into the solution.

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Figure 3.

Chemical compounds used in this study to make in-house mixed-P standard solutions. KH_2PO_4 dissociates readily to give phosphate anions that are detected in reactive P tests. $Na_3P_3O_9$ and ATP each contribute 3 phosphate equivalents, but these molecules must undergo acid digestion to release phosphate anions. $Na_3P_3O_9$ and ATP are thus detected in total P tests but are not detected in reactive P tests.

Solutions were first prepared containing only KH_2PO_4, $Na_3P_3O_9$, or ATP and analyzed using total P and reactive P Hach kits (see Supplemental Material for a complete table of all solution recipes). Figure 4 shows these results by comparing samples that contain no reactive P (top; ATP only and $Na_3P_2O_9$ only) to samples with only reactive P (bottom; P 25, P 10, WW 10). For samples with no reactive P ($Na_3P_3O_9$ and ATP), the total P results match the expected values only when the heating step was included in the protocol. When heating was omitted from the protocol, both samples showed <1.5 mg L^{-1} PO_4^{3-} for the total P measurement. As expected, the reactive P test for both $Na_3P_3O_9$ and ATP measured almost zero ($Na_3P_3O_9$ = 0.34 mg L^{-1} PO_4^{3-}; ATP = 0.08 mg L^{-1} PO_4^{3-}). Standard solutions purchased (WW 10) and prepared in-house (P 25, P 10) which only contained reactive P showed no difference in the values for the total P test when steps were omitted from the protocol. As expected, the results of the reactive P test and total P test are identical for samples containing only reactive P.

Figure 4.

Demonstration of the different results obtained from total P and reactive P tests for identical samples, including tests where steps were systematically omitted from the total P protocol. Expected values are shown as horizontal reference lines (dashed lines = ATP). As expected, samples containing condensed and organic P (top, triangles) show no response to the reactive P test (shaded column). Samples containing ATP (closed symbols) and $Na_3P_3O_9$ (open symbols) are accurately measured only when the total P test has been performed according to the protocol. Eliminating the heating step ("-heat" and "-heat, -$S_2O_8^{2-}$") results in a complete failure of the total P test. Eliminating only the persulfate pouch ("-$S_2O_8^{-2}$") shows only a small effect on the total P result for ATP. In contrast, samples that only contain reactive P (bottom, circles; WW 10, shaded symbols; P 25, open symbols; P 10, closed symbols) show nearly identical results for total P and reactive P (shaded column), and omitting steps from the total P protocol imparts no effect.

We also prepared two standard solutions with mixed-P forms that could be used to validate results from reactive P or total P tests using the same solution. Figure 5 shows total P and reactive P results for a 2-part standard solution (containing KH_2PO_4 and $Na_2P_3O_9$) and a 3-part standard solution (containing KH_2PO_4, $Na_3P_3O_9$, and ATP). Both the 2-part and 3-part standard solutions give measured total P concentrations close to their expected values. When the heating step is omitted, the total P results are nearly identical to the reactive P results.

Figure 5.

Comparison of total P and reactive P (shaded column) results for two mixed standard solutions prepared in-house. The 2-part mixed standard solution (open symbols) is a mixture of KH_2PO_4 and $Na_3P_3O_9$. The 3-part mixed standard solution (closed symbols) contains KH_2PO_4, $Na_3P_3O_9$, and ATP. Expected values are shown as horizontal lines (solid lines for 2-part standard solution; dashed lines for 3-part standard solution). Measured results for both mixed standard solutions were close to the expected values. When the heating step is omitted from the total P protocol, the measured value is the same as that for the reactive P test.

The results in Figure 5 show that a 2-part standard solution of KH_2PO_4 and $Na_3P_3O_9$ is sufficient to conclude whether the digestion heating step proceeds as necessary. These chemicals are affordable and do not require any special handling or storage considerations, so a solution of these two compounds could serve as a simple, yet robust mixed-P standard solution. The ATP salt has some disadvantages compared to $Na_3P_3O_9$; it is more expensive, requires storage under refrigeration, and can have a variable water content bottle-to-bottle, making the calculation of the expected concentration slightly more complicated (see Supplemental Material).

However, there are two advantages for why some researchers may choose to include ATP in their in-house standard-solution formulation. First, as persulfate is expected to affect hydrolysis of organic P (ATP) but is not necessary for hydrolysis of condensed P ($Na_3P_3O_9$), we hypothesized that including

ATP in the mixture could validate that the persulfate reagent is included in the total P protocol. When the $S_2O_8^{2-}$ is omitted from the solution of only $Na_3P_3O_9$ or from the WW 10 standard solution (see Figure 4), the total P result does not decrease. In fact, there is a slight increase in the measured total P values (55.6 to 56.6 mg L^{-1} PO_4^{3-} for $Na_3P_3O_9$ from 9.8 to 10.0 mg L^{-1} PO_4^{3-} for WW 10), although this change is well within the test-to-test variability demonstrated in Figure 1. On the other hand, when $S_2O_8^{2-}$ is omitted from the solution of only ATP, the total P value measured decreases from 46.4 to 43.8 mg L^{-1}. Similarly, for the 3-part mixture, omitting $S_2O_8^{2-}$ gives a small decrease in total P from 50.4 to 49.4 mg L^{-1}, however, this is on the border of the test-to-test variability of 1.0 to 1.5 mg L^{-1} shown in Figure 1. If validating inclusion of persulfate is desired, increasing the ratio of ATP in the 3-part mixed standard solution may be prudent. Alternatively, a 2-part standard solution containing only KH_2PO_4 and ATP would also be suitable. The second advantage of including ATP is that it also contains N atoms (5 N equivalents per ATP molecule) and can therefore be used to validate total nitrogen digestion protocols as well. Using the Hach low range total N kit (method 10071), we measured total N concentrations of 8.9 mg L^{-1} N in the ATP only solution and 2.0 mg L^{-1} N in the 3-part standard solution, both of which are in close agreement with the expected values of 11.4 and 2.3 mg L^{-1} N, respectively.

Finally, we evaluated the stability of the in-house mixed-P standard solutions over time, with and without refrigeration of the solutions. If either the $Na_3P_3O_9$ or ATP is susceptible to degradation over time (e.g., slow hydrolysis), then the reactive P concentration of the standard solutions should increase as the samples age. Figure 6 shows reactive P values measured for $Na_3P_3O_9$, ATP, 2-part, and 3-part mixtures after ageing for several days in sealed glass bottles. Only the 3-part mixture was stored under refrigeration; the other solutions were left in the laboratory ambient (21 °C). Small increases in the reactive P concentration were observed for all three solutions stored at room temperature (0.91, 1.98, and 1.40 mg L^{-1} PO_4^{3-} for ATP, $Na_3P_3O_9$, and the 2-part mixture, respectively) for 6 to 11 days. The 3-part mixture stored for 13 days at 4 °C increased by only 0.2 mg L^{-1} PO_4^{3-}. All of these values are within the test-to-test variability of 1.0 to 1.5 mg L^{-1} PO_4^{3-}, except for the $Na_3P_3O_9$ solution, which is only slightly higher.

Figure 6.

Comparison of reactive P results for freshly prepared samples (open symbols) and the same samples after ageing for several days (closed symbols) under laboratory conditions. Samples containing ATP and/or $Na_3P_3O_9$ show very small increases in reactive P when stored at 20 °C for 6 to 11 days. The 3-part standard solution showed no increase in reactive P after 13 days when stored at 4 °C.

In summary, solutions prepared in-house with $Na_3P_3O_9$ and/or ATP are sufficiently stable for several days without refrigeration to serve as standards for validation of total P protocols. These compounds show minimal degradation once dissolved in water and stored at room temperature. The mixed standard solutions can be used in-house or sent to third-party laboratories to confirm that the total P sample digestion protocol is followed correctly.

Conclusions

Accurate and clear reporting of P in effluent from novel wastewater treatment technologies is critical for preventing eutrophication of receiving water bodies and in protecting human health. New research into methods to mitigate nutrient discharge in effluent from non-sewered sanitation systems and the adoption of nutrient discharge standards has many research groups stepping into new territory with phosphorus measurements. We have shown that commercially-available kits are accurate tools for evaluating reactive P and total P concentrations in standard solutions, and results were consistent across the two testing methods used in-house and at third-party lab once the correct unit conversion was applied. We also described options for making a standard solution containing condensed and/or organic P compounds that can be used to validate the digestion step in total P assays. We hope that the mixed-P standard solution recipes will be useful for other research groups, as commercially-available wastewater standard solutions only contain orthophosphate.

The work described here leads us to give recommendations for best practices going forward:

1) Regularly include P standard solutions of known concentration in P analyses. We advise that both an orthophosphate and organic phosphate standard solution (or a mixture) be used to validate which P assay is being used, determine the presence of a digestion step, and confirm the reported units.

2) Clearly state the fraction of phosphorus measured—reactive P, acid-hydrolyzable P, or total P—when reporting phosphorus results. Neither the units for reporting P nor a reference to "standard method 4500-P" are sufficient to convey this important information.

3) Discontinue use of the unit "PO_4-P" in reporting results, and instead only use either PO_4^{3-} or P. We think this may be the root of much confusion in the field, as (1) "PO_4-P" and "P" units are interchangeable and therefore redundant, (2) "PO_4-P" and "PO_4^{3-}" are *not* interchangeable, yet look quite similar, and (3) "PO_4-P" is not a chemically meaningful notation (see Supplemental Material for further discussion).

We hope that these recommendations will be useful and widely adopted by groups (including ours) who are adding or continuing with total P analysis as part of their routine testing.

Supplemental Material

sj-pdf-1-ehi-10.1177_11786302211019218 – Supplemental material for Potential Pitfalls in Wastewater Phosphorus Analysis and How to Avoid Them:

Click here for additional data file. (573K, pdf)

Supplemental material, sj-pdf-1-ehi-10.1177_11786302211019218 for Potential Pitfalls in Wastewater Phosphorus Analysis and How to Avoid Them by Praveen Rosario, Ramya Viswash, Thamayanthi Seenivasan, Sudha Ramalingam, Katelyn L Sellgren, Sonia Grego and Lena Trotochaud in Environmental Health Insights

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Supplemental Material: Supplemental material for this article is available online. Supplemental tables, details of Hach kit test procedures, example soitchiometric calculations, discussion of chemically meaningful unit notations, and underlying data.

References

1. Cho K, Qu Y, Kwon D, et al.. Effects of anodic potential and chloride ion on overall reactivity in electrochemical reactors designed for solar-powered wastewater treatment. *Environ Sci Technol*. 2014;48:2377-2384. [PubMed] [Google Scholar]

2. Cid CA, Qu Y, Hoffmann MR. Design and preliminary implementation of onsite electrochemical wastewater treatment and recycling toilets for the developing world. *Environ Sci*. Water Res. Technol. 2018;4:1439-1450. [PMC free article] [PubMed] [Google Scholar]

3. Cid CA, Stinchcombe A, Ieropoulos I, Hoffmann MR. Urine microbial fuel cells in a semi-controlled environment for onsite urine pre-treatment and electricity production. *J Power Sources*. 2018;400:441-448. [PMC free article] [PubMed] [Google Scholar]

4. Huang X, Qu Y, Cid CA, et al.. Electrochemical disinfection of toilet wastewater using wastewater electrolysis cell. *Water Res*. 2016;92:164-172. [PMC free article] [PubMed] [Google Scholar]

5. Varigala SK, Hegarty-Craver M, Krishnaswamy S, et al.. Field testing of an onsite sanitation system on apartment building blackwater using biological treatment and electrochemical disinfection. *Environ Sci*. Water Res. Technol. 2020;6:1400-1411. [Google Scholar]

6. Künzle R, Pronk W, Morgenroth E, Larsen TA. An energy-efficient membrane bioreactor for on-site treatment and recovery of wastewater. *J Water Sanit Hyg Dev*. 2015;5:448-455. [Google Scholar]

7. Hanak DP, Kolios AJ, Onabanjo T, et al.. Conceptual energy and water recovery system for self-sustained nano membrane toilet. *Energy Convers Manag*. 2016;126:352-361. [PMC free article] [PubMed] [Google Scholar]

8. Tobias R, O'Keefe M, Künzle R, et al.. Early testing of new sanitation technology for urban slums: The case of the Blue Diversion Toilet. *Sci Total Environ*. 2017;576:264-272. [PMC free article] [PubMed] [Google Scholar]

9. Kamranvand F, Davey CJ, Sakar H, et al.. Impact of fouling, cleaning and faecal contamination on the separation of water from urine using thermally driven membrane separation. *Sep Sci Technol*. 2018;53:1372-1382. [PMC free article] [PubMed] [Google Scholar]

10. Rogers TW, Rogers TS, Stoner MH, et al.. A granular activated carbon/electrochemical hybrid system for onsite treatment and reuse of blackwater. *Water Res*. 2018;144:553-560. [PMC free article] [PubMed] [Google Scholar]

11. Ziemba C, Larive O, Reynaert E, Huisman T, Morgenroth E. Linking transformations of organic carbon to post-treatment performance in a biological water recycling system. *Sci Total Environ*. 2020;721:137489. [PMC free article] [PubMed] [Google Scholar]

12. Sahondo T, Hennessy S, Sindall RC, et al.. Field testing of a household-scale onsite blackwater treatment system in South Africa. *Sci Total Environ*. 2020;703:135469. [PMC free article] [PubMed] [Google Scholar]

13. Welling CM, Sasidaran S, Kachoria P, et al.. Field testing of a household-scale onsite blackwater treatment system in Coimbatore, India. *Sci Total Environ*. 2020;713:136706. [PMC free article] [PubMed] [Google Scholar]

14. Trotochaud L, Andrus RM, Tyson KJ, et al.. Laboratory demonstration and preliminary techno-economic analysis of an onsite wastewater treatment system. *Environ Sci Technol*. 2020;54:16147-16155. [PMC free article] [PubMed] [Google Scholar]

15. United National Children's Fund (UNICEF), World Health Organization. *Progress on Household Drinking Water, Sanitation and Hygiene 2000-2017: Special Focus on Inequalities*. World Health Organization; 2019. [Google Scholar]

16. WWAP (UNESCO World Water Assessment Programme). *The United Nations World Water Development Report 2019: Leaving No One Behind*. UNESCO; 2019. [Google Scholar]

17. Silbiger NJ, Nelson CE, Remple K, et al.. Nutrient pollution disrupts key ecosystem functions on coral reefs. *Proc Biol Sci*. 2018;285:20172718. [PMC free article] [PubMed] [Google Scholar]

18. Amin MN, Kroeze C, Strokal M. Human waste: an underestimated source of nutrient pollution in coastal seas of Bangladesh, India and Pakistan. *Mar Pollut Bull*. 2017;118:131-140. [PubMed] [Google Scholar]

19. Correll DL. The role of phosphorus in the eutrophication of receiving waters: A review. *J Environ Qual*. 1998;27:261-266. [Google Scholar]

20. Paerl HW, Otten TG. Harmful cyanobacterial blooms: causes, consequences, and controls. *Microb Ecol*. 2013;65:995-1010. [PubMed] [Google Scholar]

21. Desmidt E, Ghyselbrecht K, Zhang Y, et al.. Global phosphorus scarcity and full-scale P-recovery techniques: A review. *Crit Rev Environ Sci Technol*. 2015;45:336-384. [Google Scholar]

22. Sena M, Rodriguez Morris M, Seib M, Hicks A. An exploration of economic valuation of phosphorus in the environment and its implications in decision making for resource recovery. *Water Res*. 2020;172:115449. [PubMed] [Google Scholar]

23. Maxcy-Brown J, Elliott MA, Krometis LA, Brown J, White KD, Lall U. Making waves: Right in our backyard-surface discharge of untreated wastewater from homes in the United States. *Water Res*. 2021;190:116647. [PubMed] [Google Scholar]

24. Trotochaud L, Hawkins BT, Stoner BR. Non-biological methods for phosphorus and nitrogen removal from wastewater: A gap analysis of reinvented-toilet technologies with respect to ISO 30500. *Gates Open Res*. 2019;3:559. [PMC free article] [PubMed] [Google Scholar]

25. Bunce JT, Ndam E, Ofiteru ID, Moore A, Graham DW. A review of phosphorus removal technologies and their applicability to small-scale domestic wastewater treatment systems. *Front Environ Sci*. 2018;6:8. [Google Scholar]

26. ISO 030500:2018. *Non-Sewered Sanitation Systems - Prefabricated Integrated Treatment Units - General Safety And Performance Requirements For Design And Testing*. International Organization for Standardization; 2018. [Google Scholar]

27. Reynaert E, Greenwood EE, Ndwandwe B, et al.. Practical implementation of true on-site water recycling systems for hand washing and toilet flushing. *Water Res X*. 2020;7:100051. [PMC free article] [PubMed] [Google Scholar]

28. Ziemba C, Larivé O, Reynaert E, Morgenroth E. Chemical composition, nutrient-balancing and biological treatment of hand washing greywater. *Water Res*. 2018;144:752-762. [PMC free article] [PubMed] [Google Scholar]

29. Cornejo PK, Becker J, Pagilla K, et al.. Sustainability metrics for assessing water resource recovery facilities of the future. *Water Environ Res*. 2019;91:45-53. [Google Scholar]

30. Kogler A, Farmer M, Simon JA, Tilmans S, Wells GF, Tarpeh WA. Systematic evaluation of emerging wastewater nutrient removal and recovery technologies to inform practice and advance resource efficiency. *ACS EST Eng*. 2021;1:662-684. [Google Scholar]

31. Warwick C, Guerreiro A, Soares A. Sensing and analysis of soluble phosphates in environmental samples: A review. *Biosens Bioelectron*. 2013;41:1-11. [PubMed] [Google Scholar]

32. 4500-P. In: Rice EW, Baird RB, Eaton AD, Clesceri LS, eds. *Standard Methods for the Examination of Water and Wastewater*. 22nd ed. American Public Health Association; 2012. [Google Scholar]

33. Methods of sampling and test (physical and chemical) for water and wastewater, IS:3025 Part 31 - Phosphorus. *Indian Standard*. 1988. (reaffirm-ed 2003). https://law.resource.org/pub/in/bis/S02/is.3025.31.1988.pdf (accessed 18 May 2021).

34. Welling CM, Varigala S, Krishnaswamy S, et al.. Resolving the relative contributions of cistern and pour flushing to toilet water usage: Measurements from urban test sites in India. *Sci Total Environ*. 2020;730:138957. [PMC free article] [PubMed] [Google Scholar]

35. Rose C, Parker A, Jefferson B, Cartmell E. The characterization of feces and urine: A review of the literature to inform advanced treatment technology. *Crit Rev Environ Sci Technol*. 2015;45:1827-1879. [PMC free article] [PubMed] [Google Scholar]

36. Schellenberg T, Subramanian V, Ganeshan G, Tompkins D, Pradeep R. Wastewater discharge standards in the evolving context of urban sustainability - The case of India. *Front Environ Sci*. 2020;8:30. [Google Scholar]

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ORIGINAL ARTICLE

CR-Possum—Can It Be Used to Predict Morbidity? A Single-Centre Retrospective Study

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Abstract

Preoperative prediction of morbidity in colorectal cancer (CRC) surgery helps to optimize the surgical outcome. In this study, we aim to develop a dedicated equation for predicting operative morbidity using colorectal possum scoring system and also to validate the predictive accuracy of CR-POSSUM scoring system in prognosticating actual complications. We did a retrospective analysis of 322 patients undergoing colorectal cancer surgery from a single centre in South India from 2004 to 2016. Mortality and morbidity risk factors as defined by CR POSSUM were collected from 322 patients who underwent CRC surgery and were used to derive equations to predict morbidity, and the results were compared with the observed morbidity. Logistic regression analysis was used to derive the equation. The model fit and model discrimination were analysed using the Hosmer-Lemeshow statistical test for goodness of fit, the Nagelkerke R^2 and area under the receiver operating characteristic (ROC) curve respectively. Out of 322 patients, 103 (32%) patients developed complications and 10 (3%) died due to complications. The regression equation we derived has an overall correct classification of about 70% ($P < 0.01$) with positive and negative predictive value of 60% and 73% respectively. The Hosmer-Lemeshow goodness of fit was 3.147 ($P = 0.829$), and the Nagelkerke R^2 was 17% and area under ROC as model discrimination was 71.6%. Hence, CR-POSSUM scoring which was originally used for predicting mortality risk can also be extrapolated to predict morbidity.

Keywords Colorectal POSSUM · POSSUM · Colorectal surgery · SSI · Morbidity and mortality · Prediction model

Introduction

Colorectal malignancy is the third most common malignancy among men and the second most common among women around the world [1]. Fortunately, in India, the incidence is not too high. However, the incidence has been found to be rising, with the AAR (adjusted annual incidence rate) for colon cancer in men and women being 4.4 and 3.9 per 100,000, respectively [2]. Being in a country, where people living with poor access to quality healthcare, extreme presentation like advanced stage and perforation are common in tertiary care centres [3, 4]. In such scenarios, the mortality and morbidity for the patients undergoing surgery are relatively high [5]. Complications following major surgeries will proportionately increase the hospital cost and duration of hospital stay [6]. Apart from such hardships, complications also had an adverse impact on long-term survival [7]. So in a country like India, where the spending on health by the government is low and health insurance is under-penetrated, morbidity following a major surgical procedure will further drain the already constrained resources [8]. The complication rates are not uniform and cannot be generalised, as there are certain factors, such as age, patients' co-morbidity and operative severity, influencing the outcome of surgery [9]. So, putting together these factors and assessing the postoperative outcome preoperatively will help in optimizing the available resources. In such cases, the predictive scoring has to be more accurate, and its predictive ability should

Key message: CR-Possum can be used to predict morbidity in patient undergoing colorectal cancer surgery.

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be proven in multiple centres. In our study, we devised a morbidity scoring system with already established parameters in CR-Possum which influence the outcome of colorectal surgery in terms of mortality and assessed the model calibration and discrimination with statistical analysis tools.

Patient and Methods

Data of 506 patients undergoing colorectal surgery for colorectal malignancy from January 2004 to December 2017 was collected from the medical records department of a single tertiary care centre. The in-patient files were analysed and the required variables as per CR-POSSUM scoring [physiologic severity score (PSS) (age, cardiac failure if any, systolic blood pressure, pulse, urea, haemoglobin) and operative severity score (OSS) (operative severity, peritoneal soiling, operative urgency, cancer staging in Dukes)] were collected and entered in an excel data sheet. Patient files, with single missing variable, were excluded from the study. Therefore, only 322 patients with complete details were included. The dichotomous outcome of each patient, either dead/alive, with/without complication, was entered. Presence or absence of complications, such as haemorrhage, wound infection, wound dehiscence, anastomotic leak, deep vein thrombosis, pulmonary embolus, cerebrovascular accident, myocardial infarction, cardiac failure, impaired renal function and respiratory failure were also entered. Complications were also graded based on Clavien-Dindo scale of surgical morbidity, and all grades were considered. Duration of hospital stay in terms of number of days for each patient was also recorded. The study was approved by institutional ethics committee.

Statistical Analysis

The data was analysed using SPSS version 24. Mean and standard deviation were calculated for continuous variables. Chi-square test was done to check association between categorical variables. The equation to predict morbidity was derived using multiple logistic regression analysis, and the model discrimination was measured by calculating the area under ROC (receiver operating characteristic) curve. The Nagelkerke R^2 and the Hosmer-Lemeshow statistics were used for model calibration (goodness of fit). Positive and negative predictive values were also calculated. $P < 0.05$ was considered as statistically significant.

Results

Out of a total of 506 patients undergoing colorectal surgery for malignancy, 322 (63.2%) satisfied the criteria to be included in the study. Among the 322 patients, 103 (31.9%) developed complications and 10 (3%) patients died of complications. The average duration of hospital stay for all patients who underwent surgery was 15.5 days. The mean duration of in-hospital stay for patients with and without complications was 18 and 14.5 days respectively ($P = 0.01$) which was statistically significant. The proportion of patients with and without complications and in-hospital stay days are shown in Fig. 1. The summary of procedures and their associated complications were shown in Table 1. The complications rates were more in tumours located in sigmoid and rectum compared to other sites ($P < 0.05$). The complications were graded as per Clavin-Dindo classification. Wound-related complication tops the list (37%) followed by respiratory complications (18%). These are shown in Table 2. The patient's demographic characteristics and the in-hospital morbidity rates for the factors taken into consideration for CR-POSSUM scoring are shown in Table 3. Majority of the patients are in the age group less than 60 years (51%) and there is a male preponderance (61%). Table 4 summarizes the CR-POSSUM scoring system which we used for the analysis. The equation we derived using above data was $In\ (R/(1-R)) = -4.83 + 0.09(PSS) + 0.35(OSS)$($P < 0.001$). The positive and negative predictive value of the equation was 60.87% and 73.09% respectively. The overall percentage of correct classification was 70%. The goodness of fit was looked at to check whether the predicted probability was assigned accordingly to individual patient's outcome. The Hosmer-Lemeshow test statistics was 3.147 ($P = 0.829$) which was reasonably a good fit for higher the P value, more fit the model is. The Nagelkerke R^2 which was another test to check goodness of fit was fairly good (17%). Model discrimination calculated by area under ROC curve was 71.6% (Fig. 2), which was reasonably a fair discrimination. The observed and expected ratio was almost close to 1 among the different range of predictions.

Discussion

POSSUM AND P-POSSUM were original scoring systems which enumerated the mortality and morbidity in a patient undergoing major surgeries [10, 11]. These have been validated in numerous studies outside the UK, and their value in surgical audit has been proven beyond doubt [12–14]. A study conducted by the American College of Surgeons (ACS-NSQIP) in 2005 to 2006 showed that ten procedure groups among various general surgical operations accounted for 62% of complications, and colectomy was topping in the list [15]. It accounts for 24% of adverse events. CR-POSSUM, developed by Tekkis et al., a modification in the original scoring system, was designed for enumeration of mortality in patients undergoing colorectal procedures [16]. Since the mortality following colorectal surgery was brought down with

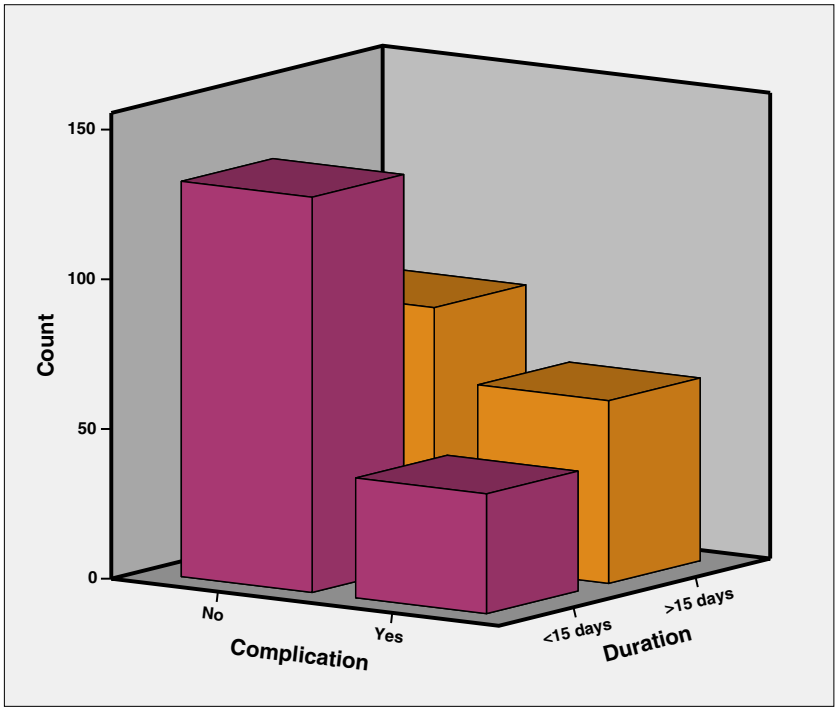


Fig. 1 Duration of hospital stay in patients with and without complications

improvement in postoperative critical care but the morbidity following such procedures especially in patients undergoing emergency surgery for perforation and obstruction was considerably high [17]. Complication rates in surgery for colorectal malignancy elective and emergency were 26 and 38% respectively. In our study, the overall complication rate was 31.9%. The original CR-POSSUM was devised by identifying odds of risk factor causing mortality. However, it was a well-known fact that mortality was the end point of severe morbidity [18, 19]. Therefore, we used the same scoring system to analyse the outcome in terms of complications in our study. The equation, though, using the same odds as for mortality did not overpredict morbidity in our study. But in the physiological severity scoring, except for preoperative cardiac status and urea level, other variables have no significant correlation

Table 1 Tumour location and procedures with in-hospital complication rates

Location of the tumour	Patients	Morbidity (%)	X^2 (P value)
Rectum	99	33(32)	14.40 ($P = 0.04$)
Sigmoid colon	77	31(30.1)	
Ascending colon	43	9(8.7)	
Transverse colon	39	9(8.7)	
Caecum	24	6(5.8)	
Descending colon	17	3(2.9)	
Rectosigmoid junction	11	7(6.8)	
Others	12	4(3.8)	
Type of procedure	Patients	Morbidity (%)	
Right hemicolectomy	99	25(24.2)	11.34 ($P = 0.18$)
Anterior resection	74	18(17.4)	
APR	46	19(18.4)	
Left hemicolectomy	34	9(8.7)	
Sigmoidectomy	31	14(13.5)	
Diversion colostomy	24	9(8.7)	
Total colectomy	5	3(2.9)	
Subtotal colectomy	2	1(0.9)	
Others	9	3(2.9)	

Table 2 Severity of complications graded as per Clavien-Dindo classification

Grade of complication	Type of complication	No. of patients (%) ($n = 103$)
I	Wound infection requiring drainage	16(15.5)
	Post op ventilation for > 1 day	6(5.8)
II	Wound infection needs resuturing	22(21.3)
	Respiratory infection	17(16.5)
	PTE/DVT	4(3.8)
III	Re-exploration due anastamotic leak	15(14.5)
	Burst abdomen/resuturing	3(2.9)
	UTI/sepsis	2(1.9)
	SMV thrombosis	1(0.9)
IV	Myocardial infarction	5(4.8)
	Bowel gangrene	1(0.9)
	Seizure	2(1.9)
	Sepsis and hypotension	1(0.9)
V	Severe sepsis	7(6.7)
	ARDS	2(1.9)
	Multivisceral metastasis	1(0.9)

Table 3 Patient demographic and in-hospital complication rates

Variables in consideration	No. of patients $n = 322$	No. of complication (n = 103)
Age		
< 60	165 (51.2%)	50 (48.5%)
61–70	104 (32.3%)	36 (35.0%)
71–80	38 (11.8%)	11 (10.7%)
> 80	15 (4.7%)	5 (4.9%)
Sex		
Male	197 (61.2%)	65 (63.1%)
Female	125 (38.8%)	37 (35.9%)
PSS		
6 to 10	273 (84.8%)	81 (78.6%)
11 to 15	49 (15.2%)	22 (21.4%)
Operative severity scoring		
Intermediate	27 (8.4%)	8 (7.8%)
Major	228 (70.8%)	61 (59.2%)
Complex major	67 (20.8%)	34 (33.0%)
Peritoneal soiling		
None	275 (85.4%)	80 (77.7%)
Local pus	6 (1.9%)	3 (2.9%)
Faceal contamination	41 (12.7%)	20 (19.4%)
Operative urgency		
Elective	280 (87%)	81 (78.6%)
Urgent	34 (10.6%)	18 (17.5%)
Emergency	8 (2.5%)	4 (3.9%)
Cancer staging		
Dukes A, B	162 (50.3%)	43 (41.7%)
Dukes C	120 (37.3%)	42 (40.8%)
Dukes D	40 (12.4%)	18 (17.5%)

with outcome. Since wound infection and its sequelae occupy a considerable share (37%) of complications in our study, the role of physiological factors influencing the result may be less significant compared to operative severity score where later affects the outcome in terms of surgical site soft tissue infection [20]. Next common complications were respiratory (16.5%) and anastamotic leak (14.5%). Regarding mortality, CR-POSSUM observed and expected mortality ratio was 1

Table 4 Colorectal POSSUM scoring system

Score	1	2	3	4	8
Physiologic score					
Age (yr)	≤ 60		61–70	71–80	≥ 81
Cardiac failure	None or mild	Moderate	Severe		
Pulse (beats/min)	40–100	101–120	> 120 or < 40		
Systolic BP (mmHg)	100–170	> 170 or 90–99	< 90		
Urea (mmol/l)	≤ 10	10.1–15	> 15		
Haemoglobin (g/dl)	13–16	10–12.9 or 16.1–18	< 10 or > 18		
Operative severity score					
Operative severity	Minor		Intermediate	Major	Complex major
Peritoneal soiling	None or serous	Local pus	Free pus/feces		
Operative urgency	Elective		Urgent		Emergency
Cancer staging (dukes)	None or dukes A, B	Dukes C	Dukes D		

and area under ROC curve was 80% showing good model discrimination. But with low mortality rate [$n = 10$ (9.7%)] in our study, the validity of it may not be reliable.

Among the various risk factors, age and cancer staging are considered non-modifiable risk factors. The rest are modifiable risk factors and give the extent of scope to which the complications can be prevented by addressing such factors. But once patients present to tertiary centres in emergency or semi-emergency conditions, most of the risk factors will be present. Tackling such elements will be time-consuming and lead to a further chance of increasing complication [21]. Surgical site-related complications top in our study (37%) and elsewhere, as well [22]. They have an adverse impact in terms of long-term survival [23]. Since operative factors play a significant role in outcome in terms of wound infection as per our study, measures that reduce the chance of acquiring surgical site infections with high level of evidence are appropriate and timely administration of antibiotic, discontinuation of prophylactic antibiotic within 24 h of surgery, optimization of blood glucose levels and avoiding spillage of bowel contents [21]. Similar to SSIs, respiratory complication and anastomotic leak leading to deep-seated infection also affect the long-term survival [24]. PSS and OSS both play a significant role in predicting the latter two complications here. Hence tackling both physiological and operative variable assumes an important role prevention of these two problems.

CR-POSSUM, which was originally developed to predict mortality, can also be used to predict morbidity. But, there are some limitations to our study. Both CR-POSSUM and POSSUM models for mortality and POSSUM model for predicting morbidity left many variables like preoperative nutrition level, operative duration, BMI and length of preoperative stay, all of which influence the eventual outcome. These were not considered while developing the equation [21]. But these scoring systems were developed 10 and 25 years ago respectively, and many new influencing variables were found in subsequent research.

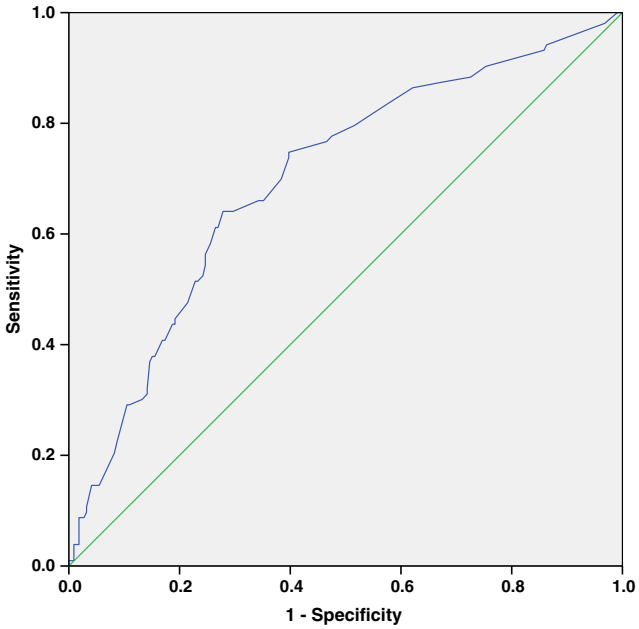


Fig. 2 Receiver operator characteristic curve (ROC) for the derived equation (Area under the curve is 71.6%)

Our results may be biased since there is a high proportion of SSI-related complication. We, therefore, validated the equation after excluding this group of patients. We excluded surgical site infection (SSI) in further analysis, to check whether physiological factors had a role in predicting non-SSI-related complications. We found that there was, indeed, a significant association ($P = 0.005$) between PSS and other non-SSI complications. The results were similar with few minor variations in predictive values. So, if a new model is developed considering the various new variables, then the predictive accuracy of such an equation can be close to reality.

To conclude, these predictive models once validated, across various centres in India, can be used as a measure of quality control in different institutions. Secondly, they can also be used to allocate resources and adequately trained professionals to deal with those patients whose expected morbidity and mortality are high.

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Compliance with Ethical Standards

Conflicting Interest The authors declare that they have no conflict of interest.

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References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008. Int J Cancer 127:2893–2917
2. Comparison of cancer incidence and patterns of all population-based cancer registries. Available from: http://www.ncrpindia.org/all_ncrp_reports/pbcr_report_2012_2014/all_content/pdf_Printed_Version/Chapter7_Printed.pdf. Assessed 26th Jan 2018
3. Sudarshan V, Hussain N, Gahine R, Mourya J (2013) Colorectal cancer in young adults in a tertiary care hospital in Chhattisgarh, Raipur. Indian J Cancer 50:337–340
4. Patil PS, Saklani A, Gambhire P, Mehta S, Engineer R, De'Souza A et al (2017) Colorectal cancer in India: an audit from a tertiary center in a low prevalence area. Indian J SurgOncol 8:484

5. MohdSuan MA, Tan WL, Soelar SA, Ismail I, Abu Hassan MR (2015) Intestinal obstruction: predictor of poor prognosis in colorectal carcinoma? Epidemiol Health 37:e2015017
6. Itani KM, Hawn MT (2013) Prevention of surgical site infection in colorectal surgery. Colorectal Cancer 2:467–475
7. Khuri SF, Henderson WG, DePalma RG, Mosca C, Healey NA, Kumbhani DJ (2005) Determinants of long-term survival after major surgery and the adverse effect of postoperative complications. Ann Surg 242:326–341
8. World Health Organisation global health expenditure database. Available from: https://data.worldbank.org/indicator/SH.XPD.TOTL.ZS. Accessed 7th Jan 2018
9. Longo WE, Virgo KS, Johnson FE, Oprian CA, Vernava AM, Wade TP, Phelan MA, Henderson WG, Daley J, Khuri SF (2000) Risk factors for morbidity and mortality after colectomy for colon cancer. Dis Colon Rectum 43:83–91
10. Prytherch DR, Whiteley MS, Higgins B, Weaver PC, Prout WG, Powell SJ (1998) POSSUM and portsmouth POSSUM for predicting mortality. Physiological and operative severity score for the enumeration of mortality and morbidity. Br J Surg 85:1217–1220
11. Copeland GP, Jones D, Walters M (1991) POSSUM: a scoring system for surgical audit. Br J Surg 78:355–360
12. Teeuwen PHE, Bremers AJA, Groenewoud JMM (2011) Predictive value of POSSUM and ACPGBI scoring in mortality and morbidity of colorectal resection: a case-control study. J Gastrointest Surg 15:294–303
13. Cengiz F, Kamer E, Zengel B, Uyar B, Tavusbay C, Unalp HR (2014) Comparison of different scoring systems in patients undergoing colorectal cancer surgery for predicting mortality and morbidity. Indian J Cancer 51:543–548
14. Baré M, Alcantara M, Gil M, Collera P, Pont M, Escobar A et al (2018) Validity of the CR-POSSUM model in surgery for colorectal cancer in Spain (CCR-CARESS study) and comparison with other models to predict operative mortality. BMC Health Serv Res 18:49
15. Schilling PL, Dimick JB, Birkmeyer JD (2008) Prioritizing quality improvement in general surgery. J Am CollSurg 207:698–704
16. Tekkis PP, Prytherch DR, Kocher HM, Senapati A, Poloniecki JD, Stamatakis JD, Windsor ACJ (2004) Development of a dedicated risk-adjustment scoring system for colorectal surgery (colorectal POSSUM). Br J Surg 91:1174–1182
17. Gallardo-Valverde JM, Calanas-Continente A, Baena-Delgado E, Zurera-Tendero L, Vazquez-Martinez C, Membrives-Obrero A, Muntane J, Arevalo-Jimenez E (2005) Obstruction in patients with colorectal cancer increases morbidity and mortality in association with altered nutritional status. Nutr Cancer 53:169–176
18. Dindo D, Demartines N, Clavien P-A (2004) Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 240:205–213
19. Petros AJ, Marshall JC, van Saene HK (1995) Should morbidity replace mortality as an endpoint for clinical trials in intensive care. Lancet 345:369–371
20. Mahajna A, Krausz M, Rosin D, Shabtai M, Hershko D, Ayalon A, Zmora O (2005) Bowel preparation is associated with spillage of bowel contents in colorectal surgery. Dis Colon Rectum 48:1626–1631
21. Pedroso-Fernandez Y, Aguirre-Jaime A, Ramos MJ, Hernández M, Cuervo M, Bravo A, Carrillo A (2016) Prediction of surgical site infection after colorectal surgery. Am J Infect Control 44:450–454
22. Young PY, Khadaroo RG (2014) Surgical site infections. Surg Clin North Am 94:1245–1264
23. Artinyan A, Orcutt ST, Anaya DA, Richardson P, Chen GJ, Berger DH (2015) Infectious postoperative complications decrease long-term survival in patients undergoing curative surgery for colorectal cancer: a study of 12,075 patients. Ann Surg 261:497–505
24. Tu R-H, Lin J-X, Li P, Xie J-W, Wang J-B, Lu J, Chen QY, Cao LL, Lin M, Zheng CH, Huang CM (2017) Prognostic significance of postoperative pneumonia after curative resection for patients with gastric cancer. Cancer Med 6:2757–2765

Predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates

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ABSTRACT

Background: Inadequate breastfeeding results in hypernatremia in otherwise healthy neonates. **Objective:** This study finds out the predisposing factors and clinical outcome of hypernatremia in breastfed late preterm and term neonates. **Materials and Methods:** A descriptive study was conducted in a tertiary hospital with retrospective data from July 2013 to June 2016. Babies with serum sodium ≥ 150 mEq/L were identified. Breastfed babies with little or no formula feeds, gestational age ≥ 34 weeks, and birth weight ≥ 2000 g were included. Babies with congenital anomalies, sepsis, prior parenteral fluid therapy and renal or endocrine diseases causing fluid and electrolyte disturbances were excluded. Total weight loss > 10% or daily weight loss > 5% from the birth weight were considered as excess weight loss. **Results:** A total of 201 babies were studied. Hypernatremia was significantly more common in babies of primiparous mothers ($p < 0.01$). There was no difference in incidence between vaginal born and cesarean-born babies. Positive correlation was seen between age at presentation, percentage of weight loss ($p = 0.001$) and serum sodium ($p = 0.01$). Significantly more cases were seen in summer season ($p < 0.01$). Excess weight loss, jaundice, fever, and dehydration were the common symptoms. Complications observed were acute kidney injury, seizures, intracranial bleed, apnea, and bradycardia. Babies with complications mostly presented after 7 days of age, had > 25% weight loss and had serum sodium ≥ 170 mEq/L. There was no death. Three babies had neurodevelopmental problems on follow-up. **Conclusion:** Breastfed babies of primiparous mothers and born in summer are predisposed to hypernatremia. Babies presenting at a later age had severe weight loss, severe hypernatremia, and complications.

Key words: *Breastfeeding, Hypernatremia, Primiparity, Summer season, Weight loss*

Exclusive breastfeeding for the first 6 months of life is recommended by the World Health Organization to optimize the growth and development of the infants [1]. Breast milk in addition to nutrients provides various immunological factors that help to reduce the diarrheal and respiratory illness in children [2]. Breastfeeding is an instinct behavior and many mother-baby dyads accomplish it successfully [3]. However, some babies may not receive enough milk from their mothers due to various reasons. If breastfeeding is inadequate, then malnutrition and hypernatremic dehydration can occur in the baby [4]. Moritz *et al*. have termed this condition as "breastfeeding associated hypernatremia" (BFAH) [5].

An increase in the incidence of BFAH in otherwise healthy neonates has been observed in the past few years [5-8]. The clinical features and complications seen in these babies have been reported in various studies [9]. It has been found that daily weighing of the babies helps in the early recognition of this illness [10,11]. Thus, the discrepancy in clinical features and complications reported in various studies were probably related to weight monitoring practices and timing of presentation of the cases [10,11]. This study was planned to find out the predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates in a hospital with regular weight monitoring practice.

MATERIALS AND METHODS

This descriptive study was done using retrospective data from July 2013 to June 2016. The study was conducted in an urban tertiary care medical college hospital with an average delivery rate of 2500 per year. Exclusive breastfeeding was promoted for all babies in the hospital. The mother-baby dyads were discharged at around 72 h after normal delivery and 120 h after cesarean delivery. As per hospital protocol, all babies were weighed daily. Excess weight loss was defined as total weight loss more than 10% from birth weight or weight loss per day more than 5% of birth weight [12]. Serum sodium level was checked for babies with excess weight loss. Hypernatremia was defined as serum sodium more than or equal to 150 mEq/L. Babies with hypernatremia were treated with liberal oral feeds, intravenous (IV) fluids, and other supportive therapies. Serum sodium <150 mEq/L was considered an end point of treatment.

The Institutional Ethics Committee approved the study. Babies admitted during the study period with hypernatremia were identified from the hospital database. Their case records were scrutinized and babies who fulfilled the following criteria were included in the study: (1) Breastfed babies with little or no formula supplementation, (2) gestational age more than or equal to 34 weeks, and (3) birth weight more than or equal to 2000 g. The babies who had major congenital anomalies, blood culture positive sepsis, received IV fluids within 48 h before diagnosis of hypernatremia and renal or endocrine disorders which may affect the fluid and electrolyte balance were excluded from the study. The following data were collected: Gestational age as completed weeks, age at diagnosis as completed days, birth weight, weight at diagnosis, maternal age, obstetric details, details about baby feeding, clinical features, treatment, complications, condition at discharge, and neurodevelopment at follow-up.

The total weight loss and weight loss per day were calculated as a percentage of the birth weight. Axillary temperature more than 38°C was considered as fever. The rate of decline in serum sodium was calculated using the sodium before and after treatment and the time taken to achieve it. Acute kidney injury (AKI) was diagnosed if serum creatinine increased by >0.3 mg/dl in 24 h or if it was >1.5 mg/dl with normal maternal renal functions [13]. Jaundice treated with phototherapy was considered as significant. At the time of discharge, babies were said to have improved if their serum sodium level was <150 mEq/L and was taking feeds well. At follow-up, their neurodevelopment was said to be normal if gross motor, fine motor, language, and social milestones were appropriate for age and had no other neurological problem.

SPSS software version 24 was used for analysis. Numerical data were presented as mean±standard deviation or median±interquartile range as appropriate. Categorical data were presented as percentages and analyzed using Chi-square test. Ordinal data were analyzed with Mann–Whitney U-test or Student's t-test. Correlation was done with Spearman rank correlation coefficient where appropriate. $p<0.05$ was considered as significant.

RESULTS

There were 213 babies with hypernatremia satisfying the inclusion criteria during the study period. 12 babies were excluded due to various reasons. The remaining 201 babies were considered for analysis (Fig. 1). During the study period, there were 7613 live births, with 4570 vaginal births, and 3043 cesarean births. There were 4463 primiparous and 3150 multiparous mothers. Hypernatremia was seen in 2.4% of vaginally born babies and 3.1% of cesarean-born babies. This difference was not statistically significant. 3.1% babies of primiparous mothers and 1.6% babies of multiparous mothers had hypernatremia, and this different was statistically significant (Chi-square 16.7, $P<0.01$). All babies were born of singleton pregnancy. Most of the (80%) mothers had no problem in breastfeeding their babies (Table 1).

Serum sodium had a negative correlation with mother's age ($r=-0.23$, $p=0.01$) and baby's gestational age ($r=-0.2$, $p=0.004$). No correlation was seen between serum sodium level and birth weight. A positive correlation was seen between age at presentation and total weight loss ($r=0.65$, $P=0.001$). Serum sodium at admission had a positive correlation with age at presentation ($r=0.704$, $p=0.01$) and percentage of total weight loss ($r=0.652$, $p=0.001$). More than 50% of the cases were seen during the summer season in this study. This seasonal variation in the incidence of cases was statistically significant (Chi-square-26.9, $p<0.01$) (Table 1).

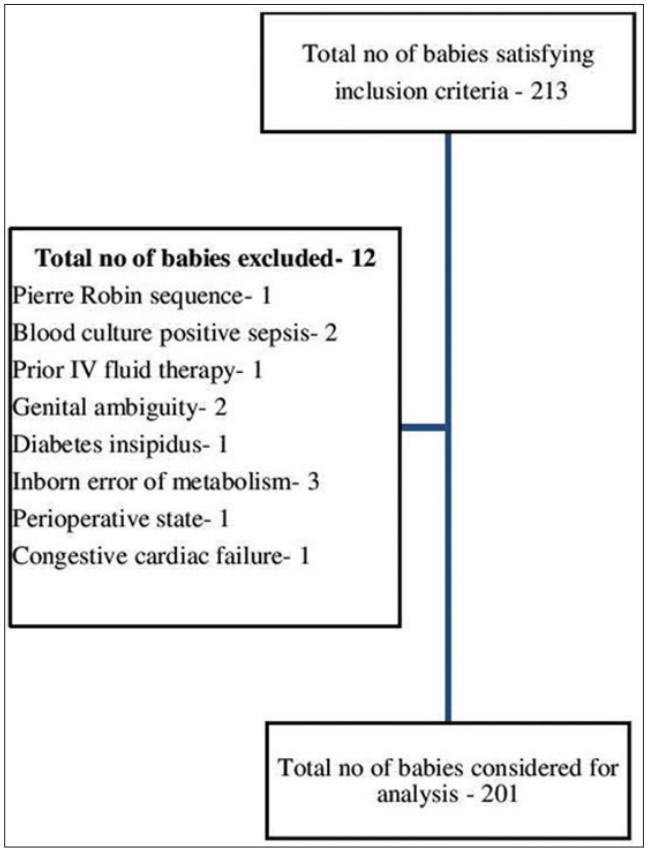


Figure 1: Flow of cases in the study

Significant jaundice was seen in 57.2% babies, but none required exchange transfusion. Phototherapy (median duration 24 h) before the onset of hypernatremia was given to 26 babies. Other common clinical findings observed were fever (33.8%) and dehydration (17.4%). No symptom other than excessive weight loss was seen in 21.4% of babies. Severe hypernatremia with serum sodium ≥170 mEq/L was seen in only five babies, and all other babies (98%) had moderate hypernatremia (serum sodium 150–169 mEq/L). About 89% of babies improved after treatment and got discharged. There was no death in the study population (Table 2). The babies who improved had a median birth weight of 3000 g (2800–3300), median admission weight of 2700 g (2460–2930), and median discharge weight of 2800 g (2540–3070).

Five babies (2.5%) had complications during the course of hypernatremia. One male baby with serum sodium of 151 mEq/L had apnea after vomiting of feeds and developed aspiration pneumonia. He improved with mechanical ventilation and antibiotics. All other four babies presented after 7 days of life

Table 1: Baseline characteristics of hypernatremic babies

Characteristics	Observation
Median maternal age (years) (n=183)*	26.9 (23-29)
Number of babies born to primiparous mothers	138/188 (73.4%)
Number of babies born to cesarean mothers	108 (53.7%)
Number of mothers reported problem in breastfeeding	42 (20.1%)
Male to female ratio	1.34:1
Median gestational age (weeks) (n=189)*	38 (37-39)
Median age at diagnosis (days)*	3 (2–18)
Number of babies presented on 2–3 days age	161 (80.1%)
Median birth weight (g)*	3080 (2810-3410)
Median weight at diagnosis (g)*	2710 (2470-3020)
Median daily weight loss (%)*	3.9 (3.3-4.7)
Median total weight loss from birth (%)*	11.3 (10-12.8)
Number of babies with 10–15% weight loss	139 (69.2%)
Season and number of babies admitted	
a. March to June (Summer)	103 (51.2%)
b. July to October	40 (19.9%)
c. November to February (Winter)	58 (28.9%)
Number of babies on exclusive breastfeeding	177 (88.1%)

*Median (IQR). IQR: Interquartile range

Table 2: Clinical features and outcome of hypernatremic babies

Characteristics	Observations
Median serum sodium at diagnosis (mEq/L)*	153 (151–155)
Serum sodium range and number of babies	
a. 150–159 mEq/L	184 (91.5%)
b. 160–169 mEq/L	12 (6%)
c. ≥170 mEq/L	5 (2.5%)
Mean time to normalize serum sodium	
a. 150–159 mEq/L (n=161)	17.8 h
b. 160–169 mEq/L (n=12)	26.6 h
c. 170–179 mEq/L (n=3)	48.8 h
d. ≥180 mEq/L (n=2)	71.3 h
Outcome	
a. Improved	178 (88.6%)
b. Left against medical advice	23 (11.4%)
Neurodevelopment problem at follow-up	3/154 (1.9%)

*Median (IQR). IQR: Interquartile range

with weight loss more than 25% of birth weight and severe hypernatremia (serum sodium ≥170 mEq/L). Complications observed were AKI (4), seizures (2), apnea (2), intracranial hemorrhage (1), and bradycardia (1). Peritoneal dialysis was done for one baby with AKI. No baby had peripheral or cerebral vessel thrombosis. Neurosonogram done for one baby with seizures showed increased white matter echogenicity. Another baby with seizures had normal neurosonogram. The treatment and outcome details of babies with severe hypernatremia are given in Table 3.

Follow-up details were available in 154 cases (76.6%) for a median duration of 1.5 months (0.5–11 months). Three babies had abnormal neurodevelopment. One female baby with serum sodium 150 mEq/L had a prolonged period of jaundice requiring phototherapy for 9 days. At follow-up, she had a global developmental delay with a seizure disorder. One male baby with serum sodium 150 mEq/L was found to have hyperactivity and aggressive behavior at 3 years. Neuroimaging was not available for these two babies. Another female baby had severe hypernatremia with AKI, seizures and normal neurosonogram in the neonatal period; and on follow-up at 1 year was found to have left hemiparesis and magnetic resonance imaging of the brain showed chronic infarct in right front parietal region and white matter changes in subcortical and deep cerebral white matter.

DISCUSSION

In this study, we have found BFAH to be more common among babies of primiparous mothers. Although breastfeeding is an instinct behavior, mothers have to learn the technique and need social support to successfully breastfeed their babies [3]. Systematic review on BFAH has found cesarean delivery, primiparity and absent previous breastfeeding experience as risk factors for BFAH [9]. However, there was no difference between cesarean babies and vaginally born babies in this study. Similarly, two studies had found no significant difference in mode of delivery among hypernatremic babies compared to control group [5,14].

It is stated that the parents of hypernatremic babies have poor recognition of their baby's illness [7,15]. About 80% of our baby's mothers have not felt any problem with their baby's feeding behavior. Excess weight loss gave a clue to identify these cases early. Thus, majority of our cases were identified at an early age, had less severe weight loss and less severe hypernatremia. Many studies report that babies presenting at a later age have higher weight loss and more severe hypernatremia [16,17]. Hence, daily weighing of babies along with appropriate lactation support during the first 4–5 days of life has been suggested for early detection of BFAH [10,11,18]. The seasonal variation in the incidence of hypernatremia was reported in some studies [12,19-21]. This was attributed to excess insensible water loss due to the hot climate in summer and overuse of room heaters in winter [9,21]. In this study, we have found significantly more number of cases during the summer season. This seasonal variation may be related to hot environmental temperature during summer seen in Tamil Nadu state of India where the study was done.

At admission, jaundice, fever, and dehydration were common symptoms in our study population. Lavagno *et al*. through systematic review have found jaundice, poor feeding, and dehydration each seen in about 45% of all reported cases [9]. Yang *et al*. have showed that percentage of weight loss within first 3 days of life has a positive correlation with jaundice at 72 h after birth in healthy term breastfed babies [22]. The hypernatremia and the resultant hyperosmolar state may damage the blood–brain barrier and facilitates bilirubin entry into nervous tissues at

Table 3: Clinical features and outcome of babies with severe hypernatremia

Gestational age (weeks)	Birth weight (g)	Total weight loss (%)	Day of diagnosis	Maximum Sr. sodium (mEq/L)	Complications	Treatment	Time to correct Sr. sodium (h)	Outcome at follow up
40	2700	25.9	12	182	Seizures, AKI, apnea, bradycardia	½ DNS+oral feeds; peritoneal dialysis; anticonvulsant	57	Normal
40	3630	14.3	4	173	Nil	½ DNS	43	Normal
37	2510	37.5	18	174	AKI, cerebral hemorrhage	NS bolus, ½ DNS	69.75	Normal
36	3060	27.5	8	173	AKI	½ DNS+oral feeds	33.5	Normal
Term	2700	24.1	17	186	AKI, seizures	NS bolus, DNS, ½ DNS; anticonvulsants	85.5	Left hemiparesis

AKI: Acute kidney injury

lower serum bilirubin level [5,23]. This has to be considered in one baby with neurodevelopmental impairment in our study who had hypernatremia and prolonged jaundice. About 20% of our babies had no symptoms at admission except for excess weight loss. Similarly Uras *et al*. and Korgali *et al*. have found that 14% and 2.4% of their hypernatremic babies were asymptomatic at admission, respectively [24,25].

Four out of five babies with severe hypernatremia had neurological and/or renal complications. Similarly, Ahmed *et al*. have found that babies with AKI presented at a later age, had higher weight loss and higher serum sodium [26]. However, less number of babies in this study had complications compared other studies [5,8,9,20,25-27]. This may be due to earlier age at presentation and a lesser degree of hypernatremia seen in the large proportion of babies in our study. There was no death in our study population and on follow-up only three babies had a neurodevelopmental problem. Mortality rate of 1-5% has been reported by various authors [8,26-28]. Koklu *et al*. have reported neurodevelopmental impairment in 47.8% cases [27]. Similarly, a high rate of neurodevelopmental impairment is also reported by Bolat *et al*. and Ergenekon *et al*. [29,30]. This emphasizes the need for formal neurodevelopmental follow-up of hypernatremic babies.

In this study, we have reported the correlation of serum sodium level with various other clinical parameters in babies with BFAH. Our study has the following limitations due to retrospective nature of the study: Some data were incomplete and long-term formal neurodevelopmental assessment was not done. Our study was hospital based, and many babies had a less severe degree of hypernatremia with fewer complications. A larger scale prospective study with neurodevelopmental follow-up is needed to have a clear understanding of the risk factors, complications and long-term effects of BFAH.

CONCLUSION

Breastfed late preterm and term babies born to primiparous mothers and born in summer season have predisposition to develop hypernatremia. These babies commonly presented with excess weight loss, jaundice, fever, and dehydration. Babies presenting beyond 7 days of age and with more than 25% weight loss are likely to have severe hypernatremia. Neurological complications and AKI were common complications observed with severe hypernatremia. These babies are at risk of neurodevelopmental impairment. Prospective study with long-term follow-up is needed.

REFERENCES

1. Kramer MS, Kakuma R. The Optimal Duration of Exclusive Breastfeeding-A Systematic Review. Geneva: World Health Organization; 2002.
2. Eidelman AI, Schanler RJ. Breastfeeding and the use of human milk. AAP policy statement. Pediatrics 2012;129:e827-41.
3. Volk AA. Human breastfeeding is not automatic: Why that's so and what it means for human evolution. J Soc Evol Cult Psychol 2009;3:305-14.
4. Livingstone VH, Willis CE, Abdel-Wareth LO, Thiessen P, Lockitch G. Neonatal hypernatremic dehydration associated with breast-feeding malnutrition: A retrospective survey. Can Med Assoc J 2000;162:647-52.
5. Moritz ML, Manole MD, Bogen DL, Ayus JC. Breastfeeding-associated hypernatremia: Are we missing the diagnosis? Pediatrics 2005;116:e343-47.
6. Laing IA, Wong CM. Hypernatraemia in the first few days: is the incidence rising? Arch Dis Child Fetal Neonatal Ed 2002;87:F158-62.
7. Cooper WO, Atherton HD, Kahana M, Kotagal UR. Increased incidence of severe breastfeeding malnutrition and hypernatremia in a metropolitan area. Pediatrics 1995;96:957-60.
8. Trotman H, Lord C, Barton M, Antoine M. Hypernatremic dehydration in Jamaican breastfed neonates: A 12-year review in a baby-friendly hospital. Ann Trop Paediatr 2004;24:295-300.
9. Lavagno C, Camozzi P, Renzi S, Lava SA, Simonetti GD, Bianchetti MG, *et al.* Breastfeeding-associated hypernatremia: A systematic review of the literature. J Hum Lact 2016;32:67-74.
10. Iyer NP, Srinivasan R, Evans K, Ward L, Cheung WY, Matthes JW. Impact of an early weighing policy on neonatal hypernatraemic dehydration and breast feeding. Arch Dis Child 2008;93:297-9.
11. Konetzny G, Bucher HU, Arlettaz R. Prevention of hypernatraemic dehydration in breastfed newborn infants by daily weighing. Eur J Pediatr 2009;168:815-8.
12. Bhat SR, Lewis P, David A, Liza SM. Dehydration and hypernatremia in breast-fed term healthy neonates. Indian J Pediatr 2006;73:39-41.
13. Subramanian S, Agarwal R, Deorari A, Paul V, Bagga A. Acute renal failure in neonates. Indian J Pediatr 2008;75:385-91.
14. Boskabadi H, Maamouri G, Ebrahimi M, Ghayour-Mobarhan M, Esmaeily H, Sahebkar A, *et al.* Neonatal hypernatremia and dehydration in infants receiving inadequate breastfeeding. Asia Pac J Clin Nutr 2010;19:301-7.

15. Oddie S, Richmond S, Coulthard M. Hypernatraemic dehydration and breast feeding: a population study. Arch Dis Child 2001;85:318-20.
16. Oddie SJ, Craven V, Deakin K, Westman J, Scally A. Severe neonatal hypernatremia: A population based study. Arch Dis Child Fetal Neonatal Ed 2013;98:F384-7.
17. Dommelen PV, Boer S, Unal S, Wouwe JP. Charts for weight loss to detect hypernatremic dehydration and prevent formula supplementation. Birth 2014;41:153-9.
18. Neifert MR. Prevention of breastfeeding tragedies. Pediatr Clin North Am 2001;48:273-97.
19. Jain S, Basu S. Hypernatremic dehydration in term and near-term neonates. Indian J Pediatr 2010;77:461.
20. Gomes SM, Fernandes A, Ramos H, Fernandes E, Santos M, Nascimento O, *et al*. Breastfeeding-associated hypernatremic dehydration. Einstein 2009;7:206-10.
21. Basiratnia M, Pishva N, Dehghani A. Prevalence of breastfeeding associated hypernatremia among hospitalized hyperbilirubinemia neonates. Iran J Neonatol 2014;5:12-6.
22. Yang WC, Zhao LL, Li YC, Chen CH, Chang YJ, Fu YC, *et al.* Bodyweight loss in predicting neonatal hyperbilirubinemia 72 hours after birth in term newborn infants. BMC Pediatr 2013;13:145.
23. Wennberg RP, Johansson BB, Folbergrová J, Siesjö BK. Bilirubin-induced changes in brain energy metabolism after osmotic opening of the blood-brain barrier. Pediatr Res 1991;30:473-8.
24. Uras N, Karadag A, Dogan G, Tonbul A, Tatli MM. Moderate hypernatremic dehydration in newborn infants: retrospective evaluation of 64 cases. J Matern Fetal Neonatal Med 2007;20:449-52.
25. Korgali EU, Cihan MK, Oguzalp T, Sahinbas A, Ekici M. Hypernatremic dehydration in breastfed term infants: Retrospective evaluation of 159 cases. Breastfeed Med 2017;12:5-11.
26. Ahmed A, Iqbal J, Ahmad I, Charoo BA, Ahmad QI, Ahmad SM. Complications due to breast-feeding associated hypernatremic dehydration. J Clin Neonatol 2014;3:153-7.
27. Koklu E, Gunes T, Ozturk MA, Kose M, Kurtoglu S, Yuksel F. A review of 116 cases of breastfeeding-associated hypernatremia in rural area of central Turkey. J Trop Pediatr 2007;53:347-50.
28. Unal S, Arhan E, Kara N, Uncu N, Alienfendioglu D. Breast-feeding-associated hypernatremia: Retrospective analysis of 169 term newborns. Pediatr Int 2008;50:29-34.
29. Bolat F, Oflaz MB, Güven AS, Özdemir G, Alaygut D, Dogan MT, *et al.* What is the safe approach for neonatal hypernatremic dehydration? A retrospective study from a neonatal intensive care unit. Pediatr Emerg Care 2013;29:808-13.
30. Ergenekon E, Unal S, Gücüyener K, Soysal SE, Koç E, Okumus N, *et al.* Hypernatremic dehydration in the newborn period and long-term follow up. Pediatr Int 2007;49:19-23.

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Prevalence and the factors influencing soil-transmitted helminths among school age children (5–14 years age) in a rural area of Coimbatore district

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Abstract

Context: Highest intensity of soil-transmitted helminthiasis (STH) is seen among school age children. **Aims**: The aim of this study is to find out the prevalence and factors associated with soil-transmitted helminthic infection among school age children (5–14 years) in a rural area of Coimbatore district. **Settings and Design:** The study was conducted in the field practice area of the Rural Health Training Centre (RHTC) Vedapatti, Coimbatore. RHTC caters to a total population of 23,841 distributed in 14 villages. After getting ethical clearance, five of the 14 villages of Vedapatti were selected by the cluster sampling method. Totally, 819 participated in the survey conducted between November 2015 and July 2016 in the field practice area. **Subjects and Methods:** Structured questionnaire was used to collect the information. Consent from parents and assent from child were obtained. Totally, 610 gave one adequate stool sample. Early morning samples were collected and transported to the laboratory within four hours. Formal ether concentration method was performed, and examination was done. **Statistical Analysis Used:** Data analysis was performed with the SPSS version 19 software. The prevalence is expressed in percentage with 95% confidence interval (CI). Univariate and multivariate analyses were performed. Strength of association was expressed in terms of odds ratio (OR) and adjusted OR with 95% CI. $P < 0.05$ was considered as statistically significant. **Results:** The prevalence of STH was 7.70% (95% CI: 5.58–9.82). *Ascaris lumbricoides* was highly prevalent 6.9% (4.89%–8.91%) followed by Hook worm 0.7% (0.04%–1.36%), and *Trichuris trichiura* 0.2% (0.15%–0.55%). Mulitivariate logistic regression analysis showed that pucca houses offered protection against STH. **Conclusions:** The prevalence of STH in a rural area of Coimbatore is 7.7% (95% CI: 5.58–9.82), and is continuing as a public health problem.

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Full Text

Introduction

The soil-transmitted helminthes causing major illness among human beings include Ascaris lumbricoides, Trichuris trichiura, Necator americanus, and Ancylostoma duodenale.[1] STH are considered neglected tropical diseases.[2] STH affects more than one fourth of the world's population, and it is reported more in tropical and subtropical areas.[3] The greatest number of cases are occurring in sub-Saharan Africa, the Americas, China, and East Asia.[4] In India, the reported prevalence of STH infection can be striked out varied from 7.8% to 92.6%.[5],[6],[7],[8],[9] The reported prevalence varies according to the place and time of the study conducted.

The highest intensity of soil-transmitted helminthes occur in school age children.[10] Children do not fully understand the connections between illness and behavior.[11] It has been demonstrated that children often than adults tend to go barefoot, resulting in more contact with soil, and fail to use sanitary facilities even when they are present.[12] STH infections are considered an important cause of sickness absenteeism. They are accounting for loss of 12.3% and 11.4% of disability adjusted life years in girls and boys, respectively.[13] Over 270 million preschool-age children, and over 600 million school-age children live in areas where sanitation is poor and these parasites are intensively transmitted.[14] In India, more than 241 million children are at the risk of parasitic worm infections. Ministry of Health and Family Welfare, Government of India launched the National Deworming Day (NDD)[15] on Feb 10, 2015. NDD aims to de-worm all children between the ages of 1–19 years. The tablets were supplied to children through government, government-aided and private schools, and Anganwadi centers.

When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent re-infection, a long-term impact can be achieved. Epidemiological studies are needed to identify the risk factors which may represent critical control points. There is no recently published article on the prevalence of soil-transmitted helminthes in Coimbatore, where open-air defecation is a common practice. As per the 2011 census, 46% of the households in Coimbatore district lack toilets. Hence, this cross-sectional study was proposed and conducted with the objective of finding the prevalence and risk factors associated with soil-transmitted helminth infections in school age children (5–14 years) in a rural area of Coimbatore district.

Subjects and Methods

The study was conducted in the field practice area of the Rural Health Training Center (RHTC) Vedapatti, Coimbatore. RHTC caters to a total population of 23,841 distributed in 14 villages.

of Thondamuthur and Madukarai block of Coimbatore district. The total number of school-aged children (aged 5–14 years) is 3266.

Ethical clearance was obtained from Institutional Human Ethics Committee, in 2014. The sample size was calculated to be 819 assuming 34.5% as prevalence, relative precision of 15%, design effect of 2, and nonresponse rate of 20%.[16] Five out of 14 villages were selected by the cluster sampling method. House-to-house survey was conducted in the five villages during November 2015–July 2016. All school-age children (aged 5–14 years) who were permanent residents of Vedapatti field practice area of RHTC were included in the study. Children who were not available on three visits within a week time were excluded from the study.

At the home setting, after getting consent from the parent and assent from the child any one of the parents of the child was interviewed in the evening hours of the day. A structured questionnaire was used to collect the information on demographic, socioeconomic, and environmental information such as availability of type of drinking water and availability of functioning sanitary toilet. Few questions were asked regarding the knowledge and attitude of the parents on STH. Respondent was asked to give comment on the personal hygiene practices of child such as washing hands with soap before taking food and after defecation, eating fallen foods on the floor, wearing foot wear outside the house and intake of de-worming tablets in the past 3 months. Observations were also made regarding the personal hygiene of the children related to STH such as clipped nails. Knowledge and attitude score was generated from the correct responses for the knowledge and attitude questions. The correct responses were given a score of one, and maximum score one could get was nine. Score five and above was considered as adequate knowledge and attitude score.

At the end of survey, a screw-capped plastic container with the identification of the child was given to collect the stool sample of the child on the next morning. Instructions regarding stool collection were given. The stool samples were collected and transported to microbiology laboratory within 4 h of sample collection. Stool samples were examined by saline wet mount and iodine wet mount.[17] Iodine preparation was done for the better visualization of morphological details of ova. All samples were re-examined after concentrating with WHO recommended.[18] Formalin-ether sedimentation method.[19] To reduce the human error, duplicate slides (both direct and after concentration method) were prepared from each sample and the slides were read by two different microbiologists and the opinion of chief microbiologist was considered as final.

Analysis

Data entry was made in the Microsoft Excel software in codes and analysis was done with SPSS-19 (IBM, Armonk, New York ,United States) computer package. The presence of ova of A. lumbricoides, Trichuris trichiura, N. americanus, and A. duodenale was considered as the dependent variable. The prevalence is expressed in percentage with 95% confidence interval (CI). Univariate analysis and logistic regression were performed to find the associations between the independent variables and dependent variables. Strength of association was expressed in terms of odds ratio (OR) and adjusted OR with 95% CI and $P < 0.05$ was considered as statistically significant.

Results

The results of this study are based on 610 children, who gave one adequate stool sample. Totally, 819 children participated in the survey; hence, the nonresponse rate was calculated as 25.52.

The distribution of the study population and their risk factors is shown in [Table 1] and [Table 2]. The mean age of the study population was 8.92 ± 2.68. Among them, children below 10 years (58.5%), female participants (52.1%), belonged to families with members four and above (54.92%), children living in kutcha and semi-pucca houses(80%), and children belonged to low socioeconomic status (52.3%) Class IV and V according to the modified Prasad classification were found in majority. Illiteracy was found among 20.2% of mothers. Open air defecation was found among 33.4% of the children. Regarding the toilet usage, only 50.7% of the children were using the toilets in the home. Around 66% did not wash their hands with soap before taking food. Moreover, 44.6% did not wash their hands with soap after defecation. Around 63% children regularly used foot wear and around 70% of the children had trimmed nail during the visit. Nearly half of the parents of children had adequate knowledge on STH. Only 59% reported intake of de-worming tablets in the past 3 months.{Table 1}{Table 2}

The prevalence of STH among 610 children who had given one adequate stool sample and whose parents participated in survey was 7.70% (95% CI: 5.58–9.82). Among all STH, A. lumbricoides was highly prevalent 6.9% (95% CI: 4.89%–8.91%) followed by Hook worm 0.7% (0.04%–1.36%), and Trichuris trichura 0.2% (0.15%–0.55%).

There was no significant association found between STH and factors influencing STH by univariate analysis. However, when all independent variables were subjected to multivariate logistic regression analysis, only pucca houses offered protection OR: 0.34(95% CI: [0.10–0.91]) against STH.

Discussion

Relatively high nonresponse rate (25.52%) was noted in this study. It is also evident from a similar study by Shubha and Fatima[20] that surveys involving bio samples shows high nonresponse rate (30.9%) when compared to simple questionnaire-based surveys.

The prevalence of STH in the study population is 7.7% (95% CI 5.58–9.82%). The results of the current study were less than other studies conducted in the nearby areas.

The explanations for the low prevalence are more number of risk group (29.13%) were found among the nonresponders compared to younger children (22.72%, P = 0.02). The study results were based on single stool sample, modified formol-ether concentration (FEC) method with low sensitivity (63.1%)[21] which could have estimated the low prevalence. The other probable explanations are Mass Drug Administration (MDA) under NDD[15] might have changed the scenario.[5],[6],[7],[8],[9] However, there are no sufficient data from to compare prevalence before de-worming in this area to support this statement. Coimbatore first round of NDD was just completed and second round was on-going at the time of the survey (2016-Feb). Kattula et al.[8] also explains the reason for the low prevalence of STH (7.8%) in Vellore area is due to Albendazole supplied in MDA along with diethylcarbamazine since 2004 Deworming using albendazole rapidly declines the overall prevalence of STH, and this is evident from Mani et al.[22] study on efficacy of albendazole in Tamil Nadu. However, there are other studies reported with a higher prevalence in the nearby areas. Kaliappan et al.[23] explains the reason for the increased prevalence (39%) in Thiruvanamalai District in the year 2013 could be due to five stool samples collected among tribal population where open-air defecation and bare foot walking were common practice. Ragunathan et al.[16] explains the reason for higher prevalence (34.56%) reported in Pudhucherry, a coastal area using FEC method in year 2006 is due to poor hygienic practices of the participants.

This study showed the most common soil-transmitted helminth as A. lumbricoides with the prevalence of 6.9% (95% CI: 4.89%–8.91%) followed by Hook worm 0.7% (95% CI: 0.04%–1.36%) and T. trichura 0.2% (95% CI: 0.15%–0.55%). In general studies showed that A. lumbricoides and T. trichura were more common among urban children and hookworm infestation among rural children.[24]

Regarding the factors influencing STH pucca houses offered protection OR: 0.34(95% CI: [0.10–0.91]) against STH, by multivariate analysis. A cross-sectional study by Kattula et al [8].showed that children living in Hut/Kutcha houses are at higher risk for STH.

Kattula et al.[8] also showed that children of more than 10 years of age, living in households without toilet, open air defecation, unhygienic practices like keeping untrimmed nails are important risk factors for STH. Kattula et al.[8] showed that the consumption of deworming tablets offered protection. However, there was no significant association noted in this study. This is a cross-sectional study; hence, the cause-effect relationship cannot be determined and is the major limitation of this study. Moreover, the other limitations are as follows: the study results were based on single stool sample which could have estimated the low prevalence. The newer techniques with higher sensitivity would have given higher prevalence. Sample size was not adequate to comment on factors influencing STH.

Conclusion

The prevalence of STH in a rural area of Coimbatore is 7.7% (95% CI: 5.58–9.82), and is continuing as a public health problem. Open-air defecation is a biggest concern (33.4%), hence, more private, public toilets should be built in this area. The children should be trained to use toilet facilities either private or public. It is very important to raise the awareness about these infections; hence, parents of children with inadequate knowledge should be given health education. When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent reinfection, a long-term impact can be achieved.

The prevalence of STH in a rural area of Coimbatore is 7.7% (95% CI: 5.58–9.82), and is continuing as a public health problem. Open-air defecation is a biggest concern (33.4%), hence, more private, public toilets should be built in this area. The children should be trained to use toilet facilities either private or public. It is very important to raise the awareness about these infections; hence, parents of children with inadequate knowledge should be given health education. When regular deworming is accompanied by appropriate sanitation and hygiene activities designed to prevent reinfection, a long-term impact can be achieved.

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Conflicts of interest

There are no conflicts of interest.

References

1 World Health Organization. Focusing on STH: Towards an Integrated Approach for STH Control. Available from: http://www.who.int/intestinal_worms/en/http://whqlibdoc.who.int/publications/2012/9789241503129_eng.pdf?ua=1. [Last accessed on 2019 Feb 24].
2 Statement Regarding Soil Transmitted Helminths, by CDC. Available from: http://www.cdc.gov/globalhealth/ntd/. [Last accessed on 2019 Feb 24].
3 Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 2014;7:37.
4 WHO. Eliminating Soil-Transmitted Helminthiasis as a Public Health Problem in Children Soil-Transmitted Helminthiasis Progress Report 2001-2010. WHO; 2012.
5 Ramesh GN, Malla N, Raju GS, Sehgal R, Ganguly NK, Mahajan RC, *et al*. Epidemiological study of parasitic infestations in lower socio-economic group in Chandigarh (North India). Indian J Med Res 1991;93:47-50.
6 Singh P, Gupta ML, Thakur TS, Vaidya NK. Intestinal parasitism in Himachal Pradesh. Indian J Med Sci 1991;45:201-4, 200.
7 Singh S, Raju GV, Samantaray JC. Parasitic gut flora in a north Indian population with gastrointestinal symptoms. Trop Gastroenterol 1993;14:104-8.
8 Kattula D, Sarkar R, Ajjampur SS, Minz S, Levecke B, Kang G. Prevalence & risk factors for soil transmitted helminth infection among school children in south India. Indian J Med Res 2014;139:76-82.
9 Naish S, McCarthy J, Williams GM. Prevalence, intensity and risk factors for soil-transmitted helminth infection in a South Indian fishing village. Acta Trop 2004;91:177-87.
10 World Bank. The global burden of disease. Appendix B. In: World Development Report: Investing in Health-World Development Indicators. New York: World Bank and Oxford University Press; 1993. p. 213-25.
11 Richmond JB, Kotelchuck M. Personal health maintenance for children. West J Med 1984;141:816-23.
12 Abera B, Alem G, Yimer M, Herrador Z. Epidemiology of soil transmitted helminths, Schistosomamansoni and haematocrit values among schoolchildren in Ethiopia. J Infect Dev Ctries 2013;7:253-60.
13 Curtale F, Pezzotti P, Sharbini AL, Al Maadat H, Ingrosso P, Saad YS, *et al*. Knowledge, perceptions and behaviour of mothers toward intestinal helminths in Upper Egypt: Implications for control. Health Policy Plan 1998;13:423-32.
14 World Health Organisation. Control of Tropical Disease. Geneva: World Health Organisation; 1998. Available from: http://www.who.int/neglected_diseases/en/. [Last accessed on 2019 Feb 24].
15 Information Regarding National Deworming Day in India from NHM Website. Available from: http://nrhm.gov.in/nrhm-components/rmnch-a/child-health-immunization/national-deworming-day-august-2016.html. [Last accessed on 2019 Feb 24].
16 Ragunathan L, Kalivaradhan SK, Ramadass S, Nagaraj M, Ramesh K. Helminthic infections in school children in Puducherry, South India. J Microbiol Immunol Infect 2010;43:228-32.
17 World Health Organization. Manual of Basic Techniques for a Health Laboratory. 2nd ed.. Geneva: World Health Organization; 2003.
18 World Health Organization. Bench Aids for the Diagnosis of Intestinal Parasites. Geneva: World Health Organization; 1994. Available from: http://apps.who.int/iris/handle/10665/37323. [Last accessed on 2019 Feb 24].
19 Chatterjee KD. Text Book of Parasitology. 13th ed. Darya Ganj - New Delhi, India: CBS Publishers & Distributors Pvt Ltd; 2015.
20 Shubha D, Fatima F. A coprological survey for assessing intensity of parasitic infection in school children: Cross-sectional study. Trop Parasitol 2011;1:88-93.
21 Yimer M, Hailu T, Mulu W, Abera B. Evaluation performance of diagnostic methods of intestinal parasitosis in school age children in Ethiopia. BMC Res Notes 2015;8:820.
22 Mani TR, Rajendran R, Munirathinam A, Sunish IP, Abdullah S, Augustin DJ. Efficacy of co-administration of albendazole and diethylcarbamazine against geohelminthiases: A study from South India. Trop Med Int Health 2002;7:541-8.
23 Kaliappan SP, George S, Francis MR, Kattula D, Sarkar R, Minz S, *et al*. Prevalence and clustering of soil-transmitted helminth infections in a tribal area in southern India. Trop Med Int Health 2013;18:1452-62.
24 Edirisuriya C, Beard FH, Hendry AJ, Dey A, Gidding HF, Hueston L, *et al*. Australian rubella serosurvey 2012-2013: On track for elimination? Vaccine 2018;36:2794-8.

Table 2 Association of soil transmitted helminthiasis with risk factors by univariate and multivariate analysis

Table 1. Association of soil transmitted helminthiasis with socioeconomic and demographic factors by univariate and multivariate analysis

			No.	Unadjusted	Adjusted	
[illegible]	<5	24	229	1.52 (0.80-2.90)	1.45 (0.76-2.77)	0.24
	>5	22	284	1	1	
[illegible]	Male	24	268	1.15 (0.60-2.18)	1.07 (0.58-2.00)	0.82
	Female	23	245	1	1	
[illegible]	Less than 5	22	253	1.18 (0.56-2.05)	1.21 (0.64-2.27)	0.55
	[illegible]	25	313	1	1	
[illegible]	Rural	1	116	0.36 (0.04-1.03)	0.34 (0.13-0.91)	0.05
	Urban Semi-permanent Mud-to-Brick	23	448	1	1	
[illegible]	Illiterate	16	116	1.08 (0.46-2.29)	1.03 (0.43-2.30)	0.94
	Literate	32	450	1	1	
[illegible]	Low SES	27	74	1.14 (0.60-2.19)	1.10 (0.56-2.20)	0.78
	High SES	21	274	1	1	

[illegible]

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ORIGINAL RESEARCH ARTICLE

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Psychological well- being of women workers in a garment factory of South India-A cross sectional study

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ABSTRACT

Background: Working women are constantly facing the problems of role conflict or dual role. Due to role conflict, there are reports of psychological distress, mental tension, anxiety, etc, which affect the psychological well being of working women. Due to outside work, they have psychological problems such as anxiety, stress, burn out, fatigue, etc. This way, the mental health status of working women is neglected and hence, the present study **aims** to assess the psychological wellbeing of women in an industrial setup. **Methods:** A cross sectional study was done among women workers of 18 years and above. A standardized questionnaire was used to collect data. The data was analyzed using SPSS version 24. A total of 136 participants completed the study. **Results:** Present study showed that majority of the workers (67%) had low level of psychological well-being. **Conclusion:** Women workers have low level of psychological well-being and further studies are needed to understand factors influencing it.

Key Words: Psychological well-being, Women workers, Garment industry.

INTRODUCTION

"The mind is everything. What you think you become." – Buddha

According to the World Health Organization (WHO), mental health includes "subjective well-being, perceived self-efficacy, autonomy, competence, inter-generational dependence, and self-actualization of one's intellectual and emotional potential, among others". WHO further states that the well-being of an individual is encompassed in the realization of their abilities, coping with normal stresses of life, productive work and contribution to their community. [1]

Psychological well-being is a very subjective term meaning contentment, satisfaction with all elements of life, self-actualization (a feeling of having achieved something with one's life), peace and happiness.[2]Psychological well-being is defined as the presence of psychological and social skills and abilities that contribute to optimal functioning in daily life. [1]

The concept of mental health is inextricably linked to that of psychological well-being. A comprehensive model developed by C. Ryff provides an integration of different viewpoints. It encompasses six inter-related psychological dimensions: positive evaluation of one's self (Self-Acceptance), a sense of continued growth and development (Personal Growth), the belief that life is purposeful and meaningful (Purpose in Life), the possession of quality relations with others (Positive Relations with Others), the capacity to manage effectively one's life (Environmental Mastery), and a sense of self-determination (Autonomy). Lack of psychological well-being has been found to be a risk factor for depression. [3]

Garment workers around the world, especially those involved in tailoring, are predominantly women. The Indian garment industry is dominated by women workers and 80% of them are young women between 21-25 years. [4]

The workplace is one of the key environments that affect our mental well-being and health. It is difficult to quantify the impact of work alone on personal identity, self-esteem and social recognition; most mental health professionals agree that the workplace environment can have a significant impact on an individual's mental well-being. [5]

Working women have many problems such as career related problems, social problems such as domestic violence, health problems such as psychological distress due to role conflict, mental stress, tension, anxiety, physical health problems such as Anemia, arthritis, etc.

As discussed above, due to outside work, they have psychological problems such as anxiety, stress, burn out, fatigue, etc. This could lead to neglect of mental health. Hence this study is proposed with the objectives of

assessing the psychological well-being of women workers in garment industries and to find out its association with socio demographic factors.

MATERIALS & METHODS

A cross sectional study was conducted in a selected garment industry in Tirupur district of Tamilnadu state. This factory is mainly involved in manufacture of garments for export. Majority of the workers are unmarried women.

Sample size: Based on 63.5% prevalence of moderate level of psychological well-being study done by K.S.Velmurugan, Dr.K.Maheswari, in Tiruchirappalli [6] sample size was calculated as 136.

Inclusion criteria: There were 200 women workers above 18 years in our study factory. Prestuctured and pre validated questionnaire was administered to 136 women workers who were selected by simple random sampling.

Study tool: Carl Ryff's 18 item psychological well-being questionnaire translated into local language was used capturing various dimensions: autonomy, environmental mastery, personal growth, positive relations and self-acceptance, purpose in life. These items were measured using Likert scales of 1 to 6, with 1 indicating strong disagree and 6 indicating strong agree. The maximum score for psychological well-being that could be obtained under each dimension is 18.The maximum score that could be obtained for overall psychological well-being is 128.

Data collection: Data collection was done using the above tool as self administered questionnaire. Data entry and analysis was done using Statistical Package for Social Sciences (SPSS) version 24. The data were analyzed by using mean, standard deviation and two-way analysis of variance (ANOVA) and p <0.05 was considered statistically significant.

Ethical Issues: Approval for the study was obtained from the Institutional Human Ethics Committee of PSG Institute of Medical Sciences and Research.

RESULTS

A total of 136 participants completed the survey. The study participants were in the age group of 18 to 25 years. The participants were representative of different sections of the factory with majority (45%) being tailors. The demographical details of the participants are shown in Table1.

Table 2 shows the mean scores of the participants under each dimension of psychological well-being. For all dimensions the mean score was 12 and below which indicates low psychological well-being. The findings indicated that workers scored high in personal growth (M= 12.02, sd= 4.05) and least in environmental mastery (M=10.10, sd=4.05).

Table 1: Demographic details of the participants

Demographic factors	Percentage
Age	
Below 20 years	58%
Above 20 years	42%
Education Qualification	
High school	48%
Hr.sec.school	40%
Diploma	4%
Graduate	8%
Work experience	
Below 1 year	57%
Above 1 year	43%
Type of family	
Nuclear family	73%
Joint family	27%
Residence	
Hostel	97%
Day scholar	3%

Table 2: Overall level of workers Psychological well-being

Indicators of Psychological well-being	Mean±SD
Autonomy	11.50±3.39
Positive relations	11.92±2.37
Self-acceptance	10.13±3.52
Environmental mastery	10.10±4.05
Purpose in life	11.66±2.24
Personal growth	12.02±4.05
Total score	67.36±13.20

Table 3: Association of various factors with psychological well-being

Socio economic Factors	Total score of Psychological well-being (Mean±SD)	p value
Age		
Below 20 years	67.69±12.74	0.728
Above 20 years	66.89±13.91	
Education Qualification		
High school	68.39±13.26	
Hr.sec.school	67.59±13.14	
Diploma	68.20±14.06	0.241
Graduate	59.63±11.84	
Work experience		
Below 1 year	67.02±12.26	0.733
Above 1 year	67.81±14.46	
Type of family		
Nuclear family	67.02±13.00	0.625
Joint family	68.27±13.84	

Table 3 shows the association of various demographic variables with psychological well-being. Age was categorized less than 20 and more than 20, also between various educational level and work experience of at least a

year and whether they belonged to nuclear or joint family. The findings indicated that there is no significant difference between demographic factors like age, education and years of education with psychological well-being.

DISCUSSION

The present study showed that overall psychological well-being of women garment workers was low (mean value 67.36). In garment industry women workers work for longer hours (10 hours). Also due to high production pressure, hourly targets are set for workers. Staying away from family and having long working hours could be reasons which need to be explored.

Our study findings revealed relatively higher score for personal growth compared to other dimensions. It indicates that women workers had more positive feelings towards their continued development and ready to experience new challenges in life. Similarly, a study done on 2015 showed that a high score in personal growth and positive relations subscales. [7]

The result of present study concurs with a similar study done in Perambalur which showed that women in higher age have low psychological well-being. In our study there is no significant difference between workers age & their psychological well-being. But a woman with higher age shows low psychological well-being. In the same study it was found that women with more work experience had higher psychological wellbeing. In our study though not significant the mean score was comparatively higher among women with more work experience. [6]

In our study we couldn't find out any reasons for poor psychological well-being. Instead, we say above mentioned reasons could be the reason for poor psychological well-being. The present study concurs with a similar study, done in Mysore where the mean score for psychological well-being was 193. [8]

Conclusion and way forward

From the study we conclude that psychological well-being is low among women workers. Management should develop policies for addressing mental health issues and creating access to counseling services.
Though quantitative analysis did not reveal statistical association between psychological well-being and demographic profile, further qualitative studies would help in better understanding the factors influencing psychological well-being.

REFERENCES

1. Mental health [internet].[Updated 2019 March 7]. Available from: https://en.wikipedia.org/wiki/Mental_health
2. Psychological well-being: Staff and organization support program [internet]. 2010 Dec. Available from:https://www.sosp.org.mt/2010/12/22/psychological-well-being/
3. Ryff CD, KeyesCLM. The structure of psychological well-being revisited. Journal of personality and social psychology 1995; 69:719.
4. Suhasini Singh. Fashionable and famous - at the garment worker's cost.Global Research Publisher 2009;Available from:https://www.globalresearch.ca/india-cheap-labor-garment-export-industry/13530
5. Mental health and work: Impact, issue and practice. Nations for mental health 2000; Available from: https://www.who.int/mental_health/media/en/712.pdf
6. Velmurugan KS, Dr.Maheswari K. A Study on Psychological Well-Being of Women Working in Southern Railways Tiruchirappalli. International Journal of Scientific Research and Management 2017; 5:7370-75.
7. Melissa Ludban, Priscilla N, Gitimu. Psychological well-being of college students. Undergraduate Research Journal for the Human Sciences 2015; 14.
8. Srimathi NL, Kiran Kumar SK. Psychological Well being of Employed Women across Different Organisations. Journal of the Indian Academy of Applied Psychology 2010; 36:89-95.
9. Appendix: Items on Well-Being Measures 2016; available from http://online.fliphtml5.com/ffch/avfx/
10. Madhuchandra MK, Srimathi NL.Psychological Well-being among Doctors and Nurses: A Comparative Study. The International Journal of Indian Psychology 2016;3:203-10
11. Alex M. Wood, Stephen Joseph. The absence of positive psychological (eudemonic) well-being as a risk factor for depression: A ten year cohort study. Journal of Affective Disorders 2010; 213–17
12. Deepthi Shanbhag, Bobby Joseph. Mental Health Status of Female Workers in Private Apparel Manufacturing Industry in Bangalore City, Karnataka, India. International Journal of Collaborative Research on Internal Medicine & Public Health 2012; 4:1893- 1900
13. Richa Aryan,Deepika Kathuria. Psychological Wellbeing at Workplace:-An Analytical Study on It Sector. International Journals of Advanced Research in Computer Science and Software Engineering 2017; 7:223-28

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Quick Response Code:

Research Priorities in Maternal, Newborn, & Child Health & Nutrition for India: An Indian Council of Medical Research-INCLEN Initiative

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In India, research prioritization in Maternal, Newborn, and Child Health and Nutrition (MNCHN) themes has traditionally involved only a handful of experts mostly from major cities. The Indian Council of Medical Research (ICMR)-INCLEN collaboration undertook a nationwide exercise engaging faculty from 256 institutions to identify top research priorities in the MNCHN themes for 2016-2025. The Child Health and Nutrition Research Initiative method of priority setting was adapted. The context of the exercise was defined by a National Steering Group (NSG) and guided by four Thematic Research Subcommittees. Research ideas were pooled from 498 experts located in different parts of India, iteratively consolidated into research options, scored by 893 experts against five pre-defined criteria (answerability, relevance, equity, investment and innovation) and weighed by a larger reference group. Ranked lists of priorities were generated for each of the four themes at national and three subnational (regional) levels [Empowered Action Group & North-Eastern States, Southern and Western States, & Northern States (including West Bengal)]. Research priorities differed between regions and from overall

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national priorities. Delivery domain of research which included implementation research constituted about 70 per cent of the top ten research options under all four themes. The results were endorsed in the NSG meeting. There was unanimity that the research priorities should be considered by different governmental and non-governmental agencies for investment with prioritization on implementation research and issues cutting across themes.

Key words Child health - Child Health & Nutrition Research Initiative - Indian Council of Medical Research - INCLEN - maternal health - Maternal, Newborn, and Child Health and Nutrition - newborn health - nutrition - research priority setting

National Steering Group[18]

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Nation-wide Network[18]

Names of participants in the Nation-wide Network are provided in Supplementary Table I (available on *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm6.pdf*) and also on *www.inclentrust.org*

Prioritization of research options in health is essential to plan for achieving efficient and impactful investment of limited resources against a large number of competing research options[1]. There is an increasing need and effort to set research priorities in health in a systematic way using a sound and transparent methodology and through engagement of the various key stakeholder constituencies[2,3]. Research priority setting (RPS) in India has traditionally been guided by a small group of experts, mostly identified from major metropolitan areas of the country. Subsequent to India's impressive yet inadequate improvement towards achieving Millennium Development Goals (MDGs) 1, 4 and 5, an expert group had convened on May 23, 2011 at the Indian Council of Medical Research (ICMR) Headquarters in New Delhi to discuss RPS. The participants observed that evidentiary gaps in the country impeded informed action in Maternal, Newborn, and Child Health and Nutrition (MNCHN) themes, and that an inclusive and transparent method should be adopted to decide the national research agenda. The agenda so developed should also identify areas for innovation and strategies to improve deliverability, efficiency, scalability and sustainability of existing interventions, avoid common blind spots in MNCHN research (*e.g*., still birth & neonatal health) and engage the multiple stakeholder constituencies including end users of any funded health research activity. This led to the formalization of the ICMR-INCLEN collaboration for undertaking the national RPS exercise for MNCHN using the Child Health and Nutrition Research Initiative (CHNRI) methodology. Since 2006-2007, the CHNRI priority setting methodology has been increasingly acknowledged as a flexible yet systematic priority-setting method for ranking competing research options using an objective, quantified and inclusive approach[2-7]. The method is opined to be effective at the national level where the results derived from inputs from national stakeholders can have a direct and prominent impact on the research investment policy[8]. As a major conceptual advancement, this method ranks the broadly defined competing research options that not only generate new knowledge but also synthesize evidence for efficient implementation of what is already known[9]. The ranking of these research options employs 'crowdsourcing' through the engagement of a wide range of stakeholder constituencies with due cognizance of their core expertise. The various dimensions of the research priorities are carefully examined by the experts using a predefined context and set of strictly defined scoring criteria. The collective optimism of the stakeholders, which can be considered as the 'wisdom of the crowd', helps in identifying research priorities through a democratic method of scoring, weighting and ranking of competing research options[1,5].

National Research Priority Setting (RPS) exercise for Maternal, Newborn, and Child Health and Nutrition (MNCHN)

The protocol was reviewed and approved by the Independent Institutional Ethics Committee of INCLEN, and the exercise was undertaken between 2012 and 2016. The context, in which the research priorities were identified in this exercise, are provided in Box 1.

The exercise was woven around four key structures, namely, the National Steering Group (NSG), the Thematic Research Subcommittees (RSCs) constituted for each of the four MNCHN themes (112 experts in India were identified based on active contribution to contemporary research), the Nation-wide Network for Crowdsourcing (experts of various disciplines related to MNCHN with due attention to regional representation

Box 1. Context of the Indian Council of Medical Research-International Clinical Epidemiology Network (ICMR-INCLEN) National Research Priority Setting Exercise for maternal, newborn, child health and nutrition

Purpose: Priority setting in maternal, newborn, and child health and nutrition for efficient and rewarding investment in research using a systematic, transparent, inclusive and consultative method.

Geography: India (National) and three regional levels *viz*., Empowered Action Group (EAG) & North-Eastern States, Southern & Western States, and Northern States & West Bengal.

Target population: Women of reproductive age (15-49 yr); pregnant women, newborns (0-28 days), under-five children (0-59 months) and children (up to the age of 18 yr).

Major areas of concern for research: Conditions that together contributed to at least 75% of the mortality and morbidity burden in Maternal, Newborn, Child Health and Nutrition in India during 2012-2013 as per the available evidence and expert opinion.

Time frame: 2016-2025.

Stakeholder constituencies (public and private sectors, health and non-health sectors): Researchers, professionals, public health functionaries, policy makers, communities and their leadership, civil society and donor agencies.

Translation and implementation context: Public and private health systems of India and their existing as well as future policies and programmes.

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from 256 institutions) and the Larger Reference Group (LRG) (84 members group) constituted with Central and State policy decision makers, politicians and bureaucrats from key ministries, MNCHN programme managers, eminent researchers and representatives from research funding organizations. The list of participants in the Nationwide Network is provided in Supplementary Table I (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm6.pdf*). [The list of all participants in the exercise with respective institutional affiliation is available on *www.inclentrust.org*].

The NSG for the exercise, co-chaired by the Secretary, Department of Health Research (DHR) and Director General (DG)-ICMR and the Executive Director of The INCLEN Trust International, was formed with key officials from concerned Ministries, namely, Ministry of Health & Family Welfare (MoHFW) (National Health Mission, Child Health, Maternal Health and Nutrition Divisions, Directorate General of Health Services and DHR-ICMR), Ministry of Women and Child Development [Integrated Child Development Services (ICDS), Food and Nutrition Board] and Ministry of Science and Technology (Department of Biotechnology, Department of Science & Technology). There was a wide range of invited subject experts, Central and State programme managers, national and international donors and multilateral agencies, who were members. Two NSG meetings were organized, one at the initiation of the exercise to ratify the context and protocol (April 18, 2013) and second, at the conclusion for reviewing and endorsing the findings (February 4, 2016).

The Thematic RSCs contributed to research ideas in the first round of crowdsourcing, helped in refining and consolidating the ideas into research options, finalizing the scoring criteria, participated in second round of crowdsourcing for scoring the research options and presented the results to the NSG for ratification.

The Nationwide Network of experts participated in the first and second round of crowdsourcing for pooling of research ideas and scoring the research options, respectively.

The LRG assigned relative weights to the five scoring criteria, namely answerability, relevance, equity, innovation and out-of-the-box thinking and investment on research.

The exercise was centrally coordinated by the RPS Management Team at the Executive Office of The INCLEN Trust International, New Delhi.

The RPS Management Team established a national network of 1066 experts from institutions across India during 2012-2013. During the same period, it completed an extensive review of literature (research, academic and policy documents) on burden of diseases of MNCHN in India and presented the summary to the NSG at its first meeting. The NSG decided that topmost causes collectively accounting for at least 75 per cent of mortality and morbidity burden (Areas of Concern) for each of the RPS themes so identified shall guide pooling of research ideas and subsequent framing of research options for priority setting. Between September and December 2013, 3498 research ideas were pooled from the nationwide network using custom-designed online software (1st round of crowdsourcing). The participation rate was 42.3 per cent [of the 1178 experts contacted (1066-national members and 112-RSC members), 498 experts contributed research ideas]. Several of these research ideas were narrations comprising more than one research idea spanning across themes, areas of concern and domains. With the help of RSC members (January 2014-February 2015), these were split and refined into 4003 research ideas that were further consolidated into 373 research options (Maternal: 122, Newborn: 56, Child: 101 and Nutrition: 94) (*http://inclentrust.org/inclen/?page_id=8666*). Five criteria (answerability, relevance, equity, innovation and out-of-the-box thinking and investment on research) were identified and defined for scoring the research options (March-June 2015) (Box 2). This was done through a comprehensive review of criteria that had been used in previous exercises at national and global levels and iterative discussion with RSC members and national and international CHNRI experts (University of Edinburgh, Johns Hopkins Bloomberg School of Public Health and the WHO).

Scoring of the research options was done independently by 893 experts (of the 1536 experts approached; Participation rate: 58.1%) using a customized online (*SurveyMonkey.com*) platform (July-October, 2015) (2nd round of crowdsourcing). The scores obtained by the research options were adjusted with criteria weights as assigned by the LRG (November, 2015-January, 2016) as per standard CHNRI data analysis practices[10]. The Average Expert Agreement (AEA) was also calculated for each research option score. The detailed methodology and process adopted for this priority setting has been published

Box 2. Criteria used for scoring the research options

Answerability: Can the research be done through ethical, transparent, well-designed, 'do-able' studies with the existing local and national capacities and or by strengthening the existing capacities through regional or global collaboration?

Relevance: Is it likely that the research would address a high burden condition and critical gap in knowledge?

Innovation and out-of-box thinking to resolve complex and refractory challenges: Does the new research have the potential for transformative change in the health system/ health care?

Equity: Is it likely that the research product will address the differences in health and nutrition that are systematically associated with social, cultural and economic hierarchies, ethnicity, gender, environment and geographic disadvantages, thereby reducing inequities?

Investment on research: Is it likely that the potential impact and benefits of the new knowledge on health/ nutrition will outweigh the considerations of investments on research?

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elsewhere[11]. The research options were categorized into four domains; description, discovery, delivery and development.

In addition to national priorities, the States and Union Territories in the country were grouped into three subnational regions to obtain regional priorities [Empowered Action Group (EAG) and North-Eastern States, States in Western and Southern India and States in Northern India (including West Bengal)] (Fig. 1).

The number of scorers ranged between 60 and 96 scorers across each region and theme. Therefore, four priority lists were obtained for each theme: one national and three regional priority lists. The NSG reviewed and discussed the ranked list of priorities and made focused observations on the patterns of research that had evolved as priorities and the possible interlinks between the priorities identified. It also discussed other potential priority research options that might not have made it to the topmost priority list. This helped enrich the structuring of the national research agenda and identify the way forward.

Outcome of the research priority setting (RPS) exercise in the four themes

About 43 per cent of all the research options scored pertained to the delivery domain and required implementation research methodology (Fig. 2). It was observed that in each of the four themes, a large



Fig. 1. Geographical context of the ICMR-INCLEN National Research Priority Setting Exercise for Maternal, Newborn, and Child Health and Nutrition (2016-2025) (Map courtesy: *www.mapsofindia.com* modified with permission).

proportion (70%) of the top ten prioritized issues were dealing with delivery domain of research and implementation of programmes. Amongst the top ten priorities identified at national level, delivery domain research options accounted for 80 per cent in maternal health, 70 per cent in newborn health, 60 per cent in child health and 70 per cent in nutrition themes. Significant differences were also observed between national and regional research priorities for all the four themes. The AEA was very high for the top ten priorities of research in the four themes both nationally and regionally. There were research options in the top ten regional priorities under various themes that did not figure in the national priority list. The Table enlists the top ten priorities identified under each theme by the exercise at the national level. Top ten priorities and their scores along with the national and region-specific ranks are provided in Supplementary Table II (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm7.pdf*). Key priority research options under each MNCHN theme are summarized below.

Maternal health

The network indicated the need for developing and evaluating screening checklists and management algorithms for severe acute maternal morbidities (SAMMs), near-miss events, high-risk pregnancies,

Table. Top ten priority research options in maternal, newborn, and child health and nutrition themes at national level

Theme	Rank	Research option
Maternal Health	1	Development and validation of algorithms for prevention, early detection and management of severe acute maternal morbidities and near-miss events in resource-constrained settings
	2	Strategies to improve quality of care during childbirth in the public health system, for example, medical practices, LSCS, active management of third stage of labour, EmOC, biophysical profiling for foetal assessment, application of epidural anaesthesia during delivery; beneficiary counselling and communication inside the labour room
	3	Early identification, referral and management of high-risk pregnancies (having maternofoetal morbidities including IUGR, stillbirths and preterms) at all levels of health care
	4	Introduction of a validated and cost-effective cancer screening programme of reproductive system for women in the community and health facilities
	5	Improving EmOC services [*e.g*., risk prediction, identification and communication; prompt referral; service availability (safe transportation, skilled personnel, capacity, logistic, blood storage); accountability; innovations]
	6	Assess blood transfusion needs, current availability and delivery mechanisms of blood for pregnant women based on PHC and CHC catchment areas in the context of prevailing burden of severe anaemia and post-partum haemorrhage
	7	Implementation research for effective delivery of evidence-based care protocols/algorithms for prevention and management of post-partum haemorrhage at different levels of care
	8	Improving maternal death audits, protocols and practices in the public health care system (body handling, support to family, communication, autopsy, death audit and causality ascertainment)
	9	Process and impact evaluation of public health programmes targeted for adolescents (ARSH, AFHS, RMNCH+A, RTI/STI screening services) in urban and rural areas
	10	Develop and validate a self-assessment check list for pregnant women to identify warning signs and need for care seeking
Newborn Health	1	Designing and evaluating curriculums for skill building and their retention for health personnel involved in newborn care in the community and at various levels of health care system (*e.g*., training in identification of warning signs, safe injection practices, administration of oxygen therapy, *etc*.)
	2	Engaging and empowering family members and community in the care of newborn (including family centred care): barriers, strategies to overcome, impact, cost-effectiveness
	3	Identifying appropriate and effective strategies (messages and channels of communication) to promote community awareness on newborn care practices and social mobilization for early healthcare seeking (including utilization of existing nutrition and health services) to prevent adverse outcomes
	4	Low cost, feasible, portable technological innovations in equipment to improve capacity (diagnosis, identification and management) and outreach for foetal & neonatal care (especially, LBW, preterm: CPAP, surfactant therapy, *etc*.,) at various levels of the health system and their impact evaluation
	5	Improving the implementation (service availability, quality, programme management and referral chain robustness) of neonate centric programmes and services (RMNCH+A, JSSK, NSSK, IMNCI & F-IMNCI, SNCUs, *etc*.)
	6	Implementation of an integrated and comprehensive maternal and newborn healthcare package for delivering continuum of care: barriers, strategies to overcome, need for governance modification, maternal and newborn outcomes
	7	Strategies to scale up home-based newborn care: Role assignment and rationalization for frontline workers, barrier identification and mitigation, cost-effectiveness, impact

Contd...

Theme	Rank	Research option
	8	Strategies for social, economic, skill and knowledge empowerment of women and its impact on newborn, child and women's health
	9	Establishing an innovative framework of monitoring and supervision with in-built mechanism of accountability to improve performance of frontline workers and health personnel involved in neonatal care (*e.g*., physical supervision; engaging PRIs and clients; use of ICT, telemedicine, maternal health)
	10	Development and validation of protocols for the management of pregnant women at risk of pre-term delivery, in the healthcare system [*e.g*., nutritional, pharmaceutical (steroids, betamimetics, progesterone, nitroglycerine patches, prophylactic antibiotics, *etc*.), surgical, exercise and lifestyle counselling]
Child Health	1	Develop locally relevant cost-effective strategies to expand the coverage of UIP by reaching segments of populations that are traditionally left out (address system 1 and community-Level 2 challenges) (*i*) VPD epidemiology, system capacity, cold chain, safety surveillance; (*ii*) Hesitancy, dropout, outreach strategies, KAP of care provider, community and clients
	2	Improving administrative data quality and strengthening data-driven child health programme monitoring, action and accountability at PHC and district levels (*e.g*., line listing of households with children with NDD, use of ICT, develop novel indicators)
	3	Development and validation of low-cost technologies for screening, referral and management of childhood pneumonia and ARI in the community and at various levels of health care (*e.g*., maternal health, point-of-care diagnostics & therapeutics, management protocols, *etc*.)
	4	Strategies to promote WASH practices in the community to improve child health and nutrition
	5	Development of cost-effective, feasible, validated point-of-care diagnostics for malaria in children for use at community and different levels of healthcare
	6	Development of evidence-based guidelines for rational use of antibiotics for childhood morbidities in India: choice of antibiotic; route and delivery systems (*e.g*., nebulizers); duration of therapy; monitoring criteria; adjunct therapies
	7	Development of an integrated child health programme for improving quality of life of children: challenges and barriers; strategies to overcome; feasibility across the country; effectiveness, cost-effectiveness
	8	Establishing an effective and sustainable vaccine preventable disease surveillance programme (especially, measles and rubella, pneumonia and diarrhoea) in India [*e.g*., defining syndromes (fever and rash) and programme thresholds, forging PPPs, building upon polio infrastructure, using technology (maternal health, GIS, *etc*.)]
	9	Identifying cost-effective strategies for supplementation of micronutrients and probiotics to prevent and control childhood diarrhoea, pneumonia and other infections
	10	To establish nationwide multicentric antimicrobial surveillance and antibiotic stewardship programme for infectious morbidities during childhood
Nutrition (Maternal and Child)	1	Identify and evaluate strategies to promote healthful lifestyle (physical activity and diet behaviour) in children through school and home-based interventions
	2	Determine characteristics of mother friendly work place policies and governance framework that enable optimal care and nutrition of pregnant and lactating women and their children: identify barriers and challenges to implement (*e.g*., financial security and compensation for loss of pay; crèches at workplaces; provision for breast milk expression and storage for working mothers)
	3	Process, impact and economic evaluation of NRCs for management of severely malnourished children (*e.g*., quality of care and client satisfaction; implementation gaps and challenges, reasons for underutilization and relapse; IEC to mothers during stay and at discharge; impact assessment, effectiveness of the RUTF used in NRCs and plausibility of indigenous preparation with the help of SHGs)

Contd...

Theme	Rank	Research option
	4	Identifying strategies for engaging the male partners, families and communities to improve the nutrition of women of reproductive age group and under-five children
	5	Process, impact, and economic evaluation of community-based management of childhood malnutrition (including SAM): role, effectiveness and accountability of various stakeholders (including frontline workers)
	6	Impact and economic evaluation of WASH practices in the community on the nutrition of women and children
	7	Cost-effective strategies to improve the quality, quantity and coverage of food supplements provided under the Mid-Day Meal Programme to improve the nutritional status of school-going children
	8	Determining optimal growth trajectory of LBW (preterm, SGA) babies: nutrient and calorie requirements; strategies to minimize, mitigate development of chronic diseases
	9	Development and popularisation of improved varieties of traditional food items rich in micronutrients (*e.g.*, iron rich millets): adoption of viable business models and modifying value and supply chains
	10	Strategies to overcome barriers and improve implementation of WASH practices in the community with particular focus on poor, socially disadvantaged groups

AFHS, Adolescent Friendly Health Services; ARI, Acute respiratory infections; ARSH, Adolescent reproductive and sexual health; CHC, Community Health Centre; CPAP, Continuous positive airway pressure; EmOC, Emergency obstetric care; F-IMNCI, Facility-based Integrated Management of Newborn and Childhood Illnesses; GIS, Geographic information system; ICT, Information and communication technology; IEC, Information, education, communication; IMNCI, Integrated Management of Newborn and Childhood Illnesses; IUGR, Intra uterine growth restriction; JSSK, *Janani Shishu Suraksha Karyakram*; KAP, Knowledge, attitude and practice; LBW, Low birth weight; LSCS, Lower segment cesarean section; NDD, Neuro-developmental disorders; NRC, Nutrition rehabilitation centres; NSSK, *Navjaat Shishu Suraksha Karyakram*; PHC, Primary Health Centre; PPP, Public private partnership; PRI, *Panchayati Raj* Institutions; RMNCH+A, Reproductive maternal newborn child and adolescent health; RTI, Reproductive tract infections; RUTF, Ready-to-use therapeutic food; SAM, Severe acute malnutrition; SGA, Small-for-gestational age; SHG, Self help group; SNCU, Special Newborn Care Units; STI, Sexually transmitted infections; UIP, Universal Immunization Programme; VPD, Vaccine preventable diseases; WASH, Water, sanitation and hygiene

post-partum haemorrhage, stillbirth, eclampsia and cancers. Development of point of care diagnostics and technological solutions for SAMMs, perinatal hypoxia and foetal distress were ranked as high priorities. Research on strategies to empower families and women for better self-care and timely care seeking, and skill enhancement (including role rationalization, task shifting and sharing) along with accountability of health providers at different levels were scored as important priorities. Process and impact evaluation of existing maternal health programmes to improve outcomes, developing implementation strategies to improve the quality of different aspects of maternal care in health system and expanding their coverage were considered important research areas. Development of pharmaceutical protocols for prevention and clinical management and novel technological solutions for identifying SAMMs, epidemiology of stillbirths and expanding coverage of reproductive tract infection (RTI)/sexually transmitted infection programmes and specific studies on symptomatic and asymptomatic RTI and its impact on stillbirth, low birth weight (LBW), intrauterine growth restriction (IUGR) and abortions were scored as important regional priorities.

Newborn health

Newborn health priorities included strategies to improve delivery and quality of care of newborn health-oriented programmes; skill and capacity enhancement of service providers including pre-service changes in the curriculum with in-built mechanisms of accountability; empowering mothers, families and communities to improve care seeking and quality of care in home environments; multicentric antimicrobial surveillance and antibiotic stewardship programme; development of point-of-care diagnostics/biomarkers for improving neonatal outcome, particularly for LBW neonates (preterm and IUGR) and neonatal sepsis; use of information and communication technology and utilization of m-Health to improve access to newborn care.

Child health

Six areas emerged prominently within child health: (*i*) issues related to coverage of Universal

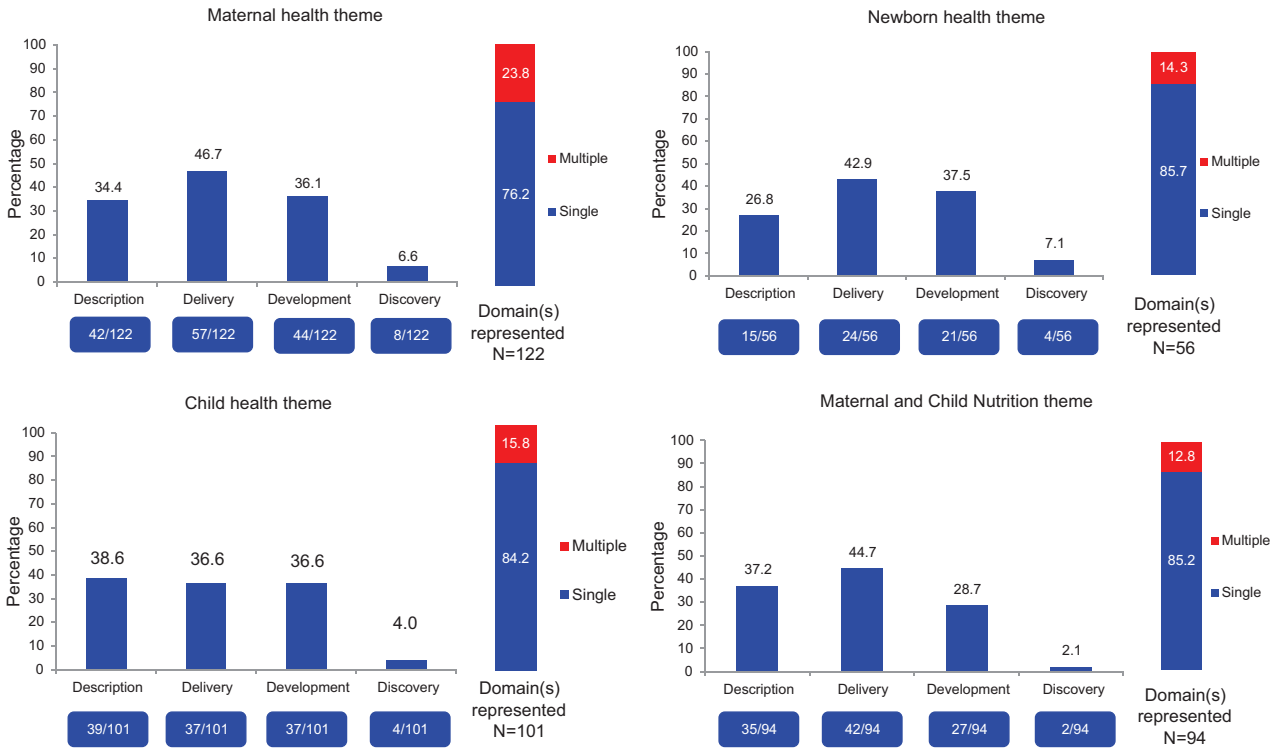


Fig. 2. Distribution of the research options in maternal, newborn, and child health and nutrition themes across the four domains of research.

Immunisation Programme (UIP) and surveillance for vaccine preventable diseases; (*ii*) point-of-care diagnostics; (*iii*) use of technology and development of biomarkers for the screening, referral and management of common childhood illnesses, namely, diarrhoea and acute respiratory infections (ARIs) along with improved control and management of malaria; (*iv*) rational use of antibiotics; (*v*) early childhood risk factors of adverse metabolic outcomes in later life, namely, obesity and metabolic syndrome; and (*vi*) water and sanitation hygiene (WASH). Operational and implementation research to effectively deliver different child health interventions in an integrated manner, including improving administrative data quality for decision-making, achieving Indian Public Health Standards at facilities, engaging community and its resources for better care seeking and care at home, strengthening curriculums for skill building and retention of personnel, was another area that scored high at both national and regional levels.

Maternal & child nutrition

Creation of mother friendly environments was an interesting and important priority to address issues of maternal and child nutrition. Research options that addressed the issues related to chronic diseases (*i.e.,* lifestyle behaviour modification interventions, point-of-care diagnostics and biomarkers for metabolic syndrome and nutrition of women around conception to prevent foetal programming and consequent risk for foetal origin of adult diseases) also scored high. Research options for enhancing skill of care providers and empowerment of families and communities including behavioural change communication were prioritized as was observed for other thematic areas. Research options around WASH (environmental and water hygiene) were scored high by experts across the country. Importantly, these research options were seen across all four themes. Research options to explore the performance and impact of various nutrition and food supplementation programmes (including community-based management of childhood malnutrition, midday meal, ICDS and food items provided under National Food Security Programme), improvement of administrative data quality for decision-making and the multisectoral governance of agriculture-nutrition-health nexus were prioritized at both national and regional levels. Anaemia (including issues related to absorption of nutrients), growth trajectory of LBW and breastfeeding were important cross-cutting areas for both mother and child perspective and were scored high.

The LRG had assigned the highest weight to the scoring criterion of 'relevance' and the lowest weight to 'investment in research'. The relative weights assigned to the five scoring criteria by different categories of members within the LRG did not vary significantly.

Discussion

The NSG strongly endorsed the research priorities identified by the exercise as appropriate for investment by different science and research departments of the Government of India and other national and international donor agencies and academic institutions over the next decade. It felt that the research agenda established with this exercise was in alignment with the national programme for achieving the recently enunciated health-related Sustainable Development Goals (SDGs) and will facilitate in accomplishing the unfinished agenda of the MDGs.

The exercise engaged experts who were well dispersed across the country. Thus, for the first time, regional MNCHN research priorities could be identified. In all the four thematic areas, there were differences between the top ten national and regional priorities. This observation made it imperative to take cognizance of the unique research needs of the regions. Although not part of the current project, the NSG suggested that a systematic effort must be made to determine factors that could explain differences in national and subnational (regional) priorities. The States falling under the three regions vary in terms of their health infrastructure, programme implementation and governance, development indicators and availability of baseline information on various health indicators. This may to some extent, explain the differences in the emerging research priorities. Better understanding of these factors will be important to design need-based research programmes and policies for different parts of the country. There are significant rural and urban differences for the health challenges and these have assumed greater significance in the light of rapid urbanization in certain parts of the country. The rural-urban divide will also have to be kept in mind when decisions on research investments are made.

In addition to the top priorities identified by the exercise, the NSG highlighted some more areas of research in MNCHN themes that deserve investment. These included role of environmental exposures in the occurrence of neurodevelopmental disorders such as autism; early childhood care and stimulation for improving cognitive outcomes; better description of childhood cancers and their risk factors; unsafe abortions; innovations to solve the 'nutrition enigma'; factors interfering with iron absorption in both women and children; impact of fluoride on health and nutrition of women and children; micronutrient deficiencies and occurrence of congenital birth defects (*e.g*., neural tube defects with folic acid deficiency); research into processes and strategies to make multisectoral governance for human nutrition workable and effective and technology innovation for developing better tools of nutrition assessment.

As part of Infant and Young Child Feeding and Complementary Feeding research agenda, NSG has also suggested that it is important to innovate strategies to promote breastfeeding; innovation is also needed in the development of low cost nutritious and healthy processed foods that require minimal cooking, are prepared from indigenous raw material and are appropriately fortified. This is important in the context of giving relief to severely time-constrained mothers who have now entered the workforce in a considerable way. Legislations affecting women and child health and nutrition are an important aspect to consider when research programmes and investment decisions are made. NSG suggested that researchers and funding agencies should work with Food Safety and Standards Authority of India in MoHFW to better understand regulatory issues related to food processing and related nutritional impacts.

Adolescents constitute almost 25 per cent of the Indian population. They suffer from several health problems that have been hitherto neglected. The foundation of several chronic diseases in the form of exposure to risk factors is laid during the adolescent years. NSG advised that adolescent-related research options were culled from the four themes and put forward separately with a caveat that scorers did not have an opportunity to see all the options in this section together. These have been put together in Supplementary Table III (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm8.pdf*). However, as adolescent health was not stated as a theme on its own for prioritization, the research options that were chosen from within the other sections may not reflect the true priorities in adolescent health. An independent exercise may be undertaken where adolescent health is given a focus (One example where a gap can easily be seen is interventions to target adolescent boys or interventions looking at accidental

injury or drowning which carry a huge burden in this age group).

The NSG acknowledged implementation research as an important area for consideration. This necessitates bridging the 'disconnect' between the research community, the policy and programme makers, implementers and the communities to achieve the desirable impact. Implementation research involves behavioural changes of health providers, and this cannot be overlooked. Some of the prioritized implementation research options can also help the national and State administrators to either commission research studies or decide to reset the way, in which the health system is working. This will also provide an opportunity for re-engineering health systems and programmes to improve their efficiencies: by dropping redundant programmes and/or making way for newer, refined and 'need of the hour' programmes. As a cross-cutting research priority, the NSG strongly pitched the imperatives of improving the quality of administrative data for better use in decision-making at ground level.

The NSG members observed that several of the priority research issues were cross-cutting and common across the four themes; these should be brought together as common research agenda for MNCHN. This will make the research investment efficient and effective by addressing more than one theme at a time and also align the research projects with the national goal to achieve universal healthcare in India. As a follow up on this suggestion, the investigator team has prepared a separate list of cross-cutting research options [Supplementary Table IV (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm9.pdf*)]. Similarly, as technology has cross-cutting usage, another list of research options related to research on the use of technology for health has been prepared for consideration by agencies with mandate to exclusively fund such research [Supplementary Table V (available at *http://www.ijmr.org.in/articles/2017/145/5/images/IndianJMedRes_2017_145_5_611_215547_sm10.pdf*)].

The NSG strongly advised that the DHR and other science ministries along with MoHFW should make efforts to pool resources for addressing priority research issues and also encourage national and international agencies to come together to invest strategically for greater and faster impact. The NSG has suggested that a number of research issues have the ability to be 'game changers' for the health systems, and for the health and nutrition of mothers and children, in general. These can be systematically identified and taken up in a mission mode by science ministries and donor agencies as per their own prioritization and investment policies. Recognizing the challenges of programme delivery in the context of weak infrastructure, national policymakers and programme planners have already identified north-eastern States and EAG States for the focus of several developmental schemes including health and nutrition (*http://nhm.gov.in/nhm/nrhm.html*). To further accelerate the developmental process, region-specific research agenda should become natural corollary. Health is a State subject in India and implementation of research issues can be integrated with the programme monitoring and evaluation frameworks existing therein. The research agenda could be accomplished in an accelerated manner and effectively with multistakeholder and multi-agency engagement through a broader convergent innovation coalition that will add to existing sectoral and cross-sectoral capacity for science, social sciences, economics, technology, innovation, strategy and policy to achieve better health and nutrition for mothers and their children in India.

Conclusion

It was concluded that the logical way forward for this exercise would be to take up these research priorities with different governmental and non-governmental agencies for investment. Various government and non-governmental funding agencies were requested to now re-align their investment with these priorities to achieve better health and nutrition for women and children of India effectively and efficiently. Further, bridging the gap between researchers and programme implementers at the grass root level would be essential along with decentralization of strategies to have State and possibly, district centric solutions for the betterment of health and nutrition scenario in India. In view of the predominance of delivery and implementation research identified as priority in all thematic areas, it was suggested to explore the possibility of supporting such research projects from the programme funds.

Acknowledgment

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References

1. Rudan I, Sridhar D. Structure, function and five basic needs of the global health research system. *J Glob Health* 2016; *6* : 010505.
2. McGregor S, Henderson KJ, Kaldor JM. How are health research priorities set in low and middle income countries? A systematic review of published reports. *PLoS One* 2014; *9* : e108787.
3. Yoshida S, Wazny K, Cousens S, Chan KY. Setting health research priorities using the CHNRI method: III. Involving stakeholders. *J Glob Health* 2016; *6* : 010303.
4. Dua T, Tomlinson M, Tablante E, Britto P, Yousfzai A, Daelmans B, *et al.* Global research priorities to accelerate early child development in the sustainable development era. *Lancet Glob Health* 2016; *4* : e887-9.
5. Yoshida S. Approaches, tools and methods used for setting priorities in health research in the 21st century. *J Glob Health* 2016; *6* : 010507.
6. Wazny K, Sadruddin S, Zipursky A, Hamer DH, Jacobs T, Kallander K, *et al.* Setting global research priorities for integrated community case management (ICCM): Results from a CHNRI (Child Health and Nutrition Research Initiative) exercise. *J Glob Health* 2014; *4* : 020413.
7. Souza JP, Widmer M, Gülmezoglu AM, Lawrie TA, Adejuyigbe EA, Carroli G, *et al.* Maternal and perinatal health research priorities beyond 2015: an international survey and prioritization exercise. *Reprod Health* 2014; *11* : 61.
8. Sharma R, Buccioni M, Gaffey MF, Mansoor O, Scott H, Bhutta ZA; Canadian Expert Group on Maternal, Newborn, Child and Adolescent Health. Setting an implementation research agenda for Canadian investments in global maternal, newborn, child and adolescent health: a research prioritization exercise. *CMAJ Open* 2017; *5* : E82-9.
9. Rudan I. Setting health research priorities using the CHNRI method: IV. Key conceptual advances. *J Glob Health* 2016; *6* : 010501.
10. Rudan I, Gibson JL, Ameratunga S, El Arifeen S, Bhutta ZA, Black M, *et al.* Setting priorities in global child health research investments: Guidelines for implementation of CHNRI method. *Croat Med J* 2008; *49* : 720-33.
11. Arora NK, Mohapatra A, Gopalan HS, Wazny K, Thavaraj V, Rasaily R, *et al*. Setting research priorities for maternal, newborn, child health and nutrition in India by engaging experts from 256 indigenous institutions contributing over 4000 research ideas: a CHNRI exercise by ICMR and INCLEN. *J Glob Health* 2017; *7* : 011003.

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Public-Private Partnership For Tuberculosis Control In India: Are The Private Practitioners Anywhere Near To Thisrntcp Sponsored Mission?

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Abstract:
Background: Revised National Tuberculosis Control Program (RNTCP) sponsored Public Private Partnership (PPP) is undermined by poor participation by private practitioners (PPs).There is an urgent need to realizefactors influencing private practitioners' preference of directly observed treatment short course (DOTS) for their patients. We attempted to study the knowledge and current practice characteristics of PPs while managing TB patients and to identify factors influencing their referral preferences for sputum microscopy and treatment under DOTS.
Methods: This was a cross-sectional survey involving 100 allopathic medical practitioners in private sector from an urban area. A self - designed and pretested questionnaire was administered to obtain data.
Results: The participants on an average managed 6.13 ± 14.87 patients with tuberculosis (TB). Most of them relied on sputum microscopy for diagnosis however only 49% referred their patients to Designated microscopy centres. A significant number participants still used serological and mantoux testing for diagnosing TB. Very few participants knew correct regimens, dosage and duration of anti TB therapy but only 46% referred their patients for DOTS based treatment. Frequent use of fluoroquinolones in anti TB therapy was noted. Factors predicting referrals to DOTS were younger age of participants,recent years in clinical practice with lesser outpatient turnover and better awareness about DOTS services. TB notification rate was low (41%) possibly due to lack of awareness about procedure involved .A lack of communication was noted between RNTCP staffs and participants indicating a significant drift in the "partnership"
Conclusion: This study has highlighted fallacies in TB management in private sector and identified correctable factors leading to sluggish involvement of PPs in DOTS based management. We recommend large scale interventional studies to evolve strategies for further strengthen the Public Private Partnership

Keywords: Public Private Partnership, Tuberculosis referral, Notification

I. Introduction

Global burden of tuberculosis is enormous and is increasing despite availability of good quality chemotherapy in organized regimens.Standardized diagnostic and therapeutic approach is the yardstick for unified struggle by mankind against Tuberculosis.World health organization declared TB as "Global Emergency" in 1993 and since then strongly advocates Directly Observed Treatment Short Course (DOTS) in standardized regimens delivered through national health programme across the world. Indiacontributes to about one third of global TB burden andhas gone a long way implementing DOTS through its Revised National Tuberculosis Control Program (RNTCP)[1]. Despite excellent coverage by RNTCP program, many patients with tuberculosis stillcontinue to seek other source of health care like practitioners modern and alternate medicine in private sector[2]. It is estimated that between 34% and 57% of tuberculosis cases are diagnosed and managed outside the public sector[3-5]. The same is also confirmed by studies on Anti TB drugs sales in India which show that the number of cases treated in the private sector alone exceeds estimated overall TB incidences[6].

While health sectors sponsored by government follow principles of DOTS, the status in private health sector is variable and often inappropriatevia improper use of diagnostic modalities, inadequate therapy and inadequate mechanisms to ensure compliance and adherence to therapy[7]. Realizing the importance of involving private health sector for effective control over tuberculosis in India, RNTCP implemented public-private partnership (PPP) during the last decade with only limited success. An attempt was made through mandatory notification since 2012, to bring into account all TB cases managed in private sector which also suffers with poor progress[8].There could be several reasons for sluggish participation of private practitioners in RNTCP which need tobe identified urgently in order to evolve strategies to improve this vital partnership. This study, using a self-designed questionnaire, attempted to study knowledge and practice characteristics of urban private medical practitioners in managing tuberculosis patients,their awareness about DOTS, aspects of notification and their level of interaction with RNTCP. The findings of this study will be useful in designing interventions to

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Original Article

Internal Medicine Section

Predicting Healthcare Utilization by Patients Admitted for COPD Exacerbation

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ABSTRACT

Introduction: Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. Acute exacerbation of COPD (AECOPD) is a frequent cause for hospitalization. Predictors of healthcare utilization by patients admitted for AECOPD in resource limited settings are not well studied.

Aim: To characterize the pattern of healthcare utilization by patients hospitalized for AECOPD and to identify clinical factors and laboratory predictors of prolonged hospital stay (PHS) and prolonged intensive care (PIC).

Materials and Methods: This study involved retrospective data analysis, to identify risk factors and evolve prediction models for increased healthcare utilization namely PHS and PIC for AECOPD. The data were extracted from 255 eligible admissions for AECOPD in 166 patients from Aug 2012 to July 2013. A logistic regression analysis was used for identification of predictors and models were tested using receiver operating characteristic curve.

Results: Independent predictors of prolonged hospital stay (> 6 days) were chronic respiratory failure at baseline, low saturation (<90%), high HbA1c level and positive isolates in sputum. Independent predictors of prolonged intensive care (for 24 hours) were past history of pulmonary tuberculosis, chronic respiratory failure at baseline, low saturation at admission, high leukocyte count and positive culture isolates in sputum. Prediction models evolved from variables available at admission had AUC of 0.805 (95% CI 0.729 – 0.881) and 0.825 (95% CI 0.752 – 0.899) for PHS and ICU admissions respectively.

Conclusion: Our prediction models derived from simple and easily available variables show good discriminative properties in predicting PHS and PIC for AECOPD. When prospectively validated, these models are useful for rational allocation of services especially in resource limited settings.

Keywords: Length of hospital stay, Intensive care

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of mortality and morbidity worldwide whose burden is projected to increase in near future [1,2]. Prevalence of COPD ranges from 0.8% to 6.0% globally [3-5]. Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD) is an event in the natural course of disease which has significant negative impact on the lung function, quality of life and survival [6-8]. All-cause mortality of these patients is as high as 49% within 3 years after hospitalization for AECOPD [9,10]. Exacerbations also involve enormous cost burden, especially in hospitalized patients [11]. These implications of AECOPD have attracted many researchers to explore ways to predict and prevent this event of paramount clinical importance. Several studies have attempted to predict and thereby prevent the occurrence of AECOPD especially severe forms leading to hospitalization [9,12]. However, when these patients get hospitalized for AECOPD, only limited data are available on how they utilize healthcare facility.

Healthcare utilization by patients admitted for AECOPD is characterized by varying periods of hospital stay, pharmacy consumption, need for various diagnostic modalities and at times ICU admissions with ventilatory or other life support measures. These characteristics, in particular length of hospital stay and ICU admissions, determine the overall outcome of AECOPD in terms of morbidity and mortality [13]. An increased healthcare utilization also means enormous burden to resource limited peripheral centres where these patients may get admitted for AECOPD. In this context, prediction of increased healthcare utilization for AECOPD at the time of admission may be useful for individualizing therapeutic approach and for rational allocation of limited resources to needy patients. This study attempted to characterize the pattern of healthcare utilization by COPD patients hospitalized for acute exacerbation, and to identify clinical and laboratory predictors of 'prolonged hospital stay'(PHS) and 'prolonged intensive care'(PIC) which is, in turn, aimed at increased health care utilization by these patients.

MATERIALS AND METHODS

The study involved retrospective analysis of data from patients admitted with a primary diagnosis of AECOPD in a tertiary care teaching hospital in southern India, during a period from August 2012 to July 2013. After obtaining approval from the institutional ethics committee, the data were extracted from electronic medical database of the hospital using ICD-10 codes. Our review of past medical records revealed that the diagnosis of COPD in all patients was made based on spirometry along with characteristic clinical and radiological findings. Admissions for AECOPD were made when the patients had worsening breathlessness along with either increased prevalence or quantity of sputum, severe enough to warrant hospitalization. This a sample of eligible admissions for AECOPD was identified for data extraction. The extracted data included demographic details, co-morbidities, clinico-laboratory and treatment related variables during admission for AECOPD. Data from hospitalizations which culminated with either of death or transfer against medical advice were excluded for evaluating predictors of PHS but included for PIC. Burden of comorbidities was quantified by Charlson's comorbidity index. Exacerbations were categorized into three types based on the presenting symptoms of worsened breathlessness, sputum purulence and increased quantity of sputum as proposed by Anthonisen NR et al., [14]. Accordingly, type 1 exacerbation was defined when all three symptoms were present at the time of admission. Type 2

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Original Article

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Effect of inhaled corticosteroids on systemic inflammation in asthma

Abstract

Background: Recent research using serum high sensitivity C-reactive protein (hs-CRP) has evidenced existence of low grade systemic inflammation in asthmatics whose correlation with various clinical indices is not fully studied. **Objective:** To investigate the relationship between systemic inflammation and various clinical and treatment characteristics of asthma. **Materials and Methods:** Forty asthmatics (22 steroid inhaling and 18 steroid naïve) and 40 healthy subjects matched for age and sex were examined cross-sectionally. Along with clinical assessment, serum hs-CRP levels were measured for all subjects using latex enhanced immunoturbidimetry method. **Results:** Serum hs CRP levels were significantly higher in steroid naïve asthmatics when compared to normal subjects (0.93 ± 1.18 vs 0.24 ± 0.31 mg/dl, respectively; Mann-Whitney U test, $P < 0.001$). This association persisted after adjusting for age, gender, body mass index (BMI), and socioeconomic status (adjusted odds ratio 10.47; 95% CI 1.88-58.3; $P < 0.01$). Steroid inhaling asthmatics had serum hs-CRP levels comparable with control group (0.17 ± 0.18 vs 0.24 ± 0.31 mg/dl respectively, $P > 0.05$). Among the clinical and treatment related variables, duration of inhaled steroids usage alone correlated significantly with serum hs-CRP levels (Pearson correlation coefficient r = 0.449, $P < 0.05$), which was independent of age, BMI, duration of illness, and frequency of emergency visits. **Conclusion:** This study confirms the existence of low grade systemic inflammation in asthma which is effectively controlled by inhaled steroids. Such an effect of inhaled steroids appears to be more pronounced in recent users than that of long-term users, possibly due to lower adherence rate among the latter.

Key words: Asthma, high sensitivity C-reactive protein, inhaled steroids, systemic inflammation

INTRODUCTION

Asthma is characterized by airway inflammation and hyperresponsiveness. Besides local inflammation, asthma is also

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[illegible] systemic inflammation was evidenced by increased levels of plasma fibrinogen and serum amyloid A.[1] Serum C-reactive protein (CRP) levels have been used extensively as a surrogate marker of systemic inflammation in several diseases as well as to predict adverse cardiovascular events.[2] Estimation of high sensitivity CRP (hs-CRP) with its enhanced sensitivity is useful in conditions with low grade systemic inflammation like asthma. Defining the role of hs-CRP in asthma has been a topic of interest in recent research.

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Is serum cholesterol a risk factor for asthma?

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ABSTRACT

Background: Proinflammatory role of serum cholesterol in asthma has been recently explored with contradicting results. Clarity on the link between serum cholesterol and asthma may lead to new evolutions in planning management strategies. The objective of our study was to examine the relationship between the serum cholesterol, asthma and its characteristics. Materials and Methods: A total of 40 asthmatics and 40 normal subjects were examined cross-sectionally and their serum fasting cholesterol and serum high sensitivity C reactive protein (hsCRP) levels were measured along with other baseline investigations. All subjects were non-smokers. Results: Serum total cholesterol (mean ± SD) among asthmatics was 176.45 ± 30.77 mgs/dL as compared to 163.33 ± 26.38 mgs/dL among normal subjects ($P < 0.05$). This higher serum cholesterol level was found to be associated with asthma independent of age, gender, body mass index (BMI), socio-economic status and serum hsCRP levels. However, the association was only modest (adjusted odds ratio 1.033; 95% confidence interval [CI] 1.008-1.059). There was no association between the serum cholesterol and asthma characteristics such as duration of illness, intake of inhaled steroids and frequency of emergency department visits. Other risk factors identified were poor ventilation (adjusted odds ratio 9.27; 95%CI 1.83-46.99) and overcrowding (adjusted odds ratio 41.9; 95% CI 3.15-557.46) at home. Conclusion: Our study found a modest but significant association between higher levels of serum cholesterol and asthma, which is independent of age, gender, BMI, socio-economic status and serum hsCRP. Future research is required in a larger population to substantiate above association and its clinical implications. Poor ventilation and overcrowding at home are risk factors for asthma possibly facilitating increased exposure to indoor allergens.

KEY WORDS: Asthma, cholesterol, high sensitivity C reactive protein, indoor hygiene, inflammation

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INTRODUCTION

Asthma currently affects about 300 million people worldwide;[1] and with this, number of patients seeking specialized asthma care has also increased.[2] Global Initiative for Asthma (GINA) 2011, has defined asthma as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role.[3] The chronic airway inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. Such a chronic inflammatory state in asthma is triggered and maintained at various levels by environmental factors. There are many host related factors that add upto inflammation and consequently increase the occurrence of asthma. One such proinflammatory host factor that has gained interest among researchers in recent years is serum cholesterol level. It is well established that hypercholesterolemia is associated with enhanced expression of proinflammatory mechanisms leading to increased levels of proinflammatory cytokines,[4] cellular adhesion molecules[5] and inflammation sensitive plasma proteins.[6]

Going by these proinflammatory properties, it has been hypothesized that serum cholesterol may also potentiate eosinophilic inflammation in those with genetic susceptibility for asthma thereby leading to its increased phenotypic expression [Figure 1]. Studies at cellular level[7] have indicated an undeniable role of

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ORIGINAL RESEARCH

An Organised Programme of Simulation-based Clinical Training for the Medical Interns

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ABSTRACT

Context: It is well-documented that high-fidelity simulated training can be used to improve very important aspects of clinical interventions which include clinical decision-making, technical skills, soft skills like communication and leadership qualities. Many of the procedural skills can also be learnt effectively through simulations, using manikins of lower fidelity. **Aim:** Present report is an attempt to substantially enhance the 'must possess' attributes of a good clinician, which are quite often difficult to achieve by conventional teaching methods. **Methods:** This programme was aimed at providing training to the medical interns in cardiopulmonary resuscitation, as well as basic and advanced cardiac life support in medical emergencies, in an attempt to help them to efficiently manage commonly encountered cardiorespiratory emergencies. The programme was evaluated by comparing their performance before and after the intervention. **Results:** Their performance scores reflected the positive effects of the simulation-based teaching. In general, in addition to improving their skills in handling certain selected medical emergencies, the programme has also proved to enhance 'entrustable professional activities' of the young medical graduates. **Conclusion:** Our study, while highlighting the desirability of utilising simulation-based clinical training (SBCT), has also sought ways and means of incorporating SBCT as a mandated programme during the medical internship at an institutional level.

Keywords: Clinical simulation, Medical interns, Medical emergencies, Clinical training, Professionalism, Team work, Enhancing skills

INTRODUCTION

The realm of medical education is undergoing significant changes globally. Some of these changes are warranted because of the great technological advances made in this field and others are due to an increasing awareness of patient safety measures warranted during the clinical procedures. The latter set of changes affects the general approach to patient care and calls for curricular changes to be brought in while training the medical undergraduate students, to effectively bring in the required attributes in the qualifying doctors. As it has been established that human error is a very significant factor as a cause for fatalities in a hospital, it is important to be able to avoid this single deterring factor while training the undergraduate medical students. There is increasing evidence to suggest that this can be achieved by effectively adopting simulation-based clinical training (SBCT), as an adjunct to conventional clinical teaching[1].

Competency-based medical education was advocated as an effective remedy for bringing in effective clinical training with consistency between different medical schools in terms of skills taught and competencies achieved[2]. In an effort to achieve this, many schools of medicine quite correctly thought of resorting to simulation-based medical education (SBME). Thus, in

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Photo Essay

Multimodal imaging of choroidal tubercles

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Key words: Choroidal tubercles, enhanced depth imaging spectral-domain optical coherence tomography, fundus autofluorescence imaging, near-infrared reflectance imaging

Case Report

A 39-year-old woman with disseminated tuberculosis and bilateral solitary choroidal tubercles [Fig. 1a and b] was subjected to multimodal imaging including enhanced depth imaging spectral-domain optical coherence tomography (EDI-OCT), near-infrared reflectance (NIR), and fundus autofluorescence (FAF) imaging. FAF imaging revealed hyperautofluorescent lesion with central hypoautofluorescence [Fig. 1c and d]. NIR imaging revealed hyperreflective lesion with a hyporeflective halo [Fig. 2a and c]. EDI-OCT [Fig. 2b and d] showed lobulated hyporeflective choroidal lesion with loss of vascular pattern and dome-shaped elevation of the overlying retina. Characteristic "increased transmission effect" was seen beneath the lesion. A small cap of subretinal fluid at the apex of lesion was noted in the right eye. Antituberculous therapy resulted in systemic improvement and regression of choroidal tubercles [Figs. 3 and 4 a–d].

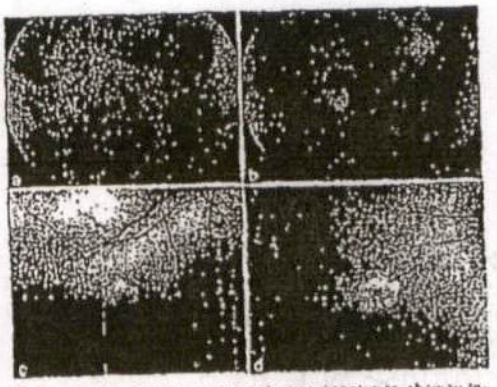


Figure 1: Color fundus photograph at presentation showing multiple yellowish nodular choroidal tubercles, temporal, in the right eye (a) nasally in the left eye (b). Corresponding fundus autofluorescence imaging showed the lesions as hyperautofluorescent with central hypoautofluorescent core (c and d)

Discussion

Characterization of choroidal lesions has evolved with the advent of modalities that enable imaging of the choroid and sclera (EDI-OCT); melanin, collagen, or fibrin (NIR imaging), and fluorophores (FAF).[1,2] EDI-OCT enables morphological evaluation of lesions, precise localization and measurement, and monitoring during treatment. NIR imaging which provides high-resolution in vivo images of subretinal pathologies demonstrated choroidal tubercles as hyperreflective due to unmasking of scleral collagen caused by defective choroidal pigmentation. There is only a single report of FAF imaging of choroidal tubercles.[3] Active lesion was hyperautofluorescent due to increased lipofuscin in the diseased retinal pigment epithelium (RPE), and the central hypoautofluorescence could be due to absent RPE, sparse lipofuscin, or secondary retinal infiltration. Posttreatment lesion may become isohypoautofluorescent. This FAF pattern is distinct from the heterogeneous appearance of choroidal metastasis, amelanotic melanomas, or nevus which results from lipofuscin, fluid, or RPE atrophy.[4,5] Thus, combined imaging helped to evaluate and monitor the chorioretinal pathology noninvasively in our patient with choroidal tubercles. Immunohistochemical studies of excised tuberculous pulmonary granulomas have shown that increased accumulation of lipid-rich caseum in the granulomas due to dysregulation in the host lipid metabolism signifies the progression of latent disease to active infection.[6] Further studies with FAF might help to detect similar changes in choroidal granulomas in vivo and thus obtain valuable insights into our understanding of ocular tuberculosis.

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Allergic Bronchopulmonary Aspergillosis Complicating Childhood Asthma

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ABSTRACT

While Allergic bronchopulmonary aspergillosis (ABPA) is known to complicate asthma in adults, its association with childhood asthma is very rare. We present two patients, a four-and half year old boy who presented with severe asthma and a 12 year-old girl whose previous chest radiographs revealed fleeting opacities. Both were diagnosed to be suffering from ABPA. [Indian J Pediatr 2009; 76 (3) : 331-332] *E-mail: skchhabra@mailcity.com*

Key words: Allergic bronchopulmonary aspergillosis; Asthma; Aspergillus

Allergic bronchopulmonary aspergillosis (ABPA), one of the spectrum of lung diseases caused by *Aspergillus* species, is a result of colonization of the fungus in the airways and the development of hypersensitivity to its antigens. It has been well documented as a complication of severe asthma in adults.[1] However, it is very rare in children except in association with cystic fibrosis[2]. Untreated or diagnosed late, ABPA may lead to parenchymal and airway damage terminating in respiratory failure and cor-pulmonale. A high degree of suspicion and application of well-defined major and minor diagnostic criteria should usually provide the diagnosis.[3] In view its rarity, we present two cases of ABPA complicating child hood asthma.

CASE REPORTS

Case 1

A four and a half year old boy presented with complaints of recurrent episodes of wheezing and cough with occasional passage of bronchial plugs of sputum since infancy. He had had frequent exacerbations sometimes with low-grade pyrexia and required hospitalizations. He also had nasal blockage and a family history of atopy. There was no history of gastrointestinal disturbances. The child was observed to be in respiratory distress with an arterial oxygen saturation of 84%. Auscultation of chest revealed bilateral diffuse polyphonic rhonchi. Treatment with oxygen and oral and nebulized corticosteroids/bronchodilators was instituted.

Total white blood count was 18,300/cmm with 7% eosinophils. PA Chest radiograph was not remarkable. In view of recurrent exacerbations of symptoms and mucoid sputum plugs, a diagnosis of bronchial asthma complicated by ABPA was considered. A contrast enhanced computed tomography (CECT) of the thorax revealed central bronchiectasis with normal tapering distally. A diagnosis of ABPA was suspected. Serum precipitins were detected against *Aspergillus Fumigatus*. Skin prick tests (1:10 dilutions) revealed positive type I reactions with *Aspergillus Niger* and *Aspergillus Fumigatus*. Serum total IgE level was 1947 IU/ml. (normal: 0-90 IU/ml). Specific IgG against *Aspergillus Fumigatus* was positive while specific IgE against the same was 4.5 (normal: 0.35 kU/L). Thus, the diagnosis of ABPA was confirmed by these criteria. Absence of eosinophilia may have been the result of treatment taken outside before presentation, possibly corticosteroids. This can produce substantial declines in eosinophil counts.

The child was started on oral prednisolone 1mg/kg/day for two weeks followed by an alternate day schedule along with a combination of inhaled formoterol and budesonide. He responded favourably to the treatment.

Case 2

A thin-built 12-year-old girl presented with recurrent wheezing with productive cough for the last two years. Family history of atopy was present. A review of serial chest radiographs done over last one year showed 'fleeting shadows'. Sputum examination for

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UNUSUAL PRESENTATION OF AN UNCOMMON LUNG MALIGNANCY

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ABSTRACT

Combined type of small cell lung carcinoma (SCLC) is a rare malignancy and the lung that is usually peripheral and diagnosed after resection. We report an unusual case of centrally located combined SCLC with squamous cell component that was diagnosed on endobronchial lung biopsy.

Lung India 2008; 25 : 132-134

Key words : Lung cancer, Combined carcinoma

INTRODUCTION

Small cell lung carcinoma (SCLC) accounting for 20-25% of all lung cancers, remains one of the most frequently diagnosed cancers and the most common cause of cancer deaths worldwide[1,2]. Combined type has been reported to occur in 1-3% of all cases of SCLC. These tumours contain of SCLC with a component of squamous cell carcinoma and/ or adenocarcinoma[3]. The rarity and heterogeneity of the tumour make the identification of combined SCLC difficult. Most are peripheral and diagnosed after resection. We report an unusual case of centrally located combined SCLC with squamous cell component that was diagnosed on endobronchial lung biopsy.

CASE REPORT

A 65 year-old male patient, current smoker (50 pack years) presented with complaints of dry cough and breathlessness for the past two months. He was also on treatment for hypertension for the last 3 years. Physical examination revealed no pallor, clubbing or lymphadenopathy. Examination of the respiratory system was not remarkable. The complete blood counts, electrocardiograph, and liver and kidney function tests (biochemistry) were within normal limits. The chest radiograph postero-anterior (PA) view taken one month apart showed a progressing mass lesion in the left paratracheal and parahilar region. Contrast enhanced computed tomography scan of the chest revealed a mass lesion with peripheral enhancement in the left hilum compressing the left main bronchus and encroaching on the wall of the descending aorta (Fig 1). Subcarinal lymph nodes were enlarged. Spirometry showed moderately

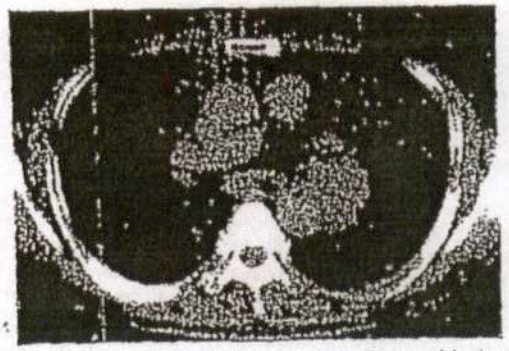


Fig.1 : Contrast enhanced computed tomography scan of the chest showing the left hilar mass compressing the left main bronchus and enlarged subcarinal lymph nodes.

severe airflow limitation with FEV_1/FVC of 68%, forced expiratory volume in one second (FEV_1) of 56% predicted and forced vital capacity (FVC) of 67% predicted. Change after bronchodilator was not significant. Fibreoptic bronchoscopy under local anesthesia revealed extramural compression superomedially in the left main bronchus. A smooth surfaced growth was seen just beyond the compression. The overlying mucosa was hyperemic and the growth bled on touch. Biopsy was taken.

Bronchial aspirate did not show any atypical cells on microscopic examination. However, post-bronchoscopy sputum showed a cluster of small atypical cells with dark blue hyperchromatic nuclei, nuclear overlapping and scant cytoplasm. Occasional singly arranged atypical keratinized squamous cells having irregular, angulated cytoplasmic

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Is Serum Cholesterol A Risk Factor For Ischemic Heart Disease In Patients With Chronic Obstructive Pulmonary Disease?

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Abstract
Background: Frequent coexistence of Chronic Obstructive Pulmonary Disease (COPD) and Ischemic Heart Disease (IHD) is often speculated to be related to lipid abnormalities. However studies reveal contradicting results, thus raising serious doubts about higher prevalence of dyslipidemia as reason for this coexistence. The objectives of this study were to examine the characteristics of lipid metabolism in COPD and to evaluate causal association between dyslipidemia and IHD among COPD subjects
Methods: This was a retrospective analysis of data from 98 COPD subjects randomly selected from the hospital medical records database. All eligible subjects were males with current or past history of smoking, with no evidence of active infections, chronic inflammatory disease or malignancy. Must had history of statin intake prior to index measurement of lipid profile. Data extracted were analyzed using SPSS 19 version. A recently published data from reference population were used for comparison.
Results: Most prevalent comorbidities were systemic hypertension (SHT), IHD and diabetes mellitus (DM). Prevalence of IHD was significantly higher than the reference population. Prevalence of hypertriglyceridemia was significantly lower in COPD subjects who also had low mean total cholesterol level than the reference population. On logistic regression analysis, elderly age and low HDL cholesterol predicted coexistence of IHD among COPD subjects independent of DM, SHT and duration of COPD. Other lipid parameters were not associated with increased prevalence of IHD among the study subjects.
Conclusion: This study has highlighted that atherogenic pattern of lipid profile among COPD subjects was comparable with that of reference population. Increased risk of IHD among COPD patients appears to be related to factors other than quantitative aspects of lipid metabolism/dyslipidemia which warrants investigations.

Keywords: Chronic obstructive pulmonary disease, ischemic heart disease, dyslipidemia.

I. Introduction

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease characterized by chronic airway inflammation in response to noxious stimuli resulting in airflow obstruction that is not fully reversible (1). World health organization predicts that by 2020, COPD will become third leading cause of death (currently fourth) and fifth leading cause of disability (currently twelfth) worldwide (2, 3). COPD has many extra-pulmonary manifestations of which ischemic heart disease (IHD) coexist frequently with COPD determining its morbidity and mortality rates. Large population based studies have also shown that COPD patients are two or three times more at risk for cardiovascular mortality which accounts for about 50% of the total number of deaths (4, 5, 6). The pathogenetic mechanism of this coexistence, though is unclear, is frequently speculated to be related to lipid metabolism in COPD patients. A deranged lipid profile is expected in COPD, for various reasons like smoking, age factor, steroid intake etc. However several studies have raised serious doubts about whether dyslipidemic state is characteristic for COPD and whether it can explain the increased risk of ischemic heart disease in these patients. This study is intended to clear these doubts with specific focus on quantitative lipid profile of COPD patients and its association with prevalence of ischemic heart disease in this subset of general population. COPD and IHD being two of top five major killer diseases, the rationale behind this study is to identify pathogenetic mechanisms linking these diseases that might open up therapeutic potential to contain the mortality and morbidity in above settings.

II. Materials And Methods

It was a retrospective analysis of data from 98 consecutive COPD subjects visiting a tertiary care chest clinic, aged 40 years and above, whose index measurement of lipid profile was documented prior to any history of statins / steroids intake. The eligible subjects registered during a period from January 2014 to December 2014, were recruited by random sampling method, whose files were then used for data extraction. All recruited subjects were free from active infections and did not have other chronic inflammatory diseases during the index

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Simulation Based Approach to Improve Competencies in Respiratory Emergencies amongst Interns

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Abstract:

Background

Medical education is rapidly evolving field with paradigm shift from information to competency based skills. Simulation has been accepted as an ethical tool, but not been incorporated into medical curriculum. We assessed the ability of residents during internship to manage respiratory emergencies and evaluated simulation as education and assessment tool

Method:

A prospective study was done with forty three residents during the internship period, which had completed emergency medicine postings. They were assessed in clinical knowledge by questionnaire and clinical skills were assessed by checklist scores in three common respiratory emergencies. The knowledge gained after the workshop was reassessed with post tests questionnaire. We evaluated student feedback with questionnaire responses

Results

43 interns were included in the study. All of them had completed respiratory medicine postings, during internship. Checklist scoring was done for five core competencies sub competencies were scored and validated with subject experts[cut off > 75%]There was remarkable difference in pre and post test scores with a mean which was statistically significant. Only 55.6% demonstrated skills in clinical knowledge with lowest scores (23%) at problem based learning. Core competencies which needed to be strengthened by practice and reinforcement were identified. Students felt that such training need to be extended for other medical emergencies and they wanted such training to be integrated in their curriculum.

Conclusion

Simulation based workshop is effective tool to teach respiratory emergencies and should be incorporated in medical curriculum as an education and assessment tool.

Keywords: Simulation based clinical training, Respiratory emergencies, Assessment tool

1. Introduction

Medical education is rapidly evolving field with paradigm shift from information to competency based skills. An important outcome of medical undergraduate training is that at the end of training, they should be able to manage range of clinical emergencies. Much evidence exists in previous studies that clinical skills may be taught poorly and students may not be able to handle demands of training during internship (1). Clinical knowledge is gained through reading, lectures, tutorials, seminars and bedside clinics. Even though clinical simulation has been accepted as an ethical tool, it has not been incorporated into medical curriculum (2-4). Simulation based workshops are effective and should be incorporated into medical curriculum which will help for

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Public-Private Partnership For Tuberculosis Control In India: Are The Private Practitioners Anywhere Near To Thisrntcp Sponsored Mission?

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Abstract:
Background: Revised National Tuberculosis Control Program (RNTCP) sponsored Public Private Partnership (PPP) is undermined by poor participation by private practitioners (PPs).There is an urgent need to realizefactors influencing private practitioners' preference of directly observed treatment short course (DOTS) for their patients. We attempted to study the knowledge and current practice characteristics of PPs while managing TB patients and to identify factors influencing their referral preferences for sputum microscopy and treatment under DOTS.
Methods: This was a cross-sectional survey involving 100 allopathic medical practitioners in private sector from an urban area. A self - designed and pretested questionnaire was administered to obtain data.
Results: The participants on an average managed 6.15 ± 14.87 patients with tuberculosis (TB). Most of them relied on sputum microscopy for diagnosis however only 49% referred their patients to Designated microscopy centres. A significant number participants still used serological and mantoux testing for diagnosing TB. Very few participants knew correct regimens, dosage and duration of anti TB therapy but only 46% referred their patients for DOTS based treatment. Frequent use of fluoroquinolones in anti TB therapy was noted. Factors predicting referrals to DOTS were younger age of participants,recent years in clinical practice with lesser outpatient turnover and better awareness about DOTS services. TB notification rate was low (41%) possibly due to lack of awareness about procedure involved. A lack of communication was noted between RNTCP staffs and participants indicating a significant drift in the "partnership".
Conclusion: This study has highlighted fallacies in TB management in private sector and identified correctable factors leading to sluggish involvement of PPs in DOTS based management. We recommend large scale interventional studies to evolve strategies for further strengthen the Public Private Partnership.
Keywords: Public Private Partnership, Tuberculosis referral, Notification

I. Introduction

Global burden of tuberculosis is enormous and is increasing despite availability of good quality chemotherapy in organized regimens.Standardized diagnostic and therapeutic approach is the yardstick for unified struggle by mankind against Tuberculosis.World health organization declared TB as "Global Emergency' in 1993 and since then strongly advocates Directly Observed Treatment Short Course (DOTS) in standardized regimens delivered through national health programme across the world. Indiacontributes to about one third of global TB burden andhas gone a long way implementing DOTS through its Revised National Tuberculosis Control Program (RNTCP)[1]. Despite excellent coverage by RNTCP program, many patients with tuberculosis stillcontinue to seek other source of health care like practitioners modern and alternate medicine in private sector[2]. It is estimated that between 34% and 57% of tuberculosis cases are diagnosed and managed outside the public sector[5-8]. The same is also confirmed by studies on Anti TB drugs sales in India which show that the number of cases treated in the private sector alone exceeds estimated overall TB incidences[9].

While health sectors sponsored by government follow principles of DOTS, the status at private health sector is variable and often inappropriatevia improper use of diagnostic modalities, inadequate therapy and inadequate mechanisms to ensure compliance and adherence to therapy[2]. Realizing the importance of involving private health sector for effective control over tuberculosis in India, RNTCP implemented public-private partnership (PPP) during the last decade with only limited success. An attempt was made through mandatory notification since 2012, to bring into account all TB cases managed in private sector which also suffers with poor progress[4].There could be several reasons for sluggish participation of private practitioners in RNTCP which need tobe identified urgently in order to evolve strategies to improve this vital partnership. This study,

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Original Article

Internal Medicine Section

Predicting Healthcare Utilization by Patients Admitted for COPD Exacerbation

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ABSTRACT

Background: Healthcare utilization, especially length of hospital stay and ICU admission, for acute exacerbation of chronic obstructive pulmonary disease (AECOPD) determine overall outcomes in terms of morbidity, mortality and cost burden. Predicting prolonged hospital stay (PHS) and prolonged intensive care (PIC) for AECOPD is useful for rational allocation of resources in healthcare centres.

Aim: To characterize the pattern of healthcare utilization by COPD patients hospitalized for acute exacerbation, and to identify clinical and laboratory predictors of 'prolonged hospital stay' (PHS) and 'prolonged intensive care' (PIC).

Materials and Methods: This study attempted through retrospective data analysis, to identify risk factors and evolve prediction models for increased healthcare utilization namely PHS and PIC for AECOPD. The data were extracted from 255 eligible admissions for AECOPD by 166 patients from Aug 2012 to July 2013. Logistic regression analysis was used for identifying predictors and models were tested with area under receiver operating characteristic curve.

Results: Independent predictors of prolonged hospital stay (≥ 6 days) were chronic respiratory failure at baseline, low saturation at admission, high HbA1c level and positive isolates in sputum culture. Independent predictors of prolonged intensive care (for ≥ 48 hours) were past history of pulmonary tuberculosis, chronic respiratory failure at baseline, low saturation at admission, high leukocyte count and positive culture isolates in sputum. Prediction models evolved from variables available at admission showed AUC 0.805 (95% CI 0.729 – 0.881) and 0.825 (95% CI 0.75 – 0.90) for PHS and ICU admissions respectively.

Conclusion: Our prediction models derived from simple and easily available variables show good discriminative properties in predicting PHS and PIC for AECOPD. When prospectively validated, these models are useful for rational allocation of services especially in resource limited settings.

Keywords: Length of hospital stay, Intensive care

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of mortality and morbidity worldwide whose burden is projected to increase in near future [1,2]. Prevalence of COPD ranges from 0.8% to 6.0% globally [3-5]. Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD) is an event in the natural course of disease which has significant negative impact on the lung function, quality of life and survival [6-8]. All-cause mortality of these patients is as high as 49% within 3 years after hospitalization for AECOPD [9,10]. Exacerbations also involve enormous cost burden, especially in hospitalized patients [11]. These implications of AECOPD have attracted many researchers to explore ways to predict and prevent this event of paramount clinical importance. Several studies have attempted to predict and thereby prevent the occurrence of AECOPD especially severe forms leading to hospitalization [9,12]. However, when these patients get hospitalized for AECOPD, only limited data are available on how they utilize healthcare facility.

Healthcare utilization by patients admitted for AECOPD is characterized by varying periods of hospital stay, pharmacy consumption, need for various diagnostic modalities and at times ICU admissions with ventilatory or other life support measures. These characteristics, in particular length of hospital stay and ICU admissions, determine the overall outcome of AECOPD in terms of morbidity and mortality [13]. An increased healthcare utilization also means enormous burden to resource limited peripheral centres where these patients may get admitted for AECOPD. of healthcare utilization by COPD patients hospitalized for acute exacerbation, and to identify clinical and laboratory predictors of 'prolonged hospital stay' (PHS) and 'prolonged intensive care' (PIC) which in this study, defined increased health care utilization by these patients.

MATERIALS AND METHODS

This study involved retrospective analysis of data from patients admitted with a primary diagnosis of AECOPD in a tertiary care teaching hospital in southern India, during a period from August 2012 to July 2013. After obtaining approval from the institutional ethics committee, the data were extracted from electronic medical database of the hospital using ICD-10 codes. Our review of past medical records revealed that the diagnosis of COPD in all patients was made based on spirometry along with characteristic clinical and radiological findings. All admissions for AECOPD were made when the patients had worsening breathlessness along with either increased purulence or quantity of sputum, severe enough to warrant hospitalization. Thus a sample of eligible admissions for AECOPD was identified for data extraction. The extracted data included demographic details, co-morbidities, clinico-laboratory and treatment related variables during admission for AECOPD. Data from hospitalizations which culminated with either of death or discharge against medical advice were excluded for evaluating predictors of PHS but included for PIC. Burden of co-morbidities was quantified by Charlson's co-morbidity index. Exacerbations were categorized into three types based on the presenting

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Effect of inhaled corticosteroids on systemic inflammation in asthma

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Abstract

Background: Recent research using serum high sensitivity C-reactive protein (hs-CRP) has evidenced existence of low grade systemic inflammation in asthmatics whose correlation with various clinical indices is not fully studied. **Objective:** To investigate the relationship between systemic inflammation and various clinical and treatment characteristics of asthma. **Materials and Methods:** Forty asthmatics (22 steroid inhaling and 18 steroid naïve) and 40 healthy subjects matched for age and sex were examined cross-sectionally. Along with clinical assessment, serum hs-CRP levels were measured for all subjects using latex enhanced immunoturbidometry method. **Results:** Serum hs CRP levels were significantly higher in steroid naïve asthmatics when compared to normal subjects (0.93 ± 1.18 vs 0.24 ± 0.31 mg/dL, respectively; Mann-Whitney U test, $P < 0.001$). This association persisted after adjusting for age, gender, body mass index (BMI), and socioeconomic status (adjusted odds ratio 10.47; 95% CI 1.88-58.3; $P < 0.01$). Steroid inhaling asthmatics had serum hs-CRP levels comparable with control group (0.17 ± 0.18 vs 0.24 ± 0.31 mg/dL respectively, $P > 0.05$). Among the clinical and treatment related variables, duration of inhaled steroids usage alone correlated significantly with serum hs-CRP levels (Pearson correlation coefficient r = 0.449, $P < 0.05$), which was independent of age, BMI, duration of illness, and frequency of emergency visits. **Conclusion:** This study confirms the existence of low grade systemic inflammation in asthma which is effectively controlled by inhaled steroids. Such an effect of inhaled steroids appears to be more pronounced in recent users than that of long-term users, possibly due to lower adherence rate among the latter.

Key words: Asthma, high sensitivity C-reactive protein, inhaled steroids, systemic inflammation

INTRODUCTION

Asthma is characterized by airway inflammation and hyperresponsiveness. Besides local inflammation, asthma is also found to have systemic inflammation as evidenced by increased levels of plasma fibrinogen and serum amyloid A.[1] Serum C reactive protein (CRP) levels have been used extensively as a surrogate marker of systemic inflammation in several chronic diseases as well to predict adverse cardiovascular events.[2-4] Estimation of high sensitivity CRP (hs-CRP) with its enhanced sensitivity is useful in conditions with low grade systemic inflammation like asthma. Defining the role of hs-CRP in asthma has been a topic of interest in recent research.

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Is serum cholesterol a risk factor for asthma?

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ABSTRACT

Background: Proinflammatory role of serum cholesterol in asthma has been recently explored with contradicting results. Clarity on the link between serum cholesterol and asthma may lead to new evolutions in planning management strategies. The objective of our study was to examine the relationship between the serum cholesterol, asthma and its characteristics. **Materials and Methods:** A total of 40 asthmatics and 40 normal subjects were examined cross-sectionally and their serum fasting cholesterol and serum high sensitivity C reactive protein (hsCRP) levels were measured along with other baseline investigations. All subjects were non-smokers. **Results:** Serum total cholesterol (mean ± SD) among asthmatics was 176.45 ± 30.77 mgs/dL as compared to 163.33 ± 26.38 mgs/dL among normal subjects ($P < 0.05$). This higher serum cholesterol level was found to be associated with asthma independent of age, gender, body mass index (BMI), socio-economic status and serum hsCRP levels. However, the association was only modest (adjusted odds ratio 1.033; 95% confidence interval [CI] 1.008-1.059). There was no association between the serum cholesterol and asthma characteristics such as duration of illness, intake of inhaled steroids and frequency of emergency department visits. Other risk factors identified were poor ventilation (adjusted odds ratio 9.27; 95% CI 1.83-46.99) and overcrowding (adjusted odds ratio 41.9; 95% CI 3.15-557.46) at home. **Conclusion:** Our study found a modest but significant association between higher levels of serum cholesterol and asthma, which is independent of age, gender, BMI, socio-economic status and serum hsCRP. Future research is required in a larger population to substantiate above association and its clinical implications. Poor ventilation and overcrowding at home are risk factors for asthma possibly facilitating increased exposure to indoor allergens.

KEY WORDS: Asthma, cholesterol, high sensitivity C reactive protein, indoor hygiene, inflammation

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INTRODUCTION

Asthma currently affects about 300 million people worldwide,[1] and with this, number of patients seeking specialized asthma care has also increased.[2] Global Initiative for Asthma (GINA) 2011, has defined asthma as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role.[3] The chronic airway inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. Such a chronic inflammatory state in asthma is triggered and maintained at various levels by environmental factors. There are many host related factors that add upto inflammation and consequently increasing the occurrence of asthma. One such proinflammatory host factor that has gained interest among researchers in recent years is serum cholesterol level. It is well established that hypercholesterolemia is associated with enhanced expression of proinflammatory mechanisms leading to increased levels

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ORIGINAL RESEARCH

An Organised Programme of Simulation-based Clinical Training for the Medical Interns

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ABSTRACT

Context: It is well-documented that high-fidelity simulated training can be used to improve very important aspects of clinical interventions which include clinical decision-making, technical skills, soft skills like communication and leadership qualities. Many of the procedural skills can also be learnt effectively through simulations, using manikins of lower fidelity. **Aim:** Present report is an attempt to substantially enhance the 'must possess' attributes of a good clinician, which are quite often difficult to achieve by conventional teaching methods. **Methods:** This programme was aimed at providing training to the medical interns in cardiopulmonary resuscitation, as well as basic and advanced cardiac life support in medical emergencies, in an attempt to help them to efficiently manage commonly encountered cardiorespiratory emergencies. The programme was evaluated by comparing their performance before and after the intervention. **Results:** Their performance scores reflected the positive effects of the simulation-based teaching. In general, in addition to improving their skills in handling certain selected medical emergencies, the programme has also proved to enhance 'entrustable professional activities' of the young medical graduates. **Conclusion:** Our study, while highlighting the desirability of utilising simulation-based clinical training (SBCT), has also sought ways and means of incorporating SBCT as a mandated programme during the medical internship at an institutional level.

Keywords: Clinical simulation, Medical interns, Medical emergencies, Clinical training, Professionalism, Team work, Enhancing skills

INTRODUCTION

The realm of medical education is undergoing significant changes globally. Some of these changes are warranted because of the great technological advances made in this field and others are due to an increasing awareness of patient safety measures warranted during the clinical procedures. The latter set of changes affects the general approach to patient care and calls for curricular changes to be brought in while training the medical undergraduate students, to effectively bring in the required attributes in the qualifying doctors. As it has been established that human error is a very significant factor as a cause for fatalities in a hospital, it is important to be able to avoid this single deterring factor while training the undergraduate medical students. There is increasing evidence to suggest that this can be achieved by effectively adopting simulation-based clinical training (SBCT), as an adjunct to conventional clinical teaching[1].

Competency-based medical education was advocated as an effective remedy for bringing in effective clinical training with consistency between different medical schools in terms of skills taught and competencies achieved[2]. In an effort to achieve this, many schools of medicine quite correctly thought of resorting to simulation-based medical education (SBME). Clinical

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Is Serum Cholesterol A Risk Factor For Ischemic Heart Disease In Patients With Chronic Obstructive Pulmonary Disease?

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Abstract
Background: Frequent coexistence of Chronic Obstructive Pulmonary Disease (COPD) and Ischemic Heart Disease (IHD) is often speculated to be related to lipid abnormalities. However studies reveal contradicting results, thus raising serious doubts about higher prevalence of dyslipidemia as reason for this coexistence. The objectives of this study were to examine the characteristics of lipid metabolism in COPD and to evaluate causal association between dyslipidemia and IHD among COPD subjects.
Methods: This was a retrospective analysis of data from 98 COPD subjects randomly selected from the hospital medical records database. All eligible subjects were males with current or past history of smoking, with no evidence of active infections, chronic inflammatory disease or malignancy. None had history of statin intake prior to index measurement of lipid profile. Data extracted were analyzed using SPSS 19 version. A recently published data from reference population were used for comparison.
Results: Most prevalent comorbidities were systemic hypertension (SHT), IHD and diabetes mellitus (DM). Prevalence of IHD was significantly higher than the reference population. Prevalence of hypertriglyceridemia was significantly lower in COPD subjects who also had low mean total cholesterol level than the reference population. On logistic regression analysis, elderly age and low HDL cholesterol predicted coexistence of IHD among COPD subjects independent of DM, SHT and duration of COPD. Other lipid parameters were not associated with increased prevalence of IHD among the study subjects.
Conclusion: This study has highlighted that atherogenic pattern of lipid profile among COPD subjects was comparable with that of reference population. Increased risk of IHD among COPD patients appears to be related to factors other than quantitative aspects of lipid metabolism (dyslipidemia) which warrants investigations.
Keywords: Chronic obstructive pulmonary disease, Ischemic heart disease, dyslipidemia.

I. Introduction

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease characterized by chronic airway inflammation in response to noxious stimuli resulting in airflow obstruction that is not fully reversible (1). World health organization predicts that by 2020, COPD will become third leading cause of death (currently fourth) and fifth leading cause of disability (currently twelfth) worldwide (2, 3). COPD has many extra-pulmonary manifestations of which ischemic heart disease (IHD) coexist frequently with COPD determining its morbidity and mortality rates. Large population based studies have also shown that COPD patients are two or three times more at risk for cardiovascular mortality which accounts for about 50% of the total number of deaths (4, 5, 6). The pathogenetic mechanism of this coexistence, though is unclear, is frequently speculated to be related to lipid metabolism in COPD patients. A deranged lipid profile is expected in COPD, for various reasons like smoking, age factor, steroid intake etc. However several studies have raised serious doubts about whether dyslipidemic state is characteristic for COPD and whether it can explain the increased risk of ischemic heart disease in these patients. This study is intended to clear these doubts with specific focus on quantitative lipid profile of COPD patients and its association with prevalence of ischemic heart disease in this subset of general population. COPD and IHD being two of top five major killer diseases, the rationale behind this study is to identify pathogenetic mechanisms linking these diseases that might open up therapeutic potential to contain the mortality and morbidity in above settings.

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Knowledge, Attitude and Practice Skills in Tuberculosis Care among Paramedical Students in South India-A Single Centre Survey

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Abstract

Background

India is the second most populous country in the world, but India has more new TB cases than any other country, with high mortality. Under Revised National Tuberculosis Control Programme, much emphasis has been laid on health education and counselling of tuberculosis patients to ensure adherence to treatment and achieve 95% cure rate. Not many studies have been done among paramedical students to assess adequacy of knowledge on tuberculosis.

Aim

The aim of this study is to evaluate knowledge, attitude and practice skills in tuberculosis care amongst final year students of undergraduate paramedical courses (pharmacy, physiotherapy and nursing) and also to evaluate differences of awareness between them.

Methodology

This study was a cross-sectional survey conducted for final year students of nursing, physiotherapy and pharmacy using a self-administered questionnaire designed and validated by a panel of subject experts. In all, 152 students were surveyed and data collected were analysed with SPSS software.

Results

The present study showed that adequacy of knowledge about tuberculosis amongst final year paramedical students was generally low. Adequacy of knowledge on tuberculosis was better amongst physiotherapy and nursing students as compared with pharmacy students. On the contrary, knowledge on how to diagnose case of tuberculosis was better amongst pharmacy students (53.3%) compared with physiotherapy and nursing students. Knowledge on special topics like multidrug-resistant tuberculosis and public health was lacking amongst all these categories.

Conclusion

Overall and domain-specific knowledge is found to be poor among final year paramedical students. Improvement in knowledge is expected to aid in primary and secondary prevention against tuberculosis.

Top

Keywords

Tuberculosis, Knowledge, Attitude, Practice skills (KAP), Paramedical students, Tuberculosis care, Revised National Tuberculosis Control Programme (RNTCP).

Top

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Tuberculosis-Associated Chylothorax: Case Report and Systematic Review of the Literature

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Established Facts

- Trauma is the most common cause of chylothorax.
- Tuberculosis (TB) is described as a cause of nontraumatic chylothorax, but several large series do not include any patients with TB-chylothorax.

Novel Insights

- TB is a very rare cause of chylothorax with only 37 cases reported in the literature.
- TB-chylothorax developed subsequent to the diagnosis of TB in 27.8% (10/36) of the patients after a median of 6.75 weeks (IQR 4–9) while on anti-TB treatment.
- TB-chylothorax developed during an immune reconstitution syndrome in 16.7% (6/36) of the patients, even in immunocompetent ones.
- TB-chylothorax developed in the absence of any mediastinal lymphadenopathy in 45.9% (17/37) of the patients; 13.5% (5/37) had isolated tubercular empyema alone.
- TB-chylothorax developed along with chylous ascites in 10.8% (4/37) of the patients.
- Most patients with TB-chylothorax responded to anti-TB treatment and dietary manipulation alone, and only 17.1% (6/35) needed thoracic duct ligation.

Keywords
Tuberculosis · Chylothorax · Pleural effusion · Chylomicron

Abstract
Tuberculosis (TB) is a rare cause of chylothorax. We describe a case and the results of a systematic review of all reported cases of TB-chylothorax. We identified 37 cases of TB-chylothorax. The symptoms at presentation were constitutional (85.7%; 30/35), dyspnea (60.6%; 20/33), and cough (54.5%; 18/33). Chylothorax developed subsequent to the diagnosis of TB in 27.8% (10/36) of the patients, after a median of 6.75 weeks (IQR 4–9). Chylothorax developed during an immune reconstitution syndrome (IRS) in 16.7% (10/36) of the patients, including immunocompetent ones. TB was disseminated in 45.9% (17/37) of the patients at the diagnosis of chylothorax. Chylothorax developed in the absence of any mediastinal lymphadenopathy in 45.9% (17/37) of the patients; 13.5% (5/37) had isolated tubercular empyema alone. The diagnosis of TB was established microbiologically in

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72.2% (26/36) and by biopsy alone in 27.8% (9/36) of the patients. Anti-TB treatment (ATT) was administered for a median of 7.57 months (IQR 6–9). Steroids were administered to 22.9% (8/35) of the patients, often for suspected IRS. Thoracic duct ligation and octreotide were required for only 17.1% (6/35) and 8.6% (3/35) of the patients, respectively. In all, 94.4% (34/36) of the patients had resolution of chylothorax and completed treatment successfully; only 5.6% (2/36) died. In conclusion, TB-chylothorax may develop without obvious mediastinal lymphadenopathy and be associated with tubercular empyema alone. TB-chylothorax can develop during treatment of TB due to IRS, even in immunocompetent patients. ATT and dietary manipulation are associated with good resolution and low mortality, and duct ligation is needed for only a small minority of patients.

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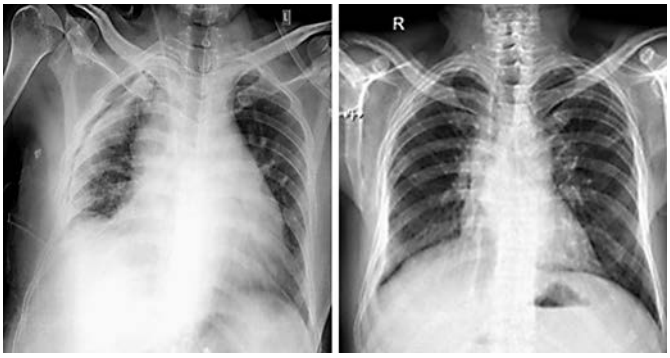


Fig. 1. Left: chest radiograph at diagnosis showing a right pyopneumothorax with pleural thickening and intercostal drain in situ. Right: chest radiograph at completion of anti-tuberculosis treatment showing significant interval resolution with minimal pleural thickening.

Introduction

Tuberculosis (TB) continues to be a global scourge. India has the highest TB burden and accounts for approximately more than one-fifth (21%) of the global disease burden, with recently revised higher estimates of disease burden [1, 2]. Chylothorax is characterized by the presence of chyle in the pleural space due to thoracic duct disruption [3, 4]. Accumulation of cholesterol or lecithin-globulin complexes in pleural fluid of long-standing effusions can occur and is described as a pseudochylothorax [3, 5]. TB is a well-described cause of pseudochylothorax [5]; the association of TB with chylothorax, however, is very rare but has been described. The mechanisms of development of chylothorax in TB (TB-chylothorax), clinical features, radiological findings, mode of diagnosis, and management aspects are unclear.

Case Report

A 50-year-old gentleman with diabetes mellitus of 5 years duration presented with productive cough for 15 days, intermittent fever, and right-sided pleuritic chest pain of 1-week duration. Chest radiography (Fig. 1, left) confirmed a right-sided pleural effusion, and diagnostic thoracocentesis showed pus-like material; an intercostal tube drain was inserted, and he was referred to this center for persisting purulent drainage from the drain. There was no history of trauma, anorexia, or weight loss. He did not report any significant illness in his family or contact with TB, did not smoke or drink alcohol, and did not raise pets. The examination showed pallor, pedal pitting edema, a regular heart rate of 72/min, and a blood pressure of 120/70 mm Hg. There was no evidence of alveolopleural fistulae, and the intercostal drain continued to drain 750 mL/day of whitish fluid.

Chest radiography showed a right pyopneumothorax (Fig. 1, left). Hemoglobin was at 6.9 g/dL, with normal leukocyte and platelet counts. Renal function testing showed renal failure with serum creatinine at 9.4 mg/dL (normal 0.8–1.0). Urine microscopy showed bland sediments without albuminuria. Ultrasound showed a normal kidney size with increased renal cortical echogenicity. Arterial blood gas showed metabolic acidosis (pH 7.25, HCO_3 14 mEq/L, pCO_2 30 mm Hg, anion gap 16 mEq/L). Fundus examination showed findings consistent with mild nonproliferative diabetic retinopathy. HbA_{1c} was at 9.2% (normal <6.5). Human immunodeficiency virus (HIV), hepatitis B, and hepatitis C tests by enzyme-linked immunosorbent assay were all negative. Two sputum smears were negative for acid-fast bacilli by Ziehl-Neelsen (ZN) staining. Liver function and thyroid function tests were normal. Pleural fluid analysis showed 99% neutrophils, with sugar <0.8 mg/dL. Pleural fluid cultures yielded *Candida albicans* twice; bacterial cultures were sterile, and the ZN smears of the pleural fluid were negative. Echocardiography showed normal left ventricular function with no valvular abnormalities, and fundoscopy was normal. A diagnosis of candidal empyema was made and fluconazole treatment was started according to sensitivity. Repeat pleural fluid cultures were sterile and showed an exudative (protein 4.1 g/dL, LDH 321 IU/mL), lymphocytic effusion (95%); however, the patient continued to have persistent drainage of purulent-looking fluid. A pleural fluid biochemical analysis was requested; it showed triglycerides at 135 mg/dL and cholesterol at 35 mg/dL. Because of diagnostic ambiguity, pleural fluid chylomicron testing was done (Christian Medical College, Vellore, India) and it was strongly positive for chylomicrons. Plain CT of the thorax (Fig. 2, 3) showed significant mediastinal adenopathy with calcification involving the pretracheal, aortopulmonary, bilateral hilar and subcarinal stations. He was planned for endobronchial ultrasound-guided needle aspiration, but, meanwhile, GeneXpert testing of the pleural fluid was positive for *Mycobacterium tuberculosis* (MTB), and *Mycobacterium* growth indicator tube cultures of the pleural fluid isolated MTB.

A final diagnosis of poorly controlled diabetes, disseminated TB with right-sided chylothorax complicated by candidal empy-

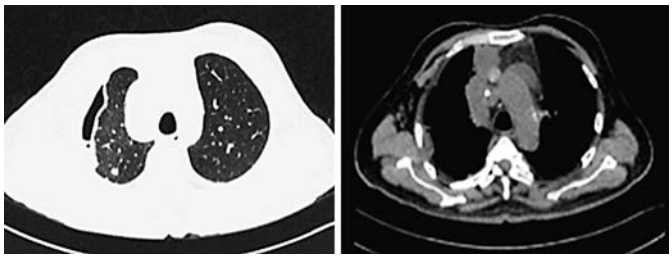


Fig. 2. Plain computed tomography images of the thorax (taken because of acute renal failure) showing moderate pericardial effusion and right mediastinal pleural collection and thickening; significant mediastinal adenopathy (largest: 2.5 × 2 cm) involving the pretracheal, aortopulmonary, bilateral hilar, and subcarinal stations was observed with calcifications.

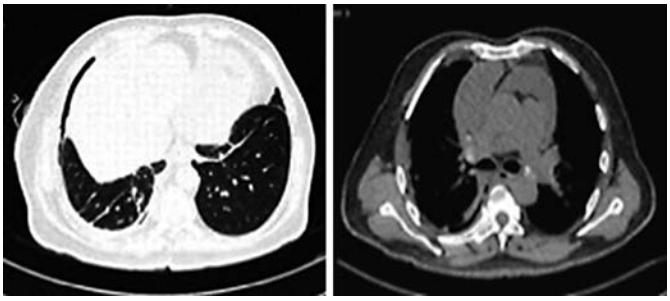


Fig. 3. Plain computed tomography images of the thorax at the level of the diaphragm indicating lack of pleural thickening and near-total lung expansion; mediastinal windows show calcified mediastinal nodes.

ema, and sepsis with acute renal failure was made. The patient underwent intermittent hemodialysis and was managed with oxygen, medium-chain fatty acid-based diet, intravenous fluids, weight-based anti-TB treatment (ATT) modified for renal failure, fluconazole, and deep venous thrombosis and stress ulcer prophylaxis. Surgery of the right trapped lung was postponed because of renal failure. His renal function gradually normalized, and the pleural drainage significantly reduced, with complete lung expansion by the second week. The intercostal drain was removed. His course was complicated by ATT-induced hepatotoxicity, necessitating initiation of levofloxacin, streptomycin, and continuation of ethambutol. He was subsequently rechallenged successfully with isoniazid and rifampicin and completed 9 months of TB treatment with clinical and radiological improvement (Fig. 1, right). He remains on follow-up and has had no further symptoms at 12 months.

Systematic Search of the Literature

Two of the authors (S.R. and R.K.) independently conducted a systematic search in PubMed (National Library of Medicine, Bethesda, MD, USA) using the terms "tuberculosis" AND "chylothorax" restricted by a publication date of 1980 onwards. These articles were screened, without blinding, by title and abstract review to identify relevant studies. Any discrepancy was resolved by consensus. This was further supplemented by a search in the Indian medical search engine IndMed, references from the retrieved articles, our personal records, and the Internet search engine Google. Full texts or abstracts were then retrieved to identify the spectrum of clinical features of chylothorax associated with TB. Articles identified in languages other than English were translated into English before data abstraction. If either the abstract or the full text was inaccessible, the corresponding author was contacted by mail requesting access to the published report. Only those articles that included patients who fulfilled the criteria (below) for chylothorax *and* TB as documented by the microbiologic or pathological criteria (below) were included for analysis.

Chylothorax was defined by (a) the presence of chylomicrons in the pleural fluid *or* (b), in the absence of an evaluation for chylomicrons, pleural fluid triglyceride >110 mg/dL *and* pleural fluid cholesterol <200 mg/dL (*or* a ratio of pleural fluid to serum cholesterol <1.0 if pleural fluid cholesterol ≥200 mg/dL). TB was defined by either (a) sputum, bronchoalveolar fluid, pleural, ascitic fluid, cerebrospinal fluid, or tissue biopsy positive for acid-fast bacilli by ZN staining *or* (b) growth of MTB by culture in the above, *or* (c) caseating granulomas *or* miliary nodules on radiology in the presence of a compatible clinical syndrome *and* a response to ATT alone.

Data Extraction

The data were abstracted in a predefined data extraction form (see online suppl. Table; for all online suppl. material, see www.karger.com/doi/10.1159/000484694). From the selected cases, the following data were gathered: (a) publication details (title, authors, year of publication, journal name, and other citation details); (b) the number of cases reported in each publication; (c) the demographic details of each case, including age and gender; (d) the duration of symptoms; (e) the symptoms at presentation, including constitutional symptoms in the form of fever, anorexia, or weight loss, dyspnea, cough, chest pain, or hemoptysis; (f) the side of the effusion; (g) details on concurrent organ system involvement; (h) the time to involvement of chylothorax, if sequential; (i) comorbidities and their details, including a diagnosis of HIV; (j) pleural fluid analysis results; (k) results of microbiologic testing of pleural and sputum samples; (l) tissue biopsy results when performed for the diagnosis of TB; (m) treatments administered, including details on steroids, ATTs, and their duration; and (n) outcome.

Informed consent was obtained from the index patient described in the paper. The institutional ethics committee approved the study.

Statistical Analysis

Continuous data are presented as mean (SD) or median (range or IQR), and categorical data as percentages and proportions. All statistical analyses were done using the statistical software SPSS IBM version 21 (PSG IMS&R).

Table 1. Clinical features of all patients with tuberculosis-associated chylothorax in the literature (n = 37 cases)[a]

Age (n = 35), years	39.27±4.13[b]
Gender (male:female) (n = 36)	20:16
Duration of symptoms prior to presentation (n = 29), months	1 (0.75–3.00)
Symptoms at presentation (n = 35)	
Constitutional	30/35 (85.7)
Dyspnea	20/33 (60.6)
Cough	18/33 (54.5)
Chest pain	10/33 (30.3)
Hemoptysis	0/33 (0)
Diagnosis concurrent (n = 36)	
Simultaneous involvement	26/36 (72.2)
Sequential	10/36 (27.8)
Time to chylothorax after diagnosis of tuberculosis (n = 10), weeks	6.75 (4–9)[c]
Clinical findings	
Chest radiography findings: effusion side (n = 37)	
Right	17/37 (45.9)
Left	8/37 (21.6)
Bilateral	12/37 (32.4)
Lung findings (n = 37)	
None	19/37 (51.4)
Consolidation	8/37 (21.6)
Nodular	8/37 (21.6)
Interstitial	2/37 (5.4)
Nodes (n = 37)	
None	11/37 (29.7)
Mediastinal	17/37 (45.9)
Abdominal	3/37 (8.1)
Both	4/37 (10.8)
Disseminated	2/37 (5.4)
Other organ involvement (n = 36)	
None	14/36 (38.9)
Multiple	11/36 (30.6)
Abdomen	5/36(13.9)
Bone	3/36 (8.1)
Pericardium	1/36 (2.8)
Comorbidities (n = 37)	
Yes	17/37 (45.9)
No	20/37 (54.1)
Renal failure	4/37 (10.8)
Human immunodeficiency virus positive	6/37 (16.2)
Reported mechanism of chylothorax (n = 35)	
Mediastinal nodes	18/35 (51.4)
Abdominal nodes	5/35 (14.3)
Extensive pleural disease	5/35 (14.3)
Constrictive pericarditis	1/35 (2.8)
Spinal abscess rupture	2/35 (5.6)
Pleural fluid analysis	
Milky appearance (n = 36)	
Yes	34/36 (94.4)
No	2/36 (5.6)
Cell count (n = 18)	
Lymphocytes	16/18 (88.9)
Neutrophils	3/18 (11.1)
Lymphocytes, %	90 (67–96)
Protein (n = 21), g/dL	4.70±0.37
Sugar (n = 14), mg/dL	107.0±11.6

Table 1 (continued)

LDH (n = 10), U/L	177 (105–461.5)
Adenosine deaminase (n = 8), U/mL	53 (35.3–87.1)
Triglyceride (n = 33), mg/dL	678 (280.25–1,154)
Cholesterol (n = 25), mg/dL	72 (35.5–106)
Chylomicrons	4/36[d]
Mode of diagnosis	
Pleural fluid AFB smear and/or NAAT positive (n = 36)	
Yes	5/36 (13.9)
No	31/36 (86.1)
Pleural fluid MTB culture positive (n = 37)	
Yes	9/37 (24.3)
No	28/37 (75.7)
Sputum or BAL AFB smear and/or NAAT positive (n = 35)	
Yes	8/35 (27)
No	27/35 (73)
Sputum/BAL AFB smear, NAAT, or sputum/BAL MTB culture positive (n = 35)	
Yes	16/35 (45.7)
No	19/35 (54.3)
Tissue diagnosis of necrotizing granulomas (n = 37)	
Yes	19/37 (51.4)
No	18/37 (48.6)
Site of tissue biopsy yielding diagnosis (n = 16)[e]	
Nodes	10/16 (62.5)
Pleura	5/16 (31.25)
Liver, marrow, spleen	5/16 (31.25)
Lung and pericardium	2/16 (12.5) each
Mode of diagnosis (n = 36)	
Microbiologic alone	18/36 (50)
Pathologic alone	10/36 (27.8)
Both	8/36 (22.2)
Treatment with ATT (n = 21), months	7.57 (6–9)
Chylothorax-specific therapy (n = 35)	
FFD/MFA	22/35 (62.9)
ICD	22/35 (62.9)
Octreotide	3/35 (8.6)
Duct ligation	6/35 (17.1)
Steroids	8/35 (22.9)
Outcome (n = 36)	
Alive	34/36 (94.4)
Dead	2/36 (5.6)

Values are presented as mean ± SE, median (IQR), or n/total n (%). AFB, acid-fast bacilli by Ziehl-Neelsen staining; ATT, anti-tuberculosis therapy; BAL, bronchoalveolar lavage; FFD, fat-free diet; ICD, intercostal tube drainage; IRS, immune reconstitution syndrome; MFA, medium-chain fatty acids; MTB, *Mycobacterium tuberculosis*; NAAT, nucleic acid amplification test. [a] Includes the current case in the analysis. [b] 6/37 (17.1%) ≤16 years. [c] 6/36 (16.7%) with IRS. [d] 11.1% positive. [e] More than one reported in some.

Results

We identified 37 reports of chylothorax associated with TB in 36 case reports, including the present case (see online suppl. Table) [6–40]; we excluded 8 reports containing 12 cases [41–49] because neither the full texts nor abstracts were available, as well as another 5 reports with 5 cases because there was no microbiologic or pathologic evidence of TB [50–54]. The cases where full information was not available were treated as "missing data." There were 20 males and 16 females with a mean age of 39.27 ± 4.13 years; 13.9% (5/36) were children [15, 22, 23, 26]. The patients had had symptoms for a median of 1 month (range 0.1–24 months) prior to presentation to the hos-

Table 2. Causes of chylothorax and expected frequencies

Traumatic chylothorax	≈50%
Noniatrogenic (blunt trauma to chest or vertebrae; knife wound to chest/gunshot wound; childbirth; forceful emesis)	≈12–15%
Iatrogenic (thoracic surgery [esophagectomy, congenital heart disease, lung/mediastinal resections]; head and neck surgery; radiation; pacemaker; central line; embolization of arteriovenous malformations)	35–38%
Nontraumatic chylothorax	≈44%
Malignancy (lymphomas [non-Hodgkin or Hodgkin]; other solid tumors) and other miscellaneous diseases (lymphangioleiomyomatosis; filariasis; sarcoidosis; amyloidosis; tuberculosis; superior vena cava obstruction; constrictive pericarditis; hemangiomatosis [Gorham's disease]/lymphangiomatosis; cardiac failure; transdiaphragmatic movement in cirrhosis)	≈15–20%
Idiopathic	≈6%

pital (Table 1). Constitutional symptoms in the form of fever, anorexia, and weight loss (85.7%; 30/35), dyspnea (60.6%; 20/33), cough (54.5%; 18/33), and chest pain (30.3%; 10/33) were the most common symptoms at presentation. Close contact with a source of pulmonary TB [22, 23, 30] or a prior history of TB [6, 12, 31] was reported by 8.3% (3/36) of the patients each.

Forty-six percent (17/37) of all chylothoraces developed in patients with TB associated with comorbidities; 16.2% (6/37) were in association with HIV infection [12, 23, 25, 26, 33, 38]. A very high proportion also had disseminated TB at diagnosis (45.9%; 17/37), including miliary TB in 18.9% (7/37) [14, 18, 26, 32, 33, 35, 36]. Chylothorax developed in association with chylous ascites in 10.8% (4/37) of the cases [14, 26, 35, 38]. Chylothorax developed on the right side or bilaterally in the majority of cases (78.3%; 29/37). Accompanying parenchymal infiltrates and lymphadenopathy were seen in 48.6% (18/37) and 70.3% (26/37) of the cases, respectively, leading to suspicion of associated TB. However, 45.9% (17/37) of the cases did not have any thoracic infiltrate or mediastinal adenopathy, including 13.5% (5/37) that presented with tubercular empyema alone [10, 21, 27, 29, 31]. The mechanism of development of chylothorax in association with TB was presumed to be due to interruption of lymphatic flow by mediastinal nodes in 51.4% (18/35), abdominal nodes in 14.3% (5/35), extensive pleural disease in 14.3% (5/35), constrictive pericarditis in 2.8% (1/35) [19], and erosion of a spinal abscess into the thoracic duct in 5.6% (2/35) of the cases [8, 12]. The diagnosis of TB was made concurrently with the diagnosis of chylothorax in 72.2% (26/36) of the cases. Chylothorax developed subsequent to a diagnosis of TB on ATT in 27.8% (10/36) of the cases at a median of 6.75 weeks (IQR 4–9) following the initiation of treatment [7, 12–14, 25, 26, 32, 36, 38, 40]. Chylothorax occurred along with an immune reconstitution syndrome (IRS) in 6 (60%; 6/10) of these cases [12, 25, 26, 32, 36, 40], including 3 in immunocompetent individuals [32, 36, 40].

The vast majority of chylothoraces were suspected because of a milky appearance of the pleural fluid (94.4%). Pleural fluid analysis showed exudative effusion in all cases described (mean protein 4.70 ± 0.37 g/dL; median LDH 177 U/L). About 88.9% of the effusions were lymphocytic predominant, with a median of 90% lymphocytes (IQR 67–97). The median triglyceride value observed was very elevated at 678 mg/dL (280.25–1,154). Consequently, chylomicrons were reported in only 11.1% (4/36) of the cases, including the index case [9, 24, 40].

The diagnosis of TB was established microbiologically in 72.2% (26/36) of the cases, with a positive pleural fluid or sputum, bronchoalveolar lavage, gastric aspirate, or tissue mycobacterial culture, or a positive nucleic acid amplification test. Tuberculous empyema was associated with chylothorax in 25% (9/36) of the cases, including the index case [10, 13, 21, 24, 27–29, 31, 35]. Pathologic biopsies of the involved organ alone established the diagnosis of TB in a further 27.8% (10/36) [6, 7, 16, 18–20, 31, 34, 37, 39].

ATT was administered for a median of 7.57 months (IQR 6–9). A fat-free or medium-chain fatty acid diet was administered in 62.9% (22/35) of the cases. Steroids were given in 22.9% (8/35) of the cases, often for suspected IRS (37.5% [3/8]) or endobronchial TB. Chylothorax increased or developed on ATT alone in 25% (9/36) of the cases, including in 16.7% (6/36) with IRS. However, tho-

racic duct ligation [12, 14, 20, 24, 36, 40] and octreotide [22, 26, 36] were required in only 17.1% (6/35) and 8.6% (3/35) of the cases, respectively. In all, 94.4% (34/36) of the cases had good resolution of chylothorax and completed treatment successfully; only 5.6% (2/36) died [18, 27].

Discussion

A wide variety of etiologies can cause nontraumatic chylothorax (Table 2). In a large series of patients with chylothorax ($n = 203$), none were reported to have chylothorax due to TB [55]. Our systematic review suggests that chylothorax is a very rare but well-described complication of TB [6–40]. The occurrence of an acute effusion with smooth unthickened pleural surfaces along with demonstration of elevated triglyceride (≥110 mg/dL) and low cholesterol levels (pleural/serum cholesterol <1) or demonstration of chylomicrons by lipoprotein electrophoresis differentiates a chylothorax from a pseudochylothorax [3]. The absence of long-standing pleural effusion prior to the current illness and clear biochemical evidence and definitive demonstration of chylomicrons along with radiological features suggestive of TB *and* microbiologic demonstration of MTB established the association of chylothorax with TB in the index case. Pleural thickening was due to TB and *Candida albicans*-related empyema; it has been reported earlier in association with TB-chylothorax [31, 40].

Several mechanisms have been described for the development of chylothorax in TB. These include (1) mediastinal adenopathy with occlusion or erosion into the thoracic duct and leakage of chyle into the pleural space, (2) abdominal lymphadenopathy with occlusion of the cisterna chyli and opening of lymphaticovenous anastomosis, and (3) constrictive pericarditis associated with elevated left subclavian venous pressures leading to increased production and reduced drainage of lymph. The observation of exudates in all cases of TB-related chylothorax reported in the present systematic review, however, suggests a direct involvement of the pleura and/or lymphatic ducts due to TB or host-immune responses as the primary cause in most patients. Other mechanisms are also evident from this systematic review; these include erosion of the thoracic duct by spinal abscesses, extensive pleural disease with lymphatic exudation, and duct damage due to IRS. The occurrence of chylothorax subsequent to initiation of ATT in 27.8% (10/36) of the patients, the absence of any associated mediastinal or abdominal adenopathy in 35%, the very high proportion of cases with disseminated TB (45.9%; 17/37) in apparently immunocompetent individuals presenting with chylothorax, including miliary TB (18.9%; 7/37), and the development of chylothorax during immune reconstitution suggest direct or host-immune damage to the thoracic duct as a major mechanism for the development of chylothorax. The median time to development of chylothorax observed in our systematic review was 6.75 weeks (IQR 4–9), which is the most common time period of immune reconstitution; IRS can occur in 2.4–28% of immunocompetent individuals with lymph nodal [40] or pulmonary TB [56].

The occurrence of chylothorax was suspected due to obvious milky pleural fluid, suggesting an underrecognition of chylothorax in TB. Tuberculous empyema was associated with chylothorax in 25% (9/36) of the cases, including 13.9% (5/36) where this was the sole manifestation. Given the disseminated paucibacillary disease in a significant majority, biopsies of involved tissues like nodes and the pleura were needed in 50% (18/36) of the cases, with 22.2% (10/36) being diagnosed only by pathology. Lymphangiograms were reported only for 8.6% (3/35) [6, 14, 37], and the site and cause of leakage of chyle was presumptive in most reported cases.

Steroids were administered in 22.9% (8/35) of the cases for IRS, bronchial stenosis, and severe disseminated TB with variable responses in reduction of chylothorax. The need for thoracic duct interruption was reported only for 17.1% (6/35) of the cases, with success in 83.3% (5/6) of these, indicating that chylothorax may resolve with a fat-free diet and ATT in the majority of cases. TB-chylothorax is associated with good prognosis with conservative treatment despite the higher proportion with disseminated disease at diagnosis and associated comorbidities. The survival rate was 94.4%.

Conclusions

Chylothorax is a very rare but well-described complication of TB. It develops with extensive mediastinal or abdominal lymphadenopathy and extensive pleural disease, but it may also develop without any obvious lymphadenopathy and as part of an IRS. ATT and dietary manipulation – followed by duct ligation if needed in a small minority of cases – are associated with good outcome and low mortality.

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Author Contributions

All authors were responsible for patient care and initiated manuscript preparation. S.R. and R.K. performed the systematic review, retrieving the articles of our systematic search and abstracting data. S.R. and R.K. take responsibility for the content of the paper, including the data and analysis.

Financial Disclosure and Conflicts of Interest

No funding was utilized for this manuscript and the authors have no conflicts of interest to disclose.

References

1 Arinaminpathy N, Batra D, Khaparde S, Vualnam T, Maheshwari N, Sharma L, Nair SA, Dewan P: The number of privately treated tuberculosis cases in India: an estimation from drug sales data. Lancet Infect Dis 2016; 16:1255–1260.
2 WHO: Global Tuberculosis Report 2016. 2016. http://apps.who.int/medicinedocs/documents/s23098en/s23098en.pdf.
3 Hillerdal G: Chylothorax and pseudochylothorax. Eur Respir J 1997;10:1157–1162.
4 Romero S: Nontraumatic chylothorax. Curr Opin Pulm Med 2000;6:287–291.
5 Ferrer J: Pleural tuberculosis. Eur Respir J 1997;10:942–947.
6 Vennera MC, Moreno R, Cot J, Marin A, Sanchez-Lloret J, Picado C, Agusti-Vidal A: Chylothorax and tuberculosis. Thorax 1983;38: 694–695.
7 Zafar A, Gul M, Zaman R: Chylothorax. J Ayub Med Coll Abbottabad 1988;3:27–28.
8 Menzies R, Hidvegi R: Chylothorax associated with tuberculous spondylitis. Can Assoc Radiol J 1988;39:238–241.
9 Haro M, Ruiz J, Álvarez J, Gallego M: Quilotórax tuberculoso. Enferm Infecc Microbiol Clin 1994;12:417–418.
10 Antón PA, Rubio J, Casán P, Franquet T: Chylothorax due to *Mycobacterium tuberculosis*. Thorax 1995;50:1019.
11 Deniel F, Rabbat A, Laaban JP: Spontaneous bilateral chylothorax revealing disseminated tuberculosis complicated by massive pulmonary embolism (in French). Rev Mal Respir 1999;16:836–838.
12 Singh S, Girod JP, Ghobrial MW: Chylothorax as a complication of tuberculosis in the setting of the human immunodeficiency virus infection. Arch Intern Med 2001;161:2621.
13 Prasad RS, Fraser MH, Urquhart GD, McLean AN: Rupture of tuberculous spinal abscess resulting in tuberculous empyema and chylothorax. Spinal Cord 2003;41:410–412.
14 Tomić I, Plavec G, Karlicić V, Spasić V, Rusović S, Stanić V, Cvijanović V, Ristanović A: Chylous effusions (in Serbian). Vojnosanit Pregl 2003;60:613–620.
15 Gie RP, Goussard P, Kling S, Schaaf HS, Beyers N: Unusual forms of intrathoracic tuberculosis in children and their management. Paediatr Respir Rev 2004;5(suppl A):S139–S141.
16 Gupta D, Maheshwari U, Aggarwal AN: Tuberculosis chylothorax. Lung India 2004;21: 54–55.
17 Chen HA, Cheng NC, Lin KP, Liao HT, Chen CH, Huang DF: Mononeuropathy multiplex and chylothorax as earlier manifestations of pulmonary tuberculosis. J Intern Med 2005; 257:561–563.
18 Saumoy M, Mirón M, Oltra C, Vidal F, Richart C: Tuberculous chylothorax: case report and review of the literature (in Spanish). An Med Interna 2005;22:238–240.
19 Singh P, Shah KV, Shah NT, Chauhan JB, Shah B, Jindal S, Gami S: Chylothorax: a rare presentation of constrictive pericarditis. Indian J Tuberc 2005;52:207–210.
20 Baig F, Khan SG, Warind J: Tuberculous chylothorax. Chest DOI: 10.1378/chest.130.4_MeetingAbstracts.304S-a.
21 Büyükşirin M, Konya A, Polat G, Ürpek G, Tibet G: Case of chylothorax due to *Mycobacterium tuberculosis*. Toraks Dergisi 2006;7: 216–217.
22 Cakir E, Gocmen B, Uyan ZS, Oktem S, Kiyan G, Karakoc F, Ersu R, Karadag B, Dagli T, Dagli E: An unusual case of chylothorax complicating childhood tuberculosis. Pediatr Pulmonol 2008;43:611–614.
23 Grobbelaar M, Andronikou S, Goussard P, Theron S, Mapukata A, George R: Chylothorax as a complication of pulmonary tuberculosis in children. Pediatr Radiol 2008;38:224–226.
24 Karapolat S, Sanli A, Onen A: Chylothorax due to tuberculosis lymphadenopathy: report of a case. Surg Today 2008;38:938–941.
25 Lin JN, Lai CH, Chen YH, Chang LL, Lee SS, Lin HH: Immune reconstitution inflammatory syndrome presenting as chylothorax in a patient with HIV and *Mycobacterium tuberculosis* coinfection: a case report. BMC Infect Dis 2010;10:321.
26 Rabie H, Lomp A, Goussard P, Nel E, Cotton M: Paradoxical tuberculosis associated immune reconstitution inflammatory syndrome presenting with chylous ascites and chylothorax in a HIV-1 infected child. J Trop Pediatr 2010;56:355–358.
27 Hijazi EM, Hamouri S: Post-coronary artery bypass grafting chylothorax with tuberculous empyema thoracis. Am J Case Rep 2011;12: 184–185.
28 Kant S, Verma SK, Anand SC, Prasad R, Verma RK: Development of bilateral chylothorax in a younger female secondary to tuberculosis. Lung India 2011;28:56–59.
29 Ananthan VS, Siva KK: A rare case of acid-fast bacilli in chylothorax. Lung India 2012;29: 166–168.
30 Tan GP, Tai DYH, Nallakaruppan VDO: A case of chylothorax. Chest DOI: 10.1378/chest.1383008.
31 Yasaratne BMGD, Madegedara RMD: Chylothorax; a description of four cases and a brief review. J Ceylon Coll Phys 2012;43:49–53.
32 Ip H, Schomberg LE, Ahmed L: Pleural effusions in a patient with tuberculosis on dialysis. Lancet 2013;382:570.
33 Bielsa S, Pardina M, Porcel JM: Chylothorax due to enlarged tuberculous lymph nodes. BMJ Case Rep 2014;2014:bcr2014204582.
34 Hamad WB, Amar JB, Dhahri B, Baccar MA, Azzabi S, Aouina H, Bouacha H: Spontaneous chylothorax revealing a mediastinal and abdominal lymph node tuberculosis. Eur Respir J 2014;44(suppl 58):P2648.
35 Kim KJ, Park DW, Choi WS: Simultaneous chylothorax and chylous ascites due to tuberculosis. Infect Chemother 2014;46:50–53.
36 Akthar M, Lardizabal A, Patrawalla AS, Emeh OK, Bolanowski PJ, Mangura B: Disseminated tuberculosis complicated by chylothorax. American Thoracic Society International Conference, May 15–20, 2015, abstract A3259. https://cms.psav.com/cPaper2015/myitinerary/session-11780.html?congress=ats2015.

37 Kutlu O, Demirbas S, Sakin A: Chylothorax due to tuberculosis lymphadenitis. North Clin Istanb 2017;3:225–228.
38 Del Cueto-Aguilera Á, Ibarra-Sifuentes H, Delgado-García G, Atilano A, Galarza-Delgado D: Chylothorax and chylous ascites due to *Mycobacterium tuberculosis* in an AIDS patient whose PCR tested negative. Pneumologia 2016;65:161–163.
39 Amar JB, Zaibi H, Dahri B, Aouina H: Spontaneous chylothorax revealing a mediastinal and abdominal lymph node tuberculosis. Indian J Tuberc 2017;64:141–143.
40 Duraikannan P, Saheer S, Balamugesh T, Christopher DJ: Rare cause of paradoxical worsening of pleural effusion in a patient with tuberculosis. Lung India 2017;34:167–169.
41 Brandt M: Ein Beitrag zur Kasuistik der Ductus Thoracicus-Tuberkulose und des Chylothorax; Inaug Diss Heidelberg, Heidelberg, Universitäts-Buchdruckerei von J Horning, 1917.
42 Kaplan M, Grumbach R, Grenier B: A case of right chylothorax during tuberculous primary infection (in French). Arch Fr Pediatr 1953;10:1060–1065.
43 Mas Ramos A: Chylothorax and primary tuberculosis infection; brief summary of the clinical entity of chylothorax and case report (in Spanish). Rev Esp Tuberc 1954;23:123–130.
44 Bujko K, Kruc S, Michalowicz R: Pleural chylothorax in a child with lymph node tuberculosis (in Polish). Pediatr Pol 1964;39:961–964.
45 Leutke HJ, Sendel A: Chylothorax due to erosion of the thoracic duct in lymph node tuberculosis (in German). Med Welt 1966;40:2117–2120.
46 Kovarik JL: Spontaneous right chylothorax with middle lobe syndrome. Report of a case. Dis Chest 1967;52:261–263.
47 van der Heijden AH, Hamersma WB: Chylothorax as a rare complication in tuberculosis (in Dutch). Ned Tijdschr Geneeskd 1984;128:2260–2262.
48 Li LY, Zhao QX, Luo WC: An analysis of 30 cases of chylothorax and chyloperitoneum (in Chinese). Zhonghua Nei Ke Za Zhi 1991;30:347–349, 382.
49 El Hammoumi MM, Drissi G, Achir A, Benchekroun A, Kabiri EH, Benosman A: Spontaneous bilateral chylothorax revealing a mediastinal and abdominal lymph node tuberculosis (in French). Rev Pneumol Clin 2014;70:173–176.
50 Menon RK, Aziah M, Kuppusamy I: Chylothorax secondary to tuberculosis. Med J Malaysia 1995;50:425–426.
51 Anwar S, Rahman AKMM, Rahman ME, Mollah AH, Matin A, Bilqis L: Bilateral chylothorax due to disseminated tuberculosis and acute lymphoblastic leukemia in a young boy: a case report and literature review. Bangladesh J Child Health 2014;38:164–168.
52 Naeem M, Sobani ZA, Zubairi A, Fatmi S, Khan JA: Constrictive pericarditis presenting as chylothorax. Singapore Med J 2011;52:e187–e189.
53 Summachiwakij S, Tungsubutra W, Koomanachai P, Charoenratanakul S: Chylous ascites and chylothorax due to constrictive pericarditis in a patient infected with HIV: a case report. J Med Case Rep 2012;6:163.
54 Büyükşirin M, Polat Gl, Usalan A, Aydoğdu Z: Chylothorax and sarcoidosis: case report. Tur Toraks Der 2011;12:117–119.
55 Doerr CH, Allen MS, Nichols FC 3rd, Ryu JH: Etiology of chylothorax in 203 patients. Mayo Clin Proc 2005;80:867–870.
56 Rajagopala S, Chandrasekharan S: Severe *Mycobacterium tuberculosis*-related immune reconstitution syndrome in an immunocompetent patient. Indian J Crit Care Med 2015;19:50–52.

ORIGINAL ARTICLE

Pulmonary Nocardiosis Presenting as Exacerbation of Chronic Pulmonary Disease

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Abstract

Background: Pulmonary nocardiosis (PN) occurs in chronic pulmonary disease (CPD) in the absence of traditional risk factors. Clinical features that differentiate bacterial exacerbations (AE-CPD_b) from PN-related exacerbations (AE-CPD_{PN}) are not well described.

Objectives: To describe a series of AE-CPD_{PN} without traditional risk factors and compare clinical features, radiology and outcomes with age, gender and CLD-type matched AE-CPD_b.

Materials and methods: Single-center retrospective review and case-control study.

Results: AE-CPD_{PN} had longer duration of symptoms and more leukocytosis at hospitalization. AE-CPD_b patients were sicker with more chronic respiratory failure (OR 33.3, $p = 0.01$), cardiac disease and pulmonary hypertension (OR 6.2, $p = 0.008$) at diagnosis. More patients with AE-CPD_b were discharged on domiciliary oxygen (OR 5.27, $p = 0.01$). On logistic regression, AE-CPD_{PN} was independently associated with mechanical ventilation (OR 22.3, $p = 0.01$), length of hospital stay (median difference, 4 days, $p = 0.016$) but not to hospital mortality. 22.7% of AE-CPD_{PN} died. Respiratory failure requiring oxygen, NIPPV or mechanical ventilation was associated with mortality in AE-CPD_{PN}.

Conclusion: PN is a rare cause of AE-CPD and can be suspected by longer symptom duration, more leukocytosis, consolidation and cavitation. AE-CPD_{PN} is associated with longer hospital stay and mechanical ventilation. Respiratory failure is associated with mortality in AE-CPD_{PN}.

Key messages:

- Pulmonary nocardiosis can present in advanced chronic lung disease as an exacerbation in the absence of traditional risk factors like immunosuppression.
- Bronchiectasis, followed by chronic obstructive pulmonary disease are the most common chronic lung disease risk factors.
- Pulmonary nocardiosis is a rare cause of acute exacerbation of chronic pulmonary disease (CPD).
- Compared to exacerbations of CPD due to bacterial infections, nocardiosis-related exacerbations (CPD_{PN}) were independently related to need for mechanical ventilation and length of hospital stay.
- Respiratory failure requiring oxygen, noninvasive ventilation and mechanical ventilation are associated with mortality in AE-CPD_{PN}.

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Introduction

Acute exacerbation of chronic pulmonary disease (AE-CPD) leads to substantial worsening in quality of life, need for additional therapies, morbidity, mortality and costs.[1] Severe exacerbations are associated with respiratory failure, need for mechanical ventilation and mortality.[2] Pulmonary nocardiosis, the most frequent presentation of human infection by *Nocardia* spp, is increasingly being recognized in patients with CPDs, including chronic obstructive pulmonary disease (COPD).[3] Predisposing factors include bacterial colonization of lower airways with advanced lung disease, oral steroid use for exacerbations and structural lung disease in the form of associated bronchiectasis in severe COPD.[4] Most series of PN in CPDs include patients with associated traditional predisposing factors like steroid therapy, solid-organ or hematopoietic cell transplantation, human immunodeficiency virus infection or malignancy; mortality determinants were thus affected by these associated risk factors.[4] The clinical features, radiologic findings, laboratory clues that should suggest the presence of PN in AE-CPD (AE-CPD_{PN}) and its outcomes are not well described. We describe a series of AE-CPD_{PN} in the absence of traditional risk factors to determine clinical features that can distinguish it from bacterial exacerbations of CPD (AE-CPD_b) and determine the association of AE-CPD_{PN} with need for mechanical ventilation, hospital length of stay and mortality.

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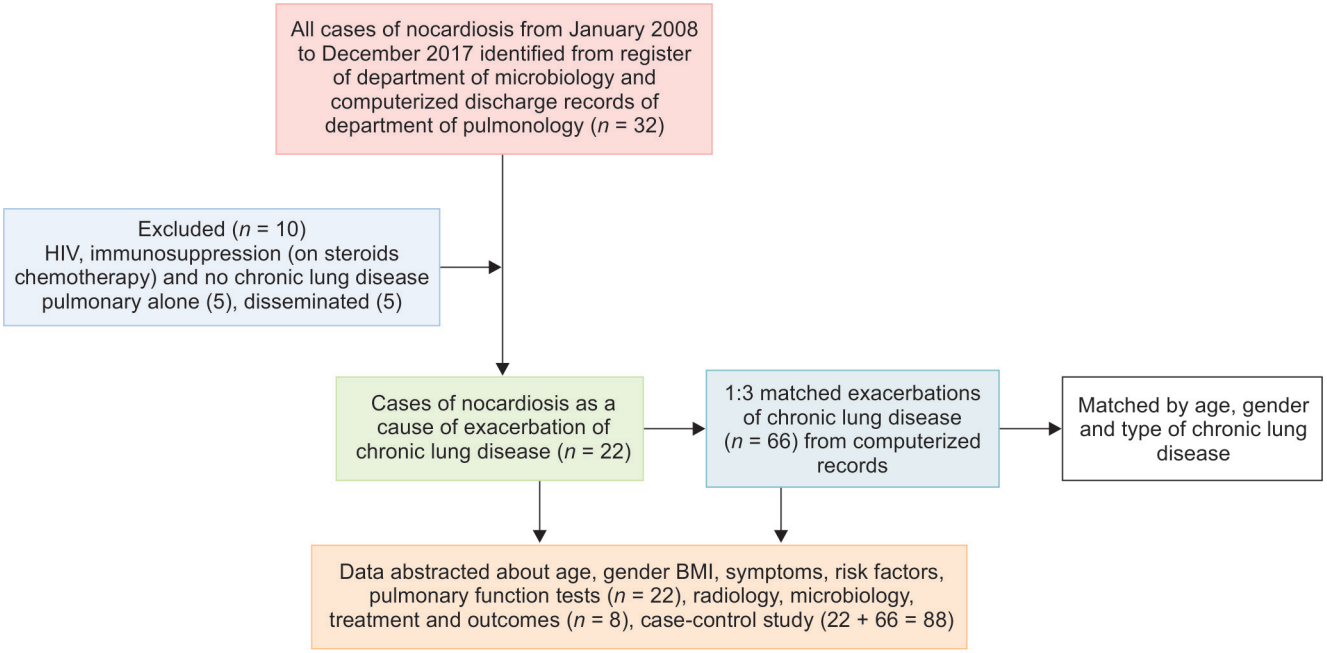
Conflict of interest: None

Materials and Methods

This is a retrospective study of patients ≥18 years and a final diagnosis of PN and AE-CPD from a large tertiary care hospital in southern India over 10 years between January 2008 and December 2017. All cases with PN were identified by an electronic search of our records and cross-referenced by a simultaneous manual search of the nocardiosis register in the department of microbiology. Clinical records of all identified cases were retrieved. Only PN presenting

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Flowchart 1: Number of enrolled patients in the study



with worsening of underlying CPD was included. PN associated with long-term steroid therapy, immunosuppressive therapy, human immunodeficiency infection (HIV) and malignancy and PN in the absence of CPD was excluded (Flowchart 1).

Study Subjects

Case Series

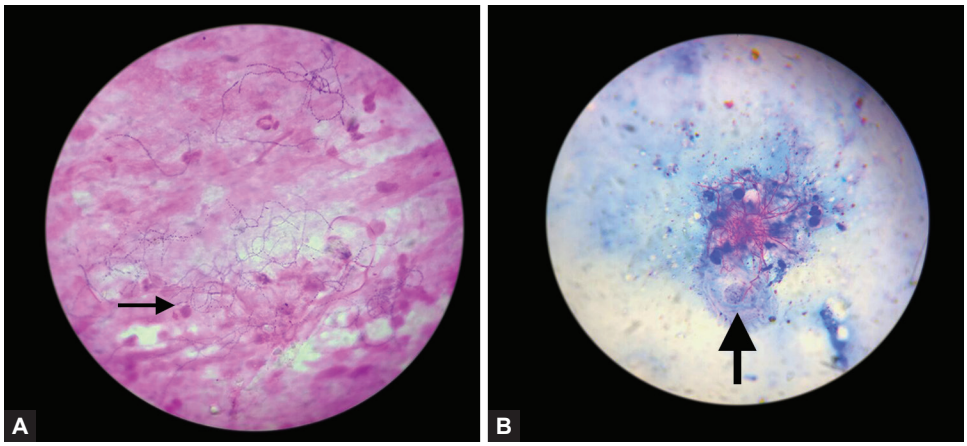
CPD was defined by the presence of one of the following conditions; COPD, bronchial asthma, bronchiectasis, interstitial lung disease (ILD) and post-tuberculosis lung disease. The diagnosis of COPD, bronchial asthma, bronchiectasis and ILD was made according to standard guidelines.[1,5,6] The diagnosis of obstruction and restriction on spirometry was according to ATS/ERS guidelines.[7] An exacerbation of CPD was defined as worsening of respiratory symptoms that resulted in additional therapy.[1] If the initial diagnosis of CPD was made during an exacerbation of CPD, spirometry performed subsequently during stable disease, where available, was taken for data abstraction. PN was diagnosed on the basis of (i) presence of compatible respiratory symptoms and (ii) isolation of *Nocardia* spp. in smears and/or cultures of respiratory samples or (iii) histopathology of transthoracic or bronchoscopic biopsies showing organisms consistent with *Nocardia* spp.[8] Respiratory samples included sputum, endotracheal aspirates, bronchoalveolar lavage fluid (BALF) and pleural fluid. Nocardiosis was diagnosed as "disseminated" if there was isolation in blood culture or demonstrable infection in other organs, including skin and central nervous system in association with respiratory infection.[8] Recent steroid intake was defined as steroid intake in the 3 months prior to diagnosis of PN. We excluded any patient with steroid intake >7.5 mg in 3 months prior to the diagnosis of pulmonary nocardiosis to eliminate oral steroid as a confounding factor for AE-$CPD_{PN.}$ Chronic respiratory failure was defined by an arterial blood gas (ABG) with PaO_2 ≤55 mm Hg or <60 mm Hg in the presence of pulmonary hypertension; $PaCO_2$ ≥45 mm Hg with or without pH ≤7.36 in the absence of symptoms suggestive of an acute deterioration.[1,9]

Controls

Controls were patients with COPD, bronchiectasis, post-tuberculosis lung disease and ILD without any Immunocompromising condition, including receipt of steroids in 3 months prior to study inclusion. Only exacerbations that were microbiologically proven or clinically presumed to be due to bacterial infection (AE-CPD_b) were included. Receipt of antibiotics was taken as a surrogate for clinical presumption of infection; any controls with suspected or proven exacerbations due to viral infections, heart failure or pulmonary embolism were excluded. Patients with a presumed bacterial infection were included only if they had a complete clinical response to antibiotics and supportive care alone; outpatient follow-up notes and radiology were retrieved and reviewed for all controls for confirmation. Controls with exacerbations and radiological infiltrates were included in an attempt to match illness severity between cases and controls. Three controls were identified for each case and matched by age, gender and type of chronic lung disease.

Microbiological Identification

Samples collected included sputum, endotracheal aspirates, BALF and pleural fluid under sterile precautions. The samples were examined by direct microscopy after Gram's staining to establish specimen quality. Modified Kinyoun's stain was also done if nocardiosis was suspected clinically or if Gram's stain showed organisms compatible with *Nocardia* spp. All samples were then homogenized with sterile glass beads in a test tube and incubated in blood agar and Sabouraud's dextrose agar at 37°C in an aerobic atmosphere for up to 4 weeks and examined every alternate day for growth. The presumptive identification of *Nocardia* spp. was based on the microscopy showing branching gram-positive thin filamentous rods (Fig. 1A), partially acid-fast with modified Kinyoun's stain (Fig. 1B) and the macroscopic morphology of buff or pigmented waxy cerebriform colonies. Identification at the species level was done using biochemical testing. From 2019 onwards, 16s rRNA based-DNA sequencing was implemented for speciation (outside study period).



Figs 1A and B: Composite image with Gram's staining of sputum sample. (A) Branching of gram-positive thin filamentous organisms consistent with *Nocardia* spp; (B) Modified acid-fast staining confirmed that these organisms were acid-fast

Treatment Protocol for PN

All admitted patients received meropenem 1 g every 8 hours for 2–4 weeks, along with oral or parenteral cotrimoxazole (15 mg/kg trimethoprim equivalent in three divided doses); cotrimoxazole alone was then continued for the remaining duration of 6 months. All patients with PN were admitted only till clinical stabilization as defined by absence of fever, normal hemodynamics and need for minimal or no oxygen; parenteral antibiotics were continued on ambulatory basis or arranged at a center of convenience for the prescribed period. In mild PN treated as outpatient basis, cotrimoxazole alone (10 mg/kg trimethoprim equivalent in three divided doses) was administered. In cases of suspected or proven disseminated nocardiosis, amikacin 15 mg/kg was added for the initial period and the initial intensive phase was extended from four to 6 weeks.[10,11]

Data Extraction

Data was abstracted in a predefined data extraction form. From the selected cases, the following data were gathered: (a) demographic details of each case including age and gender, (b) duration of symptoms, (c) symptoms at presentation, (d) type of chronic lung disease, (e) body mass index, (f) co-morbidities and their details, including smoking, alcohol abuse, diabetes, chronic kidney disease, steroid receipt in the last 6 months before diagnosis and concurrent malignancy, (g) diagnosis of chronic respiratory failure before the diagnosis of PN, (h) spirometry values, (i) laboratory investigations, including total leukocyte counts, arterial blood gas analysis, (j) radiologic findings and echocardiography, (k) microbiologic isolates, (l) treatment administered, including details of steroids, antimicrobial therapy and their duration, oxygen use, noninvasive or invasive ventilation and its duration, and (m) outcomes; discharge on domiciliary oxygen and hospital mortality.

Outcome Measures

The duration of symptoms prior to presentation, symptoms, laboratory findings, radiologic findings, treatment administered and outcomes were compared between patients with AE-CPD_b and AE-CPD_{PN}

Statistics

Statistical analyses were performed using SPSS version 14. Continuous variables were described in a descriptive fashion (mean ± SD, median, IQR) and discrete variables were described as frequency proportions. Comparisons for continuous variables were performed using the independent T test or Mann–Whitney test and proportions using the Chi-square test or Fisher's exact test, where appropriate. Statistical significance was assessed at the 2-sided $p \leq 0.05$ level. The independent association of AE-CPD_{PN} with the need for noninvasive or invasive mechanical ventilation and mortality was tested by logistic regression.

Ethics

The Research Ethics Committee of our Hospital approved this study. All data abstracted was anonymized, thus ensuring confidentiality and patient privacy.

Results

There were a total of 32 cases of PN over a 10-year period. We excluded 10 cases, including five with disseminated nocardiosis without coexisting CPD. There were a total of 22 cases of AE-CPD_{PN}, including 13 men and 9 women; all had localized pulmonary disease. The type of CPD was most commonly bronchiectasis (59%, 13/22), followed by COPD (36.4%, 8/22) and ILD (1/22, 4.5%) (Table 1A). Bronchiectasis was cystic (CB) and multilobar in all and the etiology was idiopathic, post-tuberculosis or allergic bronchopulmonary aspergillosis (ABPA)-related. 21/22 of AE-CPD_{PN} had inhaled corticosteroids (ICS) use compared to 63/66 of the controls; these were patients with advanced airway disease and were on triple therapy and ICS use was not different in cases compared to controls. The final 22 AE-CPD_{PN} included did not have any oral steroid intake in the 12 months prior to the diagnosis of PN. The patient with AE-CPD_{PN} related to ILD was steroid-naïve and the diagnosis of ILD was made concurrent with the diagnosis of PN. Two patients with AE-CPD_{PN} received steroids for severe bronchospasm with a diagnosis of AE-CPD_b before the diagnosis of PN; in both, steroids were discontinued after diagnosis. Among patients with AE-CPD_b, *Pseudomonas aeruginosa* was the most common organism isolated. 27.2% (6/22) of the patients with AE-CPD_{PN} had a presumed or proven bacterial coinfection. All patients with AE-CPD_{PN} were treated with cotrimoxazole for a median (IQR) duration of 6(2) months. Two patients were subsequently treated with other regimens after discontinuation of cotrimoxazole; reasons included inability to tolerate cotrimoxazole due to persistent severe hyperkalemia and in another, the isolate was reported as *N. farcinica* resistant to cotrimoxazole in association with clinical failure.

Table 1A: Comparison between patients with exacerbations of chronic lung disease due to Nocardia and bacterial exacerbations of chronic lung disease

Characteristics	*Nocardia related exacerbation (n = 22)*	*Bacterial exacerbations (n = 66)*	*P value, **OR 95% CI*
Age years, mean ± SE	61.36 ± 2.9	60.5 ± 1.7	0.79
Gender, male: female	13:9	39:27	1.00
Etiology of chronic lung disease before diagnosis	COPD (8/22), BXSIS (13/22), ILD (1/22)	COPD (24/66), Bronchiectasis (39/66), ILD (3/66)	1.00
Body mass Index kg/m^2 mean ± SE	18.6 ± 1.2	21.3 ± 0.9	0.08
Risk factors			
	Smoking (8/22, 36.4%), alcohol abuse (3/22, 13.6%)	Smoking (28/66, 42.4%), alcohol abuse (8/66, 12.1%)	N.S
	*Recent steroid intake (2/22, 9.1%)	Recent steroid intake (6/66, 9.1%)	1.00
	Diabetes (6/22, 27.2%)	Diabetes (16/66, 24.2%)	N.S
	Prior tuberculosis (9/22, 40.9%)	Prior tuberculosis (14/66, 21.2%)	0.08
Symptoms at presentation			
Fever	10/22 (45.4%)	19/66 (43.9%)	0.07
Cough	20/22 (90.9%)	60/66 (90.9%)	0.83
Dyspnea	16/22 (72.7%)	61/66 (92.4%)	OR 0.17 (0.04–0.69), $p = 0.01$
Chronic respiratory failure before diagnosis	0/22 (0%)	28/66 (42.4%)	OR 0.03 (0.0–0.51), $p < 0.001$
Duration before presentation, days (Median, IQR)	12, (23)	7, (8)	0.001
Spirometry			
FEV/FVC%, mean ± SE	68 ± 7.23	63.2 ± 4.95	0.65
FEV1 L, % mean ± SE	1.01 ± 0.05L, 53.6 ± 2.4%	1.1 ± 0.1 L, 45.4 ± 4.9%	0.71
FVC L, % mean ± SE	1.5 ± 0.1 L, 58.0 ± 2.1%	1.7 ± 0.2 L, 54.4 ± 5.8%	0.55
Total leukocyte count/µL, Mean ± SE	17985 ± 228/µL	12426 ± 65/µL	0.002
N%, Absolute neutrophil count/µL, mean ± SE	83.6 ± 2.8%, 14420 ± 238/µL	78.6 ± 1.4%, 10027 ± 63/µL	0.01
Hemoglobin g/dL, mean ± SE	11.8 ± 0.4	12.4 ± 0.2	0.24
Erythrocyte sedimentation rate mm/hr, mean ± SE	62.2 ± 5.9	37.1 ± 3.3	<0.001
Renal failure at diagnosis	2/22 (9.1%)	22/66 (33.3%)	OR 0.2 (0.04–0.9), $p = 0.02$
Arterial blood gas at diagnosis			
pH, mean ± SE	7.43 ± 0.01	7.36 ± 0.01	0.016
paO_2 mm Hg, mean ± SE	73.2 ± 5.3	88.8 ± 4.9	0.14
$paCO_2$ mm Hg, mean ± SE	38.9 ± 3.3	51.6 ± 2.2	0.013
HCO_3 mEq/L, mean ± SE	25.0 ± 1.5	28.3 ± 0.8	0.06

There was no difference in body-mass index, lung function at baseline, presence of obstruction on spirometry, presence of risk factors like smoking, alcohol abuse, recent steroid intake, diabetes or prior tuberculosis between AE-CPD_b, and AE-CPD_{PN} (Table 1B). When compared to age, gender and type of CPD-matched AE-CPD_b, AE-CPD_{PN} had longer symptom duration (median difference 5 days, $p = 0.014$) prior to presentation. There was more leukocytosis (mean difference 5559/µL, $p = 0.002$), neutrophilia (mean difference 4373/µL, $p = 0.01$) and higher erythrocyte sedimentation rate (mean difference 25.1 mm/hour, $p < 0.001$) at hospitalization. Patients with AE-CPD_b had higher proportion with dyspnea at presentation (OR 4.57, $p = 0.02$), chronic respiratory failure prior to diagnosis of bacterial exacerbation (OR 33.3, $p = 0.01$), and more respiratory acidosis (pH difference –0.04, $p = 0.016$, $PaCO_2$ difference 12.7 mm Hg, $p = 0.01$) at diagnosis. More patients with AE-CPD_b had acute kidney injury (AKI, O.R 5.0, 95% CI 1.07–23.3, $p = 0.04$) at diagnosis, with 22.7% (5/22) requiring dialysis. Two patients with AE-CPD_{PN} had renal failure, with both requiring dialysis. There was also higher proportion of left ventricular systolic or diastolic dysfunction (OR 5.27, 95% CI 1.34-20.7, $p = 0.01$) and pulmonary hypertension (OR 6.2, $p = 0.008$) in patients with AE-CPD_b.

On imaging, AE-CPD_{PN} had higher proportion of consolidation (95.5%) and cavitation (27.3%). Consolidation was mass-like, multilobar or multifocal in all patients with AE-CPD_{PN}. (Figs 2 and 3). On univariate analysis, there was no difference in the need for oxygen, noninvasive ventilation or mechanical ventilation between

Table 1B: Comparison of findings and clinical course between patients with exacerbations of chronic lung disease due to Nocardia and bacterial exacerbations of chronic lung disease

Characteristics	*Nocardia related exacerbation*	*Bacterial exacerbations*	*P value, OR 95% CI*
Arterial blood gas at presentation	Hypoxemia (69.2%), type 1 (23.1%), type 2 failure (7.7%)	Hypoxemia (28.6%), type 1 (16.1%), type 2 failure (55.4%)	**OR 0.05, (0.007–0.44), $p = 0.006$
Radiological findings at diagnosis			
Consolidation	21/22 (95.5%)	53/66 (80.3%)	0.06
Cavitation	6/22 (27.3%)	4/66 (6.1%)	5.81 (1.46–23.1), $p = 0.01$
Pleural effusion	2/22 (9.1%)	2/66 (3%)	0.26
Cardiac findings			
Cardiac disease	LVSD (0/17), LVDD (3/17, 17.6%)	LVSD (5/49, 10.2%), LVDD (21/49, 42.8%)	OR 0.18 (0.04–0.74), $p = 0.01$
Pulmonary hypertension	3/17 (17.6%)	28/49 (57.1%)	OR 0.16 (0.04–0.63), $p = 0.008$
Microbiological evaluation			
Etiology of exacerbations	*Nocardia* (22/22, 100%), bacterial coinfections (6/22, 27.2%)	No growth (49/66, 74.3%), *Pseudomonas* 9/66 (13.6%), others (6/66, 9.1%)	NA
Treatment details			
New respiratory failure needing oxygen	9/22 (40.9%)	21/66 (33%)	O.R 1.48, $p = 0.44$
Oxygen duration days, median, IQR	5 (10.75)	5 (3)	0.50
NIPPV	5/22 (22.7%)	23/66 (34.8%)	0.28
NIPPV duration days, median, IQR	4 (7)	6 (2.5)	0.29
Mechanical ventilation	5/22 (22.7%)	6/66 (9.1%)	O.R 2.94 (0.79–10.8), $p = 0.10$
Mechanical ventilation duration days, median, IQR	2 (5.75)	3.5 (1.75)	0.32
Hospital length of stay days, Median, IQR	8 (9)	5 (4)	0.016
Antibiotic receipt	Cotrimoxazole (20/22), minocycline (1/22), others (1/22)	Penicillins (29/66, 43.9%), Cephalosporins (25/66, 37.8%), FQ (8/66, 12.1%)	NA
Duration of cotrimoxazole months median, IQR	6 (2)	NA	NA
Steroids during exacerbation	2/22 (9.1%)	26/66 (39.4%)	OR 0.15 (0.03–0.71), $p = 0.01$
Discharged on home oxygen	3/17 (17.6%)	35/66 (53%)	OR 0.18 (0.04–0.72), $p = 0.01$
Alive at hospital discharge	17/22 (77.3%)	66/66 (100%)	OR 41.8 (2.2–792.7), $p = 0.01$

**Odds ratios are presented for significant associations

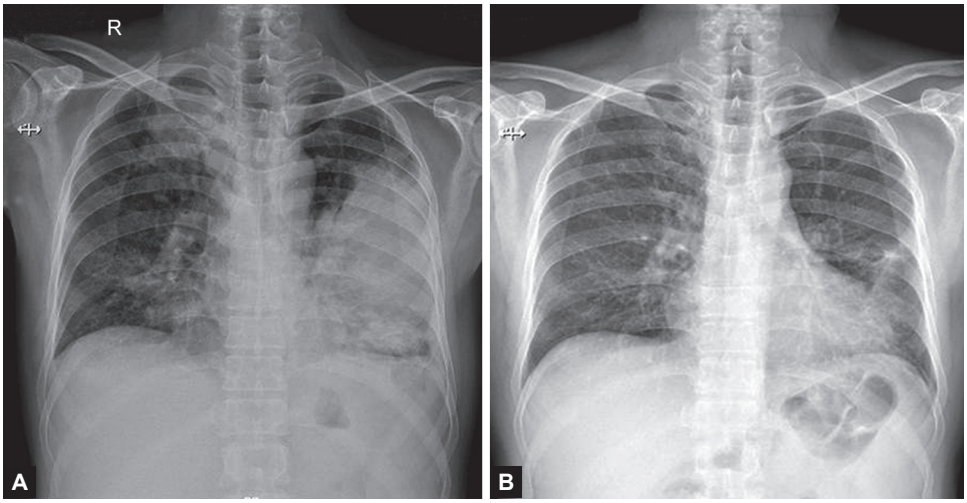
N%, percentage of neutrophils; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; pH, inverse log of proton concentration; PaO_2, partial pressure of oxygen; $PaCO_2$, partial pressure of carbon dioxide; HCO_3, serum bicarbonate; LVSD, left ventricular systolic dysfunction; LVDD, left ventricular diastolic dysfunction; ASD, atrial septal defect; PH, pulmonary hypertension; *≤3 months from diagnosis; NIPPV, noninvasive positive pressure ventilation; FQ, fluoroquinolone; SD, standard deviation; IQR, interquartile range; OR, odds ratio; CI, confidence interval

AE-CPD_{PN} and AE-CPD_b. Patients with AE-CPD_{PN} had a longer stay for clinical stabilization ($p = 0.016$) and more patients with AE-CPD_b were discharged on domiciliary oxygen (O.R 5.27, $p = 0.01$). On multiple logistic regression, after adjusting for higher illness severity of AE-CPD_b, AE-CPD_{PN} was independently related to need for mechanical ventilation (OR 22.3, $p = 0.01$), length of hospital stay (median difference, 4 days, $p = 0.016$) but not to hospital mortality (Table 2).

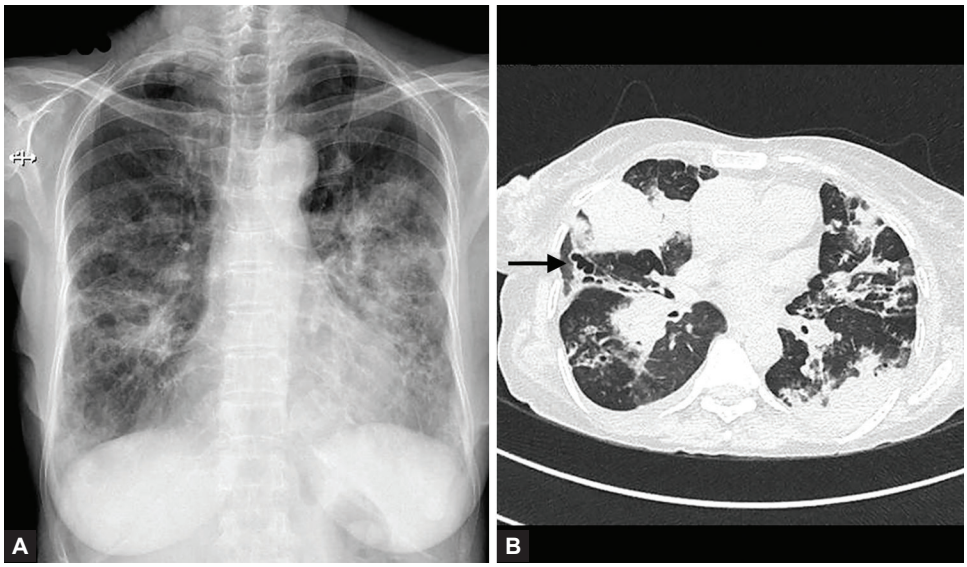
AE-CPD_{PN} was associated with a 22.7% (5/22) mortality. There was no difference in recent receipt of steroids prior to diagnosis of PN, renal failure at diagnosis, pH or hospital stay between survivors and nonsurvivors (Table 3). Respiratory failure requiring oxygen, NIPPV or mechanical ventilation was associated with mortality in AE-CPD_{PN}.

Discussion

Pulmonary nocardiosis is a rare thoracic infection predominantly affecting immunocompromised hosts.[8] The traditional risk factors for PN include long-term steroid therapy, malignancy, chemotherapy and HIV infection.[11] However, one-third of cases reportedly occur in immunocompetent patients.[12] There is increasing recognition that COPD is a risk factor for PN.[13] Data are mostly from case reports and small series, given the rarity of PN.[3,4,14-16] Notably, several of these cases have COPD associated with bronchiectasis and this is an important but under-recognized risk-factor for PN as well.[3,13] In our series, bronchiectasis was the most common underlying CPD. No clustering of cases was noted and only five of the 22 cases were diagnosed and managed in the outpatient non-CF bronchiectasis



Figs 2A and B: Composite image with chest radiograph (A) of a patient with pulmonary nocardiosis-related chronic pulmonary disease exacerbation showing multilobar consolidation, with a dense mass-like consolidation in the left lingula. Bronchoscopic biopsy from lingula confirmed nocardiosis; (B) Repeat chest radiographs at 6 months of cotrimoxazole showed resolution with a small area of residual fibrosis



Figs 3A and B: (A) Composite image with chest radiograph of a patient with pulmonary nocardiosis-related chronic pulmonary disease exacerbation showing multilobar consolidation; (B) Computed tomography confirmed extensive consolidation and also showed bronchiectasis (thin arrow)

Table 2A: Univariate logistic regression of the association of *Nocardia*-related exacerbations and need for mechanical ventilation

	Nocardia-related versus bacterial exacerbation			
	Odds ratio	*Lower*	*Upper*	*p value*
Renal failure	0.2	0.043	0.93	0.041
Respiratory failure	0.18	0.051	0.64	0.008
pH (unit change = 0.1)	3.99	1.424	15.18	0.02

Table 2B: Multiple logistic regression of the association of *Nocardia*-related exacerbations and need for mechanical ventilation (*after adjusting for renal failure and respiratory failure before diagnosis)

	Nocardia-related vs bacterial exacerbation			
	Odds ratio	*Lower*	*Upper*	*p value*
Need for mechanical ventilation	22.34	2.04	282.3	0.01*

clinic. None of the cases with AE-CPD$_{PN}$ had received long-term steroids before diagnosis, including all the patients with ABPA. In one-third of the cohort, the diagnosis of CPD was made when they presented with AE-CPD.

Thoracic involvement is the most common presentation of PN and is seen in 85% of cases at presentation. Symptoms are nonspecific and this is especially true with PN presenting in patients with CPD.[4,8] PN in CPD presents with an exacerbation of the underlying respiratory symptoms and is often similar to bacterial exacerbations of CPD (AE-CPD$_b$).[17] In fact, two cases of PN in our series were initially diagnosed as AE-CPD$_b$ and improved with antibacterials for AE-CPD; early relapse of symptoms after discontinuation of antibiotics for AE-CPD$_b$ (typically seven days in our practice) prompted extensive microbiological evaluation, yielding a diagnosis of AE-CPD$_{PN}$. This study was conducted in this background to identify clinical features that can distinguish AE-CPD$_{PN}$ from AE-CPD$_b$.

Table 3: Comparison of clinical features of AE-CPDPN between survivors and nonsurvivors

Characteristic	*Survivors (n = 17)*	*Nonsurvivors (n = 5)*	*Statistics*
Age years, mean ± S.D	60.5 ± 14.9	64.0 ± 10.7	0.64
Gender males: Total	9/17 (52.9%)	4/5 (80%)	0.36
Body mass index, Kg/m^2, mean±S.D	18.2 ± 5.2	21.4 ± 3.4	0.58
Duration before presentation, days median, range	12 (88)	8 (116)	0.57
Recent steroids (<3 months) before diagnosis	2/17 (11.8%)	0/5 (0%)	1.00
Hemoglobin g/dL	11.5 ± 2.2	12.8 ± 1.2	0.27
Total leukocyte count//µL, Mean ± S.E	16462 ± 2422	22860 ± 5630	0.34
pH, Mean ± S.D	7.44 ± 0.06	7.40 ± 0.05	0.16
paO_2 at diagnosis, Mean ± S.D	73.2 ± 18.3	73.2 ± 22.6	1.00
Chronic respiratory failure before diagnosis	0/17	0/5	NA
Renal failure at diagnosis	1/17 (5.9%)	1/5 (20%)	0.41
Cavity on chest imaging	4/17 (23.6%)	2/5 (40%)	0.58
Nocardia vs coinfection	5/17 (29.5%)	1/5 (20%)	1.00
Respiratory failure needing oxygen	4/17 (23.6%)	5/5/ (100%)	0.005
NIPPV	2/17 (11.8%)	3/5 (60%)	0.05
Mechanical ventilation	2/17 (11.8%)	3/5 (60%)	0.05
Hospital length of stay days Median, IQR	10 (9)	5 (13)	0.25

SD, standard deviation; IQR, interquartile range

We did not find additional risk-factors independent of CPD predisposing to PN in our series (Tables 1A and 1B), again establishing the independent risk of CPD for PN.[13] AE-CPD_{PN} occurred in patients with advanced lung disease (mean FEV1 1.01 ± 0.05 L, 53.6 ± 2.4% predicted) who presented with longer symptom duration, more leukocytosis and inflammatory markers when compared to AE-CPD_b. Extensive consolidation or cavitation prompted suspicion for PN in AE-CPD and cavitation was seen in 27.3% at diagnosis. Four patients with AE-CPD_b had an initial diagnosis of cavitation; one was related to an exacerbation of COPD related to previous tuberculosis and in another three, underlying bronchiectasis had not been diagnosed and consolidation around cystic bronchiectasis spaces was initially misdiagnosed as cavitation.

The cohort of AE-CPD_b was sicker with a higher proportion of cardiac disease, pulmonary hypertension, chronic respiratory failure on long-term oxygen therapy, respiratory acidosis and AKI at presentation. Consequently, more patients with AE-CPD_b were treated with oxygen, NIPPV (though statistically non-significant) and were discharged on domiciliary oxygen. After adjustment for the higher illness severity of AE-CPD_b, AE-CPD_{PN} was independently related to the need for mechanical ventilation and duration of hospital stay. The in-hospital mortality of 22.7% in AE-CPD_{PN} is similar to previous published reports of PN.[4] Respiratory failure requiring oxygen therapy, NIPPV or mechanical ventilation was associated with mortality in AE-CPD_{PN}.

Our study has several strengths. This is one of the largest series of PN in immunocompetent CPD till date. In another large series of 30 patients with PN-CPD, 23% (7/30) were related to chronic steroid therapy, chemotherapy and malignancy. Associated immune-compromise was associated with mortality and this masked the mortality associations of PN-CPD *per se*.[4] We also identified a matched cohort of AE-CPD_b to identify clinical features that could help in early diagnosis of PN in AE-CPD. There are several limitations of this study. The retrospective nature of the study resulted in an imbalance in the baseline characteristics between cases and controls; controls had more preexisting respiratory failure than cases. PN is a rare disease and conducting prospective studies is not always feasible. Second, we included patients with consolidation in the control group of AE-CPD_b. While this is debatable,[1,18] we aimed at a control group with similar severity of illness to AE-CPD_{PN} and including AE-CPD_b with consolidation was considered appropriate as most patients with AE-CPD_{PN} had consolidation at diagnosis. While some reports of AE-CPD exclude those with consolidation, this would have prevented us from identifying clinical features that help in early diagnosis of AE-CPD_{PN}. Third, data on speciation of *Nocardia* spp. is being revised with 16sRNA studies and our data is currently not available. It is well described that extensive discrepancy between biochemical testing and 16sRNA testing occurs and leads to re-speciation.[19] Fourth, most of the cases of PN (22/32, 68.75%) in the current series were related to CPD; this is possibly because of high index of suspicion in our unit and the absence of hemato-oncology and organ transplant specialty service till 2016 in our hospital. Finally, the absence of spirometry reports for the five patients who died may have lead to over-estimation of values in the AE-CPD_{PN} arm. As has been described, 29% were diagnosed with CPD when they presented with AE-CPD and it was not always feasible to do spirometry.

Conclusion

In conclusion, PN is a rare cause of AE-CPD and can be recognized by a longer symptom duration, leukocytosis, extensive consolidation and cavitation. Early recognition is important, given the high short-term mortality. AE-CPD_{PN} is independently associated with longer duration of hospital stay and mechanical ventilation. Respiratory failure requiring oxygen, NIPPV and mechanical ventilation is associated with mortality in AE-CPD_{PN}.

References

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for the Diagnosis, Management and Prevention of chronic obstructive pulmonary disease: 2018 Report. 2018. Accessed 20/7/2018.
2. Ko FW, Chan KP, Hui DS, Goddard JR, Shaw JG, Reid DW, et al. Acute exacerbation of COPD. Respirology. 2016;21(7):1152–1165.
3. Menendez R, Cordero PJ, Santos M, Gobernado M, Marco V. Pulmonary infection with *Nocardia* species: a report of 10 cases and review. Eur Respir J. 1997;10(7):1542–1546.
4. Garcia-Bellmunt L, Sibila O, Solanes I, Sanchez-Reus F, Plaza V. Pulmonary nocardiosis in patients with COPD: characteristics and prognostic factors. Arch Bronconeumol. 2012;48(8):280–285.
5. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA). 2018. Accessed 20-7-2018.
6. Travis WD, Costabel U, Hansell DM, King TE Jr, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–748.
7. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319–338.
8. Lerner PI. Nocardiosis. Clin Infect Dis. 1996;22(6):891–903.

9. Continuous or nocturnal oxygen therapy in hypoxemic chronic obstructive lung disease: a clinical trial. Nocturnal Oxygen Therapy Trial Group. Ann Intern Med. 1980;93(3):391–398.
10. Ambrosioni J, Lew D, Garbino J. Nocardiosis: updated clinical review and experience at a tertiary center. Infection. 2010;38(2):89–97.
11. Lederman ER, Crum NF. A case series and focused review of nocardiosis: clinical and microbiologic aspects. Medicine (Baltimore). 2004;83(5):300–313.
12. Beaman BL, Beaman L. Nocardia species: host-parasite relationships. Clin Microbiol Rev. 1994;7(2):213–264.
13. Riviere F, Billhot M, Soler C, Vaylet F, Margery J. Pulmonary nocardiosis in immunocompetent patients: can COPD be the only risk factor? Eur Respir Rev. 2011;20(121):210–212.
14. Aide MA, Lourenco SS, Marchiori E, Zanetti G, Mondino PJ. Pulmonary nocardiosis in a patient with chronic obstructive pulmonary disease and bronchiectasis. J Bras Pneumol. 2008;34(11):985–988.
15. Anderson M, Kuzniar TJ. Pulmonary nocardiosis in a patient with chronic obstructive pulmonary disease–case report and literature review. Pneumonol Alergol Pol. 2012;80(6):565–569.
16. Castellana G, Grimaldi A, Castellana M, Farina C, Castellana G. Pulmonary nocardiosis in chronic obstructive pulmonary disease: A new clinical challenge. Respir Med Case Rep. 2016;18:14–21.
17. Khare V, Gupta P, Himanshu D, Kumar D. Emergence of co-trimoxazole resistant Nocardia brasiliensis causing fatal pneumonia. BMJ Case Rep. 2013;2013.
18. Franssen F. The semantics of COPD exacerbations. 2017. Accessed 20-7-2018.
19. Valdezate S, Garrido N, Carrasco G, Medina-Pascual MJ, Villalon P, Navarro AM, et al. Epidemiology and susceptibility to antimicrobial agents of the main Nocardia species in Spain. J Antimicrob Chemother. 2017;72(3):754–761.

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Case Report

Effectiveness of Pulmonary Rehabilitation in Lymphangioleiomyomatosis

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Abstract

Lymphangioleiomyomatosis (LAM) is a cystic lung disease frequently associated with reduced exercise capacity, mostly affecting young women. The disease is characterized by progressive pulmonary cystic change, recurrent pneumothorax, chylous pleural collections, and in most cases, progressive respiratory failure. Diagnosis is made by a combination of clinical features and computed tomography scanning or, with lung biopsy. We present a case report of a young woman who suspected of LAM and diagnosed with cystic lung disease, admitted to the intensive care unit in view of worsening hypoxia associated with pneumothorax. She was on ventilator support and placement of repeated intercostal drains and had a tracheostomy to step down to bilevel positive airway pressure support, after the medical stabilization. She was referred for pulmonary rehabilitation, in which she showed good outcomes and recovered well.

Keywords: Hormonal therapy, lymphangioleiomyomatosis, pulmonary rehabilitation

Introduction

Lymphangioleiomyomatosis (LAM) is a rare disease of the lungs and lymphatics, which can occur sporadically or in association with tuberous sclerosis. LAM almost exclusively affects young women, generally developing before menopause. The disease is characterized by progressive pulmonary cystic changes with the accumulation of LAM cells, recurrent pneumothorax, and chylous pleural collections and, in most cases, progressive respiratory failure.[1] Along with chest X-ray changes (pneumothorax and effusions), high resolution computed tomography (HRCT) findings of multiple, small, diffusely distributed cysts, an elevated serum vascular endothelial growth factor-D (VEGF-D) testing are the most diagnostic measures. Lung biopsy is indicated only when HRCT findings and VEGF-D testing are nondiagnostic.[2]

Most patients with LAM complain of impaired quality of life and reduced exercise capacity, secondary to airflow obstruction, abnormal diffusion capacity, dynamic hyperinflation, peripheral muscle dysfunction (deconditioning), and pulmonary hypertension.[3] Available medical management like Sirolimus and hormonal therapy (Medroxyprogesterone, Gonadotropin-releasing hormone agonists) may slow down disease progression; however, the disease remains incurable. Pulmonary rehabilitation (PR) is a safe intervention and improves exercise capacity, dyspnea, daily physical activity, muscle strength, and quality of life in LAM.[4,5] After obtaining appropriate consent from the patient, we present a case report of a young female patient who suspected of LAM and diagnosed with cystic lung disease and the outcomes of PR.

Case Report

A 25-year-old female, health-care worker, reported to the emergency room with sudden onset of breathlessness and hypoxemic shock. In view of worsening conditions, she was shifted to the intensive care unit (ICU) and intubated with

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mechanical ventilation. Initial chest X-ray [Figure 1a] showed a bilateral pneumothorax with subcutaneous emphysema and it was treated by bilateral intercostals drainage (ICD). There was persistent air leak for a prolonged duration, along with inadequate lung expansion. Initial HRCT-chest [Figure 2a and 2c] showed extensive fibro-bronchiectatic changes with few cystic changes noted in bilateral lower lobes and also bronchopleural fistula (BPF). Since she was diagnosed with pulmonary tuberculosis 3 months ago with sputum positive (sputum Gene expert positive, Rifampicin sensitive), and she was started on treatment; however, Ziehl-Neelsen stain was negative after the 3 months. She had developed multiple episodes of nosocomial infection during the period of ICU. It was treated with appropriate antibiotics, and infection settled down. Repeated blood and tracheal cultures were sterile; however, her respiratory distress persisted due to recurrent pneumothorax.

A repeat HRCT-thorax was done, which showed extensive bilateral cystic changes with a right-sided loculated pneumothorax with 2 ICDs *in situ* on the right side and one on the left side, and very poor lung reserve. A possible diagnosis of Sporadic LAM was suspected with HRCT; hence cardiothoracic surgeon opinion was obtained for open lung biopsy but deferred in view of high risk. Autologous blood patch was attempted twice to seal the BPF but was a failure. In view of the difficulty in weaning from the ventilator, tracheostomy was done after 2 weeks. In view of constant ICD site pain, thoracic epidural analgesia was given. Gradually, she was weaned off the ventilator and was maintained on bilevel positive airway pressure support.

In view of possible LAM, high dose of medroxyprogesterone and doxycycline were administered with informed consent. Gradually bilateral air leak stopped, and bilateral ICDs were removed. The repeated chest X-rays [Figure 1b-d] and HRCT [Figure 2b and 2d] showed multiple cysts with surrounding multiple large bullae replacing the left upper lobe sparing the lingular segment-bulla have increased in size. Multiple cysts in the right upper lobe, right middle lobe, and anterior right lower lobe. Cysts have increased in size/parenchymal strands, small areas of pneumonitis in the right lower lobe and upper lobe [Figure 2c].

Due to prolonged ICU stays along with worsened disease condition, she developed ICU acquired critical illness myoneuropathy, and systemic deconditioning associated with proximal muscle atrophy. With the low lung reserve and cystic lung disease, the team planned and advised her family to get ready for lung transplantation. Meanwhile, PR was initiated to achieve fitness for surgery. Hence, she was enrolled in PR program with exercise-based intervention. Nutritional status was improved with dietary correction and psychological support was given, which helped her to come out of her anxiety.

Pulmonary rehabilitation program

The PR program was initiated after getting informed consent. PR program was conducted with two interventional programs; ICU-based intervention, and inpatient. The ICU-based intervention outcomes were measured by using the Standardized 5 Questions (S5Q) for assessing patients' ability to cooperate, Modified Medical Research scores for muscle strength, and Berg Balance Scale for balance. The ICU based intervention was started with the use of the Leuven protocol from "start to move."[6] It is a six-level assessment and treatment protocol, which includes basic assessment of S5Q (1). Open and close your eyes, (2). Look at me, (3). Open your mouth and stick out your tongue, (4). Shake yes and no nod your head, (5). I will count to 5, frown your eyebrows) and Modified Medical Research score (0 = no visible contraction, 1 = visible contraction without limb movements, 2 = movements of the limb in the eliminated gravity, 3 = movement against gravity

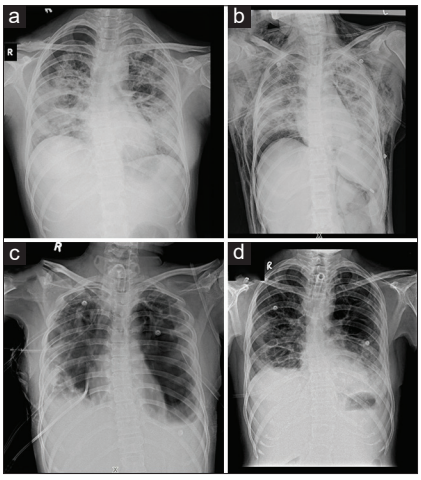


Figure 1: (a) Initial chest X-ray before admission. (b) During admission repeated chest X-rays. (c) After 1 month of hormone treatment. (d) At the time of discharge



Figure 2: (a) Initial high resolution computed tomography-chest at the time of admission. (b) At the time of discharge. (c) After 1 month of hormone treatment. (d) At the time of discharge

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in full range, 4 = movement against gravity with resistance, 5 = normal strength). If the patient passed in S5Q then go for muscle strength test (muscles to be tested deltoid, biceps, wrist extensors, quadriceps, dorsiflexors, and the total score; 60 if the score <48/60-significant muscle weakness). Berg balance scale components include sitting with back unsupported but feet supported on the floor, sitting to standing, standing unsupported (scores from 0-unable to do for 10 s to 4-able to do for 2 min comfortably). According to this assessment, the treatment protocol was administered in ICU and explained in Table 1.

In-patient PR program was initiated after shifting to the ward. Baseline assessment was collected. It was followed by 1 min sit to stand test, 6 min walk test,[7] activity questionnaire's functional independence measure (score; self-care domain), which includes eating, grooming, bathing, dressing upper and lower body and toileting (scoring starts with 1-total assistance to 7-complete independence), physical activity index (which includes poor to high active category) and modified sphygmomanometer,[8] was used to the measured hand grip. The PR program consists of exercise training (flexibility training, endurance training, strength training, airway clearance technique, and breathing retraining), educational support (about her condition, program, nutrition, advantages, and guidance to family members), psychological counseling, and nutritional intervention as per need. The detailed PR program is explained in Table 1. The PR program was continued from the day shifted from ICU and 4–6 days/week to till discharge (4 weeks). Home care program was advised to do regular exercises such as

Table 1: Protocol of pulmonary rehabilitation program

Phase I: ICU based Intervention started after hemodynamic stability (6 weeks)
Goals in the ICU
Optimizing lung expansion and secretion clearance, breathing pattern retraining, to maintain upper and lower extremity ROM, strength and basic bed mobility and transfers
Exercise program in acute phase
Assessment criteria (Level-0: No cooperation, Level-2: Moderate cooperation)
Basic assessment S5Q=0-3/5, MRC sum=36, BBS sitting=1[6]
Airway clearance
Gentle chest percussion, vibration & shaking manoeuvres, suctioning on need basis, along with manual hyperventilation
Breathing retraining
Synchronizing the breathing with abdomino thoracic movement with ventilator, 15–20 repetition/3–4 times/day, after tracheostomy with BIPAP, 10–15 times of diaphragmatic and thoracic expansion exercise with manual resistance (manual resistance was applied with hands over the xiphisternal and abdomen of the patient by the physical therapist and the patient were asked to inspire against hand resistance. Followed by sand bag with ranges from 0.5 kg to 2 kg was used for resistance)
DVT prophylaxis and ROM
Pneumatic compression device applied during night and sleep time, active ankle pump exercise, calf stretching, squeezing and other ROM exercises when the patient awakes, 15–20 repetition/3–4 times/day
Exercise program in acute maintenance phase
Assessment criteria (Level-3 close to full cooperation-Level 5 Full cooperation) Basic assessment S5Q=4–5/5, MRC sum=36, BBS sitting=1–3, standing=0–2, sit-to standing=0–1[6]
Early mobilization
Initially long sitting, bed side dangled sitting with caution about all tubes and drains 10–30 min/daily, chair sitting after drain removal done up to extubation from ventilator, and on BiPAP
Cardiopulmonary endurance training
Leg cycling with mini pedal exerciser 10–20 min once/daily, standing march 10–15 times once/daily up to weaned from ventilator. Same continued twice daily after weaning
Walking training
Initially 5–10 min with 2–4 L/min oxygen with BiPAP to maintain SpO_2 above 90%, then progressed up to 20 min, twice daily with 2–3 L/min oxygen support with BiPAP
Phase II: Inpatient rehabilitation program till discharge, 5–6 days/week (4 weeks)
Endurance training
Level walking as much as tolerated (10–20 min, according to patients walking speed or with 6MWT distance or intensity of modified Borg scale 4–6/10). Arm and leg cycle ergo meter: 10–20 min/5–6 weekly. Borg's scale 4–6/10
Strength training
Target muscles trained with dumbbells, weight cuffs (up to 2 weeks we avoided upper limb weight lifting due to repeated pig tail and ICD insertion)
Upper limb: Biceps, triceps, deltoid, pectorals. Lower limb: quadriceps, hamstrings, gluteus muscles, calf and dorsi flexors with 1–3 kg/10 rep/2 sets/ alternate days
Breathing retraining exercises
Breathing control through diaphragmatic breathing and localized thoracic expansion exercise with 15–20 rep/2–3 h once, trained breathing exercise in sitting, semi Fowler's position and side lying and supine gradually, paced breathing was trained during activities

S5Q: Standardized 5 questions, MRC: Medical research council score for muscle testing, BBS: Berg's Balance score, BiPAP: Bi-level Positive airway pressure, ICD: Intercostals drainage, 6MWT: 6-min walk test, ROM: Range of motion, ICU: Intensive care unit, DVT: Deep-vein thrombosis

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Table 2: Outcome measures

Outcome measures	Initial assessment in ICU	During inpatient rehabilitation (4 weeks)			
		First	Second	Third	Fourth
Anthropometric parameters					
Height (cm)	157				
Weight (kg)	32.6	36.4	38.5	39.7	40.4
BMI (kg/m^2)	13.23	14.77	15.62	16.11	16.39
Hand grip (mm of Hg)	R15 and L12	R30 and L25	R40 and L35	R45 and L40	R60 and L55
6-min walk distance	-	120 m (20.01%)	160 m (26.87%)	220 m (37.11%)	280 m (47.35%)
1 minute sit to stand	3 times/min	10 times/min	18 times/min	26 times/min	30 times/min
Berg balance score (3 domains)	1/12	8/12	10/12	12/12	12/12
SpO_2% desaturation during activities	−8 (98–90) 40% FiO_2	−6 (96–90%) 28% FiO_2	−3 (98–95) 4 l/min O_2	−3 (98–95) 4 l/min O_2	−3 (98–95) Room air
Δ Heart rate	24 beats (120–154 beats/min)	22 beats (126–148 beats/min)	34 beats (118–152 beats/min)	24 beats (130–154 beats/min	34 beats (130–164 beats/min)
Modified Borg scale	7/10	5/10	4/10	3/10	3/10
FIM scale (Self-care domain)	6/42 (14%) Maximal assistance	11/42 (26%) Maximal assistance	24/42 (57%) Moderate assistance	30/42 (71%) Minimal assistance	33/42 (78.5%) Supervision
PAI	6 (sedentary)	36 (poor, not good enough)	48 (fair, acceptable)	64 (very good, active, and healthy)	80 (very active lifestyle)
ABG					
pH	7.268	7.44	7.387	7.39	7.48
FiO_2 (%)	21	45	40	35	21
PCO_2 (mmHg)	64.1	47	52.4	56.0	29.3
PaO_2 (mmHg)	62.4	71.5	72.5	80.8	74.1
HCO_3 (mEql/L)	22.7	95.2	30.8	33.8	22.0
SaO_2 (%)	89.7	31.5	94.4	96.1	95.2
Lactate (mmol/L)	4.60	1.23	1.55	1.1	1.0

FIM: Functional independence measure, BMI: Body mass index, PAI: Physical activity index, ABGs: Arterial blood gases, ICU: Intensive care unit

walking, breathing exercises, resistance exercises with the use of simple object such as water bottles and sandbags. Outcome measures are explained in Table 2.

Discussion

Our patient showed significant improvements in exercise performance and quality of life following PR from day one to till discharge. Initially, the patients were hopeless towards exercise-based intervention due to long-term ventilation, repeated ICD tubes insertion, painful events during procedures, being transferred for different tests such as computed tomography and long-term ICU stay. Repeated psychological counseling, and appropriate hormone therapy with medroxyprogesterone and doxycycline the symptoms of pneumothorax and air leaks in ICDs were settled, along with adjustment of analgesics the graded mobilization was initiated. She accepted the changes and gave her full effort during the sessions. Initially, she was mobilized with maximal assistance then reduced to supervised mobilization; initially, she could participate 15–30 min in exercise sessions, while before discharge, she tolerated up to 2 h. She had showed a noticeable improvement in exercise capacity, observed in both endurance exercise time, during walking, reducing the level of dyspnea, increased daily physical activity, enhanced muscle strength, and improved quality of life. A positive trend toward symptoms of depression was also observed. The program was safe, well-tolerated and had a high rate of adherence. Her family members also showed high interest during the PR sessions and advised to follow the same at home.

This report clearly shows the importance and benefit of a PR program, even though it shows changes in patient recovery from physical illness rather than the disease state. PR program can be a good adjunct therapy to pharmacological treatment. In addition, serves as a bridging aid before lung transplantation in such chronic respiratory diseases such as LAM.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Johnson SR, Tattersfield AE. Decline in lung function in lymphangioleiomyomatosis: relation to menopause and progesterone treatment. Am J Respir Crit Care Med 1999;160:628-33.
2. McCormack FX, Gupta N, Finlay GR, Young LR, Taveira-DaSilva AM, Glasgow CG, *et al*. Official American Thoracic Society/ Japanese Respiratory Society clinical practice guidelines: Lymphangioleiomyomatosis diagnosis and management. Am J Respir Crit Care Med 2016;194:748-61.

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3. Baldi BG, Albuquerque AL, Pimenta SP, Salge JM, Kairalla RA, Carvalho CR. Exercise performance and dynamic hyperinflation in lymphangioleiomyomatosis. Am J Respir Crit Care Med 2012;186:341-8.
4. Araujo MS, Baldi BG, Freitas CS, Albuquerque AL, Marques da Silva CC, Kairalla RA, *et al*. Pulmonary rehabilitation in lymphangioleiomyomatosis: A controlled clinical trial. Eur Respir J 2016;47:1452-60.
5. Gloeckl R, Nell C, Schneeberger T, Jaroschi I, Boensch M, Watz H, *et al*. Benefits of pulmonary rehabilitation in patients with severe lymphangioleiomyomatosis: Improving our practice: Novel approaches to pulmonary rehabilitation/poster discussion. Am J Respir Crit Care Med 2020;201:A6120.
6. Gosselink R, Clerckx B, Robbeets C, Vanhullebusch T, Vanpee G, Segers J, *et al*. Physiotherapy in the intensive care unit. Neth J Crit Care. 2011;15:66-7.
7. Palaniappan Ramanathan R, Chandrasekaran B. Reference equations for 6-min walk test in healthy Indian subjects (25-80 years). Lung India 2014;31:35-8.
8. Chandrasekaran B, Ghosh A, Prasad C, Krishnan K, Chandrasharma B. Age and anthropometric traits predict handgrip strength in healthy normals. J Hand Microsurg 2010;2:58-61.

Original Article

Validity of tuberculous pleuritis diagnosed in a resource-constrained setting in Dindigul district of Tamil Nadu

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Abstract

Context: Majority of the Indians live in rural areas where resource constrained settings depend on cheaper and less invasive tests to diagnose extrapulmonary tuberculosis (TB). The decline in prevalence of TB in the country could affect the validity of the diagnosis. The aim was to measure validity of the pleural fluid study of proteins, lactate dehydrogenase (LDH), and cell counts in diagnosis of tuberculous pleuritis. **Materials and Methods:** This was a cross-sectional study conducted in a 300 bedded secondary care hospital in rural Tamil Nadu. Exhaustive sampling was performed during April 2013 to March 2014. Pleural fluid study of 54 patients with exudative pleural effusion was conducted. Diagnosis was established by closed needle pleural biopsy. Receiver operator curves were plotted and area under curve (AUC) was calculated for various parameters. Sensitivity, specificity, and predictive values were calculated for different cut-off values of the parameter with significant AUC. **Results:** Prevalence of tuberculous pleural effusion was 56% (95% confidence interval [95% CI] - 42.5–69.5%). Lymphocyte predominance in pleural fluid was the only valid test, and cut-off >80% had sensitivity of 70.0% (95% CI - 53.3–86.7%) and specificity of 70.8% (95% CI - 52.2–89.4%). Pleural fluid pH, protein or its ratio with serum protein, sugar, total leukocyte count, LDH or its ratio with serum LDH; erythrocyte sedimentation rate were not valid screening tests. **Conclusions:** Lymphocyte predominance > 80% can be used as a marker of tuberculous pleuritis. Since the prevalence of tuberculous pleuritis in India has come down considerably, newer tests need to be included to make a valid diagnosis.

Keywords: Extrapulmonary tuberculosis, pleural tuberculosis, tuberculosis, tuberculous pleuritis

Introduction

In India, one-fifth of new tuberculosis (TB) cases notified in 2014 were extrapulmonary.[1] Pleural effusion is the second common form, constituting about 28% of extrapulmonary TB (EPTB).[2,3] In 12th 5 years plan, new objective of early detection and treatment of at least 90% of all types of TB cases including EPTB was stated for Revised National Tuberculosis Control Program.[4] EPTB is known to be associated with delayed diagnosis.[5] Invasive or costly investigations are often required to diagnose EPTB.[6] In a rural area, healthcare settings are not well-equipped to diagnose EPTB. In India, 68.8% population live in rural area.[7]

In resource-constrained settings, pleural fluid study of protein and lymphocyte counts were suggested by World Health

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Organization (WHO) to diagnose pleural TB.[8] In our institution, we had been using pleural fluid cell counts and simple pleural fluid biochemical tests to diagnose tuberculous pleuritis. In the context of falling prevalence of TB in India, the validity of using these tests were questionable in diagnosing tuberculous pleuritis.[1] Adenosine deaminase (ADA) assay or pleural biopsy were not available in our setting. Hence, this study was undertaken to measure validity of the pleural fluid study of protein, lactate dehydrogenase (LDH), and cell counts for diagnosing tuberculous pleuritis which had been in use in our hospital.

Materials and Methods

Study design

The study design was cross-sectional.

Study setting

The study was carried out in Internal Medicine Department of a 300 bedded secondary care level hospital in Dindigul district of Tamil Nadu. For 1 year, we had service of a pulmonologist in our hospital when this study was conducted. Since our institution did not have facilities for histopathological examination, special arrangements were made for the purpose of the study to transport the biopsy specimen to a tertiary care teaching hospital nearly 400 km away.

Study participants

The study period was April 2013 to March 2014. Patients presenting with evidence of pleural effusion on chest X-ray and were exudative by Light's criteria were included in the study.

Sample size and sampling technique

We expected sensitivity of 93.5% for differential lymphocyte count cut-off more than 80%.[9] We expected prevalence of TB among exudative pleural effusion to be 43.8%.[10] For alpha error of 5% and precision of 10%, we needed sample size of 53. With exhaustive sampling done during the study period, we obtained a sample size of 54.

Study tools

A semi-structured performa was used to collect information regarding symptoms and co-morbidities from the patient interview as well as from hospital records. Cell counts and biochemical parameters of pleural fluid were studied. Diagnosis was established by pleural biopsy.

Pleural biopsies were performed by pulmonologist by closed needle technique. Pleural biopsy was conducted under strict aseptic conditions. The affected side of the chest was cleaned thoroughly with antiseptics and draped. Under local anesthesia, a 23-gauge needle was passed and pleural fluid aspirated to confirm the site for incision. Just above the upper border of the rib in the selected site, a 0.5 cm incision was made. Through this incision, Tru-Cut needle was introduced, and multiple biopsies were taken by multiple passes. The pleural tissue obtained was put into a formalin bottle. Biopsy specimens were sent for histopathological examination to Pathology Department of a tertiary care center.

Analysis

Data entry was carried out using EpiData software, version 3.1 (The Epidata Association, Odense, Denmark) and analysis was performed using SPSS software, version 20 (IBM corporation, New York). Mean and standards deviation (SD) were calculated for continuous variables. Proportions were calculated for categorical variables. Histopathology of pleural biopsy was considered gold standard. Receiver operator curve (ROC) was plotted for various laboratory parameters. Sensitivity, specificity, and predictive values for various cut-offs of parameter with a significant area under curve were calculated.

Ethical aspects

The study was approved by Ethical Committee of the Institution. Participants were explained possible complications of pleural biopsy procedure in detail, and an information sheet also was provided. They were informed that institution would bear the expense for the management of complication if any. Voluntary written informed consent was given by the study participants. Privacy was ensured for an interview. Confidentiality of the information is maintained.

Results

Characteristics of the study participants

There were 54 study participants. All of them had unilateral and exudative pleural effusion. Males comprised 35 (65%) of the study participants and females the rest. Mean age was 47.7 years (SD = 16.4), and 31 (57%) were above 60 years. Fourteen (25.9%) participants were tobacco smokers, 5 (9.3%) were diabetic, and 6 (11.1%) were hypertensive. None of them had cardiac failure or renal failure. Majority had one or the other of the following symptoms such as a cough, breathlessness, loss of appetite, loss of weight, or fever.

Laboratory parameters

Among hematological parameters, 42 (77.7%) had anemia, all had raised erythrocyte sedimentation rate (ESR), 18 (33.3%) had leukocytosis, 32 (59.2%) had neutrophilia, 3 (5.5%) had band forms, 34 (62.9%) had lymphocytopenia, none had lymphocytosis, 4 (7.4%) had eosinophilia and 10 (18.5%) had monocytosis. In pleural fluid study, mean LDH was 898.2 (SD = 551.2), mean total protein was 4.7 g/dl (SD = 0.9), and mean pH was 7.5 (SD = 0.4). Forty-two (77.7%) had lymphocyte dominance more than 50% in pleural fluid.

Validity of laboratory parameters

The diagnosis was established by pleural biopsy. Among the study participants, 30 (56%, 95% confidence interval [95% CI] - 42.5–69.5%) had tuberculous pleuritis, 7 (13%) had malignancy and 17 (31%) had other inflammatory conditions.

ROCs were plotted to explore valid laboratory parameters to diagnose tuberculous pleural effusion. The parameters studied were pleural fluid pH, protein, LDH, sugar, total leukocyte count, lymphocyte predominance, ratio of LDH in pleural fluid to serum, ratio of protein in pleural fluid to serum and ESR. Among these parameters shown in Table 1, only the pleural fluid differential lymphocyte count had a diagnostic value with an accuracy of 80.1% (95% CI - 67.8–92.4%, $P < 0.001$).

WHO criteria for diagnosing pleural effusion in resource-constrained settings (pleural fluid protein >3 g/dl and lymphocytes >50%) was 90% sensitive but specificity was 45.8%. Table 2 shows sensitivity, specificity, and predictive value of different cut-offs of lymphocyte predominance. With pleural fluid lymphocyte count > 50% as cut-off for TB, sensitivity was 93.3% (95% CI - 83.9–100%) and specificity was 41.7% (95% CI - 21.6–61.8%); with pleural fluid lymphocyte count >80%, sensitivity was 70.0% (95% CI - 53.3–86.7%), specificity was 70.8% (95% CI - 52.2–89.4%), positive predictive value was 75% (95% CI - 58.6–91.4%), and negative predictive value was 65.4% (95% CI - 46.7–84.1%), respectively [Table 2]. We had explored combinations of laboratory parameters for better sensitivity and specificity but, none emerged.

Discussion

Differentiating the pleural effusion as exudative or transudative using the Light's criteria was the first step toward diagnosis. The 54 samples identified as exudative may include some transudates as we know the specificity of Light's criteria can be as low as 71%.[11] The gold standard for diagnosis of tuberculous pleuritis is the demonstration of *Mycobacterium tuberculosis* in pleural fluid or biopsy specimen or demonstration of caseating granuloma in the biopsy specimen.[12] Tuberculous pleuritis was diagnosed in 30 (56%) samples, by closed needle biopsy. However, the sensitivity of closed biopsy is lower than biopsy taken by diagnostic thoracoscopy.[12]

Among the biochemical parameters and cell counts of pleural fluids, only pleural fluid differential lymphocyte count had a diagnostic value for tuberculous pleuritis. The WHO criteria of lymphocytes >50% and protein >3 g/dl in pleural fluid had high sensitivity (90%), but specificity was poor (45.8%). Pleural fluid lymphocytes >80% as a cut-off, sensitivity was 70.0%, and specificity was 70.8%. However, these estimates were not very precise. According to Pettersson and Riska, lymphocyte predominance >80% was characteristic of tuberculous pleuritis but also of malignant pleural effusion.[9]

The criteria recommended by WHO for diagnosing tuberculous pleuritis in resource-constrained setting was based on studies done in settings where 95% of the pleural effusions were tuberculous.[8] It is known that, in India, prevalence of TB had halved by 2013 compared to 1990.[1] In our study, only 56% (95% CI - 42.5–69.5%) of the pleural effusion were tuberculous. Similarly, in a tertiary care setting in Tamil Nadu, only 43.8% (95% CI - 29.5–58.1%) were tuberculous.[10] With low prevalence, false positive rates increase.

In this context, newer markers need to be considered for the diagnosis of tuberculous pleuritis. Burgess *et al.* had recommended to combine pleural fluid lymphocyte-neutrophil ratio and ADA, which would give sensitivity of 88% and specificity of 95% for tuberculous pleuritis diagnosis.[13] A meta-analysis estimate of sensitivity and specificity of ADA levels in pleural fluid were 92% and 90%, respectively.[14] Polymerase chain reaction for mycobacterial DNA had poor sensitivity.[15] Interferon-gamma, interleukin 12 (IL-12), IL-18 were identified as markers of tuberculous pleuritis.[16] Interferon-gamma is highly efficient marker of tuberculous pleuritis but costly.[15] Detection of free and immune complexed mycobacterial antigens ES-31 and EST-6 were suggested as an adjunct test to diagnose tuberculous pleuritis.[17]

Table 1: Area under the receiver operating curve of laboratory parameters for diagnosis of tuberculous pleuritis (*n*=54)

Laboratory parameters	AUC* with 95% CI	*P*
PF† pH	0.541 (0.383–0.699)	0.608
PF protein	0.603 (0.451–0.755)	0.198
PF LDH	0.535 (0.373–0.696)	0.663
PF sugar	0.559 (0.396–0.722)	0.459
PF total leukocyte count	0.431 (0.269–0.592)	0.384
PF differential count of lymphocytes	0.801 (0.678–0.924)	<0.001
Ratio of PF protein to serum protein	0.550 (0.395–0.705)	0.531
Ratio of PF LDH to serum LDH	0.492 (0.335–0.648)	0.917
ESR	0.542 (0.388–0.697)	0.614

*AUC: Area under curve; †PF: Pleural fluid. LDH: Lactate dehydrogenase; CI: Confidence interval; ESR: Erythrocyte sedimentation rate

Table 2: Sensitivity, specificity and predictive values of lymphocyte predominance in pleural fluid for tuberculous pleuritis (*n*=54)

Differential lymphocyte count (%)	With 95% CI (%)			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Above 50	93.3 (83.9–100)	41.7 (21.6–61.8)	66.7 (52.2–81.2)	83.3 (61.8–100)
Above 60	86.7 (74.3–99.1)	45.8 (25.5–66.1)	66.7 (51.6–81.8)	73.3 (50.5–96.1)
Above 70	80.0 (65.4–94.6)	66.7 (47.5–85.9)	75.0 (59.7–90.3)	72.7 (53.7–91.7)
Above 80	70.0 (53.3–86.7)	70.8 (52.2–89.4)	75.0 (58.6–91.4)	65.4 (46.7–84.1)
Above 90	60.0 (42.1–77.9)	91.7 (80.4–100)	90.0 (76.6–100)	64.7 (48.3–81.1)

CI: Confidence interval

Conclusions

Pleural fluid protein is not a valid test to diagnose TB. Lymphocyte predominance >80% in pleural fluid is a valid marker but, because of low prevalence of TB among pleural effusion, predictive values are compromised. Newer tests need to be included in pleural fluid studies with due to consideration of cost and logistics to improve validity of the diagnosis of tuberculous pleuritis in resource-constrained settings.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ministry of Health and Family Welfare. Government of India. TB India 2015 – Revised National TB Control Programme – Annual Status Report. New Delhi: Central TB Division; 2015.
2. Prakasha SR, Suresh G, D'sa IP, Shetty SS, Kumar SG. Mapping the pattern and trends of extrapulmonary tuberculosis. J Glob Infect Dis 2013;5:54-9.
3. Arora VK, Gupta R. Trends of extra-pulmonary tuberculosis under revised national tuberculosis control programme: A study from south Delhi. Indian J Tuberc 2006;53:77-83.
4. Ministry of Health and Family Welfare. Government of India. TB India 2013 – Revised National TB Control Programme – Annual Status Report. New Delhi: Central TB Division; 2013.
5. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. BMC Public Health 2008;8:15.
6. Solovic I, Jonsson J, Korzeniewska-Kosela M, Chiotan DI, Pace-Asciak A, Slump E, *et al.* Challenges in diagnosing extrapulmonary tuberculosis in the European Union, 2011. Euro Surveill 2013;18. Pii: 20432.
7. Ministry of Home Affairs. Government of India. Provisional Population Totals Paper 2 of 2011 India, Series 1. New Delhi: Office of the Registrar General and Census Commissioner; 2011. p. 8.
8. World Health Organization. Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents: Recommendations for HIV-prevalent and resource-constrained settings. Geneva: WHO Press; 2006. p. 21.
9. Pettersson T, Riska H. Diagnostic value of total and differential leukocyte counts in pleural effusions. Acta Med Scand 1981;210:129-35.
10. James P, Gupta R, Christopher DJ, Balamugesh T. Evaluation of the diagnostic yield and safety of closed pleural biopsy in the diagnosis of pleural effusion. Indian J Tuberc 2010;57:19-24.
11. Gázquez I, Porcel JM, Vives M, Vicente de Vera MC, Rubio M, Rivas MC. Comparative analysis of Light's criteria and other biochemical parameters for distinguishing transudates from exudates. Respir Med 1998;92:762-5.
12. Vorster MJ, Allwood BW, Diacon AH, Koegelenberg CF. Tuberculous pleural effusions: Advances and controversies. J Thorac Dis 2015;7:981-91.
13. Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio. Increased specificity for the diagnosis of tuberculous pleuritis. Chest 1996;109:414-9.
14. Liang QL, Shi HZ, Wang K, Qin SM, Qin XJ. Diagnostic accuracy of adenosine deaminase in tuberculous pleurisy: A meta-analysis. Respir Med 2008;102:744-54.
15. Valdés L, Pose A, San José E, Martínez Vázquez JM. Tuberculous pleural effusions. Eur J Intern Med 2003;14:77-88.
16. Hiraki A, Aoe K, Matsuo K, Murakami K, Murakami T, Onoda T, *et al.* Simultaneous measurement of T-helper 1 cytokines in tuberculous pleural effusion. Int J Tuberc Lung Dis 2003;7:1172-7.
17. Wankhade G, Majumdar A, Kamble PD, De S, Harinath BC. Multi-antigen and antibody assays (SEVA TB ELISA) for the diagnosis of tuberculous pleural effusion. Indian J Tuberc 2012;59:78-82.

An unusual cause of breathlessness and profuse micronodules

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ABSTRACT

We describe a 21-year-old male with a history of smoking and subacute onset of breathlessness with normal cardiorespiratory examination. The presence of "track marks" and digital infarcts prompted evaluation for infective endocarditis and confrontational history taking revealed anorexia, weight loss over 3 months along with intravenous drug abuse of reconstituted tablets of tapentadol. Echocardiography was normal and blood cultures were sterile; computed tomography showed bilateral, diffuse, small centrilobular nodules with "tree-in-bud" appearance. In this clinicopathologic conference, we discuss the clinical and radiological differential diagnosis of centrilobular nodules, lung biopsy findings, and management options for patients with such a presentation.

KEY WORDS: Excipient lung disease, intravascular talcosis, pulmonary foreign body angiogranulomatosis, talc granulomatosis

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PRESENTATION OF THE CASE

A 21-year-old college student presented with breathlessness and left-sided chest pain for a week. Breathlessness was insidious in onset and persisted on walking at own speed at level. Chest pain was left-sided, intermittent, pricking and non-exertional. There was no history of fever, rhinitis, headache, hemoptysis, cough, or joint pains. He did not raise pets but admitted to smoking 7–8 cigarettes/day and consuming whisky (192 g of alcohol weekly). He denied any recent worsening of educational performance. He did not report any significant prior medical illness or pertinent illness in family or occupational dust exposure. He was initially seen in cardiology and evaluation showed normal cardiac and respiratory examination without any pallor, clubbing, or organomegaly. Body mass index was 18.6 kg/m^2. The examination also showed extensive track marks over both arms with evidence of multiple peripheral digital infarcts [Figure 1], raising suspicion for infective endocarditis (IE). On confronting, he disclosed daily intravenous self-injections of three–four powdered and reconstituted 50 mg tablets of Tapentadol (Tydol, Sun Pharma) in sterile distilled water two–three times a day over the last 2 years. He also admitted to using inhaled cannabis twice weekly over the last 2 years. There was associated anorexia and unquantified weight loss over the last 3 months. Three sets of blood cultures were sterile. Electrocardiography showed T-wave inversions in leads V3–V6 and II, III, and aVF. Transthoracic echocardiography (TTE) showed normal valves without any evidence of vegetations or pulmonary hypertension and normal left ventricular function. Serum creatinine

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Figure 1: Composite image of the clinical photographs (left) of the right forearm showing "track marks," indicating healed superficial septic thrombophlebitis and (right) both hands showing multiple bilateral distal digital pulp infarcts. Similar findings were also seen in both plantar surfaces (not shown)

was 0.6 mg/dL; urine microscopy was normal without any albuminuria. Hemoglobin was 14.2 g/dL with normal leukocyte and platelet counts. He remained afebrile. Transesophageal echocardiography (TEE) was planned; in the meanwhile, review of his chest radiographs showed diffusely scattered fine nodules bilaterally [Figure 2, left]. Spirometry showed mild restriction with reduced diffusion capacity for carbon monoxide (forced expiratory volumes at 1 s 2.25 L [65%]%, forced vital capacity 2.87 L [71%], DLco 62%). There was desaturation to 92% while walking 6 minutes, with preserved walk distance (545 meters, 89% predicted). Human immunodeficiency virus (HIV), anti-nuclear antibodies, hepatitis B, and hepatitis C antibodies were negative by enzyme-linked immunosorbent assay. Immunoglobulin E levels were 9 IU/mL (normal <100 IU/mL). High-resolution computed tomography (HRCT) of the thorax showed bilateral profuse evenly distributed micronodules with "tree-in-bud" [Figure 2, right]. There was no sputum production on induction with 3% saline. A procedure was planned for diagnosis.

DIFFERENTIAL DIAGNOSIS

Important features of this case

This HIV-negative young adult with significant smoking history, cannabis use, and intravenous drug abuse with reconstituted tablets over 2 years has breathlessness, anorexia, and weight loss with radiological findings of micronodules. There is evidence of distal embolic infarcts, but initial evaluation for endocarditis was negative. The reduced DLco, restriction on spirometry and unexpectedly low walk distance in a young individual with desaturation on walking implies extensive parenchymal and/or pulmonary vascular disease. The differential diagnosis of profuse bilateral micronodules is extensive [Table 1] but can be narrowed by careful history and analysis of HRCT findings in conjunction with the expected pulmonary complications of intravenous drug abuse [Table 2].[1] The first step is to characterize the

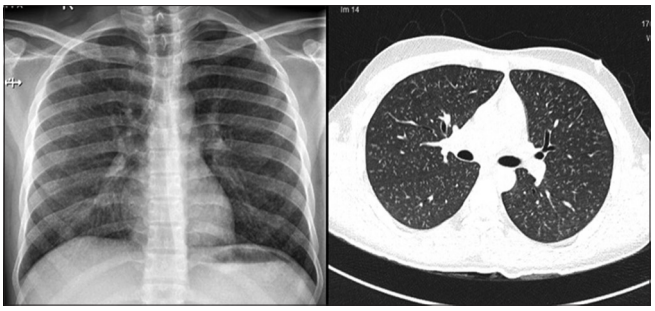


Figure 2: Composite image of the chest radiograph (left) showing small nodular opacities and high-resolution computed tomography of the thorax (right) showed bilateral profuse evenly distributed micronodules with tree-in-budding; pulmonary artery was normal in size, and there were no associated lymphadenopathy. Expiratory computed tomography did not show any air trapping (not shown)

Table 1: Causes of diffuse micronodular disease on computed tomography

With tree-in-budding
Infectious causes
Tuberculosis, nontubercular mycobacteria (typically MAC)
Bronchopneumonia: *Haemophilus influenzae*, *Mycoplasma pneumoniae*, Chlamydia
Viral bronchiolitis
Airway-invasive aspergillosis
Aspiration
Bronchiectasis: Cystic fibrosis, ciliary disorders, immunodeficiency
Immunologic
Allergic bronchopulmonary aspergillosis
Diffuse panbronchiolitis
Hematogenous: intravascular metastases (rare)
Without tree-in-budding
Infective causes
Tuberculosis
Fungi: *Histoplasma capsulatum*, blastomycosis, candidemia
Varicella zoster
Immunologic causes
Tropical pulmonary eosinophilia
Hypersensitivity pneumonitis (subacute)
Follicular bronchiolitis
Respiratory bronchiolitis
Sarcoidosis
Diffuse pulmonary amyloidosis
Allergic bronchopulmonary aspergillosis (rarely)
Occupational lung disease
Berylliosis, silicosis, coal worker's pneumoconiosis
Idiopathic
Pulmonary alveolar microlithiasis
Idiopathic pulmonary hemosiderosis
Malignancy
Metastasizing leiomyoma
Pulmonary Langerhans cell histiocytosis (early)
Metastatic adenocarcinoma, renal cell carcinoma, papillary thyroid carcinoma
Others
Metastatic calcification (renal failure)
Pulmonary arteriolar causes
Pulmonary edema and hemorrhage, excipient lung disease, plexogenic arteriopathy, veno-occlusive disease, pulmonary capillary hemangiomatosis

MAC: *Mycobacterium avium* intracellulare, *Wuchereria bancrofti*

Table 2: Pulmonary problems in intravenous drug abusers

Mechanism of Injury	Observed side-effects
Pharmacodynamic properties of injected drug	Noncardiogenic pulmonary edema (cocaine, opioids) Cocaine intoxication: pulmonary edema, pulmonary hemorrhage, cardiogenic pulmonary edema (coronary spasm) Methylphenidate intoxication: hemoptysis
Complications of unsafe intravenous injections	Lung abscess (with loss of consciousness, seizures, and aspiration) Infective endocarditis Septic pulmonary embolism Infections acquired from needle sharing (HIV, hepatitis B, hepatitis C)
Effects of contaminants	Excipient lung disease* Emphysema (lower lobe predominant "Ritalin" lung with methylphenidate) and Bullous lung disease Pulmonary vascular disease Group 1: Pulmonary hypertension (cocaine and methamphetamine) Group 3: Pulmonary hypertension (emphysema, foreign body granulomatosis, and interstitial lung disease) Interstitial lung disease Pneumothorax (subclavian injections, cocaine, and bullous lung disease), septic emboli
Related to associated abused substances, poverty, malnutrition	Pneumonia Tuberculosis More frequent exacerbations of asthma (fatal asthma)

*Talc, starch, crospovidone, and microcrystalline cellulose are used as fillers. HIV: Human Immunodeficiency Virus

distribution of the micronodules spatially within the lung and also the secondary pulmonary lobule. These nodules were uniform in size and were distributed evenly in a cephalocaudal direction, without any preferential lobar involvement. None of the nodules abut the pleural surface or the fissures [Figure 3]; there is clear evidence of extensive bilateral branching nodules in centrilobular distribution. There is no evidence of bronchiectasis; while no contrast was administered, in conjunction with echocardiography, there is no dilated pulmonary artery or lymphadenopathy. These findings limit the radiological differentials to causes of micronodules in a centrilobular distribution and extensive uniform "tree-in-bud" appearance. "Tree-in-bud" indicates the presence of dilated centrilobular bronchioles with lumen impacted by mucus, fluid, or pus and associated with peribronchiolar inflammation. "Tree-in-bud" indicates small airway disease and is associated with airway infection in the large majority but can also be rarely caused by arteriolar disease.[2] Its presence makes three common causes of centrilobular nodules unlikely: hypersensitivity pneumonitis, follicular bronchiolitis, and respiratory bronchiolitis.

Endobronchial tuberculosis

Mycobacterium tuberculosis is endemic in India, with more than 2.2 million new cases each year. Delayed recognition

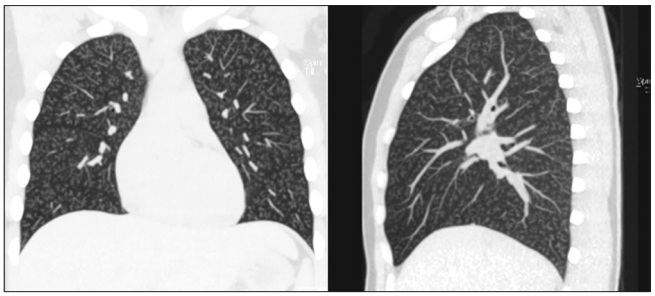


Figure 3: Coronal and sagittal reconstruction of the maximum intensity projection formatting of the high-resolution computed tomography images of the thorax showing diffuse, extensive, bilateral centrilobular nodules with branching and without any lobar predominance

and extensive disease are related to associated substance abuse, poverty, and malnutrition, all of which were present in this patient. Extensive endobronchial spread of tuberculosis can cause extensive tree-in-bud opacities although asymmetry is usually seen.[3] Tuberculosis is also the most common cause of micronodules with a random pattern (miliary). Even though the "pretest probability" of tuberculosis is high in this patient, the marked symmetry and the absence of any cavitation or consolidation is odd; digital infarcts are unusual but could be caused by an associated immune complex phenomenon that can occur with tuberculosis. *Mycobacterium avium*-intracellulare infection can also cause centrilobular nodules with "tree-in-bud," bronchiectasis, and consolidation. While it can be seen in immunocompetent patients, the classic phenotype is a middle-aged woman with slender build and without any constitutional symptoms.

Bronchopneumonia and viral bronchiolitis

Infectious bronchiolitis due to viruses, *Mycoplasma*, and chlamydia is a common cause of profuse and symmetric centrilobular nodules with "tree-in-bud;"[3] however, the prolonged illness and associated embolic phenomenon are unusual.

Allergic bronchopulmonary aspergillosis

Centrilobular nodules with "tree-in-bud" or miliary nodules can be caused by allergic bronchopulmonary aspergillosis but the absence of asthma, central bronchiectasis, and normal IgE levels rule out this diagnosis.[2,4]

Diffuse panbronchiolitis

Diffuse panbronchiolitis (DPB) is a rare disease of unknown cause presenting in males between the second and fifth decade with productive cough, diffuse bilateral centrilobular nodules with "tree-in-bud" and sinusitis. While reported in Indians,[5] it is extremely rare in our population but can explain all the clinical and radiological features.[6] Cold agglutinins are frequent in DPB and pulp infarcts may be due to an associated connective tissue disease. The absence of productive sputum, obstruction on spirometry, sinusitis, and air trapping on expiratory CT are unusual in DPB and necessitate further evaluation with surgical lung biopsy to establish the diagnosis.[6]

Excipient lung disease

Centrilobular nodules are usually caused by bronchiolar disease but can rarely be caused by arteriolar disease. Arteriolar causes include pulmonary edema and hemorrhage, excipient lung disease (ELD), granulomatosis with polyangiitis (GPA), plexogenic arteriopathy (PH), veno-occlusive disease (VOD), pulmonary capillary hemangiomatosis (PCH), and metastatic calcification.[2] There was no evidence of heart failure, renal failure or anemia and hemoptysis to suggest alveolar hemorrhage. The absence of pulmonary hypertension makes centrilobular nodules due to PH, VOD, and PCH very unlikely. Nodules due to GPA are larger, fewer, and associated with cavitation. Excipients are insoluble inert particulate filler materials that bind and protect the active drug during production as well as shape and lubricate tablets for easy swallowing. Excipients include talc (hydrated magnesium silicate), microcrystalline cellulose, crospovidone, and starch. Typically, excipients constitute 5% of a tablet's mass. On intravenous injection, excipient particles lodge in pulmonary arterioles and trigger an angiogranulomatous reaction; progressive disease is associated with dyspnea and pulmonary hypertension.[7] However, the presence of finger pulp infarcts, "tree-in-bud" appearance and absence of pulmonary hypertension are unusual in ELD.

CLINICAL DIAGNOSIS

In summary, the differential diagnosis of extensive centrilobular nodules with "tree-in-bud" without bronchiectasis or pulmonary hypertension in this young adult with smoking, cannabis use, and intravenous drug abuse of reconstituted tablets can be narrowed down to extensive endobronchial tuberculosis, diffuse panbronchiolitis, and ELD.

Dr. RM. PL. Ramanthan: With these clinicoradiological possibilities in mind, this patient underwent fundus examination and bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial lung biopsy, with a plan for surgical biopsy if evaluation was inconclusive. Can we now have the findings?

Dr. K. Divya: Dilated fundus examination confirmed refractile particles scattered along both the retinal arteries and retinal veins [Figure 4], confirming systemic embolization of small (<5 μm) talc particles, consistent with intravenous talcosis. Inhalational talc exposure does not cause such marked retinal deposition as the inhaled particles are larger and are restricted by bronchioles and pulmonary circulation.[8,9]

Dr. Nidhya Ganesan: BAL was negative for acid-fast bacilli and for *M. tuberculosis* by Xpert MTB/RIF; occasional needle-shaped crystals were seen on cytology. The transbronchial lung biopsy specimen confirmed perivascular granulomatous reaction to talc particles [Figure 5, left] that appear as birefringent needle-shaped crystals within the granulomas under polarized light [Figure 5, right]. Review of the composition of Tydol confirmed the presence of talc as excipient. Microcrystalline cellulose appears rod like while starch has a maltese cross pattern and crospovidone is nonbirefringent on polarization.[7]

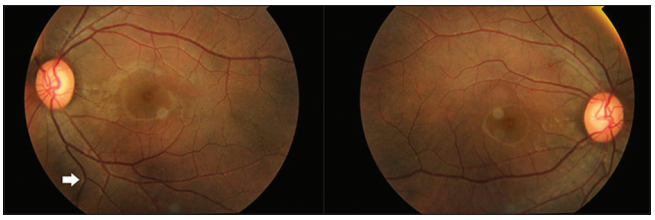


Figure 4: Fundus photograph of both eyes showing refractile particles scattered along both the retinal arteries and retinal veins, confirming the diagnosis of intravenous talcosis

Dr. Srinivas Rajagopala: A diagnosis of ELD (talc granulomatosis) was made. Psychiatry services were involved, and the patient underwent counseling, arrangement for family supervision, and daily-observed methadone replacement. He has successfully discontinued intravenous drug abuse and remains stable at 3 months of follow-up. Serial spirometry, DLco, and radiographs have been advised at 6 monthly intervals. Three unusual findings need an explantation; 1) The presence of "tree-in-bud" in a process that is hematogenous in origin 2)The absence of pulmonary hypertension in ELD and 3)The cause of the bilateral digital infarcts observed.

Dr. B. Devanand: Diseases involving pulmonary arterioles can cause centrilobular nodules as arterioles run in the peribronchovascular interstitium adjacent to bronchioles. Talcosis associated with intravenous abuse is most commonly associated with diffuse fine centrilobular nodules, ground-glass abnormalities or a combination of nodules and lower-lobe emphysema (Ritalin lung with methylphenidate).[9,10] The "tree-in-bud" pattern is unusual for arteriolar disease; it can be caused by intra-arteriolar accumulation of talc with associated adjacent perivascular granulomatous reaction giving the appearance of branching centrilobular nodules and has been described in a few cases of ELD earlier.[11]

Dr. Srinivas Rajagopala: What is the course of ELD? Can the disease progress despite cessation of abuse?

Dr. Kancherla Roopa: Lung fibrosis and pulmonary hypertension are related to the extent of vascular obstruction and granulomatous inflammation and are usually irreversible with talc, cellulose, and crospovidone as these cannot be mobilized; starch can be removed by metabolism and pulmonary hypertension can be reversible to a large degree. Disease progression can occur despite cessation due to ongoing granulomatous foreign-body reaction to the deposited, unmobilizable talc. The initial series of patients described all had progressive symptoms leading to death over 9 months to 5 years.[10,12] The index

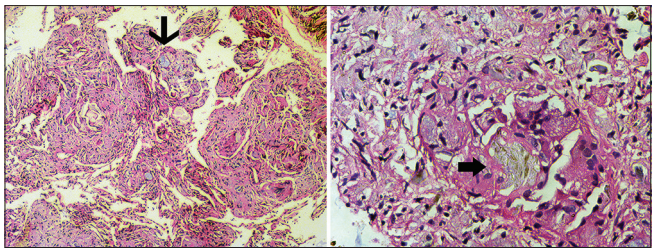


Figure 5: Photomicrographs of the transbronchial lung tissue biopsy specimen (left) demonstrating perivascular foreign body granulomatous reaction to the talc particles (thin arrow, H and E, ×100). Under polarized light, birefringent 20–95 μm needle-shaped crystals were seen in the granuloma, consistent with talc (block arrow, H and E, ×400)

patient has a large load of perivascular disease and is at risk for pulmonary hypertension and disease progression despite cessation of further abuse.

Dr. Srinivas Rajagopala: How common is systemic embolization of talc given that the lung acts as an effective filter?

Dr. K. Divya: The talc that is trapped in the pulmonary arterioles is typically around 10 μm, but smaller particles can escape to the systemic circulation and be detected in the retina, spleen, and virtually every organ on autopsy.[12] Finding excipient crystals in fundoscopy is a rapid noninvasive method to confirm intravenous talcosis.

Dr. Srinivas Rajagopala: Given the presence of digital infarcts, is TEE needed to exclude IE in this patient?

Dr. G. Rajendiran: The first consideration in this patient with possible unsafe needle practices, no murmurs, weight loss, breathlessness, and digital embolic manifestations was subacute native valve IE. TTE has low sensitivity compared to TEE (46% vs. 93%) although both are highly specific (96%).[13] Embolic manifestations are seen in about half of patients with IE.[14] He was not administered any antibiotics, three sets of blood cultures were sterile, and TTE showed all valves clearly without any vegetations. TEE is restricted in our practice to high probability for IE or in whom TTE is rendered less sensitive due to obesity, lung hyperinflation, or prosthetic valves. The index patient met only 2 minor Modified Duke's criteria and was considered low risk for IE after the initial evaluation and TEE deferred.[15] Furthermore, a clear alternative explanation for the observed distal infarcts in the form of talc deposition in the distal microcirculation with thrombosis is available and corroborated by fundoscopy. While this physical finding has not been described in ELD previously, it is consistent with the distal digital embolization and subsequent microthrombosis caused by talc.

Dr. Srinivas Rajagopala: What are the options for those with progressive breathlessness despite cessation of intravenous drug abuse?

Dr. RM. PL. Ramanthan: There are previous small series of lung allografts after a careful selection process in ELD with excellent outcomes.[16] Cadaveric bilateral sequential lung transplantation is a reality in our country today, and he can be listed for the same at our center if he meets criteria and remains free of substance abuse for at least 6 months with adequate psychiatry counseling.

SUMMARY

Clinical examination findings continue to have relevance in this era of investigation-driven medicine. Excipient lung disease can result from intravenous injection of reconstituted tablets and can present with ground glass opacities, small nodules, and emphysema. Pulmonary arteriolar diseases must be considered in the differential diagnosis of centrilobular nodules on CT.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that names, identifying marks, and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Marchiori E, Souza AS Jr., Franquet T, Müller NL. Diffuse high-attenuation pulmonary abnormalities: A pattern-oriented diagnostic approach on high-resolution CT. AJR Am J Roentgenol 2005;184:273-82.
2. Webb R, Muller NL, Naidich DP. HRCT Findings: Multiple nodules and nodular opacities. In: High-Resolution CT of the Lung. 5th ed. Philadelphia: LWW Wolters Kluwer; 2014. p. 125-9.
3. Walker CM, Abbott GF, Greene RE, Shepard JA, Vummidi D, Digumarthy SR, *et al*. Imaging pulmonary infection: Classic signs and patterns. AJR Am J Roentgenol 2014;202:479-92.
4. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, *et al*. Allergic bronchopulmonary aspergillosis: Review of literature and proposal of new diagnostic and classification criteria. Clin Exp Allergy 2013;43:850-73.
5. Nath A, Aggarwal AN, Gupta R. Diffuse panbronchiolitis: Report of a rare disease from India. Indian J Chest Dis Allied Sci 2010;52:43-5.
6. Poletti V, Casoni G, Chilosi M, Zompatori M. Diffuse panbronchiolitis. Eur Respir J 2006;28:862-71.
7. Nguyen VT, Chan ES, Chou SH, Godwin JD, Fligner CL, Schmidt RA, *et al*. Pulmonary effects of i.v. Injection of crushed oral tablets: "Excipient lung disease". AJR Am J Roentgenol 2014;203:W506-15.
8. Akira M, Kozuka T, Yamamoto S, Sakatani M, Morinaga K. Inhalational talc pneumoconiosis: Radiographic and CT findings in 14 patients. AJR

Am J Roentgenol 2007;188:326-33.
9. Ward S, Heyneman LE, Reittner P, Kazerooni EA, Godwin JD, Müller NL, *et al*. Talcosis associated with IV abuse of oral medications: CT findings. AJR Am J Roentgenol 2000;174:789-93.
10. Feigin DS. Talc: Understanding its manifestations in the chest. AJR Am J Roentgenol 1986;146:295-301.
11. Bendeck SE, Leung AN, Berry GJ, Daniel D, Ruoss SJ. Cellulose granulomatosis presenting as centrilobular nodules: CT and histologic findings. AJR Am J Roentgenol 2001;177:1151-3.
12. Sieniewicz DJ, Nidecker AC. Conglomerate pulmonary disease: A form of talcosis in intravenous methadone abusers. AJR Am J Roentgenol 1980;135:697-702.
13. Bashore TM, Cabell C, Fowler V Jr. Update on infective endocarditis. Curr Probl Cardiol 2006;31:274-352.
14. McDonald JR. Acute infective endocarditis. Infect Dis Clin North Am 2009;23:643-64.
15. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr., Ryan T, *et al*. Proposed modifications to the duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;30:633-8.
16. Weinkauf JG, Puttagunta L, Nador R, Jackson K, LaBranche K, Kapasi A, *et al*. Long-term outcome of lung transplantation in previous intravenous drug users with talc lung granulomatosis. Transplant Proc 2013;45:2375-7.

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Miliary Sarcoidosis: does it exist? A case series and systematic review of literature

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Abstract. *Background and Objectives:* Sarcoidosis typically presents with peribronchovascular and perilymphatic nodules on high-resolution computed tomography (HRCT); a miliary pattern is reported but not well described. *Design, setting:* We describe four patients with miliary sarcoidosis and results of a systematic review of all previously reported cases from 1985 onwards. *Results:* We identified only 27 cases of "miliary" sarcoidosis in the HRCT era. These patients were older (85.2% older than 40 years), had more co-morbidities (72.7%) and were symptomatic compared to "typical" sarcoidosis. Respiratory symptoms were present in 61.9% at diagnosis. Hypercalcemia was seen in 28.5%. On review of HRCT images, only 34.6% (9/26) had a "true miliary" pattern without fissural nodules. In our series, prominent perivascular granulomas were seen on histopathology in all. 44.4% (12/27) had tuberculosis preceding or concurrent to miliary sarcoidosis. Of the eight true associations, tuberculosis preceded sarcoidosis by 52 (median, IQR 36) weeks in six and occurred concurrently in another two. The diagnosis of tuberculosis was clinical in all with concurrent diagnosis of tuberculosis and sarcoidosis. Treatment with steroids had 100% response and 14.2% relapse. *Conclusions:* A true miliary pattern in the HRCT era is very rare in sarcoidosis and subtle perilymphatic pattern is nearly always seen; this should be labeled "pseudo-miliary". Prominent perivascular granulomas are associated with true miliary pattern. Miliary sarcoidosis patients are older and symptomatic, needing treatment at diagnosis. "Miliary" sarcoidosis may follow treatment for tuberculosis; concurrent cases possibly indicate the difficulty in differentiating both or a "tuberculo-sarcoid" presentation. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (1): 53-65)*

Key words: sarcoidosis, miliary, micronodules, miliary tuberculosis

Introduction

Miliary sarcoidosis has been described as a rare presentation of sarcoidosis and was reported in <1% of all cases in the pre-computed tomography (CT) era on chest radiography (1). With the advent of high-resolution-CT (HRCT), peribronchovascular and perilymphatic nodules in relation to the secondary pulmonary lobule are recognized as classic of sarcoidosis (2). However, random micronodules (miliary pattern) have been described and sarcoidosis is included in the differential diagnosis of a "miliary" pattern on HRCT (3, 4). There are no well-described series or systematic reviews of "miliary" sarcoidosis in the high-resolution CT (HRCT) era. On the other hand, tuberculosis continues to be a global pandemic with an estimated 10 million cases worldwide in 2016 (5). Miliary tuberculosis accounts for 1-2%

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of all cases of new cases of tuberculosis and 8% of all extra-pulmonary tuberculosis (6). The relationship between tuberculosis and sarcoidosis continues to be enigmatic; tuberculosis can precede, follow or present concurrently with sarcoidosis (7). In patients with miliary tuberculosis, the clinical symptoms of night sweats, weight loss and fatigue are non-specific and overlap markedly with sarcoidosis. Respiratory symptoms are often minimal in either forms of miliary disease and microbiological evidence of *M.tuberculosis* is only present in 20-30% of miliary tuberculosis (8). Sarcoidosis has a characteristic perilymphatic pattern on histopathology and radiology (2); the co-relation of random nodules seen on radiology with pathology in "miliary" sarcoidosis is unclear. We describe a series of patients with miliary sarcoidosis and the results of our systematic review on miliary sarcoidosis in the HRCT era.

Materials and methods

We describe four patients with miliary nodules and proven sarcoidosis that was managed in the authors' institution over three years and performed a systematic search of literature for miliary sarcoidosis.

Systematic search of literature

Two of the authors (R.S and R.K) conducted a systematic search of literature in PUBMED independently using the MeSH term "miliary" *AND* "sarcoidosis" limited to title/abstract and restricted the search from the year 1985 onwards (9). The choice of the year was defined by the first description of the current HRCT protocols and detailed description. This was further supplemented by search of IndMed using the same terms, the references of retrieved articles, our personal records and the Internet search engine GOOGLE. These articles were screened without blinding, by title and abstract review to identify relevant studies. Any discrepancy was resolved by consensus. Full texts or abstracts were then retrieved to identify the type of lung involvement in reported cases with miliary sarcoidosis. Only those articles that included patients with HRCT according to established protocols (9), included representative images or detailed descriptions and had a histological diagnosis compatible with sarcoidosis (10) were included for analysis. For the purpose of this systematic review, we included all descriptions that were accepted as "miliary" in published cases but reanalyzed the images independently. The diagnosis of sarcoidosis was according to previous published guidelines (10).

Data extraction

Data was abstracted from selected cases in a predefined data extraction form (Supplementary Table).

Statistical analysis

Continuous data are presented as mean (standard deviation (SD), median [range or Interquartile Range (IQR)] and categorical data as percentages and proportions. All statistical analysis was done using the Statistical software SPSS Version 14.

Ethics

The Human Ethics Committee of PSG IMS&R, Coimbatore, approved the study. All data was anonymized to maintain patient confidentiality.

Case reports

Case 1

A 65-year-old lady presented with cough, night sweats and exertional breathlessness of two months' duration. There was no history of weight loss or fever. Her past history was significant for hypertension and non-productive cough two years before that was evaluated at another center. CT had shown right paratracheal and bilateral hilar lymphadenopathy along with upper lobe-predominant micronodules. Tuberculin skin testing was negative and endobronchial ultrasound-guided needle aspiration (EBUS-FNA) and transbronchial lung biopsy (TBLB) were performed. EBUS-FNA showed few non-caseating granulomas and TBLB was non-contributory. A presumptive diagnosis of tuberculosis was made and four-drug anti-tuberculosis treatment (ATT) was administered for six months with improvement in cough. Examination was unremarkable during current evaluation. There was no exposure to pets, drugs

or occupational dusts. Chest radiography was normal. Spirometry was normal and diffusion capacity showed mild reduction. HRCT showed profuse random micronodules without lymphadenopathy and an upper-lobe and peripheral predominance that was visualized better with maximum intensity projection (MIP, Figure 1). Renal function tests, serum calcium and angiotensin-converting enzyme (ACE) levels were normal. Fibreoptic bronchoscopy (FOB) was done with bronchoalveolar lavage (BAL) and TBLB; BAL Fluid was negative for acid-fast bacilli and Xpert MTB/RIF and TBLB showed interstitial inflammation only. Surgical lung biopsy was performed and showed extensive perivascular non-caseating granulomas (Figure 2). BAL mycobacterial cultures were subsequently reported as negative. She was started on steroids with resolution in cough and breathlessness by second month of treatment. She remains asymptomatic and off steroids after one year of treatment.

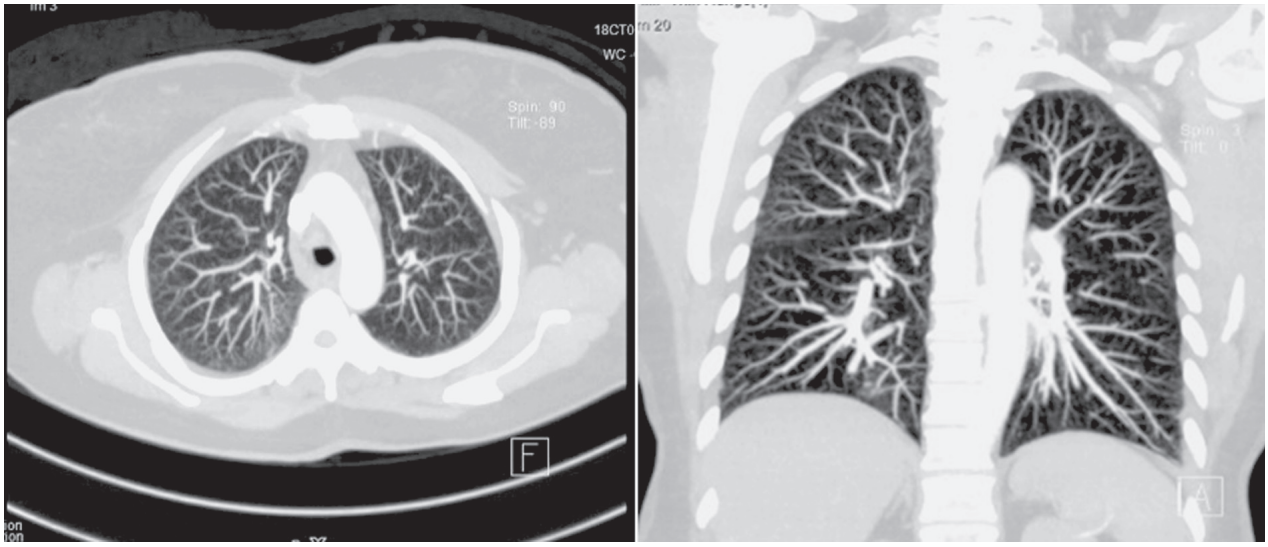


Fig. 1. Composite image of Maximal Intensity Projection images of high-resolution computed tomography (HRCT) of the thorax from patient 1 showing upper-lobe and peripheral-predominant miliary micronodules

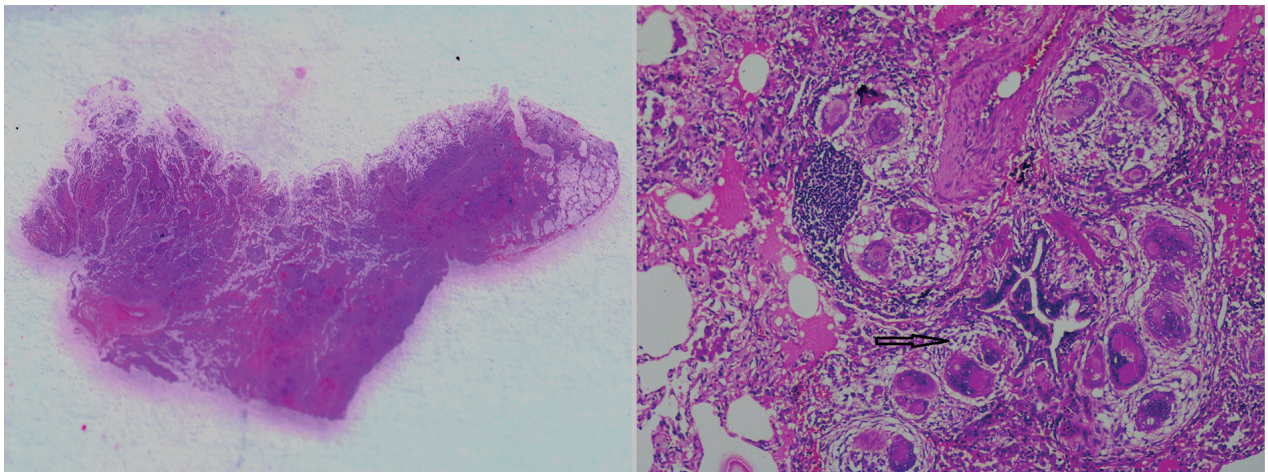


Fig. 2. Composite image of photomicrographs of surgical lung biopsy specimen of Patient 1 with left [Gross Pathological specimen, transverse plane] showing subpleural sparing and (right, Hematoxylin and Eosin stain (H & E), x 4) showing peribronchial and perivascular granulomas (arrow)

Case 2

A 40-year old gentleman presented with polyuria, fatigue and nausea of 20 days duration. There was also history of unquantified weight loss and exertional dyspnea. He did not smoke and did not raise pets, abuse drugs or have exposure to occupational dusts. Physical examination showed raised papulonodular skin lesions over hands and legs. Renal function tests showed serum creatinine of 2.65 mg/dL (normal <0.8 mg/dL); Urine microscopy showed 2+ albumin with bland sediments. Hemoglobin was 10.9 g/dL with normal leukocyte and platelet counts. Ultrasound showed normal kidney size with preserved corticomedullary distinction. Chest radiographs showed miliary nodules. Serum ionized calcium was 1.8 mg/dL (normal 1.1-1.3 mg/dL) with suppressed parathyroid hormone (1.9 pg/mL, normal 15-65) and normal vitamin D levels (23.6 ng/mL). ACE levels were elevated (98 U/mL, normal 8-53). Tuberculin skin testing was negative. CT-Chest showed random micronodules without zonal preponderance and bilateral hilar lymphadenopathy (Figure 3, left). FOB was done with BAL and TBLB; BAL was negative for acid-fast bacilli and Xpert MTB/RIF. TBLB showed perivascular non-caseating granulomas with Schaumann and asteroid bodies (Figure 4). Punch biopsy of the skin also showed non-caseating granulomas in the dermis**.** He was started on steroids with resolution of hypercalcemia and normalization of renal function. His subsequent course was complicated by steroid-aggravated diabetes presenting with hyperosmolar coma; methotrexate was added and steroids were tapered. He remained asymptomatic with near-complete radiologic clearing (Figure 3, right) after one year of stopping methotrexate but relapsed by 18 months and remains on methotrexate.

Case 3

A 55-year old gentleman with moderately controlled diabetes mellitus presented with exertional breathlessness, cough, right-sided shoulder pain and weight loss of one month's duration. There was no anorexia or expectoration. He was a farmer by occupation and did not have exposure to poultry or occupational dusts and chemicals. Physical examination showed fine bilateral crepitations without clubbing. Renal function tests, serum calcium, urine microscopy, ACE levels and hemogram were normal. Chest radiographs showed miliary nodules (Figure 5, left). Human Immunodeficiency virus was negative by enzyme-linked sorbent assay. Tuberculin skin testing was strongly positive (18 mm, 1 TU). CT-Chest showed random micronodules without lymphadenopathy (Figure 6, left). FOB was done with BAL

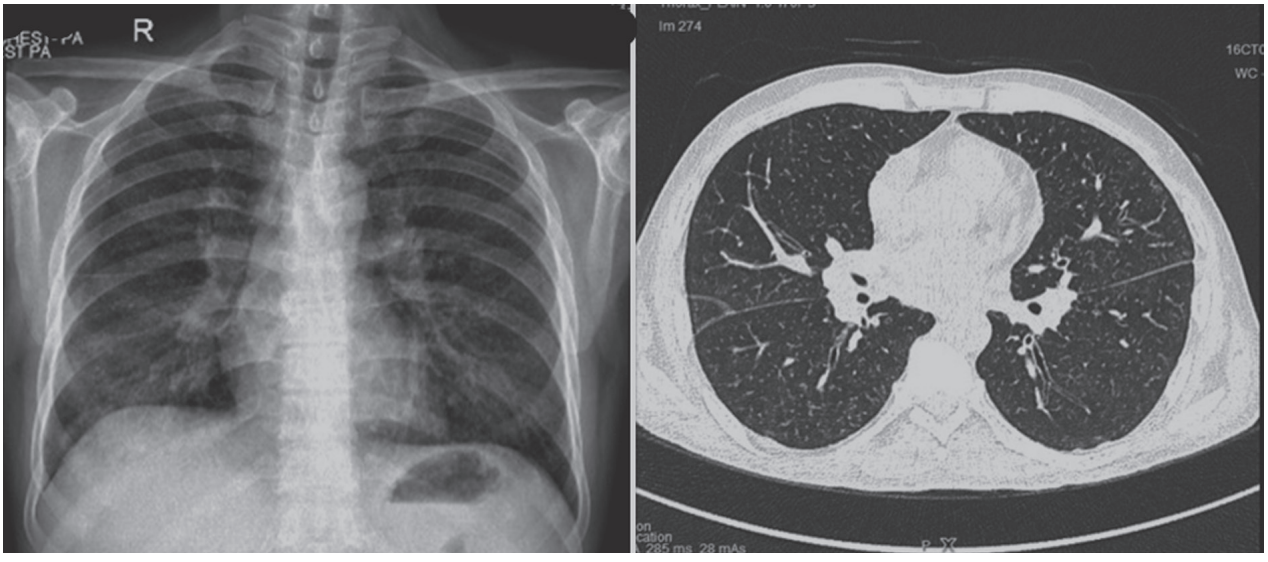


Fig. 3. Composite images of chest radiography of patient 2 showing miliary nodules (left) and HRCT images (major fissure level) showing upper lobe-predominant random nodules. No involvement of the fissure or "beading" of the fissure is seen

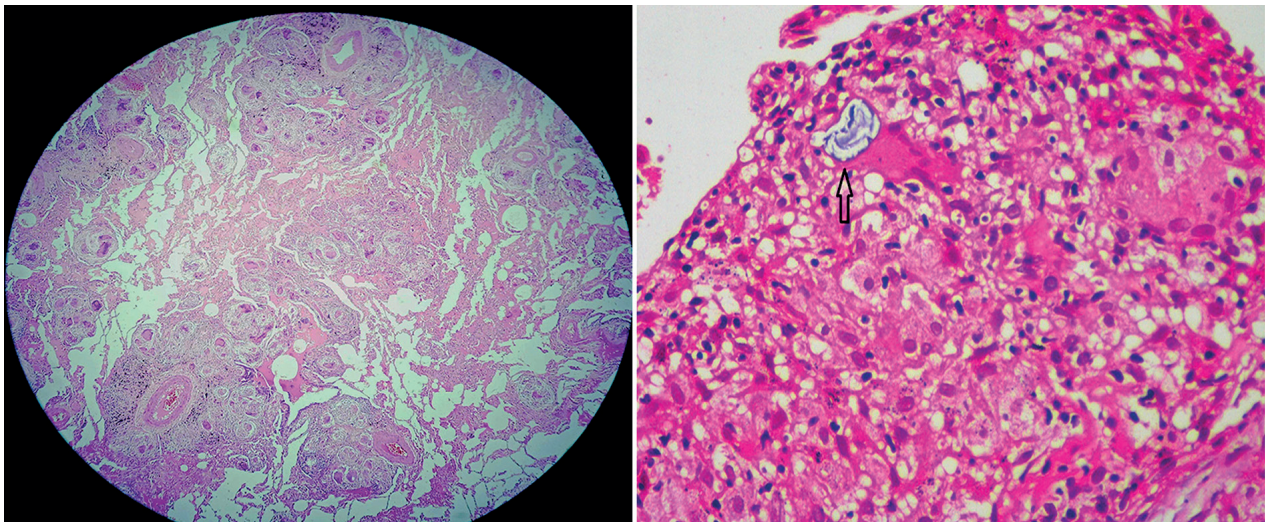


Fig. 4. Composite image of Photomicrographs of lung biopsy sample of Patient 2 with left, [Hematoxylin and Eosin stain (H & E), x scanner power] showing multiple non-necrotizing perivascular granulomas, and right, [H & E stain, x 40) showing a Schaumann body within a giant cell (arrow)

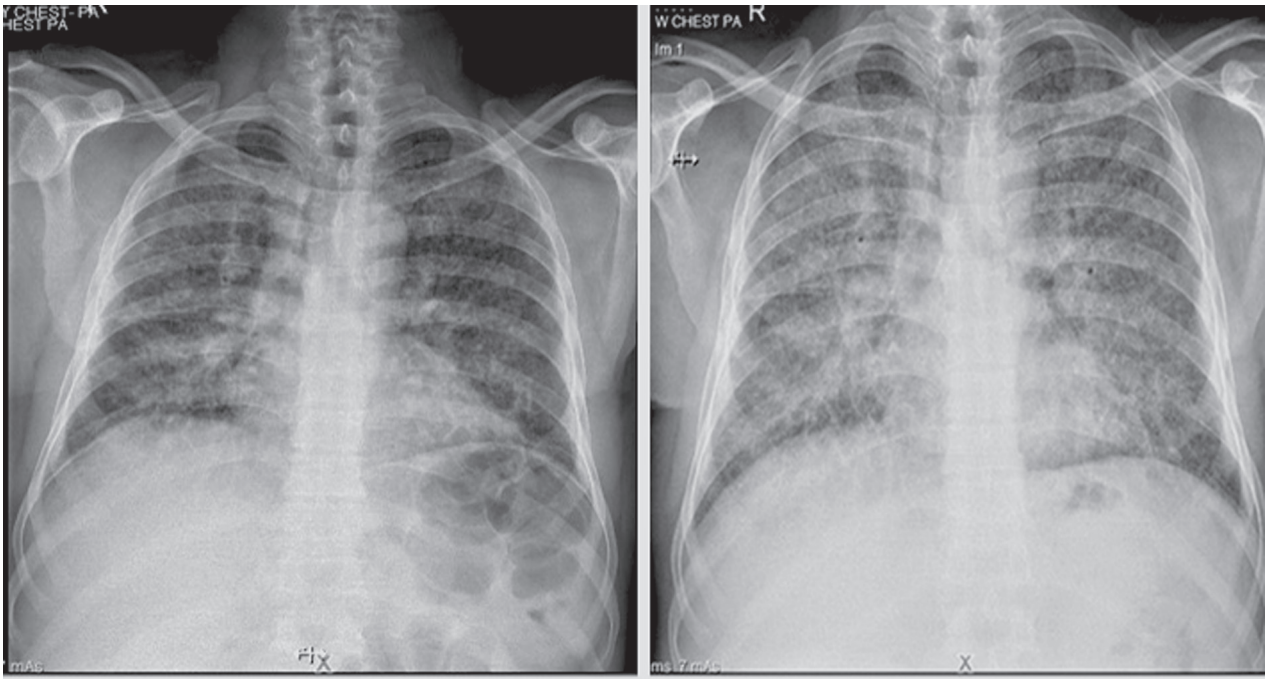


Fig. 5. Composite image of the Chest Radiographs of Patient 3 at initial evaluation (left) showing extensive miliary nodules. Subsequent Chest radiograph (right) after six weeks of anti-tuberculosis treatment alone show profusion of nodules with diffuse ground-glass opacification. Worsening hypoxemia was clinically noted

and TBLB; BAL was negative for acid-fast bacilli and Xpert MTB/RIF. TBLB showed well-formed perivascular granulomas with Langhans' giant cells, occasional fibrinoid necrosis and lymphocytes rimming (Figure 6, right). A diagnosis of miliary tuberculosis was made and four-drug weight-based ATT was started. However, his symptoms continued to worsen with worsening nodules and development

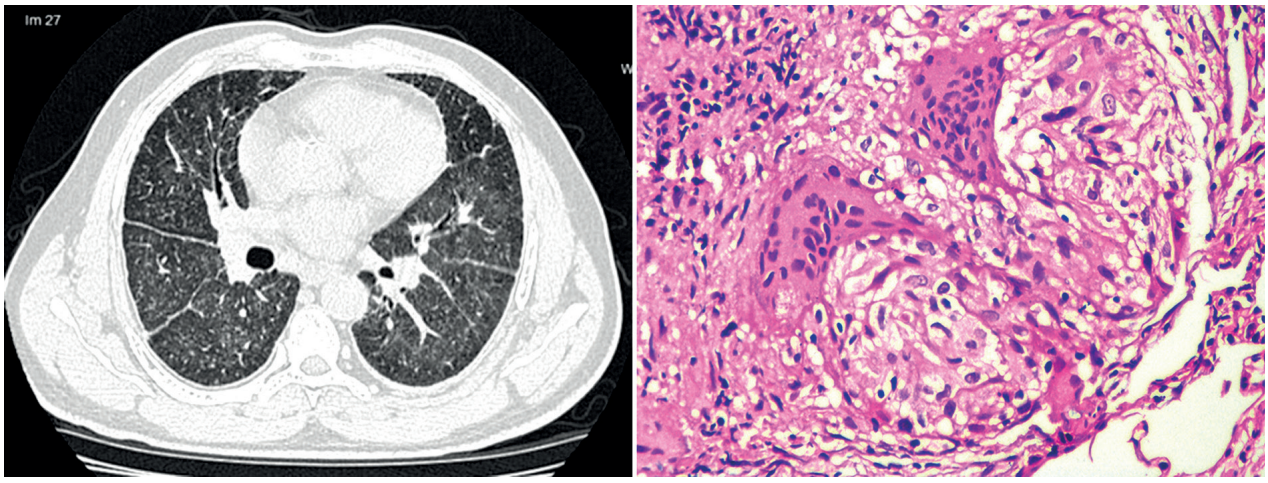


Fig. 6. Composite image of the HRCT image of Patient 3 at the level of major fissure during worsening hypoxemia corresponding to right-side of Figure 5 (left), showing random nodules with fissural prominence, with beading in both oblique fissures; some interlobular septal thickening and diffuse ground glass opacity can be seen and right, (Hematoxylin and Eosin stain, x 40) showing a non-necrotizing granuloma with peripheral lymphocyte cuffing

of hypoxemia needing oxygen at four weeks (Figure 5, right). BAL mycobacterial cultures were negative in the meantime. Steroids were added with a presumptive diagnosis of immune reconstitution syndrome, with resolution of hypoxemia and symptoms. Symptoms recurred after stopping steroids at four weeks; right-sided vision loss due to uveitis occurred. After a clinico-pathological review, a possibility of concurrent miliary tuberculosis with sarcoidosis was considered. ATT was continued and low-dose tapering steroids and methotrexate (in view of poorly controlled diabetes) were added, with complete resolution of symptoms by eight weeks. He remains asymptomatic after two years of cessation of ATT and methotrexate.

Case 4

A 48-year-old gentleman presented with fatigue for two weeks. There was no history of night sweats, weight loss or fever. He did not smoke, did not raise pets, abuse alcohol or drugs and did not report exposure to occupational dusts. Physical examination was normal. Renal function tests, urine microscopy and hemogram were normal. Serum calcium was elevated (1.43 mg/dL, normal 1.1-1.3). Chest radiographs showed miliary nodules (Figure 7, left). ACE levels were elevated (76 U/mL, normal 8-53). Tuberculin skin testing was negative. CT-Chest showed upper lobe predominant random micronodules and lower-lobe interlobular septal thickening (Figure 8, left & right). FOB was done with BAL and TBLB; BAL was negative for mycobacterial cultures and Xpert MTB/RIF. TBLB showed prominent perivascular non-necrotizing epitheloid granulomas with asteroid bodies (Figure 7, right). A diagnosis of miliary sarcoidosis was made and steroids were initiated. His symptoms and hypercalcemia resolved by four weeks and steroids were tapered over the next nine months. He remains asymptomatic on follow-up.

Results of the systematic search

Our systematic search identified 27 reports of miliary sarcoidosis in 21 reports (3, 11-30); this included 16 men and 9 women (Table 1, supplementary Table). This cohort of "miliary sarcoidosis" was older, was mostly symptomatic and had high co-morbidity burden. The mean age of the included patients was 47.5±13.2 years; 85.2% were older than 40 years. 72.7% reported at least one associated co-morbidity (11, 12, 16, 18, 21, 27, 30).

The median time to presentation was 8 (IQR 12) weeks. The majority had symptoms at the time of presentation (90.9%, 20/22); only two patients recognized by surveillance transbronchial lung biopsies post-lung transplantation were asymptomatic

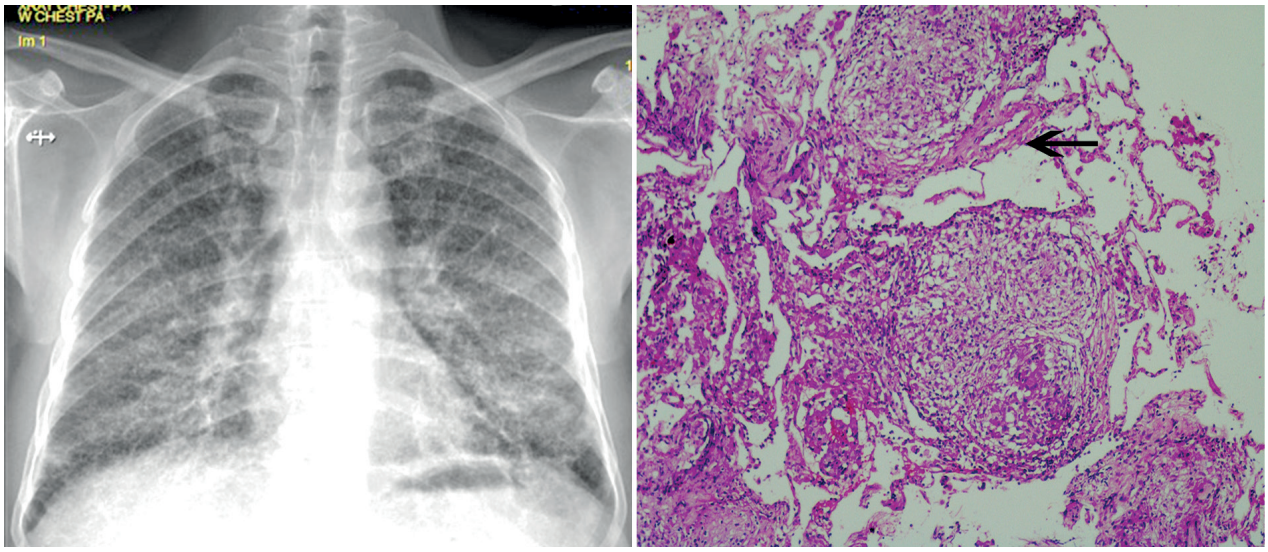


Fig. 7. Composite image of chest radiography images (left) of Patient 4 showing miliary nodules at diagnosis and photomicrographs of the lung biopsy specimen [right, Hematoxylin and Eosin stain (H & E) x 4] showing multiple perivascular granulomas (arrow)



Fig. 8. Composite image of the HRCT image of Patient 4 showing (left, upper lobes) random nodules with perilymphatic predominance, beading of the right oblique fissure and perivascular nodules and (right, lower lobes) marked interlobular septal thickening in the lower lobes associated with random nodules

at diagnosis (11, 12). Respiratory symptoms, however, were present in 63.6% only at diagnosis (13, 14, 16, 20, 21, 23-25, 28-30). In those with respiratory symptoms, breathlessness and cough were present in less than 50% (45.4%). Fever (27.3%) (13, 21-23, 29), night sweats (11.1%) (22, 27) and weight loss (22.7%)(18, 22, 27) were all seen in a significant percentage at diagnosis.

Symptomatic multi-systemic disease in association with miliary sarcoidosis was seen in 36.4%.(17, 19, 20, 23, 30) Hypercalcemia was seen in 27.3% (19, 20, 23, 27) and acute kidney injury was seen in 9.1% (19) at diagnosis. None needed dialysis but chronic kidney disease occurred in association with nephrocalcinosis (19). Ocular (13.6%, 3/22) involvement (25), bone (4.5%, 1/22) disease (17), sicca syndrome

Table 1. Summary of clinical features of all reported cases of thoracic sarcoidosis with miliary nodules on High-resolution Computed Tomography (N=27) [$]

Characteristic	Description
Age, Mean ± S.D, N=24	47.5±13.2 years; Age >40 years 85.2%
Gender (Male: Female), N=25	16:9
	In those >40 years, Male: Females O.R 0.26, p=1.00 (61.9% vs 75%)
Country of description	India (10/26), United States (7/26), Greece (5/26), Japan (3/26), Germany (1/26)
Symptoms at presentation	
Any symptoms, N=22	None, asymptomatic (9.1%, 2/22)
Symptom duration prior to presentation, Median, IQR N=19	8, (12) weeks
Symptoms	Any respiratory symptom (63.6%, 14/22)
	Dyspnea (45.4%, 10/22), cough (45.4%, 10/22)
	Fatigue (30.4%, 7/23), fever (27.3%, 6/22), weight loss (22.7%, 5/22), night sweats (11.1%, 3/22)
Acute kidney injury	9.1%, 2/22
Hypercalcemia	27.3%, 6/22
Co-morbidities, N=22	Any 72.7% (16/22)
	Smoking (18.2%, 4/22), diabetes (13.6%, 3/22), hypertension (18.2%, 4/22), lung transplantation (9.1%, 2/22), dust exposure (13.6%, 3/22)
Symptomatic disseminated sarcoidosis	8/22 (36.4%)
Other site involvement	Ocular (13.6%, 3/22), pleural effusion (9.1%, 2/22), bone (4.5%, 1/22), sicca syndrome (4.5%, 1/22), pancreas (4.5%, 1/22)
Radiological findings (N=26)	
True miliary pattern on HRCT	17/26 (65.4%) only
Perilymphatic nodules on HRCT in miliary CT	8/17 (47.1%); Truly random nodules total 9/26 (34.6%)
Zonal predominance seen	Upper & middle (7/26, 26.9%), central (7.7%, 2/26), peripheral (19.2%, 5/26)
Fissural nodules	65.4% (17/26)
Peripheral subpleural predominance	69.2% (18/26)
Interlobular septal thickening	23.1% (6/26)
Intralobular septal thickening	11.5% (3/26)
Ground glass opacity	11.5% (3/26)
Mediastinal adenopathy	Yes (50%, 13/26); Right paratracheal (23.1%, 6/23), bilateral hilar (23.1%, 9/23), others (8.7%, 2/23)
Generalized lymphadenopathy	8.7% (2/23)
Steroid treatment	Yes 21/22 (95.2%), Response 100% (21/21)
Relapse	14.2% (3/21)
Tuberculosis and miliary sarcoidosis	
Both tuberculosis and sarcoidosis	Both tuberculosis and sarcoidosis 44.4% (12/27)
	Definite or probable tuberculosis followed by sarcoidosis 29.7% (8/27)
	Probable misdiagnosis of tuberculosis 33.3%, (4/12)
	Microbiologically proven 37.5% (3/8) of all cases only
	Sequential (87.5%, 7/8), concurrent (25%, 2/8)[@@]

(continued on next page)

Characteristic	Description
Time to sequential miliary sarcoidosis in those with tuberculosis preceding sarcoidosis, (Median, IQR) N=7	52 (36) weeks
Site of tuberculosis	Pulmonary 87.5% (7/8), spinal 12.5% (1/8)
Yield of TBLB, N=21	85.7% (18/21)
Miliary tuberculosis preceding or concurrent with miliary sarcoidosis	5/8 (62.5%), 2/5 with miliary TB preceding miliary sarcoidosis
ATT-induced hepatitis	2/8 (25%)

Abbreviations: S.D standard deviation, IQR Inter-quartile range, HRCT High-resolution Computed Tomography, TBLB transbronchial lung biopsy, ATT Anti-tuberculosis treatment
[@@]One patient had both sequential and concurrent diagnosis of tuberculosis and sarcoidosis

(4.5%, 1/22) (20) and pancreatic mass (4.5%, 1/22) (28) were the other manifestations seen. Pleural effusion was seen in association in 9.1% (2/22) (24, 28).

On review of the HRCT images, a "true miliary" pattern was seen in 65.4% (17/26) of the reported cases only. In these cases, fissural nodules and prominence could still be seen in 47.1% (8/17); only 34.6% (9/26) of reported cases could be classified as random micronodules on radiologic review (12-14, 17, 18, 24, 26, 28, 30). An upper and middle lobe distribution of nodules (11-13, 15, 16, 25-27) was seen in 26.9% (7/26) and a peripheral distribution was seen in 69.2% (11, 12, 15, 24, 28). Perilymphatic clustering of nodules (3, 11-18, 22, 24-26, 28) or a perivascular dominance (3, 13, 15, 19, 22, 26-28) of nodules was seen in 65.4% and 42.3% respectively. Interlobular septal thickening, often lower-lobe in distribution, was seen in 23.1% (Table 1) (12, 14, 15, 18). Mediastinal adenopathy was observed in 50% in association with micronodules and generalized systemic lymphadenopathy was seen in 8.7% of patients (19, 20).

44.4% (12/26) of the reported patients had tuberculosis preceding or concurrent to the diagnosis of miliary sarcoidosis (13-17, 20-22, 29). In four of these patients, this was likely a misdiagnosis (13, 14, 30). In eight patients, tuberculosis occurred preceding (15-17, 21, 22) sarcoidosis in six (62.5%), concurrently in one (37.5%) (29) and both in one (23). In those with tuberculosis preceding sarcoidosis, a microbiological confirmation was made in 42.9% (15-17, 22); Others were diagnosed based on pathology and clinical response (21). In patients with concurrent diagnosis of tuberculosis and sarcoidosis, the diagnosis of tuberculosis was clinical in all; in all these patients, clinical and pathological findings, including caseous necrosis, were consistent with tuberculosis but clinical resolution occurred on initiation of steroids only (23, 29). The time to subsequent diagnosis of miliary sarcoidosis after treatment for tuberculosis was a median of 52 (IQR, 36) weeks. Miliary tuberculosis followed or concurrent with miliary sarcoidosis was reported in four patients (Table 2) (15, 16, 23, 29).

TBLB was the most common modality used for diagnosis and was reported as diagnostic in 85.7%. As most patients were symptomatic, treatment with steroids was offered in 95.2%, with 100% response. Methotrexate was administered in two patients for steroid-related severe adverse effects, with good response in both. Three patients (14.2%) relapsed after steroid taper; prompt response was noted on re-institution without further relapse.(21)

Discussion

The description of "miliary" refers to small, well-defined, 1 to 3 mm nodules on chest radiography and is derived from Latin miliarius, meaning millet seed (8). This pattern implies hematogenous dissemination of disease and is classically associated with hematogenous spread of tuberculosis, fungal infections and malignancy (31). However, more than 40 diseases have been associated with this pattern on chest radiography (Table 2). The importance of quickly establishing the diagnosis, especially in high prevalence areas of tuberculosis, is that miliary tuberculo-

Table 2. Reported causes of miliary nodules on High-resolution Computed Tomography

Classification	Cause of miliary Nodules	Site of micronodules in SPL
Infectious	Mycobacterium tuberculosis	Random
	Disseminated Bacillus-Calmette-Guerin infection	Random
	Bacterial: Mycoplasma pneumoniae, Hemophilus influenzae	Centrilobular
	Fungal: Histoplasmosis, Coccidioidomycosis, hematogenous Candida infection	Random
	Viral: Varicella-zoster, cytomegalovirus	Centrilobular
	Parasitic: Tropical pulmonary eosinophilia	Centrilobular
Immunologic	Pneumoconiosis: Silicosis	Perilymphatic
	Pneumoconiosis: Coal worker's pneumoconiosis	Perilymphatic
	Hypersensitivity pneumonitis	Centrilobular
	Sarcoidosis	Perilymphatic
	Idiopathic pulmonary hemosiderosis	Centrilobular
	Pulmonary alveolar microlithiasis	Centrilobular
	Diffuse panbronchiolitis	Centrilobular
	Allergic bronchopulmonary aspergillosis	Centrilobular
Malignancy	Secondaries from bronchogenic carcinoma	Perilymphatic
	Others: melanoma, papillary thyroid carcinoma, renal, breast, Trophoblastic tumor, osteosarcoma	Random
	Pulmonary Langerhans' cell histiocytosis	Random
	Metastasizing leimyoma	Random
	Lymphoma	Perilymphatic
Substance abuse	Excipient lung disease	Centrilobular

Abbreviations: SPL secondary pulmonary lobule

Table 3. High-resolution computed tomography findings in Thoracic Sarcoidosis (Modified from Criado et al)

Typical features	
Lymphadenopathy	Site (hilar, right paratracheal), bilateral, symmetrical, well defined. May show "cluster of black pearls" sign. If calcified, patchy or egg-shell
Nodules	Micronodules 2-4 mm, bilateral, well-defined, upper and middle zone and peri-hilar predominant. Peri-bronchovascular, subpleural, interlobular septal (peri-lymphangitic pattern)
Fibrotic changes	Interlobular septal thickening, Reticular opacities, architectural distortion, traction bronchiectasis
Atypical features	
Lymphadenopathy	Unilateral, isolated, anterior or posterior mediastinal
Nodules	Miliary
	Lower-lobe predominant, unilateral, "Halo" sign or "Atoll" sign
Consolidation	Mass-like opacities, conglomerate masses, solitary pulmonary nodules, confluent alveolar opacities and ground-glass opacities
Linear opacities	Intra-lobular septal thickening
Airway	Mosaic attenuation, trachea-bronchial abnormalities, atelectasis
Pleural	Effusion, chylothorax, pneumothorax, pleural thickening, calcification, plaque-like lesions

sis is the most common cause, has 25-30% mortality in adults and is invariably fatal if untreated (8).

The finding of a miliary pattern on chest radiographs was noted in early roentgenologic series of thoracic sarcoidosis; 1.3% (2/150) had "miliary" disease (1). Thoracic sarcoidosis has been reported to have several typical and atypical findings on HRCT; miliary is classified as an atypical finding (Table 3) (3). The description of micronodules by their distribution in relation to the "secondary pulmonary lobule" on HRCT along with the clarity of margins and attenuation further helps in narrowing the differential diagnosis of diffuse pulmonary micronodules. Micronodules can be accurately classified as having a centrilobular, perilymphatic or random distribution with their characteristic disease associations (Table 2) (4). Miliary nodules have a random distribution and can be seen in relation to pleural surfaces, small vessels and interlobular septa but do not have a consistent or predominant relationship to any of these (31). Given the reported prevalence of sarcoidosis varies from 4.5-36 per 100,000, the very small number of cases of "miliary" sarcoidosis reported in literature in the HRCT era (Table 1) suggests that this is a very rare occurrence. Most series of computed tomographic findings in sarcoidosis and a review on atypical findings in thoracic sarcoidosis did not report "miliary" sarcoidosis (32, 33).

Our systematic review suggests that this small reported number is an overestimate as a true miliary pattern was seen only in 34.6% (9/26) of the published cases of "miliary" sarcoidosis; and subtle perilymphatic pattern is nearly always seen and the term "pseudo-miliary" may be more appropriate. Distinct perilymphatic clustering of nodules or a perivascular dominance of nodules was seen in 65.4% and 42.3%; fissural nodules leading to the "beaded fissure sign" was prominent and seen in 65.4% of cases. Further, unlike true miliary nodules that lack any zonal predominance, an upper lobe and peripheral clustering was seen in 30.8% and 19.2% respectively. In all our patients with random micronodules, we observed a prominent perivascular distribution of micronodules on histopathology (Figures 2, 4, 6 and 8); two of these had associated "beaded fissure sign" on HRCT. Pathologically, sarcoidosis causes perilymphatic clustering of granulomas; hematogenous or airway spread has not been postulated (34). Profuse clustering of micronodules along the centrilobular and perilymphatic compartments gives rise to an appearance of random nodules in the majority with a "pseudo-miliary" appearance. In the few patients with a true random pattern, prominent perivascular granulomas or drainage into the pulmonary lymphatics and subsequent dissemination by hematogenous route could be postulated.

The link between mycobacteria and sarcoidosis continues to be an enigma (7, 35). In the original descriptions, about 5% of sarcoidosis had tuberculosis preceding them. Polymerase chain reaction analyses of lung biopsies of sarcoidosis have shown mycobacterial DNA in 26.4%.(35) Tuberculosis may present concurrently and confound the diagnosis and management of these patients (23, 36, 37). It has been hypothesized that the immunological responses in sarcoidosis are analogous to "tuberculoid" or "pauci-bacillary" leprosy (38); persisting immunological responses to mycobacterial antigen can explain the extraordinarily high rates of tuberculosis preceding the diagnosis of pseudo-miliary sarcoidosis in the present systematic review.

The diagnosis of sarcoidosis rests on a constellation of clinical, radiological, histopathological and laboratory findings (39). Constitutional features including significant weight loss can occur in up to 25% of patients (Table 2). Fibrinoid necrosis and rarely, caseation can occur in sarcoidosis on histopathology (40, 41). Caseation may sometimes be absent in tuberculosis (41). Radiology can help in the differentiation of sarcoidosis from tuberculosis (26). The presence of hypercalcemia, skin involvement, negative tuberculin skin-testing, upper and middle-lobe distribution of nodules, a peripheral distribution of nodules when present and interlobular septal thickening all point to the diagnosis of "pseudo-miliary" sarcoidosis alone (42). However, sarcoidosis can precede, occur concurrent with or follow tuberculosis and differentiating tuberculosis from sarcoidosis remains a major challenge, especially in high prevalence areas of tuberculosis (43, 44). When the constellation of findings are non-conclusive, a trial of anti-tuberculosis treatment is appropriate in high prevalence areas given the very small number of true miliary sarcoidosis reported in literature. Steroids could be added when there is no clear response at 4-6 weeks (39, 43).

Conclusion

A true miliary pattern in the HRCT era is very rare in sarcoidosis and subtle perilymphatic pattern is nearly always seen; this pattern should be labeled "pseudo-miliary". Prominent perivascular granulomas are associated with true miliary pattern. Miliary sarcoidosis patients are older and symptomatic and mostly need treatment at diagnosis. "Miliary" sarcoidosis may follow treatment for tuberculosis; concurrent cases possibly indicate difficulty in diagnosis or a "tuberculo-sarcoid" presentation. Transbronchial lung biopsy had a good yield for diagnosis. Most patients were symptomatic and treated with steroids, with 100% response and 14.4% relapse.

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Drs. R.S and Dr. S.S take responsibility for the content of the manuscript, including the data and analysis

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References

1. Kirks DR, McCormick VD, Greenspan RH. Pulmonary sarcoidosis. Roentgenologic analysis of 150 patients. Am J Roentgenol Radium Ther Nucl Med. 1973;117(4):777-86. PubMed PMID: 4266919.
2. Koyama T, Ueda H, Togashi K, Umeoka S, Kataoka M, Nagai S. Radiologic manifestations of sarcoidosis in various organs. Radiographics : a review publication of the Radiological Society of North America, Inc. 2004;24(1):87-104. doi: 10.1148/rg.241035076. PubMed PMID: 14730039.
3. Criado E, Sanchez M, Ramirez J, Arguis P, de Caralt TM, Perea RJ, et al. Pulmonary sarcoidosis: typical and atypical manifestations at high-resolution CT with pathologic correlation. Radiographics : a review publication of the Radiological Society of North America, Inc. 2010;30(6):1567-86. Epub 2010/11/13. doi: 10.1148/rg.306105512. PubMed PMID: 21071376.
4. Raoof S, Amchentsev A, Vlahos I, Goud A, Naidich DP. Pictorial essay: multinodular disease: a high-resolution CT scan diagnostic algorithm. Chest. 2006;129(3):805-15. doi: 10.1378/chest.129.3.805. PubMed PMID: 16537886.
5. Global tuberculosis report 2018. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO2018 10/11/2018.
6. Sharma SK, Mohan A, Sharma A. Challenges in the diagnosis & treatment of miliary tuberculosis. Indian J Med Res. 2012;135(5):703-30. PubMed PMID: 22771605; PubMed Central PMCID: PMCPMC3401706.
7. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Sarcoidosis and tuberculosis: the same disease with different manifestations or similar manifestations of different disorders. Curr Opin Pulm Med. 2012;18(5):506-16. doi: 10.1097/MCP.0b013e3283560809. PubMed PMID: 22759770.
8. Sharma SK, Mohan A, Sharma A, Mitra DK. Miliary tuberculosis: new insights into an old disease. Lancet Infect Dis. 2005;5(7):415-30. doi: 10.1016/S1473-3099(05)70163-8. PubMed PMID: 15978528.
9. Zerhouni EA, Naidich DP, Stitik FP, Khouri NF, Siegelman SS. Computed tomography of the pulmonary parenchyma. Part 2: Interstitial disease. Journal of thoracic imaging. 1985;1(1):54-64. PubMed PMID: 3843414.
10. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 1999;16(2):149-73. PubMed PMID: 10560120.
11. Kazerooni EA, Jackson C, Cascade PN. Sarcoidosis: recurrence of primary disease in transplanted lungs. Radiology. 1994;192(2):461-4. Epub 1994/08/01. doi: 10.1148/radiology.192.2.8029415. PubMed PMID: 8029415.
12. Kazerooni EA, Cascade PN. Recurrent miliary sarcoidosis after lung transplantation. Radiology. 1995;194(3):913. Epub 1995/03/01. doi: 10.1148/radiology.194.3.7863002. PubMed PMID: 7863002.
13. Gupta D, Kumar S, Jindal SK. Bilateral miliary mottling without hilar lymphadenopathy: A rare presentation of sarcoidosis. Lung India. 1996;14(2):87-8.
14. Chugh IM, Agarwal AK, Arora VK, Shah A. Bilateral miliary pattern in sarcoidosis. The Indian journal of chest diseases & allied sciences. 1997;39(4):245-9. PubMed PMID: 9654821.
15. Voloudaki AE, Tritou IN, Magkanas EG, Chalkiadakis GE, Siafakas NM, Gourtsoyiannis NC. HRCT in miliary lung disease. Acta radiologica (Stockholm, Sweden : 1987). 1999;40(4):451-6. Epub 1999/07/08. PubMed PMID: 10394878.
16. Hatzakis K, Siafakas NM, Bouros D. Miliary sarcoidosis following miliary tuberculosis. Respiration; international review of thoracic diseases. 2000;67(2):219-22. Epub 2000/04/25. doi: 10.1159/000029492. PubMed PMID: 10773799.
17. Kline MY, Leibert EM. A case of pulmonary tuberculosis and miliary sarcoidosis. Chest2005. p. 480S-1S.
18. Stroebe K, Haddadin H. Bilateral miliary sarcoidosis. Chest2012. p. 1036A.
19. Hodges HK, Lee PY, Hausmann JS, Teot LA, Sanford EL, Levin KW. Hypercalcemia and miliary sarcoidosis in a 15-year-old boy. Arthritis and rheumatism. 2013;65(8):2112. Epub 2013/05/21. doi: 10.1002/art.38019. PubMed PMID: 23686651.
20. Kumar P, Jaco MJ, Pandit AG, Shanmughanandan K, Jain A, Rajeev, et al. Miliary sarcoidosis with secondary Sjogren's syndrome. The Journal of the Association of Physicians of India. 2013;61(7):505-7. Epub 2014/04/30. PubMed PMID: 24772762.
21. Bostantzoglou C, Samitas K, Gkogkou C, Zervas E, Gaga M. Mediastinal widening and miliary chest radiograph pattern in a middle aged man: could it be sarcoidosis? BMJ case reports. 2014;2014. Epub 2014/07/19. doi: 10.1136/bcr-2014-204884. PubMed PMID: 25035447; PubMed Central PMCID: PMCPMC4112298.
22. Luetkens JA, Zoghi S, Rockstroh JK, Naehle CP. Pulmonary sarcoidosis shortly after spinal tuberculosis infection: a diagnostic challenge. BMJ case reports. 2014;2014. Epub 2014/04/15. doi: 10.1136/bcr-2013-203333. PubMed PMID: 24728896; PubMed Central PMCID: PMCPMC3987564.

23. Mandal SK, Ghosh S, Mondal SS, Chatterjee S. Coexistence of pulmonary tuberculosis and sarcoidosis: a diagnostic dilemma. BMJ case reports. 2014;2014. Epub 2014/12/21. doi: 10.1136/bcr-2014-206016. PubMed PMID: 25527682; PubMed Central PMCID: PMCPMC4275725.
24. Enomoto Y, Yokomura K, Suda T. Bilateral pleural effusion associated with miliary sarcoidosis. American journal of respiratory and critical care medicine. 2015;191(4):474-5. Epub 2015/02/14. doi: 10.1164/rccm.201411-2022IM. PubMed PMID: 25679105.
25. Taki M, Ikegami N, Konishi C, Nakao S, Funazou T, Ariyasu R, et al. Pulmonary Sarcoidosis Presenting with Miliary Opacities. Internal medicine (Tokyo, Japan). 2015;54(19):2483-6. Epub 2015/10/02. doi: 10.2169/internalmedicine.54.4681. PubMed PMID: 26424308.
26. Bhalla AS, Das A, Naranje P, Goyal A, Guleria R, Khilnani GC. Dilemma of diagnosing thoracic sarcoidosis in tuberculosis endemic regions: An imaging-based approach. Part 1. Indian J Radiol Imaging. 2017;27(4):369-79. doi: 10.4103/ijri.IJRI_200_17. PubMed PMID: 29379230; PubMed Central PMCID: PMCPMC5761162.
27. Chaddha U, English R, Daniels J, Walia R, Mehta AC, Panchabhai TS. A 58-Year-Old Man With Fatigue, Weight Loss, and Diffuse Miliary Pulmonary Opacities. Chest. 2017;151(6):e131-e4. Epub 2017/06/11. doi: 10.1016/j.chest.2016.11.015. PubMed PMID: 28599946.
28. Matsuura S, Mochizuka Y, Oishi K, Miyashita K, Naoi H, Mochizuki E, et al. Sarcoidosis with Pancreatic Mass, Endobronchial Nodules, and Miliary Opacities in the Lung. Internal medicine (Tokyo, Japan). 2017;56(22):3083-7. Epub 2017/09/26. doi: 10.2169/internalmedicine.8916-17. PubMed PMID: 28943576; PubMed Central PMCID: PMCPMC5725865.
29. Arar O, Boni F, Meschi T, Tana C. Pulmonary sarcoidosis presenting with miliary opacities. Current Medical Imaging Reviews. 2018. doi: 10.2174/1573405614666180806141415.
30. Joshi V, S. Jain, V. Sharma, N. Khippal, Chaturvedi A. Granulomatous Hepatitis with Miliary Mottling: A Rare Cause. Journal of The Association of Physicians of India. 2018;66:97-8.
31. Andreu J, Mauleon S, Pallisa E, Majo J, Martinez-Rodriguez M, Caceres J. Miliary lung disease revisited. Current problems in diagnostic radiology. 2002;31(5):189-97. PubMed PMID: 12419999.
32. Kuhlman JE, Fishman EK, Hamper UM, Knowles M, Siegelman SS. The computed tomographic spectrum of thoracic sarcoidosis. Radiographics : a review publication of the Radiological Society of North America, Inc. 1989;9(3):449-66. doi: 10.1148/radiographics.9.3.2727355. PubMed PMID: 2727355.
33. Cozzi D, Bargagli E, Calabro AG, Torricelli E, Giannelli F, Cavigli E, et al. Atypical HRCT manifestations of pulmonary sarcoidosis. La Radiologia medica. 2018;123(3):174-84. doi: 10.1007/s11547-017-0830-y. PubMed PMID: 29124658.
34. Spagnolo P, Rossi G, Trisolini R, Sverzellati N, Baughman RP, Wells AU. Pulmonary sarcoidosis. Lancet Respir Med. 2018;6(5):389-402. doi: 10.1016/S2213-2600(18)30064-X. PubMed PMID: 29625772.
35. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis. Eur Respir J. 2007;30(3):508-16. doi: 10.1183/09031936.00002607. PubMed PMID: 17537780.
36. Smith-Rohrberg D, Sharma SK. Tuberculin skin test among pulmonary sarcoidosis patients with and without tuberculosis: its utility for the screening of the two conditions in tuberculosis-endemic regions. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2006;23(2):130-4. PubMed PMID: 17937109.
37. Shah JR, Hede J, Mathur RS. Diagnostic criteria of tuberculous sarcoidosis. Lung India. 2009;26(3):86-8. doi: 10.4103/0970-2113.53232. PubMed PMID: 20442843; PubMed Central PMCID: PMCPMC2862513.
38. Agarwal R, Gupta D. Tuberculous sarcoidosis: Is it a separate entity? Lung India. 2009;26(3):61-2. doi: 10.4103/0970-2113.53225. PubMed PMID: 20442837; PubMed Central PMCID: PMCPMC2862507.
39. Jindal SK, Gupta D, Aggarwal AN. Sarcoidosis in India: practical issues and difficulties in diagnosis and management. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2002;19(3):176-84. PubMed PMID: 12405486.
40. Karkhanis V, Joshi JM. All that caseate is not TB. Lung India.2007(24):100-1.
41. Danila E, Zurauskas E. Diagnostic value of epithelioid cell granulomas in bronchoscopic biopsies. Internal medicine (Tokyo, Japan). 2008;47(24):2121-6. doi: 10.2169/internalmedicine.47.1452. PubMed PMID: 19075536.
42. Bhalla AS, Das A, Naranje P, Goyal A, Guleria R, Khilnani GC. Dilemma of diagnosing thoracic sarcoidosis in tuberculosis-endemic regions: An imaging-based approach. Part 2. Indian J Radiol Imaging. 2017;27(4):380-8. doi: 10.4103/ijri.IJRI_201_17. PubMed PMID: 29379231; PubMed Central PMCID: PMCPMC5761163.
43. Guleria R, Mahashur A, Ghoshal AG, Thomas PK, Raghu G, Baughman RP. Challenges in diagnosing Sarcoidosis in tuberculosis endemic regions: Clinical scenario in India. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG. 2016;33(4):381-4. PubMed PMID: 28079850.
44. Jain R, Mohan A, Guleria R. Sarcoidosis vs tuberculosis: Diagnostic mystery still unresolved. Indian J Tuberc. 2017;64(4):243-5. doi: 10.1016/j.ijtb.2017.09.001. PubMed PMID: 28941846.

Summary of all reported cases of thoracic sarcoidosis with miliary nodules on High-resolution Computed Tomography [$]

Author	Year	Age/ Gender	Symptom duration, Country	Clinical Features					Radiology findings			Histo-pathology site	Outcome	Comments
				Fever/ Night sweats	Weight loss	Respiratory symptoms	Renal, electrolytes	Others	Miliary	Other distributions seen	Adenopathy		Alive/dead; treatment	
Kazerroni et al	1995	43/M	No symptoms, U.S	Asymptomatic; diagnosed on surveillance TBLB post-BLTx and HRCT images. In both cases, granulomas preceded the findings on HRCT (by 1 year in patient 1 and 2 years in patient 2)					No	Y; PL, UL pred	No	TBLB	Better; steroids @ 3 months	15 months post-BLTx
		44/F							No	Y; PL, IL, Peripheral	No	TBLB	No change, asymptomatic	27 months post-HBLTx
Gupta et al	1996	24/M	2 months, India	Fever +	No	Dyspnea, cough	No	Exposure to mines	No	Y, PL, Fissural, vessels	No	TBLB	Steroids; better @2 months	Misdiagnosed at TB. HRCT clear peri-lymphatic pattern
Chugh IM et al	1997	46/F	4 months, India	No	No	Dyspnea, cough	No	HTN	NA	Could not be assessed[$]	Yes; (R) H, PT	TBLB	Steroids, Better	Misdiagnosed at TB; given ATT
Voloudaki et al	1999	Mean 45/ M (2); F(1)	This was a radiological series from Greece; clinical finding are not described. In "miliary" sarcoidosis, a clear subpleural, perilymphatic pattern with Fissural (beaded septum) is seen						No	IL+, Fissural nodules	Yes; all three	TBLB (1), SLBx (2)	3/7 with miliary nodules**, 1/3 miliary TB followed by "miliary" sarcoidosis	
Hatzakis	2000	47/M	NA, Greece	No	No	Cough	No	CAD/MI	Yes	Random nodules	SC nodes	SLBx	Steroids, better	**Miliary TB followed by miliary sarcoidosis (1 yr)
Kline et al	2005	53/F	4 months, U.S	No	No	No	No	Dizziness, Liver, skeletal lesions	No	Yes; PL	No	Calvarial Bx, Liver	Alive, better	[%]Pulmonary TB followed by multisystem sarcoidosis
Criado et al	2010	One case in a series on atypical findings of sarcoidosis; no clinical findings are reported							Yes	Y, Fissural nodules+, vessels	No	NA	NA	Image alone available
Stroebe et al	2012	53/F	4 months, U.S	Fatigue	27 Kgs	No	No	DM, HTN, COPD	NA	Y; Images not provided	Y; ML, H, HL	TBLB	Better, steroids	No images provided
Hodges et al	2013	15/M	3 months, U.S	Fatigue, nausea	No	No	AKI, Hyper-calcemia	Skin, nephrocalcinosis, Gen LNE	Yes	Random	RP LNE	TBLB, Lymph node	Better, steroids @ 4 months	Truly miliary nodules on HRCT
Kumar et al	2013	45/M	3 months, India	Fatigue	No	Cough	Hyper-calcemia	Sicca syndrome	Yes	Random	ML, HL, RP LNE	TBLB, Lip Bx	Better, steroids	Hepatic disease, glands
Bostantzoglou et al	2014	48/M	1 month, Greece	Fever	No	Cough, dyspnea	No	Thalassemia	Yes	Random	HL, (R)PT	TBLB, EBBx	Better, relapsed 18 months	[#]TB (clinical) followed by sarcoidosis
Mandal et al	2014	38/F	1 month, India	Fever	No	Cough, dyspnea	Hyper-calcemia	Concurrent TB and sarcoidosis	Yes	Random	No	TBLB, Bone marrow+	Better on ATT & steroids	[#]TB 1 yr earlier, TB concurrent with sarcoidosis
Luetkens et al	2014	37/M	2 months, Germany	Fever, night sweats	Yes	No	No	INH resistant MTB spinal abscess+	Yes	Random, along vessels, fissures+	No	TBLB	Steroids, better @ 4 months	[##]TB followed by miliary sarcoidosis (8 weeks)

Author	Year	Age/ Gender	Symptom duration	Clinical Features					Radiology findings			Histo-pathology Site of diagnosis	Outcome	Comments
				Fever/ Night sweats	Weight loss	Respiratory symptoms	Renal, electrolytes	Others	Miliary	Other distributions seen	Adenopathy			
Taki et al	2015	40/F	6 months, Japan	No	No	Dyspnea	No	Ocular	Yes	Random, upper & mid-zone, Fissural nodules +	HL, ML	TBLB	Better, steroids @1 year	Complete Clearing of nodules on CT at 1 year
Enomoto et al	2015	69/M	2 weeks, Japan	No	No	Dyspnea	No	Bilateral exudative, lymphocytic effusion	No	Upper, mid-zone, sub-pleural nodules+	No	TBLB inconclusive, thoracoscopic pleural Bx	Steroids, better	Clear subpleural pattern on CT and Pathology
Bhalla et al	2017	Radiological collection of thoracic sarcoidosis; no clinical information is provided. One image of miliary sarcoidosis included							Yes	Random, Fissure+, along vessels	BHL	NA	NA	Image alone available
Matsuura et al	2017	78/F	2 months, Japan	No	No	Cough	No	Right pleural effusion	Yes	PL, fissural, sub-pleural nodules+	No	EUS-FNA, TBLB, EBBx	No treatment, better @1 year	Pancreatic mass, endobronchial nodules, effusion
Chaddha et al	2017	58/M	6 months	Night sweats, Fatigue, no fever	18 Kgs	None	Hyper-calcemia	Arthralgia, RA, Hypothyroidism, A.F	Yes	Along vessels, Fissural nodules+	Y; SC, PT R, L	EBUS-FNA, TBLB	Better	Family history of sarcoidosis
Arar et al	2018	55/M	NA, India	Fever	No	Dyspnea	No	None	Yes	Random	No	TBLB, caseating granulomas	NA	?[#]concurrent TB, sarcoidosis
Joshi et al	2018	40/M	3 months, India	Fever	Anorexia	Cough	Hypercalciuria, normal serum Calcium	Hepatosplenomegaly, Skin; Nausea, vomiting	Yes	Fissural nodules+	BHL	Skin, BMA, TBLB negative; Liver Bx Granulomas	Better at 6 months of steroids	Diabetes, Psoriasis; empiric ATT
Rajagopala et al	2018	65/F	2 months, India	Night sweats	No	Dyspnea	No	Hypertension	Yes	Random, Fissural nodules+	No	TBLB negative; SLBx+	Better on steroids @ 1 year	[#]Prior diagnosis of tuberculosis
		40/M	20 days, India	Fatigue	Yes	Dyspnea	AKI, Hypercalcemia	Polyuria, nausea, skin lesions	Yes	Random	BHL	TBLB, Skin Bx	Better on MTX	Multi-system sarcoidosis, relapse@ 1 year
		55/M	1 month, India	No	Yes	Dyspnea	No	Ocular	Yes	Random	No	TBLB	Better on MTX @ 9 months	[#]Concurrent TB-sarcoidosis
		48/M	2 weeks, India	Fatigue	No	No	Hypercalcemia	Ocular	Yes	Random, IL+	No	TBLB	Better, steroids @8 months	

[$]Poor quality HRCT images precluded assessment of type of nodules; **In one miliary sarcoidosis followed miliary tuberculosis; [%]Concurrent tuberculosis and miliary sarcoidosis with organomegaly, neurologic and bone involvement; [#]Tuberculosis was diagnosed based on pathology, radiology and clinical response; ATT stopped at 5 months due to liver injury; [##]Tb was diagnosed microbiologically based on mycobacterial cultures

Abbreviations: M Male, F Female, U.S United States, NA Not available, BLTx Bilateral lung transplantation, HBLTx Heart and lung transplantation, HRCT high resolution computed tomography, UL Upper lobe, PL Peri-lymphatic pattern, IL Interlobular nodules, ILo, Intralobular septal thickening, CAD coronary artery disease, MI Myocardial infarction, TB tuberculosis, DM Diabetes mellitus, HTN hypertension, COPD Chronic obstructive pulmonary disease, AKI acute kidney injury, INH isoniazid, ML Mediastinal, BHL bilateral Hilar lymphadenopathy, RPT Right paratracheal, LNE lymphadenopathy, Y Yes, Kgs kilograms, Bx biopsy, TBLB Transbronchial lung biopsy, SLBx surgical lung biopsy, EBUS-FNA endobronchial ultrasound guided fine needle aspiration, ATT Anti-tuberculosis treatment, MTX-Methotrexate

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Original Research Article

Antibiotic sensitivity pattern to urinary tract infections in a tertiary care hospital in South India

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the most common bacterial illnesses and hence one of the most important indications for antibiotic treatment. Current knowledge of the common organisms implicated in causing UTI in the local community and surveillance to monitor the changes in susceptibility of uropathogens are imperative to ensure appropriate therapy. The study objectives were to assess the proportion of UTI caused by each of the common urinary pathogens, to study the antibiotic drug sensitivity patterns by analyzing the culture and sensitivity reports and to identify the drugs which would be potentially favourable candidates for empirical therapy in the study locale.

Methods: This hospital based cross sectional study was done by collecting the data from case records of patients who attended OPD or were admitted with diagnosis of UTI during the study period.

Results: *Escherichia coli* was the single most important cause of UTI, accounting for 70% of the infection among the studied subjects, amongst which 55% was extended spectrum beta-lactamase producing organisms. A pattern of increased susceptibility to higher antibiotics like ertapenem, tigecycline and decreased sensitivity to fluroquinolones was evident. In addition, nitrofurantoin was found to be one of the antibiotics to which most of the organisms were susceptible (85%) favouring its use in empirical therapy of UTI.

Conclusions: The pattern of accentuated susceptibility to higher antibiotics in the face of declining trend in the sensitivity to commonly used antibiotics necessitates promoting awareness on the need for rational prescribing of antibiotics among clinicians.

Keywords: Antibiotics, Culture and sensitivity, *Escherichia coli*, Urinary tract infection

INTRODUCTION

Urinary tract infections (UTI) remain one of the commonest infections diagnosed in out-patients and hospitalized patients. An in-depth knowledge on antimicrobial susceptibility pattern is essential for appropriate therapy, particularly with increasing resistance resulting from repeated use of the same antibiotic.[1] It is also essential to be aware of the changing patterns of antibiotic resistance in a locale.[2] Published data may not always represent the scenario prevailing in a particular location as the studies done elsewhere could vary in the pattern of microorganisms involved in causation, their sensitivity and resistance to different antibiotics which in turn depends on the antibiotic prescribing pattern in that region. This indicates that there is a need for studying the existing antibiotic sensitivity patterns in the local population.[3] Resistance significantly varies by region and the highest antibiotic resistance has been observed in developing countries. Similarly a significant rise in resistance over time is seen in studies reporting on community acquired *E. coli* UTI.[3]

Hence this study was designed to evaluate the drug sensitivity patterns to some of the commonly used antimicrobials in UTI like the beta lactams, fluroquionolones and aminoglycosides. In addition, the

antibacterial drugs commonly used in UTI like nitrofurantoin, cotrimoxazole were also studied for the sensitivity patterns. Thus, the study was done to provide an insight into the drug sensitivity patterns of common urinary pathogens to antibiotic therapy by analyzing the culture and sensitivity reports.

Our study was designed to identify the proportion of UTI caused by each of the urinary pathogens prevalent in the study locale and the likely drug candidates with the potential of being used as empirical therapy in community acquired pathogens by evaluating their sensitivity pattern, thereby enabling identification of the drugs which can effectively be used in the management of UTI.

METHODS

This was a hospital based study done at a tertiary care teaching hospital after the approval from Institutional Human Ethics Committee. The study was a cross sectional one, done using case records. The data from case records of the patients who attended OPD or admitted with diagnosis of UTI during the study period were included for the study based on inclusion and exclusion criteria.

The data from the case records of adult patients above the age of 20 years, of both sexes, diagnosed with UTI were included for the study. Patients with asymptomatic bacteriuria, those with complicated UTI, upper UTI or known malformations, paediatric and pregnant patients, immunocompromised patients like patients with HIV, on corticosteroids or immunosuppressant therapy, organ transplant recipient were excluded.

The culture and *in vitro* sensitivity testing report data were collected in addition to demographic data, co morbid conditions, symptoms and duration of symptoms and drug therapies. In addition, data on history of hospitalizations, catheterizations and frequency of recurrences were also obtained from the case sheets. Also, the details recorded on the review date regarding cure or continuing symptoms, repeat culture reports, if any, was made note of from the case record.

RESULTS

A total of 20 patients whose case records indicated that they had been diagnosed with UTI were included for the study. All these patients were urine culture positive. In our study 30% of patients were in the age group 20 -40 years and a large majority of about 45% were in the age group of 41-60 years and 25% of the patients were aged 61 -80 years (Figure 1). Male female ratio in our study population was 65:35.

Catheterization was done in 20% of the study subjects. The duration of catheterization was variable with one patient having a history of prolonged catheterization of 12 days. Concomitant renal disease was found in a high proportion of the UTI patients. Amongst the studied population, 10% had renal failure, 10% nephritis and 5% pyelonephritis with renal calculi. One patient manifested with lupus nephritis as she was a known case of systemic lupus erythematosis. One patient was a case of neurogenic bladder. About10% patients had presented with concomitant prostatitis and a similar 10% with UTI had history of recurrent episodes (Figure 2).

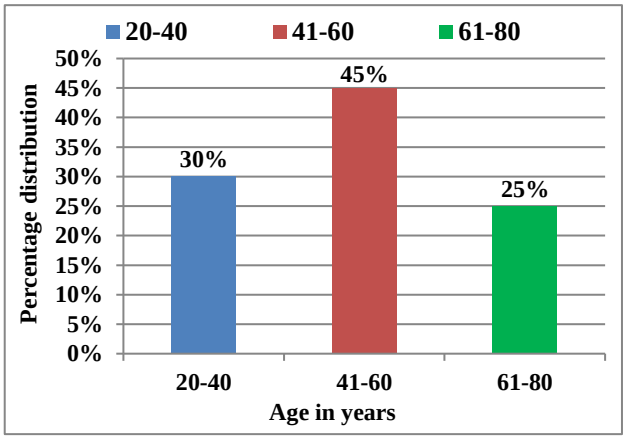


Figure 1: Frequency distribution of age in the study population.

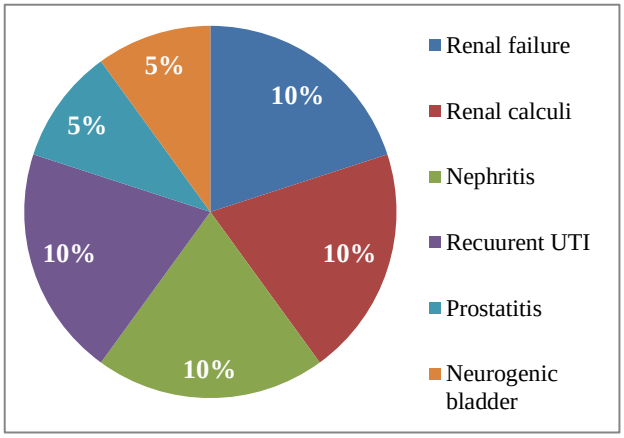


Figure 2: Frequency distribution of concomitant renal disease.

Data also demonstrated that concomitant systemic diseases were found in a significant proportion of the patients whose records were evaluated. A total of 40% had diabetes mellitus and 30% patients were hypertensives. Interestingly, amongst the 30% with hypertension, 83% were both diabetic and hypertensives. Heart disease was seen in 10%, one with diastolic dysfunction and the others with coronary artery disease. Amongst those whose data were studied, it was found that there were about five patients with other chronic systemic illnesses like chronic calcific pancreatitis, pituitary ademona, hypothyroidism, SLE, liver disease with cholelithiasis (Figure 3).

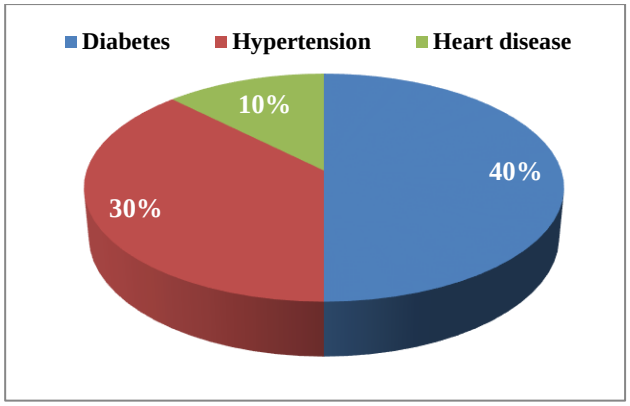


Figure 3: Frequency distribution of concomitant systemic diseases.

Escherichia coli was the single most important microorganism found to implicated in causation of UTI, accounting for 70% of the infection among the studied subjects (Table 1).

Table 1: Percentage distribution of organisms isolated in culture.

Organisms grown in culture	Percentage
Escherichia coli (ESBL)	55%
Escherichia coli	15%
Pseudomonas aeroginosa	10%
Enterococcus fecium	10%
Enterococcus fecalis	5%
Klebsiella pneumoniae	5%
Infection with dual organisms	10%

Organisms grown in urine culture in the study population revealed a high proportion of *E. coli* being Extended spectrum beta-lactamase (ESBL) producing organisms. Next in frequency was *Pseudomonas aeroginosa* and *Enterococcus fecium*, responsible for 10% each whilst *Enterococcus fecalis and Klebsiella* contributing 5% each. Co-infection with two of these organisms (dual infection) was seen in 10% (Figure 4).

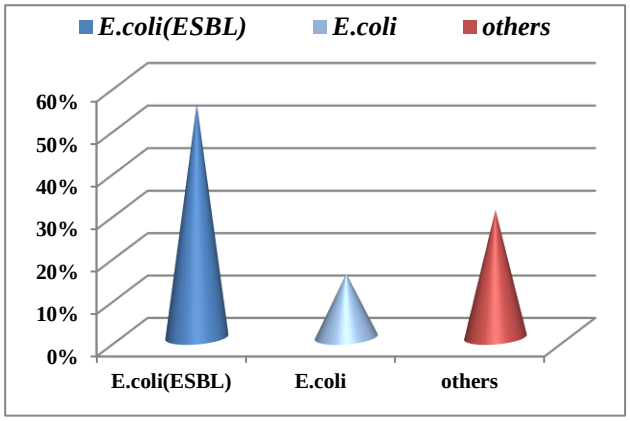


Figure 4: Frequency distribution of the urinary pathogens isolated in culture.

The sensitivity of the cultured organisms was also evaluated based on their susceptibility to different groups of antibiotics.

Aminoglycosides

The sensitivity to amikacin was found to be highest (60%) followed by netilmycin (40%) and the sensitivity for tobramycin and gentamycin were equal, each with 30% (Figure 5).

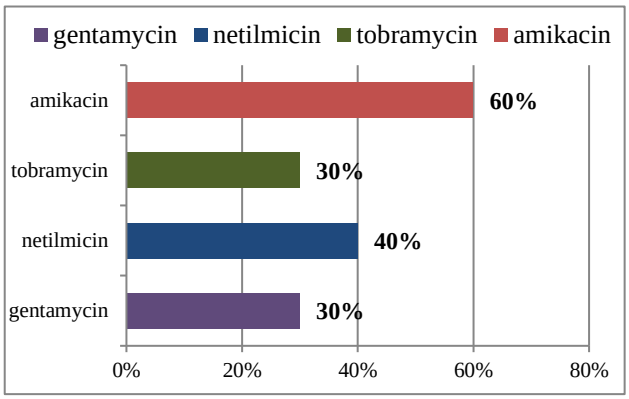


Figure 5: Frequency distribution of sensitivity to aminoglycosides.

Fluoroquinolones

The overall sensitivity to quinolones was found to be lower than that of aminoglycosides and among the quinolones, the greatest sensitivity percentage was recorded for ofloxacin (Figure 6).

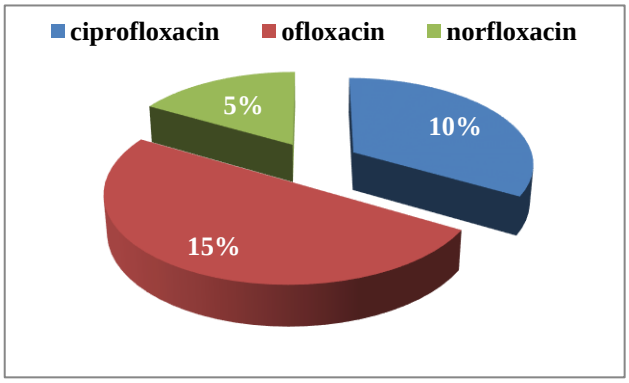


Figure 6: Frequency distribution of sensitivity to fluoroquinolones.

Beta lactams

Analyzing beta-lactams demonstrating high sensitivity, it was found that remarkably higher sensitivity was seen for beta lactams when combined with beta-lactamase inhibitors. It is quite obvious that the greater susceptibility can be accounted by the high proportion of ESBL organisms cultured. 60% of all culture reports studied revealed sensitivity of the cultured bacteria to

cefaperazone in combination with sulbactam and the next highest being piperacillin tazobactum combination with 45% and a slightly lower percentage of 40% was recorded for amoxicillin clavulanate (Figure 7).

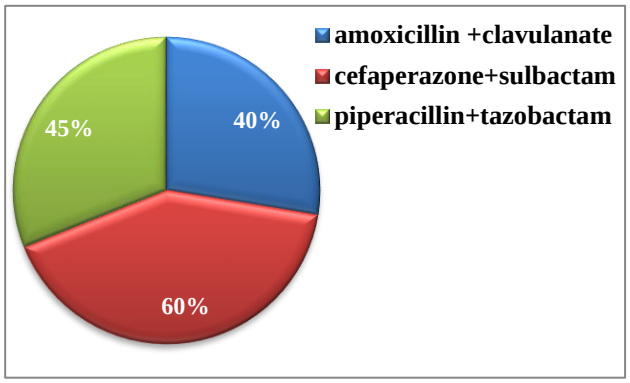


Figure 7: Frequency distribution of sensitivity to commonly used beta lactams in combination with beta-lactamase inhibitors.

Amino-penicillins

The aminopenicillins were one of the drug groups studied for bacterial sensitivity apart from ureidopencillins in the penicillin group of beta-lactam antibiotics and among the two amino-pencillins used commonly, organisms showed a marginally higher sensitivity to ampicillin (20%) as compared to amoxicillin (10%).

Cephalosporins

In the cephalosporin group, cefaperazone in combination with sulbactum was the antibiotic to which most organisms (60%) were sensitive. Apart from it, the other cephalosporins found to be useful in 10 -20% of infections based on culture and sensitivity pattern are cefepime, ceftriaxone, cephalothin and cefuroxime respectively with 30%, 20%, 15% and 10%. It was evident that sensitivity for the first and second generation was lower in comparison to third and fourth generations of cephalosporins (Figure 8).

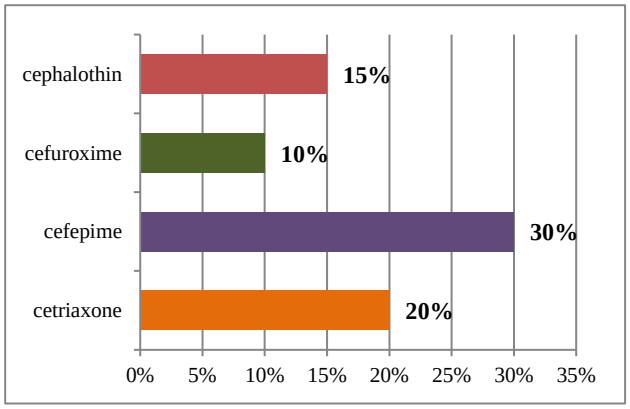


Figure 8: Frequency distribution of sensitivity to cephalosporins.

Carbapenems

Among carbapenems, greatest sensitivity was recorded for ertapenem (70%) (Figure 9).

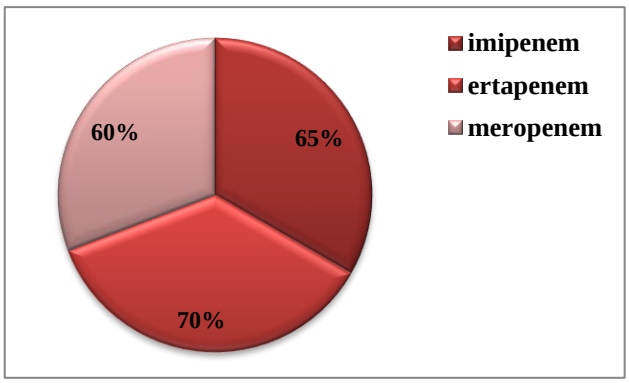


Figure 9: Frequency distribution of sensitivity to carbapenems.

Tetracycline and glycylcycline

Tigecycline was found to be the antibiotic to which 60% of the organisms studied were susceptible to and it matched that of cefaperazone combined with sulbactum in achieving the same percentage of sensitivity (Figure 10).

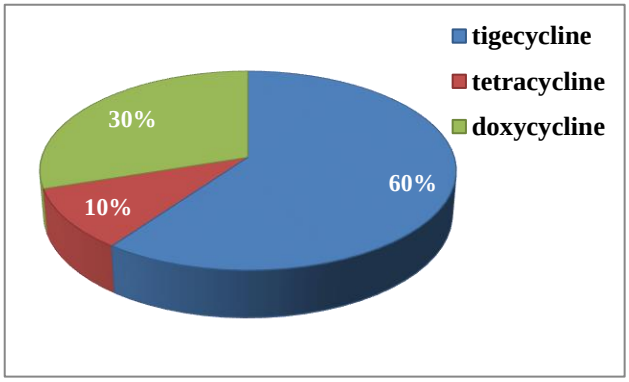


Figure 10: Frequency distribution of sensitivity to tetracyclines and glycylcycline.

Miscellaneous antibacterials

Interestingly, 85% of the cultures showed sensitivity to nitrofurantoin. Also, colistin was another antibiotic found to have similar percentage of sensitivity comparable with tigecycline and cefaperazone sulbactam combination. Sensitivity to cotrimoxazole was 40% and that for vancomycin and linezolid, with 20% each (Figure 11).

The pattern of antibiotic sensitivity of the UTI causing organisms was analyzed. Comparing the various drug groups with differential sensitivity percentages, the highest *in-vitro* sensitivity was to the beta lactam group followed by nitrofuantoin and colistin of the

miscellaneous antibacterial group and the least sensitivity was demonstrated by the fluoroquinolones (Figure 12).

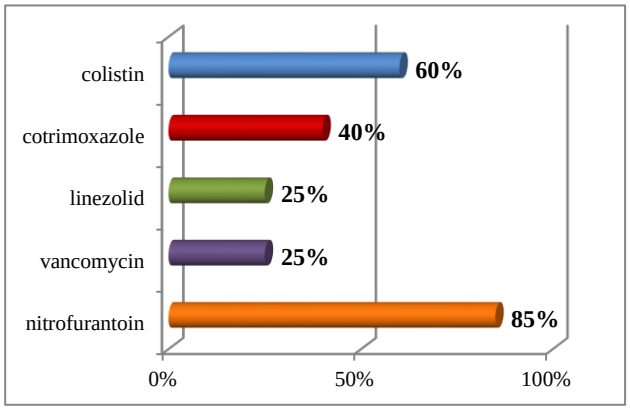


Figure 11: Frequency distribution of sensitivity to miscellaneous antibiotics.

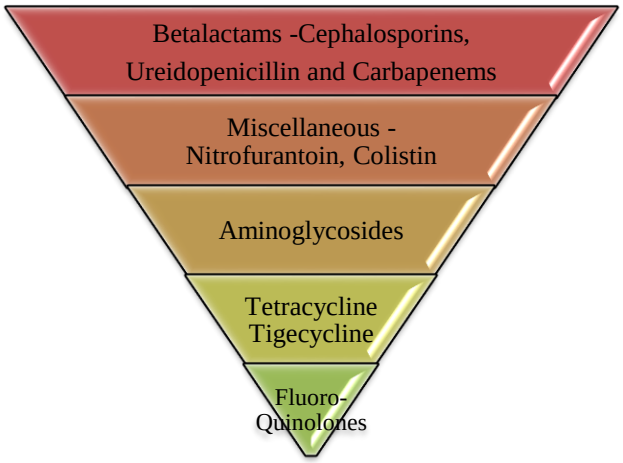


Figure 12: The pattern of drug susceptibility of urinary pathogens to different antibiotics.

DISCUSSION

Urinary tract infections (UTI) is the most common infection experienced by human after respiratory and gastrointestinal infections, accounting for 8.6 million visits (84% by women) in 2007 in the United States.[4,5] UTI is said to occur more commonly in females than in males; in males it is uncommon, but often associated with structural abnormality.[6] But in our sample, we found a greater percentage of males than females.

The highest co-morbidity noted in our study population was diabetes and hypertension. Diabetes plays a definitive role in increasing the occurrence of UTI because of the changes in immunological function and thereby increasing the susceptibility to UTI. Studies have demonstrated that the epidemiology of urinary tract infections (UTIs) among men and women with diabetes is similar to the epidemiology of those without diabetes with women having greater risk than men.[7] However, the bacteriology and antibiotic susceptibility patterns also do not, in general, differ from those without diabetes.[7,8] Our study results are well in line with these findings. In addition, a study has revealed that the virulence factors of *E. coli* affecting the diabetic individuals also remains the same as in the general population.[8]

Worldwide, the most frequent etiology is *Escherichia coli* and this is also reflected in our study results.[9] However the sensitivity and resistance patterns of *E. coli* strains causing UTI varies considerably between regions and countries.[10] In this study, we found that most *E. coli* isolated in culture were susceptible to cefaperazone-sulbactum, ertapenem, tigecycline, nitrofurantoin, colistin and amikacin. Among these nitrofurantoin and colistin are the drugs administered orally and the rest have to be administered by parenteral route. Interestingly, we found a decreasing sensitivity to fluoroquinolones which have been regarded as one of the first line drugs in UTI and therefore rampantly used which consecutively would have lead to a high degree of fluoroquinolone resistance in our population.

The study highlights the increasing trend of resistant Gram negative organism in culture necessitating the need for higher antibiotics. A study has highlighted that among tetracyclines, tigecycline has best in vitro susceptibility against ESBL producing Gram negative rods.[11] Likewise, a high cure rate, both clinical and microbiological has been recorded for ertapenem also, particularly for ESBL infections.[12] The indiscriminate use of such higher antibiotics like ertapenem and tigecycline will foster development of drug resistance, further complicating the management of urinary tract infection.

The vicious cycle wherein widespread use of newer antibiotics leading on to the organisms developing resistance to them and that in sequence, further necessitating the development of newer antibiotics ultimately results in difficult to treat infections. Such a situation can be overcome by implementing rational prescribing practices like the use of lower antibiotics with limited spectrum to which the organism is susceptible rather than resorting to prescribe higher antibiotics with a wide spectrum.[13]

This study has clearly delineated the emphatic role of nitrofurantoin in urinary tract infections. Our findings are in concordance with currently emerging trend in the use of nitrofurantoin as a key oral antibiotic option in the treatment of acute uncomplicated cystitis due to multi-drug resistant Gram negative bacilli causing UTI.[14] Studies also have acknowledged that the efficacy of nitrofurantoin for the empirical treatment is supportable from a public health perspective in an attempt to decrease uropathogen resistance.[15] Thus the current scenario based on drug sensitivity justifies the use of nitrofurantoin in the empiric management of UTI, not only due to the high susceptibility of *E. coli* to nitrofurantoin and the ease of oral administration but also owing to the fact that it lacks

cross-resistance with commonly prescribed antibiotics for UTI. However, nitrofurantoin is contraindicated in renal disease since most of it is really excreted.

Limitation

The limitation of the study is the small sample size. A larger study with a larger sample is needed to confirm the drug sensitivity pattern of UTI causing organisms in the study locale established through our study results.

CONCLUSION

The study highlights that *E. coli* contributes to the majority of the urinary tract infections diagnosed in our tertiary care hospital. In addition, the current drug sensitivity pattern signifies the need for higher antibiotics and this mandate implementing strategies of rational prescribing to avert forging resistance to these agents also. Nitrofurantoin, with good activity against a high percentage of the cultured organisms causing UTI, rationalizes its use for empirical treatment of UTI.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Human Ethics Committee (Approval number: 16/158)

REFERENCES

1. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital, Aligarh, India. Ann Clinical Antimicrob, 2007 Mar 23;6:4.
2. Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxocin resistance in community and hospital acquired Escherichia coli urinary tract infections: a systematic review and meta analysis of observational studies. BMC Infec Dis, 2015 Nov 25;15(1):545.
3. Cullen IM, Manecksha RP, McCullagh E, Ahmad S, O'Kelly F, Flynn RJ, et al. The changing pattern of antimicrobial resistance within 42,033 Escherichia coli isolates from nosocomial, community and urology patient-specific urinary tract infections, Dublin, 1999-2009. BJU Int. 2012 Apr;109(8):1198-206.
4. Najar MS, Saldanha CL, Banday KA. Approach to urinary tract infections. Indian J Nephrol. 2009 Oct;19(4):129-39.
5. Hooton TM. Clinical practice. Uncomplicated urinary tract infection. N Engl J Med. 2012;366:1028-37.
6. Ferdosi-Sh E, Javanian M, Moradian KM. Resistance patterns of Escherichia coli causing urinary tract infection Caspian J Intern Med. 2015;6(3):148-51.
7. De Lastours V, Foxman B. Urinary tract infection in diabetes: epidemiologic considerations. Curr Infect Dis Rep. 2014 Jan;16(1):389.
8. Harwalkar A, Gupta S, Rao A, Srinivasa H. Prevalence of virulence factors and phylogenetic characterization of uropathogenic Escherichia coli causing urinary tract infection in patients with and without diabetes mellitus. Trans R Soc Trop Med Hyg. 2015 Dec;109(12):769-74.
9. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015 May;13(5):269-84.
10. Ferdosi-Shahandashti E, Javanian M, Moradian-Kouchaksaraei M, Yeganeh B, Bijani A, Motevaseli E, et al. Resistance patterns of Escherichia coli causing urinary tract infection. Caspian J Intern Med. 2015;6(3):148-51.
11. Gill MM, Usman J, Hassan A, Kaleem F, Shaheen N. In vitro susceptibility pattern of extended spectrum β-lactamase producing gram negative bacilli against tetracyclines. J Ayub Med Coll Abbottabad. 2015 Oct-Dec;27(4):788-90.
12. Veve MP, Wagner JL, Kenney RM, Grunwald JL, Davis SL. Comparison of fosfomycin to ertapenem for outpatient or step-down therapy of extended-spectrum β-lactamase urinary tract infections. Int J Antimicrob Agents. 2016 Jul;48(1):56-60.
13. Beveridge LA, Davey PG, Phillips G, McMurdo ME. Optimal management of urinary tract infections in older people. Clin Interv Aging. 2011;6:173-80.
14. Cunha BA, Cunha CB, Lam B, Giuga J, Chin J, Zafonte VF, Gerson S. Nitrofurantoin safety and effectiveness in treating acute uncomplicated cystitis (AUC) in hospitalized adults with renal insufficiency: antibiotic stewardship implications. Eur J Clin Microbiol Infect Dis. 2017 Feb 2.
15. Hooton TM. The current management strategies for community-acquired urinary tract infection. Infect Dis Clin North Am. 2003 Jun;17(2):303-32.

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Hepato-Protective Effect of Folic Acid and Vitamin B12 in Comparison to N-acetylcysteine in Experimentally Induced Acetaminophen Toxicity in Rats

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ABSTRACT

To compare the effectiveness of vitamin B12 and folic acid in comparison to N-acetylcysteine (NAC) as hepato-protective agent against liver damage in experimentally induced acute acetaminophen toxicity in Sprague dawley rats. 18 male Sprague dawley rats were randomly divided into three groups of six each. Group A was administered NAC, group B - vitamin B12 and group C - folic acid intraperitoneally one hour after an acute over-dosage of acetaminophen 3mg/kg. After 48 hours, 1.5 ml of blood was withdrawn and the animals were sacrificed for histopathological examination of liver. The mean AST and ALT of vitamin B12 group did not differ statistically from that of the NAC group. The folic acid group had higher mean AST though not statistically significant whilst the mean ALT was significantly higher compared to NAC group. The reduction in the mean ALP and GGT with vitamin B12 showed statistically significant reduction whereas the folic acid group did not. Histopathology of liver revealed that the tissue architect was totally disturbed with portal tract appearing hemorrhagic and congested in addition to centrilobular necrosis in the folic acid group. In vitamin B12 group, the architecture of tissue was preserved with only mild inflammation. Vitamin B12, unlike folic acid has proved to be an efficacious hepato-protective agent in animal model of acute acetaminophen toxicity.

Keywords: Acetaminophen toxicity, Folic acid, Vitamin B12, Liver enzymes, Histopathology.

INTRODUCTION

Acetaminophen or paracetamol is one of the commonly used non-steroidal anti-inflammatory drugs. Though considered as safe analgesic-antipyretic, an overdose can lead to hepatotoxicity. Each year in the United States, approximately 6% of adults are prescribed acetaminophen doses of more than 4 g/day and 30,000 patients are hospitalized for acetaminophen toxicity.[1] Acetaminophen overdose is clinically significant as it is the most common cause of drug-induced liver failure.[2] Liver

injury occurs in 17% of adults with unintentional acetaminophen overdose.[1]Acute tubular necrosis is yet another manifestation of acute acetaminophen toxicity and it can occur either isolated or along with hepatotoxicity.[3]

N-acetylcysteine (NAC) is used as the treatment of choice for acetaminophen overdosage.[4, 5] Intravenous and oral formulations of NAC are available for the treatment of acetaminophen overdose. Though the current standard of care involves NAC, the use of this drug has many adverse effects like allergy, anaphylaxis, angioedema, bronchospasm.These reactions are dose-dependent and preceded by release of histamine. Hypotension can occur due to the vasodilatory effect and in severe cases, there can be consequent oliguria which may lead on to the issue of pre-renal pathology for an acute renal failure.[6]Animal studies and previous case reports strongly suggest that massive NAC overdose can cause cerebral edema and seizures.[7]

Though it has been well established that NAC can reduce mortality in acute overdose toxicity of acetaminophen, it is important to understand that the risk of adverse reactions to NAC do exists as is the risk of its overdosage.[6, 8] Noteworthy, the preparation of NAC for intravenous administration is quite complex.[6] Cases of iatrogenic NAC overdose have been reported in literature and it is important as this could even lead to death of the patient due to progressive hemolysis, thrombocytopenia and acute renal failure.[6, 7]

A high incidence of more than 60% patients treated with NAC has been reported to develop ADR.[8] Vitamin B12 and folic acid have been demonstrated to possess hepato-protective effect in other models of liver injury. Though one animal study indicating the effectiveness of folic acid in liver injury induced by acetaminophen has been reported in literature,[9] there are no studies evaluating the protective role of vitamin B12 in this animal model and in addition, this is the first study comparing the hepato-protective activity of vitamin B12 with folic acid in experimentally induced acute acetaminophen toxicity in rats in order to identify the potentially less toxic alternative to NAC by evaluating the effects of vitamin B12 and folic acid on liver enzymes and histopathology of liver tissue in comparison to that of NAC.

MATERIALS AND METHODS

The institutional animal ethics committee approved the protocol of the study (Approval number: 289/2015/IAEC). 18 Adult, male Sprague dawley rats weighing 200-300g were housed at constant ambient temperature in 12hr light, 12hr dark cycle. They were group housed as 3 animals per cage in polypropylene cages. Pellet diet and tap water were provided *ad libitum*. The animals were randomly allocated into the following three groups of 6 rats per group.

Group A: 6 rats in which acetaminophen at a dose of 3g/kg was given and NAC at a dose of 150mg/kg once after 1 hour of acetaminophen toxic dose.
Group B: 6 rats in which acetaminophen at a dose of 3g/kg was given and vitamin B12 at a dose 10mg/kg once after 1 hour of acetaminophen toxic dose.
Group C: 6 rats in which acetaminophen at a dose 3g/kg was given and folic acid 8mg/kg administered once after 1 hour of acetaminophen toxic dose.

The animals are weighed and the drugs were orally gavaged based on the body weight. 48 hours later, 1.5ml of blood was collected from the tail vein of the rats and liver function test (LFT) was performed. Quantitative analyses of liver enzymes namely, aspartate-aminotransferase (AST) or serum glutamic-oxaloacetic transaminase (SGOT), alanine-aminotransferase (ALT) or serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and ã-glutamyl transpeptidase (GGT) were performed. The values of these enzymes thus estimated were indicative of the severity of liver damage and were statistically analyzed.

All the rats were then sacrificed and liver specimens were taken, fixed in 10% formaldehyde and embedded in paraffin. Sections were cut from the tissue blocks, mounted on slides, and stained with Van Gieson's stain. They were examined under microscope for liver cell damage and disruption of normal architecture. [Figure1]

Statistical analysis: The data was entered in Excel and analyzed using SPSS software version

19. Comparison of the mean of liver enzymes was done using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test. The difference between the groups was considered statistically significant if $p<0.05$. Histopathological changes between groups were qualitatively analyzed.

RESULTS

The mean AST (260.2 ± 54.7) of vitamin B12 group was lower compared to that of the group which received NAC with mean AST (319.8 ± 57.6) and though the mean ALT for vitamin B12 group (114.3 ± 32.4) was higher than that of NAC

Table 1: Comparison of liver enzymes between groups using one way ANOVA

Parameter	Between groups comparison	Mean difference	p value
AST	Groups A and B	59.667	0.103
	Groups A and C	-30.167	0.393
ALT	Groups A and B	-27.167	0.348
	Groups A and C	-207.333*	0.000
ALP	Groups A and B	119.167*	0.001
	Groups A and C	-378.000*	0.000
GGT	Groups A and B	-0.400	0.372
	Groups A and C	-1.650*	0.002

*The mean difference is significant at the 0.05 level.

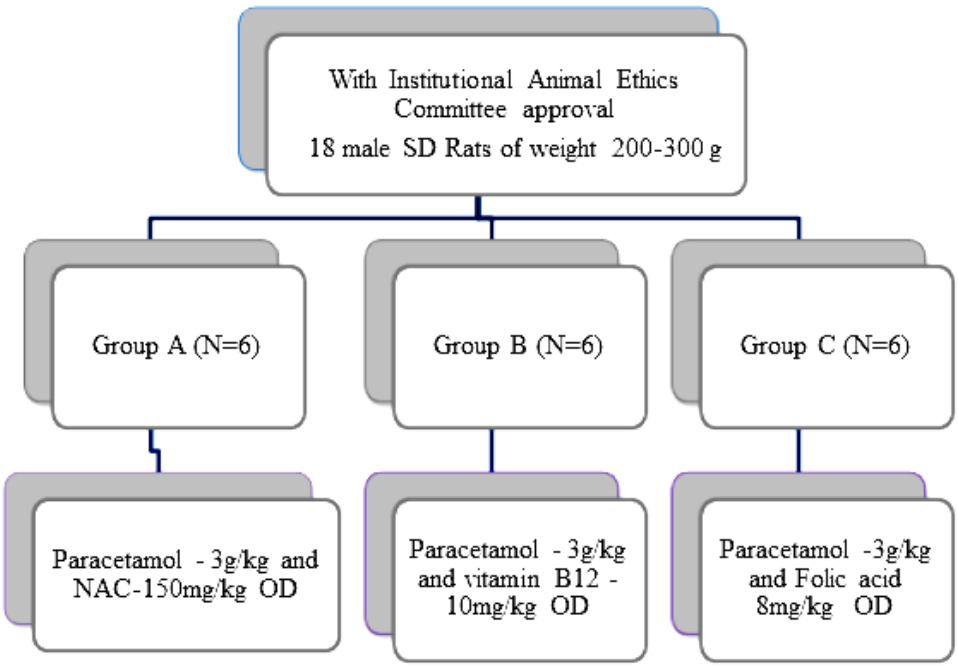


Fig. 1: Methodology

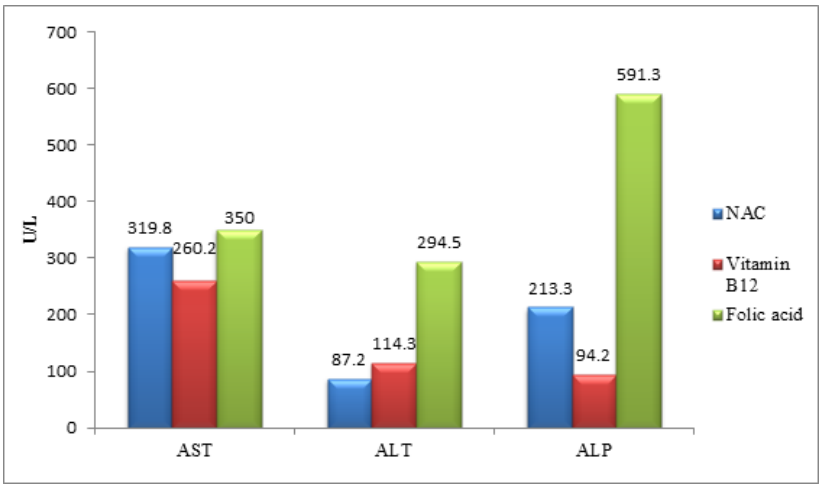


Fig. 2: Comparison of mean AST, ALT, ALP between groups

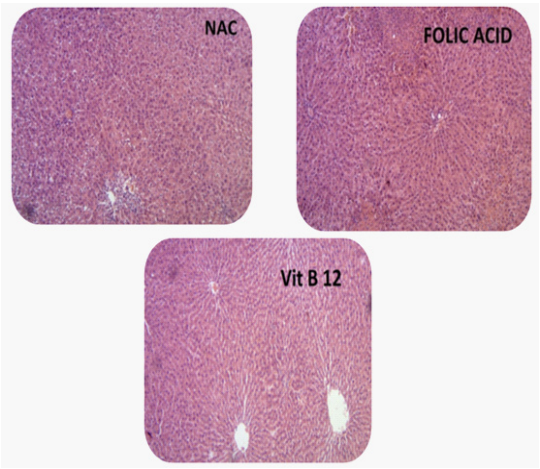


Fig. 3: Histopathology picture of the study groups

group (87.2 ± 41.5), [Figure2] the difference was not statistically significant by ANOVA with *post-hoc* LSD test either for AST (p=0.103) or ALT (p=0.348) between the two groups. [Table1]

On the contrary, folic acid group demonstrated a higher mean AST (350 ± 65.4) and mean ALT (294.5 ± 65.5) than NAC group. Comparison of the folic acid group with the group that received standard NAC treatment using ANOVA revealed that there was no statistical significance for the difference in the mean AST (p= 0.393) unlike that for mean ALT (p< 0.001). In addition, the vitamin B12 group had a significantly lower AST with mean difference of -89.8 ± 34.3 (p= 0.019) as well as for ALT with mean difference of -180.2 ± 28.0(p< 0.001) when compared to the group given folic acid. [Figure2 and table1]

Interestingly, there was a statistically greater reduction in mean ALP for vitamin B12 (94.2 ± 61.2) compared to NAC group (213.3 ± 48.2) whereas folic acid group (591.3 ± 44.8) had a statistically higher mean ALP (p< 0.001) indicating that folic acid did not demonstrate ALP reduction analogous to vitamin B12. [Table1]

Though mean GGT was slightly higher for the vitamin B12 group (1.18 ± 0.77) compared to the NAC group (0.78 ± 0.68), there was no statistical significance (p=0.372) for the difference in the mean values. However GGT for folic acid group (2.43 ± 0.79) mirrored the ALP wherein there was a statistically significant higher mean GGT (p=0.002) observed for folic acid group compared to the NAC group. [Table1]

Histopathological examination of the liver specimens revealed interface hepatitis, focal hepatic necrosis and thinning of sinusoidal plate in group A. In contrast, the group which received folic acid demonstrated tissue architect being totally disturbed with portal tract appearing hemorrhagic and congested in addition to centrilobular necrosis. Group B or vitamin B12 group had the architecture of tissue preserved with only mild inflammation. [Figure3]

DISCUSSION

This animal study has brought out the effective lowering of the liver enzymes by vitamin B12 in acute acetaminophen toxicity. The protective efficacy of vitamin B12 in reducing the liver damage after acetaminophen overdose is also evident from the histopathological changes observed in the study. It has been well established that acetaminophen induced liver damage causes centrilobular necrosis associated with a marked increase in AST, ALT activity.[10, 11, 12] Also, the increase in serum ALP activities is supported by the findings of earlier studies that, acetaminophen overdose is also accompanied by an increase in ALP activity due to hepatocellular injury. [10, 13] The reactive oxygen species-sensitive transcription factor namely nuclear factor-kappaB (NF-kappaB) is said to play a vital role in genesis of acetaminophen induced hepatotoxicity[12] and it has been reported that oxidative stress mediated by the metabolite N-acetyl-P-benzoquinone-imine (NAPQI) is considered to be the main cause of hepatic injury which involves depletion of cellular ATP, reduced Ca^{+2} ATPase activity with increased cytosolic Ca^{+2} levels, apoptosis and lipid peroxidation mediated by reactive oxygen species associated with a concomitant increase in AST, ALT, ALP.[14,15] It has also been hypothesized that reactive oxygen species induced lipid peroxidation generates peroxynitrite, a powerful oxidant and nitrating agent. Peroxynitrite can consequentially modify cellular macromolecules, aggravating mitochondrial dysfunction and ATP depletion, thus leading to cellular necrosis in hepatocytes and sinusoidal endothelial cells.[16]

The effectiveness of vitamin B12 parallels that of NAC since there is no significant difference in the mean AST, ALT and GGT values between the two groups [Table1]. In addition, the reduction in mean ALP was statistically greater for the vitamin B12 group than the NAC group. In comparison, folic acid did not demonstrate a similar effectiveness either in reduction of raised hepatic enzymes or reversal of histopathological changes induced by acute administration of high dose acetaminophen. [Figue3] This is in contrast to an earlier study which indicated lowering of liver enzymes and improvement in hepatocellular damage histopathologically following administration of folic acid in acetaminophen induced toxicity in rats. Folic acid, though has plausible hepato-protective effect attributed to its property of eliminating the changes that are related to the oxidative stress such as lipid peroxidation and oxidative DNA damage, [9, 17] our study did not replicate a similar efficacy and this can be ascribed to a lower dose of acetaminophen for induction of toxicity in the previous studies and disparity in the time duration of blood withdrawn for estimation of liver enzymes. Such hepato-protective property of folate supplementation regimens after induction of liver toxicity using either chromium in rabbits or folate deprivation with copper exposure in rats have been reported in literature.[18, 19] The contradiction in findings between different studies probably stems from the fact that there is some inherent variation in the pathways and diversity in the intensity of oxidative stress induced by different models.

On the other hand, our study has delineated a significant hepato-protective effect of vitamin B12 demonstrating its efficacy in acute acetaminophen toxicity rat model.[Table1 and Figure3] The potential mechanisms contributory to this beneficial effect is the ability of vitamin B12 to maintain the sulphydryl level under oxidative conditions despite lack of radical scavenging property.[20] Moreover, vitamin B12 has been shown to protect rat primary hepatocytes from hepatotoxin-induced cell death in *in-vitro* experiments.[20] Evidence suggests that the activity of glutathione reductase is significantly lower in B12 deficient liver[21] and that the interaction between vitamin B12 and glutathione could protect against disease related to vitamin B12 deficiency.[22] The presence of cobalt complex in vitamin B12 has been shown to effectively inhibit the hepatic inflammation and fibrosis.[20] Besides, protective effect of vitamin B12 in experimental liver injury by carbon tertrachloride[23, 24] has been well documented in literature wherein the beneficial effect has been primarily hypothesized as owing to the improvement in mitochondrial integrity and this could be another contributory factor in the hepato-protection in acetaminophen toxicity amelioration also.

CONCLUSION

This study highlights that vitamin B12 exhibits significant hepato-protective effect and hence can be considered as a potential therapeutic

agent, alternative to N-acetyl cysteine in acute acetaminophen over dosage after appropriate human trials.

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We acknowledge Dr.Pavithra and animal house technicians for their support in the conduct of the study.

REFERENCES

1. Blieden M, Paramore LC, Shah D, Ben-Joseph R. A perspective on the epidemiology of acetaminophen exposure and toxicity in the United States. *Expert Rev Clin Pharmacol.* **7**(3):341-8 (2014).
2. Yaman H, Isbilir S, Cakir E, Uysal B. Current issues with paracetamol induced toxicity. *J Exp Integr Med.* **1**(3):165-6 (2011).
3. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. *J Med Toxicol*. **4**(1):2-6 (2008).
4. Prescott LF, Illingworth RN, Critchley JA, Stewart MJ, Adam RD, Proudfoot AT. Intravenous N-acetylcystine: the treatment of choice for paracetamol poisoning. *Br Med J.* **2**(6198): 1097-100 (1979).
5. Heard K, Green J. Acetylcysteine therapy for acetaminophen poisoning. *Curr Pharm Biotechnol,* **13**(10): 1917-23 (2012).
6. Mahmoudi GA, Astaraki P, Mohtashami AZ, Ahadi M. N-acetylcysteine overdose after acetaminophen poisoning. *Int Med Case Rep J.* **8**:65-9 (2015).
7. Heard K, Schaeffer TH. Massive acetylcysteine overdose associated with cerebral edema and seizures. *ClinToxicol* (Phila). **49**(5):423-5 (2011).
8. Zyoud SH, Awang R, Syed Sulaiman SA, Sweileh WM, Al-Jabi SW. Incidence of adverse drug reactions induced by N-acetylcysteine in patients with acetaminophen overdose. *Hum Exp Toxicol.*; **29**(3):153-60 (2010).
9. AL-Sowyan NS. Efficacy and safety of folic acid during toxic hepatitis induced by acute overdose of paracetamol. *International Journal of Pharmacology.* **5**: 208-214 (2009).
10. Abdel Salam OM, Baiuomy AR, El-Shenawy SM, Hassan NS. Effect of pentoxifylline on hepatic injury caused in the rat by the administration of carbon tetrachloride or acetaminophen. *Pharmacol Rep.* **57**(5):596-603 (2005).
11. Janbaz KH, Saeed SA,Gilani AH. Studies on the protective effects of caffeic acid and quercetin on chemical-induced hepatotoxicity in rodents. *Phytomedicine.* **11**(5):424-30 (2004).
12. Oz HS, McClain CJ, Nagasawa HT, Ray MB, de Villiers WJ, Chen TS. Diverse antioxidants protect against acetaminophen hepatotoxicity. *J Biochem Mol Toxicol.;* **18**(6):361-8 (2004).
13. Shivashangari KS, Ravikumar V, Devaki T. Evaluation of the protective efficacy of Asteracantha longifolia on acetaminophen-induced liver damage in rats. *J Med Food.* **7**(2):245-51 (2004).
14. Ferret PJ, Hammoud R, Tulliez M, Tran A, Trébéden H, Jaffray P, Malassagne B, Calmus Y, Weill B, Batteux F. Detoxification of reactive oxygen species by a nonpeptidyl mimic of superoxide dismutase cures acetaminophen-induced acute liver failure in the mouse. *Hepatology.* **33**(5):1173-80 (2001).
15. Sener G, Sehirli O, Cetinel S, Yegen BG, Gedik N, Ayanoglu-Dulger G. Protective effects of MESNA (2-mercaptoethane sulphonate) against acetaminophen-induced hepatorenal oxidative damage in mice. *J Appl Toxicol;* **25**(1):20-9 (2005).
16. Jaeschke H, Knight TR, Bajt ML. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. *Toxicol Lett.;* **144**(3):279-88 (2003).
17. Ghosh A, Sil PC. Anti-oxidative effect of a protein from Cajanus indicus L against acetaminophen-induced hepato-nephro toxicity. *J Biochem Mol Biol.;* **40**(6):1039-49 (2007).
18. El-Demerdash FM, Yousef MI, Elaswad FA. Biochemical study on the protective role of folic acid in rabbits treated with chromium (VI). . *J Environ Sci Health B.* **41**(5):731-46 (2006).

19. Lin HL, Yu CC, Chou YF, Huang RF. Folate deprivation and copper exposure potentiate reactive oxygen species generation and chromosomal DNA loss but not mitochondrial DNA deletions in rat hepatocytes. *Int J Vitam Nutr Res.;* **76**(5):332-40 (2006).
20. Isoda K, Kagaya N, Akamatsu S, Hayashi S, Tamesada M, Watanabe A, Kobayashi M, Tagawa Y, Kondoh M, Kawase M, Yagi K. Hepatoprotective effect of vitamin B12 on dimethylnitrosamine-induced liver injury. *Biol Pharm Bull.* **31**(2):309-11 (2008).
21. Biswas DK, Johnson BC. Glutathione reductase and dehydrogenase activity in vitamin b12 deficiency. *Arch Biochem Biophys.* **104**: 375-80 (1964).
22. Watson WP, Munter T, Golding BT. A new role for glutathione: protection of vitamin B12 from depletion by xenobiotics. *Chem Res Toxicol;* **17**(12):1562-7 (2004).
23. Kasbekar DK, Lavate WV, Rege DV. Sreenivasan A. A study of vitamin B12 protection in experimental liver injury to the rat by carbon tetrachloride. *Biochem J.* **72**:384-9 (1959).
24. Antelava NA, Gogoluari MI, Gogoluari LI, Pirtskhalaishvili NN, Okudzava MV. Efficacy and safety of heptral, vitamin B6 and folic acid during toxic hepatitis induced by CCL4. *Georgian Med News.* **150**:53-6 (2007).

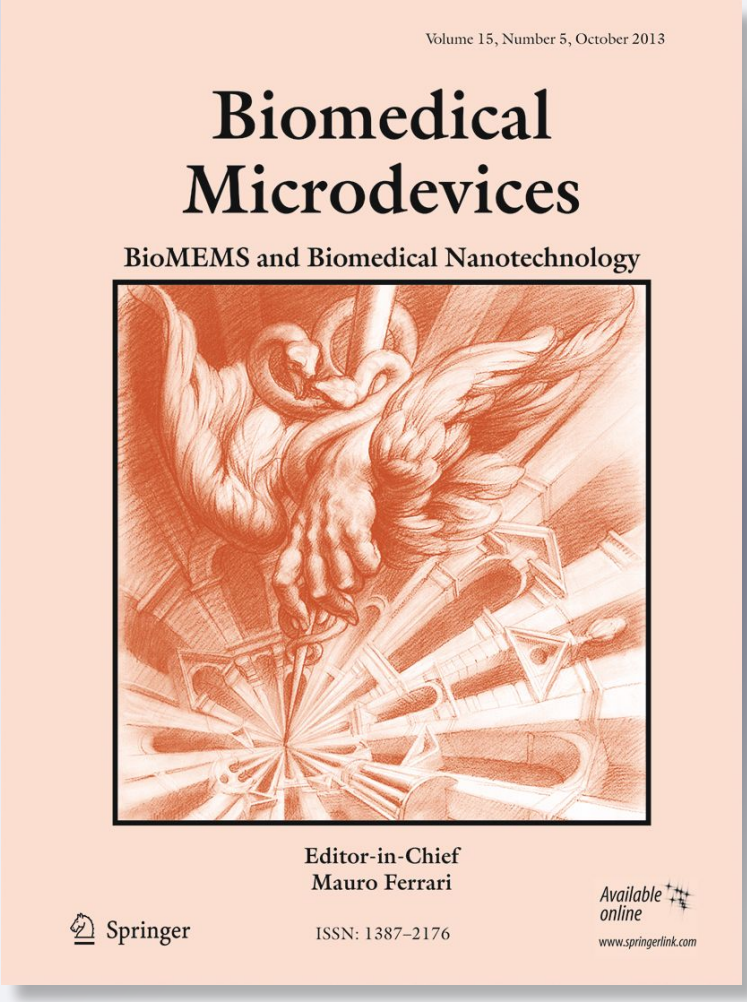
Microfluidic device for novel breast cancer screening by blood test using miRNA beacon probe

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Microfluidic device for novel breast cancer screening by blood test using miRNA beacon probe

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Abstract Breast cancer is identified as the highest cause of death in women suffering from cancer. Early diagnosis is the key to increase the survival of breast cancer victims. Molecular diagnosis using biomarkers have advanced much in the recent years. The cost involved in such diagnosis is not affordable for most of the population. The concept being investigated here is to realize a simple diagnosis system for screening cancer by way of a blood test utilizing a miRNA based biomarker with a complementary molecular beacon probe. A microfluidic platform was designed and attached with a fluorescence reader, which is portable and cost effective. Experiments were performed with 51 blood samples of which 30 were healthy and 21 were positive for breast cancer, collected against institutional human ethical clearance, IHEC 16/180–7–9-2016. miRNA 21 was chosen as the biomarker because it is overexpressed 4-fold in the serum of breast cancer patients. This work involved design of an experiment to prove the concept of miRNA over expression followed by detection of miRNA 21 using the microfluidic platform attached with a fluorescence reader and validation of the results using quantitative Real Time Polymerase Chain Reaction (qRT-PCR). The results obtained from the microfluidic device concurred with qRT-PCR results. The device is suitable for point-of-care application in a mass-screening programme. The study also has revealed that the stage of the cancer could be indicated by this test, which will be further useful for deciding a therapeutic regime.

Keywords Screening · Breast cancer · Biomarker · miRNA-21

1 Introduction

The prevalence of breast cancer is the highest among cancers in women. The World Health Organization (WHO) has placed a paper on the screening procedure evaluation for breast cancer (BC) since breast cancer has been the highest cause of female mortality (World Health Organization 2014). Though advanced cancer regimes exist, the cause of high mortality rate is due to delayed diagnosis. In young women, societal and emotional insecurity

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prevent them from disclosing the symptoms. Screening of high-risk population at regular intervals will reduce the mortality rate. Currently mammography is the screening tool which is not cost effective for a middle-income country(World Health Organization 2014). This scheme is not very convenient to address a population, since the mammogram facility is hospital centric. Histological diagnosis of cancer happens only when a symptom is reported. Biomarker based diagnosis is proved to compliment pathological examination and is capable of early detection (Emole 2012). In this work, a circulating biomarker identified is used to develop a screening tool by way of a blood test. miRNA 21 is reported to be overexpressed in the serum of breast cancer patients (Van Schooneveld et al. 2015). Molecular beacon probes are used for PCR to quantify miRNA. This molecular beacon probe is used with a microfluidic platform to read the overexpression of a specific miRNA (BETA et al. 2015). Microfluidic platform was used for detecting miRNA 18a from the serum of retinoblastoma patients (Salim 2015) which was a motivation to design a method for much prevalent and life threatening breast cancer. Based on this concept an objective for screening of breast cancer was derived which is the need of the day when breast cancer has taken highest position causing women mortality. Serum samples of BC patients were analyzed for miRNA-21 expression using qRT-PCR method (Asaga et al. 2011). Expression of miRNA-21 was found higher when compared with healthy controls as well as the level of miRNA-21 indicated difference based on cancer stages. The objectives of the study were (1) Detection of over expression of miRNA-21 in patients' sera using molecular beacon probe by allowing it to hybridize with miRNA-21 in serum (2) Development of a microfluidic platform to improve the hybridization efficiency compared to a well plate (3) Incorporation of a fluorescence reader to make the device portable. (4) Validate the test results using qRT-PCR.

Participants for this study were clinically confirmed for breast cancer. Healthy volunteers were included from master health checkup participants who were not having breast cancer. Inclusion of healthy and diseased individuals was based on need for Case-Control study to evaluate diagnostic accuracy (Rutjes et al. 2005). Diagnostic accuracy is the ability of the test proposed to distinguish between the targeted disease condition (case) and the healthy ones (control). Experiments conducted on serum of 21 breast cancer patients and 30 healthy individuals revealed that this device can be used for screening of breast cancer, which is validated against qRT-PCR results. 21 women included in the study were diagnosed with breast cancer at stage 2 or above. Statistical analysis was carried out based on the results for significance and sensitivity.

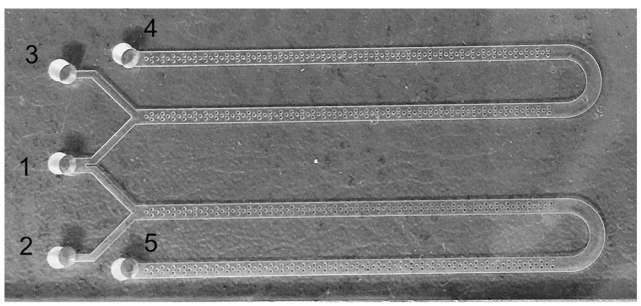
2 Materials and methods

2.1 Molecular beacon probe

Fluorescence based detection has taken molecular diagnosis to wider horizons due to its simplicity and sensitivity in quantifying molecules. Molecular beacon probes are oligonucleotides designed for target molecules with a reporter dye and a quencher (Braut-Boucher and Aubery 2010). They are hairpin shaped molecules with quenched fluorophore molecules which move away from the quencher, once the hairpin shaped molecule hybridizes with its complementary target nucleic acid molecule. While in quenched state, the excitation energy will be released through non-fluorescent means (Valeur 2001) like fluorescence resonance energy transfer (FRET) (Enderlein 2014). When the hybrid is formed, fluorophore molecules absorb excitation energy and emit fluorescence at a shifted wavelength from the exciting wavelength. miRNA-21 is the target nucleic acid in the serum. A custom miRCURY LNA™ microRNA detection probe hsa-miR-21-5p, (5TYE665/TCAACATCAGTCTGATAAGCTA) was used for the experiment with the excitation peak wavelength at 645 nm and the emission at 665 nm (Exiqon 2009) (Exiqon n.d.).

2.2 Sample collection

Ethical clearance was obtained from institutional ethical clearance committee to collect blood samples from confirmed breast cancer patients and healthy voluntary individuals as per IHEC No. 16/180 dated 07–09-2016. The inclusion criteria were based on consent by the individual with matching age group of 25–60 which is the most vulnerable age group for breast cancer. 5 ml of blood samples were collected in the BD Vacutainer SST tubes from twenty one BC patients and thirty healthy volunteers as reference or control. All the samples were in the age group of 25 to 60 years. Control blood samples were collected from healthy individuals who participated for the master health program diagnosed as non-



1. Probe inlet , 2. Control serum inlet 3. Test serum inlet , 4. Test collection well, 5. Control Collection well

Fig. 1 Standard slide size microfluidic mixer with pillar structures

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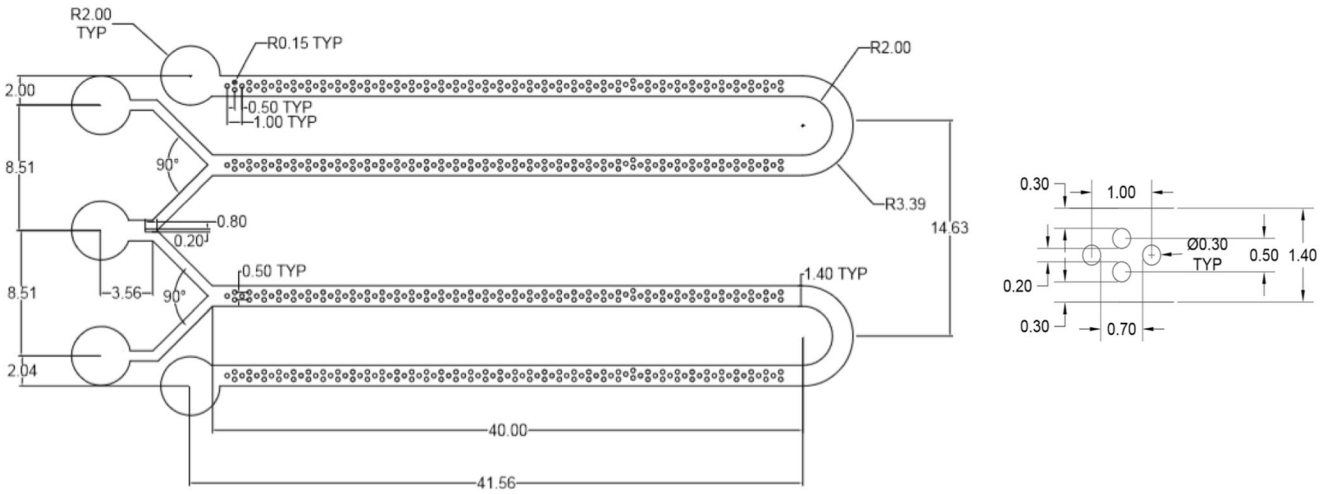


Fig. 2 Microfluidic mixer with dimensions in mm

cancerous individuals. Collected blood samples were kept at room temperature for 15 min and then the serum was separated from the blood by centrifuging at 3000 rpm for 15 min and the supernatant (serum) was transferred and stored at -80^0 C.

2.3 Microfluidic platform

The proposed screening scheme is to measure miRNA-21 over expression by hybridizing the probe with target miRNA from the serum in a microfluidic platform. The microfluidic platform should provide efficient interaction of probe molecules with the RNAs in the serum. There are two parameters to consider while using a microfluidic platform. One of them is the volume of probe required to provide a result with significance of over expression in terms of fluorescence emitted, because lower the probe-volume, lower is the cost involved. This demands a thinner device where the Reynolds number becomes low of the order of 1 to 10 or lesser, resulting in laminar flow of fluid. Therefore, there is a possibility of mixing only by way of diffusion. The other contradictory parameter is that, for diffusion to be efficient a longer path should be provided for the fluids to interact in the channel, which in turn increases the volume. Comsol Multiphysics 5.2a was used for the design and simulation of single phase fluid flow. A microfluidic mixer is designed and developed for efficient mixing of two fluids [5]. The fluids flowing are serum and probe in this case. Diffusion coefficient of serum is 61 μ m^2/s and miRNA probe in water is 100 μ m^2/s (Milo et al. 2009). These diffusion coefficients are high enough for mass transport, but in a microfluidic channel since the flow is laminar, diffusion will happen only between lamella. There was the need to increase the interacting surface of the fluids to make diffusion better and at the same time to improve the mass transport by convection also. Active microfluidic mixers are useful in such applications, but are not cost effective for single use applications. Hence a passive microfluidic device with pillar structures along the channel was proposed for effective hybridization. The device was modified with two channels to allow mixing of sample under test in one channel and a control in the second channel with the probe. The dimensions are chosen such that the whole device is of a standard slide size as shown in Fig. 1. The device was fabricated on Poly(Methyl Methacrylate) (PMMA) using micromachining. Equally important was the compatibility of the material with biological sample. Also, the material chosen needed to be fabrication process compatible. PMMA was chosen as the material for fabricating the microfluidic device which matched the requirement compared to Polydimethylsiloxane (PDMS) or

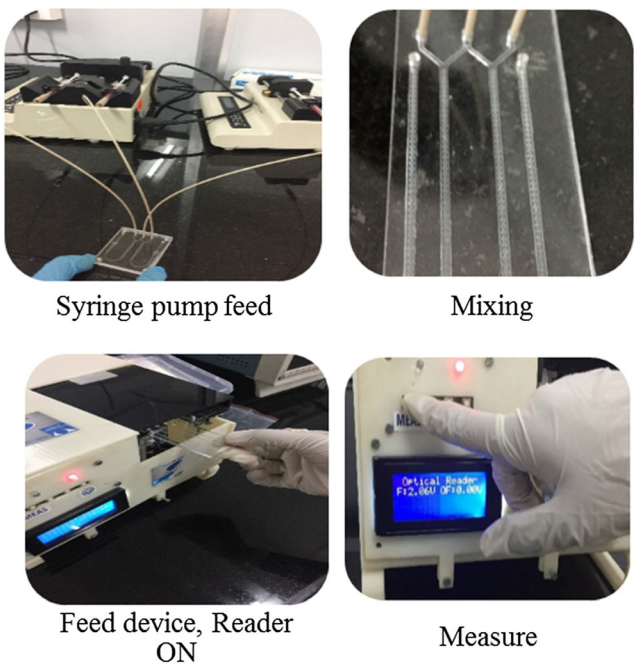


Fig. 3 Experiment set up for screening

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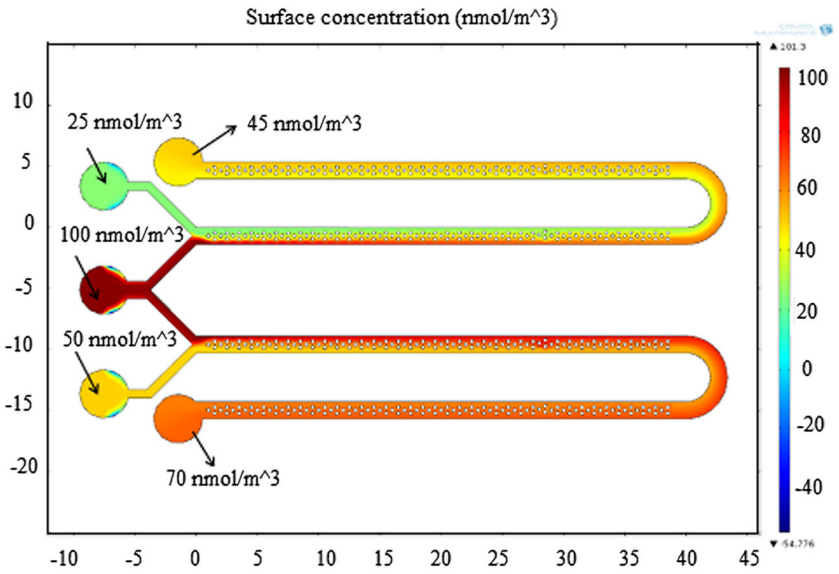


Fig. 4 COMSOL Simulated result of mixing efficiency with pillars in the channel (Salim 2015)

Polycarbonate. High transparency makes PMMA an ideal replacement for glass and is compatible with human tissue making it an important material for biomedical devices. PMMA is a clear, colorless polymer and is commonly called acrylic glass or simply acrylic (Ackermann et al. 2014). The device has 3 inlet wells - one for the molecular beacon probe, the second one for breast cancer serum (Case) and the third one for healthy serum (Control). There are two collection wells provided to measure fluorescence. The design and development of microfluidic device was done by one of the authors for a project to diagnose retinoblastoma from patients' serum using miRNA-18a (Salim 2015).

The dimensions of the microfluidic mixer are shown in Fig. 2. The overall device size is 53 mm × 25 mm as that of a standard slide. The channel depth is 0.235 mm. The inlet wells are of 4 mm diameter. The channel width is 1.4 mm. Length of the channel is 88 mm including the curved part resulting in a volume of approximately 26 μ liters. Sufficient entry length is provided so that the flow is fully developed before entering the channel. The pillars are of 300 μm diameter and placed in 3 rows spaced 1 mm apart horizontally. Vertically, split pillars are 300 μm spaced away from the channel wall and separated by 200 μm.

As the flow rate decreases, Reynolds number decreases and flow remains laminar. At the same time the mass transfer Peclet number, which is a ratio of mass transport by advection to mass transport by diffusion, decreases resulting in diffusion dominated mixing. The mass transfer Peclet number is directly proportional to hydraulic diameter and hence pillar structures added to the channel will reduce the hydraulic diameter and enhances diffusion (Madou 2002).

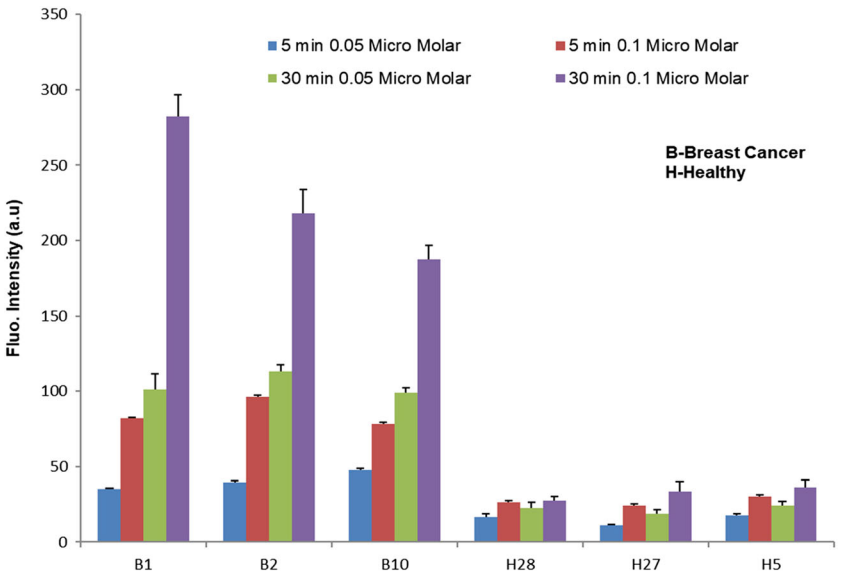


Fig. 5 Standardization of probe concentration and time

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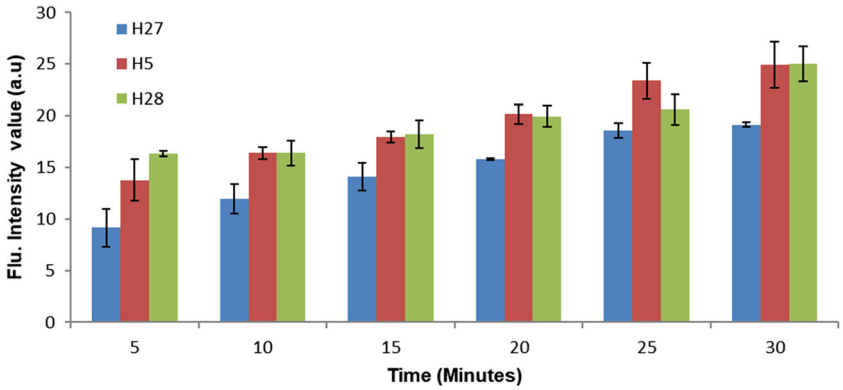


Fig. 6 Time standardization with 0.06 μM

2.4 miRNA-21 over expression using multimode fluorescence reader

Probe concentration Experiments were conducted to choose the molar concentration of probe to effectively utilize the probe, which in turn decides the cost of screening. Sensitivity was tested with probe concentration ranging from 0.1 μM to 0.5 μM initially and then molar concentration ranging from 0.01 μM to 0.09 μM. These experiments were conducted by simple mixing of serum and probe in microplate wells using a pipette. 50 μl of sample and 50 μl of probe at varying concentrations were experimented and the fluorescence was read using multimode scanning fluorescence reader. The results for 0.1 μM to 0.5 μM were discarded since 0.01 μM to 0.09 μM has proved good for measuring over expression of miRNA-21.

Fluid flow rate in microfluidic mixer To decide the flow rate in the microfluidic channel, fluorescence measurements were done at varying time interval starting from 1 min up to 30 min at an interval of 5 min with a probe concentration of 0.06 μM. This knowledge was required to design the channel length of the microfluidic mixer for effective hybridization.

Over expression of miRNA-21 Experiments were done on all the 21-breast cancer positive samples and 30 healthy samples, choosing 0.06 μM as probe concentration and 10 min as time for hybridization in the microplate wells to read the fluorescence.

2.5 Screening by microfluidic platform and built in fluorescence reader

Two micro syringe pumps were used to pump the fluids into the microfluidic platform; one of the syringe pump is dual and the other one single. The dual pump was used to pump serum and control to the inlet well at a flow rate of 1 μl/min and the single pump was pumping probe in the common inlet, feeding the channel for control and test sample. The flow rate was chosen as 2 μl/min. The designed device underwent slight modification in dimensions for the wells based on the fitting accessories used for the connecting syringe pump and due to fabrication limitations. The dimensions of the inlet wells were chosen to fit PEEK tubing to minimize the feed through volume. The fabricated device with the experiment set up is shown in Fig. 3. The microfluidic device fed to the fluorescence reader, which has a lens assembly to focus 645 nm wavelength light for exciting the hybridized sample and a photo diode to detect the emitted fluorescence at 665 nm. A dichroic lens was used to allow the excitation to the sample and return the emission on to the photo detector at an angle of 90 degrees with respect to the source. Fluorescence measurement requires low noise detection and hence a lock in amplifier in synchronous detection mode was used

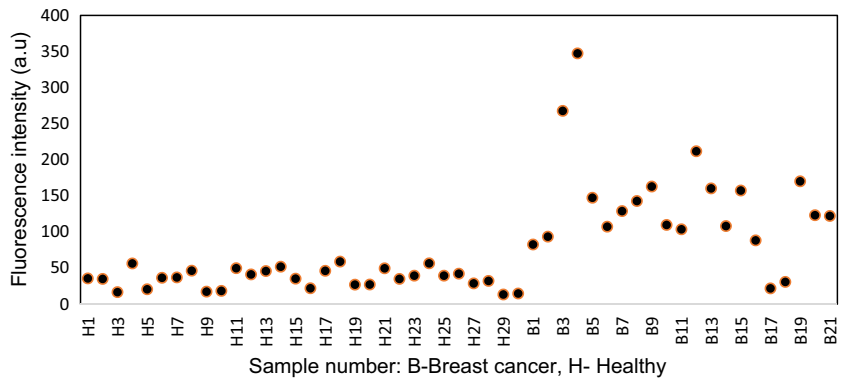


Fig. 7 miRNA-21 level for healthy and Breast cancer serum using well plate on scanning multimode reader

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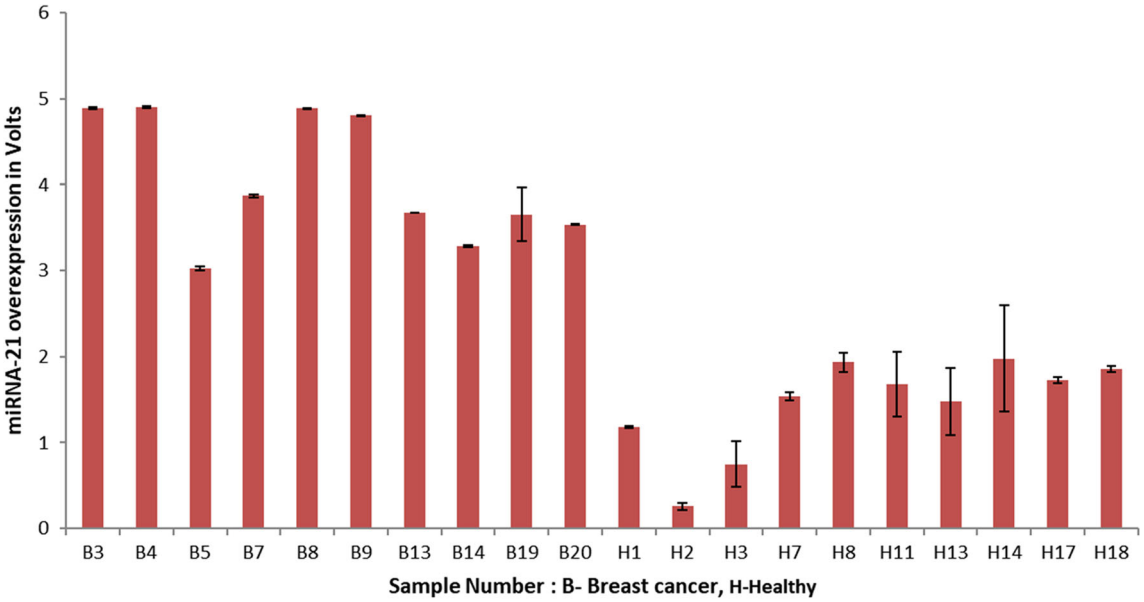


Fig. 8 miRNA 21 expression level measured on microfluidic device

(Bhagyajyoti et al. 2012) (PerkinElmer_Application_note 2000). The reader allows sliding of the microfluidic platform to read the collection wells of control and test by moving a lever. The fluorescence intensity is converted to voltage using optical detector (Transducer concept). A 16-bit analog to digital converter is used to obtain a resolution of 0.004 Volts which can represent 1026 levels of fluorescence intensity from 0 to 350 a.u, and is calibrated. The conversion to voltage is done for easy processing and developing user friendly display such as screened positive, negative etc. based on a threshold level.The result is displayed in Volts to compare the value of healthy control and BC sample under test which is calibrated for a range of 0 a.u to 350 a.u fluorescence value corresponding to 0 to 5 Volts from the fluorescence microscope.

2.6 Validation using qRT-PCR

The entire miRNA was isolated from the 10 BC and one healthy serum samples by miRNeasy plasma/serum kit (Qiagen, USA) according to the manufacturer's protocol. Healthy sample chosen was with mostly matching miRNA-21 level (36.91) corresponding to the average miRNA-21 level (35.73) obtained from 30 healthy samples. In the reverse transcription (RT) step, cDNA is reverse transcribed from total miRNA isolated from BC samples using a small RNA-specific, stem-loop RT primer from the TaqMan small RNA Assays and reagents from the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA). 5 ng of the miRNA from BC sample and Healthy sample were used for the synthesis of cDNA. The reaction parameters were 16 °C for 30 min, 42 °C for 30 min, 85 °C for 5 min and final hold at 4 °C. After the synthesis of cDNA, real time PCR (qRT-PCR) was carried out for analyzing the gene expression of the control and BC samples by using Taqman probes labeled with FAM dye. snRNA U6 was used as the internal control. The reaction parameters for the qRT-PCR were a hold at 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 60 s.

2.7 Statistical validity test

Statistical analysis was performed to understand the significance, sensitivity and specificity of the method, confidence interval for miRNA level for true positive and true negative. When a screening regime is defined, the tool becomes stronger if the specificity is high which indicates the percentage of true negative out of a non-disease population. A 2-sample T test was done to check if the mean value of miRNA-21 expression of healthy individuals group differs from the mean value of miRNA-21 expression of a breast cancer patients' group. Correlation analysis was performed to check the concurrence of microfluidic device measurements with multimode reader measurements and the gold standard measurement using qRT-PCR.

Table 1 Patient demographics

Clinical Stage of Cancer	Sample number
Stage II	B1, B5, B8, B10, B11, B13, B16, B20
Stage II-after surgery	B17, B18
Stage III	B2, B3, B9, B12, B15, B19, B21
Stage IV	B4, B6, B7, B14
Healthy	H1 -H30

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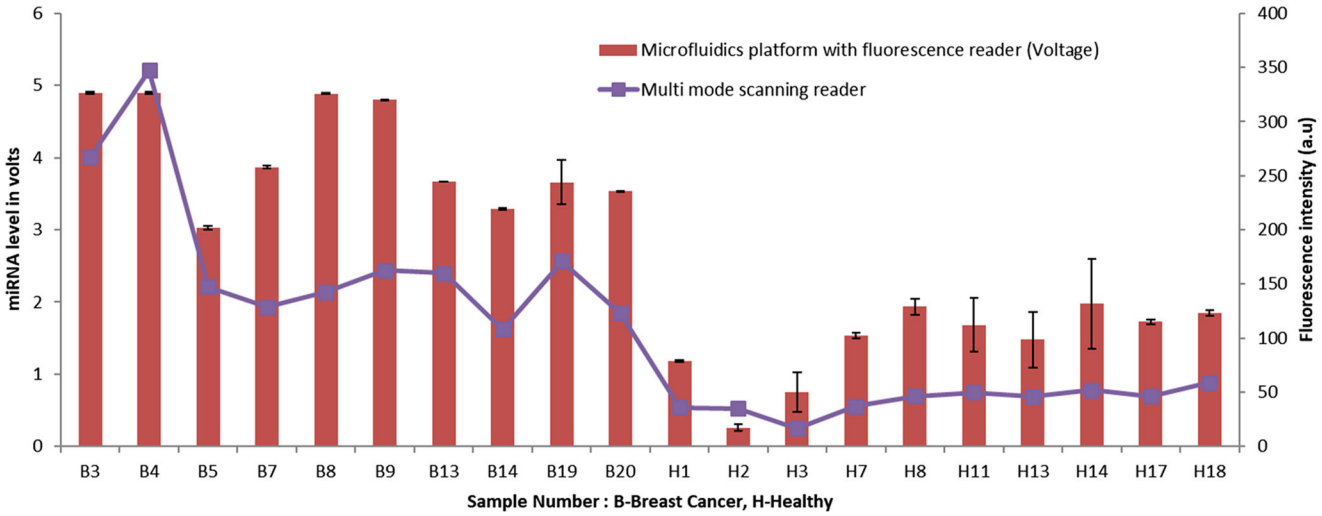


Fig. 9 Comparison of microfluidic platform results with well plate fluorescence reading using multimode fluorescence reader

3 Results and discussion

3.1 Microfluidic mixer

Mixing efficiency of the designed device was verified using COMSOL Multiphysics 5.2a. Several designs were attempted for enhancing molecular interaction of serum and probe for efficient indication of miRNA over expression. The one with pillars arranged for split-and-recombine flow pattern showed better mixing and was chosen for fabrication. The result for simulation is shown in Fig. 4. The entry length was calculated as 0.56 μ meter. Pillars are incorporated to ease mixing otherwise the flow remains laminar due to very low Reynolds number of the order of 0.028 for a flow rate of 2 μ liter /min (effective flow rate in the channel) which corresponds to flow velocity of 121 μm/s. The mass flow rate is calculated as 20.5 μ gm/s. The pillars are arranged to achieve a split and recombine pattern for the flow which enhanced molecular interaction by way of increased surface interaction. The mass transfer Peclet number of 4.86e-4 is indicating that diffusion is dominant for mass transport. By introducing the pillar structures, effective area of interaction is enhanced and hence resulted in better diffusion.

3.2 miRNA-21 over expression using multimode fluorescence reader

Time and probe concentration standardization: Time and probe concentration for hybridization were performed choosing two values each for time and probe concentration as per

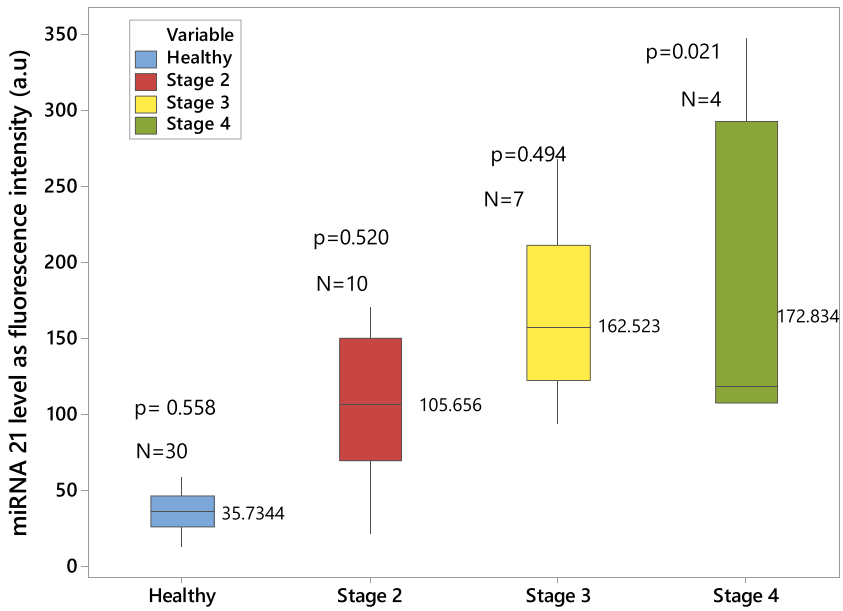


Fig. 10 Classification of stages of cancer at diagnosis and miRNA 21 expression with mean and *p*-values

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Table 2 Statistical significance of miRNA-21 expression for BC and healthy sera measured using scanning multimode reader

	N	Mean, μ	Std. Deviation	Std. Error
BC	21	137.4	72.7	16
Healthy	30	35.7	13.2	2.4

Difference = μ (BC) - μ (Healthy)

Estimate for difference: 101.7

95% CI for difference: (68.2, 135.1)

T-Test of difference = 0 (vs ≠): T-Value = 6.34 *P*-Value = 0.000 DF = 20

Taguchi design of experiments. Two values each of time and concentration were chosen for understanding the trend. Fig. 5 shows that the time required for hybridization is more if probe concentration is higher. The change in fluorescence intensity corresponding to time change from 5 to 30 min is much less for 0.05 μM concentration compared to the change in time for 0.1 μM concluding that time is not an independent variable but depends on the concentration. Lesser molar concentration required lesser hybridization time.

Figure 6 shows all the fluorescence values for time varying from 5 min to 30 min in steps of 5 carried out for 0.06 μM concentration where moving average is seen to be levelled after 20 min. Hence a time of 10 min is suitable for a sensitive read of fluorescence. With this the channel length was designed for 12 μl volume so that a 1 μl/min flow rate will take 12 min to flow till the collection well.

Choosing the molar concentration for probe as 0.06 μM and time for hybridization as 10 min, fluorescence of all the 30 samples of healthy volunteers and 21 breast cancer patients were measured using multimode fluorescence reader which showed significant difference between the miRNA-21 level of healthy individuals from breast cancer patients as seen in Fig. 7. The average value of healthy samples was 35.7. The miRNA-21 level of breast cancer patients sample had variation in intensity based on their stage of diagnosis, with an average value of 137.4. In most of the cases, diagnosis is at advanced stage as indicated from the case sheet done as per the 7th edition of TNM staging of the Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) in 2009. Two cases (B17, B18) with low miRNA-21 level seen in stage 2 had undergone surgery while blood samples were collected.

3.3 Screening experiment using microfluidic platform

Experiments conducted on microfluidic platform using 10 breast cancer samples showed difference in miRNA-21 level compared to 10 healthy samples as seen in Fig. 8. Healthy control samples have shown voltage levels below 2 V and all the breast cancer positive samples resulted in voltages of 3Volts and above. B4 was recorded as stage 4 and has shown highest level of miRNA-21. B3 was indicated as stage 3 but has shown higher level of miRNA-21 corresponding to stage 4 in all measurements. miRNA-21 level had been correlated with stage of breast cancer using qRT-PCR (Asaga et al. 2011). B17 and B18 were samples collected after surgery and showed miRNA-21 level corresponding to healthy volunteers as seen from Fig. 7. Demographics of BC patients is given in Table 1.

Figure 9 demonstrates a comparison of microfluidic device measurements with well plate measurement where mixing is done by pipetting. The readings are concurrent and hence miniaturization, which has reduced the probe

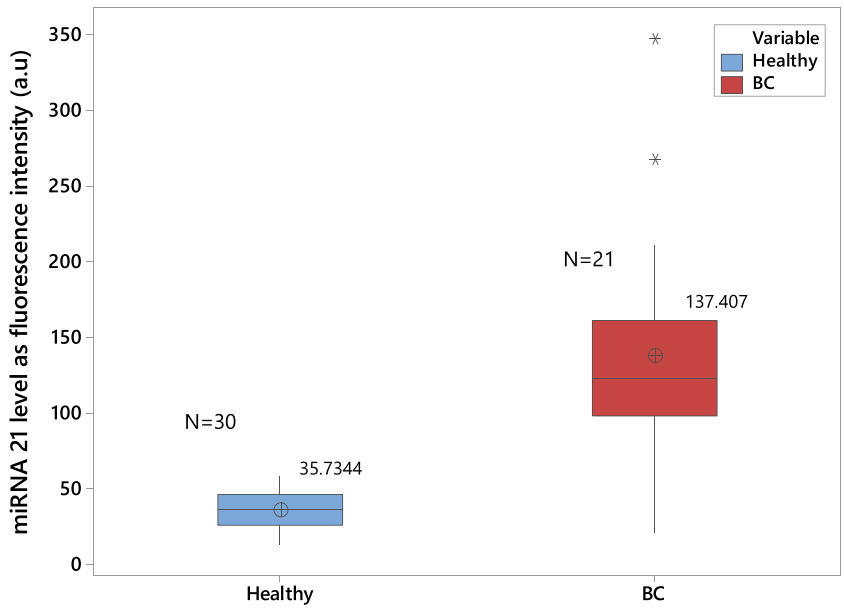
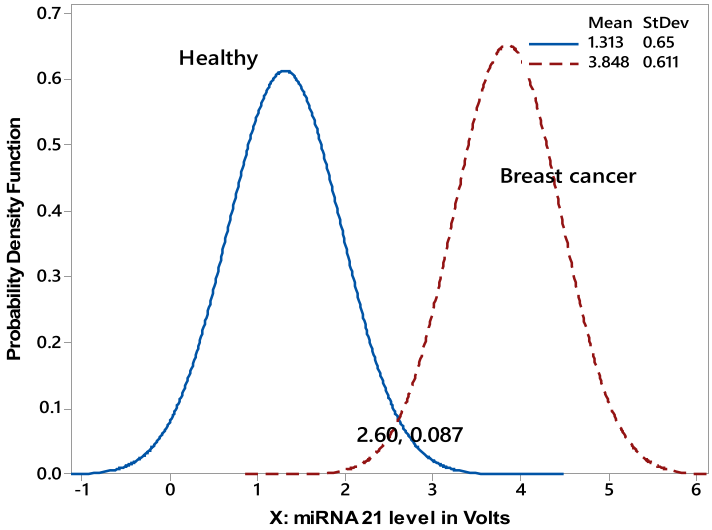


Fig. 11 Estimate of CI using 2-sample T test 95% CI for difference between 68.2 and135.1

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B3	4.90	H1	0.49
B4	4.90	H2	0.26
B5	3.03	H3	0.75
B7	3.87	H7	1.53
B8	4.89	H8	1.94
B9	4.80	H11	1.68
B13	3.67	H13	1.83
B14	3.29	H14	1.97
B19	3.66	H17	1.26
B20	3.53	H18	1.18

Fig. 12 Probability density function of Breast cancer and healthy sample miRNA 21 level measured using microfluidic device with reader. Reading above 2.6 V is screened positive with very good specificity >95%

requirement from 50 μl in well plate to 12 μl in microfluidic mixer, has not affected the accuracy of test results. Pearson correlation study was performed using Minitab, which resulted in correlation coefficient of 0.894 and *p*-value of 0. With this we are also justifying that stage indication by miRNA measurements should be more accurate than the conventional imaging methods where the skill of the technician plays a major role. Fig. 10 shows the classification of stages of detection based on the patients' files and the miRNA 21 over expression measured using the fluorescence reader. There is a marked difference in the average values corresponding to stages but for stage 4 has a spread in values with 3 of the samples resulted in miRNA level corresponding to stage 3. Three of four samples of stage 4 presented values corresponding to stage 3 which has resulted in spread of miRNA level and low p-value in stage 4 as seen in box plot of Fig. 10.

3.4 Statistical analysis

3.4.1 Two-sample T-test for BC positive vs. healthy samples

Study on 30 healthy samples to find a range of miRNA level to screen the sample as negative revealed that miRNA levels in the range 30–40 have a 95% Confident interval. The mean values for hypothesis test are shown in Table 2. The distribution of data is normal. Probability of false positive is almost 0, hence significance is extremely good (>95%). The estimated difference in mean of breast cancer and healthy control is 101.7 as seen from Fig. 11. The probability density functions of healthy and BC miRNA-21 level measured using microfluidic device attached with reader for 10 samples each are depicted in Fig. 12. Statistical significance is given in Table 3.

3.4.2 Correlations between stage demographic and measured status

Correlation study was performed for stage demographics against microfluidic based measurements. Pearson correlation coefficient of 0.444 and *p*-value of 0.044 were obtained. Linear correlation coefficient of 0.444 indicates moderate strength in linear relationship, this is due to the discrete status of stage. The *p*-value of 0.044 indicates that there is only 4.4% risk in concluding that a linear correlation exists.

The samples collected after surgery and 2 samples designated as stage 4 which resulted in miRNA level corresponding to stage 2 were removed to get the correlation between demographic and measured stage as 0.579 correlation coefficient with a p-value of 0.015.

3.5 Validation using qRT-PCR

The results of qRT-PCR showed that gene expression of miRNA-21 for 10 BC positive blood samples and a control sample (H7) concurred with the results obtained using microfluidic device as given in Fig. 13. The highest

Table 3 Statistical significance of miRNA-21 expression for BC and healthy sera measured using microfluidic device

	N	Mean, μ	Std. Deviation	Std. Error
BC	10	3.848	0.611	0.193
Healthy	10	1.313	0.65	0.206

Difference = μ (BC) - μ (Healthy)

Estimate for difference: 2.535

95% CI for difference: (1.940, 3.130)

T-Test of difference = 0 (vs ≠): T-Value = 8.99 *P*-Value = 0.000 DF = 17

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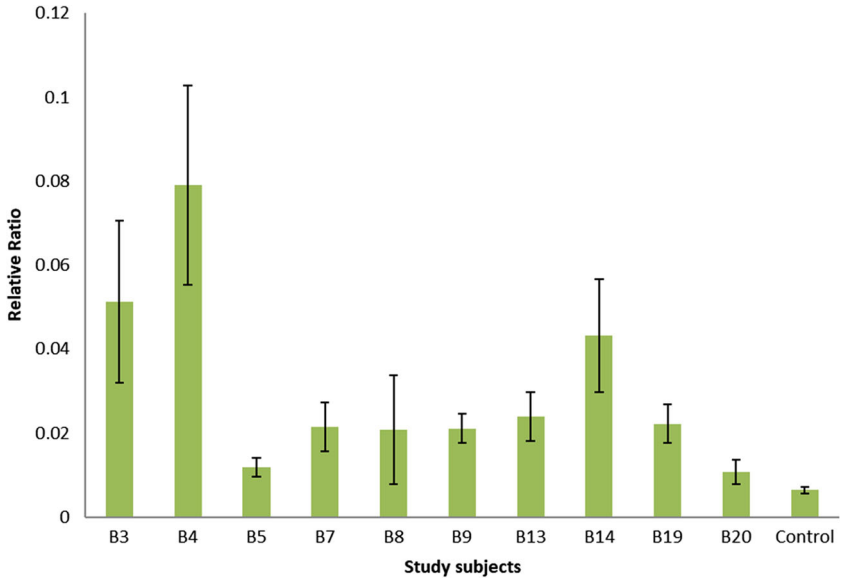


Fig. 13 Gene expression of miRNA-21 in breast cancer sample Vs control (H7) using qRT-PCR

expression is obtained for sample B4, which is reported as stage 4 at the time of diagnosis. B3 was diagnosed at stage 3 and the miRNA level of this sample was detected almost same level as B4 using fluorescence detector. Correlation analysis was performed for the results obtained by microfluidic platform with reader and qRT-PCR resulting Pearson correlation coefficient of 0.730 and p-value of 0.011.

4 Conclusion

Screening of breast cancer for a high-risk population is essential to improve the mortality rate of women and the existing accepted scheme is a mammogram. This tool is not affordable for much of the population of developing countries. Mammogram has side effects if there is the need for regular monitoring in high-risk population. The scheme proposed here where screening is done by an inexpensive blood test and moreover can reach even the population who are reluctant to come to a hospital. The method is useful even for young women with dense breasts where mammogram sometimes suggests further tests and adds to the financial burden to the individual.

The method is cost effective for mass population due to the use-and-throw microfluidic device, does not require a scientist or skilled technician, low power consumption, battery backup, portable and the short time for getting result. The platform is versatile and is useful for any biomarker-based screening. The method proposed here uses a device optimized to use the minimum quantity of probe, and the time required for the result is only 30 min. This test can be done any number of times for high-risk populations since there is no side effect like radiation hazard as in the case of mammogram and other imaging schemes. This cost-effective screening tool will have a tremendous impact on cancer diagnosis and treatment in developing countries.

Screening tools must have high specificity so as to rule out people for a disease when they do not have any symptom, whereas for a diagnostic test the tool must have high sensitivity to rule in people who has a symptom (Kumar and Indrayan 2011). The gold standard for breast cancer screening is a mammogram and therefore the adaptability of the test proposed here is compared with the mammogram and the benefits are clearly seen. Since the designed method comes under molecular diagnosis category, comparison for validation is performed using the standard test which is Real-Time PCR. From this study, a voltage level for miRNA 21 expression less than 2 Volts measured by the device could be confidently declared as a true negative case for breast cancer. In addition, molecular beacon probes can be designed to include multiple miRNA so as to detect multiple miRNA levels in the sample by a single test to enhance true positive and true negative while eliminating possibility of other diseases.

References

J. Ackermann, M. Juda, and D. Hirsch, Kunststoffe Int. 2014, 59 (2014)
S. Asaga, C. Kuo, T. Nguyen, M. Terpenning, A.E. Giuliano, D.S.B. Hoon, Clin. Chem. **57**, 84 (2011)
M. Beta, S. Krishnakumar, S.V. Elchuri, B. Salim, J. Narayanan, Anal. Sci. **31**, 231 (2015)
J. Bhagyajyoti, L. Sudheer, P. Bhaskar, C. Parvathi, Int. J. Sci. Eng. Technol. Res. **1**, 40 (2012)
F. Braut-Boucher, M. Aubery, *Encyclopedia of Spectroscopy and Spectrometry*, p. 635 (2010)
J. Emole, in *Prim. Care a Glance-Hot Top. New Insights, InTech*, pp. 175–187 (2012)

Author's personal copy

J. Enderlein, Compr. Biomed. Phys. pp. 111–151 (2014)

Exiqon, Biofluids Guidelines Analyzing microRNAs in Liquid Biopsies (2015), http://www.exiqon.com/ls/Documents/Scientific/microRNA-serum-plasma-guidelines.pdf. Accessed 26 Sept 2017

Exiqon, *Locked Nucleic Acid* (*LNA* ^{TM}) (2009), https://www.exiqon.com/ls/documents/scientific/lna_folder.pdf. Accessed 26 Sept 2017

R. Kumar, A. Indrayan, Indian Pediatr. **48**, 277 (2011)

M.J. Madou, *Fundamentals of Microfabrication: The Science of Miniaturization*, p. 49 (2002)

R. Milo, P. Jorgensen, U. Moran, G. Weber, M. Springer, Nucleic Acids Res. **38**, D750 (2009)

PerkinElmer_Application_note, *Technical Note TN1000, PerkinElmer Instruments, USA*, (2000)

A.W.S. Rutjes, J.B. Reitsma, J.P. Vandenbroucke, A.S. Glas, P.M.M. Bossuyt, Clin. Chem. **1341**, 1335 (2005)

B. Salim, J. Anal. Bioanal. Tech. **6**, 4 (2015)

B. Valeur, *Molecular Fluorescence: Principles and Applications*, p. 8 (2001)

E. Van Schooneveld, H. Wildiers, I. Vergote, P.B. Vermeulen, L.Y. Dirix, S.J. Van Laere, Breast Cancer Res. **17**, 1 (2015)

World Health Organization, *WHO Position Paper on Mammography Screening WHO Library Cataloguing-in-Publication Data WHO; (NLM classification: WP 815) World Health Organization*, (2014)

A comprehensive analysis on uses and side effects of steroids with respect to different routes

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INTRODUCTION:

Steroids are a class of chemicals that includes the hormones. There are two types of steroids namely, glucocorticoids & mineralocorticoids. Glucocorticoids such as cortisol, control carbohydrate, fat & protein metabolism and other mechanism. While the mineralocorticoids such as aldosterone control electrolyte and water levels. Most of the uses of corticosteroids in day to day practice have been in the field of Rheumatology, Orthopaedics, dermatology, oncology, respiratory medicine, otorhinolaryngology & ophthalmology.(1) Endocrinology utilizes corticosteroids for hypoadrenalism and tapering regimens in hypothalamic-pituitary-adrenal axis suppression. Glucocorticoids causes the following adverse effects such as osteoporosis, hyperglycemia, cushing syndrome, CVS disease, glaucoma, cataracts, infections. While, mineralocorticoids causes hypernatremia, hypokalemia, arterial hypertension, hyperglycemia, edema as the side effects.(2,3)

The main purpose of this review is to analyse the serious side effects caused by corticosteroids while administering in different routes of administration like topical steroids, intranasal steroids, steroid ear & eye drops, intra-articular steroid injections, oral steroids. Being very powerful anti-inflammatory agents, they were described as impressive drugs as they not only improved certain clinical conditions, but also confered a subjective sense of well-being. So, double headed action of steroids are widely discussed in this paper.

SYSTEM WISE USES AND SIDE EFFECTS OF CORTICOSTEROIDS

Corticosteroids and cardiovascular system:

Studies states that prescribed 7.5mg of prednisolone or more/ day compared to non-steroid prescription in cardio patients causes increased risk of CHF, MI, stroke, transient ischemic attack(Relative risk : 3.72, 3.26,1.73,7.41 respectively)(4).

There was an increased risk of arteriosclerosis in patients with Rheumatoid arthritis receiving long term corticosteroids therapy, shown by 3 fold increase in the incidence of lower limb calcifications.

Corticosteroids and endocrine system:

There is 1.8 fold risk of developing diabetes even at doses lower than 10mg prednisolone per day blood sugar should be monitored before and after commencement of corticosteroids as there is an increased risk of developing hyperglycemia when steroids exceed the dose equivalent to 10mg prednisolone per day.(5-7)

Corticosteroids and bone mineral density:

If steroids are required for prolonged periods, the dose should be limited to 15-20mg prednisolone per day to avert osteonecrosis. Fractures may occur in 30-50 % of patients on chronic corticosteroid therapy. Fracture risk may decrease rapidly after cessation of steroids.(8-10)

Corticosteroids and central nervous system:

At a daily dose prednisolone of 15mg per day, mood disorders have been observed in rheumatoid arthritis patients.High dose of corticosteroids taken for ocular symptoms produced hypomanic symptoms in 30% and depressive symptoms in 10% of patients within 1 week period[36] Partial memory loss was observed in patients receiving steroid doses between 5-40 mg prednisolone per day for one year or more.(11-13)

Corticosteroids and Gastro intestinal system:

Used in inflammatory bowel disease, auto immune hepatitis, severe alcoholic hepatitis.

Oral budesonide is less effective than intravenous because it is an oral non-systemic corticosteroid with only 10% systemic bioavailability.[14]

Corticosteroids and liver disease:

Steroids are used in the treatment of alcoholic hepatitis patient. they are used to suppress the activated immune response but in conditions like renal failure,active infections,gastric bleeding risk may occur.

Steroids when given in combination with NSAID increase the risk of peptic ulcer but when prescribed alone it will not cause any risk.[15-18]

Corticosteroids and hepatitis:

Initial studies had demonstrated the effectiveness of steroids for treating chronic active hepatitis in non hepatitis-B patients. Subsequent studies on patients who are hepatitis –B virus carriers have either shown no added effects or worse outcome in those treated with long term steroids.

There is an increase in hepatitis-B virus DNA levels with the use of steroids and its subsequent cessation can lead to a flare of the condition so it is therefore not advisable to start patients with hepatitis-B virus on long term systemic steroids.

The usage of corticosteroids has neither beneficial nor deleterious effects in patients with Hepatitis – C.[19-22]

Routes of corticosteroids and side effects:

Steroids administered in different routes may cause varied side effects which are discussed below:

TOPICAL ROUTE:

DRUGS	REPORTED ADVERSE EFFECTS	PERCENTAGE
Budesonide	Intra ocular pressure changes	30%
	Irritation,Itching,Bruising	1%
	Contact dermatitis	In 100 out 7238 patients
Hydrocortisone	Pseudotumour , Benign intra cranial hypertension, Cataract glaucoma, Contact dermatitis .	74 out of 7238 patients
Triamcinolone acetonide	Cushing syndrome	

A study report says that misuse of topical steroids out of 6723 patients 379 patients (5.63%) were reported ADR .Out of which 78.89% were females and the remaining were males Most common ADR: acne (37.99%), Telangiectasia(18.99%).[23]

CONDITION	NUMBER	PERCENTAGE
Facial acne	144	37.99%
Plethoric face and telangiectasia	72	18.99%
Facial hypertrichosis	70	18.46%
Hyper hypo pigmentation	60	15.83%
Cutaneous atrophy	38	10.02%
Perioral dermatitis	30	7.91%
Pyoderma	21	5.54%
Tineaincognito	17	4.48%

Side effects topical use of steroids in different age groups :

PATIENTS	**DOSE**	**SIDE EFFECTS**
Infants(210)	89.5g	Telangiectasia is prolonged effect Other effects: Atrophy of the anticubital and popliteal fossae
Children(546)	135g	
Adolescent(515)	304g	
Adult(515)	304g	

INTRANASAL ROUTE:

Used in nasal obstruction, nasal discharge, nasal itching, post nasal drip. It is better than oral histamine.Eg: Budesonide nasal spray administered for 6 weeks which does not cause HPA suppression in children (2-5 years).

Fluticasone propionate administered for one year which does not cause any effect on growth velocity in children(3.5-9 years)

Memetasone furoate administered for one year in children (3-9 years)which does not cause any growth velocity and HPA suppression.

Intranasal corticosteroids causes Osteopenia in male(18%), in female(42%)and it also causes cushing's syndrome[24-26]

INHALATION ROUTE:

The following local adverse effects are caused due to inhalation route of corticosteroids:

- Pharyngitis
- Dysphonia
- Reflex cough
- Bronchospasm
- Oropharyngeal candidiasis
- Reduction in bone markers in adults
- Acute adrenal insufficiency in >500µg / day of fluticasone propionate
- Moderate dose: Aggressiveness,Hyper anxiety, Mood changes and Excitability.
- Skin thining.

In low doses it do not cause neuropsychological and behaviourial changes.[27]

SYSTEMIC SIDE EFFECTS:

It causes the following side effects which includes:

- Suppressed HPA axis function
- Adrenal crisis
- Suppresses growth velocity in children
- Reduced bone mineral density
- Bone fractures
- Osteoporosis
- Skin thining
- Cataract
- Glaucoma

INTRAOCULAR ROUTE:

Steroid induced ocular hypertension (>21mm hg) was reported in some patients. : Total number of patients (156)

NO. OF PATIENTS	CONDITION	PERCENTAGE
36	Steroid induced ocular hypertension	24%
22	Bilateral steroid hypertension	44(eyes)
15	Vernal conjunctivitis	41.6%
16	Withdrawal effects	22(eyes)
7	Needed surgery	10(eyes)

But,It was reported that 80.5% had normal intra ocular pressure even after withdrawing steroids.[28]

ORAL ROUTE:

- It causes HPA axis suppression.
- Oral prednisolone 2 mg /kg / day for 5 days causes behavourial changes(aggression and anxiety)
- It is effective in acute asthma, Polyarticular gout,Ulcerative colitis.[29-31]

PARENTRAL ROUTE:

It is used in early treatment of lateral epicondylitis and medial epicondylitis,De quervian's tenosynovitis, polyarticular gout,adhesive capsulitis,osteoarthritis, ulcerative colitis.

ADR: Subcutaneous necrosis with local skin atophy, post injection pain,local discomfort, flare, bruising, superifical radial nerve neuropraxia and local subcutaneous fat atrophy[32-34]

CONCLUSION:

Steroids are available in different dosage form to explore its potency differently and always there is questions to answer all steroid preparations are same to produce its action and side effects. This review will help us to understand the safety and toxicity profiles of steroids in different route of administration. Selection and use of steroids is like handling double edged sword in a single hand ie., the indication will become a side effect when it is used for a long duration or with wrong therapeutic justification. The practitioner should have a thorough knowledge about the uses and toxicity profile of different steroids in different route before initiating or terminating it.

Reference :

[1] Rosen J, Miner JN. The search for safer glucocorticoid receptor ligands. Endocrine Reviews Vol 26, Number 3, May 2005.
[2] Glucocorticoid use and abuse in SLE. Ruiz-Irastorza G1, Danza A, Khamashta M.
[3] Benefits and risks of low-dose glucocorticoid treatment in the patient with rheumatoid arthritis.Kavanaugh A1, Wells AF2.
[4] Wei L, MacDonald TM, Walker BR. Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease. Ann Intern Med 2004;141(10):764-70.
[5] Gurwitz JH, Bohn RL, Glynn RJ, et al. Glucocorticoids and the risk for initiation of hypoglycemic therapy. Arch Intern Med 1994;154(1):97-101.
[6] McMahon M, Gerich J, Rizza, R. Effects of glucocorticoids on carbohydrate metabolism. Diabetes Metab Rev. 1988; 4(1):17-30.
[7] Tappy L, Randin D, Vollenweider P, et al. Mechanisms of dexamethasone-induced insulin resistance in healthy humans. J Clin Endocrinol Metab. 1994;79(4):1063-9.
[8] Van Staa TP, Leufkens HG, Abenhaim L, et al. Use of oral corticosteroids and risk of fractures. J Bone Miner Res. 2000; 15(6):993-1000.
[9] Van Staa TP, Leufkens HG, Cooper C. Use of inhaled corticosteroids and risk of fractures. J Bone Miner Res;2001:16(3):581-8.
[10] Kanis JA, Johansson H, Oden A, et al. A meta-analysis of prior corticosteroid use and fracture risk. J Bone Miner Res 2004;19(6):893-9.
[11] Jeffcoate WJ, Silverstone JT, Edwards CR, et al. Psychiatric manifestations of Cushing's Syndrome: response to lowering of plasma cortisol. Q J Med 1979;48(191):465-72.
[12] Cohen SI. Cushing's Syndrome: a psychiatric study of 29 patients. Br J Psychiatry 1980;136:120-4.
[13] Keenan PA, Jocobson MW, Soleymani RM, et al. The effect on memory of chronic prednisone treatment in patients with systemic disease. Neurology 1996;47(6):1396-402.
[14] Lofberg R, Danielsson A, Suhr O, et al. Oral budesonide versus prednisolone in patients with active extensive and left- sided ulcerative colitis. Gastroenterology 1996;110(6): 1713-8.
[15] Imperiale TF, McCullough AJ. Do corticosteroids reduce mortality from alcoholic hepatitis? A meta-analysis of the randomised trials. Ann Intern Med 1990;113(4):299-307.
[16] Christensen E, Gluud C. Glucocorticoids are ineffective in alcoholic hepatitis: A meta-analysis adjusting for confounding variables. Gut 1995;37(1):113-18.
[17] Mathurin P, Mendenhall CL, Carithers RL Jr, et al. Corticosteroids improve short-term survival in patients with severe alcoholic hepatitis (AH): individual data analysis of the last three randomized placebo controlled double blind trials of corticosteroids in severe AH. J Hepatol 2002;36(4): 480-7.
[18] Madhotra R, Gilmore IT. Recent developments in the treatment of alcoholic hepatitis. QJM 2003;96(6);391-400.
[19] Lam KC, Lai CL, Trepo C, et al. Deleterious effect of prednisolone in HBsAg – positive chronic active hepatitis. N Engl J Med 1981;304(7):380-6.
[20] Gregory PB, Knauer CM, Kempson RL, et al. Steroid therapy in severe viral hepatitis. N Engl J Med 1976;294(13): 681-7.
[21] Lee SD, Tong MJ, Wu JC, et al. A randomized double-blind placebo-controlled trial of prednisolone therapy in HBeAg and HBV DNA positive Chinese patients with chronic active hepatitis B. J Hepatol 1991;12(2):246-50.
[22] Weller IV, Bassendine MF, Murray AK, et al. Effects of prednisolone/azathioprine in chronic hepatitis B viral infection. Gut 1982;23(8):650-5.
[23] Misuse of topical corticosteroids: A clinical study of adverse effects Vivek Kumar Dey.
[24] Kim KT, Rabinovitch N, Uryniak T, et al. Effect of budesonide aqueous nasal spray on hypothalamic-pituitary-adrenal axis function in children with allergic rhinitis. Ann Allergy Asthma & Immunol 2004;93(1):61-7.
[25] Allen DB, Meltzer EO, Lemanske RF Jr, et al. No growth suppression in children treated with the maximum recommended dose of fluticasone propionate aqueous nasal spray for one year. Allergy Asthma Proc 2002;23(6):407-13.
[26] Schenkel EJ, Skoner DP, Bronsky EA, et al. Absence of growth retardation in children with perennial allergic rhinitis after one year of treatment with mometasone furoate aqueous nasal spray. Pediatrics 2000;105(2):E22.
[27] Systemic side effects of inhaled corticosteroids in patients with asthma. Dahl R1.
[28] Outcome of Ocular Steroid Hypertensive Response in Children. Kaur S1, Dhiman I, Kaushik S, Raj S, Pandav SS.
[29] Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. Br Med J 1955;(4947):1041-8.
[30] Truelove SC, Jewell DP. Intensive intravenous regimen for severe attacks of ulcerative colitis. Lancet 1974;1(7866): 1067-70.
[31] Lennard-Jones JE, Longmore AJ, Newell AC, et al. An assessment of prednisone, salazopyrin, and topical hydrocortisone hemisuccinate used as outpatient treatment for ulcerative colitis. Gut 1960;1:217-22.
[32] Kirkup ME, Birchall NM, Weinberg EG, et al. Acute and maintenance treatment of atopic dermatitis in children - two comparative studies with fluticasone propionate (0.05%) cream. J Dermatolog Treat 2003;14(3):141-8
[33] Anderson BC et al. Treatment of De Quervain's tenosynovitis with corticosteroids: A prospective study of the response to local injection. Arthritis and Rheumatism. 1991;34:793-8.
[34] McKenzie JMM. Conservative Management of de Quervain's Disease. BMJ 1972;4:659-660.

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Original Article

Drug prescription pattern of outpatients in a tertiary care teaching hospital in Tamil Nadu

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Abstract

Purpose: Drug use prescribing indicators advocated by the World Health Organization (WHO) are important tools for assessing the degree of polypharmacy, use of generic medicines, and to evaluate if there is inappropriate use of antibiotics or parenteral medications besides estimating the adherence to the essential drugs list. This study aimed to assess the WHO prescribing indicators in prescriptions given at the medical outpatient department (OPD) in a private medical college hospital in South India.

Materials and Methods: The study was done prospectively from patients when they presented for consultation at the medical OPD at our tertiary care center. Prescriptions were randomly chosen to be analyzed for the WHO prescribing indicators from September 2016 to April 2017.

Results: A total of 700 prescriptions were analyzed and the average number of drugs per prescription was 2.955 ± 1.32. 32.57% of prescriptions had fixed drug combinations and a similar value of 36% was obtained for prescriptions containing more than one drug for the same indication. Amongst the prescribing indicators, generic prescribing was appallingly low (6.42%). In contrast, antibiotic prescribing and prescription of injections showed an appreciably rational trend with 15.42% and 8.14%, respectively. Furthermore, the prescription of the drugs enlisted in the essential drugs list was determined to be 90.67%.

Discussion: The need for increase in generic prescribing and augmenting the adherence of prescriptions to the essential drugs list has been identified. This can be accomplished by multimodal approach that includes regulatory changes, conducting educational programs directed at attitudinal change among current doctors and imparting modifications in medical curriculum so as to inculcate the culture of abiding by the best prescription practices among budding doctors.

Conclusion: This study has delineated the requisite for pertinent changes in current prescribing trends in a tertiary care teaching private colleges.

Keywords: Generic drugs, polypharmacy, prescribing indicators, rational prescribing

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INTRODUCTION

Evaluation of drug prescription pattern is an important aspect of patient care, which also serves as a measure of the quality of care provided. A recent systematic analysis has ascertained that prescribing quality is a dimension requiring constant evaluation.[1] The rational use of drugs is imperative for an effective and efficient health-care system. However, irrational drug use, considered as a global menace

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is especially prevalent in the developing countries due to irrational prescribing, dispensing, and administration of medications.[2] Such irrational prescribing is unethical and greatly decrease the standard of drug therapy in addition to resulting in widespread health hazards such as increased incidence of adverse effects, drug interactions, and emergence of drug resistance, especially with antimicrobial therapy.[3] The World Health Organization (WHO) has reported that more than half of all medicines are prescribed, dispensed, or sold inappropriately.[4]

Prescription pattern monitoring studies are tools for assessing the prescribing, dispensing, and distribution of medicines prevailing in a particular locale. The main aim of such studies is to facilitate rational use of medicines.[5] The drug use indicators have been developed by the WHO in coordination with international network for rational use of drugs as an endeavor to measure the extent of rational prescribing.[5,6] According to the WHO, core drug use indicators are divided into three categories, namely, the prescribing indicators, patient care indicators, and the quality of care indicators. These are highly standardized indicators which do not need national adaptation. Although they do not measure all aspects of drug utilization which require intensive methodologies, extensive and varied sources of data, the core drug use indicators provide a simple tool for quickly and reliably assessing a few critical aspects of pharmaceuticals use in health care.

The drug use indicators collected in a cross-sectional survey or measured at different points in time to assess the change in performance are typically measured within a defined geographic or administrative area, either to describe drug use at a given point in time or to monitor changes over time. This study was designed to assess the drug prescribing practices at the medical outpatient department at our tertiary care center which is a teaching medical college hospital, using the five WHO prescribing indicators which include the average number of drugs per patient encounter, percentage of drugs prescribed by generic name, percentage of encounters with an antibiotic prescribed, percentage of encounters with an injection prescribed, and percentage of drugs prescribed from essential drugs list or formulary.[6] These WHO indicators would be instrumental in identifying the degree of polypharmacy, extent of generic prescribing, whether usage of antibiotics and parenteral medications are appropriate besides estimating the adherence to the essential drugs list.[6,7]

Treatment of diseases by the use of essential drugs, prescribed by their generic names, has been emphasized by the WHO and the National Health Policy of India. It has been highlighted that the prescription monitoring studies are imperative to bridge the areas such as rational use of drugs, pharmacovigilance, pharmacoeconomics, pharmacogenetics, and evidence-based medicine.[5] In India, it is currently essential to conduct studies on prescribing patterns in every state and utilize the data generated by such studies to improve the quality of patient care with the primary aim of promoting rational use of drugs.

Appraisal of the WHO prescribing indicators in a private health facility is less common compared to public sector hospitals. Moreover, the level of health care, wherein this study is undertaken adds to its significance. Unlike most Indian studies, the current study is aimed to analyze the entire list of prescribing indicators advocated by the WHO in a teaching medical college hospital. In such a scenario, not only are the prescribers and patients but also the medical students from one of the stakeholders who imbibe the culture of rational prescribing. Thus, this study would throw light on deficiencies which require appropriate and sustained interventions to avoid being carried onto the next generation.

Noteworthy, most studies encompass prescription monitoring of a particular group of drugs such as antiepileptics, antimicrobials, antiasthmatics, and antihypertensive drugs[5] rather than evaluating all the prescribing indicators irrespective of the diagnosis like our study, which would enable capturing a wider picture of the current trends. Medicine is an ever-evolving science and it is important to understand that conducting such prescription monitoring studies periodically as a measure to ensure rational drug use is imperative.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Ethics Committee (Approval number: 16/261), The research was conducted as a cross-sectional study in the outpatient medical department (OPD) of our tertiary care teaching hospital in South India. Based on the WHO guideline of including at least 600 patient encounters, we chose a sample size of 700. This study was done prospectively from the patients when they presented for consultation at the OPD after obtaining a written informed consent. Prescriptions were randomly chosen to be analyzed for the WHO prescribing indicators over 8 months from September 2016 to April 2017. Two well-trained clinical pharmacists were involved in collecting data on prescribing indicators. Each prescription was regarded as single-patient encounter for calculation of the required parameters. Prescriptions of patients attending medical OPD and treated on outpatient

basis for their ailments were included irrespective of the comorbidities. Data were collected on the demographic details of age, gender, diagnosis, and the treatment prescribed which were mentioned in the prescription. The prescribing indicators were studied in accordance to the standard guideline advocated by the WHO.[6]

1. Average number of drugs per encounter: Average was calculated by dividing the total number of different drug products prescribed, by the number of encounters surveyed. Whether the patient actually received the drugs was not considered relevant in calculating this indicator
2. Percentage of drugs prescribed by generic name: percentage was calculated by dividing the number of drugs prescribed by generic name, by the total number of drugs prescribed and expressed as a percentage
3. Percentage of encounters with an antibiotic prescribed: Percentage was calculated by dividing the number of patient encounters during which an antibiotic was prescribed, by the total number of encounters surveyed and expressed as a percentage
4. Percentage of encounters with an injection prescribed: percentage was calculated by dividing the number of patient encounters during which an injection was prescribed, by the total number of encounters surveyed, multiplied by 100
5. Percentage of drugs prescribed from essential drugs list or formulary: percentage was calculated by dividing the number of products prescribed which were on the essential drugs list or local formulary, by the total number of products prescribed and multiplied by 100.

RESULTS

The data were entered in Microsoft Excel 2007 and analyzed as frequency distributions and percentages to assess the prescribing indicators. A total of 700 prescriptions were analyzed over 8 months from September 2016 to April 2017. The demographic distribution of patients mirrored a rising trend with increasing age as a higher proportion of patients (295) were 60 years and above (42.1%). Among the rest, those aged 40 years and above were 36.7% (257) and 21.2% (148) were <40 years. Both males and females were almost equal in proportion with 357 males and 343 females. Diagnosis were multiple and diverse. Hence, categorization into communicable and non-communicable diseases showed that majority (68.6%) had non-communicable diseases contrary to those with communicable diseases (31.4%). It was found that a total number of 2069 drug products had been prescribed in the 700 patient encounters, and thus, the average number of drugs per prescription was 2.955 and the standard deviation was 1.32. Moreover, the median number of drugs per prescription was 3, which unlike the mean, would not be unduly influenced by the outliers. Overall, the study revealed a higher value for this indicator than the reference standard [Table 1]. Analyzing the patient encounters lacking the data for specified variables, it was found, 647 (92.42%) prescriptions did not have any generic names, whereas only 17 patient encounters lacked at least one drug from essential drugs list. It was evident that 84.57% prescriptions did not have any antibiotics and likewise, a high percentage of 91.85% did not have any injections prescribed.

It was interesting to find that among the 2069 drug products prescribed, the highest percentage (10.95%) of prescribed drugs were supplements which ranked first followed by the antidiabetic, antiplatelet, hypolipidemic, and anti-hypertensive drugs, whereas the overall percentage of antibiotics prescribed was only 15.42%, and hence lower in comparison to the former groups of drugs [Table 2].

The number of prescriptions with fixed drug combinations was 228 accounting for 32.57% [Figure 1]. The most common one being prescribed was analgesic combination (paracetamol with ibuprofen followed by tramadol with paracetamol) for pain relief. A similar value of 36% (252 out of 700) was obtained for prescriptions containing more than one drug for the same indication. Interestingly, prescriptions analyzed showed that patient encounters with two drugs (27.73%) were equal in proportion to those that included three drugs (27.28%) accounting for a total of 55% prescriptions falling under either of these two categories. An analogous high percentage (23.98%) was also recorded for prescriptions with four drugs in the treatment regimen [Figure 2].

With respect to generic prescribing, this study recorded a very low percentage of 6.42% indicating that current prescribing pattern of clinicians favored prescribing by

Table 1: Comparison of the World Health Organization prescribing indicators observed with standard reference range

Indicator	*n*	Average/percentage (SD)	Standard reference range/optimal value
Average number of drugs per patient encounter	2069	2.955 (1.32)	1.6-1.8
Percentage drugs prescribed by generic names	133	6.42% (0.34)	100%
Percentage patient encounters with an antibiotic	108	15.42% (0.38)	20.0%-26.8%
Percentage patient encounters with an injection	57	8.14% (0.27)	13.4%-24.1%
Percentage drugs from essential drugs list	1876	90.67% (1.31)	100%

SD=Standard deviation

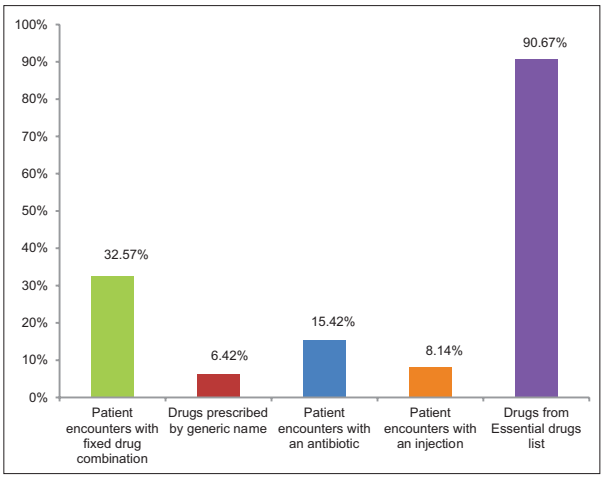


Figure 1: Measured percentages of prescribed fixed-drug combinations and the World Health Organization prescribing indicators

Table 2: Frequency distributions of commonly prescribed drugs

Name of the drug groups commonly prescribed	Name of drugs commonly prescribed	Sum (%)
Antidiabetic drugs	Metformin	161 (7.78)
Antiplatelet drugs	Aspirin	52 (2.51)
NSAIDS	Paracetamol	86 (4.15)
Supplements	Calcium and Vitamin D3	60 (2.89)
	Iron and folic acid	112 (5.41)
	Multivitamins and folic acid	55 (2.65)
Antiulcer drugs	Pantoprazole	99 (4.78)
	Ranitidine	73 (3.52)
Antihypertensive drugs	Amlodipine	36 (1.73)
	Telmisartan	21 (1.01)
Antibiotics	Levofloxacin	10 (0.48)
	Trimethoprim + sulfamethoxazole	8 (0.38)
	Azithromycin	7 (0.33)
	Amoxicillin + clavulanate	7 (0.33)
	Cefpodoxime proxetil	6 (0.28)

NSAIDS=Nonsteroidal anti-inflammatory drugs

brand names rather than using the generic ones. The percentage of patient encounters in which an antibiotic was prescribed was only 108 (15.42%) [Figure 1]. The highest prescribed antibiotic was levofloxacin followed by co-trimoxazole with azithromycin, amoxicillin-clavulanate, and cefpodoxime proxetil being next to the quinolone and the sulfomethoxazole-trimethoprim combination which were widely prescribed at our center during the study period [Table 2]. The most common indication for the antibiotic use was found to be a variety of respiratory infections ranging from pharyngitis, sinusitis to pneumonia.

About 8.14% of patient encounters had an injection being prescribed, and an overall 90.67% of adherence to essential drugs list was evident [Figure 1].

DISCUSSION

It is well known that the indicators of prescribing practices measure the performance of health-care providers in several key dimensions related to rational use of drugs. The indicators were assessed in our study based on a sample of 700 patient encounters that took place at the OPD in our hospital. The data that were collected prospectively by analyzing the prescriptions demonstrated that in our teaching hospital, the average number of drugs prescribed per encounter was 2.955. Comparison to the standard range advocated by the WHO for this indicator which estimates the degree of polypharmacy revealed that the measured average was much higher than the reference range of 1.6–1.8 considered as ideal.[6] This is also reflected in the high percentage of fixed drug combinations prescribed in addition to the use of combination of different drugs for a single indication in one patient encounter. In a similar study done in Goa,[8] the average number of drugs prescribed was 1.8 which was found to be lower than our finding and that obtained in other Indian studies on prescribing indicators by Upadhyay *et al.* (3.76) and Raj *et al.* (4.98).[9,10] In contrast, similar studies of drug use pattern in other countries such as Sudan (1.4), Zimbabwe (1.3), and Ethiopia (1.9)[11] have shown that the average number of drugs per encounter estimated in our study was higher.

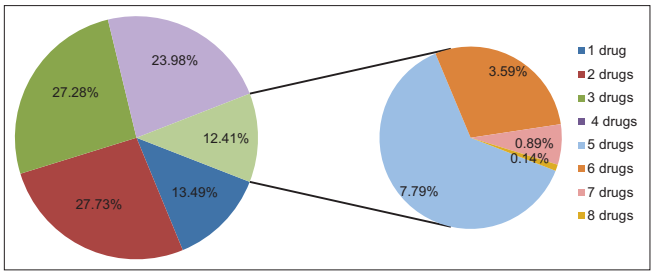


Figure 2: Percentage distribution of number of drugs in the prescriptions

The high average number of drug products per prescription exceeding the WHO reference range is demonstrative of a high degree of polypharmacy prevalent in our study locale. The reasons that could be attributed to this is the change in the epidemiological trend of rampantly increased prevalence of non-communicable diseases such as diabetes, hypertension, dyslipidemia, and coronary artery disease which are often coexistent contributing to the need for treating multiple disease entities in the same patient simultaneously.[12] Concomitance of such cardiometabolic conditions in a single patient further entails the requisite for prescribing more than a single drug for a given clinical indication which is often resistant to treatment in the face of multiple diseases presenting concurrently. Our study also stands testimony to this fact since a high proportion of participants (68.6%) had the diagnosis of non-communicable disease with diabetes ranking highest with 34.4%

Another rationalization for the high percentage of polypharmacy lies in the fact that currently there is a demographic shift with an increase in the aging population in our country.[13] This epidemiological transition is further responsible for perpetuating the current scenario prevailing. About 42.1% of the study population also was aged >60 years signifying that the greater proportion of aged population contributed to the high polypharmacy.

However, the high prescription rates for supplements recorded in our study remain unjustified and arise from two important factors potentially contributing to the irrational prescribing practice. First, the influence of pharmaceutical industries which vigorously promote supplementary products among doctors by providing biased information supporting the need for prescribing such products. Second, patients misconceptions on the health-promoting effects of supplements resulting in their persuasion and demand for such products in their prescriptions. However, the best approach toward such patients is to educate them rather than giving in to their false ideations.[3]

The percentage of drugs prescribed by generic name in our study was 6.42% which is too low compared with the standard derived to serve as ideal, which is 100%.[6] Wang *et al*. found that higher the doctor's education and training experience, the proportion of drugs that they prescribed by generic names showed a decrease.[14] Moreover, attitudinal differences have also been shown to exist among consultants in low- and middle-income countries compared to those in high-income countries.[15]

The likely explanation for the low percentage of generic prescribing could be due to repeated and persuasive promotion of the propriety products by pharmaceutical companies and in certain instances, clinicians are compelled to concede to the insistence of affluent patients demanding innovator drugs for treatment.[16] Another plausible cause for this stems from the presumed belief among a subset of prescribing physicians that the bioavailability differences between generic and brand drugs could adversely affect the therapeutic outcomes.[17] Such prejudices could unfavorably affect the tendency of prescribing generic drugs. Finally, the role played by industries in hindering generic prescribing by offering financial perks to prescribers cannot be underestimated.[18] Literature evidence states that generic prescribing is better in public centers in comparison to that in private sector hospitals.[16]

It is important to increase awareness on generic prescribing considering the high treatment costs incurred by the practice of prescribing by brand names. Nicolosi and Gray investigated the cost impact of generic and proprietary prescribing among chronic disease patients in South Africa and their findings indicated that "all generics were more than 40% cheaper, per defined daily dose per month, than the brand version."[19] An analysis of facility-based medicines price data from 17 countries by Cameron and Laing,[20] found that an average of 9%–89% could be saved by switching from brand to generic equivalents. Shift in the trend favoring generic prescribing is warranted and this can be implemented by an integrated approach of training the medical students who are future prescribers on the pharmacoeconomic significance of this practice in addition to conducting continuing medical education programs for clinicians with the focus of alleviation of their apprehension on bioequivalence regarding the use of generic drugs. Moreover, a variety of strategies have been recommended by experts to overcome the barriers to genetic prescribing and the most vital of these include enforcing statutory obligations, setting clear guidelines for generic prescribing, and legally de-incentivizing prescribing by propriety name.[21]

Interestingly, we found that the number of antibiotics prescribed (15.42%) as well as the number of encounters with an injection (8.14%) was low, indicating a positive trend toward reduction in indiscriminate use of antibiotics and unnecessary injections. An obviously rational prescribing pattern is noted for both these indicators in our tertiary care center. Noteworthy, our study duration was 8 months, and hence, the study would not have comprehensively captured the seasonal variation in antibiotic prescription pattern due to differential incidence rates of infections at different times of the year.

About 90.67% of drugs prescribed conform to the WHO essential drugs list which is lower than that obtained in other studies in India (99.6%), Tanzania (96%), and South Ethiopia (96.6%), whereas higher in comparison to that in countries like Nepal (88%). This could be attributable to the lack of sensitization of the prescribers and lack of rules enforced to mandate prescribing from the essential drugs list. Akin to generic prescribing, this indicator also has been demonstrated to vary between the private and public sectors[22] indicating the need for the practice of prescribing from essential drugs list to be more widely adopted especially in private sector hospitals.

One of the strengths of the study is that it was done prospectively using the patient prescriptions attending the OPD rather than the data being collected retrospectively from case records which is often the design adopted in prescription pattern studies as it is much easier to

collect information from patient records. In addition, the prospective design of this study positively ensures eliminating duplication of data, while retrospective analyses cannot be claimed to be completely devoid of bias such as exclusion of incomplete data from poor record keeping unlike prospective evaluation.

A major limitation of our study is that it was done in a single center which is a private medical college hospital with limited sample size, and hence, the data cannot be extrapolated to other centers lacking similar characteristics like public or government hospitals and health-care centers.

This study implies the need for implementing interventions such as programs, workshops, and symposia to impart awareness on rational prescribing among the medical fraternity. Thus this research has not only identified the pitfalls in current prescription practices, but in addition, has recommended the potential solutions to weed out irrational prescribing trends encountered.

CONCLUSION

Our study on prescribing indicators has clearly delineated that the prescribing practices for antibiotics and injections are appropriate and rational; conformity to essential drugs list is considerably good, though scope for improvising is certainly evident. However, the degree of polypharmacy is higher than the standard. Generic prescribing is also an element that needs to be vastly improved on. Therefore, appropriate measures to reduce polypharmacy and increase generic prescribing by clinicians have to be implemented by the administrative team and policymakers to ensure rational and safe prescribing.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Song J, Zhang L, Li Y, Zeng L, Hu D, Liang Y, *et al*. Indicators for assessing quality of drug use: A systematic literature review. J Evid Based Med 2017;10:222-32.
2. Enato EF, Chima IE. Evaluation of drug utilization patterns and patient care practices. West Afr J Pharm 2011;22:36-41.
3. Garg M, Vishwakarma P, Sharma M, Nehra R, Saxena KK. The impact of irrational practices: A wake up call. J Pharmacol Pharmacother 2014;5:245-7.
4. World Health Organization Medicine Strategy, 2008-2013. Geneva: WHO; 2008.
5. Jain S, Upadhyaya P, Goyal J, Kumar A, Jain P, Seth V, *et al*. A systematic review of prescription pattern monitoring studies and their effectiveness in promoting rational use of medicines. Perspect Clin Res 2015;6:86-90.
6. World Health Organization. How to Investigate Drug use in Health Facilities: Selected Drug use Indicators – EDM Research Series No. 007. WHO/DAP/93.1. Geneva: World Health Organization; 1993.
7. Hogerzeil HV, Bimo, Ross-Degnan D, Laing RO, Ofori-Adjei D, Santoso B, *et al*. Field tests for rational drug use in twelve developing countries. Lancet 1993;342:1408-10.
8. Chandelkar UK, Rataboli PV. A study of drug prescribing pattern using WHO prescribing indicators in the state of Goa, India. Int J Basic Clin Pharmacol 2014;3:1057-61.
9. Upadhyay DK, Palaian S, Ravi Shankar P, Mishara P, Sah AK. Prescribing pattern in diabetic outpatients in a tertiary care teaching hospital in Nepal. J Clin Diagn Res 2007;1:248-55.
10. Raj K, Kamlesh K, Kajal HL. A study of drug prescribing pattern and cost analysis among diabetic patients in a tertiary care teaching institute in North India. J Drug Deliv Ther 2013;3:56-61.
11. Desalegn AA. Assessment of drug use pattern using WHO prescribing indicators at Hawassa University Teaching and Referral Hospital, South Ethiopia: A cross-sectional study. BMC Health Serv Res 2013;13:170.
12. Mohan V, Venkatraman JV, Pradeepa R. Epidemiology of cardiovascular disease in type 2 diabetes: The Indian scenario. J Diabetes Sci Technol 2010;4:158-70.
13. Marasinghe KM, Lapitan JM, Ross A. Assistive technologies for ageing populations in six low-income and middle-income countries: A systematic review. BMJ Innov 2015;1:182-95.
14. Wang H, Li N, Zhu H, Xu S, Lu H, Feng Z, *et al*. Prescription pattern and its influencing factors in Chinese county hospitals: A retrospective cross-sectional study. PLoS One 2013;8:e63225.
15. Hassali MA, Wong ZY, Alrasheedy AA, Saleem F, Mohamad Yahaya AH, Aljadhey H, *et al*. Perspectives of physicians practicing in low and middle income countries towards generic medicines: A narrative review. Health Policy 2014;117:297-310.
16. Ofori-Asenso R, Brhlikova P, Pollock AM. Prescribing indicators at primary health care centers within the WHO African region: A systematic analysis (1995-2015). BMC Public Health 2016;16:724.
17. Kesselheim AS, Eddings W, Raj T, Campbell EG, Franklin JM, Ross KM, *et al*. Physicians' trust in the FDA's use of product-specific pathways for generic drug approval. PLoS One 2016;11:e0163339.
18. Qian J, Hansen RA, Surry D, Howard J, Kiptanui Z, Harris I, *et al*. Disclosure of industry payments to prescribers: Industry payments might be a factor impacting generic drug prescribing. Pharmacoepidemiol Drug Saf 2017;26:819-26.
19. Nicolosi E, Gray A. Potential cost savings from generic medicines-protecting the prescribed minimum benefits. S Afr Fam Pract 2009;51:60-3.
20. Cameron A, Laing R. Cost Savings of Switching Private Sector Consumption from Originator Brand Medicines to Generic Equivalents; World Health Report (2010) Background Paper, 35. Geneva: World Health Organization; 2010.
21. Drozdowska A, Hermanowski T. Solution-driven approaches to generic substitution challenges – A survey among international experts. Fundam Clin Pharmacol 2015;29:592-603.
22. Mohlala G, Peltzer K, Phaswana-Mafuya N, Ramlagan S. Drug prescription habits in public and private health facilities in 2 provinces in South Africa. East Mediterr Health J 2010;16:324-8.

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Original article

A study on the effectiveness of teaching the principles and methods of clinical audit to medical postgraduates

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ABSTRACT

Background: Clinical audit is based on the concept of quality assurance in health care. It is important for all health care providers especially specialists and consultants to be aware of the principles governing clinical audit in addition to the methods in conducting such an audit.
Objectives:
- To assess the knowledge and attitude of medical postgraduate students on clinical audit.
- To evaluate the effectiveness of an interventional program conducted to educate the principles and methods of conducting clinical audit to medical postgraduates.

Methods: The study was done in 92 postgraduate medical students in our institute using a pre-test and post-test questionnaire, evaluating their knowledge on different perspectives of medical audit and also assessing the change in their attitude towards audit after an educative interventional program. The questionnaire consisted of 13 knowledge questions and 3 attitude questions. Each question was scored and the overall maximum score was 35.
Results: The mean post-test score (24.45 ± 3.21) was significantly higher than the mean pre-test score (16.18 ± 3.41) using paired *t*-test ($p < 0.001$) indicating that the educational intervention was effective in improving the understanding of the candidates on clinical audit.
Conclusion: It is imperative for all medical postgraduates to realize the importance of clinical audit in addition to acquiring the skill of conducting a meaningful and successful clinical audit. Attainment of such a competency can be achieved through planned repeated interventions and help usher in an era of best quality of patient care.
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1. Introduction

It is important for all clinicians to realize the growing importance of the clinical audits. A clinical audit is considered as an integral part of the clinical practice. It is a quality improvement process that seeks to improve patient care and outcomes.[1,2] Evaluation of patient outcomes in the field of medicine have been conducted since as early as 1853.[3] The aim of the clinical audit process is to ensure adequate quality of care in the management of patients. With the current day and age of advanced medical care, it is crucial to review and constantly improve the standard of care provided. This requires sustained efforts in the unrelenting strive towards providing the best possible quality of care to patients.

The "Quality Initiative" considered forerunner of clinical audit, was launched by the Royal College of General Practitioners in 1983 to define specific objectives for patient care and monitor the extent to which those objectives were met.[4] Later, the National Institute for health and Clinical Excellence (NICE) published the paper "Principles for Best Practice in Clinical Audit", which defined clinical audit as "a quality improvement process that seeks to improve patient care and outcomes through systematic review of care against explicit criteria and the implementation of change".[2]

Hence it is necessary to understand and be familiar with the underlying principles and methods in conducting a clinical audit. This is especially important from the perspective of the medical

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post-graduate who is not only the future specialist and consultant but an important stakeholder in ascertaining whether quality care is offered to all patients in a particular health care unit. It is therefore essential for all medical postgraduates in various disciplines to have first-hand knowledge of clinical audit. It is also being increasingly recognized by the academic sectors that postgraduates should be trained to conduct clinical audit conceptualizing it as an essential component of evidence based medicine.[5,6]

Accordingly, our study was designed to assess the knowledge of postgraduate students on the principles and methods of clinical audit in our tertiary care teaching hospital and also to evaluate the effectiveness of an interventional training program in medical audit which was conducted specifically with the objective of sensitizing and imparting knowledge on how to conduct a clinical audit.

2. Materials and methods

The study protocol was approved by the institutional human ethics committee before the start of the study (Approval Number: 13/289). The study was done prospectively in a tertiary care teaching medical college hospital using a pre-test and post-test questionnaire that was validated by a team of three experts from the hospital clinical audit committee and institutional quality control committee. After obtaining a written informed consent, the questionnaire was administered to the postgraduate students at the beginning and at the end of an educational program on how to conduct clinical audit by audit experts. The questions framed evaluated the knowledge and attitude of medical postgraduates about clinical audit before and after the program. The data was collected from the participants of two consecutive programs conducted in two successive years. Each participant attended only one of the two consecutive sessions. The questionnaire consisted of 16 questions out of which 13 were knowledge and three were attitude questions.

2.1. Statistical analysis

The questions were scored and statistical difference in the mean pre-test and post-test score was analyzed using paired *t*-test. The results of the survey was also analyzed qualitatively and expressed as frequency distribution of percentages.

3. Results

A total of 92 postgraduates participated in the study. The study which was done at a single centre in two consecutive years involving two different batches of 2nd year postgraduates from various specialties, 39 students participated in the first year and the remaining 43 in the next successive session in the following year.

The first question on the need for an audit evoked diverse array of responses both in pre and post-test. The four options which were included were (1) audit has to be an integral part of clinical practice, (2) done to evaluate adverse patient outcomes, (3) as academic program in medical education and (4) administrative evaluation of patient care. Though all the four choices may be apparently appropriate, doing an audit as a mere administrative evaluation is unjustified as the objective of any clinical audit is betterment of patient care, based on which, the first three options are considered the correct responses. At the end of the session, it was observed that about 60% had clearly excluded administrative evaluation though only 20% of them had acquired complete knowledge on the need for clinical audits in contrast to only 8% who were aware of this before the program as made obvious by pre-test analysis. It was apparent that the term 'audit' had been deeply entrenched with the belief of being a tool for administrative evaluation, as made evident from a statistical comparison of pre-test and post-test scores ($p = 0.904$) (Table 1).

Answering the question on types of clinical audit which can be standard based, adverse occurrence screening or critical incident monitoring and peer reviewed, only 50% in the pre-test agreed that all three were indeed the audit types while the post-test revealed that 82.6% accepted "all the above" as the appropriate response.

In assessing the understanding of the students on the types of evaluation, design of the audit and the stages in the audit cycle, it could be demonstrated that there was definitive acquisition of knowledge on these aspects of audit based upon the increase in percentage of the responders opting for the correct answer in the post-test when compared to the pre-test (Figs. 1 and 2).

On the ethics approval perspective, a shift in the responses favoring the need for ethics committee approval before publication of the results (54.3%) rather that at the beginning of the audit (9.8%) was evident in the post-test, contrary to the impression of most students in the pre-test that ethics committee's approval had to be obtained before the start of an audit (59.8%). A considerable increase in the proportion of participants who indicated that there

Table 1
Comparison of pre-test and post-test scores on knowledge questions in clinical audit.

Knowledge/Attitude on clinical audit	Pre-test score Mean ± SD	Post-test score Mean ± SD	Mean difference of the paired sample ± SD	P value
Need for audit	0.47 ± 0.68	0.49 ± 0.68	−0.02 ± 0.85	0.904
Types of audit	2.78 ± 1.48	3.51 ± 1.11	−0.72 ± 1.85	<0.001
Types of evaluation	0.67 ± 0.95	1.37 ± 0.93	−0.69 ± 1.12	<0.001
Design	1.52 ± 0.85	1.96 ± 0.29	−0.43 ± 0.82	<0.001
Steps in audit cycle	0.44 ± 0.83	1.82 ± 0.56	−1.38 ± 1.01	<0.001
Ethical approval process	0.53 ± 0.79	1.42 ± 0.69	−0.89 ± 1.02	<0.001
Who can conduct audit	3.64 ± 0.97	3.87 ± 0.61	−0.22 ± 1.01	0.034
Who can make recommendations	1.28 ± 1.05	1.63 ± 1.06	−0.34 ± 1.50	0.029
Tool for prioritizing topic	0.61 ± 0.92	1.24 ± 0.97	−0.63 ± 1.14	<0.001
Defining aims	0.93 ± 1.00	1.52 ± 0.85	−0.58 ± 1.09	<0.001
Criteria selection	0.54 ± 0.89	1.35 ± 0.94	−0.80 ± 1.15	<0.001
Standard definition	0.76 ± 0.97	1.50 ± 0.87	−0.73 ± 1.35	<0.001
Commonest method involving service users	0.58 ± 0.49	0.78 ± 0.41	−0.20 ± 0.56	0.01
Audit is not research	0.51 ± 0.50	0.91 ± 0.28	−0.40 ± 0.51	<0.001
Professional responsibility	0.71 ± 0.45	0.82 ± 0.38	−0.11 ± 0.50	0.041
Achievement of same performance level as in research indicates lag in quality	0.20 ± 0.39	0.26 ± 0.44	−0.06 ± 0.48	0.203
Overall score	16.18 ± 3.41	24.45 ± 3.21	−8.28 ± 4.02	<0.001

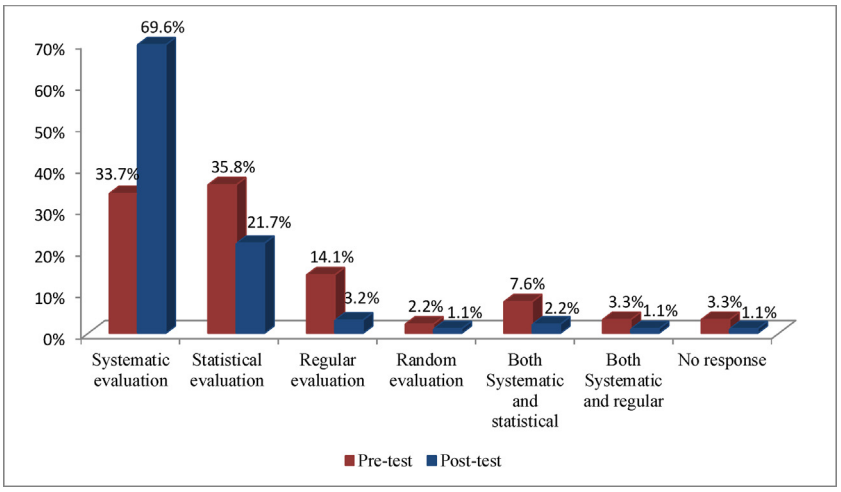


Fig. 1. Percentge of responses to types of evaluation for structure, processes and outcomes of care in clinical audit.

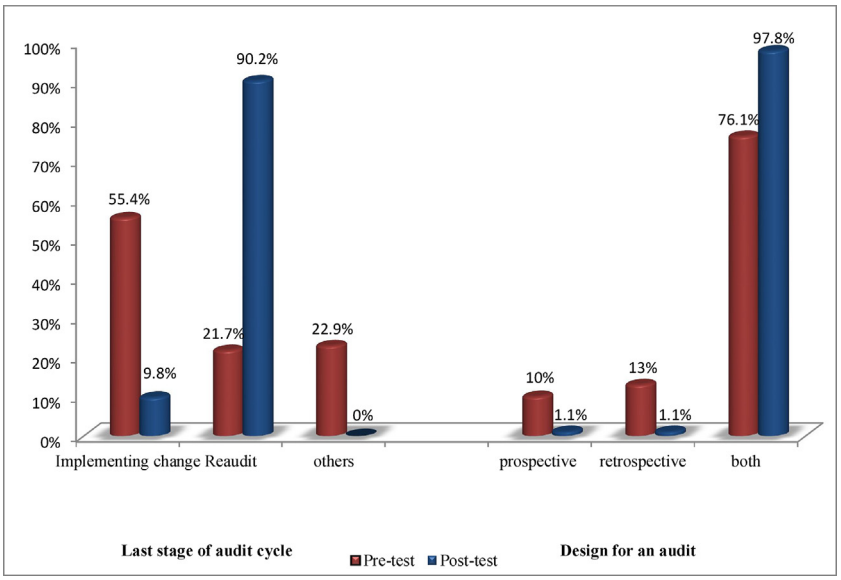


Fig. 2. Percentage of responses to steps in an audit cycle and the design for an audit.

was no need for ethics approval for the conduct of a clinical or therapeutic audit was also noted in the post-test (33.7%).

Appraisal of the postgraduate students' knowledge on who can conduct an audit and who can make recommendations based on an audit result, drafted as two separate questions made it evident that the session had enlightened 97.8% on the fact that any medical professional including an administrator can conduct an audit whilst the question on recommendation which was structured as dual correct response one, namely the PI (principal investigator) and the hospital audit team, only 5.5% responded with completely correct answer in contrast to 72.8% who had chosen only one of the correct responses at the end of the session though it was obvious that there was an increase in the percent of single correct response compared to the pre-test results (59.8%).

Knowledge on the tool for prioritizing the audit topic showed an increase, demonstrated by the rise in percentage of students opting the right response in the post-test (69.6%) compared to the pre-test (30.4%) (Fig. 3). Likewise their understanding on defining the aims, criteria selection and comprehending the standard revealed augmentation, manifested by selection of the correct choice indicating an improved knowledge on these concepts by large majority of participants (Fig. 4).

Attitude questions patterned on yes/no response ascertained that the students considering audit as yet another research was high before the program (40.2%) but they were able to discern the difference between an audit and research subsequent to the intervention (91.30%). In addition, a high proportion of participants were able to appreciate that auditing forms part of their professional accountability at the end of the program (82.6%). However, it was clear that the study subjects had not undoubtedly understood that failure to achieve the same performance level as that in research was often not possible in unselected patient populations in a practical clinical scenario; only 60.9% had chosen the right response in the post-test (Fig. 5).

Evaluating the candidates' knowledge on the commonest method of audit involving patients, 85.9% rightly agreed that it was satisfaction survey in the post-test contrary to only 57.6% who were in favor of it in the pre-test. Each question was scored with maximum total score being 35. A paired *t*-test was done to assess if the difference in mean score for post-test (24.45 ± 3.21) was statistically higher compared to that of the pretest (16.18 ± 3.41)

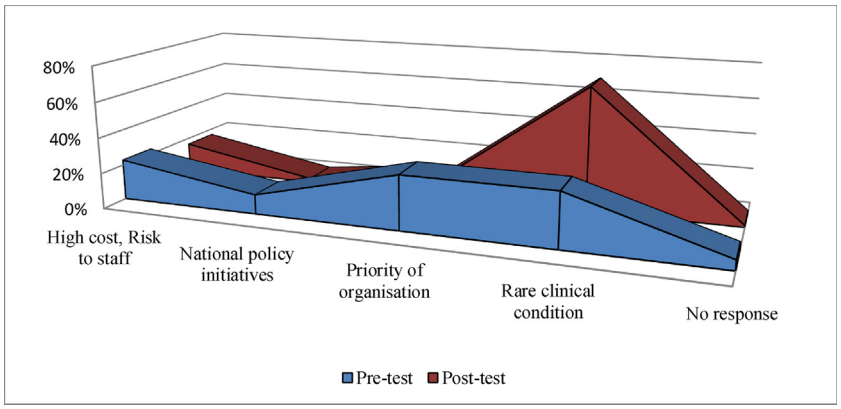


Fig. 3. Frequency distribution of responses to "the tool for prioritizing the topic for audit are all except" expressed in percentages.

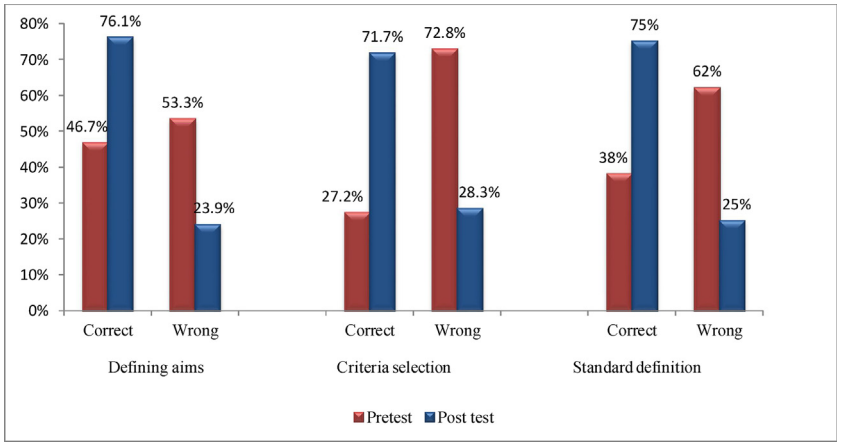


Fig. 4. Frequency distribution of the percentage of correct and wrong responses to the parameters of aims, criteria and standard in an audit.

and it was found that a statistically higher ($p < 0.001$) mean post-test score was achieved (Table 1).

4. Discussion

This study involving 92 postgraduate students in a tertiary care teaching hospital revealed that the knowledge and understanding on the principles and concepts were quite limited as was evident from the pretest analysis. However the planned intervention in the form of an educational session was effective in conveying the basic fundamentals regarding clinical audit to the participants. This was discernible from the statistically greater mean score attained in the post-test compared to the pre-test (Table 1).

In analyzing the individual components, it was demonstrable that elements such as types of audit, types of evaluation, design and stages, ethics committee approval for an audit were well comprehended by the participating candidates following the intervention. Likewise their understanding on the prioritizing tool,

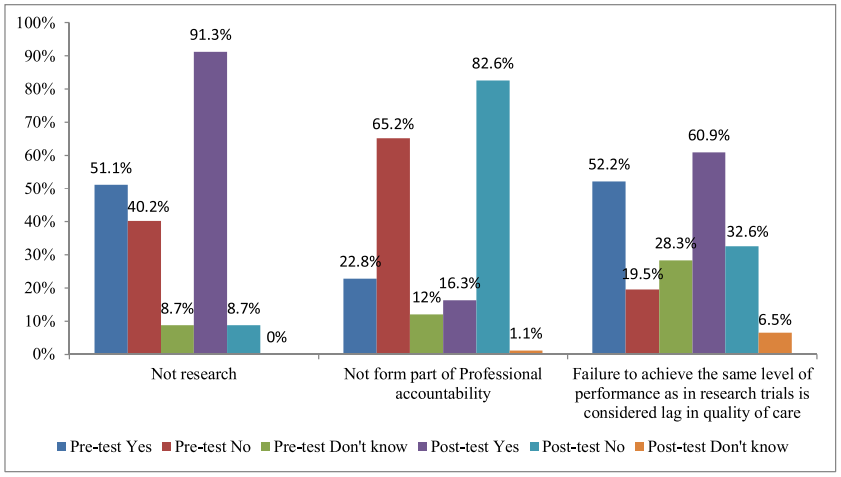


Fig. 5. Percentage distribution of responses to the attitudinal aspects of an audit.

defining aims, criteria selection and the meaning of standard showed statistically significant improvement ($p < 0.001$). A fairly good knowledge was also gained on who can conduct ($p = 0.034$) and who can make recommendations following an audit ($p = 0.029$). The participants were able to appreciate the role played by the PI in making recommendations proved by the shift in the choice of responses from the hospital audit team in the pre-test to the PI in the post-test.

An equal magnitude of improvement was not seen in the understanding on the need for an audit because a considerable majority of participants, despite the program, erroneously believed that the conduct of clinical audit was purely on administrative motive; this was made obvious by the post-test score on this element (Table 1). Also, their attitude towards achieving the same performance level as in research to ensure the best quality of care was rather unaltered (Fig. 5). Such misbelieves and misconceptions seem to be deeply rooted signifying the need for repetitive and sustained measures such as continuing medical education programs, symposia and workshops in clinical audit to enable better understanding as well as need for an attitudinal change towards such clinical audit facets.

Introspecting on the need for such programs targeting the medical postgraduates reveals that this measure not only provides a good learning experience but also would ensure ready involvement of these future consultants in the quality improvement process, as rightly pointed out by Walshe that the outcomes of an audit depend on 'who did it and how it was done'.[7] In addition, enhanced understanding of the audit concepts would pave the way for weeding out the lack of commitment, poor record keeping and inadequate data entries which are the deterrents in the path of quality improvement. Assuming the role of audit lead to facilitating teamwork are the positive attributes that would stem from the in-depth knowledge thus gained. It would not be an exaggeration to say that incorporating the competency of participation in medical audit as a module in postgraduate medical curriculum would be a step forward in implementing evidence based medicine in clinical care, since it is understandable that practicing medicine does not merely stop with updating on current research evidence of best practice but being able to show that such new standards of care is achieved in one's own practice through the clinical audit findings. Thus postgraduate training should encompass clinical audit which is considered as an integral part of clinical governance, for ascertaining the best clinical care to patients.[8]

5. Conclusion

This study has highlighted that educational programs on clinical audit with the objective to impart knowledge and improve understanding of the principles, concepts and methods should be designed and conducted for the postgraduate medical students of diverse specialties for enabling them to acquire the skill of conducting clinical audit which would ensure sustained quality control in patient care.

Authors contributions

The first author was involved in concept, design, data acquisition, interpretation, analysis, drafting and approval of manuscript.

The second author was involved in concept, design, data analysis, drafting and approval of manuscript.

Conflict of interest

The authors have none to declare.

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None.

References

1. Dixon N. *Good Practice in Clinical Audit: A Summary of Selected Literature to Support Criteria for Clinical audit*. National Centre for Clinical Audit; 1996.
2. National Institute for Clinical Excellence (Great Britain). *Principles for Best Practice in Clinical Audit*. Radcliffe Publishing; 2002.
3. Lokuarachchi S. Clinical audit-what is it and how to do it? *Galle Med J*. 2009;11:41–4310.4038/gmj.v11i1.1122.
4. Chen M. Clinical audit. *J Clin Exp Ophthalmol*. 2012;3:1000107.
5. Glasziou P, Burls A, Gilbert R. Evidence based medicine and the medical curriculum. *BMJ (Clini Res Ed)*. 2008;337:704–705.
6. Rhodes M, Ashcroft R, Atun RA, Freeman GK, Jamrozik K. Teaching evidence-based medicine to undergraduate medical students: a course integrating ethics, audit, management and clinical epidemiology. *Med Teach*. 2006;28:313–317.
7. Walshe KM. Principles for best practice in clinical audit. *Qual Saf Health Care*. 2002;11:392.
8. Straus SE, Ball C, Balcombe N, Sheldon J, McAlister FA. Teaching evidence-based medicine skills can change practice in a community hospital. *J Gen Intern Med*. 2005;20:340–343.

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A study comparing cognitive function assessment in type-2 diabetes mellitus using Rowland Universal Dementia Assessment Scale and Mini Mental State Examination

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Abstract:

BACKGROUND: Diabetes mellitus is a metabolic disorder which can predispose to cognitive impairment. It is important to screen diabetic patients for cognitive dysfunction in the routine clinical management. Our study aimed at comparative evaluation of commonly used cognition assessment tools such as Mini Mental State Examination (MMSE) and Rowland Universal Dementia Assessment Scale (RUDAS) in diabetes patients.

METHODS: The study was conducted as a prospective case–control design in a tertiary care teaching hospital from March to October 2015. After obtaining written informed consent, the data on demographic details, duration of diabetes, and glycemic control marked by glycated hemoglobin were recorded. A total of 101 previously diagnosed type-2 diabetes patients and an equal number of age-, gender-, and literacy-matched controls without diabetes were administered the MMSE and RUDAS cognition scales in a one-to-one interview by a trained diabetic educator who was blinded to the groups.

RESULTS: The study revealed that there was a significant difference in cognition scores between the diabetic patients with short duration (<5 years) and those with a longer history of diabetes (≥5 years) only in the MMSE scale using independent sample *t*-test ($P = 0.018$), unlike the RUDAS in which the difference was insignificant ($P = 0.235$). Similarly, there was also a significant correlation between glycemic control and cognition scores in the MMSE ($P = 0.03$), but not in the RUDAS scale ($P = 0.083$).

CONCLUSION: MMSE scale has been proved to be advantageous over the RUDAS as a screening tool for cognition assessment in diabetes patients.

Keywords:

Cognition score, diabetes duration, glycemic control, screening

Introduction

Diabetes mellitus is a metabolic disorder due to dysregulation of glucose homeostasis. The world prevalence of diabetes in adults has been predicted to increase from 285 million in 2010 to 439 million by 2030.[1] It is well established that diabetes is associated with significant morbidity, including neurological disability. Although it is known that diabetes can lead to cognitive deficits in the long term, elucidation of the effects on the central nervous system is still incomplete, unlike other complications such as nephropathy, retinopathy, and peripheral neuropathy. It is estimated that in 2010, 35.6 million people lived with dementia worldwide, with numbers expected to almost double every

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20 years, to 65.7 million by 2030 and 115.4 million by 2050.[2] In addition to this, the scenario of type-2 diabetes predisposing to and accelerating cognitive impairment would further increase the burden of disease. With increasing prevalence of type-2 diabetes in India, the magnitude of cognitive impairment that the disease contributes to and the factors affecting should be studied.

Studies from various populations have consistently shown that diabetes is associated with cognitive deficits and dementia; both in elderly and younger patients.[3] Various pathophysiological mechanisms have been proposed. A process similar to that of peripheral neuropathy such as alterations in Na^+K^+ATPase activity and the ensuing reduction in myoinositol and sorbitol metabolism has been suggested. These may trigger biochemical abnormalities at the neuronal level, resulting in structural and functional changes, primarily in the white matter.[4] Chronic hyperglycemia, atherosclerosis, and hemodynamic changes may lead to microinfarcts which in turn to cognitive impairment. Diabetes-induced microvascular changes, such as disruption of the blood–brain barrier, and the resultant derangement of neurovascular coupling could be partly related to cognitive dysfunction.[5-7] Hyperglycemia may also be directly toxic to the neuron, leading to its degeneration[8] and consequentially the hippocampal as well as global atrophy.[5] Thus, diabetes is considered as one of the important contributory factors in the development of cognitive deficits.

Undiagnosed cognitive impairment can lead to reduced health status,[9] implying that screening for cognitive deficits should be an integral part of patient management in diabetes. Among the neuropsychological tests for dementia, the Mini Mental State Examination (MMSE), devised by Folstein *et al*., is one of the most widely used tools for the assessment of cognitive impairment.[10] The Rowland Universal Dementia Assessment Scale (RUDAS) is yet another screening test, designed as a multicultural cognitive assessment scale relatively unaffected by gender, education, and first language, as it can easily be converted for implementation in the local community.[11] Although there are studies comparing the accuracy and reliability of these two scales in geriatric population with and without dementia,[12-15] a thorough literature review reveals a paucity of studies directly comparing the two in a diabetic population. Hence, our study aimed at evaluating the effectiveness of the two scales as tools in assessing the cognitive decline in type-2 diabetes patients by comparing the correlation of cognitive score with the glycemic control and duration of diabetes.

Methods

This was a single-center, hospital-based case–control study conducted by collecting data from study participants from March to October 2015. Approval from the Institutional Human Ethics Committee was obtained before the start of the study.

Inclusion criteria

All patients willing to give consent, both males and females aged between 30 and 60 years with previously diagnosed type-2 diabetes mellitus (case) and without diabetes mellitus (control), with or without hypertension were included for the study.

Exclusion criteria

Patients with type-1 diabetes, stroke, alcohol intake or smoking habits, total blindness in both eyes, or complete loss of hearing in both ears; and patients who were previously diagnosed with and were known case of mental retardation, psychiatric disorders, and psychoactive drug use in addition to pregnant and lactating women were excluded.

The sample size calculation was done using "Epi tools," with a prevalence of 7% which was based on the previous data, and desired precision of 0.05 at 95% confidence interval revealed the sample size estimate of 101.

After obtaining written informed consent, the data on age, sex, literacy, current fasting and postprandial blood glucose (done by enzymatic reference method with hexokinase), and glycated hemoglobin (HbA1c) values (done by immunoturbidimetry), along with history on duration of diabetes and duration of hypertension were collected. The study was conducted on a sample of 101 diabetic cases and 101 non-diabetic controls matched for age, sex, and literacy to ensure homogeneity of the sample and to avoid bias in the results, owing to these parameters. In this study, patients who exhibited the ability to both read and write the colloquial language and/or English were included as literates.

MMSE and RUDAS scales were administered in a one-to-one interview with the study participant by a trained diabetic educator who was blinded to the group to which the patient belonged to. MMSE tested global cognition function with items assessing orientation, word recall, registration, attention, language abilities, and visuospatial ability and was scored out of 30. As per the MMSE scale, 21–23, 11–20, and ≤10 scores were indicative of mild, moderate, and severe cognitive dysfunction, respectively, while a score of 24 or higher was considered normal.[10] According to the recommendations of Crum *et al*., the MMSE scores were corrected for the literacy status. Based on these criteria, a score <24 was considered as impaired cognition for literates and that <22 for illiterates.[16] RUDAS scale measured memory, gnosis, praxis, visuospatial skills, judgment, and language. The maximum RUDAS score

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was 30, for which the recommended cut point ≤22 was used to indicate cognitive impairment.[11]

Statistical analyses was done using Pearson's correlation test to analyze the correlation between mean MMSE/RUDAS score with parameters such as duration of diabetes and glycemic control. Student's *t*-test was used to identify significant difference in the mean scores between cases and controls based on age, sex, literacy, and comorbidity.

Results

The data were entered into Excel and analyzed using SPSS version 19. Among 101 cases, 4% had mild and 1% had moderate cognitive dysfunction using MMSE, whereas cognition deficit was identified in 5% cases using RUDAS. 1% controls, according to RUDAS score, had cognitive impairment while MMSE scoring revealed that 3% controls had cognitive dysfunction which was mild.

The baseline characteristics of age, sex, and literacy in both groups were matched [Table 1]. There were a higher proportion of females in both the case and the control group, with a male to female ratio of 36:65. Percentage of literates was high (94%) in both groups. The cases were arbitrarily categorized based on the observed HbA1c value as those with good control and poor control. An HbA1c value of 8.5% was taken as the cutoff value; those with <8.5% were included in the good control group and those with HbA1c values ≥8.5% were grouped as poor controllers of glycemic level. 44.6% of cases had good control, while 54.4% were included in the poor control group. The mean score was found to be less in patients who had HbA1c level ≥8.5% using both the scales. An independent sample *t*-test showed that the mean difference between the scores of patients with good and poor control was statistically significant in MMSE scale ($P = 0.046$), while in RUDAS scale, the mean difference was not statistically significant ($P = 0.372$) [Figure 1].

Cases in our study population were divided into those with duration of diabetes <5 years and those with ≥5 years history of diabetes. Accordingly, it was found that 60.4% cases had <5 years of diabetes history and the rest had ≥5 years duration. The mean scores were found to be less in people who had diabetes ≥5 years using both scales and was statistically significant in MMSE scale ($P = 0.018$) but not in RUDAS scale ($P = 0.235$) [Figure 2]. Furthermore, using Pearson's correlation test, a statistically significant negative correlation between duration of diabetes and cognitive levels ($P = 0.015$) in addition to a significant positive correlation between glycemic control and cognitive score ($P = 0.03$) was detected in testing with MMSE scale in the diabetes patients. In contrast, no significant correlation between duration of diabetes or glycemic control with cognitive dysfunction was evident while using RUDAS scale [Table 2].

In both MMSE and RUDAS scales, an independent sample *t*-test revealed that there was no statistically significant difference between the cases and controls in both categories of <50 years of age and those with ≥50 years. The difference in cognition level between the cases and controls in the two gender groups was similarly tested using independent sample *t*-tests, and it was found that there was no statistically significant difference in the mean scores based on gender using either of the scales [Table 3]. Another significant observation was that the mean score of females, in both cases and controls, were lower than that of males, even though the difference was not statistically significant, except in the diabetic group assessed using RUDAS scale, wherein the mean score

Table 1: Baseline characters of study population

Parameters	Mean±SD	
	Cases, 101 (diabetic)	Controls, 101 (non-diabetic)
Age	49.75±7.1	49.57±7.0
Sex (%)		
Male	36 (35.6)	36 (35.6)
Female	65 (64.4)	65 (64.4)
Literacy (%)		
Literate	6 (5.9)	6 (5.9)
Illiterate	95 (94.1)	95 (94.1)
MMSE	28.09±2.3	28.01±2.13
RUDAS	27.80±2.4	28.11±1.65
Duration of diabetes	5.67±5.38	-
HBA1c	9.36±2.34	-

SD = Standard deviation, MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, HBA1c = Hemoglobin A1c

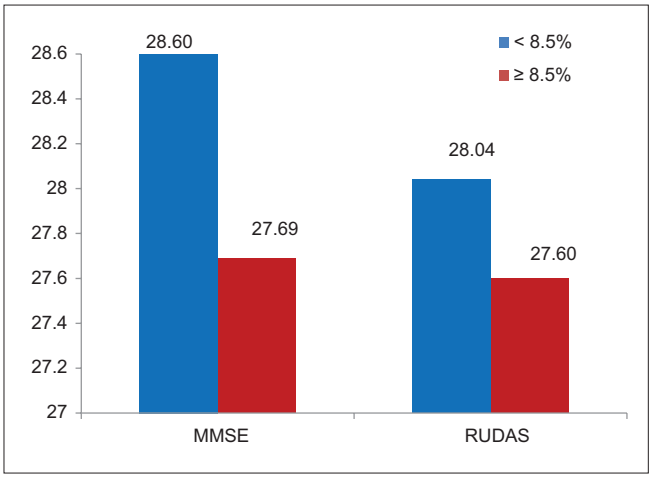


Figure 1: Association between mean cognition score expressed as mean ± standard deviation and glycated hemoglobin (glycemic control) using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

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of females was significantly higher than that of males [Figure 3].

It was found using Student's *t*-test that the illiterates had a mean score lower than that of the literates, though a statistically significant difference was observed only with the MMSE scale (P = 0.001) but not in the RUDAS scale (P = 0.085). Ninety-five percent of the study population were literates, and this precluded performing a between-group analysis for cases and controls [Figure 4].

An independent sample *t*-test revealed that there was no statistically significant difference between the mean scores of cases and controls with hypertension. Similarly, there was no statistically significant difference between the mean scores of those with diabetes alone and those with diabetes and hypertension in the case group using both MMSE and RUDAS [Table 4], indicating that coexisting hypertension did not significantly affect cognition score in patients with type-2 diabetes.

Table 2: Correlation of duration of diabetes and glycemic control with cognitive levels using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

Scale	Parameter	Correlation value	*P*
MMSE	Duration of diabetes	0.240	0.015
	HbA1c	−0.216	0.030
RUDAS	Duration of diabetes	0.163	0.103
	HbA1c	−0.173	0.083

MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, HBA1c = Hemoglobin A1c

Table 3: Independent sample *t*-test for comparing the mean scores of cases and controls across age and gender groups using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

Scale	Parameter	Study population	Mean±SD	*t*	*P*
MMSE	<50 years	Cases	28.23±2.162	0.291	0.772
		Controls	28.10±2.141		
	≥50 years	Cases	27.98±2.483	0.082	0.935
		Controls	27.94±2.138		
RUDAS	<50 years	Cases	27.76±2.496	0.065	0.073
		Controls	28.54±1.530		
	≥50 years	Cases	27.83±2.339	0.187	0.852
		Controls	27.76±1.677		
MMSE	Male	Cases	28.11±2.148	−0.634	0.528
		Controls	28.39±1.517		
	Females	Cases	28.09±2.447	0.653	0.515
		Controls	27.82±2.391		
RUDAS	Male	Cases	27.08±2.970	1.300	0.197
		Controls	28.36±1.533		
	Females	Cases	28.20±1.930	−1.594	0.250
		Controls	27.98±1.709		

MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, SD = Standard deviation

Discussion

The MMSE is a cognitive scale used for detecting cognitive impairment. Having developed in the West, it has serious limitations due to cultural and educational

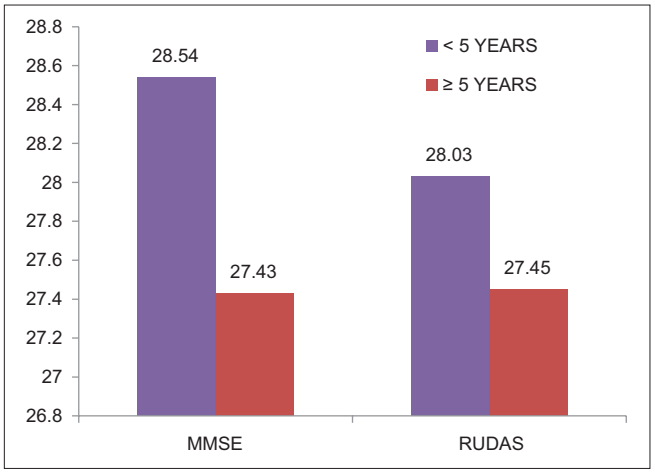


Figure 2: Association between mean cognition score expressed as mean ± standard deviation and duration of diabetes using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

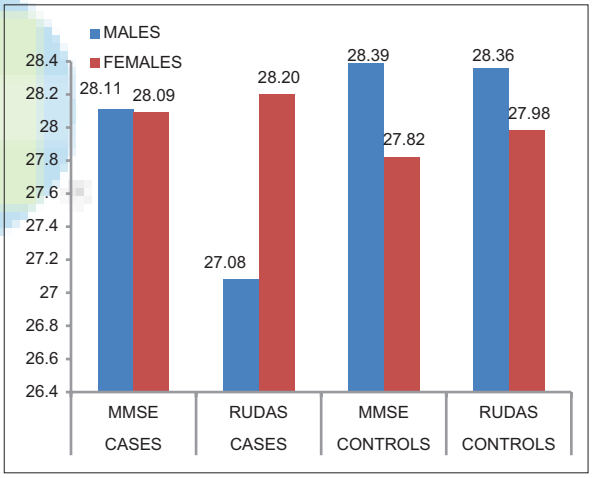


Figure 3: Comparison of mean cognition score expressed as mean ± standard deviation between males and females using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

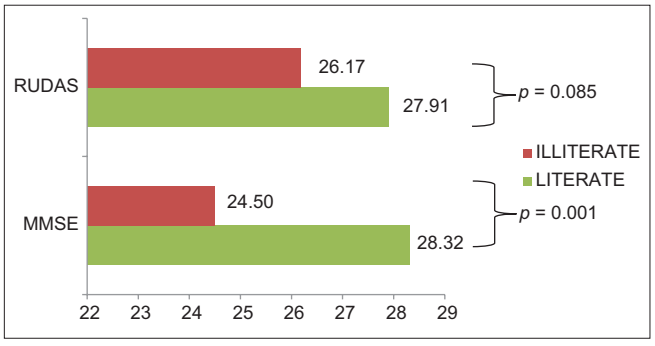


Figure 4: Association between cognition level and literacy using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

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Table 4: Independent sample *t*-test for comparing the difference in mean cognition scores in those with and without hypertension using Mini Mental State Examination and Rowland Universal Dementia Assessment Scale

Scale	Parameter	Mean±SD	*t*	*P*
MMSE	Cases with hypertension	27.81±2.212	−0.676	0.504
	Controls with hypertensive	27.17±1.472		
RUDAS	Cases with hypertension	27.55±2.908	0.091	0.928
	Controls with hypertensive	27.67±3.011		
MMSE	Diabetes with hypertension	27.81±2.212	0.837	0.405
	Diabetes only	28.23±2.391		
RUDAS	Diabetes with hypertension	27.55±2.908	0.705	0.483
	Diabetes only	27.91±2.152		

MMSE = Mini Mental State Examination, RUDAS = Rowland Universal Dementia Assessment Scale, SD = Standard deviation

bias.[16,17] It is one of the commonly used tools for cognitive assessment consisting of 11-item testing with sensitivity of 87% and specificity of 82%.[18] The RUDAS is a newer screening test developed in Australia. It is a brief six-item screening test which is short and easy to administer. It is claimed to be culturally and educationally unbiased and developed in view of application in multicultural setting.[11,12,19] It assesses body orientation, praxis, drawing, judgment, memory, and language. It has the additional advantage of being capable of assessing the impairment in executive function. With a cutoff score of 23/30, it has 88% sensitivity and 90% specificity.[11,18]

It has been well proven that age, in addition to ethnicity and education, is an important factor affecting the MMSE score. In our study, there was no statistically significant difference in the mean score between patients aged above and below 50 years. In a study done using MMSE scale, there was no quantitative difference in cognition scores above and below 70 years of age, but a significant difference between diabetic and non-diabetic patients was detected.[3] Their result indicated that in diabetic patients, diabetes could be a greater risk factor for cognitive impairment than age. However, our study results did not replicate such a significant difference between cases and controls in both the age groups studied [Table 3]. The reason attributable to this inference is that most of the earlier studies were done in a population with higher mean age,[5,20] but our study included a much younger population and that may also account for the lower percentage of cognitive deficits detected. A similar result has been demonstrated in a study with middle-aged diabetic population.[4]

The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men.[21] In our study sample, the proportion of females was much higher than the males. Using MMSE, the males scored higher than females though not statistically significant and this was in concordance with earlier studies.[3] Assessed using RUDAS, the female cases scored significantly higher than male cases, but such an observation was not seen in the control group and the reason for the result could not be explained.

Our study demonstrated that the magnitude of the difference in the mean score between literates and illiterates was significantly higher in the MMSE score as compared to the RUDAS scale, indicating that our study has also proven that RUDAS scale is free of educational barrier as described in the previous studies.[11,12] However, former studies done in Thailand and South Indian Malayalam population showed that cultural, linguistic, and education barriers were not fully overcome with the use of RUDAS scale.[13,15]

The cutoff values for glycemic control and duration of diabetes have been chosen arbitrarily and do not depend on the conventional cutoff adopted internationally for the management of clinical condition. The reason being that the duration of diabetes and the glycemic control beyond which cognitive dysfunction occurs, in comparison to other complications of diabetes, is less defined and less well understood. Hence, using the same clinical cutoff criteria used for management would obviously result in false-negative correlation. In the diabetic population studied, the association of mean cognition score with the glycemic control marked by HbA1c level was found to be statistically significant, in addition to a significant positive correlation which was evident only in the MMSE but not the RUDAS scale. Our study thus reflected the results obtained in few previous studies which have highlighted the positive association between poor glycemic control and poor cognition using MMSE,[22,23] but no such correlation detected using RUDAS.[24] Another study done in middle-aged adults with type-2 diabetes has demonstrated that there is predominantly psychomotor slowing with poor metabolic control, even though learning, memory, and problem-solving skills appear to be largely intact in such patients.[4] On the contrary, few other studies have established that the magnitude of glycemic control does not have significant correlation with the cognitive score using MMSE scale.[3,25,26] The potential explanation for the contradictory results between correlations of cognitive dysfunction with glycemic control is that only the recent HbA1c values representing short-term control were included in most analyses and not the time trend in HbA1c, indicative of long-term control as the measure to categorize patients with good and bad glycemic control.

Evaluating the association between the duration of diabetes and cognitive scores revealed a significant negative correlation. Those with a longer duration (≥5 years) of diabetes exhibited a lower mean score than those with shorter duration of diabetes (<5 years)

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using the MMSE, but not with the RUDAS. Our results were consonant with results obtained in few similar studies.[3,22,25,27] Thus, most studies have proven a definitive correlation between cognitive decline and duration of diabetes than with glycemic control.

In addition, our study has shown that there was a statistically significant difference neither between the mean score of cases with and without hypertension nor between the scores of hypertensive cases and controls using both scales. Literature evidence states that although hypertension alone may not be associated with significant cognitive decline, along with diabetes, it increases the risk of cognitive impairment.[28]

With enormously increasing prevalence of diabetes and diabetes being an important risk factor for cognitive impairment, it is the need of the hour to incorporate cognitive assessment in the routine management of diabetes patients. Ours is the first study that compared MMSE with RUDAS head-to-head in a diabetic population, and the study has proved that MMSE has a definitive advantage over RUDAS for screening diabetes-induced cognitive deficit. In patients with type-2 diabetes, increase in memory deficits with reduction in psychomotor speed and reduced executive function are strongly supported by the findings of earlier studies though there are also other cognitive domains such as verbal fluency, complex motor function, attention, and recall which are shown to be negatively affected. Hence, it is clear that diabetes indeed has composite neurocognitive effects, rather than a single one and difficulty in diagnosis of such multiple neurocognitive function deficits is well recognized.[29] RUDAS tests the executive function unlike MMSE, yet RUDAS failed to demonstrate statistical significance which could be potentially attributed to the relatively high rate of intrasubject variability which is inherent to cognitive testing, thus impairing its ability to identify executive function deficits, especially those which are mild.[29]

In our study, the actual differences in the mean scores between those with good and poor glycemic control (0.91) and those with shorter and longer duration of diabetes (1.11) in the MMSE were small despite achieving statistical significance. Since score differences in the range of 2–4 or greater in MMSE are necessary to conclude a clinically relevant difference, the clinical significance of our interpretation has to be guarded and requires further investigation involving a larger sample size.

Another limitation of the study is that a large proportion of participants were literates and this could be a potential source of bias in interpreting the results. Hence, we recommend that the effectiveness of MMSE scale between literates and illiterates should be compared in a diabetic population before it can be emphasized that the MMSE scale is best suited for screening cognitive deficit in routine clinical examination of diabetic patients for early identification of cognitive impairment.

Conclusion

Our study has truly delineated that cognition deficits based on MMSE scoring correlates with the magnitude of glycemic control as well as the duration of diabetes. The results signify that in the general population, screening for dementia may preferably be done with RUDAS scale, given the higher sensitivity, specificity and particularly as it is efficient to overcome the literacy barrier in a multicultural multi-linguistic population like the one in India. However, in type-2 diabetes patients, it is the MMSE scale that has been proved to be useful in detecting early cognitive impairment, especially in patients with poor glycemic control and longer history of diabetes.

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Conflicts of interest

There are no conflicts of interest.

References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
2. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP, *et al*. The global prevalence of dementia: A systematic review and metaanalysis. Alzheimers Dement 2013;9:63-7500.
3. Alencar RC, Cobas RA, Gomes MB. Assessment of cognitive status in patients with type 2 diabetes through the mini-mental status examination: A cross-sectional study. Diabetol Metab Syndr 2010;2:10.
4. Ryan CM, Geckle MO. Circumscribed cognitive dysfunction in middle-aged adults with type 2 diabetes. Diabetes Care 2000;23:1486-93.
5. Korf ES, White LR, Scheltens P, Launer LJ. Brain aging in very old men with type 2 diabetes: The Honolulu-Asia aging study. Diabetes Care 2006;29:2268-74.
6. Mogi M, Horiuchi M. Neurovascular coupling in cognitive impairment associated with diabetes mellitus. Circ J 2011;75:1042-8.
7. Arvanitakis Z, Schneider JA, Wilson RS, Li Y, Arnold SE, Wang Z, *et al*. Diabetes is related to cerebral infarction but not to AD pathology in older persons. Neurology 2006;67:1960-5.
8. Klein JP, Waxman SG. The brain in diabetes: Molecular changes in neurons and their implications for end-organ damage. Lancet Neurol 2003;2:548-54.
9. Koekkoek PS, Biessels GJ, Kooistra M, Janssen J, Kappelle LJ, Rutten GE, *et al*. Undiagnosed cognitive impairment, health status and depressive symptoms in patients with type 2 diabetes. J Diabetes Complications 2015;29:1217-22.
10. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-98.
11. Storey JE, Rowland JT, Basic D, Conforti DA, Dickson HG.

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The Rowland universal dementia assessment scale (RUDAS): A multicultural cognitive assessment scale. Int Psychogeriatr 2004;16:13-31.
12. Rowland JT, Basic D, Storey JE, Conforti DA. The Rowland universal dementia assessment scale (RUDAS) and the Folstein MMSE in a multicultural cohort of elderly persons. Int Psychogeriatr 2006;18:111-20.
13. Iype T, Ajitha BK, Antony P, Ajeeth NB, Job S, Shaji KS, *et al.* Usefulness of the Rowland universal dementia assessment scale in South India. J Neurol Neurosurg Psychiatry 2006;77:513-4.
14. Pang J, Yu H, Pearson K, Lynch P, Fong C. Comparison of the MMSE and RUDAS cognitive screening tools in an elderly inpatient population in everyday clinical use. Intern Med J 2009;39:411-4.
15. Limpawattana P, Tiamkao S, Sawanyawisuth K. The performance of the Rowland universal dementia assessment scale (RUDAS) for cognitive screening in a geriatric outpatient setting. Aging Clin Exp Res 2012;24:495-500.
16. Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the mini-mental state examination by age and educational level. JAMA 1993;269:2386-91.
17. Tombaugh TN, McIntyre NJ. The mini-mental state examination: A comprehensive review. J Am Geriatr Soc 1992;40:922-35.
18. Velayudhan L, Ryu SH, Raczek M, Philpot M, Lindesay J, Critchfield M, *et al.* Review of brief cognitive tests for patients with suspected dementia. Int Psychogeriatr 2014;26:1247-62.
19. Naqvi RM, Haider S, Tomlinson G, Alibhai S. Cognitive assessments in multicultural populations using the Rowland universal dementia assessment scale: A systematic review and meta-analysis. CMAJ 2015;187:E169-75.
20. Gregg EW, Yaffe K, Cauley JA, Rolka DB, Blackwell TL, Narayan KM, *et al.* Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of osteoporotic fractures research group. Arch Intern Med 2000;160:174-80.
21. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
22. Ebady SA, Arami MA, Shafigh MH. Investigation on the relationship between diabetes mellitus type 2 and cognitive impairment. Diabetes Res Clin Pract 2008;82:305-9.
23. Reaven GM, Thompson LW, Nahum D, Haskins E. Relationship between hyperglycemia and cognitive function in older NIDDM patients. Diabetes Care 1990;13:16-21.
24. Iype T, Shaji SK, Balakrishnan A, Charles D, Varghese AA, Antony TP, *et al.* Cognition in type 2 diabetes: Association with vascular risk factors, complications of diabetes and depression. Ann Indian Acad Neurol 2009;12:25-7.
25. Cosway R, Strachan MW, Dougall A, Frier BM, Deary IJ. Cognitive function and information processing in type 2 diabetes. Diabet Med 2001;18:803-10.
26. Karan NS. Assessment of the cognitive status in diabetes mellitus. J Clin Diagn Res 2012;6:1658-62.
27. Ruis C, Biessels GJ, Gorter KJ, van den Donk M, Kappelle LJ, Rutten GE, *et al.* Cognition in the early stage of type 2 diabetes. Diabetes Care 2009;32:1261-5.
28. Hassing LB, Hofer SM, Nilsson SE, Berg S, Pedersen NL, McClearn G, *et al.* Comorbid type 2 diabetes mellitus and hypertension exacerbates cognitive decline: Evidence from a longitudinal study. Age Ageing 2004;33:355-61.
29. Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. Endocr Rev 2008;29:494-511.

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Original Research Article

Can serum ferritin levels predict the severity of dengue early?: an observational study

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ABSTRACT

Background: Dengue infection is a major public health threat; early recognition is crucial to improve the survival in severe dengue. Although there are various biomarkers to predict the severity of dengue, they are not routinely used in clinical practice for prognostication. We analyzed whether serum ferritin can be used to predict the severity at an earlier stage.
Methods: A hospital based prospective observational study was done involving 119 dengue cases diagnosed by positive NS1 antigen or dengue specific serology (capture ELISA). Serum ferritin was measured in all at the time of diagnosis. Clinical and platelet count monitoring was done daily; classified as severe and non-severe according to 2009 WHO criteria.
Results: Out of 119, 5 developed severe dengue; patients with severe dengue had significantly lower median platelet count ($p<0.0001$); higher ferritin levels ($p=0.03$) and hospital stay ($p<0.0001$) than non-severe group. Age had a significant negative co-relation with platelet count ($r= -0.427$; $p<0.0001$); positive correlation with ferritin levels ($r=0.16$; $p=0.08$) and hospital stay ($r= 0.26$; $p=0.004$) indicating that elderly subjects are at risk of severe disease. Serum ferritin levels negatively correlated with the platelet count ($r= -0.51$ $p<0.001$). High ferritin levels in severe cases are noted from day 4 of clinical illness.
Conclusions: Elevated serum ferritin levels can be used as a potential early prognostic marker to predict the severity of dengue infection in clinical practice.

Keywords: Predictor of severity, Serum ferritin, Early, Severe dengue

INTRODUCTION

Dengue infection poses a major health problem in tropical and subtropical countries.[1] According to World Health Organization, around 50 to 100 million new infections are estimated to occur every year in more than 100 endemic countries across the world; of which around 500000 people with severe dengue require hospitalization each year and about 2.5% of those affected die.[2] The burden of the disease is so much that in the year 2012 World Health Organization classified the disease as "the most important viral disease that is transmitted by mosquitoes".[3] Dengue is caused by dengue virus serotypes I-IV and transmitted to human beings by the bite of Aedes mosquitoes.[4]

The clinical spectrum of dengue infection is variable from mild fever to severe forms of dengue such as dengue hemorrhagic fever and dengue shock syndrome. Severe dengue is characterized by severe thrombocytopenia with major bleeding, plasma leakage resulting in fluid accumulation, respiratory distress and

multi-organ dysfunction.[5] The presence of certain clinical warning symptoms which help to predict the severity are bleeding, skin rash, nausea, vomiting, abdominal pain and hepatosplenomegaly.[6]

Clinically severe dengue results from interplay between virus related virulence factors and host factors which include inflammatory response of the host to infection with exuberant T and B cell activation, release of cytokines (cytokine storm), altered endothelial function with increased vascular permeability and nutritional status of the host. [7]

In clinical practice, patients with dengue infection are grouped as 1) those with warning signs that need intensive monitoring and aggressive management 2) those without warning signs. Non-severe cases without warning signs can develop into severe dengue later during the course of illness. They are at risk of developing complications related to plasma leakage and thrombocytopenia often at the end of febrile stage of illness (third to fifth day of illness) which if unrecognized can be fatal.

Studies had identified various biomarkers for immune and endothelial cell activation, biochemical and genetic markers to predict the severe dengue.[8] The clinical utility of these markers is limited since measurement of these markers like soluble receptors, cytokines, growth factors, vWF; genetic profiling etc. is technically difficult and not widely available. Acute phase reactants like alpha1 anti-trypsin, ceruloplasmin and ferritin levels are elevated during dengue infection; they have a longer half-life unlike the cytokines, and it is feasible to measure their levels.[9]

Increased serum ferritin has been associated with severe dengue in children.[10] Hyperferritinemia in dengue infected subjects is associated with intense immune activation and coagulation disturbances as noted in Aruba Dengue outbreak.[11] An Indian study had shown that serum ferritin levels are significantly elevated in dengue infected cases compared to the controls; On group analysis, cases with severe dengue had higher ferritin levels than milder forms which was noted both during the febrile and defervescence stages of the illness.[9]

Results from the previous studies had shown that hyperferritinemia is associated with severe disease which is noted throughout the disease course. In a Malaysian study, a large proportion of patients developed severe dengue early during febrile stage. [12] It is very crucial to predict the risk of progression to severe dengue at the earliest, by simple measurable tests to initiate appropriate intensive, supportive therapy.

In the present study, we analysed whether serum ferritin measured during early disease course can be used as a marker to indicate the severity which helps to triage and manage them appropriately.

METHODS

This is a hospital based prospective observational study conducted from April 2015 to June 2016 in a tertiary care centre, PSG Institute of Medical Sciences and Research Centre (PSGIMS and R), Coimbatore, South India. The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Human Ethics Committee, PSGIMS and R. All adult patients who were hospitalized and diagnosed with dengue infection either by dengue NS1 antigen or positive dengue specific serology were included in the study after obtaining their informed consent for participation in the study. Patients with chronic inflammatory conditions or diseases; iron overload status; sideroblastic anemia; thalasemia; malignancy; liver disease were excluded from the study.

Study methodology

The study was based on prospective collection of data in dengue infected patients who fulfill the criteria as mentioned above. Diagnosis was based on NS1 antigen test (done with J. Mitra dengue NS1 Ag Microlisa kit) when the presentation was less than 5 days of illness or positive dengue specific serology (dengue IgM and dengue IgG done by Panbio dengue IgM; dengue IgG capture ELISA) if presented after 5 days of illness. Serum ferritin levels were measured if the presentation was less than 7 days from the onset of fever. After informed consent, 2ml of blood was collected from the study subjects; ferritin assay was done (by electrochemiluminescence immunoassay -COBAS e411) at the time of diagnosis if the diagnosis was made in the study center; or at the time of admission if referred from an outside hospital with positive test results (NS1 or dengue serology positive).

The clinical course of the disease and platelet count were monitored carefully on a daily basis and patients were classified as having severe and non-severe infection as per WHO 2009 criteria; classified as severe dengue when shock, respiratory distress, severe bleeding; organ impairment occur.[5] Mean lowest platelet count was calculated in both the subgroups from all the platelet count values obtained on a daily basis during the hospital stay. Mean ferritin levels were calculated and compared in both the subgroups.

Statistical analysis

Descriptive statistics were reported as mean±Standard deviation (SD), median with 25^{th} and 75^{th} percentiles. Mann Whitney U test was used to compare platelet count and serum ferritin between severe and non-severe dengue groups. Spearman's Rank correlation was used to assess the correlation between age with platelet count, serum ferritin and hospital stay. All analyses were done using SPSS version 21.0. P value less than 5% was considered statistically significant.

RESULTS

A total of 119 subjects were analyzed, of which there were 68 males (57.14%) and 51 (42.85%) females. The mean age of the study population was 29.1±11.4 years. About 50% of the study population was less than 25 years. There was no significant difference in the mean age between male and female. Diagnosis of dengue infection was established by positive NS1 antigen in 61 subjects while in the remaining 58, it was diagnosed by positive dengue serology.

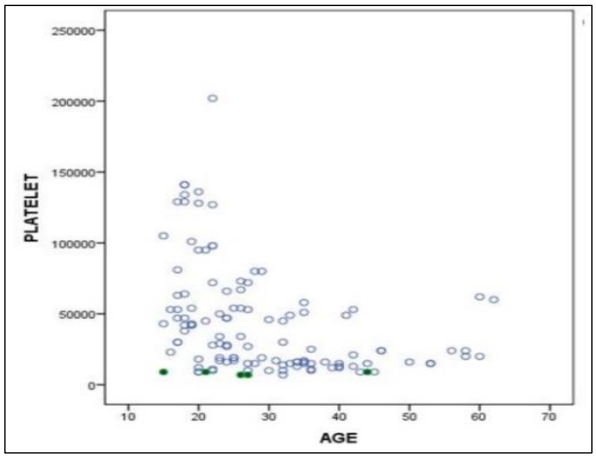
Among the total 119, only 5 (4.2%) subjects had clinical severe dengue, of which 4 were males and one female. The mean lowest platelet count was 40426.47×10^9/L in males, 45000×10^9/L in females. Around 75% of the study population stayed in the hospital for ≤ 3 days; 16% stayed for 4 days; 5% stayed for 5 days. Around 5% had a hospital stay for ≥9 days. Serum ferritin levels were measured in 14 subjects on day 3; 19 on day 4; 36 on day 5; 29 on day 6 and for the remainder 21 subjects it was measured on day 7. The mean ferritin levels measured was 1399.53±690.68ng/ml; (median-1856). The mean hospital stay was 3.11 days.

Table 1: Comparison of serum ferritin, platelet count and hospital stay in severe and non-severe dengue.

	Severe dengue	Non-severe dengue	P value
Serum ferritin ng/ml	2000 (2000, 2000)	1593 (757, 2000)	0.03*
Platelet count ×109/l	9000 (7000, 9000)	29500 (15750, 55000)	<0.0001*
Hospital stay days	16 (11, 21)	3 (2, 3)	<0.0001*

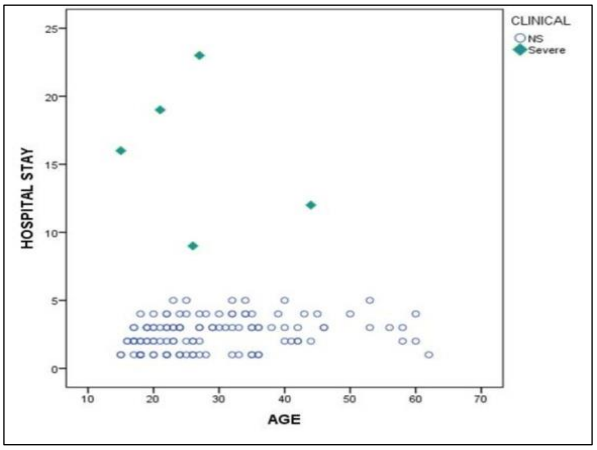
Median platelet count was significantly lower among patients who had severe dengue as compared to non-severe dengue group (Table 1). The median platelet count recorded during daily monitoring of platelets throughout the hospital stay were 9000 ×10^9/L (7000, 9000) in severe and 29500×10^9/L (15750, 55000) in non-severe dengue respectively. The median hospital stays, and serum ferritin levels were significantly higher among severe dengue subjects as compared to non-severe group (Table 1). The median serum ferritin levels and hospital stay among severe and non-severe dengue patients were 2000ng/ml (2000, 2000) vs. 1593ng/ml (757, 2000) and 16 (11, 21) vs. 3 days (2, 3) respectively.

In the present study, higher age showed a significant negative correlation with platelet count (Pearson co-efficient r= -0.427; p<0.0001). Also, higher age positively correlated with hospital stay (r= 0.26; p=0.004) and ferritin levels (r=0.16; p=0.08). The correlation with hospital stay is significant. Serum ferritin levels showed a significant negative correlation with the platelet count (Pearson co-efficient r= -o.51; p<0.001). The results are depicted in Figures 1-4.



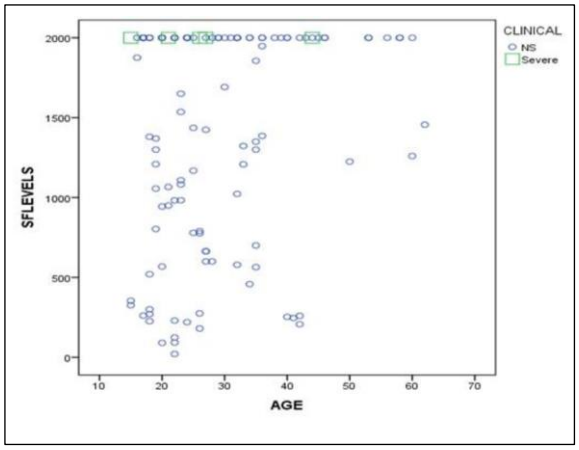
Age negatively correlated with platelet count which is significant (r= -0.427; p<0.0001*)

Figure 1: The co-relation co-efficient between age (years) and platelet count(x109/L).



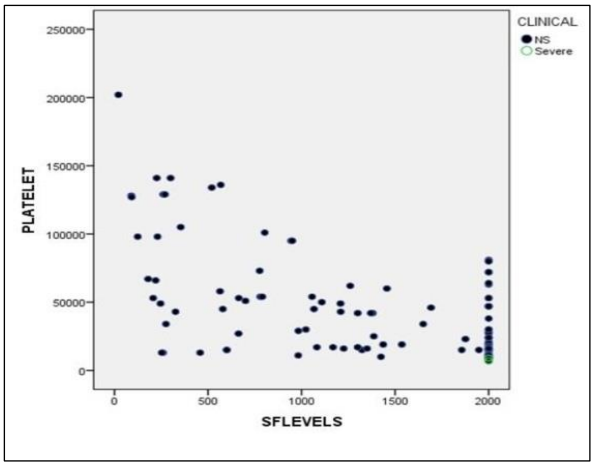
Age positively correlated with hospital stay which is significant (Pearson co-efficient r= 0.26; p=0.004*)

Figure 2: The co-relation co-efficient between age (years) and hospital stay (days).



Age positively correlated with serum ferritin levels (non significant r=0.16; p=0.08)

Figure 3: The co-relation co-efficient between age (years) and SF levels (serum ferritin in ng/ml).



Serum ferritin levels showed a significant negative correlation with the platelet count (Pearson co-efficient r= –o.51 p<0.001*)

Figure 4: The co-relation co-efficient between SF levels (serum ferritin in ng/ml) and platelet count.

Serum ferritin measured from day 3 to day 7 of illness was compared in both the severe and non-severe groups and reported in Table 2. It was observed that non-significantly, patients who had severe dengue had higher median serum ferritin levels from day 4 onwards as compared to non-severe dengue cases.

Table 2: Comparison of serum ferritin levels in severe and non-severe dengue measured from the day 3 to day 7 of illness.

Serum ferritin measurement	Ferritin level in ng/ml		P value
	Severe dengue	Non-severe dengue	
3 day	-	n=14 1486 (1013, 2000)	-
4 day	n=1 2000±0 2000 (2000, 2000)	n=18 1001±811 859 (242, 2000)	0.421
5 day	n=2 2000±0 2000 (2000, 2000)	n=34 1549±587 2000 (1183, 2000)	0.349
6 day	n=1 2000±0 2000 (2000, 2000)	n=28 1222±727 1315 (565, 2000)	0.414
7 day	n=1 2000±0 2000 (2000, 2000)	n=20 1512±722 1974 (1103, 2000)	0.571

Serum ferritin measured from day 4 to7 of illness was high in severe dengue compared to non-severe cases, although not statistically significant

DISCUSSION

Currently there is no specific drug or preventive vaccine available for dengue infection. The mainstay of management of dengue infected subjects is supportive care and close monitoring for complications.[13] Most of these patients recover within 5 to 7 days from onset of the febrile illness while only a small percentage progress to severe dengue, with a study quoting 5%.[14] Severe dengue often occurs at the end of febrile or during convalescent stage and carries a very high mortality. It is very crucial to predict the severe form well in advance even prior to the appearance of warning signs by a simple diagnostic marker so that early identification and appropriate management would improve the outcome in these patients.

Increased expression of acute phase reactants is observed in patients with severe dengue infection when compared to non-severe cases.[9] This serves to prognosticate the dengue infected patients well ahead of the appearance of clinical warning signs. One such acute phase reactant is ferritin which is produced by reticuloendothelial cells in response to infection and inflammation. Ferritin is highly elevated in dengue infected patients than in patients with other febrile illnesses.[11] Hyperferritinemia seen in these patients exhibit two opposite functions; Early in the phase of clinical illness, increased serum ferritin levels exert a protective effect by chelating the toxic free iron radicals at the site of inflammation.[15] while in severe cases, raised ferritin may assume a pathogenic role by activating immune cells resulting in cytokine storm.[9]

Males constitute 57.14% of the study population in the present study which was in accordance with other studies. The mean hospital stay was comparable with other studies. The mean duration of hospital stay was 2.73±1.41 days as observed by Ahmed at al, and 3.7±1.02 days in a study conducted in Rawalpindi.[16,17]

Results from the present study showed that hyperferritinemia is associated with severe dengue which is noted in other studies as well.[9-11, 17] To analyze whether serum ferritin can predict the severity earlier in the course of illness, day wise serum ferritin levels was compared in both the sub-groups; the serum ferritin levels were higher in severe dengue cases when compared to non-severe group and this increase is noted from day 4 of illness onwards which clearly indicates that serum ferritin can predict the severity of dengue at an earlier stage.

A study conducted during a dengue outbreak on the Caribbean island Aruba which compared the ferritin levels in dengue infected patients with those having other viral illnesses concluded that ferritin is a useful clinical marker to differentiate dengue from other febrile illnesses; serum ferritin levels >1500ng/ml is associated with severe disease ; serum ferritin levels measured on day 4-5 better predicts the outcome of dengue infection and it concluded that these patients with hyperferritinemia should be monitored carefully for complications.[11] Results from the present study also had shown that hyperferritinemia (2000ng/ml) is seen in all cases with severe dengue and this increase in serum

ferritin levels is noted from day 4 of clinical illness in severe cases when compared to non-severe cases.

Another study conducted in one hundred seventy-seven Thai children which measured serum ferritin levels during the entire clinical course (febrile, toxic and convalescent stages) showed similar results.[10] Increased serum ferritin was associated with severe dengue; patients with non-severe dengue had serum ferritin levels less than 1,200ng/ml during the entire course of illness, while severe forms recorded higher levels of serum ferritin during the febrile stage and the defevervescence stage. Patients with dengue hemorrhagic fever (DHF) grade III and IV had significantly higher median ferritin levels measured on day 5,6 and 7 of illness than those with grade I, II DHF and dengue fever. In that study it was concluded that serum ferritin level ≥1,200ng/ml from day 4 of the illness predicts the occurrence of dengue hemorrhagic fever.

In a study conducted in South India by Soundravally R et al, which included 48 dengue infected cases and 48 cases with other febrile illness as controls, serum ferritin levels were measured on the day of admission (which is a median of 4 days after the onset of fever) and day of defervescence (which is a median of 4 days after the day of admission).[9] Compared to other febrile illnesses, dengue cases demonstrated significant increase in serum ferritin levels ;also within clinical groups, severe disease exhibited higher ferritin levels compared to non-severe group throughout the febrile and defervescence stages of illness (940.09±568.31 in non-severe cases vs. 1264.71±492.59 in severe dengue on day of admission ; 418.19±404.59 vs. 1490.74±359.40 on day of defervescence) The study concluded that raised serum ferritin levels could predict the severity of dengue with a sensitivity and specificity of 76.9% and 83.3% respectively. The present study also showed similar results; hyperferritinemia was noted in severe cases compared to non-severe group from day 4 to day 7 of illness.

One another study showed significant association between raised serum ferritin levels on the day of admission and the development of severe dengue.[16] Serum ferritin was measured on the day of admission in a total of 104 dengue positive cases and they were grouped into a) Group A with normal ferritin levels (up to 100µg/dl) and b) Group B with high serum ferritin levels ; both the groups were followed up for the occurrence of severe dengue. Severe dengue was noted only in 2 out of 31 patients with normal serum ferritin levels while more than half of the patients with severe dengue (35 out of 73) had raised serum ferritin levels. The study concluded that serum ferritin can be used as an early marker to predict the severity of the disease.

Another significant observation made out in the present study was that age had a positive co-relation with the serum ferritin levels and the duration of hospital stay while it negatively co-related with the platelet count. This implies that age is an important predictor of severity of dengue infection. As age advances, there is increased risk of developing severe disease with low platelet count, high ferritin levels and prolonged hospital stay. Studies had shown that elderly individuals are at risk of developing severe disease. In a study conducted in Southern Taiwan during a dengue outbreak with 5336 dengue cases, the incidence of dengue hemorrhagic fever and dengue shock syndrome is significantly higher in persons aged 60 years and above when compared with younger age groups.[18] Similar observation was made in a Singapore study; persons aged more than 60 years had more occurrence of severe dengue (20.3% vs. 14.6%, P = 0.006) as compared to younger adults.[19]

Our study is unique in that serum ferritin was measured once at the time diagnosis whereas in most of the earlier studies serum ferritin levels were done at multiple times during various stages of clinical illness.[9-11] The results of the present study in which serum ferritin was measured either at the time of diagnosis (or at the time of admission if referred from outside) within 7 days from onset of fever, correlated with the findings of the previous studies . It would be simpler, easier and convenient to measure serum ferritin on a single day, which is practically feasible in clinical setting catering to a large population.

The present study has few limitations as well. First, is the small number of severe dengue observed in the present study; hence we were unable to derive the cut-off value of day wise ferritin levels which can predict the risk of development of severe dengue. A larger sample size with more number of severe cases would be appropriate before arriving at a definite conclusion. Second, the upper limit of serum ferritin measured in the present study was 2000ng/ml due to laboratory limitations; so higher values could not be observed which if available can enable us to better understand about its correlation with both the severity of thrombocytopenia and clinical severity with more accuracy.

Also, the nature of infection (primary or secondary); dengue serotypes were not taken into consideration. To address the limitations in the present study, future studies are needed to find out the cut off level of serum ferritin which can predict severe dengue at the earliest for effective disease management.

CONCLUSION

The present study had shown that elevated serum ferritin levels done early during the febrile stage of the illness (3rd to 7th day of illness) predict the severity of dengue.

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REFERENCES

1. World Health Organization. Working to Overcome the Global Impact of Neglected Tropical Diseases. First WHO report on Neglected Tropical Diseases, 2010. Available at: www.who.int/publications/2010/9789241564090_eng.pdf. Accessed on 29 November 2018.
2. World Health Organization. Regional Office of South-East Asia. Dengue fact sheet. Available at: http://www.searo.who.int/entity/vector_borne_tropical_diseases/data/data_factsheet/en/. Accessed on December 3, 2018
3. World Health Organization. Global Strategy for Dengue Prevention and Control, 2012-2020. Geneva: WHO Press; 2012. Available at: https://www.who.int/denguecontrol/9789241504034/en. Accessed on 1 January 2018.
4. Back AT, Lundkvist A. Dengue viruses-an overview. Infect Ecol Epidemiol. 2013;3:10-3.
5. World Health Organization (WHO) and the Special Programme for Research and Training in Tropical Diseases (TDR). Dengue guidelines for diagnosis, treatment, prevention and control, 2009. Available at: https://www.who.int/rpc/guidelines/9789241547871/en/. Accessed on 27 November 2018.
6. Zhang H, Zhou YP, Peng HJ, Zhang XH, Zhou FY, Liu ZH, et al. Predictive symptoms and signs of severe dengue disease for patients with dengue fever: a meta-analysis. Biomed Res Int. 2014;359308.
7. Martina BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev. 2009;22:564-81.
8. John DV, Lin YS, Perng GC. Biomarkers of severe dengue disease-a review. J Biomed Sci. 2015;22:83.
9. Soundravally R, Agieshkumar B, Daisy M, Sherin J, Cleetus C. Ferritin levels predict severe dengue. Infection. 2015;43(1):13-9.
10. Chaiyaratana W, Chuansumrit A, Atamasirikul K, Tangnararatchakit K. Serum ferritin levels in children with dengue infection. Southeast Asian J Trop Med Public Health. 2008;39:832-36.
11. van de Weg CA, Huits RM, Pannuti CS, Brouns RM, van den Berg RW. Hyperferritinaemia in dengue virus infected patients is associated with immune activation and coagulation disturbances. PLoS Negl Trop Dis. 2014;9:e3214.
12. Md-Sani SS, Md-Noor J, Han W, Gan SP, Rani NS, Tan HL, et al. Prediction of mortality in severe dengue cases. BMC Infectious Dis. 2018;18:232.
13. Biswas A, Pangtey G, Devgan V, Singla P, Murthy P, Dhariwal AC, et al. Indian national guidelines for clinical management of dengue fever. J Indian Medical Asso. 2015;113(12).
14. Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, et al. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. Trop Med Int Health. 2011;16(8):936-48.
15. Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood. 2002;99:3505-16.
16. Ahmed A, Alvi AH, Butt A, Nawaz AA, Hanif A. Assessment of Dengue fever severity through liver function tests. J Coll Physicians Surg Pak. 2014;24:640-44.
17. Nadeem M, Shafiq MM, Manzoor MS, Ahmed SI. JRMC. 2016;20(3):165-7.
18. Liu CC, Huang KJ, Huang MC, Lin JJ, Wang SM, Liu JJ, et al. High case-fatality rate of adults with dengue hemorrhagic fever during an outbreak in non-endemic Taiwan: risk factors for dengue-infected elders. Am J Infect Dis. 2008;4:10-17.
19. Rowe EK, Leo YS, Wong JG, Thein TL, Gan VC, Lee LK, et al. Challenges in dengue fever in the elderly: atypical presentation and risk of severe dengue and hospital-acquired infection. PLoS Negl Trop Dis. 2014;8(4).

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Case Report

N-ACETYLCYSTEINE, A BOON FOR YELLOW PHOSPHORUS-INDUCED ACUTE LIVER FAILURE? A CASE REPORT

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ABSTRACT

Rat killer, in the form of a paste, is a commonly used rodenticide in India. It contains 3% yellow phosphorus, which is a local and a systemic toxin that damages all tissues it contacts. The lethal dose of yellow phosphorus is about 1 mg/kg of body weight. We present a case report of a 30-year-old female patient with an alleged history of intake of 30 g rat killer paste mixed with one glass juice with suicidal intent. She presented with clinical features of acute liver failure (ALF) and was treated with N-acetylcysteine (NAC) infusion with other supportive therapy and recovered completely within 13 days. Poisoning with yellow phosphorus needs to be studied in the aspect of treatment, due to the lack of any specific antidote. The patient factors that help in the recovery also need to be investigated. Although highly lethal, the recovery of ALF due to yellow phosphorus-containing rodenticide is possible. Early intravenous administration of NAC, which acts as glutathione substitute, anti-inflammatory agent, and anti-oxidant could contribute to complete resolution of ALF in yellow phosphorus poisoning.

Keywords: Yellow phosphorus, Poisoning, Acute liver failure.

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INTRODUCTION

Poisoning is a major problem worldwide. Intentional and accidental ingestions of toxins in humans lead to high mortality and morbidity rates. Yellow phosphorus is a well-known hepatotoxic agent commonly found in fireworks, ammunition, agricultural dung and it is ubiquitously present in rodenticides. It is available as both paste and powder form in the open market in India, with concentrations varying from 2% to 5%. The lethal dose of yellow phosphorus is 1 mg/kg of body weight, and ingestion of amounts >780 mg is almost invariably followed by death [1,2]. It is a protoplasmic poison, which spontaneously combusts in air and causes multi-organ failure when ingested. The quantity of yellow phosphorus consumed, lack of specific antidote, and time lapse in treatment affects the outcome of poisoning. Very few case reports of yellow phosphorus poisoning have been reported worldwide. Here is one such rare case report of successful management of a 30-year-old female patient who consumed a substantial amount of yellow phosphorus in the form of rodenticide paste which is 18 times of the lethal dose.

CASE REPORT

A 30-year-old female patient, a housewife of weight 50 kg, with no medical or medication history was brought to the emergency department with complaints of loose stools (2 episodes), vomiting (7 episodes), and mild giddiness. The initial presentation of the patient depicted gastroenteritis. On personal interview of the patient, it was revealed that she consumed two packets of 15 g rodenticide paste-containing yellow phosphorus (3%), 11 hours before admission with suicidal intent.

On initial examination, she was conscious, oriented, had paled, and was psychologically depressed. There were no bleeding manifestations, jaundice, or other signs of liver failure. Laboratory investigations showed normal biochemical parameters. Electrocardiogram was within the normal limits. Ultrasonography of the abdomen showed fatty liver. She was treated with supportive measures such as intravenous pantoprazole 40 mg and intravenous ondansetron 4 mg. N-acetylcysteine (NAC) infusion was started within 1 hour of admission, and the patient was shifted to the intermediate care unit (Tables 1 and 2).

From day 2, the patient experienced a clinical deterioration with mild abdominal pain and vomiting. It was followed by two episodes of vomiting, fatigue, abdominal pain, and nausea on day 3. On day 4, she had generalized body tiredness, myalgia, and disturbed sleep due to nausea. On day 5, she experienced mild nausea. On day 6, she had 1 episode of vomiting and was shifted to the ward. On these days, her laboratory investigations showed deranged liver enzymes (Table 3).

From day 7 to day 13, the patient was asymptomatic and improved clinically. She was discharged from hospital on day 14 after 1 day of observation. The discharge medications consisted of tablet pantoprazole 40 mg O.D, tablet ursodeoxycholic acid 300 mg B.D, tablet rifaximin 550 mg B.D, tablet L-adenosyl L-methionine 200 mg BD, and syrup lactulose 20 ml TID, which was continued till review.

The patient was reviewed on the 7th day from hospital discharge, her laboratory investigations showed improvement in the liver enzymes. On the second review, the laboratory investigations were normal and the patient had completely recovered (Table 4).

DISCUSSION

Sporadic cases of ingestion of toxins on a suicidal intent are common in developing countries [3]. Our patient allegedly ingested 900 mg of yellow phosphorus in the form of a paste which is highly lethal. Orally ingested yellow phosphorus is rapidly absorbed through the gastrointestinal tract. About 69%–73% of the total ingested dose concentrates in the liver after remaining in the gut for a few hours [4].

The features of yellow phosphorus poisoning are classically divided into three stages. The first stage involves gastrointestinal irritation and shock. Features begin within minutes of ingestion, but may be delayed in some cases. Symptoms involve nausea, vomiting, diarrhea, and abdominal pain [5-7]. Our patient developed several episodes of vomiting, diarrhea, and giddiness within 11 h of ingestion.

The second stage is quiescent or asymptomatic period, which lasts for 24–74 h [8]. The patient is mistaken to be improving and is most likely to get discharged prematurely. The patient was asymptomatic and was clinically stable for 24 h after initial supportive therapy.

The third stage involves a clinical deterioration with fulminant hepatic failure, which may progress to multi-organ failure. This stage involves high rates of mortality, and death usually occurs in 4–8 days [9]. From day 2, our patient's biochemical and clinical parameters deteriorated. She was at increased risk of fulminant hepatic failure but recovered by day 13 due to prompt treatment with NAC.

The current report is in line with other reports that it is difficult to manage yellow phosphorus poisoning due to multiple factors. First due to the property of yellow phosphorus itself, that it gets rapidly absorbed and remains stable for long periods in the gut because of higher water content and low oxygen tension [10]. Second, because there are no diagnostic tests to detect or antidote available to treat yellow phosphorus poisoning. Finally, treatment of yellow phosphorus is complicated due to symptomatic improvement in stage 2.

Mechanism of toxicity of yellow phosphorus is by the production of phosphoric acid, which produces an exothermic reaction and causes direct tissue damage due to the production of free radicals. NAC, which is an antioxidant and hepatoprotectant, acts as a glutathione precursor by donating sulfhydryl groups [11]. It also improves mitochondrial energy metabolism [12]. It acts as a scavenger of oxygen free radicals and it replenishes the glutathione stores. It is found to have a good prognosis in patients with yellow phosphorus poisoning when administered early [13,14]. The patient's liver enzymes were highly elevated. The outcome of the patient after 10 days of NAC administration was excellent; highlighting the importance NAC in nonacetaminophen-related liver failure.

Table 1: Core therapy of NAC

Day	NAC dose
Day 1	NAC 10.5 g in 200 ml 5% dextrose over 1 h NAC 3.5 g in 500 ml 5% dextrose over 4 h NAC 7 g in 1000 ml 5% dextrose over 16 h
Day 2 to Day 10	NAC 3 g in 500 ml 5% dextrose over 4 h (2 doses per day)

NAC: N-acetylcysteine

CONCLUSION

Our case report highlights the importance of history taking of patients brought to emergency care. Yellow phosphorus poisoning requires more than just primary care. Early improvement in symptoms should not be mistaken as a resolution to the clinical problem. Instead, the patient has to be periodically monitored for clinical and biochemical parameters for at least 5 days before premature discharge from the hospital. Prompt use of NAC within the first 12 h of poisoning has been

Table 2: Supportive medications administered during the hospital stay

Drugs	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
Vit K 10 mg intravenous Q24H	✓	✓	✓	✓	✓								
Piperacillin-tazobactam 4.5 g intravenous Q6H					✓	✓	✓	✓	✓	✓	✓		
Tablet L-adenosyl L-methionine 200 mg Oral Q12H			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tablet ursodeoxycholic acid 300 mg Oral Q12H				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Syrup lactulose 20 ml oral Q12H			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Capsule rifaximin 550 mg–400 mg-550 mg oral Q8H				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 3: Abnormal laboratory parameters of the patient during the hospital stay

Laboratory parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 13
Direct bilirubin (mg/dl) ref: (0.0–0.2)	0.2	0.3	1.9	2.1	4.7	6.1	5.9			4.5	2
Indirect bilirubin (mg/dl) ref : (0.2–0.8)	0.4	0.5	0.5	0.9	1	0.9	0.3			0.2	0.4
Serum glutamic oxaloacetic transaminase (U/L) ref: (5–41)	17	93	652	555	460	324	333	364		219	146
Serum glutamic pyruvic transaminase (U/L) ref: (5–38)	19	263	2047	1758	1071	534	375	638		102	102
Alkaline phosphatase (U/L) ref: (40–129)	81	81	114	138	157					167	147
Platelet ($\times 10^3/\mu L$) ref: (150–400)	171	166		139		120	71		74		
Prothrombin time (s) ref: (11–15)	17.8	27.8		29.4		16	13	13.8			
Lactate (mmol/L) ref: (0.4–2.2)	0.9		2.7		4.1	3.1	3.7			2.9	3
Ammonia (mcg/dl) ref: (15–45)	20				22						22

Table 4: Comparison of laboratory parameters on review after discharge from the hospital

Laboratory parameters	First review (after 1 week)	Second review (after 1 month)
Direct bilirubin (mg/dl) ref: (0.0–0.2)	1.1	0.3
Indirect bilirubin (mg/dl) ref: (0.2–0.8)	0.4	0.7
Serum glutamic oxaloacetic transaminase (U/L) ref: (5–41)	63	37
Serum glutamic pyruvic transaminase (U/L) ref: (5–38)	41	23
Alkaline phosphatase (U/L) ref: (40–129)	124	123
Lactate (mmol/L) ref: (0.4–2.2)	2.7	2.1
Prothrombin time (s) ref: (11–15)	12.6	12.6
Platelet ($\times 10^3/\mu L$) ref: (150–400)	100	350

found to be beneficial. Due to high mortality rates, restriction of use and appropriate disposal procedures of yellow phosphorus-containing rodenticide should be developed.

AUTHORS' CONTRIBUTION

Aashiq Ahamed Shukkoor: Informed consent, maintaining patients file and master file of project, drafting final report, submission to IHEC, publication.

Nimmy Elizabeth George: Informed consent, maintaining patients file and master file of project, drafting final report, submission to IHEC, publication.

Saravanan Thangavelu: Concept, design, laboratory investigations, laboratory report interpretation, treatment decision, patient evaluation, examination of patients on follow-up, publication.

CONFLICT OF INTEREST

The authors have none to declare.

REFERENCES

1. Fernandez OU, Canizares LL. Acute hepatotoxicity from ingestion of yellow phosphorus-containing fireworks. J Clin Gastroenterol 1995;21:139-42.
2. Bayne JR, Beck JC, Lowenstein L, Browne JS. Cortisone acetate in the treatment of acute phosphorus poisoning. Can Med Assoc J 1952;67:465-7.
3. Sumalatha S, Padma D, Pai KS, Kotian SR, Kumar N, Bhat KM. Hepatoprotective activity of aqueous extract of *Caesalpinia bonduc* against CCL4 induced chronic hepatotoxicity. Int J Pharm Pharm Sci 2016;8:207-11.
4. Lakshmi CP, Goel A, Basu D. Cholestatic presentation of yellow phosphorus poisoning. J Pharmacol Pharmacother 2014;5:67-9.
5. Saoji AA, Lavekar AS, Salkar HR, Pawde GB, Tripathi SS. A case on suicidal poisoning associated with ratol and a perspective on yellow phosphorous poisoning. Int J Recent Trends Sci Technol 2014;10:223-5.
6. Hiran S. Ventricular arrhythmia due to yellow phosphorous poisoning. J Case Rep Stud 2017;5:303-6.
7. Sleeba SK, Raj M, Kabeer PA, Dipu KP. A rare case of yellow phosphorous poisoning with acute cholestatic hepatitis, bicytopenia, and impending hepatic failure. Int J Pharm Pharm Sci 2016;8:402-3.
8. McCarron MM, Gaddis GP, Trotter AT. Acute yellow phosphorus poisoning from pesticide pastes. Clin Toxicol 1981;18:693-711.
9. Manojkumar CH, Keerthivyas KS, K rishna YS. Cardiac toxicity after acute yellow phosphorous ingestion: Case report. Int J Adv Res 2017;5:2445-8.
10. Mauskar A, Mehta K, Nagotkar L, Shanbag P. Acute hepatic failure due to yellow phosphorus ingestion. Indian J Pharmacol 2011;43:355-6.
11. Kharkongor MA, Mishra AK, Ninan K F, Iyadurai R. Early use of intravenous N-acetylcysteine in treatment of acute yellow phosphorous poisoning. Curr Med Issues 2017;15:136-8.
12. Zwingmann C, Bilodeau M. Metabolic insights into the hepatoprotective role of N-acetylcysteine in mouse liver. Hepatology 2006;43:454-63.
13. Nalabothu M, Monigari N, Acharya R. Clinical profile and outcomes of rodenticide poisoning in tertiary care hospital. Int J Sci Res Publ 2015;5:1-12.
14. Priya G. Antioxidant mediated defence role of eclitaalba herbal extract against CCL4 induced toxic hepatitis. Int J Curr Pharm Res 2018;10:29-32.

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Case Report

Internal Medicine Section

Looking Beyond Lupus in Lupus

BHARGAVI KUMAR[1], SARAVANAN THANGAVELU[2]

ABSTRACT

Haematological abnormalities are a common finding in Systemic Lupus Erythematosus (SLE) patients. The wide spectrum includes anaemia, cytopenias, thrombosis as well as bleeding. Among the bleeding disorders, qualitative coagulation defects can be rarely encountered because of clotting factor inhibitors. More uncommon is the occurrence of quantitative clotting factor deficiencies. Here, we describe a rare cause of abdominal pain in a patient with SLE who developed perinephric and colonic sub mucosal haematomas owing to a rare association with acquired factor XIII deficiency which was diagnosed on quantitative assays and effectively treated with cryoprecipitate and fresh frozen plasma.

Keywords: Abdominal haematomas, Factor XIII deficiency, Systemic lupus erythematosus

CASE REPORT

A 21-year-old young lady presented to the Emergency Department with left loin pain, vomiting and loose stools for the last one day. She did not report any fever, radiation of pain, urinary symptoms, pain aggravation on food intake or any prior strenuous activity.

The patient was diagnosed with SLE 2 years ago and was on treatment with steroids, Mycophenolate Mofetil (MMF) and Hydroxychloroquine. She reported history of intermittent episodes of pain in her calf muscles in the last one year. Magnetic resonance imaging (MRI) had revealed the presence of organised haematomas in calf muscles, but the patient did not follow-up due to spontaneous resolution of symptoms.

On examination, the patient was pale, tachycardic and hypotensive. No rashes were noted. Respiratory system examination fairly ruled out pleurisy and musculoskeletal examination was normal. A non-tender firm ballotable mass was palpable in the Left lumbar region.

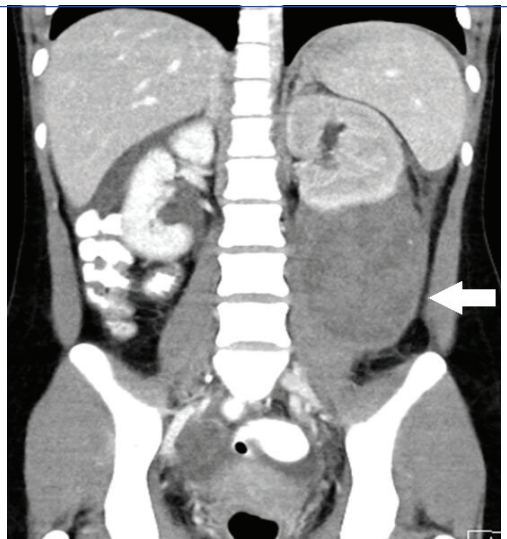
Preliminary investigations showed microcytic anaemia, (haemoglobin 6.2 g/dL), severe thrombocytopenia (22×10^9/L) and mild neutrophilic leucocytosis as shown in [Table/Fig-1]. Her renal function and complements were normal with slightly elevated Erythrocytic sedimentation rate (35 mm first hour). Ultrasonogram of the abdomen showed 9.4×7.4 cm heterogeneous lesion adjacent to the lower pole of the left kidney. A computed tomography revealed the presence of left perinephric haematoma with left hydroureteronephrosis [Table/Fig-2]. Initial coagulation profile revealed normal Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and slightly low fibrinogen levels (1.4 g/L). Direct Coombs test turned out to be positive. Von Willebrand Factor assay was normal. Antiphospholipid Antibody (APLA) work up was done in which anti beta2 glycoprotein turned out to be negative, with Lupus anticoagulant ratio of 1.6.

The differential diagnoses considered were acute SLE flare/bleeding due to thrombocytopenia/coagulation defects and MMF induced haematological crisis. However, complement activity, disease markers were normal and the patient was on irregular treatment with MMF. Since initial PT, APTT levels were normal, possible factor XIII deficiency was considered and she was evaluated accordingly.

The patient was stabilised with intravenous fluids namely Normal Saline, low dose methyl prednisolone 250 mg over 3 days and 2 units packed red blood cells transfusion. There was recurrence of vomiting and loose stools during initiation of enteral feeds and a repeat CT abdomen revealed new onset transmural haematomas in small bowel loops, distal ascending colon, transverse colon,

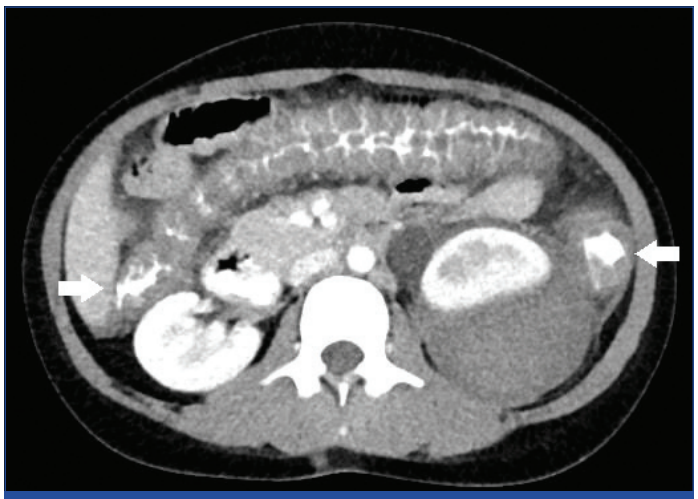
	Day 1	Day 5	Day 7	Day 11	Standard value
Haemoglobin g/dL	6.2	8.4	9.9	11.9	12-15
Mean corpuscular volume fL	73.2				80-100
White blood counts ×10^3/uL	15	10.6		9.2	4-10
Platelets x10^3/uL	22000	42000	83000	110	150-400
Prothrombin time (seconds)/INR	13.3/1.2	13.9/1.33		13.1/1.25	9-12
APTT (seconds)	29.1	22.8		28.2	25-34
Serum fibrinogen levels mg/dL	76	140		342	200-400
C3 c level/C4 mg/dL	83/21				90-180 10-40
ESR mm 1st hour	35	30		17	4-12 mm
HsCRP mg/dL	0.30				<0.5 mg/dL
Procalcitonin ng/mL	0.2				<0.5

[Table/Fig-1]: Preliminary investigations showing microcytic anaemia, severe thrombocytopenia and mild neutrophilic leucocytosis.

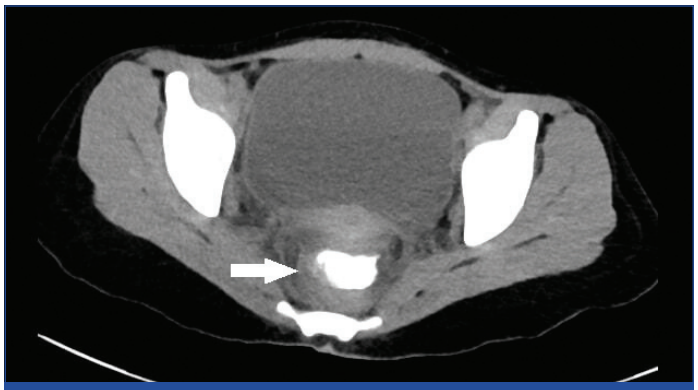


[Table/Fig-2]: CT abdomen showing perinephric haematoma over lower pole of left kidney with ureteric compression.

descending colon and rectum [Table/Fig-3,4]. The coagulation profile results reaffirmed normal APTT levels; repeat fibrinogen levels were normal (3.4 g/L) and platelet counts were improving (120×10^9/L). On performing a clot solubility test, the clot completely dissolved in 5M urea solution and on mixing studies, the clot dissolved partially. Since the results were equivocal, it was not possible to differentiate a hereditary factor XIII deficiency from a factor XIII inhibitor in this case. Subsequently, a factor XIII assay was done which turned out to be <1% thus confirming severe deficiency. Patient was transfused with a total of 18 unit's cryoprecipitate (270 mL) and 10 units of fresh frozen plasma (2400 mL total) during her entire hospital stay following which she showed steady improvement.



[Table/Fig-3]: CT abdomen depicting transmural haematoma of distal ascending, transverse and descending colon.



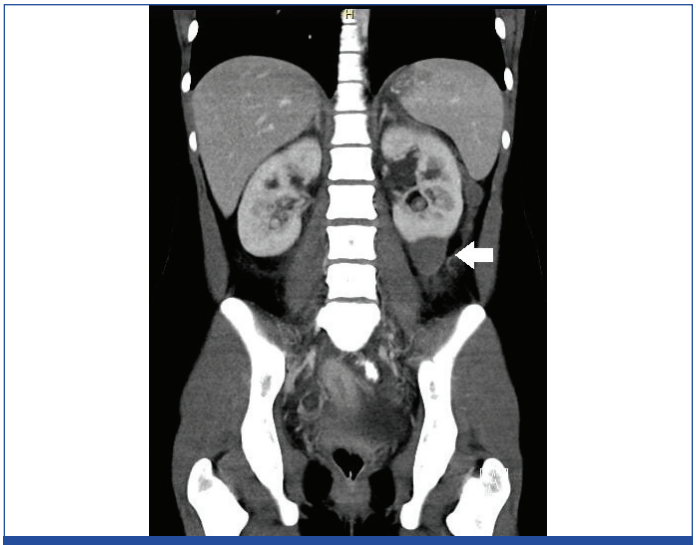
[Table/Fig-4]: CT abdomen showing transmural haematoma involving rectum.

A follow-up CT abdomen a month later showed resolving left perinephric haematomas with resolution of colonic and rectal haematomas. The patient is currently doing well on 5 mg once daily prednisolone and azathioprine [Table/Fig-5].

DISCUSSION

SLE is a challenging disease to treat. In the current era, diagnosing SLE has become common due to increasing awareness, knowledge of its varied presentations and available investigations. However, the course of the disease poses a constant challenge to the treating physician owing to the myriad of systemic manifestations and complications.

Acute abdomen in SLE patients is a challenging diagnostic and therapeutic problem. Most patients are on steroids and/or immunosuppressive agents which mask the physical findings, thus leading to a delayed definitive diagnosis. The reported incidence of abdominal pain in SLE varies from 8 to 40% [1,2]. Assuming that all are due to vasculitis may lead to diagnostic errors. In an observation of 36 active SLE patients presenting with acute abdomen by Medina F et al., 19 had vasculitis, 3 had Anti Phospho Lipid Antibody (APLA) related intra-abdominal thrombosis and the rest were non-SLE related [3]. Therefore, it is imperative to carefully evaluate such a patient based on their clinical, imaging and immunological profile.



[Table/Fig-5]: CT abdomen revealing resolving haematoma over the lower pole of left kidney.

Haematological abnormalities are common findings in patients with SLE. The prevalence is described as 47% in most studies [4]. In a study by Sasidharan PK et al., haematological manifestations were found to be the most common initial presentation of SLE and it was the presenting manifestation in 82% of the patients. The haematological spectrum encompasses anaemia, leucopenia, thrombocytopenia, autoimmune haemolytic anaemia, thrombotic thrombocytopenic purpura, myelofibrosis-hypoplastic anaemia and thrombotic manifestations if associated with APLA [5]. The index patient too had leucopenias along with cutaneous manifestations in her initial presentation leading to her diagnosis.

It is imperative to distinguish haematological abnormalities arising due to disease activity from those due to drug effects. The index patient had normal complement activity and disease markers making acute SLE flare unlikely. The patient was on irregular treatment with MMF, making drug related side effect a remote consideration. Bleeding despite normalisation of platelet count, fibrinogen levels and normal PT, APTT prompted us to investigate us for rare clotting factor abnormalities, such as Factor XIII defects. Coagulation defects in SLE could be due to inhibitors more commonly [6], and due to factor deficiencies rarely [7-9].

Factor XIII is needed to stabilise the fibrin clots and it's deficiency could be genetically acquired as Autosomal recessive disorder or could be due to acquired causes [7]. Hereditary deficiency is attributed to the F13A1 gene located on the short arm of chromosome 6 (6p24.2-p23). Acquired factor XIII deficiency occurs much less often, and usually affects middle-aged or elderly individuals [10]. It can be associated with conditions such as severe liver disease, chronic kidney failure, inflammatory bowel disease, Henoch-Schonlein purpura, autoimmune disorders such as SLE or scleroderma and certain cancers such as myeloid forms of leukaemia. It has also been linked to the use of certain medications including isoniazid, penicillin, and phenytoin.

Individuals with factor XIII deficiency form blood clots per se, but the clots are unstable and often break down, resulting in prolonged, uncontrolled mild to fatal bleeding episodes. The most common bleeding symptoms are subcutaneous bleeding (57%), delayed umbilical cord bleeding (56%), muscle haematoma (49%), haemorrhage after surgery (40%), haemarthrosis (36%) and intracerebral bleeding (34%) [11,12]. However, the index patient's presentation was unusual with perinephric, colonic submucosal and transmural haematomas.

Factor XIII deficiency is screened by clot solubility test and confirmed by quantitative assays [13]. It is treated by factor XIII concentrates,

fresh frozen plasma or cryoprecipitate. When inhibitors develop in individuals with factor XIII deficiency, additional therapy in the form of immunosuppressive agents would be required.

On review of literature, there is a reported Lupus patient with associated factor 13 deficiency who developed intracerebral haematoma [9]. There are case reports of subcutaneous and intramuscular haematomas due to factor XIII deficiency following an invasive procedure in a patient with SLE [14]. In another instance a 70-year-old man had developed spontaneous left gluteal haematoma owing to factor XIII deficiency [15]. Occurrence of gastrointestinal haematomas as in the index patient seems to be extremely rare.

CONCLUSION

It is fruitful to think beyond lupus while approaching the presenting complaints even in a diagnosed patient of SLE. Attributing all the features to the disease per se might be misleading. A high index of suspicion for rarer clotting factor deficiencies is required while evaluating the bleeding manifestations with a normal coagulation profile. There exists a lacuna in ascertaining the fact as to why in an autoimmune disease, there is associated clotting factor deficiency, unlike the expected scenario of clotting factor inhibitors. Only continued medical research can unravel such mysteries.

REFERENCES

[1] Sultan SM, Ioannou Y, Isenberg DA. A review of gastrointestinal manifestations of systemic lupus erythematosus. Rheumatology. 1999;38(10):917-32.
[2] Zizic TM, Classen JN, Stevens MB. Acute abdominal complications of systemic lupus erythematosus and polyarteritis nodosa. The American Journal of Medicine. 1982;73(4):525-31.
[3] Medina F, Ayala A, Jara LJ, Becerra M, Miranda JM, Fraga A, et al. Acute abdomen in systemic lupus erythematosus: The importance of early laparotomy. The American Journal of Medicine. 1997;103(2):100-05.
[4] Bennett JC, Claybrook J, Kinsey H, Holley HL. The clinical manifestations of systemic lupus erythematosus. A study of forty-five patients. Journal of Chronic Diseases. 1961;13(5):411-25.
[5] Sasidharan P, Bindya M, Sajeeth Kumar K. Hematological manifestations of SLE at initial presentation: Is it underestimated? ISRN Hematology. 2012:1-5.
[6] Bashal F. Hematological disorders in patients with systemic lupus erythematosus. The Open Rheumatology Journal. 2013;7(1):87-95.
[7] Chuliber F, Schutz NP, Otero V, Barrera L, Altuna D, Fantl DB, et al. Acquired Factor XIII deficiency in the inpatient setting: Clinical suspicion, impact and treatment: Retrospective case series. Blood. 2016;128(22):1415.
[8] Sawlani KK, Chaudhary SC, Roy A, Tripathi AK. Factor XIII deficiency presenting with intracerebral bleed. BMJ Case Rep. 2013;2013:bcr2012007303.
[9] Dabadghao VS, Singh VB, Meena BL, Khichar S. Systemic lupus erythematosus with intracerebral hematoma due to decreased factor XIII activity: A rare association. J Mahatma Gandhi Inst Med Sci. 2013;18:129-31.
[10] NORD (National Organization for Rare Disorders). (2019). Factor XIII Deficiency-NORD (National Organization for Rare Disorders). [online] Available at: https://rarediseases.org/rare-diseases/factor-xiii-deficiency [Accessed 6 Oct. 2019].
[11] Acharya SS, Coughlin A, DiMichele DM, The North American Rare Bleeding Disorder Study Group. Rare Bleeding Disorder Registry: deficiencies of factors II, V, VII, X, XIII, fibrinogen and dysfibrinogenemias. J Thromb Haemost. 2004;2:248-56.
[12] Ivaskevicius V, Seitz R, Kohler HP, Schroeder V, Muszbek L, Ariens RA, et al. International registry on factor XIII deficiency: a basis formed mostly on European data. Thromb Haemost. 2007;97(6):914-21. doi: 10.1160/TH07-01-0034.
[13] Kohler HP, Ichinose A, Seitz R, Ariens RAS, Muszbek L. On behalf of the Factor XIII and Fibrinogen SSC Subcommittee of the ISTH. Diagnosis and classification of factor XIII deficiencies. J Thromb Haemost. 2011;9:1404-06.
[14] Rabik CA, Atkinson MA, Sule S, Strouse JJ. Treatment of an acquired Factor XIII inhibitor in an adolescent with systemic lupus erythematosus and renal failure. Transfusion. 2017;57(9):2159-63.
[15] Fogarty H, Byrne M, O'Connell NM, Ryan K, White B, O'Donnell JS, et al. Acquired factor Xiii deficiency: an uncommon but easily missed cause of severe bleeding. Ir Med J. 2018;111(5):757.

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Case Report

Euglycemic diabetic ketoacidosis: another masquerader!

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ABSTRACT

Euglycemic ketoacidosis is defined by the triad of euglycemia, metabolic acidosis and ketonemia or ketonuria. In the current era of diabetic management, it is a serious concern with the usage of sodium glucose co-transporter 2 (SGLT2) inhibitors potentiated by a number of precipitating agents. Empagliflozin though a novel oral hypoglycemic agent in this category may also lead to this potential complication. Here we report a 59 year old male, type 2 diabetic who was on empagliflozin and presented with euglycemic ketoacidosis after a binge of alcohol.

Keywords: Empagliflozin, SGLT2 inhibitor, Type 2 diabetes, Euglycemic ketoacidosis

INTRODUCTION

Hyperglycemic states in diabetes like Diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar non ketotic coma are widely encountered, well known and therefore easily diagnosed emergencies. However euglycemic ketoacidosis is an often missed relatively less known condition and under reported scenario. A high index of clinical suspicion is warranted to arrive at this diagnosis because the normoglycemia may mask our clinical judgement of DKA.

The management of diabetes mellitus is ever evolving with oral hypoglycemic agents being an area of constant research and updates. Approved by the Food and Drug administration of USA (FDA) in 2014, Sodium glucose co-transporter 2 (SGLT2) inhibitors is a new addition to the already existing broad list of oral hypoglycemic agents for type II diabetes mellitus. Apart from reducing blood sugars, its spectrum also extends to reducing blood pressure, reduction of weight and cardio protection.[1] It is now included in American Diabetes Association treatment guidelines as one of the add-on agents in dual or triple drug combinations in diabetes as it can effectively reduce HbA1c by 0.5-0.7%. However euglycemic ketoacidosis is a rare but potential complication in this class of drugs for which FDA has released a safety alert in 2015.[2] Its diagnosis may be delayed owing to the normal sugar levels and needs a strong index of suspicion whenever there is ketonemia or ketonuria in the presence of normal blood sugar levels and high anion gap metabolic acidosis.

CASE REPORT

A 59 year old male presented to the emergency department with abdominal discomfort and orthopnea following a binge alcohol intake. Patient was a chronic smoker and alcoholic for 30 years and a known case of

type 2 diabetes mellitus and systemic hypertension for 5 years. He was on irregular treatment with tablet metformin 500 mg in once daily dosage and had started taking tablet empagliflozin 10 mg once daily for the past 2 years without prescribed medical advice. His concomitant medications included tablet nebivolol 5 mg once daily for systemic hypertension. On examination, the patient was moderately dyspnoeic, tachypnoeic, drowsy, arousable and normotensive. Respiratory system examination showed basal crepitations and abdomen examination showed mild epigastric tenderness.

Preliminary investigations revealed normal sugar levels (169 mg/dl), ketonuria (3+) and elevated HbA1c (10.8%). His complete blood counts, serum procalcitonin, liver, thyroid and renal functions were normal but for low bicarbonate (4.65 mEq/l) and a pH of 7.2. The calculated anion gap was high (27.7). Cardiac functions were normal as evidenced by echocardiogram and biomarkers.

An ultrasonography of the abdomen was done in view of epigastric tenderness and showed a bulky pancreas suggestive of acute pancreatitis. His amylase (956 U/l) and lipase (3873 U/l) were also elevated and lipid profile evaluation revealed hypertrigliceridemia (242 mg/dl). His laboratory parameters are summarised in Table 1.

Noting his ketonuria and high anion gap metabolic acidosis with normal blood sugars we arrived at a diagnosis of euglycemic ketoacidosis in the background of his medication history of empagliflozin therapy which was probably precipitated by alcohol binge and acute pancreatitis. Patient was put on ventilator support and started on intravenous fluids, insulin along with 5% dextrose and bicarbonate correction. The acidosis improved gradually and blood glucose levels were controlled with rapidly acting insulin. After the patient improved symptomatically, he was weaned off and tolerated soft diet.

Table 1: Laboratory parameters of the patient across his days of admission showing his normal sugar values at admission and metabolic acidosis which resolved following treatment.

	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 10
Sugars (mg/dl)	169	141	132	178	193	299	268
Urine for ketones	+++	+++	++	+	Nil	Nil	Nil
pH	7.2	6.9	7.47	7.4	7.48	7.40	7.41
HCO_3 (mEq/l)	4.65	7.0	14	11.3	18	23.8	24.1
Amylase (U/l)	956	-	-	145	242	-	-
Lipase (U/l)	3873	-	-	639	1158	887	637

On discharge, the patient was counselled regarding diet, insulin therapy, advised alcohol abstinence and was counselled to stop any self-medication of new oral hypoglycemic agents. The patient is now on regular follow up, maintaining average blood sugars of 200 mg/dl. As the patient was apprehensive about continuing insulin, he is now put on oral agents-insulin secretagogues alongside diet and lifestyle modification.

DISCUSSION

There are a multitude of causes leading to metabolic acidosis in diabetics. These include normal anion gap acidosis like renal failure as well as high anion gap states like lactic acidosis and ketoacidosis. Ketoacidosis could be due to diabetic ketoacidosis, starvation ketoacidosis or alcoholic ketoacidosis. Diabetic ketoacidosis may be either hyperglycemic or euglycemic. Thus analyzing the risk factors that contribute to the development of ketoacidosis is very crucial.

Euglycemic ketoacidosis was first observed and reported in a case series by Munro et al who observed that a subset of DKA patients had sugar levels <300 mg/dl.[3] Subsequently euglycemic ketoacidosis has been characterized by the triad of euglycemia (blood glucose <250 mg/dl), metabolic acidosis (pH <7.35, bicarbonate <18 mEq/l) and ketonemia or ketonuria.[4] To explain it succinctly, euglycemic ketoacidosis is DKA sans marked hyperglycemia. It is mainly described in type 1 diabetes but nevertheless not uncommon in type 2 diabetes. Possible contributing factors include starvation, alcoholism, pregnancy, pancreatitis and drugs. Among drugs, SGLT2 inhibitors are notorious to cause euglycemic ketoacidosis.

SGLT 2 inhibitors act primarily by inhibiting sodium glucose co-transporter 2 in proximal convoluting tubule which mediates 80-90% of glucose absorption in kidney. As a result of this facilitation of glucose excretion by kidney, blood glucose levels are lowered. When the blood glucose levels reduce, the insulin release from pancreas also reduces proportionately tilting the normal insulin: glucagon ratio, thereby favoring ketogenesis. Other proposed mechanisms include release of glucagon by direct SGLT2 inhibition in pancreas, release of kisseptin which inhibits insulin release and also decreased reabsorption of ketones leading to ketonemia.

Empagliflozin has shown meticulous benefits in reducing major cardiac events as evidenced in the 'EMPA-REG OUTCOME' trial.[5] Since empagliflozin's mechanism of action is independent of beta cell functioning, they are useful in all type 2 diabetes patients as long as estimated glomerular filtration rate is above 45-60 ml/min. Other potential benefits include weight loss (2-3 kg) and

reduction in blood pressure (3-6 mmHg systolic, 1-2 mmHg diastolic) due to natriuresis besides cardioprotection. Though beneficial in numerous aspects, empagliflozin also has adverse effects like genital fungal infections (3-5%), urinary tract infections (1-2%), volume depletion and hypotension. However of serious concern is the development of euglycemic ketoacidosis though reported in less than 0.1% patients. Canagliflozin and dapagliflozin also carry the risk of euglycemic ketoacidosis with reported incidence being 0.8% and less than 0.1% respectively.[6]

On literature review, we noted that Gammons and Counselman have presented a case report of a chronic alcoholic, type 2 diabetic patient on empagliflozin developing euglycemic ketoacidosis with acute pancreatitis similar to our index patient.[7] So also Candelario and Wykretowicz have presented a type 2 diabetic female who developed euglycemic ketoacidosis while on empagliflozin therapy precipitated by acute cholecystitis.[8]

In our index patient with euglycemic ketoacidosis, the clear culprits were empagliflozin overtly precipitated by his binge of alcohol and pancreatitis. A high index of suspicion alone prompted us to withdraw the offending drug and treat him with insulin and dextrose apart from intravenous fluids in correcting his metabolic acidosis.

CONCLUSION

DKA need not necessarily present with hyperglycemia. High anion gap ketoacidosis should be a red flag to look for euglycemic ketoacidosis which requires both insulin and dextrose in correcting the underlying metabolic derangement. Careful drug history and possible precipitators should be identified and promptly corrected. SGLT2 inhibitors though a novel agent in the treatment of diabetes also comes with its quantum of adverse effects like euglycemic ketoacidosis which is a potential life threatening complication. The key to a successful usage of newer exciting hypoglycemic agents lies in choosing our patients wisely and carefully avoiding those who have other contributing factors for acidosis.

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REFERENCES

1. Rosenwasser RF, Sultan S, Sutton D, Choksi R, Epstein BJ. SGLT 2 inhibitors and their potential in treatment of Diabetes, Diabet Metabol Syndrome Obes. 2013;6:453-67.
2. U.S. Food and Drug Administration. Drug Safety Communication: FDA warns that SGLT2 inhibitors for diabetes may result in a serious condition of too much acid in the blood, 2015. Available at: http://www.fda.gov/downloads/Drugs/Drugsafety/UCM446954. Accessed on 05 October 2018.
3. Munro JF, Campbell IW, McCuish AC, Duncan LJ. Euglycaemic diabetic ketoacidosis. Br Med J. 1973;2:578-80.
4. Miles JM, Gerich JE. Glucose and ketone body kinetics in diabetic ketoacidosis. Clin Endocrinol Metab. 1983;12:303-19.
5. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes (EMPA-REG OUTCOME). New Engl J Med. 2015;373:2117-28.
6. Rosenstock J, Ferrannini E. Euglycemic diabetic ketoacidosis: a predictable, detectable, and preventable safety concern with SGLT2 inhibitors. Diab Care. 2015;38(9):1638-42.
7. Gammons T, Counselman FL. Sodium-glucose cotransporter-2 induced diabetic ketoacidosis with minimal hyperglycemia. Clin Practice Cases Emerg Med. 2018;2(1):47-50.
8. Candelario N, Wykretowicz J. The DKA that wasn't: a case of euglycemic diabetic ketoacidosis due to empagliflozin. Oxford Med Case Report. 2016;2016(7):144-6.

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CASE REPORT

Dengue Fever Complicated By Hemophagocytosis – A Difficult To Manage Case

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Abstract: Dengue fever, which is transmitted by the mosquito Aedes aegypti is a common acute viral febrile illness, affecting 390 million people worldwide every year. It is endemic in several countries. Although the usual presentation is that of a self-limiting illness, its complications are protean. Infection associated with hemophagocytic syndrome (IAHS) is a rare and fatal complication of dengue fever. It should be suspected in a patient with fever beyond seven days, associated with hepatosplenomegaly, hyperferritinemia, worsening cytopenias and development of multiorgan dysfunction(MOD). We report a 19-year-old female, who presented with an acute febrile illness and was diagnosed with primary dengue fever. Despite appropriate supportive therapy, she worsened clinically during the course of hospitalisation. A disproportionately high ferritin level and persistent bicytopenia prompted investigations for hemophagocytic lymphohistiocytosis (HLH). Further evaluation revealed features of HLH, as per the diagnostic criteria laid down by the Histiocyte Society. She was successfully treated with glucocorticoids, etoposide and intravenous immunoglobin and other supportive therapy. She had severe cytopenia and cardiac dysfunction during the course of her illness and its management. This case adds to the limited adult cases of dengue-associated hemophagocytic syndrome and shows its difficulty in management due to associated extra complications.

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1. INTRODUCTION

Dengue fever which is endemic in India and several other countries can sometimes manifest with many atypical features sometimes that may be life-threatening too if not diagnosed and intervened in time **(1).** HLH is one of them, which is now being detected more readily due to increasing awareness amongst clinicians. It is a status of abnormal immune response caused by a cytokine storm induced by the uncontrolled proliferation and activation of the macrophages in the reticuloendothelial system causing a systemic inflammatory response and multi-organ dysfunction **(2)**. Reactive HLH is associated with infections, autoimmune disorders or malignancies. HLH has been reported in dengue patients **(3)**. Its incidence is estimated to be approximately 1.2 cases per 10,000,000 individuals **(4)**. Primary HLH occurs due to various genetic abnormalities and often presents during infancy and early childhood. It is associated with an autosomal recessive inheritance pattern. Secondary HLH is associated with infections, malignancy, metabolic and rheumatological conditions. Many infections are known to cause HLH and this is often mistaken for sepsis and multiorgan dysfunction syndrome(MODS). We describe the case of a 19-year-old female, who had primary dengue fever complicated by hemophagocytosis which was difficult to manage due to different drug and non-drug related complications.

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1.1. Case Report

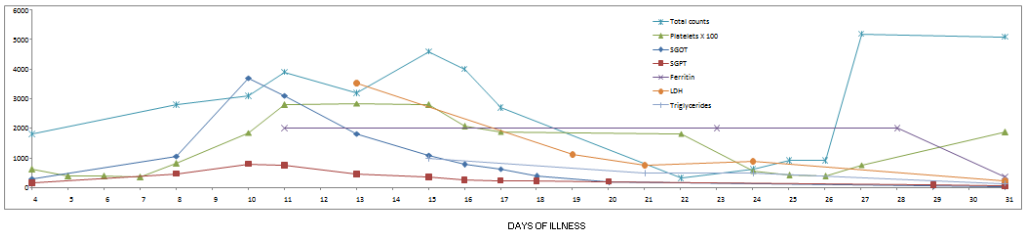
A 19-year-old female was admitted with high-grade intermittent fever for 3 days, associated with a headache and myalgia without any symptoms suggestive of a focus of infection. On the day of admission, she had mild pallor, no icterus or edema and her vitals were stable. Systemic examination did not reveal any significant finding. Her white blood cell count was 1800cells/cu.mm, hemoglobin was 8.7g/dl and platelet count was 60,000cells/cu.mm. Liver function tests were moderately deranged with elevated transaminase, alkaline phosphatase and Gama glutaryl transferase. With clinical features and preliminary reports, she was diagnosed to have dengue fever and it was confirmed with dengue IgM positivity (by ELISA). She was treated with supportive care, intravenous fluids and antipyretics. Follow up of cultures was also negative for any organisms.

But even after 6 days of illness, she continued to have a fever and general unwellness. There was an increasing trend in serial liver enzymes, with mild prolongation of prothrombin time. However, she had no bleeding manifestation. She was suspected to have hospital acquired new infection and was evaluated for the same. She started on an empirical intravenous broad-spectrum antibiotic as per hospital antibiotic policy. Urine, blood and bone marrow culture were sterile. But she continued to have a fever and general unwellness and worsening of liver function and prothrombin time as well. Serology for human immunodeficiency virus, hepatitis-B, C,

A, E and scrub typhus infections was negative. Epstein-Bar virus serology was consistent with past infection while IgM antibody for cytomegalovirus was positive. Anti-nuclear antibody profile was also negative.

On the ninth day of illness, she was shifted to the medical intensive care unit in view of desaturation and worsened sensorium. She was intubated and mechanical ventilation was given. Since she had a worsening course of illness even after on appropriate antibiotic therapy, the possibility of HLH was considered. She underwent a panel of investigations for the same, which included serum triglycerides, ferritin, lactate dehydrogenase (LDH), peripheral smear and bone marrow smear and biopsy, repeat cultures and procalcitonin (Trend of laboratory results shown in Fig. (**1**). She had high levels of serum triglyceride, ferritin, LDH and evidence of hemophagocytosis in the bone marrow examination (as shown in Figs. (**2** and **3**). Fig. (**4**) Peripheral smear showing spherocytes, suggestive of the transfused population.

Apart from supportive care, she was also started on treatment for hemophagocytic lympho- histiocytosis as per HLH 2004 protocol. She continued to have fever and biochemical parameters showed no improvement even after two days of therapy with Dexamethasone and Etoposide. Hence she was also started on Immunoglobulin therapy. Subsequently, fever and other abnormal parameters regressed. She was extubated and shifted out of the intensive care unit. During her stay in the intensive care unit, she had worsening of neutropenia secondary to Inj. Etoposide, for which she was given Inj. Granulocyte colony-stimulating factor (G-CSF). She also developed left ventricular systolic dysfunction probably as a complication of dengue and/or secondary to immunoglobulin therapy which was managed with fluid restriction and diuretic. After stabilization, she was shifted to ward where she underwent a complete rehabilitation programme. At the time of discharge, her biochemical and hematological parameters normalized. During the three months follow up, she maintains good health and blood parameters.



LAB PARAMETERS	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Total WBC counts (4,000-10,000/UL)	1800				2800		3100	3900		3200		4600	4000	2700					300		600	900	900	5200				5100
Platelets(1,50,000-4,00,000/UL)	60000	37000	37000	35000	80000		185000	280000		284000		279000	206000	188000					181000		56000	40000	38000	76000				186000
SGOT (5-38U/L)	294				1055		3700	312		1822		1082	765	623	372		176									51		27
SGPT (5-41U/L)	136				460		795	736		436		335	241	225	203		196									82		49
Ferritin (30-400 ng/mL)								>2000												>2000					>2000			361
LDH (135-225 U/L)										3517						1111		749			878							200
Triglycerides (<150 mg/dL)												966						470			479							125

Fig. (1). Showing trend of laboratory results.

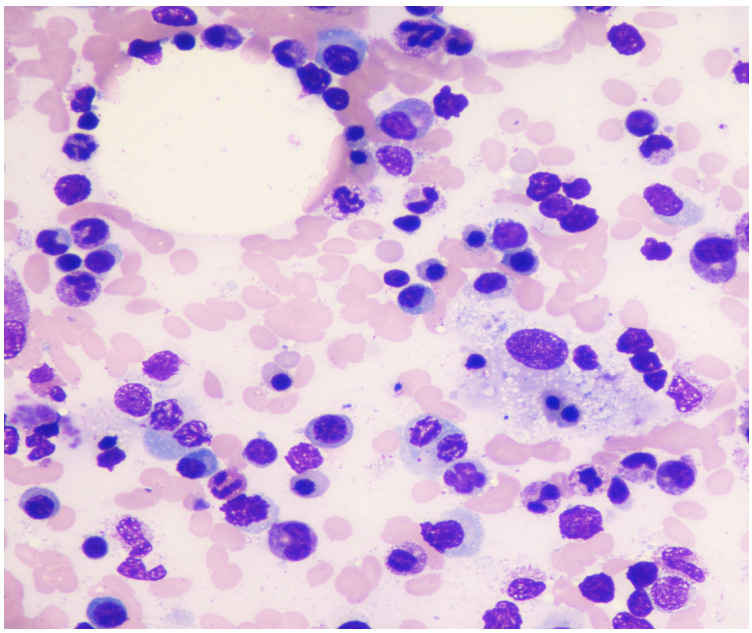


Fig. (2). Bone marrow aspirate showing prominent phagocytosis of blood elements by Histiocytes. (Low Power magnification).

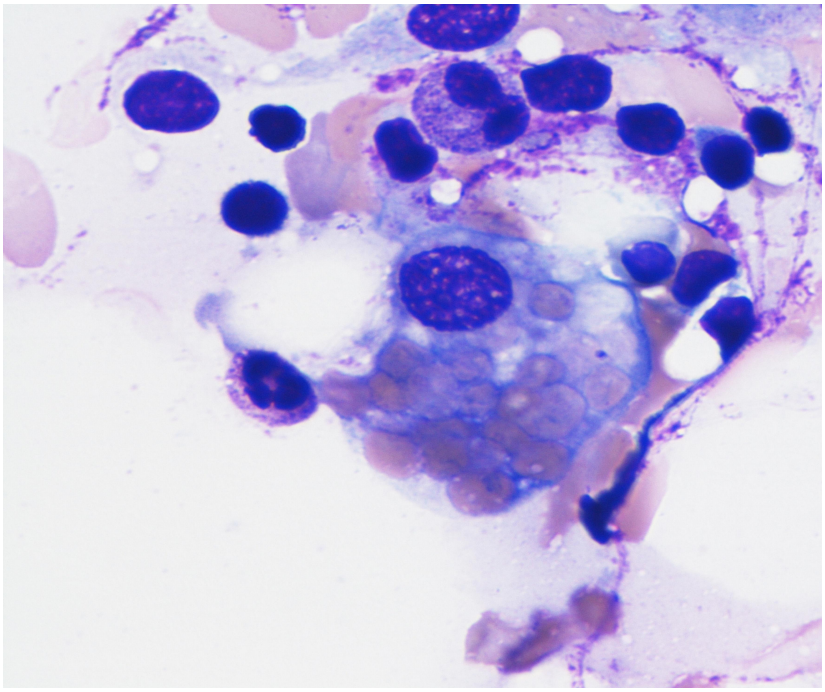


Fig. (3). Bone marrow aspirate showing erythrophagocytosis (High power magnification).

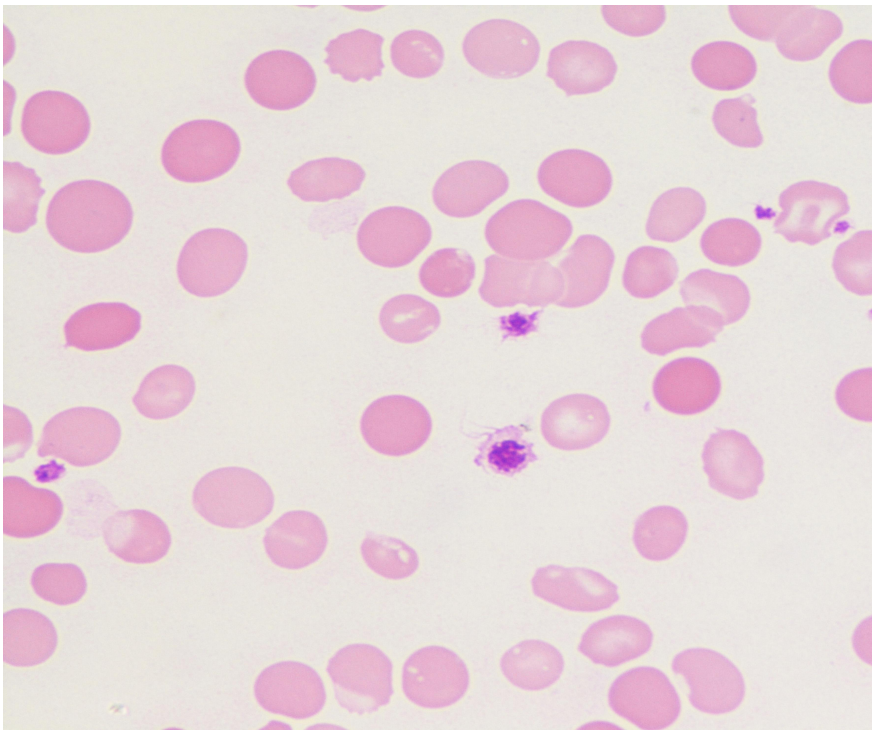


Fig. (4). Peripheral smear with spherocytes suggestive of transfused population (X100).

2. DISCUSSION

HLH is a rare and life-threatening disorder with major therapeutic and diagnostic difficulties. It causes tissue destruction due to abnormal immune activation. Clinical features of HLH, which is secondary to unrestrained immune activation are not specific and mimic sepsis with MODS. Although primary HLH is typically seen in the paediatric age group, secondary HLH can occur in adults and children in association with certain triggers which includes solid organ malignancies, infections, rheumatological disorders and

inherited or acquired immunodeficiency. Several infections like visceral leishmaniasis**(5)**, tuberculosis, dengue and scrub typhus trigger HLH.

Dengue is an uncommon cause of HLH and has been mentioned in a few case reports **(6)**. T cells infected with dengue virus produce cytokines like tumor necrosis factor (TNF)-α and interferon (IFN) gamma which possibly contribute to the development of HLH syndrome. HLH is characterized by excessive macrophage activation and cytokine release. This results in unchecked inflammation due to immune dysregulation. Patients with HLH are acutely ill with fever, hepatosplenomegaly, effusions and lymphadenopathy. Laboratory findings include bicytopenia, coagulopathy, liver dysfunction, hyperferritinemia and elevated triglycerides. Bone marrow infiltration by activated macrophages can be demonstrated, and diagnosis is made based on the HLH-2004 protocol proposed by the Histiocyte Society. HLH can be established in the presence of (i) molecular diagnosis consistent with HLH or (ii) the presence of five out of eight criteria, namely, fever, splenomegaly, cytopenias, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis in tissue, hyperferritinemia, increase in CD25/IL-2 receptor and reduced or absent NK cell activity. Our patient met 6 of the 8 criteria.

Glucocorticoids are used as the first line of treatment of HLH. Other agents as per HLH-2004 protocol **(2)** are cyclosporin, etoposide and intrathecal methotrexate (for patients with central nervous system involvement). The presence of both dengue-associated HLH and multiorgan dysfunction (MOD) in majority of the patients may indicate that they are part of an inflammatory spectrum, which also includes systemic inflammatory response syndrome **(7).** Interleukin (IL)-6 and TNF are associated in the pathogenesis of MOD. Nonetheless, treatment for each syndrome is different, as HLH is treated with chemo-immunotherapy whereas supportive therapy is a keystone in MOD.

Dengue fever, caused by a mosquito-borne virus belonging to the Flaviviridae family, is a serious public health problem in the Indian subcontinent. The year 2017 has seen a gross increase in the number of dengue cases in India. The clinical manifestations range from a self-limiting febrile illness to life-threatening disease with shock and MOD. In a study focusing on 162 adult HLH patients, 58% survival was noted **(8).** The diagnosis of dengue is based on the detection of virus non-structural protein 1, which has greater sensitivity in primary infection (90%) when compared with secondary infection (60–80%). Alternatively, real-time PCR tests are available for virus detection. Serological testing for IgM and IgG antibodies helps in differentiation between primary and secondary infections.

Dengue-associated HLH has been described commonly in children, with few reports in adults **(9).** It has been more commonly noted in patients with dengue hemorrhagic fever. But our patient didn't have any bleeding manifestations. In our patient, the diagnosis was established on Day 12 of illness. During the treatment she had cardiac dysfunction probably due to the immunoglobulin administered or dengue itself, hence fluid management was prioritised. She also developed etoposide-induced severe neutropenia which was managed with G-CSF injections. She was successfully treated with etoposide, immunoglobulin and a tapering course of corticosteroids as per the HLH-2004 protocol**(10)** and showed a good response to the same. At the first month and subsequent monthly follow up, she was well with no features of reactivation. In our patient, the initial improvement was followed by clinical worsening leading to a diagnosis of dengue-associated HLH. This case highlights the need for awareness of causes of worsening dengue, its immediate confirmation and early therapeutic intervention as this may be life-saving. This case also adds to the few adults reported HLH in dengue as there have been approximately 45 cases dengue-related HLH **(11)**.

CONCLUSION

Usually, dengue fever spontaneously subsides after five days of illness with supportive care alone. If a person continues to be symptomatic even after five to seven days of illness, along with the possibility of secondary bacterial infections there should be a high index of suspicion of its atypical and rare complications. Early diagnosis and intervention may be life-saving.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Dengue Challenges India's Health System [Internet]. [17 October 2015]; http://www.sciencedirect.com/science/article/pii/S014067361500313X

[2] Henter JI, Horne A, Aricó M, *et al.* HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48(2): 124-31. [http://dx.doi.org/10.1002/pbc.21039] [PMID: 16937360]

[3] Ramanathan M, Duraisamy G. Haemophagocytosis in dengue haemorrhagic fever: a case report. Ann Acad Med Singapore 1991; 20(6): 803-4. [PMID: 1803975]

[4] Ellis EM, Sharp TM, Pérez-Padilla J, *et al.* Incidence and Risk Factors for Developing Dengue-Associated Hemophagocytic Lymphohistiocytosis in Puerto Rico, 2008 - 2013. PLoS Negl Trop Dis 2016; 10(8): e0004939. [http://dx.doi.org/10.1371/journal.pntd.0004939] [PMID: 27556807]

[5] Rajagopala S, Dutta U, Chandra KS, Bhatia P, Varma N, Kochhar R. Visceral leishmaniasis associated hemophagocytic lymphohistiocytosis--case report and systematic review. J Infect 2008; 56(5): 381-8. [http://dx.doi.org/10.1016/j.jinf.2008.02.013] [PMID: 18405976]

[6] Vijayalakshmi AM, Ganesh VRR. Hemophagocytic syndrome associated with dengue hemorrhagic fever. Indian Pediatr 2009; 46(6): 545. [PMID: 19556676]

[7] Castillo L, Carcillo J. Secondary hemophagocytic lymphohistiocytosis and severe sepsis/ systemic inflammatory response syndrome/ multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. Pediatr Crit Care Med 2009; 10(3): 387-92. [http://dx.doi.org/10.1097/PCC.0b013e3181a1ae08] [PMID: 19325510]

[8] Rivière S, Galicier L, Coppo P, *et al*. Reactive hemophagocytic syndrome in adults: a retrospective analysis of 162 patients. Am J Med 2014; 127(11): 1118-25.
[http://dx.doi.org/10.1016/j.amjmed.2014.04.034] [PMID: 24835040]

[9] Ray S, Kundu S, Saha M, Chakrabarti P. Hemophagocytic syndrome in classic dengue Fever. J Glob Infect Dis 2011; 3(4): 399-401.
[http://dx.doi.org/10.4103/0974-777X.91068] [PMID: 22224008]

[10] Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. Blood 2011; 118(15): 4041-52.
[http://dx.doi.org/10.1182/blood-2011-03-278127] [PMID: 21828139]

[11] Srichaikul T, Punyagupta S, Kanchanapoom T, Chanokovat C, Likittanasombat K, Leelasiri A. Hemophagocytic syndrome in Dengue hemorrhagic fever with severe multiorgan complications. J Med Assoc Thai 2008; 91(1): 104-9.
[PMID: 18386553]

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CASE REPORT

Dapsone Hypersensitivity Syndrome (DHS): A Detrimental Effect of Dapsone? A Case Report

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Abstract: ***Background*:** Dapsone is a sulfone derived drug used in the treatment of leprosy and several chronic inflammatory dermatological diseases. Dapsone Hypersensitivity Syndrome (DHS) is characterized by fever, hepatitis, generalized exfoliative dermatitis and lymphadenopathy. It is rare and potentially fatal.

***Case Report*:** We present a case report of a 52 years old female with a recent history of antecedent dapsone exposure of 100 mg daily for 2 weeks. She developed fever 10 days after exposure to dapsone therapy and was treated in various primary and tertiary centers for features of sepsis. When she presented to us, clinical features of multi-organ dysfunction and intractable sepsis was evident. She was successfully managed with intravenous corticosteroids and other supportive therapy.This case of DHS is unique due to pulmonary, hepatic and colonic involvement in addition to secondary bacterial and fungal infection, which is associated with an increased risk of mortality. As dapsone is mainstay in the treatment several infections and inflammatory conditions,further research is needed to characterize markers to diagnose DHS and to develop screening policies prior to initiation of dapsone therapy.

Keywords: Dapsone, dapsone hypersensitivity, exfoliative dermatitis.

1. INTRODUCTION

Dapsone belongs to the sulfonamide drug class. It was initially used to treat leprosy and later found to have anti-inflammatory properties as well. At present, it is used in a wide variety of dermatological conditions, especially those with a neutrophil predominance, as dapsone inhibits neutrophil activation and recruitment through a number of pathways. The effective dose of dapsone is 100 mg daily, which can be increased upto a maximum of 300 mg daily [1]. Though dapsone is well tolerated, DHS is said to occur at an incidence of 0.2 to 0.5% in patients receiving dapsone therapy. This drug induced hypersensitivity syndrome also known as DRESS (Drug, rash, Eosinophilia and systemic symptoms) syndrome [2]. It typically occurs as a reaction to drugs like carbamazepine, phenytoin, phenobarbital, allopurinol, sulfasalazine and minocycline, mimicing a severe form of sepsis. DHS is characterized by fever, rash and multi- organ failure. It can cause irreversible organ damage and fatality. The mortality associated with DHS is 12-23%. DHS generally occurs within 1-8 weeks after starting dapsone, though in some cases, it can occur as late as six months after treatment initiation or as early as 6 hours in previously sensitized patients [3].

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2. CASE REPORT

A 52 years old Indian female presented with fever of twenty days duration associated with itching all over her body. She had complaints of breathing difficulty of ten days duration, not associated with cough. She also complained of abdominal pain with loose stools for the past ten days. Her medical and medication history revealed that two months prior to current admission, she was treated with dapsone external application and oral tablet 100 mg daily by a dermatologist for suspected dermatitis. She developed fever and other constitutional symptoms 10 days after exposure to dapsone, then she was referred to a general practitioner. Basic investigations were done and medications were given for symptomatic relief, but patient did not have any relief, later admitted in a tertiary care centre. She was hospitalized for one week, where she developed hepatic dysfunction, renal azotemia, respiratory distress and new exfoliative, itchy and scaly skin leisions started to develop. Later, she was shifted to our hospital.

On physical examination, she was pale, afebrile and had icterus. She was found to have hyperpigmented exfoliative skin lesions all over her body, including the face. She also had bilateral cervical and axillary lymphadenopathy and mild nontender hepatomegaly. Laboratory investigations illustrated the presence of anemia, increasing trend of leucocytes with eosinophil counts compared to prior report elsewhere, elevated liver enzymes, hypoalbuminemia in addition to

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raised lactate dehydrogenase and procalcitonin level. Blood culture was sterile but sputum culture showed moderate growth of budding yeast. Stool and urine cultures were negative. Ultra sonography of abdomen revealed mild hepatomegaly with moderate fatty infiltration, splenomegaly, and right minimal pleural effusion with consolidation. Evaluations for other infectious etiologies were negative, which consisted of hepatitis panel including hepatitis B surface antigen, human immuno-deficiency virus tests, anti-leptospira IgM antibody, weilfelix test and scrub typhus IgM antibody.

A diagnosis of DHS with co-existing secondary sepsis was made, based on the patient's treatment history, exfoliative dermatitis and multiorgan dysfunction with reasonable time relationship to dapsone exposure. As skin leisions were exfoliative and scaly, skin biopsy was not done, and due to patient's financial reasons HLA-B*13:01 analysis was not done.

Systemic hydrocortisone 50 mg every 8 hours was started by intravenous route from the second day. Bronchodilators, antihistamine, antibiotics and antifungal along with supportive therapy were also provided. Besides, she was monitored for dapsone induced secondary seizure.

The erosions on skin started healing 2 days after beginning corticosteroid therapy and no acute symptoms were noted thereafter. After 5 days of systemic corticosteroids, our patient was switched to oral prednisolone 40 mg daily. She was discharged on day 9 with oral corticosteroids, antihistamine and other supportive medications.

Prednisolone was then tapered and stopped over a period of 4 weeks. During follow-up, the patient had a relapse of itchy skin leisions and fever. Hence the oral corticosteroid was restarted at a dose of 40 mg everyday for one month and tapered the dose of 2.5 mg every week till the end of 5 months duration during which the patient had a gradual remission. Till then she was under regular periodic follow-up without any evidence of relapse of the disease.

A causality assessment of this adverse drug reaction was done, which revealed that the causality to be "probable" according to both WHO and Naranjo causality assessment scales.

3. DISCUSSION

Sulfonamides are compounds that contain sulfamoyl group ($-SO_2NH_2$). Absorption of orally administered dapsone from gastrointestinal tract occurs readily, with a bioavailability of about 86%. It is metabolized via either N-hydroxylation or N-acetylation, which leads to toxic intermediate metabolites.These metabolites form haptens resulting in the production of anti-dapsone antibodies.

DHS is thought to be an immune mediated reaction, involving T-lymphocyte activation, macrophage and cytokine release. Dapsone induced delayed hypersensitivity syndrome is directed by dapsone specific T cells. It develops typically between 2 to 8 weeks of dapsone administration and clinical features lasts even after withdrawal of the drug [4]. Our patient developed DHS after ten days from initiation of the drug and clinical features were apparent for more than a month after drug withdrawal.

The skin eruption in DHS is often initially morbilliform which later progresses into exfoliative dermatitis [5]. The skin rash severity is usually associated with fatal outcomes, especially if the damage involves more than 30 percent of the body surface [6]. Such a presentation was seen in our patient since she had lesions all over the body, including the trunk, limbs and face which is associated with an increased risk of mortality.

Hepatic involvement in DHS often demonstrates a mixed hepatocellular and cholestatic pattern. A similar pattern was evident in our patient with a high alkaline phosphatase apart from a modest increase in transaminases. Hyperbilirubinemia in DHS can be attributed to both hepatotoxicity and hemolysis, which is yet another feature of the hypersensitivity syndrome. Due to hemolysis, the lifespan of red cells is reduced and accounts for anemia that manifests in most, if not all patients [7]. Hypoalbuminemia is also reported with DHS probably due to binding of dapsone to the circulating serum albumin. Hypoalbuminemia was apparent in this patient as well [5].

Agranulocytosis caused by DHS, increases the susceptibility to infections thus, accounting for the secondary bacterial and fungal infections in DHS patients including ours [4]. Hence sepsis can be considered as an indirect consequence of the drug reaction. This element of the hypersensitivity syndrome is the one which misleads to an interpretation of infective etiology complicating the diagnosis and the subsequest management, necessitating a high index of suspicion for proper diagnosis and treatment.

Dapsone is known to produce acute eosinophilic pneumonia with hypoxia and pleural effusion [3]. This explains the presence of pleural effusion and severe hypoxemia in our patient which might as well be regarded as an interplay between drug reaction and secondary respiratory infection.

Appropriate management of DHS includes prompt discontinuation of dapsone and introduction of systemic steroids [3]. Since dapsone has a long elimination half life of 24-30 hours and it persists in organs for about 35 days, slow tapering of the corticosteroid therapy is recommended [5]. Besides, this case also bears evidence to the fact that early discontinuation may cause relapse and that adequate duration of steroid treatment is necessary to ensure prompt recovery.

The major route of elimination of dapsone is by N-acetylation which exhibits genetic polymorphism. Dapsone and sulfonamides contain an aromatic group which is readily acetylated by cytosolic hepatic acetyl transferases. Hence, fast acetylators are at a low risk of DHS as a lower proportion of the drug is available for N-hydroxylation. Thus, serious adverse drug reactions to dapsone can arise from an inter-individual pharmacogenetic variation in the drug metabolism, the sequential detoxification and elimination of metabolites [3, 7]. Recent studies show that HLA-B*13:01 allele may be used as a pharmacogenomic marker to prevent severe cutaneous adverse reactions [8]. The presence of this allele in Indians is 1-12% and its absence is associated with 7-fold risk reduction [6].

In addition, susceptibility to DHS can be increased with reduced glutathione reserve, as in conditions like AIDS (Acquired immunodeficiency syndrome). Environmental factors, such as smoking, drugs inhibiting CYP 450 and deficiency of antioxidants, such as vitamin E, vitamin C and selenium can contribute to the risk of developing DHS [9].

Evidence suggests that there is no obligatory cross-reactivity between sulfonamides and other sulfonamide non antibiotic drug, such as dapsone due to the difference in chemical structures between these compounds [10].

CONCLUSION

This case highlights that a high index of suspicion is needed to diagnose DHS. Nevertheless, physicians should be familiar with protypical features of this hypersensitivity syndrome to avoid delay in correct diagnosis. The various systemic manifestations can be easily mistaken for other disorders apart from a misinterpretation of progression of the primary skin disease. This case also delineates the significance of capturing the history of antecent drug exposure. In the current clinical scenario of dapsone being used in numerous conditions on account of its promising steroid sparing effect, it is imperative that the physicians and clinical pharmacists be aware of this potentially life threatening hypersensitivity reaction. Periodic monitoring of patients started on dapsone is necessary to detect early warning signs of DHS. It is also emphasized that early tapering of corticosteroids in DHS is not recommended as it can cause relapse of the manifestations.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This prospective case study was approved by the Institutional Human Ethics Committee PSG IMS&R, Tamil Nadu, India..

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION

Written and informed consent was obtained from the patient for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCE

[1] Evan W. Piette, Victoria P. Werth. Dapsone in the management of the autoimmune bullous diseases. Immunol Allergy Clin North Am 2012; 32(2): 561-64.

[2] Kamal KS, Shyam CC, Jitendra S, Deep CR, Sanjay M, Madhu MG. Dapsone-induced pure red cell aplasia and cholestatic jaundice: A new experience for diagnosis and management. J Res Pharm Pract 2016; 5(3): 215-18.

[3] Kolar VV, Karyampundi A, Tarun KD. Dapsone Hypersensitivity syndrome: A rare life threatening complication of dapsone therapy. J Pharmacol Pharmacother 2013; 4(2): 158-60.

[4] So YC, Ho YH, Jung HL, Jae SP, Min SJ. Severe dapsone hypersensitivity syndrome in a child. Korean J Pediatr 2013; 56(6): 260-64.

[5] Mary G. An unusual case of dapsone syndrome. Indian Dermatol Online J 2011; 2(2): 88-90.

[6] Na W, Leela P, Hong L, Furen Z. A Review on dapsone Hypersensitivity Syndrome Among Chinese Patients with an Emphasis on Preventing Adverse Drug Reaction with Genetic Testing. Am. J. Trop. Med. Hyg., 2017; 96(5): 1014-18.

[7] Coleman MD, Breckenridge AM, Park BK. Bioactivation of dapsone to a cytotoxic metabolite by human hepatic microsomal enzymes. Br. J. Clin. Pharmac 1989; 28: 389-95.

[8] Tempark T, Satapornpong P, Rerknimitr P, et al. Dapsone-induced severe cutaneous adverse drug reactions are strongly linked with HLA-B*13:01 allele in the Thai population. Pharmacogenet Genomics 2017; 27: 429-37.

[9] Semaan GK, Bhuvana G, Dima NN, David SC, Guhan K. The Dapsone Hypersensitivity Syndrome Revisited: a potentially fatal multisystem disorder with prominent hepatopulmonary manifestations. J Occup Med Toxicol 2006; 1(9).

[10] Gottfried W, Christian B. Arch Dermatol Res 2014; 306: 130-24.

To err is…Drug? Patient? Doctor? Healthcare System?

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ABSTRACT

Medicine is a practicing art. This is because we evolve constantly with everyday learning. The core of the health care system is management of patients with pharmacological measures. However the role of the health care deliverers does not stop with prescribing drugs. It is of central importance to be aware of the potential side effects of the drug including the rarest of the complications. It is equally important to discuss with our patients the available choices, the risk - benefit ratio and counsel them accordingly regarding anticipated common and rare side effects. The patient must be repeatedly reinforced about the importance of periodic follow up with the doctor, need for regular laboratory monitoring and reporting of alarm signs without any delay. Thus patient education, awareness of drug characteristics and a detailed doctor prescription are central in avoiding as well as managing drug related adverse effects. Here we present a case series of 9 different patients presenting with varied drug related adverse effects.

Key words: Adverse drug reactions, Periodic Monitoring, Patient education, Warning signs, Physician role.

INTRODUCTION

The European Medicines Agency (EMA) defines an adverse drug reaction as "a response to a medicinal product which is noxious and unintended.[1] The US Food and Drug Administration (FDA) defines an adverse drug reaction as any untoward medical occurrence associated with the use of a drug in humans "for which there is a reasonable possibility that the drug caused the adverse event."[2] Most of the drug reactions usually go underreported. Doctor prescription, drug characteristics and patient education are the three vertices of the adverse drug management triangle.

Drug reactions could be highly characteristic or nonspecific.[3] They could be dose, frequency or time dependent, occurring either almost right away or much later. Polypharmacy is a clear culprit in the occurrence of adverse drug reactions.

Patient factors like age, gender, creatinine clearance, body weight and fat distribution, race, ethnicity, genetic susceptibility, smoking and alcoholism are well known factors contributing to adverse drug reactions.[4] Patient education of drug intake, timing with other drugs, other co-morbidities, alertness in early recognition and reporting of warning signs are also of paramount importance.

A physician should be insightful in drug prescription fully taking into consideration previous drug histories and other simultaneous drugs the patient is on. It is wise to restrain hurry while diagnosing the disease or prescription even on a crowded day practice. It is prudent to refer the patient when required or have a close follow up while in doubt.

CASE REPORT

Case 1

A 58 yr old male patient presented with history of fever, loose stools and abdominal discomfort for 1 week. Patient was diagnosed

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with Myasthenia gravis 3 months ago elsewhere and started on immunosuppressants with oral Prednisolone 5mg once daily, Azathioprine 50mg once daily and Pyridostigmine 60mg thrice daily.

His examination was normal except for right eye ptosis. His initial blood counts showed pancytopenia as shown in Table 1. Bone marrow biopsy was suggestive of myelosuppression. With the diagnosis of drug induced pancytopenia, azathioprine was withheld and he was started on broad spectrum antibiotics and antifungal agents. Patient was given an injection of Granulocyte-Colony Stimulating Factor (G-CSF), following which his counts steadily improved. 2 weeks later patient reported with normal blood counts and patient was put on Mycophenolate mofetil along with pyridostigmine and steroids. Currently the patient's myasthenic symptom has improved and is maintained on oral prednisolone 10 mg once daily.

Case 2

27 year old female was admitted with history of fever for 3 days. Patient was evaluated elsewhere for a right sided neck swelling 1 month ago. A Computed Tomography (CT) Angiogram of the chest had shown Aortoarteritis changes suggestive of vasculitis for which she was treated with oral Azathioprine 50 mg once daily and oral Methotrexate 10 mg weekly.

On admission she was pale and tachypnoeoic tachypnoeic with gingival petechial petechiae. Her blood counts showed severe pancytopenia (Table 1). With a clinical diagnosis of drug induced bone marrow suppression, patient was put on strict barrier nursing with empirical antibiotics and antifungal agents. Patient required supportive transfusion with packed red cells, platelets and G - CSF in view of profound pancytopenia. However a week later, patient developed persistent dry cough, left peri orbital hematoma, hoarse voice, hypotension and stridor. A chest imaging and tracheal aspirate were suggestive of Aspergillus. Patient was put on mechanical ventilation and high end antibiotics and antifungal agents but eventually succumbed to refractory septic shock.

Case 3

58 year old female patient was admitted with history of recurrent nasal and gum bleeding for 1 month. Patient was diagnosed with *Pemphigus vulgaris* and had been taking Methotrexate 7.5mg weekly once for 10 years.

On examination, patient was pale and had non pruritic, non-blanchable multiple hyper pigmented maculae all through the body. Initial investigations as displayed in Table 2 showed pancytopenia and subsequently a bone marrow biopsy was suggestive of myeloid suppression with no atypical cells. Patient was started on intravenous antibiotics and transfused with packed red cells and platelets. Further the skin lesions were suggestive of psoriasis and patient was managed with antihistaminic drugs and topical applications. A month later patient reported with normal counts and is doing well on topical steroids and moisturizers.

Table 1: Showing the bicytopenia pattern in case 1 and subsequent improvement; case 2 showing persistent pancytopenia.

		CASE 1				CASE 2				
Lab parameter	Reference range	Day 1	After G-CSF	2 weeks later	1 year later	Day 1	Day 3	Day 5	Day 9	Day 15
Hemoglobin	13 - 17g/dL	10.2	10.9	14.8	9.6	3.8	7.5	9.5	9	6.3
Total white blood count (WBC)	4-10x10^3/uL	0.8	6.8	6.6	8.4	0.7	1.3	1.7	2.2	0.6
Platelets	150-400x10^3/uL	261	556	247	212	22	9	3	10	11

Table 2: Showing the drug induced pancytopenia in case 3, 4 and 5 with subsequent improvement.

Lab parameter	CASE 3				CASE 4					CASE 5				
	Day 1	Day 5	A month later	A year later	Day 1	Day 3	Day 6	2 weeks later	2 months later	6 months prior to treatment	Day 1	Day 2	2 days after G-CSF	1 Month later
Hemoglobin	7.1	8.2	8.4	10.0	8.2	9	10.1	8.0	13.2	8.5	4.4	5.3	6.5	9.9
White blood count	2.1	3.9	14.7	6.8	0.3	1.9	4.3	10.5	6.2	8.5	3.1	1.8	8.0	6.7
Platelets	4	86	187	211	30	37	65	617	242	246	75	53	117	153

Case 4

68 year old male patient was admitted with complaints of fever, productive cough and breathlessness for 2 days. Patient was a known case of Rheumatoid arthritis on oral Methotrexate 5 mg weekly for 8 years.

On admission, patient was severely dyspnoeic and tachypnoeic, had bilateral lung crepitations with Varicella zoster skin lesions. His initial investigations showed pancytopenia (Table 2). Patient was treated with empirical antibiotics, antifungal and antiviral drugs. He was supported with blood products transfusion and G-CSF. His tracheal and urine cultures revealed multidrug resistant *Klebsiella pneumonia*. Patient had a tumultuous hospital stay where he required reintubation after weaning owing to excessive oropharyngeal secretions which was further complicated by an episode of atrial fibrillation reverted by cardio version. After aggressive management with sensitive antibiotics and ventilator support, patient improved symptomatically and he was able to mobilize around in wheel chair. Patient was discharged with Hydroxychloroquine tablet 200 mg once daily and reported on follow up with improved counts and no active inflammatory signs.

Case 5

51 year old female patient was admitted with history of generalized fatigability, exertional dyspnoea and swelling of legs for 10 days. She was diagnosed with Rheumatoid arthritis for the last 2 years. Initially the patient had taken native medication for her joint pain and then subsequently started elsewhere on weekly Methotrexate dose of 5 mg, Leflunomide 10 mg daily and 5 mg once daily prednisolone for the last 6 months. On examination, the patient was pale, had glossitis, bilateral pitting pedal edema and crepitations in the lungs. Initial investigations (Table 2) showed pancytopenia. On the suspicion of drug induced pancytopenia, methotrexate and leflunomide were withheld. Patient was started on broad spectrum antibiotics and antifungal agents with strict barrier nursing. Bone marrow biopsy showed hypo cellular marrow with no atypical cells. The patient was administered one dose of G-CSF and 2 units packed red cells were transfused. Her blood counts gradually started improving and patient was discharged with low dose oral prednisolone and is maintained on the same on follow up.

Case 6

59 year old female patient presented with breathlessness, decreased urine output, swelling of legs for the last 2 days. Patient had started developing facial puffiness a month ago for which she was suggested thyroid function tests elsewhere. Her Thyroid Stimulating Hormone (TSH) levels were 6 microIU/ml and patient was started directly on 100 micrograms (mcg) Levothyroxine.

On examination, the patient was tachypnoeic, had anasarca and extensive bilateral lung crepitation's. Her cardiac markers were elevated and an echocardiogram showed mild pericardial effusion with preserved ejection fraction and cardiomegaly. A diagnosis of levothyroxine induced heart failure was arrived at and patient was treated with noninvasive ventilation, diuretics and her Levothyroxine dose was brought down to 50 micrograms/day. Patient gradually improved and was discharged with 50 mcg of Levothyroxine. On follow up her dose was gradually increased by 12.5 mcg and patient is doing well on tab. Levothyroxine 75 micrograms per day.

Case 7

A 29 yr old female patient with no known previous co morbidities came with complaints of generalized swelling of body, decreased urine output and orthopnea for 1 week. Patient was apparently normal 3 months back when she started having hair fall, mild facial puffiness, dyspnea on exertion and dryness of skin but was able to attend to her household work. She had consulted elsewhere, her TSH levels were 5.3 micro IU/ml and the patient was started on tablet Levothyroxine 100 mcg following which her breathlessness exaggerated.

On examination, the patient was dyspnoeic, tachypnoeic, had anasarca and oral ulcers with lung crepitations. A clinical diagnosis of a volume overload status due to Levothyroxine induced heart failure with underlying connective tissue disease was arrived at and Levothyroxine was stopped. Initial investigations showed mild neutrophilic leukocytosis, elevated ESR with severe nephrotic range proteinuria as Table 3. Her Antinuclear Antibody (ANA) pattern was 3+ with hypocomplementemia. An echocardiogram showed grade 1 diastolic dysfunction. Patient was started on diuretics, noninvasive ventilation, intravenous Methylprednisolone 250 mg injection (3 doses) and albumin infusion. Patient subsequently improved; a week later patient developed sudden breathlessness, a CT pulmonary angiogram showed a left sub segmental pulmonary thromboembolism and antiphospholipid antibodies were also positive. Patient was administered anticoagulation and improved well. Patient was discharged a week later with oral prednisolone 40 mg once daily, oral anticoagulants and Hydroxychloroquine (HCQ). A year later patient is doing well on follow up with normalized disease markers and is on oral Prednisolone 5 mg once daily and Mycophenolate mofetil 500 mg twice daily.

Case 8

45 yr old female patient was admitted with history of fever and cough for 1 week. She was a known case of endometrial carcinoma on chemotherapy. She had undergone her 5th cycle with Paclitaxel a week ago. Patient also had underlying diabetes mellitus and chronic kidney disease, which were conservatively managed.

On examination, the patient was pale, tachypnoeic and febrile with bilateral lung crepitations. Investigations showed severe metabolic acidosis, profound pancytopenia, high procalcitonin and deranged renal functions as summarized in Table 4. Chest x-ray showed left lower lobe pneumonia. A diagnosis of febrile neutropenic sepsis was made and patient was put on aseptic precautions and barrier nursing. She required aggressive management with Granulocyte Colony Stimulating Factor, antibiotics, antifungals, antivirals, antipyretics, bicarbonate infusion and other supportive measures. Patient gradually improved and was discharged with oral antibiotics and had normalized blood counts on follow up.

Case 9

50 yr old male presented with progressive difficulty in walking over the last 8 months. He was diagnosed with retroviral disease 8 years ago and was on Tenofovir, Lamivudine and Ritonavir boosted Atazanavir for the last 4 years.

On examination, the patient was emaciated and decreased range of movements in the lower limbs due to pain. Initial investigations showed hypokalemia, hypophosphatemia with significant metabolic acidosis. Urine showed albuminuria (3+) and glycosuria (3+). His liver functions also showed deranged alkaline phosphatase levels and vitamin D levels were normal (Table 5). Subsequently an X-Ray and Computed tomography of the hips showed bilateral fracture neck of femur. Hence a diagnosis of Tenofovir induced proximal renal tubular acidosis and bone toxicity was arrived at and tenofovir was promptly withheld. Patient was managed with intravenous fluids, potassium and bicarbonate supplementation with thiazide diuretics. Bilateral open reduction with internal fixation was planned, however patient opted conservative management. His HIV viral load and CD4 counts were in the improving trend compared to previous year values. After hypersensitivity testing, patient was started on Abacavir / Lamivudine and Doultegravir regimen and was discharged with oral potassium and bicarbonate supplements. Patient reported to follow up with normal electrolytes and is attending to activities of daily living.

DISCUSSION

Drug related adverse effects are thus not an uncommon entity. Rather it is grossly underreported. This case series has observed drug reactions in 9 patients who were admitted in PSG Institute of Medical sciences and

Table 3: Showing the thyroid pattern at admission, nephrotic range proteinuria and hypocomplementemia in case 7.

Lab parameter	At admission	1 month after discharge	A year later	Reference range
T3 (Serum)	0.37	1.06		0.846 - 2.02 ng/mL
FT4 (Serum)	0.98	1.12		0.932 - 1.71 ng/dL
TSH (Serum)	0.484	1.68		0.27 - 4.2 microIU/ml
FT3 (Serum)	0.64	2.92		2-4.4 Pg/ml
Urine Protein Creatinine Ratio	27.33	10.108	0.070	
Serum Creatinine	0.83			0.66 - 1.09 mg/dL
Serum albumin	0.7 g/dl	1.5	3.4	3.4 - 4.8 g/dL
Complement 3c level	28.42mg/dl	79	102	90 - 180 mg/dl
Complement 4 level	10mg/dl	14	16	10 - 40 mg/dl

Table 4: Showing the pancytopenia and subsequent improvement with treatment in case 8.

Lab parameter	Day of admission	After 1st dose G-CSF	After 3rd dose G-CSF	Day of discharge	10 days after discharge	Reference Range
Creatinine mg/dl	3.6	3.4		2.83	2.6	0.66-1.09 mg/dl
Hemoglobin g/dL	6.7	6.4	5.5	7.6	9.0	13 - 17g/dL
Total WBC 10^3/microL	0.2	0.5	5.1	10.1	5.7	4-10x10^3/uL
Absolute Neutrophil Count 10^3/uL	0.0	0.2	4.7	9.2	4.5	2 - 7x10^3/uL
Plateletsx10^3/uL	64	63	61	82	324	150-400x10^3/uL

Table 5: Showing the hypokalemia and metabolic acidosis pattern in case 9.

Lab parameter	Day 1 of admission	Day 3	Day 5	Month after discharge	Reference range
Serum Creatinine	1.75	1.26	1.6	1.43	0.8 - 1.25 mg/dl
pH	7.230	7.342	7.334	7.33	7.35-7.45
Serum Potassium	2.45	4.6	3.0	3.66	3.5 - 5.0mEq/L
Serum bicarbonate	12.5	15.9	17	21.6	22 - 30mEq/L
Serum ionised Calcium	1.202			1.2	1.1-1.3m mol/L
Serum Phosphous		2.1			2.7-4.5 mg/dl
Serum Alkaline phosphatase	459				40 – 129 U/L

Research, a tertiary care hospital in Coimbatore, Tamil Nadu. They were either referred from their primary care physician and/or admitted in our emergency department with severe drug related adverse events. The discussed 9 patients were on different class of drugs for their respective diagnosis prescribed elsewhere and presented to us with a variety of adverse effects requiring hospitalization or developing grave complications. Fortunately 8 of them recovered with prompt diagnosis and withdrawal of the offending drug.

Azathioprine is an imidazolyl derivative of mercaptopurine which acts by blocking the pathway for purine synthesis. The 6-thioguanine nucleotide metabolites appear to mediate the majority of azathioprine's immunosuppressive and toxic effects. Azathioprine, though a well-established immunosuppressive drug for myasthenia showing improvement in 70-90% patients has its share of serious complications.

Of grave concern is haematological toxicity. Dose-related immediate or delayed severe hematologic toxicities in the form of leucopenia, thrombocytopenia or pancytopenia may occur. Panda BK *et al.* have reported a case of severe pancytopenia in a patient of myasthenia gravis following azathioprine.[5] Other side effect profile includes gastrointestinal disturbances, hepatotoxicity and carcinogenic potential. Therefore it is important to periodically monitor blood counts and educate the patient about the need for the same.

In our case 1, the patient was not aware of the potential complication of his drugs, thereby delayed presenting to the hospital until he had profound leucopenia. With a proper patient education, early diagnosis can be arrived at and prompt action can avoid grave consequences. Case 2 patient was co-prescribed with azathioprine and methotrexate - both contributing to her profound pancytopenia. Patient had a tumultuous hospital course warranting intensive care admission, developed grave fungal and bacterial infections and succumbed to the same. Therefore it is crucial to carefully evaluate and closely follow up patients while co prescribing 2 potential hematotoxic drugs.

Thiopurine methyltransferase (TPMT) genotyping or phenotyping and nudix hydrolase 15 (nucleotide diphosphate [NUDT15]) genotyping may help to identify patients who are at an increased risk for developing azathioprine related hematological toxicity.[6] Once identified, patients may require dose reduction, interruption or selection of an alternate agent altogether.

Methotrexate being a folate antimetabolite inhibits dihydrofolate reductase, thereby hampering purine and thymidylic acid synthesis. DNA synthesis, repair and cellular replication are thus hindered as methotrexate targets actively proliferative tissues more and is therefore used in a number of conditions like rheumatoid arthritis, psoriasis as well as certain malignancies.

Thrombocytopenia (3% to 10%), leukopenia (1% to 3%), pancytopenia (1% to 3%), agranulocytosis, anemia, aplastic anemia, bone marrow suppression are noted hematological adverse effects of methotrexate. That is why it is pertinent to closely monitor the complete blood counts prior to initiation, weekly in the first month of initiation, every 2 to 4 weeks in the first few months and monthly thereafter. In a study of 46 patients on methotrexate in Lucknow, India, it was noted that initial WBC count was a predictor for development of pancytopenia later.[7] Patients with renal insufficiency or older age will require frequent monitoring. Case 3 (a female patient) and case 4 (older age group) had been taking only low dose methotrexate in weekly schedule for years, yet presented with pancytopenia revealing the fact that even low dose methotrexate can cause pancytopenia as a delayed presentation and periodic monitoring is indispensable. These findings are similar to a case series report on methotrexate where pancytopenia was encountered as a later manifestation[8] and another case report which observed low dose methotrexate causing

pancytopenia.[9] Once hematotoxicity is encountered, the drug is withheld, serum methotrexate levels are monitored and leucovorin rescue dosing is done to combat methotrexate effects. Granulocyte Colony stimulating factors will also be required where the patient has persistent pancytopenia. Hepatotoxicity and lung toxicity also require close attention and a baseline work up for the above is mandatory before starting methotrexate.

Leflunomide is an immunomodulatory agent that inhibits pyrimidine synthesis, resulting in its antiproliferative and anti-inflammatory effects. It acts by inhibiting dihydroorotate dehydrogenase and is converted to the active metabolite A771726 on administration.[10] Usually used in rheumatoid arthritis and systemic lupus erythematous. In November 2003, FDA issued a drug safety alert update on leflunomide, warning of rare post marketing reports of bone marrow suppression and sepsis. Though rare (incidence of 1 in 3698 patients) the incidence of pancytopenia is potentiated by concomitant administration of other myelotoxic agents as in case 5 where the patient was on both methotrexate and leflunomide. And myelosuppression can persist even after discontinuing the drug owing to its long half-life. A number of case reports reinforce the fact that hematological toxicities of methotrexate and leflunomide combination therapy are deleteriously additive.[11]

Levothyroxine (T_4) is a synthetic form of thyroxin, which is an endocrine hormone secreted by the thyroid gland. T_4 is converted to its active metabolite, L-triiodothyronine (T_3). Thyroid hormones (T_4 and T_3) then bind to thyroid receptor proteins in the cell nucleus and exhibit their metabolic actions through control of DNA transcription and protein synthesis.

While a decision to start a patient on levothyroxine is arrived at, certain characteristics should be carefully checked. These include patient's weight, lean body mass, etiology of hypothyroidism, pregnancy, age and cardiac disease. Thyroid hormone can be started at anticipated full replacement doses in individuals, who are young and otherwise healthy in most situations, a starting levothyroxine dose of 50-75 μg/day (1.6 μg/kg/day) will suffice. In patients with coronary heart disease, initial dose should be 12.5 to 50 μg/day.[12] Aggressive replacement of thyroid hormone in initial higher doses may worsen cardiac function in patients with existing cardiac disease. That is why it is imperative to start these patients on smaller doses and then titrate in small increments after no less than 4-6 weeks. Achieving a target TSH level at times may take several months owing to delayed preadaptation of the hypothalamic-pituitary axis. Therefore in patients receiving treatment with Levothyroxine, dosing changes should be made every 6-8 weeks only in usual scenarios.

In our case 6, 59 yr old woman was forth right put on high dose levothyroxine 100 micrograms for a TSH value of 6 microIU/ml exacerbating her heart failure. In case 7, though the patient had an underlying connective tissue disease, she was able to attend to her activities of daily living. However challenging her with levothyroxine 100 micrograms for a TSH value of 5.13 microIU/ml clearly exaggerated her symptoms precipitating her cardiac failure resulting in emergency presentation and subsequent prolonged hospitalization, which could have been avoided.

Treatment with levothyroxine in patients with heart failure is associated with a higher risk for all-cause mortality, cardiovascular death and major adverse cardiac events according to findings published in The Journal of Clinical Endocrinology and Metabolism.[13] These events are attributed to the low T3 syndrome. When hypothyroidism is treated with levothyroxine, there is often a relatively high thyroxin/T3 ratio in serum with relatively low liothyronine levels in blood, which could further exacerbate the T3-deficient state in patients with chronic heart failure

Paclitaxel is a natural taxane promotes microtubule assembly by enhancing the action of tubulin dimers, stabilizing existing microtubules and inhibits their disassembly. These actions ultimately halt cell replication thereby modulating the desired immune response. Paclitaxel finds usage in a variety of conditions notably breast, ovarian malignancies, lung cancer and Kaposi sarcoma. Our index patient case 8 was on paclitaxel for endometrial carcinoma. The hematological adverse effects include neutropenia (78% to 98%), leukopenia (grade 4: 17%), anemia (47% to 90%), thrombocytopenia (4% to 20%). It is therefore crucial to regularly monitor the complete and differential blood counts of the patient while on chemotherapy.

Chemotherapeutic agents do exaggerate the immune compromised state of cancer affected patients. Usually the magnitude of the neutrophil mediated component of the inflammatory response may be muted in neutropenic patients with fever sometimes being the only sign of infection. It is critical to recognize neutropenic fever early and to initiate empiric therapy promptly in order to avoid progression to a sepsis syndrome and possibly death. Neutropenic patients have the risk of developing a neutropenic fever syndrome, risk of resultant medical complications as well as risk of non-response to empiric antibiotic treatment.[14]

Certain risk factors are predictive of the development of a neutropenic fever syndrome. Patient-related predictors include age> 65 yrs, female sex, high body surface area and poor nutritional status. Disease-related predictors include elevated lactate dehydrogenase, advanced malignancy staging and myelophthisis. Treatment-related predictors of neutropenic fever include administration of high dose chemotherapy regimens and failure to administer hematopoietic growth factor support to patients receiving high-risk regimens. High risk patients are those who have other co morbidities, significant hepatic or renal impairment or those who are expected to be severely neutropenic (absolute neutrophil count [ANC] <500 cells/microL) for >7 days. The index patient case 8 was a female patient with diabetes mellitus and chronic kidney disease enhancing her risk of pancytopenia.

A number of validated tools are used to calculate the risk index for measuring the risk for neutropenic fever–related medical complications and must be put to effective use wherever possible in tertiary centres.[15] In a research article that investigated the capability of genetic variation in drug metabolizing enzymes and transporters (DMET platform) to predict haematological toxicity, the single nucleotide polymorphism SNP rs 9285726 in MAT1A (methionine adenosyl transferees) and SNP rs 776173 in CYP39A1 were found to be associated with neutropenia. These are potential areas of research in predicting paclitaxel induced hematologoxical toxicity and in picking up individual patients who are at more risk.[16]

Tenofovir disoproxil fumarate (TDF) is a nucleotide reverse transcriptase inhibitor. It interferes with the HIV viral RNA dependent DNA polymerase resulting in inhibition of viral replication exhibiting its action via active tenofovir diphosphate. Nephrotoxicity is a known and established side effect of tenofovir. One study reported that the incidence rate of moderate or severe renal insufficiency caused by tenofovir was 29.2 and 2.2 cases per 1000 person-year respectively. Apart from this tenofovir can also cause interstitial nephritis, lactic acidosis, nephrogenic diabetes insipidus and rhabdomyolysis and decreased bone mineral density.[17]

Tenofovir can rarely cause Fanconi syndrome.[18] It is a proximal tubulopathy causing impaired reabsorption of glucose, aminoacids, bicarbonate and phosphate resulting in their excretion. The presentation of fanconi syndrome can be quite delayed also in a patient on tenofovir.[19] Therefore drug related adverse effect should be considered in any patient presenting with renal tubular acidosis type 2 while on tenofovir regardless of the duration of drug exposure.

Our case 9 patient also developed toxicities 4 years after tenofovir exposure and developed Fanconi's syndrome as well as bone toxicity which is quite rare.[20] The index patient however improved remarkably after stopping Tenofovir and with calcium and phosphate supplementation. This finding is similar to a study from Christian Medical College, South India where the incidence of tenofovir induced renal tubular toxicity has been observed as quite rare (3.6%) and the average latency period between tenofovir exposure and nephrotoxicity was prolonged (42 months).[21] The study suggests that patients on tenofovir should be monitored serially and periodically for proteinuria, creatinine clearance rather than creatinine values, hypophosphatemia and alkaline phosphate levels. Tenofovir alafenamide is the pro-drug of TDF which achieves higher intracellular concentrations rather than plasma concentrations at low dose, thereby resulting in lower toxicities and similar efficacy.

CONCLUSION

Thus it is important to choose and prescribe drugs and their dosage carefully. It is fundamental for the treating doctor to be aware of drug combination/interactions leading to profound side effects. Patient education and awareness of drug related side effects is core in management and helps in averting serious side effects earlier and thereby avoiding unnecessary/prolonged hospitalization and related complications. As a physician it is pertinent to constantly update and learn from errors whether of one's self or others. After all learning and unlearning is part of the continuing medical education process.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ANA: Antinuclear antibody; **HCQ:** Hydroxychloroquine; **TSH:** Thyroid stimulating hormone; **CSF:** Colony stimulating factor; **TPMT:** Thiopurine methyltransferase; **NUDIT:** 15 nucleotide diphosphate 15; **ANC**: Absolute neutrophil count; **TDF:** Tenofovir disoproxil fumarate.

REFERENCES

1. Guideline on good pharmacovigilance practices (GVP): Annex I – Definitions (Rev 4). European Medicines Agency and Heads of Medicines Agencies. 2017. www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/05/WC500143294.pdf.[cited 2019october 19]
2. Investigational New Drug Application Safety Reporting. US Food and Drug Administration. 21CFR312.32. 2019. [cited 2019 Oct 19). Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32.
3. Ferner RE, McGettigan P. Adverse drug reactions. BMJ. 2018;363:k4051.

4. Alomar MJ. Factors affecting the development of adverse drug reactions. Saudi Pharmaceutical Journal. 2014;22(2):83-94.
5. Panda BK, Umarje S, Diwan A. Azathioprine-Induced Pancytopenia and Septic Complications: A Probable Cause of Death. J Pharm Pract. 2018;31(5):510-3.
6. Kim JC, Kim YK, Hyun HS, Park EJ, Kang HG, Ha S, *et al*. A Case of Azathioprine Induced Severe Myelosuppression and Alopecia Totalis in IgA Nephropathy. Childhood Kidney Diseases. 2017;21(1):35-9. doi: 10.3339/jkspn.2017.21.1.35
7. Ajmani, Preet YS, Prasad S, Chowdhury A, Aggarwal A, Lawrence A, *et al*. Methotrexate-induced pancytopenia: A case series of 46 patients. Int J Rheum Dis. 2017;20(7):846-51.
8. Lim AYN, Gaffney K, Scott DGI. Methotrexate-induced pancytopenia: Serious and under-reported? Our experience of 25 cases in 5 years. *Rheumatology*. 2005;44(8):1051-5.
9. Rajnics P, Kellner VS, Kellner A, Karadi E, Kollar B, Egyed M. The Hematologic Toxicity of Methotrexate in patients with Autoimmune Disorders. J Neoplasm. 2017;2(1):1-6.
10. Breedveld FC, Dayer J. Leflunomide: Mode of action in the treatment of rheumatoid arthritis. Annals of the Rheumatic Diseases. 2000;59(1):841-9.
11. Qu C, Lu Y, Liu W. Severe bone marrow suppression accompanying pulmonary infection and hemorrhage of the digestive tract associated with leflunomide and low-dose methotrexate combination therapy. J Pharmacol Pharmacother. 2017;8(1):35-7.
12. Einfeldt MN, Olsen AS, Kristensen SL, Khalid U, Faber J, Torp-Pedersen C, *et al*. Long-Term Outcome in Patients With Heart Failure Treated With Levothyroxine: An Observational Nationwide Cohort Study. The Journal of Clinical Endocrinology and Metabolism. 2019;104(5):1725-34.
13. Pearce SHS, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S, *et al*. ETA guideline: Management of subclinical hypothyroidism. Eur Thyroid J. 2013;2(4):215-28.
14. Eric B. Risk assessment of adults with chemotherapy-induced neutropenia. Up to Date. 2019. Retrieved October 20. https://www.uptodate.com/contents/risk-assessment-of-adults-with-chemotherapy-induced-neutropenia
15. Lyman GH, Kuderer NM, Crawford J, Wolff DA, Culakova E, Poniewierski MS, *et al*. Predicting individual risk of neutropenic complications in patients receiving cancer chemotherapy. Cancer. 2011;117(9):1917-27.
16. Nieuweboer AJ, Smid M, DeGraan AJ, Elbouazzaoui S, DeBruijn P, Martens JW, *et al*. Predicting paclitaxel-induced neutropenia using the DMET platform. Pharmacogenomics. 2015;16(11):1231-41.
17. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Niño MD, Izquierdo MC, Poveda J, *et al*. Tenofovir Nephrotoxicity. AIDS Research and Treatment. 2011. Article ID 354908. https://doi.org/10.1155/2011/354908.
18. Mathew G, Knaus SJ. Acquired Fanconi's syndrome associated with tenofovir therapy. J Gen Intern Med. 2006;21(11):C3-5.
19. Luni FK, Khan AR, Prashar R, Vetteth S, Duggan JM. Fanconi Syndrome and Antiretrovirals: It Is Never Too Late. Am J Ther. 2016;23(2):e558-60.
20. Lucey JM, Hsu P, Ziegler JB. Tenofovir-related Fanconi's syndrome and osteomalacia in a teenager with HIV. BMJ Case Reports. 2013;bcr2013008674.
21. Mitra S, Priscilla R, Rajeev K, Sauradeep S, Rajkumar S, Cherian AO. Renal tubular dysfunction associated with tenofovir therapy. J Assoc Physicians India. 2014;62(7):580-2.

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Tenofovir induced delayed nephro-osteo toxicity – a case report

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Abstract

Tenofovir disoproxil fumarate (TDF) is the foundation nucleotide reverse-transcriptase inhibitor and the recommended first-line regimen for all naive Human Immunodeficiency Virus-1 (HIV-1) patients whose age is more than 10 years and body weight is more than 30 kg. Although it has a good safety profile overall, nephrotoxicity is a concern and its overall incidence is 1–6% with a long period of clinical latency. Nephrotoxicity may manifest as either proximal renal tubule dysfunction in the form of a partial or complete Fanconi syndrome or as decreased renal function leading to acute or chronic kidney injury. Osteomalacia can also develop secondary to complicating hypophosphataemia and low calcitriol levels. Here we report a 50-year-old HIV-positive male on tenofovir who presented with proximal renal tubular acidosis and fracture of left neck of femur four years after initiation of the drug.

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Introduction

Tenofovir disoproxil fumarate (TDF) is an oral acyclic nucleotide analogue reverse transcriptase inhibitor used in the treatment of HIV-1 infection approved by the Food and Drug Administration (FDA) in 2001.[1] Tenofovir has found a major role in the fixed-dose combination along with lamivudine and efavirenz or doultegravir.[2] It has an added advantage in HIV-hepatitis B co-infection. It has an overall good safety profile, a relatively long half-life and allows once-daily dosing ensuring good patient compliance with relatively rare side effects.

Tenofoviris is actively transported into the mitochondria of proximal tubule cells of the kidney and thereby can lead to proximal tubular dysfunction in long-term use.[1] Proximal renal tubular acidosis is characterised by defective proximal tubular reabsorption associated with phosphaturia, glycosuria, aminoaciduria, bicarbonaturia, resulting in hypophosphataemia, hypokalaemia and metabolic acidosis. Overt Fanconi syndrome is rare, but can occur as a delayed presentation, with incidence being 1.09 per 1000 patient years.[3]

Osteotoxicity can occur either due to direct effect of the drug itself or secondary to hypophosphataemia as a result of renal toxicity.[4] Hence routine and careful monitoring for these toxicities is absolutely essential as they may occur even after a long asymptomatic period.

Case presentation

A 50-year-old male presented with poor appetite, significant weight loss of 6 kg in 2 months and progressively painful difficulty in walking over 8 months. He was diagnosed with HIV-1 8 years ago and was on regular treatment with tenofovir, lamivudine, atazanavir and ritonavir for 4 years elsewhere. CD4 count done 3 months previously was 118 cells/mm3. He took diclofenac 50 mg intermittently over the previous 2 months.

On examination, he was thin, pale and had a limited range of movements in both lower limbs and left shoulder due to pain. His body mass index (BMI) was 16.5k g/m2.

Preliminary investigations showed macrocytic normochromic anaemia, elevated creatinine levels, hypokalaemia, hypophosphataemia, low bicarbonate levels and elevated serum alkaline phosphatase (ALP) levels. Arterial blood gas analysis revealed metabolic acidosis. Urine examination showed mild albuminuria and glycosuria (serum glucose was normal) with no pus cells. Results are shown in Table 1. Ultrasonography of the abdomen and pelvis revealed chronic renal parenchymal changes and renal artery Doppler study was normal.

X-ray pelvis showed an undisplaced fracture in the left neck of femur and an old displaced fracture in right neck of femur

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Table 1

Lab parameters	DAY 1	DAY 2	DAY 3	DAY 5	2 WEEKS AFTER DISCHARGE	1 MONTH AFTER DISCHARGE	Reference range
pH	7.23	7.259	7.266		7.34		7.35-7.45
Acid base excess mmol/L	-14.4	-12.2	-11.6	-8.8			-2 - +3
Serum Sodium (mEQ/l)	139		144	143	138	143	136-146
Serum Potassium (mEQ/l)	2.45	3.22	3.24	4.60	5.5	3.66	3.5-5.0
Serum Bicarbonate (mEQ/l)	12.5	14.7	14.2	15.9	15.7	21.6	22-30
Serum Chloride (mEQ/l)	107		105			105	102-109
Serum phosphorous (mg/dl)		1.3		2.1	4.7		2.7-4.5
Serum Creatinine (mg/dl)	1.75			1.24		1.26	0.8-1.25
Creatinine clearance (mL/min)	39.29			55		54.56	97-137
Serum ALP(U/l)	459						40-129
Serum Vitamin D (ng/ml)	31.08						Deficient <20 Insufficient 21-29 Normal 30-70
Urine pH	6.8			>9.0			4.6-8.0
Urine glucose		4+			3+	1+	negative
Urine albumin		3+				1+	negative

confirmed on computed tomographic scanning. Serum vitamin D levels were normal. HIV viral load was undetermined and CD4 counts were 327.

Tenofovir-induced proximal renal tubular acidosis and pathological fracture was suspected and tenofovir withheld. He was treated with intravenous fluids, appropriate bicarbonate correction, thiazide diuretics and potassium citrate and his acidosis gradually improved. He was discharged on a high-potassium diet with oral potassium citrate, calcium and oral sodium bicarbonate supplements. At follow-up two weeks later he had an improving appetite and decreased pain in his legs. His electrolytes were normal with decreasing trend of creatinine levels. He was started on abacavir after hypersensitivity testing with HLAB5701 along with lamivudine and doultegravir. His bicarbonate, creatinine clearance and urine albuminuria and glycosuria improved significantly and he was able to attend to his activities of daily living.

Discussion

According to the India HIV Estimation 2017 report, national adult (15–49 years) HIV prevalence in India is estimated at 0.22% (0.16%–0.30%).[2] India has the third largest HIV epidemic in the world, with 2.14 million people living with HIV as per the UNAIDS 2018 data. 56% of adults are on anti-retroviral treatment.

Kidney disease in HIV-affected individuals may be attributed to a variety of causes which include: HIV-associated nephropathy (HIVAN) presenting with focal segmental glomerulosclerosis, HIV-associated immune complex kidney disease (HIVICK), acute kidney injury or chronic kidney disease.[5] The common antiretroviral therapy (ART) causes include tenofovir (tubular toxicity), protease inhibitor indinavir (nephrolithiasis, crystal nephropathy and interstitial nephritis) and enfuviritide (glomerulopathy).[6]

Tenofovir-induced nephrotoxicity is quite rare with most studies estimating it between 1–6%.[7] Tenofovir-induced nephrotoxicity could present as proximal tubular dysfunction

with preserved renal function (14%) or proximal tubular dysfunction associated with decreased renal function – which can either be acute kidney injury (1.6 %) or chronic kidney disease (8%).[1] Our patient also presented with proteinuria, glycosuria along with elevated creatinine levels and chronic renal parenchymal changes on ultrasonography.

About 20–30% of TDF is actively transported into the renal proximal tubule cells by organic anion transporters (OAT 1 and 3) located in the basolateral membrane and then subsequently secreted to the tubular lumen by the apical membrane transporters MRP4 and MRP-2 (Multidrug Resistance Proteins).[8] Any factor which causes overexpression of OAT1 and 3 or inhibits MRP-4 will cause tenofovir to accumulate within the cells, which in turn causes mitochondrial toxicity. Rarely, distal renal tubular acidosis has also been encountered (<5%), although the exact mechanism is not known.[9]

Risk factors include old age, low BMI, prior history of renal disease, reduced baseline glomerular filtration rate (eGFR), diabetes mellitus, hypertension, hepatitis C co-infection and genetic polymorphisms (APOL1G1 gene).[8] The index patient's BMI at the time of presentation was quite low. However, his BMI at the time of initiation with tenofovir was not known. Factors which inhibit MRP-4 include anti-inflammatory drugs like diclofenac, ibuprofen as well as protease inhibitors. The index patient was on concomitant ritonavir and diclofenac, both of which might have added on to the toxicity.

Nephrotoxicity can occur after years of tenofovir therapy as a delayed presentation with the median duration of presentation ranging between 16 months to 5 years.[10] Casado et al. observed a 32% prevalence of proximal tubulopathy after a median time of 5 years on tenofovir.[11] Our patient presented with nephrotoxicty 4 years after ART initiation with tenofovir. He had asthenia, loss of appetite, significant weight loss, muscle pain, easy fatigability and poor quality of life despite improvement in CD4 count, which prompted us to probe drug-related toxicities.

Osteotoxicity in HIV may be multifactorial. HIV can cause direct bone damage due to its effects on bone remodelling. Tenofovir can cause osteotoxicity through secondary effects of proximal renal tubular acidosis and its resultant hypophosphataemia. Because renal metabolism of vitamin D to its active form dihydroxyl cholecalciferol also occurs in proximal tubular cells, serum vitamin D levels can also be low in these patients. In addition, tenofovir can influence phosphate metabolism through circulating factors like phosphatonins.[4] In our patient vitamin D levels were normal but hypophosphataemia and elevated alkaline phosphate levels were noted and were probable contributors to bone toxicity and resultant pathological fracture.

This combined presentation of renal and bone toxicity with tenofovir therapy is quite rare, with few case reports available.[12,13] Lucey et al. reported a teenage boy with HIV infection on tenofovir presenting four years later with Fanconi syndrome and stress fracture of bilateral metatarsals.[13]

The early stages of proximal tubulopathy can be diagnosed by urine analysis of beta 2 microglobulin. In a study from Christian Medical College, South India, alkaline phosphatase (ALP) levels increased by nearly 200% after treatment with tenofovir. It was statistically significant thereby rendering it an important marker for monitoring of toxicity.[7] This is similar to the observation by Woodward et al. in which 89.5% of patients on tenofovir who developed hypophosphataemia had elevated ALP levels after a median time of seven years on ART.[12] It was also observed that creatinine clearance was a more valuable indicator compared to absolute creatinine values. National AIDS Control Organisation guidelines 2018 suggest measuring creatinine clearance and assessing risk factors for renal toxicity prior to starting tenofovir therapy. eGFR, urine protein/creatinine ratio, urine glucose and urine phosphates need to be monitored every three months for one year and then biannually.[2]

It is recommended to switch to alternative nucleoside reverse transcriptase inhibitors whenever tenofovir toxicity is encountered. However, even in those patients where tenofovir-related toxicities are encountered, it is still noted that the patients had a strong clinical and immunological response.

Ideally, if tenofovir entry to proximal tubule cells is prevented or exit is facilitated, renal toxicity may be averted. Drugs like probenecid which is an inhibitor of OAT1 and rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist that induces the expression of many proximal tubular cell transporters were studied in the past.[1] However, both had dose-limiting side effects.

Conclusion

Although antiretroviral therapy has given hope and life to HIV patients, it is important to be aware of the potential side effects and thereby monitor for the associated toxicities.

Early diagnosis of tubulopathy by urinary protein excretion, glycosuria assessment, creatinine clearance evaluation and alkaline phosphate assessment can be of central importance in identifying kidney and bone toxicities. Prompt recognition of toxicity and withdrawal of the drug can significantly improve or even reverse tubular damage in most patients, thereby avoiding chronic kidney damage.

References

1 National AIDS Control Organisation. National Technical Guidelines on ART October 2018. https://lms.naco.gov.in/frontend/content/NACO%20%20National%20Technical%20Guidelines%20on%20ART_October%202018%20(1).pdf (accessed 2/1/20).

2 Fernandez BF, Ferrer AM, Ana B Sanz et al. Tenofovir Nephrotoxicity: 2011 Update. *AIDS Res Treat* 2011; 2011: 354908. doi: 10.1155/2011/354908.

3 Medland NA, Chow EP, Walker RG et al. Incidence of renal Fanconi syndrome in patients taking antiretroviral therapy including tenofovir disoproxil fumarate. *Int J STD AIDS* 2018; 29: 227–36.

4 Grant PM, Cotter AG. Tenofovir and bone health. *Curr Opin HIV AIDS* 2016; 11: 326–32.

5 Jotwani V, Atta MG, Estrella MM. Kidney Disease in HIV: Moving beyond HIV-Associated Nephropathy. *J Am Soc Nephrol* 2017; 28: 3142–54.

6 Rho M, Perazella MA. Nephrotoxicity associated with antiretroviral therapy in HIV-infected patients. *Curr Drug Saf* 2007; 2: 147–54.

7 Shubhanker M , Rupali P, Rajeev K et al. Renal Tubular Dysfunction Associated with Tenofovir Therapy *J Assoc Physicians India* 2014; 62: 580–2.

8 Tourret J, Deray G and Bagnis CI et al. Tenofovir Effect on the Kidneys of HIV-Infected Patients: A Double-Edged Sword? *J Am Soc Nephrol* 2013; 24: 1519–27.

9 Iwata K, Nagata M, Watanabe S et al. Distal renal tubular acidosis without renal impairment after use of tenofovir: a case report. *BMC Pharmacol Toxicol* 2016; 17: 52.

10 Ezinga M, Wetzels J, Bosch M et al. Long-term treatment with tenofovir: prevalence of kidney tubular dysfunction and its association with tenofovir plasma concentration. *Antivir Ther* 2014; 19: 765–71.

11 Casado JL. Renal and Bone Toxicity with the Use of Tenofovir: Understanding at the End. *AIDS Rev* 2016; 18: 59–68.

12 Woodward CLN, Hall AM, Williams IG. Tenofovir-associated renal and bone toxicity. *HIV Medicine* 2009; 10: 482–7.

13 Lucey JM, Hsu P, Ziegler JB. Tenofovir-related Fanconi's syndrome and osteomalacia in a teenager with HIV. *BMJ Case Rep* 2013; 2013: bcr2013008674.

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ORIGINAL ARTICLE

Association of Gastroesophageal Reflux Disease with Anxiety, Depression, and Sleep Disorders

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Background: Gastroesophageal reflux disease (GERD) is commonly associated with impaired quality of life. Chronic symptoms in this highly prevalent disorder could potentially lead to psychological manifestations such as anxiety, depression, and sleep disorders. **Aim:** The purpose of the study was to evaluate the magnitude of association of GERD symptoms based on health-related quality of life (GERD-HRQL) with anxiety, depression, and sleep quality using Hospital Anxiety and Depression Scale (HADS) and Pittsburgh Sleep Quality Index (PSQI). **Methods:** This was a cross-sectional study done at a tertiary care hospital in 241 participants. Cases were patients diagnosed with GERD, and the control group was participants who did not have GERD, devoid of all gastrointestinal symptoms according to GERD-HRQL. Data on age, gender, body weight, smoking/alcohol intake, and medication history were obtained from 98 cases and 143 matched control subjects. The three questionnaires, namely, GERD-HRQL, HADS, and PSQI questionnaires were administered by a trained blinded interviewer. **Results:** There was a statistically significant difference ($P < 0.001$) in the mean score of cases in the HADS depression scale (7.35 ± 3.65); the HADS anxiety scale (9.33 ± 4.51) and the mean global PSQI sleep score (4.62 ± 1.96) compared to the controls. Using Pearson's correlation, heart burn ($P = 0.036$), and regurgitation scores ($P = 0.026$), including the global quality of life score ($P = 0.003$), correlated significantly with the anxiety score. We found a statistically significant correlation between heart burn and poor sleep ($P = 0.012$) but not for regurgitation ($P = 0.772$). **Conclusions:** This study highlights the increased risk of anxiety, depression, sleep disorder in GERD, and the significant correlation between HRQL, especially heartburn with anxiety and poor sleep quality. This enlightens that specific screening and treatment strategies targeting such psychological manifestations are imperative for overall improved quality of life in GERD patients.

Key words: Mood disorders, sleep quality, Hospital Anxiety and Depression Scale, Pittsburgh Sleep Quality Index, gastroesophageal reflux disease-health related quality of life

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic disorder characterized by reflux of the stomach contents, which can give rise to symptoms and complications. Typical GERD symptoms affect patients' quality of life and overall work productivity.[1] In addition, it also contributes to enormous health-care costs. It is a highly prevalent disorder, and a recent systematic review showed that the prevalence of GERD is 18.1%–27.8% in North America, 8.8%–25.9% in Europe, 2.5%–7.8% in East Asia, 8.7%–33.1% in the Middle East, 11.6% in Australia, and 23.0% in South America.[2] Although conventionally considered as a disease affecting middle age and the older population, recent studies have shown that there is a significant increase in the proportion of the disease in the younger age group.[3]

Patient's quality of life is increasingly being considered as a medical outcome index in the evaluation of the impact of GERD symptoms on patients' health status. The impact of

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GERD on quality of life can be evaluated by a detailed symptom analysis which typically includes heartburn, acid regurgitation and epigastric pain in the majority of the patients using GERD health-related quality of life (HRQL) questionnaire.[4]

Studies have shown that chronic conditions like GERD could secondarily result in psychological manifestations of anxiety, depression, and insomnia. Few studies have also demonstrated that anxiety and depression could decrease the threshold for perception of visceral stimuli, thereby exacerbating the risk of developing functional gastrointestinal (GI) disorders, thus elucidating a bidirectional cause and effect relationship.[5-7] The current research focuses on the interplay between the psychological manifestations of anxiety, depression, altered sleep, and the symptomatology of GERD with the objective to evaluate the magnitude of the correlation between the quality of life and the psychological status of patients.

Literature evidence reveals that there is a paucity of studies evaluating psychosocial impairment due to GERD symptoms in the Indian population. Hence, this study was aimed to evaluate the magnitude of association of GERD symptoms with anxiety, depression and sleep quality by assessing the correlation of the GERD-HRQL scores with that of anxiety, depression and sleep quality using the respective Hospital Anxiety and Depression scale (HADS) and Pittsburgh Sleep Quality Index (PSQI) scale in Indian patients with GERD. In addition, the study was also designed to analyze whether anxiety, depression, and sleep scores were affected by comorbidities in GERD patients.

METHODS

This was a hospital-based cross-sectional questionnaire study done at tertiary care teaching hospital after the approval by PSG Institute of Medical Sciences and Research-Institutional Human Ethics Committee (Approval no: 16/435 dated January 10, 2017). Patients attending Gastroenterology and Medicine outpatient department and diagnosed with GERD were included for the study after obtaining written informed consent. GERD was diagnosed based on the presence of the typical clinical presentation along with the endoscopic features of GERD. The control group were subjects who did not have GERD and were devoid of all GI symptoms, according to GERD-HRQL. GERD-HRQL questionnaire, a disease-specific tool, was used to measure the symptomatic outcomes and therapeutic effects in patients with GERD. The scale had 16 items that focused on heartburn symptoms, dysphagia, medication effects, and the patient's present health condition. Each item was scored from 0 to 5, with a higher score indicating a better quality of life. Two scores, namely heartburn score and regurgitation score, were obtained by the summation of the scores for questions 1–6 and 10–15 respectively in addition to the total score derived by summing all the 15 score values. The 16th question was a patient's satisfaction question based on a 3 point scale.

Velanovich *et al*. had examined the psychometric properties of the GERD-HRQL in GERD patients and reported that the Cronbach's α coefficient for each subscale ranged from 0.89 to 0.94 demonstrating a high test– retest reliability and internal consistency.[4]

The presence of anxiety and depression levels was assessed using the Anxiety and Depression Scale. The HADS, a widely used scale that had been designed for detecting depression and anxiety in out-patient clinic setting, was used to measure symptoms of anxiety and depression and consisted of 14 items, seven items for the anxiety subscale, and seven for the depression subscale. HADS Anxiety (HADS A) focused on the symptoms of generalized anxiety disorder, and HADS depression (HADS D) was focused on anhedonia, the main symptom of depression. Each item was scored on a response-scale with four alternatives ranging between 0 and 3. The overall score value 8 or higher was considered the cutoff value for the existence of anxiety and depression.[8]

The PSQI, an effective instrument was used for measuring the quality and patterns of sleep based on examination of seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the past month. Scoring of responses ranged from 0 to 3, and all the seven components were summed together with scores of 5 or greater indicating poor sleep quality.[9]

The subjects included in this study were >20 years of age, either newly diagnosed or on proton-pump inhibitor (PPI) therapy. Patients who had concomitant diabetes, hypertension, liver, pancreatic, or chronic renal disease were also included in the study. The participants included were either non-smokers or those who were current or ex-smokers with a smoking index <100 and also non-alcoholic. However, patients who had serious comorbidities such as liver failure, renal failure, morbid obesity, patients with heart disease on antiplatelet therapy, osteoporosis patients and those on long-term nonsteroidal anti-inflammatory drugs (NSAIDs) or other chronic medications which cause gastric reflux and those with documented history of psychiatric illnesses including anxiety disorders, depression or bipolar illness and sleep disorders were excluded. Subjects aged <20 years, pregnant and lactating women were also excluded from the study.

A total of 98 cases by consecutive sampling were recruited from January 2018 to May 2018. Data on age, gender, body weight, alcohol intake, smoking, NSAID intake, concomitant GI medications other than PPI, including their daily dose were obtained. Similarly, data were also obtained from 143

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control subjects who were matched so that they represented the same proportion of subjects with age, sex, and smoking status as in the case group. The three questionnaires, namely, GERD-HRQL, HADS, PSQI questionnaires, were administered by a trained interviewer blinded to the group to which the participant belongs.

Statistical analysis

All data were entered in excel, and the statistical analyses were performed using SPSS version 24 (IBM Corporation, United states). The mean HADS and PSQI scores of patients and controls were compared using an independent sample *t*-test to detect statistical significance. Similarly, Student's *t*-test was also used to analyze if the mean difference in the scores of the patients with and without comorbidities were significant. In addition, the correlation of the GERD-HRQL scores with anxiety, depression, and sleep quality scores in patients with GERD was performed using Pearson's correlation.

RESULTS

We recruited a total of 241 participants after obtaining written informed consent, among which 98 subjects were GERD patients and recruited to the case group, while the remaining 143 participants were the non-GERD control population. A total of 84 cases were on proton-pump inhibitor therapy, accounting for a percentage of 85.71%. The mean age of the cases was 46 years, whereas the mean age in the control group was 50 years. Among the cases, 55.10% were <50 years of age and 44.90% were in the age group of 50 years and above. Also, 67.35% cases were males and the rest were females with male: female ratio approximating 2:1. The analysis revealed that there was no difference in the mean scores between the cases in the age group <50 years and those who were aged 50 years and above. Among the cases, an independent sample *t*-test demonstrated that the mean score for anxiety was significantly higher in the female population compared to the males among the cases, but no such significant difference was evident for the depression or the sleep score [Table 1].

The mean score of the cases in the HADS D scale (7.35 ± 3.65) was significantly higher ($P < 0.001$) compared to the mean score of the control population (4.60 ± 3.34) using independent sample *t*-test. Likewise, the mean anxiety score of the cases (9.33 ± 4.51) was greater in comparison to the mean score of the controls (6.35 ± 3.74) using HADS A, and this difference was also statistically significant ($P < 0.001$). A similar significance in the PSQI sleep score between cases and controls was also obtained using Student's *t*-test [Table 2 and Figure 1]. A Chi-square test revealed that the proportion of cases found to have depression (50%); anxiety (66.32%), and sleep disorder (45.91%) were significantly higher compared to the control population [Table 3].

Table 1: Comparison of scores across age and gender groups using independent sample *t*-test

Scale	Age (years)/sex	Mean score	SD	*t*	*P**
GERD-HRQL	<50	24.24	11.92	0.472	0.638
	≥50	23.20	9.82		
HADS D	<50	6.88	3.47	1.399	0.165
	≥50	7.93	3.81		
HADS A	<50	9.72	4.20	0.921	0.359
	≥50	8.86	4.87		
PSQI	<50	4.77	2.10	0.883	0.379
	≥50	4.43	1.77		
GERD-HRQL	Male	23.56	10.68	0.268	0.790
	Female	24.21	11.76		
HADS D	Male	6.92	3.32	1.572	0.122
	Female	8.25	4.17		
HADS A	Male	8.66	4.26	2.067	0.043
	Female	10.71	4.76		
PSQI	Male	4.46	2.05	1.175	0.244
	Female	4.93	1.74		

*$P<0.05$ is statistically significant. SD=Standard deviation; GERD=Gastroesophageal reflux disease; HRQL=Health-related quality of life; HADS=Hospital anxiety and depression scale; HADS D=HADS depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

Table 2: Comparison of depression, anxiety, and sleep quality scores between cases and controls using independent sample *t*-test

Scale	Case/control	Mean score	SD	*t*	*P**
HADS D	Case	7.35	3.65	5.934	<0.001
	Control	4.60	3.34		
HADS A	Case	9.33	3.51	5.387	<0.001
	Control	6.35	3.74		
PSQI	Case	4.62	1.96	5.076	<0.001
	Control	3.33	1.89		

*Statistically significant. SD=Standard deviation; HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

Analysis of the GERD-HRQL scores revealed that among the 98 cases in the study population, 35 patients (35.7%) had symptomatic heartburn, whereas 45 patients (45.9%) were symptomatic for regurgitation. On the satisfaction scale, 66.35% were satisfied with their present condition and 26.50% recorded a neutral response, while 7.15% were found to be dissatisfied with their current clinical status [Figure 2]. A comparative analysis of whether the mean scores vary

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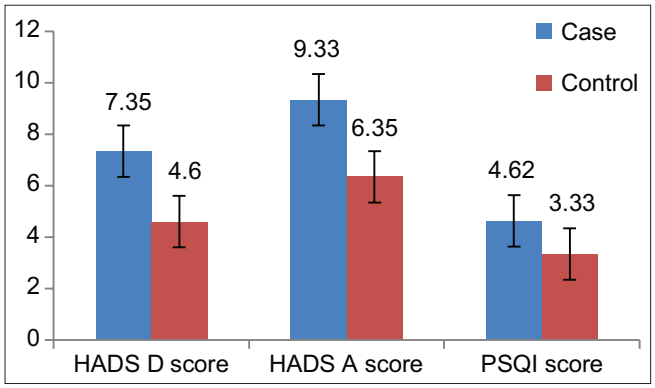


Figure 1: Chart representing mean ± standard deviation of the scores between cases and controls. The error bars represent standard deviation

Table 3: Comparison of the proportion of cases and control with and without depression, anxiety and sleep disturbances using Chi-square test

	Depression		Anxiety		Sleep disorder	
	Yes	No	Yes	No	Yes	No
Cases	49	49	65	33	45	53
Controls	35	108	58	85	26	117
Chi-square statistic	16.684		15.449		21.526	
*P**	<0.001		<0.001		<0.001	

*Statistically significant

significantly among the patients with asymptomatic (scores of ≤12 with each individual question not exceeding 2 for questions 1–6 of the GERD-HRQL indicate heartburn elimination) and symptomatic heartburn with scores of >12, it was found that the though the mean depression and anxiety scores were higher in the symptomatic patients, there was no statistical significance between the mean difference in the scores detectable. However, patients with symptomatic heartburn had a statistically significant higher mean score for sleep disturbance ($P = 0.034$) compared to the asymptomatic group. However, when a similar analysis was performed for the regurgitation, there was no significant mean difference between those with scores >12 and those with lower scores in all the three scales [Table 4].

Comparison of the mean scores of the cases with and without comorbidities, there was no statistical significance in the mean scores obtained for those with and without diabetes, hypertensive and normotensive patients, those with other comorbidities such as liver disease, pancreatic, or chronic renal disease [Table 5].

Analyzing the correlation of heartburn, regurgitation and satisfaction components of the quality of life scores with the depression, anxiety and sleep quality scores, it was found that neither the heartburn nor the regurgitation score correlated

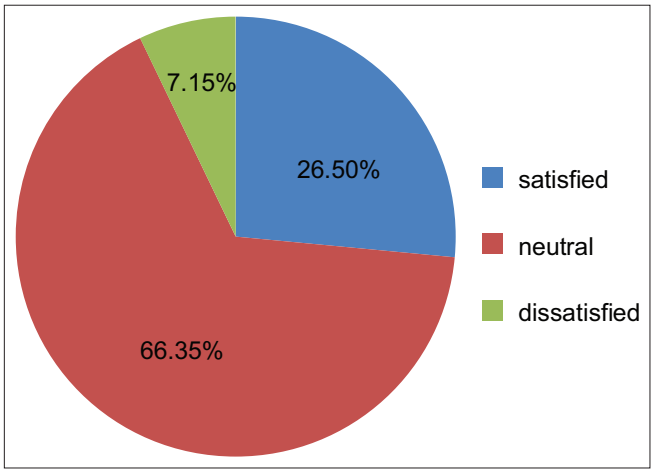


Figure 2: Chart representing satisfaction score in Gastroesophageal Reflux Disease-Health Related Quality of life Scale

with depression score whilst there was a correlation of the patient satisfaction with the depression score. However, both the heartburn and the regurgitation scores correlated with the anxiety score, including the global quality of life score. Interestingly, we found that there was a statistically significant correlation between heartburn and poor sleep, which was not detectable for regurgitation. In addition, it was found that the patients unsatisfied with their present clinical status were more likely to have depression and poor sleep quality rather than anxiety [Table 6 and Figure 3].

DISCUSSION

A statistically higher mean score for all the three clinical conditions, namely depression, anxiety, and sleep disturbance was demonstrable in the GERD cases compared to the control group indicating a high risk of these psychological manifestations in patients with reflux disease. Conceivably, impairment in quality of life was also elicited in the cases compared to the control population using GERD-HRQL.

Our study is one of the few studies evaluating the interplay between the HRQL changes in GERD with the various psychological manifestations such as anxiety, depression, and sleep manifestations in a South Indian population. Considering the cut off value of 8 for the diagnosis of anxiety and depression using the respective components of the HADS questionnaire, the study has effectively demonstrated that a high probability of anxiety disorder existed in the GERD population with a mean anxiety score of 9.33 ± 3.51 unlike with respect to depression wherein the mean score was marginally below the cut off value. A similar result was also obtained in another study done in 225 patients by Kessing *et al.*[10] However, our result was in contrast to a previous study

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Table 4: Comparison of depression, anxiety, and sleep quality scores with gastroesophageal reflux disease-health related quality of life scores of the cases

Scale	Heart burn score ≤12	Heart burn score >12	Regurgitation score ≤12	Regurgitation score >12
HADS D	7.17±3.92	7.68±3.14	7.18±3.79	7.55±3.51
HADS A	8.85±4.42	10.20±4.61	8.96±3.98	9.77±5.08
PSQI	4.26±1.56*	5.25±2.41*	4.71±2.04	4.51±1.87

**P* value significant at 0.05 level using independent sample *t*-test. HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

Table 5: Comparison of depression, anxiety, and sleep quality scores of cases with and without comorbidities

Scale	Comorbidities	*n*	Mean score	SD	*t*	*P**
HADS D	With comorbidities	70	7.04	3.25	1.181	0.245
	No comorbidities	28	8.14	4.47		
	Diabetes	22	8.68	3.83	1.872	0.070
	No diabetes	76	6.97	3.53		
	Hypertension	17	8.64	4.66	1.310	0.205
	No hypertension	81	7.08	3.37		
	Liver disease	35	7.65	2.74	0.674	0.502
	No liver disease	63	7.19	4.08		
	Pancreatic and renal disease	36	7.52	4.32	0.325	0.746
	No pancreatic and renal disease	62	7.25	3.23		
HADS A	With comorbidities	70	9.25	4.12	0.244	0.808
	No comorbidities	28	9.53	5.44		
	Diabetes	22	9.40	5.34	0.075	0.941
	No diabetes	76	9.31	4.28		
	Hypertension	17	9.58	5.88	0.203	0.842
	No hypertension	81	9.28	4.21		
	Liver disease	35	9.48	4.27	0.249	0.804
	No liver disease	63	9.25	4.67		
	Pancreatic and renal disease	36	10.02	4.99	1.105	0.273
	No pancreatic and renal disease	62	8.93	4.20		
PSQI	With comorbidities	70	4.42	1.72	1.351	0.185
	No comorbidities	28	5.10	2.42		
	Diabetes	22	5.00	2.48	0.856	0.399
	No diabetes	76	4.51	1.78		
	Hypertension	17	5.11	2.20	1.042	0.309
	No hypertension	81	4.51	1.90		
	Liver disease	35	4.57	2.01	0.189	0.851
	No liver disease	63	4.65	1.94		
	Pancreatic and renal disease	36	4.66	1.92	0.171	0.865
	Pancreatic and renal disease	62	4.59	1.99		

**P*<0.05 is significant. SD=Standard deviation; HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

in which depression was found to be one of the most prevalent comorbidities in patients with GERD.[11] Our research revealed that a high proportion of cases (71.42%) had comorbidities amongst which concomitance of liver diseases recorded the highest. However, none of the comorbidities were significantly associated with the HADS or the sleep scores.

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Table 6: Correlation between gastroesophageal reflux disease-health related quality of life score with depression, anxiety, and sleep quality scores of cases

Scale	GERD-HRQL score		Heart burn score		Regurgitation score		Satisfaction score	
	Pearson correlation	*P**	Pearson correlation	*P**	Pearson correlation	*P**	Pearson correlation	*P**
HADS D score	0.157	0.122	0.071	0.488	0.150	0.141	0.244	0.016
HADS A score	0.293	0.003	0.212	0.036	0.225	0.026	0.177	0.081
PSQI score	0.161	0.113	0.252	0.012	0.030	0.772	0.267	0.008

*Correlation is significant if $P<0.05$ (two-tailed significance). GERD=Gastroesophageal reflux disease; HRQL=Health-related quality of life; HADS D=Hospital anxiety and depression scale depression; HADS A=HADS anxiety; PSQI=Pittsburgh sleep quality index

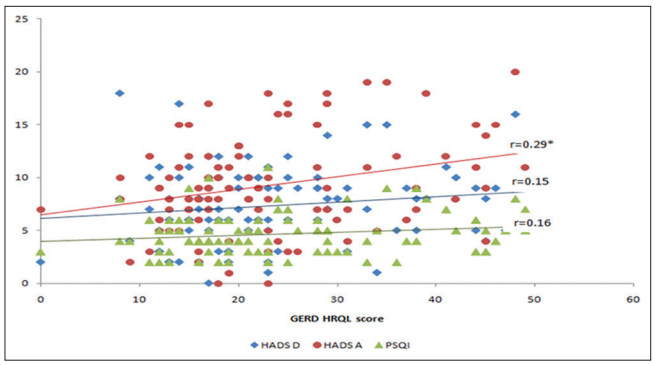


Figure 3: Chart representing the correlation of Gastroesophageal Reflux Disease-Health Related Quality of Life score with depression, anxiety, and sleep quality scores of cases. *Correlation coefficient *r* value is significant

Although the literature evidence strongly supports the association of sleep disorder manifestations with GERD[7,12] the mean global PSQI score of cases in our study did not achieve the significant cut-off level of 5 indicative of poor quality sleep despite the mean score being significantly higher than the control population. This could be potentially because a large majority of patients (85.71%) were on treatment with PPI. A systematic review has delineated that a statistically significant improvement in sleep quality occurred after initiation of PPI therapy in GERD patients though it may not be completely successful in normalizing the sleep quality to achieve a global PSQI score that is not statistically different from the control population.[13] The large proportion of cases on PPI therapy precluded a between-group analysis of the depression, anxiety, and sleep scores with the patients not on PPI in our research. Symptomatic relief with PPI therapy can consequentially alleviate the psychological manifestations in GERD though evidences support that persistent symptoms despite PPI treatment is quite common which can impede the improvement in severity of the psychological parameters.

Studies have supported that chronic and mild inflammation in the GI tract due to GERD could result in up-regulation of the central nervous system (CNS) cytokine production which in turn can affect the CNS functions, ultimately resulting in psychiatric manifestations such as depressive and anxiety disorders.[14-16] Studies in animal models have also demonstrated that chronic peripheral inflammation induces central pro-inflammatory markers like interleukin IL-1β as well as tumor necrosis factor alpha-α;[17] the long term elevation of which significantly correlate with the occurrence of psychological manifestations supporting the hypothesis.[18]

In addition, the symptom complex of GERD enables envisioning the intertwined and bidirectional nature of the relationship between sleep disorders and GERD. GERD results in heartburn, acid reflux, sinusitis or sinus problems, and frequent dry cough necessitating throat-clearing that awakens the patient during sleep. Frequent arousal during sleep activates neuroendocrine systems, including the vagal nerve and triggers bronchoconstriction. Frequent bronchoconstriction narrows the diameter of the respiratory tract and arouses patients from sleep, intensifying sleep disorders.[19-21] Furthermore, GERD-related micro-aspirations inducing bronchial exudative mucosal reaction also adds to the narrowing in diameter of the respiratory tract, accelerating sleep disorder.[22] The flip side of the coin is that for several reasons, GERD symptoms aggravate during sleep. Anti-reflux mechanisms such as swallowing rate, upper and lower esophageal sphincter pressure, gastric emptying, and the awareness of heartburn have all been found to be depressed during sleep. Also, while recumbent, the force of gravity encourages the flow of gastric content towards the esophagus. Thus, current literature supports the fact that sleep disorder has a dual cause and effect relationship with GERD.[13,23]

A differential effect of the two predominant symptoms, namely heartburn and regurgitation analyzed using GERD-HRQL, illustrated that heartburn patients had a significantly lower mean PSQI score and a positive correlation with anxiety and poor sleep quality whilst regurgitation only correlated with anxiety score. A similar result has been documented by Lee *et al*. whose study disclosed that patients with heartburn had more severely impaired daily activity, including sleep interruption compared to those with a predominant regurgitation.[24] Likewise, increased anxiety level has been found to be associated with greater heartburn but not regurgitation.[10] This discrepancy perhaps stems from the

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differences in physico-pathological mechanism underlying the key clinical presentations of GERD. Reflux episodes inducing regurgitation has been shown to have a higher proximal extent of the liquid component, but less esophageal pH drop, than episodes inducing heartburn.[25]

In the current study, a significant positive correlation between the GERD-HRQL and anxiety was demonstrable but not for depression and sleep derangements. A similar study using a different scale for evaluating depression, anxiety and HRQL was able to prove that there existed a significant correlation of the quality of life with both anxiety and depression.[26] The difference in results could be attributed to the fact that our study was done in a smaller subset of patients with GERD and that most patients were on treatment which also precluded a between-group analysis of untreated patients with those on treatment. Furthermore, the categorization of cases into the erosive and nonerosive disease was not done, and these were our study limitations.

However, the study has clearly brought out the need to understand the increased risk of psychological manifestations including depression, anxiety, and sleep disorders in GERD patients including those on treatment, which necessitates addressal by periodic assessments and redressal by specific planned psychiatric interventions, both pharmacological and behavioral, as a part of the comprehensive treatment plan.

CONCLUSIONS

The study highlights that GERD patients constitute a vulnerable population for psychological disturbances such as anxiety, depression, and sleep disorder, which may not be completely eliminated by the treatment of the underlying reflux disease. Hence, periodic evaluation and specific treatment strategies for such manifestations would be beneficial in improving the quality of life in these patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Tack J, Becher A, Mulligan C, Johnson DA. Systematic review: The burden of disruptive gastro-oesophageal reflux disease on health-related quality of life. Aliment Pharmacol Ther 2012;35:1257-66.
2. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: A systematic review. Gut 2014;63:871-80.
3. Yamasaki T, Hemond C, Eisa M, Ganocy S, Fass R. The changing epidemiology of gastroesophageal reflux disease: Are patients getting younger? J Neurogastroenterol Motil 2018;24:559-69.
4. Velanovich V, Vallance SR, Gusz JR, Tapia FV, Harkabus MA. Quality of life scale for gastroesophageal reflux disease. J Am Coll Surg 1996;183:217-24.
5. You ZH, Perng CL, Hu LY, Lu T, Chen PM, Yang AC, *et al*. Risk of psychiatric disorders following gastroesophageal reflux disease: A nationwide population-based cohort study. Eur J Intern Med 2015;26:534-9.
6. Chen JH, Wen SH, Hsu CS, Yi CH, Liu TT, Chen CL, *et al*. Clinical characteristics and psychosocial impact of different reflux time in gastroesophageal reflux disease patients. J Formos Med Assoc 2017;116:123-8.
7. Kimura Y, Kamiya T, Senoo K, Tsuchida K, Hirano A, Kojima H, *et al*. Persistent reflux symptoms cause anxiety, depression, and mental health and sleep disorders in gastroesophageal reflux disease patients. J Clin Biochem Nutr 2016;59:71-7.
8. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res 2002;52:69-77.
9. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193-213.
10. Kessing BF, Bredenoord AJ, Saleh CM, Smout AJ. Effects of anxiety and depression in patients with gastroesophageal reflux disease. Clin Gastroenterol Hepatol 2015;13:1089-950.
11. Moraes-Filho JP, Navarro-Rodriguez T, Eisig JN, Barbuti RC, Chinzon D, Quigley EM. Comorbidities are frequent in patients with gastroesophageal reflux disease in a tertiary health care hospital. Clinics (Sao Paulo) 2009;64:785-90.
12. Iwakura N, Fujiwara Y, Shiba M, Ochi M, Fukuda T, Tanigawa T, *et al*. Characteristics of sleep disturbances in patients with gastroesophageal reflux disease. Intern Med 2016;55:1511-7.
13. Regenbogen E, Helkin A, Georgopoulos R, Vasu T, Shroyer AL. Esophageal reflux disease proton pump inhibitor therapy impact on sleep disturbance: A systematic review. Otolaryngol Head Neck Surg 2012;146:524-32.
14. Salim S, Chugh G, Asghar M. Inflammation in anxiety. Adv Protein Chem Struct Biol 2012;88:1-25.
15. Bakunina N, Pariante CM, Zunszain PA. Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology 2015;144:365-73.

[Downloaded free from http://www.jmedscindmc.com on Friday, December 17, 2021, IP: 103.224.34.2]

16. Ranjbaran Z, Keefer L, Stepanski E, Farhadi A, Keshavarzian A. The relevance of sleep abnormalities to chronic inflammatory conditions. Inflamm Res 2007;56:51-7.
17. Lampa J, Westman M, Kadetoff D, Agréus AN, Le Maître E, Gillis-Haegerstrand C, *et al*. Peripheral inflammatory disease associated with centrally activated IL-1 system in humans and mice. Proc Natl Acad Sci U S A 2012;109:12728-33.
18. Kivimäki M, Shipley MJ, Batty GD, Hamer M, Akbaraly TN, Kumari M, *et al*. Long-term inflammation increases risk of common mental disorder: A cohort study. Mol Psychiatry 2014;19:149-50.
19. Jansson C, Nordenstedt H, Wallander MA, Johansson S, Johnsen R, Hveem K, *et al*. A population-based study showing an association between gastroesophageal reflux disease and sleep problems. Clin Gastroenterol Hepatol 2009;7:960-5.
20. Dubois RW, Aguilar D, Fass R, Orr WC, Elfant AB, Dean BB, *et al*. Consequences of frequent nocturnal gastro-oesophageal reflux disease among employed adults: Symptom severity, quality of life and work productivity. Aliment Pharmacol Ther 2007;25:487-500.
21. Jung HK, Choung RS, Talley NJ. Gastroesophageal reflux disease and sleep disorders: Evidence for a causal link and therapeutic implications. J Neurogastroenterol Motil 2010;16:22-9.
22. Demeter P, Pap A. The relationship between gastroesophageal reflux disease and obstructive sleep apnea. J Gastroenterol 2004;39:815-20.
23. Khoury RM, Camacho-Lobato L, Katz PO, Mohiuddin MA, Castell DO. Influence of spontaneous sleep positions on nighttime recumbent reflux in patients with gastroesophageal reflux disease. Am J Gastroenterol 1999;94:2069-73.
24. Lee SW, Lien HC, Lee TY, Yang SS, Yeh HJ, Chang CS. Heartburn and regurgitation have different impacts on life quality of patients with gastroesophageal reflux disease. World J Gastroenterol 2014;20:12277-82.
25. Bredenoord AJ, Weusten BL, Curvers WL, Timmer R, Smout AJ. Determinants of perception of heartburn and regurgitation. Gut 2006;55:313-8.
26. Yang XJ, Jiang HM, Hou XH, Song J. Anxiety and depression in patients with gastroesophageal reflux disease and their effect on quality of life. World J Gastroenterol 2015;21:4302-9.

ORIGINAL ARTICLE

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A comparative study on the effect of sodium-glucose cotransporter-2 inhibitors and dipeptidyl peptidase-4 inhibitors as an add on therapy in patients with type 2 DM

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ABSTRACT

There were about 65 million diabetic population in 2016 in India. There were studies which concluded that the Dipeptidyl Peptidase-4 Inhibitors and Sodium Glucose Co-Transportor 2 Inhibitor were providing non-glycaemic control and cardiovascular benefits. Hence, it is important to find out the effect of those drugs on various parameters among the Indian Type 2 Diabetes Mellitus population. The aim is to evaluate and compare the effects of SGLT-2 inhibitors and DPP-4 inhibitors in Type 2 DM patients. The objective is to find out &compare the effects of both class of drugs on the physical profile [Body Weight (BW), Body Mass Index (BMI), Hip-Waist ratio), Diabetic Profile [Fasting Blood Sugar] (FBS), Glycated Haemoglobin (HbA1C)] & Side Effect Profile. The patients were categorized into two Group 1 & 2. Both groups were containing 50 patients, each based on the inclusion &exclusion criteria. The collection of demographics and history were done. Group 1 patients were initiated with DPP-4 Inhibitor, Group 2 patients with SGLT-2 Inhibitors, respectively. At baseline and during follow-up, the parameters like the physical profile, Diabetic Profile were noted. In Body Weight, the mean difference between the groups was found to be 2.9 $\pm$0.79 ($p<0.05$). Similarly, for BMI, it was 2.9$\pm$1.36 ($p<0.04$). For the Hip-Waist ratio, it was 0.07 ($p<0.05$). For FBS, it was 22.08 mg/dl (0.0001), for HbA1c it was 1.09 ($p<0.0043$). On the Side effect profile, SGLT-2 Inhibitors were showing higher incidence for the occurrence of Urinary Tract Infection (UTI) while comparing with DPP-4 Inhibitors. This can be overcome by proper patient counseling. This study concluded that SGLT-2 inhibitors may serves as the best medication choice for add on therapy in Type 2 DM patients.

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INTRODUCTION

Diabetes mellitus is one of the most common chronic diseases which is present in almost all nations. It is the most common non-communicable disease (Shaw *et al.*, 2010). About 65 million diabetic population in 2016 in India, whereas it was 26 million in 1990. In India, the prevalence of Diabetes Mellitus among adults aged 20 years or older was increased from 5.5% to 7.7% between the years of 1990 to 2016, respectively. In 2016, the highest prevalence is seen in the states of Tamil Nadu

& Kerala, which were stated as High Epidemiological Transition Level (High ETL) & Delhi as Higher-Middle ETL, which is followed by Goa, Punjab & Karnataka (Tandon *et al.*, 2018). The micro and macro vascular complications will be reduced by obtaining fair glycaemia control (Alfeena Mary PS *et al.*, 2018). Early initiation of pharmacologic therapy is associated with improved glycaemic control and reduced long-term complications in type 2 DM. In the case of uncontrolled type 2 DM, the drug classes like SGLT-2 inhibitors and DPP-4 inhibitors are used as add on therapy, sometimes as monotherapy also.

DPP-4 inhibitors (Sitagliptin, Teneligliptin, Evo gliptin) produce its effect by activating incretin, which is released by the intestinal cells after food intake. It is usually given as a once-daily dose. DPP-4 is an enzyme that degrades various biologically active peptides, including the GLP-1 and GIP. GLP-1 is playing a major part in releasing insulin (Aschner *et al.*, 2010). Along with glucose control, these drugs were found to have cardiovascular benefits due to its pharmacological augmentation of GLP-1R (Dicker, 2011).

SGLT-2 inhibitors (Dapagliflozin, Canagliflozin, Empagliflozin, Remogliflozin, Ertugliflozin) are a newer class of OHAs (Oral Hypoglycaemic Agents) which are approved to be used as monotherapy or in combination with metformin, sulfonylurea, or insulin. Work by preventing the reabsorption of glucose in PCT and by facilitating its excretion in urine. Hence, the glycaemic control is achieved along with the glucose excretion. The mechanism of action of these drugs is dependent on blood glucose levels and independent of the actions of insulin. So, there are minimum incidences for the occurrence of hypoglycaemia, and there is no risk of overstimulation or fatigue of the beta cells. (Alfeena Mary PS *et al.*, 2018).

Since both classes of drugs are being used either as monotherapy or as part of dual and triple therapy in type 2 diabetes patients, it is important to compare the effects of those drugs in the Indian type 2 diabetes population. This study involves the comparison of Physical (weight, BMI, Hip-waist ratio), Diabetic (FBS, HbA1C) & Side effect profiles of both classes of drugs.

MATERIALS AND METHODS

The study was conducted in the Department of General Medicine in PSG Hospital, Coimbatore. It was approved by the Institutional Human Ethics Committee, PSG Institute of Medical Sciences and Research (Proposal no: 19/072). It is an observational study which included a total of 100 subjects who were divided into two treatment groups. Both groups contain 50 patients each. Group 1 (G1) contains 28 Males & 22 Females whereas Group 2 (G2) contains 30 Males & 20 Females.

The inclusion criteria include Type 2 DM patient of age above 18 years with poor diabetic control and on regular follow up, Patients who are going to be initiated respectively with SGLT 2 Inhibitors & DPP-4 inhibitors as add on therapy for controlling DM and the patients with normal liver function, renal function. Exclusion criteria include patients with Type 1 DM, renal failure, psychiatric, paediatric, gestational diabetes mellitus, history of diabetic ketoacidosis & primary renal glucosuria, and patients who are unwilling to participate.

Group 1 patients were initiated with DPP-4 Inhibitor, Group 2 patients with SGLT-2 Inhibitors, respectively. At baseline and during follow-up after 3 months of drug initiation, the parameters like the physical profile, Diabetic Profile, and side effects were noted and statistically compared. The effect of drugs within a group was compared by using Paired *t*-Test and between groups were by using Independent *t*-Test.

RESULTS AND DISCUSSION

Effects of DPP-4 Inhibitors

The total of 50 G1 patients who were initiated with DPP-4 inhibitors, drugs given were Teneligliptin 20mg (14 patients), Evo gliptin 5mg (07 patients), DPP-4 Inhibitor +Metformin 500mg (16 patients), DPP-4 Inhibitor +Repaglinide 0.5 mg (06 patients), DPP-4 Inhibitor +Glimepiride 1mg (07 patients).

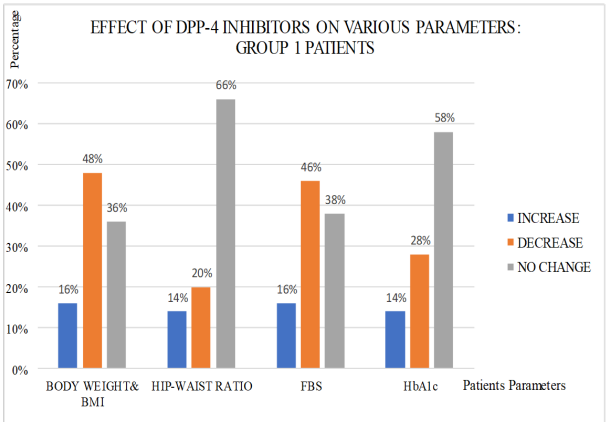


Figure 1: Cluster chart showing the effect of DPP-4 inhibitors on various parameters among Group 1 patients [BMI-Body Mass Index FBS-Fasting Blood Sugar HbA1c-Glycated Haemoglobin]

Once after the drug initiation, the patients were

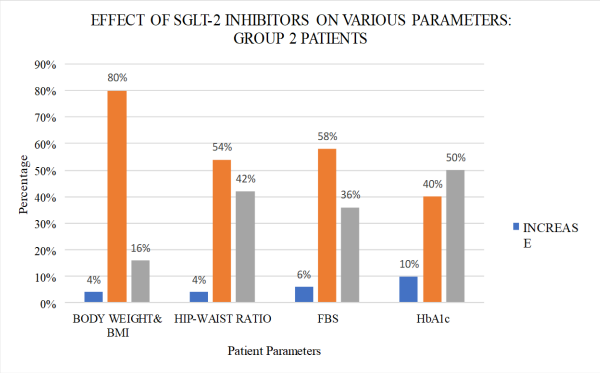


Figure 2: Cluster chart showing the effect of SGLT-2 inhibitors on various parameters among Group2 patients [BMI-Body Mass Index FBS-Fasting Blood Sugar HbA1c-Glycated Haemoglobin]

followed up for three months, and their physical and diabetic profile are monitored, and the changes were noted. According to the effect of drugs on individuals, their parameters get varied. They were depicted in Figure 1

Figure 1 showing the effect of DPP-4 Inhibitors on various parameters among G1 patients. The mean baseline weight (68.44±3.81) was reduced to 64.45±1.71) (p=0.04), the mean baseline BMI (26.73±1.31) was reduced to (25±1.38) (p=0.05), the mean difference between Hip-Waist ratio was 0.13 (p=0.05), the mean difference between FBS was 59.87 mg/dl (p=0.03), the mean difference between HbA1c was 1.2% (p=0.04).

A retrospective sub-analyzed study of sitagliptin effect on glucose and lipid metabolism, blood pressure, body weight and renal function in type 2 DM patients for a period of 6 months by dividing 173 patients of Type 2 DM into obese group (BMI $\geq$ 25) and non-obese group (BMI < 25), concluded that the body weight was significantly reduced in obese patients from the baseline values despite the ages and HbA1c also decreased in both the groups significantly (Katsuyama *et al.*, 2014)

A review of sitagliptin brought a conclusion that on the addition of sitagliptin (100mg/d) to already existing oral antidiabetic treatment produce a significant reduction of HbA1c. After 24 weeks of the addition of sitagliptin (100mg/d) to metformin produced a significant reduction of HbA1c (0.65%), FBS (25.4 mg/dl), Post Prandial Glucose (2h) (50.6 mg/dl) (Gallwitz, 2007)

GLP-1 also found have cardioprotective action in experimental models of heart failure and myocardial infarction. DPP-4 inhibitors may have cardiovascular effects either by depending or independent of GLP-1 (Jose and Inzucchi, 2012)

An Indian study reported the results of a 16-week, multicentric, double-blind, placebo-controlled, Phase 3 studies of teneligliptin 20 mg daily in drug-naive T2DM patients. This study (N = 237) reported a significant −0.55% glycated hemoglobin (HbA1c) reduction (placebo-subtracted) in teneligliptin arm (P = 0.0043) compared to control. While a significant reduction in 2 hours postprandial glucose (PPG) (−25.8 mg/dl, P = 0.0070) versus placebo was observed, an insignificant reduction in fasting plasma glucose (FPG) was seen (−8.8 mg/dl, P = 0.18) in teneligliptin 20 mg arm. Similarly, a higher percentage of patient achieved the target HbA1c of <7% in teneligliptin arm (43.4% vs. 27.3%, P = 0.026) compared to the control and "overall" the drug was well tolerated (Suryawanshi *et al.*, 2016)

Effects of SGLT-2 Inhibitors

The effects of SGLT-2 Inhibitors among various parameter of G2 patients were depicted in Figure 2

Figure 2 showing the effect of SGLT-2 Inhibitors on various parameters among G2 patients. The mean baseline weight (71.34±3.28) was reduced to 64.45±1.97) (p=0.02), the mean baseline BMI (29.93±4.32) was reduced to (27.03±2.96) (p=0.04), the mean difference between Hip-Waist ratio was 0.27 (p=0.04), the mean difference between FBS was 81.95 mg/dl (p=0.01), the mean difference between HbA1c was 2.29% (p=0.02)

A retrospective study which involves 61 patients who received 12 months of dapagliflozin concluded that After the 12 months intake of drug the body weight was -3.4±2.6 kg reduced significantly from the baseline value, the drug reduces the hyperglycemia and body weight by inhibiting the renal glucose reabsorption in a safe manner in Type 2 DM patients. (Cho *et al.*, 2017)

The study of "Efficacy and Safety of SGLT-2 Inhibitors" in Emirati patients which included 307 patients concluded that at 6 months of drug administration the baseline HbA1c was decreased from the baseline value, from 8.9±1.7% to 8±1.5% & at 1 year the mean HbA1c was 8±1.4% (p=0.0001). Likely, the bodyweight also reduced from the baseline at the sixth month, from 85.7±17.8 kg to 84±12.2 kg (p=0.0001). (Bashier *et al.*, 2017)

Comparision between the effects of DPP-4 inhibitors & SGLT-2 inhibitors

Since both categories of the drugs is used as add on therapy in the treatment of Type 2 DM, it's important to find out the effect of those drugs on various parameters among the Indian Type 2 DM population. This result may help to wisely choose the add

on therapy drugs for the treatment of Type 2 DM.

Effects of drugs on

Body weight and BMI

Table 1: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on Body Weight and BMI

Body Weight& BMI	DPP-4 Inhibitors	SGLT-2 Inhibitors
Increase (%)	16	4
Decrease (%)	48	80
No Change (%)	36	16

BMI-Body Mass Index

Hip-waist ratio

Table 2: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on Hip-Waist Ratio

Hip-waist ratio	DPP-4 inhibitors	SGLT-2 Inhibitors
Increase (%)	14	4
Decrease (%)	20	54
No change (%)	66	42

The comparison of the effect of both the drugs over the patient's body weight and Hip-Waist Ratio were depicted in Table 1 &Table 2 respectively. In Body Weight, the mean difference between the groups was found to be 2.9 ±0.79 ($p<0.05$). Similarly, for BMI, it was 2.9±1.36 ($p<0.04$). For the Hip-Waist ratio, it was 0.07 ($p<0.05$). SGLT-2 Inhibitors were showing significant reduction while comparing with DPP-4 Inhibitors.

In 2017, Min SH et al. conducted a meta-analysis study titled "Comparison between SGLT2 inhibitors and DPP4 inhibitors added to insulin therapy in type 2 diabetes: a systematic review with indirect comparison meta-analysis". Covariate-adjusted indirect comparison using meta-regression analyses revealed that SGLT2i/INS achieved greater reduction in HbA1c [weighted mean difference (WMD) -0.24%, 95% confidence interval (CI) -0.43 to -0.05%], fasting plasma glucose (WMD -18.0 mg/dL, 95% CI -28.5 to -7.6 mg/dL) and body weight (WMD -2.38 kg, 95% CI -3.18 to -1.58 kg) from baseline than DPP4i/INS without increasing the risk of hypoglycemia. (Min *et al.*, 2017)

FBS

HbA1c

The comparison of the effect of both the drugs over the patient's FBS & HbA1c were depicted in Table 3 &Table 4 respectively. The mean difference between the groups over FBS was 22.08 mg/dl (0.0001), for HbA1c it was 1.09 ($p<0.0043$). SGLT-2 Inhibitors were showing significant reduction while comparing with DPP-4 Inhibitors.

Table 3: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on FBS

FBS	DPP-4 Inhibitors	SGLT-2 Inhibitors
Increase(%)	16	6
Decrease (%)	46	58
No Change (%)	38	36

FBS- Fasting Blood Sugar

Table 4: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on HbA1c

HbA1c	DPP-4 Inhibitors	SGLT-2 Inhibitors
Increase (%)	14	10
Decrease (%)	28	40
No Change (%)	58	50

HbA1c-Glycated Haemoglobin

Table 5: Side Effect Frequency of the Drugs

Drugs Category	Side Effects (%)
DPP-4 Inhibitors	10
SGLT-2 Inhibitors	14

An observational study on "Safety and Efficacy of Sodium-glucose Cotransporter-2 Inhibitors as Adjuvant to Insulin Add-on Therapy in Uncontrolled Type 2 Diabetic Patients" concluded that the SGLT-2 Inhibitors were more effective in both glycemic control (FBS, HbA1c) and non-glycemic control (Body Weight, BMI, Blood Pressure) and acts as a well-tolerated therapy in patients. This put forth the overall reduction in cardiovascular risks (Alfeena Mary PS *et al.*, 2018)

Side effect profile of the drugs

Since 50 patients were enrolled in each group like DPP-4 Inhibitors and SGLT-2 Inhibitors, respectively, along with the effects of two categories of drugs on various patient's parameters, their Side effects are also monitored during the follow-up period of 3 months. It was given in Table 5.

Side effects of DPP-4 Inhibitors: Nausea, Diarrhoea, Stomach pain, Flu-like symptoms, Nasopharyngitis and HSR (Amori *et al.*, 2007)

Side effects of SGLT-2 Inhibitors: UTI, Hyperkalaemia, Hypotension, Hypoglycaemia and Dia-

betic Ketoacidosis (Hsia *et al.*, 2017)

On Side effect profile, SGLT-2 Inhibitors were showing higher incidence for the occurrence of UTI while comparing with DPP-4 Inhibitors

CONCLUSIONS

Both DPP-4 Inhibitors and SGLT-2 Inhibitors were producing significant impact over the Physical and Diabetic profile of Type 2 DM patients. Though while comparing with DPP-4 Inhibitors, SGLT-2 Inhibitors were producing a more prominent effect over patient's parameters, and apart from producing a significant effect on the Physical and Diabetic profile, SGLT-2 inhibitors were also found to have cardiovascular benefits. Side effects like UTI can be overcome by proper patient counseling. On an overall consideration of the effects produced by both the class of drugs, this study concluded that SGLT-2 inhibitors may serves as the best medication choice for add on therapy in Type 2 DM patients.

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REFERENCES

Alfeena Mary PS, *et al.* 2018. A study on safety and efficacy of sodium-glucose cotransporter-2 inhibitors as adjuvant to insulin add-on therapy in uncontrolled type 2 diabetic patients. Asian Journal of Pharmaceutics, 12(3):896–900. Suppl.

Amori, R. E., Lau, J., Pittas, A. G. 2007. Efficacy and Safety of Incretin Therapy in Type 2 Diabetes. JAMA, 298(2):194–194.

Aschner, P., Katzeff, H. L., Guo, H., Sunga, S., Williams-Herman, D., Kaufman, K. D., Goldstein, B. J. 2010. Efficacy and safety of monotherapy of sitagliptin compared with metformin in patients with type 2 diabetes. Diabetes, Obesity, and Metabolism, 12(3):252–261.

Bashier, A., Khalifa, A. A., Rashid, F., Abdelgadir, E. I., Qaysi, A. A., Ali, R., Alawadi, F. 2017. Efficacy and Safety of SGLT2 Inhibitors in Reducing Glycated Hemoglobin and Weight in Emirati Patients With Type 2 Diabetes. Journal of Clinical Medicine Research, 9(6):499–507.

Cho, H. A., Jung, Y. L., Lee, Y. H., Lee, Y. C., Lee, J. E., Lee, S. J., Kim, C. H. 2017. Efficacy of Body Weight Reduction on the SGLT2 Inhibitor in People with Type 2 Diabetes Mellitus. Journal of Obesity & Metabolic Syndrome, 26(2):107–113.

Dicker, D. 2011. DPP-4 Inhibitors: Impact on glycemic control and cardiovascular risk factors. Diabetes Care, 34(Supplement_2):276–278.

Gallwitz, B. 2007. Review of sitagliptin phosphate: a novel treatment for type 2 diabetes. Vascular Health and Risk Management, 3(2):203–210.

Hsia, D. S., Grove, O., Cefalu, W. T. 2017. An Update on SGLT2 Inhibitors for the Treatment of Diabetes Mellitus. Curr Opin Endocrinol Diabetes Obes, 24(1):73–79.

Jose, T., Inzucchi, S. E. 2012. Cardiovascular effects of the DPP-4 inhibitors. Diabetes and Vascular Disease Research, 9(2):109–116.

Katsuyama, H., Adachi, H., Hamasaki, H., Moriyama, S., Sako, A., Yanai, H. 2014. Significant Differences in Effects of Sitagliptin Treatment on Body Weight and Lipid Metabolism Between Obese and Non-Obese Patients With Type 2 Diabetes. Journal of Endocrinology and Metabolism, 4(5-6):136–142.

Min, S. H., Yoon, J. H., Hahn, S., Cho, Y. M. 2017. Comparison between SGLT2 inhibitors and DPP4 inhibitors added to insulin therapy in type 2 diabetes: a systematic review with indirect comparison meta-analysis. Diabetes/Metabolism Research and Reviews, 33(1).

Shaw, J. E., Sicree, R. A., Zimmet, P. Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice, 87(1):4–14.

Suryawanshi, S. Y., Bhargava, A., Agarwal, P., Chamle, V. 2016. Evaluation of safety and efficacy of teneligliptin in newly diagnosed Indian type 2 diabetes patients. In *233 Spring ST*, volume 59, pages 358–358. SPRINGER.

Tandon, N., Anjana, R. M., Mohan, V., Kaur, T., Afshin, A., Ong, K., Dandona, L. 2018.

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Case Report

Internal Medicine Section

Looking Beyond Lupus in Lupus

BHARGAVI KUMAR[1], SARAVANAN THANGAVELU[2]

ABSTRACT

Haematological abnormalities are a common finding in Systemic Lupus Erythematosus (SLE) patients. The wide spectrum includes anaemia, cytopenias, thrombosis as well as bleeding. Among the bleeding disorders, qualitative coagulation defects can be rarely encountered because of clotting factor inhibitors. More uncommon is the occurrence of quantitative clotting factor deficiencies. Here, we describe a rare cause of abdominal pain in a patient with SLE who developed perinephric and colonic sub mucosal haematomas owing to a rare association with acquired factor XIII deficiency which was diagnosed on quantitative assays and effectively treated with cryoprecipitate and fresh frozen plasma.

Keywords: Abdominal haematomas, Factor XIII deficiency, Systemic lupus erythematosus

CASE REPORT

A 21-year-old young lady presented to the Emergency Department with left loin pain, vomiting and loose stools for the last one day. She did not report any fever, radiation of pain, urinary symptoms, pain aggravation on food intake or any prior strenuous activity.

The patient was diagnosed with SLE 2 years ago and was on treatment with steroids, Mycophenolate Mofetil (MMF) and Hydroxychloroquine. She reported history of intermittent episodes of pain in her calf muscles in the last one year. Magnetic resonance imaging (MRI) had revealed the presence of organised haematomas in calf muscles, but the patient did not follow-up due to spontaneous resolution of symptoms.

On examination, the patient was pale, tachycardic and hypotensive. No rashes were noted. Respiratory system examination fairly ruled out pleurisy and musculoskeletal examination was normal. A non-tender firm ballotable mass was palpable in the Left lumbar region.

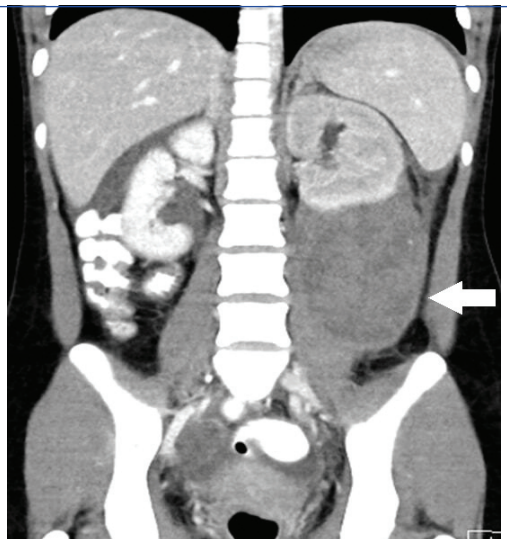
Preliminary investigations showed microcytic anaemia, (haemoglobin 6.2 g/dL), severe thrombocytopenia (22×10^9/L) and mild neutrophilic leucocytosis as shown in [Table/Fig-1]. Her renal function and complements were normal with slightly elevated Erythrocytic sedimentation rate (35 mm first hour). Ultrasonogram of the abdomen showed 9.4×7.4 cm heterogeneous lesion adjacent to the lower pole of the left kidney. A computed tomography revealed the presence of left perinephric haematoma with left hydroureteronephrosis [Table/Fig-2]. Initial coagulation profile revealed normal Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and slightly low fibrinogen levels (1.4 g/L). Direct Coombs test turned out to be positive. Von Willebrand Factor assay was normal. Antiphospholipid Antibody (APLA) work up was done in which anti beta2 glycoprotein turned out to be negative, with Lupus anticoagulant ratio of 1.6.

The differential diagnoses considered were acute SLE flare/bleeding due to thrombocytopenia/coagulation defects and MMF induced haematological crisis. However, complement activity, disease markers were normal and the patient was on irregular treatment with MMF. Since initial PT, APTT levels were normal, possible factor XIII deficiency was considered and she was evaluated accordingly.

The patient was stabilised with intravenous fluids namely Normal Saline, low dose methyl prednisolone 250 mg over 3 days and 2 units packed red blood cells transfusion. There was recurrence of vomiting and loose stools during initiation of enteral feeds and a repeat CT abdomen revealed new onset transmural haematomas in small bowel loops, distal ascending colon, transverse colon,

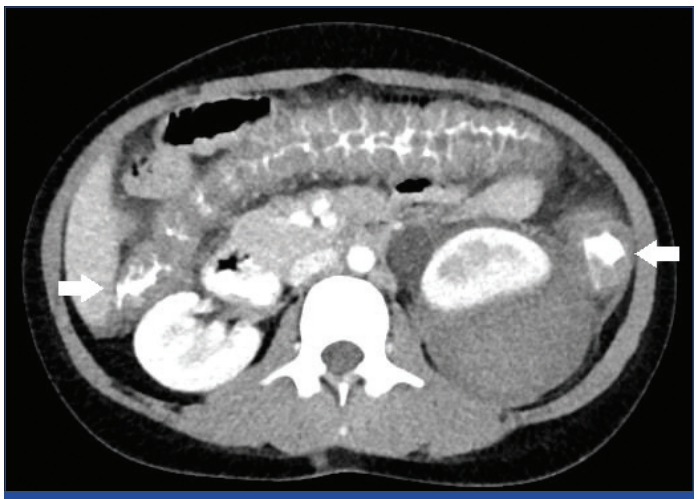
	Day 1	Day 5	Day 7	Day 11	Standard value
Haemoglobin g/dL	6.2	8.4	9.9	11.9	12-15
Mean corpuscular volume fL	73.2				80-100
White blood counts ×10^3/uL	15	10.6		9.2	4-10
Platelets x10^3/uL	22000	42000	83000	110	150-400
Prothrombin time (seconds)/INR	13.3/1.2	13.9/1.33		13.1/1.25	9-12
APTT (seconds)	29.1	22.8		28.2	25-34
Serum fibrinogen levels mg/dL	76	140		342	200-400
C3 c level/C4 mg/dL	83/21				90-180 10-40
ESR mm 1st hour	35	30		17	4-12 mm
HsCRP mg/dL	0.30				<0.5 mg/dL
Procalcitonin ng/mL	0.2				<0.5

[Table/Fig-1]: Preliminary investigations showing microcytic anaemia, severe thrombocytopenia and mild neutrophilic leucocytosis.

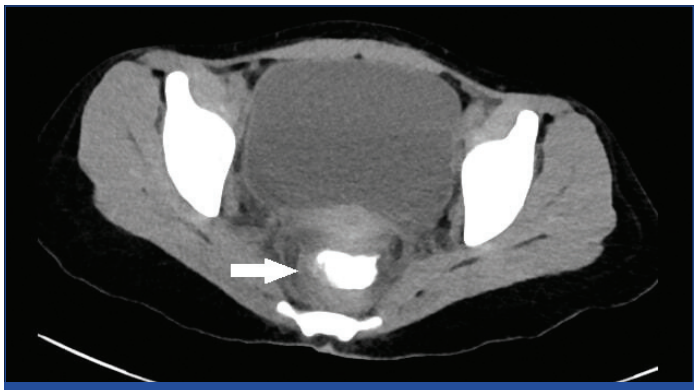


[Table/Fig-2]: CT abdomen showing perinephric haematoma over lower pole of left kidney with ureteric compression.

descending colon and rectum [Table/Fig-3,4]. The coagulation profile results reaffirmed normal APTT levels; repeat fibrinogen levels were normal (3.4 g/L) and platelet counts were improving (120×10^9/L). On performing a clot solubility test, the clot completely dissolved in 5M urea solution and on mixing studies, the clot dissolved partially. Since the results were equivocal, it was not possible to differentiate a hereditary factor XIII deficiency from a factor XIII inhibitor in this case. Subsequently, a factor XIII assay was done which turned out to be <1% thus confirming severe deficiency. Patient was transfused with a total of 18 unit's cryoprecipitate (270 mL) and 10 units of fresh frozen plasma (2400 mL total) during her entire hospital stay following which she showed steady improvement.



[Table/Fig-3]: CT abdomen depicting transmural haematoma of distal ascending, transverse and descending colon.



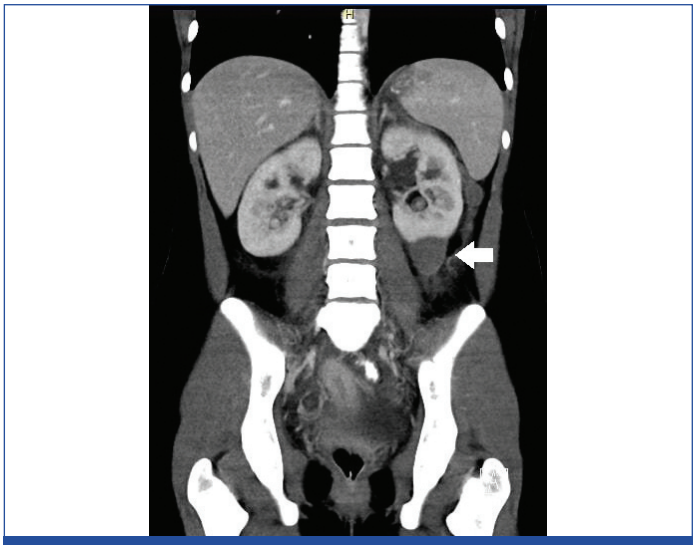
[Table/Fig-4]: CT abdomen showing transmural haematoma involving rectum.

A follow-up CT abdomen a month later showed resolving left perinephric haematomas with resolution of colonic and rectal haematomas. The patient is currently doing well on 5 mg once daily prednisolone and azathioprine [Table/Fig-5].

DISCUSSION

SLE is a challenging disease to treat. In the current era, diagnosing SLE has become common due to increasing awareness, knowledge of its varied presentations and available investigations. However, the course of the disease poses a constant challenge to the treating physician owing to the myriad of systemic manifestations and complications.

Acute abdomen in SLE patients is a challenging diagnostic and therapeutic problem. Most patients are on steroids and/or immunosuppressive agents which mask the physical findings, thus leading to a delayed definitive diagnosis. The reported incidence of abdominal pain in SLE varies from 8 to 40% [1,2]. Assuming that all are due to vasculitis may lead to diagnostic errors. In an observation of 36 active SLE patients presenting with acute abdomen by Medina F et al., 19 had vasculitis, 3 had Anti Phospho Lipid Antibody (APLA) related intra-abdominal thrombosis and the rest were non-SLE related [3]. Therefore, it is imperative to carefully evaluate such a patient based on their clinical, imaging and immunological profile.



[Table/Fig-5]: CT abdomen revealing resolving haematoma over the lower pole of left kidney.

Haematological abnormalities are common findings in patients with SLE. The prevalence is described as 47% in most studies [4]. In a study by Sasidharan PK et al., haematological manifestations were found to be the most common initial presentation of SLE and it was the presenting manifestation in 82% of the patients. The haematological spectrum encompasses anaemia, leucopenia, thrombocytopenia, autoimmune haemolytic anaemia, thrombotic thrombocytopenic purpura, myelofibrosis-hypoplastic anaemia and thrombotic manifestations if associated with APLA [5]. The index patient too had leucopenias along with cutaneous manifestations in her initial presentation leading to her diagnosis.

It is imperative to distinguish haematological abnormalities arising due to disease activity from those due to drug effects. The index patient had normal complement activity and disease markers making acute SLE flare unlikely. The patient was on irregular treatment with MMF, making drug related side effect a remote consideration. Bleeding despite normalisation of platelet count, fibrinogen levels and normal PT, APTT prompted us to investigate us for rare clotting factor abnormalities, such as Factor XIII defects. Coagulation defects in SLE could be due to inhibitors more commonly [6], and due to factor deficiencies rarely [7-9].

Factor XIII is needed to stabilise the fibrin clots and it's deficiency could be genetically acquired as Autosomal recessive disorder or could be due to acquired causes [7]. Hereditary deficiency is attributed to the F13A1 gene located on the short arm of chromosome 6 (6p24.2-p23). Acquired factor XIII deficiency occurs much less often, and usually affects middle-aged or elderly individuals [10]. It can be associated with conditions such as severe liver disease, chronic kidney failure, inflammatory bowel disease, Henoch-Schonlein purpura, autoimmune disorders such as SLE or scleroderma and certain cancers such as myeloid forms of leukaemia. It has also been linked to the use of certain medications including isoniazid, penicillin, and phenytoin.

Individuals with factor XIII deficiency form blood clots per se, but the clots are unstable and often break down, resulting in prolonged, uncontrolled mild to fatal bleeding episodes. The most common bleeding symptoms are subcutaneous bleeding (57%), delayed umbilical cord bleeding (56%), muscle haematoma (49%), haemorrhage after surgery (40%), haemarthrosis (36%) and intracerebral bleeding (34%) [11,12]. However, the index patient's presentation was unusual with perinephric, colonic submucosal and transmural haematomas.

Factor XIII deficiency is screened by clot solubility test and confirmed by quantitative assays [13]. It is treated by factor XIII concentrates,

fresh frozen plasma or cryoprecipitate. When inhibitors develop in individuals with factor XIII deficiency, additional therapy in the form of immunosuppressive agents would be required.

On review of literature, there is a reported Lupus patient with associated factor 13 deficiency who developed intracerebral haematoma [9]. There are case reports of subcutaneous and intramuscular haematomas due to factor XIII deficiency following an invasive procedure in a patient with SLE [14]. In another instance a 70-year-old man had developed spontaneous left gluteal haematoma owing to factor XIII deficiency [15]. Occurrence of gastrointestinal haematomas as in the index patient seems to be extremely rare.

CONCLUSION

It is fruitful to think beyond lupus while approaching the presenting complaints even in a diagnosed patient of SLE. Attributing all the features to the disease per se might be misleading. A high index of suspicion for rarer clotting factor deficiencies is required while evaluating the bleeding manifestations with a normal coagulation profile. There exists a lacuna in ascertaining the fact as to why in an autoimmune disease, there is associated clotting factor deficiency, unlike the expected scenario of clotting factor inhibitors. Only continued medical research can unravel such mysteries.

REFERENCES

[1] Sultan SM, Ioannou Y, Isenberg DA. A review of gastrointestinal manifestations of systemic lupus erythematosus. Rheumatology. 1999;38(10):917-32.
[2] Zizic TM, Classen JN, Stevens MB. Acute abdominal complications of systemic lupus erythematosus and polyarteritis nodosa. The American Journal of Medicine. 1982;73(4):525-31.
[3] Medina F, Ayala A, Jara LJ, Becerra M, Miranda JM, Fraga A, et al. Acute abdomen in systemic lupus erythematosus: The importance of early laparotomy. The American Journal of Medicine. 1997;103(2):100-05.
[4] Bennett JC, Claybrook J, Kinsey H, Holley HL. The clinical manifestations of systemic lupus erythematosus. A study of forty-five patients. Journal of Chronic Diseases. 1961;13(5):411-25.
[5] Sasidharan P, Bindya M, Sajeeth Kumar K. Hematological manifestations of SLE at initial presentation: Is it underestimated? ISRN Hematology. 2012:1-5.
[6] Bashal F. Hematological disorders in patients with systemic lupus erythematosus. The Open Rheumatology Journal. 2013;7(1):87-95.
[7] Chuliber F, Schutz NP, Otero V, Barrera L, Altuna D, Fantl DB, et al. Acquired Factor XIII deficiency in the inpatient setting: Clinical suspicion, impact and treatment: Retrospective case series. Blood. 2016;128(22):1415.
[8] Sawlani KK, Chaudhary SC, Roy A, Tripathi AK. Factor XIII deficiency presenting with intracerebral bleed. BMJ Case Rep. 2013;2013:bcr2012007303.
[9] Dabadghao VS, Singh VB, Meena BL, Khichar S. Systemic lupus erythematosus with intracerebral hematoma due to decreased factor XIII activity: A rare association. J Mahatma Gandhi Inst Med Sci. 2013;18:129-31.
[10] NORD (National Organization for Rare Disorders). (2019). Factor XIII Deficiency-NORD (National Organization for Rare Disorders). [online] Available at: https://rarediseases.org/rare-diseases/factor-xiii-deficiency [Accessed 6 Oct. 2019].
[11] Acharya SS, Coughlin A, DiMichele DM, The North American Rare Bleeding Disorder Study Group. Rare Bleeding Disorder Registry: deficiencies of factors II, V, VII, X, XIII, fibrinogen and dysfibrinogenemias. J Thromb Haemost. 2004;2:248-56.
[12] Ivaskevicius V, Seitz R, Kohler HP, Schroeder V, Muszbek L, Ariens RA, et al. International registry on factor XIII deficiency: a basis formed mostly on European data. Thromb Haemost. 2007;97(6):914-21. doi: 10.1160/TH07-01-0034.
[13] Kohler HP, Ichinose A, Seitz R, Ariens RAS, Muszbek L. On behalf of the Factor XIII and Fibrinogen SSC Subcommittee of the ISTH. Diagnosis and classification of factor XIII deficiencies. J Thromb Haemost. 2011;9:1404-06.
[14] Rabik CA, Atkinson MA, Sule S, Strouse JJ. Treatment of an acquired Factor XIII inhibitor in an adolescent with systemic lupus erythematosus and renal failure. Transfusion. 2017;57(9):2159-63.
[15] Fogarty H, Byrne M, O'Connell NM, Ryan K, White B, O'Donnell JS, et al. Acquired factor Xiii deficiency: an uncommon but easily missed cause of severe bleeding. Ir Med J. 2018;111(5):757.

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Case Report

Euglycemic diabetic ketoacidosis: another masquerader!

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ABSTRACT

Euglycemic ketoacidosis is defined by the triad of euglycemia, metabolic acidosis and ketonemia or ketonuria. In the current era of diabetic management, it is a serious concern with the usage of sodium glucose co-transporter 2 (SGLT2) inhibitors potentiated by a number of precipitating agents. Empagliflozin though a novel oral hypoglycemic agent in this category may also lead to this potential complication. Here we report a 59 year old male, type 2 diabetic who was on empagliflozin and presented with euglycemic ketoacidosis after a binge of alcohol.

Keywords: Empagliflozin, SGLT2 inhibitor, Type 2 diabetes, Euglycemic ketoacidosis

INTRODUCTION

Hyperglycemic states in diabetes like Diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar non ketotic coma are widely encountered, well known and therefore easily diagnosed emergencies. However euglycemic ketoacidosis is an often missed relatively less known condition and under reported scenario. A high index of clinical suspicion is warranted to arrive at this diagnosis because the normoglycemia may mask our clinical judgement of DKA.

The management of diabetes mellitus is ever evolving with oral hypoglycemic agents being an area of constant research and updates. Approved by the Food and Drug administration of USA (FDA) in 2014, Sodium glucose co-transporter 2 (SGLT2) inhibitors is a new addition to the already existing broad list of oral hypoglycemic agents for type II diabetes mellitus. Apart from reducing blood sugars, its spectrum also extends to reducing blood pressure, reduction of weight and cardio protection.[1] It is now included in American Diabetes Association treatment guidelines as one of the add-on agents in dual or triple drug combinations in diabetes as it can effectively reduce HbA1c by 0.5-0.7%. However euglycemic ketoacidosis is a rare but potential complication in this class of drugs for which FDA has released a safety alert in 2015.[2] Its diagnosis may be delayed owing to the normal sugar levels and needs a strong index of suspicion whenever there is ketonemia or ketonuria in the presence of normal blood sugar levels and high anion gap metabolic acidosis.

CASE REPORT

A 59 year old male presented to the emergency department with abdominal discomfort and orthopnea following a binge alcohol intake. Patient was a chronic smoker and alcoholic for 30 years and a known case of

type 2 diabetes mellitus and systemic hypertension for 5 years. He was on irregular treatment with tablet metformin 500 mg in once daily dosage and had started taking tablet empagliflozin 10 mg once daily for the past 2 years without prescribed medical advice. His concomitant medications included tablet nebivolol 5 mg once daily for systemic hypertension. On examination, the patient was moderately dyspnoeic, tachypnoeic, drowsy, arousable and normotensive. Respiratory system examination showed basal crepitations and abdomen examination showed mild epigastric tenderness.

Preliminary investigations revealed normal sugar levels (169 mg/dl), ketonuria (3+) and elevated HbA1c (10.8%). His complete blood counts, serum procalcitonin, liver, thyroid and renal functions were normal but for low bicarbonate (4.65 mEq/l) and a pH of 7.2. The calculated anion gap was high (27.7). Cardiac functions were normal as evidenced by echocardiogram and biomarkers.

An ultrasonography of the abdomen was done in view of epigastric tenderness and showed a bulky pancreas suggestive of acute pancreatitis. His amylase (956 U/l) and lipase (3873 U/l) were also elevated and lipid profile evaluation revealed hypertrigliceridemia (242 mg/dl). His laboratory parameters are summarised in Table 1.

Noting his ketonuria and high anion gap metabolic acidosis with normal blood sugars we arrived at a diagnosis of euglycemic ketoacidosis in the background of his medication history of empagliflozin therapy which was probably precipitated by alcohol binge and acute pancreatitis. Patient was put on ventilator support and started on intravenous fluids, insulin along with 5% dextrose and bicarbonate correction. The acidosis improved gradually and blood glucose levels were controlled with rapidly acting insulin. After the patient improved symptomatically, he was weaned off and tolerated soft diet.

Table 1: Laboratory parameters of the patient across his days of admission showing his normal sugar values at admission and metabolic acidosis which resolved following treatment.

	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 10
Sugars (mg/dl)	169	141	132	178	193	299	268
Urine for ketones	+++	+++	++	+	Nil	Nil	Nil
pH	7.2	6.9	7.47	7.4	7.48	7.40	7.41
HCO_3 (mEq/l)	4.65	7.0	14	11.3	18	23.8	24.1
Amylase (U/l)	956	-	-	145	242	-	-
Lipase (U/l)	3873	-	-	639	1158	887	637

On discharge, the patient was counselled regarding diet, insulin therapy, advised alcohol abstinence and was counselled to stop any self-medication of new oral hypoglycemic agents. The patient is now on regular follow up, maintaining average blood sugars of 200 mg/dl. As the patient was apprehensive about continuing insulin, he is now put on oral agents-insulin secretagogues alongside diet and lifestyle modification.

DISCUSSION

There are a multitude of causes leading to metabolic acidosis in diabetics. These include normal anion gap acidosis like renal failure as well as high anion gap states like lactic acidosis and ketoacidosis. Ketoacidosis could be due to diabetic ketoacidosis, starvation ketoacidosis or alcoholic ketoacidosis. Diabetic ketoacidosis may be either hyperglycemic or euglycemic. Thus analyzing the risk factors that contribute to the development of ketoacidosis is very crucial.

Euglycemic ketoacidosis was first observed and reported in a case series by Munro et al who observed that a subset of DKA patients had sugar levels <300 mg/dl.[3] Subsequently euglycemic ketoacidosis has been characterized by the triad of euglycemia (blood glucose <250 mg/dl), metabolic acidosis (pH <7.35, bicarbonate <18 mEq/l) and ketonemia or ketonuria.[4] To explain it succinctly, euglycemic ketoacidosis is DKA sans marked hyperglycemia. It is mainly described in type 1 diabetes but nevertheless not uncommon in type 2 diabetes. Possible contributing factors include starvation, alcoholism, pregnancy, pancreatitis and drugs. Among drugs, SGLT2 inhibitors are notorious to cause euglycemic ketoacidosis.

SGLT 2 inhibitors act primarily by inhibiting sodium glucose co-transporter 2 in proximal convoluting tubule which mediates 80-90% of glucose absorption in kidney. As a result of this facilitation of glucose excretion by kidney, blood glucose levels are lowered. When the blood glucose levels reduce, the insulin release from pancreas also reduces proportionately tilting the normal insulin: glucagon ratio, thereby favoring ketogenesis. Other proposed mechanisms include release of glucagon by direct SGLT2 inhibition in pancreas, release of kisseptin which inhibits insulin release and also decreased reabsorption of ketones leading to ketonemia.

Empagliflozin has shown meticulous benefits in reducing major cardiac events as evidenced in the 'EMPA-REG OUTCOME' trial.[5] Since empagliflozin's mechanism of action is independent of beta cell functioning, they are useful in all type 2 diabetes patients as long as estimated glomerular filtration rate is above 45-60 ml/min. Other potential benefits include weight loss (2-3 kg) and

reduction in blood pressure (3-6 mmHg systolic, 1-2 mmHg diastolic) due to natriuresis besides cardioprotection. Though beneficial in numerous aspects, empagliflozin also has adverse effects like genital fungal infections (3-5%), urinary tract infections (1-2%), volume depletion and hypotension. However of serious concern is the development of euglycemic ketoacidosis though reported in less than 0.1% patients. Canagliflozin and dapagliflozin also carry the risk of euglycemic ketoacidosis with reported incidence being 0.8% and less than 0.1% respectively.[6]

On literature review, we noted that Gammons and Counselman have presented a case report of a chronic alcoholic, type 2 diabetic patient on empagliflozin developing euglycemic ketoacidosis with acute pancreatitis similar to our index patient.[7] So also Candelario and Wykretowicz have presented a type 2 diabetic female who developed euglycemic ketoacidosis while on empagliflozin therapy precipitated by acute cholecystitis.[8]

In our index patient with euglycemic ketoacidosis, the clear culprits were empagliflozin overtly precipitated by his binge of alcohol and pancreatitis. A high index of suspicion alone prompted us to withdraw the offending drug and treat him with insulin and dextrose apart from intravenous fluids in correcting his metabolic acidosis.

CONCLUSION

DKA need not necessarily present with hyperglycemia. High anion gap ketoacidosis should be a red flag to look for euglycemic ketoacidosis which requires both insulin and dextrose in correcting the underlying metabolic derangement. Careful drug history and possible precipitators should be identified and promptly corrected. SGLT2 inhibitors though a novel agent in the treatment of diabetes also comes with its quantum of adverse effects like euglycemic ketoacidosis which is a potential life threatening complication. The key to a successful usage of newer exciting hypoglycemic agents lies in choosing our patients wisely and carefully avoiding those who have other contributing factors for acidosis.

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REFERENCES

1. Rosenwasser RF, Sultan S, Sutton D, Choksi R, Epstein BJ. SGLT 2 inhibitors and their potential in treatment of Diabetes, Diabet Metabol Syndrome Obes. 2013;6:453-67.
2. U.S. Food and Drug Administration. Drug Safety Communication: FDA warns that SGLT2 inhibitors for diabetes may result in a serious condition of too much acid in the blood, 2015. Available at: http://www.fda.gov/downloads/Drugs/Drugsafety/UCM446954. Accessed on 05 October 2018.
3. Munro JF, Campbell IW, McCuish AC, Duncan LJ. Euglycaemic diabetic ketoacidosis. Br Med J. 1973;2:578-80.
4. Miles JM, Gerich JE. Glucose and ketone body kinetics in diabetic ketoacidosis. Clin Endocrinol Metab. 1983;12:303-19.
5. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes (EMPA-REG OUTCOME). New Engl J Med. 2015;373:2117-28.
6. Rosenstock J, Ferrannini E. Euglycemic diabetic ketoacidosis: a predictable, detectable, and preventable safety concern with SGLT2 inhibitors. Diab Care. 2015;38(9):1638-42.
7. Gammons T, Counselman FL. Sodium-glucose cotransporter-2 induced diabetic ketoacidosis with minimal hyperglycemia. Clin Practice Cases Emerg Med. 2018;2(1):47-50.
8. Candelario N, Wykretowicz J. The DKA that wasn't: a case of euglycemic diabetic ketoacidosis due to empagliflozin. Oxford Med Case Report. 2016;2016(7):144-6.

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Tenofovir induced delayed nephro-osteo toxicity – a case report

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Abstract

Tenofovir disoproxil fumarate (TDF) is the foundation nucleotide reverse-transcriptase inhibitor and the recommended first-line regimen for all naive Human Immunodeficiency Virus-1 (HIV-1) patients whose age is more than 10 years and body weight is more than 30 kg. Although it has a good safety profile overall, nephrotoxicity is a concern and its overall incidence is 1–6% with a long period of clinical latency. Nephrotoxicity may manifest as either proximal renal tubule dysfunction in the form of a partial or complete Fanconi syndrome or as decreased renal function leading to acute or chronic kidney injury. Osteomalacia can also develop secondary to complicating hypophosphataemia and low calcitriol levels. Here we report a 50-year-old HIV-positive male on tenofovir who presented with proximal renal tubular acidosis and fracture of left neck of femur four years after initiation of the drug.

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Introduction

Tenofovir disoproxil fumarate (TDF) is an oral acyclic nucleotide analogue reverse transcriptase inhibitor used in the treatment of HIV-1 infection approved by the Food and Drug Administration (FDA) in 2001.[1] Tenofovir has found a major role in the fixed-dose combination along with lamivudine and efavirenz or doultegravir.[2] It has an added advantage in HIV-hepatitis B co-infection. It has an overall good safety profile, a relatively long half-life and allows once-daily dosing ensuring good patient compliance with relatively rare side effects.

Tenofoviris is actively transported into the mitochondria of proximal tubule cells of the kidney and thereby can lead to proximal tubular dysfunction in long-term use.[1] Proximal renal tubular acidosis is characterised by defective proximal tubular reabsorption associated with phosphaturia, glycosuria, aminoaciduria, bicarbonaturia, resulting in hypophosphataemia, hypokalaemia and metabolic acidosis. Overt Fanconi syndrome is rare, but can occur as a delayed presentation, with incidence being 1.09 per 1000 patient years.[3]

Osteotoxicity can occur either due to direct effect of the drug itself or secondary to hypophosphataemia as a result of renal toxicity.[4] Hence routine and careful monitoring for these toxicities is absolutely essential as they may occur even after a long asymptomatic period.

Case presentation

A 50-year-old male presented with poor appetite, significant weight loss of 6 kg in 2 months and progressively painful difficulty in walking over 8 months. He was diagnosed with HIV-1 8 years ago and was on regular treatment with tenofovir, lamivudine, atazanavir and ritonavir for 4 years elsewhere. CD4 count done 3 months previously was 118 cells/mm3. He took diclofenac 50 mg intermittently over the previous 2 months.

On examination, he was thin, pale and had a limited range of movements in both lower limbs and left shoulder due to pain. His body mass index (BMI) was 16.5k g/m2.

Preliminary investigations showed macrocytic normochromic anaemia, elevated creatinine levels, hypokalaemia, hypophosphataemia, low bicarbonate levels and elevated serum alkaline phosphatase (ALP) levels. Arterial blood gas analysis revealed metabolic acidosis. Urine examination showed mild albuminuria and glycosuria (serum glucose was normal) with no pus cells. Results are shown in Table 1. Ultrasonography of the abdomen and pelvis revealed chronic renal parenchymal changes and renal artery Doppler study was normal.

X-ray pelvis showed an undisplaced fracture in the left neck of femur and an old displaced fracture in right neck of femur

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Table 1

Lab parameters	DAY 1	DAY 2	DAY 3	DAY 5	2 WEEKS AFTER DISCHARGE	1 MONTH AFTER DISCHARGE	Reference range
pH	7.23	7.259	7.266		7.34		7.35-7.45
Acid base excess mmol/L	-14.4	-12.2	-11.6	-8.8			-2 - +3
Serum Sodium (mEQ/l)	139		144	143	138	143	136-146
Serum Potassium (mEQ/l)	2.45	3.22	3.24	4.60	5.5	3.66	3.5-5.0
Serum Bicarbonate (mEQ/l)	12.5	14.7	14.2	15.9	15.7	21.6	22-30
Serum Chloride (mEQ/l)	107		105			105	102-109
Serum phosphorous (mg/dl)		1.3		2.1	4.7		2.7-4.5
Serum Creatinine (mg/dl)	1.75			1.24		1.26	0.8-1.25
Creatinine clearance (mL/min)	39.29			55		54.56	97-137
Serum ALP(U/l)	459						40-129
Serum Vitamin D (ng/ml)	31.08						Deficient <20 Insufficient 21-29 Normal 30-70
Urine pH	6.8			>9.0			4.6-8.0
Urine glucose		4+			3+	1+	negative
Urine albumin		3+				1+	negative

confirmed on computed tomographic scanning. Serum vitamin D levels were normal. HIV viral load was undetermined and CD4 counts were 327.

Tenofovir-induced proximal renal tubular acidosis and pathological fracture was suspected and tenofovir withheld. He was treated with intravenous fluids, appropriate bicarbonate correction, thiazide diuretics and potassium citrate and his acidosis gradually improved. He was discharged on a high-potassium diet with oral potassium citrate, calcium and oral sodium bicarbonate supplements. At follow-up two weeks later he had an improving appetite and decreased pain in his legs. His electrolytes were normal with decreasing trend of creatinine levels. He was started on abacavir after hypersensitivity testing with HLAB5701 along with lamivudine and doultegravir. His bicarbonate, creatinine clearance and urine albuminuria and glycosuria improved significantly and he was able to attend to his activities of daily living.

Discussion

According to the India HIV Estimation 2017 report, national adult (15–49 years) HIV prevalence in India is estimated at 0.22% (0.16%–0.30%).[2] India has the third largest HIV epidemic in the world, with 2.14 million people living with HIV as per the UNAIDS 2018 data. 56% of adults are on anti-retroviral treatment.

Kidney disease in HIV-affected individuals may be attributed to a variety of causes which include: HIV-associated nephropathy (HIVAN) presenting with focal segmental glomerulosclerosis, HIV-associated immune complex kidney disease (HIVICK), acute kidney injury or chronic kidney disease.[5] The common antiretroviral therapy (ART) causes include tenofovir (tubular toxicity), protease inhibitor indinavir (nephrolithiasis, crystal nephropathy and interstitial nephritis) and enfuviritide (glomerulopathy).[6]

Tenofovir-induced nephrotoxicity is quite rare with most studies estimating it between 1–6%.[7] Tenofovir-induced nephrotoxicity could present as proximal tubular dysfunction

with preserved renal function (14%) or proximal tubular dysfunction associated with decreased renal function – which can either be acute kidney injury (1.6 %) or chronic kidney disease (8%).[1] Our patient also presented with proteinuria, glycosuria along with elevated creatinine levels and chronic renal parenchymal changes on ultrasonography.

About 20–30% of TDF is actively transported into the renal proximal tubule cells by organic anion transporters (OAT 1 and 3) located in the basolateral membrane and then subsequently secreted to the tubular lumen by the apical membrane transporters MRP4 and MRP-2 (Multidrug Resistance Proteins).[8] Any factor which causes overexpression of OAT1 and 3 or inhibits MRP-4 will cause tenofovir to accumulate within the cells, which in turn causes mitochondrial toxicity. Rarely, distal renal tubular acidosis has also been encountered (<5%), although the exact mechanism is not known.[9]

Risk factors include old age, low BMI, prior history of renal disease, reduced baseline glomerular filtration rate (eGFR), diabetes mellitus, hypertension, hepatitis C co-infection and genetic polymorphisms (APOL1G1 gene).[8] The index patient's BMI at the time of presentation was quite low. However, his BMI at the time of initiation with tenofovir was not known. Factors which inhibit MRP-4 include anti-inflammatory drugs like diclofenac, ibuprofen as well as protease inhibitors. The index patient was on concomitant ritonavir and diclofenac, both of which might have added on to the toxicity.

Nephrotoxicity can occur after years of tenofovir therapy as a delayed presentation with the median duration of presentation ranging between 16 months to 5 years.[10] Casado et al. observed a 32% prevalence of proximal tubulopathy after a median time of 5 years on tenofovir.[11] Our patient presented with nephrotoxicty 4 years after ART initiation with tenofovir. He had asthenia, loss of appetite, significant weight loss, muscle pain, easy fatigability and poor quality of life despite improvement in CD4 count, which prompted us to probe drug-related toxicities.

Osteotoxicity in HIV may be multifactorial. HIV can cause direct bone damage due to its effects on bone remodelling. Tenofovir can cause osteotoxicity through secondary effects of proximal renal tubular acidosis and its resultant hypophosphataemia. Because renal metabolism of vitamin D to its active form dihydroxyl cholecalciferol also occurs in proximal tubular cells, serum vitamin D levels can also be low in these patients. In addition, tenofovir can influence phosphate metabolism through circulating factors like phosphatonins.[4] In our patient vitamin D levels were normal but hypophosphataemia and elevated alkaline phosphate levels were noted and were probable contributors to bone toxicity and resultant pathological fracture.

This combined presentation of renal and bone toxicity with tenofovir therapy is quite rare, with few case reports available.[12,13] Lucey et al. reported a teenage boy with HIV infection on tenofovir presenting four years later with Fanconi syndrome and stress fracture of bilateral metatarsals.[13]

The early stages of proximal tubulopathy can be diagnosed by urine analysis of beta 2 microglobulin. In a study from Christian Medical College, South India, alkaline phosphatase (ALP) levels increased by nearly 200% after treatment with tenofovir. It was statistically significant thereby rendering it an important marker for monitoring of toxicity.[7] This is similar to the observation by Woodward et al. in which 89.5% of patients on tenofovir who developed hypophosphataemia had elevated ALP levels after a median time of seven years on ART.[12] It was also observed that creatinine clearance was a more valuable indicator compared to absolute creatinine values. National AIDS Control Organisation guidelines 2018 suggest measuring creatinine clearance and assessing risk factors for renal toxicity prior to starting tenofovir therapy. eGFR, urine protein/creatinine ratio, urine glucose and urine phosphates need to be monitored every three months for one year and then biannually.[2]

It is recommended to switch to alternative nucleoside reverse transcriptase inhibitors whenever tenofovir toxicity is encountered. However, even in those patients where tenofovir-related toxicities are encountered, it is still noted that the patients had a strong clinical and immunological response.

Ideally, if tenofovir entry to proximal tubule cells is prevented or exit is facilitated, renal toxicity may be averted. Drugs like probenecid which is an inhibitor of OAT1 and rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist that induces the expression of many proximal tubular cell transporters were studied in the past.[1] However, both had dose-limiting side effects.

Conclusion

Although antiretroviral therapy has given hope and life to HIV patients, it is important to be aware of the potential side effects and thereby monitor for the associated toxicities.

Early diagnosis of tubulopathy by urinary protein excretion, glycosuria assessment, creatinine clearance evaluation and alkaline phosphate assessment can be of central importance in identifying kidney and bone toxicities. Prompt recognition of toxicity and withdrawal of the drug can significantly improve or even reverse tubular damage in most patients, thereby avoiding chronic kidney damage.

References

1 National AIDS Control Organisation. National Technical Guidelines on ART October 2018. https://lms.naco.gov.in/frontend/content/NACO%20%20National%20Technical%20Guidelines%20on%20ART_October%202018%20(1).pdf (accessed 2/1/20).

2 Fernandez BF, Ferrer AM, Ana B Sanz et al. Tenofovir Nephrotoxicity: 2011 Update. *AIDS Res Treat* 2011; 2011: 354908. doi: 10.1155/2011/354908.

3 Medland NA, Chow EP, Walker RG et al. Incidence of renal Fanconi syndrome in patients taking antiretroviral therapy including tenofovir disoproxil fumarate. *Int J STD AIDS* 2018; 29: 227–36.

4 Grant PM, Cotter AG. Tenofovir and bone health. *Curr Opin HIV AIDS* 2016; 11: 326–32.

5 Jotwani V, Atta MG, Estrella MM. Kidney Disease in HIV: Moving beyond HIV-Associated Nephropathy. *J Am Soc Nephrol* 2017; 28: 3142–54.

6 Rho M, Perazella MA. Nephrotoxicity associated with antiretroviral therapy in HIV-infected patients. *Curr Drug Saf* 2007; 2: 147–54.

7 Shubhanker M , Rupali P, Rajeev K et al. Renal Tubular Dysfunction Associated with Tenofovir Therapy *J Assoc Physicians India* 2014; 62: 580–2.

8 Tourret J, Deray G and Bagnis CI et al. Tenofovir Effect on the Kidneys of HIV-Infected Patients: A Double-Edged Sword? *J Am Soc Nephrol* 2013; 24: 1519–27.

9 Iwata K, Nagata M, Watanabe S et al. Distal renal tubular acidosis without renal impairment after use of tenofovir: a case report. *BMC Pharmacol Toxicol* 2016; 17: 52.

10 Ezinga M, Wetzels J, Bosch M et al. Long-term treatment with tenofovir: prevalence of kidney tubular dysfunction and its association with tenofovir plasma concentration. *Antivir Ther* 2014; 19: 765–71.

11 Casado JL. Renal and Bone Toxicity with the Use of Tenofovir: Understanding at the End. *AIDS Rev* 2016; 18: 59–68.

12 Woodward CLN, Hall AM, Williams IG. Tenofovir-associated renal and bone toxicity. *HIV Medicine* 2009; 10: 482–7.

13 Lucey JM, Hsu P, Ziegler JB. Tenofovir-related Fanconi's syndrome and osteomalacia in a teenager with HIV. *BMJ Case Rep* 2013; 2013: bcr2013008674.

To err is…Drug? Patient? Doctor? Healthcare System?

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ABSTRACT

Medicine is a practicing art. This is because we evolve constantly with everyday learning. The core of the health care system is management of patients with pharmacological measures. However the role of the health care deliverers does not stop with prescribing drugs. It is of central importance to be aware of the potential side effects of the drug including the rarest of the complications. It is equally important to discuss with our patients the available choices, the risk - benefit ratio and counsel them accordingly regarding anticipated common and rare side effects. The patient must be repeatedly reinforced about the importance of periodic follow up with the doctor, need for regular laboratory monitoring and reporting of alarm signs without any delay. Thus patient education, awareness of drug characteristics and a detailed doctor prescription are central in avoiding as well as managing drug related adverse effects. Here we present a case series of 9 different patients presenting with varied drug related adverse effects.

Key words: Adverse drug reactions, Periodic Monitoring, Patient education, Warning signs, Physician role.

INTRODUCTION

The European Medicines Agency (EMA) defines an adverse drug reaction as "a response to a medicinal product which is noxious and unintended.[1] The US Food and Drug Administration (FDA) defines an adverse drug reaction as any untoward medical occurrence associated with the use of a drug in humans "for which there is a reasonable possibility that the drug caused the adverse event."[2] Most of the drug reactions usually go underreported. Doctor prescription, drug characteristics and patient education are the three vertices of the adverse drug management triangle.

Drug reactions could be highly characteristic or nonspecific.[3] They could be dose, frequency or time dependent, occurring either almost right away or much later. Polypharmacy is a clear culprit in the occurrence of adverse drug reactions.

Patient factors like age, gender, creatinine clearance, body weight and fat distribution, race, ethnicity, genetic susceptibility, smoking and alcoholism are well known factors contributing to adverse drug reactions.[4] Patient education of drug intake, timing with other drugs, other co-morbidities, alertness in early recognition and reporting of warning signs are also of paramount importance.

A physician should be insightful in drug prescription fully taking into consideration previous drug histories and other simultaneous drugs the patient is on. It is wise to restrain hurry while diagnosing the disease or prescription even on a crowded day practice. It is prudent to refer the patient when required or have a close follow up while in doubt.

CASE REPORT

Case 1

A 58 yr old male patient presented with history of fever, loose stools and abdominal discomfort for 1 week. Patient was diagnosed

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with Myasthenia gravis 3 months ago elsewhere and started on immunosuppressants with oral Prednisolone 5mg once daily, Azathioprine 50mg once daily and Pyridostigmine 60mg thrice daily.

His examination was normal except for right eye ptosis. His initial blood counts showed pancytopenia as shown in Table 1. Bone marrow biopsy was suggestive of myelosuppression. With the diagnosis of drug induced pancytopenia, azathioprine was withheld and he was started on broad spectrum antibiotics and antifungal agents. Patient was given an injection of Granulocyte-Colony Stimulating Factor (G-CSF), following which his counts steadily improved. 2 weeks later patient reported with normal blood counts and patient was put on Mycophenolate mofetil along with pyridostigmine and steroids. Currently the patient's myasthenic symptom has improved and is maintained on oral prednisolone 10 mg once daily.

Case 2

27 year old female was admitted with history of fever for 3 days. Patient was evaluated elsewhere for a right sided neck swelling 1 month ago. A Computed Tomography (CT) Angiogram of the chest had shown Aortoarteritis changes suggestive of vasculitis for which she was treated with oral Azathioprine 50 mg once daily and oral Methotrexate 10 mg weekly.

On admission she was pale and tachypnoeoic tachypnoeic with gingival petechial petechiae. Her blood counts showed severe pancytopenia (Table 1). With a clinical diagnosis of drug induced bone marrow suppression, patient was put on strict barrier nursing with empirical antibiotics and antifungal agents. Patient required supportive transfusion with packed red cells, platelets and G - CSF in view of profound pancytopenia. However a week later, patient developed persistent dry cough, left peri orbital hematoma, hoarse voice, hypotension and stridor. A chest imaging and tracheal aspirate were suggestive of Aspergillus. Patient was put on mechanical ventilation and high end antibiotics and antifungal agents but eventually succumbed to refractory septic shock.

Case 3

58 year old female patient was admitted with history of recurrent nasal and gum bleeding for 1 month. Patient was diagnosed with *Pemphigus vulgaris* and had been taking Methotrexate 7.5mg weekly once for 10 years.

On examination, patient was pale and had non pruritic, non-blanchable multiple hyper pigmented maculae all through the body. Initial investigations as displayed in Table 2 showed pancytopenia and subsequently a bone marrow biopsy was suggestive of myeloid suppression with no atypical cells. Patient was started on intravenous antibiotics and transfused with packed red cells and platelets. Further the skin lesions were suggestive of psoriasis and patient was managed with antihistaminic drugs and topical applications. A month later patient reported with normal counts and is doing well on topical steroids and moisturizers.

Table 1: Showing the bicytopenia pattern in case 1 and subsequent improvement; case 2 showing persistent pancytopenia.

		CASE 1				CASE 2				
Lab parameter	Reference range	Day 1	After G-CSF	2 weeks later	1 year later	Day 1	Day 3	Day 5	Day 9	Day 15
Hemoglobin	13 - 17g/dL	10.2	10.9	14.8	9.6	3.8	7.5	9.5	9	6.3
Total white blood count (WBC)	4-10x10^3/uL	0.8	6.8	6.6	8.4	0.7	1.3	1.7	2.2	0.6
Platelets	150-400x10^3/uL	261	556	247	212	22	9	3	10	11

Table 2: Showing the drug induced pancytopenia in case 3, 4 and 5 with subsequent improvement.

Lab parameter	CASE 3				CASE 4					CASE 5				
	Day 1	Day 5	A month later	A year later	Day 1	Day 3	Day 6	2 weeks later	2 months later	6 months prior to treatment	Day 1	Day 2	2 days after G-CSF	1 Month later
Hemoglobin	7.1	8.2	8.4	10.0	8.2	9	10.1	8.0	13.2	8.5	4.4	5.3	6.5	9.9
White blood count	2.1	3.9	14.7	6.8	0.3	1.9	4.3	10.5	6.2	8.5	3.1	1.8	8.0	6.7
Platelets	4	86	187	211	30	37	65	617	242	246	75	53	117	153

Case 4

68 year old male patient was admitted with complaints of fever, productive cough and breathlessness for 2 days. Patient was a known case of Rheumatoid arthritis on oral Methotrexate 5 mg weekly for 8 years.

On admission, patient was severely dyspnoeic and tachypnoeic, had bilateral lung crepitations with Varicella zoster skin lesions. His initial investigations showed pancytopenia (Table 2). Patient was treated with empirical antibiotics, antifungal and antiviral drugs. He was supported with blood products transfusion and G-CSF. His tracheal and urine cultures revealed multidrug resistant *Klebsiella pneumonia*. Patient had a tumultuous hospital stay where he required reintubation after weaning owing to excessive oropharyngeal secretions which was further complicated by an episode of atrial fibrillation reverted by cardio version. After aggressive management with sensitive antibiotics and ventilator support, patient improved symptomatically and he was able to mobilize around in wheel chair. Patient was discharged with Hydroxychloroquine tablet 200 mg once daily and reported on follow up with improved counts and no active inflammatory signs.

Case 5

51 year old female patient was admitted with history of generalized fatigability, exertional dyspnoea and swelling of legs for 10 days. She was diagnosed with Rheumatoid arthritis for the last 2 years. Initially the patient had taken native medication for her joint pain and then subsequently started elsewhere on weekly Methotrexate dose of 5 mg, Leflunomide 10 mg daily and 5 mg once daily prednisolone for the last 6 months. On examination, the patient was pale, had glossitis, bilateral pitting pedal edema and crepitations in the lungs. Initial investigations (Table 2) showed pancytopenia. On the suspicion of drug induced pancytopenia, methotrexate and leflunomide were withheld. Patient was started on broad spectrum antibiotics and antifungal agents with strict barrier nursing. Bone marrow biopsy showed hypo cellular marrow with no atypical cells. The patient was administered one dose of G-CSF and 2 units packed red cells were transfused. Her blood counts gradually started improving and patient was discharged with low dose oral prednisolone and is maintained on the same on follow up.

Case 6

59 year old female patient presented with breathlessness, decreased urine output, swelling of legs for the last 2 days. Patient had started developing facial puffiness a month ago for which she was suggested thyroid function tests elsewhere. Her Thyroid Stimulating Hormone (TSH) levels were 6 microIU/ml and patient was started directly on 100 micrograms (mcg) Levothyroxine.

On examination, the patient was tachypnoeic, had anasarca and extensive bilateral lung crepitation's. Her cardiac markers were elevated and an echocardiogram showed mild pericardial effusion with preserved ejection fraction and cardiomegaly. A diagnosis of levothyroxine induced heart failure was arrived at and patient was treated with noninvasive ventilation, diuretics and her Levothyroxine dose was brought down to 50 micrograms/day. Patient gradually improved and was discharged with 50 mcg of Levothyroxine. On follow up her dose was gradually increased by 12.5 mcg and patient is doing well on tab. Levothyroxine 75 micrograms per day.

Case 7

A 29 yr old female patient with no known previous co morbidities came with complaints of generalized swelling of body, decreased urine output and orthopnea for 1 week. Patient was apparently normal 3 months back when she started having hair fall, mild facial puffiness, dyspnea on exertion and dryness of skin but was able to attend to her household work. She had consulted elsewhere, her TSH levels were 5.3 micro IU/ml and the patient was started on tablet Levothyroxine 100 mcg following which her breathlessness exaggerated.

On examination, the patient was dyspnoeic, tachypnoeic, had anasarca and oral ulcers with lung crepitations. A clinical diagnosis of a volume overload status due to Levothyroxine induced heart failure with underlying connective tissue disease was arrived at and Levothyroxine was stopped. Initial investigations showed mild neutrophilic leukocytosis, elevated ESR with severe nephrotic range proteinuria as Table 3. Her Antinuclear Antibody (ANA) pattern was 3+ with hypocomplementemia. An echocardiogram showed grade 1 diastolic dysfunction. Patient was started on diuretics, noninvasive ventilation, intravenous Methylprednisolone 250 mg injection (3 doses) and albumin infusion. Patient subsequently improved; a week later patient developed sudden breathlessness, a CT pulmonary angiogram showed a left sub segmental pulmonary thromboembolism and antiphospholipid antibodies were also positive. Patient was administered anticoagulation and improved well. Patient was discharged a week later with oral prednisolone 40 mg once daily, oral anticoagulants and Hydroxychloroquine (HCQ). A year later patient is doing well on follow up with normalized disease markers and is on oral Prednisolone 5 mg once daily and Mycophenolate mofetil 500 mg twice daily.

Case 8

45 yr old female patient was admitted with history of fever and cough for 1 week. She was a known case of endometrial carcinoma on chemotherapy. She had undergone her 5th cycle with Paclitaxel a week ago. Patient also had underlying diabetes mellitus and chronic kidney disease, which were conservatively managed.

On examination, the patient was pale, tachypnoeic and febrile with bilateral lung crepitations. Investigations showed severe metabolic acidosis, profound pancytopenia, high procalcitonin and deranged renal functions as summarized in Table 4. Chest x-ray showed left lower lobe pneumonia. A diagnosis of febrile neutropenic sepsis was made and patient was put on aseptic precautions and barrier nursing. She required aggressive management with Granulocyte Colony Stimulating Factor, antibiotics, antifungals, antivirals, antipyretics, bicarbonate infusion and other supportive measures. Patient gradually improved and was discharged with oral antibiotics and had normalized blood counts on follow up.

Case 9

50 yr old male presented with progressive difficulty in walking over the last 8 months. He was diagnosed with retroviral disease 8 years ago and was on Tenofovir, Lamivudine and Ritonavir boosted Atazanavir for the last 4 years.

On examination, the patient was emaciated and decreased range of movements in the lower limbs due to pain. Initial investigations showed hypokalemia, hypophosphatemia with significant metabolic acidosis. Urine showed albuminuria (3+) and glycosuria (3+). His liver functions also showed deranged alkaline phosphatase levels and vitamin D levels were normal (Table 5). Subsequently an X-Ray and Computed tomography of the hips showed bilateral fracture neck of femur. Hence a diagnosis of Tenofovir induced proximal renal tubular acidosis and bone toxicity was arrived at and tenofovir was promptly withheld. Patient was managed with intravenous fluids, potassium and bicarbonate supplementation with thiazide diuretics. Bilateral open reduction with internal fixation was planned, however patient opted conservative management. His HIV viral load and CD4 counts were in the improving trend compared to previous year values. After hypersensitivity testing, patient was started on Abacavir / Lamivudine and Doultegravir regimen and was discharged with oral potassium and bicarbonate supplements. Patient reported to follow up with normal electrolytes and is attending to activities of daily living.

DISCUSSION

Drug related adverse effects are thus not an uncommon entity. Rather it is grossly underreported. This case series has observed drug reactions in 9 patients who were admitted in PSG Institute of Medical sciences and

Table 3: Showing the thyroid pattern at admission, nephrotic range proteinuria and hypocomplementemia in case 7.

Lab parameter	At admission	1 month after discharge	A year later	Reference range
T3 (Serum)	0.37	1.06		0.846 - 2.02 ng/mL
FT4 (Serum)	0.98	1.12		0.932 - 1.71 ng/dL
TSH (Serum)	0.484	1.68		0.27 - 4.2 microIU/ml
FT3 (Serum)	0.64	2.92		2-4.4 Pg/ml
Urine Protein Creatinine Ratio	27.33	10.108	0.070	
Serum Creatinine	0.83			0.66 - 1.09 mg/dL
Serum albumin	0.7 g/dl	1.5	3.4	3.4 - 4.8 g/dL
Complement 3c level	28.42mg/dl	79	102	90 - 180 mg/dl
Complement 4 level	10mg/dl	14	16	10 - 40 mg/dl

Table 4: Showing the pancytopenia and subsequent improvement with treatment in case 8.

Lab parameter	Day of admission	After 1st dose G-CSF	After 3rd dose G-CSF	Day of discharge	10 days after discharge	Reference Range
Creatinine mg/dl	3.6	3.4		2.83	2.6	0.66-1.09 mg/dl
Hemoglobin g/dL	6.7	6.4	5.5	7.6	9.0	13 - 17g/dL
Total WBC 10^3/microL	0.2	0.5	5.1	10.1	5.7	4-10x10^3/uL
Absolute Neutrophil Count 10^3/uL	0.0	0.2	4.7	9.2	4.5	2 - 7x10^3/uL
Plateletsx10^3/uL	64	63	61	82	324	150-400x10^3/uL

Table 5: Showing the hypokalemia and metabolic acidosis pattern in case 9.

Lab parameter	Day 1 of admission	Day 3	Day 5	Month after discharge	Reference range
Serum Creatinine	1.75	1.26	1.6	1.43	0.8 - 1.25 mg/dl
pH	7.230	7.342	7.334	7.33	7.35-7.45
Serum Potassium	2.45	4.6	3.0	3.66	3.5 - 5.0mEq/L
Serum bicarbonate	12.5	15.9	17	21.6	22 - 30mEq/L
Serum ionised Calcium	1.202			1.2	1.1-1.3m mol/L
Serum Phosphous		2.1			2.7-4.5 mg/dl
Serum Alkaline phosphatase	459				40 – 129 U/L

Research, a tertiary care hospital in Coimbatore, Tamil Nadu. They were either referred from their primary care physician and/or admitted in our emergency department with severe drug related adverse events. The discussed 9 patients were on different class of drugs for their respective diagnosis prescribed elsewhere and presented to us with a variety of adverse effects requiring hospitalization or developing grave complications. Fortunately 8 of them recovered with prompt diagnosis and withdrawal of the offending drug.

Azathioprine is an imidazolyl derivative of mercaptopurine which acts by blocking the pathway for purine synthesis. The 6-thioguanine nucleotide metabolites appear to mediate the majority of azathioprine's immunosuppressive and toxic effects. Azathioprine, though a well-established immunosuppressive drug for myasthenia showing improvement in 70-90% patients has its share of serious complications.

Of grave concern is haematological toxicity. Dose-related immediate or delayed severe hematologic toxicities in the form of leucopenia, thrombocytopenia or pancytopenia may occur. Panda BK *et al.* have reported a case of severe pancytopenia in a patient of myasthenia gravis following azathioprine.[5] Other side effect profile includes gastrointestinal disturbances, hepatotoxicity and carcinogenic potential. Therefore it is important to periodically monitor blood counts and educate the patient about the need for the same.

In our case 1, the patient was not aware of the potential complication of his drugs, thereby delayed presenting to the hospital until he had profound leucopenia. With a proper patient education, early diagnosis can be arrived at and prompt action can avoid grave consequences. Case 2 patient was co-prescribed with azathioprine and methotrexate - both contributing to her profound pancytopenia. Patient had a tumultuous hospital course warranting intensive care admission, developed grave fungal and bacterial infections and succumbed to the same. Therefore it is crucial to carefully evaluate and closely follow up patients while co prescribing 2 potential hematotoxic drugs.

Thiopurine methyltransferase (TPMT) genotyping or phenotyping and nudix hydrolase 15 (nucleotide diphosphate [NUDT15]) genotyping may help to identify patients who are at an increased risk for developing azathioprine related hematological toxicity.[6] Once identified, patients may require dose reduction, interruption or selection of an alternate agent altogether.

Methotrexate being a folate antimetabolite inhibits dihydrofolate reductase, thereby hampering purine and thymidylic acid synthesis. DNA synthesis, repair and cellular replication are thus hindered as methotrexate targets actively proliferative tissues more and is therefore used in a number of conditions like rheumatoid arthritis, psoriasis as well as certain malignancies.

Thrombocytopenia (3% to 10%), leukopenia (1% to 3%), pancytopenia (1% to 3%), agranulocytosis, anemia, aplastic anemia, bone marrow suppression are noted hematological adverse effects of methotrexate. That is why it is pertinent to closely monitor the complete blood counts prior to initiation, weekly in the first month of initiation, every 2 to 4 weeks in the first few months and monthly thereafter. In a study of 46 patients on methotrexate in Lucknow, India, it was noted that initial WBC count was a predictor for development of pancytopenia later.[7] Patients with renal insufficiency or older age will require frequent monitoring. Case 3 (a female patient) and case 4 (older age group) had been taking only low dose methotrexate in weekly schedule for years, yet presented with pancytopenia revealing the fact that even low dose methotrexate can cause pancytopenia as a delayed presentation and periodic monitoring is indispensable. These findings are similar to a case series report on methotrexate where pancytopenia was encountered as a later manifestation[8] and another case report which observed low dose methotrexate causing

pancytopenia.[9] Once hematotoxicity is encountered, the drug is withheld, serum methotrexate levels are monitored and leucovorin rescue dosing is done to combat methotrexate effects. Granulocyte Colony stimulating factors will also be required where the patient has persistent pancytopenia. Hepatotoxicity and lung toxicity also require close attention and a baseline work up for the above is mandatory before starting methotrexate.

Leflunomide is an immunomodulatory agent that inhibits pyrimidine synthesis, resulting in its antiproliferative and anti-inflammatory effects. It acts by inhibiting dihydroorotate dehydrogenase and is converted to the active metabolite A771726 on administration.[10] Usually used in rheumatoid arthritis and systemic lupus erythematous. In November 2003, FDA issued a drug safety alert update on leflunomide, warning of rare post marketing reports of bone marrow suppression and sepsis. Though rare (incidence of 1 in 3698 patients) the incidence of pancytopenia is potentiated by concomitant administration of other myelotoxic agents as in case 5 where the patient was on both methotrexate and leflunomide. And myelosuppression can persist even after discontinuing the drug owing to its long half-life. A number of case reports reinforce the fact that hematological toxicities of methotrexate and leflunomide combination therapy are deleteriously additive.[11]

Levothyroxine (T_4) is a synthetic form of thyroxin, which is an endocrine hormone secreted by the thyroid gland. T_4 is converted to its active metabolite, L-triiodothyronine (T_3). Thyroid hormones (T_4 and T_3) then bind to thyroid receptor proteins in the cell nucleus and exhibit their metabolic actions through control of DNA transcription and protein synthesis.

While a decision to start a patient on levothyroxine is arrived at, certain characteristics should be carefully checked. These include patient's weight, lean body mass, etiology of hypothyroidism, pregnancy, age and cardiac disease. Thyroid hormone can be started at anticipated full replacement doses in individuals, who are young and otherwise healthy in most situations, a starting levothyroxine dose of 50-75 μg/day (1.6 μg/kg/day) will suffice. In patients with coronary heart disease, initial dose should be 12.5 to 50 μg/day.[12] Aggressive replacement of thyroid hormone in initial higher doses may worsen cardiac function in patients with existing cardiac disease. That is why it is imperative to start these patients on smaller doses and then titrate in small increments after no less than 4-6 weeks. Achieving a target TSH level at times may take several months owing to delayed preadaptation of the hypothalamic-pituitary axis. Therefore in patients receiving treatment with Levothyroxine, dosing changes should be made every 6-8 weeks only in usual scenarios.

In our case 6, 59 yr old woman was forth right put on high dose levothyroxine 100 micrograms for a TSH value of 6 microIU/ml exacerbating her heart failure. In case 7, though the patient had an underlying connective tissue disease, she was able to attend to her activities of daily living. However challenging her with levothyroxine 100 micrograms for a TSH value of 5.13 microIU/ml clearly exaggerated her symptoms precipitating her cardiac failure resulting in emergency presentation and subsequent prolonged hospitalization, which could have been avoided.

Treatment with levothyroxine in patients with heart failure is associated with a higher risk for all-cause mortality, cardiovascular death and major adverse cardiac events according to findings published in The Journal of Clinical Endocrinology and Metabolism.[13] These events are attributed to the low T3 syndrome. When hypothyroidism is treated with levothyroxine, there is often a relatively high thyroxin/T3 ratio in serum with relatively low liothyronine levels in blood, which could further exacerbate the T3-deficient state in patients with chronic heart failure

Paclitaxel is a natural taxane promotes microtubule assembly by enhancing the action of tubulin dimers, stabilizing existing microtubules and inhibits their disassembly. These actions ultimately halt cell replication thereby modulating the desired immune response. Paclitaxel finds usage in a variety of conditions notably breast, ovarian malignancies, lung cancer and Kaposi sarcoma. Our index patient case 8 was on paclitaxel for endometrial carcinoma. The hematological adverse effects include neutropenia (78% to 98%), leukopenia (grade 4: 17%), anemia (47% to 90%), thrombocytopenia (4% to 20%). It is therefore crucial to regularly monitor the complete and differential blood counts of the patient while on chemotherapy.

Chemotherapeutic agents do exaggerate the immune compromised state of cancer affected patients. Usually the magnitude of the neutrophil mediated component of the inflammatory response may be muted in neutropenic patients with fever sometimes being the only sign of infection. It is critical to recognize neutropenic fever early and to initiate empiric therapy promptly in order to avoid progression to a sepsis syndrome and possibly death. Neutropenic patients have the risk of developing a neutropenic fever syndrome, risk of resultant medical complications as well as risk of non-response to empiric antibiotic treatment.[14]

Certain risk factors are predictive of the development of a neutropenic fever syndrome. Patient-related predictors include age> 65 yrs, female sex, high body surface area and poor nutritional status. Disease-related predictors include elevated lactate dehydrogenase, advanced malignancy staging and myelophthisis. Treatment-related predictors of neutropenic fever include administration of high dose chemotherapy regimens and failure to administer hematopoietic growth factor support to patients receiving high-risk regimens. High risk patients are those who have other co morbidities, significant hepatic or renal impairment or those who are expected to be severely neutropenic (absolute neutrophil count [ANC] <500 cells/microL) for >7 days. The index patient case 8 was a female patient with diabetes mellitus and chronic kidney disease enhancing her risk of pancytopenia.

A number of validated tools are used to calculate the risk index for measuring the risk for neutropenic fever–related medical complications and must be put to effective use wherever possible in tertiary centres.[15] In a research article that investigated the capability of genetic variation in drug metabolizing enzymes and transporters (DMET platform) to predict haematological toxicity, the single nucleotide polymorphism SNP rs 9285726 in MAT1A (methionine adenosyl transferees) and SNP rs 776173 in CYP39A1 were found to be associated with neutropenia. These are potential areas of research in predicting paclitaxel induced hematologoxical toxicity and in picking up individual patients who are at more risk.[16]

Tenofovir disoproxil fumarate (TDF) is a nucleotide reverse transcriptase inhibitor. It interferes with the HIV viral RNA dependent DNA polymerase resulting in inhibition of viral replication exhibiting its action via active tenofovir diphosphate. Nephrotoxicity is a known and established side effect of tenofovir. One study reported that the incidence rate of moderate or severe renal insufficiency caused by tenofovir was 29.2 and 2.2 cases per 1000 person-year respectively. Apart from this tenofovir can also cause interstitial nephritis, lactic acidosis, nephrogenic diabetes insipidus and rhabdomyolysis and decreased bone mineral density.[17]

Tenofovir can rarely cause Fanconi syndrome.[18] It is a proximal tubulopathy causing impaired reabsorption of glucose, aminoacids, bicarbonate and phosphate resulting in their excretion. The presentation of fanconi syndrome can be quite delayed also in a patient on tenofovir.[19] Therefore drug related adverse effect should be considered in any patient presenting with renal tubular acidosis type 2 while on tenofovir regardless of the duration of drug exposure.

Our case 9 patient also developed toxicities 4 years after tenofovir exposure and developed Fanconi's syndrome as well as bone toxicity which is quite rare.[20] The index patient however improved remarkably after stopping Tenofovir and with calcium and phosphate supplementation. This finding is similar to a study from Christian Medical College, South India where the incidence of tenofovir induced renal tubular toxicity has been observed as quite rare (3.6%) and the average latency period between tenofovir exposure and nephrotoxicity was prolonged (42 months).[21] The study suggests that patients on tenofovir should be monitored serially and periodically for proteinuria, creatinine clearance rather than creatinine values, hypophosphatemia and alkaline phosphate levels. Tenofovir alafenamide is the pro-drug of TDF which achieves higher intracellular concentrations rather than plasma concentrations at low dose, thereby resulting in lower toxicities and similar efficacy.

CONCLUSION

Thus it is important to choose and prescribe drugs and their dosage carefully. It is fundamental for the treating doctor to be aware of drug combination/interactions leading to profound side effects. Patient education and awareness of drug related side effects is core in management and helps in averting serious side effects earlier and thereby avoiding unnecessary/prolonged hospitalization and related complications. As a physician it is pertinent to constantly update and learn from errors whether of one's self or others. After all learning and unlearning is part of the continuing medical education process.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ANA: Antinuclear antibody; **HCQ:** Hydroxychloroquine; **TSH:** Thyroid stimulating hormone; **CSF:** Colony stimulating factor; **TPMT:** Thiopurine methyltransferase; **NUDIT:** 15 nucleotide diphosphate 15; **ANC**: Absolute neutrophil count; **TDF:** Tenofovir disoproxil fumarate.

REFERENCES

1. Guideline on good pharmacovigilance practices (GVP): Annex I – Definitions (Rev 4). European Medicines Agency and Heads of Medicines Agencies. 2017. www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/05/WC500143294.pdf.[cited 2019october 19]
2. Investigational New Drug Application Safety Reporting. US Food and Drug Administration. 21CFR312.32. 2019. [cited 2019 Oct 19). Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32.
3. Ferner RE, McGettigan P. Adverse drug reactions. BMJ. 2018;363:k4051.

4. Alomar MJ. Factors affecting the development of adverse drug reactions. Saudi Pharmaceutical Journal. 2014;22(2):83-94.
5. Panda BK, Umarje S, Diwan A. Azathioprine-Induced Pancytopenia and Septic Complications: A Probable Cause of Death. J Pharm Pract. 2018;31(5):510-3.
6. Kim JC, Kim YK, Hyun HS, Park EJ, Kang HG, Ha S, *et al*. A Case of Azathioprine Induced Severe Myelosuppression and Alopecia Totalis in IgA Nephropathy. Childhood Kidney Diseases. 2017;21(1):35-9. doi: 10.3339/jkspn.2017.21.1.35
7. Ajmani, Preet YS, Prasad S, Chowdhury A, Aggarwal A, Lawrence A, *et al*. Methotrexate-induced pancytopenia: A case series of 46 patients. Int J Rheum Dis. 2017;20(7):846-51.
8. Lim AYN, Gaffney K, Scott DGI. Methotrexate-induced pancytopenia: Serious and under-reported? Our experience of 25 cases in 5 years. *Rheumatology*. 2005;44(8):1051-5.
9. Rajnics P, Kellner VS, Kellner A, Karadi E, Kollar B, Egyed M. The Hematologic Toxicity of Methotrexate in patients with Autoimmune Disorders. J Neoplasm. 2017;2(1):1-6.
10. Breedveld FC, Dayer J. Leflunomide: Mode of action in the treatment of rheumatoid arthritis. Annals of the Rheumatic Diseases. 2000;59(1):841-9.
11. Qu C, Lu Y, Liu W. Severe bone marrow suppression accompanying pulmonary infection and hemorrhage of the digestive tract associated with leflunomide and low-dose methotrexate combination therapy. J Pharmacol Pharmacother. 2017;8(1):35-7.
12. Einfeldt MN, Olsen AS, Kristensen SL, Khalid U, Faber J, Torp-Pedersen C, *et al*. Long-Term Outcome in Patients With Heart Failure Treated With Levothyroxine: An Observational Nationwide Cohort Study. The Journal of Clinical Endocrinology and Metabolism. 2019;104(5):1725-34.
13. Pearce SHS, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S, *et al*. ETA guideline: Management of subclinical hypothyroidism. Eur Thyroid J. 2013;2(4):215-28.
14. Eric B. Risk assessment of adults with chemotherapy-induced neutropenia. Up to Date. 2019. Retrieved October 20. https://www.uptodate.com/contents/risk-assessment-of-adults-with-chemotherapy-induced-neutropenia
15. Lyman GH, Kuderer NM, Crawford J, Wolff DA, Culakova E, Poniewierski MS, *et al*. Predicting individual risk of neutropenic complications in patients receiving cancer chemotherapy. Cancer. 2011;117(9):1917-27.
16. Nieuweboer AJ, Smid M, DeGraan AJ, Elbouazzaoui S, DeBruijn P, Martens JW, *et al*. Predicting paclitaxel-induced neutropenia using the DMET platform. Pharmacogenomics. 2015;16(11):1231-41.
17. Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Niño MD, Izquierdo MC, Poveda J, *et al*. Tenofovir Nephrotoxicity. AIDS Research and Treatment. 2011. Article ID 354908. https://doi.org/10.1155/2011/354908.
18. Mathew G, Knaus SJ. Acquired Fanconi's syndrome associated with tenofovir therapy. J Gen Intern Med. 2006;21(11):C3-5.
19. Luni FK, Khan AR, Prashar R, Vetteth S, Duggan JM. Fanconi Syndrome and Antiretrovirals: It Is Never Too Late. Am J Ther. 2016;23(2):e558-60.
20. Lucey JM, Hsu P, Ziegler JB. Tenofovir-related Fanconi's syndrome and osteomalacia in a teenager with HIV. BMJ Case Reports. 2013;bcr2013008674.
21. Mitra S, Priscilla R, Rajeev K, Sauradeep S, Rajkumar S, Cherian AO. Renal tubular dysfunction associated with tenofovir therapy. J Assoc Physicians India. 2014;62(7):580-2.

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SHORT ARTICLE

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Retrospective Analysis of Demographic and Clinical Profile of Dengue Infection in a Tertiary Care Hospital

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Abstract

Background: Dengue infection is an exceedingly common cause of fever in the tropical countries. **Aim:**to study the demographic and clinical profile of Dengue patients**. Design:** Retrospective observational study of serologically proven dengue cases. **Methods and materials:** the study included Dengue patients admitted in PSG Hospital between January 2011 and December 2012. **Results :**Dengue infection was found to be more common in the younger age groups. It was more common in men. It was prevalent across all occupational profiles. The common clinical features included fever, myalgia, headache, abdominal pain, diarrhea and arthralgia. Bleeding manifestations included mucosal and skin bleeds and the commonest bleeding manifestation was melena. There was no major life threatening bleeds. Analysis of the hematological profile showed that nearly 91.5% of patients had thrombocytopenia with 48.9% having severe thrombocytopenia. **Conclusion** : Dengue infection is an important differential diagnosis for acute febrile illness in the tropics which can present with multiple non specific and systemic symptoms with a high incidence of thrombocytopenia.

Key-words: Dengue,Coimbatore,Demography,Tropical,Symptoms

Introduction

The word dengue is derived from Spanish in which it means “affectation”, “careful” or “fastidious” probably to describe the cautious stiff movements of patients suffering from it due to muscle, bone and joint pain. It was considered a sporadic disease in the 19th century. With the advent of globalization and increased travel, the incidence of dengue has increased 30-fold over the past century to become endemic in 112 countries in the world (1).About 50 million people develop dengue infection every year and 2.5 billion people live in endemic areas.(2).Dengue infection is a very important health problem affecting many tropical countries. Dengue is one of the important causes of undifferentiated fevers in India along with malaria, typhoid and other viral infections. Various endogenous studies are available in India, most of which have evaluated a few aspects of the disease spectrum. Some studies have documented the extent of epidemics with data collected during that period. There have been increasing reports of dengue infection with newer manifestations, mainly with hepatic and cerebral symptoms in the past (3,4). We designed a descriptive study of adult dengue infection to describe the demographic and clinical profile. The aim of the study is to provide data on dengue infection in a tertiary care location in Tamil Nadu.

Materials and methods

This study was a retrospective analysis of patients who were admitted in the medical wards of PSGIMSR with febrile illness and diagnosed to have dengue infection by serological analysis. These were mainly adult patients and were admitted between January 2011 and December 2012.All patients screened for this study had undergone dengue serology and only patients who were dengue IgM positive and greater than fifteen years of age were included in this study. Patients with other causes for fever and with coexisting infections were excluded from this study. Demographic data regarding age, gender, occupation and location were collected and tabulated. The spectrums of clinical features were noted. Complete blood count was assessed by coulter.

Results

Our present study was a retrospective analysis of patients presenting to our hospital in the years 2011-2012 who tested serologically positive for dengue infection .We performed a retrospective analysis of demographic and clinical profile of 235 patients with dengue infection who were admitted to our hospital. In our present study, the incidence of dengue infection had the highest incidence between 15-40 years. [FIG 1]. Of the 235 cases of dengue infection 60(25.5%) were primary dengue and 175(74.5%) cases were secondary dengue infection. Among 235 dengue patients, 144 were male and 91 were female. Occupational profile showed equal distribution of blue collar workers (unskilled workers) and students with lesser incidence in house wives and white collar (skilled) workers.[FIG 2]. In our study the commonest symptoms were fever [92%] followed by myalgia [40.4%], headache [42.4%], vomiting [40.4%].[FIG 3].Hepatomegaly seen in 6.80%, Splenomegaly1.30%, lymphadenopathy 0.40%,Plerual effusion 6.40% and abdominal findings 14.90% of individuals. Bleeding manifestations happened in 26.8 % of patients , the commonest of which was melena [7.20%], followed by gum bleeds, ecchymosis,, hematemesis , petechiae and purpura[FIG 4]. When the presence of thrombocytopenia was analyzed, it was found that 91.5% of the patients had low platelet counts [<150000 cells/mm3].

Fir.1. Prevalence of Dengue infection with age

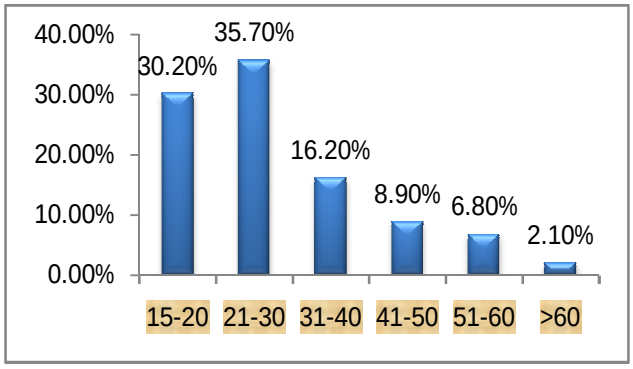


Fig 2.Occupation of Dengue infection

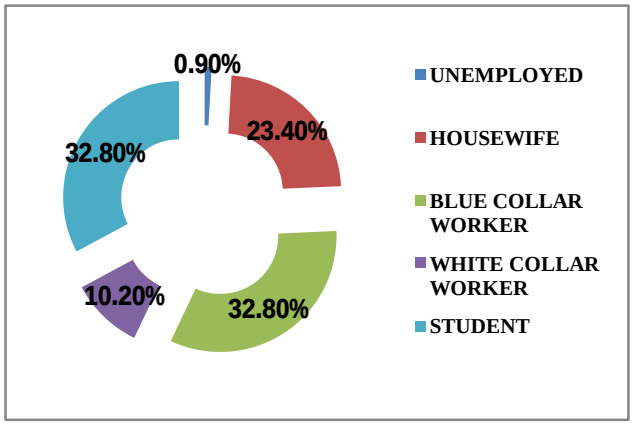


Fig 3.Symptoms of Dengue infection

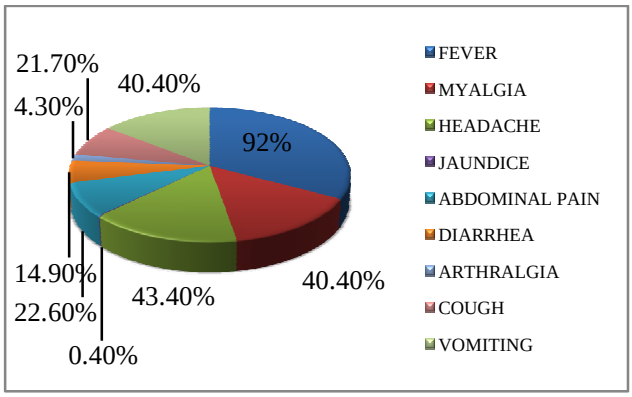
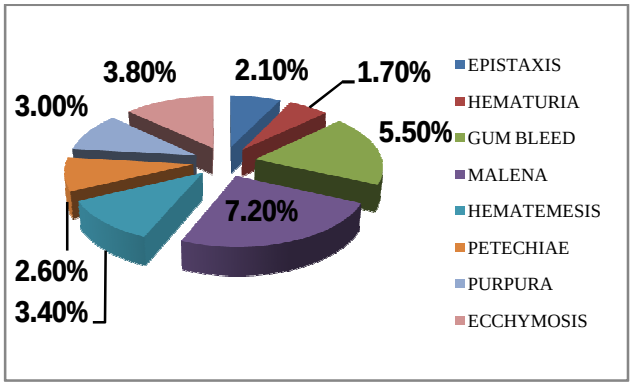


Fig 4.Bleeding Manifestation in Dengue infection



Discussion

There are 2.5 billion people living in the tropical & sub-tropical zones who are at risk of acquiring infection due to ideal environmental conditions for the transmission of dengue virus by Aedes mosquitoes. Outbreaks have occurred in five of six WHO regions over the past 50 years (1). Most of the cases of dengue infection are found in south east Asia and western pacific though it is prevalent in many countries.(2).

Our study included patients above 15 years. The incidence of dengue was least in the older age groups probably in our view because of lesser outdoor activity compared to younger people. This was similar to a study performed by Amir Khan et al (5).n a study by Khoa T.D.Thai et al (6) showed that increasing age lead to increasing risk of clinical attack in both primary and secondary dengue infection. This study was done by obtaining seroprevalence of dengue specific for age and frequency of clinical attack specific for age. This study included pediatric population and the average age of patients with primary and secondary dengue infection were 12 and 20 years respectively.

Our study showed a male preponderance in concordance with a study done by Prafulla Dutta et al (7)and Amirkhan et al(5). Another study from Prasith et al from Lao People's Democratic Republic's also showed a similar male preponderance of dengue infection (8). Majority of the patients were from Coimbatore and nearby districts.

In relation to occupational profile our study differs from the study by Prafulla Datta et al where majority of the patients were blue collar workers (7). This was because their study population was from a rural location and ours is from an urban location.

Secondary dengue infection was more common than primary dengue infection indicating that the majority of the patients had already had an attack of dengue infection.

In addition to the more common complaints such as fever, myalgia, headache, other symptoms included abdominal pain, diarrhea, arthralgia and cough .This was more or less similar to other studies from India done by Prafulla Dutta et al with slight differences (7). Their study showed a higher incidence of eye pain (68.5%) which was not present in our study. Our study showed that 20.4% of patients had cough and 40% of patients had vomiting which had not been reported in the 2003 dengue epidemic in the study by Rachel Daniel et al (10) . Our study had lesser incidence of abdominal pain when compared to the study by Ritu Karoli et al(12). Abdominal pain may be related to inflammation of serosa.This finding was also similar to another study by Muhammad H et al. (11)

There was no major intracranial bleed on our study. In the study by Rachel Daniel et al showed a lower incidence [15.2%] of bleeding manifestations but with 2 major intracranial bleeds .Their commonest bleeding manifestation was melena which was similar to our study (10). In another study by Ritu Karoli et al, hemorrhagic manifestations were higher in 40% of patients with epistaxis being the commonest symptom (12).

The incidence of thrombocytopenia in our study was 91%, which was higher compared to the study by Prafulla Dutta et al (7) where the incidence of thrombocytopenia was 71.3% and similar to the study by Rachel Daniel et al (10) where it 90%.The majority had severe thrombocytopenia [<50000 cells/mm3] (48.9%), with the rest comprising of moderate [50000-100000 cells/mm3](25.1%) and mild[100000 to 150000 cells/mm3] (17.4%) thrombocytopenia. The other 8.5% had normal platelet counts.

Conclusion: This study is a retrospective analysis capturing the demographic and clinical profile of dengue patients. Dengue infection forms an important differential diagnosis of acute febrile illness in the tropics .Since this was a retrospective analysis, not all aspects could be looked into. In future, there is a need for analysis of biochemical parameters of dengue patients.

References

1. G N Malavige1,S Fernando1, D J Fernando, S L Seneviratne. Dengue viral infections Postgrad Med J 2004;80:588-601

2. Dengue guidelines for diagnosis, treatment, prevention and control new edition. Geneva: World Health Organization; 2009. WHO.page 3

3. Cam BV, Fonsmark L, Hue NB et al. Prospective case-control study of encephalopathy in children with dengue hemorrhagic fever. American Journal of Tropical Medicine and Hygiene 65, (2001) 848–851.

4. Pancharoen C & Thisyakorn U, Neurological Manifestations in Dengue Patients. Southeast Asian Journal of Tropical Medicine and Public Health 32, (2001) 341–345.

5. Amir Khan, Shams Ur Rehman, Rizwan Ullah. Demographic profile, clinical presentation and outcome of dengue fever in mardan medical complex teaching hospital,KJMS.January-June, 2013, Vol. 6, No. 1

6.Thai, K. T. D., Nishiura, H., Hoang, P. L., Tran,et al.Age-specificity of clinical dengue during primary and secondary infections. PLoS Neglected Tropical Diseases, *5*(6), (2011). e1180.

7.Dutta, P., Khan, S., Borah, J., & Mahanta, J. Demographic and Clinical Features of Patients with Dengue in Northeastern Region of India: A Retrospective Cross-Sectional Study during 2009-2011. Journal of Virology & Microbiology, *2012*, 1–11.

8. 8 Prasith, N., Keosavanh et al. Assessment of gender distribution in dengue surveillance data, the Lao People's Democratic Republic. Western Pacific Surveillance and Response Journal : WPSAR, 4(2), . (2013). 17–24.

9. Dengue guidelines for diagnosis, treatment, prevention and control new edition. Geneva: World Health Organization; 2009. WHO. page 91

10. Rachel Daniel, Rajamohanan and Aby Zachariah Philip.A Study of Clinical Profile of Dengue Fever in Kollam, Kerala, India(2005). A Study of Clinical Profile of Dengue Fever, Dengue Bulletin Vol 29, 2005.*29*, 197–202.

11. Muhammad, H., Khan, S., Khan, B. A., Arif, M., & Raza, A. (2012). Retrospective Analysis of 68 Cases of Dengue Fever, Pak J Med Res Vol.51, No.1, 2012.

12. Karoli, R., Fatima, J., Siddiqi, Z., Kazmi, K. I., & Sultania, A. R. (2010). Original Article Clinical profile of dengue infection at a teaching hospital in North India, (October), J Infect Dev Ctries. 2012 Jul 23;6(7):551-4

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Original Research Article

Retrospective analysis of ultrasound features of dengue infection

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ABSTRACT

Background: Dengue infection is an extremely common infection in tropical countries. It is considered to be prevalent all-round the year. The aim of the study was to analyse the ultrasound features of serologically proven dengue cases. To correlate the platelet count with ultrasound features of dengue.
Methods: Study included dengue patients admitted from January 2011 to December 2012. Dengue was diagnosed by IgM antibody test. All patients underwent ultrasound evaluation and platelet count testing.
Results: most common finding was ascites in 43 cases (39.8%), splenomegaly in 41 cases (37.9%), right sided pleural effusion in 25 cases (23.4%), gall bladder wall thickening was present in 30 cases (27.7%) and hepatomegaly was present in 20 cases (18.5%). Presence of most of the features of ultrasound evaluation correlated with a platelet count of less than 40000 cells/mm^3.
Conclusions: The commonest sonographic findings were ascites and splenomegaly followed by pleural effusion and gall bladder wall thickening. Thus, presence of these ultrasound features in a febrile patient may be suggestive of dengue fever and presence of all the sonographic features may suggest a low platelet count.

Keywords: Ascites, Gall bladder wall thickening, Sonography, Splenomegaly

INTRODUCTION

Dengue is an extremely common Arboviral infection seen worldwide and highly prevalent in tropical countries. There are approximately 50 million new dengue infections occurring each year. There are about 2.5 billion people living in dengue endemic areas worldwide.[1] The incidence of dengue has been steadily increasing in the last 5 decades.

The spectrum of dengue infections includes classical dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Epidemic dengue is an emerging health problem in South East Asian countries where the vector of dengue infection Aedes Egypti is widely prevalent and is leading to increasing hospitalisation of both children and adults.[2] Our present study is a retrospective analysis of ultrasound findings in serologically proven dengue cases. It also correlates the ultrasound findings with platelet levels.

METHODS

This study was done at PSG Hospital, Coimbatore. The study consisted of a retrospective analysis of 108 serologically proven dengue cases (IgM ELISA positive) admitted between January 2011 and December 2012.

Inclusion criteria included patients above 15 years and other tropical infections including malaria, typhoid scrub typhus and hepatitis were excluded from this study. These patients had undergone platelet count testing by coulter. Ultrasound evaluation of abdomen and thorax was done using Siemens Accuson S3000 and Philips IU 22 machines with 3-5 MHz transducers. Dengue serology was done by Elisa method.

RESULTS

The spectrum of findings in our study included gall bladder wall thickening, pleural effusion, ascites, hepatomegaly and splenomegaly. Out of 108 patients, 30 had gall bladder wall thickening (27.7%), 13 had bilateral pleural effusion (12.03%), 25 had right sided pleural effusion (23.4%). There were no cases of isolated left pleural effusion. 43 patients had ascites (39.8%), 20 had sided hepatomegaly (18.5%), 41 had splenomegaly (37.9%). 40 patients had normal ultrasound studies (37.4%).

Correlation of platelet count with imaging findings

- Gall bladder wall thickening was seen in 19.4%of patients with platelet count less than 40000 and in 5.5% of patients with less than 1 lakh,
- Other common findings in cases with platelet less than 40000 included ascites (28.7%) and pleural effusion (25.8%),
- The commonest finding in our study was ascites followed by pleural effusion and gall bladder wall thickening,
- Out of 41 cases of splenomegaly 14.8%were seen in cases with platelet count less than 40000 cells/mm^3 and in 11.85% of cases with platelet counts between 40000 to 1 lakh cells/mm^3.

Table1: Incidence of different sonographic findings in dengue fever.

Ultrasound findings	Cases	Percentage
Gall bladder wall thickening	30	27.7
Bilateral pleural effusion	13	12.03
Right sided pleural effusion	25	25
Ascites	43	39.8
Splenomegaly	41	37.9
Hepatomegaly	20	18.5
Hepatosplenomegaly	8	7.4
Normal study	40	37.4

Table 2: Correlation of ultrasound findings with platelet count.

USG findings	<40000 cells/mm^3	40000-1 lakh Cells/mm^3	1-1.5 lakhs cells/mm^3	>1.5 lakhs cells/m^3
Gall bladder wall thickening	21 (19.4)	6 (5.5)	3 (2.7)	0
Pleural effusion	28 (25.9)	8 (7.4)	1 (.9)	1 (.9)
Ascites	31 (28.7)	10 (9.2)	1 (.9)	1 (.9)
Hepatomegaly	12 (11.1)	3 (2.7)	4 (2.8)	1 (.9)
Splenomegaly	16 (14.8)	2 (1.8)	9 (8.3)	4 (3.7)

DISCUSSION

Dengue is considered to be one of the most important tropical infections in the world. Dengue infection has increased by thirty-fold worldwide in the last five decades. It is considered to be endemic in at least 100 countries. Severe dengue is an important cause for morbidity and death in adults and children in Asian countries.

Dengue in India has been endemic in 16 states since the beginning. India contributes about 34% of the global burden of dengue infection.[3] From 2011-2012 dengue has spread to the remaining states.[4]

There has been a steady increase in dengue cases in India from 2010 onwards. The principal vector of dengue fever is the Aedes egypti mosquito. The other species which can transmit dengue is the Aedes albopictus mosquito. This virus can undergo transovarian transmission.[5]

Ultrasound findings in dengue infection include gallbladder wall thickening, ascites, pleural effusion and hepatospenomegaly. In very severe cases subcapsular collection of fluid in liver, spleen and fluid accumulation around the kidney can happen. Pericardial effusion can also occur.[6] Our study had gallbladder wall thickening in 27.7% of cases. The differential diagnosis of gallbladder wall thickening includes acute cholecystitis, pancreatitis, hepatitis and diverticular disease.[7] Hence it is considered to be a very nonspecific finding.

In a study by KS Vedaraju et al the incidence of gall bladder wall thickening was 83.3%, ascites was 53.9%.[8] These values were much higher compared to the results obtained in our study. In another study by Sunita Bajaj et al hepatomegaly was the most common finding in 74.5%. This was in contrast to our study which had an incidence of hepatomegaly of 18.5%.[9]

In a study by Dayanand et al, 17.9% of patients had isolated left sided pleural effusion which was absent in our study.[10] Majority of the ultrasound findings in our study was associated with a platelet count of less than 40000 cells/mm^3. This may suggest that patients with all the ultrasound findings may eventually go on to develop low platelet counts. This was similar to the study by Santosh VK et al.[6]

CONCLUSION

The most common ultrasound findings in dengue cases in our study were ascites followed by splenomegaly. Other findings included gall bladder wall thickening, pleural effusion and hepatomegaly. Presence of these ultrasound findings in a febrile patient supports the diagnosis of dengue infection. Also, a significant proportion of the ultrasound findings correlated with a platelet count of less than 40000cells/mm^3. Limitations of this study are that the ultrasound findings were not correlated with age.

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REFERENCES

1. World Health Organization. Dengue: Guidelines for Diagnosis Treatment Prevention and Control (New Edition 2009). World Health Organization; 2009. Available at http://apps.who.int/iris/handle/10665/44188.
2. Dengue guidelines for diagnosis, treatment, prevention and control new edition. Geneva: World Health Organization; 2009.
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504-7.
4. Cecilia, D. Current status of dengue and chikungunya in India, WHO South-East Asia J Public Health. 2014;3(1).
5. Joshi V, Mourya DT, Sharma RC. Persistence of dengue-3 virus through transovarial transmission passage in successive generations of Aedes aegypti mosquitoes. Am J Tropic Medic Hyg. 2002;67(2):158-61.
6. Santhosh VR, Patil PG, Srinath MG, Kumar A, Jain A, Archana M. Sonography in the diagnosis and assessment of dengue fever. J Clinic Imaging Sci. 2014;4.
7. Barbosa AB, Souza LR, Pereira RS, D'Ippolito G. Gallbladder wall thickening at ultrasonography: how to interpret it?. Radiologia Brasileira. 2011;44(6):381-7.
8. Vedaraju KS, Kumar KR, Vijayaraghavachari TV. Role of Ultrasound in the Assessment of Dengue Fever. Inter J Sci Study. 2016;3(10):59-62.
9. Dayanand KR, Kavitha K, Singh AP, Chiranth N. Correlation of ultrasound (USG) findings with serological tests in dengue fever. J Evid Based Med Health. 2016;3(12):37-374.
10. Bajaj S, Chander DR. Study of ultrasound finding in dengue fever. J Evid Based Medic Healthcare. 2016;3(86):4683-7.

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Original Research Article

Analysis of liver function tests in dengue fever

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ABSTRACT

Background: Dengue fever is an exceedingly common tropical infection in India. It can affect the liver with a wide spectrum of manifestations. The aim of the study was to analyse the liver function tests derangement in serologically proven dengue cases.
Methods: Patients who were Dengue IgM positive were included in this study. All the patients underwent liver function tests.
Results: Of 1oo patients, 33% had less than 2-fold increase in ALT levels, 18% had 2-4-fold increase, 20% had 4-10-fold increase and 11% had more than 10-fold increase. Overall 18% had normal values and 82% had values of ALT above normal. With regard to AST 8% had normal values, 26% had less than 2-fold normal, 26% had 2-4-fold increase, 25% had 4-10-fold increase and 15% had greater than 10-fold increase. 92% of patients had values above normal. Bilirubin levels were elevated in 5% of cases. Alkaline phosphatase levels were elevated in 25% of cases; serum globulins were increased in 9% of cases. Serum proteins were low in 43% of cases and serum albumin was low in 31% of cases.
Conclusions: The spectrum of hepatic involvement in dengue fever can vary from asymptomatic biochemical involvement to severe acute liver cell injury. Hyperbilirubinemia may also be observed. Low albumin levels may be present and may be a marker of the critical phase of the disease.

Keywords: ALT, AST, Dengue fever, Hyperbilirubinemia

INTRODUCTION

Dengue is one of the most important mosquito borne viral diseases which is spreading rapidly throughout the world. It has increased exponentially in the last five decades. There are about 50million new dengue infections occurring each year worldwide.[1] There are about 2.5billion people living in areas where dengue is endemic and hence potentially at risk for infection. The number of dengue cases has gone up from 15497 cases in 1960-1969 to 925896 cases in 2000-2007.[2]

Multiple outbreaks of dengue have happened in India. Reports have come from Bangalore, Punjab and Delhi.[3-5] Most of these cases have been reported in the post monsoon season from September to November. Dengue fever can affect multiple organ systems. There have been reports of dengue fever involving heart, nervous system and liver causing myocarditis, encephalitis and hepatitis. This present study is an analysis of liver function tests abnormalities in dengue fever.

METHODS

Our present study is a retrospective observational analysis of 100 serologically proven dengue patients admitted at PSG Hospital from January to September 2017. Inclusion criteria included patients more than 15

years of age, dengue IgM positive. Exclusion criteria consisted of patients with pre existing liver disease, scrub typhus, malaria and typhoid. All a patient underwent liver function testing.

Bilirubin testing was done by diazo method, alaninine aminotransferase [ALT] was tested by IFCC method, aspartate aminotransferase [AST] was tested by IFCC method. Alkaline phosphatase was tested by IFCC method. Serum proteins were tested by Biuret method, Serum albumin was tested by BCG, dyebind method and serum globulin levels were calculated. Dengue serology was done by ELISA method.

RESULTS

All patients underwent liver function tests. Of 1oo patients, 33% had less than 2 fold increase in ALT levels, 18% had 2-4 fold increase, 20% had 4-10 fold increase and 11% had more than 10 fold increase.

Overall 18% had normal values and 82% had values of ALT above normal. With regard to AST 8% had normal values, 26% had less than 2 fold normal, 26% had 2-4 fold increase, 25% had 4-10 fold increase and 15% had greater than 10 fold increase. 92% of patients had values above normal.

Bilirubin levels were elevated in 5% of cases. Alkaline phosphatase levels were elevated in 25% of cases, serum globulins were increased in 9% of cases. Serum proteins were low in 43% of cases and serum albumin was low in 31% of cases.

Table 1: Range of aminotransferase elevations in dengue fever.

	ALT number	ALT %	AST number	AST %
Normal	18	18	8	8
<Two fold rise	33	33	26	26
Two-four fold rise	18	18	26	26
Four-ten fold rise	20	20	25	25
>Ten fold rise	11	11	15	15

Table 2: Ranges of increased bilirubin, alkaline phosphatase and globulin levels.

Tests	Number	%
Bilirubin > 2mg/dl	5	5
Alkaline phosphatase increased	25	25
Serum globulins increased	9	9

Table 3: Range of protein levels in dengue fever.

Serum proteins	Number	%
Low	43	43
Normal	57	57
Increased	0	0

Table 4: Range of serum albumin levels in dengue fever.

Serum albumin	Number	%
Low	31	31
Normal	69	69
Increased	0	0

DISCUSSION

Dengue infection is known to affect the liver. The liver injury is not fully manifest in the early stages of dengue fever. The cause for the liver dysfunction is thought to be multifactorial. It is postulated that liver damage may happen secondary to hypoxia, direct effect of virus or immune mediated damage.[6] The spectrum of liver involvement in dengue fever varies from asymptomatic elevation of transaminases to severe liver involvement in the form of acute liver cell failure. Dengue virus targets the hepatocytes and the kupffer cells in the liver.[7] Virus enters the cells by binding to receptors and is taken inside the cell by endocytosis.

The final outcome of hepatic involvement is apoptosis of cells. Biopsies done on patients with dengue fever have revealed patterns such as microvascular steatosis, liver cell necrosis, councilman bodies and portal tract inflammation.[8]

Asymptomatic derangement in liver function tests are common findings. Rarely dengue fever can cause acute liver cell injury. Patients may present with pain in the right hypochondrium, hepatomegaly and jaundice.[9]

In a study by Amit Soni et al, with regards to AST levels, it was shown that 45.6% had less than 3 fold elevation, 36.7% had 3-10 fold elevation and 16.4% had greater than 10 fold elevation.[10] In the same study with regards to ALT, 27.8% had 1-3 fold elevation, 59.4% had 3-10 fold elevation and 8.9% had greater than 10 fold elevation. In this study AST elevation was more than ALT which was similar to our study. The increased AST/ALT ratios can be used to differentiate dengue infection from viral hepatitis where it is infrequently seen.[7] The incidence of AST and ALT elevations more than 10 fold were also comparable to our study.

In a study by Parkash et al, 3.1% of patients had hyperbilirubinemia which was comparable to our study which had 5 %.[11] Larreal Y et al in their study had reported hyperbilirubinemia in 2 out of 63 cases.[12]

In a study by Samatha Fernando et al, serum proteins were low in 63.6% of cases and serum albumin was low in 54.5% of cases.[6] This was in slight variance with our study where serum proteins were low in 43% and serum albumin was low in 31% of cases. In a study by Brito et al, it was shown that 71% of cases with dengue hemorrhagic fever had hypoalbuminemia concluding that serum albumin level was a marker for critical stage of the

infection where there was increased vascular permeability.[12] Our study did not differentiate between classical dengue and dengue hemorrhagic fever.

In a study by Rajoo Singh Chhina et al, alkaline phosphatase levels were elevated in 30.3% of cases with dengue fever and 40% of cases with dengue hemorrhagic fever.[13] This was comparable to our study where alkaline phosphatase was elevated in 25% of cases.

CONCLUSION

The spectrum of hepatic involvement in dengue fever can vary from asymptomatic biochemical involvement to severe acute liver cell injury. Hyperbilirubinemia may also be observed. Low albumin levels may be present and may be a marker of the critical phase of the disease. Treatment is usually supportive. Limitation of this study is that differentiation between classical dengue fever and dengue hemorrhagic fever was not done. Also, coagulation function of the liver in the form of prothrombin time was not assessed especially in patients with severe elevations of aminotransferases.

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REFERENCES

1. Dengue guidelines for diagnosis, treatment, preventionand control: new edition. Geneva, World Health Organization. 2009:3 Available at http://www.who.int/rpc/guidelines/9789241547871/en/.
2. Dengue guidelines for diagnosis, treatment, preventionand control new edition. Geneva: World HealthOrganization. WHO. 2009:5 Available at http://www.who.int/rpc/guidelines/9789241547871/en/.
3. George S, Soman RS. Studies on dengue in Bangalore city: isolation of virus from man and mosquitoes. Indian J Med Res. 1975;63:396-401.
4. Kaur H, Prabhakar H, Mathew P, Marshalla R, Arya M. Dengue haemorrhagic fever outbreak in october-november 1996 in Ludhiana, Panjab, India. Indian J Med Res. 1997;106:1-3.
5. Gupta E, Dar L, Narang P, Srivastava VK, Broor S. Serodiagnosis of dengue during an outbreak at a tertiary care hospital in Delhi. Indian J Med Res. 2005;121:36-8.
6. Fernando S, Wijewickrama A, Gomes L, Punchihewa CT, Madusanka SD, Dissanayake H, et al. Patterns and causes of liver involvement in acute dengue infection. BMC Infec Dis. 2016;16(1):319.
7. Samanta J, Sharma V. Dengue and its effects on liver. W J Clinic Cases: WJCC. 2015;3(2):125.
8. Hien TT, Vinh NN, Hien PT, Chinh NT, Simmons C, Wills B. Liver involvement associated with dengue infection in adults in Vietnam. Ame Tropic Medic Hyg. 2010;83(4):774-80.
9. Ahmed A, Alvi AH, Butt A, Nawaz AA, Hanif A. Assessment of dengue fever severity through liver function tests. J Coll Physicians Surg Pak. 2014;24(9):640-4.
10. Soni A, Patel PM, Malhi NS, Avasthi GL. Spectrum of Liver Dysfunction in Patients with Dengue Infection and the Markers of Severe Disease: Study from a Tertiary Care Centre in Punjab. J Liver Res Disord Ther. 2017;3(4):00063.
11. Parkash, O, Almas A, Jafri SW, Hameed S, Akhtar J, Alishahh. Research article Severity of acute hepatitis and its outcome in patients with dengue fever in a tertiary care hospital Karachi, Pakistan (South Asia). BMC Gasteroentrol. 2010;10:43.
12. Larreal Y, Valero N, Estevez J, Reyes I, Maldonado M, Espina LM, et al. Hepatic alterations in patients with dengue. Invest Clin. 2005;46(2):169-78.
13. Brito Carlos AA, Albuquerque Maria de Fátima MP, Lucena-Silva Norma. Plasma leakage detection in severe dengue: when serum albumin quantification plays a role?. Rev. Soc. Bras. Med. Trop. 2007;40(2):220-3.
14. Chhina RS, Goyal O, Chhina DK, Goyal P, Kumar R, Puri S. Liver function tests in patients with dengue viral infection. Dengue Bulletin. 2008;32.

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Original Research Article

Retrospective study of white blood cell count and hematocrit in dengue fever

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ABSTRACT

Background: Dengue is an extremely important tropical disease transmitted by the Aedes aegypti mosquito. Aim of the study: to study the white blood cell levels in serologically proven dengue cases; to study hematocrit levels in cases of dengue fever.
Methods: The present study is a retrospective observational analysis of white blood cell count (WBC count) and hematocrit in 235 serologically proven Dengue cases admitted in the years 2011-2012
Results: 107 cases (45.5%) had leukopenia, 111 cases (47.2%) had normal WBC counts and 12 cases had leukocytosis (5.1%). 23 cases had increased hematocrit (9.8%), 76 cases had a low hematocrit (32.3%) and 128 cases had a normal hematocrit (54.5%).
Conclusions: A significant proportion of patients had leukopenia which may be an early marker of dengue infection. Only a small percentage of patients had raised hematocrit. It may be that rise from baseline levels of haematocrit which may be more significant than absolute values.

Keywords: Dengue fever, Leukopenia, Raised hematocrit

INTRODUCTION

Dengue is a very important viral disease transmitted by the bite of the infected Aedes aegypti mosquito. It has been increasing year after year and has expanded into multiple countries all over the world. There are about 50 million new dengue infections each year worldwide.[1] World health organization has stated that dengue infection can be considered to be a public health emergency because of its capacity to spread exponentially beyond national borders.

Dengue infection shows great variability in its incidence with high epidemic and non-epidemic years. It can present as large outbreaks of infection and also shows seasonal variation. There are multiple serotypes of dengue virus circulating in India.[2]

Dengue infection can be classified into undifferentiated fever, dengue fever and dengue hemorrhagic fever.[3] Dengue hemorrhagic fever is further classified into 4 subtypes of which 3 and 4 are representative of dengue shock syndrome.

It can present with wide variety of symptoms. They include gastrointestinal symptoms such as anorexia, nausea, vomiting; skin manifestation such as rash, musculoskeletal features such as myalgia and arthralgia and laboratory manifestations such as leukopenia.[4]

METHODS

The present study is a retrospective observational analysis of white blood cell count (WBC count) and hematocrit in 235 serologically proven Dengue cases

admitted in the years 2011-2012. Inclusion criteria included patients above 15 years of age and were Dengue IgM positive.

Exclusion criteria excluded patients with other tropical infections such as malaria, typhoid and scrub typhus. Dengue serology was done by Elisa method.

WBC count and hematocrit were done by coulter technique. WBC count less than 5000 cells/mm^3 was taken as leukopenia and WBC count greater than 10000 cells/mm^3 was taken as leukocytosis.[5] Hematocrit of less than 40% was taken as low and greater than 45% was taken to be raised hematocrit.

RESULTS

235 patients were part of the study. All the patients included in our study were Dengue IgM positive. WBC count was available for 230 patients. Our analysis showed that 107 cases (45.5%) had leukopenia. About 111 cases (47.2%) had normal WBC counts and 12 cases had leukocytosis (5.1%).

The comparison of leukopenia in our study has been done with other studies in Table 2 with the values varying in different studies. Hematocrit values were available for 228 patients. 23 cases had increased hematocrit (9.8%), 76 cases had a low hematocrit (32.3%) and 128 cases had a normal hematocrit values (54.5%).

Table 1: Range of distribution of white blood cell count.

WBC count	Number	Percent
Normal	111	47.2
Low	107	45.5
High	12	5.1
Not done	5	2.1
Total	235	100.0

Table 2: Different studies showing incidence of leukopenia.

Study	Leukopenia (%)
Christopher et al	87
Shyamsundar khatroth	20
Rajesh Deshwal et al	20.1
Present study	47.2

Table 3: Range of distribution of hematocrit levels.

Hematocrit	Number	Percent
Normal	128	54.5
Low	76	32.3
High	23	9.8
Not done	8	3.4
Total	235	100.0

DISCUSSION

Dengue fever has three phases namely, the febrile phase, the critical phase and the stage of plasma leakage. The febrile phase is characterized by dehydration. In the critical phase there is plasma leakage with accumulation of fluid in third spaces such as pleural and peritoneal cavities and the recovery phase may lead to hypervolemia especially if large amounts of fluids are being administered.

The febrile phase is the symptomatic stage and leukopenia may be present in this stage. Also increasing leukopenia precedes the stage of plasma leakage. In a study by Christopher J Gregory et al which dealt with the utility of tourniquet test and WBC count as a marker to differentiate dengue fever from other febrile illnesses, the authors showed that 87% of dengue cases had leukopenia compared to 28% of non dengue cases.[6] The conclusion was that leukopenia could be taken up as one of the indicators to separate dengue from other febrile illnesses. The other indicator was a positive tourniquet test.

In another study by Kalyanarooj S et al, the authors concluded that early dengue infection was characterised by leukopenia.[7] In a study by Rajesh Deshwal et al, the incidence of leukopenia was 20.19%. In another study by Shyamsundar Khatroth showed that the incidence of leukopenia was 20%.[8]

In our study the incidence of leukopenia was 47.2% which was higher than the studies by Kalyoonrooj et al and Rajesh Deshwal. It is possible that a substantial subset of patients in our study could have presented in the febrile phase of the illness.

Rising hematocrit levels are a marker of the critical phase of dengue infection. After leukopenia there is the stage of plasma leakage where there are third space losses. Ultrasound and chest X-ray are useful tools to detect pleural effusion and ascites which are characteristic of this stage. The extent up to which hematocrit rises from the baseline can indicate the severity of plasma leakage. In a study by Francisca Raimunda et al, hematocrit was shown to be increased in patients with Dengue hemorrhagic fever.[9]

In another study by Kailash C Meena et al the percentage of patients with raised hematocrit was 9.8%.[10] The percentage of patients with raised hematocrit in our study was 9.8% which was similar to Kailash C Meena' s study.

CONCLUSION

Our study had an incidence of leukopenia of 47.2% which was higher than other comparable studies. Presence of leukopenia could be a marker of early dengue infection especially when combined with other indicators. The incidence of increased hematocrit was 9.8%. Rising

hematocrit may be a marker for the stage of plasma leakage necessitating increased fluid administration. Drawback of this study was that few patients did not undergo all the tests. More studies are required to look for serial WBC count and hematocrit levels during the course of dengue infection.

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REFERENCES

1. Dengue guidelines for diagnosis, treatment, prevention and control: new edition, Geneva. World Health Organization; 2009.
2. Dengue guidelines for diagnosis, treatment, prevention and control new edition. Geneva: World Health Organization. WHO. 2009:5.
3. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. World Health Organization. Geneva, WHO; 2009:10.
4. Ho TS, Wang SM, Lin YS, Liu CC. Clinical and laboratory predictive markers for acute dengue infection. J Biomed Sci. 2013;20(1):75.
5. Sawasdivorn S, Vibulvattanakit S, Sasavatpakdee M, Iamsirithavorn S. Efficacy of clinical diagnosis of dengue fever in paediatric age groups as determined by WHO case definition 1997 in Thailand. Dengue Bulletin 25:56-64.S.
6. Gregory CJ, Lorenzi OD, Colón L, García AS, Santiago LM, Rivera RC, et al. Utility of the tourniquet test and the white blood cell count to differentiate dengue among acute febrile illnesses in the emergency room. PLoS neglected tropical diseases. 2011;5(12):e1400.
7. Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N, et al. Early clinical and laboratory indicators of acute dengue illness. J Infec Dis. 1997;176(2):313-21.
8. Shamsunder Khatroth. A Study on Clinical and Hematological Profile of Dengue Fever in a Tertiary Care Hospital. IAIM. 2017;4(8):96-102.
9. Azin FR, Gonçalves RP, Pitombeira MH, Lima DM, Castelo Branco I. Dengue: profile of hematological and biochemical dynamics. Revista brasileira de hematologia e hemoterapia. 2012;34(1):36-41.
10. Meena KC, Jelia S, Meena S, Arif M, Ajmera D, Jatav VS. A study of hematological profile in dengue fever at tertiary care center, Kota, Rajasthan, India. Int J Adv Med. 2016;3:621-4.

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Original Research Article

Insulin resistance and significance of postprandial lipid profile in normoglycemic coronary artery disease

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Abstract

Background: Coronary artery disease is a continuum of a pathological process, in which the coronary arteries gradually thicken, harden and atherosclerotic plaques develop which further occludes the blood flow. The prevalence of coronary artery disease is rapidly rising in India. There are various risk factors of CAD which have been extensively studied, out of which Type-2 diabetes is an important etiopathogenic factor of accelerated CAD. Insulin resistance plays an important role in the pathogenesis of the development of type- 2 diabetes. Despite the clear relationship between type-2 diabetes and CAD**,** the association of insulin resistance and CAD is more obscure in people without diabetes: **Aims & Objectives:** To find the association between insulin resistance and Coronary artery disease, to evaluate the role of postprandial lipid profile and its relationship with development of CHD and the evaluation of apolipoproteins (a and b) on the same group. **Subjects and Methods:** Fifty patients of coronary artery disease admitted to ICCU/CCU of psg institute of medical sciences and research were studied. The cases of this present study were 50 non diabetic CAD patients and controls were selected based on age and sex matched non diabetic 'and non CAD patients who has to undergo similar set of investigations as cases to find out association of insulin resistance by using HOMA score and to find out the significance in abnormalities of postprandial lipid profile in these patients. **Results:** 92% of the cases had insulin resistance, 52% of the controls had normal HOMA score when compared to cases. Apolipoprotein b was significantly elevated in cases (84%) than controls**.** All patients were normoglycemic. Serum triglycerides were significantly high in cases than controls in both fasting and postprandial states. **Conclusion:** Improving insulin sensitivity would offer substantial benefits by decreasing the morbidity, mortality, and economic burden associated with CAD especially in the country like India

Key words: Insulin Resistance, Coronary Artery Disease, Homa Score

Introduction

Coronary artery disease is a continuum of a pathological process, in which the coronary arteries gradually thicken, harden and atherosclerotic plaques develop which further occludes the blood flow [1]. This leads to clinical manifestations such as angina and acute coronary events resulting from disruption of plaques, such as acute coronary syndromes, myocardial infarction and death [1,2].

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The prevalence of CAD is rising rapidly in rural India. Lifestyle changes and aggressive control of risk factors are urgently needed to reverse this trend. Asian Indians have considerably higher prevalence of premature coronary artery disease and standardized mortality rates for CAD compared with Europeans.

Within the Indian subcontinent, a dramatic increase in the prevalence of CAD has been predicted in the next 20 years due to rapid changes in demography and lifestyle consequent to economic development [3, 4].

There are various risk factors of CAD which have been extensively studied, out of which Type-2 diabetes is an important etiopathogenic factor of accelerated CAD. Insulin resistance plays an important role in the pathogenesis of the development of type- 2 diabetes. Elevated insulin levels and insulin resistance may be evident several years prior to the diagnosis of type-2 diabetes. There are evidences supporting association of Insulin and CAD which indirectly reflects glucose intolerance, hypertension and dyslipidemia. However, the results from several studies are conflicting. Despite the clear relationship between type-2 diabetes and CAD**,** the association of insulin resistance and CAD is more obscure in people without diabetes [5, 6].

It is well established that dysfunctional endothelium contributes to development and progression of atherosclerosis. Consequently, early detection and treatment of endothelial dysfunction may be an attractive strategy for preventing CAD**.** Unfortunately, established validated methods for assessment of endothelial dysfunction for chronic heart disease risk prediction in the clinical setting are not currently available. Current techniques to assess endothelial function are invasive, expensive, or suffer from lack of high sensitivity, specificity, reproducibility, or clinically defined cut-off values. Therefore, at this time, targeting established and modifiable risk factors for endothelial dysfunction and insulin resistance is the best primary strategy to prevent these conditions [7, 8].

Hypercholesterolemia and hyper triglyceridemia are considered the independent risk factors but most of the earlier studies in this area have considered only the fasting lipid profiles and lipoproteins. Recently it has been proposed that postprandial lipoproteins may be better indicators of deranged lipoprotein metabolism and hence of atherosclerosis and CHD. Postprandial hypertriglyceridemia (PHTG) and delayed triglyceride (TG) rich lipoprotein clearance have been found to impair endothelial function significantly either directly or by increasing superoxide anions.

It has also been reported that magnitude and duration of postprandial lipidemia is positively related to the pathogenesis and progression of CHD [9]. There is comparatively more transfer of cholesterol and cholesterol esters from HDL to LDL in postprandial state leading to their low levels and this along with higher triglycerides and VLDL levels are better indicators of coronary heart disease [10].

Aims & Objectives

- To find the association between insulin resistance and Coronary artery disease
- To evaluate the role of postprandial lipid profile and its relationship with development of CHD
- Evaluation of apolipoproteins (a and b) on the same group

Materials and Methods

Fifty patients of coronary artery disease admitted to ICCU/CCU of PSG Institute of medical sciences and research were studied. The cases of this present study were 50 non diabetic CAD patients and controls were selected based on age and sex matched non diabetic ‘and non CAD patients who has to undergo similar set of investigations as cases to find out association of insulin resistance by using HOMA score [11] and to find out the significance in abnormalities of postprandial lipid profile in these patients. Patients with Acute coronary event and Non diabetics were selected as cases and the patients with Diabetics on insulin/ Oral hypoglycemic agents, Severe cardiac failure (classes 3–4), Renal hepatic, and other systemic diseases, Morbid obesity, History of malignancy and Previous h/o dyslipidemia were excluded. The selected patients were studied in detail with history and physical examination. Complete blood picture, Urine analysis, Renal function tests fasting Blood sugar and postprandial blood sugar, glycosylated haemoglobin, Fasting and post prandial lipid profile. Apolipoprotein a and b Fasting insulin levels, Chest x-ray, Electrocardiogram Echocardiogram and Coronary Angiography were done in patients. The estimation of Insulin resistance by HOMA score = fasting insulin (Mu/L) × fasting glucose (mmol/L)/22.5. The study Parameters entered in Microsoft Excel spread sheet and statistically analysed using Chi-square and Fisher Exact test to find the significance of proportion of incidence of insulin between various levels of study parameters namely BMI, Age, abnormal lipid profile and complications etc. and Student t test to find the significance of mean levels of lab parameters between the presence and absence of insulin resistance.

Results

Among cases 70% of patients were males and 30% were females, Among controls 66% patient were males and 34% were females .10% of the CAD patients and 9% of the controls had habit of consuming smoking and alcohol. 72% of the cases and 68% of the control are obese. 92% of the cases had insulin resistance, 52% of the controls had normal HOMA score when compared to cases. Apolipoprotein b was significantly elevated in cases (84%) than controls**.** All patients were normoglycemic. Serum triglycerides were significantly high in cases than controls in both fasting and postprandial states.HDL and Total cholesterol was unchanged both in fasting and postprandial state LDL cholesterol was found to be mildly increased in patients but not significantly as fasting level. TG/HDL RATIO was found to be >4 in cases with insulin resistance when compared to those without insulin resistance. TC/HDL RATIO was found to be > 3.5:1 in high in cases & controls with Insulin resistance (72%, 43.2% respectively) when compared to patients without insulin resistance. Post prandial triglycerides where high when compared to fasting levels among cases when compared to controls. Percentage of postprandial hypertriglyceridemia was much higher in cases when compared to controls. Significant association of Obesity & Insulin resistance in cases and controls (Table 1& 2)

Table-1: Relationship between Insulin Resistance and BMI Fishers exact test- Cases

	Normal		Obese		Total		P value
	No	%	No	%	No	%	0.041*
Normal	4	40.0	4	10.0	8	16.0	
Abnormal	6	60.0	36	90.0	42	84.0	
Total	100	100.0	40	100.0	50	100.0	

Table-2: Relationship between Insulin Resistance and BMI Fishers exact test- Controls

	Normal		Obese		Total		P value
	No	%	No	%	No	%	0.002*
Normal	22	68.8	4	22.2	26	52.0	
Abnormal	10	31.2	14	77.8	24	48.0	
Total	16	100.0	34	100.0	50	100.0	

Discussion

Coronary artery disease (CAD) is continuums of a pathological process, in which the coronary arteries gradually thicken & harden and atherosclerotic plaques develop that further occlude blood flow. This leads to clinical manifestations such as angina and acute coronary events such as acute coronary syndromes, myocardial infarction and death, due to the disruption of plaques.

Coronary artery disease is the leading cause of death in India and is also the leading cause of death worldwide. It was previously thought to affect primarily the population of developed countries. CAD now leads to more death and disability in developing countries such as India. CAD affects people at younger ages in developing countries compared to developed countries, thereby having a greater economic impact. Effective screening, evaluation and management strategies for coronary artery disease are well established in developed countries, but these strategies are not fully implemented in India. For the development & implementation of such strategies identifying the risk factors is an important initial step.

An effort has been made to recognize insulin resistance in non-diabetic CAD patients which is a simple and non-invasive practical tool. It requires only a single sample assayed for Insulin and glucose and can be calculated by HOMA score [11].

There was significant insulin resistance (IR) (92%) among cases. This is similar to a study done by Bertoluci et al [12] from Brazil which showed 82.6% of CAD patients had insulin resistance when compared to the controls/non CAD group. Another American study by David et al [13] showed that IR was responsible (96%) for 46.8%, 6.2% and 12.5 % of the annual CHD events in diabetics, non-diabetics and total US

population respectively. Yet another study by E. Devici et al [14] which compared Insulin resistance among normoglycemic patients with CAD revealed significant association of IR. Metabolic syndrome was similar in both the control and cases and thus HOMA IR values may provide more sensitive information than the Metabolic syndrome definitions about the association of IR and CAD in normoglycemic patients. Like the above study quoted, there was significant association of obesity and Insulin Resistance in our study which was 84% In the CAD group and 77.8% in the non CAD group. Approximately 50% of patients with essential hypertension, both treated and untreated, appear to be insulin resistant based on the study by Roopa et al [15].

Significance of the postprandial lipid profile in patients with CAD is a new and emerging study which has only limited references & is not practiced routinely day to day clinical setup. In this study Serum triglycerides were significantly high in cases than controls in both fasting and postprandial states. Total cholesterol was unchanged both in fasting and postprandial state. HDL cholesterol was found to be decreased significantly in fasting state, but LDL cholesterol was found to be mildly increased. A study by Vijay et al[16] done in Haryana showed significant elevation of Serum TG, total cholesterol and VLDL – cholesterol in patients than controls in both fasting and postprandial states ($p<0.001$) and HDL-cholesterol was found to be decreased significantly in fed state only ($p < 0.05$).

It concluded that there is comparatively more transfer of cholesterol and cholesterol esters from HDL to LDL in postprandial state leading to their low levels and this along with higher triglycerides and VLDL levels are better indicators of coronary heart disease. Apoprotein b was significantly higher in CAD group when compared to controls which is similar to a study by Bertsch RA et al [17] which showed that these pro-atherogenic lipoproteins were strong predictors of CAD when compared to the non HDL cholesterol. In those controls (4 patients) in whom Apo B was elevated there was also Insulin Resistant.

Among them, 2 patients were hypertensive and their ECHO and ECG showed features of left ventricular hypertrophy. Other two were obese without pre morbidities and had non specific ST-T changes in ECG with a normal ECHO study. Apo B, TG/HDLc ratio and Tc/HDL ratio were significantly higher among patients with insulin resistance

Conclusion

There is insulin resistance in patients with coronary artery disease and also among non diabetic patient who are obese. Apolipoprotein b levels where significantly raised in CAD patients. Serum triglycerides were significantly high in cases than controls in both fasting and postprandial states. Percentage of postprandial hypertriglyceridemia was much higher in cases when compared to controls. Fed state did not alter the total cholesterol/ LDL/HDL significantly. Patients with IR had high TGL/HDLc, TC/HDLc and Apo B values. Fasting Insulin and Insulin resistance (HOMA SCORE) can be included as one of the panel of investigations to assess the risk factor of CAD among nondiabetic population. Earlier identification of these patients would help to intervene and improve the quality of life. Improving insulin sensitivity would offer substantial benefits by decreasing the morbidity, mortality, and economic burden associated with CAD, especially in the country like India.

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References

1. Joseph J, Shamburek RD, Cochran EK, Gorden P, Brown RJ. Lipid regulation in lipodystrophy versus the obesity-associated metabolic syndrome: the dissociation of HDL-C and triglycerides. J Clin Endocrinol Metab. 2014 Sep;99(9):E1676-80. doi: 10.1210/jc.2014-1878. Epub 2014 Jun 13.

2. Reaven GM, Lerner RL, Stern MP, Farquhar JW. Role of insulin in endogenous hypertriglyceridemia. J Clin Invest. 1967 Nov;46(11):1756-67.

3. Howard G, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R. Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. Circulation. 1996 May 15;93(10):1809-17.

4. Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet. 1975 Jan 4;1(7897):16-9.

5. Welborn TA, Breckenridge A, Rubinstein AH, Dollery CT, Fraser TR. Serum-insulin in essential hypertension and in peripheral vascular disease. Lancet. 1966 Jun 18;1(7451):1336-7.

6. Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia. 1991 Jul;34(7):457-62.

7. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000 Aug;106(4):473-81.

8. Gavrilova O, Marcus-Samuels B, Graham D, et al. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. Journal of Clinical Investigation. 2000;105(3):271-278.

9. Klannemark M, Orho M, Langin D, Laurell H, Holm C, Reynisdottir S, Arner P, Groop L. The putative role of the hormone-sensitive lipase gene in the pathogenesis of Type II diabetes mellitus and abdominal obesity. Diabetologia. 1998 Dec;41(12):1516-22.

10. Babirak SP, Brown BG, Brunzell JD. Familial combined hyperlipidemia and abnormal lipoprotein lipase. Arterioscler Thromb. 1992 Oct;12(10):1176-83.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412-9.

12. Bertoluci MC, Pimazoni-Netto A, Pires AC, Pesaro AE, Schaan BD, Caramelli B, Polanczyk CA, Júnior CV, Gualandro DM, Malerbi DA, Moriguchi E, Borelli FA, Salles JE, Júnior JM, Rohde LE, Canani LH, Cesar LA, Tambascia M, Zanella MT, Gus M, Scheffel RS, Dos Santos RD. Diabetes and cardiovascular disease: from evidence to clinical practice - position statement 2014 of Brazilian Diabetes Society. Diabetol Metab Syndr. 2014 May 20;6:58. doi: 10.1186/1758-5996-6-58. eCollection 2014.

13. Hadaegh F, Derakhshan A, Mozaffary A, Hasheminia M, Khalili D, Azizi F. Twelve-Year Cardiovascular and Mortality Risk in Relation to Smoking Habits in Type 2 Diabetic and Non-Diabetic Men: Tehran Lipid and Glucose Study. PLoS One. 2016 Mar 1;11(3):e0149780. doi: 10.1371/journal.pone.0149780. eCollection 2016.

14. Deveci E, Yesil M, Akinci B, Yesil S, Postaci N, Arikan E, Koseoglu M. Evaluation of insulin resistance in normoglycemic patients with coronary artery disease. Clin Cardiol. 2009 Jan;32(1):32-6. doi: 10.1002/clc.20379.

15. Roopa AN ,Reddy KSS ,Chandrasekhara P, Umabai KR .Study of microalbuminuria and insulin resistance in patients with essential hypertension and metabolic syndrome and its relationship to target organ damage.J Med Sci Health 2015;1(3):5-9

16.Shankar V, Kaur Harnam ,Dahiya K.Comparison of Fasting and Post prandial Lipoid profile in patients of coronary heart disease. Bombay Hospital Journal.2008;50(3).

17. Bertsch RA, Merchant MA. Study of the Use of Lipid Panels as a Marker of Insulin Resistance to Determine Cardiovascular Risk. The Permanente Journal. 2015;19(4):4-10.

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Original Research Article

A study of the symptoms of gastro oesophageal reflux disease and the associated risk factors in a tertiary care centre

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ABSTRACT

Background: Gastro esophageal reflux disease is the commonest pathologies encountered by gastroenterologist in day to day practice. Longstanding and untreated gastro esophageal reflux disease can lead to many complications including adenocarcinoma. This study was conducted to evaluate the various symptoms and to analyze the lifestyle and dietary factors influencing gastro esophageal reflux disease which can be modified.
Methods: Hundred patients with gastro-esophageal reflux disease of age more than 18 years were enrolled in the study. Various patient details including demographic details, lifestyle information and symptomatology data were analyzed and compared with complications.
Results: Mean age of gastro esophageal reflux disease patients was 56.09±15.93 years. Gastro esophageal reflux disease is more prevalent in males than females. Gastro esophageal reflux disease is more frequent in BMI <25, greater number of co morbidities and in non-vegetarians. Mean age of gastro esophageal reflux disease with complications was 67±11.53 years and without complications was 52.64±15.57 years. No strong association of smoking, alcohol, spicy foods, fried foods, citrus fruits, heavy meals, tea/coffee, aerated drinks, sleep disturbance and effect on work was identified in gastro esophageal reflux disease.
Conclusions: Classical symptoms of gastro esophageal reflux disease were not present in all the patients. Higher age of the patient infers higher risk of complications. Daily episodes of heartburn, regurgitation and retrosternal chest pain implies higher risk of complications. Presence of *Helicobacter pylori* in gastro esophageal reflux disease patients signify higher risk of complications.

Keywords: Adenocarcinoma, Gastro esophageal reflux, Heartburns, Regurgitation, Retrosternal chest pain

INTRODUCTION

Gastro esophageal reflux disease (GERD) is defined as a condition due to reflux of stomach content into the esophagus causing trouble symptoms or complications or both.[1] GERD is one amongst the common gastro-esophageal junction pathologies encountered by gastroenterologist in day to day practice. GERD occurs either due to patulous lower esophageal sphincter or increased pressure within the stomach. It has a wide variety of clinical presentations ranging from gastrointestinal (common) to extra-gastrointestinal (uncommon) symptoms. Common gastrointestinal symptoms are heartburn, regurgitation and retrosternal chest pain. Less common gastrointestinal symptoms are dysphagia and odynophagia. Extra-gastrointestinal symptoms are bronchial asthma, laryngitis, hoarseness of voice, chronic cough, sore throat and dental erosions. Furthermore, longstanding and untreated GERD leads to morbid complications such as esophageal ulcer, Barrett's esophagus, esophageal stricture and adenocarcinoma

Every year GERD affects approximately 4.5/1000 persons in the United Kingdom and 5.4/1000 persons in the United States.[2,3] In Western populations, the prevalence of GERD ranges from 10% to 20%. GERD symptoms occur at least once a month in 44%, once a week in 20% and daily in 7% of the adult US population.[4-6] However, studies describing the epidemiology of GERD in South-East Asia and for that matter in India are sparse; and traditionally GERD was thought to be uncommon in the developing countries, on the other hand, studies in the multiethnic population showed that people of Indian origin are at higher risk of GERD than ethnic Malay and Chinese.[7,8] And the studies done in India emphasize that the prevalence of GERD in India is likely to be between 8% and 20%, which is comparable with GERD prevalence rates published in western countries.[9] In addition, these studies highlight that the risk factors for GERD are related to diet and life-style preferences, which are both modifiable risk factors. Hence this study was conducted to evaluate the various symptoms and to analyze the lifestyle and dietary factors influencing it which can be modified. The study also evaluates the association of clinical, lifestyle and endoscopic characteristics with complications of GERD.

METHODS

It was a cross sectional observational study done between February 2016 to March 2017 at the Department of Medicine in a tertiary care hospital in south India. Total of 100 patients with upper gastrointestinal endoscopy proven Gastro-esophageal reflux disease of age more than 18 years were enrolled in the study. Patients with mass lesion in oesophagus or stomach, oesophageal varices, history of corrosive ingestion, pregnant women and terminally ill patients were excluded from the study.

Prior to the commencement of the study, ethical clearance was obtained from Institutional Human Ethical Committee. The selected patients were briefed about the nature of the study and written informed consent was obtained from them in regional language. Subsequently, patients were interviewed for demographic details, lifestyle information, and symptomatology data. The patients were divided into GERD with complications and GERD without complications. The demographic, lifestyle and symptoms of the two groups were compared.

Statistical analysis

Data was analyzed using SPSS version 20.0 statistical software. Categorical variables were expressed as percentages and the comparative analysis was done using chi-square test or Fischer exact test. Continuous variables were expressed as mean±standard deviation (SD) and the comparative analysis was done by independent sample 't' test. A probability value (p value) of less than or equal to 0.05 at 95% confidence interval was considered as statistically significant.

RESULTS

A total of 100 patients, diagnosed of Gastro-esophageal reflux disease (GERD) based on UGI scopy were enrolled in this study. Mean age of GERD patients was 56.09±15.93 years (Range: 20 - 82 years). Demographic parameters analyzed were age, sex and BMI of the patients. Lifestyle parameters assessed were smoking, alcohol, spicy food and fried food intake, intake of meat, tea / coffee, citrus fruits, aerated drinks, heavy meals, sleep disturbances and effect on daily work. Clinical symptoms evaluated were heartburn, regurgitation, retrosternal chest pain, dysphagia, odynophagia, positional variation of these symptoms. In addition, presence of *Helicobacter pylori*, GERD grades and its complications were also analyzed.

Table 1: Distribution of demographic parameters.

Sex	Distribution
Male	75
Female	25
Body mass index	
<25	70
>25	30
Number of co morbidities	
<3	48
>3	21
Absent	31

From the Table 1 it is seen that GERD is more prevalent in males than females with male to female ratio of 3:1. It is also seen that GERD is more frequent in BMI <25 and with greater number of co morbidities.

Table 2: Distribution of life style parameters.

Parameter	Present	Absent
Smoking	52	48
Alcohol	44	56
Non veg habit	75	25
Intake of spicy foods	59	41
Intake of fried foods	26	74
Intake of citrus fruits	13	87
Heavy meal intake	33	67
Sleep disturbance	32	68
Effect of work	9	9
Tea/coffee intake (>3 cups / day)	39	61
Aerated drinks intake (>5 drinks / week)	4	96

From the Table 2, it is seen that GERD is more prevalent in non-vegetarians than vegetarians. It is also seen that there was no strong association of smoking, alcohol, spicy foods, fried foods, citrus fruits, heavy meals, tea/coffee, aerated drinks, sleep disturbance or effect on work with GERD.

From the Table 3, it is seen that classical symptoms of GERD such as heartburn, regurgitation and retrosternal chest pain were not present in all the patients. It is also seen that dysphagia and odynophagia were less frequent in GERD.

Table 3: Distribution of clinical symptoms.

Clinical symptom	≤2/ Week	>2/ Week	Daily	Absent
Heart burns	11	41	22	26
Regurgitation	31	25	19	25
Retrosternal chest pain	32	9	25	34
Dysphagia	5	3	17	75
Odynophagia	1	3	14	82

Prevalence of GERD complications were 24% in present population. Most common complication was erosive esophagitis followed by Barrett's esophagus and esophageal stricture. Furthermore, we divided the total of 100 GERD patients into two groups: GERD with complications (24 patients) and GERD without complications (76 patients) and analyzed the documented variables between these two groups.

Table 4: Demographic variables between GERD with complication and without complications.

Demographic parameters	Independent 't' test		
	Complication Mean±SD	Without complication Mean±SD	P value
Age	67.00±11.78	52.64±15.68	0.000*
BMI	23.00±3.42	23.42±4.19	0.657
	Chi square test		
Sex	67% males	78% males	0.279
	33% females	22% females	

From the Table 4 it is seen that mean age of GERD with complications was 67±11.53 years (Range: 41 - 82 years) and mean age of GERD without complications was 52.64 ± 15.57 years (Range: 20 - 82 years). Age of the patient showed significant association with the presence of complications (p= 0.000). Higher the age of the patient, higher is the risk of complication. There was no significant difference in presence of complications with sex of the patient.

From the Table 5, it is seen that daily episodes of heartburn, regurgitation and retrosternal chest pain implies higher risk of complications (p=0018, 0.023, 0.016 respectively). It is also seen that, although dysphagia and odynophagia showed p value of < 0.05, they didn't exhibit strong association between these two groups due to lower likelihood ratio of 47.84 and 62.38 respectively.

Table 5: Clinical variables between GERD with complication and without complications.

Clinical parameters	Independent 't' test		
	GERD with complication Mean±SD	Without complication Mean±SD	P value
Number of co-morbidity	1.67±1.30	1.38±1.38	0.375
	Chi square test		
Heart burns	0% <2/wk	14 % <2/wk	0.018*
	17 % ≥2/wk	49 % ≥2/wk	
	83% daily	3% daily	
	0% absent	34% absent	
Regurgitation	0% <2/wk	41% <2/wk	0.023*
	25 % ≥2/wk	25% ≥ 2/wk	
	75% daily	1% daily	
	0% absent	33% absent	
Retrosternal chest pain	0% <2/wk	42 % <2/wk	0.016*
	17 % ≥2 /wk	7 % ≥ 2/wk	
	83% daily	6% daily	
	0% absent	45% absent	
Dysphagia	0% <2/wk	7 % <2/wk	0.025*
	13% ≥2/wk	0 % ≥2/wk	
	54% daily	4% daily	
	29% absent	89% absent	
Odynophagia	0% <2/wk	1 % <2/wk	0.035*
	13% ≥ 2/wk	0 % ≥ 2/wk	
	58% daily	0% daily	
	29% absent	99% absent	
Positional variation of symptoms	25% present	25% present	1.000
	75% absent	75% absent	

From the Table 6, it is seen that none of the lifestyle parameters had significant difference between two groups.

From the Table 7, it is seen that, presence of *Helicobacter pylori* in GERD patients signifies higher risk of complications (p=0.000).

DISCUSSION

In present study, authors found that out of 100 GERD patients, 75% were males and 25% were females with male to female ratio of 4:1. Present findings were in contrary to previous meta-analysis study by Kim YS et al, who inferred that GERD affects more frequently women than men.[10]

In this present study, we found that 70% of the patients had BMI <25 and 30% of the patients had BMI ≥25, contradicting the inference of previous study by Jacobson BC et al, who found that GERD symptoms exacerbation is more frequent in patients with BMI of ≥25.[11]

Table 6: Lifestyle parameters between GERD with complication and without complications.

Lifestyle parameters	Chi square test		
	GERD with complication	GERD without complication	P value
Smoking	50% present	53% present	0.822
	50% absent	47% absent	
Alcohol	37% present	46% present	0.462
	63% absent	54% absent	
Veg vs nonveg	37% Veg	21% veg	0.105
	63% Non veg	79% Non veg	
Spicy foods	50% present	62% present	0.304
	50% absent	38% absent	
Fried foods	29% present	25% present	0.685
	71% absent	75% absent	
Citrus fruits	4% present	16% present	0.140
	96% absent	84% absent	
Tea / coffee	38% frequent	39% frequent	0.978
	16% infrequent	18% infrequent	
	46% absent	43% absent	
Aerated drinks	0% frequent	5% frequent	0.305
	0% infrequent	4% infrequent	
	100% absent	91% absent	
Heavy meals	71% present	34% present	0.647
	29% absent	66% absent	
Sleep disturbance	71% present	33% present	0.733
	29% absent	67% absent	
Effect on work	4% present	11% present	0.343
	96% absent	89% absent	

Table 7: Analysis of reflux esophagitis grades and *H. pylori* between GERD with complications and without complications.

Parameters	Chi square test		
	GERD with complication	GERD without complication	P value
H. pylori	83% present	22% present	0.000*
	17% absent	78% absent	
Reflux esophagitis grades	0% A	75% A	0.000*
	8% B	16% B	
	46% C	8% C	
	46% D	1% D	

In addition, this study showed that out of 100 GERD patients, 69% had associated co-morbidities of which 48% had <3 co-morbidities and 21% had ≥3 co-morbidities. This finding supports the previous study by Moraes-Filho JPP et al, who postulated the strong association of co-morbidities with GERD and its worsening effect on GERD.[12]

Though heartburn, regurgitation and retrosternal chest pain are the classic presenting symptoms of GERD, in present study these symptoms were not seen in all the patients. Previous study by Kahrilas PJ et al, inferred that heart burn was present in nearly all GERD patients in their study and regurgitation was found in 80% of GERD patients, in contradiction to the results of our present study.[13] Furthermore, dysphagia and odynophagia have been described as third most common clinical symptom of GERD.[13] In contrary, in present study 75% of patients didn't have dysphagia and 82% of patients didn't have odynophagia. Amongst 100 GERD patients in our study, 75 patients didn't have positional variation of symptoms. In contrary to our study a recumbent (lying) posture after meals was observed as a precipitating factor by 42%of the cases as reported in one of the studies.[14]

In a previous study by Çela L et al, they inferred that the prevalence of GERD is significantly higher in smokers, alcoholics and individuals with increased frequency of consumption of meat, fried foods and spicy foods.[15] In accordance with these findings, present study showed more number of patients (75%) is non-vegetarians. Besides, we found the reverse trend in history of smoking, alcohol, intake of fried foods and spicy foods.

Literature search revealed the refluxogenic properties of the following products: citrus fruits and juices, tomatoes, coffee / tea, aerated drinks and chocolate.[16,17] Postulated mechanism by which these dietary products cause or aggravate GERD symptoms was by increased gastric juice secretion, delaying stomach evacuation and decreased LES pressure. However, current study did not show any significant association with these.

It was a common belief that eating habits such as heavy meals and eating directly before sleep might contribute to the occurrence of symptoms of GERD. Although previous studies by Iwakiri K et al, and Fujiwara et al, postulated the negative influence of these eating habits on the aggravation or occurrence of GERD symptoms. In our study we inferred that there was no significant contribution of heavy meal intake towards occurrence of GERD symptoms.[14,17]

A previous study by Gross M et al, postulated that GERD had substantial effect on work productivity in study of 249 German patients with GERD, although patients were on routine clinical care and proton pump inhibitors therapy.[18] In contrary to this finding we found that effect of work was not contributory to GERD.

In another study by Jung H et al, inferred that complex relationship between GERD and sleep exists and further they suggested modest bidirectional association of GERD and sleep disturbance.[19] In contrast to these findings, our study showed sleep disturbances were absent in 68% of the patients.

In this present study, authors found that *Helicobacter pylori* was present in 37% of the patients and was absent in 63% of the patients. Previous studies by many authors

proposed a strong argument regarding the protective role of *Helicobacter pylori* against GERD.[20,21] Prevalence of *Helicobacter pylori* was reported to be low about 5-10% in patients affected with GERD in comparison to the control groups by previous studies.[20,21] Present study findings were in contrast to these studies.

Los Angeles classification system published in 1999 was the widely accepted for categorizing reflux esophagitis on UGI scopy.[22] In this present study, 57% patients had Grade A reflux esophagitis, 14% patients had Grade B reflux esophagitis, 11% of the patients had Grade C reflux esophagitis and 12% patients had Grade D reflux esophagitis.

Complications of GERD include erosive esophagitis with ulcers, Barrett's esophagus and esophageal stricture. In previous study by Spechler SJ et al, showed that esophageal adenocarcinoma is the most common complication of GERD and warranted serial endoscopic screening for development of Barrett's esophagus.[23] In another study by Chait MM et al, postulated that 20% of the adults with GERD have serious complications.[24] Supporting this finding, our study showed that 24% of the patients had complications (12% esophageal ulcer, 6% Barrett's esophagus and 6% esophageal stricture).

Of the demographic parameters, age of the patient showed significant association with the presence of complications (p= 0.000). Higher the age of the patient, higher is the risk of complication. Reduced pain perception can increase the rate of GERD complications in the elderly, because acid injury can occur without the usual warning symptom of significant heartburn and acid reflux symptoms.[25] Another study by Thrift AP et al, inferred that risk of Barrett's Esophagus increased linearly with earlier age at onset of frequent GERD symptoms.[26] Age at symptom onset may help practitioners decide which patients with GERD symptoms to refer for endoscopic screening for Barrett's Esophagus.

Lifestyle factors can be associated with increased gastro esophageal reflux and more complications of GERD.[26] Tobacco smoking, caffeine, alcohol and fatty foods adversely affect GERD. Obesity, sedentary lifestyle and nocturnal gastro esophageal reflux are important mechanisms that are associated with more severe esophageal and extra esophageal complications of GERD in the elderly.[27,28] Obesity is a significant problem which increases acid reflux and thus increases GERD and its complications.[11] Nocturnal effects on GERD are reported by up to 78% of patients, with 75% of patients reporting that it negatively affects their ability to sleep.[28] Nocturnal gastro esophageal reflux and the recumbent, supine position remove the protective effect of gravity in GERD in the elderly patient.[29,30] Nocturnal GERD allows for more gastro esophageal reflux and further increases esophageal injury and GERD complications, especially in elderly patients who often spend more time in bed due to co morbid illness, such as dementia, Parkinson's disease, cerebrovascular disease, cardiovascular disease, pulmonary disease and diabetes mellitus. However, in the current study none of the lifestyle parameters showed any significant difference between GERD with complication and without complication.

In the present study we found that heart burn, regurgitation, retrosternal chest pain showed significant association between GERD with complications and without complications groups. It can be postulated that daily episodes of heart burn, regurgitation and retrosternal chest pain infers high risk of GERD complications.

In our study, we inferred that presence of *Helicobacter pylori* in GERD patients signifies higher risk of complications. *H. pylori* was present in 83% of patients in GERD with complications group and was absent in 78% of the patients in GERD without complications group. Among grades of reflux esophagitis, grades C and D were moderately higher in GERD with complications group. The worldwide variation in incidence of GERD may be inversely related to the prevalence of *H. pylori* infection.[27] Studies have found a negative association between the prevalence of *H. pylori* infection and GERD that is more marked with the more virulent CagA strains.[30]Additionally, they have shown a negative association of *H. pylori* status and the complications of GERD including Barrett's esophagus and esophageal adenocarcinoma.[30] A study by Labenz et al, revealed a possible protective effect of *H. pylori* infection in the subgroup analysis of patients with severe esophagitis.[31] In a study from China, a stepwise relationship was found between increasing grade of esophagitis and decreasing prevalence of *H. pylori*.[32] In a Swedish study, *H. pylori* was found to be associated with a significantly decreased risk of adenocarcinoma of the esophagus.[33] A subgroup analysis showed that the negative association was only apparent for the CagA positive strains of *H. pylori*. Another study shows that the persistent *H. pylori* infection in GERD has been reported to be a risk factor for a subtype of esophageal squamous cell carcinoma.[34]

Present study has few limitations as well. It was a single center experience. Terminally ill patients were not included in this study. Patients with esophageal mass were not included in this study, though adenocarcinoma of esophagus can occur secondary to GERD, but it has other multifactorial predispositions. Future studies with large scale, multicenter and case control design focused on this context are warranted.

CONCLUSION

Classical symptoms of GERD were not present in all the patients. Dysphagia and Odynophagia were less frequent presenting symptoms. GERD is more common in non-vegetarians than vegetarians. No strong association of smoking, alcohol, spicy foods, fried foods, citrus fruits,

heavy meals, tea/coffee, aerated drinks, sleep disturbance and effect on work was identified in GERD. Prevalence of GERD complications were 24% in studied population with order of erosive esophagitis >Barrett's esophagus and esophageal stricture. Higher age of the patient infers higher risk of complications. Daily episodes of heartburn, regurgitation and retrosternal chest pain implies higher risk of complications. Presence of *Helicobacter pylori* in GERD patients signifies higher risk of complications.

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REFERENCES

1. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R; Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: A global evidence-based consensus. Am J Gastroenterol. 2006;101:1900-20.
2. Dent J, El-Serag HB, Wallander M, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut. 2005 May 1;54(5):710-7.
3. DeVault KR, Castell DO. Updated guidelines for the diagnosis and treatment of gastroesophageal refl ux disease. The practice parameters committee of the American College of Gastroenterology. Am J Gastroenterol. 1999;94:1434-42.
4. Nebel OT, Fornes MF, Castell DO. Symptomatic gastroesophageal refl ux: Incidence and precipitating factors. Am J Dig Dis. 1976;21:953-6.
5. A Gallup Organization National Survey: Heartburn Across America. Princeton: The Gallup Organization Inc., Princeton, NJ. 1988.
6. Locke 3rd GR, Talley NJ, Fett SL, Zinsmeister AR, Melton 3rd LJ. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. Gastroenterol. 1997 May 1;112(5):1448-56.
7. Sharma P, Wani S, Romero Y, Johnson D, Hamilton F. Racial and geographic issues in gastroesophageal reflux disease. Am J Gastroenterol. 2008;103:2669-80.
8. Ho KY, Kang JY, Seow A. Prevalence of gastrointestinal symptoms in a multiracial Asian population, with particular reference to reflux-type symptoms. Am J Gastroenterol. 1998;93:1816-22.
9. Bhatia SJ, Reddy DN, Ghoshal UC, Jayanthi V, Abraham P, Choudhuri G, Broor SL, Ahuja V, Augustine P, Balakrishnan V, et al. Epidemiology and symptom profile of gastroesophageal reflux in the Indian population: report of the Indian Society of Gastroenterology Task Force. Indian J Gastroenterol. 2011 May 1;30(3):118.
10. Kim YS, Kim N, Kim GH. Sex and gender differences in gastroesophageal reflux disease. J Neurogastroenterol Motility. 2016 Oct;22(4):575.
11. Jacobson BC, Somers SC, Fuchs CS, Kelly CP, Camargo CA. Association Between Body Mass Index and Gastroesophageal Reflux Symptoms in Both Normal Weight and Overweight Women. New Eng J Med. 2006;354:2340-8.
12. Moraes-Filho JPP, Navarro-Rodriguez T, Eisig JN, Barbuti RC, Chinzon D, Quigley EMM. Comorbidities are Frequent in Patients with Gastroesophageal Reflux Disease in a Tertiary Health Care Hospital. Clinics (Sao Paulo, Brazil). 2009;64(8):785-790.
13. Kahrilas PJ. Regurgitation in Patients with Gastroesophageal Reflux Disease. Gastroenterol Hepatol. 2013;9(1):37-9.
14. Fujiwara Y, Machida A, Watanabe Y, Shiba M, Tominaga K, Watanabe T, et al. Association between dinner-to-bed time and gastro-esophageal reflux disease. Am J Gastroenterol. 2005 Dec;100(12):2633.
15. Çela L, Kraja B, Hoti K, Toçi E, Muja H, Roshi E, et al. Lifestyle characteristics and gastroesophageal reflux disease: a population-based study in Albania. Gastroenterol Res Practice. 2013;2013.
16. Myklebust S, Espelid I, Svalestad S, Tveit AB. Dental health behavior, gastroesophageal disorders and dietary habits among Norwegian recruits in 1990 and 1999. Acta Odontol Scand. 2003 Jan 1;61(2):100-4.
17. Iwakiri K, Kobayashi M, Kotoyori M, Yamada H, Sugiura T, Nakagawa Y. Relationship between postprandial esophageal acid exposure and meal volume and fat content. Dig Dis Sci. 1996 May 1;41(5):926-30.
18. Gross M, Beckenbauer U, Burkowitz J, Walther H, Brueggenjuergen B. Impact of gastro-oesophageal reflux disease on work productivity despite therapy with proton pump inhibitors in Germany. Eur J Med Res. 2010;15(3):124-30.
19. Jung H, Choung RS, Talley NJ. Gastroesophageal Reflux Disease and Sleep Disorders: Evidence for a Causal Link and Therapeutic Implications. J Neurogastroenterol Motility. 2010;16(1):22-29.
20. Labenz J, Blum AL, Bayerdorffer E, Meining A, Stolte M, Borsch G. Curing Helicobacter pylori infection in patients with duodenal ulcer may provoke reflux esophagitis. Gastroenterol. 1997 May 1;112(5):1442-7.
21. Malfertheiner P , Gerards C. Helicobacter pylori infection and gastro-oesophageal reflux disease: coincidence or association? Baillieres Best Pract Res Clin Gastroenterol. 2000;14:731-41.
22. Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, et al. Endoscopic assessment of oesophagitis: clinical and functional

correlates and further validation of the Los Angeles classification. Gut. 1999 Aug 1;45(2):172-80.
23. Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterol. 2011;140:1084-91.
24. Chait MM. Gastroesophageal reflux disease: Important considerations for the older patients. World J Gastrointestinal Endosc. 2010;2(12):388-96.
25. Chait MM. Complications of gastroesophageal reflux disease in the elderly. Ann Long Term Care. 2005;13:8-32.
26. Thrift AP, Kramer JR, Qureshi Z, Richardson PA, El-Serag HB. Age at Onset of GERD Symptoms Predicts Risk of Barrett's Esophagus. Am J Gastroenterol. 2013;108(6):915-922.
27. Chait MM. The association and clinical implications of gastroesophageal reflux disease and H pylori. Practical Gastroenterology. 2006;30:40–48.
28. Farup C, Kleinman L, Sloan S, Ganoczy D, Chee E, Lee C, Revicki D. The impact of nocturnal symptoms associated with gastroesophageal reflux disease on health-related quality of life. Arch Intern Med. 2001;161:45-52.
29. Mody R, Bolge SC, Kannan H, Fass R. Effects of gastroesophageal reflux disease on sleep and outcomes. Clin Gastroenterol Hepatol. 2009;7:953-9.
30. Sharma P. Recent advances in Barrett's esophagus: short-segment Barrett's esophagus and cardia intestinal metaplasia. Semin Gastrointest Dis. 1999;10:93-102.
31. Labenz J, Jaspersen D, Kulig M, Leodolter A, Lind T, Meyer-Sabellek W, et al. Risk factors for erosive esophagitis: a multivariate analysis based on the ProGERD study initiative. Am J Gastroenterol. 2004;99:1652-6.
32. Zhang J, Chen XL, Wang KM, Guo XD, Zuo AL, Gong J. Relationship of gastric Helicobacter pylori infection to Barrett's esophagus and gastro-esophageal reflux disease in Chinese. World J Gastroenterol. 2004;10:672-5.
33. Ye W, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK, Nyrén O. Helicobacter pylori infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. J Natl Cancer Inst. 2004;96:388-96.
34. Ye W, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK, et al. Helicobacter pylori infection andgastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. J Natl Cancer Inst. 2004;96(5):387-96.

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A prospective study on the clinical profile and outcome of meningoencephalitis in adults in a South Indian tertiary care centre

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Abstract

Background Although there are numerous studies on meningitis and encephalitis separately, literature on meningoencephalitis is sparse. In this study we analysed the clinical profile of meningoencephalitis and its clinical outcome.

Methods Fifty adults diagnosed with meningoencephalitis from July 2014 to July 2015 in a tertiary care hospital in South India were studied prospectively and their clinical presentation, aetiology and outcome were analysed.

Results Among 50 patients, 33 (66%) were male; 39 (78%) were <50 years of age. Fever was the most common presenting symptom in 41 out of 50 patients (82%), followed by headache (74%) and altered sensorium (62%); only 18 patients (36%) had all three classical symptoms. Twenty-eight out of 50 patients (56%) did not have neck stiffness. A majority of patients had acute-to-subacute clinical presentation. *Mycobacterium tuberculosis* was identified in 58% (29 out of 50). Forty-seven patients (94%) recovered completely.

Conclusion Tuberculosis was the most common cause of meningoencephalitis in the studied population, often with subacute presentation, and outcome was good with early institution of antituberculous therapy.

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Introduction

Meningitis is a clinical syndrome characterised by inflammation of the meninges surrounding the brain and the spinal cord. The classic triad of meningitis consists of fever, headache and neck stiffness.[1] Meningitis can be of infective or noninfective aetiology, occurring in any age group, with extremes of age being the most severely affected. The immune-compromised state also has high mortality and morbidity. The overall case fatality rate of bacterial meningitis in adult patients is around 30%.[2–4]

Although encephalitis by definition involves the brain parenchyma, it may also involve the meninges as well, which is termed as 'meningoencephalitis'. From an epidemiologic and pathophysiologic perspective, encephalitis is distinct from meningitis, though on clinical evaluation both can coexist. The clinical presentation is encephalopathy with diffuse or focal neurological symptoms, including behavioural and personality changes, decreased level of consciousness, neck pain/stiffness, photophobia, lethargy, generalised or focal seizures, acute confusion or amnesic states, and flaccid paralysis.[5]

Organisms responsible for bacterial meningitis are *Streptococcus pneumoniae*, *Neisseria meningitis*, Group B streptococci, *Listeria monocytogens* and *Haemophilus influenza* type b. Most patients recover completely if appropriate antibiotic therapy is instituted promptly. *Mycobacterium tuberculosis* is another major cause, especially in developing countries. Tuberculous meningitis is a critical disease in terms of fatal outcome and permanent sequelae, requiring rapid diagnosis and treatment.[6]

The term aseptic meningitis is used for all types of inflammation of the brain meninges not caused by pus-producing bacteria. It is usually a benign syndrome. Viral and aseptic meningitis are terms used interchangeably as, not only viruses are a major cause, other noninfective causes are equally attributable to the development of meningitis. Worldwide causes of viral meningitis include enterovirus, herpes, mumps, measles and HIV, with enterovirus being the most common cause of viral meningitis.

In the emergency setting differentiating bacterial meningitis from other causes, such as fungal, tuberculous, viral,

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Table 1 Age-wise distribution of the study subjects

Age (years)	Male	Female	Total
18–30	12	5	17
31–50	17	5	22
51–60	2	7	9
>60	2	0	2
Total	33	17	50

neoplastic, toxic or autoimmune causes, is extremely difficult. If a diagnosis of meningitis is made, it is prudent to start the patient on empirical antibiotics until the cultures and other results are awaited.[7] Whereas plenty of literature is available on meningitis, the clinico-aetiological profile and outcome in patients with meningoencephalitis remains not that well studied, except for in the paediatric population.[8] The present study was carried out to assess the causes, clinical profile and outcome in adults diagnosed with meningoencephalitis in a tertiary care hospital.

Methods

Patients

The study was based on prospective collection of data in adults aged 18 years and above who were diagnosed with meningoencephalitis of various aetiologies, confirmed with laboratory investigations and brain imaging. The study was conducted in a tertiary care centre, Coimbatore, South India, from July 2014 to July 2015.

Inclusion and exclusion criteria

Patients with community-acquired meningoencephalitis, those who underwent lumbar puncture for the diagnosis and those with both positive and negative cerebrospinal fluid cultures were included in the study. Patients with hospital-acquired meningitis, patients with myelitis, neurosurgical devices or a recent history of neurosurgery were excluded.

Assessments

Data were extracted using a proforma that included detailed history, clinical examination details and requisite investigations. History and clinical findings attributable to the meningitis and meningoencephalitis were collected in detail, which included fever, headache, vomiting, neck stiffness, seizures, altered sensorium, cerebrospinal fluid (CSF) rhinorrhoea and focal neurological deficits. In addition to basic investigations (which included complete blood count, blood sugar, renal and liver function tests, and chest X-ray), blood culture was also taken for all patients. All underwent lumbar puncture and CSF analysis was carried out that included sugar, protein, total count, differential count, Gram stain, bacterial culture, Ziehl Neelsen stain for acid-fast bacilli (AFB), India ink stain for *Cryptococcus,* cryptococcal antigen test and automated culture for AFB. Neuroimaging (CT or MRI brain) was carried out on all patients. CSF polymerase chain reaction (PCR) was carried out in selected patients. Herpes simplex virus (HSV) PCR was carried out in selected patients with suspected HSV encephalitis based on clinical, CSF and neuroimaging findings.

Table 2 Clinical presentation of the study subjects

Clinical variables	Number of patients	%
Headache	37	74
Fever	41	82
Seizures	6	12
Vomiting	24	48
Altered mental status	31	62
Cerebrospinal fluid rhinorrhea	5	10
Hemiparesis	4	8
Speech disturbances	2	4
Cranial nerve palsy	4	8
Neck stiffness	22	44
Papilloedema	5	10

Table 3 Aetiological profile of meningoencephalitis

Aetiology	Number of patients	%
Tuberculous meningitis	29	58
Acute pyogenic meningitis	8	16
Viral meningoencephalitis	8	16
Cryptococcal meningitis	2	4
Cerebral toxoplasmosis	2	4
Aspergillus meningitis	1	2
Total	50	100

Based on the duration of illness, patients were classified into acute (<1 week), subacute (1–4 weeks) and chronic (>4 weeks) and also grouped based on aetiology. To assess the clinical outcome of the patients the Barthel index was used.[9] Barthel index is an ordinal scale used to measure the performance in 10 activities of daily living with the score ranging between 0 and 20; functional evaluation was carried out at the end of the first month. A score of <12 is taken as a poor outcome and >12 as a good outcome; death is included in the poor outcome category. The results were analysed to assess the clinical presentation, aetiology and clinical outcome.

Ethical clearance

The study was approved by the Institutional Human Ethics Committee (Approval Number 14/193).

Results

The study included 50 consecutive patients with acute meningoencephalitis. Among 50 patients, 33 (66%) were male, 17 (34%) were female. The majority of them (78%) were <50 years of age (Table 1).

Clinical presentation

The common initial presenting symptoms were fever, headache and altered sensorium (Table 2). Among 50

Aetiology	Acute	Subacute	Chronic	Total
Tuberculous meningitis	4	21	4	29
Acute pyogenic meningitis	8	0	0	8
Viral meningoencephalitis	8	0	0	8
Cryptococcal meningitis	1	1	0	2
Cerebral toxoplasmosis	2	0	0	2
Aspergillus meningitis	0	1	0	1
Total	23	23	4	50

Acute: clinical presentation <1 week; Subacute: clinical presentation between 1–4 weeks; Chronic: >4 weeks duration

Table 4 Mode of onset of symptoms in various aetiologies of meningoencephalitis

patients, 32 (64%) had both fever and headache; only 18 (36%) had all the three classical signs of meningitis – headache, fever and altered sensorium. Twenty-eight patients (56%) did not have neck stiffness, even though there was meningeal involvement as noted in neuroimaging. Six patients (12%) had seizures; five had generalised tonic–clonic seizures, while focal motor seizures was noted in one. Among those six patients, one had a parenchymal lesion in the MRI (granuloma), meningeal enhancement was noted in three and MRI was normal in two.

Altered sensorium was observed in 31 patients; of which 10 had significant deterioration in consciousness (Glasgow coma scale <10). Among those with poor Glasgow coma scale, one had pyogenic meningitis with extensive vasculitic infarcts in the bilateral cerebral hemisphere and brainstem; seven had abnormal findings in MRI like meningeal enhancement, granuloma or hydrocephalus; MRI was normal in the remaining two. All of these 10 patients with altered sensorium required ventilatory support in intensive care unit. See online for Supplementary Tables 1–4.

Aetiology and mode of onset

The aetiological profile of meningoencephalitis is listed in Table 3. The most common cause found in the present study was *M. tuberculosis*. Among eight patients with acute pyogenic meningitis, the microbiological diagnosis was confirmed in five; three had pneumococcal growth in CSF analysis, while blood culture was positive in two (*S. aureus*; pneumococci). The remaining three patients with acute pyogenic meningitis had sterile cultures due to prior antibiotic exposure. However, very high neutrophil count in the CSF suggested the diagnosis of acute pyogenic meningitis.

Acute and subacute clinical presentation was seen in 23 each while the remaining four presented with chronic symptoms. The mode of onset of symptoms in various aetiologies is listed in Table 4.

Outcome

Clinical outcome was assessed at the end of 1 month using the Barthel index. Among 50 patients, 47 (94%) recovered completely without any deficits; two had fulminant course and succumbed to the illness by the end of 1 month; one had Barthel index score of 0 (Table 5).

Discussion

The present study included 50 adult patients with meningoencephalitis, *M. tuberculosis* was the most common agent. Tuberculosis still poses a huge health threat in developing countries like India despite advances in the detection and management of the disease.

The common presenting symptoms were fever, headache and altered sensorium. This was similar to other studies with acute bacterial meningitis.[2,10,11] In the present study, nine did not have fever (five had tuberculous aetiology, two had cryptococcal meningitis, cerebral toxoplasmosis and *Aspergillus* meningitis in one each). All patients with acute pyogenic and viral meningoencephalitis presented with fever. Only 18 out of 50 patients (36%) had all the three symptoms – fever, headache and neck stiffness; a similar observation (41%) was noted in a prospective cohort study of community-acquired bacterial meningitis in adults in the Netherlands.[11] Neck stiffness was absent in 28 patients (56%), which indicates that absence of fever or neck stiffness does not exclude the possibility of infectious meningoencephalitis.

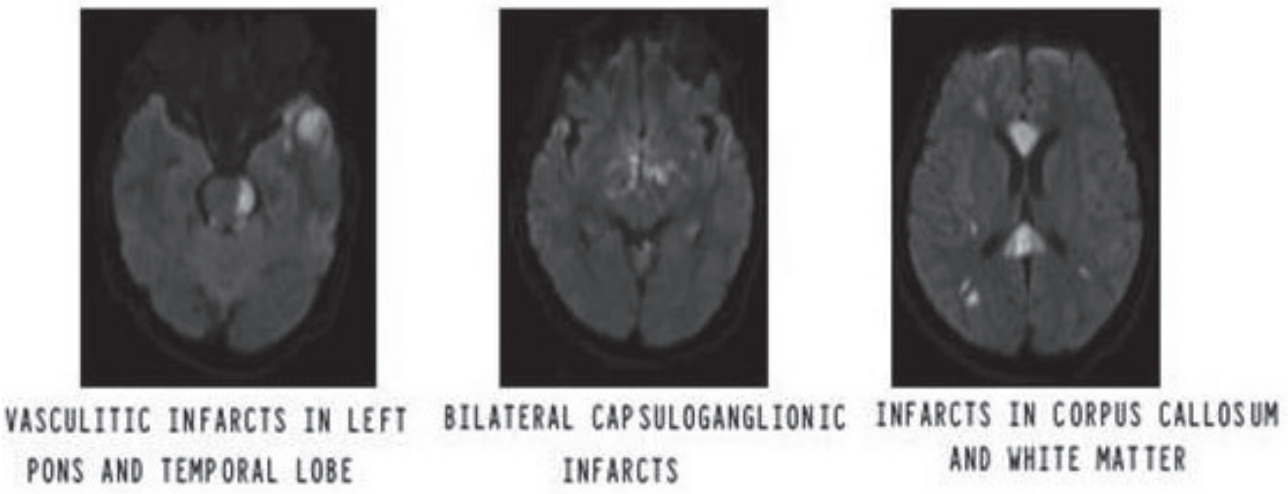
Diagnosis of pyogenic meningitis was made based on typical CSF findings (Supplementary Table 1) that showed high protein, low sugar and very high neutrophil count in the CSF (>1,000 cells/mm^3 in most of the patients). In five out of these eight patients with acute pyogenic meningitis, organisms could be isolated either in the blood or CSF. For the remaining three, the cultures were sterile possibly due to prior antibiotic exposure. In a retrospective study of 305 patients with acute bacterial meningitis, 53 (17.4%) had

Outcome	TM	APM	VME	CM	CT	AM	Total
Good (n = 47)	29	6	8	1	2	1	47
Poor (n = 3)	0	2	0	1	0	0	3
Total	29	8	8	2	2	1	50

AM: *Aspergillus* meningitis; APM: acute pyogenic meningitis; CM: cryptococcal meningitis; CT: cerebral toxoplasmosis; TM: tuberculous meningitis; VME: viral meningoencephalitis

Table 5 Clinical outcome in various aetiologies of meningoencephalitis

Figure 1 Fulminant pneumococcal meningitis with extensive vasculitic infarcts in bilateral cerebral hemisphere and brainstem



received antibiotics before admission and among these, death occurred only in one. The death rate was high (12%) in the remaining 252 patients who had not received antibiotics before admission. This study indicates the importance of the early institution of antibiotic therapy.[12,13]

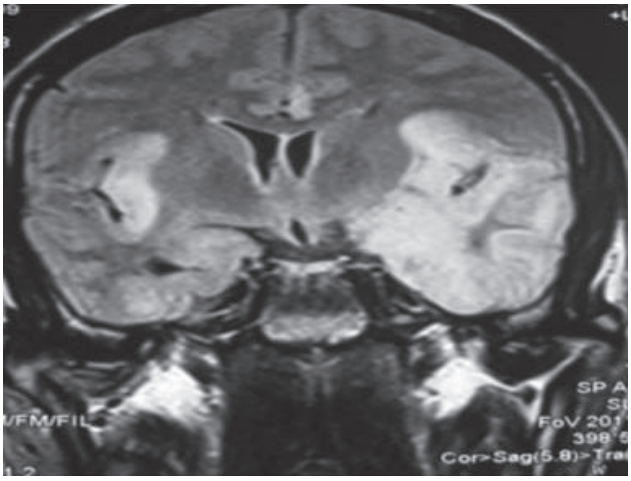
Surprisingly, four out of eight patients (50%) with acute pyogenic meningitis had a history of CSF rhinorrhoea before the onset of illness. Of these, three had a head injury in the past. MRI brain in one patient revealed a defect in the anterior cranial fossa that was the cause of CSF leak. One of them had recurrent episodes of pyogenic meningitis due to persistent CSF leak through the nose. After recovery from meningitis, he underwent a neurosurgical procedure for the correction of bony defects. This highlights the importance of elicitation of history for CSF rhinorrhoea in subjects with recurrent meningitis so that the underlying cause can be corrected. Six out of eight acute meningoencephalitis patients recovered completely without residual deficits; however, two had fulminant pneumococcal meningitis, of which one succumbed to the illness although treatment was initiated appropriately, the other survived with severe neurological disability, i.e. bilateral conjugate gaze paralysis of eyeballs and quadriparesis due to multiple infarcts (Figure 1). These outcomes highlight the fact that despite early diagnosis and appropriate treatment some patients with acute pyogenic meningitis may have a fulminant course with a severe neurological disability that can impair the quality of life.

Eight patients had acute viral meningoencephalitis, diagnosed based on CSF analysis and brain imaging features. A viral aetiology was considered when CSF had slightly elevated protein, low-to-normal blood sugar and moderately elevated lymphocyte count. Bacterial cultures, Gram stain, AFB stain and India ink stain were negative in these patients. Out of these eight patients, microbiological confirmation (positive HSV PCR in CSF) was possible in only two; both had temporal cortex hyperintense signals in MRI brain (Figure 2). All recovered completely with acyclovir and were asymptomatic during follow up. In many cases of presumed viral encephalitis (32–75%), the responsible organism could not be pin-pointed despite detailed diagnostic testing. In the California Encephalitis Project, 334 patients with encephalitis were studied from 1998 to 2000; aetiology could not be identified in 208 (62%), despite extensive testing and evaluation.[14]

Although commonly tuberculous and cryptococcal meningitis presents with subacute courses, four patients with tuberculous meningitis and one with cryptococcal meningitis presented with acute onset illness. Two patients presented with central nervous system toxoplasmosis and these patients were found to be HIV positive; they presented with hemiparesis; CSF serology for toxoplasma immunoglobulin G was positive; both the patients recovered well with treatment.

In the present study 15 had basal meningeal exudates on brain imaging. Hydrocephalus was present in five; of which two had significantly larger hydrocephalus, which progressively increased in size and both underwent ventriculoperitoneal shunt. In two large community-based series, hydrocephalus was seen in approximately 75% of

Figure 2 Herpes simplex encephalitis – T2-weighted MRI shows left temporal and right insular hyperintensity



patients, basilar meningeal enhancement in 38%, cerebral infarcts in 15–30% and tuberculomas in 5–10%.[15,16] With early appropriate antituberculous therapy and steroids, all patients with tuberculous meningitis in the present study recovered well without neurological deficits.

The present study had several limitations: PCR studies in all CSF culture-negative samples could not be performed and the study was conducted in a single centre with a limited number of patients. Further larger studies on meningoencephalitis would throw more light on the clinical profile and outcome.

In conclusion, the present study underscores the fact that the absence of fever or neck stiffness does not exclude the possibility of infectious meningoencephalitis. The focus in all cases should be on early diagnosis and appropriate treatment, though in some patients the outcome may not be uniformly good.

Online Supplementary Material

Supplementary Tables 1–4 are available with the online version of this paper, which can be accessed at https://www.rcpe.ac.uk/journal.

References

1 Ginsberg L, Kidd D. Chronic and recurrent meningitis. *Pract Neurol* 2008; 8: 348–61.
2 Aronin SI, Peduzzi P, Quagliarello VJ. Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. *Ann Intern Med* 1998; 129: 862–9.
3 Mace SE. Acute bacterial meningitis. *Emerg Med Clin North Am* 2008; 26: 281–317.
4 Roos KL, van de Beek D. Bacterial meningitis. *Handb Clin Neurol* 2010; 96: 51–63.
5 Bloch KC, Glaser C. Diagnostic approaches for patients with suspected encephalitis. *Curr Infect Dis Rep* 2007; 9: 315–22.
6 Rich AR, McCordick HA. The pathogenesis of tuberculous meningitis. *Bull John Hopkins Hospital* 1933; 52: 5–37.
7 Fitch MT, Abrahamian FM, Moran GJ et al. Emergency department management of meningitis and encephalitis. *Infect Dis Clin North Am* 2008; 22: 33–52.
8 Khajeh A, Sharifi-Mood B, Soleimani GR. Pediatric meningoencephalitis; a research on patients hospitalized in Zahedan, Southeastern Iran. *Int J Infect* 2015; 2: e23835.
9 Mahoney FI, Barthel DW. Functional evaluation: the Barthel index. *Md State Med J* 1965; 14: 61–5.
10 Van de Beek D, de Gans J, Spanjaard L et al. Clinical features and prognostic factors in adults with bacterial meningitis. *N Engl J Med* 2004; 351: 1849.
11 Bijlsma MW, Brouwer MC, Kasanmoentalib ES et al. Community-acquired bacterial meningitis in adults in the Netherlands, 2006–14: a prospective cohort study. *Lancet* Infect Dis 2016; 16: 39.
12 The Research Committee of the British Society for the Study of Infection. Bacterial meningitis: causes for concern. *J Infect* 1995; 30: 89–94.
13 Begg N, Cartwright KAV, Cohen J et al. Consensus statement on diagnosis, investigation, treatment, and prevention of acute bacterial meningitis in immunocompetent adults. *J Infect* 1999; 39: 1–15.
14 Glaser CS, Gilliam S, Schnurr D et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998–2000. *Clin Infect Dis* 2003; 36: 731–42.
15 Bhargava S, Gupta AK, Tandon PN. Tuberculous meningitis–a CT study. *Br J Radiol* 1982; 55: 189.
16 Ozates M, Kemaloglu S, Gurkan F et al. CT of the brain in tuberculous meningitis. A review of 289 patients. *Acta Radiol* 2000; 41: 13.

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Original Research Article

Evaluation of iron status in patients with end stage renal disease

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ABSTRACT

Background: Anaemia is almost universal in haemodialysis patients. Here, we evaluated the utility and clinical implications of the commonly employed tests in assessing iron status in haemodialysis patients.
Methods: In this prospective observational study, we enrolled 100 prevalent haemodialysis patients, and measured transferrin saturation (TSAT) and serum ferritin at enrolment. Patients were categorized to have normal, deficient, overload, or indeterminate status of iron based on KDOQI guidelines. To study anaemia trend, haemoglobin (Hb) level at time of enrolment, as well as levels 3-months before and 3-months after enrolment, were collected. Patient survival outcomes were also obtained till October 2016.
Results: Using serum ferritin and TSAT, iron status was determinable in 48% of the patients ie 20%-normal iron status, 25% - iron deficiency, 3% - iron overload. Fifty-two percent of the patients' iron status was indeterminate by current parameters. In spite of being on standard-of-care treatment in our centre, we observed that mean-Hb level of patients in the indeterminate group showed insignificant increases compared to normal- and deficient-groups. Mean delta-Hb levels over 6-months were 0.80±1.54 g/dl in the indeterminate group, compared to 1.87±1.95 g/dl in the deficient group (P=0.03). In our cohort, 57% had died and 5 lost follow-ups during the study (P=0.30 between groups).
Conclusions: Serum ferritin and TSAT accurately categorized the iron status of 48% haemodialysis patients in our study. Divergent serum ferritin and TSAT values, or an iron indeterminate state was prevalent in 52% of our urban dialysis cohort. The indeterminate group had inferior increases in Hb over time with current treatment, showing clinical relevance of this finding. Our data suggests the need for more sensitive indices to accurately assess iron status, and improve anaemia management.

Keywords: ESRD patients, Iron status, Serum ferritin, TSAT

INTRODUCTION

Anaemia is frequently found in chronic kidney disease (CKD) patients and is an almost universal finding in patients with end stage renal disease (ESRD).[1] Additionally, anaemia is associated with increased cardiovascular morbidity and mortality in this patient population.[2]

The pathophysiology of anaemia in CKD is multifactorial.[3] The proximate cause of the anaemia is relative deficiency of erythropoietin, a glycoprotein produced by renal tubular cells. Other factors which may cause or contribute to anaemia includes iron deficiency, vitamin B12 and/or folic acid deficiency, decreased erythrocyte survival, severe hyperparathyroidism, aluminium toxicity and hypothyroidism. The use of erythrocyte stimulating agents (ESA) such as recombinant human erythropoietin (EPO) began two decades ago and has revolutionized the treatment of anaemia in ESRD. This has decreased the need for blood transfusions and consequently the incidence of associated complications.[4]

Despite the widespread use of EPO, over 50% of the patients do not reach target haemoglobin levels.[1,5] Resistance to erythropoietin most commonly is due to iron deficiency but chronic inflammation, infection, and/or hyperparathyroidism also contribute.[6,7] Iron deficiency in ESRD is primarily due to impaired dietary intake and impaired gastrointestinal absorption but blood loss during haemodialysis and increased iron demands from ESA use also contribute.[8] Management of iron deficit is crucial in haemodialysis patients. Adequate treatment of iron deficiency is vital to attain target haemoglobin concentration and avoid excessively high EPO doses, which has been associated with increased risk of malignancy.[9] Furthermore, IV iron and blood transfusions both increase the risk of developing hospital acquired infections.[10,11] Therefore effective treatment of anaemia in haemodialysis patients requires continuous assessment of iron status.

Serum ferritin and transferrin saturation (TSAT) are the commonly used tests for the assessment of iron status in ESRD. However, measurement of these parameters in ESRD patients may be altered by inflammation, infection, or nutritional status.[12] Therefore, we conducted this study to evaluate the utility of the common tests, serum ferritin and TSAT, in assessing iron status in haemodialysis patients. In order to examine the clinical utility of iron status as defined by the current guidelines, we evaluated anaemia in this cohort, as well as patient outcomes.

METHODS

This study was conducted from August 2011 to March 2012 in a large tertiary care teaching hospital in South India. The study was conducted among the ESRD patients undergoing haemodialysis. All ESRD patients on haemodialysis for at least 3 months were enrolled in the study after the application of the exclusion criteria and after obtaining informed consent. The study was done after obtaining institutional ethical committee clearance. The exclusion criteria were: age less than 18 years old, evidence of acute or chronic infection, trauma within the previous 4 weeks, recent bleeding episodes, blood transfusion within the last one month, malignancy, post-transplant status, liver disease, haemoglobinopathies and other haematological diseases. The diagnostic biochemical tests used to assess the iron status were TSAT and serum ferritin.

Patients were categorized to have normal, deficient, overload, or indeterminate status of iron based on serum ferritin and TSAT values. Based on NKF-DOQI guidelines, iron deficiency is taken as ferritin level less than 200mg/dl and TSAT less than 20%, iron overload as ferritin level more than 500 mg/dl and TSAT more than 50%. The values in between the above ranges (ferritin between 200 and 500 mg/dl; TSAT between 20 and 50 %) are considered as normal iron status, whereas values not conforming to these categories are said to be indeterminate. The combined utility of the above tests in assessing iron status was determined by dividing the number of patients in whom iron status was detected (normal, deficient or overload) by total number of patients and expressed in percentage. The haemoglobin level of all the enrolled patients at time of enrolment was collected. To determine haemoglobin trend within each group, we collected haemoglobin levels 3-months before and 3-months after enrolment from chart review. Patient outcomes (death, alive and lost follow up) were obtained by chart review and telephone-follow up till October 2016.

Statistical analysis

The data were analysed using Statistical Package for Social Science (SPSS) Version 19.0, Graphpad Prism-6 and Microsoft Excel 2010. Non-parametric characteristics between any two groups were compared using Mann-Whitney test, and proportions using Chi-square test. For comparing more than two mean values we have used one-way analysis of variance. P value less than 0.05 was considered as statistically significant.

RESULTS

Demographics of study population

A total of 100 prevalent ESRD patients on haemodialysis were studied. The key demographics of the study cohort are summarized in Table 1. Patients ranged from 18 to 80 years. The mean age of the patients was 48.5 years. Among 100 patients, 63 were male and 37 were female.

Description by iron status

We classified these patients based on their iron status as per the NKF-KDOQI (see methods). Using serum ferritin and TSAT, iron status could be determined in 48% of the patients; 20% had normal iron status, 25% had iron deficiency, 3% had iron overload. The remaining 52% of the patient's iron status was indeterminate (Table 1 and 2). In univariate analysis, patients who were iron indeterminate were nearly indistinguishable from other groups by clinical and epidemiologic variables (as shown

in Table 1). However interestingly, iron indeterminate and overload groups had significantly greater dialysis vintage than the normal/iron-deficient groups.

Table 1: Baseline demographic characteristics.

	Total n=100	Diagnosed	n=48		Indeterminate n=52	
Groups		**Normal-1 n=20**	**Deficient-2 n=25**	**Overload-3 n=3**	**Indeterminate-4 n=52**	**P Value**
	Mean (SD)	**Mean(SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
Age (years)	48.5 (13.91)	43.85 (11.75)	48.56 (13.18)	48.66 (23.18)	50.25 (14.48)	0.38
Sex						
Male (%)	63 (63)	12 (60)	17 (68)	1 (33.3)	33 (63.5)	0.68
Female (%)	37 (37)	8 (40)	8 (32)	2 (66.7)	19 (36.5)	
Vintage (duration in months)	21 (18.76)	13.65 (11.96)	15.88 (13.09)	48.00 (41.56)	24.73 (19.75)	0.003
Diabetes mellitus (%)	30 (30)	5 (25)	9 (36)	0 (0)	16 (30.8)	0.57
Systemic Hypertension (%)	84 (84)	16 (80)	24 (96)	2 (66.7)	42 (80.8)	0.26
Cardiac disease (%)	12 (12)	2 (10)	5 (20)	0 (0)	5 (9.6)	0.51
Iron supplements (route and duration)						0.24
Oral >6months	27 (27)	2 (10)	6 (24)	2 (66.7)	17 (32.7)	
Oral < 6months	13 (13)	3 (15)	4 (16)	0 (0)	6 (11.5)	
IV > 6months	28 (28)	6 (30)	4 (16)	0 (0)	18 (34.6)	
IV<6months	16 (16)	6 (30)	5 (20)	0 (0)	5 (9.6)	
No iron supplements	16 (16)	3 (15)	6 (24)	1 (33.3)	6 (11.5)	
Haemoglobin (g/dL)	7.95 (1.74)	8.14 (1.76)	7.23 (1.60)	8.26 (2.39)	8.21 (1.71)	0.12
Ferritin (ng/mL)	423.12 (388.61)	346.40 (86.87)	114.2 (48.45)	1446.00 (549.06)	542.13 (390.31)	<0.001
TSAT (expressed in %)	23.94 (11.58)	27.68 (5.57)	14.69 (4.15)	61.27 (8.94)	24.80 (10.37)	<0.001
Iron (micro g/dL)	59.62 (25.91)	66.45 (20.86)	43.32 (12.83)	106.00 (72.95)	62.15 (23.95)	<0.001
TIBC (micro g/dL)	260.97 (63.31)	240.55 (57.80)	300.20 (71.09)	164.33 (89.01)	255.53 (48.46)	<0.001
Died (%)	57 (57)	9 (45)	14 (56)	3 (100)	31 (59.6)	0.30

TSAT: Transferrin saturation, TIBC: Total iron binding capacity.

Table 2: Iron status of patients.

Iron status	Number of patients	Percentage (%)	TSAT (expressed in %)		Ferritin (ng/mL)	
			Mean	**SD**	**Mean**	**SD**
Diagnosed	48	48	23.02	12.81	294.18	346.62
Normal	20	20	27.68	5.57	346.4	86.87
Deficient	25	25	14.69	4.15	114.2	48.45
Overload	3	3	61.27	8.94	1446	549.06
Indeterminate status	52	52	24.80	10.37	542.13	390.31
High serum ferritin-normal TSAT	22	22	29.54	8.28	805.13	413.67
High serum ferritin-low TSAT	4	4	18.49	2.24	815	195.30
High TSAT- normal ferritin	1	1	67.95		496	
High TSAT-low ferritin	0	0				
Low ferritin-normal TSAT	11	11	25.49	4.35	154.54	40.18
Low TSAT-normal ferritin	14	14	15.53	2.98	358.71	87.84

TSAT: Transferrin saturation.

Outcomes by iron status

Anaemia outcomes: To examine the haemoglobin responses and anaemia, trends of patients according to iron status, we tabulated haemoglobin levels of patients in each group by iron status at enrolment at different time points before- and after- study enrolment (Figure 1 and Figure 2).

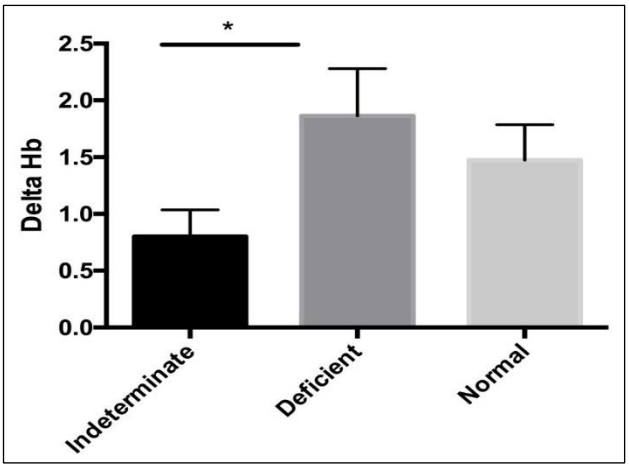


Figure 1: Compares Delta-haemoglobin in each study group, from 3-months before time of enrolment in study to 3-months after. [Columns represent means and error bars represent SEM. * = ANOVA (with post-test comparison) P< 0.05].

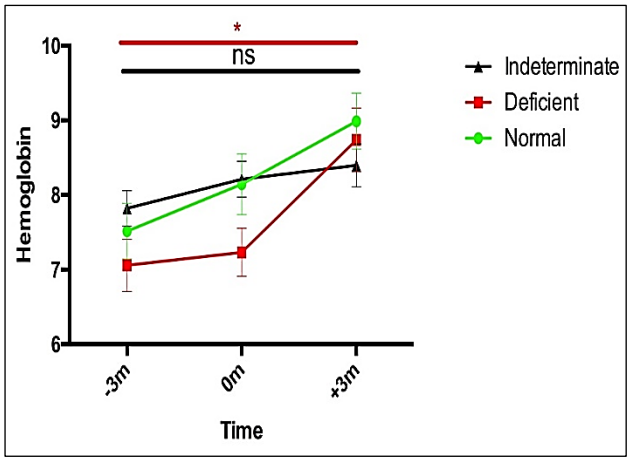


Figure 2: Trendlines of mean haemoglobin levels in each group by iron status. Indeterminate group had significantly inferior increases of haemoglobin over 6-months of follow-up. [Line represents mean, and error bars SD at each time point. * = Mann-whitney U P<0.05].

Table 3: Haemoglobin trend.

Groups	Total	Normal-1	Deficient-2	Overload-3	Indeterminate-4
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Haemoglobin 3months before (g/dL)	n=94 7.56(1.64)	n=19 7.51(1.61)	n=23 7.05 (1.67)	n=3 7.46 (1.15)	n=49 7.82 (1.66)
Haemoglobin 0 month (g/dL)	n=100 7.95(1.74)	n=20 8.14(1.76)	n=25 7.23 (1.60)	n=3 8.26 (2.39)	n=52 8.21 (1.71)
Haemoglobin 3 months after (g/dL)	n=90 8.64 (1.91)	n=19 8.98(1.63)	n=23 8.73 (2.00)	n=3 9.56 (3.13)	n=45 8.39 (1.92)

Table 3 summarizes haemoglobin levels and patients (n) in each group at each of the different time points studied. As the overload group consisted of 3 patients only, we excluded these from subsequent analysis. We interestingly observed that the mean haemoglobin level of patients in the Normal and Deficient groups showed significant increases over a 6-month time period, while those in the indeterminate group had insignificant increases in haemoglobin levels in this period (Figure 1). Mean delta haemoglobin levels over 6 months (3-months before enrolment to 3-months after enrolment) were 0.80±1.54 g/dl in the indeterminate group, compared to 1.87±1.95 g/dl in the deficient group (Mann Whitney P=0.03; Figure 2).

Patient outcomes

Overall during the follow up period, 29 patients continued on dialysis, 5 were at outside dialysis units, 8 patients received kidney transplants (4 of whom expired after transplantation). A total of 57 patients in our cohort had died during the follow up period (till October 2016). Five patients were lost to follow up in this time. There were no significant differences detectable in number of deaths between any of the groups by iron status in the duration of our follow-up, although all 3 patients in the overload group had expired (Table 1 and Figure 3).

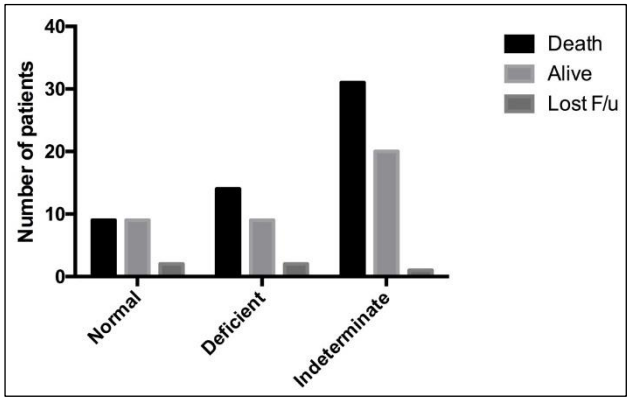


Figure 3: Patient outcomes in the study cohort till October 2016. No significant differences were observed in the proportion of patients that died in each group in this follow-up period (Chi square P=ns).

DISCUSSION

Anaemia contributes significantly to the morbidity and mortality in ESRD. ESA and iron supplementation are the most important factors in treatment of anaemia in ESRD patients.[13] Iron deficiency has been identified as a major factor for anaemia in CKD patients, with a 43-90% prevalence in different series.[6,14-16] Hence the periodical monitoring of iron status is crucial in ESRD.

There are two forms of iron deficiency, absolute iron deficiency and functional iron deficiency. Reduction of reticuloendothelial system iron in the bone marrow is referred to as absolute iron deficiency. Absolute iron deficiency may occur from impaired intestinal absorption, blood loss through the dialysis circuit, vascular access surgeries, repeated phlebotomies and increased iron utilization with erythropoietin therapy. In clinical practice, absolute iron deficiency is determined by serum ferritin less than 200ng/ml and TSAT less than 20%.[17] In the non-ESRD population, "functional" iron deficiency is ascribed when iron reserves in the bone marrow are adequate, but iron mobilization is impaired as in states of chronic inflammation.[18]

The current study assesses the utility of combining serum ferritin and TSAT to assess iron status among an Indian haemodialysis population. The evaluation of iron status is straightforward when both serum ferritin and TSAT are either high or low. But when these parameters are divergent, for instance, if the TSAT is low and serum ferritin is high, the accurate interpretation of iron status is difficult. This entity was common in our study where the iron status of only 48% of the patients were clearly determined (20% had normal iron status, 25% had iron deficiency and 3% had iron overload), and was indeterminate in the remaining 52% of patients. Interestingly in our study, the only epidemiologic factor associated significantly with indeterminate status was dialysis vintage. Why patients with indeterminate or overload status appeared to have longer durations of dialysis, is unclear but requires further exploration. Our data suggest that in a significant proportion of haemodialysis patients in a typical urban dialysis unit in India, the conventionally applied parameters were insufficient to accurately categorize iron status. Similar data has emerged from other groups.[19] Further, our data crucially demonstrates that patients with indeterminate status by conventional assessment have significantly inferior increases in haemoglobin levels with current therapy, suggesting the need for development of more specific diagnostic strategies to ascertain iron status in this group, to accurately manage iron status and anaemia.

Most studies have revealed that serum ferritin is very well related with iron stores in the bone marrow is the best surrogate of iron status in healthy individuals.[20-22] However, ferritin is an acute phase reactant increasing in states of chronic inflammation (such as dialysis), malignancy, or systemic disease, and ferritin levels alone may not reflect iron stores available for haematopoiesis. Thirty-six patients had low ferritin (<200) in our study. Only 25 of them had iron deficiency (combined with low-TSAT), whereas ~31% of them were in indeterminate group. Further, 29 patients had high serum ferritin. Only 3 of these had iron overload (combining TSAT), while 4/29 had low TSAT (<20%). Hence, decision to treat these patients with iron supplements based on these parameters would likely be imprecise. Similarly, a transferrin saturation of less than 20% in patients on haemodialysis is considered to be suggestive of iron deficiency whereas other studies have suggested that a transferrin saturation < 20% versus >20% alone may be inaccurate for assessing iron deficiency.[23-28] While most patients with TSAT less than 20% are iron deficient, few patients don't respond to an increase in iron supplements with either increase in haematocrit or maintenance of haematocrit with a decreased ESA requirement. On the other hand, numerous patients with a TSAT greater than 20% have functional iron deficiency respond to increased iron doses with elevations in the haematocrit, or maintenance of their haematocrit at decreased erythropoietin doses.[25-28] In our study, 43 patients had low TSAT (<20%); only 25 of them had iron deficiency (combined with ferritin). We found that 57 patients had TSAT values more than 20%, out of which 11 had low ferritin (<200). TSAT values in these 11 patients may be falsely high because of low total iron binding capacity (TIBC) during inflammation and infection.

The difficulties in the interpretation of iron status using TSAT and serum ferritin in ESRD patients and the consequent hematologic implications we show, warrant the application of other erythrocyte and reticulocyte based indices, such as the percentage of hypochromic red blood cells (Hypo) and the reticulocyte haemoglobin content (CHr). CHr requires a special analyser and is recommended as an alternative iron status indicator as well as an initial measure of the response to iron treatment in haemodialysis population.[29-31] Fishbane et al evaluated the reticulocyte haemoglobin content among 32 haemodialysis population and showed it to have a sensitivity of 100% and specificity of 80%.[29] Mittman et al evaluated reticulocyte haemoglobin content among 364 haemodialysis population receiving EPO and proved it to be an indicator of functional deficiency of iron.[30] Based on these and other data, NKF DOQI recommends a target of CHr greater than 29pg/cell.[32-35] The Hypo is an indicator of the percentage of red blood cells that contain low haemoglobin. Thus, it indicates only the functional iron availability and does not reflect storage iron. A Hypo level of greater than 10% indicates definitive functional iron deficiency. European Best Practice Guidelines stated that Hypo >10% is the most accurate marker for ID.[36] However, there are other studies which suggested lower threshold values for Hypo.[32,37-40] Both these tests are not widely available in India.

Here, we report that the combination of serum ferritin and TSAT accurately categorized the iron status in only 48%

of ESRD patients in our study. When serum ferritin and TSAT values diverged, they become unreliable for accurate interpretation of iron status, signaling an iron indeterminate state that was prevalent in 52% of our urban dialysis cohort. As we show here, this group of iron-indeterminate patients has inferior increases in haemoglobin over time, showing clinical relevance of this finding. Our data suggests that more sensitive indices such as Hypo and/or CHr may need to be employed (along with conventional tests) to accurately assess iron status, and improve management tools. This could lead to more accurate dosing of iron and ESAs, decrease overtreatment of anaemia, and possibly translate to cost savings from medication usage. Future studies on iron markers including both traditional and newer indices are required to improve iron management in ESRD population, especially in Indian population, where iron deficiency is common and similar studies are lacking.

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REFERENCES

1. KDOQI. Clinical Practice Guidance and Clinical Practice Recommendations for anaemia in Chronic Kidney Disease: 2007 Update of Haemoglobin target. Am J Kidney Dis. 2007;50:471-530.
2. Parfrey PS, Lauve M, Latremouille-Viau D, Lefebvre P. Erythropoietin therapy and left ventricular mass index in CKD and ESRD patients: a meta-analysis. Clin J Am Soc Nephrol. 2009;4:755-62.
3. Sonnweber T, Theurl I, Seifert M, Schroll A, Eder S, Mayer G, et al. Impact of Iron treatment on immune effector function and cellular iron status of circulating monocytes in dialysis patients. Nephrol Dial Transplant. 2011;26:977-87.
4. Feldman HI, Santanna J, Guo W, Furst H, Franklin E, Joffe M, et al. Iron administration and clinical outcomes in haemodialysis patients. J Am Soc Nephrol. 2002;13:734-44.
5. Collins AJ, Li S, Ebben J, Ma JZ, Manning W. Hematocrit levels and associated Medicare expenditures. Am J Kidney Dis. 2000;36:282-93.
6. MacDougall IC, Hutton RD, Cavill I, Coles GA, Williams JD. Poor response to treatment of renal anaemia with erythropoietin corrected by iron given intravenously. BMJ. 1989;299:157-8.
7. Sakiewicz P, Paganini E. The use of iron in patients on dialysis- mistakes and misconceptions. J Nephrol. 1998;11:5-15.
8. Horl WH. Clinical aspects of iron use in the anaemia of kidney disease. J Am Soc Nephrol. 2007;18:382-93.
9. René É, Lazrak HH, Laurin LP, Elftouh N, Vallée M, Lafrance JP. Association of erythropoiesis stimulating agents and the incidence risk of cancer diagnosis among chronic dialysis patients: a nested case-control study. Nephrol Dial Transplant. 2017;32:1047-52.
10. Brewster UC, Perazella MA. Intravenous iron and the risk of infection in end-stage renal disease patients. Semin Dial. 2004;17:57-60.
11. Rohde JM, Dimcheff DE, Blumberg N, Saint S, Langa KM, Kuhn L, et al. Health care-associated infection after red blood cell transfusion: a systematic review and meta-analysis. JAMA. 2014;311:1317-26.
12. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol. 2006;1:S4-8.
13. Nissenson AR. Hyporesponsiveness to erythropoietin: overview, 1996. Perit Dial Int. 1996;16:417-20.
14. Eschabach JW, Abdulhadi MH, Browne JK, Delano BG, Downing MR, Egrie JC, et al. Recombinant human erythropoietin in anaemic patients with end stage renal disease. Results of a phase III multicenter clinical trial. Ann Intern Med. 1989;111:992-1000.
15. Aggarwal HK, Nand N, Singh S, Singh M, Hemant, Kaushik G. Comparison of oral versus intravenous iron therapy in predialysis patients of chronic renal failure receiving recombinant human erythropoietin. J Assoc Physicians India. 2003;51:170-4.
16. Gupta M, Kannan M, Gupta S, Saxena R. Contribution of iron deficiency to anemia in chronic renal failure. Indian J Pathol Microbiol. 2003;46:563-4.
17. Fernandez-Rodriguez AM, Guindeo-Casasus MC, Molero-Labarta T, Domínguez-Cabrera C, Hortal-Casc n L, Pérez-Borges P, et al. Diagnosis of iron deficiency in chronic renal failure. Am J Kidney Dis. 1999;34:508-13.
18. Coyne DW. Hepcidin: clinical utility as a diagnostic tool and therapeutic target. Kidney Int. 2011;80:240-4.
19. Rafi A, Karkar A, Abdelrahman M. Monitoring iron status in end stage renal disease patients on haemodialysis. Saudi J Kidney Dis Transplant. 2007;18:73-8.
20. Caville I. Disorders of iron metabolism. Diagnostic methods. Clin Haematol. 1982;11:259-73.
21. Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. Annu Rev Med. 1993;44:63-74.
22. Cooper MJ, Zlotkin SH. Day-to-day variation of transferrin receptor and ferritin in healthy men and women. Am J Clin Nutr. 1996;64:738-42.

23. Macdougall IC. Monitoring of iron status and iron supplementation in patients treated with erythropoietin. Curr Opin Nephrol Hypertens. 1994;3:620-5.
24. Neff M. Algorithm for the treatment of anaemia in ESRD. Network Forum Quality Assurance Committee; 1995.
25. Fishbane S, Kowalski EA, Imbriano LJ, Maesaka JK. The evaluation of iron status in haemodialysis patients. J Am Soc Nephrol. 1996;7:2654-7.
26. Macdougall IC, Tucker B, Thompson J, Tomson CR, Baker LR, Raine AE. A randomized controlled study of iron supplementation in patients treated with erythropoietin. Kidney Int. 1996;50:1694-9.
27. Silverberg DS, Laina A, Peer G, Kaplan E, Levi BA, Frank N, et al. Intravenous iron supplementation for the treatment of the anemia of moderate to severe chronic renal failure patients not receiving dialysis. Am J Kidney Dis. 1996;27:234-8.
28. Silverberg DS, Blum M, Peer G, Kaplan F, Laina A. Intravenous ferric saccharate as an iron supplement in dialysis patients. Nephron. 1996;72:413-7.
29. Fishbane S, Galgano C, Langley RC Jr, Cranfield W, Maesaka JK. Reticulocyte haemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int. 1997;52:217-22.
30. Mittman N, Sreedhara R, Mushnick R, Chattopadhyay J, Zelmanovic D, Vaseghi M, et al. Reticulocyte haemoglobin content predicts functional iron deficiency in hemodialysis patients receiving EPO. Am J Kidney Dis. 1997;30:912-3.
31. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. Crit Rev Clin Lab Sci. 2000;37:93-130.
32. Cullen P, Söffker J, Höpfl M, Bremer C, Schlaghecken R, Mehrens T, et al. Hypochromic red cells and reticulocyte haemoglobin content as markers of iron-deficient erythropoiesis in patients undergoing chronic haemodialysis. Nephrol Dial Transplant. 1999;14:659-65.
33. Fishbane S, Galgano C, Langley RC Jr, Canfield W, Maesaka JK. Reticulocyte hemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int. 1997;52:217-22.
34. Mittman N, Sreedhara R, Mushnick R, Chattopadhyay J, Zelmanovic D, Vaseghi M, et al. Reticulocyte hemoglobin content predicts functional iron deficiency in hemodialysis patients receiving EPO. Am J Kidney Dis. 1997;30:912-22.
35. KDOQI; National Kidney Foundation. Clinical Practice Guidelines and Clinical Practice Recommendations for Anaemia in Chronic Kidney Disease. Am J Kidney Dis. 2006;47:S11-145.
36. European best practice guidelines for the management of anaemia in patients with chronic renal failure. Nephrol Dial Transplant. 1999;14:S14-15.
37. Braun J, Lindner K, Schreiber M, Heidler A, Hörl WH. Percentage of hypochromic red cells as predictor of erythropoietic and iron response after i.v. iron supplementation in maintenance haemodialysis patients. Nephrol Dial Transplant. 1997;12:1173-81.
38. Jones CH, Richardson D, Ayers S, Newstead CG, Will EJ, Davison AM. Percentage hypochromic red cells and the response to intravenous iron therapy in anaemic haemodialysis patients. Nephrol Dial Transplant. 1998;13:2873-6.
39. Tessitore N, Solero P, Gammaro, Sabato A, Maschio G. Iron status and low dose intravenous Fe gluconate in hemodialysis patients on maintenance erythropoietin (Abstract). J Am Soc Nephrol. 1998;9:272.
40. Tessitore N, Solero GP, Lippi G, Bassi A, Faccini GB, Bedogna V, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. Nephrol Dial Transplant. 2001;16:1416-23.

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Association of N-terminal-Pro-B-type Natriuretic Peptide with Localization of Thrombus and Clinical Outcome in Acute Pulmonary Embolism

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ABSTRACT

Introduction: Pulmonary Embolism is a major health problem which is associated with significant mortality and morbidity. It is a common and lethal condition. This study was undertaken to find the association between NT-Pro-BNP levels and the localisation of thrombus in acute Pulmonary Embolism and to prognosticate the patients.

Material and methods: Prospective study conducted at a tertiary care hospital.Thirty patients with Pulmonary Embolism of various etiologies, confirmed with clinical features, laboratory investigations and imaging and fulfilling the inclusion and exclusion criteria were included. After getting informed consent, Blood samples for assessment of NT-Pro-BNP levels were collected within 24 hours of admission.

Results: In 19 patients, the thrombus was located centrally (in main pulmonary artery) whereas in 11 patients, the thrombus was located in the peripherally (in segmental and subsegmental pulmonary arteries). 22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died. Patients with central pulmonary embolus had higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries had relatively less NTPro-BNP levels. Patients with high NT-Pro-BNP levels had complicated in-hospital course/ mortality whereas, patients with relatively lower NT-Pro-BNP had uncomplicated in-hospital course.

Conclusion: This study shows that higher NT-Pro-BNP levels indicate higher probability of a more central location of thrombus in pulmonary embolism and also right ventricular overload. Also patients with higher NT-Pro-BNP levels had a complicated in hospital course.

Key words: Pulmonary Embolism, Pulmonary Arteries, Right Heart Failure, NT Pro-BNP

INTRODUCTION

Pulmonary Embolism (PE) is a major health problem which is associated with significant mortality and morbidity. It is a common and lethal condition. Pulmonary Thrombo Embolism (PTE) and deep vein thrombosis (DVT) collectively known as venous thromboembolism (VTE) encompass one disease entity. Among cardiovascular diseases, VTE along with stroke and myocardial infarction rank as three big cardiovascular killers. PTE implies partial or complete occlusion of pulmonary arterial circulation by the clot formed elsewhere usually in deep veins of the leg. Less than 5% of venous thrombosis occurs in other sites. The incidence of PE in non-hospitalised patients is 3.5 per 10,000 person years while the incidence rate in hospitalised in-patients can be above 500 per 10,000 person years.[1] In India, VTE is a common cause of mortality and morbidity in patients hospitalized for medical or surgical illnesses. It is often misdiagnosed and not intervened in time. Though the exact magnitude of problem is not known in India, the clinical relevance and incidence is not expected to be different from Western countries. Acute PE has high early mortality rates. Despite the advances in diagnosis and treatment, the early mortality rates have not changed significantly. Due to the embolus obstructing the pulmonary bed, PE can result in a life threatening condition, acute right ventricular (RV) failure.[2] Early diagnosis is of paramount importance since most patients die within the first hours of presentation. Timely management is usually highly effective in reversing RV failure. The diagnosis of acute PE is based on the assessment of clinical likelihood, ECG, CXR, echocardiography, laboratory investigations, mainly D-dimer and cardiac biomarkers and imaging techniques (CT pulmonary angiogram). Several cardiac biomarkers have emerged as indicator of right ventricular dysfunction and clinical outcome predictor in patients with acute PE. Brain type natiuretic peptide (BNP) is a marker of ventricular dysfunction. Following cardiac myocyte stretch, this compound is released into the circulation. It is synthesized as an inactive pro hormone (Pro-BNP) which is split into active hormone BNP and an inactive N-terminal fragment (NT-Pro-BNP).[3] This study was undertaken to find the association between NT-Pro-BNP levels and the localisation

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of thrombus in acute PE, to monitor and prognosticate the patients closely.

Primary objectives of the study were to study the association of N-terminal-Pro-B-Type Natriuretic Peptide with localisation of thrombus in Acute Pulmonary Embolism and to study the association between NT-Pro-BNP levels and clinical outcome in patients with Acute Pulmonary Embolism and secondary objectives were to study on the various risk factors in patients diagnosed with acute pulmonary thrombo embolism and to follow up the patients at the end of 1 month to know the clinical outcome.

MATERIAL AND METHODS

Prospective study was conducted at a tertiary care hospital, PSG Hospitals, Coimbatore. Thirty consecutive patients diagnosed to have pulmonary embolism were included in this study with the following criteria.

Inclusion criteria

All patients of age more than 18 years with clinical and radiological evidence of acute PE will be included in the study.

1) Clinical evidence

Patients presenting with dyspnoea, hemoptysis and chest pain with no acute ischemic changes in ECG and with normal Ejection fraction. ECG in acute pulmonary embolism will shows S1Q3T3 pattern.

2) Radiological evidence

CT pulmonary angiogram showing filling defects within the pulmonary vasculature.

Exclusion criteria

Patients with COPD, known case of Congestive cardiac failure and chronic kidney disease are excluded from this study.

After getting informed consent, Blood samples for assessment of NT-Pro-BNP levels were collected within 24 hours of admission. Risk factors for pulmonary thrombo embolism were noted such as prolonged bed rest, hypertension, malignancy etc. Thrombi located in main/ right/left pulmonary artery were classified as central while the others at segmental/sub-segmental levels were classified as peripheral. Echocardiographic findings such as ejection fraction, functions of right and left ventricle, pulmonary artery pressure were noted. Clinical course of the patient and clinical outcome were also noted. Clinical outcome of the patient at the end of one month was recorded by following up. Then descriptive and statistical analysis of location of thrombus and clinical outcome in relation with NT-Pro-BNP levels was done.

Risk factors	Frequency (n)	Percent (%)
Prolonged immobilization	13	43.3
Carcinoma	4	13.3
Unprovoked	13	43.3
Total	30	100.0

Table-1: Risk factors of the study participants

Clinical Outcome	Frequency (n)	Percent (%)
Normal	22	73.3
Morbid	5	16.7
Death	3	10.0
Total	30	100.0

Table-2: Clinical Outcome after 30 Days

STATISTICAL ANALYSIS

The data collected from the patients was tabulated using Microsoft excel. The data are reported as mean +/- SD depending on their distribution. Risk factors will be described in numbers and percentage. Any association will be analysed using chi-square test. Correlation will be analysed by Pearsman correlation. A p value of <0.05 using two tailed test was taken as being significant for all statistical tests. All data were analysed with statistical software package (SPSS version 24.0)

RESULTS

In our study, we included 30 patients diagnosed with acute pulmonary thromboembolism. The mean age of study population is 57.6 years. 11 patients belonged to the group of 51 to 60 years. Only 2 patients were younger adults and 2 patients were old adults. Among the study group 17 patients were male (56.7%) and 13 patients were female (43.3%).In our study population following were the risk factors noted. Thirteen patients had prolonged immobilisation following road traffic accident or following major orthopaedic surgeries. 4 patients had underlying malignancy such as Ca lung or Ca breast. 13 patients had unprovoked PTE.(Table 1) The thrombus was located unilaterally in 19 patients whereas 11 patients have bilateral thrombus. In 19 patients, the thrombus was located centrally (that is, in main pulmonary artery) whereas in 11 patients, the thrombus is located in the peripherally (segmental and sub segmental pulmonary arteries).22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died.(Table 2)

Patients with central pulmonary embolus have higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries have relatively less NT-Pro-BNP levels. And the difference between the two was statistically significant. (P= 0.02)(Table 3)

Patients with high NT-Pro-BNP levels had complicated in-hospital course/mortality whereas, patients with relatively

	Localisation	N	Mean	Std. Deviation	P value
PROBNP	Central	19	6196.27	4377.75	.02*
	Peripheral	11	1529.10	2636.40	

Table-3: Association of PROBNP with localization

	Clinical Outcome	N	Mean	Std. Deviation	P value
PROBNP	Normal	22	3814.15	3860.52	.005*
	Morbid	5	3009.16	4033.77	
	Death	3	11864.00	1417.83	

Table-4: Association of PROBNP with Clinical Outcome

lower NT-Pro-BNP had uncomplicated in-hospital course. (P = 0.005)(Table 4)

DISCUSSION

Acute PE shows wide variations in clinical presentation from asymptomatic course to a massive pulmonary embolism that may be fatal. When planning treatment for this condition, risk stratification is important. In this study 30 patients diagnosed with pulmonary embolism were included. The mean age of study population is 57.6 years.17 patients were male(56.7%) and 13 patients were female (43.3%). The thrombus was located unilaterally in 19 patients whereas 11 patients had bilateral thrombus. In 19 patients, the thrombus was located centrally (main pulmonary artery) whereas in 11 patients the thrombus was located in the peripheral pulmonary artery (segmental and subsegmental pulmonary arteries). 22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died. Patients with central pulmonary embolus have higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries have relatively less NTPro-BNP levels. And the difference between the two is statistically significant. (P= 0.02).

Patients with high NT-Pro-BNP levels had complicated in-hospital course/ mortality whereas, patients with relatively lower NT-Pro-BNP had uncomplicated in hospital course. (P = 0.005).From this study, it is evident that patients with higher NT-Pro-BNP levels have more central thrombus, have complicated in-hospital course and also cause morbidity and mortality. Whereas patients with comparatively lower NT-Pro-BNP levels have thrombus in segmental or subsegmental pulmonary arteries and they have an uncomplicated course.

The findings of our study were consistent with many other similar studies.

In a prospective study conducted by Akpinar et.al in turkey in Forty-nine patients concluded that higher pro bnp levels indicate higher probability of a more central location of thrombus in pulmonary embolism, resulting a more adverse clinical course and also leading to right ventricular overload..

In a prospective study done by Pruszcyk et.al in Poland in 79 patients found NT-Pro-BNP to be a prognostic marker in acute pulmonary embolism.[5]

An Egyptian prospective study by A.Selem et al conducted in 64 patients concluded elevated NT pro-BNP in PE was associated with worse short term prognosis in terms of higher morbidity and mortality.[6] Another study, Heleder Dores et al a retrospective study also had similar results as that of our study which concluded that elevated NT pro-BNP in PE identified worse short term prognosis and also had excellent power to predict 30 day all cause mortality.[7]

In a meta analysis conducted by Frederikus et.al, in the Netherlands, they found that higher concentrations of NT-Pro-BNP distinguish patients with pulmonary embolism at higher risk of complicated in-hospital course and death from those with low NT-Pro-BNP levels.[8] In another meta analysis conducted by Cavallazzi et.al in USA, they found that NT-Pro-BNP levels are significant predictors of all cause in hospital or short term mortality.[9] A meta analysis by Guillaume Coutance et al concluded while elevated BNP levels can help to identify high risk of death and adverse outcome, the high negative predictive value of normal BNP levels is certainly more useful.[10]

From our study it is evident that NT-Pro-BNP levels are significant predictor of all cause in- hospital mortality in patients with acute PE. The potential outcome of our study is that patients with normal levels of NT-Pro-BNP have a high negative predictive value for unfavourable outcome. Patients with normal NT-Pro-BNP levels have low risk of death as well as for hemodynamic collapse.This was consistant with a study conducted by Nils Kucher in 73 patients which predicted that lower the pro-BNP levels, lower would be the hospital stay.[11]

Our study have a few limitations. First, the sample size is limited. Further studies including larger number of patients are necessary to clarify this point. Secondly, NT-pro-BNP is a precursor of BNP. The conversion of BNP to NT-Pro-BNP may take several minutes after myocardial stretch which may lead to false negative results in patients who present early. Serial monitoring of NT-Pro-BNP (eg. At admission and 6 hours later) may overcome this limitation.

CONCLUSION

This study shows that higher NT-Pro-BNP levels indicate higher probability of a more central location of thrombus in pulmonary embolism and also leading to right ventricular overload.Also patients with higher NT-Pro-BNP levels had a complicated in-hospital course.Further studies with large number of patients were undertaken to determine the exact cut off levels of NT-Pro-BNP to help in treatment decisions.

Ethical approval

The study was approved by the Institutional Ethics Committee

REFERENCES

1. Heit JA, Melton LJ, Lahose CM. Incidence of venous thromboembolism in hospitalised patients vs community residents. Mayo clin Proc 76:1102-1110
2. Fremont B, Pacouret G, Jacobi D, Puglisi R, Charbonnier B, de Labriolle A.Prognostic value of the echocardiographic right/left ventricular end diastolic diameter ratio in patients with acute pulmonary

embolism: results from a monocenter registry of 1416 patients. Chest 2008;133;358-362.
3. Christian Hall, Essential biochemistry and physiology of (NT-pro) BNP, The European journal of Heart Failure 2004;6:257-260.
4. E. Akpinar, E. Sayin, E. Buyuk and M. Gulhan. Association of Levels of N-terminal-Pro-B-Type Natriuretic Peptide with Localisation of Thrombus in Acute Pulmonary Embolism. The Indian Journal of Chest Diseases & Allied Sciences 2012;Vol.54
5. Pruszczyk P, Kostrubiec M, Bochowicz A, Styczyñski G, Szulc M,Kurzyna M, Fijałkowska A, Kuch-Wocial A, Chlewicka I, Torbicki A:N-terminal pro-brain natriuretic peptide in patients with acute pulmonary embolism. Eur Respir J 2003, 22:649-653.
6. A. Selem,H.Radwan,Abdelaziz M.Gomaa Prognostic value of N-Terminal Pro-Brain Natriuretic Peptide in Acute Pulmonary Embolism.Open journal of endocrine and metabolic disease 2012;2:58-62.
7. H. Dores, C. Fonseca, S. Leal, I. Rosário, J. Abecasis, J. Monge, M. J. Correia, L. Bronze, A. Leitão, I. Arroja, A. Aleixo and A. Silva, "NT-pro BNP for Risk Stratification of Pulmonary Embolism," Portuguese Journal of Cardiology,2011;30:881-886
8. Frederikus Klok F, Mos I, Huisman M. Brain-type natriuretic peptide levels in the prediction of adverse outcome in patients with pulmonary embolism. Am J Respir Crit Care Med. 2008;178:425-30.
9. R. Cavallazzi, A. Nair, T. Vasu and P. E. Marik. Natriuretic Peptides in Acute Pulmonary Embolism. Intensive Care Med 2008;34:2147-2156
10. Guillaume Coutance, Olivier Le Page, Ted Lo and martial Hamon prognostic value of brain natriuretic peptide in acute pulmonary embolism. Critical Care 2008,12:R109.
11. Nils Kucher, Gert printzen, Tanja Doernhoefer,Stephan Windecker, Bernhard Meier, Otto Martin Hess. Low Pro-brain natriuretic Peptide Levels predict Benign Clinical Outcome in Acute Pulmonary Embolism. Circulation 2003;107:1576-1578.

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Original Research Article

Evaluation of iron status in patients with end stage renal disease

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ABSTRACT

Background: Anaemia is almost universal in haemodialysis patients. Here, we evaluated the utility and clinical implications of the commonly employed tests in assessing iron status in haemodialysis patients.
Methods: In this prospective observational study, we enrolled 100 prevalent haemodialysis patients, and measured transferrin saturation (TSAT) and serum ferritin at enrolment. Patients were categorized to have normal, deficient, overload, or indeterminate status of iron based on KDOQI guidelines. To study anaemia trend, haemoglobin (Hb) level at time of enrolment, as well as levels 3-months before and 3-months after enrolment, were collected. Patient survival outcomes were also obtained till October 2016.
Results: Using serum ferritin and TSAT, iron status was determinable in 48% of the patients ie 20%-normal iron status, 25% - iron deficiency, 3% - iron overload. Fifty-two percent of the patients' iron status was indeterminate by current parameters. In spite of being on standard-of-care treatment in our centre, we observed that mean-Hb level of patients in the indeterminate group showed insignificant increases compared to normal- and deficient-groups. Mean delta-Hb levels over 6-months were 0.80±1.54 g/dl in the indeterminate group, compared to 1.87±1.95 g/dl in the deficient group (P=0.03). In our cohort, 57% had died and 5 lost follow-ups during the study (P=0.30 between groups).
Conclusions: Serum ferritin and TSAT accurately categorized the iron status of 48% haemodialysis patients in our study. Divergent serum ferritin and TSAT values, or an iron indeterminate state was prevalent in 52% of our urban dialysis cohort. The indeterminate group had inferior increases in Hb over time with current treatment, showing clinical relevance of this finding. Our data suggests the need for more sensitive indices to accurately assess iron status, and improve anaemia management.

Keywords: ESRD patients, Iron status, Serum ferritin, TSAT

INTRODUCTION

Anaemia is frequently found in chronic kidney disease (CKD) patients and is an almost universal finding in patients with end stage renal disease (ESRD).[1] Additionally, anaemia is associated with increased cardiovascular morbidity and mortality in this patient population.[2]

The pathophysiology of anaemia in CKD is multifactorial.[3] The proximate cause of the anaemia is relative deficiency of erythropoietin, a glycoprotein produced by renal tubular cells. Other factors which may cause or contribute to anaemia includes iron deficiency, vitamin B12 and/or folic acid deficiency, decreased erythrocyte survival, severe hyperparathyroidism, aluminium toxicity and hypothyroidism. The use of erythrocyte stimulating agents (ESA) such as recombinant human erythropoietin (EPO) began two decades ago and has revolutionized the treatment of anaemia in ESRD. This has decreased the need for blood transfusions and consequently the incidence of associated complications.[4]

Despite the widespread use of EPO, over 50% of the patients do not reach target haemoglobin levels.[1,5] Resistance to erythropoietin most commonly is due to iron deficiency but chronic inflammation, infection, and/or hyperparathyroidism also contribute.[6,7] Iron deficiency in ESRD is primarily due to impaired dietary intake and impaired gastrointestinal absorption but blood loss during haemodialysis and increased iron demands from ESA use also contribute.[8] Management of iron deficit is crucial in haemodialysis patients. Adequate treatment of iron deficiency is vital to attain target haemoglobin concentration and avoid excessively high EPO doses, which has been associated with increased risk of malignancy.[9] Furthermore, IV iron and blood transfusions both increase the risk of developing hospital acquired infections.[10,11] Therefore effective treatment of anaemia in haemodialysis patients requires continuous assessment of iron status.

Serum ferritin and transferrin saturation (TSAT) are the commonly used tests for the assessment of iron status in ESRD. However, measurement of these parameters in ESRD patients may be altered by inflammation, infection, or nutritional status.[12] Therefore, we conducted this study to evaluate the utility of the common tests, serum ferritin and TSAT, in assessing iron status in haemodialysis patients. In order to examine the clinical utility of iron status as defined by the current guidelines, we evaluated anaemia in this cohort, as well as patient outcomes.

METHODS

This study was conducted from August 2011 to March 2012 in a large tertiary care teaching hospital in South India. The study was conducted among the ESRD patients undergoing haemodialysis. All ESRD patients on haemodialysis for at least 3 months were enrolled in the study after the application of the exclusion criteria and after obtaining informed consent. The study was done after obtaining institutional ethical committee clearance. The exclusion criteria were: age less than 18 years old, evidence of acute or chronic infection, trauma within the previous 4 weeks, recent bleeding episodes, blood transfusion within the last one month, malignancy, post-transplant status, liver disease, haemoglobinopathies and other haematological diseases. The diagnostic biochemical tests used to assess the iron status were TSAT and serum ferritin.

Patients were categorized to have normal, deficient, overload, or indeterminate status of iron based on serum ferritin and TSAT values. Based on NKF-DOQI guidelines, iron deficiency is taken as ferritin level less than 200mg/dl and TSAT less than 20%, iron overload as ferritin level more than 500 mg/dl and TSAT more than 50%. The values in between the above ranges (ferritin between 200 and 500 mg/dl; TSAT between 20 and 50 %) are considered as normal iron status, whereas values not conforming to these categories are said to be indeterminate. The combined utility of the above tests in assessing iron status was determined by dividing the number of patients in whom iron status was detected (normal, deficient or overload) by total number of patients and expressed in percentage. The haemoglobin level of all the enrolled patients at time of enrolment was collected. To determine haemoglobin trend within each group, we collected haemoglobin levels 3-months before and 3-months after enrolment from chart review. Patient outcomes (death, alive and lost follow up) were obtained by chart review and telephone-follow up till October 2016.

Statistical analysis

The data were analysed using Statistical Package for Social Science (SPSS) Version 19.0, Graphpad Prism-6 and Microsoft Excel 2010. Non-parametric characteristics between any two groups were compared using Mann-Whitney test, and proportions using Chi-square test. For comparing more than two mean values we have used one-way analysis of variance. P value less than 0.05 was considered as statistically significant.

RESULTS

Demographics of study population

A total of 100 prevalent ESRD patients on haemodialysis were studied. The key demographics of the study cohort are summarized in Table 1. Patients ranged from 18 to 80 years. The mean age of the patients was 48.5 years. Among 100 patients, 63 were male and 37 were female.

Description by iron status

We classified these patients based on their iron status as per the NKF-KDOQI (see methods). Using serum ferritin and TSAT, iron status could be determined in 48% of the patients; 20% had normal iron status, 25% had iron deficiency, 3% had iron overload. The remaining 52% of the patient's iron status was indeterminate (Table 1 and 2). In univariate analysis, patients who were iron indeterminate were nearly indistinguishable from other groups by clinical and epidemiologic variables (as shown

in Table 1). However interestingly, iron indeterminate and overload groups had significantly greater dialysis vintage than the normal/iron-deficient groups.

Table 1: Baseline demographic characteristics.

	Total n=100	Diagnosed	n=48		Indeterminate n=52	
Groups		**Normal-1 n=20**	**Deficient-2 n=25**	**Overload-3 n=3**	**Indeterminate-4 n=52**	**P Value**
	Mean (SD)	**Mean(SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
Age (years)	48.5 (13.91)	43.85 (11.75)	48.56 (13.18)	48.66 (23.18)	50.25 (14.48)	0.38
Sex						
Male (%)	63 (63)	12 (60)	17 (68)	1 (33.3)	33 (63.5)	0.68
Female (%)	37 (37)	8 (40)	8 (32)	2 (66.7)	19 (36.5)	
Vintage (duration in months)	21 (18.76)	13.65 (11.96)	15.88 (13.09)	48.00 (41.56)	24.73 (19.75)	0.003
Diabetes mellitus (%)	30 (30)	5 (25)	9 (36)	0 (0)	16 (30.8)	0.57
Systemic Hypertension (%)	84 (84)	16 (80)	24 (96)	2 (66.7)	42 (80.8)	0.26
Cardiac disease (%)	12 (12)	2 (10)	5 (20)	0 (0)	5 (9.6)	0.51
Iron supplements (route and duration)						0.24
Oral >6months	27 (27)	2 (10)	6 (24)	2 (66.7)	17 (32.7)	
Oral < 6months	13 (13)	3 (15)	4 (16)	0 (0)	6 (11.5)	
IV > 6months	28 (28)	6 (30)	4 (16)	0 (0)	18 (34.6)	
IV<6months	16 (16)	6 (30)	5 (20)	0 (0)	5 (9.6)	
No iron supplements	16 (16)	3 (15)	6 (24)	1 (33.3)	6 (11.5)	
Haemoglobin (g/dL)	7.95 (1.74)	8.14 (1.76)	7.23 (1.60)	8.26 (2.39)	8.21 (1.71)	0.12
Ferritin (ng/mL)	423.12 (388.61)	346.40 (86.87)	114.2 (48.45)	1446.00 (549.06)	542.13 (390.31)	<0.001
TSAT (expressed in %)	23.94 (11.58)	27.68 (5.57)	14.69 (4.15)	61.27 (8.94)	24.80 (10.37)	<0.001
Iron (micro g/dL)	59.62 (25.91)	66.45 (20.86)	43.32 (12.83)	106.00 (72.95)	62.15 (23.95)	<0.001
TIBC (micro g/dL)	260.97 (63.31)	240.55 (57.80)	300.20 (71.09)	164.33 (89.01)	255.53 (48.46)	<0.001
Died (%)	57 (57)	9 (45)	14 (56)	3 (100)	31 (59.6)	0.30

TSAT: Transferrin saturation, TIBC: Total iron binding capacity.

Table 2: Iron status of patients.

Iron status	Number of patients	Percentage (%)	TSAT (expressed in %)		Ferritin (ng/mL)	
			Mean	**SD**	**Mean**	**SD**
Diagnosed	48	48	23.02	12.81	294.18	346.62
Normal	20	20	27.68	5.57	346.4	86.87
Deficient	25	25	14.69	4.15	114.2	48.45
Overload	3	3	61.27	8.94	1446	549.06
Indeterminate status	52	52	24.80	10.37	542.13	390.31
High serum ferritin-normal TSAT	22	22	29.54	8.28	805.13	413.67
High serum ferritin-low TSAT	4	4	18.49	2.24	815	195.30
High TSAT- normal ferritin	1	1	67.95		496	
High TSAT-low ferritin	0	0				
Low ferritin-normal TSAT	11	11	25.49	4.35	154.54	40.18
Low TSAT-normal ferritin	14	14	15.53	2.98	358.71	87.84

TSAT: Transferrin saturation.

Outcomes by iron status

Anaemia outcomes: To examine the haemoglobin responses and anaemia, trends of patients according to iron status, we tabulated haemoglobin levels of patients in each group by iron status at enrolment at different time points before- and after- study enrolment (Figure 1 and Figure 2).

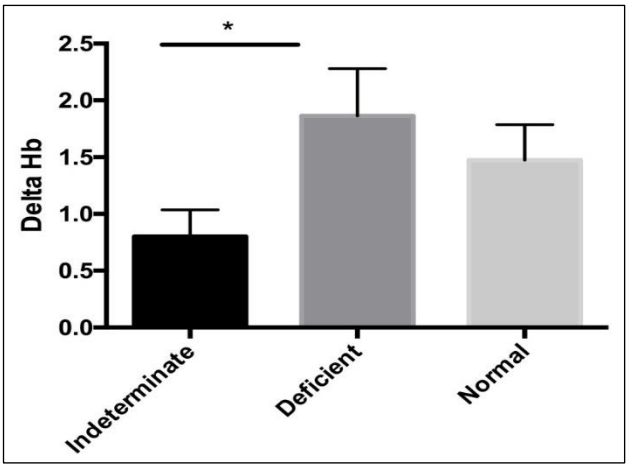


Figure 1: Compares Delta-haemoglobin in each study group, from 3-months before time of enrolment in study to 3-months after. [Columns represent means and error bars represent SEM. * = ANOVA (with post-test comparison) P< 0.05].

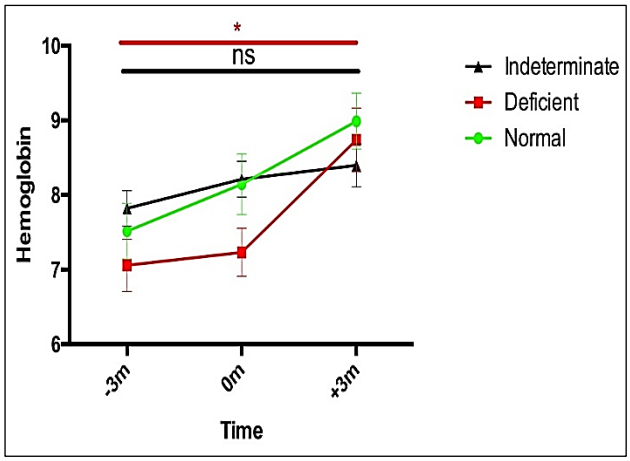


Figure 2: Trendlines of mean haemoglobin levels in each group by iron status. Indeterminate group had significantly inferior increases of haemoglobin over 6-months of follow-up. [Line represents mean, and error bars SD at each time point. * = Mann-whitney U P<0.05].

Table 3: Haemoglobin trend.

Groups	Total	Normal-1	Deficient-2	Overload-3	Indeterminate-4
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Haemoglobin 3months before (g/dL)	n=94 7.56(1.64)	n=19 7.51(1.61)	n=23 7.05 (1.67)	n=3 7.46 (1.15)	n=49 7.82 (1.66)
Haemoglobin 0 month (g/dL)	n=100 7.95(1.74)	n=20 8.14(1.76)	n=25 7.23 (1.60)	n=3 8.26 (2.39)	n=52 8.21 (1.71)
Haemoglobin 3 months after (g/dL)	n=90 8.64 (1.91)	n=19 8.98(1.63)	n=23 8.73 (2.00)	n=3 9.56 (3.13)	n=45 8.39 (1.92)

Table 3 summarizes haemoglobin levels and patients (n) in each group at each of the different time points studied. As the overload group consisted of 3 patients only, we excluded these from subsequent analysis. We interestingly observed that the mean haemoglobin level of patients in the Normal and Deficient groups showed significant increases over a 6-month time period, while those in the indeterminate group had insignificant increases in haemoglobin levels in this period (Figure 1). Mean delta haemoglobin levels over 6 months (3-months before enrolment to 3-months after enrolment) were 0.80±1.54 g/dl in the indeterminate group, compared to 1.87±1.95 g/dl in the deficient group (Mann Whitney P=0.03; Figure 2).

Patient outcomes

Overall during the follow up period, 29 patients continued on dialysis, 5 were at outside dialysis units, 8 patients received kidney transplants (4 of whom expired after transplantation). A total of 57 patients in our cohort had died during the follow up period (till October 2016). Five patients were lost to follow up in this time. There were no significant differences detectable in number of deaths between any of the groups by iron status in the duration of our follow-up, although all 3 patients in the overload group had expired (Table 1 and Figure 3).

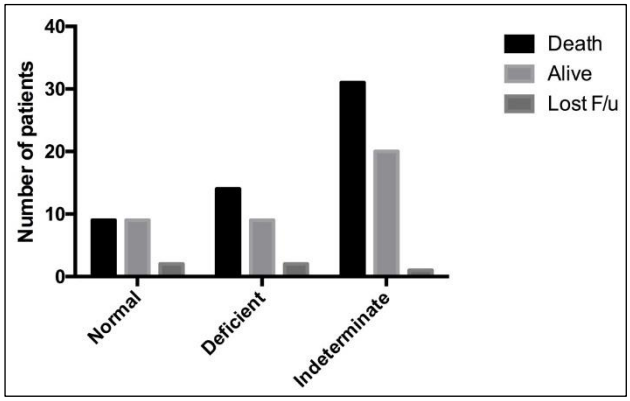


Figure 3: Patient outcomes in the study cohort till October 2016. No significant differences were observed in the proportion of patients that died in each group in this follow-up period (Chi square P=ns).

DISCUSSION

Anaemia contributes significantly to the morbidity and mortality in ESRD. ESA and iron supplementation are the most important factors in treatment of anaemia in ESRD patients.[13] Iron deficiency has been identified as a major factor for anaemia in CKD patients, with a 43-90% prevalence in different series.[6,14-16] Hence the periodical monitoring of iron status is crucial in ESRD.

There are two forms of iron deficiency, absolute iron deficiency and functional iron deficiency. Reduction of reticuloendothelial system iron in the bone marrow is referred to as absolute iron deficiency. Absolute iron deficiency may occur from impaired intestinal absorption, blood loss through the dialysis circuit, vascular access surgeries, repeated phlebotomies and increased iron utilization with erythropoietin therapy. In clinical practice, absolute iron deficiency is determined by serum ferritin less than 200ng/ml and TSAT less than 20%.[17] In the non-ESRD population, "functional" iron deficiency is ascribed when iron reserves in the bone marrow are adequate, but iron mobilization is impaired as in states of chronic inflammation.[18]

The current study assesses the utility of combining serum ferritin and TSAT to assess iron status among an Indian haemodialysis population. The evaluation of iron status is straightforward when both serum ferritin and TSAT are either high or low. But when these parameters are divergent, for instance, if the TSAT is low and serum ferritin is high, the accurate interpretation of iron status is difficult. This entity was common in our study where the iron status of only 48% of the patients were clearly determined (20% had normal iron status, 25% had iron deficiency and 3% had iron overload), and was indeterminate in the remaining 52% of patients. Interestingly in our study, the only epidemiologic factor associated significantly with indeterminate status was dialysis vintage. Why patients with indeterminate or overload status appeared to have longer durations of dialysis, is unclear but requires further exploration. Our data suggest that in a significant proportion of haemodialysis patients in a typical urban dialysis unit in India, the conventionally applied parameters were insufficient to accurately categorize iron status. Similar data has emerged from other groups.[19] Further, our data crucially demonstrates that patients with indeterminate status by conventional assessment have significantly inferior increases in haemoglobin levels with current therapy, suggesting the need for development of more specific diagnostic strategies to ascertain iron status in this group, to accurately manage iron status and anaemia.

Most studies have revealed that serum ferritin is very well related with iron stores in the bone marrow is the best surrogate of iron status in healthy individuals.[20-22] However, ferritin is an acute phase reactant increasing in states of chronic inflammation (such as dialysis), malignancy, or systemic disease, and ferritin levels alone may not reflect iron stores available for haematopoiesis. Thirty-six patients had low ferritin (<200) in our study. Only 25 of them had iron deficiency (combined with low-TSAT), whereas ~31% of them were in indeterminate group. Further, 29 patients had high serum ferritin. Only 3 of these had iron overload (combining TSAT), while 4/29 had low TSAT (<20%). Hence, decision to treat these patients with iron supplements based on these parameters would likely be imprecise. Similarly, a transferrin saturation of less than 20% in patients on haemodialysis is considered to be suggestive of iron deficiency whereas other studies have suggested that a transferrin saturation < 20% versus >20% alone may be inaccurate for assessing iron deficiency.[23-28] While most patients with TSAT less than 20% are iron deficient, few patients don't respond to an increase in iron supplements with either increase in haematocrit or maintenance of haematocrit with a decreased ESA requirement. On the other hand, numerous patients with a TSAT greater than 20% have functional iron deficiency respond to increased iron doses with elevations in the haematocrit, or maintenance of their haematocrit at decreased erythropoietin doses.[25-28] In our study, 43 patients had low TSAT (<20%); only 25 of them had iron deficiency (combined with ferritin). We found that 57 patients had TSAT values more than 20%, out of which 11 had low ferritin (<200). TSAT values in these 11 patients may be falsely high because of low total iron binding capacity (TIBC) during inflammation and infection.

The difficulties in the interpretation of iron status using TSAT and serum ferritin in ESRD patients and the consequent hematologic implications we show, warrant the application of other erythrocyte and reticulocyte based indices, such as the percentage of hypochromic red blood cells (Hypo) and the reticulocyte haemoglobin content (CHr). CHr requires a special analyser and is recommended as an alternative iron status indicator as well as an initial measure of the response to iron treatment in haemodialysis population.[29-31] Fishbane et al evaluated the reticulocyte haemoglobin content among 32 haemodialysis population and showed it to have a sensitivity of 100% and specificity of 80%.[29] Mittman et al evaluated reticulocyte haemoglobin content among 364 haemodialysis population receiving EPO and proved it to be an indicator of functional deficiency of iron.[30] Based on these and other data, NKF DOQI recommends a target of CHr greater than 29pg/cell.[32-35] The Hypo is an indicator of the percentage of red blood cells that contain low haemoglobin. Thus, it indicates only the functional iron availability and does not reflect storage iron. A Hypo level of greater than 10% indicates definitive functional iron deficiency. European Best Practice Guidelines stated that Hypo >10% is the most accurate marker for ID.[36] However, there are other studies which suggested lower threshold values for Hypo.[32,37-40] Both these tests are not widely available in India.

Here, we report that the combination of serum ferritin and TSAT accurately categorized the iron status in only 48%

of ESRD patients in our study. When serum ferritin and TSAT values diverged, they become unreliable for accurate interpretation of iron status, signaling an iron indeterminate state that was prevalent in 52% of our urban dialysis cohort. As we show here, this group of iron-indeterminate patients has inferior increases in haemoglobin over time, showing clinical relevance of this finding. Our data suggests that more sensitive indices such as Hypo and/or CHr may need to be employed (along with conventional tests) to accurately assess iron status, and improve management tools. This could lead to more accurate dosing of iron and ESAs, decrease overtreatment of anaemia, and possibly translate to cost savings from medication usage. Future studies on iron markers including both traditional and newer indices are required to improve iron management in ESRD population, especially in Indian population, where iron deficiency is common and similar studies are lacking.

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REFERENCES

1. KDOQI. Clinical Practice Guidance and Clinical Practice Recommendations for anaemia in Chronic Kidney Disease: 2007 Update of Haemoglobin target. Am J Kidney Dis. 2007;50:471-530.
2. Parfrey PS, Lauve M, Latremouille-Viau D, Lefebvre P. Erythropoietin therapy and left ventricular mass index in CKD and ESRD patients: a meta-analysis. Clin J Am Soc Nephrol. 2009;4:755-62.
3. Sonnweber T, Theurl I, Seifert M, Schroll A, Eder S, Mayer G, et al. Impact of Iron treatment on immune effector function and cellular iron status of circulating monocytes in dialysis patients. Nephrol Dial Transplant. 2011;26:977-87.
4. Feldman HI, Santanna J, Guo W, Furst H, Franklin E, Joffe M, et al. Iron administration and clinical outcomes in haemodialysis patients. J Am Soc Nephrol. 2002;13:734-44.
5. Collins AJ, Li S, Ebben J, Ma JZ, Manning W. Hematocrit levels and associated Medicare expenditures. Am J Kidney Dis. 2000;36:282-93.
6. MacDougall IC, Hutton RD, Cavill I, Coles GA, Williams JD. Poor response to treatment of renal anaemia with erythropoietin corrected by iron given intravenously. BMJ. 1989;299:157-8.
7. Sakiewicz P, Paganini E. The use of iron in patients on dialysis- mistakes and misconceptions. J Nephrol. 1998;11:5-15.
8. Horl WH. Clinical aspects of iron use in the anaemia of kidney disease. J Am Soc Nephrol. 2007;18:382-93.
9. René É, Lazrak HH, Laurin LP, Elftouh N, Vallée M, Lafrance JP. Association of erythropoiesis stimulating agents and the incidence risk of cancer diagnosis among chronic dialysis patients: a nested case-control study. Nephrol Dial Transplant. 2017;32:1047-52.
10. Brewster UC, Perazella MA. Intravenous iron and the risk of infection in end-stage renal disease patients. Semin Dial. 2004;17:57-60.
11. Rohde JM, Dimcheff DE, Blumberg N, Saint S, Langa KM, Kuhn L, et al. Health care-associated infection after red blood cell transfusion: a systematic review and meta-analysis. JAMA. 2014;311:1317-26.
12. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol. 2006;1:S4-8.
13. Nissenson AR. Hyporesponsiveness to erythropoietin: overview, 1996. Perit Dial Int. 1996;16:417-20.
14. Eschabach JW, Abdulhadi MH, Browne JK, Delano BG, Downing MR, Egrie JC, et al. Recombinant human erythropoietin in anaemic patients with end stage renal disease. Results of a phase III multicenter clinical trial. Ann Intern Med. 1989;111:992-1000.
15. Aggarwal HK, Nand N, Singh S, Singh M, Hemant, Kaushik G. Comparison of oral versus intravenous iron therapy in predialysis patients of chronic renal failure receiving recombinant human erythropoietin. J Assoc Physicians India. 2003;51:170-4.
16. Gupta M, Kannan M, Gupta S, Saxena R. Contribution of iron deficiency to anemia in chronic renal failure. Indian J Pathol Microbiol. 2003;46:563-4.
17. Fernandez-Rodriguez AM, Guindeo-Casasus MC, Molero-Labarta T, Domínguez-Cabrera C, Hortal-Casc n L, Pérez-Borges P, et al. Diagnosis of iron deficiency in chronic renal failure. Am J Kidney Dis. 1999;34:508-13.
18. Coyne DW. Hepcidin: clinical utility as a diagnostic tool and therapeutic target. Kidney Int. 2011;80:240-4.
19. Rafi A, Karkar A, Abdelrahman M. Monitoring iron status in end stage renal disease patients on haemodialysis. Saudi J Kidney Dis Transplant. 2007;18:73-8.
20. Caville I. Disorders of iron metabolism. Diagnostic methods. Clin Haematol. 1982;11:259-73.
21. Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. Annu Rev Med. 1993;44:63-74.
22. Cooper MJ, Zlotkin SH. Day-to-day variation of transferrin receptor and ferritin in healthy men and women. Am J Clin Nutr. 1996;64:738-42.

23. Macdougall IC. Monitoring of iron status and iron supplementation in patients treated with erythropoietin. Curr Opin Nephrol Hypertens. 1994;3:620-5.
24. Neff M. Algorithm for the treatment of anaemia in ESRD. Network Forum Quality Assurance Committee; 1995.
25. Fishbane S, Kowalski EA, Imbriano LJ, Maesaka JK. The evaluation of iron status in haemodialysis patients. J Am Soc Nephrol. 1996;7:2654-7.
26. Macdougall IC, Tucker B, Thompson J, Tomson CR, Baker LR, Raine AE. A randomized controlled study of iron supplementation in patients treated with erythropoietin. Kidney Int. 1996;50:1694-9.
27. Silverberg DS, Laina A, Peer G, Kaplan E, Levi BA, Frank N, et al. Intravenous iron supplementation for the treatment of the anemia of moderate to severe chronic renal failure patients not receiving dialysis. Am J Kidney Dis. 1996;27:234-8.
28. Silverberg DS, Blum M, Peer G, Kaplan F, Laina A. Intravenous ferric saccharate as an iron supplement in dialysis patients. Nephron. 1996;72:413-7.
29. Fishbane S, Galgano C, Langley RC Jr, Cranfield W, Maesaka JK. Reticulocyte haemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int. 1997;52:217-22.
30. Mittman N, Sreedhara R, Mushnick R, Chattopadhyay J, Zelmanovic D, Vaseghi M, et al. Reticulocyte haemoglobin content predicts functional iron deficiency in hemodialysis patients receiving EPO. Am J Kidney Dis. 1997;30:912-3.
31. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. Crit Rev Clin Lab Sci. 2000;37:93-130.
32. Cullen P, Söffker J, Höpfl M, Bremer C, Schlaghecken R, Mehrens T, et al. Hypochromic red cells and reticulocyte haemoglobin content as markers of iron-deficient erythropoiesis in patients undergoing chronic haemodialysis. Nephrol Dial Transplant. 1999;14:659-65.
33. Fishbane S, Galgano C, Langley RC Jr, Canfield W, Maesaka JK. Reticulocyte hemoglobin content in the evaluation of iron status of hemodialysis patients. Kidney Int. 1997;52:217-22.
34. Mittman N, Sreedhara R, Mushnick R, Chattopadhyay J, Zelmanovic D, Vaseghi M, et al. Reticulocyte hemoglobin content predicts functional iron deficiency in hemodialysis patients receiving EPO. Am J Kidney Dis. 1997;30:912-22.
35. KDOQI; National Kidney Foundation. Clinical Practice Guidelines and Clinical Practice Recommendations for Anaemia in Chronic Kidney Disease. Am J Kidney Dis. 2006;47:S11-145.
36. European best practice guidelines for the management of anaemia in patients with chronic renal failure. Nephrol Dial Transplant. 1999;14:S14-15.
37. Braun J, Lindner K, Schreiber M, Heidler A, Hörl WH. Percentage of hypochromic red cells as predictor of erythropoietic and iron response after i.v. iron supplementation in maintenance haemodialysis patients. Nephrol Dial Transplant. 1997;12:1173-81.
38. Jones CH, Richardson D, Ayers S, Newstead CG, Will EJ, Davison AM. Percentage hypochromic red cells and the response to intravenous iron therapy in anaemic haemodialysis patients. Nephrol Dial Transplant. 1998;13:2873-6.
39. Tessitore N, Solero P, Gammaro, Sabato A, Maschio G. Iron status and low dose intravenous Fe gluconate in hemodialysis patients on maintenance erythropoietin (Abstract). J Am Soc Nephrol. 1998;9:272.
40. Tessitore N, Solero GP, Lippi G, Bassi A, Faccini GB, Bedogna V, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. Nephrol Dial Transplant. 2001;16:1416-23.

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Original Article

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Correlation of Reciprocal Changes and QRS Amplitude in ECG to Left Ventricular Dysfunction, Wall Motion Score and Clinical Outcome in First Time ST Elevation Myocardial Infarction

Internal Medicine Section

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ABSTRACT

Introduction: Electrocardiogram (ECG) is the simplest tool for diagnosing ST Elevation Myocardial Infarction (STEMI). We can use a12 lead ECG for prognostication purposes also.

Aim: The aim of the study was to find out the role of ECG as a prognostic marker in terms of clinical outcome and wall motion abnormality.

Materials and Methods: It was a prospective study done in PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India, from January 2014 to September 2014. Patients aged above 18 years admitted with first episode of ST EMI as per the inclusion and exclusion criteria were recruited for the study. Presence of reciprocal changes and QRS amplitude was measured from ECG. Presence of Left Ventricular Dysfunction (LVD) and wall motion score were calculated from ECG along with clinical outcome during first follow up visit. Statistical analysis was done using SPSS software. Probability was calculated using chi-square test, independent t-test and ANOVA analysis.

Results: A total of 120 patients were recruited for the study of which six were excluded based on the exclusion criteria. Among 114 patients analysed, 55 had reciprocal changes; 38 of them developed LVD which was statistically significant (p=0.002). Of the 78 patients with Anterior Wall Myocardial Infarction (AWMI), 35 had reciprocal changes; 15 (42.9%) of them had NYHA Class 1 symptoms, 14 (40%) had Class II and 4 (11%) had class III symptoms at follow up. The association was statistically significant (p=0.001). Similar statistically significant association was found in patients with Inferior Wall Myocardial Infarction (IWMI) who had reciprocal changes and NYHA symptoms at follow up (p=0.004). The mean wall motion score in patients with AWMI and reciprocal changes was 24.83 ± 4.1; whereas, without reciprocal changes was 23.98 ± 3.6; the association was not statistically significant. The mean QRS amplitude of all patients with LVD was 33.25 ±16.34. The association between QRS amplitude and LVD was not statistically significant. The overall mean wall motion score was 24.86 ± 3.91. The association between QRS amplitude and wall motion score was statistically significant (r value = 0.210). The association between QRS amplitude and wall motion score was statistically significant when we analysed AWMI (r= -0.147, p=0.199) and IWMI (r= -0.359, p=0.031) separately.

Conclusion: ECG can be used as a tool for prognostication in acute STEMI. The presence of reciprocal changes in the ECG can signify poorer outcome on follow up. Lower QRS amplitude can be used as a predictor of larger infarct.

Keywords: Coronary artery disease, Heart failure, Prognostic marker

INTRODUCTION

Myocardial infarction is one of the most important causes for hospitalization and is commonly seen in all populations worldwide [1]. The clinical approach to a patient suspected to have an acute coronary syndrome consists of detailed history taking, ECG changes, cardiac biochemistry evidence and imaging. The reliability of each of these modalities as a diagnostic tool is dependent on multiple factors, the most significant being the window period between the time of infarction and the time the patient seeks medical attention [2]. The current approach to patients presenting with typical or atypical features of myocardial ischemia or infarction starts with making the provisional diagnosis of acute coronary syndrome [3,4]. Depending on the changes seen on the 12 lead ECG, acute coronary syndrome is classified into STEMI, Non ST Elevation Myocardial Infarction (NSTEMI) or unstable angina [5].

Inspite of major leaps in the diagnostic tools and treatment of myocardial infarction, STEMI persists as one of the leading cause for mortality and morbidity in both the developed and developing world [6]. The prevalence of coronary disease and myocardial infarction is also on rise in the developing countries, with further worsening of adverse cardiovascular events due to disadvantages like inadequate primary prevention policies and limited availability of medical help [7].

There has been extensive research done in the field of cardiovascular medicine focused on ST elevation myocardial infarction and the dynamics of complications surrounding it. One particular area of interest in recent studies appears to be the study of extent of infarct over imaging modalities and its correlation with various events in the course of infarction [8,9]. Other parameters gaining importance are non traditional ECG changes like reciprocal changes, ST depression, QRS morphology, q waves etc., [10-17].

The simplest and oldest diagnostic tool for myocardial infarction is the 12 lead ECG. In daily practice, even though the ECG remains an important test in diagnosis and detection of progression of disease, there still remains ample potential for its role as a prognostic marker [18,19]. The fundamental advantage that stands to be gained by

realizing this potential is the time it saves for immediate intervention. In addition, it can serve as an instant and cost effective method for risk assessment instead of waiting for biochemical and angiography results. The purpose of this study was to observe the association between ECG changes (reciprocal changes and QRS amplitude) with left ventricular function, wall motion score and clinical outcome in patients with first episode of STEMI.

MATERIALS AND METHODS

It was a prospective study done in PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India, from January 2014 to September 2014. The study population comprised of patients hospitalized after being diagnosed with STEMI based on ST elevation seen on ECG done at admission. All those patients aged above 18 years admitted with first episode of STEMI, who underwent uncomplicated thrombolysis, were included in the study. Those patients with bundle branch blocks, pre-existing cardiac illness, re-infarction, with clinical findings of severe LVD at presentation and who underwent angioplasty were excluded from the study. Informed consent was obtained from the patients.

The patient's age, gender, risk factors for myocardial infarction were documented at admission. The first ECG taken was studied for presence or absence of reciprocal changes. The total of R wave amplitude in mm and S wave amplitude in all the leads of the ECG was taken and the sum was noted as the QRS amplitude. Left ventricular function was assessed by 2-D echocardiogram based on ejection fraction using eyeballing method. Patients were graded as having mild LVD if the ejection fraction was 45%-50%, moderate LVD if ejection fraction was 35%-45% and severe LVD if ejection fraction was less than 35% [20].

Regional wall motion abnormality was assessed using American Society of Echocardiography (ASE) method by grading wall motion of all 17 left ventricular segments. This was represented as wall motion score. For each of the individual 17 segments, score of 1 was taken as normal regional wall motion; score of 2 was given for hypokinesia, score of 3 for akinesia, score of 4 as dyskinesia and 5 for anuerysm. So, if a patient's echo showed normal left ventricular function, the total wall motion score was 17 for all segments together [21]. Clinical outcome of the patient was assessed in terms of New York Heart Association (NYHA) classification based on symptoms at first follow up after discharge from the hospital (which was commonly three to four weeks after infarction) [22].

STATISTICAL ANALYSIS

The data collected from the patients was tabulated using Microsoft excel. Statistical analysis was done using SPSS software. Probability was calculated using chi-square test, independent t-test and ANOVA analysis. The p-value was considered significant if it was found to be less than 0.05.

RESULTS

A total of 120 patients were included in the study. Six patients were excluded because the area of STEMI was inferior wall and posterior wall. So, a total of 114 patients presenting with STEMI were included.

Out of the total 114 patients studied, 78 (68.4%) had AWMI and 36 (31.6%) had IWMI. Among the total study population, 55 (48.2%) patients had reciprocal changes in ECG. Of the 78 patients with AWMI, 35 (63.6%) had reciprocal changes in ECG. Of the 36 patients with IWMI, 20 (36.4%) had reciprocal changes.

Association between Reciprocal Changes and LVD

Out of 55 patients with reciprocal changes, 38 patients (69.1%) developed LVD [Table/ Fig-1]. The association between the two variables was found significant (chi-square test value = 0.002).

Of the 55 patients who had reciprocal changes in their ECGs, 17 (30.9%) had adequate left ventricular function, 14 (25.5%) patients had mild LVD, 19 (34.5%) had moderate LVD and the remaining 5 (9.1%) patients had severe LVD [Table/ Fig-2].

In the 78 patients with AWMI, 35 patients had reciprocal changes. The association between presence of reciprocal changes and LVD was found using Chi-square test and was insignificant (p-value = 0.843). Out of the 35 patients with reciprocal changes, 6 (40%) patients had adequate left ventricular function and out of the 43 patients without reciprocal changes, 9 (60%) patients had adequate left ventricular function [Table/ Fig-3].

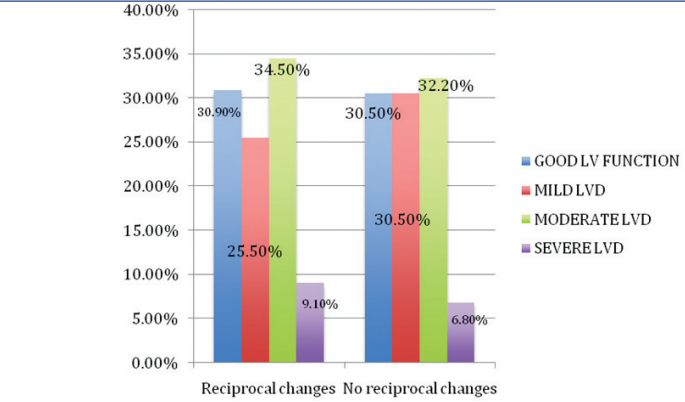
In the 36 patients with IWMI, 20 patients had reciprocal changes. The association between presence of reciprocal changes and LVD was found using chi-square test and was insignificant (p-value = 0.856). Out of these 20 patients with reciprocal changes, 11 (55%) patients had adequate left ventricular function and out of the 16 patients without reciprocal changes, 9 (56.3%) patients had adequate left ventricular function [Table/ Fig-4].

Association between Reciprocal Changes and Wall Motion Score

In the 78 patients with AWMI, the association between reciprocal changes and wall motion score was analysed. The mean wall motion score in patients with and without reciprocal changes were 24.83 ± 4.1 and 23.98 ± 3.6 respectively [Table/ Fig-5]. The difference between the two groups was not significant (p-value = 0.342).

Reciprocal Changes	Left Ventricular Dysfunction		Pearson Chi-square p-value
	Yes	No	
Yes	38(69.1%)	17 (30.9%)	0.002
No	41(69.5%)	18(30.5%)	

[Table/Fig-1]: Correlation between reciprocal changes and LV dysfunction.



[Table/Fig-2]: Reciprocal changes and LV dysfunction.

LVD	Reciprocal changes		Total
	No	Yes	
No LVD	9(60%)	6(40 %)	15(100%)
Mild LVD	13(59.1%)	9(40.9%)	22(100%)
Moderate LVD	19(52.8%)	17(47.2%)	36(100%)
Severe LVD	2(40%)	3(60%)	5(100%)
Total	43(53.1%)	35(44.9%)	78(100%)

[Table/Fig-3]: AWMI - reciprocal changes vs. LV dysfunction.

Reciprocal Changes	No LVD	Mild LVD	Moderate LVD	Severe LVD	Total
No	9 (56.3%)	5 (31.3%)	0 (0%)	2 (12.5%)	16 (100%)
Yes	11(55%)	5 (25%)	2 (10%)	2 (10%)	20 (100%)
Total	20 (55.6%)	10 (27.8%)	2 (5.6%)	4 (11.1%)	36 (100%)

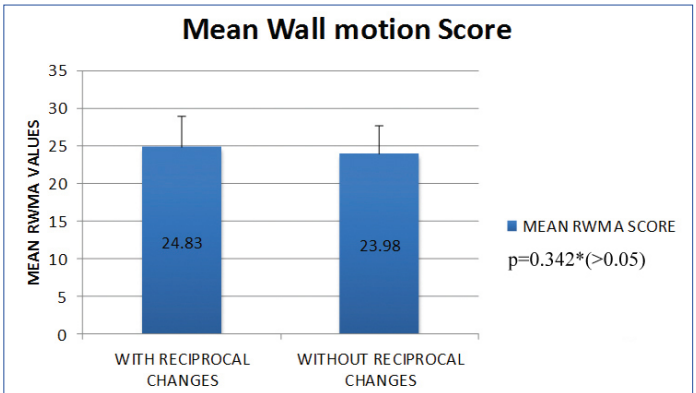
[Table/Fig-4]: IWMI - Reciprocal changes v/s LV dysfunction.

In the 36 patients with IWMI, the association between reciprocal changes and Wall motion score was analysed. The mean Wall motion score in patients with and without reciprocal changes were 22 ± 4.623 and 21.31 ± 4.99 respectively [Table/ Fig-6].The difference between the two groups was not significant (p-value = 0.431).

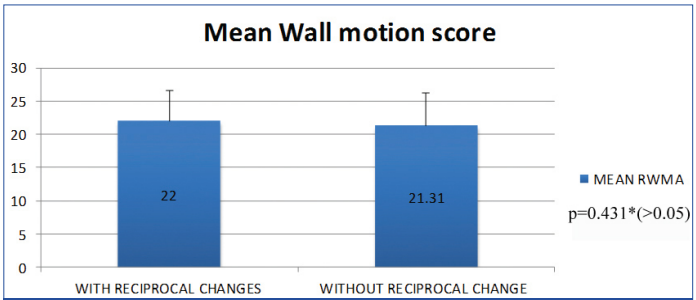
Association between Reciprocal Changes and Symptoms at Follow up

A total of 35 out of 78 patients with anterior wall infarction had reciprocal changes in their ECGs. Among these 35 patients, 15 (42.9%) had NYHA Class I symptoms, 14 (40%) patients had NYHA Class II symptoms, 4 (11.4%) patients had NYHA Class III symptoms and 2(5.7%) were lost to follow up [Table/ Fig-7]. The p-value calculated using Pearson chi-square test and was found to be statistically significant (p = 0.001). This indicates those patients who had reciprocal changes are at high risk to develop cardiac failure symptoms at follow up.

Out of the 36 patients with IWMI, 20 patients had reciprocal changes in their ECGs. Among those 20 patients, 9 (45%) had no symptoms NYHA Class I, 10 (50%) patients had NYHA Class II symptoms and 1 (5%) patient had NYHA Class III symptoms [Table/ Fig-8). The p-value calculated using Pearson Chi square test was statistically significant (p = 0.004). This also indicates those patients who had reciprocal changes are at high risk to develop cardiac failure symptoms at follow up.



[Table/Fig-5]: Mean wall motion score and reciprocal changes in AWMI.
* Independent t-test



[Table/Fig-6]: Mean wall motion score and reciprocal changes in IWMI.
* Independent t-test

Reciprocal Changes	Outcome at First Follow up			
	NYHA Class I	NYHA Class II	NYHA Class III	Lost to follow up
YES	15 (42.9%)	14 (40%)	4(11.4%)	2(5.7%)
NO	33(76.7%)	5(11.6%)	0(0%)	5(11.6%)

[Table/Fig-7]: Correlation between reciprocal changes and clinical outcome in AWMI.

Reciprocal Changes	Outcome at First Follow up			
	NYHA Class I	NYHA Class II	NYHA Class III	Lost to Follow up
YES	9(45%)	10(50%)	1(5%)	0(0%)
NO	15(93.8%)	0(0%)	0(0%)	1(6.3%)

[Table/Fig-8]: Correlation of reciprocal changes and clinical outcome in IWMI.

Association between QRS amplitude and LV Dysfunction

The mean QRS amplitude of all patients with LVD was 33.25 ±16.34.The mean QRS amplitude in patients with AWMI was 34.17 ± 16.93 and with IWMI was 35.03 ± 14.42. The association between QRS amplitude and LVD was analyzed [Table /Fig-9a] in patients with AWMI using ANOVA test and was found to be statistically insignificant (p-value = 0.307). The association between QRS amplitude and LVD was analyzed in patients with IWMI using ANOVA test and was found to be statistically insignificant (p-value 0.156) [Table /Fig-9b].

Association between QRS Amplitude and Wall Motion Score

The mean QRS amplitude in all the 114 patients was 34.17 ± 16.929. The mean wall motion score in all 114 patients was 24.36 ± 3.915. Using Pearson chi-square test, the correlation was statistically significant (r-value = - 0.210). The mean QRS amplitude in AWMI patients was 34.17 ± 16.9 [Table /Fig-10]. The mean wall motion score in AWMI patients was 24.36 ± 3.9. Using the Pearson chi-square test, the correlation was found to be statistically significant (r-value = -0.147, p= 0.199).

The mean QRS amplitude in IWMI patients was 35.03 ± 14.4. The mean wall motion score in IWMI patients was 21.69 ± 4.7 [Table /Fig-11]. Using the Pearson chi-square test, the correlation was found to be statistically significant (r-value = - 0.359, p= 0.031)

Association between QRS Amplitude and Symptoms at Follow up

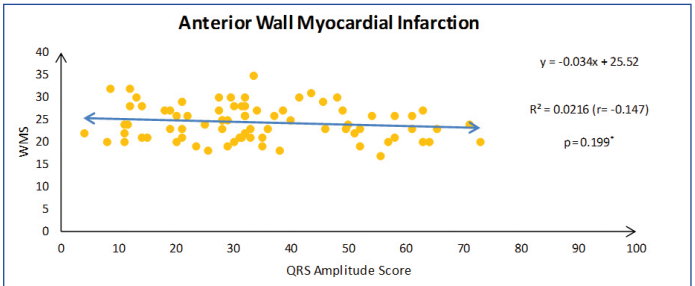
Among the 78 patients with anterior wall STEMI, 48 patients had no symptoms at first follow up (NYHA Class I), 23 patients were symptomatic (NYHA Class II and above) and seven patients lost to follow up. In these 48 patients under NYHA Class I, the mean QRS amplitude was 33.95 ± 18.17. In these 23 patients under NYHA Class II and above, the mean QRS amplitude was 35.30 ± 15.12. The difference between the two groups was statistically insignificant (p = 0.759). Among the 36 patients with IWMI, 24 patients had no symptoms at first follow up (NYHA Class I), 11 patients were symptomatic (NYHA II and above) and one patient lost to follow up. In these 24 patients under NYHA Class I, the mean QRS amplitude was 34.70 ± 13.47. In these 11 patients under NYHA Class II and above, the mean QRS amplitude was 36.72 ± 17.17. The difference between the two groups was statistically insignificant (p = 0.706).

LVD	QRS Amplitude Score				p-value (ANOVA)
	n	Mean	Std. Deviation	Std. Error of Mean	
No	15	34.03	18.564	4.793	0.307
Mild	22	39.36	19.638	4.187	
Moderate	36	32.14	14.54432	2.424	
Severe	5	26.4	13.198	5.902	

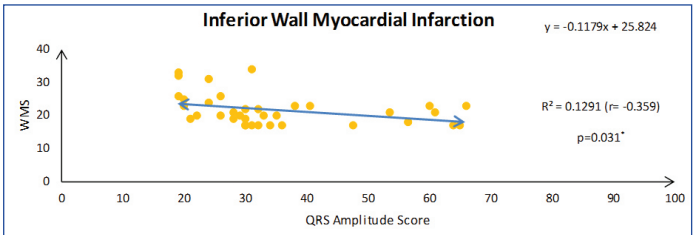
[Table/Fig-9a]: QRS amplitude and LV dysfunction in AWMI.

LVD	QRS Amplitude Score				p-value (ANOVA)
	n	Mean	Std. Deviation	Std. Error of Mean	
No	20	38.48	13.758	3.076	0.156
Mild	10	35.25	16.622	5.256	
Moderate	2	23	4.243	3	
Severe	4	23.25	5.679	2.839	

[Table/Fig-9b]: QRS amplitude and LV dysfunction in IWMI.



[Table/Fig-10]: QRS amplitude and wall motion score in AWMI.
*Pearson chi-square test



[Table/Fig-11]: QRS amplitude and wall motion score in IWMI.
*Pearson chi-square test

DISCUSSION

In our study, reciprocal changes in the ECG were seen in about half of the patients (48.2%), of which around two thirds had AWMI and the remaining had IWMI. Majority of the patients studied (69.3%) had LVD following STEMI. The presence of reciprocal changes in ECG showed significant association with development of LVD when analyzed in all the patients in total. When the subset of patients with reciprocal changes in AWMI and IWMI were considered separately, the association with LVD was statistically insignificant. This was in-contrast to the study by Peterson ED et al., which showed ST depression in precardial leads in IWMI had poor prognosis [11].

A similar result was found in a study done by Atroush HE et al., ejection fraction was not statistically different in patients with acute IWMI with and without precordial ST segment depression [23]. This was in contrast to the study by Parale GP et al., where they found that patients with AWMI and IWMI with reciprocal changes had significant LVD compared to those without [24]. Zoghi M et al., also found that patients of AWMI with reciprocal ST depression and without had significant difference with regard to ejection fraction [25]. Atroush HE et al., in their study, found that patients with AWMI infarction with precordial ST segment depression had significant complications during hospital course than patients without reciprocal changes [23]. Salcedo JR et al., also found that complications during hospital stay were more in patients of AWMI with precardial ST segment depression [26].

In AWMI patients, the presence of reciprocal changes did not reflect in an increase in the wall motion score. When a similar association was analyzed in IWMI patients, it was also found to be insignificant. The association between reciprocal changes and clinical outcome in terms of NYHA class of symptoms on first follow up was found to be significant. This may imply that the presence of reciprocal changes can spell worse outcome following STEMI when compared to those without reciprocal changes in their ECG.

On analyzing the association between QRS amplitude and wall motion score revealed that a decrease in QRS amplitude was associated with an increase in wall motion score in both AWMI and IWMI patients. When the association between QRS amplitude and degree of LVD was found, it was statistically insignificant. This meant that low QRS amplitude did not reflect the degree of LVD.

This is the first study of this kind correlating reciprocal changes and QRS amplitude with LVD and clinical outcome in STEMI.

LIMITATION

The limitation of the study is the small sample size; we need a larger study with higher sample size to extrapolate the findings. We also agree the assessment of outcome at follow up has confounding factors like compliance with medication, diet and lifestyle modification measures.

CONCLUSION

In STEMI, the patients who had reciprocal changes in their ECG were more prone to develop LVD, but this significance was lost when individual areas of infarction were considered. The presence of reciprocal changes in the ECG can signify poorer outcome on follow up at three to four weeks after infarction. In patients with lower QRS amplitudes, higher wall motion scores were observed, indicating that low voltage of QRS complexes in the ECG can be predictive of larger extent of the infarct. Even though we are in the era of primary PTCA and early re-vascularisation therapy for STEMI, ECG is a very useful tool to prognosticate where early revascularization option is not available.

REFERENCES

[1] Mendis S, Puska P, Norrving B, editors. Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization, Geneva 2011.
[2] Goldberg RJ, Yarzebski J, Lessard D, Gore JM. Decade-long trends and factors associated with time to hospital presentation in patients with acute myocardial infarction: The Worcester heart attack study. Arch Intern Med. 2000; 160: 3217–23.
[3] Pope JH, Aufderheide TP, Ruthazer R, Woolard RH, Feldman JA, Beshansky JR, et al. Missed diagnosis of acute cardiac ischemia in the emergency department. N Eng J Med. 2000;342: 1163-70.
[4] Lee TH, Goldman L. Evaluation of the patients with acute chest pain. N Eng J Med. 2000;342:1187-95.
[5] Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee Heart disease and stroke statistics—2009 update. A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee [published correction appears in Circulation. 2009;119(3):e182] Circulation 2009;119(3):480-86.
[6] Anderson JL, Adams CD, Antman EM, Bridges CR, Califf RM, Casey DE Jr, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST elevation myocardial infarction: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2007, 50: e1.
[7] Thomas A. Gaziano, Asaf Bitton, Shuchi Anand, Shafika Abrahams-Gessel, Adrianna Murphy. Growing epidemic of coronary heart disease in low- and middle-income countries. Curr Probl Cardiol. 2010; 35(2): 72-115.
[8] Cury RC, Shash K, Nagurney JT, Rosito G, Shapiro MD, Nomura CH, et al. Cardiac magnetic resonance with T2-weighted imaging improves detection of patients with acute coronary syndrome in the emergency department. Circulation 2008;118:837-44.
[9] Raman SV, Simonetti OP, Winner MW 3rd, Dickerson JA, He X, Mazzaferri EL Jr, et al. Cardiac magnetic resonance with oedema imaging identifies myocardium at risk and predicts worse outcome in patients with non-ST-segment elevation acute coronary syndrome. J Am Coll Cardiol. 2010;55:2480-88.
[10] Hathaway WR, Peterson ED, Wagner GS, Granger CB, Zabel KM, Pieper KS, et al. Prognostic significance of the initial electrocardiogram in patients with acute myocardial infarction. GUSTO-I Investigators. Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries. JAMA. 1998;279(5):387.
[11] Peterson ED, Hathaway WR, Zabel KM, Pieper KS, Granger CB, Wagner GS, et al. Prognostic significance of precordial ST segment depression during inferior myocardial infarction in the thrombolytic era: Results in 16,521 patients. J Am Coll Cardiol. 1996;28(2):305.
[12] Birnbaum Y, Kloner RA, Sclarovsky S, Cannon CP, McCabe CH, Davis VG, et al. Distortion of the terminal portion of the QRS on the admission electrocardiogram in acute myocardial infarction and correlation with infarct size and long-term prognosis (Thrombolysis in Myocardial Infarction 4 Trial). Am J Cardiol. 1996;78(4):396.
[13] Barbagelata A, Califf RM, Sgarbossa EB, Goodman SG, Stebbins AL, Granger CB, et al. Thrombolysis and Q wave versus non-Q wave first acute myocardial infarction: A GUSTO-I substudy. Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Arteries Investigators. J Am Coll Cardiol. 1997;29(4):770.
[14] Goodman SG, Langer A, Ross AM, Wildermann NM, Barbagelata A, Sgarbossa et al. Non-Q-wave versus Q-wave myocardial infarction after thrombolytic therapy: Angiographic and prognostic insights from the global utilization of streptokinase and tissue plasminogen activator for occluded coronary arteries-I angiographic substudy. GUSTO-I Angiographic Investigators. Circulation. 1998;97(5):444.

[15] Lockwood E, Fu Y, Wong B, Van de Werf F, Granger CB, Armstrong PW, et al. Does 24-hour ST-segment resolution postfibrinolysis add prognostic value to a Q wave? An ASSENT 2 electrocardiographic substudy. Am Heart J. 2003;146(4):640.
[16] Andrews J, French JK, Manda SO, White HD. New Q waves on the presenting electrocardiogram independently predict increased cardiac mortality following a first ST-elevation myocardial infarction. Eur Heart J. 2000;21(8):647.
[17] Askenazi J, Parisi AF, Cohn PF, Freedman WB, Braunwald E. Value of the QRS complex in assessing left ventricular ejection fraction. Am J Cardiol. 1978;41(3):494.
[18] Savonitto S, Ardissino D, Granger CB, Morando G, Prando MD, Mafrici A, et al. Prognostic value of the admission electrocardiogram in acute coronary syndromes. JAMA. 1999;281(8):707-13.
[19] Welch RD, Zalenski RJ, Frederick PD, Malmgren JA, Compton S, Grzybowski M, et al. Prognostic value of a normal or nonspecific initial electrocardiogram in acute myocardial infarction. JAMA. 2001;286(16):1977-84.
[20] Shahgaldi K, Gudmundsson P, Manouras A, Brodin LA, Winter R. Visually estimated ejection fraction by two dimensional and triplane echocardiography is closely correlated with quantitative ejection fraction by real-time three dimensional echocardiography. Cardiovasc Ultrasound. 2009;7:41.
[21] Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: A report from the Am Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005;18:1440-63.
[22] Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, et al. Guidelines for the diagnosis and treatment of chronic heart failure: Executive summary (update 2005): The task force for the diagnosis and treatment of chronic heart failure of the European Society of Cardiology. Eur Heart J. 2005; 26:1115–40.
[23] Atroush HE, Effat H, Shehata M, Emara H. Reciprocal ST segment changes in acute inferior myocardial infarction: Clinical, hemodynamic and angiographic implications. The Egyptian Heart Journal. 2012;64:97-103.
[24] Parale GP, Kulkarni PM, Khade SK, Athawale S, Vora A. Importance of reciprocal leads in acute myocardial infarction. J Assoc Physicians India. 2004;52:376-79.
[25] Zoghi M, Gugun C, Yavuzgi O, Turko Iu I, Kultusay H, Akilli A, et al. The angiographic correlation between ST segment depression in non infarcted leads and the extent of coronary artery disease in patients with acute inferior myocardial infarction: A clue for multivessel disease. Can J Cardiol. 2003;19:67-71.
[26] Salcedo JR, Baird MG, Chambers RJ, Beanlands DS. Significance of reciprocal ST segment depression in anterior precordial leads in acute inferior myocardial infarction: Concomitant left anterior descending coronary artery disease? Am J Cardiol. 1981;48:1003-08.

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Original Article

Physiology Section

Effects of Sleep Deprivation on the Cognitive Performance of Nurses Working in Shift

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ABSTRACT

Introduction: Sleep deprivation and altered circadian rhythm affects the cognitive performance of an individual. Quality of sleep is compromised in those who are frequently involved in extended working hours and shift work which is found to be more common among nurses. Cognitive impairment leads to fatigability, decline in attention and efficiency in their workplace which puts their health and patients' health at risk.

Aim: To find out the prevalence of sleep deprivation and its impact on cognition among shift working nurses.

Materials and Methods: Sleep deprivation among 97 female and three male healthy nurses of age 20–50 years was assessed by Epworth Sleepiness Scale (ESS). Cognition was assessed by Montreal Cognitive Assessment (MoCA) questionnaire. Mobile applications were used to test their vigilance, reaction time, photographic memory and numerical cognition. The above said parameters were assessed during end of day shift and 3-4 days after start of night shift.

Results: Poor sleep quality was observed among 69% of shift working nurses according to ESS scores. The cognitive performance was analysed using Wilcoxon signed rank test. The MoCA score was found to be lesser among 66% of nurses during night (25.72) than day (26.81). During the night, 32% made more mathematical errors. It was also found that, 71%, 83% and 68% of the nurses scored lesser during night in the Stroop's colour test, vigilance test and memory tests respectively. Thus, impairment in cognitive performance was statistically significant ($p<0.001$) among shift working nurses.

Conclusion: Cognitive performance was found to be impaired among shift working nurses, due to poor sleep quality and decreased alertness during wake state. Thus, shift work poses significant cognitive risks in work performance of nurses.

Keywords: Circadian rhythm, Night shift, Sleep quality

INTRODUCTION

Sleep plays an integral part in the health and well-being of the individual. Sleep is controlled by the Supra Chiasmatic Nucleus (SCN) of the hypothalamus, endogenous clock which regulates the production of melatonin, a hormone that induces sleep [1]. Physiological systems that follow circadian rhythm like sleep-wake cycle, hormone secretions, core body temperature are influenced by signals from the environment especially light [2]. Altered circadian rhythm results in physiological and psychological variations [3].

The recommended sleep duration for a healthy adult is seven to nine hours per day [4]. Shift work results in a conflict between a days oriented circadian physiology and requirement for work and sleep at the wrong biological time of day. Internally driven circadian biological clocks regulate the periods of sleepiness and wakefulness. Disruption of circadian rhythm and inadequate sleep are associated with derangement of physiological functions and leads to obesity, cardiovascular disease, and cognitive impairment. Thus, shift work, night work, and longer, irregular working hours are associated with reduced sleep duration and poor quality of sleep [5,6].

Sleep Deprivation (SD) is defined as, "obtaining inadequate sleep to support adequate daytime alertness" [7]. Long-term SD has a detrimental effect on cognitive function, attention, learning, and working memory and is associated with exaggerated neural reactivity [8]. Moreover, it is also associated with mal adaptations of physiological and behavioral functions. Sleep deprivation affects the ability of health workers who are involved in multitude of life saving tasks which need more attention and concentration. Extended work shifts of twelve hours or longer is common with nurses, whose insufficient sleep puts patients' health and their health at risk [9]. SD among nurses is the major cause for making an error in patient care and significant decrease in vigilance of the job [10]. The aim of this study was to assess the prevalence of SD among nurses working in shift and its impact on their cognitive performance.

MATERIALS AND METHODS

This cross-sectional study was conducted among staff nurses of Peelamedu Samanaidu Govindasamy Hospitals, Coimbatore, Tamil Nadu, India, who were on a rotating work schedule i.e., staff posted to day shift for one month and the same staff posted to night shift for the next one month during August 2016 to September 2016 after obtaining the ethical clearance from the Institution Ethical Committee.

Sample Size

Hundred staff nurses were chosen whose age ranged from 20 years to 50 years. Sample size (96), was calculated considering prevalence of sleep deprivation among staff nurses as 51% with 20 % precision [11].

Inclusion Criteria

Staff nurses of all departments like Medicine, Surgery, OBG, Paediatrics, etc., who were working eight hours shift a day for six days a week, not suffering from any known medical or mental illness and had a previous work experience of at least one year in rotating shift work, were included.

Exclusion Criteria

Staff nurses with a history of medical illness especially neurological diseases, sleep disorders (insomnia, somnolence, etc.,) and smoking, alcohol, any other drug consumption or any medications (within the last 14 days), those who do overtime and other jobs during off duty were excluded from the study.

The participants were informed about the test procedure and a written consent was obtained from them. The staff nurses were assessed for sleep deprivation using the Epworth Sleepiness Scale (ESS) [12]. The ESS was used to measure day time sleepiness and

hence to diagnose sleep deprivation. The probability of falling asleep of the subject was rated from 0 to 3 during eight different situations in their day to day activities, not necessarily every day. The total score was calculated by adding the scores for the eight questions.

The study participants were assessed for any alterations in their cognitive performance as a result of sleep deprivation by using a series of tests. These tests were conducted twice, at the end of their eight hour duration day shift work and end of eight hour duration night shift work after 3-4 days of the commencement night shift. This ensured enough time for the circadian rhythm to reset according to the changed working hours [13]. The tests assessed general intellectual function, numerical cognition, execution, vigilance and memory.

General Intellect

The Montreal Cognitive Assessment (MoCA) Version 7.3 was used to assess various cognitive domains concerned with visuospatial skill, naming, memory recall, attention, language, abstraction, delayed recall and orientation [14]. This test can be easily completed within 10 minutes. The total possible score is 30 points. The scores obtained during the day shift and night shift hours were compared.

Executive Function (Response Inhibition and Working Memory)

Executive function includes control and coordination of cognitive skills like analytical thinking, working memory, planning, cognitive flexibility, analysing the problem and as well as control over attention and inhibition. In this study, response inhibition and visual working memory was assessed. Mobile applications (Confusing colours (stroop test), Memoryze) were used for this purpose [15,16]. Response inhibition was found using an application that worked on the principle of Stroop effect [15].

Attention (Vigilance)

Attention deficit is more commonly reported in sleep deprivation. Failure of vigilant attention affects the individual's ability to respond to stimuli on time. A mobile application (Vigilance test) which works like the Mackworth clock test was used to assess sustained attention. The application only picked up accuracy in terms of number of targets detected and false alarms [17].

Simple Reaction Time

Simple reaction time is the test to assess the processing speed and motor control of the individual by providing a known stimulus to elicit the response. The participant was asked to press a button as soon as a colour change occurs. This was done using a mobile application (reaction time test) [18].

Numerical Cognition (Mental Speed)

The mental speed was assessed by asking the participants to solve a worksheet having 25 maths problems. This worksheet had problems of addition, subtraction, multiplication and division of 2-digit numbers. The time taken for solving the worksheet at different shift hours was noted and compared [19].

STATISTICAL METHODS

Data was analyzed using SPSS Version 19.0. Prevalence of sleep deprivation was expressed in percentage. A Wilcoxon Signed-Ranks test was used to compare non-parametrical values. The p value < 0.001 was considered statistically significant.

RESULTS

Of the 100 participants, 97 of them were females and the rest were male staff nurses. The mean age of the participants was 25.06 years.

The sleep deprivation was assessed at the end night shift work on day 3 or 4 of the night shift. From the scores obtained from ESS, it was found that 69% had disturbed sleep patterns due to shift work. The average ESS score obtained was 9.37 (± 4.225). Majority of the nurses (42%) had mild SD (ESS scores of 8 to 11 [Table/Fig-1].

The maximum score that was obtained in MoCA during night shift and day shift hours was 29 and 30 respectively. The minimum score was found to be 21 during night and 23 during day. The mean score for general intellect and attention was decreased during night, while the mean scores for mental speed and reaction time were increased during the night hours [Table/Fig-2].

Sleep deprivation	Epsworth Sleepiness Score	Prevalence (%)
Normal sleep	Less than 8	31
Mild	8-11	42
Moderate	12-15	17
Severe	16-24	10

[Table/Fig 1]: Prevalence of sleep deprivation (n=100).

A Wilcoxon Signed-Ranks test was used to compare these non-parametrical values. This test indicated that MoCA test score obtained during the day shift hours was statistically significantly higher than the scores of night shift hours (Z=-5.872, p<0.001). Also, the scores obtained for execution (Z=-5.056, p<0.001) and memory tests (Z=-5.241, p<0.001) during the day shift hours were significantly higher than those obtained at night. The time taken to complete the math worksheet was significantly lower during the day than at night (Z=-5.762, p<0.001). The response time for the vigilance test was statistically significantly quicker during the day than at night (Z=-7.241, p<0.001). The number of targets detected was significantly higher during the day shift hours (Z=7.163, p<0.001) and the number of false alarms was significantly higher during the night hours (Z=-7.373, p<0.001) [Table/Fig-2].

Domain		Day Shift		Night Shift		Wilcoxon Signed-Ranks Test	
		Mean	SD	Mean	SD	Z*	p
General intellect (MoCA)		26.81	1.368	25.72	1.590	-5.872	<0.001
Mental speed (Maths)		2.14	0.689	2.34	0.740	-5.672	<0.001
Response Inhibition		8.48	4.602	4.91	3.210	-5.056	<0.001
Simple Reaction time		0.33	0.142	0.54	0.148	-7.241	<0.001
Working Memory		12.66	4.848	8.85	4.398	-5.241	<0.001
Attention (Vigilance)	Targets detected	5.03	1.794	2.53	1.666	7.163	<0.001
	False alarms	1.37	1.195	3.07	0.832	-7.373	<0.001

[Table/Fig 2]: Cognitive performance.
*Based on positive ranks (night shift – day shift) p (2 – tail) < 0.001 is statistically significant.

DISCUSSION

Our present study reported 69% of shift working nurses were affected with sleep deprivation. Thus, the poor quality and inadequate quantity of sleep among the participants in the present study could be due to extended shift work (more than 13 to 14 hours for each shift, including transportation time), irregular sleep wake cycle due to their disrupted circadian rhythms. This is in line with the results observed among physicians having the similar work schedules [20].

There has been increased interest in the effects of sleep deprivation among health care personnel's as it relates to patient care. Moreover, working at night and working excessive hours restrict sleep duration, as a result of fatigue concentration decreases and there will be increase in cognitive defects and also leads to compromised health and safety at work [21,22]. Research conducted among night shift residents' and nurses' reported that poor concentration hinders their efficiency, decision making capacity and also causes increased error rates [23].

Our present study aimed to compare cognitive functions of the shift working nurses at the end of the day shift and night shift hours. Better cognitive ability was be reported among those who have never exposed to shift work compared to those who have exposed

to sleep deprivation, altered circadian rhythm and extended shift work duration [10]. Lower level of scores was observed in general intellectual ability, maths, response inhibition, attention, simple reaction time and working memory. This decline may be due to an increase in prevalence of sleep deprivation among shift working nurses, as sleep deprivation affects the frontal and prefrontal cortex [24].

Partial sleep deprivation had brought about a decline in response inhibition during night shift in the present study indicates the greater vulnerability of frontal lobe to sleep deprivation. This is in line with results of the study conducted among BPO workers in India [25].

Working memory and episodic memory was found to be associated with temporal lobe function. As the shift work is known to produce deleterious effects on mental health, which is responsible for the impairment of memory, concentration and thinking ability. We found that visual working memory was better during the day shift than at night. Thus, memory also becomes enslaved to sleep deprivation [26,27]. Moreover, we observed that the maths problems were solved at a slower pace during the night shift hours. Our findings were consistent with the results of a study conducted among a group of interns in the medical field in US [28].

Furthermore, simple reaction time was found to be delayed during the night hours as a result of SD. Vigilance test showed that there were lesser target detections and more false alarms during the night hours. There are studies which prove that tests of vigilant attention during periods of SD are sensitive to both circadian and homeostatic drives and that SD causes an overall slowing of reaction times, results in increased errors of omission and commission, and enhances the time-on-task effect [29-31].

Overall irregularities in circadian rhythm and natural sleep wake cycle resulted in the impairment of cognitive function during the night shift, than at day. Some studies state that, though day and night shift work are of the same duration, those who work night shift are more liable to have sleep deprivation and hence more vulnerable to its various deteriorating effects [32-34].

LIMITATION

ESS is quite reliable and stable measure of sleep quality, it is not as accurate as an actigraph or actimetry sensor in detection of SD. All domains of cognition were also not tested in this study. Moreover, mobile application software utilized to test cognition were subjected to internal face validation due to resource constraints. Further studies on a larger population are needed with the aid of actigraphy to assess the prevalence of SD and its impact on all the cognitive domains.

CONCLUSION

This study shows that SD is prevalent among staff nurses working in shift. The various cognitive domains like general intellect, reaction time, attention, vigilance and memory are impaired as a result of SD, which increases the chances of error at work place. Hence, it is necessary to incorporate measures like rest breaks, napping, exercise, and bright light at work place.

REFERENCES

[1] Gillette MU, Tischkau SA. Suprachiasmatic nucleus: the brain's circadian clock. Recent Progress in Hormone Research. 1999;54:33-60.
[2] Kuhn G. Circadian rhythm, shift work, and emergency medicine. Ann Emerg Med. 2001;37:88–98.
[3] Durmer JS, Dinges DF. Neurocognitive consequences of sleep deprivation. Semin Neurol. 2005;25(1):117-29.
[4] Hirshkowitz M, Whiton K, Albert SM. National sleep foundation's sleep time duration recommendations: methodology and results summary. Sleep Health. 2015;1(1):40-43.
[5] Bliwise DL. Historical change in the report of daytime fatigue. Sleep. 1996;19(6):462-64.
[6] Harma M, Kandolin I. Shift work, age and well-being: recent developments and future perspectives. Journal of Human Ergology. 2001;30(1-2):287-93.
[7] Hershner SD, Chervin RD. Causes and Consequences of sleepiness among college students. Nat Sci Sleep. 2014;6:73-84.
[8] Dai XJ, Gong HH, Wang YX. Gender differences in brain regional homogeneity of healthy subjects after normal sleep and after sleep deprivation: a resting-state fMRI study. Sleep Med. 2012;13:720–27.
[9] Stimpfel AW, Sloane DM, Aiken LH. The longer the shifts for hospital nurses, the higher the levels of burnout and patient dissatisfaction. Health Affairs. 2012;31(11):2501-09.
[10] Chang YS, Wu YH, Hsu CY, Tang SH, Yang LL, Su SF. Impairment of perceptual and motor abilities at the end of a night shift is greater in nurses working fast rotating shifts. Sleep Medicine. 2011;12(9):866–69.
[11] Saleh AM, Awadalla NJ, El-masri YM, Wafaa F. Sleep Impacts of nurses' circadian rhythm sleep disorders, fatigue, and depression on medication administration errors. Egyptian Journal of Chest Diseases and Tuberculosis. 2014;63(1):145–53.
[12] Johns MW. Reliability and factor analysis of the Epworth Sleepiness Scale. Sleep. 1992;15: 376–81.
[13] Burgess PA. Optimal shift duration and sequence: recommended approach for short-term emergency response activations for public health and emergency management. Am J Public Health. 2007;97(Suppl 1):S88–S92.
[14] Nasreddine ZS, Phillips NA, Bédirian V. The montreal cognitive assessment, moca: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53:695–99.
[15] Iljimae Apps. Confusing colours. Mobile Application Software. Version 2.2, 2014https://play.google.com/store/apps/details?id=com.ndquangr.confusing colour&hl=en. Acessed on 2016/8/01.
[16] Memoryze. Nemeean Lion. Mobile Application Software. Version 2.0, 2015https://play.google.com/store/search?q=Memoryze.%20Nemeean%20 Lion.&c=apps&hl=en. Acessed on 2016/8/01.
[17] Vigilance Test. hsapps.com. Mobile Application Software. Version 1.0, 2013https://play.google.com/store/apps/details?id=com.hsapps.vigilanztest&hl=en acesseced on 2016/8/01.
[18] Wait Now. Reaction Time Test Mobile Application Software. Version 1.09, 2015. https://play.google.com/store/search?q=Wait%20Now.%20Reaction%20 Time%20Test&c=apps&hl=en. Accessed on 2016/8/01.
[19] Veasey S, Rosen R, Barzansky B, Rosen I, Owens J. Sleep loss and fatigue in residency training. JAMA. 2002;288:1116-24.
[20] Palhares VC, Corrente JE, Matsubara BB. Association between sleep quality and quality of life in nursing professionals working rotating shifts. Rev Saúde Pública. 2014;48(4):594-601.
[21] Belayachi J, Benjelloun O, Madani N, Abidi K, Dendane T, Zeggwagh AA. Self-perceived sleepiness in emergency training physicians: prevalence and relationship with quality of life. J Occup Med Toxicol. 2013;8:24.
[22] Lockley SW, Landrigan CP, Barger LK, Czeisler CA. When policy meets physiology: the challenge of reducing resident work hours. Clinical Orthopaedics and Related Research. 2006;449:116-27.
[23] Barger LK, Ayas NT, Cade BE, Cronin JW, Rosner B, Speizer FE, et al. Impact of extended-duration shifts on medical errors, adverse events, and attentional failures. PLoS Medicine. 2006;3(12):e487.
[24] Rouch I, Wild P, Ansiau D, Marquie JC. Shiftwork experience, age and cognitive performance. Ergonomics. 2005;48(10):1282-93.
[25] Riccio CA, Reynolds CR, Lowe P Moore JJ. The continuous performance test: a window on the neural substrates for attention? Archives of Clinical Neuropsychology. 2002;17(3):235–72.
[26] Shwetha B, Sudhakar H. Influence of shift work on cognitive performance in male business process outsourcing employees. Indian Journal of Occupational and Environmental Medicine. 2012;16(3):114.
[27] Lo JC, Ong JL, Leong RL, Gooley JJ, Chee MW. Cognitive performance, sleepiness, and mood in partially sleep deprived adolescents: the need for sleep study. SLEEP. 2016;39(3): 687–98.
[28] Raidy DJ, Scharff LF. Effects of sleep deprivation on auditory and visual memory tasks. Perceptual And Motor Skills. 2005;101(2):451-67.
[29] Gunzelmann G, Gross JB, Gluck KA, Dinges DF. Sleep deprivation and sustained attention performance: Integrating mathematical and cognitive modeling. Cognitive Science. 2009;33(5):880-910.
[30] Lim J, Dinges DF. Sleep deprivation and vigilant attention. Annals of the New York Academy of Sciences. 2008;1129:305–22.
[31] Doran SM, Van Dongen HP, Dinges DF Sustained attention performance during sleep deprivation: evidence of state instability. Archives Italiennes De Biologie. 2001;139(3):253-67.
[32] Williamson AM, Feyer AM. Moderate sleep deprivation produces impairments in cognitive and motor performance equivalent to legally prescribed levels of alcohol intoxication. Occupational and Environmental Medicine. 2000;57(10):649-55.
[33] Smith MR, Eastman CI. Shift work: health, performance and safety problems, traditional counter measures, and innovative management strategies to reduce circadian misalignment. Nature and Science of Sleep. 2012;4:111–32.
[34] Akerstedt T, Wright KP. Sleep loss and fatigue in shift work and shift work disorder. Sleep Medicine Clinics. 2009;4(2):257-71.

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Original Research Article

Comparison of microalbuminuria and heart rate variability in prediction of cardiovascular complications in diabetic population: a pilot study

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ABSTRACT

Background: Microalbuminuria is considered as an early marker and strongly associated with risk of cardiovascular complications in diabetic patients. Autonomic imbalance due to micro vascular damage to parasympathetic and sympathetic fibers results in reduced heart rate variability; also predicts increased risk for cardiovascular events in diabetics. Hence it is necessary to identify an early and effective predictor of diabetic micro vascular complications. Objective of the study was to compare heart rate variability of controls with type 2 diabetes with and without microalbuminuria.
Methods: This comparative study was conducted among individuals without diabetes, hypertension and dyslipidemia (controls) and Type II diabetics with and without microalbuminuria (cases). Cases and controls were subjected to general clinical examination; microalbuminuria and HbA1C were noted. heart rate variability was assessed using digital physiograph. Frequency (HF, LF, LF/HF ratio) domain readings were noted.
Results: Mann Whitney U test was employed to analyze nonparametric data. Diabetics with microalbuminuria when compared with controls showed statistically significant (p-value=0.015) reduction in vagal activity. When compared with diabetics without microalbuminuria (180.4±151.7) they showed reduction in HF with no statistical significance. When diabetic group without microalbuminuria (0.56±0.31) was compared with controls they showed alternation in LF/HF ratio which has no statistical significance. Diabetics with microalbuminuria when compared with controls showed a statistically significant (p-value=0.009) alternation in LF /HF ratio implying an autonomic imbalance.
Conclusions: This study shows there is significant vagal inhibition and autonomic imbalance in diabetic patients with microalbuminuria compared to controls. To ascertain the role of HRV as an early predictor of cardiovascular complications we propose to conduct study with a larger sample size in future.

Keywords: Autonomic imbalance, Diabetes mellitus, Heart rate variability, Microalbuminuria

INTRODUCTION

The magnitude of non-communicable diseases (cardiovascular diseases, diabetes, malignancies and chronic respiratory diseases) has brought about a negative socioeconomic impact and sufferings to the mankind.[1] 36 million deaths have been reported every year due to diabetes and its complications. Moreover, 9 million people succumb to death prematurely.[2] Improved and effective medications have increased the lifespan of diabetic population. Hence there is a need to identify and intervene at the earliest in order to prevent complications and provide them a better quality of living. Microalbuminuria is considered as an early marker. It

causes endothelial dysfunction and predisposes atherosclerotic coronary artery disease, stroke, peripheral vascular disease, and cardiovascular mortality among diabetic population.[3,4] It was stated in heart outcomes prevention evaluation study, that diabetic patients with microalbuminuria are more prone for cardiovascular complications thus periodic screening for microalbuminuria could allow early identification of vascular disease.[5] Moreover, microvascular damage to parasympathetic and sympathetic fibers cause autonomic imbalance also predicts increased risks for cardiovascular arrhythmias, sudden death, and myocardial infarction in adults with diabetes.[6] HRV rate variability is a non-invasive indicator of cardiac autonomic function that predicts mortality and morbidity.[7]

However, the studies available till date give no conclusive answer to the concurrent association between lower cardio vascular autonomic function and microalbuminuria. Our objective was to compare heart rate variability of normal individuals with type 2 diabetics with and without microalbuminuria.

METHODS

This comparative study was conducted after obtaining clearance from institutional human ethics committee and informed consent from study participants. Individuals without diabetes, hypertension and dyslipidemia were recruited as controls (n=14). Type II diabetics with and without microalbuminuria of both sexes with age ranging from 45-65 years were included as cases (n=14). Patients with hypertension, coronary artery disease, cerebrovascular accident, family history of young MI, hyper or hypothyroidism, and beta blocker treatment were excluded. Cases and controls were subjected to general clinical examination. Presence of microalbuminuria and HbA1C values were noted from their clinical records.

Heart rate variability

Cases and controls were instructed to refrain from smoking, caffeine intake for 2 hours and alcohol intake for 36 hours. It was ensured they had adequate rest, got at least 8 hours of uninterrupted sleep on the night before the assessment of HRV and had normal breakfast on the day of assessment. They were made to lie quietly in a couch in supine position for five minutes to alleviate the anxiety in a sound attenuated room with dim lighting and the temperature ranging from 20 to 25°Celsius.

After explaining the procedure to the subject, heart rate variability, a quantitative marker of the autonomic activity was assessed using an ambulatory ECG system (INCO digital NIVIQURE, Bangalore, India) in lead II for 5 minutes. It is a multi-channel digital data acquisition system which enables to acquire, analyze and store ECG data. ECG data was obtained at a sampling rate of 1024 Hz in standard lead II configuration. The interface RS232C-compatible module was used to transfer data from the recording unit to the computer. The transferred data was analyzed using inbuilt software system. The series of RR intervals obtained was subjected to frequency domain analysis.

Frequency domain variables low frequency (LF) and high frequency (HF) power of HRV spectrum in normalized units and LF/HF ratio were measured. Descriptive statistics and Mann Whitney U test was employed to analyze nonparametric data.

RESULTS

42 individuals participated in the study, out of which 14 were non-diabetics, who acted as controls and the remaining 28 diabetics acted as cases. Out of 28 diabetics, 14 were presented without microalbuminuria and 14 with microalbuminuria. Results were expressed in mean±standard deviation. The mean age of the control group was found to be 45.78±6.8 and that of cases without microalbuminuria was 53.07±10.9 and with microalbuminuria was found to be 53±10.8.

When the diabetic group was compared with the non-diabetics HF showed statistically significant reduction (p=0.041) and the LF/HF ratio showed a significant alteration (p=0.015) (Table 1).

Table 1: Comparison between diabetics and non-diabetics.

Variables	Mean±standard deviation		P-value
	Non - diabetics N=14	Diabetics N=28	
HBA1C	5.71±0 .23	8.55±2.57	0.000*
HF	281±262	136.7±123.5	0.041*
LF	82.8±64.8	74.8±43.5	0.883
LF/HF	0.47±0.3	0.73±0.35	0.053

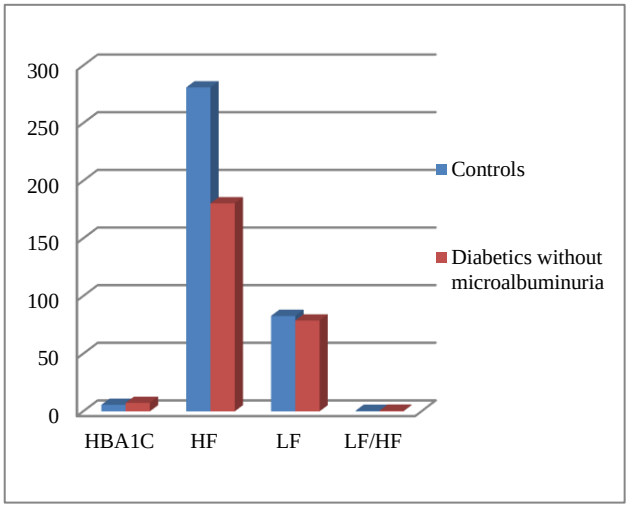


Figure 1: Comparison between the controls and diabetics without microalbuminuria.

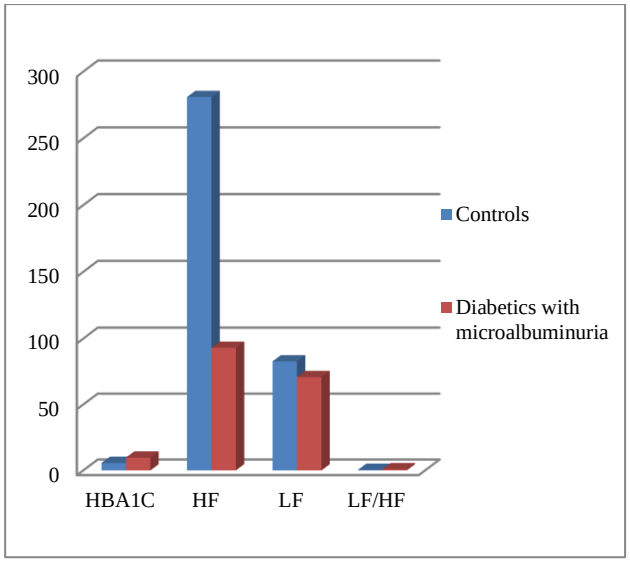


Figure 2: Comparison between the controls and diabetics with microalbuminuria.

On analysis of short term HRV, the mean HF was found to be 281±262 in controls. When the diabetic group without microalbuminuria (180.4±151.7) was compared with the controls reduction in HF was observed with no statistical significance (Figure 1). However, the diabetic group with microalbuminuria (93.05±67.6) when compared with controls showed statistically significant (p=0.015) reduction in HF (Figure 2).

LF/HF ratio was found to be 0.47±0.3 in controls. When diabetic group without microalbuminuria (0.56±0.31) was compared with controls they showed an alteration which has no statistical significance. However when the diabetic group with microalbuminuria was compared with controls they showed a statistically significant (p=0.009) alteration in LF/HF ratio (Table 2).

DISCUSSION

Microalbuminuria and heart rate variability are considered as the early predictors and prognostic markers of impending microvascular complications in diabetic population.[8,9] Increased prevalence of microalbuminuria (10 to 40%) was found to be reported in diabetic population when compared normal healthy individuals (5 to 7%).[10] This finding substantiates the presence of microalbuminuria as an early marker for diabetic complications.

Table 2: Comparison between diabetics with and without microalbuminuria.

Variables	Mean±standard deviation		p-value
	Diabetics without microalbuminuria n=14	Diabetics with microalbuminuria n=14	
HBA1C	7.37±1.25	9.72±3.02	0.021*
HF	180.42±151.71	93.05±67.6	0.08
LF	78.79±35.83	70.86±51.06	0.38
LF/HF	0.56±0.31	0.89±0.32	0.004*

HRV analysis is a noninvasive method to assess cardiac autonomic function. It is quantified in time and frequency domains. Time domain is based on statistical operation on R-R intervals and frequency domain is based on spectral analysis of an array of R-R intervals. Frequency domain measures are considered to be more sensitive according to the suggestion of Task force of the European society of Cardiology.[11] This suggestion led to the usage of frequency domain measures in our study.

The frequency domain measures such as LF indicates sympathetic activity, HF assesses cardiovagal activity and the LF/HF reflects the sympathovagal balance. In present study we observed that the frequency domain parameters (HF, LF and HF/LF) which assess the autonomic balance showed an alteration suggesting autonomic dysfunction in diabetic patients with and without microalbuminuria.

Many researchers have reported that there is 2 to 4 times increased risk for cardiac dysautonomia in a diabetic population when compared to controls.[12] In accordance with this study, our findings report a significant reduction in HF (136.7±123.5) and an alteration in LF/HF (0.73±0.35) which suggests a significant cardiovagal inhibition and sympathovagal imbalance in the diabetic group when compared with the nondiabetic population. In a comparative study conducted among diabetics and controls, Mirza M observed both sympathetic and parasympathetic dysfunction with parasympathetic dominance in diabetic group[13,14]

Furthermore, present study also revealed a nonsignificant decrease in HF among the diabetics without microalbuminuria whereas a statistically significant decrease among the diabetics with microalbuminuria. This finding proved that there exists a significant cardiovagal inhibition in the diabetics with microalbuminuria. This observation is in line with the

other studies which report a decrease in time and frequency domain parameters of HRV before the clinical evidence of autonomic neuropathy in diabetic patients.[15] Carnethon et al, in their study found out that reduced HRV leads to complications and found to be independent prognostic marker in patients with type 2 diabetes.[16]

In our study LF/HF ratio was found to be altered in diabetics without microalbuminuria with no statistical significance. However, the diabetic group with microalbuminuria showed a statistically significant alternation implying an autonomic imbalance. In another study conducted by Colhoun et al among type 1 diabetic patients power spectral analysis of HRV was found to be reduced and was associated with cardiovascular risk factors such as increased HbA1c, dyslipidemia, higher BMI hypertension and albuminuria.[17]

It was observed in previous studies that there was a higher incidence of myocardial Ischemia in Type 1 diabetes patients with microalbuminuria.[18] However, several studies confirmed the association of microalbuminuria and alteration in vascular tone are the results of endothelial dysfunction which predicts cardiovascular events in diabetic population.[19] The results of a study which employed heart rate reserve and rating of perceived exertion in prescribing intensity of exercise also strongly support the proposed role of reduced heart rate variability along with microalbuminuria as a sensitive marker in predicting cardiovascular complications.[20]

Prevention plays a key role in quality health care delivery so it is necessary to identify early, noninvasive, feasible and effective tool to predict cardiovascular complications in diabetic population. Our pilot study is in favor of the positive role of short term HRV as a tool which fulfils all the aforementioned criteria in predicting cardiovascular complications

CONCLUSION

This study shows there is significant vagal inhibition and autonomic imbalance in diabetic patients with microalbuminuria when compared to controls. To ascertain the role of HRV as an early predictor of cardiovascular complications we propose to conduct the study with a larger sample size in future.

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REFERENCES

1. United Nations General Assembly. Resolution 64/265. Prevention and control of non-communicable diseases; 2010.
2. World Health Organization. Global burden of disease. 2004 update.
3. Bianchi S, Bigazzi R, Campese VM. Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications. Am J Kidney Dis. 1999;34(6):973-95.
4. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci. 2005;109(2):143-59.
5. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA. 2001;286:421-6.
6. Gottsater A, Ahlgren AR, Taimour S, Sundkvist G. Decreased heart rate variability may predict the progression of carotid atherosclerosis in type 2 diabetes. Clin Auton Res. 2006;16:228-34.
7. Vinik AI, Maser RE, Ziegler D. Neuropathy: the crystal ball for cardiovascular disease? Diabetes Care. 2010;33:1688-90.
8. Zandbergen AA, Vogt L, de Zeeuw D, Lamberts SW, Ouwendijk RJ, Baggen MG, et al. Change in albuminuria is predictive of cardiovascular outcome in normotensive patients with T2DM and microalbuminuria. Diabetes Care. 2007;30(12):3119-21.
9. Boysen A, Lewin MA, Hecker W, Leichter HE, Uhlemann F. Autonomic function testing in children and adolescents with diabetes mellitus. Pediatr Diab. 2007;8(5):261-4.
10. de Zeeuw D, Parving HH, Henning RH. Microalbuminuria as an early marker for cardiovascular disease. J Am Soc Nephrol. 2006;17(8):2100-5.
11. Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology: Heart rate variability: standards of measurements, physiological interpretation and clinical use. Circulation. 1996;93:1043-65.
12. American Diabetes Association. Economic costs of diabetes in the US in 2002. Diabetes Care. 2003;26(3):917-32.
13. Mirza M, Lakshmi ANR. A comparative study of heart rate variability in diabetic subjects and normal subjects. Int J Biomed Adv Res. 2012;03(08):640-4.
14. Faulkner MS, Quinn L, Rimmer JH, and Rich BH. Cardiovascular endurance and heart rate variability in adolescents with Type 1 or Type 2 Diabetes. Biol Res Nurs. 2005;7(1):16-29.
15. Kudat H, Akkaya V, Sozen AB, Salman S, Demirel S, Ozcan M, et al. Heart rate variability in diabetes patient. J Int Med Res. 2006;34:291-6.
16. Carnethon MR, Liao D, Evans GW, Cascio WE, Chamless LE, Heiss G. Correlates of the shift in heart rate variability with an active postural change in a healthy population sample: the atherosclerosis risk in communities study. Am Heart J. 2002;143(5):808-13.

17. Colhoun HM, Francis DP, Rubens MB, Underwood SR, Fuller JH. The association of heart-rate variability with cardiovascular risk factors and coronary artery calcification: a study in type 1 diabetic patients and the general population. Diabetes Care. 2001;24(6):1108-14.
18. Earle KA, Mishra M, Morocutti A, Barnes D, Stephens E, Chambers J, et al. Microalbuminuria as a marker of silent myocardial ischaemia in IDDM patients. Diabetologia. 1996;39:854-6.
19. Weir MR. Microalbuminuria and cardiovascular disease. Clin J Am Soc Nephrol. 2007;2(3):581-90.
20. Colberg SR, Swain DP, Vinik AI. Use of heart rate reserve and rating of perceived exertion to prescribe exercise intensity in diabetic autonomic neuropathy. Diabetes care. 2003;26(4):986-90.

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Case Report

Eosinophilic gastroenteritis in an immunosuppressed patient: a case report

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ABSTRACT

Eosinophilic gastroenteritis is a rare disease that is characterized by Eosinophil infiltration in one or multiple segments of the gastrointestinal tract. The etiology of this condition remains unknown. Eosinophilic gastroenteritis has heterogeneous clinical manifestations that depend upon the location and depth of infiltration in the gastrointestinal tract, and eosinophilia may or may not be present. This article reports a case of Eosinophilic Gastroenteritis in a 57 years old retroviral male patient, who presented with chronic diarrhea, bilateral pedal edema and eosinophilia. Ultimately, the diagnosis of eosinophilic gastroenteritis was clinched. The rare character of eosinophilic gastroenteritis and its varied clinical presentations often lead to delayed diagnosis and complications. Case reports may help to disseminate knowledge about the disease, thereby increasing the likelihood of early diagnosis and intervention to prevent complications.

Keywords: Chronic diarrhea, Eosinophilic gastroenteritis

INTRODUCTION

Eosinophilic gastroenteritis (EG) is classified in a group of diseases known as eosinophilic gastrointestinal disorders (EGIDs). The primary EGIDs are defined as diseases that selectively affect the gastrointestinal tract with the presence of inflammatory infiltrate that is rich in eosinophils in the absence of other causes of eosinophilia, such as drug reactions, parasitic infections, or malignancies. This group of diseases, the incidence of which has increased over the last few decades, includes eosinophilic esophagitis, eosinophilic gastritis, eosinophilic enteritis, eosinophilic colitis, and EG.[1] All of these conditions exhibit significant infiltration of eosinophils in the gastrointestinal tract and a very strong association with allergies.[2] In 1970, Klein classified the disease according to the predominance of eosinophilic infiltration in different layers of the intestinal wall, namely, the mucosal, muscle, and sub serosal layers.[3]

The involvement of different layers usually gives rise to different clinical manifestations. Mucosal disease generally presents with bleeding, protein-losing enteropathy, or malabsorption. Involvement of the muscle layer may cause bowel wall thickening and subsequent intestinal obstruction. The sub serosal form usually presents with eosinophilic ascites. The diagnosis for EGIDs is established after ruling out other causes of an eosinophilic disease, particularly atopy, parasitic infestations, vasculitis, and hyper eosinophilic syndrome (HES). Authors report a typical case of widespread EG with associated involvement of colonic mucosa in which symptoms dramatically responded to a course of steroids, as evidenced by careful follow up.

CASE REPORT

57 years old male, diagnosed with HIV 1 infection 2 months back, started on anti-retroviral drugs (Tenofovir,

Lamivudine, Efavirenz) presented with complaints of loose stools (3-4 episodes/day) for 2 months and B/L swelling of legs for 4 days. He also had history of loss of weight and appetite. He had no history of atopy/ food or drug allergies. He was treated with nitazoxanide but without any improvement. His CD_4 count was 751 cells and HIV viral load was 880IU/L. HBsAg, anti HCV and TPHA were negative.

On examination he was thin built and had pitting pedal edema. Systemic examination revealed no abnormalities. Investigations showed anaemia (Hb-9.8 g%) and eosinophilia (67.1%) (Absolute eosinophil count 8,700 cells/cu.mm). Liver function test showed hypoproteinemia, elevated alkaline phosphatase and GGT levels. serum LDH, uric acid were within normal limits. Since he was an immunosuppressed host, opportunistic infections were first considered. Stool examination with modified ZN stain for microsporidium, isospora and cryptosporidium were negative.

Stool examination for other ova and parasites were also negative. Radio allegro sorbent test (RAST) for a battery of allergens including common foods were negative. As he had granulomatous hepatitis picture, CECT abdomen was done to look for intraabdominal lymphadenopathy which showed transmural thickening in caecum, ascending colon and proximal transverse colon, with few enlarged mesenteric lymph nodes. Upper GIscopy and deep duodenal biopsies were normal. Colonoscopy showed ileal nodularity, biopsies were taken from multiple sites of colon.

As a part of workup for granulomatous hepatitis, liver biopsy was done. Histopathological examination of liver biopsy showed lobular inflammation with many eosinophils, heavy inflammatory infiltrate including many eosinophils. Ileal biopsy also demonstrated tissue eosinophilia. He was started on T. prednisolone 60 mg (1mg/kg/day). Complete remission of the diarrhea occurred in about one week. One month later, the patient's bowel habits were normal, and he had gained 4 kg.

DISCUSSION

Eosinophilic gastroenteritis can involve any part of gastrointestinal tract from the esophagus down to the rectum. The stomach and duodenum are the most common sites of involvement.[4-7] Four criteria are required for the diagnosis of EG: the presence of gastrointestinal symptoms, eosinophilic infiltration of the gastrointestinal tract, exclusion of parasitic disease or other identifiable cause of eosinophilia and the absence of other systemic involvement.[8-10]

Eosinophils are normal constituents of the mucosa of the gastrointestinal tract; however, the number of eosinophils that can be defined as normal in a biopsy specimen has not been clearly defined.[10] Peripheral eosinophilia is not necessary to make a diagnosis, but it is present in approximately two-thirds of patients and is more prevalent in the serosal than in the mucosal and muscular disease forms.[11,12] The presence of peripheral eosinophilia, abundant eosinophils in the gastrointestinal tract, and a dramatic response to corticosteroid provide some support that the disease is mediated by a hypersensitivity-type reaction. Moreover, a study at Mayo Clinic showed that 50% of patients with EG give a history of allergy such as asthma, allergic rhinitis, urticaria, drug allergy, and eczema.[13]

It must be emphasized that in the mucosal form of the disease, multiple biopsies must be taken during endoscopy, because mucosal involvement is often patchy in nature. Treatment with a steroid is the mainstay in the management of EG. Dramatic clinical improvement is seen after treatment with a low dose of steroid. The duration of treatment is controversial, however. This patient was started with prednisolone 60 mg daily, then tapered over (a period of 6 weeks) and responded favourably.

Due to the positive retroviral status in present patient, authors at first, ruled out opportunistic infections. Not many cases of EG in immunosuppressed patients are reported in literature. Bacterial infections (*Helicobacter pylori*, etc) and parasitic infestations (Ascaridosis, Anisakiasis, Enterobiasis, Anchylostomiasis, Trichina, Teniasis, etc) are a well-known cause of EG, but in present patient neither bacteria in gastric biopsies or stool culture nor ova and parasites were found in the stools.[14-16]

Hypereosinophilic syndrome (HES) may be associated with gastroenteritis but was ruled out because it is an idiopathic condition characterized by marked peripheral eosinophilia exceeding 1500 cells/ml for six consecutive months and its major targets are the heart, lung, brain and kidneys, with>55% of patients presenting with a complication in≥ 1 of these sites.[17] Celiac disease was excluded since deep duodenal biopsy showed normal histologic pattern. In present case, no positive food allergen could be demonstrated, and the colonic involvement makes it similar to the pure form of EG, in which a positive RAST test is rarely found.[3]

CONCLUSION

Eosinophilic gastroenteritis is an uncommon disease with signs and symptoms similar to many other diseases. It is quite rare in immunosuppressed patients, which is why it is often not considered in the differential diagnosis. Histology is essential for the diagnosis of EG and should be considered. Making the correct diagnosis early may result in effective treatment, thereby achieving quicker remission.

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REFERENCES

1. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). J Allergy Clin Immunol. 2004;113:11-28.
2. Guajardo JR, Plotnick LM, Fende JM, Collins MH, Putnam PE, Rothenberg ME. Eosinophil-associated gastrointestinal disorders: a worldwide- web based registry. J Pediatr. 2002;141:576-81.
3. Klein NC, Hargrove RL, Sleisenger MH, Jeffries GH. Eosinophilic gastroenteritis. Medicine (Baltimore). 1970;49:299-319.
4. Daneshjoo RJ, Talley N. Eosinophilic gastroenteritis. Curr Gastroenterol Rep. 2002;4:366-72.
5. Khan S, Orenstein SR. Eosinophilic gastroenteritis. Gastroenterol Clin North Am. 2008;37:333-48.
6. Chen MJ, Chu CH, Lin SC, Shih SC, Wang TE. Eosinophilic gastroenteritis: clinical experience with 15 patients. World J Gastroenterol. 2003;9:2813-6.
7. Khan S. Eosinophilic gastroenteritis. Best Pract Res Clin Gastroenterol. 2005;19:177-98.
8. Furuta GT, Forbes D, Boey C, Dupont C, Putnam P, Roy SK, et al. EGIDs Working Group. Eosinophilic Gastrointestinal Diseases (EGIDs). J Ped Gastroenterol Nutr. 2008;47:234-8.
9. Baig MA, Qadi A, Rasheed JA. Review of Eosinophilic Gastroenteritis. J National Med Assoc. 2006;98:1616-9.
10. Straumann A. Idiopathic eosinophilic gastrointestinal diseases in adults. Best Pract Res Clinical Gastroenterol. 2008;22(3):481-96.
11. Okpara N, Aswad B, Baffy G. Eosinophilic colitis. World J Gastroenterol. 2009;15:2975-9.
12. Talley NJ, Shorter RG, Phillips SF, Zinsmeister AR. Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer and subserosal tissues. Gut 1990;31:54-8.
13. Solans R, Bosch JA, Perez-Bocanegra C, Selva A, Huguet P, Alijotas J, et al. Churg-Strauss syndrome: outcome and longterm follow up of 32 patients. Rheumatology (Oxford). 2001;40:763-71.
14. DeSchryver-Kecskemeti K, Clouse RE. A previously unrecognized subgroup of 'eosinophilic gastroenteritis'. Association with connective tissue diseases. Am J Surg Pathol. 1984;8:171-80.
15. Muller MJ, Sewell GS. Coexistence of eosinophilic gastroenteritis and Helicobacter pylori gastritis: causality versus coincidence. Dig Dis Sci. 2001;46:1784-6.
16. Macedo T, MacCarty RL. Eosinophilic ileocolitis secondary to Enterobius vermicularis: case report. Abdom Imaging. 2000;25:530-2.
17. Scudamore HH, Phillips SF, Swedlund HA, Gleich GJ. Food allergy manifested by eosinophilia, elevated immunoglobulin E level, and protein-losing enteropathy: the syndrome of allergic gastroenteropathy. J Allergy Clinical Immunol. 1982 Aug 1;70(2):129-38.

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Original Article

Internal Medicine Section

Effect of Age and Hypertension on Cognition: A Cross-sectional Study

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ABSTRACT

Introduction: Cognitive impairment has evolved as major health and social issue. As age advances cognitive tasks involving processing speed, working memory and executive function get affected. Cognitive impairment is also influenced by modifiable risk factors such as hypertension, diabetes mellitus and hypercholesterolemia.

Aim: To find out the effect of age and hypertension on cognition.

Materials and Methods: The total study participants were divided into three groups based on their age as group I (18-29) group II (30-59) and group III (above 60) with 20 participants (n= 20) in each group. Their cognition was assessed using Mini Mental State Examination, Picture word Learning test, Stroop colour word test, Letter digit coding test, Verbal fluency and Logic memory tests. Disease history and known co morbidities (hypertension) were noted. Post-hoc test (Bonferroni) was employed to assess age-wise comparison of cognition and Pearson's chi-square test was used to compare cognition between hypertensive and normotensives.

Results: Group II had impaired cognition when compared with group I (p-value- 0.000). Group III had impaired cognition when compared with group I (p-value- 0.000) and group II (p-value 0.001). The hypertensives in group II and III had statistically significant impairment in cognition when compared with normotensives (p-value -0.001) in the same group.

Conclusion: Cognitive functions are impaired as a result of increase in age. Furthermore, there is also a significant decrease in cognitive functions amongst those suffering from hypertension. Hence, we conclude there is decrease in cognitive function as age advances and the co-existence of hypertension hastens this decline.

Keywords: Cognitive impairment, Mini mental state examination, Old age

INTRODUCTION

The word cognition comes from the Latin verb *cognosco* meaning 'I know, perceive' or 'to recognise or conceptualise. Cognitive functions are those which include cerebral activities that lead to knowledge and encompass reasoning, memory, attention and language. Ageing can also have diverse effects on cognitive function. The spectrum of cognitive decline ranges from normal cognitive ageing (healthy" or "successful" Ageing), mild Cognitive impairment (does not prevent them from performing daily activities) and severe cognitive deficits (impede the ability to live independently [1]. Cognitive impairment has evolved as major health and social issue as the life span of both men and women has increased dramatically. Cognitive impairment accounts for about 40% of hospital admissions among the older individuals [2]. As age advances cognitive tasks involving rapid processing and transformation of information, which includes processing speed, working memory and executive function gets affected [3].

These age-related cognitive declining results from structural and functional changes that affect neuronal structure like loss of synapses and dysfunction of neuronal networks [4]. Cognitive impairment is also influenced by modifiable risk factors such as hypertension, diabetes mellitus and hypercholesterolemia [5]. Among these factors hypertension, which causes vascular changes resulting in cerebral infarction and diffuse ischaemic changes contribute to cognitive impairment [6].

Age related cognitive decline is already stated by several studies [7,8] earlier; but none of them used six tests which examine the different domains of cognition and there are only a few studies [9,10] that have included co-morbid condition such as hypertension to study the decline in cognition as age advances.

Hence, we aimed to conduct a study to find out the effect of age on cognition with the help of series of cognitive assessment tests. In this study we also analysed the effect of more common and prevalent (33.8% in urban parts of India) [11] co-morbid condition, hypertension on cognition especially amongst the vulnerable elderly group of individuals.

MATERIALS AND METHODS

This cross-sectional study was conducted during the month of January 2014 for the period of two months among students, staff and patients of PSG Institute of Medical Sciences and Research, Coimbatore after obtaining the ethical clearance from institutional ethical committee (Ethics registration file No 13/328). A total of 60 participants after getting their written informed consent were included in the study. Participants were grouped based on their age Group-I (18-29), Group II (30-59), Group III (above 60). Participants younger than 18 years of age and those suffering from co-morbidities apart from hypertension such as diabetes mellitus; chronic respiratory diseases, chronic liver diseases, thyroid diseases, renal and neurological disorders were excluded from the study. Hypertensive participants (Systolic BP of more than 140mm Hg, Diastolic BP of more than 90 mm Hg) for more than two years duration and on anti hypertensive treatment were included the study.

Study participants were assessed for their cognitive performance by using a series of tests. An initial interview of subjects was conducted where they were asked about their education, occupation and age at retirement for group III. Disease history and known co-morbidities (hypertension) were noted.

Mini Mental State Examination [12]

It is an 11 question measure that tests five areas of cognitive function: orientation, registration, attention and calculation, recall and language. The maximum score is 30, a score of 23 or lower is indicative of cognitive impairment.

Picture Word Learning Test [13] Verbal Learning Test of Long-Term Memory

Fifteen pictures were successively presented at a rate of 1 per 2 seconds and the subjects were asked to recall as many pictures as possible. This was carried out three times. After 20 minutes delayed recall was performed. The main outcome variables are the accumulated number of recalled pictures over the three learning trials and the number of pictures recalled at delayed recall.

Stroop Colour Word Test [13]

This test involves reading colour names printed in incongruously coloured ink for example "green" printed in blue letters where the subject is to say "blue". The main outcome variable is the time needed for the test.

Letter Digit Coding Test [14]

This test is used as a measure of the speed of processing the general information. The subject is asked to fill in digits near letters, according to a key presented on the top of the test sheet (with 9 consonants in random order), and to work as fast as possible. The outcome variable in the total number of correct variable is 60 seconds.

Verbal Fluency [15]

The participant has to say as many words as possible from a category in a given time (usually 60 seconds). This category can be phonemic for example words starting with the letter "p". The outcome measure is the total number of words in 60 seconds.

Logic Memory I [16]

A story was read out to the participants and they were asked a few questions based on it. The outcome measure was the number of correct answers to the questions.

The test results were scored as good, moderate and severe and decline in cognition was noted. Data acquisition was done in an excel sheet and the scores were analysed among different age groups to study the effect of age and hypertension on cognitive function.

STATISTICAL ANALYSIS

Data was entered, analysed using SPSS software version 19. Descriptive statistics was employed for analysis. Data was expressed in percentages for categorical variables. Post-hoc Test – Bonferroni and Pearson's chi-square test was used to analyse categorical variables.

RESULTS

Study participants were divided into three groups based on their age as group I (18-29), group II (30-59) and group III (above 60) with 20 participants in each group and their mean age in each group was found to be 19.3,40.75,67.75 years, respectively. In group I there were 12 males and 8 females, group II comprised of 11 males and 9 females and group consisted of 15 males and 5 females. Group I had nil hypertensives whereas group II and group III had three and six hypertensives respectively.

On analysing the scores of the cognitive tests in the age group I (18-29), 15% of the individuals had cognitive impairment based on Picture Word Learning test, 5% based on Letter Digit Coding, 65% based on verbal fluency and 20% based on logic memory. In group II (30-59), we observed cognitive impairment in all the individuals based on verbal fluency, 70-75% based on picture word learning test and logic memory. Less (10-40%) individuals were found to have cognitive impairment based on Mini mental state examination, stroop colour word test and logic memory. In group III (above 60 years) cognitive impairment was found to be 100% based on verbal fluency, logic memory and picture word learning test, 45% impairment based on Mini mental state examination, stroop colour word test and 65% based on letter digit coding [Table/Fig-1]. Significantly low scores of cognition were observed in group II and group III when compared between the three groups [Table/Fig-2].

When the hypertensives and normotensives were compared, group II hypertensives showed a statistically significant impaired cognition based on stroop colour word, verbal fluency and logic memory test whereas group III did not show statistically significant difference in their cognition level [Table/Fig-3].

DISCUSSION

In the present study it was observed that certain cognitive domains are subjected to the decline as age advances. This may be due to physiological process such as inflammation, neurobiological changes, diet and life style [17]. In our study, we found that there is increase in cognitive impairment among older individuals when compared to middle-aged and younger individuals as there occurs changes in sensory perception inhibitory ability, processing speed, memory and reasoning which lead to cognitive decline.

Results from this study revealed ability of processing speed starts to decline from the third decade of life and the impact of the loss of speed processing affects other cognitive domains such as memory, spatial ability, reasoning, working memory, executive functions, orientation, recall ability, registration and calculation. Salthouse TA had also suggested that processing speed affects cognitive function [7].

Tests	Group I		Group II		Group III	
	Good cognition	Impaired cognition	Good cognition	Impaired cognition	Good cognition	Impaired cognition
Picture word learning test	85% (17)	15% (3)	30% (6)	70% (14)	10% (2)	100% (18)
Stroop colour word	100% (20)	-	70%(14)	30% (6)	55% (11)	45% (9)
Letter digit coding	95% (19)	5% (1)	60% (12)	40% (8)	35% (7)	65% (13)
Mini mental state examination	100% (20)	-	90% (18)	10%(2)	55% (11)	45% (9)
Verbal fluency	35% (7)	65% (13)	-	100% (20)	25% (5)	100% (15)
Logic memory	16% (16)	20% (4)	25% (5)	75% (15)	-	100% (20)

[Table/Fig-1]: Cognitive status among different age groups.

Comparison between Age Groups		p-value					
		PWL	SCW	LDC	MMSE	VF	LM
Group I	Group II	0.004*	0.256	0.057	1.000	0.000*	0.000*
Group I	Group III	0.000*	0.001*	0.000*	0.001*	0.000*	0.000*
Group II	Group III	0.001*	0.137	0.226	0.006*	1.000	0.021*

[Table/Fig-2]: Age-wise comparison of cognition – Post-hoc Test - (Bonferroni).
* Significant (p-value <.05) PWL – Picture Word Learning test SCW- Stroop Colour Word test LDC – Letter Digit Coding MMSE – Mini Mental State Examination VF – Verbal Fluency LM – Logic Memory

Tests	Age Groups		
	Group I	Group II	Group III
PWL	[a]	0.219	0.709
SCW	[a]	0.004*	0.850
LDC	[a]	0.448	0.584
MMSE	[a]	0.531	0.692
VF	[a]	0.001*	0.394
LM	[a]	0.038*	0.143

[Table/Fig-3]: Comparison of cognition between hypertensives and normotensives in different age groups (Pearson's Chi-square test).
[a]No statistics were computed because HTN is a constant.
* Significant (p-value <.05)

Slowing of speed processing plays an integral role in age-related memory changes as well. In our study memory is found to be affected more in older and middle-aged individuals. Burke DM et al., had suggested that as age advances the word-finding difficulties may occur due to weaker memory system which is unable to link one unit to another [18]. Naveh-Benjamin M et al., had also proposed that associative memory deficit in older adults' is due to loss of episodic memory [19].

In our present study, changes were observed among group II and III in mini mental state examination, verbal fluency and stroop colour word tests which gives information on selective and divided attention of the individuals. Hasher L et al., had proposed that older adults' are unable to concentrate on irrelevant information while they are focusing attention on relevant information [20]. Attention impairment was found to be associated with increased incidence of automobile accidents among older individuals [21].

Genetic, medical, psychological, social and lifestyle factors which determines cognitive ageing that impaires people's independence and quality of life [22]. In our study there exists a significant increase in the cognitive impairment among hypertensives belonging to group II and III. It was well-supported by many longitudinal studies that elevated blood pressure is associated with cognitive decline [23].

In our study hypertensives belonging to the age group II showed a statistically significant impaired cognition based on stroop colour word test, verbal fluency and logic memory. Long standing hypertension in some patients may cause not only stroke but also chronic end organ damage of the brain in the form of demyelination of the white matter, with cognitive decline [24]. In the Framingham study, Elias MF et al., had opined that untreated BP level is inversely proportional to cognitive function [25]. Launer LJ et al., had reported in his study that midlife systolic blood pressure is a significant predictor of reduced cognitive function in later life. He also suggested that early control of systolic blood pressure levels may reduce the risk of cognitive impairment in old age [26].

Several follow-up studies conducted among hypertensives showed inverse relationship of systolic as well as diastolic blood pressure with their cognitive performance [25,27]. In a study conducted among hypertensives of various stages, Andre-Petersson L et al., observed stage 3 Hypertension was associated with lower performance on psychomotor, speed and visuo-spatial memory tests; stage 1 hypertension is associated with higher performance on tests measuring verbal and constructional ability [28].

LIMITATION

The small sample size is a limitation of this study. The factors like ethnicity, hyperinsulinaemia, homocystinaemia, duration of hypertension, level of blood pressure control and choice of antihypertensive treatment affect cognition. There are studies that have mentioned the protective effect of antihypertensive drugs over cognitive decline on hypertensive patients as age advances. In this study these factors were not taken into account and add to the limitations.

CONCLUSION

Cognitive domains such as general intellect, reaction time, memory, vigilance and attention are impaired as a result of increase in age. Furthermore, there is also a significant decrease in cognitive functions amongst those suffering from hypertension. Hence, we conclude there is decrease in cognitive function as age advances and the co existence of hypertension hastens this decline.

REFERENCES

[1] O'Caoimh R, Timmons S, Molloy DW. Screening of mild cognitive impairment: Comparison of "MCI Specific" screening instruments. J Alzheimes Dis. 2016;51(2):619-29.
[2] Gruber-Baldini AL, Zimmerman S, Morrison RS, Grattan LM, Hebel JR, Dolan MM, et al. Cognitive impairment in hip fracture patients; timing of detection and longitudinal follow up. J Am Geriatr Soc. 2003;51:1227-36.
[3] Glisky EL. Changes in cognitive function in human ageing in Brain Ageing: Models, Methods, and Mechanisms, ed. D. R. Riddle (Boca Ratón, FL: CRC Press). 2007; 3–20
[4] Murman DL. The impact of age on cognition. Seminars in Hearing. 2015;36(3):111-21.
[5] Ganguli M, Fu B, Snitz BE, Unverzagt FW, Loewenstein DA, Hughes TF, et al. Vascular risk factors and cognitive decline in a population sample. Alzheimer Disease and Associated Disorders. 2014;28(1):9-15.
[6] Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2011;42(9):2672-713.
[7] Salthouse TA. When does age-related cognitive decline begin? Neurobiology of ageing. 2009;30(4):507-14
[8] Harada CN, Natelson Love MC, Triebel K. Normal Cognitive Ageing. Clinics in geriatric medicine. 2013;29(4):737-52.
[9] Lanari A, Silvestrelli G, De Dominicis P, Tomassoni D, Amenta F, Parnetti L. Arterial hypertension and cognitive dysfunction in physiologic and pathologic ageing of the brain. Am J Geriatr Cardiol. 2007;16(3):158-64.
[10] Cherubini A, Lowenthal DT, Paran E, Mecocci P, Williams LS, Senin U. Hypertension and cognitive function in the elderly.Am J Ther. 2007;14(6): 533-54.
[11] Anchala R, Kannuri NK, Pant H, Khan H, Franco OH, Di Angelantonio E, et al. Hypertension in India: a systematic review and meta- analysis of prevalence, awareness and control of hypertension. Journal of Hypertension. 2014;32(6):1170-77.
[12] Pangman VC, Sloan J, Guse L. An Examination of psychometric properties of the Mini-Mental Status Examination and the standardized Mini – Mental Status Examination: Implications for Clinical Practice. Applied Nursing Research. 2000;13(4):209-13.
[13] Stroop JR Studies of interference in serial verbal reactions. Journal of Experimental Psychology. 1992;2(1):15-23.
[14] Nouchi R, Taki Y, Takeuchi H, Hashizume H, Nozawa T, Kambara T, et al. Brain training game boosts executive functions, working memory and processing speed in the young adults: a randomized controlled trial. PLoS ONE. 2013;8(2):e55518. https://doi.org/10.1371/journal.pone.0055518.
[15] Contador I, Almondes K, Fernndez- Calvo B, Boycheva E. Semantic verbal fluency: normative data in older Spanish adults from NEDICES population- based cohort. Archives of Neuropsychology. 2016;31(8):954-62.
[16] Rabin LA, Barr WB, Bertin LA. Assessment practices of clinical neuropsychologists in the United States and Canada: a survery of INS, NAN and APA division 40 members. Archives of Clinical Neuropsychology. 2005:20:33-65.
[17] Ian J, Deary, Janie Corley, Alan J, Sarah E, Lorna M. Age – associated cognitive decline. Br Med Bull. 2009;92:135-52.
[18] Burke DM, Shafto MA. Ageing and language production. Curr Dir Psychol Sci. 2004;13(1):21–24.
[19] Naveh Benjamin M, Brav TK, Levv O. The associative memory deficit of older adults: the role of strategy utilization. Psychol Ageing. 2007;22(1):202-08.
[20] Hasher L, Chung C, May CP, Foong N. Age, Time of Testing, and Proactive Interference. Canadian Journal of Experimental Psychology. 2002,56(3):200-07.
[21] Marottoli RA, Cooney Jr LM, Wagner DR, Doucette J, Tinetti ME. Predictors of automobile crashes and moving violations among elderly drivers. Ann Intern Med. 1994;121:842-46.
[22] Williams KN, Kemper S. Interventions to reduce cognitive decline in ageing. J Psychosoc Nurs Ment Health Serv. 2010;48(5):42.
[23] Wysocki M, Luo X, Schmeidler J, Dahlman K, Lesser GT, Grossman H, et al. Hypertension is associated with cognitive decline in elderly people at high risk for dementia. The American Journal of Geriatric Psychiatry. 2012;20(2):179-87.
[24] Van Swieten JC, Geyskes GG, Derix MM, Peeck BM, Ramos LM, Van Latum JC, et al. Hypertension in the elderly is associated with white matter lesions and cognitive decline. Ann Neurol. 1991;30(6):825-30.

[25] Elias MF, Wolf PA, D'Agostino RB, Cobb J, White LR. Untreated blood pressure level is inversely related to cognitive functioning: the framingham study. Am J Epidemiol. 1993;138(6):353-64.
[26] Launer LJ, Masaki K, Petrovitch H, Foley D, Havlik RJ. The association between midlife blood pressure levels and late life cognitive function. The Honolulu – Asia ageing study. JAMA.1995;274:1846-51.
[27] Rebecca P, Webster G, Helen P, Kamal H, Lenore J, Lon R. Antihypertensive medication use and risk of cognitive impairment: the Honolulu – Asia gaining study. Neurology. 2013;81(10):888-95.
[28] Andre- Petersson L, Hagberg B, Janzon L. a comparison of cognitive ability in normotensive and hypertensive 68-year-old men: results from population study "men born in 1914" In Malmo, Sweden. Exp Ageing Res. 2001;27(4):319-40.

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Case Report

Human brucellosis in a non-susceptible host: a case report

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ABSTRACT

Clinical human brucellosis is quite rare in India, that too in an non susceptible host. This report describes a case of clinical human brucellosis in India. This case involved a 36 years old male, a professor in an Engineering college. He presented with complaints of fever for 4 months with myalgia, generalized tiredness, loss of weight and loss of appetite. On evaluation, total counts and ESR was elevated and all other investigations were normal. Based on history, common causes like occult tuberculosis or autoimmune diseases were considered. But, blood cultures grew *Brucella melitensis* in all 2 samples and a diagnosis of brucellosis was made. He was treated with Rifampicin and Doxycycline. Suspicion of brucellosis in this patient is low because the patient has no history of contact with animals, consumption of unpasteurized milk or occupational exposure. So, the health care practitioners should be aware of this possibility of this zoonotic infection as a differential diagnosis in patients with nonspecific symptoms and unexplained prolonged fever.

Keywords: Brucellosis, Non-susceptible host, Prolonged fever

INTRODUCTION

Burcellosis is a multisystem disease with a broad spectrum of nonspecific symptoms that usually occurs within 2 weeks but up to 3 months after inoculation. It is usually due to consumption of unpasteurized contaminated goat's milk or soft cheese that had been infected with *Brucella melitensis*.[1] They are small non-motile, nonsporing, non-capsulated gram-negative coccobacilli that usually infects goats and sheep.[2] The principal organizsm causing worldwide brucellosis is *Brucella melitensis*. It is the most pathogenic and invasive one, followed by *Brucella suis*, *Brucella abortus*, *Brucella canis*, *Brucella pinnipediae* and *Brucella cetaceae*.[3]

Human brucellosis is commonly found in countries with rural communities that live with close association with animals and its prevalence in a region depends on factors such as methods of processing milk and milk products, food habits, social economic status, hygiene and climate.[4] Both human and animal brucellosis are still endemic in some parts of the world such as Pakistan, China and Srilanka.[5-7]

The clinical features of brucellosis are nonspecific and depends upon the stage of the disease and the organs and systems involved. The most commonly reported symptoms are undulating fever, fatigue, malaise, chills, sweats which may be characterized by a peculiar odour at night, insomnia, headache, myalgia, arthralgia, anorexia, weight loss, hepatomegaly and splenomegaly. Brucellosis can also manifest as a localized disease affecting the central and peripheral nervous system, gastrointestinal tract, genitourinary tract and heart.[8]

Without the critical epidemiological clue to exposure to animals or consumption of unpasteurized dairy products,

this disease can pose a great diagnostic challenge to the clinician. Authors report a case of human brucellosis in a non- susceptible host.

CASE REPORT

36 years old male presented with complaints of fever for 4 months, which was associated with myalgia, generalized tiredness, loss of weight and loss of appetite. There was no history of contact with animals, consumption of unpasteurized milk or occupational exposure. General examination was unremarkable. Abdominal palpation revealed hepatosplenomegaly. His hemoglobin and platelet levels were within normal limits, while total counts showed leucopenia with monocytosis. Erythrocyte sedimentation rate was also elevated. Other basic biochemical tests were within normal limits, except for mild transaminitis.

At this moment, either occult tuberculosis or autoimmune diseases were suspected based on the history, clinical and laboratory findings. However, blood cultures were taken, and broad-spectrum antibiotics were started. CECT chest and CECT abdomen were not suggestive of occult TB infection. Autoimmune workup was negative. As a part of workup for fever of longer duration, Brucella agglutination test and scrub IgM serology were sent. Brucella agglutination test turned out to be positive. Simultaneously, blood culture grew *Brucella melitensis* in all 2 samples.

Based on his serological test and blood cultures, a diagnosis of brucellosis was made. He was eventually prescribed Rifampicin once daily and Doxycycline BD for 6 consecutive weeks.

DISCUSSION

In this case, the patient did not have any history of living in close association with animals or consumption of unpasteurized milk. He also did not have any occupational risk of exposure to brucella. Nevertheless, human brucellosis is quite rare in South India, making the suspicion level among clinicians to be quiet low.

There was a long interval between onset of symptoms and diagnosis in our patient. A lot of health care practitioners do not consider the possibility of brucellosis, especially in patients with prolonged fever. Delay or misdiagnosis can lead to treatment failure, relapses, focal complications and high case fatality rate.[9]

This patient showed many of the symptoms of brucellosis which included undulating fever, myalgia and other clinical features such as hepatomegaly and splenomegaly.[10]

Infective endocarditis is a rare devastating complication from systemic brucellosis and could require surgical intervention. Splenic, hepatic or pulmonary abscess can also develop. Other rare complications include deep vein thrombosis, arthritis, spondylitis, meningitis and nephritis. Ocular manifestations include optic neuritis and uveitis.[11] Common hematological manifestations include leucopenia, anemia and thrombocytopenia.[12]

The standard treatment for human brucellosis is doxycycline, 100mg BD and rifampicin 600-900mg once a day for 6 consecutive weeks. A combination of doxycycline and gentamycin is also preferred. However, the standard treatment varies depending on the patients' age, pregnancy status, CNS involvement and cardiovascular involvement.[13] For children less than 8 years of age, a combination of TMP-SMX and aminoglycoside or a combination of rifampicin and TMP-SMX for 45 days is needed.[14,15]

CONCLUSION

Diagnosis of brucellosis is generally not considered in a non-endemic country. Health care practitioners should be aware of this possibility of this zoonotic infection as a differential diagnosis in patients with nonspecific symptoms and unexplained prolonged fever.

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REFERENCES

1. Ramin B, MacPherson P. Human brucellosis. Br Med J. 2013;341:c4545.
2. Cohen J, Powderly WG, Opal SM, Calandra T, Clumeck N, et al. Infectious diseases. 3rd ed. China: Mosby. 2010.
3. Mantur BG, Amarnath SK, Shinde RS. Review of clinical and laboratory features of human brucellosis. Indian J Med Microbiol. 2007;25(3):188-202.
4. Gwida M, Al Dahouk S, Melzer F, Rösler U, Neubauer H, Tomaso H. Brucellosis–regionally emerging zoonotic disease?. Croatian Med J. 2010;51(4):289-95.
5. Akhtar R, Chaudhry ZI, Shakoori AR, Ahmad M, Aslam A. Comparative efficacy of conventional diagnostic methods and evaluation of polymerase chain reaction for the diagnosis of bovine brucellosis. Vet World. 2010;3(2):53-6.
6. Zhang Z, Yu S, Wang X, Dong S, Xu J, Wang J, et al. Human brucellosis in the People's Republic of China during 2005-2010. Int J Infect Dis. 2013;17(5):e289-e292.
7. Priyantha MA. Identification of biovars of Brucella abortus in aborted cattle and buffaloes herd in Sri Lanka. Vet World. 2011;4:542-5.
8. Franco MP, Mulder M, Gilman RH, Smits HL, 2007. Human brucellosis. Lancet Infect Dis 7: 775-86.

9. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: a re-emerging zoonosis. Veterinary Microbiol. 2010;140(3-4):392-8.
10. Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, Zinsstag J. Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS neglected tropical diseases. 2012;6(12):e1929.
11. Rolando I, Olarte L, Vilchez G, Lluncor M, Otero L, Paris M, Carrillo C, Gotuzzo E. Ocular manifestations associated with brucellosis: a 26-year experience in Peru. Clin Infect Dis. 2008;46(9):1338-45.
12. Sari I, Altuntas F, Hacioglu S, Kocyigit I, Sevinc A, Sacar S, et al. A multicenter retrospective study defining the clinical and hematological manifestations of brucellosis and pancytopenia in a large series: Hematological malignancies, the unusual cause of pancytopenia in patients with brucellosis. Am J Hematol. 2008;83(4):334-9.
13. Gilbert DN, Chambers HF, Eliopolus GM, Saag MS, Pavia AT, et al. Guide to antimicrobial therapy 2016. 46th ed. USA: Antimicrobial Therapy Inc; 2016.
14. Lubani MM, Dudin KI, Sharda DC, Ndhar DS, Araj GF, Hafez HA et al. A multicenter therapeutic study of 1100 children with brucellosis. Pediatr Infectious Dis J. 1989;8(2):75-8.
15. Solera J, Martinez-Alfaro E, Espinosa A. Recognition and optimum treatment of brucellosis. Drugs. 1997;53(2):245-56.

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Non Hodgkin's lymphoma of B cell type with hemophagocytosis in a patient with HIV infection

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Abstract

Human immunodeficiency virus causes chronic infection of the immune system leading to reduction of CD4 helper cells, making the patient prone for many opportunistic infections. The incidence of opportunistic infections is increased in the presence of low CD4 count. Here, we present a case of HIV infection presenting with mediastinal lymphadenopathy, treated as disseminated tuberculosis which then developed pancytopenia with hyperbilirubinemia. He was finally diagnosed to have non Hodgkin's lymphoma of B cell type with hemophagocytic syndrome at postmortem. We emphasize that when a HIV patient has a relatively higher CD4 count non infectious causes should be kept in mind while empirically treating for opportunistic infections.

Keywords

AIDS/HIV; chronic infection; mmune system

Introduction

Acquired immuno deficiency syndrome (AIDS) is the end stage of the spectrum of disease caused by human immuno deficiency virus (HIV). Decline in the immunity is a direct result of CD4 cell destruction by the virus, leading to increase in occurrence of neoplasms and opportunistic infections. Lower CD4 counts can be correlated with higher incidence of opportunistic infections. We present a 45 year old male with HIV infection, on empirical anti-tuberculosis therapy (based on pulmonary imaging) who developed pancytopenia with indirect hyperbilirubinemia. Analysis of this case allows a focused approach to arriving at differentials in patients presenting as such. This case also stresses the importance of considering non infectious causes during evaluation of opportunistic infections in AIDS and also the consequences of delay in diagnosis, a compromise usually made in resource limited settings.

Case Report

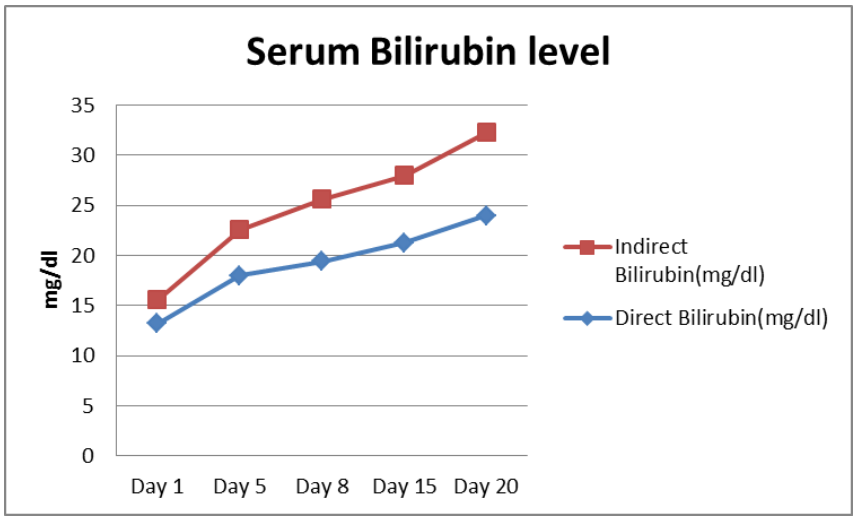
A 45 year old male with retroviral infection for six months presented with yellowish discoloration of sclera, abdominal pain for two days associated with loss of weight and appetite, pale colored stools and pruritus.

Two months prior to current admission, he presented to another center with fever of one month

duration lacking localizing symptoms. Computed Tomography study of the chest and abdomen revealed the presence of multiple retroperitoneal lymph nodes and bronchiectasis, for which antituberculous therapy was initiated. (Isoniazid + Rifampicin + Pyrazinamide + Ethambutol). Investigations done two months prior to current admission as per the records were: Hemoglobin (Hb) – 12.3 g%, Total WBC count – 6300 cells/cmm, Platelets – 1,40,000 cells/cmm, Erythrocyte Sedimentation Rate (ESR) – 88mm in 1 hour, Creatinine – 0.8mg/dl, Bilirubin – 0.6 mg%, Alanine Transaminase (ALT) – 34IU/L, Aspartate Transaminase (AST) – 24IU/L, Alkaline Phosphatase (ALP)- 258 IU/L, Gamma-Glutamyl Transpeptidase (GGTP)- 312 IU/L. His Prothrombin Time (PT) was 12 seconds and International Normalised ratio (INR) was 1.0. He had tested Human Immunodeficiency Virus (HIV) positive then with a CD4 count at that time of 326 and HIV viral load was 120000 copies. He was initiated on Anti- Retroviral Therapy (ART) with Tenofovir, Emtricitabane and Efavirenz.

He was conscious and oriented on presentation. He was found to exhibit pallor, jaundice and per abdominal examination revealed splenomegaly. He did not have asterixis and there were no focal neurological deficits.

His investigations during the current admission were: Complete haemogram (CBC) revealed pancytopenia (Hb – 8.7g/dl; Total WBC count -3000 cells/cmm; Platelets- 68,000/cmm, ESR -86mm in 1 hour). Renal function tests were normal. His liver function test (LFT) showed hyperbilirubinemia with normal ALT and AST with increase in ALP (324 IU/L) and GGTP (314IU/L). INR was 1.1. In view of hyperbilirubinemia with normal ALT, Rifampicin was stopped and other medications were continued. Despite these efforts, there was a progressive worsening of his LFT (Graph 1), hence ATT and ART drugs were withheld. Ultrasound of abdomen showed normal liver echo texture, no intra hepatic biliary radicle dilatation or common bile duct dilatation and splenomegaly. Serology for Hepatitis A, B, and C, E viruses, Cytomegalovirus (CMV) and Epstein Barr virus (EBV) were negative. Hepatitis B and Hepatitis C viruses were not detectable. Autoimmune workup (Anti-nuclear antibody (ANA), Anti Liver Kidney Microsomal (LKM) antibody) was also negative. Serum procalcitonin was carried out to rule out bacterial sepsis as a cause for cholestasis, but was found to be within normal limits. Repeated blood, urine cultures for bacteria and fungi (using Sabouraud dextrose agar incubated at 22 and 30 degree Celsius) were sterile. Finally, Magnetic resonance cholangio pancreatogram (MRCP) was done to rule out any obstructive pathology and was normal.

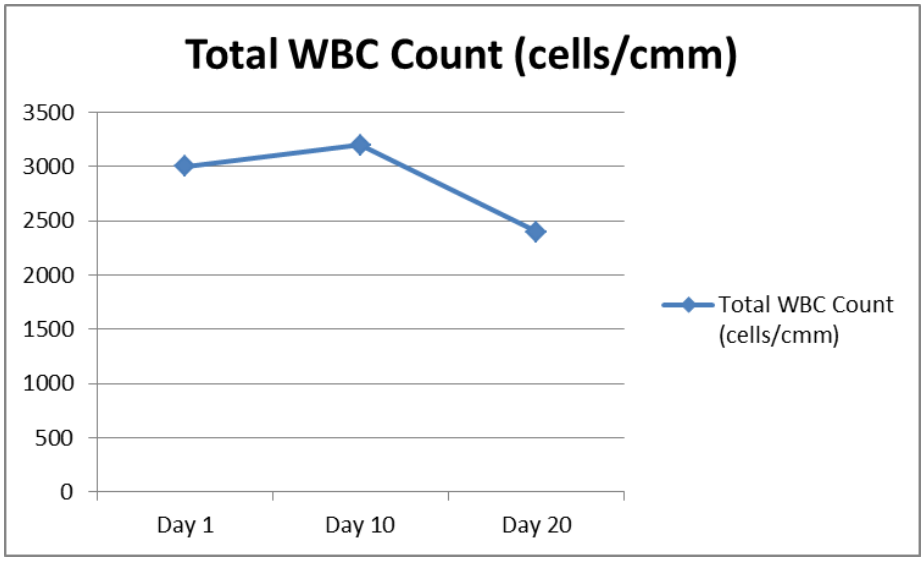


Graph 1: Serum Bilirubin level during the hospital stay.

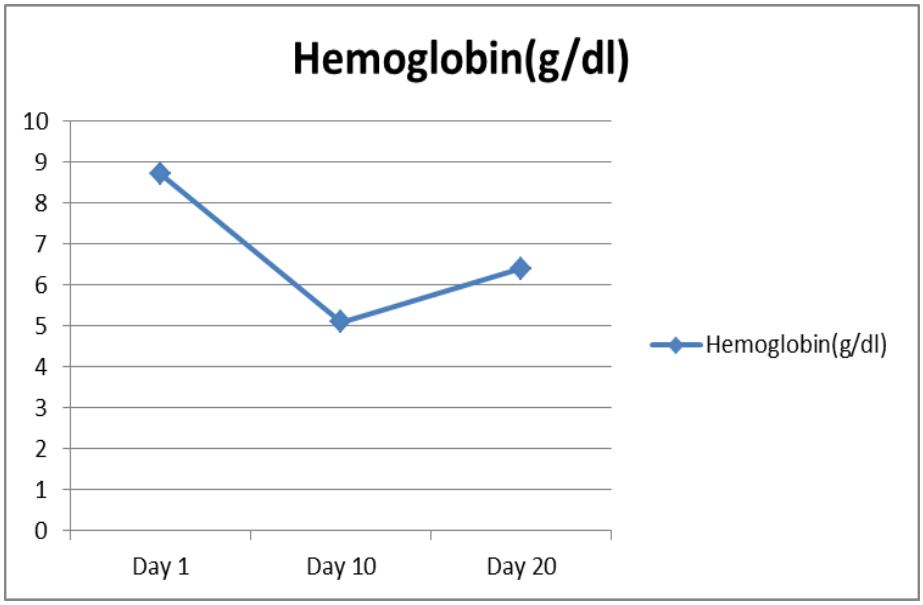
On the tenth day of admission at our centre, a complete blood count was done, showing pancytopenia (Total WBC count – 3200 cells/cmm, Hb – 5.1g/dl, Platelet count – 64,000 cells/cmm) and reticulocyte count was 14%. Serum vitamin B12 and Folic acid were within normal limits. Anaemia profile was suggestive of anaemia of chronic disease. Subsequently, bone marrow aspiration and biopsy showed a hypercellular marrow without any granuloma, fibrosis, atypical cells, hemophagocytic macrophages or infiltrative lesions.

During the course of his hospital stay, it was seen that his blood counts displayed a decreasing trend.

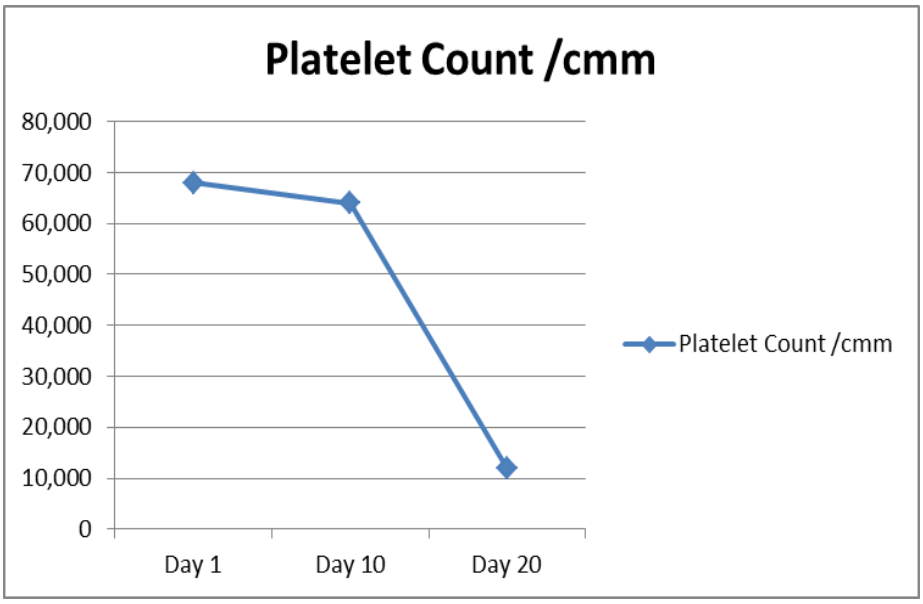
The changing trend in Total WBC counts, Hb and Platelet count during the course of hospital stay was as follows:



Graph 2: Total WBC count during hospital stay



Graph 3: Hemoglobin changes during hospital stay



Graph 4: Platelet count during hospitalization

He was treated with supportive medications including blood and blood product transfusions. Lymph node/liver biopsy was planned, but decision to carry out the procedure was deferred in view of thrombocytopenia. In spite of these efforts, the patient developed complications of intracranial cranial hemorrhage and expired. After obtaining the required permissions from his near relations, postmortem liver and spleen biopsy were done.

The sections taken from the biopsied spleen and liver showed fragments of splenic tissue and liver tissue with congestion and one fragment showed infiltration by a population of pleomorphic cells. Morphologically, the cells were round with abundant cytoplasm and irregular vesicular nuclei. Occasional giant cells were seen (Figure 1A&1B). Portal tracts were not visualised in the sections studied. In addition to these findings, the liver tissue showed intracellular cholestasis, evidence of hemophagocytosis in liver cells and sinusoidal dilation (Figure 1C&1D). The atypical cells were showing positivity for CD 45 (leukocyte common antigen) and CD 20 (Figure 2A &2B) cellular markers. These findings were consistent with non-Hodgkin's lymphoma of B-cell type with associated hemophagocytosis of liver.

Discussion

The hallmark of infection by the human immunodeficiency virus is the depletion of CD-4 T helper cells and defective macrophage and monocyte functioning. A direct consequence of this is the greater tendency of past tuberculosis reactivation with hastening of its clinical manifestations. Thus, its presentation is more sub-acute than chronic in these patients [1].

Two months prior to admission, our patient was started on ART for retroviral disease and ATT for suspected pulmonary and extra pulmonary tuberculosis. He developed cholestatic jaundice and pancytopenia and deteriorated despite discontinuing possible causative medications. In the setting of HIV infection, possible differential diagnoses for cholestatic jaundice would be medications, opportunistic infections, neoplasms, AIDS cholangiopathy and sclerosing cholangiopathy [2].

In this particular case the cause for cholestasis remained elusive despite extensive investigations.

There was no evidence of any intra-hepatic biliary radicle dilatation on ultrasonography of the abdomen, confirmed by MRCP. Hence intra-hepatic cholestasis was unlikely [3]. Then, cultures of the blood and urine including fungal cultures were done to rule out infections. Procalcitonin was also normal. These findings point towards a non-infectious cause. As certain pharmacological therapies can cause cholestatic jaundice, a detailed drug history was also elicited and none of the drugs he was taking have been implicated as causing cholestasis and pancytopenia. The possibility of HIV cholangiopathy was considered but was thought to be unlikely in view of the progressive cholestasis, no evidence of intrahepatic biliary radicle dilatation in imaging (USG or MRCP) and negative stool for Cryptosporidium parvum [4]. In fact, the presence of jaundice itself is an unusual feature of AIDS cholangiopathy [5] .

On evaluation of progressive pancytopenia, the cause was ultimately not discernible. In view of this bone marrow biopsy was done which showed the presence hypercellular marrow. The common causes for pancytopenia in HIV infection without fever diagnosed using bone marrow biopsy are [6]:

1. Infection

    Parvovirus infection

    Histoplasma capsulatum

    Mycobacterium tuberculosis

    Mycobacterium avium complex

    HIV

    Salmonella typhi

2. Miscellaneous

    Hodgkin's disease

    Hypersplenism

    Hemophagocytic syndrome

The presence of hypercellular marrow, reticulocytosis and splenomegaly was suggestive of either immune pancytopenia [7,8] or hypersplenism. Autoimmune workup (ANA, Coombs test) was negative. The possibility of haemophagocytic lymphohistiocytosis was considered in view of pancytopenia, liver involvement and increased ferritin. However this diagnosis was thought to be unlikely due to the observation that triglycerides were under normal limits, patient was afebrile and bone marrow and peripheral smear analysis did not reveal any correlative evidence. The cause for pancytopenia in this case could be explained by hypersplenism rather than the infiltrative disorder per se due to the combination of elevated reticulocyte count and splenomegaly. An additional explanation for pancytopenia is a paraneoplastic manifestation of lymphoma. However, the negative Coombs test disaffirms this as a cause for this presentation.

Hence the working diagnosis was vanishing bile duct syndrome with hypersplenism leading to pancytopenia (or a coexisting autoimmune pancytopenia). The other likelihood was lymphoma with hemophagocytosis which was ruled out in view of inconclusive bone marrow findings (no atypical cells and no evidence of hemophagocytosis). A liver biopsy was planned but was deferred in view of the

coagulopathy and thrombocytopenia. Our patient's condition continued to decline during the hospital stay and died due to intracranial bleed. Postmortem liver and spleen biopsy revealed non Hodgkin's lymphoma of B cell type with hemophagocytosis. Conclusively, this underscores the importance of arriving at a histopathological diagnosis before initiating ATT.

The final diagnosis was determined to be haemophagocytosis. Its presentation in this patient was nothing short of unusual. This patient was afebrile (although fever occurs in 70% to 100% of haemophagocytic syndromes), had hypertriglyceridemia and bone marrow aspiration and biopsy did not reveal any siginficant evidence to support the diagnosis. A study done by McCall et al has shown that few patients with HLH did not show marrow evidence of haemophagocytosis in the first biopsy but showed it only on later biopsies [9].

Diagnosis of hemophagocytic lymphohistiocytosis (HLH) syndrome relies on clinical, laboratory, and histopathological findings. Diagnostic guidelines were proposed by the Histiocytic Society in 1991 and updated in 2004 is as follows [10]:

The diagnosis of HLH can be established if one of either 1 or 2 of the criteria below is fulfilled.

1. A molecular diagnosis consistent with HLH

2. Diagnostic criteria for HLH are fulfilled (5 of the 8 criteria below):*

Fever

Splenomegaly

Cytopenias (affecting 2-3 lineages in the peripheral blood)

Hypertriglyceridemia and/or hypofibrinogenemia

Hemophagocytosis in BM, spleen, or lymph nodes

Low or absent NK-cell activity (according to local laboratory reference)

Ferritin - 500 g/L

Soluble CD25 (sIL2r) - 2400 U/ml.

Our patient fulfilled only three of the total eight criteria during the diagnostic workup but was established to have haemophagocytosis in liver biopsy at postmortem. This underscores the fact that the diagnostic criterion is primarily implied for conduct of clinical trials and it does not include all the clinical and laboratory features [11,12]. As a result of the complex diagnostic criteria and rarity of the condition the diagnosis is often delayed. Hence a high degree of clinical suspicion is very important in early diagnosis and preventing the high mortality associated with the disease [13]. There have been various causes that have been implicated in the etiology of HLH. The association of lymphoma as an etiology is well known but B cell lymphoma presenting with HLH is quite rare. The cause for pancytopenia was hemophagocytosis with or without paraneoplastic manifestation of Lymphoma [14].

The incidence of Lymphomas is much higher in HIV patients than in the general population [15]. The mode of transmission is not associated with any increase in risk of lymphomas in contrast to Kaposi sarcoma which is much more common in men who have sex with men. Most of the opportunistic

infections occur in low CD4 counts which also holds true for EBV associated Non Hodgkin's Lymphoma but HIV associated Hodgkin's lymphoma occurs in most patients with relatively intact CD4 count [16]. The initial presentation of AIDS related Lymphomas can be very subtle while in others it may be very obvious. Symptoms include but are not limited to low grade fever, unexplained fever, night sweats, adenopathy or organomegaly. The challenge lies in the exploration of various differentials that come up as a result of the incidence of these symptoms, in people diagnosed with AIDS. A high level of lactate dehydrogenase (LDH) is found in aggressive lymphomas and has prognostic implications. Excision biopsies are preferred over needle procedures as they preserve the architecture and immunophenotyping done whenever needed. Neuroimaging should be done at diagnosis as more than 17% of peripheral lymphomas in AIDS patients involve CNS. Treatment of Lymphomas is similar for AIDS and non-AIDS patients. Though with the introduction of HAART the prognosis of lymphomas has improved, the prognosis of lymphomas is worse in AIDS patients than for the same lymphomas in non AIDS patients [17,18,19].

Figures

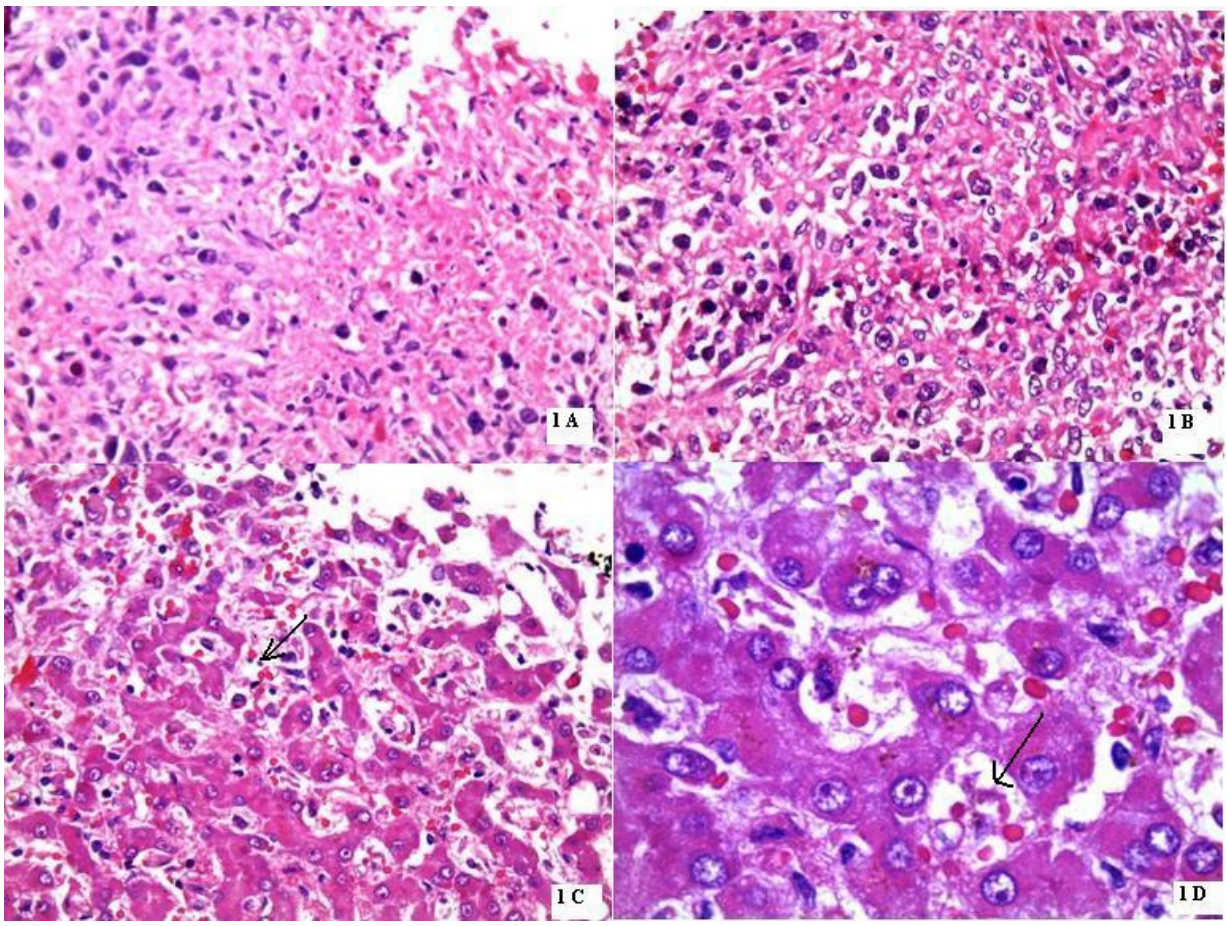


Figure 1: A: Biopsy from liver shows fragment of atypical cell collections. 100 x magnifications, H&E
B: Biopsy from spleen shows fragment of atypical cells 100x magnification, H&E
C: Liver tissue shows hemophagocytosis. 100 x magnification, H&E
D: Liver tissue reveals hemophagocytosis as pointed by the arrow. 400 x magnification, H&E

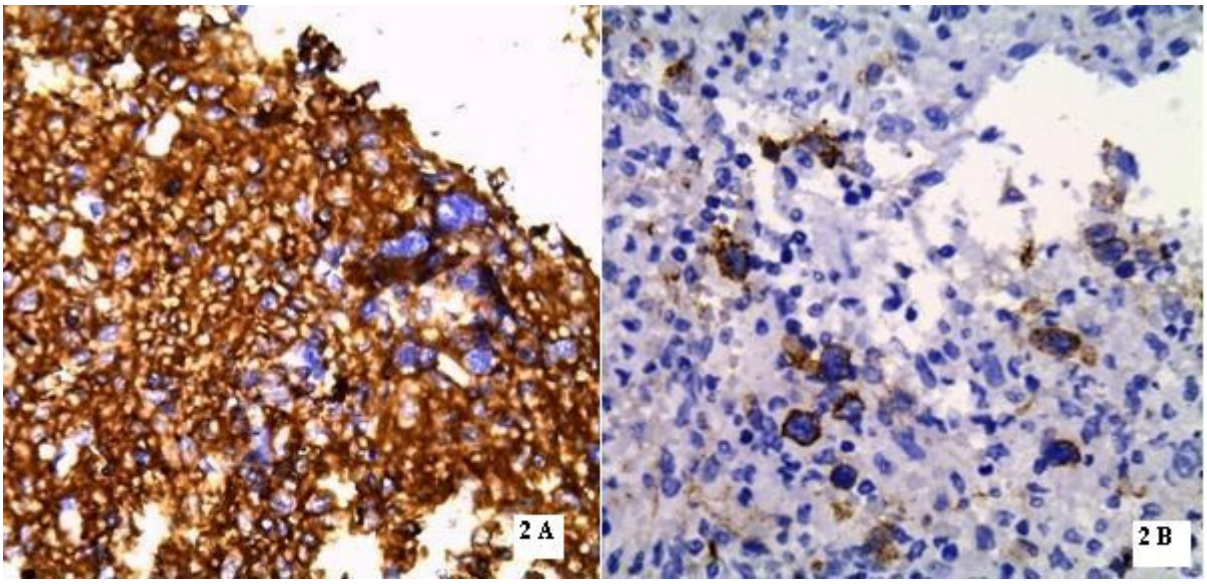


Figure 2: A: The atypical cells express strong positivity for LCA/ CD 45. 100 x magnification
B: Large cells show moderate positivity for CD 20. 100 x magnification

Conclusion

We hereby have described an unusual presentation of lymphoma in a retroviral positive patient. Hemophagocytic syndrome is known to occur with lymphomas but its association is more common with T cell lymphoma than B cell lymphoma, as was concluded in this case. The diagnostic criteria for Hemophagocytic syndrome is meant for conduct of clinical trials. It should be noted, however, that these diagnostic criteria do not reflect all of the typical clinical or laboratory features of patients with HLH, many of which are helpful in making the diagnosis. Hence treatment should not be delayed in fulfilling the criteria. A high degree of clinical suspicion is essential in early diagnosis of hemophagocytic syndrome. The microbiological diagnosis is very important when we evaluate a HIV infected patient for opportunistic infections. It is imperative that non infectious etiologies are considered during evaluation, especially when the CD4 count is not low. There is no role for empirical anti-tuberculous therapy even in resource limited settings.

References

1. Corbett EL, Charalambous S, Moloi VM, Fielding K, Grant AD, Dye C, et al. Human immunodeficiency virus and the prevalence of undiagnosed tuberculosis in African gold miners. Am J Respir Crit Care Med 2004; 170: 673–9.

2. Te HS. Cholestasis in HIV-infected patients. Clin Liver Dis 2004; 8: 213–28, viii–ix.

3. Heathcote EJ. Diagnosis and management of cholestatic liver disease. Clin Gastroenterol Hepatol 2007; 5: 776–82.

4. Bilgin M, Balci NC, Erdogan A, Momtahen AJ, Alkaade S, Rau WS. Hepatobiliary and pancreatic MRI and MRCP findings in patients with HIV infection. AJR Am J Roentgenol 2008; 191: 228–32.

5. Lefkowitch JH. The liver in AIDS. Semin Liver Dis 1997; 17: 335–44.

6. Madrigal-jiménez HM, Hernández-Rivera G. [Usefulness of bone marrow microscopic examination in HIV-infected patients with pancytopenia]. Gac Med Mex; 142: 13–7.

7. Martino R, Muñiz-Díaz E, Arilla M, Ibáñez M, Altés A, Guanyabens C, et al. Combined autoimmune cytopenias. Haematologica; 80: 305–10.

8. Michel M, Chanet V, Dechartres A, Morin A-S, Piette J-C, Cirasino L, et al. The spectrum of Evans syndrome in adults: new insight into the disease based on the analysis of 68 cases. Blood 2009; 114: 3167–72.

9. McCall CM, Mudali S, Arceci RJ, Small D, Fuller S, Gocke CD, et al. Flow Cytometric Findings in Hemophagocytic Lymphohistiocytosis. Am J Clin Pathol 2012; 137: 786–94.

10. Henter J-I, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48: 124–31.

11. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. Blood 2011; 118: 4041–52.

12. Rosado FGN, Kim AS. Hemophagocytic lymphohistiocytosis: an update on diagnosis and pathogenesis. Am J Clin Pathol 2013; 139: 713–27.

13. Weitzman S. Approach to hemophagocytic syndromes. Hematology Am Soc Hematol Educ Program 2011; 2011: 178–83.

14. Alirhayim Z, Dyal H, Alarhayem A, Donthireddy V. Non-Hodgkin's lymphoma: a cause of paraneoplastic cholestasis. *BMJ Case Reports* 2013; doi:10.1136/bcr-2013-009714

15. Crun-Cianflone N., Hullsiek K.H., Marconi V., et al: Trends in the incidence of cancers among HIV-infected persons and the impact of antiretroviral therapy; a 20-year cohort study. AIDS 2009; 2; 23(1): 41-50

16. Krishnan A., Molina A., Zaia J., et al: Durable remissions with autologous stem cell transplantation for high-risk HIV-associated lymphomas. Blood 2005; 105: 874-878.

17. Simard E.P., Pfeiffer R.M., and Engels E.A.: Cumulative incidence of cancer among individuals with AIDS in the United States. Cancer 2011; 117: 1089-1096.

18. Stebbing J., Duru O., and Bower M.: Non-AIDS-defining cancers. CurrOpin Infect Dis 2009; 22: 7-10.

19. Simard E.P., and Engels E.A.: Cancer as a cause of death among people with AIDS in the United States. Clin Infect Dis 2010; 51: 957-962.

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IRIS due to cryptococcal meningitis ten years after the initial episode in a HIV positive patient with a high CD4 count and suppressed viral load – A case report

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Abstract

Cryptococcus neoformans is a fungus which causes opportunistic infection in patients with Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS) with meningitis being the commonest presentation in HIV affected individuals. A CD4 T cell count <200 has been shown to be the strongest risk factor for meningitis, and the mean CD4 count in HIV-related cryptococcal disease is <100 with a range of 75 to 125. Affected individuals are put on secondary fluconazole prophylaxis, in the absence of which 50 to 60% develop relapse. As virtually all patients receive anti-retroviral therapy (ART), relapse and immune reconstitution inflammatory syndrome (IRIS) following ART are well described in the initial months after starting ART. We report a case of late onset IRIS in an immune reconstituted patient with a high CD4 count and suppressed viral load.

Keywords

cryptococcal meningitis relapse, CD4 counts, cryptococcus in HIV

Case Presentation

A 44 year old male was diagnosed with HIV/AIDS (CDC C, WHO 4) in 2004 after presenting with cryptococcal meningitis. His baseline CD 4 count was 16 cells/cmm and HIV viral load was 47,259 copies / ml. His serum and cerebrospinal fluid (CSF) cryptococcal antigen (CRAG) were positive and CSF India ink preparation was positive for cryptococcus. He also had hepatitis B co-infection with a hepatitis B viral load of 750000. He was treated with intravenous amphotericin B deoxycholate and high dose fluconazole. He was then started on ART (zidovudine, lamivudine and efavirenz), improved clinically and was maintained on chronic suppressive therapy with fluconazole till 2006. His serial CD4 counts and HIV viral loads are given in Table 1.

His ART was switched to Tenofovir, Emtricitabine, Efavirenz (TDF/FTC/EFV) in 2009 to treat concomitant hepatitis B. He was admitted in March 2014 with difficulty in walking, increased forgetfulness and severe headache. On examination he had mild neck stiffness. Investigations showed normal haemogram, renal and Liver Function Tests. At this time, CD4 count was 485 cells/cmm and HIV viral load was undetectable [<Less than Detectable Limit (LDL)] (Table 1). MRI brain showed leptomeningeal contrast enhancement suggestive of meningitis with non uniform hydrocephalus with

multiple segmentation (Figure 1, 2).

CSF analysis showed a WBC count of 280 (Neutrophils 1%, Lymphocytes 98%), protein level was 580mg/dl and glucose was 29mg/dl. Serum and CSF cryptococcal antigen (CRAG) were positive. CSF India ink preparation for cryptococci was negative. Gene Xpert for MTB done on CSF was negative. CSF culture for bacteria, fungi and mycobacteria were negative. CSF HIV viral load was 126 copies / ml. He was treated with intravenous amphotericin B and flucytosine, and then switched to fluconazole prophylaxis. He improved clinically and was doing well at 6 month follow up.

Discussion

AIDS related cryptococcal meningitis has been of utmost concern as it is associated with high mortality if not treated with appropriately. Though Cryptococcus affects skin and lungs, meningitis is very common in the HIV affected individuals. It chiefly chooses to affect the severely immune compromised especially in those whose CD4 count is less 100 [1]. It is the second leading cause of morbidity and mortality in AIDS patients [2,3]. Those who are already known cases of HIV, developing cryptococcal meningitis if not under treatment are associated with higher mortality. The other problem encountered in HIV is immune reconstitution inflammatory syndrome which occurs in those who are initiated on ART [4]. "Immune reconstitution inflammatory syndrome" (IRIS) is condition where paradoxical worsening of preexisting infectious processes or unmasking of hidden infections occurs after the initiation of highly active antiretroviral therapy (HAART) in HIV-infected individuals [5]. As the immune function improves after the initiation of HAART, systemic or local inflammatory reactions may occur at the site or sites of the preexisting infection. IRIS is usually a self-limiting condition; but can be life threatening if central nervous system is involved.

Two important complications of cryptococcal meningitis are relapse and IRIS. Relapse has been defined as the reappearance of clinical symptoms (symptomatic relapse) and the recovery of viable cryptococci in previously sterile CSF (microbiological relapse) [6,7] . Our patient did not fit criteria for microbiologic relapse. Reasons for relapse include absence of secondary prophylaxis after an episode of meningitis [8], poor compliance with fluconazole therapy [9] and fluconazole resistance [10,11].

An important cause of symptomatic meningitis is IRIS following immune reconstitution with ART. Fungal cultures are typically negative, and the diagnosis is made by cryptococcal antigen testing and the temporal occurrence after ART [4,7]. Typical risk factors include low CD4 count (usually <50) or early initiation of ART. As we could not see or grow cryptococci from CSF, our patient appeared to have IRIS rather than relapse.

Our patient was unusual in that he became symptomatic 10 years after initiation of ART and 8 years after secondary prophylaxis with fluconazole was discontinued and had a CD4 count of 485 when IRIS occurred. This is distinctly different from the literature which describes IRIS in the first few months after ART inititiation and at low CD4 counts, typically <100 [4,7].

Table

Table 1: CD4 count and HIV viral load over 10 year period

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Cd4 (cells/cmm)	16	85	125	215	175	183	277	300	254	485
HIV Viral load (copies/ml)	47259	<LDL	<LDL	<LDL	<LDL	<LDL	<LDL	<LDL	<LDL	<LDL

Figures



Figure 1: MRI brain showed leptomeningeal contrast enhancement suggestive of meningitis with non uniform hydrocephalus with multiple segmentation

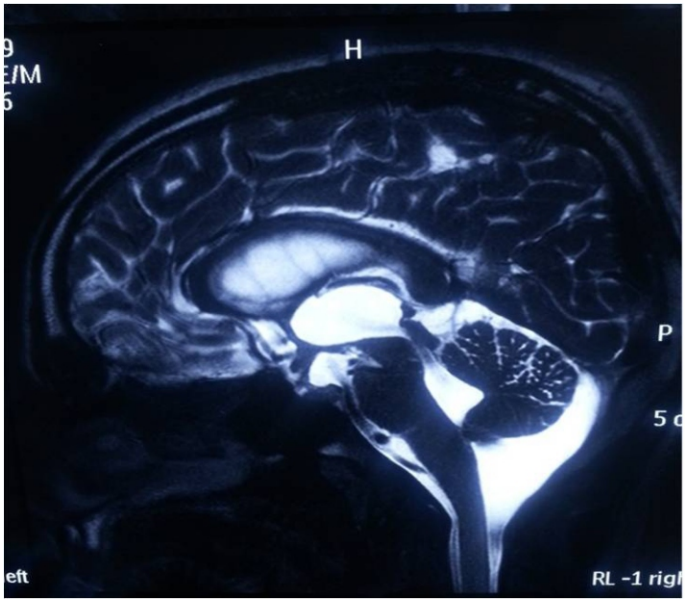


Figure 2: MRI brain showing non uniform hydrocephalus with multiple segmentation

Conclusion

Our case illustrates the importance of remaining vigilant for IRIS in patients with a history of cryptococcal meningitis, even in patients with high CD4 counts and suppressed viral loads many years after the initial episode of meningitis.

References

1. S Majumder, SK Mandal, D Bandyopadhyay. Prognostic Markers in AIDS-related Cryptococcal Meningitis. Journal of Association of Physicians of India 2011. 59; 152-154.

2. Sachdeva RK, Randev S, Sharma A, Wanchu A et al. A retrospective study of AIDS-associated crypto meningitis. AIDS Res Hum Retroviruses 2012. 28: 1220-1226.

3. Jongwutiwes U, Kiertiburanakul S, Sungkanuparph S. Impact of antiretroviral therapy on the relapse of cryptococcosis and survival of HIV infected patients with cryptococcal infection. Curr HIV Res 2007. 5: 355-360.

4. G. Nunnari, M. Gussio, M.R. Pinzone, F. Martellotta et al. Cryptococcal meningitis in an HIV-1-infected person: relapses or IRIS? European Review for Medical and Pharmacological Sciences 2013. 17: 1555-1559.

5. DeSimone JA, Pomerantz RJ, Babinchak TJ. Inflammatory reactions in HIV-1-infected persons after initiation of highly active antiretroviral therapy. Ann Intern Med 2000; 133:447.

6. Abdu K Musubire, David R Boulware, David B Meya, Joshua Rhein. Diagnosis and Management of Cryptococcal Relapse. J AIDS Clinic Res 2013, S3003.

7. Haddow LJ, Colebunders R, Meintjes G, Lawn SD et al. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. Lancet Infect Dis 2010. 10: 791-802.

8. Mussini C, Pezzotti P, Miró JM, Martinez E et al. Discontinuation of maintenance therapy for cryptococcal meningitis in patients with AIDS treated with highly active antiretroviral therapy: an international observational study. Clin Infect Dis 2004. 38: 565-571.

9. Kumar S, Wanchu A, Chakrabarti A, Sharma et al. Cryptococcal meningitis in HIV infected: experience from a North Indian tertiary center. Neurol India 2008. 56: 444-449.

10. Bicanic T, Harrison T, Niepieklo A, Dyakopu N, Meintjes G. Symptomatic relapse of HIV-associated Cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and Immune reconstitution. Clin Infect Dis 2006; 43(8): 1069-1073

11. Musubire AK, Meya BD, Mayanja-Kizza H, Lukande R et al. Challenges in diagnosis and management of Cryptococcal immune reconstitution inflammatory syndrome (IRIS) in resource limited settings. Afr Health Sci 2012. 12: 226-230.

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Basidiobolomycosis - Miss Me; It Hurts You

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Abstract

Basidiobolomycosis is a subcutaneous infection of the limbs and trunk caused by *Basidiobolus ranarum*. It is one of the under diagnosed infection which usually occurs in tropical areas. High index of clinical suspicion is needed to diagnose this condition. We hereby report a case of basidiobolomycosis in a 25 year old male who presented to us with a non healing ulcer in left thigh. Biopsy showed granulomatous inflammation with fungal infection suggestive of phycomycosis. Fungal culture grew *Basidiobolus ranarum*. Patient was treated with oral itraconazole. The ulcer resolved completely after 3 months of treatment.

Introduction

Zygomycosis is an infection caused by fungal agents belonging to the phylum Zygomycota. These are saprophytic fungi and are found ubiquitously in the environment. The class Zygomycetes includes two fungal orders: Mucorales and Entomophthorales. They have different pathogenic potentials; the order Mucorales primarily affects the immunocompromised patients causing invasive diseases like rhino cerebral or pulmonary mucormycosis with high mortality, where as the order entomophthorales, which include *Basidiobolus* and *Conidiobolus* genera, cause chronic infection in the subcutaneous tissue in the immune competent individual.[1] Basidiobolomycosis is caused by the fungus *Basidiobolus ranarum*. Here we present a case of subcutaneous basidiobolomycosis which was misdiagnosed as mucormycosis by histopathological examination.

Case Details

A 25 year old male software engineer from Coimbatore was referred to us for intravenous amphotericin B, for the treatment of his non healing ulcer over the anterior aspect of left thigh as biopsy of the lesion was reported as mucormycosis. He had this lesion for the past 3 months which started as a painless nodular swelling (Figure 1A). He had consulted many clinicians earlier and was on multiple antibiotics, but his skin lesion progressively increased in size. He had no co-morbid illness and was apparently normal 3 month back. Ultra sonogram of the thigh was suggestive of abscess in subcutaneous plane. He underwent incision and drainage (I and D) of the abscess which resulted in a deep cavitatory lesion. Culture from the material during I and D was sterile. As the lesion was worsening (Figure 1B) he underwent another debridement. Gram's stain, AFB stain of the tissue didn't reveal any organisms and bacterial culture of the pus was sterile. Biopsy of the ulcer was performed and sent for histopathological examination (HPE). The HPE was reported as extensive ulceration and dense inflammation of dermis and subcutaneous area with fat necrosis and abscess formation composed of neutrophils and eosinophils along with thin walled broad aseptate fungal hyphae branching at right angles and surrounded by fibrosis and dense inflammation with giant cells. PAS stain was positive. Final report was foreign body granuloma with many fungal hyphae resembling mucormycosis. At this point we decided to re-evaluate the patient. On examination, he had a 3×1 cm linear non tender ulcer with surrounding induration on the left thigh (Figure 1C). His complete blood count showed total leukocyte count – 7400 (Neutrophils -50 %, Lymphocytes-34%, Eosinophils-4%, Monocytes-7%), ESR – 24 mm in 1 hour. His blood glucose, renal function test and liver function test were within normal limits. Human Immunodeficiency virus 1 and 2 were non-reactive. Since the history and clinical picture didn't fit with a diagnosis of mucormycosis (the morphology of the lesion, duration of the ulcer), a second opinion of the slide was sought. The histopathology description (Figure 2A, 2B, 2C) included the presence of granulomas in dermis and subcutis associated with the presence of numerous eosinophils. There were also scattered abscesses and eosinophilic material surrounding the fungal hyphae resembling Splendore–Hoeppli phenomenon. A probable diagnosis of subcutaneous phycomycosis was suggested with a comment that fungal culture was required for confirmation. Additional biopsy specimen was collected aseptically for fungal culture from the edge of the lesion. On Sabouraud's Dextrose Agar, furrowed creamy brown, heaped up, radially folded colonies grew after 3 days of incubation (Figure 3). Lactophenol cotton blue wet mount showed aseptate hyphae and smooth walled zygospores with characteristic conjugation beaks which confirmed the fungus to be *Basidiobolus ranarum* (Figure 4). After the diagnosis of basidiobolomycosis was confirmed, the patient was started on oral Itraconazole 200 mg once daily for 3 months. The ulcer healed slowly and complete resolution of the lesion (Figure 1D) was seen after 3 months.

Discussion

Basidiobolomycosis is caused by *Basidiobolus ranarum*, a saprophytic fungus present in soil, decaying fruit and vegetable matter as well as in the gut of amphibians and reptiles.[2] It can cause a variety of clinical manifestations including subcutaneous zygomycosis, gastrointestinal zygomycosis and occasionally an acute systemic illness. The subcutaneous form is a granulomatous infection of the skin and subcutaneous tissues characterized by the formation of firm, painless, disciform nodule generally on the

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Fig. 1: (A) Nodular swelling over thigh; (B) Painless ulcer after I and D; (C) Ulcer with induration after debridement; (D) Healed lesion after treatment

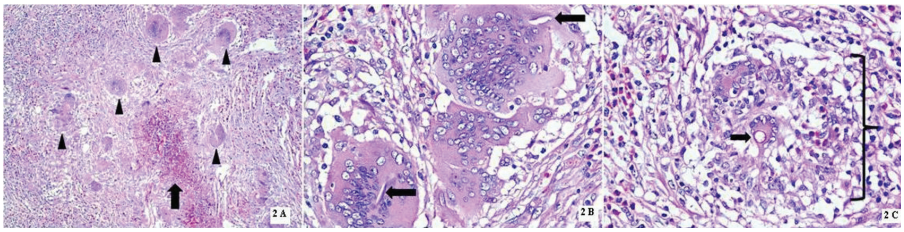


Fig. 2: (A) Low power view showing granulomas with many foreign-body type multinucleated giant cells (arrowheads) and Splendore-hoeppli phenomenon (arrow). H and E; (B) High power view showing giant cells with fragments of broad, aseptate fungal hyphae (arrows) in the cytoplasm. H and E; (C) High power view showing a granuloma (bracket) containing many Eosinophils and a giant cell with cross section of fungal hyphae (arrow) in the cytoplasm. H and E

thighs and buttocks in a "bathing suit" distribution.[1] It mainly affects young, male and immunocompetent persons. It is usually transmitted by minor trauma which may be through insect bite, intravenous catheter, or even intramuscular injection. Clinically it starts as small hardened swelling over the extremities and progressively increases in size to form a painless, non tender swelling. Macroscopically it closely mimics a soft tissue tumor, synovial sarcoma, Burkitt's lymphoma or histopathologically mimics mucormycosis. The other differential diagnosis would be atypical mycobacterial infections, sporotrichosis and parasitic infections like oncocerciais.[3]

In our patient also it started as a small painless swelling and progressively increased in size. Even though the histopathology was reported as mucormycosis, the possibility of mucormycosis could be ruled out by the absence of features such as angio/tissue invasion and necrosis; and slow growing nature of the lesion. Laboratory diagnosis is mainly based on histopathological features and culture of the organism which is the "gold standard". Histologically, basidiobolomycosis is associated with eosinophilic infiltration; with short broad aseptate hyphae.[4] Some may be surrounded by an eosinophilic material which is called as 'Splendore – Hoeppli' phenomenon.[5]

Most patients with basidiobolomycosis respond well to



Fig. 3: Growth of basidiobolus ranarum on Sabouraud's dextrose agar appearing yellowish grey rugose and smooth colonies with a waxy texture on the obverse end

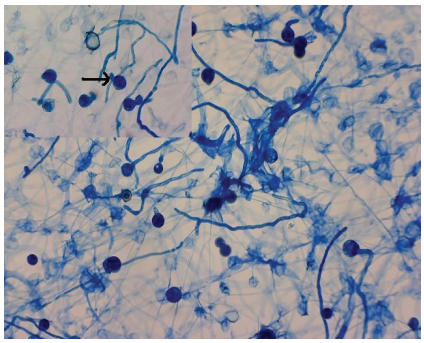


Fig. 4: Lacto phenol cotton blue staining of basidiobolus ranarum. Non septate broad hyphae and zygospores with conjugation beaks seen (Insert - arrow) X 400 magnification

oral itraconazole. The other treatment options are potassium iodide, trimethoprim-sulfamethoxazole. Some strains do not respond to amphotericin B. In the present case also the patient responded well to itraconazole and the ulcer got completely resolved after three months of treatment.

Conclusion

This case is presented to insist on the importance of clinical correlation of histopathology reports along with proper sampling and appropriate microbiological test while treating rare infections.

References

1. Anaparthy UR, Deepika G. A case of subcutaneous zygomycosis. *Indian Dermatol* 2014; 5:51–4.
2. Ellis DH. Subcutaneous zygomycosis. In: Merz WG, Hay RJ, editors. Topley and Wilson's: Microbiology and microbial infections - Medical mycology. 10 th ed. London: Hodder Arnold; 2005:348.
3. Mondal AK, Saha A, Seth J, Mukherjee S. Subcutaneous

zygomycosis: A report of one case responding excellently to potassium iodide. *Indian J Dermatol* 2015; 60:500-2.

4. Khan ZU, Khoursheed M, Makar R, Al-Waheeb S, Al-Bader I, Al-Muzaini A, et al. Basidiobolus ranarum as an etiologic agent of gastrointestinal zygomycosis. *J Clin Microbiol* 2001; 39:2360-3.

5. Anand M, Deshmukh SD, Pande DP, Naik S, Ghadage DP. Subcutaneous Zygomycosis Due to Basidiobolus ranarum: A Case Report from Maharastra, India. *Journal of Tropical Medicine* vol. 2010. Article ID 950390, 3 pages, 2010. doi:10.1155/2010/950390.

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Kikuchi Fujimoto Disease: A case report

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Abstract

Kikuchi Fujimoto Disease (KFD) is a rare clinical syndrome. Classically, KFD presents with fever and cervical lymphadenopathy. In most cases, it is a benign self limiting syndrome lasting around one to four months. It has been documented in all ethnic groups but is more prevalent in the Asian population. The diagnosis of KFD can be difficult as its clinical features can be similar to malignant lymphoma and Systemic Lupus Erythematosus (SLE) associated lymphadenitis. A strong association with SLE and sometimes other autoimmune diseases has been noted. Severe extranodal involvement can occur very rarely. A case of severe KFD presenting with features similar to secondary sepsis is unusual. In this report, we discuss a case of atypical Kikuchi's disease which presents with generalized serositis mimicking sepsis. Severe KFD has been known to resolve with corticosteroid administration. Patients with severe disease may need long term monitoring for development of autoimmune disorders.

Keywords

Kikuchi Fujimoto Disease (KFD); lymphadenopathy; sepsis

Abbreviations

KFD: Kikuchi Fujimoto Disease; SLE: Systemic Lupus Erythematosus; ESR: Erythrocyte Sedimentation Rate; INR: International Normalized Ratio; HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus; ADA: Adenosine Deaminase; FNAC: Fine Needle Aspiration Cytology; AFB: Acid Fast bacilli; ANA: Anti-nuclear Antibody; ANCA: Anti-neutrophil Cytoplasmic Antibodies; CD: Cluster of Differentiation; FDG-PET: Fluorodeoxyglucose Positron Emission Tomography

Introduction

Kikuchi Fujimoto disease (KFD) is a rare benign self limiting syndrome, classically described as cervical lymphadenopathy with low grade fever. At times, it presents with extranodal involvement which can even be life threatening. Varied presentations have been reported, emphasizing the need for accurate diagnosis and treatment [1-4]. This report aims to discuss a case of atypical Kikuchi's disease which presented with generalized serositis mimicking sepsis.

Case Presentation

A 40 year old male presented with low grade fever for one week followed by abdominal distension

and pain along with vomiting and breathlessness two days prior to admission. He also had bilateral neck swellings for the past one month. He was admitted elsewhere for his complaints and the investigations showed leucopenia, thrombocytopenia, minimally elevated liver enzymes and positive Dengue IgM antibody. Based on these investigations he was treated for dengue fever and also underwent cervical lymphnode excision biopsy. As he worsened clinically with the development of abdominal pain, vomiting and breathlessness he was referred to our center.

On examination he was febrile, tachycardic (PR- 140/min), normotensive (BP- 120/70 mm Hg), room air saturation was only 88% and kept on 4L oxygen support. The abdomen was soft but distended, bowel sounds were sluggish, clinically free fluid was appreciated. Ryle's tube aspiration produced 700 ml bilious aspirate.

Table 1: Initial Investigations

	At admission	On the day of decompensation	Post corticosteroid therapy
Complete Blood Counts			
Hemoglobin (g/dL)	13.9	12.3	9.3
WBC count (cells/µL)	6000	16000	14000
Neutrophils (%)	66.6%	85.5	83.4
Lymphocyte (%)	20.7	7.5	9.4
Monocyte (%)	12.3	6.1	4.9
Eosinophil (%)	0.3	0.9	2.2
Basophil (%)	0.1	0	0.1
Platelet (cells/µL)	7000	161,000	152,000
ESR	15	NA	NA
Prothrombin time (s)	11	12.4	NA
INR	1.05	1.18	NA
Liver Function Tests			
Total bilirubin (mg/dL)	1.9	0.6	0.8
Indirect/ direct (mg/dL)	1.6/1.3	0.4/0.2	0.4/0.4
SGPT (U/L)	46	38	130
SGOT (U/L)	30	45	43
ALP (U/L)	60	49	59
Total protein (g/dL)	6.3	6.8	5.9
A/G (g/dL)	3.3/3	3.6/3.2	3.3/2.4
GGT(U/L)	174	188	187
Procalcitonin (ng/mL)	0.250	0.255	NA
Lipase (U/L)	21	NA	NA
Amylase(U/L)	36	NA	NA
Renal function tests			
Urea (mg/dL)	52	47	34
Creatinine (mg/dL)	1.27	0.81	0.45

Table 2: Ascitic Fluid Analysis

	At admission	On the day of decompensation
Color	Pale yellow, slightly cloudy	Pale yellow slightly cloudy
Total count	430 cells/ cu.mm	790 cells/cu.mm
Neutrophils	31%	44%
Lymphocytes	13%	16%
Monocytes/ Macrophages	56%	40%
Mesothelial cells	Few	++

Table 3: Pleural Fluid Analysis

Color	Pale yellow
Total count	620 cells/ cu.mm
Neutrophils	8%
Lymphocytes	10%
Eosinophils	2%
Monocytes/ Macrophages	80%
Protein	4 g/dL
Albumin	2.45g/dL
ADA	9.6 U/L

Initial investigation (Table 1) revealed a severe thrombocytopenia, with a normal total and differential count. Urea and creatinine levels were mildly elevated. Liver function tests showed a slight elevation of serum total bilirubin levels. Amylase and lipase levels were normal. Serology was negative for hepatitis B antigen (HBsAg), Anti HCV and HIV antibodies. Dengue serology was positive for IgG antibody but negative for IgM antibody. Chest radiograph showed minimal pleural effusion. No air fluid levels seen in erect abdomen radiograph. Ultrasound abdomen showed moderate ascitis and minimal pleural effusion. Ascitic fluid total counts were elevated with atypical monocytic predominance (Table 2). It was a high protein low serum ascitic albumin gradient type of ascitis suggestive of peritoneal involvement. Pleural fluid total counts were elevated with monocytosis and presence of mesothelial cells (Table 3). Pleural fluid ADA level was normal. Pleural fluid cytology showed inflammatory cells composed of neutrophils, lymphocytes and anthracotic pigment laden macrophages, consistent with suppuration. Sinus tachycardia with low voltage complexes were seen in ECG but echocardiogram and Troponin T were normal. Serum procalcitonin was not elevated. Blood cultures, ascitic and pleural fluid cultures were sterile. AFB staining for tubercular bacilli and Gene Xpert MTB-RIF was negative ruling out tubercular cause. On further evaluation, abdominal Computed Tomography (CT) scan showed left mild to moderate pleural effusion, bilateral atelectasis and moderate fatty liver, omental fat stranding was seen and edematous wall in the distal ileal loops showing luminal narrowing.

Patient continued to have fever spikes and tachycardia was persistent throughout the hospital stay. On the 4th day of admission his clinical condition went for decompensation requiring mechanical

ventilation. He was treated with antibiotics for secondary infection and other supportive measures. Repeat procalcitonin level (Table 1) was normal, cultures continued to be sterile. This suggested a possible autoimmune cause but on further investigation ANA – immunofluorescence, ANA profile and ANCA profiles were negative, RA factor was negative. At this time the histopathology report of the lymph node biopsy showed a typical histology of Kikuchi's disease. A pulse therapy of high dose intravenous steroids was started following which patient showed a remarkable response. He was extubated and continued on nasal oxygen within two days of initiation of therapy. Platelet counts, total counts, kidney function, tachypnea and tachycardia improved and fever spikes settled. The patient was switched to oral steroid therapy and did well.

Discussion

Kikuchi Fujimoto disease (KFD) was first described by Kikuchi [1] et al and Fujimoto [2] et al independently in 1972, Japan. KFD is a perplexing disease due to its wide range of clinical manifestations and no definitive etiology. Typically, it is described as a sub-acute lymph node enlargement in association with low grade fever. It is also known as Histiocytic Necrotising Lymphadenitis for its defining histopathological features, a mononuclear hypercellular response with variable necrosis [3,4].

KFD is very rare typically said to occur in young females of Asian descent. Although more frequent in Asians, it has been reported in all population groups. The age at presentation usually is less than 40 years. There is a female predominance among adults, anywhere from 1:4 to 1:1 male to female ratio has been reported. A male predominance among children is noted in the pediatric age group [5,6,7].

In the retrospective analysis done by Dumas et al and In Young Joung et al, patients more frequently present with unilateral cervical lymphadenopathy involving the posterior cervical triangle [6,7]. Fever, myalgias, arthalgia are the most common presenting symptoms. Patients also had associated cutaneous rash, night sweats, and weight loss. Tender lymphadenopathy and autoimmune associations are more frequent in womenand severe presentations are seen more in men. Cutaneous rash, weight loss, arthalgia are associated with SLE. The syndrome in classical cases is benign, self limiting, lasting for around one to four months and requiring no more than supportive therapy alone. Recurrence is rare anywhere from 20%- 3% [5,7].

Various theories have been proposed to explain the etio-pathogenesis of the disease. It is thought to be a self limiting hyper-immune response to agents like viruses or physio-chemical agents. Viruses like Epstein Barr Virus (EBV), Herpes-viruses 6 and 8, Herpes simplex virus, Human immunodeficiency virus and other infectious agents like Yersenia pestis and Toxoplasma have been suggested as the etiology. A viral etiology has been justified based on the clinical prodrome, atypical blood picture and poor response to antibiotics but no consistent evidence has been put forth. Other theories suggest that KFD may be an incomplete autoimmune phenomenon which may or may not evolve into a full blown autoimmune disease. The association of SLE with KFD supports this theory. KFD has also been associated with some HLA (Human Leukocyte Antigen) phenotypes, which can explain a familial predisposition in rare cases and its higher frequency in some ethnic groups. Though many points support these theorized none have been established as the cause for the disease [4,5].

There is no pathognomonic feature of KFD in blood investigations, but studies have noted

leucopenia, elevated serum Lactate Dehydrogenase and thrombocytopenia in these patients [5-7]. Only definitive investigation for diagnosis is lymph node biopsy and histopathology. The typical histopathological feature in case of KFD is effacement of architecture of the lymph node, paracortical apoptotic necrosis with presence of histiocytes, plasmacytoid cells interspersed by karyorrhectic debris and without formation of granulomas. Crescentic histiocytes are present in the necrotic foci with clustering of the plasmacytoid dendritic cells at the margins of the necrotic foci. There may be a mix of lymphocytes present some of them may be large with tingible body macrophages giving a false appearance of lymphoma. Kuo et al have divided the histopathological findings in KFD into proliferative, necrotizing and xanthomatous types [3]. Immunohistochemistry is used along with histopathology for diagnosis and for differentiation from malignant lymphomas [3-5]. The predominance of CD8 positive lymphocytes, myeloperoxidase, lysozyme, CD68 and CD4 in histiocytes is considered a marker for KFD along with absence of neutrophils. FNAC can show approximately 50% accuracy in selected cases of lymphadenopathy and sometimes can be useful independently as a means of diagnosis if typical clinical features are present [5]. Imaging studies like ultrasonography, computed tomography and FDG-PET scans find their use in evaluation of lymphadenopathy especially in generalized disease. Studies to determine the characteristic imaging patterns of KFD induced lymphadenopathy have been published but have yet to be accepted as a reliable means of diagnosis [5,6].

The diagnosis of Kikuchi's disease can be confounding due to its varied presentations. The possibility of misdiagnosis is high, as other more common disease present similarly. Moreover, the only definitive means of diagnosis is lymph node biopsy. The main differentials are Tuberculosis, Malignant lymphoma and SLE lymphadenitis. Cases of KFD mimicking acute appendicitis, malignant papillary carcinoma of thyroid have been published. There have also been reports of high rates of misdiagnosis in the past. One such article reports a 40% chance of misdiagnosis, a false diagnosis of malignant lymphoma was made [8]. With greater awareness of the disease and improving imaging studies misdiagnosis may have reduced. KFD may also be under-diagnosed as a mild episode can be attributed to probable infectious or reactive cause without further evaluation.

Accurate diagnosis becomes challenging especially when the presentation is atypical. Varied atypical manifestations of KFD have been reported. Extra-nodal involvement of KFD is associated with greater severity and duration of disease. There have been reports of Kikuchi-Fujimoto disease causing pleural effusion and interstitial lung disease, cryptogenic organizing pneumonia, autoimmune hepatitis, panuveitis and aseptic meningitis [6,9]. There is a strong association of KFD with Systemic Lupus Erythematosis, 13-25% of patients with KFD also developed SLE [6]. Kikuchi's disease can occur before, during and after the onset of SLE [5]. KFD has also been reported in association with autoimmune diseases like Rheumatoid arthritis, Sjogren's syndrome, Anti-phospolipid antibody syndrome, relapsing polychondritis multiple connective tissue disorder and others [9].

In contrary to the classical, benign cases of KFD, there are reports of fatal disease. In these extremely rare cases, death occurred due to disseminated intravascular coagulopathy, pulmonary hemorrhage and hemophagocytic syndrome [10].

In this case for discussion, the main working diagnosis was sepsis of probable gastrointestinal origin. A severe case of Kikuchi's disease mimicking sepsis with generalized serositis without an

autoimmune association is unheard of. This may suggest that KFD can occur independently without an autoimmune trigger. A case was reported of a female patient with histopathology suggesting KFD developed ascitis and plural effusion which progressed into a case of SLE [11]. In another report two cases with mixed connective tissue disorder develop KFD presenting similar to sepsis [12]. This also emphasizes the need for long term follow up for this patient to review autoimmune status.

Accurate diagnosis is essential in choosing the line management, as this is hugely different from that of its differentials. Hence, a diagnosis of KFD in any case with fever and lymphadenopathy should always be kept in mind.

The treatment of KFD is mostly symptomatic. Till present no guideline has been established for treatment of Kikuchi- Fujimoto disease. Good results have been documented with use of steroids in case of severe KFD. Others such as hydroxychloroquine, intravenous immunoglobulin and a combination therapy have been tried in cases of refractory or recurrent disease [6,12]. Although, a good response to treatment and prognosis is seen, a case refractory to treatment has been reported. A quick improvement in the general condition was seen in our patient after administration of steroids and he remains sero-negative in follow-up for 6 months following remission.

Conclusion

In conclusion, Kikuchi Fujimoto disease though rare, should always be on the list of differentials for any case of fever with lymphadenopathy. Despite being benign, KFD can present with severe manifestations. So, accurate diagnosis is indispensible in treatment of such patients.

References

1. Kikuchi M. Lymphadenitis showing focal reticulum cell hyperplasia with nuclear debris and phagocytes: A clinicopathological study. Acta Haematol Jpn. 1972; 35:379–80.

2. Fujimoto Y, Kozima Y, Yamaguchi K. Cervical subacute necrotizing lymphadenitis: A new clinicopathologic entity. Naika. 1972; 20:920–27.

3. Kuo TT. Kikuchi's disease (Histiocytic Necrotising Lymphadenitis). A clinicopathologic study of 79 cases with an analysis of histologic subtypes, immunohistology and DNA ploidy. Am J Surg Pathol. 1995 Jul; 19(7):798-809.

4. Hutchinson CB, Wang E. Kikuchi- Fujimoto Disease. Arch Pathol Lab Med. 2010 Feb; 134(2): 289-93.

5. BoschX, Guilabert A, Miquel R, Campo E. Enigmatic Kikuchi-Fujimoto disease: a comprehensive review. Am J Clin Pathol. 2004 Jul; 122(1):141-52.

6. Dumas G, Prendki V, Haroche J, Amoura Z, Cacoub P, Galicier L *et al*. Kikuchi-Fujimoto Disease: Retrospective Study of 91 Cases and Review of the Literature. Medicine (Baltimore). 2014 Nov; 93(24):372-82.

7. Jung IY, Ann HW, Kim JJ, Lee SJ, Kim J, Seoung H *et* al. The incidence and clinical characteristics by gender differences in patients with Kikuchi–Fujimoto disease. Tarantino. G, ed.Medicine. 2017 Feb; 96(11):e6332.

8. Menasce LP, Banerjee SS, Edmondson D, Harris M. Histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease): continuing diagnostic difficulties. Histopathology. 1998 Sep; 33(3):248-54.

9. Garcia-Zamalloa A, Taboada-Gomez J, Bernardo-Galán P, Magdalena FM, Zaldumbide-Dueñas L, Ugarte-Maiztegui M. Bilateral pleural effusion and interstitial lung disease as unusual manifestations of Kikuchi Fujimoto Disease: A case report and literature review. BMC Pulm Med. 2010 Nov 5; 10:54.

10. Bianca Barbat, Ruby Jhaj, Daniyeh Khurram. Fatality in Kikuchi-Fujimoto disease: A rare phenomenon. World J Clin Cases. 2017 Feb 16; 5(2): 35-9.

11. Mihaela Găman, Ana-Maria Vlădăreanu, Camelia Dobrea, Minodora Onisâi, Cristina Marinescu, Irina VoicanDaniela Vasile et al. A Challenging Case of Kikuchi-Fujimoto Disease Associated with Systemic Lupus Erythematosus and Review of the Literature. Case Rep Hematol. 2018 Jan;2018:1791627.

12. Sharma V, Rankin R. Fatal Kikuchi-like lymphadenitis associated with connective tissue disease: a report of two cases and review of the literature. SpringerPlus. 2015 Apr; 4:167.

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Characterization of cardiac autonomic function in COVID-19 using heart rate variability: a hospital based preliminary observational study

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Abstract

Objectives: The novel corona virus disease, which was initially reported in China in late 2019, has become a global pandemic affecting 330 million cases. COVID-19 affects predominantly the respiratory system, in addition to other organ systems, mainly the cardiovascular system. One of the hypotheses is that virus entering the target cells by binding to angiotensin converting enzyme 2 affecting hypothalamic pituitary axis could lead to dysautonomia which is measured by heart rate variability (HRV). HRV is a non-invasive measure of autonomic function that facilitates identification of COVID-19 patients at the risk of developing cardiovascular complications. So, we aimed to assess HRV in COVID patients and compare between COVID patients and normal controls.

Methods: In a case control design, we compared 63 COVID-19 infected patients with 43 healthy controls matched for age and gender. Along with clinical characterization, heart rate variability was evaluated using ambulatory 5 min ECG in lead II and expressed in frequency and time domain measures. Statistical analysis was performed using SPSS 17.0.

Results: Mean age of the study population was 49.1 ± 14.2 years and 71 (66.9%) were males. Frequency domain measures high (HF) and low (LF) frequency powers were significantly decreased in COVID-19 patients compared to controls. HF/LF and LF/HF ratios were not different between groups. Time domain measures rMSSD (root mean square of successive RR interval differences) and SDNN (standard deviation of NN intervals) were significantly increased among COVID-19 subjects. COVID-19 infection was associated with increased parasympathetic activity as defined by rMSSD>40 {adjusted odds ratio 7.609 (95% CI 1.61–35.94); p=0.01} and SDNN>60 {adjusted odds ratio 2.620 (95% CI 1.070–6.44); p=0.035} after adjusting for age, gender and comorbidities.

Conclusions: Our study results showed increased parasympathetic tone in COVID patients. Early diagnosis of autonomic imbalance in COVID patients is needed to plan management and limit progression of disease.

Keywords: heart failure; pulmonary hypertension; right heart.

Introduction

In December 2019, a novel coronavirus disease (nCoV) epidemic emerged in Wuhan City, Hubei Province, China. Owing to its high transmissibility and infectivity rate it spread across the world and WHO declared it as a pandemic on March 11, 2020 [1, 2]. As this virus predominantly affects the respiratory system it has been renamed as severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) and the disease caused by this virus has been named as corona virus disease 19 (COVID-19) [3].

COVID-19 shows wide range of clinical presentations ranging from asymptomatic/mild symptoms (fever, cough, dyspnea, myalgia, fatigue, anosmia, dysgeusia and diarrhea) to severe illness like acute respiratory distress syndrome (ARDS), arterial and venous thrombosis, myocarditis and varieties of neurological manifestations [4–7]. A report from WHO says that “80% of infections are mild or asymptomatic, 15% are severe infections and 5% are critical infections” [8]. Co-morbid conditions like obesity, type 2 diabetes mellitus, coronary artery disease, chronic liver disease, chronic kidney disease and chronic respiratory disease are known to be risk factors for developing severe disease [9–11]. Wide spectrum of manifestations is explained by SARS CoV-2 having target cells in multiple organs and systems like respiratory tract including lung parenchyma,

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ileum, bladder, esophagus, heart, kidney, hypothalamus, pituitary, adrenal glands etc. These target cells have angiotensin converting enzyme-2 (ACE2) receptors in their cell wall. ACE2 binding affinity of the SARS CoV-2 spike protein ectodomain is one of possible mechanisms by which entry to these cells is facilitated [1, 12]. Histopathological and immune histochemical studies have clearly demonstrated a direct assault on these organs/systems by the SARS CoV-2 virus [13].

Though main portal of entry is respiratory system and hence the dominantly affected one, SARS CoV-2 reaches other systems through mechanism yet unknown. Neuroinvasive potential of SARS CoV-2 has been discussed in recent literature in the context of plausible explanation for variety of neurological manifestations of COVID-19 [14–16]. Earlier studies have demonstrated that SARS CoV-1 and MERS CoV (Middle east respiratory syndrome CoV) have the potential to enter and invade central nervous system, dominantly in brainstem structures [12, 17, 18]. Owing to the similarities in viral structure and receptor binding domain, neurotropism of SARS CoV-2 can be speculated from the evidence found from SARS CoV-1. Though sparse, direct evidence of neurotropism of SARS CoV-2 is emerging in literature. A recent report by Paniz–Mondolfi et al. [19], demonstrated by electron microscopy, presence of viral particles in postmortem brain tissues of a COVID-19 patient. Another report by Moriguchi et al. [20], evidenced the presence of SARS CoV-2 in cerebrospinal fluid. It has been postulated that SARS CoV-2 can enter the central nervous system by several mechanisms such as invasion through olfactory epithelium [21], synapse-connected route from terminal nerve endings [22] and transmission across blood-brain barrier by endothelial damage [19]. Clinical implications of neuropropensity of SARS CoV-2 range from mild symptoms like headache, dizziness etc., to severe manifestations like seizures, encephalitis, Guillain-Barre syndrome and cerebrovascular events. Involvement of autonomic nervous system (brainstem and hypothalamus) can lead to dysfunction of vital organs including cardiovascular system, thereby significantly influencing the outcome in COVID-19 patients. Cardiovascular system is also affected by the virus leading to manifestations like acute myocardial infarction, myocarditis and arterial & venous thrombosis. Monitoring the cardiac autonomic function of patients with COVID-19 can help us identify the individuals who are at risk of developing adverse cardiovascular outcomes.

Autonomic dysfunction has been reported in viral infections like retrovirus (human immunodeficiency virus, human T-lymphotropic virus), herpes viruses, flavivirus, arbovirus, enterovirus 71 and lyssavirus [23]. Current literature on autonomic dysfunction in COVID-19 patients is very sparse, barring a few case reports [24, 25]. Though various other methods were employed in these clinical settings to elicit dysautonomia, heart rate variability (HRV) measurement was seldom used for this purpose. During last two decades, HRV is considered most objective, reproducible and validated tool for demonstration of autonomic dysfunction [26]. Frequency and time domain measures of HRV have emerged as the most promising markers of balance between parasympathetic and sympathetic activity of autonomic nervous system that controls homeostasis of all vital organs.

Eliciting an association between autonomic dysfunction and COVID-19 could clarify the genesis of many clinical manifestations and importantly the unpredictable natural course of the illness. In this study, we intended to evaluate for autonomic imbalance and the direction of its deviation in COVID-19 subjects by measuring HRV and comparing the differences in HRV metrics between SARS CoV-2 positive and negative individuals.

Methods

This was an analytical cross-sectional study involving adult COVID-19 patients admitted for treatment in PSG Institute of Medical Sciences, Coimbatore, a specialized COVID-19 treating center in South India during March to June 2020. Sample frame during this period was approximately 500 inpatients whose COVID-19 status was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) test of nasopharyngeal and oropharyngeal swab. Eligible inpatients (test group) of both genders, aged between 25 and 60 years were recruited from the sample frame by convenient sampling method. Patients with uncontrolled hypertension, coronary artery disease, cerebrovascular accident, family history of young myocardial infarction were excluded. Similarly, those patients on treatment with beta-blockers, inhaled or oral beta-mimetics, theophylline and other drugs with potential chronotropic effects were excluded. Patients with severe disease (those requiring oxygen support and/or intensive care) were also excluded. The comparison group included age and gender-matched healthy individuals from among the employees of medical school who tested negative for SARS CoV-2 by RT-PCR of their nasopharyngeal and oropharyngeal swab and fulfilled above mentioned selection criteria. This study was cleared by the institutional human ethics committee, and all study participants gave written informed consent. All such subjects recruited for the study underwent a detailed clinical examination.

Assessment of HRV

Study participants in both the groups were instructed to refrain from smoking, caffeine intake for 2 h and alcohol intake for 36 h. They should have had adequate rest, at

least 8 h of uninterrupted sleep on the night before the assessment of HRV and normal breakfast on the day of assessment. They were made to lie quietly in a couch in supine position for 5 min to alleviate the anxiety in a sound attenuated room with dim lighting and the temperature ranging from 20 to 25 °C.

After explaining the procedure to the subject, HRV, a quantitative marker of the autonomic activity was assessed using an ambulatory ECG system (INCO digital NIVIQURE, Bangalore, India) in lead II for 5 min. It is a multichannel digital data acquisition system which enables to acquire, analyze and store ECG data. ECG data was obtained at a sampling rate of 1,024 Hz in standard lead II configuration. The interface RS232C-compatible module was used to transfer data from the recording unit to the computer. The transferred data was analyzed using inbuilt software system. The series of RR intervals obtained were subjected to frequency and time domain analysis. Time domain indices of HRV indicate the amount of variability in measurements of interbeat interval (IBI), which represent time period between successive heartbeats. Time domain measures include standard deviation of NN intervals (SDNN), root mean square of successive RR interval differences (rMSSD) and percentage of successive RR intervals (pNN50). Frequency domain measurements estimate the distribution of absolute or relative power into frequency bands. Frequency domain measures include low frequency (LF) and high frequency (HF) [26]. HF component indicates the parasympathetic activity, whereas LF represents both sympathetic and vagal activity, and SDNN, rMSSD and pNN50 measure parasympathetic activity [26, 27].

Statistical analysis

The data were examined initially for normality of distribution and homogeneity of variance and expressed in terms of mean ± SD or percentage. Data with wide dispersion were expressed in log transformed values. Data from continuous variables were compared between study groups using Student's *t*-test whereas categorical variables were compared using Chi-Square test. Mann–Whitney *U* test was performed for variables that were not normally distributed. HRV variables that were significantly different between the groups were used as dependent variables for logistic regression analysis. For this purpose, HRV measures were dichotomized to categorical variable (artificial categorization using median split [28]) using an arbitrary cut-off value that was close to the median as reported by Nunan et al. [27] in their systematic review of studies involving normal subjects. Stepwise logistic regression analysis was employed to identify significant covariates and to evaluate association between HRV measures and SARS CoV-2 status after adjusting for important confounders like age, gender and comorbidity. Subgroup analysis was performed to characterize clinical and HRV profiles of SARS CoV-2 infected subjects based on symptom status. A p value of <0.05 was considered significant. All statistical analyses were carried out using SPSS version 17 software.

Results

In total, 106 participants were recruited for the study, which included 63 (59.4%) COVID-19 subjects and 43 (40.6%) healthy individuals who tested negative for SARS CoV-2. Among COVID-19 patients 33 (52.3%) had mild to moderate symptoms and 30 (47.6%) were asymptomatic. Mean age of the study population was 49.09 ± 14.21 years with 71 (67%) male subjects and 35 (33%) female subjects (Figure 1).

Comparing COVID-19 patients and healthy subjects, both groups were age and gender matched. Vital parameters like heart rate, SPO_2 at room air, systolic and diastolic blood pressure showed no difference between the study groups. Among frequency domain HRV parameters, HF and LF powers were significantly decreased among COVID-19 patients as compared to healthy individuals. HF/LF ratio and LF/HF ratio were not different between the groups (Table 1). Among time domain parameters, mean rMSSD was significantly higher in the COVID-19 group compared to healthy individuals (Figure 2). Mean SDNN value was higher among COVID-19 subjects with a trend toward statistical significance.

In the above graphical representation, the box spans the interquartile range, ends of the box represent upper and lower quartiles, bold horizontal line within the box represents the median value and the whiskers show the

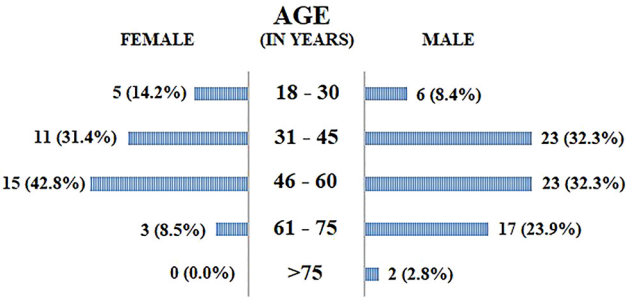


Figure 1: Age and gender distribution of study population.

Table 1: Comparison of clinical profile and heart rate variability parameters between COVID-19 subjects and healthy individuals. Data shown as mean ± SD and frequency {n (%)}.

Variables	COVID-19 subjects (n=63)	Healthy subjects (n=43)	p-Value
Age, years	48.39 ± 16.3	50.1 ± 10.5	0.517
Gender			0.449
Male	44 (69.8%)	27 (62.8%)	
Female	19 (30.2%)	16 (37.2%)	
SPO_2 in room air, %	95 ± 6	97 ± 1	0.678
Systolic BP, mmHg	124 ± 15	121 ± 6	0.386
Diastolic BP, mmHg	77 ± 7	77 ± 4	0.818
HR	84 ± 15	81 ± 15	0.151
HF[a]	1.79 ± 0.15	2.09 ± 0.39	0.0001*
LF	33.47 ± 20.31	77.46 ± 50.8	0.0001*
HF/LF ratio	4.16 ± 4.56	2.34 ± 1.89	0.248
LF/HF ratio	0.70 ± 0.71	0.64 ± 0.35	0.282
pNN50	4.45 ± 8.12	1.99 ± 3.23	0.561
rMSSD[a]	1.49 ± 0.58	1.15 ± 0.29	0.019*
SDNN[a]	1.89 ± 0.33	1.73 ± 0.10	0.10
rMSSD>40	19 (30.6%)	2 (4.7%)	0.001[b]
SDNN>60	27 (45.0%)	11(25.6%)	0.044[b]

SPO_2, oxygen saturation; BP, blood pressure; HR, heart rate; SDNN, standard deviation of NN intervals; rMSSD, root mean square of successive RR interval differences; pNN50, percentage of successive RR intervals; LF, low frequency power; HF, high frequency power. *Mann–Whitney *U* test, level of significance 0.05. [a]Log transformed values. [b]Chi square test, level of significance 0.05.

Table 2: Stepwise logistic regression analysis of association between COVID-19 infection and parasympathetic overtone as defined by rMSSD>40 or SDNN>60.

Parasympathetic overtone	Odds ratio (95% CI)	p-Value
rMSSD>40		
Unadjusted	9.058 (1.98–41.35)	0.004
Adjusted for age and gender	8.670 (1.86–40.30)	0.006
Adjusted for age, gender and diabetes mellitus type 2	8.174 (1.75–38.22)	0.008
Adjusted for age, gender and presence of comorbidities	7.609 (1.61–35.94)	0.01
SDNN>60		
Unadjusted	2.380 (1.014–5.58)	0.046
Adjusted for age and gender	2.427 (1.020–5.77)	0.045
Adjusted for age, gender and diabetes mellitus type 2	2.417 (1.002–5.83)	0.049
Adjusted for age, gender and presence of comorbidities	2.620 (1.070–6.44)	0.035

minimum and maximum values. The circles with numericals are outliers.

Based on the published data on range of observations and median value of normal subjects, we defined parasympathetic overtone when subjects had rMSSD more than 40 and SDNN more than 60 [27]. These dichotomous variables indicated that COVID-19 patients had higher parasympathetic overtone than the healthy subjects (Table 1). Stepwise logistic regression analysis revealed a significant association between SARS CoV-2 infection status and parasympathetic overtone after adjusting for age, gender, T2DM or the presence of one or more comorbidities (Table 2).

Subgroup analysis was performed comparing the characteristics between symptomatic and asymptomatic subjects with COVID-19 (Table 3). Symptomatic COVID-19 patients were elderly and predominantly males as compared to asymptomatic patients. Symptomatic patients more frequently had at least one co-morbidity. Common

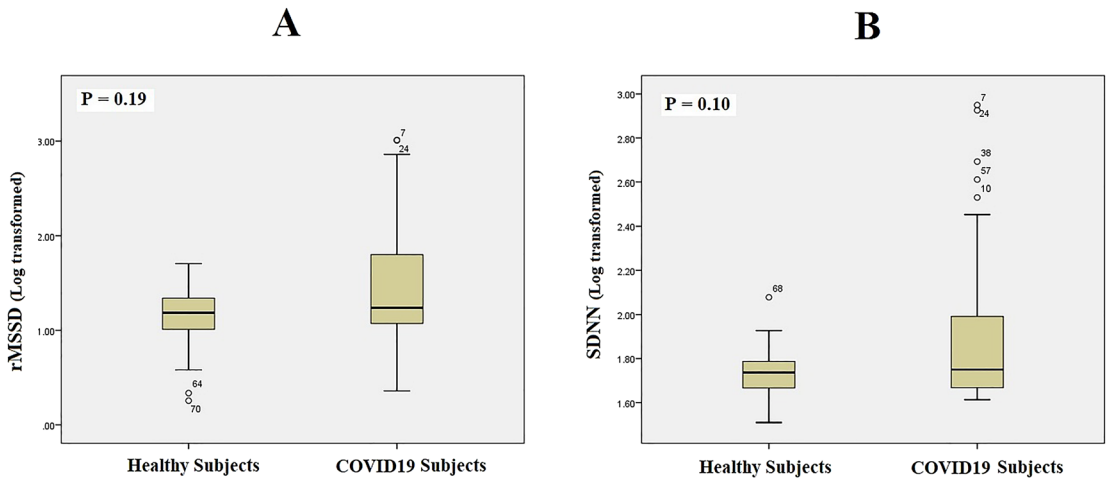


Figure 2: Boxplot graphical representation of rMSSD (A) and SDNN (B) among COVID-19 patients and healthy subjects.

Table 3: Subgroup analysis of clinical profile and heart rate variability parameters among symptomatic and asymptomatic COVID-19 subjects. Data shown as mean ± SD and frequency {n (%)}.

Variables	Symptomatic COVID-19 subjects (n=33)	Asymptomatic COVID-19 subjects (n=33)	p-Value
Age, years	57.59 ± 13.5	38.57 ± 13.1	0.0001[c]
Gender			0.006[b]
Males	28 (84.8%)	16 (53.3%)	
Females	5 (15.2%)	17 (46.7%)	
SPO_2 in room air, %	94 ± 9	97 ± 1	0.072
Systolic BP, mmHg	128 ± 16	121 ± 15	0.097
Diastolic BP, mmHg	77 ± 7	77 ± 8	0.763
Comorbidities			
DM type 2	11 (36.7%)	7 (23.3%)	0.260
Systemic hypertension	10 (33.3%)	2 (6.7%)	0.010[b]
Any one or more of above comorbidities	20 (66.7%)	8 (26.7%)	0.002[b]
HR	88.14 ± 17.11	80.55 ± 12.98	0.055
HF	64.61 ± 21.22	68.58 ± 19.33	0.442
LF	35.38 ± 21.22	31.37 ± 19.40	0.437
HF/LF ratio	3.82 ± 4.35	4.54 ± 4.83	0.518
LF/HF ratio	0.79 ± 0.83	0.61 ± 0.55	0.650
pNN50	4.58 ± 8.73	4.30 ± 7.53	0.358
rMSSD[a]	1.41 ± 0.62	1.56 ± 0.53	0.086*
SDNN[a]	1.87 ± 0.36	1.91 ± 0.3	0.135

SPO_2, oxygen saturation; BP, blood pressure; HR, heart rate; SDNN, standard deviation of NN intervals; rMSSD, root mean square of successive RR interval differences; pNN50, percentage of successive RR intervals; LF, low frequency power; HF, high frequency power. *Mann–Whitney *U* test, level of significance 0.05. [a]Log transformed values. [b]Chi square test, level of significance 0.05. [c]Student's *t*-test, level of significance 0.05.

co-morbidities among symptomatics were DM type 2 (36.7%), Systemic hypertension (33.3%), obesity (10%), hypothyroidism (13.3%) and psychomorbidity (10%). Both frequency and time domain HRV parameters showed no difference between the subgroups.

Discussion

Our study clearly demonstrated an increased parasympathetic activity in COVID-19 patients, independent of important confounders like age, gender and comorbidities including diabetes mellitus. Autonomic dysfunction in COVID-19 subjects in our study was best demonstrated by time domain measures of HRV namely rMSSD and SDNN with predefined cut-off values. HF and LF powers in frequency domain were significantly decreased in COVID-19 subjects, though their ratios were not significantly different when compared to the healthy uninfected subjects. In a subgroup analysis of COVID-19 subjects, HRV measures were not different between asymptomatics and mild to moderate symptomatic. To our knowledge, our study was the first to demonstrate higher rate of autonomic imbalance among COVID-19 patients as compared to uninfected healthy group.

Our search through literature yielded scanty reports on autonomic dysfunction among COVID-19 patients. Ritwik Ghosh et al. [24] reported in one case of COVID-19, clinical features of autonomic dysfunction in the form of sinus arrhythmia, postural hypotension, intermittent profuse sweating, constipation, erectile dysfunction and squeezing sensation in the chest. Cardiac dysautonomia was not studied in this report. In another case report, Reiner Buchhorn et al. [25], studied a 58-year-old COVID-19 patient with 24 h ECG monitoring and found a significant decrease in heart rate and a paradoxical decline in HRV. But for these two case reports suggesting a possible autonomic dysfunction, no studies have yet, evaluated the presence of this important physiological disharmony in COVID-19 patients.

Though scanty in the context of COVID-19, plethora of evidence available in the literature demonstrating autonomic dysfunction in other infectious diseases like human immunodeficiency virus [29–31] human T Cell lymphotropic virus [32], Epstein Barr virus [33], cytomegalovirus [34], West Nile virus [35], Chagas disease [36, 37], diphtheria [38], leprosy [39], dengue[40], tetanus [41] and botulism [42]. Methodological differences in evaluating autonomic function as well as variations in study design do not allow head-to-head comparison of these studies with our findings in COVID-19 patients**.** In our search through literature, we found only two reports using HRV to link autonomic dysfunction with infection. Jeevagan Vijayabala et al. [43] postulated sympathetic dysfunction as one of the pathogenetic mechanisms in Dengue shock syndrome . Robert Carter et al. [40] examined HRV of 27 children during defervescence in dengue viral infection and found that cardiac parasympathetic activity was the major cause for reduction in heart rate during this period of illness. Their observations were mainly based on frequency domain measures of HRV after correcting for baseline changes in heart rate. Contradictory to the above observations, La-Orkhun et al. [44] found no significant changes in various time and frequency domain metrics of HRV at least 24 h after defervescence, and follow-up conducted at least 14 days after defervescence. Our findings are consistent with Jeevagan Vijayabala et al. and Robert Carter et al., that we too found an increased parasympathetic activity in COVID-19 patients as demonstrated by the increase in time domain variables of HRV. This study further substantiates

two cases as cited above that reported autonomic dysfunction due to COVID-19. Proposed mechanisms of viral infection induced autonomic dysfunction include invasion of the central nervous system and the direct viral, toxin-mediated or immune-mediated involvement of the peripheral and autonomic nervous system [45].

Unlike the time domain variables, we found that frequency domains of HRV, specifically the LF/HF ratio were not different between the COVID-19 subjects and the healthy subjects. Traditionally LF/HF ratio was considered a marker of sympatho-vagal balance in cardiovascular system. However recent evidence this ratio merely indicates one part of complex non-linear interaction between sympathetic and parasympathetic nervous systems confounded by multiple physiological and mechanical factors [46]. Some of these confounders may have been missed in our study protocol. Also, smaller sample size may explain our inability to detect subtle difference in LF/HF ratio between the groups.

Our study involved a standardized measurement of heart rate variability using an ambulatory 5 min ECG recording in lead II. Precision of our evaluation was further augmented by stringent selection of subjects and optimal conditions for HRV measurement during conduct of the study. Our study is limited by participation of only asymptomatics and mild to moderate symptomatic patients with SARS CoV-2 infection. Severely ill COVID-19 patients were excluded from the study because we believed that factors like stress, cytokine storm, drugs like steroids, vasopressors etc., positive pressure ventilation, prolonged hospitalization and related psychomorbidity that typically characterize these patients may have a confounding effect on our observations. None of our study subjects had any of these factors that could have possibly altered our findings. Sample size of the study was not adequate to provide enough statistical power to our findings. But we believe that this preliminary report can serve a stimulus for future research in this direction. A larger sample size could have facilitated further evaluation of association between symptoms and autonomic function in our subjects.

In conclusion, this study has found that COVID-19 is associated with autonomic dysfunction whose mechanism and prognostic implications need to be evaluated further. HRV measurement, being a simple, noninvasive, inexpensive tool, its utility in clinical practice as a possible rapid diagnostic and prognostic marker needs to be assessed in future research.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This study was cleared by the local institutional human ethics committee and all study participants gave written informed consent.

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
2. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 2020;395:470–3.
3. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016;24:490–502.
4. Gorbalenya AE, Baker SC, Baric RS, Groot RJ, Drosten C, Gulyaeva AA, et al. Severe acute respiratory syndrome-related coronavirus: the species and its viruses: a statement of the Coronavirus Study Group. Nat Microbiol 2020;5:536–44.
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel corona virus-infected pneumonia in Wuhan, China. JAMA J Am Med Assoc 2020;323:1061–9.
6. Ellul MA, Benjamin L, Singh B, Lant S, Michael BD, Easton A, et al. Neurological associations of COVID-19. Lancet Neurol 2020;19: 767–83.
7. Vaira LA, Salzano G, Deiana G, De Riu G. Anosmia and Ageusia common findings in COVID-19 patients. Laryngoscope 2020;130: 1787.
8. Coronavirus disease 2019 (COVID-19) situation report. Available from: www.who.int›docs›20200306-sitrep-46-covid-19 [Accessed 11 Jan 2021].
9. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;3099:19–20.
10. Guan W, Ni Z, Hu Y. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708–20.
11. Zhang JJ, Dong X, Cao YY. Clinical characteristics of 140 patients infected by SARS-CoV-2 in Wuhan, China. Allergy 2020;75: 1730–41.
12. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med 2020;14:185–92.
13. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 2004;203:622–30.
14. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol 2020:1–4. https://doi.org/10.1002/jmv.25728.
15. Huang P, Tang L, Ren L, Liu L. Research progress in nervous system damage caused by SARS-CoV-2. Zhong Nan Da Xue Bao Yi Xue Ban 2020;28:1247–54.

16. Yashavantha Rao HC, Jayabaskaran C. The emergence of a novel coronavirus (SARS-CoV-2) disease and their neuroinvasive propensity may affect in COVID 19 patients. J Med Virol 2020;92: 786–90.
17. Li K, Wohlford Lenane C, Perlman S, Zhao J, Jewell AK, Reznikov LR, et al. Middle East respiratory syndrome coronavirus causes multiple organ damage and lethal disease in mice transgenic for human dipeptidyl peptidase 4. J Infect Dis 2016;213: 712–22.
18. McCray PB Jr, Pewe L, Wohlford Lenane C, Hickey M, Manzel L, Shi L, et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J Virol 2007;81: 813–21.
19. Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednicky J, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). J Med Virol 2020;92:699–702.
20. Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, Takamino J, et al. A first Case of Meningitis/Encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis 2020.
21. Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol 2008;82:7264–75.
22. Li YC, Bai WZ, Hirano N, Hayashida T, Taniguchi T, Sugita Y, et al. Neurotropic virus tracing suggests a membranous coating mediated mechanism for transsynaptic communication. J Comp Neurol 2013;521:203–12.
23. Carod-Artal FJ. Infectious diseases causing autonomic dysfunction. Clin Auton Res 2018;28:67–81.
24. Ghosh R, Roy D, Sengupta S, Benito-Leon J. Autonomic dysfunction heralding acute motor axonal neuropathy in COVID-19. J Neurovirol 2020;26:964–66.
25. Buchhorn R, Baumann C, Willaschek C. Heart rate variability in a patient with coronavirus disease 2019. Int Cardiovasc Forum J 2020. https://doi.org/10.17987/icfj.v20i0.685.
26. Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 1996;93:1043–65.
27. Nunan D, Sandercock GRH, Brodie DA. A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. Pacing Clin Electrophysiol 2010; 33:1407–17.
28. De Coster J, Gallucci M, Anne-Marie R. Iselin. Best practices for median splits, artificial categorization and their continuous alternatives. J Exp Psychopathol 2011;2:197–209.
29. DM FS, Umekawa S, Kocher M, Kallianpur KJ, Shikuma CM, Low P. Symptoms of autonomic dysfunction in human immunodeficiency virus. Open Forum Infect Dis 2015;2:ofv103.
30. Mittal CM, Wig N, Mishra S, Deepak KK. Heart rate variability in human immunodeficiency virus-positive individuals. Int J Cardiol 2004;94:1–6.
31. Becker K, Gorlach I, Frieling T, Haussinger D. Characterization and natural course of cardiac autonomic nervous dysfunction in HIV-infected patients. AIDS 1997;11:751–7.
32. Alamy AH, Menezes FB, Leite AC, Nascimento OM, Araujo AQ. Dysautonomia in human T-cell lymphotrophic virus type I-associated myelopathy/tropical spastic paraparesis. Ann Neurol 2001;50:681–5.
33. Bennett JL, Mahalingam R, Wellish MC, Gilden DH. Epstein-Barr virus-associated acute autonomic neuropathy. Ann Neurol 1996; 40:453–5.
34. Nakao K, Namekawa M, Kondo S, Ono S, Nakano I. Subacute autonomic and sensory neuropathy closely related to cytomegalovirus infection preceded by frequent syncopal attacks. Rinsho Shinkeigaku 2016;8:555–9.
35. Bode AV, Sejvar JJ, Pape WJ, Campbell GL, Marfin AA. West Nile virus disease: a descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003. Clin Infect Dis 2006;42: 1234–40.
36. Junqueira LF Jr. Insights into the clinical and functional significance of cardiac autonomic dysfunction in Chagas disease. Rev Soc Bras Med Trop 2012;45:243–52.
37. Vasconcelos DF, Junqueira LF Jr. Cardiac autonomic and ventricular mechanical functions in asymptomatic chronic chagasic cardiomyopathy. Arq Bras Cardiol 2012;98:111–9.
38. Illarramendi X, Buhrer-Sekula S, Sales AM, Bakker MI, Oliveira A, Nery JA, et al. High prevalence of vasomotor reflex impairment in newly diagnosed leprosy patients. Eur J Clin Invest 2005;35: 658–65.
39. Idiaquez J. Autonomic dysfunction in diphtheritic neuropathy. J Neurol Neurosurg Psychiatry 1992;55:159–61.
40. Carter R, III, Hinojosa-Laborde C, Convertino VA. Heart rate variability in patients being treated for dengue viral infection: new insights from mathematical correction of heart rate. Front Physiol 2014;5:46.
41. Lin TS, Chen LK, Lin TY, Wen SH, Chen MC, Jan RH, et al. Autonomic dysfunction because of severe tetanus in an unvaccinated child. PediatrNeonatol 2011;52:169–71.
42. Merz B, Bigalke H, Stoll G, Naumann M. Botulism type B presenting as pure autonomic dysfunction. Clin Auton Res 2003; 13:337–8.
43. Vijayabala J, Attapaththu M, Jayawardena P, De Silva SG, Constantine G. Sympathetic dysfunction as a cause for hypotension in dengue shock syndrome. Chin Med J 2012;125:3757–8.
44. La-Orkhun V, Supachokchaiwattana P, Lertsapcharoen P, Khongphatthanayothin A. Spectrum of cardiac rhythm abnormalities and heart rate variability during the convalescent stage of dengue virus infection: a Holter study. Ann Trop Paediatr 2011;31:123–8.
45. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol 2013;4:26.
46. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18–22.

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Post COVID 19 infections: A case series of 5 different pathogens

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Abstract

Secondary infections are commonly identified in viral respiratory infections and are an important cause of mortality and morbidity. Post Coronavirus disease 19 (COVID 19) infections significantly change the long-term outcome of COVID 19. The complexities of post COVID 19 infections are the diversity of pathogens encountered and high index of suspicion is needed to diagnose & manage these infections. But the significance of bacterial and fungal infection following COVID 19 has not been adequately elucidated in literature. We report five interesting cases of secondary infections in post COVID 19 patients which include *Pneumocystis Jirovecii* pneumonia; Mucormycosis; Pulmonary Tuberculosis; Pulmonary Nocardiosis and Pseudomonal lung abscess. We have highlighted the challenges imposed and management of such patients.

Keywords

post coronavirus disease 19; nocardia; tuberculosis; mucormycosis, *pneumocystis jirovecii*.

Introduction

Coronavirus disease 19 (COVID19) is an ongoing pandemic caused by the Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS CoV 2). This virus commonly affects the lungs causing pneumonia with acute respiratory distress syndrome, type 1 respiratory failure, pulmonary thromboembolism and other organ systems. Severe complications include respiratory failure, sepsis, acute coronary syndrome, acute renal failure and secondary infections. Those who had COVID 19 develop two types of complications. First one is post-COVID 19 sequelae where in the patients have persisting hypoxia requiring oxygen support. The second one is the post COVID 19 infections. Risk factors for secondary infections included the use of broad-spectrum antimicrobials, glucocorticoids, severe pulmonary involvement and patients on immunosuppressant drugs for other conditions [1,2]. Review of literature has revealed reports of co-infection with various microorganisms during active COVID 19. There were no reports of infections following remission

of COVID 19. Here we report 5 such cases of post COVID 19 infection with different pathogens all occurring after remission from COVID 19.

Case I

A 63-year-old male known case of ischemic heart disease was treated for COVID 19. High resolution computerized tomography of (HRCT) lungs revealed CT severity index 14/25. He was treated with Remdesivir, Low molecular weight Heparin (LMWH), glucocorticosteroid. He improved and was discharged. One month after discharge, he presented with fever, breathlessness and cough for 10 days. On examination, he maintained 90% saturation in room air and had bilateral wheeze and crepitations. Complete Blood Count (CBC) showed white blood cell (WBC) count of 12,900 with 78% neutrophils and 10% lymphocytes. His renal function test (RFT) and liver function tests (LFT) were within normal limits. HRCT chest showed evidence of resolving atypical pneumonia. Bronchoalveolar Lavage (BAL) tested positive for Pneumocystis. jirovecii by Gomori Methenamine Silver (GMS) stain (Figure 1) and PCR. He was treated with Cotrimoxazole and prednisolone. He completely recovered after the treatment.

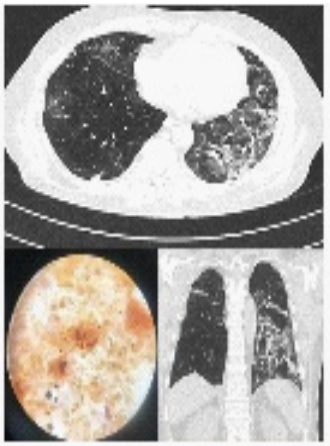


Figure 1: HRCT coronal and axial section of lung showing subtle ground glass densities with predominant interlobular septal thickening within with adjacent parenchymal strands and thin atelectasis in both lungs, predominantly in the peripheries, peri hilar regions and lower lobes. BAL- Gomori Methenamine silver staining shows cysts of *Pneumocystis Jirovecii.*

Case II

A 56-year-old male a known case of Type 2 Diabetes mellitus/ Systemic Hypertension who was treated for COVID 19 and discharged home 2 months back, presented with fever for more than 2 weeks. His vitals were stable and systemic examination showed bilateral crepitations. CBC showed a WBC count of 1,700 cells/cmm with 75% neutrophils, 19% lymphocytes and 5% monocytes. His RFT and LFT were within normal limits. Positron Emission Tomography (PET) scan showed features suggestive of COVID 19 sequelae in lungs and low metabolic activity at lymph nodes. BAL sample tested positive for *Mycobacterium Tuberculosis* (MTB) by XPERT MTB-Rifampicin (RIF) assay. He was started on Anti-Tubercular Therapy (ATT) with Rifampicin, Isoniazid, Pyrazinamide and Ethambutol. The patient gradually improved and was then discharged with ATT. On review, he had no further fever spikes and was symptomatically better.

Case III

A 61-year-old male, a known case of Type 2 Diabetes Mellitus/ Dyslipidemia, treated for COVID 19

one month back, presented with pain on the left half of the face with blurring of vision and protrusion of the left eye. On examination, he had proptosis of the left eye with left III, IV, V2, VI, VII cranial nerve palsies. CBC showed a WBC count of 8,500 with 56% neutrophils and 34% lymphocytes. RFT and LFT were within normal limits. MRI showed evidence of fungal sinusitis and orbital cellulitis. Diagnostic nasal endoscopy showed large posterior septal perforation with the involvement of the left maxillary sinus. He underwent left maxillary sinus debridement. He was started on intravenous Liposomal amphotericin B for 4 weeks and was switched to oral Isavuconazole. His tissue culture on Sabouraud dextrose agar and Lactophenol Cotton Blue stain revealed growth *Rhyzopus sp* (Figure 2). He was doing well on follow up.

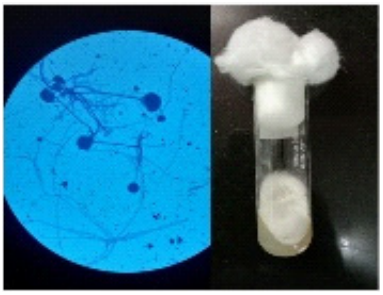


Figure 2: Tissue culture on Sabouraud dextrose agar showing black to grey tan cottony growth with salt and pepper appearance. Lactophenol Cotton Blue staining showed broad pauciseptate hyaline hyphae with right angled branching.

Case IV

A 54-year-old male known case of Type 2 diabetes mellitus had COVID 19 a month back, presented with non-productive cough and high-grade fever for 2 days. On examination, his vitals were stable and had reduced breath sounds in the left interscapular area. CBC showed a WBC count of 9,800 per cmm with 82% neutrophils and 12% lymphocytes. His RFT, LFT were within normal limits. Blood cultures were sterile. Chest x-ray showed a thick-walled cavity with an air-fluid level in the left middle and lower zone. BAL sample grew *Pseudomonas aeruginosa* (inducible AMPC). He was started on intravenous Meropenem. During the course of his stay, he developed proximal muscle weakness of all four limbs. Nerve conduction study showed demyelinating Polyradiculoneuropathy due to Guillain Barre syndrome. He was started on IV immunoglobulin and showed gradual improvement. On review, he was afebrile and chest X-ray showed complete resolution of the abscess.

Case V

A 58-year-old male with no co-morbidities admitted and treated for COVID 19 presented 2 weeks post-discharge with fever, dry cough and breathlessness for a week. On examination, he was afebrile and vitals stable. Systemic examination revealed bilateral basal crepitations. CBC showed a WBC count of 10,800 cells/cmm with 78% neutrophils and 12% lymphocytes. RFT and LFT were within normal limits. HRCT showed consolidation of the left lower lobe. BAL for modified Ziehl Neelsen stain for Nocardia was positive (Figure 3). He was started on Trimethioprim-Sulfamethoxazole. On review, the patient had no fever and was symptomatically better.

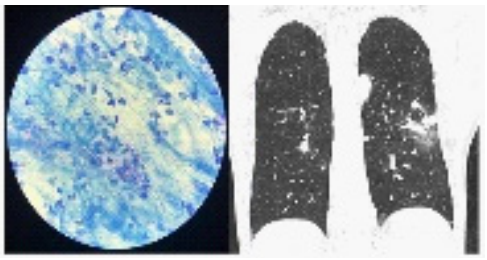


Figure 3: HRCT coronal section of lung showing consolidation in the left lower lobe. BAL – Modified Acid Fast Bacilli (AFB) stain showing the partially acid-fast beaded filamentous appearance of Nocardia.

Discussion

We report five cases of post COVID 19 sequelae that developed different secondary infections in a time span of two weeks to three months after active COVID 19 infection. All five patients were aged above 55 years and had similar severity of COVID 19 infection and treatment for the same. Four of these five patients had co morbidities.

Experiences from SARS, MERS and influenza outbreaks have evidenced co infection with other microorganisms in a subset of patients that frequently determines the morbidity and mortality [3,4]. A similar clinical pattern has been reported in COVID 19 pandemic, with concurrent secondary infections occurring during acute phase of COVID 19 infection. Lansbury L et al in their metaanalysis reviewed thirty studies and reported a pooled prevalence of 7% of co infections; most of them were of bacterial etiology [5]. Various other pathogens like invasive aspergillosis [6], Pneumocystis jirovecii [7], Mycobacterium tuberculosis [8] and mucormycosis [9] have also been implicated.

In our search through literature, we found no publications on secondary infections during the recovery phase of COVID 19 infection, similar to our report. More recently, studies have emerged in literature on the so called “Post COVID 19 syndromes” which elaborates on the clinical course after the acute phase of COVID 19 infection [10,11]. Even these studies have not commented on the incidence or characteristics of secondary infections occurring during the recovery phase of COVID 19 infection. In this context, indirect evidence comes from a study by Donnelly JP et al, who found that sepsis (8.5%) and pneumonia (3.1%) were common causes for readmission of COVID 19 patients within 60 days of discharge from initial hospitalization [12]. This study along with our report reiterates the importance of early diagnosis and management of post COVID 19 secondary infections.

Propensity for developing secondary infections ‘de novo” in late phases of COVID 19 infection could be explained by several factors like COVID 19 related damage to the lungs, immunomodulatory drugs, comorbidities etc. It is also possible that concurrent infections during acute phase of COVID 19 infection, might have remained undetected due to hesitancy for thorough microbiological evaluation using invasive “aerosol generating” procedures like bronchoscopy. Such undiagnosed concurrent infections potentiated by aforesaid factors, may have emerged in later course of recovery from COVID 19 infection.

Going by the potential impact of post COVID 19 secondary infections on morbidity and mortality, we strongly recommend future research in this subject. In an attempt to synchronize our efforts to understand this important entity we also recommend data repositories or registries to profile post COVID 19 infections. We believe that these efforts will help us evolve strategies towards better management of COVID 19.

References

1. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR et al. Bacterial co-infection and secondary infection in patients with COVID 19-19: A living rapid review and meta-analysis. Clin Microbiol Infect. 2020; 26: 1622-1629.

2. Salehi M, Ahmadikia K, Badali H, Khodavaisy S. Opportunistic Fungal Infections in the Epidemic Area of COVID 19-19: A Clinical and Diagnostic Perspective from Iran. Mycopathologia. 2020; 185: 607-611.

3. Sarah H Alfaraj, Jaffar A Al-Tawfiq, TalalAAltuwaijri, ZIadMemish. Middle East Respiratory Syndrome coronavirus and Pulmonary Tuberculosis coinfection: Implications for infection control. Intervirology. 2017; 60: 53-55.

4. Dennis W Metzger and KeerSun.Immune Dysfunction and Bacterial Co-infection following Influenza. J Immunol 2013; 191: 2047-2052.

5. Louise Lansbury, Benjamin Lim, Vadsala Baskaran, Wei Shen Lim. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect 2020; 81: 266–275.

6. Michele Bartoletti, Renato Pascale, Monica Cricca, et al for PREDICO Study Group. Epidemiology of Invasive Pulmonary Aspergillosis Among Intubated Patients With COVID-19: A Prospective Study, Clinical Infectious Diseases, 2020. ciaa1065.

7. Carlos Rubiano, Kathleen Tompkins, Subhashini A Sellers, Brian Bramson, Joseph Eron, Jonathan B Parr et al, Pneumocystis and Severe Acute Respiratory Syndrome Coronavirus 2 Coinfection: A Case Report and Review of an Emerging Diagnostic Dilemma, Open Forum Infectious Diseases 2021; 8: OFAA 633,

8. Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla A et al. Coronavirus Disease (Covid-19) Associated Mucormycosis (CAM): Case Report and Systematic Review of Literature. Mycopathologia. 2021. 5: 1–10.

9. Gao Y, Liu M, Chen Y, Shi S, Geng J, Tian J. Association between tuberculosis and COVID-19 severity and mortality: A rapid systematic review and meta-analysis. J Med Virol. 2021; 93: 194-196.

10. Chaolin Huang, Lixue Huang, Yeming Wang. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021; 397: 220–32.

11. Chopra V, Flanders SA, O'Malley M, Malani AN, Prescott HC. Sixty-Day Outcomes Among Patients Hospitalized with COVID-19. Ann Intern Med. 2020; 11: M20-5661.

12. John P. Donnelly, Xiao QingWang, Theodore J. Iwashyna, Hallie C. Prescott. Readmission and Death after Initial Hospital Discharge among Patients With COVID-19 in a Large Multihospital System. JAMA. 2021; 325: 304-6.

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Original Research Article

Prevalence of metabolic syndrome in patients with premature coronary artery disease proven by coronary angiogram

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ABSTRACT

Background: Metabolic syndrome (MS) is associated with premature coronary artery disease (CAD). The aim of this study was to evaluate the prevalence of MS and its association with severity of CAD proven by coronary angiogram (CAG) in young patients.
Methods: We included patients, aged 45 years or less, admitted with acute coronary syndrome (ACS), who had CAD confirmed by coronary angiography. They were divided into two groups according to the presence or absence of MS based on International Diabetes Federation (IDF) criteria. CAD was classified into single, double and triple vessel disease (TVD). The prevalence of MS and its individual parameters was calculated.
Results: Among 90 young patients who presented with ACS, MS was present in 67 patients (74.44%). Among those with MS, the prevalence of each individual criterion was statistically significant in MS group (P <0.05). Prevalence of pre-existing hypertension and diabetes was significantly higher in MS group (p <0.01). Smoking, alcohol consumption and family history of CAD were not statistically significant in patients with and without MS. Fifteen out of 90 patients (14 in MS group) who presented with ACS had TVD in CAG, but this was not statistically significant (p 0.06).
Conclusions: This study confirms a very high prevalence of MS in young Indian patients with premature CAD. MS was more prevalent than the conventional risk factor smoking in young CAD patients. We could not find significant difference in severity of CAD based on CAG between MS and non-MS group.

Keywords: Coronary angiogram, Coronary artery disease, Metabolic syndrome, Young patients

INTRODUCTION

Coronary artery disease (CAD) affecting younger individuals has reached enormous proportions. There is no doubt that it will become a major public health problem in the future unless we concentrate on reversible causes and prevention, through research studies. CAD is increasing in developing and transitional countries because of urbanization, demographic and lifestyle changes, whereas it is decreasing in many developed countries.[1] Studies have suggested that the risk of myocardial infarction is mostly due to modifiable risk factors like smoking, physical inactivity, poor diet, psychosocial stress, hypertension ,diabetes mellitus, dyslipidemia and increased waist-hip ratio.[2,3] Many of these risk factors are grouped or are seen at the onset of metabolic syndrome (MS).

Metabolic syndrome, a cluster of glucose intolerance, hypertension, dyslipidemia and abdominal obesity, has

been recognized as a major predeterminant of type 2 diabetes mellitus and CAD.[4,5] MS was associated with a 1.5 fold increase in all-cause mortality and a two fold increase in cardiovascular outcomes in a recent metaanalysis.[6] Presence of MS with or without diabetes was found to be an independent determinant of premature CAD.[7] CAD in younger population is an area of increasing interest because of its potential for long-term disability and premature death.

Few studies have reported MS and its association with CAD in the young.[7-9] We found only very limited studies in an Indian setting analyzing the association of MS with premature CAD.[10] The prevalence of MS in young patients with angiographically proven premature CAD has not been studied in Indians, to our knowledge. Understanding the impact of MS on CAD in younger age group is important for developing public health policy towards prevention and management. Hence this study was undertaken to know the prevalence of metabolic syndrome using International Diabetes Federation (IDF) criteria in patients with premature coronary artery disease proven by coronary angiogram (CAG). We also tried to evaluate any association between the presence of metabolic syndrome and severity of CAD.

METHODS

This cross-sectional study was conducted at our centre, a large tertiary care teaching hospital in South India. The study was done after obtaining institutional ethical committee clearance and informed consent. All patients, aged 45 years or less, admitted with acute coronary syndrome (ACS) [ST-elevation myocardial infarction (STEMI)/non-ST-elevation myocardial infarction (NSTEMI) and unstable Angina as per standard definition], in our hospital during the period March 2014-August 2014 were included in the study. We excluded patients younger than 18 years or older than 45 years of age and patients who did not undergo coronary angiogram for various reasons.

ACS was diagnosed in patients with symptoms compatible with myocardial infarction (MI), based on electrocardiogram (ECG) changes and cardiac enzymes. Ejection fraction (EF) and left ventricular systolic dysfunction (LVD) was assessed by echocardiography. CAG was done in all subjects either at admission for primary Percutaneous Coronary Intervention (PCI) or electively after initial medical stabilization with thrombolysis or conservative treatment, depending on the clinical circumstances and consent from the patient. CAD was diagnosed as a 50% or greater stenosis in one of the three major coronary arteries and classified as single vessel disease (SVD), double vessel disease (DVD) and triple vessel disease (TVD) based on the number of arteries involved. The IDF criteria were used for a diagnosis of MS, which is suitable for Indian population.[4] MS was defined as the presence of abdominal obesity (defined by waist circumference ≥ 90 cm for men and ≥ 80 cm for women) plus two or more of the following factors

- Triglycerides ≥150 mg/dL or specific treatment for this lipid abnormality,
- High density lipoprotein (HDL) cholesterol <40 mg/dL for men or <50 mg/dL for women or specific treatment for this lipid abnormality,
- Blood pressure ≥130/85 mm Hg or treatment of previously diagnosed hypertension,
- Fasting plasma glucose ≥ 100 mg/d L or previously diagnosed type 2 diabetes.

Detailed history was taken including presenting symptoms, history of diabetes/systemic hypertension/ history of CAD in the family/usage of alcohol and smoking. A careful physical examination was done with reference to waist circumference (WC) and blood pressure. About ≥3 measurements of BP at the time of entry with values greater than or equal to 130/85mmHg on an average or any medications for hypertension was considered significant. WC was recorded according to the national health and nutritional survey. Peripheral venous blood samples were drawn at the time of admission, the following morning after an overnight fast for lipid profile (total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides) and at day 5 for fasting plasma glucose.

Statistical analysis

The data were analyzed using Statistical Package for Social Science (SPSS) Version 19.0, and Microsoft Excel 2010. Continuous variables were expressed as mean/SD and compared with independent sample t-test. Categorical variables were expressed as percentage and compared with chi-square test. A predictive value of less than 0.05 was taken as statistically significant.

RESULTS

A total of 90 young patients with ACS, who underwent CAG were studied. The key demographics of the study group are summarized in Table 1. Patients ranged from 26 to 45yrs. The mean age of the patients was 39.7 yrs. Among 90 patients, 82 were male and eight were female. Out of 90 cases, 67 (74.44%) had MS according to IDF criteria and 23 (25.55%) did not. Of these 67 patients with MS, 29 (43.28%) met three, 24 (35.82%) met four and 14 (20.89%) met all five criteria for MS. MS was found in 60 men out of 82 (73.17%) and seven women out of eight (87.5%), thus a greater percentage of young women had MS than men, but this was not statistically significant.

The distribution of MS criteria as defined by IDF in those 67 patients was as follows (Figure 1).

Table 1: Baseline demographic characteristics.

Variable value	Ms		P
	Yes (n=67)	No (n=23)	
Blood pressure ≥130/85mmhg	44 (65.67%)	6 (26.08)	<0.01
Fasting blood glucose ≥100mg/dl	43 (64.17%)	9 (39.13%)	<0.05
Triglycerides (mg/dl)	179.14	115.86	<0.01
HDL-C (mg/dl)	32.36	36.78	<0.05
WC (in cms)	95.64	87.23	<0.001
Age	40.49	37.39	<0.01
Sex			
Male	60	22	0.674
Female	7	1	
Diabetes mellitus	37 (55.22%)	5 (21.73%)	<0.01
Pre-existing hypertension	31 (46.26%)	3 (13.04%)	<0.01
Family history of CAD	10 (14.92%)	2 (8.69%)	0.448
Smoking	42 (62.68%)	15 (65.21%)	0.825
Alcohol consumption	32 (47.76%)	13 (56.52%)	0.468
Dyslipidemia	15 (22.3%)	2 (8.69%)	0.148
STEMI	36 (53.7%)	18 (78.26%)	<0.05
LVD	23 (34.32%)	11 (47.82%)	0.24

MS: Metabolic syndrome, HDL-C: High density lipoprotein cholesterol, WC: Waist circumference, CAD: Coronary artery disease, STEMI: ST-elevation myocardial infarction, LVD: Left ventricular systolic dysfunction.

All (100%) had abdominal obesity, 60 (89.55%) had low HDL or treatment for lipid abnormality, 45 (67.16%) had BP≥130/85 mmHg or treatment for hypertension, 43 (64.17%) had elevated triglycerides or treatment for lipid abnormality and 43 (64.17%) had raised FBS or DM. All these criteria were statistically significant in MS group.

Table 2: Angiography results in MS/non- MS.

Angiogram result	MS		Non-MS		P value
	N= 67	%	N=23	%	
SVD	39	58.2	16	69.56	0.335
DVD	14	20.89	6	26.08	0.605
TVD	14	20.89	1	4.34	0.066

MS: Metabolic syndrome, SVD: Single vessel disease, DVD: Double vessel disease, TVD: Triple vessel disease.

MS patients had significantly higher prevalence of pre-existing hypertension and diabetes (p<0.01). Alcohol consumption, smoking status, patients receiving treatment for dyslipidemia and family history of CAD were not statistically significant in patients with and without MS (Table 1).

Thirty-six patients (53.7%) out of 67 in MS and 18(78.26%) out of 23 in non-MS cohort presented with ST elevation MI, which was statistically significant (p <0.05). Twenty-three patients (34.32%) in MS and 11(47.82%) in non-MS group had LVD with the p value of 0.24, which was not significant.

Majority of patients in both MS and non-MS groups had SVD, 58.2 % and 69.56 % respectively; it was not significant (p 0.33). Fifteen out of 90 patients who presented with ACS had TVD. Fourteen patients with TVD had MS, whereas only one patient in non-MS group had TVD, even though TVD was more common in MS, it was not statistically significant (p 0.06) (Table 2).

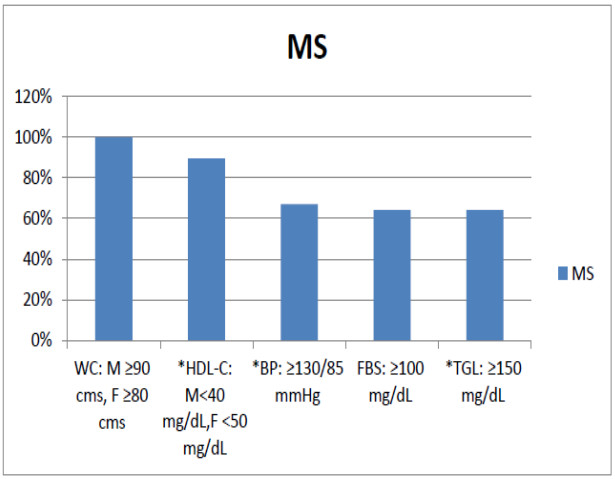


Figure 1: Prevalence of each criterion in MS group. MS: metabolic syndrome, WC: waist circumference, HDL-C: high density lipoprotein cholesterol, BP: blood pressure, FBS: fasting blood glucose or known type 2 diabetic, TGL: triglycerides. M: male, f: female, * or on treatment.

DISCUSSION

MS is characterized by a constellation of risk factors such as raised triglyceride, low HDL, glucose intolerance, hypertension and abdominal obesity that may lead to the development of CAD. Asian Indians are more prone to

diabetes and premature CAD, which is attributed to increased insulin resistance and characteristic abdominal obesity. Asian Indians have a different distribution of body fat, with abdominal obesity or a higher body fat content, even in the presence of normal body mass index.[11] Increased visceral fat is a dependable indicator of hyperinsulinemia and insulin resistance, which are risk factors for diabetes, dyslipidemia, hypertension and CAD.[12]

Hence, we have applied IDF criteria which has lower ethnic-specific waist circumference cut-offs for obesity, in our study.

We found that the prevalence of MS among patients presenting with young CAD was alarmingly high (74.44%). The prevalence of MS in young patients with CAD was similarly high in studies done by Hassanin et al. (66.39%) and Ranjith et al. (57%).[9,13] However, other studies have shown lower prevalence of MS in similar groups of patients ranging from 26% to 37%.[8,10,14]

Various studies in young CAD patients have shown a higher prevalence of MS in young women than in men.[9,10,14] In our study too the prevalence of MS was more common in women than men (87.5% versus 73.17%), but the difference was not statistically significant. This is important because Morraquin et al. have shown that women with CAD and MS have significantly higher risk of cardiovascular events and decreased survival than women without MS.[15]

Cigarette smoking was found to be the most common risk factor for premature CAD in earlier studies.[9,10,14,16] This was in contrast to our study, where we found MS was dominating with 74.44% versus smoking (63.33%), as a risk factor for premature CAD. This finding could suggest that MS may play a significantly more important role than smoking in the development of cardiovascular disease in younger age group.

While assessing the various components of MS in patients with the syndrome, we found that all patients had abdominal obesity, low HDL was the next most frequently seen risk factor (89.55%). A similar pattern of the individual components of MS in young patients with CAD was seen in a study done by Sadeghian et al.[17] Another study done in young Indians also showed a higher prevalence of low HDL (78.8%) in MS patients.[10] Higher percentage of low HDL in Indians was seen in a study conducted by Goswami et al. in MS patients with CAD.[18]

Studies have shown the predominance of dysfunctional HDL in MS patients and this proinflammatory HDL can further worsen metabolic dysfunction and hence increase the cardiovascular risk.[19] We also noted in our study that all eight women with premature CAD had low HDL. Hence, our study confirms higher prevalence of abdominal obesity and low HDL, which are well recognized risk factors for CAD.[20-23]

We also found a higher incidence of STEMI in non-MS group (78.26%) than MS (53.7%). This was unlike the results of Danciu et al. and Chung et al., where MS patients had more STEMI.[8,24] Danciu et al. found that STEMI in MS patients was statistically significant, whereas Chung et al. did not.

Although TVD was more common in the MS cohort than non-MS (14 patients versus one patient), this was not statistically significant. Thus, no significant difference in disease severity in terms of number of vessels stenosed, based on CAG, was found in our study. Goswami et al. in an Indian study found that the prevalence of MS increased significantly in patients with TVD, but they had included patients of all age groups.[18] Marroquin et al. showed that women with MS had increased risk of cardiovascular events in angiographically significant CAD patients, but again patients ranged from 21 to 86 years.[15]

There are a few limitations to our study. Those patients who met four out of five criteria for MS without the principal criteria (waist circumference more than 80 in women and more than 90 in men) were excluded from the MS group. We had only few female patients in our study. The strength of our study is that we describe the prevalence of MS in angiographically proven premature CAD, which is the gold standard to diagnose CAD.

CONCLUSION

To conclude, this study clearly indicates a very high prevalence of MS in Indian patients with premature CAD. Our study is important because we found that MS was more prevalent than the conventional risk factor of smoking in young patients with CAD. We could not find significant difference in severity of CAD between MS and non-MS group in young patients, but larger and more studies in a similar age group are required to confirm this finding.

Family physicians have an important role in identifying MS in young healthy individuals and thus, may help find people at risk for premature CAD. Healthcare professionals should routinely measure waist circumference and screen for low HDL, which is usually ignored in practice. The presence of MS or any of its components in young people should trigger an aggressive approach toward patient education and implementation of primary and secondary measures to help reduce the prevalence of premature CAD.

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REFERENCES

1. De Backer GG. The global burden of coronary heart disease. Medicographia. 2009;31(4):343-8.
2. Gupta R, Gupta VP, Sarna M, Bhatnagar S, Thanvi J, Sharma V, et al. Prevalence of coronary heart disease and risk factors in an urban Indian population: Jaipur Heart Watch-2. Ind heart J. 2002;54(1):59-66.
3. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. The lancet. 2004;364(9438):937-52.
4. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome-a new worldwide definition. The Lancet. 2005;366(9491):1059-62.
5. Reddy KK, Rao AP, Reddy TP. Socioeconomic status and the prevalence of coronary heart disease risk factors. Asia Pac J Clin Nutr. 2002;11(2):98-103.
6. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. Metabolic syndrome and the cardiovascular risk a systematic review and metaanalysis. J Am Coll Cardio. 2010;56(14):1113-32.
7. Iribarren C, Go AS, Husson G, Sidney S, Fair JM, Quertermous T, et al. Metabolic syndrome and earlyonset coronary artery disease: is the whole greater than its parts?. J Am Coll Cardiol 2006;48(9):1800-7.
8. Danciu SC, Iqbal FM, Manankil MF, Koul S, Raghuvir R, Herrera CJ. Metabolic Syndrome in Younger Patients with Acute Coronary Syndrome. Eur J Gen Med. 2012;91(1):22-6.
9. Hassanin N, Gharib S, El Ramly MZ, Meged MA, Makram A. Metabolic syndrome and coronary artery disease in young Egyptians presenting with acute coronary syndrome. Kasr Al Ainy Medic J. 2015;21(1):27.
10. Aggarwal A, Aggarwal S, Sharma V. Metabolic syndrome and coronary artery disease in Indians younger than 40 years. J Endo Meta. 2012;2(1):39-45.
11. Das UN. Metabolic syndrome X is common in South Asians, but why and how? Nutrition. 2002;18:774-6.
12. Das UN. Pathobiology of metabolic syndrome X in obese and nonobese South Asian Indians: further discussion and some suggestions. Nutrition 2003;19:560-2.
13. Ranjith N, Pegoraro RJ, Naidoo DP, Esterhuizen TM. Metabolic syndrome in young Asian Indian patients with myocardial infarction: cardiovascular topic. Cardio J Afr. 2007;18(4):228-32.
14. Turhan H, Yasar AS, Basar N, Bicer A, Erbay AR, Yetkin E. High prevalence of metabolic syndrome among young women with premature coronary artery disease. Coron Artery Dis. 2005;16(1):37-40.
15. Marroquin OC, Kip KE, Kelley DE, Johnson BD, Shaw LJ, Bairey Merz CN, et al. Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women: a report from the Women's Ischemia Syndrome Evaluation. Circulation. 2004;109(6):714-21.
16. Von Eyben FE, Bech J, Madsen JK, Efsen F. High prevalence of smoking in young patients with acute myocardial infarction. J R Soc Health 1996;116(3):153-6.
17. Sadeghian S, Darvish S, Salimi S, Esfehani FA, Fallah N, Mahmoodian M, et al. Metabolic syndrome: stronger association with coronary artery disease in young men in comparison with higher prevalence in young women. Coron Artery Dis. 2007;18(3):163-8.
18. Goswami B, Tayal D, Tyagi S, Mallika V. Prevalence of metabolic syndrome in patients with angiographically proven coronary artery disease presenting to a tertiary care hospital in Delhi, India. Diabetes Meta Synd. 2011;5(2):53-60.
19. Watson KE, Ansell BJ, Watson AD, Fonarow GC. HDL function as a target of lipid-modifying therapy. Rev Cardiovasc Med. 2007;8(1):1-8.
20. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. J Cl Endocrinol Meta. 2001;86(11):5366-71.
21. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, et al. INTERHEART Study Investigators. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet. 2005;366(9497):1640-9.
22. Jayasinghe SR, Jayasinghe SH. Variant metabolic risk factor profile leading to premature coronary disease: time to define the syndrome of accelerated athero-coronary metabolic syndrome in Asian Indians. Singapore Med J. 2009;50(10):949-55.
23. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K, et al. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. JAMA. 2007;297(3):286-94.
24. Chung EH, Curran PJ, Sivasankaran S, Chauhan MS, Gossman DE, Pyne CT, et al. Prevalence of Metabolic Syndrome in Patients < 45 Years of Age with Acute Myocardial Infarction Having Percutaneous Coronary Intervention. Am J Cardio. 2007;100(7):1052-5.

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Case Report

Human brucellosis in a non-susceptible host: a case report

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ABSTRACT

Clinical human brucellosis is quite rare in India, that too in an non susceptible host. This report describes a case of clinical human brucellosis in India. This case involved a 36 years old male, a professor in an Engineering college. He presented with complaints of fever for 4 months with myalgia, generalized tiredness, loss of weight and loss of appetite. On evaluation, total counts and ESR was elevated and all other investigations were normal. Based on history, common causes like occult tuberculosis or autoimmune diseases were considered. But, blood cultures grew *Brucella melitensis* in all 2 samples and a diagnosis of brucellosis was made. He was treated with Rifampicin and Doxycycline. Suspicion of brucellosis in this patient is low because the patient has no history of contact with animals, consumption of unpasteurized milk or occupational exposure. So, the health care practitioners should be aware of this possibility of this zoonotic infection as a differential diagnosis in patients with nonspecific symptoms and unexplained prolonged fever.

Keywords: Brucellosis, Non-susceptible host, Prolonged fever

INTRODUCTION

Burcellosis is a multisystem disease with a broad spectrum of nonspecific symptoms that usually occurs within 2 weeks but up to 3 months after inoculation. It is usually due to consumption of unpasteurized contaminated goat's milk or soft cheese that had been infected with *Brucella melitensis*.[1] They are small non-motile, nonsporing, non-capsulated gram-negative coccobacilli that usually infects goats and sheep.[2] The principal organizsm causing worldwide brucellosis is *Brucella melitensis*. It is the most pathogenic and invasive one, followed by *Brucella suis*, *Brucella abortus*, *Brucella canis*, *Brucella pinnipediae* and *Brucella cetaceae*.[3]

Human brucellosis is commonly found in countries with rural communities that live with close association with animals and its prevalence in a region depends on factors such as methods of processing milk and milk products, food habits, social economic status, hygiene and climate.[4] Both human and animal brucellosis are still endemic in some parts of the world such as Pakistan, China and Srilanka.[5-7]

The clinical features of brucellosis are nonspecific and depends upon the stage of the disease and the organs and systems involved. The most commonly reported symptoms are undulating fever, fatigue, malaise, chills, sweats which may be characterized by a peculiar odour at night, insomnia, headache, myalgia, arthralgia, anorexia, weight loss, hepatomegaly and splenomegaly. Brucellosis can also manifest as a localized disease affecting the central and peripheral nervous system, gastrointestinal tract, genitourinary tract and heart.[8]

Without the critical epidemiological clue to exposure to animals or consumption of unpasteurized dairy products,

this disease can pose a great diagnostic challenge to the clinician. Authors report a case of human brucellosis in a non- susceptible host.

CASE REPORT

36 years old male presented with complaints of fever for 4 months, which was associated with myalgia, generalized tiredness, loss of weight and loss of appetite. There was no history of contact with animals, consumption of unpasteurized milk or occupational exposure. General examination was unremarkable. Abdominal palpation revealed hepatosplenomegaly. His hemoglobin and platelet levels were within normal limits, while total counts showed leucopenia with monocytosis. Erythrocyte sedimentation rate was also elevated. Other basic biochemical tests were within normal limits, except for mild transaminitis.

At this moment, either occult tuberculosis or autoimmune diseases were suspected based on the history, clinical and laboratory findings. However, blood cultures were taken, and broad-spectrum antibiotics were started. CECT chest and CECT abdomen were not suggestive of occult TB infection. Autoimmune workup was negative. As a part of workup for fever of longer duration, Brucella agglutination test and scrub IgM serology were sent. Brucella agglutination test turned out to be positive. Simultaneously, blood culture grew *Brucella melitensis* in all 2 samples.

Based on his serological test and blood cultures, a diagnosis of brucellosis was made. He was eventually prescribed Rifampicin once daily and Doxycycline BD for 6 consecutive weeks.

DISCUSSION

In this case, the patient did not have any history of living in close association with animals or consumption of unpasteurized milk. He also did not have any occupational risk of exposure to brucella. Nevertheless, human brucellosis is quite rare in South India, making the suspicion level among clinicians to be quiet low.

There was a long interval between onset of symptoms and diagnosis in our patient. A lot of health care practitioners do not consider the possibility of brucellosis, especially in patients with prolonged fever. Delay or misdiagnosis can lead to treatment failure, relapses, focal complications and high case fatality rate.[9]

This patient showed many of the symptoms of brucellosis which included undulating fever, myalgia and other clinical features such as hepatomegaly and splenomegaly.[10]

Infective endocarditis is a rare devastating complication from systemic brucellosis and could require surgical intervention. Splenic, hepatic or pulmonary abscess can also develop. Other rare complications include deep vein thrombosis, arthritis, spondylitis, meningitis and nephritis. Ocular manifestations include optic neuritis and uveitis.[11] Common hematological manifestations include leucopenia, anemia and thrombocytopenia.[12]

The standard treatment for human brucellosis is doxycycline, 100mg BD and rifampicin 600-900mg once a day for 6 consecutive weeks. A combination of doxycycline and gentamycin is also preferred. However, the standard treatment varies depending on the patients' age, pregnancy status, CNS involvement and cardiovascular involvement.[13] For children less than 8 years of age, a combination of TMP-SMX and aminoglycoside or a combination of rifampicin and TMP-SMX for 45 days is needed.[14,15]

CONCLUSION

Diagnosis of brucellosis is generally not considered in a non-endemic country. Health care practitioners should be aware of this possibility of this zoonotic infection as a differential diagnosis in patients with nonspecific symptoms and unexplained prolonged fever.

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REFERENCES

1. Ramin B, MacPherson P. Human brucellosis. Br Med J. 2013;341:c4545.
2. Cohen J, Powderly WG, Opal SM, Calandra T, Clumeck N, et al. Infectious diseases. 3rd ed. China: Mosby. 2010.
3. Mantur BG, Amarnath SK, Shinde RS. Review of clinical and laboratory features of human brucellosis. Indian J Med Microbiol. 2007;25(3):188-202.
4. Gwida M, Al Dahouk S, Melzer F, Rösler U, Neubauer H, Tomaso H. Brucellosis–regionally emerging zoonotic disease?. Croatian Med J. 2010;51(4):289-95.
5. Akhtar R, Chaudhry ZI, Shakoori AR, Ahmad M, Aslam A. Comparative efficacy of conventional diagnostic methods and evaluation of polymerase chain reaction for the diagnosis of bovine brucellosis. Vet World. 2010;3(2):53-6.
6. Zhang Z, Yu S, Wang X, Dong S, Xu J, Wang J, et al. Human brucellosis in the People's Republic of China during 2005-2010. Int J Infect Dis. 2013;17(5):e289-e292.
7. Priyantha MA. Identification of biovars of Brucella abortus in aborted cattle and buffaloes herd in Sri Lanka. Vet World. 2011;4:542-5.
8. Franco MP, Mulder M, Gilman RH, Smits HL, 2007. Human brucellosis. Lancet Infect Dis 7: 775-86.

9. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: a re-emerging zoonosis. Veterinary Microbiol. 2010;140(3-4):392-8.
10. Dean AS, Crump L, Greter H, Hattendorf J, Schelling E, Zinsstag J. Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS neglected tropical diseases. 2012;6(12):e1929.
11. Rolando I, Olarte L, Vilchez G, Lluncor M, Otero L, Paris M, Carrillo C, Gotuzzo E. Ocular manifestations associated with brucellosis: a 26-year experience in Peru. Clin Infect Dis. 2008;46(9):1338-45.
12. Sari I, Altuntas F, Hacioglu S, Kocyigit I, Sevinc A, Sacar S, et al. A multicenter retrospective study defining the clinical and hematological manifestations of brucellosis and pancytopenia in a large series: Hematological malignancies, the unusual cause of pancytopenia in patients with brucellosis. Am J Hematol. 2008;83(4):334-9.
13. Gilbert DN, Chambers HF, Eliopolus GM, Saag MS, Pavia AT, et al. Guide to antimicrobial therapy 2016. 46th ed. USA: Antimicrobial Therapy Inc; 2016.
14. Lubani MM, Dudin KI, Sharda DC, Ndhar DS, Araj GF, Hafez HA et al. A multicenter therapeutic study of 1100 children with brucellosis. Pediatr Infectious Dis J. 1989;8(2):75-8.
15. Solera J, Martinez-Alfaro E, Espinosa A. Recognition and optimum treatment of brucellosis. Drugs. 1997;53(2):245-56.

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Case Report

Eosinophilic gastroenteritis in an immunosuppressed patient: a case report

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ABSTRACT

Eosinophilic gastroenteritis is a rare disease that is characterized by Eosinophil infiltration in one or multiple segments of the gastrointestinal tract. The etiology of this condition remains unknown. Eosinophilic gastroenteritis has heterogeneous clinical manifestations that depend upon the location and depth of infiltration in the gastrointestinal tract, and eosinophilia may or may not be present. This article reports a case of Eosinophilic Gastroenteritis in a 57 years old retroviral male patient, who presented with chronic diarrhea, bilateral pedal edema and eosinophilia. Ultimately, the diagnosis of eosinophilic gastroenteritis was clinched. The rare character of eosinophilic gastroenteritis and its varied clinical presentations often lead to delayed diagnosis and complications. Case reports may help to disseminate knowledge about the disease, thereby increasing the likelihood of early diagnosis and intervention to prevent complications.

Keywords: Chronic diarrhea, Eosinophilic gastroenteritis

INTRODUCTION

Eosinophilic gastroenteritis (EG) is classified in a group of diseases known as eosinophilic gastrointestinal disorders (EGIDs). The primary EGIDs are defined as diseases that selectively affect the gastrointestinal tract with the presence of inflammatory infiltrate that is rich in eosinophils in the absence of other causes of eosinophilia, such as drug reactions, parasitic infections, or malignancies. This group of diseases, the incidence of which has increased over the last few decades, includes eosinophilic esophagitis, eosinophilic gastritis, eosinophilic enteritis, eosinophilic colitis, and EG.[1] All of these conditions exhibit significant infiltration of eosinophils in the gastrointestinal tract and a very strong association with allergies.[2] In 1970, Klein classified the disease according to the predominance of eosinophilic infiltration in different layers of the intestinal wall, namely, the mucosal, muscle, and sub serosal layers.[3]

The involvement of different layers usually gives rise to different clinical manifestations. Mucosal disease generally presents with bleeding, protein-losing enteropathy, or malabsorption. Involvement of the muscle layer may cause bowel wall thickening and subsequent intestinal obstruction. The sub serosal form usually presents with eosinophilic ascites. The diagnosis for EGIDs is established after ruling out other causes of an eosinophilic disease, particularly atopy, parasitic infestations, vasculitis, and hyper eosinophilic syndrome (HES). Authors report a typical case of widespread EG with associated involvement of colonic mucosa in which symptoms dramatically responded to a course of steroids, as evidenced by careful follow up.

CASE REPORT

57 years old male, diagnosed with HIV 1 infection 2 months back, started on anti-retroviral drugs (Tenofovir,

Lamivudine, Efavirenz) presented with complaints of loose stools (3-4 episodes/day) for 2 months and B/L swelling of legs for 4 days. He also had history of loss of weight and appetite. He had no history of atopy/ food or drug allergies. He was treated with nitazoxanide but without any improvement. His CD_4 count was 751 cells and HIV viral load was 880IU/L. HBsAg, anti HCV and TPHA were negative.

On examination he was thin built and had pitting pedal edema. Systemic examination revealed no abnormalities. Investigations showed anaemia (Hb-9.8 g%) and eosinophilia (67.1%) (Absolute eosinophil count 8,700 cells/cu.mm). Liver function test showed hypoproteinemia, elevated alkaline phosphatase and GGT levels. serum LDH, uric acid were within normal limits. Since he was an immunosuppressed host, opportunistic infections were first considered. Stool examination with modified ZN stain for microsporidium, isospora and cryptosporidium were negative.

Stool examination for other ova and parasites were also negative. Radio allegro sorbent test (RAST) for a battery of allergens including common foods were negative. As he had granulomatous hepatitis picture, CECT abdomen was done to look for intraabdominal lymphadenopathy which showed transmural thickening in caecum, ascending colon and proximal transverse colon, with few enlarged mesenteric lymph nodes. Upper GIscopy and deep duodenal biopsies were normal. Colonoscopy showed ileal nodularity, biopsies were taken from multiple sites of colon.

As a part of workup for granulomatous hepatitis, liver biopsy was done. Histopathological examination of liver biopsy showed lobular inflammation with many eosinophils, heavy inflammatory infiltrate including many eosinophils. Ileal biopsy also demonstrated tissue eosinophilia. He was started on T. prednisolone 60 mg (1mg/kg/day). Complete remission of the diarrhea occurred in about one week. One month later, the patient's bowel habits were normal, and he had gained 4 kg.

DISCUSSION

Eosinophilic gastroenteritis can involve any part of gastrointestinal tract from the esophagus down to the rectum. The stomach and duodenum are the most common sites of involvement.[4-7] Four criteria are required for the diagnosis of EG: the presence of gastrointestinal symptoms, eosinophilic infiltration of the gastrointestinal tract, exclusion of parasitic disease or other identifiable cause of eosinophilia and the absence of other systemic involvement.[8-10]

Eosinophils are normal constituents of the mucosa of the gastrointestinal tract; however, the number of eosinophils that can be defined as normal in a biopsy specimen has not been clearly defined.[10] Peripheral eosinophilia is not necessary to make a diagnosis, but it is present in approximately two-thirds of patients and is more prevalent in the serosal than in the mucosal and muscular disease forms.[11,12] The presence of peripheral eosinophilia, abundant eosinophils in the gastrointestinal tract, and a dramatic response to corticosteroid provide some support that the disease is mediated by a hypersensitivity-type reaction. Moreover, a study at Mayo Clinic showed that 50% of patients with EG give a history of allergy such as asthma, allergic rhinitis, urticaria, drug allergy, and eczema.[13]

It must be emphasized that in the mucosal form of the disease, multiple biopsies must be taken during endoscopy, because mucosal involvement is often patchy in nature. Treatment with a steroid is the mainstay in the management of EG. Dramatic clinical improvement is seen after treatment with a low dose of steroid. The duration of treatment is controversial, however. This patient was started with prednisolone 60 mg daily, then tapered over (a period of 6 weeks) and responded favourably.

Due to the positive retroviral status in present patient, authors at first, ruled out opportunistic infections. Not many cases of EG in immunosuppressed patients are reported in literature. Bacterial infections (*Helicobacter pylori*, etc) and parasitic infestations (Ascaridosis, Anisakiasis, Enterobiasis, Anchylostomiasis, Trichina, Teniasis, etc) are a well-known cause of EG, but in present patient neither bacteria in gastric biopsies or stool culture nor ova and parasites were found in the stools.[14-16]

Hypereosinophilic syndrome (HES) may be associated with gastroenteritis but was ruled out because it is an idiopathic condition characterized by marked peripheral eosinophilia exceeding 1500 cells/ml for six consecutive months and its major targets are the heart, lung, brain and kidneys, with>55% of patients presenting with a complication in≥ 1 of these sites.[17] Celiac disease was excluded since deep duodenal biopsy showed normal histologic pattern. In present case, no positive food allergen could be demonstrated, and the colonic involvement makes it similar to the pure form of EG, in which a positive RAST test is rarely found.[3]

CONCLUSION

Eosinophilic gastroenteritis is an uncommon disease with signs and symptoms similar to many other diseases. It is quite rare in immunosuppressed patients, which is why it is often not considered in the differential diagnosis. Histology is essential for the diagnosis of EG and should be considered. Making the correct diagnosis early may result in effective treatment, thereby achieving quicker remission.

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REFERENCES

1. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). J Allergy Clin Immunol. 2004;113:11-28.
2. Guajardo JR, Plotnick LM, Fende JM, Collins MH, Putnam PE, Rothenberg ME. Eosinophil-associated gastrointestinal disorders: a worldwide- web based registry. J Pediatr. 2002;141:576-81.
3. Klein NC, Hargrove RL, Sleisenger MH, Jeffries GH. Eosinophilic gastroenteritis. Medicine (Baltimore). 1970;49:299-319.
4. Daneshjoo RJ, Talley N. Eosinophilic gastroenteritis. Curr Gastroenterol Rep. 2002;4:366-72.
5. Khan S, Orenstein SR. Eosinophilic gastroenteritis. Gastroenterol Clin North Am. 2008;37:333-48.
6. Chen MJ, Chu CH, Lin SC, Shih SC, Wang TE. Eosinophilic gastroenteritis: clinical experience with 15 patients. World J Gastroenterol. 2003;9:2813-6.
7. Khan S. Eosinophilic gastroenteritis. Best Pract Res Clin Gastroenterol. 2005;19:177-98.
8. Furuta GT, Forbes D, Boey C, Dupont C, Putnam P, Roy SK, et al. EGIDs Working Group. Eosinophilic Gastrointestinal Diseases (EGIDs). J Ped Gastroenterol Nutr. 2008;47:234-8.
9. Baig MA, Qadi A, Rasheed JA. Review of Eosinophilic Gastroenteritis. J National Med Assoc. 2006;98:1616-9.
10. Straumann A. Idiopathic eosinophilic gastrointestinal diseases in adults. Best Pract Res Clinical Gastroenterol. 2008;22(3):481-96.
11. Okpara N, Aswad B, Baffy G. Eosinophilic colitis. World J Gastroenterol. 2009;15:2975-9.
12. Talley NJ, Shorter RG, Phillips SF, Zinsmeister AR. Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer and subserosal tissues. Gut 1990;31:54-8.
13. Solans R, Bosch JA, Perez-Bocanegra C, Selva A, Huguet P, Alijotas J, et al. Churg-Strauss syndrome: outcome and longterm follow up of 32 patients. Rheumatology (Oxford). 2001;40:763-71.
14. DeSchryver-Kecskemeti K, Clouse RE. A previously unrecognized subgroup of 'eosinophilic gastroenteritis'. Association with connective tissue diseases. Am J Surg Pathol. 1984;8:171-80.
15. Muller MJ, Sewell GS. Coexistence of eosinophilic gastroenteritis and Helicobacter pylori gastritis: causality versus coincidence. Dig Dis Sci. 2001;46:1784-6.
16. Macedo T, MacCarty RL. Eosinophilic ileocolitis secondary to Enterobius vermicularis: case report. Abdom Imaging. 2000;25:530-2.
17. Scudamore HH, Phillips SF, Swedlund HA, Gleich GJ. Food allergy manifested by eosinophilia, elevated immunoglobulin E level, and protein-losing enteropathy: the syndrome of allergic gastroenteropathy. J Allergy Clinical Immunol. 1982 Aug 1;70(2):129-38.

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Association of N-terminal-Pro-B-type Natriuretic Peptide with Localization of Thrombus and Clinical Outcome in Acute Pulmonary Embolism

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ABSTRACT

Introduction: Pulmonary Embolism is a major health problem which is associated with significant mortality and morbidity. It is a common and lethal condition. This study was undertaken to find the association between NT-Pro-BNP levels and the localisation of thrombus in acute Pulmonary Embolism and to prognosticate the patients.

Material and methods: Prospective study conducted at a tertiary care hospital.Thirty patients with Pulmonary Embolism of various etiologies, confirmed with clinical features, laboratory investigations and imaging and fulfilling the inclusion and exclusion criteria were included. After getting informed consent, Blood samples for assessment of NT-Pro-BNP levels were collected within 24 hours of admission.

Results: In 19 patients, the thrombus was located centrally (in main pulmonary artery) whereas in 11 patients, the thrombus was located in the peripherally (in segmental and subsegmental pulmonary arteries). 22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died. Patients with central pulmonary embolus had higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries had relatively less NTPro-BNP levels. Patients with high NT-Pro-BNP levels had complicated in-hospital course/ mortality whereas, patients with relatively lower NT-Pro-BNP had uncomplicated in-hospital course.

Conclusion: This study shows that higher NT-Pro-BNP levels indicate higher probability of a more central location of thrombus in pulmonary embolism and also right ventricular overload. Also patients with higher NT-Pro-BNP levels had a complicated in hospital course.

Key words: Pulmonary Embolism, Pulmonary Arteries, Right Heart Failure, NT Pro-BNP

INTRODUCTION

Pulmonary Embolism (PE) is a major health problem which is associated with significant mortality and morbidity. It is a common and lethal condition. Pulmonary Thrombo Embolism (PTE) and deep vein thrombosis (DVT) collectively known as venous thromboembolism (VTE) encompass one disease entity. Among cardiovascular diseases, VTE along with stroke and myocardial infarction rank as three big cardiovascular killers. PTE implies partial or complete occlusion of pulmonary arterial circulation by the clot formed elsewhere usually in deep veins of the leg. Less than 5% of venous thrombosis occurs in other sites. The incidence of PE in non-hospitalised patients is 3.5 per 10,000 person years while the incidence rate in hospitalised in-patients can be above 500 per 10,000 person years.[1] In India, VTE is a common cause of mortality and morbidity in patients hospitalized for medical or surgical illnesses. It is often misdiagnosed and not intervened in time. Though the exact magnitude of problem is not known in India, the clinical relevance and incidence is not expected to be different from Western countries. Acute PE has high early mortality rates. Despite the advances in diagnosis and treatment, the early mortality rates have not changed significantly. Due to the embolus obstructing the pulmonary bed, PE can result in a life threatening condition, acute right ventricular (RV) failure.[2] Early diagnosis is of paramount importance since most patients die within the first hours of presentation. Timely management is usually highly effective in reversing RV failure. The diagnosis of acute PE is based on the assessment of clinical likelihood, ECG, CXR, echocardiography, laboratory investigations, mainly D-dimer and cardiac biomarkers and imaging techniques (CT pulmonary angiogram). Several cardiac biomarkers have emerged as indicator of right ventricular dysfunction and clinical outcome predictor in patients with acute PE. Brain type natiuretic peptide (BNP) is a marker of ventricular dysfunction. Following cardiac myocyte stretch, this compound is released into the circulation. It is synthesized as an inactive pro hormone (Pro-BNP) which is split into active hormone BNP and an inactive N-terminal fragment (NT-Pro-BNP).[3] This study was undertaken to find the association between NT-Pro-BNP levels and the localisation

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of thrombus in acute PE, to monitor and prognosticate the patients closely.

Primary objectives of the study were to study the association of N-terminal-Pro-B-Type Natriuretic Peptide with localisation of thrombus in Acute Pulmonary Embolism and to study the association between NT-Pro-BNP levels and clinical outcome in patients with Acute Pulmonary Embolism and secondary objectives were to study on the various risk factors in patients diagnosed with acute pulmonary thrombo embolism and to follow up the patients at the end of 1 month to know the clinical outcome.

MATERIAL AND METHODS

Prospective study was conducted at a tertiary care hospital, PSG Hospitals, Coimbatore. Thirty consecutive patients diagnosed to have pulmonary embolism were included in this study with the following criteria.

Inclusion criteria

All patients of age more than 18 years with clinical and radiological evidence of acute PE will be included in the study.

1) Clinical evidence

Patients presenting with dyspnoea, hemoptysis and chest pain with no acute ischemic changes in ECG and with normal Ejection fraction. ECG in acute pulmonary embolism will shows S1Q3T3 pattern.

2) Radiological evidence

CT pulmonary angiogram showing filling defects within the pulmonary vasculature.

Exclusion criteria

Patients with COPD, known case of Congestive cardiac failure and chronic kidney disease are excluded from this study.

After getting informed consent, Blood samples for assessment of NT-Pro-BNP levels were collected within 24 hours of admission. Risk factors for pulmonary thrombo embolism were noted such as prolonged bed rest, hypertension, malignancy etc. Thrombi located in main/ right/left pulmonary artery were classified as central while the others at segmental/sub-segmental levels were classified as peripheral. Echocardiographic findings such as ejection fraction, functions of right and left ventricle, pulmonary artery pressure were noted. Clinical course of the patient and clinical outcome were also noted. Clinical outcome of the patient at the end of one month was recorded by following up. Then descriptive and statistical analysis of location of thrombus and clinical outcome in relation with NT-Pro-BNP levels was done.

Risk factors	Frequency (n)	Percent (%)
Prolonged immobilization	13	43.3
Carcinoma	4	13.3
Unprovoked	13	43.3
Total	30	100.0

Table-1: Risk factors of the study participants

Clinical Outcome	Frequency (n)	Percent (%)
Normal	22	73.3
Morbid	5	16.7
Death	3	10.0
Total	30	100.0

Table-2: Clinical Outcome after 30 Days

STATISTICAL ANALYSIS

The data collected from the patients was tabulated using Microsoft excel. The data are reported as mean +/- SD depending on their distribution. Risk factors will be described in numbers and percentage. Any association will be analysed using chi-square test. Correlation will be analysed by Pearsman correlation. A p value of <0.05 using two tailed test was taken as being significant for all statistical tests. All data were analysed with statistical software package (SPSS version 24.0)

RESULTS

In our study, we included 30 patients diagnosed with acute pulmonary thromboembolism. The mean age of study population is 57.6 years. 11 patients belonged to the group of 51 to 60 years. Only 2 patients were younger adults and 2 patients were old adults. Among the study group 17 patients were male (56.7%) and 13 patients were female (43.3%).In our study population following were the risk factors noted. Thirteen patients had prolonged immobilisation following road traffic accident or following major orthopaedic surgeries. 4 patients had underlying malignancy such as Ca lung or Ca breast. 13 patients had unprovoked PTE.(Table 1) The thrombus was located unilaterally in 19 patients whereas 11 patients have bilateral thrombus. In 19 patients, the thrombus was located centrally (that is, in main pulmonary artery) whereas in 11 patients, the thrombus is located in the peripherally (segmental and sub segmental pulmonary arteries).22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died.(Table 2)

Patients with central pulmonary embolus have higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries have relatively less NT-Pro-BNP levels. And the difference between the two was statistically significant. (P= 0.02)(Table 3)

Patients with high NT-Pro-BNP levels had complicated in-hospital course/mortality whereas, patients with relatively

	Localisation	N	Mean	Std. Deviation	P value
PROBNP	Central	19	6196.27	4377.75	.02*
	Peripheral	11	1529.10	2636.40	

Table-3: Association of PROBNP with localization

	Clinical Outcome	N	Mean	Std. Deviation	P value
PROBNP	Normal	22	3814.15	3860.52	.005*
	Morbid	5	3009.16	4033.77	
	Death	3	11864.00	1417.83	

Table-4: Association of PROBNP with Clinical Outcome

lower NT-Pro-BNP had uncomplicated in-hospital course. (P = 0.005)(Table 4)

DISCUSSION

Acute PE shows wide variations in clinical presentation from asymptomatic course to a massive pulmonary embolism that may be fatal. When planning treatment for this condition, risk stratification is important. In this study 30 patients diagnosed with pulmonary embolism were included. The mean age of study population is 57.6 years.17 patients were male(56.7%) and 13 patients were female (43.3%). The thrombus was located unilaterally in 19 patients whereas 11 patients had bilateral thrombus. In 19 patients, the thrombus was located centrally (main pulmonary artery) whereas in 11 patients the thrombus was located in the peripheral pulmonary artery (segmental and subsegmental pulmonary arteries). 22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died. Patients with central pulmonary embolus have higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries have relatively less NTPro-BNP levels. And the difference between the two is statistically significant. (P= 0.02).

Patients with high NT-Pro-BNP levels had complicated in-hospital course/ mortality whereas, patients with relatively lower NT-Pro-BNP had uncomplicated in hospital course. (P = 0.005).From this study, it is evident that patients with higher NT-Pro-BNP levels have more central thrombus, have complicated in-hospital course and also cause morbidity and mortality. Whereas patients with comparatively lower NT-Pro-BNP levels have thrombus in segmental or subsegmental pulmonary arteries and they have an uncomplicated course.

The findings of our study were consistent with many other similar studies.

In a prospective study conducted by Akpinar et.al in turkey in Forty-nine patients concluded that higher pro bnp levels indicate higher probability of a more central location of thrombus in pulmonary embolism, resulting a more adverse clinical course and also leading to right ventricular overload..

In a prospective study done by Pruszcyk et.al in Poland in 79 patients found NT-Pro-BNP to be a prognostic marker in acute pulmonary embolism.[5]

An Egyptian prospective study by A.Selem et al conducted in 64 patients concluded elevated NT pro-BNP in PE was associated with worse short term prognosis in terms of higher morbidity and mortality.[6] Another study, Heleder Dores et al a retrospective study also had similar results as that of our study which concluded that elevated NT pro-BNP in PE identified worse short term prognosis and also had excellent power to predict 30 day all cause mortality.[7]

In a meta analysis conducted by Frederikus et.al, in the Netherlands, they found that higher concentrations of NT-Pro-BNP distinguish patients with pulmonary embolism at higher risk of complicated in-hospital course and death from those with low NT-Pro-BNP levels.[8] In another meta analysis conducted by Cavallazzi et.al in USA, they found that NT-Pro-BNP levels are significant predictors of all cause in hospital or short term mortality.[9] A meta analysis by Guillaume Coutance et al concluded while elevated BNP levels can help to identify high risk of death and adverse outcome, the high negative predictive value of normal BNP levels is certainly more useful.[10]

From our study it is evident that NT-Pro-BNP levels are significant predictor of all cause in- hospital mortality in patients with acute PE. The potential outcome of our study is that patients with normal levels of NT-Pro-BNP have a high negative predictive value for unfavourable outcome. Patients with normal NT-Pro-BNP levels have low risk of death as well as for hemodynamic collapse.This was consistant with a study conducted by Nils Kucher in 73 patients which predicted that lower the pro-BNP levels, lower would be the hospital stay.[11]

Our study have a few limitations. First, the sample size is limited. Further studies including larger number of patients are necessary to clarify this point. Secondly, NT-pro-BNP is a precursor of BNP. The conversion of BNP to NT-Pro-BNP may take several minutes after myocardial stretch which may lead to false negative results in patients who present early. Serial monitoring of NT-Pro-BNP (eg. At admission and 6 hours later) may overcome this limitation.

CONCLUSION

This study shows that higher NT-Pro-BNP levels indicate higher probability of a more central location of thrombus in pulmonary embolism and also leading to right ventricular overload.Also patients with higher NT-Pro-BNP levels had a complicated in-hospital course.Further studies with large number of patients were undertaken to determine the exact cut off levels of NT-Pro-BNP to help in treatment decisions.

Ethical approval

The study was approved by the Institutional Ethics Committee

REFERENCES

1. Heit JA, Melton LJ, Lahose CM. Incidence of venous thromboembolism in hospitalised patients vs community residents. Mayo clin Proc 76:1102-1110
2. Fremont B, Pacouret G, Jacobi D, Puglisi R, Charbonnier B, de Labriolle A.Prognostic value of the echocardiographic right/left ventricular end diastolic diameter ratio in patients with acute pulmonary

embolism: results from a monocenter registry of 1416 patients. Chest 2008;133;358-362.
3. Christian Hall, Essential biochemistry and physiology of (NT-pro) BNP, The European journal of Heart Failure 2004;6:257-260.
4. E. Akpinar, E. Sayin, E. Buyuk and M. Gulhan. Association of Levels of N-terminal-Pro-B-Type Natriuretic Peptide with Localisation of Thrombus in Acute Pulmonary Embolism. The Indian Journal of Chest Diseases & Allied Sciences 2012;Vol.54
5. Pruszczyk P, Kostrubiec M, Bochowicz A, Styczyñski G, Szulc M,Kurzyna M, Fijałkowska A, Kuch-Wocial A, Chlewicka I, Torbicki A:N-terminal pro-brain natriuretic peptide in patients with acute pulmonary embolism. Eur Respir J 2003, 22:649-653.
6. A. Selem,H.Radwan,Abdelaziz M.Gomaa Prognostic value of N-Terminal Pro-Brain Natriuretic Peptide in Acute Pulmonary Embolism.Open journal of endocrine and metabolic disease 2012;2:58-62.
7. H. Dores, C. Fonseca, S. Leal, I. Rosário, J. Abecasis, J. Monge, M. J. Correia, L. Bronze, A. Leitão, I. Arroja, A. Aleixo and A. Silva, "NT-pro BNP for Risk Stratification of Pulmonary Embolism," Portuguese Journal of Cardiology,2011;30:881-886
8. Frederikus Klok F, Mos I, Huisman M. Brain-type natriuretic peptide levels in the prediction of adverse outcome in patients with pulmonary embolism. Am J Respir Crit Care Med. 2008;178:425-30.
9. R. Cavallazzi, A. Nair, T. Vasu and P. E. Marik. Natriuretic Peptides in Acute Pulmonary Embolism. Intensive Care Med 2008;34:2147-2156
10. Guillaume Coutance, Olivier Le Page, Ted Lo and martial Hamon prognostic value of brain natriuretic peptide in acute pulmonary embolism. Critical Care 2008,12:R109.
11. Nils Kucher, Gert printzen, Tanja Doernhoefer,Stephan Windecker, Bernhard Meier, Otto Martin Hess. Low Pro-brain natriuretic Peptide Levels predict Benign Clinical Outcome in Acute Pulmonary Embolism. Circulation 2003;107:1576-1578.

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Original Research Article

Tropical sprue in megaloblastic anemia

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ABSTRACT

Background: The causes of megaloblastic anemia may vary in different geographical regions. The aim of the present study is to evaluate the utilization of bone marrow examination and upper gastrointestinal endoscopy (UGIE) in megaloblastic anemia.
Methods: This was a cross-sectional descriptive study done on 50 patients (age ≥15years) of macrocytic anemia after applying inclusion and exclusion criteria. A bone marrow aspiration with biopsy and an UGIE with duodenal biopsy were performed in consented patients with evidence of megaloblastic anemia in the peripheral smear or Vitamin B12 deficiency or folate deficiency or both.
Results: Out of 50 cases, 38 patients had pure Vitamin B12 deficiency, 2 patients had pure folate deficiency and 5 patients had combined deficiency. Among 43 patients with vitamin B12 deficiency, only four (9.3%) were vegetarians and remaining 39 (90.7%) were having non-vegetarian diet. Bone marrow study was done in 29 patients (out of 50) and all of them were found to have megaloblastic erythropoiesis in the bone marrow. Thirty three out of 50 consented for UGIE and duodenal biopsy. Out of 33, 17 patients (51.5%) had features of tropical sprue in biopsy.
Conclusions: We found a high prevalence of tropical sprue in megaloblastic anemia due to Vitamin B12 and/or folate deficiency. We recommend that UGIE with deep duodenal biopsy should be considered in all patients with megaloblastic anemia to rule out tropical sprue in India.

Keywords: Bone marrow study, Duodenal biopsy, Megaloblastic anemia, Tropical sprue, Upper gastrointestinal endoscopy

INTRODUCTION

Megaloblastic anemia is characterized by macrocytic anemia and abnormally large erythrocyte precursor cells (megaloblasts) in the bone marrow. Disorders that affect the synthesis of DNA in the precursors of erythrocytes in the bone marrow lead to megaloblastic anemia. Vitamin B12 and folate deficiencies are the most common causes of megaloblastic anemia.

The causes of megaloblastic anemia may vary in different geographical areas. Pernicious anemia is a major cause of megaloblastic anemia in western countries, but nutritional deficiencies and malabsorption could be the leading cause of megaloblastic anemia in India.[1] Studies have shown that vitamin B12 deficiency is more common in vegetarians.[2,3]

The main cause of folate deficiency is less intake of green leafy vegetables and legumes. There have been hardly any studies on the role of bone marrow and upper gastrointestinal endoscopy (UGIE) in the evaluation of megaloblastic anemia. The objective of the present study is to evaluate the utilization of bone marrow examination and UGIE in megaloblastic anemia.

METHODS

This was a cross-sectional descriptive study carried over a period of 12 months from July 2013 to June 2014 on 50 patients (age ≥15years) of macrocytic anemia treated in a tertiary care hospital of South India. Macrocytic anemia was identified when:

- A mean red blood corpuscular volume >95 fL
- Anemia with hemoglobin of
  a) <13 g/dL in male
  b) <12 g/dL in female.

Inclusion criteria

Patients above the age of 15 years with macrocytic anemia.

Exclusion criteria

Patients with liver disease, chronic kidney disease, hypothyroidism, hemolytic anemia, hemorrhagic disease, post splenectomy and pregnant patients.

The history was taken in detail; a complete and thorough physical examination was carried out in all patients. Detailed information was sought on alcohol consumption, drug intake, thyroid disorder and other comorbid illness. All patients were investigated with a hemogram that included estimation of hemoglobin level, red cell indices (MCV, MCH and MCHC), red cell distribution width, total leucocyte count, differential leucocyte count, platelet count, reticulocyte count, and peripheral smear examination.

All patients were subjected to fasting serum Vitamin B12, fasting serum folic acid, serum TSH, creatinine, liver function test and lactate dehydrogenase. A bone marrow aspiration with biopsy and an UGIE with deep duodenal biopsy were performed in patients with evidence of megaloblastic anemia in the peripheral smear or Vitamin B12 deficiency or folate deficiency or both, who gave consent for the same.

Statistical analysis

The data were analyzed using Microsoft Excel 2010 and Statistical Package for Social Science (SPSS) Version 19.0. Continuous variables were expressed as mean/SD and categorical variables were expressed as percentage. Independent student T test and Anova tests were used as described below. A predictive value of less than 0.05 was taken as statistically significant.

RESULTS

In current study population of fifty patients presenting with macrocytic anemia, 34 were male comprising about 68% and 16 were female comprising about 32%. The mean age of male and female were 53.15±15.89 and 54.37±16.58 respectively. Majority of the population belong to the age group above 40 years, both in males and females. Six patients in this study group were vegetarians. Five patients gave history of consuming alcohol. Three patients gave history of drug exposure and were on phenytoin/ sodium valproate.

The major symptom at presentation was fatigability (58%, n=29), 16% (n=8) presented with chronic diarrhea and 26% (n=13) with other symptoms like short pyrexia etc. No patient had bleeding tendencies or features of nervous system involvement.

On clinical examination, pallor was noticed in all patients in various degrees from mild to severe. Icterus was seen in 10 patients, hyperpigmentation of the knuckles in 7 patients. Organomegaly was noticed in 2 patients, among them one had hepato-splenomegaly and one had splenomegaly.

Out of 50 cases, 38 patients had low Vitamin B12, 5 patients had low levels of both folate and Vitamin B12 and 2 patients had low folate level alone. Vitamin B12 and folate levels were normal in the remaining five patients (Table1). Among 43 patients with Vitamin B12 deficiency, only four (9.3%) were vegetarians and remaining 39 (90.7%) were having non- vegetarian diet.

Table 1: Vitamin B12 and folate status in the study group.

Vitamin status	n=50	Percentage (%)
Pure vitamin B12 deficiency	38	76
Pure folate deficiency	2	4
Combined deficiency	5	10
Both normal	5	10

Table 2 shows hematological parameters of our patients. Out of 50 patients, 24 patients presented with anemia alone, 6 patients with bicytopenia (low hemoglobin and low platelets) and the remaining 20 patients with pancytopenia.

Table 2: Hematological parameters.

Hematological parameter	Mean ± SD
Hemoglobin (g%)	7.1 ± 2.5
MCV (fL)	115.1 ± 10.3
Red cell distribution width	22.71 ± 6.2
Total WBC count	4775 ± 2643
Platelet count	123686 ± 100097

Table 3: Comparison of severity of anemia with macrocytosis.

Hemoglobin (g%)	MCV(fL) Mean ± SD	n =50	P value
< 6	113.56 ± 7.83	18	>0.05
> 6	115.96 ± 11.39	32	

Study found no statistical significance (p=0.4) between the severity of anemia and the degree of macrocytosis, when the values of MCV were compared with the degree of anemia using independent student T test (Table 3). There was no statistical significance (p=>0.5) between the mean of MCV and levels of serum Vitamin B12 using Anova test (Table 4).

Table 4: Comparison of serum Vitamin B12 level and MCV.

Vitamin B12 level (pg/mL)	n=50	MCV (fL) Mean ± SD	P value
< 100	n = 28	116.69±10.89	>0.05
101-190	n = 15	112.20±6.83	
> 190	n = 7	114.92±12.50	

Out of 24 patients with anemia, 21 had low serum Vitamin B12 and 16 of 20 patients with pancytopenia had low serum Vitamin B12. All 6 patients with bicytopenia had low serum Vitamin B12. When the various levels of low serum Vitamin B12 were compared with presentation of patient with anemia, bicytopenia or pancytopenia using Anova test there was no significance (p=>0.5) (Table 5).

Table 5: Comparison of serum Vitamin B12 level and hematological presentation.

Presentation	n=50	Vitamin B-12 level (pg/mL) Mean ± SD	P value
Pancytopenia	20	64.37±31.41	>0.05
Bicytopenia	6	76.83±33.36	
Anemia	24	98.24±44.88	

The peripheral smear examination showed neutrophilic hyper segmentation and / or macro-ovalocytosis in 25 patients suggesting megaloblastic erythropoiesis. These patients and another four (Vitamin B12/folate/or both deficient) who gave consent were subjected to a bone marrow examination. Among 50 patients, bone marrow examination was done in 29 and all of them were found to have megaloblastic erythropoiesis in the bone marrow.

Thirty three out of 50 consented for UGIE and deep duodenal biopsy. Twenty-six of them showed duodenal finding of either scalloped duodenal folds or folded duodenal margins. Others showed features of erosive gastritis, atrophic gastritis or antral gastritis. One had external gastric impression and the other had gastric polyp (Table 6).

Among twenty-six patients who had abnormal duodenal features in endoscopy, fifteen showed features suggestive of tropical sprue in duodenal biopsy (Table 6). Two patients who had normal duodenal findings also had features of tropical sprue in biopsy. Hence 17 patients out of 33 (51.5%), who gave consent for endoscopy and biopsy had tropical sprue. Among 17 patients with tropical sprue, 15 had low Vitamin B12 level, one had both low vitamin B12 and folate levels and one had both normal.

Table 6: Upper gastrointestinal endoscopy and duodenal biopsy findings.

Endoscopy findings	n =33
Scalloped duodenal folds / flattened duodenal margins	26
Erosive gastritis	2
Atrophic gastritis	2
Antral gastritis	1
Others	2
Duodenal biopsy findings	**n =33**
Tropical sprue	17
Intraepithelial lymphocytosis	2
Non-specific	3
Brunner's gland hyperplasia	3
Peptic duodenitis	1
No specific pathology	7

DISCUSSION

Megaloblastic anemia has variable etiological factors. In current study population 76% had Vitamin B12 deficiency, 10% had deficiency of both Vitamin B12 and folate and 4% had pure folate deficiency. Our findings were consistent with a study by Khanduri and Sharma, who have reported Vitamin B12 deficiency to be 65% in 120 patients of megaloblastic anemia, combined Vitamin B12 and folate deficiency in 12% and folate deficiency in 6% .[4] A similar high prevalence of Vitamin B12 deficiency was reported by Sarode et al, in 102 cases (76%) of nutritional megaloblastic anaemia.[5] In contrast to this finding, Haq et al, found that folic acid deficiency was the commonest cause (62.5%) of megaloblastic anemia in her patients.[6]

The predominant symptoms in this study were fatigability (58%) and chronic diarrhea (16%). No patient presented with bleeding manifestation or had neurological symptoms. Unnikrishnan et al, found 91.7% of their patients presenting with fatigability, but in contrast to present study, 36.7% had bleeding manifestation and 10% presented with neurological symptoms.[7] In another study by Haq et al, in a population of 80 patients, 84% had fatigability and 27.5% had bleeding tendency.[6] Bleeding manifestations in thrombocytopenia due to Vitamin B12 or folate deficiency are typically mild and this may be the reason why no patient presented with bleeding tendency in the study.

In the present study, clinical examination revealed pallor in 100%, icterus in 20%, skin hyperpigmentation in 14% and organomegaly in 4%. Haq et al, found pallor in 84% and splenomegaly in 48%.[6] In a study by Unnikrishnan et al, 35% had jaundice, 31.7% had splenomegaly, 28.3% had hepatomegaly and 23.3% had skin changes.[7] Thus

when compared to the above studies we found lesser percentage of patients with organomegaly and skin changes.

Among 43 patients with Vitamin B12 deficiency, only four (9.3%) were vegetarians and remaining 39 (90.7%) were having non-vegetarian diet. 39 out of 44 (88.6%) non-vegetarians had Vitamin B12 deficiency in present study group. In concordance with present study, Unnikrishnan et al, and Iqbal et al, also reported a higher proportion of non-vegetarians having megaloblastic anemia and Vitamin B12 deficiency.[7,8] In contrast to our finding, Liggy et al and Kankonkar et al, showed Vitamin B12 deficiency higher in vegetarians than non-vegetarian. But the study cohort was different, Liggy et al, included all the patients and not only anemic patients, Kankonkar studied B12 deficiency in different diseases.[9,10] In current study 40% of patients had pancytopenia. Other studies have also found that megaloblastic anemia can present with pancytopenia.[5,11]

Bone marrow study was done in 29 out of 50 patients by us. All patients had megaloblastic erythropoiesis in the bone marrow. Khanduri and Sharma have suggested that bone marrow study should be done in selected patients when myelodysplasia is a differential diagnosis.[4] We also agree with them that bone marrow examination need not be done in all patients initially with megaloblastic anemia or Vitamin B12/folate deficiency, as it did not contribute to identifying a different etiology. In present study population of 50 patients, 33 (66%) underwent endoscopy and biopsy. 17 patients out of 33 (51.5%) had tropical sprue (TS). Among 17 patients with TS, 15 had low Vitamin B12 level, one had both low Vitamin B12 and folate levels and one had both normal.

An interesting observation in this study was that tropical sprue was more prevalent in megaloblastic anemia due to Vitamin B12 and/or folate deficiency. This may be because of endemic nature of TS in India. We found that Vitamin B12 deficiency was more in non-vegetarians and this may be due to underlying TS associated malabsorption.

TS is a malabsorption syndrome of unknown etiology or due to a probable persistent infection. TS cause malabsorption of folic acid and Vitamin B12 leading to megaloblastic anemia. A study done in West India in 1968 has reported that majority of patients with TS have Vitamin B12 deficiency.[12] After extensive literature search, we could not find many studies of endoscopic biopsy in megaloblastic anemia. There are few studies in malabsorption syndrome. Dutta et al. in a south Indian study and Ranjan et al. in a north Indian study found that TS was the commonest cause of malabsorption syndrome.[13,14]

There are a few limitations to current study. The sample size was small and we could not do complete work up for megaloblastic anemia and malabsorption due to cost factor.

CONCLUSION

To conclude, we found a high prevalence of TS in megaloblastic anemia due to Vitamin B12 and/or folate deficiency. We observed in thisstudy that being a non-vegetarian does not protect against Vitamin B12 deficiency, hence we recommend that all patients with megaloblastic anemia (including vegetarians) in India should be subjected to UGIE with deep duodenal biopsy to rule out TS, as the prevalence is high and treatment is different.

Study also found that bone marrow study did not yield added information and hence need not be considered in all patients with megaloblastic anemia or Vitamin B12/folate deficiency at the time of presentation, unless clinically indicated. We need larger and more studies to confirm our finding.

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REFERENCES

1. Hoffbrand V, Provan D. ABC of clinical haematology. Macrocytic anaemias. BMJ. 1997;314:430-3.
2. Antony AC. Prevalence of cobalamin (vitamin B-12) and folate deficiency in India-audi alteram partem. Am J Clin Nutr. 2001;74:157-9.
3. Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. Natl Med J India. 2005;18:182-3.
4. Khanduri U, Sharma A. Megaloblastic anameia: prevalence and causative factors. Natl Med J India. 2007;20:172-5.
5. Sarode R, Garewal G, Marwaha N, Marwaha RK, Varma S, Ghosh K, et al. Pancytopenia in nutritional megaloblastic anaemia: a study from north-west India. Trop Geogr Med. 1989;41:331-6.
6. Haq S, Iqbal N, Fayyaz F, Tasneem A. Serum B12 and folate levels in patients with megaloblastic change in the bone marrow. Biomedica. 2012;28:35-9.
7. Unnikrishnan V, Dutta TK, Badhe BA, Bobby Z, Panigrahi AK. Clinico-aetiologic profile of macrocytic anemias with special reference to megaloblastic anemia. Indian J Hematol Blood Transfus. 2008;24:155-65.
8. Iqbal SP, Kakepoto GN, Iqbal SP. Vitamin B12 deficiency- a major cause of megaloblastic anaemia in patients attending a tertiary care hospital. J Ayub Med Coll Abbottabad. 2009;21:92-4.

9. Liggy A, Thomas T, Nambudiri H. Vitamin B12 status in a tertiary care center in central Gujarat. National J Community Med. 2012;3:414-6.
10. Kankonkar SR, Joshi SV, Tijoriwala SJ, Prabhu RV, Raikar SC, Kankonkar RC, et al. A study of Vitamin B12 deficiency in different diseases. Available at www.bhj.org/journal/2004_4602_april/studyofvitam in _110.htm.
11. Kumar R, Kalra SP, Kumar H, Anand AC, Madan N. Pancytopenia--a six-year study. J Assoc Physicians India. 2001;49:1078-81.
12. Jeejeebhoy KN, Desai HG, Borkar AV, Deshpande V, Pathare SM. Tropical malabsorption syndrome in West India. Am J Clin Nutr. 1968;21:994-1006.
13. Dutta AK, Balekuduru A, Chacko A. Spectrum of malabsorption in India-tropical sprue is still the leader. J Assoc Physicians India. 2011;59:420-2.
14. Ranjan P, Ghoshal UC, Aggarwal R, Pandey R, Misra A, Naik S, et al. Etiological spectrum of sporadic malabsorption syndrome in northern Indian adults at a tertiary hospital. Indian J Gastroenterol. 2004;23:94-8.

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Assessment of Handgrip Strength in Patients with Rheumatoid Arthritis and Fibromyalgia-Do They Differ? -An Observational Study

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Abstract

Background: Rheumatoid arthritis (RA) and Fibromyalgia (FM) are chronic illnesses with pain as the common component. Hand grip function is limited in both RA and FM patients, the assessment of which might help in important decision making.

Methods and findings: We analyzed the hand grip strength in RA and FM patients with simple inexpensive hand dynamometer and compared it with age and sex matched healthy controls. Our study had 16 RA and 14 FM patients. The mean hand grip force in dominant right hand was 87.8 ± 22.7 kilograms in fibromyalgia while in RA it was 60.3 ± 21.0 kilograms. Non-dominant hand grip was 72.5 ± 25.7 kilograms in fibromyalgia while in RA it was 52.2 ± 29.3 kilograms. On comparison of the hand grip strength between RA and FM patients, we found that RA patients had a significantly lower dominant and non -dominant hand grip strength than FM patients ($p=0.002$ and $p=0.055$ respectively). When compared with age and sex matched healthy controls both dominant and non-dominant hand grip was significantly lower in RA group ($p<0.001$) while non-dominant hand grip strength in FM was significantly lower than controls ($p<0.001$).

Conclusion: Rheumatoid arthritis patients have poor handgrip than Fibromyalgia and both have poor hand grip than age matched controls.

Keywords: Hand grip strength; Grip strength in rheumatoid arthritis; Hand grip strength in fibromyalgia; Dynamometer; Rheumatoid arthritis; Fibromyalgia

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory systemic disorder of autoimmune etiology which causes progressive joint inflammation, destruction and limitation of joint function if left untreated. Hand and wrist joint involvement is noticed in 80 to 90% of RA patients [1] leading to hand deformities and limitation of the hand function. The most commonly affected hand joints are the metacarpophalangeal (MCP), proximal interphalangeal (ICP) and wrist joints. Reduced flexion or extension of PCP and MCP joints affect the fine movements of the hand and grasp function [2] causing functional disability which in turn affects the activities of daily living (ADL).

Impairment of hand function in RA is due to multiple factors. Apart from hand pain, deformities and loss of range of joint movements, the decreased muscle strength or fatigue also plays an important role in the limitation of joint function. Most of the patients with RA have reduced handgrip strength and impaired hand function [3-7] which has implications on the overall morbidity and mortality.

The effect of handgrip strength on total locomotion [8] was studied by Jonsson et al. and found it to be highly significant. Also one of the predictors of mortality in RA was reduced handgrip [9] as noted by Pincus et al. All these indicate that it is all the more important to assess the hand function in RA patients and initiate appropriate early treatment strategies to preserve the hand function.

Another rheumatological disease which causes chronic widespread pain, fatigue, limitation of physical activities, functional and emotional impairment is Fibromyalgia syndrome [10-14]. Chronic pain seen in fibromyalgia is non-inflammatory.

Alteration in the central processing of pain input and defect in endogenous inhibition of pain is responsible for the chronic widespread pain and increased pain sensitivity [15]. Difficulty in performing the daily routine tasks such as walking, reaching for high shelves, lifting objects and washing own hair is due to decrease in the muscle strength and flexibility. Numerous studies have shown reduced handgrip in patients with FM [16-20].

In fact RA and FM are two commonly encountered rheumatological diseases which can cause limitation of hand

function. Both can co-exist in the same patient and can have masking clinical features. In view of increased pain sensitivity seen in FM patients, they might exhibit tenderness on palpation which can be mistaken for hand involvement in RA. This necessitates the objective measurement of hand function by using a valid tool to assess the hand function.

Assessment of handgrip by dynamometer is a well-established measure to assess the hand function in both the diseases [16,21]. With this background, we intended to assess the handgrip strength in RA and FM patients and to compare it with the healthy subjects to find out the degree of impairment of hand strength in both the study groups.

Aim

The aim of the study is to assess the handgrip strength in RA and FM patients and to compare it with the age and sex matched controls.

Patients and Methods

The study included a total of 30 study subjects, out of which 16 had Rheumatoid arthritis diagnosed as per 2010 ACR classification criteria; 14 had Fibromyalgia diagnosed according to ACR revised diagnostic criteria 2016.

A group of 30 age and sex matched controls were chosen in the study. Subjects more than 17 years were included in the study. Patients with hand deformities or anomalies, blindness, neurological or muscular diseases which affect the performance of the test were excluded from the study.

Methodology

This is a cross sectional observational study conducted in patients attending Rheumatology OP during June 2013-March 2014 in a tertiary care center in Coimbatore, South India. The study design was approved by the Ethics committee and informed consent obtained from all the participants. The handgrip strength of the dominant and non–dominant hand was assessed in both the study and control groups by the same examiner to avoid the inter-observer error.

The hand grip power assessment was done by using a country made handheld plastic dynamometer with the readings from 0 to 110 kilograms. This hand held dynamometer has the advantage of easy availability and reduced cost. The assessment was done with the subjects seated in a chair, shoulders adducted and neutrally rotated, elbows flexed at 90 degrees, forearm in neutral position; wrist between 0 to 30 degrees dorsiflexion and 0 to 15 degrees ulnar deviation. After an initial demonstration of the test, the subjects were asked to squeeze the instrument gradually and continuously for a period of at least two seconds; two recordings were taken in each hand and the mean score was calculated.

Statistical Analysis

Descriptive statistics were reported using mean and SD, median with 25^{th}, 75^{th} percentiles. Handgrip strength of right and left sides were compared between FM and RA group using independent t test. Handgrip strength between FM, RA and control group was compared using ANOVA. Bonferroni correction test was done for multiple comparisons. Paired t test was used to compare the right and left side handgrip strength in each group separately. p value less than 5% was considered as statistically significant.

Results

Among 30 patients, 16 had RA with a mean age of 53.19 ± 6.12 years; 14 had FM with a mean age of 45.36 ± 13.48 years.

The mean age of the control group was 48.03 ± 11.07 years. There were 13 females each in the RA and FM groups; while there were 3 males in RA and 1 male in FM group. There were 4 male and 26 females in the control group.

The results of mean, median with 25^{th} and 75^{th} percentiles of hand grip in RA, FM and control groups were listed in **Table 1**. The mean hand grip in dominant hand was 87.8 ± 22.7 kilograms in fibromyalgia while in RA it was 60.3 ± 21.0 kilograms. Non-dominant hand grip was 72.5 ± 25.7 kilograms in fibromyalgia while in RA it was 52.2 ± 29.3 kilograms. In healthy controls the grip strength was 89.4 ± 11.7 in dominant hand and 81.2 ± 14.1 kilograms in non-dominant hand.

On comparison of the hand grip strength between RA and FM patients **(Table 1)**, we found that RA patients had a significantly lower hand grip in both dominant and non-dominant hands than FM patients (p=0.002 and p=0.055 respectively).

Table 1: Comparison of hand grip in rheumatoid arthritis (RA) and fibromyalgia (FM) patients.

Variables		FM	RA	P-value
Dominant Hand	Mean	87.8 ± 22.7	60.3 ± 21.0	0.002*
	Median with 25^{th} and 75^{th} percentiles	90 (70, 110)	70 (40, 77)	
Non-dominant Hand	Mean	72.5 ± 25.7	52.2 ± 29.2	0.055*
	Median with 25^{th} and 75^{th} percentiles	70 (53.7, 92.5)	45 (31, 70)	

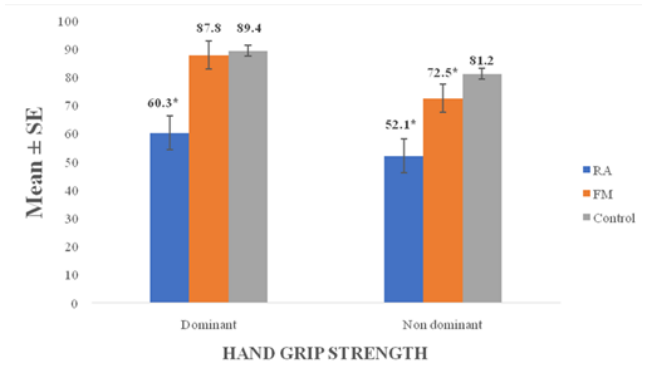


Figure 1: Graphical comparison.

When compared with healthy age and gender matched controls **(Figure 1)**, results showed that RA group had significantly lower dominant hand grip than FM and healthy controls ($p<0.001$). While comparing the non-dominant handgrip strength, it was observed that both FM and RA groups had significantly lower strength as compared to health control groups **(Table 2)**.

Within group comparison of dominant and non-dominant hand grip, results showed that in FM group, hand grip strength was significantly higher in dominant hand ($p=0.003$) but there was no significant difference in RA group **(Table 3)**.

Table 2: Comparison of hand grip in rheumatoid arthritis (RA), fibromyalgia (FM) and healthy controls.

Variables		FM	RA	Control	P-value
Dominant Hand	Mean	87.8 ± 22.6	60.3 ± 21.0*	89.4 ± 11.7	<0.001
	Median with 25th and 75th percentiles	90 (70, 110)	70 (40, 78)	85 (80, 100)	
Non-dominant Hand	Mean	72.50 ± 25.70*	52.1 ± 29.2*	81.2 ± 14.1	<0.001
	Median with 25th and 75th percentiles	70 (54, 93)	45 (31, 70)	80 (70, 90)	
*Significantly lower than control group.					

Table 3: Comparison of dominant and non-dominant handgrip in rheumatoid arthritis (RA) and fibromyalgia (FM) patients.

Variables	Dominant Hand	Non-dominant Hand	P-value
FM	87.8 ± 22.6	72.5 ± 25.7	0.003*
RA	60. 3 ± 21.0	52.1 ± 29.2	0.403

Discussion

While there are various studies on RA and FM separately which had compared the hand grip strength with that of healthy controls, the literature is sparse regarding head-on comparison of handgrip in FM and RA patients. Our study is one of its kinds which assessed the handgrip force in RA and FM patients and compared it with the healthy controls. The present study showed that both the dominant and non-dominant hand grip was less in RA group when compared with the FM and healthy controls. Our observation of reduced hand grip in RA patients when compared with the healthy controls was similar to that observed in other studies [17,22,23].

Our results concur with the other studies which had reported lower hand grip in FM patients when compared with the healthy women [17,18]. Nordenskiold et al. [17] reported 40% reduction in the handgrip in FM patients than healthy adults. Sahin et al. [18] compared the handgrip of 41 fibromyalgia patients with 40 years of age and BMI matched healthy women and noted lower grip force in FM patients. Similar observation of reduced grip force in FM patients than healthy controls was noted in other studies as well [19,20]. Also patients with severe FM had lower grip force than those with moderate FM [16,24] which we have not analyzed in our study.

In the present study we noted that the dominant hand grip is significantly higher than the non-dominant hand in FM group while we did not observe any significant difference in RA group. This might possibly be due to symmetrical hand involvement limiting the hand grip function equally in Rheumatoid arthritis. The data from the literature is conflicting regarding the influence of dominance on hand grip in RA patients. Iop et al. [25] did not observe a significant difference in hand grip between the right and left hand in the Rheumatoid arthritis group which was similar to our observation. Fraser et al. [26] observed that dominant hand in RA patients was on average 20% weaker than the non-dominant hand while another study [27] reported greater hand grip in the dominant hands of RA patients.

To the best of our knowledge, we found only one study which had assessed the hand grip strength in RA and FM patients and compared it with the healthy controls. Our observation of reduced grip force in both the study groups was similar to that observed by Nordenskjold [17] who had studied 19 RA, 14 FM and 18 healthy subjects. The hand grip was assessed by Grippit instrument and compared with the normative data from 169 healthy subjects.

We used a simple, easily available, non-expensive hand held dynamometer to assess the hand grip while most of the reported studies worldwide had used Jamar dynamometer, a standard instrument to measure the hand grip. The need for assessment of chronic diseases through simple means is more important in the follow up of patients; having expensive and

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complicated assessment tools may not be always feasible in routine clinical practice. One such kind of instrument is the simple country made hand held dynamometer which is widely available but lacks the reliability. Also the normative data of hand grip as measured by Jamar dynamometer is available across a wide range of population [28]. There is one Indian study with normative data for hand grip in young adults [29] while the data across various age groups is lacking. Hence we compared our study results with the age and sex matched controls.

We have not taken into account the physique and the work pattern of the study population which might have an influence in the assessment of hand grip strength. Manual workers and labors which require lot of muscular action may have a greater hand grip strength than people who are not used to do such activities. The disease activity assessment was not done in our study patients which can have an influence of the hand function. Ours is a small observational study but worth mentioning which can stimulate further research in this field.

Conclusion

RA patients have poor hand grip than fibromyalgia and both RA and FM have low hand grip when compared to controls.

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Competing and Conflicting Interests

The authors declare no competing and conflicting interest.

References

1. Durmus D, Uzuner B, Durmaz Y, Bilgici A, Kuru O (2013) Michigan hand outcomes questionnaire in rheumatoid arthritis patients: Relationship with disease activity, quality of life, and hand grip strength. J Back Musculoskelet Rehabil 26: 467-473.
2. Melvin JL (1982) Rheumatoid disease, occupational therapy and rehabilitation (2nd ed.), Philadelphia, FA Davis: 32.
3. Myers DB, Grennan DM, Palmer DG (1980) Hand grip function in patients with rheumatoid arthritis. Arch Phys Med Rehabil 61: 369-373.
4. MacBain KP (1970) Assessment of function in the rheumatoid hand. Can J Occup Ther 37: 95-103.
5. An KN, Chao EYS, Askew LJ (1980) Hand strength measurement instruments. Arch Phys Med Rehabil 61: 366-368.
6. Spiegel JS, Paulus HE, Ward NB, Spiegel TM, Leake B, et al. (1987) What are we measuring? An examination of walk time and grip strength. J Rheumatol 14: 80-86.
7. Jones E, Hanly JG, Mooney R, Rand LL, Spurway PM, et al. (1991) Strength and function in the normal and rheumatoid hand. J Rheumatol 18: 1313-1318.
8. Jonsson B, Larsson SE (1990) Hand function and total locomotion status in rheumatoid arthritis-an epidemiologic study. Acta Orthop Scand 61: 339-343.
9. Pincus T, Callahan LF, Vaughn WK (1987) Questionnaire, walking time and button test measures of functional capacity as predictive markers for mortality in rheumatoid arthritis. J Rheumatol 14: 240-251.
10. Wilson HD, Robinson JP, Turk DC (2009) Toward the identification of symptom patterns in people with fibromyalgia. Arthritis Rheum 61: 527-534.
11. Belt NK, Kronholm E, Kauppi MJ (2009) Sleep problems in fibromyalgia and rheumatoid arthritis compared with the general population. Clin Exp Rheumatol 27: 35-41.
12. Gormsen L, Rosenberg R, Bach FW, Jensen TS (2010) Depression, anxiety, health-related quality of life and pain in patients with chronic fibromyalgia and neuropathic pain. Eur J Pain 14: 127.
13. Salaffi F, Sarzi-puttini P, Girolometti R, Atzeni F, Gasparini S, et al. (2009) Health-related quality of life in fibromyalgia patients: A comparison with rheumatoid arthritis patients and the general population using the SF-36 health survey. Clin Exp Rheumatol 27: S67-S74.
14. Mas AJ, Carmona L, Valverde M, Ribas B, EPISER Study group (2008) Prevalence and impact of fibromyalgia on function and quality of life in individuals from the general population: Results from a nationwide study in Spain. Clin Exp Rheumatol 26: 519-526.
15. Bradley LA (2009) Pathophysiology of fibromyalgia. Am J Med 122: S22-30.
16. Aparicio VA, Ortega FB, Heredia JM, Carbonell-Baeza A, Sjostrom M, et al. (2011) Handgrip strength test as a complementary tool in the assessment of fibromyalgia severity in women. Arch Phys Med Rehabil 92: 83-88.
17. Nordenskiold UM, Grimby G (1993) Grip force in patients with rheumatoid arthritis and fibromyalgia and in healthy subjects: A study with the Grippit instrument. Scand J Rheumatol 22: 14-19.
18. Sahin G, Ulubas B, Calikoglu M, Erdogan C (2004) Handgrip strength, pulmonary function tests, and pulmonary muscle strength in fibromyalgia syndrome: Is there any relationship? South Med J 97: 25-29.
19. Maquet D, Croisier JL, Renard C, Crielaard JM (2002) Muscle performance in patients with fibromyalgia. Joint Bone Spine 69: 293-299.
20. Mengshoel AM, Forre O, Komnaes HB (1990) Muscle strength and aerobic capacity in primary fibromyalgia. Clin Exp Rheumatol 8: 475-479.
21. Santos MR, Moro CM, Vosgerau DS (2014) Protocol for physical assessment in patients with fibromyalgia syndrome. Rev Bras Reumatol 54: 117-123
22. Bjork MA, Thyberg ISM, Skogh T, Gerdle BUC (2007) Hand function and activity limitation according to health assessment questionnaire in patients with rheumatoid arthritis and healthy referents: 5-year follow up of predictors of activity limitation (the Swedish TIRA project). J Rheumatol 34: 296-302.
23. Brorsson S, Nilsdotter A, Pedersen E, Bremander A, Thorstensson C (2012) Relationship between finger flexion and extension force in healthy women and women with rheumatoid arthritis. J Rehabil Med 44: 605-608.
24. Aparicio VA, Carbonell A, Ortega FB, Ruiz-Ruiz J, Heredia JM, et al. (2010) Handgrip strength in men with fibromyalgia. Clin Experiment Rheumatol 28: S78-S81.

25. Iop RR, Shiratori AP, Ferreira L, Junior NGB, Domenech SC, et al. (2015) Production capacity of maximal isometric grip strength in women with rheumatoid arthritis: A pilot study. Fisioter Pesq 22: 11-16.

26. Fraser A, Vallow J, Preston A, Cooper RG (1999) Predicting 'normal' grip strength for rheumatoid arthritis patients. Rheumatol 38: 521-528.

27. Nordenskiold U, Grimby G (1997) Assessments of disability in women with rheumatoid arthritis in relation to grip force and pain. Disabil Rehabil 19: 13-19.

28. Leong DP, Teo KK, Rangarajan S, Kutty VR, Lanas F, et al. (2016) Reference ranges of handgrip strength from 125,462 healthy adults in 21 countries: A prospective urban rural epidemiologic (PURE) study. J Cachexia Sarcopenia Muscle 7: 535-546.

29. Bansal N (2008) Hand grip strength: Normative data for young adults. Indian J Physiother Occup Ther 2: 29-33.

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ORIGINAL ARTICLE

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A comparative study on the effect of sodium-glucose cotransporter-2 inhibitors and dipeptidyl peptidase-4 inhibitors as an add on therapy in patients with type 2 DM

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ABSTRACT

There were about 65 million diabetic population in 2016 in India. There were studies which concluded that the Dipeptidyl Peptidase-4 Inhibitors and Sodium Glucose Co-Transportor 2 Inhibitor were providing non-glycaemic control and cardiovascular benefits. Hence, it is important to find out the effect of those drugs on various parameters among the Indian Type 2 Diabetes Mellitus population. The aim is to evaluate and compare the effects of SGLT-2 inhibitors and DPP-4 inhibitors in Type 2 DM patients. The objective is to find out &compare the effects of both class of drugs on the physical profile [Body Weight (BW), Body Mass Index (BMI), Hip-Waist ratio), Diabetic Profile [Fasting Blood Sugar] (FBS), Glycated Haemoglobin (HbA1C)] & Side Effect Profile. The patients were categorized into two Group 1 & 2. Both groups were containing 50 patients, each based on the inclusion &exclusion criteria. The collection of demographics and history were done. Group 1 patients were initiated with DPP-4 Inhibitor, Group 2 patients with SGLT-2 Inhibitors, respectively. At baseline and during follow-up, the parameters like the physical profile, Diabetic Profile were noted. In Body Weight, the mean difference between the groups was found to be 2.9 $\pm$0.79 ($p<0.05$). Similarly, for BMI, it was 2.9$\pm$1.36 ($p<0.04$). For the Hip-Waist ratio, it was 0.07 ($p<0.05$). For FBS, it was 22.08 mg/dl (0.0001), for HbA1c it was 1.09 ($p<0.0043$). On the Side effect profile, SGLT-2 Inhibitors were showing higher incidence for the occurrence of Urinary Tract Infection (UTI) while comparing with DPP-4 Inhibitors. This can be overcome by proper patient counseling. This study concluded that SGLT-2 inhibitors may serves as the best medication choice for add on therapy in Type 2 DM patients.

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INTRODUCTION

Diabetes mellitus is one of the most common chronic diseases which is present in almost all nations. It is the most common non-communicable disease (Shaw *et al.*, 2010). About 65 million diabetic population in 2016 in India, whereas it was 26 million in 1990. In India, the prevalence of Diabetes Mellitus among adults aged 20 years or older was increased from 5.5% to 7.7% between the years of 1990 to 2016, respectively. In 2016, the highest prevalence is seen in the states of Tamil Nadu

& Kerala, which were stated as High Epidemiological Transition Level (High ETL) & Delhi as Higher-Middle ETL, which is followed by Goa, Punjab & Karnataka (Tandon *et al.*, 2018). The micro and macro vascular complications will be reduced by obtaining fair glycaemia control (Alfeena Mary PS *et al.*, 2018). Early initiation of pharmacologic therapy is associated with improved glycaemic control and reduced long-term complications in type 2 DM. In the case of uncontrolled type 2 DM, the drug classes like SGLT-2 inhibitors and DPP-4 inhibitors are used as add on therapy, sometimes as monotherapy also.

DPP-4 inhibitors (Sitagliptin, Teneligliptin, Evo gliptin) produce its effect by activating incretin, which is released by the intestinal cells after food intake. It is usually given as a once-daily dose. DPP-4 is an enzyme that degrades various biologically active peptides, including the GLP-1 and GIP. GLP-1 is playing a major part in releasing insulin (Aschner *et al.*, 2010). Along with glucose control, these drugs were found to have cardiovascular benefits due to its pharmacological augmentation of GLP-1R (Dicker, 2011).

SGLT-2 inhibitors (Dapagliflozin, Canagliflozin, Empagliflozin, Remogliflozin, Ertugliflozin) are a newer class of OHAs (Oral Hypoglycaemic Agents) which are approved to be used as monotherapy or in combination with metformin, sulfonylurea, or insulin. Work by preventing the reabsorption of glucose in PCT and by facilitating its excretion in urine. Hence, the glycaemic control is achieved along with the glucose excretion. The mechanism of action of these drugs is dependent on blood glucose levels and independent of the actions of insulin. So, there are minimum incidences for the occurrence of hypoglycaemia, and there is no risk of overstimulation or fatigue of the beta cells. (Alfeena Mary PS *et al.*, 2018).

Since both classes of drugs are being used either as monotherapy or as part of dual and triple therapy in type 2 diabetes patients, it is important to compare the effects of those drugs in the Indian type 2 diabetes population. This study involves the comparison of Physical (weight, BMI, Hip-waist ratio), Diabetic (FBS, HbA1C) & Side effect profiles of both classes of drugs.

MATERIALS AND METHODS

The study was conducted in the Department of General Medicine in PSG Hospital, Coimbatore. It was approved by the Institutional Human Ethics Committee, PSG Institute of Medical Sciences and Research (Proposal no: 19/072). It is an observational study which included a total of 100 subjects who were divided into two treatment groups. Both groups contain 50 patients each. Group 1 (G1) contains 28 Males & 22 Females whereas Group 2 (G2) contains 30 Males & 20 Females.

The inclusion criteria include Type 2 DM patient of age above 18 years with poor diabetic control and on regular follow up, Patients who are going to be initiated respectively with SGLT 2 Inhibitors & DPP-4 inhibitors as add on therapy for controlling DM and the patients with normal liver function, renal function. Exclusion criteria include patients with Type 1 DM, renal failure, psychiatric, paediatric, gestational diabetes mellitus, history of diabetic ketoacidosis & primary renal glucosuria, and patients who are unwilling to participate.

Group 1 patients were initiated with DPP-4 Inhibitor, Group 2 patients with SGLT-2 Inhibitors, respectively. At baseline and during follow-up after 3 months of drug initiation, the parameters like the physical profile, Diabetic Profile, and side effects were noted and statistically compared. The effect of drugs within a group was compared by using Paired *t*-Test and between groups were by using Independent *t*-Test.

RESULTS AND DISCUSSION

Effects of DPP-4 Inhibitors

The total of 50 G1 patients who were initiated with DPP-4 inhibitors, drugs given were Teneligliptin 20mg (14 patients), Evo gliptin 5mg (07 patients), DPP-4 Inhibitor +Metformin 500mg (16 patients), DPP-4 Inhibitor +Repaglinide 0.5 mg (06 patients), DPP-4 Inhibitor +Glimepiride 1mg (07 patients).

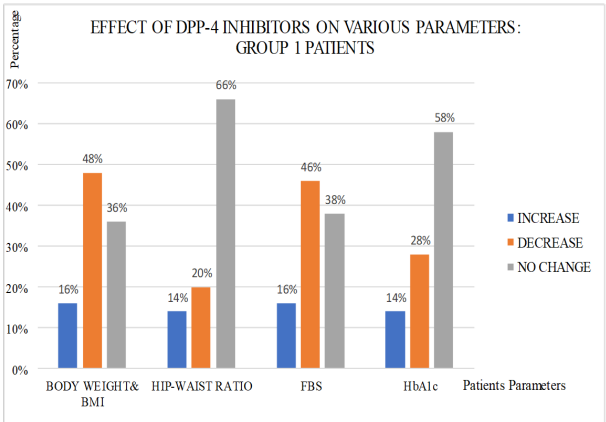


Figure 1: Cluster chart showing the effect of DPP-4 inhibitors on various parameters among Group 1 patients [BMI-Body Mass Index FBS-Fasting Blood Sugar HbA1c-Glycated Haemoglobin]

Once after the drug initiation, the patients were

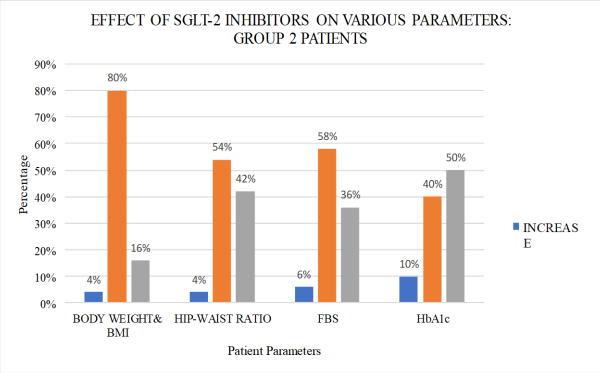


Figure 2: Cluster chart showing the effect of SGLT-2 inhibitors on various parameters among Group2 patients [BMI-Body Mass Index FBS-Fasting Blood Sugar HbA1c-Glycated Haemoglobin]

followed up for three months, and their physical and diabetic profile are monitored, and the changes were noted. According to the effect of drugs on individuals, their parameters get varied. They were depicted in Figure 1

Figure 1 showing the effect of DPP-4 Inhibitors on various parameters among G1 patients. The mean baseline weight (68.44±3.81) was reduced to 64.45±1.71) (p=0.04), the mean baseline BMI (26.73±1.31) was reduced to (25±1.38) (p=0.05), the mean difference between Hip-Waist ratio was 0.13 (p=0.05), the mean difference between FBS was 59.87 mg/dl (p=0.03), the mean difference between HbA1c was 1.2% (p=0.04).

A retrospective sub-analyzed study of sitagliptin effect on glucose and lipid metabolism, blood pressure, body weight and renal function in type 2 DM patients for a period of 6 months by dividing 173 patients of Type 2 DM into obese group (BMI $\geq$ 25) and non-obese group (BMI < 25), concluded that the body weight was significantly reduced in obese patients from the baseline values despite the ages and HbA1c also decreased in both the groups significantly (Katsuyama *et al.*, 2014)

A review of sitagliptin brought a conclusion that on the addition of sitagliptin (100mg/d) to already existing oral antidiabetic treatment produce a significant reduction of HbA1c. After 24 weeks of the addition of sitagliptin (100mg/d) to metformin produced a significant reduction of HbA1c (0.65%), FBS (25.4 mg/dl), Post Prandial Glucose (2h) (50.6 mg/dl) (Gallwitz, 2007)

GLP-1 also found have cardioprotective action in experimental models of heart failure and myocardial infarction. DPP-4 inhibitors may have cardiovascular effects either by depending or independent of GLP-1 (Jose and Inzucchi, 2012)

An Indian study reported the results of a 16-week, multicentric, double-blind, placebo-controlled, Phase 3 studies of teneligliptin 20 mg daily in drug-naive T2DM patients. This study (N = 237) reported a significant −0.55% glycated hemoglobin (HbA1c) reduction (placebo-subtracted) in teneligliptin arm (P = 0.0043) compared to control. While a significant reduction in 2 hours postprandial glucose (PPG) (−25.8 mg/dl, P = 0.0070) versus placebo was observed, an insignificant reduction in fasting plasma glucose (FPG) was seen (−8.8 mg/dl, P = 0.18) in teneligliptin 20 mg arm. Similarly, a higher percentage of patient achieved the target HbA1c of <7% in teneligliptin arm (43.4% vs. 27.3%, P = 0.026) compared to the control and "overall" the drug was well tolerated (Suryawanshi *et al.*, 2016)

Effects of SGLT-2 Inhibitors

The effects of SGLT-2 Inhibitors among various parameter of G2 patients were depicted in Figure 2

Figure 2 showing the effect of SGLT-2 Inhibitors on various parameters among G2 patients. The mean baseline weight (71.34±3.28) was reduced to 64.45±1.97) (p=0.02), the mean baseline BMI (29.93±4.32) was reduced to (27.03±2.96) (p=0.04), the mean difference between Hip-Waist ratio was 0.27 (p=0.04), the mean difference between FBS was 81.95 mg/dl (p=0.01), the mean difference between HbA1c was 2.29% (p=0.02)

A retrospective study which involves 61 patients who received 12 months of dapagliflozin concluded that After the 12 months intake of drug the body weight was -3.4±2.6 kg reduced significantly from the baseline value, the drug reduces the hyperglycemia and body weight by inhibiting the renal glucose reabsorption in a safe manner in Type 2 DM patients. (Cho *et al.*, 2017)

The study of "Efficacy and Safety of SGLT-2 Inhibitors" in Emirati patients which included 307 patients concluded that at 6 months of drug administration the baseline HbA1c was decreased from the baseline value, from 8.9±1.7% to 8±1.5% & at 1 year the mean HbA1c was 8±1.4% (p=0.0001). Likely, the bodyweight also reduced from the baseline at the sixth month, from 85.7±17.8 kg to 84±12.2 kg (p=0.0001). (Bashier *et al.*, 2017)

Comparision between the effects of DPP-4 inhibitors & SGLT-2 inhibitors

Since both categories of the drugs is used as add on therapy in the treatment of Type 2 DM, it's important to find out the effect of those drugs on various parameters among the Indian Type 2 DM population. This result may help to wisely choose the add

on therapy drugs for the treatment of Type 2 DM.

Effects of drugs on

Body weight and BMI

Table 1: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on Body Weight and BMI

Body Weight& BMI	DPP-4 Inhibitors	SGLT-2 Inhibitors
Increase (%)	16	4
Decrease (%)	48	80
No Change (%)	36	16

BMI-Body Mass Index

Hip-waist ratio

Table 2: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on Hip-Waist Ratio

Hip-waist ratio	DPP-4 inhibitors	SGLT-2 Inhibitors
Increase (%)	14	4
Decrease (%)	20	54
No change (%)	66	42

The comparison of the effect of both the drugs over the patient's body weight and Hip-Waist Ratio were depicted in Table 1 &Table 2 respectively. In Body Weight, the mean difference between the groups was found to be 2.9 ±0.79 ($p<0.05$). Similarly, for BMI, it was 2.9±1.36 ($p<0.04$). For the Hip-Waist ratio, it was 0.07 ($p<0.05$). SGLT-2 Inhibitors were showing significant reduction while comparing with DPP-4 Inhibitors.

In 2017, Min SH et al. conducted a meta-analysis study titled "Comparison between SGLT2 inhibitors and DPP4 inhibitors added to insulin therapy in type 2 diabetes: a systematic review with indirect comparison meta-analysis". Covariate-adjusted indirect comparison using meta-regression analyses revealed that SGLT2i/INS achieved greater reduction in HbA1c [weighted mean difference (WMD) -0.24%, 95% confidence interval (CI) -0.43 to -0.05%], fasting plasma glucose (WMD -18.0 mg/dL, 95% CI -28.5 to -7.6 mg/dL) and body weight (WMD -2.38 kg, 95% CI -3.18 to -1.58 kg) from baseline than DPP4i/INS without increasing the risk of hypoglycemia. (Min *et al.*, 2017)

FBS

HbA1c

The comparison of the effect of both the drugs over the patient's FBS & HbA1c were depicted in Table 3 &Table 4 respectively. The mean difference between the groups over FBS was 22.08 mg/dl (0.0001), for HbA1c it was 1.09 ($p<0.0043$). SGLT-2 Inhibitors were showing significant reduction while comparing with DPP-4 Inhibitors.

Table 3: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on FBS

FBS	DPP-4 Inhibitors	SGLT-2 Inhibitors
Increase(%)	16	6
Decrease (%)	46	58
No Change (%)	38	36

FBS- Fasting Blood Sugar

Table 4: DPP-4 Inhibitors Vs SGLT-2 Inhibitors on HbA1c

HbA1c	DPP-4 Inhibitors	SGLT-2 Inhibitors
Increase (%)	14	10
Decrease (%)	28	40
No Change (%)	58	50

HbA1c-Glycated Haemoglobin

Table 5: Side Effect Frequency of the Drugs

Drugs Category	Side Effects (%)
DPP-4 Inhibitors	10
SGLT-2 Inhibitors	14

An observational study on "Safety and Efficacy of Sodium-glucose Cotransporter-2 Inhibitors as Adjuvant to Insulin Add-on Therapy in Uncontrolled Type 2 Diabetic Patients" concluded that the SGLT-2 Inhibitors were more effective in both glycemic control (FBS, HbA1c) and non-glycemic control (Body Weight, BMI, Blood Pressure) and acts as a well-tolerated therapy in patients. This put forth the overall reduction in cardiovascular risks (Alfeena Mary PS *et al.*, 2018)

Side effect profile of the drugs

Since 50 patients were enrolled in each group like DPP-4 Inhibitors and SGLT-2 Inhibitors, respectively, along with the effects of two categories of drugs on various patient's parameters, their Side effects are also monitored during the follow-up period of 3 months. It was given in Table 5.

Side effects of DPP-4 Inhibitors: Nausea, Diarrhoea, Stomach pain, Flu-like symptoms, Nasopharyngitis and HSR (Amori *et al.*, 2007)

Side effects of SGLT-2 Inhibitors: UTI, Hyperkalaemia, Hypotension, Hypoglycaemia and Dia-

betic Ketoacidosis (Hsia *et al.*, 2017)

On Side effect profile, SGLT-2 Inhibitors were showing higher incidence for the occurrence of UTI while comparing with DPP-4 Inhibitors

CONCLUSIONS

Both DPP-4 Inhibitors and SGLT-2 Inhibitors were producing significant impact over the Physical and Diabetic profile of Type 2 DM patients. Though while comparing with DPP-4 Inhibitors, SGLT-2 Inhibitors were producing a more prominent effect over patient's parameters, and apart from producing a significant effect on the Physical and Diabetic profile, SGLT-2 inhibitors were also found to have cardiovascular benefits. Side effects like UTI can be overcome by proper patient counseling. On an overall consideration of the effects produced by both the class of drugs, this study concluded that SGLT-2 inhibitors may serves as the best medication choice for add on therapy in Type 2 DM patients.

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REFERENCES

Alfeena Mary PS, *et al.* 2018. A study on safety and efficacy of sodium-glucose cotransporter-2 inhibitors as adjuvant to insulin add-on therapy in uncontrolled type 2 diabetic patients. Asian Journal of Pharmaceutics, 12(3):896–900. Suppl.

Amori, R. E., Lau, J., Pittas, A. G. 2007. Efficacy and Safety of Incretin Therapy in Type 2 Diabetes. JAMA, 298(2):194–194.

Aschner, P., Katzeff, H. L., Guo, H., Sunga, S., Williams-Herman, D., Kaufman, K. D., Goldstein, B. J. 2010. Efficacy and safety of monotherapy of sitagliptin compared with metformin in patients with type 2 diabetes. Diabetes, Obesity, and Metabolism, 12(3):252–261.

Bashier, A., Khalifa, A. A., Rashid, F., Abdelgadir, E. I., Qaysi, A. A., Ali, R., Alawadi, F. 2017. Efficacy and Safety of SGLT2 Inhibitors in Reducing Glycated Hemoglobin and Weight in Emirati Patients With Type 2 Diabetes. Journal of Clinical Medicine Research, 9(6):499–507.

Cho, H. A., Jung, Y. L., Lee, Y. H., Lee, Y. C., Lee, J. E., Lee, S. J., Kim, C. H. 2017. Efficacy of Body Weight Reduction on the SGLT2 Inhibitor in People with Type 2 Diabetes Mellitus. Journal of Obesity & Metabolic Syndrome, 26(2):107–113.

Dicker, D. 2011. DPP-4 Inhibitors: Impact on glycemic control and cardiovascular risk factors. Diabetes Care, 34(Supplement_2):276–278.

Gallwitz, B. 2007. Review of sitagliptin phosphate: a novel treatment for type 2 diabetes. Vascular Health and Risk Management, 3(2):203–210.

Hsia, D. S., Grove, O., Cefalu, W. T. 2017. An Update on SGLT2 Inhibitors for the Treatment of Diabetes Mellitus. Curr Opin Endocrinol Diabetes Obes, 24(1):73–79.

Jose, T., Inzucchi, S. E. 2012. Cardiovascular effects of the DPP-4 inhibitors. Diabetes and Vascular Disease Research, 9(2):109–116.

Katsuyama, H., Adachi, H., Hamasaki, H., Moriyama, S., Sako, A., Yanai, H. 2014. Significant Differences in Effects of Sitagliptin Treatment on Body Weight and Lipid Metabolism Between Obese and Non-Obese Patients With Type 2 Diabetes. Journal of Endocrinology and Metabolism, 4(5-6):136–142.

Min, S. H., Yoon, J. H., Hahn, S., Cho, Y. M. 2017. Comparison between SGLT2 inhibitors and DPP4 inhibitors added to insulin therapy in type 2 diabetes: a systematic review with indirect comparison meta-analysis. Diabetes/Metabolism Research and Reviews, 33(1).

Shaw, J. E., Sicree, R. A., Zimmet, P. Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice, 87(1):4–14.

Suryawanshi, S. Y., Bhargava, A., Agarwal, P., Chamle, V. 2016. Evaluation of safety and efficacy of teneligliptin in newly diagnosed Indian type 2 diabetes patients. In *233 Spring ST*, volume 59, pages 358–358. SPRINGER.

Tandon, N., Anjana, R. M., Mohan, V., Kaur, T., Afshin, A., Ong, K., Dandona, L. 2018.

J R Coll Physicians Edinb 2019; 49: 282–6 | doi: 10.4997/JRCPE.2019.406 ORIGINAL RESEARCH PAPER

A prospective study on the clinical profile and outcome of meningoencephalitis in adults in a South Indian tertiary care centre

Velammal Petchiappan[1], Divya Pedireddy[2], Santni Manickam[3], Jayachandran Kuppusamy[4]

Abstract

Background Although there are numerous studies on meningitis and encephalitis separately, literature on meningoencephalitis is sparse. In this study we analysed the clinical profile of meningoencephalitis and its clinical outcome.

Methods Fifty adults diagnosed with meningoencephalitis from July 2014 to July 2015 in a tertiary care hospital in South India were studied prospectively and their clinical presentation, aetiology and outcome were analysed.

Results Among 50 patients, 33 (66%) were male; 39 (78%) were <50 years of age. Fever was the most common presenting symptom in 41 out of 50 patients (82%), followed by headache (74%) and altered sensorium (62%); only 18 patients (36%) had all three classical symptoms. Twenty-eight out of 50 patients (56%) did not have neck stiffness. A majority of patients had acute-to-subacute clinical presentation. *Mycobacterium tuberculosis* was identified in 58% (29 out of 50). Forty-seven patients (94%) recovered completely.

Conclusion Tuberculosis was the most common cause of meningoencephalitis in the studied population, often with subacute presentation, and outcome was good with early institution of antituberculous therapy.

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Introduction

Meningitis is a clinical syndrome characterised by inflammation of the meninges surrounding the brain and the spinal cord. The classic triad of meningitis consists of fever, headache and neck stiffness.[1] Meningitis can be of infective or noninfective aetiology, occurring in any age group, with extremes of age being the most severely affected. The immune-compromised state also has high mortality and morbidity. The overall case fatality rate of bacterial meningitis in adult patients is around 30%.[2–4]

Although encephalitis by definition involves the brain parenchyma, it may also involve the meninges as well, which is termed as 'meningoencephalitis'. From an epidemiologic and pathophysiologic perspective, encephalitis is distinct from meningitis, though on clinical evaluation both can coexist. The clinical presentation is encephalopathy with diffuse or focal neurological symptoms, including behavioural and personality changes, decreased level of consciousness, neck pain/stiffness, photophobia, lethargy, generalised or focal seizures, acute confusion or amnesic states, and flaccid paralysis.[5]

Organisms responsible for bacterial meningitis are *Streptococcus pneumoniae*, *Neisseria meningitis*, Group B streptococci, *Listeria monocytogens* and *Haemophilus influenza* type b. Most patients recover completely if appropriate antibiotic therapy is instituted promptly. *Mycobacterium tuberculosis* is another major cause, especially in developing countries. Tuberculous meningitis is a critical disease in terms of fatal outcome and permanent sequelae, requiring rapid diagnosis and treatment.[6]

The term aseptic meningitis is used for all types of inflammation of the brain meninges not caused by pus-producing bacteria. It is usually a benign syndrome. Viral and aseptic meningitis are terms used interchangeably as, not only viruses are a major cause, other noninfective causes are equally attributable to the development of meningitis. Worldwide causes of viral meningitis include enterovirus, herpes, mumps, measles and HIV, with enterovirus being the most common cause of viral meningitis.

In the emergency setting differentiating bacterial meningitis from other causes, such as fungal, tuberculous, viral,

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Table 1 Age-wise distribution of the study subjects

Age (years)	Male	Female	Total
18–30	12	5	17
31–50	17	5	22
51–60	2	7	9
>60	2	0	2
Total	33	17	50

neoplastic, toxic or autoimmune causes, is extremely difficult. If a diagnosis of meningitis is made, it is prudent to start the patient on empirical antibiotics until the cultures and other results are awaited.[7] Whereas plenty of literature is available on meningitis, the clinico-aetiological profile and outcome in patients with meningoencephalitis remains not that well studied, except for in the paediatric population.[8] The present study was carried out to assess the causes, clinical profile and outcome in adults diagnosed with meningoencephalitis in a tertiary care hospital.

Methods

Patients

The study was based on prospective collection of data in adults aged 18 years and above who were diagnosed with meningoencephalitis of various aetiologies, confirmed with laboratory investigations and brain imaging. The study was conducted in a tertiary care centre, Coimbatore, South India, from July 2014 to July 2015.

Inclusion and exclusion criteria

Patients with community-acquired meningoencephalitis, those who underwent lumbar puncture for the diagnosis and those with both positive and negative cerebrospinal fluid cultures were included in the study. Patients with hospital-acquired meningitis, patients with myelitis, neurosurgical devices or a recent history of neurosurgery were excluded.

Assessments

Data were extracted using a proforma that included detailed history, clinical examination details and requisite investigations. History and clinical findings attributable to the meningitis and meningoencephalitis were collected in detail, which included fever, headache, vomiting, neck stiffness, seizures, altered sensorium, cerebrospinal fluid (CSF) rhinorrhoea and focal neurological deficits. In addition to basic investigations (which included complete blood count, blood sugar, renal and liver function tests, and chest X-ray), blood culture was also taken for all patients. All underwent lumbar puncture and CSF analysis was carried out that included sugar, protein, total count, differential count, Gram stain, bacterial culture, Ziehl Neelsen stain for acid-fast bacilli (AFB), India ink stain for *Cryptococcus,* cryptococcal antigen test and automated culture for AFB. Neuroimaging (CT or MRI brain) was carried out on all patients. CSF polymerase chain reaction (PCR) was carried out in selected patients. Herpes simplex virus (HSV) PCR was carried out in selected patients with suspected HSV encephalitis based on clinical, CSF and neuroimaging findings.

Table 2 Clinical presentation of the study subjects

Clinical variables	Number of patients	%
Headache	37	74
Fever	41	82
Seizures	6	12
Vomiting	24	48
Altered mental status	31	62
Cerebrospinal fluid rhinorrhea	5	10
Hemiparesis	4	8
Speech disturbances	2	4
Cranial nerve palsy	4	8
Neck stiffness	22	44
Papilloedema	5	10

Table 3 Aetiological profile of meningoencephalitis

Aetiology	Number of patients	%
Tuberculous meningitis	29	58
Acute pyogenic meningitis	8	16
Viral meningoencephalitis	8	16
Cryptococcal meningitis	2	4
Cerebral toxoplasmosis	2	4
Aspergillus meningitis	1	2
Total	50	100

Based on the duration of illness, patients were classified into acute (<1 week), subacute (1–4 weeks) and chronic (>4 weeks) and also grouped based on aetiology. To assess the clinical outcome of the patients the Barthel index was used.[9] Barthel index is an ordinal scale used to measure the performance in 10 activities of daily living with the score ranging between 0 and 20; functional evaluation was carried out at the end of the first month. A score of <12 is taken as a poor outcome and >12 as a good outcome; death is included in the poor outcome category. The results were analysed to assess the clinical presentation, aetiology and clinical outcome.

Ethical clearance

The study was approved by the Institutional Human Ethics Committee (Approval Number 14/193).

Results

The study included 50 consecutive patients with acute meningoencephalitis. Among 50 patients, 33 (66%) were male, 17 (34%) were female. The majority of them (78%) were <50 years of age (Table 1).

Clinical presentation

The common initial presenting symptoms were fever, headache and altered sensorium (Table 2). Among 50

Aetiology	Acute	Subacute	Chronic	Total
Tuberculous meningitis	4	21	4	29
Acute pyogenic meningitis	8	0	0	8
Viral meningoencephalitis	8	0	0	8
Cryptococcal meningitis	1	1	0	2
Cerebral toxoplasmosis	2	0	0	2
Aspergillus meningitis	0	1	0	1
Total	23	23	4	50

Acute: clinical presentation <1 week; Subacute: clinical presentation between 1–4 weeks; Chronic: >4 weeks duration

Table 4 Mode of onset of symptoms in various aetiologies of meningoencephalitis

patients, 32 (64%) had both fever and headache; only 18 (36%) had all the three classical signs of meningitis – headache, fever and altered sensorium. Twenty-eight patients (56%) did not have neck stiffness, even though there was meningeal involvement as noted in neuroimaging. Six patients (12%) had seizures; five had generalised tonic–clonic seizures, while focal motor seizures was noted in one. Among those six patients, one had a parenchymal lesion in the MRI (granuloma), meningeal enhancement was noted in three and MRI was normal in two.

Altered sensorium was observed in 31 patients; of which 10 had significant deterioration in consciousness (Glasgow coma scale <10). Among those with poor Glasgow coma scale, one had pyogenic meningitis with extensive vasculitic infarcts in the bilateral cerebral hemisphere and brainstem; seven had abnormal findings in MRI like meningeal enhancement, granuloma or hydrocephalus; MRI was normal in the remaining two. All of these 10 patients with altered sensorium required ventilatory support in intensive care unit. See online for Supplementary Tables 1–4.

Aetiology and mode of onset

The aetiological profile of meningoencephalitis is listed in Table 3. The most common cause found in the present study was *M. tuberculosis*. Among eight patients with acute pyogenic meningitis, the microbiological diagnosis was confirmed in five; three had pneumococcal growth in CSF analysis, while blood culture was positive in two (*S. aureus*; pneumococci). The remaining three patients with acute pyogenic meningitis had sterile cultures due to prior antibiotic exposure. However, very high neutrophil count in the CSF suggested the diagnosis of acute pyogenic meningitis.

Acute and subacute clinical presentation was seen in 23 each while the remaining four presented with chronic symptoms. The mode of onset of symptoms in various aetiologies is listed in Table 4.

Outcome

Clinical outcome was assessed at the end of 1 month using the Barthel index. Among 50 patients, 47 (94%) recovered completely without any deficits; two had fulminant course and succumbed to the illness by the end of 1 month; one had Barthel index score of 0 (Table 5).

Discussion

The present study included 50 adult patients with meningoencephalitis, *M. tuberculosis* was the most common agent. Tuberculosis still poses a huge health threat in developing countries like India despite advances in the detection and management of the disease.

The common presenting symptoms were fever, headache and altered sensorium. This was similar to other studies with acute bacterial meningitis.[2,10,11] In the present study, nine did not have fever (five had tuberculous aetiology, two had cryptococcal meningitis, cerebral toxoplasmosis and *Aspergillus* meningitis in one each). All patients with acute pyogenic and viral meningoencephalitis presented with fever. Only 18 out of 50 patients (36%) had all the three symptoms – fever, headache and neck stiffness; a similar observation (41%) was noted in a prospective cohort study of community-acquired bacterial meningitis in adults in the Netherlands.[11] Neck stiffness was absent in 28 patients (56%), which indicates that absence of fever or neck stiffness does not exclude the possibility of infectious meningoencephalitis.

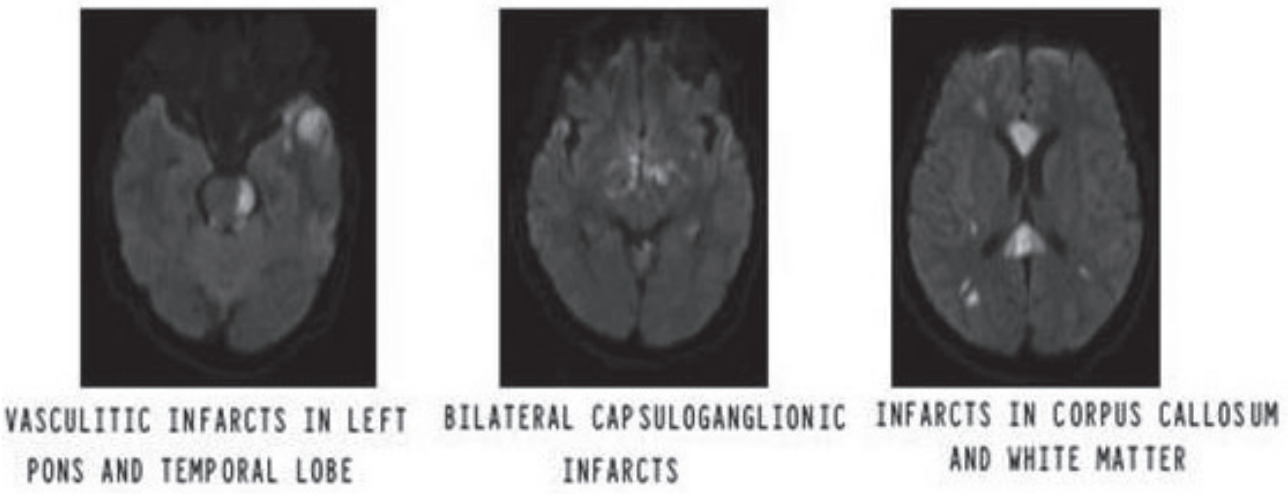
Diagnosis of pyogenic meningitis was made based on typical CSF findings (Supplementary Table 1) that showed high protein, low sugar and very high neutrophil count in the CSF (>1,000 cells/mm^3 in most of the patients). In five out of these eight patients with acute pyogenic meningitis, organisms could be isolated either in the blood or CSF. For the remaining three, the cultures were sterile possibly due to prior antibiotic exposure. In a retrospective study of 305 patients with acute bacterial meningitis, 53 (17.4%) had

Outcome	TM	APM	VME	CM	CT	AM	Total
Good (n = 47)	29	6	8	1	2	1	47
Poor (n = 3)	0	2	0	1	0	0	3
Total	29	8	8	2	2	1	50

AM: *Aspergillus* meningitis; APM: acute pyogenic meningitis; CM: cryptococcal meningitis; CT: cerebral toxoplasmosis; TM: tuberculous meningitis; VME: viral meningoencephalitis

Table 5 Clinical outcome in various aetiologies of meningoencephalitis

Figure 1 Fulminant pneumococcal meningitis with extensive vasculitic infarcts in bilateral cerebral hemisphere and brainstem



received antibiotics before admission and among these, death occurred only in one. The death rate was high (12%) in the remaining 252 patients who had not received antibiotics before admission. This study indicates the importance of the early institution of antibiotic therapy.[12,13]

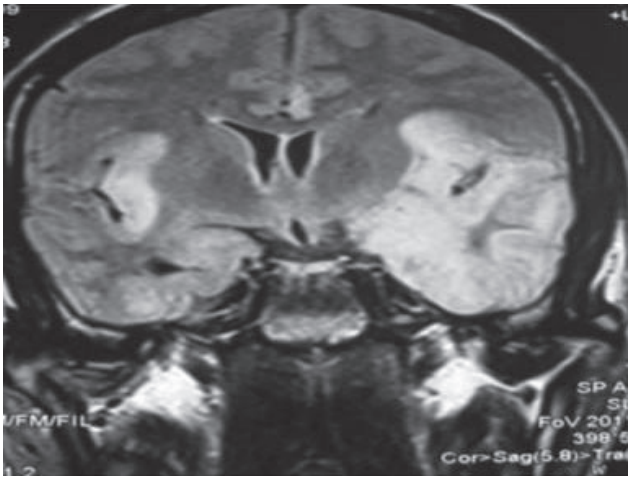
Surprisingly, four out of eight patients (50%) with acute pyogenic meningitis had a history of CSF rhinorrhoea before the onset of illness. Of these, three had a head injury in the past. MRI brain in one patient revealed a defect in the anterior cranial fossa that was the cause of CSF leak. One of them had recurrent episodes of pyogenic meningitis due to persistent CSF leak through the nose. After recovery from meningitis, he underwent a neurosurgical procedure for the correction of bony defects. This highlights the importance of elicitation of history for CSF rhinorrhoea in subjects with recurrent meningitis so that the underlying cause can be corrected. Six out of eight acute meningoencephalitis patients recovered completely without residual deficits; however, two had fulminant pneumococcal meningitis, of which one succumbed to the illness although treatment was initiated appropriately, the other survived with severe neurological disability, i.e. bilateral conjugate gaze paralysis of eyeballs and quadriparesis due to multiple infarcts (Figure 1). These outcomes highlight the fact that despite early diagnosis and appropriate treatment some patients with acute pyogenic meningitis may have a fulminant course with a severe neurological disability that can impair the quality of life.

Eight patients had acute viral meningoencephalitis, diagnosed based on CSF analysis and brain imaging features. A viral aetiology was considered when CSF had slightly elevated protein, low-to-normal blood sugar and moderately elevated lymphocyte count. Bacterial cultures, Gram stain, AFB stain and India ink stain were negative in these patients. Out of these eight patients, microbiological confirmation (positive HSV PCR in CSF) was possible in only two; both had temporal cortex hyperintense signals in MRI brain (Figure 2). All recovered completely with acyclovir and were asymptomatic during follow up. In many cases of presumed viral encephalitis (32–75%), the responsible organism could not be pin-pointed despite detailed diagnostic testing. In the California Encephalitis Project, 334 patients with encephalitis were studied from 1998 to 2000; aetiology could not be identified in 208 (62%), despite extensive testing and evaluation.[14]

Although commonly tuberculous and cryptococcal meningitis presents with subacute courses, four patients with tuberculous meningitis and one with cryptococcal meningitis presented with acute onset illness. Two patients presented with central nervous system toxoplasmosis and these patients were found to be HIV positive; they presented with hemiparesis; CSF serology for toxoplasma immunoglobulin G was positive; both the patients recovered well with treatment.

In the present study 15 had basal meningeal exudates on brain imaging. Hydrocephalus was present in five; of which two had significantly larger hydrocephalus, which progressively increased in size and both underwent ventriculoperitoneal shunt. In two large community-based series, hydrocephalus was seen in approximately 75% of

Figure 2 Herpes simplex encephalitis – T2-weighted MRI shows left temporal and right insular hyperintensity



patients, basilar meningeal enhancement in 38%, cerebral infarcts in 15–30% and tuberculomas in 5–10%.[15,16] With early appropriate antituberculous therapy and steroids, all patients with tuberculous meningitis in the present study recovered well without neurological deficits.

The present study had several limitations: PCR studies in all CSF culture-negative samples could not be performed and the study was conducted in a single centre with a limited number of patients. Further larger studies on meningoencephalitis would throw more light on the clinical profile and outcome.

In conclusion, the present study underscores the fact that the absence of fever or neck stiffness does not exclude the possibility of infectious meningoencephalitis. The focus in all cases should be on early diagnosis and appropriate treatment, though in some patients the outcome may not be uniformly good.

Online Supplementary Material

Supplementary Tables 1–4 are available with the online version of this paper, which can be accessed at https://www.rcpe.ac.uk/journal.

References

1 Ginsberg L, Kidd D. Chronic and recurrent meningitis. *Pract Neurol* 2008; 8: 348–61.
2 Aronin SI, Peduzzi P, Quagliarello VJ. Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. *Ann Intern Med* 1998; 129: 862–9.
3 Mace SE. Acute bacterial meningitis. *Emerg Med Clin North Am* 2008; 26: 281–317.
4 Roos KL, van de Beek D. Bacterial meningitis. *Handb Clin Neurol* 2010; 96: 51–63.
5 Bloch KC, Glaser C. Diagnostic approaches for patients with suspected encephalitis. *Curr Infect Dis Rep* 2007; 9: 315–22.
6 Rich AR, McCordick HA. The pathogenesis of tuberculous meningitis. *Bull John Hopkins Hospital* 1933; 52: 5–37.
7 Fitch MT, Abrahamian FM, Moran GJ et al. Emergency department management of meningitis and encephalitis. *Infect Dis Clin North Am* 2008; 22: 33–52.
8 Khajeh A, Sharifi-Mood B, Soleimani GR. Pediatric meningoencephalitis; a research on patients hospitalized in Zahedan, Southeastern Iran. *Int J Infect* 2015; 2: e23835.
9 Mahoney FI, Barthel DW. Functional evaluation: the Barthel index. *Md State Med J* 1965; 14: 61–5.
10 Van de Beek D, de Gans J, Spanjaard L et al. Clinical features and prognostic factors in adults with bacterial meningitis. *N Engl J Med* 2004; 351: 1849.
11 Bijlsma MW, Brouwer MC, Kasanmoentalib ES et al. Community-acquired bacterial meningitis in adults in the Netherlands, 2006–14: a prospective cohort study. *Lancet* Infect Dis 2016; 16: 39.
12 The Research Committee of the British Society for the Study of Infection. Bacterial meningitis: causes for concern. *J Infect* 1995; 30: 89–94.
13 Begg N, Cartwright KAV, Cohen J et al. Consensus statement on diagnosis, investigation, treatment, and prevention of acute bacterial meningitis in immunocompetent adults. *J Infect* 1999; 39: 1–15.
14 Glaser CS, Gilliam S, Schnurr D et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998–2000. *Clin Infect Dis* 2003; 36: 731–42.
15 Bhargava S, Gupta AK, Tandon PN. Tuberculous meningitis–a CT study. *Br J Radiol* 1982; 55: 189.
16 Ozates M, Kemaloglu S, Gurkan F et al. CT of the brain in tuberculous meningitis. A review of 289 patients. *Acta Radiol* 2000; 41: 13.

Is Serum Uric Acid an Added Risk Factor for Micro-Vascular Complications of Diabetes Mellitus? – A Prospective Study

Santni Manickam[1], Prashanth Arun[2], Velammal Petchiappan[3], Sujaya Menon[4]

ABSTRACT

Introduction: Diabetes is the leading cause of adult blindness due to retinopathy, end stage renal disease due to nephropathy and foot ulcers and lower limb amputation due to neuropathy. Serum uric acid levels independently predict the development of micro vascular complications. In this study, we analyse the association of serum uric acid with the micro vascular complications of diabetes.

Material and methods: In this hospital based observational study, hundred diabetic patients were included; of which fifty had micro vascular complications, the remaining fifty had no complications. Blood investigations including fasting(FBS) and post prandial blood sugars(PPBS), glycosylated haemoglobin (HbA1C), and serum creatinine and serum uric acid were done in all patients. All the parameters were compared between the two groups.

Results: Females presented with significantly higher complications as compared to males. The mean age of the patients presented with and without complications was 56.4 ± 9.3 and 59.9 ± 10.3 respectively. HbA1C had a positive correlation with the serum uric acid (SUA) (r=0.327, *P* = 0.001). Mean uric acid levels were higher among patients with complications (5.96 ± 2.16) compared to patients without complications (4.95 ± 2.04) which was statistically significant (*P* value =0.021). Patients with nephropathy and neuropathy had significant elevation in serum uric acid levels than those without; however this significance was not noted in those with retinopathy.

Conclusion: Patients with micro vascular complications had higher levels of serum uric acid compared to those without complications. Also there was positive correlation between HbA1C and serum uric acid levels.

Keywords: Serum Uric Acid, Micro Vascular Complications, Diabetic Retinopathy, Diabetic Neuropathy, Diabetic Nephropathy, Glycosylated Haemoglobin.

INTRODUCTION

Diabetes mellitus is the most challenging health problem in this century. Diabetes is the leading cause of adult blindness due to retinopathy, end stage renal disease due to nephropathy and foot ulcers and lower limb amputation due to neuropathy.[1]

Uric acid (UA) is primarily a purine metabolic waste product. About 70% of it gets excreted by the kidneys. Hyperuricemia has gained importance as many studies have reported that it not only has an important role in the development of metabolic syndrome but also micro vascular risk factor.[2-5] Elevated serum uric acid is a feature of hyperinsulinemia or insulin resistance.[6]

Many studies have suggested that inflammation and oxidative stress results from the metabolism of uric acid, leading to vascular injury. The rate limiting step of uric acid production is an enzymatic reaction of the xanthine oxidase (XO) enzyme that oxidises hypoxanthine – xanthine into uric acid. UA synthesis is accompanied by the generation of reactive oxygen species (ROS). XO in the vascular endothelium is associated with ischemia reperfusion injury. It has also been suggested that XO inhibitors improve endothelium-dependent vascular relaxation in blood vessels of hyperlipidemic rabbits.[7] XO as the source of ROS in ischemia/reperfusion injury has been discovered 30 years ago.[8,9]

In recent years there has been a debate regarding the association of hyperuricemia with diabetic complications. If this association is conclusively established, therapeutic interventions aiming to reduce uric acid synthesis might help to retard the progression of micro vascular complications of diabetes. Hence this study was conducted aiming to show the association of uric acid levels with diabetic complications.

MATERIAL AND METHODS

This is a hospital based observational study conducted in a tertiary care centre at Coimbatore from April 2015 to April 2016. Patients with Type 2 Diabetes Mellitus of the age group of 20 to 80 years admitted in the medical wards were included in the study. Patients admitted with acute complications like diabetic ketoacidosis and hyperosmolar coma and patients with gestational diabetes were excluded

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from the study. Informed consent was obtained from the patients. Before commencement of the study, approval was obtained from the Institutional Ethics Committee.

A detailed history and physical examination was done. Baseline investigations including fasting and post prandial blood sugars, HbA1c and serum creatinine were done. The diagnosis of diabetes was based on American Diabetic Association criteria. The presence of micro vascular complications was established by fundus examination by an ophthalmologist to assess retinopathy, Urine spot Protein/ creatinine ratio (PCR) to assess nephropathy and nylon monofilament test to assess neuropathy. Serum uric acid was done in all patients. Hyperuricemia was defined as serum uric acid greater than 7 mg/dl.

The study population was divided into two groups: group I patients with diabetes and without any vascular complications; group II patients with diabetes and any one or more of vascular complications.

STATISTICAL ANALYSIS

Descriptive statistics were reported as mean and standard deviation; categorical variables were presented with number and percentages. Chi-square test was used to test the association between the variables. Independent t test was used to compare the blood parameters between diabetes with complications and without complications. P value less than 0.05 was considered statistically significant.

RESULTS

Among the 100 diabetic patients studied, there were 62 males and 28 females; 50 had presented with complications and 50 had no complications in the study group. Out of 50 with diabetes related micro-vascular complications, there were 24 males and 26 females. There was a significant difference in the presence of complications and sex; Females presented with significantly higher complications as compared to males (P= 0.004). The mean age of the patients presented with and without complications was 56.4 ± 9.3 and 59.9 ± 10.3 years respectively; although not significant (P= 0.084), patients who presented with complications were younger than those without complications.

	Present (%)	Absent (%)
Diabetic Retinopathy	19(38)	31(62)
Diabetic Neuropathy	16(32)	34(68)
Diabetic Nephropathy	19(38)	31(62)

Table-1: Distribution of Micro vascular complications

	Diabetes with complications	Diabetes without complications	P value
Sex Male Female	 24 (39) 26 (68)	 38 (61) 12 (32)	0.004*
Age	56.4 ± 9.3	59.9 ± 10.3	0.084
HbA1c	8.28 ± 2.00	7.55 ± 1.50	0.044*
FBS	137.8 ± 44.5	133.5 ± 46.6	0.644
PPBS	210.1 ± 60.3	187.7± 52.7	0.028*
Uric acid	5.96 ± 2.16	4.95 ± 2.04	0.021*

Table-2: Comparison of age, sex, HbA1c and uric acid levels in diabetic patients with and without micro-vascular complications.

Microvascular complication		Serum uric acid level (mg/dL)	P value
Diabetic retinopathy	Present	5.97 ± 2.14	0.97
	Absent	5.95 ±2.20	
Diabetic neuropathy	Present	6.56 ± 2.30	0.004
	Absent	4.73 ± 1.10	
Diabetic nephropathy	Present	7.05 ± 2.13	0.006
	Absent	5.33 ± 1.94	

Table-3: Serum uric acid levels in micro vascular complications

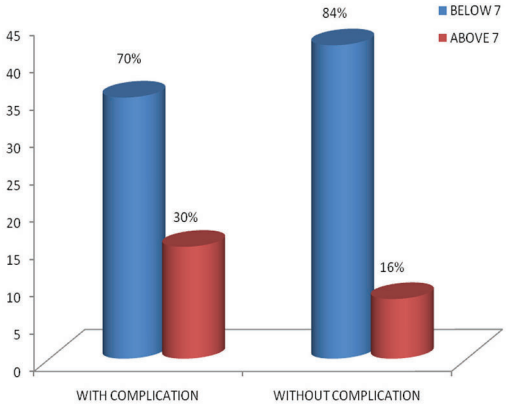


Figure-1: Frequency distribution of serm uric acid(mg/dl) in Diabetes mellitus with and without complications.

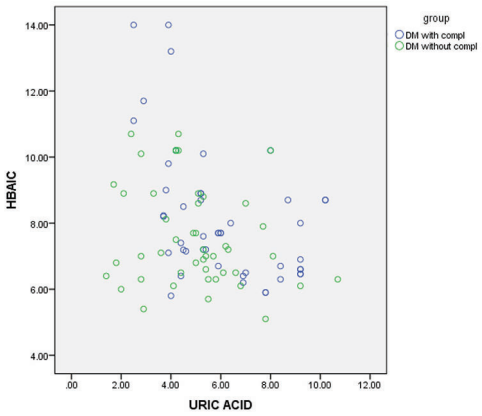


Figure-2: Co-relation of HbA1C with the Serum uric acid levels

The distribution of micro vascular complications is shown in Table 1. Both retinopathy and nephropathy were equally prevalent and slightly more than neuropathy.

Hyperuricemia (Serum uric acid >7 mg/dl) was more prevalent among patients with complications (30%) compared to patients without complications (Fig 1). As shown in Table 2, mean uric acid levels were higher among patients with complications (5.96 ± 2.16 mg/dl) compared to patients without complications (4.95 ± 2.04 mg/dl) which was statistically significant (*P* value = 0.021). HbA1C had a positive correlation with the serum uric acid levels (r=0.327, *P* = 0.001) as shown in Fig 2.

Table 3 represents the prevalence of hyperuricemia in patients with retinopathy, neuropathy and nephropathy, as compared to those without these complications. It can be seen that nephropathy and neuropathy patients had significantly more hyperuricemia than those without. However there was no statistically significant difference seen in the group with retinopathy. This suggests that high uric acid levels are associated with microangiopathies in Type 2 Diabetes.

DISCUSSION

Increasing amounts of uric acid in the serum causes Gout and this is the most significant cause of lifestyle-related disorder. Uric acid is primarily a product of purine metabolism. Serum uric acid (UA) levels are determined by a balance between Uric acid production and excretion. The rate-limiting step of UA production is an enzymatic reaction of the xanthine dehydrogenase/xanthine oxidase (XDH/XO) enzyme that oxidizes hypoxanthine-xanthine into UA. It is expressed in the liver and small intestine of XDH/XO-rich parenchyma cells[10] and is thought to be the major source for UA. The enzyme is also expressed in adipose tissue, the vascular endothelium, and macrophages, all of which are implicated in lifestyle-related diseases.[11] The UA production rate is based on the amount of substrate and/or XO activity. Since the generation of reactive oxygen species (ROS) depends on XO activity, XO is one of the major sources of oxidative stress in cells along with nicotinamide adenine dinucleotide phosphate oxidase, myeloperoxidase, lipoxygenase, and nitric oxide synthase.[12]

The kidney is an important regulator of circulating UA levels and is responsible for 60%-70% of total body UA excretion.[13] The remaining UA is secreted into the intestine, followed by bacterial uricolysis.[13] UA excretion in the kidney consists of urate secretion and reabsorption, and earlier research suggests the involvement of hyperfiltration.[6] Ninety percent of UA filtered by the kidney is reabsorbed.[13]

In patients with diabetes, the SUA level is low due to increased urate clearance.[14] In these patients, hypouricemia is associated with glycosuria[15], decreased metabolic control, hyperfiltration, and a late onset of disease, while elevated SUA is a feature of hyperinsulinemia or insulin resistance.[6]

Development of vascular complications was predicted independently by serum uric acid.[16] In a study conducted by Poonam Agrawal et al., it was concluded that the Uric acid levels are raised in patients with diabetic retinopathy when compared to those without retinopathy.[17] A study conducted by Ching-Chao Liang et al., concluded that there were increased serum uric acid levels which correlated with the severity of diabetic Retinopathy in patients with type 2 DM.[18] Vitreous UA and glucose concentrations were higher in proliferative than in non-proliferative DR. Focal UA production in the vitreous is thought to be involved in the pathogenesis and progression of DR.[19]

UA is lowered in Diabetes mellitus (DM) due to hyperfiltration[20], but decreased UA excretion during renal dysfunction raises SUA levels. Nazir Shah et al.[21] conducted a study in 2013 in 163 patients of diabetes mellitus (type 2) and proposed that elevation in the levels of uric acid was more in the diabetic patients with nephropathy (50%). In a study conducted in 60 diabetic patients by Nasri et al.[22], it was proved that there was a significant association between diabetic nephropathy and the levels of uric acid in the patient's serum. Petter Bjornstadet al[23] stated that there is a relation between the uric acid level elevation and development of diabetic nephropathy. Su-Mi Kim et al.[24] demonstrated that high UA contributes to the development of nephropathy in diabetic patients.

Several recent studies have been investigating therapeutic interventions to delay nephropathy progression.[25-27] Allopurinol therapy significantly decreases serum UA levels in hyperuricemic patients with mild to moderate CKD. Its use is safe and has been shown to help preserve kidney function when used for a duration of 12 months.[25] Febuxostat has a higher renoprotective effect than allopurinol, inhibits oxidative stress, has anti-atherogenic activity, reduces blood pressure, and decreases pulse wave velocity and left ventricular mass index, most likely due to a strong serum UA lowering effect.[27] In animal diabetic nephropathy model, allopurinol attenuated transforming growth factor-beta1-induced Smad pathway activation in tubular cells.[28]

The prevalence of diabetic peripheral neuropathy shows a significant correlation with increased UA levels.[29] Several studies demonstrated that, when controlled for confounding factors such as age, gender, BMI, renal function, and/or diabetic duration, Serum UA levels were high in patients with diabetic polyneuropathy and sudomotor dysfunction.[30,32]

The pathophysiology of diabetic neuropathy is not completely understood, and multiple metabolic imbalances underlie the development of diabetic neuropathy.[33] Hyperglycemia, dyslipidemia, and cardiovascular dysfunction are all independent risk factors for neuropathy. Probable etiologic factors include the polyol pathway, non-enzymatic glycation, free radicals, oxidative stress, and inflammation. Oxidative stress and inflammation are involved in XDH/XO activity. It is therefore speculated that UA generation by XDH/XO plays a role in diabetic neuropathy.

In a study conducted by Yili Xu et al., it was concluded that the patients with vascular complications had increased serum uric acid levels. He also said that this could be an independent predictor of vascular complications.[34] We found similar results in our study.

CONCLUSION

Diabetic patients with micro vascular complications have higher serum uric acid levels than those without complications. Also there was positive correlation between HbA1C and serum uric acid levels.

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REFERENCES

1. Sarah S Premraj et al. Correlation between serum uric acid level and microangiopathic complications

in patients with type 2 diabetes mellitus. MedPulse – International Medical Journal. January 2018; 5: 18-22.
2. Cai Z, Xu X, Wu X, Zhou C, Li D. Hyperuricemia and the metabolic syndrome in Hangzhou. Asia Pac J Clin Nutr. 2009; 18:81-7.
3. Nakagawa T, Cirillo P, Sato W, Gersch M, Sautin Y, Roncal C (et al). The conundrum of hyperuricemia, metabolic syndrome, and renal disease. Intern Emerg Med. 2008;3:313-8.
4. Takahashi MM, de Oliveira EP, de Carvalho AL, de SouzaDantas LA, Burini FH, Portero-McLellan KC et al. Metabolic syndrome and dietary components are associated with coronary artery disease risk score in free-living adults: a cross-sectional study. Diabetol Metab Syndr. 2011; 3:7.
5. Chen JH, Chuang SY, Chen HJ, Yeh WT, Pan WH. Serum uric acid level as an independent risk factor for all-cause cardiovascular, and ischemic stroke mortality: a Chinese cohort study. Arthritis Rheum. 2009; 61:225-32.
6. Bo S, Cavallo-Perin P, Gentile L, Repetti E, Pagano G. Hypouricemia and hyperuricemia in type 2 diabetes: two different phenotypes. Eur J Clin Invest 2001; 31: 318-321
7. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest. 1993; 91:2546–2551.
8. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985; 312:159–163.
9. Ono T, Tsuruta R, Fujita M, Aki HS, Kutsuna S, Kawamura Y, et al. Xanthine oxidase is one of the major sources of superoxide anion radicals in blood after reperfusion in rats with forebrain ischemia/reperfusion. Brain Res. 2009; 1305:158–167.
10. Pritsos CA. Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system. Chem Biol Interact. 2000; 129:195–208.
11. Moriwaki Y, Yamamoto T, Suda M, Nasako Y, Takahashi S, Agbedana OE, et al. Purification and immunohistochemical tissue localization of human xanthine oxidase. Biochim Biophys Acta. 1993; 1164:327–330.
12. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress." Histol Histopathol. 2008; 23:381–390.
13. Bobulescu IA, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. Adv Chronic Kidney Dis. 2012; 19:358–371.
14. Tuomilehto J, Zimmet P, Wolf E, Taylor R, Ram P, King H. Plasma uric acid level and its association with diabetes mellitus and some biologic parameters in a biracial population of Fiji. Am J Epidemiol. 1988; 127:321–336.
15. Andrade JA, Kang HC, Greffin S, Garcia Rosa ML, Lugon JR. Serum uric acid and disorders of glucose metabolism: the role of glycosuria. Braz J Med Biol Res. 2014; 0:0.
16. Bjornstad P, Maahs DM, Rivard CJ, Pyle L, Rewers M, Johnson RJ et al. Serum uric acid predicts vascular complications in adults with type 1 diabetes: the coronary artery calcification in type 1 diabetes study. Acta Diabetol 2014; 51: 783-791.
17. Agrawal P, Sethi S, Reddy VS, Lamba S. Serum uric acid and lipid profile in diabetic retinopathy in rural Haryana, India. Int J Adv Med 2016;3:309-312
18. Ching-Chao Liang et al. Association of serum uric acid concentration with Diabetic Nephropathy and Albuminuria in Taiwan. Int. J. Mol. Sci. 2016, 17, 1248
19. Krizova L, Kalousova M, Kubena A, Benakova H, Zima T, Kovarik Z, et al. Increased uric acid and glucose concentrations in vitreous and serum of patients with diabetic macular oedema. Ophthalmic Res. 2011; 46:73–79.
20. Shichiri M, Iwamoto H, Marumo F. Diabetic hypouricemia as an indicator of clinical nephropathy. Am J Nephrol. 1990; 10:115–122.
21. Nazir Shah, Amanullah, Sheraz Jamal and Mumtaz Ali Marwat. Association Of Hyperuricemia With Diabetic Nephropathy Intype 2 Diabetes Mellitus. Khyber Journal of Medical Sciences.
22. Behradmanesh S, Horestani MK, Baradaran A, Nasri H. Association of serum uric acid with proteinuria in type 2 diabetic patients. J Res Med Sci 2013; 18:44-6.
23. Petter Bjornstad et al. Fructose and uric acid in diabetic nephropathy. Diabetologia. 2015; 58:1993–2002
24. Su-Mi, Kim et al. Hyperuricemia-induced NLRP3 activation of macrophages contributes to the progression of diabetic nephropathy. Am J Physiol Renal Physiol 2015; 308: F993–F1003.
25. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. Am J Kidney Dis. 2006; 47:51–59.
26. Maahs DM, Caramori L, Cherney DZ, Galecki AT, Gao C, Jalal D, et al. Uric acid lowering to prevent kidney function loss in diabetes: the preventing early renal function loss (PERL) allopurinol study. Curr Diab Rep. 2013; 13:550–559.
27. Sezai A, Soma M, Nakata K, Hata M, Yoshitake I, Wakui S et al Comparison of febuxostat and allopurinol for hyperuricemia in cardiac surgery patients (NU-FLASH Trial). Circ J. 2013;77:2043–2049.
28. Kim SM, Choi YW, Seok HY, Jeong KH, Lee SH, Lee TW et al. Reducing serum uric acid attenuates TGF-β1-induced profibrogenic progression in type 2 diabetic nephropathy. Nephron Exp Nephrol. 2012; 121:e109–e121.
29. Chuengsamarn S, Rattanamongkolgul S, Jirawatnotai S. Association between serum uric acid level and microalbuminuria to chronic vascular complications in Thai patients with type 2 diabetes. J Diabetes Complications. 2014; 28:124–129.
30. Kiani J, Habibi Z, Tajziehchi A, Moghimbeigi A, Dehghan A, Azizkhani H. Association between serum uric acid level and diabetic peripheral neuropathy (A case control study). Caspian J Intern Med. 2014; 5:17–21.
31. Papanas N, Katsiki N, Papatheodorou K, Demetriou M, Papazoglou D, Gioka T, et al. Peripheral neuropathy is associated with increased serum levels of uric acid in type 2 diabetes mellitus. Angiology. 2011; 62:291–295.

32. Papanas N, Demetriou M, Katsiki N, Papatheodorou K, Papazoglou D, Gioka T et al. Increased serum levels of uric acid are associated with sudomotor dysfunction in subjects with type 2 diabetes mellitus. Exp Diabetes Res. 2011; 2011:346051.
33. Vincent AM, Callaghan BC, Smith AL, Feldman EL. Diabetic neuropathy: cellular mechanisms as therapeutic targets. Nat Rev Neurol. 2011; 7:573–583.
34. Xu Y, Zhu J, Gao L, Liu Y, Shen J, et al. Hyperuricemia as an Independent Predictor of Vascular Complications and Mortality in Type 2 Diabetes Patients: A Meta-Analysis. PLoS ONE 2013; 8: e78206.

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A Prospective Study of Correlatio of Magnetic Resonance Imaging of Brain Including MR Angiogram and Carotid Vertebral Arterial Doppler Study with the Clinical Features in Ischemic Stroke

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ABSTRACT

Introduction: Stroke is a global health problem. In this study we evaluated the risk factors, clinical features and their correlation with Magnetic Resonance Imaging (MRI) brain including Magnetic Resonance Angiogram (MRA) and Carotid Verteberal (CV) Doppler findings in patients presenting with acute ischemic stroke.

Material and methods: Seventy five patients of the age group 20 to 80 years admitted with acute ischemic stroke were enrolled for the study. A detailed history, clinical examination and blood investigations were done. MRI, MRA and CV Doppler were done in all patients. Serum Homocysteine levels and Anti-nuclear antibody assay (ANA) by Immunofluorescence (IF) were done in selected patients. Statistical analysis was done using SSPS version 16.0 for microsoft windows.

Results: In this study, there were more males than females. The mean age was 59 years. Young stroke population (<40 years) was 5%. Anterior circulation was predominantly involved than posterior circulation. Right Middle Cerebral Artery was the most common artery involved. There was positive correlation for clinical presentation and area of infarct and for areas of infarct and the vessels involved. Most common clinical presentations were weakness and slurring of speech. There were 3% intracranial anomalies. 56% had significantly elevated levels of homocysteine. 24% had ANA- IF positivity. Recurrence of stroke was seen in 17%; National Institute of Health Stroke Scale (NIHSS) score was higher in patients with recurrent stroke and right hemisphere infarcts.

Conclusion: There was positive correlation for clinical presentation and area of infarct and for areas of infarct and the vessels involved.

Keywords: Stroke, Anterior Circulation, Clinical co-relation, MRI, MRA Brain, Homocysteine, Area of Infarct.

INTRODUCTION

Stroke is a worldwide concern. Approximately two hundred lakh people every year suffer from stroke and of these fifty lakh do not survive.[1,2] In the developed nations, stroke is the leading cause for disability, second cause of dementia and third cause of death. Stroke is the second common cause of death and fourth leading cause of disability globally.[3] Stroke is an important cause for epilepsy, falls and depression in developed nations.[4] It is the leading cause of functional impairments, with 20% of survivors required hospital and nursing care for a prolonged period of more than 3 months duration and 15% - 30% of patient develop permanent disability.[5] Stroke is a life-changing event both financially and physically.

Stroke is caused by acute interruption of blood supply to the brain region it supplies, usually because of the blockade of a cerebral artery by a thrombus or emboli or by rupture of a vessel. This cuts off the supply of vital oxygen and nutrients to the brain, accumulation of free radicals causing damage to the brain tissue. An acute total occlusion of the vessels to the brain can cause death of the brain tissue within four to ten minutes. WHO clinically defines stroke as the rapid development of clinical signs and symptoms of a focal neurological disturbance lasting more than 24 hours or leading to death with no apparent cause other than vascular origin.[6] Among CVA, ischemic stroke constitutes 50%–85%[6,7] Hemorrhagic strokes - Subarachnoid hemorrhage and intra-cerebral hemorrhage, account for 1%-7% and 7%-27% respectively of all strokes worldwide.[8,9] In a study done, hemorrhagic stroke has significantly higher mortality when compared with ischemic stroke.

This study was conducted to evaluate various parameters like the risk factors of stroke, areas of brain involvement including the vessels involved and their clinical correlation in patients with acute ischemic stroke.

MATERIAL AND METHODS

This is a prospective observational study conducted in a tertiary hospital at Coimbatore between June 2015 and June 2016. A total of 75 patients in the age group of 20 to

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80 years admitted with acute ischemic stroke to the medical and neurology wards were enrolled in the study. The study was initiated after obtaining clearance from the Institutional Ethical Committee. After obtaining informed consent, a detailed history attributable to the risk factors of stroke was obtained. A detailed clinical examination including baseline NIHSS score was obtained.

Investigations like complete hemogram, urine analysis, blood sugar, serum electrolytes, serum creatinine, blood urea, chest X-ray, fasting lipid profile, echocardiogram, electrocardiogram, MRI brain with MRA intracranium, CV Doppler were done in all patients. HbA1C was done if patient was found to be Diabetic based on random blood sugars. Investigations like ANA- IF and serum Homocysteine were done in selected patients.

STATISTICAL ANALYSIS

The data was reported as the mean +/- SD or the median, depending on their distribution. Frequencies were reported in percentages. The differences in quantitiative variables between groups were assessed by means of the unpaired t test. Non parametric test Wilcoxon signed test was used to assess the qualitative variables. To assess the difference in categoric variables between groups chi square test was used. Two tailed test was considered significant for all statistical tests when *P* value is <0.05. All data were analysed with a statistical software package (SSPS version 16.0 for microsoft windows).

Symptoms	Number of patients
Headache	6
Slurring of speech	33
Gait	16
Giddiness	17
Deviation of mouth	33
Weakness	51
Vomiting	8
Urinary incontinence	1
Decreased sensation	12
Total loss of speech	4
Double vision	5
Hiccups	1
Blurring of vision	1
Altered sensorium	1
Seizures	3
Pin prick sensation	1
Pain	1
Dysphagia	1
Loc	

Table-1: Clinical Presentation

RESULTS

Among the study population of 75 patients, 62 (83%) were males and 13 (17%) were females. 77% of total patients were aged more than 50 years. The mean age in the study population was 59 years. The young stroke population (<40 years) in this study was 5%.

The percentage of smokers, alcoholics and both was 17%, 5% and 24% respectively. In the study population the percentage of Type 2 Diabetes mellitus, Systemic hypertension, Dyslipidemia and combined was 11%, 9%, 35% and 41% respectively.

The most common symptoms were weakness, slurring of speech, deviation of angle of mouth as listed in Table 1.

In the study population, 47 patients (63%) had anterior circulation stroke and rest twenty eight patients (37%) had posterior circulation involvement. The most commonly involved arteries in the present study were Middle Cerebral Artery (MCA). Forty three out of seventy five patients (57%) had MCA involvement. Among the 43 patients with MCA involvement, right MCA was involved in 24 patients and 19 patients had diseased left MCA. Anterior cerebral artery (ACA) was involved in 4 patients. Posterior cerebral artery (PCA) was involved in twenty eight patients (Figure 1)

31% of the patients had intracranial obstruction due to atherothrombotic process which was classically seen in MRA intracraium. 11% of the patients had only extracranial occlusion which was seen in CV doppler studies. Twenty one percentage of the study population had both intracranial and extracranial leisions and 37% had normal IC & EC studies, probably related to small vessel ischemia.

Forty five patients (60%) had positive correlation of location of the infarct in the brain with either MRA or CV Doppler findings *P* <0.001. 37% had normal MRA & CV Doppler findings. Only in 3% of the study population there was no

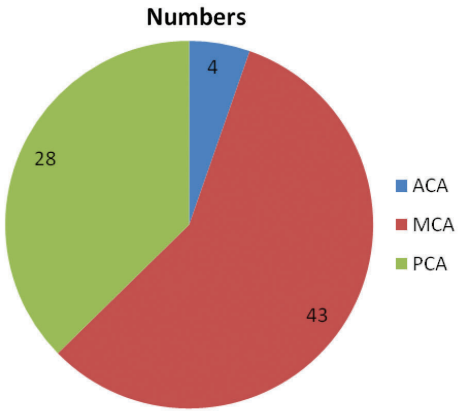


Figure-1: Most commonly involved arteries

			95% CI of mean				
	Mean	SD	Lower	Upper	Minimum	Maximum	Sig
Right	5.5	2.9	4.5	6.5	1	11	<0.05
Left	5.2	2.3	4.4	6.0	1	10	
Bilateral	4.8	3.3	2.0	7.5	2	12	
Total	5.3	2.7	4.7	5.9	1	12	

Table-2: Mean NIHSS score with Laterality

correlation of the same.

The clinical presentation was correlating with infarct in MRI in seventy patients (93%) P <0.05. There was no correlation in 7% of the study population due to bilateral involvement.

In this study population, recurrent CVA was seen in thirteen patients (17%). The mean age of recurrent CVA was 62 yrs and average NIHSS was 6.5 which were significantly higher than patients who had CVA for the first time. NIHSS was more in those with right hemisphere involvement when compared with left and bilateral involvement. The average NIHSS for right, left, bilateral involvement was 5.5, 5.2, and 4.8 respectively (Table 2). The maximum NIHSS in this study population was 12 which were noted with bi-hemisphere involvement.

Fasting serum Homocysteine levels and ANA –IF was done in 46 patients. Elevated homocysteine and ANA –IF positivity was noted in 56% and 24% respectively. Serum homocystiene was elevated in 36% of the alcoholics and 64% of non alcoholics. The highest value was 160 against <12 of the normal reference range.

In the study population, 2 (3%) had congenital anomalies. They were Frontal arteriovenous malformation and fetal PCA. None of the patients in the study population underwent thrombolysis.

DISCUSSION

Stroke among adults is rapidly increasing in the recent times. A systematic approach with good clinical history and examiantion, evaluation of risk factors, investigations and proper management of stroke is the need of the hour for acute ischemic stroke (AIS) patients.

In this study, males were more when compared with females. The male: female sex ratio among affected individuals is 7:1 in India. Among the females high risk groups are those taking oral contraceptives and pregnancy.[10,11]

The mean age was 59 years. In our study young stroke population (<40 years) was 5%. In a study at All India Institute of Medical Sciences (AIIMS), out of the 2,634 patients admitted with AIS, 440 patients (16.7%) were in the age group of 18-45 years and majority (83.4%) were male.[12] Smoking, alcoholism and dyslipidemia have been found to be significantly associated with stroke and its recurrence. Poor control of Diabetes Mellitus and systemic hypertension were associated with stroke recurrence.

Anterior circulation was predominantly involved than posterior circulation. Right MCA was the most common artery involved. There was positive correlation for clinical presentation and area of infarct and for areas of infarct and the vessels involved. Most common clinical presentation was weakness and slurring of speech. There were 3% intracranial anomalies

Eleven percent of patients had only extracranial occlusion. Extracranial occlusion that is carotid artery disease constitutes 10-20% of the strokes; stroke risk is directly proportional to the degree of occlusion. Carotid endarterectomy should be considered within 2 weeks if carotid stenosis of more than 70% involvement. There is less benefit of end carotidectomy and carotid stenting in patients with stenosis of carotids 50 to 69% and in asymptomatic patients, and there is literally no benefit in patients with stenosis of less than 50%

Hyperhomo-cysteinemia emerged as an important independent risk factor for ischemic stroke.[13] 56% had significantly elevated levels of homocystiene. The levels ideally have to be assessed 6 weeks later, for ideally diagnosing hyperhomocystinemia. Vitamins such as B12, B6 and folate play a vital role in the Homocysteine - methionine cycle[14], thus alcoholics are at risk of developing lower levels of folate and hence the increased Homocysteine levels. In contrary the proportion of elevated levels of Homocysteine was more in the non alcoholics when compared with alcoholics. There is correlation made in few studies that higher the Homocysteine values higher the mortality and recurrence of stroke.

24% had ANA- IF positivity but none of them had history of connective tissue disease. Incidental ANA- IF positivity in 24% of the study population raises concern whether it should be done routinely in all stroke patients and the same should be repeated to confirm the autoimmune etiology 6 weeks later.

Recurrence of stroke though was seen in 17%, but no mortality was observed among these patients. Relapse of alcohol, hard to kickoff smoking, poor adherence of medications was prime important in the stroke recurrence. NIHSS score was comparatively higher in these patients than in patients who had stroke for the first time. Similarly Right sided infarcts had higher NIHSS

None of the patients was thrombolysed due to varied reasons like exclusion criteria, after explaining the risks involved and mainly the time lapse in reaching the hospital. None of the patients was treated surgically.

CONCLUSION

There was positive correlation for the clinical presentation with area of infarct and for areas of infarct with the vessels involved. Elevated serum homocystiene levels and incidental positivity of ANA-IF noted in the present study raises concern; further studies are needed which will throw light in this area and indicate their significance in patients with acute ischemic stroke.

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REFERENCES

1. Dalal PM, Bhattacharjee M. Stroke Epidemic in India: Hypertension-Stroke Control Programme is Urgently Needed. JAPI 2007. Vol 55.
2. Dalal P, Bhattacharjee M, and Vairale J, Bhat P. UN millennium development goals: can we halt the stroke epidemic in India? Ann Indian Acad Neurol 2007; 10: 130-6.
3. Strong K, Mathers C, Bonita R. Preventing stroke: saves lives around the world. Lancet Neurol 2007; 6: 182-7.
4. Marc Fisher MD, Bo Norrving. 1st Global Conference

on Healthy. Lifestyles and Non communicable diseases Control. Moscow, April 28-29, 2011.

5. Steinwachs DM, Collins-Nakai RL, Cohn LH, Garson A Jr, Wolk MJ.J The future of cardiology: utilization23 and costs of care. Am Coll Cardiol. 2000;35:91B-98B.
6. Mackay J, Mensah G, Mendis S, Greenlund K. Atlas of heart disease and stroke. WHO 2004.
7. Jamieson DG. Diagnosis of Ischemic Stroke. Am J Med. 2009;122(4 SUPPL. 2).
8. Feigin V, Lawes C, Bennet D, Barker Cello S, Parag V. Worldwide stroke incidence and early case fatality in 56 population based studies: a systematic review. Lancet Neurology 2009; 8: 355- 369.
9. Smith SD, Eskey CJ. Hemorrhagic stroke. Radiologic Clinics of North America. 2011. p. 27–45.
10. Sethi PK. Stroke - Incidence in India and Management of Ischaemic stroke. Neurosci Today [Internet]. 2002;6:139–43. Available from: http://openmed.nic.in/1753/01/SGRH_p202-206_EDIT.pdf
11. Das S, Hazra A, Ray BK, Ghosal M, Banerjee TK, Roy T, et al. Burden among stroke caregivers: Results of a community-based study from Kolkata, India. Stroke. 2010; 41:2965–8.
12. Dash D, Bhashin A, Pandit AK, Tripathi M, Bhatia R, Prasad K, et al. Risk factors and etiologies of ischemic strokes in young patients: A tertiary hospital study in north India. J Stroke. 2014; 16:173–7
13. Modi M, Prabhakar S, Majumdar S, Khullar M, Lal V, Das C P. Hyperhomocysteinemia as a risk factor for ischemic stroke: An Indian scenario. Neurol India 2005; 53:297-301
14. Yakub M, Iqbal MP, Kakepoto GN, Rafique G, Memon Y, Azam I, et al. High prevalence of mild hyperhomocysteinemia and folate, B 12 and B 6 deficiencies in an urban population in Karachi, Pakistan. Pakistan Journal of Medical Sciences. 2010; 26:923–929.

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Original Research Article

Glycaemic control and C- reactive protein levels in type 2 diabetes mellitus -how well they co-relate?: a prospective study

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ABSTRACT

Background: Poorly controlled diabetes mellitus as indicated by elevated glycated haemoglobin (HbA1c) levels is associated with increased cardiovascular risk. C–reactive protein (CRP), an important cardiovascular risk factor, is elevated in diabetics with poor glycaemic control than those with good control. The present study assessed the correlation between HbA1c and CRP levels.
Methods: A prospective study was conducted in thirty type 2 diabetic patients irrespective of the disease duration and treatment; those with established target organ damage were excluded. HbA1c and hsCRP levels were measured at baseline; sugars were monitored monthly and medications optimised; at the end of six months HbA1c and hsCRP levels were measured.
Results: Mean age of the study subjects was 58.7±8.6 years; At the baseline, all had poor glycaemic control (HbA1c >7%); 15 had hsCRP >3 mg/L. At the end of 6 months, 5 achieved good glycaemic control (HbA1c <7%); 10 had hsCRP >3 mg/L. Baseline median hsCRP was 3.33 mg/L (0.68, 15.9) and at the end of 6 months it was 2.08 mg/L (0.48, 9.12). Mean HbA1c at baseline and end line was 10.6±1.55% and 8.43±1.84% respectively. There was significant reduction in both the mean HbA1c and median hsCRP at the end of 6 months (p <0.001). Positive correlation was observed between HbA1c and CRP at baseline (r=0.32, p=0.10). However, this was not observed at the end of 6 months.
Conclusions: There is positive correlation between the level of glycaemic control (HbA1c) and CRP levels; Better glycaemic control results in significant reduction in the hsCRP levels.

Keywords: C-reactive protein, Correlation, Glycaemic control, hsCRP, HbA1c

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by the defects in insulin secretion or action; chronic hyperglycaemia can lead to microvascular and macrovascular complications if the blood sugars are not under optimal control. The glycaemic control is assessed by the measurement of glycated haemoglobin (HbA1c) which has its own advantages and disadvantages.[1] Till date HbA1c is the widely used tool to assess the glycaemic status. Poor glycaemic control as indicated by elevated HbA1c levels accelerates the atherosclerosis process and significantly increases the risk of cardiovascular events.[2] C-reactive protein measured by highly sensitive assays (hsCRP), is a very sensitive marker of the inflammatory activity in the arterial wall.[3,4] It is an important predictor of cardiovascular risk apart from the traditional risk factors.[5,6] It is interesting to note that chronic hyperglycaemia stimulates the release of various inflammatory cytokines (IL 6; TNF α) and induces the secretion of acute phase reactants by liver, which in turn results in elevation of CRP in association

with elevated fasting plasma glucose.[7] Studies had shown that elevated CRP levels is associated with an increased risk of future development of diabetes mellitus.[8] Also, people with diabetes mellitus had elevated levels of CRP than non -diabetics.[9,10] We understand that both chronic systemic inflammation and hyperglycaemia contribute to the development and progression of atherosclerotic cardiovascular disease. Experimental and clinical studies have confirmed the inter-relationship between CRP, hyperglycaemia and atherosclerosis.[11,12] In states of elevated CRP, hyperglycaemia exaggerates the pro-atherogenic effects of CRP.[12,13] Few studies which had assessed the relationship between CRP levels and the level of glycaemic status showed conflicting results; some studies had proven the positive co-relation between glycaemic control and CRP levels.[14-16] while some failed to do so.[6] Also, the effect of good glycaemic control on CRP levels is not clear. The aim of this study is to determine the co-relation of glycaemic control as measured by HbA1c levels with hsCRP in patients with type 2 diabetes mellitus and to determine if better glycaemic control reduces CRP levels.

METHODS

The study was conducted in the Department of Medicine, PSG Institute of Medical Sciences, Coimbatore. Patients with type 2 diabetes mellitus diagnosed on the basis of WHO criteria were prospectively enrolled in the study from September 2014 to May 2015 irrespective of disease duration and treatment. Patients with established cardiovascular disease, chronic kidney disease, infections, haemolytic anaemia, on drug therapy with statins and non-steroidal anti -inflammatory drugs were excluded from the study. The study was approved by the Institutional Ethics Committee (approval number: 14/184); informed consent was obtained from all the study participants.

At the time of enrolment in the study, blood samples were collected from the participants for measurement of HbA1c and hsCRP levels. HbA1c was measured by high pressure liquid chromatography (Variant II Turbo - HbA1c kit - 2.0). High sensitive CRP was measured by particle enhanced turbidometry assay (Cobas Integra 400 plus). They were followed up on a monthly basis, clinical evaluation was done; fasting and postprandial blood sugars were monitored and adjustment of anti-diabetic medications done on an individual basis in addition to re-enforcement on life style modification (diet and exercise). At 6 months, blood samples were collected for HbA1c and CRP levels.

Statistical analysis

Descriptive statistics were reported as mean ± SD for normally distributed data, median with 25th and 75th for the non-normal data. Paired t test or Wilcoxon Signed rank test was used to test the significance for the change in HBA1c and CRP after 6 months from baseline. Spearman's Rank correlation is used to assess the correlation between the variables. P value less than 5% was considered statistically significant. All the analyses were done using SPSS version 23.0.

RESULTS

Among the 30 subjects studied, 70% of people were between the age group of 50-70 years; the mean age of the study subjects was 58.7±8.6 years; there were 17 females (57%) and 13 males (43%).

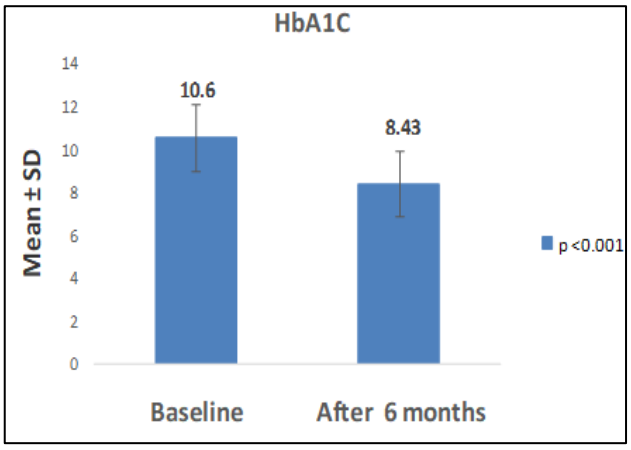


Figure 1: Mean HbA1c levels in % at baseline and at the end of 6 months.

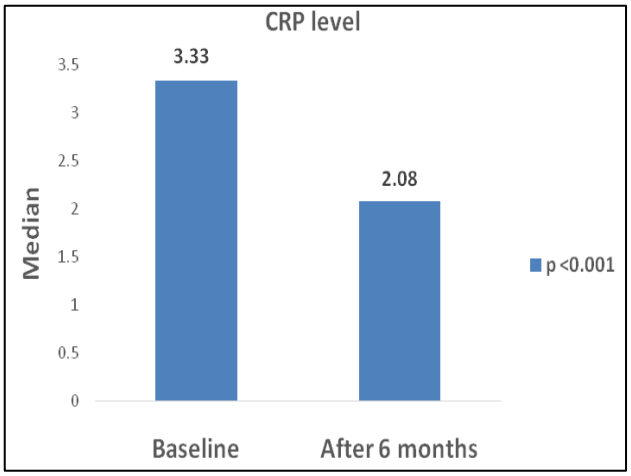


Figure 2: Median CRP levels in mg/L at baseline and at the end of 6 months.

At the baseline, all of them had poor glycaemic control (HbA1c >7%); 15 had hs CRP >3 mg/L. At the end of 6 months, 6 patients lost to follow up; HbA1c and hsCRP levels were measured for the remaining subjects; 5 achieved good glycaemic control (HbA1c <7%); 10 had hs CRP >3 mg/L. The mean hsCRP at baseline and at the end of 6 months was 7.85±9.75 and 4.88 ±5.41 mg/L respectively. Baseline median hsCRP was 3.33 (0.68, 15.9) and at the end of 6 months it was 2.08 (0.48, 9.12). The mean HbA1c at baseline and end line was

10.6±1.55% and 8.43±1.84% respectively. There was statistically significant reduction in both the mean HbA1c and median hsCRP values at the end of 6 months with effective individual based anti-diabetic treatment (p <0.001). The results are depicted in Figure 1 and 2. Positive co-relation was observed between HbA1c and CRP at baseline (r=0.32, p=0.10) although it was not statistically significant. However, this correlation was not observed at the end of 6 months.

DISCUSSION

The role of chronic low-grade inflammation contributing to the pathogenesis of diabetes and its related complications is well known. Chronic hyperglycaemia induces oxidative stress and chronic inflammatory state, which jointly contribute to the pathogenesis of atherosclerosis. C-reactive protein levels more than 3.0 mg/L is associated with worse cardiovascular outcome.[17] Various studies had demonstrated significantly elevated serum hsCRP levels in diabetics than non-diabetics.[15,16,18] Also, significant elevation was noted in those with poor glycaemic control compared to those with good glycaemic control.[15] Recent research has shown that HbA1c and CRP jointly contribute to the increased cardiovascular risk in patients with advanced cardiovascular disease.[19]

The mean hsCRP levels in the study population at baseline was 7.85±9.75 mg/l which is in accordance to that observed in another Indian study conducted in Bangalore (6.9±9.3 mg/l); while Saudi Arabian and Bangladesh studies had shown mean hsCRP levels lower than that observed in the present study, 1.13 and 2.3 mg/L respectively.[6,18] The reason for this difference in CRP levels is unclear; further studies are needed to assess the differences in ethnic groups; standardisation of the techniques used for measurement of hsCRP.

A positive co-relation was observed between HbA1c and hsCRP levels at the baseline in the present study indicating that the change in HbA1c affects hsCRP levels in the same direction. There are other studies which support our results. In a cross-sectional study conducted by Gohel et al, a significant positive linear relationship was observed between hsCRP and HbA1c.[15] Li et al, Khan DA et al, and Sarinnapakorn V et al, also had made similar observation in their studies.[20-22] Kashinakunti et al, in their case control study noted significantly high hsCRP levels in diabetics than controls; also noted positive co-relation between hsCRP and HbA1c, although non-significant.[16] Low HbA1c levels strongly related to negative hs-CRP levels as observed by Amanullah S et al.[23] Another study conducted in Sudan showed significant correlation between CRP levels with fasting plasma glucose and HbA1c.[24] All the above-mentioned studies were cross-sectional observational studies which assessed the relationship between Hba1c and CRP.

On literature search authors could find only one study conducted in Mexico which had assessed prospectively the effects of good glycaemic control on the CRP levels. The study was conducted on newly diagnosed type 2 diabetes patients; they were followed up for 6 months by intensive blood sugar management. There was significant reduction in HbA1c from baseline value of 13.0±4.9 to 8.9±2.9% at the end of 6 months, p <0.00001 which resulted in significant reduction in hsCRP levels (9.6±6.2 to 6.3±3.0 mg/l, p <0.00001). The present study also had shown similar results; significant reduction in hsCRP levels at the end of 6 months compared to baseline was observed following better glycaemic control and reduction in HbA1c levels. In that study, although glycaemic control was achieved in 71 patients at the end of 6 months, only 29 (40.8%) of them showed a reduction in CRP levels to 3 mg/l or less and the remaining 42 (59.2%) maintained a high CRP level. The study concluded that CRP is moderately influenced by glycaemic control.[25] Similar to that observation, the present study also showed that only 41.6% of the study subjects achieved CRP levels <3 mg/L by 6 months.

The positive co-relation observed between HbA1c and CRP at the baseline was not observed at 6 months; this may be because CRP is moderately influenced by glycaemic control.25 There are other variables which affect CRP levels like age, gender, body weight, LDL cholesterol levels. The present study did not take into considerations those variables which is a limitation of the present study.

CONCLUSION

There is positive correlation between the level of glycemic control (HbA1c) and CRP levels; Better glycemic control results in significant reduction in the hsCRP levels.

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REFERENCES

1. Saudek CD, Kalyani RR, Derr RL. Assessment of Glycemia in Diabetes Mellitus: Hemoglobin A. JAPI. 2005 Apr; 53.
2. Cavero-Redondo I, Peleteiro B, Álvarez-Bueno C, et alGlycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and non-diabetic populations: a systematic review and meta-analysis. BMJ Open. 2017;7:e015949.

3. Kamath DY, Xavier D, Sigamani A, Pais P. High sensitivity C-reactive protein (hsCRP) & cardiovascular disease: An Indian perspective. Indian J Med Res. 2015;142:261-8.
4. Pfützner A, Forst T. High-sensitivity Creactive protein as cardio-vascular risk marker in patients with diabetes mellitus. Diabetes Technol Ther. 2006; 8(1):28-36.
5. Asegaonkar SB, Marathe A, Tekade ML, Cherekar L, Bavikar J, Bardapurkar J et al.High-sensitivity C-reactive protein: a novel cardiovascular risk predictor in type 2 diabetics with normal lipid profile. J Diab Complications. 2011;25(6):368-70.
6. Mohieldein AH, Hasan M, El-Habiby MI.High Sensitivity C-Reactive Protein as Atherogenic Marker Among Type 2 Diabetes. Eur Sci J. 2017(13);33:403.
7. Lin Y, Rajala MW, Berger JP. Hyperglycaemia-induced production of acute phase reactants in adipose tissue. J Biol Chem. 2001; 276(45):42077-83.
8. Freeman DJ, Norrie J, Caslake MJ, et al. West of Scotland Coronary Prevention Study: C-reactive protein is an independent predictor of riskfor the development of diabetes in the West of Scotland Coronary Prevention Study. Diab. 2002;51:1596-1600.
9. Tutuncu Y, Satman I, Celik S, Dinccag N, Karsidag K, Telci A, et al. A comparison of hs-CRP levels in new diabetes groups diagnosed based on FPG, 2-hPG, or HbA1c criteria. J Diabe Res. 2016.
10. Satman I, Omer B, Tutuncu Y, Kalaca S, Gedik S, Dinccag N, et al. Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. Eur J Epidemiol. 2013;28(2):169-80.
11. Ridker PM, Buring JE, Cook NR. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy Am women. Circulation. 2003;107:391-7.
12. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. Circulation. 2003;107:398-404.
13. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation. 2000;102:2165-8.
14. King DE, Mainous AG, Buchanan TA, Pearson WS.C-reactive protein and glycemic control in adults with diabetes. Diabe Care. 2003;26(5):1535-9
15. Gohel MG, Chacko AN. Serum GGT activity and hs-CRP level in patients with type 2 diabetes mellitus with good and poor glycemic control: An evidence linking oxidative stress, inflammation and glycemic control. J Diabe Metabolic Dis. 2013;12:56.
16. Kashinakunti SV, Rangappa M, Kallaganada GS. International J Biochem Res Rev. 2016;11(4): 1-8
17. Cardoso CR, Leite NC, Salles GF. Prognostic Importance of C-Reactive Protein in High Cardiovascular Risk Patients with Type 2 Diabetes Mellitus: The Rio de Janeiro Type 2 Diabetes Cohort Study. J Am Heart Assoc. 2016;5(11):e004554.
18. Fazlul Haque AKM, Saifuddin Ekram ARM, Islam QT, Jahan MS, Haque MZ. Evaluation of serum high sensitivity c -reactive protein (hs-crp) in type-2 diabetic patient. J Med. 2010;11:20-3.
19. Schillinger M, Exner M, Amighi J, Mlekusch W, Sabeti S, Rumpold H. et al. Joint effects of C-reactive protein and glycated hemoglobin in predicting future cardiovascular events of patients with advanced atherosclerosis. Circulation. 2003; 108(19):2323-8.
20. Li CZ, Xue YM, Gao F, Wang M. Determination of serum hs-CRP in patientswith type 2 diabetes mellitus. Di Yi Jhun Yi Da Xne Xne Bao. 2004;24(7):791-3.
21. Dilshad Ahmed K, Shazia Q. Evaluation of cardiac risk by oxidative stress and inflammatory markers in diabetic patients. Pak J Med Sci. 2009;25:5.
22. Sarinnapakorn V, Wanicagool W: Association between hs-CRP and Hba1c in overweight type 2 diabetic female patients. J Med Assoc Thai. 2013;96(3):S54-8.
23. Amanullah S, Jarari A, Govindan M, Basha MI, Khatheeja S. Association of hsCRP with diabetic and non-diabetic individuals. Jordan J Biol Sci. 2010;3(1):7-12.
24. Abdrabo AA. Association between fasting plasma glucose and highly sensitive C-reactive protein in a Sudanese population. Sudan Med J. 2012 August;48(2).
25. Rodríguez-Morán M, Guerrero-Romero F. Elevated concentrations of C-reactive protein in subjects with type 2 diabetes mellitus are moderately influenced by glycemic control. J Endocrinol Invest. 2003;26(3):216-21.

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A Study of Serum Albumin Levels in Acute Ischemic Stroke and its Correlation with Clinical Outcome

Santni Manickam[1], Joel Franklin[2], Velammal Petchiappan[3], Sujaya Menon[4]

ABSTRACT

Introduction:: Stroke is a global health problem. Studies have shown that serum albumin level is an independent predictor of ischemic stroke outcome. In the present study we compared the serum albumin and the volume of infarct with the clinical outcome.

Material and methods: Fifty patients of age more than 18 years with clinical and radiological evidence of acute ischemic stroke were included in the study. The severity of stroke at admission was assessed by National Institute of Health stroke scale (NIHSS). Serum albumin levels were measured at admission within 36 hours after stroke onset. Functional outcome was measured 1 week post admission and after 3 months during follow up using modified Rankin scale (mRs). A score of mRs >3 or death was taken as poor outcome. Statistical analysis and interpretation of the data was done. *P* value of <0.05 was taken as statistically significant.

Results: The mean age group of the study population was 53 years, male patients had more severe stroke compared to female patients. Most common risk factors were diabetes mellitus and systemic hypertension. Patients with a good outcome had lower NIHSS score, low mRS score, high albumin and low volume of infarct. Those with worst outcome had higher NIHSS, high mRS and high volume of infarct.

Conclusion: Relatively high serum albumin level in acute stroke patients decreases the risk of poor outcome. It is not only serum albumin but volume of infarct also does play a role in the final outcome of the patient.

Keywords: Acute Ischemic Stroke, Serum Albumin, Volume of Infarct, Functional Outcome

INTRODUCTION

Stroke is a global health problem. Around 20 million people suffer from stroke every year and about 5 million people among them do not survive.[1,2] It is the second most common cause of death and fourth leading cause of disability worldwide.[3] Stroke is also a predisposing factor for epilepsy, falls and depression in developed countries.[4] It is a leading cause of functional impairments, with 20% of survivors requiring institutional care after 3 months and 15% - 30% being permanently disabled.[5]

It is a well known fact that if stroke patients have associated protein energy malnutrition they tend to have poor outcome.[6] Studies have shown that serum albumin level is an independent predictor of ischemic stroke outcome.[7,8] Experimental studies have shown that human albumin infusion after stroke onset significantly improves the neurological function and reduces the infarct volume.[9] Although low serum albumin is associated with poor neurological outcome, albumin therapy in acute stroke patients is not beneficial as shown by the ALIAS (Albumin in Acute Ischemic Stroke) part 1 and 2 trials which evaluated the efficacy and safety of human albumin therapy in acute ischemic stroke.[10,11] The volume of infarct and its co-relation with the serum albumin was not studied in any of the previous studies. In the present study we compared the serum albumin and also the volume of infarct with the clinical outcome.

MATERIAL AND METHODS

The study was a Hospital based prospective study which included a total of fifty patients of age more than 18 years with clinical and radiological evidence of acute ischemic stroke admitted to the department of General Medicine and Neurology in a tertiary care centre in South India. Patients with Cerebral haemorrhage of any aetiology, Liver disease; Cardiac failure, Nephrotic syndrome, Diabetic nephropathy, Protein losing enteropathies and Malignancies were excluded from the study. Diagnosis of ischemic stroke is based on clinical observation confirmed by radiological imaging.

Prior to the commencement of the study, ethical clearance was obtained from Institutional Human Ethical Committee. The selected patients were briefed about the nature of the study and written informed consent was obtained from them in regional language. Patients were examined clinically in detail and the severity of stroke was assessed by National Institute of Health stroke scale (NIHSS) at baseline. Blood samples for assessment of albumin were collected at admission within 36 hours after stroke onset. Serum albumin estimation was done using Bromo Cresol Green (BCG) end point method. Functional outcome was measured 1 week post admission and after 3 months during follow up using

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modified Rankin scale (mRs). This scale runs from 0-6, running from perfect health without symptoms to death. A score of mRs: 0-3 was taken as favourable score and mRs: 4-6 was taken as unfavourable score. Serum albumin level <3.5 g% is considered as low.

The data were reported as the mean +/- SD or the median, depending on their distribution. The differences in quantitative variables between groups were assessed by means of the unpaired t test. Comparison between groups was made by the non-parametric Mann- Whitney test. ANOVA was used to assess the variables. The chi square test was used to assess the difference in categoric variables between groups.

Statistical analysis and interpretation of the data was done by using SPSS version 20. *P* value of <0.05 using two tailed test was taken as being of significance for all statistical tests

Serum albumin g/dl	NIHSS score		Total	*P* value
	≤10	>10		
3-3.4	0	1	1	0.124
3.5-3.9	10	3	13	
4-4.4	24	3	27	
>4.4	7	2	9	
Total	41	9	50	

Table-1: Serum Albumin with NIHSS Score

RESULTS

Among the study population of 50 patients, 44(88%) were males and 6(12%) were females. Out of 50 patients, 3 patients (1 male, 2 females) lost to follow-up. Number of patients with NIHSS score less than or equal to 10 was 41 and more than 10 was 9. Among those with NIHSS more than 10, there were seven males and two females. NIHSS score of 10 or less than 10 was seen in high numbers in 56-65 and >65 years age group. The risk factors of the study population and the nature of the ischemic stroke were tabulated in Figure 1and Figure 2 respectively.

Among the 47 patients, number of patients who had significant disability was 15. Among them 6 were in the age group of 56 to 65. Number of patients who had mild disability was 32.Among them about 11 patients were in the age group more than 65. After 3 months, number of patients with significant disability were 4 and number of patients with mild disability were 43.Significant improvement was seen in the age group of 56 to 65 (9 to 13).

Out of the 43 male patients, 14 patients had significant disability (mRs >3) and 29 patients had mild disability (mRs ≤3). Among the 14 with significant disability, 3 had death as outcome. Out of the 4 female patients, 1 had significant disability and died during the first week. Remaining 3

Serum albumin g/dl	mRs at 1 week		Total	P value	mRs at 3 months		Total	*P* value
	≤3	>3			≤3	>3		
<3.5	0	1	1	0.319	1	0	1	0.894
≥3.5	32	14	46		41	5	46	
Total	32	15	47		42	5	47	

Table-2: Serum Albumin with mRs score

mRs at 1 week	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	*P* value
					Lower Bound	Upper Bound			
≤3	32	5.5948	12.00600	2.12238	1.2662	9.9234	.11	52.54	0.001
>3	14	26.5182	26.47135	7.07477	11.2341	41.8023	.30	81.60	
Total	46	11.9628	19.91184	2.93584	6.0497	17.8759	.11	81.60	

Table-3: Volume of Infarct in CT scan with mRs after 1 week

mRs at 3 months	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	*P* value
					Lower Bound	Upper Bound			
≤3	42	10.6385	17.63608	2.72131	5.1428	16.1343	.11	62.70	0.146
>3	4	25.8675	37.60486	18.80243	-33.9702	85.7052	1.57	81.60	
Total	46	11.9628	19.91184	2.93584	6.0497	17.8759	.11	81.60	

Table-4: Volume of Infarct in CT Scan with mRs after 3 months

Study	No of Patients	Mean Albumin	*P* Value
Idicula et al[18]	444	3.76	<0.05
Dziedzic et al[7]	759	3.55	<0.01
Gaurav et al[19]	50	3.81	<0.001
Present study	50	3.85	0.894

Table-5: Comparison with other studies

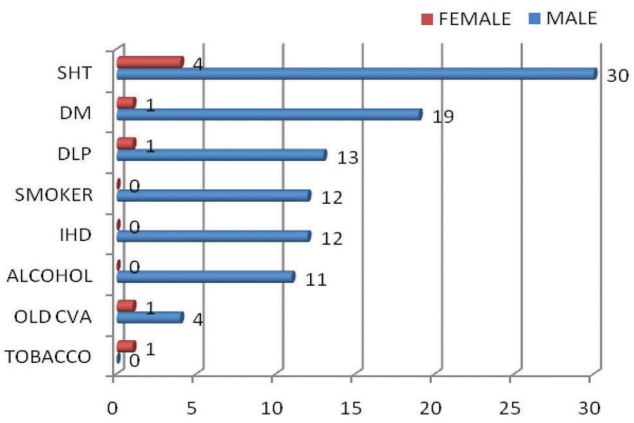


Figure-1: Risk factors of stroke in the study population

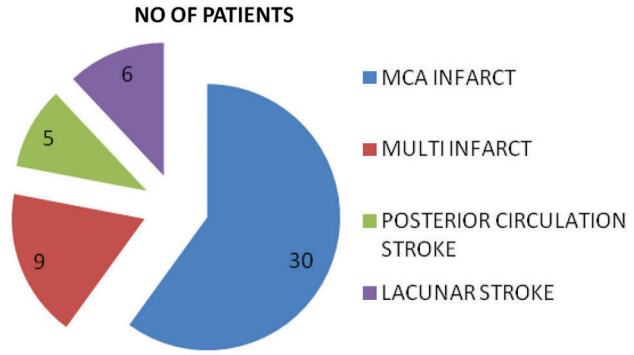


Figure-2: Frequency of lesion in MRI / CT brain

patients had mild disability. At 3 months follow-up, among the male patients, number of patients with significant disability dropped from 14 to 4. In the female group, the 3 patients with mild disability had improvement in the mRS score and didn't worsen.

Among 50 patients, only one patient in the age group of 46 - 55 had low serum albumin levels (<3.4 g/dl)). Mean albumin was 3.8. Majority of patients had albumin levels between 4 and 4.4. Both males and females showed similarity in this.

Table 1 showed that out of 9 patients with NIHSS score >10, only one patient had low serum albumin level<3.5 g/dl. The remaining 41 patients with a NIHSS score of less than 10 had serum albumin level above 3.5 g/dl.

On co-relating serum albumin level with mRS score at 1 week after stroke onset, while all the 32 patients with mRS ≤3 had serum albumin levels ≥3.5 g/dl, only one out of 15 with mRS > 3 had low serum albumin levels, the rest had serum albumin levels ≥3.5 g/dl. *P* value was 0.319. Hence there was no significant correlation. (Table 2) On co-relating serum albumin level with mRS score at 3 months after stroke, it was noted that out of 14 patients with high albumin levels who initially had mRS > 3 at 1 week, 9 had clinical improvement and the remaining 5 persisted to have mRS > 3 at 3 months post stroke. Also, a single patient with serum albumin <3.5 had an improvement in mRS score from baseline.

Out of 47 patients, volume of infarct could not be calculated for one patient. In the rest, the mean volume of infarct is significantly higher in those with mRs >3 than those ≤3 score at 1 week after stroke. (*P* value was 0.001) The results are tabulated in Table 3

The volume of infarct and mRS scale was compared during 3rd month using ANOVA method. *P* value was 0.146. This showed there was no correlation between volume of infarct and outcome during 3rd month. (Table 4)

DISCUSSION

It is well known that serum albumin plays a major role in the clinical outcome of vascular diseases. The neuroprotective effect of albumin is due to its various properties like anti-inflammatory and anti-oxidant effects, inhibition of thrombosis in microcirculation.[12-14] It reduces hematocrit level and also plays a vital role in aggregation of erythrocytes[15] by increasing low shear viscosity and decreasing erythrocyte sedimentation under no-flow conditions. Albumin has a good antioxidant property. Other neuroprotective effect of albumin in stroke includes Prevention of thrombosis and Prevention of leukocyte adhesion within post capillary microcirculation in the early reperfusion phase.

Albumin captures the oxygen free radicals and slows the production of reactive hydroxyl radical species. Albumin has a peculiar property of binding to copper ions by doing so it inhibits the process of copper ion dependent lipid per oxidation at cell membrane. It has also been postulated that albumin exerts neuroprotection by binding to lysophosphatidylcholine. Free lysophosphatidylcholine increases leukocyte adhesion molecules which lead to inflammatory mediated damage on vascular endothelium. It also causes apoptosis when it is present in high concentration. Based on the above said properties it was postulated that albumin infusion post ischemic stroke may be beneficial in long term outcome.

Experimental studies in rats done by Belayev et al. showed that high-dose human albumin therapy, if administered within 2 to 4 hours after stroke onset, is highly effective in improving neurological status and in reducing infarction volume and extent of brain swelling.[16]

Based on all the evidences human trial known as ALIAS (Albumin In Acute Ischemic Stroke) was conducted. It showed that treatment with intravenous albumin 25% at 2 g/kg was not associated with improved outcome at 90 days and was associated with increased incidence of intracerebral haemorrhage and pulmonary oedema. Further research is required to come to conclusive evidence that human albumin infusion can improve the clinical outcome of stroke patients as there were good results in rat models of stroke.

The mean age group of the study population was 53 years, predominantly middle aged group which is a decade lower than the peak age group of stroke in western countries.[17] In the present study, male patients had more severe stroke compared to female patients. Most common risk factors noticed in this study were diabetes mellitus and systemic hypertension.

Around 42 patients had good outcome in this study and only 5 patients had poor outcome. Most of the previous studies showed that patients with good outcome had lower NIHSS

score and high serum albumin level on admission and those with worst outcome showed high NIHSS score and low serum albumin level. In our study also patient with low NIHSS and high albumin had good neurological outcome. Only one patient had low albumin and high NIHSS score had unfavourable mRs score after 1 week; however his mRs score improved after 3 months

The studies which were done earlier didn't take volume of infarct into account. The patients with poor outcomes had a mean volume of infarct of around 26.51 and those with good outcome had a mean volume of infarct of around 5.59. Even though there was significant correlation between volume of infarct and mRS score at 1 week there was no significant correlation between them at 3 months

In this study, most patients with a good outcome had lower NIHSS score, low mRS score, high albumin and low volume of infarct. Those with worst outcome had higher NIHSS, high mRS and high volume of infarct. The one patient with low serum albumin also had a good outcome in 3 months. It is not only serum albumin but volume of infarct also do play a role in the final outcome of the patient which was not considered in other studies. Those who had poor outcome had a mean volume of infarct of around 25 cm^3 and also there was statistical significance between volume of infarct and mRS at 1 week. Even though clinically most of the patients had good outcome after 3 months based on mRS score, statistically *P* value was not significant. Hence this study is contrary to the evidence that serum albumin is a long term predictor of ischemic stroke outcome. The disadvantage of this study was that not enough number of patients could be obtained with low serum albumin levels.

CONCLUSION

Though the risk of stroke increases with age, our patients were predominantly in the middle aged group. Male patients had more severe stroke compared to female patients. Most common risk factors noticed in this study were diabetes mellitus and systemic hypertension. Most patients with a good outcome had lower NIHSS score, low mRS score, high albumin and low volume of infarct. Those with worst outcome had higher NIHSS, high mRS and high volume of infarct. The one patient with low serum albumin also had a good outcome in 3 months. It is not only serum albumin but volume of infarct also does play a role in the final outcome of the patient.

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REFERENCES

1. Warlow C, Sudlow C, Dennis M, Wardlaw J, Sandercock P. Stroke. Lancet. 2003. p. 1211–24.
2. Strong K, Mathers C, Bonita R. Preventing stroke: saving lives around the world. Lancet Neurology. 2007. p. 182–7.
3. WHO. Stroke, Cerebrovascular accident [Internet]. Health topics. 2013. Available from: http://www.who.int/topics/cerebrovascular_accident/en/
4. WHO. Global burden of stroke. atlas Hear Dis stroke. 2004;15:50–1.
5. Lipska K, Sylaja PN, Sarma PS, Thankappan KR, Kutty VR, Vasan RS, et al. Risk factors for acute ischaemic stroke in young adults in South India. J Neurol Neurosurg Psychiatry 2007;78:959–63.
6. Davalos A, Ricart W, Gonzalez-Huix F, Soler S, Marrugat J, Molins A, et al. Effect of malnutrition after acute stroke on clinical outcome. Stroke. 1996; 27: 1028–1032.
7. Dziedzic T, Slowik A, Szczudlik A. Serum albumin level as a predictor of ischemic stroke outcome.Stroke. 2004; 35: 156–158.
8. Babu MS, Kaul S, Dadheech S, Rajeshwar K, Jyothy A, Munshi A. Serum albumin levels in ischemic stroke and its subtypes: correlation with clinical outcome. Nutrition. 2013;29:872-5.
9. Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD. Human albumin therapy of acute ischemic stroke. Marked neuroprotective efficacy at moderate doses and with a broad therapeutic window. Stroke. 2001;32:553–560.
10. Ginsberg MD, Palesch YY, Martin RH, Hill MD, Moy CS, Waldman BD, et al.The Albumin in Acute Stroke (ALIAS) Multicenter Clinical Trial. Safetyanalysis of part 1 and rationale and design of part 2. Stroke 2011;42:119–27.
11. Hill MD, Martin RH, Palesch YY, Tamariz D, Waldman B, Ryckborst KJ, et al.The Albumin in Acute Stroke Part 1 Trial. An exploratory efficacy analysis.Stroke 2011;42:1621–5.553–560.
12. Jorgensen KA, Stoffersen E. On the inhibitory effect of albumin on platelet aggregation. Thromb Res 1980;17:13–8.
13. Halliwell B. Albumin: an important extracellular antioxidant? Biochem Pharmacol. 1998;37:569–571.
14. Belayev L, Pinard E, Nallet H, Seylaz J, Liu Y, Riyamongkol P, Zhao W, Busto R, Ginsberg MD. Albumin therapy of transient focal cerebral ischemia. In vivo analysis of dynamic microvascular responses. Stroke. 2002;33:1077–1084.
15. Reinhart WH, Nagy C. Albumin affects erythrocyte aggregation and sedimentation. Eur J Clin Invest.1995;25:523–528.
16. Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD. Human Albumin Therapy of Acute Ischemic Stroke Marked Neuroprotective Efficacy at Moderate Doses and With a Broad Therapeutic Window. Stroke. 2001; 32:553-560.
17. Hankey JK, Jamrozik K, Broadhurst RJ et al. Long-term disability after first-ever stroke and related prognostic factors in The Perth Community Stroke Study 1989-1990. Stroke. 2002;33:1034-1040.
18. Idicula TT, Waje-Andreassen U, Brogger J, NaessH, Thomassen L. Serum albumin in ischemic stroke patients: the higher the better. The Bergen Stroke Study. Cerebrovasc Dis. 2009; 28:13-17.
19. Kasundra G, Sood I. Prognostic Significance Of Serum Albumin Levels In Acute Ischemic Stroke. Natl J Integr Res Med. 2014; 5: 1-4.

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Original Research Article

Can serum ferritin levels predict the severity of dengue early?: an observational study

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ABSTRACT

Background: Dengue infection is a major public health threat; early recognition is crucial to improve the survival in severe dengue. Although there are various biomarkers to predict the severity of dengue, they are not routinely used in clinical practice for prognostication. We analyzed whether serum ferritin can be used to predict the severity at an earlier stage.
Methods: A hospital based prospective observational study was done involving 119 dengue cases diagnosed by positive NS1 antigen or dengue specific serology (capture ELISA). Serum ferritin was measured in all at the time of diagnosis. Clinical and platelet count monitoring was done daily; classified as severe and non-severe according to 2009 WHO criteria.
Results: Out of 119, 5 developed severe dengue; patients with severe dengue had significantly lower median platelet count ($p<0.0001$); higher ferritin levels ($p=0.03$) and hospital stay ($p<0.0001$) than non-severe group. Age had a significant negative co-relation with platelet count ($r= -0.427$; $p<0.0001$); positive correlation with ferritin levels ($r=0.16$; $p=0.08$) and hospital stay ($r= 0.26$; $p=0.004$) indicating that elderly subjects are at risk of severe disease. Serum ferritin levels negatively correlated with the platelet count ($r= -0.51$ $p<0.001$). High ferritin levels in severe cases are noted from day 4 of clinical illness.
Conclusions: Elevated serum ferritin levels can be used as a potential early prognostic marker to predict the severity of dengue infection in clinical practice.

Keywords: Predictor of severity, Serum ferritin, Early, Severe dengue

INTRODUCTION

Dengue infection poses a major health problem in tropical and subtropical countries.[1] According to World Health Organization, around 50 to 100 million new infections are estimated to occur every year in more than 100 endemic countries across the world; of which around 500000 people with severe dengue require hospitalization each year and about 2.5% of those affected die.[2] The burden of the disease is so much that in the year 2012 World Health Organization classified the disease as "the most important viral disease that is transmitted by mosquitoes".[3] Dengue is caused by dengue virus serotypes I-IV and transmitted to human beings by the bite of Aedes mosquitoes.[4]

The clinical spectrum of dengue infection is variable from mild fever to severe forms of dengue such as dengue hemorrhagic fever and dengue shock syndrome. Severe dengue is characterized by severe thrombocytopenia with major bleeding, plasma leakage resulting in fluid accumulation, respiratory distress and

multi-organ dysfunction.[5] The presence of certain clinical warning symptoms which help to predict the severity are bleeding, skin rash, nausea, vomiting, abdominal pain and hepatosplenomegaly.[6]

Clinically severe dengue results from interplay between virus related virulence factors and host factors which include inflammatory response of the host to infection with exuberant T and B cell activation, release of cytokines (cytokine storm), altered endothelial function with increased vascular permeability and nutritional status of the host. [7]

In clinical practice, patients with dengue infection are grouped as 1) those with warning signs that need intensive monitoring and aggressive management 2) those without warning signs. Non-severe cases without warning signs can develop into severe dengue later during the course of illness. They are at risk of developing complications related to plasma leakage and thrombocytopenia often at the end of febrile stage of illness (third to fifth day of illness) which if unrecognized can be fatal.

Studies had identified various biomarkers for immune and endothelial cell activation, biochemical and genetic markers to predict the severe dengue.[8] The clinical utility of these markers is limited since measurement of these markers like soluble receptors, cytokines, growth factors, vWF; genetic profiling etc. is technically difficult and not widely available. Acute phase reactants like alpha1 anti-trypsin, ceruloplasmin and ferritin levels are elevated during dengue infection; they have a longer half-life unlike the cytokines, and it is feasible to measure their levels.[9]

Increased serum ferritin has been associated with severe dengue in children.[10] Hyperferritinemia in dengue infected subjects is associated with intense immune activation and coagulation disturbances as noted in Aruba Dengue outbreak.[11] An Indian study had shown that serum ferritin levels are significantly elevated in dengue infected cases compared to the controls; On group analysis, cases with severe dengue had higher ferritin levels than milder forms which was noted both during the febrile and defervescence stages of the illness.[9]

Results from the previous studies had shown that hyperferritinemia is associated with severe disease which is noted throughout the disease course. In a Malaysian study, a large proportion of patients developed severe dengue early during febrile stage. [12] It is very crucial to predict the risk of progression to severe dengue at the earliest, by simple measurable tests to initiate appropriate intensive, supportive therapy.

In the present study, we analysed whether serum ferritin measured during early disease course can be used as a marker to indicate the severity which helps to triage and manage them appropriately.

METHODS

This is a hospital based prospective observational study conducted from April 2015 to June 2016 in a tertiary care centre, PSG Institute of Medical Sciences and Research Centre (PSGIMS and R), Coimbatore, South India. The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Human Ethics Committee, PSGIMS and R. All adult patients who were hospitalized and diagnosed with dengue infection either by dengue NS1 antigen or positive dengue specific serology were included in the study after obtaining their informed consent for participation in the study. Patients with chronic inflammatory conditions or diseases; iron overload status; sideroblastic anemia; thalasemia; malignancy; liver disease were excluded from the study.

Study methodology

The study was based on prospective collection of data in dengue infected patients who fulfill the criteria as mentioned above. Diagnosis was based on NS1 antigen test (done with J. Mitra dengue NS1 Ag Microlisa kit) when the presentation was less than 5 days of illness or positive dengue specific serology (dengue IgM and dengue IgG done by Panbio dengue IgM; dengue IgG capture ELISA) if presented after 5 days of illness. Serum ferritin levels were measured if the presentation was less than 7 days from the onset of fever. After informed consent, 2ml of blood was collected from the study subjects; ferritin assay was done (by electrochemiluminescence immunoassay -COBAS e411) at the time of diagnosis if the diagnosis was made in the study center; or at the time of admission if referred from an outside hospital with positive test results (NS1 or dengue serology positive).

The clinical course of the disease and platelet count were monitored carefully on a daily basis and patients were classified as having severe and non-severe infection as per WHO 2009 criteria; classified as severe dengue when shock, respiratory distress, severe bleeding; organ impairment occur.[5] Mean lowest platelet count was calculated in both the subgroups from all the platelet count values obtained on a daily basis during the hospital stay. Mean ferritin levels were calculated and compared in both the subgroups.

Statistical analysis

Descriptive statistics were reported as mean±Standard deviation (SD), median with 25^{th} and 75^{th} percentiles. Mann Whitney U test was used to compare platelet count and serum ferritin between severe and non-severe dengue groups. Spearman's Rank correlation was used to assess the correlation between age with platelet count, serum ferritin and hospital stay. All analyses were done using SPSS version 21.0. P value less than 5% was considered statistically significant.

RESULTS

A total of 119 subjects were analyzed, of which there were 68 males (57.14%) and 51 (42.85%) females. The mean age of the study population was 29.1±11.4 years. About 50% of the study population was less than 25 years. There was no significant difference in the mean age between male and female. Diagnosis of dengue infection was established by positive NS1 antigen in 61 subjects while in the remaining 58, it was diagnosed by positive dengue serology.

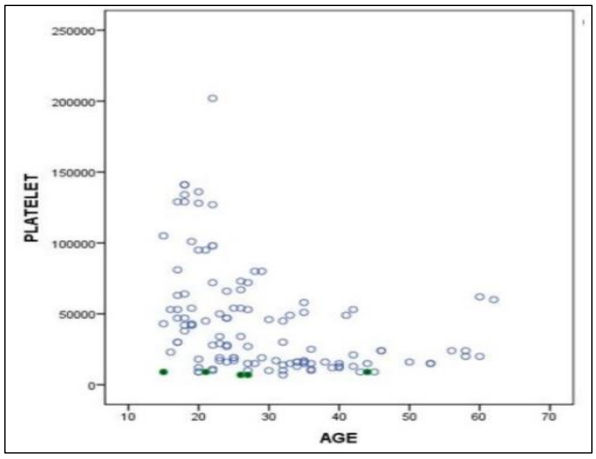
Among the total 119, only 5 (4.2%) subjects had clinical severe dengue, of which 4 were males and one female. The mean lowest platelet count was 40426.47×10^9/L in males, 45000×10^9/L in females. Around 75% of the study population stayed in the hospital for ≤ 3 days; 16% stayed for 4 days; 5% stayed for 5 days. Around 5% had a hospital stay for ≥9 days. Serum ferritin levels were measured in 14 subjects on day 3; 19 on day 4; 36 on day 5; 29 on day 6 and for the remainder 21 subjects it was measured on day 7. The mean ferritin levels measured was 1399.53±690.68ng/ml; (median-1856). The mean hospital stay was 3.11 days.

Table 1: Comparison of serum ferritin, platelet count and hospital stay in severe and non-severe dengue.

	Severe dengue	Non-severe dengue	P value
Serum ferritin ng/ml	2000 (2000, 2000)	1593 (757, 2000)	0.03*
Platelet count ×109/l	9000 (7000, 9000)	29500 (15750, 55000)	<0.0001*
Hospital stay days	16 (11, 21)	3 (2, 3)	<0.0001*

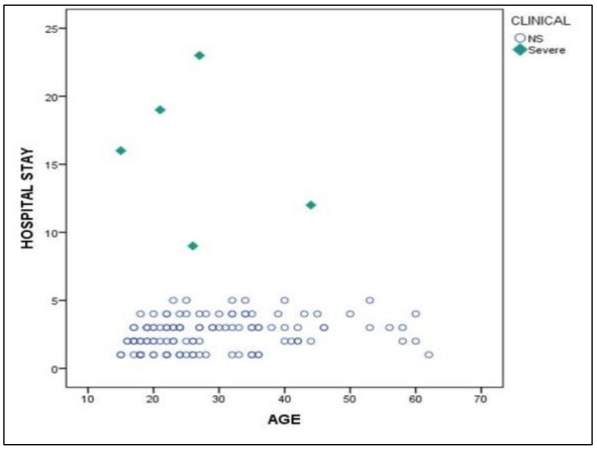
Median platelet count was significantly lower among patients who had severe dengue as compared to non-severe dengue group (Table 1). The median platelet count recorded during daily monitoring of platelets throughout the hospital stay were 9000 ×10^9/L (7000, 9000) in severe and 29500×10^9/L (15750, 55000) in non-severe dengue respectively. The median hospital stays, and serum ferritin levels were significantly higher among severe dengue subjects as compared to non-severe group (Table 1). The median serum ferritin levels and hospital stay among severe and non-severe dengue patients were 2000ng/ml (2000, 2000) vs. 1593ng/ml (757, 2000) and 16 (11, 21) vs. 3 days (2, 3) respectively.

In the present study, higher age showed a significant negative correlation with platelet count (Pearson co-efficient r= -0.427; p<0.0001). Also, higher age positively correlated with hospital stay (r= 0.26; p=0.004) and ferritin levels (r=0.16; p=0.08). The correlation with hospital stay is significant. Serum ferritin levels showed a significant negative correlation with the platelet count (Pearson co-efficient r= -o.51; p<0.001). The results are depicted in Figures 1-4.



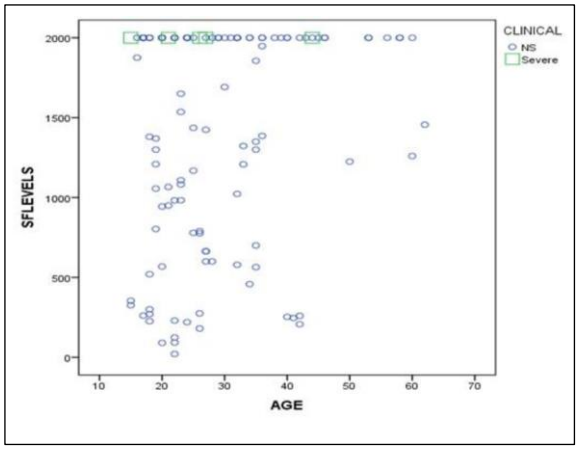
Age negatively correlated with platelet count which is significant (r= -0.427; p<0.0001*)

Figure 1: The co-relation co-efficient between age (years) and platelet count(x109/L).



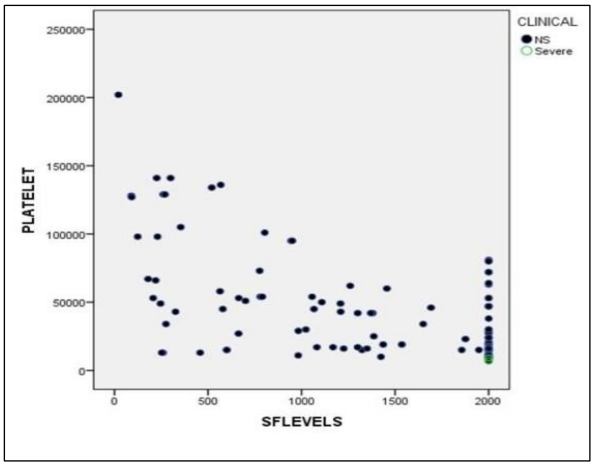
Age positively correlated with hospital stay which is significant (Pearson co-efficient r= 0.26; p=0.004*)

Figure 2: The co-relation co-efficient between age (years) and hospital stay (days).



Age positively correlated with serum ferritin levels (non significant r=0.16; p=0.08)

Figure 3: The co-relation co-efficient between age (years) and SF levels (serum ferritin in ng/ml).



Serum ferritin levels showed a significant negative correlation with the platelet count (Pearson co-efficient r= –o.51 p<0.001*)

Figure 4: The co-relation co-efficient between SF levels (serum ferritin in ng/ml) and platelet count.

Serum ferritin measured from day 3 to day 7 of illness was compared in both the severe and non-severe groups and reported in Table 2. It was observed that non-significantly, patients who had severe dengue had higher median serum ferritin levels from day 4 onwards as compared to non-severe dengue cases.

Table 2: Comparison of serum ferritin levels in severe and non-severe dengue measured from the day 3 to day 7 of illness.

Serum ferritin measurement	Ferritin level in ng/ml		P value
	Severe dengue	Non-severe dengue	
3 day	-	n=14 1486 (1013, 2000)	-
4 day	n=1 2000±0 2000 (2000, 2000)	n=18 1001±811 859 (242, 2000)	0.421
5 day	n=2 2000±0 2000 (2000, 2000)	n=34 1549±587 2000 (1183, 2000)	0.349
6 day	n=1 2000±0 2000 (2000, 2000)	n=28 1222±727 1315 (565, 2000)	0.414
7 day	n=1 2000±0 2000 (2000, 2000)	n=20 1512±722 1974 (1103, 2000)	0.571

Serum ferritin measured from day 4 to7 of illness was high in severe dengue compared to non-severe cases, although not statistically significant

DISCUSSION

Currently there is no specific drug or preventive vaccine available for dengue infection. The mainstay of management of dengue infected subjects is supportive care and close monitoring for complications.[13] Most of these patients recover within 5 to 7 days from onset of the febrile illness while only a small percentage progress to severe dengue, with a study quoting 5%.[14] Severe dengue often occurs at the end of febrile or during convalescent stage and carries a very high mortality. It is very crucial to predict the severe form well in advance even prior to the appearance of warning signs by a simple diagnostic marker so that early identification and appropriate management would improve the outcome in these patients.

Increased expression of acute phase reactants is observed in patients with severe dengue infection when compared to non-severe cases.[9] This serves to prognosticate the dengue infected patients well ahead of the appearance of clinical warning signs. One such acute phase reactant is ferritin which is produced by reticuloendothelial cells in response to infection and inflammation. Ferritin is highly elevated in dengue infected patients than in patients with other febrile illnesses.[11] Hyperferritinemia seen in these patients exhibit two opposite functions; Early in the phase of clinical illness, increased serum ferritin levels exert a protective effect by chelating the toxic free iron radicals at the site of inflammation.[15] while in severe cases, raised ferritin may assume a pathogenic role by activating immune cells resulting in cytokine storm.[9]

Males constitute 57.14% of the study population in the present study which was in accordance with other studies. The mean hospital stay was comparable with other studies. The mean duration of hospital stay was 2.73±1.41 days as observed by Ahmed at al, and 3.7±1.02 days in a study conducted in Rawalpindi.[16,17]

Results from the present study showed that hyperferritinemia is associated with severe dengue which is noted in other studies as well.[9-11, 17] To analyze whether serum ferritin can predict the severity earlier in the course of illness, day wise serum ferritin levels was compared in both the sub-groups; the serum ferritin levels were higher in severe dengue cases when compared to non-severe group and this increase is noted from day 4 of illness onwards which clearly indicates that serum ferritin can predict the severity of dengue at an earlier stage.

A study conducted during a dengue outbreak on the Caribbean island Aruba which compared the ferritin levels in dengue infected patients with those having other viral illnesses concluded that ferritin is a useful clinical marker to differentiate dengue from other febrile illnesses; serum ferritin levels >1500ng/ml is associated with severe disease ; serum ferritin levels measured on day 4-5 better predicts the outcome of dengue infection and it concluded that these patients with hyperferritinemia should be monitored carefully for complications.[11] Results from the present study also had shown that hyperferritinemia (2000ng/ml) is seen in all cases with severe dengue and this increase in serum

ferritin levels is noted from day 4 of clinical illness in severe cases when compared to non-severe cases.

Another study conducted in one hundred seventy-seven Thai children which measured serum ferritin levels during the entire clinical course (febrile, toxic and convalescent stages) showed similar results.[10] Increased serum ferritin was associated with severe dengue; patients with non-severe dengue had serum ferritin levels less than 1,200ng/ml during the entire course of illness, while severe forms recorded higher levels of serum ferritin during the febrile stage and the defevervescence stage. Patients with dengue hemorrhagic fever (DHF) grade III and IV had significantly higher median ferritin levels measured on day 5,6 and 7 of illness than those with grade I, II DHF and dengue fever. In that study it was concluded that serum ferritin level ≥1,200ng/ml from day 4 of the illness predicts the occurrence of dengue hemorrhagic fever.

In a study conducted in South India by Soundravally R et al, which included 48 dengue infected cases and 48 cases with other febrile illness as controls, serum ferritin levels were measured on the day of admission (which is a median of 4 days after the onset of fever) and day of defervescence (which is a median of 4 days after the day of admission).[9] Compared to other febrile illnesses, dengue cases demonstrated significant increase in serum ferritin levels ;also within clinical groups, severe disease exhibited higher ferritin levels compared to non-severe group throughout the febrile and defervescence stages of illness (940.09±568.31 in non-severe cases vs. 1264.71±492.59 in severe dengue on day of admission ; 418.19±404.59 vs. 1490.74±359.40 on day of defervescence) The study concluded that raised serum ferritin levels could predict the severity of dengue with a sensitivity and specificity of 76.9% and 83.3% respectively. The present study also showed similar results; hyperferritinemia was noted in severe cases compared to non-severe group from day 4 to day 7 of illness.

One another study showed significant association between raised serum ferritin levels on the day of admission and the development of severe dengue.[16] Serum ferritin was measured on the day of admission in a total of 104 dengue positive cases and they were grouped into a) Group A with normal ferritin levels (up to 100µg/dl) and b) Group B with high serum ferritin levels ; both the groups were followed up for the occurrence of severe dengue. Severe dengue was noted only in 2 out of 31 patients with normal serum ferritin levels while more than half of the patients with severe dengue (35 out of 73) had raised serum ferritin levels. The study concluded that serum ferritin can be used as an early marker to predict the severity of the disease.

Another significant observation made out in the present study was that age had a positive co-relation with the serum ferritin levels and the duration of hospital stay while it negatively co-related with the platelet count. This implies that age is an important predictor of severity of dengue infection. As age advances, there is increased risk of developing severe disease with low platelet count, high ferritin levels and prolonged hospital stay. Studies had shown that elderly individuals are at risk of developing severe disease. In a study conducted in Southern Taiwan during a dengue outbreak with 5336 dengue cases, the incidence of dengue hemorrhagic fever and dengue shock syndrome is significantly higher in persons aged 60 years and above when compared with younger age groups.[18] Similar observation was made in a Singapore study; persons aged more than 60 years had more occurrence of severe dengue (20.3% vs. 14.6%, P = 0.006) as compared to younger adults.[19]

Our study is unique in that serum ferritin was measured once at the time diagnosis whereas in most of the earlier studies serum ferritin levels were done at multiple times during various stages of clinical illness.[9-11] The results of the present study in which serum ferritin was measured either at the time of diagnosis (or at the time of admission if referred from outside) within 7 days from onset of fever, correlated with the findings of the previous studies . It would be simpler, easier and convenient to measure serum ferritin on a single day, which is practically feasible in clinical setting catering to a large population.

The present study has few limitations as well. First, is the small number of severe dengue observed in the present study; hence we were unable to derive the cut-off value of day wise ferritin levels which can predict the risk of development of severe dengue. A larger sample size with more number of severe cases would be appropriate before arriving at a definite conclusion. Second, the upper limit of serum ferritin measured in the present study was 2000ng/ml due to laboratory limitations; so higher values could not be observed which if available can enable us to better understand about its correlation with both the severity of thrombocytopenia and clinical severity with more accuracy.

Also, the nature of infection (primary or secondary); dengue serotypes were not taken into consideration. To address the limitations in the present study, future studies are needed to find out the cut off level of serum ferritin which can predict severe dengue at the earliest for effective disease management.

CONCLUSION

The present study had shown that elevated serum ferritin levels done early during the febrile stage of the illness (3rd to 7th day of illness) predict the severity of dengue.

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REFERENCES

1. World Health Organization. Working to Overcome the Global Impact of Neglected Tropical Diseases. First WHO report on Neglected Tropical Diseases, 2010. Available at: www.who.int/publications/2010/9789241564090_eng.pdf. Accessed on 29 November 2018.
2. World Health Organization. Regional Office of South-East Asia. Dengue fact sheet. Available at: http://www.searo.who.int/entity/vector_borne_tropical_diseases/data/data_factsheet/en/. Accessed on December 3, 2018
3. World Health Organization. Global Strategy for Dengue Prevention and Control, 2012-2020. Geneva: WHO Press; 2012. Available at: https://www.who.int/denguecontrol/9789241504034/en. Accessed on 1 January 2018.
4. Back AT, Lundkvist A. Dengue viruses-an overview. Infect Ecol Epidemiol. 2013;3:10-3.
5. World Health Organization (WHO) and the Special Programme for Research and Training in Tropical Diseases (TDR). Dengue guidelines for diagnosis, treatment, prevention and control, 2009. Available at: https://www.who.int/rpc/guidelines/9789241547871/en/. Accessed on 27 November 2018.
6. Zhang H, Zhou YP, Peng HJ, Zhang XH, Zhou FY, Liu ZH, et al. Predictive symptoms and signs of severe dengue disease for patients with dengue fever: a meta-analysis. Biomed Res Int. 2014;359308.
7. Martina BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev. 2009;22:564-81.
8. John DV, Lin YS, Perng GC. Biomarkers of severe dengue disease-a review. J Biomed Sci. 2015;22:83.
9. Soundravally R, Agieshkumar B, Daisy M, Sherin J, Cleetus C. Ferritin levels predict severe dengue. Infection. 2015;43(1):13-9.
10. Chaiyaratana W, Chuansumrit A, Atamasirikul K, Tangnararatchakit K. Serum ferritin levels in children with dengue infection. Southeast Asian J Trop Med Public Health. 2008;39:832-36.
11. van de Weg CA, Huits RM, Pannuti CS, Brouns RM, van den Berg RW. Hyperferritinaemia in dengue virus infected patients is associated with immune activation and coagulation disturbances. PLoS Negl Trop Dis. 2014;9:e3214.
12. Md-Sani SS, Md-Noor J, Han W, Gan SP, Rani NS, Tan HL, et al. Prediction of mortality in severe dengue cases. BMC Infectious Dis. 2018;18:232.
13. Biswas A, Pangtey G, Devgan V, Singla P, Murthy P, Dhariwal AC, et al. Indian national guidelines for clinical management of dengue fever. J Indian Medical Asso. 2015;113(12).
14. Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, et al. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. Trop Med Int Health. 2011;16(8):936-48.
15. Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood. 2002;99:3505-16.
16. Ahmed A, Alvi AH, Butt A, Nawaz AA, Hanif A. Assessment of Dengue fever severity through liver function tests. J Coll Physicians Surg Pak. 2014;24:640-44.
17. Nadeem M, Shafiq MM, Manzoor MS, Ahmed SI. JRMC. 2016;20(3):165-7.
18. Liu CC, Huang KJ, Huang MC, Lin JJ, Wang SM, Liu JJ, et al. High case-fatality rate of adults with dengue hemorrhagic fever during an outbreak in non-endemic Taiwan: risk factors for dengue-infected elders. Am J Infect Dis. 2008;4:10-17.
19. Rowe EK, Leo YS, Wong JG, Thein TL, Gan VC, Lee LK, et al. Challenges in dengue fever in the elderly: atypical presentation and risk of severe dengue and hospital-acquired infection. PLoS Negl Trop Dis. 2014;8(4).

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Letter to the Editor

Comment on "Long-Term Dietary Changes after Diagnosis of Rheumatoid Arthritis in Swedish Women: Data from a Population-Based Cohort"

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We read with great interest the article published in International Journal of Rheumatology, "Long-Term Dietary Changes after Diagnosis of Rheumatoid Arthritis in Swedish Women: Data from a Population-Based Cohort" by Cecilia Lourdudoss et al. [1]. The role of diet in rheumatoid arthritis (RA) remains an ever-burning question for both the patient and the treating physician and, while it is quite acceptable for the lay person to think that the occurrence of the arthritis is influenced by the diet, unfortunately the scientific community is yet to produce a definite answer.

In a systematic review of dietary intervention studies in RA, it was concluded that the effect of various dietary manipulations on the disease is uncertain [2]. The potential risks associated with such intervention were also highlighted in that review. The present study is among the important observations in this field and the authors give specific dietary recommendations for patients with RA in the future. The authors noted that there was no change in dietary pattern in RA patients, whereas those without RA made certain changes to their dietary pattern during the study period. This might be because of the relatively small RA population when compared to the controls in this cohort. The duration of the disease, the disease activity, and the occurrence of comorbid illness also were not taken into consideration.

We did a study that assessed the role of diet in RA patients and its impact on arthritis-related symptoms as perceived by the patients themselves, using a simple questionnaire in their own language [3]. We categorized them as diet restrictors and nonrestrictors based on whether they are following any change in dietary pattern depending upon the aggravation of joint symptoms following a specific food intake. We also considered associated comorbidities and alternative medicine intake, which can influence the dietary pattern. Out of a total of 101 patients with an average disease duration of 50.2 ± 65.4 months, 44 (43.6%) followed dietary restriction while 57 (56.4%) did not observe any restriction. 20 of the 44 patients who restricted their diet (45.5%) felt a difference in joint symptoms on exposure to particular diet; 52 out of 57 (91.2%) nonrestrictors did not experience any influence of diet on their symptoms. Patients who observed changes in their joint symptoms on exposure to a particular diet adopted dietary restriction and we observe this association as statistically significant in our study ($p < 0.0001$). 20 out of the 44 and 52 out of 57 patients in either group appeared to be correctly judging whether or not to restrict their diets.

We concluded that the decision of modifying diet should be individual case-based rather than a generalized dietary restriction, which lacks a solid scientific proof as of now and might deprive the individual of the benefits of a nutritious diet. The voluntary addition or deletion of part of the diet might well have an influence on the overall dietary habits of the entire family, as most often RA affects women who

are often the main homemaker. Unlike gouty arthritis where there are definite dietary triggers, rheumatoid arthritis is a disease with complex pathogenesis and variable course and hence needs to be managed in a multifactorial manner with the basic recommendation for a healthy and nutritious diet till we have convincing data regarding the benefits of dietary manipulation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

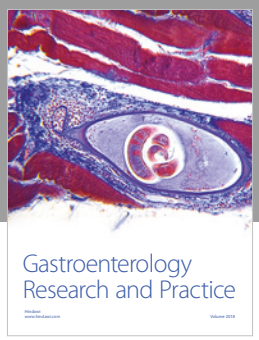
References

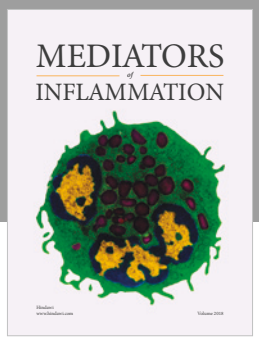
[1] C. Lourdudoss, L. Arnaud, A. Wolk, R. F. van Vollenhoven, and D. Di Giuseppe, "Long-term dietary changes after diagnosis of rheumatoid arthritis in swedish women: data from a population-based cohort," *International Journal of Rheumatology*, vol. 2018, Article ID 9152480, 7 pages, 2018.

[2] K. B. Hagen, M. G. Byfuglien, L. Falzon, S. U. Olsen, and G. Smedslund, "Dietary interventions for rheumatoid arthritis," *Cochrane Library: Cochrane Reviews*, vol. 1, 2009.

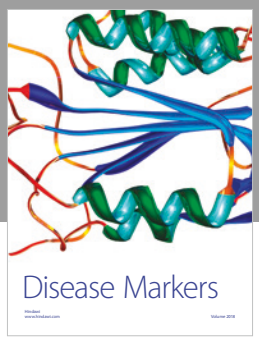
[3] V. Petchiappan, P. Priyadarshini, and V. N. Prabhu, "Dietary restrictions in rheumatoid arthritis-what the patient?s feel: A questionnaire based study," *Archives of General Internal Medicine*, vol. 02, no. 03, 2018.



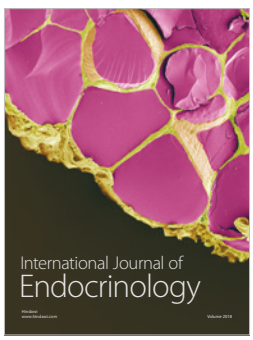






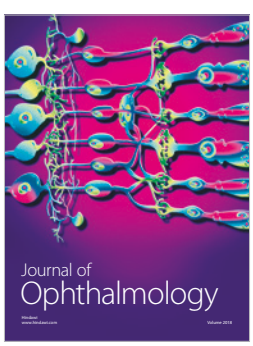


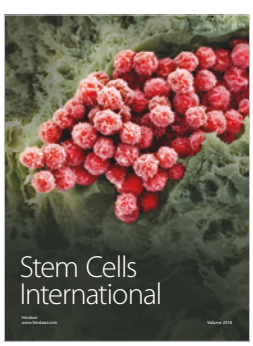


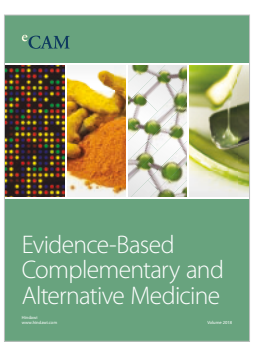






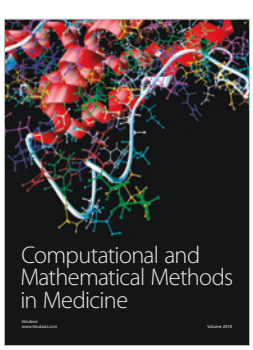


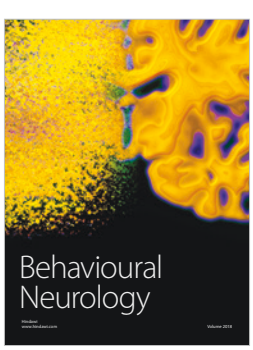




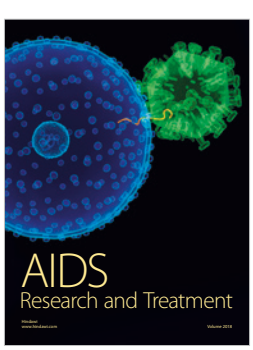


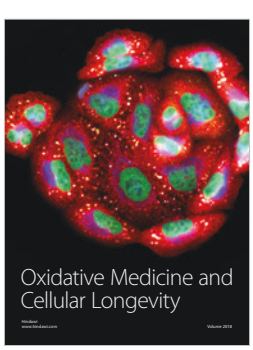












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Dietary restrictions in rheumatoid arthritis-what the patient's feel: A questionnaire based study.

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Abstract

Background: There is increasing evidence for the role of diet in the etiopathogenesis of Rheumatoid Arthritis (RA) while it is not clear how it modifies the disease activity in view of inconclusive results of various dietary intervention studies. We intend to study the influence of diet on RA patients by a simple questionnaire.

Methods and findings: Descriptive cross-sectional study was carried out among 101 consecutive RA patients with simple questionnaire and analysed whether they had dietary restriction or not and how it affected their symptoms. There were 44 patients who restricted their diet and 57 who did not observe dietary restriction. Of the 44 dietary restrictors, 20 (44.5%) had reported worsening of joint pains after a particular food while 52 out of 57 non dietary restrictors (91.2%) did not feel a difference in the joint symptoms with food intake which was statistically significant ($p<0.0001$). Just like patient reported disease outcome measures which are less complicated and time consuming has emerged as a promising tool in the disease management, this study also observed that majority of our patients are right in restricting their diet based on their joint symptoms.

Conclusion: The patient has to decide on their own basis whether or not to restrict a particular food item based on their symptoms instead of imposing a specific dietary pattern until we get more evidence to prove the contrary.

Keywords: Dietary restriction for arthritis, Diet and rheumatoid arthritis, Diet in early rheumatoid arthritis, Diet in established rheumatoid arthritis, Questionnaire for diet in rheumatoid arthritis.

Abbreviations: DMARDS: Disease Modifying Anti-Rheumatic Drugs; RA: Rheumatoid Arthritis.

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Introduction

Rheumatoid arthritis (RA) is one of the commonest debilitating arthritis which has seen a sea change in the diagnosis and management over the past few decades. While pharmacological therapies receive prime importance in the management plan, non- pharmacological measures are often neglected, but the scenario is changing, and more focus is made on the non–pharmacological intervention measures including diet, exercise and healthy life habits.

The pathogenesis of RA is complex which includes genetic and non –genetic factors. Non-genetic factors account for around 40% of the risk for RA [1]. Among the non–genetic factors cigarette smoking is a well-established risk factor for the development of inflammatory polyarthritis [2-5]. Diet is another well studied risk factor [6,7]; it can influence the body weight which has a bearing on the individual's mobility; also it can affect the inflammatory process by altering the gut micro flora, anti-oxidant activity. The presence of altered intestinal micro flora is implicated in the pathogenesis as well as the disease progression in RA [8-10]. Diet can have positive and negative impact on the disease. High protein diets is implicated in the disease pathogenesis [11,12] while low protein diets can improve RA related symptoms [13-16]. A very high prevalence of RA is noted in countries with greater consumption of red meat [17]. Certain food items like fish [18,19], cooked vegetables, and β cryptoxanthin found in fruits and vegetables [20-22] have a possible protective effect while increased coffee intake is associated with the greater risk of RA [23,24]. Patients with RA were subjected to various dietary interventions to improve or lessen the burden of the disease. Studies done so far to find out the impact of the diet on disease activity and symptoms have shown variable results.

In a study by Proudman et al. [25], consumption of fish rich in omega 3 fatty acids, in combination with conventional Disease Modifying Anti –Rheumatic Drugs (DMARDS) is more likely to achieve remission than DMARDS alone. There are conflicting results with regard to protein intake. Pattison et al. [12] studied the intake of red meat and the occurrence of inflammatory polyarthritis and concluded that increased intake of red meat is associated with higher risk of developing inflammatory polyarthritis, whereas Nurses' Health Study [26] has given contradictory results.

In real life scenario, many patients with RA report that there is increase in their joint symptoms after taking a particular food and they adjust their dietary pattern based on their experience by restricting that particular diet in future and gets benefited by their own dietary manipulation. The question about the influence of diet on the occurrence of arthritis related symptoms remain

large for the patients. With this background, we intended to study the role of diet in RA patients and its impact on the arthritis related symptoms as perceived by the patients themselves using a simple questionnaire in their own language.

Aim

To study the influence of diet on the joint related symptoms in patients with Rheumatoid arthritis as perceived by them.

Methodology

Descriptive study was carried out among 101 consecutive Rheumatoid arthritis patients attending the out-patient services of Rheumatology Department during September 2012-March 2013 in a tertiary care centre, Coimbatore, Southern India. Patients aged more than 17 years of age with a diagnosis of RA as per American College of Rheumatology 2010 criteria and on standard DMARDS (Methotrexate 10 -25 mg/week; Hydroxychloroquine 200-400 mg/day; Leflunomide 10-20 MG/day; Prednisolone <7.5 mg/day) were included in the study however among them subjects were excluded if other Connective tissue diseases like Systemic lupus erythematosus, Fibromyalgia and Spondyloarthropathies, those who require frequent change in dose of DMARDS and not willing to participate in the study.

The study design was approved by the Institutional Ethics committee. After getting the consent, the participants were given a questionnaire in their local language which includes key details like disease duration, dietary pattern, history regarding co-morbidities and exposure to alternative medicine therapy; their regular diet patterns, whether they are restricting any particular food item; have they felt any change in the joint symptoms (new onset pain or stiffness, involvement of multiple joints) after a particular food intake and if so, they were asked to list down the name of food items which they think is responsible for the change in symptom.

Statistical analysis

Disease duration was calculated from the time of first symptom occurrence till the date of sampling and categorised as ≤ 6 months and >6 months. Diet restriction was categorised as diet restrictors and non-diet restrictors.

Descriptive statistics were reported using mean with standard deviation for continuous variables and percentages for categorical variables. Independent t test was used to compare the mean age between two groups. Chi-square test was used to test the association between diet restriction and disease duration with other study variables. P value less than 5% was considered as statistically significant. All the analyses were done using SPSS version 23.0 software.

Results

The characteristics of the study population are listed in Table 1. There were 85 female patients and 16 were male with ratio of 5.3:1. Mean age of the study subjects was 48.3 ± 11.6 years. The average disease duration of the study population was 50.2 ± 65.4 months. When we divided the patient into early with disease duration of ≤ 6 months and established RA with more than 6 months there were 80 with established disease while 21 had early disease.

Of the 101 patients 40(39.6%) had been exposed to complementary therapies in the past in the form of Ayurveda, Siddha and Homeopathy. 18 patients had associated co-morbid illness; most commonly reported was Systemic hypertension which was seen in 12 patients and 5 patients had diabetes while one had both Diabetes mellitus and Systemic hypertension. The diet pattern of the patient population is quite variable with 66 of them taking mixed diet (both vegetarian and non-vegetarian) and 35 were lacto-vegetarians.

There were a total of 44 patients (43.6%) who were following some kind of dietary restriction which was self-imposed in 5; by relatives and friends in 7 while 32 were advised by their primary care physicians itself. The remaining 57(56.4%) did mention that they did not follow any dietary restriction.

Of the total 101 patients, only 25 (24.8%) felt there was a change in the joint symptoms on taking particular diet, of this 20 were following diet restriction and the remaining 5 belonged to the non-diet restrictors group. The list of food items which worsened the joint symptoms is mentioned in the Table 2.

Disease duration and diet restriction

The mean disease duration in the early RA was 4.28 months and in established RA was 62.68 months. Although not statistically significant, the proportion of patients who had been exposed to alternative medicine was more in established RA (42.5%) than in early RA (28.6%).

In the early RA subgroup, seven patients (33.3%) had felt a need for diet restriction; the remaining fourteen (66.7%) did not have any restriction in the food intake. Eighteen among the early disease group (85.7%) did not observe any change in their joint symptoms after a particular food while three (14.3%) noticed worsening of their symptoms.

Table 1. *Characteristics of the study population.*

Characteristics	Number	Percentage
Age in years	48.3 ± 11.6	
Sex		
Female	85	84.2
Male	16	15.8
Duration of disease		
<= 6months	21	20.8
>6 months	80	79.2
Alternative medications		
Yes	40	39.6
No	61	60.4
Co morbid conditions		
Yes	18	17.8
No	83	82.2
Diet Restriction for co-morbid		
Yes	8	7.9
No	93	92.1
Diet restriction for Arthritis		
Yes	44	43.6
No	57	56.4
Any difference in arthritis		
Yes	25	24.8
No	76	75.2
Type of food		
Vegetarian	35	34.7
Mixed	66	65.3

Table 2. *Food items reported to worsen the joint symptoms.*

Chicken, Meat, Raw rice		
Bajji (snack item made of gram flour deep fried in oil)	Uzhundu vada (snack item made of Bengal gram flour deep fried in oil)	Lemon pickles
Potato	Pumpkin	Unripe banana
Curd	Milk	Egg

Table 3. *Comparison of characteristics by disease duration: Reported as number within parenthesis percentages; p value using chi-square test; * - p value using independent t test.*

	Disease Duration		
	≤ 6 months n-21	>6 months n-80	P value
Age in years	44.1 ± 10.6	49.4 ± 11.8	0.06*
Sex Female Male	 17 (81.0) 4 (19.0)	 68 (85.0) 12 (15.0)	0.65
Alternative Medications Yes No	 6 (28.6) 15 (71.4)	 34 (42.5) 46 (57.5)	0.24
Co morbid conditions Yes No	 5 (23.8) 16 (76.2)	 13 (16.3) 67 (83.8)	0.32
Any difference in arthritis Yes No	 3 (14.3) 18 (85.7)	 22 (27.5) 58 (72.5)	0.21
Diet restriction Yes No	 7 (33.3) 14 (66.7)	 37 (46.3) 43 (53.8)	0.28
Type of food Veg Mixed	 5 (23.8) 16 (76.2)	 30 (37.5) 50 (62.5)	 0.49

In established RA, thirty seven (46.3%) had diet restriction; while the remaining forty three (53.8%) did not. A majority of them (72.5%) did not feel any influence of diet on their joint symptoms while change in their symptoms in response to some dietary habits was observed in twenty two patients (27.5%). The results are summarised in Table 3.

Diet restrictors versus non restrictors

There were a total of 44 patients who restricted their diet while 57 of them have been not on any kind of diet restriction for arthritis.

Of the diet restrictors majority was female (81.8%); majority of them (84.1%) had established disease more than 6 months; 50% had been exposed to alternative medicine. The exposure to alternative form of medicine was slightly higher in diet restrictor as compared to non-restrictor groups (p=0.06). A majority (77.3%) of the diet restrictor group did not have any co-morbidities.

A significant association was observed between diet restriction and any difference/worsening of joint symptoms (p<0.0001). Twenty of the 44 patients who restricted their diet (45.5%) felt a difference in joint symptoms on exposure to particular diet while the remaining 24 patients (54.5%) did not feel so. 52 out of 57 (91.2%) non restrictors did not experience any influence of diet on their symptoms while 5 (8.8%) felt a change in symptoms even though they did not restrict any dietary foods in particular. The results are summarised in Table 4.

Discussion

In this study we analysed the influence of diet on joint symptoms in a group of patients by a simple questionnaire.

The mean age of our study population was 48.33 ± 11.74 years which is in comparable with the RA study population worldwide. Although our study did not target on established disease alone, we had more number of patients with established disease by consecutive picking. The mean disease duration in the study group was 50.22 ± 65.39 months which is important because this gives enough time for the patients to test various foods and to arrive at a conclusion regarding the influence of diet on their disease pattern.

The percentage of patients with associated co-morbid diseases like diabetes and hypertension requiring dietary restriction was less in our study population making this observation more relevant.

Almost all the alternative form of therapies mandates a specific dietary restriction. In our study, 50% of dietary restrictors had alternative therapy exposure previously; although this association is not statistically significant which indicates that alternative medicine exposure does not influence the dietary patterns for our study population.

Patients who observed any changes in the joint symptoms on exposure to a particular diet adapt dietary restriction and we observe this association as statistically significant in our study (p<0.0001). This emphasise the influence of diet on the disease activity as noted in other studies.20 out of the 44 and 52 out of 57 patients in either groups were rightly deciding what to eat and what not to eat.

When we searched for similar studies, we could find one such questionnaire based study [27] which assessed diet and disease symptoms in rheumatic diseases. The study included a total of 742 patients which consisted of 290 patients with RA; 51 with Juvenile Rheumatoid arthritis, 87 with Ankylosing spondylitis, 51 with psoriatic arthropathy, 65 with primary fibromyalgia and 34 with osteoarthritis. The median disease duration of RA patients in this study was 11 months (0-55) which is shorter than our present study (50.22 ± 65.39 months). One third of

Table 4. *Comparison of characteristics by diet restriction for arthritis: Reported as number within parenthesis percentages; p value using chi-square test; * - p value using independent t test.*

	Diet Restriction for Arthritis		
	Yes n-44	No n-57	P value
Age in years	49.9 ± 11.3	47.1 ± 11.9	0.22*
Sex Female male	 36 (81.8) 8 (18.2)	 49 (86.0) 8 (14.0)	0.57
Duration of disease ≤ 6 months >6 months	 7 (15.9) 37 (84.1)	 14 (24.6) 43 (75.4)	0.28
Alternative Medications Yes No	 22 (50.0) 22 (50.0)	 18 (31.6) 39 (68.4)	0.06
Co morbid conditions Yes No	 10 (22.7) 34 (77.3)	 8 (14.0) 49 (86.0)	0.26
Any difference in joint pains Yes No	 20 (45.5) 24 (54.5)	 5 (8.8) 52 (91.2)	 <0.0001
Type of food Veg Mixed	 19 (43.2) 25 (56.8)	 16 (28.0) 41 (72.0)	 0.15

Rheumatoid arthritis patients reported worsening of symptoms after a particular food intake which is comparable to our observation (one fourth). But there was not any definite answer for the same.

Understanding the association between diet and RA disease activity, numerous studies had been conducted in various parts of the world to ascertain whether dietary manipulation is rewarding in the management of RA patients in terms of improvement in joint pain, stiffness and physical function. Dietary intervention measures followed in various studies include fasting followed by vegetarian diet [14,28], vegan diet [16,29], elemental diet [30,31], Mediterranean diet [32,33], Elimination diet [34] and the results were compared with those put on ordinary diet but the results of these studies were variable.

Hagen et al. [35] did a systemic review of all these dietary intervention studies which included 14 randomised controlled trials, 1 case control study with a total of 837 patients and concluded that the effect of various forms of dietary manipulation on RA patients is uncertain. It was also noted that there was a high risk of dropout rates and weight loss in those subjected to dietary manipulation or restriction which can cause adverse potential effects.

Our study is one of its kinds which assessed the real life situation of patients with RA and their ever burning question of the influence of diet on the disease. It is easier to assess the role of drugs in the disease management and various trials can be formulated for the same while it is tough to assess the role of dietary factors and its influence on the disease course. Since RA as such has a variable course and each patient behaves in a different manner with periods of spontaneous remissions and exacerbations. Not all patients with RA are capable of sticking to a particular diet regimen. Most of the real life patients are in the lower to middle socio-economic class and following a special diet pattern will only add to the financial burden for their families.

We did make out from our study that the patients are the best judges of their symptoms and the influence of diet on their disease pattern. Our advice for the patients is to decide on their own basis whether or not to restrict a particular food item based on their symptoms instead of giving a dietary pattern for them.

So, till we have convincing data regarding the benefits of dietary manipulation in Rheumatoid arthritis patients, we encourage them to have a healthy, balanced diet with individual modifications in their diet pattern based on their own judgement regarding the influence of diet on their joint related symptoms.

Limitations of our Study

This is a cross-sectional study; we have not objectively assessed the influence of diet on the disease activity; instead our study was based on the assessment on arthritis related symptoms by patients themselves.

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We have not received any funds from any agencies or sponsors.

Competing and Conflicting Interest

The authors disclose no conflicting or competing interest.

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References

1. Silman A, MacGregor A, Thomson W, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol. 1993;32:903-7.
2. Heliovaara M, Aho K, Aromaa A, et al. Smoking and risk of rheumatoid arthritis. J Rheumatol. 1993;20:1830-5.
3. Voigt LF, Koepsell TD, Nelson JL, et al. Smoking, obesity, alcohol consumption, and the risk of rheumatoid arthritis. Epidemiology. 1994;5:525-32.
4. Symmons DP, Bankhead CR, Harrison BJ, et al. Blood transfusion, smoking, and obesity as risk factors for the development of rheumatoid arthritis: results from a primary care–based incident case–control study in Norfolk, England. Arthritis Rheum. 1997;40:1955-61.
5. Hutchinson D, Shepstone L, Moots R, et al. Heavy cigarette smoking is strongly associated with rheumatoid arthritis (RA), particularly in patients without a family history of RA. Ann Rheum Dis. 2001;60:223-7.
6. Choi HK: Dietary risk factors for rheumatic diseases. Curr Opin Rheumatol. 2005;17:141-6.
7. Beri D, Malaviya AN, Shandilya R, et al. Effect of dietary restrictions on disease activity in rheumatoid arthritis. An Rheum Dis. 1988;47:69-72.
8. Vaahtovuo J, Munukka E, Korkeamäki M, et al. Fecal microbiota in early rheumatoid arthritis. J Rheumatol. 2008;35:1500-5.
9. Toivanen P. Normal intestinal micro biota in the etiopathogenesis of rheumatoid arthritis. Ann Rheum Dis. 2003;62:807-11.
10. Scher JU, Sczesnak A, Longman RS, et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife. 2013;2:e01202.
11. Buchanan WW, Laurent RM. Rheumatoid arthritis: an example of ecological succession? Can Bull Med Hist. 1990;7:77-91.
12. Pattison DJ, Symmons DP, Lunt M, et al. Dietary risk factors for the development of inflammatory polyarthritis: evidence for a role of high level of red meat consumption. Arthritis Rheum. 2004;50:3804-12.
13. Skoldstam L. Fasting and vegan diet in rheumatoid arthritis. Scand J Rheumatol. 1986;15:219-21.
14. Kjeldsen-Kragh J, Haugen M, Borchgrevink CF, et al. Controlled trial of fasting and one year vegetarian diet in rheumatoid arthritis. Lancet. 1991;338:899-902.
15. Kjeldsen-Kragh J, Haugen M, Forre O, et al. Vegetarian diet for patients with rheumatoid arthritis: can the clinical effects be explained by the psychological characteristics of the patients? Br J Rheumatol. 1994;33:569-75.

16. Nenonen MT, Helve TA, Rauma AL, et al. Uncooked, lactobacilli-rich, vegan food and rheumatoid arthritis. Br J Rheumatol. 1998;37:274-81.
17. Grant WB. The role of meat in the expression of rheumatoid arthritis. Br J Nutr. 2000;84:589-95.
18. Linos A, Kaklamanis E, Kontomerkos A, et al. The effect of olive oil and fish consumption on rheumatoid arthritis: a case control study. Scand J Rheumatol. 1991;20:419-26.
19. Shapiro JA, Koepsell TD, Voigt LF, et al. Diet and rheumatoid arthritis in women: a possible protective effect of fish consumption. Epidemiology. 1996;7:256-63.
20. Linos A, Kaklamani VG, Kaklamani E, et al. Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables? Am J Clin Nutr. 1999;70:1077-82.
21. Cerhan JR, Saag KG, Merlino LA, et al. Mikuls TR, Criswell LA. Antioxidant micronutrients and risk of rheumatoid arthritis in a cohort of older women. Am J Epidemiol. 2003;157:345-54.
22. Bendich A, Olson JA. Biological actions of carotenoids. FASEB J. 1989;3:1927-32.
23. Heliovaara M, Aho K, Knekt P, et al. Coffee consumption, rheumatoid factor, and the risk of rheumatoid arthritis. Ann Rheum Dis. 2000;59:631-5.
24. Mikuls TR, Cerhan JR, Criswell LA, et al. Coffee, tea, and caffeine consumption and risk of rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheum. 2002;46:83-91.
25. Proudman SM, James MJ, Spargo LD, et al. Fish oil in recent onset rheumatoid arthritis: a randomised, double-blind controlled trial within algorithm-based drug use. Ann Rheum Dis. 2015;74:89-95.
26. Benito-Garcia E, Feskanich D, Hu FB, et al. Protein, iron, and meat consumption and risk for rheumatoid arthritis: a prospective cohort study. Arthritis Res Ther. 2007;9:R16.
27. Haugen M, Kjeldsen-Kragh J, Nordvåg BY, et al. Diet and disease symptoms in rheumatic diseases-results of a questionnaire based survey. Clin Rheumatol. 1991;10:401-7.
28. Sköldstam L, Larsson L, Lindström FD. Effects of fasting and lactovegetarian diet on rheumatoid arthritis. Scandinavian J Rheumatol. 1979;8:249-55.
29. Hafström I, Ringertz B, Spångberg A, et al. A vegan diet free of gluten improves the signs and symptoms of rheumatoid arthritis: the effects on arthritis correlate with a reduction in antibodies to food antigens. Rheumatology. 2001;40:1175-9.
30. Holst-Jensen SE, Pfeiffer-Jensen M, Monsrud M, et al. Treatment of rheumatoid arthritis with a peptide diet. A randomized, controlled trial. Scandinavian J Rheumatol. 1998;27:329-36.
31. Kavanagh R, Workman E, Nash P, et al. The effects of elemental diet and subsequent food reintroduction on rheumatoid arthritis. British J Rheumatology. 1995;34:270-3.
32. McKellar G, Morrison E, McEntegart A, et al. A pilot study of a Mediterranean type diet intervention in female patients with rheumatoid arthritis living in areas of social deprivation in Glasgow. Annals of the Rheumatic Diseases. 2007;66:139-43.
33. Sköldstam L, Hagfors L, Johansson G. An experimental study of Medieterranean diet intervention for patients with rheumatoid arthritis. Annals of the Rheumatic Diseases. 2003;62:208-14.
34. Darlington LG, Ramsey NW, Mansfield JR. Placebo controlled, blind study of dietary manipulation therapy in rheumatoid arthritis. Lancet. 1986; February:236-8.
35. Hagen KB, Byfuglien MG, Falzon L, et al. Dietary interventions for rheumatoid arthritis. Cochrane Database Syst Rev. 2009;1:CD006400.

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Original Research Article

ANA immunofluorescence versus profile-how well they perform in autoimmune diseases: an analysis of their clinical utility in a tertiary care centre

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ABSTRACT

Background: While Immunofluorescence assay remains the gold standard for the detection of ANA, Immunoprofile by ELISA is being increasingly utilized in view of easy availability and quick results. The study was done to find out whether ANA profile results are comparable with IFA.
Methods: About 100 patients who had undergone both immunofluorescence and Immunoprofile were included. Immunofluorescence correlation with profile and their correlation with the disease were analyzed; sensitivity, specificity and predictive values were calculated.
Results: ANA was positive in 78% by immunofluorescence; 73% by ANA profile. 22 patients in whom ANA IFA was negative were picked up by ANA profile. 27 patients who were not detected by ANA profile were tested positive by IFA. ANA testing by immuno profile had a sensitivity of 65% with a positive predictive value of 69% when compared with IFA. Immunofluorescence pattern and ANA profile correlated with the diagnosed disease in 63% and 49% respectively. Immunofluorescence pattern correlated with the ANA profile in only 35% of the study subjects. On correlation with the disease, ANA profile scored less compared to ANA-IFA with a sensitivity and specificity of 46% each; positive predictive value of 59%; negative predictive value of 33%. On analysis of individual disease, ANA profile is as good as IFA in SLE and scleroderma in terms of sensitivity. In Sjogren's syndrome and MCTD, specificity and positive predictive value of ANA profile is high.
Conclusions: ANA IFA performs better than immunoprofile in the diagnosis of autoimmune diseases.

Keywords: Antinuclear antibody, ANA immunoprofile, Immunofluorescence, Immunofluorescence versus profile

INTRODUCTION

Connective tissue diseases (CTD) are a group of autoimmune disorders characterized by the presence of antinuclear antibodies (ANA). The detection of these auto antibodies in the sera of the affected persons is very much essential to diagnose these diseases. The history of ANA testing dates back to 1948 with the observation of LE cell by Hargraves et al.[1] in the bone marrow of Systemic Lupus Erythematosus (SLE) patients. Although they are called as anti-nuclear antibodies, these antibodies exhibit reactivity not only against nuclei but also against various other cellular structures like cell surface, nucleoli and cytoplasm.[2] Despite its varied sensitivity in various

connective tissue diseases and false positivity results in numerous conditions, ANA is still widely used as a screening test for connective tissue disorders.

The two broad categories of ANA include a) auto antibodies against double stranded DNA (ds -DNA) seen in SLE and against histones seen in drug induced lupus. 3, 4 b) auto antibodies to extractable nuclear antigens (ENA) like antibody to Smith (Sm) antigen which is specific for SLE; other subtypes of ENAs include antibodies against ribonucleoproteins (RNP), SSA/Ro, SSB/La, Scl-70, Jo-1 and PM1.5 The sensitivity and specificity of ANA and its clinically important specific antigen subtypes is listed in Table 1.[6,7]

Table 1: The sensitivity and specificity of ANA and its clinically important antigenic subtypes.

ANA	**SLE**	**93**	**57**
	Sjogren's syndrome	48	52
	Systemic sclerosis	85	54
	Polymyositis/ dermatomyositis	61	63
	MCTD		
Antigen subtypes			
Anti - dsDNA	SLE	57	97
Anti - Sm	SLE	25-30	High
Anti -SS A/Ro	Sjogren's syndrome, Subacute cutaneous SLE, Neonatal lupus syndrome	8-70	87
Anti-SSB/La	Sjogren's syndrome, Subacute cutaneous SLE, Neonatal lupus syndrome	16-40	94
Anti-U3-RNP	Systemic sclerosis	12	96
Anti-centromere	Limited cutaneous systemic sclerosis	65	99.9
Scl-70	Systemic sclerosis	20	100
Jo-1	Polymyositis	30	95

The most commonly used laboratory tests to detect anti-nuclear antibodies include immunofluorescence (IF) assay and enzyme linked immunosorbent assay (ELISA). In immunofluorescence assay (IFA), the presence of a specific staining pattern indicates the presence of certain auto antibodies which in turn is associated with certain clinical states.[8-10] In antigen specific ELISA assays, we can detect the presence of various auto antibodies directed against specific antigens (ANA profile). Also, it is simple to perform, automated and does not require skilled personnel to interpret the results unlike IF assays.

The interpretation of ANA by immunofluorescence assay which is the gold standard is limited by the cost, requirement of skills and proper handling of the specimen. The detection of ANA by IFA does not always yield the specific clinical diagnosis; most often specific antibody assay is required to diagnose the clinical syndrome. Also, the borderline ANA-IF staining (like 1+ positive; weak positive results) creates confusion which demands further diagnostic testing. It is noteworthy to understand that while certain diseases like MCTD can be diagnosed by the presence of a specific antibody anti - U1RNP, other autoimmune diseases have overlapping ANA IFA patterns and antibody profile. Detection of ANA by both immunofluorescence and ANA profile increases the cost and the time interval for diagnosis as each laboratory has its own turnaround times. Simply doing an ANA profile directly might cut short the time but sometimes it may not detect the antibodies when that specific antigen is not included in the kit in which case the diagnosis and thereby the treatment of the disease might get delayed. Each test has its own advantages and disadvantages. With this background, we intended to correlate the ANA results of 100 patients with autoimmune connective diseases who had simultaneously underwent ANA testing by both immunofluorescence (IF) and Immunoprofile (ANA profile) methods. These results were finally correlated with the disease diagnosed.

The aim of the study is to analyze the correlation between a) ANA- IFA pattern and ANA profile sub typing; b) ANA- IFA pattern and the disease diagnosed and c) ANA profile sub typing, and the disease diagnosed.

METHODS

The present study was a laboratory based observational study conducted in the adult patients who attended the Rheumatology OPD in a tertiary care centre in South India from August 2014 to June 2015. A total of 100 patients were included in the study.

Inclusion criteria

- Adults greater than 17 years and less than 60 years of age
- Who had underwent both ANA IFA and ANA profile tests.

Exclusion criteria

- Who had viral infections in the recent past
- Those on medications known to cause ANA positivity
- Those previously diagnosed to have rheumatic diseases
- Those above 60 years of age.

Thorough history and general physical examination were done for all the patients and diagnosis was made based on American College of Rheumatology (ACR) criteria.

Five milliliters venous blood was collected in a clean Wassermann tube, serum was separated from the clotted blood samples by centrifugation as per standard protocol. The serum was stored at -20°C until the time of assay. Determination of ANA was done by indirect

immunofluorescent techniques using HEp-2 Cell Line Substrate as per the standard recommendation; a titer of ≥1:100 was taken as positive for IF. ANA profile was done by using IMTEC-ANA-LIA MAXX for detecting antibodies against ds DNA, nucleosomes, histones, Sm D1, PCNA, SSA/Ro 60, SS A/Ro 52, SSB/La, CENP - B, Scl 70, U1sn RNP, AMA, M2, Jo 1, PM-Scl, Mi 2 and Ku as per the kit manual.

Table 2: Clinical significance of common Immunofluorescence patterns.

ANA –IF pattern	Antigen	Associated disease
Speckled	ENA, RNP, Sm, SSA/Ro, SSB/La, Scl-70, Jo-1, ribosomal-P	SLE, Mixed CTD, SS, Primary Sjogren's syndrome, PM
Homogenous	dsDNA, Histones	SLE, Drug induced SLE
Peripheral (rim)	RNP, Sm, SSA/Ro	SLE, SS
Nucleolar	Anti-PM-Scl, anti-RNA polymerase I-III, anti-U3-RNP, To RNP	SS, PM (polymyositis scleroderma)
Centromere	CENP A-E	Limited SS

The detection of ANA by profile method was compared with the standard IF assay. Also, the results of these two diagnostic assays (IFA and ANA profile) were subjected to correlation with the disease diagnosed. For example, when those patients diagnosed with SLE have homogenous pattern detected by IF method; double stranded DNA (dsDNA); histone antibodies in the ANA immunoprofile method, it is considered to be co-relating. The clinical significance of common ANA -IFA patterns and antibodies in ANA profile is listed in Table 2.

Statistical analysis

Descriptive statistics were reported using mean and standard deviation for the continuous variables, number and percentages for the categorical variables. Cross tabulation was done between ANA IFA (Gold standard) and ANA profile (ELISA). The sensitivity, specificity, positive and negative predictive values (PPV) and (NPV) were calculated. These values were calculated for both the ANA indices analysis.

RESULTS

Authors analyzed the ANA test results detected by both immunofluorescence and ANA profile method of 100 patients in whom diagnosis of rheumatic disease was made based on clinical and immunological criteria. Sjogren's syndrome and Mixed Connective tissue Disease (MCTD) accounted for two-thirds of the study population; the distribution of various diseases in the study population is listed in Table 3. There were 72 females and 28 males with the mean age of 34.23±5.8 years. The female preponderance was noted in the disease subsets as well.

Table 3: Distribution of various rheumatic diseases in the study population.

Disease	Total	Female	Male
Systemic Lupus Erythematosus (SLE)	20	15	5
Sjogren's syndrome (SS)	32	21	11
Scleroderma	10	7	3
Mixed Connective tissue Disease (MCTD)	30	22	8
Myositis	4	3	1
CTD associated ILD (CTD – ILD)	4	4	0

ANA was positive in 78% of our subjects by immunofluorescence method and in 73% by ANA profile. The IF pattern observed were homogenous in 11; speckled in 48; nucleolar in 17; centromere and cytoplasmic pattern one in each. 51 patients (51%) were found to be positive by both IFA and immuno-profile methods. 22 patients in whom ANA IFA was negative were picked up by ANA profile. Also 27 patients who were not detected by ANA profile were tested positive by IFA method. We found ANA testing by immunoprofile has a sensitivity of 65% with a positive predictive value of 69% when compared to standard IFA method. The results were tabulated in Table 4. Specificity and negative predictive value could not be calculated since all our study subjects had established rheumatic autoimmune disease.

Table 4: Comparison of ANA IFA and ANA profile results.

	ANA IFA	
	Positive	**Negative**
ANA profile	51	22
	27	0
	78	22

Correlation of ANA results with the disease

In 63% of the subjects, the pattern observed in the immunofluorescence correlated with the diagnosed disease; whereas ANA profile correlated with the disease in 49%.

Both the tests correlated with the disease in 29 patients. On correlating IFA pattern with ANA profile, we found that IFA pattern correlated with the observed antibodies in ANA profile in only 35% of the study subjects.

On cross tabulation of correlation with the disease, we found that ANA profile scored less compared to ANA-IFA with a sensitivity and specificity of 46% each; positive predictive value of 59% and negative predictive value of 33% (Table 5).

Table 5: ANA profile vs. IFA correlation with the disease.

	Immunofluorescence pattern correlation with the disease	
ANA profile correlation with the disease	Present	Absent
	29	20
	34	17
	63	37

Sub analysis based on individual disease

On comparison of ANA results with respect to individual disease, we found that in SLE, ANA profile is as good as Immunofluorescence assay in picking up the disease with a sensitivity of 90% although positive predictive value (PPV) was 47%. Out of 10 patients in whom ANA-IFA was negative, 9 were picked up by ANA profile. We noted a high PPV of 82% in Sjogren's syndrome with a sensitivity of 64% when compared with IFA method. All the scleroderma patients were picked up by ANA profile while IFA method fails to detect the disease in one. The performance of ANA profile in other diseases is less compared to standard IF method. The results are tabulated in Table 6.

Table 6: Comparison of ANA immunofluorescence assay (IFA) and ANA profile results in disease subsets.

		ANA IFA					
		Positive	**Negative**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
SLE	ANA profile Positive	9	10	-	-	-	0
	Negative	1	0	90%	0	47%	0
Sjoren's syndrome	Positive	18	4	-	-	-	-
	Negative	10	0	64%	0	82%	0
Scleroderma	Positive	9	1	-	-	-	-
Myositis	Positive	1	2	-	-	-	-
	Negative	1	0	50%	0	33%	0
MCTD	Positive	13	4	-	-	-	-
	Negative	13	0	50%	0	76%	0
CTD	Positive	1	1	-	0	50%	0
	Negative	2	0	33%	-	-	-

PPV - positive predictive value; NPV- Negative Predictive value

Table 7: ANA immunofluorescence assay (IFA) and ANA profile correlation with the disease.

		ANA IFA correlation with disease					
		Present	**Absent**	**sensitivity**	**Specificity**	**PPV**	**NPV**
SLE	Present	8	10	80%	0	44%	0
	Absent	2	0	-	-	-	-
Sjoren's syndrome	Present	8	2	44%	86%	80%	55%
	Absent	10	12	-	-	-	-
Scleroderma	Present	5	3	100%	40%	62%	100%
	Absent	0	2	-	-	-	-
Myositis	Present	1	2	50%	0	33%	0
	Absent	1	0	-	-	-	-
MCTD	Present	6	2	24%	60%	75%	13%
	Absent	19	3	-	-	-	-
CTD	Present	1	1	33%	0	50%	0
	Absent	2	0	-	-	-	-

ANA profile correlation with the disease

PPV - positive predictive value; NPV- Negative Predictive value

Correlation with the disease

In SLE, ANA profile correlated well with the disease with a sensitivity of 80% and PPV of 44%. In Sjogren's syndrome, authors found that ANA profile scored well in ruling in the disease with a high specificity of 86% and NPV of 55%; also, its sensitivity is 44% with a very good PPV 80%. With respect to Scleroderma, ANA profile

performed well with 100% sensitivity and NPV; although specificity is 40%. Similarly, although sensitivity is low in MCTD, in 75% of the cases ANA profile is correct in predicting the disease. The results are tabulated in Table 7.

DISCUSSION

Very few investigations in the field of Medicine have such an important role in the diagnosis of diseases like the detection of anti-nuclear antibodies (ANA) for autoimmune diseases. Majority of referrals to a tertiary care centre for positive results following unnecessary ANA testing are often based on ELISA results rather than IFA; studies have revealed that majority of them are false positives.[11] Each method has its own advantages and disadvantages.

The present study analyzed the correlation between ANA results detected by IFA and ANA profile in 100 patients with autoimmune diseases.

There are many studies which had compared ANA results detected by IFA vs. ELISA in patients with suspected autoimmune disease.[12-14] But there were only few studies which had assessed the results in patients with established autoimmune disease.[15-17] The present study is one of its kind which had utilized both the tests (IFA and ANA profile) for establishing the diagnosis and then compared the performance of ANA profile with IFA pattern; correlated both the test results with the disease diagnosed.

Female preponderance is often observed in autoimmune diseases; the present study observed a female preponderance of 72% similar to that noted in other studies.[18-20]

ANA IFA was found positive in 78 patients (78%); of these ANA - IFA positives, 51 were found to be positive by ANA profile as well. The present study findings were similar to that observed by Latha M et al, who had reported that ANA IFA was positive in 71.3% out of 279 samples; 159(56.9%) were both ANA IF and Line Immuno assay (LIA) positive.[18] In another study conducted in East India, among 394 samples tested for both ANA IFA and ANA profile, 138 were ANA IFA positive (35.02%) in 1 in 40 dilution; of which 114 (82.6%) were also line immune assay positive.[19]

Positive ANA IFA with negative ANA profile is noted in 27% cases. This may be attributed to the fact that some rare auto antibodies may be missed by ANA profile if the kit fails to incorporate that specific antigenic substrate, which could be detected by IF staining. Review of the ANA pattern found in these 27 cases had shown speckled in 13; nucleolar in 12, homogenous and cytoplasmic pattern in 1 each. Similar observation was noted by Baronaite et al, who had studied the ANA results in 400 patients using IFA and Elisa immuno assay (EIA) techniques and found that the EIA method was less reliable for assessing nuclear and speckled reactivity patterns.[13]

22 patients who were ANA IFA negative were picked up by ANA profile in the present study. Almost half of them had dsDNA antibodies (12 patients); Ro 52 and Scl 70 antibodies in 3; Sm antibodies in 3; nRNP/Sm+RibP in 3; PCNA/ histone antibodies in 1. The negative immunofluorescence could be because SSA and dsDNA antibodies were not detected by ANA -IFA method. The negative immunofluorescence may be due to the low concentration of auto antigens or its destruction during the preparation process.[21]

Surprisingly SLE, which is largely diagnosed by ANA positivity, would have been missed in 50 percent of the cases if only IFA assay is done. This showcases few interesting thoughts, where in the past, ANA negative lupus had been diagnosed based on simple assays, when this advanced ANA profile was not available. This was also noted in other studies, where significant number of IFA assays missed reactivity to dsDNA which was picked by the sub typing analysis.[13,18]

Similar to present study observation, there are various studies, which have shown inconsistencies between ANA IFA and ANA profile results. Latha M et al, noted that 40 ANA IFA positive samples (20.1%) were negative by ANA profile. Of the 80 ANA-IFA negative samples, 14 (17.5%) were detected to be positive by IF method.[18] In one study, positive result by IFA was noted in 17.3 % cases although they were negative for line immunoassay; The pattern observed in that study was mostly homogenous (66%) while in present study it was speckled followed by nucleolar pattern. In the same study, 14.8% of the ANA negative samples were tested positive by line immunoassay.[19] Sebastian et al, noted that 17.5% of ANA IFA positive samples were not detected by LIA method while 13.5% which were negative by IF method were found positive by LIA.[22]

This observation stresses the importance of further investigation with immunoprofile although IFA is negative if the clinical features are strongly suggestive in a given clinical setting. So, it is noteworthy to understand that there are certain auto antibodies which may escape staining by IFA method while certain auto antibodies present in the patient's serum may not be detected by ANA profile if it does not contain that specific antigenic substrate. Hence ANA -IFA is considered to be the gold standard screening test while ELISA profile is taken as the confirmatory test.[23]

The results of various studies which had compared ANA -IFA with ANA profile are inconsistent with regards to sensitivity, specificity and predictive values.[24-26] Some studies have shown moderate agreement between Enzyme immune assay and IFA.[15,24,26] While some have found that IFA is better others have shown that ELISA is superior.[20,22,27,28]

In the present study, we found that ANA IFA pattern co-related with the disease in 63%; ANA profile with the disease only in 49%; On cross tabulation, we observed that ANA testing by immunoprofile has a sensitivity of 65% with a positive predictive value of 69% when compared to standard IFA. Present study observation was similar to that noted by Priyadarshini et al, who compared ANA profile with standard IFA and reported ELISA has a sensitivity of 71.43% and specificity of 86.84% when compared with IFA12. There are studies which had reported that ELISA with improved profile is as good as IFA.

In a study by Kolahi et al, the sensitivity and specificity of immunoblot assay (ANA profile) in comparison with IF assay was 98.65% and 90.91% respectively.[17] Another study which had compared ANA detection by ELISA kit with IFA had reported that ELISA showed a reasonably good sensitivity (90.7%) and positive predictive value (89.1%) when compared to IFA method.[28] Raman et al, reported that sensitivity of LIA when compared to IFA was 89% and specificity was 42%.[19]

The present study also observed that IFA correlated well with the disease than ANA profile. Compared to standard IFA, the sensitivity and specificity of ANA profile in correlating with the disease was 46% each; positive predictive value of 59% and negative predictive value of 33%.

The present study is unique in that we assessed the results of ANA profile and IFA with respect to individual disease. We found that in SLE, ANA profile is as good as Immunofluorescence assay in picking up the disease with a sensitivity of 90%. Authors noted a high PPV of 82% in Sjogren's syndrome; 76% in MCTD by ANA profile when compared with IF method. All the scleroderma patients were picked up by ANA profile while IFA fails to detect the disease in one.

This observation tells that ANA profile is a very useful test in SLE and scleroderma. When the specific antibodies are positive in Sjogren's syndrome and MCTD, it is very likely that person is suffering from that specific autoimmune disease.

On correlation of ANA profile and IFA with the disease, we found that in SLE, ANA profile correlated well with the disease with a sensitivity of 80% and PPV of 44%. In Sjogren's syndrome, we found that ANA profile scored well in ruling in the disease with a high specificity of 86% and a very good PPV 80%. Authors also found a very good correlation of ANA profile with the disease in Scleroderma, with 100% sensitivity and NPV; Similarly, in MCTD, in 75% of the cases ANA profile is correct in predicting the disease.

Authors had used a mixed population of autoimmune diseases with small numbers which might again differ when used in larger sample.

CONCLUSION

ANA profile correlation with the disease is less when compared to Immunofluorescence assay. Also, ANA profile has a very low sensitivity and specificity when compared to IFA. ANA detection by immunofluorescence remains the investigation of choice when compared to ANA profile in the diagnosis of autoimmune disorders. In certain diseases like SLE and Scleroderma, ANA profile is as good as IF assay in picking up the disease and correlation with the disease.

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REFERENCES

1. Hargraves MM, Richmond H, Morton R. Presentation of two bone marrow elements: the 'tart' cell and the LE cell. Proceedings of the Mayo Clinic. 1948;23:25-8.
2. Satoh M, Chan EKL, Sobel ES, Kimpel DL, Yamasaki Y, Narain S, et al. Clinical implication of autoantibodies in patients with systemic rheumatic diseases. Exp Rev Clin Immunol. 2007;3:721-38.
3. Monestier M, Kotzin BL. Antibodies to histones in systemic lupus erythematosus and drug-induced lupus syndromes. Rheum Dis Clin North Am. 1992;18:415-36.
4. Fishbein E, Alarcon-Segovia D, Vega JM. Antibodies to histones in systemic lupus erythematosus. Clin Exp Immunol. 1979 Apr;36(1):145.
5. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagnostic Pathology. 2009 Dec;4(1):1.
6. Colglazier CL, Sutej PG. Laboratory testing in the rheumatic diseases: a practical review. South Med J. 2005 Feb 1;98(2):185-91.
7. Habash-Bseiso DE, Steven HY, Glurich I, Goldberg JW. Serologic testing in connective tissue diseases. Clin Med Res. 2005;3:190-3.
8. Nakamura RM, Peebles CL, Rubin RL, Molden DP, Tan EM. Autoantibodies to nuclear antigens (ANA), 2nd ed. American Society of Clinical Pathologists Press, Chicago, Ill. 1985.
9. White RH, Robbins DL. Clinical significance and interpretation of antinuclear antibodies. West J Med. 1987 Aug;147(2):210-13.
10. Zweiman, B, Lisak RP. Autoantibodies: autoimmunity and immune complexes, p. 885–911. In J. B. Henry (ed.), Clinical and diagnosis management by laboratory methods, 18th ed. W. B. Saunders Company, Philadelphia, Pa. 1991.
11. Abeles AM, Abeles M. The clinical utility of a positive antinuclear antibody test result. Am J Med. 2013 Apr;126(4):342-8.

12. Priyadarshini S, Meenakshisundaram J, Pothini J. A Comparative study of enzyme linked immunosorbent assay (ELISA) with immunofluorescence assay (IFA) for the detection of antinuclear antibodies. Int J Pharm Bio Sci. 2013 Oct;4(4):(B)75-80.
13. Baronaite R, Engelhart M, Mørk Hansen T, Thamsborg G, Slott Jensen H, Stender S, et al. A comparison of anti-nuclear antibody quantification using automated enzyme immunoassays and immunofluorescence assays. Autoimmune Dis. 2014:534759.
14. Angel J, Thomas M, Appalaraju B. Evaluation of ELISA and indirect immunofluorescence in the diagnosis of autoimmune diseases and their interpretation in the clinical situation. J Acad Clin Microbiol. 2015 Jan 1;17(1):7.
15. Gniewek RA, Stites DP, Thomas MM, Joan FH, Mayumi Nakagawa. Comparison of antinuclear antibody testing methods: immunofluorescence assay versus enzyme immunoassay. Clin Diag Lab Immunol. 1997;4(2):185-8.
16. Minz RW, Kumar Y, Saikia B, Anand S, Varma S, Singh S. Use of panel testing for detection of antinuclear antibody in a resource-limited setting: an appraisal. Postgrad Med. 2016 Nov;128(8):869-74.
17. Kolahi S, Bonyadi MR, Safarzadeh E, Ghojazadeh M, Pishahang E. Diagnostic value of Immunoblotting assay for Determination of anti-nuclear antibodies in rheumatic diseases. Adv Biores. 2017 Jul 1;8(4):153-8.
18. Madhavi Latha B, Anil Kumar B. Detection of antinuclear antibodies by indirect immunofluorescence method and its comparison with line immunoassay in a tertiary care hospital: A laboratory based observational study. J Med Sci Res. 2014;2(4):194-9.
19. Raman S, Adhya AK, Pradhan P, Dash K, Senapati U. Correlation of indirect immunofluorescence and line immunoassay method in detection of autoimmune diseases: an observational study at a tertiary care teaching hospital. Sch J App Med Sci. 2017;5(7A):2520-6.
20. Hayashi N, Kawamoto T, Mukai M, Morinobu A, Koshiba M, Kondo S, et al. Detection of antinuclear antibodies by use of an enzyme immunoassay with nuclear HEp-2 cell extract and recombinant antigens: comparison with immunofluorescence assay in 307 patients. Clin Chem. 2001 Sep 1;47(9):1649-59.
21. Tanaka N, Muro Y. Anti-SSA/Ro antibody determination by indirect immunofluorescence and comparison of different methods of antinuclear antibody screening. Mod Rheumtol. 2008;18(6):585-92.
22. Sebastian W, Roy A, Kini U, Mullick S. Correlation of antinuclear antibody immunofluorescence patterns with immune profile using line immunoassay in Indian scenario. Indian J Pathol Microbiol. 2010;53(3):427-32.
23. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis. 2010;69(8):1420-2.
24. James K1, Meek G. Evaluation of commercial enzyme immunoassays compared to immunofluorescence and double diffusion for autoantibodies associated with autoimmune diseases. Am J Clin Pathol. 1992 Apr;97(4):559-65.
25. González C, Martin T, Arroyo T, García-Isidoro M, Navajo JA, González-Buitrago JM. Comparison and variation of different methodologies for the detection of autoantibodies to nuclear antigens (ANA). J Clin Lab Anal. 1997;11:388-92.
26. Homburger HA1, Cahen YD, Griffiths J, Jacob GL. Detection of antinuclear antibodies: comparative evaluation of enzyme immunoassay and indirect immunofluorescence methods. Arch Pathol Lab Med. 1998 Nov;122(11):993-9.
27. Nossent H1, Rekvig OP. Antinuclear antibody screening in this new millennium: farewell to the microscope? Scand J Rheumatol. 2001;30(3):123-6.
28. Divate S1, Hardikar P, Bichile LS, Rajadhyaksha A. Clinical utility of screening for antinuclear antibodies by enzyme immunoassay-a preliminary study. J Assoc Physicians India. 2004 Apr;52:290-3.

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Urinary Tract Infection in Diabetics - A Five Year Retrospective Study on the Prevalence of Bacterial Isolates and its Antibiotic Susceptibility Patterns in A Tertiary Care Hospital in South India

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ABSTRACT

Introduction: Urinary tract infection (UTI) is almost most common seen infection in the diabetic patient due to various local and systemic immunopathogenetic reasons. This causes multiple renal and extrarenal complications and even death if not intervened early. We aimed to find out the prevalence of different bacteria in urine culture and the antibiotic susceptibility pattern amongst diabetic patients admitted in our hospital and to start empirical antibiotics for early recovery and to prevent complications.

Material and Methods: Retrospectively, 651 culture positive urine samples were taken for study with inclusion and exclusion criteria. We saw all the case records and investigations of every subject to correlate with criteria of selection. Our laboratory follows the standard protocols for urine sampling, timely transportation and microbiological study. Antibiotic susceptibly was tested by Kirby Bauer method and interpretations were done by clinical laboratory and standards institute (CLSI) guidelines.

Results: Out of total 651 patients most commonly affected age group was 51-60 years (30.26%).Here males (53.3%) dominated females (46.7%) in number with little difference. Most common Gram-negative bacteria were Escherichia coli (69.12%) and Klebsiella pneumoniae (8.60%) while most common Gram-positive bacteria were Enterococcus faecalis (10.90%) and Staphylococcus aureus (2.91%).Most of the gram-negative bacteria were having a good susceptibility to amikacin, piperacillin/tazobactam, cefoperazone/sulbactam and carbapenems while Gram-positive bacteria were mostly sensitive to nitrofurantoin, linezolid and vancomycin.

Conclusion: in our study, the occurrence of UTI among the diabetic is commoner in the sixth decade with a male predominance. We found E.coli, E.faecalis, and K.pneumoniae as the most commonly grown organisms in descending order. Most of the Gram-negative isolates were susceptible to amikacin, piperacillin/tazobactam, cefoperazone/sulbactam and carbapenems, while most of the Gram-positive cocci were sensitive to nitrofurantoin, linezolid, and vancomycin. Antibiotic resistance is seen very commonly in our setup. We cannot depend upon frequently used oral antibiotics and certain groups like fluoroquinolones and cephalosporin (except for sulbactam containing ones) and ampicillin for empirical treatment in UTI especially the complicated ones.

Keywords: Urinary Tract Infection, Diabetics, Prevalence, Isolates, Antibiotic Susceptibility

INTRODUCTION

The urinary tract is a principal site of infection in diabetics.[1] UTI (Urinary Tract Infection) encompasses a variety of clinical entities, including ABU (asymptomatic bacteriuria), cystitis, prostatitis, and pyelonephritis. There can be an increased risk of ABU or symptomatic UTI in diabetics.[2]

Urinary tract infection can be uncomplicated or complicated. Uncomplicated UTI can be asymptomatic bacteriuria, cystitis, pyelonephritis, in men and nonpregnant women, also prostatitis in men. Complicated UTI can be symptomatic episodes of cystitis or pyelonephritis in men or women with an anatomic predisposition to infection, with a foreign body in the urinary tract, or factors predisposing to a delayed response to therapy. There can be a relapse or reinfection. A relapse is a recurrent infection with an organism similar to the pretherapy isolate, usually following persistence of the organism in the genitourinary tract. A reinfection is a recurrent infection with a new organism. The quantitative criteria of at least 10^5 CFU (colony forming units)/mL are generally appropriate for the microbiological identification of complicated urinary infection. Bacteriuria is more common in diabetics than in non-diabetics due to a combination of host and local risk factors.[3] Some microorganisms become more virulent in a high glucose environment. Therefore, screening for UTI in diabetic patients is very important to enable bacteruria to be properly treated.[4] There is a significant correlation between duration of diabetes and UTI.[5] Changes in host defense mechanisms, the presence of diabetic cystopathy and of microvascular disease in the kidneys may play a role in the higher incidence of UTI in diabetic patients. As diabetics are more prone to UTI, it is necessary to pay special attention to early diagnosis and treatment.[6]

The aim of this study was to determine the causative bacteria, culture and sensitivity pattern in diabetics with Urinary tract infection in our setup. This will guide us to start an

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appropriate timely empirical antibiotic which will prevent the diabetic patients from the complications of UTI like pyelonephritis, urosepsis, renal failure etc. This can decrease the hospital stay, morbidity, and mortality.

MATERIAL AND METHODS

This was a 5 year (August 2010 to August 2015) study done in PSG Hospital, Peelamedu, Coimbatore and was based on the retrospective collection of data from case records of patients who were admitted under medicine department of PSG Hospital. Only those cases that fulfill the inclusion criteria were analyzed. To know the reliability of causative organism, we went through the case records and looked into the various clinical and laboratory parameters of the Diabetic patients who have culture positive UTI.

As per the standard protocol clean voided midstream urine samples were collected in sterile containers. In sampling procedure, all the patients were given proper information while collecting the urine sample. Urine culture reports that exhibited CFU of more than 10^5/ml were considered positive. Only single isolates were taken for study purpose and mixed growths were excluded. The pathogens were isolated and identified using phenotypic methods and biochemical testing. Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI). We took nine types of isolates and went through their susceptibility patterns to 26 antibiotics. Ethical clearance was obtained before the start of the study.

Inclusion criteria:

1. Diabetic patients
2. Sex: Both male and female
3. Age: 18 years and above
4. Urine culture positive cases

Exclusion criteria:

1. Nondiabetic
2. Pregnancy
3. Known congenital/acquired urogenital anomaly
4. Patients on chronic catheterization
5. Candiduria

STATISTICAL ANALYSIS

The data was descriptively analyzed using Microsoft Excel 2007 software.

RESULT

As per our finding depicted in Table 1 most of the patients were in the age range 51 to 60 years (30.26%) followed by 61 to 70 years (22.27%) and next 41 to 50 years (20.89%) while lower and higher of age groups are lesser affected.

In figure 1 regarding the gender predilection, males were more affected than females (53.3% vs 46.7%).

From the distribution pattern seen in figure 2, it is clear that most common Gram-negative bacteria were E.coli (69.12%) followed by Klebsiella pneumoniae (8.60%). Out of Gram-positive organisms most frequently seen bacteria (figure 3), were Enterococcus faecalis (10.90%) followed by Staphylococcus aureus (2.91%). So, the actual second most common bacteria seen were E.faecalis.

Age	No.of patients	Percentage
10-20	2	0.3
21 -30	18	2.76
31 – 40	39	5.99
41 – 50	136	20.89
51– 60	197	30.26
61– 70	145	22.27
71 – 80	87	13.36
81 – 90	27	4.14
90– 100	0	0
Total	651	100

Table-1: Distribution of patients by age

Age group	In male	In female
10-20	1	1
21-30	10	8
31-40	20	19
41-50	69	67
51-60	100	97
61-70	81	64
71-80	48	39
81-90	18	9
91-100	0	0

Table-2: Number of urine samples with single isolate in various age groups

S.N.	Bacteria	AK	GM	VCM	NA	LOX	NOX	COX	CPH	CTZ	CTX	CEP	CEF-S	AMP	PIP-T
1	E.coli	96	46	-	7	31	18	19	16	20	22	28	71	10	77
2	E.faecium	-	14	100	-	-	-	-	-	-	-	-	-	43	-
3	E. faecalis	75	28	100	17	11	11	-	-	-	-	-	-	54	-
4	K.pneumonaie	81	56	-	42	54	45	37	0	34	35	39	54	2	59
5	P. aeruginosa	72	69	-	-	-	50	40	-	68	-	61	67	-	82
6	Staph.aureus	-	86	100	-	-	20	33	-	-	100	-	-	-	-
7	Citrobacter spp	50	50	-	-	100	47	-	-	51	-	39	-	-	50
8	Proteus spp	91	80	-	20	60	82	40	50	89	67	78	80	-	100
9	Acinetobacter spp.	20	25	-	-	-	-	50	-	25	-	26	-	-	60

AK=amikacin, GM=gentamycin, VCM=vancomycin, LOX=levofloxacin, NOX=norfloxacin, COX=ciprofloxacin, CPH=cephalexin, CTZ=ceftazidime, CTX=ceftriaxone, CEP=cefipime, CEFS=cefoperazone/sulbactam, AMP=ampicillin, PIP-T, piperacillin/tazobactam

Table-3: (part-1) Antibiotic sensitivity in percentage

S.N.	Bacteria	MNM	INM	ENM	CTMX	NIT	TET	LNZ	COLI	RIF	TIGE	CLIND	AMXCL
1	E.coli	95	96	96	34	81	38	-	99	-	97	100	33
2	E. faecium	-	-	-	-	77	40	100	-	-	-	-	29
3	E. faecalis	-	-	-	-	82	47	100	-	-	-	-	-
4	K.pneumonaie	79	83	81	56	28	-	-	91	-	63	39	-
5	P.aeruginosa	50	78	-	0	20	-	-	82	-	-	-	0
6	Staph.aureus	-	-	-	57	100	-	100	-	10	-	50	40
7	Citrobacter spp.	54	59	-	-	-	-	-	-	-	-	-	-
8	Proteus spp.	88	71	75	50	20	-	-	-	-	-	-	50
9	Acinetobacter spp.	-	40	-	80	-	-	-	50	-	-	-	-

(MNM=meropenem, INM=imipenem, ENM=ertapenem, CTMX=cotrimoxazole, NIT=nitrofurantoin, TET=tetracycline, LNZ=linezolid, COLI=colistin, RIF=rifampicin, TIGE=tigecycline, CLIND=clindamycin, AMXCL=amoxicillin/clavulinic acid, CXIM=cefixime)

Table-3: (part-2) Antibiotic sensitivity in percentage

S.N.	Organisms	AK	GM	NA	CXIM	CTX	AMP	CTMX	AMXCL
1	E.coli	3	51	93	100	78	90	66	52
2	K.pneumonaie	16	44	58	100	65	98	44	56
3	P.aeruginosa	33	33	-	-	-	-	-	-
4	Citobacter spp.	50	50	100	-	-	-	100	-
5	Proteus spp.	9	20	80	-	33	30	50	50
6	E.faecalis	25	97	83	-	-	46	-	40
7	Staphylococcus aureus	-	14	-	-	0	100	43	60
8	E.faecium	-	80	-	-	-	57	-	71

Table-4: Resistance to commonly used antibiotics (in percentage)

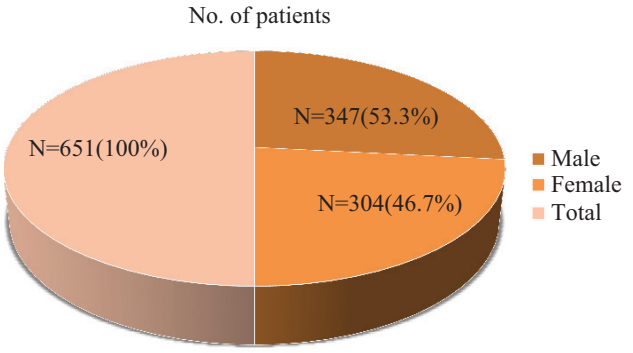


Figure-1: Distribution of patients by gender

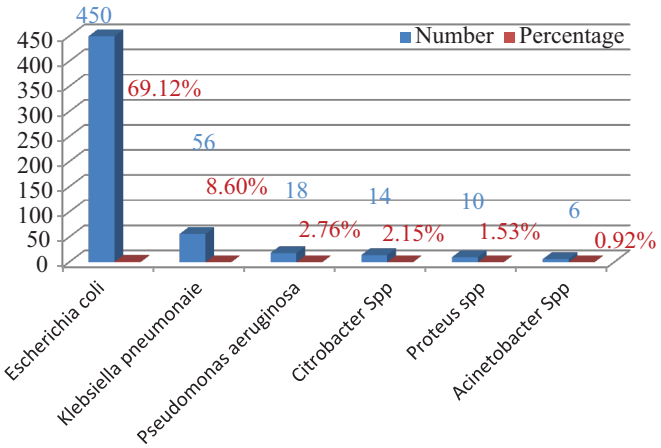


Figure-2: Distribution of Gram –negative bacteria

From table 2 we observed that males had higher prevalence of bacteriuria compared to females in all the age groups. There were only two young patients above 18 and below 20 years age group.

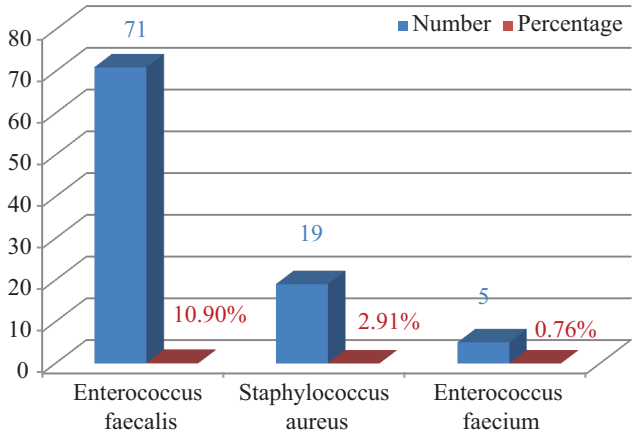


Figure-3: Distribution of Gram-positive bacteria

As shown in table 3 and 4, good susceptibility was seen to amikacin by E.coli (96%) K.pneumoniae (81%), P.aeruginosa (72%) and Proteus spp. (91%). Linezolid and Vancomycin showed 100% activity against Enterococci and Staphylococcus aureus. Amongst fluoroquinolones, ciprofloxacin has low activity (40%) against Pseudomonas while levofloxacin stands good (100%) for Citrobacter and lower (60%) for Proteus. We saw poor response to norfloxacin against most bacteria grown except Proteus spp. which has good (82%). From amongst the cephalosporins, cephalexin, cefixime and ceftazidime are not good options for most bacteria but Cefoperazone-sulbactam stands good (>65%) against E.coli, P.aeruginosa and Proteus spp. Ceftazidime shows 68% and 89% sensitivity against P.aeruginosa and Proteus spp.respectively. ceftriaxone which is very commonly used injectable to start empirically in inpatient setup is not a good choice due to very low sensitivity in E.coli (22%) and

K.pneumoniae (35%). High level Ampicillin resistant is seen in both Gram-positive and negative bacteria.

If we look at E.coli –ESBL (71.42%) and Klebsiella pneumoniae - ESBL(56.90%), best options were carbapenems, colistin, tigecycline, amikacin, piperacillin-tazobactam and nitrofurantoin. There were carbapenemase producers also like in E.coli (4-5%) and Klebsiella (20%). Cotrimoxazole and amoxicillin-clavulanic acid have no good response (<60% sensitivity) to any of these bacteria. Linezolid is good for both the species of Enterococcus (100% susceptible). Nitrofurantoin sensitivity was good in Staph. aureus (96%), E. faecalis (82%) and E.coli (81%). Best response giving carbapenems against K.pneumonaie and P.aeruginosa was by imipenem (83% and 78% respectively) while against Proteus spp.meropenem has good activity (88%). Acinetobacter spp. Which was seen in 6 patients has poor sensitivity to amikacin (20%), imipenem (40%) and colistin (50%) but good sensitivity to cotrimoxazole (80%). We found 3 MRSA out of 19 isolates.

High-grade resistance was seen for many commonly used antibiotics like ampicillin, cotrimoxazole, cefixime, nalidixic acid and amoxicillin/clavulanate (table: 5).

DISCUSSION

In this study, we were supposed to see the prevalence of various bacteria in diabetics with proven UTI and antimicrobial susceptibility patterns. We found that Diabetic males are outnumbering females in terms of prevalence of UTI. This is in contrast to an Indian study[7], a Nigerian study[8] but in favor of study in Sudan[9] and east India.[10]

Most frequently patients were in the affected age group from 51 to 60 years(30.26%).This was Similar to a study by R. Simkhada.[11] but dissimilar to a study in Kuwait by May Sewify et al[12] and in Nepal by PK Jha et al.[13]

Like in almost all the studies from international or national locations[14,15], the most common bacterial isolate was E.coli(59.12%). It was similar to a case-control study done in New Delhi[16] which showed E.coli as most common bacteria in UTI (64.3%). In a study from Nepal[11], it was 52.38%. While in Romanian study[17] it was 70.4%.

In our study, we found Klebsiella pneumoniae (8.60%) and Pseudomonas aeruginosa (2.76%) as next common isolate amongst Gram-negative while Enterococcus faecalis (10.90%) and Staphylococcus aureus (2.91%) are most common Gram-positive isolates. In a study done in Turkey, K.pneumoniae and P.aeruginosa were 9.1% and 3% respectively[18] which was in the same line as ours. Overall in our study second most common isolate is E.faecalis (10.90%) after E.coli (59.12%) which is on the same trend as seen by N.Chatterjee et al.[10] In contrast, second most common isolate in the above mentioned Turkish study was CoNS (24.2%). If we see a retrospective study in Nepal, there was a similar trend of the frequency of Enterococcus spp.(13.84%), K.pneumoniae (8.3%) and S.aureus (7.11%). We also found Citrobacter spp, Proteus spp, and Acinetobacter spp though less frequently.

In this study, seeing antibiotic resistance we found that ampicillin and cefixime are not good options as high-level resistance is seen for commonly grown isolates of E.coli and K.pneumonaie. Citrobacter spp.is 100% resistant to nalidixic acid and cotrimoxazole and equally resistant to amikacin and gentamycin i.e 50%.There is high resistance to gentamycin, ampicillin and amoxicillin-clavulanic acid against Gram-positive organisms especially Enterococcus spp.Fluoroquinolones and cephalosporins are resistant to most of the Gram-negative isolates. Cephalosporin resistance was similarly seen in Nepal[13] and Bangladesh.[19] Good options for them are amikacin, piperacillin/tazobactam, and cefoperazone/sulbactam. Our study has a high prevalence of ESBL in E.coli (71.42%) and K.pneumonaie (56.90%) which needs to be managed with carbapenems as first-line drugs and if not feasible, second-line drugs should be piperacillin/tazobactam or cefoperazone /sulbactam. Though ESBL organisms are in similar frequency as shown in many other studies it was lower compared to a study from Kerala[20] which showed very high-level ESBL growth (approximately 90%) and from Manipal[21] by M.Srinivas et al which showed it 78.6%. Though amikacin is a cost-effective injectable for both non-ESBL and ESBL isolates, its renal toxicity restricts its widespread use.

In our findings, nitrofurantoin had good activity against E.coli and Gram-positive isolates similar to as seen by PK Jha et al[13] and Citrobacter spp. had approximately 50% susceptibility to most of the tested antibiotics but 100% for levofloxacin.

If we see options with antipseudomonal activity in our set up and susceptibility pattern, best antibiotics are Piperacillin / tazobactam and colistin with equal efficacy (82%) followed by imipenem and amikacin (78% and 72% respectively). Here ciprofloxacin has low efficacy (40%) and cotrimoxazole was 100% resistant to P.aeruginosa. Similar findings were seen by M.Srinivas et al[21] with 82% susceptibility to Piperacillin/ tazobactam and 68.2% to cefoperazone/ sulbactam but a very good response to amikacin (93.8%). Meropenem activity against P.aeruginosa in our study was only 50% while M.Srinivas et al[21] found it 87.5%.

In our findings, Gram-positive isolates have shown 100% sensitivity to vancomycin while an article from south India shows 81% and 94% susceptibility respectively by Enterococcus and Staphylococcus.[22] We have less nephrotoxic linezolid with 100% sensitivity against Gram-positive isolates. In an Indian study, Enterococcus faecalis was the most commonly isolated Gram-positive organism with 3.2 percent resistance to vancomycin.[23] We found Acinetobacter spp.in 6 cases showing multidrug-resistant with very low sensitivity even to carbapenems and colistin.

Multi-drug resistant bacteria commonly arise due to the prolonged use of cephalosporins and other antibiotics, particularly their use as prophylaxis treatment, inadequate dosage or inaccurate frequency of administration. This also produces class and cross-resistance. Pseudomonas spp. has become a big challenge to treat. Plasmid-mediated resistance due to the usage of broad-spectrum antibiotics has been considered as one of the factors associated with this

phenomenon.[24]

Amikacin which is easily available and cost-effective remains a good choice for most of the uropathogens if renal status allows and there is a facility to follow renal function test regularly. There is good sensitivity even in current era due to its ability to evade attacks by all antibiotic-inactivating enzymes that are responsible for antibiotic resistance in bacteria, except for aminoacetyltransferase and nucleotidyltransferase.[25] This is accomplished by the L-hydroxyaminobuteroyl amide (L-HABA) moiety attached to N-1 (compare to kanamycin, which simply has a hydrogen), which blocks the access and decreases the affinity of aminoglycoside-inactivating enzymes.[26,27] Amikacin ends up with only one site where these enzymes can attack, while gentamicin and tobramycin have six.

Enterococci are getting commoner in many culture samples especially from urine due to inappropriate and intensive use of broad-spectrum antibiotics.[28] Same are the reasons in cases of other microorganisms. Poultry farms and human foods of animal origin are also contributing significantly to the increasing multidrug resistance amongst isolates.

These are the reasons, why to have knowledge of the pattern of local microbial prevalence and antimicrobial susceptibility to start the most appropriate empirical drug depending on clinical and laboratory profile of patients.

CONCLUSION

Most commonly affected age group with UTI amongst diabetics was from 51 to 60 years where males exceeded females in prevalence. We found E.coli, E.faecalis, and K.pneumoniae as the most commonly grown organisms in descending order. Most of the Gram-negative isolates were susceptible to amikacin, piperacillin/tazobactam, cefoperazone/sulbactam and carbapenems, while Gram-positive cocci were mostly sensitive to nitrofurantoin, linezolid, and vancomycin. Ampicillin, cephalosporins (with the exception of sulbactam combination), cotrimoxazole, and fluoroquinolones are not good choices to start on an empirical basis.

REFERENCES

1. Bryan CS, Reynolds KL, Metzger WT. Bacteremia in diabetic patients: Comparison of incidence and mortality with non-diabetic patients. Diabetic Care 1985; 244-249.
2. Muller LMAJ, Gorter KJ, Hak E et al.Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005;41:281-288.
3. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus. FEMS Immunol Med Microbiol. 1999; 26:259–65.
4. Bahl AL, Chugh RN, Sharma KB. Asymptomatic bacteremia in diabetics attending a diabetic clinic. Indian J Med Sci. 1970;24:1–6.
5. Patil, N. R., Mali, U. S., Ramtirthkar, M. N., Bhave (Sule), A. P., Mali, S. S., Mane, V. S. Bacteriuria in diabetic patients. World Journal of Science and Technology 2012;2:25-27.
6. Geerlings SE, Stolk RP, Camps MJ, Netten PM, Hoekstra JB, Bouter KP, et al. Asymptomatic bacteriuria may be considered a complication in women with diabetes. Diabetes Mellitus Women Asymptomatic Bacteriuria Utrecht-Study Group. Diab Care. 2000;23:744–9.
7. Janifer J, Geethalakshmi S, Satyavani K, Viswanathan V. Prevalence of lower urinary tract infection in South Indian type 2 diabetic subjects. Indian J Nephrol 2009; 19:107-11.
8. Ifediora, A.C., Obeagu, E.I., Akahara, Ijeoma Chukwudi and Eguzouwa Uloma Priscilla-J.Bio.Innov 2016; 5: 68-82.
9. Hamdan et al. Annals of ClinicalMicrobiology and Antimicrobials 2015;14:26.
10. Nandini Chatterjee, Chandan Chatterjee, Sinjan Ghosh, Mainak, Mukhopadhyay, Ramkrishna Brahmachari,Kartik Patar-The Journal of the Association of Physicians of India 2016;64:26-30.
11. R. Simkhada. Nepal Med Coll J 2013;15:1-4.
12. May Sewify, Shinu Nair, Samia Warsame, Mohamed Murad, Asma Alhubail, Kazem Behbehani, Faisal Al-Refaei, and Ali Tiss- Journal of Diabetes Research Volume 2016 .
13. Jha P K, Baral R, Khanal B. International Journal of Biomedical Laboratory Science 2014;3:29-34.
14. Baloch G. H., Jeffery, M. H., Madhudas, C., Devrajani, B. R., and Ali Shah, S. Z.Frequency and pattern in patients with diabetes mellitus. Professional Med J 2011;18: 466-69.
15. Chukwuocha, U. M., Emerole, C. O., Njokuobi, T.N., and Nwawume, I. C.Urinary tract infections (UTIs) associated with diabetic patients in the Federal Medical Center Owerri, Nigeria. Global Advanced Research Journal of Microbiology 2012;1:062-066.
16. Goswami R, Bal CS, Tejaswi S, Punjabi GV, Kapil A, Kochupillai N. . Prevalence of urinary tract infection and renal scars in patients with diabetes mellitus. Diabetes Res Clin Pract. 2001; 53:181-6.
17. Teodora Chiţă, Bogdan Timar, Delia Muntean, Luminiţa Bădiţoiu, Florin Horhat, Elena Hogea, Roxana Moldovan, Romulus Timar, Monica Licker-Dove medical press 2016; 2017:13 Pages 1—7.
18. Demiss Nigussie, Anteneh Amsalu, Turk J Urol 2017; 43: 85-92.
19. Shill MC. Prevalence of Uropathogens in Diabetic Patients and Their Corresponding Resistance Pattern: Results of a Survey Conducted at Diagnostic Centers in Dhaka, Bangladesh. Oman Medical Journal 2010;25:282-285.
20. Prabhu H, Oommen AT, Henry R. Clinico-Microbiological Profile of Urinary Tract Infections in Diabetic Patients in a Tertiary Centre in Kerala. Kerala Medical Journal. 2016; 9:97–104.
21. Srinivas M Aswani, UK Chandrashekar, KN Shivashankara, and BC Pruthvi. Australas Med J. 2014; 7: 29–34.
22. Abdulla MC, Feroz P. Jenner, Jemshad Alungal- Int J Res Med Sci. 2015; 3:2576-2579.
23. Mandal J, Acharya SN, Buddhapriya D, Parija SC. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant Escherichia coli. Indian J Med Res. 2012;

136:842.

24. Lister P.D., Wolter D.J., Hanson N.D. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev. 2009;22:582–610.
25. Mudd, Efrain (7 August 2017). O Aminoglycosides. Pharmacological Sciences.
26. Kondo, Shinichi; Hotta, Kunimoto. Semisynthetic aminoglycoside antibiotics: Development and enzymatic modifications. Journal of Infection and Chemotherapy. 1999; 5: 1–9.
27. Park, Je Won; Ban, Yeon Hee; Nam, Sang-Jip; Cha, Sun-Shin; Yoon, Yeo Joon. Biosynthetic pathways of amino glycosides and their engineering. Current Opinion in Biotechnology. Chemical biotechnology: Pharmaceutical biotechnology. 2017; 48: 33–41.
28. Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: Results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis. 2001;32: S133–45.

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A Study of Prevalence of Depression in Type 2 Diabetic Patients in a Tertiary Care Centre

Tolstoy Rajangam[1], Mritheika Priyashini R[2], Santni Manickam[3]

ABSTRACT

Introduction: Diabetes mellitus and depression are mutually aggravating conditions. Here we present a study on the prevalence of depression and their association with the control of the Type 2 diabetes patients above 40 years of age in a tertiary care center.

Material and Methods: It was a cross-sectional study involving 100 patients with Type 2 Diabetes mellitus above the age of 40 years with HbA1c of < 8% was taken as the controlled group and with HbA1c of ≥ 8% as the uncontrolled group. 'The Hamilton's Depression Scoring' questionnaire was used to assess their level of depression. Both the groups were correlated statistically.

Results: There was a statistically significant association between the depression and the control of the diabetes mellitus in patients aged above 40 years ($p<0.0016$). There was also a statistically significant correlation between the suicidal tendency with the control of the diabetes mellitus in those patients ($p<0.05$). However, there was no positive correlation between the control of diabetes and anxiety.

Conclusion: People with uncontrolled diabetes were more prone to depression and suicidal tendencies than the controlled diabetics.

Keywords: Diabetes Mellitus, Depression, Suicidal Tendency, Anxiety, Elderly Men

INTRODUCTION

One of the greatest challenges of medicine in the 21st century is co morbidity, where two or more disease occurs together in the same individual.[1] Diabetes mellitus is a chronic disease which affects virtually every organ in the human system. The World Health Organization projected that 300 million people will suffer from diabetes by 2025.[2] Globally, an estimated 43 million diabetics have symptoms of depression.[3] People with diabetes who have depression often find it more difficult to follow diabetes treatment recommendations and have poor metabolic control. Thus depression can further aggravate the blood sugar levels and hence 'a vicious cycle' occurs. These patients also have higher complication rates, increased health care use and increased disability, lost productivity, lower quality of life and increased risk of death.[4]

There are several international studies evaluating the prevalence of depression in the diabetic patient. A study on Diabetes and Depressive Symptoms in Korean Women was performed for 6,572 women obtained from the Fifth Korean National Health and Nutrition Examination Survey conducted in 2010 to 2011 which concluded saying that physicians should manage diabetes in consideration to depression, as depressive symptoms were observed in 22.6% of subjects with diabetes.[5] The prevalence of Depression, anxiety and stress symptoms among diabetics was studied by a cross-sectional study in Malaysia which concluded that these symptoms were prevalent among Type 2 diabetics, with a third being classified as anxious.[6] A study on the bidirectional association between depression and Type 2 diabetes in Women available on Arch Intern Med suggests that the relative risk of developing depression is greater in diabetics when compared to non-diabetics.[7]

In patients with Diabetes and essential hypertension, the prevalence of depression and suicidal tendencies was studied and was found that depression and suicidal tendencies are found prominent in the diabetic patient as well as hypertensive, more being in the uneducated side.[8] Diabetes may be diagnosed and treated, but the depression in these patients goes unnoticed. Most of the time, depression is not considered an important factor, often ignored and left untreated.

The available data regarding the prevalence of depression in type 2 diabetes patients in India are limited. We investigated the prevalence of depression in patients with Type 2 Diabetes attending a tertiary care hospital in south India and its relationship with glycemic control was examined.

MATERIAL AND METHODS:

It was a cross-sectional study of 2 months duration involving 100 patients with Type 2 Diabetes of more than 5 years duration above the age of 40 years who presented to the Department of General Medicine and Endocrinology. The controlled group was taken as the one with HbA1c of < 8% and the uncontrolled group was with HbA1c of ≥ 8%. Patients with gestational diabetes, cardiac failure, severe renal failure, stroke and other such complications may be excluded.

A standard questionnaire for depression, 'The Hamilton's Depression Scoring' questionnaire was given to each patient. The questionnaire was given to the patients to assess their

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level of depression. The glycemic control of each patient was verified by their HbA1c results. Statistical analysis was performed using SPSS version 20. Prevalence of depression in both populations of controlled and uncontrolled diabetic patients was presented in percentage. Major determining factors like suicidal thoughts and anxiety are elaborated in addition to the depression score.

Ethical clearance was obtained from the institutional Human Ethics Committee and Informed consent was obtained from the respondents before administering the questionnaire.

STATISTICAL ANALYSIS

Descriptive statistics like mean and percentages were used for the analysis for the interpretation of results. Comparision of groups was done by chi sqaure test.

RESULTS

The study was conducted to find out the prevalence of depression in Type 2 Diabetic people who are over 40 years of age and the relation with controlled vs. uncontrolled diabetic status. A total of around 100 people were involved in this study and the results are tabulated below based on Hamilton's scoring.

Table-1 shows that when compared to the controlled diabetic patients, the uncontrolled patients have more prevalence of depression in each of the mild, moderate and severe segment. On doing a statistical analysis comparing the controlled and the uncontrolled diabetic groups using Chi-square, the p-value was found to be p<0.0016 which is statistically significant. The Odds ratio obtained was 2.79 which imply that the uncontrolled diabetics have twice the risk of confronting with depression when compared to the patients with good glycemic control.

	Frequency (Percentage)	
	Controlled diabetes	**Uncontrolled diabetes**
Normal (0-5)	17 (38%)	9 (18%)
Mild (6-8)	8 (16%)	11 (22%)
Moderate (8-11)	11 (22%)	16 (32%)
Severe (>12)	12 (24%)	14 (28%)

Table-1: Depression

	Frequency (Percentage)	
	Controlled diabetes	**Uncontrolled diabetes**
Normal (0-5)	47 (94%)	34 (68%)
Mild (6-8)	3 (6%)	6 (12%)
Moderate (8-11)	0 (0%)	6 (12%)
Severe (>12)	0 (0%)	4 (8%)

Table-2: Suicidal tendencies

	Frequency (Percentage)	
	Controlled diabetes	**Uncontrolled diabetes**
Normal (0-5)	11 (22%)	9 (18%)
Mild (6-8)	22 (44%)	25 (50%)
Moderate (8-11)	15 (30%)	14 (28%)
Severe (>12)	2 (4)	2 (4%)

Table-3: Anxiety

Table 2 also shows the similar pattern that the uncontrolled diabetic patients exhibit more suicidal tendencies than the controlled group in all segments – mild, moderate and severe. Comparing the two groups by the Chi-square test, it was found to be statistically significant (P< 0.05). Suicidal tendencies, like the depression score, are associated with the uncontrolled diabetic status.

Table 3 shows that in both the controlled as well as the uncontrolled diabetic group, the anxiety is variable in its severity and there is no statistical correlation between either of the two groups with anxiety (P-value - 0.935).

DISCUSSION

Our study confirms that people with uncontrolled diabetes have increased chances of depression when compared with the controlled diabetics. The uncontrolled diabetics have twice the risk of confronting with depression when compared to the patients with good glycemic control. Our results were similar to many other studies. Anderson et al summarised 20 cross-sectional reports and that the odds of depression in the diabetic group were twice that of the non-diabetic comparison group.[9]

A study by Rajesh Rajput et al showed that depression and anxiety symptoms were two-fold higher in diabetic patients as compared to healthy controls.[10] In a population-based study in Chennai, it was found that the prevalence of depression was 23.4%.[11] Raval *et al*. found a very high prevalence (41%) of depression in 300 patients with type 2 diabetes in a tertiary care hospital in Northern India.[2] Another study conducted at a tertiary care center found the prevalence of depression in T2DM patients to be 16.9%.[12] A multi-center study done in Pakistan found prevalence at 43.5%.[13]

In the present study, 94% of the patients with controlled diabetes do not exhibit suicidal tendencies and 6% of them exhibit mild symptoms. Whereas in uncontrolled group, the suicidal tendencies are nil in 68% of the patients while 12% has mild and moderate symptoms. There is a severe tendency of suicidal tendencies in 8% of uncontrolled group. According to Subramani et al the percentage of subjects with suicidal thoughts is 12.4%.[14] It was 3.1 % in study by Kessler et al.[15] It was 9.2% according to Nock M K et al.[16] According to study by Siddharth Sarkar et al suicidal ideas and attempts are more frequent in patients with diabetes mellitus than healthy or medically ill controls, Psychological morbidity, including depression, precedes suicidal ideas and attempts.[17] Thus a detailed psychiatric analysis to rule out depression is mandatory for all diabetic patients as each influences the other significantly.

CONCLUSION

In our study, we found that people with uncontrolled diabetes were more prone to depression and suicidal tendencies than the controlled diabetics. However, there is no statistical correlation between either of the two groups with anxiety. Interventional studies would bring out the effect of treating

diabetes as well the depression on each other. As the association between diabetes and depression is bidirectional, a proper approach towards both is required in order to prevent them from becoming worse.

REFERENCES

1. Graham CC, Sartorius N, Cimino LCGL Diabetes and depression in general practice: meeting the challenges of managing comorbidity. Br J Gen Pract 2014; 64: 386-387.
2. Raval A, Dhanaraj E, Bhansali A, Tiwari P Prevalence & determinants of depression in type 2 diabetes patients in a tertiary care centre. Indian J Med Res 2010; 132: 195-200.
3. Mental health and chronic physical illnesses. The need for continued and integrated care. Vancouver: World Federation for Mental Health; 2010.
4. Kaveeshwar SAJC The current state of diabetes mellitus in India. Australas Med J 2014;7: 45-48.
5. Han Na Sung, Hong Seok Chae, Eung Soo Kim, Jong Sung Kim Korean J Fam Med. Diabetes and Depressive Symptoms in Korean Women: The Fifth Korean National Health and Nutrition Examination Survey (2010-2011) 2014; 35: 127–135.
6. Gurpreet Kaur, Guat Hiong Tee, Suthahar Ariaratnam, Ambigga S Krishnapillai, Karuthan China. Depression, anxiety and stress symptoms among Diabetics in Malaysia: a cross sectional study in an urban primary care setting. BMC Fam Pract. 2013; 14: 69.
7. An Pan, Michel Lucas, Qi Sun, Rob M. van Dam, Oscar H. Franco, JoAnn E. Manson, Walter C. Willett, Alberto Ascherio, Frank B. Hu Bidirectional Association between Depression and Type 2 Diabetes in Women. . 2010; 170: 1884–1891.
8. MN Igwe, R Uwakwe, CA Ahanotu, GM Onyeama, MO Bakare, AC Ndukuba Factors associated with depression and suicide among patients with diabetes mellitus and essential hypertension in a Nigerian teaching hospital Afr Health Sci. 2013; 13: 68–77.
9. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care. 2001; 24:1069–1078.
10. Rajesh Rajput, Pratibha Gehlawat, Deepak Gehlan, Rajiv Gupta, Meena Rajput Prevalence and predictors of depression and anxiety in patients of diabetes mellitus in a tertiary care center; Indian journal of endocrinology and metabolism:2016;20:746-751.
11. PoongothaiS, AnjanaRM, PradeepaR, GanesanA, UnnikrishnanR, RemaM,et al. Association of depression with complications of type 2 diabetes – The Chennai Urban Rural Epidemiology Study (CURES-102). J Assoc Physicians India 2011;59:644-8.
12. Balhara YP, Sagar R. Correlates of anxiety and depression among patients with type 2 diabetes mellitus. Indian J Endocrinol Metab 2011;15 Suppl 1:S50-4.
13. Khuwaja AK, Lalani S, Dhanani R, Azam IS, Rafique G, White F. Anxiety and depression among outpatients with type 2 diabetes: A multi-centre study of prevalence and associated factors. Diabetol Metab Syndr 2010;2:72.
14. Poongothai S, Pradeepa R, Ganesan A, Mohan V Prevalence of Depression in a Large Urban South Indian Population — The Chennai Urban Rural Epide-miology Study 2009 (Cures – 70). PLoS ONE 4(9): e7185.
15. RC Kessler RC, Berglund P, Borges G, Nock M, Wang PS Trends in suicide ideation, plans, ges-tures, and attempts in the United States, 1990-1992 to 2001-2003. JAMA 2005;293: 2487–95.
16. Nock MK, Borges G, Bromet EJ, Alonso J, Anger-meyer M, et alCrossnational prevalence and risk factors for suicidal ideation, plans and attempts. Br J Psychiatry. 2008;192: 98–105.
17. Sarkar S, Balhara YP. Diabetes mellitus and sui-cide. Indian journal of endocrinology and metabolism. 2014;18:468.

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Research Article

Effect of Dietary Calcium Intake on Bone Mineral Density in Elderly South Indian Men

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ABSTRACT

Background and Objective: There is a higher prevalence of vitamin D deficiency and associated osteoporosis in south Indian men despite abundant sunlight. Here we present a study on the effect of dietary calcium on bone mineral density in elderly men. **Methods:** This study was done as a part of an original study on the prevalence of osteoporosis and vitamin D deficiency. One hundred twenty elderly men were included. The dietary calcium intake was determined by a trained dietician. Quantitative and qualitative aspects of food intake were accessed and the Published data on Indian food was used to estimate dietary calcium intake. Phytate to calcium ratio was calculated. Bone mineral density (BMD) was done for all the subjects in the lumbar spine, hip and forearm. We also studied sunlight exposure pattern and its effect on vitamin D level. **Results:** The Mean calcium intake of the study population was 455 mg/day which was lower than recommended dietary value. There was a significant positive correlation between calcium intake and BMD at all three sites. The mean phytate to calcium ratio was 1.0643. Phytate to calcium ratio was negatively associated with hip and lumbar spine BMD. The mean vitamin D level was 20.4 ng/dl and the mean BMI (Body mass index) was 23.3 kg/m^2. **Conclusion**: The calcium intake in south Indian elderly men was lower than the recommended allowance and there was a positive correlation between calcium intake and bone mineral density.

Keywords: Bone mineral density, Dietary calcium, Elderly men, South India, Osteoporosis, Phytate, Vitamin D

INTRODUCTION

Calcium and vitamin D play an important role in bone mass accrual[1]. Normal calcium intake varies in different geographical regions[2]. Adequate calcium intake has shown a positive effect on bone mineral density (BMD) and vertebral fracture reduction in post-menopausal women[3]. Vitamin D plays an important role in calcium absorption from the intestines and suppresses parathyroid hormone production. Dietary calcium intake is associated with greater BMD in post-menopausal women than calcium supplementation[4]. Low calcium intake is also associated with increased bone turnover markers and osteoporosis[5]. Adequate dietary calcium intake during adolescence increases BMD. Vitamin D deficiency among Indian population is high even with adequate sunlight exposure[6]. This may be due to skin pigmentation and dressing habits among certain communities[7]. Because vitamin D is essential for calcium absorption, communities with poor calcium intake may be further compromised by vitamin D deficiency. We have already reported a higher prevalence of vitamin D deficiency and osteoporosis in South Indian men[8]. Hence, we undertook this study to find the effect of calcium intake on BMD in elderly South Indian men. We also studied sunlight exposure pattern and its effect on vitamin D level.

METHOD AND MATERIALS

This study was done as a part of an original study of the prevalence of osteoporosis. It is a cross-sectional prevalence study of osteoporosis in South Indian men and the influence of lifestyle factors on BMD, done after getting the permission from the Institutional Ethics Committee. One hundred twenty men of the original study were analysed.

This study was done in an urban area of Vellore, South India (12.9202°N, 79.1333°E), for 1 year duration from August 2010 to July 2011. After the initial survey, men above the age of 50 years were recruited by the cluster sampling method. Men with a history of chronic liver disease, renal disease or drugs that can affect bone health were excluded from the study.

The dietary calcium intake was determined by a trained dietician. Quantitative and qualitative aspects of food intake were accessed using semi-quantitative food frequency questionnaire. It includes a meal plan recall that covers the menu for a week. Published data on Indian food were utilised for estimating dietary calcium intake[9]. As a part of the study, phytate intake was assessed, and the phytate-to-calcium ratio was calculated because it is more predictive of calcium absorption[10]. History of sunlight exposure and clothing patterns were also assessed. The study was done during summer to minimise the effect of sunlight on vitamin D status. Twenty-four-hour urinary calcium excretion was done to assess the adequacy of nutritional calcium intake.

Biochemical analysis was done as a part of the original study. Fasting sample was obtained for estimation of serum calcium (8.6–10.2 mg/dl), phosphorus (2.5–5 mg/dl), albumin (3.5–5 g/ml), alkaline phosphatise (40–125 IU/L), creatinine (0.6–1.2 mg/dl), 25-hydroxy (25-OH) vitamin D (30–75 ng/ml), intact parathyroid hormone (iPTH)(8–50 pg/ml) and testosterone (300–1000 ng/dl). BMD was assessed at the lumbar spine, hip and forearm using Hologic DXAQDR 4500 Discovery A machine. Precision was 2% at both the measured sites (the spine and neck of the femur).

Statistical analysis was done using SPSS V.16.O Software (IBM Corp., USA). The independent sample 't' test was used to compare the measures of two continuous variables if they were normally distributed and nonparametric test was used if it was not normal.

RESULTS

One hundred twenty men from the original study were the part of this sub-study. The mean age of the study population was 58 years. The mean 25-OH vitamin D level of the study population was 20.4 ng/ml (range 4–58 ng/ml), the mean PTH was 44.5 pg/ml (range 14.5–151 pg/ml) and mean body mass index was 23.3 kg/m^2 (range 16.2–41.6 kg/m^2). The mean testosterone level was 620 ng/dl (range 270–980 ng/dl).

The mean calcium intake of the study population was 455 mg/day (range 131–1565 mg/day), and the mean urinary calcium excretion was 185 mg/24 hours (range 26–558 mg/24 hours). Out of 120 subjects, only one subject had a documented calcium intake of 1,000 mg and above. About 62% of the study population consumed less than 500 mg of calcium per day (Table 1).

Table 1: Mean Calcium Intake and Urinary Calcium Excretion

Characteristic	Mean (Std Deviation)	Range
Calcium intake (mg/24 hours)	455.9 (184.2)	131–1565
24-hour urine calcium (mg/24 hours)	185(108)	26–558

There was a positive correlation between calcium intake and BMD in all three sites, which is statistically significant (Figures 1–3).

In our study, the mean phytate-to-calcium ratio was 1.0643 (SD 0.616). The phytate-to-calcium ratio was negatively associated with hip and lumbar spine BMD. About 64% of the study population had adequate sunlight exposure of at least 20 minutes per day during daytime (between 10 a.m. to 3p.m.). Mean vitamin D level was higher in subjects with adequate sunlight exposure (21.1ng/ml vs.18.9 ng/ml).

DISCUSSION

The aim of this study was to evaluate the dietary calcium intake and its effect on BMD in men above 50 years of

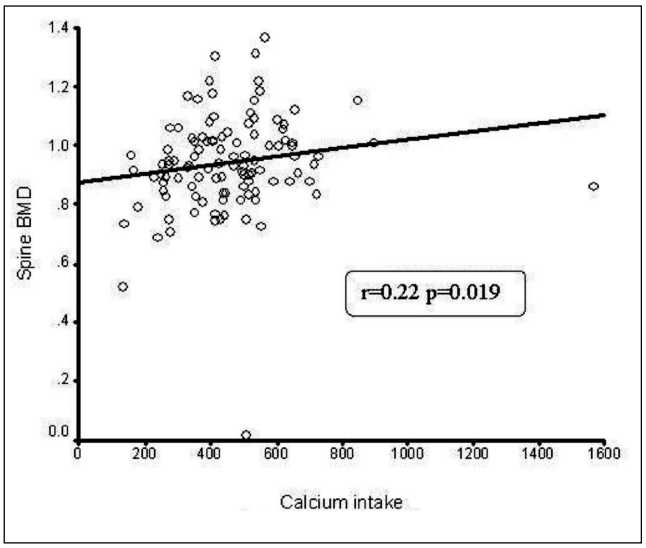


Figure 1: Correlation between Calcium Intake and Lumbar Spine BMD

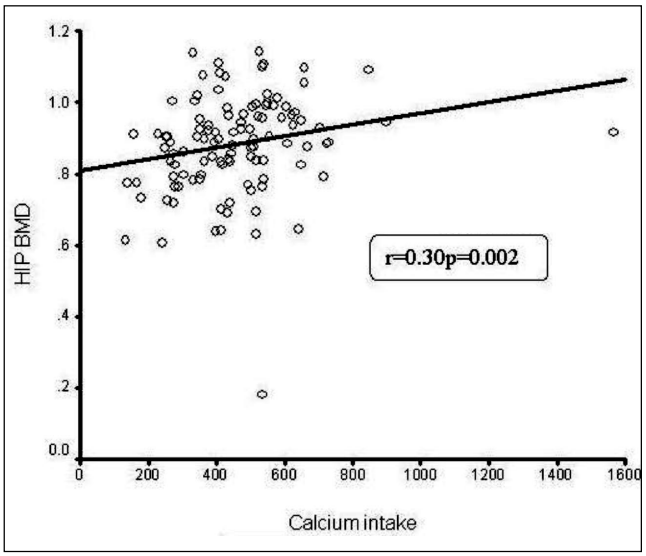


Figure 2: Correlation between Calcium Intake and Total Hip BMD

age. Dietary calcium intake plays an important role in bone health. Calcium and phosphorous are principle constituents of bones accounting for 65% of its weight[11]. Vitamin D plays an important role in calcium absorption and bone health by suppressing PTH level[12]. Hence, optimal vitamin D is necessary for calcium absorption. Even under normal conditions, only about 30%–40% of dietary calcium is absorbed[13].

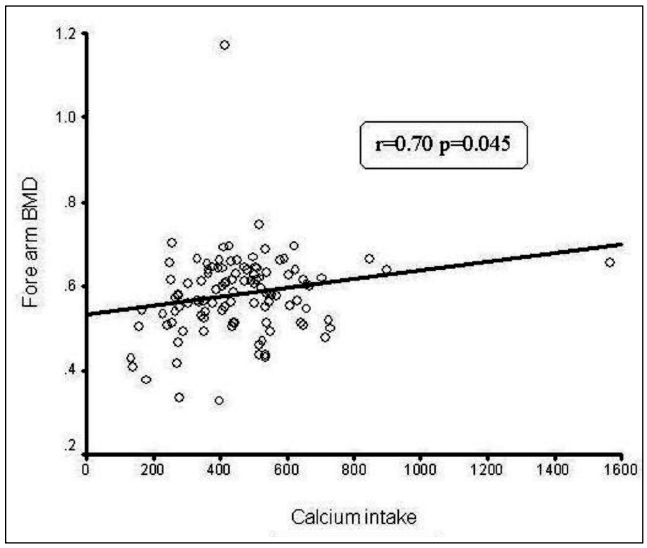


Figure 3: Correlation between Calcium Intake and Forearm BMD

Absorption of calcium is affected by dietary phytate, oxalate and sodium. Myo-inositol hexaphosphate (phytate), a saturated cyclic acid, is a storage form of phosphorus in cereals and legumes[14]. When consumed, it forms a complex with minerals such as zinc, calcium and iron and retards their intestinal absorption[15–17].

Effect of calcium intake on BMD and fracture risk is not consistent. In a large epidemiological study, reduced calcium intake was not associated with increased incidence of the forearm or hip fracture in men[18].

The mean calcium intake in our study was 455 mg/day (range 131–1565 mg/day). This is lower than the recommended intake as per Indian Council of Medical Research, which suggested at least 600 mg/day[19]. In several studies, it was shown that dietary calcium intake is lower in the Indian subcontinent[20–22]. It was shown that in subjects with low calcium intake, there is an accelerated conversion of vitamin D to its inactive polar metabolite, mediated by 1,25-dihydroxyvitamin D [23]. This may explain the widely prevalent vitamin D deficiency in the Indian subcontinent. It was postulated in UK Asians that inadequate sunlight exposure, low calcium intake and high cereal consumption may lead to secondary hyperparathyroidism, which may lead to progressive vitamin D reduction[24].

Harinarayan *et al*. from the same geographical area published data regarding dietary intake of calcium in both urban and rural subjects. In that study, the mean dietary intake of calcium in urban and rural men were 322±8 and 306±2, respectively, which is lower than that in our study[25].

In our study, there was a positive correlation between calcium intake and BMD (spine, total hip and forearm) with a respective r value of 0.22 (p value 0.019), 0.30 (p value 0.002) and 0.70 (p value 0.045). The mean phytate-to-calcium ratio was 1.0643 (SD 0.616).

A low calcium intake is considered as a risk factor for fracture because it is one of the principle constituents of the bone. In a study by Lee *et al.*, there was a positive relationship between dietary calcium intake, calcium/phosphorus intake ratio and femoral neck BMD in men older than 50 years[26]. Tandon *et al.* have shown that dietary calcium intake was positively associated with BMD and negatively associated with dietary phytate intake[27]. In that study, even with adequate calcium intake, about 10% of subjects had osteoporosis at the lumbar spine. Even though large epidemiology studies have shown no association between calcium intake and fracture risk, in our study, increased calcium intake was associated with increased BMD.

Dietary phytate impairs the absorption of calcium. In our study population where dietary calcium intake was low, this might further impair the calcium availability[28]. Harinaryan *et al*. published a study from a similar geographical area. They showed that low calcium and high phytate consumption in both rural and urban subjects. These studies clearly highlight the need for an increase in recommended dietary allowance of calcium intake in elderly Indian subjects.

The vitamin D levels are lower even in subjects with optimal sunlight exposure (21.1 ng/ml vs. 18.9 ng/ml). This may be due to dressing habits among certain communities, skin pigmentation and environmental pollution[29].

CONCLUSION

As there is a positive correlation between calcium intake and BMD and the calcium intake in the South Indian elderly men is low, this study clearly highlights the need to take measures to increase the calcium intake in elderly subjects to improve the bone health and to prevent osteoporotic fractures. The recommended dietary allowance of calcium is also to be increased, at least in the elderly population, in view of consumption of dietary phytates. In addition, the study also necessitates the need for screening of vitamin D deficiency in elderly Indian subjects even with adequate sunlight exposure. An interventional study with calcium and Vitamin D supplementation can be done as a follow-up.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Parfitt AM, Gallagher JC, Heaney RP, Johnston CC, Neer R, Whedon GD. Vitamin D and bone health in the elderly. American Journal of Clinica l Nutrition 1982;(35):1014–31.

[2] Parr RM, Dey A, McCloskey EV, Aras N, Balogh A, Borelli A, *et al.* Contribution of calcium and other dietary components to global variations in bone mineral density in young adults. Food and Nutrition Bulletin 2002; 23(3, Suppl.):180.

[3] Shea B, Wells G, Cranney A, *et al*. Meta-analyses of therapies for postmenopausal osteoporosis. VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. Endocrine Reviews 2002;23(4):552–9.

[4] Napoli N, Thompson J, Civitelli R, Armamento-Villareal RC. Effects of dietary calcium compared with calcium supplements on estrogen metabolism and bone mineral density. American Journal of Clinical Nutrition 2007;(85):1428–33.

[5] Pongchaiyakul C, Kosulwat V, Charoenkiatkul S, Chailurkit LO, Rojroongwasinkul N, Rajatanavin R. The association of dietary calcium, bone mineral density and biochemical bone turnover markers in rural Thai women. Journal of Medical Association Thailand 2008;91(3):295–302.

[6] Harinarayan CV, Joshi SR. Vitamin D status in India-its implications and remedial measures. Journal of Association of Physicians of India 2009;(57):40–8.

[7] Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesis vitamin D3. Lancet 1982;(1):74–6.

[8] Shetty S, Kapoor N, Naik D, *et al.* Osteoporosis in healthy South Indian males and the influence of life style factors and vitamin D status on bone mineral density. *Journal of Osteoporosis* 2014;2014:Article ID 723238, 5 pages.

[9] Gopalan C, Sastri BVR, Balasubramanyam SC. Nutritive value of Indian foods. *Food composition table nutritive value of Indian foods.* Hyderabad (Andhra Pradesh): National Institute of Nutrition ICMR; 1996; Appendix 1: 92–4.

[10] Guéguen L, Pointillart A. The bioavailability of dietary calcium. Journal of American College of Nutrition 2000;(19):119S–36.

[11] Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocrine Reviews 2001;22(4):477–501.

[12] Christakos S, Dhawan P, Liu Y, Peng X, Porta A. New insights into the mechanisms of vitamin D action. Journal of Cell Biochemistry 2003;(88):695–705.

[13] Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. American Journal of Clinical Nutrition 2004;80(6):1706S–9S.

[14] Harland BF, Oberleas D. Phytate in foods. World Reviews on Nutrition and Diet 1987;52:235–59.

[15] Na¨vert B, Sandström B, Cederblad A. Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. British Journal of Nutrition 1985;53:47–53.

[16] Heaney RP, Weaver CM, Fitzsimmons ML. Soybean phytate content: effect on calcium absorption. American Journal of Clinical Nutrition 1991;53:745–7.

[17] Hallberg L, Rossander L, Skanberg AB. Phytates and the inhibitory effect of bran on iron absorption in man. American Journal of Clinical Nutrition 1987;45:988–96.

[18] Owusu W, Willett WC, Feskanich D, Ascherio A, Spiegelman D, Colditz GA. Calcium intake and the incidence of forearm and hip fractures among men. Journal of Nutrition 1997;127:1782–7.

[19] Standing Committee. Scientific evaluation of dietary reference intakes. Calcium. In: *Dietary reference intakes for calcium*. Food and Nutrition Board, National Academy Press; 1999. pp. 71–145.

[20] Harinarayan CV, Ramalakshmi T, Venkataprasad U. High prevalence of low dietary calcium and low vitamin D status in healthy south Indians. Asia Pacific Journal of Clinical Nutrition 2004;13(4):359–6.

[21] Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney RC, *et al.* Vitamin D and bone mineral density status of healthy school children in northern India. American Journal of Clinical Nutrition 2005;(82):477–82.

[22] Balasubramanian K, Rajeswari J, Gulab, Govil YC, Agarwal AK, Kumar A, *et al.* Varying role of vitamin D deficiency in the etiology of rickets in young children vs. adolescents in northern India. Journal of Tropical Pediatrics 2003;(49):201–6.

[23] Clements MR, Johnson L, Fraser DR. A new mechanism for induced vitamin D deficiency in calcium deprivation. Nature 1987;(325):62–5.

[24] Clements MR. The problem of rickets in UK Asians. Journal of Human Nutrition and Diet 1989;(2):105–16.

[25] Harinarayan CV, Ramalakshmi T. Patterns of dietary calcium intake in south Indian rural, urban and metropolitan city subjects. Journal of Clinical Science Research 2015;(4):143.

[26] Lee K-J, Kim K-S, Kim H-N, Seo J-A. Association between dietary calcium and phosphorus intakes, dietary calcium/phosphorus ratio and bone mass in the Korean population. Sang-Wook Song Nutrition Journal 2014;(13):114.

[27] Tandon N, Marwaha RK, Kalra S, Gupta N, Dudha A, Kochupillai N. Bone mineral parameters in healthy young Indian adults with optimal vitamin D availability. National Medical Journal of India 2003;(16):298–302.

[28] Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PV, Sarma KV, Kumar E. High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. American Journal of Clinical Nutrition 2007;(85):1062–67.

[29] Goswami R, Kochupillai N, Gupta N, Goswami D, Singh N, Dudha A. Presence of 25(OH) D deficiency in a rural North Indian village despite abundant sunshine. Journal of Association of Physicians of India 2008;(56):755–7.

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A Rare Etiology of Severe Thrombocytopenia in Patient with Chronic Liver Disease

Mohammad Zeya Ansari, R Tolstoy, G Jagadeeswaran

Abstract

Thrombocytopenia is a common complication in patients with chronic liver disease and is multi-factorial. It complicates the management and worsens the prognosis. Treatment options are costly and include platelet transfusion, splenectomy, splenic artery embolization, TIPPS and thrombopoietin (TPO) agonists.

Here we are presenting a patient with decompensated liver disease with known chronic alcoholism and profound thrombocytopenia despite multiple platelet transfusions. On further work up we found a coexistent autoimmune etiology. Thrombocytopenia promptly responded to steroids, a cost effective option.

Introduction

Thrombocytopenia, a common complication in patients with chronic liver disease, is seen in nearly 75% of cirrhotic patients.[1] The pathogenesis of thrombocytopenia in chronic liver disease is multi-factorial.[1] Initially it was thought that thrombocytopenia in CLD is only due to portal hypertension where there will be an enlargement of spleen which causes destruction of platelets. So initially treatment for thrombocytopenia was aimed at reversing portal hypertension. But persistently decreased platelets were noted even in patients without splenomegaly.[2] So other factors like intrasplenic production of autoantibodies and plasma expansion resulting in hemodilution also contribute to thrombocytopenia. Suppression of platelet production in bone marrow is also another factor which is seen in HCV infection and alcohol related chronic liver disease. Antiviral therapy like interferon alfa also induces thrombocytopenia.[3] In some patients of CLD there is an evidence based autoantibodies related destruction of platelets and it is mostly observed in HCV related cases.[4] Here autoantibodies are directed against platelet surface antigens and enhance the removal of platelets by splenic and hepatic reticuloendothelial systems there by triggering rapid destruction as seen in chronic ITP. TPO is a potent cytokine produced by liver, bone marrow and kidney which regulates megkaryocytes and platelet production.[5] In cirrhotic patients with thrombocytopenia, low circulating levels of TPO is seen compared to cirrhotic patient with normal platelet count.[6] The treatment options available for severe thrombocytopenia are platelet transfusion, splenic artery embolization, splenectomy and TIPS. Platelet transfusion is indicated in patients with less than 10,000-20,000/µl platelets in uncomplicated patients and less than 50,000/µl in patients undergoing procedures.[7] Newly derived options include recombinant interleukin-11, eltrombopag and recombinant TPO.

Case Summary

A, 50 year old male patient with ethanol related chronic liver disease and no other comorbidities, came to EMD with the complaints of melaena, gum bleed and haematuria for 10 days, fever with chills, abdominal pain and distention for 3 days.

On examination, patient was pale and febrile with stable vital parameters. He had minimal ascites and epigastric tenderness.

Serial CBC, LFT and RFT are summarized in Table.1. Upper GI endoscopy showed esophageal varices and portal gastropathy and EVL done immediately. Ultrasound abdomen Showed features of CLD and portal hypertension with medical renal disease. Ascitic fluid analysis had no evidence of peritonitis. Urine culture showed significant bacteria (E.coli) and managed accordingly. He had severe thrombocytopenia which was managed with platelet transfusions. Bone marrow aspiration and biopsy showed dimorphic anemia with thrombocytopenia, megaloblastic erythroid hyperplasia and mild megakaryocytic hyperplasia. His serum B12 and folate level were within normal range. Hepatis B, C, HIV, Dengue virus, *Helicobacter pylori*, scrub typhus, leptospirosis infection and Coomb's test were negative. Renal failure was stabilized with hemodialysis.

As his thrombocytopenia persisted despite multiple platelet transfusions (total 19 units) and other common causes of thrombocytopenia being ruled out, we suspected of some uncommon etiology including an autoimmune condition. ANA tested was positive with fine granular++, cytoplasmic+ pattern. He was positive for smooth muscle antibody and negative for anti-ds DNA Ab and hence he was diagnosed to have autoimmune hepatitis type-1 with associated ITP. We started steroids (Inj. methylprednisolone 125 mg IV OD for 3 days followed by oral prednisolone 60mg OD). His platelet count started improving from 2nd day of IV steroid.

On follow-up visits, his platelet count was in safe range (> 1 lakh / cu.mm3) with low dose oral steroid and is currently being managed as ITP.

Discussion

The causes of thrombocytopenia in chronic liver disease may be due to sequestrating function of spleen, presence of antiplatelet antibodies and impaired thrombopoietin production in cirrhotic patients.[6] It is very important to differentiate ITP from common cause of thrombocytopenia in CLD as the management differs. Hypersplenism in liver disease due to portal hypertension

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Table 1: Blood investigation (CBC, LFT, RFT) reports

	Normal	Day1	Day3	Day5	Day7	Day10
Hb (g/dl)	13-17	7.1	6.4	6.0	7.8	9.6
PCV (%)	40-50	21.3				
WBC count/µl	4-10X10^3	5.4X10	5.0X10^3			76X10^3
MCV (fl)	80-100	96.6				
Neutrophils (%)	40-80	70.7				
Lymphocytes (%)	20-40	17.6				
Monocytes (%)	2-10	10.8				
Eosinophils (%)	1-6	0.9				
Basophils (%)	0-2	0.0				
Platelet count/µl	150-400X10^3	6X10^3	3X10^3	4X10^3	36X10^3	125X10^3
ESR (AEFH)	3-10	106	98	78	78	45
Serum Protein (g/dl)	6.4-8.3	6.5		6.8		6.9
Serum Albumin (g/dl)	3.4-4.8	2.3		2.6		2.9
Serum globulin (g/dl)	1.8-3.6	4.2		4.2		4.0
Total bilirubin (mg/dl)	0.2-1.0	1.2				1.1
Direct/indirect	0-0.2/0.2-0.8	0.8/0.4				
SGOT (u/l)	5-38	62				54
SGPT (u/l)	5-41	15				14
ALP (u/l)	40-129	205				154
GGT (u/l)	10-66	409				126
Serum creatinine (mg/dl)	0.8-1.25	2.3	4.43	3.95	3.4	2.1

causes increased sequestration of platelets within spleen leaving less in circulation. However it is also to be clear that thrombocytopenia can occur in liver cirrhosis without splenomegaly.[2] Thrombopoietin is a cytokine produced by the liver which plays a key role in platelet production and subsequent function may be low in cirrhosis. Thrombocytopenia in liver disease without evidence of cirrhosis, splenomegaly and interferon is recognized especially in patients with hepatitis C and auto immune hepatitis.

Based on persistent thrombocytopenia, ANA and anti SMA positivity we diagnosed him as AIH type-1 and associated ITP. The International Autoimmune Hepatitis Group proposed a scoring system to standardize the diagnosis, useful for clinical trials, but that may be inaccurate in individual cases.[8] Liver biopsy was not done for two reasons-severe thrombocytopenia and patient refusal.

Autoimmune hepatitis (AIH) is a chronic and progressive necroinflammatory and fibrotic process of the liver of unknown cause that occurs in children and adults, usually associated with the presence of autoantibodies and Hypergammaglobulinemia.[8] Affected individuals often have concurrent extrahepatic autoimmune disorder like rheumatoid arthritis, Sjogren's syndrome and auto immune thyroiditis but association with idiopathic (immune) thrombocytopenic purpura has been rarely reported.[9-13]

ITP is an autoimmune disease characterized by a low platelet count, mucocutaneous bleeding, normal bone marrow findings and the absence of other causes of thrombocytopenia in which anti-platelet autoantibodies such as PAIgG induces platelet destruction.[14] There is a case series of 5 patients with AIH type-2 where patients had at least one associated autoimmune disorder including IgE-induced IgA deficiency, ITP, and arthritis.[15] In an Indian case series of 10 patients with AIH, three had underlying rheumatic disease (SLE, Sjogren's and Rheumatoid arthritis each one), while others had primary AIH. In the same report, 2 patients were found to have autoimmune thrombocytopenia and were managed by immunosuppressant.[16] Indeed prednisolone administration is a treatment option for AIH and is also effective for ITP. Therefore ITP should be considered when liver dysfunction is accompanied by severe thrombocytopenia particularly in the autoimmune types of liver disease.

Though in our patient there was normal bilirubin, SGOT and SGPT (which is usually elevated in hepatitis) but persistent thrombocytopenia and positive ANA with SMA made us to consider it AIH type-1.

Conclusion

It is worthy to evaluate for an alternative autoimmune etiology for CLD if thrombocytopenia is severe or persisting as there are better and cost effective treatment options i.e. steroids.

Learning points

1. All CLD with history of alcohol ingestion is not purely alcohol related.
2. Thrombocytopenia in CLD should be evaluated for causes other than alcohol and hypersplenism.
3. All autoimmune hepatitis may not have gross LFT derangement.

References

1. Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Weksler B, Esteban R. *J Hepatol* 2008; 48:1000-7.
2. García-Suárez J, Burgaleta C, Hernanz N, Albarran F, Tobaruela P, Alvarez-Mon M. HCV-associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha2b-interferon therapy. *Br J Haematol* 2000; 110: 98–103.
3. Fried MW. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347:975-82.
4. Weksler BB. *Aliment Pharmacol Ther* 2007; 26Suppl1:13-9.
5. Qian S, Fu F, Li W, Chen Q, de Sauvage FJ. Primary role of the liver in thrombopoietin production shown by tissue-specific knockout. *Blood* 1998; 92:2189–2191.
6. Koruk M, Onuk MD, Akcay F, Savas MC. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis, and its relationship with circulating thrombocyte counts. *Hepatogastroenterology* 2002; 49:1645-8.
7. Liumbruno G, Bennardello F, Lattanzio A, Piccoli P, Rossetti G; Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) Work Group. Recommendations for the transfusion of plasma and platelets. *Blood Transfus* 2009; 7:132–150.
8. Krawitt EL. Autoimmune hepatitis. *N Engl J Med* 1996; 334:897-903.
9. Manns MP, Strassburg CP. Autoimmune hepatitis: clinical challenges. *Gastroenterology* 2001; 120:1502-17.
10. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; 31:929-38.
11. Yonetsu M, Kakizaki S, Katakai K, et al. A case of autoimmune hepatitis associated with idiopathic thrombocytopenic purpura. *Jpn J Gastroenterol* 1997; 94:610-4.
12. Omagari K, Kinoshita H, Kato Y, et al: Clinical features of 89 patients with autoimmune hepatitis in Nagasaki Prefecture. *Japan J Gastroenterol* 1999; 34:221-6.
13. Pfueller SL, Firkin BG, Kerlero de Rosbo N, Riglar A, Mackay IR. Association of increased immune complexes, platelet IgG and serum IgG in chronic active hepatitis. *Clin Exp Immunol* 1983; 54:655- 60.
14. Pamuk GE, Pamuk ON, Baslar Z, el at. Overview of 321 patients with idiopathic thrombocytopenic purpura. Retrospective analysis of the clinical features and response to therapy. *Ann Hematol* 2002; 81:436-40.
15. Duchini A, McHutchison JG, Pockros PJ. LKM-positive autoimmune hepatitis in the western United States: a case series. *Am J Gastroenterol* 2001; 96:3447-8.
16. Balakrishnan C, Mangat G, Kalke S,Desai D, Joshi A, Deshpande RB, Joshi VR. The spectrum of chronic autoimmune hepatitis. *J Assoc Physicians India* 1998; 46:431-5.

A Tertiary Care Hospital based Study on the Prevalence of Pulmonary Hypertention in Patients with Glomerular Filtration Rate less than 30 Ml /Min Per 1.73 Meter Square on Dialysis

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ABSTRACT

Introduction: Pulmonary artery hypertension (PAH) which can be primary or secondary, has been found to be associated with chronic kidney disease, especially end stage renal disease. Finding prevalence of pulmonary hypertension in early stages of chronic kidney disease is important because it creates very high burden of cardio vascular morbidity and mortality. In this study we have assessed various factors associated with prevalence of pulmonary hypertension in patients with glomerular filtration rate less than 30ml/min per 1.73 meter square on dialysis.

Material and methods: This is a one year, hospital based, prospective observational study of patients aged more than 18 years with GFR less than 30 ml/min per 1.73-meter square on dialysis, who were found to have pulmonary hypertension on echocardiography. The prevalence of pulmonary hypertension in this group of patients and the risk factors in CKD associated with it are calculated followed by a descriptive analysis and interpretation of the data.

Results: The various factors we analyzed and p value for association of pulmonary hypertension in CKD for the percentage of male and female in our study group is 0.241, for distribution of the pulmonary hypertension among different age groups is 0.503, for the significance of diabetic mellitus is 0.595,for systemic hypertension is 0.206, for arterial venous fistula is 0.780,for superimposed infections 0.166, for volume overload is 0.560, for anemia is 0.780, for left ventricular diastolic dysfunction is 0.662, for creatinine clearance is 0.717,for duration of dialysis is 0.000. With above results, only association with significant p-value (0.000) in our study population with pulmonary hypertensions longer duration.

Conclusion: Prevalence of pulmonary hypertension in our study is 22%. The risk factors like age,sex, diabetes, systemic hypertension, AVF, superimposed infection, volume overload, anaemia, LVDD has no influence on pulmonary hypertension in our study, only strong association that we have in our study population with pulmonary hypertension with CKD is longer duration of dialysis.

Keywords: Pulmonary Hypertension (PH), CKD

INTRODUCTION

Though, there has been fast advancement in the medical field which helps in the early and accurate diagnosis of health status of a patient, pulmonary hypertension remains a hidden problem that takes plenty of time in diagnosis from the presence of first symptom. Many patients are diagnosed only in advanced stage of disease.[1] Normal pressure in pulmonary artery is 25/10 mmHg and if the pulmonary artery pressure exceeds 40/20 mmHg or average pressure exceeds 25 mmHg, then the pulmonary hypertension is said to be present.Though the cut off value varies in many studies. If the pressure in the pulmonary artery remains persistently high then the right ventricle of the heart may get hypertrophied,so right ventricle will not be able to pump properly and the symptoms of right heart failure will occur. Pulmonary artery pressure is increased by many conditions and so pulmonary hypertension is classified accordingly. Prevalence of pulmonary artery hypertension inWHO's class1 which is caused mainly by connective tissue disorder, drug, and toxic agents is 15 cases/million adult population.[2,3] Prevalence of idiopathic pulmonary artery hypertension is 5.9 cases/million adult population.[4] Pulmonary hypertension due to systemic sclerosis, portal hypertension, congenital heart disease, and sleep apnea is 7-12%[5,6], 2-16%,[7] 30%[8], and 15-20%[9] respectively. Up to 60%of the patients with severe left ventricular systolic dysfunction and 70% of those with the heart failure with preserved ejection fraction may present with pulmonary hypertension. Almost all the patients with mitral valve diseases have pulmonary hypertension and 65% of those with symptomatic aortic stenosis also will have pulmonary hypertension.[10,11] Chronic thrombo-embolic pulmonary hypertension (CTEPH) prevalence was 3.2 cases/million/year and incidents were 0.9 cases/million/year. A large survey that is conducted in united states that registered information from all form of pulmonary hypertension from 1980-2002 documented that death rate in patients with pulmonary hypertension during these times were stable and ranging from 5.2-5.4deaths/1,00,000.[12,13,14,15]

Chronic kidney disease(CKD) is the global health burden with high economic cost to health system and is an

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independent risk factor for coronary vascular disease. All stages of CKD are associated with increased risk of cardio vascular premature mortality, morbidity and decreased quality of life.

Pulmonary artery hypertension in renal disease is an on-going research topic since it has very limited data. Prevalence of pulmonary hypertension in patients on hemodialysis, peritoneal dialysis and in stage - 5 CKD, ranges from 18.8 - 68.8%, 0.4-2% and 9-39% respectively. No proper epidemiological data are available yet for early stages of CKD. Pulmonary hypertension has direct association with mortality in end stage renal disease. Pulmonary hypertension in end stage renal disease is associated with the worst outcomes.[16-22] It is important to prevent pulmonary hypertension in patient with ESRD in early stages where even kidney transplantation may not reverse the condition,it alsohas high risk of mortality.

Study aimed to find the prevalence of pulmonary hypertension in patients with glomerular filtration rate less than 30ml/min per 1.73-meter square on dialysis in tertiary care hospital, to find the percentage of male and female in our study group and to find the significance of their association with pulmonary hypertension, to find the distribution of the pulmonary hypertension among different age groups and to find the significance of association pulmonary hypertension with diabetic mellitus, systemic hypertension, arterial venous fistula, superposed infection, volume overload, anaemia, left ventricular diastolic dysfunction, creatinine clearance, and duration of dialysis.

MATERIALS AND METHODS

In this prospective observational study conducted during the period of August 2016 to August 2017, we included total of 50 patients who were admitted in hospital fulfilling inclusion criteria. A proforma was made which included the detailed history, clinical examinations, and requisite investigations. After taking informed consent from each patient, detailed history was obtained and complete examination was done. Investigations like complete hemogram, routine urine analysis, blood sugar, serum electrolyte, serum creatinine, blood urea, thyroid function test, liver function test, ultrasound abdomen and pelvis, retroviral serology, hepatitis B and C serology, chest X-ray, echocardiogram, and electrocardiogram were done. GFR is calculated with the help of Cockcroft-Gault formula. Finally, the prevalence of pulmonary hypertension and the risk factors association with it were calculated and statistical analysis was done with the available data.

Inclusion criteria

-Age more than 18years

-Patients with GFR less than 30 ml/minper 1.73-meter square on haemodialysis

Exclusion criteria

Patients who has ejection fraction less than 45%

Patients who are known case of chronic lung disease, thrombo-embolic disease, sarcoidosis, sickle cell disease, HIV, connective tissue disorder, sleep disordered breathing, portal hypertension, congenital heart disease.

STATISTICAL ANALYSIS

The data collected from the patients is tabulated using Microsoft Excel. The chi square test was used to assess the difference in categorical variables between groups. Variables were presented as frequencies (numbers) and proportions (%) and Chi square test was used to compare frequencies. Results were presented in tables with an explanatory paragraph for each table by using Microsoft Office word software version 2010. Level of significance (P value) was considered at 0.05 as cut-off point for significant association.

RESULTS

Distribution of PH in various Age Groups

As seen in table -1, out of the total of number of 50 patients those belonging to age groups less than 40 years, 41-50 years, 51-60years,61-70 years and above 70 years were 6(12%), 7(14%), 24(48%), 9 (18%) and 4(8%) respectively. Patients who were found to have PH in these age groups were 3(50%), 0(0%), 6(25%), 1(11.1%), 1(25%) respectively. So,

Age (in years)	Pulmonary hypertension		Total	P value
	Yes	No		
<=40	3(50%)	3(50%)	6(100%)	0.241
41-50	0(0%)	7(100%)	7(100%)	
51-60	6(25%)	18(75%)	24(100%)	
61-70	1(11.1%)	8(88.9)%	9(100%)	
>70yr	1(25%)	3(75%)	4(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-1: Distribution of PH in various age groups

Sex	Pulmonary hypertension		Total	P Value
	Yes	No		
Male	5(17.9%)	23(82.1%)	28(100%)	0.503
Female	6(27.3%)	16(72.2%)	22(100%)	
Total	11(22.0%)	39(78%%)	50(100%)	

Table-2: Sex distribution of pulmonary hypertension

	Pulmonary HTN			
DM	Yes	No	Total	P value
Yes	7(22.6%)	24(77.4%)	31(100%	0.595
No	4(21.1%)	15(78.9%)	19(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-3: Association of Diabetes with pulmonary hypertension

		Pulmonary hypertension			p value
		Yes	No	Total	
Systemic hypertension	Yes	9(19.6%)	37(80.4%)	46(100%)	0.206
	No	2(50.0%)	2(50%)	4(100%)	
	Total	11(22.0%)	39(78.0%)	50(100%)	

Table-4: Distributionof systemic hypertension in pulmonary hypertension

	Pulmonary HTN			p-value
	Yes	No	Total	
AVF Yes	11(22.4%)	38(77.6%)	49(100%)	0.780
No	0 (0%)	1(100%)	1(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-5: Arteriovenous fistula in CKD and pulmonary hypertension

	Pulmonary htn			P value
	Yes	No	Total	
Superimposed infection				
yes	7(33.3%)	14(66.7%)	21(100%)	0.166
No	4(13.8%)	25(86.2%)	29(100%)	
Total	11(22.0%)	39(78.0%)	50(100%)	

Table-6: Superimposed infection and pulmonary hypertension

	Pulmonary HTN			P value
	Yes	No	Total	
Volume overload				
Yes	6(23.1%)	20(76.9%)	26(100.0%)	0.560
No	5(20.8%)	19(79.2%)	24(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-7: Association of volume overload with pulmonary hypertension

	Pulmonary HTN			P value
	Yes	No	Total	
Anaemia				
Yes	11(22.4%)	38(77.6%)	49(100%)	0.780
No	0(0%)	1(100%)	1(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-8: Anaemia with pulmonary hypertension

	Pulmonary HTN			P value
	Yes	No	Total	
LVDD				
Yes	10(24.4%)	31(75.6%)	41(100%)	0.662
No	1(11.1%)	8(88.9%)	9(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-9: LVDD (with EF>45%) and pulmonary hypertension

	Pulmonary htn			P value
Creatine clearence in GFR in ml	Yes	No	Total	
0-5	1(16.7%)	5(83.3%)	6(100%)	0.717
5-10	7(21.2%)	26(78.8%)	33(100%)	
10-15	3(33.3%)	6(66.7%)	9(100%)	
15-20	0(0%)	2(100%)	2(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-10: Creatinine clearance and pulmonary hypertension

maximum number of patients with PH were in the age group 51-50 and less than 40 years but the p-value was 0.241 which

	Pulmonary htn			P value
Duration of dialysis	Yes	No	Total	
0-5 years	0 (0%)	15 (100%)	15 (100%)	0.000
5-10 years	6 (20.0%)	24 (80.0%)	30 (100%)	
10-15 years	5(100.0%)	0(0%)	5(100%)	
15-20 years	11(22.0%)	39(78.0%)	50(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-11: Duration of Dialysis and pulmonary Hypertension

was not significant.

In the table 2 we compared the PH distribution among male and female population, in which males 5(17.9%) patients are found to have pulmonary hypertension and among females 6 (27.3%) patients had pulmonary hypertension. There were no significant association of pulmonary hypertension among male and female, since the p value was 0.503.

In the table 3 we analysed significance of association of diabetic mellitus with pulmonary hypertension. In total of 50 patients, 31(62%) patients were found to have DM, and among them 7(22.6%) patients were found to have PH. P value was 0.595, hence there was no significance of DM association with PH.

In the table 4 we analysed significance of association of systemic hypertension with pulmonary hypertension. In total of 50 patients, 46(92%) patients were found to have SHTN, and among them 9(19.6%) patients were found to have PH. P value was 0.206, hence there was no significance of SHTN association with PH.

In the table 5 we analysed significance of association of AVF with pulmonary hypertension. In total of 50 patients, 49(98%) patients were on AVF and one patient was on peritoneal dialysis, and among patients on arteriovenous fistula 11(22.4%) patients were found to have PH, and patient with peritoneal dialysis had no PH and P value was 0.780,which is not significance of AVF association with PH.

In the table 6 we analysed significance of association of super imposed infection with pulmonary hypertension. In total of 50 patients, 21(42%) patients were found to have super imposed infection, and among them 7(33.3%) patients were found to have PH. P value was 0.166, which also no significance of super imposed infection association with PH.

In the table 7 we analysed significance of association of volume overload with pulmonary hypertension. In total of 50 patients, 26(52%) patients were found to have volume overload, and among them 6(23.1%) patients were found to have PH. P value was 0.560, hence there was no significance of volume overload association with PH.

In the table 8 we analysed significance of association of anaemia with pulmonary hypertension. In total of 50 patients, 49(98%) patients were found to have anaemia, and among them 11(22.4%) patients were found to have PH. P value was 0.780, hence there was no significance of anaemia association with PH.

In the table 9 we analysed significance of association of LVDD with pulmonary hypertension. In total of 50 patients,

41(82%) patients were found to have LVDD, and among them 10(24.4%) patients were found to have PH. P value was 0.662, hence there was no significance of LVDD association with PH.

In the table 10 we analysed the pulmonary hypertension distribution and significance of association among the patients with varying range of creatinine clearance. In our study group 6(12%), 33(66%), 9(18%), 2(4%) patients were found to have creatinine clearance between 0-5%, 5-10%, 10-15%, 15-30% respectively and among them pulmonary hypertension was found in 1(16.7%), 7(21.2%), 3(33.3%), 0(0%) respectively. P value is 0.717 insignificant.

In the table 11 we analysed significance of association of duration of dialysis with pulmonary hypertension. In total of 50 patients, 15(30%), 30(60%), 5(10%), 15(30%) patients were found to be between 0-5, 5-10,10-15 years of dialysis respectively, and among them 0(0%), 6(20%), 5(100%), 11(22%) patients were found to have PH respectively. P value was 0.00, hence association of duration of dialysis with pulmonary hypertension is statistically significant.

DISCUSSION

There is no availability of epidemiological data in many developing countries including India for cause of pulmonary hypertension, but in India most common causes of PH are due to rheumatic heart disease, obstructive airway disease and congenital heart disease unlike western countries where idiopathic pulmonary artery hypertension and PH due to right heart disease are common.

Craddock et al say that exposure of dialysis membrane to blood leads to activation of neutrophils which leads to sequestration of neutrophils in the lungs. This phenomenon, is more common if cellulose membranes are used and it causes worsening of lung disease in HD patients.[23]

Presence of connective tissue disease, super imposed infections, haematological and liver diseases can cause pulmonary hypertension in patients with CKD. These conditions cause PH mainly by affecting micro vascular tone in lungs. Endothelial dysfunction has association with pulmonary hypertension in patients with CKD on HD, Main mechanism behind that includes imbalance between vasoconstrictors and vasodilators and high endothelin-1 and low nitric oxide levels affect vascular tone leading to PH.[24,25,26]

The prevalence of PH in CKD in our study was 22%, and the maximum affected age group was 51-60 years.In the study by Qian Zhang et al[27], the total prevalence of PH in CKD was 47.38% with the maximum number of patients seen in age 46.44(±14.63) years. Similarly another study from Iraq[28], showed maximum patients in 50 to 60 years and 60 to 70 years age group (35.3% in both groups).Male and female patients had prevalence of 55.32% and 44.68% respectively and their total number of patients compared to ours was large (n=705) while we had male and female prevalence of 17.95 and 27.3% respectively.

As noted by Qian Zhang et al[27], multivariate odds ratio(with 95%CI) for prevalence of PH in CKD showed that gender, diabetes, systemic hypertension had no significant correlation with PH while eGFR was contributing significantly to it. But, in our study all these parameters were insignificant for PH. Similarly, Ali Abdul Majid Dyab Allawi et al[28] and Thenappan T[29] also found no association between PH in CKD and hypertension even though hypertension is an established risk factor in for PH. Chronic volume overload leads to increased venous return in CKD patients may induce PH but as such we did not get any association between them. Severe anaemia is a well-known cardiovascular risk factor in CKD patients as low haemoglobin leads to hypoxic injury in tissues including pulmonary vasculature.[30] In our study we found no significant association of PH with anaemia while In a retrospective study low haemoglobin had significant impact on PH(P-value-0.000).[27]

In our study, we did not find a correlation between Left ventricular diastolic dysfunction and PH although LV end - diastolic pressure is taken as gold standard for its definition. Measuring LVDD in cases where pulmonary systolic pressure may not be estimated may help in predicting a relation with PH.[31]

According to Yigla M et al, pulmonary hypertension (PH) is a progressive, fatal pulmonary circulatory disease that accompanies many conditions (including left to right side shunt) with compensatory elevated cardiac output. PH also complicates chronic haemodialysis (HD) therapy immediately after the creation of an arteriovenous (AV) access, even before starting HD therapy. It tends to regress after temporary AV access closure and after successful kidney transplantation. This syndrome is associated with a statistically significant survival disadvantage. The laboratory hallmark of this syndrome is reduced basal and stimulatory nitric oxide (NO) levels. It appears that patients with end-stage renal disease (ESRD) acquire endothelial dysfunction that reduces the ability of their pulmonary vessels to accommodate the AV access-mediated elevated cardiac output, exacerbating the PH.[32]

We found 22.4% patients with AV fistula who had PH but this was not significant(p-value>0.780) while flow dependent significance of AV fistula was seen by Magdy M Emara et al.[33] In our study we have not measured AVF flow rate so could not assess its significance.

Comparing our study with Domenici et al[34], who noticed PH in 58.9% cases on HD and 22.2% on PD, we found 22.2% patients on HD having PH but no PH in patient on PD. Similarly another study by Yigla et al[35], found PH 39.7% in patients on HD but none on PD. An study in Iran, based on echocardiographic evaluations, found PH in 30-40% patients on chronic dialysis.[36]

Duration of dialysis significantly added to the development of PH which is evident from our study. This is well supported by Magdy et al[33], who reported duration of dialysis to be quite significantly associated with PH(P-value<0.001 and Pearson correlation 0.780).

Compared with previous studies, risk factors like age, sex, diabetes, systemic hypertension, AVF, superimposed infection, volume overload, anaemia, LVDD are not

associated with pulmonary hypertension in our study. But we found that the longer the duration of dialysis, the higher the possibility of pulmonary hypertension.

Limitations

A larger population based study may provide better data and correlations of above mentioned risk factors.

We have excluded secondary causes of PH in those who are known cases of chronic illness from available information only but have not investigated completely.

CONCLUSION

Prevalence of pulmonary hypertension in our study is 22 percentages. The risk factors like age, sex, diabetes, systemic hypertension, AVF, superimposed infection, volume overload, anaemia, LVDD has no influence on PH in our study. only association that we have in our study population in patients with PH is longer duration of dialysis.

ABBREVIATIONS

PH - Pulmonary hypertension, CKD - Chronic kidney disease, AVF - Arteriovenous fistula, LVDD - Left ventricular diastolic dysfunction, EF - Ejection fraction, ESRD - End stage renal disease, HD - Haemodialysis, PD - Peritoneal dialysis, GFR - Glomerular filtration rate, DM - Diabetes mellitus, SHTN - Systemic hypertension

REFERENCES

1. Dennis Kasper; Anthony Fauci; Stephen Hauser; Dan Longo; J Jameson. Pulmonary hypertension. Dennis L. Kasper, MD, Stephen L. Hauser, MD, J. Larry Jameson, MD, PhD, anthony S. Fauci, MD, Dan L. Longo, MD, Joseph Loscalzo, Ph. D. (eds). Harrison's Principles of Internal Medicine, 19 th ed.; 2015. pp. 1655-1660.
2. R.EDavide Bolignano, Stefania Rastelli, Rajiv Agarwal, Danilo Fliser, Ziad Massy, Alberto Ortiz, Andrzej Wiecek, Alberto Martinez-Castelao, Adrian Covic, David Goldsmith, Gultekin Suleymanlar, Bengt Lindholm, Gianfranco Parati, Rosa Sicari, Luna Gargani, Francesca Mallamaci, Gerard London, and Carmine Zoccali. Pulmonary Hypertension in CKD. American Journal Of Kidney Disease 2013; 61: 612-622.
3. Simonneau, G., Robbins, I.M., Beghetti, M. et al. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2009; 54: S43–S54
4. Nazzareno Galiè Marc Humbert Jean-Luc Vachiery Simon Gibbs Irene Lang Adam Torbicki Gérald Simonneau Andrew Peacock Anton Vonk Noordegraaf Maurice Beghetti Ardeschir Ghofrani Miguel Angel Gomez Sanchez Georg Hansmann Walter Klepetko Patrizio Lancellotti Marco Matucci Theresa McDonagh Luc A. Pierard Pedro T. Trindade Maurizio Zompatori Marius Hoeper Authors/Task Force Members Document Reviewers: Victor Aboyans, Antonio Vaz Carneiro, Stephan Achenbach, Stefan Agewall, Yannick Allanored, Riccardo Asteggiano, Luigi Paolo Badano, Joan Albert Barberà a, Hélène Bouvaist, Héctor Bueno, Robert A. Byrne, Scipione Carerj, Graça Castro, Çetin Erol, Volkmar Falk, Christian Funck-Brentano, Matthias Gorenflob, John Grantonc, Bernard Iung, David G. Kiely, Paulus Kirchhof, Barbro Kjellstrom, Ulf Landmesser, John Lekakis, Christos Lionis, Gregory Y. H. Lip, Sty. 2015 ESC/ERS
5. Hachulla E, Gressin V, Guillevin L, et al. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. Arthritis Rheum. 2005;52:3792-3800.
6. Mukerjee D, St George D, Coleiro B, et al. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hyper- tension: application of a registry approach. Ann Rheum Dis. 2003;62:1088-1093.2
7. Krowka MJ, Swanson KL, Frantz RP, McGoon MD, Wiesner RH. Portopulmonary hypertension: results from a 10-year screening algorithm. Hepatology. 2006;44:1502-1510.
8. Friedman WF. Proceedings of National Heart, Lung, and Blood Institute Pediatric Cardiology Workshop: pulmonary hyper- tension. Pediatr Res. 1986;20:8-11
9. Kessler R, Chaouat A, Weitzenblum E, et al. Pulmonary hypertension in the obstructive sleep apnoea syndrome: preva- lence, causes and therapeutic consequences. Eur Respir J. 1996;9: 787.
10. Oudiz RJ. Pulmonary hypertension associated with left-sided heart disease. Clin Chest Med 2007;28:233–241.
11. Vahanian AAlfieri OAndreotti FAntunes MJBaron-Esquivias GBaumgartner HBorger MACarrel TPDe Bonis MEvangelista AFalk VIung BLancellotti PPierard LPrice SSchafers HJSchuler GStepinska JSwedberg KTakkenberg JVon Oppell UOWindecker SZamorano JLZembala M. Guidelines on the management of valvular heart disease (version 2012). Eur Heart J 2012;33:2451–2496.
12. Seeger WAdir YBarberà JAChampion HCoghlan JGCottin VDe Marco TGaliè NGhio SGibbs SMartinez FJSemigran MJSimonneau GWells AUVachiéry JL. Pulmonary hypertension in chronic lung diseases. J Am Coll Cardiol 2013;62:D109–D116.
13. Hurdman Jcondliffe, Relliot, CASwift, ARajaram Sdavies, CHill Chamilton, NArmstrong IJBillings, Cpollard, LWild JMLawrie, ALawson Rsabroe, IKiely DG. Pulmonary hypertension in COPD: results from the ASPIRE registry. Eur Respir J 2013;41:1292–1301.
14. Cottin Vnunes, Hbrillet, PY Delaval Pdevouassoux, GTillie-Leblond IIsrael-Biet DCourt-FortuneValeyre DCordier JF. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J 2005;26:586–593.
15. Escribano-Subias PBlanco ILopez-Meseguer MLopez-Guarch CJRoman AMorales PCastillo-Palma MJSegovia JGomez-Sanchez MABarbera JA. Survival in pulmonary hypertension in Spain: insights from the Spanish registry. Eur Respir J 2012;40:596–603.
16. Mark J. Sarnak, Kari E. Roberts. Pulmonary Hypertension in CKD: Some Answers, Yet More Questions. Journal Of American Society of Nephrology 2015; 27():
17. Agarwal R. Prevalence, determinants and prognosis of pulmonary hypertension among hemodialysis patients. Nephrol Dial Transplant 27: 3908–3914, 2012
18. Havlucu Y, Kursat S, Ekmekci C, Celik P, Serter S, et al. Pulmonary hypertension in patients with chronic renal failure. Respiration. 2007;74:503–510.

19. Abdelwhab S, Elshinnawy S. Pulmonary hypertension in chronic renal failure patients. Am J Nephrol. 2008;28:990–997.
20. Yigla M, Fruchter O, Aharonson D, Yanay N, Reisner SA, et al. Pulmonary hypertension is an independent predictor of mortality in hemodialysis patients. Kidney Int. 2009;75:969–975
21. Nickel NGolpon HGreer MKnudsen LOlsson KWesterkamp VWelte THoeper MM. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. Eur Respir J 2012;39:589–596.
22. Fritz JSBlair COudiz RJDufton COlschewski HDespain DGillies HKawut SM. Baseline and follow-up 6-min walk distance and brain natriuretic peptide predict 2-year mortality in pulmonary arterial hypertension. Chest. 2013;143:315–323.
23. Craddock PR, Fehr J, Brigham KL, Kronenberg RS, Jacob HS. Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. N Engl J Med. 1977;296:769–774.
24. Giaid A. Nitric oxide and endothelin-1 in pulmonary hypertension. Chest. 1998;114:208S-212S.
25. Zoccali C. The endothelium as a target in renal diseases. J Nephrol. 2007;20:39–44.
26. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. N Engl J Med. 1995;333:214–221.
27. Zhang Q, Wang L, Zeng H, Lv Y, Huang Y. Epidemiology and risk factors in CKD patients with pulmonary hypertension: a retrospective study. BMC Nephrol. 2018;19:70.
28. Ali Abdul Majid Dyab Allawi, Mohammed Hannon Alsodani, Ahmed Mushhin Hardan Alqaisi. Prevalence and Risk of Pulmonary Hypertension in Chronic Kidney Disease. International Journal of Science and Research (IJSR). Volume 6 Issue 7, July 2017
29. Thenappan T. Pulmonary hypertenson in chronic kidney disease: a hemodynamic characterization. Pulmonary circulation 2017;7:567-568.
30. van Loon RL, Bartelds B, Wagener FA, Affara N, Mohaupt S, Wijnberg H, Pennings SW, Takens J, Berger RM. Erythropoietin Attenuates Pulmonary Vascular Remodeling in Experimental Pulmonary Arterial Hypertension through Interplay between Endothelial Progenitor Cells and Heme Oxygenase. Front Pediatr. 2015;3:71.
31. Miller WL, Mahoney DW, Michelena HI, Pislaru SV, Topilsky Y, Enriquez-Sarano M. Contribution of ventricular diastolic dysfunction to pulmonary hypertension complicating chronic systolic heart failure. JACC Cardiovasc Imaging. 2011;4:946-54
32. Yigla M, Abassi Z, Reisner SA, Nakhoul F. Pulmonary hypertension in hemodialysis patients: an unrecognized threat. Semin Dial. 2006;19:353–357.
33. Magdy M.Emara,Mohamad A.Habeb Alsayed Ahmed Alnahal,Tarek A.Elshazly, Faisal O.Alatawi,Amer S.Masoud.Prevalence of pulmonary hypertension in patients with chronic kidney disease on and without dialysis.Egyptian Journal of Chest Diseases and Tuberculosis. 2013;62:761-768.
34. Domenici A, Luciani R, Principe F. Pulmonary hypertension in dialysis patients.Perit Dial Int. 2010;30:251-2.
35. Yigla M1, Nakhoul F, Sabag A, Tov N, Gorevich B, Abassi Z, Reisner SA. Pulmonary hypertension in patients with end-stage renal disease.Chest. 2003;123:1577-82.
36. Mitra Mahdavi-Mazdeh, Seyed Alijavad-Mousavi, Hooman Yahyazadeh, Mitra Azadi, Hajar Yoosefnejad, Yoosef Ataiipoor. Pulmonary Hypertention in hemodialysis patients. Saudi J Kidney Dis Transpl 2008;19:189-93

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ORIGINAL RESEARCH

An Organised Programme of Simulation-based Clinical Training for the Medical Interns

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ABSTRACT

Context: It is well-documented that high-fidelity simulated training can be used to improve very important aspects of clinical interventions which include clinical decision-making, technical skills, soft skills like communication and leadership qualities. Many of the procedural skills can also be learnt effectively through simulations, using manikins of lower fidelity. **Aim:** Present report is an attempt to substantially enhance the 'must possess' attributes of a good clinician, which are quite often difficult to achieve by conventional teaching methods. **Methods:** This programme was aimed at providing training to the medical interns in cardiopulmonary resuscitation, as well as basic and advanced cardiac life support in medical emergencies, in an attempt to help them to efficiently manage commonly encountered cardiorespiratory emergencies. The programme was evaluated by comparing their performance before and after the intervention. **Results:** Their performance scores reflected the positive effects of the simulation-based teaching. In general, in addition to improving their skills in handling certain selected medical emergencies, the programme has also proved to enhance 'entrustable professional activities' of the young medical graduates. **Conclusion:** Our study, while highlighting the desirability of utilising simulation-based clinical training (SBCT), has also sought ways and means of incorporating SBCT as a mandated programme during the medical internship at an institutional level.

Keywords: Clinical simulation, Medical interns, Medical emergencies, Clinical training, Professionalism, Team work, Enhancing skills

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INTRODUCTION

The realm of medical education is undergoing significant changes globally. Some of these changes are warranted because of the great technological advances made in this field and others are due to an increasing awareness of patient safety measures warranted during the clinical procedures. The latter set of changes affects the general approach to patient care and calls for curricular changes to be brought in while training the medical undergraduate students, to effectively bring in the required attributes in the qualifying doctors. As it has been established that human error is a very significant factor as a cause for fatalities in a hospital, it is important to be able to avoid this single deterring factor while training the undergraduate medical students. There is increasing evidence to suggest that this can be achieved by effectively adopting simulation-based clinical training (SBCT), as an adjunct to conventional clinical teaching[1].

Competency-based medical education was advocated as an effective remedy for bringing in effective clinical training with consistency between different medical schools in terms of skills taught and competencies achieved[2]. In an effort to achieve this, many schools of medicine quite correctly thought of resorting to simulation-based medical education (SBME). This, in

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turn, saw the usurping of skill laboratories using manikins of varying fidelities being established to train the medical, nursing and physiotherapy students at undergraduate and postgraduate levels of training. SBME has been practiced in many medical schools abroad and there are reports concerning their success in achieving the goals[3]. It is well-documented that a high fidelity simulator can be used to improve very important aspects of clinical interventions which include clinical decision making, technical skills, soft skills like communication and leadership qualities. Procedural skills like administering intravenous drugs, urinary catheterisation, endotracheal intubation and many other practical skills can be taught and learnt effectively through simulations, using manikins of lower fidelity. It is not surprising to realise that simulated learning is becoming an integral part of the curriculum in many medical schools as these modules enhance learning in cognitive, psychomotor and affective domains[4]. These exercises can also be used to assess the student performance in all the domains of learning[5–7].

Establishment of a standard skills laboratory involves many challenges which include organisation of the equipments required and huge finances for these infrastructural facilities. In addition, this also involves careful training of enough resource personnel[8–10].

Internship is a critical and final stage of medical training, wherein a candidate is expected to acquire all the skills to practice medicine under supervision, ensuring that at the end of the training, he/she becomes capable of executing the functions of a doctor independently[11]. Considering the importance of the internship period, it would be desirable to incorporate simulated learning into this programme. As of now, since Medical Council of India (MCI) has not stipulated a compulsory posting for the conduct of simulation-based training, it is not easy to find the appropriate time for this programme[12].

In our medical school, which is a 30-year-old teaching institution, and which is equipped with the state-of-the-art centre of clinical simulation, an organised programme of SBCT is being conducted for the last 2 years. This is an attempt to substantially enhance the 'must possess' attributes of a good clinician, which is quite often difficult to achieve by conventional teaching methods.

All the Compulsory Rotatory Residential Interns (CRRIs) need to have training in cardiopulmonary resuscitation, as well as basic and advanced cardiac life support (ACLS). This will enable them to efficiently manage commonly encountered cardiorespiratory emergencies.

SBCT is relevant for the training of medical interns to function as inter-professional health team member and also as an individual learner.

METHODOLOGY

This study had the approval of Institutional Ethics Committee vide 14/167. Medical interns underwent this training in groups of 10–12 per batch.

Programme Design

1. Clinical material which dealt with evidence-based guidelines for simulated scenario and ACLS protocol was distributed a week prior to the workshop.
2. The participants took a pretest in the MCQ (multiple-choice questions) format which was designed to assess their basic knowledge of Basic Life Support (BLS) protocol, respiratory emergencies and fundamentals of cardiac life support scenarios. Participants took this test a day prior to the workshop.
3. Students who failed to score 75% in the test were asked to revise the study material and appear for training on a subsequent session. This made sure that the participants knew the background material of the course.
4. The programme started with a brief introduction on the logistics and sequence of the programme. They were then introduced to the features of the high-fidelity simulator, SimMan 3G – 15 min.
5. The clinical scenario was presented with volunteers helping to act as the attendant to the patient and the skilled assistant in the ward. Students were

summoned to treat a patient brought to the emergency ward.

The clinical scenario started with a patient presenting signs of respiratory emergency. The scenario included an episode of cardiac arrest, followed by cardiac symptoms of arrhythmias, which called for intervention through ACLS algorithms. They handled the scenario, and their performance was recorded on a video, to be used during debriefing – 20 min.

6. Global rating scales in form of checklists which were designed to assess clinical skills, communication and problem solving skills were used to rate the performance of the students. Debriefing was done which focussed on clinical knowledge, team performance, constructive feedback, communication, interpersonal and leadership skills – 45 min; students' discussion and interactions – 30 min.
7. Debriefing was followed by a brief session of power point presentations by the faculty on the relevant theory underlying the respiratory and cardiovascular scenarios, presented on that day – 30 min.
8. Students had another chance to go through the scenario – 15 min.
9. Students' feedback – 15 min.

Data analysis was done by comparison of their performance before and after the debriefing, using the checklist. Data was analysed using the software SPSS 19.0.

RESULTS

A total of 122 medical interns participated in this study which was conducted over 1-year period from January 2016 to January 2017 in clinical simulation laboratory of our tertiary care teaching hospital. The participants had to qualify in a pretest with a minimum of 75% marks. This test was designed to assess their basic knowledge of BLS protocol, respiratory emergency scenarios and fundamentals of cardiac life support, in an effort to make sure that the participants are familiar with the background information. In the pretest which was conducted to assess the clinical knowledge in simulated scenarios with MCQ, 98% students scored more than 75% which qualified them to undergo simulated training.

In the scenario of respiratory emergency (COPD – chronic obstructive pulmonary disease), only 19% of students could ask relevant question in clinical history which improved to 35% after the programme. 26% of students were able to apply clinical knowledge gained by traditional learning methods, which improved to 41%. Ability of problem-based learning also improved remarkably. Only about 9% students exhibited proven ability to effectively communicate well amongst their team members. By the end of the programme, they recorded an improvement showing that 30% of the participants effectively communicated during the scenarios.

In the segment of cardiac arrhythmias, requiring ACLS scenario protocol, there was significant difference amongst team dynamics, inter-communication skills and professionalism after debriefing session. Patient care and clinical knowledge which were earlier 33% and 26%, respectively, improved remarkably to 48% and 74% subsequently after the session of simulated learning.

The differences observed in all six domains of simulated COPD scenario showed a *p* value of *p* value (0.0009), which is less than 0.05 and hence statistically significant. Similarly, the five domains of ACLS scenario were also statistically significant with the *p* value of 0.01, which is less than 0.05; therefore, training does have significant impact on the students.

Qualitative data analysis was done from feedback questionnaire student compared traditional methods with simulation-based training. They felt that there is a need to have SBCT training as evinced by the positive effects the programme in their general behaviour and competence and were of the opinion that SBCT improved their decision-making skills and was helpful in improving their confidence in handling clinical emergencies. They appreciated the infrastructural facilities and qualities of the instructors (Table 1). CRRIs, as a group, felt that this programme need to be made

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Table 1: Results of the COPD scenario

Domain	Debriefing	
	Pre	Post
Patient care (PC)	47	84
Clinical knowledge (CK)	54	61
System-based practice (SBP)	03	40
Problem-based learning (PBL)	15	49
Inter-communication skills (ICS)	10	33
Professionalism (PR)	08	25

mandatory for the medical interns and felt they could have more occasions to go through this drill.

DISCUSSION

End of the medical graduate training is marked by a year-long internship, which is an intense training period, wherein the young doctors learn to confidently administer healthcare interventions, under supervision. It is extremely important for these graduates to possess the requisite level of clinical knowledge, skills and qualities that help them to don the role of a healer. Competencies are achieved, skills are learnt and correct attitudes are imbibed during this crucial period of their training. For all these to happen, the medical school and the management/administration need to gear-up training programmes which equip the young doctors with 'entrustable professional activities', as suggested by MCI[13,14]. Successful adoption of such programmes is restricted mainly to reports from United Kingdom, USA and Western Australia.

Considering the fact that establishment of a standard skills laboratory involves huge funding for infrastructural facilities and training of required resource personnel, MCI has recommended a phase-wise establishment of skill laboratories to enable the young doctors to master various levels of competence in the medical colleges (MCI). MCI has suggested that in the initial phase, facilities to impart basic life support, surgical skills and conducting normal labour can be provided, subsequently followed by the establishment of advanced life support, laparoscopic skills, neonatal and paediatric resuscitation, leaving the facilities to impart microsurgical and advanced laparoscopic skills along with human patient simulator for critical training in anaesthesiology for the final phase[13]. A few medical schools with awareness of the significance of clinical simulation and who have the required resources have made attempts to incorporate clinical simulation at various stages of undergraduate medical education, realising that clinical simulation of an appropriate nature can be incorporated into the curriculum at all stages of medical education[15]. However, it appears that most of the medical schools in India are yet to gear up to accept these norms for SBME. Hence, there is a paucity of information regarding organised implementation of such programmes in India.

Even though a few institutions in India have started employing SBME in their routine training of medical undergraduates, this trend is yet to gain acceptance. It is assumed that first and foremost for the acceptance of a new method of educational tool will require acceptance of the same by the faculty and administrators. Lack of expensive equipment and the required infrastructural facilities for this interesting and academically rewarding programme could also pose as a significant factor for lack of implementation of SBME in Indian medical colleges. Yet another very important reason could be the difficulty in identifying a time for this exercise, which is not yet strictly mandated by the regulatory authorities. SBME requires faculty specially trained in this method of instruction. Faculty need to be fully trained to run the scenario, to impart the skills and to debrief. They need to feel confident in extending health care at primary level and also need to be aware of their limitations. Our attempts of training them using simulated clinical scenarios have proved to be of significance in inculcating 'must possess' competencies in an intern.

Our programme had scenarios selected from more than one speciality. For instance, a patient with respiratory emergency (COPD) was shown to go in for cardiac arrest which then followed with a couple of episodes of arrhythmias. Thus, the clinical case scenario was made to be 'holistic' and appeared as realistic as possible. The participants handled the situation as a team. It is important that the exercises provide for simulation is as close to the reality as possible. The scenarios were

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planned to include all the domains of competencies expected out of the interns. Thus, scenarios had highlights on patient care, clinical knowledge and system-based practices. In addition, scenarios had enough scope to address issues of including team dynamics, interpersonal communication skills. When all components are put together, successful management of the scenarios also required substantial degree of professionalism. The learning objectives from the scenarios were noted. These objectives constituted the content for debriefing session (Table 2).

Table 2: Results of ACLS scenario

Domain	Debriefing	
	Pre	Post
Patient care (PC)	24	48
Clinical knowledge (CK)	42	74
Team dynamics (TD)	02	36
Inter-communication skills (ICS)	11	19
Professionalism (PR)	03	18

Results of evaluation of our current programme indicate that the majority of the participants improved their scores on all the competencies analysed after the session, and this reiterates the role of clinical scenarios in enhancing the clinical competencies of the participants and subsequently the success of this programme.

To address some of the comments raised by the participants, it would certainly be a right move to offer this course at the beginning of their internship, probably during the CRRI induction programme. The practical difficulty would be to find enough time for all the interns to go through this programme. As the benefits of such a programme are well-felt, efforts could be taken to make SBCT mandatory for the internship. As success of such an event would be dependent on the providers of the course, necessary efforts need to be taken to train the trainers for this programme. Making SBCT programme as a part of training of postgraduate students in general medicine, emergency medicine and respiratory medicine would be a welcome idea to get enough number of

Table 3: Catalogued set of suggestions from the CRRI's feedback

Frequency	More often – varying from 'twice a month' onwards
Choice of clinical scenarios	More clinical scenarios in emergency medicine required
Benefits	Improved our confidence of handling emergency care
	Ideal for inculcating team dynamics, communication skill and professionalism
	Helps us to face the reality
	Helps us to be open-minded in our approach in dealing with a clinical scenario
	SBCT helps to make decisions logically and quicker
	Realised the value of 'debriefing'
	Helps in gaining confidence in clinical management
Timing	Programme to be before posting in EMD
	Introduce SBCT during the orientation programme of the CRRIs
Mandate	Should be made mandatory for the interns
	For the pre-final and final year UG students
	Must be included in the UG curriculum
General	Very informative and useful
	Demonstration of a scenario before SBCT desirable
	The concept of SBCT is 'great' – learning outcome is 'superb'
	Equipment and infrastructural facilities were of the 'top' order
	Sim lab experience will help us save lives
	Considered introducing a 'credit point system' for SBCT
	Learnt 'what not to do'

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trainers for this course. Training of the postgraduate students in surgical specialities also could be included in this programme. It is to be understood that the medical interns are hard pressed for time and to get them to participate in a programme of this nature has not been easy. We were able to mobilise the interns during their postings in ENT and ophthalmology, when they are relatively less busy. It certainly required the co-operation and the concurrence of the heads of these two departments to depute the interns to take part in this important programme (Table 3).

Faculty members from the departments of respiratory medicine, general medicine and emergency medicine volunteered to be resource persons for this programme. They, in turn, managed to train their postgraduate students to utilise simulated clinical training as an effective method of teaching learning activity which needs to be nurtured further.

Postgraduate students in the specialities were requested to help in conducting this programme. This has facilitated in training of the postgraduate students of the concerned specialities in the upcoming realm of clinical simulation, which is gaining importance and significance in medical education.

Challenges involved mainly were concerned with identifying the time for SBCT. Training the resource persons from relevant specialities is yet another thrust area. Institutions with the infrastructural facilities for simulations will find it not too hard to embark on the emerging trend of simulated teaching.

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REFERENCES

[1] Jones F, Passos-Neto CE, Braghiroli OF. Simulation in medical education: brief history and methodology. Principles and Practice of Clinical Research 2015;1(2):56–63.

[2] Sanson-Fisher RW, Rolfe IE, Williams N. Competency based teaching: the need for a new approach to teaching clinical skills in the undergraduate medical education course. Medical Teacher 2005;27(1):29–36.

[3] Dillon GF, Boulet JR, Hawkins RE, Swanson DB. Simulations in the United States Medical Licensing Examination (USMLETM). Quality and Safety in Health Care 2003;13(Suppl 1):PP41–5.

[4] McGaghie WC, Issenberg SB, Petrusa ER, Scalese RJ. Effect of practice on standardised learning outcomes in simulation-based medical education. Medical Education 2006; 40(8):792–7.

[5] Berkenstadt H, Zia V, Gafni N, Sidi A. The validation process of incorporating simulation-based accreditation into the anaesthesiology Israeli national board exams. The Israel Medical Association Journal 2006;8(10):728–33.

[6] Scalese RJ, Obeso VT, Issenberg SB. Simulation technology for skills training and competency assessment in medical education. Journal of General Internal Medicine 2008; 23(Suppl 1):46–9.

[7] Hardie EM. Current methods in use for assessing clinical competencies: what works? Journal of Veterinary Medical Education 2008;35(3):359–68.

[8] Small SD, Wuerz RC, Simon R, Shapiro N, Conn A, Setnik G. Demonstration of high-fidelity simulation team training for emergency medicine. Academic Emergency Medicine 1999;6(4):312–23.

[9] Akaike M, Fukotomi M, Nagamune M, Fujimotot A, Tsuji A, Ishida K, et al. Simulation-based medical education in clinical skills laboratory. The Journal of Medical Investigation 2012;59:28–34.

[10] Welter JM, Nestel D, Brooks PM, Conn JJ. Simulation in clinical teaching and learning. The Medical Journal of Australia 2012;196(9):1–5.

[11] Kulkarni M. Medical internship training – challenges and possible solutions. Journal of Education Technology in Health Sciences 2017;4(1):5–6.

[12] Medical Council of India Vision. 2015. Available from: https://www.mciindia.org/documents/rulesAndRegulations/GME_REGULATIONS.pdf

[13] Zodpey S, Sharma A, Zahirrudin QS, Gaidhane A, and Shrikande S. Faculty development programs for medical teachers in India. Journal of advances in medical education and professionalism 2016;4(2):97-101.

[14] Chacko TV. Simulation-based medical education: using best practices and curriculum mapping to maximize educational benefits in the context of shift toward competency-based medical education. Archives of Medicine and Health Sciences 2017;5:9–15.

[15] Datta R, Upadhyay KK, Jaideep CN. Simulation and its role in medical education. Medical Journal Armed Forces India 2012;68(2):167–72.

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Urinary Tract Infection in Diabetics - A Five Year Retrospective Study on the Prevalence of Bacterial Isolates and its Antibiotic Susceptibility Patterns in A Tertiary Care Hospital in South India

Jagadeeswaran G[1], Mohammad Zeya Ansari[2], Tolstoy Rajangam[3]

ABSTRACT

Introduction: Urinary tract infection (UTI) is almost most common seen infection in the diabetic patient due to various local and systemic immunopathogenetic reasons. This causes multiple renal and extrarenal complications and even death if not intervened early. We aimed to find out the prevalence of different bacteria in urine culture and the antibiotic susceptibility pattern amongst diabetic patients admitted in our hospital and to start empirical antibiotics for early recovery and to prevent complications.

Material and Methods: Retrospectively, 651 culture positive urine samples were taken for study with inclusion and exclusion criteria. We saw all the case records and investigations of every subject to correlate with criteria of selection. Our laboratory follows the standard protocols for urine sampling, timely transportation and microbiological study. Antibiotic susceptibly was tested by Kirby Bauer method and interpretations were done by clinical laboratory and standards institute (CLSI) guidelines.

Results: Out of total 651 patients most commonly affected age group was 51-60 years (30.26%).Here males (53.3%) dominated females (46.7%) in number with little difference. Most common Gram-negative bacteria were Escherichia coli (69.12%) and Klebsiella pneumoniae (8.60%) while most common Gram-positive bacteria were Enterococcus faecalis (10.90%) and Staphylococcus aureus (2.91%).Most of the gram-negative bacteria were having a good susceptibility to amikacin, piperacillin/tazobactam, cefoperazone/sulbactam and carbapenems while Gram-positive bacteria were mostly sensitive to nitrofurantoin, linezolid and vancomycin.

Conclusion: in our study, the occurrence of UTI among the diabetic is commoner in the sixth decade with a male predominance. We found E.coli, E.faecalis, and K.pneumoniae as the most commonly grown organisms in descending order. Most of the Gram-negative isolates were susceptible to amikacin, piperacillin/tazobactam, cefoperazone/sulbactam and carbapenems, while most of the Gram-positive cocci were sensitive to nitrofurantoin, linezolid, and vancomycin. Antibiotic resistance is seen very commonly in our setup. We cannot depend upon frequently used oral antibiotics and certain groups like fluoroquinolones and cephalosporin (except for sulbactam containing ones) and ampicillin for empirical treatment in UTI especially the complicated ones.

Keywords: Urinary Tract Infection, Diabetics, Prevalence, Isolates, Antibiotic Susceptibility

INTRODUCTION

The urinary tract is a principal site of infection in diabetics.[1] UTI (Urinary Tract Infection) encompasses a variety of clinical entities, including ABU (asymptomatic bacteriuria), cystitis, prostatitis, and pyelonephritis. There can be an increased risk of ABU or symptomatic UTI in diabetics.[2]

Urinary tract infection can be uncomplicated or complicated. Uncomplicated UTI can be asymptomatic bacteriuria, cystitis, pyelonephritis, in men and nonpregnant women, also prostatitis in men. Complicated UTI can be symptomatic episodes of cystitis or pyelonephritis in men or women with an anatomic predisposition to infection, with a foreign body in the urinary tract, or factors predisposing to a delayed response to therapy. There can be a relapse or reinfection. A relapse is a recurrent infection with an organism similar to the pretherapy isolate, usually following persistence of the organism in the genitourinary tract. A reinfection is a recurrent infection with a new organism. The quantitative criteria of at least 10^5 CFU (colony forming units)/mL are generally appropriate for the microbiological identification of complicated urinary infection. Bacteriuria is more common in diabetics than in non-diabetics due to a combination of host and local risk factors.[3] Some microorganisms become more virulent in a high glucose environment. Therefore, screening for UTI in diabetic patients is very important to enable bacteruria to be properly treated.[4] There is a significant correlation between duration of diabetes and UTI.[5] Changes in host defense mechanisms, the presence of diabetic cystopathy and of microvascular disease in the kidneys may play a role in the higher incidence of UTI in diabetic patients. As diabetics are more prone to UTI, it is necessary to pay special attention to early diagnosis and treatment.[6]

The aim of this study was to determine the causative bacteria, culture and sensitivity pattern in diabetics with Urinary tract infection in our setup. This will guide us to start an

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appropriate timely empirical antibiotic which will prevent the diabetic patients from the complications of UTI like pyelonephritis, urosepsis, renal failure etc. This can decrease the hospital stay, morbidity, and mortality.

MATERIAL AND METHODS

This was a 5 year (August 2010 to August 2015) study done in PSG Hospital, Peelamedu, Coimbatore and was based on the retrospective collection of data from case records of patients who were admitted under medicine department of PSG Hospital. Only those cases that fulfill the inclusion criteria were analyzed. To know the reliability of causative organism, we went through the case records and looked into the various clinical and laboratory parameters of the Diabetic patients who have culture positive UTI.

As per the standard protocol clean voided midstream urine samples were collected in sterile containers. In sampling procedure, all the patients were given proper information while collecting the urine sample. Urine culture reports that exhibited CFU of more than 10^5/ml were considered positive. Only single isolates were taken for study purpose and mixed growths were excluded. The pathogens were isolated and identified using phenotypic methods and biochemical testing. Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI). We took nine types of isolates and went through their susceptibility patterns to 26 antibiotics. Ethical clearance was obtained before the start of the study.

Inclusion criteria:

1. Diabetic patients
2. Sex: Both male and female
3. Age: 18 years and above
4. Urine culture positive cases

Exclusion criteria:

1. Nondiabetic
2. Pregnancy
3. Known congenital/acquired urogenital anomaly
4. Patients on chronic catheterization
5. Candiduria

STATISTICAL ANALYSIS

The data was descriptively analyzed using Microsoft Excel 2007 software.

RESULT

As per our finding depicted in Table 1 most of the patients were in the age range 51 to 60 years (30.26%) followed by 61 to 70 years (22.27%) and next 41 to 50 years (20.89%) while lower and higher of age groups are lesser affected.

In figure 1 regarding the gender predilection, males were more affected than females (53.3% vs 46.7%).

From the distribution pattern seen in figure 2, it is clear that most common Gram-negative bacteria were E.coli (69.12%) followed by Klebsiella pneumoniae (8.60%). Out of Gram-positive organisms most frequently seen bacteria (figure 3), were Enterococcus faecalis (10.90%) followed by Staphylococcus aureus (2.91%). So, the actual second most common bacteria seen were E.faecalis.

Age	No.of patients	Percentage
10-20	2	0.3
21 -30	18	2.76
31 – 40	39	5.99
41 – 50	136	20.89
51– 60	197	30.26
61– 70	145	22.27
71 – 80	87	13.36
81 – 90	27	4.14
90– 100	0	0
Total	651	100

Table-1: Distribution of patients by age

Age group	In male	In female
10-20	1	1
21-30	10	8
31-40	20	19
41-50	69	67
51-60	100	97
61-70	81	64
71-80	48	39
81-90	18	9
91-100	0	0

Table-2: Number of urine samples with single isolate in various age groups

S.N.	Bacteria	AK	GM	VCM	NA	LOX	NOX	COX	CPH	CTZ	CTX	CEP	CEF-S	AMP	PIP-T
1	E.coli	96	46	-	7	31	18	19	16	20	22	28	71	10	77
2	E.faecium	-	14	100	-	-	-	-	-	-	-	-	-	43	-
3	E. faecalis	75	28	100	17	11	11	-	-	-	-	-	-	54	-
4	K.pneumonaie	81	56	-	42	54	45	37	0	34	35	39	54	2	59
5	P. aeruginosa	72	69	-	-	-	50	40	-	68	-	61	67	-	82
6	Staph.aureus	-	86	100	-	-	20	33	-	-	100	-	-	-	-
7	Citrobacter spp	50	50	-	-	100	47	-	-	51	-	39	-	-	50
8	Proteus spp	91	80	-	20	60	82	40	50	89	67	78	80	-	100
9	Acinetobacter spp.	20	25	-	-	-	-	50	-	25	-	26	-	-	60

AK=amikacin, GM=gentamycin, VCM=vancomycin, LOX=levofloxacin, NOX=norfloxacin, COX=ciprofloxacin, CPH=cephalexin, CTZ=ceftazidime, CTX=ceftriaxone, CEP=cefipime, CEFS=cefoperazone/sulbactam, AMP=ampicillin, PIP-T, piperacillin/tazobactam

Table-3: (part-1) Antibiotic sensitivity in percentage

S.N.	Bacteria	MNM	INM	ENM	CTMX	NIT	TET	LNZ	COLI	RIF	TIGE	CLIND	AMXCL
1	E.coli	95	96	96	34	81	38	-	99	-	97	100	33
2	E. faecium	-	-	-	-	77	40	100	-	-	-	-	29
3	E. faecalis	-	-	-	-	82	47	100	-	-	-	-	-
4	K.pneumonaie	79	83	81	56	28	-	-	91	-	63	39	-
5	P.aeruginosa	50	78	-	0	20	-	-	82	-	-	-	0
6	Staph.aureus	-	-	-	57	100	-	100	-	10	-	50	40
7	Citrobacter spp.	54	59	-	-	-	-	-	-	-	-	-	-
8	Proteus spp.	88	71	75	50	20	-	-	-	-	-	-	50
9	Acinetobacter spp.	-	40	-	80	-	-	-	50	-	-	-	-

(MNM=meropenem, INM=imipenem, ENM=ertapenem, CTMX=cotrimoxazole, NIT=nitrofurantoin, TET=tetracycline, LNZ=linezolid, COLI=colistin, RIF=rifampicin, TIGE=tigecycline, CLIND=clindamycin, AMXCL=amoxicillin/clavulinic acid, CXIM=cefixime)

Table-3: (part-2) Antibiotic sensitivity in percentage

S.N.	Organisms	AK	GM	NA	CXIM	CTX	AMP	CTMX	AMXCL
1	E.coli	3	51	93	100	78	90	66	52
2	K.pneumonaie	16	44	58	100	65	98	44	56
3	P.aeruginosa	33	33	-	-	-	-	-	-
4	Citobacter spp.	50	50	100	-	-	-	100	-
5	Proteus spp.	9	20	80	-	33	30	50	50
6	E.faecalis	25	97	83	-	-	46	-	40
7	Staphylococcus aureus	-	14	-	-	0	100	43	60
8	E.faecium	-	80	-	-	-	57	-	71

Table-4: Resistance to commonly used antibiotics (in percentage)

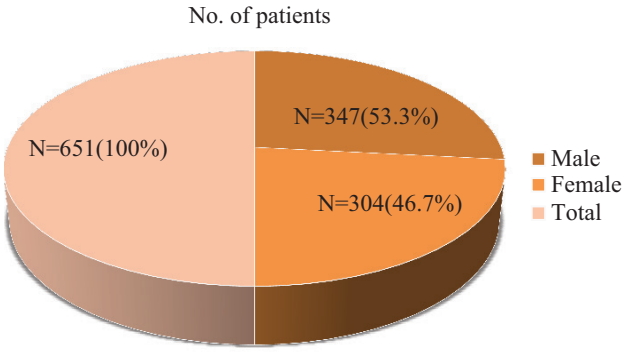


Figure-1: Distribution of patients by gender

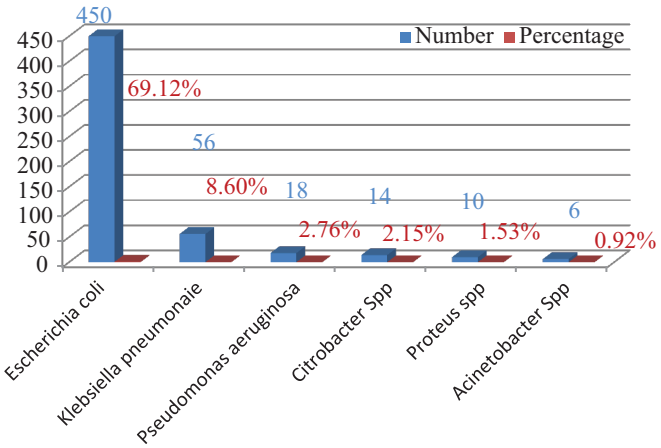


Figure-2: Distribution of Gram –negative bacteria

From table 2 we observed that males had higher prevalence of bacteriuria compared to females in all the age groups. There were only two young patients above 18 and below 20 years age group.

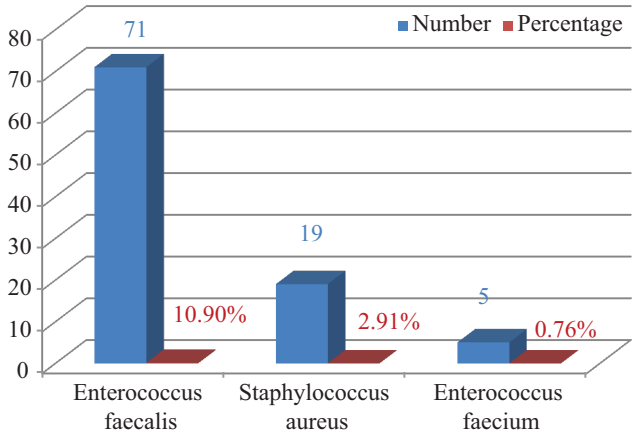


Figure-3: Distribution of Gram-positive bacteria

As shown in table 3 and 4, good susceptibility was seen to amikacin by E.coli (96%) K.pneumoniae (81%), P.aeruginosa (72%) and Proteus spp. (91%). Linezolid and Vancomycin showed 100% activity against Enterococci and Staphylococcus aureus. Amongst fluoroquinolones, ciprofloxacin has low activity (40%) against Pseudomonas while levofloxacin stands good (100%) for Citrobacter and lower (60%) for Proteus. We saw poor response to norfloxacin against most bacteria grown except Proteus spp. which has good (82%). From amongst the cephalosporins, cephalexin, cefixime and ceftazidime are not good options for most bacteria but Cefoperazone-sulbactam stands good (>65%) against E.coli, P.aeruginosa and Proteus spp. Ceftazidime shows 68% and 89% sensitivity against P.aeruginosa and Proteus spp.respectively. ceftriaxone which is very commonly used injectable to start empirically in inpatient setup is not a good choice due to very low sensitivity in E.coli (22%) and

K.pneumoniae (35%). High level Ampicillin resistant is seen in both Gram-positive and negative bacteria.

If we look at E.coli –ESBL (71.42%) and Klebsiella pneumoniae - ESBL(56.90%), best options were carbapenems, colistin, tigecycline, amikacin, piperacillin-tazobactam and nitrofurantoin. There were carbapenemase producers also like in E.coli (4-5%) and Klebsiella (20%). Cotrimoxazole and amoxicillin-clavulanic acid have no good response (<60% sensitivity) to any of these bacteria. Linezolid is good for both the species of Enterococcus (100% susceptible). Nitrofurantoin sensitivity was good in Staph. aureus (96%), E. faecalis (82%) and E.coli (81%). Best response giving carbapenems against K.pneumonaie and P.aeruginosa was by imipenem (83% and 78% respectively) while against Proteus spp.meropenem has good activity (88%). Acinetobacter spp. Which was seen in 6 patients has poor sensitivity to amikacin (20%), imipenem (40%) and colistin (50%) but good sensitivity to cotrimoxazole (80%). We found 3 MRSA out of 19 isolates.

High-grade resistance was seen for many commonly used antibiotics like ampicillin, cotrimoxazole, cefixime, nalidixic acid and amoxicillin/clavulanate (table: 5).

DISCUSSION

In this study, we were supposed to see the prevalence of various bacteria in diabetics with proven UTI and antimicrobial susceptibility patterns. We found that Diabetic males are outnumbering females in terms of prevalence of UTI. This is in contrast to an Indian study[7], a Nigerian study[8] but in favor of study in Sudan[9] and east India.[10]

Most frequently patients were in the affected age group from 51 to 60 years(30.26%).This was Similar to a study by R. Simkhada.[11] but dissimilar to a study in Kuwait by May Sewify et al[12] and in Nepal by PK Jha et al.[13]

Like in almost all the studies from international or national locations[14,15], the most common bacterial isolate was E.coli(59.12%). It was similar to a case-control study done in New Delhi[16] which showed E.coli as most common bacteria in UTI (64.3%). In a study from Nepal[11], it was 52.38%. While in Romanian study[17] it was 70.4%.

In our study, we found Klebsiella pneumoniae (8.60%) and Pseudomonas aeruginosa (2.76%) as next common isolate amongst Gram-negative while Enterococcus faecalis (10.90%) and Staphylococcus aureus (2.91%) are most common Gram-positive isolates. In a study done in Turkey, K.pneumoniae and P.aeruginosa were 9.1% and 3% respectively[18] which was in the same line as ours. Overall in our study second most common isolate is E.faecalis (10.90%) after E.coli (59.12%) which is on the same trend as seen by N.Chatterjee et al.[10] In contrast, second most common isolate in the above mentioned Turkish study was CoNS (24.2%). If we see a retrospective study in Nepal, there was a similar trend of the frequency of Enterococcus spp.(13.84%), K.pneumoniae (8.3%) and S.aureus (7.11%). We also found Citrobacter spp, Proteus spp, and Acinetobacter spp though less frequently.

In this study, seeing antibiotic resistance we found that ampicillin and cefixime are not good options as high-level resistance is seen for commonly grown isolates of E.coli and K.pneumonaie. Citrobacter spp.is 100% resistant to nalidixic acid and cotrimoxazole and equally resistant to amikacin and gentamycin i.e 50%.There is high resistance to gentamycin, ampicillin and amoxicillin-clavulanic acid against Gram-positive organisms especially Enterococcus spp.Fluoroquinolones and cephalosporins are resistant to most of the Gram-negative isolates. Cephalosporin resistance was similarly seen in Nepal[13] and Bangladesh.[19] Good options for them are amikacin, piperacillin/tazobactam, and cefoperazone/sulbactam. Our study has a high prevalence of ESBL in E.coli (71.42%) and K.pneumonaie (56.90%) which needs to be managed with carbapenems as first-line drugs and if not feasible, second-line drugs should be piperacillin/tazobactam or cefoperazone /sulbactam. Though ESBL organisms are in similar frequency as shown in many other studies it was lower compared to a study from Kerala[20] which showed very high-level ESBL growth (approximately 90%) and from Manipal[21] by M.Srinivas et al which showed it 78.6%. Though amikacin is a cost-effective injectable for both non-ESBL and ESBL isolates, its renal toxicity restricts its widespread use.

In our findings, nitrofurantoin had good activity against E.coli and Gram-positive isolates similar to as seen by PK Jha et al[13] and Citrobacter spp. had approximately 50% susceptibility to most of the tested antibiotics but 100% for levofloxacin.

If we see options with antipseudomonal activity in our set up and susceptibility pattern, best antibiotics are Piperacillin / tazobactam and colistin with equal efficacy (82%) followed by imipenem and amikacin (78% and 72% respectively). Here ciprofloxacin has low efficacy (40%) and cotrimoxazole was 100% resistant to P.aeruginosa. Similar findings were seen by M.Srinivas et al[21] with 82% susceptibility to Piperacillin/ tazobactam and 68.2% to cefoperazone/ sulbactam but a very good response to amikacin (93.8%). Meropenem activity against P.aeruginosa in our study was only 50% while M.Srinivas et al[21] found it 87.5%.

In our findings, Gram-positive isolates have shown 100% sensitivity to vancomycin while an article from south India shows 81% and 94% susceptibility respectively by Enterococcus and Staphylococcus.[22] We have less nephrotoxic linezolid with 100% sensitivity against Gram-positive isolates. In an Indian study, Enterococcus faecalis was the most commonly isolated Gram-positive organism with 3.2 percent resistance to vancomycin.[23] We found Acinetobacter spp.in 6 cases showing multidrug-resistant with very low sensitivity even to carbapenems and colistin.

Multi-drug resistant bacteria commonly arise due to the prolonged use of cephalosporins and other antibiotics, particularly their use as prophylaxis treatment, inadequate dosage or inaccurate frequency of administration. This also produces class and cross-resistance. Pseudomonas spp. has become a big challenge to treat. Plasmid-mediated resistance due to the usage of broad-spectrum antibiotics has been considered as one of the factors associated with this

phenomenon.[24]

Amikacin which is easily available and cost-effective remains a good choice for most of the uropathogens if renal status allows and there is a facility to follow renal function test regularly. There is good sensitivity even in current era due to its ability to evade attacks by all antibiotic-inactivating enzymes that are responsible for antibiotic resistance in bacteria, except for aminoacetyltransferase and nucleotidyltransferase.[25] This is accomplished by the L-hydroxyaminobuteroyl amide (L-HABA) moiety attached to N-1 (compare to kanamycin, which simply has a hydrogen), which blocks the access and decreases the affinity of aminoglycoside-inactivating enzymes.[26,27] Amikacin ends up with only one site where these enzymes can attack, while gentamicin and tobramycin have six.

Enterococci are getting commoner in many culture samples especially from urine due to inappropriate and intensive use of broad-spectrum antibiotics.[28] Same are the reasons in cases of other microorganisms. Poultry farms and human foods of animal origin are also contributing significantly to the increasing multidrug resistance amongst isolates.

These are the reasons, why to have knowledge of the pattern of local microbial prevalence and antimicrobial susceptibility to start the most appropriate empirical drug depending on clinical and laboratory profile of patients.

CONCLUSION

Most commonly affected age group with UTI amongst diabetics was from 51 to 60 years where males exceeded females in prevalence. We found E.coli, E.faecalis, and K.pneumoniae as the most commonly grown organisms in descending order. Most of the Gram-negative isolates were susceptible to amikacin, piperacillin/tazobactam, cefoperazone/sulbactam and carbapenems, while Gram-positive cocci were mostly sensitive to nitrofurantoin, linezolid, and vancomycin. Ampicillin, cephalosporins (with the exception of sulbactam combination), cotrimoxazole, and fluoroquinolones are not good choices to start on an empirical basis.

REFERENCES

1. Bryan CS, Reynolds KL, Metzger WT. Bacteremia in diabetic patients: Comparison of incidence and mortality with non-diabetic patients. Diabetic Care 1985; 244-249.
2. Muller LMAJ, Gorter KJ, Hak E et al.Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005;41:281-288.
3. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus. FEMS Immunol Med Microbiol. 1999; 26:259–65.
4. Bahl AL, Chugh RN, Sharma KB. Asymptomatic bacteremia in diabetics attending a diabetic clinic. Indian J Med Sci. 1970;24:1–6.
5. Patil, N. R., Mali, U. S., Ramtirthkar, M. N., Bhave (Sule), A. P., Mali, S. S., Mane, V. S. Bacteriuria in diabetic patients. World Journal of Science and Technology 2012;2:25-27.
6. Geerlings SE, Stolk RP, Camps MJ, Netten PM, Hoekstra JB, Bouter KP, et al. Asymptomatic bacteriuria may be considered a complication in women with diabetes. Diabetes Mellitus Women Asymptomatic Bacteriuria Utrecht-Study Group. Diab Care. 2000;23:744–9.
7. Janifer J, Geethalakshmi S, Satyavani K, Viswanathan V. Prevalence of lower urinary tract infection in South Indian type 2 diabetic subjects. Indian J Nephrol 2009; 19:107-11.
8. Ifediora, A.C., Obeagu, E.I., Akahara, Ijeoma Chukwudi and Eguzouwa Uloma Priscilla-J.Bio.Innov 2016; 5: 68-82.
9. Hamdan et al. Annals of ClinicalMicrobiology and Antimicrobials 2015;14:26.
10. Nandini Chatterjee, Chandan Chatterjee, Sinjan Ghosh, Mainak, Mukhopadhyay, Ramkrishna Brahmachari,Kartik Patar-The Journal of the Association of Physicians of India 2016;64:26-30.
11. R. Simkhada. Nepal Med Coll J 2013;15:1-4.
12. May Sewify, Shinu Nair, Samia Warsame, Mohamed Murad, Asma Alhubail, Kazem Behbehani, Faisal Al-Refaei, and Ali Tiss- Journal of Diabetes Research Volume 2016 .
13. Jha P K, Baral R, Khanal B. International Journal of Biomedical Laboratory Science 2014;3:29-34.
14. Baloch G. H., Jeffery, M. H., Madhudas, C., Devrajani, B. R., and Ali Shah, S. Z.Frequency and pattern in patients with diabetes mellitus. Professional Med J 2011;18: 466-69.
15. Chukwuocha, U. M., Emerole, C. O., Njokuobi, T.N., and Nwawume, I. C.Urinary tract infections (UTIs) associated with diabetic patients in the Federal Medical Center Owerri, Nigeria. Global Advanced Research Journal of Microbiology 2012;1:062-066.
16. Goswami R, Bal CS, Tejaswi S, Punjabi GV, Kapil A, Kochupillai N. . Prevalence of urinary tract infection and renal scars in patients with diabetes mellitus. Diabetes Res Clin Pract. 2001; 53:181-6.
17. Teodora Chiţă, Bogdan Timar, Delia Muntean, Luminiţa Bădiţoiu, Florin Horhat, Elena Hogea, Roxana Moldovan, Romulus Timar, Monica Licker-Dove medical press 2016; 2017:13 Pages 1—7.
18. Demiss Nigussie, Anteneh Amsalu, Turk J Urol 2017; 43: 85-92.
19. Shill MC. Prevalence of Uropathogens in Diabetic Patients and Their Corresponding Resistance Pattern: Results of a Survey Conducted at Diagnostic Centers in Dhaka, Bangladesh. Oman Medical Journal 2010;25:282-285.
20. Prabhu H, Oommen AT, Henry R. Clinico-Microbiological Profile of Urinary Tract Infections in Diabetic Patients in a Tertiary Centre in Kerala. Kerala Medical Journal. 2016; 9:97–104.
21. Srinivas M Aswani, UK Chandrashekar, KN Shivashankara, and BC Pruthvi. Australas Med J. 2014; 7: 29–34.
22. Abdulla MC, Feroz P. Jenner, Jemshad Alungal- Int J Res Med Sci. 2015; 3:2576-2579.
23. Mandal J, Acharya SN, Buddhapriya D, Parija SC. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant Escherichia coli. Indian J Med Res. 2012;

136:842.

24. Lister P.D., Wolter D.J., Hanson N.D. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev. 2009;22:582–610.
25. Mudd, Efrain (7 August 2017). O Aminoglycosides. Pharmacological Sciences.
26. Kondo, Shinichi; Hotta, Kunimoto. Semisynthetic aminoglycoside antibiotics: Development and enzymatic modifications. Journal of Infection and Chemotherapy. 1999; 5: 1–9.
27. Park, Je Won; Ban, Yeon Hee; Nam, Sang-Jip; Cha, Sun-Shin; Yoon, Yeo Joon. Biosynthetic pathways of amino glycosides and their engineering. Current Opinion in Biotechnology. Chemical biotechnology: Pharmaceutical biotechnology. 2017; 48: 33–41.
28. Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: Results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis. 2001;32: S133–45.

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A Rare Etiology of Severe Thrombocytopenia in Patient with Chronic Liver Disease

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Abstract

Thrombocytopenia is a common complication in patients with chronic liver disease and is multi-factorial. It complicates the management and worsens the prognosis. Treatment options are costly and include platelet transfusion, splenectomy, splenic artery embolization, TIPPS and thrombopoietin (TPO) agonists.

Here we are presenting a patient with decompensated liver disease with known chronic alcoholism and profound thrombocytopenia despite multiple platelet transfusions. On further work up we found a coexistent autoimmune etiology. Thrombocytopenia promptly responded to steroids, a cost effective option.

Introduction

Thrombocytopenia, a common complication in patients with chronic liver disease, is seen in nearly 75% of cirrhotic patients.[1] The pathogenesis of thrombocytopenia in chronic liver disease is multi-factorial.[1] Initially it was thought that thrombocytopenia in CLD is only due to portal hypertension where there will be an enlargement of spleen which causes destruction of platelets. So initially treatment for thrombocytopenia was aimed at reversing portal hypertension. But persistently decreased platelets were noted even in patients without splenomegaly.[2] So other factors like intrasplenic production of autoantibodies and plasma expansion resulting in hemodilution also contribute to thrombocytopenia. Suppression of platelet production in bone marrow is also another factor which is seen in HCV infection and alcohol related chronic liver disease. Antiviral therapy like interferon alfa also induces thrombocytopenia.[3] In some patients of CLD there is an evidence based autoantibodies related destruction of platelets and it is mostly observed in HCV related cases.[4] Here autoantibodies are directed against platelet surface antigens and enhance the removal of platelets by splenic and hepatic reticuloendothelial systems there by triggering rapid destruction as seen in chronic ITP. TPO is a potent cytokine produced by liver, bone marrow and kidney which regulates megkaryocytes and platelet production.[5] In cirrhotic patients with thrombocytopenia, low circulating levels of TPO is seen compared to cirrhotic patient with normal platelet count.[6] The treatment options available for severe thrombocytopenia are platelet transfusion, splenic artery embolization, splenectomy and TIPS. Platelet transfusion is indicated in patients with less than 10,000-20,000/µl platelets in uncomplicated patients and less than 50,000/µl in patients undergoing procedures.[7] Newly derived options include recombinant interleukin-11, eltrombopag and recombinant TPO.

Case Summary

A, 50 year old male patient with ethanol related chronic liver disease and no other comorbidities, came to EMD with the complaints of melaena, gum bleed and haematuria for 10 days, fever with chills, abdominal pain and distention for 3 days.

On examination, patient was pale and febrile with stable vital parameters. He had minimal ascites and epigastric tenderness.

Serial CBC, LFT and RFT are summarized in Table.1. Upper GI endoscopy showed esophageal varices and portal gastropathy and EVL done immediately. Ultrasound abdomen Showed features of CLD and portal hypertension with medical renal disease. Ascitic fluid analysis had no evidence of peritonitis. Urine culture showed significant bacteria (E.coli) and managed accordingly. He had severe thrombocytopenia which was managed with platelet transfusions. Bone marrow aspiration and biopsy showed dimorphic anemia with thrombocytopenia, megaloblastic erythroid hyperplasia and mild megakaryocytic hyperplasia. His serum B12 and folate level were within normal range. Hepatis B, C, HIV, Dengue virus, *Helicobacter pylori*, scrub typhus, leptospirosis infection and Coomb's test were negative. Renal failure was stabilized with hemodialysis.

As his thrombocytopenia persisted despite multiple platelet transfusions (total 19 units) and other common causes of thrombocytopenia being ruled out, we suspected of some uncommon etiology including an autoimmune condition. ANA tested was positive with fine granular++, cytoplasmic+ pattern. He was positive for smooth muscle antibody and negative for anti-ds DNA Ab and hence he was diagnosed to have autoimmune hepatitis type-1 with associated ITP. We started steroids (Inj. methylprednisolone 125 mg IV OD for 3 days followed by oral prednisolone 60mg OD). His platelet count started improving from 2nd day of IV steroid.

On follow-up visits, his platelet count was in safe range (> 1 lakh / cu.mm3) with low dose oral steroid and is currently being managed as ITP.

Discussion

The causes of thrombocytopenia in chronic liver disease may be due to sequestrating function of spleen, presence of antiplatelet antibodies and impaired thrombopoietin production in cirrhotic patients.[6] It is very important to differentiate ITP from common cause of thrombocytopenia in CLD as the management differs. Hypersplenism in liver disease due to portal hypertension

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Table 1: Blood investigation (CBC, LFT, RFT) reports

	Normal	Day1	Day3	Day5	Day7	Day10
Hb (g/dl)	13-17	7.1	6.4	6.0	7.8	9.6
PCV (%)	40-50	21.3				
WBC count/µl	4-10X10^3	5.4X10	5.0X10^3			76X10^3
MCV (fl)	80-100	96.6				
Neutrophils (%)	40-80	70.7				
Lymphocytes (%)	20-40	17.6				
Monocytes (%)	2-10	10.8				
Eosinophils (%)	1-6	0.9				
Basophils (%)	0-2	0.0				
Platelet count/µl	150-400X10^3	6X10^3	3X10^3	4X10^3	36X10^3	125X10^3
ESR (AEFH)	3-10	106	98	78	78	45
Serum Protein (g/dl)	6.4-8.3	6.5		6.8		6.9
Serum Albumin (g/dl)	3.4-4.8	2.3		2.6		2.9
Serum globulin (g/dl)	1.8-3.6	4.2		4.2		4.0
Total bilirubin (mg/dl)	0.2-1.0	1.2				1.1
Direct/indirect	0-0.2/0.2-0.8	0.8/0.4				
SGOT (u/l)	5-38	62				54
SGPT (u/l)	5-41	15				14
ALP (u/l)	40-129	205				154
GGT (u/l)	10-66	409				126
Serum creatinine (mg/dl)	0.8-1.25	2.3	4.43	3.95	3.4	2.1

causes increased sequestration of platelets within spleen leaving less in circulation. However it is also to be clear that thrombocytopenia can occur in liver cirrhosis without splenomegaly.[2] Thrombopoietin is a cytokine produced by the liver which plays a key role in platelet production and subsequent function may be low in cirrhosis. Thrombocytopenia in liver disease without evidence of cirrhosis, splenomegaly and interferon is recognized especially in patients with hepatitis C and auto immune hepatitis.

Based on persistent thrombocytopenia, ANA and anti SMA positivity we diagnosed him as AIH type-1 and associated ITP. The International Autoimmune Hepatitis Group proposed a scoring system to standardize the diagnosis, useful for clinical trials, but that may be inaccurate in individual cases.[8] Liver biopsy was not done for two reasons-severe thrombocytopenia and patient refusal.

Autoimmune hepatitis (AIH) is a chronic and progressive necroinflammatory and fibrotic process of the liver of unknown cause that occurs in children and adults, usually associated with the presence of autoantibodies and Hypergammaglobulinemia.[8] Affected individuals often have concurrent extrahepatic autoimmune disorder like rheumatoid arthritis, Sjogren's syndrome and auto immune thyroiditis but association with idiopathic (immune) thrombocytopenic purpura has been rarely reported.[9-13]

ITP is an autoimmune disease characterized by a low platelet count, mucocutaneous bleeding, normal bone marrow findings and the absence of other causes of thrombocytopenia in which anti-platelet autoantibodies such as PAIgG induces platelet destruction.[14] There is a case series of 5 patients with AIH type-2 where patients had at least one associated autoimmune disorder including IgE-induced IgA deficiency, ITP, and arthritis.[15] In an Indian case series of 10 patients with AIH, three had underlying rheumatic disease (SLE, Sjogren's and Rheumatoid arthritis each one), while others had primary AIH. In the same report, 2 patients were found to have autoimmune thrombocytopenia and were managed by immunosuppressant.[16] Indeed prednisolone administration is a treatment option for AIH and is also effective for ITP. Therefore ITP should be considered when liver dysfunction is accompanied by severe thrombocytopenia particularly in the autoimmune types of liver disease.

Though in our patient there was normal bilirubin, SGOT and SGPT (which is usually elevated in hepatitis) but persistent thrombocytopenia and positive ANA with SMA made us to consider it AIH type-1.

Conclusion

It is worthy to evaluate for an alternative autoimmune etiology for CLD if thrombocytopenia is severe or persisting as there are better and cost effective treatment options i.e. steroids.

Learning points

1. All CLD with history of alcohol ingestion is not purely alcohol related.
2. Thrombocytopenia in CLD should be evaluated for causes other than alcohol and hypersplenism.
3. All autoimmune hepatitis may not have gross LFT derangement.

References

1. Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Weksler B, Esteban R. *J Hepatol* 2008; 48:1000-7.
2. García-Suárez J, Burgaleta C, Hernanz N, Albarran F, Tobaruela P, Alvarez-Mon M. HCV-associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha2b-interferon therapy. *Br J Haematol* 2000; 110: 98–103.
3. Fried MW. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347:975-82.
4. Weksler BB. *Aliment Pharmacol Ther* 2007; 26Suppl1:13-9.
5. Qian S, Fu F, Li W, Chen Q, de Sauvage FJ. Primary role of the liver in thrombopoietin production shown by tissue-specific knockout. *Blood* 1998; 92:2189–2191.
6. Koruk M, Onuk MD, Akcay F, Savas MC. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis, and its relationship with circulating thrombocyte counts. *Hepatogastroenterology* 2002; 49:1645-8.
7. Liumbruno G, Bennardello F, Lattanzio A, Piccoli P, Rossetti G; Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) Work Group. Recommendations for the transfusion of plasma and platelets. *Blood Transfus* 2009; 7:132–150.
8. Krawitt EL. Autoimmune hepatitis. *N Engl J Med* 1996; 334:897-903.
9. Manns MP, Strassburg CP. Autoimmune hepatitis: clinical challenges. *Gastroenterology* 2001; 120:1502-17.
10. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; 31:929-38.
11. Yonetsu M, Kakizaki S, Katakai K, et al. A case of autoimmune hepatitis associated with idiopathic thrombocytopenic purpura. *Jpn J Gastroenterol* 1997; 94:610-4.
12. Omagari K, Kinoshita H, Kato Y, et al: Clinical features of 89 patients with autoimmune hepatitis in Nagasaki Prefecture. *Japan J Gastroenterol* 1999; 34:221-6.
13. Pfueller SL, Firkin BG, Kerlero de Rosbo N, Riglar A, Mackay IR. Association of increased immune complexes, platelet IgG and serum IgG in chronic active hepatitis. *Clin Exp Immunol* 1983; 54:655- 60.
14. Pamuk GE, Pamuk ON, Baslar Z, el at. Overview of 321 patients with idiopathic thrombocytopenic purpura. Retrospective analysis of the clinical features and response to therapy. *Ann Hematol* 2002; 81:436-40.
15. Duchini A, McHutchison JG, Pockros PJ. LKM-positive autoimmune hepatitis in the western United States: a case series. *Am J Gastroenterol* 2001; 96:3447-8.
16. Balakrishnan C, Mangat G, Kalke S,Desai D, Joshi A, Deshpande RB, Joshi VR. The spectrum of chronic autoimmune hepatitis. *J Assoc Physicians India* 1998; 46:431-5.

A Tertiary Care Hospital based Study on the Prevalence of Pulmonary Hypertention in Patients with Glomerular Filtration Rate less than 30 Ml /Min Per 1.73 Meter Square on Dialysis

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ABSTRACT

Introduction: Pulmonary artery hypertension (PAH) which can be primary or secondary, has been found to be associated with chronic kidney disease, especially end stage renal disease. Finding prevalence of pulmonary hypertension in early stages of chronic kidney disease is important because it creates very high burden of cardio vascular morbidity and mortality. In this study we have assessed various factors associated with prevalence of pulmonary hypertension in patients with glomerular filtration rate less than 30ml/min per 1.73 meter square on dialysis.

Material and methods: This is a one year, hospital based, prospective observational study of patients aged more than 18 years with GFR less than 30 ml/min per 1.73-meter square on dialysis, who were found to have pulmonary hypertension on echocardiography. The prevalence of pulmonary hypertension in this group of patients and the risk factors in CKD associated with it are calculated followed by a descriptive analysis and interpretation of the data.

Results: The various factors we analyzed and p value for association of pulmonary hypertension in CKD for the percentage of male and female in our study group is 0.241, for distribution of the pulmonary hypertension among different age groups is 0.503, for the significance of diabetic mellitus is 0.595,for systemic hypertension is 0.206, for arterial venous fistula is 0.780,for superimposed infections 0.166, for volume overload is 0.560, for anemia is 0.780, for left ventricular diastolic dysfunction is 0.662, for creatinine clearance is 0.717,for duration of dialysis is 0.000. With above results, only association with significant p-value (0.000) in our study population with pulmonary hypertensions longer duration.

Conclusion: Prevalence of pulmonary hypertension in our study is 22%. The risk factors like age,sex, diabetes, systemic hypertension, AVF, superimposed infection, volume overload, anaemia, LVDD has no influence on pulmonary hypertension in our study, only strong association that we have in our study population with pulmonary hypertension with CKD is longer duration of dialysis.

Keywords: Pulmonary Hypertension (PH), CKD

INTRODUCTION

Though, there has been fast advancement in the medical field which helps in the early and accurate diagnosis of health status of a patient, pulmonary hypertension remains a hidden problem that takes plenty of time in diagnosis from the presence of first symptom. Many patients are diagnosed only in advanced stage of disease.[1] Normal pressure in pulmonary artery is 25/10 mmHg and if the pulmonary artery pressure exceeds 40/20 mmHg or average pressure exceeds 25 mmHg, then the pulmonary hypertension is said to be present.Though the cut off value varies in many studies. If the pressure in the pulmonary artery remains persistently high then the right ventricle of the heart may get hypertrophied,so right ventricle will not be able to pump properly and the symptoms of right heart failure will occur. Pulmonary artery pressure is increased by many conditions and so pulmonary hypertension is classified accordingly. Prevalence of pulmonary artery hypertension inWHO's class1 which is caused mainly by connective tissue disorder, drug, and toxic agents is 15 cases/million adult population.[2,3] Prevalence of idiopathic pulmonary artery hypertension is 5.9 cases/million adult population.[4] Pulmonary hypertension due to systemic sclerosis, portal hypertension, congenital heart disease, and sleep apnea is 7-12%[5,6], 2-16%,[7] 30%[8], and 15-20%[9] respectively. Up to 60%of the patients with severe left ventricular systolic dysfunction and 70% of those with the heart failure with preserved ejection fraction may present with pulmonary hypertension. Almost all the patients with mitral valve diseases have pulmonary hypertension and 65% of those with symptomatic aortic stenosis also will have pulmonary hypertension.[10,11] Chronic thrombo-embolic pulmonary hypertension (CTEPH) prevalence was 3.2 cases/million/year and incidents were 0.9 cases/million/year. A large survey that is conducted in united states that registered information from all form of pulmonary hypertension from 1980-2002 documented that death rate in patients with pulmonary hypertension during these times were stable and ranging from 5.2-5.4deaths/1,00,000.[12,13,14,15]

Chronic kidney disease(CKD) is the global health burden with high economic cost to health system and is an

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independent risk factor for coronary vascular disease. All stages of CKD are associated with increased risk of cardio vascular premature mortality, morbidity and decreased quality of life.

Pulmonary artery hypertension in renal disease is an on-going research topic since it has very limited data. Prevalence of pulmonary hypertension in patients on hemodialysis, peritoneal dialysis and in stage - 5 CKD, ranges from 18.8 - 68.8%, 0.4-2% and 9-39% respectively. No proper epidemiological data are available yet for early stages of CKD. Pulmonary hypertension has direct association with mortality in end stage renal disease. Pulmonary hypertension in end stage renal disease is associated with the worst outcomes.[16-22] It is important to prevent pulmonary hypertension in patient with ESRD in early stages where even kidney transplantation may not reverse the condition,it alsohas high risk of mortality.

Study aimed to find the prevalence of pulmonary hypertension in patients with glomerular filtration rate less than 30ml/min per 1.73-meter square on dialysis in tertiary care hospital, to find the percentage of male and female in our study group and to find the significance of their association with pulmonary hypertension, to find the distribution of the pulmonary hypertension among different age groups and to find the significance of association pulmonary hypertension with diabetic mellitus, systemic hypertension, arterial venous fistula, superposed infection, volume overload, anaemia, left ventricular diastolic dysfunction, creatinine clearance, and duration of dialysis.

MATERIALS AND METHODS

In this prospective observational study conducted during the period of August 2016 to August 2017, we included total of 50 patients who were admitted in hospital fulfilling inclusion criteria. A proforma was made which included the detailed history, clinical examinations, and requisite investigations. After taking informed consent from each patient, detailed history was obtained and complete examination was done. Investigations like complete hemogram, routine urine analysis, blood sugar, serum electrolyte, serum creatinine, blood urea, thyroid function test, liver function test, ultrasound abdomen and pelvis, retroviral serology, hepatitis B and C serology, chest X-ray, echocardiogram, and electrocardiogram were done. GFR is calculated with the help of Cockcroft-Gault formula. Finally, the prevalence of pulmonary hypertension and the risk factors association with it were calculated and statistical analysis was done with the available data.

Inclusion criteria

-Age more than 18years

-Patients with GFR less than 30 ml/minper 1.73-meter square on haemodialysis

Exclusion criteria

Patients who has ejection fraction less than 45%

Patients who are known case of chronic lung disease, thrombo-embolic disease, sarcoidosis, sickle cell disease, HIV, connective tissue disorder, sleep disordered breathing, portal hypertension, congenital heart disease.

STATISTICAL ANALYSIS

The data collected from the patients is tabulated using Microsoft Excel. The chi square test was used to assess the difference in categorical variables between groups. Variables were presented as frequencies (numbers) and proportions (%) and Chi square test was used to compare frequencies. Results were presented in tables with an explanatory paragraph for each table by using Microsoft Office word software version 2010. Level of significance (P value) was considered at 0.05 as cut-off point for significant association.

RESULTS

Distribution of PH in various Age Groups

As seen in table -1, out of the total of number of 50 patients those belonging to age groups less than 40 years, 41-50 years, 51-60years,61-70 years and above 70 years were 6(12%), 7(14%), 24(48%), 9 (18%) and 4(8%) respectively. Patients who were found to have PH in these age groups were 3(50%), 0(0%), 6(25%), 1(11.1%), 1(25%) respectively. So,

Age (in years)	Pulmonary hypertension		Total	P value
	Yes	No		
<=40	3(50%)	3(50%)	6(100%)	0.241
41-50	0(0%)	7(100%)	7(100%)	
51-60	6(25%)	18(75%)	24(100%)	
61-70	1(11.1%)	8(88.9)%	9(100%)	
>70yr	1(25%)	3(75%)	4(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-1: Distribution of PH in various age groups

Sex	Pulmonary hypertension		Total	P Value
	Yes	No		
Male	5(17.9%)	23(82.1%)	28(100%)	0.503
Female	6(27.3%)	16(72.2%)	22(100%)	
Total	11(22.0%)	39(78%%)	50(100%)	

Table-2: Sex distribution of pulmonary hypertension

	Pulmonary HTN			
DM	Yes	No	Total	P value
Yes	7(22.6%)	24(77.4%)	31(100%	0.595
No	4(21.1%)	15(78.9%)	19(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-3: Association of Diabetes with pulmonary hypertension

		Pulmonary hypertension			p value
		Yes	No	Total	
Systemic hypertension	Yes	9(19.6%)	37(80.4%)	46(100%)	0.206
	No	2(50.0%)	2(50%)	4(100%)	
	Total	11(22.0%)	39(78.0%)	50(100%)	

Table-4: Distributionof systemic hypertension in pulmonary hypertension

	Pulmonary HTN			p-value
	Yes	No	Total	
AVF Yes	11(22.4%)	38(77.6%)	49(100%)	0.780
No	0 (0%)	1(100%)	1(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-5: Arteriovenous fistula in CKD and pulmonary hypertension

	Pulmonary htn			P value
	Yes	No	Total	
Superimposed infection				
yes	7(33.3%)	14(66.7%)	21(100%)	0.166
No	4(13.8%)	25(86.2%)	29(100%)	
Total	11(22.0%)	39(78.0%)	50(100%)	

Table-6: Superimposed infection and pulmonary hypertension

	Pulmonary HTN			P value
	Yes	No	Total	
Volume overload				
Yes	6(23.1%)	20(76.9%)	26(100.0%)	0.560
No	5(20.8%)	19(79.2%)	24(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-7: Association of volume overload with pulmonary hypertension

	Pulmonary HTN			P value
	Yes	No	Total	
Anaemia				
Yes	11(22.4%)	38(77.6%)	49(100%)	0.780
No	0(0%)	1(100%)	1(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-8: Anaemia with pulmonary hypertension

	Pulmonary HTN			P value
	Yes	No	Total	
LVDD				
Yes	10(24.4%)	31(75.6%)	41(100%)	0.662
No	1(11.1%)	8(88.9%)	9(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-9: LVDD (with EF>45%) and pulmonary hypertension

	Pulmonary htn			P value
Creatine clearence in GFR in ml	Yes	No	Total	
0-5	1(16.7%)	5(83.3%)	6(100%)	0.717
5-10	7(21.2%)	26(78.8%)	33(100%)	
10-15	3(33.3%)	6(66.7%)	9(100%)	
15-20	0(0%)	2(100%)	2(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-10: Creatinine clearance and pulmonary hypertension

maximum number of patients with PH were in the age group 51-50 and less than 40 years but the p-value was 0.241 which

	Pulmonary htn			P value
Duration of dialysis	Yes	No	Total	
0-5 years	0 (0%)	15 (100%)	15 (100%)	0.000
5-10 years	6 (20.0%)	24 (80.0%)	30 (100%)	
10-15 years	5(100.0%)	0(0%)	5(100%)	
15-20 years	11(22.0%)	39(78.0%)	50(100%)	
Total	11(22%)	39(78%)	50(100%)	

Table-11: Duration of Dialysis and pulmonary Hypertension

was not significant.

In the table 2 we compared the PH distribution among male and female population, in which males 5(17.9%) patients are found to have pulmonary hypertension and among females 6 (27.3%) patients had pulmonary hypertension. There were no significant association of pulmonary hypertension among male and female, since the p value was 0.503.

In the table 3 we analysed significance of association of diabetic mellitus with pulmonary hypertension. In total of 50 patients, 31(62%) patients were found to have DM, and among them 7(22.6%) patients were found to have PH. P value was 0.595, hence there was no significance of DM association with PH.

In the table 4 we analysed significance of association of systemic hypertension with pulmonary hypertension. In total of 50 patients, 46(92%) patients were found to have SHTN, and among them 9(19.6%) patients were found to have PH. P value was 0.206, hence there was no significance of SHTN association with PH.

In the table 5 we analysed significance of association of AVF with pulmonary hypertension. In total of 50 patients, 49(98%) patients were on AVF and one patient was on peritoneal dialysis, and among patients on arteriovenous fistula 11(22.4%) patients were found to have PH, and patient with peritoneal dialysis had no PH and P value was 0.780,which is not significance of AVF association with PH.

In the table 6 we analysed significance of association of super imposed infection with pulmonary hypertension. In total of 50 patients, 21(42%) patients were found to have super imposed infection, and among them 7(33.3%) patients were found to have PH. P value was 0.166, which also no significance of super imposed infection association with PH.

In the table 7 we analysed significance of association of volume overload with pulmonary hypertension. In total of 50 patients, 26(52%) patients were found to have volume overload, and among them 6(23.1%) patients were found to have PH. P value was 0.560, hence there was no significance of volume overload association with PH.

In the table 8 we analysed significance of association of anaemia with pulmonary hypertension. In total of 50 patients, 49(98%) patients were found to have anaemia, and among them 11(22.4%) patients were found to have PH. P value was 0.780, hence there was no significance of anaemia association with PH.

In the table 9 we analysed significance of association of LVDD with pulmonary hypertension. In total of 50 patients,

41(82%) patients were found to have LVDD, and among them 10(24.4%) patients were found to have PH. P value was 0.662, hence there was no significance of LVDD association with PH.

In the table 10 we analysed the pulmonary hypertension distribution and significance of association among the patients with varying range of creatinine clearance. In our study group 6(12%), 33(66%), 9(18%), 2(4%) patients were found to have creatinine clearance between 0-5%, 5-10%, 10-15%, 15-30% respectively and among them pulmonary hypertension was found in 1(16.7%), 7(21.2%), 3(33.3%), 0(0%) respectively. P value is 0.717 insignificant.

In the table 11 we analysed significance of association of duration of dialysis with pulmonary hypertension. In total of 50 patients, 15(30%), 30(60%), 5(10%), 15(30%) patients were found to be between 0-5, 5-10,10-15 years of dialysis respectively, and among them 0(0%), 6(20%), 5(100%), 11(22%) patients were found to have PH respectively. P value was 0.00, hence association of duration of dialysis with pulmonary hypertension is statistically significant.

DISCUSSION

There is no availability of epidemiological data in many developing countries including India for cause of pulmonary hypertension, but in India most common causes of PH are due to rheumatic heart disease, obstructive airway disease and congenital heart disease unlike western countries where idiopathic pulmonary artery hypertension and PH due to right heart disease are common.

Craddock et al say that exposure of dialysis membrane to blood leads to activation of neutrophils which leads to sequestration of neutrophils in the lungs. This phenomenon, is more common if cellulose membranes are used and it causes worsening of lung disease in HD patients.[23]

Presence of connective tissue disease, super imposed infections, haematological and liver diseases can cause pulmonary hypertension in patients with CKD. These conditions cause PH mainly by affecting micro vascular tone in lungs. Endothelial dysfunction has association with pulmonary hypertension in patients with CKD on HD, Main mechanism behind that includes imbalance between vasoconstrictors and vasodilators and high endothelin-1 and low nitric oxide levels affect vascular tone leading to PH.[24,25,26]

The prevalence of PH in CKD in our study was 22%, and the maximum affected age group was 51-60 years.In the study by Qian Zhang et al[27], the total prevalence of PH in CKD was 47.38% with the maximum number of patients seen in age 46.44(±14.63) years. Similarly another study from Iraq[28], showed maximum patients in 50 to 60 years and 60 to 70 years age group (35.3% in both groups).Male and female patients had prevalence of 55.32% and 44.68% respectively and their total number of patients compared to ours was large (n=705) while we had male and female prevalence of 17.95 and 27.3% respectively.

As noted by Qian Zhang et al[27], multivariate odds ratio(with 95%CI) for prevalence of PH in CKD showed that gender, diabetes, systemic hypertension had no significant correlation with PH while eGFR was contributing significantly to it. But, in our study all these parameters were insignificant for PH. Similarly, Ali Abdul Majid Dyab Allawi et al[28] and Thenappan T[29] also found no association between PH in CKD and hypertension even though hypertension is an established risk factor in for PH. Chronic volume overload leads to increased venous return in CKD patients may induce PH but as such we did not get any association between them. Severe anaemia is a well-known cardiovascular risk factor in CKD patients as low haemoglobin leads to hypoxic injury in tissues including pulmonary vasculature.[30] In our study we found no significant association of PH with anaemia while In a retrospective study low haemoglobin had significant impact on PH(P-value-0.000).[27]

In our study, we did not find a correlation between Left ventricular diastolic dysfunction and PH although LV end - diastolic pressure is taken as gold standard for its definition. Measuring LVDD in cases where pulmonary systolic pressure may not be estimated may help in predicting a relation with PH.[31]

According to Yigla M et al, pulmonary hypertension (PH) is a progressive, fatal pulmonary circulatory disease that accompanies many conditions (including left to right side shunt) with compensatory elevated cardiac output. PH also complicates chronic haemodialysis (HD) therapy immediately after the creation of an arteriovenous (AV) access, even before starting HD therapy. It tends to regress after temporary AV access closure and after successful kidney transplantation. This syndrome is associated with a statistically significant survival disadvantage. The laboratory hallmark of this syndrome is reduced basal and stimulatory nitric oxide (NO) levels. It appears that patients with end-stage renal disease (ESRD) acquire endothelial dysfunction that reduces the ability of their pulmonary vessels to accommodate the AV access-mediated elevated cardiac output, exacerbating the PH.[32]

We found 22.4% patients with AV fistula who had PH but this was not significant(p-value>0.780) while flow dependent significance of AV fistula was seen by Magdy M Emara et al.[33] In our study we have not measured AVF flow rate so could not assess its significance.

Comparing our study with Domenici et al[34], who noticed PH in 58.9% cases on HD and 22.2% on PD, we found 22.2% patients on HD having PH but no PH in patient on PD. Similarly another study by Yigla et al[35], found PH 39.7% in patients on HD but none on PD. An study in Iran, based on echocardiographic evaluations, found PH in 30-40% patients on chronic dialysis.[36]

Duration of dialysis significantly added to the development of PH which is evident from our study. This is well supported by Magdy et al[33], who reported duration of dialysis to be quite significantly associated with PH(P-value<0.001 and Pearson correlation 0.780).

Compared with previous studies, risk factors like age, sex, diabetes, systemic hypertension, AVF, superimposed infection, volume overload, anaemia, LVDD are not

associated with pulmonary hypertension in our study. But we found that the longer the duration of dialysis, the higher the possibility of pulmonary hypertension.

Limitations

A larger population based study may provide better data and correlations of above mentioned risk factors.

We have excluded secondary causes of PH in those who are known cases of chronic illness from available information only but have not investigated completely.

CONCLUSION

Prevalence of pulmonary hypertension in our study is 22 percentages. The risk factors like age, sex, diabetes, systemic hypertension, AVF, superimposed infection, volume overload, anaemia, LVDD has no influence on PH in our study. only association that we have in our study population in patients with PH is longer duration of dialysis.

ABBREVIATIONS

PH - Pulmonary hypertension, CKD - Chronic kidney disease, AVF - Arteriovenous fistula, LVDD - Left ventricular diastolic dysfunction, EF - Ejection fraction, ESRD - End stage renal disease, HD - Haemodialysis, PD - Peritoneal dialysis, GFR - Glomerular filtration rate, DM - Diabetes mellitus, SHTN - Systemic hypertension

REFERENCES

1. Dennis Kasper; Anthony Fauci; Stephen Hauser; Dan Longo; J Jameson. Pulmonary hypertension. Dennis L. Kasper, MD, Stephen L. Hauser, MD, J. Larry Jameson, MD, PhD, anthony S. Fauci, MD, Dan L. Longo, MD, Joseph Loscalzo, Ph. D. (eds). Harrison's Principles of Internal Medicine, 19 th ed.; 2015. pp. 1655-1660.
2. R.EDavide Bolignano, Stefania Rastelli, Rajiv Agarwal, Danilo Fliser, Ziad Massy, Alberto Ortiz, Andrzej Wiecek, Alberto Martinez-Castelao, Adrian Covic, David Goldsmith, Gultekin Suleymanlar, Bengt Lindholm, Gianfranco Parati, Rosa Sicari, Luna Gargani, Francesca Mallamaci, Gerard London, and Carmine Zoccali. Pulmonary Hypertension in CKD. American Journal Of Kidney Disease 2013; 61: 612-622.
3. Simonneau, G., Robbins, I.M., Beghetti, M. et al. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2009; 54: S43–S54
4. Nazzareno Galiè Marc Humbert Jean-Luc Vachiery Simon Gibbs Irene Lang Adam Torbicki Gérald Simonneau Andrew Peacock Anton Vonk Noordegraaf Maurice Beghetti Ardeschir Ghofrani Miguel Angel Gomez Sanchez Georg Hansmann Walter Klepetko Patrizio Lancellotti Marco Matucci Theresa McDonagh Luc A. Pierard Pedro T. Trindade Maurizio Zompatori Marius Hoeper Authors/Task Force Members Document Reviewers: Victor Aboyans, Antonio Vaz Carneiro, Stephan Achenbach, Stefan Agewall, Yannick Allanored, Riccardo Asteggiano, Luigi Paolo Badano, Joan Albert Barberà a, Hélène Bouvaist, Héctor Bueno, Robert A. Byrne, Scipione Carerj, Graça Castro, Çetin Erol, Volkmar Falk, Christian Funck-Brentano, Matthias Gorenflob, John Grantonc, Bernard Iung, David G. Kiely, Paulus Kirchhof, Barbro Kjellstrom, Ulf Landmesser, John Lekakis, Christos Lionis, Gregory Y. H. Lip, Sty. 2015 ESC/ERS
5. Hachulla E, Gressin V, Guillevin L, et al. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. Arthritis Rheum. 2005;52:3792-3800.
6. Mukerjee D, St George D, Coleiro B, et al. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hyper- tension: application of a registry approach. Ann Rheum Dis. 2003;62:1088-1093.2
7. Krowka MJ, Swanson KL, Frantz RP, McGoon MD, Wiesner RH. Portopulmonary hypertension: results from a 10-year screening algorithm. Hepatology. 2006;44:1502-1510.
8. Friedman WF. Proceedings of National Heart, Lung, and Blood Institute Pediatric Cardiology Workshop: pulmonary hyper- tension. Pediatr Res. 1986;20:8-11
9. Kessler R, Chaouat A, Weitzenblum E, et al. Pulmonary hypertension in the obstructive sleep apnoea syndrome: preva- lence, causes and therapeutic consequences. Eur Respir J. 1996;9: 787.
10. Oudiz RJ. Pulmonary hypertension associated with left-sided heart disease. Clin Chest Med 2007;28:233–241.
11. Vahanian AAlfieri OAndreotti FAntunes MJBaron-Esquivias GBaumgartner HBorger MACarrel TPDe Bonis MEvangelista AFalk VIung BLancellotti PPierard LPrice SSchafers HJSchuler GStepinska JSwedberg KTakkenberg JVon Oppell UOWindecker SZamorano JLZembala M. Guidelines on the management of valvular heart disease (version 2012). Eur Heart J 2012;33:2451–2496.
12. Seeger WAdir YBarberà JAChampion HCoghlan JGCottin VDe Marco TGaliè NGhio SGibbs SMartinez FJSemigran MJSimonneau GWells AUVachiéry JL. Pulmonary hypertension in chronic lung diseases. J Am Coll Cardiol 2013;62:D109–D116.
13. Hurdman Jcondliffe, Relliot, CASwift, ARajaram Sdavies, CHill Chamilton, NArmstrong IJBillings, Cpollard, LWild JMLawrie, ALawson Rsabroe, IKiely DG. Pulmonary hypertension in COPD: results from the ASPIRE registry. Eur Respir J 2013;41:1292–1301.
14. Cottin Vnunes, Hbrillet, PY Delaval Pdevouassoux, GTillie-Leblond IIsrael-Biet DCourt-FortuneValeyre DCordier JF. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J 2005;26:586–593.
15. Escribano-Subias PBlanco ILopez-Meseguer MLopez-Guarch CJRoman AMorales PCastillo-Palma MJSegovia JGomez-Sanchez MABarbera JA. Survival in pulmonary hypertension in Spain: insights from the Spanish registry. Eur Respir J 2012;40:596–603.
16. Mark J. Sarnak, Kari E. Roberts. Pulmonary Hypertension in CKD: Some Answers, Yet More Questions. Journal Of American Society of Nephrology 2015; 27():
17. Agarwal R. Prevalence, determinants and prognosis of pulmonary hypertension among hemodialysis patients. Nephrol Dial Transplant 27: 3908–3914, 2012
18. Havlucu Y, Kursat S, Ekmekci C, Celik P, Serter S, et al. Pulmonary hypertension in patients with chronic renal failure. Respiration. 2007;74:503–510.

19. Abdelwhab S, Elshinnawy S. Pulmonary hypertension in chronic renal failure patients. Am J Nephrol. 2008;28:990–997.
20. Yigla M, Fruchter O, Aharonson D, Yanay N, Reisner SA, et al. Pulmonary hypertension is an independent predictor of mortality in hemodialysis patients. Kidney Int. 2009;75:969–975
21. Nickel NGolpon HGreer MKnudsen LOlsson KWesterkamp VWelte THoeper MM. The prognostic impact of follow-up assessments in patients with idiopathic pulmonary arterial hypertension. Eur Respir J 2012;39:589–596.
22. Fritz JSBlair COudiz RJDufton COlschewski HDespain DGillies HKawut SM. Baseline and follow-up 6-min walk distance and brain natriuretic peptide predict 2-year mortality in pulmonary arterial hypertension. Chest. 2013;143:315–323.
23. Craddock PR, Fehr J, Brigham KL, Kronenberg RS, Jacob HS. Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. N Engl J Med. 1977;296:769–774.
24. Giaid A. Nitric oxide and endothelin-1 in pulmonary hypertension. Chest. 1998;114:208S-212S.
25. Zoccali C. The endothelium as a target in renal diseases. J Nephrol. 2007;20:39–44.
26. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. N Engl J Med. 1995;333:214–221.
27. Zhang Q, Wang L, Zeng H, Lv Y, Huang Y. Epidemiology and risk factors in CKD patients with pulmonary hypertension: a retrospective study. BMC Nephrol. 2018;19:70.
28. Ali Abdul Majid Dyab Allawi, Mohammed Hannon Alsodani, Ahmed Mushhin Hardan Alqaisi. Prevalence and Risk of Pulmonary Hypertension in Chronic Kidney Disease. International Journal of Science and Research (IJSR). Volume 6 Issue 7, July 2017
29. Thenappan T. Pulmonary hypertenson in chronic kidney disease: a hemodynamic characterization. Pulmonary circulation 2017;7:567-568.
30. van Loon RL, Bartelds B, Wagener FA, Affara N, Mohaupt S, Wijnberg H, Pennings SW, Takens J, Berger RM. Erythropoietin Attenuates Pulmonary Vascular Remodeling in Experimental Pulmonary Arterial Hypertension through Interplay between Endothelial Progenitor Cells and Heme Oxygenase. Front Pediatr. 2015;3:71.
31. Miller WL, Mahoney DW, Michelena HI, Pislaru SV, Topilsky Y, Enriquez-Sarano M. Contribution of ventricular diastolic dysfunction to pulmonary hypertension complicating chronic systolic heart failure. JACC Cardiovasc Imaging. 2011;4:946-54
32. Yigla M, Abassi Z, Reisner SA, Nakhoul F. Pulmonary hypertension in hemodialysis patients: an unrecognized threat. Semin Dial. 2006;19:353–357.
33. Magdy M.Emara,Mohamad A.Habeb Alsayed Ahmed Alnahal,Tarek A.Elshazly, Faisal O.Alatawi,Amer S.Masoud.Prevalence of pulmonary hypertension in patients with chronic kidney disease on and without dialysis.Egyptian Journal of Chest Diseases and Tuberculosis. 2013;62:761-768.
34. Domenici A, Luciani R, Principe F. Pulmonary hypertension in dialysis patients.Perit Dial Int. 2010;30:251-2.
35. Yigla M1, Nakhoul F, Sabag A, Tov N, Gorevich B, Abassi Z, Reisner SA. Pulmonary hypertension in patients with end-stage renal disease.Chest. 2003;123:1577-82.
36. Mitra Mahdavi-Mazdeh, Seyed Alijavad-Mousavi, Hooman Yahyazadeh, Mitra Azadi, Hajar Yoosefnejad, Yoosef Ataiipoor. Pulmonary Hypertention in hemodialysis patients. Saudi J Kidney Dis Transpl 2008;19:189-93

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Is Serum Uric Acid an Added Risk Factor for Micro-Vascular Complications of Diabetes Mellitus? – A Prospective Study

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ABSTRACT

Introduction: Diabetes is the leading cause of adult blindness due to retinopathy, end stage renal disease due to nephropathy and foot ulcers and lower limb amputation due to neuropathy. Serum uric acid levels independently predict the development of micro vascular complications. In this study, we analyse the association of serum uric acid with the micro vascular complications of diabetes.

Material and methods: In this hospital based observational study, hundred diabetic patients were included; of which fifty had micro vascular complications, the remaining fifty had no complications. Blood investigations including fasting(FBS) and post prandial blood sugars(PPBS), glycosylated haemoglobin (HbA1C), and serum creatinine and serum uric acid were done in all patients. All the parameters were compared between the two groups.

Results: Females presented with significantly higher complications as compared to males. The mean age of the patients presented with and without complications was 56.4 ± 9.3 and 59.9 ± 10.3 respectively. HbA1C had a positive correlation with the serum uric acid (SUA) (r=0.327, *P* = 0.001). Mean uric acid levels were higher among patients with complications (5.96 ± 2.16) compared to patients without complications (4.95 ± 2.04) which was statistically significant (*P* value =0.021). Patients with nephropathy and neuropathy had significant elevation in serum uric acid levels than those without; however this significance was not noted in those with retinopathy.

Conclusion: Patients with micro vascular complications had higher levels of serum uric acid compared to those without complications. Also there was positive correlation between HbA1C and serum uric acid levels.

Keywords: Serum Uric Acid, Micro Vascular Complications, Diabetic Retinopathy, Diabetic Neuropathy, Diabetic Nephropathy, Glycosylated Haemoglobin.

INTRODUCTION

Diabetes mellitus is the most challenging health problem in this century. Diabetes is the leading cause of adult blindness due to retinopathy, end stage renal disease due to nephropathy and foot ulcers and lower limb amputation due to neuropathy.[1]

Uric acid (UA) is primarily a purine metabolic waste product. About 70% of it gets excreted by the kidneys. Hyperuricemia has gained importance as many studies have reported that it not only has an important role in the development of metabolic syndrome but also micro vascular risk factor.[2-5] Elevated serum uric acid is a feature of hyperinsulinemia or insulin resistance.[6]

Many studies have suggested that inflammation and oxidative stress results from the metabolism of uric acid, leading to vascular injury. The rate limiting step of uric acid production is an enzymatic reaction of the xanthine oxidase (XO) enzyme that oxidises hypoxanthine – xanthine into uric acid. UA synthesis is accompanied by the generation of reactive oxygen species (ROS). XO in the vascular endothelium is associated with ischemia reperfusion injury. It has also been suggested that XO inhibitors improve endothelium-dependent vascular relaxation in blood vessels of hyperlipidemic rabbits.[7] XO as the source of ROS in ischemia/reperfusion injury has been discovered 30 years ago.[8,9]

In recent years there has been a debate regarding the association of hyperuricemia with diabetic complications. If this association is conclusively established, therapeutic interventions aiming to reduce uric acid synthesis might help to retard the progression of micro vascular complications of diabetes. Hence this study was conducted aiming to show the association of uric acid levels with diabetic complications.

MATERIAL AND METHODS

This is a hospital based observational study conducted in a tertiary care centre at Coimbatore from April 2015 to April 2016. Patients with Type 2 Diabetes Mellitus of the age group of 20 to 80 years admitted in the medical wards were included in the study. Patients admitted with acute complications like diabetic ketoacidosis and hyperosmolar coma and patients with gestational diabetes were excluded

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from the study. Informed consent was obtained from the patients. Before commencement of the study, approval was obtained from the Institutional Ethics Committee.

A detailed history and physical examination was done. Baseline investigations including fasting and post prandial blood sugars, HbA1c and serum creatinine were done. The diagnosis of diabetes was based on American Diabetic Association criteria. The presence of micro vascular complications was established by fundus examination by an ophthalmologist to assess retinopathy, Urine spot Protein/ creatinine ratio (PCR) to assess nephropathy and nylon monofilament test to assess neuropathy. Serum uric acid was done in all patients. Hyperuricemia was defined as serum uric acid greater than 7 mg/dl.

The study population was divided into two groups: group I patients with diabetes and without any vascular complications; group II patients with diabetes and any one or more of vascular complications.

STATISTICAL ANALYSIS

Descriptive statistics were reported as mean and standard deviation; categorical variables were presented with number and percentages. Chi-square test was used to test the association between the variables. Independent t test was used to compare the blood parameters between diabetes with complications and without complications. P value less than 0.05 was considered statistically significant.

RESULTS

Among the 100 diabetic patients studied, there were 62 males and 28 females; 50 had presented with complications and 50 had no complications in the study group. Out of 50 with diabetes related micro-vascular complications, there were 24 males and 26 females. There was a significant difference in the presence of complications and sex; Females presented with significantly higher complications as compared to males (P= 0.004). The mean age of the patients presented with and without complications was 56.4 ± 9.3 and 59.9 ± 10.3 years respectively; although not significant (P= 0.084), patients who presented with complications were younger than those without complications.

	Present (%)	Absent (%)
Diabetic Retinopathy	19(38)	31(62)
Diabetic Neuropathy	16(32)	34(68)
Diabetic Nephropathy	19(38)	31(62)

Table-1: Distribution of Micro vascular complications

	Diabetes with complications	Diabetes without complications	P value
Sex Male Female	 24 (39) 26 (68)	 38 (61) 12 (32)	0.004*
Age	56.4 ± 9.3	59.9 ± 10.3	0.084
HbA1c	8.28 ± 2.00	7.55 ± 1.50	0.044*
FBS	137.8 ± 44.5	133.5 ± 46.6	0.644
PPBS	210.1 ± 60.3	187.7± 52.7	0.028*
Uric acid	5.96 ± 2.16	4.95 ± 2.04	0.021*

Table-2: Comparison of age, sex, HbA1c and uric acid levels in diabetic patients with and without micro-vascular complications.

Microvascular complication		Serum uric acid level (mg/dL)	P value
Diabetic retinopathy	Present	5.97 ± 2.14	0.97
	Absent	5.95 ±2.20	
Diabetic neuropathy	Present	6.56 ± 2.30	0.004
	Absent	4.73 ± 1.10	
Diabetic nephropathy	Present	7.05 ± 2.13	0.006
	Absent	5.33 ± 1.94	

Table-3: Serum uric acid levels in micro vascular complications

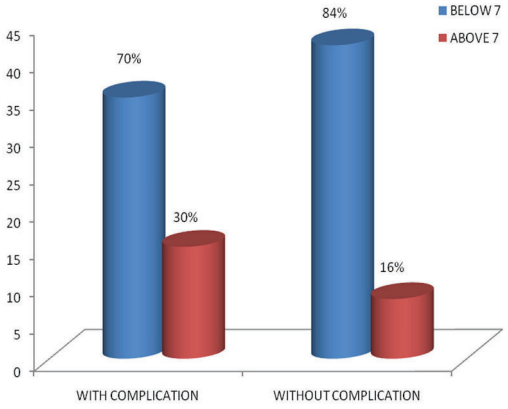


Figure-1: Frequency distribution of serm uric acid(mg/dl) in Diabetes mellitus with and without complications.

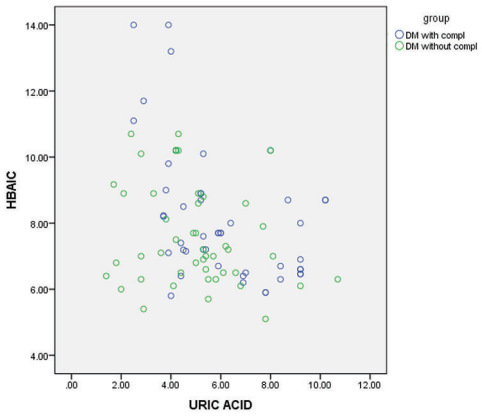


Figure-2: Co-relation of HbA1C with the Serum uric acid levels

The distribution of micro vascular complications is shown in Table 1. Both retinopathy and nephropathy were equally prevalent and slightly more than neuropathy.

Hyperuricemia (Serum uric acid >7 mg/dl) was more prevalent among patients with complications (30%) compared to patients without complications (Fig 1). As shown in Table 2, mean uric acid levels were higher among patients with complications (5.96 ± 2.16 mg/dl) compared to patients without complications (4.95 ± 2.04 mg/dl) which was statistically significant (*P* value = 0.021). HbA1C had a positive correlation with the serum uric acid levels (r=0.327, *P* = 0.001) as shown in Fig 2.

Table 3 represents the prevalence of hyperuricemia in patients with retinopathy, neuropathy and nephropathy, as compared to those without these complications. It can be seen that nephropathy and neuropathy patients had significantly more hyperuricemia than those without. However there was no statistically significant difference seen in the group with retinopathy. This suggests that high uric acid levels are associated with microangiopathies in Type 2 Diabetes.

DISCUSSION

Increasing amounts of uric acid in the serum causes Gout and this is the most significant cause of lifestyle-related disorder. Uric acid is primarily a product of purine metabolism. Serum uric acid (UA) levels are determined by a balance between Uric acid production and excretion. The rate-limiting step of UA production is an enzymatic reaction of the xanthine dehydrogenase/xanthine oxidase (XDH/XO) enzyme that oxidizes hypoxanthine-xanthine into UA. It is expressed in the liver and small intestine of XDH/XO-rich parenchyma cells[10] and is thought to be the major source for UA. The enzyme is also expressed in adipose tissue, the vascular endothelium, and macrophages, all of which are implicated in lifestyle-related diseases.[11] The UA production rate is based on the amount of substrate and/or XO activity. Since the generation of reactive oxygen species (ROS) depends on XO activity, XO is one of the major sources of oxidative stress in cells along with nicotinamide adenine dinucleotide phosphate oxidase, myeloperoxidase, lipoxygenase, and nitric oxide synthase.[12]

The kidney is an important regulator of circulating UA levels and is responsible for 60%-70% of total body UA excretion.[13] The remaining UA is secreted into the intestine, followed by bacterial uricolysis.[13] UA excretion in the kidney consists of urate secretion and reabsorption, and earlier research suggests the involvement of hyperfiltration.[6] Ninety percent of UA filtered by the kidney is reabsorbed.[13]

In patients with diabetes, the SUA level is low due to increased urate clearance.[14] In these patients, hypouricemia is associated with glycosuria[15], decreased metabolic control, hyperfiltration, and a late onset of disease, while elevated SUA is a feature of hyperinsulinemia or insulin resistance.[6]

Development of vascular complications was predicted independently by serum uric acid.[16] In a study conducted by Poonam Agrawal et al., it was concluded that the Uric acid levels are raised in patients with diabetic retinopathy when compared to those without retinopathy.[17] A study conducted by Ching-Chao Liang et al., concluded that there were increased serum uric acid levels which correlated with the severity of diabetic Retinopathy in patients with type 2 DM.[18] Vitreous UA and glucose concentrations were higher in proliferative than in non-proliferative DR. Focal UA production in the vitreous is thought to be involved in the pathogenesis and progression of DR.[19]

UA is lowered in Diabetes mellitus (DM) due to hyperfiltration[20], but decreased UA excretion during renal dysfunction raises SUA levels. Nazir Shah et al.[21] conducted a study in 2013 in 163 patients of diabetes mellitus (type 2) and proposed that elevation in the levels of uric acid was more in the diabetic patients with nephropathy (50%). In a study conducted in 60 diabetic patients by Nasri et al.[22], it was proved that there was a significant association between diabetic nephropathy and the levels of uric acid in the patient's serum. Petter Bjornstadet al[23] stated that there is a relation between the uric acid level elevation and development of diabetic nephropathy. Su-Mi Kim et al.[24] demonstrated that high UA contributes to the development of nephropathy in diabetic patients.

Several recent studies have been investigating therapeutic interventions to delay nephropathy progression.[25-27] Allopurinol therapy significantly decreases serum UA levels in hyperuricemic patients with mild to moderate CKD. Its use is safe and has been shown to help preserve kidney function when used for a duration of 12 months.[25] Febuxostat has a higher renoprotective effect than allopurinol, inhibits oxidative stress, has anti-atherogenic activity, reduces blood pressure, and decreases pulse wave velocity and left ventricular mass index, most likely due to a strong serum UA lowering effect.[27] In animal diabetic nephropathy model, allopurinol attenuated transforming growth factor-beta1-induced Smad pathway activation in tubular cells.[28]

The prevalence of diabetic peripheral neuropathy shows a significant correlation with increased UA levels.[29] Several studies demonstrated that, when controlled for confounding factors such as age, gender, BMI, renal function, and/or diabetic duration, Serum UA levels were high in patients with diabetic polyneuropathy and sudomotor dysfunction.[30,32]

The pathophysiology of diabetic neuropathy is not completely understood, and multiple metabolic imbalances underlie the development of diabetic neuropathy.[33] Hyperglycemia, dyslipidemia, and cardiovascular dysfunction are all independent risk factors for neuropathy. Probable etiologic factors include the polyol pathway, non-enzymatic glycation, free radicals, oxidative stress, and inflammation. Oxidative stress and inflammation are involved in XDH/XO activity. It is therefore speculated that UA generation by XDH/XO plays a role in diabetic neuropathy.

In a study conducted by Yili Xu et al., it was concluded that the patients with vascular complications had increased serum uric acid levels. He also said that this could be an independent predictor of vascular complications.[34] We found similar results in our study.

CONCLUSION

Diabetic patients with micro vascular complications have higher serum uric acid levels than those without complications. Also there was positive correlation between HbA1C and serum uric acid levels.

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REFERENCES

1. Sarah S Premraj et al. Correlation between serum uric acid level and microangiopathic complications

in patients with type 2 diabetes mellitus. MedPulse – International Medical Journal. January 2018; 5: 18-22.
2. Cai Z, Xu X, Wu X, Zhou C, Li D. Hyperuricemia and the metabolic syndrome in Hangzhou. Asia Pac J Clin Nutr. 2009; 18:81-7.
3. Nakagawa T, Cirillo P, Sato W, Gersch M, Sautin Y, Roncal C (et al). The conundrum of hyperuricemia, metabolic syndrome, and renal disease. Intern Emerg Med. 2008;3:313-8.
4. Takahashi MM, de Oliveira EP, de Carvalho AL, de SouzaDantas LA, Burini FH, Portero-McLellan KC et al. Metabolic syndrome and dietary components are associated with coronary artery disease risk score in free-living adults: a cross-sectional study. Diabetol Metab Syndr. 2011; 3:7.
5. Chen JH, Chuang SY, Chen HJ, Yeh WT, Pan WH. Serum uric acid level as an independent risk factor for all-cause cardiovascular, and ischemic stroke mortality: a Chinese cohort study. Arthritis Rheum. 2009; 61:225-32.
6. Bo S, Cavallo-Perin P, Gentile L, Repetti E, Pagano G. Hypouricemia and hyperuricemia in type 2 diabetes: two different phenotypes. Eur J Clin Invest 2001; 31: 318-321
7. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest. 1993; 91:2546–2551.
8. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985; 312:159–163.
9. Ono T, Tsuruta R, Fujita M, Aki HS, Kutsuna S, Kawamura Y, et al. Xanthine oxidase is one of the major sources of superoxide anion radicals in blood after reperfusion in rats with forebrain ischemia/reperfusion. Brain Res. 2009; 1305:158–167.
10. Pritsos CA. Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system. Chem Biol Interact. 2000; 129:195–208.
11. Moriwaki Y, Yamamoto T, Suda M, Nasako Y, Takahashi S, Agbedana OE, et al. Purification and immunohistochemical tissue localization of human xanthine oxidase. Biochim Biophys Acta. 1993; 1164:327–330.
12. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress." Histol Histopathol. 2008; 23:381–390.
13. Bobulescu IA, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. Adv Chronic Kidney Dis. 2012; 19:358–371.
14. Tuomilehto J, Zimmet P, Wolf E, Taylor R, Ram P, King H. Plasma uric acid level and its association with diabetes mellitus and some biologic parameters in a biracial population of Fiji. Am J Epidemiol. 1988; 127:321–336.
15. Andrade JA, Kang HC, Greffin S, Garcia Rosa ML, Lugon JR. Serum uric acid and disorders of glucose metabolism: the role of glycosuria. Braz J Med Biol Res. 2014; 0:0.
16. Bjornstad P, Maahs DM, Rivard CJ, Pyle L, Rewers M, Johnson RJ et al. Serum uric acid predicts vascular complications in adults with type 1 diabetes: the coronary artery calcification in type 1 diabetes study. Acta Diabetol 2014; 51: 783-791.
17. Agrawal P, Sethi S, Reddy VS, Lamba S. Serum uric acid and lipid profile in diabetic retinopathy in rural Haryana, India. Int J Adv Med 2016;3:309-312
18. Ching-Chao Liang et al. Association of serum uric acid concentration with Diabetic Nephropathy and Albuminuria in Taiwan. Int. J. Mol. Sci. 2016, 17, 1248
19. Krizova L, Kalousova M, Kubena A, Benakova H, Zima T, Kovarik Z, et al. Increased uric acid and glucose concentrations in vitreous and serum of patients with diabetic macular oedema. Ophthalmic Res. 2011; 46:73–79.
20. Shichiri M, Iwamoto H, Marumo F. Diabetic hypouricemia as an indicator of clinical nephropathy. Am J Nephrol. 1990; 10:115–122.
21. Nazir Shah, Amanullah, Sheraz Jamal and Mumtaz Ali Marwat. Association Of Hyperuricemia With Diabetic Nephropathy Intype 2 Diabetes Mellitus. Khyber Journal of Medical Sciences.
22. Behradmanesh S, Horestani MK, Baradaran A, Nasri H. Association of serum uric acid with proteinuria in type 2 diabetic patients. J Res Med Sci 2013; 18:44-6.
23. Petter Bjornstad et al. Fructose and uric acid in diabetic nephropathy. Diabetologia. 2015; 58:1993–2002
24. Su-Mi, Kim et al. Hyperuricemia-induced NLRP3 activation of macrophages contributes to the progression of diabetic nephropathy. Am J Physiol Renal Physiol 2015; 308: F993–F1003.
25. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. Am J Kidney Dis. 2006; 47:51–59.
26. Maahs DM, Caramori L, Cherney DZ, Galecki AT, Gao C, Jalal D, et al. Uric acid lowering to prevent kidney function loss in diabetes: the preventing early renal function loss (PERL) allopurinol study. Curr Diab Rep. 2013; 13:550–559.
27. Sezai A, Soma M, Nakata K, Hata M, Yoshitake I, Wakui S et al Comparison of febuxostat and allopurinol for hyperuricemia in cardiac surgery patients (NU-FLASH Trial). Circ J. 2013;77:2043–2049.
28. Kim SM, Choi YW, Seok HY, Jeong KH, Lee SH, Lee TW et al. Reducing serum uric acid attenuates TGF-β1-induced profibrogenic progression in type 2 diabetic nephropathy. Nephron Exp Nephrol. 2012; 121:e109–e121.
29. Chuengsamarn S, Rattanamongkolgul S, Jirawatnotai S. Association between serum uric acid level and microalbuminuria to chronic vascular complications in Thai patients with type 2 diabetes. J Diabetes Complications. 2014; 28:124–129.
30. Kiani J, Habibi Z, Tajziehchi A, Moghimbeigi A, Dehghan A, Azizkhani H. Association between serum uric acid level and diabetic peripheral neuropathy (A case control study). Caspian J Intern Med. 2014; 5:17–21.
31. Papanas N, Katsiki N, Papatheodorou K, Demetriou M, Papazoglou D, Gioka T, et al. Peripheral neuropathy is associated with increased serum levels of uric acid in type 2 diabetes mellitus. Angiology. 2011; 62:291–295.

32. Papanas N, Demetriou M, Katsiki N, Papatheodorou K, Papazoglou D, Gioka T et al. Increased serum levels of uric acid are associated with sudomotor dysfunction in subjects with type 2 diabetes mellitus. Exp Diabetes Res. 2011; 2011:346051.
33. Vincent AM, Callaghan BC, Smith AL, Feldman EL. Diabetic neuropathy: cellular mechanisms as therapeutic targets. Nat Rev Neurol. 2011; 7:573–583.
34. Xu Y, Zhu J, Gao L, Liu Y, Shen J, et al. Hyperuricemia as an Independent Predictor of Vascular Complications and Mortality in Type 2 Diabetes Patients: A Meta-Analysis. PLoS ONE 2013; 8: e78206.

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Original Research Article

Glycaemic control and C- reactive protein levels in type 2 diabetes mellitus -how well they co-relate?: a prospective study

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ABSTRACT

Background: Poorly controlled diabetes mellitus as indicated by elevated glycated haemoglobin (HbA1c) levels is associated with increased cardiovascular risk. C–reactive protein (CRP), an important cardiovascular risk factor, is elevated in diabetics with poor glycaemic control than those with good control. The present study assessed the correlation between HbA1c and CRP levels.
Methods: A prospective study was conducted in thirty type 2 diabetic patients irrespective of the disease duration and treatment; those with established target organ damage were excluded. HbA1c and hsCRP levels were measured at baseline; sugars were monitored monthly and medications optimised; at the end of six months HbA1c and hsCRP levels were measured.
Results: Mean age of the study subjects was 58.7±8.6 years; At the baseline, all had poor glycaemic control (HbA1c >7%); 15 had hsCRP >3 mg/L. At the end of 6 months, 5 achieved good glycaemic control (HbA1c <7%); 10 had hsCRP >3 mg/L. Baseline median hsCRP was 3.33 mg/L (0.68, 15.9) and at the end of 6 months it was 2.08 mg/L (0.48, 9.12). Mean HbA1c at baseline and end line was 10.6±1.55% and 8.43±1.84% respectively. There was significant reduction in both the mean HbA1c and median hsCRP at the end of 6 months (p <0.001). Positive correlation was observed between HbA1c and CRP at baseline (r=0.32, p=0.10). However, this was not observed at the end of 6 months.
Conclusions: There is positive correlation between the level of glycaemic control (HbA1c) and CRP levels; Better glycaemic control results in significant reduction in the hsCRP levels.

Keywords: C-reactive protein, Correlation, Glycaemic control, hsCRP, HbA1c

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by the defects in insulin secretion or action; chronic hyperglycaemia can lead to microvascular and macrovascular complications if the blood sugars are not under optimal control. The glycaemic control is assessed by the measurement of glycated haemoglobin (HbA1c) which has its own advantages and disadvantages.[1] Till date HbA1c is the widely used tool to assess the glycaemic status. Poor glycaemic control as indicated by elevated HbA1c levels accelerates the atherosclerosis process and significantly increases the risk of cardiovascular events.[2] C-reactive protein measured by highly sensitive assays (hsCRP), is a very sensitive marker of the inflammatory activity in the arterial wall.[3,4] It is an important predictor of cardiovascular risk apart from the traditional risk factors.[5,6] It is interesting to note that chronic hyperglycaemia stimulates the release of various inflammatory cytokines (IL 6; TNF α) and induces the secretion of acute phase reactants by liver, which in turn results in elevation of CRP in association

with elevated fasting plasma glucose.[7] Studies had shown that elevated CRP levels is associated with an increased risk of future development of diabetes mellitus.[8] Also, people with diabetes mellitus had elevated levels of CRP than non -diabetics.[9,10] We understand that both chronic systemic inflammation and hyperglycaemia contribute to the development and progression of atherosclerotic cardiovascular disease. Experimental and clinical studies have confirmed the inter-relationship between CRP, hyperglycaemia and atherosclerosis.[11,12] In states of elevated CRP, hyperglycaemia exaggerates the pro-atherogenic effects of CRP.[12,13] Few studies which had assessed the relationship between CRP levels and the level of glycaemic status showed conflicting results; some studies had proven the positive co-relation between glycaemic control and CRP levels.[14-16] while some failed to do so.[6] Also, the effect of good glycaemic control on CRP levels is not clear. The aim of this study is to determine the co-relation of glycaemic control as measured by HbA1c levels with hsCRP in patients with type 2 diabetes mellitus and to determine if better glycaemic control reduces CRP levels.

METHODS

The study was conducted in the Department of Medicine, PSG Institute of Medical Sciences, Coimbatore. Patients with type 2 diabetes mellitus diagnosed on the basis of WHO criteria were prospectively enrolled in the study from September 2014 to May 2015 irrespective of disease duration and treatment. Patients with established cardiovascular disease, chronic kidney disease, infections, haemolytic anaemia, on drug therapy with statins and non-steroidal anti -inflammatory drugs were excluded from the study. The study was approved by the Institutional Ethics Committee (approval number: 14/184); informed consent was obtained from all the study participants.

At the time of enrolment in the study, blood samples were collected from the participants for measurement of HbA1c and hsCRP levels. HbA1c was measured by high pressure liquid chromatography (Variant II Turbo - HbA1c kit - 2.0). High sensitive CRP was measured by particle enhanced turbidometry assay (Cobas Integra 400 plus). They were followed up on a monthly basis, clinical evaluation was done; fasting and postprandial blood sugars were monitored and adjustment of anti-diabetic medications done on an individual basis in addition to re-enforcement on life style modification (diet and exercise). At 6 months, blood samples were collected for HbA1c and CRP levels.

Statistical analysis

Descriptive statistics were reported as mean ± SD for normally distributed data, median with 25th and 75th for the non-normal data. Paired t test or Wilcoxon Signed rank test was used to test the significance for the change in HBA1c and CRP after 6 months from baseline. Spearman's Rank correlation is used to assess the correlation between the variables. P value less than 5% was considered statistically significant. All the analyses were done using SPSS version 23.0.

RESULTS

Among the 30 subjects studied, 70% of people were between the age group of 50-70 years; the mean age of the study subjects was 58.7±8.6 years; there were 17 females (57%) and 13 males (43%).

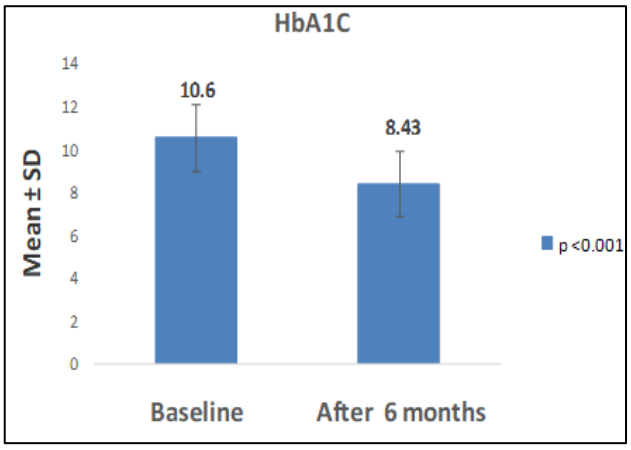


Figure 1: Mean HbA1c levels in % at baseline and at the end of 6 months.

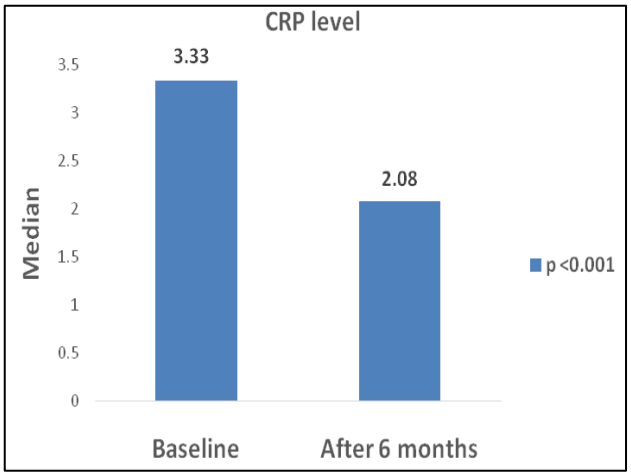


Figure 2: Median CRP levels in mg/L at baseline and at the end of 6 months.

At the baseline, all of them had poor glycaemic control (HbA1c >7%); 15 had hs CRP >3 mg/L. At the end of 6 months, 6 patients lost to follow up; HbA1c and hsCRP levels were measured for the remaining subjects; 5 achieved good glycaemic control (HbA1c <7%); 10 had hs CRP >3 mg/L. The mean hsCRP at baseline and at the end of 6 months was 7.85±9.75 and 4.88 ±5.41 mg/L respectively. Baseline median hsCRP was 3.33 (0.68, 15.9) and at the end of 6 months it was 2.08 (0.48, 9.12). The mean HbA1c at baseline and end line was

10.6±1.55% and 8.43±1.84% respectively. There was statistically significant reduction in both the mean HbA1c and median hsCRP values at the end of 6 months with effective individual based anti-diabetic treatment (p <0.001). The results are depicted in Figure 1 and 2. Positive co-relation was observed between HbA1c and CRP at baseline (r=0.32, p=0.10) although it was not statistically significant. However, this correlation was not observed at the end of 6 months.

DISCUSSION

The role of chronic low-grade inflammation contributing to the pathogenesis of diabetes and its related complications is well known. Chronic hyperglycaemia induces oxidative stress and chronic inflammatory state, which jointly contribute to the pathogenesis of atherosclerosis. C-reactive protein levels more than 3.0 mg/L is associated with worse cardiovascular outcome.[17] Various studies had demonstrated significantly elevated serum hsCRP levels in diabetics than non-diabetics.[15,16,18] Also, significant elevation was noted in those with poor glycaemic control compared to those with good glycaemic control.[15] Recent research has shown that HbA1c and CRP jointly contribute to the increased cardiovascular risk in patients with advanced cardiovascular disease.[19]

The mean hsCRP levels in the study population at baseline was 7.85±9.75 mg/l which is in accordance to that observed in another Indian study conducted in Bangalore (6.9±9.3 mg/l); while Saudi Arabian and Bangladesh studies had shown mean hsCRP levels lower than that observed in the present study, 1.13 and 2.3 mg/L respectively.[6,18] The reason for this difference in CRP levels is unclear; further studies are needed to assess the differences in ethnic groups; standardisation of the techniques used for measurement of hsCRP.

A positive co-relation was observed between HbA1c and hsCRP levels at the baseline in the present study indicating that the change in HbA1c affects hsCRP levels in the same direction. There are other studies which support our results. In a cross-sectional study conducted by Gohel et al, a significant positive linear relationship was observed between hsCRP and HbA1c.[15] Li et al, Khan DA et al, and Sarinnapakorn V et al, also had made similar observation in their studies.[20-22] Kashinakunti et al, in their case control study noted significantly high hsCRP levels in diabetics than controls; also noted positive co-relation between hsCRP and HbA1c, although non-significant.[16] Low HbA1c levels strongly related to negative hs-CRP levels as observed by Amanullah S et al.[23] Another study conducted in Sudan showed significant correlation between CRP levels with fasting plasma glucose and HbA1c.[24] All the above-mentioned studies were cross-sectional observational studies which assessed the relationship between Hba1c and CRP.

On literature search authors could find only one study conducted in Mexico which had assessed prospectively the effects of good glycaemic control on the CRP levels. The study was conducted on newly diagnosed type 2 diabetes patients; they were followed up for 6 months by intensive blood sugar management. There was significant reduction in HbA1c from baseline value of 13.0±4.9 to 8.9±2.9% at the end of 6 months, p <0.00001 which resulted in significant reduction in hsCRP levels (9.6±6.2 to 6.3±3.0 mg/l, p <0.00001). The present study also had shown similar results; significant reduction in hsCRP levels at the end of 6 months compared to baseline was observed following better glycaemic control and reduction in HbA1c levels. In that study, although glycaemic control was achieved in 71 patients at the end of 6 months, only 29 (40.8%) of them showed a reduction in CRP levels to 3 mg/l or less and the remaining 42 (59.2%) maintained a high CRP level. The study concluded that CRP is moderately influenced by glycaemic control.[25] Similar to that observation, the present study also showed that only 41.6% of the study subjects achieved CRP levels <3 mg/L by 6 months.

The positive co-relation observed between HbA1c and CRP at the baseline was not observed at 6 months; this may be because CRP is moderately influenced by glycaemic control.25 There are other variables which affect CRP levels like age, gender, body weight, LDL cholesterol levels. The present study did not take into considerations those variables which is a limitation of the present study.

CONCLUSION

There is positive correlation between the level of glycemic control (HbA1c) and CRP levels; Better glycemic control results in significant reduction in the hsCRP levels.

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REFERENCES

1. Saudek CD, Kalyani RR, Derr RL. Assessment of Glycemia in Diabetes Mellitus: Hemoglobin A. JAPI. 2005 Apr; 53.
2. Cavero-Redondo I, Peleteiro B, Álvarez-Bueno C, et alGlycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and non-diabetic populations: a systematic review and meta-analysis. BMJ Open. 2017;7:e015949.

3. Kamath DY, Xavier D, Sigamani A, Pais P. High sensitivity C-reactive protein (hsCRP) & cardiovascular disease: An Indian perspective. Indian J Med Res. 2015;142:261-8.
4. Pfützner A, Forst T. High-sensitivity Creactive protein as cardio-vascular risk marker in patients with diabetes mellitus. Diabetes Technol Ther. 2006; 8(1):28-36.
5. Asegaonkar SB, Marathe A, Tekade ML, Cherekar L, Bavikar J, Bardapurkar J et al.High-sensitivity C-reactive protein: a novel cardiovascular risk predictor in type 2 diabetics with normal lipid profile. J Diab Complications. 2011;25(6):368-70.
6. Mohieldein AH, Hasan M, El-Habiby MI.High Sensitivity C-Reactive Protein as Atherogenic Marker Among Type 2 Diabetes. Eur Sci J. 2017(13);33:403.
7. Lin Y, Rajala MW, Berger JP. Hyperglycaemia-induced production of acute phase reactants in adipose tissue. J Biol Chem. 2001; 276(45):42077-83.
8. Freeman DJ, Norrie J, Caslake MJ, et al. West of Scotland Coronary Prevention Study: C-reactive protein is an independent predictor of riskfor the development of diabetes in the West of Scotland Coronary Prevention Study. Diab. 2002;51:1596-1600.
9. Tutuncu Y, Satman I, Celik S, Dinccag N, Karsidag K, Telci A, et al. A comparison of hs-CRP levels in new diabetes groups diagnosed based on FPG, 2-hPG, or HbA1c criteria. J Diabe Res. 2016.
10. Satman I, Omer B, Tutuncu Y, Kalaca S, Gedik S, Dinccag N, et al. Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. Eur J Epidemiol. 2013;28(2):169-80.
11. Ridker PM, Buring JE, Cook NR. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy Am women. Circulation. 2003;107:391-7.
12. Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. Circulation. 2003;107:398-404.
13. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation. 2000;102:2165-8.
14. King DE, Mainous AG, Buchanan TA, Pearson WS.C-reactive protein and glycemic control in adults with diabetes. Diabe Care. 2003;26(5):1535-9
15. Gohel MG, Chacko AN. Serum GGT activity and hs-CRP level in patients with type 2 diabetes mellitus with good and poor glycemic control: An evidence linking oxidative stress, inflammation and glycemic control. J Diabe Metabolic Dis. 2013;12:56.
16. Kashinakunti SV, Rangappa M, Kallaganada GS. International J Biochem Res Rev. 2016;11(4): 1-8
17. Cardoso CR, Leite NC, Salles GF. Prognostic Importance of C-Reactive Protein in High Cardiovascular Risk Patients with Type 2 Diabetes Mellitus: The Rio de Janeiro Type 2 Diabetes Cohort Study. J Am Heart Assoc. 2016;5(11):e004554.
18. Fazlul Haque AKM, Saifuddin Ekram ARM, Islam QT, Jahan MS, Haque MZ. Evaluation of serum high sensitivity c -reactive protein (hs-crp) in type-2 diabetic patient. J Med. 2010;11:20-3.
19. Schillinger M, Exner M, Amighi J, Mlekusch W, Sabeti S, Rumpold H. et al. Joint effects of C-reactive protein and glycated hemoglobin in predicting future cardiovascular events of patients with advanced atherosclerosis. Circulation. 2003; 108(19):2323-8.
20. Li CZ, Xue YM, Gao F, Wang M. Determination of serum hs-CRP in patientswith type 2 diabetes mellitus. Di Yi Jhun Yi Da Xne Xne Bao. 2004;24(7):791-3.
21. Dilshad Ahmed K, Shazia Q. Evaluation of cardiac risk by oxidative stress and inflammatory markers in diabetic patients. Pak J Med Sci. 2009;25:5.
22. Sarinnapakorn V, Wanicagool W: Association between hs-CRP and Hba1c in overweight type 2 diabetic female patients. J Med Assoc Thai. 2013;96(3):S54-8.
23. Amanullah S, Jarari A, Govindan M, Basha MI, Khatheeja S. Association of hsCRP with diabetic and non-diabetic individuals. Jordan J Biol Sci. 2010;3(1):7-12.
24. Abdrabo AA. Association between fasting plasma glucose and highly sensitive C-reactive protein in a Sudanese population. Sudan Med J. 2012 August;48(2).
25. Rodríguez-Morán M, Guerrero-Romero F. Elevated concentrations of C-reactive protein in subjects with type 2 diabetes mellitus are moderately influenced by glycemic control. J Endocrinol Invest. 2003;26(3):216-21.

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A Study of Serum Albumin Levels in Acute Ischemic Stroke and its Correlation with Clinical Outcome

Santni Manickam[1], Joel Franklin[2], Velammal Petchiappan[3], Sujaya Menon[4]

ABSTRACT

Introduction:: Stroke is a global health problem. Studies have shown that serum albumin level is an independent predictor of ischemic stroke outcome. In the present study we compared the serum albumin and the volume of infarct with the clinical outcome.

Material and methods: Fifty patients of age more than 18 years with clinical and radiological evidence of acute ischemic stroke were included in the study. The severity of stroke at admission was assessed by National Institute of Health stroke scale (NIHSS). Serum albumin levels were measured at admission within 36 hours after stroke onset. Functional outcome was measured 1 week post admission and after 3 months during follow up using modified Rankin scale (mRs). A score of mRs >3 or death was taken as poor outcome. Statistical analysis and interpretation of the data was done. *P* value of <0.05 was taken as statistically significant.

Results: The mean age group of the study population was 53 years, male patients had more severe stroke compared to female patients. Most common risk factors were diabetes mellitus and systemic hypertension. Patients with a good outcome had lower NIHSS score, low mRS score, high albumin and low volume of infarct. Those with worst outcome had higher NIHSS, high mRS and high volume of infarct.

Conclusion: Relatively high serum albumin level in acute stroke patients decreases the risk of poor outcome. It is not only serum albumin but volume of infarct also does play a role in the final outcome of the patient.

Keywords: Acute Ischemic Stroke, Serum Albumin, Volume of Infarct, Functional Outcome

INTRODUCTION

Stroke is a global health problem. Around 20 million people suffer from stroke every year and about 5 million people among them do not survive.[1,2] It is the second most common cause of death and fourth leading cause of disability worldwide.[3] Stroke is also a predisposing factor for epilepsy, falls and depression in developed countries.[4] It is a leading cause of functional impairments, with 20% of survivors requiring institutional care after 3 months and 15% - 30% being permanently disabled.[5]

It is a well known fact that if stroke patients have associated protein energy malnutrition they tend to have poor outcome.[6] Studies have shown that serum albumin level is an independent predictor of ischemic stroke outcome.[7,8] Experimental studies have shown that human albumin infusion after stroke onset significantly improves the neurological function and reduces the infarct volume.[9] Although low serum albumin is associated with poor neurological outcome, albumin therapy in acute stroke patients is not beneficial as shown by the ALIAS (Albumin in Acute Ischemic Stroke) part 1 and 2 trials which evaluated the efficacy and safety of human albumin therapy in acute ischemic stroke.[10,11] The volume of infarct and its co-relation with the serum albumin was not studied in any of the previous studies. In the present study we compared the serum albumin and also the volume of infarct with the clinical outcome.

MATERIAL AND METHODS

The study was a Hospital based prospective study which included a total of fifty patients of age more than 18 years with clinical and radiological evidence of acute ischemic stroke admitted to the department of General Medicine and Neurology in a tertiary care centre in South India. Patients with Cerebral haemorrhage of any aetiology, Liver disease; Cardiac failure, Nephrotic syndrome, Diabetic nephropathy, Protein losing enteropathies and Malignancies were excluded from the study. Diagnosis of ischemic stroke is based on clinical observation confirmed by radiological imaging.

Prior to the commencement of the study, ethical clearance was obtained from Institutional Human Ethical Committee. The selected patients were briefed about the nature of the study and written informed consent was obtained from them in regional language. Patients were examined clinically in detail and the severity of stroke was assessed by National Institute of Health stroke scale (NIHSS) at baseline. Blood samples for assessment of albumin were collected at admission within 36 hours after stroke onset. Serum albumin estimation was done using Bromo Cresol Green (BCG) end point method. Functional outcome was measured 1 week post admission and after 3 months during follow up using

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modified Rankin scale (mRs). This scale runs from 0-6, running from perfect health without symptoms to death. A score of mRs: 0-3 was taken as favourable score and mRs: 4-6 was taken as unfavourable score. Serum albumin level <3.5 g% is considered as low.

The data were reported as the mean +/- SD or the median, depending on their distribution. The differences in quantitative variables between groups were assessed by means of the unpaired t test. Comparison between groups was made by the non-parametric Mann- Whitney test. ANOVA was used to assess the variables. The chi square test was used to assess the difference in categoric variables between groups.

Statistical analysis and interpretation of the data was done by using SPSS version 20. *P* value of <0.05 using two tailed test was taken as being of significance for all statistical tests

Serum albumin g/dl	NIHSS score		Total	*P* value
	≤10	>10		
3-3.4	0	1	1	0.124
3.5-3.9	10	3	13	
4-4.4	24	3	27	
>4.4	7	2	9	
Total	41	9	50	

Table-1: Serum Albumin with NIHSS Score

RESULTS

Among the study population of 50 patients, 44(88%) were males and 6(12%) were females. Out of 50 patients, 3 patients (1 male, 2 females) lost to follow-up. Number of patients with NIHSS score less than or equal to 10 was 41 and more than 10 was 9. Among those with NIHSS more than 10, there were seven males and two females. NIHSS score of 10 or less than 10 was seen in high numbers in 56-65 and >65 years age group. The risk factors of the study population and the nature of the ischemic stroke were tabulated in Figure 1and Figure 2 respectively.

Among the 47 patients, number of patients who had significant disability was 15. Among them 6 were in the age group of 56 to 65. Number of patients who had mild disability was 32.Among them about 11 patients were in the age group more than 65. After 3 months, number of patients with significant disability were 4 and number of patients with mild disability were 43.Significant improvement was seen in the age group of 56 to 65 (9 to 13).

Out of the 43 male patients, 14 patients had significant disability (mRs >3) and 29 patients had mild disability (mRs ≤3). Among the 14 with significant disability, 3 had death as outcome. Out of the 4 female patients, 1 had significant disability and died during the first week. Remaining 3

Serum albumin g/dl	mRs at 1 week		Total	P value	mRs at 3 months		Total	*P* value
	≤3	>3			≤3	>3		
<3.5	0	1	1	0.319	1	0	1	0.894
≥3.5	32	14	46		41	5	46	
Total	32	15	47		42	5	47	

Table-2: Serum Albumin with mRs score

mRs at 1 week	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	*P* value
					Lower Bound	Upper Bound			
≤3	32	5.5948	12.00600	2.12238	1.2662	9.9234	.11	52.54	0.001
>3	14	26.5182	26.47135	7.07477	11.2341	41.8023	.30	81.60	
Total	46	11.9628	19.91184	2.93584	6.0497	17.8759	.11	81.60	

Table-3: Volume of Infarct in CT scan with mRs after 1 week

mRs at 3 months	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	*P* value
					Lower Bound	Upper Bound			
≤3	42	10.6385	17.63608	2.72131	5.1428	16.1343	.11	62.70	0.146
>3	4	25.8675	37.60486	18.80243	-33.9702	85.7052	1.57	81.60	
Total	46	11.9628	19.91184	2.93584	6.0497	17.8759	.11	81.60	

Table-4: Volume of Infarct in CT Scan with mRs after 3 months

Study	No of Patients	Mean Albumin	*P* Value
Idicula et al[18]	444	3.76	<0.05
Dziedzic et al[7]	759	3.55	<0.01
Gaurav et al[19]	50	3.81	<0.001
Present study	50	3.85	0.894

Table-5: Comparison with other studies

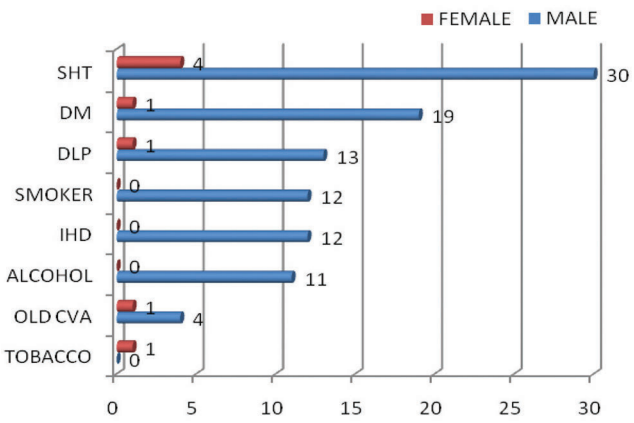


Figure-1: Risk factors of stroke in the study population

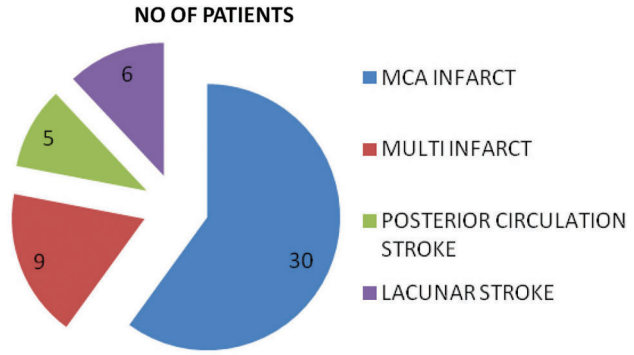


Figure-2: Frequency of lesion in MRI / CT brain

patients had mild disability. At 3 months follow-up, among the male patients, number of patients with significant disability dropped from 14 to 4. In the female group, the 3 patients with mild disability had improvement in the mRS score and didn't worsen.

Among 50 patients, only one patient in the age group of 46 - 55 had low serum albumin levels (<3.4 g/dl)). Mean albumin was 3.8. Majority of patients had albumin levels between 4 and 4.4. Both males and females showed similarity in this.

Table 1 showed that out of 9 patients with NIHSS score >10, only one patient had low serum albumin level<3.5 g/dl. The remaining 41 patients with a NIHSS score of less than 10 had serum albumin level above 3.5 g/dl.

On co-relating serum albumin level with mRS score at 1 week after stroke onset, while all the 32 patients with mRS ≤3 had serum albumin levels ≥3.5 g/dl, only one out of 15 with mRS > 3 had low serum albumin levels, the rest had serum albumin levels ≥3.5 g/dl. *P* value was 0.319. Hence there was no significant correlation. (Table 2) On co-relating serum albumin level with mRS score at 3 months after stroke, it was noted that out of 14 patients with high albumin levels who initially had mRS > 3 at 1 week, 9 had clinical improvement and the remaining 5 persisted to have mRS > 3 at 3 months post stroke. Also, a single patient with serum albumin <3.5 had an improvement in mRS score from baseline.

Out of 47 patients, volume of infarct could not be calculated for one patient. In the rest, the mean volume of infarct is significantly higher in those with mRs >3 than those ≤3 score at 1 week after stroke. (*P* value was 0.001) The results are tabulated in Table 3

The volume of infarct and mRS scale was compared during 3rd month using ANOVA method. *P* value was 0.146. This showed there was no correlation between volume of infarct and outcome during 3rd month. (Table 4)

DISCUSSION

It is well known that serum albumin plays a major role in the clinical outcome of vascular diseases. The neuroprotective effect of albumin is due to its various properties like anti-inflammatory and anti-oxidant effects, inhibition of thrombosis in microcirculation.[12-14] It reduces hematocrit level and also plays a vital role in aggregation of erythrocytes[15] by increasing low shear viscosity and decreasing erythrocyte sedimentation under no-flow conditions. Albumin has a good antioxidant property. Other neuroprotective effect of albumin in stroke includes Prevention of thrombosis and Prevention of leukocyte adhesion within post capillary microcirculation in the early reperfusion phase.

Albumin captures the oxygen free radicals and slows the production of reactive hydroxyl radical species. Albumin has a peculiar property of binding to copper ions by doing so it inhibits the process of copper ion dependent lipid per oxidation at cell membrane. It has also been postulated that albumin exerts neuroprotection by binding to lysophosphatidylcholine. Free lysophosphatidylcholine increases leukocyte adhesion molecules which lead to inflammatory mediated damage on vascular endothelium. It also causes apoptosis when it is present in high concentration. Based on the above said properties it was postulated that albumin infusion post ischemic stroke may be beneficial in long term outcome.

Experimental studies in rats done by Belayev et al. showed that high-dose human albumin therapy, if administered within 2 to 4 hours after stroke onset, is highly effective in improving neurological status and in reducing infarction volume and extent of brain swelling.[16]

Based on all the evidences human trial known as ALIAS (Albumin In Acute Ischemic Stroke) was conducted. It showed that treatment with intravenous albumin 25% at 2 g/kg was not associated with improved outcome at 90 days and was associated with increased incidence of intracerebral haemorrhage and pulmonary oedema. Further research is required to come to conclusive evidence that human albumin infusion can improve the clinical outcome of stroke patients as there were good results in rat models of stroke.

The mean age group of the study population was 53 years, predominantly middle aged group which is a decade lower than the peak age group of stroke in western countries.[17] In the present study, male patients had more severe stroke compared to female patients. Most common risk factors noticed in this study were diabetes mellitus and systemic hypertension.

Around 42 patients had good outcome in this study and only 5 patients had poor outcome. Most of the previous studies showed that patients with good outcome had lower NIHSS

score and high serum albumin level on admission and those with worst outcome showed high NIHSS score and low serum albumin level. In our study also patient with low NIHSS and high albumin had good neurological outcome. Only one patient had low albumin and high NIHSS score had unfavourable mRs score after 1 week; however his mRs score improved after 3 months

The studies which were done earlier didn't take volume of infarct into account. The patients with poor outcomes had a mean volume of infarct of around 26.51 and those with good outcome had a mean volume of infarct of around 5.59. Even though there was significant correlation between volume of infarct and mRS score at 1 week there was no significant correlation between them at 3 months

In this study, most patients with a good outcome had lower NIHSS score, low mRS score, high albumin and low volume of infarct. Those with worst outcome had higher NIHSS, high mRS and high volume of infarct. The one patient with low serum albumin also had a good outcome in 3 months. It is not only serum albumin but volume of infarct also do play a role in the final outcome of the patient which was not considered in other studies. Those who had poor outcome had a mean volume of infarct of around 25 cm^3 and also there was statistical significance between volume of infarct and mRS at 1 week. Even though clinically most of the patients had good outcome after 3 months based on mRS score, statistically *P* value was not significant. Hence this study is contrary to the evidence that serum albumin is a long term predictor of ischemic stroke outcome. The disadvantage of this study was that not enough number of patients could be obtained with low serum albumin levels.

CONCLUSION

Though the risk of stroke increases with age, our patients were predominantly in the middle aged group. Male patients had more severe stroke compared to female patients. Most common risk factors noticed in this study were diabetes mellitus and systemic hypertension. Most patients with a good outcome had lower NIHSS score, low mRS score, high albumin and low volume of infarct. Those with worst outcome had higher NIHSS, high mRS and high volume of infarct. The one patient with low serum albumin also had a good outcome in 3 months. It is not only serum albumin but volume of infarct also does play a role in the final outcome of the patient.

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REFERENCES

1. Warlow C, Sudlow C, Dennis M, Wardlaw J, Sandercock P. Stroke. Lancet. 2003. p. 1211–24.
2. Strong K, Mathers C, Bonita R. Preventing stroke: saving lives around the world. Lancet Neurology. 2007. p. 182–7.
3. WHO. Stroke, Cerebrovascular accident [Internet]. Health topics. 2013. Available from: http://www.who.int/topics/cerebrovascular_accident/en/
4. WHO. Global burden of stroke. atlas Hear Dis stroke. 2004;15:50–1.
5. Lipska K, Sylaja PN, Sarma PS, Thankappan KR, Kutty VR, Vasan RS, et al. Risk factors for acute ischaemic stroke in young adults in South India. J Neurol Neurosurg Psychiatry 2007;78:959–63.
6. Davalos A, Ricart W, Gonzalez-Huix F, Soler S, Marrugat J, Molins A, et al. Effect of malnutrition after acute stroke on clinical outcome. Stroke. 1996; 27: 1028–1032.
7. Dziedzic T, Slowik A, Szczudlik A. Serum albumin level as a predictor of ischemic stroke outcome.Stroke. 2004; 35: 156–158.
8. Babu MS, Kaul S, Dadheech S, Rajeshwar K, Jyothy A, Munshi A. Serum albumin levels in ischemic stroke and its subtypes: correlation with clinical outcome. Nutrition. 2013;29:872-5.
9. Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD. Human albumin therapy of acute ischemic stroke. Marked neuroprotective efficacy at moderate doses and with a broad therapeutic window. Stroke. 2001;32:553–560.
10. Ginsberg MD, Palesch YY, Martin RH, Hill MD, Moy CS, Waldman BD, et al.The Albumin in Acute Stroke (ALIAS) Multicenter Clinical Trial. Safetyanalysis of part 1 and rationale and design of part 2. Stroke 2011;42:119–27.
11. Hill MD, Martin RH, Palesch YY, Tamariz D, Waldman B, Ryckborst KJ, et al.The Albumin in Acute Stroke Part 1 Trial. An exploratory efficacy analysis.Stroke 2011;42:1621–5.553–560.
12. Jorgensen KA, Stoffersen E. On the inhibitory effect of albumin on platelet aggregation. Thromb Res 1980;17:13–8.
13. Halliwell B. Albumin: an important extracellular antioxidant? Biochem Pharmacol. 1998;37:569–571.
14. Belayev L, Pinard E, Nallet H, Seylaz J, Liu Y, Riyamongkol P, Zhao W, Busto R, Ginsberg MD. Albumin therapy of transient focal cerebral ischemia. In vivo analysis of dynamic microvascular responses. Stroke. 2002;33:1077–1084.
15. Reinhart WH, Nagy C. Albumin affects erythrocyte aggregation and sedimentation. Eur J Clin Invest.1995;25:523–528.
16. Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD. Human Albumin Therapy of Acute Ischemic Stroke Marked Neuroprotective Efficacy at Moderate Doses and With a Broad Therapeutic Window. Stroke. 2001; 32:553-560.
17. Hankey JK, Jamrozik K, Broadhurst RJ et al. Long-term disability after first-ever stroke and related prognostic factors in The Perth Community Stroke Study 1989-1990. Stroke. 2002;33:1034-1040.
18. Idicula TT, Waje-Andreassen U, Brogger J, NaessH, Thomassen L. Serum albumin in ischemic stroke patients: the higher the better. The Bergen Stroke Study. Cerebrovasc Dis. 2009; 28:13-17.
19. Kasundra G, Sood I. Prognostic Significance Of Serum Albumin Levels In Acute Ischemic Stroke. Natl J Integr Res Med. 2014; 5: 1-4.

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Original Research Article

Prevalence of metabolic syndrome in patients with premature coronary artery disease proven by coronary angiogram

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ABSTRACT

Background: Metabolic syndrome (MS) is associated with premature coronary artery disease (CAD). The aim of this study was to evaluate the prevalence of MS and its association with severity of CAD proven by coronary angiogram (CAG) in young patients.
Methods: We included patients, aged 45 years or less, admitted with acute coronary syndrome (ACS), who had CAD confirmed by coronary angiography. They were divided into two groups according to the presence or absence of MS based on International Diabetes Federation (IDF) criteria. CAD was classified into single, double and triple vessel disease (TVD). The prevalence of MS and its individual parameters was calculated.
Results: Among 90 young patients who presented with ACS, MS was present in 67 patients (74.44%). Among those with MS, the prevalence of each individual criterion was statistically significant in MS group (P <0.05). Prevalence of pre-existing hypertension and diabetes was significantly higher in MS group (p <0.01). Smoking, alcohol consumption and family history of CAD were not statistically significant in patients with and without MS. Fifteen out of 90 patients (14 in MS group) who presented with ACS had TVD in CAG, but this was not statistically significant (p 0.06).
Conclusions: This study confirms a very high prevalence of MS in young Indian patients with premature CAD. MS was more prevalent than the conventional risk factor smoking in young CAD patients. We could not find significant difference in severity of CAD based on CAG between MS and non-MS group.

Keywords: Coronary angiogram, Coronary artery disease, Metabolic syndrome, Young patients

INTRODUCTION

Coronary artery disease (CAD) affecting younger individuals has reached enormous proportions. There is no doubt that it will become a major public health problem in the future unless we concentrate on reversible causes and prevention, through research studies. CAD is increasing in developing and transitional countries because of urbanization, demographic and lifestyle changes, whereas it is decreasing in many developed countries.[1] Studies have suggested that the risk of myocardial infarction is mostly due to modifiable risk factors like smoking, physical inactivity, poor diet, psychosocial stress, hypertension ,diabetes mellitus, dyslipidemia and increased waist-hip ratio.[2,3] Many of these risk factors are grouped or are seen at the onset of metabolic syndrome (MS).

Metabolic syndrome, a cluster of glucose intolerance, hypertension, dyslipidemia and abdominal obesity, has

been recognized as a major predeterminant of type 2 diabetes mellitus and CAD.[4,5] MS was associated with a 1.5 fold increase in all-cause mortality and a two fold increase in cardiovascular outcomes in a recent metaanalysis.[6] Presence of MS with or without diabetes was found to be an independent determinant of premature CAD.[7] CAD in younger population is an area of increasing interest because of its potential for long-term disability and premature death.

Few studies have reported MS and its association with CAD in the young.[7-9] We found only very limited studies in an Indian setting analyzing the association of MS with premature CAD.[10] The prevalence of MS in young patients with angiographically proven premature CAD has not been studied in Indians, to our knowledge. Understanding the impact of MS on CAD in younger age group is important for developing public health policy towards prevention and management. Hence this study was undertaken to know the prevalence of metabolic syndrome using International Diabetes Federation (IDF) criteria in patients with premature coronary artery disease proven by coronary angiogram (CAG). We also tried to evaluate any association between the presence of metabolic syndrome and severity of CAD.

METHODS

This cross-sectional study was conducted at our centre, a large tertiary care teaching hospital in South India. The study was done after obtaining institutional ethical committee clearance and informed consent. All patients, aged 45 years or less, admitted with acute coronary syndrome (ACS) [ST-elevation myocardial infarction (STEMI)/non-ST-elevation myocardial infarction (NSTEMI) and unstable Angina as per standard definition], in our hospital during the period March 2014-August 2014 were included in the study. We excluded patients younger than 18 years or older than 45 years of age and patients who did not undergo coronary angiogram for various reasons.

ACS was diagnosed in patients with symptoms compatible with myocardial infarction (MI), based on electrocardiogram (ECG) changes and cardiac enzymes. Ejection fraction (EF) and left ventricular systolic dysfunction (LVD) was assessed by echocardiography. CAG was done in all subjects either at admission for primary Percutaneous Coronary Intervention (PCI) or electively after initial medical stabilization with thrombolysis or conservative treatment, depending on the clinical circumstances and consent from the patient. CAD was diagnosed as a 50% or greater stenosis in one of the three major coronary arteries and classified as single vessel disease (SVD), double vessel disease (DVD) and triple vessel disease (TVD) based on the number of arteries involved. The IDF criteria were used for a diagnosis of MS, which is suitable for Indian population.[4] MS was defined as the presence of abdominal obesity (defined by waist circumference ≥ 90 cm for men and ≥ 80 cm for women) plus two or more of the following factors

- Triglycerides ≥150 mg/dL or specific treatment for this lipid abnormality,
- High density lipoprotein (HDL) cholesterol <40 mg/dL for men or <50 mg/dL for women or specific treatment for this lipid abnormality,
- Blood pressure ≥130/85 mm Hg or treatment of previously diagnosed hypertension,
- Fasting plasma glucose ≥ 100 mg/d L or previously diagnosed type 2 diabetes.

Detailed history was taken including presenting symptoms, history of diabetes/systemic hypertension/ history of CAD in the family/usage of alcohol and smoking. A careful physical examination was done with reference to waist circumference (WC) and blood pressure. About ≥3 measurements of BP at the time of entry with values greater than or equal to 130/85mmHg on an average or any medications for hypertension was considered significant. WC was recorded according to the national health and nutritional survey. Peripheral venous blood samples were drawn at the time of admission, the following morning after an overnight fast for lipid profile (total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides) and at day 5 for fasting plasma glucose.

Statistical analysis

The data were analyzed using Statistical Package for Social Science (SPSS) Version 19.0, and Microsoft Excel 2010. Continuous variables were expressed as mean/SD and compared with independent sample t-test. Categorical variables were expressed as percentage and compared with chi-square test. A predictive value of less than 0.05 was taken as statistically significant.

RESULTS

A total of 90 young patients with ACS, who underwent CAG were studied. The key demographics of the study group are summarized in Table 1. Patients ranged from 26 to 45yrs. The mean age of the patients was 39.7 yrs. Among 90 patients, 82 were male and eight were female. Out of 90 cases, 67 (74.44%) had MS according to IDF criteria and 23 (25.55%) did not. Of these 67 patients with MS, 29 (43.28%) met three, 24 (35.82%) met four and 14 (20.89%) met all five criteria for MS. MS was found in 60 men out of 82 (73.17%) and seven women out of eight (87.5%), thus a greater percentage of young women had MS than men, but this was not statistically significant.

The distribution of MS criteria as defined by IDF in those 67 patients was as follows (Figure 1).

Table 1: Baseline demographic characteristics.

Variable value	Ms		P
	Yes (n=67)	No (n=23)	
Blood pressure ≥130/85mmhg	44 (65.67%)	6 (26.08)	<0.01
Fasting blood glucose ≥100mg/dl	43 (64.17%)	9 (39.13%)	<0.05
Triglycerides (mg/dl)	179.14	115.86	<0.01
HDL-C (mg/dl)	32.36	36.78	<0.05
WC (in cms)	95.64	87.23	<0.001
Age	40.49	37.39	<0.01
Sex			
Male	60	22	0.674
Female	7	1	
Diabetes mellitus	37 (55.22%)	5 (21.73%)	<0.01
Pre-existing hypertension	31 (46.26%)	3 (13.04%)	<0.01
Family history of CAD	10 (14.92%)	2 (8.69%)	0.448
Smoking	42 (62.68%)	15 (65.21%)	0.825
Alcohol consumption	32 (47.76%)	13 (56.52%)	0.468
Dyslipidemia	15 (22.3%)	2 (8.69%)	0.148
STEMI	36 (53.7%)	18 (78.26%)	<0.05
LVD	23 (34.32%)	11 (47.82%)	0.24

MS: Metabolic syndrome, HDL-C: High density lipoprotein cholesterol, WC: Waist circumference, CAD: Coronary artery disease, STEMI: ST-elevation myocardial infarction, LVD: Left ventricular systolic dysfunction.

All (100%) had abdominal obesity, 60 (89.55%) had low HDL or treatment for lipid abnormality, 45 (67.16%) had BP≥130/85 mmHg or treatment for hypertension, 43 (64.17%) had elevated triglycerides or treatment for lipid abnormality and 43 (64.17%) had raised FBS or DM. All these criteria were statistically significant in MS group.

Table 2: Angiography results in MS/non- MS.

Angiogram result	MS		Non-MS		P value
	N= 67	%	N=23	%	
SVD	39	58.2	16	69.56	0.335
DVD	14	20.89	6	26.08	0.605
TVD	14	20.89	1	4.34	0.066

MS: Metabolic syndrome, SVD: Single vessel disease, DVD: Double vessel disease, TVD: Triple vessel disease.

MS patients had significantly higher prevalence of pre-existing hypertension and diabetes (p<0.01). Alcohol consumption, smoking status, patients receiving treatment for dyslipidemia and family history of CAD were not statistically significant in patients with and without MS (Table 1).

Thirty-six patients (53.7%) out of 67 in MS and 18(78.26%) out of 23 in non-MS cohort presented with ST elevation MI, which was statistically significant (p <0.05). Twenty-three patients (34.32%) in MS and 11(47.82%) in non-MS group had LVD with the p value of 0.24, which was not significant.

Majority of patients in both MS and non-MS groups had SVD, 58.2 % and 69.56 % respectively; it was not significant (p 0.33). Fifteen out of 90 patients who presented with ACS had TVD. Fourteen patients with TVD had MS, whereas only one patient in non-MS group had TVD, even though TVD was more common in MS, it was not statistically significant (p 0.06) (Table 2).

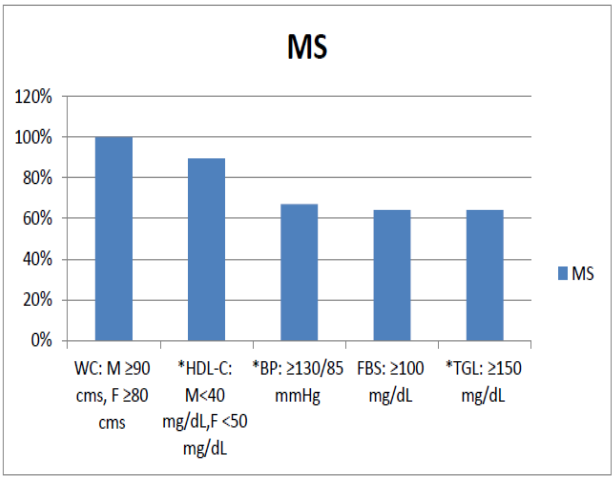


Figure 1: Prevalence of each criterion in MS group. MS: metabolic syndrome, WC: waist circumference, HDL-C: high density lipoprotein cholesterol, BP: blood pressure, FBS: fasting blood glucose or known type 2 diabetic, TGL: triglycerides. M: male, f: female, * or on treatment.

DISCUSSION

MS is characterized by a constellation of risk factors such as raised triglyceride, low HDL, glucose intolerance, hypertension and abdominal obesity that may lead to the development of CAD. Asian Indians are more prone to

diabetes and premature CAD, which is attributed to increased insulin resistance and characteristic abdominal obesity. Asian Indians have a different distribution of body fat, with abdominal obesity or a higher body fat content, even in the presence of normal body mass index.[11] Increased visceral fat is a dependable indicator of hyperinsulinemia and insulin resistance, which are risk factors for diabetes, dyslipidemia, hypertension and CAD.[12]

Hence, we have applied IDF criteria which has lower ethnic-specific waist circumference cut-offs for obesity, in our study.

We found that the prevalence of MS among patients presenting with young CAD was alarmingly high (74.44%). The prevalence of MS in young patients with CAD was similarly high in studies done by Hassanin et al. (66.39%) and Ranjith et al. (57%).[9,13] However, other studies have shown lower prevalence of MS in similar groups of patients ranging from 26% to 37%.[8,10,14]

Various studies in young CAD patients have shown a higher prevalence of MS in young women than in men.[9,10,14] In our study too the prevalence of MS was more common in women than men (87.5% versus 73.17%), but the difference was not statistically significant. This is important because Morraquin et al. have shown that women with CAD and MS have significantly higher risk of cardiovascular events and decreased survival than women without MS.[15]

Cigarette smoking was found to be the most common risk factor for premature CAD in earlier studies.[9,10,14,16] This was in contrast to our study, where we found MS was dominating with 74.44% versus smoking (63.33%), as a risk factor for premature CAD. This finding could suggest that MS may play a significantly more important role than smoking in the development of cardiovascular disease in younger age group.

While assessing the various components of MS in patients with the syndrome, we found that all patients had abdominal obesity, low HDL was the next most frequently seen risk factor (89.55%). A similar pattern of the individual components of MS in young patients with CAD was seen in a study done by Sadeghian et al.[17] Another study done in young Indians also showed a higher prevalence of low HDL (78.8%) in MS patients.[10] Higher percentage of low HDL in Indians was seen in a study conducted by Goswami et al. in MS patients with CAD.[18]

Studies have shown the predominance of dysfunctional HDL in MS patients and this proinflammatory HDL can further worsen metabolic dysfunction and hence increase the cardiovascular risk.[19] We also noted in our study that all eight women with premature CAD had low HDL. Hence, our study confirms higher prevalence of abdominal obesity and low HDL, which are well recognized risk factors for CAD.[20-23]

We also found a higher incidence of STEMI in non-MS group (78.26%) than MS (53.7%). This was unlike the results of Danciu et al. and Chung et al., where MS patients had more STEMI.[8,24] Danciu et al. found that STEMI in MS patients was statistically significant, whereas Chung et al. did not.

Although TVD was more common in the MS cohort than non-MS (14 patients versus one patient), this was not statistically significant. Thus, no significant difference in disease severity in terms of number of vessels stenosed, based on CAG, was found in our study. Goswami et al. in an Indian study found that the prevalence of MS increased significantly in patients with TVD, but they had included patients of all age groups.[18] Marroquin et al. showed that women with MS had increased risk of cardiovascular events in angiographically significant CAD patients, but again patients ranged from 21 to 86 years.[15]

There are a few limitations to our study. Those patients who met four out of five criteria for MS without the principal criteria (waist circumference more than 80 in women and more than 90 in men) were excluded from the MS group. We had only few female patients in our study. The strength of our study is that we describe the prevalence of MS in angiographically proven premature CAD, which is the gold standard to diagnose CAD.

CONCLUSION

To conclude, this study clearly indicates a very high prevalence of MS in Indian patients with premature CAD. Our study is important because we found that MS was more prevalent than the conventional risk factor of smoking in young patients with CAD. We could not find significant difference in severity of CAD between MS and non-MS group in young patients, but larger and more studies in a similar age group are required to confirm this finding.

Family physicians have an important role in identifying MS in young healthy individuals and thus, may help find people at risk for premature CAD. Healthcare professionals should routinely measure waist circumference and screen for low HDL, which is usually ignored in practice. The presence of MS or any of its components in young people should trigger an aggressive approach toward patient education and implementation of primary and secondary measures to help reduce the prevalence of premature CAD.

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REFERENCES

1. De Backer GG. The global burden of coronary heart disease. Medicographia. 2009;31(4):343-8.
2. Gupta R, Gupta VP, Sarna M, Bhatnagar S, Thanvi J, Sharma V, et al. Prevalence of coronary heart disease and risk factors in an urban Indian population: Jaipur Heart Watch-2. Ind heart J. 2002;54(1):59-66.
3. Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. The lancet. 2004;364(9438):937-52.
4. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome-a new worldwide definition. The Lancet. 2005;366(9491):1059-62.
5. Reddy KK, Rao AP, Reddy TP. Socioeconomic status and the prevalence of coronary heart disease risk factors. Asia Pac J Clin Nutr. 2002;11(2):98-103.
6. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. Metabolic syndrome and the cardiovascular risk a systematic review and metaanalysis. J Am Coll Cardio. 2010;56(14):1113-32.
7. Iribarren C, Go AS, Husson G, Sidney S, Fair JM, Quertermous T, et al. Metabolic syndrome and earlyonset coronary artery disease: is the whole greater than its parts?. J Am Coll Cardiol 2006;48(9):1800-7.
8. Danciu SC, Iqbal FM, Manankil MF, Koul S, Raghuvir R, Herrera CJ. Metabolic Syndrome in Younger Patients with Acute Coronary Syndrome. Eur J Gen Med. 2012;91(1):22-6.
9. Hassanin N, Gharib S, El Ramly MZ, Meged MA, Makram A. Metabolic syndrome and coronary artery disease in young Egyptians presenting with acute coronary syndrome. Kasr Al Ainy Medic J. 2015;21(1):27.
10. Aggarwal A, Aggarwal S, Sharma V. Metabolic syndrome and coronary artery disease in Indians younger than 40 years. J Endo Meta. 2012;2(1):39-45.
11. Das UN. Metabolic syndrome X is common in South Asians, but why and how? Nutrition. 2002;18:774-6.
12. Das UN. Pathobiology of metabolic syndrome X in obese and nonobese South Asian Indians: further discussion and some suggestions. Nutrition 2003;19:560-2.
13. Ranjith N, Pegoraro RJ, Naidoo DP, Esterhuizen TM. Metabolic syndrome in young Asian Indian patients with myocardial infarction: cardiovascular topic. Cardio J Afr. 2007;18(4):228-32.
14. Turhan H, Yasar AS, Basar N, Bicer A, Erbay AR, Yetkin E. High prevalence of metabolic syndrome among young women with premature coronary artery disease. Coron Artery Dis. 2005;16(1):37-40.
15. Marroquin OC, Kip KE, Kelley DE, Johnson BD, Shaw LJ, Bairey Merz CN, et al. Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women: a report from the Women's Ischemia Syndrome Evaluation. Circulation. 2004;109(6):714-21.
16. Von Eyben FE, Bech J, Madsen JK, Efsen F. High prevalence of smoking in young patients with acute myocardial infarction. J R Soc Health 1996;116(3):153-6.
17. Sadeghian S, Darvish S, Salimi S, Esfehani FA, Fallah N, Mahmoodian M, et al. Metabolic syndrome: stronger association with coronary artery disease in young men in comparison with higher prevalence in young women. Coron Artery Dis. 2007;18(3):163-8.
18. Goswami B, Tayal D, Tyagi S, Mallika V. Prevalence of metabolic syndrome in patients with angiographically proven coronary artery disease presenting to a tertiary care hospital in Delhi, India. Diabetes Meta Synd. 2011;5(2):53-60.
19. Watson KE, Ansell BJ, Watson AD, Fonarow GC. HDL function as a target of lipid-modifying therapy. Rev Cardiovasc Med. 2007;8(1):1-8.
20. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. J Cl Endocrinol Meta. 2001;86(11):5366-71.
21. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, et al. INTERHEART Study Investigators. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet. 2005;366(9497):1640-9.
22. Jayasinghe SR, Jayasinghe SH. Variant metabolic risk factor profile leading to premature coronary disease: time to define the syndrome of accelerated athero-coronary metabolic syndrome in Asian Indians. Singapore Med J. 2009;50(10):949-55.
23. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K, et al. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. JAMA. 2007;297(3):286-94.
24. Chung EH, Curran PJ, Sivasankaran S, Chauhan MS, Gossman DE, Pyne CT, et al. Prevalence of Metabolic Syndrome in Patients < 45 Years of Age with Acute Myocardial Infarction Having Percutaneous Coronary Intervention. Am J Cardio. 2007;100(7):1052-5.

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Original Research Article

Tropical sprue in megaloblastic anemia

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ABSTRACT

Background: The causes of megaloblastic anemia may vary in different geographical regions. The aim of the present study is to evaluate the utilization of bone marrow examination and upper gastrointestinal endoscopy (UGIE) in megaloblastic anemia.
Methods: This was a cross-sectional descriptive study done on 50 patients (age ≥15years) of macrocytic anemia after applying inclusion and exclusion criteria. A bone marrow aspiration with biopsy and an UGIE with duodenal biopsy were performed in consented patients with evidence of megaloblastic anemia in the peripheral smear or Vitamin B12 deficiency or folate deficiency or both.
Results: Out of 50 cases, 38 patients had pure Vitamin B12 deficiency, 2 patients had pure folate deficiency and 5 patients had combined deficiency. Among 43 patients with vitamin B12 deficiency, only four (9.3%) were vegetarians and remaining 39 (90.7%) were having non-vegetarian diet. Bone marrow study was done in 29 patients (out of 50) and all of them were found to have megaloblastic erythropoiesis in the bone marrow. Thirty three out of 50 consented for UGIE and duodenal biopsy. Out of 33, 17 patients (51.5%) had features of tropical sprue in biopsy.
Conclusions: We found a high prevalence of tropical sprue in megaloblastic anemia due to Vitamin B12 and/or folate deficiency. We recommend that UGIE with deep duodenal biopsy should be considered in all patients with megaloblastic anemia to rule out tropical sprue in India.

Keywords: Bone marrow study, Duodenal biopsy, Megaloblastic anemia, Tropical sprue, Upper gastrointestinal endoscopy

INTRODUCTION

Megaloblastic anemia is characterized by macrocytic anemia and abnormally large erythrocyte precursor cells (megaloblasts) in the bone marrow. Disorders that affect the synthesis of DNA in the precursors of erythrocytes in the bone marrow lead to megaloblastic anemia. Vitamin B12 and folate deficiencies are the most common causes of megaloblastic anemia.

The causes of megaloblastic anemia may vary in different geographical areas. Pernicious anemia is a major cause of megaloblastic anemia in western countries, but nutritional deficiencies and malabsorption could be the leading cause of megaloblastic anemia in India.[1] Studies have shown that vitamin B12 deficiency is more common in vegetarians.[2,3]

The main cause of folate deficiency is less intake of green leafy vegetables and legumes. There have been hardly any studies on the role of bone marrow and upper gastrointestinal endoscopy (UGIE) in the evaluation of megaloblastic anemia. The objective of the present study is to evaluate the utilization of bone marrow examination and UGIE in megaloblastic anemia.

METHODS

This was a cross-sectional descriptive study carried over a period of 12 months from July 2013 to June 2014 on 50 patients (age ≥15years) of macrocytic anemia treated in a tertiary care hospital of South India. Macrocytic anemia was identified when:

- A mean red blood corpuscular volume >95 fL
- Anemia with hemoglobin of
  a) <13 g/dL in male
  b) <12 g/dL in female.

Inclusion criteria

Patients above the age of 15 years with macrocytic anemia.

Exclusion criteria

Patients with liver disease, chronic kidney disease, hypothyroidism, hemolytic anemia, hemorrhagic disease, post splenectomy and pregnant patients.

The history was taken in detail; a complete and thorough physical examination was carried out in all patients. Detailed information was sought on alcohol consumption, drug intake, thyroid disorder and other comorbid illness. All patients were investigated with a hemogram that included estimation of hemoglobin level, red cell indices (MCV, MCH and MCHC), red cell distribution width, total leucocyte count, differential leucocyte count, platelet count, reticulocyte count, and peripheral smear examination.

All patients were subjected to fasting serum Vitamin B12, fasting serum folic acid, serum TSH, creatinine, liver function test and lactate dehydrogenase. A bone marrow aspiration with biopsy and an UGIE with deep duodenal biopsy were performed in patients with evidence of megaloblastic anemia in the peripheral smear or Vitamin B12 deficiency or folate deficiency or both, who gave consent for the same.

Statistical analysis

The data were analyzed using Microsoft Excel 2010 and Statistical Package for Social Science (SPSS) Version 19.0. Continuous variables were expressed as mean/SD and categorical variables were expressed as percentage. Independent student T test and Anova tests were used as described below. A predictive value of less than 0.05 was taken as statistically significant.

RESULTS

In current study population of fifty patients presenting with macrocytic anemia, 34 were male comprising about 68% and 16 were female comprising about 32%. The mean age of male and female were 53.15±15.89 and 54.37±16.58 respectively. Majority of the population belong to the age group above 40 years, both in males and females. Six patients in this study group were vegetarians. Five patients gave history of consuming alcohol. Three patients gave history of drug exposure and were on phenytoin/ sodium valproate.

The major symptom at presentation was fatigability (58%, n=29), 16% (n=8) presented with chronic diarrhea and 26% (n=13) with other symptoms like short pyrexia etc. No patient had bleeding tendencies or features of nervous system involvement.

On clinical examination, pallor was noticed in all patients in various degrees from mild to severe. Icterus was seen in 10 patients, hyperpigmentation of the knuckles in 7 patients. Organomegaly was noticed in 2 patients, among them one had hepato-splenomegaly and one had splenomegaly.

Out of 50 cases, 38 patients had low Vitamin B12, 5 patients had low levels of both folate and Vitamin B12 and 2 patients had low folate level alone. Vitamin B12 and folate levels were normal in the remaining five patients (Table1). Among 43 patients with Vitamin B12 deficiency, only four (9.3%) were vegetarians and remaining 39 (90.7%) were having non- vegetarian diet.

Table 1: Vitamin B12 and folate status in the study group.

Vitamin status	n=50	Percentage (%)
Pure vitamin B12 deficiency	38	76
Pure folate deficiency	2	4
Combined deficiency	5	10
Both normal	5	10

Table 2 shows hematological parameters of our patients. Out of 50 patients, 24 patients presented with anemia alone, 6 patients with bicytopenia (low hemoglobin and low platelets) and the remaining 20 patients with pancytopenia.

Table 2: Hematological parameters.

Hematological parameter	Mean ± SD
Hemoglobin (g%)	7.1 ± 2.5
MCV (fL)	115.1 ± 10.3
Red cell distribution width	22.71 ± 6.2
Total WBC count	4775 ± 2643
Platelet count	123686 ± 100097

Table 3: Comparison of severity of anemia with macrocytosis.

Hemoglobin (g%)	MCV(fL) Mean ± SD	n =50	P value
< 6	113.56 ± 7.83	18	>0.05
> 6	115.96 ± 11.39	32	

Study found no statistical significance (p=0.4) between the severity of anemia and the degree of macrocytosis, when the values of MCV were compared with the degree of anemia using independent student T test (Table 3). There was no statistical significance (p=>0.5) between the mean of MCV and levels of serum Vitamin B12 using Anova test (Table 4).

Table 4: Comparison of serum Vitamin B12 level and MCV.

Vitamin B12 level (pg/mL)	n=50	MCV (fL) Mean ± SD	P value
< 100	n = 28	116.69±10.89	>0.05
101-190	n = 15	112.20±6.83	
> 190	n = 7	114.92±12.50	

Out of 24 patients with anemia, 21 had low serum Vitamin B12 and 16 of 20 patients with pancytopenia had low serum Vitamin B12. All 6 patients with bicytopenia had low serum Vitamin B12. When the various levels of low serum Vitamin B12 were compared with presentation of patient with anemia, bicytopenia or pancytopenia using Anova test there was no significance (p=>0.5) (Table 5).

Table 5: Comparison of serum Vitamin B12 level and hematological presentation.

Presentation	n=50	Vitamin B-12 level (pg/mL) Mean ± SD	P value
Pancytopenia	20	64.37±31.41	>0.05
Bicytopenia	6	76.83±33.36	
Anemia	24	98.24±44.88	

The peripheral smear examination showed neutrophilic hyper segmentation and / or macro-ovalocytosis in 25 patients suggesting megaloblastic erythropoiesis. These patients and another four (Vitamin B12/folate/or both deficient) who gave consent were subjected to a bone marrow examination. Among 50 patients, bone marrow examination was done in 29 and all of them were found to have megaloblastic erythropoiesis in the bone marrow.

Thirty three out of 50 consented for UGIE and deep duodenal biopsy. Twenty-six of them showed duodenal finding of either scalloped duodenal folds or folded duodenal margins. Others showed features of erosive gastritis, atrophic gastritis or antral gastritis. One had external gastric impression and the other had gastric polyp (Table 6).

Among twenty-six patients who had abnormal duodenal features in endoscopy, fifteen showed features suggestive of tropical sprue in duodenal biopsy (Table 6). Two patients who had normal duodenal findings also had features of tropical sprue in biopsy. Hence 17 patients out of 33 (51.5%), who gave consent for endoscopy and biopsy had tropical sprue. Among 17 patients with tropical sprue, 15 had low Vitamin B12 level, one had both low vitamin B12 and folate levels and one had both normal.

Table 6: Upper gastrointestinal endoscopy and duodenal biopsy findings.

Endoscopy findings	n =33
Scalloped duodenal folds / flattened duodenal margins	26
Erosive gastritis	2
Atrophic gastritis	2
Antral gastritis	1
Others	2
Duodenal biopsy findings	**n =33**
Tropical sprue	17
Intraepithelial lymphocytosis	2
Non-specific	3
Brunner's gland hyperplasia	3
Peptic duodenitis	1
No specific pathology	7

DISCUSSION

Megaloblastic anemia has variable etiological factors. In current study population 76% had Vitamin B12 deficiency, 10% had deficiency of both Vitamin B12 and folate and 4% had pure folate deficiency. Our findings were consistent with a study by Khanduri and Sharma, who have reported Vitamin B12 deficiency to be 65% in 120 patients of megaloblastic anemia, combined Vitamin B12 and folate deficiency in 12% and folate deficiency in 6% .[4] A similar high prevalence of Vitamin B12 deficiency was reported by Sarode et al, in 102 cases (76%) of nutritional megaloblastic anaemia.[5] In contrast to this finding, Haq et al, found that folic acid deficiency was the commonest cause (62.5%) of megaloblastic anemia in her patients.[6]

The predominant symptoms in this study were fatigability (58%) and chronic diarrhea (16%). No patient presented with bleeding manifestation or had neurological symptoms. Unnikrishnan et al, found 91.7% of their patients presenting with fatigability, but in contrast to present study, 36.7% had bleeding manifestation and 10% presented with neurological symptoms.[7] In another study by Haq et al, in a population of 80 patients, 84% had fatigability and 27.5% had bleeding tendency.[6] Bleeding manifestations in thrombocytopenia due to Vitamin B12 or folate deficiency are typically mild and this may be the reason why no patient presented with bleeding tendency in the study.

In the present study, clinical examination revealed pallor in 100%, icterus in 20%, skin hyperpigmentation in 14% and organomegaly in 4%. Haq et al, found pallor in 84% and splenomegaly in 48%.[6] In a study by Unnikrishnan et al, 35% had jaundice, 31.7% had splenomegaly, 28.3% had hepatomegaly and 23.3% had skin changes.[7] Thus

when compared to the above studies we found lesser percentage of patients with organomegaly and skin changes.

Among 43 patients with Vitamin B12 deficiency, only four (9.3%) were vegetarians and remaining 39 (90.7%) were having non-vegetarian diet. 39 out of 44 (88.6%) non-vegetarians had Vitamin B12 deficiency in present study group. In concordance with present study, Unnikrishnan et al, and Iqbal et al, also reported a higher proportion of non-vegetarians having megaloblastic anemia and Vitamin B12 deficiency.[7,8] In contrast to our finding, Liggy et al and Kankonkar et al, showed Vitamin B12 deficiency higher in vegetarians than non-vegetarian. But the study cohort was different, Liggy et al, included all the patients and not only anemic patients, Kankonkar studied B12 deficiency in different diseases.[9,10] In current study 40% of patients had pancytopenia. Other studies have also found that megaloblastic anemia can present with pancytopenia.[5,11]

Bone marrow study was done in 29 out of 50 patients by us. All patients had megaloblastic erythropoiesis in the bone marrow. Khanduri and Sharma have suggested that bone marrow study should be done in selected patients when myelodysplasia is a differential diagnosis.[4] We also agree with them that bone marrow examination need not be done in all patients initially with megaloblastic anemia or Vitamin B12/folate deficiency, as it did not contribute to identifying a different etiology. In present study population of 50 patients, 33 (66%) underwent endoscopy and biopsy. 17 patients out of 33 (51.5%) had tropical sprue (TS). Among 17 patients with TS, 15 had low Vitamin B12 level, one had both low Vitamin B12 and folate levels and one had both normal.

An interesting observation in this study was that tropical sprue was more prevalent in megaloblastic anemia due to Vitamin B12 and/or folate deficiency. This may be because of endemic nature of TS in India. We found that Vitamin B12 deficiency was more in non-vegetarians and this may be due to underlying TS associated malabsorption.

TS is a malabsorption syndrome of unknown etiology or due to a probable persistent infection. TS cause malabsorption of folic acid and Vitamin B12 leading to megaloblastic anemia. A study done in West India in 1968 has reported that majority of patients with TS have Vitamin B12 deficiency.[12] After extensive literature search, we could not find many studies of endoscopic biopsy in megaloblastic anemia. There are few studies in malabsorption syndrome. Dutta et al. in a south Indian study and Ranjan et al. in a north Indian study found that TS was the commonest cause of malabsorption syndrome.[13,14]

There are a few limitations to current study. The sample size was small and we could not do complete work up for megaloblastic anemia and malabsorption due to cost factor.

CONCLUSION

To conclude, we found a high prevalence of TS in megaloblastic anemia due to Vitamin B12 and/or folate deficiency. We observed in thisstudy that being a non-vegetarian does not protect against Vitamin B12 deficiency, hence we recommend that all patients with megaloblastic anemia (including vegetarians) in India should be subjected to UGIE with deep duodenal biopsy to rule out TS, as the prevalence is high and treatment is different.

Study also found that bone marrow study did not yield added information and hence need not be considered in all patients with megaloblastic anemia or Vitamin B12/folate deficiency at the time of presentation, unless clinically indicated. We need larger and more studies to confirm our finding.

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REFERENCES

1. Hoffbrand V, Provan D. ABC of clinical haematology. Macrocytic anaemias. BMJ. 1997;314:430-3.
2. Antony AC. Prevalence of cobalamin (vitamin B-12) and folate deficiency in India-audi alteram partem. Am J Clin Nutr. 2001;74:157-9.
3. Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. Natl Med J India. 2005;18:182-3.
4. Khanduri U, Sharma A. Megaloblastic anameia: prevalence and causative factors. Natl Med J India. 2007;20:172-5.
5. Sarode R, Garewal G, Marwaha N, Marwaha RK, Varma S, Ghosh K, et al. Pancytopenia in nutritional megaloblastic anaemia: a study from north-west India. Trop Geogr Med. 1989;41:331-6.
6. Haq S, Iqbal N, Fayyaz F, Tasneem A. Serum B12 and folate levels in patients with megaloblastic change in the bone marrow. Biomedica. 2012;28:35-9.
7. Unnikrishnan V, Dutta TK, Badhe BA, Bobby Z, Panigrahi AK. Clinico-aetiologic profile of macrocytic anemias with special reference to megaloblastic anemia. Indian J Hematol Blood Transfus. 2008;24:155-65.
8. Iqbal SP, Kakepoto GN, Iqbal SP. Vitamin B12 deficiency- a major cause of megaloblastic anaemia in patients attending a tertiary care hospital. J Ayub Med Coll Abbottabad. 2009;21:92-4.

9. Liggy A, Thomas T, Nambudiri H. Vitamin B12 status in a tertiary care center in central Gujarat. National J Community Med. 2012;3:414-6.
10. Kankonkar SR, Joshi SV, Tijoriwala SJ, Prabhu RV, Raikar SC, Kankonkar RC, et al. A study of Vitamin B12 deficiency in different diseases. Available at www.bhj.org/journal/2004_4602_april/studyofvitam in _110.htm.
11. Kumar R, Kalra SP, Kumar H, Anand AC, Madan N. Pancytopenia--a six-year study. J Assoc Physicians India. 2001;49:1078-81.
12. Jeejeebhoy KN, Desai HG, Borkar AV, Deshpande V, Pathare SM. Tropical malabsorption syndrome in West India. Am J Clin Nutr. 1968;21:994-1006.
13. Dutta AK, Balekuduru A, Chacko A. Spectrum of malabsorption in India-tropical sprue is still the leader. J Assoc Physicians India. 2011;59:420-2.
14. Ranjan P, Ghoshal UC, Aggarwal R, Pandey R, Misra A, Naik S, et al. Etiological spectrum of sporadic malabsorption syndrome in northern Indian adults at a tertiary hospital. Indian J Gastroenterol. 2004;23:94-8.

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A Prospective Study of Correlatio of Magnetic Resonance Imaging of Brain Including MR Angiogram and Carotid Vertebral Arterial Doppler Study with the Clinical Features in Ischemic Stroke

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ABSTRACT

Introduction: Stroke is a global health problem. In this study we evaluated the risk factors, clinical features and their correlation with Magnetic Resonance Imaging (MRI) brain including Magnetic Resonance Angiogram (MRA) and Carotid Verteberal (CV) Doppler findings in patients presenting with acute ischemic stroke.

Material and methods: Seventy five patients of the age group 20 to 80 years admitted with acute ischemic stroke were enrolled for the study. A detailed history, clinical examination and blood investigations were done. MRI, MRA and CV Doppler were done in all patients. Serum Homocysteine levels and Anti-nuclear antibody assay (ANA) by Immunofluorescence (IF) were done in selected patients. Statistical analysis was done using SSPS version 16.0 for microsoft windows.

Results: In this study, there were more males than females. The mean age was 59 years. Young stroke population (<40 years) was 5%. Anterior circulation was predominantly involved than posterior circulation. Right Middle Cerebral Artery was the most common artery involved. There was positive correlation for clinical presentation and area of infarct and for areas of infarct and the vessels involved. Most common clinical presentations were weakness and slurring of speech. There were 3% intracranial anomalies. 56% had significantly elevated levels of homocysteine. 24% had ANA- IF positivity. Recurrence of stroke was seen in 17%; National Institute of Health Stroke Scale (NIHSS) score was higher in patients with recurrent stroke and right hemisphere infarcts.

Conclusion: There was positive correlation for clinical presentation and area of infarct and for areas of infarct and the vessels involved.

Keywords: Stroke, Anterior Circulation, Clinical co-relation, MRI, MRA Brain, Homocysteine, Area of Infarct.

INTRODUCTION

Stroke is a worldwide concern. Approximately two hundred lakh people every year suffer from stroke and of these fifty lakh do not survive.[1,2] In the developed nations, stroke is the leading cause for disability, second cause of dementia and third cause of death. Stroke is the second common cause of death and fourth leading cause of disability globally.[3] Stroke is an important cause for epilepsy, falls and depression in developed nations.[4] It is the leading cause of functional impairments, with 20% of survivors required hospital and nursing care for a prolonged period of more than 3 months duration and 15% - 30% of patient develop permanent disability.[5] Stroke is a life-changing event both financially and physically.

Stroke is caused by acute interruption of blood supply to the brain region it supplies, usually because of the blockade of a cerebral artery by a thrombus or emboli or by rupture of a vessel. This cuts off the supply of vital oxygen and nutrients to the brain, accumulation of free radicals causing damage to the brain tissue. An acute total occlusion of the vessels to the brain can cause death of the brain tissue within four to ten minutes. WHO clinically defines stroke as the rapid development of clinical signs and symptoms of a focal neurological disturbance lasting more than 24 hours or leading to death with no apparent cause other than vascular origin.[6] Among CVA, ischemic stroke constitutes 50%–85%[6,7] Hemorrhagic strokes - Subarachnoid hemorrhage and intra-cerebral hemorrhage, account for 1%-7% and 7%-27% respectively of all strokes worldwide.[8,9] In a study done, hemorrhagic stroke has significantly higher mortality when compared with ischemic stroke.

This study was conducted to evaluate various parameters like the risk factors of stroke, areas of brain involvement including the vessels involved and their clinical correlation in patients with acute ischemic stroke.

MATERIAL AND METHODS

This is a prospective observational study conducted in a tertiary hospital at Coimbatore between June 2015 and June 2016. A total of 75 patients in the age group of 20 to

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80 years admitted with acute ischemic stroke to the medical and neurology wards were enrolled in the study. The study was initiated after obtaining clearance from the Institutional Ethical Committee. After obtaining informed consent, a detailed history attributable to the risk factors of stroke was obtained. A detailed clinical examination including baseline NIHSS score was obtained.

Investigations like complete hemogram, urine analysis, blood sugar, serum electrolytes, serum creatinine, blood urea, chest X-ray, fasting lipid profile, echocardiogram, electrocardiogram, MRI brain with MRA intracranium, CV Doppler were done in all patients. HbA1C was done if patient was found to be Diabetic based on random blood sugars. Investigations like ANA- IF and serum Homocysteine were done in selected patients.

STATISTICAL ANALYSIS

The data was reported as the mean +/- SD or the median, depending on their distribution. Frequencies were reported in percentages. The differences in quantitiative variables between groups were assessed by means of the unpaired t test. Non parametric test Wilcoxon signed test was used to assess the qualitative variables. To assess the difference in categoric variables between groups chi square test was used. Two tailed test was considered significant for all statistical tests when *P* value is <0.05. All data were analysed with a statistical software package (SSPS version 16.0 for microsoft windows).

Symptoms	Number of patients
Headache	6
Slurring of speech	33
Gait	16
Giddiness	17
Deviation of mouth	33
Weakness	51
Vomiting	8
Urinary incontinence	1
Decreased sensation	12
Total loss of speech	4
Double vision	5
Hiccups	1
Blurring of vision	1
Altered sensorium	1
Seizures	3
Pin prick sensation	1
Pain	1
Dysphagia	1
Loc	

Table-1: Clinical Presentation

RESULTS

Among the study population of 75 patients, 62 (83%) were males and 13 (17%) were females. 77% of total patients were aged more than 50 years. The mean age in the study population was 59 years. The young stroke population (<40 years) in this study was 5%.

The percentage of smokers, alcoholics and both was 17%, 5% and 24% respectively. In the study population the percentage of Type 2 Diabetes mellitus, Systemic hypertension, Dyslipidemia and combined was 11%, 9%, 35% and 41% respectively.

The most common symptoms were weakness, slurring of speech, deviation of angle of mouth as listed in Table 1.

In the study population, 47 patients (63%) had anterior circulation stroke and rest twenty eight patients (37%) had posterior circulation involvement. The most commonly involved arteries in the present study were Middle Cerebral Artery (MCA). Forty three out of seventy five patients (57%) had MCA involvement. Among the 43 patients with MCA involvement, right MCA was involved in 24 patients and 19 patients had diseased left MCA. Anterior cerebral artery (ACA) was involved in 4 patients. Posterior cerebral artery (PCA) was involved in twenty eight patients (Figure 1)

31% of the patients had intracranial obstruction due to atherothrombotic process which was classically seen in MRA intracraium. 11% of the patients had only extracranial occlusion which was seen in CV doppler studies. Twenty one percentage of the study population had both intracranial and extracranial leisions and 37% had normal IC & EC studies, probably related to small vessel ischemia.

Forty five patients (60%) had positive correlation of location of the infarct in the brain with either MRA or CV Doppler findings *P* <0.001. 37% had normal MRA & CV Doppler findings. Only in 3% of the study population there was no

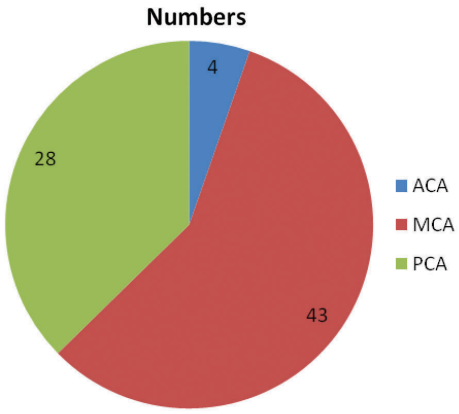


Figure-1: Most commonly involved arteries

			95% CI of mean				
	Mean	SD	Lower	Upper	Minimum	Maximum	Sig
Right	5.5	2.9	4.5	6.5	1	11	<0.05
Left	5.2	2.3	4.4	6.0	1	10	
Bilateral	4.8	3.3	2.0	7.5	2	12	
Total	5.3	2.7	4.7	5.9	1	12	

Table-2: Mean NIHSS score with Laterality

correlation of the same.

The clinical presentation was correlating with infarct in MRI in seventy patients (93%) P <0.05. There was no correlation in 7% of the study population due to bilateral involvement.

In this study population, recurrent CVA was seen in thirteen patients (17%). The mean age of recurrent CVA was 62 yrs and average NIHSS was 6.5 which were significantly higher than patients who had CVA for the first time. NIHSS was more in those with right hemisphere involvement when compared with left and bilateral involvement. The average NIHSS for right, left, bilateral involvement was 5.5, 5.2, and 4.8 respectively (Table 2). The maximum NIHSS in this study population was 12 which were noted with bi-hemisphere involvement.

Fasting serum Homocysteine levels and ANA –IF was done in 46 patients. Elevated homocysteine and ANA –IF positivity was noted in 56% and 24% respectively. Serum homocystiene was elevated in 36% of the alcoholics and 64% of non alcoholics. The highest value was 160 against <12 of the normal reference range.

In the study population, 2 (3%) had congenital anomalies. They were Frontal arteriovenous malformation and fetal PCA. None of the patients in the study population underwent thrombolysis.

DISCUSSION

Stroke among adults is rapidly increasing in the recent times. A systematic approach with good clinical history and examiantion, evaluation of risk factors, investigations and proper management of stroke is the need of the hour for acute ischemic stroke (AIS) patients.

In this study, males were more when compared with females. The male: female sex ratio among affected individuals is 7:1 in India. Among the females high risk groups are those taking oral contraceptives and pregnancy.[10,11]

The mean age was 59 years. In our study young stroke population (<40 years) was 5%. In a study at All India Institute of Medical Sciences (AIIMS), out of the 2,634 patients admitted with AIS, 440 patients (16.7%) were in the age group of 18-45 years and majority (83.4%) were male.[12] Smoking, alcoholism and dyslipidemia have been found to be significantly associated with stroke and its recurrence. Poor control of Diabetes Mellitus and systemic hypertension were associated with stroke recurrence.

Anterior circulation was predominantly involved than posterior circulation. Right MCA was the most common artery involved. There was positive correlation for clinical presentation and area of infarct and for areas of infarct and the vessels involved. Most common clinical presentation was weakness and slurring of speech. There were 3% intracranial anomalies

Eleven percent of patients had only extracranial occlusion. Extracranial occlusion that is carotid artery disease constitutes 10-20% of the strokes; stroke risk is directly proportional to the degree of occlusion. Carotid endarterectomy should be considered within 2 weeks if carotid stenosis of more than 70% involvement. There is less benefit of end carotidectomy and carotid stenting in patients with stenosis of carotids 50 to 69% and in asymptomatic patients, and there is literally no benefit in patients with stenosis of less than 50%

Hyperhomo-cysteinemia emerged as an important independent risk factor for ischemic stroke.[13] 56% had significantly elevated levels of homocystiene. The levels ideally have to be assessed 6 weeks later, for ideally diagnosing hyperhomocystinemia. Vitamins such as B12, B6 and folate play a vital role in the Homocysteine - methionine cycle[14], thus alcoholics are at risk of developing lower levels of folate and hence the increased Homocysteine levels. In contrary the proportion of elevated levels of Homocysteine was more in the non alcoholics when compared with alcoholics. There is correlation made in few studies that higher the Homocysteine values higher the mortality and recurrence of stroke.

24% had ANA- IF positivity but none of them had history of connective tissue disease. Incidental ANA- IF positivity in 24% of the study population raises concern whether it should be done routinely in all stroke patients and the same should be repeated to confirm the autoimmune etiology 6 weeks later.

Recurrence of stroke though was seen in 17%, but no mortality was observed among these patients. Relapse of alcohol, hard to kickoff smoking, poor adherence of medications was prime important in the stroke recurrence. NIHSS score was comparatively higher in these patients than in patients who had stroke for the first time. Similarly Right sided infarcts had higher NIHSS

None of the patients was thrombolysed due to varied reasons like exclusion criteria, after explaining the risks involved and mainly the time lapse in reaching the hospital. None of the patients was treated surgically.

CONCLUSION

There was positive correlation for the clinical presentation with area of infarct and for areas of infarct with the vessels involved. Elevated serum homocystiene levels and incidental positivity of ANA-IF noted in the present study raises concern; further studies are needed which will throw light in this area and indicate their significance in patients with acute ischemic stroke.

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REFERENCES

1. Dalal PM, Bhattacharjee M. Stroke Epidemic in India: Hypertension-Stroke Control Programme is Urgently Needed. JAPI 2007. Vol 55.
2. Dalal P, Bhattacharjee M, and Vairale J, Bhat P. UN millennium development goals: can we halt the stroke epidemic in India? Ann Indian Acad Neurol 2007; 10: 130-6.
3. Strong K, Mathers C, Bonita R. Preventing stroke: saves lives around the world. Lancet Neurol 2007; 6: 182-7.
4. Marc Fisher MD, Bo Norrving. 1st Global Conference

on Healthy. Lifestyles and Non communicable diseases Control. Moscow, April 28-29, 2011.

5. Steinwachs DM, Collins-Nakai RL, Cohn LH, Garson A Jr, Wolk MJ.J The future of cardiology: utilization23 and costs of care. Am Coll Cardiol. 2000;35:91B-98B.
6. Mackay J, Mensah G, Mendis S, Greenlund K. Atlas of heart disease and stroke. WHO 2004.
7. Jamieson DG. Diagnosis of Ischemic Stroke. Am J Med. 2009;122(4 SUPPL. 2).
8. Feigin V, Lawes C, Bennet D, Barker Cello S, Parag V. Worldwide stroke incidence and early case fatality in 56 population based studies: a systematic review. Lancet Neurology 2009; 8: 355- 369.
9. Smith SD, Eskey CJ. Hemorrhagic stroke. Radiologic Clinics of North America. 2011. p. 27–45.
10. Sethi PK. Stroke - Incidence in India and Management of Ischaemic stroke. Neurosci Today [Internet]. 2002;6:139–43. Available from: http://openmed.nic.in/1753/01/SGRH_p202-206_EDIT.pdf
11. Das S, Hazra A, Ray BK, Ghosal M, Banerjee TK, Roy T, et al. Burden among stroke caregivers: Results of a community-based study from Kolkata, India. Stroke. 2010; 41:2965–8.
12. Dash D, Bhashin A, Pandit AK, Tripathi M, Bhatia R, Prasad K, et al. Risk factors and etiologies of ischemic strokes in young patients: A tertiary hospital study in north India. J Stroke. 2014; 16:173–7
13. Modi M, Prabhakar S, Majumdar S, Khullar M, Lal V, Das C P. Hyperhomocysteinemia as a risk factor for ischemic stroke: An Indian scenario. Neurol India 2005; 53:297-301
14. Yakub M, Iqbal MP, Kakepoto GN, Rafique G, Memon Y, Azam I, et al. High prevalence of mild hyperhomocysteinemia and folate, B 12 and B 6 deficiencies in an urban population in Karachi, Pakistan. Pakistan Journal of Medical Sciences. 2010; 26:923–929.

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Association of N-terminal-Pro-B-type Natriuretic Peptide with Localization of Thrombus and Clinical Outcome in Acute Pulmonary Embolism

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ABSTRACT

Introduction: Pulmonary Embolism is a major health problem which is associated with significant mortality and morbidity. It is a common and lethal condition. This study was undertaken to find the association between NT-Pro-BNP levels and the localisation of thrombus in acute Pulmonary Embolism and to prognosticate the patients.

Material and methods: Prospective study conducted at a tertiary care hospital.Thirty patients with Pulmonary Embolism of various etiologies, confirmed with clinical features, laboratory investigations and imaging and fulfilling the inclusion and exclusion criteria were included. After getting informed consent, Blood samples for assessment of NT-Pro-BNP levels were collected within 24 hours of admission.

Results: In 19 patients, the thrombus was located centrally (in main pulmonary artery) whereas in 11 patients, the thrombus was located in the peripherally (in segmental and subsegmental pulmonary arteries). 22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died. Patients with central pulmonary embolus had higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries had relatively less NTPro-BNP levels. Patients with high NT-Pro-BNP levels had complicated in-hospital course/ mortality whereas, patients with relatively lower NT-Pro-BNP had uncomplicated in-hospital course.

Conclusion: This study shows that higher NT-Pro-BNP levels indicate higher probability of a more central location of thrombus in pulmonary embolism and also right ventricular overload. Also patients with higher NT-Pro-BNP levels had a complicated in hospital course.

Key words: Pulmonary Embolism, Pulmonary Arteries, Right Heart Failure, NT Pro-BNP

INTRODUCTION

Pulmonary Embolism (PE) is a major health problem which is associated with significant mortality and morbidity. It is a common and lethal condition. Pulmonary Thrombo Embolism (PTE) and deep vein thrombosis (DVT) collectively known as venous thromboembolism (VTE) encompass one disease entity. Among cardiovascular diseases, VTE along with stroke and myocardial infarction rank as three big cardiovascular killers. PTE implies partial or complete occlusion of pulmonary arterial circulation by the clot formed elsewhere usually in deep veins of the leg. Less than 5% of venous thrombosis occurs in other sites. The incidence of PE in non-hospitalised patients is 3.5 per 10,000 person years while the incidence rate in hospitalised in-patients can be above 500 per 10,000 person years.[1] In India, VTE is a common cause of mortality and morbidity in patients hospitalized for medical or surgical illnesses. It is often misdiagnosed and not intervened in time. Though the exact magnitude of problem is not known in India, the clinical relevance and incidence is not expected to be different from Western countries. Acute PE has high early mortality rates. Despite the advances in diagnosis and treatment, the early mortality rates have not changed significantly. Due to the embolus obstructing the pulmonary bed, PE can result in a life threatening condition, acute right ventricular (RV) failure.[2] Early diagnosis is of paramount importance since most patients die within the first hours of presentation. Timely management is usually highly effective in reversing RV failure. The diagnosis of acute PE is based on the assessment of clinical likelihood, ECG, CXR, echocardiography, laboratory investigations, mainly D-dimer and cardiac biomarkers and imaging techniques (CT pulmonary angiogram). Several cardiac biomarkers have emerged as indicator of right ventricular dysfunction and clinical outcome predictor in patients with acute PE. Brain type natiuretic peptide (BNP) is a marker of ventricular dysfunction. Following cardiac myocyte stretch, this compound is released into the circulation. It is synthesized as an inactive pro hormone (Pro-BNP) which is split into active hormone BNP and an inactive N-terminal fragment (NT-Pro-BNP).[3] This study was undertaken to find the association between NT-Pro-BNP levels and the localisation

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of thrombus in acute PE, to monitor and prognosticate the patients closely.

Primary objectives of the study were to study the association of N-terminal-Pro-B-Type Natriuretic Peptide with localisation of thrombus in Acute Pulmonary Embolism and to study the association between NT-Pro-BNP levels and clinical outcome in patients with Acute Pulmonary Embolism and secondary objectives were to study on the various risk factors in patients diagnosed with acute pulmonary thrombo embolism and to follow up the patients at the end of 1 month to know the clinical outcome.

MATERIAL AND METHODS

Prospective study was conducted at a tertiary care hospital, PSG Hospitals, Coimbatore. Thirty consecutive patients diagnosed to have pulmonary embolism were included in this study with the following criteria.

Inclusion criteria

All patients of age more than 18 years with clinical and radiological evidence of acute PE will be included in the study.

1) Clinical evidence

Patients presenting with dyspnoea, hemoptysis and chest pain with no acute ischemic changes in ECG and with normal Ejection fraction. ECG in acute pulmonary embolism will shows S1Q3T3 pattern.

2) Radiological evidence

CT pulmonary angiogram showing filling defects within the pulmonary vasculature.

Exclusion criteria

Patients with COPD, known case of Congestive cardiac failure and chronic kidney disease are excluded from this study.

After getting informed consent, Blood samples for assessment of NT-Pro-BNP levels were collected within 24 hours of admission. Risk factors for pulmonary thrombo embolism were noted such as prolonged bed rest, hypertension, malignancy etc. Thrombi located in main/ right/left pulmonary artery were classified as central while the others at segmental/sub-segmental levels were classified as peripheral. Echocardiographic findings such as ejection fraction, functions of right and left ventricle, pulmonary artery pressure were noted. Clinical course of the patient and clinical outcome were also noted. Clinical outcome of the patient at the end of one month was recorded by following up. Then descriptive and statistical analysis of location of thrombus and clinical outcome in relation with NT-Pro-BNP levels was done.

Risk factors	Frequency (n)	Percent (%)
Prolonged immobilization	13	43.3
Carcinoma	4	13.3
Unprovoked	13	43.3
Total	30	100.0

Table-1: Risk factors of the study participants

Clinical Outcome	Frequency (n)	Percent (%)
Normal	22	73.3
Morbid	5	16.7
Death	3	10.0
Total	30	100.0

Table-2: Clinical Outcome after 30 Days

STATISTICAL ANALYSIS

The data collected from the patients was tabulated using Microsoft excel. The data are reported as mean +/- SD depending on their distribution. Risk factors will be described in numbers and percentage. Any association will be analysed using chi-square test. Correlation will be analysed by Pearsman correlation. A p value of <0.05 using two tailed test was taken as being significant for all statistical tests. All data were analysed with statistical software package (SPSS version 24.0)

RESULTS

In our study, we included 30 patients diagnosed with acute pulmonary thromboembolism. The mean age of study population is 57.6 years. 11 patients belonged to the group of 51 to 60 years. Only 2 patients were younger adults and 2 patients were old adults. Among the study group 17 patients were male (56.7%) and 13 patients were female (43.3%).In our study population following were the risk factors noted. Thirteen patients had prolonged immobilisation following road traffic accident or following major orthopaedic surgeries. 4 patients had underlying malignancy such as Ca lung or Ca breast. 13 patients had unprovoked PTE.(Table 1) The thrombus was located unilaterally in 19 patients whereas 11 patients have bilateral thrombus. In 19 patients, the thrombus was located centrally (that is, in main pulmonary artery) whereas in 11 patients, the thrombus is located in the peripherally (segmental and sub segmental pulmonary arteries).22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died.(Table 2)

Patients with central pulmonary embolus have higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries have relatively less NT-Pro-BNP levels. And the difference between the two was statistically significant. (P= 0.02)(Table 3)

Patients with high NT-Pro-BNP levels had complicated in-hospital course/mortality whereas, patients with relatively

	Localisation	N	Mean	Std. Deviation	P value
PROBNP	Central	19	6196.27	4377.75	.02*
	Peripheral	11	1529.10	2636.40	

Table-3: Association of PROBNP with localization

	Clinical Outcome	N	Mean	Std. Deviation	P value
PROBNP	Normal	22	3814.15	3860.52	.005*
	Morbid	5	3009.16	4033.77	
	Death	3	11864.00	1417.83	

Table-4: Association of PROBNP with Clinical Outcome

lower NT-Pro-BNP had uncomplicated in-hospital course. (P = 0.005)(Table 4)

DISCUSSION

Acute PE shows wide variations in clinical presentation from asymptomatic course to a massive pulmonary embolism that may be fatal. When planning treatment for this condition, risk stratification is important. In this study 30 patients diagnosed with pulmonary embolism were included. The mean age of study population is 57.6 years.17 patients were male(56.7%) and 13 patients were female (43.3%). The thrombus was located unilaterally in 19 patients whereas 11 patients had bilateral thrombus. In 19 patients, the thrombus was located centrally (main pulmonary artery) whereas in 11 patients the thrombus was located in the peripheral pulmonary artery (segmental and subsegmental pulmonary arteries). 22 participants of the study had normal clinical outcome. 5 patients were morbid (oxygen dependant/ heart failure) whereas 3 patients died. Patients with central pulmonary embolus have higher NT-Pro-BNP levels whereas patients with pulmonary embolus in the segmental or subsegmental pulmonary arteries have relatively less NTPro-BNP levels. And the difference between the two is statistically significant. (P= 0.02).

Patients with high NT-Pro-BNP levels had complicated in-hospital course/ mortality whereas, patients with relatively lower NT-Pro-BNP had uncomplicated in hospital course. (P = 0.005).From this study, it is evident that patients with higher NT-Pro-BNP levels have more central thrombus, have complicated in-hospital course and also cause morbidity and mortality. Whereas patients with comparatively lower NT-Pro-BNP levels have thrombus in segmental or subsegmental pulmonary arteries and they have an uncomplicated course.

The findings of our study were consistent with many other similar studies.

In a prospective study conducted by Akpinar et.al in turkey in Forty-nine patients concluded that higher pro bnp levels indicate higher probability of a more central location of thrombus in pulmonary embolism, resulting a more adverse clinical course and also leading to right ventricular overload..

In a prospective study done by Pruszcyk et.al in Poland in 79 patients found NT-Pro-BNP to be a prognostic marker in acute pulmonary embolism.[5]

An Egyptian prospective study by A.Selem et al conducted in 64 patients concluded elevated NT pro-BNP in PE was associated with worse short term prognosis in terms of higher morbidity and mortality.[6] Another study, Heleder Dores et al a retrospective study also had similar results as that of our study which concluded that elevated NT pro-BNP in PE identified worse short term prognosis and also had excellent power to predict 30 day all cause mortality.[7]

In a meta analysis conducted by Frederikus et.al, in the Netherlands, they found that higher concentrations of NT-Pro-BNP distinguish patients with pulmonary embolism at higher risk of complicated in-hospital course and death from those with low NT-Pro-BNP levels.[8] In another meta analysis conducted by Cavallazzi et.al in USA, they found that NT-Pro-BNP levels are significant predictors of all cause in hospital or short term mortality.[9] A meta analysis by Guillaume Coutance et al concluded while elevated BNP levels can help to identify high risk of death and adverse outcome, the high negative predictive value of normal BNP levels is certainly more useful.[10]

From our study it is evident that NT-Pro-BNP levels are significant predictor of all cause in- hospital mortality in patients with acute PE. The potential outcome of our study is that patients with normal levels of NT-Pro-BNP have a high negative predictive value for unfavourable outcome. Patients with normal NT-Pro-BNP levels have low risk of death as well as for hemodynamic collapse.This was consistant with a study conducted by Nils Kucher in 73 patients which predicted that lower the pro-BNP levels, lower would be the hospital stay.[11]

Our study have a few limitations. First, the sample size is limited. Further studies including larger number of patients are necessary to clarify this point. Secondly, NT-pro-BNP is a precursor of BNP. The conversion of BNP to NT-Pro-BNP may take several minutes after myocardial stretch which may lead to false negative results in patients who present early. Serial monitoring of NT-Pro-BNP (eg. At admission and 6 hours later) may overcome this limitation.

CONCLUSION

This study shows that higher NT-Pro-BNP levels indicate higher probability of a more central location of thrombus in pulmonary embolism and also leading to right ventricular overload.Also patients with higher NT-Pro-BNP levels had a complicated in-hospital course.Further studies with large number of patients were undertaken to determine the exact cut off levels of NT-Pro-BNP to help in treatment decisions.

Ethical approval

The study was approved by the Institutional Ethics Committee

REFERENCES

1. Heit JA, Melton LJ, Lahose CM. Incidence of venous thromboembolism in hospitalised patients vs community residents. Mayo clin Proc 76:1102-1110
2. Fremont B, Pacouret G, Jacobi D, Puglisi R, Charbonnier B, de Labriolle A.Prognostic value of the echocardiographic right/left ventricular end diastolic diameter ratio in patients with acute pulmonary

embolism: results from a monocenter registry of 1416 patients. Chest 2008;133;358-362.
3. Christian Hall, Essential biochemistry and physiology of (NT-pro) BNP, The European journal of Heart Failure 2004;6:257-260.
4. E. Akpinar, E. Sayin, E. Buyuk and M. Gulhan. Association of Levels of N-terminal-Pro-B-Type Natriuretic Peptide with Localisation of Thrombus in Acute Pulmonary Embolism. The Indian Journal of Chest Diseases & Allied Sciences 2012;Vol.54
5. Pruszczyk P, Kostrubiec M, Bochowicz A, Styczyñski G, Szulc M,Kurzyna M, Fijałkowska A, Kuch-Wocial A, Chlewicka I, Torbicki A:N-terminal pro-brain natriuretic peptide in patients with acute pulmonary embolism. Eur Respir J 2003, 22:649-653.
6. A. Selem,H.Radwan,Abdelaziz M.Gomaa Prognostic value of N-Terminal Pro-Brain Natriuretic Peptide in Acute Pulmonary Embolism.Open journal of endocrine and metabolic disease 2012;2:58-62.
7. H. Dores, C. Fonseca, S. Leal, I. Rosário, J. Abecasis, J. Monge, M. J. Correia, L. Bronze, A. Leitão, I. Arroja, A. Aleixo and A. Silva, "NT-pro BNP for Risk Stratification of Pulmonary Embolism," Portuguese Journal of Cardiology,2011;30:881-886
8. Frederikus Klok F, Mos I, Huisman M. Brain-type natriuretic peptide levels in the prediction of adverse outcome in patients with pulmonary embolism. Am J Respir Crit Care Med. 2008;178:425-30.
9. R. Cavallazzi, A. Nair, T. Vasu and P. E. Marik. Natriuretic Peptides in Acute Pulmonary Embolism. Intensive Care Med 2008;34:2147-2156
10. Guillaume Coutance, Olivier Le Page, Ted Lo and martial Hamon prognostic value of brain natriuretic peptide in acute pulmonary embolism. Critical Care 2008,12:R109.
11. Nils Kucher, Gert printzen, Tanja Doernhoefer,Stephan Windecker, Bernhard Meier, Otto Martin Hess. Low Pro-brain natriuretic Peptide Levels predict Benign Clinical Outcome in Acute Pulmonary Embolism. Circulation 2003;107:1576-1578.

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ORIGINAL ARTICLE

Contact sensitivity in patients with venous leg ulcer: A multi-centric Indian study

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Venous leg ulcers are the most common form of non-healing leg ulcers. They are subjected to treatments such as topical medications, dressings, and compression therapies. This can lead to exposure to a number of allergens with subsequent sensitisation and contact dermatitis of the regional skin. This may contribute to the poor ulcer healing. To detect the various contact sensitisers in patients with venous leg ulcers through patch testing, patients from 6 centres across India with venous leg ulcers of longer than 6 weeks duration were enrolled for the study. They were patch tested using a special parch test kit with 27 antigens. A total of 172 patients were included in the study; 82 (48.2%) tested positive for at least 1 antigen. Among them, polyvalent sensitisation was noted in 71% of patients. Wood tar mix (10.4%) and the framycetin (8.7%) were the most common allergens. There is a high frequency of allergic sensitisation to various ingredients of topical therapies used in the venous ulcer management, which may interfere with wound healing. Avoiding them can help obtain a better therapeutic outcome.

KEYWORDS
contact dermatitis, patch test, venous ulcer

1 | INTRODUCTION

Venous ulcers are an important sequel of chronic venous insufficiency (CVI). They are known for protracted course and refractory healing. A multidisciplinary approach is required for their assessment and treatment, including proper wound management, compression, and surgical interventions. Persistence of the ulcer for a longer duration leads to exposure to numerous topical applicants and medicament-incorporated dressings. Contact with these medicaments by the barrier-disrupted skin and occlusive wound dressings can lead to contact sensitisation. This allergic contact dermatitis of the skin surrounding the leg ulcers is undesirable because it delays wound healing.

Contact sensitisation in leg ulcer patients is very frequently reported in published studies.[1–7] The spectrum of allergens causing contact dermatitis in chronic leg ulcer patients depends on the wound care practices in a given area. In the majority of studies, a major group of sensitisers include fragrances, lanolin, antimicrobial agents, and rubber-related allergens. There is a high probability of polyvalent sensitisation, especially in ulcers of a longer duration. Patients with chronic leg ulcers, with or without associated dermatitis, should hence be patch tested to detect sensitisation to various allergens. This is an Indian study on the pattern of contact sensitisation in patients with chronic leg ulcers using special patch test series.

2 | METHODS

This is a prospective, multicentre, cross-sectional study conducted in 6 centres across India. Approval to conduct study was obtained from the respective institutional ethics committees. Consecutive patients who presented with chronic venous ulcers due to CVI persisting for 6 weeks and who consented to participate in the study were recruited. Patients with and without dermatitis of the surrounding skin were recruited for the study. Relevant clinical and demographic details were obtained. Local examination, including the morphology of ulceration, presence of dermatitis or other signs of CVI, and other relevant clinical findings, were also recorded. Those who were on immunosuppressive drugs, a steroid dose of 20 mg of prednisolone or equivalent, and those with dermatitis involving the back were excluded from the study. The study was conducted for a period of 1 year.

All patients included were patch tested using patch test series for leg ulcers obtained from the Chemotechnique Diagnostics, Modemgatan 9, SE-235 39, Vellinge, Sweden, which includes 27 allergens consisting of medicaments, preservatives, steroids, etc. Application of patch test series and patch test reading was performed according to the guidelines of the International Contact Dermatitis Research Group. The Finn chamber method was used to apply the allergens. Test patches were removed at 48 hours, and a patch test reading was performed. Readings were also taken at 72 to 96 hours and on the 7th day. Readings were recorded as negative (−), doubtful (?), weak/non-vesicular reaction (+), strong reaction (oedematous or vesicular) (++) and extreme reaction (bulla or ulceration) (+++). Patch testing was considered positive when recorded as (+), (++), or (+++) reactions during any reading. Findings from all 6 centres were obtained and tabulated. Univariate analysis was the statistical method followed.

3 | RESULTS

A total of 172 subjects participated in the study. Details of the subjects from various centres are given in Table 1. Most patients were middle aged or elderly; almost half of the patients were aged between 50 and 70 years (Table 2). Majority of the patients were males (142 out of 172). A total of 52% of our patients had an ulcer duration of less than a year (Table 3). Of 172 patients, 83 (48.2%) showed positive patch test reactions to 1 or more antigens (Table 3). Almost 50% of those showing positive reactions had disease duration of less than 1 year. A total of 29 % of the subjects demonstrated positive reaction to only 1 antigen and 27% to 2 antigens (Figure 1). One patient had positive reactions to 7 antigens.

Table 4 shows the various antigens that were patch tested. There were 183 positive reactions. Clinical relevance of the positive patch test reactions in these cases often remains questionable, but most positive reactions included antibiotics, antiseptics, solvents, and emulsifiers, which are components of the topical applicants. The most common allergen in our study was wood tar mix (19 patients). This was followed by framycetin sulphate (17 patients) and thiomersal and eosin (13 patients each). Twelve patients each

Key Messages

- venous leg ulcers therapies, such as topical medications, dressings, and compressions, expose the patients to allergens, followed by sensitisation and contact dermatitis; patch testing them can identify these allergens, and avoiding these allergens is an integral part of successful wound healing
- venous leg ulcer therapies, such as topical medications, dressings, and compressions, expose the patients to various allergens leading to contact dermatitis; it can contribute to poor wound healing
- patch testing patients can identify these allergens; avoiding these allergens is an integral part of successful wound healing

TABLE 1 Subject enrolment

Centre 1	50
Centre 2	37
Centre 3	32
Centre 4	32
Centre 5	11
Centre 6	10
Total	172

TABLE 2 Demographic profile of the subjects

	Gender		
Age (years)	Male	Female	Total
18-20	1	2	3
21-30	10	1	11
31-40	20	7	27
41-50	29	4	33
51-60	34	7	41
61-70	35	8	43
71-80	9	1	10
81-90	4	0	4
Total	142	30	172

TABLE 3 Duration of disease with patch test positivity

Duration of ulcer (months)	Number of patients (%)	Number of patients with positive patch test	Percentage positivity ($N = 83$)
0-3	34 (19.8)	18	21.7
3-6	30 (17.4)	15	18.1
6-12	26 (15.1)	10	12.0
12-60	54 (31.4)	27	32.5
>60	28 (16.3)	13	15.7
Total	172 (100)	83 (48.2%)	100

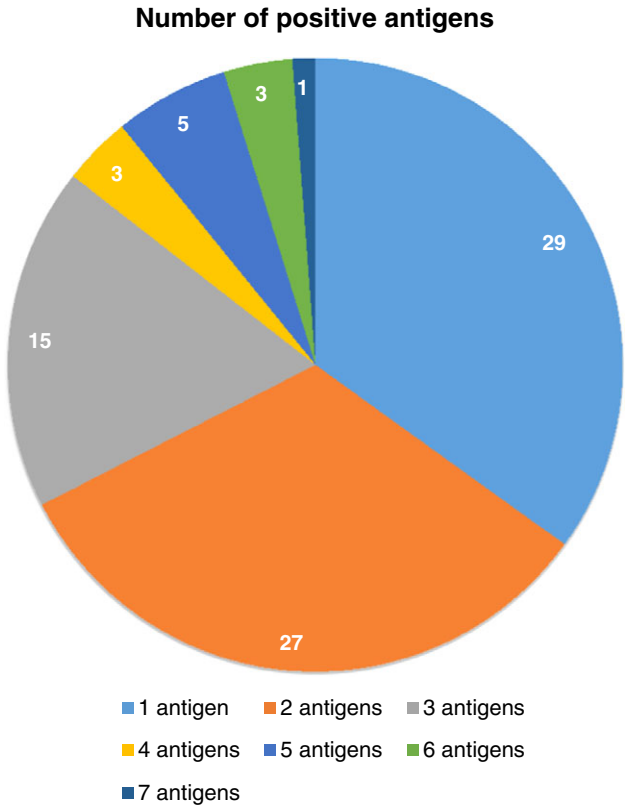


FIGURE 1 Number of positive antigens

showed positive patch test reactions to benzalkonium chloride and p-CHLORO-m-CRESOL (PCMC). All allergens except tixocortol pivolate showed at least 1 positive test reaction.

4 | DISCUSSION

Chronic venous ulcers are 1 of the common problems, with prevalence rates for open ulcers ranging from 0.12% to 1.1% of the population.[8] Venous disease is the most frequent aetiology of leg ulcers, followed by arterial disease and other causes, such as diabetes.[9] Prevalence of venous ulcers has reduced due to better management of CVI, but it continues to be a major health problem. Chronic course of the ulcers, local inflammation, skin barrier disruption, use of topical medicaments including "over the counter" preparations, and the use of occlusive bandages are the reasons patients get sensitised to many antigens.[10] This leads to the onset of dermatitis, maceration of the surrounding skin, delayed wound healing, and bacterial infection. Allergic contact dermatitis may develop at distant sites other than the legs due to allergen exposure, dissemination from the ulcer site, or systemic spread.[11] Apart from poor wound healing, the presence of contact dermatitis of the surrounding skin will reduce the choices of therapy and local wound care.

Eczema was the most frequent manifestation of CVI, followed by leg ulceration.[12] Ulceration is an important determinant of the poor quality of life in venous disease. Our study included all patients presenting with leg ulcers irrespective of the presence of eczema. We used the leg ulcer series commercially supplied by the Chemotechnique Diagnostics.

Our study included 172 patients; 83 (48.2%) patients demonstrated positive patch test reactions to at least 1 allergen (Table 3). Our study results are almost at par with the results of Smart et al, who reported positive patch test results in 46% of their patients.[6] Higher positive results have been reported by Tavadia et al (68%), Saap et al (63%), Machet et al (75%), Lim et al (61.4%), and Jasmina et al (75%).[1–5] Some of these studies tested a larger number of antigens, which could be the reason for higher positive reactions. For example, in the study by Saap et al, a standard North American Contact Dermatitis Group series and 54 supplemental antigens were used.[2] Contact sensitivity to various allergens was higher in their general population, thus yielding higher results. Artüz et al. recently reported their findings in a study involving 40 patients and 20 controls; reported patch test positivity in these groups was 77.5% and 50%, respectively.[13] Management practices in our population may expose our patients to a lesser number of allergens, and this could have led to a lower percentage of positive reactions. In spite of all these factors, 48% positivity appears to be a high value and could play a role in the non-healing nature of the ulcers.

Among the 172 patients, 60% belonged to the age group of 30 to 60 years; another 30% were aged over 60 years. According to a population survey, 22% of the leg ulcers began before 40 years of age.[14] Only 30 (17.4%) of our patients were females, which may be due to decreased hospital reporting by women. In a population-based study, women outnumbered men by a ratio of 2.8:1.[15]

The duration of the leg ulcer is an important factor in the development of contact sensitisation. Venous ulcers are usually chronic; 33% to 60% of the ulcers can persist beyond 6 weeks;[16] 40 % of the ulcers may remain open for over 1 year.[17] One can predict that there will be more patch test-positive reactions with increased duration of the ulcer. In our study, 48% of those who tested positive had an ulcer duration of 2 years or more (Table 3). Polyvalent sensitisation and higher mean number of allergen sensitisations were found more frequently in ulcers with a duration of more than 5 years.[5]

Polyvalent sensitisation has been reported consistently with leg ulcers.[1–3,5] Studies have proven that the common sensitisers are the various ingredients of topical applicants, dressings, and bandages.[18] Lanolin, antibiotics, antiseptics, preservatives, emulsifiers, resins, and latex were the principal sensitisers. A total of 71% of our patients were positive for more than 1 allergen (Table 4). One patient was found to have positive reactions to 11 allergens. A total of 183 positive reactions were found in our study (Table 4). The most common allergen in our patients was the wood tar mix, with 19 patients (10.4% of all positive reactions) showing

TABLE 4 List of allergens and positive reactions

S. No.	Antigens	Patients with positive reaction	Percentage ($N = 183$)
1.	Control	0	0
2.	Amerchol L 101	5	2.7
3.	Fusidic acid sodium salt	4	2.2
4.	Chlorhexidine digluconate	5	2.7
5.	Benzalkonium chloride	12	6.6
6.	Nitrofurazone	6	3.3
7.	Bacitracin	4	2.2
8.	Cetearyl alcohol	4	2.2
9.	2,6-Ditert-butyl-4-cresol (BHT)	3	1.6
10.	Chloramphenicol	7	3.8
11.	Benzoyl peroxide	2	1.1
12.	Propylene glycol	9	4.9
13.	Propolis	6	3.3
14.	Thimerosal (Merthiolate)	13	7.1
15.	Sorbic acid	3	1.6
16.	Eosin	13	7.1
17	4-Chloro-3-cresol (PCMC)	12	6.6
18	Budesonide	3	1.6
19	Triethanolamine	8	4.4
20	Framycetin sulphate	16	8.7
21	Sorbitan sesquioleate	4	2.2
22	Tixocortol-21-pivalate	0	0
23	Sorbitan monoleate (span 80)	5	2.7
24	Phenyl mercuric acetate	6	3.3
25	Chloroacetamide	2	1.1
26	Diazolidinyl urea (Germall ii)	4	2.2
27	Imidazolidinyl urea (Germall 115)	7	3.8
28	Wood tar mix	19	10.4
	Total	183	100

positive reactions. This was followed by framycetin (16 patients, 9%), eosin (13 patients, 7.5%), *p*-CHLORO-*m*-CRESOL or PCMC (12, 7%), and benzalkonium chloride (12 patients, 7%). Other important allergens include propylene glycol, triethanolamine, chloramphenicol, imidazolidinyl urea, and phenyl mercuric acetate (Table 4). The majority of these reactions were relevant. There was no sensitisation to tixocortol pivolate. A venous eczema study utilising Indian standard series has been published by Jindal et al; they found 50% of positive results, with fragrance mix, *p*-phenylene diamine, wool alcohol, and nickel being the other common detected allergens.[19]

Wood tar mix was 1 of the most frequent (20%) allergens also found in the study by Saap et al.[2] In their study, sensitivity to balsum of Peru and bacitracin were the most frequent. Wood tar sensitisation was also 1 of the common sensitisers in a large study involving 1883 patients with allergic contact dermatitis.[20] Wood tar is used in the flavouring of certain food. A high degree of cross-allergy between wood tars and fragrance mix can occur. Framycetin is a common antibacterial agent used for ulcer care in India; hence, sensitisation to this agent is highly relevant. In spite of lack of evidences of the efficacy of framycetin or other antibiotics, the practice of topical antibiotic usage widely prevails in wound care.[21] We have known about very high framycetin sensitisation in leg ulcer patients for decade.[22,23] A number of antibiotic- and antiseptic-impregnated dressings are also used in ulcer management.[24] Eosin sensitisation has been reported in 2 studies.[25,26] In one of those studies, a very high rate of contact sensitivity (82.5%) has been reported, with eosin being positive in 5%. Eosin is an acid red dye used in lipsticks, nail polish, textiles, and antiseptics. Patch test positivity is thus relevant in patients with leg ulcers. PCMC and benzalkonium chloride are compounds used as an antiseptic and disinfectant. Both are known for their potential for allergic contact dermatitis.[27,28-] Saap et al. have reported 13% patch test positivity to benzalkonium chloride in their study.[2] We detected many other allergens with low frequency, and the relevance of such positive reactions cannot be determined.

Compression therapy using various bandages and stockings is an integral part of management and prevention of venous ulcers.[29,30] Allergic contact dermatitis to synthetic rubber, neoprene, in compression stockings has been reported.[32,33] Modern dressing-related contact dermatitis is infrequent but has also been reported.[31–34] Usage of these modern dressings is infrequent in Indian patients, and hence, sensitisation may be a rare occurrence. Many indigenous and traditional home remedies are used, and their sensitisation potential has also been explored in a few studies.[10,35]

The most important limitation of this study was the noninclusion of a control group. Inclusion of a control group with healthy volunteers was not considered in order to avoid the discomfort involved in patch testing. There was no uniformity in the number of patients recruited from the various centres. Indian standard series patch testing could have been performed in addition to the leg ulcer series.

Venous insufficiency and ulcers are problems Indian patients experience because of occupations that involve prolonged standing. There may be a genetic predisposition to varicose veins in many patients. It affects people during their prime of life as our study inferred. It is more common in men, who are the breadwinners in most Indian households, thus causing health as well as economic burden. The pattern of sensitisation appears to be related to treatment practices. Allergens and their frequencies in Indian patients with venous disease were almost similar to those reported from other parts of the world.[1–4,19] Common allergens in many of those studies were the fragrance mix, antibacterials, wood tar mix, and lanolins. Sensitisation to fragrance mix was very high, and a significant number were sensitive to topical corticosteroids in the study by Tavadia et al. We found wood tar mix and the antibacterials (framycetin) among the common allergens, and we found no sensitisation to corticosteroids.

Studies have inferred that patients with chronic wounds of the lower extremity experience functional disability and

emotional distress, with negative influences on quality of life. Apart from physical and psychosocial functioning, treatment-related issues also can have an impact.[36,37] A review of the literature and calculated sensitisation rates of a pool of 3043 patients extracted from 24 series, and their own, has shown a significant increase in sensitisation in recent times.[3] Contact allergy to the various topical treatments employed in leg ulcer management can contribute to or worsen eczema and can impede ulcer healing. Thus, it can be a major reason of poor quality of life. Modern dressings with lower allergenic potential may be used as wound dressing. It is better to avoid antiseptics and antibiotics in the absence of infection, and it should be used for a limited period. This should be known by all health care personnel involved in managing venous leg ulcers.

REFERENCES

1. Tavadia S, Bianchi J, Dawe RS, et al. Allergic contact dermatitis in venous leg ulcer patients. *Contact Dermatitis*. 2003;48:261-265.
2. Saap L, Fahim S, Arsenault E, et al. Contact sensitivity in patients with leg ulcerations: a North American study. *Arch Dermatol*. 2004;140:1241-1246.
3. Machet L, Couhe'C, Perrinaud A, Hoarau C, Lorette G, Vaillant L. A high prevalence of sensitization still persists in leg ulcer patients: a retrospective series of 106 patients tested between 2001 and 2002 and a meta-analysis of 1975–2003 data. *Br J Dermatol*. 2004;150:929-935.
4. Lim KS, Tang MB, Goon AT, Leow YH. Contact sensitization in patients with chronic venous leg ulcers in Singapore. *Contact Dermatitis*. 2007;56:94-98.
5. Jankićević J, Vesić S, Vukićević J, Gajić M, Adamic M, Pavlović MD. Contact sensitivity in patients with venous leg ulcers in Serbia: comparison with contact dermatitis patients and relationship to ulcer duration. *Contact Dermatitis*. 2008;58:32-36.
6. Smart V, Alavi A, Coutts P, et al. Contact allergens in persons with leg ulcers: a Canadian study in contact sensitization. *Int J Lower Extrem Wounds*. 2008;7:120-125.
7. Barbaud A, Collet E, Le Coz JC, Meaume S, Gillois P. Contact allergy in chronic leg ulcers: results of a multicentre study carried out in 423 patients and proposal for an updated series of patch tests. *Contact Dermatitis*. 2009;60:279-287.
8. Graham ID, Harrison MB, Nelson EA, Lorimer K, Fisher A. Prevalence of lower-limb ulceration: a systematic review of prevalence studies. *Adv Skin Wound Care*. 2003;16:305-316.
9. Forssgren A, Fransson I, Nelzen O. Leg ulcer point prevalence can be decreased by broad-scale intervention: a follow-up cross-sectional study of a defined geographical population. *Acta Derm Venereol*. 2008;88:252-256.
10. Lim KS, Tang MB, Goon AT, Leow YH. The role of topical traditional ramyce medicaments as contact sensitisers in chronic venous leg ulcer patients. *Ann Acad Med Singapore*. 2007;36:942-946.
11. Beliauskienė A, Valiukevičienė S, Sitkauskienė B, Schnuch A, Uter W. Contact sensitization to the allergens of European baseline series in patients with chronic leg ulcers. *Medicina (Kaunas)*. 2011;47:480-485.
12. Joseph NBA, Faizan Thouseef M, Devi MU, Abna A, Juneja I. A ramycetin review of epidemiology and management of varicose veins for national guidance. *Ann Med Surg*. 2016;8:21-27.
13. Artüz F, Yılmaz E, Külcü Çakmak S, Polat Düzgün A. Contact sensitisation in patients with chronic leg ulcers. *Int Wound J*. 2016;13:1190-1192.
14. Callam MJ, Harper DR, Dale JJ, Ruckley CV. Chronic ulcer of the leg: clinical history. *Br Med J (Clin Res Ed)*. 1987;294:1389-1391.
15. Callam MJ, Ruckley CV, Harper DR, Dale JJ. Chronic ulceration of the leg: extent of the problem and provision of care. *R Med J (Clin Res Ed)*. 1985;22:1855-1856.
16. Vasudevan B. Venous leg ulcers: pathophysiology and classification. *Ind Dermatol Online J*. 2014;5:366-370.
17. Briggs M, Flemming K. Living with leg ulceration: a synthesis of qualitative research. *J Adv Nurs*. 2007;59:319-328.
18. Dogra S, Sarangal R. Summary of recommendations for leg ulcers. *Ind Dermatol Online J*. 2014;5:400-407.
19. Jindal R, Sharma NL, Mahajan VK, Tegta GR. Contact sensitization in venous eczema: preliminary results of patch testing with Indian standard series and topical medicaments. *Ind J Dermatol Venereol Leprol*. 2009;75:136-141.
20. Roesyanto ID, van den Akker TW, van Joost TW. Wood tars allergy, cross-sensitization and coal tar. *Contact Dermatitis*. 1990;22:95-98.
21. O'Meara S, Al-Kurdi D, Ologun Y, Ovington LG, Martyn-St James M, Richardson R. Antibiotics and antiseptics for venous leg ulcers. *Cochrane Database Syst Rev*. 2014;10(1):CD003557.
22. Fräki JE, Peltonen L, Hopsu-Havu VK. Allergy to various components of topical preparations in stasis dermatitis and leg ulcer. *Contact Dermatitis*. 1979;5:97-100.
23. Kirton V, Munro-Ashman D. Contact dermatitis from neomycin and ramycetin. *Lancet*. 1965;16:138-139.
24. Dogra S, Rai R. Venous leg ulcer: topical treatment, dressings and surgical debridement. *Ind Dermatol Online J*. 2014;5:371-373.
25. Le Coz CJ, Scrivener Y, Santinelli F, Heid E. Contact sensitization in leg ulcers. *Ann Dermatol Venereol*. 1998;125:694-699.
26. Reichert-Pénétrat S, Barbaud A, Weber M, Schmutz JL. Leg ulcers. Allergologic studies of 359 cases. *Ann Dermatol Venereol*. 1999;126:131-135.
27. Yamano T, Shimizu M, Noda T. Allergenicity evaluation of p-chloro-m-cresol and p-chloro-m-xylenol by non-radioactive murine local lymph-node assay and multiple-dose guinea pig maximization test. *Toxicology*. 2003;28(190):259-266.
28. Wentworth AB, Yiannias JA, Davis MD, Killian JM. Benzalkonium chloride: a known irritant and novel allergen. *Dermatitis*. 2016;27:14-20.
29. Nair B. Compression therapy for venous leg ulcers. *Ind Dermatol Online J*. 2014;5:378-382.
30. Shenoy MM. Prevention of venous leg ulcer recurrence. *Ind Dermatol Online J*. 2014;5:386-389.
31. Valois A, Waton J, Avenel-Audran M, et al. Contact sensitization to modern dressings: a multicentre study on 354 patients with chronic leg ulcers. *Contact Dermatitis*. 2015;72:90-96.
32. Raju S, Hollis K, Neglen P. Use of compression stockings in chronic venous disease: patient compliance and efficacy. *Ann Vasc Surg*. 2007;21:790-795.
33. Renner R, Simon JC, Treudler R. Contact sensitization to modern wound dressings in 70 patients with chronic leg ulcers. *Dermatitis*. 2013;24:60-63.
34. D'Erme AM, Iannone M, Dini V, Romanelli M. Contact dermatitis in patients with chronic leg ulcers: a common and neglected problem: a review 2000-2015. *J Wound Care*. 2016;25 (suppl 9):S23-S29.
35. Vishwanath V. Venous leg ulcer: management aspect in Indian scenario. *Ind Dermatol Online J*. 2014;5:396.
36. Harlin SL, Harlin RD, Sherman TI, Rozsas CM, Shafqat MS, Meyers W. Using a structured, computer-administered questionnaire for evaluating health-related QOL with chronic lower extremity wounds. *Ostomy Wound Manage*. 2009;55:30-39.
37. Vishwanath V. Quality of life: venous leg ulcers. *Ind Dermatol Online J*. 2014;5:397-399.

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COVID-19 Presenting as Enanthem and Itchy Exanthem

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Sir,

Dermatologists can play a very important role in coronavirus disease 2019 (COVID-19) pandemic as early recognition of skin lesions are important in diagnosing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The various cutaneous lesions that have been described so far with COVID-19 falls into four categories: exanthema (varicella-like, papulo-vesicular and morbilliform rash), vascular (chilblain-like, purpuric /petechial and livedoid lesions), urticarial and acro-papular eruption.[1]

A 36 year old male, known case of decompensated chronic liver disease (ethanol related cirrhosis) came to the emergency department with history of itchy rash in the trunk and limbs for 7 days. There was no history of fever, cough, sore throat, loose stools or drug intake. Cutaneous examination revealed multiple maculopapular exanthematous rash with excoritation over chest, abdomen, back, arms, thighs and both lower legs. Examination of the oral cavity revealed multiple tiny erythematous macules and pinpoint enanthems over soft palate and the tongue showed evidence of candidiasis. Since endoscopy was suggested by the gastroenterologist, swabs were taken from the nasopharynx and oropharynx for COVID-19. Real-time reverse transcriptase–polymerase chain reaction for SARS-CoV-2 turned out to be positive. Most of the skin manifestations reported in literature coexist with other symptoms of COVID-19. Many patients who are confirmed or suspected to have

COVID-19 do not have their oral cavity examined due to safety concerns. In our patient, we did not suspect, as the patient did not have symptoms suggestive of COVID-19. Exanthem can occur in patients with viral infections and drug reactions. Usually, exanthem presenting as pustules and dusky lesions suggest drug etiology, whereas, petechial or vascular pattern with enanthem point towards an infectious etiology.[2]

In a study done by Dr Jimenez-Cauhe et al, oral cavity of 21 patients with COVID-19 and skin rash were examined, among which 6 patients had enanthem. The mean duration between onset of COVID-19 symptoms and appearance of enanthem is 12.3 days.[3] Enanthem can present as petechial, macular, macular with petechiae, or erythematovesicular. Our patient had macular enanthem and itchy exanthem without other symptoms of COVID-19.

Physicians should have a high degree of suspicion when patients present with skin manifestation of exanthematous rash and oral enanthem as it may be a presenting feature of COVID-19.

References

1. Gisondi P, Plaserico S, Bordin C, Alaibac M, Girolomoni G, Naldi L. Cutaneous manifestations of SARS-CoV-2 infection: a clinical update. *J Eur Acad Dermatol Venereol* 2020; 34:2499-2504. doi:10.1111/jdv.16774.
2. Drago F, Paolino S, Rebora A, et al. The challenge of diagnosing atypical exanthems: a clinico-laboratory study. *J Am Acad Dermatol* 2012; 67:1282-8.
3. Jimenez-Cauhe J, Ortega-Quijano D, de Perosanz-Lobo D, et al. Enanthem in Patients with COVID-19 and Skin Rash. *JAMA Dermatol* 2020; 156:1134–1136. doi:10.1001/jamadermatol.2020.2550.

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Original Article

Effectiveness of Dual Sequential Wavelength Laser in the Treatment of Portwine Stains – A Retrospective Study

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Abstract

Background: Port Wine Stain (PWS) is only partially and superficially treated with the Pulsed dye laser (PDL) because of its limited depth of penetration. The 1064-nm long pulsed Nd:YAG laser has greater depth of penetration and is used to treat deeper vessels. The dual sequential wavelength laser (DSWL) which combines PDL/Nd:YAG (595/1064 nm) can be more effective for the treatment of deeper, nodular portwine stains due to its synergistic effect. The purpose of this study is to evaluate the efficacy and safety of DSWL in the treatment of portwine stains after five treatment sessions. **Materials and Methods:** A total of 11 patients with PWS lesions on the head and neck, who were treated with DSWL for at least five sittings at monthly interval were included in this study. The assessment of the therapeutic response and grading of improvement was done with a scale of 0 to 4 by comparing the photographs taken before treatment and after 5 sittings of laser treatment. **Results:** Out of the 11 patients, 5 patients (45.45%) had more than 70% improvement. Six out of eleven patients (54.54%) had 40 to 70% improvement at the end of five sittings with no adverse effects including purpura. **Conclusion:** Dual Sequential Wavelength Laser is a good modality of treatment for portwine stains. It has enhanced penetration and effective clearance of thicker, nodular, recalcitrant lesions of PWS. DSWL is safe and efficacious and it can be recommended as a therapeutic modality for portwine stains.

Keywords: *Dual sequential wavelength laser Nd:YAG laser, PDL, portwine stains, vascular lasers*

Introduction

Pulsed dye laser (PDL) is the "gold standard" for treating portwine stains (PWS).[1] PDL is used for many years to treat vascular lesions. It destroys the target tissues by the principle of selective photothermolysis.[2] Nearly 25–50% of the lesions treated with PDL alone do not demonstrate a significant improvement even after many sessions of treatment. The 1064 nm-long pulsed Nd:YAG laser has greater depth of penetration of upto 5 to 6 mm and greater affinity for deeper large vessels.[3] Vascular lesions of varying size, density, color, flow and depth may not respond to either PDL or Nd:YAG alone.[4] The dual sequential wavelength laser (DSWL) which combines PDL/Nd:YAG with 595/1064 nm has been proposed as an alternative treatment for PWS. DSWL is effective for the treatment of portwine stains. It produces two widely separated laser wavelengths in a single device[5] and at a lower fluence, it gives good therapeutic response with lesser side effects.[6]

Materials and Methods

This is a retrospective study conducted in our department of dermatology after getting approval from our institutional ethics committee. Eleven patients with PWS lesions on the head and neck, who were treated with DSWL (Cynergy system, Cynosure Corp, Westford, MA, USA) for at least five sittings at monthly interval were included in this study.

All the patients had been treated with a fluence ranging from 4 to 10 J/cm^2 and pulse duration between 0.5 and 40 ms for PDL and a fluence ranging from 20 to 50 J/cm^2 with pulse duration between 15 and 40 ms for Nd:YAG. The spot size of 7 or 10 mm were used with inter-pulse delay of 1 second for sufficient cooling. The air cooling device (Cynosure smart cool, USA) was attached to the laser hand piece for epidermal cooling. The assessment of the therapeutic response and grading of improvement was done with a scale of 0 to 4 by comparing the photographs taken before treatment and after five sittings of

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laser treatment. Grade 0 is less than 20% improvement, grade 1 is 21 to 40% improvement, grade 2 is 41 to 60% improvement, grade 3 is 61 to 80% improvement, grade 4 is more than 80% of improvement.

Results

Of the 11 patients, 8 were females and 3 were male patients with age groups ranging from 18 to 44 years. One patient had lesions over the neck, 10 patients had facial lesions [Figure 1a]. The improvement of lesions after five laser sittings is shown in Table 1. Out of the 11 patients, 5 patients (45.45%) had more than 70% improvement, i.e., 1 patient had 90% improvement [Figure 1b], 2 patients had 80% improvement, 2 patients had 70% improvement. Totally, 6 out of 11 patients (54.54%) had 40–70% improvement i.e., 4 patients had 50% improvement and 2 patients had 40% improvement.

Out of the 11 patients, seven patients had flat reddish lesions and four patients had thick, elevated lesions of PWS over face [Figure 2a] and neck. Of the four patients who had thick, elevated lesions, 1 patient had 80% improvement [Figure 2b], 1 patient had 70% improvement**,** 2 patients had 50% improvement. One patient who had thick nodular lesions over the neck [Figure 3a] had 70% improvement [Figure 3b] after 5 sittings of laser treatment at monthly intervals. No adverse effects were recorded including purpura after DSWL treatment.

Discussion

Portwine stains (PWS) present as light pink macules due to progressive vessel ectasia and can progress into dark purple lesions. PWS are usually located in the papillary and reticular dermis. If hypertrophy of soft tissues occurs, it can become thicker and more nodular over a period of time. Various modalities of treatment are available for portwine stains, including Pulse Dye Laser, Nd:YAG lasers and Intense Pulsed Light (IPL). Several vascular lasers like argon, tunable dye, copper vapor, krypton lasers, which were also used in the past with the risk of dyschromia and scarring.[7]

Table 1: Percentage of improvement after 5 sittings

Age/Sex	Site of the lesion	Improvement (%)
23 yrs/M	Right side of the scalp and face	80
18 yrs/F	Right side of the face	90
34 yrs/M	Left side of the face	40
20 yrs/F	Right side of the face	50
33 yrs/F	Right side of the forehead and upper eyelid	40
21 yrs/F	Left side of the cheek and upper lip	70
28 yrs/M	Left side of the face	50
18 yrs/F	Left side of the cheek and upper lip	50
22 yrs/F	Left side of the face	80
44 yrs/F	Left side of the face	50
42 yrs/F	Right side of the neck	70

DSWL works under the principle of selective photothermolysis. It is the process by which a target tissue absorbs photons, becoming heated and destroyed totally, while structures surrounding it are relatively spared. In case of PWS, the desired targets are 10–500 μm dilated capillaries and postcapillary venules in the superficial and deep dermis.[8] PDL has shallow depth of penetration and hence it is not effective for treating deeper, nodular lesions of PWS. Over the years, modifications to the original PDL system have been made in order to overcome these disadvantages. Usually PWS lesions extend 3 to 5 mm into the dermis and the PDL is effective only up to

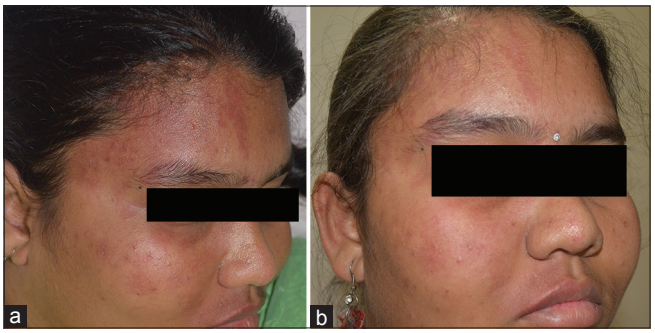


Figure 1: PWS lesions over the right half of face. (a) Before treatment. (b) After five sessions of treatment with DSWL at monthly intervals, she had 90% improvement

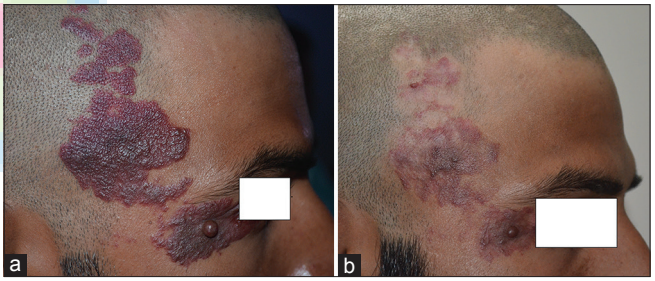


Figure 2: PWS lesions over the right side of scalp and face. (a) Before treatment. (b) After five sessions of treatment with DSWL at monthly intervals, he had 80% improvement

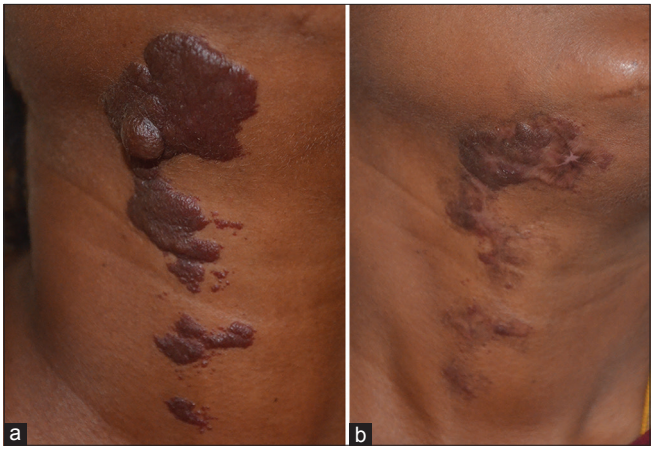


Figure 3: PWS lesions over the right side of neck. (a) Before treatment. (b) After five sessions of treatment with DSWL at monthly intervals, she had 70% improvement

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2 mm depth. Deeper vessels that are not damaged fully with PDL therapy may recover in future. High energy is required to thermo-coagulate the deep vessels and hence, epidermis should be protected to minimize the damage to keratinocytes and melanocytes by means of epidermal cooling. Cooling is therefore an integral part of all vascular lasers.[8]

DSWL is a new concept that sequentially emits two wavelengths from the same delivery fiber with a pre-selected delay between the two pulses. The PDL energy emits first, converts oxy-hemoglobin to met-hemoglobin. Met-hemoglobin has three times higher absorption co-efficient to Nd:YAG wavelength than oxy-hemoglobin. Pre-selected delay will allow the met-hemoglobin to penetrate deep into the blood vessels. Nd:YAG laser emits few milliseconds later following PDL, allows the reduction of treatment fluence and thereby reduces the adverse effects.[1]

Usually PWS appears pink during infancy period, which becomes red during adulthood. Without treatment, PWS typically darkens as age increases and may develop nodular thickened lesions. Early treatment gives more optimal results in all patients. Much quicker results can be visualized when the laser treatment is begun at an earlier age.[8]

Zoran Barcot *et al*. stated that, after PDL treatment nearly 65% of patients achieved between 50% and 90% lightening of their lesions and 15% achieved greater than 90% lightening with individual treatment sessions spaced 2 to 3 months apart; 8 to 10 serial treatments were needed to achieve significant lightening of the lesions.[9] Tina Alster *et al*. proved that the use of dual 595/1,064-nm wavelength laser provided continued improvement of PWS that were previously recalcitrant to PDL therapy. Moderate clinical improvement was observed in 12 out of 25 subjects (48%), and mild improvement was observed in 13/25 subjects (52%). The total number of treatments ranged from 2 to 7 sessions. Side effects were limited to erythema, edema, and purpura. Rarely vesicle formation was observed, with no scarring or pigmentary changes.[10]

Badawi *et al*. conducted a study with 20 patients of nodular and thick port wine stains, stated that the average improvement was 70% following 4 treatment sessions at 3–4 weeks interval.[11] Barton *et al*. has demonstrated that the sequential application of lasers at 532- and 1,064-nm wavelength caused permanent vessel damage at which the two pulses individually had little or no effect.[12]

Alster *et al*. stated that the DSWL is best used for darker, thicker, nodular, recalcitrant PWS, whereas the PDL can be used alone for superficial, early, pink lesions of PWS, and the Nd:YAG laser can be used alone in patients with darker skin tones. The frequency of laser treatments in the literature showed more variations, ranged between few weeks to few months. Patients with dark skin types have increased risk for hypo or hyper-pigmentation, may benefit more from this DSWL technology.[13]

Koster *et al*. demonstrated that studies show 50–90% overall clearance of PWS, with approximately 10% improvement per treatment session. Superior results had been observed with the DSWL approach when compared to PDL alone in PWS cases which was resistant to other treatment modalities.[14] In this study, out of the 11 patients 5 patients (45.45%) had more than 70% improvement, 6 patients (54.54%) had 40–70% improvement at the end of 5 sittings and there was a progressive lesional lightening noted after each laser treatment in all patients without any adverse effects.

Conclusion

Dual sequential wavelength laser (DSWL) is a good modality of treatment for portwine stains. It has enhanced penetration and effective clearance of thicker, nodular, recalcitrant lesions of PWS. DSWL is safe and efficacious and it can be recommended as a therapeutic modality for portwine stains.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Taylor MB, Samolitis NJ. MultiPlex 1064/585 nm laser treatment of vascular lesions. Lasers Surg Med 2007;1:79-9.
2. Wang T, Chen D, Yang J, Ma G, Yu W, Lin X. Safety and efficacy of dual wavelength laser (1064+595 nm) for treatment of non treated portwine stains. J Eur Acad Dermatol Venereol 2018;32:260-4.
3. Borges da Costa J, Boixeda P, Moreno C, Santiago J. Treatment of resistant port-wine stains with a pulsed dual wavelength 595 and 1064 nm laser: A histochemical evaluation of the vessel wall destruction and selectivity. Photomed Laser Surg 2009;27:599-605.
4. Chang HS, Kim YG, Lee JH. Treatment using a long pulsed Nd:YAG laser with a pulsed dye laser for four cases of blebbed

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port wine stains. Ann Dermatol 2011;1:75-8.
5. Walsh BM. Dual wavelength lasers. Laser Physics 2010;20:622-34.
6. Li G1, Sun J, Shao X, Sang H, Zhou Z. The effects of 595- and 1064-nm lasers on rooster comb blood vessels using dual-wavelength and multi-pulse techniques. Dermatol Surg 2011;37:1473-9.
7. Srinivas CR, Kumaresan M. Lasers for vascular lesions: Standard guidelines of care. Indian J Dermatol Venereol Leprol 2011;77:349-68.
8. Brightman LA, Geronemus RG, Reddy KK. Laser treatment of port-wine stains. Clin Cosmet Investig Dermatol 2015;8:27-33.
9. Barčot Z, Župančić B. Pulsed dye laser treatment of vascular lesions in childhood. Acta Dernatovenerol Croat 2010;18:201-8.
10. Alster TS, Tanzi EL. Combined 595-nm and 1,064-nm laser irradiation of recalcitrant and hypertrophic port-wine stains in children and adults. Dermatol Surg 2009;35:914-8.
11. Badawi A, Atteya A, Salah M, Samy N, Rafei M. The combination of pulsed dye and neodemium yag lasers in treatment of nodular and thick port-wine stains. Lasers Surg Med 2007;1:39.
12. Barton JK, Frangineas G, Pummer H, Black JF. Cooperative phenomena in two-pulse, two-color laser photocoagulation of cutaneous blood vessels. Photochem Photobiol 2001;73:642-50.
13. Alster TS, Railan D. Laser treatment of vascular birthmarks. J Craniofac Surg 2006;17:720-3.
14. Koster PH, van der Horst CM, Bossuyt PM, van Gemert MJ. Prediction of portwine stain clearance and required number of flashlamp pumped pulsed dye laser treatments. Lasers Surg Med 2001;29:151-5.

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Original Article

Efficacy of Topical Timolol versus Saline in Chronic Venous Ulcers: A Randomized Controlled Trial

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Abstract

Background: Chronic venous ulcers sometimes fail to heal in spite of adequate treatment. Nonhealing is possibly because of chronic activation of β-2 adrenergic receptor (B2-AR) in the keratinocytes by endogenously generated catecholamines, which inhibits keratinocyte migration. Blocking of B2-AR using β-blockers has been reported to promote wound healing through keratinocyte migration, angiogenesis, and fibroblast migration. Topical timolol, a B2-AR antagonist, is used to promote wound healing. **Aims and Objectives:** The aim of this study was to compare the efficacy of topical timolol versus saline in chronic venous leg ulcers and to compare the mean reduction in ulcer area at the end of 4 weeks. **Materials and Methods:** Twenty patients were randomized into two groups. Patients in Group 1 were treated with one drop of 0.5% topical timolol every alternate days for 4 weeks. Ulcer margins were measured and ulcer area calculated every week for 4 weeks. Similar dressing and measurement were carried out for patients with saline for 4 weeks. Healing rate was assessed by the percentage of reduction of ulcer area of both groups at week 4. **Results:** The mean reduction in the ulcer size in timolol group was 86.80%, and in saline group 43.82% at the end of 4 weeks. **Conclusion:** Topical timolol is an easy, noninvasive therapy that can be recommended for chronic ulcer.

Keywords: Healing, timolol, venous leg ulcer

Introduction

Venous stasis ulcers account for 80%–90% of all leg ulcers. The current treatment for venous ulcers is compression and wound care with dressings. All of which have not showed any significant improvement in healing. Keratinocyte migration is a key component in wound healing and it is required for skin reepithelization and complete wound repair. β-2 adrenergic receptor (B2-AR) is expressed primarily on human keratinocytes.[1] Activation of this receptor inhibits keratinocyte migration. Blocking of B2-AR pathway using B2-AR antagonists (β-blockers) has been reported to promote wound healing through different mechanisms, including keratinocyte migration, promotion of angiogenesis, increased dermal fibroblast migration, and epidermal differentiation.[2] Timolol, a commercially available B2-AR antagonist, used as an intraocular glaucoma medication has been shown to promote wound healing when instilled in chronic leg ulcers. The purpose of this study was to assess the efficacy of topical timolol in wound healing.

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Materials and Methods

Twenty patients diagnosed with chronic venous ulcer of lower extremities, presenting to the OPD over the study period and satisfying the inclusion and exclusion criteria, were included in the study after obtaining informed consent.

The inclusion criteria of the study included patients who were over 18 years old, venous ulcers lasting for at least 4 weeks with no improvement with good wound care, dressings, and compression stockings.

The exclusion criteria of the study included infected ulcers, patients on systemic β-blockers, patients with asthma and COPD, and patients with venous leg ulcers on systemic

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treatment. Each patient after randomization was allotted into treatment or control group.

Group 1 (treatment group). Ten patients with chronic venous leg ulcer receiving topical timolol

Group 2 (control group). Ten patients with chronic venous ulcer receiving saline dressing.

All patients were advised to continue compression stockings.

Group 1. One milliliter of timolol contains 5 mg of the drug. Each milliliter contains 20 drops. Hence, each drop contains 0.25 mg of the drug. To each patient, one drop was instilled every 2 cm^2 of wound edge and then covered with sterile dressing. This was repeated every alternate days.

Group 2. One drop of saline was instilled every 2 cm^2 of wound and then covered with sterile dressing every alternate days.

Ulcer margin was measured by measuring the greatest length and the greatest width, perpendicular to the greatest length every week for a maximum period of 4 weeks. Digital photographs were taken during each visit.

The primary end point is to evaluate the ulcer reduction rate at the end of 4 weeks. The improvement was also evaluated with photography and the ulcer reduction rate in both groups was calculated at the end of 1 month after beginning the treatment. The healing rate between the study and control group was compared statistically using Satistical Package for Social Sciences Software Version 24.0. Armonk, NY: IBM Corp.

Results

A total of 20 patients meeting the inclusion and exclusion criteria were enrolled in the study. All the patients were suffering from chronic venous ulcers. The result was assessed on the basis of ulcer area reduction. The mean ulcer area reduction after 1 week in the timolol group was 23.06%, after 2 weeks it was 36.57%, at 3 weeks it was 67.18%, and after 4 weeks it was 86.80% [Table 1]. However in the saline group, it was 11.01%, 17.53%, 31.78%, and 43.82%, respectively [Table 2]. The overall mean reduction

Table 1: Percentage of improvement in patients treated with timolol dressing

S. no. of patients	Initial measurement in cm^2	Measurement after 1 week	Measurement after 2 weeks	Measurement after 3 weeks	Final measurement	Percentage of improvement
1	10	6.6	4	1.2	0	100
2	6.21	4.12	2.56	1	0	100
3	2.52	2.17	1.54	1.2	0.7	72.2
4	9	7.3	6.4	4.2	1.2	86.66
5	3.5	3.1	2.76	2	1.12	68
6	24.36	18.75	14.06	7.36	5.8	76.19
7	9.24	7.3	7.2	2.04	0	100
8	19.25	17.27	16.83	14.1	6.16	65.71
9	31.5	22	19.25	2.7	0	100
10	4.16	2.58	2.4	0.6	0	100
Mean percentage of improvement	0%	23.06%	36.57%	67.184%	86.8%	86.8%

Table 2: Percentage of improvement in patients treated with saline dressing

S. no. of patients	Initial measurement in cm^2	Measurement after 1 week	Measurement after 2 weeks	Measurement after 3 weeks	Final measurement	Percentage of improvement
1	1.4	1.2	1.0	0.8	0.55	60.71
2	3.8	3.1	2.7	2.1	1.72	54.73
3	31.35	30	28.5	23	20	36.2
4	1.6	1.5	1.4	1.25	1.06	33.75
5	6.21	5.94	5.8	5.5	4.40	29.14
6	13.6	13.1	12.8	12.5	11.9	12.5
7	5.4	4.7	4.5	3.8	3.12	42.2
8	9.5	8.1	7.1	5.25	3.9	58.94
9	3.7	3.1	3.0	2.1	1.63	55.94
10	7.4	6.3	5.7	4.1	3.4	54.05
Mean percentage of improvement	0%	11.01%	17.53%	31.78%	43.82%	43.82%

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in the ulcer area was 86.80% in the patients with timolol dressing as compared to 43.82% in the patients treated with saline dressing [Figure 5]. There was a complete closure of the ulcer in five patients (50%) in the timolol group [Figures 1 and 2]. There was no case of complete closure of the ulcer in the saline group [Figures 3 and 4].

Although there was a reduction in the ulcer size in the timolol group as compared to the saline group at the end of 4weeks, it was not statistically significant at $P = 0.104$ [Table 3].

There were no local or systemic side effects noted.

Discussion

Chronic ulcers are a common problem, particularly in older people resulting in repeated admissions. The treatment of chronic ulcer presents a therapeutic challenge and includes good wound care, dressings, compression therapy, and lifestyle modifications. Dressings often used for chronic ulcers include hydrocolloid, hydrogel foams, pastes, silver impregnated dressings, and simple nonadherent dressings. A meta-analysis of 42 randomized controlled trials with more than 1000 patients showed no significant difference among dressing types.[3] Hydrocolloid dressings that are expensive are not shown to have a healing benefit over simple nonadherent dressings. There is no clear evidence to support the use of one dressing over the other and so the choice of dressing depends on the cost and ease of application.[3]

B2-AR receptors are present on cells in multiple organ systems, including the skin. Within skin, receptors are present on keratinocytes, fibroblasts, and melanocytes. B2-AR is the dominant subtype expressed on keratinocyte surfaces.[1] The influence of this receptor has been studied by observing the effects of agonist and antagonist at the receptor. In addition, the epidermis contains the full range of enzymes necessary to synthesize catecholamines such as norepinephrine and epinephrine to bind to various adrenergic receptors and delay wound healing.[4] B2-AR antagonists have been reported to promote wound healing, and one potential mechanism of action via keratinocyte migration. Keratinocyte migration occurs by the facilitation of chemotaxis, the polarization of cells, and activation of extracellular signal-related kinases essential in the signaling of promigratory pathways. The B2-AR activation inhibits keratinocyte migration by activating the serine/threonine phosphatase 2A, which downregulates phosphorylation of extracellular signal-related kinases necessary for migration.[5] Therefore, B2-AR antagonists prevent the phosphorylation of phosphatase 2A and downstream extracellular signal-related kinase promotion, inducing a promigratory pathway in keratinocytes. The B2-AR antagonists improve the ability of keratinocytes to respond to such migratory signals, whereas the B2-AR agonists decrease keratinocytes ability to respond.[6] Angiogenesis and dermal fibroblast proliferation are also regulated by B2-AR. The B2-AR antagonists have been found to promote angiogenesis in chick chorioallantoic membrane assays and *in vivo* murine wound models. Dermal fibroblast migration is also increased when exposed to B2-AR antagonists, and epidermal differentiation is improved with B2-AR antagonists.[7,8] Catecholamines, such as epinephrine and norepinephrine, are endogenously generated agonists, and these are also generated by the wound epithelium. Therefore, it is possible that one pathogenic mechanism contributing to nonhealing skin wounds is the chronic activation of B2-AR by endogenously generated catecholamines.[9] Blocking of B2-AR pathway using pharmacological antagonists promotes keratinocyte migration and re-epithelization *in vitro*, in human skin, in an *ex vivo* skin wound model, and *in vivo* in animal preclinical studies.[10,11]

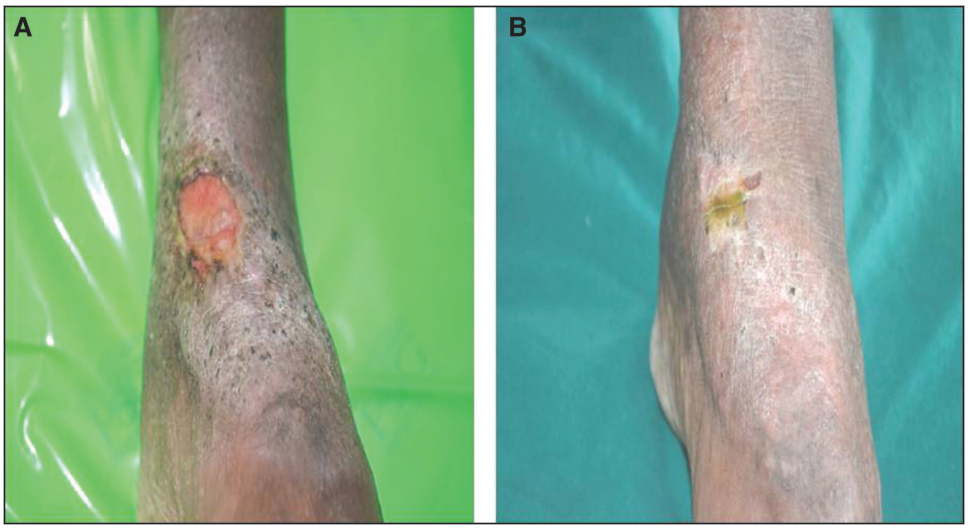


Figure 1: (A) Before application of timolol dressing. (B) After the application of timolol dressing at the end of 4 week

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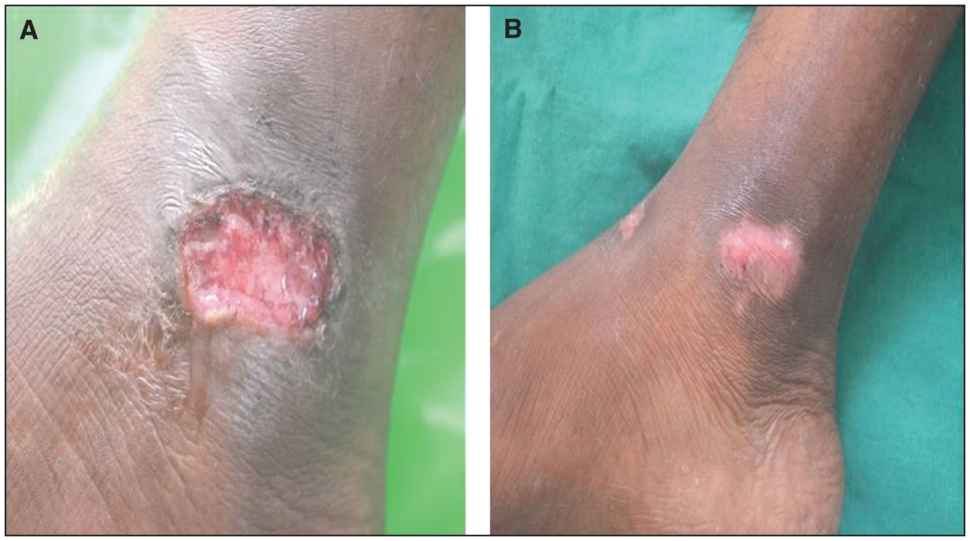


Figure 2: (A) Before application of timolol dressing. (B) After the application of timolol dressing at the end of 4 weeks

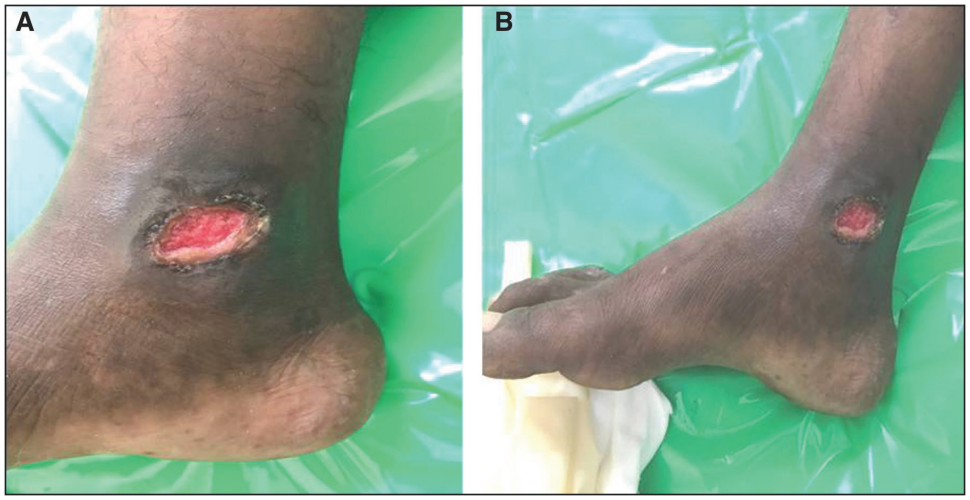


Figure 3: (A) Before application of saline dressing. (B) After application of saline dressing at the end of 4 weeks

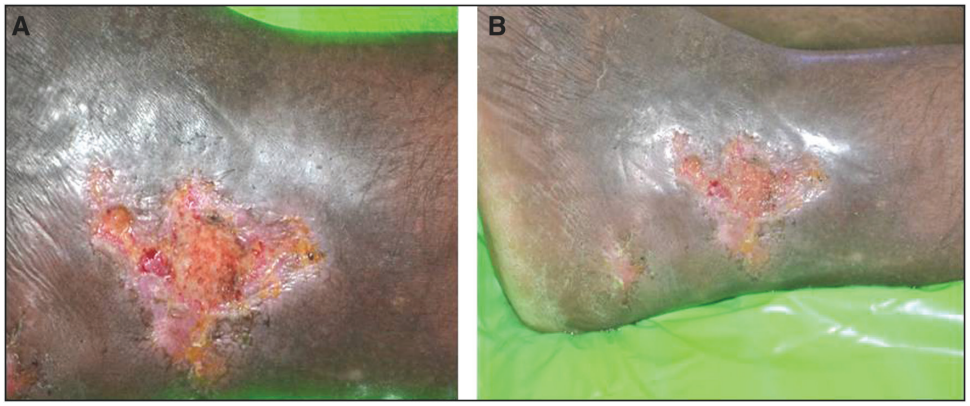


Figure 4: (A) Before application of saline dressing. (B) After application of saline dressing at the end of 4 weeks

People with burns have been treated with systemic B2-AR antagonist proponalol to diminish the effect of excessive catecholamine response after burn injury. Mohammadi *et al.*[12] carried out a randomized double-blind trial in burn patients. They included 79 burn patients, 37 in the study group and 42 in the control group. The participants in the study group who received oral propranolol had better wound healing.

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Table 3: Group statistics showing standard deviation (SD) and *P* value

	Groups	*N*	Mean	SD	*P* value
Initial measurement	Timolol	10	11.9	9.7	0.519
	Saline	10	8.4	8.8	
Measurement after first week	Timolol	10	9.1	7.3	0.806
	Saline	10	7.7	8.5	
Measurement after second week	Timolol	10	7.7	6.5	0.963
	Saline	10	7.2	8.2	
Measurement after third week	Timolol	10	3.6	4.1	0.340
	Saline	10	6.0	6.8	
Final measurement	Timolol	10	1.4	2.4	0.104
	Saline	10	5.1	6.1	

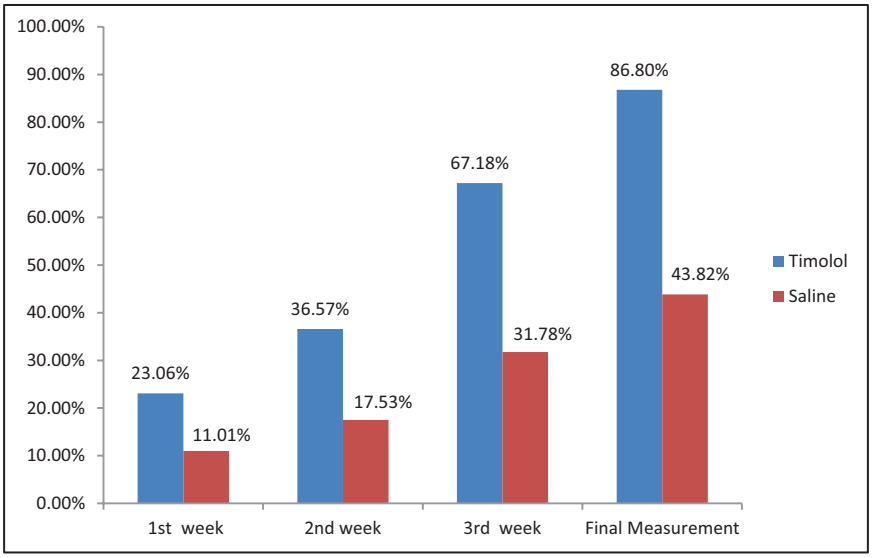


Figure 5: Bar diagram showing the comparison of the mean improvement in timolol group and saline group after every week and also after the treatment for 4 weeks

A case report by Tang *et al.*[13] showed that topical timolol application produced complete wound healing in a refractory wound on the mid back.

A cohort study by Chakkittakandiyil *et al.*[14] showed the efficacy, tolerability, and safety of timolol maleate 0.5% solution in treating infantile hemangiomas. Thus, Manahan *et al.*[15] used topical timolol as a safe and inexpensive drug to treat chronic ulcer. Braun *et al.*[16] used topical timolol in five patients with recalcitrant wounds; 0.5% topical timolol was instilled in chronic, recalcitrant wounds of these five patients, and improvement was noted in all five, including three patients who had complete healing. The mean reduction in wound size after 7 weeks was 78.2%. Lev-Tov *et al.*[17] successfully treated chronic venous leg ulcer with topical timolol.

A study by Thomas *et al.*[18] showed that the patients treated with topical timolol had a significantly higher ulcer healing rate of 61.79% in the study group and 29.62% in the control group at the end of 12 weeks. Analysis showed that there were significant differences in percentage change in ulcer of the study and control groups. Thus, treatment using topical application of timolol resulted in faster healing rate compared with conventional treatment.

Our study showed that patients in timolol group (86.80%) had significant reduction in the size of the ulcer as compared to the saline group (43.82%) after 4 weeks of treatment. There was no side effects and the treatment was well tolerated.

Our research attempts to study the efficacy of topical timolol on healing of chronic venous leg ulcer. Topical timolol is cost-effective, easily available as a 0.5% solution, and is therefore a treatment option in chronic ulcers. This is the first randomized controlled study comparing timolol and saline on healing rate of chronic venous ulcers. The limitation of our study is the sample size. However, more studies are needed.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal.

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The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Sivamani RK, Lam ST, Isseroff RR. Beta adrenergic receptors in keratinocytes. Dermatol Clin 2007;25:643-53, x.
2. Pullar CE, Chen J, Isseroff RR. PP2A activation by beta2-adrenergic receptor agonists: novel regulatory mechanism of keratinocyte migration. J Biol Chem 2003;278:22555-62.
3. Palfreyman S, Nelson EA, Michaels JA. Dressings for venous leg ulcers: systematic review and meta-analysis. BMJ 2007; 335:244.
4. Grando SA, Pittelkow MR, Schallreuter KU. Adrenergic and cholinergic control in the biology of epidermis: physiological and clinical significance. J Invest Dermatol 2006;126:1948-65.
5. Pullar CE, Chen J, Isseroff RR. PP2A activation by beta2-adrenergic receptor agonists: novel regulatory mechanism of keratinocyte migration. J Biol Chem 2003;278:22555-62.
6. Pullar CE, Zhao M, Song B, Pu J, Reid B, Ghoghawala S, *et al*. Beta-adrenergic receptor agonists delay while antagonists accelerate epithelial wound healing: evidence of an endogenous adrenergic network within the corneal epithelium. J Cell Physiol 2007; 211:261-72.
7. Zhao M, Bai H, Wang E, Forrester JV, McCaig CD. Electrical stimulation directly induces pre-angiogenic responses in vascular endothelial cells by signaling through VEGF receptors. J Cell Sci 2004;117:397-405.
8. Pullar CE, Isseroff RR. The beta 2-adrenergic receptor activates pro-migratory and pro-proliferative pathways in dermal fibroblasts via divergent mechanisms. J Cell Sci 2006;119:592-602.
9. Sivamani RK, Shi B, Griffiths E, Vu SM, Lev-Tov HA, Dahle S, *et al*. Acute wounding alters the beta2-adrenergic signaling and catecholamine synthetic pathways in keratinocytes. J Invest Dermatol 2014;134:2258-66.
10. Pullar CE, Rizzo A, Isseroff RR. Beta-adrenergic receptor antagonists accelerate skin wound healing: evidence for a catecholamine synthesis network in the epidermis. J Biol Chem 2006;281:21225-35.
11. Sivamani RK, Pullar CE, Manabat-Hidalgo CG, Rocke DM, Carlsen RC, Greenhalgh DG, *et al*. Stress-mediated increases in systemic and local epinephrine impair skin wound healing: potential new indication for beta blockers. PLoS Med 2009; 6:e12.
12. Mohammadi AA, Bakhshaeekia A, Alibeigi P, Hasheminasab MJ, Tolide-ei HR, Tavakkolian AR, *et al*. Efficacy of propranolol in wound healing for hospitalized burn patients. J Burn Care Res 2009;30:1013-7.
13. Tang JC, Dosal J, Kirsner RS. Topical timolol for a refractory wound. Dermatol Surg 2012;38:135-8.
14. Chakkittakandiyil A, Phillips R, Frieden IJ, Siegfried E, Lara-Corrales I, Lam J, *et al*. Timolol maleate 0.5% or 0.1% gel-forming solution for infantile hemangiomas: a retrospective, multicenter, cohort study. Pediatr Dermatol 2012;29:28-31.
15. Manahan MN, Peters P, Scuderi S, Surjana D, Beardmore GL. Topical timolol for a chronic ulcer–a case with its own control. Med J Aust 2014;200:49-50.
16. Braun LR, Lamel SA, Richmond NA, Kirsner RS. Topical timolol for recalcitrant wounds. JAMA Dermatol 2013;149:1400-2.
17. Lev-Tov H, Dahle S, Moss J, Isseroff RR. Successful treatment of a chronic venous leg ulcer using a topical beta-blocker. J Am Acad Dermatol 2013;69:e204-5.
18. Thomas B, Kurien JS, Jose T, Ulahannan SE, Varghese SA. Topical timolol promotes healing of chronic leg ulcer. J Vasc Surg Venous Lymphat Disord 2017;5:844-50.

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Erythrokeratoderma en cocardes – A rare phenotype

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Case Report

Erythrokeratoderma En Cocardes – A Rare Phenotype

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Abstract

Erythrokeratodermas are disorders of keratinization with two major types, namely erythrokeratodermia variabilis and symmetrical progressive erythrokeratoderma. Apart from the two main types, rare variants include erythrokeratoderma en cocardes (EEC), erythokeratoderma with erythema gyratum repens-like lesions, erythema annulare centrifugum-like lesions, and reticulate erythrokeratoderma. Herein, we report a rare case of EEC with associated palmoplantar keratoderma, the association which has not been described in previous reports.

Keywords: *En cocardes, erythrokeratoderma, palmoplantar keratoderma*

Introduction

Erythrokeratodermas are disorders of keratinization which encompass two main subtypes, namely erythrokeratodermia variabilis (EKV) and symmetric progressive erythrokeratoderma (SPEK) and other atypical rare variants.[1] Erythrokeratoderma en cocardes (EEC) is a rare variant characterized by polycyclic, concentric, and targetoid lesions along with erythematous plaques.[2]

Case Report

A 4-year-old boy born out of third-degree consanguineous marriage presented with multiple hyperpigmented scaly lesions involving the arms, forearms, back, gluteal region, thighs, and legs since 6 months of his age. There was a history of aggravation of lesions during cold season and improvement during summer. There was no history of atopy and other systemic symptoms. Milestones were achieved normally. There was no history of similar skin lesions in siblings or other family members.

Cutaneous examination revealed multiple scaly plaques with polycyclic borders involving the arms, forearms, thighs, and legs [Figures 1a,b and 2]. In the posterior aspect of the thigh, there were scaly plaques with polycyclic borders sparing of the popliteal fossa [Figure 3]. There were multiple discrete annular scaly plaques with concentric rings involving the forearms and thighs. Anterior aspect of legs showed diffuse ichthyosis extending to the dorsum of the feet [Figure 4]. Palms and soles revealed diffuse keratoderma with fissuring and scaling [Figure 5]. Examination of hair, nails, and teeth was normal. Systemic examination was essentially normal. Hematological examination did not reveal any abnormality. Scrapings of skin for potassium hydroxide mount were negative for fungal elements.

Histopathology revealed hyperkeratosis, focal mild parakeratosis, conspicuous granular layer, and irregular acanthosis. There were mild spongiosis and lymphocytic exocytosis. The papillary and upper dermis showed pigment incontinence, prominent capillaries, and a mild perivascular lymphohistiocytic infiltrate, a picture compatible with erythokeratoderma [Figure 6].

Owing to the distinct clinical morphology with annular lesions, polycyclic borders, and histopathological features, a diagnosis of EEC was entertained.

Discussion

Erythrokeratoderma has two variants: SPEK and EKV. SPEK is characterized by well-demarcated, hyperkeratotic plaques distributed symmetrically over the elbows, knees, dorsal aspect of the hands, feet, and buttocks, sparing the trunk. SPEK has its onset in infancy or early childhood.[3] EKV, on the other hand, manifests as two distinct clinical pictures: with generalized, erythematous, hyperkeratotic plaques in one and localized sharply demarcated

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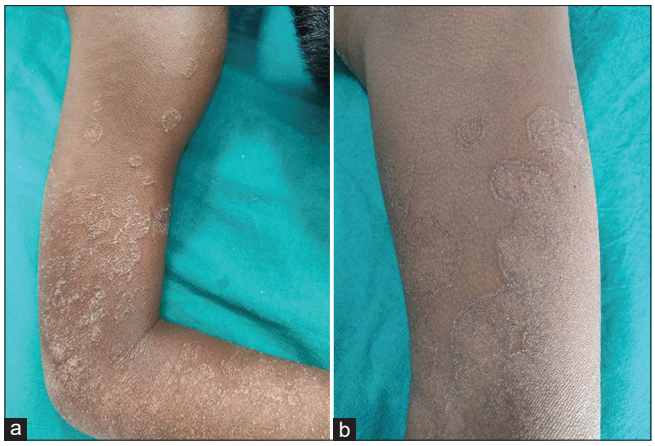


Figure 1: (a) Multiple scaly plaques with polycyclic borders involving the arm and forearm, (b) Scaly plaques with polycyclic borders involving the arm



Figure 2: Scaly plaques with polycyclic borders involving the anterior aspect of thighs

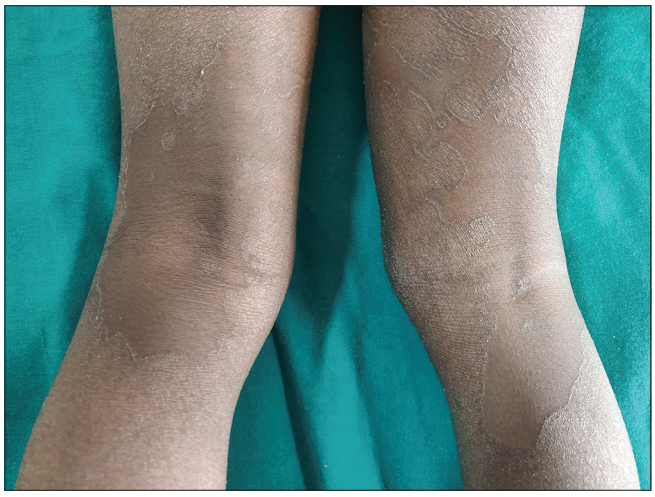


Figure 3: Scaly plaques with polycyclic borders involving the posterior aspect of thighs sparing the popliteal fossa



Figure 4: Diffuse ichthyosis of legs extending to dorsum of feet



Figure 5: Diffuse palmoplantar keratoderma

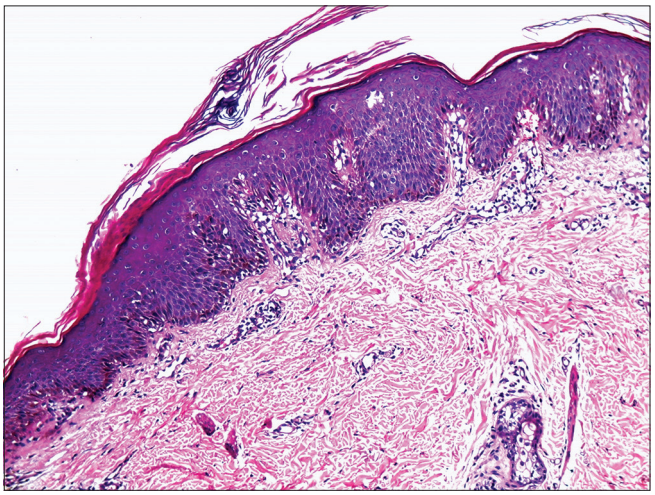


Figure 6: Epidermis with hyperkeratosis, focal mild parakeratosis, conspicuous granular layer, and irregular acanthosis. Dermis with mild perivascular lymphohistiocytic infiltrates.(H and E, ×100)

hyperkeratotic plaques in the other. EKV has its onset in birth or in neonatal period in 50% of patients and within 1st year of life in 90% of patients.[4]

Rare variants of EKV described include EEC, EKV with erythema gyratum repens-like lesions, EKV with

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erythema annulare centrifugum-like lesions, and reticulate erythrokeratoderma.[4,5] Degos *et al*., in 1947, described EEC in a 13-year-old girl, a rare and atypical variant of EKV with polycyclic plaques, with concentric layers of scaling and erythema giving a targetoid, or "en cocardes" appearance.[6]

The index case described by Degos had an onset at birth, remissions and recurrences, desquamation of palm, similar skin lesions in father and paternal uncle pointing to autosomal-dominant inheritance, and improvement of skin lesions during summer.[7] The present case had onset during 6 months of age, without any family history and improvement during summer. Unlike the index case, the cases reported by Rajagopalan *et al*. and López-Ferrer *et al*. did not show improvement of lesions during the summer.[1,2] Furthermore, the present case showed prominent palmoplantar keratoderma, which has not been described in previous reports.[1,2,8]

Apart from EEC, differential diagnoses considered include annular type of epidermolytic ichthyosis, X-linked recessive ichthyosis, and SPEK. Unlike, in annular epidermolytic ichthyosis, there was no history of blisters and erosions at birth, and histopathology did not revealed any epidermolytic hyperkeratosis. X-linked recessive ichthyosis presents with dark brown ichthyotic scales with sparing of popliteal and antecubital fossa with associated cryptorchidism, autism, and deep stromal corneal opacity.[4] Although the present case had ichthyosis of legs with sparing of popliteal fossa, there were no associated systemic abnormalities. Tinea imbricata can be a morphological differential diagnosis, but the presence of skin lesions since 6 months of age and negative test for skin scrapings for fungus preclude the condition.

Histopathology cannot distinguish between the variants of erythrokeratodermas. Genetic studies show mutation in connexin genes in cases of EKV. Connexins are a family of transmembrane proteins that form gap junctions in the cells. These intercellular channels play an important role in cell growth, homeostasis, and epidermal differentiation.[4] Mutations in genes, namely GJB3, GJB4, and rarely GJA1 encoding the connexins, have been identified as the underlying pathogenesis in EKV.[3,9] In SPEK apart from GJB4 mutation, mutations reported include a frameshift mutation in loricrin gene, homozygous loss of function mutation in KRT83 gene, and mutation in KDSR gene which codes for an enzyme involved in ceramide synthesis.[3] López-Ferrer *et al*. have reported a case of EEC with missense mutation R32W in GJB3 gene.[1] Similar mutation in GJB3 gene had been reported in patients with palmoplantar involvement and deafness.[10] The present case had clinical lesions analogous to EEC and palmoplantar involvement but without any hearing impairment. However, genetic study for mutation analysis was not done due to resource constraints.

Treatment options include topical emollients, retinoids, salicylic acid, and corticosteroids. Systemic retinoid such as acitretin and isotretinoin shows good improvement, but with recurrence of lesions once the treatment is stopped.[8] Improvement with retinoids with psoralen and ultraviolet A therapy has been reported.[11] Since the disease is a genodermatoses, parents should be counseled regarding the chronic nature of the disease and the need for long-term follow-up. Treatment plan with acitretin was considered, but the patient did not report for follow-up.

The present case is highlighted for its distinct clinical picture and its association with palmoplantar keratoderma.

Declaration of patient consent

Author certify that they have obtained all appropriate patient consent forms. In the form the patients/parents have given their images and other clinical informations to be reported in the journal. The patients & parents understand that their/their children's names and initials will not be published and due efforts will be made to conceal their identity.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. López-Ferrer A, Puig L, Nakamura M, Fernández-Figueras MT, Alomar A. Erythrokeratoderma en cocardes with R32W mutation in GJB3. J Clin Exp Dermatol Res 2010;1:113.
2. Rajagopalan B, Pulimood S, George S, Jacob M. Erythrokeratoderma en cocardes. Clin Exp Dermatol 1999;24:173-4.
3. Richard G, Ringpfeil F. Ichthyoses, Erythrokeratodermas, and Related Disorders. In: Bolognia JL, Schaffer JV, Cerroni L, editors. Dermatology. 4th ed. Philadelphia: Mosby Elsevier; 2018. p. 912-6.
4. Fleckman P, DiGiovanna JJ. The Ichthyoses. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, editors. Fitzpatrick's Dermatology in General Medicine. 8th ed. New York: McGraw Hill; 2012. p. 530-1.
5. Karadag AS, Bilgili SG, Calka O, Bayram I. Erythrokeratodermia variabilis: Two case reports. Indian Dermatol Online J 2013;4:340-3.
6. Degos R, Delzant O, Morival H. Erytheme desquamatif en plaques, congenital et familial (genodermatose nouvelle?). Bull Soc Fr Dermatol Syphiligr 1947;54:442.
7. Cram DL. Erythrokeratoderma variabilis and variable circinate erythrokeratodermas. Arch Dermatol 1970;101:68-73.
8. Srinivas SM, Dhar S. Erythrokeratodermia variabilis and erythrokeratoderma en cocardes: Case series with review of literature. Indian J Paediatr Dermatol 2016;17:202-5.
9. Richard G, Brown N, Smith LE, Terrinoni A, Melino G, Mackie RM, *et al*. The spectrum of mutations in erythrokeratodermias—novel and de novo mutations in GJB3. Hum Genet 2000;106:321-9.
10. Kelsell DP, Wilgoss AL, Richard G, Stevens HP, Munro CS, Leigh IM. Connexin mutations associated with palmoplantar keratoderma and profound deafness in a single family. Eur J Hum Genet 2000;8:469-72.
11. Yüksek J, Sezer E, Köseoğlu D Markoç F, Yildiz H. Erythrokeratodermia variabilis: Successful treatment with retinoid plus psoralen and ultraviolet A therapy. J Dermatol 2011;38:725-7.

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Case Report

Hemophagocytic Lymphohistiocytosis Complicating Methotrexate Toxicity

Abstract

Hemophagocytic syndrome or hemophagocytic lymphohistiocytosis (HLH) is a rare but potentially fatal disease caused by dysregulated activation of macrophages against one's blood cells. Major pathologic feature of HLH is hemophagocytosis. We present a case of HLH complicating methotrexate toxicity in a 65-year-old psoriatic patient with history of renal disease. Diagnosis of HLH was established as he fulfilled five out of eight HLH diagnostic criteria. This case report is presented to enlighten clinicians about the clinical entity of HLH and to suspect and recognize this rare and generally fatal disease at the earliest.

Keywords: *Cytopenias, hemophagocytic lymphohistiocytosis, hemophagocytic syndrome*

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Introduction

Hemophagocytic syndrome or hemophagocytic lymphohistiocytosis is a clinical syndrome of hyperinflammation resulting in uncontrolled and ineffective immune response.[1,2] It may be primary or secondary. Primary HLH is a genetic entity. Secondary HLH is associated with infections, malignancies, immune disorders, or drugs.[2-5] In both types, activated macrophages engulf erythrocytes, leukocytes, platelets, and precursor cells in bone marrow, liver, and lymph nodes.[6] Most severe forms of the disease lead to a sepsis like picture and multiorgan dysfunction. HLH is a disease of rapid progression and has high mortality despite treatment.[3,7-9] We report a case of secondary HLH complicating methotrexate toxicity and sepsis.

Case History

A 65-year-old male, a case of chronic plaque psoriasis on intermittent therapy with methotrexate since 15 years and chronic kidney disease since 5 years presented with fever, severe body ache, and ulcerative lesions over lips and trunk. He developed these symptoms 5 days after inadvertent intake of tablet methotrexate 5 mg for 5 consecutive

days. Examination showed tender inguinal and axillary lymphadenopathy, hemorrhagic crusting of lips, and necrotic ulcers over psoriatic plaques and as well as normal skin with severe skin tenderness and positive Nikolsky's sign. Since ulcers were present over psoriatic plaques, methotrexate-induced skin necrosis was suspected. Histopathology findings were skin with detached stratum corneum, large subcorneal pustule [Figure 1a], mild spongiosis, marked regenerative atypia, basal vacuolar degeneration, pigment incontinence, and apoptotic keratinocytes [Figure 1b]. Routine investigations showed bicytopenia with hemoglobin of 9.6 g/dl, WBC of 600 cells/mm^3 with normal platelet count of 120,000 cells/mm^3. His renal function tests were altered with elevated urea (106 mg/dl) and creatinine levels (3.16 mg/dl). The patient was started on antibiotics, leucovorin rescue therapy (folinic acid 10 mg every 6th hourly), and injection Filgrastim (Granulocyte colony-stimulating factor) 300 mg subcutaneously daily. There was worsening pancytopenia and renal function and further developed splenomegaly, features of sepsis. Blood culture was positive for Group B streptococci and methicillin sensitive *staphylococcus aureus* and procalcitonin levels were elevated (24.5 ng/ml). His antibiotics were upgraded and platelet transfusion given in spite of which patient developed fever, tachycardia, hypotension with further decrease in platelet count to 6,000 cells/mm^3 on

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5th day. Secondary HLH was suspected and a workup was rapidly initiated based on HLH 2004 diagnostic criteria. Peripheral smear revealed pancytopenia [Figure 2]. Bone marrow aspiration was done which showed megaloblastic erythroid maturation, maturation arrest in the myeloid series, and hemophagocytosis [Figure 3a and b]. Patient had hyperferritinemia (>500 mcg/L) with normal fibrinogen (522 mg/dl) and triglycerides (50 mg/dl) levels. He thus fulfilled 5 out of 8 diagnostic criteria of HLH and was treated as per HLH 2004 protocol with systemic antifungals and injection dexamethasone 10 mg/m^2. Despite all supportive measures, patient developed multiorgan dysfunction and succumbed.

Discussion

HLH is a rare disease characterized by cytokine overproduction and tissue infiltration with cytotoxic lymphocytes and activated macrophages resulting in fever, hepatosplenomegaly, and cytopenias.[1,2,9] A strict diagnostic criteria of HLH was given in 2004 by Histiocyte Society and is as follows.[9,10] Molecular diagnosis consistent with HLH that is the identification of mutations of PRF1, UNC13D, or STX11 or patient should fulfill five out of the eight criteria: fever (>100.4°F), splenomegaly, cytopenia affecting at least two of three lineages: Hb <9 g/dl (in infants <4 weeks: Hb <10 g/100 ml, platelets <100 × 10^9/L, Neutrophils <1 × 10^9/L), hypertriglyceridemia (fasting >265 mg/dl) and or hypofibrinogenemia (≤150 mg/dl), ferritin >500 ng/ml, hemophagocytosis in bone marrow, spleen, or lymph nodes, low or absent NK cell activity, soluble CD25 (soluble IL-2 receptor) >2400 U/ml and in the case of familial HLH there should be no evidence of malignancy.

This patient presented with features of methotrexate toxicity with skin necrosis, bone marrow suppression, and renal failure. His progressive pancytopenia was initially thought to be due to high dose methotrexate. He did not respond to leucovorin rescue therapy or injection Filgrastim and worsened clinically with features suggestive of sepsis due to which antibiotics were upgraded. He was evaluated for HLH as he had progressive pancytopenia unresponsive to treatment and decreased counts in spite of platelet transfusions. The major pathologic feature of HLH is hemophagocytosis as seen in our patient. It is also seen in conditions like sepsis and multiorgan failure.[3] There is considerable clinical and laboratory overlap between sepsis and HLH.[8,9] Diagnosis of HLH was established in our patient as he fulfilled five out of eight diagnostic criteria of HLH. This is a rare presentation of HLH complicating methotrexate toxicity. This case report is presented to enlighten clinicians about HLH inorder to reduce the mortality associated with the disease.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

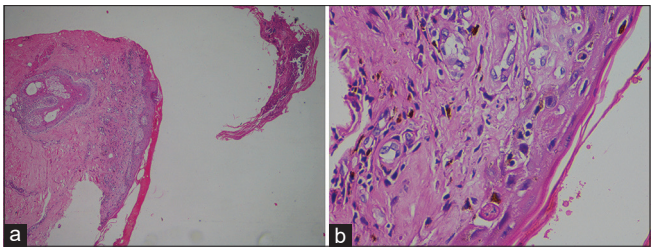


Figure 1: (a) Skin with detached stratum corneum, large subcorneal pustule (H and E, ×4). (b) Mild spongiosis, marked regenerative atypia, basal vacuolar degeneration, pigment incontinence and apoptotic keratinocytes (H and E, ×40)

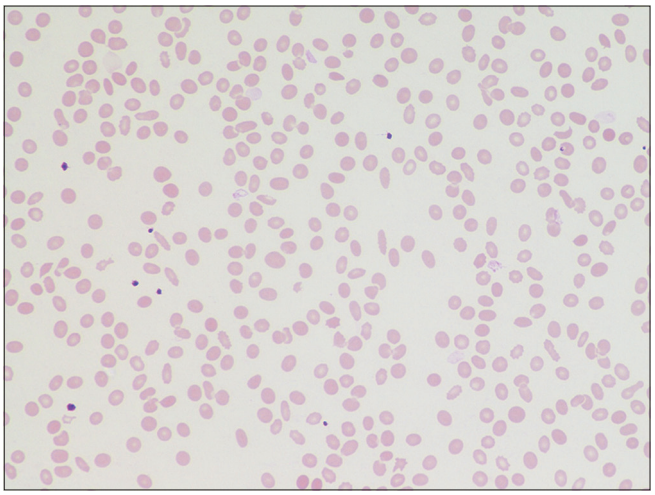


Figure 2: Peripheral smear showing pancytopenia (Giemsa ×40)

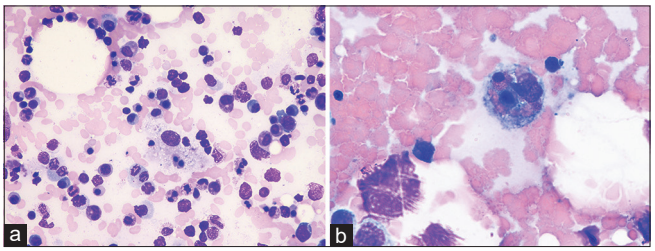


Figure 3: Bone marrow showing hemophagocytosis. (a) Giemsa ×40. (b) Oil immersion ×100)

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Janka G. Hemophagocytic lymphohistiocytosis: When the immune system runs amok. Klin Padiatr 2009;221:278-85.

2. Rosado FG, Kim AS. Hemophagocytic lymphocytosis: An update on diagnosis and pathogenesis. Am J Clin Pathol 2013;139:713-27.
3. Gupta S, Weitzman S. Primary and secondary hemophagocytic lymphohistiocytosis: Clinical features, pathogenesis and therapy. Expert Rev Clin Immunol 2010;6:137-54.
4. Mehta RS, Smith RE. Hemophagocytic lymphohistiocytosis (HLH): A review of literature. Med Oncol 2013;30:740.
5. Freeman HR, Ramanan AV. Review of haemophagocytic lymphohistiocytosis. Arch Dis Child 2011;96:688-93.
6. Favara BE. Hemophagocytic lymphohistiocytosis: A hemophagocytic syndrome. Semin Diagn Pathol 1992;9:63-74.
7. Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C. Infections associated with haemophagocytic syndrome. Lancet Infect Dis 2007;7:814-22.
8. Price B, Lines J, Lewis D, Holland N. Haemophagocytic lymphohistiocytosis: A fulminant syndrome associated with multiorgan failure and high mortality that frequently masquerades as sepsis and shock. S Afr Med J 2014;104:401-6.
9. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, *et al*. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124-31.
10. Henter JI, Tondini C, Pritchard J. Histiocyte disorders. Crit Rev Oncol Hematol 2004;50:157-74.

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Original Article

Comparison of Direct Immunofluorescence of Plucked Hair and Skin for Evaluation of Immunological Remission in Pemphigus

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Abstract

Background: Pemphigus is a chronic autoimmune bullous disorder characterized by autoantibodies directed against desmoglein 3 and/or 1. Demonstration of intercellular deposition of IgG on the cell surface of keratinocytes by direct immunofluorescence (DIF) of the skin is the gold standard in the diagnosis of pemphigus. Recently, DIF of plucked hair demonstrating intercellular deposition of IgG in the outer root sheath (ORS) has shown to be useful. **Objective:** To compare the DIF of plucked hair and skin for the evaluation of immunological remission in pemphigus vulgaris patients in clinical remission. **Materials and Methods:** A total of 30 patients with pemphigus vulgaris with positive DIF of the skin and hair at baseline were included, and DIF of skin and hair was repeated after 6 months or more of clinical remission (with no new/non-healing lesions). Presence of intercellular deposits of IgG and or C3 in skin and ORS of the hair was considered as positive. **Results:** Of the 30 patients, DIF of skin was positive in 10 patients and hair was positive 14 patients. The findings of hair and skin DIF correlated with each other in 22 patients. In 6 (20%) patients DIF of hair was positive even though the DIF of skin was negative. The sensitivity of hair DIF was 80% and specificity was 70%. **Limitations:** Small sample size. **Conclusion:** DIF of hair is a simple, non-invasive, and cost effective procedure and can be used as an additional procedure for the assessment of immunological remission in patients with pemphigus vulgaris.

Keywords: *Hair immunofluorescence, immunological remission, pemphigus*

Introduction

Pemphigus is a chronic autoimmune bullous disorder characterized by autoantibodies directed against desmoglein (Dsg) 3 and/or 1.[1] Direct immunofluorescence (DIF) of the skin or mucosa is considered as the gold standard in the diagnosis of pemphigus. Systemic steroids alone or in combination with other immunosuppressive agents are the main stay of treatment, and long-term administration of the same is associated with significant adverse effects, morbidity, and mortality.

Therefore, a system is required to monitor disease activity to lower the dosage of the drugs and eventually withdraw treatment.Various methods for assessment of immunological remission include DIF, indirect immunofluorescence, and anti-desmoglein enzyme-linked immunosorbent assay (ELISA) titres.[2-7]

DIF of the skin or mucosa is an invasive and expensive procedure and the patient may not be willing for the same. Recently, pemphigus-specific immunofluorescence pattern has been demonstrated in the outer root sheath (ORS) of hair follicles, which is structurally analogous to epidermal keratinocytes, with a sensitivity ranging from 85–100%.[8-11]

Hence, DIF of hair may be an ideal substrate for assessment of immunological remission because it is a simple, non-invasive, and cost effective procedure. We conducted this study to compare the DIF of plucked hair and skin for the evaluation of immunological remission in pemphigus vulgaris patients in clinical remission.

Materials and Methods

After obtaining approval from the institutional ethics committee and consent from the patients, a prospective study was conducted in the Department of Dermatology in the PSG institute of medical sciences and research (PSG IMS&R) over a period of 1 year.

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A total of 30 patients with pemphigus vulgaris who showed intercellular deposits of IgG antibodies against Dsg3 and/orDsg1 and/or Complement 3 (C3) in the DIF of skin and hair during the active stage of disease fulfilling the following inclusion criteria were included in the study: (a) patients in clinical remission, defined as those who had no new or non-healing skin or mucosal lesions for the past 6 months or more and (b) with or without daily prednisolone dosage equal to or less than 10 mg with or without adjuvant immunosuppressive therapy such as azathioprine 50mg or cyclophosphamide 50 mg daily.

Patients with new or non-healing skin or mucosal lesions in the preceding 6 months and patients with other bullous disorders were excluded from the study.

The presence of lace-like pattern in DIF in the ORS of the hair follicle and in the epidermis was considered as positive [Figure 1].

Skin and hair DIF was performed in all the patients. The skin sample was obtained from the upper trunk. Trichogram was done and anagen hair was collected. The samples were transported in the Michels medium to the Department of Pathology in PSG IMS&R and subjected to DIF staining with fluorescent isothiocyanate (FITC) labeled rabbit anti IgG and C3 antibodies. The samples were washed in PBS three times, each wash taking 10 minutes. Following which they were incubated with FITC labeled anti-IgG and anti-C3 for 1 hour at 37°C and once again washed in PBS three times for 10 minutes each, and then air dried and mounted on slide with PBS-glycerol solution for examination. The PBS contained propidium bromide as a counterstain.

Patients demographic parameters, phenotype of pemphigus vulgaris, history of scalp involvement, duration of disease, duration of remission, and past and current treatment were recorded.

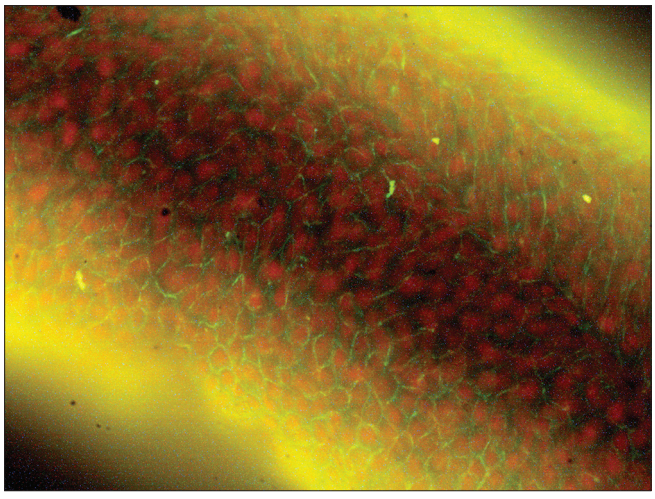


Figure 1: Fish net-like pattern of immunofluorescence in the outer root sheath of hair on DIF

Results

Patient characteristics and hair and skin DIF findings are shown in Tables 1 and 2, respectively. The age of patients in our study ranged from 17–69 years, and majority (33.3%) of the patients were in the age group of 41–50 years. All the 30 patients had a history of scalp involvement. Of the 30 patients, DIF of skin was positive in 10 patients and hair was positive 14 patients. The findings of hair and skin DIF correlated with each other in 22 patients. In 6 patients, DIF of hair was positive and skin was negative. Whereas in 2 patients, DIF of the skin was positive and hair was negative, as seen in Table 2.

The skin DIF was considered as the gold standard, and the sensitivity and specificity of hair DIF was calculated. The sensitivity (true positive/true positive + false negative) of hair DIF was 80% and specificity (true negative/true negative + false positive) was 70%.There was a statistically significant association between skin and hair DIF positivity with a *P* value of 0.010 [Table 2]. Positive predictive value of hair DIF (true positive/true positive + false positive), which refers to the percentage of patients with a positive test who actually have the disease was 57.14%, and negative predictive value (true negative/true negative + false negative), which refers to the percentage of patients with a negative test who do not have the disease was 87.5%.

A total of 18 patients were undergoing treatment in our study. In these patients, the hair and skin DIF was negative in 10 and 13 patients, respectively, which was higher than that in patients not undergoing treatment. However, the findings were not statistically significant. There was no

Table 1:Patient characteristics

Total Number of patients (*n*)	30
Age (years) Mean±SD	44.83±13.27
Sex – female:male	19:11
Phenotype of disease Mucocutaneous (*n*)	30
History of scalp involvement (*n*)	30
Duration of disease (months) Mean±SD	45±16.87
Duration of remission (months) Mean±SD	32.512±23.61
DIF positive with either substrate	16
Number of patients on treatment	18
Number of patients not on treatment	12
Number of positive hair DIF	14
Number of positive skin DIF	10

Table 2: Hair and skin DIF skin findings in pemphigus patients in clinical remission

Hair test	Skin test		*P* (Chi square test)
	Positive	Negative	
Positive	8(57.1%)	6(42.9%)	0.010 Significant
Negative	2(12.5%)	14(87.5%)	

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significant association between hair DIF and duration of disease, remission, and treatment.

Discussion

Desmogleins are the main target antigens in pemphigus. These desmogleins are calcium dependent transmembrane adhesion proteins belonging to the cadherin family. In the epidermis and hair, the distribution of desmogleins correlates with the degree of differentiation. Desmoglein 1 is expressed in the suprabasal cells of the epidermis and inner root sheath as well as the innermost layers of the ORS of the hair follicle.[12] Desmoglein 3 is present in the basal and suprabasal cells of the epidermis and throughout the ORS of the hair follicle except in the areas of epidermal-like keratinization such as in the infundibulum, where its confined to the basal layer of ORS.[12] Desmoglein 3 is also responsible for anchoring the telogen hair in the follicle.[13]

Wilson *et al*. in 1991 demonstrated that the human hair follicle is rich in the target antigens of pemphigus.[14] In 2003, Schaerer and Trueb first reported positive DIF findings in the ORS and the matrix of hair follicle in 100% of pemphigus patients.[8] Subsequently, Rao *et al*. reported 80% positivity and Daneshpazhooh *et al*. reported 91% positivity of DIF in the anagen hair.[9,10] Kumaresan *et al*. and Tanasilovic *et al*. also demonstrated positive DIF findings in 100% of their patients with active pemphigus in ORS of both anagen and telogen hair.[11,15]

David *et al*. in 1989, based on their study suggested that repeated negative DIF in pemphigus patients on clinical remission could be a sign of immunological remission.[16] Similar findings were also reported by Balighi *et al*. and Ratnam *et al*.[6,17]

Ratnam *et al*. also noted that the patients with positive DIF findings during clinical remission had a significantly higher relapse rate after the discontinuation of treatment.[17]

Various studies have shown that the rate of relapse was approximately 44–100% in patients with positive DIF findings during remission and 13–27% in patients with negative DIF.[16,18]

Rao *et al*. in 2012 conducted a study to assess the role of hair DIF in monitoring the disease activity in pemphigus; they suggested that, in patients in clinical remission, DIF of hair could be an ideal substrate for assessment of immunological remission.[19]

Only limited studies are available till date to analyze the role of hair DIF for the assessment of immunological remission. Apart from the study published by Rao *et al*., one study by Daneshpazhooh *et al*.in 2013 demonstrated the usefulness of hair DIF in the assessment of immunological remission in pemphigus patients.[20]

In their study, the sensitivity and specificity of hair DIF was 79% and 48%, respectively. Similarly, our study had a sensitivity of 80%, however, the specificity was 70% which is comparatively higher.

Even though skin or mucosal DIF is considered as the ideal substrate for the assessment of immunological remission, 6 patients in our study had positive hair DIF even though skin DIF was negative, as seen in Table 1. Thus indicating that these patients were not yet in immunological remission. Had the clinician relied only on the DIF of skin, this finding could have been missed. The sensitivity of hair DIF in our study was not high enough to suggest it as an alternative to skin DIF for the assessment of immunological remission. However, hair DIF, being a non-invasive procedure can be used initially for patients in clinical remission to assess immunological status and if it is positive then the DIF of skin could be avoided.

Conclusion

Hence, DIF of hair is a simple, non-invasive, and cost effective procedure, and can be used as an additional procedure for assessment of immunological remission in pemphigus vulgaris. However, further studies are needed to validate the same.

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Conflicts of interest

There are no conflicts of interest.

References

1. Absaq C, Mouquet H, Gilbert D, Tron F, Grassi V, Musette P, *et al*. ELISA testing of anti-desmoglein 1 and 3 antibodies in the management of pemphigus. Arch Dermatol 2009;145:585-7.
2. Cheng SW, Amagai M, Nishikawa T. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3. Br J Dermatol 2002;147:261-5.
3. Beutner EH, Jordon RE, Chorzelski TP. The immunopathology of pemphigus and bullous pemphigoid. J Invest Dermatol 1968;51:63-80.
4. Sams WM, Jordan RE. Correlation of pemphigoid and pemphigus antibody titres with activity of disease.Br J Dermatol 1971;84:7-13.
5. Chorzelski TP, Von Weiss JF, Lever WF. Clinical significance of autoantibodies in pemphigus. Arch Dermatol 1966;93:570-6.
6. O'Loughlin S, Goldman GC, Provost TT. Fate of pemphigus antibody following successful therapy. Preliminary evaluation of pemphigus antibody determinations to regulate therapy. Arch Dermatol1978;114:1769-72.
7. Beutner EH, Chorzelski TP, Jablonska S. Immunofluorescence tests. Clinical significance of sera and skin in bullous diseases. Int J Dermatol 1985;24:405-21.
8. Schaerer L, Trüeb RM. Direct immunofluorescence of plucked hair in pemphigus. Arch Dermatol 2003;139:228-9.
9. Rao R, Shenoi SD, Balachandran C. Demonstration of pemphigus specific immunofluorescence pattern by direct immunofluorescence of plucked hair. J Am Acad Dermatol 2008;58:AB85.

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10. Daneshpazhooh M, Asgari M, Naraghi ZS, Barzgar MR, Akhyani M, Balighi K, *et al*. A study on plucked hair as a substrate for direct immunofluorescence in pemphigus vulgaris. J Eur Acad Dermatol Venereol 2009;23:129-31.
11. Kumaresan M, Rai R, Sandhya V. Immunofluorescence of the outer root sheath in anagen and telogen hair: An aid to diagnosis in pemphigus. Int J Trichol 2009;1:138-9.
12. Wu H, Stanley JR, Cotsarelis G. Desmoglein isotype expression in the hair follicle and its cysts correlates with type of keratinization and degree of differentiation. J Invest Dermatol 2003;120:1052-7.
13. Koch PJ, Mahoney MG, Cotsarelis G, Rothenberger K, Lavker RM, Stanley JR. Desmoglein 3 anchors telogen hair in the follicle. J Cell Sci 1998;111:2529-37.
14. Wilson CL, Dean D, Wojnarowska F. Pemphigus and the terminal hair follicle.J Cutan Pathol 1991;18:428-31.
15. Tanasilovic S, Medenica L, Popadic S. Direct immunofluorescence of the outer root sheath in anagen and telogen hair in pemphigus vulgaris and pemphigus foliaceus. Australas J Dermatol2014;55:74-6.
16. David M, Weissman-Katzenelson V, Ben-Chetrit A, Hazaz B, Ingber A, Sandbank M. The usefulness of immunofluorescent tests in pemphigus patients in clinical remission. Br J Dermatol 1989;120:391-5.
17. Balighi K, Taheri A, Mansoori P, Chams C. Value of direct immunofluorescence in predicting remission in pemphigus vulgaris. Int J Dermatol 2006;45:1308-11.
18. Ratnam KV, Pang BK. Pemphigus in remission: Value of negative direct immunofluorescence in management. J Am Acad Dermatol 1994;30:547-50.
19. Rao R, Dasari K, Shenoi SD, Balachandran C, Dinesh P. Monitoring the disease activity in pemphigus by direct immunofluorescence of plucked hair: Apilot study. Indian J Dermatol 2013;58:164.
20. Daneshpazhooh M, Naraghi ZS, Ramezani A, Ghanadan A, Esmaili N, Chams-Davatchi C. Direct immunofluorescence of plucked hair for evaluation of immunologic remission in pemphigus vulgaris. JAm Acad Dermatol 2011;65:173-7.

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SHORT COMMUNICATION

Papillon–Lefèvre syndrome (PLS) with novel compound heterozygous mutation in the exclusion and Peptidase C1A domains of Cathepsin C gene

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Abstract

Papillon Lefevre syndrome (PLS) manifests with palmoplantar keratoderma, combined with a rapidly progressive periodontitis associated with mutations in Cathepsin C (*CTSC*) gene. This article reports a 15-year old male proband with typical PLS traits having a novel compound heterozygote with p.Q49X mutation in exon 1 and p.Y259C missense mutation in exon 6 of *CTSC* gene respectively. The exon 1 mutation, p.Q49X, (found in proband's mother) was located in exclusion domain and exon 6 mutation, p.Y259C (found in proband's father), was present in peptidase C1A, papain C-terminal domain. Interestingly, missense mutation p.Y259C identified in this study was found to be not reported so far. Upon computational analysis, this missense mutation was found to be lethal. Moreover, our protein modelling approach using mutant protein revealed the presence of monomeric structure on contrary to the tetrameric structure of the wild type protein. In addition, in vitro functional characterization of mutant p.Y259C expressed in HEK293 cells showed a significant reduction in CTSC activity (0.015 ± 0.009 mU/ml) when compared with wild type protein (0.21 ± 0.008 mU/ml). Thus, in this study, we have demonstrated that the pathogenic missense mutant p.Y259C might cause PLS by impaired CTSC function.

Keywords Papillon Lefevre syndrome · Cathepsin C · Compound heterozygote · Missense mutation · Palmoplantar keratoderma.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11033-020-05622-0) contains supplementary material, which is available to authorized users.

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Abbreviations

PLS Papillon lefevre syndrome
CTSC Cathepsin C

Introduction

Papillon–Lefèvre syndrome (PLS) is an autosomal recessive disorder characterized by palmoplantar keratoderma in association with aggressively progressing periodontitis. Keratosis can occur in the knees and elbows [1, 2]. Mutations in *CTSC* gene predisposes to PLS [3] with an increased risk of medical manifestations in consanguineous marriages. Haim munk syndrome and PLS share common features but with supplementing components like arachnodactyly, acroosteolysis and onychogryphosis is found only in the former.

CTSC gene finds its locus on chromosome 11q14-q21 [4, 5] and encodes a lysosomal cysteine protease involved in activation of many serine proteinases within immune cells. It is involved in cell-mediated cytotoxicity, phagocytic destruction of bacteria, local activation or deactivation of cytokines. Many reports on *CTSC* mutation leading to loss of function

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of CTSC protein and development of PLS has been reported [6, 7].

In our study, the proband suspected to be a PLS case was screened for *CTSC* gene mutation and found to have a compound heterozygous mutation, p.Q49X in exon 1 and p.Y259C in exon 6 of *CTSC* respectively. p.Y259C is a novel missense mutation not reported so far in association with PLS. It is a pathogenic mutation with a monomeric structure and had reduced enzyme activity.

Materials and methods

Patient and family unit

A 15-year old boy who attended the Dermatology outpatient clinic of PSG Hospitals, Coimbatore, India was clinically examined for dermatological, dental and radiological findings. The patient and his parents consented for genetic analysis and therefore, blood samples were drawn from them. Third degree parental consanguinity was documented. The phenotypic characteristics were then compared with the genotype results.

Mutation analysis of *CTSC*

Genomic DNA was isolated using EZ-10 Spin Column Blood Genomic DNA MiniPreps Kit (Bio Basic Canada Inc). The seven exons of *CTSC* gene were amplified using the primer sets reported [6]. The PCR reaction mixture of 20 µl containing 50 ng DNA as the template, 50 nM each of the forward and the reverse primer, 1 mM each of dNTPs, in a 1X PCR buffer (with1.5 mM $MgCl_2$) with 1U of Taq Polymerase (Ampliquon, Germany) was incubated in a thermal cycler (Eppendorf, USA). The cycling parameters included 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 60 °C annealing for 40 s and extension of 72 °C for 1 min. The amplified exons were sequenced by Sanger's sequencing and analysed.

Computational analysis

The CTSC protein was analysed using InterProscan and PROSITE database tools to identify significant domains and functional active sites in the protein respectively. In order to predict the potential pathogenecity of *CTSC* mutations, two bioinformatics tools were used, PolyPhen-2 (Polymorphism Phenotyping v2) in a scale from 0.00 (for benign) to 1.00 (for deleterious) (http://genetics.bwh.harvard.edu/pph2) and Mutation Taster (http://www.mutationtaster.org/). Multiple protein sequence alignments were performed using COBALT (Constraint-based Multiple Alignment Tool). Moreover, the wild type and the mutant proteins were modelled using SWISS MODEL (https://swissmodel.expasy.org) tool to obtain the comparative 3D structure of both the proteins.

Site directed mutagenesis

The Q5 Site-Directed Mutagenesis Kit (NEB, USA) was used to construct the missense mutant, Y259C. The kit manufacturer's instructions were followed to obtain the mutant clones and were confirmed through sequencing.

Cell culture and transfection

HEK293T cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma Aldrich, USA) at 37 °C in a 5% CO_2 atmosphere. The cells were transiently transfected with the Lipofectamine 3000 reagent (Invitrogen, USA) and Opti-MEM Reduced Serum Medium in 24 well plates. After 48 h, the medium was replaced with complete DMEM with 100 µg Hygromycin to obtain the cells bearing the plasmid (pCMV3) with Hygromycin resistance.

Mutant and wild type CTSC protein expression and purification

The transfected cells were disrupted in 100 µl lysis buffer (10 mM Na_3PO_4, 0.1% Triton X-100) and centrifuged at 2500 g for 5 min at 4 °C. The supernatant (5 µg per lane) was separated on 10% SDS PAGE. Proteins were quantified using the Bradford assay. The His—CTSC protein fractions were selectively purified using Ni-NTA column (Qiagen, Germany). The protein mixture was incubated in the binding buffer (20 mM Tris/HCl, 200 mM NaCl; pH 7.5), washed (20 mM Tris/HCl, 200 mM NaCl, 5 mM imidazole; pH 7.5) and eluted (20 mM Tris/HCl, 200 mM NaCl, 200 mM imidazole; pH 7.5) to obtain pure protein fractions. The protein fractions were allowed to undergo activation by with 20 µg/ml papain at 37 °C, pH 6.5 for 6 h to generate active peptides of CTSC. To inhibit the activity of the mature enzyme, 1 mM iodoacetamide (Sigma Aldrich, USA) was used.

Recombinant CTSC enzyme activity assay

Enzymatic activity was assayed by mixing 20 µl of the purified protein with 30 µl reaction buffer (100 mM Na_3PO_4, 2 mM NaCl, 2 mM DTT), 30 µl dilution buffer (10 mM Na_3PO_4, 0.1% Triton -X-100), and 100 µl of chromogenic CTSC substrate (1.5 mM gly-phe-para-nitroanilide). Absorbencies were measured at 405 nm, samples were incubated at 37 °C for 1 h, and read again. Cathepsin C activity of samples, expressed as para-nitroanilide released (nmol/mg of protein * hour), was determined interpolating

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the difference between first and second reads in a standard curve (0.0351–2.24 nmoles) of pure 4 para-nitroaniline (4 pNA) (Sigma-Aldrich, USA) [8].

Statistical analyses

Data were expressed as mean ± standard error of the mean (SEM) from three independent experiments. The data were evaluated for statistical significance with unpaired *t* test. $p < 0.05$ was considered statistically significant. All the statistical analyses were carried out using GraphPad Prism software version 5.0 Windows (GraphPad Software, USA).

Results

Clinical manifestations of the patient with PLS

A 15 year old boy, born out of third degree consanguineous marriage, presented to the dermatology OPD with complaints of thickened skin involving the palms and soles since infancy. He had erythematous lesions involving elbows, knees and few areas of trunk. The patient gave history of failure of eruption of lower incisors following the fall of primary teeth. The patient had developed multiple furuncles in the past. On examination, the patient had diffuse palmoplantar keratoderma with multiple erythematous papules and plaques seen involving the dorsum of hands, feet, elbows, knees and few areas of the trunk. On examining the teeth, the lower central incisors were absent (Fig. 1a). The nails showed multiple transverse ridging. Biospy done from the cutaneous lesions showed evidence of psoriasiform hyperplasia of epidermis with dermis being unremarkable. Routine blood investigations and X ray of the hands were normal.

CTSC mutation analysis

The seven exons of the *CTSC* gene from the proband and parents were sequenced using Sanger sequencing method. Further, *CTSC* gene analysis (NCBI reference sequences for mRNA: NM_001814.6, Concensus CDS: CCDS44693.1 and Protein: NP_001805.4) of the proband showed a compound heterozygous mutation, Q49X in exon1 and Y259C in exon 6 (Fig. 1b, c). The parents were heterozygous carriers of the mutations and were unimpacted. The nonsense mutation *c.145C > T* (p.Q49X) in exon 1 was inherited from mother while missense mutation *c.776A > G* (p.Y259C) in exon 6 was from father (ESM_1).

Evolutionary conservation of *CTSC* and in silico analysis

The mutations, p.Q49X and p.Y259C belonged to the exclusion domain (25-142 aminoacids) and Peptidase C1A, papain C-terminal domain (231-458 aminoacids)

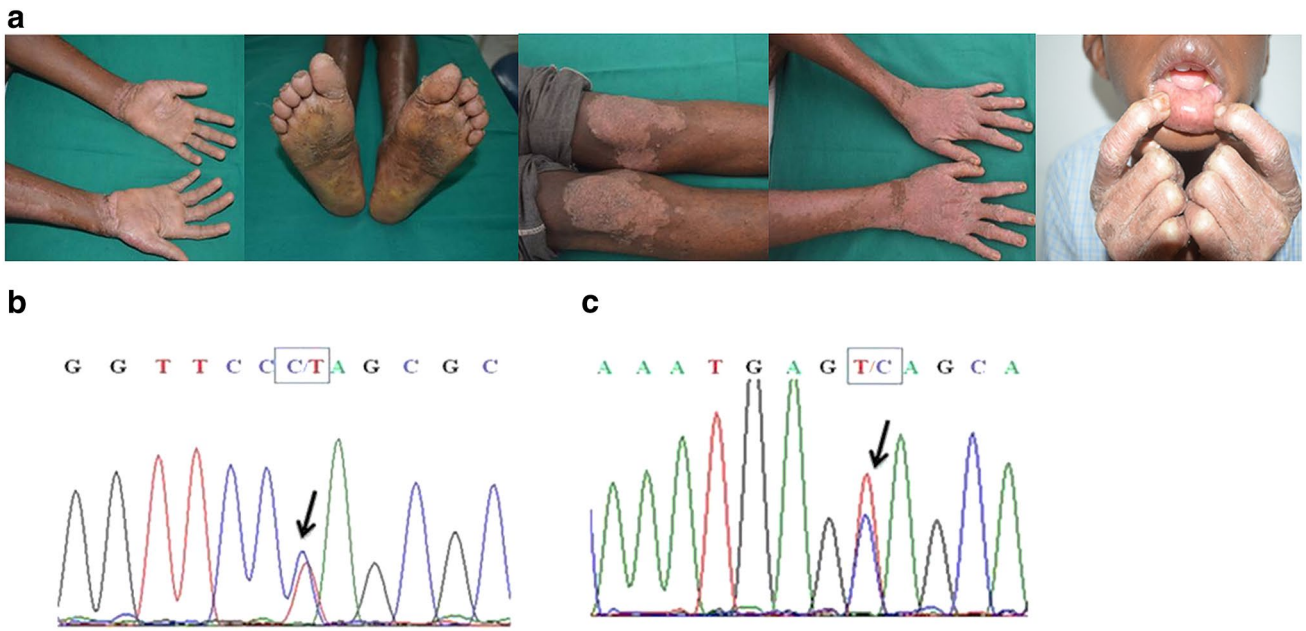


Fig. 1 Clinical observations and sequence results of *CTSC* in the patient. **a** Clinical examinations in the patient revealed multiple erythematous papules and plaques seen involving the dorsum of hands, feet, elbows, knees and few areas of the trunk. The nails showed multiple transverse ridging. The lower central incisor teeth were absent. Compound heterozygous mutation in the *CTSC* gene of the proband revealing **b** heterozygous mutation (*c.145C > T*) in exon 1 and **c** heterozygous mutation (*c.776A > G*) in exon 6

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respectively as identified from InterProscan analyses. The PROSITE database search revealed that cathepsin C protein has a thiol (cysteine) protease enzyme whose active sites are composed of Cys258, His405 and Asn427. PolyPhen2 predictions of the functional effect of the missense mutation proved that mutation was predicted to be damaging with a score of 1.00. Another tool, Mutation Taster also predicted this mutation to be disease causing. Moreover, the aminoacid Y (tyrosine) at 259th position was found to be highly conserved among different species like house mouse, rat, zebra fish, dog, cattle, chimpanzee, monkey, chicken and frog (Fig. 2a).

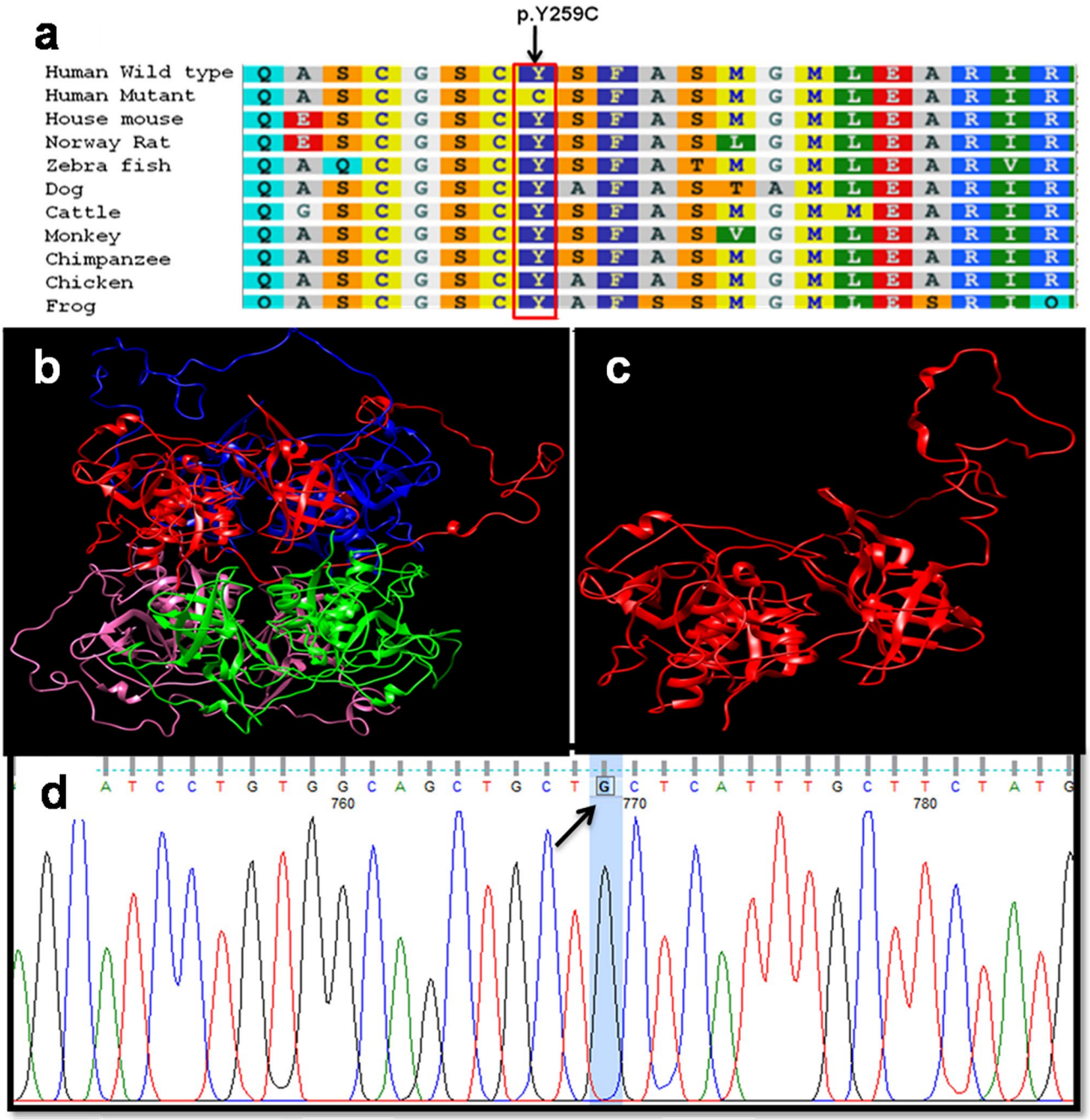


Fig. 2 Confirmation of pathogenecity of p.Y259C mutation of *CTSC* gene. **a** Cross-species multiple sequence alignment showing the high conservation of the aminoacid Y at 259th position. 3D structure of the proteins modelled using SWISS MODEL software showing the **b** tetrameric structure of the wild type protein-A chain (red), B chain (blue), C chain (green), D chain (pink) and **c** monomeric structure of the mutant protein (p.Y259C)-A chain (red) as predicted by the software. **d** Electropherogram of the mutant clone confirming *c.776A > G* mutation in CTSC gene to construct pCMV3-CTSC Y259C vector

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The protein sequence derived from the wild type and mutant protein (Y259C) were subjected to sequence based structure prediction using SWISS MODEL protein modelling software. The wild type protein was predicted by the SWISS MODEL as a tetramer having A, B, C and D chains (Fig. 2b). On the other hand, the mutant protein carrying the novel mutation p.Y259C was predicted to be only a monomer having the A chain (Fig. 2c).

In vitro expression of mutant p.Y259C CTSC protein

The pCMV3-CTSC vector was subjected to site directed mutagenesis to create a p.Y259C mutation. After mutagenesis, the clones were confirmed by Sanger's sequencing (Fig. 2d). To obtain the rCTSC protein, the following vectors were transfected into HEK 293T cell lines; pCMV3-CTSC, pCMV3-CTSC Y259C and pCMV3 negative control vector. After transfection, the cells bearing hygromycin resistant phenotypes were selectively harvested. DNA was isolated from the harvested cells and *CTSC* gene *was* amplified (Fig. 3a). The cells were lysed to obtain the protein fractions which were purified. The purified protein (inactive) was of size 55 kDa which is a propeptide. It was activated by papain treatment which yielded bands of sizes 25 kDa and 6 kDa (Fig. 3b).

Mutant p.Y259C CTSC shows reduced activity

The mutant p.Y259C CTSC protein showed a statistically significant reduction in its activity (0.015 ± 0.009 mU/ml) when compared to wild type CTSC protein (0.21 ± 0.008 mU/ml). Upon treatment with 1 mM iodoacetamide, a cysteine protease inhibitor, the activity of both mutant and wild type protein was reduced by 10 folds (Fig. 3c).

Discussion

PLS is caused by mutation in cathepsin C (*CTSC*) gene which encodes a lysosomal enzyme expressed predominantly in palms, soles, elbow, knee and gingiva. This explains the disordered keratinization leading to cutaneous lesions in these areas. Cathepsin C also plays an important role in the function of polymorphonuclear cells and macrophages, thus explaining the reason for recurrent pyoderma and gingivitis in PLS [9].

Among the PLS cases, missense mutations predominates nonsense and frameshift mutations in *CTSC* gene. The first compound heterozygous individual with PLS was reported in 2000 [10]. In the current study, a nonsense mutation p.Q49X ($c.145C > T$) and a missense mutation p.Y259C ($c.776A > G$) was present in a compound heterozygous state in the proband. This nonsense mutation truncated the protein to a 49 amino acid polypeptide as a nonfunctional enzyme resulting in PLS phenotype. p.Q49X has been reported in an Indian family coupled with PLS [11] and Haim Munk syndrome [12].

A missense mutation $c.776A > G$ (p.Y259C) observed in the affected patient was located in Peptidase C1A, papain C-terminal domain which is important for the intracellular protein degradation activity of the enzyme as this domain corresponds to the 233 amino acids mature enzyme unit [13] of cathepsin C. Moreover, this mutation Y259C lies next to the active site Cys258, thus, raising a suspicion of any possible Cys-Cys disulfide bond formation between Cys258 and Cys259 in the mutant protein and abolishing the active site availability. The 3D structure prediction results show that the p.Y259C mutant protein lacked the tetrameric structure unlike the wild type protein and

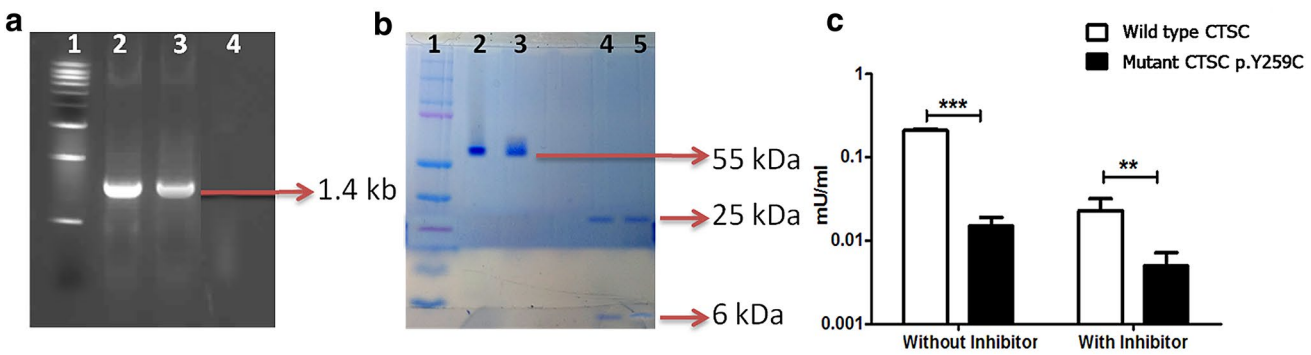


Fig. 3 Cloning, expression and enzyme activity of wild type and p.Y259C mutant CTSC protein. **a** *CTSC* amplified from cells transfected with wild type (lane 2), mutant plasmid (lane 3) and negative control vector (lane 4). **b** The image shows propeptide form of His-CTSC (lane 2) and His-CTSC Y259C (lane 3) purified from the cell lysate of wild type and mutant plasmid transfected HEK 293T cell lines. The active form of His-CTSC (lane 4) and His-CTSC Y259C (lane 5) after papain treatment is also shown. 5 µg of the protein was loaded onto 10% SDS PAGE and followed by Coomassie blue staining. Lane 1: Prestained protein ladder (Biorad, USA). **c** Mutant CTSC (p.Y259C) protein showed a statistically significant 14 fold reduction in the enzyme activity when compared to wild type CTSC protein. 1 mM iodoacetamide inhibited the CTSC enzyme activity proportionally in mutant CTSC (p.Y259C) and wild type CTSC by tenfolds

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had a monomeric structure (A chain). It is reported that tetramerization is crucial for the enzymatic activity [14]. Therefore, the structure modelled by SWISS MODEL substantiates the fact that this missense mutation has resulted in the development of PLS phenotype by abolishing the formation of tetrameric structure. Similarly, structural effects leading to functional abnormalities were also reported as a result of P35delL and R272P mutations in CTSC gene [15]. The inactive form of CTSC protein was of 55 kDa which after maturation yielded bands of molecular weights of 25 kDa and 6 kDa observed in earlier studies [16].

This missense mutation identified in our study, p.Y259C, was found as a novel pathogenic mutation associated with PLS syndrome In addition, there was a statistically significant 14 fold reduction in CTSC enzyme activity in the mutant protein when compared to the wild type protein. The presence of cysteine protease inhibitor, iodoacetamide further reduced the enzyme activity in both the wild type and the mutant proteins by 10 folds as observed in other studies [17]. Similarly, 85% reduction in the enzyme activity was observed in Mexican patients with PLS in a study [18]. Recently, many reports on requirement of early identification of PLS and proper dental management was emphasized [19–22]. Therefore, our study has helped to broaden the list of reported mutations associated with PLS.

Author contributions SM and TS: Conceptualization, design of study and interpretation; SM: Laboratory experimentation and Writing—original draft preparation; BP and RR: Clinical data collection interpretations; SR, TS and RS: Writing—review and editing; SR and RS: Funding acquisition and supervision; All the authors reviewed and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors did not declare any conflict of interest.

Ethical approval This study was approved by the Institutional Human Ethics Committee (IHEC), PSG Instituteof Medical Sciences and Research, Coimbatore, India and the study procedures were in accordance with the1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The patient and his parents gave written informed consent for geneticanalysis and for a future possible publication.

References

1. Papillon MN, Lefevre B (1924) Two cases of familial symmetric palmoplanter keratosis (Maleda's disease) in a brother and his sister. Alterations in both cases (in French). Bull Soc Francaise Dermatologie Syphiligraphie 31:81–84
2. Fageeh HN (2018) Papillon-Lefèvre syndrome: a rare case report of two brothers and review of the literature. Int J Clin Pediatr Dent 1:352–355. https://doi.org/10.5005/jp-journals-10005-1523
3. Korkmaz B, Caughey GH, Chapple I et al (2018) Therapeutic targeting of cathepsin C: from pathophysiology to treatment. Pharmacol Ther 190:202–236. https://doi.org/10.1016/j.pharmthera.2018.05.011
4. Hart TC, Bowden DW, Ghaffar KA, Wang W, Cutler CW, Cebeci I et al (1998) Sublocalization of the Papillon-Lefevre syndrome on chromosome 11q14-q21. Am J Med Genet 79(19980901):134–139
5. Lingeswaran A, Gopal SD (2018) A rare presentation of psychotic depression with suicidality in a case of papillon–lefèvre syndrome. Indian J Psychol Med 40:372–374
6. Toomes C, James J, Wood AJ, McCormick D, Lench N, Hewitt CH et al (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. Nat Genet 23:421–424. https://doi.org/10.1038/70525
7. Chedid JA, Salameh M, El-Outa A, Noujeim ZEF (2019) Papillon-Lefèvre Syndrome: diagnosis, dental management, and a case report. Case Rep Dent. https://doi.org/10.1155/2019/4210347
8. Bullón P, Castejón-Vega B, Román-Malo L et al (2018) Autophagic dysfunction in patients with Papillon-Lefèvre syndrome is restored by recombinant cathepsin C treatment. J Allergy Clin Immunol 142:1131–1143
9. Hattab FN (2019) Papillon-Lefèvre syndrome: from then until now. Stomatol Dis Sci 3:1. https://doi.org/10.20517/2573-0002.2018.22
10. Allende LM, García-Pérez MA, Moreno A, Corell A, Carasol M, Martínez-Canut P et al (2001) Cathepsin C gene: first compound heterozygous patient with Papillon-Lefèvre syndrome and a novel symptomless mutation. Hum Mutat 17:152–153
11. Selvaraju V, Markandaya M, Prasad PV, Sathyan P, Sethuraman G, Srivastava SC (2003) Mutation analysis of the cathepsin C gene in Indian families with Papillon-Lefèvre syndrome. BMC Med Genet 4:5. https://doi.org/10.1186/1471-2350-4-5
12. Rai R, Thiagarajan S, Mohandas S, Natarajan K, Shanmugasekar C, Ramalingam S (2010) Haim Munk syndrome and Papillon Lefevre syndrome–allelic mutations in cathepsin C with variation in phenotype. Int J Dermatol 49:541–543. https://doi.org/10.1111/j.1365-4632.2010.04300.x
13. Nakano A, Nomura K, Nakano H, Ono Y, LaForgia S, Pulkkinen L, Hashimoto I, Uitto J (2001) Papillon-Lefèvre syndrome: mutations and polymorphisms in the cathepsin C gene. J Invest Dermatol 116:339–343. https://doi.org/10.1186/1471-2350-4-5
14. Nagy N, Vályi P, Csoma Z, Sulák A, Tripolszki K, Farkas K et al (2014) CTSC and Papillon-Lefèvre syndrome: detection of recurrent mutations in Hungarian patients, a review of published variants and database update. Mol Genet Genomic Med 2:217–228. https://doi.org/10.1002/mgg3.61
15. Moghaddasian M, Arab H, Dadkhah E, Boostani H, Babak AR, Abbaszadegan MR (2014) Protein modeling of cathepsin C mutations found in Papillon-Lefèvre syndrome. Gene 538:182–187. https://doi.org/10.1016/j.gene.2013.11.079
16. Yang W, Xia W, Mao J, Xu D, Chen J, Feng S et al (2011) High level expression, purification and activation of human dipeptidyl peptidase I from mammalian cells. Protein Expr Purif 76:59–64. https://doi.org/10.1016/j.pep.2010.09.001
17. Dağlioğlu C (2017) Cloning, expression, and activity analysis of human cathepsin C in the yeast Pichia pastoris. Turk J Biol 41:746–753. https://doi.org/10.3906/biy-1704-4
18. Romero-Quintana JG, Frías-Castro LO, Arámbula-Meraz E, Aguilar-Medina M, Dueñas-Arias JE, Melchor-Soto JD et al (2013) Identification of novel mutation in cathepsin C gene causing

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Papillon-Lefèvre Syndrome in Mexican patients. BMC Med Genet 14:7. https://doi.org/10.1186/1471-2350-14-7
19. Yousry YM, El-Latif AEA, El-Gawad RYA (2018) Case report: clinical manifestation and dental management of Papillon-Lefèvre syndrome. F1000Res 7:1420. https://doi.org/10.12688/f1000research.16042.1
20. Tyagi S, Thomas AM, Balla V, Kundra R (2019) Cu-sil dentures: a novel approach of Papillon-Lefèvre syndrome management. J Indian Soc Pedod Prev Dent 37:303–307. https://doi.org/10.4103/JISPPD.JISPPD_256_18
21. Millet C, Virard F, Lienhart G, Ducret M (2020) Digital prosthodontic management of a young patient with Papillon-Lefèvre syndrome: a clinical report. J Prosthet Dent 123:548–552. https://doi.org/10.1016/j.prosdent.2019.03.013
22. Giannetti L, Apponi R, Dello Diago AM, Jafferany M, Goldust M, Sadoughifar R (2020) Papillon-Lefèvre syndrome: oral aspects and treatment. Dermatol Ther. https://doi.org/10.1111/dth.13336

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Pirfenidone-Induced Dose-Dependent Phototoxicity - A Distinct Drug Reaction

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Pirfenidone-Induced Dose-Dependent Phototoxicity – A Distinct Drug Reaction

P Arunprasath, Reena Rai, Chaitra Venkataswamy[1]

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Sir,

Pirfenidone is a novel drug with anti-fibrotic and anti-inflammatory properties used in the treatment of idiopathic lung fibrosis.[1] Herein, we report a case of pirfenidone-induced phototoxicity.

A 70-year-old male presented with hyperpigmented itchy skin lesions involving the face, forearms, and thighs for 10 days. His skin lesions were associated with photosensitivity, swelling of face, redness, and watering of eyes. History revealed that he was a recently diagnosed case of idiopathic pulmonary fibrosis and was started on treatment with pirfenidone 200mg two tablets thrice daily. Two months later the dose of pirfenidone was stepped-up to 200 mg three tablets thrice daily. One month after dose increment, the patient started developing itching and redness involving the photoexposed areas.

Cutaneous examination revealed hyperpigmentation with erythematous borders involving the face, "v"area of neck, forearms, dorsum of hands, upper back, thighs, and legs. There was a clear cut demarcation between the photoexposed and nonphotoexposed areas [Figures 1 and 2]. Examination of palms, soles, oral cavity, and genitalia did not reveal any abnormality. Routine hematological and biochemical investigations were within normal limits. Antinuclear antibody by immunofluorescence method was negative. Histopathological examination revealed epidermis exhibiting hyperkeratosis, parakeratosis, and large subcorneal clefting with extensive epidermal necrosis. There was also subepidermal blister formation containing fibrin and mixed inflammatory infiltrate. Dermis showed lichenoidinfiltrate composed of lymphocytes, plasma cells, occasional eosinophils, and neutrophils [Figures 3 and 4]. Naranjo algorithm showed a score of 6 with probable association of drug with the reaction. A diagnosis of pirfenidone-induced phototoxic drug reaction was entertained based on the distinct photodistributed skin lesions and extensive epidermal necrosis with dermal inflammatory infiltrate in histopthology.

Photosensitivity following drug intake occurs in two different patterns: phototoxicity and photoallergy. Phototoxicity is a nonimmunologic reaction following absorption of ultraviolet radiation by drug metabolites. On the contrary, photoallergic reaction is a cell-mediated immune reaction occurring in a sensitized host. Clinically phototoxic reaction mimics sunburn with sharp delineation to the sun exposed areas, whereas photoallergic reaction mimics eczema and it involves nonphotoexposed skin as well. Other patterns described in drug-induced photosensitivity include lichenoid skin lesions, pseudoporphyria, pellagra reaction, and photoonycholysis.[2]

Pirfenidone is an anti-fibrotic agent approved for treatment of idiopathic lung fibrosis. It inhibits transforming growth factor (TGF) -β stimulated collagen production by reducing fibroblast proliferation and production of fibrogenic mediators. Anti-inflammatory action is attributed to its inhibition of inflammatory cytokines like tumor necrosis factor-α, interleukin-1, and production of anti-inflammatory cytokine interleukin-10.[1]

In a study by Taniguchi *et al.* photosensitivity was the major adverse event observed in 51% of the patients in the high-dose group and 53% in low-dose group.[3] Apart from phototoxicity; pirfenidone-induced photoallergy and photoleucomelanoderma have been reported.[1,4]

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Figure 1: Hyperpigmentation with erythematous borders involving the "v"area of neck, forearms

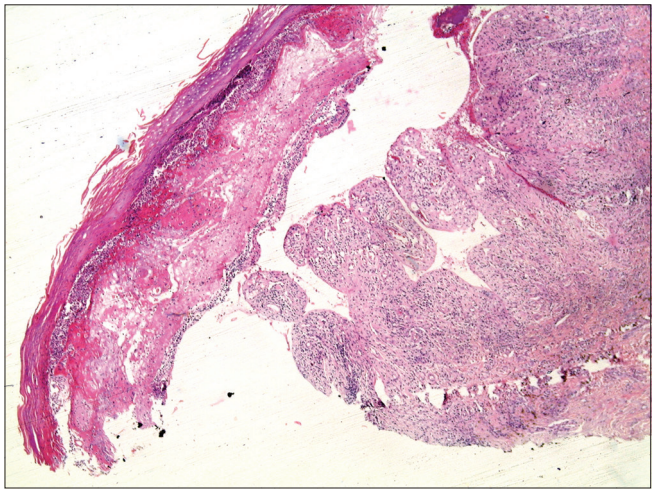


Figure 3: Epidermis with hyperkeratosis, parakeratosis, subcorneal clefting, extensive epidermal necrosis, and subepidermal blister formation with fibrin and mixed inflammatory infiltrate. Dermis showing inflammatory infiltrate composed of lymphocytes, plasma cells, occasional eosinophils, and neutrophils [H&E 100×]

Seto *et al.* in their study assessed the photosafety of pirfenidone and concluded that there was production of singlet oxygen and superoxide radicals from drug metabolites of pirfenidone on exposure to sunlight pointing to its phototoxic potential. The study also showed that phototoxicity testing using high doses of pirfenidone in rats caused photosensitivity compared to low doses, pointing to the factthat phototoxic reaction parallels concentration of drug levels in skin.[5] Likewise in the present case skin lesions appeared after increment of the dose.

Diagnosis in drug induced photosensitivity can be established with histopathological examination, phototesting in case of phototoxic reaction, and photopatch testing in photoallergic reaction. Guillén *et al.* reported a similar case, where they conducted phototesting which showed a reduced minimal erythema dose and an aberrant response to ultraviolet A starting from 2 J/cm^2.[6] In our case, histopathology revealed a picture consistent with phototoxic reaction, however phototesting was not done.



Figure 2: Hyperpigmentation with erythematous borders involving thighs and legs

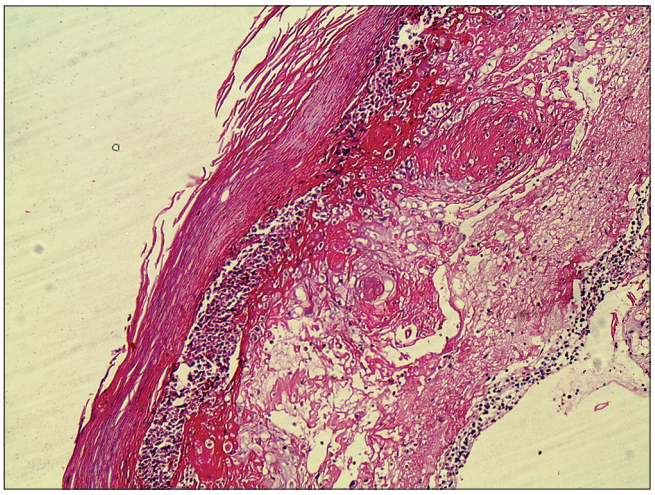


Figure 4: Closer view showing keratinocyte necrosis and inflammatory cells in epidermis [H&E 400×]

Treatment options include photoprotection, broad spectrum sunscreens, short course of steroids, and dose reduction

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or stopping of drug. With resolution of skin lesions reintroduction of the drug can be attempted with low doses. However in photoallergic reactions drug reintroduction should be avoided.[7,8] The present case was treated with short course of steroids, sunscreens, and stopping of the drug with improvement of skin lesions. The present case is highlighted for the distinct side effect profile of a new therapeutic agent.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Kim HJ, Seo JW, Kim TH, Choi SH, Song KH, Kim KH. Pirfenidone-induced photosensitivity in a patient with idiopathic pulmonary fibrosis. Ann Dermatol 2018;30;614-6.
2. Pandhi D. Phototoxic and photoallergic drug reactions. In: Gupta LK, Martin AM, editors. IADVL's textbook on cutaneous adverse drug reactions-A comprehensive guide. 1st edition. India: Bhalani Publishing House; 2018.p. 140-8.
3. Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M, *et al.* Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J 2010;35:821-9.
4. Tsuruta A, Washio K, Fukunaga A, Nishigori C. Pirfenidone induced photoleukomelanoderma in a patient with idiopathic pulmonary fibrosis. J Dermatol 2016;43:207-9.
5. Seto Y, Inoue R, Kato M, Yamada S, Onoue S. Photosafety assessments on pirfenidone: Photochemical, photobiological, and pharmacokinetic characterization. J Photochem Photobiol B 2013;120:44-51.
6. Guillén BF, Giácaman MM, Oñate CV, Tapial JM, Bel PH, Ferriols AP. Pirfenidone-induced photosensitivity confirmed by pathological phototest. Photodiagnosis Photodyn Ther 2019;25:103-5.
7. Costabel U, Bendstrup E, Cottin V, Dewint P, Egan JJ, Ferguson J, *et al.* Pirfenidone in idiopathic pulmonary fibrosis: Expert panel discussion on the management of drug-related adverse events. Adv Ther 2014;31:375-91.
8. Gaikwad RP, Mukherjee SS. Pirfenidone induced phototoxic reaction in an elderly man. Indian J Dermatol Venereol Leprol 2016;82:101-3.

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CASE REPORT

Thrombocytopenia induced by intermittent therapy with Rifampicin – A case report

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Accepted for publication 2 May 2018

Introduction

Rifampicin is one of the most widely used and effective antituberculosis/antileprosy drugs. Adverse effects are uncommon, except for occasional hepatotoxicity, skin-rash, gastrointestinal upsets, and flu-like syndrome.[1] It is rarely allergic, and other autoimmune manifestations including thrombocytopenia are seen especially with intermittent treatment.[2] Here we report a case of thrombocytopenia caused by intermittent administration of rifampicin in view of its rare occurrence.

Case Report

A 50 year old male diagnosed with Hansen's disease was administered with multibacillary multidrug therapy (MB-MDT) in August 2017. After a month when he took the second dose of monthly MB-MDT he developed a few purpuric lesions over the inner aspect of his lower lip, left arm and right leg associated with fever, myalgia and cough with hemoptysis.

On Investigation it was discovered that he had thrombocytopenia (8000), other essential baseline investigations were normal including Prothrombin time and INR.

With the differential diagnosis of viral fever induced thrombocytopenia or rifampicin induced thrombocytopenia, the patient was admitted and evaluated. Dengue (rapid card test) and antiplatelet antibody test for rifampicin (Ranbaxy lab) was negative. Six units of platelets were transfused. Since he had hemoptysis sputum culture & sensitivity, chest X ray and HRCT lung was normal. Post transfusion his platelets improved to 1,01,000 hence he was discharged and advised to continue MB MDT after 1 week.

A month later he presented to the emergency room, with severe body pain, chills, sweating, nausea, and one episode of vomiting after taking rifampicin.

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On examination he had hypotension, tachycardia, ECG was in sinus rhythm. On investigation he again had severe thrombocytopenia (4000) and mild hyperbilirubinemia without any purpuric lesions. He was admitted in intermediate care and was started on platelet transfusion (4 units). On day 2 his platelet count further dropped to 3000. He developed numerous purpuric lesions over hand and legs, along with hemoptysis. He was treated with short course of steroids. After 17 units of platelets transfusion platelets improved to 84,000. The patient improved symptomatically. Hence he was diagnosed as Rifampicin induced thrombocytopenia. Clarithromycin was replaced in the place of rifampicin in MB MDT and the patient was discharged. On follow-up his platelet count was normal and he had no bleeding manifestations.

Discussion

Rifampicin is a semisynthetic derivative of rifamycin B which is an antibiotic produced by Streptomyces meditteranei. It is a potent bactericidal drug which has broad antibacterial spectrum including mycobacterium species. It has a rapid action on *Mycobacterium leprae* by inhibiting DNA dependent RNA polymerase.

Rifampicin is commonly known to cause discoloration of body fluids like tears and urine to an orange or red colour. The most serious complications are hepatotoxicity, cutaneous syndrome, abdominal syndrome, respiratory syndrome, and flu like symptoms. Thrombocytopenia is rarely seen.

Rifampicin induced thrombocytopenia is an uncommon but life threatening complication. Only one case has been reported with anti-leprosy treatment.[3] Rifampicin binds non-covalently to specific membrane glycoprotein IX present on the platelets and induces conformational changes thereby activating the complement pathway which leads to destruction of the platelets.

Rifampicin-induced thrombocytopenia is more common in intermittent therapy than in continuous therapy because in continuous therapy there is neutralisation of antibodies, and antigen – antibody complex is being continuously removed without causing an allergic reaction, whereas intermittent therapy allows a sufficient quantity of antibody to be built up during the drug free interval.

Drug induced throbocytopenia is assessed by using standard criteria to define three levels of severity of bleeding.[4]

1. Major bleeding – defined as intracranial or retroperitoneal bleed or overt bleed with decrease in haemoglobin by more than 2 gm% or require two or more units of blood transfusion.
2. Minor bleeding – Overt bleeding that does not meet the criteria of major bleeding (Malena, gross hemoturia epistaxis, gingival bleeding that is prolonged for 30 mins or requires medical intervention) excessive menstrual bleeding or vaginal bleeding other than menses.
3. Trivial bleeding – Petechiae, purpurae, brief epistaxis or gingival bleed, guaic test positive stool or microscopic hematuria.

The direct assay for measurement of platelet bound antibodies can be performed but its sensitivity is 49–66% and specificity is 78–92%. In our case this test was negative so we

restarted Rifampicin again and the patient had a severe episode of thrombocytopenia. Hence confirming our diagnosis of rifampicin induced thrombocytopenia. So the negative test cannot be used to rule out the diagnosis.

In these patients Rifampicin should be stopped. Platelet counts should be monitored regularly, and most of the patients recover with platelet transfusion. Occasionally patients with severe thrombocytopenia and hemorrhagic manifestations may require temporary support with corticosteroids.[5] Plasmapheresis can be helpful in some cases. The offending drug should not be reused as only minute quantities of the drug are needed to set up a subsequent immune reaction. Even death has been reported.

References

[1] Girling DJ. Adverse effect of anti mycobacterial drugs. *Drugs*, 1982; **23**: 56–74. [PubMed: 6459920].
[2] Blajchman MA, Lowry RC, Pettit JE, Stradling P. Rifampicin – induced immune thrombocytopenia. *Br Med J*, 1970; **3**: 24–26. [PMCID: PMC1700974][PubMed: 5427483].
[3] Das S, Roy AK, Maiti A. Rifampicin induced thrombocytopenia. *Indian J Dermatol*, 2006; **51**: 222.
[4] Garg R, Gupta N, Mehra S *et al.* Rifampicin induced thrombocytopenia. *Indian J TB*, 2007; **54**: 94.
[5] Dixit R, George J, Sharma AK. Thrombocytopenia due to rifampicin. *Lung India: official organ of Indian Chest Society*, 2012; **29**: 90.

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Original Article

Analysis of Hypersensitivity in Fragrance Series by Patch Testing

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Abstract

Introduction: Allergic contact dermatitis (ACD) is an inflammatory disorder, which occurs as a result of repeated contact with an allergen. Fragrances are the products obtained naturally or produced synthetically. The purpose of this study is to detect the fragrance allergens producing ACD. **Materials and Methods:** This is an open-label prospective observational study. Patients of age above 18 years with history of using cosmetic products with dermatitis lesions over face, neck, axilla and hands were included. Patch testing was done with fragrance series by using the standard technique. The results were interpreted on day 2 and day 4 as recommended by International Contact Dermatitis Research Group criteria. **Results:** Totally 27 patients were included in this study. Of them, 12 were males and 15 were females; the mean age was 43 years. The mean duration of symptoms was 12.5 months. The most common site of involvement was hands. Housewives and office workers were the most commonly affected occupational groups. The most commonly used category of cosmetic product was talcum powders by 13 (48.1%) patients. In this study, 85.18% patients showed at least one fragrance antigen positivity. Fragrance mix II is the most frequent allergen in this study. **Discussion:** We conclude that the fragrance mix II is an important marker to find out fragrance allergy. Hand dermatitis is the most common presentation in patients with fragrance allergy. Perfumed talcum powders, soaps and perfumes are the leading sources of sensitization to fragrance allergens.

Keywords: *Allergic contact dermatitis, cosmetics, fragrances, hand dermatitis, patch testing*

Introduction

Allergic contact dermatitis (ACD) is an inflammatory disorder, which occurs as a result of repeated contact with an allergen, leading to the rapid activation of T cells and further release of cytokines.[1] In the sixth century, Aetius Amidenus, a physician to the Byzantine court first used the word "eczema". Eczema may present clinically as scaling, clustered papulovesicles, associated with erythema, pruritus and fissuring. It is caused by a different variety of external and internal factors.[2] Patch testing is considered as the gold standard and only reliable method to identify the contact allergens.[3]

Cosmetics is defined as the substances which are intended to be poured, rubbed, sprinkled or sprayed on skin, applied to a normal or previously sensitized skin for cleansing, promoting attractiveness, beautifying, or to alter the appearance of the human skin or body.[4] Cosmetics and toiletries are being used by most of the people for hygiene and personal care of the body to enhance the attractiveness of consumers, get pleasant smell, protection and masking the defects present in the skin.[5] Cosmetics are complex mixtures made up of preservatives, perfumes, emulsifiers, stabilizing agents, various types of lipids, alcohols and so on.[6]

Fragrances are the products obtained naturally or produced synthetically. Natural fragrances, such as balsams, concretes, essential oils and absolutes are available, with a few animal products such as musk, civet and ambergris, which also can be manufactured synthetically.[7] Masking fragrances are used mainly in topical medicaments and cosmetic products by labeling them as "fragrance free". But they contain original fragrances.[8] The purpose of this study is to detect all the fragrance allergens which cause ACD.

Materials and Methods

This is an open-label prospective observational study conducted from March 2017 to July 2018 in a tertiary care hospital. All patients of age above 18 years who

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attended our dermatology outpatient department with the history of using various topical cosmetic products such as perfumes, detergent soap, after shave lotion, moisturizers, facial makeup creams, with dermatitis lesions over face, neck, axilla and hands were included in the study. Age below 18 years, who have not used any cosmetic products, who are on systemic corticosteroids, immunosuppressants, pregnant, lactating females, were excluded from the study.

The study was reviewed and approved by an institutional ethical committee, and all patients gave their voluntary informed consent to participate in this study. Detailed history regarding duration, occupation, systemic illness was obtained and recorded [Table 1]. Patch testing with fragrance series [Figure 1] was done by using the standard technique, that is, 0.1 ml of each antigen was placed in an aluminum Finn chamber, mounted on an adhesive tape [Figure 2], which was applied over the back of all the patients [Figure 3]. A total of 42 patch test antigens [Table 2] are present in the fragrance series obtained from chemotechnique diagnostics, AB Sweden. The results were interpreted on days 2 and 4 as recommended by International Contact Dermatitis Research Group (ICDRG) criteria [Table 3].

Results

A total of 27 patients were included in this study and patch testing was done with fragrance series. Of them, 12 (44.4%) were males and 15 (55.5%) were females; the mean age was 43 years (range 18-68 years). The mean duration of symptoms was 12.5 months (range 1-24 months). The most common site of involvement was hands [Figure 4], which



Figure 1: Fragrance series antigens (Chemotechnique diagnostics, AB Sweden)

Table 1: Demographic data of all 27 patients

Age/sex	Occupation	Areas involved	Duration of symptoms	Cosmetic products used	Total number of antigens positive
54/F	Housewife	Face, neck	1 month	Giordani gold cream	2
64/M	Navy man	Face, neck,	2 months	Yardley lavender powder	9
36/M	Office worker	Axilla, hands	8 months	Dermicool powder	1
37/M	Painter	Neck	6 months	Power detergent soap	4
34/M	Painter	Hand, face	3 months	Dermicool powder, perfume	0
21/F	Office worker	Face, hand	1 month	Ponds powder, enchanter spray	3
18/M	Textile labour	Face, hands	1 year	Fair & Lovely cream, Savlon soap, aftershave lotion	2
20/F	Office worker	Hands	4 months	Venusia max cream, Rin detergent soap	2
46/F	Housewife	Hands, neck	6 months	Fair & Lovely cream, Gokul sandal powder, Mysore sandal soap	2
55/F	Office worker	Hands	6 months	Anti-hair fall shampoo, Vim bar soap	0
50/M	Business	Neck	3 months	Jasmine perfumes, Fogg body spray	1
18/F	Office worker	Hands, neck	1.5 years	Saffron face wash, Pond's powder, Mysore sandal soap	0
54/F	Agriculture	Face	8 months	Pond's powder, Hamam soap	4
40/F	Housewife	Neck	1 month	Surf Excel detergent, Mysore sandal soap	6
60/M	Agriculture	Face, neck	2 years	Gokul sandal powder, perfumes	1
31/F	Housewife	Face, hands	6 months	Gokul sandal powder, perfumes	3
36/F	Housewife	Face, hands	6 months	Mysore sandal soap, perfumes	4
54/F	Painter	Hands	1 month	Moisturex cream, herbal soap	5
30/F	Office worker	Hands	8 months	Moisturizing cream, perfumes, sunscreen lotion	4
65/M	Agriculture	Axilla, hands	2 months	Gokul sandal powder, Fair & Lovely cream	4
26/M	Business	Face, hands	2 years	Keraglo ad shampoo, Fair And Handsome cream	0
60/F	Cement worker	Hands, neck	3 months	Gokul sandal powder, Lifebouy soap, Arasan soap	3
55/F	Agriculture	Hands, neck	1 month	Hamam soap, Arasan soap	4
59/M	Cement worker	Hands, axilla	1 month	Lifebouy soap, body spray, moisturizers	2
40/M	Agriculture	Hands, neck, axilla	1.5 years	Perfumes, body spray, Pond's powder	3
55/M	Machine operator	Hands, axilla	2 years	Mysore sandal soap, sunscreen lotion	3
68/F	Housewife	Face, neck	6 months	Moisturizers, sunscreen lotion, Mysore sandal soap	3

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Figure 2: Patch testing: Antigens are placed in aluminum Finn chambers mounted over an adhesive tape



Figure 4: Hyperpigmented scaly lesions over the hands



Figure 3: Patch test antigens applied over the back of patients



Figure 5: Erythema and scaling seen over the neck

was observed in 19 (70.3%) patients [Table 4], followed by neck [Figure 5] in 12 (44.4%) patients.

The most commonly seen occupational groups with dermatitis features were housewives 6 (22.2%) and office workers 6 (22.2%) patients [Table 5]. The most commonly used category of cosmetic products by these patients were talcum powders by 13 (48.1%) patients [Table 6] followed by scented soaps in 12 (44.4%) patients.

Out of the 27 patients, 8 (29.6%) were atopic individuals and 19 (70.3%) were non-atopic individuals. Of the 27 patients, 23 (85.18%) patients showed at least one antigen positivity, and 4 (14.8%) patients were negative to all antigens. The most frequent allergen to become positive in this study is fragrance mix II [Figure 6] in eight (29.6%) patients [Table 7], followed by cinnamic aldehyde and cinnamic alcohol in seven (25.9%) patients each [Figure 7].

Discussion

ACD is one of the commonest examples of type IV hypersensitivity reaction which usually affects the previously sensitized persons. The contact allergens are very small molecules which are able to penetrate deeper layers of the skin and produce sensitization.[2] Fragrances are the most common cause of allergic reactions to

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Table 2: Fragrance series antigens (Chemotechnique Diagnostics, AB Sweden)

Antigen	Concentration (%)
Control	
Cinnamic aldehyde	1
Cinnamic alcohol	2
Amyl cinnamaldehyde	2
Eugenol	2
Isoeugenol	2
Geraniol	2
Oakmoss absolute	2
Hydroxycitronellal	2
Narcissus Absolute	2
Musk xylene	1
Methyl anthranilate	5
Musk moskene	1
Musk ketone	1
Jasmine synthetic	2
Benzyl salicylate	1
Benzyl alcohol	10
Vanillin	10
Lavender absolute	2
Cananga oil	2
Rose oil. Bulgarian	2
Ylang-Ylang oil	2
Geranium oil Bourbon	2
Jasmine absolute, Egyptian	2
Sandalwood oil	2
Lyral	5
Citral	2
Farnesol	5
Citronellol	1
Hexyl Cinnamic aldehyde	10
Coumarin	5
Fragrance mix II	14
Amyl cinnamic alcohol	5
Anise alcohol	10
Benyl benzoate	10
Benzyl cinnamate	10
Butyl phenyl methyl propional	10
Evernia furfuracea	1
Alpha-iso methyl ionone	10
d-Limonene	10
Linalool	10
Methyl-2-octynoate	0.2

cosmetics. Fragrances can enter into the body through nose into lungs, upper airways, ingestion, skin, and it can cause irritation of eyes, throat and nose, headaches, dizziness, forgetfulness and easy fatigability.[9]

In our study, females outnumbered males in fragrance hypersensitivity reactions by nearly 10%. The fact that there is a high prevalence of fragrance allergy in women than men could be due to the frequent use of skin care, personal hygiene and face care products by the female population.

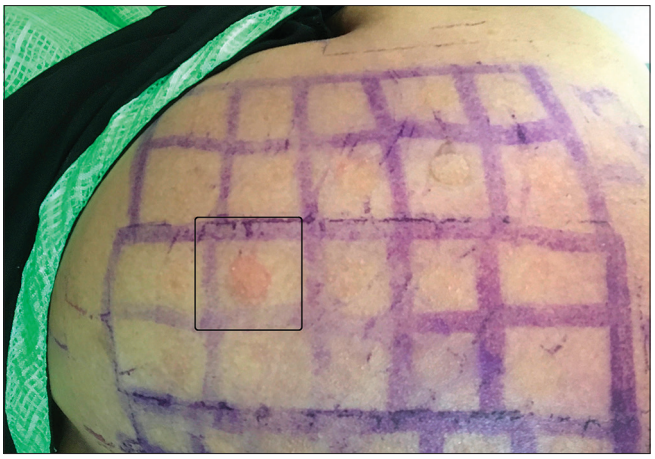


Figure 6: Patch test: Same patient shown in Figure 4, showed positive reaction to fragrance mix II

In this study, housewives and office workers are the occupational groups who developed fragrance allergy most frequently, which is seen in six (22.2%) patients in each occupational group. DeGroot *et al*.[7] found that fragrance allergy from perfumes, deodorants and aftershave lotions is commonly encountered in Swedish college students.

In this study, most commonly 19 (70.3%) patients had lesions over the hands, and next frequent site was over the neck in 12 (44.4%) patients. Our findings correlate well with the studies conducted by Santucci and Malten;[7] they found that hand dermatitis was the most common presentation with 41% and 52% positivity, respectively, because of frequent contact of the fragrance products such as soaps, fairness creams, shampoos and topical medications with hands before application at various sites of the body.[7]

In this study, the most common category of cosmetic products used were talcum powders by 13 (48.1%) patients and scented soaps by 12 (44.4%) patients. An observational study found that the skin care products such as lotions, creams were the cosmetic categories which were blamed for the positive reactions in patch testing in 37% of patients; 30% of positive cases had used personal care products and 13% used deodorants and antiperspirants.[5] According to Cornelis *et al*., soaps were used by 87% of the people, 82% people used toothpaste, shampoos were used by 80%, deodorants and antiperspirants by 61%, talcum powder and body spray were used by nearly 45% of the study population.[5]

In this study, we found that the most frequent allergen to show positivity was fragrance mix II in eight (29.6%) patients. The second most common allergens were cinnamic aldehyde and cinnamic alcohol in seven (25.9%) patients, which is followed by Geranium oil Bourbon and Lavender absolute in five (18.5%) patients. Our observations are similar to a study conducted by Johansen *et al*.: who stated that the most common allergens are fragrance mix II and

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Table 3: Interpretation of Patch Test Results (ICDRG Criteria)

Notation	Description
Negative	No changes in the tested skin area
?+	Non-palpable faint erythema
1+	Palpable erythema with moderate edema or infiltrate, no papules or vesicles
2+	Strong infiltrate and erythema, numerous papules or vesicles
3+	Many vesicles coalesced to form bulla or ulceration
NT	Not tested
IR	Inflammation is sharply limited to an exposed area, lack of infiltrate, small petechiae, pustules with papules and vesicles

ICDRG=International Contact Dermatitis Research Group

Table 4: Location of dermatitis lesions

Location	Number of patients	Percentage
Neck	12	44.4
Face	11	40.7
Hands	19	70.3
Axilla	5	18.5

Table 5: Various occupational groups presented with dermatitis

Occupation	Number of patients	Percentage
Housewife	6	22.2
Navy man	1	3.7
Office worker	6	22.2
Painter	3	11.1
Business	2	7.4
Textile laborer	1	3.7
Agriculture	5	18.5
Cement worker	2	7.4
Machine operator	1	3.7

Table 6: Categories of the cosmetic products used by the patients

Category	Number of patients	Percentage
Perfumes	9	33.3
Soaps	12	44.4
Detergents	5	18.5
Moisturizers	5	18.5
Talcum powders	13	48.1
Fairness creams	5	18.5
Sunscreens	3	11.1
Shampoo	2	7.4
Aftershave lotion	1	3.7

balsam of peru.[10] Santucci *et al*. identified 54 out of 92 patients were sensitive to fragrance mix II.[7]

Various studies have been conducted in various countries to know the incidence of fragrance allergy, but only a few studies are available for our Indian skin type. This

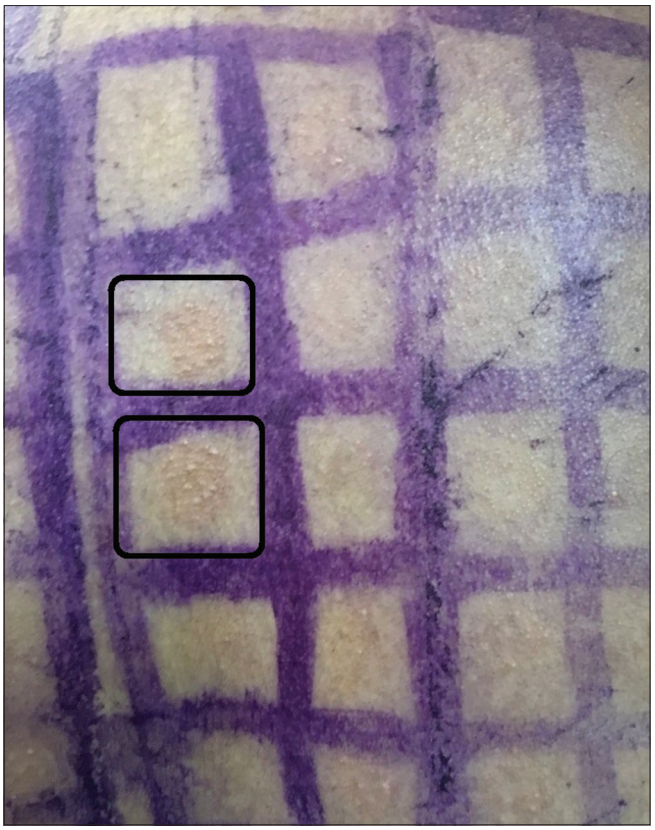


Figure 7: Patch test: Same patient shown in Figure 5, showed positive reaction to cinnamic alcohol and cinnamic aldehyde

is because the fragrance allergy is usually not reported to the dermatologists by the patients. In USA, the most frequent sensitizer was fragrance mix II. In Netherlands, isoeugenol is followed by oak moss absolute. In a European study, they found that oak moss absolute is the most frequently positive fragrance allergen followed by isoeugenol.[10]

In this study, a total of 27 patients were included with dermatitis. Of them, 8 (29.6%) patients were known atopic and 19 (70.3%) patients were non-atopic individuals. But Katsarma *et al*.[10] stated that more than 50% of ACD patients were with atopic diathesis in their study. Caress and Maria *et al*.[11] also concluded that the fragrance-induced ACD is most commonly seen in atopic eczema cases.

In this study, we did not experience any adverse events or intolerability to patch testing. All the 27 patients came for regular follow-up and also for the readings taken on day 2 and day 4. Once the patient is diagnosed as a case of ACD by patch testing, pamphlets were issued regarding the awareness of how to avoid contact with the particular allergens. Cosmetics are not very safe as claimed by the manufacturing companies, and it may contain many fragrance allergens. ACD induced by cosmetics was more prevalent in urban people than rural because of the health awareness and attitude towards cosmetics in urban people.[12]

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Table 7: Frequency of fragrance allergen positivity

Antigens	Number of patients showed positivity	Percentage
Cinnamic aldehyde	7	25.9
Cinnamic alcohol	7	25.9
Isoeugenol	4	14.8
Oakmoss absolute	4	14.8
Lavender absolute	5	18.5
Geranium oil Bourbon	5	18.5
Fragrance mix II	8	29.6
Benzyl cinnamate	4	14.8

Conclusion

Patch testing is a simple method to diagnose the causative agent of ACD. It shortens the time lapse from the first visit to final diagnosis and increases the period of remission, which in turn reduces the cost of treatment. We conclude that fragrance mix II is an important marker to find out fragrance allergy. Hand dermatitis is the most common presentation in patients with fragrance allergy. Perfumed talcum powders, soaps and perfumes are the leading source of sensitization to fragrance allergens producing fragrance allergy.

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Conflicts of interest

There are no conflicts of interest.

References

1. El-Nour H, Lundeberg L, Abdel-Magid N, Lonne-Rahm SB, C Azmitia E, Nordlind K. Serotonergic mechanisms in human allergic contact dermatitis. Acta Derm Venereol 2007;87:390-6.
2. Agarwal US, Besarwal RK, Gupta R, Agarwal P, Napalia S. Hand eczema. Indian J Dermatol 2014;59:213-24.
3. Goossens A. Art and science of patch testing. Indian J Dermatol Venereol Leprol 2007;73:289-91.
4. Dogra A, Dua A. Cosmetic dermatitis. Indian J Dermatol 2005;50:191-195.
5. de Groot AC. Adverse Reactions to Cosmetics. Thesis, State University of Groningen, 1988.
6. Dogra A, Minocha YC, Sood VK, Dewan SP. Contact dermatitis due to cosmetics and their ingredients. Indian J Dermatol Venereol Leprol 1994;60:72-5.
7. de Groot AC, Frosch PJ. Adverse reactions to fragrances: A clinical review. Contact Dermatitis 1997;36:57-86.
8. Arribas MP, Soro P, Silvestre JF. Allergic contact dermatitis to fragrances. Part 1. Actas Dermosifiliogr 2012;103:874-9.
9. Shehade SA, Beck MH, Hillier VF. Epidemiological survey of standard series patch test results and observations on day 2 and day 4 readings. Contact Dermatitis 1991;24:119-22.
10. Katsarma G, Gawkrodger DJ. Suspected fragrance allergy requires extended patch testing to individual fragrance allergens. Contact Dermatitis 1999;41:193-7.
11. Heisterberg MV, Andersen KE, Avnstorp C, Kristensen B, Kristensen O, Kaaber K, *et al*. Fragrance mix II in the baseline series contributes significantly to detection of fragrance allergy. Contact Dermatitis 2010;63:270-6.
12. Vij A, Murthy PM, Ravindra K. Study of contact dermatitis due to topical medicaments and cosmetics by patch testing. Indian J Dermatol 2007;52:194-7.

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Original Article

Comparative Analysis of BIOCHIP Mosaic-Based Indirect Immunofluorescence with Direct Immunofluorescence in Diagnosis of Autoimmune Bullous Diseases: A Cross-Sectional Study

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**Both authors have contributed equally in this study.*

Abstract

Background: Autoimmune bullous diseases (AIBD) are a heterogeneous group of diseases characterized by autoantibodies against desmosomal proteins in the pemphigus group of disorders and adhesion molecules of the dermal-epidermal junction in pemphigoid group of diseases. Direct immunofluorescence (DIF) establishes the diagnosis of AIBD by demonstrating intercellular deposits of IgG and C3 in case of pemphigus and linear deposits of IgG and C3 along the basement membrane zone (BMZ) in bullous pemphigoid (BP). BIOCHIP mosaic-based indirect immunofluorescence (IIF), a novel diagnostic approach employs detection of characteristic staining pattern and target antigens in a single miniature incubation field. **Aim:** To compare the BIOCHIP mosaic-based IIF with DIF in the diagnosis of AIBD. **Materials and Methods:** A total of 40 patients of AIBD in the active phase of the disease were included in the study. Skin biopsy was done in these patients for DIF study and serum was subjected to BIOCHIP mosaic-based IIF assay. The results were then compared. **Results:** DIF revealed a diagnosis of Pemphigus in 18 patients and BP in 22 patients. BIOCHIP showed a diagnosis of pemphigus in 18 patients, BP in 18 patients and floor pattern staining in four patients, which could be attributed to any of the floor pattern staining subepidermal blistering disease. **Limitations:** Small sample size, lack of control group and no comparison made with ELISA. **Conclusion:** This study concludes that the result of BIOCHIP showed a significant correlation with the DIF and can be used as a first line-screening tool in the diagnosis of AIBD.

Keywords: *Autoimmune bullous skin diseases, BIOCHIP mosaic, direct immunofluorescence, indirect immunofluorescence*

Introduction

Autoimmune bullous diseases (AIBD) include diverse group of skin diseases characterized by autoantibodies against desmosomal proteins in case of pemphigus group of diseases and components of basement membrane zone (BMZ) in pemphigoid diseases. Diagnosis of AIBD is based on the combination of characteristic clinical features, histopathological findings, direct immunofluorescence (DIF), indirect immunofluorescence (IIF) or enzyme-linked immunosorbent assay (ELISA) for target antigens. BIOCHIP mosaic, a novel diagnostic technique employs detection of target antigens and characteristic-staining pattern, in a single miniature incubation field.[1]

Materials and Methods

The present study was a cross-sectional study conducted during September 2018-July 2019 in the department of dermatology and pathology in a tertiary care hospital. Institutional ethics committee approval was obtained prior to the commencement of the study. A total of 40 patients with suspected AIBD were included in the study. AIBD patients under treatment and clinical remission were excluded from the study. All the participants were informed regarding the study and samples were collected after written consent. Skin biopsy was obtained from uninvolved perilesional skin for DIF and 5 ml of blood was obtained for BIOCHIP mosaic evaluation. Subsequently, the results of DIF and BIOCHIP mosaic were compared.

BIOCHIP mosaic

Procedure

The Dermatology mosaic 7 BIOCHIP (Euroimmun, Germany) was

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used in this study. The incubation field in BIOCHIP slide has mosaic of six different substrates: primate oesophagus, primate salt split skin, transfected cells with desmoglein 1 (Dsg1), desmoglein 3 (Dsg3), C-terminal globular domain of the bullous pemphigoid antigen 230 (BP230) and recombinant antigenic dots of tetrameric bullous pemphigoid antigen 180–non-collagenous 16 A domain (BP180-NC16A). For BIOCHIP mosaic, 5 ml of blood is taken by venipuncture in a plain test tube. The blood sample is then centrifuged at the rate of 3000 rotations per minute for 10 minutes for serum separation. 50 µL of serum is mixed with 450 µL of buffer [1:10 dilution]. 30 µL of the above mixture is incubated in substrate wells for 30 minutes and washed with a buffer for 5 minutes. After that, fluorescent conjugate 25 µL is added and incubated again for 30 minutes and washed with a buffer for 5 minutes. The slide is then mounted for interpretation under a fluorescent microscope. All the tests were run with the positive controls provided with the kit.

Interpretation

Primate oesophagus shows fine granular fluorescence of intercellular space (ICS) staining pattern in case of pemphigus and linear basement membrane zone (BMZ) staining pattern in pemphigoid diseases. In salt split skin substrate there will be a linear fluorescence pattern in the roof of the split in bullous pemphigoid and floor of the split in epidermolysis bullosa acquisita and rare variants like anti-laminin 332 pemphigoid and anti-P200 pemphigoid. Dsg 1, Dsg 3 and BP 230 transfected cells show fine granular cytoplasmic fluorescence and BP 180 substrate shows diamond-shaped fluorescence in positive cases.

In the case of DIF, an ICS pattern with IgG and/or C3 is diagnostic of pemphigus. A linear BMZ-staining pattern with predominantly IgG and/or C3 points to the diagnosis of pemphigoid. Standards for reporting of diagnostic accuracy for DIF included type of antibody (IgG, IgA or IgM), intensity (1+ to 3+) and pattern of staining (ICS or BMZ pattern). For BIOCHIP reporting was done as per the interpretation described above.

Results

During the study period, 40 patients fulfilled the inclusion criteria, which included 16 males and 24 females. The age of these patients ranged between 19 and 80 years with a mean age of 53.8 years. Among the study participants, a clinical diagnosis of pemphigus was entertained in 18 patients (16 pemphigus vulgaris and 2 pemphigus foliaceus) and bullous pemphigoid in 22 patients based on characteristic clinical findings.

In pemphigus group, DIF showed characteristic ICS pattern with IgG and C3 in 9 patients and IgG alone in 9 patients. In the pemphigoid group, DIF showed a linear BMZ pattern with IgG and C3 in 14 patients, IgG alone in 4 patients and C3 alone in 4 patients [Figure 1a and b].

BIOCHIP mosaic was then probed with sera from these patients. In the pemphigus group, primate oesophagus showed ICS pattern in all 18 patients, Dsg 1 and 3 were positive in 13 patients, Dsg 3 was positive in 3 patients and Dsg 1 was positive in 2 patients. In general, Dsg 1 was positive in 15 patients and Dsg 3 was positive in 16 patients [Figure 2a and b]. In pemphigoid group, primate oesophagus showed linear BMZ pattern in all 22 patients, BP 180 and BP 230 were positive in 14 patients, BP 180 in 3 patients, and BP 230 in 1 patient. Overall BP 180 was positive in 17 patients and BP 230 in 15 patients [Figure 3a and b]. Salt split skin substrate showed a roof pattern in 18 patients and floor pattern in 4 patients [Figure 4a, b and Tables 1, 2].

BIOCHIP mosaic results showed a concordance of 100% in the diagnosis of pemphigus with DIF. In case of the pemphigoid group in comparison with DIF, BIOCHIP showed the diagnosis of bullous pemphigoid in 18 patients. The remaining four patients showed a floor pattern in salt

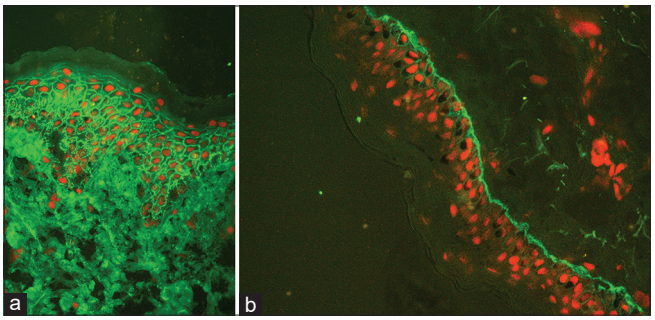


Figure 1: (a) DIF – IgG Intercellular staining pattern in the pemphigus vulgaris (× 200). (b) DIF–IgG linear Basement membrane zone-staining pattern in bullous pemphigoid (×200)

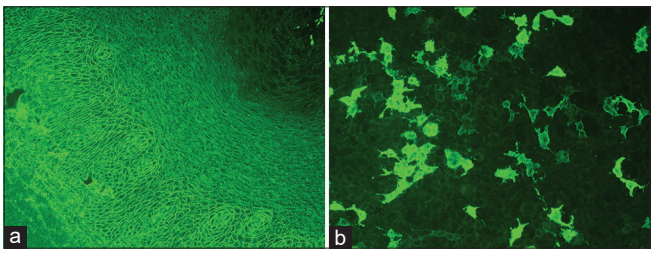


Figure 2: (a) Primate Oesophagus – Intercellular staining pattern (×200). (b) Positive fluorescence in Dsg 3 Transfected cells (×200) (similar fluorescence will be seen in Dsg 1, BP 230 transfected cells)

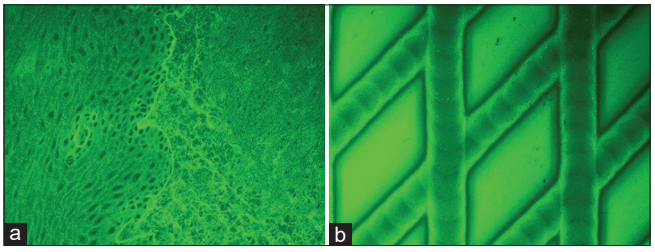


Figure 3: (a) Primate Oesophagus – Basement membrane zone-staining pattern (×200). (b) Positive fluorescence in BP 180 Tetrameric dots (×200)

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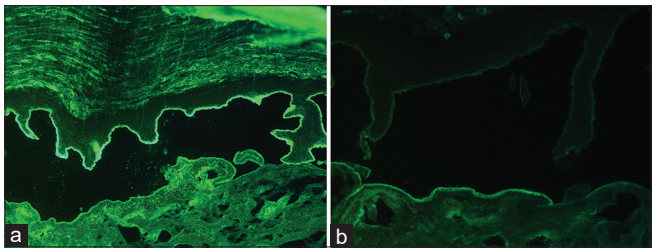


Figure 4: (a) Salt split skin – Roof pattern (×200). (b) Salt split skin – Floor pattern (×200)

split skin substrate and was negative for any of the target antigens.

The correlation between BIOCHIP mosaic and DIF in the diagnosis of AIBD was assessed using Spearman's correlation coefficient and it was found to be statistically significant.

Discussion

The diagnosis of AIBD involves a multistep approach-combining clinical feature, histopathology, DIF, IIF or ELISA. However, the gold standard remains the visualisation of autoantibodies in the skin or mucosa by DIF of perilesional skin biopsy.[1] BIOCHIP mosaic-based IIF is a new diagnostic technique that combines a simultaneous assessment of the staining pattern and identification of target antigens in a single field.[2] Studies assessing the validity of biochip have been conducted in Germany, Italy, Turkey, Poland and Australia. There is a need to assess the diagnostic value of the BIOCHIP in population groups of various ethnicities.[2]

Van Beek *et al*. from Germany were the first to evaluate the utility of BIOCHIP mosaic in the diagnosis of AIBD by comparing it with the multistep algorithm described by Schimdt and Zillikens.[1,3] The results of their study concluded that the diagnostic efficacy of the BIOCHIP mosaic was comparable with the conventional multistep procedure in the diagnosis of AIBD.[1]

Tampoia *et al.* investigated the value of the BIOCHIP method by comparing it with two commercially available ELISA tests (MBL, Japan and EuroImmun, Germany) in the diagnosis of pemphigus and pemphigoid.[4] Later Russo *et al.*from Italy and Özkesici *et al.* from Turkey evaluated the utility of BIOCHIP in the diagnosis of AIBD by comparing it with ELISA.[5,6] These studies concluded that the BIOCHIP method has a diagnostic accuracy comparable to ELISA.[4-6] However in the present study, no such comparison was made with ELISA.

Prussmann and co-workers from Germany studied the prevalence of pemphigus autoantibodies in the general population with a total of 7063 participants using the BIOCHIP method. Their study revealed a very low prevalence of autoantibodies in a large cohort of healthy individuals. Also functional analysis revealed differences between pathogenic autoantibodies in diseased individuals and antibodies detected from healthy donors.[7] The present study did not include any healthy controls and samples were collected only from cases of suspected AIBD.

Table: 1 Results of BIOCHIP mosaic in the pemphigus group (*n*=18)

Substrate	Result	Number of patients	Percentage
Primate oesophagus	Intercellular staining pattern	18	100
Dsg 1	Positive	15	83.3
Dsg 3	Positive	16	88.8

Dsg – Desmoglein

Table: 2 Results of BIOCHIP mosaic in pemphigoid group (*n*=22)

Substrate	Result	Number of patients	Percentage
Primate oesophagus	Linear basement membrane zone pattern	22	100
Salt split skin	Roof staining	18	81.8
Salt split skin	Floor staining	04	18.2
BP 180	Positive	17	77.3
BP 230	Positive	15	68.2

BP – Bullous pemphigoid antigen

Russo *etal.* in their study used the serum and salivary samples to detect anti-Dsg autoantibodies for diagnosis of pemphigus and concluded that saliva is not a suitable sample for BIOCHIP.[8] Gornowicz-Porowska *et al.* from Poland compared the original BIOCHIP method with modified BIOCHIP method using monoclonal IgG instead of routine IgG and concluded that modified BIOCHIP has as higher sensitivity and specificity.[9] The present study utilized routine commercial IgG provided with the kit.

Similar to the previous studies, the current study also revealed that Dsg 3 and BP 180 were the commonly detected antigens using BIOCHIP in pemphigus and pemphigoid groups, respectively [Table 3].[1,4-6,8-13]

The higher correlation in pemphigus group could be attributed to the fact that in pemphigus diseases only two main target antigens are there, both of which are present in BIOCHIP substrates; whereas in the pemphigoid group apart from BP 180 and 230, other antigens in the basement membrane zone may be the target, which needs evaluation with immunoblotting.

In the present study, out of 22 patients in the pemphigoid group, four patients showed a floor pattern of staining in salt split skin.These four patients revealed a linear BMZ pattern in primate oesophagus and were negative for any of the target antigens. These cases could be attributed to floor pattern-staining diseases like epidermolysis bullosa acquisita, anti-laminin 332 pemphigoid or anti-P-200 pemphigoid, which needs further evaluation with

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Table 3: Percentage of positivity of target antigens in the diagnosis of AIBD by BIOCHIP mosaic in previous studies

Authors	Country	Year	Pemphigus (*n*)	Bullous Pemphigoid (*n*)	Dsg 1 (%)	Dsg 3 (%)	BP180 (%)	BP230 (%)
Van Beek *et al*.[1]	Germany	2012	65	42	52.3	98.5	100	54.8
Tampoia *et al*.[4]	Italy	2012	36	40	33.3	100	90	40
Damoiseaux *et al*.[11]	Netherlands	2012	-	60	-	-	88	38
Zarian *et al*.[12]	Italy	2012	-	18	-	-	83.3	39
Russo *et al*.[5]	Italy	2014	42	-	19	97.62	-	-
Özkesici *et al*.[6]	Turkey	2017	45	18	37.8	86.7	88.9	66.7
Russo *et al*.[8]	Italy	2017	8	-	12.5	100		
Gornowicz-Porowska *et al*.[9]	Poland	2017	19	37	68.4	63.2	89.2	24.3
Yang *et al*.[10]	Australia	2019	23	28	75	67.5	55.3	65.8
Tirumalae *et al*.[13]	India	2019	37	29	73	86	95	14
Current study	India	2019	18	22	83.3	88.8	77.3	68.3

AIBD=Autoimmune bullous disease, Dsg=Desmoglein, BP=Bullous pemphigoid antigen.

immunoblotting.[14-16] This finding of floor pattern in 4 out of 22 cases of pemphigoid group using BIOCHIP is a significant observation. In an Indian study by Tirumalae *etal.*, similar findings were noted in four cases that showed either roof or floor positivity on salt split skin substrate with negative results in other substrates. They categorized these cases as unclassified subepidermal diseases.[13]

The advantage of BIOCHIP is that the combination of different substrates in the same field allows for concurrent evaluation of characteristic-staining pattern, identification of target antigens at once. Also, it facilitates distinction among the various types of AIBD. Further, this multiparametric technique is cost- and time-effective compared to the conventional multi-step approach.[17] However, it has limitations in categorisation of pemphigoid diseases due to restricted antibody coating. This could be overcome by testing additional target antigens or immunoblotting.

The literature search revealed only a few studies investigating the validity of the BIOCHIP IIF in the diagnosis of AIBD. These studies had concluded that the BIOCHIP method has a high degree of sensitivity and specificity in the diagnosis of AIBD.[2] The results of the present study show that the diagnosis of AIBD by BIOCHIP is showing a statistically significant correlation with that of DIF.

Limitations of this study include small sample size, lack of control group and no comparison made with ELISA.

Conclusion

BIOCHIP mosaic shows a good correlation with DIF. BIOCHIP mosaic is a non-invasive, rapid diagnostic technique that can detect the characteristic-staining pattern and target antigens in a single miniature incubation field and can be used as firstline tool in the diagnosis of AIBD.

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Conflicts of interest

There are no conflicts of interest.

References

1. Van Beek N, Rentzsch K, Probst C, Komorowski L, Kasperkiewicz M, Fechner K, *et al.* Serological diagnosis of autoimmune bullous skin diseases: prospective comparison of the BIOCHIP mosaic-based indirect immunofluorescence technique with the conventional multi-step single test strategy. Orphanet J Rare Dis 2012;7:49.
2. Xuan RR, Yang A, Murrell DF. New biochip immunofluorescence test for the serological diagnosis of pemphigus vulgaris and foliaceus: A review of the literature. Int J Womens Dermatol 2018;4:102-8.
3. Schmidt E, Zillikens D. Diagnosis and treatment of patients with autoimmune bullous disorders in Germany. Dermatol Clin 2011;29:663-71.
4. Tampoia M, Zucano A, Villalta D, Antico A, Bizzaro N. Anti-skin specific autoantibodies detected by a new immunofluorescence multiplex biochip method in patients with autoimmune bullous diseases. Dermatology 2012;225:37-44.
5. Russo I, Saponeri A, Peserico A, Alaibac M. The use of biochip immunofluorescence microscopy for the diagnosis of Pemphigus vulgaris. Acta Histochem 2014;116:713-6.
6. Özkesici B, Mutlu D, Dönmez L, Uzun S. The value of the BIOCHIP mosaic-based indirect immunofluorescence technique in the diagnosis of pemphigus and bullous pemphigoid in Turkish patients. Acta Dermatovenerologica Croat 2017;25:202-9.
7. Prussmann W, Prussmann J, Koga H, Recke A, Iwata H, Juhl D, *et al.* Prevalence of pemphigus and pemphigoid autoantibodies in the general population. Orphanet J Rare Dis 2015;10:63.
8. Russo I, Saponeri A, Michelotto A, Alaibac M. Salivary samples for the diagnosis of Pemphigus vulgaris using the BIOCHIP approach: A pilot study. *In Vivo* 2017;31:97-9.
9. Gornowicz-Porowska J, Seraszek-Jaros A, Bowszyc-Dmochowska M, Kaczmarek E, Pietkiewicz P, Bartkiewicz P, *et al.* Accuracy of molecular diagnostics in pemphigus and bullous pemphigoid: Comparison of commercial and modified mosaic indirect immunofluorescence tests as well as enzyme-linked immunosorbent assays. Postepy Dermatol Alergol 2017;34:21-7.
10. Yang A, Xuan R, Melbourne W, Tran K, Murrell DF. Validation of the BIOCHIP test for the diagnosis of bullous pemphigoid, pemphigus vulgaris and pemphigus foliaceus. J Eur Acad

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Dermatol Venereol 2020;34:153-60.
11. Damoiseaux J, van Rijsingen M, Warnemunde N, Dahnrich C, Fechner K, Tervaert JW. Autoantibody detection in bullous pemphigoid: Clinical evaluation of the EUROPLUS™ Dermatology Mosaic. J Immunol Methods 2012;382:76-80.
12. Zarian H, Saponeri A, Michelotto A, Zattra E, Belloni-Fortina A, Alaibac M. Biochip technology for the serological diagnosis of bullous pemphigoid. ISRN Dermatol 2012;2012:237802.
13. Tirumalae R, Kalegowda IY. Role of BIOCHIP Indirect Immunofluorescence Test in Cutaneous Vesiculobullous Diseases. Am J Dermatopathol 2020;42:322-8.
14. Schmidt E, Zillikens D. Pemphigoid diseases. Lancet 2013;381:320-32.
15. Domloge-Hultsch N, Anhalt GJ, Gammon WR, Lazarova Z, Briggaman R, Welch M, *et al.* Antiepiligrin cicatricial pemphigoid. A subepithelial bullous disorder. Arch Dermatol 1994;130:1521-9.
16. Zillikens D, Kawahara Y, Ishiko A, Shimizu H, Mayer J, Rank CV, *et al.*A novel subepidermal blistering disease with autoantibodies to a 200-kDa antigen of the basement membrane zone. J Invest Dermatol 1996;106:1333-8.
17. Saschenbrecker S, Karl I, Komorowski L, Probst C, Dähnrich C, Kai Fechner K, *et al.*Serological diagnosis of autoimmune bullous skin diseases. Front Immunol 2019;10:1974.

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Brief Report

Comparison of Efficacy of Autologous Platelet-Rich Fibrin versus Unna's Paste Dressing in Chronic Venous Leg Ulcers: A Comparative Study

G. Yuvasri, Reena Rai

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Abstract

Background: Chronic venous leg ulcers are difficult to treat and recurrences are common. Among various modalities of treatment, platelet-rich fibrin (PRF) has shown promising results in ulcer healing by providing necessary growth factors. Unna's paste dressing has also proven to enhance wound healing. **Aim:** The aim of this study is to compare the efficacy of autologous PRF versus Unna's paste dressing in chronic venous leg ulcer patients and to compare the mean reduction in ulcer area at the end of 4 weeks. **Materials and Methods:** Twenty patients with chronic venous leg ulcers were randomly divided into two groups. Group 1: Patients received PRF dressing which was repeated every week for 4 weeks. Group 2: Patients received Unna's paste dressing once a week for 4 weeks. The ulcer size was assessed with the help of photographs, and ulcer area was measured. **Results:** The mean reduction in the area of the ulcer size in the PRF group was 86.03% and in the Unna's paste group was 71.97%, which was not statistically significant with a *P* value of 0.223. **Conclusion:** We conclude that though there was no statistical significance between the groups, PRF showed a significant reduction in ulcer size.

Keywords: *Platelet-rich fibrin, Unna's paste, venous leg ulcer*

Introduction

Venous ulcers are the most common cause of chronic leg ulcers. The chronicity and high recurrence rate of venous ulcer are the two fundamental factors causing grave impact on patients' well-being.[1] One of the reasons for chronicity of the ulcer has been attributed to lack of growth factors and nutrients which are essential for wound healing.

A major revolution in the treatment of leg ulcer is cellular therapy, where the patient's own blood is used, which contains various cytokines and growth factors. Platelet-derived products such as platelet-rich fibrin (PRF) have shown promising results in wound healing.

Another dressing for chronic leg ulcer is zinc oxide paste (Unna's paste). It has re-epithelialization and anti-inflammatory properties which can enhance wound healing. Hence, we conducted an open-label randomized study to compare the efficacy of PRF and Unna's paste dressing in chronic venous leg ulcers.

Materials and Methods

A total of 20 patients were randomly divided into two groups based on the inclusion and exclusion criteria. Patients suffering from chronic venous ulcers of the lower extremity for more than 6 months, attending the outpatient department of Dermatology Venereology and Leprosy, and having an ulcer area of 1 cm × 1 cm to 5 cm × 5 cm were included in the study.[2] A thorough clinical examination and ultrasound venous Doppler were performed to confirm the diagnosis of venous ulcer. Ethical clearance was taken from the Institutional Ethics Committee.

Patients with ulcers of less than 6 months duration and of other etiology such as arterial ulcer, diabetic ulcer, neuropathic and vasculitic ulcers; patients with osteomyelitis affecting the area of the ulcer; patients with exposed tendons or bones; patients with infection; and patients receiving anticoagulants/antiplatelet drugs/bleeding diathesis were excluded from this study.

All patients in our study continued using compression stockings.

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Preparation of platelet-rich fibrin

Procedure

After obtaining informed consent, 10 ml of the patient's blood was withdrawn into the vacutainer without any anticoagulant and immediately centrifuged (to prevent blood from clotting) at 3000 rpm for 15 mins. After 15 mins, a fibrin gel appears in the center of the vacutainer with red blood cells (RBCs) at the base and acellular plasma above. The PRF obtained was removed with the help of a toothed forcep and placed on the ulcer after removing the adherent RBCs. On an average, 10 ml of whole blood yields about 2.5 ml of the clot.[2]

Dressing procedure

Group 1: The measurements of the ulcer were taken. The PRF gel was then placed on the ulcer floor and covered with a sterile gauze piece (primary dressing), which was, in turn, covered with a sterile gauze pad (secondary dressing) held in place with a sterile roller bandage. The dressing was removed after 1 week. This treatment was repeated every week for 4 weeks.[2]

Preparation of Unna's paste

Procedure

Sixty grams of zinc oxide was mixed with 100 ml of glycerine and stirred well. This mixture was heated for 10–20 mins.

Dressing procedure

The measurements of the ulcer were taken. Ulcers were covered with a gauze impregnated with Unna's paste which was in turn covered with a sterile gauze-pad followed by a sterile roller bandage. This dressing was left in place for 1 week. This treatment was repeated every week for 4 weeks.

Measurements documentation

The greatest length and the greatest breadth were measured using a thread and a scale (the clock face method). This was done before starting the treatment, before repeating the treatment each time at weekly intervals, and after the treatment was completed (the final measurement). Digital photographs in both groups were taken before starting the treatment and after the treatment was completed.[2]

Results

A total of 20 patients enrolled in the study met the inclusion and exclusion criteria. The result was assessed on the basis of the ulcer area reduction. The mean reduction in the ulcer area in the PRF group was 7.50% after the 1st week, 35.04% after the 2nd week, 62.23% after the 3rd week, and 86.03% after the 4th week [Table 1]. However, in the Unna's paste group, the mean reduction in the ulcer size was 14.80%, 29.57%, 56.16%, and 71.97%, respectively [Table 2]. The overall mean reduction in the ulcer area was 86.03% in the patients treated with PRF dressing compared to 71.97% in the patients treated with Unna's paste dressing [Figure 1]. There was a complete closure of the ulcer in four patients (40%) in the PRF group [Figure 2a and b; 3a and b]. There was no case of complete closure of the ulcer in the Unna's paste group [Figure 4a and b; 5a and b].

Statistical analysis using independent *t*-test and Chi-square test showed no significant reduction of the ulcer size between both groups [Table 3].

Discussion

Wound healing is a complicated process which involves a cascade of events such as clotting, neutrophil chemotaxis, epithelialization, angiogenesis, and scar formation. Any interruption in these events leads to failure of wound closure whereby an acute wound is converted to a chronic one. Chronic ulcers are difficult to treat.[3] Therefore, an ideal dressing which offers nutritional support has a significant impact on wound repair leading to complete closure of the wound.

PRF offers the growth factors necessary for wound healing, and have been used in various fields of medicine and surgery. The derived PRF acts as a biodegradable scaffold for cell migration, proliferation, and differentiation, and

Table 1: Patients treated with PRF dressing

Sr. no of patients	Initial measurement of ulcer size (cm^2)	Measurement of ulcer size after 1st week (cm^2)	Measurement of ulcer size after 2nd week (cm^2)	Measurement of ulcer size after 3rd week (cm^2)	Final measurement of ulcer size (cm^2)	Percentage of improvement
1	4.76	4	3.84	3.68	2.8	41.17%
2	0.96	0.96	0.8	0.25	0	100%
3	5.25	5	3.96	3.6	1.84	64.95%
4	3.5	3.5	2	1.125	0.5	85.71%
5	1.25	1.25	0.75	0.5	0.25	80%
6	2.4	2.2	1	0.25	0	100%
7	5.72	5.5	3.5	1.25	0.5	91.25%
8	8.9	5.6	3.97	3	0.25	97.19%
9	5.8	5.5	3.8	1.15	0	100%
10	10.5	10.5	8.4	5	0	100%
Mean % of improvement	0	7.50%	35.04%	62.23%	86.03%	86.03%

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Table 2: Patients treated with Unna's paste dressing

S. no of patients	Initial measurement of ulcer size (cm^2)	Measurement of ulcer size after 1st week (cm^2)	Measurement of ulcer size after 2nd week (cm^2)	Measurement of ulcer size after 3rd week (cm^2)	Final measurement of ulcer size (cm^2)	Percentage of improvement
1	10	9	5.4	3.75	1	90%
2	0.25	0.25	0.15	0.1	0.06	76%
3	6.75	6.5	1	0.3	0.3	95.55%
4	3.75	3.5	3.45	2.64	3.23	13.86%
5	5	4.75	4.4	3.23	0.25	95%
6	13.5	12.5	8.4	5	2	85.18%
7	13.5	12.5	9	5.6	0.5	96.29%
8	22.5	20.5	15.6	9	3	86.66%
9	5	3.75	5	5.75	3	40%
10	2.55	2.55	2.25	1.5	1.5	41.17%
Mean % of improvement	0	14.80%	29.57%	56.16%	71.97%	71.97%

Table 3: Statistical analysis

Variables	No. of Patients	Mean	Std. Deviation	*P*
PRF	10	86.03	19.510	0.223
Unna's paste	10	71.97	29.358	

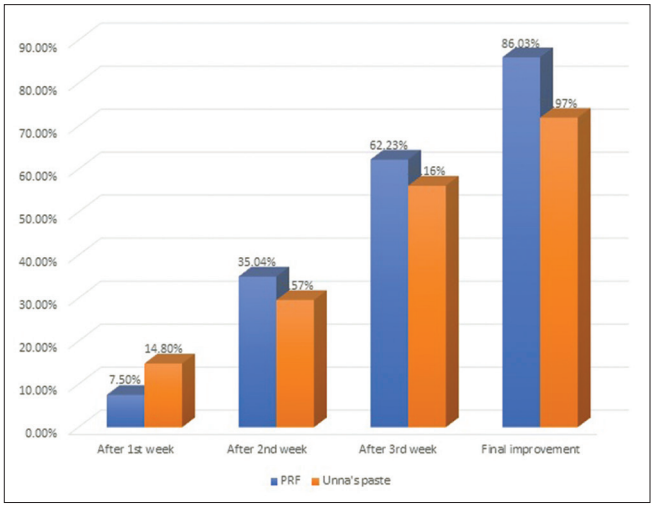


Figure 1: Bar diagram showing percentage of size reduction with platelet-rich fibrin and Unna's paste dressing

delivers growth factors.[4] Platelets trapped within the three-dimensional mesh ensures slow and continuous release of growth factors over time which lasts until the 28th day.[5] Various growth factors such as platelet-derived growth factor help in angiogenesis, neutrophilic chemotaxis, and collagen synthesis; transforming growth factor-beta helps in regulating fibroblast proliferation and deposition of bone matrix; insulin-like growth factor-1 and fibroblast growth factor enhance proliferation and differentiation of osteoblast and activate protein synthesis; vascular endothelial growth factor aids angiogenesis and endothelial cell migration; epidermal growth factor exerts collagen secretion and epithelial mitogenesis; and connective tissue growth factor helps in cartilage regeneration.[6,7]

Unna's paste dressing has a dual action, one by its anti-inflammatory and antibacterial action and offers graded compression.

Various studies with PRF and Unna's paste dressing for chronic venous leg ulcers have shown good results.

A randomized controlled trial conducted by Somani *et al*.[2] compared the efficacy of PRF and saline dressing in chronic venous leg ulcers concluded that the mean reduction in the area of the ulcer size in PRF group was 85.51% and in the saline group was 42.74% which was statistically significant.

Similarly in another randomized controlled study, Goda[8] compared PRF dressing and conventional dressing for venous ulcers. He concluded that the mean percentage of wound reduction was higher in the PRF group than the control group which was statistically significant.

A randomized double-blinded study was conducted by Stromberg and Agren[9] to compare the efficacy of local zinc oxide gauze compress and a placebo. It showed 83% improvement in the zinc oxide group when compared to the placebo group which showed only 42% improvement. Therefore, they concluded that zinc oxide accelerates wound healing.

A case series by Parboteeah and Brown[10] concluded that zinc paste therapy along with compression bandage is more efficacious in the treatment of venous leg ulcers that are difficult to treat with other therapies.

Luz *et al*.[11] conducted a study wherein 43 venous ulcer patients were randomized into two groups. Out of 43 patients, 32 patients in Unna's boot group showed a significant reduction in the ulcer when compared to 11 patients in the control group who received simple dressing. They concluded that though there was no statistical significance between the groups, Unna's boot group showed better improvement at the end of 3 months.

Conclusion

In conclusion, the reduction in ulcer size was better in PRF group (86.03%) when compared to Unna's paste

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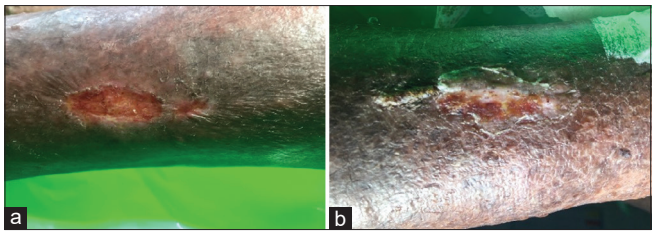


Figure 2: (a) Patient 1: A 54-year-old female with a venous ulcer over the left leg before treatment with platelet-rich fibrin dressing; (b) 80% improvement after 4 weeks of treatment with platelet-rich fibrin dressing



Figure 4: (a) Patient 3: A 50-year-old female with an ulcer over the right leg before treatment with Unna's paste dressing; (b) 90% improvement after 4 weeks of treatment with Unna's paste dressing

group (71.97%), but it was not statistically significant. PRF dressing is simple, cost-effective, and patient-friendly. However, the sample size was small and so large-scale studies are needed.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.



Figure 3: (a) Patient 2: A 62-year-old male presented with an ulcer over the right leg before treatment with platelet-rich fibrin dressing, (b) showing complete closure after 4 weeks of treatment with platelet-rich fibrin dressing

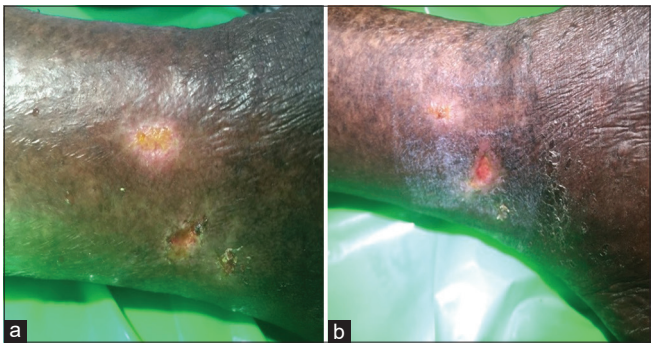


Figure 5: (a) Patient 4- 49-year-old male with ulcer near right lateral malleolus before treatment with Unna's paste dressing, (b) showing 70% improvement after 4 weeks of treatment with Unna's paste dressing

References

1. Vasudevan B. Venous leg ulcers: Pathophysiology and classification. Indian Dermatol Online J 2014;5:366-70.
2. Somani A, Rai R. Comparison of efficacy of autologous platelet-rich fibrin versus saline dressing in chronic venous leg ulcers: A randomized controlled trial. J Cutan Aesthet Surg 2017;10:8–12.
3. Ashcroft GS, Jeong MJ, Ashworth JJ, Hardman M, Jin W, Moutsopoulos N, *et al*. Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. Wound Repair Regen 2012;20:38–49.
4. Borie E, Oliví DG, Orsi IA, Garlet K, Weber B, Beltrán V, *et al*. Platelet-rich fibrin application in dentistry: A literature review. Int J Clin Exp Med 2015;8:7922-9.
5. Chignon-Sicard B, Georgiou CA, Fontas E, David S, Dumas P, Ihrai T, *et al*. Efficacy of leukocyte-and platelet-rich fibrin in wound healing: A randomized controlled clinical trial. Plast Reconstr Surg 2012;130:819-29.
6. Raja VS, Naidu EM. Platelet rich fibrin: Evolution of a second-generation platelet concentrate. Indian J Dent Res 2008;19:42-6.
7. Hotwani K, Sharma K. Platelet rich fibrin-a novel acumen into regenerative endodontic therapy. Restor Dent Endod 2014;39:1-6.
8. Goda AA. Autogenous leucocyte-rich and platelet-rich fibrin for the treatment of leg venous ulcer: A randomized control study. Egypt J Surg 2018;37:316-21.
9. Stromberg, HE, Agren, MS. Topical zinc oxide treatment improves arterial and venous leg ulcers. Br J Dermatol 1984;111:461-8.
10. Parboteeah S, Brown A. Managing chronic venous leg ulcers with zinc oxide paste bandages. Br J Nurs 2008;17:S30, S32, S34-6.
11. Luz BS, Araujo CS, Atzingen DA, Mendonca AR, Filho MM, Medeiros ML. Evaluating the effectiveness of the customized Unna boot when treating patients with venous ulcers. An Bras Dermatol 2013;88:41-9.

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Abstract

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Keywords: *Platelet-rich fibrin, Unna's paste, venous leg ulcer*

Introduction

Venous ulcers are the most common cause of chronic leg ulcers. The chronicity and high recurrence rate of venous ulcer are the two fundamental factors causing grave impact on patients' well-being.[1] One of the reasons for chronicity of the ulcer has been attributed to lack of growth factors and nutrients which are essential for wound healing.

A major revolution in the treatment of leg ulcer is cellular therapy, where the patient's own blood is used, which contains various cytokines and growth factors. Platelet-derived products such as platelet-rich fibrin (PRF) have shown promising results in wound healing.

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All patients in our study continued using compression stockings.

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Preparation of platelet-rich fibrin

Procedure

After obtaining informed consent, 10 ml of the patient's blood was withdrawn into the vacutainer without any anticoagulant and immediately centrifuged (to prevent blood from clotting) at 3000 rpm for 15 mins. After 15 mins, a fibrin gel appears in the center of the vacutainer with red blood cells (RBCs) at the base and acellular plasma above. The PRF obtained was removed with the help of a toothed forcep and placed on the ulcer after removing the adherent RBCs. On an average, 10 ml of whole blood yields about 2.5 ml of the clot.[2]

Dressing procedure

Group 1: The measurements of the ulcer were taken. The PRF gel was then placed on the ulcer floor and covered with a sterile gauze piece (primary dressing), which was, in turn, covered with a sterile gauze pad (secondary dressing) held in place with a sterile roller bandage. The dressing was removed after 1 week. This treatment was repeated every week for 4 weeks.[2]

Preparation of Unna's paste

Procedure

Sixty grams of zinc oxide was mixed with 100 ml of glycerine and stirred well. This mixture was heated for 10–20 mins.

Dressing procedure

The measurements of the ulcer were taken. Ulcers were covered with a gauze impregnated with Unna's paste which was in turn covered with a sterile gauze-pad followed by a sterile roller bandage. This dressing was left in place for 1 week. This treatment was repeated every week for 4 weeks.

Measurements documentation

The greatest length and the greatest breadth were measured using a thread and a scale (the clock face method). This was done before starting the treatment, before repeating the treatment each time at weekly intervals, and after the treatment was completed (the final measurement). Digital photographs in both groups were taken before starting the treatment and after the treatment was completed.[2]

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A total of 20 patients enrolled in the study met the inclusion and exclusion criteria. The result was assessed on the basis of the ulcer area reduction. The mean reduction in the ulcer area in the PRF group was 7.50% after the 1st week, 35.04% after the 2nd week, 62.23% after the 3rd week, and 86.03% after the 4th week [Table 1]. However, in the Unna's paste group, the mean reduction in the ulcer size was 14.80%, 29.57%, 56.16%, and 71.97%, respectively [Table 2]. The overall mean reduction in the ulcer area was 86.03% in the patients treated with PRF dressing compared to 71.97% in the patients treated with Unna's paste dressing [Figure 1]. There was a complete closure of the ulcer in four patients (40%) in the PRF group [Figure 2a and b; 3a and b]. There was no case of complete closure of the ulcer in the Unna's paste group [Figure 4a and b; 5a and b].

Statistical analysis using independent *t*-test and Chi-square test showed no significant reduction of the ulcer size between both groups [Table 3].

Discussion

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1	4.76	4	3.84	3.68	2.8	41.17%
2	0.96	0.96	0.8	0.25	0	100%
3	5.25	5	3.96	3.6	1.84	64.95%
4	3.5	3.5	2	1.125	0.5	85.71%
5	1.25	1.25	0.75	0.5	0.25	80%
6	2.4	2.2	1	0.25	0	100%
7	5.72	5.5	3.5	1.25	0.5	91.25%
8	8.9	5.6	3.97	3	0.25	97.19%
9	5.8	5.5	3.8	1.15	0	100%
10	10.5	10.5	8.4	5	0	100%
Mean % of improvement	0	7.50%	35.04%	62.23%	86.03%	86.03%

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Table 2: Patients treated with Unna's paste dressing

S. no of patients	Initial measurement of ulcer size (cm^2)	Measurement of ulcer size after 1st week (cm^2)	Measurement of ulcer size after 2nd week (cm^2)	Measurement of ulcer size after 3rd week (cm^2)	Final measurement of ulcer size (cm^2)	Percentage of improvement
1	10	9	5.4	3.75	1	90%
2	0.25	0.25	0.15	0.1	0.06	76%
3	6.75	6.5	1	0.3	0.3	95.55%
4	3.75	3.5	3.45	2.64	3.23	13.86%
5	5	4.75	4.4	3.23	0.25	95%
6	13.5	12.5	8.4	5	2	85.18%
7	13.5	12.5	9	5.6	0.5	96.29%
8	22.5	20.5	15.6	9	3	86.66%
9	5	3.75	5	5.75	3	40%
10	2.55	2.55	2.25	1.5	1.5	41.17%
Mean % of improvement	0	14.80%	29.57%	56.16%	71.97%	71.97%

Table 3: Statistical analysis

Variables	No. of Patients	Mean	Std. Deviation	*P*
PRF	10	86.03	19.510	0.223
Unna's paste	10	71.97	29.358	

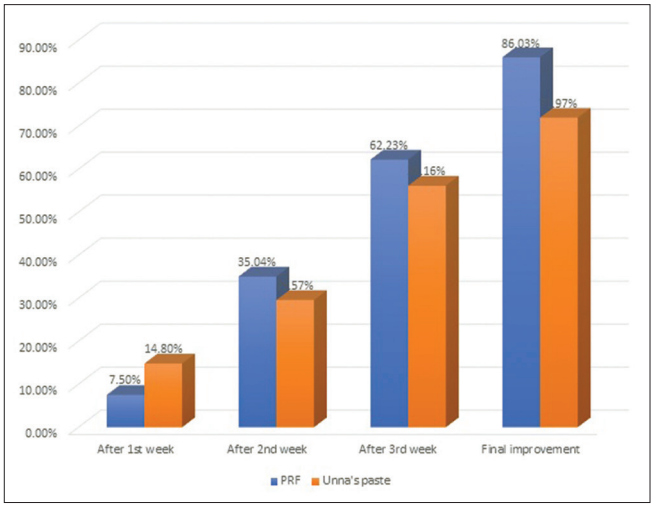


Figure 1: Bar diagram showing percentage of size reduction with platelet-rich fibrin and Unna's paste dressing

delivers growth factors.[4] Platelets trapped within the three-dimensional mesh ensures slow and continuous release of growth factors over time which lasts until the 28th day.[5] Various growth factors such as platelet-derived growth factor help in angiogenesis, neutrophilic chemotaxis, and collagen synthesis; transforming growth factor-beta helps in regulating fibroblast proliferation and deposition of bone matrix; insulin-like growth factor-1 and fibroblast growth factor enhance proliferation and differentiation of osteoblast and activate protein synthesis; vascular endothelial growth factor aids angiogenesis and endothelial cell migration; epidermal growth factor exerts collagen secretion and epithelial mitogenesis; and connective tissue growth factor helps in cartilage regeneration.[6,7]

Unna's paste dressing has a dual action, one by its anti-inflammatory and antibacterial action and offers graded compression.

Various studies with PRF and Unna's paste dressing for chronic venous leg ulcers have shown good results.

A randomized controlled trial conducted by Somani *et al*.[2] compared the efficacy of PRF and saline dressing in chronic venous leg ulcers concluded that the mean reduction in the area of the ulcer size in PRF group was 85.51% and in the saline group was 42.74% which was statistically significant.

Similarly in another randomized controlled study, Goda[8] compared PRF dressing and conventional dressing for venous ulcers. He concluded that the mean percentage of wound reduction was higher in the PRF group than the control group which was statistically significant.

A randomized double-blinded study was conducted by Stromberg and Agren[9] to compare the efficacy of local zinc oxide gauze compress and a placebo. It showed 83% improvement in the zinc oxide group when compared to the placebo group which showed only 42% improvement. Therefore, they concluded that zinc oxide accelerates wound healing.

A case series by Parboteeah and Brown[10] concluded that zinc paste therapy along with compression bandage is more efficacious in the treatment of venous leg ulcers that are difficult to treat with other therapies.

Luz *et al*.[11] conducted a study wherein 43 venous ulcer patients were randomized into two groups. Out of 43 patients, 32 patients in Unna's boot group showed a significant reduction in the ulcer when compared to 11 patients in the control group who received simple dressing. They concluded that though there was no statistical significance between the groups, Unna's boot group showed better improvement at the end of 3 months.

Conclusion

In conclusion, the reduction in ulcer size was better in PRF group (86.03%) when compared to Unna's paste

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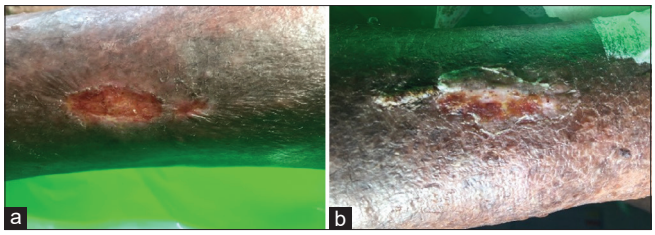


Figure 2: (a) Patient 1: A 54-year-old female with a venous ulcer over the left leg before treatment with platelet-rich fibrin dressing; (b) 80% improvement after 4 weeks of treatment with platelet-rich fibrin dressing



Figure 4: (a) Patient 3: A 50-year-old female with an ulcer over the right leg before treatment with Unna's paste dressing; (b) 90% improvement after 4 weeks of treatment with Unna's paste dressing

group (71.97%), but it was not statistically significant. PRF dressing is simple, cost-effective, and patient-friendly. However, the sample size was small and so large-scale studies are needed.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.



Figure 3: (a) Patient 2: A 62-year-old male presented with an ulcer over the right leg before treatment with platelet-rich fibrin dressing, (b) showing complete closure after 4 weeks of treatment with platelet-rich fibrin dressing

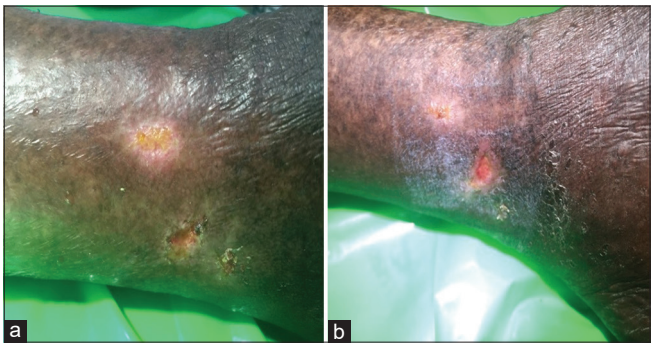


Figure 5: (a) Patient 4- 49-year-old male with ulcer near right lateral malleolus before treatment with Unna's paste dressing, (b) showing 70% improvement after 4 weeks of treatment with Unna's paste dressing

References

1. Vasudevan B. Venous leg ulcers: Pathophysiology and classification. Indian Dermatol Online J 2014;5:366-70.
2. Somani A, Rai R. Comparison of efficacy of autologous platelet-rich fibrin versus saline dressing in chronic venous leg ulcers: A randomized controlled trial. J Cutan Aesthet Surg 2017;10:8–12.
3. Ashcroft GS, Jeong MJ, Ashworth JJ, Hardman M, Jin W, Moutsopoulos N, *et al*. Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. Wound Repair Regen 2012;20:38–49.
4. Borie E, Oliví DG, Orsi IA, Garlet K, Weber B, Beltrán V, *et al*. Platelet-rich fibrin application in dentistry: A literature review. Int J Clin Exp Med 2015;8:7922-9.
5. Chignon-Sicard B, Georgiou CA, Fontas E, David S, Dumas P, Ihrai T, *et al*. Efficacy of leukocyte-and platelet-rich fibrin in wound healing: A randomized controlled clinical trial. Plast Reconstr Surg 2012;130:819-29.
6. Raja VS, Naidu EM. Platelet rich fibrin: Evolution of a second-generation platelet concentrate. Indian J Dent Res 2008;19:42-6.
7. Hotwani K, Sharma K. Platelet rich fibrin-a novel acumen into regenerative endodontic therapy. Restor Dent Endod 2014;39:1-6.
8. Goda AA. Autogenous leucocyte-rich and platelet-rich fibrin for the treatment of leg venous ulcer: A randomized control study. Egypt J Surg 2018;37:316-21.
9. Stromberg, HE, Agren, MS. Topical zinc oxide treatment improves arterial and venous leg ulcers. Br J Dermatol 1984;111:461-8.
10. Parboteeah S, Brown A. Managing chronic venous leg ulcers with zinc oxide paste bandages. Br J Nurs 2008;17:S30, S32, S34-6.
11. Luz BS, Araujo CS, Atzingen DA, Mendonca AR, Filho MM, Medeiros ML. Evaluating the effectiveness of the customized Unna boot when treating patients with venous ulcers. An Bras Dermatol 2013;88:41-9.

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Original Article

Comparison of Efficacy of Autologous Platelet-rich Fibrin versus Saline Dressing in Chronic Venous Leg Ulcers: A Randomised Controlled Trial

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Abstract

Background: Venous leg ulcer is a chronic condition, and various treatment modalities are available. Platelet-rich fibrin (PRF) is one of the newer modalities and it contains fibroblast growth factor (GF), vascular endothelial GF, angiopoitin and platelet-derived GF which enhances the wound healing. Hence, we conducted a randomised controlled trial to compare the efficacy of PRF versus saline dressing in chronic venous leg ulcers. **Aim:** This study aims to compare the efficacy of autologous PRF with saline dressing in patients with chronic venous leg ulcer and to compare the mean reduction in ulcer area at the end of 4 weeks. **Materials and Methods:** Fifteen patients with chronic venous leg ulcers of >6 months duration having an ulcer area of 1 cm × 1 cm to 5 cm × 5 cm were taken into the study and were randomly divided into two groups. Group 1: Patients received PRF dressing. Ten millilitres of patient's blood was taken and centrifuged at 3000 rpm for 15 min. A fibrin clot obtained in the middle of the tube was removed and used for dressing over the wound surface. It was repeated every week for 4 weeks. Group 2: Patients received saline dressings once a week for 4 weeks. The assessment of the ulcer size was done with the help of photographs, and ulcer area was measured. **Results:** The mean reduction in the area of the ulcer size in PRF group was 85.51%, and the mean reduction in the area of the ulcer size in Saline group was 42.74% which was statistically significant with a $P < 0.001$ and $t = 4.11$. **Conclusion:** We conclude that PRF dressing can be used as it is effective, inexpensive, safe and an outpatient procedure.

Keywords: Platelet-rich fibrin, saline, venous ulcers

Introduction

Various modalities of treatment for venous ulcers include compression stockings, good wound care and sometimes surgical therapies. The treatment is often difficult and is generally associated with high recurrence rate.[1-3] Dressings play a major role in healing of these ulcers. Moist occlusive dressings with saline are known to improve wound healing. Platelet concentrates have been widely used in regenerative medicine to promote wound healing as they contain transforming growth factor (GF) *F*, platelet-derived GF, vascular-endothelial GF, platelet derived epidermal GF, insulin-like GF-I and basic fibroblast GF. Hence, we conducted a study to compare the efficacy of autologous platelet rich fibrin (PRF) over saline dressing in chronic venous leg ulcers.

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Materials and Methods

Selection and description of participants

A total of 15 patients were randomly divided into two groups on the basis of inclusion and exclusion criteria.

Inclusion criteria

Patients suffering from chronic venous ulcers of the lower extremity for more than 6 months, with an ulcer area varying from 1 × 1 cm to 5 × 5 cm attending the out-patient Department of Dermatology Venereology and Leprosy were included.

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Exclusion criteria

Ulcers of >6 months duration, size less than 1 sq cm or more than 5 sq cm and neuropathic, arterial, diabetic or vasculitic ulcers vasculitis. Patients with infected ulcers, osteomyelitis affecting the area of the ulcer , ulcers with exposed tendons or bones Ulcers with exposed tendons or bones.Patients receiving anticoagulants/antiplatelet drugs/bleeding diathesis were also excluded from the study.

Materials and Methods

The patients were divided into two groups; Group 1: Nine patients with chronic venous ulcer receiving PRF dressing weekly for 4 weeks. Group 2: Patients with chronic venous ulcer receiving saline dressings weekly for 4 weeks.

Preparation of platelet rich fibrin

Procedure

After obtaining consent, 10 ml of the patient's own blood was withdrawn into the vacutainer without any anticoagulant and immediately centrifuged (to prevent blood from clotting) at 3000 rotations per minute for 15 min. After 15 min, a fibrin gel appears in the centre of the vacutainer [Figure 1], in between the red blood cells (RBCs) which are settled at the base and acellular plasma above. The PRF obtained was removed with the help of a toothed forceps and was placed on the ulcer after removing the adherent RBC's [Figure 2]. On an average, 10 ml of whole blood yields about 2.5 ml of clot.

Dressing procedure

Group 1: The measurements of the ulcer were taken. After 15 min, the vacutainer was removed from the centrifuge machine. A sterile non-toothed forceps was used to remove the PRF gel. With the help of an 11 number blade, the RBC clot adhering to the PRF gel was scraped off. The PRF gel was then placed on the ulcer floor and covered with a sterile gauze piece (primary dressing), which was, in turn, was covered with a sterile gauze pad (secondary dressing) held in place with a sterile roller bandage. The dressing was removed after 1 week. The PRF remnants were removed with water and sterile gauze. This treatment was repeated every week for 4 weeks.

Group 2: The measurement of the ulcer was taken. The ulcer was covered with a sterile gauze soaked in saline (primary dressing), which was in turn covered with a sterile gauze pad (secondary dressing) and was covered with a sterile roller bandage. This dressing was left in place for 1 week. This treatment was repeated every week for 4 weeks.

Measurements documentation

The greatest length and the greatest breadth were measured using a thread and a scale (the clock face method). This was done before starting the treatment, and before repeating the treatment each time at weekly intervals and after the treatment was completed (the final measurement). Digital photographs were taken before starting the treatment, before repeating the treatment each time at weekly intervals and after the treatment was completed.

Results

A total of 15 patients meeting the inclusion and exclusion criteria were enrolled in the study. All the patients were suffering from chronic venous ulcers. The result was assessed on the basis of ulcer area reduction. The mean ulcer area reduction after 1 week in the PRF group was 26.27%, after 2 weeks it was 46.25%, at 3 weeks it was 77.08% and after 4 weeks it was 85.51% [Table 1]. Whereas in the saline group, it was 14.55%, 23.24%, 34.78% and 42.74%, respectively [Table 2]. The overall mean reduction in the ulcer area was 85.51% in the patients with PRF dressing compared to 42.74% in the patients treated with saline dressing [Bar Diagram 1]. There was a complete closure of the ulcer in five patients (55.55%) in the PRF group [Figures 3-6]. There was no case of complete closure of the ulcer in the saline group [Figures 7-10].

Statistical analysis showed a significant reduction in the ulcer size in the PRF group compared to the saline group [Table 3].

Discussion

PRF is an autologous platelet and leucocyte-rich fibrin material and is an important advancement in regenerative medicine. It

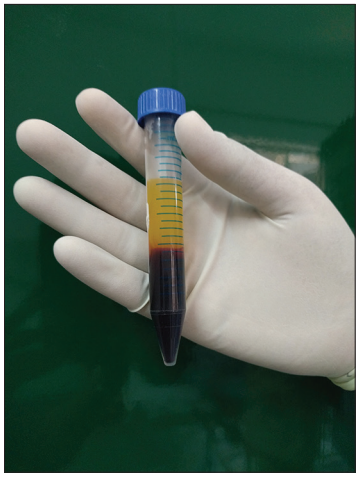


Figure 1: Vacutainer with platelet-rich fibrin clot

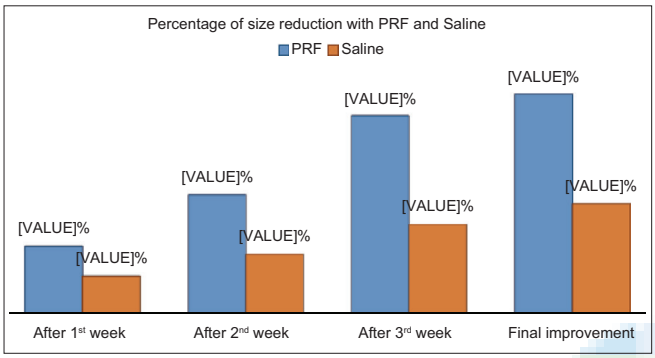


Figure 2: Platelet-rich fibrin placed on the ulcer

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forms an organised network where the platelets and leucocytes are concentrated leading to the sustained release of various GFs, resulting in wound healing. Hence, it can also be used for the treatment of venous ulcers.[4]

A study conducted by Margolis *et al*. which included 26,599 patients, concluded that patients who were treated with products derived from platelets, tend to heal faster than patients who were treated without the products derived from platelets. He also concluded that even though the ulcers that were treated with these derivatives were bigger and deeper than the other groups these showed better improvement at the end of 12 weeks.[5] Our study showed similar results where ulcers treated with PRF showed better and faster improvement when compared to saline dressing.



Bar Diagram 1: The bar diagram above shows the comparison of the mean improvement in PRF group and Saline group after every week and also after the treatment for 4 weeks

In another study, Anitua *et al*. showed that healing increased significantly with the help of PRF. She also concluded that it not only helps in supplying the required GFs but also by forming fibrin matrix which helps in cell migration, it also helps in neo-vascularisation.[6] In this study, there was a mean reduction of ulcer size of 85.51% in the group treated with PRF at the end of 4 weeks.

In another study Mazzucco *et al*. concluded that healing is improved and is much faster when the wound is treated with platelet-rich gel. He stated that this also helps to reduce the hospital stay. In his study, he demonstrated that in patients who were treated with platelet-rich gel the wound healed in 3.5 weeks and the wounds which were not treated with platelet-rich gel took 6 weeks.[7] In our study, 55.55% of patients treated with PRF had complete closure at the end of 4 weeks, whereas no patient treated with saline had complete closure.

In another conducted by Saldalamacchia *et al*., it was concluded that the use of PRF had significant effectiveness in the treatment of ulcers. He stated that the use of PRF had better reduction of area of ulcer when compared with the basic wound care without the use of PRF. He concluded that though in his study he treated the patients with PRF for a short time it showed good improvement.[8] Similarly, in our study, we found mean reduction

Table 1: Patients treated with platelet rich fibrin dressing

Patient number	Initial measurement in cm^2	Measurement after 1st week	Measurement in cm^2 after 2nd week	Measurement in cm^2 after 3rd week	Final measurement in cm^2	Percentage of improvement
1	3.5	2.64	2.64	1.0	1.0	71.42
2	4	3.24	1.96	0.0	0	100
3	15.2	9.92	7.29	5.94	0	100
4	6.16	3.57	0	0	0	100
5	16.96	16.64	13.92	11.44	9.43	44.39
6	5.06	3.80	2.24	0.0	0	100
7	2.0	1.6	1.6	0.0	0	100
8	11.47	10.8	10.08	6.82	3.96	65.47
9	8.91	3.25	1.52	1.04	1.04	88.32
Mean percentage of improvement	0	26.27	46.25	77.08	85.51	85.51

Table 2: Patients treated with saline dressing

Patient number	Initial measurement in cm^2	Measurement after 1st week	Measurement in cm^2 after 2nd week	Measurement in cm^2 after 3rd week	Final measurement in cm^2	Percentage of improvement
1	1.4	1.3	1.2	0.99	0.99	29.28
2	5.7	4.86	4.25	4.08	3.3	42.10
3	13.8	12.6	12.6	12.0	12.0	13.04
4	3.6	2.64	2.4	1.87	1.6	55.55
5	3.68	2.94	2.66	2.21	1.6	56.52
6	1.0	0.9	0.7	0.5	0.4	60.00
Mean percentage of improvement	0	14.55	23.24	34.78	42.74	42.74

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Figure 3: Patient 1 before treatment with platelet-rich fibrin dressing



Figure 4: Patient 1 after 4 weeks of treatment with platelet-rich fibrin dressing



Figure 5: Patient 2 before treatment with platelet-rich fibrin dressing



Figure 6: Patient 2 after 4 weeks of treatment with platelet-rich fibrin dressing



Figure 7: Patient 3 before treatment with saline dressing



Figure 8: Patient 3 after 4 weeks of treatment with saline dressing

of 85.51% in ulcer area at the end of 4 weeks in patients treated with PRF which is faster when compared to saline group.

A study carried out by O'Connell *et al.*, concluded that the treatment of venous and non-venous ulcers had different outcomes. He showed that the patients who had venous ulcers and were treated with PRF membrane had a total closure of wound in 66.7% of patients. Whereas patients with ulcers of non-venous

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Figure 9: Patient 4 before treatment with saline dressing



Figure 10: Patient 4 after 4 weeks of treatment with Saline dressing

Table 3: Group statistics

Variables	Number of patients	Mean	SD	*t*	*P*
Saline	6	42.74	18.52	4.11	<0.001
PRF	9	85.51	20.45		

The study was statistically significant with the $P<0.001$. SD: Standard deviation, PRF: Platelet rich fibrin

origin had total closure in only 44% of patients. Hence, he proved that PRF was very efficacious in the treatment of venous ulcers.[4]

In many studies, there was no significant improvement in the ulcers treated with saline dressing.[5,7-9] Our study showed 42.74% reduction in ulcer size when treated with saline dressing, however, this was less when compared to the 85.51% ulcer area reduction in the PRF dressing group.

Although we had good reduction in the ulcer size in the patients treated with PRF, we need to validate the results with a larger sample size.

Conclusion

This procedure is simple, patient-friendly, cost-effective, painless and can be performed as an outpatient procedure. We would like to conclude that the use of PRF dressings as an adjuvant therapy in the treatment of chronic venous ulcer shows great potential to achieve complete closure of ulcers and can successfully be used as a routine procedure in the management.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bergqvist D, Lindholm C, Nelzén O. Chronic leg ulcers: The impact of venous disease. J Vasc Surg 1999;29:752-5.
2. Evans CJ, Fowkes FG, Ruckley CV, Lee AJ. Prevalence of varicose veins and chronic venous insufficiency in men and women in the general population: Edinburgh Vein Study. J Epidemiol Community Health 1999;53:149-53.
3. Mayer W, Jochmann W, Partsch H. Varicose ulcer: Healing in conservative therapy. A prospective study. Wien Med Wochenschr 1994;144:250-2.
4. O'Connell SM, Impeduglia T, Hessler K, Wang XJ, Carroll RJ, Dardik H. Autologous platelet-rich fibrin matrix as a stimulator of healing of chronic lower extremity ulcers. Wound Repair Regen 2006;14:A76.
5. Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. Diabetes Care 2001;24:483-8.
6. Anitua E, Sanchez M, Nurden AT, Zalduendo M, de la Fuente M, Orive G, *et al.* Autologous fibrin matrices: A potential source of biological mediators that modulate tendon cell activities. J Biomed Mater Res A 2006;77:285-93.
7. Mazzucco L, Medici D, Serra M, Panizza R, Rivara G, Orecchia S, *et al.* The use of autologous platelet gel to treat difficult-to-heal wounds: A pilot study. Transfusion 2004;44:1013-8.
8. Saldalamacchia G, Lapice E, Cuomo V, De Feo E, D'Agostino E, Rivellese AA, *et al.* A controlled study of the use of autologous platelet gel for the treatment of diabetic foot ulcers. Nutr Metab Cardiovasc Dis 2004;14:395-6.
9. Steed DL, Goslen JB, Holloway GA, Malone JM, Bunt TJ, Webster MW. Randomized prospective double-blind trial in healing chronic diabetic foot ulcers. CT-102 activated platelet supernatant, topical versus placebo. Diabetes Care 1992;15:1598-604.

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ORIGINAL RESEARCH ARTICLE

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Depression among elderly diabetics at Peripheral health centres attached to a private medical college in Coimbatore: a cross sectional study

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ABSTRACT

Introduction; Depression is an illness that may affect and be affected by diabetes mellitus. The clinical guidelines for older adults with diabetes mellitus have recognized this by recommending screening for and management of depression in diabetic elderly people. The aim of this study was to find out the prevalence of depression using PHQ -9 questionnaire among elderly diabetics in peripheral primary health care centres attached to a Private medical college. **Objectives:** 1. To find out the prevalence of depression among elderly diabetics 2. To find out the factors associated with depression among elderly diabetics **Methodology:** This cross sectional study was conducted among known diabetics above 60 years of age attending the NCD clinic of Peripheral health centres at Peelamedu, Vedapatti, Neelambur during December 2017 – April 2018. The socio demographic factors and morbidity factors such as fasting blood sugar, duration of diabetes, history of hypertension, past history of diabetes related complications, hours of sleep were included to find out the association with levels of depression. **Results:** Among the study population, 40.4 % were having the signs of depression in which the majority have only minimal symptoms of depression. The factors such as Female Gender, Fasting blood sugar >126 mg/dl and hours of sleep < 7 hours found to be statistically significant association with depression. **Conclusion:** The prevalence of depression using PHQ 9 is 40.4% and the factors such as female gender, fasting blood sugar more than 127 mg/dl and sleep at night less than 7 hours were found to have statistically significant association with depression.

Key word: Depression, PHQ-9, Screening, Prevalence, Elderly diabetics.

INTRODUCTION

Depression is an illness that may affect and be affected by diabetes mellitus. Depression is an independent risk factor for the onset of type 2 diabetes mellitus. It negatively affects the course of diabetes and is associated with increased risk of complications, hyperglycemia, and mortality. Depression may exert its negative effect through hormonal, neuronal, or immune system changes that directly affect the body's ability to produce or use insulin or, the effect of depression may be indirect, by resulting in poor self-care behaviours, such as overeating, drinking alcohol, not exercising, skipping medications, or failing to keep medical appointments. Thus, identifying and treating depression in diabetes is strongly recommended.[1]

Evidence suggests that depression may relate to poorer glycaemic control[2] and that poorer control predicts a poorer course of depression in adult samples.[3]There is some evidence that depression does not prospectively predict worsening of blood glucose control in the elderly, but more research needs to be done to understand this relationship in this group.

In interactions with the health care system, the presence of depression may influence decisions made by health care providers concerning medical outcomes (e.g., blood glucose and blood pressure). For example, providers may set goals that are easier to achieve, yet are less stringent, when they believe that depression will interfere with a patient's ability to adhere to a stricter regimen. Depression can also have an effect on patients' own goals and preferences for treatment, because depressed patients may feel too hopeless or helpless to embark on the lifestyle changes that are most beneficial for diabetes self-care.

Thus, in many different ways, diabetes may become more difficult to manage for elderly people who are also depressed, and depression may become more difficult to resolve in those who have diabetes, resulting in higher health care utilization and medical costs for those with this comorbidity.[4] The clinical guidelines for older adults with diabetes mellitus have recognized this by recommending screening for and management of depression in diabetic elderly people.

Tools such as the short form of the Geriatric Depression Scale, the Patient Health Questionnaire-9, and the newly

developed Depression and Suicide Screen are useful. [5] The present study used PHQ – 9 questionnaire to arrive at a provisional diagnosis of depression as patients with minimal symptoms (score 5-9), minor depression (score 10-14), moderate Major depression (score 15-19), severe major depression (score >20).

The objectives of this study were to find out the prevalence of depression using PHQ -9 questionnaire among elderly diabetics in peripheral primary health care centres attached to a Private medical college and to find out the factors associated with depression.

METHODOLOGY

The Department of community medicine of PSG Institute of Medical sciences and Research has one Urban (Peelamedu) and Two Rural health training centres (Vedapati and Neelambur) to deliver primary health care to people. These centres have basic laboratory services to manage Non communicable diseases (NCD). All these three centres run NCD clinic on a particular day of the week and maintain NCD register. This cross sectional study was conducted among known diabetics above 60 years of age attending the NCD clinic of Peripheral health centres at Peelamedu, Vedapatti, Neelambur during December 2017 – April 2018. By purposive sampling, 104 elderly diabetics who came for review during the study period at NCD clinic were selected. All registered diabetics above 60 years of age attending the NCD clinic were included and those who were on treatment for pre-existing major and minor mental illness were excluded.

After obtaining approval from Institutional Human Ethics Committee, informed consent obtained from the patients at NCD clinics of peripheral health centres. Study Questionnaire with PHQ-9 administered to the patients till the sample size of 100 reached. Questionnaire contains the following items i.e. Socio demographic variables, fasting blood sugar, presence of hypertension, complications related to diabetes and hours of sleep at past night to find out the association of above factors on depression among the study population.

Those patients found to be having depression will be followed up based on degree of depression at the peripheral health centres. Proportions, test of statistical significance (Chi square) and odds ratio with 95% confidence interval (CI) using SPSS version 24 were calculated.

RESULTS

Table 1: Distribution of study population at Peripheral Health Centres

Peripheral Health centres	Study population	Percent (%)
UHTC (Peelamedu)	46	44.2
VHTC (Vedapatti)	29	27.9
NHTC (Neelambur)	29	27.9
Total	104	100

The present study intended to find out the prevalence of depression among elderly diabetics and the factors associated with depression. There were 104 elderly

Table2: Prevalence of depression among the study population

Depression	Study population	Percent (%)
NO DEPRESSION	62	59.6
DEPRESSION	42	40.4
Total	104	100

Table 3: PHQ 9 Grading of Depression among the study population

PHQ 9 Grading	Study population	Percent (%)
NO DEPRESSION	62	59.6
MINIMAL SYMPTOMS	25	24
MINOR DEPRESSION	9	8.7
MODERATE MAJOR DEPRESSION	5	4.8
SEVERE MAJAOR DEPRESSION	3	2.9
Total	104	100

Table 4: Socio Demographic and co-morbidity variables of the study population

Variables	Category	Study population (n=104)	Percent (%)
Age in years	60-69	73	70.2
	70-79	27	26
	>80	4	3.8
Gender	Male	47	45.2
	Female	57	54.8
Educational status	Illiterate	32	30.8
	Primary	39	37.5
	Secondary	26	25
	Intermediate	4	3.8
	College	3	2.9
Marital status	Married	76	73.1
	Divorced	1	1
	Widow	27	26
Socio-economic status (modified Prasad scale August 2017)	CLASS I Rs>5910	22	21.2
	CLASS II Rs 2960 - 5909	34	32.7
	CLASS III Rs 1770-2959	16	15.4
	CLASS IV Rs 890-1769	22	21.2
	CLASS V Rs <890	10	9.6
Duration of Diabetes	<10 YEARS	81	77.9
	>11 YEARS	23	22.1
Fasting blood sugar level	FBS <126 mg/dl	31	29.8
	FBS >127 mg/dl	73	70.2
Hours of sleep	<7 hours	83	79.8
	>8 hours	21	20.2
Hypertension	Yes	53	51
	No	51	49
Complications	Yes	47	45.2
	No	57	54.8

Table 5: Factors associated with depression in the study population by univariate analysis

Study variables	Category	Depression	No depression	Unadjusted Odds Ratio 95% CI	P Value
Age	60-70 years	32(39.5%)	49(60.5%)	1.178	
	>71 years	10(43.5%)	13(56.5%)	(0.46-3.00)	0.732
Gender	Male	12(25.5%)	35(74.5%)	3.241	
	Female	30(52.6%)	27(47.4%)	(1.40-7.48)	**0.005***
Educational status	Up to Primary	30(42.3%)	41(57.7%)	0.781	
	Secondary and above	12(36.4%)	21(63.6%)	(0.33-1.83)	0.569
Marital status	Married	31(40.8%)	45(59.2%)	0.939	
	Divorced/widow	11(39.3%)	17(60.7%)	(0.38-2.27)	0.89
Socioeconomic status	Class I,II & III	27(37.5%)	45(62.5%)	1.471	
	Class IV& V	15(46.9%)	17(53.1%)	(0.63-3.41)	0.368
Duration of Diabetes	<10 YEARS	33(40.7%)	48(59.3%)	0.935	
	>11 YEARS	9(39.1%)	14(60.9%)	(0.36-2.41)	0.544
Fasting blood sugar level	FBS ≤126 mg/dl	8(25.8%)	23(74.2%)	2.506	
	FBS ≥127 mg/dl	34(46.6%)	39(53.4%)	(0.99-6.33)	**0.038***
Hours of sleep	<7 hours	38(45.8%)	45(54.2%)	0.279	
	>8 hours	4(19.0%)	17(81.0%)	(0.08-0.89)	**0.028***
Hypertension	Yes	18(34.0%)	35(66.0%)	0.579	
	No	24(47.1%)	27(52.9%)	(0.26-1.27)	0.174
Complications	Yes	16(34.0%)	31(66.0%)	0.615	
	No	26(45.6%)	31(54.4%)	(0.27-1.36)	0.231

* Statistically significant (p< 0.05)

diabetics (Table. 1) included in the study. Among the study population, 40.4 % were having the signs of depression (Table.2), of which 24% have only minimal symptoms of depression (Table.3).

The socio demographic factors i.e. age, gender, education, income per capita per month and morbidity factors such as fasting blood sugar, duration of diabetes, history of hypertension, past history of diabetes related complications, hours of sleep and PHQ -9 score are shown in Table.4.

Each of the above variables were categorized into two to find out the statistically significant (p< 0.05) association with depression among the study population using chi square test (Table. 5) in SPSS version 24. The factors such as Female Gender, fasting blood sugar >126 mg/dl and hours of sleep < 7 hours found to be statistically significant association with depression in the present study.

DISCUSSION

In the present study the prevalence of depression among elderly with diabetes mellitus was found to be 40.4%. The study conducted by SA Black et al showed that 31.1% of the older diabetic individuals reported high levels of depressive symptoms. [6] Prevalent major depressive episodes were present in 15.4% of the subjects with diabetes, which was significantly higher than in nondiabetic subjects in a study conducted among community-dwelling elderly persons with diabetes mellitus by P. de Jonge et. al. [7] In a study conducted by Manjiri D Pawaskar et al. among Medicare enrolled elderly population with type 2 diabetes mellitus, 17% had depressive symptoms. [8] In a study conducted by Kee-Lee Chou et al, on Prevalence of depression among elderly Chinese with diabetes, 26% of elderly diabetics reported elevated levels of depressive symptoms. [9] Elderly female diabetics are more among depressed (OR 3.2, 95%CI: 1.40-7.48) than men in this study which is statistically significant. Finkelstein EA et al reported diagnosed annual prevalence rates of major depression more among elderly female diabetics. In our study there is no significant association between the subjects with duration of diabetes less than 10 years and more than 10 years whereas in a study done by Yu-Ling Bai et al. a significant difference was found for depression scores between subjects with diabetes for <1 year and those with diabetes for >10 years.[10] Elderly with high Fasting blood sugar >127 mg/dl have higher risk of depression (OR 2.5, 95%CI: 0.99-6.33) than those with Fasting blood sugar <126 mg/dl in the present study. PJ Lustman et al reported in the meta analytic review of 24 studies that Depression is associated with hyperglycemia in patients with type 1 or type 2 diabetes[3]. However, association of depression with hyperglycemia in elderly diabetics is not documented. Elderly diabetics who slept more than 8 hours at night had lesser risk of depression (OR 0.279, 95%CI: 0.08-0.89) than those who slept less than 7 hours at night in this study. The factors such as age, education,

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income, marital status, past history of diabetes related complications and hypertension were not found to be significant in this study. This study has few limitations like very small sample size, FBS is taken as an indicator for glycemic control instead of Hb A1C and PHQ 9 administered by different doctors at three health centres.

Conclusion:

This cross sectional study among elderly diabetics in primary health centre setting of a private medical college revealed that prevalence of depression using PHQ 9 is 40.4% and the factors such as female gender, fasting blood sugar more than 127 mg/dl and sleep at night less than 7 hours were found to have statistically significant association with depression.

REFERENCES

1. Trief PM. Depression in elderly diabetes patients. Diabetes spectrum. 2007 Apr 1;20(2):71-5
2. Finkelstein EA, Bray JW, Chen H, Larson MJ, Miller K, Tompkins C, Keme A, Manderscheid R: Prevalence and costs of major depression among elderly claimants with diabetes. Diabetes Care 2003Feb 1:26:415–420
3. Lustman PJ, Anderson RJ, Freedland KE, DeGroot MK, Carney RM, Clouse RE: Depression and poor glycemic control: a meta-analytic review of the literature. Diabetes Care 2000 23:934 –942,
4. Finkelstein EA, Bray JW, Chen H, Larson MJ, Miller K, Tompkins C, Keme A, Manderscheid R: Prevalence and costs of major depression among elderly claimants with diabetes. Diabetes Care 2003Feb 1:26:415–420,
5. Hoyl MT, Alessi CA, Harker JO, Josephson KR, Piertruszka FM, Koelfgen M, Mervis JR, Fitten LJ, Rubenstein LZ: Development and testing of a five-item version of the Geriatric Depression Scale. J Am Geriatr Soc 1999 47 :873–878,
6. S.A.Black, Increased health burden associated with comorbid depression in older diabetic Mexican Americans. Results from the Hispanic Established Population for the Epidemiologic Study of the Elderly survey; Diabetes Care 1999 Jan;22(1):56-64.
7. De Jonge P, Roy JF, Saz P, Marcos G, Lobo A. Prevalent and incident depression in community-dwelling elderly persons with diabetes mellitus: results from the ZARADEMP project. Diabetologia. 2006 Nov 1;49(11):2627-33
8. Pawaskar MD, Anderson RT, Balkrishnan R. Self-reported predictors of depressive symptomatology in an elderly population with type 2 diabetes mellitus: a prospective cohort study. Health and quality of life outcomes. 2007 Dec;5(1):50
9. Chou KL, Chi I. Prevalence of depression among elderly Chinese with diabetes. International Journal of Geriatric Psychiatry. 2005 Jun;20(6):570-5
10. Bai YL, Chiou CP, Chang YY, Lam HC. Correlates of depression in type 2 diabetic elderly patients: A correlational study. International journal of nursing studies. 2008 Apr 1;45(4):571-9.

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Comparing 24-hour symptom triggered therapy and fixed schedule treatment for alcohol withdrawal symptoms – A randomized control study

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1. Introduction

Acute alcohol withdrawal symptoms are common in patients who are dependent on alcohol. While most of them develop only mild withdrawal symptoms like anxiety, insomnia, tremors, nausea, tachycardia, and diaphoresis, 5–15% develop withdrawal seizures and about 5 % develop delirium tremens (Lerner and Fallon, 1985; Guthrie, 1989). It is estimated that 2–10% of patients with delirium tremens die due to metabolic, cardiovascular or infectious complications. Hence it is prudent to manage alcohol withdrawal symptoms effectively.

As supported by meta-analyses reviews, benzodiazepine is the most effective medication in treating alcohol withdrawal symptoms and preventing complications (Mayo-Smith, 1997; Holbrook et al., 1999). The severity of withdrawal varies among patients, which warrant appropriate benzodiazepine dosing. Also, many patients have only minimal withdrawal symptoms which could be managed safely without using benzodiazepines (Naranjo et al., 1983; Shaw et al., 1981). However, in clinical practice, benzodiazepines are empirically predetermined and administered for all the patients. A better approach of management is symptom-triggered therapy, where patients are monitored using a structured scale (Clinical Institute Withdrawal Assessment for Alcohol scale - CIWA-Ar), and benzodiazepines are administered according to severity of the withdrawal symptoms (Sullivan et al., 1991; Wartenberg et al., 1990).

Symptom-triggered therapy reduces the risk of under medicating or overmedicating a patient with benzodiazepines and results in less total benzodiazepine dosage and shorter duration of treatment. This has been confirmed by 3 randomized controlled trials (Saitz et al., 1994; Daeppen et al., 2002; Sachdeva et al., 2014). In all these trials, the investigators used CIWA-Ar assessments throughout the detoxification phase which was more than 3 days. In resource crunch settings, 1–4 hourly rating with CIWA-Ar for the entire detoxification phase may not be feasible. As withdrawal symptoms start emerging within a few hours after stopping alcohol, we hypothesize that structured 1–4 hourly assessments with CIWA-Ar during the first day of detoxification alone could predict the required effective dose of benzodiazepine and the amount received on the first day could be effectively tapered down in the subsequent days.

We conducted a randomized controlled trial comparing the efficacy of 2 detoxification regimens: 1) 24 h structured assessment with CIWA-Ar scale to initiate Symptom Triggered Therapy and 2) Fixed Schedule Treatment.

2. Methods

2.1. Study population

This study was conducted in the de-addiction ward which is part of the psychiatry department of PSG Institute of Medical Science and Research, a tertiary teaching medical college hospital. All consecutive patients between the ages of 16 and 65 years, who got admitted and met the DSM IV criteria for alcohol dependence syndrome when interviewed using Structured Clinical Interview, 1998 schedule for Diagnostic and Statistical Manual IV (SCID IV), were included in the study.

Patients with delirium which was not due to alcohol withdrawal were excluded from the study. Patients with co-morbid Axis I psychiatric disorders and with other co-morbid substance use disorders except nicotine were also excluded. We got written informed consent from the patients and the family members who had accompanied the patients. Patients who were not having capacity to give consent because of delirium were included only if their key family members gave valid consent. Our study was approved by the human ethics board of our institution.

2.2. Measurements

The eligible patients were personally interviewed by one of the postgraduate trainees (SS or S) to get details regarding their demographic characteristics, medical comorbidities, and use of substances by using a semi-structured proforma. We used a translated Tamil version of Severity of Alcohol Dependence Questionnaire (SADQ) to assess the severity of dependence (Stockwell et al., 1994).

We randomized the consecutive recruited patients using computer generated randomization technique into 2 detoxification regimes: 1) 24 h Symptom Triggered Treatment (24 HR -STT) and 2) Fixed Schedule Treatment (FST). We used Tamil translated version of the CIWA-Ar scale for administering benzodiazepines in the 24 HR-STT

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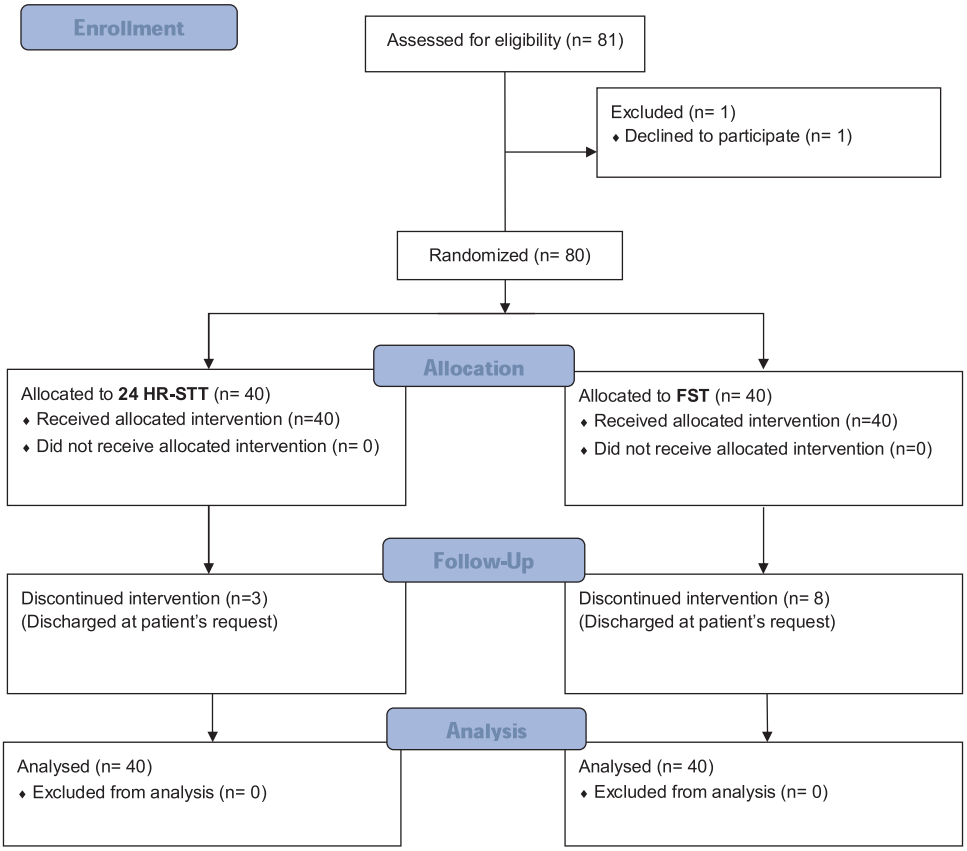


Fig. 1. CONSORT Flow Diagram.

group.

Detoxification regimes:

1) 24 h Symptom Triggered Treatment (24 HR -STT):

We gave a structured training to all the nursing staff in the psychiatry ward in administering CIWA – Ar scale. A didactic lecture was followed by a live demonstration of administering CIWA – Ar on 3 patients with different levels of withdrawal symptoms. Later, the nursing staff were shown a video showing an interview conducted to rate CIWA – Ar on 2 different patients in withdrawal state, and the nurses were asked to rate on CIWA – Ar independently. We found that the single measure intra class correlation co-efficient was 0.898 indicating a high level of agreement for all the items of CIWA – Ar (Venkateswaran Rajaraman, 2017).

For the first 24 h, a nursing staff or a resident on the ward administered CIWA-Ar every one hour and if the score came below 10 for three consecutive ratings, then the rating was done once in 4 h. Whenever the score was greater than or equal to 10, that patient was given chlordiazepoxide 20 mg (or lorazepam 1 mg, if there was liver dysfunction). The total amount of benzodiazepine received on the first day was given on the 2nd day as divided doses and from 3rd day onwards chlordiazepoxide was tapered by 10 mg every day (1 mg per day, in case of lorazepam).

2) Fixed Schedule treatment (FST):

The clinician who admitted the patient, based on the clinical factors, decided the initial dose of benzodiazepine (chlordiazepoxide or lorazepam) and from 3rd day onwards the benzodiazepine dosage was tapered (chlordiazepoxide: 10 mg per day/ lorazepam: 1 mg per day) and stopped.

2.3. Outcome measures

The total dose of benzodiazepines administered and the duration of detoxification in both the treatment groups were our primary outcome measures. An independent investigator, who was blind to the treatment allocation, administered CIWA-Ar rating for both the groups, every morning between 8 a.m. and 10 a.m., for a period of 7 days. This measured how effectively withdrawal symptoms were treated in both the groups. We also estimated for any new onset delirium tremens and seizures during the detoxification period.

2.4. Sample size calculation

Before launching the current study, we did a pilot study on a group of 25 patients who got admitted with alcohol dependence syndrome. We found that for the entire detoxification period, they needed average139.6 mg (SD = 70.44) of chlordiazepoxide, which was decided by Fixed Schedule Treatment method, to manage withdrawal symptoms, over the mean duration of 6.11 ± 1.9 days. We proposed that by adopting 24 h Symptom Triggered Treatment the total dose of chlordiazepoxide should be reduced by at least 50 mg to be considered as clinically meaningful. Using the formula, $2 \times [SD] \times (Z\alpha + Z\beta) /$ Mean difference (Kim, 2016), we found that to observe a significant group difference with 90 % power and an alpha of 0.05 (two-tailed), we needed a sample size of 31 patients in each group. Considering a drop-out rate of about 30 %, we estimated that we would require a sample size of 40 in each group.

2.5. Statistical analyses

We tested for normality of continuous variables by examining their histograms and with Shapiro-Wilks test. Student *t*-test was used to compare continuous variables like age, monthly income, SADQ total scores, and number of detoxification days between the two regimens. We used Mann Whitney *U* test to compare CIWA-Ar baseline scores,

total benzodiazepine dose, daily benzodiazepine doses, duration of detoxification and daily CIWA-Ar scores between the two treatment regimens. Chi-square test was used to examine associations between categorical variables. All the patients who were randomized were included in the analysis and for the patients who dropped out, we used last observation carried forward analysis.

3. Results

3.1. Sample characteristics

All the patients who were screened for this study were males and spoke Tamil language. At the stage of screening one patient refused to give consent; we recruited consecutive 80 patients who fulfilled our inclusion criteria and randomized into the 2 detoxification groups: 24 h Symptom triggered treatment (24 HR –STT) = 40 and Fixed Schedule treatment (FST) = 40. Eleven patients (24 HR –STT = 3 and FST = 8) dropped out of the study as they got discharged before completion of their detoxification and their data till drop out were used in the analyses (Fig. 1).

Mean age of the subjects was 40.21 years (SD = 9.54), 83.8 % were married and were earning on an average Rs. 14,805(SD = 13,019.27) per month. The mean score on severity of alcohol dependence questionnaire (SADQ) was 29.65(S.D = 9.83), which amounts to moderate dependence. Demographic characteristics like age, marital status and monthly earnings were similar in both 24 HR –STT and FST groups. The last intake of alcohol was within 12 h prior to the initial assessment and all patients were within the withdrawal period. At the baseline assessment, there were also no differences in severity of dependence, severity of withdrawal symptoms and number of patients with delirium tremens between the 2 groups. None of the patients had withdrawal seizures or hallucinations. All the patients received Thiamine (oral and intramuscular) and oral vitamin B complex. None of the patients received antipsychotics or benzodiazepines other than chlordiazepoxide and lorazepam (Table 1).

3.2. Benzodiazepine and treatment outcome

Lorazepam was used for detoxification in 5 patients (24 HR –STT = 1 and FST = 4) and for the rest of the patients chlordiazepoxide was used and all of them received the same brand of benzodiazepines by oral route. While all the patients in the FST group received benzodiazepines, 12 patients (30 %) in the 24 HR –STT group didn't need any benzodiazepine. Among patients who received benzodiazepines, the median total dose (chlordiazepoxide equivalence) was 95 mg lower in 24 HR –STT group than FST group, which was statistically significant. During the first 6 days of detoxification, the amount of benzodiazepine received on each day was significantly lower in 24 HR –STT group than FST group. Also, the duration of detoxification was significantly shorter in 24 HR –STT than in FST. Median scores of CIWA Ar, as assessed by an independent assessor, in both the groups were similar and low suggesting that the withdrawal symptoms were effectively managed (Table 2).

3.3. Delirium tremens

The clinical diagnosis of Delirium Tremens was made based on ICD 10 Classification of Mental and Behavioural disorders: Clinical descriptions and diagnostic guidelines. At baseline 9 patients had delirium tremens (24 HR –STT = 5 and FST = 4) and during the detoxification phase, 2 patients developed delirium tremens (24 HR –STT = 1 on 3rd day; FST = 1 on 2nd day). Among patients with delirium tremens, those who were in 24 HR –STT group needed 135 mg more total chlordiazepoxide mean dose than FST group; but this difference didn't reach statistical significance. Among patients without delirium tremens, 24 HR –STT group needed 168.18 mg lesser total chlordiazepoxide mean dose than FST group, and the difference was statistically significant (Table 3).

4. Discussion

Our data supported our hypothesis that using 24 h Symptom Triggered Treatment, in patients who are withdrawing from alcohol, can result in: less benzodiazepine dose, shorten detoxification period and avoid unnecessary benzodiazepines, without incidence of any withdrawal related complications.

4.1. Benzodiazepine use

FST and 24 HR –STT groups were similar in demographic and alcohol related variables (severity of dependence and withdrawal symptoms as measured by SADQ and CIWA-Ar scales respectively).

24 HR –STT group received significantly less chlordiazepoxide equivalence dose (median 115 mg) than FST group (median 210 mg). The results are consonant with other studies which had compared Symptom triggered treatment with Fixed schedule treatment: Saitz et al. (1994) (100 mg versus 425 mg of chlordiazepoxide), Daeppen et al. (2002) (37.5 mg versus 231.4 mg of oxazepam) and Sachdeva et al. (2014) (9.5 mg versus 19.9 mg of lorazepam). In our study, patients in the 24 HR – STT group had significantly shorter duration of detoxification (120 h) than patients in the FST group (144 h) ($p = 0.0001$). Again, this finding is consonant with the earlier studies: Daeppen et al. (2002) (20 versus 62.7 h), Saitz et al. (1994) (9 versus 68 h) and Sachdeva et al. (2014) (47.8 versus 146 h). While these studies (Saitz et al., 1994; Daeppen et al., 2002; Sachdeva et al., 2014) had used the CIWA-Ar rating for first 3 days, we had used it only for the first 24 h, but we were able to achieve similar results. Thus restricting CIWA-Ar rating to only first 24 h of detoxification is more time efficient and effective at the same time.

Another statistically significant finding favouring the 24 HR-STT

Table 1
Baseline demographic characteristics and alcohol history by treatment group.

Characteristics	24 Hrs -Symptom Triggered Treatment (24 HR –STT) N = 40	Fixed Schedule Treatment (FST) N = 40	Statistics
Demographic and clinical characteristics			
Age (Mean)	40.05 ± 8.66	40.38 ± 10.46	$t(78) = 0.151, p = 0.88^a$
Marital status Married	32(80 %)	35(87.5 %)	$X^2(1) = 0.83, p = 0.36^b$
Monthly income (Rs.) (Mean)	15,225 ± 13,700.99	14,385 ± 12,460.73	$t(78) = -0.287, p = 0.76^a$
Baseline features of alcohol dependence syndrome			
SADQ score (Mean)	29.63 ± 9.86	29.68 ± 9.92	$t(78) = 0.023, p = 0.98^a$
Baseline CIWA-Ar (Mean (Median) ± SD)	10.40(10) ± 7.46	7.83 (6) ± 6.53	$U = 620.5, p = 0.084^c$
Delirium tremens n (%)	5(12.8 %)	4(10.0 %)	$X^2(1) = 0.156, p = 0.69^b$

SADQ: Severity of alcohol dependence questionnaire, CIWA-Ar: Clinical Institute Withdrawal Assessment of Alcohol scale, a: student *t*-test, b: chi-square test, c: mann whitney *U* test.

Table 2
Comparison of Treatment Outcomes by Treatment Group.

Characteristics	24 Hrs Symptom Triggered Treatment (24 HR –STT) N = 40	Fixed Schedule Treatment (FST) N = 40	Statistics
Chlordiazepoxide equivalent dose (mg) Mean (Median) ± SD			
Total	170.50 (115) ± 227.21	286.25 (210) ± 258.06	$U = 406, p < 0.0001^c$
Day 1	41.03 (40) ± 48.98	66.50 (60) ± 35.77	$U = 360.5, p < 0.0001^c$
Day 2	38.72 (40) ± 43.84	58.50 (50) ± 34.83	$U = 433.5, p = 0.001^c$
Day 3	31.54 (30) ± 37.53	48.50 (40) ± 33.86	$U = 446, p = 0.001^c$
Day 4	23.95 (20) ± 32.09	38.25 (30) ± 32.57	$U = 451, p = 0.002^c$
Day 5	18.86 (10) ± 26.21	29.21 (20) ± 30.44	$U = 444, p = 0.013^c$
Day 6	12.19 (0) ± 21.21	20.00 (10) ± 27.39	$U = 417.5, p = 0.026^c$
Day 7	7.50 (0) ± 14.59	13.06 (0) ± 23.88	$U = 498, p = 0.26^c$
No. of patients who didn't receive any BZ (%)	12 (30)	0 (0)	$X^2(1) = 14.12, p < 0.0001^b$
Duration of detoxification (days) (Mean (Median) ± SD)	3.90 (5) ± 3.19	6.35 (6) ± 2.05	$U = 427.5, p = 0.0001^c$
CIWA –AR ratings Mean (Median) ± SD			
Day 2	4.51 (4) ± 4.88	6.62 (4) ± 6.55	$U = 618.5, p = 0.11^c$
Day 3	3.54 (2) ± 4.87	3.75 (3) ± 5.49	$U = 702, p = 0.44^c$
Day 4	3.26 (2) ± 4.59	2.15 (2) ± 3.17	$U = 677.5, p = 0.30^c$
Day 5	2.00 (1) ± 3.56	1.39 (1) ± 2.00	$U = 6655\ p = 0.83^c$
Day 6	1.72 (0) ± 2.79	1.24 (0) ± 1.99	$U = 564, p = 0.72^c$
Day 7	1.26 (0) ± 2.24	1.14 (0) ± 2.47	$U = 534.5, p = 0.74^c$

CIWA-Ar: Clinical Institute Withdrawal Assessment of Alcohol scale, b:chi-square test, c: mannwhitney *U* test.

Table 3
Treatment outcome among patients with and without delirium tremens.

	24 Hrs Symptom Triggered Treatment (24 HR –STT)	Fixed Schedule Treatment (FST)	Statistic Mann-Whitney U test
Total Chlordiazepoxide equivalent dose (mg) Mean (Median) ± SD			
Patients with Delirium Tremens (n = 11)	635 (560.00) ± 184.15	500 (620.00) ± 237.38	U = 12 P = 0.583
Patients without Delirium Tremens (n = 69)	88.53 (50.00) ± 98.60	255.71 (210.00) ± 249.16	U = 181.000 P < .0001

group was that 30 % of patients in this group did not require any benzodiazepine, where as all the patients in the Fixed Schedule Treatment group received benzodiazepines during detoxification even though the severity of baseline withdrawal score in both these groups were comparable. Two studies have reported even a higher percentage of patients who didn't require benzodiazepines: Daeppen et al. (2002) - 60.7 % and Foy et al. (1988) - 46 %.Thus using symptom triggered treatment helps to identify patients who can be safely managed without prescribing benzodiazepines.

Using benzodiazepines for alcohol withdrawal, by whatever method, doesn't seem to result in benzodiazepine abuse or dependence. In our study, benzodiazepines were stopped in a very short period in both the groups (24 HR –STT: 3.90 (5) ± 3.19 days and FST: 6.35 (6) ± 2.05 days). Shree Shilpa et al. (2019) found that, among 109 Benzodiazepine abusers in a psychiatric setting, none of them were prescribed for alcohol withdrawal.

4.2. Course of withdrawal

Even though the median dose and duration of detoxification was lesser in the 24 HR-STT group, the withdrawal symptoms were effectively controlled in both the treatment groups as shown by very low CIWA-Ar ratings from the 2nd day as assessed by an independent rater, who was masked about the treatment allocation.

4.3. Implementation of STT

Administration of CIWA-Ar is simple to learn and we have successfully trained nursing staff and residents in using this (Venkateswaran Rajaraman, 2017). A physician could advice nurses to assess patients using CIWA-Ar at regular intervals on the first day of hospital admission.

The implementation of symptom triggered treatment for alcohol withdrawal in an in-patient unit includes not only training of the personnel but also the choice of patients who need symptom triggered treatment. Studies have shown inappropriate use of symptom triggered treatment in general hospital settings due to indiscriminate use of CIWA-Ar without properly establishing two important selection criteria for STT, such as the diagnosis of alcohol dependence and the communication ability of the patient (Hecksel KA et al., 2008).The authors in the study had identified that patients with medical comorbidities were more likely to be wrongly initiated on symptom triggered treatment when the above mentioned criteria were overlooked. Also, Bostwick and Lapid (2004) had reported in their case studies of 4 medically complicated patients, in which physicians had overlooked the period of abstinence in patients with remote history of alcohol use. This had resulted in inappropriate use of CIWA-Ar scale when the patient was not in withdrawal. Therefore, even though the CIWA-Ar scale is easy to administer and nurses can be trained to administer benzodiazepines according to symptom triggered regimen, physicians have to meticulously assess and establish the alcohol dependence criteria and current state of withdrawal in the patient before initiating symptom triggered treatment protocol.

4.4. Limitations

Generally studies exclude patients younger than 18 years; in our study, in the inclusion criteria we had set the age range as 16–65, however the age of recruited patients ranged from 24 to 65 (Mean: 40.21 ± 9.55). Not being a double blind study was our biggest limitation; but by carefully adhering to the study protocol, we tried to overcome any investigator bias. Also, in the Fixed Schedule Treatment group, the doses of benzodiazepines were decided by the primary treating psychiatrists who were not part of the investigative team and they were unaware of our hypotheses. The tapering of doses were different for chlordiazepoxide (10 mg per day) and lorazepam (1 mg per day); however it is unlikely to affect the result as out of 80 patients, only 5 had received lorazepam (24 HR –STT = 1 and FST = 4). During

the study, none of the patients suffered from any known adverse effects due to chlordiazepoxide or lorazepam; however not having a structured assessment for adverse effects is a limitation. But, dropout rates were less in the 24-HRSTT group than the FST group thus favoring the symptom triggered treatment.

To assess the severity, we used Severity of Alcohol Dependence Questionnaire (SADQ); however we didn't capture the type and exact quantity of alcohol consumed. Complicated withdrawal symptoms like delirium tremens, withdrawal seizures and alcoholic hallucinosis were poorly represented in our sample and thus findings may not be generalizable for use in these conditions, for which further studies are needed. We had not captured the physical parameters in a meaningful way which could be analysed and presented.

The study setting happened in a psychiatry ward where we have psychiatry residents and nursing staff with psychiatry skills. Hence, the efficacy of such interventions may not be the same in other medical settings.

5. Conclusion

Symptom triggered treatment using CIWA-Ar scale for the first 24 h seems to be a favourable, safe and effective method of detoxification for alcohol related withdrawal symptoms among hospitalized patients, where nursing staff or residents are trained in using the CIWA-Ar to administer the benzodiazepines.

Declaration of Competing Interest

None.

References

Bostwick, J.M., Lapid, M.I., 2004. False positives on the Clinical Institute Withdrawal Assessment for Alcohol-Revised: is this scale appropriate for use in the medically ill? Psychosomatics 45 (3), 256–261.

Daeppen, J.-B., Gache, P., Landry, U., Sekera, E., Schweizer, V., Gloor, S., Yersin, B., 2002. Symptom-triggered vs fixed-schedule doses of benzodiazepine for alcohol withdrawal. Arch. Intern. Med. 162, 1117–1121.

Foy, A., March, S., Drinkwater, V., 1988. Use of an objective clinical scale in the assessment and management of alcohol withdrawal in a large hospital. Alcohol. Clin. Exp. Res. 12, 360–364.

Guthrie, S.K., 1989. The treatment of alcohol withdrawal. Pharmacotherapy 9, 131–143.

Hecksel KA, J., Bostwick, J.M., Jaeger, T.M., Cha, S.S., 2008. Inappropriate use of symptom-triggered therapy for alcohol withdrawal in the general hospital. Mayo Clin. Proc. 83 (March 3), 274–279.

Holbrook, A.M., Crowther, R., Lotter, A., Cheng, C., King, D., 1999. Meta-analysis of Benzodiazepineuse in the treatment of acute alcolol withdrawal. CMAJ 160, 649–655.

Kim, H.Y., 2016. Statistical notes for clinical researchers: sample size calculation 1. Comparison of two independent sample means. Restor. Dent. Endod. 41 (1), 74–78. https://doi.org/10.5395/rde.2016.41.1.74.

Lerner, W.D., Fallon, H.J., 1985. The alcohol withdrawal syndrome. N. Engl. J. Med. 313 915-2.

Mayo-Smith, M.F., 1997. For the American Society of Addiction Medicine. Pharmacologicalmanagement of alcohol withdrawal: a meta-analysis and evidence-basedpractice guideline. JAMA 278, 144–151.

Naranjo, C.A., Sellers, E.M., Chater, K., Iversen, P., Roach, C., Sykora, K., 1983. Nonpharmacologic interventionin acute alcohol withdrawal. ClinPharmacolTher. 34, 214–219.

Sachdeva, A., Chandra, M., Deshpande, S.N., 2014. A comparative study of fixed tapering dose regimen versus symptom-triggered regimen of lorazepam for alcohol detoxification. Alcohol Alcohol. 49 (3), 287–291.

Saitz, R., Mayo-Smith, M.F., Roberts, M.S., Redmond, H.A., Bernard, D.R., Calkins, D.R., 1994. Individualized treatment for alcohol withdrawal: a double-blind controlled trial. JAMA. 272, 519–523.

Shaw, J.M., Kolesar, G.S., Sellers, E.M., Kaplan, H.L., Sandor, P., 1981. Development of optimal treatment tacticsfor alcohol withdrawal, I: assessment and effectivenessof supportive care. J ClinPsychopharmacol 1, 382–389.

Shree Shilpa, H.A., Kumar, Naveen, Maheswari, E., Virupaksha, H.S., Subeesh, Viswam, Rajalekshmi Saraswathy, G., Kunnavil, Radhika, 2019. Deprescribing of benzodiazepines and Z-drugs amongst the psychiatric patients of a tertiary care Hospital. Asian J Psychiatry 2019 (44), 189–194.

Structured Clinical Interview, 1998. Version 2.0 for DSM-IV Alcohol Use Disorders; Revised August.

Stockwell, T., Sitharan, T., McGrath, D., 1994. Lang; the measurement of alcohol dependence and impaired control in community samples. Addiction 89, 167–174.

Sullivan, J.T., Swift, R.M., Lewis, D.C., 1991. Benzodiazepinerequirements during alcohol withdrawal syndrome: clinical implications of using a standardizedwithdrawal scale. J ClinPsychopharmacol. 11, 291–295.

Venkateswaran Rajaraman, 2017. A training program for nursing staff to improve their knowledge and skills in the assessment of alcohol dependence syndrome. University Journal of Medicine and Medical Specialities 3 (5) The TN Dr MGR medical university; 2017.

Wartenberg, A.A., Nirenberg, T.D., Liepman, M.R., Silvia, L.Y., Begin, A.M., Monti, P.M., 1990. Detoxification of alcoholics: improving care by symptom-triggered sedation. Alcohol ClinExp Res. 14, 71–75.

Research Report

PHENOMENOLOGY OF DELIRIUM IN DECOMPENSATED LIVER DISEASE PATIENTS-A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: Decompensated liver disease (DCLD) is associated with various cognitive changes. The present study aimed to estimate the prevalence, phenomenology, and course of delirium in decompensated liver disease patients. **Methods**: This prospective observational study was done on 111 patients admitted to the gastroenterology department with the diagnosis of DCLD. Richmond Agitation Sedation Scale (RASS) and confusion assessment method (CAM) were used to detect delirium for the first five consecutive days. Patients detected to be having delirium were administered a delirium rating scale (DRS) for the next seven days to evaluate the features of delirium. Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. For normally distributed quantitative parameters, the mean values were compared using an independent sample t-test. Categorical outcomes were compared between study groups using the chi-square test. coGuide version V.1.0 was used for statistical analysis. **Results:** The prevalence of delirium among patients with DCLD was 34 (30.63%). All delirious patients continued to have delirium symptoms for the initial three days. Six patients remitted as early as the fourth day of DRS assessment, while nine patients continued to have delirium symptoms even by the seventh day of evaluation. **Conclusion:** Nearly one-third of patients with DCLD presented with delirium. They presented with insomnia, cognitive deficits, motor retardation, and minimal psychotic symptoms. Many patients completely recovered from delirium within a week, but a few had delirium up to the seventh day.

Keywords: delirium, Hepatitis, hepatic encephalopathy, cirrhosis

Introduction

Delirium is a complex neuropsychiatric disorder and a potentially life-threatening acute brain dysfunction. Among the various causes of delirium, decompensated liver disease (DCLD) is an important one. [1] The literature on DCLD has dealt in-depth with various cognitive changes associated with it. Various terms are given for it as hepatic encephalopathy, minimal hepatic encephalopathy, episodic and persistent encephalopathy.[2] During the natural course of DCLD like fatty liver (steatosis), followed by inflammation ofthe liver tissue (hepatitis), scar tissue formation (fibrosis), and finally, destruction of liver architecture (cirrhosis) cause a decrease in the number of functional liver cells resulting in an inability to remove toxic substances from the blood. As a result, ammonia and manganese accumulate in the brain leading to structural

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changes in the astrocytes. These factors contribute to altered neurotransmission and brain dysfunction.

Based on psychomotor features, two clinical subtypes of delirium have been described; hyperactive and hypoactive.[3] Some patients fluctuate between these two and fall into the third subtype called mixed delirium.[4] Hyperactive delirium is characterized by agitated behaviour with increased psychomotor activity.[5] Alcohol withdrawal is a classic example of hyperactive delirium. Patients with hypoactive delirium are quiet and lethargic with decreased psychomotor activity.[5] Probably, these phenotypes signify different forms of neurologic dysfunction. However, as of now, clinically, there is no substantial understanding to pinpoint the aetiology.[6]

The prevalence of cognitive changes among patients with decompensated liver disease (DCLD) ranges from 30%-60%. The annual risk of a patient with DCLD developing hepatic encephalopathy is 20%, and 60%-80% of the patients with DCLD have some form of cognitive impairment during their lifetime.[7] Survival probability among DCLD patients who developed encephalopathy was 42% at one year and 23% at three years.[8] Prompt identification and management of this condition reduces mortality, morbidity and also shortens hospital stay.[9] Literature available includes studies on clinical profile, plasma ammonia levels in patients with liver failure with or without hepatic encephalopathy (HE) and prognostic factors associated with HE.[10–12] Longitudinal studies are found to be looking into the phenomena of all-cause delirium.[13] Though there are Indian studies on all-cause delirium [14], studies looking specifically at hepatic encephalopathy (HE) are rare. Hence, the current study aimed to study the phenomenology and course of delirium in decompensated liver disease patients. The objectives were to estimate the prevalence of delirium in patients admitted with decompensated liver disease and determine the association between delirium with clinical and laboratory parameters.

MATERIALS AND METHODS

Study population and study site: The study was conducted on patients admitted in the ward and ICU of a PSG Institute of Medical Science and Research, Coimbatore, with decompensated liver disease (DCLD) (based on clinical examination and supportive laboratory test reports).

Study design: Prospective observational study.

Study duration: Eight months from May 2013 to January 2014.

Inclusion Criteria:

1. Patients who were diagnosed with decompensated liver disease (DCLD).
2. Age between15 to 80 years.

Exclusion criteria:

1. Patients who were having severe mental disorders and mental retardation.
2. Patients on ventilators.
3. Patients were having severe impairment in vision (blindness) and hearing as they could not undergo the psychological tests.

Sample size: The sample size was calculated assuming the prevalence of delirium as 20% as per the study by FF Poordad et al.[15] The other parameters considered for sample size calculation were 8% absolute precision and a 95% confidence level. The following formula was used for sample size calculation.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n = Sample size, Z= Z statistic for the level of confidence, P= Expected prevalence of proportion (if the expected prevalence is 20%, then P= 0.2), and d = Absolute precision (if the precision is 8%, then d=0.08). The required number of subjects as per the calculation mentioned above was 97. To account for a non-participation rate of about 4% (four subjects), it was decided to sample about 111 subjects into the study.

Sampling method: All the eligible subjects were recruited into the study by consecutive sampling until the sample size was reached.

Ethical considerations: The Institutional Human Ethics Committee, PSG Institute of Medical Sciences & Research , Coimbatore approved the study, and the proposal approval number is 13/064. Written informed consent was obtained from the patient, and most were not capable of giving informed consent due to episodes of delirium at entry. Consent from the nearest key family member was taken in these cases.

Data collection tools and clinical examination

Patient proforma: Consists of data regarding socio-demographic profile, substance use details, clinical and laboratory parameters. A single examiner (psychiatrist) administered all the scales.

Various scales used for measurement:

SADQ (severity of alcohol dependence questionnaire)

The Severity of Alcohol dependence questionnaire was developed by Addiction Research Unit at Maudsley Hospital in 1983. It covers five aspects of alcohol dependence like Physical withdrawal symptoms, Affective withdrawal symptoms, relief in drinking, the frequency of alcohol consumption, and the speed with which onset of withdrawal symptoms occurs. It focuses on the last six months of heavy alcohol drinking. It takes 2-5 minutes to administer this questionnaire.[16]

RASS (Richmond Agitation Sedation Scale)

In our study, we use this scale to assess the level of consciousness of the patient. The Richmond Agitation Sedation Scale was developed at Virginia Commonwealth University in Richmond by a multidisciplinary team, and it can be performed in less than 20 seconds and requires minimal training.

CAM-ICU (confusion assessment method)

Confusion Assessment Method- ICU was developed by Sharon Inouye et al. in 1990, subsequently revised in October 2010. It is a bedside assessment tool, even usable by non-psychiatrists. This was designed mainly for ICU patients who are critically ill, with or without a ventilator, and unable to talk. It takes 5-8 minutes to assess the presence or absence of Delirium.[17]

The Delirium Rating Scale-Revised 98 (DRS-R-98) and administration of various scales:

The timings for data collection were scheduled between 5-7 pm because it is well known that delirium worsens during evening hours. The severity of alcohol dependence questionnaire (SADQ) was used on patients with a history of alcohol use. Each of the patients was administered RASS and CAM-ICU scales daily for five days to detect delirium. If a patient was detected to be having delirium on any of those five days, from that day onwards DRS-R-98 scale was administered. The onset of delirium was considered as the primary outcome variable. Data collection was scheduled between 5-7 pm of all the seven days of the study period (every 24 hours) to avoid overlap of symptoms and better capture of signs. DRS-R-98 scale is a validated scale with a high inter-rater reliability score ranging from 0.66–0.99, specificity ranging from 79%-88%, and sensitivity of 83%-88%. It is a 16-item clinician-rated scale, and it has two sections. First, 13 items are for assessing delirium symptoms severity, and the next 3 are diagnostic items. First, 13 items are rated from 0-3 points, and diagnostic items are rated from 0-2.[18]

Statistical Methods: The onset of delirium was considered as the primary outcome variable. Symptoms at different follow-up periods were considered as the primary explanatory variable. Demographic variables (like age, gender), clinical variables, and laboratory parameters were considered other study-relevant variables. Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. For normally distributed quantitative parameters, the mean values were compared using an independent sample t-test. Categorical outcomes were compared between study groups using the chi-square test. P-value <0.05 was considered statistically significant. The coGuide version V.1.0 was used for statistical analysis.[19]

RESULTS

In our study, the prevalence of delirium among patients with DCLD was 30.63% (34). 94 (84.7%) were males in the study sample, and 17(15.3%) were females. More than half of the study group had been diagnosed with alcohol-induced DCLD 64(57.7%). The majority of patients with DCLD had portal HTN91(82%), ascites 43(38.7%), and oesophagal varices 36 (32.4%) were almost equally distributed in the entire sample. Among111 patients, 71 patients (64%) had a history of alcohol consumption in their lifetime. The number of patients using nicotine in the form of smoking was 48(43.2%), and in the form of tobacco, chewing was noted only in 10 (9%) of patients. Among the patients who had a history of alcohol consumption in their lifetime, around 36 (50%) consumed alcohol within one month before hospital admission. (Table 1)

Patients continued to have sleep disturbance throughout one week of delirium. Sleep disturbances seen were sleep-wake cycle disruption, insomnia, day-night

reversal, and frequent daytime naps. From the fourth day onwards, psychotic symptoms (delusions, perceptual abnormalities, and thought disorder) started

Table 1: Summary of demographic variables and clinical parameters (N=111)

Petameters	Summary
Gender	
Male	94 (84.7%)
Female	17 (15.3%)
Age	53.6 ± 8 years
DCLD subtypes	
DCLDalcohol-induced	64 (57.7%)
DCLD-cryptogenic	15 (13.5%)
DCLD- other types	32 (28.8%)
Manifestations of DCLD	
Portal hypertension	91 (82%)
Ascites	43 (38.7%)
Esophageal varices	36 (32.4%)
Mean total bilirubin	6.58 ± 0.25
Mean SGPT	49.31 ± 5.33
Mean SGOT	84.38 ± 9.45
Mean alkaline phosphatase	133 ± 20.15
Mean proteins total	6.27 ± 0.18
Substance use	
Alcohol	71 (64%)
Nicotine (smoking)	48 (43.2%)
Nicotine (Chewable)	10 (9%)
Last drink of Alcohol consumption (n=71)	
0 to 30 days of admission	36 (50.7%)
between 30 to 90 days of admission	8 (11.2%)
>90 days of admission	27 (38.0%)
SADQ severity of alcohol dependence (no=51)	
Mild	3 (5.8%)
Moderate	28 (54.9%)
Severe	20 (39.2%)
The onset of Delirium (no=34)	
Day 1	23 (67.6%)
Day 2	7 (20.5%)
Day 3	4 (11.7%)
Last drink (N=71)	
<7 days	26 (36.62%)
>7 days	45 (63.38%)

Patients continued to have sleep disturbance throughout one week of delirium. Sleep disturbances seen were sleep-wake cycle disruption, insomnia, day-night reversal, and frequent daytime naps. From the fourth day onwards, psychotic symptoms (delusions, perceptual abnormalities, and thought disorder) started reducing and were absent on the sixth and seventh days. On day 7, patients continued to have the following symptoms; sleep disturbance 9 (100%), motor retardation 4(44.4%). The cognitive deficits which continued were disorientation, inattention, visuospatial deficits, short-term memory loss, and long-term memory loss. (Table 2)

We looked for association with hepatic encephalopathy by considering various parameters like age, gender, the severity of DCLD presentation, alcohol use, and LFT values. We compared these parameters between delirious and non-delirious patients. There were no statistically significant differences between delirious and non-delirious in various parameters like age, gender, the severity of DCLD presentation, and LFT parameters like (SGPT, SGOT, alkaline phosphatase, total protein, GGT) (p-value >0.05) except for bilirubin, which showed statistical significance. (Table 3)

DISCUSSION

In our study, the prevalence of delirium in patients with decompensated liver disease was 30.63%. Among the various causes of decompensated liver disease, alcohol-induced DCLD was seen in most patients (57%). While analyzing the association, none of the factors like age, gender, alcohol, or severity of liver dysfunction was associated with delirium except for bilirubin in DCLD patients.

The mean age of the participants was 53.6±8 years, and 84.7% were males, and 15.3% were females. A study on cirrhotic hepatitis C patients had a mean age of 57.8 ± 7 and 63%, and 37% were males and females, respectively.[20]

Our study was comparable to other studies on cognitive changes in decompensated liver disease (30%-60%) in the prevalence of delirium.[21] Overt hepatic encephalopathy occurs in approximately 30%–45% of cirrhotic patients and 10%–50% of patients with the transjugular intrahepatic portosystemic shunt.[22] Minimal hepatic encephalopathy, characterized by

Table 2: Course of each symptom of delirium in 7days period

SL. No	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Insomnia	34(100%)	29 (96.6%)	25 96.1%)	17 (94.4%)	9 (81.8%)	10 (90.9%)	9 (100%)
Perceptual isturbance	10 (29.4%)	7 (23.3%)	4 (15.3%)	2 (11.1%)	0	0	0
Delusions	10 (29.4%)	7 (23.3%)	3 (11.5%)	0	0	0	0
Affect disturbances	31 (91.1%)	23 (76.6%)	12 46.1%)	10 (55.5%)	5 (45.4%)	3 (27.2%)	1 (11.1%)
Language deficits	18 (52.9%)	12 (40%)	4 (15.3%)	2 (11.1%)	1 (9%)	0	0
Thought disorder	11 (32.3%)	6 (20%)	4 (15.3%)	1 (5.5%)	1 (9%)	0	0
Motor agitation	18 (52.9%)	16 (53.3%)	10 38.4%)	8 (44.4%)	5 (45.4%)	2 (18.1%)	2(2 2.2%)
Motor retardation	32 (94.1%)	22 (73.3%)	16 61.5%)	10 (55.5%)	5 (45.4%)	4 (36.3%)	4 (44.4%)
Disorientation	34 (100%)	26 (86.6%)	14 53.8%)	8 (44.4%)	4 (36.3%)	2 (18.1%)	2 (22.2%)
Inattention	30(88.2%)	21 (70%)	11 42.3%)	4 (22.2%)	4 (36.3%)	2 (18.1%)	2 (22.2%)
Short-term memory	25 (73.5%)	15 (50%)	9 (34.6%)	5 (27.7%)	3 (27.2%)	2 (18.1%)	2 (22.2%)
Long-term memory	19 (55.8%)	13 (43.3%)	6 (23%)	4 (22.2%)	1 (9%)	1 (9%)	1 (11.1%)
Visuo-spatial deficits	17 (50%)	12 (40%)	6 (23%)	4 (22.2%)	1 (9%)	1 (9%)	1 (11.1%)
Total D-pts	34	30	26	18	11	11	9 (26.4%)

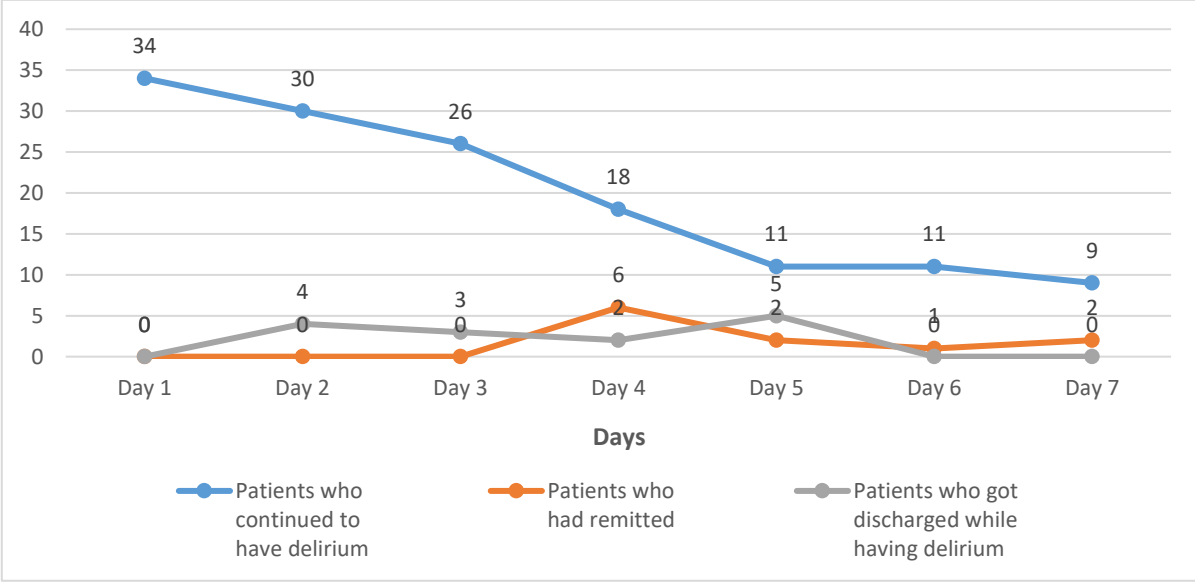
Table 3: Comparison of demographic variables and clinical parameters between delirium and non-delirium (N=111)

Parameters	Delirium (N=34)	Non-delirium (n=77)	P value
Mean age	55.09	52.96	0.372‡
Gender			
Male	30(88.2%)	64(83.1%)	0.476†
Female	4(11.8%)	13(16.9%)	
The severity of DCLD presentation			
Portal HTN	26(76.5%)	65(84.4%)	0.422†
Esophageal varices	13(38.2%)	23(29.9%)	0.382†
Ascites	11(32.4%)	32(41.6%)	0.404†
LFT parameters			
Total Bilirubin	8.65 ± 0.13	5.67 ± 0.09	0.010‡
SGPT	38.53 ± 3.08	51.88 ± 5.11	0.908‡
SGOT	77.88 ± 8.90	87.25 ± 7.78	0.482‡
Alkaline Phosphatase	144.26 ± 12.33	128.16 ± 11.45	0.435‡
Total Protein	6.30 ± 1.01	6.26 ± 0.99	0.828‡
GGT	96.68 ± 3.34	141.61 ± 2.56	0.954‡
Last drink			
<7days	9(34.6%)	17(65.4%)	0.797†
>7days	14(31.1%)	31(68.9%)	

‡*Independent sample t-test, † chi-square test*

subtle motor and cognitive deficits, affects approximately 20%–60% of patients with liver disease.[8] The true incidence and prevalence of hepatic encephalopathy is difficult to establish because of

Figure 1: Course of Delirium as evaluated by Delirium rating scale (DRS)



considerable differences in the aetiology and severity of hepatic encephalopathy and the varying tools in diagnosing.

Unlike the previously used scales like West-Haven Criteria, PSE-syndrome test (Portosystemic encephalopathy Syndrome Test and Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) test, which detects cognitive changes rather than delirium, we have used RASS and CAM-ICU, which detect delirium due to any cause. Unlike previous scales (which need physicians and psychologists to assess), nursing staff can administer these scales with minimal time and monetary resources.

To the best of our knowledge, this is the first study to look into the breadth of the phenomenology of delirium in patients with DCLD. Our study describes delirium in detail by tracing out and defining 13 of its symptoms over seven days (Table 3). By unfolding how exactly each of these symptoms remitted in a week, this study also portrays the course of delirium.

On meticulously observing the symptoms of delirium, sleep disturbance is present in all patients, and it is present throughout delirium. Sleep disturbance includes sleep-wake cycle disturbance, day-night reversal, nocturnal disturbance only, or frequent daytime naps. Sleep-wake inversion or the combination of restless nights and excessive daytime sleepiness were first described as a sign of overt HE by Sherlock et al.[23] Similar findings were recorded in studies with both insomnia and fragmented sleep with multiple night awakening sin cirrhosis patients.[24]

The next glaring symptom is disorientation, where the person is disoriented to time, place, and person in increasing order of severity. While recovering, the patient first gets oriented to person, and the last thing he gets oriented to is time. Almost 100% of the patient had disorientation on day one. Similar results were shown in studies where almost all participants showed disorientation features.[25] A recent longitudinal study of the phenomenology of delirium showed the prevalence of disorientation in all visits to be 81% with DRS-R-98 and 69% with the cognitive test for delirium (CTD).[13] Another longitudinal study (Fan et al. 2005) has suggested that psychomotor abnormalities and sleep-wake cycle disturbances present mainly in the early course of a delirium.[26] In contrast, disorientation, inattention, impaired memory, and sleep disturbances are present throughout delirium (McCusker et al., 2003).[27]

Another important area in delirium symptoms is cognitive deficits. Cognitive deficits consist of inattention, memory impairment, visuospatial disturbances. The cognitive symptom was one of the persistent symptoms patients had while getting discharged. Studies were done in the past, giving weightage on how to detect delirium in patients with a

subclinical presentation, mainly in cognitive deficits.[9] A study done to assess all-cause delirium's phenomenology using the MDAS scale showed an increased prevalence of perceptual disturbances (e.g., hallucinations) and delusions. In contrast, the present study showed less prevalent symptoms for perceptual disturbances and delusions.[28] The difference may be due to the scales used in both studies and the latter used only for DCLD patients.

Psychotic symptoms experienced were thought disorder, delusions, and perceptual abnormalities. Delusions predominantly seen were persecutory. Perceptual abnormalities ranged from illusions to hallucinations, predominantly auditory. We also found that psychotic symptoms were the first to remit among all the delirium symptoms. More prevalent symptoms such as hallucinations, illusions, and delusions were seen in patients with the activated subtype in a study done by Ross et al.[14]

Delirium was fully remitted in 11 patients within a week, but 14 patients got discharged within seven days while still having some features of delirium. Three of these patients requested discharge for personal reasons, and four patients got discharged against medical advice. The remaining seven patients were discharged from the hospital side when they still had minimal delirious features (like motor retardation and cognitive deficits).

This study also tried to analyze the association of delirium following hepatic encephalopathy with other factors. Age, gender, marital status, socio-economic status, and education did not significantly affect delirium. Moreover, on generally comparing LFT results between delirious and non-delirious patients, patients with delirium showed significantly more deranged bilirubin values. This is also supported by previous studies.[29]

The main strength of our study was that the participants were followed up by a week to assess the course of delirium in decompensated liver disease in detail.

Limitations: We don't have data on patients who had a more extended hospital stay in which delirium could have developed after five days (stopped visiting DCLD patients on the fifth day itself according to our study protocol). We did not consider other parameters associated with delirium, like biochemical parameters (electrolyte, ammonia, arterial blood gas, etc.), cardiac or renal parameters. Also, pharmacotherapy was not assessed, such as benzodiazepines for alcohol withdrawal that could have precipitated encephalopathy in some patients.

Conclusions: We conclude by saying that a significant number of patients having DCLD present with delirium. They present with insomnia, cognitive deficits, motor retardation, and minimal psychotic symptoms. Many patients completely recovered from delirium within a week, but few had delirium for more than one week. No significant association was seen in delirium patients with liver function tests and alcohol consumption except for bilirubin.

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Conflict of interest:

None declared.

REFERENCES

1. Mardini H, Saxby BK, Record CO. Computerized Psychometric Testing in Minimal Encephalopathy and Modulation by Nitrogen Challenge and Liver Transplant. Gastroenterology. 2008;135:1582–90.
2. Córdoba J, Sanpedro F, Alonso J, Rovira A. 1H magnetic resonance in the study of hepatic encephalopathy in humans. Metab Brain Dis. 2002;17:415–29.
3. Sanders RD. Delirium, neurotransmission, and network connectivity: The search for a comprehensive pathogenic framework. Anesthesiology. 2013;118:494–6.
4. Morandi A, Pandharipande P, Trabucchi M, Rozzini R, Mistraletti G, Trompeo AC, et al. Understanding international differences in terminology for delirium and other types of acute brain dysfunction in critically ill patients. Intensive Care Med. 2008;34(10):1907–15.
5. Meyer T, Eshelman A, Abouljoud M. Neuropsychological Changes in a Large Sample of Liver Transplant Candidates. Transplant Proc. 2006;38(10):3559–60.
6. Reinehr R, Görg B, Becker S, Qvartskhava N, Bidmon HJ, Selbach O, et al. Hypoosmotic swelling and ammonia increase oxidative stress by NADPH oxidase in cultured astrocytes and vital brain slices. Glia. 2007;55(7):758–71.
7. Perry W, Hilsabeck RC, Hassanein T. Cognitive dysfunction in chronic hepatitis C: A review. Dig Dis Sci. 2008;53(2):307–21.

8. Bajaj JS, Saeian K, Verber MD, Hischke D, Hoffmann RG, Franco J, et al. Inhibitory control test is a simple method to diagnose minimal hepatic encephalopathy and predict development of overt hepatic encephalopathy. Am J Gastroenterol. 2007;102(4):754–60.
9. Bajaj JS. Review article: The modern management of hepatic encephalopathy. Aliment Pharmacol Ther. 2010;31(5):537–47.
10. Butterworth RF. Hepatic encephalopathy - A serious complication of alcoholic liver disease. Alcohol Res Heal. 2003;27(2):143–5.
11. Kundra A, Jain A, Banga A, Bajaj G, Kar P. Evaluation of plasma ammonia levels in patients with acute liver failure and chronic liver disease and its correlation with the severity of hepatic encephalopathy and clinical features of raised intracranial tension. Clin Biochem. 2005;38(8):696–9.
12. Onyekwere CA, Ogbera AO, Hameed L. Chronic liver disease and hepatic encephalopathy: Clinical profile and outcomes. Niger J Clin Pract. 2011;14(2):181–5.
13. Leonard M, Adamis D, Saunders J, Trzepacz P, Meagher D. A longitudinal study of delirium phenomenology indicates widespread neural dysfunction. Palliat Support Care. 2015;13(2):187–96.
14. Ross CA, Peyser CE, Shapiro I, Folstein MF. Delirium: Phenomenologic and Etiologic Subtypes. Int Psychogeriatrics. 1991;3(2):135–47.
15. Poordad FF. The burden of hepatic encephalopathy. Aliment Pharmacol Ther. 2007;25:3–9.
16. Stockwell T, Murphy D, Hodgson R. The Severity of Alcohol Dependence Questionnaire: Its Use, Reliability and Validity. Br J Addict. 1983;78(2):145–55.
17. Inouye SK, Van Dyck CH, Alessi CA, Balkin S, Siegal AP, Horwitz RI. Clarifying confusion: The confusion assessment method: A new method for detection of delirium. Ann Intern Med. 1990;113(12):941–8.
18. Ortiz M, Córdoba J, Doval E, Jacas C, Pujadas F, Esteban R, et al. Development of a clinical hepatic encephalopathy staging scale. Aliment Pharmacol Ther. 2007;26(6):859–67.
19. BDSS Corp. Released 2020. coGuide Statistics software, Version 1.0, India: BDSS corp. Available from: https://www.coguide.in. [Last accessed on 2021 Aug 10].
20. Barboza KC, Salinas LM, Sahebjam F, Jesudian AB, Weisberg IL, Sigal SH. Impact of depressive symptoms and hepatic encephalopathy on health-related quality of life in cirrhotic hepatitis C patients. Metab Brain Dis. 2016;31(4):869–80.
21. Bustamante J, Rimola A, Ventura PJ, Navasa M, Cirera I, Reggiardo V, et al. Prognostic significance of hepatic encephalopathy in patients with cirrhosis. J Hepatol. 1999;30(5):890–5.
22. Papatheodoridis G V., Goulis J, Leandro G, Patch D, Burroughs AK. Transjugular intrahepatic portosystemic shunt compared with endoscopic treatment for prevention of variceal rebleeding: A meta-analysis. Hepatology. 1999;30(3):612–22.
23. Sherlock S, Summerskill WHJ, White LP, Phear EA. Portal-Systemic Encephalopathy Neurological Complications of Liver Disease. Lancet. 1954;264:453–7.
24. Montagnese S, Middleton B, Skene DJ, Morgan MY. Night-time sleep disturbance does not correlate with neuropsychiatric impairment in patients with cirrhosis. Liver Int. 2009;29(9):1372–82.
25. Grover S, Agarwal M, Sharma A, Mattoo SK, Avasthi A, Chakrabarti S, et al. Symptoms and aetiology of delirium: A comparison of elderly and adult patients. East Asian Arch Psychiatry. 2013;23(2):56–64.
26. Fann JR, Alfano CM, Burington BE, Roth-Roemer S, Katon WJ, Syrjala KL. Clinical presentation of delirium in patients undergoing hematopoietic stem cell transplantation. Cancer. 2005;103:810–20.
27. McCusker J, Cole M, Dendukuri N, Han L BÉ. The course of delirium in older medical inpatients: A prospective study. J Gen Intern Med. 2003;18:696–704.
28. Boettger S, Breitbart W. Phenomenology of the subtypes of delirium: Phenomenological differences between hyperactive and hypoactive delirium. Palliat Support Care. 2011;9(2):129–35.
29. Krieger D, Krieger S, Theilmann L, Jansen O, Gass P, Lichtnecker H. Manganese and chronic hepatic encephalopathy. Lancet. 1995;346:270–4.

Original Research Article

2nd to 4th Digit Ratio (2D:4D) – Is It a Marker for Dyslexia ?

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ABSTRACT

Background: Specific learning disorder in general called as dyslexia is a neuro developmental disorder, which occurs more commonly in males (Male:Female ratio is 4:1), there are evidence to suggest role of prenatal gonadal hormone exposure in neurodevelopment. However, studies on association between 2nd to 4th digit ratio and dyslexia are mixed. Objective: To compare the 2nd and 4th digit ratio (2D:4D) of both hands between children with dyslexia and children without dyslexia.
Methodology: A case control study, recruited 30 children with dyslexia and 30 age and education matched normal controls, after getting informed consent from their parents. Collected socio demographic details, applied CARS, Schonell's reading and spelling test, followed by detailed evaluation by psychiatrist and clinical psychologist, including psychological testing to confirm the diagnosis of dyslexia. The digit length of index and ring finger on both hands of all children were measured using Vernier Calliper by direct method.
Results: In both the hands 2D:4D ratio was more in children with dyslexia than normal children, but the difference was not significant (p – 0.146). Analysis of only male samples showed digit ratio is higher in dyslexic children than normal children; which were significant (p – 0.03).
Conclusion: Our study does not support an association between digit ratios and dyslexia; however, trend towards higher ratio in dyslexia especially in male children are interesting and needs further exploration. Need more studies in this area to form a definite conclusion.

Keywords: Dyslexia, Specific learning disorder, Digit ratio, 2D:4D, Biomarker.

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INTRODUCTION

Dyslexia is a neuro - developmental disorder, Prevalence of dyslexia ranges from 4 % to 17%. The prevalence is higher in males (Male: Female ratio is 4:1),[1]. This suggests there could be some factors that is different in either sex which plays a role in the development of this disorder. The neurobiology of dyslexia remains largely unknown but evidence suggests that neuronal migration and axon guidance pathways may be disrupted in dyslexia [2].

Normal brain is sexually dimorphic, due to the influence of gonadal hormones on neurodevelopment during antenatal period. Aberrations in neurodevelopment due to gonadal hormonal imbalance are one of the mechanisms for developing dyslexia. The relation between imbalance in gonadal hormones and dyslexia is very complex.

The absence of testosterone leads the central nervous system to develop passively, in a primarily female fashion, while the presence of testosterone induces the masculinization of the brain, indicating that the sexual dimorphism of the brain is mediated by the epigenetic action of gonadal steroids [3]. Geschwind and Galaburda suggested that testosterone inhibited the growth of certain areas of the left hemisphere and

facilitated the growth of the same areas in the right hemisphere. As a result, language skills would be negatively affected and hand preference would tend to shift toward the left. In extreme forms, this abnormal cortical development would render an individual incapable of handling effectively the linguistic tasks required for reading and writing [4]. Ramus, based on animal studies demonstrated that rodents with induced ectopias and microgyri exhibit learning deficits. Focal cortical abnormalities in specific areas of left perisylvian cortex are the primary cause of dyslexia. When foetal testosterone exposure is high, cortical disruption propagates to the thalamus, provoking additional sensory impairments as a consequence of the conjunction of high fetal testosterone exposure and ectopias or microgyri [5]. A study showed high prenatal testosterone may impair developing auditory perceptual processes and phonological processing in the left hemisphere. These impairments in phonology in turn can adversely affect development in reading [6].

But it's not possible to measure the prenatal gonadal hormone exposure directly. Hence, we need to evaluate it indirectly using an indicator which could predict gonadal hormones concentration in utero. The digit ratio is the ratio of the lengths of different digits, typically as measured from the basal / proximal crease of the finger to the tip of the finger. Androgen and Estrogen differentially regulate the network of genes that controls condrocyte proliferation, leading to differential growth of digits in males and females [7].

The ratio between the length of the 2nd and 4th digits is: (a) fixed in utero; (b) lower in men than in women; (c) negatively related to testosterone and sperm counts; and (d) positively related to estrogen concentrations [8]. 2D:4D ratio may be used as an indicator and predictive factor in a variety of disorders associated with a disturbed testosterone/estrogen hormone balance like Gender identity disorder, Autism, Dyslexia, Migraine, Stammering, Immune dysfunction, Infertility [8].

The existing literature shows mixed results regarding the relation between digit ratio and dyslexia; there is also ongoing debate whether 2nd digit to 4th digit ratio is a reliable indicator of prenatal gonadal hormones exposure. Few researchers even question the proposed role of gonadal hormones in the development of dyslexia. This is an interesting area and has a wide scope for research. There are many studies on digit ratio in autism, but only very few studies in dyslexia. Hence this study is planned to examine the relationship between 2nd digit to 4th digit ratio and dyslexia. A reliable finding in this area of research would have a very significant clinical implication; this has prompted us to carry out this study.

The objective of the study was to compare the 2^{nd} to 4^{th} digit ratio (2D:4D) of both hands between children with dyslexia and children without dyslexia.

METHODOLOGY

Hypothesis

Children with specific learning disability (Dyslexia) will have smaller 2^{nd} digit to 4^{th} digit ratio compared to children without dyslexia.

Subjects and Procedure

We screened all children aged between 5 and 18 years old, who attended psychiatric outpatient department in PSG institute of medical sciences and research, between May 1st 2014 and July 31st 2014. PSG institute of medical sciences and research is a tertiary care general hospital. A final year MBBS student who got trained in applying Schonell reading test, Schonell spelling test and Childhood Autism Rating Scale administered these tests on all children. All children were evaluated in detail by a psychiatrist; the diagnosis of dyslexia and the type of dyslexia was confirmed only after further psychological testing by the child psychologist. We consecutively recruited 30 children with dyslexia who fulfilled the inclusion and exclusion criteria.

Control group had 30 age and education matched children who visited hospital for non psychiatric problems or were recruited from the community. Control group didn't had any academic difficulties or psychiatric problem according to parents, Schonell reading test, Schonell spelling test and childhood autism rating scale was administered to all children in control group as well, child suspected to have dyslexia or autism were referred to psychiatry for further evaluation.

Children were included in the study after getting informed consent from their parents and only if the child was willing to participate in the study. Children with autism, Asperger's syndrome, mental retardation,

attention deficit hyperactivity disorder, schizophrenia, medical or surgical condition which would interfere with measurement of digit length e.g., contractures, trauma, amputation etc, were excluded from the study. Children who were recruited for the study, the length of their index finger and ring finger (from the basal crease to the tip of the finger) of both the hands is measured using Vernier Calliper by direct method, which is an accurate as well as easy method to measure the digit length [9].
The study was approved by Institutional human ethics committee, PSG institute of medical sciences and research.

Materials

1. **Semi structured proforma:** This Proforma is used to get basic details of the cases and controls. It includes the name, age, sex, education medium, syllabus, socio economic status, height, weight, IQ, co morbidity.
2. **Childhood Autism Rating Scale (CARS):** It is a tool for assessing autism. This is scored based on the interview with the primary care giver and observation of the child. The child is diagnosed as having autism if they score above 30 [10].
3. **Schonell reading test:** A reading test that determines the reading age, however it does not test reading comprehension. The reading age is calculated from the number of words read correctly and compared to the student's chronological age to see if the student is reading at the level for their age, or are reading either below or above level for their age [11].
4. **Schonell spelling test:** A spelling test that determines the spelling age. It comprises of 100 words, and the child is asked to write 30 words (applicable for their class).The spelling age is calculated from the number of words wrote correctly and compared to the student's chronological age to see if the student is writing at the level for their age, or are writing either below or above level for their age [12].

STATISTICAL ANALYSIS

Statistical analysis was done using the Statistical package for social sciences (SPSS) version-18. Continuous variables were assessed using the unpaired t-test and categorical variables were assessed using the chi-square test. P value of less than 0.05 is considered as statistically significant.

RESULTS

Table 1 – Socio-demographic variables

		CASE (N=30)	CONTROL (N=30)	p Value
Age (Mean, SD) in years		11.60 ± 3.328	13.40 ± 2.568	0.092
Education (Mean, SD) in years		6.90 ± 2.998	8.50 ± 2.610	0.470
Sex	**Female**	3	18	0.001
	Male	27	12	
Medium	**English**	28	27	0.640
	Tamil	2	3	
Syllabus	**CBSE**	1	29	0.313
	Samachir	0	30	
SES	**Lower middle**	0	6	0.010
	Upper middle	27	24	
	Upper	3	0	

The mean age of cases and controls were 11.6 years and 13.4 years respectively, the difference in age between the 2 groups was not statistically significant. There is no statistical difference between the 2 groups on other socio demographic profiles like education, medium, syllabus and socioeconomic status. Most of the children

on either group were from upper middle socioeconomic class, studied in English medium and their syllabus was samachir, which is the most popular syllabus in the state of Tamil Nadu, India.
The children with dyslexia were predominantly males (90% of the cases) were as in control group only 40 % were males; this was statistically significant (p = 0.001).

Table 2: 2D:4D ratio between male and female children

Male Vs Female (n-39) (n-21)		t	df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	-0.761	58	0.450	-0.01239	-0.04498	0.02020
	Equal variances not assumed	-0.674	29.596	0.506	-0.01239	-0.04994	0.02517
Lt hand 2D:4D	Equal variances assumed	-0.761	58	0.450	-0.01239	-0.04498	0.02020
	Equal variances not assumed	-0.674	29.596	0.506	-0.01239	-0.04994	0.02517

Taken all 60 children together and divided them into 2 groups based on sex, male children had lower 2nd to 4th digit ratio on either hand when compared to controls, shown in (table 2), but the difference is not significant (p = 0.506).

Table 3: 2D:4D ratio between SLD and control groups

SLD Vs Control (n-30) (n-30)		t	Df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	1.490	58	0.142	0.02283	-0.00784	0.05349
	Equal variances not assumed	1.490	33.222	0.146	0.02283	-0.00833	0.05398
Lt hand 2D:4D	Equal variances assumed	1.490	58	0.142	0.02283	-0.00784	0.05349
	Equal variances not assumed	1.490	33.222	0.146	0.02283	-0.00833	0.05398

The comparison of 2nd to 4th digit ratio using independent t test between children with dyslexia and normal children showed that the mean digit ratio on both hands is more in children with dyslexia than normal children, which is in contrary to our hypothesis, however this was not statistically significant (p = 0.146). Our sample predominantly contained children with writing disorder (23/30 children). So, we decided to compare the 2D: 4D ratios between children with writing disorder only and controls.
As shown above in (table 4) the 2nd digit to 4th digit ratio in both right and left hand was higher in children with writing disorder than control group, but this again was not significant (p = 0.134). The sub-analysis comparing children with writing disorder and controls yielded a result similar to dyslexia group in general. Since the number of children with reading and mathematics disorder were very few, it was not possible to do a separate analysis on them. As dyslexia is more common in males and testosterone is expected to play a major role, a separate analysis was done comparing the digit ratio between male children with and without dyslexia.
The 2nd to 4th digit ratio on either hand was higher in male children with dyslexia compared to male children without dyslexia; the difference was statistically significant with a p value of 0.03. This is shown in (table 5).

Table 4: 2D:4D Ratio between dysgraphia children and controls

Dysgraphia Vs Control (n-23) (n-30)		T	Df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	1.372	51	0.176	0.02404	-0.01115	0.05923
	Equal variances not assumed	1.533	35.936	0.134	0.02404	-0.00776	0.05585
Lt hand 2D:4D	Equal variances assumed	1.372	51	0.176	0.02404	-0.01115	0.05923
	Equal variances not assumed	1.533	35.936	0.134	0.02404	-0.00776	0.05585

Table 5: 2D:4D Ratio in male children

Male children Case Vs Control (n-27) (n-12)		T	Df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	3.442	37	0.001	0.05263	0.02165	0.08361
	Equal variances not assumed	2.456	12.001	0.030	0.05263	0.00594	0.09932
Lt hand 2D:4D	Equal variances assumed	3.442	37	0.001	0.05263	0.02165	0.08361
	Equal variances not assumed	2.456	12.001	0.030	0.05263	0.00594	0.09932

DISCUSSION

There are very few studies in this area, to best of our knowledge this is the first study in India looking into the relation between 2nd digit to 4th digit ratio and dyslexia. We have taken 30 children with dyslexia (cases) and 30 normal age and education matched controls. There was a significant difference between the two groups with respect to gender distribution, predominance of male children in dyslexia group could be explained by the general prevalence of dyslexia, which is more common in males (Male: Female ratio is 4:1) and ours being a consecutive sampling.

The current study did not show any difference in the 2nd to 4th digit ratio between children with dyslexia and normal children, neither the right hand comparison nor the left hand comparison between groups showed significant difference, which is in contrast to the observation made by Manning et al 2002 who reported 2nd digit to 4th digit ratio is low in dyslexia compared to Non dyslexic individuals. But our finding is in concurrence with the findings of two other studies, which also reported no association between 2D:4D ratio and dyslexia. A study done in Netherland on children with dyslexia with a phonological deficit showed no evidence for an association between reading disabilities and the 2nd digit to 4th digit ratio, also showed no support for the assumed relation between the 2nd digit to 4th digit ratio and prenatal testosterone[10]. Another study showed digit ratios were sexually dimorphic 'male lower than female' pattern, no difference in digit ratio between dyslexic and normal reading children. Digit ratios were not related to any sensory measure.[11]

The present study showed that 2nd to 4th digit was lower in children without dyslexia than in children with dyslexia, which is contrary to our own hypothesis that 2nd to 4th digit ratio will be low in dyslexic children compared to normal children, and contradicts those studies which shows an association between digit ratio and dyslexia. This finding is difficult to explain by any existing literature on dyslexia and digit ratio. However similar finding was reported in a study on 2nd to 4th digit ratio among 107 individual across all departments and all academic grades, who completed an Internet-based questionnaire on autism or dyslexia in self and family members, the study showed an association between autism and lower 2nd digit to 4th digit ratio, whereas interestingly dyslexia was associated with a higher 2nd to 4th digit ratio in that study [12].

Males had a lower digit ratio compared to females irrespective of being dyslexic or not, points in favour of the concept of sexual dimorphism in digit ratio as suggested by a study [13], however could not agree fully because the difference was not significant.

Some of the theories put forward to explain dyslexia and digit ratio are testosterone theory in the context of cerebral lateralization with testosterone suppressing certain areas in left hemisphere, Extreme male brain theory which suggests excess masculinization of brain leading to dyslexia.

In this study there is no difference in the digit ratio between left hand and right hand nor there do any association of digit ratio in either of this hand and dyslexia. Comparison of only male samples between groups also revealed a higher ratio in children with dyslexia than normal children; this was significant ($p = 0.03$). These findings are in contrast to theories explaining dyslexia and digit ratio.

Our finding does not support an association between digit ratio and dyslexia; neither has it supported the cerebral lateralization theory or extreme male brain theory. The reasons could be digit ratio depends on the production of testosterone and sensitivity of digits to foetal testosterone, which can vary across different ethnic groups, this can explain why there is no difference in digit ratio between groups but this can't explain the reason for lower ratio in controls.

May be there is no real association between dyslexia and digit ratio as supported by studies, 2nd to 4th digit length may not be an indicator of prenatal gonadal hormone exposure 14 and most of the studies supporting the association has not directly compared dyslexia with normal children, it was either done on children with family history of dyslexia or comparing individuals in different academic fields [12]. So, findings in these studies could be influenced by some confounding factors.

Strength of the study

The sample in our study is homogenous and it's a direct comparison between children with dyslexia and without dyslexia. We recruited the children consecutively so any bias in sampling is avoided. Exclusion of comorbid condition like autism, Asperger's syndrome etc which could have confounded the findings if present, as studies also show an association between these disorders and digit ratio.15 All children in dyslexia group were evaluated in detail by a psychiatrist and clinical child psychologist. Using direct method to measure digit ratio by Vernier Calliper, which is an accurate as well as easy method to measure digit length.

Limitations of the study

The findings of this study should be interpreted with caution because of small sample size, possibility of type II error could not be ruled out. The investigator was not blind to the diagnosis, however any bias in measurement was unlikely as the results contradicts our own hypothesis. Measurement was done by a single investigator; having 2 or 3 persons to measure the digit could have added more reliability and validity to the findings. Controls were recruited based on the parents report on their children's academic performance, physical and mental status, not evaluated by psychiatrist or clinical psychologist, it's possible that children with subtle psychological disturbance are missed and mistakenly recruited as normal controls. Despite all these limitations, the finding of this study is unique and cannot be dismissed.

CONCLUSION

This study does not support the association of 2nd digit to 4th digit ratio and dyslexia; neither do the theories put forward to support such an association. However, this study has certain limitations, we need more studies in this area with a larger sample size to overcome above limitations and form a definite conclusion.

The finding children with dyslexia having a higher digit ratio than controls especially in male samples are interesting and needs further exploration.

REFERENCES

1. Ruiz P. Comprehensive textbook of psychiatry. Sadock BJ, Sadock VA, editors. Philadelphia: Lippincott Williams & Wilkins; 2000.
2. Galaburda AM. Ordinary and extraordinary brain development: Anatomical variation in developmental dyslexia. Ann Dyslexia 1989;39(1):65-80.
3. Arnold AP, Gorski RA. Gonadal steroid induction of structural sex differences in the central nervous system. Ann Rev Neurosci 1984;7(1):413-42.
4. Geschwind N, Galaburda AM. Cerebral lateralization: Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. Arch Neurol 1985;42(5):428-59.
5. Ramus F. Neurobiology of dyslexia: A reinterpretation of the data. Trends Neurosci 2004;27(12):720-6.
6. Beech JR, Beauvois MW. Early experience of sex hormones as a predictor of reading, phonology, and auditory perception. Brain Lang 2006;96(1):49-58.
7. Manning JT, Scutt D, Wilson J, Lewis-Jones DI. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Human Reprod 1998;13(11):3000-4.
8. Manning JT, Bundred PE. The ratio of 2nd to 4th digit length: a new predictor of disease predisposition?. Med Hypoth 2000;54(5):855-7.
9. Kemper CJ, Schwerdtfeger A. Comparing indirect methods of digit ratio (2D: 4D) measurement. Am J Hum Biol 2009;21(2):188-91.
10. van Gelder M, Tijms J, Hoeks J, Manning JT. 'Second to fourth digit ratio and dyslexia: no evidence for an association between reading disabilities and the 2D: 4D ratio'/'Manning replies'. Dev Med Child Neurol 2005;47(10):718.
11. Boets B, De Smedt B, Wouters J, Lemay K, Ghesquière P. No relation between 2D: 4D fetal testosterone marker and dyslexia. Neuroreport 2007;18(14):1487-91.
12. Brosnan MJ. Digit ratio and faculty membership: Implications for the relationship between prenatal testosterone and academia. Br J Psychol 2006;97(4):455-66.
13. Manning JT, Barley L, Walton J, Lewis-Jones DI, Trivers RL, Singh D, Thornhill R, Rohde P, Bereczkei T, Henzi P, Soler M. The 2nd: 4th digit ratio, sexual dimorphism, population differences, and reproductive success: evidence for sexually antagonistic genes?. Evol Hum Behav 2000;21(3):163-83.
14. Kratochvíl L, Flegr J. Differences in the 2nd to 4th digit length ratio in humans reflect shifts along the common allometric line. Biol Letters 2009;5(5):643-6.
15. Manning JT, Baron-Cohen S, Wheelwright S, Sanders G. The 2nd to 4th digit ratio and autism. Dev Med Child Neurol 2001;43(3):160-4.

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ORIGINAL ARTICLE

Trajectory of insight on various dimensions among bipolar disorder in-patients

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ABSTRACT

Background: Insight is awareness of one's symptoms, illness, and need for treatment. In bipolar disorder, insight is better in bipolar depression and nonpsychotic depression when compared with mania and psychotic depression. Insight impacts on medication adherence. In our study, we measured and compared with various clinical parameters of insight. The aim of this study is to assess various dimensions of insight recovery prospectively in bipolar affective disorder patients with treatment and drug compliance. **Materials and Methods:** Patient's insight was assessed using Mood Disorder Insight Scale (MDIS) at baseline, 1st, 3rd, and 6th months. Their insight was then compared with various sociodemographic profiles and correlated with number of mood episodes, family history of mental illness, and 6-month MDIS scores. **Results:** Depression patients scored better in insight components ($P = 0.001$). The good compliance group attributed their symptoms to their illness than the poor compliance group ($P = 0.013$). The MDIS scores were gradually improving from baseline to 6 months ($P \leq 0.001$). There was no relationship between insight and the number of episodes ($P = 0.788$). **Conclusion:** Depressive episode patients had better insight during the baseline, which improved during 6 months follow-up compared with manic patients. Among various components of insight, insight on the attribution of symptoms was a predictor of good compliance. Progression of insight was steady and proportionate to the duration of treatment in depressive episode patients.

Keywords: Bipolar affective disorder, depression, insight, mania

The bipolar affective disorder is common and disabling disorder and is associated with considerable morbidity.[1] It is a mood disorder that is characterized by episodes of depression, mania, hypomania, and mixed episodes, with a period of inter-episodic recovery. The World Health Organization estimated that bipolar disorder was the 46th greatest cause of disability and mortality in the world among 291 diseases and causes of injuries. Insight is defined as the awareness of one's illness, the ability to recognize the symptoms of the illness and the need for treatment. Insight of a patient can be categorized into absent insight, partial insight, and full insight.[2-5]

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Insight is being highlighted as state-dependent component in a few studies. In disorder like bipolar affective illness, impairment in insight fully resolves. There are few studies which highlight that insight does not resolve completely even in the euthymic state. The more the number of episodes especially manic episodes, the lesser is the insight recovery. However, psychotic depression is associated with less insight than nonpsychotic depression. Insight continues to be poor in chronic illness like schizophrenia. Nowadays, more attention has been given to insight of the patient toward mental illness. There are many

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studies, which were being done which evaluated the relationship between insight and course and outcome of the illness, compliance to medications and the severity of psychopathology. The prevalence of good insight in remitted bipolar affective disorder patients is only 47%.[6] In another study, the prevalence of absent insight was 39.09% in the schizophrenia group, 36.36% in the bipolar disorder with psychotic symptoms and 9.37% in bipolar disorder without psychotic symptoms.[7]

The bipolar affective disorder is usually associated with noncompliance and poor outcome.[8,9] Insight and quality of life, cognitive functions, psychotic symptoms, and the number of episodes in mood disorders are interrelated.[10] Many studies have shown that insight is impaired in the acute phase of illness and insight improves as the patient improves symptomatically. Insight was more impaired during manic episodes when compared with depression.[11] Lack of insight in mania was present for all three components of insight-awareness of illness, social consequences of illness, and therapy benefits.[12] A study has showed that insight of bipolar affective disorder patients and schizophrenia patients were the same during admission. At 6 month follow-up, there was a large difference between both the groups. Bipolar disorder patients had better insight when compared with schizophrenia patients.[13]

Adherence to medications plays a key role in the effectiveness of any treatment. Research on the relationship between insight and adherence to treatment is important because adherence to medication can be modified. Meta-analysis that was done to study the relationship between insight and treatment compliance showed poor insight was associated with poor adherence to medications, which was similar to the literature on schizophrenia patients.[14] Studies on schizophrenia patients showed that patients with more insight have a better long-term outcomes.[15] There are similar researches in bipolar disorder, which showed improvement of insight from baseline and follow-up was related to better outcomes of illness and quality of life.[16]

Various scales are available for the assessment of insight. However, most of the scales were validated to measure insight in psychotic illness. Mood Disorder Insight Scale (MDIS) is a short and self-report instrument, includes all the components of insight and it is specifically modified for affective disorder patients. The MDIS was derived from the insight Scale for psychosis which was developed by Birchwood *et al.*, 1994. This scale measures three aspects of insight-awareness of mental disorder, attribution of symptoms, and need for treatment. Hence, we used this scale, which specifically measures insight in bipolar affective disorder in our study.

Most of the studies on insight were done in schizophrenia patients. Very few studies were done on bipolar disorder patients. There are only very few studies which have studied insight in various phases of illness. In disorder like bipolar affective illness impairment in insight fully resolves. The more the number of episodes especially manic episodes, the lesser is the insight recovery. Insight continues to be poor in chronic illness like schizophrenia.

An earlier study reported a 94% prevalence of impaired insight in symptomatic patients in various psychiatric illness such as depression, manic, and bipolar I patients. The prevalence of insight in remitted patients is 47%.[6]

A prospective study on insight in various phases of bipolar illness, observed that insight was impaired in mania when compared with the euthymic state. Insight was better in patients who had single episode of mania or depression; this insight was similar to preepisode insight. However, patients with multiple episodes of illness in 2 years had poorer insight when compared with insight of patients with less number of episodes.[7,17,18]

Very limited number of studies had compared insight with sociodemographic and clinical variables such as age, educational status, gender, marital status, and family history of mental illness. Most of the studies that have been done in insight are cross-sectional study. There are very few prospective studies on insight in bipolar disorder patients.

Therefore, the aim of this study is to assess insight recovery in bipolar affective disorder patients admitted in the psychiatry ward. We also assessed the correlation of insight about awareness of illness, attribution of symptoms to illness and insight recovery with various phases of bipolar affective illness.

MATERIALS AND METHODS

Participants and procedures

This is a prospective study. Participants of our study were both male and female gender for >12 years. These patients were admitted and were undergoing in-patient treatment in PSG Hospitals, a 2000-bedded tertiary hospital at Coimbatore, for Bipolar affective disorder either in depression or manic phase. These patients were admitted between the period of April 2016 and 2017. The participants were diagnosed with bipolar affective disorder using the International Statistical Classification of Diseases (ICD-10) criteria by the treating consultant. Patients having comorbid mental retardation and other Axis 1 psychiatric disorder except nicotine dependence, patients who did not respond for at least three telephonic calls in

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the follow-up and patients <12 years old were excluded from our study.

We recruited 50 consecutive in-patient bipolar affective disorder patients based on the ICD-10 criteria. Nine patients were excluded as per the exclusion criteria. Five of them had follow-up elsewhere, two patients did not give consent and two patients had mental retardation. Informed consent in the local language (Tamil) was obtained from primary caregiver for those patients who were not able to give consent. Once the patient became euthymic informed consent was obtained from the patient as well. The personal identification data such as name, age, gender, educational qualifications, and socioeconomic data of all the participants were recorded. Data related to psychiatry illness such as the number of past episodes and family history of mental illness was also recorded.

Once the patient got admitted, the severity of the present episode was measured using rating scales. The severity of affective symptoms was assessed by the Youngs Mania Rating Scale (YMRS) and Hamilton Depression Rating Scale (HAMD) for mania and depression, respectively. Baseline Insight was assessed using MDIS. Then, the patient was treated according to their clinical diagnosis by the consulting psychiatrist. One month after discharge, the insight was assessed using the MDIS scale and recorded. Then again, at the 3rd and 6th months of follow-up insight was measured using MDIS. The total scores of insight were calculated at baseline, 1st month, 3rd month, and 6th month and compared for improvement in insight.

Drug compliance was assessed clinically from the patient and reliable informant. During follow-up, the course of the illness was assessed, and the escalation of the dose of psychotropics or addition of a new psychotropic medication is also assessed. Patients who missed the follow-up are contacted through telephonic calls, encouraged to attend outpatient department and Insight assessed. Patients who did not respond for at least three telephonic calls were considered as dropouts [Figure 1]. The study was approved by the Institute Human Ethics committee of PSG IMSR and Hospital.

Assessment

Data were collected using the following instruments.

Semi-structured pro forma

The sociodemographic profile of the patient, details of substance use, diagnosis, and clinical details (compliance of medications, the severity of illness using YMRS and HAMD scales, number of past episodes, and family history of psychiatry illness) were assessed.

Mood Disorder Insight Scale

MDIS is a short and self-report instrument. This scale has eight questions which are read by the interviewer and the patient is asked whether their response is agree, disagree, or unsure for each of the eight items in the questionnaire. Depending on the response, the scores are calculated. The total score is 16. The MDIS was derived from the Insight Scale for psychosis, which was developed by Birchwood. This scale has good test–retest reliability and internal consistency. The test–retest reliability (coefficient alpha) of the MDIS was 0.95 ($P < 0.01$). This scale for insight includes all the components of insight and it is specifically modified for affective disorder patients. The administration of this scale does not require any special training as this is a self-report questionnaire. This scale measures three aspects of insight-awareness of mental disorder, attribution of symptoms, and need for treatment.

Data analysis

Data were entered into MS Excel and analyzed using SPSS version 20. Qualitative data were summarized as frequencies and percentages. Quantitative data were checked for normality. Normally distributed data were summarized using mean and standard deviation. Nonnormally distributed data were summarized using Median and inter-quartile range. Association between qualitative variables was tested using Chi-square test. Mann–Whitney U test was used to compare the distribution of MDIS scores by level of compliance and phase of disease; mania or depression. Friedman's test was used to check if the MDIS scores changed significantly with time. Correlation between the YMRS score and MDIS at baseline was tested using the Spearman rank correlation test. Statistical significance was interpreted using an arbitrary cut-off of $P = 0.05$.

RESULTS

Descriptive statistics for the study variables and demographics data are shown in Table 1.

The number of episodes ranged from 2 to 7. Fifteen patients had psychotic symptoms in their mood episodes and twenty-six patients had no psychotic symptoms. Family history of psychiatry illness was present in 26.8% ($n = 11$) and absent in remaining 73.2% ($n = 30$). Twenty-three patients (56.1%) of the participants had good compliance and the remaining 43.9% ($n = 18$) had poor compliance [Table 2].

The insight scores between different age group were compared, the *P* value was not statistically significant ($P = 0.318$). The insight scores, according to educational status, were not

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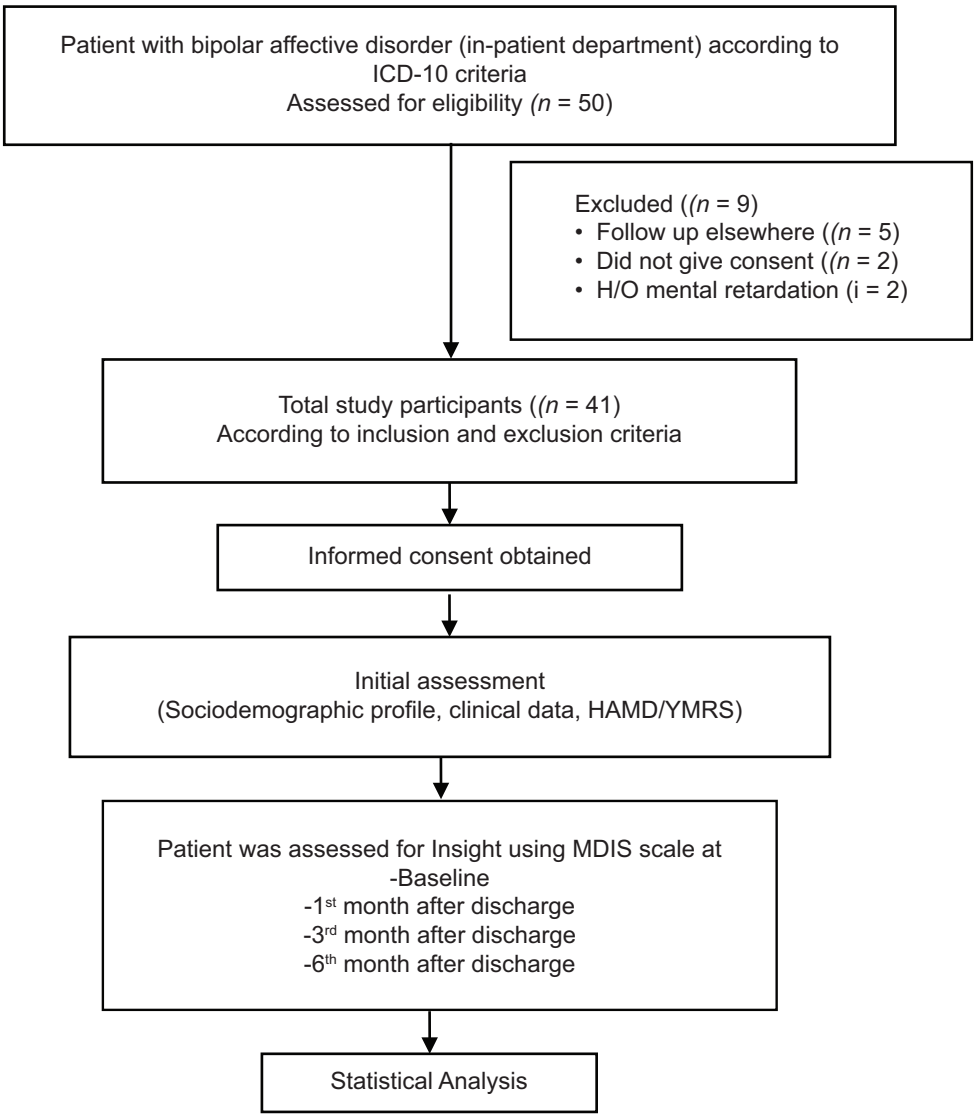


Figure 1: Flow chart

statistically significant ($P = 0.75$). Insight scores did not differ statistically between males and females, P value was 0.552. Insight scores of participants who had family history of mental illness were compared with participants who did not have family history of mental illness also did not have any statistical significance ($P = 0.124$) [Table 2].

The overall scores of the MDIS scale were compared between mania and depression groups, depression patients scored more when compared with manic patients in the insight scale. The more the MDIS score, the better the insight. The P value was also significant $P = 0.001$ [Table 3].

The total scores of MDIS between good compliance and poor compliance group were statistically significant, P value was 0.012. Good compliance group scored better in insight scale than the poor compliance group [Table 4].

In our study, we assessed for the insight score of the participants at baseline, 1st month, 3rd month and 6th month. When these scores were compared from baseline to the end of 6 months, we found that the insight scores were gradually improving. This was statistically significant and P value was < 0.001 [Table 5].

Figure 2 shows the MDIS scores at baseline, 1st month, 3rd month, and 6th month in depressive patients. The MDIS scores of the depressive patient were higher when compared with manic patients. This shows that depressive patients have more insight than manic patients. Figure 3 shows the MDIS scores at baseline, 1st month, 3rd month, and 6th month in manic patients. The MDIS scores of the manic patient were lower when compared with depressive patients indicating manic patients have less insight about illness than depressive patients.

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Table 1: Sociodemographic profile (*n*=41)

Variable	*n* (%)
Age (years)	
<18	2 (4.9)
18-65	38 (92.7)
>65	1 (2.4)
Sex	
Males	25 (61)
Females	16 (39)
Education status	
Primary	7 (17.1)
Secondary	7 (17.1)
Higher secondary	8 (19.5)
Diploma/graduate	19 (46.3)
Marital status	
Married	29 (70.7)
Single	12 (29.3)
Socioeconomic status	
Upper	8 (19.5)
Middle	27 (65.8)
Lower	6 (14.7)
Occupation	
Employed	6 (14.6)
Unemployed	29 (70.8)
Home maker	6 (14.6)

Table 2: Comparing sociodemographic profile and Mood Disorder Insight Scale Scores

Factors	Baseline MDIS	*P*
Age		
<18	4 (1)	0.318
18-65	6 (6)	
>65	4 (0)	
Education status		0.750
Primary	6 (5)	
Secondary	9 (13)	
Higher secondary	7 (6)	
Diploma/graduate	6 (5)	
Marital status		0.195
Married	6 (4)	
Single	4 (5)	
Sex		0.552
Males	6 (5)	
Females	6 (8)	
Family history		0.124
No	5 (8)	
Yes	6 (6)	

MDIS - Mood disorder insight scale

In Figure 4, MDIS scores were plotted against the period of assessment. The MDIS scores have an increasing trend from baseline to 6 months in the depression group. The progression of insight reached a plateau after 3 months for manic patients.

The number of episodes of illness was compared with MDIS scores. The *P* value was not significant ($P = 0.788$).

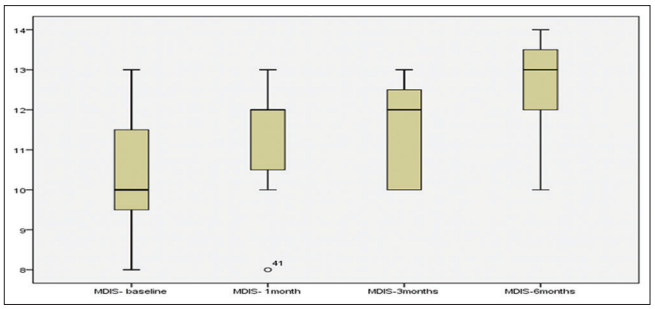


Figure 2: Progression of insight based on Mood Disorder Insight Scale scores at baseline, 1, 3 and 6 months in participants with depression

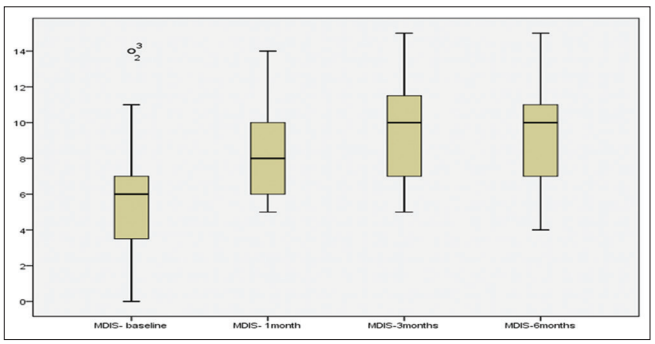


Figure 3: Progression of Mood Disorder Insight Scale from baseline to 6 months in patients with mania

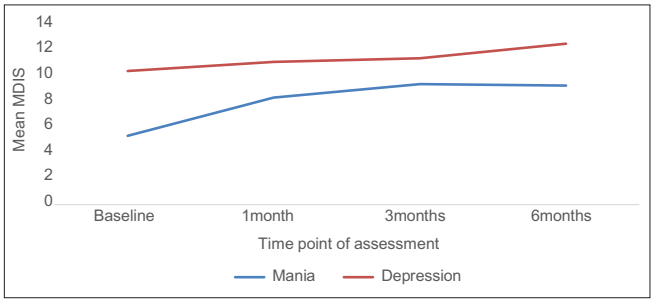


Figure 4: Progression of Mood Disorder Insight Scale from baseline to 6 months in patients with mania and depression

The presence of family history of mental illness was compared with MDIS scores. The *P* value was not significant ($P = 0.701$) [Table 6].

MDIS scores were negatively correlated with both YMRS and HAMD scores, which implies less severe the illness, the better the insight. The correlation with YMRS was statistically significant ($P = 0.003$), whereas with HAMD the correlation was not statistically significant ($P = 0.640$) [Table 7].

DISCUSSION

Insight is a multidimensional and complex phenomenon (Amador *et al.*, 1993). We compared sociodemographic profile (name, age, gender, educational

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Table 3: Comparison of insight measured using Mood Disorder Insight Scale Scores between depression and mania at baseline

Score	Median (IQR)		Mann-Whitney U	*P*
	Mania	Depression		
Awareness of mental disorder	2 (2)	4 (2)	37.5	0.003
Attribution of symptoms	2 (2)	2 (1)	53	0.021
Need for treatment	3 (2)	4 (3)	60	0.041
Total	6 (4)	10 (4)	28.5	0.001

IQR - Interquartile quartile range

Table 4: Comparison of insight measured using Mood Disorder Insight Scale Scores between patients with different levels of compliance

Score	Median (IQR)		Mann-Whitney U	*P*
	Good compliance	Poor compliance		
Awareness of mental disorder	2 (4)	2 (2)	163.5	0.225
Attribution of symptoms	2 (2)	2 (2)	118.5	0.013
Need for treatment	4 (4)	2 (2)	147.5	0.106
Total	7 (5)	4 (5)	112	0.012

IQR - Interquartile quartile range

Table 5: Progression of insight based on Mood Disorder Insight Scale Scores at baseline, 1, 3 and 6 months

MDIS	Median	IQR	*P**
Baseline	6	5	<0.001
1 month	8	4	
3 months	10	5	
6 months	10	6	

*Friedman test. MDIS - Mood disorder insight scale; IQR - Interquartile quartile range

Table 6: Correlation of number of episodes, family history of mental illness, and 6 months Mood Disorder Insight Scale Scores

Factors	MDIS 6 months	*P*
Number of episodes		
1	9 (3)	0.788
2	10 (6)	
3	9 (7)	
Family history		
Yes	10 (4)	0.701
No	10 (6)	

MDIS - Mood disorder insight scale

Table 7: Correlation of mood disorder insight scale baseline total score with Youngs mania rating scale and Hamilton depression rating scale

	YMRS score	HAMD score
Spearman correlation coefficient	−0.488**	−0.287
P	0.003	0.640

YMRS - Youngs mania rating scale; HAMD - Hamilton depression rating scale

qualifications, and socioeconomic data) of the participants with MDIS scores. There was no difference in insight between different groups.

In this study, insight scores were lower in females than males ($P = 0.08$), but the P value was not significant. The insight scores were compared with different educational status groups. There was no statistically significant ($P = 0.75$) difference within these groups. On the contrary to our understanding insight did not differ according to the educational status in our study. To our knowledge, no other studies on insight had compared the relationship between educational status and marital status.

Insight scores of participants who had family history of mental illness were compared with participants who did not have family history of mental illness. Though the scores of the participants who had family history of mental illness were higher than the other group, it was not statistically significant.

The overall MDIS scores, when compared between mania and depression groups, showed that depressive patients scored more when compared with manic patients in the insight scale ($P = 0.001$). The individual dimensions of scale were also better among depression than mania, which was statistically significant. Another study which was done to evaluate insight in mania, depression and euthymia in 95 bipolar affective disorder patients showed poor insight among mania patients when compared with depressive patients.[19] The P value was significant 0.001.

Twenty-three patients that is 56.1% of the participants had good compliance and the remaining 43.9% ($n = 18$) had poor compliance. Compliance is a very important part to consider in illness like bipolar affective disorder. Literature on the prevalence of nonadherence also had similar values. The median prevalence of nonadherence is 44.7% in

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patients with bipolar affective disorder.[19] In one study that was done to assess the predictors of nonadherence in bipolar affective disorder patients showed treatment nonadherent individuals had more negative attitudes toward medications and reduced insight into illness when compared with treatment adherent individuals.[20] We also compared the MDIS scores between good compliance and poor compliance group. The total scores of MDIS between good compliance and poor compliance group were statistically significant and *P* value was 0.012. Yen *et al.* study[21] showed insight about illness and treatment was positively correlating with adherence to medication in the bipolar affective disorder group and schizophrenia group. So interventions, which concentrated on building insight play a vital role in ensuring good compliance among bipolar patients.

In our study, we assessed for the insight score of the participants at baseline, 1st month, 3rd month, and 6th month. When these scores were compared from baseline to 6 months, we found that the insight scores were gradually improving. This was statistically significant and *P* value was < 0.001. In another prospective study which evaluated the correlation of insight in 65 bipolar disorder patients for 2 years showed insight of the manic patients gradually improved as they returned to the euthymic state.[21]

The average number of episodes in our study among the participants was two. The number of episodes ranged from 2 to 7. In our study, the number of episodes did not make any significant difference in insight of the participants ($P = 0.788$). Similar findings were seen in Cassidy study; on the contrary, showed more the number of episodes, poorer the insight.[22]

Limitations

The sample size is very small in our study, and hence the results cannot be generalized. The initial assessment of insight was not assessed on specifically on the same day after admission for all patients. The treating consultant psychiatrist made a diagnosis of bipolar affective disorder. No separate research diagnostic criteria were used to recruit our patients. With the help of insight scale we have assessed whether the patient is aware of their symptoms in general but have not analyzed about the awareness of individual symptoms. MDIS scale gives only qualitative assessment and not a quantitative assessment.

CONCLUSION

Depressive episode patients had better insight during the baseline, which improved during 6 months follow-up compared with manic episode patients. Among various components of Insight, insight on the attribution of symptoms was a predictor of good compliance during 6 months follow-up than the awareness of mental disorder or need for treatment. Progression of insight was steady and proportionate to the duration of treatment in depressive episode patients. More severe the manic episode poorer was the insight.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kathleen RM, Robert J, Jian-Ping H, Ronald CK, Sing L, Nancy AS, *et al*. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. Arch Gen Psychiatry 2011;68:241.
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed.. Arlington, VA: (DSM-5), American Psychiatric Association; 2013.
3. Pedersen CB, Mors O, Bertelsen A, Waltoft BL, Agerbo E, McGrath JJ, *et al*. A comprehensive nationwide study of the incidence rate and lifetime risk for treated mental disorders. JAMA Psychiatry 2014;71:573-81.
4. Soraya S, Kate MS, Matthias CA, Patricia B, Evelyn JB, Traolach SB, *et al*. Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys. Arch Gen Psychiatry 2009;66:785.
5. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, *et al.* Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2197-223.
6. Varga M, Magnusson A, Flekkoy K, Ronneberg U, Opjordsmoen S. Insight, symptoms and neurocognition in bipolar I patients. J Affect Disord 2006;91:1-9.
7. Yen CF, Chen CS, Yang SJ, Ko CH, Yen JY, Huang CF. Relationships between insight and psychosocial adjustment in patients with bipolar I disorder. Bipolar Disord 2007;9:737-42.
8. Ghaemi SN, Hebben N, Stoll AL, Pope HG Jr. Neuropsychological aspects of lack of insight in bipolar disorder: A preliminary report. Psychiatry Res 1996;65:113-20.
9. Ghaemi SN, Pope HG Jr., Lack of insight in psychotic and affective disorders: A review of empirical studies. Harv Rev Psychiatry 1994;2:22-33.
10. American Psychiatric Association. Mood disorder due to a general medical condition. In: American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Washington DC: American Psychiatric Publishing; 2000. p. 401-4.
11. Peralta V, Cuesta MJ. Lack of insight in mood disorders. J Affect Dis 1998;49:55-8.
12. Dell'Osso L, Pini S, Cassano GB, Mastrocinque C, Seckinger RA, Saettoni M, *et al*. Insight into illness in patients with mania, mixed mania, bipolar depression and major depression with psychotic features. Bipolar Disord 2002;4:315-22.
13. Fennig S, Everett E, Bromet EJ, Jandorf L, Fennig SR, Tanenberg-Karant M, *et al.* Insight in fi Insight in psychotic patients. Schizophrenia Res 1996;22:257-63.
14. Sadock BJ, Sadock VA, editors. Kaplan and Sadock's

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Synopsis of Psychiatry: Behavioral Sciences, Clinical Psychiatry. Philadelphia: Lippincott Willimas& Wilkins; 2007.
15. Schwartz RC, Cohen BN, Grubaugh A. Does insight affect long-term impatient treatment outcome in chronic schizophrenia? Comp Psychiatry 1997;38:283-8.
16. Ghaemi SN, Boiman E, Goodwin FK. Insight and outcome in bipolar, unipolar, and anxiety disorders. Comp Psychiatry 2000;41:167-71.
17. Yen CF, Cheng CP, Huang CF, Ko CH, Yen JY, Chang YP, *et al.* Relationship between psychosocial adjustment and executive function in patients with bipolar disorder and schizophrenia in remission: The mediating and moderating effects of insight. Bipolar Disord 2009;11:190-7.
18. Yen CA, Chen CA, Yang SA, Ko CA, Yen JA, Huang CÄ. Relationships between insight and psychosocial adjustment in patients with bipolar I disorder. Bipolar Disord 2007a; 9:737-42.
19. de Assis da Silva R, Mograbi DC, Silveira LA, Nunes AL, Novis FD, Landeira-Fernandez J, *et al*. The reliability of self-assessment of affective state in different phases of bipolar disorder. J Nerv Ment Dis 2014;202:386-90.
20. Scott J, Pope M. Nonadherence with mood stabilizers: Prevalence and predictors. J Clin Psychiatry 2002;63:384-90.
21. Yen CF, Chen CS, Ko CH, Yeh ML, Yang SJ, Yen JY, *et al*. Relationships between insight and medication adherence in outpatients with schizophrenia and bipolar disorder: Prospective study. Psychiatry Clin Neurosci 2005;59:403-9.
22. Cassidy F. Insight in bipolar disorder: Relationship to episode subtypes and symptom dimensions. Neurop Disord Treatment 2010;6:627-31.

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Asian pearls

Clinical characteristics of patients with non-affective, non-organic, late onset psychosis

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ABSTRACT

Objective: Schizophrenia and related psychotic disorders are predominantly studied in young population. However some individuals do develop psychotic disorder for the first time during their old age. The aim of this study is to look at the clinical characteristics of non-affective, non-organic, late onset psychosis.
Participants and methods: Retrospective chart review study, medical records of all patients registered between 1st of January 2006 and 31st May 2011 at geriatric clinic in NIMHANS, Bangalore was screened, 83 files with a diagnosis of late onset psychosis and meeting the study criteria were systematically analyzed.
Results: The mean age at onset of illness was 67 ± 10 years; 98.8% were married, females formed 67.5% of the sample, commonest phenomenon was delusions followed by hallucinations, 80.5% of the subjects had delusion of persecution, 38.5% had referential delusion, 51.8% had accusative and derogatory auditory hallucinations, negative symptoms was seen only in 2.4% of subjects, none had formal thought disorder. 3.6% had co-morbid Axis II diagnosis.
Conclusion: Despite its rarity non affective, non organic, late onset psychosis forms a distinct group with unique manifestation. Further systematic research is needed for better understanding of this condition.
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1. Introduction

Schizophrenia and related Non-Affective psychotic disorders are predominantly studied in adolescence and young adults. However some individuals do develop psychotic disorder for the first time during their old age. Community prevalence estimates for schizophrenia range from 0.1% to 0.5% (Castle and Murray, 1993; Copeland et al., 1992, 1998; Kua, 1992). 1-year prevalence rate of schizophrenia in individuals between ages 45 and 64 is 0.6% (Keith et al., 1991). For individuals over 65 years of age, incidence rate of schizophrenia with first onset after 44 years is 12.6 per 100,000 population per year (Copeland et al., 1998). The Studies on late-onset schizophrenia began with Manfred Bleuler, Late onset schizophrenia constituted 15% of patient whom he examined; 4% of the patients had an onset after 60 (Bleuler, 1943). About 50% of the patients with late-onset schizophrenia had symptoms that were similar to those seen in early onset schizophrenic patients. However he did report certain striking differences; more than 50% of patients had Paraphrenia like symptoms, depressive, anxious, catatonic or confused agitated symptoms, but with less affective flattening (Harris and Jeste, 1988; Jeste et al., 1988, 1995; Mayer et al., 1993; Pearlson et al., 1989; Rabins et al., 1984). Subsequently there are lot of studies on late onset psychosis (LOP), mostly from developed countries of Europe and America, but the research findings in this area is struck with difficulties because neither ICD 10 nor DSM V contain separate codes of diagnosis for 'late onset schizophrenia'; different terms being used to describe a similar illness occurring in old age and using different age cut-off to be called late onset psychosis (LOP) (Howard et al., 2000). But other limitations of existing literature in this field are some studies include patients who had early onset psychosis but grown old, including patients with affective disorder and organic psychosis. Some of these confusions were cleared after The International Late Onset Schizophrenia group came up in the year 2000 with consensus on nomenclature defining Late Onset Schizophrenia and Very Late Onset Schizophrenia like Psychosis. For developing countries like India and China with their large population and increasing geriatric population, late onset psychosis is a major concern. Socio cultural factors like globalization, migration, breaking up of joint families, poorer healthcare facilities etc in developing countries in Asia are certainly different to that of developed countries in Europe and America. There are many

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factors that increase the risk of developing psychiatric disorder in elderly like loss of close relatives, social network, previous status in society, sensory functions, functional ability, and health (Skoog, 2008).These psycho socio cultural factors can influence the presentation of individuals with late onset psychosis. Currently there is a paucity of literature on the clinical manifestation of late onset psychosis in Asia especially from India. So the aim of this study is to study the clinical characteristics of non-affective, non-organic, late onset psychosis in an Indian set-up.

2. Methods

2.1. Study design & setting

This is a retrospective chart review study conducted at the Geriatric clinic & Services in National institute of mental health and neuro sciences (NIMHANS) Bangalore, a tertiary care hospital in India, which caters to individuals with psychiatric, neurology and neurosurgery problems. Geriatric clinic is run by a multi disciplinary team which includes psychiatrists, neurologists, neuro psychologists and social workers. All the patients attending geriatric clinic are assessed using a semi structured workup proforma, thorough physical examination, Hindi Mental State Examination scale (HMSE) which is an Indian adaptation of Mini Mental State Examination, Geriatric Depression Scale (GDS), Clinical Dementia Rating Scale (CDR), Neuro Psychiatric Inventory (NPI) and Everyday Abilities Scale for India (EASI). They also undergo basic investigations like complete haemogram with peripheral smear, random blood sugar test, liver function test, renal function test, serum electrolytes and Computerized tomography of brain. Other investigations are done if necessary.

2.2. Study participants

We retrieved and screened medical records of all patients who got registered in the geriatric clinic between 1st of January 2006 and 31st of May 2011. In this study we included medical records of patients diagnosed to have psychosis (Schizophrenia, delusional disorder, acute psychosis, nonspecific psychotic disorder, other psychotic disorder) as per International classification of disease 10th version (ICD-10), who were above 50 years of age at the time of onset of psychosis and those who followed up in geriatric clinic atleast for 3 months. We excluded files of patients whose diagnosis was other than psychosis, who developed psychotic symptoms secondary to affective disorder or substance use or organicity (epilepsy, cerebral tumors etc), dementia, whose age at onset of psychosis was below 50 years, those who had major mental illness in the past and medical records that lacked sufficient data. Finally we selected 83 medical records which satisfied our inclusion and exclusion criteria, these records were analyzed.

This study was approved by NIMHANS Institute ethics committee, written informed consent was not taken as it is a retrospective chart review, and anonymity of all patients was maintained.

2.3. Materials

1. Hindi Mental Status Examination (HMSE) **–** Is an Indian adaptation of *Mini Mental Status Examination* (MMSE) that is being used in epidemiological studies of dementia in India. It contains 23 items which test different components of intellectual capability. The scale has a total score of 31, a score less than 19 is indicative of dementia and warrants a detailed cognitive assessment (Ganguli et al., 1995).

2. Everyday Abilities Scale for India (EASI) –This is brief, reliable and valid scale to assess activities of daily living, with norms, which is appropriate for use in assessing dementia in elderly people in India. This is a 12-item uni-dimensional scale which measures mobility, instrumental and personal care activities, the scale has good internal consistency (Pandav et al., 2002). Each item is scored either 0 or 1, less number of 1 score, in other words lower total scores indicates better functioning.

3. Neuropsychiatric Inventory (NPI) – The purpose of NPI is to obtain information on the presence of psychopathology. The NPI was developed for application to patients with Alzheimer's disease and other dementias, but it may be useful in the assessment of behavioral changes in other conditions aswell. It assesses ten behavioral symptoms including psychotic and affective symptoms and two neurovegetative symptoms. The NPI has been proven to be sensitive to change with treatment, it is available in many languages, has been shown to be valid and reliable in cross-cultural studies (Wood et al., 2000).

4. Geriatric Depression Scale (GDS) – Short version – GDS short version is a 15 item scale.The GDS was found to have a high sensitivity and specificity. The validity and reliability of the tool have been supported through both clinical practice and research. In a validation study comparing the Long and Short Forms of the GDS for self-rating of symptoms of depression, both were successful in differentiating depressed from non-depressed adults with a high correlation (Sheikh and Yesavage, 1986). Scores of 0–4 are considered normal; 5–8 indicate mild depression; 9–11 indicate moderate depression; and 12– 15 indicate severe depression.

2.4. Statistical analysis

Descriptive statistics (frequency, ratio, mean, standard deviation) was used to define the socio demographics and clinical characteristics of the patients. Chi square test and Fisher's exact tests were conducted for to compare the symptomatology between different age groups. Person's correlation test was used to analyze the association between cognitive functions and different variables, with p value of <0.05 considered as significant. The data was analyzed using SPSS version 19.

3. Results

Among the 83 medical records that were reviewed, the mean age of the patient was 69 ± 9 years at the time of initial consultation, and age ranging between 51 and 91 years. The average age at the time of onset of illness was 67 ± 10 years. The mean duration of illness was 2.5 years (SD $\pm$ 2.2 years); 55.4% of the patients were uneducated and 54.2% belonged to low socio economic class.

3.1. Socio demographics and clinical characteristics

The socio demographics and clinical characteristics of the patients are depicted in (Table 1). The sample had female preponderance; with number of females being almost double that of males. Except one person all others were married, most of them followed Hindu religion. Psychosis not otherwise specified (psychosis NOS) was the most common diagnosis in these patients, followed by schizophrenia, delusional disorder and acute and transient psychosis. Interestingly all patients diagnosed with acute and transient psychosis developed illness only at $\geq$60 years. Only 3/83 (3.6%) patients had Axis II disorder (Anankastic personality disorder – 1; Anxious avoidant personality disorder – 1; and Paranoid personality disorder –1) Precipitating factor was noticed in 12% of these patients, with some developing psychosis following a loss of family member, some following hearing or visual impairment etc. Positive family history was found in more than 20% of patients, with nearly 11% of them had either a first or second

Table 1
Socio-demographics and Clinical characteristics.

Variables	Mean	SD
Current age (years)	69	±9
Age at onset of illness (years)	67	± 10
Education (years)	3.4	±4.6
Duration of illness (years)	2.5	±2.2
	N = 83	%
Sex		
Female	56/83	67.5
Male	27/83	32.5
Socioeconomic status		
Low	45/83	54.2
Middle	35/83	42.2
High	3/83	3.6
Married	82/83	98.8
Diagnosis		
Psychosis NOS	39/83	46.9
Schizophrenia	15/83	18.0
Delusional disorder	15/83	18.0
Acute and transient psychosis	14/83	16.8
Schizoaffective disorder	0/83	0
Precipitating Factor	10/83	12
Entry event	3/83	3.6
Exit event	2/83	2.4
Sensory impairment	2/83	2.4
Other	5/83	6
Positive family history of psychiatric disorders	17/83	20.5
Psychosis	9/83	10.8
Mood disorder	4/83	4.8
Dementia	3/83	3.6
Suicide	1/83	1.2
Other disorders	1/83	1.2
Co-morbid medical illness	56/83	67.4

SD: Standard deviation, N: total number, Psychosis NOS – Psychosis not otherwise specified.

degree relative with psychosis. Co-morbid medical illness was found in 67.46% of patients; with most common being hypertension followed by Type 2 diabetes mellitus.

3.2. Symptomatology

Delusions and hallucinations dominated their clinical presentation, with acting out behavior secondary to delusions being the commonest reason for bringing the patient for consultation. Thought alienation phenomenon noted in 15.7%, none of the patients reported delusional perception, made phenomenon or somatic passivity. Affective symptoms (sadness, irritability, fear, anxiety, dysphoria and rarely elation) were seen in nearly half of the patients (Table 2**).** Only two patients had negative symptoms, one with apathy, anhedonia, and another patient had asocialization, apathy and anhedonia. One third of patients had verbal/physical aggression and other behavioral disturbance like restlessness, aimless wandering, and disorganization. One third had subjective or objective memory disturbances. No patients had formal thought disorder or expressed any bizarre delusions. Among delusions, delusions of persecution followed by referential delusion were the commonest. 64% of patients had hallucination, out of which half of them had 2nd person auditory hallucination, very few patients had hallucination in other modality (Table 3**).** There was no significant difference in the symptomatology between those with late onset psychosis and very late onset psychoses (where the onset of psychosis was ≥60years) (N = 16 & 67 respectively).

Table 2
Symptomatology.

Symptoms	N = 83 n/N	%
Delusion	73/83	88
Hallucination	53/83	63.9
Thought alienation	13/83	15.7
Made phenomenon	0/83	0
Somatic passivity	0/83	0
Formal thought disorder	0/83	0
Negative symptom	2/83	2.4
Affective symptom	43/83	51.8
Aggression	29/83	34.9
Other behavioral disturbance	28/83	33.7
Sleep disturbance	48/83	57.8
Memory disturbance	27/83	32.5
Suicide attempt	3/83	3.6

Table 3
Type of Delusion & Hallucination.

	N = 83 n/N	%
Delusion		
Persecutory	67/83	80.7
Reference	32/83	38.5
Misinterpretation	10/83	12.0
Infidelity	3/83	3.6
Grandiose	2/83	2.4
Infestation	1/83	1.2
Bizarre	0/83	0
Other	0/83	0
Hallucination		
2nd person auditory hallucination	43/83	51.8
3rd person auditory hallucination	12/83	14.4
Running commentary	0/83	0
Visual hallucination	10/83	12.0
Tactile hallucination	2/83	2.4
Extracampine hallucination	1/83	1.2
Intracorporeal hallucination	1/83	1.2
Pseudo hallucination	1/83	1.2

3.3. Cognitive function and activities of daily living

Although one third of the patients had some memory disturbances, their cognitive functions and their ability to function independently remained largely intact (Table 4**)**, with mean HMSE and EASI scores being 24.5 ± 4.9 & 2.3 ± 2.8 respectively. Most patients had an EASI score of 0 indicating they were functioning independently. Cognitive functions were slightly lower in females

Table 4
Cognitive function & Activities of daily living.

	Mean	SD
HMSE (score out of 31)	24.50	±4.9
Female	23.34	±4.4
Male	26.50	±3.5
EASI (score out of 15)	2.3	±2.8
Female	2.5	±2.9
Male	2.1	±2.7
HMSE &	Pearson's correlation P* value	
Current age	0.74	
Age at onset of illness	0.67	
Duration of illness	0.83	

HMSE: Hindi Mental Status Examination, EASI: Everyday Abilities. Scale for India, Correlation significant at p* value < 0.05.

compared to males. There is no significant association between cognitive functions and age, age at onset of illness or duration of illness (P values – 0.74; 0.67; 0.83 respectively).

4. Discussion

4.1. Sociodemographics and clinical characteristics

This study evaluated the clinical characteristics of non-affective, non-organic, late onset psychosis in a sample of Indian population seeking care in special clinic. A minimum of 3 month follow up was included as a part of study criteria in order to validate the diagnosis and to allow for any revision after initial consultation. Most participants in this study were above 60 years of age at the time of onset of psychosis, the "age of onset" is defined as a change from a state without psychotic features to a clearly abnormal psychotic state as evidenced by the presence of any of the characteristic symptoms of psychosis according to ICD-10. Female preponderance was noted in overall sample which is comparable to other studies (Castle and Murray, 1993; Harish et al., 1996). Many were uneducated in our sample which could be due to female preponderance and also most of them were from rural background, according to Indian government census in 2001 on literacy rate in India, overall literacy rate works out to be 64.8%, the male literacy rate is 75.3% and that for females is 53.7%, showing a gap of 21.6 percentage points between the sexes at the national level and the gap is more in the rural areas. In our sample almost all were married and had a stable occupation suggesting a good pre-morbid functioning in these patients, in concurrence with other studies done in western countries (Alici-Evcimen et al., 2003; Lacks et al., 2006), the higher marriage rate and fairly good adjustment in occupation may also merely reflect the late onset of illness in this group, this finding good pre-morbid functioning is in contrast to early onset group, with some researchers speculating early onset patients perhaps represents pernicious form of the illness particularly associated with pre-morbid dysfunction (Castle and Howard, 1992; Pearlson et al., 1989). In our study co-morbid Axis II disorder was found only in 3.6% of participants and with only one patient diagnosed to have paranoid personality disorder, our study contradicts other studies which reported as high as 45% of patient with late onset psychosis having paranoid or schizotypal personality (Jeste et al., 1988; Kay and Roth, 1961; Kay et al., 1976). Family history of psychiatric disorder was noted in just over 20% of patients, among which family history of psychosis and mood disorder are 10.8% and 4.8% respectively, family history studies almost all show a familial risk of late-onset schizophrenia lower than that of early onset patients but greater than that of the general population (Howard et al., 1997).

4.2. Symptomatology

In our population delusions and hallucinations are the common symptoms, which are in concordance to other studies (Castle and Howard, 1992; Pearlson et al., 1989) which also reported delusions and hallucinations being common among late onset psychosis. On further analysis of type of delusions we found certain differences when compared to other studies. Though delusion of persecution was the commonest (88%) none of the patients had partition delusion which is in contrast to other studies that reported a high prevalence of this delusion (Howard et al., 1997; Pearlson et al., 1989).In addition only 2.4% had grandiose delusion, whereas other studies reported grandiose delusion in 14%-17% of subjects (Howard et al., 1997; Pearlson et al., 1989).Though accusatory, abusive, 3rd person auditory hallucination and hallucinations in other modalities were noted in our sample, none had running commentary type hallucination which is not in accordance with other studies (Harish et al., 1996; Howard et al., 1997).

In our study negative symptoms was reported in 2.4% of subjects and none had any formal thought disorder which is consistent with an overall lower prevalence of these symptoms in other published reports (Almeida et al., 1995; Howard et al., 1994, 1997; Pearlson et al., 1989). Some affective symptoms was noted in half of these subjects, but they appeared secondary to psychotic symptoms and were not severe enough or lacked associated symptoms that would warrant a diagnosis of mood disorder or schizoaffective disorder. As we all know well even in core schizophrenia it is not uncommon to have depressive or anxiety symptoms, which could in fact be secondary to psychotic symptoms. It is possible to misdiagnose patients with severe depression with psychotic symptoms as late onset psychosis, but in this study we recruited only those patients who followed up for at least 3 months, and we excluded those patients if diagnosis was revised to affective disorder. 3.6% of patients attempted suicide which is a cause for concern, as the intentionality and lethality of such attempts are very high in geriatric population. There was no difference in Symptomatology between sexes. This perhaps suggests that the clinical presentation of late onset schizophrenia is similar in both sexes. Diagnosis wise most patients would fall under the category of Psychosis Not otherwise Specified (Psychosis NOS) as per ICD-10, as our sample is not restricted to schizophrenia.

Memory disturbance either subjective or objective was reported in one third of the subjects despite having a stable cognitive functions and ability to take care of themselves as indicated by HMSE (Mean-24.5) and EASI (Mean-2.3) scores, in our study there was no correlation between cognitive functioning and age, age of onset of illness or duration of illness, this could be because of our strict selection criteria this in turn can validate our initial diagnosis. However it's worth evaluating the longitudinal course of these patients as two of these patients diagnosis was revised to fronto temporal dementia after nearly 1.5 years of follow-up, and there are many studies that suggest late onset psychosis is a prodrome of dementia (Brodaty et al., 1999).

4.3. Strengths

Strengths of this study are it adds to the existing knowledge on late onset psychosis in this part of the world as there is paucity of research in India on this area, relatively large sample size compared to many earlier studies done in west (Jeste et al., 1988, 1995; Pearlson et al., 1989)as well as in India (Harish et al., 1996), stricter diagnostic criteria making the sample more homogenous. We felt compared to late onset psychosis as such (which could be secondary to other cause) non-affective non-organic late onset psychosis forms a distinct group with unique manifestations which warrants further research for better understanding.

4.4. Limitations

Some of the major limitations of this study are its retrospective study design, reliance on medical records to collect details; lack of control groups (like early onset group, late onset group with organic psychosis) that would have helped us to distinguish the clinical features of these groups. One can always question the reliability of determination of symptoms from medical records, as interpretation of psychopathology can vary between individuals, the symptoms which were not mentioned in the medical records were considered as absent, No inter-rater reliability test done, which could have made the data more reliable, and the severity of symptoms could not be assessed. However since we use validated

scales and follow a standard protocol at geriatric clinic in NIMHANS, Bangalore, we hope some of these limitations were reduced.

5. Conclusion

Despite its rarity non affective, non organic, late onset psychosis forms a distinct group and validity of this psychiatry entity is undeniable. Though our study has certain striking similarities with other published research like female preponderance, delusions and hallucinations being the common symptoms, lack of negative symptoms and formal thought disorder, there are subtle variations with respect to the type of delusion and hallucination. Further systematic research is needed for better understanding of phenomenology, epidemiology, biological underpinnings, psychosocial issues, as well as course and outcome of this condition.

References

Alici-Evcimen, Y., Ertn, T., Eker, E., 2003. Case series with late-onset psychosis hospitalized in geriatric psychiatry unit in Turkey: experience in 9 years. Int. Psychogeriatr. 15, 69–72.

Almeida, O., Howard, R., Levy, R., David, A.S., 1995. Psychotic states arising in late life (late paraphrenia): psychopathology and nosology. Br. J. Psychiatry. 166, 205–214.

Bleuler, M., 1943. Die spatschizophrenenKrankheitsbilder. Fortschritte der Neurologie und Psychiatrie 15, 259–290.

Brodaty, H., Sachdev, P., Rose, N., Rylands, K., Prenter, L., 1999. Schizophrenia with onset after age 50 years. I: Phenomenology and risk factors. Br. J. Psychiatry 175, 410–415.

Castle, D.J., Howard, R., 1992. What do we know about the aetiology of late onset schizophrenia? Eur. Psychiatry 7, 99–108.

Castle, D.J., Murray, R.M., 1993. The epidemiology of late-onset schizophrenia. Schizophr. Bull. 19, 691–700.

Copeland, J.R.M., Davidson, I.A., Dewey, M.E., Gilmore, C., Larkin, B.A., McWilliam, C., et al., 1992. Alzheimer's disease, other dementias, depression and pseudodementia: prevalence: incidence and three-year outcome in Liverpool. Br. J. Psychiatry. 161, 230–239.

Copeland, J.R.M., Dewey, M.E., Scott, A., Gilmore, C., Larkin, B.A., Cleave, N., et al., 1998. Schizophrenia and delusional disorder in older age: community prevalence incidence, comorbidity and outcome. Schizophr. Bull. 24, 153–161.

Ganguli, M., Ratcliff, G., Chandra, V., Sharma, S., Gilby, J., Pandav, R., et al., 1995. A hindi version of the MMSE: The development of a cognitive screening instrument for a largely illiterate rural elderly population in India. Int. J. Geriatr. Psychiatry 10, 367–377.

Harish, M.G., Suresh, K., Rajan, I., Janardhanreddy, Y.C., Sumant, K., 1996. Phenomenological study of late-onset schizophrenia. Indian J. Psychiatary 38, 231–235.

Harris, M.J., Jeste, D.V., 1988. Late-onset schizophrenia: an overview. Schizophr. Bull. 14, 39–45.

Howard, R., Almeida, O.P., Levy, R., 1994. Phenomenology, demography and diagnosis in late paraphrenia. Psychol. Med. 24, 397–410.

Howard, R., Graham, C., Sham, P., Dennehey, J., Castle, D.J., Levy, R., et al., 1997. A controlled family study of late-onset nonaffective psychosis (late paraphrenia). Br. J. Psychiatry 170, 511–514.

Howard, R., Rabins, P.V., Seeman, M.V., Jeste, D.V., 2000. International Late-Onset Schizophrenia Group: late-onset schizophrenia and very-late-onset schizophrenia-like psychosis: an international consensus. Am. J. Psychiatry 157, 172–178.

Jeste, D.V., Harris, M.J., Pearlson, G.D., Rabins, P.V., Lesser, I., Miller, B.L., et al., 1988. Late-onset schizophrenia: studying clinical validity. Psychiatric Clin. N. Am. 11, 1–13.

Jeste, D.V., Harris, M.J., Krull, A., Kuck, J., McAdams, L.A., Heaton, R., 1995. Clinical and neuropsychological characteristics of patients with late-onset schizophrenia. Am. J. Psychiatry 152, 722–730.

Kay, D.W.K., Roth, M., 1961. Environmental and hereditary factors inthe schizophrenias of old age (late paraphrenia) and theirbearing on the general problem of causation in schizophrenia. J. MentSci. 107, 649–686.

Kay, D.W.K., Cooper, A.F., Garside, R.F., Roth, M., 1976. The differentiation of paranoid from affective psychoses by patients' premorbid characteristics. Br. J. Psychiatry 129, 207–215.

Keith, S.J., Regier, D.A., Rae, D.S., 1991. Schizophrenic disorders. In: Robins, L.N., Regier, D.A. (Eds.), Psychiatric Disorders in America: The Epidemiologic Catchment Area Study. Free Press, New York, pp. 33–52.

Kua, E.H.A., 1992. Community study of mental disorders in elderly Singaporean Chinese using the GMS-AGECAT package. Aust. N. Z. J. Psychiatry 26, 502–506.

Lacks, J., Fontenelle, L.F., Chalita, A., Mendlowicz, M.V., 2006. Absence of dementia in late-onset schizophrenia: a one year follow-up of Brazilian case series. Arquivos de Neuro-Psichiatra 64, 946–949.

Mayer, C., Kelterborn, G., Naber, D., 1993. Age of onset in schizophrenia: relations to psychopathology and gender. Br. J. Psychiatry 162, 665–671.

Pandav, R., Fillenbaum, G., Ratcliff, G., Dodge, H., Ganguli, M., 2002. Sensitivity and specificity of cognitive and functional screening instruments for dementia: the Indo-U.S. Dementia Epidemiology Study. J. Am. GeriatrSoc. 50 (3), 554–561.

Pearlson, G.D., Kreger, L., Rabins, P.V., Chase, G.A., Cohen, B., Wirth, J.B., et al., 1989. A chart review study of late-onset and early-onset schizophrenia. Am. J. Psychiatry 146, 1568–1574.

Rabins, P.V., Pauker, S., Thomas, J., 1984. Can schizophrenia begin after age 44? Compr. Psychiatry 25, 290–293.

Sheikh, J.I., Yesavage, J.A., 1986. Geriatric Depression Scale (GDS). Recent evidence and development of a shorter version. In: Brink, T.L. (Ed.), Clinical Gerontology: A Guide to Assessment and Intervention. The Haworth Press, Inc, New York, pp. 165–173.

Skoog, I., 2008. Mental Disorders Associated with Aging. In: Heggenhougen, K., Quah, S. (Eds.), International encyclopedia of public health, vol. 6. Academic Press, San Diego (CA), pp. 174–182.

Wood, S., Cummings, J.L., Hsu, M.-A., Barclay, T., Wheatley, M.V., Yarema, K.T., et al., 2000. The use of the Neuropsychiatric Inventory in nursing home residents, characterization and measurement. Am. J. Geriatr. Psychiatry 8, 75–83.

Original Research Article

A study on correlation of lithium and renal dysfunction with estimated glomerular filtration rate

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Lithium has been used in the treatment of mood disorders but its use is limited for its narrow therapeutic window and renal dys function. We aimed to correlate severity of renal dysfunction with duration of lithium therapy and serum lithium levels. We also analysed patients with Lithium induced renal dysfunction with normal creatinine values, but with abnormal eGFR. **M** **d** We included Bipolar patients who are on atleast 2 years of Lithium therapy & with minimum of 2 serum Creatinine values during treatment, baseline & 6months post-baseline. We recruited 906 patients, only 55 patients qualified for our inclusion criteria. We had 110 creatinine values & we calculated eGFR based on Modification of Diet in Renal Disease study group formula. We correlated duration of lithium therapy, serum lithium levels with average S.Creatinine levels and average eGFR. There was no correlation between duration of lithium treatment with average serum creatinine values (r = -0.17) or average eGFR (r = +0.24).There was no correlation between serum lithium levels with average serum creatinine values (r = -0.04) and average eGFR (r = -0.06).Among 19 patients with high serum creatinine values , almost all except one had Grade 2 renal dysfunction eGFR value Neither the duration of lithium therapy (upto 17 years) , nor serum lithium levels correlated with renal dysfunction. Fifteen percent of patients with normal creatinine levels had early renal dysfunction on eGFR, indicating probably eGFR as an early indicator than creatinine.

rd fLithium, Creatinine, Renal dysfunction, eGFR, Estimated Glomerular filtration rate

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Introduction

Lithium has been used in the treatment of mood disorders for several decades. But its use is limited for its narrow therapeutic window and multiple systemic side effects. Various studies were done to find the relationship between the serum lithium levels, duration of therapy, and dosage of lithium carbonate with its clinical responses. Among the available psychotrophics, Lithium remains a key drug especially in maintenance therapy. Lithium needs regular monitoring of serum lithium levels and renal dysfunction because of its narrow therapeutic window, as it is excreted nearly exclusively through the kidneys. The effect on Lithium induced renal dysfunction remains a controversy for more than four decades. Histopathological changes have been demonstrated way back in 1977, concluding chronic renal disease may develop only in patients with renal concentration of lithium reaches toxic level- either periodically or continuously.[1]

The duration of lithium treatment and cumulative dose has been suggested as major determinants of nephrotoxicity, but were only from small case series with

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multiple methodological issues.[2,3] Previous meta- analysis have shown poor quality, emphasizing the need for large scale studies. [4,5] In the study by Stephen Clos *et al*, no effect was found on rate of eGFR change over time in euthymic BPAD patients on Lithuim maintenance therapy.[6] Lithium causes a modest decline in renal function. However, the available research suggests that ESRD is a very rare complication of long-term lithium treatment, affecting ~1% of patients who have taken lithium for over 15 years.[7] With this above background, we planned for a study aiming for correlation of severity of renal dysfunction with duration of lithium therapy and serum lithium levels. We also did a correlation between socio-demographic variable and severity of renal dysfunction. We also analysed patients with Lithium induced renal dysfunction with normal creatinine values, but with abnormal eGFR.

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We included patients with bipolar affective disorder who are on atleast 2 years of Lithium therapy & with minimum of 2 serum Creatinine values during the treatment, one at baseline & atleast one after>= 6 months post-baseline. We excluded patients who were on short duration of lithuim treatment (< 2 years) or who had only one/no serum creatinine value. We also excluded patients who were on not on follow-up at PSG. We retrieved 1296 Serum lithium values of Bipolar affective disorder patients from PSG Hospitals MRD records (2013 – 2017). 522 values were repeat serum lithium values of same patient. Hence 774 patients with all the available lithium values were included. 295 patients excluded , as they were all inpatients and they had another out-patient file. Among the remaining 499 patients, 339 patients were excluded (160 patients were left), as they had one or no serum creatinine value.105 patients were excluded as they did not have serum creatinine values both at baseline and 6months post-baseline. Finally, 55 Patients were analysed for the study. We had 121□creatinine values & we calculated corresponding eGFR based on Modification of Diet in Renal Disease (MDRD) study group formula,[8] eGFR (mL × min^{-1}× [1.73 m^2] $^{-1}$)= 175 × (S_{Cr} standardized [mg × dL^{-1}]) $^{-1.154}$× (age [years])$^{-0.203}$Correction factor: for women × 0.742 for blacks × 1.18 186 x [Plasma Creatinine (µmol/L) x 0.0011312] $^{-1.154}$ x [age (years)] $^{-0.203}$ x [0.742 if female] x [1.212 if black] The following categories of eGFR were considered, as per KDOQI criteria. [9] Grade 1 -> 90 ml/min/1.73 m^2: Grade 2 – 60 to 89 ml/min/1.73 m^2: Grade 3a – 45 to 59 ml/min/1.73 m^2: Grade 3b–30 to 44 ml/min/1.73 m^2: Grade 4– 15 to 29 ml/min/1.73 m^2 : Grade 5–<15 ml/min/1.73 m^2.We analyzed baseline socio-demographic variables , namely, age & sex .We categorized age into adolescence, adults & geriatric population. We also analysed the co-morbidities, duration of follow-up after initiation of study, serum Lithium Level, total serum Creatinine values & total eGFR values. We correlated duration of lithium therapy with patients average S.Creatinine levels and average eGFR. We also correlated serum lithium levels with patients average S.Creatinine levels and average eGFR. We compared patient's serum creatinine level and the corresponding eGFR. We analysed patients having normal serum creatinine (<=1 mg/dl) values with patients having abnormal eGFR.

□□□□□□□□□□□□□□□□□□□□□□□□We analysed duration of lithium therapy, serum lithium levels, average serum creatinine levels , eGFR as a continuous measures. We used Pearsons correlation co-efficient to correlate duration of lithium therapy, serum lithium levels with serum creatinine and eGFR. If the r value is +1, it means a positive correlation and if it is1, it means a negative correlation. We also tabulated normal serum creatinine (<=1 mg/dl) values with abnormal eGFR in varying ranges.

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Majority of the patients were men & in adult age group. Majority of patients followed upto 5 years & more than two-fifth followed up more than 5 years. Diabetes was the commonest co-morbidity. Six percentage of patients had serum lithium level in toxic range. Seventeen percentage of patients had serum creatinine value more than one. Thirty percentage of patients had eGFR less than 90.

Table 1: Socio-demographic variables at baseline and Lithium parameters

Socio-demographic parameters & Lithium variables			Mean
Age(yrs)	<18	1 (1.8%)	44.93
	18-65	50 (90.9)	
	>65	4 (7.3)	
Sex	Male	32 (58.2)	-
	Female	23 (41.8)	
Duration of Lithium(yrs)	<2	0(0%)	
	2-5	32 (58.1%)	5

Co-morbidity	>5	23 (41.9%)	-
	DM	11 (20%)	
	HT	1 (1.8%)	
	DM & HT	4 (7.3%)	
	CVA	1 (1.8%)	
	Nil	38 (69.1%)	
Duration of Follow-up after initiation of study(yrs)	<2	2(3.6%)	3.2
	2-5	53 (96.4%)	
Average serum Lithium Level(mg/dl)	<0.8	27 (49.1%)	0.75
	0.8 – 1.2	24.5 (44.1%)	
	>1.2	3.5 (6.4%)	
Total Serum Creatinine values	≤ 1	91 (82.8%)	0.78
	>1	19(17.2%)	
Total eGFR values	≥90	77 (70%)	102
	60-89	32 (29%)	
	45-59	1 (1%)	

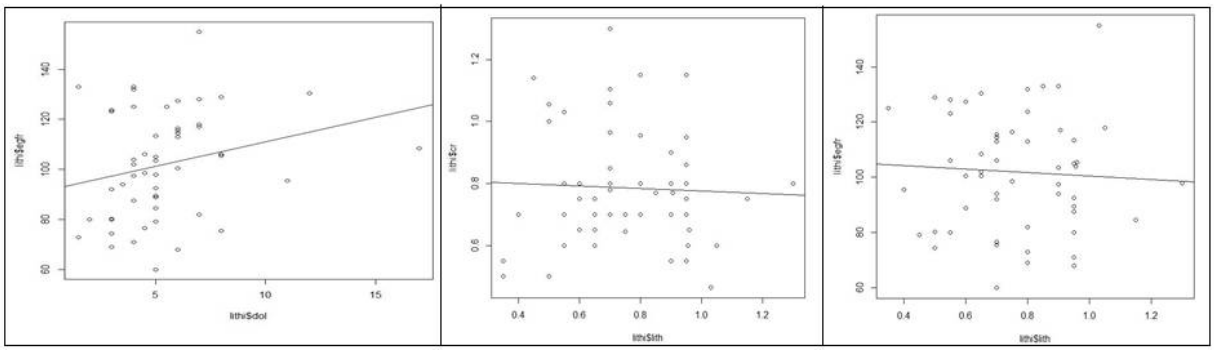


Figure 1 **Figure 2** **Figure 3**

Figure 1: Pearsons Correlation curve comparing duration of Lithium therapy with average serum creatinine values: **Figure 2:** Pearsons Correlation curve comparing serum lithium levels with average serum creatinine values; **Figure 3:** Pearsons Correlation curve comparing serum lithium levels with average estimated Glomerular filtration rate

Figure 1, The above curve denotes no correlation between duration of lithium treatment with average eGFR (r =+0.24).
Figure 2, The above curve denotes no correlation between serum lithium levels with average serum creatinine level (r = -0.04).
Figure 3, The above curve denotes no correlation between serum lithium levels with average eGFR (r = -0.06).

Table 2: Comparison of patients with normal creatinine levels with low eGFR

Sr. No	Sr. Creat. Values (mg/dl)	eGFR Values (ml / min / 1.73 m²)
1	0.8	76
2	0.8	77
3	0.8	80
4	0.8	74
5	0.8	74
6	0.8	80
7	0.9	77
8	1.0	87
9	0.9	85
10	0.7	86
11	1.0	85
12	0.8	68
13	0.9	89
14	0.8	72
15	0.98	87

Among 102 (102 / 121) normal serum creatinine values, 15 serum creatinine values (14.7 %) had low corresponding eGFR values.

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Table 3: Comparison of patients with high creatinine levels with eGFR values.

Sr .No	Sr. Creat. Values (mg/dl)	eGFR Values (ml / min / 1.73 m²)
1	1.01	79
2	1.09	72
3	1.24	63
4	1.02	66
5	1.10	75.9
6	1.20	64
7	1.03	79
8	1.09	72
9	1.01	82
10	1.21	64
11	1.38	60
12	1.36	**57**
13	1.20	66
14	1.11	75
15	1.10	74
16	1.09	74
17	1.02	77
18	1.05	78

All the patients, except one had Grade 1 renal dysfunction, eGFR between 60 to 98.

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We did a retrospective study on effect of lithium levels and duration of lithium therapy on renal dysfunction. Majority of the recruited were adult men with diabetes as the common co-morbidity. The duration of lithium therapy, did not correlate with renal dysfunction in-terms of serum creatinine values. Three patients were on treatment for more than ten years, one for even seventeen years. The corresponding eGFR values also did not correlate with duration of lithium therapy. Previous studies and meta-analysis also concluded the same result. In the study by Stefan Clos *et al*, both, duration of exposure to lithium and mean serum lithium level were not significant predictors for eGFR decline. Our study also reveals serum lithium level (which is a marker of adequacy of lithium dose), did not correlate with renal dysfunction, either by creatinine levels or eGFR levels. Previous studies have shown that exposure to lithium (in the absence of episodes of toxicity) is a causal factor and the length of lithium exposure as a predictor of renal dysfunction.[10,11] In contrary, our study did not show correlation. A large UK community sample study by Miney *et al*[12] had a short observation period of 2 years and did not analyse longitudinal changes. In our study patients were on lithium, even for 17 years and the mean duration of lithium was five years. In a UK cohort study, Close and colleagues 13 used diagnostic codes for adverse renal outcomes rather than eGFR. We preferred eGFR as an indicator of renal dysfunction as it is a better predictor than serum creatinine. In an observational study by, Aprahamian and colleagues,[14] a single-blind follow-up study , no significant change in renal function of elderly patients after 4 years of chronic low-dose lithium use , as evidenced by our study. About fifteen patients (15 %), with normal creatinine levels had low eGFR values, indicating eGFR could pick-up renal dysfunction early. Among 19 patients with high serum creatinine values, almost all except one had Grade 2 renal dysfunction eGFR value .A single patient had grade 3 renal dysfunction 9 (eGFR –57).Our study limitations are , retrospective study design which has its own critics. The sample size was small and hence results can't be generalized. Mean duration on lithium was five years; a longer study would be of more credit. We could have had a control population, with which we would have analyzed in a better way. Our study was an observational study , hence only the available creatinine & lithium level were used for analysis.Almost one-third of patients had a co-morbid physical illness , disorders like diabetes and hypertension is known to cause renal dysfunction. Cockcroft-Gault equation is well suited for estimation of GFR changes during pharmacotherapy, instead we used MDRD equation, which is applied in adults with chronic renal disease. The National Kidney Foundation grades chronic kidney disease based on kidney damage (for ≥ 3 months, as defined by structural of functional abnormalities of the kidney, with or without decreased GFR, manifest by either : Pathological abnormalities; or markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests) and eGFR. In our study, we used only eGFR as an indicator of renal dysfunction. The strengths of our study are, we had a strict inclusion criteria, thereby excluded patients an acute phase of lithium treatment

(less than two years of treatment). We included patients only with post-baseline creatinine levels, atleast after 6 months, hence had a minimum renal exposure to lithium.

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In our study, neither the dosage (analysed with serum lithium level), nor the duration of lithium treatment, caused renal dysfunction. Hence, clinicians prescribing lithium need not be anxious on renal parameters, but need to monitor as per guidelines. We would also recommend clinicians to monitor eGFR than creatinine, to identify renal dysfunction early.

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1. Hestbech J, Hansen HE, Amdisen A, Olsen S. Chronic renal lesions following long-term treatment with lithium. *Kidney Int* 1977;12:205–13.□
2. Nolin T, Himmelfarb J. Mechanisms of drug-induce nephrotoxicity Adverse drug reactions. 2010:111–30.□
3. Presne C, Fakhouri F, Noel LH. Lithium-induced nephropathy: rate of progression and prognostic factors. *Kidney Int* 2003;64: 585–92.□
4. Paul R, Minay J, Cardwell C, Fogarty D, Kelly C. Meta-analysis of the effects of lithium usage on serum creatinine levels. *JPsychopharmacol* 2010; 24: 1425–31.□
5. McKnight RF, Adida M, Budge K, Stockton S, Goodwin GM, Geddes JR. Lithium toxicity profile: a systematic review and meta-analysis. *Lancet* 2012; 379: 721–28.□
6. Stefen Clos , Long-term effect of lithium maintenance therapy on estimated glomerular filtration rate in patients with affective disorders: a population-based cohort study. Lancet Psychiatry 2015 : 2:1075-83□
7. Stevens LA , Effects of chronic lithium treatment on renal function : Journal of Affective disorders 2010 : 126, 436-440.□
8. Stevens LA, Zhang Y, Schmid CH: Evaluating the performance of equations for estimating glomerular filtration rate. J Nephrol 2008;21:797–807.□
9. NKF- KDOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification , 2016.□
10. Nolin T,Himmelfarb J. Mechanisms of drug-induced nephrotoxicity. Adverse drug reactions, 2010: 111–30.□
11. Presne C, Fakhouri F, Noel LH. Lithium-induce nephropathy: rate of progression and prognostic factors. *Kidney Int* 2003;64□585–92□
12. Minay J, Paul R, McGarvey D. Lithium usage and renal function testing in a large UK community population;a case-control study. *Gen Hosp Psychiatry* 2013; 35□631–35.□
13. Close H, Reilly J, Mason JM. Renal failure in lithium-treated bipolar disorder: a retrospective cohort study. J.Nephrol. 2014;9: 560-565.□
14. Aprahamian I, Santos FS, dos Santos B. Long-term, low-dose lithium treatment does not impair renal function in the elderly: a 2-year randomized, placebo-controlled trial followed by single-blind extension. *J Clin Psychiatry* 2014;75□72–78.

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Original Research Article

Effectiveness of mood stabilizer in euthymic BPAD patients: An one year prospective observational study, comparing Lithium v/s Divalproate sodium

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Abstract Bipolar disorder is potentially a lifelong and disabling condition, which requires long-term pharmacological therapy as maintenance treatment. Lithium is considered as first-line mood stabilizer, followed by Divalproate, both are known to reduce the risk of relapse. However, relapses do occur and these medications are also associated with side effects. We aimed to compare the mood stabilizing effect between Lithium and Divalproate in our prospective study. **Methods** 104 bipolar patients who were euthymic (52 patients each on Lithium and Divalproate therapy) were followed up for one year with periodic evaluation done during initial, 3rd, 6th, 9th and 12th month. Time taken to relapse, severity of mood episode, suicidal risk and functioning were assessed using standardized scales. Compliance to medications and adverse effects were also assessed. **Results** The duration of treatment was for a longer period in Lithium group (p=0.001) and this was the only confounding variable which was significant. Patients who are taking Divalproate had more severe manic episode at the end of 1 year when compared to patients taking Lithium (p=0.041). Patients on Lithium also had lesser suicidal risk (trending towards significance). **Conclusion** There was no difference in terms of frequency of depressive episode, adherence, adverse effects and global functioning between the two groups. But Lithium group patients had less frequent and less severe manic episodes and lower suicidal risk, favoring Lithium to be a better mood stabilizer, with less adverse effects and better quality of life.

Key words Bipolar disorder; Mood stabilizer; Maintenance treatment; Quality of life.

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Introduction

Bipolar Affective Disorder (BPAD) is one of the most disabling mental illnesses affecting most productive period of life at the age 15-45years.[1] Lithium carbonate has been a gold standard treatment for past five decades. But it has a narrow therapeutic index and significant adverse effects.[2] Anticonvulsants such as Divalproate sodium, Carbamazepine and Oxcarbamazepine, have been proposed as an alternative, also have more adverse effect profile and their comparative efficacy with Lithium is uncertain.[3-8] Lithium Carbonate as a mood stabilizer has been a superior agent to reduce the risk of relapse and to prevent suicidal behaviours.[2-7] In view of its adverse effects, tolerance becomes an issue; which can interfere with adherence.[4-7] Lithium causes multiple skin reactions, the most common are acne and psoriasis. The prevalence of skin reaction with Lithium ranges between 3-34 %. Some studies have shown high chance of discontinuation of Lithium is due to adverse skin reactions.[9] Anticonvulsants approved by FDA, has the next level of

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evidence as a mood stabilizer but their long term safety and efficacy in comparison with Lithium remains uncertain[7] Randomised controlled trails have shown superiority of Divalproate sodium to placebo.[10-11] Divalproate sodium has been comparable with Lithium in Manic episode[11] FDA approved mood stabilisers for the treatment of bipolar affective disorder are Lithium, Divalproate sodium, Carbamazepine and Lamotrigine.[17-20] Mood stabilisers can also be used as monotherapy which was approved by FDA.[21-22] Mania with two or more episodes of depression showed a good improvement with Divalproate sodium than Lithium.[23-24] Lithium and Divalproate sodium showed more effect than any other mood stabilisers during acute phase of mania and in maintenance phase.[25-27] Olanzapine, Risperidone and Quetiapine are FDA approved atypical antipsychotics for the acute phase of mania[17,18,20] According to BALANCE study both Lithium monotherapy and combination therapy with Lithium and Divalproate sodium are more likely to prevent relapse than Divalproate sodium monotherapy, irrespective of baseline severity of illness and is maintained for up to 2 years.[12] Bowden *et al*, in his randomized placebo controlled 12 months trial of Lithium v/s Divalproate sodium, has shown no significant difference between the two groups in terms of time to recurrence of mood episode during maintenance therapy. Compared to placebo, Divalproate sodium has lesser discontinuation rate[13.] Even though open labelled trails favours Divalproate sodium in reducing the frequency and intensity of further episodes, there are less comparative study with Lithium in maintenance therapy.[14-16] Long term outcomes are often poor in patients with bipolar disorder despite treatment; therefore more effective treatments are needed to reduce recurrences and morbidity. Hence, we proposed a prospective comparison study of Lithium and Divalproate sodium in euthymic BPAD patients for a period of at least 1year during the maintenance phase. We aimed to compare the mood stabilizing properties of Lithium and Divalproate sodium in BPAD patients. Our other objectives are to evaluate the time taken for relapse of any mood episodes (mania/depression/mixed episodes), assess the severity of the mood episodes, episodes of deliberate self harm, adherence to study treatment, adverse effects of medications and global assessment of functioning.

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This is a prospective observational study done in patients with bipolar affective disorder who came to the psychiatry department in PSG Institute of Medical Sciences and Research, Coimbatore, India. A total of 104 bipolar affective disorder patients were recruited from out-patient setting. The study protocol was explained to the participants, and a written informed consent was obtained from the patient. If patient could not consent, it was obtained from the family member and later from the patient when he/she could consent. The study was approved by Institutional Human Ethics Committee.

Men and women aged 18 years and above who received a clinical diagnosis of BPAD (as per ICD-10 criteria) in psychiatry out-patient department were eligible for the study. Patients were initiated or continued on a single mood stabilizer either on Lithium or Divalproate sodium. Only patients who achieved remission within 2 months of follow-up were included in the study. Patients who were already on more than one mood stabilizers (Lithium, Valproate, Carbamazepine, Lamotrigine, Gabapentin and Topiramate) during index diagnosis and medically unstable patients with conditions such as renal failure, liver cirrhosis, severe diabetes, arrhythmia etc. were excluded from the study.

After recruiting the patients as per our inclusion and exclusion criteria, 52 patients who were on Lithium therapy and 52 patients who were on Divalproate therapy, were prospectively followed up for 1 year period. Patients were evaluated by the investigator, following 2 months of euthymic period (index evaluation). During follow up patients were evaluated at 3rd, 6th, 9thand 12th month respectively (periodic evaluation). Patients who are missing on follow up are contacted through telephone and requested to come for follow-up and assessed. As it is an observational study, investigators took no role in modifying the dosage of medications, but it was taken care by the primary incharge treating team. During initial evaluation, a semi-structured proforma (socio-demographic details and other confounding variables) is administered. Diagnosis was confirmed by applying a SCID (Structured Clinical Interview for DSM-IV) version for mood disorder. As per earlier literature available, remission is a state that comes close to being symptom free. By which the euthymic status of the patient was ascertained by applying HAM-D and YMRS rating scales. Typically remission should have a score of ≤ 7 on HAM-D and ≤ 2 on YMRS. Severity of suicidal ideas is assessed by Modified SADPERSONS Scale. Global Assessment of Functioning was assessed using a GAF scale. The dosage of the mood stabilizer could be altered by the primary in-charge team based on serum concentration of the drug/adverse effects, during 1 year maintenance period. Participants who remain on the allotted treatment for 1 year of study were periodically evaluated. Use of other psychotropics was allowed during the study trial (antipsychotics, benzodiazepines).Periodic assessment was done as per the figure 1 given below, for confounding variables, SCID-mood disorder sub-scale,

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Young Mania Rating Scale (YMRS)/ Hamilton rating scale for Depression (HAM-D) appropriate to the patient (to access the severity of episode if any), Modified SADPERSONS scale-to see suicidal score, adverse drug reaction and Global assessment of functioning (GAF) scale.

Statistical analyses were conducted using software package SPSS version 20. We compared the efficacy of Lithium and Divalproate sodium with the following variables such as age, gender, marital status and education status, which were expressed in percentage and the presence of any statistically significant difference, was analysed using Chi-square test. Also statistically significant difference between the age of onset, number of previous episodes, previous hospitalisation, polarity of previous episodes, psychotropics and duration of mood stabiliser with that of Lithium and Divalproate sodium was done using Chi-square test. Comparison of duration of illness, time taken for any mood episodes, severity of manic episode/depressive episode, suicidal risk, adherence to medications, adverse effects, global assessment functioning between Lithium and Divalproate sodium was done using analysis of variance; p-values <0.05 was considered as significant.

Table 1: Comparison of socio-demographic and confounding variable between Lithium and *Divalproate sodium group*:

Variables		Lithium	Divalproate sodium	p value
Age at recruitment	18- 35 years	31(59.6%)	25(48.1%)	0.392
	36- 59 years	18(34.6%)	21(40.4%)	
	> 60 years	3(5.8%)	6(11.5%)	
Gender	Male	32(61.5%)	39(75.0%)	0.140
	Female	20(38.5%)	13(25.0%)	
Educational status	Illiterate	7(13.5%)	14(26.9%)	0.101
	Up to 10th std	27(51.9%)	20(38.5%)	
	11th-12th	5(9.6%)	10(19.2%)	
	Graduate	13(25.0%)	8(15.4%)	
Marital status	Unmarried	15(28.8%)	11(21.2%)	0.169
	Married, living together	32(61.5%)	26(50.0%)	
	Married, living separately	3(5.8%)	7(13.5%)	
	Married, divorced	1(1.9%)	4(7.7%)	
	Widow/widower	1(1.9%)	4(7.7%)	
Age of onset	< 35 years	19(36.5%)	9(17.3%)	0.085
	36- 59 years	27(51.9%)	36(69.2%)	
	> 60 years	6(11.5%)	7(13.5%)	
Number of episodes	1episode	0(0.0%)	3(5.8%)	0.145
	2episode	18(34.6%)	13(25.0%)	
	>=3episodes	34(65.4%)	36(69.2%)	
Previous hospitalisations	No hospitalisation	7(13.5%)	6(11.5%)	0.594
	1 hospitalisation	9(17.3%)	5(9.6%)	
	2 hospitalisations	15(28.8%)	19(36.5%)	
	>=3hospitalisations	20(38.5%)	22(42.3%)	
Polarity of previous episodes	1 Depressive episode	1(1.9%)	0(0.0%)	0.373
	1 Manic episode	4(7.7%)	6(11.5%)	
	Depression = Mania	18(34.6%)	14(26.9%)	
	Depression > Mania	11(21.2%)	7(13.5%)	
	Mania > Depression	12(23.1%)	12(23.1%)	
	>=2 mania episodes	6(11.5%)	13(25.0%)	
Psychotropics	No drugs	23(44.5%)	13(25.0%)	0.982
	Typical antipsychotics	7(13.5%)	15(28.8%)	
	Atypical antipsychotics	19(36.5%)	21(40.4%)	
	Antidepressants	2(3.8%)	2(3.8%)	
	Benzodiazepines	1(1.9%)	1(1.9%)	
Duration of Mood Stabilizer	<6months	1(1.9%)	0(.0%)	**0.001**
	6months-1year	7(13.5%)	1(1.9%)	
	1- 2years	5(9.6%)	20(38.5%)	
	>2years	39(75.0%)	31(59.6%)	

There was no significant difference between the two groups in socio-demographic variables (Table 1) such as age (p=0.392), gender (p=0.14), educational qualification (p=0.10) and marital status (p=0.17).There is no statistical significance among the confounding variables such as; age of onset (p=0.085), number of episodes (p=0.145), previous hospitalisations (p=0.594), polarity of episodes (p=0.373), psychotropics (p=0.982). Between the two groups, the duration of illness (p=0.001) was the only confounding variable which was significant between the two groups. The number of mood episodes that occurred was; among Lithium group patients 13 had manic episodes and 8 had depressive episodes. Among *Divalproate sodium group* patients 12 had manic episodes and 6 had depressive episodes. In both the groups' manic episodes were more common than depressive episodes.

For a manic episode relapse; in the *Lithium group*, 39 patients had no episodes of mania, 5 patients developed mania in less than three months, 4 patients between three to six months and 4 patients between six to nine months. In the *Divalproate sodium group*, 40 patients had no episodes of mania, 2 patients developed mania in less than three months, 3 patients between three to six months, 3 patients between six to nine months and 4 patients between nine to twelve months. The time taken for manic episode was not statistically significant between Lithium and *Divalproate sodium group* (p=0.339). For a depressive episode to occur; in the *Lithium group*, 45 patients had no episodes of depression, 2 patients developed depression between three to six months, 2 patients between six to nine months and 3 patients between nine to twelve months. In the *Divalproate sodium group*, 46 patients had no episodes of depression, 1 patient developed depression in less than three months, 3 patients between three to six months and 2 patients between nine to twelve months. The time taken for depressive episode was also not statistically significant between Lithium and *Divalproate sodium group* (p=0.240).

Comparison of severity of manic episode between Lithium and *Divalproate sodium group* using YMRS

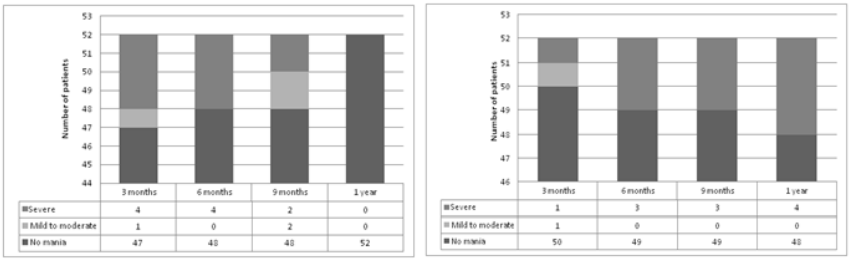


Figure 2A: Figure 2B:

Figure 2 A: Severity of mania in patients taking Lithium; **Figure 2B:** Severity of mania in patients taking Divalproate sodium

The severity of mania was assessed using The Young Mania Rating Scale (YMRS) in Figure2. Patients who are taking Divalproate sodium had more severe manic episode at the end of one year when compared to patients taking Lithium (4 patients v/s none – p=0.041), but the comparison was not significant during initial 3 follow ups at 3, 6 and 9 months (p=0.388, 0.696, 0.331 respectively)

Comparison of severity of depressive episode between Lithium and *Divalproate sodium group* using HDRS:

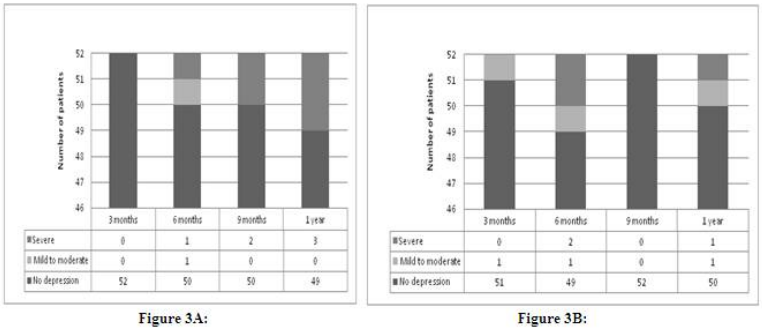


Figure 3A: Figure 3B:

Figure 3A: Severity of depression in patients taking Lithium; **Figure 3B:** Severity of depression in patients taking Divalproate

The severity of depression was assessed using Hamilton Depression Rating Scale (HDRS) in Figure 3. On comparison with patients who are taking Lithium and Divalproate sodium. There was no statistically significant difference in the severity of depressive episode during all four follow ups (3rd, 6th, 9th and 12th month periodic evaluation; p = 0.315, 0.842, 0.153, 0.366 respectively).

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Table 2: Suicidal risk assessment between Lithium and *Divalproate sodium group* using Modified SADPERSONS Scale

Groups	No risk	Moderate risk	Severe risk	p Value
		At Baseline		
Lithium	37 (71.2%)	15 (28.8%)	0	
Divalproate sodium	35 (67.3%)	17 (32.7%)	0	0.671
		During *3 months* follow-up		
Lithium	35 (67.3%)	14 (26.9%)	3 (5.8%)	
Divalproate sodium	34 (65.4%)	18 (34.6%)	0	0.173
		During *6 months* follow-up		
Lithium	39(67.3%)	9(26.9%)	4(7.7%)	
Divalproate sodium	34(65.4%)	15(28.8%)	3 (5.8%)	0.371
		During *9 months* follow-up		
Lithium	42(80.8%)	8(15.4%)	2(3.8%)	
Divalproate sodium	40(76.9%)	9(17.3%)	3 (5.8%)	0.857
		During *12 months* follow-up		
Lithium	47(90.4%)	3(5.8%)	2(3.8%)	
Divalproate sodium	38(73.1%)	9(17.3%)	5 (9.6%)	**0.073**

Modified SADPERSONS scale was applied to assess suicidal risk. The severity of suicidal risk scale (Table 2) was not statistically significant during initial and all four follow-ups, but was trending towards significance during the 12th month follow-up (baseline, 3rd, 6th, 9th and 12th month periodic evaluation; p=0.671, 0.173, 0.371, 0.857, 0.0 respectively) In the Lithium group of patients; 4 (7.7%) had no follow-up, 5 (9.6%) have followed-up once, 8 (15.4%) have followed-up twice, 10 (9.2%) have followed-up thrice and 25 (48.1%) have been completely followed-up till the end of one year. In the *Divalproate sodium group* of patients; 5 (9.6%) had no follow-up, 4 (7.7%) have followed-up once, 8 (15.4%) have followed-up twice, 13 (25%) have followed-up thrice and 22 (42.3%) have been completely followed-up till the end of one year. Among 104 recruited patients, 25 patients of Lithium group had completed all four follow-ups as compared to 22 patients of *Divalproate sodium group*. But on comparison between the two groups, they were not statistically significant (p=0.938).

Table 3: Adverse effects of the treatment

Groups	No drug reaction	Minimal reaction	More reaction	p value
		At Baseline		
Lithium	28(53.8%)	24(46.2%)	0	
Divalproate sodium	24(46.2%)	26 (50%)	2(3.8%)	0.303
		During *3rd month*follow-up		
Lithium	23(44.2%)	29(55.8%)	0	
Divalproate sodium	24(46.2%)	23(44.2%)	5(9.6%)	0.057
		During*6th month* follow-up		
Lithium	27(51.9%)	25(48.1%)	0	
Divalproate sodium	26 (50%)	24(46.2%)	2(3.8%)	0.361
		During *9th month* follow-up		
Lithium	30(57.7%)	22(42.3%)	0	
Divalproate sodium	35(67.3%)	16(30.8%)	1(1.9%)	0.312
		During *12th month* follow-up		
Lithium	42(80.8%)	10(19.2%)	0	
Divalproate sodium	39(75.0%)	11(21.2%)	2(3.8%)	0.340

The adverse drug effect (categorised as nausea, diarrhoea, tremors, weight gain, sedation, polydipsia, polyuria, tachycardia, alopecia, any major skin lesions, hypothyroid symptoms, signs of renal dysfunction) profile (Table 3) was the same between Lithium and *Divalproate sodium group* during all four visits except during initial follow up (3months) in which Lithium group was better than *Divalproate sodium group* (five patients on *Divalproate sodium group* had >=3 adverse drug reaction compared to none in the Lithium group), which was statistically significant.

Table 4: Global Assessment Functioning

Groups	Good functioning	Mild impairment	Moderate impairment	Severe impairment	p value
		At *Baseline*			
Lithium	51 (98.1%)	1 (1.9%)	0	0	0.315
Divalproate sodium	52 (100%)	0	0	0	
		During *3rd month* follow-up			
Lithium	46(88.5%)	2(3.8%)	3(5.8%)	1(1.9%)	0.553
Divalproate sodium	49(94.2%)	2(3.8%)	1(1.9%)	0	
		During *6th month* follow-up			
Lithium	46(88.5%)	1(1.9%)	3(5.8%)	2(3.8%)	
Divalproate sodium	46(88.5%)	2(3.8%)	2(3.8%)	2(3.8%)	0.912
		During *9th month* follow-up			
Lithium	46(88.5%)	2(3.8%)	3(5.8%)	1(1.9%)	
Divalproate sodium	49(94.2%)	0	1 (1.9%)	2(3.8%)	0.330
		During *12th month* follow-up			
Lithium	49(94.2%)	0	3(5.8%)	0	
Divalproate sodium	46(88.5%)	0	2(3.8%)	4(7.7%)	0.117

On assessment of social, psychological and occupational functioning by using GAF scale (Table 4). During the initial follow ups Lithium group patients had more functional impairment than *Divalproate sodium group* patients (initial and 3rd follow up). During 6th month follow up two patients in each group had severe impairment. During 9th month and 1year follow up, *Divalproate sodium group* had more severe functional impairment than Lithium group, but was not statistically significant.

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In our prospective study of one year duration, we compared the mood stabilising effect of Lithium and Divalproate sodium in euthymic bipolar patients. The socio-demographic and confounding variables did not differ between the two groups except that the Lithium group patients had longer duration of treatment than those treated with Divalproate sodium. As a primary outcome of the study, we compared the frequency of manic and depressive episode between two groups. There were similar number of episodes and the predominant mood episode was mania in both the groups. The above finding could also be because the polarity of previous episode in both the groups was predominantly mania. During the period of 9th to12th month follow up, Divalproate sodium had more manic episodes. The above finding emphasises Lithium to be a better long term mood stabiliser than Divalproate sodium. Previous studies have shown that Lithium treatment reduces the risk of relapse in bipolar disorder. The preventive effect is clear for manic episodes, although it is equivocal for depressive episodes.[5] Lithium appears to be the most effective treatment to prevent recurrence or relapse of bipolar disorder and may prolong the time before adjunctive prescribing is necessary.[28] This finding is further supported by the result of recent meta-analysis[29] suggest that Lithium is superior in prevention of manic episodes, in comparison to anticonvulsants. However, there was no significant difference regarding prevention of overall mood episodes, depressive episodes or study completion. Based on the severity of mood episode; patients on Divalproate arm, had more severe manic episodes as the duration of follow up increased. This again emphasises that Lithium is a better anti-manic agent for long-term therapy. But, the frequency and severity of depressive episodes did not differ between both the groups. On comparison of suicidal risk, bipolar patients who were on Lithium had lower suicidal risk than Divalproate patients, especially with prolonged duration of treatment. Both Lithium and *Divalproate sodium group* patients had almost equal follow ups and were equally adherent to

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treatment. Two patients on Lithium were switched to Divalproate sodium as they had severe skin reaction which affected the study adherence. Adverse effect profile did not differ during initial and periodic assessment between the two groups. Bipolar patients on Divalproate sodium had more adverse effects during initial follow ups, which were not seen during further follow up. Global assessment of functioning was done considering psychological, social and occupational functioning on a hypothetical continuum of mental-health illness. Patients on *Divalproate sodium group* had more severe impairment in functioning, than patients in Lithium group. The serum Lithium level was less than adequate in majority of new onset manic/depressive episode patients, signifying that majority of the patients who developed new onset manic/depressive episode were on sub therapeutic dose. Further emphasising the relationship between plasma levels of the drug and relapse prevention.

Our sample size was smaller and hence the results cannot be generalised. Telephonic assessments for patients, who missed follow ups, cannot be considered as reliable as face to face interview. Use of other psychotropics (antipsychotics, benzodiazepines) was allowed. We know that medication like Olanzapine, Risperidone and Quetiapine can have a mood stabilising effect, which could have affected the outcome of the study. A follow-up more than 1 year is needed in order to strongly support our finding that, bipolar patients on Lithium were better than Divalproate sodium in preventing the manic episode.

Bipolar patients on Lithium had lesser frequent and less severity of manic episode over one year follow up. The frequency of depressive episode was similar between the Lithium and *Divalproate sodium group*s. Lithium exhibited better anti-suicidal properties. Lithium continues to be a gold standard in spite of certain cutaneous side effects establishing its seven decades of dominance as a mood stabilizer agent. In addition, long-term use of Lithium also ensures better quality of life.

r SU and SA designed this study. SA managed the data collection. SU, SA and KSR analysed the data. KSR wrote the draft of the manuscript under the supervision of SU and SA. All authors contributed to and have approved the the final manuscript.

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1. Murray CJL, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet* 1997; 1436–42.
2. American Psychiatric Association. Practice guideline for the treatment of patients with bipolar disorder (revision).*Am J Psychiatry* 2002; **1** (4 suppl) 1–50.
3. Goodwin GM. Evidence-based guidelines for treating bipolar disorder: revised second edition—recommendations from the British Association for Psychopharmacology. *J Psychopharmacol*2009; **2** 346–88.
4. National Institute for Health and Clinical Excellence. Bipolar disorder: the management of bipolar disorder in adults, children and adolescents, in primary and secondary care. http://guidance.nice.org.uk/CG38 (accessed Nov 19, 2009).
5. Geddes JR, Burgess S, *et al.* Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. *Am J Psychiatry* 2004; **161** 217–22.
6. Cipriani A, Pretty H, *et al.* Lithium in the prevention of suicidal behaviour and all-cause mortality in patients with mood disorders: a systematic review of randomised trials.*Am J Psychiatry* 2005; **162** 1805–19.
7. Soares-Weiser K, Bravo Vergel Y, *et al.*. A systematic review and economic model of the clinical effectiveness and cost effectiveness of interventions for preventing relapse in people with effectiveness of interventions for preventing relapse in people with bipolar disorder. *Health Technol Assess* 2007; **11** 1–226.
8. Coryell W, Winokur G, *et al.* Lithium and recurrence in long term follow up of bipolar affective disorder.psychol med.1997;27:281-289.
9. Chan HH, Wing Y, *et al.*A control study of the cutaneous side effects of chronic lithium therapy. J Affect Disord. 2000 57:107–113
10. Bowden CL, Brugger AM, *et al.*, for the Depakoke Mania Study Group. Efficacy of divalproex vs lithium and placebo in the treatment of mania. JAMA. 1994;271:918-924.
11. Pope HG Jr, McElroy SL,*et al.*. Valproate in the treatment o acute mania: a placebo-controlled study. Arch Gen Psychiatry. 1991;48:62-68.
12. John R Geddes, Guy M Goodwin, *et al.* Lithium plus valproate combination therapy versus monotherapy for relapse prevention in bipolar I disorder(BALANCE): a randomised open-label trial. *Lancet* 2010; 375: 385–95
13. Charles L. Bowden, Joseph R.Calabrese, *et al.* A Randomized, Placebo-Controlled 12-Month Trial of Divalproex and Lithium in Treatment of Outpatients With Bipolar I Disorderfor Divalproex Maintenance Study GroupArch Gen Psychiatry. 2000;57:481-48
14. Lambert PA, Venaud G. Comparative study of valpromide versus lithium in the treatment of affective disorders. Nervure. 1992;5:57
15. Calabrese JR, Delucchi GA. Spectrum of efficacy of valproate in 55 patients with rapid-cycling bipolar disorder. AM J Psychiatry, 1990;147:431-434
16. McElroy SL, Keck PE Jr. Pope HG Jr. Sodium Valproate :its use in primary psychiatric disorders. J.clinPsychopharmacol. 1987; 7; 16-24.

17. Bowden CL, Ketter TA, Sachs GS, Thase ME. Focus on bipolar disorder treatment. J. Clin. Psychiatry.2005b; 66: 1598–1609.
18. FDA FDA approved drug products. 2009. [May 27]. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm.
19. Fountoulakis KN, Grunze H, Panagiotidis P, Kaprinis G. Treatment of bipolar depression: an update. J Affect Disord. 2008; 109: 21–34.
20. Ketter TA, Wang PW, Nowakowska C, editors. Treatment of acute mania in bipolar disorder. American Psychiatric Publishing; Washington, DC: 2005.
21. Bowden CL. Acute and maintenance treatment with mood stabilizers. Int J Neuropsychopharmacol.2003;6: 269–75
22. Bowden CL. Predictors of response to divalproex and lithium. J ClinPsychiatry. 1995; 56(Suppl 3):25–30.
23. Swann AC, Bowden CL, *et al.* Depression during mania. Treatment response to lithium or divalproex. Arch Gen Psychiatry. 1997; 54: 37–42.
24. Kleindienst N, Greil W. Differential efficacy of lithium and carbamazepine in the prophylaxis of bipolar disorder: results of the MAP study. Neuropsychobiology . 2000;42(Suppl 1):2–10.
25. Lerer B, Moore N, Meyendorff E, *et al.* Carbamazepine versus lithium in mania: a double-blind study. J Clin Psychiatry. 1987; 48: 89–93.
26. Vasudev K, Goswami U, Kohli K. Carbamazepine and valproate monotherapy: feasibility, relative safety and efficacy, and therapeutic drug monitoring in manic disorder. Psychopharmacology (Berl) 2000;150:15–23.
27. Young RC, Biggs JT, *et al.* A rating scale for mania reliability, validity and sensitivity. Br.J Psychiatry. 1978; 133;429-435.
28. Hayes JF, Marston L, Walters *et al.*Lithium vs. valproate vs. olanzapine vs. quetiapine as maintenance monotherapy for bipolar disorder: a population-based UK cohort study using electronic health records. World Psychiatry. 2016 Feb; 15(1):53–8.
29. Severus E, Taylor MJ *et al.*Lithium for prevention of mood episodes in bipolar disorders: systematic review and meta-analysis. Int J Bipolar Disord. 2014 Dec 20; 2(1):15.

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Background: Lithium is a commonly used drug with a narrow therapeutic index, it has significant adverse effects on kidney and thyroid, and is life threatening during intoxication. Maudsley guidelines on managing bipolar affective disorder patients (BPAD) with lithium recommends checking weight at baseline and every 3rd month, serum lithium after 7th day thereafter 3 months once, renal function test and serum thyroid stimulating hormone (TSH) at baseline and 6 months once. ***Aim:*** To evaluate whether Pre-Lithium workup and monitoring is done in Bipolar affective disorder (BPAD) patients initiated on Lithium as per Maudsley prescribing guidelines in psychiatry. ***Method:*** Retrospective audit conducted in a tertiary care hospital on lithium monitoring in BPAD patients treated with lithium atleast for six months compared against Maudsley prescribing guidelines. ***Results:*** Among medical records of 114 bipolar patients fulfilling study criteria, weight and serum lithium at baseline was checked in 100% of patients; serum creatinine and TSH at baseline along with lithium at 3rd month was done in 83.3%, 78.9% and 68.4% of patients respectively. Maudsley guidelines for serum creatinine, TSH and lithium level at 6th month were met only in 43.8%, 43.8% and 72.8% of patients respectively. ***Conclusion:*** The quality of lithium monitoring in bipolar patients falls well short of accepted standards; Hence addressing the issues in monitoring and following a standard protocol can improve the effectiveness of treatment and quality of life of patients.

Keywords: *Lithium Monitoring, Lithium, Audit, Bipolar Affective Disorder.*

□ipolar affective disorder (BPAD) is a mood disorder characterized by episodes of mania, hypomania and depression and the lifetime prevalence ranges between 0.3% and 4.8%.[1] Bipolar affective disorder is almost always recurrent. Lithium is approved by FDA and commonly used for treating mood disorders. Lithium is a drug with wide therapeutic applications but with a narrow therapeutic index. The accepted serum lithium level for

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treating patients is 0.6-1.2 meq/L.[2] Some of the common side effects encountered with lithium are weight gain, sedation, nausea, vomiting, diarrhea, tremors, acne, hair loss, dysphoria, reduced creativity, dysarthria, hypothyroidism, renal disease and during lithium toxicity patients may develop coarse tremor, dysarthria, ataxia, seizures, coma and death. Toxic effects of lithium become more likely as the serum level rises above 1.5 meq/L.[3] But patients can develop lithium toxicity signs at any serum lithium levels depending on their tolerability.[2] Up to 75% of patients treated with lithium experience some side effects.[4] Hypothyroidism occurs in 5% - 35% of patients treated with lithium. It occurs more frequently in women.[5,6] Approximately 10% -20% of patients receiving long term lithium treatment display morphological kidney changes, usually interstitial fibrosis, tubular atrophy and sometimes glomerular sclerosis.[7]

Appropriate baseline assessment prior to lithium treatment will identify patients at significant risk. Also periodic monitoring will help us for early detection of side effects thereby preventing any serious complications. Maudsley prescribing guidelines is one among the various standard guidelines which is accepted widely for monitoring patients on lithium.

The National Institute for Health and Care Excellence (NICE) defines clinical audit as a 'quality improvement process that seeks to improve patient care and outcomes through systematic review of care against explicit criteria and the implementation of change'. By auditing clinical practice against standards derived from evidence-based guidelines, defined aspects of the quality of care delivered to patients can be measured and monitored.[8]For trainees, participation in audit can allow a number of intended learning outcomes to be achieved, these include gaining an understanding of how clinical governance can be applied in practice, and the development of leadership skills related to monitoring performance and effecting change.[9]

Hence we wanted to evaluate whether a standard protocol is being followed in our set up for monitoring patients on lithium treatment.

Aim:

- To evaluate whether Pre-Lithium workup and monitoring is done in Bipolar affective disorder (BPAD) patients initiated on Lithium as per Maudsley prescribing guidelines in psychiatry.

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Study Design & Participants

This was a retrospective study looking at pre lithium workup and lithium monitoring in bipolar affective disorder patients treated with lithium. This study was conducted in a tertiary care medical college hospital in India. We screened medical records of all patients registered in psychiatry department in our hospital between January 2011 and March 2014. We included both out-patients and in-patients aged between 18 years and 65 years, who were

diagnosed to have bipolar affective disorder as per international classification of disease 10th edition (ICD-10).We included only those patients who were started on tablet lithium by the department of psychiatry in our hospital and followed up in our department for at least 6 months duration after initiating lithium.

We excluded patients whose diagnosis is other than bipolar affective disorder and who are not on lithium. We also excluded patients who were already on lithium before visiting our department or patients who were started on lithium by an outside psychiatrist. Among the 451 medical records retrieved with a diagnosis of bipolar affective disorder, 114 patients fulfilled the inclusion and exclusion criteria and were recruited for further analysis. Information were gathered regarding age, gender, primary diagnosis, baseline values of weight, serum creatinine, serum thyroid stimulating hormone level (TSH) and periodic monitoring of serum Lithium, serum creatinine and TSH up to 6 months using a data collection form. In case of any discrepancy regarding the information recorded in the file, it was sorted out after discussion between the authors.

Materials:

Maudsley prescribing guidelines in psychiatry[10] is a standard evidence based and widely accepted guidelines, which provides clinicians with practical and useful advice on prescribing psychotropic agents in commonly encountered clinical situation.

Maudsley guidelines on monitoring patients on lithium recommends checking weight at baseline, 3rd and 6th month, serum lithium to be checked after 7th day of starting lithium or change of dose thereafter every 3 months, estimated glomerular filtration rate (eGFR) and serum thyroid stimulating hormone (TSH) to be done at baseline and 6 months once.

The gathered information was compared with the Maudsley guidelines for identifying whether the baseline work up and periodic monitoring of laboratory values that were carried out during that period was optimal or not. For the purpose of this study we have slightly modified Maudsely guidelines as in instead of eGFR we have chosen serum creatinine levels, as eGFR is not done in our set up.

The study was approved by Institutional human ethics committee.

Statistical analysis*:*

Each of the four outcomes of interest (measures of serum lithium level, serum creatinine, thyroid stimulating hormone and body weight) was measured dichotomously; whether or not the standard had been met. Percentage of people who underwent these assessments was calculated. The data was entered and analyzed using Microsoft Excel worksheet for windows 7.

[illegible]

A total of 114 medical records fulfilling study criteria were identified and reviewed to check whether standard protocol is followed while monitoring patients on lithium.

The mean age of people in our study was 35.3 years (age range: 18 – 62 years) and sample predominantly consisted of male patients (N=41; 65%).

Base line weight was checked in 97.3% of patients, weight at 3rd and 6th month as well as serum lithium at baseline was done in all patients, serum lithium at 3rd month & 6th month was done only in 68.4% & 72.8% of patients respectively, serum creatinine at baseline & at 6th month was checked in 83.3% & 43.8% of patients respectively and serum TSH levels at baseline & at 6th month was done only in 78.9% & 43.8% of patients respectively ([illegible]1).

Table 1: Percentage of people in whom monitoring was carried out as per Maudsley guidelines

[illegible]	[illegible]		3rd [illegible]		6th [illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	111/114	97.3%	114/114	100%	114/114	100%
[illegible]	114/114	100%	78/114	68.4%	83/114	72.8%
[illegible]	95/114	83.3%			50/114	43.8%
[illegible]	90/114	78.9%			50/114	43.8%

[illegible]

Good doctors - Safer patients calls for the reinvigoration of clinical audit to support better patient care and service improvement. The Royal College of Psychiatrists' guidance emphasizes that it is not the direct involvement in data collection that is important, but rather the review of the audit evidence for the quality of care provided, and taking the lead in implementing strategies to improve quality where these are required.[9] Such reflection on the data by clinicians is perhaps the most potent aspect of clinical audit. It is important to note that although audit can generate new knowledge, its primary purpose is to compare practice against pre-determined standards. To our knowledge, this is the first study done in India to audit lithium monitoring practice in BPAD patients receiving lithium. In this study, we selected only those patients who are diagnosed to have bipolar affective disorder, firstly as in these patients lithium is usually started not only for acute management as in 1st episode of mania, but also as a prophylaxis to prevent future episodes wherein lithium is given for long duration. Secondly, recruiting bipolar affective disorder patients alone makes the sample homogenous, where a standard protocol can be followed. For the same reason, patients in extremes of age category were excluded, as these patients may require more frequent assessments and additional investigations.

Though there are various standard guidelines for monitoring patients on lithium, Maudsley practice guideline appears much more simpler, but at the same time evidence based and feasible in our set up, given the fact that health care system especially mental health care in developing countries like India is below compared to developed countries like USA and UK. In our study, we found that weight is checked in 97.3% of patients at baseline and 100% during review which could be explained by the fact that it is easy to check and has been routinely done for all the patients irrespective of the diagnosis, and there is no cost involved. Serum lithium is done for all patients at baseline which is a welcome thing, but subsequently lithium level was not done for more than $1/4^{th}$ of the patients, the reason could be serum lithium at baseline is usually done to adjust the dosage rather than for monitoring side effects. Serum creatinine and TSH at baseline was done in around 80% of patients, but subsequent monitoring of these parameters at 6 month was carried out only in 43.8% of patients. Our study shows quality of lithium monitoring in patients falls well short of accepted standards and targets which could be compared with the similar findings noted in audits done in the west. An audit done in UK on lithium monitoring against recommended standard NICE guidelines in patients recently starting lithium, showed a documented baseline measure of renal or thyroid function in 84% and 82% respectively. For patients prescribed lithium for a year or more, the NICE standards for monitoring lithium serum levels, and renal and thyroid function were met in 30%, 55% and 50% of cases respectively.[11]Despite the existence of explicit standards for monitoring patients who are prescribed lithium, a number of local audits conducted in different areas of the UK over the last 20 years have found this monitoring to be suboptimal.[12,13,14,15]These findings suggest that there is no standard protocol which is being followed in monitoring patients on lithium treatment.

Possible explanation for sub optimal monitoring:

There could be various reasons for this like patient related factors and clinician related factors. Patient related factors:

Previous studies done in western countries have identified certain factors that may influence monitoring rates like willingness of patients to have blood tests and either receiving inadequate information about lithium treatment and the need for regular blood tests or not assimilating the information given,[16,17]Other factors are patient not being compliant to treatment, unaffordability and patient unable to review on time due to personal reasons or simply forgetting to do the test.

Clinician related factors:

Certain clinician related factors that can influence lithium monitoring are clinician unable to spend adequate time with the patient, forgetting to ask for investigation, clinician's perception that patient may not do it; clinicians inadequate knowledge regarding standard protocols for monitoring patients on lithium or despite being aware of standard guidelines clinician may not feel any need to follow the guidelines.

Ours being a tertiary care teaching hospital, where these standard guidelines are often discussed and taught to postgraduate students, the possibility that doctors not being aware of existence of any standard protocol is less likely. But looking at our findings which show a sub optimal standard of monitoring patients on lithium; it appears that theoretical knowledge has not actually translated into clinical practice, which could also be due to various logistic reasons.

Few clinicians may disagree with the recommendations given by Maudsley guidelines, as every patient is different and it is the clinical judgment based on one's own clinical experience that could help in treating these patients, rather than doing serum creatinine and TSH every 6 months. But we should keep in mind, in a country like India where people tend to ignore early symptoms leading to delayed identification of even common medical illness like diabetes; there is always a possibility that one can miss early warning signs of serious adverse effects. Also, given the fact that lithium can cause serious side effects and has a narrow therapeutic index following a standard protocol is worthwhile.

Our findings cannot be generalized to other set up, however one may not be absolutely wrong in assuming that the findings might be similar in other tertiary or non-tertiary care centers in India.

Strengths of the study are the topic itself which needs to be addressed, homogenous sample wherein a standard protocol can be followed, using a simple and feasible guidelines and auditing helps to improve the clinical service provided.

Limitations of the study are the retrospective design of the study itself, relying solely on medical records to collect details (in our study, missing reports and nil documentation were taken as "test not done"), small sample size which is due to strict selection criteria in our study, short term study – however, looking at the findings one can easily assume that the rate of monitoring must be similar or much worse after 1 to 2 years of starting lithium, gathering additional information on patient variables and reasons for asking or not asking an investigation would have given some more insight into issues related to monitoring, and poor documentation in medical records.

Few steps that can be taken to improve the monitoring are discussing with colleagues about the issues in following existing protocols, to formulate own standard protocol which is not only feasible to their setup but also evidence based, attaching such a protocol to the medical records of patients on lithium, insisting on strictly adhering to it and proper documentation.

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Lithium is one of the commonly used drugs in psychiatry, which has a wide therapeutic use but can be life threatening at toxic levels and can cause serious side effects if not monitored properly. Despite existence of standard guidelines the monitoring in lithium treated patients is

still at a sub optimal level; hence addressing the issues in monitoring and following a standard protocol can improve the effectiveness of treatment and quality of life of patients.

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1. Rihmer Z, Angst J. Mood disorders epidemiology. In: Sadock BJ, Sadock VA, editors. Kaplan &Sadock's Comprehensive textbook of psychiatry. 8th ed. Philadelphia: LWW; 2005.pp. 1575-82.
2. Jefferson JW, Greist JH. Lithium. In:Sadock BJ, Sadock VA, editors. Kaplan &Sadock's Comprehensive textbook of psychiatry. 8th ed. Philadelphia: LWW; 2005.pp. 2839-51.
3. Ellenhorn MJ, Lithium. In: Ellenhorn's medical toxicology: diagnosis and treatment of Human poisoning. Baltimore: Williams & Wilkins; 1997. pp 1579- 85.
4. Goodwin FK, Jamison KR, editors. Manic-Depressive Illness. New york: Oxford University Press; 1990.
5. Johnston AM, Eagles JM. Lithium – associated clinical hypothyroidism: prevalence and risk factors. Br J psychiatry 1999; 175:336-39.
6. Anon. Drug treatments for bipolar disorder: maintenance, prevention and special situations. Drug and Therapeutics Bulletin 2005; 43:33-37.
7. Bendz H, Sjodin I, Aurell M. Renal function on and off lithium in patients treated with lithium for 15 years or more: acontrolled, prospective lithium – withdrawal study. Nephrol Dial Transplant 1996; 11:457-60.
8. National Institute for Clinical Excellence. Principles for Best Practice in Clinical Audit. Radcliffe Medical Press; 2002. Available from: http://www.nice.org.uk/media/796/23/BestPracticeClinicalAudit.pdf.
9. Royal College of Psychiatrists. A Competency Based Curriculum for Specialist Training in Psychiatry: Specialist Module in General Psychiatry. Royal College of Psychiatrists, 2009.
10. Taylor D, Paton C, Kapur S, editors. The Maudsley prescribing guidelines. 10th edition. London: Informa health care; 2009.
11. Collins N, Barnes TRE, Smith AS, Gerrett D, Paton C. Standards of lithium monitoring in mental health trusts in the UK. BMC Psychiatry 2010; 10:80.
12. Glover KJ, Lawley D. How safe is lithium prescribing? Audit of a local prescribing framework and patient survey. Psychiatr Bull 2005; 29:98-100.
13. Kehoe RF, Mander AJ. Lithium treatment: prescribing and monitoring habits in hospital and general practice. BMJ 1992; 304:552-54.
14. Fielding S, Kerr S, Godber C. Lithium in the over-65s-a dedicated monitoring service leads to a better quality of treatment supervision. Int J Geriatr Psychiatry 1999; 14:985-87.

15. Eagles JM, McCann I, MacLeod TN, Paterson N. Lithium monitoring before and after the distribution of clinical practice guidelines. ActaPsychiatrScand2000; 101:349-53.
16. Holmes B. If you want a job done properly, do it yourself - setting up a county-wide lithium database. Bipolar Disorders 2003; (5, Suppl 1):53.
17. Holmes B. The Norfolk-wide lithium database: the answer, or more questions? Bipolar Disorders 2005; (7,Suppl 2):65.

Ramasamy S, Srinivasan S, & Ramanathan S A (2017). Standards of Lithium Treatment Monitoring In a Teaching Hospital in South India among Bipolar Affective Disorder. *International Journal of Indian Psychology*, Vol. 5, (1), DIP: 18.01.098/20170501, DOI: 10.25215/0501.098

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Managing levels of insomnia in psychiatric patients

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Abstract
Sleep is important in homeostasis, memory, learning and wellbeing. Objectives were to assess the level of insomnia among psychiatric patients, to evaluate the effectiveness of structured teaching programme regarding management of level of insomnia among psychiatric patients and to associate the level of insomnia of psychiatric patients with selected demographic variables. Pre Experimental Design with One group pre-test and post-test design was used. Purposive sampling technique was applied and thirty psychiatric patients were selected. Insomnia severity index tool was used for data collection. Structured teaching programme on management of insomnia for a period of 30 minutes included sleep wave cycle, factors affecting sleep, sleep disturbances and sleep promotion measures. In the pre-test, 46.6% patients have sub threshold, 36.6% have moderate level and 16.7% have severe level of insomnia. In the post-test, 20% patients have no significant insomnia, 60% have sub threshold and 20% have moderate insomnia. Table 4 shows that statistically signified improvement in psychiatric patients after the intervention. The mean and the standard deviation of pre-test level of insomnia is 15.16± 2.78 respectively whereas in the psot-test it was 10.53±2.65 respectively. The t-value was 8.0793 at p>0.001. It shows that structured teaching programme on mangement of level of insomnia was effective to reduce the level of insomnia among psychiatric patients. Thus, there was a statistically significant improvement between pre and post-test level of insomnia among psychiatric patients.

Keywords: sleep, insomnia, structured teaching programme, psychiatric patients.

Introduction
"Sleep is the golden chain that ties health and our bodies together"-Thomas Dekker.
Everyone sleeps! Sleep is familiar to us all, yet still remains a medical mystery. Sleep is a fascinating and the daily experience of every human being. It occupies almost one-third of a person's life and undergoes considerable variation in its pattern as a person grows and matures. Sleep is a state of consciousness, distinct from working, during which we are generally unaware of our bodies and minds.
Sleep is a naturally recurring state of mind, characterized by altered consciousness, relatively inhibiting sensory activity, reduced muscle activity and inhibition of nearly all voluntary muscles during rapid eye movement (REM) sleep and reduced interaction with the surroundings.
Sleep is important in homeostasis, memory, learning and wellbeing. Sleep is essential to survival. It increases in slow wave activity after learning a task have been associated with improved performance of the task. Although the mechanism is not entirely clear, numerous studies support the instrumental role sleep plays in memory consolidation and learning.

Background of the study
Chronic sleep problems affect 50%to 80% of patients in a typical psychiatric practice, compared with 10%to 18% of adults in general US population. (Ford, 2009)
The overall prevalence of symptoms of sleep disorders in the psychiatric outpatients sample was 40.75%.The prevalence of symptoms of narcolepsy, sleep breathing disorder, PLM/RLS, circadian rhythm disorder and parasomnia were 12.5%,14.5%,14.8%,4.5% and 13.8% respectively. (Adhiti Hombali, 2019)
Lack of sleep may lead to a number of problems. Patients who have poor night time sleeps are more likely to have confusion, frustration, depressed mood, attention and memory problems excessive day time sleepiness, more night time falls, and use more over the counter or prescription sleep aids. Disturbed sleep is also associated with a poor quality of life.

Body chronically deprived of sleep is a walking time bomb.17 hours of sustained wakefulness leads to a decreasing performance. Reducing a person's normal sleep time at night by half decreases the activity of T-cells, the cells that destroys bacteria, virus and tumour cells. Better management of sleeping problems in patients can improve these patients health and quality of life.

A study was conducted on promoting rest and sleep with non-pharmacological program was designed to enhance rest and sleep among 250 patients which was implemented in a hospital medical unit. Nursing assistants provided patients with various modalities to improve sleep, including back rubs, warm drinks, blankets warmed in a blanket warmer, aromatherapy, relaxation music, and earplugs. Additional interventions were taken to reduce noise. The outcomes of the study showed 63.45% patients had improved sleep quality and quantity. (Holzinger, Brigitte, 2019).

Effectiveness of sleep management strategies was studied on residents of aged care facilities for the promotion of sleep. Findings suggested that multidisciplinary strategies such as combining a reduction in environmental noise, reduction of night-time nursing care and promotion of daytime activity, were 69% more effective for promoting sleep in the population of interest. Wrist actigraphy was found to be the most accurate objective sleep assessment tool. It was found that lack of sleep, disturbed sleep and the overuse of medications especially sedations reduced the quality of life in older people. Effective safe sleep interventions should be promoted and practiced by nurses. (Matsuda, Risa; Shun, 2019).

A study was conducted on hospitalized patients for the frequency of sleep disruption and daytime function and effectiveness of cognitive behavior therapy and exercise on sleep. There was good evidence supporting the effectiveness of cognitive behavior therapy on sleep. Results of the study showed that exercise improves sleep as effectively as benzodiazepines. (Navarro-Bravo, 2019)

Sleep disturbance is the most common cause of insomnia in psychiatric patients. So, good quality sleep is important to body and mind's ability to renew and refresh. Role of nurse includes preventing the factors that disturbs the sleep and provide environment that is congenial for good sleep. They should provide sufficient information regarding quality of sleep to the patients by sleep promotional measures such as bedtime rituals which help in improving sleep pattern.

Problem Statement: Effectiveness of structured teaching programme on management of level of insomnia among psychiatric patients in a tertiary care Hospital, Coimbatore.

Objectives

- To assess the level of insomnia among psychiatric patients.
- To evaluate the effectiveness of structured teaching programme regarding management of level of insomnia among psychiatric patients.
- To associate the level of insomnia of psychiatric patients with selected demographicvariables.

Methodology

Research design

Pre Experimental Design

One group pre-test and post-test design

⇩

Pre-test (O_1) administration of insomnia severity index to the psychiatric patients

⇩

Intervention (X) administration of structured teaching program on management of level of insomnia

⇩

Post-test (O_2) administration of insomnia severity index to psychiatric patients.

Setting of the study: Psychiatric wards of PSG Hospitals.

Population: Psychiatric patients of both sex with psychiatric disturbance from PSG Hospitals, Coimbatore.

Sampling technique: Purposive sampling technique

Sampling selection criteria

Inclusive criteria

- Those who are cooperative to participate in the study.
- Those have partial/total insight present.
- Those who stay more than a week.
- Both male and females.

Exclusive criteria

- Psychiatric patients who are aggressive.
- Psychiatric patients who are taking sleep medications.

Instrument and tools for data collection

Section-A: Demographic variables : Baseline data information such as name, age, education, religion, occupation and family data like type of family, member of family, income of family of psychiatric patients.

Section-B: Insomnia severity index: Insomnia severity index is a brief self-report instrument measuring the patients perception of both nocturnal and diurnal symptoms of insomnia.

The insomnia severity index has 7 questions. The 7 answers are added up to get a total score. When the patients have total score, should look at the guidelines of scoring/interpretation to check the sleep difficulties.

Scoring

Score	Interpretation
0 – 7	No clinically significant insomnia
8 – 14	Sub threshold insomnia
15 – 21	Moderate insomnia
22 – 28	Severe insomnia

Section-C: Structured teaching programme on management insomnia for a period of 30 minutes. It includes sleep wave cycle, factors affecting sleep, sleep disturbances and sleep promotion measures.

Findings

Tables 1: Frequency and percentage distribution of demographic variables of psychiatric patients n=30

S. No	Demographic variables	Frequency	Percentage (%)
1.	**Age in years**		
	a) Below 40	9	30
	b) Above 40	21	70
2.	**Educational status**		
	a) Illiterate	10	33.33
	b) Higher education	9	30
	c) Graduate	8	26.66
	d) post graduate	3	10
3.	**Occupational status**		
	a) Private	10	33.33
	b) Government	5	16.66
	c) House wife	10	33.33
	d) Business	5	16.66
4.	**Monthly family income**		
	a) 3000	0	0
	b) 4000-6000	11	36.66
	c) 7000-12000	19	63.33
5.	**Type of family**		
	a) Nuclear family	24	80
	b) Joint family	6	20

Table 1 shows the 21 patients (70%) were at the age group of >40 years, 10(33.3%) patients were illiterate 10 patients (33.3%) were working at private sector, 19 patients (63.3%) income were above 10000, 24 patients (80%) belongs to a nuclear family.

Table 2: Assessment of pre-test level of insomnia among psychiatric patients n=30

S. No	Level of sleep disturbance	Pre-test	
		Frequency	Percentage (%)
1.	No significant insomnia	0	0%
2.	Sub threshold insomnia	14	46.6
3.	Moderate insomnia	11	36.7
4.	Severe insomnia	5	16.7

Table 2 revealed with respect that 14 patients (46.6%) have sub threshold insomnia, 11 patients (36.6%) have moderate level of insomnia, 5 patients (16.7%) have severe level of insomnia.

Table 3: Assessment of post level of insomnia among psychiatric patients. n=30

S. No	Level of Insomnia	Post Test	
		Frequency	Percentage (%)
1.	No significant insomnia	6	20
2.	Sub threshold insomnia	18	60
3.	Moderate insomnia	6	20
4.	Severe insomnia	0	0

Table 3 shows that 6 psychiatric patients (20%) have no significant insomnia,18 psychiatric patients(60%) have sub threshold insomnia and 6 psychiatric patients (20%) have moderate insomnia in post-test.

Table 4: Comparison of pre-test and post-test level of insomnia among psychiatric patients n=30

S. No		Mean	S.D	t-value
1.	Pre-test	15.16	2.78	8.0793
2.	Post test	10.53	2.65	

Note: Statistically significant-***p>0.001

Table 4 shows that statistically signified improvement in psychiatric patients after the intervention. The mean and the standard deviation of pre-test level of insomnia is 15.16± 2.78 respectively after providing management of insomnia the level of insomnia is reduced whereas the mean and standard deviation of post-test level of insomnia among psychiatric patients is 10.53±2.65 respectively. The t-value is 8.0793 at p>0.001. It shows that management of insomnia is effective to reduce the level of insomnia among psychiatric patients. Thus, there was a statistically significant improvement between pre and post-test level of insomnia after the management of level of insomnia among psychiatric patients.

Table 5: Association of pre-test level of management of insomnia with selected demographic variables n=30

S. No	Demographic variables	Sub threshold insomnia	Moderate insomnia	Severe insomnia	Calculated value	Tabulated value
1.	**Age in years**					
	a. Below 40	3	4	2	0.2380	3.841 **NS**
	b. Above 40	11	7	3		
2.	**Educational status**					
	a. Illiterate	6	3	1	2.454	7.815 **NS**
	b. Higher education	4	3	2		
	c. Graduate	3	4	1		
	d. post graduate	1	1	1		
3.	**Occupational status**					
	a. Private	3	2	2	4.5359	7.815 **NS**
	b. Government	3	3	1		
	c. House wife	3	3	3		
	d. Business	4	1	2		
4.	**Monthly family income**					
	a. 3000	0	0	0	3.394	5.991 **NS**
	b. 4000-6000	9	2	0		
	c. 7000-12000	5	9	5		
5.	**Type of family**					
	a. Nuclear family	12	8	4	0.035	3.841 **NS**
	b. Joint family	2	3	1		

Note:**p< 0.005, S-significant, NS-Non significant

Table 5 shows that there is no significant association between insomnia and selected demographic variables like age, education, occupation, income and type of family.

Nursing implications

Nursing Education

- Nursing students should explore more on various approaches in management of insomnia.
- Conferences, workshops, seminar and symposium can be held for all health personnel in bringing about behavioural changes among psychiatric patients towards sleep hygiene.
- In-Service education can be given to the staff nurses and faculty members regarding management of level of insomnia in order to improve the good sleeping pattern among psychiatric patients.

Nursing practice

- The nurses working in the health services should be equipped with the knowledge on management of level of insomnia.
- Planned structured teaching programmes on management of level of insomnia to be scheduled for health care workers.
- Pamphlets can be provided with appropriate pictures and explanations to improve sleeping pattern.

Nursing Administration

- Advanced clinical nursing should focus on normal sleeping pattern for patients in any ward setting in hospital
- The administrator shall initiate educating the psychiatric patients and family members about sleep hygiene.
- Promote awareness about management of insomnia to public

Nursing Research

Nurse researchers can do similar study on patients with medical illnesses

Recommendations

- Similar studies can be conducted for large sample of psychiatric patients.
- Similar studies can be conducted among IT professionals and older adults

Conclusion

Structured teaching programme helped to reduce the level of insomnia, from severe level to sub threshold level of insomnia among majority of the psychiatric patients.

References

1. Armenti KR. Prevention of sleep disorders. New Solutions 2003;13(3):241:259. Baywood Publishing Co.,Inc.
2. Berkley JA, Lowe BS, Mwangi I *et al*. Sleep Disturbances in Kenya.NEngl J Med 2005;3(52):39-47.
3. Celik S, Oztekin D, Akyoleu N, Issever H. Sleep Disturbance:the patient care activities applied at the night shift in the intensive care unit. Journal of Clinical Nursing 2005;14(1):102-106.
4. Denise Polit F, Cheryl Tatano Beck. Nursing Research Principles and Method. 7 th edition. Philadelphia: Lippincott Williams and Wilkins.
5. Gaudreau JD, Gagnon P, *et al*. Association between psychoactive medications and sleep in hospitalized patients: International Journal of a critical review. Psychosomatics 2005;46(4):302-1.
6. Goodburn EA, Chowdhury M *et al*. Sleep Complications. Department of Medicine. College of Health Sciences, Obafemi University. Nigeria 2003.
7. Kryger Roth, Dement. Principals and practice sleep medicine.6th Edition. Elsevier publications 2017.
8. Nau SD, McCare CS, Cook KG, Lichstein KL. Treatment of insomnia in older adults. Clinical Psychology Review 2005;25(5):645-672.
9. Newman N, Long L. Psychological factors affecting sleep, The Mount Sinai Journal Of Medicine 2017;77(1):114-23.
10. Potter, Pery. Fundamentals of nursing. South Asian edition. Delhi: Elsevier Publications 2013.
11. Sharma, Thomas. Potter and Perry's fundamentals of nursing (South Asian edition).New Delhi: Elevier publications.
12. Swank C, Christianson CA, *et al*. Effect of a Information Booklet on sleep promotion involved in egg donor screening. Journal of Medicine 2001;30(6):P.686-9.
13. Thomason JW. Ely EW. Delirium in the intensive care unit is bad:what is the sleep pattern? Critical Care Medicine 2004;33(11):2352-64.
14. Villegas H, Villanueva C, *et al*. Department of psychology 1992, 177-82.
15. Wittbrodt ET. Daily interruption of continuous sleep general medicine 2005;25(5):3-6.

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ORIGINAL ARTICLE

Trajectory of insight on various dimensions among bipolar disorder in-patients

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ABSTRACT

Background: Insight is awareness of one's symptoms, illness, and need for treatment. In bipolar disorder, insight is better in bipolar depression and nonpsychotic depression when compared with mania and psychotic depression. Insight impacts on medication adherence. In our study, we measured and compared with various clinical parameters of insight. The aim of this study is to assess various dimensions of insight recovery prospectively in bipolar affective disorder patients with treatment and drug compliance. **Materials and Methods:** Patient's insight was assessed using Mood Disorder Insight Scale (MDIS) at baseline, 1st, 3rd, and 6th months. Their insight was then compared with various sociodemographic profiles and correlated with number of mood episodes, family history of mental illness, and 6-month MDIS scores. **Results:** Depression patients scored better in insight components ($P = 0.001$). The good compliance group attributed their symptoms to their illness than the poor compliance group ($P = 0.013$). The MDIS scores were gradually improving from baseline to 6 months ($P \leq 0.001$). There was no relationship between insight and the number of episodes ($P = 0.788$). **Conclusion:** Depressive episode patients had better insight during the baseline, which improved during 6 months follow-up compared with manic patients. Among various components of insight, insight on the attribution of symptoms was a predictor of good compliance. Progression of insight was steady and proportionate to the duration of treatment in depressive episode patients.

Keywords: Bipolar affective disorder, depression, insight, mania

The bipolar affective disorder is common and disabling disorder and is associated with considerable morbidity.[1] It is a mood disorder that is characterized by episodes of depression, mania, hypomania, and mixed episodes, with a period of inter-episodic recovery. The World Health Organization estimated that bipolar disorder was the 46th greatest cause of disability and mortality in the world among 291 diseases and causes of injuries. Insight is defined as the awareness of one's illness, the ability to recognize the symptoms of the illness and the need for treatment. Insight of a patient can be categorized into absent insight, partial insight, and full insight.[2-5]

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Insight is being highlighted as state-dependent component in a few studies. In disorder like bipolar affective illness, impairment in insight fully resolves. There are few studies which highlight that insight does not resolve completely even in the euthymic state. The more the number of episodes especially manic episodes, the lesser is the insight recovery. However, psychotic depression is associated with less insight than nonpsychotic depression. Insight continues to be poor in chronic illness like schizophrenia. Nowadays, more attention has been given to insight of the patient toward mental illness. There are many

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studies, which were being done which evaluated the relationship between insight and course and outcome of the illness, compliance to medications and the severity of psychopathology. The prevalence of good insight in remitted bipolar affective disorder patients is only 47%.[6] In another study, the prevalence of absent insight was 39.09% in the schizophrenia group, 36.36% in the bipolar disorder with psychotic symptoms and 9.37% in bipolar disorder without psychotic symptoms.[7]

The bipolar affective disorder is usually associated with noncompliance and poor outcome.[8,9] Insight and quality of life, cognitive functions, psychotic symptoms, and the number of episodes in mood disorders are interrelated.[10] Many studies have shown that insight is impaired in the acute phase of illness and insight improves as the patient improves symptomatically. Insight was more impaired during manic episodes when compared with depression.[11] Lack of insight in mania was present for all three components of insight-awareness of illness, social consequences of illness, and therapy benefits.[12] A study has showed that insight of bipolar affective disorder patients and schizophrenia patients were the same during admission. At 6 month follow-up, there was a large difference between both the groups. Bipolar disorder patients had better insight when compared with schizophrenia patients.[13]

Adherence to medications plays a key role in the effectiveness of any treatment. Research on the relationship between insight and adherence to treatment is important because adherence to medication can be modified. Meta-analysis that was done to study the relationship between insight and treatment compliance showed poor insight was associated with poor adherence to medications, which was similar to the literature on schizophrenia patients.[14] Studies on schizophrenia patients showed that patients with more insight have a better long-term outcomes.[15] There are similar researches in bipolar disorder, which showed improvement of insight from baseline and follow-up was related to better outcomes of illness and quality of life.[16]

Various scales are available for the assessment of insight. However, most of the scales were validated to measure insight in psychotic illness. Mood Disorder Insight Scale (MDIS) is a short and self-report instrument, includes all the components of insight and it is specifically modified for affective disorder patients. The MDIS was derived from the insight Scale for psychosis which was developed by Birchwood *et al.*, 1994. This scale measures three aspects of insight-awareness of mental disorder, attribution of symptoms, and need for treatment. Hence, we used this scale, which specifically measures insight in bipolar affective disorder in our study.

Most of the studies on insight were done in schizophrenia patients. Very few studies were done on bipolar disorder patients. There are only very few studies which have studied insight in various phases of illness. In disorder like bipolar affective illness impairment in insight fully resolves. The more the number of episodes especially manic episodes, the lesser is the insight recovery. Insight continues to be poor in chronic illness like schizophrenia.

An earlier study reported a 94% prevalence of impaired insight in symptomatic patients in various psychiatric illness such as depression, manic, and bipolar I patients. The prevalence of insight in remitted patients is 47%.[6]

A prospective study on insight in various phases of bipolar illness, observed that insight was impaired in mania when compared with the euthymic state. Insight was better in patients who had single episode of mania or depression; this insight was similar to preepisode insight. However, patients with multiple episodes of illness in 2 years had poorer insight when compared with insight of patients with less number of episodes.[7,17,18]

Very limited number of studies had compared insight with sociodemographic and clinical variables such as age, educational status, gender, marital status, and family history of mental illness. Most of the studies that have been done in insight are cross-sectional study. There are very few prospective studies on insight in bipolar disorder patients.

Therefore, the aim of this study is to assess insight recovery in bipolar affective disorder patients admitted in the psychiatry ward. We also assessed the correlation of insight about awareness of illness, attribution of symptoms to illness and insight recovery with various phases of bipolar affective illness.

MATERIALS AND METHODS

Participants and procedures

This is a prospective study. Participants of our study were both male and female gender for >12 years. These patients were admitted and were undergoing in-patient treatment in PSG Hospitals, a 2000-bedded tertiary hospital at Coimbatore, for Bipolar affective disorder either in depression or manic phase. These patients were admitted between the period of April 2016 and 2017. The participants were diagnosed with bipolar affective disorder using the International Statistical Classification of Diseases (ICD-10) criteria by the treating consultant. Patients having comorbid mental retardation and other Axis 1 psychiatric disorder except nicotine dependence, patients who did not respond for at least three telephonic calls in

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the follow-up and patients <12 years old were excluded from our study.

We recruited 50 consecutive in-patient bipolar affective disorder patients based on the ICD-10 criteria. Nine patients were excluded as per the exclusion criteria. Five of them had follow-up elsewhere, two patients did not give consent and two patients had mental retardation. Informed consent in the local language (Tamil) was obtained from primary caregiver for those patients who were not able to give consent. Once the patient became euthymic informed consent was obtained from the patient as well. The personal identification data such as name, age, gender, educational qualifications, and socioeconomic data of all the participants were recorded. Data related to psychiatry illness such as the number of past episodes and family history of mental illness was also recorded.

Once the patient got admitted, the severity of the present episode was measured using rating scales. The severity of affective symptoms was assessed by the Youngs Mania Rating Scale (YMRS) and Hamilton Depression Rating Scale (HAMD) for mania and depression, respectively. Baseline Insight was assessed using MDIS. Then, the patient was treated according to their clinical diagnosis by the consulting psychiatrist. One month after discharge, the insight was assessed using the MDIS scale and recorded. Then again, at the 3rd and 6th months of follow-up insight was measured using MDIS. The total scores of insight were calculated at baseline, 1st month, 3rd month, and 6th month and compared for improvement in insight.

Drug compliance was assessed clinically from the patient and reliable informant. During follow-up, the course of the illness was assessed, and the escalation of the dose of psychotropics or addition of a new psychotropic medication is also assessed. Patients who missed the follow-up are contacted through telephonic calls, encouraged to attend outpatient department and Insight assessed. Patients who did not respond for at least three telephonic calls were considered as dropouts [Figure 1]. The study was approved by the Institute Human Ethics committee of PSG IMSR and Hospital.

Assessment

Data were collected using the following instruments.

Semi-structured pro forma

The sociodemographic profile of the patient, details of substance use, diagnosis, and clinical details (compliance of medications, the severity of illness using YMRS and HAMD scales, number of past episodes, and family history of psychiatry illness) were assessed.

Mood Disorder Insight Scale

MDIS is a short and self-report instrument. This scale has eight questions which are read by the interviewer and the patient is asked whether their response is agree, disagree, or unsure for each of the eight items in the questionnaire. Depending on the response, the scores are calculated. The total score is 16. The MDIS was derived from the Insight Scale for psychosis, which was developed by Birchwood. This scale has good test–retest reliability and internal consistency. The test–retest reliability (coefficient alpha) of the MDIS was 0.95 ($P < 0.01$). This scale for insight includes all the components of insight and it is specifically modified for affective disorder patients. The administration of this scale does not require any special training as this is a self-report questionnaire. This scale measures three aspects of insight-awareness of mental disorder, attribution of symptoms, and need for treatment.

Data analysis

Data were entered into MS Excel and analyzed using SPSS version 20. Qualitative data were summarized as frequencies and percentages. Quantitative data were checked for normality. Normally distributed data were summarized using mean and standard deviation. Nonnormally distributed data were summarized using Median and inter-quartile range. Association between qualitative variables was tested using Chi-square test. Mann–Whitney U test was used to compare the distribution of MDIS scores by level of compliance and phase of disease; mania or depression. Friedman's test was used to check if the MDIS scores changed significantly with time. Correlation between the YMRS score and MDIS at baseline was tested using the Spearman rank correlation test. Statistical significance was interpreted using an arbitrary cut-off of $P = 0.05$.

RESULTS

Descriptive statistics for the study variables and demographics data are shown in Table 1.

The number of episodes ranged from 2 to 7. Fifteen patients had psychotic symptoms in their mood episodes and twenty-six patients had no psychotic symptoms. Family history of psychiatry illness was present in 26.8% ($n = 11$) and absent in remaining 73.2% ($n = 30$). Twenty-three patients (56.1%) of the participants had good compliance and the remaining 43.9% ($n = 18$) had poor compliance [Table 2].

The insight scores between different age group were compared, the *P* value was not statistically significant ($P = 0.318$). The insight scores, according to educational status, were not

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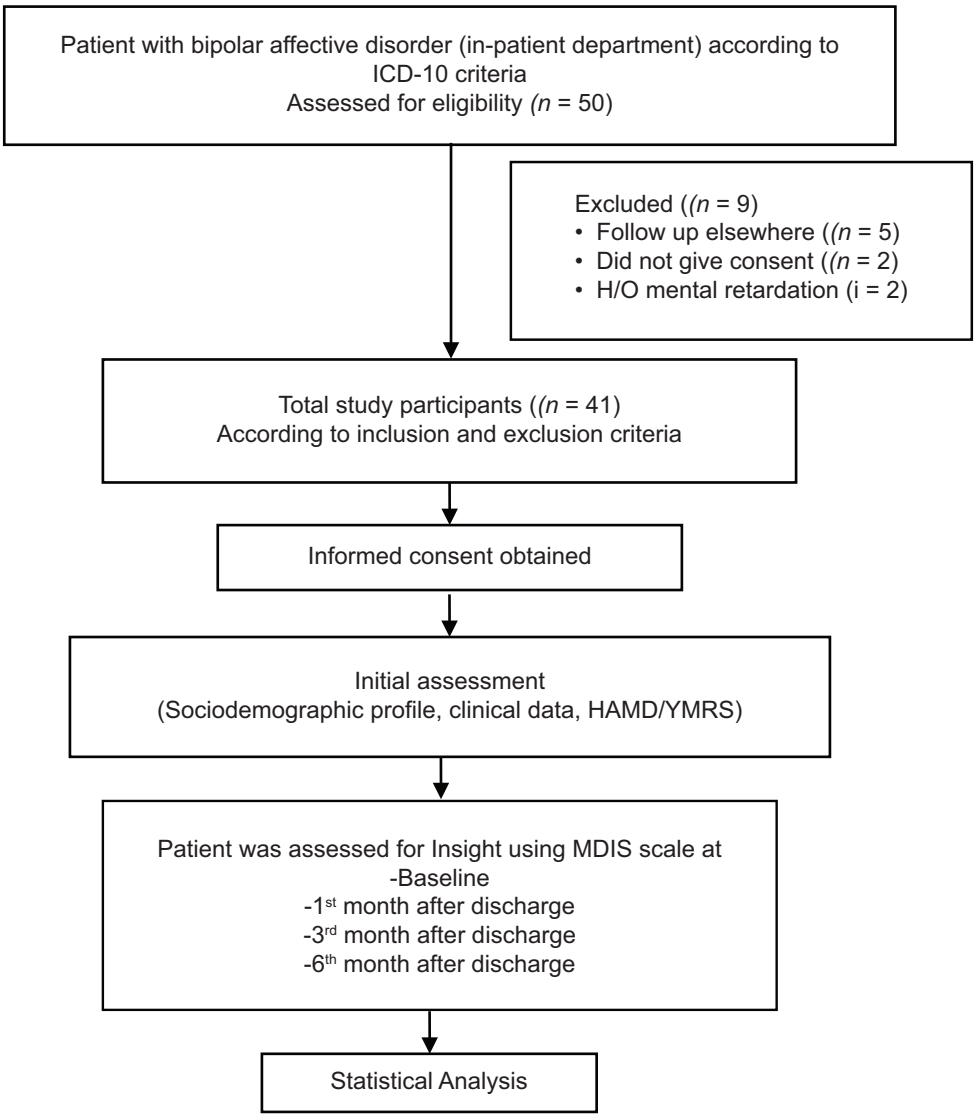


Figure 1: Flow chart

statistically significant ($P = 0.75$). Insight scores did not differ statistically between males and females, P value was 0.552. Insight scores of participants who had family history of mental illness were compared with participants who did not have family history of mental illness also did not have any statistical significance ($P = 0.124$) [Table 2].

The overall scores of the MDIS scale were compared between mania and depression groups, depression patients scored more when compared with manic patients in the insight scale. The more the MDIS score, the better the insight. The P value was also significant $P = 0.001$ [Table 3].

The total scores of MDIS between good compliance and poor compliance group were statistically significant, P value was 0.012. Good compliance group scored better in insight scale than the poor compliance group [Table 4].

In our study, we assessed for the insight score of the participants at baseline, 1st month, 3rd month and 6th month. When these scores were compared from baseline to the end of 6 months, we found that the insight scores were gradually improving. This was statistically significant and P value was < 0.001 [Table 5].

Figure 2 shows the MDIS scores at baseline, 1st month, 3rd month, and 6th month in depressive patients. The MDIS scores of the depressive patient were higher when compared with manic patients. This shows that depressive patients have more insight than manic patients. Figure 3 shows the MDIS scores at baseline, 1st month, 3rd month, and 6th month in manic patients. The MDIS scores of the manic patient were lower when compared with depressive patients indicating manic patients have less insight about illness than depressive patients.

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Table 1: Sociodemographic profile (*n*=41)

Variable	*n* (%)
Age (years)	
<18	2 (4.9)
18-65	38 (92.7)
>65	1 (2.4)
Sex	
Males	25 (61)
Females	16 (39)
Education status	
Primary	7 (17.1)
Secondary	7 (17.1)
Higher secondary	8 (19.5)
Diploma/graduate	19 (46.3)
Marital status	
Married	29 (70.7)
Single	12 (29.3)
Socioeconomic status	
Upper	8 (19.5)
Middle	27 (65.8)
Lower	6 (14.7)
Occupation	
Employed	6 (14.6)
Unemployed	29 (70.8)
Home maker	6 (14.6)

Table 2: Comparing sociodemographic profile and Mood Disorder Insight Scale Scores

Factors	Baseline MDIS	*P*
Age		
<18	4 (1)	0.318
18-65	6 (6)	
>65	4 (0)	
Education status		0.750
Primary	6 (5)	
Secondary	9 (13)	
Higher secondary	7 (6)	
Diploma/graduate	6 (5)	
Marital status		0.195
Married	6 (4)	
Single	4 (5)	
Sex		0.552
Males	6 (5)	
Females	6 (8)	
Family history		0.124
No	5 (8)	
Yes	6 (6)	

MDIS - Mood disorder insight scale

In Figure 4, MDIS scores were plotted against the period of assessment. The MDIS scores have an increasing trend from baseline to 6 months in the depression group. The progression of insight reached a plateau after 3 months for manic patients.

The number of episodes of illness was compared with MDIS scores. The *P* value was not significant ($P = 0.788$).

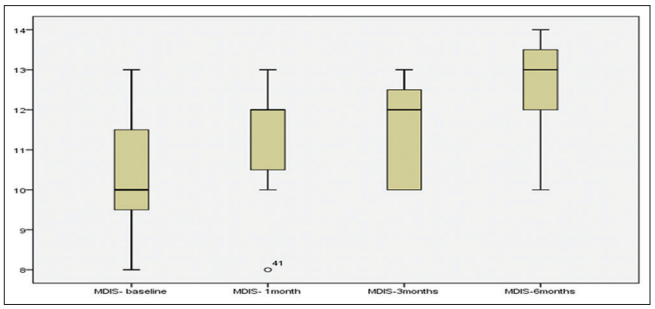


Figure 2: Progression of insight based on Mood Disorder Insight Scale scores at baseline, 1, 3 and 6 months in participants with depression

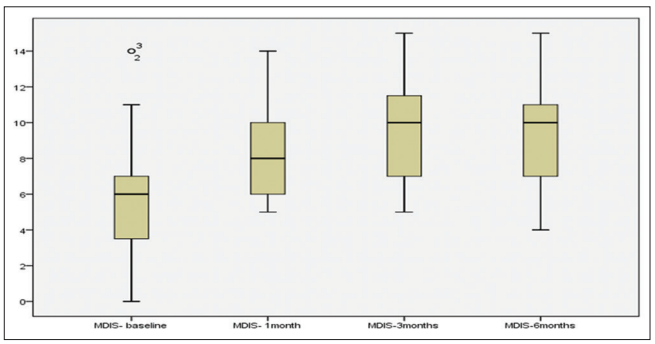


Figure 3: Progression of Mood Disorder Insight Scale from baseline to 6 months in patients with mania

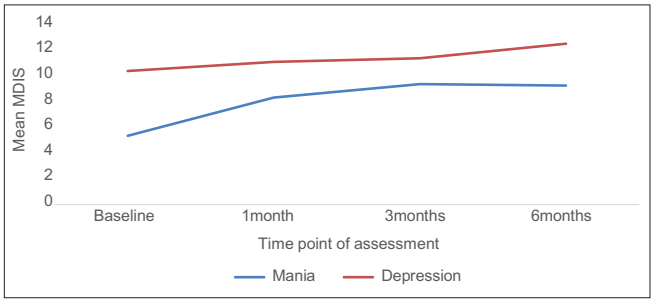


Figure 4: Progression of Mood Disorder Insight Scale from baseline to 6 months in patients with mania and depression

The presence of family history of mental illness was compared with MDIS scores. The *P* value was not significant ($P = 0.701$) [Table 6].

MDIS scores were negatively correlated with both YMRS and HAMD scores, which implies less severe the illness, the better the insight. The correlation with YMRS was statistically significant ($P = 0.003$), whereas with HAMD the correlation was not statistically significant ($P = 0.640$) [Table 7].

DISCUSSION

Insight is a multidimensional and complex phenomenon (Amador *et al.*, 1993). We compared sociodemographic profile (name, age, gender, educational

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Table 3: Comparison of insight measured using Mood Disorder Insight Scale Scores between depression and mania at baseline

Score	Median (IQR)		Mann-Whitney U	*P*
	Mania	Depression		
Awareness of mental disorder	2 (2)	4 (2)	37.5	0.003
Attribution of symptoms	2 (2)	2 (1)	53	0.021
Need for treatment	3 (2)	4 (3)	60	0.041
Total	6 (4)	10 (4)	28.5	0.001

IQR - Interquartile quartile range

Table 4: Comparison of insight measured using Mood Disorder Insight Scale Scores between patients with different levels of compliance

Score	Median (IQR)		Mann-Whitney U	*P*
	Good compliance	Poor compliance		
Awareness of mental disorder	2 (4)	2 (2)	163.5	0.225
Attribution of symptoms	2 (2)	2 (2)	118.5	0.013
Need for treatment	4 (4)	2 (2)	147.5	0.106
Total	7 (5)	4 (5)	112	0.012

IQR - Interquartile quartile range

Table 5: Progression of insight based on Mood Disorder Insight Scale Scores at baseline, 1, 3 and 6 months

MDIS	Median	IQR	*P**
Baseline	6	5	<0.001
1 month	8	4	
3 months	10	5	
6 months	10	6	

*Friedman test. MDIS - Mood disorder insight scale; IQR - Interquartile quartile range

Table 6: Correlation of number of episodes, family history of mental illness, and 6 months Mood Disorder Insight Scale Scores

Factors	MDIS 6 months	*P*
Number of episodes		
1	9 (3)	0.788
2	10 (6)	
3	9 (7)	
Family history		
Yes	10 (4)	0.701
No	10 (6)	

MDIS - Mood disorder insight scale

Table 7: Correlation of mood disorder insight scale baseline total score with Youngs mania rating scale and Hamilton depression rating scale

	YMRS score	HAMD score
Spearman correlation coefficient	−0.488**	−0.287
P	0.003	0.640

YMRS - Youngs mania rating scale; HAMD - Hamilton depression rating scale

qualifications, and socioeconomic data) of the participants with MDIS scores. There was no difference in insight between different groups.

In this study, insight scores were lower in females than males ($P = 0.08$), but the P value was not significant. The insight scores were compared with different educational status groups. There was no statistically significant ($P = 0.75$) difference within these groups. On the contrary to our understanding insight did not differ according to the educational status in our study. To our knowledge, no other studies on insight had compared the relationship between educational status and marital status.

Insight scores of participants who had family history of mental illness were compared with participants who did not have family history of mental illness. Though the scores of the participants who had family history of mental illness were higher than the other group, it was not statistically significant.

The overall MDIS scores, when compared between mania and depression groups, showed that depressive patients scored more when compared with manic patients in the insight scale ($P = 0.001$). The individual dimensions of scale were also better among depression than mania, which was statistically significant. Another study which was done to evaluate insight in mania, depression and euthymia in 95 bipolar affective disorder patients showed poor insight among mania patients when compared with depressive patients.[19] The P value was significant 0.001.

Twenty-three patients that is 56.1% of the participants had good compliance and the remaining 43.9% ($n = 18$) had poor compliance. Compliance is a very important part to consider in illness like bipolar affective disorder. Literature on the prevalence of nonadherence also had similar values. The median prevalence of nonadherence is 44.7% in

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patients with bipolar affective disorder.[19] In one study that was done to assess the predictors of nonadherence in bipolar affective disorder patients showed treatment nonadherent individuals had more negative attitudes toward medications and reduced insight into illness when compared with treatment adherent individuals.[20] We also compared the MDIS scores between good compliance and poor compliance group. The total scores of MDIS between good compliance and poor compliance group were statistically significant and *P* value was 0.012. Yen *et al.* study[21] showed insight about illness and treatment was positively correlating with adherence to medication in the bipolar affective disorder group and schizophrenia group. So interventions, which concentrated on building insight play a vital role in ensuring good compliance among bipolar patients.

In our study, we assessed for the insight score of the participants at baseline, 1st month, 3rd month, and 6th month. When these scores were compared from baseline to 6 months, we found that the insight scores were gradually improving. This was statistically significant and *P* value was < 0.001. In another prospective study which evaluated the correlation of insight in 65 bipolar disorder patients for 2 years showed insight of the manic patients gradually improved as they returned to the euthymic state.[21]

The average number of episodes in our study among the participants was two. The number of episodes ranged from 2 to 7. In our study, the number of episodes did not make any significant difference in insight of the participants ($P = 0.788$). Similar findings were seen in Cassidy study; on the contrary, showed more the number of episodes, poorer the insight.[22]

Limitations

The sample size is very small in our study, and hence the results cannot be generalized. The initial assessment of insight was not assessed on specifically on the same day after admission for all patients. The treating consultant psychiatrist made a diagnosis of bipolar affective disorder. No separate research diagnostic criteria were used to recruit our patients. With the help of insight scale we have assessed whether the patient is aware of their symptoms in general but have not analyzed about the awareness of individual symptoms. MDIS scale gives only qualitative assessment and not a quantitative assessment.

CONCLUSION

Depressive episode patients had better insight during the baseline, which improved during 6 months follow-up compared with manic episode patients. Among various components of Insight, insight on the attribution of symptoms was a predictor of good compliance during 6 months follow-up than the awareness of mental disorder or need for treatment. Progression of insight was steady and proportionate to the duration of treatment in depressive episode patients. More severe the manic episode poorer was the insight.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kathleen RM, Robert J, Jian-Ping H, Ronald CK, Sing L, Nancy AS, *et al*. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. Arch Gen Psychiatry 2011;68:241.
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed.. Arlington, VA: (DSM-5), American Psychiatric Association; 2013.
3. Pedersen CB, Mors O, Bertelsen A, Waltoft BL, Agerbo E, McGrath JJ, *et al*. A comprehensive nationwide study of the incidence rate and lifetime risk for treated mental disorders. JAMA Psychiatry 2014;71:573-81.
4. Soraya S, Kate MS, Matthias CA, Patricia B, Evelyn JB, Traolach SB, *et al*. Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys. Arch Gen Psychiatry 2009;66:785.
5. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, *et al.* Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2197-223.
6. Varga M, Magnusson A, Flekkoy K, Ronneberg U, Opjordsmoen S. Insight, symptoms and neurocognition in bipolar I patients. J Affect Disord 2006;91:1-9.
7. Yen CF, Chen CS, Yang SJ, Ko CH, Yen JY, Huang CF. Relationships between insight and psychosocial adjustment in patients with bipolar I disorder. Bipolar Disord 2007;9:737-42.
8. Ghaemi SN, Hebben N, Stoll AL, Pope HG Jr. Neuropsychological aspects of lack of insight in bipolar disorder: A preliminary report. Psychiatry Res 1996;65:113-20.
9. Ghaemi SN, Pope HG Jr., Lack of insight in psychotic and affective disorders: A review of empirical studies. Harv Rev Psychiatry 1994;2:22-33.
10. American Psychiatric Association. Mood disorder due to a general medical condition. In: American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Washington DC: American Psychiatric Publishing; 2000. p. 401-4.
11. Peralta V, Cuesta MJ. Lack of insight in mood disorders. J Affect Dis 1998;49:55-8.
12. Dell'Osso L, Pini S, Cassano GB, Mastrocinque C, Seckinger RA, Saettoni M, *et al*. Insight into illness in patients with mania, mixed mania, bipolar depression and major depression with psychotic features. Bipolar Disord 2002;4:315-22.
13. Fennig S, Everett E, Bromet EJ, Jandorf L, Fennig SR, Tanenberg-Karant M, *et al.* Insight in fi Insight in psychotic patients. Schizophrenia Res 1996;22:257-63.
14. Sadock BJ, Sadock VA, editors. Kaplan and Sadock's

[Downloaded free from http://www.industrialpsychiatry.org on Tuesday, March 16, 2021, IP: 59.92.30.139]

Synopsis of Psychiatry: Behavioral Sciences, Clinical Psychiatry. Philadelphia: Lippincott Willimas& Wilkins; 2007.
15. Schwartz RC, Cohen BN, Grubaugh A. Does insight affect long-term impatient treatment outcome in chronic schizophrenia? Comp Psychiatry 1997;38:283-8.
16. Ghaemi SN, Boiman E, Goodwin FK. Insight and outcome in bipolar, unipolar, and anxiety disorders. Comp Psychiatry 2000;41:167-71.
17. Yen CF, Cheng CP, Huang CF, Ko CH, Yen JY, Chang YP, *et al.* Relationship between psychosocial adjustment and executive function in patients with bipolar disorder and schizophrenia in remission: The mediating and moderating effects of insight. Bipolar Disord 2009;11:190-7.
18. Yen CA, Chen CA, Yang SA, Ko CA, Yen JA, Huang CÄ. Relationships between insight and psychosocial adjustment in patients with bipolar I disorder. Bipolar Disord 2007a; 9:737-42.
19. de Assis da Silva R, Mograbi DC, Silveira LA, Nunes AL, Novis FD, Landeira-Fernandez J, *et al*. The reliability of self-assessment of affective state in different phases of bipolar disorder. J Nerv Ment Dis 2014;202:386-90.
20. Scott J, Pope M. Nonadherence with mood stabilizers: Prevalence and predictors. J Clin Psychiatry 2002;63:384-90.
21. Yen CF, Chen CS, Ko CH, Yeh ML, Yang SJ, Yen JY, *et al*. Relationships between insight and medication adherence in outpatients with schizophrenia and bipolar disorder: Prospective study. Psychiatry Clin Neurosci 2005;59:403-9.
22. Cassidy F. Insight in bipolar disorder: Relationship to episode subtypes and symptom dimensions. Neurop Disord Treatment 2010;6:627-31.

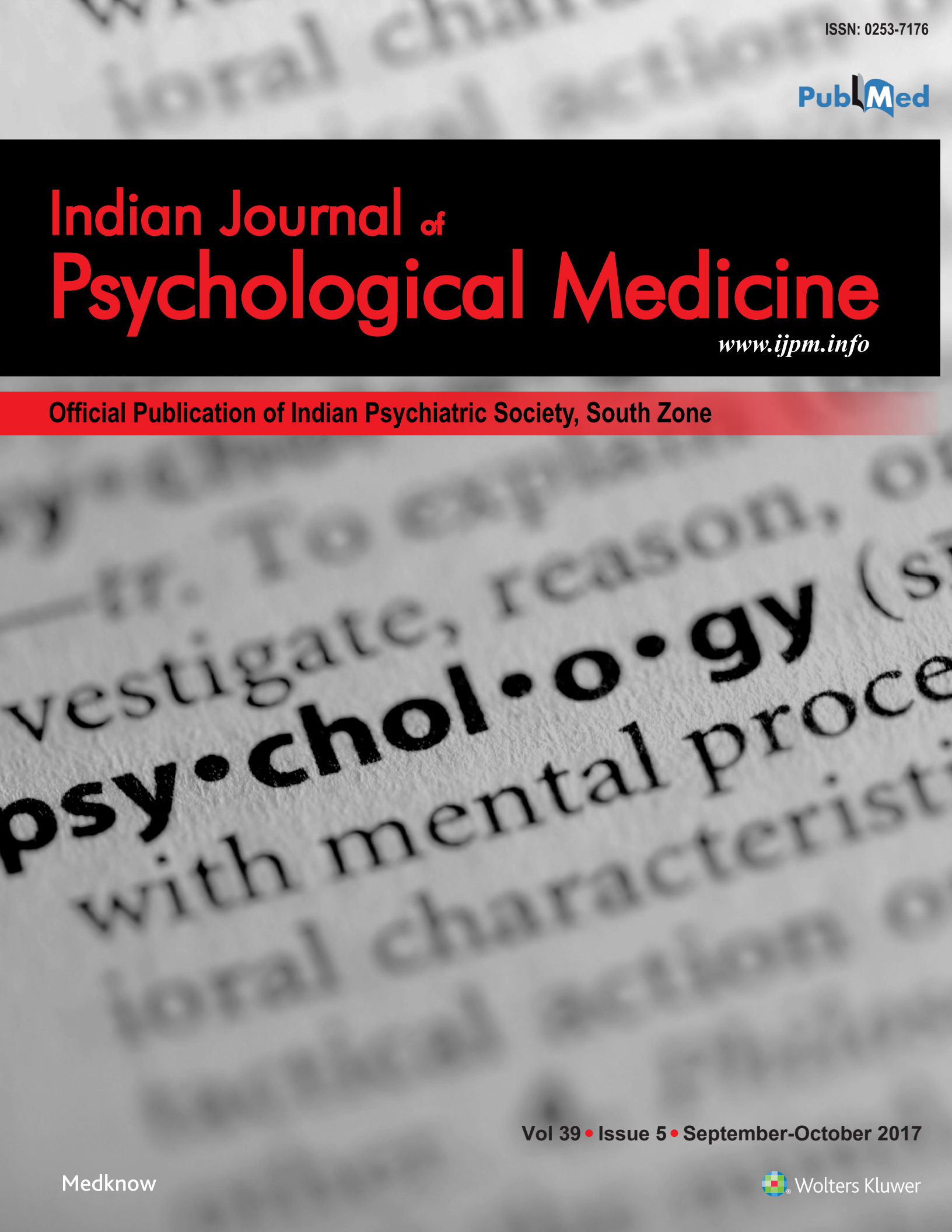
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Original Article

Prevalence and Sociodemographic Profile of Lithium-induced Cutaneous Side Effects in Bipolar Affective Disorder Patients: A 1-year Prospective Observational Study in South India

B. S. Suganya Priyadharshini, I. Syed Ummar

ABSTRACT

Objectives: Objective in our study is i) To assess the prevalence and sociodemographic profile of cutaneous side effects in bipolar affective disorder patients (BPAD) on lithium. ii) To assess the course of pre-existing skin reactions, when patient is initiated on lithium therapy. **Methods:** Lithium induced cutaneous side effects were assessed at baseline and monthly for 6 months followed by every 2 months over a year. Dermatologist opinion obtained to diagnose and treat for each patient who developed cutaneous side effects. Data were analyzed in percentage. **Results:** The prevalence of lithium induced cutaneous side effects in our study is 38.46%. On analyzing the individual subtype of cutaneous lesions, acneiform eruption and hairfall were most common. The cutaneous adverse reactions on lithium are more common in early adulthood than in the late adulthood and geriatric age group and among the subtypes of skin lesion acneiform eruptions are more common among males than in females. On analyzing the course of pre-existing lesions, severe alopecia developed with pre-existing systemic lupus erythematosis. **Conclusion:** These novel finding indicate that prevalence of lithium induced cutaneous side effects continues to be high. Hence, clinician should educate the patients before initiating lithium to improve attrition rate.

Key words: *Bipolar disorder, cutaneous sideeffects, Lithium*

INTRODUCTION

Lithium has been used in the treatment of mood disorders for more than five decades[1] and it has been the focus of numerous investigations for its systemic side-effects. Although lithium is notorious to cause cutaneous lesions[2] the literature on skin disorders secondary to lithium therapy is limited.

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Lithium is known to cause multiple cutaneous side effects, and the most common side effects are an acneiform and psoriasiform eruption.[3] It is known to cause psoriasis and also exacerbate preexisting psoriasis. Lithium-induced psoriasis are most distressing, and it is associated with the increased risk of suicide and substance use.[4]

It causes specific manifestations such as acneiform eruptions, seborrheic dermatitis, hyperpigmentation, and follicular keratitis. Other cutaneous manifestations includes mucosal and vaginal ulceration, edema, purpura, lupus erythematosus such as syndrome, urticaria, pretibial ulceration, dermatitis herpetiformis, eczema, exfoliative dermatitis, folliculitis, alopecia, allergic vasculitis, hidradenitis suppurativa, lichenoid stomatitis, exacerbation of Darier's disease, palmoplantar hyperkeratosis with ichthyosiform features, increased growth of wart, mycosis fungoides, and hair loss.[5,7-9]

Lithium is also suggested to induce lupus erythematosus. Lithium-induced lupus was more common among females and among adolescence (20–40 years).[6]

In the previous study, females showed increased incidence of cutaneous side effects compared to males. The prevalence of lithium-induced skin reactions is reported to range from 3% to 34%.[7] In another study, the prevalence of lithium-induced skin reactions is reported to be 45%.[8]

Lithium-induced lesions cause disturbances in the self-image and affect their social, occupational, and interpersonal functioning which may contribute to the risk of noncompliance. Even though its cutaneous side effects results in poor compliance, studies in these area are very few hence we have decided to do study with the primary aim is to find the prevalence of lithium-induced skin reactions. Our other objectives are to find the sociodemographic profile and its correlate with lithium-induced skin side effects and the course of preexisting skin reactions with lithium therapy.

MATERIALS AND METHODS

Study design

This is a prospective observational study done in bipolar affective disorder patients who came to our psychiatry department. A total of 52 bipolar affective disorder patients were recruited both from in- and out-patient settings by convenient sampling. The study protocol was explained to the participants, and a written informed consent was obtained from the patient. If patient could not consent, it was obtained from the family member and later from the patient when he/she could consent. The study was approved by Institutional Human Ethics Committee with the reference number 14/147.

Participants

Men and women aged 18–65 years with the primary diagnosis of bipolar affective disorder who were either newly initiated or already on lithium therapy were eligible for the study. Diagnosis of bipolar affective disorder was made by a qualified psychiatrist based on the International Classification of Disease criteria.

Patient on other mood stabilizers and on other medication known to cause cutaneous reactions other than antipsychotics were excluded from the study.

Assessments

Initial assessment

we designed a semi-structured pro forma and dermatological assessment sheet which were used for the initial assessment. It included sociodemographic details of the patient such as age, sex, socioeconomic status and education, details of history of skin lesions, and family history of skin lesion. We arbitrarily divided age group into early adulthood <40 years, late adulthood between 40 and 60 years and more than 60 years. All the patients were asked for the presence of skin lesions currently and in the past through history and by complete physical examination. Semi-structured dermatological assessment sheet includes the details about the type of skin lesions and questionnaire covering the details of skin lesions and its relationship to treatment lithium. These patients were examined by the dermatologist and diagnosis was made. The dermatologist would also determine that the lesion was related to the drug lithium. Necessary treatment for the concerned dermatological diagnosis was given by the dermatologist.

Follow-up assessment

We did follow-up assessments monthly for 6 months and thereafter every 2 months for a year. During each follow-up, patients were examined for the presence of skin lesions. Follow-up assessment was done using semi-structured pro forma which included the course of individual skin lesions and preexisting skin lesions as persisted, remitted or exacerbated, and for the presence of new lesions. If the patient develops any new lesions dermatologist opinion was sought each time and they were given required medications. If the patient missed a follow-up, they were contacted over phone and enquired for the presence of any skin lesions and its course both from the patient and the caregiver. Even if they did not come for follow-up, they were included in the study.

Statistical analyses

The prevalence of cumulative subtype of cutaneous lesions and total prevalence of cutaneous lesions were calculated.

Table 1: Baseline sociodemographic details of the sample study

Variables	Number of patients (%)
Age (years)	
<40	31 (59.6)
40-60	18 (34.6)
>60	3 (5.8)
Gender	
Female	25 (48.1)
Male	27 (51.9)
Socioeconomic status	
Upper middle	49 (94.2)
Lower middle	3 (5.8)
Education	
Illiterate	15 (28.8)
Mid school	30 (57.7)
High school	7 (13.5)
Postgraduate	0

The prevalence of individual lesions is compared with the age and gender.

RESULTS

General characteristics of study sample

In the study sample, the majority were in the age group of <40 years (59.6%), those between 40 and 60 years are 34.6%. Gender distribution was almost equal where females are 48.1% and males are 51.9%. The majority were from upper middle socioeconomic status (94.2%) as per modified Kuppusamy scale and 57.7% had finished their mid school level [Table 1].

Analysis of cumulative prevalence of cutaneous lesion during 1-year follow-ups

The prevalence of lithium-induced skin reactions in our study was 38.46%. On analyzing the cumulative prevalence, acneiform eruption peaked during the 2nd month (n = 11/52), and it abated at the end of 1 year. Hair fall had peaked at the 4th month (n = 6/52) later has gradually came down and again increased at the end of 1 year. Hyperpigmentation occurred from the 3rd month; it peaked in the 5th month and persisted throughout the study. Only one patient had developed seborrheic dermatitis during 1st month, but it abated in between for 4 months with dermatological treatment and reappeared during 8th month and persisted till the end of the study.

Lithium-induced ulcer (n = 1/52) developed during the 1st month, and it persisted throughout. It developed in the right index and middle fingers with multiple ulcers gradually it has increased its size and number. But after the end of the study, lithium was stopped and was changed over to other mood stabilizers.

Combined lesions such as acne with hair fall, acne with hyperpigmentation, and acne with seborrheic dermatitis had occurred. One patient developed acne with hair fall and acne with hyperpigmentation, they cleared up at the end of the study. As shown in Table 2, one patient had acne with seborrheic dermatitis waned off for a month and reappeared.

Prevalence of cutaneous lesions among various age groups

Acneiform eruptions (n = 11/52) and hyperpigmentation (n = 2/52) are common in early adulthood, whereas lithium-induced ulcer (n = 1/52) seen in late adulthood. Hair fall is equal in both early and late adulthood (5.7%). In geriatrics, 1.9% developed acneiform eruptions with hyperpigmentation [Table 3].

Gender difference in the prevalence of cutaneous skin lesions

15.2% (n = 8/52) of males and 11.4% (n = 6/52) females had developed acneiform eruptions, whereas 3.8% males and 7.6% females developed hair fall. Seborrheic dermatitis occurred in males and lithium-induced ulcer in females. Hyperpigmentation has seen in 3.8% males and 1.9% females.

About 3.8% of females had developed acneiform eruption with hair fall whereas acneiform eruption with hyperpigmentation (3.8%) and seborrheic dermatitis (1.9%) occurred in males [Table 4].

Course of preexisting skin reaction

Three patients had preexisting skin lesions, of which one had tinea during initial assessment which gradually subsided with treatment during 3rd month, and one had candidiasis improved and remitted in the 4th month.

One patient had systemic lupus erythematosus, developed severe hair fall at a 2nd month, and they stopped treatment with lithium therapy on their own at the end of 3rd month.

Drop outs

After recruitment of 52 patients, 10 were dropped out at the end of the study. Of which five were dropped out because of poor compliance. One dropped out due to lithium toxicity. One dropped out at the end of the 3rd month due to persistent vomiting, and he was changed over to another mood stabilizer. One dropped out at the end of 5th month due to nonaffordability to do routine serum lithium level. One dropped out due to severe hair fall.

DISCUSSION

In this study, the mean age was 38.6 years and most common age group was early adulthood (18–40 years).

Table 2: Prevalence of cumulative subtype of skin lesions

Type of skin lesion	Initial assessment	Follow ups 1	Follow ups 2	Follow ups 3	Follow ups 4	Follow ups 5	Follow ups 6	Follow ups 7	Follow ups 8	Follow ups 9
	Initial (%)	1st month (%)	2nd month (%)	3rd month (%)	4th month (%)	5th month (%)	6th month (%)	8th month (%)	10th month (%)	12th month (%)
Acneiform eruption	13.3	19	20	17.3	13.3	7.6	11.5	9.6	5.8	1.9
Hair fall	1.9	3.8	7.7	7.7	11.4	9.5	7.7	7.7	7.7	13.3
Seborrheic dermatitis	0	1.9	1.9	0	0	0	0	1.9	1.9	1.9
Hyperpigmentation	0	0	0	1.9	1.9	3.8	3.8	3.8	3.8	3.8
Acne and hyperpigmentation	1.9	1.9	1.9	1.9	0	0	0	0	0	0
Lithium-induced ulcer	0	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Acne and hair fall	0	1.9	1.9	0	0	0	0	0	0	0
Acne and seborrheic dermatitis	0	0	0	0	1.9	1.9	0	1.9	1.9	1.9

Our sample had an equal gender distribution. Most were in the upper middle socioeconomic status and had completed up to middle school education.

The prevalence of lithium-induced cutaneous side effects in our study was 38.46% which is comparable to the prevalence reported by Sarantidis and Waters and Chan *et al*. Acneiform eruptions and hair fall are most common lesion in our study whereas study by Sarantidis and Waters showed acneiform eruption and psoriasis as the most common secondary cutaneous lesions. The incidence of hyperpigmentation was found significant in our study compared to previous studies.

The course of acneiform eruption showed that the lesions were more during the initial period of follow-ups and gradually improved on treatment with topical retinoids.[10-12] The patient did not have the classical type of distribution but occurred over the extremities (mostly in the back of the shoulders) as reported in previous studies by Jennis and Kerbs *et al*.

Hyperpigmentation, hair fall and seborrheic dermatitis coexist with acneiform eruptions during the course. Seborrheic dermatitis has reduced along the course with treatment but reappears on discontinuation of treatment for the same but when compared with the previous study by Dreno *et al*. where lithium is used as the treatment for seborrheic dermatitis.

In this study, one patient had developed lithium-induced ulcers. She developed multiple small pustular ulcers over the index and middle fingers which was found to be most distressing for the patient. Even with dermatological treatment using topical applications patient continued to have repeated pustular ulcers in hands with fissure.

The cutaneous adverse reactions on lithium are more common in early adulthood than in the late adulthood and geriatric age group and among the subtypes of skin lesion acneiform eruptions are more common among males than in females which is supported by previous study by Chan *et al*. Acneiform eruptions coexists with hair fall and is more common among females. An acneiform eruption with hyperpigmentation is more common among males.

Patient with pre-existing severe skin reactions are prone for new skin reaction and may contribute to poor attrition. Interestingly, patients with milder skin reactions like tinea and candidiasis improved over the course of time. But patient with systemic lupus erythematosis developed severe hairfall, resulting in dropout, which is supported by the previous study.[13,14]

The strength of our study is prospective study, and the recruited patients are studied at multiple intervals for a

Table 3: Percentage of cutaneous skin lesion among various age groups

Age (years)	Acneiform Eruption (%)	Hair fall (%)	Seborrheic dermatitis (%)	Hyperpigmentation (%)	Lithium induced ulcers (%)	Acne, hyperpigmentation (%)	Acne, hair fall (%)	Acne, seborrheic dermatitis (%)
Early adulthood (<40)	20	5.7	1.9	3.8	0	0	1.9	1.9
Late adulthood (40-60)	3.8	5.7	0	1.9	1.9	0	1.9	0
Geriatrics (>60)	1.9	0	0	1.9	0	1.9	1.9	0

Table 4: Gender difference in prevalence of cutaneous skin lesions

Age	Acneiform eruption (%)	Hair fall (%)	Seborrheic dermatitis (%)	Hyperpigmentation (%)	Lithium-induced ulcers (%)	Acne, hyperpigmentation (%)	Acne, hair fall (%)	Acne, seborrheic dermatitis (%)
Female	11.4	7.6	0	1.9	1.9	0	3.8	0
Male	15.2	3.8	1.9	3.8	0	3.8	0	1.9

long duration. Dermatologist opinion obtained for each patient who developed lesions during each follow-up.

Our limitations are small sample size to be generalized. Few patients were on lithium with psychotropic medications which would also contribute to the skin lesions. We had taken convenience sampling. A single dermatologist assessing all the cohorts would have made the study better, and it was not possible due to practical reasons. Naranjo scale which is used to assess the association between the drugs-induced reaction and drug is not used in our study. No blinding was done for the primary therapist nor for the dermatologist, and we followed the patients through telephonic calls which may not be correct as direct observation.

CONCLUSION

The prevalence of lithium-induced cutaneous lesions continuous to be high. Clinician should educate the patient before initiating lithium to improve the attrition rate. Physician need not be apprehensive about changing to other mood stabilizers as lithium-induced skin reactions could be managed with dermatological treatment. Physician should also be cautious in starting lithium in selective cohort of sample.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Shorter E. The history of lithium therapy. Bipolar Discord 2009;11:4-9.
2. Albrecht G. Undesirable skin reactions to lithium. Hautarzt 1985;36:77-82.
3. Chan HH, Wing Y, Su R. A control study of the cutaneous side effects of chronic lithium therapy. J Affect Disord 2000;57:107-13.
4. Sarantidis D, Waters B. A review and controlled study of cutaneous conditions associated with lithium carbonate. Br J Psychiatry 1983;143:42-50.
5. Gupta MA, Gupta AK, Haberman HF. Psoriasis and psychiatry: An update. Gen Hosp psychiatry 1987;9:156-66.
6. KB Shreedhar, J Madhukara, J Jessy, SM Manohari, K Srinivasan. Drug hypersensitivity syndrome with lithium 2010;76:426-7.
7. Posey RE. Lithium carbonate dermatitis. JAMA 1972;221:1517.
8. Meinhold JM,West D,Gurwich E,Spunt A,KoyaD.Cutaneous reaction to lithium carbonate: A case report. J Clin Psychiatry 1980;41:395-6.
9. Kanzaki T. Acneiform eruption induced by lithium carbonate. J Dermatol 1991;18:481-3.
10. Kansal NK, Chawla O, Singh A, Matreja PS. The cutaneous adverse effects of Lithium. J Clinical and Diagnostic Research 2011;5:570-2.
11. Sharma A, Padala PR. Lithium induced rash. Prim care companion. J clin Psychiatry 2006:8:377.
12. Deandrea D, Walker N, Mehlmauer M. Dermatological reactions to lithium: A critical review of the literature. J Clin Psychopharmacol 1982;2:199-204.
13. Antonov D, Djiev JK, Tsankov N. Drug induced lupus erythematosis. Clinics in Dermatology 2004;22:157-66.
14. Orwin A: Hair loss following lithium therapy.Br J Psychiatry 1988;143;42-50.

Original Research Article

Effectiveness of mood stabilizer in euthymic BPAD patients: An one year prospective observational study, comparing Lithium v/s Divalproate sodium

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Abstract Bipolar disorder is potentially a lifelong and disabling condition, which requires long-term pharmacological therapy as maintenance treatment. Lithium is considered as first-line mood stabilizer, followed by Divalproate, both are known to reduce the risk of relapse. However, relapses do occur and these medications are also associated with side effects. We aimed to compare the mood stabilizing effect between Lithium and Divalproate in our prospective study. **Methods** 104 bipolar patients who were euthymic (52 patients each on Lithium and Divalproate therapy) were followed up for one year with periodic evaluation done during initial, 3rd, 6th, 9th and 12th month. Time taken to relapse, severity of mood episode, suicidal risk and functioning were assessed using standardized scales. Compliance to medications and adverse effects were also assessed. **Results** The duration of treatment was for a longer period in Lithium group (p=0.001) and this was the only confounding variable which was significant. Patients who are taking Divalproate had more severe manic episode at the end of 1 year when compared to patients taking Lithium (p=0.041). Patients on Lithium also had lesser suicidal risk (trending towards significance). **Conclusion** There was no difference in terms of frequency of depressive episode, adherence, adverse effects and global functioning between the two groups. But Lithium group patients had less frequent and less severe manic episodes and lower suicidal risk, favoring Lithium to be a better mood stabilizer, with less adverse effects and better quality of life.

Key words Bipolar disorder; Mood stabilizer; Maintenance treatment; Quality of life.

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Introduction

Bipolar Affective Disorder (BPAD) is one of the most disabling mental illnesses affecting most productive period of life at the age 15-45years.[1] Lithium carbonate has been a gold standard treatment for past five decades. But it has a narrow therapeutic index and significant adverse effects.[2] Anticonvulsants such as Divalproate sodium, Carbamazepine and Oxcarbamazepine, have been proposed as an alternative, also have more adverse effect profile and their comparative efficacy with Lithium is uncertain.[3-8] Lithium Carbonate as a mood stabilizer has been a superior agent to reduce the risk of relapse and to prevent suicidal behaviours.[2-7] In view of its adverse effects, tolerance becomes an issue; which can interfere with adherence.[4-7] Lithium causes multiple skin reactions, the most common are acne and psoriasis. The prevalence of skin reaction with Lithium ranges between 3-34 %. Some studies have shown high chance of discontinuation of Lithium is due to adverse skin reactions.[9] Anticonvulsants approved by FDA, has the next level of

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evidence as a mood stabilizer but their long term safety and efficacy in comparison with Lithium remains uncertain[7] Randomised controlled trails have shown superiority of Divalproate sodium to placebo.[10-11] Divalproate sodium has been comparable with Lithium in Manic episode[11] FDA approved mood stabilisers for the treatment of bipolar affective disorder are Lithium, Divalproate sodium, Carbamazepine and Lamotrigine.[17-20] Mood stabilisers can also be used as monotherapy which was approved by FDA.[21-22] Mania with two or more episodes of depression showed a good improvement with Divalproate sodium than Lithium.[23-24] Lithium and Divalproate sodium showed more effect than any other mood stabilisers during acute phase of mania and in maintenance phase.[25-27] Olanzapine, Risperidone and Quetiapine are FDA approved atypical antipsychotics for the acute phase of mania[17,18,20] According to BALANCE study both Lithium monotherapy and combination therapy with Lithium and Divalproate sodium are more likely to prevent relapse than Divalproate sodium monotherapy, irrespective of baseline severity of illness and is maintained for up to 2 years.[12] Bowden *et al*, in his randomized placebo controlled 12 months trial of Lithium v/s Divalproate sodium, has shown no significant difference between the two groups in terms of time to recurrence of mood episode during maintenance therapy. Compared to placebo, Divalproate sodium has lesser discontinuation rate[13.] Even though open labelled trails favours Divalproate sodium in reducing the frequency and intensity of further episodes, there are less comparative study with Lithium in maintenance therapy.[14-16] Long term outcomes are often poor in patients with bipolar disorder despite treatment; therefore more effective treatments are needed to reduce recurrences and morbidity. Hence, we proposed a prospective comparison study of Lithium and Divalproate sodium in euthymic BPAD patients for a period of at least 1year during the maintenance phase. We aimed to compare the mood stabilizing properties of Lithium and Divalproate sodium in BPAD patients. Our other objectives are to evaluate the time taken for relapse of any mood episodes (mania/depression/mixed episodes), assess the severity of the mood episodes, episodes of deliberate self harm, adherence to study treatment, adverse effects of medications and global assessment of functioning.

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This is a prospective observational study done in patients with bipolar affective disorder who came to the psychiatry department in PSG Institute of Medical Sciences and Research, Coimbatore, India. A total of 104 bipolar affective disorder patients were recruited from out-patient setting. The study protocol was explained to the participants, and a written informed consent was obtained from the patient. If patient could not consent, it was obtained from the family member and later from the patient when he/she could consent. The study was approved by Institutional Human Ethics Committee.

Men and women aged 18 years and above who received a clinical diagnosis of BPAD (as per ICD-10 criteria) in psychiatry out-patient department were eligible for the study. Patients were initiated or continued on a single mood stabilizer either on Lithium or Divalproate sodium. Only patients who achieved remission within 2 months of follow-up were included in the study. Patients who were already on more than one mood stabilizers (Lithium, Valproate, Carbamazepine, Lamotrigine, Gabapentin and Topiramate) during index diagnosis and medically unstable patients with conditions such as renal failure, liver cirrhosis, severe diabetes, arrhythmia etc. were excluded from the study.

After recruiting the patients as per our inclusion and exclusion criteria, 52 patients who were on Lithium therapy and 52 patients who were on Divalproate therapy, were prospectively followed up for 1 year period. Patients were evaluated by the investigator, following 2 months of euthymic period (index evaluation). During follow up patients were evaluated at 3rd, 6th, 9thand 12th month respectively (periodic evaluation). Patients who are missing on follow up are contacted through telephone and requested to come for follow-up and assessed. As it is an observational study, investigators took no role in modifying the dosage of medications, but it was taken care by the primary incharge treating team. During initial evaluation, a semi-structured proforma (socio-demographic details and other confounding variables) is administered. Diagnosis was confirmed by applying a SCID (Structured Clinical Interview for DSM-IV) version for mood disorder. As per earlier literature available, remission is a state that comes close to being symptom free. By which the euthymic status of the patient was ascertained by applying HAM-D and YMRS rating scales. Typically remission should have a score of ≤ 7 on HAM-D and ≤ 2 on YMRS. Severity of suicidal ideas is assessed by Modified SADPERSONS Scale. Global Assessment of Functioning was assessed using a GAF scale. The dosage of the mood stabilizer could be altered by the primary in-charge team based on serum concentration of the drug/adverse effects, during 1 year maintenance period. Participants who remain on the allotted treatment for 1 year of study were periodically evaluated. Use of other psychotropics was allowed during the study trial (antipsychotics, benzodiazepines).Periodic assessment was done as per the figure 1 given below, for confounding variables, SCID-mood disorder sub-scale,

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Young Mania Rating Scale (YMRS)/ Hamilton rating scale for Depression (HAM-D) appropriate to the patient (to access the severity of episode if any), Modified SADPERSONS scale-to see suicidal score, adverse drug reaction and Global assessment of functioning (GAF) scale.

Statistical analyses were conducted using software package SPSS version 20. We compared the efficacy of Lithium and Divalproate sodium with the following variables such as age, gender, marital status and education status, which were expressed in percentage and the presence of any statistically significant difference, was analysed using Chi-square test. Also statistically significant difference between the age of onset, number of previous episodes, previous hospitalisation, polarity of previous episodes, psychotropics and duration of mood stabiliser with that of Lithium and Divalproate sodium was done using Chi-square test. Comparison of duration of illness, time taken for any mood episodes, severity of manic episode/depressive episode, suicidal risk, adherence to medications, adverse effects, global assessment functioning between Lithium and Divalproate sodium was done using analysis of variance; p-values <0.05 was considered as significant.

Table 1: Comparison of socio-demographic and confounding variable between Lithium and *Divalproate sodium group*:

Variables		Lithium	Divalproate sodium	p value
Age at recruitment	18- 35 years	31(59.6%)	25(48.1%)	0.392
	36- 59 years	18(34.6%)	21(40.4%)	
	> 60 years	3(5.8%)	6(11.5%)	
Gender	Male	32(61.5%)	39(75.0%)	0.140
	Female	20(38.5%)	13(25.0%)	
Educational status	Illiterate	7(13.5%)	14(26.9%)	0.101
	Up to 10th std	27(51.9%)	20(38.5%)	
	11th-12th	5(9.6%)	10(19.2%)	
	Graduate	13(25.0%)	8(15.4%)	
Marital status	Unmarried	15(28.8%)	11(21.2%)	0.169
	Married, living together	32(61.5%)	26(50.0%)	
	Married, living separately	3(5.8%)	7(13.5%)	
	Married, divorced	1(1.9%)	4(7.7%)	
	Widow/widower	1(1.9%)	4(7.7%)	
Age of onset	< 35 years	19(36.5%)	9(17.3%)	0.085
	36- 59 years	27(51.9%)	36(69.2%)	
	> 60 years	6(11.5%)	7(13.5%)	
Number of episodes	1episode	0(0.0%)	3(5.8%)	0.145
	2episode	18(34.6%)	13(25.0%)	
	>=3episodes	34(65.4%)	36(69.2%)	
Previous hospitalisations	No hospitalisation	7(13.5%)	6(11.5%)	0.594
	1 hospitalisation	9(17.3%)	5(9.6%)	
	2 hospitalisations	15(28.8%)	19(36.5%)	
	>=3hospitalisations	20(38.5%)	22(42.3%)	
Polarity of previous episodes	1 Depressive episode	1(1.9%)	0(0.0%)	0.373
	1 Manic episode	4(7.7%)	6(11.5%)	
	Depression = Mania	18(34.6%)	14(26.9%)	
	Depression > Mania	11(21.2%)	7(13.5%)	
	Mania > Depression	12(23.1%)	12(23.1%)	
	>=2 mania episodes	6(11.5%)	13(25.0%)	
Psychotropics	No drugs	23(44.5%)	13(25.0%)	0.982
	Typical antipsychotics	7(13.5%)	15(28.8%)	
	Atypical antipsychotics	19(36.5%)	21(40.4%)	
	Antidepressants	2(3.8%)	2(3.8%)	
	Benzodiazepines	1(1.9%)	1(1.9%)	
Duration of Mood Stabilizer	<6months	1(1.9%)	0(.0%)	**0.001**
	6months-1year	7(13.5%)	1(1.9%)	
	1- 2years	5(9.6%)	20(38.5%)	
	>2years	39(75.0%)	31(59.6%)	

There was no significant difference between the two groups in socio-demographic variables (Table 1) such as age (p=0.392), gender (p=0.14), educational qualification (p=0.10) and marital status (p=0.17).There is no statistical significance among the confounding variables such as; age of onset (p=0.085), number of episodes (p=0.145), previous hospitalisations (p=0.594), polarity of episodes (p=0.373), psychotropics (p=0.982). Between the two groups, the duration of illness (p=0.001) was the only confounding variable which was significant between the two groups. The number of mood episodes that occurred was; among Lithium group patients 13 had manic episodes and 8 had depressive episodes. Among *Divalproate sodium group* patients 12 had manic episodes and 6 had depressive episodes. In both the groups' manic episodes were more common than depressive episodes.

For a manic episode relapse; in the *Lithium group*, 39 patients had no episodes of mania, 5 patients developed mania in less than three months, 4 patients between three to six months and 4 patients between six to nine months. In the *Divalproate sodium group*, 40 patients had no episodes of mania, 2 patients developed mania in less than three months, 3 patients between three to six months, 3 patients between six to nine months and 4 patients between nine to twelve months. The time taken for manic episode was not statistically significant between Lithium and *Divalproate sodium group* (p=0.339). For a depressive episode to occur; in the *Lithium group*, 45 patients had no episodes of depression, 2 patients developed depression between three to six months, 2 patients between six to nine months and 3 patients between nine to twelve months. In the *Divalproate sodium group*, 46 patients had no episodes of depression, 1 patient developed depression in less than three months, 3 patients between three to six months and 2 patients between nine to twelve months. The time taken for depressive episode was also not statistically significant between Lithium and *Divalproate sodium group* (p=0.240).

Comparison of severity of manic episode between Lithium and *Divalproate sodium group* using YMRS

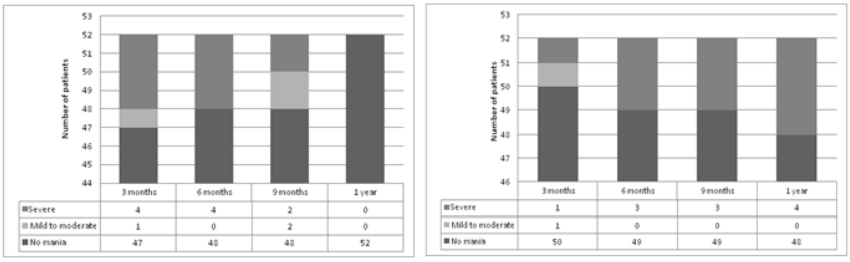


Figure 2A: Figure 2B:

Figure 2 A: Severity of mania in patients taking Lithium; **Figure 2B:** Severity of mania in patients taking Divalproate sodium

The severity of mania was assessed using The Young Mania Rating Scale (YMRS) in Figure2. Patients who are taking Divalproate sodium had more severe manic episode at the end of one year when compared to patients taking Lithium (4 patients v/s none – p=0.041), but the comparison was not significant during initial 3 follow ups at 3, 6 and 9 months (p=0.388, 0.696, 0.331 respectively)

Comparison of severity of depressive episode between Lithium and *Divalproate sodium group* using HDRS:

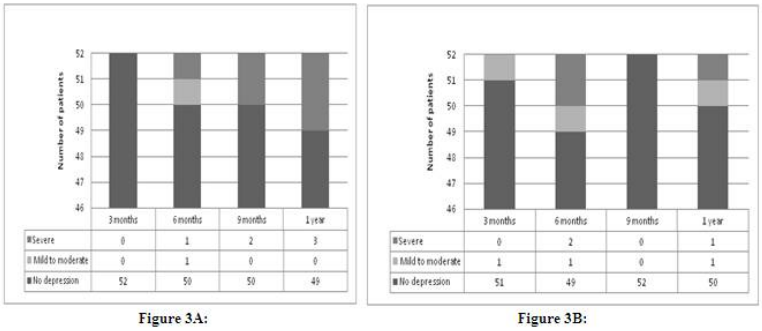


Figure 3A: Figure 3B:

Figure 3A: Severity of depression in patients taking Lithium; **Figure 3B:** Severity of depression in patients taking Divalproate

The severity of depression was assessed using Hamilton Depression Rating Scale (HDRS) in Figure 3. On comparison with patients who are taking Lithium and Divalproate sodium. There was no statistically significant difference in the severity of depressive episode during all four follow ups (3rd, 6th, 9th and 12th month periodic evaluation; p = 0.315, 0.842, 0.153, 0.366 respectively).

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Table 2: Suicidal risk assessment between Lithium and *Divalproate sodium group* using Modified SADPERSONS Scale

Groups	No risk	Moderate risk	Severe risk	p Value
		At Baseline		
Lithium	37 (71.2%)	15 (28.8%)	0	
Divalproate sodium	35 (67.3%)	17 (32.7%)	0	0.671
		During *3 months* follow-up		
Lithium	35 (67.3%)	14 (26.9%)	3 (5.8%)	
Divalproate sodium	34 (65.4%)	18 (34.6%)	0	0.173
		During *6 months* follow-up		
Lithium	39(67.3%)	9(26.9%)	4(7.7%)	
Divalproate sodium	34(65.4%)	15(28.8%)	3 (5.8%)	0.371
		During *9 months* follow-up		
Lithium	42(80.8%)	8(15.4%)	2(3.8%)	
Divalproate sodium	40(76.9%)	9(17.3%)	3 (5.8%)	0.857
		During *12 months* follow-up		
Lithium	47(90.4%)	3(5.8%)	2(3.8%)	
Divalproate sodium	38(73.1%)	9(17.3%)	5 (9.6%)	**0.073**

Modified SADPERSONS scale was applied to assess suicidal risk. The severity of suicidal risk scale (Table 2) was not statistically significant during initial and all four follow-ups, but was trending towards significance during the 12th month follow-up (baseline, 3rd, 6th, 9th and 12th month periodic evaluation; p=0.671, 0.173, 0.371, 0.857, 0.0 respectively) In the Lithium group of patients; 4 (7.7%) had no follow-up, 5 (9.6%) have followed-up once, 8 (15.4%) have followed-up twice, 10 (9.2%) have followed-up thrice and 25 (48.1%) have been completely followed-up till the end of one year. In the *Divalproate sodium group* of patients; 5 (9.6%) had no follow-up, 4 (7.7%) have followed-up once, 8 (15.4%) have followed-up twice, 13 (25%) have followed-up thrice and 22 (42.3%) have been completely followed-up till the end of one year. Among 104 recruited patients, 25 patients of Lithium group had completed all four follow-ups as compared to 22 patients of *Divalproate sodium group*. But on comparison between the two groups, they were not statistically significant (p=0.938).

Table 3: Adverse effects of the treatment

Groups	No drug reaction	Minimal reaction	More reaction	p value
		At Baseline		
Lithium	28(53.8%)	24(46.2%)	0	
Divalproate sodium	24(46.2%)	26 (50%)	2(3.8%)	0.303
		During *3rd month*follow-up		
Lithium	23(44.2%)	29(55.8%)	0	
Divalproate sodium	24(46.2%)	23(44.2%)	5(9.6%)	0.057
		During*6th month* follow-up		
Lithium	27(51.9%)	25(48.1%)	0	
Divalproate sodium	26 (50%)	24(46.2%)	2(3.8%)	0.361
		During *9th month* follow-up		
Lithium	30(57.7%)	22(42.3%)	0	
Divalproate sodium	35(67.3%)	16(30.8%)	1(1.9%)	0.312
		During *12th month* follow-up		
Lithium	42(80.8%)	10(19.2%)	0	
Divalproate sodium	39(75.0%)	11(21.2%)	2(3.8%)	0.340

The adverse drug effect (categorised as nausea, diarrhoea, tremors, weight gain, sedation, polydipsia, polyuria, tachycardia, alopecia, any major skin lesions, hypothyroid symptoms, signs of renal dysfunction) profile (Table 3) was the same between Lithium and *Divalproate sodium group* during all four visits except during initial follow up (3months) in which Lithium group was better than *Divalproate sodium group* (five patients on *Divalproate sodium group* had >=3 adverse drug reaction compared to none in the Lithium group), which was statistically significant.

Table 4: Global Assessment Functioning

Groups	Good functioning	Mild impairment	Moderate impairment	Severe impairment	p value
		At *Baseline*			
Lithium	51 (98.1%)	1 (1.9%)	0	0	0.315
Divalproate sodium	52 (100%)	0	0	0	
		During *3rd month* follow-up			
Lithium	46(88.5%)	2(3.8%)	3(5.8%)	1(1.9%)	0.553
Divalproate sodium	49(94.2%)	2(3.8%)	1(1.9%)	0	
		During *6th month* follow-up			
Lithium	46(88.5%)	1(1.9%)	3(5.8%)	2(3.8%)	
Divalproate sodium	46(88.5%)	2(3.8%)	2(3.8%)	2(3.8%)	0.912
		During *9th month* follow-up			
Lithium	46(88.5%)	2(3.8%)	3(5.8%)	1(1.9%)	
Divalproate sodium	49(94.2%)	0	1 (1.9%)	2(3.8%)	0.330
		During *12th month* follow-up			
Lithium	49(94.2%)	0	3(5.8%)	0	
Divalproate sodium	46(88.5%)	0	2(3.8%)	4(7.7%)	0.117

On assessment of social, psychological and occupational functioning by using GAF scale (Table 4). During the initial follow ups Lithium group patients had more functional impairment than *Divalproate sodium group* patients (initial and 3rd follow up). During 6th month follow up two patients in each group had severe impairment. During 9th month and 1year follow up, *Divalproate sodium group* had more severe functional impairment than Lithium group, but was not statistically significant.

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In our prospective study of one year duration, we compared the mood stabilising effect of Lithium and Divalproate sodium in euthymic bipolar patients. The socio-demographic and confounding variables did not differ between the two groups except that the Lithium group patients had longer duration of treatment than those treated with Divalproate sodium. As a primary outcome of the study, we compared the frequency of manic and depressive episode between two groups. There were similar number of episodes and the predominant mood episode was mania in both the groups. The above finding could also be because the polarity of previous episode in both the groups was predominantly mania. During the period of 9th to12th month follow up, Divalproate sodium had more manic episodes. The above finding emphasises Lithium to be a better long term mood stabiliser than Divalproate sodium. Previous studies have shown that Lithium treatment reduces the risk of relapse in bipolar disorder. The preventive effect is clear for manic episodes, although it is equivocal for depressive episodes.[5] Lithium appears to be the most effective treatment to prevent recurrence or relapse of bipolar disorder and may prolong the time before adjunctive prescribing is necessary.[28] This finding is further supported by the result of recent meta-analysis[29] suggest that Lithium is superior in prevention of manic episodes, in comparison to anticonvulsants. However, there was no significant difference regarding prevention of overall mood episodes, depressive episodes or study completion. Based on the severity of mood episode; patients on Divalproate arm, had more severe manic episodes as the duration of follow up increased. This again emphasises that Lithium is a better anti-manic agent for long-term therapy. But, the frequency and severity of depressive episodes did not differ between both the groups. On comparison of suicidal risk, bipolar patients who were on Lithium had lower suicidal risk than Divalproate patients, especially with prolonged duration of treatment. Both Lithium and *Divalproate sodium group* patients had almost equal follow ups and were equally adherent to

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treatment. Two patients on Lithium were switched to Divalproate sodium as they had severe skin reaction which affected the study adherence. Adverse effect profile did not differ during initial and periodic assessment between the two groups. Bipolar patients on Divalproate sodium had more adverse effects during initial follow ups, which were not seen during further follow up. Global assessment of functioning was done considering psychological, social and occupational functioning on a hypothetical continuum of mental-health illness. Patients on *Divalproate sodium group* had more severe impairment in functioning, than patients in Lithium group. The serum Lithium level was less than adequate in majority of new onset manic/depressive episode patients, signifying that majority of the patients who developed new onset manic/depressive episode were on sub therapeutic dose. Further emphasising the relationship between plasma levels of the drug and relapse prevention.

Our sample size was smaller and hence the results cannot be generalised. Telephonic assessments for patients, who missed follow ups, cannot be considered as reliable as face to face interview. Use of other psychotropics (antipsychotics, benzodiazepines) was allowed. We know that medication like Olanzapine, Risperidone and Quetiapine can have a mood stabilising effect, which could have affected the outcome of the study. A follow-up more than 1 year is needed in order to strongly support our finding that, bipolar patients on Lithium were better than Divalproate sodium in preventing the manic episode.

Bipolar patients on Lithium had lesser frequent and less severity of manic episode over one year follow up. The frequency of depressive episode was similar between the Lithium and *Divalproate sodium group*s. Lithium exhibited better anti-suicidal properties. Lithium continues to be a gold standard in spite of certain cutaneous side effects establishing its seven decades of dominance as a mood stabilizer agent. In addition, long-term use of Lithium also ensures better quality of life.

r SU and SA designed this study. SA managed the data collection. SU, SA and KSR analysed the data. KSR wrote the draft of the manuscript under the supervision of SU and SA. All authors contributed to and have approved the the final manuscript.

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1. Murray CJL, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet* 1997; 1436–42.
2. American Psychiatric Association. Practice guideline for the treatment of patients with bipolar disorder (revision).*Am J Psychiatry* 2002; **1** (4 suppl) 1–50.
3. Goodwin GM. Evidence-based guidelines for treating bipolar disorder: revised second edition—recommendations from the British Association for Psychopharmacology. *J Psychopharmacol*2009; **2** 346–88.
4. National Institute for Health and Clinical Excellence. Bipolar disorder: the management of bipolar disorder in adults, children and adolescents, in primary and secondary care. http://guidance.nice.org.uk/CG38 (accessed Nov 19, 2009).
5. Geddes JR, Burgess S, *et al.* Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. *Am J Psychiatry* 2004; **161** 217–22.
6. Cipriani A, Pretty H, *et al.* Lithium in the prevention of suicidal behaviour and all-cause mortality in patients with mood disorders: a systematic review of randomised trials.*Am J Psychiatry* 2005; **162** 1805–19.
7. Soares-Weiser K, Bravo Vergel Y, *et al.*. A systematic review and economic model of the clinical effectiveness and cost effectiveness of interventions for preventing relapse in people with effectiveness of interventions for preventing relapse in people with bipolar disorder. *Health Technol Assess* 2007; **11** 1–226.
8. Coryell W, Winokur G, *et al.* Lithium and recurrence in long term follow up of bipolar affective disorder.psychol med.1997;27:281-289.
9. Chan HH, Wing Y, *et al.*A control study of the cutaneous side effects of chronic lithium therapy. J Affect Disord. 2000 57:107–113
10. Bowden CL, Brugger AM, *et al.*, for the Depakoke Mania Study Group. Efficacy of divalproex vs lithium and placebo in the treatment of mania. JAMA. 1994;271:918-924.
11. Pope HG Jr, McElroy SL,*et al.*. Valproate in the treatment o acute mania: a placebo-controlled study. Arch Gen Psychiatry. 1991;48:62-68.
12. John R Geddes, Guy M Goodwin, *et al.* Lithium plus valproate combination therapy versus monotherapy for relapse prevention in bipolar I disorder(BALANCE): a randomised open-label trial. *Lancet* 2010; 375: 385–95
13. Charles L. Bowden, Joseph R.Calabrese, *et al.* A Randomized, Placebo-Controlled 12-Month Trial of Divalproex and Lithium in Treatment of Outpatients With Bipolar I Disorderfor Divalproex Maintenance Study GroupArch Gen Psychiatry. 2000;57:481-48
14. Lambert PA, Venaud G. Comparative study of valpromide versus lithium in the treatment of affective disorders. Nervure. 1992;5:57
15. Calabrese JR, Delucchi GA. Spectrum of efficacy of valproate in 55 patients with rapid-cycling bipolar disorder. AM J Psychiatry, 1990;147:431-434
16. McElroy SL, Keck PE Jr. Pope HG Jr. Sodium Valproate :its use in primary psychiatric disorders. J.clinPsychopharmacol. 1987; 7; 16-24.

17. Bowden CL, Ketter TA, Sachs GS, Thase ME. Focus on bipolar disorder treatment. J. Clin. Psychiatry.2005b; 66: 1598–1609.
18. FDA FDA approved drug products. 2009. [May 27]. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm.
19. Fountoulakis KN, Grunze H, Panagiotidis P, Kaprinis G. Treatment of bipolar depression: an update. J Affect Disord. 2008; 109: 21–34.
20. Ketter TA, Wang PW, Nowakowska C, editors. Treatment of acute mania in bipolar disorder. American Psychiatric Publishing; Washington, DC: 2005.
21. Bowden CL. Acute and maintenance treatment with mood stabilizers. Int J Neuropsychopharmacol.2003;6: 269–75
22. Bowden CL. Predictors of response to divalproex and lithium. J ClinPsychiatry. 1995; 56(Suppl 3):25–30.
23. Swann AC, Bowden CL, *et al.* Depression during mania. Treatment response to lithium or divalproex. Arch Gen Psychiatry. 1997; 54: 37–42.
24. Kleindienst N, Greil W. Differential efficacy of lithium and carbamazepine in the prophylaxis of bipolar disorder: results of the MAP study. Neuropsychobiology . 2000;42(Suppl 1):2–10.
25. Lerer B, Moore N, Meyendorff E, *et al.* Carbamazepine versus lithium in mania: a double-blind study. J Clin Psychiatry. 1987; 48: 89–93.
26. Vasudev K, Goswami U, Kohli K. Carbamazepine and valproate monotherapy: feasibility, relative safety and efficacy, and therapeutic drug monitoring in manic disorder. Psychopharmacology (Berl) 2000;150:15–23.
27. Young RC, Biggs JT, *et al.* A rating scale for mania reliability, validity and sensitivity. Br.J Psychiatry. 1978; 133;429-435.
28. Hayes JF, Marston L, Walters *et al.*Lithium vs. valproate vs. olanzapine vs. quetiapine as maintenance monotherapy for bipolar disorder: a population-based UK cohort study using electronic health records. World Psychiatry. 2016 Feb; 15(1):53–8.
29. Severus E, Taylor MJ *et al.*Lithium for prevention of mood episodes in bipolar disorders: systematic review and meta-analysis. Int J Bipolar Disord. 2014 Dec 20; 2(1):15.

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Original Research Article

A study on correlation of lithium and renal dysfunction with estimated glomerular filtration rate

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Lithium has been used in the treatment of mood disorders but its use is limited for its narrow therapeutic window and renal dys function. We aimed to correlate severity of renal dysfunction with duration of lithium therapy and serum lithium levels. We also analysed patients with Lithium induced renal dysfunction with normal creatinine values, but with abnormal eGFR. **M** **d** We included Bipolar patients who are on atleast 2 years of Lithium therapy & with minimum of 2 serum Creatinine values during treatment, baseline & 6months post-baseline. We recruited 906 patients, only 55 patients qualified for our inclusion criteria. We had 110 creatinine values & we calculated eGFR based on Modification of Diet in Renal Disease study group formula. We correlated duration of lithium therapy, serum lithium levels with average S.Creatinine levels and average eGFR. There was no correlation between duration of lithium treatment with average serum creatinine values (r = -0.17) or average eGFR (r = +0.24).There was no correlation between serum lithium levels with average serum creatinine values (r = -0.04) and average eGFR (r = -0.06).Among 19 patients with high serum creatinine values , almost all except one had Grade 2 renal dysfunction eGFR value Neither the duration of lithium therapy (upto 17 years) , nor serum lithium levels correlated with renal dysfunction. Fifteen percent of patients with normal creatinine levels had early renal dysfunction on eGFR, indicating probably eGFR as an early indicator than creatinine.

rd fLithium, Creatinine, Renal dysfunction, eGFR, Estimated Glomerular filtration rate

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Introduction

Lithium has been used in the treatment of mood disorders for several decades. But its use is limited for its narrow therapeutic window and multiple systemic side effects. Various studies were done to find the relationship between the serum lithium levels, duration of therapy, and dosage of lithium carbonate with its clinical responses. Among the available psychotrophics, Lithium remains a key drug especially in maintenance therapy. Lithium needs regular monitoring of serum lithium levels and renal dysfunction because of its narrow therapeutic window, as it is excreted nearly exclusively through the kidneys. The effect on Lithium induced renal dysfunction remains a controversy for more than four decades. Histopathological changes have been demonstrated way back in 1977, concluding chronic renal disease may develop only in patients with renal concentration of lithium reaches toxic level- either periodically or continuously.[1]

The duration of lithium treatment and cumulative dose has been suggested as major determinants of nephrotoxicity, but were only from small case series with

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multiple methodological issues.[2,3] Previous meta- analysis have shown poor quality, emphasizing the need for large scale studies. [4,5] In the study by Stephen Clos *et al*, no effect was found on rate of eGFR change over time in euthymic BPAD patients on Lithuim maintenance therapy.[6] Lithium causes a modest decline in renal function. However, the available research suggests that ESRD is a very rare complication of long-term lithium treatment, affecting ~1% of patients who have taken lithium for over 15 years.[7] With this above background, we planned for a study aiming for correlation of severity of renal dysfunction with duration of lithium therapy and serum lithium levels. We also did a correlation between socio-demographic variable and severity of renal dysfunction. We also analysed patients with Lithium induced renal dysfunction with normal creatinine values, but with abnormal eGFR.

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We included patients with bipolar affective disorder who are on atleast 2 years of Lithium therapy & with minimum of 2 serum Creatinine values during the treatment, one at baseline & atleast one after>= 6 months post-baseline. We excluded patients who were on short duration of lithuim treatment (< 2 years) or who had only one/no serum creatinine value. We also excluded patients who were on not on follow-up at PSG. We retrieved 1296 Serum lithium values of Bipolar affective disorder patients from PSG Hospitals MRD records (2013 – 2017). 522 values were repeat serum lithium values of same patient. Hence 774 patients with all the available lithium values were included. 295 patients excluded , as they were all inpatients and they had another out-patient file. Among the remaining 499 patients, 339 patients were excluded (160 patients were left), as they had one or no serum creatinine value.105 patients were excluded as they did not have serum creatinine values both at baseline and 6months post-baseline. Finally, 55 Patients were analysed for the study. We had 121□creatinine values & we calculated corresponding eGFR based on Modification of Diet in Renal Disease (MDRD) study group formula,[8] eGFR (mL × min^{-1}× [1.73 m^2] $^{-1}$)= 175 × (S_{Cr} standardized [mg × dL^{-1}]) $^{-1.154}$× (age [years])$^{-0.203}$Correction factor: for women × 0.742 for blacks × 1.18 186 x [Plasma Creatinine (µmol/L) x 0.0011312] $^{-1.154}$ x [age (years)] $^{-0.203}$ x [0.742 if female] x [1.212 if black] The following categories of eGFR were considered, as per KDOQI criteria. [9] Grade 1 -> 90 ml/min/1.73 m^2: Grade 2 – 60 to 89 ml/min/1.73 m^2: Grade 3a – 45 to 59 ml/min/1.73 m^2: Grade 3b–30 to 44 ml/min/1.73 m^2: Grade 4– 15 to 29 ml/min/1.73 m^2 : Grade 5–<15 ml/min/1.73 m^2.We analyzed baseline socio-demographic variables , namely, age & sex .We categorized age into adolescence, adults & geriatric population. We also analysed the co-morbidities, duration of follow-up after initiation of study, serum Lithium Level, total serum Creatinine values & total eGFR values. We correlated duration of lithium therapy with patients average S.Creatinine levels and average eGFR. We also correlated serum lithium levels with patients average S.Creatinine levels and average eGFR. We compared patient's serum creatinine level and the corresponding eGFR. We analysed patients having normal serum creatinine (<=1 mg/dl) values with patients having abnormal eGFR.

□□□□□□□□□□□□□□□□□□□□□□□□We analysed duration of lithium therapy, serum lithium levels, average serum creatinine levels , eGFR as a continuous measures. We used Pearsons correlation co-efficient to correlate duration of lithium therapy, serum lithium levels with serum creatinine and eGFR. If the r value is +1, it means a positive correlation and if it is1, it means a negative correlation. We also tabulated normal serum creatinine (<=1 mg/dl) values with abnormal eGFR in varying ranges.

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Majority of the patients were men & in adult age group. Majority of patients followed upto 5 years & more than two-fifth followed up more than 5 years. Diabetes was the commonest co-morbidity. Six percentage of patients had serum lithium level in toxic range. Seventeen percentage of patients had serum creatinine value more than one. Thirty percentage of patients had eGFR less than 90.

Table 1: Socio-demographic variables at baseline and Lithium parameters

Socio-demographic parameters & Lithium variables			Mean
Age(yrs)	<18	1 (1.8%)	44.93
	18-65	50 (90.9)	
	>65	4 (7.3)	
Sex	Male	32 (58.2)	-
	Female	23 (41.8)	
Duration of Lithium(yrs)	<2	0(0%)	
	2-5	32 (58.1%)	5

Co-morbidity	>5	23 (41.9%)	-
	DM	11 (20%)	
	HT	1 (1.8%)	
	DM & HT	4 (7.3%)	
	CVA	1 (1.8%)	
	Nil	38 (69.1%)	
Duration of Follow-up after initiation of study(yrs)	<2	2(3.6%)	3.2
	2-5	53 (96.4%)	
Average serum Lithium Level(mg/dl)	<0.8	27 (49.1%)	0.75
	0.8 – 1.2	24.5 (44.1%)	
	>1.2	3.5 (6.4%)	
Total Serum Creatinine values	≤ 1	91 (82.8%)	0.78
	>1	19(17.2%)	
Total eGFR values	≥90	77 (70%)	102
	60-89	32 (29%)	
	45-59	1 (1%)	

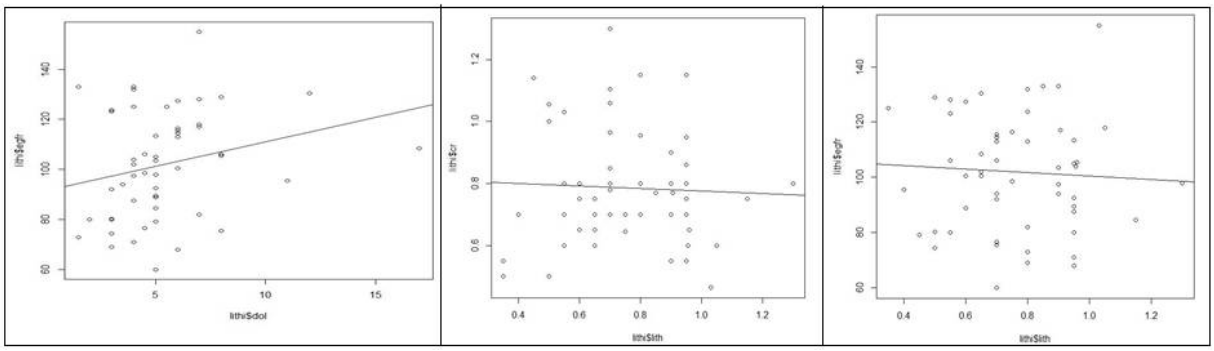


Figure 1 **Figure 2** **Figure 3**

Figure 1: Pearsons Correlation curve comparing duration of Lithium therapy with average serum creatinine values: **Figure 2:** Pearsons Correlation curve comparing serum lithium levels with average serum creatinine values; **Figure 3:** Pearsons Correlation curve comparing serum lithium levels with average estimated Glomerular filtration rate

Figure 1, The above curve denotes no correlation between duration of lithium treatment with average eGFR (r =+0.24).
Figure 2, The above curve denotes no correlation between serum lithium levels with average serum creatinine level (r = -0.04).
Figure 3, The above curve denotes no correlation between serum lithium levels with average eGFR (r = -0.06).

Table 2: Comparison of patients with normal creatinine levels with low eGFR

Sr. No	Sr. Creat. Values (mg/dl)	eGFR Values (ml / min / 1.73 m²)
1	0.8	76
2	0.8	77
3	0.8	80
4	0.8	74
5	0.8	74
6	0.8	80
7	0.9	77
8	1.0	87
9	0.9	85
10	0.7	86
11	1.0	85
12	0.8	68
13	0.9	89
14	0.8	72
15	0.98	87

Among 102 (102 / 121) normal serum creatinine values, 15 serum creatinine values (14.7 %) had low corresponding eGFR values.

Table 3: Comparison of patients with high creatinine levels with eGFR values.

Sr .No	Sr. Creat. Values (mg/dl)	eGFR Values (ml / min / 1.73 m²)
1	1.01	79
2	1.09	72
3	1.24	63
4	1.02	66
5	1.10	75.9
6	1.20	64
7	1.03	79
8	1.09	72
9	1.01	82
10	1.21	64
11	1.38	60
12	1.36	**57**
13	1.20	66
14	1.11	75
15	1.10	74
16	1.09	74
17	1.02	77
18	1.05	78

All the patients, except one had Grade 1 renal dysfunction, eGFR between 60 to 98.

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We did a retrospective study on effect of lithium levels and duration of lithium therapy on renal dysfunction. Majority of the recruited were adult men with diabetes as the common co-morbidity. The duration of lithium therapy, did not correlate with renal dysfunction in-terms of serum creatinine values. Three patients were on treatment for more than ten years, one for even seventeen years. The corresponding eGFR values also did not correlate with duration of lithium therapy. Previous studies and meta-analysis also concluded the same result. In the study by Stefan Clos *et al*, both, duration of exposure to lithium and mean serum lithium level were not significant predictors for eGFR decline. Our study also reveals serum lithium level (which is a marker of adequacy of lithium dose), did not correlate with renal dysfunction, either by creatinine levels or eGFR levels. Previous studies have shown that exposure to lithium (in the absence of episodes of toxicity) is a causal factor and the length of lithium exposure as a predictor of renal dysfunction.[10,11] In contrary, our study did not show correlation. A large UK community sample study by Miney *et al*[12] had a short observation period of 2 years and did not analyse longitudinal changes. In our study patients were on lithium, even for 17 years and the mean duration of lithium was five years. In a UK cohort study, Close and colleagues 13 used diagnostic codes for adverse renal outcomes rather than eGFR. We preferred eGFR as an indicator of renal dysfunction as it is a better predictor than serum creatinine. In an observational study by, Aprahamian and colleagues,[14] a single-blind follow-up study , no significant change in renal function of elderly patients after 4 years of chronic low-dose lithium use , as evidenced by our study. About fifteen patients (15 %), with normal creatinine levels had low eGFR values, indicating eGFR could pick-up renal dysfunction early. Among 19 patients with high serum creatinine values, almost all except one had Grade 2 renal dysfunction eGFR value .A single patient had grade 3 renal dysfunction 9 (eGFR –57).Our study limitations are , retrospective study design which has its own critics. The sample size was small and hence results can't be generalized. Mean duration on lithium was five years; a longer study would be of more credit. We could have had a control population, with which we would have analyzed in a better way. Our study was an observational study , hence only the available creatinine & lithium level were used for analysis.Almost one-third of patients had a co-morbid physical illness , disorders like diabetes and hypertension is known to cause renal dysfunction. Cockcroft-Gault equation is well suited for estimation of GFR changes during pharmacotherapy, instead we used MDRD equation, which is applied in adults with chronic renal disease. The National Kidney Foundation grades chronic kidney disease based on kidney damage (for ≥ 3 months, as defined by structural of functional abnormalities of the kidney, with or without decreased GFR, manifest by either : Pathological abnormalities; or markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests) and eGFR. In our study, we used only eGFR as an indicator of renal dysfunction. The strengths of our study are, we had a strict inclusion criteria, thereby excluded patients an acute phase of lithium treatment

(less than two years of treatment). We included patients only with post-baseline creatinine levels, atleast after 6 months, hence had a minimum renal exposure to lithium.

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In our study, neither the dosage (analysed with serum lithium level), nor the duration of lithium treatment, caused renal dysfunction. Hence, clinicians prescribing lithium need not be anxious on renal parameters, but need to monitor as per guidelines. We would also recommend clinicians to monitor eGFR than creatinine, to identify renal dysfunction early.

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1. Hestbech J, Hansen HE, Amdisen A, Olsen S. Chronic renal lesions following long-term treatment with lithium. *Kidney Int* 1977;12:205–13.□
2. Nolin T, Himmelfarb J. Mechanisms of drug-induce nephrotoxicity Adverse drug reactions. 2010:111–30.□
3. Presne C, Fakhouri F, Noel LH. Lithium-induced nephropathy: rate of progression and prognostic factors. *Kidney Int* 2003;64: 585–92.□
4. Paul R, Minay J, Cardwell C, Fogarty D, Kelly C. Meta-analysis of the effects of lithium usage on serum creatinine levels. *JPsychopharmacol* 2010; 24: 1425–31.□
5. McKnight RF, Adida M, Budge K, Stockton S, Goodwin GM, Geddes JR. Lithium toxicity profile: a systematic review and meta-analysis. *Lancet* 2012; 379: 721–28.□
6. Stefen Clos , Long-term effect of lithium maintenance therapy on estimated glomerular filtration rate in patients with affective disorders: a population-based cohort study. Lancet Psychiatry 2015 : 2:1075-83□
7. Stevens LA , Effects of chronic lithium treatment on renal function : Journal of Affective disorders 2010 : 126, 436-440.□
8. Stevens LA, Zhang Y, Schmid CH: Evaluating the performance of equations for estimating glomerular filtration rate. J Nephrol 2008;21:797–807.□
9. NKF- KDOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification , 2016.□
10. Nolin T,Himmelfarb J. Mechanisms of drug-induced nephrotoxicity. Adverse drug reactions, 2010: 111–30.□
11. Presne C, Fakhouri F, Noel LH. Lithium-induce nephropathy: rate of progression and prognostic factors. *Kidney Int* 2003;64□585–92□
12. Minay J, Paul R, McGarvey D. Lithium usage and renal function testing in a large UK community population;a case-control study. *Gen Hosp Psychiatry* 2013; 35□631–35.□
13. Close H, Reilly J, Mason JM. Renal failure in lithium-treated bipolar disorder: a retrospective cohort study. J.Nephrol. 2014;9: 560-565.□
14. Aprahamian I, Santos FS, dos Santos B. Long-term, low-dose lithium treatment does not impair renal function in the elderly: a 2-year randomized, placebo-controlled trial followed by single-blind extension. *J Clin Psychiatry* 2014;75□72–78.

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Original Research Article

2nd to 4th Digit Ratio (2D:4D) – Is It a Marker for Dyslexia ?

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ABSTRACT

Background: Specific learning disorder in general called as dyslexia is a neuro developmental disorder, which occurs more commonly in males (Male:Female ratio is 4:1), there are evidence to suggest role of prenatal gonadal hormone exposure in neurodevelopment. However, studies on association between 2nd to 4th digit ratio and dyslexia are mixed. Objective: To compare the 2nd and 4th digit ratio (2D:4D) of both hands between children with dyslexia and children without dyslexia.
Methodology: A case control study, recruited 30 children with dyslexia and 30 age and education matched normal controls, after getting informed consent from their parents. Collected socio demographic details, applied CARS, Schonell's reading and spelling test, followed by detailed evaluation by psychiatrist and clinical psychologist, including psychological testing to confirm the diagnosis of dyslexia. The digit length of index and ring finger on both hands of all children were measured using Vernier Calliper by direct method.
Results: In both the hands 2D:4D ratio was more in children with dyslexia than normal children, but the difference was not significant (p – 0.146). Analysis of only male samples showed digit ratio is higher in dyslexic children than normal children; which were significant (p – 0.03).
Conclusion: Our study does not support an association between digit ratios and dyslexia; however, trend towards higher ratio in dyslexia especially in male children are interesting and needs further exploration. Need more studies in this area to form a definite conclusion.

Keywords: Dyslexia, Specific learning disorder, Digit ratio, 2D:4D, Biomarker.

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INTRODUCTION

Dyslexia is a neuro - developmental disorder, Prevalence of dyslexia ranges from 4 % to 17%. The prevalence is higher in males (Male: Female ratio is 4:1),[1]. This suggests there could be some factors that is different in either sex which plays a role in the development of this disorder. The neurobiology of dyslexia remains largely unknown but evidence suggests that neuronal migration and axon guidance pathways may be disrupted in dyslexia [2].

Normal brain is sexually dimorphic, due to the influence of gonadal hormones on neurodevelopment during antenatal period. Aberrations in neurodevelopment due to gonadal hormonal imbalance are one of the mechanisms for developing dyslexia. The relation between imbalance in gonadal hormones and dyslexia is very complex.

The absence of testosterone leads the central nervous system to develop passively, in a primarily female fashion, while the presence of testosterone induces the masculinization of the brain, indicating that the sexual dimorphism of the brain is mediated by the epigenetic action of gonadal steroids [3]. Geschwind and Galaburda suggested that testosterone inhibited the growth of certain areas of the left hemisphere and

facilitated the growth of the same areas in the right hemisphere. As a result, language skills would be negatively affected and hand preference would tend to shift toward the left. In extreme forms, this abnormal cortical development would render an individual incapable of handling effectively the linguistic tasks required for reading and writing [4]. Ramus, based on animal studies demonstrated that rodents with induced ectopias and microgyri exhibit learning deficits. Focal cortical abnormalities in specific areas of left perisylvian cortex are the primary cause of dyslexia. When foetal testosterone exposure is high, cortical disruption propagates to the thalamus, provoking additional sensory impairments as a consequence of the conjunction of high fetal testosterone exposure and ectopias or microgyri [5]. A study showed high prenatal testosterone may impair developing auditory perceptual processes and phonological processing in the left hemisphere. These impairments in phonology in turn can adversely affect development in reading [6].

But it's not possible to measure the prenatal gonadal hormone exposure directly. Hence, we need to evaluate it indirectly using an indicator which could predict gonadal hormones concentration in utero. The digit ratio is the ratio of the lengths of different digits, typically as measured from the basal / proximal crease of the finger to the tip of the finger. Androgen and Estrogen differentially regulate the network of genes that controls condrocyte proliferation, leading to differential growth of digits in males and females [7].

The ratio between the length of the 2nd and 4th digits is: (a) fixed in utero; (b) lower in men than in women; (c) negatively related to testosterone and sperm counts; and (d) positively related to estrogen concentrations [8]. 2D:4D ratio may be used as an indicator and predictive factor in a variety of disorders associated with a disturbed testosterone/estrogen hormone balance like Gender identity disorder, Autism, Dyslexia, Migraine, Stammering, Immune dysfunction, Infertility [8].

The existing literature shows mixed results regarding the relation between digit ratio and dyslexia; there is also ongoing debate whether 2nd digit to 4th digit ratio is a reliable indicator of prenatal gonadal hormones exposure. Few researchers even question the proposed role of gonadal hormones in the development of dyslexia. This is an interesting area and has a wide scope for research. There are many studies on digit ratio in autism, but only very few studies in dyslexia. Hence this study is planned to examine the relationship between 2nd digit to 4th digit ratio and dyslexia. A reliable finding in this area of research would have a very significant clinical implication; this has prompted us to carry out this study.

The objective of the study was to compare the 2^{nd} to 4^{th} digit ratio (2D:4D) of both hands between children with dyslexia and children without dyslexia.

METHODOLOGY

Hypothesis

Children with specific learning disability (Dyslexia) will have smaller 2^{nd} digit to 4^{th} digit ratio compared to children without dyslexia.

Subjects and Procedure

We screened all children aged between 5 and 18 years old, who attended psychiatric outpatient department in PSG institute of medical sciences and research, between May 1st 2014 and July 31st 2014. PSG institute of medical sciences and research is a tertiary care general hospital. A final year MBBS student who got trained in applying Schonell reading test, Schonell spelling test and Childhood Autism Rating Scale administered these tests on all children. All children were evaluated in detail by a psychiatrist; the diagnosis of dyslexia and the type of dyslexia was confirmed only after further psychological testing by the child psychologist. We consecutively recruited 30 children with dyslexia who fulfilled the inclusion and exclusion criteria.

Control group had 30 age and education matched children who visited hospital for non psychiatric problems or were recruited from the community. Control group didn't had any academic difficulties or psychiatric problem according to parents, Schonell reading test, Schonell spelling test and childhood autism rating scale was administered to all children in control group as well, child suspected to have dyslexia or autism were referred to psychiatry for further evaluation.

Children were included in the study after getting informed consent from their parents and only if the child was willing to participate in the study. Children with autism, Asperger's syndrome, mental retardation,

attention deficit hyperactivity disorder, schizophrenia, medical or surgical condition which would interfere with measurement of digit length e.g., contractures, trauma, amputation etc, were excluded from the study. Children who were recruited for the study, the length of their index finger and ring finger (from the basal crease to the tip of the finger) of both the hands is measured using Vernier Calliper by direct method, which is an accurate as well as easy method to measure the digit length [9].
The study was approved by Institutional human ethics committee, PSG institute of medical sciences and research.

Materials

1. **Semi structured proforma:** This Proforma is used to get basic details of the cases and controls. It includes the name, age, sex, education medium, syllabus, socio economic status, height, weight, IQ, co morbidity.
2. **Childhood Autism Rating Scale (CARS):** It is a tool for assessing autism. This is scored based on the interview with the primary care giver and observation of the child. The child is diagnosed as having autism if they score above 30 [10].
3. **Schonell reading test:** A reading test that determines the reading age, however it does not test reading comprehension. The reading age is calculated from the number of words read correctly and compared to the student's chronological age to see if the student is reading at the level for their age, or are reading either below or above level for their age [11].
4. **Schonell spelling test:** A spelling test that determines the spelling age. It comprises of 100 words, and the child is asked to write 30 words (applicable for their class).The spelling age is calculated from the number of words wrote correctly and compared to the student's chronological age to see if the student is writing at the level for their age, or are writing either below or above level for their age [12].

STATISTICAL ANALYSIS

Statistical analysis was done using the Statistical package for social sciences (SPSS) version-18. Continuous variables were assessed using the unpaired t-test and categorical variables were assessed using the chi-square test. P value of less than 0.05 is considered as statistically significant.

RESULTS

Table 1 – Socio-demographic variables

		CASE (N=30)	CONTROL (N=30)	p Value
Age (Mean, SD) in years		11.60 ± 3.328	13.40 ± 2.568	0.092
Education (Mean, SD) in years		6.90 ± 2.998	8.50 ± 2.610	0.470
Sex	**Female**	3	18	0.001
	Male	27	12	
Medium	**English**	28	27	0.640
	Tamil	2	3	
Syllabus	**CBSE**	1	29	0.313
	Samachir	0	30	
SES	**Lower middle**	0	6	0.010
	Upper middle	27	24	
	Upper	3	0	

The mean age of cases and controls were 11.6 years and 13.4 years respectively, the difference in age between the 2 groups was not statistically significant. There is no statistical difference between the 2 groups on other socio demographic profiles like education, medium, syllabus and socioeconomic status. Most of the children

on either group were from upper middle socioeconomic class, studied in English medium and their syllabus was samachir, which is the most popular syllabus in the state of Tamil Nadu, India.
The children with dyslexia were predominantly males (90% of the cases) were as in control group only 40 % were males; this was statistically significant (p = 0.001).

Table 2: 2D:4D ratio between male and female children

Male Vs Female (n-39) (n-21)		t	df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	-0.761	58	0.450	-0.01239	-0.04498	0.02020
	Equal variances not assumed	-0.674	29.596	0.506	-0.01239	-0.04994	0.02517
Lt hand 2D:4D	Equal variances assumed	-0.761	58	0.450	-0.01239	-0.04498	0.02020
	Equal variances not assumed	-0.674	29.596	0.506	-0.01239	-0.04994	0.02517

Taken all 60 children together and divided them into 2 groups based on sex, male children had lower 2nd to 4th digit ratio on either hand when compared to controls, shown in (table 2), but the difference is not significant (p = 0.506).

Table 3: 2D:4D ratio between SLD and control groups

SLD Vs Control (n-30) (n-30)		t	Df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	1.490	58	0.142	0.02283	-0.00784	0.05349
	Equal variances not assumed	1.490	33.222	0.146	0.02283	-0.00833	0.05398
Lt hand 2D:4D	Equal variances assumed	1.490	58	0.142	0.02283	-0.00784	0.05349
	Equal variances not assumed	1.490	33.222	0.146	0.02283	-0.00833	0.05398

The comparison of 2nd to 4th digit ratio using independent t test between children with dyslexia and normal children showed that the mean digit ratio on both hands is more in children with dyslexia than normal children, which is in contrary to our hypothesis, however this was not statistically significant (p = 0.146). Our sample predominantly contained children with writing disorder (23/30 children). So, we decided to compare the 2D: 4D ratios between children with writing disorder only and controls.
As shown above in (table 4) the 2nd digit to 4th digit ratio in both right and left hand was higher in children with writing disorder than control group, but this again was not significant (p = 0.134). The sub-analysis comparing children with writing disorder and controls yielded a result similar to dyslexia group in general. Since the number of children with reading and mathematics disorder were very few, it was not possible to do a separate analysis on them. As dyslexia is more common in males and testosterone is expected to play a major role, a separate analysis was done comparing the digit ratio between male children with and without dyslexia.
The 2nd to 4th digit ratio on either hand was higher in male children with dyslexia compared to male children without dyslexia; the difference was statistically significant with a p value of 0.03. This is shown in (table 5).

Table 4: 2D:4D Ratio between dysgraphia children and controls

Dysgraphia Vs Control (n-23) (n-30)		T	Df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	1.372	51	0.176	0.02404	-0.01115	0.05923
	Equal variances not assumed	1.533	35.936	0.134	0.02404	-0.00776	0.05585
Lt hand 2D:4D	Equal variances assumed	1.372	51	0.176	0.02404	-0.01115	0.05923
	Equal variances not assumed	1.533	35.936	0.134	0.02404	-0.00776	0.05585

Table 5: 2D:4D Ratio in male children

Male children Case Vs Control (n-27) (n-12)		T	Df	Sig. (2-tailed)	Mean Difference	95% CI of Difference	
						Lower	Upper
Rt hand 2D:4D	Equal variances assumed	3.442	37	0.001	0.05263	0.02165	0.08361
	Equal variances not assumed	2.456	12.001	0.030	0.05263	0.00594	0.09932
Lt hand 2D:4D	Equal variances assumed	3.442	37	0.001	0.05263	0.02165	0.08361
	Equal variances not assumed	2.456	12.001	0.030	0.05263	0.00594	0.09932

DISCUSSION

There are very few studies in this area, to best of our knowledge this is the first study in India looking into the relation between 2nd digit to 4th digit ratio and dyslexia. We have taken 30 children with dyslexia (cases) and 30 normal age and education matched controls. There was a significant difference between the two groups with respect to gender distribution, predominance of male children in dyslexia group could be explained by the general prevalence of dyslexia, which is more common in males (Male: Female ratio is 4:1) and ours being a consecutive sampling.

The current study did not show any difference in the 2nd to 4th digit ratio between children with dyslexia and normal children, neither the right hand comparison nor the left hand comparison between groups showed significant difference, which is in contrast to the observation made by Manning et al 2002 who reported 2nd digit to 4th digit ratio is low in dyslexia compared to Non dyslexic individuals. But our finding is in concurrence with the findings of two other studies, which also reported no association between 2D:4D ratio and dyslexia. A study done in Netherland on children with dyslexia with a phonological deficit showed no evidence for an association between reading disabilities and the 2nd digit to 4th digit ratio, also showed no support for the assumed relation between the 2nd digit to 4th digit ratio and prenatal testosterone[10]. Another study showed digit ratios were sexually dimorphic 'male lower than female' pattern, no difference in digit ratio between dyslexic and normal reading children. Digit ratios were not related to any sensory measure.[11]

The present study showed that 2nd to 4th digit was lower in children without dyslexia than in children with dyslexia, which is contrary to our own hypothesis that 2nd to 4th digit ratio will be low in dyslexic children compared to normal children, and contradicts those studies which shows an association between digit ratio and dyslexia. This finding is difficult to explain by any existing literature on dyslexia and digit ratio. However similar finding was reported in a study on 2nd to 4th digit ratio among 107 individual across all departments and all academic grades, who completed an Internet-based questionnaire on autism or dyslexia in self and family members, the study showed an association between autism and lower 2nd digit to 4th digit ratio, whereas interestingly dyslexia was associated with a higher 2nd to 4th digit ratio in that study [12].

Males had a lower digit ratio compared to females irrespective of being dyslexic or not, points in favour of the concept of sexual dimorphism in digit ratio as suggested by a study [13], however could not agree fully because the difference was not significant.

Some of the theories put forward to explain dyslexia and digit ratio are testosterone theory in the context of cerebral lateralization with testosterone suppressing certain areas in left hemisphere, Extreme male brain theory which suggests excess masculinization of brain leading to dyslexia.

In this study there is no difference in the digit ratio between left hand and right hand nor there do any association of digit ratio in either of this hand and dyslexia. Comparison of only male samples between groups also revealed a higher ratio in children with dyslexia than normal children; this was significant ($p = 0.03$). These findings are in contrast to theories explaining dyslexia and digit ratio.

Our finding does not support an association between digit ratio and dyslexia; neither has it supported the cerebral lateralization theory or extreme male brain theory. The reasons could be digit ratio depends on the production of testosterone and sensitivity of digits to foetal testosterone, which can vary across different ethnic groups, this can explain why there is no difference in digit ratio between groups but this can't explain the reason for lower ratio in controls.

May be there is no real association between dyslexia and digit ratio as supported by studies, 2nd to 4th digit length may not be an indicator of prenatal gonadal hormone exposure 14 and most of the studies supporting the association has not directly compared dyslexia with normal children, it was either done on children with family history of dyslexia or comparing individuals in different academic fields [12]. So, findings in these studies could be influenced by some confounding factors.

Strength of the study

The sample in our study is homogenous and it's a direct comparison between children with dyslexia and without dyslexia. We recruited the children consecutively so any bias in sampling is avoided. Exclusion of comorbid condition like autism, Asperger's syndrome etc which could have confounded the findings if present, as studies also show an association between these disorders and digit ratio.15 All children in dyslexia group were evaluated in detail by a psychiatrist and clinical child psychologist. Using direct method to measure digit ratio by Vernier Calliper, which is an accurate as well as easy method to measure digit length.

Limitations of the study

The findings of this study should be interpreted with caution because of small sample size, possibility of type II error could not be ruled out. The investigator was not blind to the diagnosis, however any bias in measurement was unlikely as the results contradicts our own hypothesis. Measurement was done by a single investigator; having 2 or 3 persons to measure the digit could have added more reliability and validity to the findings. Controls were recruited based on the parents report on their children's academic performance, physical and mental status, not evaluated by psychiatrist or clinical psychologist, it's possible that children with subtle psychological disturbance are missed and mistakenly recruited as normal controls. Despite all these limitations, the finding of this study is unique and cannot be dismissed.

CONCLUSION

This study does not support the association of 2nd digit to 4th digit ratio and dyslexia; neither do the theories put forward to support such an association. However, this study has certain limitations, we need more studies in this area with a larger sample size to overcome above limitations and form a definite conclusion.

The finding children with dyslexia having a higher digit ratio than controls especially in male samples are interesting and needs further exploration.

REFERENCES

1. Ruiz P. Comprehensive textbook of psychiatry. Sadock BJ, Sadock VA, editors. Philadelphia: Lippincott Williams & Wilkins; 2000.
2. Galaburda AM. Ordinary and extraordinary brain development: Anatomical variation in developmental dyslexia. Ann Dyslexia 1989;39(1):65-80.
3. Arnold AP, Gorski RA. Gonadal steroid induction of structural sex differences in the central nervous system. Ann Rev Neurosci 1984;7(1):413-42.
4. Geschwind N, Galaburda AM. Cerebral lateralization: Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. Arch Neurol 1985;42(5):428-59.
5. Ramus F. Neurobiology of dyslexia: A reinterpretation of the data. Trends Neurosci 2004;27(12):720-6.
6. Beech JR, Beauvois MW. Early experience of sex hormones as a predictor of reading, phonology, and auditory perception. Brain Lang 2006;96(1):49-58.
7. Manning JT, Scutt D, Wilson J, Lewis-Jones DI. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Human Reprod 1998;13(11):3000-4.
8. Manning JT, Bundred PE. The ratio of 2nd to 4th digit length: a new predictor of disease predisposition?. Med Hypoth 2000;54(5):855-7.
9. Kemper CJ, Schwerdtfeger A. Comparing indirect methods of digit ratio (2D: 4D) measurement. Am J Hum Biol 2009;21(2):188-91.
10. van Gelder M, Tijms J, Hoeks J, Manning JT. 'Second to fourth digit ratio and dyslexia: no evidence for an association between reading disabilities and the 2D: 4D ratio'/'Manning replies'. Dev Med Child Neurol 2005;47(10):718.
11. Boets B, De Smedt B, Wouters J, Lemay K, Ghesquière P. No relation between 2D: 4D fetal testosterone marker and dyslexia. Neuroreport 2007;18(14):1487-91.
12. Brosnan MJ. Digit ratio and faculty membership: Implications for the relationship between prenatal testosterone and academia. Br J Psychol 2006;97(4):455-66.
13. Manning JT, Barley L, Walton J, Lewis-Jones DI, Trivers RL, Singh D, Thornhill R, Rohde P, Bereczkei T, Henzi P, Soler M. The 2nd: 4th digit ratio, sexual dimorphism, population differences, and reproductive success: evidence for sexually antagonistic genes?. Evol Hum Behav 2000;21(3):163-83.
14. Kratochvíl L, Flegr J. Differences in the 2nd to 4th digit length ratio in humans reflect shifts along the common allometric line. Biol Letters 2009;5(5):643-6.
15. Manning JT, Baron-Cohen S, Wheelwright S, Sanders G. The 2nd to 4th digit ratio and autism. Dev Med Child Neurol 2001;43(3):160-4.

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Whites Science
A Scientific Erudition

Pre experimental study to assess the effectiveness of structured teaching program on knowledge regarding management of violent behaviour among primary care givers of mentally ill in tertiary care hospital in Coimbatore India

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ABSTRACT

Introduction: In India, the family is the main support system for people with mental illnesses. It has been observed that >90% of patients with chronic mental illness live with their families and continue to take care of basic necessities [1-3]. Most often, the patients who have mental illness are brought for psychiatric help by the family. The family members take care of their medications, earn a living for them, stay with them, and most importantly, offer them emotional and psychological support. This also has a significant role in the rehabilitation of the patient and in bringing him or her to the mainstream. This family support system, which is unique to India, is supposed to be one of the good prognostic indicators for mental illness [4]. Family means to feel secure and to be loved and cared for. Conventionally, joint family setups in India have taken care of the older adults and sick people with utmost attention. Thus, the family has always taken the major share in responsibilities of mental health care compared to the government.

Aim: The aim of this study is to evaluate the effectiveness of structured teaching program on management of violent behaviour among primary care givers of mentally ill.

Methodology: The pre experimental study was undertaken on management of violent behaviour among primary caregivers of mentally ill clients. Convenient sampling technique was used. The data was collected through structured questionnaire. Descriptive and inferential statistics were used to analyze the data.

Results: Majority of the 17 primary care givers (85%) had adequate knowledge and 3 primary care givers (15%) had moderately adequate knowledge regarding the management of mentally ill.

Conclusion: The results showed high significant level with mean difference score of 23.55 and the 't' value was 2.81 ($p<0.05$). Hence, null hypothesis is rejected and H_1 research hypothesis is accepted. The study found that structured teaching program helped the primary care givers in caring with the mental ill.

Keywords: Effectiveness, Violent Behaviour, Mentally ill, Primary Care Givers, Structured Teaching Program

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INTRODUCTION

The family in India is the primary support mechanism for mentally ill individuals. It was noted that > 90% of chronic mental ill patients live with their families and continue to take care of fundamental necessities. A global program of deinstitutionalization and gradual transfer of inpatient care to community settings has meant that caregivers are taking on increasing levels of care giving duties and are

widely recognized for their input to patient care and recovery [4]. The intrinsic factors are a combination of personality characteristics, current intense mental distress and problems in dealing with anger. The extrinsic factors are more varied including the physical and social settings where violence and aggressive characteristics of the victims the experience and training of health and social professionals and the perceived risk of danger to others. Understanding how such variable contextual factors interacts with historical behaviour in the aetiology of violence and aggression is important in informing evidence based approaches to the prevention of violence and aggression that would otherwise emerge and aggression that has already occurred or is still in progress [5]. This approach is anomalous because the impact of violence and aggression in mental health, health and community setting is significant and diverse, adversely affecting the health and safety of service users, care givers and staff [6]. Critically, the management of violence and aggression may itself be hazardous to those exhibiting violent or aggressive behaviour and accentuate risks to their health and safety [7].

Statement of the Problem

Pre experimental study to assess the effectiveness of structured teaching program on knowledge regarding management of violent behaviour among primary care givers of mentally ill in tertiary care hospital in Coimbatore India.

OBJECTIVES

1. To assess the management of violent behaviour among primary caregivers of mentally ill.
2. To evaluate the effectiveness of structured teaching program on management of violent behaviour among primary caregivers of mentally ill.
3. To associate the management of violent behaviour among primary caregivers of mentally ill with selected demographic variables.

Assumptions

1. Primary caregivers may not have adequate knowledge on management of violent behaviour of mentally ill.
2. Administration of structured teaching program may enhance management of violent behaviour among primary caregivers of mentally ill.

Research Hypotheses

H_1: There will be a significant difference in the pre and post test of management of violent behaviour among primary caregivers of mentally ill**.**

H_2: There will be a significant association of the post test of management of violent behaviour with selected demographic variables among primary caregivers of mentally ill.

Research Variables

Independent Variable: Structured teaching program on management of violent behaviour.
Dependant Variable: Knowledge on management of violent behaviour among primary caregivers of mentally ill.

Research approach and design: Pre experimental research design.

Setting of the study

The study was conducted in PSG Hospital. PSG Hospital is a tertiary care hospital which has bed strength of 1400 and 19 specialty OPDs. This study was conducted among am 20 participants who were admitted in psychiatric ward at selected PSG Hospital at Coimbatore.

Population and Sampling

The population of the study consists of primary caregivers of patients on mentally ill. The sample consists of primary caregivers of patient who stay with the patient during hospitalization. The total number of patients with mentally ill from January 2018 to December 2018 was used as the baseline data to determine the sample size**.** Total of 1274 patients were with the diagnosis of affective disorders.

Sample size: Sample size has been calculated by using allowable error method and 20 samples of patient's primary caregiver were selected.

$$n = \frac{4pq}{L^2}$$

P =mean/total population in a year×100
Mean = 1274/12= 106
P = (106/1274×100) =8.32
q =100-p
q= 100-8.32=91.66
L =allowable error=13

$$n = \frac{4x8.32x91.66}{13^2}$$

=18.05
Estimated sample size is 20

Sampling technique: Convenient sampling technique was used in this study. Sample size of the study was 20 primary caregivers of mentally ill [8,9].

Sample Selection Criteria

Inclusive Criteria:

- Primary care givers who could read and understand English/Tamil.
- Primary care givers who were willing to participate.

Exclusive Criteria:

- Primary caregivers such as health professionals and home nurses.
- Primary care givers who are below the age of 15 years.

Technique of data collection if interval, give detailed description of intervention performed

The tool constructed in this study consists of 3 parts.

Section A: Demographic profile of the primary caregivers:
Includes age, relationship with the patient, educational status, occupational status, family income and previous information regarding violent behaviour.

Section B: Demographic profile of mentally ill:
Age, diagnosis, duration of illness, duration of violent behaviour and previous information regarding violent behaviour.

Section C: The Management of Aggression Violence Attitude Scale (MAVAS) was modified as knowledge questionnaire with the expert content validity. It is self administered and consists of 27 questions. Thirteen were related to the causes of aggression, violence and reflected the internal, external, and situational/interactional models of violent behaviour. And 14 items represented different approaches to the management of aggression.

Reliability of Scale for the Management of Aggressive and Violent Behaviour (C_MAVAS): Psychometric properties testing in mental health nurses were assessed in China among mental health nurses. A convenient sample of 262 qualified mental health nurses working in a local psychiatric hospital was recruited. The result showed that the content validity of the C_MAVAS was very satisfactory with validity indices of 97.4% for the overall scale and 90% to 100% for individual items. Exploratory factor analysis yielded a four-factor solution: 'interactional perspectives on patient violence', 'best ways perceived for violence management', 'internal or biomedical perspectives on patient violence', and 'external perspectives on patient aggression and violence', were important in shaping their attitudes towards managing violence and patient disruptiveness. Internal consistency of the Chinese version was barely satisfactory (Cronbach's alpha=0.51-0.67) for the four factors/subscales and its test-retest reliability was good (Pearson's coefficient=0.84). The findings suggests the C_MAVAS is a valid and reliable tool to measure mental health nurses' attitudes towards patient violence/aggression in a mental hospital setting [8,9].

Scoring	
Level of Knowledge	**Percentage**
Inadequate knowledge	< 50%
Moderately adequate knowledge	51-75%
Adequate knowledge	>75%

INTERVENTION

Structured teaching program on management of violent behaviour in the aspect of definition, causes, symptoms and management of violent behaviour. It includes communication strategies, environmental strategies, behavioural strategies, therapeutic strategies, and ways to protect you and stress management.

Ethical issues: The project was approved by the Intuitional human ethics committee (IHEC), PSG Institute of Medical Science and Research. Detailed information on the research project was communicated to caregivers, including procedures for protecting interviewees' and patients' privacy; arrangements for secure access to and storage of data; confidentiality arrangements for the one-on-one interviews in a private room; and the non-recording of patients' names.

Plans for data analysis: The data analysis plan includes descriptive and inferential statistics.

Descriptive Statistics

- Percentage and frequency to analyze demographic variables.
- Mean and standard deviation to analyze the pre and post test scores of management of violent behaviour among primary caregivers.

Inferential Statistics

Paired "t" test was used to compare the pre and post test scores of management of violent behaviour among primary caregivers of mentally ill.

- Chi-Square test was used to find out the association between demographic data and management of violent behaviour among primary caregivers.

Table 1: Assessment of post test level of management of violent behaviour among primary care givers (n=20).

S. No	Knowledge level	Category	Frequency	Percentage
1.	Inadequate knowledge	< 50%	3	15
2.	Moderately adequate knowledge	51-75	16	80
3.	Adequate knowledge	>75%	1	5

Table 2: Frequency and percentage distribution of post test of violent behaviour among primary care givers (n=20).

S. No	Knowledge level	Category	Frequency	Percentage
1.	Inadequate knowledge	<50%	-	-
2.	Moderately adequate knowledge	51-75%	3	15
3.	Adequate knowledge	>75%	17	85

Table 3: Comparison of pre test and post test violent behaviour among primary care givers (n=20).

S. No	Characteristic	Pre test		Post test		Mean Difference	t-value
		Mean	S.D	Mean	S.D		
1.	Primary care givers knowledge assessment	57.25	7.785	80.8	19.3	23.55	t=2.81 S*

DISCUSSION

In the present study the demographic variables of patient shows the 7 samples (35%) were at the age group 21-30 years, 12 samples (60%) were male, and 18 samples (90%) were Hindu, 14 samples (70%) were 1-3 years of duration of illness, 15 samples (75%) were married patients.12 samples (60%) were nuclear family, 14 samples (70%) were urban people.

A similar study was conducted in violence behaviour in adolescents and the results shows that the mean frequency 60.2% and fighting injury and (non injured: 84.5%) and the bullying frequency varied widely cross sectional from 57.0% for fighting was most highly associated with smoking, drinking, feeling irritable or bad tempered and having been bullied [10].

In the present study the effectiveness of structured teaching program on violent behaviour among primary care givers were showed the mean value of pre test was 57.25 and was increased in post test to 80.8. The difference between the pre test and post test mean was 23.6. The 't' value was 3.84, which had

high statistical significance at p<0.05 level and which confirms that there was a statistically significant difference between pre test and post test knowledge of primary care givers in the management of mentally ill patients.

The present study results were consistent with the findings of the following studies carried out on caregiver reports of patient-initiated violence in psychosis. Patient violence was unrelated to carergiver burden or carergiver distress ($P > 0.05$). Self-esteem was significantly lower in carergivers reporting patient violence ($t = 2.199$, $df = 60$, $P = 0.03$, mean difference = −3.10 [95% CI −5.92 to −0.281]), while levels of functional coping through emotional support were significantly higher ($t = 2.902$, $df = 61$, $P = 0.005$, mean difference = −0.56 [95% CI = −0.956 to −0.176]) [11].

In the present study the association between selected demographic variables and effectiveness of structured teaching program on violent behaviour among primary care givers showed that the there is significant association with the effectiveness of structured teaching program and religion $\chi^2 = 3.95$, marital status $\chi^2 = 3.95$ and the occupational status $\chi^2 = 3.95$. It was inferred that religion, marital status and occupational status had association with the effectiveness of structured teaching program on violent behaviour among primary care givers mentally ill.

A similar study was conducted on caregiver reports of patient-initiated violence in psychosis. The association of the study showed that regarding gender ($\chi^2 = 8.23$, $df = 1$, $P = 0.004$), with a history of voluntary admissions ($\chi^2 = 17.2$, $df = 1$, $P < 0.01$). Fewer had a history of violence ($\chi^2 = 11.3$, $df = 1$, $P = 0.001$) or sexual offences ($\chi2 = 7.43$, $df = 1$, $P = 0.006$) [12-14].

CONCLUSION

The study was conducted to assess the effectiveness of structured teaching program on management of violent behaviour among primary care givers of individual with mental health problem. A questionnaire based on violent behaviour among primary care givers. In the overall majority of pre test of 16 primary care givers (80%) had moderately adequate knowledge, 3 primary care givers (15%) had inadequate knowledge and 1 primary care giver (5%) had adequate knowledge. In the with respect to majority of post test of 3 primary care givers (15%) had moderately adequate knowledge and 17 primary care givers (85%) had adequate knowledge. The mean difference score was 23.55 and the ‘t’ value was 2.81 which had high statistical significance at p<0.05 level. Hence the null hypothesis is rejected and H_1 the research hypothesis is accepted. The study found that education had helped to develop knowledge and increase ability of the primary care givers in caring for patient who are on mentally ill.

REFERENCES

1. Trivedi JK. Mental health Act: salient features, objectives, critique and future directions. Indian J Psychiatry 2009:51:11-9.
2. Thara, R., Henrietta, M., Joseph, A., Rajkumar, S., & Eaton, W. W. (1994). Ten-year course of schizophrenia?the Madras longitudinal study. Acta Psychiatrica Scandinavica, 90(5), 329–336. doi:10.1111/j.1600-0447.1994.tb01602.x
3. Chadda R. K. (2014). Caring for the family caregivers of persons with mental illness. Indian journal of psychiatry, 56(3), 221–227. doi:10.4103/0019-5545.140616
4. Wig NN. Keynote address. In: Kulhara P, Avasthi A, Verma S, editors. Schizophrenia-Indian Scene. Mumbai, Searle (India); 1997.
5. National Institute of Clinical Excellence (NICE) Schizophrenia guideline (update) [Internet] London (GB): NICE; 2009. [Cited 2013 Oct 1]. Available

6. Llor-Esteban, B., Sánchez-Muñoz, M., Ruiz-Hernández, J. A., & Jiménez-Barbero, J. A. (2017). User violence towards nursing professionals in mental health services and emergency units. The European Journal of Psychology Applied to Legal Context, 9(1), 33–40. doi:10.1016/j.ejpal.2016.06.002
7. Violence and Aggression Short-term management in mental health, health and community settings Updated edition.2015. NICE Guideline NG10. The British Psychological Society and The Royal College of Psychiatrists.
8. Cheung, T., Mui, J., Ho, Y., & Chien, W. (2018). A Scale for the Management of Aggressive and Violent Behaviour (C_MAVAS): Psychometric Properties Testing in Mental Health Nurses. International Journal of Environmental Research and Public Health, 15(7), 1496. doi:10.3390/ijerph15071496
9. Yiannakoulias, N., Sanchez-Ramirez, D., Svenson, L. W., & Voaklander, D. C. (2016). A cohort study of regional migration and the risks of attempted suicide and violent assault injury. Injury Prevention, 22(6), 407–411. doi:10.1136/injuryprev-2015-041932
10. Jain, S., & Cohen, A. K. (2013). Fostering Resilience Among Urban Youth Exposed to Violence. Health Education & Behavior, 40(6), 651–662. doi:10.1177/1090198113492761
11. Polit, D.F. Cheryl Tatano Beck. 2003. Nursing Research: Principles and Methods (Nursing Research: Principles & Practice). LWW; Seventh edition.
12. Smith-Khuri, E., Iachan, R., Scheidt, P. C., Overpeck, M. D., Gabhainn, S. N., Pickett, W., & Harel, Y. (2004). A Cross-national Study of Violence-Related Behaviors in Adolescents. Archives of Pediatrics & Adolescent Medicine, 158(6), 539. doi:10.1001/archpedi.158.6.539
13. Jay P Signh, Helen Doll, Martin. 2012. "The prediction of violence and antisocial behaviour; A systemic review and meta- analysis of the utility of risk assessment in individual". Journal of medical 345, e 4692.
14. Grassi, L., Peron, L., Marangoni, C., Zanchi, P., & Vanni, A. (2008). Characteristics of violent behaviour in acute psychiatric in-patients: a 5-year Italian study. Acta Psychiatrica Scandinavica, 104(4), 273–279. doi:10.1111/j.1600-0447.2001.00292.x

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Research Paper

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Dr. Syed Ummer. I[1]*, Dr. Sushith Sugathan. C[2]

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Background: Suicide has a strong association with mental disorder and contributes to the excess mortality of the mentally ill. Suicidal ideation is prevalent and appears to be a precondition for suicide attempts among psychiatric patients with Major Depressive disorder. Though ideas and attempts may overlap there are studies that show the two are separate clinical entities with unique psycho-socio demographic profile. ***Aim:*** To study the correlation of psychotic symptoms with suicidal ideation and with suicidal attempt. ***Methodology:*** Cross sectional study of patients consecutively admitted with major depressive disorder. Severity of suicidal ideations and attempts were rated on validated scales and the sociodemographic and clinical correlates were analyzed. ***Results:*** There were more suicide attempts in patients without psychotic symptoms than those with psychotic symptoms with various levels of severity. ***Conclusion:*** There was no correlation between severity of psychotic symptoms and suicidal attempt in the sample.

Keywords: *Psychotic symptoms, Severe depression, Suicide*

□sychological autopsy studies show that considerable number of patients with mental illness committed suicide. These studies show that 90% of people who committed suicide fulfilled criteria for mental illness. Therefore it is important to identify people at risk for suicide, especially those with mental illness(1)(2)(3).

Patients with affective disorders and alcoholism carry the highest risk of suicide. Unipolar depression can present as either with or without psychotic symptoms. Severe depression may or may not be associated with psychotic symptoms, whereas the mild and moderate forms of depression is never associated with psychotic symptoms(4). Studies predict a prevalence of 0.4% for psychotic symptoms in patients with major depressive disorder.

There have been studies looking at differences between psychotic and non psychotic depression, even beyond the presence of psychotic symptoms(5)(6)(7)(8) (9)(10). That is they have

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studied differences in heritability, environmental and genetic risk factors, treatment response and mortality.Some studies also say that risk of suicide is higher in patients with psychotic depression than those in non-psychotic depression but there are other studies that refute these findings too(11)(12).

Rationale for study

In a register based prospective cohort study on 34,671 patients with severe depression in Danish psychiatry hospitals out of which 12,101were psychotic depression patients, 755 completed suicide during follow up. But psychotic depression was not found to be an independent risk factor for suicide in severe depression (AOR=0.97 [0.83-1.15]) though other risk factors like older age, male gender, and a previous incident of self-harm were significant risk factors for both groups (psychotic and non-psychotic depression)(1).

But in yet another study which was a prospective case study of records of 61psychotic depression and 59 non psychotic depression cases analysed for mortality outcomes at 15 years after initial assessment, they found that patients with psychotic depression had a two-fold higher risk of mortality than those with severe depression without psychotic symptoms. Butthere was no statistically significant difference in the number of patients who had died by suicide between the two groups(13).

On reviewing literature on Indian studies on the topic, we were not able to come across similar studies looking into risk of suicide in patients with psychotic depression, as compared to non-psychotic depression. Thus we wanted to carry out this study in an Indian population in south India to see if we are able to replicate the findings in the western studies and we wanted to analyse further the correlation between psychotic symptoms and various aspects of suicide such as suicidal idea or attempt and method of suicide.

Aim

Primary aim was to study the correlation of psychotic symptoms with suicidal ideation and with suicidal attempt. Secondary objectives were to study the correlation between the severity of psychotic symptoms and method of suicide and to study the correlation between the content of psychotic symptoms and method of suicide.

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This study employed a cross sectional consecutive sampling methodology. Study was conducted in the psychiatry in-patient ward at a private medical college hospital in Coimbatore, Tamil Nadu. Study duration was 1 year (from January 2016 to December 2016) and all the consecutive patients, between the ages of 18 and 65 years, admitted in the psychiatry ward with the diagnosis of major depressive disorder were screened for study inclusion after getting informed consent.

Inclusion criteria consisted of patients whose age was between 18 and 65 yrs and with a diagnosis of major depressive disorder diagnosed by their respective consulting psychiatrist in the department who was blind to the study protocol. The study researcher administered the SCID for DSM IV interview schedule and if the diagnosis was confirmed for Major depressive disorder then the patient was included in the study.

Exclusion criteria consist of patients who refused to give consent and patients who had co morbid mental retardation or neuro-psychiatric co morbidity like dementia. Patients with

Bipolar depression were excluded from the study as we wanted to study specifically unipolar depressive disorder group of patients. Patients with co morbid physical illness, substance abuse and other psychiatric co morbidities were also excluded from the study. Total sample size for the study was 53.

Instruments:

Once the patient was recruited into the study, the researcher had collected details about the socio demographic and clinical variables by using a semi structured profoma and then administered the Hamilton depression rating scale(HAMD-17) to assess the severity of depression. The researcher then administered the following scales for assessment of severity of suicidal behaviour:

- a scale that was developed in order to assess the severity of suicide attempts. The scale consists of 15 questions which are scaled from 0-2, which take into account both the logistics of suicide attempt as well as the intent. The scale has high reliability and validity[16].
- this scale was developed for assessing severity of suicide ideas. Principal component analysis revealed a bi dimensional structure, with factors corresponding to (1) Desire and Ideation and (2) Plans and Preparations. Each factor displayed acceptable internal consistency and expected patterns of convergent validity via associations with hopelessness, depressive symptoms, impulsivity, and a self-report measure of suicidal behaviors[19].

Statistical analysis

We performed statistical analysis with PASW (SPSS) version 19.0 for Windows. The correlation of socio-demographic variables with suicide ideation and depressive patients who attempted suicide, patient history with suicidal attempts, severity of depression with suicidal ideation and suicide attempt were determined using The correlation of suicidal ideation and suicide attempt were determined using P-value less than 0.05 was considered as statistically significant.

1

Majority of the patients belonged to early adulthood age group of 18 to 40 years (47.2%). Both genders were equally represented among the sample. Majority of the patients belonged to middle and low socio economic status (90.6%), were married (75.5%), residing in rural community (69.8%) and were graduates (35.8%) and were living in a nuclear family (71.7%). Majority of the patients did not have family history of suicide (84.9%).

Three-fourth of the patients did not have any past suicide attempts. One patient had attempted suicide three times (1.9%). 12 patients had attempted once (22.6%). 80% of the patients had very severe depressive episode (42/53) as per HAM-D rating scale.

Among 53 recruited depression patients, 35 patients (66%) had suicidal ideas and 13 patients attempted suicide (24.5%). Among the patients who had suicidal ideas (35/53), majority of the patients (41.5%) had severe suicidal ideas as per MSIS scale (total score >20) - table 4.

Poisoning was the commonest method of suicide (11.3%) followed by hanging (5.7%). Electric shock, burns, wrist slash were other methods of suicidal attempts.(Table 1).

Depressive patients who had attempted suicide before had severe suicidal ideas than patients without previous suicidal attempt which was statistically significant (table 2; p-value- **0.01**) Patients who had past history of suicide attempt had higher intent for current suicide attempt which was statistically highly significant (table 3; p-value-**0.000**)

Table 1: Method of suicide attempt

M	POISON	6	11.3
	ELECTRIC SHOCK	1	1.9
M	HANGING	3	5.7
	BURN	1	1.9
	WRIST CUT	1	1.9
	MULTIPLE	1	1.9

Table 2: Correlation of patient history with suicidal ideation

			M		
	NO	18 (45.0%)	12 (30.0%)	10 (25.0%)	**0.01**
	ONCE	0 (0.0%)	1 (8.3%)	11 (91.7%)	
M	MORE THAN ONCE	0 (0.0%)	0 (0.0%)	1 (100.0%)	
M	NO	15 (33.3%)	12 (26.7%)	18 (40.0%)	0.686
	YES	3 (37.5%)	1 (12.5%)	4 (50.0%)	

Table 3: Correlation of patient history with suicidal attempts

				M		
				M	**M**	
	NO	40 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**0.000**
	ONCE	0 (0.0%)	2 (16.7%)	4 (33.3%)	6 (50.0%)	
M	MORE THAN ONCE	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	
M	NO	34 (75.6%)	2 (4.4%)	4 (8.9%)	5 (11.1%)	0.560
	YES	6 (75.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)	

2 **rr** **r** **d** **r** **d** **d** **d** **d**

The depressive patients in whom the severity of depression was very severe, had higher suicidal ideas, which was statistically significant (table 4; p-value-**0.020**). The severity of depressive symptoms did not correlate with severity of suicide attempt (table 4; p-value-0.608).

Table 4: Correlation of severity of depression with suicidal ideation and attempt

			M		
	M	4 (100.0%)	0 (0.0%)	0 (0.0%)	**0.020**
		3 (42.9%)	3 (42.9%)	1 (14.3%)	
		11 (26.2%)	10 (23.8%)	21 (23.8%)	

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There were more suicide attempts in patients without psychotic symptoms than those with psychotic symptoms with various levels of severity (Table 5). There was no correlation between severity of psychotic symptoms and suicidal attempt in the sample. Considering the content of psychotic symptoms, we found that one patient who had auditory hallucination, had multiple suicide attempts. Poisoning was the common method of suicide, both in patients with delusions and hallucination (Table 6).

Table 5: Correlation of psychotic symptoms with method of suicide

		18 (69.2%)	3 (11.5%)	1 (3.8%)	2 (7.7%)	1 (3.8%)	1 (3.8%)	0 (0.0%)
		7 (87.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0	0	0 (0.0%)
		2 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0	0	0 (0.0%)
		11 (91.7%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0	0	0 (0.0%)
		2 (40.0%)	1 (20%)	0 (0.0%)	1 (20.0%)	0	0	1 (20%)

Table 6: Correlation of psychotic symptoms with method of suicide

		18 (69.2%)	8 (61.5%)	3 (11.5%)	1 (3.8%)	2 (7.7%)	1 (3.8%)	1 (3.8%)	0 (0.0%)
		7 (87.5%)	1 (7.7%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		11 (91.7%)	1 (7.7%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		2 (40.0%)	3 (23.0%)	1 (20.0%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	1 (20.0%)

From our study we found that majority of the depressive patients had suicidal ideas (66.0%) and one-fourth of the depressive patients had attempted suicide. The above results emphasises the need to address and how common the prevalence of suicide idea and suicide attempts among depressive patients.

In our study, the suicidal idea was more common in early adulthood age group, which was not found in previous studies(14). In our study, the over-represented groups in the sample with attempted suicide were men of the older age groups, especially men between 45 and 64, and women over 65, who also had more severe suicide attempt in the sample. The severity of suicide ideas was high among low socio-economic status and married group. This is in contrary to previous studies in which suicidal ideas were common among the un-married patients (15). Further in our sample we found that unemployed and rural inhabitants had more severe suicide ideas. In previous studies, unemployment remained a risk factor, but urban background was also a risk factor. Living in nuclear family was a high risk factor in our sample which is in accordance with previous studies. Also a history of past suicide attempt, remains a risk factor for more severe suicide ideas for current episode.

Presence of psychotic symptoms in depressive patients did not correlate with suicide idea or attempt. All the patients with suicidal attempt in the sample were patients who had very severe depression on HAMD rating scale. Suicidal attempt has been found to be positively correlated with severity of depression in other Indian studies also(16). Also the severity of suicidal ideation was positively correlated with the severity of depression on HAMD rating scale. ($p = 0.020$).

The type of positive symptoms (delusions and hallucinations) did not correlate with method of suicide. Poisoning was the commonest method of suicide in both men and women. Men had attempted more lethal methods for committing suicide such as hanging or electric shock. Almost all women show poisoning as method who attempted suicide. Benzodiazepine poisoning was the most common method used for poisoning by women.

We used validated scales and we stuck to a homogenous sample analyzing subjects with diagnosis of major depressive disorder alone and excluded subjects with substance abuse and other psychiatric co morbidities that could influence suicidal behavior such as personality disorders or adjustment disorder etc. In our sample the patients with severe depression was represented adequately and there were no dropouts, thus were able to assess suicidal behavior true to our study objective.

Some of the limitations of our study are that the researcher may have been biased when assessing the suicidal behavior as the severity rating was also done by the same interviewer. Since it was a cross sectional study we have not analyzed the course of the suicidal behavior in these group of patients and their response to treatment. We have not studied the correlation of the presence of any recent life events with severity of suicidal behavior. Further studies are needed to compare whether the correlations with severity of suicidal behaviour differ in other psychiatric illnesses like schizophrenia, bipolar disorder, personality disorder etc.

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There was no correlation between severity of psychotic symptoms and suicidal attempt in the sample. The sample was too small to analyse for the correlation of content of psychotic symptoms with suicidal attempt.□

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1. Anne Katrine K; AnthonyJ; Jimmi Nielsen et al; Risk factors for suicide among 34,671 patients with psychotic and non-psychotic severe depression; Journal ofAffectiveDisorders156(2014)119–125.

2. Cavanagh J.T, Carson A.J, Sharpe M, Lawrie S.M; Psychological autopsy studies of suicide: a systematic review; Psychol.Med; 2003; 33, 395–405
3. Erlangsen A, Nordentoft M, Conwell Y et al; Key considerations for preventing suicide in older adults: consensus opinions of an expert panel; Crisis; 2011: 32,106–109
4. Ohayon, Maurice M et al; Prevalence of depressive episodes with psychotic features in the general population; The American Journal of Psychiatry; Washington 159.11 (Nov 2002): 1855-6.
5. Domschke, K; Clinical and molecular genetics of psychotic depression; Schizophrenia Bulletin; ,2013; 39,766–775.
6. Ostergaard, S.D, Waltoft,B.L., Mortensen, P.B., Mors, O; Environmental and familial risk factors for psychotic and non-psychotic severe depression; J.Affect. Disord; .,2013; 147,232–240.
7. Leadholm,A.K.,Rothschild,A.J.,Nolen,W.A.,Bech,P.,Munk-Jorgensen,P.,Oster-gaard, S.D; The treatment of psychotic depression: is there consensus among guidelines and psychiatrists? J.Affect.Disord; ,2013; 145,214–220.
8. Loo, C.K., Mahon, M., Katalinic, N., Lyndon, B., Hadzi-Pavlovic, D; Predictors of response to ultra brief right unilateral electroconvulsive therapy; J.Affect. Disord; 2011; 130,192–197.
9. Coryell,W.,Leon,A.,Winokur,G.,Endicott,J.,Keller,M.,Akiskal,H.,Solomon,D;.Import ance of psychotic features to long-term course in major depressive disorder; Am.J.Psychiatry; 1996; 153,483–489.
10. Vythilingam, M., Chen,J., Bremner, J.D., Mazure, C.M., Maciejewski, P.K., Nelson,J.C., 2003.Psychoticdepressionandmortality.Am.J.Psychiatry160,574–576.
11. Park M.H;Kim T.S; Yim, et al; Clinical characteristics of depressed patients with a history of suicide attempts: results from the CRESCEND study in South Korea; Journal of Nerv.Mental Disorder; 2010; 198,748–754.
12. Rothschild, A.J; Clinical Manual for Diagnosis and Treatment of Psychotic Depression; American Psychiatric Publishing, Inc., Washington DC, USA.
13. Meena Vythilingam, Joyce Chen B.S et al; Psychotic depression and mortality; American Journal of Psychiatry; 160:574-576
14. B. M. Barraclough, J. Bunch, B. Nelson and P. Sainsbury; A Hundred Cases of Suicide: Clinical Aspects; October 1974;Volume 125, Issue 587, pp. 355-373.
15. Simon, Robert; Suicide risk assessment: is clinical experience enough? Journal of the American Academy of Psychiatry and the law; 2006; 34 (3): 276-8.
16. Srivastava S, Kulshreshtha N; Expression of suicidal intent in depressives; Indian J Psychiatry 2000;42:184

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Conflict of Interest

The authors colorfully declare this paper to bear not conflict of interests □

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Study on Correlation between Psychotic Symptoms and Suicide in a Cohort of Patients with Severe Depression

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Background: Suicide has a strong association with mental disorder and contributes to the excess mortality of the mentally ill. Suicidal ideation is prevalent and appears to be a precondition for suicide attempts among psychiatric patients with Major Depressive disorder. Though ideas and attempts may overlap there are studies that show the two are separate clinical entities with unique psycho-socio demographic profile. ***Aim:*** To study correlates in patients with suicidal ideation and those with suicide attempts in a cohort of major depressive disorder patients. ***Methodology:*** Study was conducted at the Inpatient psychiatry unit at a private medical college in Coimbatore, Tamil Nadu. It was a Cross sectional study design. Patients consecutively admitted with major depressive disorder (DSM IV TR) were recruited. Severity of suicidal ideations and attempts were rated on validated scales and the socio demographic and clinical correlates were analyzed. ***Results:*** Clinical correlates like severity of depression, severity of suicidal ideation and history of past suicidal attempt were positively correlated with severity of suicidal ideation as well as severity of current suicidal attempts in the cohort of depressive disorder patients. ***Conclusion:*** Assessment of current episode severity of both suicidal ideation as well as suicidal attempt is important in identifying high risk patients with major depressive disorder.

Keywords: *Clinical, Socio-Demographic, Correlates, Depressive Patients, Suicidal Ideas, Suicidal Attempts*

□ccording to World health organization (WHO), over 800,000 people die due to suicide every year. Also for every suicide there are many more people who attempt suicide every year. WHO defines suicidal act as the injury with varying degree of lethal intent and suicide as, such acts with fatal outcome? Suicidal acts with no fatal outcome are defined as suicide attempts, attempted suicide, Para suicide, or act of deliberate self-harm.

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A prior suicide attempt is the single most important risk factor for suicide in the general population. In 2004, suicide was the 16th leading cause of death worldwide. Also suicide is the second leading cause of death among 15–29 year olds and this is an alarming finding. It is not only in the developed countries that suicide has been studied, but 75% of global suicides occur in low and the middle income countries.

There are several studies that have been done on incidence of suicide in India. Over the years studies have reported incidence rates that range from 2.36 to 42 per 100,000 populations. Majority of these studies have been done in hospital based setting and few in community based samples. In one of the earliest article on attempted suicide by Venkoba Rao, an incidence rate of 43/100,000 in Madurai was reported. Also in the same study, 1 in 12 cases of the suicide attempts were fatal (4). According to the NCRB report in 2010 the rate of suicide was 11.4 per 100,000 populations; an increase of 5.9% when taking into accounts the rate from the year 2008 onwards.

Suicide has a strong association with mental disorder and contributes to the excess mortality of the mentally ill. A systematic review and meta-analysis, by Harris and Barraclough et al, which included 249 reports on the mortality of mental disorders, comparing the relative risk of suicide for a given psychiatric disorder with the expected suicide rate in the general population, all psychiatric diagnoses (except mental retardation), had an increased mortality rate underscoring the importance of making a correct psychiatric diagnosis in suicide risk assessment.

Suicidal ideation is prevalent and appears to be a precondition for suicide attempts among psychiatric patients with Major Depressive disorder. The overall level of psychopathology of suicide attempters is higher compared with that in patients with ideation. Another important aspect to look at is whether suicide and attempted suicide along with other suicidal behavior form a single entity. Previous studies on suicide and attempted suicide reveals that these two phenomena tended to be regarded as one, directed at death, which succeeded in some cases and failed in others. Whereas there are recent investigators who have suggested that suicide and attempted suicide are two separate clinical entities with unique psycho-socio demographic profile, though there may be some overlap.

In the Weissman et al study they had conducted nine cross-national epidemiological surveys using similar diagnostic assessment and criteria (DSM 3) and reported lifetime prevalence rates per 100 for □□□□d□□d□□□□□ that ranged from 2.09 (Beirut) to 18.51 (Christchurch, New Zealand). Lifetime prevalence rates per 100 for □□□□d□□□□□□ □□□ranged from 0.72 (Beirut) to 5.93 (Puerto Rico).

Other studies have analyzed suicidal behavior and its correlates in specific psychiatric disorder as well. In a study by Srivastava et al they had found a positive correlation between suicide attempt and factors like the severity of depression, male gender, and age less than 35 years, being married, and being employed.

In another study done at Varanasi, on a sample of 60 out-patient psychiatry patients with diagnosis of Major depressive disorder (DSM IV-TR), they reported that all the patients who express suicidal ideation did not attempt suicide. Suicidal attempts were reported in only 16.6% of patients with major depressive episode with suicidal ideation. Also young patients with depression, unmarried men, married women and students, having severe suicidal ideation with agitation or paranoid symptoms were more likely to attempt suicide.

Rationale for study

There are no Indian studies comparing the clinical and socio demographic variables with the severity of suicidal ideation and suicide attempt as distinct entities in major depressive disorder. Most of the studies had considered both these groups as a continuum, whereas we want to study these two groups as distinct factors influencing suicidal behavior in depression. Also though many studies had studied correlation of suicidal behavior with severity of depression, they had not analyzed the socio demographic and clinical correlations with the severity of suicidal ideation or severity of attempt.

Aim

To compare the socio demographic and clinical variables between the patients with suicidal ideation and those with suicide attempt in a cohort of major depressive disorder patients.

Objectives

1. To compare the correlation of socio demographic and clinical variables with the severity of suicidal ideation.
2. To compare the correlation of socio demographic and clinical variables with the severity of suicidal attempt.

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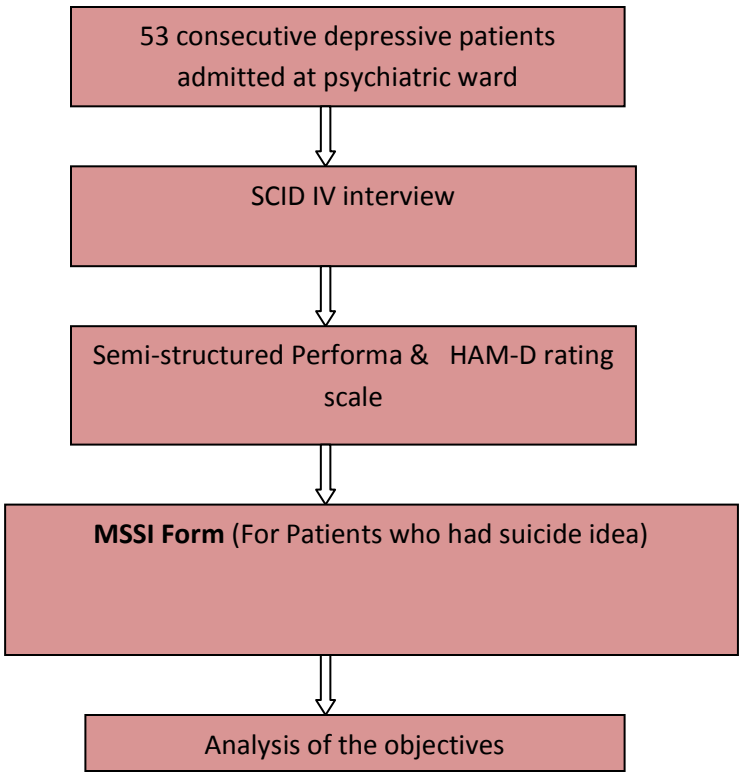
This study was a cross sectional consecutive sampling study that was conducted in the psychiatry In-patient ward at a private medical college and hospital in Coimbatore, Tamil Nadu. Study duration was 1 year (from January 2016 to December 2016) and all the consecutive patients, between the ages of 18 and 65 years, admitted in the psychiatry ward with the diagnosis of major depressive disorder were screened for study inclusion (Fig. 1).

After explaining the study protocol, we got written informed consent from the patient and the family member who had accompanied the patient. Consenting patients were interviewed by the researcher.

Inclusion criteria consisted of patients whose age was between 18 and 65 yrs and should have been admitted in the psychiatry ward with diagnosis of Major depressive disorder by their respective consulting psychiatrist in the department who was blind to the study protocol. The study researcher administers the SCID for DSM IV interview schedule and if the diagnosis is confirmed for Major depressive disorder the patient is included in the study.

Exclusion criteria consists of patients who refused to give consent and patients who with mental retardation or neuro-psychiatric co morbidity like dementia were also excluded as they would not be able to accurately respond to the severity assessment scales being administered. Patients with Bipolar depression were excluded from the study as we wanted to study specifically unipolar depressive disorder group of patients. Patients with co morbid physical illness, substance abuse and other psychiatric co morbidities were also excluded from the study. Total sample size for the study was 53.

Fig 1. Flow chart for study recruitment and methodology



Measurements

Once the patient was recruited into the study, the researcher had collected details about the socio demographic and clinical variables by using a semi structured profoma. The researcher then administered the Hamilton depression rating scale (HAMD-17) to assess the severity of depression. The researcher then administered the following scales for assessment of severity of suicidal behaviour:

1. **□ □□□□d □□□ □□□□□ □□□□□□ □□□□□r □ □□□□ □ □□□□** a scale that was developed in order to assess the severity of suicide attempts. The scale consists of 15 questions which are scaled from 0-2, which take into account both the logistics of suicide attempt as well as the intent. The scale has high reliability and validity[16].
2. **M □d□□d □□□□□□□□r □□□□□d □□□d □□□□□□M □□□□□** this scale was developed for assessing severity of suicide ideas. Principal component analysis revealed a bi dimensional structure, with factors corresponding to (1) Desire and Ideation and (2) Plans and Preparations. Each factor displayed acceptable internal consistency and expected patterns of convergent validity via associations with hopelessness, depressive symptoms, impulsivity, and a self-report measure of suicidal behaviors.

□

Statistical analysis

We performed statistical analysis with PASW (SPSS) version 19.0 for Windows. The correlation of socio-demographic variables with suicide ideation and depressive patients who attempted suicide, patient history with suicidal attempts, severity of depression with suicidal ideation and suicide attempt were determined using □□□□□□□r □□□□□□□ The correlation of suicidal ideation and suicide attempt were determined using □□r□□□□□rr□□□□□□□ P-value less than 0.05 were considered as statistically significant.

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Socio-demographic and clinical variables in the whole sample

Most of the patients belong to early adulthood age group (47.2%). Out of the total sample of 53 patients, 24 were males and 29 were females. Majority of the patients belong to middle and low socio economic status (90.6%), were married (75.5%) residing in rural community (69.8%) and were graduates (35.8%) and were living in a nuclear family (71.7%). Most of the patients did not have family history of suicide (84.9%).

The mean severity of depression on HAMD-17 rating scale in the sample was 28.72 (+/- 6.3), thus indicating severe depression. 79.2% of the patients had very severe depressive episode (42/53) as per HAM-D rating scale.

Three-fourth (75%) of the patients did not have any past suicide attempts. Out of the total patients who had attempted suicide (N=13), one patient had attempted suicide three times in the

past (1.9%) and 12 patients had attempted once (22.6%). Among 53 recruited depression patients 35 patients (66%) had suicidal ideas and 13 patients had attempted suicide (24.5%). Among the patients who had suicidal ideas, majority (62%) of the patients had severe suicidal ideas as per MSIS scale (i.e, total score >20). Among patients who did attempt suicide (13/53), majority of them (53.8%) had high suicide attempt severity (SRAF total score>20), 30.8 % had medium severity and 15.4 % had low severity of suicide attempt.

Correlation of Socio demographic variables with severity of suicidal ideas

Suicidal ideas were common and were more severe, in age group 18-40 years than middle and late adulthood age group. The severity of suicidal ideas was high among lower socioeconomic status patients and who were married. Unemployment and patients residing in rural background and patients in nuclear families had more severe suicidal ideas. But none the above socio demographic variables were statistically significant when compared across the grades of severity of suicidal ideation (Table 1)

Table 1: Correlation of socio-demographic variables with severity of suicide ideation

			M		
	18-40	6 (24.0%)	6 (24.0%)	13 (52%)	0.256
	41-60	6 (35.3%)	6 (35.3%)	5 (29.4%)	
	61-65	6 (54.5%)	1 (9.1%)	4 (36.4%)	
	MALE	8 (33.8%)	4 (16.7%)	12 (50.0%)	0.392
	FEMALE	10 (34.5%)	9 (31.0%)	10 (34.5%)	
	HIGH	2 (40.0%)	1 (20.0%)	2 (40.0%)	0.699
	MIDDLE	9 (45.0%)	4 (20.0%)	7 (35.0%)	
	LOW	7 (25.0%)	8 (28.62%)	13 (46.4%)	
M	MARRIED	14 (35.0%)	10 (25.0%)	16 (40.0%)	0.620
	UNMARRIED	2 (20.0%)	3 (30.0%)	5 (50.0%)	
	WIDOW	2 (66.7%)	0 (0.0%)	1 (33.3%)	
	EMPLOYED	8 (36.4%)	6 (27.3%)	8 (36.4%)	0.834
	UNEMPLOYED	9 (34.6%)	5 (19.2%)	12 (46.2%)	
	STUDENT	1 (20.0%)	2 (40.0%)	2 (40.0%)	
	URBAN	4 (25.0%)	3 (18.8%)	9 (56.3%)	0.358
	RURAL	14 (37.8%)	10 (27.0%)	13 (35.1%)	
	ILLITERATE	4 (40.0%)	3 (30.0%)	3 (30.0%)	0.941
	MIDDLE SCHOOL	3 (42.9%)	1 (14.3%)	3 (42.9%)	
	HIGH SCHOOL	3 (25.0%)	3 (25.0%)	6 (50.0%)	
	HIGHER SECONDAR	2 (40.0%)	2 (40.0%)	1 (20.0%)	

	Y SCHOOL				
	COLLEGE	6 (31.6%)	4 (21.1%)	9 (47.4%)	
	NUCLEAR	13 (34.2%)	10 (26.3%)	15 (39.5%)	0.856
	JOINT	5 (33.3%)	3 (20.0%)	7 (46.7%)	
	NO	15 (33.3%)	12 (26.7%)	18 (40.0%)	0.686
	YES	3 (37.5%)	1 (12.5%)	4 (50.0%)	

Correlation of socio-demographic variables with severity of suicidal attempt

Depressive patients who are in early adulthood age group (18-40 years) had a more severe suicide attempt than other age group which was statistically significant (p-value-**0.026**). None of the other variables were statistically significant in their correlation with severity of suicidal attempt (Table 2).

Poisoning was the commonest method of suicide in both men and women. Men had attempted more lethal methods (hanging/electric shock). Almost all women show poisoning as method who attempted suicide. Benzodiazepine poisoning was the most common method used for poisoning by women (Table 2a).

TABLE 2: Correlation of socio-demographic variables with severity of suicidal attempt

	18-40	17 (68.0%)	0 (0.0%)	4 (16.0%)	4 (16.0%)	**0.026**
	41-60	14 (82.4%)	0 (0.0%)	0 (0.0%)	3 (17.6%)	
	61-65	9 (81.8%)	2 (18.2%)	0 (0.0%)	0 (0.0%)	
	Male	16 (66.7%)	2 (8.3%)	2 (8.3%)	4 (16.7%)	0.348
	Female	24 (82.8%)	0 (0.0%)	2 (6.9%)	3 (10.3%)	
	High	4 (80.0%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	0.797
	Middle	14 (70.0%)	1 (5.0%)	1 (5.0%)	4 (20.0%)	
	Low	22 (78.6%)	1 (3.6%)	2 (7.1%)	3 (10.7%)	
	Married	30 (75.0%)	2 (5.0%)	2 (5.0%)	6 (15.0%)	0.660
	Unmarried	7 (70.0%)	0 (0.0%)	2 (20.0%)	1 (10.0%)	
	Widow	3 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Employed	17 (77.3%)	0 (0.0%)	1 (4.5%)	4 (18.2%)	0.579
	Unemployed	20 (76.9%)	2 (7.7%)	2 (7.7%)	2 (7.7%)	
	Student	3 (60.0%)	0 (0.0%)	1 (20.0%)	1 (20.0%)	
	Urban	11 (68.8%)	0 (0.0%)	2 (12.5%)	3 (18.8%)	0.516
	Rural	29 (78.4%)	2 (5.4%)	2 (5.4%)	4 (10.8%)	
	Illiterate	9 (90.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0.611

	Middle School	6 (85.7%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	
	High School	8 (66.7%)	0 (0.0%)	1 (8.3%)	3 (25.0%)	
	Higher Secondary School	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	College	12 (63.2%)	1 (5.3%)	3 (15.8%)	3(15.8%)	
	Nuclear	30 (78.9%)	2 (5.3%)	1 (2.6%)	5 (13.2%)	0.151
	Joint	10 (66.7%)	0 (0.0%)	3 (20.2%)	2 (13.3%)	
	No	34 (75.6%)	2 (4.4%)	4 (8.9%)	5 (11.1%)	0.560
	Yes	6 (75.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)	

Table 2a: Correlation of gender and method of suicide

		8 (61.5%)	16 (66.7%)	2 (8.3%)	1 (4.2%)	3 (12.5%)	1 (4.2%)	1 (4.2 %)	0 (0.0%)
		5 (38.5%)	24 (82.8%)	4 (13.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0 %)	1. (3.4%)

Correlation of history of previous suicide attempts with severity of suicide ideation & current severity of suicidal attempt

Depressive patients who had attempted suicide before had more severe suicidal ideas than patients without previous suicidal attempt which was statistically significant p-value-**0.01**).
Patients who had past history of suicide attempt had higher severity of current suicide attempt which was statistically highly significant (p-value-**0.000**). (Table 3)

Table 3: Correlation of suicide attempt history with severity of suicide ideation and suicidal attempt

	NONE	18 (45.0%)	12 (30.0%)	10 (25.0%)	**0.01**
	ONCE	0 (0.0%)	1 (8.3%)	11 (91.7%)	
	MORE THAN ONCE	0 (0.0%)	0 (0.0%)	1 (100.0%)	

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In the other studies they have looked into only the incidence of suicidal thoughts and attempts, whereas in our study the correlation of socio demographic and clinical variables with the severity of suicidal ideation and attempts were analyzed.

Gender

Though there were higher no. of males with severe suicidal ideation (50%) compared to females (34.5%), but this difference was not statistically significant. In the cross-national study by Weissman et al, also there was no statistical significant difference in the suicidal ideation between the genders. In other Indian studies also no significant difference has been identified.

When taking the suicidal attempters group, more number of males (33.3%) had attempted suicide than females (17.2%). Even when considering severity of suicidal attempt across from low to high it were the males who were consistently higher represented across the severity of the attempt but not statistically significant. This is comparable to findings by Narang et al (sample size 208), that found that the prevalence of suicidal attempt was high among males with psychiatric illness (ICD 10 criteria), when compared to females. In contrast the western studies have shown that there is significantly higher risk in women than in men for suicidal attempt. Our study is unique in that other Indian or western studies have not compared the correlations with the severity of suicidal attempts and ideation in patients with major depressive disorder without co morbidities.

Socio economic status and place of living

In our sample the lower socioeconomic status patients were represented the most (52.8%), followed by the middle class (37.7%) and higher socioeconomic status were only 9.4%. When considering severe suicidal ideation, all the 3 groups were comparable. Whereas compared to the high and middle income groups, the low income group had most persons with suicidal ideation but these differences were not statistically significant. But when considering rural versus urban, though rural population had the most representation (69.8%), the no. of patients who had the most severe suicidal ideation were seen in the urban group (56.3%) versus the rural group (35.1%), but again this was not statistically significant. But when looking at patients with suicidal attempts the proportion were almost equal in the rural versus urban and in the various socioeconomic strata. This finding is comparable to findings from Narang et al

Marital, Literacy and employment

In our sample the proportion of patients representing unmarried population was very less (24.5%) compared to married (75.5%), thus not allowing for meaningful comparison. When considering the employment status, there was no statistically significant difference in the severity of suicidal ideation with both employed and unemployed having proportions of 36.4% and 46.2% respectively showing severe levels of suicidal ideation. But when we compared the

severity of suicidal attempt, the proportion of employed patients (18.2%) had more severe suicidal attempt than unemployed (7.7%), but the difference did not reach statistical significance. Also when comparing illiterate versus literate patients, the severity of suicidal ideation was comparable across the levels of literacy studied. But in severity of suicidal attempt, the proportion of literate patients had more severe attempt than the illiterate group (in which only 1 patient had a low severe suicidal attempt). These findings are comparable to the Srivastava et al study[7] that found that more educated patients in the group were more likely to attempt suicide. However, Kessler et al had found that poorly educated subjects were more vulnerable to suicidal attempts, but here we also have to note that this study included various psychiatry diagnoses whereas the srivastava et al study included only major depressive disorder patients similar to our current study.

Correlation of Type of family and family h/o suicide with severity of suicidal idea and attempt

Though the proportion of patients who had severe suicidal ideation was slightly higher in joint family (46.7%) group compared with nuclear family (39.5%), it was not statistically significant. Concordantly the proportion of patients with suicidal attempt were also higher in joint family group (33.3%), compared to the nuclear family group. But these differences were not statistically significant same as even reported in study by Narang et al, in which they had a larger sample size (100).

Only 8 patients out of the whole sample had reported family history of suicide. But the proportion of these patients with severe suicidal ideation was 50%, which was slightly higher than the patients without family history of suicide who had severe suicidal ideation (40%). Similarly even though the sample size was very less in the patients with family history of suicide, 25% of them had severe suicidal attempt which was greater than when compared with the patients without family history in whom the proportion of patients with severe suicidal attempt was only 11 %. But again these differences were not statistically significant. Indian studies have shown that family history of attempted suicide or completed suicide was common among patients suffering from depression.

Correlation of history of previous suicide attempts with severity of suicide ideation & attempt

12 patients (22.6%) in the sample had at least one history of previous suicidal attempt and one patient had previous history of more than once. The proportion of patients who had severe suicidal ideation was higher in the group of patients with previous suicidal attempt (92.3%) than those without (25%). This difference was statistically significant ($p = 0.01$)

All the patients who attempted suicide during the current episode (13) had a previous history of suicide attempt, compared to those without suicide attempt during current episode that had none. This difference was also statistically significant ($p = 0.000$). Thus from this study we further confirm to the understanding that the history of previous suicide attempt in major depressive

disorder appears to be a major risk factor for suicidal attempt or severe suicidal ideation during the subsequent episodes also. This finding has been replicated in previous studies that have found attempted suicide to be one of the predictors of future suicide. Studies in India shows that about 18% of suicide victims had a previous suicide attempt.

Correlation between Severity of depression and suicide ideation and attempt in the sample

All the patients with suicidal attempt in the sample were patients who had very severe depression on HAMD rating scale. Suicidal attempt has been found to be positively correlated with severity of depression in other Indian studies also[15]. Also the severity of suicidal ideation was positively correlated with the severity of depression on HAMD rating scale. (*p = 0.020*)

Correlation of severity of suicide idea and severity of suicide attempt

Depressive patients who had higher suicidal ideas in our sample had attempted suicide with higher intentionality which was statistically significant (*R value= 0.364, p-value-**0.007***). Study by Kessler et al had shown that cumulative probabilities for transition from suicidal idea to plan were 34 % and for transition from plan to attempt was 72% [21]. But they had included various co morbidities and whereas our study is specifically on major depressive disorder and also we have not differentiated between suicidal ideators and suicidal plan in our study. We have not analyzed the individual factors in the rating scales for suicidal ideation and attempts across the various variables studied.

Strengths and limitations

This was a cross sectional study in the inpatient ward during the patient's current depressive episode thus we were able to assess in cross sectionally, the suicidal behavior without any recall bias. In our sample the patients with severe depression was represented adequately and there were no dropouts, thus were able to assess suicidal behavior true to our study objective. We used validated scales and we stuck to a homogenous sample analyzing subjects with diagnosis of major depressive disorder alone and excluded subjects with substance abuse and other psychiatric co morbidities that could influence suicidal behavior such as personality disorders or adjustment disorder etc.

Some of the limitations of our study are that the researcher may have been biased when assessing the suicidal behavior as the severity rating was also done by the same interviewer. Since it was a cross sectional study we have not analyzed the course of the suicidal behavior in these group of patients and their response to treatment. We have not studied the correlation of the presence of any recent life events with severity of suicidal behavior. Further studies are needed to compare whether the correlations with severity of suicidal behavior differ in other psychiatric illnesses like schizophrenia, bipolar disorder, personality disorder etc.

Severity of depression was correlated with severity of suicidal ideations as well as severity of suicidal attempts. In addition to identifying suicidal behavior, the assessment of severity of suicidal ideations and past suicidal attempts is of great importance in identifying high risk patients presenting with major depressive disorder.

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A.S. Srivastava, Rakesh Kumar; Suicidal ideation and attempts in patients with major depression: Sociodemographic and clinical variables; *Indian Journal of Psychiatry* 2005;47:225–228

Aponte (1969) Epidemiological aspects of suicide and attempted suicide. Proceeding of the 5th *International Conference for Suicide Prevention* (Ed.) Fox, R., Vienna.

Beck, R.W Morris, J.B, and Beck, A.T (1974) cross- validation of the suicidal intent scale. Psychological reports, 34, 445-446

Bhatia MS, Aggarwal NK, Aggarwal BB. Psychosocial profile of suicide ideators, attempters and completers in India. *Int J Soc Psychiatry* 2000;46:155‑63.

David, L. (1970) Relationship between attempted suicides and completed suicides. *Psychological Reports*, 27(3), 719-722.

E C Harris and B Barraclough; Suicide as an outcome for mental disorders. *A meta-analysis; BJP* 1997, 170:205-228

Gelder, M., Gath, D., Mayou, R. & Cowen, P. (1996). Oxford Textbook of Psychiatry, 3rd edition: *Oxford University Press*, 414-455.

Harris EC, Barraclough B: Excess mortality of mental disorder. *Br J Psychiatry*. 1998, 173: 11-53. 10.1192/bjp.173.1.11

Hawton K, Fagg J. Suicide, and other causes of death, following attempted suicide. *Br J Psychiatry* 1988;152:359‑66.

Kessler RC, Borges G, Walters EE. Prevalence of and risk factors for lifetime suicide attempts in the National Comorbidity Survey. *Arch Gen Psychiatry* 1999;56:617–26.

M.M. Weissman et al; Prevalence of suicide ideation and suicide attempts in nine countries; *Psychological Medicine*, 1999, 29, 9-17

Malone KM, Oquendo MA, Haas GL, et al. Protective factors against suicidal acts in major depression: Reasons for living. *Am J Psychiatry* 2000;157:1084–8.

Mathers C, Fat DM, Boerma JT, et al. The global burden of disease: 2004 update; Geneva, Switzerland: *World Health Organization*; 2008.

Nandi, D.N., Banerjee, G & Boral, G.C. (1978). Suicide in West Bengal; *Indian Journal of Psychiatry*, 20, 155-60.

Narang RL, Mishra BP, Mohan N. Attempted suicide in Ludhiana. *Indian J Psychiatry* 2000;42:83–7.

National Crime Records Bureau. Accidental Deaths and Suicides in India 2008. Ministry of Home Affairs, *Government of India New Delhi*; 2010.

P.N Suresh Kumar; An Analysis of Suicide Attempters Versus Completers in Kerala; *Indian Journal of Psychiatry*, 2004, 46(2)144-149.

Pettit JW et al; Factor structure and psychometric properties of the Modified Scale for Suicidal Ideation among suicidal youth; *Depress Anxiety*. 2009;26(8): 769-74.

Phillips, M.R., Xianylun, Li & Zhang, Y (2003) Suicide rate in China, 1995- 1999. In: Vijayakumar, L. Suicide Prevention - Meeting the challenge together, Orient Longman, 214-231.

Rao VA; Attempted suicide; *Indian J Psychiatry*; 1965;7: 253-64

Singh, A.N. (1972). *The study of 38 cases of suicides and attempted suicides and their causation in teenagers.* Proceedings of the 6th International Conference for Suicide prevention (Ed.). Litman, Mexico.

Sokero TP, Melartin TK, Rytsala HJ, et al. Suicidal ideation and attempts among psychiatric patients with major depressive disorder. *J Clin Psychiatry* 2003;64:1094–100.

Srivastava S, Kulshreshtha N. Expression of suicidal intent in depressives. *Indian J Psychiatry* 2000;42:184

Thomas E. Joiner Jr., M. David Rudd et al The Modified Scale for Suicidal Ideation: Factors of Suicidality and Their Relation to Clinical and Diagnostic Variables; *Journal of Abnormal Psychology* 1997. Vol. 106, No. 2, 260-265

Vijayakumar L; Indian research on suicide; *Indian J Psychiatry*; 2010;52:S291-6.

World Health Organisation; WHO Fact sheet; reviewed September 2016; http://www.who.int/mediacentre/factsheets/fs398/en/

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Biological dysrhythm in remitted bipolar I disorder

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ABSTRACT

Background: Recent treatment guidelines support treatment of biological rhythm abnormalities as a part of treatment of bipolar disorder, but still, literature examining various domains (Sleep, Activity, Social, and Eating) of biological rhythm and its clinical predictors are less.

Objectives: The main aim of our study is to compare various domains of biological rhythm among remitted bipolar I subjects and healthy controls. We also explored for any association between clinical variables and biological rhythm among bipolar subjects.

Methods: 40 subjects with Bipolar I disorder and 40 healthy controls who met inclusion and exclusion criteria were recruited for the study. Diagnoses were ascertained by a qualified psychiatrist using MINI 5.0. Sociodemographic details, biological rhythm (BRIAN—Biological Rhythm Interview of assessment in Neuropsychiatry) and Sleep functioning (PSQI- Pittsburgh Sleep Quality Index) were assessed in all subjects.

Results: Mean age of the Bipolar subjects and controls were 41.25 ± 11.84 years and 38.25 ± 11.25 years respectively. Bipolar subjects experienced more biological rhythm disturbance when compared to healthy controls (total BRIAN score being 34.25 ± 9.36 vs 28.2 ± 6.53) ($p = 0.002$). Subsyndromal depressive symptoms (HDRS) had significant positive correlation with BRIAN global scores($r = 0.368$, $p = 0.02$). Linear regression analysis showed that number of episodes which required hospitalization ($\beta = 0.601$, $t = 3.106$, $P = 0.004$), PSQI ($\beta = 0.394$, $t = 2.609$, $p = 0.014$), HDRS ($\beta = 0.376$, $t = 2.34$, $t = 0.036$) explained 31% of variance in BRIAN scores in remitted bipolar subjects.

Conclusion: Biological rhythm disturbances seem to persist even after clinical remission of bipolar illness. More studies to look into the impact of subsyndromal depressive symptoms on biological rhythm are needed.

1. Introduction

In humans, periodic fluctuations in levels of melatonin, growth hormone, prolactin, cortisol and thyrotropin show a circadian pattern. This circadian rhythm is governed by the master clock Suprachiasmatic Nucleus which synchronizes environmental cues with endogenous timing system (Roenneberg et al., 2003), the major environmental cue (zeitgeber) being the timing of sunlight. Synchronization of these circadian rhythms was found to be disturbed in subjects with bipolar disorder (Chung et al., 2012). Social *zeitgebers* like timing of meals, work schedules and schedules of other family members can influence social rhythm and thereby, circadian rhythm (Carney et al., 2006). Social zeitgeber theory states that in vulnerable individuals, circadian rhythm abnormalities can lead to mood episodes (Grandin et al., 2006). Moreover, in the expert guidelines, interventions stabilizing circadian rhythms, especially Interpersonal social rhythm therapy, have been recommended for a long time, as a treatment during maintenance phase of major depression and bipolar disorder (Yatham et al., 2013).

Biological rhythm disturbance especially sleep, has been well studied in bipolar disorder. These studies had established persistent sleep disturbance during remitted phase especially delayed sleep onset, more sleep awakenings after sleep onset, poor sleep efficiency and high sleep wake variability (Ng et al., 2015). These disturbances not only impair the functioning in remitted phase of the bipolar illness (Giglio et al., 2010), but also increase the risk of relapse of mood disorder (Gruber et al., 2011). Growing evidence also points to baseline social rhythm irregularity in mood disorders (Yatham et al., 2013). Subjects with Bipolar spectrum disorders were found to have less social rhythm regularity, which had been shown to be predictive of shorter time to relapse with manic or depressive episode (Shen et al., 2008). Though studies focusing on biological rhythm abnormalities during interepisode period had targeted sleep and activity rhythm using actigraphy (McKenna et al., 2014), social rhythm had not been always studied. Moreover, social rhythm in bipolar disorder had been extensively

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studied in isolation in the context of Interpersonal social rhythm therapy (Van Tienoven et al., 2014).

Growing literature especially in the last 4 years, had looked into the association between Bipolar disorder and Biological rhythm using a validated scale, Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) to look into sleep, activity, social and eating rhythms (Mondin et al., 2017; Cudney et al., 2016; Cardosa Tde et al., 2015; Duarte Faria et al., 2015; Rosa et al., 2013). Cross sectional assessment of Biological rhythm using BRIAN in 1023 young adults (18–24 years) showed that subjects with euthymic bipolar disorder had more biological rhythm disturbance when compared to healthy controls. Interestingly, bipolar subjects had more biological rhythm disturbance during depressive phase, followed by hypomanic/Manic/mixed phase and euthymic phase of illness (Mondin et al., 2017). Similar finding was also observed in drug naïve Bipolar subjects (18–24 years), where it was observed that biological rhythm continued to be disturbed even during the euthymic phase of illness when compared to healthy community controls (Duarte Faria et al., 2015, Rosa et al., 2013). In another prospective study in young adults with bipolar disorder, biological rhythm disturbance tend to reduce significantly, at 6th and 12th month follow-ups when compared to its baseline (Cardoso Tde et al., 2015).

Though the existing studies had found that biological rhythm was more disrupted in euthymic Bipolar disorder than healthy controls, the recruited subjects in those studies also had another axis I comorbidity along with bipolar disorder (Mondin et al., 2017; Duarte Faria et al., 2015; Rosa et al., 2013). In light of finding that disorders like Substance use disorders (Logan et al., 2014), Post traumatic stress disorder (Agorastos et al., 2014) and Attention deficit hyperactivity disorder (Kooij and Bijlenga, 2013) have been found to be independently associated with persistent chrono-disruption, the findings of the above study could not be entirely attributed to bipolar disorder itself. Moreover, the above studies ((Mondin et al., 2017; Cardosa Tde et al., 2015; Duarte Faria et al., 2015; Rosa et al., 2013) were mostly focused on young adults (18–29 years) who were predominantly from urban background and thus we need studies with different population characteristics to evaluate the generalizability of these findings. Also, by examining biological rhythm in bipolar subjects (without any psychiatric comorbidities), any observed biological rhythm disturbances can be more likely due to bipolar disorder.

So, the primary aim of the study is to compare the biological rhythm (sleep, social, activity and eating domains) between remitted bipolar I subjects (without any psychiatric comorbidities) and healthy controls. Our secondary aim is to explore for any association between clinical variables (sociodemographic details, illness variables, quality of sleep) and biological rhythm in subjects with bipolar disorder.

2. Methodology

2.1. Recruitment of participants

With prior informed consent, subjects attending outpatient services of department of Psychiatry in a tertiary medical college hospital, Coimbatore, India were screened for inclusion and exclusion criteria for a period of 4 months in 2014. None of the subjects attending outpatient services received any structured program focusing on regulation of circadian rhythm. 40 subjects who met the below mentioned inclusion and exclusion criteria were recruited by consecutive sampling. [3-didn't consent for the study, 6- illiterate in Tamil(local language), 4-medically very sick for evaluation].

Subjects of 18 to 60 years of age, of either gender, diagnosed to have Bipolar affective disorder type 1 (as per DSM IV TR criteria by a qualified psychiatrist) who were euthymic (with a score of ≤ 8 as per Young Mania Rating Scale and a score of ≤ 8 as per 17 item Hamilton Depression Rating Scale) for the last 6 months (as corroborated by medical records) were included into the study. Additionally, these subjects should have consented for the study and should be able to read and write Tamil, the predominant language prevalent in this part of the country. Each subject was independently assessed by the psychiatrist to rule out associated comorbidities and if needed, the details were cross-checked with the medical records as well.

We had excluded subjects who had brief period of euthymia (less than 6 months), current mood episode [hypomania, Mania (YMRS score > 8) or depression (17 item HDRS > 8)], Mental retardation, comorbid axis I disorders (using MINI 5.0) like substance use disorders (except nicotine dependence), Post traumatic stress disorder and Attention deficit hyperactivity disorder (independently evaluated by the psychiatrist), Bipolar disorder secondary to general medical condition, medical conditions which induce sleep disturbance like Obstructive sleep apnoea and neurodegenerative conditions.

Age, gender & occupation matched healthy controls (n = 40) who were 18–60 years old, of either gender and literate in Tamil were recruited for the study with prior informed consent. Controls were meticulously screened for psychiatric illness like Bipolar disorder, Depression, Schizophrenia, Anxiety disorder, Mental retardation and Substance use disorder using structured assessment tool (MINI 5.0). Similarly, controls who had medical disorders like Obstructive Sleep apnea and who were illiterate in Tamil were also excluded. The entire protocol adhered to Indian Council of Medical Research (ICMR) Ethical guidelines for biomedical research on human participants (2006) which was reviewed and approved by the Institutional hospital ethics committee.

2.2. Administration of tools

Evaluation was done by two Investigators, one being psychiatrist and other being a medical graduate who was trained in psychiatric evaluation. The following tools were administered: (1) MINI 5.0 (Mini neuropsychiatric interview) to evaluate for BPAD and other axis I comorbidities (2) 17 item Hamilton depression rating scale (HDRS) (3) Young Mania rating scale (YMRS) (4) Biological Rhythm Interview of Assessment in Neuropsychiatry (BRIAN) (Giglio et al., 2009) (5) Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) (7) Semi structured assessment tool to collect details about sociodemographic variables and clinical variables like age of onset of illness, number of episodes, rapid cycling, number of episodes, duration of the episodes and others. To reduce the risk of recall bias, these details were cross checked with the clinical data in the file and other informants if necessary.

BRIAN scale, developed by Bipolar Disorder Program in Brazil (Giglio et al., 2009), contains 18 items focusing on three important areas related to biological rhythm disturbance in psychiatric patients, namely sleep/activity, social rhythms and eating pattern. It measures the frequency of problems related to the maintenance of circadian rhythm regularity in the last 14 days. For example, in social rhythm, "Do you have difficulties in synchronizing daily routines and sleep patterns with significant others (family, friends, spouse)? How frequently?". Items are rated using a 4-point scale: (1) = not at all, (2) = seldom, (3) = sometimes, and (4) = often or in some items 1 = never, 2 = seldom, 3 = often, 4 = always. The total BRIAN scores ranges from 1 to 72, where the higher scores suggest severe biological/circadian rhythm disturbance. The scores were regrouped and scored as 5 domains namely, sleep, activity, social, eating and chronotype. Validity and reliability of BRIAN had been established not only in English but also in Portuguese version (Giglio et al., 2009).

PSQI (Pittsburgh Sleep Quality Index) (Buysse et al., 1989) is a self-administered instrument for measuring sleep quality in the last 30 days. The instrument has 19 questions which are grouped in to 7 sub-components, generating a score range 0–21. PSQI global score > 5 indicates poor sleep quality implying clinically significant sleep disturbance. As the scales like BRIAN and PSQI were self-administered, these were translated to the local language (Tamil) and back translated

to English. In the pilot study, these translated (Tamil) scales were administered to a group of bipolar subjects to assess the feasibility of the scales and necessary changes were made based on their feedback. The Tamil version of the BRIAN has been validated as part of this study, which is discussed in further sections of the study. Any doubts raised by subjects during assessment, were clarified by the investigators now and then.

2.3. Statistical analysis

Statistical analysis was conducted using SPSS for Windows – Version 19.0. Analysis of the socio demographic and clinical data using Shapiro-Wilk test of normality showed non–normal distribution and thus non parametric tests were used. Comparison of Socio demographic data, BRIAN scores and PSQI scores among cases and controls was performed using Mann – Whitney *U* test for continuous variables and $\chi 2$ test for discrete variables. Correlation among variables like, BRIAN, PSQI and clinical variables in bipolar subjects was done using Spearman's test of correlation. In order to find the predictors of biological rhythm disturbance, linear regression was performed with BRIAN scores as the dependent variable. Internal consistency reliability of the translated scale was measured using cronbach's α.

3. Results

3.1. Demographics and illness description

The mean age of the Bipolar subjects and controls were 41.25 ± 11.84 years and 38.25 ± 11.25 years respectively. Comparison of socio demographic details between bipolar subjects and controls didn't show any statistically significant difference in age, gender, socio economic status and occupational status (Table 1). The mean age of onset of Bipolar disorder was 27.38 ± 10.55 years (Table 2), with a median of 25 years. Mean HDRS and YMRS score in our subjects were 3.05 ± 1.12 and 3.75 ± 0.72, respectively, indicating that these subjects were euthymic at the time of evaluation.

3.2. Comparison of biological rhythm among the groups

Bipolar subjects (34.25 ± 9.36) experienced more biological rhythm disturbance when compared to controls (28.2 ± 6.53), which was statistically significant ($p = 0.002$) as measured by Mann- Whitney *U* test (Table 3). Activity ($p = 0.001$), eating ($p = 0.001$) and social ($p = 0.012$) domains of biological rhythm were more impaired in bipolar subjects when compared to controls. No statistically significant difference was found in sleep and chronotype domains of biological rhythm between bipolar subjects and controls. Analysis of PSQI scores revealed delayed sleep latency ($p < 0.001$) in bipolar subjects when compared to healthy controls, but they didn't differ on sleep efficiency, sleep quality, sleep duration and days disturbed due to sleepiness.

3.3. Clinical correlates of biological rhythm

As the parameters studied were not normally distributed in bipolar subjects, Spearman's test of correlation was used. Based on the correlation analysis, it was found that higher the number of mixed episodes, more worse were the biological rhythm disturbances (total BRIAN score $r = 0.291$, $p = 0.06$, trend towards of significance) (Table 4). Age(in years), duration of illness(in months), age of onset of bipolar disorder(in years), time elapsed since last episode(in months) number of manic episodes, number of depressive episodes, number of episodes requiring hospitalization and number of episodes with suicidal attempts did not correlate significantly with BRIAN total score and its domain scores(sleep, activity, eating and social). As depicted in Fig. 1, there was a near significant positive correlation between total BRIAN score and persisting sleep disturbances (PSQI score) ($r = 0.298$, $p = 0.06$, trend towards significance) in bipolar subjects (Table 5).

3.4. Subsyndromal mood symptoms and biological rhythm

There was significant positive correlation between BRIAN Global score ($r = 0.368$, $p = 0.02$), activity domain ($r = 0.359$, $p = 0.023$), sleep domain ($r = 0.344$, $p = 0.03$) and depressive symptoms (HDRS

Table 1
Comparison of socio demographic variables between remitted bipolar subjects and healthy controls.

Variables	Remitted Bipolar Subjects	Healthy Controls	P value (Mann– Whitney *U* test)/$\chi 2$
Age[a]	38.25 ± 11.25(Years) (Mean ± S.D)	41.25 ± 11.849(Years) (Mean ± S.D)	0.268
Gender[b]			
Male	21	21	0.262
Female	19	19	
SES [b]			
LSES	3	2	0.644
MSES	37	38	
Marital status[b]			
Unmarried	9	4	0.315
Married	30	35	
Widowed	1	1	
Occupation[b]			
Unemployed	8	4	0.455
Semiskilled	22	26	
Skilled	9	8	
Professional	0	0	
Buisness	0	2	
Residence[b]			
Urban	28	25	0.375
Rural	12	15	
Education(based on years of schooling)[b]			
Primary	10	12	0.755
Secondary	23	24	
Higher	7	4	

SES- Socio Economic class, LSES- Lower socioeconomic class, MSES- Middle Socio economic class.
[a] Mann– Whitney *U* test.
[b] - Chi- square test.

Table 2
Clinical Variables in Remitted Bipolar group(n = 40).

Variables	Mean ± S.D	N(%)
Age of Onset of BPAD(years)	27.38 ± 10.55	
Polarity of the first Episode		
Mania		29 (72.5%)
Depression		10 (25%)
Mixed		1 (2.5%)
Number of episodes of Mania	3.40 ± 1.45	
Number of episodes of depression	1.90 ± 0.93	
Number of episodes of Mixed state	0.45 ± 0.13	
Rapid cycling		5(12.5%)
Number of episodes requiring hospitalization	1.35 ± 1.07	
Number of episodes with suicidal attempt	1.93 ± 0.10	
Family history of BPAD		5(12.5%)
Duration of illness(months)	139.75 ± 99.04	
Time elapsed after the last episode(months)	25.4 ± 11.06	
Medications		
Lithium 800–1200 mg (1012.5 ± 245.05)		28 (70%)
Divalproex sodium 1000 − 1500 mg (1456.45 ± 122.4)		12(30%)
Antipsychotics (Olanzapine 2.5 mg, Quetiapine 25 mg)		9 (22%)
Benzodiazepines (Clonazepam 0.25 mg)		0 (0%)

Table 3
Comparison of BRIAN scores between remitted bipolar subjects(n = 40) and Healthy controls(n = 40).

Domains	Remitted Bipolar subjects (Mean ± S.D)	Healthy Controls (Mean ± S.D)	P value (Mann-Whitney *U* test)
Sleep Domain	8.70 ± 3.413	7.93 ± 2.674	0.309
Activity domain*	8.60 ± 3.433	6.28 ± 2.320	**0.001**
Social Domain*	5.70 ± 2.233	4.75 ± 1.706	**0.012**
Eating domain*	6.78 ± 2.904	4.80 ± 1.800	**0.001**
Chronotype	4.47 ± 0.877	4.58 ± 0.781	0.440
Total BRAIN score*	34.25 ± 9.364	28.20 ± 6.533	**0.002**

score). But, there was no significant correlation between eating domain (r = 0.009, p = 0.95) or social domain(r = 282, p = 0.07) of the BRIAN and depressive symptoms (HDRS). But subsyndromal manic symptoms (YMRS) didn't have any statistically significant correlation with global BRIAN scores (r = − 0.178, p = 0.272).

3.5. Predictors of biological rhythm disturbance

In order to find the predictors of biological rhythm in remitted bipolar subjects, we performed linear regression with age, number of Manic episodes, number of Depressive episodes, number of Mixed episodes, number of episodes which required hospitalization, number of episodes with suicidal attempt, mean duration of episode, HDRS, YMRS, PSQI scores as independent variables and BRIAN global score as the dependent variable. Results of linear regression shows that number of episodes which required hospitalization (β = 0.601, t = 3.106, P = 0.004), HDRS(β = 0.376, t = 2.34, t = 0.036) and PSQI (β = 0.394, t = 2.609, p = 0.014) predicted 31% of variance in BRIAN sores(R^2 = 0.310, F = 2.594, P = 0.02).

3.6. Psychometric properties of BRIAN

Cronbach α for all five items of the translated BRIAN was 0.83 which showed good internal consistency reliability among the items of the scale. Concurrent validity of translated BRIAN was measured using Spearman's correlation, which showed statistically significant correlation between total BRIAN scores and PSQI items, especially total PSQI scores (r = 0.459, p = 0.001), PSQI daytime dysfunction(r = 0.324, P < 0.001), PSQI sleep disturbance (r = 0.498, P < 0.001), and PSQI sleep quality (r = 0.311, p = 0.003), which suggests good concurrent validity.

4. Discussion

4.1. Comparison of biological rhythm among the groups

Our study finding showed that bipolar subjects experienced greater biological rhythm disturbances than healthy controls especially in respect to activity, eating and social domains. This finding holds more significance as none of the recruited bipolar subjects had any axis I comorbidity and thus impaired biological rhythm disturbance can be attributed more likely to bipolar disorder in this study. Though previous studies have (Mondin et al., 2017; Duarte Faria et al., 2015; Rosa et al., 2013) showed more disturbances in social, sleep and activity rhythms in bipolar subjects, our study didn't find any difference in sleep rhythm

Table 4
Correlation between Clinical variables and Biological rhythm in bipolar subjects(n = 40).

Clinical variables	BRIAN Sleep	BRIAN Activity	BRIAN Social	BRAIN Eating	BRIAN Total
Age	0.086	−0.025	0.028	0.113	0.262
Age of onset of bipolar disorder	−0.030	0.142	0.012	−0.066	0.191
Duration of illness(months)	0.028	−0.0183	0.017	0.200	0.013
Time elapsed since last episode(months)	−0.177	0.170	0.082	−0.195	−0.259
Number of Manic episodes	−0.101	−0.040	0.046	−0.087	0.102
Number of Depressive episodes	0.262	0.033	0.121	0.316	−0.062
Number of Mixed episodes	0.256	0.043	0.018	0.356†	−0.291*
Number of episodes requiring hospitalization	0.276	0.186	0.188	0.178	−0.061
Number of episodes with suicidal attempts	0.196	0.145	−0.055	0.279	−0.140

*Statistically significant trend towards significance(P = 0.06).

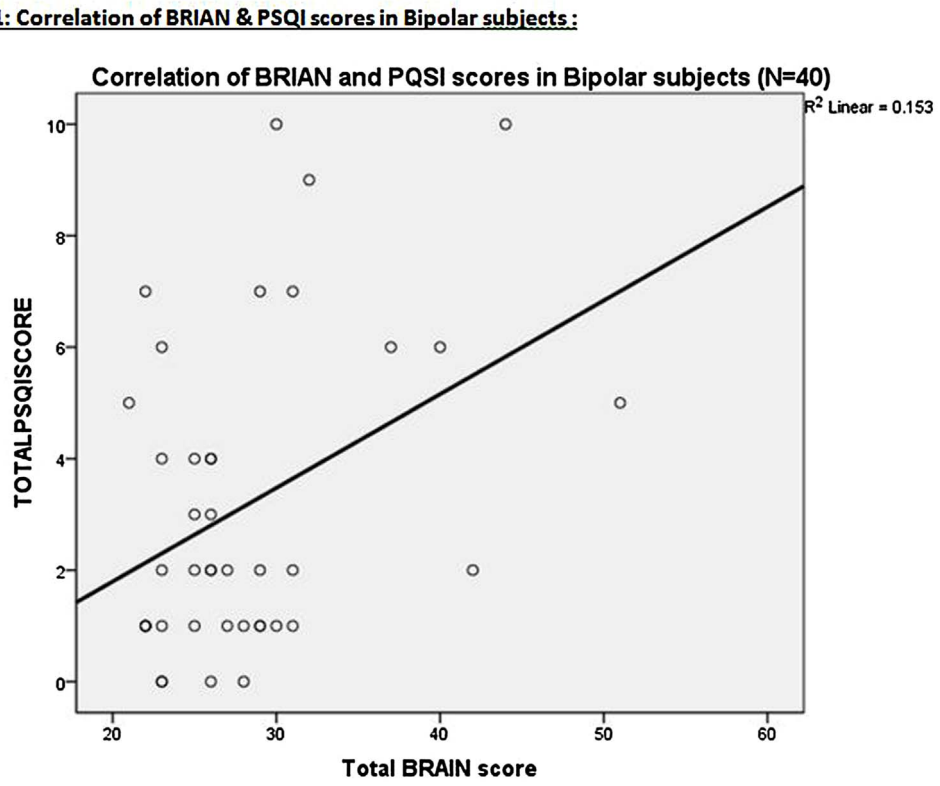


Fig. 1. Correlation of BRIAN & PSQI scores in Bipolar subjects.

between bipolar subjects and healthy controls.

4.2. Subsyndromal depressive symptoms and biological rhythm

Interestingly, mean HDRS score in remitted bipolar subjects was low (3.05 ± 1.12), yet these subsyndromal depressive symptoms had a significant association with biological rhythm even during the euthymic phase of the illness. This finding is in line with the results of study by Cardoso Tde et al. (2015), in which serial prospective assessments of biological rhythm, depressive and anxiety symptoms were done at baseline, 6th month & 12th month. This study emphasized that significant positive correlation existed between lesser Biological rhythm disturbances and lesser depressive and anxiety symptoms at 6th month & 12th month follow up. Recent meta-analysis comprising 12 studies involving 5837 patients showed that subsyndromal depressive symptoms during the euthymic phase of bipolar disorder, doubled the risk of having a subsequent mood episode (Radua et al., 2017). But, it is not yet clear whether the association between subsyndromal depressive symptoms and increased risk for subsequent mood episode is mediated by persisting biological rhythm disturbances in bipolar disorder (Levenson et al., 2015).

4.3. Sleep rhythm and bipolar disorder

In our study, bipolar subjects had increased sleep onset latency as measured by PSQI when compared to healthy controls. Sleep rhythm abnormalities such as increased sleep latency measured by actigraphy during euthymic phase of illness in bipolar I disorder have been shown to have high negative impact on the course of illness (Saunders et al., 2013; Gershon et al., 2012). Based on the observation that seasonal depression occurs when circadian rhythms are phase delayed relative to external light and/or sleep/wake cycle, Lewy et al. proposed a phase shift hypothesis of Seasonal affective disorder (Lewy et al., 1988). This hypothesis emphasized the use of light therapy for the treatment of seasonal affective disorder, which synchronizes internal circadian rhythm with external light and/or sleep/wake cycle rhythm.

In the present study, there was no statistically significant difference in sleep rhythm between bipolar subjects and controls which could be secondary to medications especially lithium (70%). Lithium had been shown to increase behavioral circadian length across many species (Abe

Table 5
Clinical predictors of Biological dysrhythm (Global BRIAN scores) in remitted bipolar subjects (n = 40).

S. No	Variables	Unstandardized β	Std. Error	Standardized β	t	P	95% confidence interval
1.	Age	−0.058	0.09	−0.099	−0.645	0.524	−0.241 to 0.126
2.	Number of Manic episodes	−1.117	0.738	−0.247	−1.514	0.141	−2.629 to 0.394
3.	Number of Depressive episodes	−0.424	0.767	−0.125	−0.553	0.585	−1.995 to 1.147
4.	Number of Mixed episodes	−1.285	1.858	−0.126	−0.691	0.495	−5.090 to 2.521
5.	Number of episodes which required hospitalization	3.365	1.083	0.601	3.106	0.004*	1.146–5.584
6.	Number of episodes with suicidal attempts	0.763	1.076	0.128	0.709	0.484	−1.442 to 2.968
7.	Duration of illness	0.004	0.099	0.005	0.036	0.971	0.199–0.206
8.	HDRS	2.493	1.063	0.376	2.344	0.026*	0.315–4.671
9.	YMRS	0.456	0.857	0.082	0.532	0.599	−1.299 to 2.212
10.	Total PSQI	0.916	0.351	0.394	2.609	0.014*	0.197–1.636

* Statistically significant = $p < 0.05$

et al., 2000). In mice, Lithium was shown to increase amplitude of oscillations in the central and peripheral oscillators, by enhancing PER 2 protein expression through GSK −3β signaling pathway (Li et al., 2012). In skin fibroblasts of bipolar patients, presence of PER3 and RORA clock gene variants predicted period lengthening effect of lithium and GSK3β clock gene variants predicted rhythm effects of lithium (McCarthy et al., 2013). In view of possible role of psychotropics on biological rhythm, future studies focusing on medication-free bipolar subjects are required.

4.4. Clinical predictors of biological dysrhythms

In our study, the regression analysis showed that number of episodes which required hospitalization, subsyndromal depressive symptoms and poor sleep quality (PSQI) predicted biological rhythm disturbances during the euthymic phase of illness. Prospective studies will be able to explain whether these clinical variables predicted more severe persisting biological rhythm abnormalities (Pinho et al., 2015) or whether the persistent rhythm abnormalities predicted depressive and mixed symptoms (Alloy et al., 2015).This in light of growing evidence for social/circadian rhythm dysregulation model of development of bipolar spectrum disorders (Alloy et al., 2015), assumes more importance.

4.5. Regulation of social rhythm as a treatment strategy

Circadian rhythm dysregulation model hypothesizes that disruptions in social rhythm have a pivotal role in course of bipolar spectrum disorders (Alloy et al., 2015). Social rhythm irregularity had been consistently found in bipolar subjects (Ashman et al., 1999) even during euthymic phase (Shen et al., 2008). Recently, Interpersonal social rhythm therapy had been shown to have good adjunctive effect in reducing depressive symptoms or hypomanic symptoms by increasing social rhythm regularity and reducing potential life event triggers (Nusslock and Frank, 2012). But interventions purely focusing on improving social rhythm, increased the regularity of daily routines only, but didn't predict changes in depressive or hypomanic symptoms (Faria et al., 2014). In view of inconsistencies in efficacy, interventions focusing on regularizing social rhythm, also need more research in remitted bipolar disorder.

4.6. Limitations

However, following limitations need to be considered 1) As the scales used were self-administered questionnaires, there could have been some amount of recall bias. This has been ameliorated to some extent by cross verification with file reports and multiple informants. 2) Our sample size was small. Thus these results need to be replicated in a larger sample in future studies. 3) We had not used objective assessment tools like actigraphy for measurement of sleep and activity rhythm. 4) Since the study purely focused on subjects with Bipolar I disorder, the results may not be generalizable to other forms of bipolar disorder. 5) Since all patients were on maintenance medications, their sleep parameters could have been influenced by those drugs. Thus, prospective naturalistic follow up studies could help us in understanding the persistent chronobiological disturbance in these patients, more conclusively.

5. Conclusion

Our study has shown that Biological rhythm disturbance seems to persist even after clinical remission of bipolar illness. Though our study has found higher biological rhythm disturbances in remitted bipolar subjects, the findings are preliminary in view of small sample size. Further studies on impact of biological rhythm on onset and course of bipolar illness may help us in understanding pathophysiology of bipolar disorder and thereby, may pave the way for new therapeutic targets for treating bipolar disorder.

Conflicts of interest

Nil.

Declaration of interests

The authors, A.I and P.P have no conflicts of interest relating to the subject matter of this article.

References

Abe, M., Herzog, E.D., Block, G.D., 2000. Lithium lengthens the circadian period of individual suprachiasmatic nucleus neurons. Neuroreport 11, 3261–3264.

Agorastos, A., Kellner, M., Baker, D.G., Otte, C., 2014. When time stands still: an integrative review on the role of chronodisruption in posttraumatic stress disorder. Curr. Opin. Psychiatry 27 (5), 385–392.

Alloy, L.B., Nusslock, R., Boland, E.M., 2015. The development and course of bipolar spectrum disorders: an integrated reward and circadian rhythm dysregulation model. Annu. Rev. Clin. Psychol. 213–250.

Ashman, S.B., Monk, T.H., Kupfer, D.J., Clark, C.H., Myers, F.S., Leibenluft, Frank E., 1999. Relationship between social rhythms and mood in patients with rapid cycling bipolar disorder. Psychiatr. Res. 86, 1–8.

Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213.

Cardoso Tde, A., Campos Mondin, T., Reyes, A.N., Zeni, C.P., Souza, L.D., da Silva, R.A., Jansen, K., 2015. Biological rhythm and bipolar disorder: twelve-month follow-up of a randomized clinical trial. J. Nerv. Ment. Dis. 203 (October (10)), 792–797.

Carney, C.E., Edinger, J.D., Meyer, B., Lindman, L., Istre, T., 2006. Daily activities and sleep quality in college students. Chronobiol. Int. 23, 623–637.

Chung, J.K., Lee, K.Y., Kim, S.H., Kim, E.J., Jeong, S.H., Jung, H.Y., Choi, J.E., Ahn, Y.M., Kim, Y.S., Joo, E.J., 2012. Circadian rhythm characteristics in mood disorders: comparison among bipolar I disorder, bipolar II disorder and recurrent major depressive disorder. Clin. Psychopharmacol. Neurosci. 10 (2), 110–116 (PubMed: 23430379).

Cudney, L.E., Frey, B.N., Streiner, D.L., Minuzzi, L., Sassi, R.B., 2016. Biological rhythms are independently associated with quality of life in bipolar disorder. Int. J. Bipolar Disord. 4 (December (1:9)). http://dx.doi.org/10.1186/s40345-016-0050-8. (Epub 2016 Mar 16).

Duarte Faria, A., Cardoso Tde, A., Campos Mondin, T., Souza, L.D., Magalhaes, P.V., Patrick Zeni, C., Silva, R.A., Kapczinski, F., Jansen, K., 2015. Biological rhythms in bipolar and depressive disorders: a community study with drug-naïve young adults. J. Affect Disord. 1 (November (186)), 145–148. http://dx.doi.org/10.1016/j.jad.2015.07.004. (Epub 2015 Jul 21).

Faria, A.D., de Mattos Souza, L.D., de Azevedo Cardoso, T., Pinheiro, K.A., Pinheiro, R.T., da Silva, R.A., Jansen, K., 2014. The influence of psychoeducation on regulating biological rhythm in a sample of patients with bipolar II disorder: a randomized clinical trial. Psychol. Res. Behav. Manage. 7, 167–174. http://dx.doi.org/10.2147/PRBM.S52352. (eCollection 2014).

Gershon, A., Thompson, W., Eidelman, P., McGlinchey, E.L., Kaplan, K.A., Harvey, A.G., 2012. Restless pillow, ruffled mind: sleep and affect coupling in interepisode bipolar disorder. J. Abnorm. Psychol. 121, 863–873 (PubMed: 22845651).

Giglio, L.M., Magalhaes, P.V., Andreazza, A.C., Walz, J.C., Jakobson, L., Rucci, P., Rosa, A.R., Hidalgo, M.P., Vieta, E., Kapczinski, F., 2009. Development and use of a biological rhythm interview. J. Affect. Disord. 118, 161–165.

Giglio, L.M., Magalhaes, P.V., Kapczinski, N.S., Walz, J.C., Kapczinski, F., 2010. Functional impact of biological rhythm disturbance in bipolar disorder. J. Psychiatr. Res. 44, 220–223.

Grandin, L.D., Alloy, L.B., Abramson, L.Y., 2006. The social zeitgeber theory, circadian rhythms, and mood disorders: review and evaluation. Clin. Psychol. Rev. 26, 679–694.

Gruber, J., Miklowitz, D.J., Harvey, A.G., Frank, E., Kupfer, D., Thase, M.E., Sachs, G.S., Ketter, T.A., 2011. Sleep matters: sleep functioning and course of illness in bipolar disorder. J. Affect. Disord. 134, 416–420.

Kooij, J.J., Bijlenga, D., 2013. The circadian rhythm in adult attention-deficit/hyperactivity disorder: current state of affairs. Expert Rev. Neurother. 13 (10), 1107–1116.

Levenson, J.C., Wallace, M.L., Anderson, B.P., Kupfer, D.J., Frank, E., 2015. Social rhythm disrupting events increase the risk of recurrence among individuals with bipolar disorder. Bipolar Disord. Dec; 17 (8), 869–879. http://dx.doi.org/10.1111/bdi.12351. (Epub 2015 Nov 28).

Lewy, A.J., Sack, R.L., Singer, C.M., White, D.M., Hoban, T.M., 1988. Winter depression and the phase-shift hypothesis for bright light's therapeutic effects: history, theory, and experimental evidence. J. Biol. Rhythyms 3, 121–134.

Li, J., Lu, W.Q., Beesley, S., Loudon, A.S., Meng, Q.J., 2012. Lithium impacts on the amplitude and period of the molecular circadian clockwork. PLoS One 7 (3), e33292.

Logan, R.W., Williams, W.P., McClung, C.A., 2014. Circadian rhythms and addiction: mechanistic insights and future directions. Behav. Neurosci. 128 (3), 387–412.

McCarthy, M.J., Wei, H., Marnoy, Z., Darvish, R.M., McPhie, D.L., Cohen, B.M., Welsh, D.K., 2013. Genetic and clinical factors predict lithium's effects on PER2 gene expression rhythms in cells from bipolar disorder patients. Transl. Psychiatry 3, e318.

McKenna, B.S., Drummond, S.P., Eyler, L.T., 2014. Associations between circadian activity rhythms and functional BRIAN abnormalities among euthymic bipolar patients: a preliminary study. J. Affect. Disord. 164, 101–106.

Mondin, T.C., Cardoso, T.A., Souza, L.D., Jansen, K., Magalhães da Silva, P.V., Kapczinski, F., da Silva, R.A., 2017. Mood disorders and biological rhythms in young adults: a large population-based study. J. Psychiatr. Res. 84 (January), 98–104.

Ng, T.H., Chung, K.F., Ho, F.Y., Yeung, W.F., Yung, K.P., Lam, T.H., 2015. Sleep-wake disturbance in interepisode bipolar disorder and high-risk individuals: a systematic review and meta-analysis. Sleep Med. Rev. 20, 46–58.

Nusslock, R., Frank, E., 2012. Interpersonal social rhythm therapy for bipolar disorder: an empirical review and case conceptualization. In: Markowitz, J.C., Weisman, M. (Eds.), Casebook of Interpersonal Psychotherapy. Oxford University Press, New York, pp. 103–121.

Pinho, M., Sehmbi, M., Cudney, L.E., Kauer-Sant'anna, M., Magalhães, P.V., Reinares, M., Bonnín, C.M., Sassi, R.B., Kapczinski, F., Colom, F., Vieta, E., Frey, B.N., Rosa, A.R., 2015. The association between biological rhythms, depression, and functioning in bipolar disorder: a large multi-center study. Acta Psychiatr. Scand.. http://dx.doi.org/10.1111/acps.12442. (Epub ahead of print).

Radua, J., Grunze, H., Amann, B.L., 2017. Meta-analysis of the risk of subsequent mood episodes in bipolar disorder. Psychother. Psychosom. 86 (2), 90–98. http://dx.doi.org/10.1159/000449417. (Epub 2017 Feb 10).

Roenneberg, T., Daan, S., Merrow, M., 2003. The art of entrainment. J. Biol. Rhythms 18 (3), 183–194.

Rosa, A.R., Comes, M., Torrent, C., Solè, B., Reinares, M., Pachiarotti, I., Salamero, M., Kapczinski, F., Colom, F., Vieta, E., 2013. Biological rhythm disturbance in remitted bipolar patients. Int. J. Bipolar Disord. 13 (June (1)), 6. http://dx.doi.org/10.1186/2194-7511-1-6. (eCollection 2013).

Saunders, E.F., Novick, D.M., Fernandez-Mendoza, J., Kamali, M., Ryan, K.A., Langenecker, S.A., Gelenberg, A.J., McInnis, M.G., 2013. Sleep quality during euthymia in bipolar disorder: the role of clinical features, personality traits, and stressful life events. Int. J. Bipolar Disord. 1, 16.

Shen, G.C., Alloy, L.B., Abramson, L.Y., Sylvia, L.G., 2008. Social rhythm regularity and the onset of affective episodes in bipolar spectrum individuals. Bipolar Disord. 10, 520–529 (PubMed: 18452448).

Van Tienoven, T.P., Minnen, J., Daniels, S., Weenas, D., Raaijmakers, A., Glorieux, I., 2014. Calculating the social rhythm metric (SRM) and examining its use in interpersonal social rhythm therapy (IPSRT) in a healthy population study. Behav. Sci. (Basel) 4 (3), 265–277. http://dx.doi.org/10.3390/bs4030265. (eCollection 2014).

Yatham, L.N., Kennedy, S.H., Parikh, S.V., Schaffer, A., Beaulieu, S., Alda, M., O'Donovan, C., Macqueen, G., McIntyre, R.S., Sharma, V., Ravindran, A., Young, L.T., Milev, R., Bond, D.J., Frey, B.N., Goldstein, B.I., Lafer, B., Birmaher, B., Ha, K., Nolen, W.A., Berk, M., 2013. Canadian Network for Mood and Anxiety Treatments(CANMAT) and International Society for Bipolar Disorders (ISBD) collaborative updateof CANMAT guidelines for the management of patients with bipolar disorder: update 2013. Bipolar Disord. 15 (1), 1–44.

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Social cue recognition and attributional bias in remitted bipolar disorder: Impact on social functioning

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ABSTRACT:

The role of social cue recognition and attributional bias on social functioning has been sparsely studied in remitted bipolar disorder (BD). In view of this, we evaluated thirty subjects with BD (without a history of psychotic symptoms) who were in remission and thirty age and, gender-matched healthy controls for social cue recognition [using SoCueReTI (Social Cue Recognition Test- Indian Setting)], and attributional bias (using an Attributional style questionnaire). Social functioning was assessed in subjects with BD using the Functional assessment short test (FAST) - Interpersonal relationships. Subjects with BD had significant deficits in recognizing social cues in low-intensity and high-intensity vignettes when compared to healthy controls. Deficits in recognizing social cues in low-intensity vignettes were significantly correlated with the FAST scores, even after controlling for the number of episodes and duration of illness. Further replication studies are needed to ascertain the association between social cue recognition deficits and social functioning in BD.

1. Introduction

Social cognition refers to the ability to perceive, understand, and respond to the intentions, behaviors, and dispositions of others (Green et al., 2008). It is a multifaceted construct involving multiple domains like social cue recognition, attributional bias, Theory of mind (ToM), and emotional perception (Green et al., 2008). Pooled estimates from various studies showed that ToM and emotional perception were significantly impaired in remitted bipolar disorder (BD) when compared to healthy controls (Samamé et al., 2015). Various studies that investigated the association between social cognition and functional outcome in BD have consistently found an association between emotional perception deficits and poor social functioning. Most of these studies had examined ToM and emotional perception. Only a few studies looked into other domains of social cognition, namely, social cue recognition and attributional bias (Vlad et al. 2018).

Social cue recognition (or social perception) helps to understand and appraise social roles, rules, and context. It involves using verbal and nonverbal cues to make inferences about a social situation. It thereby helps in establishing relationships and facilitating social interaction (Corrigan and Green, 1993; Savla et al. 2013). In subjects with BD, social cue recognition deficits were found, not only during the symptomatic phase of BD (Bazin et al. 2009) but also during the euthymic phase of the illness (Cusi et al. 2012). In the study by Cusi and colleagues, it was found that social cue recognition deficits negatively correlated with work/ school and marital role functioning (Cusi et al. 2012). Interestingly, BD subjects with a history of psychotic symptoms had intermediate deficits in auditory, visual, and visual-auditory decoding (emotional perception) tasks when compared to subjects with schizophrenia and to subjects with BD without a history of psychotic symptoms (Zenisek et al. 2015). Similarly, in another study, BD subjects with a history of psychotic symptoms had severe emotion recognition deficits, when compared to BD subjects without a history of psychotic symptoms. However, higher-order social cognitive domains like ToM and interpersonal problem-solving skills were not significantly different between BD subjects with or without psychotic symptoms (Thaler et al. 2013). In this context, we wished to examine inherent social cue recognition deficits of BD in those without a history of psychotic symptoms.

Attributional style/ bias, a social cognitive ability, typically helps to make inferences about the causes of positive and negative social events, linking them to internal (personal), external (other person), or situational factors (Savla et al. 2013). Externalizing bias is the tendency to over attribute positive rather than negative events to oneself, and personalizing bias is the tendency to attribute negative events to others

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rather than to situational factors (Kinderman and Bentall, 1996). Earlier studies that compared subjects with or without psychiatric illness had found attenuated externalizing bias in subjects with psychiatric illness (Bhojak et al. 1989; Mezulis et al. 2004). These studies included subjects with various psychiatric illnesses, like depression, anxiety disorder, bipolar disorder, and psychosis. Subsequently, a few studies examined attributional bias exclusively in BD (Lahera et al. 2012, 2015; Lyon et al. 1999; Stange et al. 2013a, 2013b). It is noteworthy that higher anger and intentionality biases were significantly associated with sub-threshold depressive symptoms and poor psychosocial functioning in subjects with BD (Lahera et al. 2012, 2015).

As the bulk of the research was in the western population, there were major concerns about the universality of externalizing bias in the non-western populations (Mezulis et al., 2004). Interestingly, a cross-cultural study had found a large externalizing bias among students studying in the USA (all ethnicities), but the bias was small or attenuated or even absent in students studying in the Asian countries like India and Japan (Chandler et al. 1981, Mezulis et al. 2004). Similarly, many cross-cultural studies reported that emotional recognition was better among members of the same national, ethnic, or regional groups (Elfenbein and Ambady, 2002). These cross-cultural differences could be explained by diverse individual and interpersonal cultural schemata, which influences the self-other constructs and thereby, the way one person may interpret another's actions (Green et al. 2008; Vogeley and Roepstorff, 2009). These findings highlight a strong influence of culture on the understanding and processing of attributional bias. Thus, it is clear that the tools that are used to assess social cognition in one culture may not be useful in another culture. The tools which are adapted to the Indian culture might be able to assess social cognition in the Indian population in a better way. In this context, Social Cognition Rating Tools in the Indian Setting (SOCRATIS), a tool that is adapted to the Indian context, had shown good content validity and known-groups validity to assess social cognition (Mehta et al. 2011).

We conducted this study with the following aims: 1. to compare the social cue recognition ability and attributional bias between subjects with BD (in remission) without a history of psychotic symptoms and age and gender-matched healthy controls 2. to determine whether any association exists between social cognition measures (social cue recognition and attributional bias) and social functioning in subjects with BD. We hypothesized that subjects with BD (in remission) will have social cue recognition deficits and attenuated externalizing bias when compared to healthy controls. We also hypothesized that in subjects with BD, social cue recognition deficits and attenuated externalizing bias will be directly associated with social functioning.

2. Methods

2.1. Subjects

Subjects were recruited from the outpatient services of the Department of Psychiatry in a tertiary care medical college and hospital (PSG Institute of Medical Sciences and Research, Coimbatore, India) from May 2017 to September 2018, by consecutive sampling. We included subjects with bipolar disorder ($n = 30$) and healthy controls ($n = 30$), who satisfied the following inclusion and exclusion criteria.

We included subjects with BD between the ages of 18 and 60 years, of either gender, fulfilling the DSM 5 criteria for bipolar affective disorder Type 1 using MINI plus 7.0.2 (Sheehan et al. 1998). Only those subjects with BD who were in remission as defined by scores on the 17-item Hamilton Rating Scale for Depression (HDRS) ≤ 8 Hamilton (1960) and Young Mania Rating Scale (YMRS) ≤ 6 (Young et al. 1978) for at least 8 weeks (Masand et al. 2008) were included. The symptomatic remission for the previous 8 weeks was confirmed based on clinical health records and corroboration from reliable informants. This stringent definition of euthymia was entertained because of the known influence of subsyndromal depressive symptoms over attributional bias (Lahera et al., 2012) and social functioning (Ospino et al. 2018). We only included those subjects with BD who could read and understand Tamil (the local vernacular language). Subjects with BD were excluded if they had a lifetime history of psychotic symptoms during the episodes, substance use disorders (except nicotine), and other DSM V disorder classes (assessed using MINI plus 7.0.2). The lifetime history of psychotic symptoms was carefully ascertained from the subjects with BD (using MINI plus 7.0.2) and from their caregivers whose information was assessed to be reliable. Additionally, clinical health records of all recruited subjects with BD were carefully scrutinized for a lifetime history of psychotic symptoms. Those who had severe cognitive impairment, intellectual disability, neurodegenerative disorders, general medical illness that could impair cognitive performance, hearing or visual impairment which could interfere with test performance, and those who had received electroconvulsive therapy in the previous 6 months were also excluded from the study. Age and gender-matched healthy controls were included after ascertaining the absence of a lifetime diagnosis of any DSM V disorder classes (assessed using MINI plus 7.0.2) and of neurological illness or severe cognitive impairment which could impair the test performance.

Ethics clearance was obtained from the Institutional Human Ethics Committee as per the Indian Council of Medical Research (ICMR)'s national ethical guidelines for biomedical and health research involving human participants (2017). The investigator (E.K) underwent a supervised 3-day training focusing on administration and scoring of the SOCRATIS tool.

2.2. Procedures

After obtaining informed written consent, socio-demographic data and relevant illness variables were collected from the participants and cross-checked with health records. In the case of any discrepancy, the author (E.K) cross-checked with the treating psychiatrist and the query was resolved. Socio-demographic details were assessed using the modified Kuppuswamy scale, updated for the year 2018 (Saleem, 2018). Among subjects with BD and healthy controls, the current severity of depressive symptoms was assessed using the 17-item Hamilton Depression Rating Scale (HDRS) and the current severity of mania was assessed using the Young's Mania Rating Scale (YMRS).

2.2.1. Assessment of social cue recognition

In subjects with BD and healthy controls, social cue recognition was assessed using SoCueReTI (Social Cue Recognition Test- Indian setting), a part of the SOCRATIS tool. SoCueReTI was modified and culturally adapted from the original Social cue recognition test (Corrigan and Green, 1993). SoCueReTI had shown good content and known groups validity. As shown by Mehta et al. 2011, SoCueReTI could also classify 82.5% of the subjects (patient vs controls) correctly ($p < 0.05$) with 95% confidence intervals of 0.78–0.92. A cut-off value of 0.87 had 84.2% sensitivity and 81% specificity in precisely classifying subjects. The mean Cronbach's alpha coefficient of SoCueReTI was found to be 0.78, indicating good internal consistency. The intraclass correlation coefficient for the total scores in original and Indian versions was > 0.7 for social cues in low-intensity and high-intensity vignettes/ videos, indicating good concurrent validity with the original social cue recognition test (Mehta et al. 2011). The SoCueReTI videos were enacted by Indian actors, with attire and physical setting appropriate to the Indian context. The scripts for each of the videos were translated into Tamil by a bilingual translator, followed by a back translation by another bilingual translator to ensure the quality and accuracy of the translation. In this assessment tool, four high–intensity, and four low-intensity vignettes were played for the participants depicting various social situations. An example of a low-intensity vignette - a friend who becomes socially embarrassed and leaves, when his repeated attempts to join a casual chat are not acknowledged by his friends. An example of a high-intensity vignette - a father scolding his adolescent son for having caused an

accident while speeding on his new motorbike. The low-intensity vignettes/ videos will have a lesser degree of emotional arousal. Hence it will be difficult to perceive and recognize the social cues in the low-intensity vignettes when compared to the high-intensity vignettes. During each video, the participants were presented with 18 cues - 9 abstract or social cues and 9 concrete or non-social cues. At the end of each video, the participants were asked to respond to a series of 'true or false' questions related to the enacted social situation in the video. Every correct response to the 'true or false' questions pertaining to recognition of a social cue earned a score of 1 and every incorrect response received no score. If a participant correctly recognized all the 9 social cues in each of the eight videos, he/she could receive a maximum score of 72. The total score thus received by each participant was divided by the maximum possible score (i.e. 72) to obtain a social perception index . Thus, lower social perception index scores indicate higher social cue recognition deficits.

2.2.2. Assessment of attributional style

Attributional bias was assessed in subjects with BD, and healthy controls, using the Attributional style questionnaire, a part of SOCRATIS. This questionnaire was modified from the Internal, Personal, and Situational Attributions Questionnaire (IPSAQ) (Kinderman and Bentall, 1996) and was culturally adapted to the Indian context (Mehta et al. 2011). The Attribution style questionnaire has shown good content validity and known-groups validity. The mean attribution scores were much lower in subjects with schizophrenia when compared to the healthy controls. Among subjects with schizophrenia, the mean Cronbach's alpha coefficient was found to be 0.71 for all the items, 0.75 for the positive items, and 0.57 for the negative items (Mehta et al. 2011). In our study, a total of 32 hypothetical social situations were read to the participants (e.g., a friend refused to talk to you). The participants were asked to make causal attributions that were classified as internal (related to self) and external (personal or situational). Two bias scores, namely externalizing bias and personalizing bias were calculated from the responses.

Externalizing bias (EB) scores were calculated as follows:

$EB = P_i\text{-}N_i$, where P_i represents the number of positive events, for which the subject made internal attributes (i.e., attributed them to something within himself/ herself) and N_i represents the number of negative events, for which the participant made internal attributes. A positive EB score indicates that the participant attributes more positive events preferentially to himself/ herself in contrast to negative events, indicating a strong self-serving/ externalizing bias.

Personalizing bias (PB) scores were calculated as follows:

$PB = N_{ep} / N_{ep} + N_{es}$, where N_{ep} represents the number of negative events which the participants attribute to people (external personal attributes), and N_{es} represents the number of negative events which the participants attribute to situations (external situational attributes). A PB score over 0.5 indicated a tendency to use personal rather than situational external attributions for negative events.

2.2.3. Assessment of social functioning

Social functioning was assessed in subjects with BD using the Interpersonal relationships sub-item of the Functional Assessment Short Test (FAST). This sub-item assesses 6 areas, namely, maintaining a friendship, participating in social activities, having a good relationship with people close to them, living together in family, having satisfactory sexual relationships and being able to defend their interests, on a four point Likert scale. A score of 0 indicates no difficulty and a score of 3 indicates severe difficulty. Higher scores indicate higher impairment in social functioning (Rosa et al. 2007).

2.3. Statistical analysis

The normality of data was checked using the Shapiro-Wilk test, which showed that data was non-normally distributed. Between-group differences (BD vs healthy controls) among socio-demographic variables were tested with the Mann-Whitney *U* test for continuous variables and Fisher's exact test for discrete variables. Among the two groups, scores of social cue recognition and attributional bias were tested for any statistically significant difference, using the nonparametric Mann-Whitney *U* test with the significance level set at $p<0.05$. Effect size (for the nonparametric tests) was calculated using Cohen $r = z / \sqrt{N}$ (Fritz et al. 2012).The effect size was considered large, medium and small if Cohen's r was 0.5, 0.3, and 0.1, respectively Coolican (2009). Spearman's correlation was used to find out the correlation among illness variables, social cue recognition, attributional bias, and social functioning (FAST–interpersonal relationships) in subjects with BD. All statistical analyses were performed using IBM SPSS statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Socio-demographic and illness details

The median age of subjects with BD and healthy controls was 36 years (IQR = 32–40.5) and 34.5 years (IQR = 29.5–38), respectively, with no significant difference between the groups ($U = 375$, $p = 0.27$). A higher percentage of subjects with BD were unmarried and belonged to low and middle socioeconomic status when compared to healthy controls. There was no statistically significant difference in gender, education, and occupation between subjects with BD and healthy controls (Table 1). The average number of episodes was 3.63 ± 1.67 and the average duration of illness was approximately 6.5 years. While the mean

Table 1
Socio-demographic characteristics of subjects with Bipolar disorder (*n*=30) and Healthy controls (*n*=30):

S No.	Characteristics	Subjects with BD			Healthy Controls			*p*
		Q1	Q2 (M)	Q3	Q1	Q2 (M)	Q3	
1.	**Age**†	32	36	40.5	29.5	34.5	38	0.266
			***n* (%)**			***n* (%)**		
2.	**Gender**‡							0.76
	Male		15 (50)			17(56.7)		
	Female		15 (50)			13(43.3)		
3.	**Education**‡$							0.11
	Professional		11(36.7)			11(36.7)		
	Graduate		3(10)			4(13.3)		
	Intermediate		3(10)			4(13.3)		
	High-school		13(43.3)			10(33.3)		
	Illiterate		-			1(3.3)		
4.	**Occupation**‡$							0.22
	Professional		5(16.7)			8 (26.7)		
	Semi - professional		1(3.3)			5 (16.7)		
	Clerical /shopowner/ farmer		4(13.3)			3(10.0)		
	Skilled		11(36.7)			11(36.7)		
	Unskilled		1(3.3)			-		
	Unemployed		8(26.7)			3(10.0)		
5.	**Socio – economic status**‡ $							0.003*
	Upper		-			4(13.3)		
	Middle		11(36.7)			11(36.6)		
	Lower		19(63.3)			15(50)		
6.	**Marital status** ‡							0.001*
	Unmarried		7(23.3)			1(3.3)		
	Married		23 (76.6)			29(96.7)		

* = significant if $p<0.05$, as measured by Fisher's exact test; Q1 - first quartile, Q2(M) - Median, Q3- third quartile
†= tested using Mann- Whitney *U* test,
‡ = tested using fisher's exact test,
$- based on modified Kuppuswamy scale, updated for the year 2018

HDRS and YMRS scores in subjects with BD were 3.67 ± 1.24 and 4.10 ± 1.19 respectively, the mean HDRS and YMRS scores in healthy controls were 0.78 ± 0.34 and 1.2 ±0.42, respectively. Twenty-four (80%) subjects with bipolar disorder were on mood stabilizers (lithium or divalproex sodium or a combination of the two), while six (20%) were on antipsychotic monotherapy (Table 2).

3.2. Comparison of social cue recognition between subjects with BD and healthy controls

Subjects with BD had statistically significant deficits in recognizing social cues (abstract) in both high-intensity ($p < 0.001$, ES = 0.29) and low-intensity ($p < 0.001$, ES = 0.31) vignettes when compared to healthy controls, with a medium effect size (ES). With respect to recognition of non-social (concrete) cues, subjects with BD had statistically significant deficits only in low-intensity vignettes ($p = 0.005$, ES = 0.31), but not in high-intensity vignettes (Table 3). None of the illness variables like age, the total number of episodes, age of onset of illness, duration of illness, HDRS scores, and YMRS scores had any statistically significant correlation with social cue recognition (Table 7).

3.3. Comparison of attributional bias between subjects with BD and healthy controls

Subjects with BD attributed less positive events (P_i) ($U = 197.5$, $p < 0.01$) and more negative events (N_i) ($U = 158$, $p < 0.01$) to something within oneself, when compared to healthy controls, which suggested attenuated externalizing bias or self-serving bias in subjects with BD ($U = 150.8$, $p < 0.01$) (Table 4). Subjects with BD were found to attribute negative events more to situational external factors than to personal external factors (personalizing bias), though the difference was not statistically significant as compared to healthy controls ($p = 0.119$). In subjects with BD, gender (males being 50%) did not have any significant influence on the scores of P_i ($p = 0.74$), N_i ($p = 0.74$), externalizing bias ($p = 0.266$) or personalizing bias ($p = 0.706$) (Table 6). Neither the illness variables like duration of illness, the total number of episodes, age of onset nor the social cue recognition scores had a significant correlation with attributional bias scores (Table 7). In subjects with BD, age was positively correlated only with P_i ($r = 0.37$, $p = 0.04$), which suggested that higher age was associated with attributing positive events to something within oneself.

Table 2
Illness characteristics in subjects with bipolar disorder (n=30):

S No.	Characteristics	Mean ± S.D	n(%)
1.	Age (years)	35 ± 8.477	
2.	Total number of episodes	3.63 ± 1.671	
3.	Number of manic episodes	2.13 ± 1.074	
4.	Number of depressive episodes	1.87 ± 1.359	
5.	Duration of illness (months)	77.13 ± 57.52	
6.	Number of episodes requiring hospitalization	2.25 ± 0.989	
7.	YMRS Scores	4.10 ± 1.185	
8.	HAMD Scores	3.67 ± 1.241	
9.	Current medications		
	Mood stabilizers *		24 (80)
	Lithium (812.43 ± 155.4 mg)		16 (53.33)
	Divalproex sodium (1234.45 ± 323.22 mg)		10 (33.33)
	Antipsychotics		22 (73.33)
	Mean chlorpromazine equivalents(mg)	37.5 ± 10.5	
	Benzodiazepines		0(0)

* = As some subjects were on combination of lithium and divalproex sodium, the percentage will not match with mood stabilizers

3.4. Correlation among illness variables, social cue recognition, attributional biases, and social functioning in subjects with bipolar disorder

In subjects with BD, the median value of their social functioning score was 6 (IQR = 6-12). Social functioning impairment was found to be significantly associated with longer duration of illness ($r = 0.434$, $p = 0.017$), higher number of episodes ($r = 0.41$; $p = 0.048$) (Table 7) and deficits in social cue recognition in low-intensity vignettes ($r = -0.47$, $p = 0.02$) (Table 5). The other illness variables (total number of manic episodes, total number of depressive episodes, and total number of episodes that required hospitalization) and attributional biases (externalizing and personalizing biases) did not correlate significantly with social functioning (Table 7). There was a small but significant negative partial correlation between social functioning and social cue recognition deficits in low-intensity vignettes ($r = -0.27$, df = 25, $p = 0.04$), after controlling for illness variables, namely duration of illness and the total number of episodes.

4. Discussion

This study found statistically significant deficits in social cue recognition and attenuated externalizing bias among remitted BD type 1 subjects (without a history of psychotic symptoms) when compared to age and gender-matched healthy controls. Social cue recognition deficits, but not externalizing bias, were significantly associated with social functioning impairment among subjects with BD.

4.1. Social cue recognition deficits in bipolar disorder

The finding of significant deficits in social cue recognition in euthymic BD is in line with previous studies (Bazin et al. 2009; Cusi et al. 2012). Bazin and colleagues had previously evaluated social cue recognition in symptomatic subjects with BD. They had found that subjects with mania had more deficits in attributing intention to implicit cues (Bazin et al. 2009). Cusi and colleagues in their study found that asymptomatic subjects with BD also had significant impairment in making judgments about social situations (Cusi et al. 2012). In line with the previous studies, there was no significant relationship among affective disorder burden indicators (earlier age of onset, longer duration of illness, a greater number of affective episodes), YMRS, HDRS, and social cue recognition (Bora et al. 2005; Cusi et al. 2012). Since the present study included only the subjects with BD without a history of psychotic symptoms, it may not be able to clarify whether these deficits were in anyway different from the subjects with BD who have a history of psychotic symptoms.

In this study, subjects with BD without a history of psychotic symptoms had significant deficits in decoding and reasoning of social and non-social cues when compared to healthy controls. The deficits in decoding social cues were significant in both low & high-intensity vignettes, while deficits in decoding non-social cues were evident only in low-intensity vignettes. The relationship between social cue recognition and the level of situational arousal can be explained based on the limited capacity model of information processing (Nuechterlein and Dawson, 1984) The recognition of social cues requires a greater degree of processing capacity when compared to the recognition of non-social cues. Similarly, processing capacity is more efficient during the middle levels of arousal, when compared to extreme levels of arousal. Overall, the deficits could be more evident in high attention-demanding tasks, namely, social cue recognition in low-intensity situations. Thus, the social cue recognition deficits seen in subjects with BD might imply that they were less conservative in considering rules, goals, and affect when asked to recognize cues on the more difficult cognitive tasks. This could represent a deficit in over-inclusive thinking (Corrigan and Nelson, 1998).

The presence of social cue recognition deficits in the remitted phase of BD, raises a question: whether the observed deficits in social cue

Table 3
Comparison of social cue recognition scores between subjects with bipolar disorder ($n = 30$) and healthy controls ($n = 30$):

S.no	Social cue recognition subitems	Subjects with BD			Healthy controls			*U*	*z*	*p*	Effect size, cohen *r* =z / √N
		Q1	**Q2(M)**	**Q3**	**Q1**	**Q2(M)**	**Q3**				
1.	Social cues - high intensity vignettes	0.69	0.83	0.89	0.82	0.89	0.92	297	-2.31	0.021	0.29*
2.	Social cues - low Intensity vignettes	0.74	0.81	0.86	0.82	0.86	0.89	288.5	-2.43	0.015	0.31*
3.	Social cues -total	0.72	0.81	0.89	0.82	0.89	0.89	291.5	-2.363	0.019	0.30*
4.	Non-social cues – high intensity vignettes	0.75	0.83	0.89	0.86	0.92	0.94	241.5	-2.13	0.23	0.27
5.	Non-social cues – low intensity vignettes	0.75	0.82	0.89	0.83	0.89	0.89	264.5	-2.80	0.005	0.36*
6.	Non-social cues - total	0.75	0.82	0.89	0.86	0.89	0.90	233	-2.23	0.12	0.28

* - if *p* value is < 0.05, it is statistically significant, *N* = number of participants in both groups; Q1 - first quartile, Q2(M) - Median, Q3 - third quartile

Table 4
Comparison of Attributional bias (AB) scores between subjects with bipolar disorder ($n = 30$) and healthy controls ($n = 30$):

S.no	AB sub items	Subjects with BD			Healthy controls			*U*	z	*p*	Effect size cohen *r* =z / √N
		Q1	**Q2(M)**	**Q3**	**Q1**	**Q2(M)**	**Q3**				
1.	P_i	5	6.5	8	6.75	15.5	16	197.5	-3.781	<0.001	0.48*
2.	N_i	3	4	5	0	1	1.25	158	-4.38	<0.001	0.56*
3.	EB	1	2	5	6	15	16	150.8	-4.6	<0.001	0.59*
4.	PB	0.3	0.5	0.7	0.4	0.5	0.8	345.5	-1.56	0.119	0.20

P_i = number of positive events, for which the subject made internal attributes, N_i= number of negative events, for which the subject made internal attributes, EB = externalizing bias, PB= personalizing bias. * - if *p* value is < 0.05, it is statistically significant, N= number of participants in both groups. Q1 - first quartile, Q2(M) - Median, Q3 - third quartile

Table 5
Correlation among attributional bias (AB) scores, social cue recognition scores (SCR), and social functioning scores (FAST – interpersonal relationships) in subjects with bipolar disorder ($n = 30$):

Characteristics	P_i	N_i	EB	PB	SCR high intensity	SCR low intensity	SCR total	Social functioning
P_i	1	-0.50*	0.16	0.22	-0.06	0.07	0.01	0.17
N_i		1	0.14	0.22	-0.26	-0.15	-0.23	-0.31
EB			1	0.33	-0.28	-0.21	-0.28	0.16
PB				1	-0.24	-0.28	-0.31	-0.18
SCR high intensity					1	0.69	0.94	-0.02
SCR low intensity						1	0.85	-0.47*
SCR total							1	-0.34
Social functioning								1

P_i = number of positive events, for which the subject made internal attributes, N_i= number of negative events, for which the subject made internal attributes, EB = externalizing bias, PB= personalizing bias, SCR= social cue recognition scores,* - if *p* value is < 0.05, it is statistically significant, N= number of participants in both groups

Table 6
Attributional bias in subjects with BD, stratified by gender ($n = 30$):

S.no	Characteristics	Males			Females			*U*	*p*
		Q1	**Q2(M)**	**Q3**	**Q1**	**Q2(M)**	**Q3**		
1.	Pi	6	6	8	5	7	7	104.5	0.74
2.	Ni	3	5	5	2	4	5	104.5	0.74
3.	Externalizing bias	1	2	7	0.3	1	5	86	0.27
4.	Personalizing bias	3	5	7	0.2	0.5	0.7	103.5	0.71

M – median; Q1 - first quartile, Q2(M) - Median, Q3 - third quartile, *U* – Mann Whitney *U* statistic, P_i = number of positive events, for which the subject made internal attributes, N_i= number of negative events, for which the subject made internal attributes.

recognition existed even before the onset of BD (as an endophenotype) or whether it is due to the cumulative disease burden? Seidel and colleagues found a trend towards significant difference in the accuracy of emotion recognition, among subjects with BD, their unaffected first-degree relatives, and healthy controls (Seidel et al. 2012). In contrast, de Brito Ferreira Fernandes and colleagues did not find any significant difference in emotional recognition among unaffected first-degree relatives and healthy controls (de Brito Ferreira Fernandes et al. 2016). A few other studies found no significant difference in emotional recognition between youth with familial risk of BD and healthy controls (Brotman et al. 2008; Christiani et al. 2019; McCormack et al. 2016; Meluken et al. 2019). To date, there are no studies that have probed into social cue recognition in unaffected first-degree relatives of BD or youth with familial risk of BD. Thus, prospective cohort studies in at-risk populations might be able to decipher whether social cue recognition deficits exist even before the onset of the illness or appear during the course of the illness.

There is accumulating evidence that culture can influence social cue recognition. The social situations are processed in a contextual, interpersonal way in the Chinese population rather than individualistic way as in the western population (Hong et al. 2003; Triandis, 2001). A cross-cultural study found that participants from Japan tended to interpret social situations and make social judgments based more on relationships when compared to participants from America (Matsuda

Table 7
Correlation among illness variables, social cue recognition, attributional bias and social functioning

	Age	Total no. of episodes (TE)	No. of manic episodes (ME)	No.of depressive episodes(DE)	Duration of illness (DILL)	No. of Episodes which required hospitalisation (DurHosp)	YMRS	HAMD	NonSCR	SCR	EB	PB	FAST-IPR
Age	1	0.45*	0.07	0.42*	0.26	0.34	0.10	0.27	-0.06	-0.02	-0.12	-0.08	0.23
TE		1	0.62*	0.89**	0.80**	0.73**	0.15	0.29	0.06	0.16	0.13	-0.17	0.41*
ME			1	0.28	0.62**	0.67	0.23	-0.02	0.04	0.21	0.24	0.07	0.09
DE				1	0.60**	0.55**	-0.004	0.30	0.21	0.19	0.12	-0.38	0.39
DILL					1	0.61**	0.17	0.27	-0.01	0.25	0.10	-0.25	0.43*
DurHosp						1	-0.13	0.14	0.11	0.20	-0.06	-0.21	0.11
YMRS							1	0.38*	-0.35	-0.19	-0.05	0.02	0.06
HAMD								1	-0.19	0.04	-0.16	-0.14	0.27
NonSCR									1	-0.81**	0.21	-0.15	0.07
SCR										1	-0.28	-0.31	0.34
EB											1	0.33	0.16
PB												1	-0.18
FAST-IPR													1

YMRS- Young Mania Rating Scale, HAMD – Hamilton Depression Rating Scale, SCR- social/abstract cue recognition scores, NonSCR – non-social/ concrete cue recognition scores, EB = externalizing bias, PB= personalizing bias, FAST- IPSR- Interpersonal relationship sub item of Functioning Short Assessment Test,* - if p value is < 0.05, it is statistically significant

and Nisbett, 2001). In this context, the use of a valid instrument that is culturally adapted to the Indian context, like SoCueReTI, can be considered as a strength of this study.

In this study, all subjects with BD were on medications, predominantly lithium, divalproex sodium, or low dose antipsychotics (mean chlorpromazine equivalents being 37.5 ± 10.5 mg). It is not clear whether the observed social cue recognition deficits are a result of the direct influence of pharmacological agents or secondary to the effect of these agents on the control of mood symptoms. Given the absence of an unmedicated remitted BD group, the exclusive effect of medications on social cue recognition could not be measured in this study. At the same time, it can be quite challenging to find unmedicated remitted subjects with BD. Additionally, in schizophrenia, the evidence for antipsychotic related improvement in social cue recognition was inconclusive. While a study with a small sample size showed a differential improvement in social cue recognition with Olanzapine when compared to typical antipsychotics (Littrell et al. 2004), another study with a large sample size showed no such association (Sergi et al. 2007). To our knowledge, there are no studies that systematically compared the distinctive effects of antipsychotics or mood stabilizers on social cue recognition in BD.

4.2. Attributional bias in bipolar disorder

We found attenuated externalizing bias in remitted BD. It had no association with any of the illness burden variables or social cue recognition deficits. A previous study had compared attributional bias among 3 groups, namely, euthymic, subsyndromal depressed, and clinically depressed (based on HDRS) subjects with BD. It showed that euthymic subjects obtained much lower scores in aggression bias when compared to subjects with subsyndromal depression and clinical depression. It was also found that there was no significant difference in attributional bias between subsyndromal depressed and clinically depressed subjects with BD, but the severity of depression significantly correlated with global functional impairment (Lahera et al. 2012). Similarly, a positive association was found between the severity of subsyndromal depressive symptoms and impaired ToM in subjects with BD (McKinnon et al. 2010). Taking all this into consideration, exclusion of the subjects who had subsyndromal or syndromal depression using a narrow definition of euthymia (HDRS ≤ 8, YMRS ≤ 6), could be considered as a strength of our study. Interestingly, studies that had examined attributional bias in BD reported that extreme attributions (positively and negatively valenced) were found to be associated with a longer recovery time, lower likelihood of recovery, and a higher likelihood for a transition from depression to hypomania or mania (Stange et al. 2013a, Stange et al. 2013b).

In a meta-analysis, it was found that women had a smaller self-serving attributional bias when compared to men during adulthood (Mezulis et al. 2004). But in this study, neither age nor gender showed any trend for such association. It should be noted that the self-serving bias was found to be smaller in the individuals of the Asian culture (Japan, Pacific Islander, India, China and Korea) when compared to those of the USA or western culture, though there was a significant variability in the magnitude of bias among the different Asian cultures (Bond et al. 1982; Mezulis et al. 2004). In the Asian culture, self is perceived as interdependent; sharing responsibility for success and assuming responsibility for failure is considered the most adaptive way to maintain self-esteem. Moreover, there is a higher likelihood of being liked by others if they have more self-effacing or self-enhancing attributions rather than self-serving attributions. Hence studies in the future should also consider this cultural context in the interpretation of attributional bias.

4.3. Social functioning in remitted bipolar disorder

In the present study, social functioning was negatively correlated with social cue recognition deficits, especially in low-intensity vignettes, even after controlling for duration of illness and number of episodes. It has also been reported previously that marital and work/ school role domain functioning was negatively correlated with social cue recognition deficits (Cusi et al. 2012). It was found that subjects need to have a higher degree of processing capacity to identify social cues in high attention-demanding tasks, namely low-intensity vignettes (Corrigan and Green, 1993) . Probably, the insensitivity to subtle covert or overt cues about the goal, rules, and affect of a social situation, could have a substantial role in guiding complex interpersonal interactions.

In our study, there was no association between attributional bias and social functioning which is in contrast to a previous study that had reported a significant association (Lahera et al. 2015). It should be noted that in this study, the selected subjects with BD had a lesser number of mood episodes and duration of illness when compared to the study by Lahera and colleagues. A study carried out by our research group in the same study population, showed that ToM did not predict social functioning in remitted BD (Palaniappan and Easwaran, 2020).

Subjects with BD have a greater degree of deficits in identifying negatively valenced emotions when compared to positively valenced emotions (Vogel et al. 2016). Various studies have demonstrated that accurate identification of facial affect especially happiness, fear, and disgust, are associated with better social functioning. Deficits in

emotional discrimination had been associated with impairments in daily life, autonomy, relationships, and occupational areas of functioning during the remitted phase of BD (Vlad et al. 2018). Thus, facial affect recognition could be one important prerequisite or even a part of the ability to identify the social cues correctly in a complex social situation.

A meta-analysis carried out in euthymic BD reported impairment in multiple neuropsychological domains like executive functions, working memory, verbal memory, and psychomotor speed, with relative preservation of verbal ability (Mann-Wrobel et al. 2011). While there was one study that did not find any association between neurocognition and community functioning in euthymic BD (Malhi et al. 2007), a meta-analysis found a weak association (Depp et al. 2012). In schizophrenia, social cognition seems to mediate the relationship between neurocognition and functioning (Schmidt et al. 2011), but in BD, social cognition moderated the relationship between neurocognition and functioning. This implies that neurocognition contributed to a better community functioning in subjects with BD who had poor social cognition (scoring 1 S.D below the mean) but not in those with good social cognition (Ospino et al. 2018). It is noteworthy that these studies have explored the role of emotion perception and ToM on functioning but have not looked into the role of social cue recognition on social functioning in euthymic BD.

In subjects with BD, deficits in neurocognition and emotional recognition were found to moderate or even be a prerequisite for a certain aspect of social cognition processing, especially ToM (Olley et al. 2005; Wolf et al. 2010). At this juncture, we need studies to explore the roles of these factors on social cue recognition and social functioning in euthymic BD (Cusi et al. 2012; Green et al. 2008). A few studies that evaluated the effects of social cognitive treatments like Social Cognition and Interaction Training (SCIT) (Lahera et al. 2013; Zhang et al. 2019), had reported improvement in attributional bias, emotional processing, ToM, and functioning. However, these studies had not examined social cue recognition. So, interventional studies, incorporating strategies to improve social cue recognition, are needed in the near future.

4.4. Limitations

The present study has not examined the influence of confounding variables like neurocognition, emotional perception, and medications on social functioning . Similarly, this study did not assess for the presence of personality disorders and thus its influence on the attributional style cannot be ruled out. Additionally, since the present study was done on subjects with bipolar disorder type 1 (in remission), without a history of psychotic symptoms, the findings cannot be generalized to those with a history of psychotic symptoms. Though the history of psychotic symptoms was looked for meticulously, we cannot rule out the possibility of recall bias. However, this study has given a preliminary inference that social cue recognition deficits and attributional bias may be independent of the lifetime history of psychotic symptoms, and thereby inherent to BD itself. Apart from this, the remission status was ascertained based on clinical health records and caregivers who had reliable information regarding their health status. It would have been more methodologically rigorous if the subjects with BD were recruited after ascertaining 8 weeks of remission status based on serial administration of HAMD & YMRS. In addition, though the psychometric properties of FAST are well established in BD, a scale that measures functional impairment based not only on the information from the subjects with BD but also from other corroborating reliable informants, would have added credibility to the findings. Also, social functioning was not measured in healthy controls in this study, which may be a notable limitation. Finally, the small sample size could have limited the interpretation of study findings.

Conclusion

Social cue recognition deficits and attenuated externalizing bias were found during the remitted phase of BD, even in those who did not have a history of psychotic symptoms. Further studies comparing BD subjects with or without a history of psychotic symptoms might help us to explain the influence of psychotic symptoms over these deficits. Though there seems to be an association between social cue recognition deficits and social functioning impairment in BD (even during remission), further replication studies may be required to understand the role of social cue recognition in social functioning, thereby aiding in the development of effective psychosocial interventions in bipolar disorder.

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Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

Authorship

All listed authors meet the ICMJE criteria. We attest that all authors contributed significantly to the creation of this manuscript, each having fulfilled criteria as established by the ICMJE. We believe these individuals should be listed as authors because:

1. Krishnapriya Easwaran was involved in conceptualization, design & data collection. She was instrumental in writing draft and gave valuable inputs for framing the paper. She was also involved in final approval of the version and will take responsibility for integrity of the contents and data.

2. Pradeep Palaniappan had been involved in conceptualization, design of the study; analyzed the results; and had given valuable inputs to content of the paper. He also had involved in final approval of the version and take responsibility for integrity of the contents.

We confirm that the manuscript has been read and approved by all named authors. We confirm that the order of authors listed in the manuscript has been approved by all named authors.

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References

Bazin, N., Brunet-Gouet, E., Bourdet, C., et al., 2009. Quantitative assessment of attribution of intentions to others in schizophrenia using an eological video-based task: a comparison with manic and depressed patients. Psychiatry Res 167 (1-2), 28–35. https://doi.org/10.1016/j.psychres.2007.12.010.

Bhojak, M., Vyas, J., Nathawat, S., Vijayvergia, D., 1989. A study of attributional style in depression compared with schizophrenic and normal patients. Indian J. Psychiatry 31, 116–121.

Bond, M.H., Leung, K., Wan, K.C., 1982. The social impact of self effacing attributions: the Chinese case. J. Soc. Psychol. 118, 157–166.

Bora, E., Vahip, S., Gonul, A.S., et al., 2005. Evidence for theory of mind deficits in euthymic patients with bipolar disorder. Acta Psychiatr Scand. 112 (2), 110–116. https://doi.org/10.1111/j.1600-0447.2005.00570.x.

Brotman, M.A., Guyer, A.E., Lawson, E.S., Horsey, S.E., Rich, B.A., Dickstein, D.P., Pine, D.S., Leibenluft, E., 2008. Facial emotion labeling deficits in children and adolescents at risk for bipolar disorder. Am. J. Psychiatry 165 (3), 385–389. https://doi.org/10.1176/appi.ajp.2007.06122050. MarEpub 2008 Feb 1. PMID: 18245180.

Christiani, C.J., Jepsen, J.R.M., Thorup, A., Hemager, N., Ellersgaard, D., Spang, K.S., Burton, B.K., Gregersen, M., Søndergaard, A., Greve, A.N., Gantriis, D.L., Poulsen, G., Uddin, M.J., Seidman, L.J., Mors, O., Plessen, K.J., Nordentoft, M., 2019. Social cognition, language, and social behavior in 7-year-old children at familial high-risk of developing schizophrenia or bipolar disorder: the Danish high risk and resilience study VIA 7-A population-based cohort study. Schizophr Bull. Oct 24 (6), 1218–1230. https://doi.org/10.1093/schbul/sbz001, 45PMID: 30852621; PMCID: PMC6811824.

Chandler, T., Shama, D., Wolf, F., Planchard, S., 1981. Multiattributional causality: A five cross-national samples study. J. Cross-Cult. Psychol. 12, 207–221.

Coolican, H., 2009. Research Methods and Statistics In Psychology. Hodder, London, United Kingdom.

Corrigan, P.W., Green, M.F., 1993. Schizophrenic patients' sensitivity to social cues: the role of abstraction. Am. J. Psychiatry 150 (4), 589–594. https://doi.org/10.1176/ajp.150.4.589.

Corrigan, P.W., Nelson, D.R., 1998. Factors that affect social cue recognition in schizophrenia. Psychiatry Res. 78 (3), 189–196. https://doi.org/10.1016/s0165-1781(98)00013-4.

Cusi, A.M., Macqueen, G.M., McKinnon, M.C., 2012. Patients with bipolar disorder show impaired performance on complex tests of social cognition. Psychiatry Res. 200 (2-3), 258–264. https://doi.org/10.1016/j.psychres.2012.06.021.

de Brito Ferreira Fernandes, F., Gigante, A.D., Berutti, M., Amaral, J.A., de Almeida, K. M., de Almeida Rocca, C.C., Lafer, B., Nery, F.G., 2016. Facial emotion recognition in euthymic patients with bipolar disorder and their unaffected first-degree relatives. Compr. Psychiatry 68, 18–23. https://doi.org/10.1016/j.comppsych.2016.03.001. JulEpub 2016 Mar 10. PMID: 27234178.

Depp, C.A., Mausbach, B.T., Harmell, A.L., Savla, G.N., Bowie, C.R., Harvey, P.D., Patterson, T.L., 2012. Meta-analysis of the association between cognitive abilities and everyday functioning in bipolar disorder. Bipolar Disord 14 (3), 217–226. Maydoi: 10.1111/j.1399-5618.2012.01011.x. PMID: 22548895; PMCID: PMC3396289.

Elfenbein, H.A., Ambady, N., 2002. On the universality and cultural specificity of emotion recognition: a meta-analysis. Psychol. Bull. 128 (2), 203–235. https://doi.org/10.1037/0033-2909.128.2.203. MarPMID: 11931516.

Fritz, C.O., Morris, P.E., Richler, J.J., 2012. Effect size estimates: current use, calculations, and interpretation. J. Exp. Psychol. Gen. 141 (1), 2–18.

Green, M.F., Penn, D.L., Bentall, R., et al., 2008. Social cognition in schizophrenia: an NIMH workshop on definitions, assessment, and research opportunities. Schizophr Bull. 34 (6), 1211–1220. https://doi.org/10.1093/schbul/sbm145.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry 23 (1), 56–62. https://doi.org/10.1136/jnnp.23.1.56. FebPMID: 14399272; PMCID: PMC495331.

Hong, Y.-Y., Benet-Martinez, V., Chiu, C.-Y., Morris, M.W, 2003. Boundaries of cultural influence: Construct activation as a mechanism for cultural differences in social perception. J. Cross. Cult. Psychol. 34, 453–464. https://doi.org/10.1177/0022022103034004005.

Kinderman, P., Bentall, R.P., 1996. A new measure of causal locus: the internal, personal and situational attributions questionnaire. Pers. Individ. Differ. 261–264.

Lahera, G., Benito, A., González-Barroso, A., et al., 2012. Social-cognitive bias and depressive symptoms in outpatients with bipolar disorder. Depress Res. Treat. 2012, 670549 https://doi.org/10.1155/2012/670549.

Lahera, G., Benito, A., Montes, J.M., Fernández-Liria, A., Olbert, C.M., Penn, D.L., 2013. Social cognition and interaction training (SCIT) for outpatients with bipolar disorder. J. Affect. Disord. 146 (1), 132–136. https://doi.org/10.1016/j.jad.2012.06.032.

Lahera, G., Herrera, S., Reinares, M., et al., 2015. Hostile attributions in bipolar disorder and schizophrenia contribute to poor social functioning. Acta Psychiatr. Scand. 131 (6), 472–482. https://doi.org/10.1111/acps.12399.

Littrell, K.H., Petty, R.G., Hilligoss, N.M., Kirshner, C.D., Johnson, C.G., 2004. Improvement in social cognition in patients with schizophrenia associated with treatment with olanzapine. Schizophr Res. Feb 1 (2-3), 201–202. https://doi.org/10.1016/S0920-9964(03)00185-3, 66PMID: 15061258.

Lyon, H., Startup, M., Bentall, R., 1999. Social cognition and the manic defense: Attributions, selective attention, and self-schema in bipolar affective disorder. J. Abnorm. Psychol. 108, 273–282.

Malhi, G.S., Ivanovski, B., Hadzi-Pavlovic, D., Mitchell, P.B., Vieta, E., Sachdev, P., 2007. Neuropsychological deficits and functional impairment in bipolar depression, hypomania and euthymia. Bipolar Disord. 9 (1-2), 114–125. Feb-Mardoi: 10.1111/j.1399-5618.2007.00324.x. PMID: 17391355.

Mann-Wrobel, M.C., Carreno, J.T., Dickinson, D., 2011. Meta-analysis of neuropsychological functioning in euthymic bipolar disorder: an update and investigation of moderator variables. Bipolar Disord. 13 (4), 334–342. https://doi.org/10.1111/j.1399-5618.2011.00935.x. JunPMID: 21843273.

Masand, P.S., Eudicone, J., Pikalov, A., et al., 2008. Criteria for defining symptomatic and sustained remission in bipolar I disorder: a post-hoc analysis of a 26-week aripiprazole study (study CN138-010). Psychopharmacol Bull. 41 (2), 12–23. PMID: 18668014.

McKinnon, M.C., Cusi, A.M., Macqueen, G.M., 2010. Impaired theory of mind performance in patients with recurrent bipolar disorder: moderating effect of cognitive load. Psychiatry Res. 177 (1-2), 261–262. https://doi.org/10.1016/j.psychres.2010.02.004.

McCormack, C., Green, M.J., Rowland, J.E., et al., 2016. Neuropsychological and social cognitive function in young people at genetic risk of bipolar disorder. Psychol Med. 46 (4), 745–758. https://doi.org/10.1017/S0033291715002147. MarEpub 2015 Dec 1. PMID: 26621494.

Mehta, U.M., Thirthalli, J., Naveen Kumar, C., et al., 2011. Validation of social cognition rating tools in Indian Setting (SOCRATIS): a new test-battery to assess social cognition. Asian J. Psychiatr 4 (3), 203–209. https://doi.org/10.1016/j.ajp.2011.05.014.

Meluken, I., Ottesen, N.M., Harmer, C.J., et al., 2019. Is aberrant affective cognition an endophenotype for affective disorders? - A monozygotic twin study. Psychol. Med. 49 (6), 987–996. https://doi.org/10.1017/S0033291718001642. AprEpub 2018 Jul 2. PMID: 29962367.

Mezulis, A.H., Abramson, L.Y., Hyde, J.S., Hankin, B.L., 2004. Is there a universal positivity bias in attributions? A meta-analytic review of individual, developmental, and cultural differences in the self-serving attributional bias. Psychol. Bull. 130 (5), 711–747. https://doi.org/10.1037/0033-2909.130.5.711.

Matsuda, T., Nisbett, R.E., 2001. Attending holistically versus analytically: comparing the context sensitivity of Japanese and Americans. J. Pers. Soc. Psychol. 81 (5), 922–934. https://doi.org/10.1037/0022-3514.81.5.922.

Nuechterlein, K.H., Dawson, M.E., 1984. Information processing and attentional functioning in the developmental course of schizophrenic disorders. Schizophr Bull. 10 (2), 160–203. https://doi.org/10.1093/schbul/10.2.160. PMID: 6729409.

Olley, A.L., Malhi, G.S., Bachelor, J., Cahill, C.M., Mitchell, P.B., Berk, M., 2005. Executive functioning and theory of mind in euthymic bipolar disorder. Bipolar Disord. 7, 43–52. https://doi.org/10.1111/j.1399-5618.2005.00254.x. Suppl.

Ospina, L.H., Nitzburg, G.C., Shanahan, M., Perez-Rodriguez, M.M., Larsen, E., Latifoglu, A., Burdick, K.E., 2018. Social cognition moderates the relationship between neurocognition and community functioning in bipolar disorder. J. Affect. Disord. 235, 7–14. https://doi.org/10.1016/j.jad.2018.03.013. Aug 1Epub 2018 Mar 29. PMID: 29631204; PMCID: PMC6082404.

Palaniappan, P., Easwaran, K., 2020. Theory of mind deficits and their influence on functional impairment in remitted phase of bipolar disorder. Indian J. Psychol. Med. 42, 1. https://doi.org/10.1177/0253717620930315. Published online first.

Rosa, A.R., Sánchez-Moreno, J., Martínez-Aran, A., et al., 2007. Validity and reliability of the functioning assessment short test (FAST) in bipolar disorder. Clin. Pract. Epidemiol. Ment. Health 3, 5. https://doi.org/10.1186/1745-0179-3-5. Published 2007 Jun 7.

Saleem, S.M., 2018. Modified Kuppuswamy scale updated for the year 2018. Paripex-Indian J. Res. 7 (3). March 2018.

Samamé, C., Martino, D.J., Strejilevich, S.A., 2015. An individual task meta-analysis of social cognition in euthymic bipolar disorders. J. Affect. Disord. 173, 146–153. https://doi.org/10.1016/j.jad.2014.10.055.

Savla, G.N., Vella, L., Armstrong, C.C., Penn, D.L., Twamley, E.W., 2013. Deficits in domains of social cognition in schizophrenia: a meta-analysis of the empirical evidence. Schizophr Bull. 39 (5), 979–992. https://doi.org/10.1093/schbul/sbs080.

Schmidt, S.J., Mueller, D.R., Roder, V., 2011. Social cognition as a mediator variable between neurocognition and functional outcome in schizophrenia: empirical review and new results by structural equation modeling. Schizophr Bull. 37, S41–S54. https://doi.org/10.1093/schbul/sbr079. SepSuppl 2(Suppl 2)PMID: 21860046; PMCID: PMC3160114.

Sergi, M.J., Green, M.F., Widmark, C., Reist, C., Erhart, S., Braff, D.L., Kee, K.S., Marder, S.R., Mintz, J., 2007. Social cognition [corrected] and neurocognition: effects of risperidone, olanzapine, and haloperidol. Am. J. Psychiatry 164 (10), 1585–1592. https://doi.org/10.1176/appi.ajp.2007.06091515. OctErratum in: Am J Psychiatry. 2007 Nov;164(11):1766. PMID: 17898351.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (20), 22–33. Supplquiz 34-57. PMID: 9881538.

Seidel, EM, Habel, U, Finkelmeyer, A, Hasmann, A, Dobmeier, M, Derntl, B., 2012. Risk or resilience? Empathic abilities in patients with bipolar disorders and their first-

degree relatives. J. Psychiatr Res. 46 (3), 382–388. https://doi.org/10.1016/j.jpsychires.2011.11.006. MarEpub 2011 Nov 30. PMID: 22133461.

Stange, J.P., Sylvia, L.G., da Silva Magalhães, P.V., et al., 2013a. Extreme attributions predict the course of bipolar depression: results from the STEP-BD randomized controlled trial of psychosocial treatment. J. Clin. Psychiatry 74 (3), 249–255. https://doi.org/10.4088/JCP.12m08019.

Stange, J.P., Sylvia, L.G., Magalhães, P.V., et al., 2013b. Extreme attributions predict transition from depression to mania or hypomania in bipolar disorder. J. Psychiatr Res. 47 (10), 1329–1336. https://doi.org/10.1016/j.jpsychires.2013.05.016.

Thaler, N.S., Allen, D.N., Sutton, G.P., Vertinski, M., Ringdahl, E.N., 2013. Differential impairment of social cognition factors in bipolar disorder with and without psychotic features and schizophrenia. J. Psychiatr Res. 47 (12), 2004–2010. https://doi.org/10.1016/j.jpsychires.2013.09.010. DecEpub 2013 Sep 30. PMID: 24112946.

Triandis, H.C., 2001. Individualism-collectivism and personality. J. Pers. 69 (6), 907–924. https://doi.org/10.1111/1467-6494.696169.

Vlad, M., Raucher-Chéné, D., Henry, A., Kaladjian, A., 2018. Functional outcome and social cognition in bipolar disorder: is there a connection? Eur. Psychiatry 52, 116–125. https://doi.org/10.1016/j.eurpsy.2018.05.002. AugEpub 2018 May 19. PMID: 29787961.

Vogel, B.D., Brück, C., Jacob, H., Eberle, M., Wildgruber, D., 2016. Effects of cue modality and emotional category on recognition of nonverbal emotional signals in schizophrenia. BMC Psychiatry 16, 218. https://doi.org/10.1186/s12888-016-0913-7. Jul 7PMID: 27388011; PMCID: PMC4936116.

Vogeley, K., Roepstorff, A., 2009. Contextualising culture and social cognition. Trends Cogn. Sci. 13 (12), 511–516. https://doi.org/10.1016/j.tics.2009.09.006. Dec.

Wolf, F., Brüne, M., Assion, H.J, 2010. Theory of mind and neurocognitive functioning in patients with bipolar disorder. Bipolar Disord. 12 (6), 657–666. https://doi.org/10.1111/j.1399-5618.2010.00854.x. SepPMID: 20882710.

Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. Br. J. Psychiatry 133, 429–435. https://doi.org/10.1192/bjp.133.5.429. NovPMID: 728692.

Zenisek, R., Thaler, N.S., Sutton, G.P., Ringdahl, E.N., Snyder, J.S., Allen, D.N., 2015. Auditory processing deficits in bipolar disorder with and without a history of psychotic features. Bipolar Disord. 17 (7), 769–780. https://doi.org/10.1111/bdi.12333.

Zhang, Y., Ma, X., Liang, S., et al., 2019. Social cognition and interaction training (SCIT) for partially remitted patients with bipolar disorder in China. Psychiatry Res. 274, 377–382. https://doi.org/10.1016/j.psychres.2019.03.002.

Theory of Mind Deficits and Their Influence on Functional Impairment in Remitted Phase of Bipolar Disorder

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ABSTRACT

Background: Functional impairment has been convincingly established in the euthymic/ remitted phase of bipolar disorder (BD). Though deficits in social cognition, especially theory of mind (ToM), predict functional impairment, the association has not been consistently proven.

Methods: Thirty remitted subjects with BD (as per DSM 5) and 30 age- and gender-matched healthy controls were screened for eligibility and the sociodemographic details and ToM scores, that is, first-order ToM, second-order ToM, and Faux pas, were collected. In subjects with BD, functioning was assessed using Functioning Assessment Short Test (FAST) and illness variables were collected.

Results: No significant difference was found in occupation or education between the groups. Remitted subjects with BD had statistically significant deficits in all domains of ToM, that is, first-order ToM (r = 0.65), second-order ToM (r = 0.69), and Faux pas (r = 0.75). Significant correlations existed between first-order ToM and FAST total score, as well as second-order ToM and FAST total score, but the correlations dropped after controlling for duration of illness and number of depressive episodes. Quantile regression analysis showed that the only factors which predicted global functional impairment was a higher number of episodes ($\beta\tau$ = −0.45, SE = 3.51, t = 0.13, P = 0.04), while all other illness variables and ToM failed to predict the global functioning.

Conclusion: Though there seems to be an association between ToM and functioning, only illness variables predicted functional impairment in subjects with BD. We need prospective studies to delineate the contributors to functional impairment.

Keywords: theory of mind, functioning, bipolar disorder, euthymic

Key Messages: Subjects with BD continue to have significant ToM deficits even during the remitted phase; the only factor which predicted global functioning was a higher number of episodes; first-order ToM, second-order ToM, or faux pas did not predict global functioning.

Social cognition is a multifaceted construct concerned with the cognitive processes involved in knowing about themselves and understanding other people's behavioral intentions. Theory of mind (ToM) is one such social cognition construct (also called mental state attribution), which involves the ability to understand other's behavior by assessing self and other's mental states such as feelings, desires, and opinions and to act accordingly.[1] Typically, ToM is subdivided as first order and second order, based on the complexity of inference. In a first-order "false-belief" task, the ability to understand that someone can hold a belief that is different from the actual state of affairs is assessed. In a second-order false-belief task, participants have to infer the (false) beliefs of one character about the (false) beliefs of a second character.

ToM deficits were evident among individuals in all the three phases of bipolar disorder (BD), namely manic, depressive, and euthymic phases.[2,3] While one might expect relatively lesser ToM impairments in the remitted phase, the deficits observed were certainly not negligible. Three meta-analyses[4–6] estimated the effect sizes of ToM deficits in the remitted state to be in the moderate range

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(0.5 <*d* <0.8), with no significant difference when compared to subsyndromal mood state, but lesser than the deficits seen during acute mood (mania) state (*d* =1.23).[4] Though the influence of illness variables over ToM vary across studies, in some studies, global cognitive impairment and history of psychotic symptoms seem to worsen the degree of ToM deficits.[7–10]

Multiple studies had consistently shown that the unemployment rate was as high as 55% in BD.[11] Functional outcomes in BD are affected by various factors such as personality, current level of depressive symptoms[12] as well as age, course, insight into positive symptoms, and lifetime substance use disorders.[13] However, there is increasing evidence indicating that social cognitive deficits contribute substantially to functional outcomes in individuals with schizophrenia,[14] but there is less research evidence in BD. While some studies have shown positive association [15,16] between ToM deficits and functional impairment in BD, others have not.[17–20,21] Among ToM tasks, a strong association was found between poorer faux pas performance and lower Functional Assessment Short Test scores in one study,[22] but such association was not consistent in other studies.[17,23] It is also possible that the association was significantly influenced by subsyndromal depressive features.[24]

Social cognition, especially ToM, seems to be under a substantial cultural influence. Individual cultural schemata and interpersonally shared cultural models determine how humans interpret each other's experiences and guide their actions.[25,26] Functional magnetic resonance imaging studies demonstrate that, based on the cultural background of the test subject, neural correlates of ToM abilities may differ significantly.[27,28] As tools developed to assess social cognition in one culture may not be useful in another culture, assessment of ToM using culturally validated tools in the Indian population is necessary.[29]

In view of multiple methodological issues like administration of different tests to assess functioning and social cognition, heterogeneous populations, low sample size, assessment at different stages of illness, presence of subsyndromal mood symptoms, and paucity of data on neurocognition, more research in this area will improve our understanding about ToM and its effect on functioning in remitted BD.[21] Thus, our primary aim was to compare the ToM abilities among remitted subjects with BD and healthy controls. The secondary aim was to find any association among illness variables, the ToM, and functioning in subjects with BD, and if any exist, then to find out which factors predict the functioning in BD. We hypothesize that subjects with BD have ToM deficits even during the remitted phase of illness and that ToM will predict functional impairment in BD.

Materials and Methods

This is a cross-sectional study with a control group, in which we recruited euthymic/remitted subjects with BD and healthy controls based on eligibility criteria.

Recruitment of Participants

We recruited 30 subjects with BD (diagnosed by DSM 5) from the outpatient psychiatric services of a tertiary care medical college, during the period of May 2017 to September 2018, with prior informed written consent. We included subjects with BD who were 18 to 60 years old; of either gender; fulfilling DSM 5 criteria for bipolar affective disorder, either Type 1 or Type 2, using structured clinical interview for DSM5 (SCID 5); who are able to read and understand Tamil (local vernacular language) and who are currently in remission as defined by scores on the 17-item Hamilton Rating Scale for Depression (HAMD; score ≤8) and Young Mania Rating Scale (YMRS; score ≤ 6) for a period of eight weeks. We included this stricter definition of euthymia to minimize the influence of subsyndromal mood symptoms on the ToM tasks.[21,30] We excluded subjects with BD if they had history of psychotic symptoms during the episodes, substance use disorders (except nicotine), or other psychiatric axis I comorbidities (using Mini International Neuropsychiatric Interview, MINI plus 5.0); clinically determined severe cognitive impairment, mental retardation or neurodegenerative disorders; general medical illness that could impair cognitive performance; received electro convulsive therapy in the past six months, or severe visual or hearing impairment impairing test performance. Relatives of patients who were hospitalized for medical illness were screened for eligibility as controls. Age-, gender-, and education-matched healthy controls were included who did not have any lifetime psychiatric diagnosis (as established by MINI plus 5.0), neurological illness, or severe cognitive impairment that could impair the test performance. Ethics clearance was obtained from the Institutional Ethics Committee. The investigator (E.K) had undergone supervised three-day training organized by the authors of the culturally validated social cognition tool, The Social Cognition Rating Tools in Indian Setting (SOCRATIS), focusing on tool administration and scoring.

Assessments

With written informed consent, sociodemographic data and relevant illness variables were collected from the participants and cross-checked with the health records. Current mood symptoms were assessed using the 17-item HAMD and YMRS.

SOCRATIS, well-validated in Indian settings,[29] was used to measure three areas of social cognition, namely ToM, social perception, and attribution bias. The ToM task included two first-order and two second-order false belief picture stories, two metaphor-irony stories, and five faux pas recognition stories, which were presented as cartoon sequences. These story-based tasks examine the ability, at different complexity levels, to "meta-represent" mental states of others. Based on test performance, the tool scores ToM as first-order ToM (FOT) index (FOTI), second-order ToM (SOT) index (SOTI), and faux pas composite index (FPCI). If the subject scores less index scores, it suggests severe ToM deficits.

Functioning status was assessed in subjects with BD using Functional Assessment Short Test (FAST),[31] which assesses six specific areas of functioning: autonomy, occupational functioning, cognitive functioning, financial issues, leisure time, and interpersonal relation-

ships, with higher scores indicating higher functional impairment.

Statistical Analysis

All calculations were performed using R software. The normality of data was checked using the Shapiro–Wilk test, which showed that the data were non-normally distributed. Between-group differences (BD vs. healthy controls) among sociodemographic variables were tested with the Mann–Whitney *U* test for continuous variables and Fisher's exact test for discrete variables. Between-group ToM scores (FOTI, SOTI, FPCI) were tested for any statistically significant difference using Mann-Whitney *U* test, with the significance level set at P =0.05. Effect size (for nonparametric tests)[32] was calculated using Cohen $r = z/\sqrt{N}$. In order to understand the correlation among illness variables, ToM, and functioning (FAST domains and global), Spearman's correlation was used. Partial correlation was used to find the degree of correlation between ToM and functioning (FAST scores) in subjects with BD, after controlling for illness variables of interest. Quantile regression was carried out with illness variables and ToM variables as independent variables while using FAST (individual and total scores) as the dependent variable. The goodness of fit of the quantile regression model was assessed by comparison with the null/base model (statistical significance set at P < 0.05). Based on $\beta\tau$ coefficients, the relative contribution of each independent variable over the FAST scores was deciphered.

Results

Sociodemographic and Illness Details

The mean age was not statistically different (U = 375, P = 0.27) between BD (35 ± 8.48) subjects (n = 30) and healthy controls (36.27 ± 7.01) (n = 30) (**Table 1**). There was no statistically significant difference in gender, education, or occupation between subjects with BD and healthy controls. The average duration of illness was approximately 6.5 years, with time spent in depressive episodes (40.05 ± 26.85 weeks, median = 32) being longer when compared to manic episodes (18.17 ± 18.28 weeks, median = 12). The mean HAMD scores (3.67 ± 1.24) and YMRS scores (4.10 ± 1.19) suggested the remitted/euthymic status of BD (**Table 2**).

TABLE 1.

Sociodemographic Profile of Subjects with BD (n = 30) and Healthy Controls (n = 30)

	Characteristics	Subjects with BD n (%)	Healthy Controls n (%)	P
Gender	Male	15 (50 %)	17(56.7 %)	0.76
	Female	15 (50 %)	13(43.3 %)	
Occupation	Professional	5(16.7 %)	8 (26.7 %)	0.22
	Semi-professional	1(3.3 %)	5 (16.7 %)	
	Clerical /shopowner/farmer	4(13.3 %)	3(10.0 %)	
	Skilled	11(36.7 %)	11(36.7 %)	
	Unskilled	1(3.3 %)	–	
	Unemployed	8(26.7 %)	3(10.0 %)	
Education	Professional	11(36.7 %)	11(36.7 %)	0.11
	Graduate	3(10 %)	4(13.3 %)	
	Intermediate	3(10 %)	4(13.3 %)	
	High-school	13(43.3 %)	10(33.3 %)	
	Illiterate	–	1(3.3 %)	
Socioeconomic status	Upper	–	4(13.3 %)	0.003*
	Middle	11(36.7 %)	11(36.6 %)	
	Lower	19(63.3 %)	15(50 %)	
Marital status	Unmarried	7(23.3 %)	1(3.3%)	0.001*
	Married	23 (76.6 %)	29(96.7 %)	
Medical comorbidities	Diabetes mellitus	2 (6.7 %)	–	
	Hypertension	2(6.7 %)	–	
	Dyslipidemia	1(3.3 %)	–	
	Hypothyroidism	4(13.3 %)	–	
	Nil	21 (70.0 %)	–	

*significant if P < 0.05, as measured by Fisher's exact test.
Source: From the current study.

ToM Scores

In subjects with BD (**Table 3**), the mean score of FPCI (0.30 ± 0.37) was the lowest, followed by SOTI (0.55 ± 0.29), then by FOTI (0.64 ± 0.29). Mann–Whitney *U* test indicated that subjects with BD had deficits in FOT (U = 152, P < 0.001), SOT (U = 128, P < 0.001), and faux pas recognition (U = 77, P < 0.001) when compared to healthy controls, which were statistically significant. Effect size estimates ($r = z/\sqrt{N}$) showed large effect ($r \geq 0.5$)[32] for all the three domains of ToM, namely, FOT (r = 0.65), SOT (r = 0.69), and faux pas recognition (r = 0.75).

Functioning in Subjects with BD

Mean FAST scores indicated that interpersonal (9.17 ± 4.2), occupational (7.37 ± 4.38), and cognitive (7.40 ± 4.28) domains of functioning were more affected than financial (1 ± 1.11), autonomy (1.30 ± 1.91), and leisure (1.30 ± 1.02) domains of functioning. Correlation between illness variables, ToM variables, and FAST (individual item based and total) scores was evaluated using Spearman's rho correlation (**Table 4**). There seems to be a significant (P < 0.05) negative correlation between FOT and all domains of FAST, indicating that more the FOT deficits (lesser FOTI), more is the functional impairment in all domains (higher FAST domain scores) (**Table 4**). But such a significant correlation was not observed for

TABLE 2.

Illness Variables in Subjects with BD (*n* = 30)

Characteristics	Mean ± SD
Age	35 ± 8.48
Total number of episodes	3.63 ± 1.67
Number of manic episodes	2.13 ± 1.07
Number of depressive episodes	1.87 ± 1.36
Number of mixed episodes	0
Duration of illness (months)	77.13 ± 57.52
Time spent in mania (weeks)	18.17 ± 18.28
Time spent in depressive episodes (weeks)	40.05 ± 26.85
Number of episodes with suicidal attempts	0
Number of episodes requiring hospitalization	2.25 ± 0.99
YMRS scores	4.10 ± 1.19
HAMD scores	3.67 ± 1.24

YMRS: Young Mania Rating Scale, HAMD: Hamilton Depression Rating Scale.
Source: From the current study.

TABLE 3.

Comparison of ToM Scores Among Subjects with BD (*n* = 30) and Healthy Controls (*n* = 30)

ToM Indices	Subjects with BD M ± SD (Mdn)	Healthy Controls M ± SD (Mdn)	U	Z	P	ES Cohen r = Z /√N
FOT index	0.64 ± 0.29 (0.50)	0.96 ± 0.93 (1.00)	152	−5.05	<0.001	0.65
SOT index	0.55 ± 0.29(0.50)	3.43 ± 13.51(1.00)	128	−5.38	<0.001	0.69
FPCI	0.30± 0.37 (0.168)	0.96 ± 0.10(1.00)	77	−5.86	<0.001	0.75

FOT index: first-order ToM index, SOT index: second-order ToM index, FPCI: faux pas composite index, ES: effect size, Mdn: median.
Source: From the current study.

FPCI and FAST domains. While the duration of illness (months) was positively correlated significantly with all domains of functioning (P < 0.05), the number of depressive episodes was positively correlated with only four domains of FAST, namely, autonomy, occupational, interpersonal, and cognitive domains. In view of the significant correlation between functioning and multiple variables (illness variables and ToM variables), we performed partial correlation analysis. After controlling for illness variables (i.e., number of episodes, number of depressive episodes, and duration of illness), the significance of the correlation between FOT and functioning (total score) dropped, but still remained significant (r = −0.44, P = 0.05), while it lost significance for SOT (r = −0.17, P = 0.48).

Predictors of Functioning in Subjects with BD During the Remitted Phase

In order to improve the model accuracy and prevent dilution of the model, we used the significantly correlated variables (total number of episodes, number of depressive episodes, duration of illness, FOTI, SOTI) as independent variables and FAST (total and sub-items) as a dependent variable into the final models (**Table 5**), while controlling for all other variables of interest. With respect to global functioning (FAST – total), the model showed goodness of fit (ΔF (5, 24) = 10.04, P = 0.03) in which total number of episodes ($\beta\tau$ = −0.45, SE = 3.51, t = 0.13, P = 0.04) had shown statistically significant contribution, while FOTI had shown trend towards significance ($\beta\tau$ = −22.57, SE = 14.36, t = −1.57, P = 0.06).

With regard to interpersonal functioning (FAST), the model showed goodness of fit (ΔF (5, 24) = 5.18, P = 0.002), in which all independent variables lost significance, except FOTI, which showed trend towards significance ($\beta\tau$ = −12, SE= 6.2, t = −1.93, P = 0.06). Among other models, the models which showed goodness of fit were FAST occupational (ΔF (5, 24)= 6.60, P = 0.0005), FAST cognitive (ΔF (5, 24) = 12.26, P = 0.0003), and FAST leisure (ΔF (5, 24) = 6.18, P = 0.0008) as dependent variables, in which none of the independent variables showed a significant contribution (**Table 6**).

Discussion

Our findings suggest that significant ToM deficits (FOT, SOT, and faux pas) are present during euthymic/remitted state of BD when compared to healthy controls. A significant association existed between global functioning (FAST–Total) and most of ToM variables, that is, with FOT (P < 0.001), SOT (P = 0.003), but not with faux pas (P = 0.17). A higher number of episodes predicted poor global functioning, while ToM did not predict global functioning in subjects with BD during the remitted phase.

ToM (first-order, second-order, and faux pas recognition) deficits seem to persist even during the remitted phase of the BD. This is in line with multiple metanalyses[4–6] that had also shown that ToM deficits persist irrespective of the form (cognitive or affective) and type of assessment tools.

The link between ToM and functioning has been explored in all the symptomatic phases of BD, that is, depressive,[18,33–35] manic,[18,33], and mixed phases.[35] Studies that explored the association between ToM and functioning in euthymic/remitted BD had shown mixed results, with some reporting significant association[15,16,18,22,24,36] and others not.[17,20] A possible link exists between depressive symptomatology and global functioning, where depressive symptoms explain 17%–35% of the variance in the global functioning.[18,33]

Our results show that FOT is most consistently correlated with all the domains

TABLE 4.

Correlation Among Illness Variables, ToM Scores and Functioning (Individual Item and Total–FAST Scores) Among Subjects with BD (*n* = 30)

	Age	TE	ME	DE	Duration	Hosp	YMRS	HAMD	FOT	SOT	FPCI	FAST A	FAST OF	FAST C	FAST F	FAST IPR	FAST L	FAST TO
Age	1	0.44	0.07	0.35	0.26	0.34	0.10	0.28	−0.16	−0.22	−0.46	−0.05	0.38	0.45	0.17	0.23	0.07	0.30
TE		1	0.62*	0.80*	0.80*	0.73*	0.15	0.29	−0.37**	−0.35	−0.50*	0.35	0.64**	0.56*	0.40**	0.35	0.34	0.57*
ME			1	0.00	0.62*	0.67*	0.23	−0.20	−0.24	0.05	−0.31	0.22	0.38**	0.12	0.09	0.10	0.11	0.18
DE				1	0.46**	0.42	0.03	0.22	−0.62	−0.24	−0.46	0.20**	0.48**	0.57*	0.41	0.43**	0.38	0.59*
Duration					1	0.61*	0.17	0.27	−0.56*	−0.35	−0.38	0.39**	0.79**	0.01*	0.43**	0.43**	0.43**	0.68*
Hosp						1	−0.13	0.14	−0.23	−0.03	−0.29	0.29	0.52**	0.16	0.65	0.11	-0.23	0.23
YMRS							1	0.38**	−0.12	−0.20	−0.27	−0.7	0.21	0.27	−0.21	0.06	0.03	0.05
HAMD								1	−0.46**	−0.46*	−0.15	0.38**	0.43**	0.35	0.23	0.27	0.07	0.37**
FOT									1	0.08*	0.67*	−0.47**	−0.63*	−0.54*	−0.61*	−0.42*	−0.38**	−0.66*
SOT										1	−0.58*	−0.18	−0.50*	−0.53*	−0.36	−0.32	−0.46*	−0.52*
FPCI											1	−0.16	−0.26	−0.32	0.09	−0.28	0.05	0.29
FAST A												1	0.37**	0.35	0.51*	0.38**	0.19	0.58*
FAST OF													1	0.66*	0.43**	0.51*	0.39**	0.82*
FAST C														1	0.60*	0.77*	0.52*	0.78*
FAST F															1	0.50*	0.55*	0.72*
FAST IPR																1	0.32	0.90*
FAST L																	1	0.59*
FAST TO																		1

TE: total number of episodes, ME: number of manic episodes, DE: number of depressive episodes, Duration: duration of illness, Hosp: number of episodes requiring hospitalization, YMRS: Young Mania Rating Scale scores, HAMD: Hamilton Depression Rating Scale scores, FOT index: first-order ToM index, SOT index: second-order ToM index, FPCI: faux pas composite index, FAST A: functional assessment short test—autonomy, FAST OF: functional assessment short test—occupational functioning, FAST F: functional assessment short test—finances, FAST IPR: functional assessment short test—interpersonal relationships, FAST C: functional assessment short test—cognition, FAST L: functional assessment short test—leisure, FAST TO: functional assessment short test—total. * P < 0.01, ** P < 0.05.
Source: From the current study.

of functioning (autonomy, financial, occupational, cognitive, interpersonal, and leisure). But there was no consistent correlation with higher-order ToM such as faux pas stories and SOT tasks in our study. These results are in line with existing literature that shows that out of 12 studies, only two[16,22] showed a positive association between faux pas (higher-order ToM) and functioning, especially social functioning.[21] In our study, the only illness variable which significantly predicted global functioning was a higher number of episodes, while all other illness variables and ToM tasks (FOT, SOT, faux pas) lost their contribution to variance in global functioning impairment. The interesting question which arises now is which factor(s) mediates the influence of illness variables over functional impairment in BD. The absence of any significant prediction of functional impairment by ToM[17,23] makes us wonder whether alternate mediators like neurocognition would mediate the influence of illness variables on functioning.[37] On the contrary, a recent study has shown that social cognition moderates the effect of neurocognitive deficits over community functioning.[38] In view of the mixed results, prospective studies looking into the association of illness variables, social cognition, neurocognition, and functioning needs to be done.

Recently, social cognition and interaction training (SCIT) has been investigated in schizophrenia[39,40] and has shown promising preliminary results. But to our knowledge, only two studies exist in BD.[41,42] The study which compared the effects of SCIT and treatment as usual on social cognition in outpatient subjects with BD[42] had shown that SCIT caused small but significant improvement in ToM, facial emotional recognition, and

TABLE 5.

Predictors of Global and Interpersonal Functioning (FAST) in Subjects with BD (*n* = 30)

FAST	Variables of Interest	$\beta\tau$ Co-efficient	SE	t	p [Pr(>|t|)]
Global	Intercept	−41.99	15.23	2.76	0.011
	Total number of episodes	−0.45	3.51	−0.13	0.04*
	Number of depressive episodes	2.58	3.71	0.70	0.49
	Duration of illness	0.03	0.08	0.38	0.71
	FOT index	−22.57	14.36	−1.57	0.06†
	SOT index	−5.34	13.48	−0.40	0.70
Interpersonal	Intercept	18	6.34	2.84	0.01
	Total number of episodes	0.00	1.30	0.00	1.00
	Number of depressive episodes	0.00	1.28	0.00	1.00
	Duration of illness	0.00	0.03	0.00	1.00
	FOT index	−12	6.20	−1.93	0.06†
	SOT index	0.00	4.69	0.00	1.00

Pr(>|*t*|) means the probability that the absolute value of *t*-distribution is greater than the calculated value. FAST: Functioning Assessment Short Test, FOT: first-order ToM, SOT: second-order ToM. *Statistically significant = P < 0.05. †Trend toward significance.
Source: From the current study.

hostility attribution biases, but not in social functioning. The authors proposed that improvement in BD may be better if SCIT is modified by including tasks involving comprehension of more complex social situations rather than lower order facial emotional recognition.

Though many studies had shown that subsyndromal depressive symptoms influence the social and occupational functioning,[16,17,33] our study did not show such a correlation (FAST-interpersonal or FAST-occupational), possibly because of our stricter definition of euthymia and the higher HAMD scores in other studies. In one such study,[22] low functioning (FAST > 11) and high functioning (FAST ≤ 11) remitted bipolar groups were compared for verbal social cognition using comprehension faux pas test, which showed that verbal social cognition and subsyndromal depressive symptoms significantly predicted poor functioning in the low functioning group but not in the high functioning and control groups. Such an association between subsyndromal depression and functioning was not universally proven in all studies.[43,44] At the same time, though subsyndromal depressive symptoms may have a lesser influence on ToM tasks, in many studies it has been found that subsyndromal depressive symptoms have a mediating effect between emotional perception deficits and social functioning.[22–24,33,36,43–45]

Based on the correlation between functional impairment and illness variables like longer duration of illness,[30] higher number of episodes, especially depressive episodes, and longer time spent in depressive episodes in our study, we hypothesize that functional impairment could be a consequence of the progressive nature of the illness rather than subsyndromal depressive symptoms. There is some evidence in the short- or long-term (3 to 10 years) prospective studies that the severity and frequency of bipolar depressive episodes contribute to cognitive decline and poor psychosocial functioning,[46] possibly through dysregulated neuroinflammation.[47] Similar inconsistent evidence also suggests that the frequency and severity of manic episodes may lead to poor psychosocial functioning and cognitive impairment,[48] but our study did not find such association. It is possible that this causal relationship could not be established with the cross-sectional nature of the current study and thus, it may need longitudinal studies using more objective rating scales rather than subjective, self-report scales like FAST.[33]

Limitations

This study was less powered to detect a statistically significant association, and this could have influenced our statistical interpretations. Second, confounding variables such as intelligence, which could influence the social cognition scores, were not formally assessed. This could have been partly mitigated by the use of SOCRATIS, which assesses intellect and memory by use of control questions, which are scored during the final scoring of the tool. This is also mitigated by a careful, clinical evaluation of premorbid intelligence. Third, we did not evaluate the neurocognitive variables like processing speed, attention, and verbal recall which could interfere or influence the social cognition task performance. Fourth, the above findings are restricted to patients in the remitted phase of bipolar I disorder without a history of psychotic symptoms. Though subjects with BD with a history of psychotic symptoms have been known to have more ToM deficits when compared to those without a history of psychotic symptoms,[8,9] we intentionally excluded them to avoid contamination of results and thereby to find the deficits that are exclusive to BD. Thus, the generalization of our findings to other types/phases of BD may not be possible and that would require a study that looks into differential social cognition findings across other bipolar diagnoses/phases. Finally, it is known that accurate predictions cannot be guaranteed in a cross-sectional study and rather should be based on a longitudinal study. Temporality assumption (dependent variable has to occur after the independent variable) cannot be assured in a cross-sectional study. But still, findings of a cross-sectional study can pave the way for testing those variables in later longitudinal cohort studies.

Conclusions

There seems to be an association between ToM deficits and global functioning in subjects with BD during

TABLE 6.

Predictors of Occupational, Autonomy, Cognitive, Financial, and Leisure Components of Functioning in Subjects with BD (*n* = 30)

FAST	Variables of Interest	$\beta\tau$ Co-efficient	SE	t	Pr(> | t |)
Occupational	Intercept	7.56	5.55	1.36	0.19
	Total number of episodes	0.46	1.43	0.32	0.75
	Number of depressive episodes	0.06	1.57	0.04	0.97
	Duration of illness	0.03	0.03	0.82	0.42
	FOT index	−3.55	5.64	−0.63	0.54
	SOT index	−3.80	5.90	−0.64	0.53
Autonomy	Intercept	0.63	3.80	0.16	0.87
	Total number of episodes	0.00	1.09	0.00	1.00
	Number of depressive episodes	0.74	1.21	0.61	0.54
	Duration of illness	0.00	0.02	0.00	1.00
	FOT index	−2.88	3.94	−0.72	0.47
	SOT index	2.24	3.90	0.58	0.57
Cognitive	Intercept	6.77	5.82	1.16	0.25
	Total number of episodes	0.69	1.52	0.05	0.96
	Number of depressive episodes	1.29	1.63	0.79	0.43
	Duration of illness	0.02	0.03	0.48	0.63
	FOT index	−4.86	6.06	−0.80	0.43
	SOT index	−1.17	5.92	−0.20	0.84
Financial	Intercept	2.67	1.35	1.97	0.05
	Total number of episodes	0.00	0.32	0.00	1.00
	Number of depressive episodes	0.00	0.36	0.00	1.00
	Duration of illness	0.00	0.01	0.00	1.00
	FOT index	−2.67	1.35	−1.97	0.06
	SOT index	0.00	1.42	0.00	1.00
Leisure	Intercept	1.96	1.3	1.51	0.144
	Total number of episodes	0.13	0.39	0.34	0.73
	Number of depressive episodes	0.03	0.040	0.07	0.94
	Duration of illness	0.00	0.01	0.00	1.00
	FOT index	−0.70	1.22	−0.57	0.57
	SOT index	−1.40	1.10	−1.26	0.22

Pr(>|*t*|) means the probability that the absolute value of *t*-distribution is greater than the calculated value. FAST: Functioning Assessment Short Test, FOT: first-order ToM, SOT: second-order ToM.
Source: From the current study.

the remitted phase, but they lose their ability to predict when illness variables such as a higher number of episodes are taken into account. Caution needs to be exercised in interpreting our findings, in view of the cross-sectional nature of the study and the low sample size. Thus, prospective studies on the effect of ToM deficits on functioning are needed to understand the role of preexisting social cognition deficits, especially ToM deficits, if any, in BD.

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Declaration of Conflicting Interests

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References

1. Green M, Penn D, and Bentall R. Social cognition in schizophrenia: An NIMH Workshop on definitions, assessment, and research opportunities. Schizophr Bull 2008; 34(6): 1211–1220.
2. Mitchell R and Young A. Theory of mind in bipolar disorder, with comparison to the impairments observed in schizophrenia. Front Psychiatry 2016 Jan 18; 6: 188. eCollection 2015.
3. Samamé C. Social cognition throughout the three phases of bipolar disorder: A state-of-the-art overview. Psychiatry Res 2013; 210(3): 1275–1286.
4. Bora E, Bartholomeusz C, and Pantelis C. Meta analysis of theory of mind (ToM) impairments in bipolar disorder. Psychol Med 2016 Jan; 46(2): 253–264.
5. Samamé C, Martino DJ, and Strejilevich SA. An individual task meta-analysis of social cognition in euthymic bipolar disorders. J Affect Disord 2015 Mar 1; 173: 146–153.
6. Samamé C, Martino DJ, and Strejilevich SA. Social cognition in euthymic bipolar disorder: Systematic review and meta-analytic approach. Acta Psychiatr Scand 2012 Apr 1; 125(4): 266–280.
7. Lee J, Altshuler L, Glahn DC, et al. Social and nonsocial cognition in bipolar disorder and schizophrenia: Relative levels of impairment. Am J Psychiatry 2013 Mar; 170(3): 334–341.
8. Thaler NS, Allen DN, Sutton GP, et al. Differential impairment of social cognition factors in bipolar disorder with and without psychotic features and schizophrenia. J Psychiatr Res 2013 Dec; 47(12): 2004–2010.
9. Lahera G, Montes JM, Benito A, et al. Theory of mind deficit in bipolar disorder: Is it related to a previous history of psychotic symptoms? Psychiatr Res 2008 Dec 15; 161(3): 309–317.
10. Bora E, Vahip S, Gonul AS, et al. Evidence for theory of mind deficits in euthymic patients with bipolar disorder. Acta Psychiatr Scand 2005 Aug; 112(2): 110–116.
11. Wingo A, Harvey P, and Baldessarini R. Neurocognitive impairment in bipolar

disorder patients: functional implications. Bipolar Disord 2009; 11(2): 113–125.
12. Pope M, Dudley R, and Scott J. Determinants of social functioning in bipolar disorder. Bipolar Disord 2007; 9(1–2): 38–44.
13. Waghorn G, Chant D, and Jaeger J. Employment functioning and disability among community residents with bipolar affective disorder: Results from an Australian community survey. Bipolar Disord 2007; 9(1–2): 166–182.
14. Green MF, Horan WP, and Lee J. Social cognition in schizophrenia. Nat Rev Neurosci 2015; 16(10): 620–631.
15. Purcell AL, Phillips M, and Gruber J. In your eyes: Does theory of mind predict impaired life functioning in bipolar disorder? J Affect Disord 2013; 151(3): 1113–1119.
16. Caletti E, Paoli RA, Fiorentini A, et al. Neuropsychology, social cognition and global functioning among bipolar, schizophrenic patients and healthy controls: Preliminary data. Front Hum Neurosci 2013; 17(7): 661.
17. Barrera A, Vázquez G, Tannenhaus L, et al. Theory of mind and functionality in bipolar patients with symptomatic remission. Rev Psiquiatr Salud Ment 2013; 6(2): 67–74.
18. Lahera G, Herrera S, Reinares M, et al. Hostile attributions in bipolar disorder and schizophrenia contribute to poor social functioning. Acta Psychiatr Scand 2015 Jun; 131(6): 472–482.
19. Benito A, Lahera G, Herrera S, et al. Deficits in recognition, identification, and discrimination of facial emotions in patients with bipolar disorder. Braz J Psychiatr 2013; 35(4): 435–438.
20. Olley AL, Malhi GS, Bachelor J, et al. Executive functioning and theory of mind in euthymic bipolar disorder. Bipolar Disord 2005; 7 (Suppl. 5): 43–52.
21. Vlad M, Raucher-Chéné D, Henry A, et al. Functional outcome and social cognition in bipolar disorder: Is there a connection? Eur Psychiat 2018 Aug; 52: 116–125.
22. Lahera G, Ruiz-Murugarren S, Iglesias P, et al. Social cognition and global functioning in bipolar disorder. J Nerv Ment Dis 2012 Feb; 200(2): 135–141.
23. Martino DJ, Strejilevich SA, Fassi G, et al. Theory of mind and facial emotion recognition in euthymic bipolar I and bipolar II disorders. Psychiatr Res 2011; 189(3): 379–384.
24. Lahera G, Benito A, González-Barroso A, et al. Social-cognitive bias and depressive symptoms in outpatients with bipolar disorder. Depress Res Treat 2012; 2012: 670549.
25. D'Andrade RG. *The development of cognitive anthropology*. Cambridge, NY: Cambridge University Press, 1995.
26. Shore B. *Culture in mind: Cognition, culture and the problem of meaning*. Oxford, NY: Oxford University Press, 1996.
27. Kobayashi C, Glover GH, and Temple E. Cultural and linguistic effects on neural bases of 'Theory of Mind' in American and Japanese children. Brain Res 2007 Aug; 1164: 95–107.
28. Zhu Y, Zhang L, Fan J, et al. Neural basis of cultural influence on self representation. Neuroimage 2007; 34: 1310–1316.
29. Mehta UM, Thirthalli J, Naveen Kumar C, et al. Validation of Social Cognition Rating Tools in Indian Setting (SOCRATIS): A new test-battery to assess social cognition. Asian J Psychiatr 2011Sep; 4(3): 203–209.
30. McKinnon MC, Cusi AM, Macqueen GM. Impaired theory of mind performance in patients with recurrent bipolar disorder: Moderating effect of cognitive load. Psychiatr Res 2010; 177(1–2): 261–262.
31. Rosa AR, Sánchez-Moreno J, Martínez-Aran A, et al. Validity and reliability of the Functioning Assessment Short Test (FAST) in bipolar disorder. Clin Pract Epidemiol Ment Health 2007 Jun 7; 3: 5.
32. Fritz CO, Morris PE, and Richler JJ. Effect size estimates: Current use, calculations, and interpretation. J Exp Psychol Gen 2012; 141(1): 2–18.
33. Van Rheenen TE and Rossell SL. Objective and subjective psychosocial functioning in bipolar disorder: An investigation of the relative importance of neurocognition, social cognition and emotion regulation. J Affect Disord 2014 Jun; 162: 134–141.
34. Aydemir O, Akkaya C, Uykur B, et al. Effect of facial emotion recognition on subjective psychosocial functioning in bipolar patients. Acta Psychiatr Scand 2013; 127(5): 412–413.
35. Vierck E and Joyce PR. Influence of personality and neuropsychological ability on social functioning and self-management in bipolar disorder. Psychiatr Res 2015; 229(3): 715–723.
36. Aparicio A, Santos J, Jimenez-Lopez E, et al. Emotion processing and psychosocial functioning in euthymic bipolar disorder. Acta Psychiatr Scand 2017; 135(4): 339–350.
37. Duarte W, Becerra R, and Cruise K. The relationship between neurocognitive functioning and occupational functioning in bipolar disorder: A literature review. Eur J Psychol 2016; 12(4): 659–678.
38. Ospina LH, Nitzburg GC, Shanahan M, et al. Social cognition moderates the relationship between neurocognition and community functioning in bipolar disorder. J Affect Disord 2018 Aug 1; 235: 7–14.
39. Gordon A, Davis PJ, Patterson S, et al. A randomized waitlist control community study of social cognition and interaction training for people with schizophrenia. Br J Clin Psychol 2018 Mar; 57(1): 116–1130.
40. Roberts DL, Combs DR, Willoughby M, et al. A randomized, controlled trial of Social Cognition and Interaction Training (SCIT) for outpatients with schizophrenia spectrum disorders. Br J Clin Psychol 2014 Sep; 53(3): 281–298.
41. Zhang Y, Ma X, Liang S, et al. Social cognition and interaction training (SCIT) for partially remitted patients with bipolar disorder in China. Psychiat Res 2019 Apr; 274: 377–382.
42. Lahera G, Benito A, Montes JM, et al. Social cognition and interaction training (SCIT) for outpatients with bipolar disorder. J Affect Disord 2013 Mar 20; 146(1): 132–136.
43. Mur M, Portella MJ, Martinez-Aran A, et al. Influence of clinical and neuropsychological variables on the psychosocial and occupational outcome of remitted bipolar patients. Psychopathology 2009; 42(3): 148–156.
44. Tabare´s-Seisdedos R, Balanza´-Martínez V, Sánchez-Moreno J, et al. Neurocognitive and clinical predictors of functional outcome in patients with schizophrenia and bipolar I disorder at one-year follow-up. J Affect Disord 2008 Aug; 109(3): 286–299.
45. Yalcin-Siedentopf N, Hoertnagl CM, Biedermann F, et al. Facial affect recognition in symptomatically remitted patients with schizophrenia and bipolar disorder. Schizophr Res 2014; 152: 440–445.
46. Vierck E and Joyce PR. Influence of personality and neuropsychological ability on social functioning and self-management in bipolar disorder. Psychiatr Res 2015; 229(3): 715–723.
47. Depp CA, Dev S, and Eyler LT. Bipolar depression and cognitive impairment: Shared mechanisms and new treatment avenues. Psychiatr Clin North Am 2016 Mar; 39(1): 95–109.
48. Robinson LJ and Ferrier IN. Evolution of cognitive impairment in bipolar disorder: A systematic review of cross-sectional evidence. Bipolar Disord 2006 Apr; 8(2): 103–116.

RESEARCH PAPER

Epinephrine Plus Vasopressin vs Epinephrine Plus Placebo in Pediatric Intensive Care Unit Cardiopulmonary Resuscitation: *A Randomized Double Blind Controlled Clinical Trial*

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Objective: To compare the efficacy of epinephrine plus vasopressin vs epinephrine plus placebo in the pediatric intensive care unit (PICU) cardiopulmonary resuscitation (CPR).

Design: Randomized, double-blind controlled clinical trial.

Setting: PICU in a tertiary care institute from February, 2019 to May, 2020.

Participants: Children aged one month to 13 years who required CPR during PICU stay. Patients in whom vascular access was not available or return of spontaneous circulation (ROSC) was achieved by defibrillation without epinephrine were excluded.

Intervention: Patients were randomized to receive vasopressin 0.1 mL per kg (=0.8 unit per kg) or placebo (0.1 mL per kg normal saline) in addition to epinephrine (1:10000) 0.1 mL per kg. The drugs were given as bolus doses every three minutes until the ROSC or up to a maximum of five doses, whichever was earlier.

Outcome Measure: The primary outcome was the proportion of patients who achieved ROSC. The secondary outcomes were survival rate and functional status (at 24-hour, PICU, hospital, and 90-day post-discharge), need for organ supports, length of stay (PICU and hospital), and adverse effect(s) of the study drugs.

Results: 90 patients (epinephrine plus vasopressin group, *n*=45 and epinephrine plus placebo group, *n*=45) were analyzed on intention-to-treat basis. There was no significant difference in the primary outcome between epinephrine plus vasopressin (*n*=25, 55.5%) and epinephrine plus placebo groups (*n*=24, 53.3%) (Relative risk 1.04, 95% CI 0.71 to 1.52). There was no significant difference in survival rate at 24-hour (*n*=7, 15.6% vs. *n*=8, 17.8%), at PICU, hospital, and 90-day post-discharge (*n*=1, 2.2% vs *n*=1, 2.2%). There was no difference in other secondary outcomes. No trial drug-related serious adverse events were observed.

Conclusion: A combination of epinephrine plus vasopressin did not improve the rate of return of spontaneous circulation in the pediatric intensive care unit cardiopulmonary resuscitation as compared with epinephrine plus placebo.

Keywords: *In-hospital cardiac arrest*.

Trial Registration: CTRI/2019/01/017200.

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Cardiac arrest is a dreadful event in pediatric intensive care unit (PICU). Cardiopulmonary resuscitation (CPR) involves chest compression and manual ventilation at appropriate intervals. Return of spontaneous circulation (ROSC) is the initial therapeutic goal in cardiac arrest and is a measure of initial success. Vasopressor medications are often used during CPR. These medications increase aortic diastolic pressure, thereby improving coronary perfusion pressure, which facilitates ROSC [1]. Epinephrine is the most widely studied and the first line vasoactive drug as per the pediatric advanced life support (PALS) guidelines [2]. Vasopressin, a potent vasoconstrictor, is well studied in adult cardiac arrest [3]. The recent advanced cardio-vascular life support guideline recommends that Vaso-pressin, combined with epinephrine, may be considered in adult cardiac arrest resuscitation [3]. Though the pediatric in-hospital cardiac arrest (IHCA) outcome has improved from 39% to 77% in high-income countries, data from low-and middle-income countries are lacking or are under-reported [2]. Animal studies, case series, and feasibility pilot studies have shown encouraging results for the use of vasopressin in pediatric cardiac arrest [4-6]. This study hypothesized that epinephrine plus vasopressin would be associated with a higher rate of ROSC as compared to epinephrine plus placebo in the pediatric intensive care unit cardiopulmonary resuscitation.

METHODS

This randomized, double-blind controlled clinical trial was undertaken in PICU of a tertiary care academic hospital from February, 2019 to May, 2020. Ours is a 19 bedded

level-III PICU, receiving critically ill children 24 hours a day throughout the year. Though our PICU is a predominantly medical ICU, it also receives complicated surgical and trauma patients. The PICU has facilities for providing multimodal hemodynamic and neuromonitoring, mechanical ventilation, and high-frequency ventilation. It is also equipped with an in-house blood gas analyzer with a co-oximeter module, osmometer, therapeutic plasma exchange, and renal replacement therapy. During the study period, the baseline mortality in our PICU was 20%, and the average length of PICU stay was six days, with a bed occupancy rate of 80%.

The trial was approved, and its progress was reviewed yearly by the institute ethics committee. Written informed consent was obtained from the parent/legally authorized representatives of all patients getting admitted to PICU at the time of transfer-in, stating that their child might be enrolled in the study if the child required CPR during the PICU stay. Children aged one month to 13 years, admitted in PICU, and who required CPR during their PICU stay were enrolled. Children who had a cardiac arrest outside of PICU and were shifted to PICU for post-cardiac arrest care were not enrolled. Children with either of the following conditions were also excluded (*i*) patients in whom vascular access was not available (*ii*) ROSC was achieved by defibrillation without the requirement of Epinephrine.

A computer-generated, unstratified, block randomization with variable block sizes of four, six, and eight was used with an allocation ratio of 1:1 by a person not involved in the study. Individual assignments were kept in serially numbered boxes. Each box contained ten identically looking one mL ampoules of either vasopressin or placebo (normal saline). The original label in each ampoule was removed and replaced by an opaque paper. Each box was serially numbered and allocated to the patient according to the random sequence. The serially-numbered trial drug boxes were kept in a separate place in PICU to avoid the wrong allocation in the stressful environment. Only one trial drug box was kept in the crash cart, which contained all the emergency drugs and equipment required for CPR. The nurses were instructed to open the trial drug box, which was kept in the crash cart during CPR. The investigator ensured the replacement of the trial drug box in the crash cart according to the serial number once the trial drug was used. Multiple simulation sessions were carried out and discussed before the start of the study. Injection normal saline (sodium chloride 0.9%, 1 mL, Serum Institute of India Pvt Ltd), injection epinephrine (Bioaderna, 1 mg per 1 mL, Health Biotech Ltd) and injection vasopressin (Vascel 20, 20 Unit per mL, CELON laboratories Pvt Ltd) were used in this study. The institute's central pharmacy supplied the trial drugs. The participants, treating team and nurses administering the medications, and the investigators, were unaware of the treatment assignments. The person who collected and entered the data into the datasheet and the study statistician were unaware of the treatment assign-ment throughout the analyses. The treatment assignment was disclosed, after the first draft of the result was finalized.

All patients received CPR in accordance with the PALS-2015 guidelines established by the American Heart Association (AHA) [2]. This includes the support of airway, breathing, including supplemental oxygen, evaluation of cardiac rhythm, high-quality CPR with minimally interrupted chest compressions, electrical defibrillation if appropriate, and medications except for the trial drugs. The resuscitation team members were trained to provide CPR as per the PALS 2015 guidelines [2]. The facility for standby extracorporeal membrane oxygenation (ECMO) is not available in the study site (PICU). Our hospital has no approved guidelines for 'Do not resuscitate' instructions. Epinephrine plus vasopressin group received intravenous epinephrine (1:10000) 0.1 mL per kg and vasopressin (1:1.5 dilution in normal saline) 0.1 mL per kg (=0.8 unit per kg; maximum dose of 5 mL, 40 unit). Epinephrine plus placebo group received intravenous epinephrine (1:10000) 0.1 mL per kg and placebo (1:1.5 dilution in normal saline) 0.1 mL per kg. The trial drugs were given as bolus doses, concurrently if two vascular accesses were available or within 10 seconds gap if one vascular access was available. The trial drugs were given at an interval of every three minutes until ROSC or a maximum of five doses, whichever was earlier. Three mL normal saline flush was given after adminis-tration of each dose of the trial drug. Subsequently, if needed, epinephrine was continued as per protocol. Post-resuscitation care was provided to the patients who achieved ROSC as per the unit protocol (from PALS-2015 guidelines) [2]. All patients were followed up until death or 90 day post-discharge. The functional status of the survivor was assessed by using the pediatric cerebral performance category (PCPC) scale and pediatric overall performance category (POPC) scale (lower the score, better the neurological outcome) [7]. Data regarding the cardiac arrest events and their outcomes were collected as per the Utstein style template and in the predesigned proforma [8-10].

The primary outcome was the proportion of patients who achieved ROSC. The secondary outcomes were (*i*) survival rate (at 24 hours, PICU, hospital, and 90-day of discharge), (*ii*) functional status (at PICU, hospital, and 90-day of discharge), (*iii*) need for organ support(s), (*iv*) length of stay in PICU and hospital, and (*v*) adverse effect(s) of the study drugs if any. ROSC was defined as the restoration of a spontaneous perfusing rhythm that results

in more than an occasional gasp, fleeting palpable pulse, or arterial waveform [2,3,10]. Sustained ROSC was defined as not requiring chest compressions for 20 consecutive minutes after obtaining ROSC and signs of perfusion [2,3,10]. The probability of adverse trial drug reaction was assessed by Naranjo algorithm [11].

The ROSC rate varies between 47% and 64.6%, as reported by previous studies [12,13]. We assumed that the primary outcome of interest in the control group was 50%. We calculated the sample size based upon the assumption of 30% improvement in the primary outcome by the intervention with 80% power at the 5% significance (two-sided) and 1:1 allocation. Thirty-nine patients were required in each group by calculation. With a 10% attrition rate, the final sample size was estimated as 86 [12-14]. The sample size was calculated using the software nQuery version 4.0.

Statistical analysis: Data were analyzed according to their assigned groups (intention to treat analysis). The distribution of data was checked with the Kolmogorov-Smirnov Z test. Continuous variables were compared between the two groups by Student's *t*-test for normally distributed or by the Mann-Whitney *U* test for skewed data. Proportions were compared by the Chi-square test (or Fisher's exact test if expected cell frequencies were less than five). Kaplan-Meier curve and log-rank test were used to analyze 'time to event' data followed by Cox proportional hazard regression analysis to adjust for the prespecified baseline factors (age, sex, and PRISM-III score). The relative risk and hazard ratio, with a 95% confidence interval, was calculated as appropriate. All tests were two-tailed, and a *P* value of less than 0.05 was considered statistically significant. IBM SPSS software 20.0 (IBM Corp) and Epi Info 7 (7.0.9.7, CDC) were used for data analysis.

RESULTS

The study flow is depicted in **Fig. 1**. Ninety patients were enrolled (epinephrine plus vasopressin, *n*=45, and epinephrine plus placebo *n*=45) after the screening of 118 patients. The baseline characteristics and clinical variables are described in **Table I**. The median (IQR) time to first cardiac arrest since admission was similar between groups [2 (1-7) vs 2 (1-5) day; *P*=0.75]. The most common (80%) arrest rhythm was pulseless electrical activity (PEA). Hemodynamic abnormality (67.8%) was the most common event that led to arrest, followed by respiratory events (23.3%). Respiratory failure was an underlying illness in 76 (84.4%) patients and sepsis in 60 (66.7%) patients. The median (IQR) duration of CPR was similar between groups [18 (10-30) vs 15 (6–30) minutes; *P*=0.96].

The proportion of patients who achieved ROSC was similar in epinephrine plus vasopressin group and epinephrine plus placebo group [RR (95% CI) 1.04 (0.71-1.52); *P*=0.83]. The time to achieve ROSC and the proportion of patients requiring ongoing CPR was similar between two groups during the first 30 minutes of CPR [Log rank *P*=0.99] **(Fig. 2)**. Among ROSC achieved patients (*n*=49), the median (IQR) time taken to ROSC was similar between two groups [10 (4-14) vs 6 (5-10) minutes, *P*=0.21]. The proportion of patients who underwent CPR beyond 30 minutes was also similar between two groups [RR (95% CI) 0.50 (0.19-1.35); *P*=0.16] and none achieved ROSC. There was no significant difference in the proportion of patients who achieved sustained ROSC in the study groups [44.4% vs 53.3%; *P*=0.40]. The survival to hospital discharge was similar in both groups [*n*=1 each]. Mean (SD) diastolic blood pressure (DBP) was similar in epinephrine plus vasopressin group as compared to epinephrine plus placebo group during CPR (38.1 (11.5) mm Hg vs 37.1 (13.4) mm Hg, *P*=0.77). There was no significant difference in the other secondary outcomes between study groups **(Table II)**. In epinephrine plus vasopressin group, one patient developed pulseless ventricular tachycardia which converted into asystole

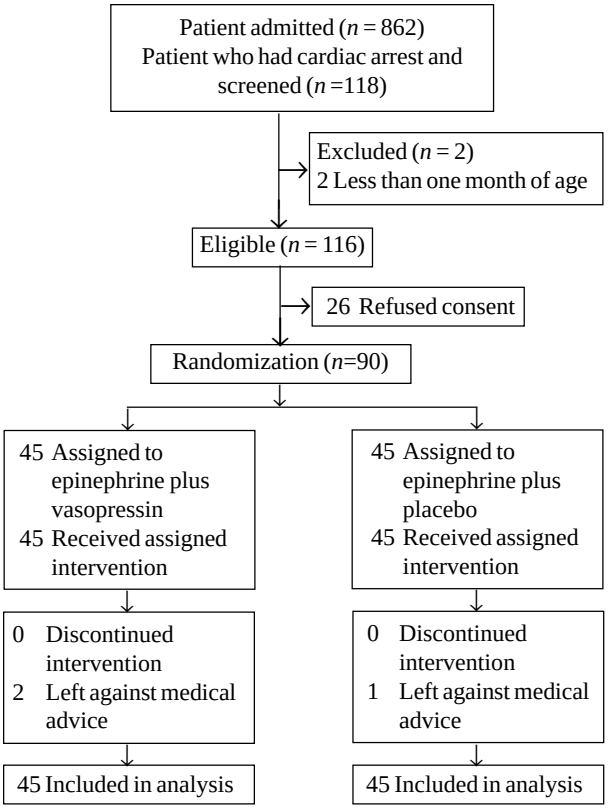


Fig. 1 Study flow chart.

Table I Baseline Characteristics and Clinical Variables of the Two Study Groups

Variables	*Epinephrine plus vasopressin group (n = 45)*	*Epinephrine plus placebo group (n = 45)*
Age, y[a]	2.5 (3.3)	3 (4.4)
Male: female	25:20	28:17
Body mass index[a]	– 2 (1.9)	– 1.9 (2.0)
Pediatric risk of mortality - III score[a]	19.6 (9.6)	18 (8.6)
Arrest rhythm		
Pulseless electrical activity	38 (84.5)	34 (75.6)
Asystole	6 (13.3)	10 (22.2)
Pulseless ventricular tachycardia	1 (2.2)	1 (2.2)
Events leading to arrest		
Hemodynamic abnormality	31 (68.9)	30 (66.7)
Respiratory events	11 (24.4)	10 (22.2)
Rhythm disturbance	3 (6.7)	5 (11.1)
Illness category		
Medical condition	40 (89)	42 (93.3)
Surgical condition	5 (11)	3 (6.7)
Diagnosis and underlying illness[b]		
Respiratory failure	38 (84.4)	38 (84.4)
Sepsis and shock[d]	37 (82.2)	23 (51.1)
CNS illness	19 (42.2)	22 (49)
Pneumonia	24 (53.3)	17 (37.8)
Congenital heart disease	7 (15.6)	10 (22.2)
Renal insufficiency	21 (46.7)	16 (35.6)
Hepatic insufficiency	21 (46.7)	14 (31.1)
Malignancy	5 (11)	4 (9)
Intervention in place at the time of event[c]		
Mechanical ventilation	44 (97.8)	43 (95.6)
$EtCO_2$ monitoring	44 (97.8)	43 (95.6)
Arterial line	37 (82.2)	34 (75.6)
Central venous access	43 (95.6)	42 (93.3)
Vasoactive drug infusion	40 (89)	39 (86.7)
Renal replacement therapy	8 (17.8)	6 (13.3)
Intervention done during CPR		
Sodium bicarbonate	14 (31.1)	23 (51.1)
Calcium gluconate	8 (17.8)	14 (31.1)
Atropine	1 (2.2)	2 (4.4)
Defibrillation	1 (2.2)	1 (2.2)
Doses of study drug[a]	3.6 (1.6)	3.5 (1.6)

Data in no. (%) or [a]mean (SD). CNS: central nervous system; SD: standard deviation; $EtCO_2$: end-tidal carbon dioxide; CPR: cardiopulmonary resuscitation; [b]Patient had one or more conditions; [c]had one or more interventions. Hence, the cumulative totals do not necessarily equal. Three patients also received EtCO2 monitoring after placement of endotracheal tube during CPR; [d]P=0.002.

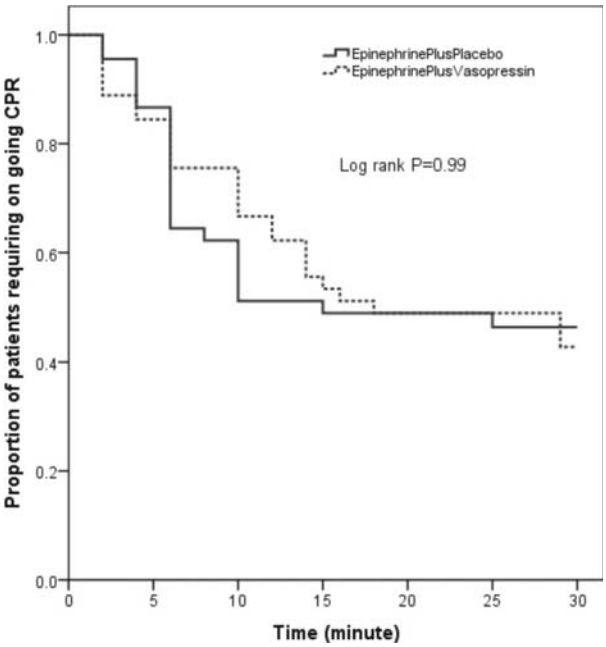


Fig. 2 Kaplan Meier curves showing time to return of spontaneous circulation (ROSC) and the proportion of patients requiring on-going cardio pulmonary resuscitation (CPR) between the two study groups.

during the third cycle of CPR. There were no serious trial drug-related adverse events observed.

DISCUSSION

This randomized controlled trial enrolled 90 patients who underwent CPR in PICU. We found no significant difference in the proportion of patients who achieved ROSC and survival rate between the Epinephrine plus Vasopressin group and Epinephrine plus Placebo group. In our study, the overall rate of achieving ROSC was 54.4%, and survival to hospital discharge was 2.2%. This observation contrasts with the data from high-income countries, where the rates were more than 80% and 40%, respectively [15]. The potential reasons could be the uniform reporting registries, universal healthcare programs, training of health care workers, and accessibility to extracorporeal membrane oxygenation (ECMO).

The outcome of a pediatric cardiac arrest depends upon many factors, including the initial presenting rhythm, the place of cardiac arrest, early recognition of the arrest, and the underlying conditions. In the previous studies done in high-income countries, asystole (55%) was the initial arrest rhythm, and respiratory failure was the common precipitating factor [1,9]. Nevertheless, they enrolled patients not only from PICU but also from the emergency department and general ward [1,9]. In contrast, this study

Table II Primary and Secondary Outcomes of the Study Groups

Variables	*Epinephrine plus vasopressin group (n = 45)*	*Epinephrine plus placebo group (n =45)*	*Relative risk (95% CI)*	*P value*
Primary outcome				
Proportion of patients achieved ROSC	25 (55.5)	24 (53.3)	1.04 (0.71-1.52)	0.83[a]
Proportion of patients achieved sustained ROSC	20 (44.4)	24 (53.3)	0.83 (0.54-1.28)	0.40[a]
Secondary outcomes				
Survival rate at 24 – hour	7 (15.6)	8 (17.8)	0.88 (0.35-2.21)	0.78[a]
At PICU discharge	1 (2.2)	1 (2.2)	1.00 (0.06-15.50)	1.00[c]
At Hospital discharge	1 (2.2)	1 (2.2)	1.00 (0.06-15.50)	1.00[c]
At 90-day post-discharge	1 (2.2)	1 (2.2)	1.00 (0.06-15.50)	1.00[c]
Functional status				
PCPC score – 1 (mild)	-	1 (2.2)	-	-
POPC score – 4 (severe)	1 (2.2)	-	-	-
Organ support therapy among patients achieved ROSC[a,b]				
Mechanical ventilation, h	1.5 (0.5-12)	5 (1.3-33)	-	0.07[b]
Vasoactive therapy, h	1.5 (0.4-8)	4 (1.3-19)	-	0.07[b]
RRT, h[c]	1 (1-14.3)	50 (1-137)	-	0.13[b]
PICU stay, h	1.5 (0.5-12)	5 (1.3-33)	-	0.08[b]
Hospital stay, h	1.5 (0.5-12)	5 (1.3-33)	-	0.08[b]

Data are presented as no.(%) except [a]median (IQR). ROSC: return of spontaneous circulation; CI: confidence interval; IQR: interquartile range; RRT: renal replacement therapy; PCPC: pediatric cerebral performance category; POPC: pediatric overall performance category. [b]25 in epinephrine plus vasopressin group and 24 in epinephrine plus placebo group; [c]seven in epinephrine plus vasopressin and six in epinephrine plus placebo group received RRT support after ROSC.

enrolled patients only from PICU, where stringent monitoring helped identify the arrest much earlier, before progressing to asystole. Similar to our study setting, Rathore, et al. [12] reported bradycardia (52.2%) and sepsis (71%) as the initial arrest rhythm and underlying diagnosis, respectively. They reported a higher ROSC rate (64.6%) and survival to hospital discharge (14%). However, only 21% of CPR occurred in PICU in that study. In our study, patients were enrolled only from PICU. So, the study population was different. Generally, PICU patients are sicker and the majority of them have multiple organ dysfunction requiring organ support. Also, the initial rhythm is an important factor in predicting the outcome; bradycardia rhythm with a pulse is more likely to recover than pulseless non-shockable rhythms [12].

At present, only a limited number of vasopressors are available for use in pediatric CPR, and insufficient data supporting their use [2]. The pediatric guidelines were extrapolated from adult clinical trials and animal studies. Vasopressin acts via the V-1 receptor in the arterial wall and increases the aortic diastolic pressure, thereby improving coronary perfusion pressure. In contrast to epinephrine, there are no β_1 mediated chronotropic and inotropic actions; hence it enhances the myocardial oxygen delivery and reduces the myocardial oxygen consumption during CPR and in the post-resuscitation period [1,16]. Another advantage of vasopressin includes the continuation of vasoconstrictive effects, even in severe acidosis, accompanying cardiac arrest. Hence, vasopressin can act as a better vasopressor during CPR, particularly in patients with sepsis-associated myocardial dysfunction and severe acidosis [16]. However, vasopressin has a longer duration of action than epinephrine, where the persistent vasoconstriction may worsen the myocardial dysfunction in the immediate post-resuscitation period. Post cardiac arrest myocardial dysfunction can be caused by various factors, including the underlying pre-arrest cardiac status, duration and quality of CPR, and the presence of other organ dysfunction(s). So, it is difficult to establish the causal relationship between post-cardiac arrest myocardial dysfunction and vasopressin use. However, no probable serious adverse event due to the trial drug was observed in this study.

The feasibility pilot study in pediatric cardiac arrest by Carroll, et al. [6] reported no significant difference in ROSC, survival to hospital discharge, and neurological

WHAT IS ALREADY KNOWN?

- Few studies have shown promising results of vasopressin use in pediatric in-hospital cardiopulmonary resuscitation.

WHAT THIS STUDY ADDS?

- The combination of cpinephrine and vasopressin did not improve the rate of return of spontaneous circulation, survival, and favorable neurological outcome as compared to Epinephrine alone.

outcome at discharge between vasopressin and control groups (who did not receive vasopressin). Nevertheless, they reported a higher survival rate at 24 hours in the vasopressin group. Their study was limited by non-randomization, small sample size, and addition of vasopressin only after non-response to epinephrine.

Similarly, Duncan, et al. [1] explored the use of vasopressin in pediatric in-hospital arrest from the American Heart Association National Registry of CPR data [1]. Patients who received vasopressin had a longer median arrest duration as compared to those who did not. They also noted that, on multivariate analysis, those who received vasopressin had a reduced ROSC; however, there was no difference in survival at 24 hours. Vasopressin was used as a "drug of last resort" for many of their patients [1], in contrast to our study, where it was used from the time CPR was initiated.

In comparison with an adult, children often present with a non-shockable rhythm, which requires high-quality chest compressions [15]. A systematic review that included 26 RCTs and 21704 participants found that vasopressin did not improve the ROSC rate but improved the survival to hospital admission compared to epinephrine [17]. However, the combination of epinephrine and vasopressin did not show any significant outcome benefits as compared to epinephrine alone [17]. However, most of the included studies were conducted over two decades back. Hence, these findings may not reflect the current practice in the growing era of extracorporeal life support (ECLS) availability.

Though all healthcare providers in our study have been trained in CPR, the intra- and inter-personal variations in chest compression were not monitored objectively. The temporal profiles of end-tidal carbon dioxide and DBP were not analyzed with the outcome of the study. Though our study found similar DBP in both the groups, the pediatric-specific target DBP during CPR is yet to be studied. However, evidence suggests that those who achieve DBP of 25 to 30 mm Hg during CPR have a higher chance of ROSC and survival [15]. Hence, goal-directed CPR targeting the end-tidal carbon dioxide and DBP needs to be considered in future study design. The availability of ECMO service during CPR or after achieving ROSC could have improved the survival to discharge. Recent studies showed that extracorporeal CPR (E-CPR) in pediatric cardiac arrest was associated with shorter resuscitation time and higher survival rate, ranging from 33-64% [18-20]. The AHA recommends considering E-CPR during in-hospital pediatric cardiac arrest, when standard resuscitation has failed, especially in a potentially reversible cause of cardiac arrest [2].

The study concludes that a combination of epinephrine and vasopressin did not improve the rate of return of spontaneous circulation, survival, and favorable neurological outcomes in pediatric intensive care unit cardiac arrest resuscitation as compared to epinephrine and placebo.

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Contributors: RR: had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; RR: Study concept and design; AS, MC, KM, RSK, AJ, RB: acquisition, analysis, or interpretation of data and drafting of the first manuscript; MC, RB, NB, SM: protocol development and revision of the manuscript; RR, SM: critical revision of the manuscript for important intellectual content; RR, NB: study supervision. RR: is the guarantor of the paper. All authors approved the final version of the manuscript.

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REFERENCES

1. Duncan JM, Meaney P, Simpson P, et al. Vasopressin for in-hospital pediatric cardiac arrest: results from the American Heart Association National Registry of Cardiopulmonary Resuscitation. Pediatr Crit Care Med.

2009;10: 191-5.
2. de Caen AR, Berg MD, Chameides L, et al. Part 12: Pediatric Advanced Life Support: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2015;132:S526-42.
3. Panchal AR, Berg KM, Hirsch KG, et al. 2019 American Heart Association Focused Update on Advanced Cardiovascular Life Support: Use of Advanced Airways, Vasopressors, and Extracorporeal Cardiopulmonary Resuscitation During Cardiac Arrest: An Update to the American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2019;140:e881-e94.
4. Voelckel WG, Lurie KG, McKnite S, et al. Effects of epinephrine and vasopressin in a piglet model of prolonged ventricular fibrillation and cardiopulmonary resuscitation. Crit Care Med. 2002;30:957-62.
5. Mann K, Berg RA, Nadkarni V. Beneficial effects of vasopressin in prolonged pediatric cardiac arrest: a case series. Resuscitation. 2002;52:149-56.
6. Carroll TG, Dimas VV, Raymond TT. Vasopressin rescue for in-pediatric intensive care unit cardiopulmonary arrest refractory to initial epinephrine dosing: A prospective feasibility pilot trial. Pediatr Crit Care Med. 2012;13: 265-72.
7. Fiser DH, Long N, Roberson PK, Hefley G, Zolten K, Brodie-Fowler M. Relationship of pediatric overall performance category and pediatric cerebral performance category scores at pediatric intensive care unit discharge with outcome measures collected at hospital discharge and 1- and 6-month follow-up assessments. Crit Care Med. 2000; 28:2616-20.
8. Zaritsky A, Nadkarni V, Hazinski MF, et al. Recommended Guidelines for Uniform Reporting of Pediatric Advanced Life Support: The Pediatric Utstein Style. Ann Emerg Med. 1995; 26:487-503.
9. Reis AG, Nadkarni V, Perondi MB, Grisi S, Berg RA. A prospective investigation into the epidemiology of in-hospital pediatric cardiopulmonary resuscitation using the international Utstein reporting style. Pediatrics. 2002;109: 200-9.
10. Jacobs I, Nadkarni V, Bahr J, et al. Cardiac Arrest and Cardiopulmonary Resuscitation Outcome Reports: Update and Simplification of the Utstein Templates for Resuscitation Registries: A Statement for Healthcare Professionals from a Task Force of the International Liaison Committee on Resuscitation (American Heart Association, European Resuscitation Council, Australian Resuscitation Council, New Zealand Resuscitation Council, Heart and Stroke Foundation of Canada, Inter American Heart Foundation, Resuscitation Councils of Southern Africa). Circulation. 2004;110:3385-97.
11. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther. 1981;30:239-45.
12. Rathore V, Bansal A, Singhi SC, Singhi P, Muralidharan J. Survival and neurological outcome following in-hospital paediatric cardiopulmonary resuscitation in North India. Paediatr Int Child Health. 2016;36:141-7.
13. Donoghue AJ, Abella BS, Merchant R, et al. Cardiopulmonary resuscitation for in-hospital events in the emergency department: A comparison of adult and pediatric outcomes and care processes. Resuscitation. 2015;92: 94-100.
14. Ridgeon EE, Bellomo R, Aberegg SK, et al. Effect sizes in ongoing randomized controlled critical care trials. Crit Care. 2017;21:132.
15. Skellett S, Biarent D, Nadkarni V. What works in paediatric CPR? Intensive Care Med. 2018;44:223-6.
16 Agrawal A, Singh VK, Varma A, Sharma R. Therapeutic applications of vasopressin in pediatric patients. Indian Pediatr. 2012;49:297-305.
17. Finn J, Jacobs I, Williams TA, Gates S, Perkins GD. Adrenaline and vasopressin for cardiac arrest. Cochrane Database Syst Rev. 2019;1:CD003179.
18. Duncan BW, Ibrahim AE, Hraska V, et al. Use of rapid-deployment extracorporeal membrane oxygenation for the resuscitation of pediatric patients with heart disease after cardiac arrest. J Thorac Cardiovasc Surg. 1998;116: 305-11.
19. Morris MC, Wernovsky G, Nadkarni VM. Survival outcomes after extracorporeal cardiopulmonary resuscitation instituted during active chest compressions following refractory in-hospital pediatric cardiac arrest. Pediatr Crit Care Med. 2004;5:440-46.
20. Thourani VH, Kirshbom PM, Kanter KR, et al. Venoarterial extracorporeal membrane oxygenation (VA-ECMO) in pediatric cardiac support. Ann Thorac Surg. 2006;82:138-44; discussion 144-5.

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RESEARCH ARTICLE

Serotype-specific differences in the laboratory parameters among hospitalized children with dengue and genetic diversity of dengue viruses circulating in Tamil Nadu, India during 2017

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Abstract

In 2017, Tamil Nadu, a southern state, had the second highest number of dengue cases from India. In the present study, the serotype-specific differences in the clinical manifestations and laboratory parameters among hospitalized children with dengue were investigated and molecular characterization of the circulating dengue virus (DENV) serotypes during 2017 in Tamil Nadu was performed. Eighty children with dengue-like symptoms consecutively admitted to a tertiary care hospital and positive for DENV NS1 antigen were investigated for DENV serotype utilizing a real-time reverse transcriptase based polymerase chain reaction assay. Complete envelope (E) gene sequencing of the DENV strains was performed. Seventy samples were positive for serotyping (25 DENV-1, 17 DENV-2, six DENV-3, and 22 DENV-4). DENV-4 infections were associated with elevated levels of liver enzymes; Alanine aminotransferase ($P = .021$) and aspartate aminotransferase ($P = .001$). However, none of the serotype was associated with any specific clinical features and severe dengue. Asian and American/African genotypes of DENV-1 were cocirculating. The circulating genotype was cosmopolitan for DENV-2 with multiple lineages, genotype III for DENV-3 and genotype I for DENV-4. Unique mutations were present in the 2017 DENV-4 isolates. The present study suggests the association of DENV-4 with elevated liver enzymes in children hospitalized for dengue. Further, the study reports the genetic diversity of DENV circulating in Tamil Nadu during 2017. The study calls for continuous monitoring of the circulating serotypes and genotypes at regional level in India which might result in a region wise database useful in predicting future outbreaks.

KEYWORDS

alanine aminotransferase, aspartate aminotransferase, dengue, genotypes, serotypes, severe dengue

N. T. Rajesh and K. Alagarasu contributed equally.

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1 | INTRODUCTION

Dengue is the commonest arthropod borne viral disease that affect millions of individuals globally each year and has been listed by the World Health Organization (WHO) as one of the major threats to mankind.[1] Infection with any of the four serotypes of dengue virus (DENV) results in a range of clinical presentations. Symptomatic dengue infections are categorized into nonsevere dengue and severe dengue (SD). The nonsevere dengue cases are again classified into those without warning signs (DwoWS) and with warning signs (DwWS). The case fatality rate is less than 1% if proper intervention is initiated at an early stage of the disease.[2] Disease severity is influenced by both virus and host factors. Immune status determined as primary infection or secondary infection with a different serotype of DENV has been reported to be associated with dengue severity by various epidemiological studies. Age, gender, nutritional status, host immune response, and host genetics contribute to differences in the clinical outcomes.[3,4] Among the viral factors, infecting serotype and the genotype within each serotype also contributes to disease severity.[3] Replacement of the circulating serotype/genotype of DENV with a different one contributes to outbreaks of higher magnitude.[5,6]

In the last decade, highest number of dengue cases were reported during 2017 in India. Within India, West Bengal and Tamil Nadu accounted for approximately 30% of the reported dengue cases.[7] Before 2017, Tamil Nadu witnessed large number of cases during 2012 and DENV-1 was the most common serotype despite the presence of other serotypes. Replacement of American/African (AM/AF) genotype with Asian genotype of DENV-1 was observed during 2012 in Tamil Nadu.[8] However, the serotypes and genotypes circulating during 2017 were not known. The present study was aimed at identifying the serotype-specific differences in the clinical symptoms and laboratory parameters in DENV infected children. Moreover, the study also intended to identify the dominant circulating serotype(s) and genotype(s) associated with dengue in Tamil Nadu during 2017.

2 | MATERIALS AND METHODS

2.1 | Study subjects

The study was a cross-sectional prospective study conducted at PSG institute of Medical Science and Research, a tertiary care teaching hospital located in western Tamil Nadu between August and December 2017. Children aged between 6 months to 15 years who fulfilled the inclusion criteria were consecutively enrolled in the study. Children admitted in the hospital with undifferentiated fever of less than 5 days duration with clinical suspicion of dengue and positive for the presence of NS1 antigen were included in the study. Exclusion criteria include: negative test for the presence of NS1 antigen, fever beyond 5 days, unwillingness to participate in the study and patients who were not admitted. The parents or the legal guardians were explained about the study purpose and an informed consent was obtained from them. The parents or the caretakers of the patients were interviewed for their demographic characteristics such as gender, age, and previous dengue episodes. The patients were observed for clinical symptoms including warning signs till their discharge and clinical features and lab data were collected in a predesigned data collection tool. Based on the clinical symptoms and laboratory parameters, the patients were categorized into dengue without warning signs (DwoWS), dengue with warning signs (DwWS), and severe dengue (SD) based on the WHO 2009 guidelines.[9] Plasma or serum samples collected from the participants at the time of entry into the study, were separated and stored at −80°C. The samples were transported in dry ice to ICMR-National Institute of Virology for identifying the serotype. The study was approved by the institutional human ethics committee (IHEC) of PSGIMS&R, Coimbatore, Tamil Nadu. Molecular characterization of the circulating DENV was performed at ICMR-NIV as a part of Apex referral laboratory activity for the year 2017 which was approved by the IHEC of ICMR-NIV.

2.2 | Serological assays

Serological assays were performed in the hospital laboratory. Presence of DENV NS1 antigen was tested by a antigen capture ELISA kit (Panbio Dengue Early ELISA) as per the manufacturer's protocol. Dengue-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies were tested using ELISA kits (Panbio IgM [01PE20] and Panbio IgG capture ELISA kit [01PE10]) according to the instructions provided with the kit. The IgG capture ELISA kit used in the study detects high-titer IgG antibodies which is usually present in secondary infections. Test positive for IgG indicates secondary infection while test negative indicates primary infection.

2.3 | Detection of dengue virus serotypes

RNA was extracted using an RNA extraction kit (QIAamp viral RNA mini kit, Qiagen, Hilden, Germany) according to the manufacturer's protocol and used for DENV serotyping. Serotyping was performed by using an in-house real-time reverse transcription polymerase chain reaction (RT-PCR) assay. The sequences of the primers and probes were given in Table S1. Primer and probe sequences, the reaction mixture composition and cycling conditions for real-time RT-PCR have been reported previously.[10] The PCR cycle at which the fluorescence signals crossed the threshold values were detected for each sample and samples that had a cycle threshold (C_t) value ≤37 were considered as positive.[10]

2.4 | Isolation of dengue virus from clinical samples

Virus isolation was performed in samples which were positive for DENV RNA with increased viral load (C_t value ≤25) using C6/36 cell

line as reported previously.[10] Virus stocks were stored at −80°C in aliquots for further use.

2.5 | Sequencing of complete envelope gene of dengue virus

Randomly selected representative virus isolates from each serotype were used for sequencing of complete E gene (seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4 isolates) using primers reported earlier.[11-13] The sequences of the primers were provided in Table S1. RNA extraction, cDNA synthesis, reverse transcription, PCR amplification, gel purification, and sequencing was carried out as described earlier.[10]

2.6 | Phylogenetic analyses of dengue virus isolates

MEGA7.0 was used for multiple sequence alignment and phylogenetic analyses.[14] Seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4 sequences (GenBank accession number for the virus isolates provided in Table S2) from the current study along with the earlier Indian (44 DENV-1, 79 DENV-2, 39 DENV-3, and 31 DENV-4) and global sequences (36 DENV-1, 43 DENV-2, 54 DENV-3, and 47 DENV-4) from GenBank representing different genotypes were used for the phylogenetic analyses. Kimura-2 Parameter model was used to estimate the genetic differences and maximum likelihood (ML) method was used for the construction of the phylogenetic trees. A bootstrap test with 1000 replications was used to evaluate the reliability of position of the branches.[10]

2.7 | Statistical analyses

To compare univariate categorical data, Fisher's Exact tests or Pearson's X^2 was used. Parametric and nonparametric data from subjects infected with different dengue serotypes were compared using one-way analysis of variance and Kruskal-Wallis tests, respectively. Modified Poisson regression with adjustment for relevant clinical covariates, that is, age, gender, and fever at total day was utilized for performing the regression analyses. Data for one serotype was compared with other three serotypes. Except platelet count, the values of other laboratory parameters at the time of admission were used for comparison. The nadir for platelet count was used for comparison. A probability (*P*) value of <.05 was considered statistically significant. All the statistical analyses were carried out using statistical software R version 3.4.4.

3 | RESULTS

3.1 | Demographic profile of the study subjects

A total of 430 children with undifferentiated fever were admitted with clinical diagnosis of dengue fever of which 236 children fulfilled the inclusion criteria, 80 consecutively admitted children were enrolled in the study and were followed up prospectively. On analyzing for the serotype, 10 samples were negative and hence excluded and only 70 subjects were analyzed. Almost equal proportion of boys and girls were observed and the median age was 9 years (6 months to 15 years) (Table 1).

TABLE 1 Demographic profile of the dengue cases infected with different dengue virus (DENV) serotypes

Patient Demographics									
	DENV–1 (n = 25)		DENV–2 (n = 17)		DENV–3 (n = 6)		DENV–4 (n = 22)		*P* value*
Variables	N	%	N	%	N	%	N	%	
Gender									
Male	9	36	11	64.7	3	50	15	68.2	.441
Female	16	64	6	35.3	3	50	7	31.8	
Locality									
Coimbatore	7	28	3	17.6	1	16.6	4	19	.832
Dharapuram	5	20	–	–	–	–	3	14.3	
Dindigul	2	8	–	–	–	–	–	–	
Karur	1	4	–	–	–	–	1	4.8	
Palani	1	4	3	17.6	1	16.6	3	14.3	
Pollachi	1	4	–	–	0	–	–	0	
Tiruchi	0	–	–	–	0	–	1	4.8	
Tirupur	8	32	11	64.7	4	66.6	10	47.6	
Age, median (range)	9 (0.9–16)	–	6 (0.3–15)	–	9 (6–15)	–	10 (4–14)	–	.236
Fever day at presentation, median (range)	4 (1–8)	–	5 (2–9)	–	4.5(3–5)	–	4.5 (1–8)	–	.442
Fever at total days, median (range)	5 (4–8)	–	5 (3–9)	?	4.5 (4–5)	–	5 (3–8)	–	.188

*X^2 test was used to compare categorical variables (gender and locality) and one–way analysis of variance was used to compare the continuous variables (age, fever day at presentation and fever at total days).

3.2 | Serotype-specific differences in the clinical features, lab parameters and disease severity

All four serotypes were observed to be circulating in the study population with the prevalent serotype being DENV-1, (n = 25, 35.7%), followed by DENV-4 (n = 22, 31.4%), and DENV-2 (n = 17, 24.3%). DENV-3 was identified in 8.6% (n = 6) of the subjects. Apart from a slight female preponderance among DENV-1 infected children, gender, age, and duration of fever were not different between the children infected with different serotypes (Table 1).

A similar modified Poisson regression analysis adjusted for age, gender, and day of fever was performed for various lab parameters. A statistically significant increase in liver enzymes, aspartate aminotransferase (median with range 131.5 [22-1156] $P = .001$) and alanine aminotransferase (median with range 63 [14-594] $P = .021$) were found in DENV-4 infected children compared to children infected with other serotypes. There was no significant difference in the level of liver enzymes between primary and secondary infections. The mean ± standard deviation of liver enzymes, SGPT and SGOT in children with primary and secondary dengue were 99.18 ± 123.91, 66.83 ± 79.09 ($P = .255$) and 172.25 ± 225.03, 139.38 ± 164.15 ($P = .443$). Other hematological parameters including platelet count during the hospital period were not significantly different among children infected with different serotypes (Table 2).

After adjustment for age, gender, and duration of fever, we observed no statistically significant correlation between the infecting serotype and the clinical features (Table 3). A majority of the children had secondary dengue infection (n = 52 [67.5%]). However, immune status was not associated with disease severity (Table 3).

Classifying the children based on disease severity according to WHO 2009 criteria, there were 38 cases with DwoWS, 26 with DwWS, and 6 had SD. There was no significant association between disease severity and infecting serotype (Table 3).

3.3 | Results of virus isolation

A total of 51 samples were used for virus isolation. Virus isolation was positive in 34 samples (seven DENV-1, 13 DENV-2, two DENV-3, and 12 DENV-4). Complete E gene of the representative virus isolates (seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4) was sequenced.

3.4 | Circulating genotypes of DENV-1

DENV-1 was classified into five genotypes as described earlier.[15] Since there were confusions with regard to the Roman number based nomenclature, geographical names were provided.[16] Seven DENV-1 virus isolates were sequenced. The ML method-based phylogenetic tree (Figure 1) revealed that five isolates grouped with the Asian genotype (GI). Two isolates clustered with the American African (AM/AF) genotype (GV). Asian genotype isolates obtained during

TABLE 2 Association of laboratory/hematological parameters with infecting dengue virus (DENV) serotype

Variables	DENV-1 (n = 25)			DENV-2 (n = 17)			DENV-3 (n = 6)			DENV-4 (n = 22)		
	Median	Range	*P* value*	Median	Range	*P* value*	Median	Range	*P* value*	Median	Range	*P* value
Hemoglobin, g/dL	12	9.7–14.6	.971	12.1	9.5–15.4	.455	12.2	10.7–17	.557	12.5	8.4–17.3	.898
Hematocrit, %	36.4	31.1–44.5	.837	36.5	30–46.2	.433	37.1	33–50.4	.562	37.15	27.2–50.2	.829
Total count of white blood cells/mm^3	3600	2920–9300	.739	4050	1900–8500	.708	3950	2600–8000	.336	4200	1700–9100	.491
Neutrophils, %	45	13.6–80	.751	42.7	11–91.7	.321	72.8	40.4–79.6	.517	52.95	7–73.9	.382
Lymphocytes, %	42	11–79.4	.849	46.4	5–79	.276	46.1	9–49	.476	34.5	20.5–83.4	.277
Erythrocyte sedimentation rate, mm/h	12	2–68	.769	10	2–44	.819	8.5	2–44	.112	15	1–59	.366
Alanine aminotransferase, IU/L	32	11–324	.491	30	12–150	.251	34	14–167	.451	63	14–594	**.021**
Aspartate aminotransferase, IU/L	87	26–566	.235	78	17–318	.845	81.5	26–208	.321	131.5	22–1156	**.001**
Minimum platelet count observed/mm^3	48 000	11 000–215 000	.258	69 000	23 000–220 000	.190	92 500	24 000–114 000	.805	58 500	13 000–173 000	.832

Note: Kruskal–Wallis test was used for comparison between different serotypes.

TABLE 3 Clinical presentations, immune status, and disease severity in children infected with different DENV serotypes

	DENV–1 (n = 25)		DENV–2 (n = 17)		DENV–3 (n = 6)		DENV–4 (n = 22)	
	N	%	N	%	N	%	N	%
Clinical variables								
Vomiting	6	24.1	6	35.3	2	33.5	5	22.7
Rash	1	4	–	–	1	16.7	–	–
Skin/mucosal bleeds	–	–	1	5.9	–	–	–	–
Myalgia	4	16	2	11.8	2	33.3	8	36.4
Abdominal pain	11	44	8	47.1	3	50	9	40.9
GI/GU bleeds	1	4	–	–	–	–	–	–
Hypotension	2	8	–	–	–	–	–	–
Respiratory distress	3	12	–	–	–	–	1	4.5
Pleural effusion	2	8	–	–	1	16.7	1	4.5
Hepatomegaly	9	36	7	41.2	3	50	10	45.5
Immune status and disease severity								
DwoWS								
Primary	11	68.7	5	55.6	1	100	7	58.3
Secondary	5	31.3	4	44.4	0	–	5	41.7
DwWs and SD								
Primary	3	33.3	3	37.5	3	60.0	6	60.0
Secondary	6	66.7	5	62.5	2	40.0	4	40.0
Disease severity and serotypes								
DwoWS	16	64.0	9	52.9	1	16.7	12	54.5
DwWS	7	28.0	6	35.3	5	83.3	8	36.4
Severe dengue	2	8.0	2	11.8	–	–	2	9.1
Relative risk adjusted for age, gender and fever days at admission[a]	1.1 (0.76–1.6)		1.03 (0.43–2.46)		0.79 (0.22–2.89)		1.41 (0.66–3.0)	
DwWS + SD vs DwoWS								

Note: X^2 test was used for comparison between different serotypes.
Abbreviations: DENV, dengue virus; DwoWS, dengue without warning signs; DwWs, dengue with warning signs; SD, severe dengue.
[a]Modified Poisson regression was used for calculating the relative risk adjusted for age, gender, and fever days at admissionadmission.

2017 from Tamil Nadu clustered with Indian strains isolated during 2012-2017 from Kerala, Maharashtra, and Tamil Nadu and strains from Sri Lanka and Singapore. Five lineages, India-I to India-IV and Afro India, have been described within the AM/AF genotype earlier.[12] The present study isolates clustered with India-IV lineage strains. Comparison of amino acid sequences of the Indian isolates belonging to Asian genotype with the isolates from Singapore and Sri Lanka revealed that most of the 2017 isolates from Tamil Nadu had three conservative changes (T329S, L475P, and L484M) and one nonconservative change (F337I) that were not observed in earlier isolates (Table S3).

3.5 | Circulating genotype of DENV-2

DENV-2 was classified into five genotypes as described earlier.[17] Further, subclassifications within the cosmopolitan genotype was performed as described recently.[18] For DENV-2, ten isolates were sequenced for E gene. All sequenced isolates grouped within the cosmopolitan genotype. Within cosmopolitan genotype, Tamil Nadu isolates clustered with strains from two lineages—Cosmopolitan I (C-I) and C-III. Within C-I, five isolates were part of C-IA sublineage, and one isolate was part of C-IB (Figure 2). Amino acid sequence analysis within the 2017 Tamil Nadu isolates revealed that C-I strains had Q at 52nd position while it was H in C-III isolates. C-I had a V at 141st position of E protein while isolates from C-IB and C-III had I. Isolates from C-IB and C-III had I at 322nd position while V was present in C-IA (Table S4).

3.6 | Circulating genotypes of DENV-3 and DENV-4

Five genotypes have been reported for DENV-3.[19] One DENV-3 isolate was sequenced which clustered with earlier isolates from India, China, Pakistan, Singapore, and Senegal into GIII genotype and lineage C (Figure S1). For DENV-4, there were five genotypes.[20] Recently, an additional genotype (GVI) has been proposed.[21] Nine DENV-4 isolates were sequenced. All the 2017 isolates from Tamil Nadu grouped within the GI genotype. In the G1 genotype, 2017 isolates from Tamil Nadu along with the strains from India, Pakistan, and Sri Lanka formed lineage C. Strains from Cambodia, China, Singapore, and Thailand formed the lineage B while those from China, Japan, Philippines, and South Korea formed lineage A (Figure 3). Comparison of amino acid sequences of the E protein from the current study isolates with earlier India isolates revealed the presence of altogether 53 unique nonconservative changes in the nine 2017 isolates from Tamil Nadu (Table S5).

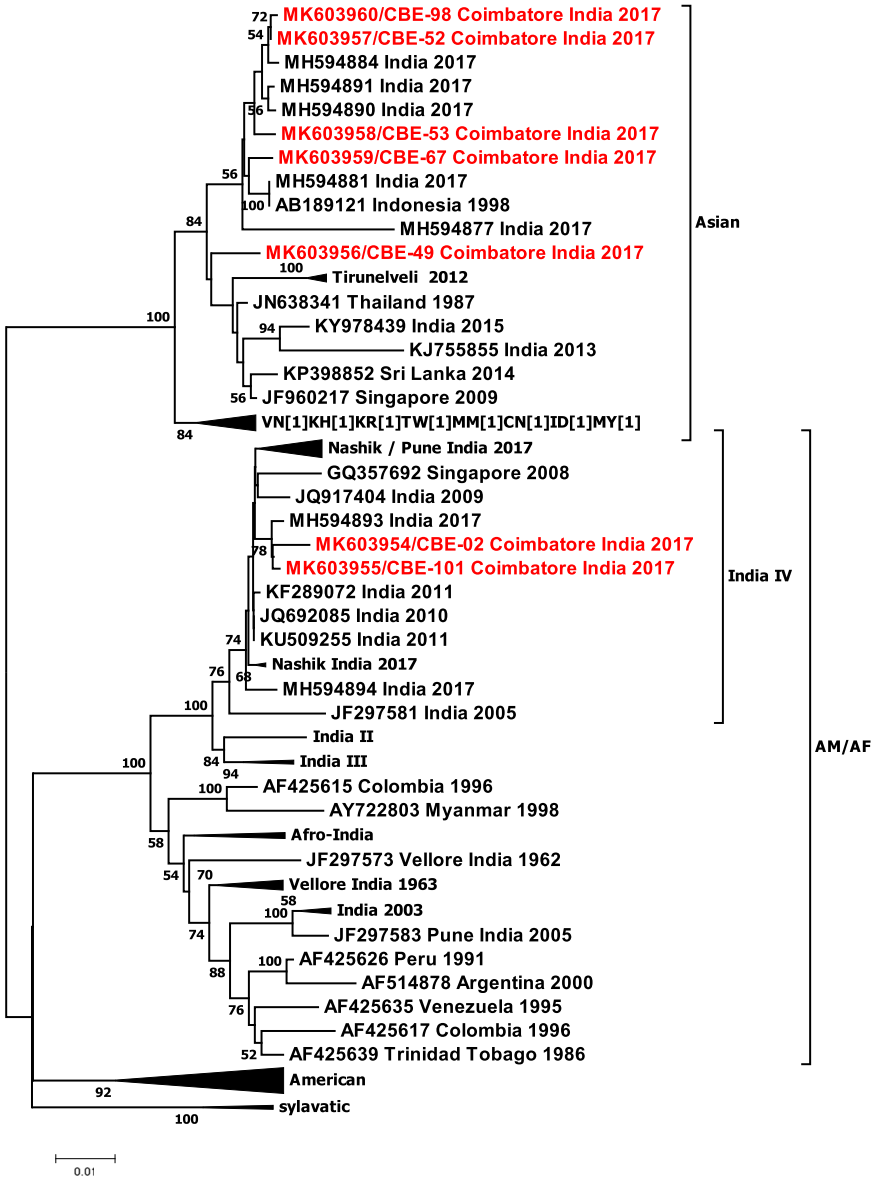


FIGURE 1 Phylogenetic tree of dengue virus-1 (DENV-1) based on E gene sequences All the current study sequences (n = 7) highlighted in Red. Apart from the current sequences, 36 global sequences and 44 Indian sequences were used for generating the phylogenetic tree KH Cambodia/VN-Vietnam/KR-Korea, Republic of/TW-Taiwan/MM-Myanmar/CN-China/ID-Indonesia/MY-Malaysia. Among the five genotypes, Asian, American/African (AM/AF) and Sylavatic genotypes were only shown

4 | DISCUSSION

Forty percent of world's population lives in places endemic to DENV infection and at least 12 000 to 24 000 deaths happen annually in children aged below 15 years.[2] During the study period, admission of 430 undifferentiated fever cases with clinical features suggesting dengue in the pediatric services of one tertiary care hospital alone explains the magnitude of the disease in this endemic region. Circulation of all the four serotypes of DENV has been reported from different regions of India. In 2017, DENV-1 and DENV-3 were more prevalent in Maharashtra, Western India.[10] DENV-1 was the most prevalent serotype while DENV-4 was the least observed one in the 2012 dengue season in Tamil Nadu.[8] In contrast to 2012, DENV-1, DENV-2, and DENV-4 were the prevalent serotypes during 2017 in the study region and could have accounted for the large number of cases from the state. Persistent circulation of DENV-1 might have led to herd immunity permitting the emergence of other serotype viruses. Circulation of multiple serotypes suggests the hyperendemic nature of dengue in this region. The study also suggests the emergence of DENV-4 along with DENV-1 and DENV-2 in Tamil Nadu during 2017.

Studies in the past on disease severity and infecting serotypes have discordant results. A study based on the data from 1994-2006 in Thai children has suggested the association of DENV-2 infection with severe dengue.[22] Further the same study revealed that the risk of SD was more in secondary infections with DENV-2 and DENV-3.[22] A study on adult patients from Singapore revealed that infection with DENV-1 had an increased risk of dengue hemorrhagic fever than DENV-2, whereas a study from Vietnam revealed no difference in the risk of developing severe disease among hospitalized adults infected with DENV-1 and DENV-2.[23,24] A study from Brazil reported that patients infected with DENV-1 serotype had higher risk of severe clinical and laboratory features compared to those infected with DENV-4.[25] A systematic review analyzing the factors associated with



FIGURE 2 Phylogenetic tree of dengue virus-2 (DENV-2) based on E gene sequences All the current study sequences (n = 7) highlighted in Red. Apart from the current sequences, 79 global sequences and 43 Indian sequences were used for generating the phylogenetic tree

dengue shock syndrome, the most severe form of dengue, has revealed DENV-2 as a risk factor.[3] Contradicting all the studies, a study from Bali reported lack of association between DENV serotypes and disease severity.[26] In the present study, as there were less number of children who progressed to SD during hospital stay, for the purpose of analysis, children with SD and DwWS were grouped together and compared with DwoWS. Overall, there were more children with DwoWS than DwWS/SD and none of the serotypes showed any statistically significant increased risk for developing DwWS/SD. Immune status was also not associated with DwWS/SD. However, DENV-4 infections were significantly associated with elevated levels of liver enzymes. A study on pediatric dengue cases from Thailand also has reported that DENV-3 and DENV-4 infection tended to have abnormal elevation of liver enzymes.[27] This observation was contrary to a study from Northern India which has reported an association of elevated levels of liver enzymes with DENV-2.[28] Whether this association of elevated enzymes with DENV-4 can generalized to other regions depends on multiple factors such as the circulating genotype of DENV-4, host immune status, and age of the patients and a study with larger sample size is needed to further confirm the finding.

Phylogenetic analyses indicated the circulation of DENV-1 Asian and AM/AF genotypes, multiple lineages of cosmopolitan genotype of DENV-2, DENV-3 GIII genotype and DENV-4 GI genotype with unique mutations in Tamil Nadu. Cocirculation of DENV-1 Asian and AM/AF genotypes has been reported from Maharashtra in recent years while cocirculation of multiple lineages of DENV-2 cosmopolitan genotype has been reported from Northern India.[10,29,30] A recent study reported that the proportion of Asian genotype among the DENV-1-positive cases in Tamil Nadu had decreased from 90% in 2012 to 57% in 2017.[29] It is possible the DENV-1 Asian genotype viruses might have evolved over the years to become a virus with less epidemic potential phenotype. However, further comparative susceptibility studies in vectors and animal models are needed. In

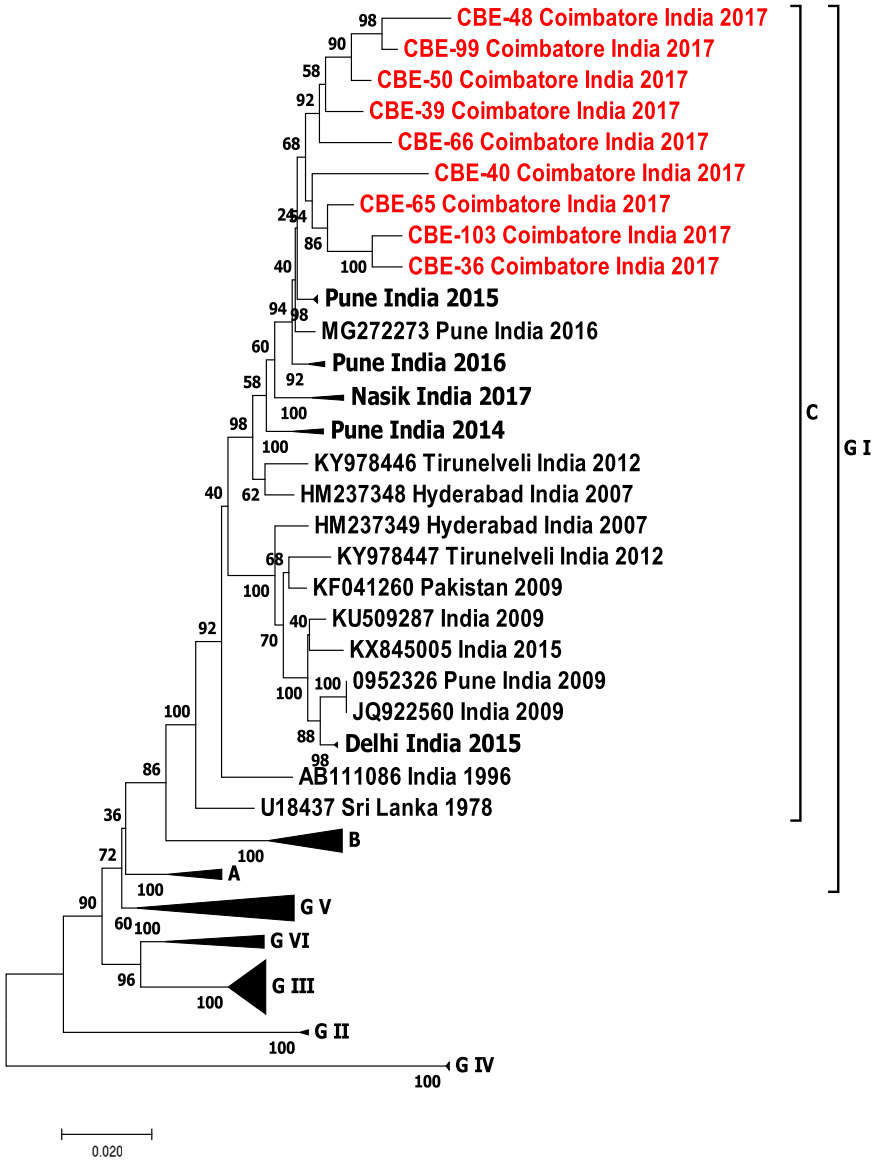


FIGURE 3 Phylogenetic tree of dengue virus-4 (DENV-4) based on E gene sequences. All the current study sequences (n = 9) highlighted in Red. Apart from the current sequences, 47 global sequences and 31 Indian sequences were used for generating the phylogenetic tree

the present study, E gene sequencing of DENV-4 isolates revealed unique mutations which were not observed in earlier Indian DENV-4 strains. The envelope protein has three structural domains I-III. Mutations in the domain II and domain III have more significance since domain II contains the fusion loop which is essential for the interaction between the host endosomal membrane and the virus envelope leading to virus membrane fusion while domain III contains epitopes that are essential for serotype-specific neutralization. Mutations in these domains might affect virus entry as well as the neutralization by serotype/genotype-specific antibodies.[31,32] DENV evolution is shaped by purifying selection in which deleterious mutations affecting viral fitness are removed with time. Further, mutations which confers fitness advantage to DENV in nature were positively selected and contributes to adaptive evolution of DENV.[33] Further follow up studies in the region are needed to discern how these mutations shape up the DENV-4 evolution. It is possible that differences in the viral factors as well as host factors might influence the variable outcomes in different studies. Monitoring the circulating serotypes and genotypes in different regions might result in a region wise database useful in predicting future outbreaks of higher magnitude when changes are observed with regard to the dominant serotypes and new genotypes.

The strength of the present study is that the subjects enrolled were belonging to only to pediatric age, they were prospectively followed up for the outcome and disease severity was classified based on latest guidelines recommended by WHO. Few limitations are, the small sample size, study participants were only hospitalized children from a particular geographic area, hence cannot be extrapolated to general population or other parts of the country. All the patients were recruited within 5 days of fever onset and hence early hospitalization and effective fluid management might have influenced the final outcome. Nonhospitalized cases with undifferentiated febrile illness, which forms the major proportion of DENV cases, were not included in the study and investigation of both hospitalized as well as nonhospitalized cases might help in

identifying the DENV serotypes associated with DwWS and severe dengue.

To conclude, the study reports the activity of all the four serotypes in Tamil Nadu during 2017 with the dominance of DENV-1, DENV-2, and DENV-4. Persistent cocirculation of DENV-1 Asian and AM/AF genotypes, cocirculation of multiple lineages of DENV-2 cosmopolitan genotype and emergence of DENV-4 GI genotype with unique mutations were observed. DENV-4 infection was associated with elevated levels of liver enzymes among hospitalized children. Serotypes and immune status were not associated with disease severity among children hospitalized for dengue.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. World Health Organization. Ten threats to global health in 2019. https://wwwwhoint/emergencies/ten-threats-to-global-health-in-2019. Accessed June 24, 2019.
2. World Health Organization. Dengue: call for urgent interventions for a rapidly expanding emerging disease. 2011. http://applicationsemrowhoInt/docs/RC_technical_papers_2011_3_14217
3. Huy NT, Van Giang T, Thuy DHD, et al. Factors associated with dengue shock syndrome: a systematic review and meta-analysis. *PLOS Negl Trop Dis*. 2013;7:e2412. https://doi.org/101371/journalpntd0002412
4. Alagarasu K. Genetics of susceptibility to severe dengue virus infections: an update and implications for prophylaxis, prognosis and therapeutics. *Dengue Bulletin*. 2016;39:1-18.
5. Ocwieja KE, Fernando AN, Sherrill-Mix S, et al. Phylogeography and molecular epidemiology of an epidemic strain of dengue virus type 1 in Sri Lanka. *Am J Trop Med Hyg*. 2014;91:225-234. https://doi.org/104269/ajtmh13-0523
6. Teoh B-T, Sam S-S, Tan K-K, et al. Dengue virus type 1 clade replacement in recurring homotypic outbreaks. *BMC Evol Biol*. 2013;13:213. https://doi.org/101186/1471-2148-13-213
7. National vector borne disease control programme. Dengue cases and deaths in the country since 2015. 2019. http://nvbdcpgovin. Accessed June 24, 2019.
8. Cecilia D, Patil JA, Kakade MB, et al. Emergence of the Asian genotype of DENV-1 in South India. *Virology*. 2017;510:40-45. https://doi.org/101016/jvirol201707004
9. World Health Organization. Special programme for research, training in tropical diseases. WHO Epidemic, Pandemic Alert Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. World Health Organization Department of Control of Neglected Tropical Diseases, World Health Organization; 2009.
10. Patil JA, Alagarasu K, Kakade MB, et al. Emergence of dengue virus type 1 and type 3 as dominant serotypes during 2017 in Pune and Nashik regions of Maharashtra, Western India. *Infect Genet Evol*. 2018;66:272-283. https://doi.org/101016/jmeegid201810016
11. Kumar SR, Patil JA, Cecilia D, et al. Evolution, dispersal and replacement of American genotype dengue type 2 viruses in India (1956-2005): selection pressure and molecular clock analyses. *J Gen Virol*. 2010;91:707-720. https://doi.org/101099/vir0017954-0
12. Patil JA, Cherian S, Walimbe AM, et al. Evolutionary dynamics of the American African genotype of dengue type 1 virus in India (1962-2005). *Infect Genet Evol*. 2011;11:1443-1448. https://doi.org/101016/jmeegid201105011
13. Patil JA, Cherian S, Walimbe AM, et al. Influence of evolutionary events on the Indian subcontinent on the phylogeography of dengue type 3 and 4 viruses. *Infect Genet Evol*. 2012;12:1759-1769. https://doi.org/101016/jmeegid201207009
14. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 70 for bigger datasets. *Mol Biol Evol*. 2016;33:1870-1874. https://doi.org/101093/molbev/msw054
15. Goncalvez AP, Escalante AA, Pujol FH, et al. Diversity and evolution of the envelope gene of dengue virus type 1. *Virology*. 2002;303:110-119.
16. Domingo C, Palacios G, Jabado O, et al. Use of a short fragment of the C-terminal E gene for detection and characterization of two new lineages of dengue virus 1 in India. *J Clin Microbiol*. 2006;44:1519-1529.
17. Twiddy SS, Woelk CH, Holmes EC. Phylogenetic evidence for adaptive evolution of dengue viruses in nature. *J Gen Virol*. 2002;83:1679-1689.
18. Ali A, Ali I. The complete genome phylogeny of geographically distinct dengue virus serotype 2 isolates (1944-2013) supports further groupings within the cosmopolitan genotype. *PLOS One*. 2015;10:e0138900. https://doi.org/101371/journalpone0138900
19. Lanciotti RS, Gubler DJ, Trent DW. Molecular evolution and phylogeny of dengue-4 viruses. *J Gen Virol*. 1997;78:2279-2284.
20. Lanciotti RS, Lewis JG, Gubler DJ, Trent DW. Molecular evolution and epidemiology of dengue-3 viruses. *J Gen Virol*. 1994;75:65-75.
21. Shrivastava S, Tiraki D, Diwan A, et al. Co-circulation of all the four dengue virus serotypes and detection of a novel clade of DENV-4 (genotype I) virus in Pune, India during 2016 season. *PLOS One*. 2018;13:e0192672. https://doi.org/101371/journalpone0192672
22. Fried JR, Gibbons RV, Kalayanarooj S, et al. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. *PLOS Negl Trop Dis*. 2010;4:e617. https://doi.org/101371/journalpntd0000617
23. Yung C-F, Lee K-S, Thein T-L, et al. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. *Am J Trop Med Hyg*. 2015;92:999-1005. https://doi.org/104269/ajtmh14-0628
24. Fox A, Le NMH, Simmons CP, et al. Immunological and viral determinants of dengue severity in hospitalized adults in Ha Noi, Viet Nam. *PLOS Negl Trop Dis*. 2011;5:e967. https://doi.org/101371/journalpntd0000967
25. Rocha BAM, Guilarde AO, Argolo AFLT, et al. Dengue-specific serotype related to clinical severity during the 2012/2013 epidemic in centre of Brazil. *Infect Dis Poverty*. 2017;6:116. https://doi.org/101186/s40249-017-0328-9
26. Megawati D, Masyeni S, Yohan B, et al. Dengue in Bali: clinical characteristics and genetic diversity of circulating dengue viruses. *PLOS Negl Trop Dis*. 2017;11:e0005483. https://doi.org/101371/journalpntd0005483
27. Kalayanarooj S, Nimmannitya S. Clinical and laboratory presentations of dengue patients with different serotypes. *Dengue Bulletin*. 2000;24:53-59.

28. Kumaria R. Correlation of disease spectrum among four Dengue serotypes: a five years hospital based study from India. *Braz J Infect Dis*. 2010;14:141-146.
29. Alagarasu K, Patil JA, Kakade MB, et al. Spatio-temporal distribution analysis of circulating genotypes of dengue virus type 1 in western and southern states of India by a one-step real-time RT-PCR assay. *Infect Genet Evol*. 2019;75:103989. https://doi.org/101016/jmeegid2019103989
30. Kar M, Nisheetha A, Kumar A, et al. Isolation and molecular characterization of dengue virus clinical isolates from pediatric patients in New Delhi. *Int J Infect Dis*. 2018;84S:S25-S33. https://doi.org/101016/jijid201812003
31. Modis Y, Ogata S, Clements D, Harrison SC. Structure of the dengue virus envelope protein after membrane fusion. *Nature*. 2014;427:313-319.
32. Gallichotte EN, Baric TJ, Nivarthi U, et al. Genetic variation between dengue virus type 4 strains impacts human antibody binding and neutralization. *Cell Reports*. 2018;25:1214-1224. https://doi.org/101016/jcelrep201810006
33. Pollett S, Melendrez MC, Maljkovic Berry I, et al. Understanding dengue virus evolution to support epidemic surveillance and counter-measure development. *Infect Genet Evol*. 2018;62:279-295. https://doi.org/101016/jmeegid201804032

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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[illegible]

[illegible] Coronavirus disease 2019 (COVID-19), in children has varied clinical presentations from asymptomatic infection to severe pneumonia, multi system inflammatory syndrome in children (MIS-C) and rarely death. To date, limited data has been published on the profile of COVID-19 infection among Indian children. The objective of this study was to analyze the clinical spectrum, laboratory parameters, treatment and it's outcome in children less than 15 years admitted with COVID-19 infection.

[illegible] This was a cross sectional study done in a tertiary care hospital. Children with COVID-19 infection detected by Reverse transcriptase polymerase chain reaction (RT-PCR) were included.

[illegible] Out of 167 children, 93 were males (55.6%) and median (Interquartile range- IQR) age of children admitted were 9 (4-13) years. Family cluster (71%) was the dominant source of infection. Majority were asymptomatic (60%, N=100). The predominant symptoms were fever (29%), cough (16%) and rhinorrhea (4.1%). Seven (4.1%) children had comorbidities and two (28%) among them had moderate to severe disease. Median (IQR) total leucocyte count was 7 (4-10) ×103 µl and platelet count was 390 (275-500) ×103 µl. Leukopenia was observed in 58% (N=81), lymphocytosis in 32.8% (N=46) and thrombocytopenia in 3.5% (N=5). Inflammatory markers were raised in 10-15% of children. Peri-bronchial cuffing was the common abnormality (N=14, 17.5%) in chest X-ray (CXR) and peripheral heterogeneous opacities were noted in severe disease. Children with moderate to severe symptoms were treated with remdesivir and steroids.

[illegible] COVID-19 infection in children was often benign and increased severity was noted among those with comorbidities. Leukopenia, lymphocytosis were commonly observed. High inflammatory markers were noted in severe disease. Nil fatality among our study group.

[illegible] COVID-19, Children, Chest X-ray, Leukopenia, Lymphocytosis, Laboratory profile

[illegible]

In the pre-coronavirus disease-19 (COVID-19) era human coronavirus (HCoV) was one among the causes of bronchiolitis in infants and upper respiratory infection in children.[1] Previous outbreaks of Severe acute respiratory syndrome (SARS) and Middle east respiratory syndrome (MERS) caused by coronavirus were associated with less mortality in children.[2] Similarly COVID-19 which causes severe acute respiratory syndrome (SARS-CoV2) in adults causes milder illness in children. The incidence in children is around 8.7% in below 19 years according to CDC.[3] The hypothesis behind the less severe disease in children being differences in innate and adaptive immunity, pre-existing antibodies to corona virus, more frequent respiratory infections, protective effects of other live vaccines, different expression of angiotensin converting enzyme-2 (ACE-2) receptors and less intense exposure.[4] Very rarely it may lead to Acute respiratory distress syndrome (ARDS), Multi organ dysfunction (MODS) and death. Multi system inflammatory syndrome in children (MIS-C), a post viral immune dysregulation following SARS-

CoV-2 infection is common among children and adolescents which may rarely lead to significant morbidity and mortality if not treated early.[5]

Hematological and biochemical markers of COVID-19 were well studied in adults. This cannot be extrapolated as such for children. The aim of the study was to present the detailed clinical, laboratory profile, treatment and outcome of 167 children below 15 years of age admitted with COVID-19 infection.

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This was a descriptive observational study and followed the Strengthening the reporting of observational studies in epidemiology (STROBE) guideline.

This study was done at PSG Institute of Medical Sciences and Research hospital which is one of the largest designated tertiary care hospitals in Coimbatore, India which caters to patients from around 250 km radius. Institutional ethics committee approval was obtained. Informed consent was taken from parents or patients included in prospective analysis (N=107) and waiver of consent was obtained for those taken retrospectively (N=60). All children less than 15 years admitted with COVID-19 infection between March 2020 to February 2021 were included in our study. Positive cases were confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) validated as per ICMR standards. Both nasopharyngeal and oropharyngeal swabs were taken for confirmation. The severity of illness were categorized into mild, moderate, severe and critical as per the COVID-19 clinical management-Interim guidance.[6] Mild illness is defined as symptomatic COVID-19 positive patients without evidence of pneumonia, moderate as with clinical signs of pneumonia and no signs of hypoxia and severe as clinical signs of pneumonia and SpO_2<90%. Critical disease is with ARDS, sepsis or septic shock and MODS.

Fever was defined when the temperature was >38°C, the source of infection was identified by recording which family member developed the symptoms first. Basic hematological and radiological investigations were taken for most of the patients admitted except in few because of logistical issues. Investigations were repeated in patients showing persistent symptoms.

All the necessary information was collected from electronic records on the hospital database. Chest X-ray findings were analyzed and reported by a single radiologist. Information on baseline demographics, clinical features, symptoms, signs, laboratory reports, imaging, treatment and outcome were recorded. Laboratory values were interpreted with age specific reference ranges. If the patient had a serial investigation for a particular parameter, the highest value was taken into consideration. Asymptomatic and mildly symptomatic children were given supportive care. Those presented with sore throat, fever beyond 3 days, lower respiratory symptoms, pneumonia (confirmed by clinical and radiological signs) were treated with antibiotics. Steroids were used only in severe cases.

Statistical analysis

Data was entered in Microsoft excel (Microsoft corporation, Redmond, USA) sheet and analyzed using SPSS software version 24. Descriptive statistics were used to summarize the data. Continuous variables were described as median and interquartile range. Categorical variables were described as frequencies and percentages. Chi square test was used to assess the relationship between gender and incidence of COVID-19. Mann-Whitney U-test was used to assess the correlation between severity of illness and inflammatory markers. A p value of <0.05 was considered significant.

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A total of 167 children admitted with COVID-19 positive reports were included in our analysis. The baseline demographic and clinical characteristics of these children are shown in Table 1.□

Demographic and clinical characteristics

The median age (IQR) of children admitted were 9 (4-13 years) and male children (N=93, 55.6%) outnumber females with a male: female ratio of 1.25:1. There was no relationship between gender and incidence of COVID infection in children (p=0.12, Chi square test). Family cluster as the source of infection was identified in 71% (N=119) (Table 1).

Fever was the predominant symptom in 29.3% (N=49) followed by cough (N=27, 16.1%) and rhinorrhea (N=7, 4.1%). Diarrhoea was noted in 3.5%, abdominal pain in 1.1% and loss of taste was present in 1.1%. Very few had fever beyond 24-48 hours after hospital admission. Among the admitted, 7 (4.1%) had preexisting comorbid conditions (Table 2). In them, a child with a germ cell tumor and a hemolytic anemia had moderate to severe illness. More than half were asymptomatic, 38% (N=64) had mild symptoms, 0.5% (N=1) had moderate symptoms and 1.1% (N=2) had severe symptoms. Out of 4 children admitted in ICU, 2 had comorbidities (hemolytic anemia and germ cell tumor) one among them required oxygen support by mask (germ cell tumor), a 2-months-old without any comorbidity had respiratory distress and hypoxemia supported by mechanical ventilation and a child with head injury was admitted for monitoring (Table 3). There was a significant association between comorbidity and severe illness (p=0.014, Fisher's exact test).

Radiological and laboratory finding

CXR was performed in 48% (N=80) of children and out of which 78% (N=63) were normal. Few had prominent

central peri bronchial cuffing (N=14, 17.5%) and peripheral heterogeneous opacities were noted in 3.7% (N=3). CT was done only in patients with moderate to severe illness which showed ground glass opacities (Table 4). On admission, the median (IQR) WBC count was 7 (4-10)×10^3/µl. Leukopenia, lymphopenia and lymphocytosis were noted in 58%, 11.4% and 32.8% respectively. Thrombocytopenia was seen only in 3.5%. Inflammatory markers IL-6, ferritin, D-dimer were raised in 10%, 6% and 13% respectively (Table 5). Moderate to severe group exhibited significantly higher IL-6, ferritin and D-dimer than milder group (p=0.04, p=0.04, p=0.06, Mann-Whitney U-test) (Figure 1-3). Hypoalbuminemia was noted in 3.5% and liver enzymes were elevated in 7-8%.

Treatment and outcome

Intravenous antibiotics and remdesivir were given in 3 children. Steroid was given only in a child with a Germ cell tumor. An infant with severe respiratory distress (0.5%) was treated with mechanical ventilatory support considering High frequency nasal cannula (HFNC) having high aerosolization risk during the initial phase of pandemic. In all others symptomatic treatment was given accordingly. Those with severe illness have recovered and discharged by 2nd to 3rd week of admission. There was nil mortality among our study group (Table 6).

Table 1: Demographic and clinical characteristics of children with COVID-19

Characteristics	n (%)
Median (IQR) age in years	9 (4-13)
Age group	
<1	19/167 (11)
1-5	39/167 (23.3)
6-10	47/167 (28.5)
10-15	62/167 (37)
Gender	
Male	93/167 (55.6)
Female	74/167 (44.3)
Exposure	
Family cluster	119/167 (71)
Other exposure	28/167 (17)
Unknown	20/167 (12)

Note: Data presented are in numbers (%) or median (interquartile range).

Table 2: Symptoms and signs in children with COVID-19

Symptoms	n (%)
Fever	49 (29.3)
Cough	27 (16.1)
Breathlessness	4 (2.3)
Sore throat	7 (4.1)
Headache	1 (0.5)
Vomiting	3 (1.7)
Abdominal pain	3 (1.7)
Diarrhea	6 (3.5)
Myalgia	2 (1.1)
Rash	2 (1.1)
Coryza	6 (3.5)
Asymptomatic	2 (1.1)

Table 3: Co-morbidities in children with COVID-19

Co-morbidities	n
Asthma	1
VSD	1
Hemolytic anemia	1
Seizure disorder	1
Germ cell tumor on chemotherapy	1
Congenital hypothyroid	1
Hirschsprung disease with dysmorphic facies	1
Severity	
Asymptomatic	100/167 (60)
Mild	64/167 (38)
Moderate	1/167 (0.5)
Severe	2/167 (1.1)

Table 4: Chest radiograph findings in children with COVID-19

Chest radiograph findings	n (%)
Normal	80/167 (48)
Normal	63/80 (78)
Peribronchial cuffing	14/80 (17.5)
Peripheral heterogeneous opacity	3/80 (3.7)
Ground glass opacity	3/167 (1.7)
Ground glass opacity	3/3 (100)

Note: CXR- Chest X-ray, CT-Computed tomography.

Table 5: Laboratory findings in children with COVID-19

Parameters	Median (IQR)	Normal range
Total leucocyte count (10^3/µl)	7 (4-10)	4-10×10^3 cells/µl
Leucocytosis	17/140 (12.1)	>10×10^3 cells/µl
Leucopenia	81/140 (58)	<4×10^3 cells/µl
Absolute neutrophil count (10^3/µl)	3.0 (0.8-5.2)	1.5-3 cells/µl
Lymphopenia	16/140 (11.4)	ALC <1.5×10^3 cells/µl
Lymphocytosis	46/140 (32.8)	>4×10^3 cells/µl
Platelet count (10^3/µl)	2.5(0.5-4.5)	1-8 ×10^3 cells/µl
Thrombocytosis (10^3/µl)	390 (275-500)	150-400×10^3 cells/µl
Thrombocytopenia	5/140 (3.5)	<150 ×10^3 cells/µl

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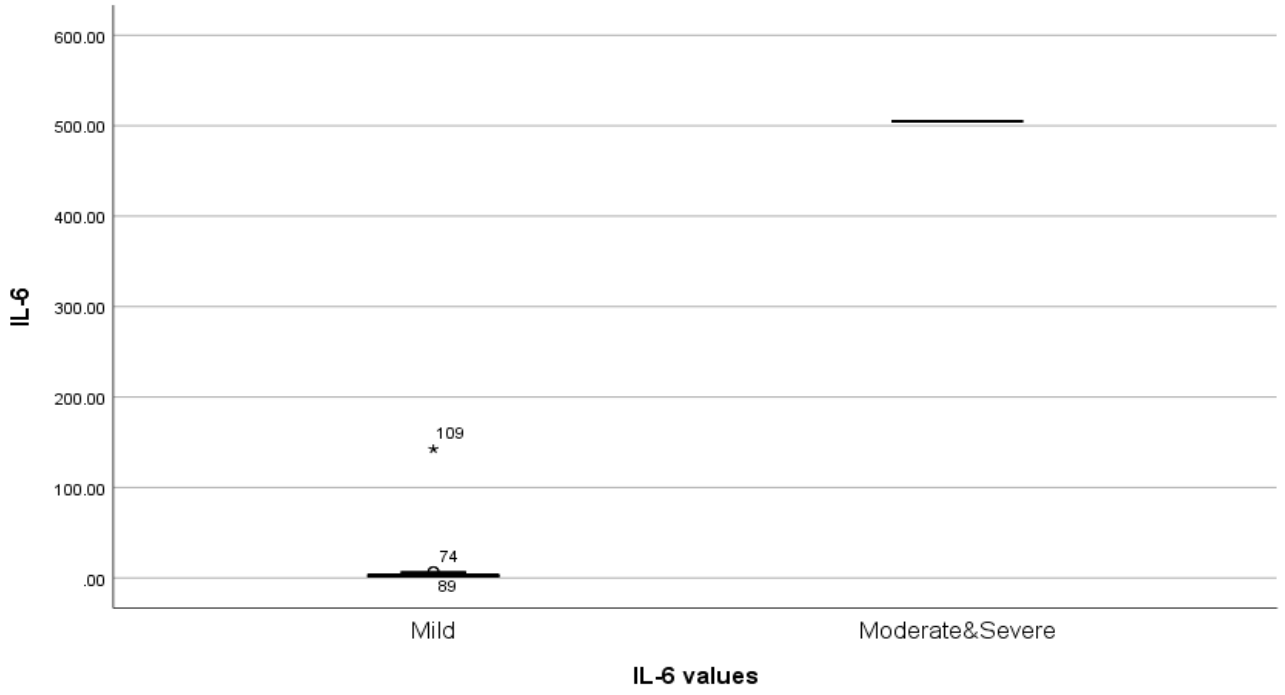
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□□□□□□□d□□ □ □□	4/38 (10.5)	>0.6 mg/dl
□□□□□□□d□□ □□ □	4/30 (10)	≥40 mm/hour
□□□□□□□d□□ □ □ □	1/40 (2.5)	>500 u/l
□ □□□□r□□□□□□□ □□□□□□□□	1/85 (1.2)	<4 mg/dl
□ □□□□□□□□ □□□□ □□□□□□□	3/85 (3.5)	<3.5 mg/dl
□□□□□□□d□□ □□ □	7/85 (8)	>50 u/l
□□□□□□□d□□ □ □ □	6/85 (7)	>45 u/l
□□□□□□□d□□r□□□□□□□□	3/106 (2.8)	As per age-based cut-off mg/dl
□□□□□□□d□□□6□	5/50 (10)	≥7 pg/ml
□□□□□□□d□□rr□□□□□□□□□	3/50 (6)	>500 ng/ml
□□□□□□□d□□ □d□□ □r□	4/31 (13)	≥0.5 mg/l FEU

Note: IQR- interquartilerange, CRP- C-reactiveprotein, ESR- Erythrocyte sedimentation rate, LDH-lactatedehydrogenase, AST- aspartate amino transferase, ALT- alanineaminotransferase, IL-6-interleukin 6.

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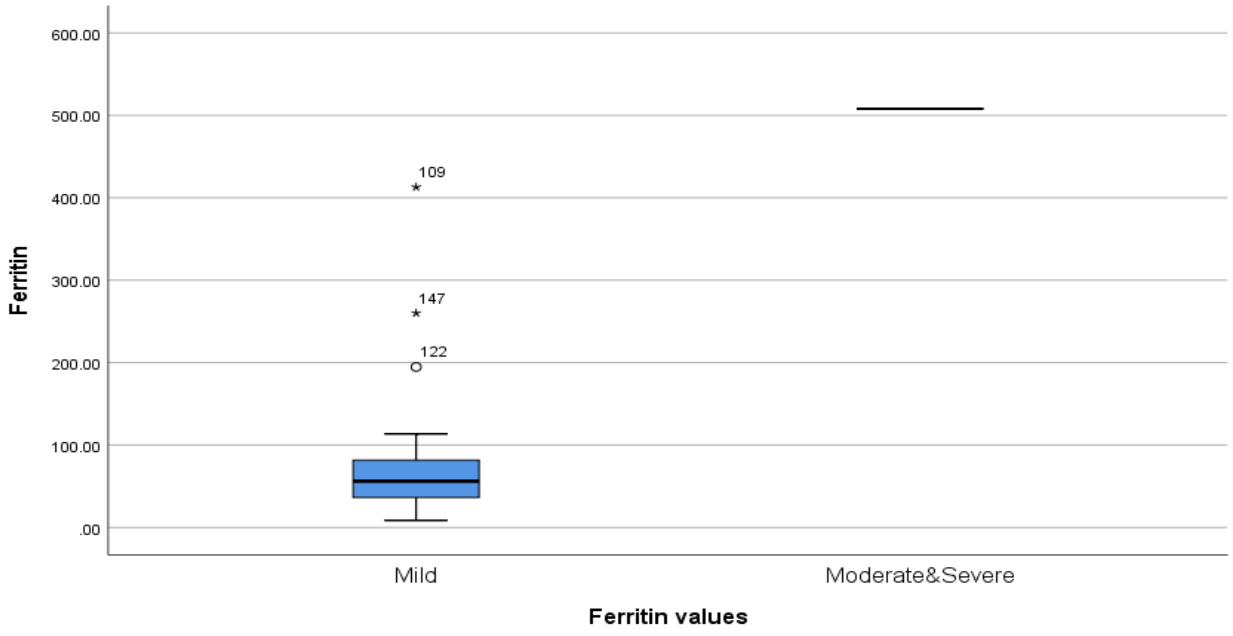
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Respiratory failure	1/167(0.5)
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IV antibiotics	4/167 (2.3)
Remdesivir	3/167 (1.8)
Oral azithromycin	10/167 (5.9)
Systemic glucocorticoid	1/167 (0.5)
Oxygen by mask	1/167 (0.5)
Mechanical ventilation	1/167 (0.5)
Admission to ICU	4/167 (2.3)
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Discharge	167/167 (100)
Mortality	Nil

Note: Reference ranges as per from Nelson textbook of paediatrics.



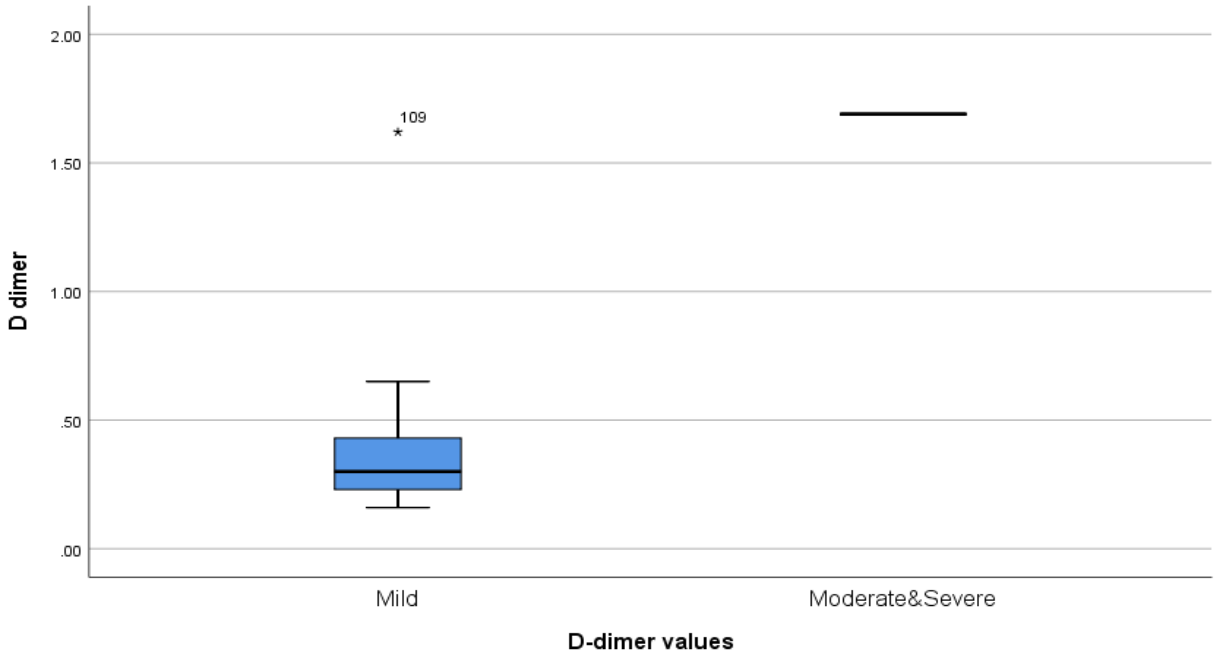
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Note: Data were expressed in median. P=0.04 by Mann-Whitney U test.□



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Note: Data were expressed in median. P=0.04 by Mann-Whitney U test. □



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Note: Data were expressed in median. P=0.06 by Mann-Whitney U test.

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The main focus of this study was to analyze the clinical, laboratory profile, treatment and it's outcome of 167 children admitted in the designated tertiary care COVID unit which admits children from asymptomatic infection to critical disease. When compared to adults, paediatric age groups were reported with less severe disease. As the schools were closed and outdoor activities were restricted the primary source of infection in children were household contacts. Majority of children in our cohort were asymptomatic (60%), and few had variable severity of symptoms from mild to severe. As per WHO-China joint mission on COVID-19, 2.5% of cases under 19 years had severe infection and 0.2% had critical disease.[7] A large

report from China stated that 10.6% ,7.3% and 4.2% children between 0-1, 1-5, 6-10 years had severe infection respectively.[8] High risk groups for severe infection are infants below 1 year and those with underlying medical conditions like neurological problems, metabolic disorders, congenital heart disease, diabetes, asthma, chronic lung disease, sickle cell disease and obesity.[9] Similarly, in this study a 2-months old infant and a child with comorbidity developed severe disease. Incidence of severe disease among comorbid children was high (14%) in this study. This is in accordance with a systematic review of 42 studies on severe COVID-19 infection where the clinical manifestations were severe in 5.1% of children with comorbidities and 0.2% without comorbidities.[10] Among children admitted in US and Canadian PICU with COVID-19, 83% had pre-existing comorbidities and out of which 73% had respiratory symptoms and 38% required mechanical ventilation.[11] Hence, children with comorbidities need careful monitoring during the first 2 weeks of illness.

Most common symptoms in our patients were fever, cough and rhinorrhoea followed by gastrointestinal symptoms which were consistent with previous reports.[6,12] Dermatological and neurological manifestations were not observed in our children.

Central peri bronchial cuffing was the common CXR abnormality noted in our study. 92% of them with the above finding neither had respiratory symptoms at admission nor during hospital stay. Hence, the central peri bronchial cuffing may be a nonspecific finding and further evaluation may be required only if there are worsening symptoms and signs. Another study from Spain had shown similar findings in CXR in 86% of it's patients.[13] In our study those with peripheral heterogeneous opacity in CXR had significant clinical symptoms and ground glass opacities in CT. This was identical to COVID pneumonia causing diffuse interstitial infiltrates involving the periphery of the lung.[14]

Similar to few meta-analysis and systematic review on COVID-19, leukopenia (58%) and lymphocytosis (32.8%) were most commonly observed in our study.[15] Lymphopenia (11.4%) and thrombocytopenia (3.5%) were comparable to that of study by Meena et al.[12] Though lymphopenia was frequently seen in adults with severe disease and high mortality, it was rarely observed in children. It may be because of milder illnesses in children.[16] Hence, these hematological findings along with clinical features similar to COVID should raise a strong suspicion of COVID-19 infection and it will be a useful predictor for planning treatment in children with moderate to severe illness.[12,16]

Inflammatory markers were raised in 15-25% of our children. Studies found that higher the CRP, IL-6 and ferritin more severe was the disease.[16] Liver enzymes were increased in 7-8% which is consistent with other studies and its prognostic significance is still uncertain.[17]

Children with severe infection can develop serious complications like acute respiratory distress syndrome, renal failure, myocarditis, shock, acute renal failure, coagulopathy, MODS and later MIS-C. In our study population only a single child developed respiratory failure and none had MODS. Antivirals and steroids were used in very few. Guidelines on COVID-19 treatment in children evolved only later and most were extrapolated from adult studies during our study period. Two children were readmitted with MIS-C manifestations after 3-4 weeks and both had very minimal symptoms during the 1st admission. More studies are needed to determine the children at risk for MIS-C. No mortality was encountered among the admissions in our study.

Hence our present study included the majority of asymptomatic children, these clinical and laboratory data may be helpful in supporting COVID-19 diagnosis in children when diagnostic tests are not available and it provides the true representation of disease spectrum of paediatric population. Limitations of this study was small sample size and single centre study.

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COVID-19 infection was milder among the majority. Infants and children with comorbidities had increased risk of severe disease. Inflammatory markers were helpful in predicting the severity of illness. Antivirals and steroids were needed in very few children. Since the disease has many new variants it might present differently in future. Hence, clinicians need to have a high index of suspicion for early diagnosis and management to have a good outcome and recovery.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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1. Varghese L, Zachariah P, Vargas C, LaRussa P, Demmer RT, Furuya YE, et al. Epidemiology and Clinical Features of Human Coronaviruses in the Pediatric Population. J Pediatr Infect Dis Soc. 2018;7(2):151-8.
2. Memish ZA, Tawfiq JA, Assiri A, Rabiah FA, Hajjar S, Albarrak A, et al. Middle East respiratory syndrome coronavirus disease in children. Pediatr Infect Dis J. 2014;33(9):904-6.

3. CDC. Healthcare Workers, 2020. Available at: https://www.cdc.gov/coronavirus/2019ncov/hcp/pediatric-hcp.html. Accessed on 25 May 2021.
4. Zimmermann P, Curtis N. Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. Arch Dis Child. 2020;320338.
5. CDC. Multisystem Inflammatory Syndrome (MIS), 2021. Available at: https://www.cdc.gov/mis/index.html. Accessed on 25 May 2021.
6. WHO. Clinical management of COVID-19: interim guidance, 2020. Available at: https://apps.who.int/iris/handle/10665/3. Accessed on 25 May 2021.
7. WHO. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), 2020. Available at: https://www.who.int/docs/default-source/coronaviruse/who-chinajointmissiononcovid-19-final-report.pdf. Accessed on 25 May 2021.
8. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiology of COVID-19 Among Children in China. Pediatrics. 2020;145(6):20200702.
9. CDC. COVID-19 and Your Health, 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/your-health/index.html. Accessed on 25 May 2021.
10. Tsankov BK, Allaire JM, Irvine MA, Lopez AA, Sauvé LJ, Vallance BA, et al. Severe COVID-19 Infection and Pediatric Comorbidities: A Systematic Review and Meta-Analysis. Int J Infect Dis. 2021;103:246-56.
11. Shekerdemian LS, Mahmood NR, Wolfe KK, Riggs BJ, Ross CE, McKiernan CA, et al. Characteristics and Outcomes of Children With Coronavirus Disease 2019 (COVID-19) Infection Admitted to US and Canadian Pediatric Intensive Care Units. JAMA Pediatr. 2020;174(9):868-73.
12. Meena J, Yadav J, Saini L, Yadav A, Kumar J. Clinical Features and Outcome of SARS-CoV-2 Infection in Children: A Systematic Review and Meta-analysis. Indian Pediatr. 2020;57(9):820-6.
13. Serrano C, Alonso E, Andrés M, Buitrago NM, Pérez VA, Parrón PM, et al. Pediatric chest x-ray in covid-19 infection. Eur J Radiol. 2020;131:109236.
14. Kumar J, Meena J, Yadav A, Yadav J. Radiological Findings of COVID-19 in Children: A Systematic Review and Meta-Analysis. J Trop Pediatr. 2021;67(3):45.
15. Ma X, Liu S, Chen L, Zhuang L, Zhang J, Xin Y. The clinical characteristics of pediatric inpatients with SARS-CoV-2 infection: A meta-analysis and systematic review. J Med Virol. 2021;93(1):234-40.
16. Terpos E, Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. Am J Hematol. 2020;95(7):834-47.
17. Moon AM, Barritt AS. Elevated Liver Enzymes in Patients with COVID-19: Look, but Not Too Hard. Dig Dis Sci. 2021;66(6):1767-9.

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Research Paper

Apolipoprotein-E Gene Polymorphism and Lipid Composition among IUGR and AGA Neonates

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Abstract

Objective The objective of this study is to study the profile of apolipoprotein E (*APOE*) gene polymorphism and lipid profile among intrauterine growth restriction (IUGR) and appropriate for gestational age (AGA) neonates. This is an observational study. This study was done at the neonatal unit of a teaching hospital in South India. All consecutively born IUGR neonates (cases) of more than 32 weeks' gestational age and AGA neonates (controls) were enrolled for the study. Genomic DNA extraction was done from a total of 102 peripheral venous blood samples. Genotyping of the APOE rs429358 and rs7412 defining the ε2, ε3, and ε4 alleles was done by polymerase chain reaction–restriction fragment length polymorphism method. Prefeed venous blood was collected and analyzed for lipid profile estimation. The allelic frequencies of cases versus control were ε2—9 (8.7%) versus 3 (2.9%); ε3—88 (84.6%) versus 81 (79.4%); and ε4–7 (6.7%) versus 18 (17.6%). The frequency of ε4 isoform allele, associated with adult onset of metabolic diseases was less among the IUGR group. The mean total cholesterol (TC), Low-Density Lipoprotein (LDL), High-Density Lipoprotein, and triglyceride (TG) were 107.59 ± 35.99, 51.69 ± 24.68, 21.75 ± 9.58, and 151.22 ± 61.84 mg/dL, respectively, in the IUGR group. The mean TC and LDL levels in IUGR group were marginally higher than AGA neonates (107 ± 35.99 vs. 100.37 ± 22.69 mg/dL and 51.69 ± 24.68 versus 46.9 ± 19.51 mg/dL, $p > 0.05$). In both groups, the mean TC and TGL levels were elevated in the ε4 isoform subgroup ($p > 0.05$). In our study, the ε2 allele was the second most predominant APOE isoform and the ε4 allele of the *APOE* gene associated with adult-onset diseases was not increased among IUGR neonates. Neonates with ε4 allele showed an abnormal lipid profile in both study groups suggesting a possible association.

Keywords

IUGR neonates - *APOE* gene polymorphism - lipid profile

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Apolipoprotein-E Gene Polymorphism and Lipid Composition among IUGR and AGA Neonates

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Abstract

Objective The objective of this study is to study the profile of apolipoprotein E (*APOE*) gene polymorphism and lipid profile among intrauterine growth restriction (IUGR) and appropriate for gestational age (AGA) neonates. This is an observational study. This study was done at the neonatal unit of a teaching hospital in South India. All consecutively born IUGR neonates (cases) of more than 32 weeks' gestational age and AGA neonates (controls) were enrolled for the study. Genomic DNA extraction was done from a total of 102 peripheral venous blood samples. Genotyping of the APOE rs429358 and rs7412 defining the ε2, ε3, and ε4 alleles was done by polymerase chain reaction–restriction fragment length polymorphism method. Prefeed venous blood was collected and analyzed for lipid profile estimation. The allelic frequencies of cases versus control were ε2—9 (8.7%) versus 3 (2.9%); ε3—88 (84.6%) versus 81 (79.4%); and ε4–7 (6.7%) versus 18 (17.6%). The frequency of ε4 isoform allele, associated with adult onset of metabolic diseases was less among the IUGR group. The mean total cholesterol (TC), Low-Density Lipoprotein (LDL), High-Density Lipoprotein, and triglyceride (TG) were 107.59 ± 35.99, 51.69 ± 24.68, 21.75 ± 9.58, and 151.22 ± 61.84 mg/dL, respectively, in the IUGR group. The mean TC and LDL levels in IUGR group were marginally higher than AGA neonates (107 ± 35.99 vs. 100.37 ± 22.69 mg/dL and 51.69 ± 24.68 versus 46.9 ± 19.51 mg/dL, $p > 0.05$). In both groups, the mean TC and TGL levels were elevated in the ε4 isoform subgroup ($p > 0.05$). In our study, the ε2 allele was the second most predominant APOE isoform and the ε4 allele of the *APOE* gene associated with adult-onset diseases was not increased among IUGR neonates. Neonates with ε4 allele showed an abnormal lipid profile in both study groups suggesting a possible association.

Keywords
- IUGR neonates
- *APOE* gene polymorphism
- lipid profile

Introduction

The term intrauterine growth restriction (IUGR) has been defined as the subnormal rate of fetal growth with respect to the growth potentials of a specific fetus in accordance to the gender and race. These neonates are born with clinical features of in utero malnutrition and may or may not have a birth weight less than 10th centile for the particular gestational age.[1] Approximately 78% of all IUGR infants delivered globally are seen in Asian continent with highest incidence seen in India.[2] The IUGR infants are prone for short-term and long-term complications making them high-risk neonates. Few of the short complications include perinatal asphyxia, persistent pulmonary hypertension,

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Clinical features and laboratory parameters in children with severe dengue and scrub typhus

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Background: Objective of study was to compare the clinical features and laboratory parameters at admission and differentiate severe dengue from scrub typhus in children.

Methods: Retrospective analysis of case records and comparison of clinical and laboratory parameters at admission of all children with a diagnosis of severe dengue and scrub typhus was done.

Results: A total of 72 children were included (severe dengue =40; scrub typhus =32) during the study period. The mean (SD) age of children with severe dengue and scrub typhus was 7.9(3.8) and 11.8(5.8) years, respectively. Majority of children with severe dengue presented with hypotension, 21(52.5%) vs 3(9.4%) in scrub typhus. Children with severe dengue had a relatively low ANC ($2.6\pm1.97x10^3/mm^3$ vs $3.9\pm2.06x10^3/mm^3$), low platelet count ($50.23\pm35.55x10^3/mm^3$ vs $140\pm95.0x10^3/mm^3$) and low mean ESR at 1hour (8.1±6.82mm vs 33.88±13.79mm) than scrub typhus.

Conclusions: Compared to scrub typhus, severe dengue was significantly associated with hypotension, lower ANC, ESR and platelets.

Keywords: Eschar, Fever, Low ESR, Rash, Thrombocytopenia, Scrub typhus, Severe dengue

Introduction

Globally 390 million dengue infections per year are reported, of which 96 million manifests clinically carrying high morbidity.[1] Dengue viruses (DV) belong to the family flaviviridae and is transmitted by vectors Aedes aegypti and Aedes albopictus.[2] Scrub typhus (ST) is another hemorrhaegic fever caused by Orientia tsutsugamushi that is spread by the bite of trombiculid mite. ST is increasingly recognized in tropical countries contributing upto 50% of patients with undiagnosed acute febrile illnesses.[3] In many parts of India, these two infections together comprise more than half of all acute undifferentiated febrile illnesses, more so in south Indian states due to the favorable tropical climatic conditions. Their similar clinical features and laboratory findings often pose diagnostic difficulty for the treating physician.[4] Early diagnosis of the illnesses is life saving to improve patient outcomes and promote timely public health interventions. In this retrospective study we have compared the clinical features and laboratory parameters of children with severe dengue and scrub typhus and attempted to derive the parameters that differentiate severe dengue from scrub typhus.

Methods

This retrospective study conducted over a period of 1 and ½ year (January 2015 to June 2016) in the department of pediatrics of PSG Institute of Medical sciences and research, a tertiary care teaching hospital in South India. Case records of all children (≤18 years) discharged with a

diagnosis of severe dengue and scrub typhus were identified, and those who had a serological confirmation were included for analysis. Severe dengue was diagnosed based on revised WHO criteria and included in the study.[5] Children with or without warning signs, without serological confirmation were excluded. The data was recorded on specified data collection form. The data included demographic parameters, clinical features, and laboratory parameters (CBC, RFT, LFT, serology, culture if any). The serological diagnosis of Dengue fever was based on dengue IgM-ELISA (Panbio dengue IgM & IgG capture ELISA) where values <9 PanBio ELISA units were considered negative, 9-11 equivocal, and more than 11 positive, as per the manufacturer's instructions). Similarly, serological diagnosis of scrub typhus was based on scrub typhus detect^{TM}IgM ELISA system. Children with SD were treated with normal saline, monitored for complications and treated according to the physician's discretion. Children with ST were treated with antibiotics and offered symptomatic care for the illness. The Institution's Human ethical committee approved the study.

Statistical analysis

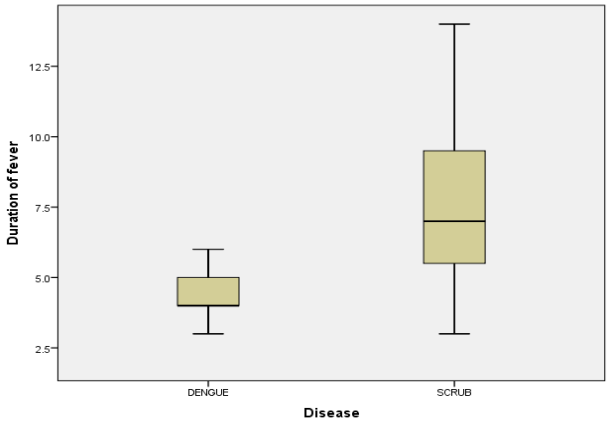
Kolmogorov-Smirnov test was used for testing normality of the data. Normally distributed continuous variables were compared using *t*-test and non-parametric variables using Wilcoxon signed-rank and Mann-Whitney U tests. Chi-square analysis was done for comparisons of proportional data among groups. Multiple logistic regression models were used to assess the association of severe dengue and scrub typhus with relevant clinical and laboratory parameters.

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A total of 7926 children got admitted as inpatients during the study period of which 6737 children were admitted for evaluation of fever. 747 children were serologically confirmed as dengue fever of which a total of 40 children were diagnosed with severe dengue fever with mean (±SD) age 7.9 (±3.80) years, and male: female ratio was 1.1:1. The mean (±SD) duration of fever at admission was 4.5(±1.13) and the mean (±SD) duration of hospital stay was 5.58 (±1.13) as in Figure 1.

Among children with severe dengue, 31(77.5%) had vomiting, 27(67%) had abdominal pain and 23(57.5%) had oliguria as presenting complaints. A majority of them (31; 77.5%) had evidence of plasma leakage, and 17(42.5%) had skin and mucosal bleeds at admission. None of the children had any alternate provisional diagnosis at admission other than severe dengue based on the clinical presentation.

A total of 32 children were serologically diagnosed as scrub typhus with mean (±SD) age of 11.8 (±5.83) years, and male:female ratio was 2.2:1. The mean (±SD) duration of fever at admission was 7.34 (±2.46) days. Of 32 children, 14 (43.8%) presented with vomiting and 11 (34.4%) presented with abdominal pain at admission. On examination, 5 (15.6%) had maculopapular rash and 15 (46.9%) had hepatosplenomegaly. Only 8 (25%) were found to have eschar to support the clinical diagnosis of scrub typhus. Other clinical parameters are presented in Table 1.



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Age#	7.97±3.8	11.8±5.83	0.001
Gender(M:F)	1.1:1	2.2:1	NS
Duration of fever at admission#	4.5±1.13	7.34±2.46	<0.001
Vomiting	31(77.5%)	14(4.38%)	0.007
Myalgia	17(42.5%)	8(25%)	NS
Rash	6(15%)	5(15.6%)	NS
Abdomen pain	27(67.5%)	11(34.4%)	0.009
Oliguria	23(57.5%)	3(9.4%)	<0.001
Respiratory distress	7(17.5%)	3(9.4%)	NS
Skin/mucosal bleeds	17(42.5%)	2(6.3%)	<0.001
Haematemesis	2(5%)	2(6.3%)	NS
Edema/plasma leakage	31(77.5%)	3(9.4%)	<0.001
Hypotension	21(52.5%)	3(9.4%)	<0.001
Hepatomegaly	37(92.5%)	15(46.9%)	<0.001
Spleen	nil	15(46.9%)	<0.001

□Expressed as Mean ±SD, NS-Non significant

We compared the clinical and laboratory characteristics among children with severe dengue and scrub typhus as shown in Table 2. We found that the mean age at presentation and duration of fever was higher among scrub typhus compared to severe dengue, 7.97±3.8 years

vs 11.8±5.83 years (p = 0.001) and 4.5±1.13 days vs 7.34±2.46 days (p<0.001), respectively. A greater proportion of children with dengue presented with vomiting, abdominal pain, oliguria, skin bleeds, edema and hypotension. There existed a statistically significant difference in the proportions of the above-mentioned variables between both groups (p<0.05). Interestingly erythematous rash was present in equal proportions between both groups (15% vs 15.6%, p = 0.14).

Table 2 Comparison of clinical features between dengue and scrub typhus

	Dengue fever	Scrub typhus	p value
Hemoglobin# (g/dL)	13.3±1.70	12.24±1.63	0.003
Hematocrit#	41.45±5.78	37.6±5.14	0.001
TLC #(x10^3/cu.mm)	5.19±3.03	7.64±3.45	0.002
ANC# (x10^3/cu.mm)	2.6±1.97	3.9±2.06	0.002
Platelet# (x10^3/cu.mm)	50.23±35.55	140±95.0	<0.001
ESR#	8.1±6.82	33.88±13.79	<0.001
SGPT#	138.75±213.75	91.9	0.31

Expressed as Mean ±SD

The mean haemoglobin (and hematocrit) values were higher among children with dengue (13.3±1.7 vs 12.24±1.63, p = 0.003). The white blood cell (WBC) count and absolute neutrophil count (ANC) were markedly different in both groups. The children with dengue had a relatively low WBC count and ANC (5.19±3.03x10^3/mm^3, 2.6±1.97x10^3/mm^3 respectively) on comparing children with scrub. The platelet count at admission was lower in children with dengue when compared to scrub typhus (50.23±35.55x10^3/mm^3 vs 140±95x10^3/mm^3, p<0.001). Also, the mean Erythrocyte Sedimentation Rate (ESR)at 1hour was lower in children with dengue fever compared to scrub typhus (8.1±6.82mm vs 33.88±13.79mm, p<0.001). The mean serum Alanine Transaminase (ALT) levels were higher in children with severe dengue compared to scrub typhus (138.75±213.75 vs 91.9IU/L). But this difference did not achieve statistical significance (p = 0.19).

From the data obtained at admission, variables between these two illnesses that had statistically significant difference were applied to a logical regression model. We found a high probability of severe dengue compared to scrub typhus was associated independently with hypotension, lower ANC, low ESR and platelets as in Table 3. With a probability cut-off of >90%, the predictive model gave a sensitivity of 100%, specificity of 87.5%, positive predictive value of 88.8% and negative predictive value of 100%, for dengue compared to scrub typhus with ANC 0.93 (0.79-0.99) as shown in Figure 2.

Table 3 Multivariate logistic regression analysis of clinical and laboratory parameters between dengue and scrub typhus

Parameter	Increase/decrease	Multivariate odds ratio (95% CI)	p value
ANC	2x10^3/cu.mmincrease	1.98 (1.18-3.30)	0.012
Platelet count	40000/cu.mmdecrease	2.47 (1.63-4.23)	<0.001
ESR	6mm decrease	5.69 (2.88-15.97)	<0.001

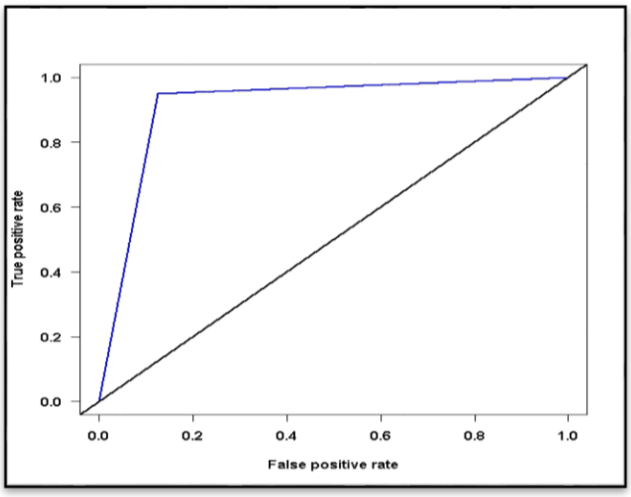


Figure 2 Receiver operating characteristic curve for ANC in predicting dengue fever from scrub typhus

Discussion

Dengue fever and scrub typhus caused by different vectors present in similar demographic distribution and clinical features. Any delay in recognition of these infectious diseases results in significant morbidity and mortality as well. Management of severe dengue is largely supportive with emphasis on fluid resuscitation and hemodynamics monitoring, whereas in scrub typhus the early initiation of antibiotics is mainstay.

Making the correct diagnosis with the available preliminary investigations while awaiting confirmatory test results might help in better management and relieve parent's anxiety. In this retrospective study we attempted to compare the clinical and laboratory parameters between these two similar diseases at initial presentation to the health care facility.

The mean age at presentation of children with severe dengue was less as compared to scrub typhus (7.9 +/- 3.80 vs 11.8 +/- 5.83) and the mean age at presentation of the latter was comparable to that reported previously by Kumar M et al.[6]

The male:female ratio of children with severe dengue was less than that of scrub typhus (1.1:1 vs 2.2:1). This difference in gender ratio could probably due to similar exposure risk for mosquito bites in-doors and higher prevalence of chigger exposure while playing outdoors by boys. A similar observation was made by Bhat NK et al, who documented a higher male to female ratio (1.44:1) in scrub typhus. Among the symptoms and signs between the study groups at presentation, mean duration of fever, abdominal pain, edema, oliguria and hypotension were strikingly different. Though these differences have been witnessed in children with scrub typhus in previous studies their incidence was much lesser in the present study, when compared to children with severe dengue.[7]

In our present study, there was significant difference in clinical features among the study groups. Children with severe dengue had a short mean duration of fever (4.5 +/- 1.13 vs 7.34 +/- 2.46 days) at presentation. Majority of children (77.5%) with severe dengue in our study presented with vomiting and edema. Two third had abdominal pain and oliguria and almost half of them presented with skin bleeds and hypotension at admission. Almost ninety percent of children presented with significant hepatomegaly and none had splenomegaly. Pothapregada S et al, in his clinical profile of children with dengue also observed a similar occurrence with fever (94.6%) being the commonest symptom and persistent vomiting as the frequent warning sign (75.1%).[8] Among children with scrub typhus, almost half of them had hepatosplenomegaly. Clinical features like rash, edema, respiratory distress, oliguria was less observed in children with scrub typhus. Only twenty-five percent of children with scrub typhus had eschar in our study population. Rose W et al, found 40.8% of their children having the characteristic eschar at the presentation but most studies project a lesser percentage.[9] Babu et al, in their prospective study found only 34.6% of serologically confirmed scrub typhus to have eschar.[10] Absence of eschar could be secondary to previous exposure to ricketssial antigen and variation in cutaneous immune response and that could be possible reason for absence of eschar in majority of our study children.

In our study, we found that the striking difference in clinical parameters between the two groups was hypotension, lower ANC, low ESR and significantly low platelet values and thus provides the best ability to differentiate between severe dengue fever and scrub typhus. ANC has been statistically high in children with scrub typhus than severe dengue. The predominance of lymphocytes with leucopenia and severe thrombocytopenia might be an additional pointer to the diagnosis of severe dengue. In the guideline of Indian academy of pediatrics on ricketssial diseases, marked leukocytosis with the shift to the left in later stages, raised ESR and thrombocytopenia were laboratory features suggestive of these infections, as noted in children with ST in the present study.[11] Liver dysfunction has been observed in children with severe dengue and scrub typhus.[12] Similar to the present study, Kumar J et al noted a 10-fold increase in the mean serum ALT levels in children with DF and could be used in differentiating these diseases.[13]

Few limitation of our study is the retrospective model, small sample size and restricted to a hospital – based population. The observation and predictive model may not be applicable to differentiate other febrile illnesses in children and the clinical utility the model needs to be tested prospectively and also validated. In a resource limited setting among endemic areas, our predictive model could be utilized to differentiate hemorrhagic fever due to scrub typhus and severe dengue, helping to initiate appropriate treatment.

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1. World Health Organization. Dengue and dengue haemorrhagic fever. Factsheet no. 117, 2008. Available at: https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue.
2. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Indian J Med Res. 2012;136(3):373-90.
3. KendreVarsharani V, Shekde SD, Chinte LT. The Study of Clinico-epidemiological Features of Dengue Cases Admitted in Tertiary Care Hospital, Latur, Maharashtra. Int J Recent Trends Sci Technol. 2014;10(2):369-72.
4. Chrispal A, Boorugu H, Gopinath KG, Chandy S, Prakash JA, Thomas EM, et al. Acute undifferentiated febrile illness in adult hospitalized patients: The disease spectrum and diagnostic predictors-an experience from a tertiary care hospital in South India. Trop Doct. 2010;40:230-4.
5. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. New edition. Geneva: WHO, 2009. Available at: http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf.
6. Kumar M, Krishnamurthy S, Delhikumar CG, Narayanan P, Biswal N, Srinivasan S. Scrub typhus in children at a tertiary hospital in southern India: clinical profile and complications. J Infect Public Health. 2012 Mar;5(1):82-8.
7. Bhat NK, Dhar M, Mittal G, Shirazi N, Rawat A, Kalra BP, et al. Scrub typhus in children at a tertiary hospital in north India: clinical profile and

complications. Iranian J Pediatrics. 2014 Aug;24(4):387.
8. Pothapregada S, Kamalakannan B, Thulasingham M, Sampath S. Clinically profiling pediatric patients with dengue. J Glob Infect Dis. 2016;8(3):115-20.
9. Rose W, Rajan RJ, Punnen A, Ghosh U. Distribution of eschar in pediatric scrub typhus. J Trop Pediatr. 2016;62(5):415-20.
10. ArunBabu T, Vijayadevagaran V, Ananthakrishnan S. Characteristics of pediatric scrub typhus eschar in south Indian children. Pediatr Dermatol. 2017;34(2):124-7.
11. Palanivel S, Nedunchelian K, Poovazhagi V, Raghunadan R, Ramachandran P. Clinical profile of scrub typhus in children. Indian J Pediatr. 2012 Nov;79(11):1459-62.
12. Chanta C, Triratanapa K, Ratanasirichup P, Mahaprom W. Hepatic dysfunction in pediatric scrub typhus: role of liver function test in diagnosis and marker of disease severity. J Med Assoc Thai. 2007 Nov;90(11):2366-9.
13. Jagadishkumar K, Jain P, Manjunath VG, Umesh L. Hepatic involvement in dengue fever in children. Iran J Pediatr. 2012 Jun;22(2):231-6.

r Rajesh NT, Elangovan B. Clinical and laboratory predictors to differentiate severe dengue from scrub typhus in children. Int J Contemp Pediatr 2019;6:1223-7.



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Gallbladder motility in children with chronic functional abdominal pain

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Abstract

Background: Chronic abdominal pain is a common gastrointestinal symptom in children that significantly lowers their quality of life. In adults, Gall bladder (GB) hypomotility / dyskinesia is associated with many functional abdominal disorders but there is scarcity of evidence on its role in childhood gastro-intestinal disorders. Aim of the study is to evaluate the GB motility in children with chronic functional abdominal pain (FAP).

Methods: Children aged 5-15 years with chronic abdominal pain fulfilling ROME-III criteria and healthy controls were included and all study participants were subjected to ultrasonographic evaluation of the gall bladder volume in fasting state and post - Fatty test meal (FTM) and its ejection fraction was calculated.

Results: Sixtysix children including 31 with chronic FAP underwent sonographic evaluation for GB motility. The mean ejection fraction (EF) of cases and controls were 51.72±17.76% and 57.3±23.26% (p value - 0.158). The mean EF of cases with upper abdominal pain and lower/peri-umbilical abdominal pain were 41.7±17.1% and 57.2±15.9% respectively (*p* value < 0.0001). Increasing BMI had no significant association with GB motility among the participants.

Conclusions: There is lack of significant association between children with chronic FAP and GB hypomotility. Children with upper abdominal pain have lesser GB EF, suggesting a possible abnormal GB motility.

Keywords: Childhood functional abdominal pain, Gallbladder, Ultrasonography

Introduction

Chronic abdominal pain is a common gastrointestinal symptom in children presenting to outpatient department. In United states and Europe, its prevalence rates ranges from 0.3 – 19% among school-going children and accounts for about 2% to 4% of all pediatric office visits.[1,2] Though it is not associated with any organic gastrointestinal pathology, children with FAP have a significantly lower quality of life and it contributes for school – absenteeism.[3] In 1999, the term abdominal pain related-functional gastrointestinal disorders in pediatric ROME II criteria, which included functional dyspepsia, functional abdominal pain (FAP), irritable bowel syndrome and abdominal migraine. These terminologies were further redefined based on duration of symptoms before diagnosis in ROME III criteria.[3]Many functional gastrointestinal disorders like functional dyspepsia, irritable bowel syndrome and disorders of Gall bladder (GB) has been studied for any possible association with abnormalities in GB motility, but the results are inconclusive.[4] In adults, GB hypomotility/dyskinesia is well established in idiopathic constipation and functional dyspepsia, but there is scarcity of evidence on its role in childhood functional abdominal disorders.[5,6] Our study

was designed to study the GB motility in children with functional abdominal pain.

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This crosssectional study was done in the outpatient department of pediatrics, PSG institute of medical sciences from January 2016 to August 2017. Children aged 5-15 years attending the OPD of the institution with chronic abdominal pain fulfilling ROME-III criteria, without any obvious cause for abdominal pain and with no previous abdominal surgeries were included in the study. Healthy children who visited the hospital for routine health visit or vaccination were included as controls. Institutional Human ethical committee approved the study and all study participants were included after obtaining informed consent. A detailed history including dietary and allergy history was obtained and clinical assessment was done. They were documented in a pre-designed data collection tool.

A total of 31 cases and 35 controls were recruited in the study and all study participants were subjected to ultrasonographic evaluation of the gall bladder in fasting state. A radiologist experienced in pediatric Sonography performed the evaluation using Philips iU22 xMATRIX ultrasound system. The Gall bladder was assessed for its area, thickness and volume, where area was derived by trace method and maximum GB width and length were measured using transverse and longitudinal images of the GB.

The volume of GB was calculated in fasting and 30-minutes following a fatty test meal using Dodd's formula, Volume (in cm^3) = 0.52 x [L x W x A], where L is the greatest length, W- greatest transverse width, and A-greatest anteroposterior diameter of the GB. The gallbladder ejection fraction (EF) for each examination was obtained from two-volume data using the following equation. Where V_0 = gallbladder volume in fasting state, Vn = gallbladder volume after the fatty test meal,

□□□□□□□□□□□□□□□EF (%) = [(Vo-Vn) / Vo] X 100

For the purpose of this study, Children with ejection fraction of less than 40% were considered to have gallbladder hypomotility.

Statistical analysis was done using statistical package for the social science version-19 (SPSS) software. The fasting and post-prandial GB volume was expressed as mean ± SD and students t test was used to compare their means between groups. Chi-squared testing was applied to look for possible association between EF and BMI. A p-value less than 0.05 was considered significant.

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There were a total of 66 children included in this study. The mean age of cases and controls were 10.14±2.71 years and 11.54±1.94 years respectively. The mean height of the cases group was 132.9±15.9 (range – 100 to 165cms) and that of controls was 145.14±11.6 (range – 110 to 159cms). The mean weight of cases and controls were 27.3 ± 10.5 Kg and 33.8 ± 6.6 Kg respectively. The mean body mass index (BMI) was 14.9±2.8 kg/m^2 (range of 11.3 to 26.3 kg/m^2) in case group and 15.9±1.8 kg/m^2 (range from 11.9 to 19.5 kg/m^2) in the control group. Out of 31 cases there were 11 boys (35.5%) and 20 (64.5%) girls respectively and in 35 controls there were 23 (65.7%) boys and 12 (34.3%) girls respectively (Table 1).□

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Age (in years)	10.14±2.71	11.54±1.94	0.08
Gender ratio	1:2	2:1	-
Height (in m)	1.34±0.15	1.43±0.11	0.06
Weight (in Kg)	27.3±10.8	34.31±6.3	0.03
BMI (Kg/m^2)	14.60±3.1	16.59±1.60	0.03
□□d□□ □□□□□□□□□□			
Predominantly Upper (%)□	11 (40%)	-	
Predominantly Lower (%)	19 (60%)		

*Student's T-Test

The mean fasting and post-prandial gallbladder volumes of cases and controls were 8.20±3.97 (cm^3), 7.52±3.13(cm^3) and 3.51±1.57 (cm^3) and 2.94±1.59 (cm^3) respectively. Though the fasting GB volumes were greater than post-test meal, in both groups, yet there was no statistical significance (P value – 0.390) (Table 2). The mean ejection fraction of cases and controls were 51.72±17.76% and 57.37±23.26% (p value – 0.158).

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Cases	8.20±3.97	7.52±3.13	51.95±18.02
Controls	3.51±1.57	2.94±1.59	57.04±23.03
P-value*	0.797	0.127	0.239

*Student's T-Test

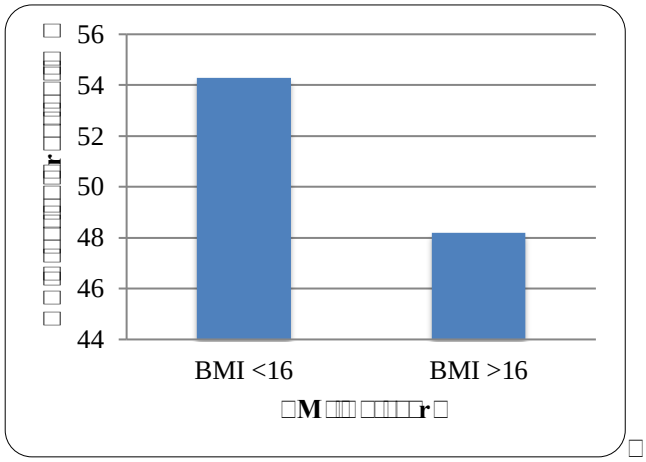
The ejection fraction of GB were compared among children with functional abdominal pain who presented predominantly with upper or lower / peri-umbilical regions, to identify any possible association between the site of abdominal pain with GB motility. The mean ejection fractions of cases with upper abdominal pain and lower/peri-umbilical abdominal pain were 41.7±17.1% and 57.2±15.9% respectively (p value <0.0001), but the former did not qualify for the definition of GB hypomotility i.e. EF<40% (Table 3).

[illegible]

[illegible]	[illegible]		
	[illegible]	[illegible]	[illegible]
Predominantly Upper	7.41±3.13	4.17±2.15	41.73±17.13
Predominantly Lower	8.67±4.40	3.13±1.0	57.87±16.12
P-value*	0.497	0.25	0.019

*Student's T-Test

In order to identify any possible association between increasing BMI and GB contractility among cases, they were sub-grouped as, BMI<16 and BMI ≥ 16. There was equal number of children (n=4) in each subgroup with EF<40%, suggestive of GB dysmotility and their mean EF were 54.28 ± 17.99 % and 48.19 ± 17.53 % respectively (*p*-value < 0.001) (Figure 1).



[illegible]

[illegible]

In our study, we found no significant difference in the gall bladder volume (in fasting state and post Fatty test meal) among the cases and controls with equal number of children in both groups with GB ejection fraction <40%. Children who presented with upper abdominal pain had significantly lesser ejection fraction than those with lower abdominal pain. Many adult studies have recognized GB hypomotility in obese patients than non-obese patients. Sari et al found a positive correlation between fasting gallbladder volume and body mass index and body fat weight in obese women than non-obese women, but no such studies were available for comparison in children.[7] In the present study there existed no significant association in GB dysmotility and increasing BMI among cases.

The exact pathogenesis for FAP remains unclear, but visceral hypersensitivity, abnormal brain - gut interaction, immune dysregulation and altered gut motility has been postulated for the occurrence of the symptoms.[8,9] Gall bladder dyskinesia has been associated with various gastrointestinal symptoms like bloating, dyspepsia, post prandial epigastric pain and upper abdominal fullness.[10]

Mehra et alm observed gall bladder hypomotility in children with functional constipation, thus strengthening the claim of GB dyskinesia as a possible claim for functional abdominal symptoms in children.[11] Several imaging modalities like oral choledochocystography and syntigraphy are utilized to assess GB contraction but Sonography has been reliably used for assessing the GB area and volume, their changes following a fatty meal.[12] Ultrasonography could be preferred for its noninvasive nature and no radiation exposure that accompanies other modalities.

In our study we found 8 (26.7%) of the children with FAP healthy and 9 (25%) controls with mean GB Ejection fraction <40% suggesting that GB hypomotility could possibly be unrelated for the abdominal pain. Interestingly children who had predominant upper abdominal pain had low GB Ejection fraction compared to the children with lower abdominal pain (41.73±17.13% vs 57.87±16.12%) as noted by Hofeltd et al, in their study where a majority of children (96.67%) who underwent laproscopic cholecystectomy for biliary dyskinesia had upper quadrant pain.[13] A similar study with more subjects with FAP localized predominantly to upper abdominal could establish an association with GB motility.

Few limitations in our study are firstly the children were consecutively enrolled and sample size of the study is too small for drawing strong conclusions. Secondly the co-existing infection like H. pylori or parasitic infection of gut could possibly have an impact on the abdominal pain that was under evaluation. Nonetheless the present study is the first of its kind that has meticulously evaluated the GB volume and contractility in children with FAP and has found a possible association of GB dyskinesis with upper abdominal pain.

[illegible]

To conclude, abnormality in GB motility could be a cause for FAP presenting predominantly as upper abdominal pain in children thus necessitating its evaluation and a larger well-controlled study might help in better understanding of the entity.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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1. Chitkara DK, Rawat DJ, Talley NJ. The epidemiology of childhood recurrent abdominal

pain in Western countries: a systematic review. The Am J gastroenterol. 2005;100(8):1868.
2. Spee LA, Lisman-Van Leeuwen Y, Benninga MA, Bierma-Zeinstra SM, Berger MY. Prevalence, characteristics, and management of childhood functional abdominal pain in general practice. Scandinavian journal of primary health care. 2013;31(4):197-202.
3. Youssef NN, Atienza K, Langseder AL, Strauss RS. Chronic abdominal pain and depressive symptoms: analysis of the national longitudinal study of adolescent health. Clinic Gastroenterol Hepatol. 2008;6(3):329-32.
4. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterol. 2006;130(5):1527-37.
5. Hemingway D, Neilly JB, Finlay IG. Biliary dyskinesia in idiopathic slow-transit constipation. Diseases of the colon & rectum. 1996;39(11):1303-7.
6. Velanovich V. Biliary dyskinesia and biliary crystals: a prospective study. The Am surg. 1997;63(1):69-74.
7. Sari R, Balci MK, Coban E, Karayalcin U. Sonographic evaluation of gallbladder volume and ejection fraction in obese women without gallstones. J clinic ultrasound. 2003;31(7):352-7.
8. Halac U, Noble A, Faure C. Rectal sensory threshold for pain is a diagnostic marker of irritable bowel syndrome and functional abdominal pain in children. J ped. 2010;156(1):60-5.
9. Bakker MJ, Boer F, Benninga MA, Koelman JH, Tijssen MA. Increased auditory startle reflex in children with functional abdominal pain. J ped. 2010;156(2):285-91.
10. Carr JA, Walls J, Bryan LJ, Snider DL. The treatment of gallbladder dyskinesia based upon symptoms: results of a 2-year, prospective, nonrandomized, concurrent cohort study. Surg Laparoscop Endoscop Percutaneous Tech. 200;19(3):222-6.
11. Mehra R, Sodhi KS, Saxena A, Thapa BR, Khandelwal N. Sonographic evaluation of gallbladder motility in children with chronic functional constipation. Gut and liver. 2015;9(3):388.
12. Dodds WJ, Groh WJ, Darweesh RM, Lawson TL, Kishk SM, Kern MK. Sonographic measurement of gallbladder volume. Am J roentgenol. 1985;145(5):1009-11.
13. Hofeldt M, Richmond B, Huffman K, Nestor J, Maxwell D. Laparoscopic cholecystectomy for treatment of biliary dyskinesia is safe and effective in the pediatric population. The Am surg. 2008;74(11):1069-72.

r Raaghul C, Rajesh NT, Kanagaraju V, Elangovan B. Gallbladder motility in children with chronic functional abdominal pain. Int J Contemp Pediatr 2019;6:2042-5.

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Analysis of Incidence and Risk Factors for Retinopathy of Prematurity among Preterm Neonates in a Tertiary Care Neonatal Unit.

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ABSTRACT

Advances in neonatology has achieved several milestones in reducing the morbidity and mortality among high risk newborns. Preterm care is one such major domain where expanding knowledge and technology has ensured a higher survival chance. Reduced mortality comes but with a greater co incidence of diseases exclusive in preterm neonates like Retinopathy of prematurity, anaemia of prematurity, bronchopulmonary displasia etc. In our study we have made an attempt to identify the incidence and risk factors in preterm neonates associated with the development of ROP.100 preterm neonates who satisfied the inclusion criteria of birth weight ≤1750 grams and ≤34 weeks GA with or without risk factors and birth weight between 1750-2000 grams and 34-36 weeks GA with additional risk factors for ROP were screened in the Neonatal intensive care unit. Out of the 100 babies screened, only 21 babies developed ROP. Lower birth weight and gestational age were significantly associated with the incidence of ROP. Other risk factors that were found to be of statistical significance were oxygen administration, RDS, sepsis, shock and phototherapy by Chi Square analysis. Using multivariate analysis, shock, gestational age and APH were found to be significant factors.

Keywords: retinopathy of prematurity, risk factors, screening, treatment

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INTRODUCTION

Retinopathy of prematurity (ROP), previously known as 'retrolental fibroplasia' because of its end stage appearance is a developmental vaso proliferative disorder that occurs in the retina of preterm infants with incomplete vascular proliferation. Incidence of ROP in India is reported to vary between 38 – 51.9%.[1-3] Out of the approximate 26 million annual live births in India, approximately 8.7 % of newborns are < 2000 grams in weight.[4]

There are about 45 million blind people in India out of which nearly 30 % are in Asia. Of the total blindness, childhood blindness accounts for 4%. India shares 20% of world's childhood blindness.[5] ROP affects 3,00,000 infants worldwide. The incidence of ROP in developing countries like India is reported to be around 24 – 47% among high risk preterms.[6]It is important not only in terms of economic burden but also in its severe social implication, which is very long in terms of blind years.

As regards our country, we are sitting at the summit of two volcanoes – one where all the latest state of the art health care facilities are available and the other, where even the basic health care is unavailable. ROP is known to grow in both these situations. Among the preventable causes of blindness in India, ROP figures very high in the agenda. Low birth weight and gestational age were found to be the most significant risk factor associated with ROP.

With neonatal units being equipped with state of the art technical support and well trained health care providers to provide quality care to the preterm neonates, ROP incidence is on the rise. By recognition of risk factors, early detection and timely intervention, blindness due to ROP is preventable.

Purpose of this study is to know the incidence of ROP and to correlate it with maternal and neonatal risk factors.

MATERIALS AND METHODOLOGY

Inclusion Criteria [7]

Preterm low birth weight babies admitted to NICU at CMCH with

- Gestational age < / = 34 weeks
- Birth weight < / = 1750 gms
- Gestational age between 34 – 36 weeks with risk factors for ROP
- Birth weight between 1750 – 2000 gms with risk factors for ROP

Exclusion Criteria

- Preterm babies who die before reaching the age for screening
- Babies who did not complete the follow up protocol
- Babies with major congenital anomalies / chromosomal anomalies

Study Design

Longitudinal study with short follow up done from October 2012 to September 2013. Data was collected by interviewing parents, from hospital records and by examination of the neonate. Sample size was 100. Variables studied were gestational age, postconceptional age, sex, birth weight, oxygen administration and modes, presence of RDS, surfactant administration, sepsis, shock, anaemia, phototherapy, blood transfusion, exchange transfusion, maternal history of PIH and APH.

Statistical Methods Used

The data are reported as mean ± SD or as median, depending on their distribution. Differences in the quantitative variables between groups were assessed by means of unpaired t test. Comparison between

groups were made by non parametric Mann-Whitney test. Chi square test was used to analyze categorical variables and multivariate analysis was done to test dependent variables. P value of < 0.05 using a two tailed test was taken as statistically significant. All data were analyzed using SPSS software, version 16.0 for windows.

Definition of Risk Factors

Respiratory Distress Syndrome [8]

- Premature infant with clinical signs soon after birth including tachypnoea, retractions, alar nasal flaring, grunting, cyanosis
- X ray evidence : low volume lungs, diffuse reticulo nodular pattern, air bronchogram, white out lung

Apnoea [9]

Cessation of respiration for > 20 seconds or cessation of respiration of any duration when accompanied by bradycardia (< 100 bpm) and/ or cyanosis

Sepsis

- Clinical sepsis : lethargy, refusal feeds, apnoea, hypoglycemia, sclerema, seizures, respiratory distress, altered nasogastric tube aspirate, abdominal distension, shock etc
- Laboratory evidence : positive sepsis screen, culture positive sepsis, thrombocytopenia

Shock

- Pallor in the absence of bleeding, skin mottling, cold and cyanosed extremities, capillary refilling time > 3 seconds, absent peripheral pulses, tachycardia, narrow pulse pressure, fall in mean arterial pressure

Anaemia [10]

- Hematocrit < 40% in ventilated babies, <30% in babies requiring CPAP and <20% in babies breathing room air is taken as anaemia.

Blood Transfusion

- Transfusion of packed cells, platelets, fresh frozen plasma or whole blood

Procedure

After obtaining oral informed consent from the parents, neonate is screened in the NICU using an indirect ophthalmoscope. Baby is preferably fed one hour prior to examination in order to avoid vomiting and aspiration. Pupils are dilated using a combination of cyclopentolate 0.5 % and phenylephrine 2.5 % drops two to three times about 10 – 15 minutes apart. Excess eye drops must be wiped off to avoid systemic absorption. The changes in the retina were graded according to the International Committee for Classification of Retinopathy of Prematurity (ICROP) guidelines 2005.[11](table 1)

Timing of Screening [7]

- First retinal examination is performed not later than 4 weeks postnatal age in infants born > 28 weeks of gestation or 31 weeks of age whichever is later. (table 3)
- Infants born < 28 weeks of gestation and birth weight < 1200 grams are examined at 2 – 3 weeks of postnatal age

Follow Up Protocol [12]

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If no ROP was detected at initial examination, the infants were re-evaluated once every 2 weeks until vascularization is complete. If ROP was detected, the examinations were performed weekly for stage 1-2 disease and more frequently for stage 3 disease, till the disease started resolving or progressed to threshold stage. Babies showing evidence of regression were followed up till vascularization was complete.

RESULTS

Results obtained by Chi Square analysis are shown in table 2. Multivariate analysis is shown in table 3.

Table 1: Classification of ROP

Location	Zone I	Circle with optic nerve in centre and a radius of twice the distance between optic nerve and macula
	Zone II	From edge of Zone 1 to nasal oraserrata nasally and equator temporally
	Zone III	Lateral most cresent shaped area from Zone II to oraserrata temporally
Severity	Stage 1	Presence of thin white demarcation line between the vascular and avascular retina
	Stage 2	The line becomes prominent to become a ridge with length and width
	Stage 3	Presence of extra retinal fibrovascular proliferation arising from the ridge extending into the vitreous
	Stage 4	Partial retinal detachment: not involving the macula (4A) or involving the macula (4B)
	Stage 5	Complete retinal detachment
Plus disease		Presence of dilatation and tortuosity of posterior retinal vessels; associated with vitreous haze and papillary rigidity
Extent		Extent of involvement of retina expressed as clock hours

Table 3: Multivariate Analysis

								95% C.I.for EXP(B)	
		B	S.E.	Wald	Df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	SH(1)	2.480	.777	10.185	1	.001	11.943	2.604	54.777
	Constant	.611	.285	4.596	1	.032	1.842		
Step 2[b]	SH(1)	2.789	.830	11.301	1	.001	16.264	3.199	82.679
	APH(1)	1.513	.684	4.888	1	.027	4.539	1.187	17.351
	Constant	-.626	.635	.974	1	.324	.534		
Step 3[c]	CGA			6.015	2	.049			
	CGA(1)	2.038	.852	5.714	1	.017	7.673	1.443	40.787
	CGA(2)	.982	1.231	.637	1	.425	2.671	.239	29.796
	SH(1)	3.280	1.006	10.634	1	.001	26.565	3.700	190.704
	APH(1)	1.909	.718	7.060	1	.008	6.746	1.650	27.581
	Constant	-2.632	1.074	6.008	1	.014	.072		

a. Variable(s) entered on step 1: SH.
b. Variable(s) entered on step 2: APH.
c. Variable(s) entered on step 3: CGA.

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	ROP PRESENT	ROP ABSENT	P value	ODDS RATIO	CONFIDENCE INTERVAL	
					Low 95% CI	Hi 95% CI
SEX						
Male	43%	57%	0.249(ns)			
Female	57%	43%				
GESTATIONAL AGE						
<30 weeks	24%	8%				
30-34 weeks	67%	72%	0.023(s)			
34 weeks	10%	20%				
BIRTH WEIGHT						
<1000 grams	14%	5%				
1001 - 1500 grams	48%	57%	0.007(s)			
>1501 grams	38%	38%				
OXYGEN						
Bubble CPAP	57%	58%				
Mechanical ventilation	43%	19%	0.013(ns)			
Not given	0%	23%				
RDS						
Present	90%	47%	0.0003(s)	10.784(OR)	2.353	49.432
Absent	10%	53%		7.464(RR)	1.836	30.345
SURFACTANT						
Present	67%	33%	0.005(s)	4.077(OR)	1.468	11.323
Absent	33%	67%		3.000(RR)	1.329	6.773
APNOEA						
Present	33%	22%	0.0259(s)	1.824(OR)	0.636	5.232
Absent	67%	78%		1.583(RR)	0.724	3.463
SEPSIS						
Present	81%	54%	0.027(s)	3.558(OR)	0.997	13.844
Absent	19%	46%		2.833(RR)	0.998	9.640
SHOCK						
Present	90%	44%	0.0002(s)	11.943(OR)	2.604	54.777
Absent	10%	56%		8.093(RR)	1.990	32.914
ANAEMIA						
Present	14%	4%	0.072(ns)	4.222(OR)	0.786	22.672
Absent	86%	96%		2.611(RR)	1.060	6.432
PHOTOTHERAPY						
Present	90%	67%	0.034(s)	4.660(OR)	1.008	21.538
Absent	10%	33%		3.694(RR)	0.920	14.834
BLOOD TRANSFUSION						
Present	62%	44%	0.151(ns)	2.043(OR)	0.691	6.145
Absent	38%	56%		1.760(RR)	0.746	4.343
EXCHANGE TRANSFUSION						
Present	29%	13%	0.077(ns)	2.760(OR)	0.869	8.768
Absent	71%	87%		2.100(RR)	0.961	4.587
PIH						
Present	62%	54%	0.539(ns)	1.360(OR)	0.508	3.646
Absent	38%	46%		1.2779RR)	0.581	2.806
APH						
Present	33%	16%	0.086(ns)	2.538(OR)	0.858	7.511
Absent	67%	84%		2.000(RR)	0.932	4.292

Table 2

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DISCUSSION

The present study reflects the problem of ROP in a tertiary care neonatal unit. Incidence of ROP in the present study is 21%. Out of the 21 babies, only one baby developed ROP requiring surgery and remaining 20 babies developed ROP not requiring surgery (includes both regressing ROP and ROP requiring follow up). Birth weight of ROP babies ranged from 930 – 1750 grams (mean 1363.81±251.41) while that of non ROP babies ranged from 900 – 1900 grams (mean 1396.33±277.42). Lower birth weight was significantly associated with the incidence of ROP ($p = 0.007$). Incidence of ROP was 20 % among babies ≤1500 grams.Incidence of ROP in babies < 34 weeks was found to be 23%. Lower gestational age was found to significantly associated ($p =0.023$) with development of ROP. Gestational age, birth weight, oxygen, respiratory distress syndrome, surfactant, sepsis, shock and phototherapy were found to be significantly associated with ROP by Chi Square analysis. Shock, gestational age and antepartum hemorrhage were found to be significant through multivariate analysis. These results show that ROP incidence is influenced by almost every illness and treatment carried out in the preterm neonate. Effective management of ROP requires a team effort by a pediatrician, ophthalmologist and the NICU staff. Thus implementing an intelligent screening policy in every NICU will help to reduce effectively one of the preventable causes of childhood blindness.

CONCLUSION

Regular screening program that includes all preterm neonates satisfying the screening guidelines should be followed strictly. Along with regular screening, effective control of administration of oxygen, monitoring of oxygen saturation, management of RDS, following strict asepsis and prompt recognition and management of shock with fluids and inotropes and careful administration of phototherapy based on the bilirubin normogram must be followed.

REFERENCES

[1] Charan R,Dogra MR,Gupta A,Narang A. Indian J Ophthalmol 1995;43:123-26.
[2] Gopal L, Sharma T, Ramchandran S, Shanmugasundaram R, Asha V. Indian J Ophthalmol 1995; 43:50-61.
[3] Varughese S, Jain S, Gupta N, Singh S, Tyagi V, Puliyel JM. Indian J Ophthalmol 2001:49:187-88.
[4] National Neonatalogy Forum of India. National Neonatal Perinatal Database. Report for 2003-2003. New Delhi 2005.
[5] Global Initiative for the elimination of Avoidable Blindness Action Plan. 2006-2007.
[6] Seyedeh Khatami et al. Iran J Pediatr 2008;18(2) :137-142.
[7] Retinopathy of Prematurity. Clinical practice guidelines. National neonatology forum. October 2010 ; 253-55
[8] John P Cloharty. Respiratory distress syndrome. Manual of Neonatal Care. 7th Edition; 406-407
[9] John P Cloharty. Apnea. Manual of Neonatal Care. 7th Edition; 397
[10] Janie M Rennie. Rennie and Roberton's Textbook of Neonatology, 5th edition. Chapter 30
[11] Arch Ophthalmol 2005;123:991–999
[12] American Academy of Pediatrics, Screening examination of premature infants for Retinopathy of Prematurity. Pediatrics; 117: 572-576

Original article

Sleep pattern and problems in healthy toddlers

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Abstract

Objective: Sleep habits and sleep disturbances vary with age biological, psychological and socio-cultural environment. To understand this normality we conducted a questionnaire based survey among parents of healthy toddlers in Coimbatore city.

Method: A questionnaire cum interview based study elicited responses from the parents. This questionnaire focused on demographic factors, sleep pattern, practices and related problems with open ended and close ended questions**.**

Key words: sleep pattern, sleep problems, toddlers

Introduction

Sleep related problems and are not uncommon in children and may persist in adulthood (1). Disrupted or deficient sleep impacts every aspect of a child's growth and development.(2-5) sleep problems can be a result of both types of sleep issues (disorders and problems), it is important to have a better understanding of sleep pattern issues prevalent in pediatric community . Sleep disorders are readily identified if a child has prominent snoring or excessive daytime sleepiness, yet many sleep related problems may go unrecognized. One reason is that sleep issues are not frequently raised by care givers during health visits (6, 7). In addition, pediatric health care providers have limited training in pediatric sleep (8), cursory questions related sleep and limited patient contact time. One study of two general pediatric clinics found that a discussion of sleep issues is rare, diagnosis uncommon, and treatment guidelines lacking (9). Many studies suggest that night-time sleep pattern in childhood is mainly determined by the environment (10, 11). Multiple factors within the home have been shown to influence the sleep duration and pattern. In an sample of over 5000 children aged from birth to 36 months, a regular bedtime routine and limited TV exposure were among the strongest predictors of longer sleep at night (12). As there are also marked ethnic and socio-economic differences in the sleep duration of young children (13), it is essential to know the sleep pattern of the community. This study therefore aimed to analyze the sleep pattern of healthy toddlers in Coimbatore city.

Material and methods

Healthy toddlers (1-3 years) attending PSG well baby/vaccination clinic at PSG hospitals Coimbatore, India were included for this cross sectional prospective study. Toddlers with chronic medical illness and on any sedative drugs were not included. The Institutional ethics committee approved the study. Written informed consent was taken from parents / caregivers. A questionnaire is adapted and translated in Tamil from two previous studies (14, 15). This questionnaire focused on demographic factors, sleep duration, bedtime rituals and sleep related disturbances with open ended and close ended

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questions among participants. Parents are asked to recall the child's sleep behaviors over a "typical" recent week. Items are rated on a 3-point scale: "usually" if the sleep behavior occurred 5 to 7 times per week; "sometimes" for 2 to 4 times per week; and "rarely" for 0 to 1 time per week. The questionnaire was administered via interview by trained health assistant.

Categorical variables were summarized by proportions while the continuous variables were summarized by means and standard deviations. Frequency data applied for responses from open ended questions. Data analysis was done using SPSS15.

Results

Demographic factors

A total of 186 completed responses from parents of toddlers were collected over six month period. Responses from parents only were taken final statistical analysis. The mean age of the toddlers was 20(+ 4.5) months with sex ratio of 55:45 of boys and girls.

Sleep duration

The total reported average duration of sleep in the study population was 11.5+2.5 hours including day time sleep. Mean duration of day time sleep was about 1.30+30minutes.18 % of the kids had no regular day time sleep. The median time for falling asleep 8.30 pm (range 7 to 11.30pm) and wake up time 6.30am (range 4.30 to 8.30am). Early sleep onset time reported in children who are not taking regular day time nap. Only 30 % of kids usually had regular sleep duration and timings.

Sleep practices

Co -sleeping with either one or both parents was prevalent among 82% of the total sample. About 12% of kids were sleeping with grant parents and others. About 6 % of parents allowed their kids to sleep in separate bed in the same room. Working parent's babies slept late in the night (median time 9.30pm) and woke up late in the morning (median time 7.40am) due to more playing in the night and undisturbed sleep in the morning hours. Commonly reported bedtime routines are bed time stories 40%, songs with patting (28%), swaddling and rocking (30%). Significant number of babies (45 %) watching television before falling asleep.

Table -1 sleep problems

Sleep problems	Boys N=102 (54.8%)	Girls N=84 (45.2%)	Total (N=186)
Over all	45	40	85 (45.6%)
Bed time resistance	23	27	50 (27%)
Irregular timings	19	23	42(23%)
Fragmented sleep	15	18	33(18%)
Frequent Night waking	24	20	44(24%)
Bruxism	10	7	17(9%)
Day time drowsiness	30	20	50(27%)
Restless leg syndrome	10	6	16(9%)
No sleep problem	52	49	101

Quality of sleep and sleep disturbances

About 20% of parents perceived that their child is not sleeping well due to reasons such as bed time resistance, irregular timings, fragmented sleep, frequent awakenings due to probable night terrors and night mares , teeth grinding and difficulty in falling asleep and getting up. They encountered one or more such issues regularly. The reported above sleep disturbances (one or more) in the study group were 45.6% though many parents felt such disturbances were not significant (refer table no-1). Bruxism was reported in 9 % of children. About 25% children reported to frequent night waking without any obvious reasons.Only 15 percent of babies

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between one to two years required 1 or 2 night feeds and bowel movements which were rare after two years. Day time drowsiness reported in 27%. As co-sleeping with parents either single or both parents observed in 83 percent of samples sub group comparisons were not done.There were no significant sex differences in the study group.

Discussion

Sleep plays vital role in the neuro-cognitive development of children. Enquiring parents about their children's sleep pattern should be part of every child care visits. Evaluating sleep pattern and disorders requires an understanding of changes in sleep occurs with developmental maturation. Children sleep pattern also varies with age, ethnicity and cultural background. There are only few studies from India evaluated sleep pattern among preschool children. This study was done in Coimbatore a tier 2 city where in both parents are mostly working. In the past, children were generally considered to be so called "good-sleepers", but recent reports do not support this myth (16).Most sleep problems may start in late infancy and become a major issue for toddlers. Adequate and sound sleep is crucial for successful learning in children. Daytime behaviors in children may also be influenced by nighttime problems.The overall prevalence of sleep disorders in children of our study is 45 %.The sleep problems reported in similar studies from other countries varies from 20-50%.Bed time resistance and irregular timings were the most common concerns reported in this study. Frequent night waking lead to increased day time drowsiness in this group (50%). Our study results were comparable to a sleep study from Iran using BEARS tool in Preschool and School-aged children (17). A Similar study from Singapore reported identical pattern of sleep duration and sleep related problems in mixed Asian population.

The prevalence of co-sleeping among Indian children is comparable to studies from all Asian and culturally similar countries (19, 20). As children's sleep patterns are more likely to be influenced by the sleep patternsof co-sleeping parents, it is essential that co- sleeping adults to practice well sleep hygiene.Sleep problems were more common in developing countries than develope countries. The lower rate of sleep problems reported from developed countries may be explained in view of higher levels of knowledge of sleep hygiene and screening programs. (19, 21).The following are the limitations. Small cross-sectional questionnaire based study and may not represent entire community. Accuracy of self reporting is not clear in this study.

Conclusion

Toddlers sleep largely depends with parent's child rearing practices and sleep ecology. Sleep related problems are common and most issues are not perceived as serious illness warranting medical attention. Addressing babies sleep pattern and educating sleep hygiene in early years of life should be a part of health care visits as sleep determines development and behavior.

References

1. Byars K.C., Yolton K., Rausch J., Lanphear B., Beebe D.W. Prevalence, patterns, and persistence of sleep problems in the first 3 years of life. Pediatrics. 2012;129:e276–e284.
2. Beebe DW. Cognitive, behavioral, and functional consequences of inadequate sleep in children and adolescents. Pediatr Clin North Am. 2011;58:649–65.

3. Fallone G, Acebo C, Arnedt JT, et al. Effects of acute sleep restriction on behavior, sustained attention, and response inhibition in children. Percept Mot Skills. 2001;93:213–29.
4. Sadeh A, Gruber R, Raviv A. The effects of sleep restriction and extension on school-age children: What a difference an hour makes. Child Dev. 2003;74:444–55.
5. McKnight-Eily LR, Eaton DK, Lowry R, et al. Relationships between hours of sleep and health-risk behaviors in US adolescent students. Prev Med. 2011;53:271–3.
6. Blunden S, Lushington K, Lorenzen B, et al. Are sleep problems under-recognised in general practice?Arch Dis Child. 2004;89:708–12.
7. Owens JA, Spirito A, McGuinn M, et al. Sleep habits and sleep disturbance in elementary school-aged children. J Dev Behav Pediatr. 2000;21:27–36.
8. Mindell JA, Moline ML, Zendell SM, et al. Pediatricians and sleep disorders: Training and practice.Pediatrics. 1994;94:194–200.
9. Chervin RD, Archbold KH, Panahi P, et al. Sleep problems seldom addressed at two general pediatric clinics. Pediatrics. 2001;107:1375–80. .
10. Fisher A, van Jaarsveld CHM, Llewellyn CH, Wardle J. Genetic and environmental influences on infant sleep. Pediatrics. 2012;129:1091–1096.
11. Touchette E., Dionne G., Forget-Dubois N., Petit D., Perusse D., Falissard B. Genetic and environmental influences on daytime and nighttime sleep duration in early childhood. Pediatrics.2013;131:e1874–e1880. .
12. Sadeh A., Mindell J.A., Luedtke K., Wiegand B. Sleep and sleep ecology in the first 3 years: a web-based study. J Sleep Res. 2009;18:60–73.
13. Mindell J, Sadeh A, Wiegand B, How TH, Goh DYT. Cross-cultural differences in infant and toddler sleep. Sleep Med. 2010;11:274–280.
14. Lam P, Hiscock H, Wake M. Outcomes of infant sleep problems: a longitudinal study of sleep, behavior, and maternal well-being. Pediatrics 2003;111:e203–7.
15. Sadeh A. A brief screening questionnaire for infant sleep problems: validation and findings for an Internet sample. Pediatrics. 2004;113(6).
16. Riter S, Wills L. Sleep wars: research and opinion. Pediatr Clin North Am 2004 Feb; 51(1):1-13.
17. Mohammadi M, Ghalebaghi B, Bandi MFG, Amintehrani E, Khodaie S, Shoaee S, et al. Sleep patterns and sleep problems among preschool and school-aged group children in a primary care setting. Iran J Pediatr. 2007;17:213-21.
18. Aishworiya R, Chan P, Kiing J, Chong SC, Laino AG, Tay SKh. Sleep behaviour in a sample of preschool children in Singapore. Ann Acad Med Singapore. 2012 Mar;41(3):99-104.
19. Li S, Jin X, Yan C, Wu S, Jiang F, Shen X. Bed- and room-sharing in Chinese school-aged children: prevalence and association with sleep behaviors. Sleep Med 2008;9:555-63.
20. . Cortesi F, Giannotti F, Sebastiani T, Vagnoni C. Cosleeping and sleep behavior in Italian school-aged children. J Dev Behav Pediatr 2004; 25:28-33.
21. Latz S, Wolf AW, Lozoff B. Cosleeping in Context - sleep practices and problems in young children in Japan and the United States. Arch Pediatr Adolesc Med 1999;153:339-46.

Original article:

Current pattern of Salmonella Typhi antimicrobial susceptibility in the era of antibiotic abuse

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Abstract

Multidrug resistance in Salmonella Typhi has emerged as a significant problem. Given the difference in the sensitivity patterns reported for Salmonella Typhi, this study aimed to evaluate antimicrobial susceptibility patterns of Salmonella Typhi in a tertiary care hospital, coimbatore in south India. Salmonella Typhi positive blood cultures from children up to 14 years of age, over a period of 18 months from June 2011 to November 2012 were analyzed . Kirby-Bauer disc diffusion method was used to evaluate antimicrobial susceptibility. Results: Of the total of 50 isolates studied, all the isolates were susceptible to ceftriaxone, most were responsive to Ampicillin, Chloramphenicol, Cotrimoxazole, and Ciprofloxacin. Out of 50, only 4 cases were sensitive to Nalidixic acid. Isolates that showed high nalidixic acid resistant (*34/50*) had elevated minimum inhibitory concentration (MIC) for ciprofloxacin, ranging from 0.135 to 0.91 µg/mL (average 0.25 ± 0.11 µg/mL) RSC. Only 2 isolates were multi drug resistant. Though multi drug resistance is less common, high level of nalidixic acid resistance and elevated MIC for fluoroquinolones is a concern in this population.

Key words: Antimicrobial susceptibility, Salmonella typhi, multidrug resistant typhoid fever

Introduction

Typhoid fever is a bacterial disease, caused by Salmonella typhi. It is transmitted through the ingestion of food or drink contaminated by the faeces or urine of infected people. It is a worldwide problem and widely prevalent in the tropical developing countries with an estimated 12-33 million cases (1) and 6 lakhs death occurring annually (2). Major contribution is from Asia (3) and it is endemic in the most parts of Indian subcontinent (4). Appropriate antibiotic therapy reduces mortality from 30% to less than 1% (5). Multidrug resistant (MDR) enteric fever (resistant to chloramphenicol, ampicillin and co-trimoxazole) has emerged across the world in the recent times (6). Salmonella Isolates with reduced susceptibility to fluoroquinolones have now reported in the Indian subcontinent and other regions (7, 8).The current worldwide increase in antimicrobial resistance and lack in the development of new antibiotics have serious public health and economic implications. The reason for increasing resistance is multifactorial, but the main cause is the high level of inappropriate antibiotic usage. About 80% of antibiotics are used in the community and the rest are used in hospitals (9, 10). It is estimated that 20-50% of all antibiotics use is inappropriate, resulting in an increased risk of side effects, higher costs and higher rates of resistance in other community pathogens (11). In this context, it was essential to find out the current sensitivity pattern of the Salmonella strains isolated from blood in our region.

Materials and Methods

It is a cross-sectional, hospital based study spanning over a period of 18 months from June 2011 to November 2012. Inpatients and outpatients of department of Paediatrics of PSG IMSR with confirmed diagnosis of Salmonella typhi including locales in and around Coimbatore including referred cases up to 14 years of age were included. Institution human ethics committee approved the study. All the suspected cases of typhoid fever on the basis of clinical features of high grade fever, headache, hepatosplenomegaly, and with other Constitutional symptoms were investigated. Blood culture was performed by collecting 5cc venous blood and diluting it 4 times under normal circumstances and 10 times if treated with antibiotics, and then injecting into a bulb containing 20-30cc of 5% bile broth. The bulb is incubated for 24-48 hrs at 37^0C and subcultures were made on McConkey's medium. Cultures were declared negative after incubation for 10 days. Antibiotic sensitivity pattern was assessed by the Kirby-baeur disc diffusion method where discs containing antibiotics were placed onto an agar plate upon which bacteria were growing. If the bacteria is sensitive to the antibiotic a zone of inhibition is seen around the disc. Minimum inhibitory concentrations (MIC) of isolates resistant to chloramphenicol, ampicillin and nalidixic acid were determined by agar dilution test using purified antibiotic powders. Data were evaluated using descriptive statistics.

Results

A total of 50 cases of salmonella typhi isolates from blood culture were included for analysis. Out of these, 36 (72%) were males and 14(28%) were females. Of the 50 cases 39(78%) were between 5-14 years and 11(22%) cases were less than 5 years. Fever was noted in all the cases (100%). Average duration of symptoms before admission was 4.5 days. Other predominant symptoms were pain abdomen (26%), diarrhea (28%), vomiting (22%) and cough (14%). Hepatomegaly(60%) and Splenomegaly(68%) were the major clinical findings. Toxic look was present in 18 % of cases. Leucopenia (<4000cumm) was present in only 5 cases. In the present study (table-1), all the isolates were susceptible to ceftriaxone(100%) , most were responsive to Ampicillin (48/50,96%), Chloramphenicol (47/50,94%),Cotrimoxazole (45/50,90%) and Ciprofloxacin (48/50,96%).Out of 50,only 4 cases were sensitive to Nalidixic acid. Isolates that showed high nalidixic acid resistant (*34/50*) had elevated MIC for ciprofloxacin, ranging from 0.135 to 0.91 µg/mL (average 0.25 ± 0.11 µg/mL) RSC. Only 2 isolates were multi drug resistant (simultaneous resistance of bacteria to chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole) in this study. No mortality in the study group. Mean duration of hospital stay was 6.5$\pm$ 3days. Mean defervescence time after initiation of antibiotic therapy in ceftriaxone treated group (36/50) was mean 4.5+2 days and ciprofloxacin (14/50) treated group was 5$\pm$3 days.

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Table -1, showing antibiotic sensitivity pattern

Antibiotics	Sensitive N=50	Intermediate	Resistant
chloramphenicol;	46(92%)	1(2%)	3(6%)
ampicillin;	45(90%)	3(6%)	2(4%)
ceftriaxone;	50(100%)	0	0
ciprofloxacin;	34(68%)	14(28%)	2(4%)
trimethoprim-sulfamethoxazole	40(80%)	5(10%)	5(10%)
nalidixic acid;	4(8%)	12(24%)	34(68%)
multidrug resistance	-	-	2(4%)

Discussion

Typhoid fever is endemic in most parts of the India and it remains a major public health problem in many similar developing countries. This organism is highly adaptable and has remarkable mechanisms of survival and transmission in the host (12). Growing drug resistance is an important factor in the morbidity and mortality of the typhoid fever. Reports from different parts of India documented an increasing resistance of *S*. Typhi strains to commonly used first line drugs especially ciprofloxacillin (13, 14, 15). The resistance pattern varies with geographical locations. This study was done in tertiary care teaching hospital Coimbatore, India.

All the isolates in our study were sensitive to ceftriaxone in contrast to the recent studies (16, 17) that recorded resistance to ceftriaxone. Nalidixic acid sensitivity has been validated as a screening test for susceptibility to ciprofloxacin. High level of nalidixic acid resistance is associated with a high MIC of ciprofloxacin, which is associated with treatment failure(18,19). In this present study, 34of 50 isolates were nalidixic acid resistant and were associated increased resistance to ciprofloxacin. The fluoroquinolones resistance is said to be due to altered DNA gyrase sub-unit but, recently plasmid-mediated resistance pattern has also been reported (20). In developing countries such as India, ciprofloxacin continues to be the mainstay in the treatment of typhoid fever as it is orally effective and economical.

The emergence of ciprofloxacin resistance is a concern in many developing countries. There is no significant difference in response to fever in children treated with ceftriaxone and ciprofloxacillin in this study. Only 4% multidrug resistant isolates in this region is notable. This study has few limitations. As it was small hospitals based study, these findings may not truly reflect the sensitivity pattern in the community. Despite the fact that multi drug resistance is less common, high level of nalidixic acid resistance and elevated MIC for fluoroquinolones in the pediatric age group warrants rational prescription of antibiotics.

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References

1. Miller SI, Pegeus DA, Principles and practice of infectious diseases, 6th ed, Churchill Livingstone, New York, 2002, 2346.
2. Ivannoff B. Typhoid fever-global situation and WHO recommendations. Southeast Asian J of Tropical Medicine and Public Health 1995; 26(2):1-6.
3. Park K, Park's textbook preventive and social medicine, 20th ed, M/S Banarsidas Bhanot, Jabalpur, 2009, 249.
4. Richens J, Weatherale DJ, Ledingham J and Warell DA, Oxford textbook of Medicine, 3rd ed, Oxford Medical publication, London, 1996, 560-8.
5. Jenkins C and Gillespie SH. Manson's tropical diseases, 22nd ed, Saunders Elsevier, Amsterdam, 2009, 931.
6. Madhulika U, Harish BN, Parija SC. Current pattern in antimicrobial susceptibility of Salmonella Typhi isolates in Pondicherry. Indian J Med Res. 2004;120:111–4.
7. Brown JC, Shanahan PM, Jesudason MV, Thomson CJ, Amyes SG. Mutations responsible for reduced susceptibility to 4-quinolones in clinical isolates of multi-resistant Salmonella Typhi in India. J Antimicrob Chemother. 1996;37:891–900.
8. Effa EE, Bukirwa H. Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever) Cochrane Database Syst Rev. 2008;(4):CD006083.
9. Wise R, Hart T, Cars O, *et al.*: Antimicrobial resistance. Is a major threat to public health**.***BMJ* 1998, **317:**609-10.
10. Cars O, Mosltad S, Melander A: Variation in antibiotic use in the European Union.*Lancet* 2001, **357:**1851-53.
11. Cizman M: The use and resistance to antibiotics in the community**.***Int J Microb Agents* 2003, **21:**297-307.
12. Parry CM, Hien TT, Dougan G, et al: Typhoid fever**.***New Engl J Med* 2002, **347:**1770-82.
13. Sen B, Dutta S, Sur D, Manna B, Deb AK, Bhattacharya SK, Niyogi SK. Phage typing, biotyping & antimicrobial resistance profile of Salmonella enterica serotype Typhi from Kolkata. Indian J Med Res 2007, 125: 685-8.
14. Choudhary A, Gopalakrishnan R, Nambi P, et al.Antimicrobial susceptibility of Salmonella enterica serovars in a tertiary care hospital in southern India. Indian J Med Res2013, 137: 800-2
15. Jog S, Soman R, Singhal T, Rodrigues C, Mehta A, Dastur FD. Enteric fever in Mumbai--clinical profile, sensitivity patterns and response to antimicrobials. J Assoc Physicians India2008, 56: 237-40.
16. Molloy A, Nair S, Cooke FJ, Wain J, Farrington M, Lehner PJ, et al. First report of Salmonella enterica serotype paratyphi A arzithromycin resistance leading to treatment failure. J Clin Microbiol 2010; 48 : 4655-7.
17. Gokul BN, Menezes GA, Harish BN. Acc-1 β-lactamase– producing Salmonella enterica serovar Typhi, India. Emerg Infect Dis 2010; 16 : 1170-1.

18. Ray P, Sharma J, Marak RS, Garg RK. Predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance in Salmonella enterica var Typhi. Indian J Med Res 2006;124:105-8.
19. Roew B, Ward LR, Threlfall EJ. Multidrug resistant Salmonella Typhi, a worldwide epidemic. Clin Infect Dis 1997;24:S106-9.
20. Tran JH, Jacoby GA. Mechanism of plasmid mediated quinolone resistance Proc Natl Acad Sci U S A 2002;89:5639-41.

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PREVALENCE OF SEROPOSITIVITY AGAINST HEPATITIS B VIRUS IN PREVIOUSLY IMMUNIZED ADOLESCENT CHILDREN

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ABSTRACT

BACKGROUND

Less is known regarding the duration and long term protection offered by Hepatitis B vaccination and whether there is a need for booster doses of hepatitis B vaccine. This study determines the level of antibodies to Hepatitis B surface antigen and analyses the prevalence of seropositivity.

METHODS

A total of 30 children in the age group of 10-15 years were included for anti-HBs titres assessment. All were vaccinated for hepatitis B (3 doses) during infancy.

RESULTS

Only 30% of the children had anti-HBs titres in the protective range, majority are between 10 and 100 mIU/mL. Remaining 70% of the children had anti-HBs titres in the subprotective range (less than 10 mIU/mL).

CONCLUSION

This small study warrants further research on the necessity of booster doses of Hepatitis B and seroprotective levels of titres in Indian population.

KEYWORDS

Hepatitis B Vaccine, Seropositivity, Adolescents.

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INTRODUCTION: India is a country with intermediate prevalence of Hepatitis B infection. The Hepatitis B virus apart from causing a serious infection which may be fatal also causes a chronic carrier state and also increases the risk of cirrhosis and hepatocellular carcinoma.[1] The Government of India with a view to bring down the incidence of this infection has introduced the Hepatitis B vaccine in the national immunisation schedule. There are around 110 countries which have included the vaccine in their vaccination schedules. The introduction of the vaccine has brought down the incidence of the disease and also the incidence of cirrhosis and hepatocellular carcinoma.

The longterm effects of the vaccination and the need for further boosters are still under study and various studies have given a wide range of results.[2,3,4,5,6,7] While some studies say the anamnestic response is good and rule out the need for boosters,[2,4,5,6] some have come out that the anamnestic response may not be so good in all the vaccines and stresses the need for pre-vaccination testing and booster doses.[7,8] There are no studies from India in this regard. This study tries to throw light on the protectiveness and seropositivity of the Hepatitis B vaccine in the Indian children aged 10–15 years who were vaccinated with the three primary doses of the vaccine before 1 year of age. This study may be a starting point for further studies in this regard from India.

MATERIALS AND METHODS: This descriptive, hospital-based study was done at PSG Hospitals, Coimbatore, India during September 2014 to August 2015. Institutional human ethics committee approved the study. Children aged 10-15 years who had received three hepatitis B vaccination doses before one year of age and who had records of the same were recruited for the study. Since this was a pilot study a convenient sample of 30 subjects were included for final analysis from 40 recruits. Records were verified for the immunisation status and confirmed. After getting proper consent for the study from the parent and obtaining assent from the children, 3 mL of blood sample was taken and anti-HBs levels were determined by Chemiluminescence immunoassay technique. Children with history of Hepatitis B infection, Hepatitis B infection in the parents, children with chronic renal failure on dialysis, history of blood transfusion and immunocompromised children status were excluded from study. A total of 30 samples were collected for study purpose. An anti-HBs level of ≥10 mIU/mL was considered to be a protective antibody level. Children with values <10 mIU/mL were considered to have inadequate antibody titres.

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Data were collected and stored in Microsoft Excel and results were given in proportions.

RESULTS: Out of the 30 subjects included in this study, 14 were males (table-1). The mean age was 12.2 years (Figure-1). Of the 30 subjects, 9 had anti-HBs levels greater than 10 mIU/mL (30%) out of which 5 had titres between 10 and 100 mIU/mL (Figure-2). These 30% are considered to be having protective titres. The rest 21 have titres less than 10 mIU/mL and are considered to have inadequate protective antibody levels (70%). One subject had a titre of 0 mIU/mL. Hence, among the study population of 30 children aged 10 to 15 years who were vaccinated for hepatitis B during infancy, only 30% had protective titres of anti-HBs antibody. Of the 9 subjects who had protective levels of anti-HBs antibody, 4 were males and 5 were females (Table 1). Of the protected, 1 was in the age group on 10–11 years, 4 between 11–12 years, one between 12–13 years, 2 between 14–15 years and one was between 15–16 years (Figure 2).

Subjects (n=30)	Anti-HBs >10 mIU/mL	Anti-HBs <10 mIU/mL
Male (n=14)	4	10
Female (n=16)	5	11

Table 1: Sex wise Distribution of Subjects and anti-HBs Titres

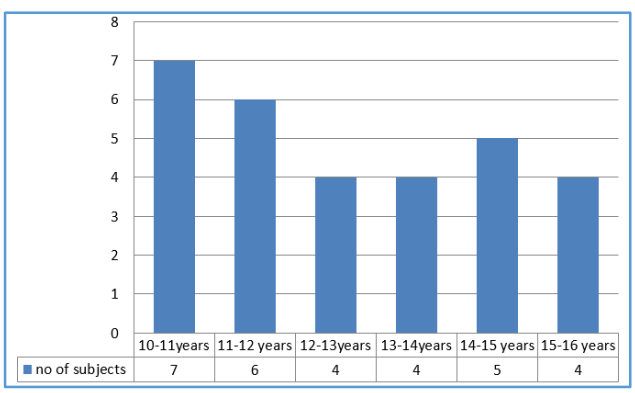


Figure 1: Age wise Distribution of Subjects

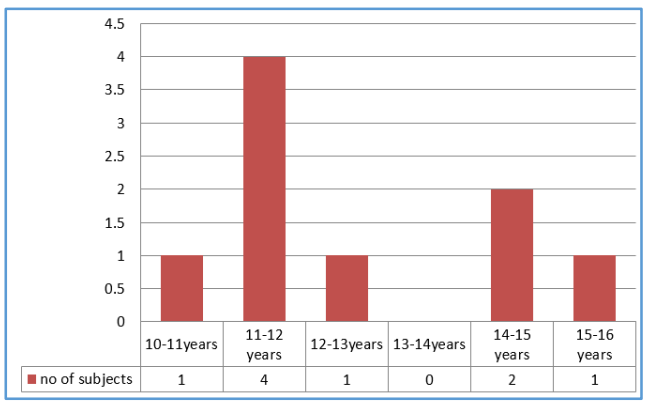


Figure 2: Age wise Distribution of Protective anti-HBs titres (>10 mIU/mL)

DISCUSSION: This study aimed at finding out the protectiveness against hepatitis B infection 10-15 years after complete vaccination and whether there is a need for further booster doses. Though there are only a very few studies available in this regard, none has come out from India. The studies from various regions have found out a variable percentage of protection by estimating the anti-HBs levels in the serum.[2,3,4,5,6,7] Since the vaccine failure has a genetic determinant also, it is necessary to know the protective levels of anti-HBs in Indian children ten years after completing the three doses of hepatitis B vaccine.

Our study has found that only 30% of the children who have completed the three doses of vaccine at infancy have protective titres of anti-HBs antibodies after 10 years of age. This is lower than the percentage of protection found in many studies.[2,4,5] Table-2 gives comparison of seroprotection against Hepatitis B across various countries.

Sl. No.	Study done by	Place	Year	Age group	Protection percentage
1	Reza Norouzirad et al	Iran	2011	1-18 years	40.10%
2	Amy B. Middleman et al	Houston	2011	16-19 years	24%
3	E. Spada et al	Italy	2014	10-17 years	60.30%
4	Hiva Saffar et al	Iran	2014	20 years	42.60%
5	Paul K. S. Chan	China	2010	17-23 years	18.90%
6	Hammitt et al	Alaska	2007	14-15 years	5%
7	Present study	India	2015	10-15 years	30%

Table 2: Seroprotection against Hepatitis B 10 years after Primary Vaccination

A study from Ahvaz city in Iran by Reza Norouzirad et al evaluated the anti-HBs levels in 840 healthy individuals in the age group between 1 and 18 years who were vaccinated during infancy.[2] The percentage of study group who had protective levels of anti-HBs (>10 mIU/mL) 1 year after vaccination was 90%. The average number of subjects having protective levels of antibody titres between 1 and 5 years was 72.1% which declined to 40.1% in the age group between 11 and 15 years. In another study among 420 healthy children aged 16 to 19 years in Houston metropolitan area by Amy B. Middleman et al, it was found that only 24% of the children had anti-HBs levels in the protective range

>10 mIU/mL.[3] But this study further checked the anamnestic response by giving one booster dose of Hepatitis B vaccine and re-checking antibody titres. 92% of children in the study group developed protective antibody titres after receiving one booster dose of the Hepatitis B vaccine. This study thus concluded that though the antibody levels were less, there was an intact immune memory which was responsible for the rapid response after receiving the booster dose of vaccination. The study also compared two groups of vaccinees – one in whom the first dose of Hepatitis B vaccine was given within 7 days and another group in whom the first dose was given after one month and found statistically significant variation in the baseline anti-HBs antibody values between the two groups with the early vaccinated group having decreased titres. This is of interest to us because many of our children are given the first dose of Hepatitis B vaccination at birth.

In a study in Italy by E. Spada et al, 571 teenagers were included and their anti-HBs levels were tested at 10 and 17 years of age.[4] 60.3% of the subjects had protective antibody levels to HBsAg. Out of the remaining 227 subjects, 199 were administered a booster dose of Hepatitis B vaccine. The protective levels of antibody were found in 72.9% subjects at 17 years which included the persons who received the booster dose of the vaccine. Out of the 155 subjects who had anti-HBs levels <10 mIU/mL, 96 were given booster dose of the vaccine to which all except two responded. The remaining two subjects received also responded after three doses of the vaccine. This study also concludes that though the antibody titres may be less, the immune response is good after a booster dose.

Saffar et al conducted a study in Iran in which 176 individuals vaccinated for Hepatitis B at infancy were administered one booster dose of Hepatitis B vaccine at 20 years of age.[5] Pre-booster anti-HBs values and the pre and post booster cytokine values were taken. It was found that only 42.6% had protective pre-booster values of anti-HBs of more than 10 mIU/mL. Among the non-protected, the cytokine values showed a two-fold increase after a booster dose in around 80% of individuals confirming preserved cells mediate immunity.

The effect of giving three doses of boosters was studied in a study conducted in China by Paul K. S. Chan which found that out of 212 students aged 17 to 23 years, only 18.9% had protective anti-HBs titres. One month after a single booster, this went up to 85.5% and after three booster doses, this number reached 100% with 97.1% having anti-HBs titres above 100 mIU/mL.[6] This study demonstrates a declining immune memory in a substantial group of individuals and the requirement of three doses of booster vaccination to achieve protective antibody titres. This study recommends pre-booster testing of anti-HBs titres and vaccinate accordingly.

These studies give a wide range of protection against Hepatitis B infection and the durability of this anamnestic response is not completely understood. A variable proportion of the vaccinated persons over a period of time lose both the protective antibody titres and the anamnestic response. The 30% protective efficacy obtained in our study is line with that obtained in many of the studies. The low levels of protection in the present study may be due to genetically determined lack of response to vaccination or due to technical errors such as intragluteal injections and inappropriate storage conditions. Some of the studies have assessed the immune memory by giving a booster dose of Hepatitis B vaccine and found that most of the subjects had a good immune memory and responded very well to a single booster dose of the vaccine.

Interestingly, there is a study from Alaska which gives a considerably lower percentage of anamnestic response. This is particularly significant since this study included a significant number of American Indians along with Alaskan natives.

One of these studies included 37 children who were vaccinated with all three primary doses of Hepatitis B before 1 year and who had documented anti-HBs titres >10 mIU/mL before 18 months of age.[7] The anti-HBs levels of these subjects were done at 14 to 15 years of age and it was found that only 5% had protective levels of anti-HBs. The subjects with anti-HBs antibody levels <10 mIU/mL were boosted with a dose of vaccine and the titres of anti-HBs rechecked after 15 days. Anamnestic response to booster dose was only 51%.

The difference in the protective titre levels of antibodies in different populations studied, the waning of anamnestic response with age in certain populations and lack of studies in the Indian population warrants further studies in this regard. Our study gives a protective level of antibodies to HBs in only 30% of the studied subjects who completed three doses of Hepatitis B vaccine before one year of age. A larger study with a larger sample and also studies assessing the anamnestic response is required in Indian population to find out the necessity of booster doses in the general Indian population.

The study also exposes the need for testing anti-HBs titres and the need for booster doses of vaccination. If not possible in all, at least in the more vulnerable groups like health care workers and contacts of HBsAg positive individuals. First of this kind of study among south Indian population, estimating the protectiveness and seropositivity of the Hepatitis B vaccine is the major strength of this study. No estimation of HBsAg antigens and core antibodies levels and small number of subjects were limitations of the study.

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REFERENCES

1. Tandon BN, Acharya SK, Tandon A. Epidemiology of hepatitis B virus infection in India. Gut 1996;38(Suppl 2):S56-S59.
2. Norouzirad R, Shakurnia AH, Assarehzadegan M-A, et al. Serum levels of anti-hepatitis B surface antibody among vaccinated population aged 1 to 18 years in Ahvaz City, southwest of Iran. Hepat Mon 2014;14(1):e13625.

3. Middleman AB, Baker CJ, Kozinetz CA, et al. Duration of protection after infant hepatitis B vaccination series. Pediatrics 2014;133(6):e1500-e1507.
4. Spada E, Romanò L, Tosti ME, et al. Hepatitis B immunity in teenagers vaccinated as infants: an Italian 17-year follow-up study. Clint Microbiol Infect 2014;20(10):O680–O686.
5. Saffar H, Saffar MJ, Ajami A, et al. Long-term T-cell-mediated immunologic memory to hepatitis B vaccine in young adults following neonatal vaccination. Hepat Mon 2014;14(9):e22223.
6. Chan PK, Ngai KL, Lao TT, et al. Response to booster doses of hepatitis B vaccine among young adults who had received neonatal vaccination. PLOS One 2014;9(9):e107163.
7. Hammitt LL, Hennessy TW, Fiore AE, et al. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine beginning at birth: a follow-up study at 15 years. Vaccine 2007;25(39-40):6958-6964.
8. Samandari T, Fiore AE, Negus S, et al. Differences in response to a hepatitis B vaccine booster dose among Alaskan children and adolescents vaccinated during infancy. Pediatrics 2007;120(2):e373–e381.

THE IMPACT OF PREMATURITY ON INFANT BRAINSTEM AUDITORY EVOKED RESPONSES

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ABSTRACT

BACKGROUND

Recent advances in perinatology have resulted in increased survival rate of preterm infants over the past two decades. Sensorineural hearing loss represent one of the serious neurodevelopmental sequelae among preterm infants. Brainstem Auditory Evoked Responses (BAER) is a noninvasive electrophysiological method for assessing the maturation of auditory system in newborn.

MATERIALS AND METHODS

Brainstem auditory evoked responses of 70 infants (35 preterm and 35 term) were recorded to analyse the impact of prematurity. Absolute latency and interpeak latencies were recorded using "Intelligent Hearing System Smart-EP". Student's t-test was used for statistical analysis.

RESULTS

The absolute latencies I, III, V and interpeak I-III, I-V were significantly prolonged among preterm babies (P value <0.05). There was a significant increase in absolute latencies I, III, V and IPL I-III among preterm babies with comorbidities (P value <0.05). Such difference in latencies was not seen among term babies with comorbidities.

CONCLUSION

Hence, it may be worthwhile to perform BAER recording in preterm babies and all babies with comorbid conditions to identify hearing abnormities at the earliest.

KEYWORDS

Preterm Infants, Brainstem Auditory Evoked Responses, Comorbid Conditions.

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BACKGROUND

In India, around 8 million Low Birth Weight (LBW) infants are born each year.[1] The LBW infants include both preterm babies and Intrauterine Growth Retardation (IUGR) babies. Recent advances in perinatal care have resulted in increased survival rates of preterm infants. However, the preterm infants are at risk of developing several immaturity related problems. Sensorineural hearing loss represents one of the serious neurodevelopmental disorder among the high-risk newborns. The incidence is ten times higher in preterm babies than the overall incidence of 1 to 3 per 1000 live births in normal babies.[2] This undetected hearing loss can result in serious impairment in language and communication skills, cognitive development, social and emotional development. Number of methods have been evaluated to search for reliable and effective technique for determining auditory functions in the neonates. Brainstem Auditory Evoked Responses (BAER) records potentials from the ear and vertex in response to brief auditory stimulation. BAER is one of the noninvasive electrophysiological method in clinical practice to detect neonatal deafness.[3] The BAER is the best tool available to assess the hearing impairments in infants.

BAER depicts the conduction along the brain stem auditory pathway; hence, comparison of auditory evoked responses of preterm infants with term infants is expected to provide insights about the maturational-related response changes during neonatal period and pathological changes due to associated comorbid conditions.

AIMS AND OBJECTIVES

This study aimed at analysing the impact of prematurity and its comorbid conditions on infant brainstem auditory evoked responses. The objectives were to record brainstem auditory evoked responses of preterm and term infants with or without comorbid conditions.

MATERIALS AND METHODS

This prospective observational study was conducted in a Tertiary Care Teaching Hospital, Coimbatore. A total of 70 infants were included in the study of which 35 were preterm infants and 35 were term infants.

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Inclusion Criteria

All the infants were selected from high-risk infant follow up clinic and Well-Baby Clinic. Preterm infants who have completed the post conceptional age of 40 weeks to 3 months of life and term infants up to 3 months of age were included.

Exclusion Criteria

Babies with family history of hearing loss, ear malformations and syndromic conditions were not included. Both preterm and term group were further divided into two subgroups based on the presence and absence of comorbidities.

The comorbid conditions in preterm included those babies who suffered from Respiratory Distress Syndrome, Neonatal Jaundice, Birth Asphyxia and Sepsis. All babies with comorbid conditions were treated in neonatal intensive care unit at Coimbatore Medical College as per the standard protocols by neonatologists. Similarly, term group babies were further divided into two based on the presence of comorbid conditions such as sepsis, birth asphyxia, neonatal jaundice and meconium aspiration syndrome. The study was carried out after explaining the procedures in detail and getting informed written consent from the parents or caregivers of the babies. The study was approved by the Institutional Ethical Committee. Intelligent Hearing System (Florida) [Smart EP, Universal Smart box Jr.,™ Opti-Amp 8002] equipment was used for recording BAER in infants.

Procedure

The procedure was done while infant sleeping naturally, usually after a feeding. Some of the babies who had difficulty in going to sleep were given the sedative promethazine orally of dose 0.5 mg/kg body weight. The babies were made to lie comfortably on his or her mother's or grandmother's lap while doing the recording. The recording was made in the sound proof BAER recording room. The skin at the site of placement of surface electrode was prepared well. The conducting gel was applied and the surface electrodes were fixed at appropriate sites with the help of adhesive plasters.

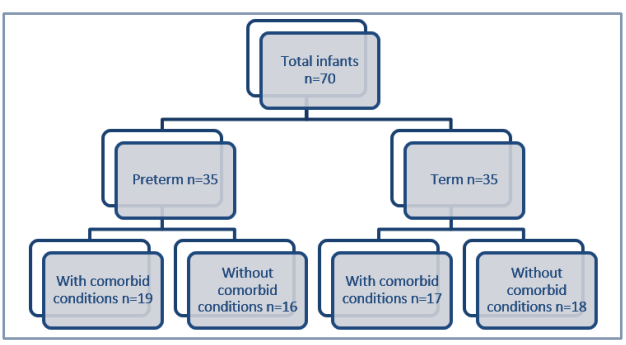
Site of placement of surface electrodes include active electrode on forehead reference electrode over the right (M1) and left (M2) mastoid processes and ground electrode on the cheek. At all the above sites, the impedance was kept below 5W. The earpiece was inserted into the corresponding ear (right or left) of infants while recording. Each ear was tested separately. The acoustic stimuli were given in the form of broadband clicks. The restriction filters were set between 100-3000 Hz. A total of 2000 stimuli were given with the repetition rate of 10 stimuli/seconds. The analysis window was 10 milliseconds. Each recording was made in duplicate to ensure reproducibility. Every time when the acoustic stimuli was given, the auditory system in the infant generated an electrical response. These evoked responses were detected by the surface electrodes. The recordings were analysed by the equipment Intelligent Hearing System and recorded as waveforms. The absolute latencies of the BAER waveforms I, III, V and the interpeak latencies I-III, III-V, I-V were marked and the values were noted down.

Statistical Analysis

Independent t-test has been used for intergroup and subgroup comparison of the BAER parameters. Statistical software SPSS 17 version was used for the analysis of the data.

RESULTS

A total of 70 infants were studied, which had 35 preterm babies and 35 term babies (flowchart-1). The mean birth weight of preterm group is 1.53 (±0.260) kg and 2.84 (±0.360) kg in term babies. The mean age was 2.03 months for preterm, 2.61 months for term (at the time of study) and sex ratio among two groups were comparable.



Flowchart 1. Depicting Study Population

The distribution of comorbid conditions such as HIE, jaundice, respiratory distress, sepsis and multiple risk factors in the study group is depicted in Table 1.

Group With Comorbidities	Comorbid Conditions				
	HIE	Jaundice	Respiratory Distress	Sepsis	Multiple Risk Factors
Term (n=17)	2	3	4	4	4
Preterm (n=19)	4	4	3	2	6

Table 1. The distribution of co-morbid conditions

Absolute latencies and interpeak latencies were compared between the two groups. To find out the impact of comorbidities, subgroup analysis was done in both term and preterm groups. The BAER parameters of preterm and term infants are depicted in Table 2 and 3.

Absolute Latency ms	Preterm Right Ear	Term Right Ear	P-value	Preterm Left Ear	Term Left Ear	P-value
I	2.02±0.35	1.73±0.23	0.000*	1.99±0.37	1.78±0.32	0.017*
III	4.74±0.28	4.53±0.29	0.003*	4.82±0.63	4.54±0.25	0.000*
V	8.16±0.42	6.63±0.31	0.000*	7.99±0.83	6.60±0.30	0.000*

Table 2. Absolute Latency in Preterm and Term Babies

***P - value less than 0.05 is significant**

Interpeak Latency	Preterm Right Ear	Term Right Ear	P-value	Preterm Left Ear	Term Left Ear	P-value
I-III	2.97±0.32	2.61±0.32	0.000*	2.98±0.33	2.55±0.26	0.000*
III-V	2.67±0.27	2.43±0.34	0.303	2.65±0.26	2.50±0.33	0.35
I-V	5.78±0.34	5.05±0.51	0.002*	5.84±0.51	4.75±0.45	0.000*

Table 3. Interpeak Latency in Preterm and Term Babies

*P-value less than 0.05 is significant.

BAER waveform I, III, V latencies were significantly prolonged among preterm babies than term babies. The interpeak latencies I-III, I-V were also significantly increased in preterm infants. The associated comorbid conditions significantly altered the wave latencies in preterm babies. The absolute latencies I, III, V and IPL I-III were significantly prolonged in preterm babies with comorbidities than in those without comorbidities. Though some latencies were prolonged in term babies with comorbid conditions, it had no statistical significance. The wave latencies among preterm group are depicted in Table 4.

Absolute Latency ms	With Comorbid R	Without Comorbid R	P value	With Comorbid L	Without Comorbid L	P-value
I	2.17±0.37	1.84±0.19	0.002*	2.18±0.37	1.75±0.20	0.000*
III	5.17±0.62	4.40±0.56	0.003*	5.13±0.64	4.45±0.37	0.001*
V	8.34±0.31	7.95±0.44	0.004*	8.43±0.37	7.47±0.93	0.000*
Interpeak Latency						
I-III	3.13±0.27	2.78±0.74	0.001*	3.15±0.30	2.77±0.25	0.000*
III-V	2.66±0.29	2.68±0.27	0.858	2.67±0.26	2.63±0.26	0.650
I-V	5.86±0.43	5.72±0.59	0.363	5.91±0.50	5.77±0.54	0.429

Table 4. Preterm With and Without Comorbidities in Both Ears

DISCUSSION

This study was done to compare the BAER parameters between preterm and term infants and also to find out the impact of comorbid conditions on waveforms. A recognisable BAER waveform can be recorded in preterm infants above 28 weeks of gestation.[4] The BAER waveform I arises from peripheral part of cochlear nerve.[4] The wave III originates from superior olivary nucleus in pons whereas wave V from Inferior Colliculus in midbrain.[4] The prolongation of BAER values in this study group was comparable with earlier studies.[5,6] We found that while comparing the BAER parameters among preterm and term babies, there was a significant prolongation of absolute latencies of wave I, III, V, interpeak latencies among preterm infants. Similarly, the interpeak latencies I-III, I-V were also significantly prolonged in preterm babies, which may be due to incomplete myelination of central brainstem auditory pathway in preterm infants.[5] In a similar comparative study done by Roopakala et al have found an increase in wave V latency, which they attributed to delay in maturation of central brainstem pathway in preterm infants.[7] The maturational defects in preterm infants they attributed are incomplete myelination of auditory nerves and pathway, decrease in axonal diameter, immaturity of neuronal synapses.[4] There is no sex difference in values in this present study as seen in many earlier studies except one from Pakistan by Shahid et al,[8] which suggested that high-risk male infants were more prone for auditory defects.[8] There is no significant BAER parameters difference between right and left ears in this study as observed in earlier studies.[5,9]

It has been found in our study that the association with comorbid conditions caused significant increase in all the absolute latencies I, III, V and IPL I-III in preterm infants. This impact of comorbidities on BAER waveforms was not present among term babies. This was probably due to prolonged exposure to extrauterine high-risk environmental conditions in preterm infants.[10,11] Babies who suffered from birth asphyxia and/or respiratory distress are exposed to prolonged periods of hypoxia, which may affect the myelination process. Studies conducted in animals have found that cochlear hair cells are sensitive to hypoxia.[12]

A study done by Agarwal among normal babies and babies with neonatal jaundice have found prolonged

absolute and interpeak latencies among 25% of the study group. The bilirubin specifically causes damage to the cell bodies of auditory nerve in spiral ganglion, brainstem cochlear nuclei.[12] These abnormalities had significant correlation with total bilirubin level.[13]

Neonatal sepsis and its related conditions like meningitis, ototoxic medications [aminoglycosides] may also lead to sensorineural hearing loss. These effects were demonstrated by Sun JH et al in a study among 248 infants using BAER.[14] The cytokines and toxin released during sepsis may cause damage to the developing auditory system.[15] Dowley et al noticed that neonatal sepsis was significantly associated with auditory neuropathy among high-risk newborn babies.[15] This impact of comorbidities is not seen in term infants and much of the changes in them are reversible.[16] Further evaluation of interaction between risk factors and neonatal hearing loss is warranted to implement more time weighted measures.

CONCLUSION

In this study, the brainstem evoked responses were found to be significantly prolonged among the preterm infants when compared to term infants. The associated comorbid conditions had great impact on the BAER latencies among preterm infants, but not in term infants. Regular follow up services should be done to assess the auditory maturation in such high-risk infants.

REFERENCES

1. State of India's newborns. Available at: http://WWW.healthynewbornnetwork.org/sites/default/files/resources/India-SOIN.pdf. Accessed on 2013.
2. American academy of pediatrics, task force on newborn and infant hearing. Newborn and infant hearing loss: detection and intervention. Pediatrics 1999;103(2):527-530.
3. Ilknur K, Hakan K, Tulay K, et al. Brainstem evoked response audiometry and risk factors in premature infants. Marmara Medical Journal 2007;20(1):59-65.
4. Gregory LH, Solmon LM, Royden J. Brainstem auditory evoked potentials in pediatrics-normal. In: Sandra LH, ed. Clinical neurophysiology of infancy, childhood and adolescence. 1st edn. New York: Butterworth-Heinemann Publishers 2005:188-191.
5. Casali RL, Santos MF. Auditory brainstem evoked responses: response patterns of full-term and premature infants. Braz J Otorhinologyngol 2010;76(6):729-738.
6. Jiang ZD, Brosi DM, Wang J, et al. Time course of brainstem pathophysiology during first month in term infants after perinatal asphyxia, revealed by MLS BAER latencies and intervals. Pediatric Research 2003;54:680-687.
7. Roopakala MS, Dayananda G, Manjula P, et al. A comparative study of brainstem auditory evoked potentials in preterm and full-term infants. Indian J Physiol Pharmacol 2011;55(1):44-52.
8. Naqqash S, Zaman S, Hamid KA. Early detection of hearing impairment among high-risk neonates. Ann Pak Inst Med Sci 2013;9(1):22-25.
9. Hasani S, Jafari Z. Effect of pre-term birth on auditory responses at the brainstem level. Journal of Otorhinolaryngilogy 2013;25(2):107-114.
10. Poblano A, Mendiola-Bonaga H, Valdez-Cárdenas H, et al. Brainstem auditory evoked potentials in low and high risk newborns. Bol Med Hosp Infant Mex 1993;50(8):551-556.
11. Jiang ZD, Wilkinson AR. Brainstem auditory evoked response at term in preterm infants after perinatal hypoxia-ischaemia. Acta Paediatr 2006;95(11):1400-1404.
12. Sawada S, Mori N, Mount RJ, et al. Differential vulnerability of inner and outer hair cell systems to chronic mild hypoxia and glutamate ototoxicity: insights into the cause of auditory neuropathy. J Otolaryngol 2001;30(2):106-114.
13. Agrawal VK, Shukla R, Misra PK, et al. Brainstem auditory evoked response in newborns with hyperbilirubinemia. Indian Pediatrics 1998;35:513-518.
14. Sun JH, Li J, Huang P, et al. Early detection of hearing impairment in high- risk infants of NICU. Zhonghua Er Ke Za Zhi 2003;41:357-359.
15. Dowley AC, Whitehouse WP, Mason SM, et al. Auditory neuropathy: unexpectedly common in a screened newborn population. Dev Med Child Neurol 2009;51(8):642-646.
16. Misra PK, Katiyar CP, Kapoor RK, et al. Brainstem auditory evoked response in neonates with birth asphyxia. Indian Pediatrics 1997;34:199-205.

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A Study on Community - Acquired Methicillin Resistant Staphylococcus Aureus Infections in Coimbatore Children

KEYWORDS: Staphylococcus aureus; Community-acquired; Methicillin-resistant; Children

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ABSTRACT

Introduction

Community acquired Methicillin-resistant Staphylococcus aureus (CA-MRSA) isolates are increasingly reported in many countries. Local epidemiological data are sparse.

Objective

To determine the frequency, clinical features, antibiotic sensitivity and outcome of infections caused by Community acquired Methicillin-resistant Staphylococcus aureus among children in a territory care teaching hospital, Coimbatore (south India)

Material and methods

Children(less than 15 years) with infections meeting the criteria of community-acquired were included. Demographic and clinical data were collected. Antibiotic susceptibilities were determined in the microbiology laboratory.

Results

The prevalence of CA-MRSA was around 35% among MRSA isolates. Skin and subcutaneous infections were the most common site (74%). All isolates were 100% sensitive to Vancomycin, Linezolid and Doxycycline. Drugs with poor sensitivity (< 5%) were Cefazolin, Penicillin, Ampicillin and Ceftriaxone. All cases except one recovered completely.

Conclusion

CA-MRSA isolates accounted for a high percentage of infections among Staphylococcus aureus in children. Community surveillance of these infections is essential to decide the appropriate empiric antibiotic treatment.

Introduction

Infections caused by Staphylococcus aureus, especially methicillin-resistant S. aureus (MRSA), are emerging as a major public health issue[1]. Methicillin-resistance of Staphylococcus aureus infections is reported to be a nosocomial origin[2]. Recent reports suggest that the epidemiology of MRSA is changing and community acquired methicillin resistance(CA-MRSA) is on the rise[3]. The risk factors for acquisition of MRSA are intensive care unit stay, prolonged hospitalization, severe chronic illness, invasive procedures, indwelling devices and long term antibiotics therapy[4,5]. The first case of community acquired methicillin resistant (CA-MRSA) in children was reported in 1988[6]. The emergence of MRSA as a cause of infection in the community in patients who have never been hospitalized and who have no other known risk factors for MRSA infection is a significant concern. Recent studies from India also confirmed the prevalence of CA- MRSA[7,8,9]. In this context we conducted a study on community acquired methicillin resistance in Staphylococcal aureus infections in Coimbatore children.

MATERIALS AND METHODS

This Indian council of medical research (ICMR) - STS funded study was conducted in Department pediatrics , PSGIMS&R, Coimbatore. The Institutional Human Ethics Committee has approved the study protocol and written informed consent was obtained. All children aged less than 15 years with any culture (pus or blood) positive for MRSA is included for analysis. MRSA identification and antimicrobial drug susceptibility was done in department of microbiology (NABH accredited laboratory) at PSG hospitals. The probable reasons for the methicillin resistance were analyzed clinically. Data extracted from the case records included demographics, Information on close contacts, infection type, culture site, antibiotic susceptibility, treatment, interventions, outcome and presence of risk factors for methicillin resistance. The duration of study period was one year (2011).

The infection is considered community acquired MRSA if the patient met all the following criteria.

1. The sample for culture is obtained during an outpatient visit or within 48 hrs of hospitalization.
2. The subject had no history within the past year of known risk factors for MRSA, including hospital admission, current intravenous drug use, surgery, dialysis, an indwelling catheter, or a percutaneous medical device.

Culture method

The samples were streaked on Blood and Mac-conkey agar. The plates were read after an incubation of 24 hours at 37 degree Celsius. The colonies suspected to be staphylococcus were subjected to catalase test, tube coagulase, mannitol fermentation, bacitracin and furazolidone sensitivity. Antibiotic susceptibility is tested on Muller Hinton agar by making lawn culture. The antibiotics tested were Cefoxitin, Erythromycin, Clindamycin, Co-trimoxole, Ciprofloxacin, Vancomycin, Linezolid, Gentamicin, Doxycycline, Rifampicin, Furazolidone, Novobiocin and Penicillin. All Staphylococcus species are catalase positive, furazolidone sensitive and bacitracin resistant. Those that are tube coagulase positive and mannitol positive are considered as S.aureus. The S.aureus those are resistant to Cefoxitin in zone size less than 22mm are considered as MRSA in this study.

Descriptive statistics were used in this study.

Results

Patient characteristics: During the one year period 290 cultures were positive for staphylococcus aureus including colonization. Of the 290 cultures, 140 isolates were MRSA infections. Among the 140 cases, 50 were identified as CA-

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MRSA and included for final analysis. The remaining 90 cases had one or more risk factors for methicillin resistance. There were 32 boys and 18 girls (sex ratio: 1.78:1) in the final study group. The median age was 3.6 years. All children were from Coimbatore region and they had no identifiable risk factors for methicillin resistance. About the sites of isolation, 20 isolates from pus, 21 from blood and in 9 cases staphylococcus aureus was isolated from both.

Clinical presentation: Localized Skin infections were the most common sites of infection, accounting for 74% (37/50) of CA-MRSA infections. Septic arthritis (4/50) and lung infections (4/50) were involved in 16 % of cases. Scalded skin syndrome in 3 (6%) cases. Osteomyelitis and toxic shock syndrome contributed each case(2%). The other non specific symptoms observed were fever (26/50),vomiting (8/50),diarrhoea (7/50) and septic shock in one case.

Antimicrobial susceptibility: all isolates were 100% sensitive to Vancomycin, Linezolid and Doxycycline (Table-1). The drugs such as Rifampin, Ciprofloxacin, Cephalothin and Clindamycin had sensitivity around 80-90% of the tested isolates. The sensitivity rate was around 55-65 % for the following drugs Co-trimoxazole, Cloxacillin, Gentamycin and Erythromycin. Drugs had poor sensitivity were Cefazolin, Penicillin, Ampicillin and Ceftriaxone.

Outcome: All but one patient recovered without any major complications. The one patient who had toxic shock syndrome died of multi-organ dysfunction syndrome. Patients with skin and soft tissue infections were treated with topical mupiroicin and oral cloxacillin and erythromycin. Those who had systemic infections were treated with either vancomycin or linezolid.

Discussion

Our study revealed CA- MRSA infections in previously healthy children without known risk factors for MRSA infection in a territory care hospital in south India. In this study among the MRSA isolates 35% of cases were community acquired as those case did not have any risk factors for nosocomial spread. The overall prevalence among all Staphylococcus aureus infections was 17 % in children. MRSA is now endemic in many parts of India. The incidence of MRSA varies from 25 per cent in western part of India to 50 per cent in South India[10]. CA-MRSA has been increasingly reported from India[11]. Clinically, CA-MRSA usually causes skin and soft tissue infection, often with abscess and furuncle formation. However, it can cause serious life-threatening conditions, which in addition to necrotizing pneumonia include necrotizing fasciitis, joint infections bloodstream infection, and septic shock[12,13]. CA-MRSA may arise in either of 2 ways: hospital strains may be carried into the community, where they then spread person to person, or community MRSA may arise de novo when the methicillin-resistance gene complex is acquired by a methicillin-susceptible strain [14]. CA-MRSA strains were recognized as a novel pathogen that is genetically different from healthcare-associated MRSA with regard to genetic resistance determinants [14]. The genetic resistance determinants are mobile staphylococcal cassette chromosome mec type IV, and certain virulence factors, such as the Panton-Valentine leukocidin. Panton-Valentine leukocidin-positive S. aureus strains have been associated with severe SSTIs and pulmonary infections [15]. In this study we have done only risk factors analysis for Methicillin resistance. We could not do molecular analysis of MRSA isolates to determine their genotypes due to various reasons. In our study all the isolates were 100% sensitive for Vancomycin, Linezolid and Doxycycline as with earlier studies[16,17]. Strains of MRSA circulating in the community generally are susceptible to a number of non beta-lactam antimicrobial agents, although resistance patterns may vary temporally and geographically[17]. Antibiotic choice is more crucial for management of these infections and should be guided by the prevalence rate and its antibiotic susceptibility profile in the community[17,18].

Conclusion

In Coimbatore city , MRSA was prevalent among pathogens of community acquired infections in children, In areas with high prevalence of methicillin resistance, even community-acquired, a glycopeptide-containing regimen should be started as initial empirical antibiotic therapy.

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Conflicts of interest: none
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Table -1: depicts antibiotic sensitivity

Drug	Sensitive	Resistant	Total cases tested	Percentage of sensitivity
Cefazolin	0	1	1	0%
Penicillin	4	46	50	0.08%
Ampicillin	1	4	5	20%
Ceftriaxone	1	1	2	50%
Co-trimoxazole	29	21	50	58.00%
Cloxacillin	23	15	38	60.53%
Gentamycin	33	17	50	66.00%
Erythromycin	28	14	42	67%
Rifampin	4	1	5	80.00%
Ciprofloxacin	44	6	50	88.00%
Cephalothin	35	9	44	88.63%
Clindamycin	39	5	44	88.63%
Doxycycline	50	0	50	100%
Levofloxacin	5	0	5	100%
Linezolid	50	0	50	100%
Vancomycin	50	0	50	100%

REFERENCE
1. Maple PA, Hamilton-Miller JM, Brumfitt W. Worldwide antibiotic resistance in methicillin resistant Staphylococcus aureus. Lancet. 1989;1:537–40. | 2. Davis SL, Perri M B, Donabedian SM, et al. Epidemiology and outcomes of community-associated methicillin-resistant Staphylococcus aureus infection. J Clin Microbiol. 2007 Jun;45(6):1705-11. | 3. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998;339:520–532. | 4. Dunkle LM, Naqvi SH, McCallum R, Lofgren IP. Eradication of epidemic methicillin-gentamicin-resistant Staphylococcus aureus in an intensive care nursery. Am J Med. 1981;70:455–458. | 5. Wijaya L, Hsu LY, Kurup A. Community-associated methicillin-resistant Staphylococcus aureus; Overview and local situation. Ann Acad Med Singapore 2006;35:479-86. | 6. Boxerbaum B, Jacobs MR, Cechner RL. Prevalence and significance of methicillin-resistant Staphylococcus aureus in patients with cystic fibrosis. Pediatr Pulmonol. 1988;4:159–163 | 7. Phakade R S, Nataraj G, Kuyare S K, Khopkar U S, Mehta P R. Is methicillin-resistant Staphylococcus aureus involved in community acquired skin and soft tissue infections?: Experience from a tertiary care centre in Mumbai. J Postgrad Med 2012;58:3-7. | 8. Thind P, Prakash S K, Wadhwa A, Garg V K, Pati B. Bacteriological profile of community-acquired pyodermas with special reference to methicillin resistant Staphylococcus aureus. Indian J Dermatol Venereol Leprol 2010;76:572-4. | 9. D'Souza N, Rodrigues C, Mehta A. Molecular characterization of Methicillin-resistant Staphylococcus aureus with emergence of epidemic clones of sequence type (ST)22 and ST 772 in Mumbai, India. J Clin Microbiol. 2010;48:1806–11. | 10. Patel AK, Patel KK, Patel KR, Shah S, Dileep P. Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. J Assoc Physicians India. 2010;58(Suppl):37–40. | 11. Gopalakrishnan R, Sureshkumar D. Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India 2010;58(Suppl):25–31. | 12. John CC, Schreiber JR. Therapies and vaccines for emerging bacterial infections: learning from methicillin-resistant Staphylococcus aureus. Pediatr. Clin. N. Am. 2006;53:699-713. | 13. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant Staphylococcus aureus. Lancet 2006;367:731-739. | 14. Charlebois ED, Perdreau-Remington F, Kreiswirth B, et al. Origins of c ommunity strains of methicillin-resistant Staphylococcus aureus. Clin Infect Dis 2004;39:47-54. | 15. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton- Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 1999;29:1128-32. | 16. Pichereau S, Rose WE . Invasive community-associated MRSA infections: epidemiology and antimicrobial management. Expert Opin Pharmacother. 2010 Dec;11(18):3009-25. | 17. Rossini CJ, Moriarty KP, Tashjian DB, Garb JL, Wait RB. Geographic distribution of community-acquired methicillin-resistant Staphylococcus aureus soft tissue infections. J Pediatr Surg. 2011 Jun;46(6):1089-92. | 18. Odell CA. Community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) skin infections. Curr Opin Pediatr 2010 Jun;22(3):273-7. |

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Parental Perception on Pediatric Obesity- Implications on Early Recognition of Obesogenic Factors and Management.

KEYWORDS: parental perception , child hood obesity, body mass index.

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ABSTRACT *OBJECTIVES: This study elicited parental perception of obesity in children as well as their understanding of the risk factors and morbidity associated.*

METHODOLOGY: Parents of children aged between 2 – 15 years with body mass index (BMI) more than 85th percentile for the age and sex according to Center for Disease Control (CDC) growth charts were chosen. Informed written consent was obtained. Child's height, weight and BMI were noted. Parents were administered a self reported Questionnaire, which included items in 3 areas namely Perception and concern about child's weight, child eating behavior and issues on weight control.

RESULTS: We analyzed the responses from parents of 60 children with BMI more than 85th percentile. Sex ratio male (53%): female (47%). Mean age -10 years, Median BMI is 28. 78% of the parents perceived their child as overweight and mothers perceived better than fathers. Of the 78%, 68% were obese and 10% were overweight. Parents attributed overeating (21%), reduced physical activity including television watching (30%) and familial (11%) for the cause of obesity. Parents were aware of complications to be hypertension (27%), diabetes (19%) and cardiac problems (21%). 45% of the parents were willing to adapt life style modification recommended for their children. Parents practiced some measures (diet - 20% & exercise -29%) to reduce weight whereas 19% believed only in medical treatment. Despite the awareness of obesity and its complications 21% of the parents were not taking any steps to reduce their ward's weight. Inconsistency in diet and physical activity (39%) were the common problems parents encountered while controlling child's weight.29% of the parents believed it is mother's responsibility to reduce child's weight and only 16% of the parents understood that it is a team effort involving parents, doctors and children.

CONCLUSION: Though 3/4th of the parents were aware about their child's overweight and the complications, their attitude and practices towards weight reduction were limited. As parents play a crucial role in preventing childhood obesity, education and motivation of the parents through family health education is essential.

Introduction

Obesity is one of the most prevalent forms of malnutrition. As a chronic disease, it is prevalent in both developed and developing countries and affecting children as well as adults. It is now so common that it is replacing the major traditional public health concerns including under nutrition.

The World Health Organization (WHO) estimates globally over 40 million under 5 children are overweight in 2011[1]. The International Obesity Task Force review of obesity in children and young people has documented the rise in prevalence of overweight and obesity from 102 countries using their own conservative definitions. The latest survey in India was conducted during 2007-2008 revealed that: 15.2 percent of boys and 14.4 percent of girls were overweight,5.4 percent of boys and 3.9 percent of girls (aged 2-17) were obese.[2] The same trend is noted in globally also: 32% were overweight in the Americas, including 8% who were obese; the corresponding prevalence was 20% and 4% in Europe; 16% and 6% in the Near and Middle East; and 5% and 1% in the Asia-Pacific region.[2]

A recent study conducted among 24,000 school children in south India showed that the proportion of overweight children increased from 4.94 percent of the total students in 2003 to 6.57 percent in 2005 demonstrating the time trend of this rapidly growing epidemic. At least 30% of obesity begins in childhood. Childhood obesity itself a predictor of adult obesity and morbidities due to it[4]. In children, a life style behavior which contributes to and perpetuates obesity is more open to change. Early reorganization and adequate treatment of this will prevent the globalization of obesity. Intervening with families is significant as parents play a crucial role in bringing a change. Therefore it is important to know the parental views about childhood obesity, their knowledge levels on obesogenic factors, complications and management. It is also beneficial for families with overweight children to seek professional help on time.

There have been a number of surveys[5-9] addressing attitudes and practices towards childhood obesity in western populations. However, studies related to parental attitudes and practices towards childhood in Indian population is lacking. Since the attitudes towards children are likely to be very different compared with adults, their management also varies. So we designed and conducted a study in our pediatric unit, on children of Body Mass Index (BMI) more than 85th percentile to assess the parental perception of their obesity.

Materials and methods

This Prospective cross sectional study was done in PSG hospitals Coimbatore. Parents of children aged between 2 – 15 years with body mass index (BMI) more than 85th percentile for the age and sex according to Center for Disease Control (CDC) growth charts were chosen. Child with obesity due to any known risk factors like drugs and syndromes were excluded. Informed written consent was obtained. Child's height, weight and BMI were noted. Parents were administered a self reported Questionnaire, which included items in 3 areas namely Perception and concern about child's weight, child eating behavior and issues on weight control. Descriptive statistics were used in this study.

Results

We analyzed the responses from parents of 60 children with BMI more than 85th percentile. The Sex ratio: males (53%): females (47%). Mean age -10 years, Median BMI is 28. Of the 60 children 54 were obese and 6 were overweight. The correct perception rate was 78% (either of the parents or care givers). The correct perception rate was more in mothers(46%) than fathers(27%).

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Table – 1 shows perception rate .

S.no	Category	Father	Mother	Others
1.	Perceived child's overweight correctly (78%)	27%	46%	5%
2.	Not perceived correctly (22%)	5%	17%	0%

The BMI results in percentage among the study population were shown in Table-2.

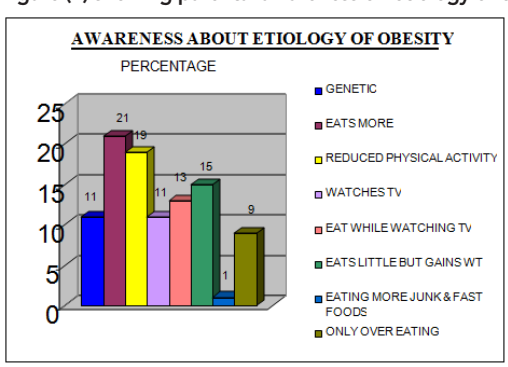
Table (2) showing the BMI results among the study population: (n=60)

S. no	Category	Obese	Overweight
1.	BMI of the children who are perceived correctly by their parents (78%)	68%	10%
2.	BMI of the children who are not perceived correctly by their parents (22%)	22%	0%

Knowledge about etiology

We studied about parent's/care givers knowledge about etiological factors for obesity in children. 10% of the respondents stated that only overeating causes obesity in children. Remaining was aware that more than one risk factor for causing obesity. 11% of the participants attributed obesity to genetic factors.

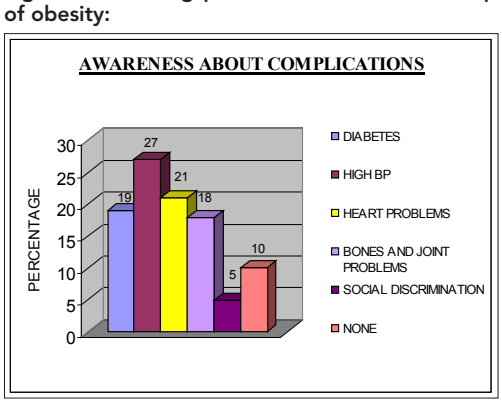
Figure (1) showing parental awareness on etiology of obesity:



Complications of obesity

When enquired about the complications of obesity in children, many were aware the common complication such as diabetes (19%), hypertension (27%), cardiac problems (18%). 10 % of the participants said that there was no major complications related to obesity (figure-2). Only 20% of parents were aware that their child can become obese adult in future.

Figure -2, showing parental awareness on complications of obesity:



Measures taken to reduce obesity

We evaluated the participant's attitude towards preventing obesity. None said that child's weight cannot be controlled and most of them thinks that it is mother's (29%) responsibility to control weight (figure-3). 16 % of participants felt that it is a team effort. While attempting to reduce their ward's weight 74% of participants encountered some difficulties. Consistently controlling the diet (39%) was the most difficult task, followed by restriction of television watching(32%) and increasing physical activity(25%). 10 % of parents felt that even after following physicians recommendations there was no significant improvement in weight reduction. 17% felt that child itself also has responsibility in weight reduction. However, 55% of the parents think that it gives good results if they follow the lifestyle changes recommended for children.

Figure -3, showing the attitude on responsibility in controlling obesity:

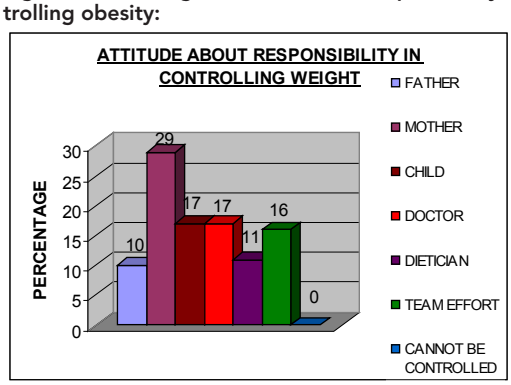
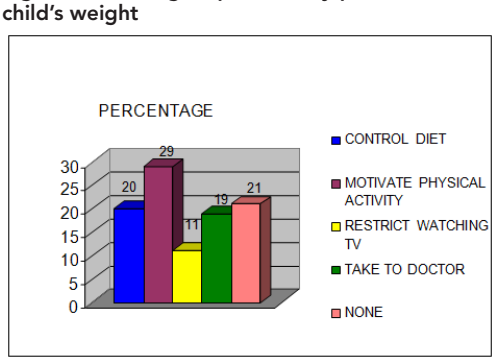


Figure -4 showing steps taken by parents to reduce their child's weight



Discussion

Obesity in children and adolescents is a serious issue with many health and social consequences that often continue into adulthood. Implementing prevention programs and getting a better understanding of treatment for children is important to controlling the obesity epidemic. Many parents are rightly concerned about their child's weight and how it affects them. They look for specific answers for prevention and treatment options. Unfortunately, the state of the science is a lot less precise than we would like. The first and foremost step in preventing obesity is early recognition of it among parents. The parents should acknowledge obesity in their wards for optimal control measures.

In children, the normal range for BMI changes with age and gender. We used the Centers for Disease Control (CDC)[10] definition; a child whose BMI is between the 85th and 95th percentile for age as being "at risk" for obesity. A child who has a BMI at or above the 95th percentile for age is considered obese.

In our study, about 78% of the parents were aware their child was overweight and mothers perceived better than others. 74% are taking steps to control weight. These results are in

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contrary with earlier studies [6,7,8] where about only 1/4th of obese children were perceived correctly. The high level of correct perception may be due to the sample selection as samples taken from a territory care teaching hospital. Routine nutritional assessment could have sensitized the parents. Among 22% parents those who have not perceived correctly, all children were obese. The inability to recognize it clearly reflects their reluctant to acknowledge concern for their obese children.. This is because overweight children are identified as healthier and better nourished and perceived as "better eaters. It also carries the risk of perceiving overweight child as normal and normal weight child as underweight child. BMI results showed obese children are perceived more correctly than overweight children. Our study findings also showed parents are aware of eating behavior and reduced physical activity. But they fail to recognize junk food and fast foods as a factor which also contributes to obesity.

Complications are well known to them mainly as hypertension and cardiac problems than other problems. Unfortunately 10% were not aware of complications. Most of them think its mothers responsibility to control weight and only 15% have understood it is a team effort of all involving parents, children, doctor and dietician. The parents are taking measures like motivating physical activity, control diet and restrict watching television to reduce their child's weight. Parents are the most important role models for children. Half of the parents are ready to adapt the life style changes recommended for their children as they think it will give better results. 25% of the parents are not facing any problem in reducing weight whereas 40% of them are encountering inconsistency in maintaining diet as the major problem.

Teaching healthy behaviors at a young age is important since change becomes more difficult with age. Behaviors involving physical activity and nutrition are the cornerstone of preventing obesity in children and adolescents[11]. Families and schools are the two most critical links in providing the foundation for those behaviors.

An important part of treating obesity among children and adolescents is for parents and healthcare professionals. Health care professionals' lack of attention to weight related concerns in childhood may be contributing to parental misperceptions of children's weight status. Involvement of the entire family is also a motivating factor. Weight control programs that involve both parents and the child have shown improvement in long-term effectiveness compared to directing the program only to the child [11]. This study has following limitations;1. Small sample size, 2. Not a community based study, 3.parents educational status were taken for analysis

Conflicts of interest: none
Data collection was sponsored by ICMR(STS –study).

REFERENCE

1. Obesity and overweight,Fact sheet N°311(Updated March 2013). http://www.who.int/mediacentre/factsheets/fs311/en. | 2. Khadilkar VV, Khadilkar AV, Cole TJ, Chiplonkar SA, Deepa Pandit. Overweight and obesity prevalence and body mass index trends in Indian children. International Journal of Pediatric Obesity, Early Online 2010.1-9. | 3. Raj M, Sundaram KR, Paul M, Deepa AS, Kumar RK. Obesity in Indian children: time trends and relationship with hypertension. Natl Med J India 2007;20:288-93. | 4. Styne DM. Childhood and Adolescent obesity PCNA 2002;48:823-847. | 5. Taveras EM, Gortmaker SL, Mitchell KF, Gillman MW. Parental perceptions of overweight counseling in primary care: the roles of race/ethnicity and parent overweight.Obesity (Silver Spring). 2008 Aug; 16(8):1794-801. Epub 2008 May 22. | 6. Nsiah-Kumi PA, Ariza AJ, Mikhail LM, Feinglass J, Binns HJ, Pediatric Practice Research Group. Family history and parents' beliefs about consequences of childhood overweight and their influence on children's health behaviors.Acad Pediatr. 2009 Jan-Feb; 9(1):53-9. | 7. Eckstein KC, Mikhail LM, Ariza AJ, Thomson JS, Millard SC, Binns HJ, Pediatric Practice Research Group.Parents' perceptions of their child's weight and health.Pediatrics. 2006 Mar; 117(3):681-90. | 8. Carnell S, Edwards C, Croker H, Boniface D, Wardle J. Parental perceptions of overweight in 3-5 y olds.Int J Obes (Lond). 2005 Apr; 29(4):353-5. | 9. He M, Evans A. Are parents aware that their children are overweight or obese? Do they care? Can Fam Physician. September 2007;53(9):1493–1499. | 10. Barlow SE and the Expert Committee. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics 2007;120 Supplement December, S164—S192. | 11. Waters E, de Silva-Sanigorski A, Burford BJ, Brown T, Campbell KJ, Gao Y, Armstrong R, Prosser L, Summerbell CD. Interventions for preventing obesity in children. Cochrane Database of Systematic Reviews 2011, Issue 12. Art. No.: CD001871. DOI: 10.1002/14651858.CD001871.pub3. |

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Risk of intussusception after monovalent rotavirus vaccine (Rotavac) in Indian infants: A self-controlled case series analysis

The INCLEN Intussusception Surveillance Network Study Group

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ABSTRACT

Background: An association between rotavirus vaccination and intussusception has been documented in post-licensure studies in some countries. We evaluated the risk of intussusception associated with monovalent rotavirus vaccine (Rotavac) administered at 6, 10 and 14 weeks of age in India.

Methods: Active prospective surveillance for intussusception was conducted at 22 hospitals across 16 states from April 2016 through September 2017. Data on demography, clinical features and vaccination were documented. Age-adjusted relative incidence for 1–7, 8–21, and 1–21 days after rotavirus vaccination in children aged 28–364 days at intussusception onset was estimated using the self-controlled case-series (SCCS) method. Only Brighton Collaboration level 1 cases were included.

Results: Out of 670 children aged 2–23 months with intussusception, 311 (46.4%) children were aged 28–364 days with confirmed vaccination status. Out of these, 52 intussusception cases with confirmed receipt of RVV were included in the SCCS analysis. No intussusception case was observed within 21 days of dose 1. Only one case occurred during 8–21 days after the dose 2. Post-dose 3, two cases in 1–7 days and 7 cases during 8–21 days period were observed. There was no increased risk of intussusception during 1–7 days after the doses 1 and 2 (zero cases observed) or dose 3 (relative incidence [RI], 1.71 [95% confidence interval {CI} 0.0–5.11]). Similarly, no increased risk during 8–21 days after the dose 1 (zero cases observed), dose 2 (RI, 0.71 [95% CI, 0.0–3.28]) or dose 3 (RI, 2.52 [95% CI, 0.78–5.61]). The results were similar for 1–21 day periods after the doses separately or pooled.

Conclusions: The risk of intussusception during the first 21 days after any dose of rotavirus vaccine (Rotavac) was not higher among the Indian infants than the background risk, based on limited SCCS analysis of 52 children.

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1. Introduction

To prevent diarrhoea related deaths, 107 countries (103 nationally and 4 sub-nationally) have introduced rotavirus vaccine (RVV) into their national immunization program (NIP) as of April 2020 [1]. The impact of RVV on the illness episodes, hospitalisations and deaths due to rotavirus and all-cause diarrhoeas has been documented from post-licensure studies from various countries [2–5].

Following the increased risk of intussusception documented with the first licensed rotavirus vaccine (RotaShield™, Wyeth-Lederle Laboratories) [6] and its withdrawal, all the clinical trials of RVV captured intussusception as an adverse event. No increased risk of intussusception was observed in large scale multicountry clinical trials of two RVVs (Rotarix™, RV1; GlaxoSmithKline Biologicals and RotaTeq™, RV5; Merck & Co, Inc) [7,8]. However, several postlicensure studies have identified some increased risk of intussusception during 1–7 days after the first (relative risk, RR: 5.3–9.9) and second (RR: 1.3–2.8) doses of these two RVVs in different countries (Mexico, Brazil, Australia, the United Kingdom, and the United States) [9–11,13,14]. Notably, the impact of RVV on morbidity and mortality outweigh the risk of intussusception and associated mortality [15].

Intussusception incidence varies widely across the countries [16]. In India, information on incidence of intussusception is lim-

Abbreviations: CRF, Case record form; CAC, Case Adjudication Committee; CI, Confidence interval; ICD, International Classification of Diseases; IQR, Interquartile range; LMIC, Low and middle income countries; MIC, Middle income countries; NIP, National immunization programmes; SCCS, Self-controlled case series; RI, Relative incidence; RR, Relative risk; RV1, Monovalent rotavirus vaccine (Rotarix™); RV5, Pentavalent rotavirus vaccine (Rotateq™); RVV, Rotavirus vaccine; TAG, Technical Advisory Group; WHO, World Health Organization.

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ited and it varies from 17.7 (Delhi, North India) to 254 (Vellore, South India) cases per 100,000 child-years [17]. Although the exact causes of the variation in intussusception rate remain unknown, ethnicity, dietary pattern, breastfeeding practices, gut microbial environment, and vertical transmission of rotavirus antibodies have been proposed as the possible risk factors [16].

Out of the four RVVs licensed in India, two Indian (Rotavac™, RV1-116E; Bharat Biotech and Rotasill™, RV5; Serum. Institute of India) have undergone efficacy trials in India and remaining two (Rotarix™ and RotaTeq™) were licensed based on evidence from other countries. Clinical trials of the Indian-manufactured rotavirus vaccines enrolled 6799–7500 infants each and had inadequate sample size for documenting the risk of intussusception [18,19]. Under the NIP, India introduced Rotavac™ in March 2016 and Rotasill™ in April 2018 in different states in a phased manner [20]. All four licensed vaccines are being used in private sector. In view of the variable risks of intussusception after RVV doses across the countries, it is necessary to generate evidence from the Indian context to sustain confidence of the professionals and public on the vaccine and program.

As part of the vaccine safety surveillance linked to RVV introduction in India, a nationally representative sentinel surveillance network was established. We used the self-controlled case series (SCCS) method to evaluate the risk of intussusception following Rotavac vaccination in Indian infants.

2. Methods

2.1. Study area and participating hospitals

This active prospective surveillance was conducted during April 2016 to September 2017 at 23 tertiary care hospitals across 20 cities and 17 states in India representing different regions. The list and location of these hospitals are given in Supplementary Figure SF1. These hospitals were selected through a systematic process and the study protocol has been published earlier [21].

2.2. Case recruitment and data collection

Children aged >1 month and <24 months admitted to these hospitals were screened to identify the suspected cases (any of these diagnoses: intussusception, intestinal obstruction- acute or subacute, acute abdomen, pain abdomen, abdominal distension, and blood in stool with vomiting). These suspected cases tracked for final diagnosis and confirmed intussusception cases meeting the Brighton collaboration level 1 criteria for diagnostic certainty were recruited. The cases were recruited irrespective of the immunization exposure and data availability. For the recruited cases, data on clinical features, investigations, treatment, outcome, and socio-demography were obtained from the hospital records and parent interview. Immunization data were collected from the immunization cards and a copy of which was obtained, whenever possible. An independent Case Adjudication Committee (CAC), with a paediatrician, paediatric surgeon, and radiologist as members, reviewed the documents to assign the diagnostic certainty levels, according to Brighton Collaboration criteria [22]. Independently the suspected and intussusception cases were verified from the medical records using diagnoses and/or International Classification of Diseases (ICD) codes (ICD-9/10, codes listed in Supplementary Table ST1), to identify any missed cases.

2.3. Statistical analysis

We used the SCCS method to estimate the age-adjusted relative incidence of intussusception for the periods 1–7, 8–21 and 1–21 days after each dose of the RVV among the children aged 28–364 days at onset of symptoms (primary analysis). In the SCCS method, each case acts as its own control and the frequency of the outcome (intussusception) that occurred in the exposure periods after vaccination is compared with the frequency in the unexposed period [23,24]. As no external controls are used, this method automatically controls for time-invariant confounding [25]. The SCCS has been used in several studies investigating the risk of intussusception with RVV vaccine [9–11,14,23,26–29] with the pseudo-likelihood method [30]. We limited the analysis to the first occurrences of intussusception that met the level-1 Brighton collaboration criteria and children aged 28–364 days at the onset of symptoms, considering the minimum and maximum ages at which RVV could be given in India. Days 22 days after the RVV dose till 364 days of age were considered as the control period. Onset of first compatible symptom was considered the date of intussusception onset. Cases with confirmed vaccine exposure information were included in analysis and those without vaccination card were excluded. The relative incidence (RI) for each case was calculated comparing the incidence of intussusception within the risk window with the incidence in all other observation widows using conditional Poisson regression. Because the background incidence of intussusception varies substantially by age in the first year of life, age was controlled using 14-day intervals in the model and confidence intervals (CIs) were derived by bootstrapping with 1000 iterations and all cases were included. Sensitivity analysis was done considering the admission date as the date of intussusception onset. P values < 0.05 were considered to be statistically significant and all reported P values are two-sided. Statistical analysis was performed using STATA version 15.0 (StataCorp LLC, Texas, USA).

2.4. Sample size

To detect a relative incidence of 1.75 within 1–7 days of after any of the 3 RVV doses with 80% power and 95% confidence level, the sample size needed was 220 using the signed root likelihood ratio [31].

2.5. Ethical issues

Informed written consent was obtained for all the eligible cases from parent or legal guardian before recruitment and data collection. Confidentiality in data handling was maintained. The study protocol was reviewed and approved by all the institute ethics committees of participating institutes.

3. Results

A total of 670 children aged >1 month and <24 months with intussusception were recruited at these study sites. Among these, 420 (62.7%) children were aged 28–364 days. Out of the infants, 86.4% (364/420) had vaccine exposure information and 80.5% (338/420) had the first episode of intussusception. The 27 (4%) children who resolved with conservative management (i.e. no radiologic or surgical intervention) were excluded. The 19 (2.8%) children who received either Rotarix™ or Rotateq™ were also excluded. Thus data for 311 children (52 with and 259 without RVV) were analysed. The Fig. 1 shows the selection of cases for SCCS analysis.

There were no significant differences in the age at onset of symptoms, gender and interval between the onset and hospitalisation between the cases included in analysis and excluded (Supplementary Table ST2). The median age of cases was 28 weeks (interquartile range [IQR], 22–35 weeks) and 68.2% were boys (Supplementary Table ST2). Vomiting (78.8%), blood in stool (71.1%), excessive crying (62.4%) and abdominal pain (49.8%) were

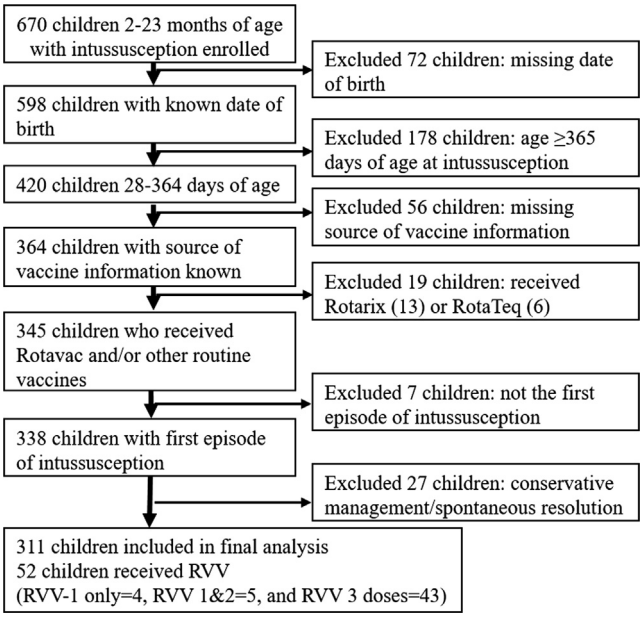


Fig. 1. Flow of case selection for the self-controlled case series analysis. *Note: RVV: Rotavirus vaccine.*

the commonest presenting features (Supplementary Table ST3). The median interval between onset of symptoms and hospitalization was 3-days (IQR, 2–6 days). Overall, RVV was received by 52 (16.7%) cases including three doses by 43, two doses by 5 and one dose by 4 cases. Among the 53 cases recruited from the five sites from states in Phase-1 RVV national introduction, 34 (64.1%) received RVV-1. Out of the 258 children recruited from the 15 sites in the states without RVV in the NIP, 18 (7%) received RVV-1. The median ages at intussusception were comparable for the children who received RVV (26.5 weeks; IQR 19.5–32.5 weeks) and who did not receive (28 weeks; IQR 22–36 weeks) (Supplementary Table ST5). The median ages of vaccination in children with and without RVV were comparable; dose 1 (with RVV: median age 7.1 weeks, IQR 6.6–8.8 weeks and without RVV: median age 7 weeks, IQR 6.6–8.4 weeks), dose 2 (with RVV: median age 12 weeks, IQR 11.3–13.8 weeks and without RVV: median age 12.3 weeks, IQR 11.3–14.1 weeks) and dose 3 (with RVV: median age 16.6 weeks, IQR 15.7–18.3 weeks and without RVV: median age 17.1 weeks, IQR 16–19.1 weeks). The Fig. 2 shows the ages at vaccination for the children with RVV and the ages of vaccination for children without RVV is given as Supplementary Figure SF2. There was no significant difference in the characteristics between the children who did or didn't receive RVV (Supplementary Table ST5).

After the first RVV dose, no case of intussusception was observed during the 1–7 days and 8–21 days risk periods (Fig. 3A). After the second RVV dose, no case of intussusception during the 1–7 days and one case during 8–21 days risk periods were observed (Fig. 3B). After the third RVV dose, two cases of intussusception during the 1–7 days and seven cases during 8–21 days risk periods were observed (Fig. 3C). One case occurred on day zero after RVV dose 3.

Table 1 shows the RI estimates using the date of symptom onset (primary analysis) and date of admission (sensitivity analysis) for intussusception in different risk periods (1–7 days or 8–21 days or 1–21 days) after the RVV doses compared to the background risk. There was no increased risk of intussusception during 1–7 days after the first and second doses (no cases observed) or third dose (relative incidence, RI, 1.03; 95% CI 0–5.11) of RVV. Similarly, no increased risk of intussusception was observed during 8–21 risk period after the first dose (no case observed), second dose (RI 0.71; 95% CI 0–3.28) and third dose (RI 2.52; 95% CI 0.78–5.61) of RVV. There was no increased risk of intussusception observed for any RVV dose at pooled level for 1–7, 8–21 and 1–21 days risk periods.

4. Discussion

This study was conducted at 20 sites as part of the vaccine safety surveillance linked to rotavirus vaccine introduction. Out of the 20 sites, five sites were in the states where the RVV was introduced in Phase-1 of the national introduction. According to the WHO and UNICEF estimates of immunization coverage using the administrative report for 2017, the RVV (Rotavac™) coverage in these Phase-1 states was 69% [32]. The RVV coverage (64.1%) among the study subjects from the study sites in Phase-1 states was comparable to the administrative coverage data. Out of the

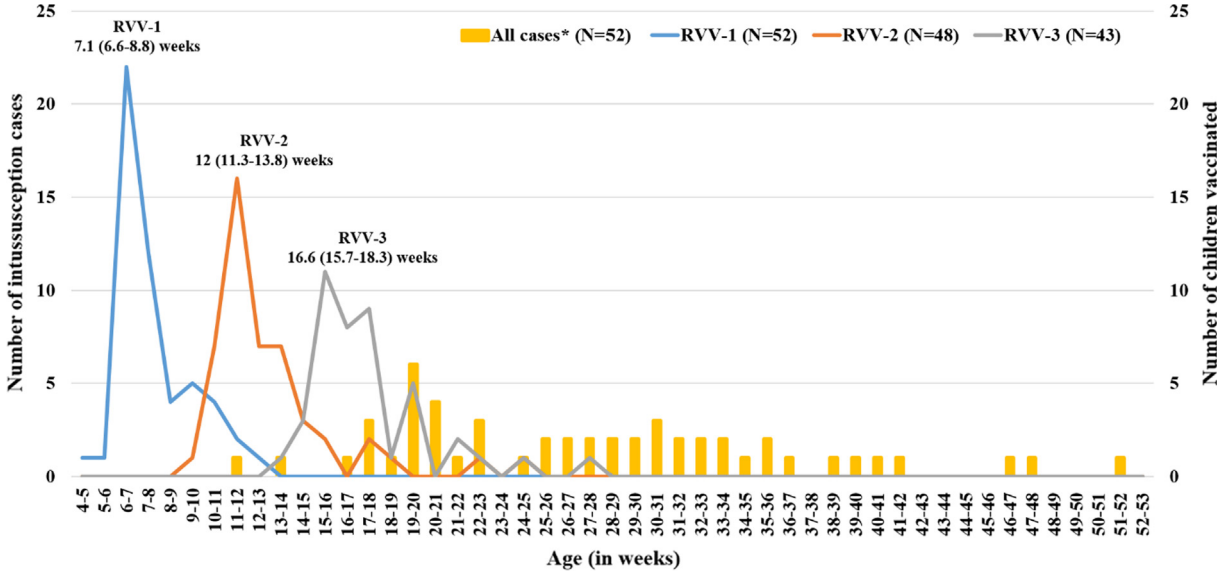


Fig. 2. The age at rotavirus vaccination and occurrence of intussusception during the first year. *Note: The ages at rotavirus vaccination given in weeks as median with interquartile range (IQR); RVV-1: Rotavirus vaccine first dose; RVV-2: Rotavirus vaccine second dose; RVV-3: Rotavirus vaccine third dose.*

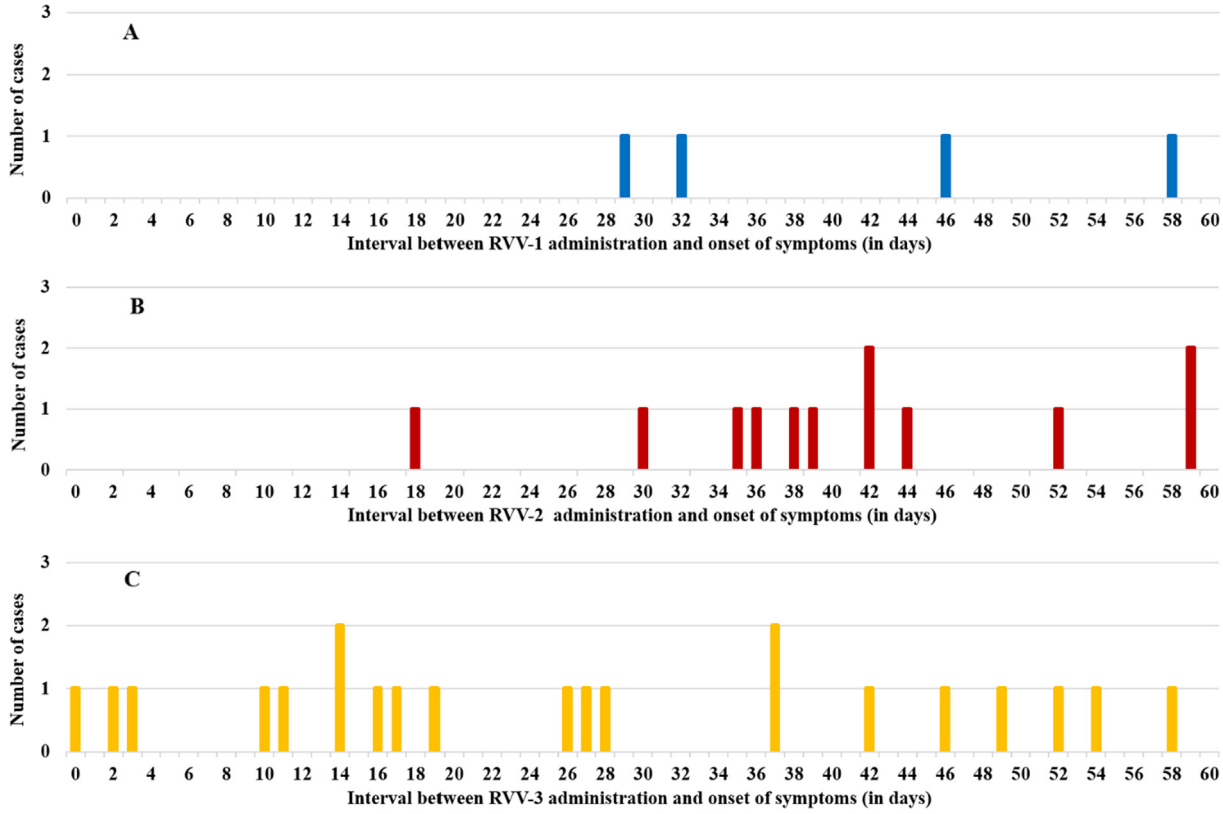


Fig. 3. The number of intussusception cases in the first 60 days after rotavirus vaccine dose 1 (3A), dose 2 (3B) and dose 3 (3C). *Note: RVV-1: Rotavirus vaccine first dose; RVV-2: Rotavirus vaccine second dose; RVV-3: Rotavirus vaccine third dose.*

Table 1
Relative incidence of intussusception in the risk periods after the rotavirus vaccine in Indian infants.

Dose	Risk period	Primary analysis*				Sensitivity analysis**			
		IS cases (n)	Relative incidence (RI with 95% CI)@			IS cases (n)	Relative incidence (RI with 95% CI)@		
	1–7 days	0	0.00	0.00	0.00	0	0.00	0.00	0.00
Dose-1	8–21 days	0	0.00	0.00	0.00	0	0.00	0.00	0.00
	1–21 days	0	0.00	0.00	0.00	0	0.00	0.00	0.00
Dose-2	1–7 days	0	0.00	0.00	0.00	0	0.00	0.00	0.00
	8–21 days	1	0.71	0.00	3.28	1	0.74	0.00	3.66
	1–21 days	1	0.50	0.00	2.37	1	0.52	0.00	2.59
Dose-3	1–7 days	2	1.71	0.00	5.11	3	2.56	0.00	6.92
	8–21 days	7	2.52	0.78	5.61	6	2.11	0.61	4.72
	1–21 days	9	2.23	0.81	4.58	9	2.20	0.88	4.41
All 3-doses	1–7 days	2	1.03	0.00	3.14	3	1.61	0.00	4.43
	8–21 days	8	1.65	0.61	3.49	7	1.46	0.51	3.15
	1–21 days	10	1.48	0.67	3.02	10	1.50	0.70	2.92

Notes: Among children aged 28–364 days; Risk period is number of days prior to the reference date; IS: Intussusception; RI: Relative Incidence.
* Using the symptom onset date for calculating the risk periods.
** Using the admission date as onset of intussusception for calculating the risk periods.
@ 95% confidence intervals bootstrapped with 1000 iterations.

258 children recruited from other 15 sites (from the states without RVV in the NIP), 18 (7%) children received RVV. We did not observe an increased risk of intussusception after any of the doses of the monovalent 116E RVV (Rotavac™) in Indian infants. No clustering of the intussusception cases in either 1–7 days or 8–21 days or 1–21 days risk windows after any RVV doses was observed. However, the study was under powered due to the low number of intussusception cases having received RVV (n = 52).

Our results are comparable to the reports of intussusception after RV1 (Rotarix™) vaccination in several African countries (Ethiopia, Ghana, Kenya, Malawi, Tanzania, Zambia, Zimbabwe and South Africa), where no increased risk of intussusception after any dose of RV1 were observed [23,28]. However, increased risks of intussusception were documented in the 1–7 days following dose 2 of RV1 in Brazil (IR 2.6; 95% CI 1.3–5.2; OR 1.9; 95% CI 1.1–3.4) and after dose 2 (IR 5.3; 95% CI 3.0–9.3 and OR 5.8; 95% CI 2.6–13) and dose 1 (IR 6.49; 95% CI 4.17–10.09) in Mexico [9,10]. Similarly, increased risks of intussusception were documented in high income countries, United States (first dose, RR 7–8.8; second dose, RR 1.8–8.1), United Kingdom (first dose, RR 13.8; second dose, RR 2.2), and Australia (first dose, RR 6.8; second dose, RR 2.8) in the 1–7 day period with RV1 vaccine [11-14]. Increased risks of intus-

susception were documented in high income countries in United States (first dose, RR 2.6–9.1; second dose, RR 1.8; third dose, RR 1.2–2.2), Finland (first dose, IRR 2) and Australia (first dose, RR 9.9; second dose, RR 2.8) in the 1–7 day period with RV5 vaccine (Rotateq™) [11–13,29]. In Singapore (first dose, RR 8.3; second dose, RR 3.0)and Spain (first dose, RR 4.7; second dose, RR 1.6) also the intussusception risk was higher where both RV1 and RV5 were used [26,27]. The RV1 doses are administered at 2 and 3 or 4 months schedule. The RV5 doses are administered at 2, 4 and 5 or 6 months schedule.

Although the exact reasons of increased risk of intussusception with RVV in some countries but not in others are not clear, some possible factors like age at vaccination, coadministration of oral polio vaccine (OPV), gut replication of RVV virus, intestinal microbiota environment, breastfeeding and dietary patterns, maternal antibody transfer and ethnicity may be considered [33]. In this study, the median age of the intussusception cases is 28 weeks (IQR, 22–35 weeks), which is comparable to the reports for Indian infants without RVV exposure [34–36]. In this study no case was observed before 6 weeks, one case before 10 weeks and 11 cases before 14 weeks of ages. This observation is similar to that from African countries [23,28]. The median or peak ages of intussusception in children from the countries with increased risk were also comparable to the Indian and African children. It was interesting to note that the countries following 6 and 10 weeks (African countries) and 6, 10 and 14 weeks (India) schedules have no or lower risk of intussusception [23,28]. But the countries following 2 and 3 months or 2 and 4 months schedules for RV1 and 2, 3 and 5 months or 2, 4 and 6 months schedules for RV5 had higher risk of intussusception [9–14,26–29]. Risk of intussusception was highest in 1–7 days after the first dose or either RV1 or RV5. In this study, we observed one case on day-zero of RVV dose-3. Apart from the schedule, while no increased risk of intussusception was observed in the low and middle income countries (LMICs) from Africa and India, the middle income countries (MICs) from Latin America and developed countries from North America, Europe and Australia had increased risks of intussusception. So, the earlier age of administration of first RVV dose may have lower risk of intussusception, when the background occurrence is low. The RVV administered at 10–14 weeks schedule was observed to have higher seroconverion compared to 6–10 weeks schedule [37]. In India and the African countries OPV is coadministered with RVV. In this study, 47 children received RVV and OPV simultaneously. The co-administration of RVV and OPV was observed to have lower rotavirus seroconversion in South Africa, Bangladesh and Chile infants [37–39]. The lower seroconversion may be due to suboptimal RVV virus replication in the intestine.

Lower efficacy (49.7–64.5%) in clinical trials and effectiveness (18–69%) of the RVVs have been observed in LMICs from Africa, Latin America and Asia compared to the middle and high income countries [5,40]. The lower immune responses to the RVVs may be due to lower replication of the RVV viruses in the intestine of infants in LMICs, which is reflected in lower fecal shedding [33]. The lower replication of RVV viruses may be dependent on the intestinal microbiota and other competing microbes of the infants in these LMICs [33,41]. The intestinal replication of virus may be influenced by the breastfeeding pattern, immunoglobulin and non-specific antibodies content in the breastmilk, dietary pattern, environmental sanitation and nutritional status of the infants [33,41]. Apart from these the maternal infection with rotavirus and vertical transfer to infants may also affect the vaccine virus replication, and thereby the immunogenicity and risk of intussusception [33,41]. The variation in the intussusception rates across the different regions globally may also be due to genetic and/or ethnic risk factors [16,41]. With these possible factors influencing the intussusception risk after RVV, the subnational variations of the risks in India should also be explored, considering the wide variation in the dietary, sociocultural practices, sanitation and environmental risk factors.

Our study had some limitations. First, the number of children who received RVV was small and is underpowered to observe significant risk of intussusception. However, we did not observe any case of intussusception during 1–7 days after the first and second doses of RVV, which was reassuring. There were two cases of intussusception (ages 18.2 and 25.3 weeks) during 1–7 days after third dose of RVV, which overlaps with the age of natural occurrence of intussusception in infants, even without RVV. The observations are similar to a recent report from India on intussusception after Rotavac, where out of 104 intussusception cases, none occurred during 1–7 days after RVV1 and RVV2 while one case occurred during 1–7 days after RVV3. The relative incidences of intussusception during 1–21 days after RVV1 and RVV2 (RI: 1.56; 95% CI: 0.0–5.28) and any RVV dose (RI: 1.51; 95% CI: 0.58–3.23). The findings from our study are comparable to observations from this report [41,42]. Second, only cases from selected hospitals were included and no definite catchment area or population base could be ascertained to derive the incidence rate. Third, all the children with intussusception were recruited at the sites irrespective of the immunization exposure status. Several of the infants were from outside the city and their parents were not carrying the vaccine cards with them. We surveillance team tried to collect the vaccination information to their best. The vaccine exposure information was available for 78.4% of infants, which corroborates with the immunization coverage status of infants in India and there was no significant difference in the age distribution between the infants who were included in analysis and those who were not. The key parameters for the vaccinated and unvaccinated infants appeared similar. We had study sites across 17 states of India and had mix of public and private hospitals and we believe that the findings are generalizable to other areas of India.

In conclusion, this SCCS analysis did not observe any increased risk of intussusception in the 1–7 and 8–21 days periods after the any dose of the oral monovalent 116E RVV (Rotavac™) among Indian infants compared to the background risk. This observation is similar to the risks observed among African infants following oral RV1 administration from post-licensure studies [23,28]. Although no increased risk of intussusception with the RVV in Indian infants is encouraging, continued documentation over longer period and sub-national risk assessment should also be considered. Such evidence will also be useful for other counties in the region and globally considering use of these RVVs for preventing diarrhoea morbidity and deaths.

5. Consent for publication

Not applicable.

6. Availability of data and materials

All data is available with the investigators and can be provided by the corresponding author upon reasonable request.

7. Disclosure statement

None. There is no financial interest or benefit for the authors arisen from this project or its direct application.

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9. Authors' contributions xxx

MKD and NKA conceptualised the framework for the study protocol, training, data analysis and interpretation. All TAG members provided input for finalisation of the study protocol and provided quality assurance oversight. All site investigators supervised the data collection at respective study site institutes. BG and AS coordinated the data collection and collation. MKD, RR, JET and BG analysed the data. MKD and RR wrote the first draft of the manuscript. JET and UP reviewed and revised the manuscript. All authors reviewed, provided critical input and approved the final version.

10. Disclaimer

The content represents the views of the authors alone and do not necessarily represent the official positions of their organizations, World Health Organization or Ministry of Health and Family Welfare, Government of India or the US Centers for Disease Control and Prevention or Bill and Melinda Gates Foundation.

CRediT authorship contribution statement

Manoja Kumar Das and Narendra Kumar Arora: Study conceptualisation, study design, protocol development, training, data analysis, interpretation, manuscript preparation.

Bini Gupta and Apoorva Sharan: Study coordination, monitoring, data cleaning, data analysis.

Ramesh Poluru, Jacqueline E. Tate and Umesh D. Parashar: Protocol development, data analysis and manuscript preparation.

Mahesh K. Aggarwal, Pradeep Haldar, Patrick L F Zuber, Jan Bonhoeffer, Arindam Ray and Christine G. Maure: Protocol development, quality assurance and monitoring.

Ashish Wakhlu, Bhadresh R Vyas, Javeed Iqbal Bhat, Jayanta K. Goswami, John Mathai, Kameswari K., Lalit Bharadia, Lalit Sankhe, Ajayakumar M.K., Neelam Mohan, Pradeep K. Jena, Rachita Sarangi, Rashmi Shad, Sanjib K. Debbarma, Shyamala J., Simmi K. Ratan, Suman Sarkar, Vijayendra Kumar, Anand P. Dubey, Atul Gupta, Cenita J. Sam, Gowhar Nazir Mufti, Harsh Trivedi, Jimmy Shad, Kaushik Lahiri, Krishnaswamy R., Meera Luthra, Narendra Behera, Padmalatha P., Rajamani G., Rakesh Kumar, Ruchirendu Sarkar, Santosh Kumar A., Subrat Kumar Sahoo, Sunil K. Ghosh, Sushant Mane, Arun Kumar Dash, Bashir Ahmad Charoo, Bikasha Bihary Tripathy, Rajendra Prasad G., Harish Kumar S., Jothilakshmi K., Nihar Ranjan Sarkar, Pavai Arunachalam, Satya Sundar G. Mohapatra, and Saurabh Garge: Participant recruitment and data collection.

All authors reviewed, provided critical input and approved the final version. The content represents the views of the authors alone and do not necessarily represent the official positions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.09.019.

References

[1] ROTA Council. Global Introduction Status [Internet]. 2020 [cited 2020 May 30]. Available from: https://rotacouncil.org/vaccine-introduction/global-introduction-status/.

[2] Quintanar-Solares M, Yen C, Richardson V, Esparza-Aguilar M, Parashar UD, Patel MM. Impact of rotavirus vaccination on diarrhea-related hospitalizations among children <5 years of age in Mexico. Pediatr Infect Dis J 2011;30:S11–5.

[3] Pendleton A, Galic M, Clarke C, Ng SP, Ledesma E, Ramakrishnan G, et al. Impact of rotavirus vaccination in Australian children below 5 years of age: a database study. Hum Vaccines Immunother 2013;9(8):1617–25.

[4] Burnett E, Jonesteller CL, Tate JE, Yen C, Parashar UD. Global impact of rotavirus vaccination on childhood hospitalizations and mortality from diarrhea. J Infect Dis 2017;215(11):1666–72.

[5] Jonesteller CL, Burnett E, Yen C, Tate JE, Parashar UD. Effectiveness of rotavirus vaccination: a systematic review of the first decade of global postlicensure data, 2006–2016. Clin Infect Dis 2017;65(5):840–50.

[6] Murphy TV, Gargiullo PM, Massoudi MS, Nelson DB, Jumaan AO, Okoro CA, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344(8):564–72.
[7] Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006;354(1):23–33.
[8] Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006;354(1):11–22.
[9] Patel MM, López-Collada VR, Bulhões MM, De Oliveira LH, Márquez AB, Flannery B, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. N Engl J Med 2011;364(24):2283–92.
[10] Velázquez FR, Colindres RE, Grajales C, Hernández MT, Mercadillo MG, Torres FJ, et al. Postmarketing surveillance of intussusception following mass introduction of the attenuated human rotavirus vaccine in Mexico. Pediatr Infect Dis J 2012;31(7):736–44.
[11] Carlin JB, Macartney KK, Lee KJ, Quinn HE, Buttery J, Lopert R, et al. Intussusception risk and disease prevention associated with rotavirus vaccines in australia's national immunization program. Clin Infect Dis 2013;57(10):1427–34.
[12] Yih WK, Lieu TA, Kulldorff M, Martin D, McMahill-Walraven CN, Platt R, et al. Intussusception risk after rotavirus vaccination in U.S. Infants. N Engl J Med 2014;370(6):503–12.
[13] Weintraub ES, Baggs J, Duffy J, Vellozzi C, Belongia EA, Irving S, et al. Risk of intussusception after monovalent rotavirus vaccination. N Engl J Med 2014;370(6):513–9.
[14] Stowe J, Andrews N, Ladhani S, Miller E. The risk of intussusception following monovalent rotavirus vaccination in England: a self-controlled case-series evaluation. Vaccine 2016;34(32):3684–9.
[15] Yen C, Tate JE, Hyde TB, Cortese MM, Lopman BA, Jiang B, et al. Rotavirus vaccines: current status and future considerations. Hum Vaccines Immunother 2014;10(6):1436–48.
[16] Jiang J, Jiang B, Parashar U, Nguyen T, Bines J, Patel MM. Childhood intussusception: a literature review. Cameron DW, editor. PLoS ONE. 2013;8(7):e68482.
[17] Jehangir S, John J, Rajkumar S, Mani B, Srinivasan R, Kang G. Intussusception in southern India: comparison of retrospective analysis and active surveillance. Vaccine 2014;32:A99–A103.
[18] Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. The Lancet 2014;383(9935):2136–43.
[19] Kulkarni PS, Desai S, Tewari T, Kawade A, Goyal N, Garg BS, et al. A randomized Phase III clinical trial to assess the efficacy of a bovine-human reassortant pentavalent rotavirus vaccine in Indian infants. Vaccine 2017;35(45):6228–37.
[20] Press Informationn Beureau. Shri J P Nadda launches Rotavirus vaccine as part of Universal Immunization Programme; terms it a "historic moment". [Internet]. Ministry of Health & Family Welfare Government of India; 2016 [cited 2019 Jan 10]. Available from: http://pib.nic.in/newsite/PrintRelease.aspx?relid=138342.
[21] Das M, Arora N, Bonhoeffer J, Zuber P, Maure C. Intussusception in young children: protocol for multisite hospital sentinel surveillance in India. Methods Protoc 2018;1(2):11.
[22] Bines JE, Kohl KS, Forster J, Zanardi LR, Davis RL, Hansen J, et al. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine 2004;22(5–6):569–74.
[23] Tate JE, Mwenda JM, Armah G, Jani B, Omore R, Ademe A, et al. Evaluation of intussusception after monovalent rotavirus vaccination in Africa. N Engl J Med 2018;378(16):1521–8.
[24] Whitaker HJ, Paddy Farrington C, Spiessens B, Musonda P. Tutorial in biostatistics: the self-controlled case series method. Stat Med 2006;25(10):1768–97.
[25] Whitaker HJ, Hocine MN, Farrington CP. The methodology of self-controlled case series studies. Stat Methods Med Res 2009;18(1):7–26.
[26] Yung C-F, Chan SP, Soh S, Tan A, Thoon KC. Intussusception and monovalent rotavirus vaccination in Singapore: self-controlled case series and risk-benefit study. J Pediatr 2015;167(1):163–168.e1.
[27] Pérez-Vilar S, Díez-Domingo J, Puig-Barberà J, Gil-Prieto R, Romio S. Intussusception following rotavirus vaccination in the Valencia Region, Spain. Hum Vaccines Immunother 2015;11(7):1848–52.
[28] Groome MJ, Tate JE, Arnold M, Chitnis M, Cox S, de Vos C, et al. Evaluation of intussusception after oral monovalent rotavirus vaccination in South Africa. Clin Infect Dis 2020;70(8):1606–12.
[29] Leino T, Ollgren J, Strömberg N, Elonsalo U. Evaluation of the intussusception risk after pentavalent rotavirus vaccination in Finnish infants. Kirk M, editor. PLOS ONE. 2016;11(3):e0144812.
[30] Farrington CP, Whitaker HJ, Hocine MN. Case series analysis for censored, perturbed, or curtailed post-event exposures. Biostatistics 2008;10(1):3–16.
[31] Musonda P, Paddy Farrington C, Whitaker HJ. Sample sizes for self-controlled case series studies. Stat Med 2006;25(15):2618–31.
[32] WHO and Unicef. India: WHO and UNICEF estimates of immunization coverage: 2019 revision [Internet]. World Health Organization; 2020 [cited 2020 Aug 12]. Available from: https://www.who.int/immunization/monitoring_surveillance/data/ind.pdf?ua=1).
[33] Patel M, Shane AL, Parashar UD, Jiang B, Gentsch JR, Glass RI. Oral rotavirus vaccines: how well will they work where they are needed most? J Infect Dis 2009;200(s1):S39–48.
[34] Srinivasan R, Girish Kumar CP, Naaraayan SA, Jehangir S, Thangaraj JWV, Venkatasubramanian S, et al. Intussusception hospitalizations before rotavirus vaccine introduction: retrospective data from two referral hospitals in Tamil Nadu, India. Vaccine 2018;36(51):7820–5.
[35] Gupta M, Kanojia R, Singha R, Tripathy JP, Mahajan K, Saxena A, et al. Intussusception rate among under-five-children before introduction of rotavirus vaccine in North India. J Trop Pediatr 2018;64(4):326–35.
[36] Singh JV, Kamath V, Shetty R, Kumar V, Prasad R, Saluja T, et al. Retrospective surveillance for intussusception in children aged less than five years at two tertiary care centers in India. Vaccine 2014;32:A95–8.
[37] Steele AD, De Vos B, Tumbo J, Reynders J, Scholtz F, Bos P, et al. Co-administration study in South African infants of a live-attenuated oral human rotavirus vaccine (RIX4414) and poliovirus vaccines. Vaccine 2010;28(39):6542–8.
[38] Emperador DM, Velasquez DE, Estivariz CF, Lopman B, Jiang B, Parashar U, et al. Interference of monovalent, bivalent, and trivalent oral poliovirus vaccines on monovalent rotavirus vaccine immunogenicity in rural Bangladesh. Clin Infect Dis 2016;62(2):150–6.
[39] Ramani S, Mamani N, Villena R, Bandyopadhyay AS, Gast C, Sato A, et al. Rotavirus serum IgA immune response in children receiving rotarix coadministered with bOPV or IPV. Pediatr Infect Dis J 2016;35(10):1137–9.
[40] Carvalho MF, Gill D. Rotavirus vaccine efficacy: current status and areas for improvement. Hum Vaccines Immunother 2019;15(6):1237–50.
[41] Parker EP, Ramani S, Lopman BA, Church JA, Iturriza-Gómara M, Prendergast AJ, et al. Causes of impaired oral vaccine efficacy in developing countries. Future Microbiol 2018;13(1):97–118.
[42] Bhandari N, Antony K, Balraj V, Rongsen-Chandola T, Kumar T, Sinha B, et al. Assessment of risk of intussusception after pilot rollout of rotavirus vaccine in the Indian public health system. Vaccine 2020;38(33):5241–8.

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Intussusception in children aged under two years in India: Retrospective surveillance at nineteen tertiary care hospitals

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ABSTRACT

Objective: Intussusception has been linked with rotavirus vaccine (RVV) as a rare adverse reaction. In view of limited background data on intussusception in India and in preparation for RVV introduction, a surveillance network was established to document the epidemiology of intussusception cases in Indian children.

Methods: Intussusception in children 2–23 months were documented at 19 nationally representative sentinel hospitals through a retrospective surveillance for 69 months (July 2010 to March 2016). For each case clinical, hospital course, treatment and outcome data were collected.

Results: Among the 1588 intussusception cases, 54.5% were from South India and 66.3% were boys. The median age was 8 months (IQR 6, 12) with 34.6% aged 2–6 months. Seasonal variation with higher cases were documented during March-June period. The most common symptoms and signs were vomiting (63.4%), bloody stool (49.1%), abdominal pain (46.9%) and excessive crying (42.8%). The classical triad (vomiting, abdominal pain, and blood in stools) was observed in 25.6% cases. 96.4% cases were diagnosed by ultrasound with ileocolic location as the commonest (85.3%). Management was done by reduction (50.8%) and surgery (41.1%) and only 1% of the patients' died. 91.1% cases met Brighton criteria level 1 and 3.3% Level 2. Between 2010 and 2015, the case load and case ratio increased across all regions.

Conclusion: Intussusception cases have occurred in children across all parts of the country, with low case fatality in the settings studied. The progressive rise cases could indicate an increasing awareness and availability of diagnostic facilities.

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1. Introduction

Intussusception is the most common bowel obstruction in young children, usually occurring between 4 and 10 months of age [1,2]. Some of these may be transient and resolve spontaneously. If the intussusception is not relieved early, it may lead to bowel ischemia and perforation and may be fatal [2]. Ileocecal region is the most common site documented. Some anatomical lead points may trigger in 10–15% cases [2]. Intussusception has been linked as an adverse reaction with rotavirus vaccine (RVV). An increased rate of intussusception (1 additional case per 10000 vaccinated children) following introduction of RVV, Rotashield™ (Wyeth Lederle, Marietta, PA, USA) in 1999 forced its withdrawal from the market [3–5]. Recognising the intussusception risk, subsequent two candidate RVVs underwent clinical trials with over 60,000 participants documented no significant increased risk [6,7]. The post-licensure surveillance with two RVVs (Rotarix™, GlaxoSmithKline Biologicals, Rixensart, Belgium and Rotateq™, Merck & Co. Inc., Kenilworth, USA) reported variable risks of intussusception across countries; ranging from no increased risk [USA [8] and Brazil [9] to a low risk of 1–2 additional cases per 100,000 vaccinated infants [Mexico [9] and Australia [10]. Introduction of RVV in national immunization programmes (NIPs) has been recommended by World Health Organization (WHO) to reduce the rotavirus diarrhoea related deaths. Also, WHO has recommended documentation of baseline rates of intussusception to allow for estimation of potential attributable risks associated with vaccine, to enable a quantitative assessment of the potential risks and benefits of a vaccine programme [11].

According to WHO, 96 countries have introduced RVV in their NIP till August 2018. India has introduced the RVV in NIP since April 2016 in a phased manner. Monitoring the risk of intussusception related to RVV requires reliable surveillance systems. Currently available studies from India have assessed the risk of intussusception from single or few medical centres. The studies use different case definitions and age groups, reference periods, methodology and case management protocols [12–14]. Measured intussusception incidence in children from published reports varied between 17.7 (95% CI 5.9, 41.4) to 254 (95%CI 5.9, 41.4) cases per 100,000 child-years [15,16].

Wide variations in incidence and case load exist across sites in India and globally [17] although reasons for those variations are not known. There is limited information about regional differences in intussusception incidence among children from India. In view of the limited background data, we established a network of tertiary care hospitals to monitor trends of intussusception over time to identify any vaccine associated risk [18,19]. In this study we report on the epidemiology of intussusception in under-two children prior to RVV introduction in India.

2. Methods

2.1. Study area and participating hospitals

This retrospective study of hospital-based sentinel surveillance for intussusception in under-two children was conducted at 19 Indian major tertiary care hospitals. Data were collected for a 69-months period (July 2010 to March 2016) at all these sites. The sites were selected from four regions (north, south, east, and west) of the country with three to six hospitals per region with a mix of medical colleges and private-sector hospitals (North region- 5 sites, 3 public and 2 private; South region- 5 sites, 2 public and 3 private; East region- 6 sites, 5 public and 1 private; West region- 3, public 2, and private 1). The data was collected during January 2016- September 2017. The method of selection of the sites are described in the protocol published [20].

2.2. Case definition, case selection and data collection

Children aged >1 month and <24 months (2–23 months) admitted to these hospitals with intussusception were reviewed. At the institutions using international classification of diseases (ICD), cases with one of the clinical conditions as per ICD-10 (K56.1, K56.2, K56.3, K56.4, K56.5, K56.6, K56.7 and K56.0) or ICD 9 (560.0, 560.2, 560.31, 560.30, 560.81, 560.9 and 560.1) were identified. At the institutes not using ICD classification, cases with exit diagnoses including intussusception, acute intestinal obstruction, subacute intestinal obstruction, acute abdomen, and blood in stool with vomiting were identified. Additionally, all the registers from clinical (pediatrics, pediatric surgery and emergency) wards, operation theatres, radiol-

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ogy (ultrasound, barium studies and CT scan) and pathology departments were screened to identify any cases. The case records of these suspected patients were extracted and reviewed to identify intussusception cases. For confirmed cases, data including demography, clinical features, management, and final outcome were abstracted using case record form (CRF). The data including symptoms, signs and other relevant information as documented in the case records were captured. A record of cases screened, identified as suspected and confirmed cases was maintained. For the suspected and confirmed cases with incomplete information from case records, the other sources of information were searched and supplemented. The modes of treatment cases as documented in case record were recorded (surgical- laparotomy with or without resection, reduction- hydrostatic or barium enema under ultrasound guidance or conservatively- managed for intestinal obstruction without reduction or surgery). The cases with documented findings compatible with intussusception on ultrasound, CT scan, barium enema or surgery were considered as confirmed cases. An independent case adjudication committee (CAC), including paediatrician, paediatric surgeon and radiologist, reviewed and assigned diagnostic certainty level using Brighton Collaboration criteria [1].

2.3. Quality assurance

To ascertain protocol adherence, rigor and completion of surveillance, multilevel quality assurance processes were implemented. Technical Advisory Group (TAG) members visited sites to assess the suspected cases identification, record retrieval and data abstraction for confirmed cases. Data team members visited all the sites on a second occasion, repeated the case identification process for 2014, 2015, and 2016 using the protocol to assess the completeness of data collection and identify any missed cases. The data abstracted for confirmed intussusception cases were verified from case records. Any additional data query was clarified with reference to the source documents.

2.4. Data management and analysis

Double data entry was done for the CRFs using a customised data entry platform with inbuilt data matching feature. The verified data were stored in a server with authorised access and daily backup. The descriptive analysis was included proportions for categorical; means and standard deviations for normally distributed continuous variables, and median with IQR for continuous variables with skewed distributions. The values between groups were compared for statistical significance using Student's *t* test or Mann–Whitney *U* test depending on the skewness. Statistical significance was considered if $p < 0.05$. Statistical analysis was performed using STATA version 15.0 (Stata Corp LLC, Texas, USA). Due to the lack of well-defined population catchment area for the hospitals, the estimation of intussusception incidence was not feasible. Instead, we estimated intussusception case ratio per 1000 paediatric hospitalisations at each hospital for comparing the case volumes by sites and years. On review, it was observed that the paediatric surgery admission numbers were relatively stable compared to the medicine department and the intussusception cases were primarily admitted to the paediatric surgery wards. Thus the intussusception case ratio was estimated per 1000 paediatric surgery admissions at the hospitals for comparison and trend analysis. The detailed methodology can be referred from the published protocol [20].

2.5. Ethical issues

The study protocol was reviewed and approved by all the ethics review committees of each participating institute. Confidentiality in data handling was maintained.

3. Results

A total of 1588 children aged under two years (2–23 months) with intussusception were admitted to these hospitals between July 2010 and March 2016. Out of these 96 (6%) (North region, n = 13; South region, n = 68; East region, n = 10 and West region, n = 5) cases had past history of intussusception documented in the case records. Over half of the cases (n = 865, 54.5%) were documented from the hospitals in Southern region. Figures for other regions were: Eastern (n = 381, 24%), Northern (n = 254, 16%) and Western (n = 88, 5.5%). Demographic and clinical characteristics of the intussusception patients are summarised in Table 1. A consistent male predominance was observed across all regions

Table 1
Demographic and clinical characteristics of children aged 2-23 months with intussusception in India (region wise and pooled).

Variable	Category	North (n = 254) n (%)	South (n = 865) n (%)	East (n= 381) n (%)	West (n= 88) n (%)	Total (n= 1588) n (%)	P value
Age (in months)	2–6	109 (42.9)	265 (30.6)	158 (41.5)	18 (20.5)	550 (34.6)	0.00
	7–12	92 (36.2)	357 (41.3)	165 (43.3)	37 (42.1)	651 (41)	0.35
	13–18	25 (9.8)	141 (16.3)	31 (8.1)	16 (18.2)	213 (13.4)	0.00
	19–23	28 (11)	102 (11.8)	27 (7.1)	17 (19.3)	174 (11)	0.01
Gender	Male	191 (75.2)	539 (62.3)	263 (69)	60 (68.2)	1053 (66.3)	0.00
	Female	63 (24.8)	326 (37.7)	118 (31)	28 (31.8)	535 (33.7)	0.00
Place of residence	Same district	89 (35)	500 (57.8)	48 (12.6)	62 (70.5)	699 (44)	0.00
	Other districts[@]	127 (50)	342 (39.5)	331 (86.9)	24 (27.3)	824 (51.9)	0.00
	Outside state	38 (15)	23 (2.7)	2 (0.5)	2 (2.3)	65 (4.1)	0.00
Referral status	Primary[*]	188 (74)	625 (72.3)	334 (87.7)	72 (81.8)	1219 (76.8)	0.00
	Referred[**]	66 (26)	240 (27.8)	47 (12.3)	16 (18.2)	369 (23.2)	0.00
Symptoms	Vomiting[¥]	164 (64.6)	631 (73)	145 (38.1)	66 (75)	1006 (63.4)	0.00
	Bilious vomiting[¥¥]	28 (11)	100 (11.6)	24 (6.3)	14 (15.9)	166 (10.5)	0.01
	Abdominal pain	144 (56.7)	412 (47.6)	136 (35.7)	53 (60.2)	745 (46.9)	0.00
	Excessive crying	42 (16.5)	538 (62.2)	70 (18.4)	30 (34.1)	680 (42.8)	0.00
	Abdominal distension	95 (37.4)	60 (6.9)	85 (22.3)	15 (17.1)	255 (16.1)	0.00
	Rectal bleeding	135 (53.2)	456 (52.7)	141 (37)	48 (54.6)	780 (49.1)	0.00
	Diarrhoea	33 (13)	177 (20.5)	53 (13.9)	30 (34.1)	293 (18.5)	0.00
	Constipation	47 (18.5)	84 (9.7)	25 (6.6)	12 (13.6)	168 (10.6)	0.00
	Fever	49 (19.3)	151 (17.5)	41 (10.8)	19 (21.6)	260 (16.4)	0.01

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Table 1 (*continued*)

Variable	Category	North (n = 254) n (%)	South (n = 865) n (%)	East (n= 381) n (%)	West (n= 88) n (%)	Total (n= 1588) n (%)	P value
	Lethargy	9 (3.5)	50 (5.8)	14 (3.7)	8 (9.1)	81 (5.1)	0.09
	Classical triad[#]	64 (25.2)	255 (29.5)	65 (17.1)	23 (26.1)	407 (25.6)	0.00
Signs	Pallor	29 (11.4)	22 (2.5)	34 (8.9)	11 (12.5)	96 (6.1)	0.00
	Dehydration	27 (10.6)	66 (7.6)	44 (11.6)	8 (9.1)	145 (9.1)	0.13
	Fever	16 (6.3)	92 (10.6)	36 (9.5)	9 (10.2)	153 (9.6)	0.23
	Lethargy	22 (8.7)	53 (6.1)	21 (5.5)	4 (4.6)	100 (6.3)	0.38*
	Abdominal distension	96 (37.8)	74 (8.6)	84 (22.1)	20 (22.7)	274 (17.3)	0.00
	Abdominal tenderness	46 (18.1)	69 (8)	35 (9.2)	12 (13.6)	162 (10.2)	0.00
	Abdominal mass	41 (16.1)	376 (43.5)	24 (6.3)	15 (17.1)	456 (28.7)	0.00
	Bowel sound (absent/ abnormal)	35 (13.8)	17 (2)	18 (4.7)	6 (6.8)	76 (4.8)	0.00
	Rectal prolapse	51 (20.1)	0 (0.0)	4 (1.1)	0 (0.0)	55 (3.5)	0.00*
	Rectal mass	23 (9.1)	8 (0.9)	6 (1.6)	1 (1.1)	38 (2.4)	0.00*
	Blood on rectal examination	80 (31.5)	107 (12.4)	75 (19.7)	31 (35.2)	293 (18.5)	0.00
	Triad (sign/symptom) [##]	147 (57.9)	464 (53.6)	148 (38.9)	48 (54.6)	807 (50.8)	0.00
Diagnosis	Ultrasound	221 (87)	862 (99.7)	364 (99.5)	84 (99.5)	1531 (96.4)	0.00*
	CT scan	1 (0.4)	0 (0.0)	0 (0.0)	1 (1.1)	2 (0.1)	0.02*
	Barium enema	0 (0.0)	0 (0.0)	2 (0.5)	1 (1.1)	3 (0.2)	0.03*
	Surgery	32 (12.6)	3 (0.4)	15 (3.9)	2 (2.3)	52 (3.3)	0.00*
Intussusception location	Colo-colic	7 (2.8)	22 (2.5)	5 (1.3)	10 (11.4)	44 (2.8)	0.00
	Ileo-colo-colic	18 (7.1)	11 (1.3)	6 (1.6)	3 (3.4)	38 (2.4)	0.00*
	Ileo-ileal	40 (15.8)	18 (2.1)	7 (1.8)	10 (11.4)	75 (4.7)	0.00
	Ileo-ileo-colic	9 (3.5)	27 (3.1)	9 (2.4)	2 (2.3)	47 (3)	0.83*
	Ileo-colic	173 (68.1)	775 (89.6)	348 (91.3)	58 (65.9)	1354 (85.3)	0.00
	Jejuno-jejunum	1 (0.4)	4 (0.5)	0(0.0)	2 (2.3)	7 (0.4)	0.06*
	>1 location[€]	6 (2.4)	8 (0.9)	6 (1.6)	3 (3.4)	23 (1.5)	0.10*
Pathological lead point (PLP)	Lymph node	66 (26)	74 (8.6)	15 (3.9)	33 (37.5)	188 (11.8)	0.00*
	Appendix	8 (3.1)	5 (0.6)	39 (10.2)	2 (2.3)	54 (3.4)	0.00*
	Payer's patch	2 (0.8)	10 (1.2)	2 (0.5)	0 (0)	14 (0.9)	0.06*
	Polyp	0 (0)	2 (0.2)	1 (0.3)	0 (0)	3 (0.2)	0.65*
	Others[&]	9 (3.6)	12 (1.3)	3 (0.8)	1 (1.1)	25 (1.5)	0.17
	Any lead point	85 (33.5)	103 (11.9)	60 (15.8)	36 (40.9)	284 (17.9)	0.00
Treatment	Reduction	47 (18.5)	686 (79.3)	34 (8.9)	39 (44.3)	806 (50.8)	0.00
	Surgery	187 (73.6)	165 (19.1)	273 (71.7)	28 (31.8)	653 (41.1)	0.00
	Conservative	19 (7.5)	14 (1.6)	61 (16)	20 (22.7)	114 (7.2)	0.00
	None[$]	1 (0.4)	0 (0.0)	13 (3.4)	1 (1.1)	15 (0.9)	0.00*
Surgery type[£]	No resection	77 (30.3)	118 (13.6)	202 (53)	21 (23.9)	418 (26.3)	0.00
	With resection	110 (43.3)	47 (5.4)	71 (18.6)	7 (8)	235 (14.8)	0.00
Outcome	Discharged	248 (97.6)	863 (99.8)	341 (89.5)	87 (98.9)	1539 (96.9)	0.00*
	Referred	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-
	LAMA[^]	3 (1.2)	0 (0.0)	29 (7.6)	1 (1.1)	33 (2.1)	0.00*
	Died	3 (1.2)	2 (0.2)	11 (2.9)	0 (0.0)	16 (1)	0.00*
Brighton Criterial level	Level 1	234 (92.1)	851 (98.4)	307 (80.6)	67 (76.1)	1459 (91.9)	0.00
	Level 2	10 (3.9)	9 (1)	23 (6)	11 (12.5)	53 (3.3)	0.00
	Level 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
	Not fitting any level	10 (3.9)	5 (0.6)	51 (13.4)	10 (11.4)	76 (4.8)	0.00

Notes:
PR: Per-rectal
The p values for some parameters could not be estimated due to no value in at least two groups.
[*] The patient presented to the study hospital as the first institute.
[**] The patient referred to the study hospital from another hospital.
[¥] Vomiting includes bilious vomiting.
[¥¥] Includes only bilious vomiting.
[#] Classical triad include abdominal pain, vomiting and blood in stools
[##] Triad include abdominal pain, vomiting and rectal bleeding (detected either as blood in stool or blood on per-rectal examination)
[$] None, as the patient(s) were referred or died before any definite treatment.
[£] The denominators used for percentage estimation are patients undergone surgery only.
[^] LAMA: Left against medical advice.
[@] The residence of the patient is in other district in the state where the study hospital is based.
[€] The location of intussusception is at more than one site; such as Ileo-colic + Colo-colic, Ileo-olic + Ileo-colo-colic, Ileo-colic + Ileo-ileo-colic, Ileo-colic+ Ileo-ileal.
[&] Others including cyst, Meckel's diverticulum, incisional hernia, intraluminal growth and malrotation.

(male: female ratios: pooled 1.96; north 3.0; south 1.65, east 2.2; and west 2.1). The majority of cases were aged <12 months, with 34.6% in the 2–6 months and 41% in the 7–12 months age bands. Nearly half (48.1%) of the patients were aged 4–8 months. Over one-third (34.7%) children were aged >1–6 months, the age for administration of routine RVV doses. The overall median age at presentation was 8 months (Inter Quartile Range, IQR 6, 12) with 8 months (IQR 6, 12) for boys and 8 months (IQR 6, 15) for girls (p = 0.09). The median age was higher for Western (11 months, IQR 7, 17) and Southern (9 months, IQR 6, 14) regions compared to Northern (7 months, IQR 5, 12) and Eastern (7 months, IQR 6, 10) regions (p = 0.00) (Table 1 and Fig. 1). Additional peaks in case load was observed around the 12th and 23rd months of age across all regions.

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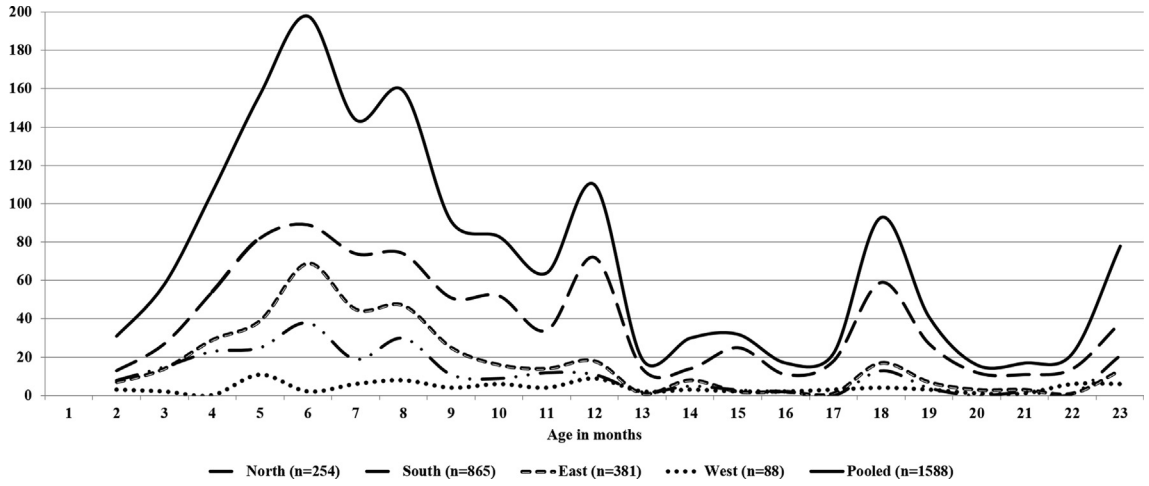


Fig. 1. Age distribution of children aged 2–23 months with intussusception in India (region wise and pooled).

Seasonal variation was observed with higher number of intussusception cases and case ratio during March to June months over each individual year studied (Fig. 2).

While the pooled intussusception case ratio was 15.5 (IQR 14.8, 16.3) per 1000 paediatric surgery admissions, the case ratio for Northern, Southern, Eastern and Western regions were observed to be 8.8 (IQR 7.8, 9.9), 36.2 (IQR 33.9, 38.6), 9.9 (IQR 8.9, 10.9) and 8 (IQR 6.5, 9.9) respectively. Between 2010 and 2015, rises in both absolute number of intussusception cases and case ratio (per 1000 paediatric surgery admissions) was observed across all the regions, but the rise was relatively higher for Eastern and Northern than the Southern and Western regions (see Fig. 3 and 4).

While 51.9% of hospitalized patients resided outside the hospital district, 44% were from the same district and 4.1% came from other states. Overall 76.8% cases presented directly to hospitals and 23.2% patients were referred from other hospitals. The median duration of symptoms to admission was 3 days (IQR 1, 5 days), but a shorter interval was observed in Southern (median 1 day, IQR 1, 5 days) and Western (median 2 days, IQR 1, 6 days) than in Northern and Eastern (median 3 days, IQR 1, 10 days) ($p = 0.04$) regions.

The most common symptoms were vomiting (63.4%), passage of blood stained stool (49.1%), abdominal pain (46.9%) and excessive crying (42.8%). Bilious vomiting was documented in 10.5% cases, more frequently Western region (15.9%). Higher proportions of abdominal pain in Western (60.2%) and Northern (56.7%); excessive crying in Southern (62.2%); blood in stool in Northern, Southern and Western (52.7%-54.9%) regions were documented. The intussusception classical symptoms triad (abdominal pain, vomiting and blood in stools) was documented in 25.6% cases, which varies from 25.2% to 29.5% in in Northern, Southern and Eastern regions, but was quite lower (17.1%) in the Western region. There was no variation in the presence of the symptoms triad with the interval between onset of symptoms and presentation to the hospital. On examination children had abdominal mass (28.7%), abdominal distention (17.3%), blood on per-rectal examination (18.5%), and abdominal tenderness (10.2%). The prevalence of findings varied across regions for abdominal distention (Northern 37.8% to Southern 8.6%), abdominal mass (Southern 43.5% to Eastern 6.3%), and blood on per-rectal examination (Western 35.2% to Northern 12.4%). On considering presence of blood on per-rectal examination, presence of triad of intussusception (abdominal pain, vomiting and blood in stools or per-rectal examination) was present in 50.8% and ranged from 57.9% in Northern to 38.9% in Eastern regions.

Most cases were diagnosed by ultrasound (96.4%) and few by laparotomy (3.3%). The most common site of intussusception was

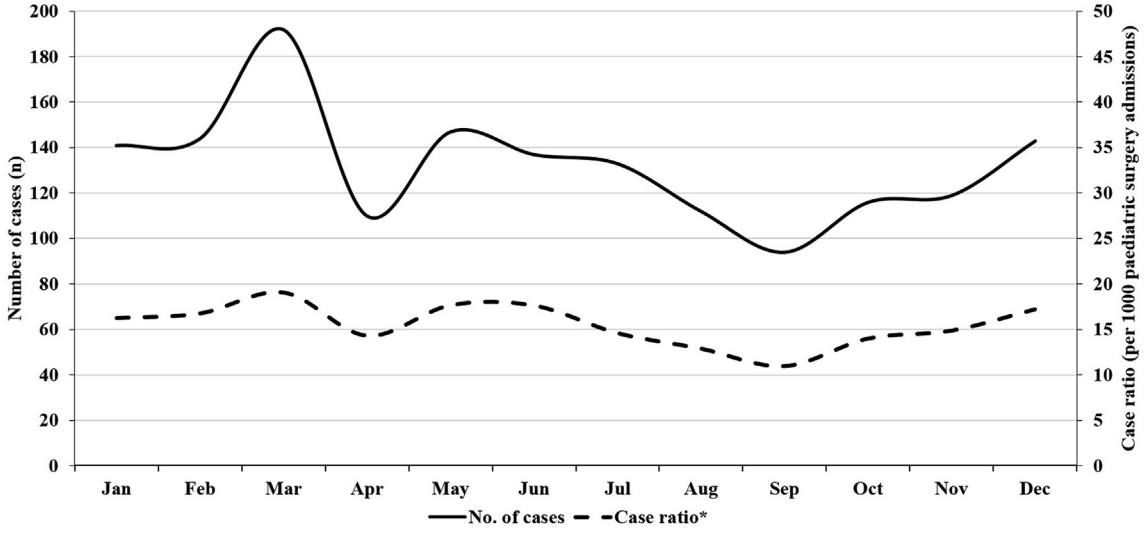


Fig. 2. Calendar monthly distribution of intussusception case load and case ratio in children aged 2–23 months in India (pooled).

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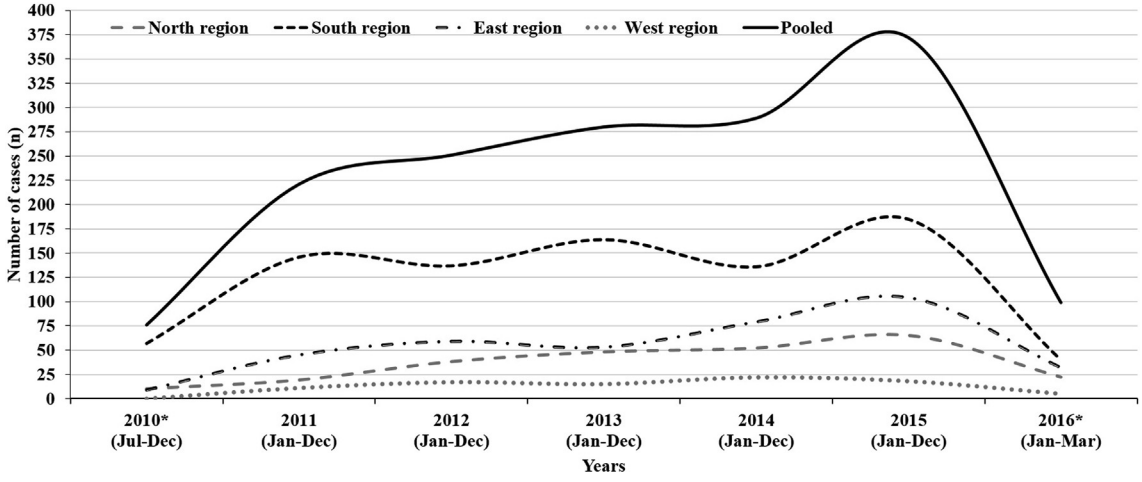


Fig. 3. Trend of intussusception case load in children aged 2–23 months in India during 2010–2016 (region wise and pooled).

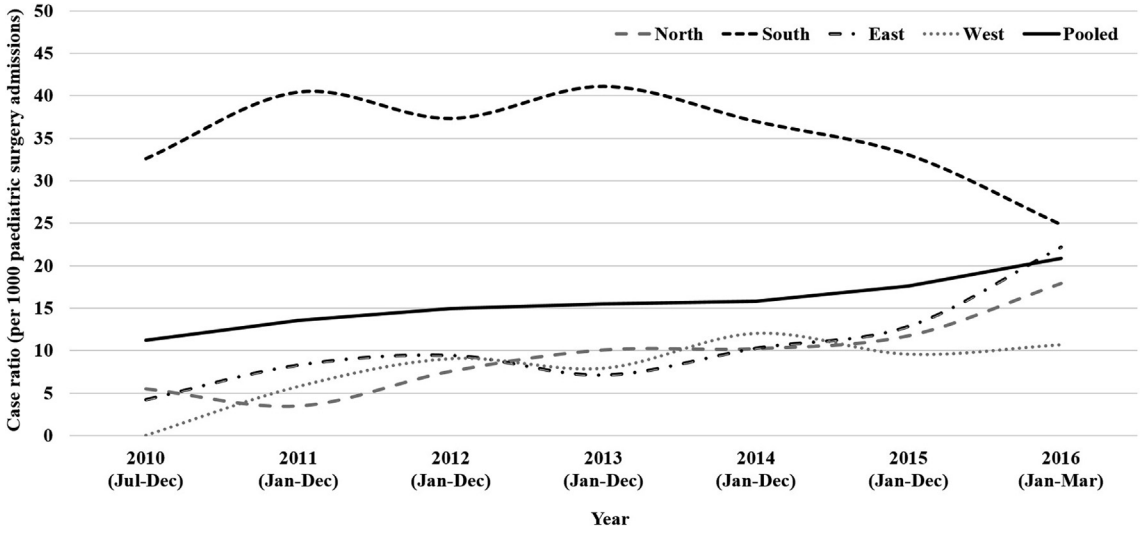


Fig. 4. Trend of intussusception case ratio in children aged 2–23 months in India during 2010–2016 (region wise and pooled).

ileocolic (85.3%) across all regions, age groups and gender. Other intussusception sites included ileo-ileal (north 15.8% and west 11.4%), colo-colic (west 11.4%) and ileo-colo-colic (north 7.1%). Double site intussusception was documented in few (1.5%) cases. Pathological lead point (PLP) was documented in 91 (17.9%) cases with lymph nodes (11.8%) as the most common one and higher proportion in Western region (37.5%) followed by Northern region (26%).

Most patients were treated by reduction (50.8%) and surgery (41.1%), while 7.2% children were managed conservatively. Among children managed by surgery, 14.8% required bowel resection. The median interval to presentation was not associated with different treatment choices: surgery (1 day; IQR 0, 3), reduction (1 day; IQR 1, 2) and conservative treatment (1 day; IQR 0, 3). Higher proportion of patients underwent surgery in Northern (73.6%) and Eastern (71.7%) regions compared to Western (31.8%) and Southern (19.1%) regions. Five hospitals did not use reduction (3 in Eastern, 1 in Northern and 1 in Southern regions) but, instead, use exclusive surgery. At the hospitals with both surgical and reduction facilities for management, surgery was done in 28.3% (341/1205) cases with 18.1% cases reduced under anaesthesia and the remainder required bowel resection. The indications for surgery were failed reduction (33.9%), late presentation (23%) and associated complications (43.1%). Only 1% of the cases died and most of these presented late (median interval 3 days, IQR 1, 6 days). The causes of death were due to complications (sepsis, shock, and gangrene) associated with delayed presentation and after surgery. Thirty three cases left against medical advice before definite treatment. The overall median period of hospitalisation was 3 days (IQR 2, 6 days), which varied from 2 to 6 days; there were important regional variations, from 2 days in the Southern to 6 days in the Northern and Eastern regions. The median hospitalisation periods were 7 days (IQR 5, 9 days), 2 days (IQR 2, 3 days) and 2 days (IQR 2, 4 days) for surgery, reduction and conservative management respectively. Using Brighton diagnostic criteria, 91.1% cases were categorised as Level 1, 3.3% as Level 2 and none in Level 3. No level could be assigned for 4.8% cases.

4. Discussion

In preparation for RVV introduction, assessing the background rate of intussusception helps prepare monitoring vaccine safety [21,22]. The global average rate of intussusception is about 74 (range 9–328) per 100,000 child years based on review of 82 studies [17]. Very high incidences have been measured in South Korea

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[23], Vietnam [24], Israel [25], and Japan [26] with 328, 302, 219, and 185 per 100,000 child years respectively [17]. Reported intussusception rates in India vary from 17.7 to 254 per 100,000 child years, [15,16]. Most reports from India are hospital-based passive surveillance and few are population-based active surveillance. These studies were of limited geographical representation to derive any national interpretation. The RVV pre-licensure studies in India (for Rotavac™ and Rotasiil™) have not shown increased intussusception risk.

This study was undertaken to document the intussusception case load in India through a nationally representative sentinel surveillance network of tertiary care hospitals to assist monitoring the trend following RVV introduction. We observed important regional difference in intussusception case load and case ratio. The Southern region documented higher absolute case number (3–10 times) and case ratio (4–5 times). The higher number of cases and case ratio in southern region sites could be due to better access to diagnostic facilities and timely referral. Additionally presence of pediatric surgeon and management facility could be the other reasons for higher referral to some of these hospitals. Higher case load and case ratio were observed during March to June, as previously reported from India [14,16,27,28].

Actual case incidence could not be estimated due to lack of defined draining population base. Instead, we used the intussusception case ratio per 1000 paediatric surgery admissions which were comparable to absolute case load. This proxy could be useful for comparison across the sites or regions and tracking in future studies. Three-fourth of the diagnosed cases were during the first year of life, with a majority of male patients aged 4–8 months, which was similar to other reports from India [12,14,16,27] and globally [1,12,14,16,17,27,29,30]. The variations in the symptoms and signs may be due to the interval between onset and admission time, longer in north and east regions. In 25.6% cases, the intussusception triad (abdominal pain, vomiting and blood in stools) was documented, which was higher than reports from other parts of India [14,27]. When either symptom (blood in stool) or sign (blood on per-rectum examination) was considered, the prevalence of a triad was 50.8%. There was regional variation in the observation of a triad which also varied with the interval prior to presentation. The most common site for intussusception was ileocolic, which matched with reports from India [12,16,27] and globally [17]. PLPs were documented in 17.9% patients and lymph nodes were the most common (11.8%). Cases that presented early had shorter hospital stay. While in several hospitals reduction was the leading treatment modality, surgery was the only treatment method at some of the hospitals. A high degree of suspicion and early investigation for detection with appropriate referral is crucial for reducing surgical intervention. The proportion of cases with level 1 certainty according to BC criteria was high and similar to other reports from India [5,16,27]. For some cases Level 1 certainty could not be achieved due to non-documentation of reduction. Based on the findings of this study, the available information with the risk of intussusception with the RVVs in market and age pattern from literature, the pattern (age of occurrence, seasonality and sites) should remain similar. The studies after RVV introduction in India shall inform on this.

The study has several limitations. These sentinel surveillance sites were tertiary care hospitals and had no definite draining population territory. Thus estimation of incidence or a case rate was not possible. Several of the institutions used manual and paper record keeping methods and were not practicing the ICD classification system. This study being retrospective in nature, could have missed the cases, if they were not listed or available in the record section. The data collection may have missed the data including symptoms, signs and other information, if not documented in the case records. The number of institutes across the regions, the case load at the hospitals, the methods of case record archival and listing of the diagnoses might have influenced the case yield.

This study documented the intussusception case load, their distribution, clinical features, management and outcome across different sites and regions in India before RVV introduction. This may provide a reasonable background information. More importantly it establishes the methodological basis for active surveillance of intussusception following RVV introduction. The private and public mix of hospitals and regional representation will allow for a more representative evaluation of the vaccines in the whole population. Intussusception is a common condition among children under-two years of age across in India with majority of cases during infancy. There were regional variations in the case load and ratio observed. An increasing secular trend of intussusceptions has been documented among children across all regions prior to the beginning of the rotavirus vaccination program. Case management was dependent on facilities available at the hospitals. Although mortality was low, it can be further prevented by early case detection, referral and timely management. Studies of intussusception following vaccine introduction will require RVV exposure documentation. A periodic monitoring of the intussusception should be undertaken to interpret the trend over time. It may also be useful to further investigate the potential risk factors for the regional variations and increasing trend.

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Ethics approval

The study protocol was reviewed and approved by INCLEN Ethics Committee (Ref: IIEC 023) and all participating Study Site Institute Ethics Committees.

Data availability

The data can be made available in request from the corresponding author.

Authors' contributions

MKD and NKA conceptualised the framework for the study protocol, training, data analysis and interpretation. All TAG members provided input for finalisation of the study protocol and provided quality assurance oversight. All study site investigators led and supervised the data collection. BG and AS coordinated the data collection and collation. MKD and BG analysed the data. MKD and BG wrote the first draft of the manuscript. All authors reviewed, provided critical input and approved the final version. The content represents the views of the authors alone and do not necessarily represent the official positions of their organizations, World Health Organization or Ministry of Health and Family Welfare, Government of India.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.04.059.

References

[1] Bines JE, Kohl KS, Forster J, Zanardi LR, Davis RL, Hansen J, et al. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine 2004;22(5–6):569–74.

[2] Bines JE, Ivanoff B. Acute intussusception in infants and children: Incidence, clinical presentation and management: a global perspective [Internet]. World Health Organisation; 2002 [cited 2017 May 10]. Available from: http://apps.who.int/iris/bitstream/handle/10665/67720/WHO_V-B_02.19_eng.pdf;jsessionid=74671C3CD99ED55137202F8D3D07CB73?sequence=1.

[3] Murphy TV, Gargiullo PM, Massoudi MS, Nelson DB, Jumaan AO, Okoro CA, et al. Intussusception among Infants Given an Oral Rotavirus Vaccine. N Engl J Med 2001;344(8):564–72.

[4] Patel MM, Haber P, Baggs J, Zuber P, Bines JE, Parashar UD. Intussusception and rotavirus vaccination: a review of the available evidence. Expert Rev Vaccines 2009;8(11):1555–64.

[5] World Health Organization. Rotavirus vaccines. Releve Epidemiol Hebd. 2007;82(32):285–95.

[6] Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006;354(1):23–33.

[7] Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006;354(1):11–22.

[8] Yen C, Tate JE, Steiner CA, Cortese MM, Patel MM, Parashar UD. Trends in intussusception hospitalizations among US infants before and after implementation of the rotavirus vaccination program, 2000–2009. J Infect Dis 2012;206(1):41–8.

[9] Patel MM, López-Collada VR, Bulhões MM, De Oliveira LH, Márquez AB, Flannery B, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. N Engl J Med 2011;364(24):2283–92.

[10] Buttery JP, Danchin MH, Lee KJ, Carlin JB, McIntyre PB, Elliott EJ, et al. Intussusception following rotavirus vaccine administration: Post-marketing surveillance in the National Immunization Program in Australia. Vaccine. 2011;29(16):3061–6.

[11] World Health Organization. Post-marketing surveillance of rotavirus vaccine safety [Internet]. World Health Organization, Geneva; 2009. Available from: https://apps.who.int/iris/bitstream/handle/10665/70017/WHO_IVB_09.01_eng.pdf?sequence=1.

[12] Bhowmick K, Kang G, Bose A, Chacko J, Boudville I, Datta SK, et al. Retrospective surveillance for intussusception in children aged less than five years in a South Indian tertiary-care hospital. J Health Popul Nutr 2009 Oct;27(5):660–5.

[13] Raman T, Mukhopadhyaya A, Eapen CE, Aruldas V, Bose A, Sen S, et al. Intussusception in southern Indian children: lack of association with diarrheal disease and oral polio vaccine immunization. Indian J Gastroenterol Off J Indian Soc Gastroenterol 2003;22(3):82–4.

[14] Singh JV, Kamath V, Shetty R, Kumar V, Prasad R, Saluja T, et al. Retrospective surveillance for intussusception in children aged less than five years at two tertiary care centers in India. Vaccine 2014;32:A95–8.

[15] Bahl R, Saxena M, Bhandari N, Taneja S, Mathur M, Parashar UD, et al. Population-based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among indian children. J Infect Dis 2009;200(s1):S277–81.

[16] Jehangir S, John J, Rajkumar S, Mani B, Srinivasan R, Kang G. Intussusception in southern India: comparison of retrospective analysis and active surveillance. Vaccine. 2014;32:A99–A103.

[17] Jiang J, Jiang B, Parashar U, Nguyen T, Bines J, Patel MM. Childhood Intussusception: A Literature Review. Cameron DW, editor. PLoS ONE. 2013 Jul 22;8(7):e68482.

[18] Rotavirus vaccines WHO position paper. January 2013 – recommendations. Vaccine. 2013;31(52):6170–1.

[19] Tate JE, Steele AD, Bines JE, Zuber PLF, Parashar UD. Research priorities regarding rotavirus vaccine and intussusception: a meeting summary. Vaccine 2012;30:A179–84.

[20] Das M, Arora N, Bonhoeffer J, Zuber P, Maure C. Intussusception in young children: protocol for multisite hospital sentinel surveillance in India. Methods Protoc. 2018;1(2):11.

[21] World Health Organisation. Post-marketing surveillance of rotavirus vaccine safety [Internet]. [cited 2017 Sep 18]. Available from: http://apps.who.int/iris/bitstream/10665/70017/1/WHO_IVB_09.01_eng.pdf.

[22] Mwenda JM, Tate JE, Steele AD, Parashar UD. Preparing for the scale-up of rotavirus vaccine introduction in Africa: establishing surveillance platforms to monitor disease burden and vaccine impact. Pediatr Infect Dis J 2014;33:S1–5.

[23] Jo DS, Nyambat B, Kim JS, Jang YT, Ng TL, Bock HL, et al. Population-based incidence and burden of childhood intussusception in Jeonbuk Province, South Korea. Int J Infect Dis. 2009;13(6):e383–8.

[24] Van Trang N, Le Nguyen NT, Dao HT, Ho VL, Tran DT, Loewen J, et al. Incidence and epidemiology of intussusception among infants in Ho Chi Minh City. Vietnam. J Pediatr. 2014;164(2):366–71.

[25] Greenberg D, Givon-Lavi N, Newman N, Wheeler J, Cohen Z, Dagan R. Intussusception in children in Southern Israel: disparity between 2 populations. Pediatr Infect Dis J 2008;27(3):236–40.

[26] Nakagomi T, Takahashi Y, Arisawa K, Nakagomi O. A high incidence of intussusception in Japan as studied in a sentinel hospital over a 25-year period (1978–2002). Epidemiol Infect 2005;134(01):57.

[27] Srinivasan R, Girish Kumar CP, Naaraayan SA, Jehangir S, Thangaraj JWV, Venkatasubramanian S, et al. Intussusception hospitalizations before rotavirus vaccine introduction: retrospective data from two referral hospitals in Tamil Nadu. India. Vaccine. 2018;36(51):7820–5.

[28] Gupta M, Kanojia R, Singha R, Tripathy JP, Mahajan K, Saxena A, et al. Intussusception rate among under-five-children before introduction of rotavirus vaccine in North India. J Trop Pediatr 2018;64(4):326–35.

[29] Patel MM, Clark AD, Sanderson CFB, Tate J, Parashar UD. Removing the age restrictions for rotavirus vaccination: a benefit-risk modeling analysis. von Seidlein L, editor. PLoS Med. 2012 Oct 23;9(10):e1001330.

[30] Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. The Lancet. 2014;383(9935):2136–43.

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Case Report

A Rare Case of Pediatric Osteochondroma Presenting as Hemothorax

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ABSTRACT

Isolated osteochondroma presenting as hemothorax is a rare entity. A 7-year-old boy presented with respiratory distress and diagnosed with hemothorax, and computed tomography showed osteochondroma and removal with resection of the rib was curative.

KEYWORDS: *Curative resection, hemothorax, osteochondroma*

INTRODUCTION

Osteochondroma is the most common benign lesion of the bones, commonly arising from the growing end of long bones. It is characterized as bony outgrowths from the surface with a cartilaginous cap. It can be solitary or multiple, with the former most commonly seen in clinical practice. Osteochondromas are benign developmental anomalies, characterized by the separation of epiphysis growth-plate cartilage from the main epiphysis.[1]

It is commonly seen on the long bones. However, the index child had an osteochondroma of rib presenting as hemothorax which is exceedingly rare, and hence, presented as a case report.

CASE REPORT

The index case was a 7-year-old boy admitted with complaints of difficulty in breathing along with left-sided chest pain of 2-day duration. There was no history of trauma or infections or any other respiratory symptomatology. The child had no swelling anywhere else in the body, and the parents were normal.

The clinical examination revealed evidence of left pleural effusion. Complete blood count within normal parameters and Mantoux test was negative, and the patient reported no history of recent contact with tuberculosis patients. Coagulation profile was also normal. Chest X-ray [Figure 1] and ultrasound showed collection of moderate amounts of pleural fluid associated with collapse of the left lower lobe.

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Diagnostic aspiration under ultrasound guidance revealed uniformly bloody fluid from the pleural cavity. Computed tomography (CT) of the chest [Figure 2] was done which revealed a speculated tumor mass over the left seventh rib near the anterior end, protruding toward the left lower lobe of the lung which is clearly evident upon three dimensional (3-D) reconstruction. Thus, a diagnosis of bony tumor causing perforative injury to the left lower lobe of the lung causing hemothorax was made.

The child was taken up for the surgery, and thoracoscopy was done initially to identify and localize the lesion and to rule out any complications. The bony spicule over the left seventh rib was resected, which was a 2 cm × 2 cm hard spicule with a smooth surface from the inner surface of the rib injuring the adjacent pleura and lung parenchyma.

The histopathological examination revealed nodular lesion comprising external fibrous tissue, middle cartilaginous tissue, and inner bony trabeculae enclosing the marrow cavity [Figure 3]. There was endochondral ossification with proliferating capillaries, hematopoietic and fatty marrow elements within the lesion. The outer fibrous layer is seen continuous with the periosteum of underlying bony tissue.

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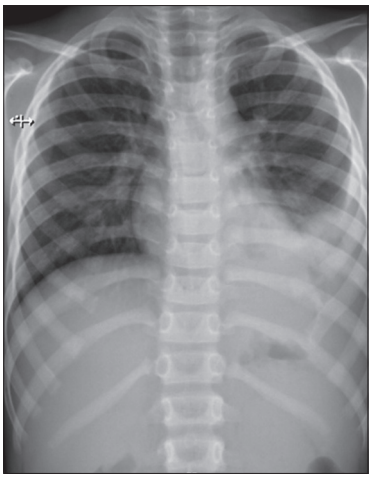


Figure 1: X-ray showing left-sided pleural effusion

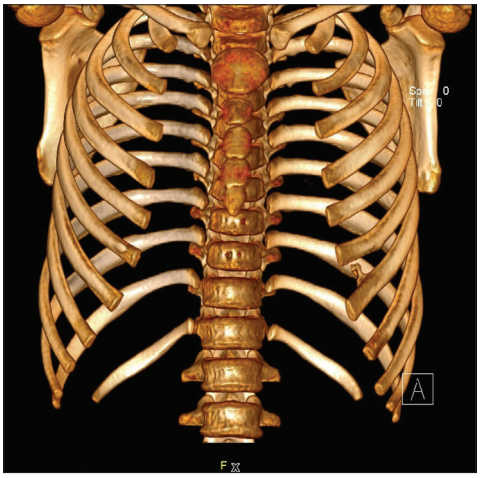


Figure 2: Three-dimensional computed tomography reconstruction showing the osteochondroma involving the anterior portion of the left seventh rib on the inner aspect

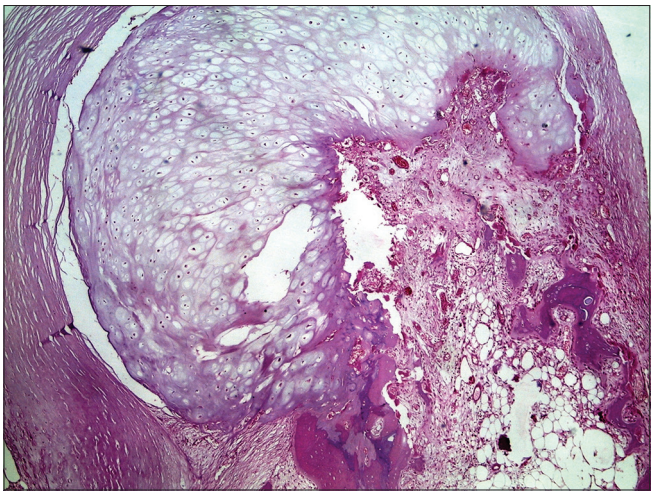


Figure 3: Histopathological examination showing external fibrous tissue, middle cartilaginous tissue, and inner bony trabeculae

These confirmed the diagnosis of osteochondroma of rib, in benign status.

Discussion

Being relatively common tumor of long bones, the incidence of osteochondroma of ribs is exceedingly rare, with an incidence of 1 in 50,000 only.[2] Even rarer is the osteochondroma causing hemothorax in the pediatric age group with only <5 cases reported in previous literature. The rarity of the case was the main reason for reporting it.

Kadu *et al*., have previously reported a similar case of 9-year-old boy with osteochondroma of the right 6th rib. During his follow-up of 3-year period, the tumor did not increase in size or developed any complications and hence was managed conservatively.[3]

Most of the patients are asymptomatic which makes it hard to diagnose. Only very few cases are observed with symptoms such as chest pain.

Osteochondroma starts early in childhood and grows till skeletal maturity is reached. It is asymptomatic when benign and small. The main complications include fracture of rib, vascular injury, osseous deformities, neural compression, and malignant transformation.

Our patient had a rare complication of hemothorax caused by the lesion, possibly due to chronic irritation of the chondroma spicule growing inward into the pleura, causing bleeding from pleural vessels.

The tumor peaks around the age group of 10–30 years with a 3% thoracic origin, which rises to 10% in hereditary multiple exostosis,[4] as evident by the case report submitted by Maeda *et al*., where the children suffering from hereditary multiple exostosis and strong family history, eventually developed osteochondroma in the ribs at latter part of life.[5,6] Hence, it is considered worthwhile to closely follow-up such patients with hereditary multiple exostosis.

Bess *et al*. emphasized the importance of preoperative radiological evaluation to provide optimal data about the lesions such as erosion, internal lytic areas, and destruction of adjacent structures, calcifications, and soft-tissue masses.[6] The size of the cartilaginous cap serves as the best indicator of malignant transformation in osteochondroma.[7]

However, according to Naidu *et al*., like in our index case, CT scan when coupled with reconstruction technique can help to localize the lesion, but the size

is often underestimated because of cartilaginous cap, which can be better visualized by magnetic resonance imaging.[8]

Malignant transformation of osteochondroma is very rare and has been seen only in <1% of solitary and 2% of hereditary multiple exostosis cases.[9]

Few other adult cases of osteochondroma of rib have been reported by Phatak *et al*., but all of them were asymptomatic and were incidentally found, unlike our index child who presented with hemothorax due to osteochondroma.[4]

The presence of complications favored surgical excision as the mainstay of treatment unlike previously reported cases, which were asymptomatic and hence managed conservatively.

This emphasized the importance of osteochondroma as an etiological factor in hemothorax and should be given due consideration while considering a diagnosis in the pediatric age group.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Shim JH, Park CK, Shin SH, Jeong HS, Hwang JH. Solitary osteochondroma of the twelfth rib with intraspinal extension and cord compression in a middle-aged patient. BMC Musculoskelet Disord 2012;13:57.
2. Tateishi U, Yamaguchi U, Miyake M, Maeda T, Chuman H, Arai Y. Primary bone tumours. In: Imaging of the Sternocostoclavicular Region. Germany: Springer, Berlin, Heidelberg; 2007. p. 207-28.
3. Kadu VV, Saindane KA, Goghate N, Goghate N. Osteochondroma of the rib: A rare radiological apeareance. J Orthop Case Rep 2015;5:62-4.
4. Phatak SV, Kolwadkar PK, Rajderkar D. Solitary osteochondroma of rib: A case report. Indian J Radiol Imaging 2006;16:339.
5. Maeda K, Watanabe T, Sato K, Takezoe T, Migita M, Takahashi M, *et al*. Two cases of asymptomatic rib exostosis treated by prophylactic surgical excision. J Pediatr Surg Case Rep 2017;20:24-8.
6. Bess RS, Robbin MR, Bohlman HH, Thompson GH. Spinal exostoses: Analysis of twelve cases and review of the literature. Spine (Phila Pa 1976) 2005;30:774-80.
7. Naidu MC, Rao SS, Krishna CV, Rao S, Kumar PA. Costal osteochondroma of rib: Case report. J Evid Based Med Healthc 2015;2:2624-27.
8. Quirini GE, Meyer JR, Herman M, Russell EJ. Osteochondroma of the thoracic spine: An unusual cause of spinal cord compression. AJNR Am J Neuroradiol 1996;17:961-4.
9. Lee CY, Ham SY, Oh YW, Lee SH, Kim KT. Osteochondroma arising from a rib mimicking a calcifying anterior mediastinal mass. J Korean Radiol Soc 2007;57:533-5.

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BMC Pediatrics

RESEARCH ARTICLE Open Access

Prospective surveillance for intussusception in Indian children aged under two years at nineteen tertiary care hospitals

The INCLEN Intussusception Surveillance Network Study Group

Abstract

Background: India introduced rotavirus vaccines (RVV, monovalent, Rotavac™ and pentavalent, Rotasiil™) in April 2016 with 6, 10 and 14 weeks schedule and expanded countrywide in phases. We describe the epidemiology of intussusception among children aged 2–23 months in India.

Methods: The prospective surveillance at 19 nationally representative sentinel hospitals from four regions recruited children with intussusception from April 2016 to September 2017. Data on sociodemography, immunization, clinical, treatment and outcome were collected. Along with descriptive analysis, key parameters between four regions were compared using Chi-Square/Fisher's exact/Mann–Whitney U/Kruskal-Wallis tests. The pre- and post-RVV periods were compared to estimate the risk ratios.

Results: Six hundred twenty-one children with intussusception from South ($n = 262$), East ($n = 190$), North ($n = 136$) and West ($n = 33$) regions were recruited. Majority ($n = 465$, 74.8%) were infants (40.0% aged 4–7 months) with median age 8 months (IQR 5, 13 months), predominantly males ($n = 408$, 65.7%) and half ($n = 311$, 50.0%) occurred during March–June months. A shorter interval between weaning and intussusception was observed for ragi based food (median 1 month, IQR 0–4.2 months) compared to rice (median 4 months, IQR 1–9 months) and wheat (median 3 months, IQR 1–7 months) based food ($p < 0.01$). Abdominal pain or excessive crying (82.8%), vomiting (72.6%), and bloody stool (58.1%) were the leading symptoms. Classical triad (abdominal pain, vomiting and bloody stool) was observed in 34.8% cases (24.4 to 45.8% across regions). 95.3% of the cases were diagnosed by ultrasound. 49.3% (10.5 to 82.4% across regions) cases were managed by reduction, 39.5% (11.5 to 71.1% across regions) cases underwent surgery and 11.1% spontaneously resolved. Eleven (1.8%) cases died. 89.1% cases met Brighton criteria level 1 and 7.6% met Level 2. RVV was received by 12 cases within 1–21 days prior to intussusception. No increase in case load (RR = 0.44; 95% CI 0.22–1.18) or case ratio (RR = 0.5; 95% CI 0.3–1.2) was observed after RVV introduction in select sites.

Conclusions: Intussusception cases were observed across all sites, although there were variations in cases, presentation and mode of management. The high case load age coincided with age of the RVV third dose. The association with ragi based weaning food in intussusception needs further evaluation.

Keywords: Intussusception, Prospective surveillance, Children, Epidemiology, Weaning food, Sociodemography, Rotavirus vaccine, India

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Background

To reduce the rotavirus diarrhoea related childhood deaths, the World Health Organization (WHO) has recommended introduction of rotavirus vaccine (RVV) in national immunization programmes (NIPs). Some increased risk of intussusception after the first (relative risk, RR: 4.7–13.8) and second (RR: 1.3–5.3) doses of RVVs was reported from several countries (Mexico, Brazil, Australia, United Kingdom, United States, Spain and Singapore) [1–8]. But no increased risk of intussusception after any dose of RVV was observed in some countries (Ethiopia, Ghana, Kenya, Malawi, Tanzania, Zambia, Zimbabwe and South Africa) [9, 10]. The impact of RVV on diarrhoea morbidity and mortality outweigh the risk of intussusception and associated mortality [11]. In view of the concern about intussusception, documentation of baseline and monitoring following RVV introduction have been recommended [12]. India introduced RVV into the NIP in April 2016 and by 2019 expanded countrywide in four phases [13]. Two types of RVV are used in NIP, Rotavac™ (RV1-116E; Bharat Biotech) in 26 states/union territories and Rotasill™ (RV5; Serum. Institute of India) in 11 states/union territories, both follow 6, 10, 14 weeks schedule. Intussusception is an acute severe clinical condition occurring mostly during infancy, which overlaps with the age of primary vaccinations. The nationally representative background epidemiology of intussusception in India is not clear. The reports from India primarily included retrospective data and considerably varied in the epidemiology, presentation and management [14, 15]. Information on population level rate in India is limited and the reported incidences of intussusception requiring hospitalization varied from 17.7 (95% CI 5.9–41.4) in Delhi (North India) to 254 (95% CI 102, 524) cases per 100,000 child-years in Vellore (South India) [16, 17]. The incidence of intussusception vary widely globally, across the different high and low-middle income countries [18]. The reasons for the variations are unknown. There is no information from India regarding the regional variation in intussusception epidemiology. Thus, documentation of the intussusception epidemiology prior to RVV introduction to establish a reliable baseline for monitoring the trend over time and identify potential risk factors was needed to support the vaccine safety surveillance efforts [19, 20]. Under the vaccine safety surveillance effort, we describe the epidemiology, clinical characteristics of intussusception among children aged under-2 years seeking hospital care in India and initial changes with RVV introduction documented through a nationally representative sentinel surveillance network.

Methods

Study area and participating hospitals

This prospective active hospital-based sentinel surveillance was conducted over 18 months, at 19 nationally representative tertiary care hospitals (Supplementary Figure 1). From the four regions, 3–6 hospitals per region including medical colleges and private-sector hospitals (North region- 5 sites, 3 public and 2 private; South region- 5 sites, 2 public and 3 private; East region- 6 sites, 5 public and 1 private; West region- 3 sites, 2 public and 1 private) were selected through a systematic process. Out of these sites, four sites were located in the states where RVV was introduced in April 2017 under phase 1. At all sites the RVVs were available in private market during the study period, even prior to the introduction in NIP.

Case definition, case selection and data collection

The children aged 2–23 months admitted to these hospitals with diagnosis of intussusception were eligible. All the age-eligible patients admitted were screened to identify the suspected cases (any of the diagnoses: intussusception, acute or subacute intestinal obstruction, acute abdomen, abdominal pain, abdominal distension, and blood in stool with vomiting). These suspected cases were tracked till final diagnosis and all confirmed intussusception cases were recruited after written informed consent from parent or legally authorised representative. A log of screened, suspected and confirmed cases was maintained. The data on socio-demography, feeding and immunization (from immunization card), clinical features, investigation findings, hospital course, treatment and outcome were collected using common case record form (CRF). The symptoms were captured as recorded in the case sheets and reported by the parents. An independent Case Adjudication Committee (CAC) comprised of a paediatrician, paediatric surgeon, and radiologist reviewed the CRFs and investigations to assign the diagnostic certainty levels, according to Brighton Collaboration case definition (BCCD) [21].

Quality assurance

Multilevel quality assurance and data quality-checking processes were put in place to ascertain protocol adherence, rigor and completion of surveillance at all sites. A data team reviewed all the CRFs and data-related query was resolved at the earliest. Each site was visited by external experts (Technical Advisory Group, TAG) to assess the case surveillance and tracking, consent, and data extraction quality and completeness in the CRFs. The TAG members checked CRFs completeness and quality for few randomly identified cases with the case records. Subsequently members from the data team visited the study sites and checked the admissions for the study period from the medical records section using diagnoses and International Classification of Diseases (ICD) codes (ICD-9/10, codes listed in Supplementary Table 1), to identify any missed cases.

Data management and analysis

Double data entry was done for the CRFs using a customised data entry platform. The matched and verified data were stored in the server with authorised access

and daily backup. The standard of living index (SLI), representing the socioeconomic status was estimated using the scores for household assets ownership, with reference to the National Family and Health Survey for India [22]. The SLI was categorised into high, medium and low categories. The cases were categorised into levels 1 to 3 based on the BCCD criteria by the CAC [21]. In view of the number of sites, for representation we grouped the sites into four regions, North (5 sites), South (5 sites), East (6 sites) and West (3 sites) regions. The parameters (sociodemographic, feeding, clinical, intervention and outcomes were compared between the regions to detect variations. The intussusception classical triad includes three features; abdominal pain, vomiting and blood in stools. We considered intussusception modified triad with abdominal pain, vomiting and rectal bleeding, detected either as blood in stool or blood on per-rectal examination. The descriptive analysis findings expressed the outputs as proportions, means and standard deviations, or median and interquartile range (IQR), as appropriate. The values between regions and groups were compared for statistical significance using Chi-Square or Fisher's exact tests for the proportions and Mann–Whitney U or Kruskal-Wallis tests for the medians depending on the skewness, sample and number of groups. The missing data were excluded from analysis. The statistical significance was considered if $p < 0.05$. The statistical analysis was performed using STATA version 15.0 (StataCorp LLC, Texas, USA). In the Indian context, there is no definite population catchment area for hospitals and no definite referral chain, which makes estimation of the intussusception incidence difficult. For comparison of the data across sites and intussusception time trend, in addition to case load (absolute number of cases), we attempted deriving the intussusception case rate per 1000 paediatric hospitalisations at the hospitals. On review, while the admission numbers in the paediatric medicine wards varied widely, the admission numbers in the paediatric surgery wards were relatively stable and the intussusception cases were primarily managed in the paediatric surgery wards. Thus we estimated the intussusception case rate per 1000 paediatric surgery admissions at these hospitals for comparison and trend analysis. For the four sites in three states (Odisha, Andhra Pradesh and Haryana), where RVV (monovalent Rotavac™, 3 doses at 6, 10, 14 weeks age) was introduced under NIP (in April 2017), the data for the post-introduction period (April to September 2017, 6 months) was compared with the pre-introduction periods (first: October 2016 to March 2017, immediate 6 months pre-introduction period and second: April 2016 to September 2016, calendar matched 6 months during the previous year) to document the risk ratio (95% confidence interval, CI). The detailed methodology of the site selection and study implementation has been published as protocol [23].

Ethical issues

Informed written consent was obtained for all the eligible cases before recruitment and data collection. Confidentiality in data handling was maintained. The study protocol was reviewed and approved by the ethics committees of all participating institutes.

Results

Between April 2016 and September 2017, out of the 182,824 children (including 32,910 paediatric surgery admissions) admitted to the network hospitals, 1203 suspected intussusception cases were identified and 621 eligible children were recruited (Supplementary Figure 2). More cases were recruited from Southern region (42.2%; 262/621) followed by East (30.6%; 190/621), North (21.9%; 136/621) and West (5.3%; 33/621) regions. Past history of intussusception was present in 24 (3.8%) cases. Table 1 shows the sociodemographic characteristics of children with intussusception. A male predominance (male-female ratio: 1.9:1) was consistent across all regions. The median age at presentation was 8 months (IQR 5, 13 months) (Supplementary Figure 3). Three quarters of the cases were infants with equal share from the age bands of 2–6 months (37.2%) and 7–12 months (37.7%) ($p < 0.01$). Children aged 4–7 months contributed to 40.0% of the total cases. The pooled intussusception case rate per 1000 paediatric surgery admission was 18.4 (IQR 14.7, 23.4). The intussusception case rate per 1000 paediatric surgery admission was highest for South (25.3, IQR 23.4, 32.7) followed by North (14.5, IQR 11, 19.7), East (13.8, IQR 10.5, 22) and West (6.6, IQR 1.2, 19.2) regions respectively. The Fig. 1 shows monthly trends of pooled intussusception case load and case rates per 1000 paediatric surgery admission (Fig. 1a) and the regional case rates per 1000 paediatric surgery admission (Fig. 1b). More cases ($n = 311$, 50.0%) were seen during March to June months, in the summer season (Fig. 1). While 42.8% of the patients were resident of the same district where the hospital was based, 41.9% of the patients presented directly to the hospital. Among the children aged > 6 months, 23.2% were exclusively breastfed for 6 months and the median duration of breastfeeding was 4 months. Mixed feeding was initiated before 6 months of age in 53.9% children. The median weaning age was 6 months and 61.0% received rice based food. Ragi (finger millet) was given to 22.8% children, only in the South region. The interval between weaning and intussusception was significantly shorter for ragi (median 1 month, IQR 0–4.2 months) than rice (median 4 months, IQR 1–9 months) and wheat (median 3 months, IQR 1–7 months) based food (Table 1) ($p < 0.01$). On analysis for the South

Table 1 The sociodemographic parameters and dietary practices of children with intussusception in India

Variable Category		North (*n* = 136)	South (*n* = 262)	East (*n* = 190)	West (*n* = 33)	Total (*n* = 621)	*P* value
Age (in months)	2–6, n (%)	55 (40.4)	88 (33.6)	78 (41.1)	10 (30.3)	231 (37.2)	< 0.01
	7–12, n (%)	57 (41.9)	92 (35.1)	73 (38.4)	12 (36.4)	234 (37.7)	< 0.01
	13–18, n (%)	16 (11.8)	40 (15.3)	25 (13.2)	3 (9.1)	84 (13.5)	< 0.01
	19–23, n (%)	8 (5.9)	42 (16.0)	14 (7.4)	8 (24.2)	72 (11.6)	< 0.01
Gender	Male, n (%)	86 (63.2)	170 (64.9)	128 (67.4)	24 (72.7)	408 (65.7)	< 0.01
	Female, n (%)	50 (36.8)	92 (35.1)	62 (32.6)	8 (24.2)	212 (34.1)	< 0.01
	Other, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)	1 (0.2)	–
Family	Nuclear, n (%)	89 (65.4)	171 (65.2)	65 (34.2)	18 (54.5)	343 (55.2)	0.06
	Joint/extended, n (%)	47 (34.6)	91 (34.7)	125 (65.8)	15 (45.5)	278 (44.8)	0.06
Religion	Hindu, n (%)	48 (35.3)	179 (68.3)	136 (71.6)	22 (66.6)	385 (62.0)	< 0.01
	Muslim, n (%)	88 (64.7)	41 (15.6)	52 (27.3)	11 (33.3)	192 (30.9)	< 0.01
	Christian, n (%)	0 (0.0)	42 (16.0)	1 (0.5)	0 (0.0)	43 (7.0)	–
SLI status[a]	High, n (%)	56 (41.2)	93 (35.5)	19 (10.0)	6 (18.2)	174 (28.0)	< 0.01
	Medium, n (%)	33 (24.3)	124 (47.3)	65 (34.2)	6 (18.2)	228 (36.7)	< 0.01
	Low, n (%)	47 (34.5)	45 (17.2)	106 (55.8)	21 (63.6)	219 (35.3)	< 0.01
Place of residence	Same district, n (%)	49 (36.0)	162 (61.8)	35 (18.4)	20 (60.6)	266 (42.8)	< 0.01
	Other districts[b], n (%)	74 (54.4)	86 (32.8)	151 (79.5)	13 (39.4)	324 (52.2)	< 0.01
	Outside state, n (%)	13 (9.6)	14 (5.3)	4 (2.1)	0 (0.0)	31 (5.0)	< 0.01
Referral status	Primary[c], n (%)	91 (66.9)	64 (24.4)	87 (45.8)	18 (54.6)	260 (41.9)	< 0.01
	Referred[d], n (%)	45 (33.1)	198 (75.6)	103 (54.2)	15 (45.5)	361 (58.1)	< 0.01
Feeding practices	Exclusively breastfed for 6 months[e], n/N (%)	3/26 (11.5)	16/52 (30.7)	18/85 (21.2)	1/1 (100)	38/164 (23.2)	< 0.01
	Mixed food before 6 months age[e], n/N (%)	25/78 (32.0)	130/197 (66.0)	46/100 (46.0)	12/20 (60.0)	213/395 (53.9)	0.08
	Exclusive breastfeeding period (in months), median (IQR)	5 (4–6)	4 (2–5)	4 (3–5)	5 (3.5–5)	4 (3–7)	–
	Mixed food initiation age in months, median (IQR)	6 (5–8)	4 (2–6)	5 (3–6)	5 (4–6)	5 (3–7)	–
	Weaning age in months, median (IQR)	7 (6–8.2)	6 (4–6)	6 (5–7)	6 (5–7)	6 (5–7)	–
Weaning food type	Rice, n (%)	48 (68.6)	116 (53.0)	80 (88.9)	5 (18.5)	249 (61.0)	< 0.01
	Wheat n (%)	12 (17.1)	5 (23.0)	3 (3.3)	11 (40.7)	31 (8.0)	< 0.01
	Ragi based[f], n (%)	0 (0.0)	50 (22.8)	0 (0.0)	0 (0.0)	50 (12.0)	–
	Other, n (%)	4 (5.7)	7 (3.2)	3 (3.3)	4 (14.8)	18 (4.0)	0.08
	Mixed n (%)	6 (8.6)	41 (18.7)	4 (4.4)	7 (25.9)	58 (14.0)	0.05
Weaning initiation to illness onset interval (in months)	Rice based, median (IQR)	3 (0–6)	3 (1–9)	4 (1–9)	4 (2–11.5)	4 (1–9)	0.08
	Wheat based, median (IQR)	3 (2.5–7)	2.5 (1.2–5.2)	0 (0–1)	3 (2–7)	3 (1–7)	0.05
	Ragi[f], median (IQR)	–	1 (0–4.2)	–	–	1 (0–4.2)	–
	Other food, median (IQR)	4 (1.5–6)	1 (0–3)	0 (0–1)	9.5 (5.2–13)	2.5 (0.7–5.5)	0.09
	Multiple food, median (IQR)	7 (2.5–9)	6 (2.7–12)	6 (3–12)	6 (3–12)	6 (3–12)	0.1
	Any food, median (IQR)	3 (1–7)	3 (1–8)	3.5 (1–8.2)	3 (1–9)	3.5 (1–8.2)	0.1

Notes:
Mixed food: Food used for weaning included at least two of the ingredients- rice, wheat, lentil and vegetables & fruits
[a]SLI: Standard of living index, was estimated using the household assets ownership (22)
[b]The residence of the patient was in other district in the state where the study hospital was based
[c]The patient presented to the study hospital as the first institute
[d]The patient was referred to the study hospital from another hospital
[e]The denominators (N) indicate valid responses received for the parameters
[f]Ragi: finger millet
The p values for some parameters could not be estimated due to small or no value in at least two groups

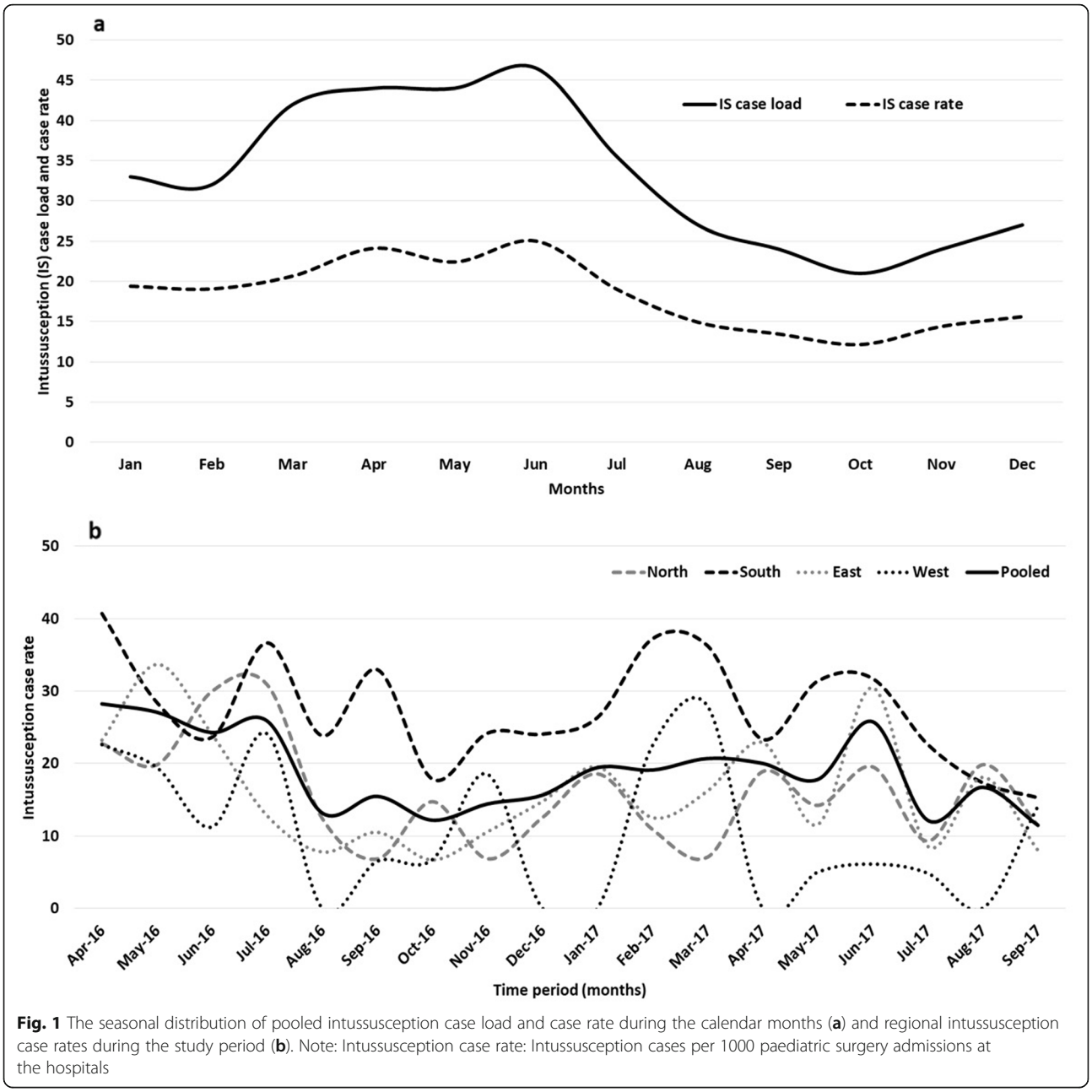


Fig. 1 The seasonal distribution of pooled intussusception case load and case rate during the calendar months (**a**) and regional intussusception case rates during the study period (**b**). Note: Intussusception case rate: Intussusception cases per 1000 paediatric surgery admissions at the hospitals

region only (where ragi based weaning practice was observed), the median interval between weaning and intussusception for ragi based food was significantly shorter (median 1 month, IQR 0–4 months) than rice (median 5 months, IQR 2–9 months) and wheat (median 3 months, IQR 2–8 months) based food ($p < 0.01$).

The pooled median interval between the onset to hospital admission was 2 days (IQR 1, 3), which varied across the regions; North- 2 days (IQR 1, 3), South- 1 day (IQR 1, 3), East- 2 days (IQR 1, 3) and West- 2 days (IQR 1, 2) ($p = 0.07$). Table 2 summarises the clinicopathological parameters and management for the recruited children. Abdominal pain or excessive crying (82.6%) was the most common symptom followed by vomiting (72.6%) and blood in stool (58.1%), as reported by the parents. More children had abdominal pain or excessive crying in North (84.6%) and East (72.6%) regions and blood in stool in the East region (73.2%). The classical triad (abdominal pain, vomiting and blood in stool) was observed in 34.8% cases, with 24.4 and 45.8% in the South and in East regions, respectively ($p < 0.01$). When blood on per-rectal examination was included, the modified intussusception triad (abdominal pain, vomiting and blood in stool or blood on per-rectal examination) was

Table 2 The clinicopathological characteristics and management of the children with intussusception in India

Variable Category		North (*n* = 136)	South (*n* = 262)	East (*n* = 190)	West (*n* = 33)	Total (*n* = 621)	*P* value
Symptoms, n (%)	Vomiting[a]	100 (73.5)	187 (71.4)	137 (72.1)	27 (81.8)	451 (72.6)	0.64
	Bilious vomiting[b]	21 (15.4)	26 (9.9)	34 (17.9)	9 (27.3)	90 (14.5)	0.02
	Abdominal pain	102 (75.0)	118 (45.0)	138 (72.6)	10 (30.3)	368 (59.3)	< 0.01
	Excessive crying	49 (36.0)	172 (65.7)	109 (57.4)	17 (51.5)	347 (55.9)	< 0.01
	Abdominal distension	34 (25.0)	29 (11.1)	69 (36.3)	3 (9.1)	135 (21.7)	< 0.01
	Rectal bleeding	81 (59.6)	123 (47.0)	139 (73.2)	18 (54.6)	361 (58.1)	< 0.01
	Diarrhoea	23 (16.9)	69 (26.3)	37 (19.5)	9 (27.3)	138 (22.2)	0.11
	Constipation	18 (13.2)	43 (16.4)	12 (6.3)	3 (9.1)	76 (12.2)	0.01
	Fever	34 (25.0)	64 (24.4)	31 (16.3)	13 (39.4)	142 (22.9)	0.02
	Lethargy	2 (1.5)	16 (6.1)	16 (8.4)	2 (6.1)	36 (5.8)	0.07
	Altered sensorium	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	–
	Classical triad[c]	52 (38.2)	64 (24.4)	87 (45.8)	13 (39.4)	216 (34.8)	< 0.01
Signs, n (%)	Pallor	11 (8.1)	14 (5.3)	18 (9.5)	3 (9.1)	46 (7.4)	0.39
	Dehydration	7 (5.2)	21 (8.0)	43 (22.6)	2 (6.1)	73 (11.8)	< 0.01
	Fever	4 (2.9)	23 (8.8)	23 (12.1)	5 (15.2)	55 (8.9)	0.02
	Lethargy	11 (8.1)	14 (5.3)	20 (10.5)	3 (9.1)	48 (7.7)	0.23
	Abdominal distension	22 (16.2)	25 (9.5)	62 (32.6)	3 (9.1)	112 (18.0)	< 0.01
	Abdominal tenderness	13 (9.6)	26 (9.9)	26 (13.7)	5 (15.2)	70 (11.3)	0.48
	Abdominal mass	13 (9.6)	91 (34.7)	24 (12.6)	4 (12.1)	132 (21.3)	< 0.01
	Bowel sound (absent/ abnormal)	11 (8.1)	10 (3.8)	43 (22.6)	2 (6.1)	66 (10.6)	< 0.01
	Rectal prolapse	15 (11.0)	2 (0.8)	1 (0.5)	0 (0.0)	18 (2.9)	< 0.01
	Rectal mass	3 (2.2)	4 (1.5)	11 (5.8)	0 (0.0)	18 (2.9)	0.04
	Blood on PR examination	19 (14.0)	31 (11.8)	94 (49.5)	19 (57.6)	163 (26.3)	< 0.01
	Modified triad[d]	83 (61.0)	125 (47.7)	143 (75.3)	21 (63.6)	372 (59.9)	< 0.01
Diagnosis method, n (%)	Ultrasound	121 (89.0)	262 (100)	179 (94.2)	30 (90.9)	592 (95.3)	< 0.01
	CT scan	2 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.3)	–
	Barium enema	1 (0.7)	0 (0.0)	1 (0.5)	1 (3.0)	3 (0.5)	–
	Surgery	12 (8.8)	0 (0.0)	10 (5.3)	2 (6.1)	24 (3.9)	< 0.01
Intussusception location, n (%)	Colo-colic	3 (2.2)	10 (3.8)	5 (2.6)	3 (9.1)	21 (3.4)	< 0.01
	Ileo-colo-colic	8 (5.9)	7 (2.7)	4 (2.1)	2 (6.1)	21 (3.4)	< 0.01
	Ileo-ileal	11 (8.1)	3 (1.2)	10 (5.3)	6 (18.2)	30 (4.8)	< 0.01
	Ileo-ileo-colic	4 (2.9)	5 (1.9)	6 (3.2)	0 (0.0)	15 (2.4)	< 0.01
	Ileocolic	109 (80.2)	234 (89.3)	163 (85.8)	20 (60.1)	526 (84.7)	< 0.01
	Jejuno-jejunum	0 (0.0)	0 (0.0)	1 (0.9)	1 (3.0)	2 (0.3)	–
	> 1 location[e]	1 (0.7)	3 (1.2)	1(0.5)	1 (3.0)	6 (1.0)	–
Pathological lead point, n (%)	Lymph node	41 (30.1)	19 (7.2)	3 (1.6)	15 (45.4)	78 (12.6)	< 0.01
	Appendix	0 (0.0)	0 (0.0)	2 (1.0)	1 (3.0)	3 (0.4)	–
	Payer's patch	1 (0.7)	1 (0.3)	1 (0.5)	0 (0.0)	3 (0.4)	–
	Polyp	1 (0.7)	0 (0.0)	0 (0.0)	1 (3.0)	2 (0.3)	–
	Others[f]	2 (1.4)	0 (0.0)	3 (1.6)	0 (0.0)	5 (0.8)	–
	Any lead point	45 (33.0)	20 (7.6)	9 (4.7)	17 (51.5)	91 (14.6)	< 0.01
Treatment modality, n (%)	Reduction	56 (41.2)	216 (82.4)	20 (10.5)	14 (42.4)	306 (49.3)	< 0.01
	Surgery	68 (50.0)	30 (11.5)	135 (71.1)	12 (36.4)	245 (39.5)	< 0.01

Table 2 The clinicopathological characteristics and management of the children with intussusception in India *(Continued)*

Variable Category		North (*n* = 136)	South (*n* = 262)	East (*n* = 190)	West (*n* = 33)	Total (*n* = 621)	*P* value
	Spontaneously resolved[g]	12 (8.8)	16 (6.1)	34 (17.9)	7 (21.2)	69 (11.1)	< 0.01
	None[h]	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.2)	–
Surgery type, n (%)	No resection[i]	41 (60.3)	25 (83.3)	106 (78.6)	9 (75)	181 (73.9)	< 0.01
	With resection[i]	27 (39.7)	5 (16.7)	29 (21.5)	3 (25.0)	64 (26.1)	< 0.01
Outcome, n (%)	Discharged	129 (94.9)	262 (100)	181 (95.3)	31 (93.9)	603 (97.1)	< 0.01
	Referred	0 (0.0)	0 (0.0)	1 (0.5)	2 (6.1)	3 (0.5)	–
	LAMA[j]	3 (2.2)	0 (0.0)	1 (0.5)	0 (0.0)	4 (0.6)	–
	Died	4 (2.9)	0 (0.0)	7 (3.7)	0 (0.0)	11 (1.8)	–
Brighton Collaboration Criteria level, n (%)	Level 1	125 (91.9)	246 (93.9)	156 (82.1)	26 (78.8)	553 (89.1)	< 0.01
	Level 2	5 (3.7)	15 (5.7)	24 (12.6)	3 (9.1)	47 (7.6)	< 0.01
	Level 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	–
	No level	6 (4.4)	1 (0.4)	10 (5.3)	4 (12.1)	21 (3.4)	< 0.01
Illness in last 4 weeks, n (%)	Diarrhoea	10 (7.4)	55 (21.0)	11 (5.8)	4 (12.1)	80 (12.9)	0.04
	ARI	17 (12.5)	104 (39.7)	21 (11.1)	5 (15.2)	147 (23.7)	0.04

Notes: *PR* Per-rectal, *ARI* Acute respiratory illnesses
[a]Includes all vomiting including bilious vomiting
[b]Includes only bilious vomiting
[c]Classical triad includes abdominal pain, vomiting and blood in stool
[d]Modified triad includes abdominal pain, vomiting and rectal bleeding (detected either as blood in stool or blood on per-rectal examination)
[e]The location of intussusception at more than one site; such as Ileo-colic + Colo-colic, Ileo-colic + Ileo-colo-colic, Ileo-colic + Ileo-ileo-colic, Ileo-colic + Ileo-ileal
[f]Others include cyst, Meckel's diverticulum, incisional hernia, intraluminal growth and malrotation
[g]Spontaneously resolved: The patient was managed with IV fluids and nil orally, not required any additional intervention
[h] None- No definite treatment could be provided, as the patient(s) was referred or died before any definite treatment
[i] The denominators used for percentage estimation include patients undergone surgery only
[j]LAMA: Left against medical advice
The p values for some parameters could not be estimated due to no value in at least two groups

present in 59.9% cases; 47.7% in South to 75.3% in East regions ($p < 0.01$).

Most of the cases (95.3%) were diagnosed by ultrasound. Ileocolic (84.7%) was the most common site of intussusception. A pathological lead point (PLP) was documented in 91 (14.6%) cases and lymph nodes or Payer's patch were the commonest (13.0%) and few had appendix or polyps (Table 2). While 49.3% cases were reduced, 39.5% underwent surgery and 11.1% resolved spontaneously. More children in East region (71.1%) underwent surgery followed by North region (50.0%) ($p < 0.01$). Among those who underwent surgery, 26.1% children required bowel resection, and 39.5% were from the North region. The onset-admission interval was longer for the cases who underwent surgery (median 2 days; IQR 1, 3) than reduction (median 1 day; IQR 1, 3) ($p < 0.01$) (Supplementary Table 2). Among the children who presented on the day of onset, 62.3% were managed by reduction (28.9% required surgery), which declined to 35.1% when they presented after 3 days (48.3% required surgery) ($p < 0.01$) (Supplementary Table 2). Seven hospitals (East-1, North-1, and South-1) were conducting only surgical management. At the hospitals with both facilities (reduction and surgery), 31.0% (127/404) children underwent surgery for indications: failed reduction (32.2%), late presentation (> 3 days since onset, 28.3%) and associated complications (39.3%) ($p < 0.01$). Most of the cases (97.1%) recovered and were discharged. Eleven cases (North-4 and East-7) died of post-surgery sepsis, shock and multiorgan failure. The median hospitalisation period was 3 days (IQR 2, 6 days) ranging from 2 to 5 days (South: 2 days, IQR 2, 3 days; North: 3 days, IQR 1, 7 days; West: 4 days, IQR 2, 6 days; and East: 5 days, IQR 3, 8 days; $p < 0.01$). The median hospital stay periods for children who underwent surgery, reduction and spontaneously resolved were 7 days (IQR 5, 9 days), 2 days (IQR 1, 2 days) and 3 days (IQR 2, 5 days), respectively ($p < 0.01$) (Supplementary Table 2). CAC assigned 89.1% (553/621) and 7.6% cases as Level 1 and 2 respectively according to the BCCD. Diarrhoea and acute respiratory illnesses (ARIs) within 4 weeks prior to intussusception was reported in 12.9 and 23.7% children respectively. More children from South region had history of diarrhoea (21%) and ARIs (39.7%) than other regions ($p < 0.05$).

Vaccination information was available for 487 (78.4%) children and 391 (80.2%) of those with vaccination information had no RVV exposure. Out of 96 (15.4%) children who received any RVV (RVV-1, $n = 96$; RVV-2, $n = 88$; and RVV-3, $n = 65$), 12 children received RVV in

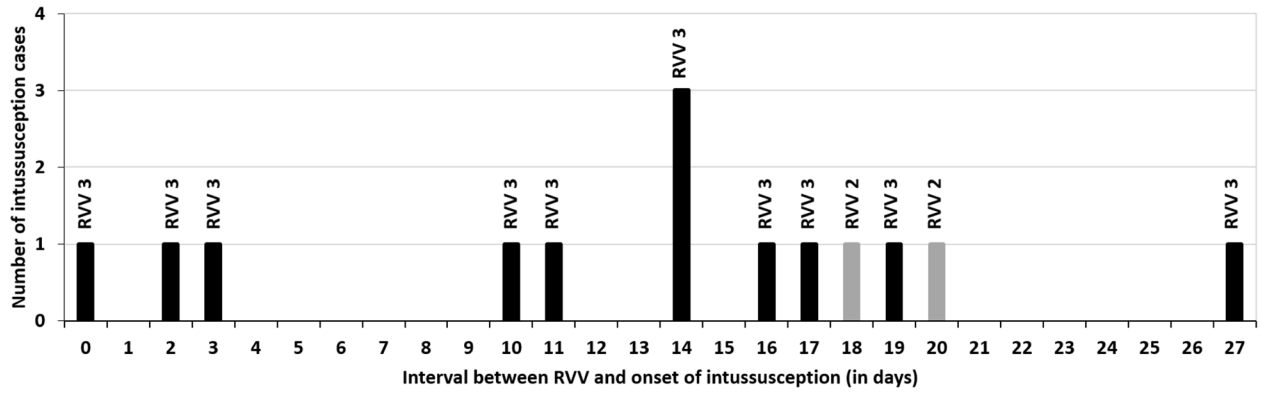


Fig. 2 Interval between last rotavirus vaccination and onset of intussusception within 28 days in the children. Note: RVV: Rotavirus vaccine

the 1–21 days preceding onset of intussusception and most ($n = 10$) after RVV-3 (median age 3.8 months, IQR 3.6, 4.2 months) (Supplementary Table 3). As shown in Fig. 2, only two cases occurred during 1–7 days after the third dose RVV and one case occurred on the vaccination day. At the four sites from three states where RVV (Rotavac™) was introduced, no increase in either intussusception case load (RR = 0.44; 95% CI 0.22, 1.18) or case rate per 1000 paediatric surgery admission (RR = 0.5; 95% CI 0.3, 1.2) during the post-RVV introduction period were observed (Table 3).

Discussion

We observed regional variances in the intussusception case load and case rate per 1000 paediatric surgery admission, high in the South region and low in the West region. Higher number of cases were observed during March to June months, which was comparable to other reports from India [14, 15, 17, 24, 25]. The male predominance (65.7%), median age at 8 months and high case load at 4–7 months of age (40.0%) were similar to other reports from India and globally (proportion of male: 63.0–77.2%; median age: 5–8 months and high case load age: 4–9 months) [14, 15, 17, 18, 21, 24, 26, 27]. About 37.0% of cases occurred during 2nd to 6th month of age, the usual age for administration of RVV. The children came from all socioeconomic categories and in all the regions. The proportion of exclusively breastfed children (< 6 months) was lower than national average (52.1%, 2015–16) [22]. The children weaned with ragi food (only in South region) had intussusception earlier (median 1 month) than those weaned with wheat (median 3 months) and rice (median 4 months) based food. A shorter exclusive breastfeeding duration with mixed feeding and some weaning foods may have some role in intussusception occurrence. Exposure to foreign proteins and enteric infections are potential risk factors for intussusception, but we couldn't find any report on association with any specific diet. There were significant variations for several sociodemographic and dietary practice parameters across the regions (Table 1). The classical triad was documented in 34.8% cases, which was higher than reports

Table 3 The intussusception cases and case ratios during post-rotavirus vaccine introduction period compared to pre-introduction periods

	Post-RVV period (Apr- Sep 2017)		Pre-RVV period (Oct 2016 - Mar 2017)				Pre-RVV period (Apr- Sep 2016)			
Region (number of sites)[a]	No of cases	IS case rate	No of cases	IS case ratio	RR (95% CI) for cases	RR (95% CI) for IS case rate	No of cases	IS ratio	RR (95% CI) for cases	RR (95% CI) for IS case rate
East ($n = 2$)[a]	20	20.4	29	29.9	0.69 (0.33–1.2)	0.67 (0.3–1.28)	45	34.6	0.44 (0.2–1.15)	0.58 (0.32–1.2)
North ($n = 1$)[a]	0	0	2	27.4	–	–	3	37.5	–	–
South ($n = 1$)[a]	6	13.3	8	20.4	0.75 (0.45–1.24)	0.65 (0.48–1.23)	11	26.4	0.55 (0.28–1.2)	0.51 (0.3–1.22)
Pooled ($n = 4$)[a]	26	16.3	39	27.2	0.67 (0.34–1.19)	0.6 (0.3–1.18)	59	32.8	0.44 (0.22–1.18)	0.5 (0.3–1.2)

Note: *IS* Intussusception, *Intussusception (IS) case rate* Intussusception cases per 1000 paediatric surgery admission, *RVV* Rotavirus vaccine, *RR* risk ratio with 95% confidence interval (CI), *RVV*: Rotavirus vaccine

The comparison includes the data from four study sites in three states

[a]Numbers in the bracket with the regions indicate the numbers of study sites per region where RVV was introduced

Post-RVV period: April 2017 to September 2017 (6 months)

Pre-RVV period: Comparison has been done for two pre-intervention periods of 6 months each; October 2016 to March 2017- the immediate pre-RVV introduction period and April 2016 to September 2016- the calendar months comparable period during the previous year

from India (19.0%) and South Korea (7.6%), but lower than Tanzania (42.5%) [15, 24, 28]. The regional variation in the classical triad appeared to parallel with the interval between illness onset and hospitalization. Ultrasound was the commonest (95.3%) mode of diagnosis, as reported (72.0–100%) from India and other countries [15, 18, 28–30]. Ileocolic was the most common location, similar to reports (68.0–79.0%) from India and globally [3, 7, 8, 14]. PLPs observed in this study was higher than reported in studies (8.0–9.0%) from India and Tanzania with appendix and lymph nodes as the common [31, 32]. The cases who presented early were more frequently managed non-surgically with shorter stay (2–3 days) compared to those with surgical intervention (7 days). There were significant variations in the clinical features among the children across the regions, which may be due to the interval for presentation or clinical practices followed at the hospitals (Table 2). Death was observed in 1.7% cases, which was comparable to reports from India (1.0%) and other countries from Asia (0.25–6.0%), Latin America (1.0–5.0%), but lower than the African countries (2.0–25.0%) [17, 18, 24, 28, 29, 31–33]. Level 1 BCCD was met by 89.1% of the cases, similar to other reports from India [5, 7, 14]. Failed documentation of reduction prevented some cases meeting the BCCD level 1 certainty.

From the limited period of post-RVV (Rotavac™) introduction at four sites (from three states), no increase in the intussusception case load and case rate per 1000 paediatric surgery admission were observed. Intussusception within 1–21 days was observed mostly after the third RVV dose, which also overlaps with the age of natural occurrence. Under the NIP, RVV is given at 6, 10, 14 weeks of age and allowed before 12 months of age [34]. A delay in RVV administration may coincide with the high case load age of natural intussusception (4–7 months age), which may make the interpretation of association with the vaccine difficult. In routine practice, documentation of vaccine exposure should improve and appropriate guidance to parents be given regarding the subsequent RVV vaccination. Monitoring of intussusception risk is recommended for countries as part of the RVV introduction program [35, 36]. In the absence of any specific population denominator for tertiary care hospitals, estimation of incidence in Indian context is difficult. The intussusception case rate per 1000 paediatric surgery admissions may be a proxy indicator for monitoring and comparison across the sites and regions with RVV introduction.

Early suspicion, case detection and referral to appropriate hospital are critical for minimizing the surgical interventions and favourable outcomes. Efforts are needed to equip and enable the public health facilities for non-surgical management in children, though this depends on the timing of the presentation to hospital.

The epidemiology, clinical presentation and management of intussusception in children across the regions may serve as baseline for future studies. The regional representation and mix of private- and public-sector hospitals were the advantages for this surveillance.

This study had some limitations. In absence of definite catchment population and referral pattern, population-based incidence or case rate estimation was not possible. The etiologies and risk factors for intussusception were not studied.

Conclusion

To conclude, the intussusception cases were seen all across the country and the majority of the cases occurred during the first year of life. The high case load age (4–7 months) for intussusception in children coincided with the age of RVV third dose. Some variations in case loads across the different regions and seasonality were observed. The potential role of diet exposure, weaning practices and food like ragi in intussusception needs further evaluation. No increased occurrence of intussusception cases was observed during the limited RVV post-introduction period. Early case detection, prompt referral and appropriate management are needed to avoid surgical intervention and complications. Immunization exposure must be documented to assess the vaccine associated risk. In absence of population-based incidence or case rate, the intussusception case rate per 1000 paediatric surgery admission may be used for inter-regional comparison and trend monitoring.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12887-020-02293-5.

Additional file 1. Supplementary document 1- Supplementary Figure 1: The study sites and their locations according to the regions. Supplementary document 2- Supplementary Table 1: The International Classification of Diseases (ICD) codes for review of the cases from medical records. Supplementary document 3- Supplementary Figure 2: The flow chart for case screening and recruitment. Supplementary document 4- Supplementary Figure 3: Age distribution of children with intussusception in India (region wise and pooled). Supplementary document 5- Supplementary Table 2: The mode of treatment according to the interval between onset, admission and intervention for the children with intussusception. Supplementary document 6- Supplementary Table 3: The intussusception cases during risk periods after rotavirus vaccine exposure.

Abbreviations

CRF: Case record form; CAC: Case Adjudication Committee; ICD: International Classification of Diseases; IQR: Interquartile range; TAG: Technical Advisory Group; WHO: World Health Organization; RVV: Rotavirus vaccine; SLI: Standard of living index; NIP: National immunization programmes.

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Study conceptualisation, study design, protocol development, training, data analysis, interpretation: MKD and NKA. Study coordination, monitoring, and data cleaning: MKD, BG, and AS. Data analysis, interpretation, and manuscript preparation: MKD and BG. Protocol development, quality assurance and monitoring: MKA, PH, PLFZ, JB, AR, and CGM. Participant recruitment and data collection: AW, BRV, JIB, JKG, JM, KK, LB, LS, AMK, NM, PKJ, RS-1, RS-2, SKD, SJ, SKR, SS, VK, YN, APD, AG, BBT, CJS, GRP, GNM, HKS, HT, JS, KJ, KS, KL, ML, NRS, PP, PA, RK, RS-3, SSGM, SKA, SG, SKS, SKG, and SM. All authors reviewed, provided critical input and approved the final version. The content represents the views of the authors alone and do not necessarily represent the official positions of their organizations, World Health Organization or Ministry of Health and Family Welfare, Government of India.

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References
1. Patel MM, López-Collada VR, Bulhões MM, De Oliveira LH, Márquez AB, Flannery B, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. N Engl J Med. 2011;364(24):2283–92.
2. Velázquez FR, Colindres RE, Grajales C, Hernández MT, Mercadillo MG, Torres FJ, et al. Postmarketing surveillance of intussusception following mass introduction of the attenuated human rotavirus vaccine in Mexico. Pediatr Infect Dis J. 2012;31(7):736–44.
3. Carlin JB, Macartney KK, Lee KJ, Quinn HE, Buttery J, Lopert R, et al. Intussusception risk and disease prevention associated with rotavirus vaccines in Australia's National Immunization Program. Clin Infect Dis. 2013; 57(10):1427–34.
4. Yih WK, Lieu TA, Kulldorff M, Martin D, McMahill-Walraven CN, Platt R, et al. Intussusception risk after rotavirus vaccination in U.S. infants. N Engl J Med. 2014;370(6):503–12.
5. Weintraub ES, Baggs J, Duffy J, Vellozzi C, Belongia EA, Irving S, et al. Risk of intussusception after monovalent rotavirus vaccination. N Engl J Med. 2014; 370(6):513–9.
6. Stowe J, Andrews N, Ladhani S, Miller E. The risk of intussusception following monovalent rotavirus vaccination in England: a self-controlled case-series evaluation. Vaccine. 2016;34(32):3684–9.
7. Yung C-F, Chan SP, Soh S, Tan A, Thoon KC. Intussusception and monovalent rotavirus vaccination in Singapore: self-controlled case series and risk-benefit study. J Pediatr. 2015;167(1):163–168.e1.
8. Pérez-Vilar S, Díez-Domingo J, Puig-Barberà J, Gil-Prieto R, Romio S. Intussusception following rotavirus vaccination in the Valencia region, Spain. Hum Vaccines Immunother. 2015;11(7):1848–52.
9. Tate JE, Mwenda JM, Armah G, Jani B, Omore R, Ademe A, et al. Evaluation of intussusception after monovalent rotavirus vaccination in Africa. N Engl J Med. 2018;378(16):1521–8.
10. Groome MJ, Tate JE, Arnold M, Chitnis M, Cox S, de Vos C, et al. Evaluation of intussusception after oral monovalent rotavirus vaccination in South Africa. Clin Infect Dis. 2020;70(8):1606–12.
11. Yen C, Tate JE, Hyde TB, Cortese MM, Lopman BA, Jiang B, et al. Rotavirus vaccines: current status and future considerations. Hum Vaccines Immunother. 2014;10(6):1436–48.
12. World Health Organization. Post-marketing surveillance of rotavirus vaccine safety. Geneva: World Health Organization; 2009. Available from: https://apps.who.int/iris/bitstream/handle/10665/70017/WHO_IVB_09.01_eng.pdf?sequence=1.
13. Press Informationn Beureau. Shri J P Nadda launches Rotavirus vaccine as part of Universal Immunization Programme; terms it a "historic moment": Ministry of Health & Family Welfare Government of India; 2016. [cited 2019 Jan 10]. Available from: http://pib.nic.in/newsite/PrintRelease.aspx?relid=138342.
14. Bhowmick K, Kang G, Bose A, Chacko J, Boudville I, Datta SK, et al. Retrospective surveillance for intussusception in children aged less than five years in a south Indian tertiary-care hospital. J Health Popul Nutr. 2009;27(5): 660–5.
15. Singh JV, Kamath V, Shetty R, Kumar V, Prasad R, Saluja T, et al. Retrospective surveillance for intussusception in children aged less than five years at two tertiary care centers in India. Vaccine. 2014;32:A95–8.
16. Bahl R, Saxena M, Bhandari N, Taneja S, Mathur M, Parashar UD, et al. Population-based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among Indian children. J Infect Dis. 2009;200(s1):S277–81.
17. Jehangir S, John J, Rajkumar S, Mani B, Srinivasan R, Kang G. Intussusception in southern India: comparison of retrospective analysis and active surveillance. Vaccine. 2014;32:A99–103.
18. Jiang J, Jiang B, Parashar U, Nguyen T, Bines J, Patel MM. Childhood intussusception: a literature review. Cameron DW, editor. PLoS ONE. 2013; 8(7):e68482.
19. World Health Organisation. Rotavirus vaccines WHO position paper: January 2013 - Recommendations. Vaccine. 2013;31(52):6170–1. https://doi.org/10.1016/j.vaccine.2013.05.037.
20. Tate JE, Steele AD, Bines JE, Zuber PLF, Parashar UD. Research priorities regarding rotavirus vaccine and intussusception: a meeting summary. Vaccine. 2012;30:A179–84.
21. Bines JE, Kohl KS, Forster J, Zanardi LR, Davis RL, Hansen J, et al. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine. 2004;22(5–6):569–74.
22. International Institute for Population Sciences. National Family Health Survey, India: International Institute for Population Sciences; 2017. [cited 2019 Apr 10]. Available from: http://rchiips.org/nfhs/data/bh/bhchap2.pdf.
23. Das M, Arora N, Bonhoeffer J, Zuber P, Maure C. Intussusception in young children: protocol for multisite hospital sentinel surveillance in India. Methods Protoc. 2018;1(2):11.
24. Srinivasan R, Girish Kumar CP, Naaraayan SA, Jehangir S, Thangaraj JWV, Venkatasubramanian S, et al. Intussusception hospitalizations before rotavirus vaccine introduction: retrospective data from two referral hospitals in Tamil Nadu, India. Vaccine. 2018;36(51):7820–5.
25. Gupta M, Kanojia R, Singha R, Tripathy JP, Mahajan K, Saxena A, et al. Intussusception rate among under-five-children before introduction of rotavirus vaccine in North India. J Trop Pediatr. 2018;64(4):326–35.
26. Patel MM, Clark AD, Sanderson CFB, Tate J, Parashar UD. Removing the age restrictions for rotavirus vaccination: a benefit-risk modeling analysis. von Seidlein L, editor. PLoS Med. 2012;9(10):e1001330.
27. Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. Lancet. 2014;383(9935):2136–43.
28. Jo DS, Nyambat B, Kim JS, Jang YT, Ng TL, Bock HL, et al. Population-based incidence and burden of childhood intussusception in Jeonbuk Province, South Korea. Int J Infect Dis. 2009;13(6):e383–8.
29. Phua KB, Lee B-W, Quak SH, Jacobsen A, Teo H, Vadivelu-Pechai K, et al. Incidence of intussusception in Singaporean children aged less than 2 years: a hospital-based prospective study. BMC Pediatr. 2013;13(1):161.
30. Mansour AM, El Koutby M, El Barbary MM, Mohamed W, Shehata S, El Mohammady H, et al. Enteric viral infections as potential risk factors for intussusception. J Infect Dev Ctries. 2013;7(01):028–35.
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33. Tagbo BN, Mwenda J, Eke C, Oguonu T, Ekemze S, Ezomike UO, et al. Retrospective evaluation of intussusception in under-five children in Nigeria. World J Vaccines. 2014;04(03):123–32.
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36. Mwenda JM, Tate JE, Steele AD, Parashar UD. Preparing for the scale-up of rotavirus vaccine introduction in Africa: establishing surveillance platforms to monitor disease burden and vaccine impact. Pediatr Infect Dis J. 2014;33:S1–5.

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Experience of establishing and coordinating a nationwide network for bidirectional intussusception surveillance in India: lessons for multisite research studies

The INCLEN Intussusception Surveillance Network Study Group

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ABSTRACT

Objectives To document and share the process of establishing the nationally representative multisite surveillance network for intussusception in India, coordination, data management and lessons learnt from the implementation.

Design This study combined both retrospective and prospective surveillance approaches.

Setting 19 tertiary care institutions were selected in India considering the geographic representation and public and private mix

Participants All children under-2 years of age with intussusception

Primary and secondary outcome measures The experience of site selection, regulatory approvals, data collection, quality assurance and network coordination were documented.

Results The site selection process involved systematic and objective four steps including shortlisting of potential institutions, information seeking and telephonic interaction, site visits and site selection using objective criteria. Out of over 400 hospitals screened across India, 40 potential institutions were shortlisted and information was sought by questionnaire and interaction with investigators. Out of these, 25 institutes were visited and 19 sites were finally selected to participate in the study. The multistep selection process allowed filtering and identification of sites with adequate capacity and motivated investigators. The retrospective surveillance documented 1588 cases (range: 14–652 cases/site) and prospective surveillance recruited 621 cases (range: 5–191 cases/site). The multilayer quality assurance measures monitored and ensured protocol adherence, complete record retrieval and data completeness. The key challenges experienced included time taken for obtaining regulatory and ethical approvals, which delayed completion of the study. Ten sites continued with another multisite vaccine safety surveillance study.

Conclusion The experience and results of this systematic and objective site selection method in India are promising. The systematic multistep site selection and data quality assurance methods presented here are feasible and practical. The lessons from the establishment and coordination of this surveillance network can be useful in planning, selecting the sites and conducting multisite and surveillance studies in India and developing countries.

Strengths and limitations of this study

- ► Provides a systematic study site selection process using objective methodology.
- ► Documents the experience with prospective and retrospective surveillance data collection with multilayered data quality assurance process.
- ► The study site selection and quality assurance measures may serve as reference for suitable adaptation in different research topics and contexts.
- ► The lessons are applicable only to the multisite studies.
- ► The documentation of contractual and financial management challenges may be limited.

INTRODUCTION

Intussusception is an acute emergency in children and most commonly occurs in infants aged 4–10 months.[1 2] Although some are transient and resolve spontaneously, if not intervened timely, it may lead to bowel ischaemia and perforation and may even be fatal.[1] Intussusception has been reported as an adverse reaction with rotavirus vaccines (RVV) with variable risks ranging from no increased risk to low risk (1–2 additional cases per 100 000 vaccinated infants) across different countries with different RVVs.[3–12] The intussusception background rate varies widely across different countries, 9 (Bangladesh) to 328 (South Korea) per 100 000 infants.[13] Limited information from India reported its incidence between 17.7 and 254 cases per 100 000 child years.[14 15] Several other studies were single or few centre studies and varied in case definitions, age groups, reference periods and methodology.[12–14]

In view of the vaccine safety concern and limited information on intussusception in India, the National Technical Advisory Group (TAG) on Immunisation recommended

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vaccine safety surveillance along with RVV introduction.[16] This surveillance network aimed to generate background information on intussusception epidemiology Indian children to inform the policy and programme related to RVV introduction and serve as baseline for future surveillance to identify any change after vaccine introduction and address the vaccine safety concerns. The objectives were to: (1) establish a surveillance network of public and private hospitals ensuring regional representation and data capturing system; (2) undertake retrospective surveillance to document the intussusception epidemiology over past 5 years; (3) undertake prospective surveillance to document the intussusception epidemiology over 18 months and potential linkage with RVVs and (4) build capacity of the investigators and institutions.

Multicentre studies face several challenges related to design, site selection, regulatory approvals, study conduct, coordination, data management and dissemination.[17–20] The lessons from multisite studies in India are limited. The intussusception surveillance study has been successfully completed and the network has evolved over time to undertake more vaccine safety studies. The purpose of this paper is to share the process of network establishment, coordination, data management and lessons learnt from such nationwide network research.

METHODS

Study design

This study combined both retrospective and prospective surveillance. The experiences and lessons presented here are based on the concurrent documentation of processes and retrospective review of the study documents.

Study governance

The Central Coordinating Unit (CCU) constituted investigators, research staffs and administrative and finance team. For technical integrity and implementation monitoring, a TAG was constituted with 17 Indian and international experts in vaccine safety, surveillance, immunisation programme, public health, child health, paediatric surgery, radiology and medical record system, representation from Ministry of Health and Indian Council of Medical Research.

Site selection

We followed a four-step systematic study site selection process (figure 1). *Step 1 (Shortlisting):* We categorised the states into four regions (north, south, east and west) and screened the tertiary care hospitals (medical colleges and private hospitals) in these regions from the websites. *Step 2 (Screening):* We solicited information about the case load, clinical and diagnostic capacities (paediatrics, paediatric surgery and radiology), medical record system, ethical and administrative approvals from the shortlisted institutions using questionnaire and telephonic interactions (online supplemental document 1). *Step 3 (Evaluation):* A TAG member visited the institutions to assess the institution capacity (clinical facility, case documentation, research support system and medical record-keeping system) and interacted with the potential investigator(s), department and institution leaderships to assess the commitment, institution support and research environment (online supplemental document 2). *Step 4 (Site finalisation):* Based on the questionnaire, interaction with investigators and TAG member feedback, the study sites were finalised.

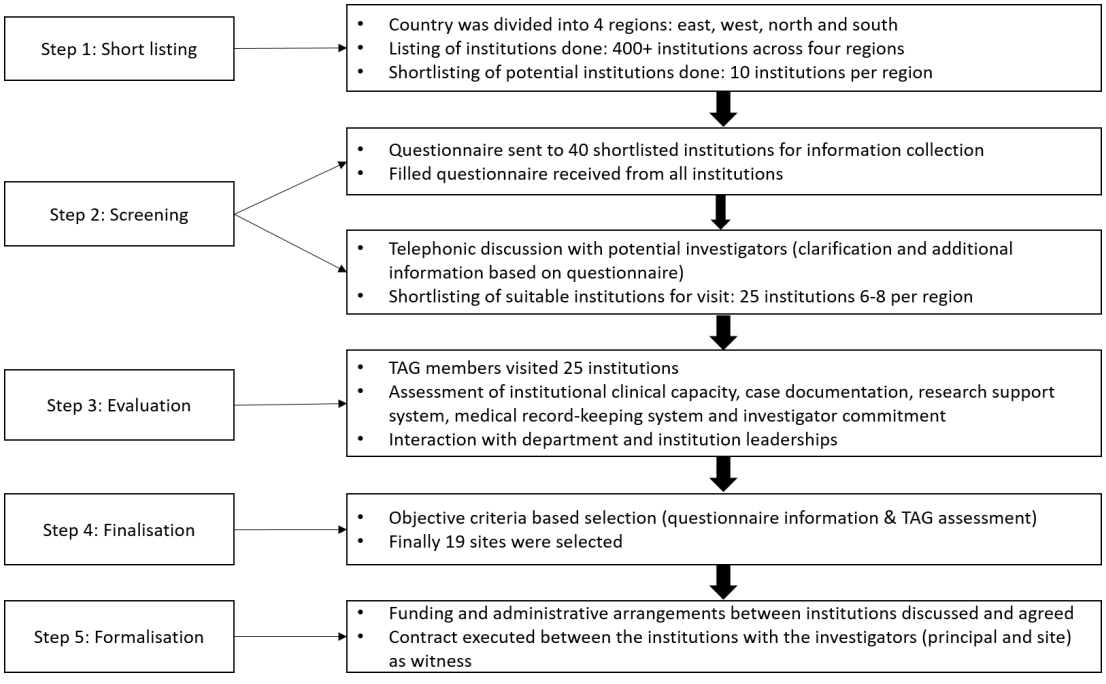


Figure 1 The steps of the site selection process. TAG, Technical Advisory Group.

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Protocol finalisation and study tool development

In multisite research projects, consensus building among the investigators on the study protocol, case record forms (CRFs), data management and monitoring are critical. The study protocol and CRFs were shared and piloted at the study sites. The protocol, study tools, implementation, data management and monitoring were discussed in detail during protocol finalisation workshop involving lead investigators from all sites and TAG members.

Regulatory procedures

As this study involved international funding, the approval from Health Ministry Screening Committee (HMSC) was obtained. The study protocol documents were submitted to the participating institutes for ethical approvals. Following the ethical approval, the study was implemented at the sites.

Study implementation process

Before implementation of the study at the sites, the following four items had to be completed: (1) institute administrative approval; (2) ethical approval; (3) agreement executed with the institution and (4) research staff selected and trained.

Data collection, management and monitoring

Separate CRFs and log sheets were prepared for the retrospective and prospective data collection. The retrospective data collection involved multiple sources; case records, registers from different departments and operation theatres and medical records section. For case record retrieval, the sites following International Classification of Diseases (ICD, ICD-9 or ICD-10) system, the ICD codes for intussusception or acute abdomen conditions were used (online supplemental document 3) (published in methodology paper).[21] At the other sites, cases were identified as per the diagnoses. For the prospective data collection, all admitted children were screened to identify the suspected cases, who were followed till final diagnosis. The children with intussusception were recruited and data collected. A weekly reporting from the sites was solicited for tracking progress. Initially, a weekly call with the study teams and later fortnightly to monthly calls were done to monitor the progress and address challenges. Based on the data collection progress reports, a monthly bulletin was prepared indicating the progresses and data quality. The completed study tools were sent periodically to CCU. The data received at CCU were reviewed for completeness, correctness and queries were resolved with reference to the source documents. Double data entry was done using customised data entry platform with inbuilt data matching programme. The data were archived in the server with authorised access and regular backup. Data analysis was done by under guidance of TAG. The data collection, flow and management followed for prospective and retrospective surveillance are shown in figures 2 and 3.

Quality assurance measures

Multiple quality assurance measures focused on protocol adherence and data quality. After initiation of data collection, TAG members visited each study site during the retrospective surveillance to verify: (a) protocol adherence; (b) identification of suitable cases and (c) data abstraction. The corrections/clarifications and repeat training was imparted as per TAG observations. After 6 months, during the prospective surveillance, TAG member again visited to review the: (a) patient screening; (b) tracking and eligible patients identification; (c) obtaining consent; and (d) data abstraction.

Patient and public involvement

No patients or the public were involved in the design, conduct, reporting and dissemination of research.

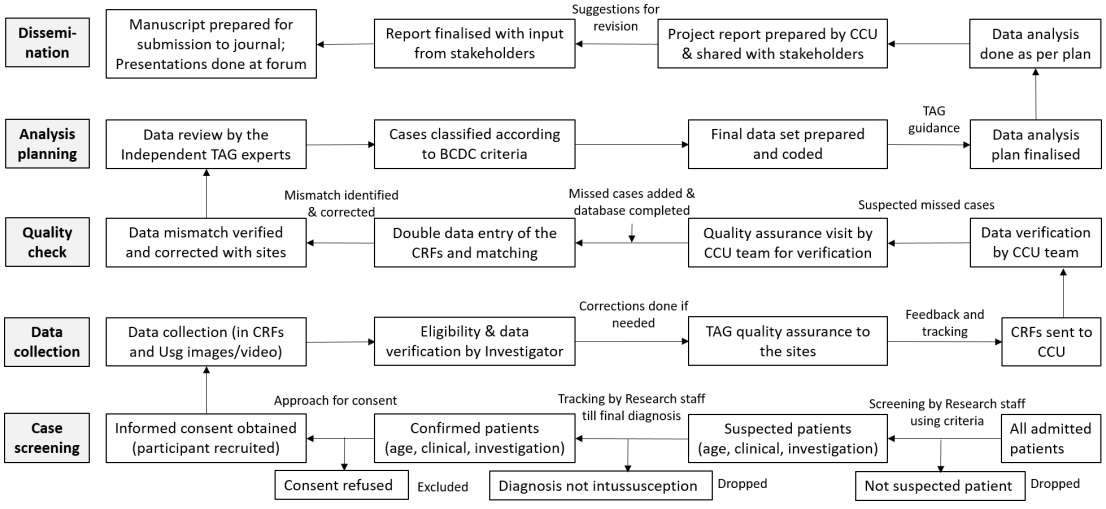


Figure 2 The data collection and management flow for prospective surveillance.BCDC, Brighton Collaboration diagnostic criteria; CCU, Central Coordinating Unit; CRF, Case record form; TAG, Technical Advisory Group; Usg, Ultrasound.

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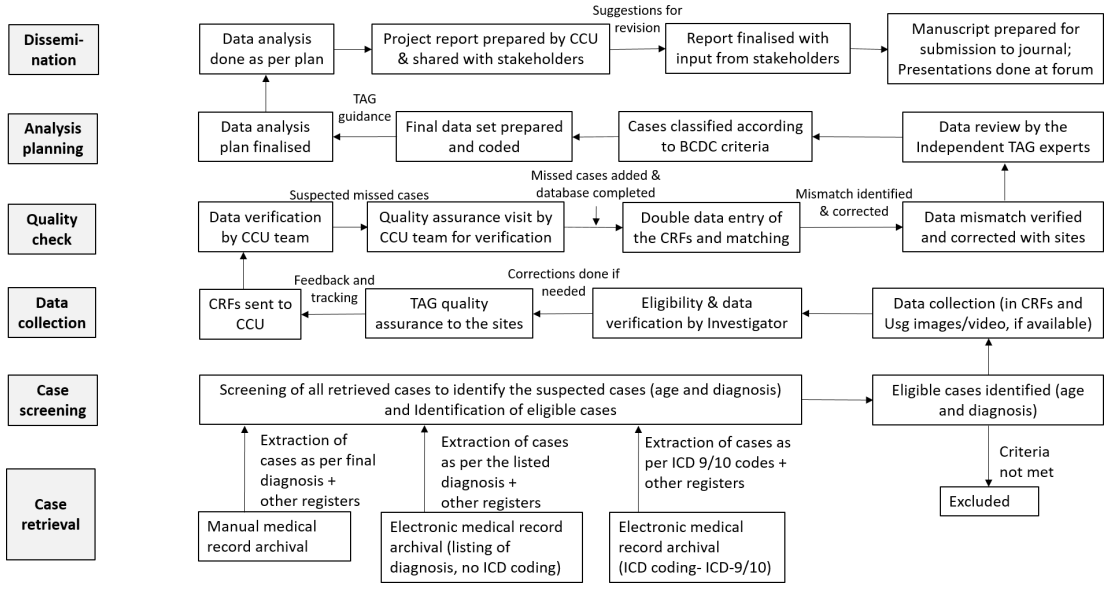


Figure 3 The data collection and management flow for retrospective surveillance. BCDC, Brighton Collaboration diagnostic criteria; CCU, Central Coordinating Unit; CRF, Case record form; ICD, International Classification of Diseases; TAG, Technical Advisory Group; Usg, Ultrasound.

RESULTS

Study timelines

The study was sanctioned in November 2014. The HMSC application was submitted in December 2014 and approved in May 2015. The formal study site selection initiated after the HMSC approval and was completed by August 2015. The protocol finalisation workshop was held in October 2015. Following the ethical approvals, the retrospective surveillance was initiated at majority of the sites in February 2016. The prospective surveillance was initiated in April 2016 and continued through September 2017. The data entry, cleaning and analysis and report drafting were completed by June 2018.

Study site selection

Out of over 400 hospitals screened across regions, 40 potential hospitals/institutions (10 per region) were shortlisted. While preparing the database, many institutions did not have adequate information on their websites about the facilities and faculty members or specialists. The potential investigators from these 40 shortlisted institutions were invited to submit desired information using a questionnaire via email and all responded. Telephonic discussions was held with the potential investigator(s) from these institutions (45–60 min) for additional information or clarifications. Based on the criteria and consultation with TAG, 25 institutes were visited. Based on the questionnaire, interactions and TAG member assessment, 19 institutions (north region: 5 sites, 3 public and 2 private; south region: 5 sites, 2 public and 3 private; east region: 6 sites, 5 public and 1 private; west region: 3 sites, 2 public and 1 private) were selected (figure 4). The study originally planned for 17 sites. Two more sites from the states where RVV was scheduled for introduction were added later. Several potential investigators requested the CCU team to discuss with their institute leadership for permission and allow him/her to lead the project. The CCU team succeeded in all expect two institutions, which could not be included. The site selection process was delayed by 7 months due to the delay in HMSC approval.

Regulatory approvals

The ethics approvals from the sites needed average 4 months (range: 1–8 months). No protocol amendment was needed.

Data collection

During July 2010 and March 2016 (retrospective surveillance period), out of 42 866 admitted under-2 years children, 2092 suspected cases were identified and 1588 confirmed intussusception cases were recruited.[22] The cases recruited at study sites ranged from 14 to 652. While five sites documented <20 cases each, two sites contributed >100 cases each. During April 2016 and September 2017 (prospective surveillance period), out of 6300 hospitalised under-2 years children, 1203 suspected cases were identified and 621 confirmed intussusception cases were recruited.[23] The cases recruited at the study sites ranged from 5 to 191 cases. While seven sites recruited <10 cases each, three sites contributed >50 cases each. At one study site, very few cases were retrieved from the retrospective and prospective surveillance, contrary to the anticipation. The descriptive data analysis was done at pooled and regional levels to document the epidemiology of intussusception. The variables and data parameters between the regions were compared with identify the similarities and differences.

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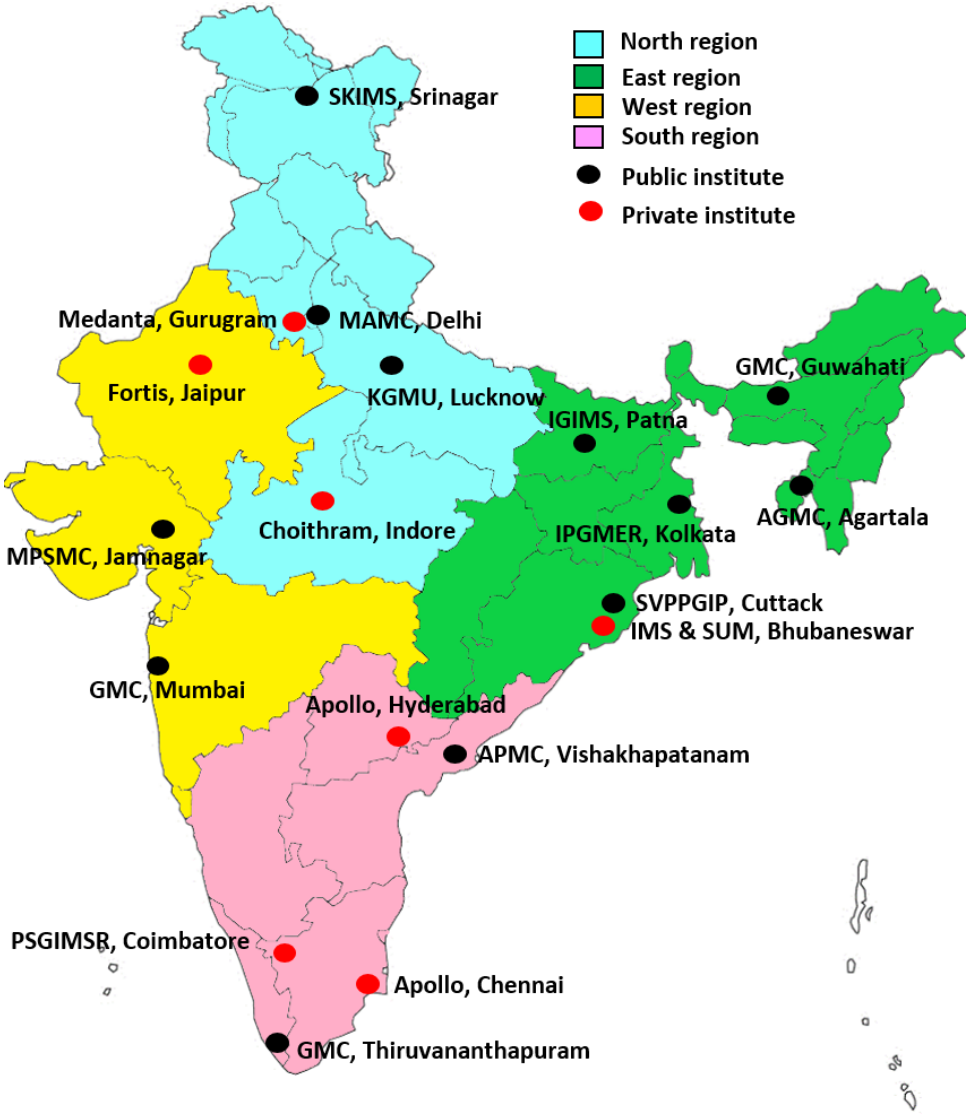


Figure 4 The geographic distribution of the network study sites. Note: The image was created for the research project by the investigators.

Medical record retrieval

Twelve (seven private and five public) institutions had electronic medical record (EMR) system. The ICD system was used at all sites except the two private hospitals, where the diagnosis was used for listing and archival. The medical records were accessible at all study sites except one site, where the research staff faced challenge in timely and adequate access to the medical records. The medical records required organisation at this site and thus the retrospective data collection took longer. The record retrieval was quicker at institutions with EMR system and retrospective data collection was completed in 2–4 months. At two sites, the retrospective data collection took longer 6 and 10 months, due to higher case load and challenge in record retrieval, respectively.

Radiology documentation

The ultrasound digital images and report were available for all prospectively recruited cases. There were variations in the ultrasound finding documentations and details in the reports, which required clarification from the radiologist for data capturing. Ultrasound reports were available at all but one hospital, where the findings were recorded in the case sheet. Digital records of ultrasounds were stored for minimum 3 months to 5 years. These ultrasound reports, digital films and clinical records were reviewed by independent TAG members for confirmation and classification according to Brighton Collaboration Criteria.[1]

Vaccination information

Majority of the parents were not carrying the vaccination card at the time of hospitalisation and about half of them came from outside the city. The research staffs pursued to obtain the vaccination card during hospitalisation and after discharge. The vaccination card after discharge was obtained through email, mobile message and also self-addressed and stamped envelopes handed over to the parents. The vaccination information was available for 78.4% of the prospective cases.

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Clinical case management

At five sites only surgery was done. At two sites, reduction was available during daytime only and surgery during other period and at 12 sites, both methods were available always.

Data quality assurance

TAG members made two quality assurance visits, first during the retrospective surveillance period, second during the prospective surveillance period and provided written feedback. The retrospective surveillance was at risk of missing cases. As it was difficult to compare the absolute case numbers across the centres and periods, we derived intussusception case rate per 1000 paediatric surgery admissions. After completion of 1 year of prospective surveillance, third quality assurance visit was made by the data management team to 11 sites with >30% differences in the case rates between retrospective and prospective periods. The team targeted verifying the confirmed cases, suspected cases and total admissions during 2015, 2016 and 2017 (until visit). The team identified three missed cases (one each at three sites).

Investigators commitment and transition

Despite the systematic selection process, the involvement of the investigators in the day-to-day operations and data verification was lesser than expected at three sites. At three sites, the lead investigators transitioned without any impact on the study activities.

Research staff issues

Although one research staff per site was planned, at seven sites, two research staffs were engaged considering the efforts needed for data retrieval and case load. These research staffs were trained through regional training workshops followed by hands-on training. At eight sites, the research staffs changed during the study period, once at six sites and twice at two sites. All the new staffs were trained by the CCU team member through site visit followed by virtual support. The investigators swiftly ensured replacements and no loss of cases. At CCU, there were three rounds of research staff transition.

Intradepartment and interdepartment coordination

At all institutions the nurses, residents and faculty members supported the surveillance. The nurse and residents at most places informed the research staff about any confirmed case, even on holidays. If the patient was discharged on any holiday, the research staffs attended and collected the data. Intradepartmental and interdepartmental coordination for data collection were smooth at all sites except two sites, where the participation from paediatric department was limited. As most of the intussusception cases were directly admitted to or transferred to paediatric surgery department on diagnosis, it did not affect the case screening and recruitment.

Financial management

In view of the foreign funding source and Foreign Contribution Regulation Act (FCRA) regulation, the funds could not be transferred to most institutes without FCRA approval. Thus, the fund was managed by the CCU for most of the institutes. Despite no fund transferred to the institutes, agreements were executed between the institutes with the investigators as the witnesses. We experienced challenges with some institutes in explaining the FCRA obligations prior to agreement execution.

Study tenure

Although planned for 30 months, the study was completed in 43 months. The delays in HMSC and ethics approval at sites were the key reasons for the delay.

Dissemination

Five peer-reviewed manuscripts have been published and seven manuscripts are under review or in preparation. The manuscripts have all the site investigators as authors, either individually or as group. The findings have been shared with the Ministry of Health and Family Welfare, WHO and other key partners. Over 22 oral presentations had been made at national, regional and international meetings.

Capacity building and transition

After this study, several sites were included in other studies coordinated by the CCU, 10 sites in one study and four sites in another study. Four site investigators collaborated in other research projects. Several of the research staffs at these sites continued with the new studies.

DISCUSSION

This multicentre sentinel surveillance successfully generated clinical and sociodemographic epidemiology information on intussusception in children including the regional the variations. The network adopted a systematic site selection process. A common understanding among the investigators was ensured and transmitted to the research staffs for appropriate implementation. The effective coordination, data management and quality assurance measures ensured high-quality data. Despite the efforts, there were challenges related to the regulatory and administrative challenges, which affected the implementation timelines. The continuation of several sites and investigators in other studied demonstrated the strong collaboration and confidence coupled with capacity building.

Poor site selection can lead to delay in completion, rise in cost, protocol amendment, implementation variations or study failure.[24] Studies have used different site selection processes: active hunting, peer referrals, inviting expression of interests, engaging research organisations, etc.[25–27] Our organisation has been conducting multisite network studies (ranging from 5 to 84 partner institutions) over last 15 years. We have adopted variable combination of

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processes for the study site and investigators selection, which have evolved over time. For this study, we adopted a systematic site selection process, which appeared lengthy and required several rounds of interactions. The four-step process assisted in identifying the highly motivated investigators and study sites with desired capacity. The potential investigators who failed to return the questionnaire with desired information or participate in the interaction are unlikely to devote time for study conduct and supervision. The systematic selection process allowed reducing the potential sites to a manageable number of suitable sites that were further assessed through visit. While the process was lengthy, it allowed also to build common understanding and commitment for the study. These experiences were similar to some multicounty studies.[25 27 28] The experiences from longer tenure clinical trial network (CTN) suggest that adoption of objective, standardised and systematic approach of site selection has better performance than an informal process. A CTN experimenting interventions for substance abuse moved from informal site selection process initially to five-step process including identification of potential sites, site selection surveys, pilot simulation data abstraction, blinded review, site selection interviews or site visits.[27] A CTN experimenting surgical interventions adopted five-step site selection process including open call, site capacity survey and pilot simulation data abstraction, criteria-based evaluation, telephonic interview and final assessment for selection.[25] The steps adopted were comparable to the steps adopted in our study.

The procedural efforts and time taken for ethical approval from the site institutes are well known. A review observed that the study site ethics approval took from 5 to 798 days and the review process and contents varied widely.[29] The ethics review process consumed sizeable staff hours and budget, forcing timeline extension and budget shortfall.[29–31] In our study also, the documentation and formats used for submission varied widely, apart from the study protocol, CRFs and consent forms. Some of the committees asked the investigators to present their protocol, while others did not.

Although the investigators were responsible for the conduct of the study, the agreements with institutions assisted in implementation of the study. The involvement of external experts TAG as in study site selection and monitoring facilitated standardisation and quality assurance. The additional data retrieval and verification effort at the sites documented the protocol adherence and robustness of data collection.

This is the first documentation of systematic site selection process for a multisite network in India. The positive experience from this study encouraged adoption of similar systematic site selection and data quality assurance mechanism for subsequent two vaccine network studies in India.

There are some limitations in the current study and documentation. While many of the lessons may be generic and have relevance for most of the multisite studies, some of them may be relevant for India and the developing countries. The documentation of contractual and financial management challenges may be limited. The study was not a clinical trial and did not involve any laboratory procedures. This was a descriptive study and had no comparison or control arm to compare the experience.

CONCLUSIONS

In conclusion, our experience with this systematic study site selection process was positive and satisfying. It used a four-step selection process using questionnaire, objective criteria for assessment and interactions with the investigators and institution stakeholders with site visits to identify suitable sites with adequate capacity. The participation of motivated instigators, agreement with the institutions and contributory protocol and tool development facilitated successful completion. Difficulties and delays in site initiation were primarily due to the regulatory and administrative approvals. The multistep site selection adopted is feasible and practical even in Indian context. The quality assurance processes also assisted in high-quality data collection. We hope that the site selection and quality assurance processes would be informative for multisite studies in India and developing countries and appropriate adaptation or modifications may be needed as per the objectives and implementation protocol of the network studies.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study protocol was reviewed and approved by all the participating institutes. The list of ethics committees of the participating institutes include the following: The INCLEN Independent Ethics Committee, The INCLEN Trust International, New Delhi, India (Ref No: IIEC 23; Dated 30 June 2015); Institutional Ethics Committee, King George's Medical University, Lucknow, Uttar Pradesh, India (Ref no: 7951/Ethics/R.Cell-15; Dated 4 December 2015); Institutional Ethics Committee, MP Shah Government Medical College, Jamnagar, Gujarat, India (ref no: 01/46/2016; Dated 6 January 2016); Institutional Ethics Committee, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu & Kashmir, India (Ref no: 42/2015; Dated 29 October 2015); Institutional Ethics Committee, Gauhati Medical College, Guwahati, Assam, India (Ref no: MC/02/2015/274; Dated 30 May 2016); Institutional Human Ethics Committee, PSG Institute of Medical Sciences, Coimbatore, Tamil Nadu, India (Ref no 15/294; Dated 16 November 2015); Institutional Ethics Committee, King George Hospital, Andhra Medical College, Vishakhapatnam, Andhra Pradesh, India (Dated 26 October 2015); Institutional Ethics Committee, Fortis Escorts Hospital, Jaipur, Rajasthan, India (Ref no: FEHJ/IEC/15/0023; Dated 14 September 2015); Institutional Ethics Committee, Grant Medical College & JJ Hospital, Mumbai, Maharashtra, India (Ref no: IEC/Pharm/288/15; Dated 19 November 2015); Institutional Ethics Committee, Government Medical College & SAT Hospital, Thiruvananthapuram, Kerala, India (Ref no: 06/05/2015/MCT; Dated 9 December 2015); Medanta Institutional Ethics Committee, Medanta-The Medicity, Gurgaon, Haryana, India (MICR 559/2015; Dated 21 January 2016); Institutional Ethics Committee, SCB Medical College, Cuttack, Odisha, India; Institutional Ethics Committee, IMS & SUM Medical College & Hospital, Bhubaneswar, Odisha, India (Ref no 210/5/10/2015; Dated 14 October 2015); Institutional Ethics Committee; Choithram Hospital and Research Centre, Indore, Madhya Pradesh, India (Ref no: EC/Oct/15/20; Dated 27 October 2015); Institutional Ethics Committee, Agartala Government Medical College, Agartala, Tripura, India; Institutional Ethics Committee- Clinical Studies, Apollo Hospitals, Chennai, Tamil Nadu (Dated 14 October 2015); Institutional Ethics Committee, Maulana Azad Medical College, Delhi, India (Ref no: F.1/IEC/MAMC/50/4/2015/308; Dated 20 November 2015); Institutional Ethics Committee, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India (Ref no: Inst/IEC/2016/197; Dated 1 March 2016); Institutional Ethics Committee, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India (Ref no: 1256/Acad; Dated 11 November 2016) and Ethics Committee, Apollo Hospital, Hyderabad, Telengana, India (Dated 13 October 2015). The interviews with stakeholders were done after obtaining written informed consent.

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Data availability statement This manuscript reports the processes and experience from the multisite network research. All relevant data has been reported. All data is available with the investigators and can be provided by the corresponding author upon reasonable request.

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REFERENCES

1 Bines JE, Kohl KS, Forster J, *et al*. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. *Vaccine* 2004;22:569–74.
2 World Health Organisation. Post-marketing surveillance of rotavirus vaccine safety [Internet], 2017. Available: http://apps.who.int/iris/bitstream/10665/70017/1/WHO_IVB_09.01_eng.pdf
3 Tate JE, Mwenda JM, Armah G, *et al*. Evaluation of intussusception after monovalent rotavirus vaccination in Africa. *N Engl J Med* 2018;378:1521–8.
4 Groome MJ, Tate JE, Arnold M, *et al*. Evaluation of intussusception after oral monovalent rotavirus vaccination in South Africa. *Clin Infect Dis* 2020;70:1606–12.
5 Patel MM, López-Collada VR, Bulhões MM, *et al*. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med* 2011;364:2283–92.
6 Velázquez FR, Colindres RE, Grajales C, *et al*. Postmarketing surveillance of intussusception following mass introduction of the attenuated human rotavirus vaccine in Mexico. *Pediatr Infect Dis J* 2012;31:736–44.
7 Carlin JB, Macartney KK, Lee KJ, *et al*. Intussusception risk and disease prevention associated with rotavirus vaccines in Australia's national immunization program. *Clin Infect Dis* 2013;57:1427–34.
8 Yih WK, Lieu TA, Kulldorff M, *et al*. Intussusception risk after rotavirus vaccination in U.S. infants. *N Engl J Med* 2014;370:503–12.
9 Weintraub ES, Baggs J, Duffy J, *et al*. Risk of intussusception after monovalent rotavirus vaccination. *N Engl J Med* 2014;370:513–9.
10 Stowe J, Andrews N, Ladhani S, *et al*. The risk of intussusception following monovalent rotavirus vaccination in England: a self-controlled case-series evaluation. *Vaccine* 2016;34:12:3684–9.
11 Pérez-Vilar S, Díez-Domingo J, Puig-Barberà J, *et al*. Intussusception following rotavirus vaccination in the Valencia region, Spain. *Hum Vaccin Immunother* 2015;11:1848–52.
12 Koch J, Harder T, von Kries R. Risk of intussusception after rotavirus vaccination. *Dtsch Arzteblatt Int* 2017;114:255–62.
13 Jiang J, Jiang B, Parashar U, *et al*. Childhood intussusception: a literature review. *PLoS One* 2013;8:e68482.
14 Bahl R, Saxena M, Bhandari N, *et al*. Population-Based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among Indian children. *J Infect Dis* 2009;200:S277–81.
15 Jehangir S, John J, Rajkumar S, *et al*. Intussusception in southern India: comparison of retrospective analysis and active surveillance. *Vaccine* 2014;32 Suppl 1:A99–103.
16 Ministry of Health and Family Welfare, Government of India. Meeting minutes: meeting of the national technical advisory group on immunization (India) [Internet], 2014. Available: https://www.nitag-resource.org/media-center/document/2220-minutes-of-the-meeting-of-ntagi-held-in-2014
17 Coates EC, Mann-Salinas EA, Caldwell NW, *et al*. Challenges associated with managing a multicenter clinical trial in severe burns. *J Burn Care Res* 2020;41:681–9.
18 Hagen NA, Stiles CR, Biondo PD, *et al*. Establishing a multicentre clinical research network: lessons learned. *Curr Oncol* 2011;18:243–9.
19 Dewa CS, Durbin J, Wasylenki D, *et al*. Considering a multisite study? how to take the leap and have a soft landing. *J Community Psychol* 2002;30:173–87.
20 Forjuoh SN. Challenges associated with multi-institutional multi-site clinical trial collaborations: lessons from a diabetes self-management interventions study in primary care. *J Clin Trials* 2015;05.
21 Das M, Arora N, Bonhoeffer J, *et al*. Intussusception in young children: protocol for multisite Hospital sentinel surveillance in India. *Methods Protoc*;1:201822:11.
22 Das MK, Arora NK, Gupta B, *et al*. Intussusception in children aged under two years in India: retrospective surveillance at nineteen tertiary care hospitals. *Vaccine* 2020;38:6849–57.
23 INCLEN Intussusception Surveillance Network Study Group. Prospective surveillance for intussusception in Indian children aged under two years at nineteen tertiary care hospitals. *BMC Pediatr* 2020;20:413.
24 Fogel DB. Factors associated with clinical trials that fail and opportunities for improving the likelihood of success: a review. *Contemp Clin Trials Commun* 2018;11:156–64.
25 Hurtado-Chong A, Joeris A, Hess D, *et al*. Improving site selection in clinical studies: a standardised, objective, multistep method and first experience results. *BMJ Open* 2017;7:e014796.
26 Gheorghiade M, Vaduganathan M, Greene SJ, *et al*. Site selection in global clinical trials in patients hospitalized for heart failure: perceived problems and potential solutions. *Heart Fail Rev* 2014;19:135–52.
27 Potter JS, Donovan DM, Weiss RD, *et al*. Site selection in community-based clinical trials for substance use disorders: strategies for effective site selection. *Am J Drug Alcohol Abuse* 2011;37:400–7.
28 Language and Reading Research Consortium (LAARC), Farquharson K, Murphy KA. Ten steps to conducting a large, multi-site, longitudinal investigation of language and reading in young children. *Front Psychol* 2016;7:419.
29 Greene SM, Geiger AM. A review finds that multicenter studies face substantial challenges but strategies exist to achieve institutional review board approval. *J Clin Epidemiol* 2006;59:784–90.
30 Green LA, Lowery JC, Kowalski CP, *et al*. Impact of institutional review board practice variation on observational health services research. *Health Serv Res* 2006;41:214–30.
31 Maskell NA, Jones EL, Davies, RJO. Variations in experience in obtaining local ethical approval for participation in a multi-centre study. *QJM Mon J Assoc Physicians* 2003;96:305–7.

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Association of meteorological parameters with intussusception in children aged under 2 years: results from a multisite bidirectional surveillance over 7 years in India

The INCLEN Intussusception Surveillance Network Study Group

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ABSTRACT

Objectives The study aimed to document the association between intussusception in Indian children and meteorological parameters and examine regional variations.

Design A bidirectional (retrospective and prospective) surveillance between July 2010 and September 2017.

Setting At 20 hospitals in India, retrospective case record review during July 2010 and March 2016 and prospective surveillance during April 2016 and September 2017 were performed.

Participants 2161 children aged 2–24 months with first intussusception episode were included.

Interventions The monthly mean meteorological parameters (temperature, sunshine, rainfall, humidity and wind speed) for the study sites were collected.

Methods The association between monthly intussusception cases and meteorological parameters was examined at pooled, regional and site levels using Pearson (r) and Spearman's rank-order (ρ) correlation, factorial analysis of variance, and Poisson regression or negative binomial regression analyses.

Results The intussusception cases were highest in summer and lowest in autumn seasons. Pearson correlation analysis showed that temperature (r=0.056; p<0.05), wind speed (r=0.134; p<0.01) and humidity (r=0.075; p<0.01) were associated with monthly intussusception cases. Spearman's rank-order correlation analysis found that temperature (ρ=0.049; p<0.05), wind speed (ρ=0.096; p<0.01) and sunshine (ρ=0.051; p<0.05) were associated with monthly intussusception cases. Poisson regression analysis resulted that monthly intussusception case was associated with rising temperature (North region, p<0.01 and East region, p<0.05), sunshine (North region, p<0.01), humidity (East region, p<0.01) and wind speed (East region, p<0.01). Factorial analysis of variance revealed a significant seasonal difference in intussusception cases for pooled level (p<0.05), 2–6 months age group (p<0.05) and North region (p<0.01). Significant differences in intussusception cases between summer and autumn seasons were observed for pooled (p<0.01), children aged 2–6 months (p<0.05) and 7–12 months (p<0.05).

Conclusions Significant correlations between intussusception cases and temperature, humidity, and wind speed were observed at pooled and regional level in India. A peak in summer months was noted, which may be used for prediction, early detection and referral for appropriate management of intussusception.

Strengths and limitations of this study

- ► This study included intussusception cases in children from 20 sites in India representing different geographical and climate regions.
- ► A relatively large number of intussusception cases in children over 7 years were analysed at pooled, regional and site levels using multiple statistical tests for consistency and triangulation.
- ► Consistency in the correlation between meteorological parameters and intussusception using multiple statistical tests was reassuring.
- ► Data for over 5 years, collected by retrospective surveillance, may be a limitation.

INTRODUCTION

Intussusception is the most common acute abdomen with intestinal obstruction in children, mostly occurring in infants.[1 2] The incidence of intussusception varies widely with a mean of 74 per 100 000 (range: 9–328) among infants with the peak during 5–7 months of age.[3] The available reports from India suggest that intussusception incidence varies from 17.7 to 254 per 100 000 child-years.[4 5] These children usually present with abdominal pain or excessive crying, vomiting, bloody stools and may have abdominal mass. Ultrasound is the leading diagnostic tool. The majority of the patients can be managed by reduction method and some may need surgery. The aetiology of intussusception remains unknown in the majority of cases.[6]

Intussusception has attracted attention due to its association with the rotavirus vaccine (RVV).

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India introduced RVV under the universal immunisation programme in 2016 and it expanded country-wide by 2019. A recent publication by our group reported regional variation in intussusception cases among Indian children under 2 years of age.[7] More number of cases were observed during March–June (summer months), suggesting seasonal variation. Other studies from India also reported seasonal variations.[5 8–11] There is variation in the seasonality of intussusception occurrence globally. A higher number of intussusception cases have been reported during warmer months from some countries,[12–16] while no seasonal variation was reported from other countries.[17–23] Reports from China found the intussusception caseload to be associated with meteorological parameters like temperature, sunshine and humidity.[12 24] In several reports from different countries, the association between meteorological factors and intussusception remains unclear, due to various reasons, including shorter study duration, smaller sample size and no definite statistical methods to explore the association. Although seasonal variations have been observed, no association between intussusception and meteorological parameters has been reported from India.

Multisite surveillance in India documented the epidemiology and trend of intussusception in children as part of the vaccine safety surveillance linked to the RVV introduction. Using the data collected by the Indian surveillance network, this study investigated the possible association between intussusception cases and various meteorological parameters and seasonality at the site, regional and national levels.

METHODS

Study area and participating hospitals

This hospital-based sentinel surveillance for intussusception in under 2-year-old children at 20 tertiary care hospitals in India included both prospective and retrospective periods. This bidirectional surveillance was comprised of retrospective (1 July 2010–31 March 2016) and prospective (1 April 2016–30 September 2017) periods of data collection. The study sites were selected from four regions (North, South, East and West) of the country, ensuring a mix of public and private tertiary care hospitals (North: three public and two private; South: two public and three private; East: five public and one private; West: two public and one private) (online supplemental figure 1). The study sites represented different climate zones in India. The method of study site selection has been detailed in the protocol published.[25]

Case definition, case selection and data collection

Children aged 2–24 months admitted to these hospitals with intussusception were eligible. For the retrospective surveillance period, the potential cases were identified using the International Classification of Diseases 9/10 or the diagnoses in the medical record section (online supplemental table 1). These case records were reviewed to identify the intussusception cases and all identified cases were recruited. For the prospective surveillance period, all age-eligible children were screened and tracked until final diagnosis, and all intussusception cases were recruited after written informed consent. Only the first intussusception cases were included in the analysis. The cases with the first episode of intussusception were identified from the history and/or from parents. For the confirmed cases, data including demography, clinical features, management and outcome were abstracted using case record forms. External experts visited the study sites to assess the protocol adherence for case selection, record retrieval and data abstraction. Data team members visited the sites and repeated the case retrieval process for the years 2014, 2015 and 2016 to assess the retrieval completeness. The data queries were clarified referring to the source documents.

Meteorological data collection

For the study sites, monthly mean meteorological data including temperature (°C), sunshine (total hours), rainfall (mm), humidity (%) and wind speed (km/hour) were collected from the website, World Weather Online (https://www.worldweatheronline.com/). These meteorological data collected for the period July 2010 through September 2017 were available in the public domain.

Data management and analysis

The descriptive analysis included proportions, means (with SDs) and median (with IQR). There was no definite population catchment area for the hospitals and the estimation of intussusception incidence was not possible. The calendar months were grouped into seasons (summer, rainy, autumn, winter and spring) for the individual sites and regions (online supplemental table 2). Chi-squared goodness-of-fit test was used to examine the periodicity of intussusception and monthly differences. Kruskal-Wallis H test (χ^2) was performed to document the differences between >3 groups (years, months/seasons and temperature ranges: <20°C, 21°C–25°C, 26°C–30°C and >30°C). Pearson correlation (r) analysis explored the association between monthly intussusception cases (dependent variable) and monthly mean meteorological parameters separately (independent variables). Spearman's rank-order correlation (ρ) analysis was also conducted to explore whether the monthly intussusception and monthly mean meteorological parameters covary. Factorial analysis of variance (F) was used to analyse the variations in the intussusception cases during the different seasons (dependent variable: seasonal intussusception cases and independent variables: season and year). Poisson regression or negative binomial regression (β) (for parameters with wide dispersion) analyses were performed to evaluate the relationships between the monthly intussusception cases (dependent variable) and the monthly mean meteorological parameters (independent variables). The association between intussusception and meteorological parameters was examined at pooled, regional and site levels with 95%

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Table 1 Distribution and demography of children with intussusception in India

Pooled	Years								
Years	2010*	2011	2012	2013	2014	2015	2016	2017†	Total
Number of cases, n	68	209	244	260	278	365	429	308	2161
Gender									
Male, n (%)	45 (66.2)	142 (67.9)	155 (63.5)	171 (65.8)	194 (69.8)	243 (66.6)	283 (66)	198 (64.3)	1431 (66.2)
Female, n (%)	23 (33.8)	67 (32.1)	89 (36.5)	89 (34.2)	84 (30.2)	122 (33.4)	146 (34)	110 (35.7)	730 (33.8)
Age groups									
2–6 months, n (%)	24 (35.3)	73 (34.9)	89 (36.5)	86 (33.1)	91 (32.7)	134 (36.7)	159 (37.1)	122 (39.6)	778 (36)
7–12 months, n (%)	33 (48.5)	92 (44)	108 (44.3)	122 (46.9)	113 (40.6)	135 (37)	155 (36.1)	117 (38)	875 (40.5)
13–24 months, n (%)	11 (16.2)	44 (21.1)	47 (19.3)	52 (20)	74 (26.6)	96 (26.3)	115 (26.8)	69 (22.4)	508 (23.5)
Regions									
North, n (%)	9 (13.2)	19 (9.1)	37 (15.2)	44 (16.9)	48 (17.3)	61 (16.7)	91 (21.2)	61 (19.8)	370 (17.1)
South, n (%)	50 (73.5)	134 (64.1)	130 (53.2)	149 (57.3)	124 (44.6)	175 (47.9)	183 (42.7)	136 (44.2)	1081 (50)
East, n (%)	9 (13.2)	45 (21.5)	61 (25)	54 (20.8)	84 (30.2)	112 (30.7)	131 (30.5)	97 (31.5)	593 (27.4)
West, n (%)	0 (0)	11 (5.3)	16 (6.6)	13 (5)	22 (7.9)	17 (4.7)	24 (5.6)	14 (4.5)	117 (5.4)

*The period of data collection was 6 months (July–December).
†The period of data collection was 9 months (January–September).

CI estimation. Statistical significance was considered if p<0.05. Statistical analysis was performed using STATA V.15.0 (Stata Corp, Texas, USA).

Patient and public involvement

The study participants or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research. The findings would be relevant for care-seeking and case management for intussusception in children. The results published shall be disseminated to the public through social media and healthcare providers.

RESULTS

Demography data

From July 2010 through September 2017, 2279 children aged 2–24 months with intussusception were recruited. Out of these, 118 children with a history of intussusception were excluded. Thus, 2161 children with the first intussusception were analysed. The demography and regional distribution of the intussusception cases across the years are shown in table 1. There were 1431 (66.2%) boys and 730 (33.8%) girls (male–female ratio, 1.96). Majority (n=1653, 76.5%) were infants. The number of cases increased across the years with the highest (31%) jump between 2014 and 2015. During 2010–2014, children aged 7–12 months had a higher share, which changed during 2015–2017. The South region contributed the highest (50%), followed by East (27.4%), North (17.1%) and West (5.4%) regions, which were consistent across the years.

Meteorological data

The monthly meteorological parameters for the regions are shown in online supplemental table 3. The monthly meteorological parameters for each site are given in online supplemental table 4. Across the regions, April–June and December–February had the highest and lowest temperatures, respectively. The pattern of meteorological parameters across the regions is shown in online supplemental figure 2.

Monthly and seasonal cycles of intussusception

The Chi-squared goodness-of-fit test revealed obvious monthly (χ^2-statistic=71.36; p<0.01) and seasonal differences (χ^2-statistic=382.28; p<0.01) in the intussusception caseload. Also, the Kruskal-Wallis H test revealed significant differences in the intussusception cases for months (p<0.01) and seasons (p<0.01). As shown in figure 1, more intussusception cases were observed during March in the South region, June in North and East regions, all during the summer season. Not much variation in cases was observed in the West region. The intussusception cases were lowest in September and October during these years. The peak and lowest seasons were summer (March–June; n=661, 30.6% cases) and autumn (September–October; 14.5% cases), respectively (online supplemental figure 3).

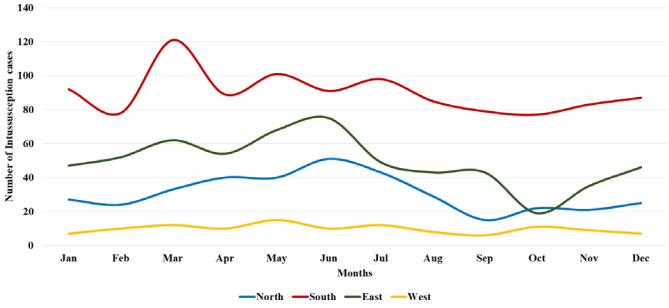


Figure 1 Monthly distribution of intussusception cases in children during 2010–2017.

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Table 2 Pearson correlation analysis between monthly intussusception cases and monthly mean meteorological parameters

	Pearson correlation coefficient (*r*) for meteorological parameters				
Parameter	**Temperature (°C)**	**Rainfall (mm)**	**Wind speed (m/s)**	**Humidity (%)**	**Sunshine (hour)**
Pooled (n=2161)	0.056*	−0.025	0.134†	0.075†	0.045
Regions					
North (n=370)	0.018	−0.103†	−0.119†	−0.373†	0.163†
South (n=1081)	0.022	−0.023	0.210†	0.161†	−0.018
East (n=593)	0.052*	0.072†	−0.084†	0.109†	0.024
West (n= (117)	0.046	−0.019	0.107†	−0.029	−0.012
Age groups					
2–6 months (n=778)	0.025	−0.022	0.092†	0.040	0.037
7–12 months (n=875)	0.068†	−0.012	0.115†	0.069†	0.042
13–24 months (n=508)	0.040	−0.030	0.125†	0.076†	0.029

Pearson correlation coefficient (*r*); significance two tailed.
*Significance level—p value of <0.05.
†Significance level—p value of <0.01.

Correlation between intussusception and meteorological factors

Table 2 shows Pearson correlation analysis for pooled, regions and age groups. For pooled data, the temperature (r=0.056; p<0.05), wind speed (r=0.134; p<0.01) and humidity (r=0.075; p<0.01) had significant association with intussusception. At regional level, intussusception cases had association with temperature for East region (r=0.052; p<0.05); with rainfall for North (r=−0.103; p<0.01) and East regions (r=0.073; p<0.01); with wind speed for all regions (r=-0.084–0.21; p<0.01); with humidity for three regions except West region (r=-0.373–0.161; p<0.01); and with sunshine for North region (r=0.163; p<0.01). For the age groups, intussusception cases had association with temperature for 7–12 months (r=0.068; p<0.01), with wind speed for all age groups (r=-0.092–0.125; p<0.01), with humidity for 7–12 months (r=0.069; p<0.01) and 13–24 months (r=0.076; p<0.01) age groups. The Pearson correlation analysis for intussusception cases with the meteorological parameters for the study sites is given in online supplemental table 5. Significant correlations for intussusception cases with temperature were observed for three sites, with rainfall for two sites, with wind speed and humidity for five sites each, and with sunshine for three sites.

Table 3 shows Spearman's rank-order correlation analysis for pooled, regions and age groups. For pooled data, the temperature (ρ=0.049; p<0.05), wind speed (ρ=0.096; p<0.01) and sunshine (ρ=0.051; p<0.05) were associated with intussusception. At regional level, intussusception

Table 3 Spearman's rank-order correlation analysis between monthly intussusception cases and monthly mean meteorological parameters

	Spearman's rank-order correlation coefficient (ρ) for meteorological parameters				
Parameter	**Temperature (°C)**	**Rainfall (mm)**	**Wind speed (m/s)**	**Humidity (%)**	**Sunshine (hour)**
Pooled (n=2161)	0.049*	0.012	0.096†	0.005	0.051*
Regions					
North (n=370)	0.110*	−0.014	0.183†	−0.114*	0.119*
South (n=1081)	0.028	−0.031	−0.024	−0.027	0.036
East (n=593)	0.061	−0.042	0.109†	−0.102*	0.038
West (n= (117)	0.052	−0.044	0.000	−0.067	0.076
Age groups					
2–6 months (n=778)	0.035	0.031	0.063†	0.007	0.044
7–12 months (n=875)	0.064†	0.036	0.103†	0.049*	0.048*
13–24 months (n=508)	0.028	0.019	0.106†	0.043	0.016

Spearman's rank-order correlation coefficient (ρ); significance two tailed.
*Significance level—p value of <0.05.
†Significance level—p value of <0.01.

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cases had association with temperature for North region (ρ=0.110; p<0.05); with wind speed for North (ρ=0.183; p<0.01) and East (ρ=0.109; p<0.01) regions; with humidity for North (ρ=−0.114; p<0.05) and East (ρ=−0.102; p<0.05) regions; and with sunshine for North region (ρ=0.119; p<0.05). For the age groups, intussusception cases had association with temperature for 7–12 months (ρ=0.064; p<0.01), with wind speed for all age groups (ρ=0.063–0.106; p<0.01), with humidity (ρ=0.049; p<0.05) and sunshine (ρ=0.048; p<0.05) for 7–12 months age group. The Spearman's rank correlation analysis for intussusception cases with the meteorological parameters for the study sites is given in online supplemental table 6. Significant correlations for intussusception cases with temperature were observed for one site, with rainfall for two sites, with wind speed for three sites, with humidity for five sites and with sunshine for two sites.

Regression analyses (Poisson regression and negative binomial regression) revealed significant association between intussusception cases and temperature for pooled level (β=0.026; 95% CI 0.011 to 0.041; p<0.01), North (β=0.027; 95% CI 0.007 to 0.047; p<0.01) and East (β=0.029; 95% CI 0.001 to 0.057; p<0.05) regions; wind speed for pooled level (β=0.082; 95% CI 0.061 to 0.103; p<0.01), North (β=0.208; 95% CI 0.101 to 0.315; p<0.01) and East (β=0.091; 95% CI 0.042 to 0.141; p<0.01) regions; humidity for pooled level (β=0.011; 95% CI 0.007 to 0.016; p<0.01) and East (β=−0.021; 95% CI −0.035 to −0.007; p<0.01) region; and sunshine for pooled level (β=0.002; 95% CI 0 to 0.003; p<0.01) and North (β=0.004; 95% CI 0.002 to 0.007; p<0.01) region (table 4). Associations between intussusception cases and temperature and humidity were evident across different age groups (p<0.01). The association was also significant at study sites level for temperature (three sites), humidity (six sites) and sunshine (five sites) (online supplemental table 7).

The monthly average temperatures at the sites and regions varied widely: 14°C–35°C in North, 23°C–31°C in South, 20°C–32°C in East and 22°C–31°C in West regions. χ^2 goodness-of-fit test showed significant difference at 20°C–30°C for pooled, North and South regions (p<0.01), indicating that intussusception cases occurred more during this temperature (online supplemental table 8/13). Kruskal-Wallis H test found significant variation in intussusception cases between different temperature categories for pooled (χ^2=15.933; p<0.01), North (χ^2=21.054; p<0.01) and South (χ^2=50.625; p<0.01) regions (online supplemental table 9/14). The number of intussusception cases increased when the mean daily temperature and the wind speed increased and mean daily humidity decreased at the pooled level and for North and East regions (online supplemental figures 4–8).

Correlation between intussusception, seasons and age groups

Factorial analysis of variance revealed significant difference in seasonal intussusception cases at pooled level (F=1.442; p<0.05), for 2–6 months age group (F=1.579; p<0.05) and for North region (F=1.809; p<0.01) (online supplemental tables 8 and 10 & 9 and 11). Multiple comparisons revealed significant difference in intussusception cases between summer and autumn seasons (mean difference=0.569; 95% CI 0.11 to 1.03; p<0.01) at the pooled level (online supplemental table 10 and 12). Significant differences in intussusception cases between summer and monsoon (mean difference=0.165; 95% CI 0.04 to 0.29, p<0.01), summer and autumn (mean difference=0.179; 95% CI 0.03 to 0.32, p<0.01), spring and monsoon (mean difference=0.276; 95% CI 0.09 to 0.46, p<0.01), and spring and autumn (mean difference=0.290; 95% CI 0.09 to 0.49, p<0.01) were observed for North region (online supplemental table 11 and 13). Significant differences in intussusception cases were observed between summer and autumn for children aged 2–6 months (mean difference=0.195; 95% CI 0.01 to 0.38, p<0.05) and for children aged 7–12 months (mean difference=0.231; 95% CI 0.01 to 0.45, p<0.05) (online supplemental table 12 and 14).

DISCUSSION

The seasonal influence on intussusception cases has been proposed, but with inconsistent findings from different countries and different parts of India. This study is the first to explore the association between intussusception and monthly mean meteorological parameters (temperature, rainfall, wind speed, humidity and sunshine) in India. This study, comprising 2161 children with the first intussusception from 20 sites in India, documented monthly and seasonal variations. Intussusception case peaks were observed during summer months (March–June) and troughs during autumn months (September–October). There was a positive correlation between intussusception cases and meteorological parameters like temperature, humidity, wind speed, and sunshine at pooled and regional levels and among children aged 7–12 months. The associations were consistent across analysis tests used (Pearson correlation, Spearman's rank correlation tests, Poisson regression or negative binomial regression). The association was more pronounced for North and East regions, where the meteorological parameter fluctuation across months, were widest. Further analysis using factorial analysis of variance also demonstrated seasonal inclination, peaking in the summer season (March–June) in India and significant difference from the autumn season (with lowest cases) (p<0.01). A significant difference in the number of intussusception cases between the temperature ranges (<20°C, 21°C–25°C, 26°C–30°C and >30°C) was observed at pooled and regional (North and South regions) levels, which indicated a rise in intussusception cases with the rise in monthly mean temperature. The intussusception cases increased with the rise in daily temperature and wind speed and fall in humidity.

The findings are consistent with the report from China in 5994 children (aged 0–12 years) with intussusception, where more cases were documented in summer months with a significant positive association with mean monthly meteorological parameters (temperature, sunshine and

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Table 4 Regression analysis of the relationship between monthly intussusception cases and monthly mean meteorological parameters

Variables	Regression coefficient, β (95% CI)	SE, S_β	χ2 value	P value
Pooled*				
Temperature (°C)	0.026 (0.011 to 0.041)	0.008	12.098	<0.01
Rainfall (mm)	−0.001 (−0.003 to 0)	0.001	2.462	0.117
Wind speed (km/hour)	0.082 (0.061 to 0.103)	0.011	58.449	<0.01
Humidity (%)	0.011 (0.007 to 0.016)	0.002	21.903	<0.01
Sunshine (hour)	0.002 (0 to 0.003)	0.001	7.000	<0.01
According to regions				
North region*				
Temperature (°C)	0.027 (0.007 to 0.047)	0.0102	7.195	<0.01
Rainfall (mm)	0.001 (−0.005 to 0.007)	0.0029	0.169	0.681
Wind speed (km/hour)	0.208 (0.101 to 0.315)	0.0545	14.556	<0.01
Humidity (%)	−0.010 (−0.020 to 0.001)	0.0052	3.448	0.063
Sunshine (hour)	0.004 (0.002 to 0.007)	0.0013	11.006	<0.01
South region*				
Temperature (°C)	0.010 (−0.037 to 0.058)	0.0243	0.174	0.676
Rainfall (mm)	−0.004 (−0.009 to 0.000)	0.0024	3.251	0.071
Wind speed (km/hour)	−0.011 (−0.045 to 0.023)	0.0173	0.401	0.527
Humidity (%)	−0.033 (−0.070 to 0.005)	0.0190	2.949	0.086
Sunshine (hour)	0.002 (−0.001 to 0.004)	0.0014	1.213	0.271
East region*				
Temperature (°C)	0.029 (0.001 to 0.057)	0.0141	4.245	0.039
Rainfall (mm)	−0.001 (−0.004 to 0.001)	0.0014	0.766	0.381
Wind speed (km/hour)	0.091 (0.042 to 0.141)	0.0251	13.244	<0.01
Humidity (%)	−0.021 (−0.035 to −0.007)	0.0070	9.188	<0.01
Sunshine (hour)	0.002 (−6.100 to 0.005)	0.0012	3.822	0.051
West region†				
Temperature (°C)	0.052 (−0.02 to 0.124)	1.987	0.036	0.159
Rainfall (mm)	−0.002 (−0.006 to 0.002)	0.855	0.002	0.355
Wind speed (km/hour)	0.022 (−0.042 to 0.087)	0.463	0.032	0.496
Humidity (%)	−0.002 (−0.016 to 0.011)	0.105	0.006	0.746
Sunshine (hour)	0.003 (−0.001 to 0.007)	2.429	0.002	0.119
According to age groups				
2–6 months†				
Temperature (°C)	0.012 (−0.004 to 0.028)	0.008	2.192	0.139
Rainfall (mm)	−0.001 (−0.003 to 0.001)	0.001	1.599	0.206
Wind speed (km/hour)	0.059 (0.037 to 0.081)	0.011	28.241	<0.01
Humidity (%)	0.006 (0.001 to 0.011)	0.003	5.276	0.022
Sunshine (hour)	0.002 (0.000 to 0.003)	0.001	4.615	0.032
7–12 months*				
Temperature (°C)	0.038 (0.018 to 0.057)	0.010	14.288	<0.01
Rainfall (mm)	−0.001 (−0.003 to 0.002)	0.001	0.447	0.504
Wind speed (km/hour)	0.08 (0.054 to 0.107)	0.013	36.037	<0.01
Humidity (%)	0.012 (0.006 to 0.018)	0.003	14.660	<0.01
Sunshine (hour)	0.002 (0.000 to 0.004)	0.001	5.144	0.023

Continued

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Table 4 Continued

Variables	Regression coefficient, β (95% CI)	SE, S_β	χ2 value	P value
13–24 months†				
Temperature (°C)	0.026 (0.006 to 0.047)	0.010	6.460	0.011
Rainfall (mm)	−0.002 (−0.005 to 0.000)	0.001	3.564	0.059
Wind speed (km/hour)	0.102 (0.077 to 0.128)	0.013	60.620	<0.01
Humidity (%)	0.017 (0.010 to 0.024)	0.004	22.599	<0.01
Sunshine (hour)	0.002 (0.000 to 0.004)	0.001	3.211	0.073

*Tests used for regression analysis: negative binomial regression.
†Tests used for regression analysis: Poisson regression.

precipitation).[12] Modelling using the data from 13 887 Chinese children with intussusception and applying the meteorological variation assumptions achieved a considerable agreement with the data collected.[24] These reports from China did not include humidity as a variable.

Studies on intussusception in children from India have reported higher cases during summer (March–July) and lowest during autumn (September–November) seasons. In three studies from South India, the highest and lowest intussusception cases were observed during March–June and September–October, respectively.[5 9 10] A multisite study from India reported the highest and lowest cases during March and September, respectively.[8] A study from North India observed the highest and lowest cases during June and September.[11] A report from our group on the retrospective surveillance observed a higher number of intussusception cases during the summer months (March–June) over the year.[7]

Seasonal patterns of intussusception occurrence have been observed from different countries. A higher number of intussusception cases were observed during the warmer months (May–October) than cooler months (November–April) in Taiwan.[14] Among Hong Kong children with intussusception, higher cases were observed during the summer months (May–July).[26] Seasonality in intussusception case occurrence was observed in Korean children with highest during warmer months (July–August) and lowest during cooler months (February).[27] Among Ethiopian children, higher intussusception cases were observed during June and lower during November.[13] Higher intussusception cases were observed during summer months and lower during winter months among South African children.[15] Among Israeli children, more intussusception cases were observed during the summer months and lower during winter.[16] No seasonal variation was also observed in some studies from different countries. Studies from Latin American countries and North America did not observe visible seasonal variation in cases.[17 23] Studies from Switzerland, France, New Zealand, Singapore and China also had no sizeable seasonal variations.[18–22]

The inconsistencies in the seasonal variations may be due to the sample size, age groups included and stratification in the analysis. Three studies that observed definite seasonal variation[12 14 24] and two that observed a statistically significant association between meteorological parameters and intussusception have a larger sample size (>5000 participants). The association observed in our study may be also due to the higher sample size. The reasons for the higher intussusception cases during summer are not known and the seasonality does not match with the rotavirus infection seasonality in India.[28] According to the reports from some developed countries (the USA and Australia), the intussusception incidence has declined over decades, and also the prevalence of prodrome infection symptoms (gastrointestinal and respiratory infections) in these children over the same period.[29–31] Further studies are needed to confirm the seasonality, association with meteorological parameters and variations in seasonality across the regions in India, along with the influences of the preventive healthcare services coverage, care-seeking practices, access to sanitation and hygiene, and changes in dietary practices.

A seasonal variation in another acute intestinal condition, acute appendicitis in children, has been reported. More acute appendicitis cases in children during summer months and lower during spring/autumn seasons have been reported from India, Pakistan, Iraq, China, Taiwan, Italy, Germany, Canada and the USA.[32–39] Among Chinese children, associations between acute appendicitis and temperature (p<0.01), humidity (p<0.01) and sunshine (p<0.01) were documented to support the seasonal variation.[35] Similar to intussusception, the appendicitis incidence has also declined in developed countries over decades, possibly due to changes in hygiene, sanitation, dietary practices, access to healthcare and reduction in infectious diseases.[40] Intussusception and acute appendicitis are intestinal problems and some of intussusceptions have associated appendicitis.[41–43] The climate factors are unlikely to directly cause intussusception or intussusception in children. But the linkage between seasonality and meteorological parameters and acute intestinal conditions appears to be plausible, which may be mediated by intestinal and non-intestinal infections (including activation of latent viral infections), mucosal inflammatory changes, lymphoid hyperplasia, or motility alterations and dietary intake changes. Although no direct relationship with the rotavirus infection could be established, higher gastrointestinal infection during summer and early rainy

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seasons may be triggers for higher intussusception in the Indian context. The changes in intussusception and appendicitis incidence over time in parallel with industrialisation, economic improvement, improved access to vaccination, healthcare, and sanitation and hygiene practices suggest a possible association. Such information in the Indian and developing country context is not available. Thus, the seasonal variation in these conditions and association between meteorological parameters appear epidemiologically and biologically plausible, which needs further evaluation. We used Pearson correlation coefficient as the primary analysis to document the strength and direction of the relationship between meteorological parameters and intussusception. As a secondary analysis and checking for consistency in the findings, we used Spearman's rank-order correlation analysis to identify the strength of the association between ranked parameters and intussusception variables.

The Pearson correlation coefficient (r) and Spearman's correlation coefficient (ρ) values for different meteorological parameters were similar, although the r values for temperature, rainfall, wind speed, and humidity were higher than the ρ values, but had r value lower for sunshine than the ρ value at the pooled level. The differences between the r and ρ values were relatively small, which supports the consistent association between the variables. The consistency in findings from factorial analysis of variance and regression analysis with the correlation coefficients further confirms the association between meteorological parameters and intussusception.

The study had several strengths. It included a relatively large number of intussusception cases over a 7-year period recruited from 20 sites representing different regions. This study explored the association between intussusception cases and meteorological parameters at site, regional and pooled national levels. There was consistency in the correlation between intussusception cases and meteorological parameters across multiple statistical methods used, which was convincing. There are several limitations in the study. A major part of this study was retrospective. The sites belong to different climate zones and had variations in the meteorological parameters. The lack of a definite catchment population and referral pattern made it difficult to estimate the intussusception incidence rate.

In conclusion, this study documented higher intussusception cases during the summer months with a positive association between the number of cases with temperature and sunshine. Also, a significant positive correlation with humidity and wind speed was observed. The association was significant for the North and East regions with wider variations in the meteorological parameters across seasons. A higher degree of suspicion for infants with compatible clinical presentation for intussusception during the summer months may improve diagnosis and clinical care to prevent complications. The study provides evidence for predicting the intussusception pattern in children and sensitising the healthcare providers at community and peripheral facilities for early detection and quick referral of patients to minimise the surgical interventions and avoid fatalities. Further studies are needed to confirm the observation and improve the evidence base on the potential risk factors for the season variations and the aetiologies of intussusception.

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Ethics approval The study protocol was reviewed and approved by all the ethics review committees of each participating institute. The institute ethics committees of the following participating institutes approved the protocol: The INCLEN Trust International, New Delhi, India; King George's Medical University, Lucknow, Uttar Pradesh, India; MP Shah Government Medical College, Jamnagar, Gujarat, India; Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu & Kashmir, India; Gauhati Medical College, Guwahati, Assam, India; PSG Institute of Medical Sciences, Coimbatore, Tamil Nadu, India; King George Hospital, Andhra Medical College, Vishakhapatnam, Andhra Pradesh, India; Fortis Escorts Hospital, Jaipur, Rajasthan, India; Grant Medical College & JJ Hospital, Mumbai, Maharashtra, India; Government Medical College & SAT Hospital, Thiruvananthapuram, Kerala, India; Medanta—The Medicity, Gurgaon, Haryana, India; SCB Medical College, Cuttack, Odisha, India; IMS & SUM Medical College & Hospital, Bhubaneswar, Odisha, India; Choithram Hospital and Research Centre, Indore, Madhya Pradesh, India; Agartala Government Medical College, Agartala, Tripura, India; Apollo Hospitals, Chennai, Tamil Nadu, India; Maulana Azad Medical College, Delhi, India; Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India; Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India; Apollo Hospital, Hyderabad, Telengana, India; and MKCG Medical College, Berhampur, Odisha, India.Confidentiality in data handling was maintained. The participants under the prospective component were recruited after written informed consent from the parent/legal guardian.

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Data availability statement All data are available with the investigators and can be provided by the corresponding author upon reasonable request.

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REFERENCES

1 Bines JE, Kohl KS, Forster J, *et al*. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. *Vaccine* 2004;22:569–74.
2 Bines JE, Ivanoff B. *Acute intussusception in infants and children: Incidence, clinical presentation and management: a global perspective [online]*. World Health Organisation, 2002. http://apps.who.int/iris/bitstream/handle/10665/67720/WHO_V-B_02.19_eng.pdf;jsessionid=74671C3CD99ED55137202F8D3D07CB73?sequence=1
3 Jiang J, Jiang B, Parashar U, *et al*. Childhood intussusception: a literature review. Cameron DW, editor. *PLoS ONE* 2013;8:e68482.
4 Bahl R, Saxena M, Bhandari N, *et al*. Population-based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among Indian children. *J Infect Dis* 2009;200:S277–81.
5 Jehangir S, John J, Rajkumar S, *et al*. Intussusception in southern India: comparison of retrospective analysis and active surveillance. *Vaccine* 2014;32:A99–103.

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6 Jain S, Haydel MJ. Child Intussusception. In: *StatPearls [online]*. Treasure Island, FL: StatPearls Publishing, 2020. http://www.ncbi.nlm.nih.gov/books/NBK431078/
7 Das MK, Arora NK, Gupta B, *et al*. Intussusception in children aged under two years in India: retrospective surveillance at nineteen tertiary care hospitals. *Vaccine* 2020;38:6849–57.
8 Singh JV, Kamath V, Shetty R, *et al*. Retrospective surveillance for intussusception in children aged less than five years at two tertiary care centers in India. *Vaccine* 2014;32:A95–8.
9 Bhowmick K, Kang G, Bose A, *et al*. Retrospective surveillance for intussusception in children aged less than five years in a South Indian tertiary-care hospital. *J Health Popul Nutr* 2009;27:660–5.
10 Srinivasan R, Girish Kumar CP, Naaraayan SA, *et al*. Intussusception hospitalizations before rotavirus vaccine introduction: retrospective data from two referral hospitals in Tamil Nadu, India. *Vaccine* 2018;36:7820–5.
11 Gupta M, Kanojia R, Singha R, *et al*. Intussusception rate among Under-Five-Children before introduction of rotavirus vaccine in North India. *J Trop Pediatr* 2018;64:326–35.
12 Guo W-liang, Zhang S-feng, Li J-en, *et al*. Association of meteorological factors with pediatric intussusception in subtropical China: a 5-year analysis. *PLoS One* 2014;9:e90521.
13 Gadisa A, Tadesse A, Hailemariam B. Patterns and seasonal variation of intussusception in children: a retrospective analysis of cases operated in a tertiary hospital in Ethiopia. *Ethiop Med J* 2016;54:9–15.
14 Chen SC-C, Wang J-D, Hsu H-Y, *et al*. Epidemiology of childhood intussusception and determinants of recurrence and operation: analysis of national health insurance data between 1998 and 2007 in Taiwan. *Pediatr Neonatol* 2010;51:285–91.
15 Moore SW, Kirsten M, Müller EW, *et al*. Retrospective surveillance of intussusception in South Africa, 1998-2003. *J Infect Dis* 2010;202:S156–61.
16 Greenberg D, Givon-Lavi N, Newman N, *et al*. Intussusception in children in southern Israel: disparity between 2 populations. *Pediatr Infect Dis J* 2008;27:236–40.
17 Sáez-Llorens X, Velázquez FR, Lopez P, *et al*. A multi-country study of intussusception in children under 2 years of age in Latin America: analysis of prospective surveillance data. *BMC Gastroenterol* 2013;13:95.
18 Buettcher M, Baer G, Bonhoeffer J, *et al*. Three-year surveillance of intussusception in children in Switzerland. *Pediatrics* 2007;120:473–80.
19 Seraysso C, Abbo O, Mouttalib S, *et al*. [Seasonal pattern of intussusceptions in infants and children: is fall/winter predominance still worth consideration? A 10-year retrospective epidemiological study]. *Arch Pediatr* 2014;21:476–82.
20 Chen YE, Beasley S, Grimwood K, *et al*. Intussusception and rotavirus associated hospitalisation in New Zealand. *Arch Dis Child* 2005;90:1077–81.
21 Boudville IC, Phua KB, Quak SH, *et al*. The epidemiology of paediatric intussusception in Singapore: 1997 to 2004. *Ann Acad Med Singap* 2006;35:674–9.
22 Liu N, Yen C, Huang T, *et al*. Incidence and epidemiology of intussusception among children under 2 years of age in Chenzhou and Kaifeng, China, 2009-2013. *Vaccine* 2018;36:7862–7.
23 Chang HG, Smith PF, Ackelsberg J, *et al*. Intussusception, rotavirus diarrhea, and rotavirus vaccine use among children in New York state. *Pediatrics* 2001;108:54–60.
24 Guo W-L, Geng J, Zhan Y, *et al*. Forecasting and predicting intussusception in children younger than 48 months in Suzhou using a seasonal autoregressive integrated moving average model. *BMJ Open* 2019;9:e024712.
25 Das MK, Arora NK, Bonhoeffer J, *et al*. Intussusception in young children: protocol for multisite Hospital sentinel surveillance in India. *Methods Protoc* 2018;1:11.
26 Nelson EAS, Tam JS, Glass RI, *et al*. Incidence of rotavirus diarrhea and intussusception in Hong Kong using standardized hospital discharge data. *Pediatr Infect Dis J* 2002;21:701–3.
27 Jo S, Lim IS, Chae SA, *et al*. Characteristics of intussusception among children in Korea: a nationwide epidemiological study. *BMC Pediatr* 2019;19:211.
28 Giri S, Nair NP, Mathew A, *et al*. Rotavirus gastroenteritis in Indian children < 5 years hospitalized for diarrhoea, 2012 to 2016. *BMC Public Health* 2019;19:69.
29 Parashar UD, Holman RC, Cummings KC, *et al*. Trends in intussusception-associated hospitalizations and deaths among US infants. *Pediatrics* 2000;106:1413–21.
30 Desai R, Curns AT, Patel MM, *et al*. Trends in intussusception-associated deaths among US infants from 1979-2007. *J Pediatr* 2012;160:456–60.
31 Justice FA, Auldist AW, Bines JE. Intussusception: trends in clinical presentation and management. *J Gastroenterol Hepatol* 2006;21:842–6.
32 Jangra B, Jangra MS, Rattan KN, *et al*. Seasonal and day of week variations in acute appendicitis in North Indian children. *J Indian Assoc Pediatr Surg* 2013;18:42.
33 Ahmed W, Akhtar MS, Khan S. Seasonal variation of acute appendicitis. *Pak J Med Sci* 2018;34.
34 Al-Mendalawi M, Lattuf M. Is there a seasonal distribution of acute appendicitis in Iraqi children? *Menoufia Med J* 2017;30:572.
35 Zhang Y, Lyu F-X, Kang Q, *et al*. Association of meteorological factors with pediatric acute appendicitis in China: a 7-year retrospective analysis. *Medicine* 2018;97:e12913.
36 Hsu Y-J, Fu Y-W, Chin T. Seasonal variations in the occurrence of acute appendicitis and their relationship with the presence of Fecaliths in children. *BMC Pediatr* 2019;19:443.
37 Gallerani M, Boari B, Anania G, *et al*. Seasonal variation in onset of acute appendicitis. *Clin Ter* 2006;157:123–7.
38 Reinisch A, Heil J, Woeste G, *et al*. The Meteorological influence on seasonal alterations in the course of acute appendicitis. *J Surg Res* 2017;217:137–43.
39 Fares A. Summer appendicitis. *Ann Med Health Sci Res* 2014;4:18.
40 Ferris M, Quan S, Kaplan BS, *et al*. The global incidence of appendicitis: a systematic review of population-based studies. *Ann Surg* 2017;266:237–41.
41 Atkinson GO, Gay BB, Naffis D. Intussusception of the appendix in children. *Am J Roentgenol* 1976;126:1164–8.
42 Ozkisacik SK, Erdem AO, Coskun O, *et al*. Small bowel intussusception together with appendicitis in childhood: a case report. *J Pediatr Surg Case Rep* 2015;3:25–6.
43 Marjon L, Hull N, Thomas K. Concurrent acute appendicitis and ileocolic intussusception in a 1-year-old child. *Radiol Case Rep* 2018;13:655–7.

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ORIGINAL ARTICLE

Profile and Epidemiology of Intussusception in Children Under-Two Years of Age: A Prospective Surveillance

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Abstract

Objective To report background intussusception epidemiology in children under-two years in Coimbatore district of Tamil Nadu state, India, prior to rotavirus vaccine (RVV) introduction.

Methods Prospective sentinel surveillance was done at four hospitals in Coimbatore during April 2016 to September 2017. The children aged >1 mo and < 24 mo with diagnosed intussusception were recruited and data on clinical, treatment, diet, immunization, and sociodemography were collected.

Results Sixty-eight children with intussusception were recruited. Among them 76.5% were infants with male predominance (57.4%) and 48.5% were 4–8 mo old. Vomiting (83.8%), pain in abdomen (including excessive crying) (72.1%), and bloody stools (45.6%) were the main symptoms. Reduction was tried in 92.6% cases with success in 83.8%. Surgery was needed by 16.2% children. No child died. Any RVV dose was received by 24.1% children and 2 children received RVV in 8–21 d and none within 1–7 d window. The incidence of intussusception requiring hospitalization was estimated to be 36.4 and 11.7 cases/100,000 children/y for the first and second years, respectively.

Conclusion Intussusception occurred in infants even without RVV exposure. Continued surveillance is needed to document the changes in intussusception epidemiology after RVV introduction.

Keywords Intussusception · Prospective surveillance · Incidence · Children · Coimbatore · South India

Introduction

Intussusception was documented as an adverse event associated with rotavirus vaccine (RVV) in 1999, following Rotashield® (Wyeth Lederle, Marietta, PA, USA) introduction in the United States, forcing withdrawal of the vaccine [1]. Subsequently, large RVV efficacy trials for Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) and Rotateq® (Merck & Co. Inc., Kenilworth, USA) across multiple countries attempted documentation of intussusception risk following RVV [2, 3]. There have been also continued efforts for post-marketing surveillance to document the association and risk of intussusception in different countries. Studies have observed no additional risk in United States and Brazil to 1–2 additional intussusception cases in Mexico and Australia per 100,000 vaccinated infants [4–6].

Intussusception commonly presents as acute abdomen and intestinal obstruction. Available literature suggests that majority of these cases are infants and between 4 and 10 mo. Although some cases resolve spontaneously (transient intussusception), most require early diagnosis and reduction, since obstruction and edema can lead to ischemia and intestinal perforation, which may be fatal. Ileocecal type is the most common [7]. There are several hypothesis postulated

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regarding the etiology and risk factors, which include neuromuscular incoordination, intestinal edema, mechanical lead points, as well as intestinal and extraintestinal viral and bacterial infections [7–9].

To reduce child mortality and morbidity, World Health Organization (WHO) has recommended introduction of RVV into the national immunization programmes. In view of the associated risk, WHO recommends surveillance for intussusception as part of the vaccine safety monitoring linked to the vaccine introduction. RVV was inducted into India's Universal Immunization Program in April 2016 and scaled up countrywide in four phases by 2019. Two RVVs, Rotavac® (Bharat Biotech, Hyderabad, India) and Rotasiil® (Serum Institute of India, Pune, India) are being used at 6, 10, 14 wk in different states.

The intussusception incidence and case load vary widely, globally, from 9 (Bangladesh) to 328 (Korea) per 10,000 infants [10–12]. According to the population-based and sentinel hospital based-surveillance studies, the intussusception incidence among Indian infants vary from 17.7–20 (95% CI 5.9–41.4) in North India to 254 (95% CI 102–514) in South India per 100,000 children per year [13–15]. The information on intussusception incidence from many parts of India are not available. Retrospective and prospective surveillances have highlighted regional variations in intussusception case load across India [16–18]. There is a need for information on intussusception epidemiology and characteristics from different states of India to support the vaccine safety monitoring and associated risk factors identification. With this background, the authors present the epidemiology, incidence, clinical features of intussusception in children aged under-two years through sentinel hospital surveillance in Coimbatore district, Tamil Nadu, India.

Material and Methods

This prospective hospital-based sentinel surveillance was conducted at four tertiary care hospitals (three from private sector and one government medical college) in Coimbatore district, Tamil Nadu, India. All these four hospitals were located in Coimbatore city, the district headquarter. These were the leading hospitals in the city and district with functional pediatric surgery facility. Most of the patients from the district attended one of these hospitals. The district has a population of 3,654,026 with over 25% of the population living in the Coimbatore city. RVV was introduced in Tamil Nadu under immunization program in September 2017. These four hospitals were part of a nationally representative sentinel surveillance for intussusception involving prospective and retrospective components conducted to support the vaccine safety surveillance effort in India. The pooled results for the retrospective and prospective surveillance components from the larger intussusception surveillance network have been published [16, 17].

Prospective surveillance system was conducted at these hospitals during April 2016 to September 2017.

All the children aged >1 mo and < 24 mo admitted to these hospitals under pediatrics or pediatric surgery units were screened daily. These age-eligible children were actively tracked till the final diagnosis. All children with intussusception were recruited with written informed consent taken from their parent or legally authorized representative. The data including sociodemography, dietary practices, immunization exposure (from immunization card or register), clinical features, treatment, and final outcome were collected. A dedicated research staff screened the cases and collected the data. Based on the information, an independent expert group including pediatrician, pediatric surgeon, and radiologist assigned diagnostic certainty level according to the Brighton Collaboration case definition (BCCD) [19].

The research staff was trained on the study protocol and data collection. The data collected were sent to the central coordinating unit, where the data were reviewed and queries were resolved with reference to the source documents. Site visits by the Central Coordinating Unit team were made to check protocol adherence and data quality. Site visit was made by the data team, which cross-checked the cases with the medical records to identify missing case.

Double data entry was done at the central unit and the data entered were matched with the hard copies to ensure correctness. The entered data were stored in the server with authorized access and daily backup was taken. The authors conducted descriptive data analysis and expressed the findings as proportions, means (with standard deviations), or medians [with interquartile range, (IQR)] as appropriate. The socioeconomic status was represented as standard of living index (SLI), as used in the National Family and Health Survey for India, using the scores for household assets ownership. SLI was categorized as high, medium, and low categories. The intussusception diagnostic certainty levels were categorized into levels 1 to 3 using the BCCD criteria [19]. The missing data were excluded from analysis. For incidence calculation, the census (2011) population data for Coimbatore district was used as the base population and growth rate (1.85%) as per the Sample Registration System (SRS) data was applied to estimate the population for 2016–2017. For estimating the number of children in the corresponding years, the live birth rate and infant mortality data from the state notification were used (Supplementary Table 1). The incidence of hospitalized intussusception was estimated as number of hospitalized cases among infants or under-two-children per 100,000 infants or under-two-children per year in Coimbatore district. The statistical analysis was performed using STATA version 15.0 (StataCorp LLC, Texas, USA).

The ethics committees of all participating institutes reviewed and approved the study protocol. Prior to recruitment of the

Table 1 The sociodemographic parameters and dietary practices of children with intussusception in Coimbatore, India

Variable	Category	Total (n=68)
Age	2–6 mo, n (%)	27 (39.7)
	7–12 mo, n (%)	25 (36.8)
	13–18 mo, n (%)	7 (10.3)
	19–23 mo, n (%)	9 (13.2)
	Median (IQR)	8 (5–12)
Gender	Male, n (%)	39 (57.4)
	Female, n (%)	29 (42.6)
Family	Nuclear, n (%)	46 (67.6)
	Joint/Extended, n (%)	22 (32.4)
Religion	Hindu, n (%)	53 (77.9)
	Muslim, n (%)	11 (16.2)
	Christian, n (%)	4 (5.9)
Standard of living status[a]	High, n (%)	22 (32.4)
	Medium, n (%)	35 (51.5)
	Low, n (%)	11 (16.2)
Place of residence	Coimbatore district, n (%)	36 (52.9)
	Other districts in the same state[b], n (%)	28 (41.2)
	Outside the state	4 (5.9)
Referral status	Primary presentation[c], n (%)	25 (36.8)
	Referred from another hospital[d], n (%)	43 (63.2)
Feeding practices	Exclusively breast-fed for ≥ 6 mo[e], n (%)	12/41 (29.3)
	Exclusive breast-feeding period, median (IQR) in mo	5 (3–5)
	Mixed feeding before 6 mo age[e], n (%)	22/54 (40.7)
	Mixed feed initiation age, median (IQR) in mo	6 (4–6)
	Weaning age, median (IQR) in mo	6 (6–7)
Weaning foods type[e]	Rice[f], n (%)	25/52 (67.3)
	Wheat[f], n (%)	3/52 (5.8)
	Other cereals[e], n (%)	2/52 (3.8)
	Fruits/Vegetables[e], n (%)	12/52 (23.1)
Weaning initiation to illness onset interval	Rice, median (IQR) in mo	7 (6–7)
	Wheat, median (IQR) in mo	6 (6–8)
	Other cereals, median (IQR) in mo	6 (5–6)

Mixed feed: Food used for weaning included at least two of the ingredients - rice, wheat, other cereals, lentil and vegetables & fruits

[a] SLI was estimated using the household assets ownership, as per the National Family Health Survey

[b] From the districts in the same state other than where the study hospital was situated

[c] The children presented to the hospital for the first time (not referred from any hospital)

[d] Referred from another hospital to the study hospital

[e] Estimated for children aged > 6 mo

[f] The denominators indicate valid responses received for the parameters

IQR Interquartile range; *SLI* Standard of living index

participants, written informed consent was obtained. Confidentiality in data handling was maintained at all levels.

Results

Between April 2016 and September 2017, 68 children with intussusception admitted to these hospitals were recruited. The sociodemographic characteristics and dietary practices for the children with intussusception are summarized in Table 1.

Over three-fourth (76.5%) of these children were infants (ages 2–6 mo: 39.7% and 7–12 mo: 36.8%) with median age of 8 mo (IQR 5–12 mo). The children aged 4–8 mo contributed to 48.5% of the cases (Fig. 1). A male predominance with male-female ratio of 1:3:1 was observed. No definite

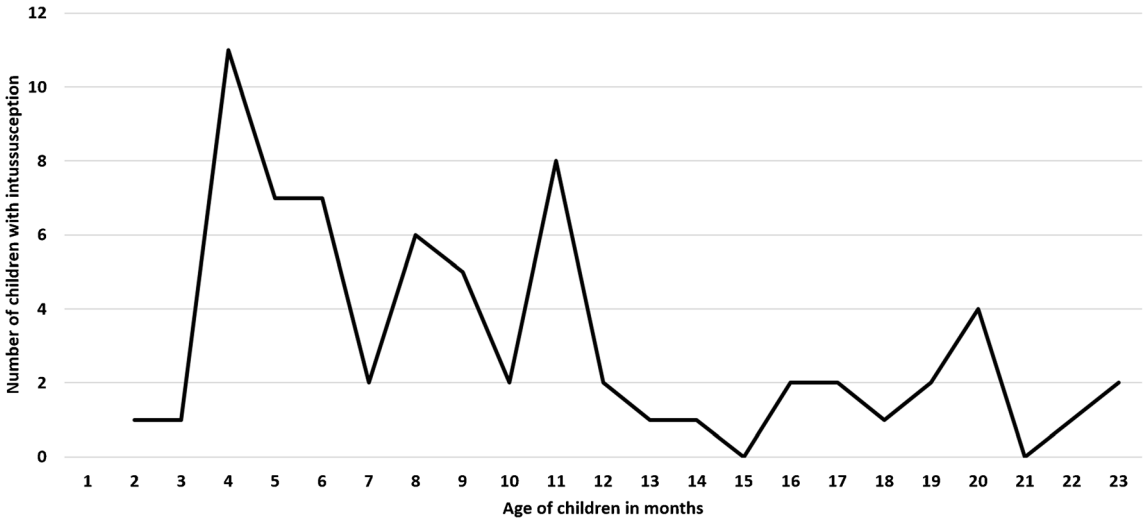


Fig. 1 Age distribution of the children with intussusception in Coimbatore district

seasonal variation was observed (Fig. 2). The patients belonged to all socioeconomic classes. About half (52.9%) of the patients were from the Coimbatore district. Overall, all children were initiated on breast-feeding, but only 29.3% of the children received exclusive breast-feeding for minimum 5 mo. Mixed feeding (animal milk and other feed) was initiated before 6 mo in 40.7% children. The median age of weaning was 6 (IQR 6–7) mo. Over two-third (67.3%) of the children received rice as main weaning food, while 23.1% received fruits or vegetables for weaning. The onset of weaning and intussusception intervals were comparable for the different types of weaning food.

Table 2 presents the clinical findings and management of these cases. Vomiting (83.8%), pain in abdomen (including excessive crying) (72.1%), and bloody stools (45.6%) were the leading symptoms. Classical intussusception triad (pain in abdomen, vomiting, and bloody stools) was observed in 26.5% patients. Abdominal mass was documented in 27.9% children. The diagnosis was made by ultrasonography in all cases with 79.4% had intussusception at ileocolic site. Only 1.5% had lymph nodes as pathological lead points (PLPs). Hydrostatic reduction was attempted in 63 (92.6%) cases and failed in 6 cases. Surgery was needed for 11 (16.2%) patients with bowel resection in 4 cases. No death was observed.

The intussusception onset-to-hospitalization interval was marginally shorter for children managed by reduction compared to those underwent surgery. The period of hospitalization was shorter with reduction (median 2, IQR 2–3 d) than surgery (median 6, IQR 4–7 d). Few children had history of diarrhea (7.3%) and respiratory illnesses (8.8%) within 4 wk of intussusception. All cases were labelled as level-1 using the BCCD criteria.

Immunization exposure information was documented for 85.3% (58/68) of the cases from a definite source. Out of the

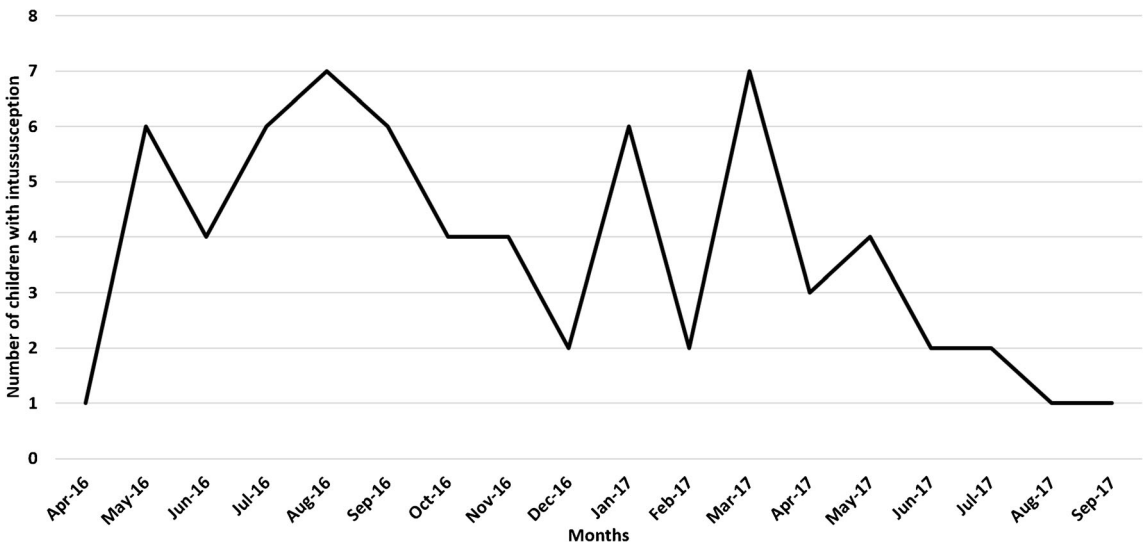


Fig. 2 Seasonal distribution of the intussusception cases in children from Coimbatore district

Table 2 The clinicopathological characteristics and management of children with intussusception in Coimbatore, India

Variable	Category	Total (n=68)
Symptoms	Vomiting[a], n (%)	57 (83.8)
	Bilious vomiting[b], n (%)	5 (8.8)
	Abdominal pain[c], n (%)	49 (72.1)
	Abdominal distension, n (%)	7 (10.3)
	Blood in stool, n (%)	31 (45.6)
	Diarrhea, n (%)	9 (13.2)
	Constipation, n (%)	8 (11.8)
	Fever, n (%)	16 (23.5)
	Lethargy, n (%)	7 (10.3)
	Classical triad[d], n (%)	18 (26.5)
Signs	Pallor, n (%)	2 (2.9)
	Dehydration, n (%)	3 (4.4)
	Abdominal distension, n (%)	5 (7.4)
	Abdominal mass, n (%)	19 (27.9)
	Rectal prolapse, n (%)	1 (1.5)
	Blood on per-rectal examination, n (%)	6 (8.8)
Diagnosis modality	Ultrasound, n (%)	68 (100)
Intussusception location	Ileocolic, n (%)	54 (79.4)
	Other locations, n (%)	14 (20.6)
Pathological lead point	Lymph node, n (%)	1 (1.5)
Treatment	Reduction, n (%)	57 (83.8)
	Surgery, n (%)	11 (16.2)
Surgery type	No resection[e], n (%)	7 (63.6)
	With resection[e], n (%)	4 (36.4)
Outcome	Discharged, n (%)	68 (100)
Intussusception onset-hospitalization interval	Reduction, median (IQR) in days	1 (1–2)
	Surgery, median (IQR) in days	2 (1–2)
Hospitalization duration	Reduction, median (IQR) in days	2 (2–3)
	Surgery, median (IQR) in days	6 (4–7)
Illness in last 4 wk	Diarrhea, n (%)	5 (7.3)
	Acute respiratory illnesses, n (%)	6 (8.8)

[a] Includes all vomiting including bilious vomiting

[b] Includes only bilious vomiting

[c] Includes excessive crying also

[d] Classical triad includes abdominal pain, vomiting and blood in stools

[e] The denominators used for percentage estimation include patients who underwent surgery only

children with known immunization exposure, 24.1% (14/58) received any RVV (Roravac®, Rotateq®, and Rorarix®) and all of them received it from the private hospitals. The median ages of primary doses of vaccination were 48 (IQR 44–57 d), 84 (IQR 77–99 d) and 119.5 (IQR 111–136 d) for first, second, and third doses, respectively (Fig. 3). Only 2 children received RVV (second dose-1 and third dose-1) within 8–21 d of last dose and none within the 1–7 d window (Supplementary Fig. 1).

The incidence of intussusception requiring hospitalization among children during first and second year of life in Coimbatore was estimated to be 36.4 (range 28.3–44.5) and 11.7 cases (range 5.2–18.3) per 100,000 children per year. The pooled incidence of intussusception requiring hospitalization in children under-two years was 23.4 (15.5–31.4) per 100,000 children per year (Supplementary Table 1).

Discussion

This multihospital sentinel study documented the epidemiology, case load, and incidence of intussusception among children under-two years in Coimbatore, Tamil Nadu prior to introduction of RVV in the immunization program. More

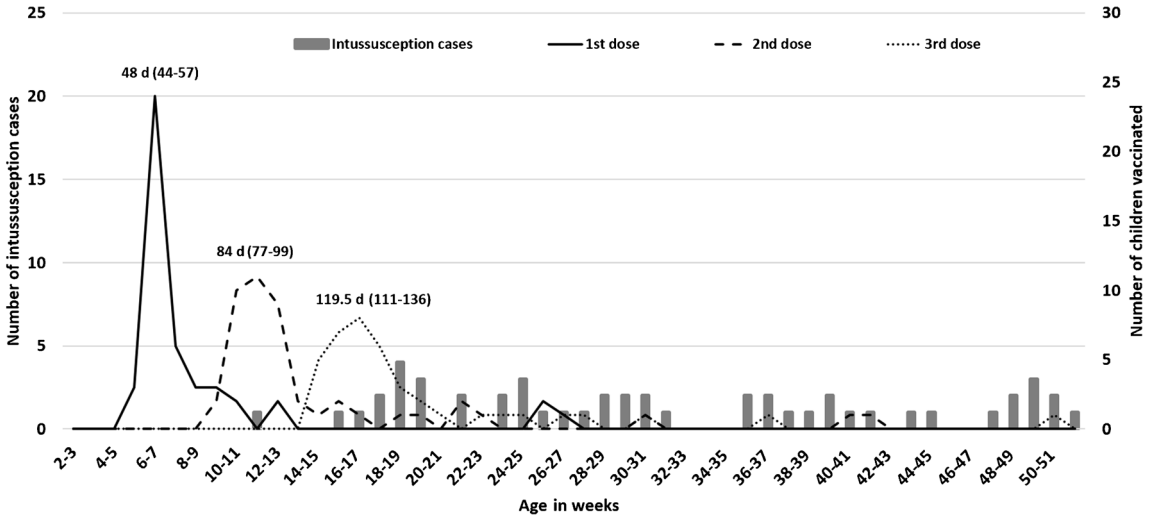


Fig. 3 Ages at vaccination and occurrence of intussusception in infants from Coimbatore. Note: The ages at vaccination are given in days as median [interquartile range (IQR)]

cases were observed among infants (76.5%) with median age of 8 mo (IQR 5–12 mo) and children aged 4–8 mo contributed nearly half of the cases. These observations were comparable to the earlier reports from other parts of India including Tamil Nadu and globally [12, 14–22]. Although males contributed more (57.4%), the male:female ratio was lesser than other reports [12, 14–22].

The children had vomiting, pain in abdomen, and bloody stools as the three top symptoms. These were similar to the reports from India and globally [12, 14–22]. The symptom triad was observed in 26.5% cases, which was comparable to some reports from India (19%–34.8%) [16–18]. Majority (79.4%) had ileocolic type Intussusception, similar (68–85.3%) to observations from India and globally [12, 14–22]. The proportion of children with PLP was comparable to some (2.1%–3.7%) [14, 21] and lesser (14.6%–41%) [16, 17, 23] than other reports from India. All cases met level-1 BCCD criteria indicating better documentation, especially post-reduction.

Majority (83.8%) of the cases were treated by hydrostatic reduction and the success rate of reduction (90.5%) was comparable to other reports from India [21, 24] and globally (67.7%–91.1%) [25]. The lower proportion of patients with triad, surgical intervention, and no mortality indicate early case detection, referral, and response for management.

As three hospitals were from private sector, the socioeconomic class of the patients were more from middle and high category, which is also reflected with the RVV receipt prior to introduction in the public health system. The exclusive breast-feeding rate (29.3%) was lower than the State average (47.8%, 2015–16) and 40.7% children received mixed feeding prior to 6 mo of age. Higher proportion of cases during 4–8 mo, which overlaps with mixed (top) feeding and early weaning suggest possible role of dietary practices in intussusception. Studies have shown increased risk and shorter interval for intussusception following oral vaccines [1]. Feeding cow's milk, weaning with some types of food, and allergy from external food have also been postulated to trigger intussusception in infants [16, 26, 27]. There is limited information regarding association of feeding and intussusception. In a study from Kerala, 78.8% of the children with intussusception were given complementary feeding during 2–4 mo of age [28].

The estimated incidence of intussusception requiring hospitalization was 35.1 per 100,000 infants and 11.7 per 100,000 children in the second year, which was lower than the report from Vellore district, Tamil Nadu (254 per 100,000 children) as part of a Phase-III trial for rotavirus vaccine [15]. The report from Vellore district included all intussusception cases, hospitalized and transient ones (those who did not require any intervention), detected on ultrasonography, which can explain the higher incidence rate [15]. The observed incidence in Coimbatore was a little higher than the reported intussusception incidence rates from North India (17.7–20) [13, 14], but was lower than another report (62, 95% CI 39.8–112.9) from Kerala (neighboring state) [29]; all of these included the hospitalized cases. The estimated intussusception incidence was comparable to the observations from Asian (Thailand - 36), Latin American (Panama - 30, Venezuela - 35) and North American (United States - 33-34) countries [12].

The ages at intussusception overlapped with the third dose of routine vaccination, when the RVV was also expected as part of the immunization schedule. Although among the children who received RVV, none had intussusception within 1–7 d after vaccination, 2 cases had it within 8–21 d window. Such overlap of ages of background intussusception in infants and RVV administration, especially third dose, is expected to make causality assessment difficult. The clinical trials conducted for the RVVs in use under the immunization program

in India were underpowered to detect any risk for intussusception [30, 31]. A recent study did not observe any increased risk of intussusception with Rotavac™ among Indian children [32]. To confirm the intussusception-RVV association in Indian context, longer surveillance and comparison with background incidence rates and analysis using the special methodologies shall be needed. Additionally, there is also need for better documentation of clinical features, reduction outcome, and vaccine exposure in routine clinical practice. In view of the diversities in dietary and sociocultural practices and geographic variations in the intussusception cases, there is a need for documentation of the epidemiology and trends from different parts of the country.

Involvement of multiple hospitals (both from private and public sector) from the district, data collection by separate research staffs following common protocol and standard case definition, and quality assurance measures are the strengths of this study. For immunization exposure, only definite source documents were accepted. There were several limitations also. This was a hospital-based surveillance. Usually hospital attendance and patient referral do not follow any definite catchment area norm. Thus, the observed incidence might be an underestimation, as some cases might have been managed at other facilities in the state. We did not explore any etiological or associated risk factors (including rotavirus vaccine) for intussusception. The clinical feature like abdominal pain has been captured based on the history and assessment of the treating doctors, which could have been under-or overrepresented.

Conclusion

This study documented the clinical features and incidence of intussusception among children aged under-two years prior to RVV introduction in Tamil Nadu, a southern state in India. The intussusception occurred in children, especially infants even without rotavirus vaccination. The intussusception incidence needs to be compared with the post-RVV introduction to document any change. For examining the association with the RVV, especially following the third dose, background rates of intussusception in the age group must be considered. There is a need for better documentation of immunization exposures and other possible risk factors for intussusception, including dietary practices, intestinal and extraintestinal infections and age-related gut motility functions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12098-021-03776-8.

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Availability of Data and Materials All data are available with the investigators and can be provided by the corresponding author upon reasonable request.

Authors' Contributions MKD and NKA conceptualized the framework for the study protocol, training, data analysis, and interpretation. All the site investigators contributed to the protocol development and finalization. JM, CJS, RG, KR, JK, and PA supervised and coordinated the data collection at study site institutes. MKD and BG coordinated data quality assurance, data cleaning and analysis of the data. MKD wrote the first draft of the manuscript and will act as guarantor for this paper. All authors reviewed, provided critical input, and approved the final version.

Declarations

Ethics Committee Approval The study protocol was reviewed and approved by INCLEN Ethics Committee (Ref: IIEC 23), Institutional Human Ethics Committee, PSG Institute of Medical Sciences and Research, Coimbatore (Ref: 15/295); Ethics Committee, Coimbatore Medical College, Coimbatore (Ref: 5/7/2016); Institutional Ethics Committee, Masonic Medical Centre for Children, Coimbatore (Ref: 28/4/2016) and Institutional Ethics Committee, GKNM Hospital, Coimbatore (Ref: 25/2016). The information about the children were collected after obtaining written informed consent.

Conflict of Interest None.

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References

1. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med. 2001;344:564–72.
2. Vesikari T, Matson DO, Dennehy P, et al. Safety and efficacy of a pentavalent human–bovine (WC3) reassortant rotavirus vaccine. N Engl J Med. 2006;354:23–33.
3. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 2006;354:11–22.
4. Yen C, Tate JE, Steiner CA, Cortese MM, Patel MM, Parashar UD. Trends in intussusception hospitalizations among US infants before and after implementation of the rotavirus vaccination program, 2000–2009. J Infect Dis. 2012;206:41–8.
5. Patel MM, López-Collada VR, Bulhões MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. N Engl J Med. 2011;364:2283–92.
6. Buttery JP, Danchin MH, Lee KJ, et al. Intussusception following rotavirus vaccine administration: post-marketing surveillance in the national immunization program in Australia. Vaccine. 2011;29:3061–6.
7. Bines JE, Ivanoff B. Acute intussusception in infants and children: incidence, clinical presentation and management: a global perspective.

World Health Organisation; 2002. Available at: http://apps.who.int/iris/bitstream/handle/10665/67720/WHO_V-B_02.19_eng.pdf;jsessionid=74671C3CD99ED55137202F8D3D07CB73?sequence=1. Accessed 10 May 2017.
8. Bines JE, Liem NT, Justice FA, et al. Risk factors for intussusception in infants in Vietnam and Australia: adenovirus implicated, but not rotavirus. J Pediatr. 2006;149:452–60.
9. Nylund CM, Denson LA, Noel JM. Bacterial enteritis as a risk factor for childhood intussusception: a retrospective cohort study. J Pediatr. 2010;156:761–5.
10. Zaman K, Breiman RF. Yunus md, et al. intussusception surveillance in a rural demographic surveillance area in Bangladesh. J Infect Dis. 2009;200(s1):S271–6.
11. Jo DS, Nyambat B, Kim JS, et al. Population-based incidence and burden of childhood intussusception in Jeonbuk Province. South Korea Int J Infect Dis. 2009;13:e383–8.
12. Jiang J, Jiang B, Parashar U, Nguyen T, Bines J, Patel MM. Childhood intussusception: a literature review. PLoS One. 2013;8:e68482.
13. Bahl R, Saxena M, Bhandari N. Et al; Delhi intussusception study hospital group. Population-based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among Indian children. J Infect Dis. 2009;200(s1):S277–81.
14. Gupta M, Kanojia R, Singha R, et al. Intussusception rate among under-five-children before introduction of rotavirus vaccine in North India. J Trop Pediatr. 2018;64:326–35.
15. Jehangir S, John J, Rajkumar S, Mani B, Srinivasan R, Kang G. Intussusception in southern India: comparison of retrospective analysis and active surveillance. Vaccine. 2014;32:A99–103.
16. The INCLEN Intussusception Surveillance Network Study Group. Prospective surveillance for intussusception in Indian children aged under two years at nineteen tertiary care hospitals. BMC Pediatr. 2020;20:413.
17. Das MK, Arora NK, Gupta B, et al. Intussusception in children aged under two years in India: retrospective surveillance at nineteen tertiary care hospitals. Vaccine. 2020;38:6849–57.
18. Singh JV, Kamath V, Shetty R, et al. Retrospective surveillance for intussusception in children aged less than five years at two tertiary care centers in India. Vaccine. 2014;32:A95–8.
19. Bines JE, Kohl KS, Forster J, et al. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine. 2004;22:569–74.
20. Bhowmick K, Kang G, Bose A, et al. Retrospective surveillance for intussusception in children aged less than five years in a south Indian tertiary-care hospital. J Health Popul Nutr. 2009;27:660–5.
21. Fahiem-Ul-Hassan M, Mufti G, Bhat N, et al. Management of intussusception in the era of ultrasound-guided hydrostatic reduction: a 3-year experience from a tertiary care center. J Indian Assoc Pediatr Surg. 2020;25:71–5.
22. Srinivasan R, Girish Kumar CP, Naaraayan SA, et al. Intussusception hospitalizations before rotavirus vaccine introduction: retrospective data from two referral hospitals in Tamil Nadu. India Vaccine. 2018;36:7820–5.
23. Raman T, Mukhopadhyaya A, Eapen CE, et al. Intussusception in southern Indian children: lack of association with diarrheal disease and oral polio vaccine immunization. Indian J Gastroenterol. 2003;22:82–4.
24. Singh AP, Tanger R, Mathur V, Gupta AK. Pneumatic reduction of intussusception in children. Saudi Surg J. 2017;5:21–5.
25. Gluckman S, Karpelowsky J, Webster AC, McGee RG. Management for intussusception in children. Cochrane Colorectal Cancer Group, editor. Cochrane Database Syst Rev . 2017. Available at: http://doi.wiley.com/10.1002/14651858.CD006476.pub3. Accessed 16 Sept 2020.
26. Johnson B, Gargiullo P, Murphy TV, Parashar UD, Patel MM. Sociodemographic and dietary risk factors for natural infant intussusception in the United States. J Pediatr Gastroenterol Nutr. 2010;51:458–63.
27. Aydin E, Beşer O, Ozek E, Sazak S, Duras E. Is there a causal relationship between intussusception and food allergy? Children (Basel). 2017;4:89.
28. John M, Siji CR. A clinical study of children with intussusception. Int J Contemp Pediatr. 2016;3:1083–8.
29. Mathew MA, Venugopal S, Arora R, Kang G. Leveraging the national rotavirus surveillance network for monitoring intussusception. Indian Pediatr. 2016;53:635–8.
30. Bhandari N, Rongsen-Chandola T, Bavdekar A, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. Lancet. 2014;383:2136–43.
31. Kulkarni PS, Desai S, Tewari T, et al. A randomized phase III clinical trial to assess the efficacy of a bovine-human reassortant pentavalent rotavirus vaccine in Indian infants. Vaccine. 2017;35:6228–37.
32. Early Rollout of ROTAVAC® India Network. Assessment of risk of intussusception after pilot rollout of rotavirus vaccine in the Indian public health system. Vaccine. 2020;38:5241–8.

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Original article

Clinical spectrum and short-term outcomes of multisystem inflammatory syndrome in children in a south Indian hospital

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Background: Multisystem inflammatory syndrome in children (MIS-C) is a new hyperinflammatory variant that evolved during the coronavirus disease 2019 pandemic. Although the precise pathophysiology of MIS-C is uncertain, it is thought to be due to immune dysregulation occurring after recovery from acute infection.

Purpose: Our study aimed to analyze the clinical spectrum, laboratory parameters, imaging characteristics, treatment strategies, and short-term outcomes of children with a diagnosis of MIS-C.

Methods: This retrospective and prospective observational study included children less than 16 years of age who were admitted to the pediatric unit of a tertiary care teaching hospital in south India between August 2020 to January 2021 with a diagnosis of MIS-C according to World Health Organization criteria.

Results: Twenty-one children were included in the analysis; all had fever with variable combinations of other symptoms. The mean age was 6.9 years; 71.4% were male. Gastrointestinal (80.9%) and cardiovascular (80.9%) systems were the most commonly affected. The majority of children had elevated inflammatory markers, and 16 (76.2%) had echocardiographic abnormalities mimicking Kawasaki disease. Eleven children (52.4%) required intensive care admission, 3 (14.3%) required supplemental oxygen, and 4 (19%) required inotropes. Nine (42.9%) were treated with intravenous immunoglobulin alone, 6 (28.6%) with steroids alone, and 3 (14.3%) with steroids and immunoglobulin. The median hospital stay was 6 days; there were no fatalities. Overweight/obesity, elevated ferritin, and mucocutaneous involvement were significantly associated with a prolonged hospital stay (≥7 days). Sixteen children (76.2%) were followed up till now and all of them had no clinical concerns.

Conclusion: MIS-C is an emerging disease with variable presentation. A high index of suspicion is necessary for its early identification and appropriate management. Further research is essential for developing optimal treatment strategies.

Key words: Clinical spectrum, Coronavirus disease 2019, Kawasaki disease, Multisystem inflammatory syndrome in children

Key message

- **Question:** What are the clinical spectrum, course, and short-term outcomes of multisystem inflammatory syndrome in children (MIS-C)?
- **Finding:** MIS-C can have variable clinical manifestations. Fever is most common, followed by gastrointestinal and cardiovascular symptoms. Early identification and appropriate management lead to favorable outcomes.
- **Meaning:** MIS-C can present in a myriad of ways and severities. High suspicion is necessary to ensure its early identification and appropriate management and favorable patient outcomes.

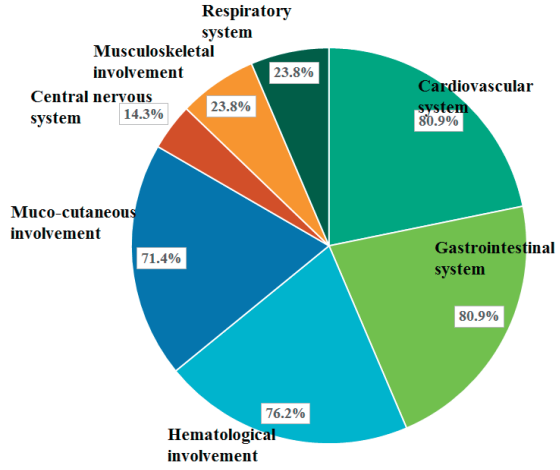
Introduction

The coronavirus disease 2019 (COVID-19) pandemic in India is a part of the global pandemic caused by severe acute respiratory syndrome corona virus-2 (SARS-CoV-2). India is one of the countries with the highest burden of confirmed COVID-19 cases in the world and ranks second in the list of countries with a maximum number of cases.[1] Earlier reports suggested that children infected with COVID-19 were highly resilient and could present with only a mild upper respiratory illness.[2-4] But in April 2020, clinicians in the United Kingdom reported a cluster of previously healthy children presenting with shock, fever, and hyper inflammation.[5] The Royal College of Paediatrics and Child Health referred to this acute condition as pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2.[6] As the pandemic progressed and the number of such cases increased globally the illness was labelled as multisystem inflammatory syndrome in children (MIS-C) by the Centers for Disease Control and Prevention and

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Clinical Spectrum of Multisystem Inflammatory Syndrome in Children



- Intensive care admission – 52.4 %
- Supplemental oxygen requirement – 14.3%
- Inotrope requirement – 19 %
- Abnormal ECHO – 76.2%
- Mortality - 0%
- Follow-up – 76.2%

Graphical abstract

the World Health Organization (WHO).[7,8] Although the exact pathophysiology of MIS-C is uncertain, it is thought to be due to immune dysregulation occurring after recovery from acute infection. As the burden of COVID-19 infection is increasing in India, more children are being recognized with this condition. Although many articles are reported from western countries there is a dearth of published reports from Asian countries.

Our study aimed at analyzing the clinical spectrum, laboratory parameters, and imaging characteristics of children less than 16 years admitted with a diagnosis of MIS-C. In addition, this study aimed to analyze the treatment strategies and short-term outcomes.

Methods

1. Study design and setting

This study was a partly retrospective and partly prospective observational study done in children admitted with a diagnosis of MIS-C in a tertiary care teaching hospital in south India from August 2020 to January 2021. The hospital is one of the largest COVID-19 care centers in south India and receives referrals from a radius of 200 kilometers. The study was approved by the Institutional Human Ethics Committee of PSGIMS&R (project No. 20/262 dated 27th November, 2020). Informed written consent was obtained from parents prior to the inclusion of children in the prospective part of the study. Waiver of consent was obtained for the retrospective collection of data.

2. Participants

Children less than 16 years of age diagnosed with MIS-C as per WHO criteria were included in the study. The following parameters were considered for diagnosis: (1) fever ≥3 days, (2) 2 of the following: a) rash or bilateral nonpurulent conjunctivitis or mucocutaneous inflammation signs (oral mucosa, hands, or feet), b) hypotension or shock, c) features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiography findings or elevated troponin/N-terminal pro-B-type natriuretic peptide [NT-proBNP]), d) evidence of coagulopathy (by prothrombin time, partial thromboplastin time, elevated D-dimers), e) Acute gastrointestinal problems [diarrhea, vomiting, or abdominal pain], (3) elevated markers of inflammation such as erythrocyte sedimentation rate, C-reactive protein, or procalcitonin, (4) no other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal toxic shock syndromes, (5) evidence of COVID-19 (real-time reverse transcription polymerase chain reaction [RT-PCR], antigen test or serology positive), or likely contact with patients with COVID-19.

3. Data collection

Data on demographics, clinical features, laboratory results, imaging studies, treatment details, outcomes, and follow-up were collected. All the investigations and treatment were carried out as per institutional protocol and treating physician's discretion.

COVID-19 infection was confirmed by testing for SARS-CoV-2 antibody by chemiluminescence immunoassay (VITROS 3600 immunodiagnostic system, Ortho Clinical Diagnostics, Raritan, NJ, USA) and/or RT-PCR as recommended by Indian Council of Medical Research. The laboratory values were interpreted as either increased or decreased based on the age-specific normal reference ranges. Organ system involvement was described based on clinical features, laboratory reports, and imaging studies. Echocardiography was performed in all children by the pediatric cardiologist to identify the following abnormalities; (a) left ventricular dysfunction (ejection fraction

less than 55%), (b) pericarditis or pericardial effusion, (c) coronary artery abnormalities (dilatation, aneurysm, or irregular nontapering coronary arteries), or (d) valvular regurgitation. The dimension of the coronary artery was measured as per standard criteria and a coronary z score above 2 was reported as dilated. Other imaging studies were done as clinically indicated.

4. Statistical analysis

Data analysis was done using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). Descriptive statistics were used to summarize population characteristics. Categorical variables were expressed as frequency or percentage and continuous variables were expressed as mean with standard deviation or median with interquartile range. IBM SPSS Statistics ver. 26.0 (IBM Co., Armonk, NY, USA) was used to perform univariate analysis and a *P* value of <0.05 was considered significant.

Results

From August 2020 to January 2021, twenty-one children met the WHO MIS-C criteria and were included in the analysis. Among them, only 2 children were admitted with active coronavirus-2 infection and were subsequently diagnosed as MIS-C. The demographic and clinical characteristics of the study population are mentioned in Table 1. The mean age of the study population was 6.9 years and 71.4% were male. All children were previously healthy and 9.5% were either obese or overweight.

All patients had fever with a variable combination of other symptoms. Gastrointestinal symptoms like abdominal pain, diarrhea, and vomiting were the most common presenting symptoms. The mean duration of symptoms prior to admission was 4.8 days and the mean total duration of fever was 7.9 days. The majority of children in this series had multiorgan involvement. Gastrointestinal (80.9%) and cardiovascular (80.9 %) systems were the most commonly affected organ systems. This was followed by hematological (76.2%), mucocutaneous (71.4%), and central nervous system (14.2%) involvement. None of the children had acute kidney injury in this series. Two children in this series had been treated for COVID-19 3 weeks prior to the diagnosis of MIS-C, and 5 children had history of confirmed exposure to COVID-19 patients in the preceding 3 to 4 weeks. In other children, no history associated with COVID-19 was revealed.

In the study population, 19 children (90.4%) had positive COVID-19 antibody tests, 1 (4.8%) had positive COVID-19 RT-PCR test, and another (4.8%) had both positive COVID-19 RT-PCR and antibody tests. The results of laboratory tests and imaging studies are summarized in Tables 2 and 3. Lymphopenia and thrombocytopenia were seen in 47.6% and 38.1% of cases, respectively. Inflammatory markers were elevated in the majority of cases. Specifically, D-dimer and interleukin 6 (IL-6) were elevated in 95.2% and 90.5% of cases, respectively. Children with cardiovascular system involvement showed elevation of

Table 1. Patients' demographic and clinical characteristics

Population characteristic	Value
Age (yr)	6.9±4
Male sex	15 (71.4)
Weight (kg), median (IQR)	20 (12.8–35.5)
Overweight/obesity	2 (9.5)
Duration of symptoms at admission (day)	4.8±2.1
Contact with COVID-19 patient	5 (23.8)
Past COVID-19 infection	2 (9.5)
Clinical features	
General	
Fever	21 (100)
Total duration of fever (day)	7.9±3.1
Highest temperature recorded in hospital admission (°F)	102.4±1.8
Giddiness	1 (4.8)
Fatigue±lethargy	13 (61.9)
Headache	5 (23.8)
Respiratory	
Cough	2 (9.5)
Sore throat/ throat pain	3 (14.3)
Runny nose±sneezing	1 (4.8)
Fast breathing	4 (19)
Gastrointestinal	
Nausea	3 (14.3)
Vomiting	12 (57.1)
Diarrhea	11 (52.4)
Abdominal pain	11 (52.4)
Abdominal distension	5 (23.8)
Musculoskeletal	
Joint pain	1 (4.8)
Myalgia	7 (33.3)
Mucocutaneous	
Rash	8 (38.1)
Bilateral non-exudative conjunctivitis	12 (57.1)
Lymphadenopathy	3 (14.3)
Strawberry tongue±cracked lips	7 (33.3)
Oedema/erythema of hands and feet	6 (28.6)
Cardiovascular	
Shock requiring inotropes	4 (19)
Neurological	
Seizures	1 (4.8)
Altered level of consciousness	3 (14.3)
Cerebellar signs	1 (4.8)
Focal neurological deficit	1 (4.8)
Organ system involvement[a)]	
One	1 (4.8)
Two	3 (14.3)
Three	4 (19)
Four or more	13 (61.9)

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
IQR, interquartile range; COVID-19, coronavirus disease 2019.
[a)]Organ system involvement was classified as cardiovascular, gastrointestinal, respiratory, nervous, musculoskeletal, hematological, or renal based on clinical features and investigations.

Table 2. Laboratory test results

Parameter	Number (%)	Definition
Blood counts		
Anemia	4/21 (19)	Hb <10 g/dL in <1 yr Hb <9 g/dL in ≥1 yr
Neutrophilia	16/21 (76.2)	ANC >7.7×10^3 cells/μL
Lymphopenia	10/21 (47.6)	ALC <1.5×10^3 cells/μL in child >8 mo ALC <4.5×10^3 cells/μL in child <8 mo
Thrombocytopenia	8/21 (38.1)	Platelet <150×10^3 cells/mm^3
Thrombocytosis	6/21 (28.6)	Platelet >500×10^3 cells/mm^3
Inflammatory markers		
Elevated ESR	17/20 (85)	≥40 mm/hr
Elevated CRP	20/20 (100)	≥0.6 mg/dL
Elevated procalcitonin	5/5 (100)	≥0.5 ng/mL
Elevated ferritin	9/21 (42.9)	>500 ng/mL
Elevated IL-6	19/21 (90.5)	≥7 pg/mL
Elevated LDH	11/16 (68.8)	LDH >300 U/L
Electrolytes		
Hyponatremia	12/17 (70.6)	Sodium <135 mEq/L
Hypokalemia	8/17 (47.1)	Potassium <3.5 mEq/L
Liver function test		
Elevated AST	6/17 (35.3)	AST >40 U/L
Elevated ALT	5/17 (29.4)	ALT >40 U/L
Hypoalbuminemia	9/17 (52.9)	Albumin <3 g/dL
Cardiac biomarkers		
Elevated troponin-T	4/13 (30.8)	≥14 pg/mL
Elevated NT-proBNP	12/15 (80)	>450 pg/mL
Coagulation profile		
Elevated D-dimer	20/21 (95.2)	≥0.5 mg/L FEU
Deranged coagulation	2/8 (25)	INR ≥1.2

Hb, hemoglobin; ANC, absolute neutrophil counts; ALC, absolute lymphocyte counts; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL, interleukin; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; FEU, fibrinogen equivalent units; INR, international normalized ratio.

cardiac biomarkers, and NT-proBNP (80%) was found to be more commonly elevated than troponin-T (30.8%). Electrolyte imbalances like hyponatremia and hypokalemia were seen in 70.6% and 47.1% of cases, respectively, which were managed appropriately.

Echocardiography was performed in all the children, and abnormality was noted in 16 children (76.2%). A greater number of children had a combination of abnormalities rather than a single abnormal finding. Coronary artery abnormality (57.1%) was the most common finding, followed by pericardial effusion (52.4%), left ventricular dysfunction (23.8%), and mitral regurgitation (23.8%). Gastrointestinal imaging studies like x-ray, ultrasound, and computed tomography were done as indicated, and ascites (50%) was the most common abnormality, followed by dilated bowel loops (35.7%), and mesenteric lymphadenitis (35.7%). Of the 17 patients who underwent chest imaging (x-ray or computed tomography), pleural effusion and lung infiltrates were observed in 3 (17.6%) and 1 patient (5.9%),

Table 3. Results of COVID-19 tests and imaging studies

Variable	Number (%)
COVID-19 test results	
Positive serology alone	19 (90.4)
Positive RT-PCR alone	1 (4.8)
Both serology and RT-PCR positive	1 (4.8)
Chest imaging (n=17)	
Pleural effusion	3 (17.6)
Lung infiltrates	1 (5.9)
Gastrointestinal imaging (n=14)	
Ascites	7 (50)
Mesenteric lymphadenitis	5 (35.7)
Dilated bowel loops	5 (35.7)
Appendicular inflammation	3 (21.4)
Appendicular abscess	1 (7.1)
Hepatomegaly	3 (21.4)
Gallbladder wall thickening	1 (7.1)
Echocardiography findings (n=21)	
Abnormal ECHO	16 (76.2)
Coronary artery abnormality	12 (57.1)
Pericardial effusion	11 (52.4)
Left ventricular dysfunction	5 (23.8)
Mitral regurgitation	5 (23.8)

COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription polymerase chain reaction; ECHO,

Table 4. Treatment details and outcomes

Variable	Value
Highest level of care	
PICU admission	11 (52.4)
Duration of PICU stay (hr)	74.8±41.6
Respiratory support	
Supplemental oxygen requirement	3 (14.3)
Duration of oxygen requirement (hr)	39.6±28.6
High flow nasal cannula	1 (4.8)
Circulatory support	
Inotropic support	4 (19)
Duration of inotrope requirement (hr)	37±24.5
Pharmacotherapy	
Antibiotics	18 (85.7)
Intravenous immunoglobulin alone	9 (42.9)
Steroids (intravenous or oral) alone	6 (28.6)
Both intravenous immunoglobulin and steroids	3 (14.3)
Aspirin	14 (66.7)
Anticoagulant	3 (14.3)
Antiepileptics	1 (4.8)
Outcomes	
Length of hospital stay (day), median (IQR)	6 (5–8)
Mortality	0 (0)
Follow-up	16 (76.2)

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
PICU, pediatric intensive care unit; IQR, interquartile range.

respectively. Of the 3 children with central nervous system involvement, 1 had encephalopathy with features of cerebellar involvement, 1 had seizures, and another had headache with bilateral papilledema. All 3 children with central nervous system involvement showed normal neuroimaging. Two children underwent cerebrospinal fluid analysis, and findings of aseptic meningitis were seen in the patient who presented with encephalopathy.

Treatment details and outcomes are summarized in Table 4. Eleven children (52.4%) required pediatric intensive care unit (PICU) admission and the mean duration of PICU stay was 74.8 hours. Three children (14.3%) required supplemental oxygen, and none of the children required mechanical ventilation. Four children (19%) required inotropic support for hemodynamic instability, and the mean duration of inotrope requirement was 37 hours. Nine children (42.9%) were treated with intravenous immunoglobulin (IVIG) alone, 6 (28.6%) were treated with steroids alone and 3 (14.3%) were treated with both IVIG and steroids. None of the cases required other immunomodulators for treatment.

An 8-year-old girl presented with features of acute appendicitis, and an emergency surgery was done, which revealed a perforated appendix with abscess collection. She was subsequently diagnosed as MIS-C and was treated with IVIG.

The median length of hospitalization was 6 days in this series. Table 5 shows the results of univariate analysis for prolonged hospital stay (≥7 days). Children who were either overweight or obese (P=0.035), had elevated ferritin (P=0.019), or had mucocutaneous involvement (P=0.040) were found to be significantly associated with prolonged hospital stay. Further extensive analysis could not be performed due to small sample size. There was no mortality in patients of our study, and all patients were discharged home. At the time of writing this article, 16 patients (76.2%) had attended follow-up visits.

Table 5. Results of univariate analysis of factors associated with prolonged hospital stay (n=21)

Variable	Hospital stay<7 days (n=14), n (%)	Hospital stay≥7 days (n=7), n (%)	*P* value	OR (95% CI)
Age groups			0.376	-
Less than 5 yr (n=7)	6 (85.7)	1 (14.3)		
5 to 9 yr (n=6)	3 (50.0)	3 (50.0)		
10 to 15 yr (n=8)	5 (62.5)	3 (37.5)		
Sex			1.000	1.000 (0.134–7.451)
Male (n=15)	10 (66.7)	5 (33.3)		
Female (n=6)	4 (66.7)	2 (33.3)		
Overweight/obesity (n=2)	0 (0)	2 (100)	**0.035**	0.263 (0.124–0.558)
Temperature > 38.9℃ (n=11)	5 (45.5)	6 (54.5)	0.089	10.80 (0.997–116.998)
Anemia (n=4)	2 (50.0)	2 (50.0)	0.432	2.400 (0.261–22.105)
Lymphopenia (n=10)	5 (50.0)	5 (50.0)	0.122	4.500 (0.627–32.295)
Thrombocytopenia (n=8)	6 (75.0)	2 (25.0)	0.525	0.533 (0.076–3.755)
Elevated ESR (n=17)	12 (70.6)	5 (29.4)	0.212	0.208 (0.015–2.854)
Elevated ferritin (n=9)	3 (33.3)	6 (66.7)	**0.019**	22.0 (1.857–260.648)
Elevated IL-6 (n=19)	12 (63.2)	7 (33.3)	0.293	-
Elevated D-dimer (n=20)	13 (65.0)	7 (35.0)	0.469	-
Hyponatremia (n=12)	6 (50.0)	6 (50.0)	0.252	4.000 (0.340–47.112)
Hypokalemia (n=8)	4 (50.0)	4 (50.0)	0.486	2.000 (0.282–14.198)
Hypoalbuminemia (n=9)	5 (55.6)	4 (44.4)	0.772	1.333 (1.191–9.311)
Abnormal ECHO (n=16)	10 (62.5)	6 (37.5)	0.469	2.400 (0.215–26.822)
PICU admission (n= 12)	7 (58.3)	5 (41.7)	0.350	2.500 (0.357–17.500)
Supplemental oxygen requirement (n=3)	1 (33.3)	2(66.7)	0.186	5.200 (0.381–70.903)
Inotropic support (n=4)	2 (50.0)	2 (50.0)	0.432	2.400 (0.261–22.105)
Organ system involvement				
Cardiovascular system (n=17)	10 (58.8)	7 (41.2)	0.116	-
Respiratory system (n=5)	2 (40.0)	3 (60.0)	0.147	4.500 (0.542–37.378)
Gastrointestinal system (n=17)	11 (64.7)	6 (35.3)	0.694	1.636 (0.138–19.387)
Central nervous system (n=3)	2 (66.7)	1 (33.3)	1.000	1.000 (0.075–13.367)
Hematological system (n=16)	9 (56.3)	7 (43.8)	0.070	1.778 (1.154–2.379)
Mucocutaneous involvement (n=15)	12 (80.0)	3 (20.0)	**0.040**	0.125 (0.15–1.038)
Musculoskeletal system (n=5)	3 (60.0)	2 (40.0)	0.717	1.467 (0.184–11.718)
Multiorgan involvement (≥4 organs) (n=13)	8 (61.5)	5 (38.5)	0.525	1.875 (0.266–13.202)

OR, odds ratio; CI, confidence interval; ESR, erythrocyte sedimentation rate; IL, interleukin; ECHO, :PICU, pediatric intensive care unit.
Boldface indicates a statistically significant difference with P<0.05.

Table 6. Common clinical characteristics in our study versus those of other studies

Clinical feature	Our study (n=21)	Whittaker et al.[10] (n=58)	Dhanalakshmi et al.[11] (n=19)
Fever	21 (100)	58 (100)	19 (100)
Vomiting	12 (57.1)	26 (45)	6 (31.5)
Abdominal pain	11 (52.4)	31 (53)	8 (42)
Diarrhea	11 (52.4)	30 (52)	3 (15.8)
Rash	8 (38.1)	30 (52)	12 (63.2)
Bilateral conjunctival congestion	12 (57.1)	26 (45)	9 (47.4)
Cervical lymphadenopathy	3 (14.3)	9 (16)	6 (31.5)
Strawberry tongue±cracked lips	7 (33.3)	17 (29)	9 (47.4)
Oedema/erythema of hands and feet	6 (28.6)	9 (16)	10 (52.6)

Values are presented as number (%).

Among them, 11 children (52.4%) had attended one follow-up visit, and 5 (23.8%) had attended 2 follow-up visits, all of whom had no clinical concerns. Among 16 children with abnormal echocardiography, 12 (75%) underwent follow-up echocardiography, and their cardiac imaging showed partial or complete resolution of previous abnormalities. Further follow-up echocardiography was recommended to some of them.

Discussion

MIS-C is an evolving concept of disease and its exact incidences in different geographic areas are uncertain. The focus of this study was to analyze the clinical spectrum and short-term outcomes of children with MIS-C managed in a tertiary care teaching hospital in south India. We described 21 children under the age of 16 years with MIS-C associated with SARS-CoV-2 infection. All these children had evidences of previous or active COVID-19 infection. The spectrum of clinical presentations in our study was broadly consistent with available literatures. The mean age of children in this study was 6.9 years, and 61.9% of affected children were above 5 years, which was similar to studies by Kaushik et al.,[9] Whittaker et al.,[10] and Dhanalakshmi et al.[11] The male preponderance in this study was also identical to previous reports by Kaushik et al.,[9] Whittaker et al.,[10] and Gupta et al.[12] Table 6 shows the comparison between common clinical features of children in this study and 2 previous studies.[10,11] Concordant to previous reports, a greater number of children in our series had multiorgan involvement with gastrointestinal and cardiovascular systems being the most commonly affected systems.[11,13-15]

The incidence of hematological abnormalities like lymphopenia (47.6%) and thrombocytopenia (38.1%) in our study was in agreement with previous reports.[11,14,16] The majority of children in this study had raised inflammatory markers, which was similar to previous reports by Kaushik et al.,[9] Dhanalakshmi et al.,[11] Feldstein et al.,[13] and Mamishi et al.[16] In contrast to previous studies by Dhanalakshmi et al.,[11] Feldstein et al.,[13] Toubiana et al.,[15] and Miller et al.,[17] none of the children in this series had acute kidney injury. The incidence of hyponatremia (70.6%) in this study was higher than in previous reports by Dhanalakshmi et al.[1] and Mamishi et al.[16] Hypokalemia was found in 47.1% of children, and it was more common in children with gastrointestinal manifestations, which was not reported in any previous studies. Hypoalbuminemia was noted in 52.9% of children in this study, which was almost similar to previous reports by Dhanalakshmi et al.[1] and Mamishi et al.[16] The elevation of cardiac biomarkers was also concordant to previous reports by Whittaker et al.[10] and Mamishi et al.[16] The incidence of echocardiographic abnormalities in this study was comparable to previous reports by Ramcharan et al.[15] and Toubiana et al.[18] The abnormalities seen in gastrointestinal imaging studies in this study were comparable to the previous report by Miller et al.[17]

Since MIS-C is an emerging disease, guidelines regarding the management are still evolving and there is a lack of consistency in management. The majority of patients in our study were treated with either IVIG, steroids, or a combination of both and, none required other immunomodulators. The treatment decision for the patients in our study was made based on severity of the illness and the treating physician's discretion. Significant differences in treatment strategies were noted among studies from different parts of the world, which could be due to lack of standard treatment protocols in the initial phase of disease evolution.[9-11,14,16] However, a considerable number of studies would be required for developing standard treatment protocols. Concordant to previous reports by Torres et al.[14] and Dhanalakshmi et al.,[11] 52.4% of children in this series required PICU admission. The median length of hospital stay was 6 days which was comparable to previous reports.[9,14,15] Although previous studies showed variable rates of mortality, no death was reported in this study.[11,14,15,17] The follow-up rate in this study was 76.2%. All children had no health concerns at the time of follow-up, and most echocardiographic abnormalities had resolved. However, these children should be followed up at regular intervals to know the long-term consequences of the disease.

A major limitation of this study is that it was conducted in a single center over a limited period of time, and has a small sample size. Another limitation is that this study only included children under the age of 16 years, because our institutional policy defined children under 16 years to be admitted to the pediatric department. It might have missed MIS-C patients older than 16

years of age.

In conclusion, MIS-C is a novel disease that can present in a myriad of ways with variable severity, and a high index of suspicion is necessary in this era of COVID-19 pandemic for early identification, appropriate management, and favorable outcomes of patients. Further research is essential to develop optimal treatment strategies.

Footnotes

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

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References

1. WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. Geneva (Switzerland): World Health Organization; 2021 [cited 2021 Feb 24]. Available from: https://covid19.who.int/.
2. Castagnoli R, Votto M, Licari A, Brambilla I, Bruno R, Perlini S, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. JAMA Pediatr 2020;174:882-9.
3. Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis 2020;34:101623.
4. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr 2020;109:1088-95.
5. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet 2020;395:1607-8.
6. Paediatric multisystem inflammatory syndrome temporally associated with COVID-19 (PIMS) - guidance for clinicians [Internet]. London: Royal College of Paediatrics and Child Health; 2021 [cited 2021 Feb 24]. Available from: https://www.rcpch.ac.uk/resources/paediatric-multi-system-inflammatory-syndrome-temporally-associated-covid-19-pims-guidance.
7. Emergency preparedness and response. Multisystem inflammatory syndrome in children associated with coronavirus disease 2019 [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2020 [cited 2021 Feb 24]. Available from: https://emergency.cdc.gov/han/2020/han00432.asp.
8. Multisystem inflammatory syndrome in children and adolescents with COVID-19 [Internet]. Geneva (Switzerland): World Health Organization [cited 2021 Feb 24]. Available from: www.who.int/publicationsdetail/multisystem-inflammatory-syndrome-in-children-and-adolescents-withcovid-19.
9. Kaushik S, Aydin SI, Derespina KR, Bansal PB, Kowalsky S, Trachtman R, et al. Multisystem inflammatory syndrome in children associated with severe acute respiratory syndrome coronavirus 2 infection (MIS-C): a multi-institutional study from New York City. J Pediatr 2020;224:24-9.
10. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. JAMA 2020;324:259-69.
11. Dhanalakshmi K, Venkataraman A, Balasubramanian S, Madhusudan M, Amperayani S, Putilibai S, et al. Epidemiological and clinical profile of pediatric inflammatory multisystem syndrome - temporally associated with SARS-CoV-2 (PIMS-TS) in Indian children. Indian Pediatr 2020;57:1010-4.
12. Gupta Dch S, Chopra Md N, Singh Md A, Gera R, Chellani Md H, Pandey PhD R, et al. Unusual clinical manifestations and outcome of multisystem inflammatory syndrome in children (MIS-C) in a tertiary care hospital of North India. J Trop Pediatr 2021;67:fmaa127.
13. Feldstein LR, Rose EB, Horwitz SM, Collins JP, Newhams MM, Son MBF, et al. Multisystem inflammatory syndrome in U.S. children and adolescents. N Engl J Med 2020;383:334-6.
14. Torres JP, Izquierdo G, Acuña M, Pavez D, Reyes F, Fritis A, et al. Multisystem inflammatory syndrome in children (MIS-C): report of the clinical and epidemiological characteristics of cases in Santiago de Chile during the SARS-CoV-2 pandemic. Int J Infect Dis 2020;100:75-81.
15. Toubiana J, Poirault C, Corsia A, Bajolle F, Fourgeaud J, Angoulvant F, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. BMJ 2020;369:m2094.
16. Mamishi S, Movahedi Z, Mohammadi M, Ziaee V, Khodabandeh M, Abdolsalehi MR, et al. Multisystem inflammatory syndrome associated with SARS-CoV-2 infection in 45 children: a first report from Iran. Epidemiol Infect 2020;148:e196.
17. Miller J, Cantor A, Zachariah P, Ahn D, Martinez M, Margolis KG. Gastrointestinal symptoms as a major presentation component of a novel multisystem inflammatory syndrome in children that is related to coronavirus disease 2019: a single center experience of 44 cases. Gastroenterology 2020;159:1571-4.e2.
18. Ramcharan T, Nolan O, Lai CY, Prabhu N, Krishnamurthy R, Richter AG, et al. Paediatric inflammatory multisystem syndrome: temporally associated with SARS-CoV-2 (PIMS-TS): cardiac features, management and short-term outcomes at a UK tertiary paediatric hospital. Pediatr Cardiol 2020;41:1391-401.

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RESEARCH ARTICLE

Serotype-specific differences in the laboratory parameters among hospitalized children with dengue and genetic diversity of dengue viruses circulating in Tamil Nadu, India during 2017

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Abstract

In 2017, Tamil Nadu, a southern state, had the second highest number of dengue cases from India. In the present study, the serotype-specific differences in the clinical manifestations and laboratory parameters among hospitalized children with dengue were investigated and molecular characterization of the circulating dengue virus (DENV) serotypes during 2017 in Tamil Nadu was performed. Eighty children with dengue-like symptoms consecutively admitted to a tertiary care hospital and positive for DENV NS1 antigen were investigated for DENV serotype utilizing a real-time reverse transcriptase based polymerase chain reaction assay. Complete envelope (E) gene sequencing of the DENV strains was performed. Seventy samples were positive for serotyping (25 DENV-1, 17 DENV-2, six DENV-3, and 22 DENV-4). DENV-4 infections were associated with elevated levels of liver enzymes; Alanine aminotransferase ($P = .021$) and aspartate aminotransferase ($P = .001$). However, none of the serotype was associated with any specific clinical features and severe dengue. Asian and American/African genotypes of DENV-1 were cocirculating. The circulating genotype was cosmopolitan for DENV-2 with multiple lineages, genotype III for DENV-3 and genotype I for DENV-4. Unique mutations were present in the 2017 DENV-4 isolates. The present study suggests the association of DENV-4 with elevated liver enzymes in children hospitalized for dengue. Further, the study reports the genetic diversity of DENV circulating in Tamil Nadu during 2017. The study calls for continuous monitoring of the circulating serotypes and genotypes at regional level in India which might result in a region wise database useful in predicting future outbreaks.

KEYWORDS

alanine aminotransferase, aspartate aminotransferase, dengue, genotypes, serotypes, severe dengue

N. T. Rajesh and K. Alagarasu contributed equally.

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1 | INTRODUCTION

Dengue is the commonest arthropod borne viral disease that affect millions of individuals globally each year and has been listed by the World Health Organization (WHO) as one of the major threats to mankind.[1] Infection with any of the four serotypes of dengue virus (DENV) results in a range of clinical presentations. Symptomatic dengue infections are categorized into nonsevere dengue and severe dengue (SD). The nonsevere dengue cases are again classified into those without warning signs (DwoWS) and with warning signs (DwWS). The case fatality rate is less than 1% if proper intervention is initiated at an early stage of the disease.[2] Disease severity is influenced by both virus and host factors. Immune status determined as primary infection or secondary infection with a different serotype of DENV has been reported to be associated with dengue severity by various epidemiological studies. Age, gender, nutritional status, host immune response, and host genetics contribute to differences in the clinical outcomes.[3,4] Among the viral factors, infecting serotype and the genotype within each serotype also contributes to disease severity.[3] Replacement of the circulating serotype/genotype of DENV with a different one contributes to outbreaks of higher magnitude.[5,6]

In the last decade, highest number of dengue cases were reported during 2017 in India. Within India, West Bengal and Tamil Nadu accounted for approximately 30% of the reported dengue cases.[7] Before 2017, Tamil Nadu witnessed large number of cases during 2012 and DENV-1 was the most common serotype despite the presence of other serotypes. Replacement of American/African (AM/AF) genotype with Asian genotype of DENV-1 was observed during 2012 in Tamil Nadu.[8] However, the serotypes and genotypes circulating during 2017 were not known. The present study was aimed at identifying the serotype-specific differences in the clinical symptoms and laboratory parameters in DENV infected children. Moreover, the study also intended to identify the dominant circulating serotype(s) and genotype(s) associated with dengue in Tamil Nadu during 2017.

2 | MATERIALS AND METHODS

2.1 | Study subjects

The study was a cross-sectional prospective study conducted at PSG institute of Medical Science and Research, a tertiary care teaching hospital located in western Tamil Nadu between August and December 2017. Children aged between 6 months to 15 years who fulfilled the inclusion criteria were consecutively enrolled in the study. Children admitted in the hospital with undifferentiated fever of less than 5 days duration with clinical suspicion of dengue and positive for the presence of NS1 antigen were included in the study. Exclusion criteria include: negative test for the presence of NS1 antigen, fever beyond 5 days, unwillingness to participate in the study and patients who were not admitted. The parents or the legal guardians were explained about the study purpose and an informed consent was obtained from them. The parents or the caretakers of the patients were interviewed for their demographic characteristics such as gender, age, and previous dengue episodes. The patients were observed for clinical symptoms including warning signs till their discharge and clinical features and lab data were collected in a predesigned data collection tool. Based on the clinical symptoms and laboratory parameters, the patients were categorized into dengue without warning signs (DwoWS), dengue with warning signs (DwWS), and severe dengue (SD) based on the WHO 2009 guidelines.[9] Plasma or serum samples collected from the participants at the time of entry into the study, were separated and stored at −80°C. The samples were transported in dry ice to ICMR-National Institute of Virology for identifying the serotype. The study was approved by the institutional human ethics committee (IHEC) of PSGIMS&R, Coimbatore, Tamil Nadu. Molecular characterization of the circulating DENV was performed at ICMR-NIV as a part of Apex referral laboratory activity for the year 2017 which was approved by the IHEC of ICMR-NIV.

2.2 | Serological assays

Serological assays were performed in the hospital laboratory. Presence of DENV NS1 antigen was tested by a antigen capture ELISA kit (Panbio Dengue Early ELISA) as per the manufacturer's protocol. Dengue-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies were tested using ELISA kits (Panbio IgM [01PE20] and Panbio IgG capture ELISA kit [01PE10]) according to the instructions provided with the kit. The IgG capture ELISA kit used in the study detects high-titer IgG antibodies which is usually present in secondary infections. Test positive for IgG indicates secondary infection while test negative indicates primary infection.

2.3 | Detection of dengue virus serotypes

RNA was extracted using an RNA extraction kit (QIAamp viral RNA mini kit, Qiagen, Hilden, Germany) according to the manufacturer's protocol and used for DENV serotyping. Serotyping was performed by using an in-house real-time reverse transcription polymerase chain reaction (RT-PCR) assay. The sequences of the primers and probes were given in Table S1. Primer and probe sequences, the reaction mixture composition and cycling conditions for real-time RT-PCR have been reported previously.[10] The PCR cycle at which the fluorescence signals crossed the threshold values were detected for each sample and samples that had a cycle threshold (C_t) value ≤37 were considered as positive.[10]

2.4 | Isolation of dengue virus from clinical samples

Virus isolation was performed in samples which were positive for DENV RNA with increased viral load (C_t value ≤25) using C6/36 cell

line as reported previously.[10] Virus stocks were stored at −80°C in aliquots for further use.

2.5 | Sequencing of complete envelope gene of dengue virus

Randomly selected representative virus isolates from each serotype were used for sequencing of complete E gene (seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4 isolates) using primers reported earlier.[11-13] The sequences of the primers were provided in Table S1. RNA extraction, cDNA synthesis, reverse transcription, PCR amplification, gel purification, and sequencing was carried out as described earlier.[10]

2.6 | Phylogenetic analyses of dengue virus isolates

MEGA7.0 was used for multiple sequence alignment and phylogenetic analyses.[14] Seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4 sequences (GenBank accession number for the virus isolates provided in Table S2) from the current study along with the earlier Indian (44 DENV-1, 79 DENV-2, 39 DENV-3, and 31 DENV-4) and global sequences (36 DENV-1, 43 DENV-2, 54 DENV-3, and 47 DENV-4) from GenBank representing different genotypes were used for the phylogenetic analyses. Kimura-2 Parameter model was used to estimate the genetic differences and maximum likelihood (ML) method was used for the construction of the phylogenetic trees. A bootstrap test with 1000 replications was used to evaluate the reliability of position of the branches.[10]

2.7 | Statistical analyses

To compare univariate categorical data, Fisher's Exact tests or Pearson's X^2 was used. Parametric and nonparametric data from subjects infected with different dengue serotypes were compared using one-way analysis of variance and Kruskal-Wallis tests, respectively. Modified Poisson regression with adjustment for relevant clinical covariates, that is, age, gender, and fever at total day was utilized for performing the regression analyses. Data for one serotype was compared with other three serotypes. Except platelet count, the values of other laboratory parameters at the time of admission were used for comparison. The nadir for platelet count was used for comparison. A probability (*P*) value of <.05 was considered statistically significant. All the statistical analyses were carried out using statistical software R version 3.4.4.

3 | RESULTS

3.1 | Demographic profile of the study subjects

A total of 430 children with undifferentiated fever were admitted with clinical diagnosis of dengue fever of which 236 children fulfilled the inclusion criteria, 80 consecutively admitted children were enrolled in the study and were followed up prospectively. On analyzing for the serotype, 10 samples were negative and hence excluded and only 70 subjects were analyzed. Almost equal proportion of boys and girls were observed and the median age was 9 years (6 months to 15 years) (Table 1).

TABLE 1 Demographic profile of the dengue cases infected with different dengue virus (DENV) serotypes

Patient Demographics									
	DENV–1 (n = 25)		DENV–2 (n = 17)		DENV–3 (n = 6)		DENV–4 (n = 22)		*P* value*
Variables	N	%	N	%	N	%	N	%	
Gender									
Male	9	36	11	64.7	3	50	15	68.2	.441
Female	16	64	6	35.3	3	50	7	31.8	
Locality									
Coimbatore	7	28	3	17.6	1	16.6	4	19	.832
Dharapuram	5	20	–	–	–	–	3	14.3	
Dindigul	2	8	–	–	–	–	–	–	
Karur	1	4	–	–	–	–	1	4.8	
Palani	1	4	3	17.6	1	16.6	3	14.3	
Pollachi	1	4	–	–	0	–	–	0	
Tiruchi	0	–	–	–	0	–	1	4.8	
Tirupur	8	32	11	64.7	4	66.6	10	47.6	
Age, median (range)	9 (0.9–16)	–	6 (0.3–15)	–	9 (6–15)	–	10 (4–14)	–	.236
Fever day at presentation, median (range)	4 (1–8)	–	5 (2–9)	–	4.5(3–5)	–	4.5 (1–8)	–	.442
Fever at total days, median (range)	5 (4–8)	–	5 (3–9)	?	4.5 (4–5)	–	5 (3–8)	–	.188

*X^2 test was used to compare categorical variables (gender and locality) and one–way analysis of variance was used to compare the continuous variables (age, fever day at presentation and fever at total days).

3.2 | Serotype-specific differences in the clinical features, lab parameters and disease severity

All four serotypes were observed to be circulating in the study population with the prevalent serotype being DENV-1, (n = 25, 35.7%), followed by DENV-4 (n = 22, 31.4%), and DENV-2 (n = 17, 24.3%). DENV-3 was identified in 8.6% (n = 6) of the subjects. Apart from a slight female preponderance among DENV-1 infected children, gender, age, and duration of fever were not different between the children infected with different serotypes (Table 1).

A similar modified Poisson regression analysis adjusted for age, gender, and day of fever was performed for various lab parameters. A statistically significant increase in liver enzymes, aspartate aminotransferase (median with range 131.5 [22-1156] $P = .001$) and alanine aminotransferase (median with range 63 [14-594] $P = .021$) were found in DENV-4 infected children compared to children infected with other serotypes. There was no significant difference in the level of liver enzymes between primary and secondary infections. The mean ± standard deviation of liver enzymes, SGPT and SGOT in children with primary and secondary dengue were 99.18 ± 123.91, 66.83 ± 79.09 ($P = .255$) and 172.25 ± 225.03, 139.38 ± 164.15 ($P = .443$). Other hematological parameters including platelet count during the hospital period were not significantly different among children infected with different serotypes (Table 2).

After adjustment for age, gender, and duration of fever, we observed no statistically significant correlation between the infecting serotype and the clinical features (Table 3). A majority of the children had secondary dengue infection (n = 52 [67.5%]). However, immune status was not associated with disease severity (Table 3).

Classifying the children based on disease severity according to WHO 2009 criteria, there were 38 cases with DwoWS, 26 with DwWS, and 6 had SD. There was no significant association between disease severity and infecting serotype (Table 3).

3.3 | Results of virus isolation

A total of 51 samples were used for virus isolation. Virus isolation was positive in 34 samples (seven DENV-1, 13 DENV-2, two DENV-3, and 12 DENV-4). Complete E gene of the representative virus isolates (seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4) was sequenced.

3.4 | Circulating genotypes of DENV-1

DENV-1 was classified into five genotypes as described earlier.[15] Since there were confusions with regard to the Roman number based nomenclature, geographical names were provided.[16] Seven DENV-1 virus isolates were sequenced. The ML method-based phylogenetic tree (Figure 1) revealed that five isolates grouped with the Asian genotype (GI). Two isolates clustered with the American African (AM/AF) genotype (GV). Asian genotype isolates obtained during

TABLE 2 Association of laboratory/hematological parameters with infecting dengue virus (DENV) serotype

Variables	DENV-1 (n = 25)			DENV-2 (n = 17)			DENV-3 (n = 6)			DENV-4 (n = 22)		
	Median	Range	*P* value*	Median	Range	*P* value*	Median	Range	*P* value*	Median	Range	*P* value
Hemoglobin, g/dL	12	9.7–14.6	.971	12.1	9.5–15.4	.455	12.2	10.7–17	.557	12.5	8.4–17.3	.898
Hematocrit, %	36.4	31.1–44.5	.837	36.5	30–46.2	.433	37.1	33–50.4	.562	37.15	27.2–50.2	.829
Total count of white blood cells/mm^3	3600	2920–9300	.739	4050	1900–8500	.708	3950	2600–8000	.336	4200	1700–9100	.491
Neutrophils, %	45	13.6–80	.751	42.7	11–91.7	.321	72.8	40.4–79.6	.517	52.95	7–73.9	.382
Lymphocytes, %	42	11–79.4	.849	46.4	5–79	.276	46.1	9–49	.476	34.5	20.5–83.4	.277
Erythrocyte sedimentation rate, mm/h	12	2–68	.769	10	2–44	.819	8.5	2–44	.112	15	1–59	.366
Alanine aminotransferase, IU/L	32	11–324	.491	30	12–150	.251	34	14–167	.451	63	14–594	**.021**
Aspartate aminotransferase, IU/L	87	26–566	.235	78	17–318	.845	81.5	26–208	.321	131.5	22–1156	**.001**
Minimum platelet count observed/mm^3	48 000	11 000–215 000	.258	69 000	23 000–220 000	.190	92 500	24 000–114 000	.805	58 500	13 000–173 000	.832

Note: Kruskal–Wallis test was used for comparison between different serotypes.

TABLE 3 Clinical presentations, immune status, and disease severity in children infected with different DENV serotypes

	DENV–1 (n = 25)		DENV–2 (n = 17)		DENV–3 (n = 6)		DENV–4 (n = 22)	
	N	%	N	%	N	%	N	%
Clinical variables								
Vomiting	6	24.1	6	35.3	2	33.5	5	22.7
Rash	1	4	–	–	1	16.7	–	–
Skin/mucosal bleeds	–	–	1	5.9	–	–	–	–
Myalgia	4	16	2	11.8	2	33.3	8	36.4
Abdominal pain	11	44	8	47.1	3	50	9	40.9
GI/GU bleeds	1	4	–	–	–	–	–	–
Hypotension	2	8	–	–	–	–	–	–
Respiratory distress	3	12	–	–	–	–	1	4.5
Pleural effusion	2	8	–	–	1	16.7	1	4.5
Hepatomegaly	9	36	7	41.2	3	50	10	45.5
Immune status and disease severity								
DwoWS								
Primary	11	68.7	5	55.6	1	100	7	58.3
Secondary	5	31.3	4	44.4	0	–	5	41.7
DwWs and SD								
Primary	3	33.3	3	37.5	3	60.0	6	60.0
Secondary	6	66.7	5	62.5	2	40.0	4	40.0
Disease severity and serotypes								
DwoWS	16	64.0	9	52.9	1	16.7	12	54.5
DwWS	7	28.0	6	35.3	5	83.3	8	36.4
Severe dengue	2	8.0	2	11.8	–	–	2	9.1
Relative risk adjusted for age, gender and fever days at admission[a]	1.1 (0.76–1.6)		1.03 (0.43–2.46)		0.79 (0.22–2.89)		1.41 (0.66–3.0)	
DwWS + SD vs DwoWS								

Note: X^2 test was used for comparison between different serotypes.
Abbreviations: DENV, dengue virus; DwoWS, dengue without warning signs; DwWs, dengue with warning signs; SD, severe dengue.
[a]Modified Poisson regression was used for calculating the relative risk adjusted for age, gender, and fever days at admissionadmission.

2017 from Tamil Nadu clustered with Indian strains isolated during 2012-2017 from Kerala, Maharashtra, and Tamil Nadu and strains from Sri Lanka and Singapore. Five lineages, India-I to India-IV and Afro India, have been described within the AM/AF genotype earlier.[12] The present study isolates clustered with India-IV lineage strains. Comparison of amino acid sequences of the Indian isolates belonging to Asian genotype with the isolates from Singapore and Sri Lanka revealed that most of the 2017 isolates from Tamil Nadu had three conservative changes (T329S, L475P, and L484M) and one nonconservative change (F337I) that were not observed in earlier isolates (Table S3).

3.5 | Circulating genotype of DENV-2

DENV-2 was classified into five genotypes as described earlier.[17] Further, subclassifications within the cosmopolitan genotype was performed as described recently.[18] For DENV-2, ten isolates were sequenced for E gene. All sequenced isolates grouped within the cosmopolitan genotype. Within cosmopolitan genotype, Tamil Nadu isolates clustered with strains from two lineages—Cosmopolitan I (C-I) and C-III. Within C-I, five isolates were part of C-IA sublineage, and one isolate was part of C-IB (Figure 2). Amino acid sequence analysis within the 2017 Tamil Nadu isolates revealed that C-I strains had Q at 52nd position while it was H in C-III isolates. C-I had a V at 141st position of E protein while isolates from C-IB and C-III had I. Isolates from C-IB and C-III had I at 322nd position while V was present in C-IA (Table S4).

3.6 | Circulating genotypes of DENV-3 and DENV-4

Five genotypes have been reported for DENV-3.[19] One DENV-3 isolate was sequenced which clustered with earlier isolates from India, China, Pakistan, Singapore, and Senegal into GIII genotype and lineage C (Figure S1). For DENV-4, there were five genotypes.[20] Recently, an additional genotype (GVI) has been proposed.[21] Nine DENV-4 isolates were sequenced. All the 2017 isolates from Tamil Nadu grouped within the GI genotype. In the G1 genotype, 2017 isolates from Tamil Nadu along with the strains from India, Pakistan, and Sri Lanka formed lineage C. Strains from Cambodia, China, Singapore, and Thailand formed the lineage B while those from China, Japan, Philippines, and South Korea formed lineage A (Figure 3). Comparison of amino acid sequences of the E protein from the current study isolates with earlier India isolates revealed the presence of altogether 53 unique nonconservative changes in the nine 2017 isolates from Tamil Nadu (Table S5).

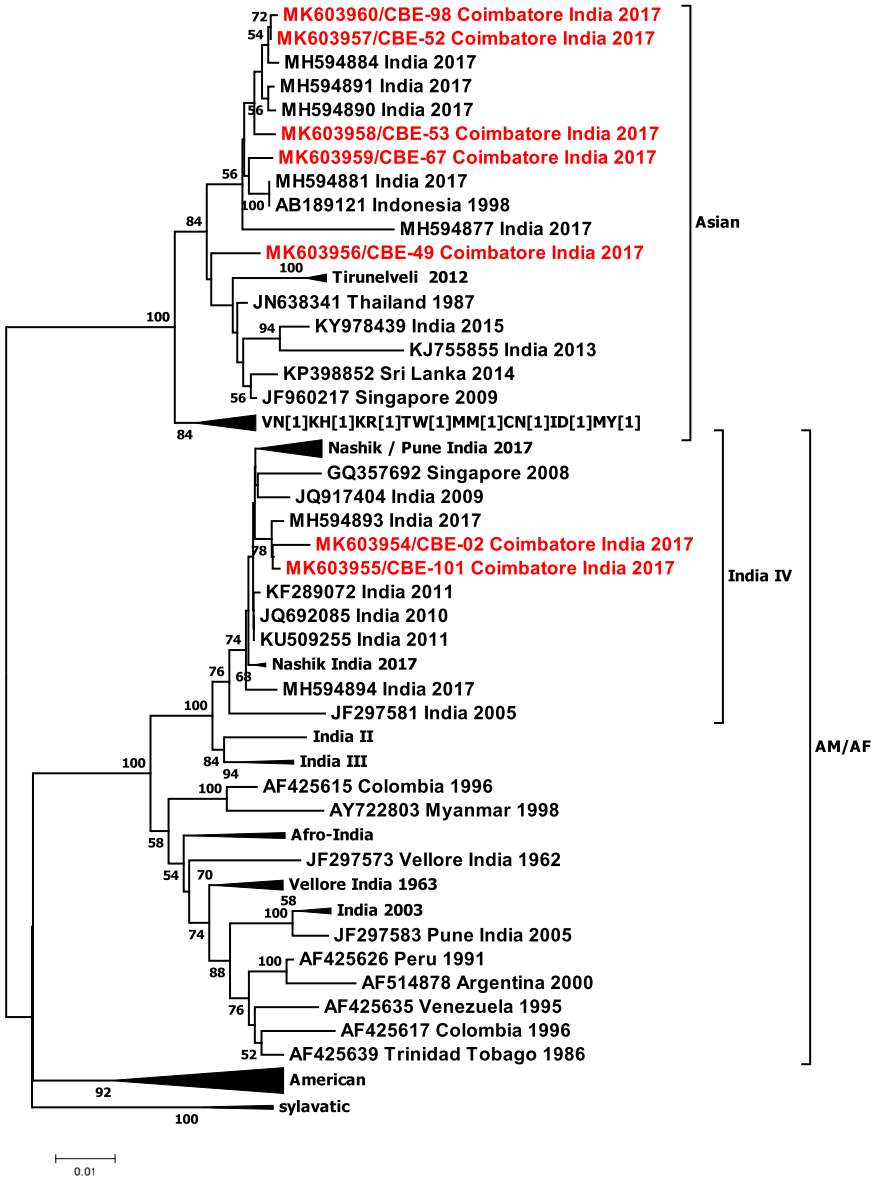


FIGURE 1 Phylogenetic tree of dengue virus-1 (DENV-1) based on E gene sequences All the current study sequences (n = 7) highlighted in Red. Apart from the current sequences, 36 global sequences and 44 Indian sequences were used for generating the phylogenetic tree KH Cambodia/VN-Vietnam/KR-Korea, Republic of/TW-Taiwan/MM-Myanmar/CN-China/ID-Indonesia/MY-Malaysia. Among the five genotypes, Asian, American/African (AM/AF) and Sylavatic genotypes were only shown

4 | DISCUSSION

Forty percent of world's population lives in places endemic to DENV infection and at least 12 000 to 24 000 deaths happen annually in children aged below 15 years.[2] During the study period, admission of 430 undifferentiated fever cases with clinical features suggesting dengue in the pediatric services of one tertiary care hospital alone explains the magnitude of the disease in this endemic region. Circulation of all the four serotypes of DENV has been reported from different regions of India. In 2017, DENV-1 and DENV-3 were more prevalent in Maharashtra, Western India.[10] DENV-1 was the most prevalent serotype while DENV-4 was the least observed one in the 2012 dengue season in Tamil Nadu.[8] In contrast to 2012, DENV-1, DENV-2, and DENV-4 were the prevalent serotypes during 2017 in the study region and could have accounted for the large number of cases from the state. Persistent circulation of DENV-1 might have led to herd immunity permitting the emergence of other serotype viruses. Circulation of multiple serotypes suggests the hyperendemic nature of dengue in this region. The study also suggests the emergence of DENV-4 along with DENV-1 and DENV-2 in Tamil Nadu during 2017.

Studies in the past on disease severity and infecting serotypes have discordant results. A study based on the data from 1994-2006 in Thai children has suggested the association of DENV-2 infection with severe dengue.[22] Further the same study revealed that the risk of SD was more in secondary infections with DENV-2 and DENV-3.[22] A study on adult patients from Singapore revealed that infection with DENV-1 had an increased risk of dengue hemorrhagic fever than DENV-2, whereas a study from Vietnam revealed no difference in the risk of developing severe disease among hospitalized adults infected with DENV-1 and DENV-2.[23,24] A study from Brazil reported that patients infected with DENV-1 serotype had higher risk of severe clinical and laboratory features compared to those infected with DENV-4.[25] A systematic review analyzing the factors associated with



FIGURE 2 Phylogenetic tree of dengue virus-2 (DENV-2) based on E gene sequences All the current study sequences (n = 7) highlighted in Red. Apart from the current sequences, 79 global sequences and 43 Indian sequences were used for generating the phylogenetic tree

dengue shock syndrome, the most severe form of dengue, has revealed DENV-2 as a risk factor.[3] Contradicting all the studies, a study from Bali reported lack of association between DENV serotypes and disease severity.[26] In the present study, as there were less number of children who progressed to SD during hospital stay, for the purpose of analysis, children with SD and DwWS were grouped together and compared with DwoWS. Overall, there were more children with DwoWS than DwWS/SD and none of the serotypes showed any statistically significant increased risk for developing DwWS/SD. Immune status was also not associated with DwWS/SD. However, DENV-4 infections were significantly associated with elevated levels of liver enzymes. A study on pediatric dengue cases from Thailand also has reported that DENV-3 and DENV-4 infection tended to have abnormal elevation of liver enzymes.[27] This observation was contrary to a study from Northern India which has reported an association of elevated levels of liver enzymes with DENV-2.[28] Whether this association of elevated enzymes with DENV-4 can generalized to other regions depends on multiple factors such as the circulating genotype of DENV-4, host immune status, and age of the patients and a study with larger sample size is needed to further confirm the finding.

Phylogenetic analyses indicated the circulation of DENV-1 Asian and AM/AF genotypes, multiple lineages of cosmopolitan genotype of DENV-2, DENV-3 GIII genotype and DENV-4 GI genotype with unique mutations in Tamil Nadu. Cocirculation of DENV-1 Asian and AM/AF genotypes has been reported from Maharashtra in recent years while cocirculation of multiple lineages of DENV-2 cosmopolitan genotype has been reported from Northern India.[10,29,30] A recent study reported that the proportion of Asian genotype among the DENV-1-positive cases in Tamil Nadu had decreased from 90% in 2012 to 57% in 2017.[29] It is possible the DENV-1 Asian genotype viruses might have evolved over the years to become a virus with less epidemic potential phenotype. However, further comparative susceptibility studies in vectors and animal models are needed. In

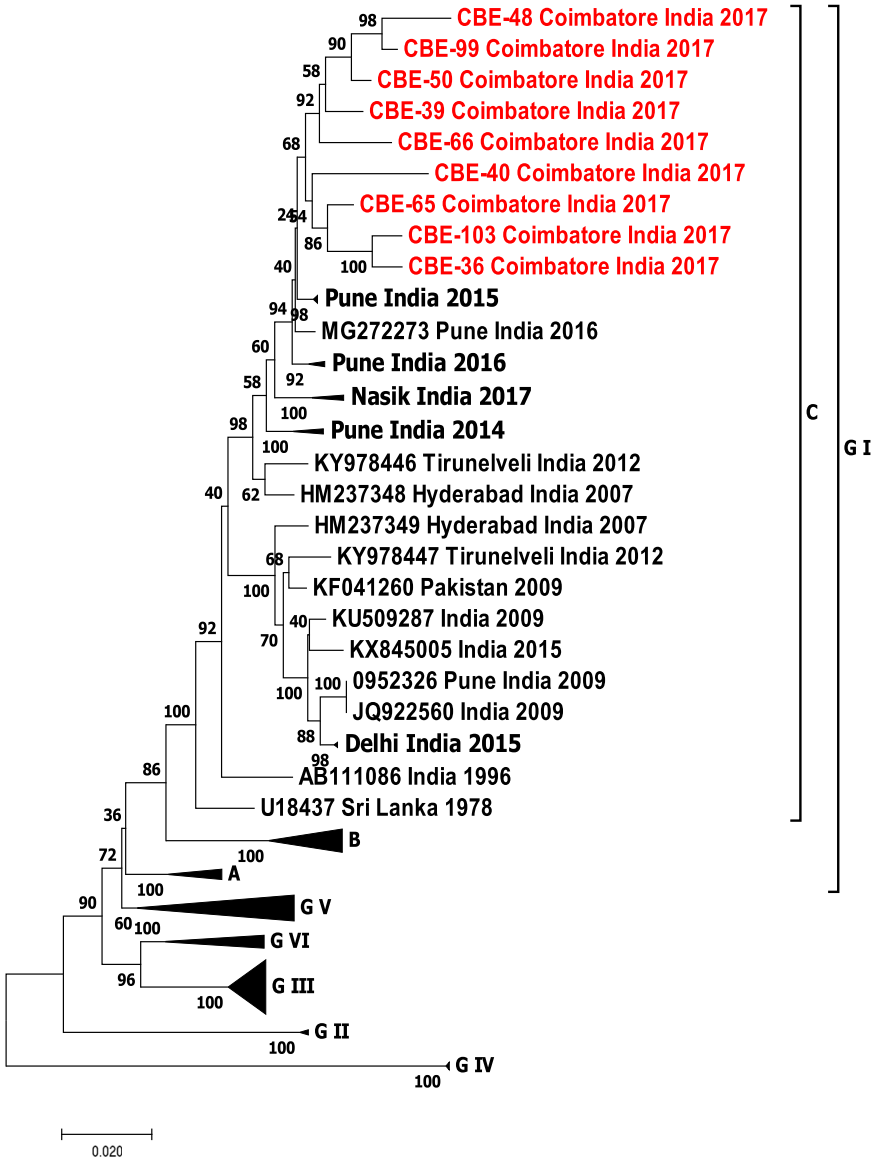


FIGURE 3 Phylogenetic tree of dengue virus-4 (DENV-4) based on E gene sequences. All the current study sequences (n = 9) highlighted in Red. Apart from the current sequences, 47 global sequences and 31 Indian sequences were used for generating the phylogenetic tree

the present study, E gene sequencing of DENV-4 isolates revealed unique mutations which were not observed in earlier Indian DENV-4 strains. The envelope protein has three structural domains I-III. Mutations in the domain II and domain III have more significance since domain II contains the fusion loop which is essential for the interaction between the host endosomal membrane and the virus envelope leading to virus membrane fusion while domain III contains epitopes that are essential for serotype-specific neutralization. Mutations in these domains might affect virus entry as well as the neutralization by serotype/genotype-specific antibodies.[31,32] DENV evolution is shaped by purifying selection in which deleterious mutations affecting viral fitness are removed with time. Further, mutations which confers fitness advantage to DENV in nature were positively selected and contributes to adaptive evolution of DENV.[33] Further follow up studies in the region are needed to discern how these mutations shape up the DENV-4 evolution. It is possible that differences in the viral factors as well as host factors might influence the variable outcomes in different studies. Monitoring the circulating serotypes and genotypes in different regions might result in a region wise database useful in predicting future outbreaks of higher magnitude when changes are observed with regard to the dominant serotypes and new genotypes.

The strength of the present study is that the subjects enrolled were belonging to only to pediatric age, they were prospectively followed up for the outcome and disease severity was classified based on latest guidelines recommended by WHO. Few limitations are, the small sample size, study participants were only hospitalized children from a particular geographic area, hence cannot be extrapolated to general population or other parts of the country. All the patients were recruited within 5 days of fever onset and hence early hospitalization and effective fluid management might have influenced the final outcome. Nonhospitalized cases with undifferentiated febrile illness, which forms the major proportion of DENV cases, were not included in the study and investigation of both hospitalized as well as nonhospitalized cases might help in

identifying the DENV serotypes associated with DwWS and severe dengue.

To conclude, the study reports the activity of all the four serotypes in Tamil Nadu during 2017 with the dominance of DENV-1, DENV-2, and DENV-4. Persistent cocirculation of DENV-1 Asian and AM/AF genotypes, cocirculation of multiple lineages of DENV-2 cosmopolitan genotype and emergence of DENV-4 GI genotype with unique mutations were observed. DENV-4 infection was associated with elevated levels of liver enzymes among hospitalized children. Serotypes and immune status were not associated with disease severity among children hospitalized for dengue.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. World Health Organization. Ten threats to global health in 2019. https://wwwwhoint/emergencies/ten-threats-to-global-health-in-2019. Accessed June 24, 2019.
2. World Health Organization. Dengue: call for urgent interventions for a rapidly expanding emerging disease. 2011. http://applicationsemrowhoInt/docs/RC_technical_papers_2011_3_14217
3. Huy NT, Van Giang T, Thuy DHD, et al. Factors associated with dengue shock syndrome: a systematic review and meta-analysis. *PLOS Negl Trop Dis*. 2013;7:e2412. https://doi.org/101371/journalpntd0002412
4. Alagarasu K. Genetics of susceptibility to severe dengue virus infections: an update and implications for prophylaxis, prognosis and therapeutics. *Dengue Bulletin*. 2016;39:1-18.
5. Ocwieja KE, Fernando AN, Sherrill-Mix S, et al. Phylogeography and molecular epidemiology of an epidemic strain of dengue virus type 1 in Sri Lanka. *Am J Trop Med Hyg*. 2014;91:225-234. https://doi.org/104269/ajtmh13-0523
6. Teoh B-T, Sam S-S, Tan K-K, et al. Dengue virus type 1 clade replacement in recurring homotypic outbreaks. *BMC Evol Biol*. 2013;13:213. https://doi.org/101186/1471-2148-13-213
7. National vector borne disease control programme. Dengue cases and deaths in the country since 2015. 2019. http://nvbdcpgovin. Accessed June 24, 2019.
8. Cecilia D, Patil JA, Kakade MB, et al. Emergence of the Asian genotype of DENV-1 in South India. *Virology*. 2017;510:40-45. https://doi.org/101016/jvirol201707004
9. World Health Organization. Special programme for research, training in tropical diseases. WHO Epidemic, Pandemic Alert Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. World Health Organization Department of Control of Neglected Tropical Diseases, World Health Organization; 2009.
10. Patil JA, Alagarasu K, Kakade MB, et al. Emergence of dengue virus type 1 and type 3 as dominant serotypes during 2017 in Pune and Nashik regions of Maharashtra, Western India. *Infect Genet Evol*. 2018;66:272-283. https://doi.org/101016/jmeegid201810016
11. Kumar SR, Patil JA, Cecilia D, et al. Evolution, dispersal and replacement of American genotype dengue type 2 viruses in India (1956-2005): selection pressure and molecular clock analyses. *J Gen Virol*. 2010;91:707-720. https://doi.org/101099/vir0017954-0
12. Patil JA, Cherian S, Walimbe AM, et al. Evolutionary dynamics of the American African genotype of dengue type 1 virus in India (1962-2005). *Infect Genet Evol*. 2011;11:1443-1448. https://doi.org/101016/jmeegid201105011
13. Patil JA, Cherian S, Walimbe AM, et al. Influence of evolutionary events on the Indian subcontinent on the phylogeography of dengue type 3 and 4 viruses. *Infect Genet Evol*. 2012;12:1759-1769. https://doi.org/101016/jmeegid201207009
14. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 70 for bigger datasets. *Mol Biol Evol*. 2016;33:1870-1874. https://doi.org/101093/molbev/msw054
15. Goncalvez AP, Escalante AA, Pujol FH, et al. Diversity and evolution of the envelope gene of dengue virus type 1. *Virology*. 2002;303:110-119.
16. Domingo C, Palacios G, Jabado O, et al. Use of a short fragment of the C-terminal E gene for detection and characterization of two new lineages of dengue virus 1 in India. *J Clin Microbiol*. 2006;44:1519-1529.
17. Twiddy SS, Woelk CH, Holmes EC. Phylogenetic evidence for adaptive evolution of dengue viruses in nature. *J Gen Virol*. 2002;83:1679-1689.
18. Ali A, Ali I. The complete genome phylogeny of geographically distinct dengue virus serotype 2 isolates (1944-2013) supports further groupings within the cosmopolitan genotype. *PLOS One*. 2015;10:e0138900. https://doi.org/101371/journalpone0138900
19. Lanciotti RS, Gubler DJ, Trent DW. Molecular evolution and phylogeny of dengue-4 viruses. *J Gen Virol*. 1997;78:2279-2284.
20. Lanciotti RS, Lewis JG, Gubler DJ, Trent DW. Molecular evolution and epidemiology of dengue-3 viruses. *J Gen Virol*. 1994;75:65-75.
21. Shrivastava S, Tiraki D, Diwan A, et al. Co-circulation of all the four dengue virus serotypes and detection of a novel clade of DENV-4 (genotype I) virus in Pune, India during 2016 season. *PLOS One*. 2018;13:e0192672. https://doi.org/101371/journalpone0192672
22. Fried JR, Gibbons RV, Kalayanarooj S, et al. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. *PLOS Negl Trop Dis*. 2010;4:e617. https://doi.org/101371/journalpntd0000617
23. Yung C-F, Lee K-S, Thein T-L, et al. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. *Am J Trop Med Hyg*. 2015;92:999-1005. https://doi.org/104269/ajtmh14-0628
24. Fox A, Le NMH, Simmons CP, et al. Immunological and viral determinants of dengue severity in hospitalized adults in Ha Noi, Viet Nam. *PLOS Negl Trop Dis*. 2011;5:e967. https://doi.org/101371/journalpntd0000967
25. Rocha BAM, Guilarde AO, Argolo AFLT, et al. Dengue-specific serotype related to clinical severity during the 2012/2013 epidemic in centre of Brazil. *Infect Dis Poverty*. 2017;6:116. https://doi.org/101186/s40249-017-0328-9
26. Megawati D, Masyeni S, Yohan B, et al. Dengue in Bali: clinical characteristics and genetic diversity of circulating dengue viruses. *PLOS Negl Trop Dis*. 2017;11:e0005483. https://doi.org/101371/journalpntd0005483
27. Kalayanarooj S, Nimmannitya S. Clinical and laboratory presentations of dengue patients with different serotypes. *Dengue Bulletin*. 2000;24:53-59.

28. Kumaria R. Correlation of disease spectrum among four Dengue serotypes: a five years hospital based study from India. *Braz J Infect Dis*. 2010;14:141-146.
29. Alagarasu K, Patil JA, Kakade MB, et al. Spatio-temporal distribution analysis of circulating genotypes of dengue virus type 1 in western and southern states of India by a one-step real-time RT-PCR assay. *Infect Genet Evol*. 2019;75:103989. https://doi.org/101016/jmeegid2019103989
30. Kar M, Nisheetha A, Kumar A, et al. Isolation and molecular characterization of dengue virus clinical isolates from pediatric patients in New Delhi. *Int J Infect Dis*. 2018;84S:S25-S33. https://doi.org/101016/jijid201812003
31. Modis Y, Ogata S, Clements D, Harrison SC. Structure of the dengue virus envelope protein after membrane fusion. *Nature*. 2014;427:313-319.
32. Gallichotte EN, Baric TJ, Nivarthi U, et al. Genetic variation between dengue virus type 4 strains impacts human antibody binding and neutralization. *Cell Reports*. 2018;25:1214-1224. https://doi.org/101016/jcelrep201810006
33. Pollett S, Melendrez MC, Maljkovic Berry I, et al. Understanding dengue virus evolution to support epidemic surveillance and counter-measure development. *Infect Genet Evol*. 2018;62:279-295. https://doi.org/101016/jmeegid201804032

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Original Research Article

Pattern and prevalence of metabolic syndrome in obese adolescents aged 10-18 years: a community based study

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ABSTRACT

Background: The prevalence and magnitude of childhood obesity is increasing in pediatric age group. Incidence of metabolic syndrome is high among obese children and adolescents leading to increased risk of cardiovascular morbidity in long term. For this reason, recognition of metabolic syndrome in obese children is of great importance.
Methods: This study was a cross-sectional study carried out among children aged 10-18 years from both urban and rural schools in Coimbatore. Children were screened, sample for metabolic studies collected after consent and analyzed.
Results: Total 1582 children in the age group of 10-18 years were screened. 300 children were recruited. Among the studied group, 19.3% were overweight, 30.7% were obese. Metabolic syndrome was present in 55.1% of obese and overweight children. Hyperinsulinemia was present in 30% of obese children.
Conclusions: This study done in adolescent school children showed a strong association between obesity and early onset of metabolic syndrome. Early intervention with lifestyle modifications is strongly recommended to prevent long term cardiovascular morbidity.

Keywords: Adolescent children, Metabolic syndrome, Obesity

INTRODUCTION

The prevalence and magnitude of childhood obesity is alarmingly increasing in pediatric population. Incidence of metabolic syndrome is high among obese children and adolescents, and it proportionately increases with worsening obesity. There is significantly increased risk of coronary morbidity and mortality in early adulthood among obese adolescents.[1] For this reason, the recognition of the metabolic syndrome in obese children is of great importance from a clinical and public health perspective. There are only few studies regarding the prevalence of the metabolic syndrome in children and adolescents in India.[2-4] This cross-sectional study was done to evaluate the pattern and prevalence of metabolic syndrome among adolescents aged between 10-18 years in Coimbatore, India. Metabolic syndrome was defined by using WHO and modified National Cholesterol Education Program Adult Treatment Panel III [NCEP ATP III] criteria.

In 1988, Reaven and colleagues coined the term Metabolic Syndrome (MetS) that refers to a combination of hypertension, abdominal obesity, insulin resistance, and hyperlipidemia and it was closely associated with obesity and increased risk for atherosclerotic cardiovascular disease.[5,6] What was first considered as adult condition has now been recognized in children.[7] With increasing prevalence of childhood obesity, it is very likely that pediatric metabolic syndrome will also be more prevalent recently, with implications for future increase in cardiovascular morbidity and mortality.

The International Association for the Study of Obesity (IASO) and International Obesity Task Force (IOTF) estimate that 200 million school children are either overweight or obese.[8]

A recent study among 38,296 children aged 8-18 y from five cities of India reported the prevalence of overweight and obesity (based on IOTF reference) as 14.4% and 2.8% respectively.[9] This may have major implications towards increasing prevalence of non-communicable diseases like diabetes, hypertension and cardiovascular disease in early adulthood.[10] Worldwide estimates of the prevalence of MetS range from 1.2% to 22.6% for youth and 9.0% to 35.0% for adults, depending on the definition of MetS used, the region, the study design, the years of the study, and the age group and study population.[11-15] Only very few data are available in India in this context.

METHODS

This study was a cross-sectional study and was carried out among children aged 10-18 years from schools in Coimbatore, including urban and rural areas. Children were screened for overweight and obesity based on BMI and waist circumference.

Weight was measured while the subjects were minimally clothed without shoes in upright position using digital scales and recorded to the nearest 0.1 kg. Height was measured in a standing position, without shoes, using a stadiometer and inch tape to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in centimeters to the nearest 0.1 cm using a non-stretchable measuring tape, without any pressure to body surface at the narrowest level between the lower border of ribcage and iliac crest. Those adolescents classified under overweight and obesity are further evaluated for the presence of metabolic syndrome. Age and sex matched normal children were taken as control. They were selected by simple random sampling.

Informed consent was obtained from parent/guardians and assent from the children. A qualified physician measured blood pressure two times with the subject in a seated position during physical examinations after one initial measurement for determining peak inflation level using a standard mercury sphygmomanometer. The mean of two measurements was considered to be the participant's blood pressure. The systolic blood pressure was defined as the appearance of the first sound (Korotkoff phase 1) and diastolic blood pressure was defined as the disappearance of the sound (Korotkoff phase 5) during deflation of the cuff at a 2 - 3 mm/s decrement rate of the mercury column.

Fasting blood samples for the serum glucose and lipid profile was drawn after the subjects had fasted overnight. Samples were collected for rural children from rural health centre and urban children at PSG Hospital respectively. Fasting plasma glucose was measured on the day of blood collection by the glucose oxidase peroxide method, and lipid profile was measured by enzymatic assay on a Hitchi 704 system auto-analyzer. Lipid profiles [(triglyceride (TG), total cholesterol (C), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and very-low-density lipoprotein-cholesterol (VLDL-C)] were determined in the fasting blood samples.

Fasting insulin was measured. Pubertal stage was determined in each patient using Tanner's criteria and hyperinsulinism (H1) was defined according to the norms for fasting insulin according to pubertal stage: prepubertal ≥ 15 mIU/L, mid-puberty (breast, genital stages 2-4)≥30 mIU/L post-pubertal≥20 mIU/L. Insulin resistance (IR) was analyzed using the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR was calculated by the following formula: fasting insulin* fasting glucose/22.5. An HOMA-IR value greater than 3.16 was used to determine IR in pubertal students.

Definition of metabolic syndrome

For the diagnosis of MetS, the presence of at least three of the following criteria (in accordance with the WHO and National Cholesterol Education Program Adult Treatment Panel III [NCEP ATP III] recommendations) was considered. These criteria included:

- Obesity: BMI≥95th percentile for age and gender (6-18 years, WHO growth charts)
- Abnormal glucose homeostasis: the presence of one of the following:
  - Hyperinsulinemia and insulin resistance (using fasting blood insulin appropriate for pubertal stage or sum of insulin levels in the first 120 min of oral glucose tolerance test)
  - Elevated fasting glucose (EPG): FG≥110 mg/dL
  - Impaired glucose tolerance (IGT), glucose at 120 min: 140-200 mg/dL
- Hypertension: systolic/diastolic blood pressure ≥95th percentile for age and sex.
- Dyslipidemia: presence of one of the following: (AAP Clinical Report on Lipid Screening in children).
  - High TG (≥95th percentile for age and sex)
  - Low HDL-C levels (<5th percentile for age and sex).
  - High total or LDL-C (≥95th percentile for age and sex).

Inclusion criteria

Children and adolescents aged between 10-18 years of age studying in urban and rural schools in Coimbatore, India. Study was done over a period of one year.

Sample size

Previous studies have estimated the prevalence of metabolic syndrome to be 4%. With this prevalence, the power of 80% and excluding the underweight children (prevalence of 30%), the screening sample size calculated as 1582. Considering the prevalence of 8% for overweight and obese children, sample size for second stage was calculated to be 150 with equal number of normal children. The samples were distributed evenly through urban and rural areas.

The data were entered in individual proforma. The collected data were then converted to excel format.

Data analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) version 19. Data expressed as percentage. Chi square test was used to find out association between categorical variables.

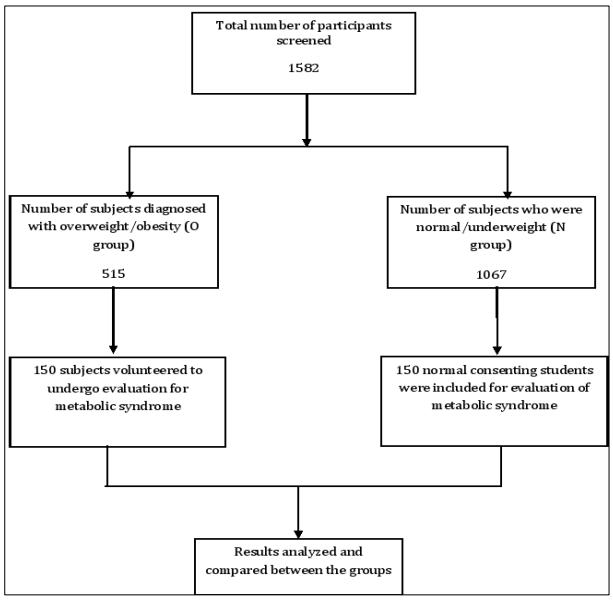


Figure 1: Flow diagram of subject recruitment.

RESULTS

Total number of school children recruited for this study in the age group of 10-18 years were 300, among whom 149 (49.7%) were boys and 151 (50.3%) were girls; in our study, there was almost equal distribution of boys and girls. Among the studied group 58 (19.3%), 92 (30.7%), 124 (41.3%), 26 (8.7%) children were overweight, obese and normal weight and underweight children respectively. Abnormal FBS (>110mg/dL) was seen in 5% of obese children and 2.1% in normal weight group, which was statistically significant. Abnormal total cholesterol level was found in 6% of obese and overweight group and 3.3% in normal group. (p value = 0.27) Triglyceride level was abnormal in 40.6% of O group children and 12.6% in N group children which was statistically significant (p=0.0001%). HDL was abnormal in 24.6% of O group children and 9.3% in N group children also had statistically significant difference (p=0.0001%). Hyperinsulinemia was present in 30% of O group children and 9.3% of N group children. HOMA - IR elevated in 60.6% of O group children and 22% in N group children. Both are statistically significant. Elevated BP was observed in 4.6% of N group children and 7.3% of O group children. Stage 1 hypertension seen in 3.3% of N group children and 14% of O group children which was statistically significant (p=0.002%). Among studied group MS was present in 55.1% in obese, overweight children. MS was more evidenced in the age group of 10-12 years (38.3%) as compared to the other age groups.

Table 1: Baseline characteristics of the study population.

Parameters		Number	Percentage
Age in years	10-12	122	40.7
	13-15	121	40.3
	16-17	57	19
Sex	Males	149	49.7
	Females	151	50.3
Overweight		58	19.3
Obese		92	30.7
Normal		124	41.3
Underweight		26	8.7

Table 2: Lipid profile, HOMA-IR, insulin levels, blood pressure between normal and overweight/obese children.

Variables		Overweight and obese(%)	Normal and underweight(%)	Chi square and p-value
TC		6	3.3	1.19,0.27
TGL		40.6	12.6	30.06,0.0001*
HDL		24.6	9.3	12.49,0.0001*
LDL		11.3	8.6	0.793,0.441
HOMA-IR		60.6	22	46,2,0.0001*
Insulin		30	9.3	20.27,0.0001*
BP	Normal	78.6	92	12.29, p- 0.002
	Elevated	7.3	4.6	
	Stage1	14	3.3	

Table 1 indicating baseline characteristics of the study population including 1. Age group, majority of the children studied were between 10-15 years of age (81%). 2. Equally distributed between both sexes. 3. 30.7% children were obese and 19.3% were overweight.

Table 2 depicting the variables of lipid profile, insulin levels and blood pressure between normal and overweight, obese group.

Table 3 depicts that 50% of children with obesity in the study population had criteria to fulfill metabolic syndrome. 5.1% of children in the overweight group and 3.3% in the normal weight group had metabolic syndrome.

Table 3: Metabolic syndrome in the study population.

Category	Number	Percentage
Normal and under weight	5	3.3
Overweight	3	5.1
Obese	46	50

Table 4 shows the distribution of metabolic syndrome, age wise amongst overweight and obese children. 38.3%between 10-12 years, 28.5% between 13-15 years and 21.4 percent 16 and 17 years fulfilled criteria for metabolic syndrome. This indicates that screening and interventions should be planned early.

Table 4: Distribution of metabolic syndrome age-wise among overweight and obese children.

Age	Number	Percentage
10-12	28	38.3
13-15	18	28.5
16-17	3	21.4

DISCUSSION

Metabolic syndrome is a group of risk factors which has been increasingly prevalent concurrent with obesity.[16] Consistently high prevalence of MS has been recorded in obese and overweight adolescents across the globe varying from 24% to 38.8% of obese children (Turkey). These figures have varied in accordance with the criteria used. Using a combination of WHO and ATP-III criteria in the present study, we found that 50% of obese and 5.1% of overweight adolescent had MS respectively. Such figures in children is a potential harbinger of adult onset cardiovascular morbidity and mortality thereon. In this study, we found that there was equal preponderance of male and female adolescents for MS.

The children between 10-12 years had higher percentage of MS as compared to older adolescents.[17] This fact highlights early screening and dietary and lifestyle modifications for younger adolescents.

Authors observed that there was no statistically significant difference in total cholesterol and LDL levels between the 2 groups. However, the circulatory triglyceride levels were statistically more in the overweight and obese children with MS. High TG has been reported in studies.[2,17,18] The significance of hypertriglyceridemia in adolescents as a harbinger of adult onset of cardiovascular disease is gradually being appreciated. (Another factor noted in this study was that a small subsect of normal weight adolescents had triglyceride values above the age specific cutoff with other parameters remaining normal.) Levels of HDL were statistically significantly lower in the overweight and obese groups as quoted by other studies.[17,19,2] Hyperinsulinemia was present in 30% of obese and overweight adolescents qualifying for MS as observed by Mehmet Atabek.[20] Acanthosis nigricans as an external marker for hyperinsulinemia was seen in 3% of obese adolescents. High FBS (>110mg/dL) was found in 5% of obese and overweight children in the study. HOMA - IR was found to be elevated in 60.6% of the overweight and obese group which was statistically significant (p value 0.0001).11 adolescents (7.3%) in the overweight and obese group had elevated BP and 14% of children in the group and high BP qualifying for stage I hypertension.

Limitation of the study was that detailed dietary assessment could not be done as it was a school based study. Exact duration of physical activity was not included in the proforma.

CONCLUSION

This study done in children between 10-18 years has shown a strong association between obesity and incidence of metabolic syndrome. Early intervention with lifestyle modifications is strongly recommended to prevent long-term cardiovascular morbidity.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Misra A, Shah P, Goel K, Hazra DK, Gupta R, Seth P, et al. The high burden of obesity and abdominal obesity in urban Indian schoolchildren: a

multicentric study of 38,296 children. Ann Nutr Metab. 2011;58(3):203-11.
2. Andrabi SM, Bhat MH, Andrabi SR, Kamili MM, Imran A, Nisar I, et al. Prevalence of metabolic syndrome in 8–18-year-old school-going children of Srinagar city of Kashmir India. Indian J Endocrinol Metab. 2013 Jan;17(1):95.
3. Singh N, Parihar RK, Saini G, Mohan SK, Sharma N, Razaq M. Prevalence of metabolic syndrome in adolescents aged 10-18 years in Jammu, J and K. Indian J Endocrinol Metab. 2013 Jan;17(1):133.
4. Bhalavi V. International Journal of Biomedical Research. Age (years).;15(15):159.
5. Reaven GM. Banting Lecture 1988: role of insulin resistance in human disease. Diab. 1988;37:1595-607.
6. Levitt NS, Lambert EV. The foetal origins of the metabolic syndrome -a South African perspective. Cardiovasc J S Afr. 2002;13:179-80.
7. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. Jama. 2002 Dec 4;288(21):2709-16.
8. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004 Jun 3;350(23):2362-74.
9. International Obesity Task Force. Available at: http://www. iaso.org/iotf/obesity/obesitytheglobalepidemic/, for Saudi, Canada, South Africa, Australia and NZ estimates. Accessed 23 December 2019.
10. Chakraborty P, Dey S, Pal R, Kar S, Zaman FA, Pal S. Obesity in Kolkata children: Magnitude in relationship to hypertension. J Natural Sci Bio Med. 2012;2:101-6.
11. Shah B, Anand K, Joshi P, Mahanta J, Mohan V, Thankappan K, et al. Report of the Surveillance of Risk Factors of Non-communicable Diseases (STEPS 1 and 2) From Five Centers in India – WHO India – ICMR initiative. New Delhi.
12. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;364(9468):1415-28.
13. Goodman E, Daniels SR, Morrison JA, Huang B, Dolan LM. Contrasting prevalence of and demographic disparities in the world health organization and national cholesterol education program adult treatment panel III definitions of metabolic syndrome among adolescents. J Pediatr. 2004;4(59): 445-51.
14. Tailor AM, Peeters PH, Norat T, Vineis P, Romaquera D. An update on the prevalence of the metabolic syndrome in children and adolescents. Int J Pediatr Obes. 2010;5(3):202-13.
15. Cameron AJ, Shaw JE, Zimmet PZ. The metabolic syndrome: prevalence in worldwide populations. Endocrinol Metab Clin North Am. 2004;33(2):351-75.
16. Sangun Ö, Dündar B, Köşker M, Pirgon Ö, Dündar N. Prevalence of metabolic syndrome in obese children and adolescents using three different criteria and evaluation of risk factors. J Clini Res Pediatr Endocrinol. 2011 Jun;3(2):70.
17. Invitti C, Maffeis C, Gilardini L, Pontiggia B, Mazzilli G, Girola A, et al. Metabolic syndrome in obese Caucasian children: prevalence using WHO-derived criteria and association with nontraditional cardiovascular risk factors. Inter J Obesity. 2006 Apr;30(4):627-33.
18. Jacob AS, Reetha G. Prevalence of metabolic comorbidities in obese children. Int J Contemp Pediatr. 2017;4(4):1450-5.
19. Singh R, Bhansali A, Sialy R, Aggarwal A. Prevalence of metabolic syndrome in adolescents from a north Indian population. Diab Med. 2007;24:2.
20. Atabek ME, Pirgon O, Kurtoglu S. Prevalence of metabolic syndrome in obese Turkish children and adolescents. Diab Res Clini Pract. 2006 Jun 1;72(3):315-21.

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Original article

Clinical spectrum and short-term outcomes of multisystem inflammatory syndrome in children in a south Indian hospital

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Background: Multisystem inflammatory syndrome in children (MIS-C) is a new hyperinflammatory variant that evolved during the coronavirus disease 2019 pandemic. Although the precise pathophysiology of MIS-C is uncertain, it is thought to be due to immune dysregulation occurring after recovery from acute infection.

Purpose: Our study aimed to analyze the clinical spectrum, laboratory parameters, imaging characteristics, treatment strategies, and short-term outcomes of children with a diagnosis of MIS-C.

Methods: This retrospective and prospective observational study included children less than 16 years of age who were admitted to the pediatric unit of a tertiary care teaching hospital in south India between August 2020 to January 2021 with a diagnosis of MIS-C according to World Health Organization criteria.

Results: Twenty-one children were included in the analysis; all had fever with variable combinations of other symptoms. The mean age was 6.9 years; 71.4% were male. Gastrointestinal (80.9%) and cardiovascular (80.9%) systems were the most commonly affected. The majority of children had elevated inflammatory markers, and 16 (76.2%) had echocardiographic abnormalities mimicking Kawasaki disease. Eleven children (52.4%) required intensive care admission, 3 (14.3%) required supplemental oxygen, and 4 (19%) required inotropes. Nine (42.9%) were treated with intravenous immunoglobulin alone, 6 (28.6%) with steroids alone, and 3 (14.3%) with steroids and immunoglobulin. The median hospital stay was 6 days; there were no fatalities. Overweight/obesity, elevated ferritin, and mucocutaneous involvement were significantly associated with a prolonged hospital stay (≥7 days). Sixteen children (76.2%) were followed up till now and all of them had no clinical concerns.

Conclusion: MIS-C is an emerging disease with variable presentation. A high index of suspicion is necessary for its early identification and appropriate management. Further research is essential for developing optimal treatment strategies.

Key words: Clinical spectrum, Coronavirus disease 2019, Kawasaki disease, Multisystem inflammatory syndrome in children

Key message

- **Question:** What are the clinical spectrum, course, and short-term outcomes of multisystem inflammatory syndrome in children (MIS-C)?
- **Finding:** MIS-C can have variable clinical manifestations. Fever is most common, followed by gastrointestinal and cardiovascular symptoms. Early identification and appropriate management lead to favorable outcomes.
- **Meaning:** MIS-C can present in a myriad of ways and severities. High suspicion is necessary to ensure its early identification and appropriate management and favorable patient outcomes.

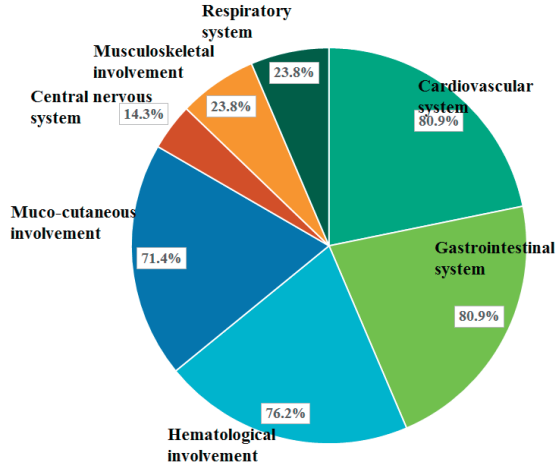
Introduction

The coronavirus disease 2019 (COVID-19) pandemic in India is a part of the global pandemic caused by severe acute respiratory syndrome corona virus-2 (SARS-CoV-2). India is one of the countries with the highest burden of confirmed COVID-19 cases in the world and ranks second in the list of countries with a maximum number of cases.[1] Earlier reports suggested that children infected with COVID-19 were highly resilient and could present with only a mild upper respiratory illness.[2-4] But in April 2020, clinicians in the United Kingdom reported a cluster of previously healthy children presenting with shock, fever, and hyper inflammation.[5] The Royal College of Paediatrics and Child Health referred to this acute condition as pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2.[6] As the pandemic progressed and the number of such cases increased globally the illness was labelled as multisystem inflammatory syndrome in children (MIS-C) by the Centers for Disease Control and Prevention and

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Clinical Spectrum of Multisystem Inflammatory Syndrome in Children



- Intensive care admission – 52.4 %
- Supplemental oxygen requirement – 14.3%
- Inotrope requirement – 19 %
- Abnormal ECHO – 76.2%
- Mortality - 0%
- Follow-up – 76.2%

Graphical abstract

the World Health Organization (WHO).[7,8] Although the exact pathophysiology of MIS-C is uncertain, it is thought to be due to immune dysregulation occurring after recovery from acute infection. As the burden of COVID-19 infection is increasing in India, more children are being recognized with this condition. Although many articles are reported from western countries there is a dearth of published reports from Asian countries.

Our study aimed at analyzing the clinical spectrum, laboratory parameters, and imaging characteristics of children less than 16 years admitted with a diagnosis of MIS-C. In addition, this study aimed to analyze the treatment strategies and short-term outcomes.

Methods

1. Study design and setting

This study was a partly retrospective and partly prospective observational study done in children admitted with a diagnosis of MIS-C in a tertiary care teaching hospital in south India from August 2020 to January 2021. The hospital is one of the largest COVID-19 care centers in south India and receives referrals from a radius of 200 kilometers. The study was approved by the Institutional Human Ethics Committee of PSGIMS&R (project No. 20/262 dated 27th November, 2020). Informed written consent was obtained from parents prior to the inclusion of children in the prospective part of the study. Waiver of consent was obtained for the retrospective collection of data.

2. Participants

Children less than 16 years of age diagnosed with MIS-C as per WHO criteria were included in the study. The following parameters were considered for diagnosis: (1) fever ≥3 days, (2) 2 of the following: a) rash or bilateral nonpurulent conjunctivitis or mucocutaneous inflammation signs (oral mucosa, hands, or feet), b) hypotension or shock, c) features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiography findings or elevated troponin/N-terminal pro-B-type natriuretic peptide [NT-proBNP]), d) evidence of coagulopathy (by prothrombin time, partial thromboplastin time, elevated D-dimers), e) Acute gastrointestinal problems [diarrhea, vomiting, or abdominal pain], (3) elevated markers of inflammation such as erythrocyte sedimentation rate, C-reactive protein, or procalcitonin, (4) no other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal toxic shock syndromes, (5) evidence of COVID-19 (real-time reverse transcription polymerase chain reaction [RT-PCR], antigen test or serology positive), or likely contact with patients with COVID-19.

3. Data collection

Data on demographics, clinical features, laboratory results, imaging studies, treatment details, outcomes, and follow-up were collected. All the investigations and treatment were carried out as per institutional protocol and treating physician's discretion.

COVID-19 infection was confirmed by testing for SARS-CoV-2 antibody by chemiluminescence immunoassay (VITROS 3600 immunodiagnostic system, Ortho Clinical Diagnostics, Raritan, NJ, USA) and/or RT-PCR as recommended by Indian Council of Medical Research. The laboratory values were interpreted as either increased or decreased based on the age-specific normal reference ranges. Organ system involvement was described based on clinical features, laboratory reports, and imaging studies. Echocardiography was performed in all children by the pediatric cardiologist to identify the following abnormalities; (a) left ventricular dysfunction (ejection fraction

less than 55%), (b) pericarditis or pericardial effusion, (c) coronary artery abnormalities (dilatation, aneurysm, or irregular nontapering coronary arteries), or (d) valvular regurgitation. The dimension of the coronary artery was measured as per standard criteria and a coronary z score above 2 was reported as dilated. Other imaging studies were done as clinically indicated.

4. Statistical analysis

Data analysis was done using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). Descriptive statistics were used to summarize population characteristics. Categorical variables were expressed as frequency or percentage and continuous variables were expressed as mean with standard deviation or median with interquartile range. IBM SPSS Statistics ver. 26.0 (IBM Co., Armonk, NY, USA) was used to perform univariate analysis and a *P* value of <0.05 was considered significant.

Results

From August 2020 to January 2021, twenty-one children met the WHO MIS-C criteria and were included in the analysis. Among them, only 2 children were admitted with active coronavirus-2 infection and were subsequently diagnosed as MIS-C. The demographic and clinical characteristics of the study population are mentioned in Table 1. The mean age of the study population was 6.9 years and 71.4% were male. All children were previously healthy and 9.5% were either obese or overweight.

All patients had fever with a variable combination of other symptoms. Gastrointestinal symptoms like abdominal pain, diarrhea, and vomiting were the most common presenting symptoms. The mean duration of symptoms prior to admission was 4.8 days and the mean total duration of fever was 7.9 days. The majority of children in this series had multiorgan involvement. Gastrointestinal (80.9%) and cardiovascular (80.9 %) systems were the most commonly affected organ systems. This was followed by hematological (76.2%), mucocutaneous (71.4%), and central nervous system (14.2%) involvement. None of the children had acute kidney injury in this series. Two children in this series had been treated for COVID-19 3 weeks prior to the diagnosis of MIS-C, and 5 children had history of confirmed exposure to COVID-19 patients in the preceding 3 to 4 weeks. In other children, no history associated with COVID-19 was revealed.

In the study population, 19 children (90.4%) had positive COVID-19 antibody tests, 1 (4.8%) had positive COVID-19 RT-PCR test, and another (4.8%) had both positive COVID-19 RT-PCR and antibody tests. The results of laboratory tests and imaging studies are summarized in Tables 2 and 3. Lymphopenia and thrombocytopenia were seen in 47.6% and 38.1% of cases, respectively. Inflammatory markers were elevated in the majority of cases. Specifically, D-dimer and interleukin 6 (IL-6) were elevated in 95.2% and 90.5% of cases, respectively. Children with cardiovascular system involvement showed elevation of

Table 1. Patients' demographic and clinical characteristics

Population characteristic	Value
Age (yr)	6.9±4
Male sex	15 (71.4)
Weight (kg), median (IQR)	20 (12.8–35.5)
Overweight/obesity	2 (9.5)
Duration of symptoms at admission (day)	4.8±2.1
Contact with COVID-19 patient	5 (23.8)
Past COVID-19 infection	2 (9.5)
Clinical features	
General	
Fever	21 (100)
Total duration of fever (day)	7.9±3.1
Highest temperature recorded in hospital admission (°F)	102.4±1.8
Giddiness	1 (4.8)
Fatigue±lethargy	13 (61.9)
Headache	5 (23.8)
Respiratory	
Cough	2 (9.5)
Sore throat/ throat pain	3 (14.3)
Runny nose±sneezing	1 (4.8)
Fast breathing	4 (19)
Gastrointestinal	
Nausea	3 (14.3)
Vomiting	12 (57.1)
Diarrhea	11 (52.4)
Abdominal pain	11 (52.4)
Abdominal distension	5 (23.8)
Musculoskeletal	
Joint pain	1 (4.8)
Myalgia	7 (33.3)
Mucocutaneous	
Rash	8 (38.1)
Bilateral non-exudative conjunctivitis	12 (57.1)
Lymphadenopathy	3 (14.3)
Strawberry tongue±cracked lips	7 (33.3)
Oedema/erythema of hands and feet	6 (28.6)
Cardiovascular	
Shock requiring inotropes	4 (19)
Neurological	
Seizures	1 (4.8)
Altered level of consciousness	3 (14.3)
Cerebellar signs	1 (4.8)
Focal neurological deficit	1 (4.8)
Organ system involvement[a)]	
One	1 (4.8)
Two	3 (14.3)
Three	4 (19)
Four or more	13 (61.9)

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
IQR, interquartile range; COVID-19, coronavirus disease 2019.
[a)]Organ system involvement was classified as cardiovascular, gastrointestinal, respiratory, nervous, musculoskeletal, hematological, or renal based on clinical features and investigations.

Table 2. Laboratory test results

Parameter	Number (%)	Definition
Blood counts		
Anemia	4/21 (19)	Hb <10 g/dL in <1 yr Hb <9 g/dL in ≥1 yr
Neutrophilia	16/21 (76.2)	ANC >7.7×10^3 cells/μL
Lymphopenia	10/21 (47.6)	ALC <1.5×10^3 cells/μL in child >8 mo ALC <4.5×10^3 cells/μL in child <8 mo
Thrombocytopenia	8/21 (38.1)	Platelet <150×10^3 cells/mm^3
Thrombocytosis	6/21 (28.6)	Platelet >500×10^3 cells/mm^3
Inflammatory markers		
Elevated ESR	17/20 (85)	≥40 mm/hr
Elevated CRP	20/20 (100)	≥0.6 mg/dL
Elevated procalcitonin	5/5 (100)	≥0.5 ng/mL
Elevated ferritin	9/21 (42.9)	>500 ng/mL
Elevated IL-6	19/21 (90.5)	≥7 pg/mL
Elevated LDH	11/16 (68.8)	LDH >300 U/L
Electrolytes		
Hyponatremia	12/17 (70.6)	Sodium <135 mEq/L
Hypokalemia	8/17 (47.1)	Potassium <3.5 mEq/L
Liver function test		
Elevated AST	6/17 (35.3)	AST >40 U/L
Elevated ALT	5/17 (29.4)	ALT >40 U/L
Hypoalbuminemia	9/17 (52.9)	Albumin <3 g/dL
Cardiac biomarkers		
Elevated troponin-T	4/13 (30.8)	≥14 pg/mL
Elevated NT-proBNP	12/15 (80)	>450 pg/mL
Coagulation profile		
Elevated D-dimer	20/21 (95.2)	≥0.5 mg/L FEU
Deranged coagulation	2/8 (25)	INR ≥1.2

Hb, hemoglobin; ANC, absolute neutrophil counts; ALC, absolute lymphocyte counts; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL, interleukin; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; FEU, fibrinogen equivalent units; INR, international normalized ratio.

cardiac biomarkers, and NT-proBNP (80%) was found to be more commonly elevated than troponin-T (30.8%). Electrolyte imbalances like hyponatremia and hypokalemia were seen in 70.6% and 47.1% of cases, respectively, which were managed appropriately.

Echocardiography was performed in all the children, and abnormality was noted in 16 children (76.2%). A greater number of children had a combination of abnormalities rather than a single abnormal finding. Coronary artery abnormality (57.1%) was the most common finding, followed by pericardial effusion (52.4%), left ventricular dysfunction (23.8%), and mitral regurgitation (23.8%). Gastrointestinal imaging studies like x-ray, ultrasound, and computed tomography were done as indicated, and ascites (50%) was the most common abnormality, followed by dilated bowel loops (35.7%), and mesenteric lymphadenitis (35.7%). Of the 17 patients who underwent chest imaging (x-ray or computed tomography), pleural effusion and lung infiltrates were observed in 3 (17.6%) and 1 patient (5.9%),

Table 3. Results of COVID-19 tests and imaging studies

Variable	Number (%)
COVID-19 test results	
Positive serology alone	19 (90.4)
Positive RT-PCR alone	1 (4.8)
Both serology and RT-PCR positive	1 (4.8)
Chest imaging (n=17)	
Pleural effusion	3 (17.6)
Lung infiltrates	1 (5.9)
Gastrointestinal imaging (n=14)	
Ascites	7 (50)
Mesenteric lymphadenitis	5 (35.7)
Dilated bowel loops	5 (35.7)
Appendicular inflammation	3 (21.4)
Appendicular abscess	1 (7.1)
Hepatomegaly	3 (21.4)
Gallbladder wall thickening	1 (7.1)
Echocardiography findings (n=21)	
Abnormal ECHO	16 (76.2)
Coronary artery abnormality	12 (57.1)
Pericardial effusion	11 (52.4)
Left ventricular dysfunction	5 (23.8)
Mitral regurgitation	5 (23.8)

COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription polymerase chain reaction; ECHO,

Table 4. Treatment details and outcomes

Variable	Value
Highest level of care	
PICU admission	11 (52.4)
Duration of PICU stay (hr)	74.8±41.6
Respiratory support	
Supplemental oxygen requirement	3 (14.3)
Duration of oxygen requirement (hr)	39.6±28.6
High flow nasal cannula	1 (4.8)
Circulatory support	
Inotropic support	4 (19)
Duration of inotrope requirement (hr)	37±24.5
Pharmacotherapy	
Antibiotics	18 (85.7)
Intravenous immunoglobulin alone	9 (42.9)
Steroids (intravenous or oral) alone	6 (28.6)
Both intravenous immunoglobulin and steroids	3 (14.3)
Aspirin	14 (66.7)
Anticoagulant	3 (14.3)
Antiepileptics	1 (4.8)
Outcomes	
Length of hospital stay (day), median (IQR)	6 (5–8)
Mortality	0 (0)
Follow-up	16 (76.2)

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
PICU, pediatric intensive care unit; IQR, interquartile range.

respectively. Of the 3 children with central nervous system involvement, 1 had encephalopathy with features of cerebellar involvement, 1 had seizures, and another had headache with bilateral papilledema. All 3 children with central nervous system involvement showed normal neuroimaging. Two children underwent cerebrospinal fluid analysis, and findings of aseptic meningitis were seen in the patient who presented with encephalopathy.

Treatment details and outcomes are summarized in Table 4. Eleven children (52.4%) required pediatric intensive care unit (PICU) admission and the mean duration of PICU stay was 74.8 hours. Three children (14.3%) required supplemental oxygen, and none of the children required mechanical ventilation. Four children (19%) required inotropic support for hemodynamic instability, and the mean duration of inotrope requirement was 37 hours. Nine children (42.9%) were treated with intravenous immunoglobulin (IVIG) alone, 6 (28.6%) were treated with steroids alone and 3 (14.3%) were treated with both IVIG and steroids. None of the cases required other immunomodulators for treatment.

An 8-year-old girl presented with features of acute appendicitis, and an emergency surgery was done, which revealed a perforated appendix with abscess collection. She was subsequently diagnosed as MIS-C and was treated with IVIG.

The median length of hospitalization was 6 days in this series. Table 5 shows the results of univariate analysis for prolonged hospital stay (≥7 days). Children who were either overweight or obese (P=0.035), had elevated ferritin (P=0.019), or had mucocutaneous involvement (P=0.040) were found to be significantly associated with prolonged hospital stay. Further extensive analysis could not be performed due to small sample size. There was no mortality in patients of our study, and all patients were discharged home. At the time of writing this article, 16 patients (76.2%) had attended follow-up visits.

Table 5. Results of univariate analysis of factors associated with prolonged hospital stay (n=21)

Variable	Hospital stay<7 days (n=14), n (%)	Hospital stay≥7 days (n=7), n (%)	*P* value	OR (95% CI)
Age groups			0.376	-
Less than 5 yr (n=7)	6 (85.7)	1 (14.3)		
5 to 9 yr (n=6)	3 (50.0)	3 (50.0)		
10 to 15 yr (n=8)	5 (62.5)	3 (37.5)		
Sex			1.000	1.000 (0.134–7.451)
Male (n=15)	10 (66.7)	5 (33.3)		
Female (n=6)	4 (66.7)	2 (33.3)		
Overweight/obesity (n=2)	0 (0)	2 (100)	**0.035**	0.263 (0.124–0.558)
Temperature > 38.9℃ (n=11)	5 (45.5)	6 (54.5)	0.089	10.80 (0.997–116.998)
Anemia (n=4)	2 (50.0)	2 (50.0)	0.432	2.400 (0.261–22.105)
Lymphopenia (n=10)	5 (50.0)	5 (50.0)	0.122	4.500 (0.627–32.295)
Thrombocytopenia (n=8)	6 (75.0)	2 (25.0)	0.525	0.533 (0.076–3.755)
Elevated ESR (n=17)	12 (70.6)	5 (29.4)	0.212	0.208 (0.015–2.854)
Elevated ferritin (n=9)	3 (33.3)	6 (66.7)	**0.019**	22.0 (1.857–260.648)
Elevated IL-6 (n=19)	12 (63.2)	7 (33.3)	0.293	-
Elevated D-dimer (n=20)	13 (65.0)	7 (35.0)	0.469	-
Hyponatremia (n=12)	6 (50.0)	6 (50.0)	0.252	4.000 (0.340–47.112)
Hypokalemia (n=8)	4 (50.0)	4 (50.0)	0.486	2.000 (0.282–14.198)
Hypoalbuminemia (n=9)	5 (55.6)	4 (44.4)	0.772	1.333 (1.191–9.311)
Abnormal ECHO (n=16)	10 (62.5)	6 (37.5)	0.469	2.400 (0.215–26.822)
PICU admission (n= 12)	7 (58.3)	5 (41.7)	0.350	2.500 (0.357–17.500)
Supplemental oxygen requirement (n=3)	1 (33.3)	2(66.7)	0.186	5.200 (0.381–70.903)
Inotropic support (n=4)	2 (50.0)	2 (50.0)	0.432	2.400 (0.261–22.105)
Organ system involvement				
Cardiovascular system (n=17)	10 (58.8)	7 (41.2)	0.116	-
Respiratory system (n=5)	2 (40.0)	3 (60.0)	0.147	4.500 (0.542–37.378)
Gastrointestinal system (n=17)	11 (64.7)	6 (35.3)	0.694	1.636 (0.138–19.387)
Central nervous system (n=3)	2 (66.7)	1 (33.3)	1.000	1.000 (0.075–13.367)
Hematological system (n=16)	9 (56.3)	7 (43.8)	0.070	1.778 (1.154–2.379)
Mucocutaneous involvement (n=15)	12 (80.0)	3 (20.0)	**0.040**	0.125 (0.15–1.038)
Musculoskeletal system (n=5)	3 (60.0)	2 (40.0)	0.717	1.467 (0.184–11.718)
Multiorgan involvement (≥4 organs) (n=13)	8 (61.5)	5 (38.5)	0.525	1.875 (0.266–13.202)

OR, odds ratio; CI, confidence interval; ESR, erythrocyte sedimentation rate; IL, interleukin; ECHO, :PICU, pediatric intensive care unit.
Boldface indicates a statistically significant difference with P<0.05.

Table 6. Common clinical characteristics in our study versus those of other studies

Clinical feature	Our study (n=21)	Whittaker et al.[10] (n=58)	Dhanalakshmi et al.[11] (n=19)
Fever	21 (100)	58 (100)	19 (100)
Vomiting	12 (57.1)	26 (45)	6 (31.5)
Abdominal pain	11 (52.4)	31 (53)	8 (42)
Diarrhea	11 (52.4)	30 (52)	3 (15.8)
Rash	8 (38.1)	30 (52)	12 (63.2)
Bilateral conjunctival congestion	12 (57.1)	26 (45)	9 (47.4)
Cervical lymphadenopathy	3 (14.3)	9 (16)	6 (31.5)
Strawberry tongue±cracked lips	7 (33.3)	17 (29)	9 (47.4)
Oedema/erythema of hands and feet	6 (28.6)	9 (16)	10 (52.6)

Values are presented as number (%).

Among them, 11 children (52.4%) had attended one follow-up visit, and 5 (23.8%) had attended 2 follow-up visits, all of whom had no clinical concerns. Among 16 children with abnormal echocardiography, 12 (75%) underwent follow-up echocardiography, and their cardiac imaging showed partial or complete resolution of previous abnormalities. Further follow-up echocardiography was recommended to some of them.

Discussion

MIS-C is an evolving concept of disease and its exact incidences in different geographic areas are uncertain. The focus of this study was to analyze the clinical spectrum and short-term outcomes of children with MIS-C managed in a tertiary care teaching hospital in south India. We described 21 children under the age of 16 years with MIS-C associated with SARS-CoV-2 infection. All these children had evidences of previous or active COVID-19 infection. The spectrum of clinical presentations in our study was broadly consistent with available literatures. The mean age of children in this study was 6.9 years, and 61.9% of affected children were above 5 years, which was similar to studies by Kaushik et al.,[9] Whittaker et al.,[10] and Dhanalakshmi et al.[11] The male preponderance in this study was also identical to previous reports by Kaushik et al.,[9] Whittaker et al.,[10] and Gupta et al.[12] Table 6 shows the comparison between common clinical features of children in this study and 2 previous studies.[10,11] Concordant to previous reports, a greater number of children in our series had multiorgan involvement with gastrointestinal and cardiovascular systems being the most commonly affected systems.[11,13-15]

The incidence of hematological abnormalities like lymphopenia (47.6%) and thrombocytopenia (38.1%) in our study was in agreement with previous reports.[11,14,16] The majority of children in this study had raised inflammatory markers, which was similar to previous reports by Kaushik et al.,[9] Dhanalakshmi et al.,[11] Feldstein et al.,[13] and Mamishi et al.[16] In contrast to previous studies by Dhanalakshmi et al.,[11] Feldstein et al.,[13] Toubiana et al.,[15] and Miller et al.,[17] none of the children in this series had acute kidney injury. The incidence of hyponatremia (70.6%) in this study was higher than in previous reports by Dhanalakshmi et al.[1] and Mamishi et al.[16] Hypokalemia was found in 47.1% of children, and it was more common in children with gastrointestinal manifestations, which was not reported in any previous studies. Hypoalbuminemia was noted in 52.9% of children in this study, which was almost similar to previous reports by Dhanalakshmi et al.[1] and Mamishi et al.[16] The elevation of cardiac biomarkers was also concordant to previous reports by Whittaker et al.[10] and Mamishi et al.[16] The incidence of echocardiographic abnormalities in this study was comparable to previous reports by Ramcharan et al.[15] and Toubiana et al.[18] The abnormalities seen in gastrointestinal imaging studies in this study were comparable to the previous report by Miller et al.[17]

Since MIS-C is an emerging disease, guidelines regarding the management are still evolving and there is a lack of consistency in management. The majority of patients in our study were treated with either IVIG, steroids, or a combination of both and, none required other immunomodulators. The treatment decision for the patients in our study was made based on severity of the illness and the treating physician's discretion. Significant differences in treatment strategies were noted among studies from different parts of the world, which could be due to lack of standard treatment protocols in the initial phase of disease evolution.[9-11,14,16] However, a considerable number of studies would be required for developing standard treatment protocols. Concordant to previous reports by Torres et al.[14] and Dhanalakshmi et al.,[11] 52.4% of children in this series required PICU admission. The median length of hospital stay was 6 days which was comparable to previous reports.[9,14,15] Although previous studies showed variable rates of mortality, no death was reported in this study.[11,14,15,17] The follow-up rate in this study was 76.2%. All children had no health concerns at the time of follow-up, and most echocardiographic abnormalities had resolved. However, these children should be followed up at regular intervals to know the long-term consequences of the disease.

A major limitation of this study is that it was conducted in a single center over a limited period of time, and has a small sample size. Another limitation is that this study only included children under the age of 16 years, because our institutional policy defined children under 16 years to be admitted to the pediatric department. It might have missed MIS-C patients older than 16

years of age.

In conclusion, MIS-C is a novel disease that can present in a myriad of ways with variable severity, and a high index of suspicion is necessary in this era of COVID-19 pandemic for early identification, appropriate management, and favorable outcomes of patients. Further research is essential to develop optimal treatment strategies.

Footnotes

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

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References

1. WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. Geneva (Switzerland): World Health Organization; 2021 [cited 2021 Feb 24]. Available from: https://covid19.who.int/.
2. Castagnoli R, Votto M, Licari A, Brambilla I, Bruno R, Perlini S, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. JAMA Pediatr 2020;174:882-9.
3. Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis 2020;34:101623.
4. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr 2020;109:1088-95.
5. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet 2020;395:1607-8.
6. Paediatric multisystem inflammatory syndrome temporally associated with COVID-19 (PIMS) - guidance for clinicians [Internet]. London: Royal College of Paediatrics and Child Health; 2021 [cited 2021 Feb 24]. Available from: https://www.rcpch.ac.uk/resources/paediatric-multi-system-inflammatory-syndrome-temporally-associated-covid-19-pims-guidance.
7. Emergency preparedness and response. Multisystem inflammatory syndrome in children associated with coronavirus disease 2019 [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2020 [cited 2021 Feb 24]. Available from: https://emergency.cdc.gov/han/2020/han00432.asp.
8. Multisystem inflammatory syndrome in children and adolescents with COVID-19 [Internet]. Geneva (Switzerland): World Health Organization [cited 2021 Feb 24]. Available from: www.who.int/publicationsdetail/multisystem-inflammatory-syndrome-in-children-and-adolescents-withcovid-19.
9. Kaushik S, Aydin SI, Derespina KR, Bansal PB, Kowalsky S, Trachtman R, et al. Multisystem inflammatory syndrome in children associated with severe acute respiratory syndrome coronavirus 2 infection (MIS-C): a multi-institutional study from New York City. J Pediatr 2020;224:24-9.
10. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. JAMA 2020;324:259-69.
11. Dhanalakshmi K, Venkataraman A, Balasubramanian S, Madhusudan M, Amperayani S, Putilibai S, et al. Epidemiological and clinical profile of pediatric inflammatory multisystem syndrome - temporally associated with SARS-CoV-2 (PIMS-TS) in Indian children. Indian Pediatr 2020;57:1010-4.
12. Gupta Dch S, Chopra Md N, Singh Md A, Gera R, Chellani Md H, Pandey PhD R, et al. Unusual clinical manifestations and outcome of multisystem inflammatory syndrome in children (MIS-C) in a tertiary care hospital of North India. J Trop Pediatr 2021;67:fmaa127.
13. Feldstein LR, Rose EB, Horwitz SM, Collins JP, Newhams MM, Son MBF, et al. Multisystem inflammatory syndrome in U.S. children and adolescents. N Engl J Med 2020;383:334-6.
14. Torres JP, Izquierdo G, Acuña M, Pavez D, Reyes F, Fritis A, et al. Multisystem inflammatory syndrome in children (MIS-C): report of the clinical and epidemiological characteristics of cases in Santiago de Chile during the SARS-CoV-2 pandemic. Int J Infect Dis 2020;100:75-81.
15. Toubiana J, Poirault C, Corsia A, Bajolle F, Fourgeaud J, Angoulvant F, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. BMJ 2020;369:m2094.
16. Mamishi S, Movahedi Z, Mohammadi M, Ziaee V, Khodabandeh M, Abdolsalehi MR, et al. Multisystem inflammatory syndrome associated with SARS-CoV-2 infection in 45 children: a first report from Iran. Epidemiol Infect 2020;148:e196.
17. Miller J, Cantor A, Zachariah P, Ahn D, Martinez M, Margolis KG. Gastrointestinal symptoms as a major presentation component of a novel multisystem inflammatory syndrome in children that is related to coronavirus disease 2019: a single center experience of 44 cases. Gastroenterology 2020;159:1571-4.e2.
18. Ramcharan T, Nolan O, Lai CY, Prabhu N, Krishnamurthy R, Richter AG, et al. Paediatric inflammatory multisystem syndrome: temporally associated with SARS-CoV-2 (PIMS-TS): cardiac features, management and short-term outcomes at a UK tertiary paediatric hospital. Pediatr Cardiol 2020;41:1391-401.

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Cord blood vitamin D levels in preterm infants and correlation with clinical outcome

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Abstract

Background: The association of serum vitamin D levels to clinical outcome in VLBW infants has not been studied. Our objective was to measure the cord blood levels, and the dose response for two doses of vitamin D in preterm infants and correlate the relationship of vitamin D levels to the clinical outcome.

Methods: We prospectively obtained cord blood levels in 80 preterm infants under 34 weeks gestation (mean gestation age 29±2 weeks and BW: 1210±350 gms). Infants were supplemented with 400 IU or 800-1000 IU vitamin D daily. Serun vitamin D levels were obtained at 2 - 3 weeks after supplementation and levels were correlated to clinical outcome.

Results: The mean cord blood vitamin D level was 12±8.5 ng/ml. Babies who developed sepsis and compared to those who did not develop these morbidities, ROP had vitamin D levels: 13.5±6 (ng/ml) versus 30.5±10 (ng/ml) ($p < 0.01$) and 15.7±11 (ng/ml) versus 34±18 (ng/ml) ($p <0.03$) respectively. Supplementation with 400 IU vitamin D resulted in levels of 17±8.6 (ng/ml) and infants given 800-1000 IU vitamin D had levels 46±17(ng/ml) ($p <0.001$).

Conclusions: These data suggest that cord blood vitamin D levels are low in preterm infants and 800-1000 IU vitamin D supplementation is advisable to achieve levels >30 ng/ml. Infants with low levels of vitamin D have higher incidence of sepsis, and ROP.

Keywords: Morbidity, Preterm Infants, Vitamin D levels

Introduction

Although there is no consensus on optimum levels of serum vitamin D levels, vitamin D deficiency is defined as a 25-hydroxy vitamin D level of less than 20 ng per millilitre (50 nmol per litre).[1-4] No specific definition exists for preterm infants. With the use of such definitions, it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency.[5-7] High prevalence of physiologically significant hypovitaminosis D among pregnant women and their term newborns have been reported in India.[8] The active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), exerts its bio-logical actions by binding to a nuclear receptor, the vitamin D receptor (VDR) and these are present in almost every organ system in the body. It is speculated that there is a physiologic role for vitamin D and its metabolites in general health.[9,12,13]

The awareness of a role for vitamin D in the regulation of immune responses was triggered by the discovery of VDRs in almost all immune cells of the innate and adaptive immune system.[10] Moreover, immune signals can regulate expression levels of the VDR and the enzymes involved in vitamin D metabolism.[11]

The VDRs and vitamin D metabolizing enzymes have also been identified in both the vasculature and the heart

including cardiac myocytes, vascular smooth muscle cells, and endothelial cells. 1,25(OH)2D3 also inhibits angiogenesis, by inhibiting the proliferation of endothelial cells and/or by repressing the release of angiogenic factors such as TGF-a, epidermal growth factor, and vascular endothelial growth factor (VEGF).[20] The association of vitamin D levels to clinical outcome in VLBW infants has not been well studied. Objectives of the study were to determine the vitamin D levels in the cord blood of preterm infants, supplement vitamin D in two doses 400 IU daily and 800-1000 IU daily to randomly assigned preterm infants and to correlate the serum vitamin D levels to neonatal morbidity and outcome.

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The study was conducted in the neonatal intensive care unit of a tertiary care hospital in south India. The study was approved by the medical ethics committee. The criterion for enrolment was gestational age of ≤34 weeks. Infants born with severe asphyxia, chromosomal disorders and congenital infections were excluded from enrolment.

Previously we have noticed that several convalescent preterm infants had very low vit. D levels ranging from 3 to 10 ng/ml, although they were receiving 400 IU of vit. D daily. In order to determine vit. D levels at birth, we obtained cord blood levels of 25 hydroxy Vitamin D3 and followed their serum vitamin D levels 2 to 3 weeks after vitamin D supplementation. When infants were getting enteral feedings of 120ml/kg/day, they were randomly assigned to receive vitamin D supplementation of 400 IU (Group 1) or 800-1000 IU (Group 2) orally daily. Vitamin D levels were measured by chemiluminescence assay (Roche Diagnostics Limited, Sussex, UK). The baseline characteristics of these groups before supplementation with vitamin D are given in Table 1.

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give rise to scarring fibrous tissue resulting in retinal detachment in severe cases. Therefore, there is great interest in the development and identification of agents that can inhibit the growth of new blood vessels. The anti-angiogenic properties of calcitriol (1,25-dihydroxyvitamin D3), has been studied in the mouse model and it has been mentioned as a potent inhibitor of retinal neovascularization and may be of benefit in the treatment of a variety of eye diseases with a neovascular component like ROP.[14,15,21] How calcitriol affects angiogenesis has been unclear.

It was also found that there is an association an association of vitamin D deficiency and late onset sepsis. Vitamin D has a role in regulating inflammation and chemokine production as well as an important role in immunomodulation.[16-18,22] Nearly all cells display a specific vitamin D receptor (VDR), including B and T lymphocytes (both resting and activated), monocytes and dendritic cells.[23] Vitamin D also facilitates neutrophil motility and phagocytic function. A major component of the antimicrobial action of Vitamin D is through the production of peptides which have antimicrobial as well as anti-endotoxin activity. Vitamin D stimulates the expression of potent antimicrobial peptides, such as cathelicidin and β defensin which exist in neutrophils, monocytes, natural killer (NK) cells and epithelial cells lining the respiratory tract.[24,25] Macrophages, lymphocytes and monocytes have VDRs that, with 25(OH)D stimulation, increase the expression of these antimicrobial peptides.[26,27] Jeng et al. noted a positive relationship between vitamin D levels and cathelicidin levels in acutely ill patients.[28] Cathelicidin is effective against gram-positive and gram-negative bacteria, fungi and mycobacteria at a variety of pathogen entry sites, including the skin and the mucosal linings of the respiratory and gastrointestinal sys-tems.[24] Patients with 25(OH) D levels less than 20 ng/mL may be unable to fully express cathelicidin, which could be associated with increased susceptibility to nosocomial infec-tions such as pneumonia, sepsis and central line infections.[28,29] A randomized controlled trial involving Japanese schoolchildren found a relative risk of influenza of 0.36 in those taking 1,200 IU/day compared with those taking 200 IU/day.[30] In-vitro studies have shown that vit-amin D3 has inhibitory activity on strains of *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli* (E. coli) and other bacteria. In the presence of 50,000-90,000 IU/mL of vitamin D_3, the organisms were killed or demonstrated marked growth inhibition.[31] The risk of diseases comorbid with septicemia are generally inversely correlated with serum 25(OH)D levels.[32] Grant had reported that vitamin D supplementation of mother and infant can reduce the risk of sepsis in infants and neonates.[33,34]

The results of our study also indicate that the usual recommended dose of 400 IU Vitamin D in preterms is not sufficient to maintain normal physiological levels in our cohort of Indian infants. This has been observed in other Indian studies also.[35-37] It is quite possible that these infants had very low vitamin D levels at birth perhaps due to maternal vitamin D deficiency and required a higher dose of vitamin D supplementation postnatally. Although none of the infants in our study who received 800-1000 IU of vitamin D very high levels of serum vita-min D we recommend that serum vitamin D levels should be monitored at least once within a month of supplementation of this high dose of vitamin D. We did not correlate the cord blood vitamin D levels to postnatal vitamin D requirement. It is quite possible that the mothers of these infants might have low vitamin D levels during pregnancy which might have contributed to low cord blood levels of vitamin D in these infants. Whether if pregnant mothers are adequately supplemented with vitamin D leading to higher cord blood levels of vitamin D may lead to lower requirements of vitamin D postnatally in these infants is not known. We realize that this is not a randomized controlled study and the sample size is rather small but the results are very convincing that a larger multicenter trial may be warranted to confirm these findings.

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These data suggest that severe vit.D deficiency is common in preterm newborns at birth and postnatally. In infants with low levels of vit D: incidences of late onset sepsis and ROP are significantly higher. Retinal maturation of infants with low levels of vitamin D is delayed perhaps increasing their risk for development of ROP even in the absence of supplemental oxygen administration. AAP guidelines of supplementation with 400 IU is insufficient and most infants require 800-1000 IU vitamin D soon after birth to maintain vitamin D levels ≥ 30 ng/ml in developing countries especially if they are exclusively breast fed. These preliminary results will assist in planning a larger multicenter trial in this field.

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1. Hollick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-81.
2. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006;81:353-73.
3. Ferrari HA, Giovannucci E, Willet WC, Dietrich T, Hughes DB. Estimation of optimal serum concentration of 25-hydroxy vitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84:18-28.
4. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet. 1998;351:805-6.

5. Thomas KK, Jones DM, Thadhani RI. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998;338:777-83.
6. Chapuy MC, Preziosi P, Maamer M. Prevalence of vitamin D in adult normal population. Osteoporosis Int. 1997;7:439-43.
7. Holick MF, Siris ES, Binkley N. Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab. 2005;90:3215-24.
8. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. Am J Clin Nutr. 2005;81:1060-4.
9. Verstuyf A, Carmeliet G, Bouillon R. Vitamin D: a pleiotropic hormone. Kidney Int. 2010;78:140-5.
10. Baeke F, Takiishi T, Korf H. Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010;10:482-96.
11. Overbergh L, Stoffels K, Valckx D. Regulation of 25-hydroxyvitamin d-1 alpha hydroxylase by IFN gamma in human monocytic THP1 cells. J Steroid Biochem Mol Biol. 2004;89:453-5.
12. Judd SE, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. Am J Med Sci. 2009;338:40-4.
13. Berthold GI, Krone W, Berthold HK. Vitamin D and cardiovascular disease. Curr Vasc Pharmacol. 2009;7:414-22.
14. Shoshan BM, Amir S, Dang DT. 1alpha,25-dihydroxyvitamin D3 (calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factorpathway in human cancer cells. Mol Cancer Ther. 2007;6:1433-9.
15. Albert DM, Scheef EA, Wang S. Calcitriol is a potent inhibitor of retinal neovascularization. Invest Ophthalmol Vis Sci. 2007;48:2327-34.
16. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. J Mol Med. 2010;88:441-50.
17. Veldman CM, Cantorna MT, Deluca HF. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Arch Biochem Biophys. 2000;374:334-8.
18. Bartley J. Vitamin D: emerging roles in infection and immunity. Expert Rev Anti Infect Ther. 2010;8:1359-69.
19. Ginde AA, Mansbach JM, Camargo CA. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the third national health and nutrition examination survey. Arch Intern Med. 2009;169:384-90.
20. Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Mol Nutr Food Res. 2011;55:96-108.
21. Alitalo A. Human anti-infectious defence may be enhanced by vitamin D. Duodecim. 2010;126:1127-34.
22. Jeng L, Yamshchikov AV, Judd SE, Blumberg HM, Martin GS, Ziegler TR, et al. Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. J Transl Med. 2009;7:28.
23. Bikle DD. Vitamin D and the immune system: role in protection against bacterial infection. Curr Opin Nephrol Hypertens. 2008;17:348-52.
24. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr. 2010;91:1255-60.
25. Feindt E, Stroder J. Studies on the antimicrobial effect of vitamin D. Klin Wochenschr. 1977;55:507-8.
26. Grant WB. Solar ultraviolet-B irradiance and vitamin D may reduce the risk of septicemia. Dermatoendocrinol. 2009;1:37-42.
27. Grant WB. Vitamin D supplementation could reduce risk of sepsis in infants. World J Pediatr 2010;6:185.
28. Grant WB. Vitamin D supplementation of mother and infant could reduce risk of sepsis in premature infants. Early Hum Dev. 2010;86:133.
29. Calcium and vitamin D requirements of enterally fed preterm infants. Available at https:// pediatrics. aappublications.org/content/pediatrics/early/2013/04/24/peds.2013-0420.full.pdf. Accessed on 14 July 2016.
30. Agarwal R, Virmani D, Jaipal ML, Gupta S, Gupta N, Sankar MJ. Vitamin D status of low birth weight infants in Delhi: a comparative Study. J Trop Pediatr. 2012;58(6):446-50.
31. Natarajan CK, Sankar J, Agarwal R. Trial of daily Vitamin D supplementation in preterm infants. Pediatrics. 2014;133;628.

r Srinivasan N, Chandramathi J, Prabhu AS, Ponthenkandath S. Vitamin D deficiency and morbidity among preterm infants in a developing country. Int J Contemp Pediatr 2017;4:499-502.

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RESEARCH ARTICLE

Serotype-specific differences in the laboratory parameters among hospitalized children with dengue and genetic diversity of dengue viruses circulating in Tamil Nadu, India during 2017

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Abstract

In 2017, Tamil Nadu, a southern state, had the second highest number of dengue cases from India. In the present study, the serotype-specific differences in the clinical manifestations and laboratory parameters among hospitalized children with dengue were investigated and molecular characterization of the circulating dengue virus (DENV) serotypes during 2017 in Tamil Nadu was performed. Eighty children with dengue-like symptoms consecutively admitted to a tertiary care hospital and positive for DENV NS1 antigen were investigated for DENV serotype utilizing a real-time reverse transcriptase based polymerase chain reaction assay. Complete envelope (E) gene sequencing of the DENV strains was performed. Seventy samples were positive for serotyping (25 DENV-1, 17 DENV-2, six DENV-3, and 22 DENV-4). DENV-4 infections were associated with elevated levels of liver enzymes; Alanine aminotransferase ($P = .021$) and aspartate aminotransferase ($P = .001$). However, none of the serotype was associated with any specific clinical features and severe dengue. Asian and American/African genotypes of DENV-1 were cocirculating. The circulating genotype was cosmopolitan for DENV-2 with multiple lineages, genotype III for DENV-3 and genotype I for DENV-4. Unique mutations were present in the 2017 DENV-4 isolates. The present study suggests the association of DENV-4 with elevated liver enzymes in children hospitalized for dengue. Further, the study reports the genetic diversity of DENV circulating in Tamil Nadu during 2017. The study calls for continuous monitoring of the circulating serotypes and genotypes at regional level in India which might result in a region wise database useful in predicting future outbreaks.

KEYWORDS

alanine aminotransferase, aspartate aminotransferase, dengue, genotypes, serotypes, severe dengue

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1 | INTRODUCTION

Dengue is the commonest arthropod borne viral disease that affect millions of individuals globally each year and has been listed by the World Health Organization (WHO) as one of the major threats to mankind.[1] Infection with any of the four serotypes of dengue virus (DENV) results in a range of clinical presentations. Symptomatic dengue infections are categorized into nonsevere dengue and severe dengue (SD). The nonsevere dengue cases are again classified into those without warning signs (DwoWS) and with warning signs (DwWS). The case fatality rate is less than 1% if proper intervention is initiated at an early stage of the disease.[2] Disease severity is influenced by both virus and host factors. Immune status determined as primary infection or secondary infection with a different serotype of DENV has been reported to be associated with dengue severity by various epidemiological studies. Age, gender, nutritional status, host immune response, and host genetics contribute to differences in the clinical outcomes.[3,4] Among the viral factors, infecting serotype and the genotype within each serotype also contributes to disease severity.[3] Replacement of the circulating serotype/genotype of DENV with a different one contributes to outbreaks of higher magnitude.[5,6]

In the last decade, highest number of dengue cases were reported during 2017 in India. Within India, West Bengal and Tamil Nadu accounted for approximately 30% of the reported dengue cases.[7] Before 2017, Tamil Nadu witnessed large number of cases during 2012 and DENV-1 was the most common serotype despite the presence of other serotypes. Replacement of American/African (AM/AF) genotype with Asian genotype of DENV-1 was observed during 2012 in Tamil Nadu.[8] However, the serotypes and genotypes circulating during 2017 were not known. The present study was aimed at identifying the serotype-specific differences in the clinical symptoms and laboratory parameters in DENV infected children. Moreover, the study also intended to identify the dominant circulating serotype(s) and genotype(s) associated with dengue in Tamil Nadu during 2017.

2 | MATERIALS AND METHODS

2.1 | Study subjects

The study was a cross-sectional prospective study conducted at PSG institute of Medical Science and Research, a tertiary care teaching hospital located in western Tamil Nadu between August and December 2017. Children aged between 6 months to 15 years who fulfilled the inclusion criteria were consecutively enrolled in the study. Children admitted in the hospital with undifferentiated fever of less than 5 days duration with clinical suspicion of dengue and positive for the presence of NS1 antigen were included in the study. Exclusion criteria include: negative test for the presence of NS1 antigen, fever beyond 5 days, unwillingness to participate in the study and patients who were not admitted. The parents or the legal guardians were explained about the study purpose and an informed consent was obtained from them. The parents or the caretakers of the patients were interviewed for their demographic characteristics such as gender, age, and previous dengue episodes. The patients were observed for clinical symptoms including warning signs till their discharge and clinical features and lab data were collected in a predesigned data collection tool. Based on the clinical symptoms and laboratory parameters, the patients were categorized into dengue without warning signs (DwoWS), dengue with warning signs (DwWS), and severe dengue (SD) based on the WHO 2009 guidelines.[9] Plasma or serum samples collected from the participants at the time of entry into the study, were separated and stored at −80°C. The samples were transported in dry ice to ICMR-National Institute of Virology for identifying the serotype. The study was approved by the institutional human ethics committee (IHEC) of PSGIMS&R, Coimbatore, Tamil Nadu. Molecular characterization of the circulating DENV was performed at ICMR-NIV as a part of Apex referral laboratory activity for the year 2017 which was approved by the IHEC of ICMR-NIV.

2.2 | Serological assays

Serological assays were performed in the hospital laboratory. Presence of DENV NS1 antigen was tested by a antigen capture ELISA kit (Panbio Dengue Early ELISA) as per the manufacturer's protocol. Dengue-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies were tested using ELISA kits (Panbio IgM [01PE20] and Panbio IgG capture ELISA kit [01PE10]) according to the instructions provided with the kit. The IgG capture ELISA kit used in the study detects high-titer IgG antibodies which is usually present in secondary infections. Test positive for IgG indicates secondary infection while test negative indicates primary infection.

2.3 | Detection of dengue virus serotypes

RNA was extracted using an RNA extraction kit (QIAamp viral RNA mini kit, Qiagen, Hilden, Germany) according to the manufacturer's protocol and used for DENV serotyping. Serotyping was performed by using an in-house real-time reverse transcription polymerase chain reaction (RT-PCR) assay. The sequences of the primers and probes were given in Table S1. Primer and probe sequences, the reaction mixture composition and cycling conditions for real-time RT-PCR have been reported previously.[10] The PCR cycle at which the fluorescence signals crossed the threshold values were detected for each sample and samples that had a cycle threshold (C_t) value ≤37 were considered as positive.[10]

2.4 | Isolation of dengue virus from clinical samples

Virus isolation was performed in samples which were positive for DENV RNA with increased viral load (C_t value ≤25) using C6/36 cell

line as reported previously.[10] Virus stocks were stored at −80°C in aliquots for further use.

2.5 | Sequencing of complete envelope gene of dengue virus

Randomly selected representative virus isolates from each serotype were used for sequencing of complete E gene (seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4 isolates) using primers reported earlier.[11-13] The sequences of the primers were provided in Table S1. RNA extraction, cDNA synthesis, reverse transcription, PCR amplification, gel purification, and sequencing was carried out as described earlier.[10]

2.6 | Phylogenetic analyses of dengue virus isolates

MEGA7.0 was used for multiple sequence alignment and phylogenetic analyses.[14] Seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4 sequences (GenBank accession number for the virus isolates provided in Table S2) from the current study along with the earlier Indian (44 DENV-1, 79 DENV-2, 39 DENV-3, and 31 DENV-4) and global sequences (36 DENV-1, 43 DENV-2, 54 DENV-3, and 47 DENV-4) from GenBank representing different genotypes were used for the phylogenetic analyses. Kimura-2 Parameter model was used to estimate the genetic differences and maximum likelihood (ML) method was used for the construction of the phylogenetic trees. A bootstrap test with 1000 replications was used to evaluate the reliability of position of the branches.[10]

2.7 | Statistical analyses

To compare univariate categorical data, Fisher's Exact tests or Pearson's X^2 was used. Parametric and nonparametric data from subjects infected with different dengue serotypes were compared using one-way analysis of variance and Kruskal-Wallis tests, respectively. Modified Poisson regression with adjustment for relevant clinical covariates, that is, age, gender, and fever at total day was utilized for performing the regression analyses. Data for one serotype was compared with other three serotypes. Except platelet count, the values of other laboratory parameters at the time of admission were used for comparison. The nadir for platelet count was used for comparison. A probability (*P*) value of <.05 was considered statistically significant. All the statistical analyses were carried out using statistical software R version 3.4.4.

3 | RESULTS

3.1 | Demographic profile of the study subjects

A total of 430 children with undifferentiated fever were admitted with clinical diagnosis of dengue fever of which 236 children fulfilled the inclusion criteria, 80 consecutively admitted children were enrolled in the study and were followed up prospectively. On analyzing for the serotype, 10 samples were negative and hence excluded and only 70 subjects were analyzed. Almost equal proportion of boys and girls were observed and the median age was 9 years (6 months to 15 years) (Table 1).

TABLE 1 Demographic profile of the dengue cases infected with different dengue virus (DENV) serotypes

Patient Demographics									
	DENV–1 (n = 25)		DENV–2 (n = 17)		DENV–3 (n = 6)		DENV–4 (n = 22)		*P* value*
Variables	N	%	N	%	N	%	N	%	
Gender									
Male	9	36	11	64.7	3	50	15	68.2	.441
Female	16	64	6	35.3	3	50	7	31.8	
Locality									
Coimbatore	7	28	3	17.6	1	16.6	4	19	.832
Dharapuram	5	20	–	–	–	–	3	14.3	
Dindigul	2	8	–	–	–	–	–	–	
Karur	1	4	–	–	–	–	1	4.8	
Palani	1	4	3	17.6	1	16.6	3	14.3	
Pollachi	1	4	–	–	0	–	–	0	
Tiruchi	0	–	–	–	0	–	1	4.8	
Tirupur	8	32	11	64.7	4	66.6	10	47.6	
Age, median (range)	9 (0.9–16)	–	6 (0.3–15)	–	9 (6–15)	–	10 (4–14)	–	.236
Fever day at presentation, median (range)	4 (1–8)	–	5 (2–9)	–	4.5(3–5)	–	4.5 (1–8)	–	.442
Fever at total days, median (range)	5 (4–8)	–	5 (3–9)	?	4.5 (4–5)	–	5 (3–8)	–	.188

*X^2 test was used to compare categorical variables (gender and locality) and one–way analysis of variance was used to compare the continuous variables (age, fever day at presentation and fever at total days).

3.2 | Serotype-specific differences in the clinical features, lab parameters and disease severity

All four serotypes were observed to be circulating in the study population with the prevalent serotype being DENV-1, (n = 25, 35.7%), followed by DENV-4 (n = 22, 31.4%), and DENV-2 (n = 17, 24.3%). DENV-3 was identified in 8.6% (n = 6) of the subjects. Apart from a slight female preponderance among DENV-1 infected children, gender, age, and duration of fever were not different between the children infected with different serotypes (Table 1).

A similar modified Poisson regression analysis adjusted for age, gender, and day of fever was performed for various lab parameters. A statistically significant increase in liver enzymes, aspartate aminotransferase (median with range 131.5 [22-1156] $P = .001$) and alanine aminotransferase (median with range 63 [14-594] $P = .021$) were found in DENV-4 infected children compared to children infected with other serotypes. There was no significant difference in the level of liver enzymes between primary and secondary infections. The mean ± standard deviation of liver enzymes, SGPT and SGOT in children with primary and secondary dengue were 99.18 ± 123.91, 66.83 ± 79.09 ($P = .255$) and 172.25 ± 225.03, 139.38 ± 164.15 ($P = .443$). Other hematological parameters including platelet count during the hospital period were not significantly different among children infected with different serotypes (Table 2).

After adjustment for age, gender, and duration of fever, we observed no statistically significant correlation between the infecting serotype and the clinical features (Table 3). A majority of the children had secondary dengue infection (n = 52 [67.5%]). However, immune status was not associated with disease severity (Table 3).

Classifying the children based on disease severity according to WHO 2009 criteria, there were 38 cases with DwoWS, 26 with DwWS, and 6 had SD. There was no significant association between disease severity and infecting serotype (Table 3).

3.3 | Results of virus isolation

A total of 51 samples were used for virus isolation. Virus isolation was positive in 34 samples (seven DENV-1, 13 DENV-2, two DENV-3, and 12 DENV-4). Complete E gene of the representative virus isolates (seven DENV-1, ten DENV-2, one DENV-3, and nine DENV-4) was sequenced.

3.4 | Circulating genotypes of DENV-1

DENV-1 was classified into five genotypes as described earlier.[15] Since there were confusions with regard to the Roman number based nomenclature, geographical names were provided.[16] Seven DENV-1 virus isolates were sequenced. The ML method-based phylogenetic tree (Figure 1) revealed that five isolates grouped with the Asian genotype (GI). Two isolates clustered with the American African (AM/AF) genotype (GV). Asian genotype isolates obtained during

TABLE 2 Association of laboratory/hematological parameters with infecting dengue virus (DENV) serotype

Variables	DENV-1 (n = 25)			DENV-2 (n = 17)			DENV-3 (n = 6)			DENV-4 (n = 22)		
	Median	Range	*P* value*	Median	Range	*P* value*	Median	Range	*P* value*	Median	Range	*P* value
Hemoglobin, g/dL	12	9.7–14.6	.971	12.1	9.5–15.4	.455	12.2	10.7–17	.557	12.5	8.4–17.3	.898
Hematocrit, %	36.4	31.1–44.5	.837	36.5	30–46.2	.433	37.1	33–50.4	.562	37.15	27.2–50.2	.829
Total count of white blood cells/mm^3	3600	2920–9300	.739	4050	1900–8500	.708	3950	2600–8000	.336	4200	1700–9100	.491
Neutrophils, %	45	13.6–80	.751	42.7	11–91.7	.321	72.8	40.4–79.6	.517	52.95	7–73.9	.382
Lymphocytes, %	42	11–79.4	.849	46.4	5–79	.276	46.1	9–49	.476	34.5	20.5–83.4	.277
Erythrocyte sedimentation rate, mm/h	12	2–68	.769	10	2–44	.819	8.5	2–44	.112	15	1–59	.366
Alanine aminotransferase, IU/L	32	11–324	.491	30	12–150	.251	34	14–167	.451	63	14–594	**.021**
Aspartate aminotransferase, IU/L	87	26–566	.235	78	17–318	.845	81.5	26–208	.321	131.5	22–1156	**.001**
Minimum platelet count observed/mm^3	48 000	11 000–215 000	.258	69 000	23 000–220 000	.190	92 500	24 000–114 000	.805	58 500	13 000–173 000	.832

Note: Kruskal–Wallis test was used for comparison between different serotypes.

TABLE 3 Clinical presentations, immune status, and disease severity in children infected with different DENV serotypes

	DENV–1 (n = 25)		DENV–2 (n = 17)		DENV–3 (n = 6)		DENV–4 (n = 22)	
	N	%	N	%	N	%	N	%
Clinical variables								
Vomiting	6	24.1	6	35.3	2	33.5	5	22.7
Rash	1	4	–	–	1	16.7	–	–
Skin/mucosal bleeds	–	–	1	5.9	–	–	–	–
Myalgia	4	16	2	11.8	2	33.3	8	36.4
Abdominal pain	11	44	8	47.1	3	50	9	40.9
GI/GU bleeds	1	4	–	–	–	–	–	–
Hypotension	2	8	–	–	–	–	–	–
Respiratory distress	3	12	–	–	–	–	1	4.5
Pleural effusion	2	8	–	–	1	16.7	1	4.5
Hepatomegaly	9	36	7	41.2	3	50	10	45.5
Immune status and disease severity								
DwoWS								
Primary	11	68.7	5	55.6	1	100	7	58.3
Secondary	5	31.3	4	44.4	0	–	5	41.7
DwWs and SD								
Primary	3	33.3	3	37.5	3	60.0	6	60.0
Secondary	6	66.7	5	62.5	2	40.0	4	40.0
Disease severity and serotypes								
DwoWS	16	64.0	9	52.9	1	16.7	12	54.5
DwWS	7	28.0	6	35.3	5	83.3	8	36.4
Severe dengue	2	8.0	2	11.8	–	–	2	9.1
Relative risk adjusted for age, gender and fever days at admission[a]	1.1 (0.76–1.6)		1.03 (0.43–2.46)		0.79 (0.22–2.89)		1.41 (0.66–3.0)	
DwWS + SD vs DwoWS								

Note: X^2 test was used for comparison between different serotypes.
Abbreviations: DENV, dengue virus; DwoWS, dengue without warning signs; DwWs, dengue with warning signs; SD, severe dengue.
[a]Modified Poisson regression was used for calculating the relative risk adjusted for age, gender, and fever days at admissionadmission.

2017 from Tamil Nadu clustered with Indian strains isolated during 2012-2017 from Kerala, Maharashtra, and Tamil Nadu and strains from Sri Lanka and Singapore. Five lineages, India-I to India-IV and Afro India, have been described within the AM/AF genotype earlier.[12] The present study isolates clustered with India-IV lineage strains. Comparison of amino acid sequences of the Indian isolates belonging to Asian genotype with the isolates from Singapore and Sri Lanka revealed that most of the 2017 isolates from Tamil Nadu had three conservative changes (T329S, L475P, and L484M) and one nonconservative change (F337I) that were not observed in earlier isolates (Table S3).

3.5 | Circulating genotype of DENV-2

DENV-2 was classified into five genotypes as described earlier.[17] Further, subclassifications within the cosmopolitan genotype was performed as described recently.[18] For DENV-2, ten isolates were sequenced for E gene. All sequenced isolates grouped within the cosmopolitan genotype. Within cosmopolitan genotype, Tamil Nadu isolates clustered with strains from two lineages—Cosmopolitan I (C-I) and C-III. Within C-I, five isolates were part of C-IA sublineage, and one isolate was part of C-IB (Figure 2). Amino acid sequence analysis within the 2017 Tamil Nadu isolates revealed that C-I strains had Q at 52nd position while it was H in C-III isolates. C-I had a V at 141st position of E protein while isolates from C-IB and C-III had I. Isolates from C-IB and C-III had I at 322nd position while V was present in C-IA (Table S4).

3.6 | Circulating genotypes of DENV-3 and DENV-4

Five genotypes have been reported for DENV-3.[19] One DENV-3 isolate was sequenced which clustered with earlier isolates from India, China, Pakistan, Singapore, and Senegal into GIII genotype and lineage C (Figure S1). For DENV-4, there were five genotypes.[20] Recently, an additional genotype (GVI) has been proposed.[21] Nine DENV-4 isolates were sequenced. All the 2017 isolates from Tamil Nadu grouped within the GI genotype. In the G1 genotype, 2017 isolates from Tamil Nadu along with the strains from India, Pakistan, and Sri Lanka formed lineage C. Strains from Cambodia, China, Singapore, and Thailand formed the lineage B while those from China, Japan, Philippines, and South Korea formed lineage A (Figure 3). Comparison of amino acid sequences of the E protein from the current study isolates with earlier India isolates revealed the presence of altogether 53 unique nonconservative changes in the nine 2017 isolates from Tamil Nadu (Table S5).

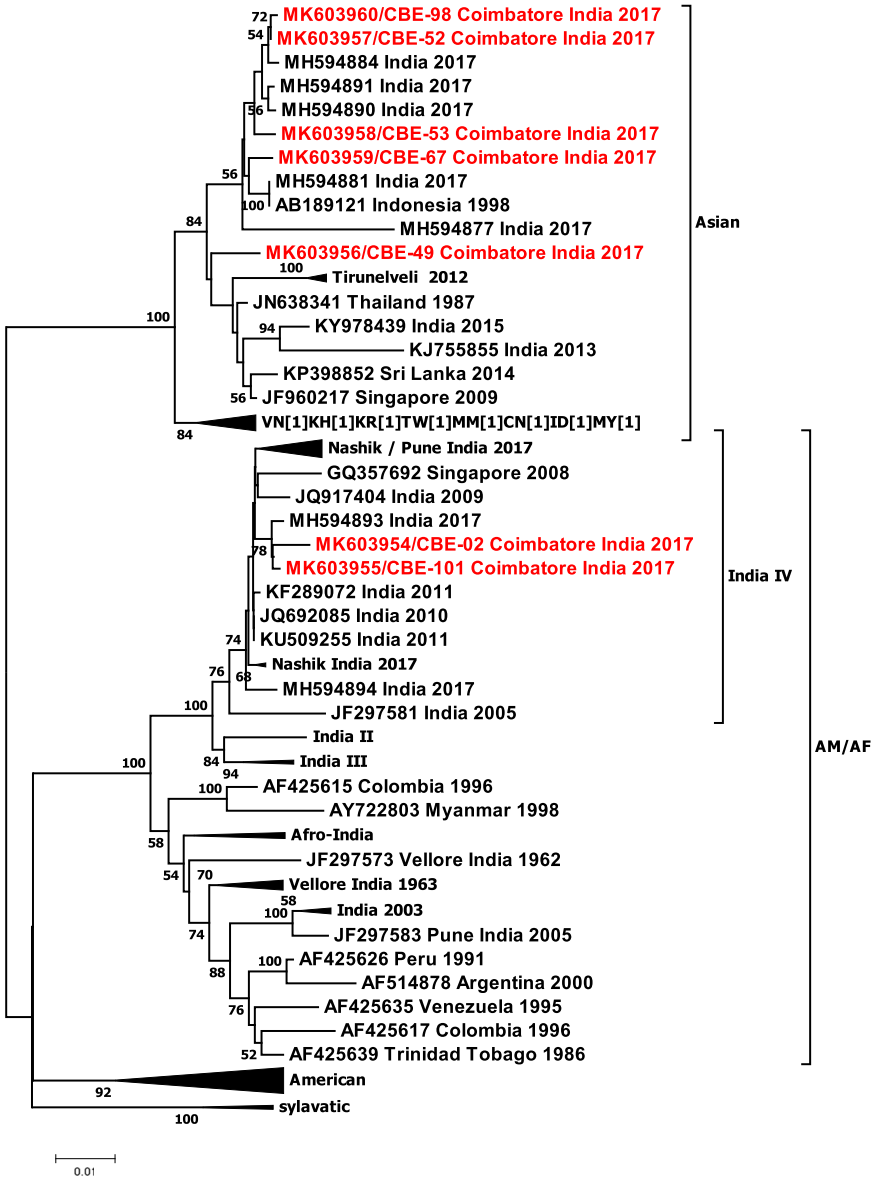


FIGURE 1 Phylogenetic tree of dengue virus-1 (DENV-1) based on E gene sequences All the current study sequences (n = 7) highlighted in Red. Apart from the current sequences, 36 global sequences and 44 Indian sequences were used for generating the phylogenetic tree KH Cambodia/VN-Vietnam/KR-Korea, Republic of/TW-Taiwan/MM-Myanmar/CN-China/ID-Indonesia/MY-Malaysia. Among the five genotypes, Asian, American/African (AM/AF) and Sylavatic genotypes were only shown

4 | DISCUSSION

Forty percent of world's population lives in places endemic to DENV infection and at least 12 000 to 24 000 deaths happen annually in children aged below 15 years.[2] During the study period, admission of 430 undifferentiated fever cases with clinical features suggesting dengue in the pediatric services of one tertiary care hospital alone explains the magnitude of the disease in this endemic region. Circulation of all the four serotypes of DENV has been reported from different regions of India. In 2017, DENV-1 and DENV-3 were more prevalent in Maharashtra, Western India.[10] DENV-1 was the most prevalent serotype while DENV-4 was the least observed one in the 2012 dengue season in Tamil Nadu.[8] In contrast to 2012, DENV-1, DENV-2, and DENV-4 were the prevalent serotypes during 2017 in the study region and could have accounted for the large number of cases from the state. Persistent circulation of DENV-1 might have led to herd immunity permitting the emergence of other serotype viruses. Circulation of multiple serotypes suggests the hyperendemic nature of dengue in this region. The study also suggests the emergence of DENV-4 along with DENV-1 and DENV-2 in Tamil Nadu during 2017.

Studies in the past on disease severity and infecting serotypes have discordant results. A study based on the data from 1994-2006 in Thai children has suggested the association of DENV-2 infection with severe dengue.[22] Further the same study revealed that the risk of SD was more in secondary infections with DENV-2 and DENV-3.[22] A study on adult patients from Singapore revealed that infection with DENV-1 had an increased risk of dengue hemorrhagic fever than DENV-2, whereas a study from Vietnam revealed no difference in the risk of developing severe disease among hospitalized adults infected with DENV-1 and DENV-2.[23,24] A study from Brazil reported that patients infected with DENV-1 serotype had higher risk of severe clinical and laboratory features compared to those infected with DENV-4.[25] A systematic review analyzing the factors associated with



FIGURE 2 Phylogenetic tree of dengue virus-2 (DENV-2) based on E gene sequences All the current study sequences (n = 7) highlighted in Red. Apart from the current sequences, 79 global sequences and 43 Indian sequences were used for generating the phylogenetic tree

dengue shock syndrome, the most severe form of dengue, has revealed DENV-2 as a risk factor.[3] Contradicting all the studies, a study from Bali reported lack of association between DENV serotypes and disease severity.[26] In the present study, as there were less number of children who progressed to SD during hospital stay, for the purpose of analysis, children with SD and DwWS were grouped together and compared with DwoWS. Overall, there were more children with DwoWS than DwWS/SD and none of the serotypes showed any statistically significant increased risk for developing DwWS/SD. Immune status was also not associated with DwWS/SD. However, DENV-4 infections were significantly associated with elevated levels of liver enzymes. A study on pediatric dengue cases from Thailand also has reported that DENV-3 and DENV-4 infection tended to have abnormal elevation of liver enzymes.[27] This observation was contrary to a study from Northern India which has reported an association of elevated levels of liver enzymes with DENV-2.[28] Whether this association of elevated enzymes with DENV-4 can generalized to other regions depends on multiple factors such as the circulating genotype of DENV-4, host immune status, and age of the patients and a study with larger sample size is needed to further confirm the finding.

Phylogenetic analyses indicated the circulation of DENV-1 Asian and AM/AF genotypes, multiple lineages of cosmopolitan genotype of DENV-2, DENV-3 GIII genotype and DENV-4 GI genotype with unique mutations in Tamil Nadu. Cocirculation of DENV-1 Asian and AM/AF genotypes has been reported from Maharashtra in recent years while cocirculation of multiple lineages of DENV-2 cosmopolitan genotype has been reported from Northern India.[10,29,30] A recent study reported that the proportion of Asian genotype among the DENV-1-positive cases in Tamil Nadu had decreased from 90% in 2012 to 57% in 2017.[29] It is possible the DENV-1 Asian genotype viruses might have evolved over the years to become a virus with less epidemic potential phenotype. However, further comparative susceptibility studies in vectors and animal models are needed. In

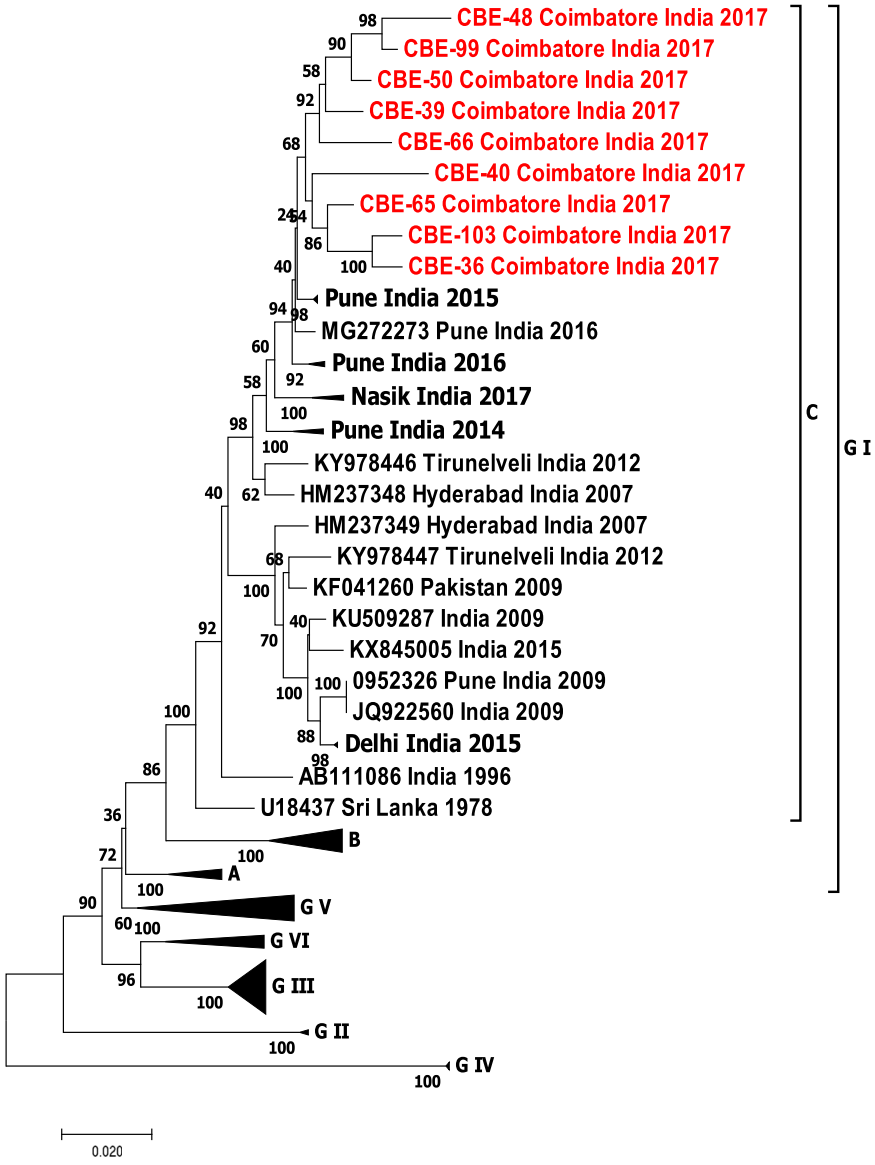


FIGURE 3 Phylogenetic tree of dengue virus-4 (DENV-4) based on E gene sequences. All the current study sequences (n = 9) highlighted in Red. Apart from the current sequences, 47 global sequences and 31 Indian sequences were used for generating the phylogenetic tree

the present study, E gene sequencing of DENV-4 isolates revealed unique mutations which were not observed in earlier Indian DENV-4 strains. The envelope protein has three structural domains I-III. Mutations in the domain II and domain III have more significance since domain II contains the fusion loop which is essential for the interaction between the host endosomal membrane and the virus envelope leading to virus membrane fusion while domain III contains epitopes that are essential for serotype-specific neutralization. Mutations in these domains might affect virus entry as well as the neutralization by serotype/genotype-specific antibodies.[31,32] DENV evolution is shaped by purifying selection in which deleterious mutations affecting viral fitness are removed with time. Further, mutations which confers fitness advantage to DENV in nature were positively selected and contributes to adaptive evolution of DENV.[33] Further follow up studies in the region are needed to discern how these mutations shape up the DENV-4 evolution. It is possible that differences in the viral factors as well as host factors might influence the variable outcomes in different studies. Monitoring the circulating serotypes and genotypes in different regions might result in a region wise database useful in predicting future outbreaks of higher magnitude when changes are observed with regard to the dominant serotypes and new genotypes.

The strength of the present study is that the subjects enrolled were belonging to only to pediatric age, they were prospectively followed up for the outcome and disease severity was classified based on latest guidelines recommended by WHO. Few limitations are, the small sample size, study participants were only hospitalized children from a particular geographic area, hence cannot be extrapolated to general population or other parts of the country. All the patients were recruited within 5 days of fever onset and hence early hospitalization and effective fluid management might have influenced the final outcome. Nonhospitalized cases with undifferentiated febrile illness, which forms the major proportion of DENV cases, were not included in the study and investigation of both hospitalized as well as nonhospitalized cases might help in

identifying the DENV serotypes associated with DwWS and severe dengue.

To conclude, the study reports the activity of all the four serotypes in Tamil Nadu during 2017 with the dominance of DENV-1, DENV-2, and DENV-4. Persistent cocirculation of DENV-1 Asian and AM/AF genotypes, cocirculation of multiple lineages of DENV-2 cosmopolitan genotype and emergence of DENV-4 GI genotype with unique mutations were observed. DENV-4 infection was associated with elevated levels of liver enzymes among hospitalized children. Serotypes and immune status were not associated with disease severity among children hospitalized for dengue.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. World Health Organization. Ten threats to global health in 2019. https://wwwwhoint/emergencies/ten-threats-to-global-health-in-2019. Accessed June 24, 2019.
2. World Health Organization. Dengue: call for urgent interventions for a rapidly expanding emerging disease. 2011. http://applicationsemrowhoInt/docs/RC_technical_papers_2011_3_14217
3. Huy NT, Van Giang T, Thuy DHD, et al. Factors associated with dengue shock syndrome: a systematic review and meta-analysis. *PLOS Negl Trop Dis*. 2013;7:e2412. https://doi.org/101371/journalpntd0002412
4. Alagarasu K. Genetics of susceptibility to severe dengue virus infections: an update and implications for prophylaxis, prognosis and therapeutics. *Dengue Bulletin*. 2016;39:1-18.
5. Ocwieja KE, Fernando AN, Sherrill-Mix S, et al. Phylogeography and molecular epidemiology of an epidemic strain of dengue virus type 1 in Sri Lanka. *Am J Trop Med Hyg*. 2014;91:225-234. https://doi.org/104269/ajtmh13-0523
6. Teoh B-T, Sam S-S, Tan K-K, et al. Dengue virus type 1 clade replacement in recurring homotypic outbreaks. *BMC Evol Biol*. 2013;13:213. https://doi.org/101186/1471-2148-13-213
7. National vector borne disease control programme. Dengue cases and deaths in the country since 2015. 2019. http://nvbdcpgovin. Accessed June 24, 2019.
8. Cecilia D, Patil JA, Kakade MB, et al. Emergence of the Asian genotype of DENV-1 in South India. *Virology*. 2017;510:40-45. https://doi.org/101016/jvirol201707004
9. World Health Organization. Special programme for research, training in tropical diseases. WHO Epidemic, Pandemic Alert Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. World Health Organization Department of Control of Neglected Tropical Diseases, World Health Organization; 2009.
10. Patil JA, Alagarasu K, Kakade MB, et al. Emergence of dengue virus type 1 and type 3 as dominant serotypes during 2017 in Pune and Nashik regions of Maharashtra, Western India. *Infect Genet Evol*. 2018;66:272-283. https://doi.org/101016/jmeegid201810016
11. Kumar SR, Patil JA, Cecilia D, et al. Evolution, dispersal and replacement of American genotype dengue type 2 viruses in India (1956-2005): selection pressure and molecular clock analyses. *J Gen Virol*. 2010;91:707-720. https://doi.org/101099/vir0017954-0
12. Patil JA, Cherian S, Walimbe AM, et al. Evolutionary dynamics of the American African genotype of dengue type 1 virus in India (1962-2005). *Infect Genet Evol*. 2011;11:1443-1448. https://doi.org/101016/jmeegid201105011
13. Patil JA, Cherian S, Walimbe AM, et al. Influence of evolutionary events on the Indian subcontinent on the phylogeography of dengue type 3 and 4 viruses. *Infect Genet Evol*. 2012;12:1759-1769. https://doi.org/101016/jmeegid201207009
14. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 70 for bigger datasets. *Mol Biol Evol*. 2016;33:1870-1874. https://doi.org/101093/molbev/msw054
15. Goncalvez AP, Escalante AA, Pujol FH, et al. Diversity and evolution of the envelope gene of dengue virus type 1. *Virology*. 2002;303:110-119.
16. Domingo C, Palacios G, Jabado O, et al. Use of a short fragment of the C-terminal E gene for detection and characterization of two new lineages of dengue virus 1 in India. *J Clin Microbiol*. 2006;44:1519-1529.
17. Twiddy SS, Woelk CH, Holmes EC. Phylogenetic evidence for adaptive evolution of dengue viruses in nature. *J Gen Virol*. 2002;83:1679-1689.
18. Ali A, Ali I. The complete genome phylogeny of geographically distinct dengue virus serotype 2 isolates (1944-2013) supports further groupings within the cosmopolitan genotype. *PLOS One*. 2015;10:e0138900. https://doi.org/101371/journalpone0138900
19. Lanciotti RS, Gubler DJ, Trent DW. Molecular evolution and phylogeny of dengue-4 viruses. *J Gen Virol*. 1997;78:2279-2284.
20. Lanciotti RS, Lewis JG, Gubler DJ, Trent DW. Molecular evolution and epidemiology of dengue-3 viruses. *J Gen Virol*. 1994;75:65-75.
21. Shrivastava S, Tiraki D, Diwan A, et al. Co-circulation of all the four dengue virus serotypes and detection of a novel clade of DENV-4 (genotype I) virus in Pune, India during 2016 season. *PLOS One*. 2018;13:e0192672. https://doi.org/101371/journalpone0192672
22. Fried JR, Gibbons RV, Kalayanarooj S, et al. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. *PLOS Negl Trop Dis*. 2010;4:e617. https://doi.org/101371/journalpntd0000617
23. Yung C-F, Lee K-S, Thein T-L, et al. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. *Am J Trop Med Hyg*. 2015;92:999-1005. https://doi.org/104269/ajtmh14-0628
24. Fox A, Le NMH, Simmons CP, et al. Immunological and viral determinants of dengue severity in hospitalized adults in Ha Noi, Viet Nam. *PLOS Negl Trop Dis*. 2011;5:e967. https://doi.org/101371/journalpntd0000967
25. Rocha BAM, Guilarde AO, Argolo AFLT, et al. Dengue-specific serotype related to clinical severity during the 2012/2013 epidemic in centre of Brazil. *Infect Dis Poverty*. 2017;6:116. https://doi.org/101186/s40249-017-0328-9
26. Megawati D, Masyeni S, Yohan B, et al. Dengue in Bali: clinical characteristics and genetic diversity of circulating dengue viruses. *PLOS Negl Trop Dis*. 2017;11:e0005483. https://doi.org/101371/journalpntd0005483
27. Kalayanarooj S, Nimmannitya S. Clinical and laboratory presentations of dengue patients with different serotypes. *Dengue Bulletin*. 2000;24:53-59.

28. Kumaria R. Correlation of disease spectrum among four Dengue serotypes: a five years hospital based study from India. *Braz J Infect Dis*. 2010;14:141-146.
29. Alagarasu K, Patil JA, Kakade MB, et al. Spatio-temporal distribution analysis of circulating genotypes of dengue virus type 1 in western and southern states of India by a one-step real-time RT-PCR assay. *Infect Genet Evol*. 2019;75:103989. https://doi.org/101016/jmeegid2019103989
30. Kar M, Nisheetha A, Kumar A, et al. Isolation and molecular characterization of dengue virus clinical isolates from pediatric patients in New Delhi. *Int J Infect Dis*. 2018;84S:S25-S33. https://doi.org/101016/jijid201812003
31. Modis Y, Ogata S, Clements D, Harrison SC. Structure of the dengue virus envelope protein after membrane fusion. *Nature*. 2014;427:313-319.
32. Gallichotte EN, Baric TJ, Nivarthi U, et al. Genetic variation between dengue virus type 4 strains impacts human antibody binding and neutralization. *Cell Reports*. 2018;25:1214-1224. https://doi.org/101016/jcelrep201810006
33. Pollett S, Melendrez MC, Maljkovic Berry I, et al. Understanding dengue virus evolution to support epidemic surveillance and counter-measure development. *Infect Genet Evol*. 2018;62:279-295. https://doi.org/101016/jmeegid201804032

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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J Pediatr Genet
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Research Paper

Apolipoprotein-E Gene Polymorphism and Lipid Composition among IUGR and AGA Neonates

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Abstract

Objective The objective of this study is to study the profile of apolipoprotein E (*APOE*) gene polymorphism and lipid profile among intrauterine growth restriction (IUGR) and appropriate for gestational age (AGA) neonates. This is an observational study. This study was done at the neonatal unit of a teaching hospital in South India. All consecutively born IUGR neonates (cases) of more than 32 weeks' gestational age and AGA neonates (controls) were enrolled for the study. Genomic DNA extraction was done from a total of 102 peripheral venous blood samples. Genotyping of the APOE rs429358 and rs7412 defining the ε2, ε3, and ε4 alleles was done by polymerase chain reaction–restriction fragment length polymorphism method. Prefeed venous blood was collected and analyzed for lipid profile estimation. The allelic frequencies of cases versus control were ε2—9 (8.7%) versus 3 (2.9%); ε3—88 (84.6%) versus 81 (79.4%); and ε4–7 (6.7%) versus 18 (17.6%). The frequency of ε4 isoform allele, associated with adult onset of metabolic diseases was less among the IUGR group. The mean total cholesterol (TC), Low-Density Lipoprotein (LDL), High-Density Lipoprotein, and triglyceride (TG) were 107.59 ± 35.99, 51.69 ± 24.68, 21.75 ± 9.58, and 151.22 ± 61.84 mg/dL, respectively, in the IUGR group. The mean TC and LDL levels in IUGR group were marginally higher than AGA neonates (107 ± 35.99 vs. 100.37 ± 22.69 mg/dL and 51.69 ± 24.68 versus 46.9 ± 19.51 mg/dL, $p > 0.05$). In both groups, the mean TC and TGL levels were elevated in the ε4 isoform subgroup ($p > 0.05$). In our study, the ε2 allele was the second most predominant APOE isoform and the ε4 allele of the *APOE* gene associated with adult-onset diseases was not increased among IUGR neonates. Neonates with ε4 allele showed an abnormal lipid profile in both study groups suggesting a possible association.

Keywords

IUGR neonates - *APOE* gene polymorphism - lipid profile

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Apolipoprotein-E Gene Polymorphism and Lipid Composition among IUGR and AGA Neonates

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Abstract

Objective The objective of this study is to study the profile of apolipoprotein E (*APOE*) gene polymorphism and lipid profile among intrauterine growth restriction (IUGR) and appropriate for gestational age (AGA) neonates. This is an observational study. This study was done at the neonatal unit of a teaching hospital in South India. All consecutively born IUGR neonates (cases) of more than 32 weeks' gestational age and AGA neonates (controls) were enrolled for the study. Genomic DNA extraction was done from a total of 102 peripheral venous blood samples. Genotyping of the APOE rs429358 and rs7412 defining the ε2, ε3, and ε4 alleles was done by polymerase chain reaction–restriction fragment length polymorphism method. Prefeed venous blood was collected and analyzed for lipid profile estimation. The allelic frequencies of cases versus control were ε2—9 (8.7%) versus 3 (2.9%); ε3—88 (84.6%) versus 81 (79.4%); and ε4–7 (6.7%) versus 18 (17.6%). The frequency of ε4 isoform allele, associated with adult onset of metabolic diseases was less among the IUGR group. The mean total cholesterol (TC), Low-Density Lipoprotein (LDL), High-Density Lipoprotein, and triglyceride (TG) were 107.59 ± 35.99, 51.69 ± 24.68, 21.75 ± 9.58, and 151.22 ± 61.84 mg/dL, respectively, in the IUGR group. The mean TC and LDL levels in IUGR group were marginally higher than AGA neonates (107 ± 35.99 vs. 100.37 ± 22.69 mg/dL and 51.69 ± 24.68 versus 46.9 ± 19.51 mg/dL, $p > 0.05$). In both groups, the mean TC and TGL levels were elevated in the ε4 isoform subgroup ($p > 0.05$). In our study, the ε2 allele was the second most predominant APOE isoform and the ε4 allele of the *APOE* gene associated with adult-onset diseases was not increased among IUGR neonates. Neonates with ε4 allele showed an abnormal lipid profile in both study groups suggesting a possible association.

Keywords
- IUGR neonates
- *APOE* gene polymorphism
- lipid profile

Introduction

The term intrauterine growth restriction (IUGR) has been defined as the subnormal rate of fetal growth with respect to the growth potentials of a specific fetus in accordance to the gender and race. These neonates are born with clinical features of in utero malnutrition and may or may not have a birth weight less than 10th centile for the particular gestational age.[1] Approximately 78% of all IUGR infants delivered globally are seen in Asian continent with highest incidence seen in India.[2] The IUGR infants are prone for short-term and long-term complications making them high-risk neonates. Few of the short complications include perinatal asphyxia, persistent pulmonary hypertension,

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Clinical features and laboratory parameters in children with severe dengue and scrub typhus

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Background: Objective of study was to compare the clinical features and laboratory parameters at admission and differentiate severe dengue from scrub typhus in children.

Methods: Retrospective analysis of case records and comparison of clinical and laboratory parameters at admission of all children with a diagnosis of severe dengue and scrub typhus was done.

Results: A total of 72 children were included (severe dengue =40; scrub typhus =32) during the study period. The mean (SD) age of children with severe dengue and scrub typhus was 7.9(3.8) and 11.8(5.8) years, respectively. Majority of children with severe dengue presented with hypotension, 21(52.5%) vs 3(9.4%) in scrub typhus. Children with severe dengue had a relatively low ANC ($2.6\pm1.97x10^3/mm^3$ vs $3.9\pm2.06x10^3/mm^3$), low platelet count ($50.23\pm35.55x10^3/mm^3$ vs $140\pm95.0x10^3/mm^3$) and low mean ESR at 1hour (8.1±6.82mm vs 33.88±13.79mm) than scrub typhus.

Conclusions: Compared to scrub typhus, severe dengue was significantly associated with hypotension, lower ANC, ESR and platelets.

Keywords: Eschar, Fever, Low ESR, Rash, Thrombocytopenia, Scrub typhus, Severe dengue

Introduction

Globally 390 million dengue infections per year are reported, of which 96 million manifests clinically carrying high morbidity.[1] Dengue viruses (DV) belong to the family flaviviridae and is transmitted by vectors Aedes aegypti and Aedes albopictus.[2] Scrub typhus (ST) is another hemorrhaegic fever caused by Orientia tsutsugamushi that is spread by the bite of trombiculid mite. ST is increasingly recognized in tropical countries contributing upto 50% of patients with undiagnosed acute febrile illnesses.[3] In many parts of India, these two infections together comprise more than half of all acute undifferentiated febrile illnesses, more so in south Indian states due to the favorable tropical climatic conditions. Their similar clinical features and laboratory findings often pose diagnostic difficulty for the treating physician.[4] Early diagnosis of the illnesses is life saving to improve patient outcomes and promote timely public health interventions. In this retrospective study we have compared the clinical features and laboratory parameters of children with severe dengue and scrub typhus and attempted to derive the parameters that differentiate severe dengue from scrub typhus.

Methods

This retrospective study conducted over a period of 1 and ½ year (January 2015 to June 2016) in the department of pediatrics of PSG Institute of Medical sciences and research, a tertiary care teaching hospital in South India. Case records of all children (≤18 years) discharged with a

diagnosis of severe dengue and scrub typhus were identified, and those who had a serological confirmation were included for analysis. Severe dengue was diagnosed based on revised WHO criteria and included in the study.[5] Children with or without warning signs, without serological confirmation were excluded. The data was recorded on specified data collection form. The data included demographic parameters, clinical features, and laboratory parameters (CBC, RFT, LFT, serology, culture if any). The serological diagnosis of Dengue fever was based on dengue IgM-ELISA (Panbio dengue IgM & IgG capture ELISA) where values <9 PanBio ELISA units were considered negative, 9-11 equivocal, and more than 11 positive, as per the manufacturer's instructions). Similarly, serological diagnosis of scrub typhus was based on scrub typhus detect^{TM}IgM ELISA system. Children with SD were treated with normal saline, monitored for complications and treated according to the physician's discretion. Children with ST were treated with antibiotics and offered symptomatic care for the illness. The Institution's Human ethical committee approved the study.

Statistical analysis

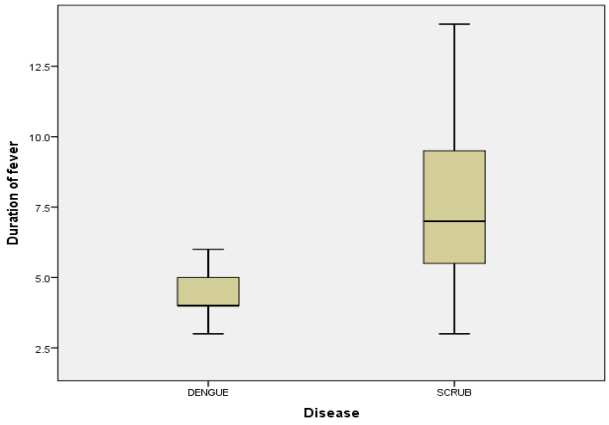
Kolmogorov-Smirnov test was used for testing normality of the data. Normally distributed continuous variables were compared using *t*-test and non-parametric variables using Wilcoxon signed-rank and Mann-Whitney U tests. Chi-square analysis was done for comparisons of proportional data among groups. Multiple logistic regression models were used to assess the association of severe dengue and scrub typhus with relevant clinical and laboratory parameters.

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A total of 7926 children got admitted as inpatients during the study period of which 6737 children were admitted for evaluation of fever. 747 children were serologically confirmed as dengue fever of which a total of 40 children were diagnosed with severe dengue fever with mean (±SD) age 7.9 (±3.80) years, and male: female ratio was 1.1:1. The mean (±SD) duration of fever at admission was 4.5(±1.13) and the mean (±SD) duration of hospital stay was 5.58 (±1.13) as in Figure 1.

Among children with severe dengue, 31(77.5%) had vomiting, 27(67%) had abdominal pain and 23(57.5%) had oliguria as presenting complaints. A majority of them (31; 77.5%) had evidence of plasma leakage, and 17(42.5%) had skin and mucosal bleeds at admission. None of the children had any alternate provisional diagnosis at admission other than severe dengue based on the clinical presentation.

A total of 32 children were serologically diagnosed as scrub typhus with mean (±SD) age of 11.8 (±5.83) years, and male:female ratio was 2.2:1. The mean (±SD) duration of fever at admission was 7.34 (±2.46) days. Of 32 children, 14 (43.8%) presented with vomiting and 11 (34.4%) presented with abdominal pain at admission. On examination, 5 (15.6%) had maculopapular rash and 15 (46.9%) had hepatosplenomegaly. Only 8 (25%) were found to have eschar to support the clinical diagnosis of scrub typhus. Other clinical parameters are presented in Table 1.



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Age#	7.97±3.8	11.8±5.83	0.001
Gender(M:F)	1.1:1	2.2:1	NS
Duration of fever at admission#	4.5±1.13	7.34±2.46	<0.001
Vomiting	31(77.5%)	14(4.38%)	0.007
Myalgia	17(42.5%)	8(25%)	NS
Rash	6(15%)	5(15.6%)	NS
Abdomen pain	27(67.5%)	11(34.4%)	0.009
Oliguria	23(57.5%)	3(9.4%)	<0.001
Respiratory distress	7(17.5%)	3(9.4%)	NS
Skin/mucosal bleeds	17(42.5%)	2(6.3%)	<0.001
Haematemesis	2(5%)	2(6.3%)	NS
Edema/plasma leakage	31(77.5%)	3(9.4%)	<0.001
Hypotension	21(52.5%)	3(9.4%)	<0.001
Hepatomegaly	37(92.5%)	15(46.9%)	<0.001
Spleen	nil	15(46.9%)	<0.001

□Expressed as Mean ±SD, NS-Non significant

We compared the clinical and laboratory characteristics among children with severe dengue and scrub typhus as shown in Table 2. We found that the mean age at presentation and duration of fever was higher among scrub typhus compared to severe dengue, 7.97±3.8 years

vs 11.8±5.83 years (p = 0.001) and 4.5±1.13 days vs 7.34±2.46 days (p<0.001), respectively. A greater proportion of children with dengue presented with vomiting, abdominal pain, oliguria, skin bleeds, edema and hypotension. There existed a statistically significant difference in the proportions of the above-mentioned variables between both groups (p<0.05). Interestingly erythematous rash was present in equal proportions between both groups (15% vs 15.6%, p = 0.14).

Table 2 Comparison of clinical features between dengue and scrub typhus

	Dengue fever	Scrub typhus	p value
Hemoglobin# (g/dL)	13.3±1.70	12.24±1.63	0.003
Hematocrit#	41.45±5.78	37.6±5.14	0.001
TLC #(x10^3/cu.mm)	5.19±3.03	7.64±3.45	0.002
ANC# (x10^3/cu.mm)	2.6±1.97	3.9±2.06	0.002
Platelet# (x10^3/cu.mm)	50.23±35.55	140±95.0	<0.001
ESR#	8.1±6.82	33.88±13.79	<0.001
SGPT#	138.75±213.75	91.9	0.31

Expressed as Mean ±SD

The mean haemoglobin (and hematocrit) values were higher among children with dengue (13.3±1.7 vs 12.24±1.63, p = 0.003). The white blood cell (WBC) count and absolute neutrophil count (ANC) were markedly different in both groups. The children with dengue had a relatively low WBC count and ANC (5.19±3.03x10^3/mm^3, 2.6±1.97x10^3/mm^3 respectively) on comparing children with scrub. The platelet count at admission was lower in children with dengue when compared to scrub typhus (50.23±35.55x10^3/mm^3 vs 140±95x10^3/mm^3, p<0.001). Also, the mean Erythrocyte Sedimentation Rate (ESR)at 1hour was lower in children with dengue fever compared to scrub typhus (8.1±6.82mm vs 33.88±13.79mm, p<0.001). The mean serum Alanine Transaminase (ALT) levels were higher in children with severe dengue compared to scrub typhus (138.75±213.75 vs 91.9IU/L). But this difference did not achieve statistical significance (p = 0.19).

From the data obtained at admission, variables between these two illnesses that had statistically significant difference were applied to a logical regression model. We found a high probability of severe dengue compared to scrub typhus was associated independently with hypotension, lower ANC, low ESR and platelets as in Table 3. With a probability cut-off of >90%, the predictive model gave a sensitivity of 100%, specificity of 87.5%, positive predictive value of 88.8% and negative predictive value of 100%, for dengue compared to scrub typhus with ANC 0.93 (0.79-0.99) as shown in Figure 2.

Table 3 Multivariate logistic regression analysis of clinical and laboratory parameters between dengue and scrub typhus

Parameter	Increase/decrease	Multivariate odds ratio (95% CI)	p value
ANC	2x10^3/cu.mmincrease	1.98 (1.18-3.30)	0.012
Platelet count	40000/cu.mmdecrease	2.47 (1.63-4.23)	<0.001
ESR	6mm decrease	5.69 (2.88-15.97)	<0.001

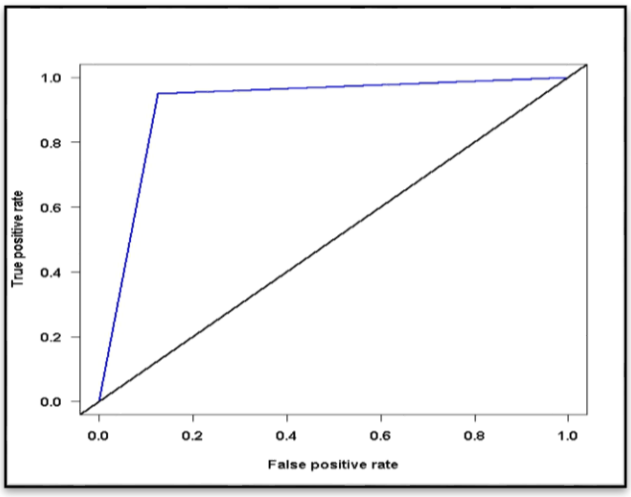


Figure 2 Receiver operating characteristic curve for ANC in predicting dengue fever from scrub typhus

Discussion

Dengue fever and scrub typhus caused by different vectors present in similar demographic distribution and clinical features. Any delay in recognition of these infectious diseases results in significant morbidity and mortality as well. Management of severe dengue is largely supportive with emphasis on fluid resuscitation and hemodynamics monitoring, whereas in scrub typhus the early initiation of antibiotics is mainstay.

Making the correct diagnosis with the available preliminary investigations while awaiting confirmatory test results might help in better management and relieve parent's anxiety. In this retrospective study we attempted to compare the clinical and laboratory parameters between these two similar diseases at initial presentation to the health care facility.

The mean age at presentation of children with severe dengue was less as compared to scrub typhus (7.9 +/- 3.80 vs 11.8 +/- 5.83) and the mean age at presentation of the latter was comparable to that reported previously by Kumar M et al.[6]

The male:female ratio of children with severe dengue was less than that of scrub typhus (1.1:1 vs 2.2:1). This difference in gender ratio could probably due to similar exposure risk for mosquito bites in-doors and higher prevalence of chigger exposure while playing outdoors by boys. A similar observation was made by Bhat NK et al, who documented a higher male to female ratio (1.44:1) in scrub typhus. Among the symptoms and signs between the study groups at presentation, mean duration of fever, abdominal pain, edema, oliguria and hypotension were strikingly different. Though these differences have been witnessed in children with scrub typhus in previous studies their incidence was much lesser in the present study, when compared to children with severe dengue.[7]

In our present study, there was significant difference in clinical features among the study groups. Children with severe dengue had a short mean duration of fever (4.5 +/- 1.13 vs 7.34 +/- 2.46 days) at presentation. Majority of children (77.5%) with severe dengue in our study presented with vomiting and edema. Two third had abdominal pain and oliguria and almost half of them presented with skin bleeds and hypotension at admission. Almost ninety percent of children presented with significant hepatomegaly and none had splenomegaly. Pothapregada S et al, in his clinical profile of children with dengue also observed a similar occurrence with fever (94.6%) being the commonest symptom and persistent vomiting as the frequent warning sign (75.1%).[8] Among children with scrub typhus, almost half of them had hepatosplenomegaly. Clinical features like rash, edema, respiratory distress, oliguria was less observed in children with scrub typhus. Only twenty-five percent of children with scrub typhus had eschar in our study population. Rose W et al, found 40.8% of their children having the characteristic eschar at the presentation but most studies project a lesser percentage.[9] Babu et al, in their prospective study found only 34.6% of serologically confirmed scrub typhus to have eschar.[10] Absence of eschar could be secondary to previous exposure to ricketssial antigen and variation in cutaneous immune response and that could be possible reason for absence of eschar in majority of our study children.

In our study, we found that the striking difference in clinical parameters between the two groups was hypotension, lower ANC, low ESR and significantly low platelet values and thus provides the best ability to differentiate between severe dengue fever and scrub typhus. ANC has been statistically high in children with scrub typhus than severe dengue. The predominance of lymphocytes with leucopenia and severe thrombocytopenia might be an additional pointer to the diagnosis of severe dengue. In the guideline of Indian academy of pediatrics on ricketssial diseases, marked leukocytosis with the shift to the left in later stages, raised ESR and thrombocytopenia were laboratory features suggestive of these infections, as noted in children with ST in the present study.[11] Liver dysfunction has been observed in children with severe dengue and scrub typhus.[12] Similar to the present study, Kumar J et al noted a 10-fold increase in the mean serum ALT levels in children with DF and could be used in differentiating these diseases.[13]

Few limitation of our study is the retrospective model, small sample size and restricted to a hospital – based population. The observation and predictive model may not be applicable to differentiate other febrile illnesses in children and the clinical utility the model needs to be tested prospectively and also validated. In a resource limited setting among endemic areas, our predictive model could be utilized to differentiate hemorrhagic fever due to scrub typhus and severe dengue, helping to initiate appropriate treatment.

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1. World Health Organization. Dengue and dengue haemorrhagic fever. Factsheet no. 117, 2008. Available at: https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue.
2. Gupta N, Srivastava S, Jain A, Chaturvedi UC. Dengue in India. Indian J Med Res. 2012;136(3):373-90.
3. KendreVarsharani V, Shekde SD, Chinte LT. The Study of Clinico-epidemiological Features of Dengue Cases Admitted in Tertiary Care Hospital, Latur, Maharashtra. Int J Recent Trends Sci Technol. 2014;10(2):369-72.
4. Chrispal A, Boorugu H, Gopinath KG, Chandy S, Prakash JA, Thomas EM, et al. Acute undifferentiated febrile illness in adult hospitalized patients: The disease spectrum and diagnostic predictors-an experience from a tertiary care hospital in South India. Trop Doct. 2010;40:230-4.
5. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. New edition. Geneva: WHO, 2009. Available at: http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf.
6. Kumar M, Krishnamurthy S, Delhikumar CG, Narayanan P, Biswal N, Srinivasan S. Scrub typhus in children at a tertiary hospital in southern India: clinical profile and complications. J Infect Public Health. 2012 Mar;5(1):82-8.
7. Bhat NK, Dhar M, Mittal G, Shirazi N, Rawat A, Kalra BP, et al. Scrub typhus in children at a tertiary hospital in north India: clinical profile and

complications. Iranian J Pediatrics. 2014 Aug;24(4):387.
8. Pothapregada S, Kamalakannan B, Thulasingham M, Sampath S. Clinically profiling pediatric patients with dengue. J Glob Infect Dis. 2016;8(3):115-20.
9. Rose W, Rajan RJ, Punnen A, Ghosh U. Distribution of eschar in pediatric scrub typhus. J Trop Pediatr. 2016;62(5):415-20.
10. ArunBabu T, Vijayadevagaran V, Ananthakrishnan S. Characteristics of pediatric scrub typhus eschar in south Indian children. Pediatr Dermatol. 2017;34(2):124-7.
11. Palanivel S, Nedunchelian K, Poovazhagi V, Raghunadan R, Ramachandran P. Clinical profile of scrub typhus in children. Indian J Pediatr. 2012 Nov;79(11):1459-62.
12. Chanta C, Triratanapa K, Ratanasirichup P, Mahaprom W. Hepatic dysfunction in pediatric scrub typhus: role of liver function test in diagnosis and marker of disease severity. J Med Assoc Thai. 2007 Nov;90(11):2366-9.
13. Jagadishkumar K, Jain P, Manjunath VG, Umesh L. Hepatic involvement in dengue fever in children. Iran J Pediatr. 2012 Jun;22(2):231-6.

r Rajesh NT, Elangovan B. Clinical and laboratory predictors to differentiate severe dengue from scrub typhus in children. Int J Contemp Pediatr 2019;6:1223-7.



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Gallbladder motility in children with chronic functional abdominal pain

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Abstract

Background: Chronic abdominal pain is a common gastrointestinal symptom in children that significantly lowers their quality of life. In adults, Gall bladder (GB) hypomotility / dyskinesia is associated with many functional abdominal disorders but there is scarcity of evidence on its role in childhood gastro-intestinal disorders. Aim of the study is to evaluate the GB motility in children with chronic functional abdominal pain (FAP).

Methods: Children aged 5-15 years with chronic abdominal pain fulfilling ROME-III criteria and healthy controls were included and all study participants were subjected to ultrasonographic evaluation of the gall bladder volume in fasting state and post - Fatty test meal (FTM) and its ejection fraction was calculated.

Results: Sixtysix children including 31 with chronic FAP underwent sonographic evaluation for GB motility. The mean ejection fraction (EF) of cases and controls were 51.72±17.76% and 57.3±23.26% (p value - 0.158). The mean EF of cases with upper abdominal pain and lower/peri-umbilical abdominal pain were 41.7±17.1% and 57.2±15.9% respectively (*p* value < 0.0001). Increasing BMI had no significant association with GB motility among the participants.

Conclusions: There is lack of significant association between children with chronic FAP and GB hypomotility. Children with upper abdominal pain have lesser GB EF, suggesting a possible abnormal GB motility.

Keywords: Childhood functional abdominal pain, Gallbladder, Ultrasonography

Introduction

Chronic abdominal pain is a common gastrointestinal symptom in children presenting to outpatient department. In United states and Europe, its prevalence rates ranges from 0.3 – 19% among school-going children and accounts for about 2% to 4% of all pediatric office visits.[1,2] Though it is not associated with any organic gastrointestinal pathology, children with FAP have a significantly lower quality of life and it contributes for school – absenteeism.[3] In 1999, the term abdominal pain related-functional gastrointestinal disorders in pediatric ROME II criteria, which included functional dyspepsia, functional abdominal pain (FAP), irritable bowel syndrome and abdominal migraine. These terminologies were further redefined based on duration of symptoms before diagnosis in ROME III criteria.[3]Many functional gastrointestinal disorders like functional dyspepsia, irritable bowel syndrome and disorders of Gall bladder (GB) has been studied for any possible association with abnormalities in GB motility, but the results are inconclusive.[4] In adults, GB hypomotility/dyskinesia is well established in idiopathic constipation and functional dyspepsia, but there is scarcity of evidence on its role in childhood functional abdominal disorders.[5,6] Our study

was designed to study the GB motility in children with functional abdominal pain.

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This crosssectional study was done in the outpatient department of pediatrics, PSG institute of medical sciences from January 2016 to August 2017. Children aged 5-15 years attending the OPD of the institution with chronic abdominal pain fulfilling ROME-III criteria, without any obvious cause for abdominal pain and with no previous abdominal surgeries were included in the study. Healthy children who visited the hospital for routine health visit or vaccination were included as controls. Institutional Human ethical committee approved the study and all study participants were included after obtaining informed consent. A detailed history including dietary and allergy history was obtained and clinical assessment was done. They were documented in a pre-designed data collection tool.

A total of 31 cases and 35 controls were recruited in the study and all study participants were subjected to ultrasonographic evaluation of the gall bladder in fasting state. A radiologist experienced in pediatric Sonography performed the evaluation using Philips iU22 xMATRIX ultrasound system. The Gall bladder was assessed for its area, thickness and volume, where area was derived by trace method and maximum GB width and length were measured using transverse and longitudinal images of the GB.

The volume of GB was calculated in fasting and 30-minutes following a fatty test meal using Dodd's formula, Volume (in cm^3) = 0.52 x [L x W x A], where L is the greatest length, W- greatest transverse width, and A-greatest anteroposterior diameter of the GB. The gallbladder ejection fraction (EF) for each examination was obtained from two-volume data using the following equation. Where V_0 = gallbladder volume in fasting state, Vn = gallbladder volume after the fatty test meal,

□□□□□□□□□□□□□□□EF (%) = [(Vo-Vn) / Vo] X 100

For the purpose of this study, Children with ejection fraction of less than 40% were considered to have gallbladder hypomotility.

Statistical analysis was done using statistical package for the social science version-19 (SPSS) software. The fasting and post-prandial GB volume was expressed as mean ± SD and students t test was used to compare their means between groups. Chi-squared testing was applied to look for possible association between EF and BMI. A p-value less than 0.05 was considered significant.

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There were a total of 66 children included in this study. The mean age of cases and controls were 10.14±2.71 years and 11.54±1.94 years respectively. The mean height of the cases group was 132.9±15.9 (range – 100 to 165cms) and that of controls was 145.14±11.6 (range – 110 to 159cms). The mean weight of cases and controls were 27.3 ± 10.5 Kg and 33.8 ± 6.6 Kg respectively. The mean body mass index (BMI) was 14.9±2.8 kg/m^2 (range of 11.3 to 26.3 kg/m^2) in case group and 15.9±1.8 kg/m^2 (range from 11.9 to 19.5 kg/m^2) in the control group. Out of 31 cases there were 11 boys (35.5%) and 20 (64.5%) girls respectively and in 35 controls there were 23 (65.7%) boys and 12 (34.3%) girls respectively (Table 1).□

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Age (in years)	10.14±2.71	11.54±1.94	0.08
Gender ratio	1:2	2:1	-
Height (in m)	1.34±0.15	1.43±0.11	0.06
Weight (in Kg)	27.3±10.8	34.31±6.3	0.03
BMI (Kg/m^2)	14.60±3.1	16.59±1.60	0.03
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Predominantly Upper (%)□	11 (40%)	-	
Predominantly Lower (%)	19 (60%)		

*Student's T-Test

The mean fasting and post-prandial gallbladder volumes of cases and controls were 8.20±3.97 (cm^3), 7.52±3.13(cm^3) and 3.51±1.57 (cm^3) and 2.94±1.59 (cm^3) respectively. Though the fasting GB volumes were greater than post-test meal, in both groups, yet there was no statistical significance (P value – 0.390) (Table 2). The mean ejection fraction of cases and controls were 51.72±17.76% and 57.37±23.26% (p value – 0.158).

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Cases	8.20±3.97	7.52±3.13	51.95±18.02
Controls	3.51±1.57	2.94±1.59	57.04±23.03
P-value*	0.797	0.127	0.239

*Student's T-Test

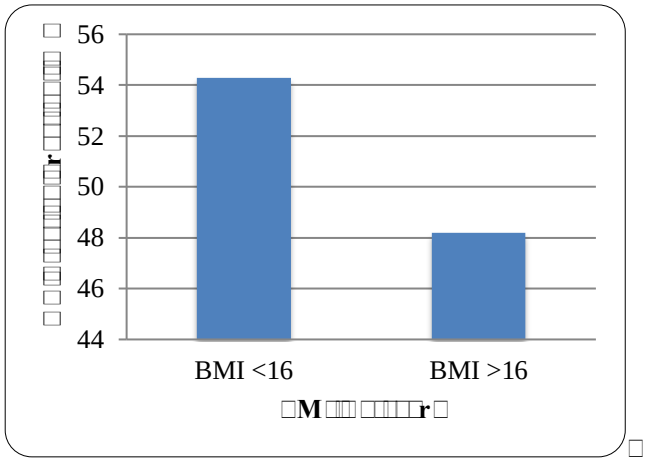
The ejection fraction of GB were compared among children with functional abdominal pain who presented predominantly with upper or lower / peri-umbilical regions, to identify any possible association between the site of abdominal pain with GB motility. The mean ejection fractions of cases with upper abdominal pain and lower/peri-umbilical abdominal pain were 41.7±17.1% and 57.2±15.9% respectively (p value <0.0001), but the former did not qualify for the definition of GB hypomotility i.e. EF<40% (Table 3).

[illegible]

[illegible]	[illegible]		
	[illegible]	[illegible]	[illegible]
Predominantly Upper	7.41±3.13	4.17±2.15	41.73±17.13
Predominantly Lower	8.67±4.40	3.13±1.0	57.87±16.12
P-value*	0.497	0.25	0.019

*Student's T-Test

In order to identify any possible association between increasing BMI and GB contractility among cases, they were sub-grouped as, BMI<16 and BMI ≥ 16. There was equal number of children (n=4) in each subgroup with EF<40%, suggestive of GB dysmotility and their mean EF were 54.28 ± 17.99 % and 48.19 ± 17.53 % respectively (*p*-value < 0.001) (Figure 1).



[illegible]

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In our study, we found no significant difference in the gall bladder volume (in fasting state and post Fatty test meal) among the cases and controls with equal number of children in both groups with GB ejection fraction <40%. Children who presented with upper abdominal pain had significantly lesser ejection fraction than those with lower abdominal pain. Many adult studies have recognized GB hypomotility in obese patients than non-obese patients. Sari et al found a positive correlation between fasting gallbladder volume and body mass index and body fat weight in obese women than non-obese women, but no such studies were available for comparison in children.[7] In the present study there existed no significant association in GB dysmotility and increasing BMI among cases.

The exact pathogenesis for FAP remains unclear, but visceral hypersensitivity, abnormal brain - gut interaction, immune dysregulation and altered gut motility has been postulated for the occurrence of the symptoms.[8,9] Gall bladder dyskinesia has been associated with various gastrointestinal symptoms like bloating, dyspepsia, post prandial epigastric pain and upper abdominal fullness.[10]

Mehra et alm observed gall bladder hypomotility in children with functional constipation, thus strengthening the claim of GB dyskinesia as a possible claim for functional abdominal symptoms in children.[11] Several imaging modalities like oral choledochocystography and syntigraphy are utilized to assess GB contraction but Sonography has been reliably used for assessing the GB area and volume, their changes following a fatty meal.[12] Ultrasonography could be preferred for its noninvasive nature and no radiation exposure that accompanies other modalities.

In our study we found 8 (26.7%) of the children with FAP healthy and 9 (25%) controls with mean GB Ejection fraction <40% suggesting that GB hypomotility could possibly be unrelated for the abdominal pain. Interestingly children who had predominant upper abdominal pain had low GB Ejection fraction compared to the children with lower abdominal pain (41.73±17.13% vs 57.87±16.12%) as noted by Hofeltd et al, in their study where a majority of children (96.67%) who underwent laproscopic cholecystectomy for biliary dyskinesia had upper quadrant pain.[13] A similar study with more subjects with FAP localized predominantly to upper abdominal could establish an association with GB motility.

Few limitations in our study are firstly the children were consecutively enrolled and sample size of the study is too small for drawing strong conclusions. Secondly the co-existing infection like H. pylori or parasitic infection of gut could possibly have an impact on the abdominal pain that was under evaluation. Nonetheless the present study is the first of its kind that has meticulously evaluated the GB volume and contractility in children with FAP and has found a possible association of GB dyskinesis with upper abdominal pain.

[illegible]

To conclude, abnormality in GB motility could be a cause for FAP presenting predominantly as upper abdominal pain in children thus necessitating its evaluation and a larger well-controlled study might help in better understanding of the entity.

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1. Chitkara DK, Rawat DJ, Talley NJ. The epidemiology of childhood recurrent abdominal

pain in Western countries: a systematic review. The Am J gastroenterol. 2005;100(8):1868.
2. Spee LA, Lisman-Van Leeuwen Y, Benninga MA, Bierma-Zeinstra SM, Berger MY. Prevalence, characteristics, and management of childhood functional abdominal pain in general practice. Scandinavian journal of primary health care. 2013;31(4):197-202.
3. Youssef NN, Atienza K, Langseder AL, Strauss RS. Chronic abdominal pain and depressive symptoms: analysis of the national longitudinal study of adolescent health. Clinic Gastroenterol Hepatol. 2008;6(3):329-32.
4. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterol. 2006;130(5):1527-37.
5. Hemingway D, Neilly JB, Finlay IG. Biliary dyskinesia in idiopathic slow-transit constipation. Diseases of the colon & rectum. 1996;39(11):1303-7.
6. Velanovich V. Biliary dyskinesia and biliary crystals: a prospective study. The Am surg. 1997;63(1):69-74.
7. Sari R, Balci MK, Coban E, Karayalcin U. Sonographic evaluation of gallbladder volume and ejection fraction in obese women without gallstones. J clinic ultrasound. 2003;31(7):352-7.
8. Halac U, Noble A, Faure C. Rectal sensory threshold for pain is a diagnostic marker of irritable bowel syndrome and functional abdominal pain in children. J ped. 2010;156(1):60-5.
9. Bakker MJ, Boer F, Benninga MA, Koelman JH, Tijssen MA. Increased auditory startle reflex in children with functional abdominal pain. J ped. 2010;156(2):285-91.
10. Carr JA, Walls J, Bryan LJ, Snider DL. The treatment of gallbladder dyskinesia based upon symptoms: results of a 2-year, prospective, nonrandomized, concurrent cohort study. Surg Laparoscop Endoscop Percutaneous Tech. 200;19(3):222-6.
11. Mehra R, Sodhi KS, Saxena A, Thapa BR, Khandelwal N. Sonographic evaluation of gallbladder motility in children with chronic functional constipation. Gut and liver. 2015;9(3):388.
12. Dodds WJ, Groh WJ, Darweesh RM, Lawson TL, Kishk SM, Kern MK. Sonographic measurement of gallbladder volume. Am J roentgenol. 1985;145(5):1009-11.
13. Hofeldt M, Richmond B, Huffman K, Nestor J, Maxwell D. Laparoscopic cholecystectomy for treatment of biliary dyskinesia is safe and effective in the pediatric population. The Am surg. 2008;74(11):1069-72.

r Raaghul C, Rajesh NT, Kanagaraju V, Elangovan B. Gallbladder motility in children with chronic functional abdominal pain. Int J Contemp Pediatr 2019;6:2042-5.

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Original Article

Oral Feeds with or without Dextrose Half Normal Saline for Treatment of Hypernatremia in Breastfed Late Preterm and Term Neonates: A Comparison Study

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Abstract

Aim: The aim of this study was to compare the effect of treatment with oral feeds alone or oral feeds and 5% dextrose in 0.45% normal saline (½ dextrose normal saline [DNS]) on the rate of decline in serum sodium in breastfed late preterm and term neonates with hypernatremia. **Materials and Methods:** The study was conducted using retrospective data from July 2013 to June 2016. Babies with gestational age ≥ 34 weeks, birth weight ≥2000 g, age at diagnosis ≤5 days, and serum sodium 150–160 mEq/L were included in the study. Babies with congenital anomalies, sepsis, prior intravenous (IV) fluid therapy, or diseases-causing dyselectrolytemias were excluded from the study. Oral feeds group (Group 1) received breastfeeds, and formula feeds liberally. Oral feeds and IV fluid group (Group 2) received ½ DNS in addition to liberal oral feeds. Decline in serum sodium ≤0.5 mEq/L/h was considered as safe. **Results:** The study population consisted of 77 babies in Group 1 and 78 in Group 2. Baseline characteristics and serum sodium at admission were comparable. Group 1 had slower decline in serum sodium (P = 0.002), more babies with safe decline in serum sodium (P = 0.02), and lesser weight gain (P = 0.000). A similar result was observed in babies with serum sodium 150–155 mEq/L. No difference in treatment outcome was seen in babies with serum sodium 156–160 mEq/L. No complications or death were seen. About three fourth of cases came for follow-up, and one baby in Group 1 had a developmental delay with seizure disorder. **Conclusion:** In hypernatremic breastfed neonates with serum sodium 150–155 mEq/L, more babies are likely to have a safe decline in serum sodium on treatment with liberal oral feeds alone.

Keywords: *Breastfeeding, hypernatremia, intravenous fluids, oral feeds, rate of decline in serum sodium*

Introduction

Exclusive breastfeeding for the first 6 months of life is recommended for optimal growth and development of infants.[1] However, neonates who receive inadequate milk from their mother, are likely to develop hypernatremic dehydration and malnutrition.[2] Hypernatremic dehydration is a medical emergency and is considered as a dangerous type of dehydration due to the complications associated with disease evolution and its therapy.[3] Until date, there is no consensus on the treatment of hypernatremic dehydration in breastfed neonates. A systematic review of breastfeeding-associated hypernatremia has found that the available data about the management of this condition is inadequate.[4]

The management of hypernatremia in breastfed neonates is clouded with controversies. Although intravenous (IV) fluids were commonly used for hypernatremia correction, the volume of fluid needed and sodium content of the IV fluid was not well defined.[3-6] Oral rehydration

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for correcting hypernatremic dehydration in breastfed babies was also reported. Oral feeds were given either alone or along with IV fluids for correction.[7] However, irrespective of the nature of treatment, all authors uniformly recommend frequent monitoring of serum sodium during correction as the rapid fall in serum sodium is associated with complications.[3,6] The usual recommended rate of fall in serum sodium during correction is not to exceed 0.5 mEq/L/h.[3,6]

In our neonatal unit, oral feeding is allowed liberally to all hypernatremic babies along with 5% dextrose in 0.45% normal saline (½ Dextrose Normal Saline [DNS]). Some babies had received only oral feeds for correction due to the refusal of consent for IV fluid therapy by the parents. This study was planned to compare the outcome among babies treated with oral feeds alone or oral feeds and ½ DNS. The primary objective of this study was to compare the effect of treatment with oral feeds alone or with oral feeds and ½ DNS on the rate of decline in serum sodium in otherwise healthy breastfed late preterm and term neonates with hypernatremia. The secondary objectives were to compare serum sodium at the end of treatment, percentage of weight gain at the end of treatment, complications during therapy, duration of hospital stay, and neurodevelopmental impairment at follow-up.

Materials and Methods

The study was conducted at a tertiary care teaching hospital in Tamil Nadu state of India. The institution has a policy of exclusive breastfeeding for all healthy babies. As per the unit protocol, all babies were weighed daily and any baby with total weight loss of more than 10% from birth weight or weight loss per day of more than 5% were considered to have excess weight loss, and their serum sodium was measured. Serum sodium ≥150 mEq/L was considered as hypernatremia.

All babies with hypernatremia were encouraged to take liberal oral feeds. This consists of counseling and supporting the mothers to adequately breastfeed their babies and to give top up formula feeds (sodium content-7 mEq/L) only if the baby does not get satisfied after completing breastfeeds. Breast milk sodium was not checked in any mother. ½ DNS was used for IV fluid therapy. The volume of IV fluid was based on the severity of dehydration and is usually administered at half of the daily maintenance fluid requirement. The treatment target was to reduce serum sodium below 150 mEq/L.

This comparative study was conducted using the retrospective data collected from July 2013 to June 2016. The Institutional Ethics Committee approved the study. Babies with hypernatremia during the study period, were identified from the hospital database, and their case records scrutinized. Since the response to treatment and complications depends on the severity and duration of hypernatremia, only babies with similar serum sodium level and age at diagnosis were considered for comparison. The inclusion criteria consist of (1) gestational age ≥ 34 weeks, (2) birth weight ≥ 2000 g, (3) age at diagnosis ≤ 5 days, (4) serum sodium 150–160 mEq/L, and (5) treated with oral feeds alone or with oral feeds and ½ DNS. A subgroup analysis for babies with serum sodium of 150–155 mEq/L and 156–160 mEq/L was also planned. The babies, with major congenital anomalies, culture-positive sepsis, received IV fluid within 48 h before the diagnosis of hypernatremia, suspected endocrine disorders or any other significant illness which may affect adequate oral feeding or fluid and electrolyte balance, were excluded from the study.

The following details were noted: maternal age, parity, mode of delivery, gender, gestational age as completed weeks, age at diagnosis as completed days of life, birth weight, weight at diagnosis, feeding problem reported by mother, nature of feed, clinical features, infusion rate of ½ DNS, serum sodium level during treatment, duration of hospital stay, condition of the baby at discharge, and condition at follow-up. The complications such as seizures, acute kidney injury, intracranial bleed, vascular thrombosis, apnea, and bradycardia, were noted.

The total weight loss, weight loss per day, and weight gain after treatment were calculated as a percentage of birth weight. The rate of decline in sodium was calculated with the serum sodium at the beginning and end of the treatment and the time taken to attain it. The rate of decline in serum sodium ≤ 0.5 mEq/L/h was considered as safe decline, and > 0.5 mEq/L/h was considered as nonsafe decline. Acute kidney injury was defined as an increase in serum creatinine by more than 0.3 mg/dl in 24 h or serum creatinine more than 1.5 mg/dl with normal maternal serum creatinine levels. Babies were said to be normal at the time of discharge if their serum sodium was < 150 mEq/L and were taking feeds well. At follow-up, the neurodevelopment of these babies was screened with Trivandrum development screening chart (TDSC), as per the unit protocol. The babies were said to be normal neurologically, if they have attained age-appropriate developmental milestones as per TDSC and had no other neurological problem.

IBM SPSS statistics for Windows- Version 24.0. (Released in 2016. IBM Corp., Armonk, N.Y., USA) was used for analysis. Numerical data were presented as mean ± standard deviation or median ± interquartile

range as appropriate. Categorical data were presented as percentages and analyzed using the Chi-square test. Ordinal data were analyzed with Mann–Whitney U-test or Student's *t*-test. P <0.05 was considered as statistically significant.

Results

During the study period, there were 155 babies satisfying the inclusion and exclusion criteria. Among them, 77 babies were treated with oral feeds alone (Group 1), and 78 babies were treated with oral feeds and ½ DNS (Group 2) [Figure 1]. The baseline characteristics between the two groups were comparable [Table 1]. Serum sodium at diagnosis was comparable between the two groups in analysis of both the whole study population and the subgroups.

In the whole study population, the median rate of sodium decline was significantly slower and within the safer limits in Group 1 babies. About 68% of babies had a safer decline in serum sodium in Group 1 compared to 49% in Group 2, and this difference was statistically significant. Although the median serum sodium at the end of treatment in Group 1 was slightly higher than Group 2, this was not statistically significant. The median weight gain percentage after treatment was significantly less in Group 1 [Table 2].

In babies with serum sodium 150–155 mEq/L, the median rate of sodium decline was within the safer limits in both the groups; however, Group 1 babies had a significantly slower decline compared to Group 2 babies. Babies, with safer decline in serum sodium, were significantly more in Group 1 (73%) than in Group 2 (52%). Median serum sodium at the end of treatment was slightly more in Group 1 compared to Group 2, and this was not statistically significant. The median weight gain percentage was significantly less in Group 1 [Table 3]. Eight (10%) babies in Group 1 left against medical advice due to personal reasons, before achieving targeted serum sodium.

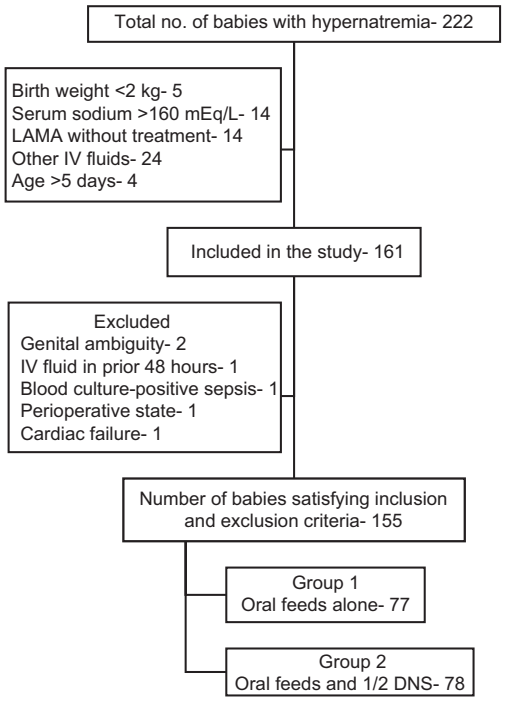


Figure 1: Flow of cases in the study. *½ DNS - 5% dextrose in 0.45% normal saline; IV fluid – Intravenous fluid; LAMA – Left against medical advice

In babies with serum sodium 156–160 mEq/L, there was no difference between the two groups in serum sodium at the end of treatment, number of babies with safer decline in serum sodium, and median weight gain percentage. Although the median rate of sodium decline was significantly higher in Group 1 compared to Group 2, it was in the nonsafe limits in both the groups.

Complications during treatment, were not seen in any baby. The median duration of hospital stay was similar in both the groups. There was no death in the study population. The number of babies followed up was similar in both the groups across the whole study population and in the subgroups. On follow-up, only one female baby in Group 1 had developmental delay, nystagmus, and seizure disorder. This baby's serum sodium at admission was 150 mEq/L, and the rate of sodium decline was 0.32 mEq/L/h. She also had prolonged jaundice requiring phototherapy for 9 days in the neonatal period.

Discussion

In this study, we have found that significantly more number of hypernatremic babies treated with oral feeds had safer fall in serum sodium compared to babies treated with oral feeds and ½ DNS. In one of the early reports on oral rehydration, Ng *et al*. have successfully treated four out of five babies with serum sodium of 151–154 mEq/L using only oral feeds. The authors have commented that their infants were less symptomatic, and oral therapy with breast milk and formula milk have corrected the dehydration in most of their cases.[7] Cagler *et al*. recommend using breast milk or formula milk or both, as the first-line therapy in well-appearing hypernatremic neonates.[8] Greenbaum suggests oral rehydration for mild-to-moderate hypernatremia but cautions that infant formulas, if given with IV fluids, may cause a rapid fall in serum sodium because of their low sodium content.[3]

Erdemir *et al*. have compared oral feeds and IV fluids for correcting hypernatremia in breastfed neonates. Babies with no feeding difficulties, were given breast

Table 1: Baseline characteristics of the compared population

Characteristics	Orals feeds alone (*n*=77)	Oral feeds + ½ DNS (*n*=78)	*P*
Male-to-female ratio	1:1	1.3:1	0.52
Median gestational age (weeks)	38 (37-39) (*n*=74)	38 (37-39) (*n*=77)	0.28
Mean birth weight (g)	3101±0.46	3085±0.44	0.883
Median age at diagnosis (days)	3 (2-3)	3 (2-3)	0.74
Mean weight at admission (g)	2756±412	2733±390	0.713
Median total weight loss (%)	11 (0.1-12.1)	11.3 (10.1-12.4)	0.154
Median weight loss per day (%)	3.9 (3.5-4.5)	4.1 (3.4-5.2)	0.06
Median maternal age (years)	26 (24-29) (*n*=76)	27 (24-30) (*n*=73)	0.34
Primiparity (%)	54 (n=76) (71)	58 (n=75) (77.3)	0.35
Cesarean delivery (%)	33 (42.9)	40 (51.3)	0.95
No feeding problem reported (%)	64 (83.1)	65 (83.3)	0.94
Exclusive breastfeeding (%)	69 (89.6)	70 (89.7)	0.95

Mean is given with ± SD; median is given with interquartile range; ½ DNS – 5% dextrose in 0.45% normal saline; *P*<0.05 is statistically significant. SD – Standard deviation

Table 2: Comparison of treatment outcomes of the whole study population

Characteristics	Oral feeds alone (*n*=77)	Oral feeds + ½ DNS (*n*=78)	*P*
Median serum sodium at diagnosis (mEq/L)	152 (151-154)	153 (151-155)	0.389
Median IV fluid rate (ml/kg/h)	0	3.3 (2.9-5.4)	
Median serum sodium at end of treatment (mEq/L)	147 (143-147)	146 (144-147)	0.000
Median rate of decline in serum sodium (mEq/L/h)	0.36 (0.22-0.63)	0.51 (0.33-0.77)	0.002
Rate of sodium decline and number of babies (%)			
≤0.5 mEq/L	52 (67.5)	38 (48.7)	0.02
0.51-1.0 mEq/L	18 (23.4)	31 (39.7)	0.03
≥1.1 mEq/L	7 (9.1)	9 (11.5)	0.63
Median percentage of weight gain	2.2 (0.9-3.3)	5 (2.5-6.5)	0.000
Median duration of hospital stay (days)	5 (4-5)	5 (4-6)	0.053
Follow-up details			
Number of babies (%)	59 (76.6)	55 (70.5)	
Mean duration (months)	1.5 (0.5-12)	2.5 (0.5-12)	
Neurologically abnormal	1	0	

Mean is given with ± SD; Median is given with interquartile range; ½ DNS – 5% dextrose in 0.45% normal saline; *P*<0.05 is statistically significant. IV – Intravenous; SD – Standard deviation

Table 3: Comparison of treatment outcome of babies with serum sodium 150-155 mEq/L

Characteristics	Oral feeds alone (*n*=67)	Oral feeds + ½ DNS (*n*=65)	*P*
Median serum sodium at diagnosis (mEq/L)	152 (151-153)	152 (151-154)	0.124
Mean IV fluid rate (ml/kg/h)	0	3.3 (2.9-6.1)	
Median serum sodium at end of treatment (mEq/L)	147 (145-149)	145 (143-147)	0.001
Median rate of decline in serum sodium (mEq/L/h)	0.32 (0.22-0.51)	0.46 (0.33-0.73)	0.000
Rate of sodium decline and number of babies (%)			
≤0.5 mEq/L	49 (73.1)	34 (52.3)	0.01
0.51-1.0 mEq/L	14 (20.9)	26 (40)	0.02
≥1.1 mEq/L	4 (6)	5 (7.7)	0.74
Median percentage of weight gain	2.1 (0.9-3.2)	5.05 (2.5-6.5)	0.000
Median duration of hospital stay (days)	5 (3-7)	5 (4-7)	0.103
Follow-up details			
Number of babies	51	45	
Mean duration (months)	1.5 (1-12)	2 (0.5-12)	
Neurologically abnormal	1	0	

Mean is given with ± SD; Median is given with interquartile range; ½ DNS – 5% dextrose in 0.45% normal saline; *P*<0.05 is statistically significant. IV – Intravenous; SD – Standard deviation

milk/formula, and those with poor feeding or vomiting received only IV fluids. At 24 h of therapy, 80% of oral feeds group had a safe decline in serum sodium compared to only 36% in IV fluid group.[9] In this study, a safe decline in serum sodium was seen in 68% of babies on oral feeds alone and in 49% of babies on IV fluids + oral feeds. In Erdemir *et al*. study, among babies with serum sodium 156–168 mEq/L, 69% had a safe decline in serum sodium with oral feeds, and none had safe decline with IV fluids.[9] Whereas in our study, among babies with serum sodium 156–160 mEq/L, the safe decline in serum sodium was seen in about 30% of babies in both the groups. In this study, ½ DNS was used for correction along with oral feeds whereas Erdemir *et al*. have used 1/5 normal saline along with daily maintenance fluids.[9] It was also observed in our study that the percentage of babies with rate of decline in serum sodium ≤ 1 mEq/L/h was almost the same in both groups across all serum sodium ranges.

Oral therapy for hypernatremia correction may be complicated by feed intolerance, necrotizing enterocolitis, and spontaneous intestinal perforation.[10,11] However, all babies in this study tolerated the feeds well. Bolat *et al*., in their study, on the treatment of hypernatremia using IV fluids, have concluded that serum sodium correction rate more than 0.5 mEq/l/h is an independent risk factor for death or convulsion.[12] However, in this study, no death or therapy-related complications were seen in babies with both safe and unsafe decline in serum sodium. Bilgin *et al*. have treated 149 babies with oral feeds or IV fluids depending on the severity, and no death was reported.[13] Similarly, Ozdogan *et al*. have treated 29 babies with oral feeds or IV fluids. There was no death, and all babies were neurologically normal at follow-up.[14] Yaseen *et al*. have treated 27 babies with oral feeds or IV fluids or both. Only one baby on IV fluids had seizures due to fast rehydration and had abnormal neurological examination at follow-up.[15]

On long-term follow up of our study population, only one baby in oral feeds alone group had neurodevelopmental impairment. This baby had safe decline in serum sodium but had a prolonged period of jaundice. It has been found in animal studies that hyperosmolar conditions can disrupt the blood–brain barrier and potentiate the neurotoxic effect of bilirubin.[16] Ergenekon *et al*. have shown that there is no correlation between serum sodium at admission and neurodevelopmental test scores at follow-up.[17] Bolat *et al*. have found no significant difference in neurodevelopmental outcome by the rate of correction of serum sodium.[12]

A recent systematic review by Bischoff *et al*. has found only two retrospective studies reporting the outcome of various therapies for hypernatremia in breastfed neonates, of which one has compared exclusive oral feeds with exclusive IV fluids.[5] We have tried to feed all the babies irrespective of IV fluid administration as babies with breastfeeding-associated hypernatremia are not only dehydrated but also starving.[18] We have shown that oral feeds can be safely given to all clinically stable babies with less severe hypernatremia.

This study has the following limitations. The volume of feeds ingested by the babies was not measured. Although all babies could have been fed with measured volume of expressed breast milk, this was not done since it may interfere with the assessment of breastfeeding and may affect the bonding between the mother and baby. Weighing the baby before and after direct breastfeed might have helped in roughly estimating the volume of milk ingested by the baby.[19] There is a chance for attrition bias in the study due to eight babies in Group 1 leaving the hospital before their serum sodium normalized. Follow-up details were not available for all cases and for one baby with developmental delay identified through TDSC, the diagnosis could not be refined using a confirmatory test such as Bayley scale of infant development. A randomized controlled trial comparing oral feeds with and without IV fluids administration may help to find the correct strategy for managing hypernatremia in otherwise healthy breastfed neonates.

Conclusion

In hypernatremic breastfed neonates with serum sodium 150–155 mEq/L, the treatment with liberal oral feeds alone using breastfeeds and/or formula feeds can cause safe decline in serum sodium in more number of babies compared to babies receiving IV fluids in addition. However, careful monitoring of the serum sodium is needed during hypernatremia correction irrespective of the nature of treatment given. A randomized control trial comparing oral feeds alone with oral feeds and IV fluids are needed to provide concrete evidence.

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Conflicts of interest

There are no conflicts of interest.

References

1. Kramer MS, Kakuma R. The Optimal Duration of Exclusive Breastfeeding-A Systematic Review. Geneva: World Health Organization; 2002.
2. Livingstone VH, Willis CE, Abdel-Wareth LO, Thiessen P, Lockitch G. Neonatal hypernatremic dehydration associated with breast-feeding malnutrition: A retrospective survey. CMAJ 2000;162:647-52.

3. Greenbaum LA. Deficit therapy. In: Kliegman RM, editor. Nelson Textbook of Pediatrics. 20th ed. Philadelphia, PA: Elsevier; 2016. p. 388-90.
4. Lavagno C, Camozzi P, Renzi S, Lava SA, Simonetti GD, Bianchetti MG, *et al*. Breastfeeding-associated hypernatremia: A systematic review of the literature. J Hum Lact 2015;32:67-74.
5. Bischoff AR, Dornelles AD, Carvalho CG. Treatment of hypernatremia in breastfeeding neonates: A systematic review. Biomed Hub 2017;2:454980.
6. Posencheg MA, Evans JR. Acid-base, fluid and electrolyte management. In: Gleason CA, Devaskar SU, editors. Avery's Diseases of the Newborn. 9th ed. Philadelphia, PA: Elsevier Saunders; 2012. p. 367-89.
7. Ng PC, Chan HB, Fok TF, Lee CH, Chan KM, Wong W, *et al*. Early onset of hypernatremic dehydration and fever in exclusively breast-fed infants. J Paediatr Child Health 1999;35:585-7.
8. Cagler MK, Altugan FS, Ozer I. Exclusive breastfeeding and hypernatremic dehydration. Iran J Publ Health 2005;34:1-11.
9. Erdemir A, Kahramaner Z, Turkoglu E, Kanik A, Sutcuoglu S, Ozer EA. Comparison of oral and intravenous fluid therapy in newborns with hypernatremic dehydration. J Matern Fetal Neonatal Med 2014;27:491-4.
10. Parrish CR, Rosner MH. Clinical observations correcting hypernatremia: Enteral or intravenous hydration? Pract Gastroenterol 2014;137:68-72.
11. Huston RK, Dietz AM, Campbell BB, Dolphin NG, Sklar RS, Wu YX. Enteral water for hypernatremia and intestinal morbidity in infants less than or equal to 1000 g birth weight. J Perinatol 2007;27:32-8.
12. Bolat F, Oflaz MB, Guven AS, Özdemir G, Alaygut D, Doğan MT, *et al*. What is the safe approach for neonatal hypernatremic dehydration? A retrospective study from a neonatal intensive care unit. Pediatr Emer Care 2013;29:808-13.
13. Bilgin LK, Akcay F, Altinkaynak K, Altindag H. Hypernatremia in breastfed newborns: A review of 149 cases. J Trop Pediatr 2011;58:332-4.
14. Ozdogan T, Iscan M, Ellikcioglu C, Yildiz E. Hypernatremic dehydration in breast-fed neonates. Arch Dis Child 2006;91:1041-7.
15. Yaseen H, Salem M, Darwich M. Clinical presentation of hypernatremic dehydration in exclusively breast-fed neonates. Indian J Pediatr 2004;71:1059- 62.
16. Wennberg RP, Johansson BB, Folbergova J, Siesjo BK. Bilirubin induced changes in brain energy metabolism after osmotic opening of the blood brain barrier. Pediatr Res 1991;30:473-8.
17. Ergenekon E, Unal S, Gucuyener K, Soysal SE, Koç E, Okumus N, *et al*. Hypernatremic dehydration in the newborn period and long-term follow up. Pediatr Int 2007;49:19-23.
18. Moritz ML. Preventing breastfeeding-associated hypernatremia: An argument for supplemental feeding. Arch Dis Child Fetal Neonatal 2013;98:F378-9.
19. Neifert MR. Prevention of breastfeeding tragedies. Pediatr Clin North Am 2001;48:273-97.

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The Effect of Mode of Delivery on Breastfeeding Outcomes: A Hospital Based Study

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ABSTRACT

Background: Some studies suggested that a high rate of caesarean deliveries results in suboptimal breastfeeding outcomes. The relationship between mode of delivery and breastfeeding outcomes has been considered important given the rising prevalence of caesarean deliveries globally. The present study is to examine the relationship of mode of delivery with breastfeeding outcomes. **Materials and Methods:** This study was conducted at PSG Institute of Medical Science and Research, Coimbatore in the month of July 2018. Ethical clearance was obtained from the institution prior to the study. Chi square test was done to assess the association between socio-demographic variables, timely initiation of breastfeeding and exclusive breastfeeding with mode of delivery. The effect of caesarean on duration of breastfeeding was obtained by applying Kaplan-Meier method. **Result:** Prevalence of caesarean delivery was 36.2% and of vaginal delivery was 63.8%. A statistically significant association was obtained between caesarean deliveries and timely initiation of breastfeeding. However mode of delivery was not significantly associated with exclusive breastfeeding and duration of breastfeeding. **Conclusion:** The findings of our study indicates that caesarean deliveries have been associated with lower timely initiation n rates compared to vaginal deliveries.

Keywords: caesarean delivery, duration of breast feeding, exclusive breastfeeding, initial breastfeeding, vaginal delivery.

Introduction

Breastfeeding is a dynamic process comprising regulation between the mother's and infant's interconnected physiological, psychological, and behavioural systems [1]. Optimal infant and young child feeding is crucial for health and development.

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Women are recommended to breastfeed exclusively for 6 months and to continue breastfeeding until the child is 2 years. Breastfeeding is widely acknowledged as the best and safest form of infant feeding [2].

The global average caesarean delivery rate is 12.4% with an average annual rate of increase of 4.4%.The use of caesarean delivery has increased in the last few decades particularly in middle- and high-income countries [3]. According to a recent analysis of national health survey data, caesarean deliveries in India has gone up from 10% to 30 % raising the country's average caesarean section rates from 5% to 18%. An independent analysis of data from the National Family Health Survey (NFHS) (199293 to 2015-16) and Rapid Survey on Children 2013-14,India's caesarean-section rate was at 18 per cent which was higher than the

Lancet report's estimate of 10 per cent[4]. It is necessary to find whether the mode of delivery affect the breastfeeding outcomes.

The primary aim of the study was to examine the association between mode of delivery and subsequent breastfeeding outcomes – initiation of breastfeeding within one hour of birth, exclusive breastfeeding up to six months and duration of breastfeeding.

Materials and Methods

This study was conducted at PSG Institute of Medical Science and Research, Coimbatore in the month of July 2018. Ethical clearance was obtained from the institution prior to the study. Informed consent was obtained from all mothers interested in participating. Questions about mode of delivery and breastfeeding were collected using questionnaire. A total of 527 mothers were studied. Eligible mother include those who with the children age below four years attending paediatric outpatient department. Babies with congenital anomalies that interfere with breastfeeding were excluded from the study. Socio economic status was measured using Prasad classification. The classification is done based on the Consumer Price Index (CPI) value of 279 for Coimbatore [5]. Class 1 include people having per-capita income greater than or equal to Rs.6180. Class 2, class 3, class 4 include the people having range of pre-capita income Rs.3090-6179, Rs.1850-3089, Rs.930-1849 respectively and class 4 include people having per-capita income less than Rs.930.

WHO recommends that for those children less than six months of age, who fed only on mother's milk in the 24 hours preceding the survey were considered as exclusive breastfeeding.

Chi square test was done to assess the association between mode of delivery with socio-demographic variables, initiation of breastfeeding and exclusive breastfeeding. p value<0.05 was considered as statistically significant.

The effect of caesarean on duration of breastfeeding was obtained by using Kaplan-Meier method. The duration of termination of breastfeeding was the outcome event and those mothers who are continuing the breastfeeding during the survey were considered as censored. Total probability of termination of breastfeeding till that time interval was calculated by multiplying all the probabilities of termination of breastfeeding at all time intervals preceding that time. The log rank test was used to assess the equality of survival curves of vaginal and caesarean delivery. The data were analysed using SPSS[5].

Results

The sample included 527 mothers. 36.2% of mothers were delivered by caesarean and 63.8% of mothers gave birth by vaginal delivery.

Table 1 depicts the various socio-demographic and clinical variables according to mode of delivery. Increased number of antenatal visits (p<0.05) and term delivery (p<0.01) were negatively associated with caesarean delivery.

Table 2 describes the association of mode of delivery with timely initiation of breastfeeding, exclusive breastfeeding and duration of breastfeeding. Timely initiation of breastfeeding was lower among caesarean deliveries compared to vaginal deliveries and the difference was statistically significant (p<0.001).The prevalence of exclusive breastfeeding was 60.1% for vaginal delivery and 55.5% for caesarean. However the difference was not statistically significant (p=0.301).There was no difference between duration of breastfeeding and mode of delivery (p=0.851).

Table1: Distribution of socio-demographic variables by modes of delivery

Characteristics		**Vaginal delivery (%)**	**Caesarean (%)**	**p value**
Mother's age	15-24	76(22.6)	32(16.8)	0.190
	25-34	243(72.3)	145(75.9)	
	35-44	17(5.1)	14(7.3)	
Mother's education	Below 12^{th}	82(24.4)	56(29.3)	0.217
	Above 12^{th}	254(75.6)	135(70.7)	
Father's education	Below 12^{th}	79(23.5)	50(26.2)	0.494
	Above 12^{th}	257(76.5)	141(73.8)	

Number of antenatal visits	>8	253(75.3)	126(66.0)	p<0.05
	≤8	83(24.7)	65(34.0)	
Condition of mother after delivery	Well	319(94.9)	180(94.2)	0.731
	Sick	17(5.1)	11(5.8)	
Gestational age	Term	310(92.3)	159(83.2)	p<0.01
	Pre term	26(7.7)	32(16.8)	
Socio economic status	Class 1&2	273(81.2)	153(80.1)	0.748
	Class 3,4 &5	63(18.8)	38(19.9)	
parity	1	242(72)	123(64)	0.068
	≥2	94(28)	68(35.6)	

Table2: Breastfeeding outcomes by of mode of delivery

Breastfeeding pattern		**Vaginal (%)**	**Caesarean (%)**	**Chi-square**	**P value**
Timely initiation of breastfeeding	Yes	252(75.0)	46(24.1)	1.285	p<0.001
	No	84(25.0)	145(75.9)		
Exclusive breastfeeding	yes	202(60.1)	106(55.5)	1.071	0.301
	no	134(39.9)	85(44.5)		
Duration of breastfeeding	Mean Duration (Months)	18.357	18.680	0.035 *	0.851

*chi-square mentioned as log rank chi-square.

Discussion

We found that 56.5% of mothers surveyed reported initiating breastfeeding within one hour of birth and 58.4% reported exclusive breastfeeding for 6 months. The median duration of breastfeeding was 18 months. We observed a negative association between breastfeeding initiation within one hour and caesarean delivery. This contradicts the findings of Sara N K et al 2017[6] and supports the findings of Prior et al 2012 [7] and Hira Palla et al 2017 [8].

Despite the well-known benefits of nourishing infants exclusively with mother's milk, the rates of breastfeeding initiation, continuation and exclusive breastfeeding are still lower in India. Mothers and infants experiences during labour and delivery may affect lactation and breastfeeding outcomes. In this study we observed that caesarean deliveries have been associated with lower initiation rates compared to undergoing vaginal delivery. This may be due to the delay in mother-infant skin to skin contact, mother's post-surgery physical complications and effects of anaesthesia.

Research evidences showed that delayed onset of lactation is significantly higher among women with caesarean delivery compared to those with vaginal delivery. In addition, early contact with between mothers and infants during the first few hours after birth is important for forming a mother-infant bond that can lead to breastfeeding successful outcomes. Apart from it, operative care routines for post caesarean delivery can delay mothers from holding their infants and disrupt bonding between mother and infant. The mothers who undergo caesarean delivery stay longer in

hospital than mothers who experience vaginal delivery. Therefore a negative impact of surgical procedure delays breastfeeding initiation and babies were used to give formula feed from hospital.We did not find any association between mode of delivery and exclusive breast feeding. This supports the findings of Sara N K et al 2017[6] and contradicts the findings of Emily prior et al [7]. We did not find any association between mode of delivery and duration of breastfeeding which contradict the finding of Perez-Escamilla et al [9] and Amy J. Hobbs [10]. It has been suggested in these studies that lower duration of breastfeeding rates on women with caesarean delivery might be associated with physiologic mechanism that affect lactogens. However, in our study we could not establish a statistically significant association between type of delivery and duration of breastfeeding. The limitation of our study include response bias that contribute inaccurate information due to retrospective reporting. Another limitation is that the region of study has a generally higher socio-economic status and it was hospital based study. Further studies should address the obstacles based on delivery mode and other factors such as medical problems, women's knowledge around breastfeeding benefits, latching, and lifestyle changes on initiation of breastfeeding, exclusive breast feeding and duration of breastfeeding. In addition to mode of delivery, other factors that may influence breastfeeding initiation includes challenges encountered during nursing, availability of support systems, recommendation and attitudes of family, peers and health care professionals. Furthermore, large scale prospective cohort studies can provide more light in this area.

Conclusion

The findings of our study indicates that caesarean deliveries have been associated with lower timely breastfeeding initiation rates compared to mothers undergoing vaginal delivery.

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References

1. Kristin P. Tully, Helen L. Ball. Trade-offs underlying maternal breastfeeding decisions: A conceptual model. Matern Child Nutr. 2013. 9(1): 90–98.
2. Inger Aakre, Anne Marie Lilleengen, Marie LersethAarsand, Tor A.Strand, Ingrid Barikmo and SigrunHenjum. Infant feeding practices in the Saharawi refugee camps Algeria, a cross-sectional study among children from birth to six months of age. International Breastfeeding Journal. 2017.
3. Ana Pilar Betrán, Jianfeng Ye, Anne-Beth Moller, Jun Zhang, A. MetinGülmezoglu, Maria Regina Torloni. The Increasing Trend in Caesarean Section Rates: Global, Regional and National Estimates 1990-2014. 2016.
4. Caesarean section deliveries up from 10% to 30% percent in India. https://www.news18.com/news/lifestyle/health-andfitnesscaesareansectiondeliveries-up-from-10-to-30-percent-in-india-1347492.html. February 10, 2017, 2:05 PM IST
5. Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers. Available from: http://labourbureaunew.gov.in/UserContent/Linking_factor_CPI_IW.pdf?pr_id. Accessed August 10, 2018.
6. Sara N. Kiani, Katherine M. Rich, Darby Herkert, Cara Safon, Rafael Perez Escamilla. Delivery mode and breastfeeding outcomes among new mothers in Nicaragua. Matern Child Nutr. https://doi.org/10.1111/mcn.12. Wileyonlinelibrary.com/journal/mcn. 2017.
7. Emily Prior, Shalini Santhakumaran, Chris Gale, Lara H Philipps, Neena Modi, and Matthew J Hyde. Breastfeeding after cesarean delivery: a systematic review and meta-analysis of world literature. American Journal of Clinical Nutrition.https://doi.org/10.3945/ajcn.111.030254 .2012. 1113–1135.
8. Hira Palla and PanagiotaKitsantas. Mode of delivery and breastfeeding practices. International Journal of Pregnancy & Child Birth.2017.167-172.
9. RafaelPerez-Escamilla, Irene Maulen-Radovan, and Kathryn G. Dewey. The Association between Cesarean Delivery and Breast-Feeding Outcomes among Mexican Women. American Journal of Public Health.1996. 832-836.
10. Amy J. Hobbs, Cynthia A. Mannion, Sheila W. McDonald, Meredith Brockway and Suzanne C.

Tough. The impact of caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. BMC Pregnancy and Childbirth. 2016. 16-90.

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Factors Associated with Timely Initiation of Breast Feeding: a Hospital Based Study

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Abstract

Background: Early or timely initiation of breastfeeding, specifically within 1 h of birth, refers to the best practice recommendation by the World Health Organization (WHO). Timely initiation of breastfeeding has the potential to prevent 22 % of neonatal deaths if all infants were breastfed within an hour after birth [1]. The primary objective of the paper is to estimate the prevalence of breast feeding initiation within first hour of life and the clinical correlates and socio demographic factors. **Material and methods:** This study was conducted at PSG Institute of Medical Science and Research, Coimbatore in the month of July 2018. Mothers with the children age below four years attending paediatric outpatient department were included in the study. Multivariate logistic analysis was usedto determine the factors associated with timely initiation breastfeeding practice. **Results:** The prevalence of timely initiation of breastfeeding within one hour was 56.5%. It was observed that the caesarean delivery, preterm delivery, sick condition of the baby, earlier breast surgery, lack of counselling after delivery and lack of skin to skin contact at birth were negatively associated with timely initiation of breastfeeding within one hour. **Conclusion:** The findings clearly indicated that nearly half of the mothers did not initiate breastfeeding within one hour of birth.

Key Words: breastfeeding, prevalence, sociodemographic factors, timely initiation.

Introduction

Timely initiation of breastfeeding is defined as putting the new born to the breast within one hour of birth [1] .Early or timely initiation of breastfeeding, specifically within 1 h of birth, refers to the best practice recommendation by the World Health Organization (WHO) [1]. Few studies revealed that breastfeeding initiation after the first hour of birth doubles the risk of neonatal mortality [2] and the promotion of breast feeding within one hour is a key component in child survival.

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In addition it has also been shown that a total of 71% of neonatal mortality could be prevented by using antenatal, intra-partum and post natal interventions related to breastfeeding. In addition, many studies reported that the initiation of breast feeding within 24 hours of birth was significantly associated with reduction in 'all cause of mortality'. Recognizing the importance of timely initiation of breastfeeding, the Ethiopian government had developed infant and young child feeding guidelines giving appropriate emphasis to key messages on timely initiation of breastfeeding in 2004. Early initiation of breastfeeding (EIBF) promotes the release of oxytocin that enables contraction of the uterus and decreases postpartum haemorrhage [3]. Though the breast feeding within first hour of life is considered as an indicator of excellence of breast feeding and only 39% of the new born in the

developing countries are put to breast within one hour of birth. According to national family health survey 3, in India, the rate of early initiation of breast feeding stands low at 24.5% [4]. The characteristics associated with the practice have been little investigated. Hence the current aim is to estimate the prevalence of breastfeeding initiation within first hour of life and examine the factors associated with timely initiation of breastfeeding.

Materials and Methods

Study methods and participants

This study was conducted in the month of July 2018 at PSG Institute of Medical Science and Research, Coimbatore. Ethical clearance from the institution was obtained prior to the study. A total of 527 mothers of children under four years were participated in the study. Mothers with the children age below four years attending paediatric outpatient department were included in the study. Babies with congenital anomalies that interfere with breastfeeding were excluded from the study. A structured interviewer administered questionnaire was used to collect data from mothers about timely initiation of breast feeding related with youngest child. The mothers were asked how long after birth their babies were first put to the breast. Initiating breastfeeding within one hour was categorized as "yes", whereas later than one hour was categorized as "no". The socio-demographic characteristics considered were as education of the mother, age of the mother, occupation of the mother, education of the father, socio economic status, gender of the child, gestational age of the child, birth weight of the child, mode of delivery, condition of the child soon after delivery and counselling for breastfeeding after delivery. Modified BG Prasad socioeconomic scale has been inuse for determining the socio-economic status with people having per-capita income greater than or equal to Rs.6180 is included in class 1, class 2 ranging from Rs.3090-6179, class 3 ranging from Rs.1850-3089, class 4 ranging from Rs.930-1849 and below Rs.930 is included in class 5. The classification is done based on the Consumer Price Index (CPI) value of 279 for Coimbatore [5]. Each study participant was adequately informed about the objectives of the study and obtained consent for ensuring their confidentiality.

Statistical methods

The collected data were checked manually for completeness and entered in to SPSS (23) for analysis. Descriptive statistics was used to summarize and analyse the socio-demographic characteristics of the study participants and the prevalence of timely initiation of breast feeding. Proportions were compared by timely initiation of breast feeding using Chi-square test.To determine the factors associated with timely initiation breastfeeding practice, binary logistic regression analysis carried out. Variables that showed significant association with timely initiation of breastfeeding in the bivariate models were entered in a multivariable logistic model. Strength of association was measured using odds ratio and 95 % confidence intervals. p value < 0.05 was considered as statistically significant.

Results

A total of 527 women were studied. The socio-demographic characteristics of children and families were given in Table 1. The prevalence of timely initiation of breastfeeding within one hour was 56.5% (Table 2).

Table 1: Socio demographic characteristics of infants and Families

Characteristics		Number (%)
Infant characteristics Child's age(months) (Mean ± SD)		15.55 ± 11.92
Education of the Mother	Below 12th standard	138(26.2)
	Above 12th standard	389(73.8)
Mother's age (years)	15-24	108(20.5)
	25-34	388(73.6)
	35-44	31(5.9)

Mother's Occupation	House wife	403(76.5)
	Paid worker	124(23.5)
Education of the Father	Below 12th standard	129(24.5)
	Above 12th standard	398(75.5)
Socio economic status	Class 1 & 2	426(80.8)
	Class 3,4 & 5	101(19.2)
Gender of the child	Female	246(46.7)
	Male	281(53.3)
Gestational age of the child	Term	469(89.0)
	Pre term	58(11.0)
Birth weight of the child (gm)	<=2500	126(23.9)
	>2500	401(76.1)
Mode of delivery	Vaginal delivery	336(63.8)
	Caesarean	191(36.2)
Condition of the child	Well	454(86.1)
	Sick	73(13.9)
Counselling after delivery	Yes	326(61.9)
	No	201(38.1)

Table 2: Timely initiation of Breast Feeding (Hours)

Brest feeding in hours	**Number (%)**
Within one hour	298 (56.5)
Between 1-4hr	78(14.8)
After 4hr	151 (28.7)
Total	527 (100)

In logistic regression analysis, (Table 3) it was observed that the timely initiation of breast feeding was lower among caesarean delivery compared to vaginal delivery and the difference was statistically significant ($p<0.001$). The timely initiation of breastfeeding was more when the condition of mother was well as compared to those mothers with sick condition ($p<0.05$). Timely initiation of breastfeeding was lower among mothers having low birth weight children than children having normal birth weight ($p<0.001$). Late initiation of breast feeding was higher for pre- term babies than term babies($p<0.001$). It was also observed that the mothers with the well children tend to initiate breastfeeding within one hour than sick children ($p<0.001$). Increased number of antenatal visits had significantly higher prevalence of timely initiation of breastfeeding than less antenatal visits ($p<0.01$). The timely initiation of breastfeeding was less in mothers who had earlier breast surgery compared to mothers who had no earlier breast surgery ($p<0.05$). It was also observed that counselling about timely initiation of breastfeeding after delivery ($p<0.001$) and skin to skin contact at birth ($p<0.001$) were significantly associated with timely initiation of breastfeeding.

Table 3: Factors associated with timely initiation of breast feeding in univariate analysis

Variable		Total	Feeding started within one hour (%)	Feeding started after one hour (%)	odds ratio	p value
Total		527	298(56.5)	229(43.5)		
Age (mean, SD)		27.72,3.95	27.69,4.06	27.75,3.82		
Age of the mother (years)	35-44	31(5.9)	15(5)	16(7)	1.676	0.403
	25-34	388(73.6)	217(72.8)	171(74.7)	1.238	
	15-24	108(70.5)	66(22.2)	42(18.3)	1	
Education of the mother	Above 12th	389(73.8)	226(75.8)	163(71.2)	0.787	0.228
	Below 12th	138(26.2)	72(24.2)	66(28.8)	1	
Occupation of the mother	Employed	124(23.5)	73(24.5)	51(22.3)	0.883	0.550
	House wife	403(76.5)	225(75.5)	178(77.7)	1	
Mode of delivery	caesarean	191(36.2)	46(15.4)	145(63.3)	9.457	P<0.001
	vaginal	336(63.8)	252(84.6)	84(36.7)	1	
Condition of mother	Sick	28(5.3)	11(3.7)	17(7.4)	2.092	P<0.05
	Well	499(94.7)	287(96.6)	212(92.2)	1	
Use of medication	Yes	66(12.5)	35(11.7)	31(13.5)	1.176	0.538
	No	461(87.5)	263(88.3)	198(86.5)	1	
Religion	others	2(0.4)	2(7.0)	0(0)	0.000	0.513
	Muslim	28(5.3)	18(6.1)	10(4.3)	0.704	
	Christian	48(9.1)	27(9.1)	21(9.1)	0.986	
	Hindu	449(85.2)	251(84.2)	198(86.5)	1	
Gender of the baby	female	246(46.7)	150(50.3)	96(41.9)	0.712	0.055
	male	281(53.3)	148(49.7)	133(58.1)	1	
Birth weight of the baby(Kg) (mean, SD)		2.88,0.56	2.97,0.45	2.76,0.65		
Birth weight of the baby(gram)	<=2500	126(23.9)	53(17.8)	73(31.9)	0.462	P<0.001
	>2500	401(76.1)	245(82.2)	156(68.1)	1	
Gestational age at delivery	Pre term	58(11.0)	12(4.0)	46(20.1)	5.991	P<0.001
	Term	469(89.0)	286(96.0)	183(79.9)	1	
Condition of baby	Sick	73(13.9)	26(8.7)	47(20.4)	2.702	p<0.001
	Well	454(86.1)	272(91.3)	182(79.6)	1	
Education of the father	Above 12th	398(75.5)	232(77.9)	166(72.5)	0.750	0.156
	Below 12th	129(24.5)	66(22.1)	63(27.5)	1	
Parity	>=2	162(30.7)	98(32.9)	64(27.9)	.792	0.223
	1	365(69.3)	200(67.1)	165(72.1)	1	
Past experience in breast feeding	No	366(69.4)	200(67.1)	166(72.5)	1.291	0.184
	Yes	161(30.6)	98(32.9)	63(27.5)	1	
Antenatal visits	>8	379(71.9)	231(77.5)	148(64.6)	1	p<0.01
	≤8	148(28.1)	67(22.5)	81(35.4)	1.887	
Earlier Breast surgery	Yes	6(1.1)	1(0.3)	5(2.2)	6.629	p<0.05
	No	521(98.9)	297(99.7)	224(97.8)	1	

Counselling during antenatal visits	No	268(50.9)	146(49)	122(53.3)	1.187	0.330
	Yes	259(49.1)	152(51)	107(46.7)	1	
Counselling after delivery	No	201(38.1)	82(27.5)	119(52)	2.85	p<0.001
	Yes	326(61.9)	216(72.5)	110(48)	1	
skin to skin contact at birth	No	311(59.0)	132(44.3)	179(78.2)	4.502	p<0.001
	Yes	216(41.0)	166(55.7)	50(21.8)	1	
Socio-economic status	Class 5	4(0.8)	1(0.3)	3(1.3)	3.649	0.498
	Class 4	25(4.7)	12(4)	13(5.7)	1.318	
	Class 3	72(13.7)	42(14.1)	30(13.1)	0.869	
	Class 2	180(38.0)	122(36.2)	78(31.4)	0.811	
	Class 1	246(42.5)	135(45.3)	111(48.5)	1	

Inthe multivariate logistic regression analysis (Table 4)it was observed that caesarean delivery (p<0.001), preterm delivery (p<0.01), sick condition of the baby (p<0.01), earlier breast surgery (p<0.01), lack of counselling after delivery (p<0.001) and lack of skin to skin contact at birth (p<0.05) were negatively associated with timely initiation of breastfeeding within one hour.

Table 4: Factors associated with delayed initiation of breast feeding in multivariate analysis

Variables		**Adjusted Odds ratio**	**95% CI**	**p value**
Mode of delivery	caesarean	7.908	4.905-12.748	p<0.001
	vaginal	1		
Condition of mother	Sick	2.189	0.814-5.888	0.121
	Well	1		
Gestational age at delivery	Pre term	3.344	1.447-7.728	p<0.01
	Term	1		
Condition of the baby	Sick	2.943	1.280-4.858	p<0.01
	Well	1		
Antenatal visits	≤8	1.406	0.869-2.275	0.165
	>8	1		
Earlier breast surgery	Yes	20.378	1.962-211.695	p<0.01
	No	1		
Counselling after delivery	No	2.626	1.676-4.115	p<0.001
	Yes	1		
skin to skin contact at birth	No	1.847	1.144-2.983	p<0.05
	Yes	1		
Birth weight of the baby	>2500	0.736	0.428-1.265	0.267
	<=2500	1		

Discussion

Globally, around four million new born die, most from preventable causes each year. Death in the neonatal period accounts for 41 % of all deaths in children below four years. Timely initiation of breastfeeding can help to prevent neonatal deaths caused by infections such as sepsis, pneumonia and diarrhoea[6].In India, a multipronged strategy including large scale programmes, effective capacity building initiatives, strong partnerships, community-

based action, and strategic mass media communication led to an increase in the rates of early initiation of breastfeeding from 24.5% in 2006 to 44.6% in 2014 (i.e. a 1.8-fold increase) [7]. More recently, analyses on a large cohort of almost 100 000 new born from three large trials in Ghana, India and Tanzania has shown that, compared with infants who initiated breast feeding within the first hour of life, the risk of neonatal death among children who initiated breast feeding between 2 and 23 hours after birth was 41% higher (RR 1.41; 95% CI 1.24 to 1.62), and 79% higher among those who initiated breast feeding at 24–96 hours of birth (RR 1.79; 95% CI 1.39 to 2.30) [7].

In this study, caesarean delivery was associated with delayed breastfeeding initiation compared to vaginal delivery. Caesarean delivery is the most consistent predictor of late initiation of breastfeeding reported by many studies conducted in Ethiopia and abroad [8]. Mothers might be uncomfortable to breastfeed due to the pain experienced after surgery and the related factors associated with caesarean delivery.

It was observed that the gestational age of the child is a predictor of timely initiation of breastfeeding. The timely initiation of breastfeeding was significantly lower in preterm children. This result may be explained by the common problems, such as limited oral-motor skills, delayed lactogenesis in prematurely delivered mothers and babies and babies were used to give formula feed from hospital. Thus, effective antenatal care to prevent premature births will contribute to early breastfeeding initiation. The observational prospective study conducted on 500 new born delivered at Department of Rajkiya Mahila Chikitsalaya (RMC), Ajmer and out born section of Department of paediatrics, JLN MC, Ajmer reported that preterm infants had significantly lower rate of early initiation of breastfeeding than term infants [9].

Furthermore, condition of the baby was significantly associated with timely initiation of breastfeeding. It was observed that sick condition of the baby adversely affect the initiation of breastfeeding within one hour. Babies who may have sick at birth include those who experienced a difficult delivery, jaundice after delivery, those with birth defects, those born prematurely and these problems can lead to late initiation of breastfeeding. Fortunately for these babies special care is available. New born babies who need intensive medical attention are often admitted in to Neonatal intensive care unit (NICU) for hours after delivery. So mothers failed to initiate breastfeeding in the first hour after delivery.

Earlier breast surgery was found to be statistically significant with timely initiation of breastfeeding. Women undergo breast surgery for many reasons. Augmentations, reductions, mastectomies, lumpectomies, and biopsies, are often carried out on women of childbearing age.

These surgeries can adversely affect timely initiation of breastfeeding and the ability to make healthy breast milk supply for a child.

In addition we have also observed a significant association between timely initiation of breast feeding and counselling about timely initiation after delivery. Counselling about timely initiation has a major impact on timely initiation of breastfeeding. The findings suggest that more efforts are needed to create awareness in providing counselling for the timely initiation during antenatal visits and delivery time. This might be related to the fact that mothers received breastfeeding information/counselling immediately after delivery which is the most appropriate time for delivering key messages about breastfeeding[10].

Baby put on mother's abdomen (skin to skin contact between mother and baby) is found to be an important factor to initiate breast feeding early. WHO recommends that early skin to skin contact between mother and baby helps to initiate early breast feeding.

Birth weight of the child is a predictor in the association of timely initiation of breast feeding in univariate analysis. Socio economic status did not have any association with timely initiation of breast feeding. Previous studies showed that initiations of breastfeeding in low birth weight children were significantly delayed [9]. Also, here educational status of mother did not have any significant association with timely initiation of breast feeding ($p>0.05$). Prior findings showed that education was found to have predominant importance for timely initiation of breastfeeding. Parity and initiation of breast feeding were found to have a lack of association in this study.

This study involves several limitations. One limitation is the recall bias caused by difference in the accuracy or completeness of the recollections retrieved by study participants regarding the breastfeeding practice from the past (retrospective reporting). Another limitation is that the region of study has a generally higher socio-economic status and it was a hospital based study. Community based study program is an approach that emphasize on community empowerment as an important tool in health promotion. Even though the study has several strengths. We have obtained a large number of demographic and clinical data which can influence the association of timely initiation of breastfeeding. All respondents are asked the same basic questions in the same order by the same interviewer to minimize the potential observer bias. Advanced statistical analysis was used to analyse the association

of socio demographic and clinical correlates with timely initiation of breastfeeding.

Conclusion

This study revealed that the prevalence of timely initiation of breastfeeding was significantly lower than the present WHO recommendations [11]. The findings clearly indicate that appropriate strategies are needed to minimize delays in initiation of breastfeeding and for practising better timely initiation of breastfeeding within one hour of birth.

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References

1. World Health Organization, UNICEF." Indicators for assessing infant and young child feeding practices" part 3: country profiles. Geneva: WHO. Available at http://apps.who.int/iris/bitstream/handle/10665/44368/%20 9789241599757_eng.pdf;jsessionid=47902C5018B04906526317E6DB2699D7?sequence=1.
2. AnimutAlebel, GetiyeDejenu, GetachewMullu et al. Timely initiation of breastfeeding and its association with birth place in Ethiopia: a systematic review and Metaanalysis,International Breastfeeding Journal 2017;12-44
3. Khanal V, Scott J, Binns CW. Factors associated with early initiation of breastfeeding in Western Nepal, International journal of environment research and public health. 2015;12(8):9562-9574.
4. Manthan Patel, Shetal Prajapti. A comparative analytical study of knowledge, attitude and practice of breastfeeding in pri,I and multipara women at a tertiary care centre in Gujarat, India, International journal of research in medical science. 2016;4(10):4403-4407.
5. Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers. Available from: http://labourbureaunew.gov.in/UserContent/Linking_factor_CPI_IW.pdf?pr_id. Accessed August 10, 2018.
6. Ekubay M, Berhe A, Yism E. Initiation of breastfeeding within one hour of birth among mothers with infants younger than or equal to 6 months of age attending public health institutions in Addis Ababa, Ethiopia Int Breastfeed J. 2018; 13
7. Víctor M Aguayo, Gagan Gupta, Gayatri Singh et al. Early initiation of breast feeding on the rise in India,BMJ Glob Health2016; 1(2): e000043.
8. Abdul basit Musa Seid. Vaginal Delivery and Maternal Knowledge on Correct Breastfeeding Initiation Time as Predictors of Early Breastfeeding Initiation, Lesson from a Community-Based Cross-Sectional Study. ISRN Epidemiology. 2014, 3–5.
9. Navneet Badaya,Sanjiv Jain, Nayan Kumar. Time of initiation of breastfeeding in various modes of delivery and to observe the effect of low birth weight and period of gestation on initiation of breastfeeding,Int J Contemp Pediatr 2018;5(4): 1509-1517.
10. Gelete BW, Addisu YK, Haile MT. Timely Initiation of Breastfeeding and Its Associated Factors among Mothers in Tiyo Woreda, Arsi Zone, Ethiopia: A Community- Based Cross Sectional Study,Clinics Mother Child Health. 2016;13-221.
11. Tiwari, Bharadva, Yadav, Malik, Gangal, et al. Infant and Young Child Feeding Guidelines, Indian paediatrics. 2016; 53:703-713.

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RESEARCH PAPER

Pediatric Appropriate Evaluation Protocol for India (PAEP-India): Tool for Assessing Appropriateness of Pediatric Hospitalization

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Objectives: To develop and assess Pediatric Appropriateness Evaluation Protocol for India (PAEP-India) for inter-rater reliability and appropriateness of hospitalization.

Design: Cross-sectional study.

Setting: The available PAEP tools were reviewed and adapted for Indian context by ten experienced pediatricians following semi-Delphi process. Two PAEP-India tools; newborn (≤28 days) and children (>28 days-18 years) were developed. These PAEP-India tools were applied to cases to assess appropriateness of admission and inter-rater reliability between assessors.

Participants: Two sets of case records were used: (*i*) 274 cases from five medical colleges in Delhi-NCR [≤28 days (*n*=51); >28 days to 18 years (*n*=223)]; (*ii*) 622 infants who were hospitalized in 146 health facilities and were part of a cohort (*n*= 30688) from two southern Indian states.

Interventions: Each case-record was evaluated by two pediatricians in a blinded manner using the appropriate PAEP-India tools, and 'admission criteria' were categorized as appropriate, inappropriate or indeterminate.

Main outcome measures: The proportion of appropriate hospitalizations and inter-rater reliability between assessors (using kappa statistic) were estimated for the cases.

Results: 97.8% hospitalized cases from medical colleges were labelled as appropriate by both reviewers with inter-rater agreement of 98.9% (k=0.66). In the southerm Indian set of infants, both reviewers labelled 80.5% admissions as appropriate with inter-rater agreement of 96.1% (*k*= 0.89).

Conclusions: PAEP-India (newborn and child) tools are simple, objective and applicable in diverse settings and highly reliable. These tools can potentially be used for deciding admission appropriateness and hospital stay and may be evaluated later for usefulness for cost reimbursements for insurance proposes.

Keywords: *Bed use, Cost, Hospital stay, In-patient, Utilization*.

Efficient and rationale allocation of health resources in India and other developing countries is essential. The recent National Health Policy of India and Ayushman Bharat Yojana aim at healthcare universalization and improving both out-patient and in-patient accessibility [1,2]. Hospitalizations consume a major proportion of healthcare resources in India and 50.9% of the resources are utilized for secondary (34.8%) and tertiary (16.1%) level services [3]. There is severe shortage of in-patient beds in India (0.7 beds/1000 population vs. world average of 3.96) with long waiting period for hospitalization particularly at government hospitals [4]. Standardizing admission and discharge processes has improved utilization, patient-flow and waiting time in several countries, and appropriateness evaluation protocols (AEP) are in wide use [5-7]. At present no Pediatric

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Appropriateness Evaluation Protocol (PAEP) tool is available for use in India.

We describe the development and pilot-testing of the PAEP-India tool to determine the inter-rater reliability for appropriateness of two sets of hospital admissions: (*i*) 274 in-patients from five medical colleges from Delhi and surrounding states and (*ii*) 622 hospitalized infants from a southern Indian cohort.

METHODS

Development of PAEP Tool

The tool development process used semi-Delphi technique [8]. The PAEP tool adaptation for India (PAEP-India) process followed the steps: (*i*) an expert group of ten experienced pediatricians from ten medical colleges, each with >10 years of clinical experience was constituted considering the differences in symptomology, diseases and threshold for hospitalization, the experts felt need for developing separate tools for newborns (≤28 days) and children (>28 days-18 years); (*ii*) review of the available literature was done and seven PAEPs (from 6 high- and 3 middle-income countries) were sourced [9-19]; (*iii*) two rounds of review and pilot testing of the tools was done by the experts; (*iv*) a face-to-face meeting was held for finalization of tools.

Each tool has two sections; 'admission criteria' to assess the appropriateness at admission and 'day of care' criteria for the appropriateness of hospitalization duration. For the PAEP- India (child) tool, the group made some amendments in both the admission criteria and day of care criteria for children (***Box* 1**).

The PAEP-India (newborn) tool was drafted with reference to the available management protocols for newborns [21-23]. The group did not suggest any change in the draft and finalized it. Finally, 'admission criteria' section comprised of 44 items in both the tools, and the 'day of care' for newborn and child tools comprised of 27 and 29 items, respectively (***Web Annexure* I** and **II**).

The group of ten pediatricians who participated in the development of the PAEP-India tools were invited as raters. Five pairs of raters were made and each pair was assigned the same set of case sheets in a blinded manner; raters in a pair were not from the same institution and did not know the other member of their pair. All the raters underwent orientation to have common understanding of using the PAEP reviewers' manual.

We had two sets of case-records drawn from different settings for inter-rater reliability assessment: 274 pediatric cases records drawn from five medical colleges located in Delhi and surrounding states (admitted during July-September 2015), and 622 cases-records from Kollam and Coimbatore, who were part of a cohort of 30688 infants recruited and followed-up in another study [24]. The case-records were anonymized and assigned unique study numbers.

The Ethics Committees of participating Institutes reviewed and approved the study. Waiver of informed consent was granted for data collection from the case records from five medical colleges. Informed consent was obtained for recruiting the infant cohort from Kollam and Coimbatore.

Statistical analysis: Inter-rater agreement was evaluated by the Cohen's kappa (*k*) statistic [25]. Landis and Koch guidelines were adopted as benchmark scales of κ coefficients (moderate: 0.41-0.60, substantial: 0.61-0.80, and almost perfect: 0.81-1.0) [26]. Statistical analyses was performed using STATA version 15.0 (StataCorp LLC, Texas, USA). Overall agreement was the proportion of judgements in which two raters agreed on categorizing as appropriate, inappropriate and indeterminate. We assessed the inter-rater reliability only for the 'admission criteria'; the 'day of care criteria' section could not be evaluated as complete clinical, nursing and laboratory assessment records were not available for most of the days in most of the case sheets in both datasets.

Box 1 ADMISSION CRITERIA AND DAY OF CARE CRITERIA FOR DEVELOPMENT OF PAEP-INDIA*(CHILD) TOOL

- "severity of illness" section, "any fever for >48 hours when a diagnosis has not been reached" was revised to ">72 hours".
- Considering accidents, "burns/inhalational injury" and "exposure to poison and snake/ scorpion bite" were added.
- Under the "severe electrolyte/acid base/ hematological abnormality" section, "hypocalcaemia", "raised creatinine", "thrombocytopenia", "increased respiratory rate" were added, modified "total leukocyte count cut-off to <5000/ mm^3" and added "raised diastolic blood pressure" to hypertension.
- Under the "intensity of services" section, revised "nebulisation use at least every 4 hours".
- Day of care criteria: added "lack of suitable care taker availability (for abandoned child)/protected place" under "patient condition" section; and clarified "unstable vitals in last 48 hours" under the "within 48 hours of the day reviewed" section.

* *PAEP-India: Pediatric Appropriateness Evaluation Protocol*

TABLE I CLINICAL DIAGNOSES IN THE TWO DATA SETS USED FOR INTER-RATER AGREEMENT FOR APPROPRIATENESS OF ADMISSION USING PAEP-INDIA TOOLS

Characteristic	*Delhi and Surroundings (n=274)*	*Kollam and Coimbatore (n=622)*
Children >28 d -18 y		
Infections	122	524
Acute respiratory infections	23	427
Acute gastroenteritis	26	33
CNS infections	20	
Urinary tract infections	3	19
Acute febrile illness[@]	28	25
Other infections[#]	22	20
Congenital diseases[€]	4	26
Other systemic diseases	43	68
Seizure/CNS disorders	15	25
Other medical disorders*	28	43
Surgical conditions	54	4
Gastrointestinal	19	4[^]
Urological	12	0
Other surgical conditions	23	0
Neonates (<28 d)		_
Medical problems	42	_
Neonatal sepsis	24	_
Other medical disorders[$]	18	_
Surgical conditions	9	_

*[@]Acute febrile illness including malaria, dengue, enteric fever, urinary tract infection, and other for evaluation; [#]Multiple system infection involves infection of more than one organ, may be with features of sepsis; [€]Congenital diseases including cardiac and central nervous system (CNS) malformations and other parts; *Other medical conditions including malignancy, coagulopathy, severe acute malnutrition, drug reaction, poisoning, constipation, nephrotic syndrome and hematemesis; [^]All 4 children had intussusception; [$]Other medical conditions in neonates included preterm care, respiratory distress syndrome, hyperbilirubinemia, hypo/hyper-glycemia, and birth asphyxia.*

RESULTS

The age strata of 274 case-records from Delhi and surrounding states were: ≤28 days (*n*=51), >28 days-12 months (*n*=48), 13-59 months (*n*=67), and >5-18 years (*n*=108). There were 54 surgical cases (20.8%). Out of 622 cases from southern India cohort, 471 (75.7%) were from Kollam and 151 (24.3%) were from Coimbatore. The median age at entry and exit were 48 days (range 32-175 days) and 153 days (range: 67-562 days), respectively. There were equal proportion of boys (50.3%) and girls (49.7%) in the cohort (***Table* I**). Out of 622 cases hospitalized to 146 hospitals, 50% (*n*=311) were admitted to tertiary (level 3), 49% (*n*=304) to secondary (level 2) and 1% (*n*=7) were admitted to primary care (level 1) care facilities respectively.

TABLE II INTER-RATER AGREEMENT FOR APPROPRIATENESS OF HOSPITALIZATION USING PAEP-INDIA TOOLS

Reviewer 1	*Reviewer 2*		
	Appropriate	*Inappropriate*	*Indeterminate*
Medical colleges in Delhi and surrounding areas (n=274)			
Appropriate	268	0	1
Inappropriate	2	1	0
Indeterminate	0	0	2
Hospitals in Kollam and Coimbatore (infants) (n=622)			
Appropriate	480	13	0
Inappropriate	9	68	2
Indeterminate	0	0	50

Kappa (*k*) coefficient was 0.66 (95% CI 0.30, 1.0) for the overall dataset for hospitalized cases in medical college. Both raters categorized 97.8% admissions as appropriate. The observed inter-rater agreements were >98% for aggregated and the disaggregated data according to age and gender. The agreement for PAEP appropriate cases and inappropriate cases were >98% with values of 0.66 and 0.49, respectively (***Table* II**).

The *k* for overall dataset was 0.89 (95% CI 0.84, 0.93) for the Kollam and Coimbatore infants. Overall both raters categorized 80.5% admissions as appropriate. The appropriate admissions in public and private hospitals were 84.5% and 78.5%, respectively. The observed agreement was >90% in most categories except for the level 1 hospitals (85.7%) which had just seven admissions.

DISCUSSION

This is the first effort to develop a tool for assessing the appropriateness of pediatric admission and hospitalization duration in India. The PAEP-India tools performed well for admission appropriateness assessment, both for newborns and children and across different levels of hospitals.

In the absence of a gold standard and true valid measure of appropriateness, the consensual validity is reflected through inter-rater agreement. Studies on admission appropriateness using the PAEP tools in different countries have reported variable levels of both observed agreement and kappa statistic (0.29-0.89) [9-12,14,16,19]. With PAEP-India tools, observed agreements were uniformly high with both the datasets.

WHAT IS ALREADY KNOWN?

- Country- and context-specific Pediatric Appropriateness Evaluation Protocol (PAEP) tools are in use for rational use of in-patient facilities.

WHAT THIS STUDY ADDS?

- India-specific PAEP tools for the assessment of appropriateness of pediatric and newborn hospitalizations were developed and tested for reliability with two sets of hospitalized children.

Despite a high observed agreement, lower kappa values may be observed, when the marginal values are imbalanced. On the contrary, higher kappa value may be observed for asymmetrical imbalanced marginal totals. Kappa is affected by prevalence and may not be reliable for rare observations. Thus very low kappa values may not necessarily reflect low overall agreement. Decision on performance of a tool should also consider the observed *versus* expected agreement, consistency across contexts, and suitability of the criteria for specific settings besides kappa statistics [27,28].

The reported proportion of appropriate pediatric admissions reported in literature range between 68%-89.5% and 59.3%-98% in high- and middle-income countries, respectively [9-12,14-16,19]. The proportion of appropriate admissions in the present study was high, particularly in medical colleges, in view of the higher demand and pressure for admission. A previous study reported that one-third of the adult patients overstay in hospitals [29]; which was triangulated by the perceptions of 83% of resident doctors and 43% of nurses in another study [30].

Awareness of the raters about the source of cases in first dataset might have influenced the high expected and observed agreements (>90%). For the second dataset (southern Indian infant cohort), the raters were neither aware about the hospitals nor involved in the study implementation or patient care. We could not assess the appropriateness of the duration of hospitalization due to lack of necessary information at the time of discharge. In the first dataset, the experts involved in development of PAEP-India tools also applied the tools in cases drawn from some medical colleges where they worked. These factors might have increased observed agreement and categorization of cases as appropriate. The findings for the level one health facilities from southern India may not be generalized as the number of hospitalizations were too small.

In conclusion, the PAEP-India tools performed consistently in two different settings demonstrating consensual validity. The advantages of the PAEP-India tools is their simplicity, objectivity and applicability in different hospitals. Further application and evaluation of these tools is required in diverse settings across India including health facilities of all levels for triangulating the evidence of its utility. Meanwhile the PAEP-India tools have potential application in insurance systems, quality assessment processes, and resource-allocation.

Contributors: MKD, NKA: planning, tool development, data analysis, manuscript writing; RP: data analysis, manuscript review; AS, AA, APD, PCG, GG, AM, AKG, AC, SA, AU, TM, RKP, JM, SM, SR: tool development, data collection, manuscript review; MG, MKA, CGM, PLFZ: planning, technical inputs, manuscript review.

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REFERENCES

1. Ministry of Health and Family Welfare, Government of India. Ayushman Bharat Yojana (2018-2022). Government of India; 2018. Available from: *https://www.pradhanmantriyojana.in/ayushman-bharat-yojana/*. Accessed March 26, 2018.
2. Ministry of Health and Family Welfare, Government of India. National Health Policy, 2017. Government of India; 2017. Available from: *https://mohfw.gov.in/sites/default/files/9147562941489753121.pdf.* Accessed July 19, 2017.
3. National Health Systems Resource Centre. National Health Accounts Estimates for India (2013-14). Ministry of Health and Family Welfare Government of India; 2016. Available from:*https://mohfw.gov.in/sites/default/files/89498311221471416058.pdf.* Accessed July 19, 2017.
4. Planning Commission (Government of India). Report of the Working Group on Tertiary Care Institutions for 12th Five Year Plan 2012-2017. Government of India; 2011. Available from: *http://planningcommission.nic.in/aboutus/committee/wrkgrp12/health/WG_2tertiary.pdf.* Accessed July 19, 2017.
5. Ortiga B, Salazar A, Jovell A, Escarrabill J, Marca G, Corbella X. Standardizing admission and discharge processes to improve patient flow: A cross sectional study. BMC Health Serv Res. 2012;12:180.
6. Strumwasser I, Paranjpe NV, Ronis DL, Share D, Sell LJ. Reliability and validity of utilization review criteria. Appropriateness evaluation protocol, standardized

medreview instrument, and intensity-severity-discharge criteria. Med Care. 1990;28:95-111.
7. Kemper KJ, Fink HD, McCarthy PL. The reliability and validity of the pediatric appropriateness evaluation protocol. QRB Qual Rev Bull. 1989;15:77-80.
8. McMillan SS, King M, Tully MP. How to use the nominal group and Delphi techniques. Int J Clin Pharmacy. 2016;38:655-62.
9. Werneke U, Smith H, Smith IJ, Taylor J, MacFaul R. Validation of the paediatric appropriateness evaluation protocol in British practice. Arch Dis Child. 1997;77:294-8.
10. Esmail A. Development of the paediatric appropriateness evaluation protocol for use in the United Kingdom. J Public Health Med. 2000;22:224-30.
11. Esmaili A, Seyedin H, Faraji O, Arabloo J, Qahraman Bamdady Y, Shojaee S, *et al*. A pediatric appropriateness evaluation protocol for Iran children hospitals. Iran Red Crescent Med J. 2014;16:e16602.
12. Smith HE, Sheps S, Matheson DS. Assessing the utilization of in-patient facilities in a Canadian pediatric hospital. Pediatrics. 1993;92:587-93.
13. Gloor JE, Kissoon N, Joubert GI. Appropriateness of hospitalization in a Canadian pediatric hospital. Pediatrics. 1993;91:70-4.
14. Henley L, Smit M, Roux P, Zwarenstein M. Bed use in the medical wards of Red Cross War Memorial Children's Hospital, Cape Town. S Afr Med J. 1991;80:487-90.
15. Shafik MH, Seoudi TMM, Raway TS, Al Harbash NZ, Ahmad MMA, Al Mutairi HF. Appropriateness of pediatric hospitalization in a general hospital in Kuwait. Med Princ Pract. 2012;21:516-21.
16. Kreger BE, Restuccia JD. Assessing the need to hospitalize children: Pediatric appropriateness evaluation protocol. Pediatrics. 1989;84:242-7.
17. Werneke U, MacFaul R. Evaluation of appropriateness of paediatric admission. Arch Dis Child. 1996;74:268-73.
18. Bianco A, Pileggi C, Trani F, Angelillo IF. Appropriateness of admissions and days of stay in pediatric wards of Italy. Pediatrics. 2003;112:124-8.
19. Formby DJ, McMullin ND, Danagher K, Oldham DR. The appropriateness evaluation protocol: application in an Australian children's hospital. Aust Clin Rev. 1991;11:123-31.
20. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? Lancet. 2003;361: 2226-34.
21. Agarwal R, Deorari A, Paul VK. AIIMS Protocols in Neonatology. 1st ed. New Delhi: CBS Publishers; 2015.
22. National Neonatology Forum, India. Evidence based clinical practice guidelines. National Neonatology Forum; 2010. Available from: *http://www.nnfi.org/*. Accessed July 19, 2017.
23. Ministry of Health and Family Welfare, Government of India. Facility based new born care training packages. Government of India; 2014. Available from: *http://nhm.gov.in/nrhm-components/rmnch-a/child-health-immunization/child-health/guidelines.html*. Accessed April 16, 2016.
24. World Health Organization. Global Advisory Committee on Vaccine Safety, 15-16 June 2016. Wkly Epidemiol Rec. 2016;91:341-8.
25. Cohen J. A Coefficient of agreement for nominal scales. Educ Psychol Meas. 1960;20:37-46.
26. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159-74.
27. Feinstein AR, Cicchetti DV. High agreement but low kappa: I. The problems of two paradoxes. J Clin Epidemiol. 1990;43:543-9.
28. Cicchetti DV, Feinstein AR. High agreement but low kappa: II. Resolving the paradoxes. J Clin Epidemiol. 1990;43:551-8.
29. Chakravarty A, Parmar N, Bhalwar R. Inappropriate use of hospital beds in a tertiary care service hospital. Med J Armed Forces India. 2005;61:121-4.
30. Kanwar V, Salaria N, Khanduja P. How do nurses perceive hospital bed utilization: A prerequisite for quality improvement in nursing care. Int J Health Sci Res. 2014;4135-41.

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Original Article

MII RetCam assisted smartphone based fundus imaging for retinopathy of prematurity

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Purpose: The gold standard for evaluating Retinopathy of prematurity (ROP), a potentially blinding disease in preterm babies, is by indirect ophthalmoscopy which is quite subjective. Digital imaging with RetCam, an advanced wide field imaging system is more precise but it is not easily available or affordable. Smartphones are being explored as an alternate cost effective and accessible imaging tool. This is possible because of the good illumination and the built-in high resolution cameras available in modern smartphones. The aim of this study is to illustrate the utility of MII RetCam assisted smartphone based fundus imaging (MSFI) in the documentation and monitoring of ROP. **Methods:** Single-centre, retrospective observational study of all the preterm babies subjected to MSFI as part of ROP screening from September 2017 to November 2018. iPhone 4S and + 20 Diopter lens attached to the MII RetCam device was used for fundus imaging at baseline and during follow up. The statistical analysis used for the same is the SPSS statistical software. **Results:** Good quality images of central and peripheral retina could be captured in 33 out of 42 babies (78.57%) with ROP. Serial imaging done in 24 babies with ROP helped in precise monitoring of the disease and planning management. Incidentally detected non-ROP findings were also documented. Unique design of the device enabled imaging by a single examiner. Image database created was useful for academic and counselling purposes. Smaller field images which can cause difficulty in distinguishing the zones is a limitation. **Conclusion:** MSFI is a potential alternate imaging tool enabling objective documentation and monitoring of ROP in low resource settings.

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Key words: Fundus imaging, MII RetCam, retinopathy of prematurity, smartphone

The gold standard for evaluating Retinopathy of prematurity (ROP)—a potentially blinding disease in preterm babies—is by indirect ophthalmoscopy which is quite subjective.[1] Digital documentation with RetCam (Natus USA, formerly Clarity, MSI, USA) is more accurate but expensive.[2-4] Smartphones equipped with good illumination and high-resolution cameras have emerged as an alternate, affordable and reliable imaging tool.[5-7] Smartphone-based fundus imaging utilizing an innovative device "Make In India (MII) RetCam" has been found to achieve good quality imaging of central and peripheral retina.[8,9] We aim to demonstrate the utility of MII RetCam assisted smartphone-based fundus imaging (MSFI) in the documentation and monitoring of a peripheral retinal pathology like ROP, in resource limited settings.

Methods

This is a single-centre, retrospective observational study of the utility of MSFI done as part of the ROP screening from September 2017 to November 2018. Babies were referred for ocular evaluation by Neonatologist as per the screening guidelines for ROP.[10] All examinations were done by a single ophthalmologist trained in ROP (TL). Examination was done either in the ophthalmology department or in the neonatal intensive care unit (NICU). All babies remained awake during the procedure and a trained nurse assisted to hold the baby and keep the head steady. Mydriasis was achieved with Tropicamide (0.5%) with Phenylephrine (2.5%) eye drops and binocular indirect ophthalmoscopy (BIO) with scleral depression was done for complete evaluation of retinal periphery. Lid speculum placed under topical anesthesia with 0.5% Proparacaine eye drops ensured good separation of the eyelids and cornea was kept moist by applying normal saline.

This was followed by MSFI and video recording of retinal features. Smartphone used was iPhone 4S (Apple Inc., Cupertino, CA, USA) with its original settings. It was done as described below:

1. iPhone and Volk + 20 Dioptre (D) lens were inserted into the respective slots at either ends of the MII RetCam device. This enabled holding both with a single hand at appropriate distance in the same axis. The other hand was therefore free to manoeuvre the eyes and to perform scleral indentation
2. The device was held in front of the patient's eye by holding the handle near the smartphone end between

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the palm and fingers in such a way that the screen of the smartphone is facing up and thumb of the same hand was used to turn on/off the video mode of the camera during recording [Fig. 1a]
3. The constant illumination in the video mode was switched on, and once the red glow was seen through the dilated pupil, it was slowly withdrawn till a clear image of the fundus was visualized on the smartphone screen
4. The video mode of the camera was then switched on with the thumb and then recorded until all the desired areas were imaged. The video mode was then similarly switched off
5. To get a good quality image, it is important to ensure that the image fills the entire 20D lens without any light reflection. This was achieved by slight back and forth movement or tilting of the device and stabilizing the baby's eyes with infant depressor. Good mydriasis also helped to reduce reflections
6. The smartphone camera lens and the 20 D lens should be cleaned well before the procedure to remove any dirt which can cause artefacts
7. The posterior pole was examined first followed by the entire peripheral retina in a sequential manner which was facilitated with the eye ball manoeuvred towards the desired direction using the scleral depressor. Tilting the baby's head slightly to the same side made it easier to visualize the extreme periphery
8. After completion of recording, the chosen good quality stills were extracted from the video sequence using the screenshot option in the smartphone (click and hold the top power button and the round menu button at the bottom simultaneously). The right hand of the examiner holding the device is not supported on the baby's face and can shake in between. Hence, capturing videos rather than still images were preferred
9. The snapshot images and video recordings were then transferred to a laptop which had a larger storage space. They were then archived in a separate folder along with the demographic data for each baby
10. Babies with immature retina and ROP were followed up as per the guidelines and the same protocol was repeated at each visit.

Study was approved by institutional human ethics committee and written informed consent was obtained from the parents of babies.

Results

132 babies with gestational age (GA) ranging from 27 to 39 weeks and birth weight (BW) ranging from 800 to 3400 grams were screened for ROP during the study period. Babies having GA more than 35 weeks and BW more than 1700 grams were included for screening at neonatologist's discretion. Fundus examination revealed mature retina in 52 babies. There were 36 babies with immature retina in zone 3, 19 babies with immature retina in zone 2, and 25 babies with ROP. They were all monitored according to the guidelines. During follow up, 17 more babies developed ROP resulting in a total of 42 babies (31.81%) with ROP. Types 1 ROP developed in 5 babies (3.79%) and were treated with laser.

MSFI captured good quality retinal images in 33 out of 42 babies with ROP (78.57%). In the remaining 9 babies image clarity was not good enough to assess the retinal changes. Serial imaging was done for 24 out of 33 babies with ROP, ranging from 2 to 11 imaging sessions. Imaging was also done for 4 babies with immature retina and 6 babies with mature retina which included 3 babies with incidental retinal findings. A total of 129 imaging sessions were done for 43 babies (mean 3). Posterior pole images helped to assess the disc, macula and plus disease [Fig. 1b and c]. None of the babies had Zone 1 disease. Peripheral images especially of the nasal and temporal periphery helped in accurate classification of ROP [Fig. 2]. Scleral depression made it possible to image the extreme peripheral retina till the ora. [Fig. 2 g and h]. Serial recordings during follow up helped to objectively monitor the disease and to study the changes associated with progression or regression of the disease [Figs. 3 and 4]. In eyes which were lasered, it was helpful to document the changes pre and post treatment [Fig. 3]. Imaging details of all the babies with ROP and their disease pattern at the time of initial and final diagnosis are shown in the Table 1. Photo documentation was comparable to clinical evaluation with BIO except in babies with stage 1 disease in zone 3. Other posterior segment findings detected in 3 babies during evaluation could also be documented which were bilateral preretinal haemorrhage, intraretinal hemorrhage, and cystoid edema at fovea, respectively. Imaging required an additional time of approximately 5 minutes and none of the babies had any systemic issues during the procedure.

Discussion

Modern smartphones with its constant, coaxial and powerful light source, coupled with 20D lens, creates a BIO like optical system enabling visualization of the fundus and the built-in automatic focus camera enables imaging of the fundus. The utility of smartphone imaging in ROP was first reported by Lin *et al.*, who recorded fundus images of 6 infants with ROP using the original settings of the camera in the smartphone.[7]

Table 1: The imaging data of babies with ROP

Zone/Stage of ROP	No of babies evaluated with BIO at initial diagnosis (*n*=42)	No of babies evaluated by MSFI at initial diagnosis (*n*=33)	No of babies evaluated with BIO at final diagnosis (*n*=42)	No of babies evaluated by MSFI at final diagnosis (*n*=33)
Zone 2 Stage 1	5	5	0	0
Zone 2 Stage 2	5	4	1	0
Zone 2 Stage 3	1	1	3	3
Zone 3 Stage 1	20	13	19	12
Zone 3 Stage 2	11	10	17	16
Zone 3 Stage 3	0	0	2	2

ROP: Retinopathy of prematurity; BIO: Binocular indirect ophthalmoscopy; n: Number; MSFI: MII RetCam assisted smartphone based fundus imaging

Biju Raju *et al*. and Oluleye TS *et al*., have documented ROP by smartphone based imaging and have found it to be effective.[11,12] Oluleye *et al*. used the app Filmic Pro to capture the images and app Aviary to edit the images. In all these techniques, the examiner holds the mobile phone in one hand and 20D lens in the other hand, and so an assistant is required for eye ball manipulation. The technique is thus more challenging to visualize the periphery, which is crucial in ROP. Also, all reports are based on single imaging session on a smaller number of babies.

In our study, MSFI was successful in objectively documenting ROP in 78.57% of patients. Though this is slightly less than that reported with the professional wide field imaging systems,[3] it has several practical advantages. The device is more affordable[8] compared to the RetCam, and a smartphone and 20D lens are available with any ophthalmologist. The technique can be learned with minimal practice within a week's time if one is well-versed with BIO and smartphone photography. As both imaging and globe manipulation is done by the same person, there is better control and coordination than the other smartphone-based models. The unique design of the device automatically ensured that smartphone and 20D lens were aligned in the same axis as the patient's eyes especially when eyeballs are rotated to the sides for peripheral examination. This ability to visualize the peripheral retina to the same extent as BIO is one of the distinct advantages of MSFI, making it superior to RetCam in evaluating the Zone 3 in premature babies. It has been found that, in comparison to BIO, RetCam had more difficulty in imaging peripheral retina till ora due to the restricted mobility of the equipment.[13]

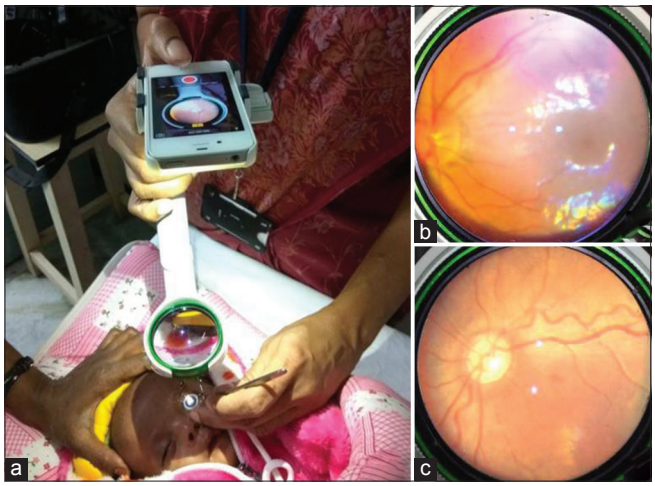


Figure 1: Showing (a) the technique of MII RetCam assisted fundus imaging. Fundus image is seen on the screen of the smartphone (b) Image of normal posterior pole (c) Image with plus disease

Serial imaging helped in improved monitoring of the course of disease over time by comparing with baseline photographs and to plan appropriate management. It also helped in more precise evaluation of plus disease, which is often inaccurately assessed by the limited bedside examination with BIO. To the best of our knowledge, serial imaging of ROP by smartphone fundoscopy has not been previously documented. This simple, non-contact, portable and user-friendly innovation can be practiced in any setting, irrespective of the systemic status of the baby or the location. Another advantage is the option to share these digital images directly from the smartphone because of its connectivity, for second opinion, in difficult situations avoiding direct referral of babies. The only other report of fundus photo documentation using MII RetCam for ROP is by Behera *et al*.[9] They used it for adult and pediatric cases and found it particularly useful in pediatric cases due to its portability. Till date, there is no published data comparing

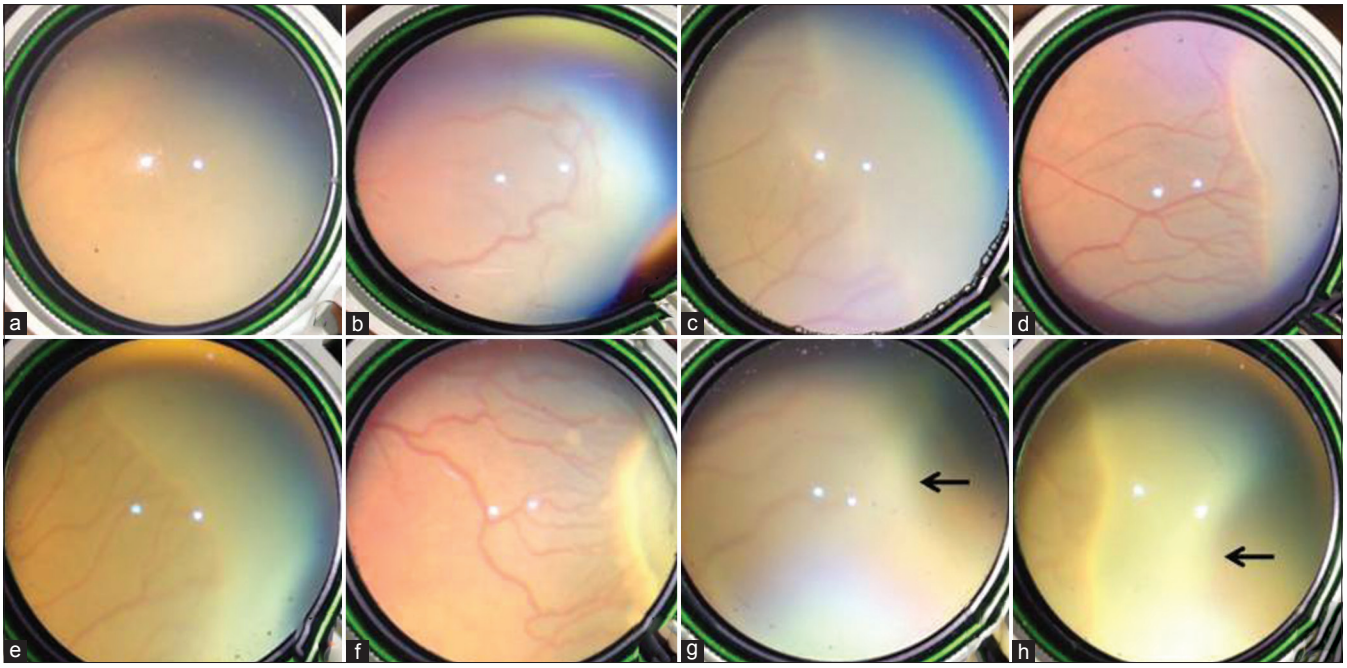


Figure 2: Showing (a) Immature retina (b) Vascular loops (c) Stage 1 ROP (d, e) Stage 2 ROP in the temporal and nasal periphery (f) Stage 3 ROP with plus disease and " popcorn" lesions (g and h) extreme periphery till ora (black arrow) in an eye (g) with mature retina and (h) with Stage 2 ROP

the quality of images obtained by smartphone in ROP to that with RetCam images or clinical examination. However, in a recent meta-analysis, smartphone image quality was found to agree substantially with the gold standards of clinical or photographic exams for evaluating other posterior segment pathologies like diabetic retinopathy.[14] We could also document other incidental posterior segment pathologies like retinal hemorrhages and foveal cystic changes. Routine ocular digital imaging in preterm babies have been emphasized by other authors who serendipitously detected significant and even life threatening conditions during ROP screening enabling timely intervention for treatable conditions.[15]

We used the iPhone 4S as it has been proved to be safe in retinal imaging.[16] Moreover, the technical feasibility of an iPhone as an ocular imaging device was found to be comparable to that of RetCam in a study by Jalil *et al*., and the authors recommend iPhone imaging in situations where expensive and complicated imaging equipment is unavailable.[17] They opine that flash illumination and autofocus in iPhones is well designed for ophthalmic imaging without the need for additional apps or much manual adjustment. Several apps like MII RetCam App, HopeScope App and Ullman Indirect App are available with different functionalities like data storage, brightness control and even short video recording, but we preferred the original settings in the iPhone and so no additional app was tried. Other authors have reported satisfactory imaging with different smartphones like iPhone 5, HTC One, Blackberry Z-10, Samsung S3 and Note3.[7,11,12,18] Another recommendation is to use micropore tapes to reduce unwanted reflections.[7]

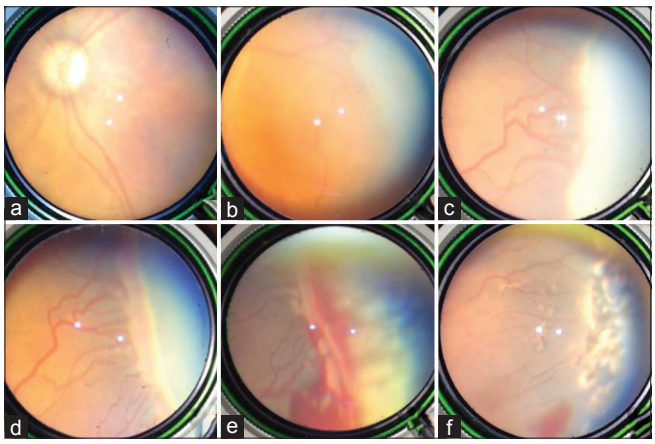


Figure 3: Serial imaging of a baby lasered for Type 1 ROP showing (a) posterior pole (b-d) progression of ROP from Stage 1 to 3 with plus disease (e) Immediate post laser with hemorrhage over ridge (f) 1 month post laser with decrease in plus disease and haemorrhage

Similar to previous reports,[8] we found that real time display of the fundus features on the smartphone screen during video recording was superior to conventional theoretical teaching of the residents in the evaluation of ROP. It was especially useful to demonstrate the peripheral retinal changes in ROP to the residents who were not competent in performing BIO in neonates. It also avoided too many doctors from directly handling the preterm babies and thus reduced the duration of examination as well as the risk of infection. Incorporating MSFI as a part of ROP screening will help to create a large image library useful for presentations, publications and research activities. It can be used as an easily available and inexpensive tool to train eye care workers.[18] It was extremely helpful in parental counselling, convincing them regarding treatment when needed and ensuring better compliance with follow up. Thus, it is a real asset in the practice of evidence-based medicine and can be a valuable proof in medico-legal situations.[8]

Our study has certain limitations as well. One of the important drawbacks of this technique is the limited field

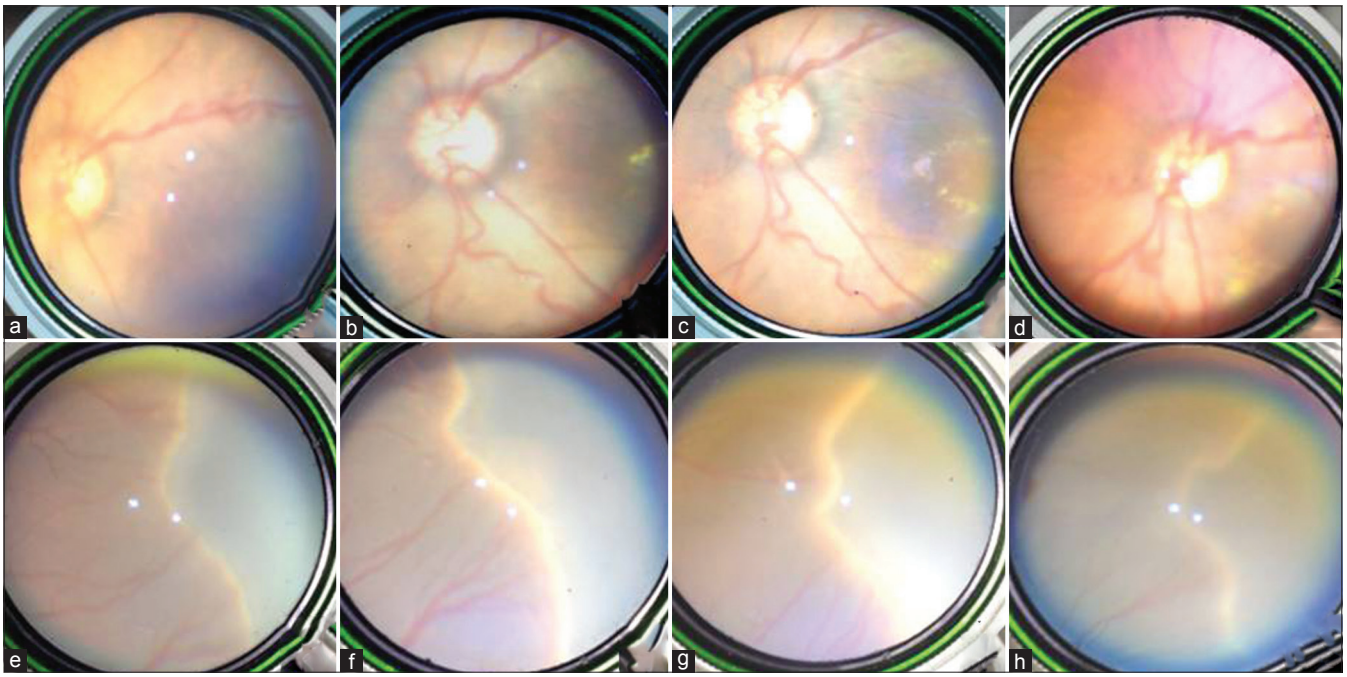


Figure 4: Serial imaging of the central (a-d) and peripheral retina (e-h) showing delayed natural regression. Stage 2 ROP noted at 36 weeks post conceptual age, worsened to Stage 3 with plus disease by 38 weeks, early regression by 40 weeks and further regression by 49 weeks

of view (<45°) when using the 20D lens, unlike the RetCam which provides a wide 130° field. This can cause difficulty in distinguishing the zones from a single static image. Using higher plus power lenses like 40 or 60D lens can increase the field up to 90°.[14] A superior option would be to use montage software programs for automated construction of mosaic photographs from multiple individual images as has been reported by Patel *et al*.[19] In their study, ROP experts interpreted individual fundus photographs and mosaic photographs of wide-angle retinal images and found that mosaic photographs were more accurate in image based diagnosis of clinically significant ROP. This is because mosaic photographs not only provides high resolution wide field images but also allows a continuous view with improved visualization of the ridge and vascular changes. In a novel study by Kim TN *et al*., software intelligence and automation-assisted smartphone based retinal imaging produced high quality 100° photomontages from 5 overlapping images in under one minute fully on-phone revealing various retinal abnormalities.[20] It was validated against clinical examination in diabetic retinopathy screening and found to have a sensitivity of 93.3% and specificity of 56.8%. More studies are needed to know if such automation techniques can enhance smartphone based imaging in ROP. Another deficiency of our study is the lack of objective grading of the quality of images by masked observers and validating it against the gold standard of BIO. The technique was more challenging in eyes with poor mydriasis, hazy media and in older babies due to the more active movements by the baby and these factors accounted for the difficulty in obtaining good images in 9 of our babies with ROP. Better case selection and availability of smartphones/apps with option of small pupil imaging can help to overcome these difficulties.

Smartphones have been successfully used as a diagnostic interface in teleophthalmology for ROP wherein retinal images taken using RetCam Shuttle at remote locations are transmitted electronically and viewed on smartphone using viewer software by experts.[21,22] Though telemedicine has tremendously improved the screening and management of ROP, it involves a significant initial investment to implement the telemedicine system (RetCam costs USD 83,500/- whereas MII RetCam device costs USD 380/-).[4,23] The availability of easily accessible and good quality imaging using smartphone offers a low cost substitute for RetCam imaging, adding another dimension to telescreening for ROP.

Conclusion

MSFI is a potential alternate imaging tool enabling objective documentation and monitoring of ROP in low resource settings. Creation of an image data base is useful for academic and research purposes. Prospective, double blinded study with a larger sample size and with comparison to RetCam will help to assess the sensitivity and specificity of this imaging modality. Improvements in software and automation techniques have the potential to widen its utility as a teleROP screening tool in future.

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Nil.

Conflicts of interest

One of the co-authors (Dr Ashish Sharma) has financial/proprietary interest in MII RetCam.

References

1. Trese MT. What is the real gold standard for ROP screening? Retina Phila Pa 2008;28(3 Suppl):S1-2.
2. Chiang MF, Wang L, Busuioc M, Du YE, Chan P, Kane SA, *et al.* Telemedical retinopathy of prematurity diagnosis: Accuracy, reliability, and image quality. Arch Ophthalmol 2007;125:1531-8.
3. Chiang MF, Melia M, Buffenn AN, Lambert SR, Recchia FM, Simpson JL, *et al.* Detection of clinically significant retinopathy of prematurity using wide-angle digital retinal photography. Ophthalmology 2012;119:1272-80.
4. Chee R-I, Patel SN, Jonas KE, Gupta MP, Chiang MF, Chan RVP, *et al.* Current trends in telemedicine for retinopathy of prematurity. Vis Pan-Am Pan-Am J Ophthalmol 2017;16:7-11.
5. Lord RK, Shah VA, Filippo ANS, Krishna R. Novel uses of smartphones in ophthalmology. Ophthalmology 2010;117:1274-1274.e3.
6. Bastawrous A. Smartphone fundoscopy. Ophthalmology 2012;119:432-433.e2; author reply 433.
7. Lin SJ, Yang CM, Yeh PT, Ho TC. Smartphone fundoscopy for retinopathy of prematurity. Taiwan J Ophthalmol 2014;4:82-5.
8. Sharma A, Subramaniam SD, Ramachandran KI, Lakshmikanthan C, Krishna S, Sundaramoorthy SK. Smartphone-based fundus camera device (MII Ret Cam) and technique with ability to image peripheral retina. Eur J Ophthalmol 2016;26:142-4.
9. Behera S, Pradhan A. MII Ret Cam: A boon towards retinal disease imaging and documentation. J Clin Exp Ophthalmol 2017;8:1-3.
10. Jalali S, Anand R, Kumar H, Dogra MR, Azad R, Gopal L. Programme planning and screening strategy in retinopathy of prematurity. Indian J Ophthalmol 2003;51:89-99.
11. Raju B, Raju NSD, Akkara JD, Pathengay A. Do it yourself smartphone fundus camera – DIYretCAM. Indian J Ophthalmol 2016;64:663-7.
12. Oluleye TS, Rotimi-Samuel A, Adenekan A. Mobile phones for retinopathy of prematurity screening in Lagos, Nigeria, sub-Saharan Africa. Eur J Ophthalmol 2016;26:92-4.
13. Shah PK, Narendran V, Saravanan VR, Raghuram A, Chattopadhyay A, Kashyap M. Screening for retinopathy of prematurity--A comparison between binocular indirect ophthalmoscopy and RetCam 120. Indian J Ophthalmol 2006;54:35-8.
14. Vilela MA, Valença FM, Barreto PK, Amaral CE, Pellanda LC. Agreement between retinal images obtained via smartphones and images obtained with retinal cameras or fundoscopic exams-systematic review and meta-analysis. Clin Ophthalmol Auckl NZ 2018;12:2581-9.
15. Jayadev C, Vinekar A, Bauer N, Mangalesh S, Mahendradas P, Kemmanu V, *et al.* Look what else we found--Clinically significant abnormalities detected during routine ROP screening. Indian J Ophthalmol 2015;63:373-7.
16. Kim DY, Delori F, Mukai S. Smartphone photography safety. Ophthalmology 2012;119:2200-1; author reply 2201.
17. Jalil M, Ferenczy SR, Shields CL. iPhone 4s and iPhone 5s imaging of the eye. Ocul Oncol Pathol 2017;3:49-55.
18. Ademola-Popoola DS, Olatunji VA. Retinal imaging with smartphone. Niger J Clin Pract 2017;20:341-5.
19. Patel SN, Klufas MA, Douglas CE, Jonas KE, Ostmo S, Berrocal A, *et al.* Influence of computer-generated mosaic photographs on retinopathy of prematurity diagnosis and management. JAMA Ophthalmol 2016;134:1283-9.

20. Kim TN, Myers F, Reber C, Loury PJ, Loumou P, Webster D, *et al.* A smartphone-based tool for rapid, portable, and automated wide-field retinal imaging. Transl Vis Sci Technol 2018;7:21.
21. Vinekar A, Gilbert C, Dogra M, Kurian M, Shainesh G, Shetty B, *et al.* The KIDROP model of combining strategies for providing retinopathy of prematurity screening in underserved areas in India using wide-field imaging, tele-medicine, non-physician graders and smart phone reporting. Indian J Ophthalmol 2014;62:41-9.
22. Kulkarni S, Kakade N, Khandekar R, Narwadkar P, Deshpande M. Use of the smart phone as a diagnostic interface for detecting severe retinopathy of prematurity: A pilot study. J Ophthalmic Vis Res 2017;12:447-8.
23. Sharma A. Emerging simplified retinal imaging. Dev Ophthalmol 2017;60:56-62.

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Background: According to the recommendations from The World Health Organization, exclusive breast feeding (EBF) should be given to infants for the first six months for healthy growth and development. However, developing countries have a wide range of variation in the practice of EBF. From this study, we aim to estimate the duration of EBF and find the impact of socio-demographic characteristics on EBF.

Materials and Methods: This study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore in the month of July 2018. Mothers with children aged below 4 years attending the paediatric outpatient department were included in the study. Kaplan-Meier curve analysis and logistic regression analysis were employed.

Results: Our analysis indicates that the prevalence of EBF was 58.4% among infants up to 6 months of age with mean duration of EBF 4.52 months. It was observed that mothers of a younger age (15-24 years) and with lower socio-economic status were negatively associated with the practice of providing EBF to infants up to six months of age.

Conclusion: The findings clearly indicate that the interventions related to EBF up to six months of age should be targeted at women in the lower socio-economic status and at younger ages.

Keyword: Exclusive breast feeding, socio-economic status, mother's age.

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Introduction

According to the recommendation of the World Health Organization (WHO), exclusive breastfeeding (EBF) should be given to infants for the first six months of life and continued breastfeeding up to two years of age or beyond for the healthy growth and development [1-3]. Recent literatures have suggested exclusive breastfeeding as a cost-effective intervention to reduce infant mortality rate in less developed countries [4, 5]. Globally, it is estimated that the lack of providing exclusive breast feeding to infants in the first 6 months of life results in 1.4 million deaths and 10% of disease in under-five children [6]. On the other hand, poor school performance, reduced productivity and impaired intellectual and social development are reported to be much higher due to the lack of EBF in the first 6 months of life.

In India, a large number of children, nearly 20 million, were not able to receive EBF for the first six months, according to the reports from National Family Health Survey (NFHS) [7]. Over the past several years, India has failed to witness any remarkable achievements in infant feeding practices. Only a small increment was recorded in EBF rates amongst infants in the 0-6 month age group from 41.2% in 1998-99 (NFHS 2) to 46.3% in 2005-2006 (NFHS 3). The mean duration of EBF among last born children is as brief as two months.

In 2016, the Government of India started various initiatives to tackle the health issues among children due to inappropriate breast-feeding practices. The Government of India developed the infant and young child feeding guidelines in 2016 in view of this initiative. However, more studies are needed to explore the factors associated with the low proportion of EBF. The present study aims to estimate the duration of EBF and to find the impact of socio-demographic characteristics influences on EBF practice.

Methodology

Study design and setting

A cross-sectional study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore in the month of July 2018. The mothers of infants less than four years of age, who attended the paediatric outpatient department, were included in the study. The selected mothers were broadly classified into two groups: EBF and non-EBF. Mothers having children aged below 6 months and who were given only breast milk in the 24 hours preceding the survey were considered as EBF and others were in non-EBF group [8].

Sample size estimation

In order to estimate the EBF with the precision of 20% of the expected prevalence of 30%, with 95% CI, we require a minimum sample size of 300 for the study.

Data collection

Data were collected using direct interview method. Mothers were interviewed about the duration of EBF in months with respect to youngest child. The socio-demographic characteristics and breast-feeding related information were elicited from the selected mothers. The characteristics studied were educational status of the mother, age of the mother, working status of the mother, educational status of father, socio-economic status of the household, child's gender, gestational age of the child, birth weight of the child, mode of delivery, condition of the child soon after delivery, and counselling for breastfeeding after delivery. The socio-economic status was calculated using Prasad classification with class 1 as the person having per-capita income greater than or equal to Rs.6180, class 2 ranging from Rs.3090-6179, class 3 ranging from Rs.1850-3089, class 4 ranging from Rs.930-1849, and class 5 as person having per-capita income below Rs.930. The classification was done based on the Consumer Price Index (CPI) value of 279 for Coimbatore [9].

Exclusion criteria

Babies with congenital anomalies that interfered with breastfeeding were not included in the study.

Ethical approval

The study was approved by the Institutional Human Ethics Committee, PSG Institute of Medical Sciences and Research, Coimbatore.

Outcome variable

The outcome variable was the exclusive breast feeding (EBF) status of infants up to six months of age.

Explanatory variables

Mother's age, educational status of mother and father, working status of mother, gender, gestational age and birth weight of the child and socio-economic status of the household were the explanatory variables.

Statistical analysis

To estimate the mean and median duration of exclusive breast feeding among infants under study, the Kaplan-Meier Survival analysis method was applied and conditional probability of survival at monthly interval of time was then obtained. The termination of EBF was considered as outcome of the event of the occurrence. Accordingly, those women who fed their babies exclusively with breast feeding on the date of interview were considered as censored cases. In such cases, the duration of EBF was recorded as it was not known when they would discontinue EBF and start introducing adult diet. The duration of exclusive breastfeeding was sub grouped into smaller time intervals and for each interval all those who have been observed at least that long were used to calculate the probability of a terminal event occurrence in the interval. Then the overall probability of the event occurrence at different time

periods were estimated using the probabilities estimated from each interval. Survival probabilities, standard error, hazard rate, and mean and median survival time have been calculated. Log rank test was applied to compare the statistical significance for the equality of duration of exclusive breast feeding for different groups. In addition, logistic regression analysis was done to find the association of socio-demographic characteristics with EBF categorized as Yes or No. Odds ratio and then 95% confidence intervals were then calculated. Multivariate logistic regression analysis was done for those variables found significantly associated with the outcome variable of interest in the Univariate analysis. $p<0.05$ was considered as statistically significant. The data were analysed using SPSS (Ver. 23).

Results

A total of 527 women were studied. The socio-demographic characteristics of infants and families are given in Table 1. The prevalence of exclusive breast feeding was 58.4% among infants up to 6 months of age with the mean duration of EBF as 4.52 months.

Table 1: Socio-demographic characteristics of infants and families

Characteristics	Number (%)
Child's age (months) (Mean±SD)	15.55 ± 11.92
Mother's education	
Below 12th standard	138 (26.2)
Above 12th standard	389 (73.8)
Mother's age (years)	
15-24	108 (20.5)
25-34	388 (73.6)
35-44	31 (5.9)
Mother's Occupation	
House wife	403 (76.5)
Paid worker	124 (23.5)
Father's education	
Below 12th standard	129 (24.5)
Above 12th standard	398 (75.5)
Socio-economic class	
Class 1 and 2	426 (80.8)
Class 3,4 and 5	101 (19.2)
Child's gender	
Female	246 (46.7)
Male	281 (53.3)
Gestational period	
Term	469 (89.0)
Preterm	58 (11.0)
Birth weight	
<=2500	126 (23.9)
>2500	401 (76.1)
Mode of delivery	
Vaginal delivery	336 (63.8)
Caesarean	191 (36.2)
Child's health	
Well	454 (86.1)
Sick	73 (13.9)
Received breast feeding counselling	
Yes	326 (61.9)
No	201 (38.1)

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Below 12th standard	4.33	5.00	0.89	0.345
Above 12th standard	5.57	6.00		
Mother's age □ □□□□r □□□□				
15-24	4.15	5.00	7.44	P<0.05
25-34	4.55	6.00		
35-44	5.04	6.00		
Mother's Occupation □				
House wife	4.49	6.00	0.19	0.663
Paid worker	4.58	6.00		
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Below 12th standard	4.15	5.00	4.49	P<0.05
Above 12th standard	4.62	6.00		
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Class 1 and 2	4.61	6.00	5.45	P<0.05
Class 3,4 and 5	4.10	5.00		
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Female	4.58	6.00	0.15	0.690
Male	4.44	6.00		
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Term	4.58	6.00	3.88	0.143
Preterm	4.02	6.00		
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≤2500	4.33	6.00	0.11	0.772
>2500	4.58	6.00		
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Vaginal delivery	4.58	6.00	0.20	0.654
Caesarean	4.41	6.00		
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Well	4.63	6.00	5.07	P<0.05
Sick	3.84	5.00		
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Yes	4.54	6.00	0.05	0.860
No	4.493	6.00		
□□□□□□	4.523	6.00		

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Yes	37 (8.7)	10 (9.9)	0.482
No	389 (91.3)	91 (90.1)	
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Yes	56 (13.1)	21 (20.8)	P<0.05
No	370 (86.9)	80 (79.2)	
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Yes	111 (26.1)	13 (12.9)	P<0.01
No	315 (73.9)	88 (87.1)	
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Yes	303 (80.6)	73 (72.3)	0.818
No	123 (19.4)	28 (27.7)	□

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On testing equality of the duration of EBF, it was observed that the mean duration of EBF was higher in mothers of age group 35-44 years than those aged 15-24 years and 25-34 years ($p<0.05$). The mean duration of EBF was more in class 1 and 2 of the socio-economic status than class 3,4, and 5 of the socio-economic status ($p<0.05$). The mean duration of EBF was more in children whose father's education was above 12th standard than those less than 12th standard ($p<0.05$). It was also observed that the mothers with the sick children tend to do less EBF than those with well children ($p<0.05$). The findings are presented in Table 2.

In the logistic regression analysis (Table 3), the prevalence of EBF was higher among the mothers of age group 25-34 years than those aged 15-24 years ($p<0.05$) and it was significantly higher among class 1 and 2 of the socio-economic status than class 3, 4, and 5 of socio-economic status ($p<0.01$). The multivariate logistic regression analysis also shows that the socio-economic status of class 1 and 2 had significantly higher prevalence of exclusive breast feeding than class 3, 4, and 5 of the socio-economic status ($p<0.01$) with an odds ratio of 1.788 (95% CI: 1.153-2.775). In addition, the prevalence of EBF was higher among the age group 25-34 years than in the aged group 15-24 years ($p<0.05$) with an odds ratio of 1.572 (95% CI: 1.021-2.421).

During the interviews, 47 (8.9%) mothers reported the problem of inverted nipple as a constraint for EBF. Seventy-seven (14.6%) mothers perceived to have the problem of low breast milk and 124 (23.5%) mothers had employment outside home. In addition, 151 (28.6%) mothers had no supportive family members at home (Table 4). It was also observed that there was a statistically significant association between perception of low breast milk and lower socio-economic status ($p<0.05$) (Table 4).

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Breast feeding is universally recognized as the best way of feeding infants which helps in their healthy growth and development. Therefore, several countries, especially developing nations, have started implementing health promotion interventions to support successful breastfeeding by taking into account the factors influencing breast feeding outcomes. For infants till the first six months of life, the WHO strongly recommends EBF without any additional food or water. The WHO also suggests providing complementary diet at 6 months along with breast milk and sustaining breast feeding until the age of 2 years. However, whether an infant is breastfed or formula fed depends on several factors like social, psychological, and environmental scenarios.

Despite several efforts made for EBF up to six months, in our study, 42% of the mothers were still not following EBF up to six months. The mean duration of EBF was 4.52 months which is much lower than the findings reported by Chudsama et al. [5]. Infants not exclusively breast fed up to six months are at risk of higher occurrence of associated infections, malnutrition deficiencies, and under-development [10, 11]. Most of the recent studies regarding EBF come out with the similar fact that the practice of EBF tends to decline and highlight the tremendous progress in the marketing of industrial milk products as well as the lack of awareness of this serious issue among mothers.

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A significantly lower prevalence of EBF was observed among mothers with lower socio-economic status, which is controversial with the result obtained from an Ethiopian demographic health survey that explained a declining trend of EBF with the higher maternal educational and socio-economic

status [12]. Furthermore, our study found that the duration of EBF was significantly lower among mothers with lower socio-economic status. The findings of our study clearly acknowledge socio-economic status of the household as a major influencing factor for longer breastfeeding practice among mothers in a higher income bracket. In addition, we have also observed a significant association between the perception of low breast milk and lower socio-economic status. The findings clearly suggest that interventions should be tailored to target women with lower socio-economic status.

[illegible]

It was observed that age of the mother is a predictor of EBF. The mean duration of EBF was significantly lower in the age group of mothers with 15-24 years. The findings suggest that more efforts are needed to create awareness about the need for EBF in younger aged mothers.

Education and occupation of the parent's and its [illegible]

We could not find statistical significance between the father's education and increased prevalence of EBF in the multivariate analysis though we observed a statistical significance in the univariate analysis. Maternal educational status was also found statistically not significant with EBF, which is a controversial observation with the findings of the Ethiopian demographic health survey which indicated a declining trend of EBF practices with higher maternal educational status but supports the findings observed by Tesfaye Setegn et al. [10].

[illegible]

The findings clearly indicate that the interventions related to EBF up to six months of age should be targeted to women in the lower socio-economic status and at younger ages.

[illegible]

Our study relies on retrospective reporting, which may result in inaccurate recall and this can be point out as a major limitation. However, most of the recent literature also followed the similar maternal recall mode for elucidating breast-feeding practices. Since this was a hospital-based study, the study population was representative of generally higher socio-economic status and which also reflects as a limitation of our work. A community-based cross-sectional study with a large sample size can provide more light in this field. Despite these limitations, the study has several strengths. We have elicited a large number of demographic and clinical data which can influence the association of duration of EBF. The same interviewer collected all information which reduces the inter observer bias. Advanced statistical methods were employed to analyse the association of socio-demographic and clinical correlates with EBF.

[illegible]

Our results can help to create more awareness about the need for EBF in the younger age group and the mothers in lower socio-economic status.

[illegible]

To the best of our knowledge, not many studies were conducted in Tamil Nadu to examine the socio-demographic and clinical correlates of exclusive breastfeeding. Results of this study indicate a need to create awareness to mothers regarding the need of providing EBF to infants up to six months of age.

[illegible]

India has undergone a rapid transition. Contradictory to our expectations, higher socio-economic status mothers are now aware about the need for EBF. More efforts are needed for those mothers in lower socio-economic status and those at younger ages. Secondly, apart from mother's education, father's education also plays a great role in breast feeding practices. In conclusion, it was observed that lower socio-economic status and young age of the mother have negative impact on EBF for six months of age.

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Author's Contribution:

ACM Design of the study, preparation of manuscript and interpretation of data, revising critically for intellectual content and final approval of the version to be published.

JKB, DMP and CD. Data collection, analysis, preparation of the manuscript, drafting the article and final approval of the version to be published.

MJ. Revision of the manuscript

VS, NK and RS. Design of the study, technical guidance and revising critically for intellectual content and final approval of the version to be published.

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All authors have declared that they have no conflict of interest.

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1.World Health Organization: The optimal duration of exclusive breastfeeding: report of an expert consultation. Geneva: World Health Organization, Department of nutrition for health and development and department of child and adolescent health and development; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

2.World Health Organization: Global strategy for infant and young child feeding. The optimal duration of exclusive breastfeeding. Geneva: World Health Organization; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

3.World Health Organization: Infant and young child feeding (IYCF) Model Chapter for textbooks for medical students and allied health professionals. Switzerland: World Health Organization: 2009,pp 19-29.
https://apps.who.int/iris/bitstream/handle/10665/44117/9789241597494_eng.pdf;jsessionid=D51E85BC3FEFDD64E24D3B3CA0B10287?sequence=1

4.Setegn T, Belachew T, Gerbaba M, Deribe K, Deribew A, Biadgilign S. Factors associated with exclusive breastfeeding practices among mothers in Goba district, south east Ethiopia: a cross-sectional study. International Breastfeeding Journal 2012; 7:17.
https://internationalbreastfeedingjournal.biomedcentral.com/articles/10.1186/1746-4358-7-17

5.Chudasama RK, Patel PC, Kavishwar AB. Factors Associated with Duration of Exclusive Breastfeeding. The Internet Journal of Pediatrics and Neonatology 2007; 9(1):1-6.
https://print.ispub.com/api/0/ispub-article/4871

6.Oweis A, Tayem A, Froelicher ES. Breastfeeding practices among Jordanian women. Int J NursPrac 2009; 15:32-40.
https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1440-172X.2008.01720.x

7.Tiwari S, Bharadva K, Yadav B, Malik S, Gangal P, Banapurmath CR, et al. Infant and Young Child Feeding Guidelines.IndianPediatrics 2016; 53:703-713.

8.WHO Division of Child Health and Development. Indicators for assessing breastfeeding practices.Geneva: WHO; 1991. WHO/CDD/SER/91.14.
https://apps.who.int/iris/bitstream/handle/10665/62134/WHO_CDD_SER_91.14.pdf?sequence=1

9.Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers [online] 2018 [cited 2018 Aug 10]. Available from:
URL:http://labourbureaunew.gov.in/UserContent/Linkingfactor_CPI_IW.pdf?pr_id .

10.SetegnT, Gerbaba M, Belachew T. Determinants of timely initiation of breastfeeding among mothers in GobaWoreda. South east Ethiopia: a cross-sectional study. BMC Public Health 2011; 11:217.
https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-11-217

11.Habibi M, Laamiri FZ, Aguenaou H, Doukkali L, Mrabet M, Barkat A. The impact of maternal socio demographic characteristics on breast feeding knowledge and practice: An experience from Casablanca, Morocco. International Journal of Paediatrics and Adolescent Medicine 2018;5: 39-48
https://www.sciencedirect.com/science/article/pii/S2352646717301576

12.Alemayehu T, Haidar J, Habte D. Determinants of exclusive breast feeding practices in Ethiopia. Ethio. J Health Dev 2009; 23: 12-18
https://www.ajol.info/index.php/ejhd/article/viewFile/44832/28326

1.World Health Organization: The optimal duration of exclusive breastfeeding: report of an expert consultation. Geneva: World Health Organization, Department of nutrition for health and development and department of child and adolescent health and development; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

2.World Health Organization: Global strategy for infant and young child feeding. The optimal duration of exclusive breastfeeding. Geneva: World Health Organization; 2001.
https://www.who.int/nutrition/publications/optimal_duration_of_exc_bfeeding_review_eng.pdf

3.World Health Organization: Infant and young child feeding (IYCF) Model Chapter for textbooks for medical students and allied health professionals. Switzerland: World Health Organization: 2009,pp 19-29.
https://apps.who.int/iris/bitstream/handle/10665/44117/9789241597494_eng.pdf;jsessionid=D51E85BC3FEFDD64E24D3B3CA0B10287?sequence=1

4.Setegn T, Belachew T, Gerbaba M, Deribe K, Deribew A, Biadgilign S. Factors associated with exclusive breastfeeding

practices among mothers in Goba district, south east Ethiopia: a cross-sectional study. International Breastfeeding Journal 2012; 7:17.
https://doi.org/10.1186/1746-4358-7-17
PMid:23186223 PMCid:PMC3560275
5.Chudasama RK, Patel PC, Kavishwar AB. Factors Associated with Duration of Exclusive Breastfeeding. The Internet Journal of Pediatrics and Neonatology 2007; 9(1):1-6.
https://doi.org/10.5580/734
6.Oweis A, Tayem A, Froelicher ES. Breastfeeding practices among Jordanian women. Int J NursPrac 2009; 15:32-40.
https://doi.org/10.1111/j.1440-172X.2008.01720.x
PMid:19187167
7.Tiwari S, Bharadva K, Yadav B, Malik S, Gangal P, Banapurmath CR, et al. Infant and Young Child Feeding Guidelines. IndianPediatrics 2016; 53:703-713.
https://doi.org/10.1007/s13312-016-0914-0
PMid:27567645
8.WHO Division of Child Health and Development. Indicators for assessing breastfeeding practices.Geneva: WHO; 1991. WHO/CDD/SER/91.14.
https://apps.who.int/iris/bitstream/handle/10665/62134/WHO_CDD_SER_91.14.pdf?sequence=1
9.Labour Bureau, Government of India. All India and Centre-wise Linking factors between New Series of Consumer Price Index Numbers [online] 2018 [cited 2018 Aug 10]. Available from:
URL:http://labourbureaunew.gov.in/UserContent/Linkingfactor_CPI_IW.pdf?pr_id.
10.SetegnT, Gerbaba M, Belachew T. Determinants of timely initiation of breastfeeding among mothers in GobaWoreda. South east Ethiopia: a cross-sectional study. BMC Public Health 2011; 11:217.
https://doi.org/10.1186/1471-2458-11-217
PMid:21473791 PMCid:PMC3088906
11.Habibi M, Laamiri FZ, Aguenaou H, Doukkali L, Mrabet M, Barkat A. The impact of maternal socio demographic characteristics on breast feeding knowledge and practice: An experience from Casablanca, Morocco. International Journal of Paediatrics and Adolescent Medicine 2018;5: 39-48
https://doi.org/10.1016/j.ijpam.2018.01.003
PMid:30805532 PMCid:PMC6363246
12.Alemayehu T, Haidar J, Habte D. Determinants of exclusive breast feeding practices in Ethiopia. Ethio. J Health Dev 2009; 23: 12-18
https://doi.org/10.4314/ejhd.v23i1.44832

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A Prospective Cohort Study on the Safety of Infant Pentavalent (DTwP-HBV-Hib) and Oral Polio Vaccines in Two South Indian Districts

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Background: Safety of pentavalent (DTwP-HBV-Hib) vaccine has been a public concern in India and other countries. This study attempted to document the association of serious adverse events following immunization (AEFI, including hospitalizations and deaths of all causes) with the 3 doses of pentavalent and oral poliovirus (OPV) vaccines.

Methods: A cohort of 30,688 infants in 2 south Indian districts were enrolled and followed-up between October 2014 and May 2016, following their first vaccination with DTwP-HBV-Hib and OPV at public health facilities. During weekly follow-ups, by telephone or home visits, the serious AEFIs (hospitalizations and deaths) occurring any time after each vaccination until 4 weeks after third dose were documented. The incidence risk ratios (IRRs) of serious AEFIs in the first (days 0–6) and fourth weeks (days 21–27) after the vaccine doses were compared using the poisson regression analysis.

Results: Of the 30,688 infants enrolled, 30,208 received their third doses of vaccines. During the 4-week periods following each vaccination, there were 365 hospitalizations and 17 deaths. Adjusted incidence risk ratio of 3 doses combined for post-vaccination serious AEFIs during the first week compared with fourth week was 0.8 [95% confidence interval: 0.6–1.0].

Conclusions: There was no increased risk of a serious AEFIs during the first week after any of the 3 doses of pentavalent and OPV vaccination compared with the fourth week. In the absence of any temporal clustering, mortality and hospitalization rates observed in vaccinated infants probably reflects the natural occurrence of such events.

Key Words: pentavalent vaccine, oral polio vaccine, vaccine safety, serious adverse events, hospitalization, death

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Vaccination has been one of the major success stories in global health, leading to the eradication of deadly diseases like smallpox and possibly the virtual disappearance of some diseases from several regions.[1] Over the last 25 years, effective vaccination programs have resulted in a >90% decrease in the global disease burdens for diphtheria, pertussis, and tetanus, including India.[2–4] However, in spite of the extensive evidence of their benefits, concerns and controversies about the value and safety of vaccines continue to impact adversely their acceptance in diverse contexts. Public concerns over reports linking autism with measles, mumps, and rubella vaccine in the United Kingdom had negative impact on vaccine coverage causing several disease outbreaks,[5] which spread to other countries, such as the United States, France, Italy, and the Netherlands.[6–9] This misinformation has spread globally. The rumor mongering through social media considerably affected vaccine coverage during the MR campaigns in south India.[10] The growing global challenge of vaccine hesitancy relates largely to vaccine safety concerns, leading to public confidence erosion, which in turn dampens the vaccine coverage.[11] The Global Advisory Committee on Vaccine Safety emphasized the need for thorough investigation and causality assessment of serious adverse events following immunization (AEFI) as a quality assurance mechanism for immunization programs.[12]

The decreased disease burdens in the developed countries have largely been due to the successful implementation of immunization programs using multivalent combination vaccines. The vaccines against diphtheria, tetanus, and pertussis combined (DTwP) are in use for several decades under universal immunization program. Recently the HBV and *Haemophilus influenza* type b (Hib) have been added to DTwP, known as the pentavalent (DTwP-HBV-Hib) vaccine. Over the past 15 years Global Alliance for Vaccines and Immunizations has supported the introduction of pentavalent vaccine in developing countries for protection against invasive Hib disease in universal immunization program.[13] However, there were temporary interruptions and introduction delays in Sri Lanka,

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Bhutan, Vietnam[14] and Pakistan[15] during 2011–2016 due to reports of hospitalizations and deaths following vaccination after national launch of the pentavalent vaccine. Temporal association of vaccinations and sudden infant death syndrome (SIDS) has been the subject of extensive speculation, despite several studies having refuted any causal association,[16–18] particularly after the diphtheria-pertussis-tetanus immunization.[19]

In India, the reports of 32 infant deaths following the pentavalent vaccination as part of the national AEFI surveillance between 2011 and 2013 raised concerns, although no causal association could be identified.[20] Some professionals and civil society groups, however, appealed before the Supreme Court of India to ban the pentavalent vaccine.[21,22] Although the safety of pentavalent vaccine has been documented in the pre-licensure studies,[23,24] these studies were too small to identify rare adverse events. Thus, it was deemed necessary to carry out further investigations on this issue. This study was designed in consultation with all key stakeholders including the Ministry of Health and Family Welfare, Government of India and WHO (advised by Global Advisory Committee on Vaccine Safety), to assess the risk of serious AEFIs (hospitalizations and deaths) following pentavalent vaccination in an infant cohort from 2 Indian states, where pentavalent vaccine was first introduced.

MATERIALS AND METHODS

The pentavalent vaccine (manufactured by Serum Institute of India Ltd., Pune, India) was first introduced in the states of Kerala and Tamil Nadu in December 2011 with a 6, 10 and 14 weeks schedule for 3 primary doses. Both states have efficient health systems with high vaccination coverage[25] and low infant-mortality rates (IMR): 12 and 21 per 1000 live births in 2013 in Kerala and Tamil Nadu, respectively, compared with an overall IMR of 40 across India.[26] This study was conducted in 2 districts, Kollam (Kerala State) and Coimbatore (Tamil Nadu State).

Study Design

This prospective self-controlled cohort study enrolled infants who received their first dose of pentavalent and oral poliovirus (OPV) vaccine from the public health system between October 2014 and November 2015 in Kollam district, and between May 2015 and February 2016 in Coimbatore district.

Recruitment and Data Collection

Infants who received their first dose of pentavalent and OPV vaccines at all public health facilities in the 2 districts (District, Special and Taluk Hospitals, Community Health Centers, Primary Health Centers, Sub-centers) and out-reach points of rural and urban areas were traced based on the contact details recorded in the immunization registers. Home visits were made to confirm eligibility (received first dose of vaccine, expected to stay in the district for the 6 months follow-up period and contactable by telephone/mobile) and to enroll infants after obtaining informed written consent. Data on maternal care, infant gender, date of birth, birth weight, pregnancy-related and neonatal illnesses were collected at enrollment. The parent(s) were contacted weekly by telephone or home visit to document the health of the infant including any illness, hospitalization or death. The follow-up contact was continued even if the second and third vaccine doses were delayed due to any reason. Study staff confirmed receipt of the second and third vaccine doses from the vaccination card and the health facility register. If infants received their second and third vaccine doses at private facilities, a copy of the vaccination card was collected as proof of date of exposure. For each dose, the vaccine batch number, date and place of administration was documented. Recruited infants were followed up to 4 weeks after the third dose of the vaccines or death, whichever was earlier. Data were collected on tablets using a customized program developed with open source platforms (Android, PHP and MySQL version 5.1) and data including other materials uploaded, synchronized in the server and stored/deposited in a central data repository. An independent International Advisory Group (IAG) with vaccine safety experts and Brighton Collaboration representation supervised the study implementation including field visits. The IAG reanalyzed the data for validation.

Outcomes

This study captured the serious AEFIs which included both hospitalizations and deaths due to any cause during the follow-up period. The details about the events such as clinical features, diagnosis, treatment, period and place of hospitalization were captured. For hospitalizations, the study team visited the hospital for confirmation and followed till outcome to document the information. Hospitalization episodes of at least 24-hours as inpatient were further categorized as "appropriate," "inappropriate" and "indeterminate" using the Pediatric Appropriateness Evaluation Protocol–India criteria. The PAEP-India tool for child and newborn were adapted from the available tools and validated in hospitalized patients from multiple hospitals in India.[27] Each case was assessed by 2 independent pediatricians using the PAEP-India tool for child and categorized the case based on checklist criteria. A third pediatrician was consulted in cases of mismatch between the 2 reviewers. For deaths, the study confirmed the event by home visit, followed by verbal autopsy by senior investigator 4 weeks later. Causes of death were determined independently by 3 pediatricians trained in review of verbal autopsies and also referred to available hospital records.

Data Analysis

The 4 weeks following every dose of vaccination were considered to be the observation period with weekly intervals post-vaccination as separate risk periods (Figure, Supplemental Digital Content 1, http://links.lww.com/INF/D758). As almost all the serious AEFI (deaths and hospitalizations) reported in the passive national AEFI surveillance program occurred during the first week after any vaccine dose, the first week was considered as the high-risk period and the fourth week as the comparison period. It was hypothesized that, the risks of serious AEFI due to any cause, during the first and fourth weeks post-vaccination remain similar for each dose of the vaccines.

Person-time of observation was calculated based on the time from vaccine administration and the date of occurrence of an event, the date of administration of the next vaccine dose (if <28 days), the last contact date (if lost to follow-up), date of death or the first 28 days after vaccination when the next dose was delayed, according to the recommended schedule, whichever came first. Follow-up was defined by weekly risk windows after each vaccine dose split into first (0–6 days), second (7–13 days), third (14–20 days) and fourth (21–27 days) weeks. If a subsequent dose was given before the end of the fourth week, the follow-up time was censored and follow-up period was assigned to the subsequent dose. If the next dose was delayed, the person-time beyond 27 days after each dose were excluded from the analysis. Poisson regression analysis was used to estimate the incidence rates (IRs) and incidence rate ratios (IRRs) for serious AEFIs (hospitalization and all-cause deaths) during the 4-consecutive weekly risk periods after each dose separately and for all 3-doses combined. IRs were calculated by dividing the number of events by the corresponding person-time denominator and IRRs were calculated by dividing the IR in the risk period of interest by the IR in the fourth week. In a multivariate model, the IRR

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was adjusted for age at start of risk period (continuous, in days) and seasonality (quarterly; January–March, April–June, July–September, and October–December). To assess the potential of healthy vaccinee bias, we performed a sensitivity analysis to compare the risks in the first week after vaccination following the second and third doses with the risks in the fourth weeks of the preceding doses. All statistical analyses were performed using Stata version 15.0 (StataCorp LLC, TX). In addition, all deaths documented were reviewed by the national AEFI committee for causality assessment according to WHO published methodology.[20]

Sample Size

Considering the IMR in the study states to be 12–21 per 1000 live births and a neonatal mortality rate (0–28 days) of 6–15 per 1000 live births,[26] the post-neonatal mortality (29–365 days) was expected to be 6 per 1000 live births. This translated to ~0.125 infant deaths per 1000 live births per week. As there was no information about the hospitalization:death ratio in this age group from India, we assumed that for every infant death there would be ~4 hospitalizations. The required sample size was 27,422 infants to detect an IRR for hospitalizations and or deaths of 1.8 in the analyses pooling the different doses, assuming a 5% significance level ($\alpha = 0.05$) and 80% power ($\beta = 0.2$).[28] With the available sample (30,688), the observed results show that between the risk periods, we are able to detect a risk-ratio of 2.5 and 1.5 (at a 5% significance level and 80% power) of serious AEFIs (hospitalization and deaths), for individual doses and all 3-doses combined, respectively.

Ethical Approvals

The study protocol and related documents were approved by ethics committees of participating institutes. The infants were recruited after obtaining informed written consent from parents or legally authorized representatives. The study protocol and related documents were approved by Institute Ethics committees at The INCLEN Trust International (INCLEN) (vide IIEC-021 dated October 14, 2013), Government Medical College, Thiruvananthapuram (vide 01/01/2014 dated January 17, 2014), PSG Institute of Medical Sciences and Research, Coimbatore (vide 13/351 dated March 5, 2014) and World Health organization, Geneva (vide RPC625 dated January 22, 2014).

RESULTS

During the study period, 34,914 infants received their first dose of pentavalent and OPV vaccines at the public health facilities. Of these, 1377 (3.9%) infants could not be traced, 2818 (8.1%) were not eligible (1287 were not staying in the district for 6 months, and 1531 were from other districts) and consent was refused for 31 (0.1%) infants. Of the 30,688 infants recruited, 30,208 (98.4%) received their third vaccine dose and 29,728 (96.9%) completed the scheduled follow-up (Fig. 1). Of 479,523 scheduled weekly follow-up contacts (including the contacts during the intervals between the different vaccine doses till completion of 4 weeks after third dose), 89.9% were completed with averages of 4.4 (dose 1), 4.8 (dose 2) and 4.9 (dose 3) contacts for the vaccine doses (data not shown).

Baseline characteristics of the cohort are summarized in Table 1. No age or sex differences were observed across the districts. The Kollam population had a higher proportion of low and median socio-economic status and joint or extended families. Median ages of infants were 48, 83 and 118 days at the times of the first, second and third vaccine doses, respectively. A total of 622 hospitalizations and 32 deaths were documented during the follow-up period.

Within the 4 weeks after the 3 vaccine doses (per protocol study risk periods), a total of 365 hospitalizations (120, 137 and 108 after doses 1, 2 and 3, respectively) were documented. Of these 365 hospitalizations, 288 were classified as appropriate (96, 106 and 86 after doses 1, 2 and 3, respectively). A total of 257 hospitalizations (92, 136 and 29 after doses 1, 2 and 3, respectively) occurred after fourth weeks of the first and second doses of vaccination (Fig. 1). Pneumonia/acute respiratory infection (70.1%) was the leading cause followed by diarrhea (6.4%), fever (4.3%) and urinary tract diseases (3.1%) during the 4 week and post-4 week periods after the vaccine doses (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759).

Of the 32 total deaths observed, 17 deaths (8,7 and 2 after doses 1, 2 and 3, respectively) occurred within 4 weeks after 3 vaccine doses, while 15 deaths (11, 3 and 1 after doses 1, 2 and 3, respectively) occurred during post-4 weeks period, (Fig. 1). The leading causes of death included infections (31.3%), congenital malformations (28.1%), congenital heart diseases with infections (15.6%), SIDS (15.6%) and other causes (9.4%) (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759). Almost all congenital malformations were diagnosed prior to death. The SIDS was made based on Brighton Collaboration Criteria.[29] Causality assessment by the National AEFI committee classified all deaths as coincidental. Differentials in causes of hospitalization and death during the first 4-weeks (0–27 days) and post-4 weeks (28+ days) after vaccination were small (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D759). Figures 2 and 3 show the IRs of serious AEFIs (hospitalization and deaths) by age and seasonality. Higher serious AEFI (hospitalization and deaths) rates were observed during 91 and 146 days of age (Fig. 2); between October and December, with a peak in December due to higher prevalence of respiratory illnesses (Fig. 3).

Serious AEFIs (Hospitalization and Deaths) Within 4 Risk-periods

The occurrence of serious AEFIs (hospitalization and deaths) across the risk-periods for the 3 doses are summarized in Table 2. Within the 4 weeks after the 3 vaccine doses, a total of 375 serious AEFIs (365 hospitalizations, 17 deaths and 7 hospitalization and deaths) occurred (125, 140 and 110 after doses 1, 2 and 3, respectively) (Fig. 1). For all-3 doses combined, the adjusted IRRs of serious AEFIs within the first week compared with the fourth week were 0.8 [95% confidence interval, (CI): 0.6–1.0]. Compared with the fourth week, the adjusted IRRs of serious AEFIs for the first week after first, second and third doses were 1.2 (95% CI: 0.8–1.9), 0.9 (95% CI: 0.6–1.5) and 0.5 (95% CI: 0.3–0.9), respectively. IRRs for serious AEFIs were not significantly higher in any of the other risk periods (second or third weeks) either for the 3 doses separately or for all doses pooled. Analyses of hospitalizations according to appropriateness did not show any increased risk in any week compared with the fourth week after any dose (Tables, Supplemental Digital Content 3, http://links.lww.com/INF/D760, and Supplemental Digital Content 4, http://links.lww.com/INF/D761). The sensitivity analysis comparing the rates of serious AEFI in the first week after the second or third doses with the fourth week after the first or second doses did not show any differences, suggesting the absence of a healthy vaccinee effect (Table 2).

DISCUSSION

This is the first large study to examine the association between administration of routine infant pentavalent and OPV vaccines and serious AEFIs (all-cause hospitalizations and/or deaths) in India, using a self-controlled prospective cohort design. We have shown that the unadjusted and adjusted risks of hospitalizations

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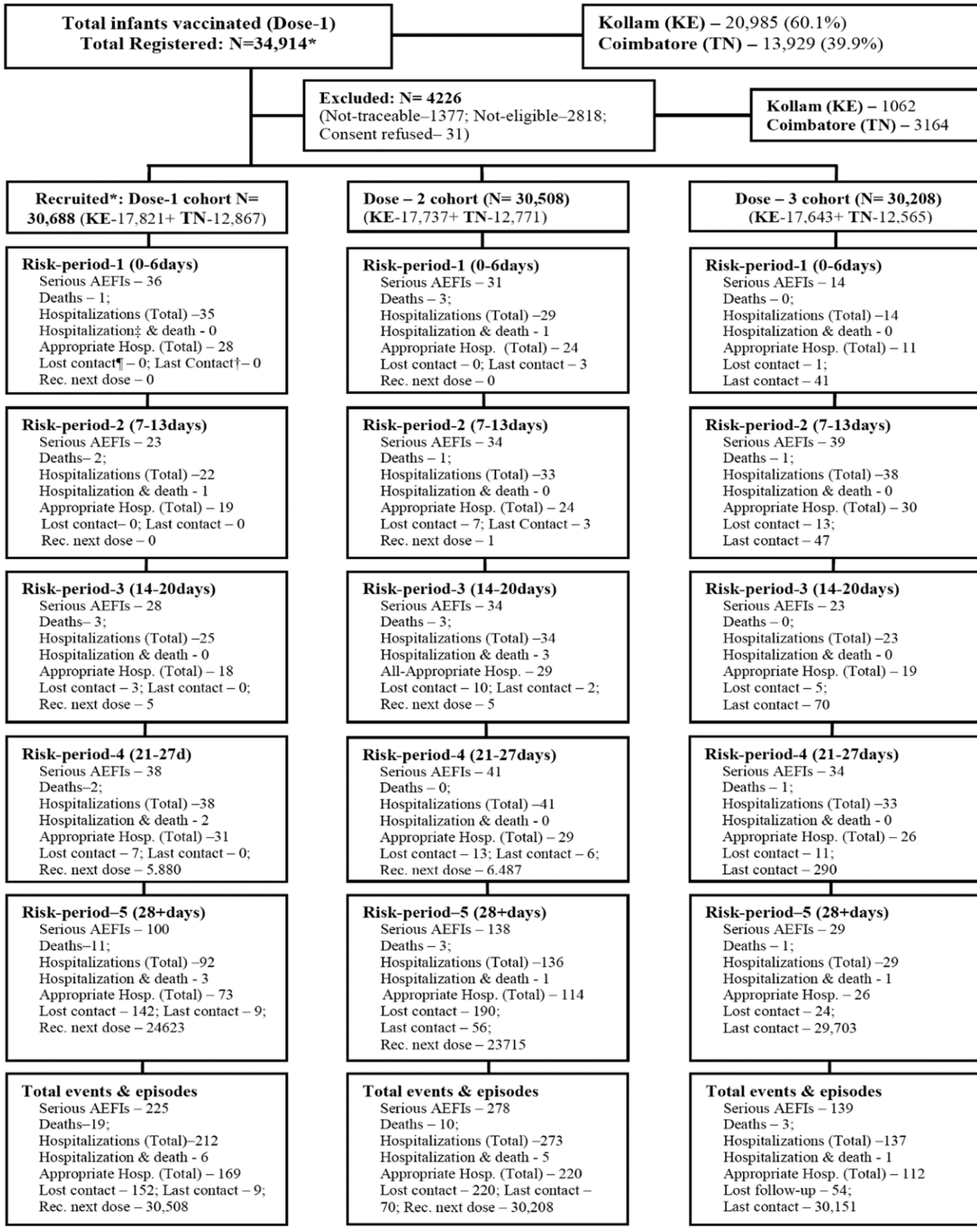


FIGURE 1. Selection and follow-up of the study population in the study districts and distribution of events. *Received first dose at public health facilities; ¶ lost contact is "Lost contact during the period"; †last contact defined as "Received respective vaccination and time period at last follow-up contact"; ‡appropriate hospitalizations as per expert opinion and identified using PAEP criteria. KE-Kerala; TN-Tamil Nadu.

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TABLE 1. Characteristics of Study Infants and Their Households

Characteristic	Kollam (N = 17,821)	Coimbatore (N = 12,867)	Combined (N = 30,688)*
Sex of the child (%)			
Males	50.3	50.4	50.3
Females	49.7	49.6	49.7
Median age at vaccination in days (P25, P75)			
Dose 1	47 (45, 50)	49 (46, 53)	48 (45, 51)
Dose 2	83 (79, 88)	83 (77, 90)	83 (78, 89)
Dose 3	118 (113, 127)	117 (109, 127)	118 (111, 127)
Birth order (%)			
1	58.7	49.4	54.8
2	37.0	43.0	39.5
≥3	4.3	7.6	5.7
Household standard of living index (%)			
Low	26.0	7.7	18.3
Middle	56.8	45.3	52.0
High	17.2	47.0	29.7
Type of family (%)			
Nuclear/elementary family	32.7	57.0	42.9
Joint/extended	67.3	43.0	57.1
Age of mother in years (mean ± SD)	25.9±4.3	24.9±4.2	25.5±4.3
Education of mother (%)			
Illiterate	0.1	2.0	0.9
Literate	99.9	98.0	99·1
Mean ± SD (in years)	12.9±2.7	11.1±3.3	12·1±3.1
Age of father in years (Mean ± SD)	32.2±4.5	30.2±4.7	31.4±4.7
Education of father (%)			
Illiterate	0.1	3.0	1.3
Literate	99.9	97.0	98.7
Mean ± SD (in years)	11.8±2.7	10.5±3.4	11.1±3.3
Received any ANC care (%)	98.9	92.5	96.2

*Total includes a small number of cases information missing for type of family, standard of living index (2 each in Kollam); Age of mother (20 in Kollam and 12 in Coimbatore); education of mother (14 in Kollam and 6 in Coimbatore); education of father (36 in Kollam and 16 in Coimbatore) and birth order (53 in Kollam and 40 in Coimbatore, respectively). Standard of living index (a proxy to household socio-economic status), constructed based on household assets and durable goods.

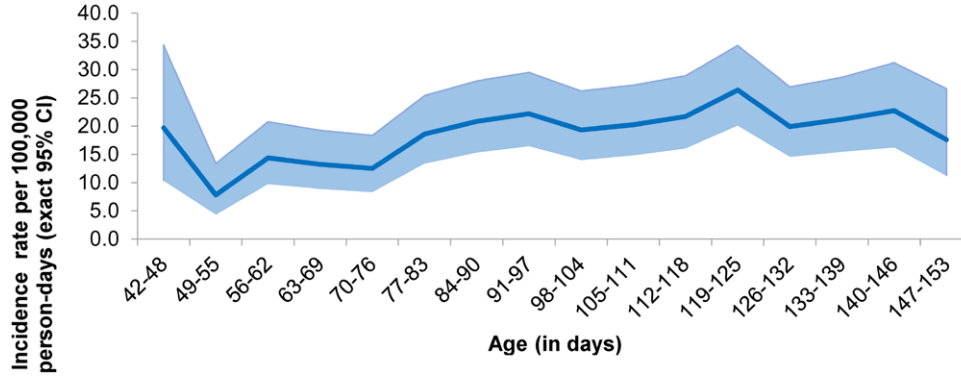


FIGURE 2. Serious AEFI (hospitalization and all-cause deaths) incidence rates (per 100,000 person-days with exact 95% CI as shaded area) by age (independent of vaccination windows). Incidence per 100,000 person-days (with exact 95% CI as shaded area); Week defined as 6-days period, starting on day-0; Person-time starts from age at first dose of vaccination and duration until the first and last day of the age period or until the Serious AEFI (hospitalization/death) events (succeeding vaccinations not considered). The hospitalization incidence presented in the figure is between 42 and 153 days of age, where 98.6% [(3,402,860/3,451,027)*100] of follow-up calls/visits were made. full color online

and deaths after any of the 3 scheduled doses of routine vaccines were similar during the first and fourth weeks post-vaccination, for either each dose separately, or all doses combined. Due to low death rates,we could not perform analyses for each individual dose but the combined analysis and analyzing the first dose only did not indicate any death clusters following vaccination.

In a highly immunized population, self-controlled study designs with defined risk windows for outcome assessment are best suited to assess possible effects during a specific time period as they are not susceptible to control selection.[30] Activation of the immune response to different vaccines occurs at different time points according to the nature of the antigen.[18,31–34] Hence, in the current study, we considered the first week after vaccination to represent the period of highest risk and the fourth week to be the comparison period. The findings of our study refute the concern of an association between serious adverse events and the pentavalent vaccine.

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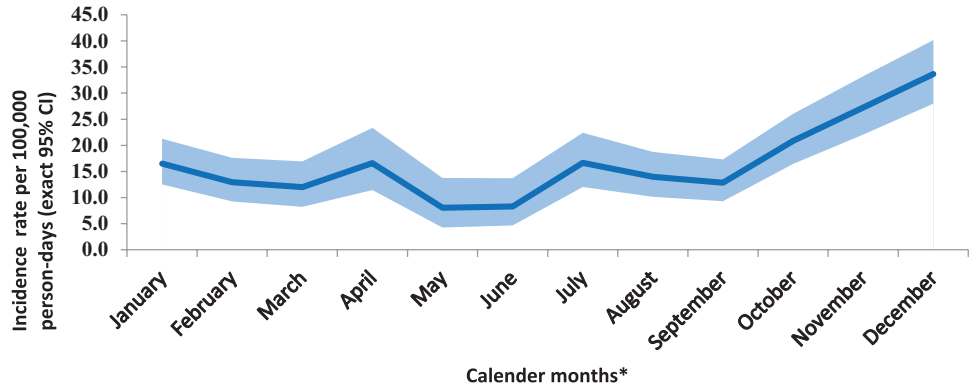


FIGURE 3. Serious AEFIs (hospitalization and all-cause deaths) incidence rates (per 100,000 person-days with exact 95% CI) by calendar months. Incidence per 100,000 person-days (with exact 95% CI); *Calendar month with 30-days period, irrespective of study year period; Person-time starts from the first day of the month and until the last day of the month period or until the Serious AEFI events (hospitalization/death). full color online

TABLE 2. Serious AEFIs (Hospitalization and All-Cause Deaths) IRs and IRRs (Unadjusted and Adjusted) After Pentavalent and Oral Polio Vaccines: Combined for Both Districts, Kollam (Kerala) and Coimbatore (Tamil Nadu), India

Vaccine dose/risk period (in days)*	Total number of		Incidence-rate (IR, with exact 95% CI)	IRRs, with 95% CI			
	Events	Person-time (in days)		Unadjusted	*P*	Adjusted†	*P*
Dose-1							
0–6 days	36	214,702	16.8 (11.7–23.2)	0.9 (0.6–1.5)	0.808	1.2 (0.8–1.9)	0.407
7–13 days	23	214,740	10.7 (6.8–16.1)	0.6 (0.4–1.0)	0.056	0.7 (0.4–1.2)	0.206
14–20 days	28	214,693	13.0 (8.7–18.9)	0.7 (0.5–1.2)	0.216	0.8 (0.5–1.3)	0.368
21–27 days‡	38	214,158	17.7 (12.6–24.4)	Reference		Reference	
Dose-2							
0–6 days	31	213,472	14.5 (9.9–20.6)	0.8 (0.5–1.2)	0.233	0.9 (0.6–1.5)	0.740
7–13 days	34	213,400	15.9 (11.0–22.3)	0.8 (0.5–1.3)	0.409	0.9 (0.6–1.5)	0.811
14–20 days	34	213,306	15.9 (11.0–22.3)	0.8 (0.5–1.3)	0.411	0.9 (0.6–1.4)	0.592
21–27 days‡	41	212,523	19.3 (13.8–26.2)	Reference		Reference	
Dose-3							
0–6 days	14	211,294	6.6 (3.6–11.1)	0.4 (0.2–0.8)	0.005	0.5 (0.3–0.9)	0.026
7–13 days	39	210,847	18.5 (13.2–25.3)	1.1 (0.7–1.8)	0.582	1.3 (0.8–2.0)	0.294
14–20 days	23	210,418	10.9 (6.9–16.4)	0.7 (0.4–1.1)	0.142	0.7 (0.4–1.2)	0.207
21–27 days‡	34	209,168	16.3 (11.3–22.7)	Reference		Reference	
All 3 doses							
0–6 days	81	639,468	12.7 (10.1–15.7)	0.7 (0.5–0.9)	0.020	0.8 (0.6–1.0)	0.068
7–13 days	96	638,987	15.0 (12.2–18.4)	0.8 (0.6–1.1)	0.226	0.9 (0.7–1.2)	0.374
14–20 days	85	638,417	13.3 (10.6–16.5)	0.7 (0.6–1.0)	0.044	0.8 (0.6–1.0)	0.062
21–27 days‡	113	635,849	17.8 (14.7–21.4)	Reference		Reference	
Sensitivity analyses							
0–6 days of dose-2	31	213,472	14.5 (9.9–20.6)	0.8 (0.5–1.3)	0.408	0.7 (0.4–1.1)	0.117
21–27 days of dose-1‡	38	214,158	17.7 (12.6–24.4)	Reference		Reference	
0–6 days of dose-3	14	211,294	6.6 (3.6–11.1)	0.3 (0.2–0.6)	0.001	0.3 (0.2–0.6)	0.001
21–27 days of dose-2‡	41	212,523	19.3 (13.8–26.2)	Reference		Reference	

*Risk period corresponds to date of hospitalization (considered as at least 24-hours stay in the hospital) or all-cause death after administration of vaccination (in days); The day of vaccination defined as day-0.

†Adjusted for seasonality (January–March, April–June, July–September, October–December); age at start of risk period (in days), using Poisson regression model.

‡Twenty-one to 27 days as reference category; IR: Number of events per 100,000 person-days of follow-time, calculated as the ratio of the hospitalizations of the person-time at risk.

The main strength of the study is the representativeness of the study cohort with respect to the population in the study area due to the high rates of participation and completion of follow-up. The socioeconomic status of the population enrolled in the study was highly representative of the overall population in the 2 districts. Use of electronic data collection enabled timely follow-up and accurate data capture with real-time monitoring. Documentation of hospitalization from both participants and hospitals allowed confirmation of causes of hospitalizations and deaths. In addition, the availability of source documents and vaccination records ensured accurate confirmation of vaccine exposure.

The main limitation was possible selection bias, as infants received first vaccine dose in the private system were not represented in the study. However, according to recent National Family Health Survey (NFHS-4, 2015–2016) data, 78% and 86% of children 12–23 months of age in Kerala and Tamil Nadu, received their vaccines from a public health facility.[25] We could not trace 3.9% infants due to inaccurate address information and 8.1% could not

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be included due to moving out or residing outside of the 2 districts. To control for confounding by contraindication to vaccination, pre-vaccination time intervals were not included in the control periods in the main analysis. Sensitivity analyses performed to assess the potential of a healthy vaccinee bias did not suggest presence of this. The study was also limited in detecting small changes for deaths, despite the large sample size.

The study found that 128 serious adverse events (120 hospitalizations and 8 deaths) occurred among 30,000 infants during the 4 weeks after receiving their first dose of pentavalent vaccine. Calculating based on Black et al[35] and projected on a 26 million annual Indian birth cohort, it is estimated that with national coverage of 100% at a minimum 26,500 infants per year will suffer a serious health event within 1 week of receiving their first dose of pentavalent vaccine, or there will be at least 21,200 events with the current 3-dose coverage of ~80% for pentavalent vaccine. These events reflect prevailing morbidities and mortality that would have occurred regardless of vaccination as documented in 2 Indian districts with low infant mortality. Investigating these events to ensure that the vaccine product or its administration are safe remains a fundamental function of the vaccine pharmacovigilance system. This highlights the importance of continued monitoring of common serious health problems in the infant population to measure the health burden and provide new epidemiologic information on the true causes of morbidity and mortality in this group.

The study did not find any increased risk of serious AEFIs (all-cause hospitalizations or deaths) in the first week following pentavalent (and OPV) vaccination and the occurrence of these AEFIs were just coincidental. That is, in the absence of temporal clustering, mortality and hospitalization rates observed in vaccinated children reflect the natural occurrence of such events in that age range in this part of India. The study findings should help to address the vaccine safety concerns and controversies around the pentavalent vaccine in India and other low- and middle-income countries, and so boost public confidence in the program. Suspicion about a possible relationship between the vaccination and serious AEFIs among parents, communities and the health providers may not only threaten the success of immunization programs but also potentially hinder introduction of newer vaccines.

CONCLUSIONS

Close monitoring of over 30,000 infants during and after receiving their first 3 doses of DTwP-HBV-Hib and OPV did not identify any relationship between the vaccinations and the occurrence of serious AEFIs (hospitalization and/or death). The study did document the huge number of serious coincidental events that can be expected even when vaccines are properly administered. In view of the recent inclusion of several new vaccines into the immunization program in India, the findings of this study will be very useful in addressing vaccine hesitancy and safety concerns to increase public acceptance and coverage.

APPENDIX

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REFERENCES

1. Roush SW, Murphy TV; Vaccine-Preventable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA*. 2007;298:2155–2163.
2. Kyu HH, Mumford JE, Stanaway JD, et al. Mortality from tetanus between 1990 and 2015: findings from the global burden of disease study 2015. *BMC Public Healt*. 2017;17:179.

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3. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375:1969–1987.
4. India State-Level Disease Burden Initiative Collaborators. Nations within a nation: variations in epidemiological transition across the states of India, 1990–2016 in the Global Burden of Disease Study. *Lancet*. 2017;390: 2437–2460.
5. Fitzpatrick M. MMR: risk, choice, chance. *Br Med Bull*. 2004;69:143–153.
6. Rosen JB, Arciuolo RJ, Khawja AM, et al. Public health consequences of a 2013 measles outbreak in New York City. *JAMA Pediatr*. 2018;172: 811–817.
7. Béraud G, Abrams S, Beutels P, et al. Resurgence risk for measles, mumps and rubella in France in 2018 and 2020. *Euro Surveill*. 2018;23 :pii=1700796.
8. Siani A. Measles outbreaks in Italy: a paradigm of the re-emergence of vaccine-preventable diseases in developed countries. *Prev Med*. 2019;121: 99–104.
9. Lisowski B, Yuvan S, Bier M. Outbreaks of the measles in the Dutch Bible Belt and in other places - new prospects for a 1000 year old virus. *Biosystems*. 2019;177:16–23.
10. Rao M, Govindarajan V. WhatsApprumours about vaccinations hamper India's drive to halt measles and rubella, The government plans to extend the campaign by a week to achieve targets. scroll.in Media report, [Internet]. Feb 24 2017; Available from: https://scroll.in/pulse/830129/rumours-about-measles-rubella-vaccine-hit-coverage.
11. Larson HJ, de Figueiredo A, Xiahong Z, et al. The state of vaccine confidence 2016: global insights through a 67-country survey. *EBioMedicine*. 2016;12:295–301.
12. World Health Organization. *Global Manual on Surveillance of Adverse Events Following Immunization*. Geneva: World Health Organization; 2014.
13. Centers for Disease Control and Prevention (CDC). Progress toward introduction of Haemophilusinfluenzae type b vaccine in low-income countries--worldwide, 2004-2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:148–151.
14. World Health Organization. Global Advisory Committee on Vaccine Safety (GACVS), Report of meeting 12–13 June 2013. *ReleveEpidemiolHebd*. 2013;88:301–312.
15. TVR Staff (Media report). Three infants in Pakistan die after receiving polio vaccine. The Vaccine Reaction [Internet]. May 13, 2018; Available from: https://thevaccinereaction.org/2018/05/three-children-die-after-receiving-polio-vaccine-in-pakistan/#_edn1.
16. Chen RT, Shimabukuro TT, Martin DB, et al. Enhancing vaccine safety capacity globally: a lifecycle perspective. *Vaccine*. 2015;33(suppl 4): D46–D54.
17. Vennemann MM, Höffgen M, Bajanowski T, et al. Do immunisations reduce the risk for SIDS? A meta-analysis. *Vaccine*. 2007;25:4875–4879.
18. Kuhnert R, Schlaud M, Poethko-Müller C, et al. Reanalyses of case-control studies examining the temporal association between sudden infant death syndrome and vaccination. *Vaccine*. 2012;30:2349–2356.
19. Walker AM, Jick H, Perera DR, et al. Diphtheria-tetanus-pertussis immunization and sudden infant death syndrome. *Am J Public Health*. 1987;77:945–951.
20. Ministry of Health and Family Welfare (MoHFW),Government of India. Results of causality assessment for reported AEFIs [Internet]. New Delhi: Ministry of Health and Family Welfare, Government of India; 2018. Available from: https://mohfw.gov.in/node/107.
21. Moneylife Digital Team Media reports. Stop unnecessary vaccination: eminent doctors, scientists to the PM. [Internet]. Dec 1, 2014; Available from: https://www.moneylife.in/article/stop-unnecessary-vaccination-eminent-doctors-scientists-to-the-pm/39645.html.
22. Nair H, Hazarika I, Patwari A. A roller-coaster ride: introduction of pentavalent vaccine in India. *J Glob Health*. 2011;1:32–35.
23. Sharma HJ, Yadav S, Lalwani SK, et al. Immunogenicity and safety of an indigenously manufactured reconstituted pentavalent (DTwP-HBV+Hib) vaccine in comparison with a foreign competitor following primary and booster immunization in Indian children. *Hum Vaccin*. 2011;7:451–457.
24. Dutta AK, Verghese VP, Pemde HK, et al. Immunogenicity and safety of a pentavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Haemophilus influenzae type B conjugate combination vaccine (Pentaxim) with hepatitis B vaccine. *Indian Pediatr*. 2009;46:975–982.
25. International Institute for Population Sciences (IIPS) and ICF. National Family Health Survey (NFHS-4), 2015-16: India [Internet]. Mumbai: IIPS; 2017. Available from: http://rchiips.org/nfhs/NFHS-4Reports/India.pdf.
26. Ministry of Home Affairs, Government of India. SRS Statistical Report 2013, Report No. 1 of 2014:75-76 [Internet]. Ministry of Home Affairs, Government of India; 2014. Available from: http://www.censusindia.gov.in/vital_statistics/SRS_Reports_2013.html.
27. Das MK, Arora NK, Poluru R, et al. Pediatric Appropriate Evaluation Protocol for India (PAEP-India): tool for assessing appropriateness of pediatric hospitalization. *Indian Pediatr*. 2018;55:1041–1045.
28. Lwanga S, Lemeshow S. *Sample Size Determination in Health Studies: A Practical Manual*. Geneva: World Health Organization; 1991.
29. Jorch G, Tapiainen T, Bonhoeffer J, et al.; Brighton Collaboration Unexplained Sudden Death Working Group. Unexplained sudden death, including sudden infant death syndrome (SIDS), in the first and second years of life: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*. 2007;25:5707–5716.
30. Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. *Epidemiol Infect*. 2011;139:1805–1817.
31. Gold M, Dugdale S, Woodman RJ, et al. Use of the Australian Childhood Immunisation Register for vaccine safety data linkage. *Vaccine*. 2010;28:4308–4311.
32. Cameron JC, Walsh D, Finlayson AR, et al. Oral polio vaccine and intussusception: a data linkage study using records for vaccination and hospitalization. *Am J Epidemiol*. 2006;163:528–533.
33. Stowe J, Andrews N, Ladhani S, et al. The risk of intussusception following monovalent rotavirus vaccination in England: a self-controlled case-series evaluation Ref. No: JVAC-D-16-01124. *Vaccine*. 2016;34:6115.
34. Patel MM, López-Collada VR, Bulhões MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med*. 2011;364:2283–2292.
35. Black S, Eskola J, Siegrist C-A, et al. Importance of background rates of disease in assessment of vaccine safety during mass immunisation with pandemic H1N1 influenza vaccines. *Lancet*. 2009;374:2115–2122.

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Incidence and etiology of congenital hypothyroidism identified by neonatal screening program in a tertiary care hospital in South India

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Abstract

Background: Congenital hypothyroidism is one of the most common preventable causes of mental retardation. The incidence and etiology of congenital hypothyroidism varies significantly across the globe.

Methods: In this retrospective observational study we aimed to find out the incidence and etiology of congenital hypothyroidism identified by neonatal screening program. We included all neonates who had their thyroid stimulating hormone screening done in a tertiary care hospital of South India between January 2014 and June 2020 and were diagnosed as a case of congenital hypothyroidism. The growth patterns, clinical features, thyroxine dose requirement during follow-up were also studied.

Results: There were 23 babies diagnosed with congenital hypothyroidism during the study period. The incidence rate was 1 in 917 live births for inborn babies, and dyshormogenesis (60%) was the most common etiology. Two babies had clinical features associated with congenital hypothyroidism. None of the babies had clinical features of congenital hypothyroidism during follow-up and their growth & development were normal for age. Babies who required dose increments of thyroxine supplement turned out to be permanent congenital hypothyroidism.

Conclusions: Incidence of congenital hypothyroidism was high in our population. Dyshormogenesis was the most common etiology. Majority of congenital hypothyroidism babies were asymptomatic at diagnosis, so screening is a vital tool for early diagnosis. Babies who required thyroxine dose increment with age tend to be permanent congenital hypothyroidism.

Keywords: Congenital hypothyroidism, Thyroid dyshormogenesis, Thyroid dysgenesis, Dose increments, Newborn screening

Introduction

Congenital hypothyroidism (CH) is an important cause of mental retardation in children.[1] With the introduction of newborn screening, most of the babies with CH are being diagnosed during the neonatal period even before the onset of symptoms. The most commonly used screening protocol is serum thyroid stimulating hormone (TSH) measurement owing to its superior sensitivity and specificity. With the increasing neonatal screening coverage all over the world, the incidence of CH is also increasing.[2]

The incidence rate of CH varies by geographic location and ethnicity.[3] There is also significant difference in the etiology of CH based on the population being studied. Our country being a mixed population with lot of inbreeding, it will be useful if the local incidence of CH and its etiology is known for better management.

Methods

We conducted this retrospective study in a tertiary level neonatal unit at PSG institute of medical science and research hospital, Coimbatore in South India. The primary

objective of the study was to find out the incidence and etiology of CH identified through newborn screening program. Our secondary objective was to follow-up the growth & development and to find out whether the dose requirement of thyroxine in these babies during follow-up can help us to differentiate permanent from transient CH cases. The study was approved by hospital ethical committee. All neonates (<30 days of life) from January 2014 to June 2020 (78 months) for whom TSH screening was done in the hospital were included in the study. We had excluded babies who were diagnosed and referred as a case of CH from other hospitals.

Serum TSH was used as screening tool for congenital hypothyroidism, which is measured by taking a venous blood, usually around 72 hours of life. In case of early discharge, samples were taken prior to discharge. Serum TSH, free T4 (fT4) and free T3 (fT3) were measured in hospital's biochemistry laboratory using sandwich electroluminescence immunoassay using cobas e601 (Roche Ltd). All neonates with TSH values <10 mIU/L were considered screen negative. If the initial TSH screening value was in the range of 10-39 mIU/L, then TSH value was repeated at around 14 days of age. All babies with serum TSH ≥40 mIU/L at any time or ≥10 mIU/L on two occasions were considered screen positive. Babies with very high TSH values were given a value of >100 mIU/L by the hospital biochemistry lab instead of absolute value.

All screen positive cases were evaluated with thyroid hormone profile, ultrasound (USG) thyroid and technetium (Tc-99m) pertechnetate thyroid scintigraphy. Etiological diagnosis was primarily based on scintigraphy results. If it shows increased tracer uptake, then it was considered to suggest organification defect and a diagnosis of dyshormogenesis was made. If there was ectopic tracer uptake, then ectopic thyroid was diagnosed and if no tracer uptake was seen then agenesis was considered. If only USG thyroid report was available, etiology of dyshormogenesis was made if the gland was present and dysgenesis was considered if it was absent. Treatment with levothyroxine was started as early as possible after evaluation. During follow-up, growth and development of these babies were monitored using World Health Organization (WHO) growth charts and Trivandrum development screening chart respectively.

Babies with CH were identified by scrutinizing the screen positive babies' case records from the medical records department. The clinical, demographic and laboratory data of these babies were obtained from the hospital's computerized database and baby's case records. Few babies had some data missing which were obtained through telephonic conversation with the parents. The follow up data including growth & development, clinical features of CH, TSH & fT4 values, the dose adjustments of thyroxine were all collected.

Data were entered in Microsoft excel and analyzed by Epi Info™ software. Results were presented as mean±SD or median with IQR for continuous variables and as percentage for discrete variables.

Results

A total of 19282 babies were born during the study period of 78 months, among them 18349 babies (95%) had their TSH screening done and 20 babies were diagnosed as CH giving an incidence rate of 1 in 917 live births. Among the 133 out-born neonates screened in our hospital, three babies were diagnosed as CH. Thus 23 babies were diagnosed as CH overall. The baseline characteristics of these babies are given in table 1.

Table 1: Baseline characteristics of the babies diagnosed with CH

Characteristics	Value
Male:Female	1:1.6
Consanguinity	3 (2,4)
Gestational age (weeks)	38.3±1.6
Birth weight (grams)	2969±449
Mode of delivery	n (%)
Vaginal	15 (65)
Caesarean	8 (35)
Family history of CH	3 (2,4)
Clinical features suggestive of CH	4 (17)
Cord TSH >200 mIU/L	3 (13)

GA – Gestational age; LBW- Low birth weight.

The clinical features, thyroid function test values, USG thyroid and Tc99 radio nucleotide isotope scan reports at diagnosis are provided (table 2). Two babies had clinical features associated with CH at presentation. One baby had Down syndrome and the other presented with delayed passage of meconium. In two babies USG thyroid report showed gland in situ but did not have scintigraphy report available and an etiological diagnosis of dyshormogenesis was made. A total of twenty babies had the etiology of CH available and among them more than half of the babies (60%) were diagnosed with dyshormogenesis. Among the babies with thyroid dysgenesis (40%), ectopic thyroid was the most common etiology and all ectopic thyroids were lingual thyroid.

Only seventeen babies had both etiological diagnosis and complete follow-up details (table 3) and among them none of the babies had any growth or developmental abnormalities. More than half of these babies (59%) did not require dose increment during follow-up and among them dyshormogenesis (90%) was the most common etiology. Those babies who required dose increments during follow-up (41%) had dysgenesis as the most common etiology (72%). Among those babies who had crossed three years of age (n=8), the thyroxine

supplements were stopped in four babies and all of them had dyshormogenesis as the etiology of CH.

Table 2: Clinical, biochemical and radiological profile of the babies with congenital hypothyroidism

Serum TSH level at diagnosis (μIU/ml)	>100		14 (61)
	40-100		4 (17)
	10-39		5 (22)
Mean serum TSH level at 21 days of life (μIU/ml)			1.1±0.6
Mean serum TSH level at 1 year of life (μIU/ml)			2.25±0.89
Goitre on USG	Absent		3 (38)
	Present		5 (63)
Tc99 scan findings	Dyshormogenesis		10 (56)
	Dysgenesis (n=8)	Agenesis	3 (17%)
		Lingual thyroid	5 (28%)
Clinical features at diagnosis	Asymptomatic		21 (91)
	delayed passage of meconium		1 (5)
	down syndrome		1 (5)

TSH- Thyroid stimulating hormone; USG- Ultrasonogram; Tc99- Technetium-99 isotope.

Table 3: Thyroxine dose requirement during follow-up and its association with aetiology of congenital hypothyroidism

Thyroxine dose requirement at 3 years of age	Not required dose increment (n=10)	Dyshormogenesis	9 (90)
		Dysgenesis	1 (10)
	Dose increment at least once during follow-up (n=7)	Dyshormogenesis	2 (28)
		Dysgenesis	5 (72)
Thyroxine supplements stopped at 3 years of age	Successfully stopped treatment (n=4/8)	Dyshormogenesis	4 (100)
		Dysgenesis	0
	Continued treatment (n=4/8)	Dyshormogenesis	1 (25)
		Dysgenesis	3 (75)

Discussion

In our study, the incidence of CH was 1 in 917 and dyshormogenesis was the commonest cause. Several studies have arrived at a different incidence rate for CH. In the study conducted in United States by Waller et al,4 non-Hispanic Black had lower incidence (1 in 11494) while Asian had the higher incidence (1 in 2506) of congenital hypothyroidism. Similar difference among ethnic groups were obtained in other studies.[5,6] In the study done by Arasar Seeralar et al in Chennai, the incidence rate of CH was 1.7 in 1000.[7] Similar high incidence rates were reported in other Indian studies done in different parts of the country.[8,9] High incidence rate found in population of Iran was attributed to a closed community with high inbreeding rate.[10] In India too this could be the reason for higher incidence due to marriage within the caste/community leading to inbreeding.

This study showed that, dyshormogenesis (60%) was the most common etiology of CH in our population. On the contrary, in most of the studies done in other parts of the world, dysgenesis contributed to more than 80% of the CH cases.[11] Since most of the cases of dyshormogenesis are inherited conditions, the high incidence is expected in a closed community like our population. Among those with dysgenesis, almost two third of the babies were diagnosed as Lingual thyroid, similar to the finding reported by Castanet et al.[12] This is in contrast to the study by Devi Dayal et al, where agenesis was reported more common than ectopia.[13]

Considering the clinical features at diagnosis, most of the babies were asymptomatic (91%) underscoring the importance of screening. Two babies had clinical features associated with CH, one with delayed passage of meconium and the other had Down syndrome. Though there were three babies who had neonatal jaundice requiring treatment with phototherapy, none had prolonged jaundice which is classically described in CH. Except these two babies, rest of them would have been missed otherwise and could have potentially ended up with morbidity without the screening process.

Among the babies who required thyroxine dose increment (41%) during the follow-up, dysgenesis (72%) was the most common etiology and these babies are likely to require lifelong thyroxine supplements. In those babies where thyroxine dose requirement did not increase with age (59%), dyshormogenesis (90%) was the most common etiology. All the babies in whom the thyroxine supplements were successfully stopped at 3 years of age belonged to this group. This shows that babies who don't require dose increment turn out to be transient CH and tend to be having dyshormogenesis. A similar finding was arrived in other studies where permanent CH had increased dose requirement as age increases compared to transient CH.[14,15] In our study, the etiological diagnosis of dyshormogenesis were made based on increased iodine trapping seen in the radionucleotide studies but confirmation with genetic studies could not be done. The fallacy of this approach is that there are several causes of transient CH which presents with increased thyroid trapping by scintigraphy at diagnosis. These include maternal iodine deficiency, transient dyshormogenesis due to haplodeficiency mutation affecting DUOX2 and DUOXA2 and other genes.[16-18] In developing country like

India where nutritional deficiency is very common, one can expect that a significant proportion of these babies with increased iodine trapping in radionucleotide scintigraphy may actually be having transient CH due to maternal iodine deficiency. One baby diagnosed as agenesis by scintigraphy (USG thyroid not available) had decreased dose requirement as age increased which is not expected in a case of true agenesis. The baby's mother was diagnosed as Hashimoto's thyroiditis since her adolescence. So the initial scintigraphy finding was likely due to the TSH blocking effect of trans placentally transferred maternal antibodies.[19] So in resource limited settings where genetic diagnosis could not be done, USG thyroid along with radio isotope scan will give a reliable etiological diagnosis as compared to either of it done alone.

□□□□□□□□□□

To conclude, the incidence of CH in our population was 1 in 917 live births. The most common etiology was dyshormogenesis followed by dysgenesis. Almost all the babies with CH were asymptomatic during neonatal period which makes screening a vital tool for diagnosis. Those babies who required increment in thyroxine dose as age increases tend to be permanent CH.

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1. Bongers-Schokking JJ, Koot HM, Wiersma D, Verkerk PH, de Muinck Keizer-Schrama SM. Influence of timing and dose of thyroid hormone replacement on development in infants with congenital hypothyroidism. J Pediatr. 2000;136(3):292-7.
2. McGrath N, Hawkes CP, McDonnell CM, Cody D, O'Connell SM, Mayne PD, et al. Incidence of Congenital Hypothyroidism Over 37 Years in Ireland. Pediatrics. 2018;142(4):e20181199.
3. Olney RS, Grosse SD, Vogt RF. Prevalence of congenital hypothyroidism--current trends and future directions: workshop summary. Pediatrics. 2010;125:31-6.
4. Waller DK, Anderson JL, Lorey F, Cunningham GC. Risk factors for congenital hypothyroidism: an investigation of infant's birth weight, ethnicity, and gender in California, 1990-1998. Teratology. 2000;62(1):36-41.
5. Hinton CF, Harris KB, Borgfeld L, Drummond-Borg M, Eaton R, Lorey F, et al. Trends in Incidence Rates of Congenital Hypothyroidism Related to Select Demographic Factors: Data From the United States, California, Massachusetts, New York, and Texas. Pediatrics. 2010;125(2):37-47.
6. Albert BB, Cutfield WS, Webster D, Carll J, Derraik JG, Jefferies C, et al. Etiology of increasing incidence of congenital hypothyroidism in New Zealand from 1993-2010. J Clin Endocrinol Metab. 2012;97(9):3155-60.
7. Seeralar AT, Arasar J, Ganesh S. Newborn screening for congenital hypothyroidism in a tertiary care centre. Int J Contemp Pediatr. 2016;3(2):456-60.
8. Kumar K, Ranieri E, Fletcher J. Newborn Screening for Congenital Hypothyroidism in India- Is overdue. J Neonatal Biol. 2014;3(2):1-3.
9. Sanghvi U, Diwakar KK. Universal newborn screening for congenital hypothyroidism. Indian Pediatr. 2008;45(4):331-2.
10. Dorreh F, Chaijan PY, Javaheri J, Zeinalzadeh AH. Epidemiology of congenital hypothyroidism in Markazi Province, Iran. J Clin Res Pediatr Endocrinol. 2014;6(2):105-10.
11. Rastogi MV, LaFranchi SH. Congenital hypothyroidism. Orphanet J Rare Dis. 2010;5(1):17.
12. Castanet M, Polak M, Bonaïti-Pellié C, Lyonnet S, Czernichow P, Léger J, et al. Nineteen years of national screening for congenital hypothyroidism: familial cases with thyroid dysgenesis suggest the involvement of genetic factors. J Clin Endocrinol Metab. 2001;86(5):2009-14.
13. Dayal D, Sindhuja L, Bhattacharya A, Sodhi KS, Sachdeva N. Agenesis and not ectopia is common in North Indian children with thyroid dysgenesis. Indian J Endocrinol Metab. 2014;18(1):97-9.
14. Ford GA, Denniston S, Sesser D, Skeels MR, LaFranchi SH. Transient versus Permanent Congenital Hypothyroidism after the Age of 3 Years in Infants Detected on the First versus Second Newborn Screening Test in Oregon, USA. Horm Res Paediatr. 2016;86(3):169-77.
15. Oron T, Lazar L, Ben-Yishai S, Tenenbaum A, Yackobovitch-Gavan M, Meyerovitch J, et al. Permanent vs Transient Congenital Hypothyroidism: Assessment of Predictive Variables. J Clin Endocrinol Metab. 2018;103(12):4428-36.
16. Imge Hulur I, Hermanns P, Nestoris C, Heger S, Refetoff S, Pohlenz J, et al. A single copy of the recently identified dual oxidase maturation factor (DUOXA) 1 gene produces only mild transient hypothyroidism in a patient with a novel biallelic DUOXA2 mutation and monoallelic DUOXA1 deletion. J Clin Endocrinol Metab. 2011;96(5):E841-5.
17. Nagasaki K, Minamitani K, Anzo M, Adachi M, Ishii T, Onigata K, et al. Guidelines for Mass Screening of Congenital Hypothyroidism (2014 revision). Clin Pediatr Endocrinol. 2015;24(3):107-33.
18. 1Secchi LA de A, Mazzeu JF, Córdoba MS, Ferrari Í, Ramos HE, Neves F de AR. Transient neonatal hypothyroidism in a boy with unbalanced translocation t(8;16). Arq Bras Endocrinol Amp Metabol. 2012;56(8):564-9.

19. Brown RS, Bellisario RL, Botero D, Fournier L, Abrams CA, Cowger ML, et al. Incidence of transient congenital hypothyroidism due to maternal thyrotropin receptor-blocking antibodies in over one million babies. J Clin Endocrinol Metab. 1996;81(3):1147-51.

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SCIENTIFIC LETTER

Serum Thyroid Stimulating Hormone Screening and Influence of Perinatal Factors

Sudhakar Palanisamy[1] · Ramesh Srinivasan[1] · Suvetha Kannappan[2] · JayaChandran Ganesan[3]

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To the Editor: Congenital hypothyroidism (CH) screening using thyroid stimulating hormone (TSH) is being done worldwide but the cutoff values vary widely in different countries and is influenced by perinatal factors [1–3]. We aimed to analyze the TSH value distribution in venous blood sampled for newborn screening and to identify the perinatal factors influencing it.

This retrospective observational study was done between January 2014 and June 2019 in south India. Newborns with their TSH screening value available were included. Babies who were < 33 wk and < 1500 g were excluded. Venous blood TSH cut-off < 10 mIU/L was considered screen negative. Institutional ethical committee approved the study.

Among a total of 15,586 babies born, analysis was done with 14,738 babies. Median gestational age and birth weight were 38 wk (37–39) and 2950 g (2670–3230), respectively. There were 51.5% male, 89.2% term babies, and 61.5% vaginally born. Median TSH value was 2.81mIU/L with 95th percentile of 11.33 mIU/L. A higher median TSH value was seen in term babies ($p < 0.001$), vaginal birth ($p < 0.001$), male gender ($p < 0.001$), birth weight ≥ 2500 g ($p < 0.004$) and babies sampled at 24–47 h of life ($p < 0.001$). Majority of population (88%) was represented by term babies between 48 and 96 h of life and this subset of population had a median TSH value of 2.85 mIU/L (1.42, 5.41) with 95th percentile being 11.33 mIU/L.

1072 (7.2%) babies were screen positive and 19 were confirmed to have CH. Since our TSH screening cutoff was < 10 mIU/L but 95th percentile in our study was 11.33 mIU/L, we followed up babies with TSH value between 10 and 11.33 mIU/L. Among 339 babies with TSH between 10 and 11.33 mIU/L, follow-up was available for 305 (90%). Repeat TSH at 2 wk of age was normal for 118 babies (34.8%). Parents were contacted for 187 babies (55.2%), aged 1–6 y and were reported to be normal.

A TSH screening cutoff of >11.33 mIU/L may be considered as an appropriate threshold for babies screened with venous blood between 48 and 96 h of age. In our study population this would have resulted in 31.6% reduction in recall rate without missing a case of CH. However a large-scale prospective population-based study is needed.

Compliance with Ethical Standards

Conflict of Interest None.

References

1. American Academy of Pediatrics, Rose SR, Section on Endocrinology and Committee on Genetics, American Thyroid Association, Brown RS, Public Health Committee, Lawson Wilkins Pediatric Endocrine Society, Foley T, et al. Update of newborn screening and therapy for congenital hypothyroidism. Pediatrics. 2006;117:2290–303.
2. Sudhanshu S, Riaz I, Sharma R, Desai MP, Parikh R, Bhatia V. Newborn screening guidelines for congenital hypothyroidism in India: recommendations of the Indian Society for Pediatric and Adolescent Endocrinology (ISPAE) - part II: imaging, treatment and follow-up. Indian J Pediatr. 2018;85:448–53.
3. Léger J, Olivieri A, Donaldson M, et al. European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. Horm Res Paediatr. 2014;81:80–103.

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Case Report

Late-Onset Hyporegenerative Anemia in an Infant with Rhesus Isoimmunization

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Abstract

Late-onset anemia is a complication of hemolytic disease of fetus and newborn. It includes late hemolytic anemia and late hyporegenerative anemia. This case report is to discuss an Rh isoimmunized infant who presented with late-onset hyporegenerative anemia. The infant had low reticulocyte count, negative direct antiglobulin test, high erythropoietin, and high ferritin levels. She was treated with repeated blood transfusions and improved after 4 months of age. The follow-up of infants with Rh isoimmune hemolytic disease is needed for identification of late-onset anemia.

Keywords: *Hydrops fetalis, hyporegenerative anemia, late-onset anemia, Rh hemolytic disease*

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Introduction

Rhesus D isoimmunization is a common cause for severe hemolytic disease of fetus and newborn (HDFN). Late-onset anemia occurring after 1st week of life is found in 71%–83% of neonates with Rh hemolytic disease, and the incidence is highest among infants who received intrauterine transfusion. It commonly develops between 2 and 6 weeks after birth and usually resolves by the 3rd month of life.[1] This case report discusses an Rh isoimmunized infant who presented during the 2nd month of life with severe anemia.

Case Report

A female neonate was delivered at 35 weeks gestational age by cesarean section to a fourth gravida mother. The mother's blood group was A-negative with positive indirect antiglobulin test (IAT). Anti-D was given in the first two pregnancies but not in the third pregnancy. In the current pregnancy, antenatal ultrasound scan at 31 weeks of gestational age showed features of hydrops fetalis with ascites, pleural effusion, and hepatosplenomegaly. Intrauterine transfusion was done twice at 32 and 34 weeks of gestational age. The baby had normal transition at birth. On examination, the baby had hepatosplenomegaly but no pallor or icterus. Double-volume exchange transfusion was done at 7 h of life in view of *in utero* hydrops fetalis. Serum bilirubin sequentially monitored was not in treatment range. Packed red blood cell (PRBC) transfusion was done on day 3 of life for anemia with hemoglobin 9.1 g/dl, and the baby was discharged on day 8 of life [Table 1].

At review on day 50 of life, the infant showed adequate weight gain but had hemoglobin of 4.2 g/dl. Peripheral smear showed severe normocytic normochromic anemia with normal leukocyte and platelets. Reticulocyte count was low (0.2%). Direct antiglobulin test (DAT) was negative, but IAT was positive. Serum erythropoietin level was high. Parvovirus B19 DNA polymerase chain reaction was negative. Work-up for iron deficiency and megaloblastic anemia showed normal reports, except for increased serum ferritin level. Bone marrow study showed normoblastic erythroid hyperplasia; the myeloid series and megakaryocytes were normal. Hemoglobin electrophoresis was normal. Hence, late-onset hyporegenerative anemia of rhesus hemolytic disease was diagnosed. The baby received PRBC transfusions on day 52, 60, and 84 of life. The baby was

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Table 1: Hematological parameters during the course of illness

	Hemoglobin (g/dl)	Treatment given	Reticulocyte count (%)	Erythropoietin (mIU/ml)	DAT	IAT	Serum ferritin (ng/ml)
D1	14.9	DVET	0.3		Positive		
D2	10.1						
D3	9.1	PRBC	0.2				
D5	12.1						
D50	4.2	PRBC	0.2	840	Negative	Positive	673
D52	7.9						
D57	6.3		0.3				
D59	6.3	PRBC	0		Negative	Positive	
D60	11.3						
D67	9.1						
D75	7.9						
D82	6.2	PRBC + IVIg	0.1	477	Negative	Positive	740
D84	12.3						
D99	10						
D113	9.4		6				760
D142	10.3		1.2		Positive	Positive	454
D203	12				Positive	Negative	258

D – Day; DVET – Double-volume exchange transfusion; PRBC – Packed red blood cells; IVIg – Intravenous immunoglobulin; DAT – Direct antiglobulin test; IAT – Indirect antiglobulin test

on folate and multivitamin supplements. Intravenous immunoglobulin (IVIg) was given on day 85 of life. At 4 months of age, the baby's hemoglobin level stabilized with increase in reticulocyte count. Liver span became normal, while spleen was not palpable. The infant did not require further transfusions. Follow-up showed hemoglobin in improving trend. At 7 months of age, IAT became negative, and DAT was positive. The Rh type that was hitherto reported as equivocal due to mixed field reaction was now reported as O-positive.

Discussion

HDFN is caused by the destruction of red blood cells (RBCs) of the fetus or newborn by maternal IgG alloantibodies that have crossed the placenta. Rh isoimmunization may lead to late hyporegenerative anemia. Pathogenesis implicated is antibody-dependent intramedullary destruction of RBCs and its precursors and bone marrow suppression by intrauterine/postnatal RBC transfusion. This anemia is characterized by normal bilirubin level and low or absent reticulocyte count. In contrast, late hemolytic anemia following Rh isoimmunization is characterized by active bone marrow erythropoiesis and elevated reticulocyte counts associated with elevated bilirubin levels.[2]

Low reticulocyte count in the peripheral smear with erythroid hyperplasia in bone marrow suggests antibody-mediated intramedullary destruction of red cell precursors. In addition, negative DAT with positive IAT in the baby suggests severe hemolysis because of the mother's Rh antibody, with the presence of free circulating maternal antibodies and absent baby's native red cells for forming antigen antibody complex.[3] Thus, the baby's circulating RBCs were probably only transfused compatible cells.

Serum erythropoietin level may be low relative to the degree of anemia in babies with late hyporegenerative anemia, but it may not be so always. Moreover, treatment with recombinant erythropoietin (rEPO) may be ineffective when anti-Rh (D) antibody titers are high. Thus, in late hyporegenerative anemia in HDFN, available data are not sufficient to support beneficial effects of rEPO.[4] Hence, erythropoietin therapy may not be essential in all such cases.

IVIg reduces the immune-mediated hemolysis by blocking Fc receptors in the reticuloendothelial system of neonate and enhances maternal antibody clearance. A meta-analysis by Louis *et al*. on the role of IVIg in isoimmune hemolytic disease of newborn showed that IVIg did not reduce the need for top-up transfusion in both Rh and ABO isoimmunization.[5] A meta-analysis by Li *et al*. has found that IVIg therapy did not cause any significant difference in the incidence of late anemia among babies with isoimmune hemolytic disease.[6] A study by Al-Alaiyan *et al*. on effects of IVIg on late hyporegenerative anemia due to rhesus HDFN has stated that there was no significant difference on the incidence of late anemia and requirement of top-up transfusion.[7] Hence, routine use of IVIg should not be encouraged in managing HDFN.

High iron storage as evidenced by increase in serum ferritin level occurs in hyporegenerative anemia due

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to treatment with multiple intrauterine transfusion and postnatal RBC transfusion. Rath *et al*. have reported that in neonates with red cell isoimmune hemolytic disease, iron overload occurs in 70% of neonates at birth, and the incidence of iron overload gradually decreases within the first 3 months, once the blood transfusions are discontinued.[8] Hence, iron essential for hemoglobin synthesis by erythroblast has to be supplemented only if serum ferritin levels are low or in normal range.

Conclusion

In infants with HDFN, regular follow-up of hemoglobin, reticulocyte count, and serum ferritin level are advisable till hematological parameters stabilize in normal range. Late-onset anemia can be managed with regular top-up transfusions and the role of rEPO and IVIg is to be decided on case-to-case basis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the legal guardian has given his consent for images and other clinical information to be reported in the journal. The guardian understands that names and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Al-Alaiyan S, Al Omran A. Late hyporegenerative anemia in neonates with rhesus hemolytic disease. J Perinat Med 1999;27:112-5.
2. Ree IM, Smits-Wintjens VE, van der Bom JG, van Klink JM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease. Expert Rev Hematol 2017;10:607-16.
3. Parker V, Tormey CA. The direct antiglobulin test: Indications, interpretation, and pitfalls. Arch Pathol Lab Med 2017;141:305-10.
4. Pessler F, Hart D. Hyporegenerative anemia associated with Rh hemolytic disease: Treatment failure of recombinant erythropoietin. J Pediatr Hematol Oncol 2002;24:689-93.
5. Louis D, More K, Oberoi S, Shah PS. Intravenous immunoglobulin in isoimmune haemolytic disease of newborn: An updated systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed 2014;99:F325-31.
6. Li ZH, Wang J, Chen C. Meta analysis of the effect of immunoglobulin infusion on neonatal isoimmune hemolytic disease caused by blood group incompatibility. Zhonghua Er Ke Za Zhi 2010;48:656-60.
7. Al-Alaiyan S, Ahmad HA, Al-Hazzani F, AlHasan M, Dawoud M, Khadawardi E, *et al*. Effects of intravenous human immunoglobulin on late hyporegenerative anemia secondary to rhesus hemolytic disease of the newborn. Int J Pediatr Adolesc Med 2014;1:73-7.
8. Rath ME, Smits-Wintjens VE, Oepkes D, Walther FJ, Lopriore E. Iron status in infants with alloimmune haemolytic disease in the first three months of life. Vox Sang 2013;105:328-33.

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Case Report

Unusual Presentation of Cow's Milk Protein Allergy in a Neonate

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Abstract

Cow's milk protein allergy is the most common food protein allergy in children. We report a neonate on breast feeds and formula feeds presenting on day 9 of life with blood in stools, anemia, shock, respiratory failure with multiorgan dysfunction, and recovered with intensive care. Colonoscopy and biopsy confirmed the diagnosis as allergic colitis. Neonate improved with cow's milk protein-eliminated diet. Food protein allergy can present with protean signs and symptoms; high index of suspicion is needed for the prompt early diagnosis.

Keywords: *Colonoscopic biopsy, cow's milk protein allergy, food protein-induced allergic proctocolitis, food protein-induced enterocolitis syndrome*

Introduction

Cow's milk protein allergy (CMPA) is the most common food protein allergy in children.[1-4] Cow's milk-based infant formula is the most common allergen triggering food protein allergy in infants. Although maternal cow's milk intake can lead to secretion of cow's milk protein in breast milk, it is rarely the sole trigger for CMPA.[5] The current case report is to discuss an unusually severe presentation of CMPA in a neonate.

Case Report

A 9-day-old girl presented with blood in stools and marked pallor. She was born at term with birth weight of 2600 g. Vitamin K was given at birth. She was on breastfeeding with occasional formula feeds. She was passing small volume stools 4–5 times a day which was blood stained for the past few days. This was associated with progressive pallor in the baby. There was no significant vomiting. She had good weight gain and crossed her birth weight.

She was lethargic with poor respiratory efforts at admission and started on mechanical ventilation. She had shock requiring fluid boluses and inotrope. There was no hepatosplenomegaly or other bleeding manifestations. The perianal region was normal. Admission hemoglobin was 2.2 g/dl, and packed red blood cell (PRBC) transfusion was done. Hemolytic workup was negative. She had elevated liver enzymes, coagulopathy, thrombocytopenia, hypoglycemia, acute kidney injury, and seizures during the course of hospitalization and was managed appropriately. Her blood culture was negative. Echocardiography initially showed mild ventricular dysfunction which improved later. Ultrasound cranium and abdomen were normal. Barium study ruled out malrotation with intermittent volvulus. PRBC transfusions were repeated on days 2, 5, and 10 of hospitalization. Baby improved and was started on breast milk feeds on day 3. She was discharged on day 10 with provisional diagnosis of clinical sepsis with multiorgan dysfunction. Her discharge hemoglobin was 10.7 g/dl.

During the follow-up on day 37 of life, her weight gain was inadequate and hemoglobin was 7.6 g/dl. After 1 week, mother reported the frequent episodes of blood in stools and was continuing on formula feeds once or twice a day. She was admitted for poor weight gain with hemoglobin 5.9 g/dl and high reticulocyte count. There were no features suggestive of hemolysis. Radionucleotide scan for Meckel's diverticulum was normal. Ultrasound abdomen ruled out duplication cyst. Serum immunoglobulin E was 45 IU/ml (normal <1.5 IU/ml). Esophago-gastro-duodeno (OGD) scopy was normal. Colonoscopy showed the features

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suggestive of severe allergic colitis. Histopathology of colonoscopic biopsy showed lymphoid hyperplasia with focal tissue eosinophilia (50/high-power field) and occasional microgranulomas suggestive of allergic colitis.

Breastfeeds were continued for the baby with dairy products eliminated from mother's diet. Mother was supplemented with calcium and Vitamin D. Elemental aminoacid-based formula was used for top up feeds. Baby was transfused PRBC on day 2 of admission and was discharged on day 8 with hemoglobin of 9 g/dl. Subsequent follow-up showed improvement in hemoglobin with adequate weight gain. Complementary feeds were started at 5 months of age. Cow's milk-related products were eliminated from baby's diet and Vitamin D with calcium supplements was added. At 10 months' follow-up, she had good weight gain and normal development.

Discussion

Food protein allergies are classified broadly into food protein-induced enterocolitis syndrome (FPIES), food protein-induced allergic protocolitis (FPIAP), food protein-induced enteropathy, and eosinophilic gastroenteritis. FPIAP seems to be the most common type of food protein allergy.[1]

FPIAP is a clinical diagnosis with predominant manifestation being blood in stools. Among infants presenting with blood in stools, 18%–65% might be having FPIAP.[2] Most of the babies appear clinically well though failure to thrive and irritability can occur rarely. Usually, they present within a few months of age and sometimes even as early as 1 week of age. Vitamin K deficiency, malrotation with volvulus, intussusception, and Meckel's diverticulum can also present with blood in stools at this early age and must be ruled out before considering allergic colitis.[6] The case reported here presented with blood in stools but also had life-threatening anemia and shock which is unusual in FPIAP.

FPIES is known to present with hemodynamic instability and hypotension in severe cases. However, as per the international consensus guidelines, the major criteria for diagnosing FPIES are vomiting within 1–4 h of ingestion of cow's milk protein. Extreme lethargy, diarrhea, pallor, hypotension, hypothermia, and need for intravenous fluid support are considered as minor criteria. The diagnosis of FPIES requires the presence of one major criterion with at least three minor criteria.[4] It is also reported that infants <2 months of age with cow's milk-related FPIES are more likely to present with diarrhea and blood in stools, whereas infants older than 2 months of age are more likely to present with vomiting.[4,7]

OGD scopy in FPIES is likely to show gastric erythema and erosion only in the presence of significant vomiting. Colonoscopy in FPIES with bloody diarrhea shows diffuse inflammation of colon. In FPIAP, colonoscopy shows focal or diffuse colitis with edema and erosion. Colonoscopic biopsy in both the conditions will show the features of inflammation with increased number of eosinophils.[8,9] Since colonoscopy and colonic biopsy findings are similar in both the conditions, clinical presentation is the main clue to the diagnosis.[10] Based on the clinical features, this baby is more likely to have FPIES even in the absence of significant vomiting. However, the management of both these pathological entities remains the same with the prescription of cow's milk protein eliminated diet.[2,4]

CMPA should be considered in the differential diagnosis of babies presenting with blood in stool, especially if they are receiving formula feeds. Response to elimination diet with recurrence of symptoms on challenge with cow's milk based diet helps in confirming the diagnosis. The prognosis is good with the early diagnosis and institution of therapeutic elimination diet.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the legal guardian has given his consent for images and other clinical information to be reported in the journal. The guardian understands that names and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ben-Shoshan M. Food protein-induced allergic proctocolitis: Over- or underdiagnosed? J Allergy Clin Immunol Pract 2020;8:1700-1.
2. Mennini M, Fiocchi AG, Cafarotti A, Montesano M, Mauro A, Villa MP, *et al.* Food protein-induced allergic proctocolitis in infants: Literature review and proposal of a management protocol. World Allergy Organ J 2020;13:100471.
3. Petrosino MI, Scaparrotta A, Di Filippo P, Attanasi M, Di Pillo S, Chiarelli F, *et al.* Food protein-induced enterocolitis syndrome in children: What's known? What's new? Eur Ann Allergy Clin Immunol 2018;50:99-107.
4. Nowak-Węgrzyn A, Chehade M, Groetch ME, Spergel JM, Wood RA, Allen K, *et al.* International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary-Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma and Immunology. J Allergy Clin Immunol 2017;139:1111-26.e4.

5. Tan J, Campbell D, Mehr S. Food protein-induced enterocolitis syndrome in an exclusively breast-fed infant-an uncommon entity. J Allergy Clin Immunol 2012;129:873, author reply 873-4.
6. Dupont C. Food protein-induced enterocolitis syndrome and proctocolitis. Ann Nutr Metab 2018;73:8-16.
7. Nomura I, Morita H, Hosokawa S, Hoshina H, Fukuie T, Watanabe M, *et al.* Four distinct subtypes of non-IgE-mediated gastrointestinal food allergies in neonates and infants, distinguished by their initial symptoms. J Allergy Clin Immunol 2011;127:685-8.e1.
8. Mehr S, Brown-Whitehorn T. What do allergists in practice need to know about non-IgE-mediated food allergies. Ann Allergy Asthma Immunol 2019;122:589-97.
9. Nowak-Węgrzyn A. Food protein-induced enterocolitis syndrome and allergic proctocolitis. Allergy Asthma Proc 2015;36:172-84.
10. Sánchez-Salguero CA. Food protein-induced proctocolitis. The shadow of allergic disorders. Allergol Immunopathol (Madr) 2018;46:1-2.

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Predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates

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ABSTRACT

Background: Inadequate breastfeeding results in hypernatremia in otherwise healthy neonates. **Objective:** This study finds out the predisposing factors and clinical outcome of hypernatremia in breastfed late preterm and term neonates. **Materials and Methods:** A descriptive study was conducted in a tertiary hospital with retrospective data from July 2013 to June 2016. Babies with serum sodium ≥ 150 mEq/L were identified. Breastfed babies with little or no formula feeds, gestational age ≥ 34 weeks, and birth weight ≥ 2000 g were included. Babies with congenital anomalies, sepsis, prior parenteral fluid therapy and renal or endocrine diseases causing fluid and electrolyte disturbances were excluded. Total weight loss > 10% or daily weight loss > 5% from the birth weight were considered as excess weight loss. **Results:** A total of 201 babies were studied. Hypernatremia was significantly more common in babies of primiparous mothers ($p < 0.01$). There was no difference in incidence between vaginal born and cesarean-born babies. Positive correlation was seen between age at presentation, percentage of weight loss ($p = 0.001$) and serum sodium ($p = 0.01$). Significantly more cases were seen in summer season ($p < 0.01$). Excess weight loss, jaundice, fever, and dehydration were the common symptoms. Complications observed were acute kidney injury, seizures, intracranial bleed, apnea, and bradycardia. Babies with complications mostly presented after 7 days of age, had > 25% weight loss and had serum sodium ≥ 170 mEq/L. There was no death. Three babies had neurodevelopmental problems on follow-up. **Conclusion:** Breastfed babies of primiparous mothers and born in summer are predisposed to hypernatremia. Babies presenting at a later age had severe weight loss, severe hypernatremia, and complications.

Key words: *Breastfeeding, Hypernatremia, Primiparity, Summer season, Weight loss*

Exclusive breastfeeding for the first 6 months of life is recommended by the World Health Organization to optimize the growth and development of the infants [1]. Breast milk in addition to nutrients provides various immunological factors that help to reduce the diarrheal and respiratory illness in children [2]. Breastfeeding is an instinct behavior and many mother-baby dyads accomplish it successfully [3]. However, some babies may not receive enough milk from their mothers due to various reasons. If breastfeeding is inadequate, then malnutrition and hypernatremic dehydration can occur in the baby [4]. Moritz *et al*. have termed this condition as "breastfeeding associated hypernatremia" (BFAH) [5].

An increase in the incidence of BFAH in otherwise healthy neonates has been observed in the past few years [5-8]. The clinical features and complications seen in these babies have been reported in various studies [9]. It has been found that daily weighing of the babies helps in the early recognition of this illness [10,11]. Thus, the discrepancy in clinical features and complications reported in various studies were probably related to weight monitoring practices and timing of presentation of the cases [10,11]. This study was planned to find out the predisposing factors and outcome of hypernatremia in breastfed late preterm and term neonates in a hospital with regular weight monitoring practice.

MATERIALS AND METHODS

This descriptive study was done using retrospective data from July 2013 to June 2016. The study was conducted in an urban tertiary care medical college hospital with an average delivery rate of 2500 per year. Exclusive breastfeeding was promoted for all babies in the hospital. The mother-baby dyads were discharged at around 72 h after normal delivery and 120 h after cesarean delivery. As per hospital protocol, all babies were weighed daily. Excess weight loss was defined as total weight loss more than 10% from birth weight or weight loss per day more than 5% of birth weight [12]. Serum sodium level was checked for babies with excess weight loss. Hypernatremia was defined as serum sodium more than or equal to 150 mEq/L. Babies with hypernatremia were treated with liberal oral feeds, intravenous (IV) fluids, and other supportive therapies. Serum sodium <150 mEq/L was considered an end point of treatment.

The Institutional Ethics Committee approved the study. Babies admitted during the study period with hypernatremia were identified from the hospital database. Their case records were scrutinized and babies who fulfilled the following criteria were included in the study: (1) Breastfed babies with little or no formula supplementation, (2) gestational age more than or equal to 34 weeks, and (3) birth weight more than or equal to 2000 g. The babies who had major congenital anomalies, blood culture positive sepsis, received IV fluids within 48 h before diagnosis of hypernatremia and renal or endocrine disorders which may affect the fluid and electrolyte balance were excluded from the study. The following data were collected: Gestational age as completed weeks, age at diagnosis as completed days, birth weight, weight at diagnosis, maternal age, obstetric details, details about baby feeding, clinical features, treatment, complications, condition at discharge, and neurodevelopment at follow-up.

The total weight loss and weight loss per day were calculated as a percentage of the birth weight. Axillary temperature more than 38°C was considered as fever. The rate of decline in serum sodium was calculated using the sodium before and after treatment and the time taken to achieve it. Acute kidney injury (AKI) was diagnosed if serum creatinine increased by >0.3 mg/dl in 24 h or if it was >1.5 mg/dl with normal maternal renal functions [13]. Jaundice treated with phototherapy was considered as significant. At the time of discharge, babies were said to have improved if their serum sodium level was <150 mEq/L and was taking feeds well. At follow-up, their neurodevelopment was said to be normal if gross motor, fine motor, language, and social milestones were appropriate for age and had no other neurological problem.

SPSS software version 24 was used for analysis. Numerical data were presented as mean±standard deviation or median±interquartile range as appropriate. Categorical data were presented as percentages and analyzed using Chi-square test. Ordinal data were analyzed with Mann–Whitney U-test or Student's t-test. Correlation was done with Spearman rank correlation coefficient where appropriate. $p<0.05$ was considered as significant.

RESULTS

There were 213 babies with hypernatremia satisfying the inclusion criteria during the study period. 12 babies were excluded due to various reasons. The remaining 201 babies were considered for analysis (Fig. 1). During the study period, there were 7613 live births, with 4570 vaginal births, and 3043 cesarean births. There were 4463 primiparous and 3150 multiparous mothers. Hypernatremia was seen in 2.4% of vaginally born babies and 3.1% of cesarean-born babies. This difference was not statistically significant. 3.1% babies of primiparous mothers and 1.6% babies of multiparous mothers had hypernatremia, and this different was statistically significant (Chi-square 16.7, $P<0.01$). All babies were born of singleton pregnancy. Most of the (80%) mothers had no problem in breastfeeding their babies (Table 1).

Serum sodium had a negative correlation with mother's age ($r=-0.23$, $p=0.01$) and baby's gestational age ($r=-0.2$, $p=0.004$). No correlation was seen between serum sodium level and birth weight. A positive correlation was seen between age at presentation and total weight loss ($r=0.65$, $P=0.001$). Serum sodium at admission had a positive correlation with age at presentation ($r=0.704$, $p=0.01$) and percentage of total weight loss ($r=0.652$, $p=0.001$). More than 50% of the cases were seen during the summer season in this study. This seasonal variation in the incidence of cases was statistically significant (Chi-square-26.9, $p<0.01$) (Table 1).

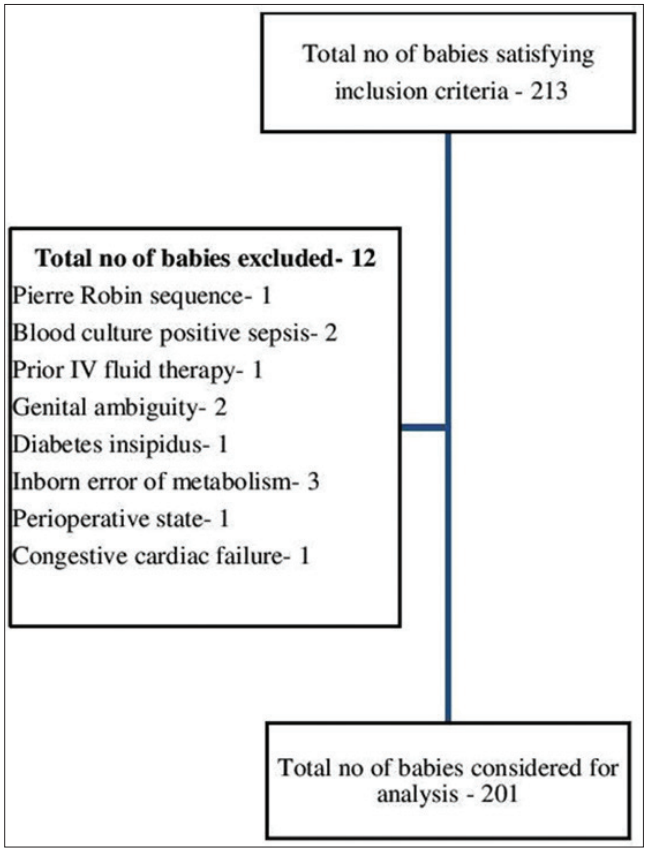


Figure 1: Flow of cases in the study

Significant jaundice was seen in 57.2% babies, but none required exchange transfusion. Phototherapy (median duration 24 h) before the onset of hypernatremia was given to 26 babies. Other common clinical findings observed were fever (33.8%) and dehydration (17.4%). No symptom other than excessive weight loss was seen in 21.4% of babies. Severe hypernatremia with serum sodium ≥170 mEq/L was seen in only five babies, and all other babies (98%) had moderate hypernatremia (serum sodium 150–169 mEq/L). About 89% of babies improved after treatment and got discharged. There was no death in the study population (Table 2). The babies who improved had a median birth weight of 3000 g (2800–3300), median admission weight of 2700 g (2460–2930), and median discharge weight of 2800 g (2540–3070).

Five babies (2.5%) had complications during the course of hypernatremia. One male baby with serum sodium of 151 mEq/L had apnea after vomiting of feeds and developed aspiration pneumonia. He improved with mechanical ventilation and antibiotics. All other four babies presented after 7 days of life

Table 1: Baseline characteristics of hypernatremic babies

Characteristics	Observation
Median maternal age (years) (n=183)*	26.9 (23-29)
Number of babies born to primiparous mothers	138/188 (73.4%)
Number of babies born to cesarean mothers	108 (53.7%)
Number of mothers reported problem in breastfeeding	42 (20.1%)
Male to female ratio	1.34:1
Median gestational age (weeks) (n=189)*	38 (37-39)
Median age at diagnosis (days)*	3 (2–18)
Number of babies presented on 2–3 days age	161 (80.1%)
Median birth weight (g)*	3080 (2810-3410)
Median weight at diagnosis (g)*	2710 (2470-3020)
Median daily weight loss (%)*	3.9 (3.3-4.7)
Median total weight loss from birth (%)*	11.3 (10-12.8)
Number of babies with 10–15% weight loss	139 (69.2%)
Season and number of babies admitted	
a. March to June (Summer)	103 (51.2%)
b. July to October	40 (19.9%)
c. November to February (Winter)	58 (28.9%)
Number of babies on exclusive breastfeeding	177 (88.1%)

*Median (IQR). IQR: Interquartile range

Table 2: Clinical features and outcome of hypernatremic babies

Characteristics	Observations
Median serum sodium at diagnosis (mEq/L)*	153 (151–155)
Serum sodium range and number of babies	
a. 150–159 mEq/L	184 (91.5%)
b. 160–169 mEq/L	12 (6%)
c. ≥170 mEq/L	5 (2.5%)
Mean time to normalize serum sodium	
a. 150–159 mEq/L (n=161)	17.8 h
b. 160–169 mEq/L (n=12)	26.6 h
c. 170–179 mEq/L (n=3)	48.8 h
d. ≥180 mEq/L (n=2)	71.3 h
Outcome	
a. Improved	178 (88.6%)
b. Left against medical advice	23 (11.4%)
Neurodevelopment problem at follow-up	3/154 (1.9%)

*Median (IQR). IQR: Interquartile range

with weight loss more than 25% of birth weight and severe hypernatremia (serum sodium ≥170 mEq/L). Complications observed were AKI (4), seizures (2), apnea (2), intracranial hemorrhage (1), and bradycardia (1). Peritoneal dialysis was done for one baby with AKI. No baby had peripheral or cerebral vessel thrombosis. Neurosonogram done for one baby with seizures showed increased white matter echogenicity. Another baby with seizures had normal neurosonogram. The treatment and outcome details of babies with severe hypernatremia are given in Table 3.

Follow-up details were available in 154 cases (76.6%) for a median duration of 1.5 months (0.5–11 months). Three babies had abnormal neurodevelopment. One female baby with serum sodium 150 mEq/L had a prolonged period of jaundice requiring phototherapy for 9 days. At follow-up, she had a global developmental delay with a seizure disorder. One male baby with serum sodium 150 mEq/L was found to have hyperactivity and aggressive behavior at 3 years. Neuroimaging was not available for these two babies. Another female baby had severe hypernatremia with AKI, seizures and normal neurosonogram in the neonatal period; and on follow-up at 1 year was found to have left hemiparesis and magnetic resonance imaging of the brain showed chronic infarct in right front parietal region and white matter changes in subcortical and deep cerebral white matter.

DISCUSSION

In this study, we have found BFAH to be more common among babies of primiparous mothers. Although breastfeeding is an instinct behavior, mothers have to learn the technique and need social support to successfully breastfeed their babies [3]. Systematic review on BFAH has found cesarean delivery, primiparity and absent previous breastfeeding experience as risk factors for BFAH [9]. However, there was no difference between cesarean babies and vaginally born babies in this study. Similarly, two studies had found no significant difference in mode of delivery among hypernatremic babies compared to control group [5,14].

It is stated that the parents of hypernatremic babies have poor recognition of their baby's illness [7,15]. About 80% of our baby's mothers have not felt any problem with their baby's feeding behavior. Excess weight loss gave a clue to identify these cases early. Thus, majority of our cases were identified at an early age, had less severe weight loss and less severe hypernatremia. Many studies report that babies presenting at a later age have higher weight loss and more severe hypernatremia [16,17]. Hence, daily weighing of babies along with appropriate lactation support during the first 4–5 days of life has been suggested for early detection of BFAH [10,11,18]. The seasonal variation in the incidence of hypernatremia was reported in some studies [12,19-21]. This was attributed to excess insensible water loss due to the hot climate in summer and overuse of room heaters in winter [9,21]. In this study, we have found significantly more number of cases during the summer season. This seasonal variation may be related to hot environmental temperature during summer seen in Tamil Nadu state of India where the study was done.

At admission, jaundice, fever, and dehydration were common symptoms in our study population. Lavagno *et al*. through systematic review have found jaundice, poor feeding, and dehydration each seen in about 45% of all reported cases [9]. Yang *et al*. have showed that percentage of weight loss within first 3 days of life has a positive correlation with jaundice at 72 h after birth in healthy term breastfed babies [22]. The hypernatremia and the resultant hyperosmolar state may damage the blood–brain barrier and facilitates bilirubin entry into nervous tissues at

Table 3: Clinical features and outcome of babies with severe hypernatremia

Gestational age (weeks)	Birth weight (g)	Total weight loss (%)	Day of diagnosis	Maximum Sr. sodium (mEq/L)	Complications	Treatment	Time to correct Sr. sodium (h)	Outcome at follow up
40	2700	25.9	12	182	Seizures, AKI, apnea, bradycardia	½ DNS+oral feeds; peritoneal dialysis; anticonvulsant	57	Normal
40	3630	14.3	4	173	Nil	½ DNS	43	Normal
37	2510	37.5	18	174	AKI, cerebral hemorrhage	NS bolus, ½ DNS	69.75	Normal
36	3060	27.5	8	173	AKI	½ DNS+oral feeds	33.5	Normal
Term	2700	24.1	17	186	AKI, seizures	NS bolus, DNS, ½ DNS; anticonvulsants	85.5	Left hemiparesis

AKI: Acute kidney injury

lower serum bilirubin level [5,23]. This has to be considered in one baby with neurodevelopmental impairment in our study who had hypernatremia and prolonged jaundice. About 20% of our babies had no symptoms at admission except for excess weight loss. Similarly Uras *et al*. and Korgali *et al*. have found that 14% and 2.4% of their hypernatremic babies were asymptomatic at admission, respectively [24,25].

Four out of five babies with severe hypernatremia had neurological and/or renal complications. Similarly, Ahmed *et al*. have found that babies with AKI presented at a later age, had higher weight loss and higher serum sodium [26]. However, less number of babies in this study had complications compared other studies [5,8,9,20,25-27]. This may be due to earlier age at presentation and a lesser degree of hypernatremia seen in the large proportion of babies in our study. There was no death in our study population and on follow-up only three babies had a neurodevelopmental problem. Mortality rate of 1-5% has been reported by various authors [8,26-28]. Koklu *et al*. have reported neurodevelopmental impairment in 47.8% cases [27]. Similarly, a high rate of neurodevelopmental impairment is also reported by Bolat *et al*. and Ergenekon *et al*. [29,30]. This emphasizes the need for formal neurodevelopmental follow-up of hypernatremic babies.

In this study, we have reported the correlation of serum sodium level with various other clinical parameters in babies with BFAH. Our study has the following limitations due to retrospective nature of the study: Some data were incomplete and long-term formal neurodevelopmental assessment was not done. Our study was hospital based, and many babies had a less severe degree of hypernatremia with fewer complications. A larger scale prospective study with neurodevelopmental follow-up is needed to have a clear understanding of the risk factors, complications and long-term effects of BFAH.

CONCLUSION

Breastfed late preterm and term babies born to primiparous mothers and born in summer season have predisposition to develop hypernatremia. These babies commonly presented with excess weight loss, jaundice, fever, and dehydration. Babies presenting beyond 7 days of age and with more than 25% weight loss are likely to have severe hypernatremia. Neurological complications and AKI were common complications observed with severe hypernatremia. These babies are at risk of neurodevelopmental impairment. Prospective study with long-term follow-up is needed.

REFERENCES

1. Kramer MS, Kakuma R. The Optimal Duration of Exclusive Breastfeeding-A Systematic Review. Geneva: World Health Organization; 2002.
2. Eidelman AI, Schanler RJ. Breastfeeding and the use of human milk. AAP policy statement. Pediatrics 2012;129:e827-41.
3. Volk AA. Human breastfeeding is not automatic: Why that's so and what it means for human evolution. J Soc Evol Cult Psychol 2009;3:305-14.
4. Livingstone VH, Willis CE, Abdel-Wareth LO, Thiessen P, Lockitch G. Neonatal hypernatremic dehydration associated with breast-feeding malnutrition: A retrospective survey. Can Med Assoc J 2000;162:647-52.
5. Moritz ML, Manole MD, Bogen DL, Ayus JC. Breastfeeding-associated hypernatremia: Are we missing the diagnosis? Pediatrics 2005;116:e343-47.
6. Laing IA, Wong CM. Hypernatraemia in the first few days: is the incidence rising? Arch Dis Child Fetal Neonatal Ed 2002;87:F158-62.
7. Cooper WO, Atherton HD, Kahana M, Kotagal UR. Increased incidence of severe breastfeeding malnutrition and hypernatremia in a metropolitan area. Pediatrics 1995;96:957-60.
8. Trotman H, Lord C, Barton M, Antoine M. Hypernatremic dehydration in Jamaican breastfed neonates: A 12-year review in a baby-friendly hospital. Ann Trop Paediatr 2004;24:295-300.
9. Lavagno C, Camozzi P, Renzi S, Lava SA, Simonetti GD, Bianchetti MG, *et al.* Breastfeeding-associated hypernatremia: A systematic review of the literature. J Hum Lact 2016;32:67-74.
10. Iyer NP, Srinivasan R, Evans K, Ward L, Cheung WY, Matthes JW. Impact of an early weighing policy on neonatal hypernatraemic dehydration and breast feeding. Arch Dis Child 2008;93:297-9.
11. Konetzny G, Bucher HU, Arlettaz R. Prevention of hypernatraemic dehydration in breastfed newborn infants by daily weighing. Eur J Pediatr 2009;168:815-8.
12. Bhat SR, Lewis P, David A, Liza SM. Dehydration and hypernatremia in breast-fed term healthy neonates. Indian J Pediatr 2006;73:39-41.
13. Subramanian S, Agarwal R, Deorari A, Paul V, Bagga A. Acute renal failure in neonates. Indian J Pediatr 2008;75:385-91.
14. Boskabadi H, Maamouri G, Ebrahimi M, Ghayour-Mobarhan M, Esmaeily H, Sahebkar A, *et al.* Neonatal hypernatremia and dehydration in infants receiving inadequate breastfeeding. Asia Pac J Clin Nutr 2010;19:301-7.

15. Oddie S, Richmond S, Coulthard M. Hypernatraemic dehydration and breast feeding: a population study. Arch Dis Child 2001;85:318-20.
16. Oddie SJ, Craven V, Deakin K, Westman J, Scally A. Severe neonatal hypernatremia: A population based study. Arch Dis Child Fetal Neonatal Ed 2013;98:F384-7.
17. Dommelen PV, Boer S, Unal S, Wouwe JP. Charts for weight loss to detect hypernatremic dehydration and prevent formula supplementation. Birth 2014;41:153-9.
18. Neifert MR. Prevention of breastfeeding tragedies. Pediatr Clin North Am 2001;48:273-97.
19. Jain S, Basu S. Hypernatremic dehydration in term and near-term neonates. Indian J Pediatr 2010;77:461.
20. Gomes SM, Fernandes A, Ramos H, Fernandes E, Santos M, Nascimento O, *et al*. Breastfeeding-associated hypernatremic dehydration. Einstein 2009;7:206-10.
21. Basiratnia M, Pishva N, Dehghani A. Prevalence of breastfeeding associated hypernatremia among hospitalized hyperbilirubinemia neonates. Iran J Neonatol 2014;5:12-6.
22. Yang WC, Zhao LL, Li YC, Chen CH, Chang YJ, Fu YC, *et al.* Bodyweight loss in predicting neonatal hyperbilirubinemia 72 hours after birth in term newborn infants. BMC Pediatr 2013;13:145.
23. Wennberg RP, Johansson BB, Folbergrová J, Siesjö BK. Bilirubin-induced changes in brain energy metabolism after osmotic opening of the blood-brain barrier. Pediatr Res 1991;30:473-8.
24. Uras N, Karadag A, Dogan G, Tonbul A, Tatli MM. Moderate hypernatremic dehydration in newborn infants: retrospective evaluation of 64 cases. J Matern Fetal Neonatal Med 2007;20:449-52.
25. Korgali EU, Cihan MK, Oguzalp T, Sahinbas A, Ekici M. Hypernatremic dehydration in breastfed term infants: Retrospective evaluation of 159 cases. Breastfeed Med 2017;12:5-11.
26. Ahmed A, Iqbal J, Ahmad I, Charoo BA, Ahmad QI, Ahmad SM. Complications due to breast-feeding associated hypernatremic dehydration. J Clin Neonatol 2014;3:153-7.
27. Koklu E, Gunes T, Ozturk MA, Kose M, Kurtoglu S, Yuksel F. A review of 116 cases of breastfeeding-associated hypernatremia in rural area of central Turkey. J Trop Pediatr 2007;53:347-50.
28. Unal S, Arhan E, Kara N, Uncu N, Alienfendioglu D. Breast-feeding-associated hypernatremia: Retrospective analysis of 169 term newborns. Pediatr Int 2008;50:29-34.
29. Bolat F, Oflaz MB, Güven AS, Özdemir G, Alaygut D, Dogan MT, *et al.* What is the safe approach for neonatal hypernatremic dehydration? A retrospective study from a neonatal intensive care unit. Pediatr Emerg Care 2013;29:808-13.
30. Ergenekon E, Unal S, Gücüyener K, Soysal SE, Koç E, Okumus N, *et al.* Hypernatremic dehydration in the newborn period and long-term follow up. Pediatr Int 2007;49:19-23.

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Predisposing factors and outcome of acute kidney injury in neonates

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ABSTRACT

Introduction: Acute kidney injury (AKI) influences the risk of mortality in sick neonates. Predicting AKI on the basis of risk factors may help in early intervention and improved outcome. **Objective:** The aim of this study is to identify the predisposing factors, clinical features, and outcome of AKI in neonates. **Methodology:** A retrospective study was carried out from September 2011 to August 2015. AKI was diagnosed if any one of the following is present: (1) Baby's serum creatinine more than 1.5 mg/dl for at least 24–48 h with normal maternal renal function, (2) baby's serum creatinine increases by more than 0.3 mg/dl over 48 h. All babies with AKI during the study were included in the study. **Result:** Twenty-five babies with AKI were studied. Male predominance was seen. Babies presented at a median age of 3 days interquartile range ([IQR] 2–5) with the median gestational age of 36 weeks (IQR 28–40) and median birth weight of 2490 g (IQR 1885–2875). Perinatal asphyxia (44%), blood culture positive sepsis (24%) and excess weight loss due to inadequate feeds (20%) were common causes for AKI. About half of the cases have received mechanical ventilation and/or inotropes. Nephrotoxic drug exposure was seen in 44% of cases. Only one-third of the babies had oliguria. Fluid boluses, diuretics, and sodium bicarbonate to correct acidosis were commonly used for medical management. Only 12% of cases required peritoneal dialysis. One baby expired. **Conclusion:** Perinatal asphyxia, sepsis, excess weight loss, and cardiopulmonary compromise predisposes to AKI in neonates. Many babies improve with medical management.

Key words: *Acute kidney injury, Cardiopulmonary compromise, Excess weight loss, Perinatal asphyxia, Sepsis*

Acute kidney injury (AKI) is commonly seen in newborns admitted to neonatal intensive care unit. It was found to have an independent influence on the risk of mortality in sick neonates [1]. The terminology "AKI" has now replaced "acute renal failure." AKI is a disorder of renal function with clinical manifestations ranging from mild dysfunction to complete a uric kidney failure [2]. Thus, the concept of AKI includes not only established renal failure but also functional impairment relative to the physiological demand [3].

Predicting AKI on the basis of risk factors may help in prompt identification and early treatment of this condition in critically ill neonates. Jetton and Askenazi have recognized newborns with the following conditions as being at increased risk for AKI: Perinatal hypoxia, prematurity and very low birthweight (VLBW), congenital heart disease, especially requiring cardiopulmonary bypass, requiring extracorporeal membrane oxygenation, sick near-term/term infants, sepsis, and congenital anomalies of kidney and urinary tract [4]. Thus, understanding the etiological profile and outcome of AKI in our neonatal population is of utmost importance to reduce the disease burden in our country. Hence, this study was conducted to describe the predisposing factors, clinical features, and outcome of AKI in newborn babies.

MATERIALS AND METHODS

The study was done in a level 3 neonatal unit of a teaching hospital in Tamil Nadu state of India. This study was done using retrospective data from September 2011 to August 2015 over a period of 4 years. A baby was said to have AKI if any one of the following two conditions is present: (1) Baby's serum creatinine more than 1.5 mg/dl for at least 24–48 h if mother's renal function is normal, (2) baby's serum creatinine increases by more than 0.3 mg/dl over 48 h [3]. All babies admitted during the study with the diagnosis of AKI were included in the study. The study was approved by the Institutional Ethics Committee.

The babies with AKI were identified by screening the diagnosis list of cases admitted during the study. Their case records were retrieved from the medical records department and scrutinized for their eligibility. Babies who satisfied the inclusion criteria were included in the study. The following baseline data were collected as follows: gender, gestational age as completed weeks, birth weight in grams, and age at diagnosis of AKI. The presence of predisposing condition for AKI such as perinatal asphyxia, sepsis, excess weight loss, renal anomalies, exposure to nephrotoxic drugs, patent ductus arteriosus, cardiac failure, umbilical arterial line, and necrotizing enterocolitis were noted.

Clinical parameters such as hydration status, urine output, blood pressure, hemodynamic compromise, respiratory problem, and any surgical procedure done were also recorded. Laboratory data collected include maximum serum creatinine, maximum blood urea, electrolyte abnormalities, acid-base status, and blood cell count and blood culture. The following serum electrolyte values were considered as normal: Sodium 135–145 mEq/L, potassium 3.5–5.5 mEq/L, ionized calcium 1–1.4 mEq/L, and bicarbonate 20–28 mEq/L. The treatment received for AKI, duration of hospital stay, and outcome were also entered in the pro forma.

Perinatal asphyxia was defined as requirement of positive pressure ventilation at birth. Babies with microbial growth in blood culture were considered to have sepsis. Any baby with weight loss more than 10% of their birth weight was said to have excess weight loss. Aminoglycosides, vancomycin, and amphotericin were considered as nephrotoxic drugs. Oliguria was defined as urine output <1 ml/kg/h. The data were collected in the designed pro forma and entered in Microsoft Excel sheet 2007. Epi info 2017 statistical software was used for analysis. The descriptive data were presented as a median ± interquartile range (IQR), and numerical data were presented as percentage.

RESULT

During the study, there were 2876 admissions to the neonatal unit and twenty-five babies had AKI. All 25 babies were included in the study. Male predominance was seen in the study population. Median gestational age was 36 weeks (IQR 28–40), and median birth weight was 2490 g (IQR 1885–2875). One-third of the babies were born preterm. Median age at presentation with AKI was 3 days (IQR 2–5) (Table 1).

Perinatal asphyxia was the underlying cause in about half of the cases in the study population. Blood culture positive sepsis was observed in about one-fourth of the babies. One-fifth of the cases had dehydration, excess weight loss, and hypernatremia due to inadequate feeds. The renal anomaly was seen in two babies as follows: One had hypoplastic kidneys and other had medullary nephron calcinosis. Cardiac failure due to supraventricular tachycardia was seen in one baby. Exposure to nephrotoxic drugs was observed in about half of the cases. Three babies had patent ductus arteriosus and two had an umbilical arterial catheter (Table 2).

One-fourth of the cases had dehydration and 12% had edema. Almost one-third of the babies had oliguria. About 50% of the cases were on mechanical ventilator. Hemodynamic compromise requiring inotropic support was observed in about 50% of the cases. Parenteral nutrition was given for one-fourth of the cases and blood component therapy was given to about half of the babies (Table 3).

Hypocalcemia, low serum bicarbonate, metabolic acidosis, and hypernatremia each were seen in about half of the cases. About one-third of the babies had hyperkalemia. Anemia and thrombocytopenia each were seen in about 30% of the cases (Table 4). Fluid bolus (40%), diuretic therapy (36%), and sodium bicarbonate infusion (36%) for acidosis correction were commonly used as medical management. Only three babies

Table 1: Baseline characteristics of the study population

Characteristics	Observations
Male: female ratio	1.78:1
Median gestational age (weeks) (n=15)	36 (28–40)
Number of preterm babies (%)	9 (36)
Median birth weight (g)	2490(1885–2875)
Median age at diagnosis (days)	3 (2–5)

Table 2: Predisposing factors for AKI in the study population

Characteristics	Observations (%)
Perinatal asphyxia	11 (44)
Sepsis	6 (24)
Dehydration due to inadequate feeds	5 (20)
Renal anomaly	2 (8)
Nephrotoxic drugs exposure	11 (44)
Patent ductus arteriosus	3 (12)
Congestive cardiac failure	1 (4)
Umbilical arterial catheter	2 (8)

AKI: Acute kidney injury

Table 3: Clinical characteristics of the study population

Characteristics	Observations (%)
Hydration status	
Dehydration	6 (24)
Edema	3 (12)
Oliguria	9 (36)
Respiratory support	
Oxygen	4 (16)
CPAP	3 (12)
Mechanical ventilation	13 (52)
Circulatory support	
On single inotrope	7 (28)
On two or more Inotropes	5 (20)
Parenteral nutrition	7 (28)
Blood component therapy	14 (56)

CPAP: Continuous positive airway pressure

Table 4: Abnormal laboratory parameters in study population

Characteristics	Observations
Median maximum serum creatinine (mg/dl)	1.73(1.56–2.29)
Median maximum blood urea (mg/dl)	61 (41.5–112)
Hypernatremia (%)	10 (40)
Hyponatremia (%)	7 (28)
Hyperkalemia (%)	9 (36)
Hypokalemia (%)	3 (12)
Hypercalcemia (%)	3 (12)
Hypocalcemia (%)	14 (56)
Low serum bicarbonate (%)	14 (56)
Metabolic acidosis (%)	8/15 (53)
Anemia (%)	8 (32)
Thrombocytopenia (%)	7 (28)

(12%) required peritoneal dialysis. About three-fourth of cases improved and got discharged. Seven cases left against medical advice and one expired (Table 5).

DISCUSSION

In this study, perinatal asphyxia, culture positive sepsis, and excess weight loss were the common causes for AKI in neonates. A study from Thailand has found that sepsis was the most common cause of AKI followed by hypovolemia, renal and urinary tract anomalies, congestive heart failure, and birth asphyxia [5]. In a prospective study from Egypt, Youssef *et al*. have found sepsis, respiratory distress syndrome, mechanical ventilation, perinatal asphyxia, and dehydration as the common predisposing factors for AKI in neonates [6]. Sepsis was identified as a predisposing factor in about three-fourth of the cases with AKI by Momtaz *et al*. [7]. Askenazi *et al*. have found that babies with AKI had low Apgar score at 5 min, low cord blood pH and required mechanical ventilation in delivery room [8]. Selewski *et al*. in a review on neonatal AKI has suggested that babies with birth weight <1500 g, perinatal asphyxia, low apgar scores, on extracorporeal membrane oxygenation and undergoing cardiac surgery need closer surveillance of renal function to identify AKI early [9].

About half of the babies in this study were sick requiring mechanical ventilation and/or inotropic support and 44% of the babies have been exposed to nephrotoxic drugs. Critically ill neonates are at risk of having AKI, as they are commonly exposed to nephrotoxic medications and have frequent infections that lead to multi-organ failure [2]. Youssef *et al*. have found that 51.9% of their AKI babies were on mechanical ventilator [6]. Respiratory support with high mean airway pressure and low mean arterial pressure were associated with renal failure in extremely low birthweight babies in a study by Viswanathan *et al*. [10]. Bansal *et al*. had observed an association between mortality and need for mechanical ventilation and shock in babies with AKI [11].

Male predominance observed in this study was also noted by Askenazi *et al*. and Bansal *et al.* [8,11]. Male gender was found to have statistically significant correlation with the occurrence of AKI [11]. However, Momtaz *et al*. had found female predominance in their study [7]. Oliguria was observed in only one-third of the study population. Similarly, Youssef *et al.* have found oliguria in only 29.6% of their babies [6]. Infants are commonly stated to have nonoliguric renal failure [2]. However, Momtaz *et al*. have found oliguria in 77.6% of their cases [7]. Bezerra *et al.* have observed an association between reduced urine output and mortality in neonates admitted to intensive care units [12]. Most of the cases in this study improved with conservative management which includes fluid boluses, diuretics, and sodium bicarbonate. Jetton and Askenazi state that currently there are little data on treatment options for AKI in neonates [13]. A trial of diuretics can be tried in oliguric neonates with AKI. The primary therapy for severe AKI is renal replacement therapy, and the indications are refractory acidosis, uremia, electrolyte disturbances, and fluid overload [9]. Only 12% of babies in this study required peritoneal dialysis. However, Momtaz *et al*. state that 36.7% of their babies required peritoneal dialysis [7].

Table 5: Treatment and outcome of the study population

Characteristics	Observation (%)
Treatment	
Fluid bolus	10 (40)
Diuretics	9 (36)
Sodium bicarbonate infusion	9 (36)
Peritoneal dialysis	3 (12)
Median duration of hospital stay (days)	7 (3–13)
Outcome	
Improved	18 (72)
Left against medical advice	7 (28)
Expired	1 (4)

AKI has an independent impact on survival after correction for comorbidities, complications, and severity of illness [2]. Chertow *et al*. have found that an increase of serum creatinine of >0.3 mg/dl was independently associated with mortality [14]. In a study by Vachvanichsanong *et al*., sepsis-induced AKI had the highest mortality rate with an overall mortality rate of 38.8% and nearly 14 times the risk of death compared to hypovolemia-induced AKI [5]. Among babies with perinatal asphyxia and AKI, the mortality rate was 71.4% [15]. VLBW babies with AKI had 2.4 times higher chance of death compared to those babies without AKI [16]. However, there was only one death in this study population. Premature infants who developed AKI in the neonatal period are at risk of hypertension, chronic kidney disease, and metabolic syndrome [2,17]. Thus, long-term follow-up these cases are required.

This study has identified the important predisposing factors for AKI in neonates of our geographical region. However, the study is limited by its retrospective nature and small sample size. A large-scale prospective study is needed to have a clear understanding of the epidemiology and outcome of AKI in neonates.

CONCLUSION

Perinatal asphyxia, sepsis, excess weight loss, and cardiopulmonary compromise requiring mechanical ventilation and/or inotropes were the common predisposing factors for AKI in neonates. Many babies improved with conservative medical management. Vigilant monitoring of renal function is required in babies with these predisposing factors.

REFERENCES

1. Askenazi DJ, Griffin R, McGwin G, Carlo W, Ambalavanan N. Acute kidney injury is independently associated with mortality in very low birthweight infants: A matched case-control analysis. Pediatr Nephrol 2009;24:991-7.
2. Askenazi DJ, Ambalavanan N, Goldstein SL. Acute kidney injury in critically ill newborns: What do we know? What do we need to learn? Pediatr Nephrol 2009;24:265-74.
3. Subramanian S, Agarwal R, Deorari AK, Paul VK, Bagga A. Acute renal failure in neonates. Indian J Pediatr 2008;75:385-91.
4. Jetton JG, Askenazi DJ. Acute kidney injury in the neonate. Clin Perinatol 2014;41:487-502.

5. Vachvanichsanong P, McNeil E, Dissaneevate S, Dissaneewate P, Chanvitan P, Janjindamai W, *et al.* Neonatal acute kidney injury in a tertiary center in a developing country. Nephrol Dial Transplant 2012;27:973-7.
6. Youssef D, Abd-Elrahman H, Shehab MM, Abd-Elrheem M. Incidence of acute kidney injury in the neonatal intensive care unit. Saudi J Kidney Dis Transpl 2015;26:67-72.
7. Momtaz HE, Sabzehei MK, Rasuli B, Torabian S. The main etiologies of acute kidney injury in the newborns hospitalized in the neonatal intensive care unit. J Clin Neonatol 2014;3:99-102.
8. Askenazi DJ, Koralkar R, Hundley HE, Montesanti A, Patil N, Ambalavanan N, *et al.* Fluid overload and mortality are associated with acute kidney injury in sick near-term/term neonate. Pediatr Nephrol 2013;28:661-6.
9. Selewski DT, Charlton JR, Jetton JG, Guillet R, Mhanna MJ, Askenazi DJ, *et al.* Neonatal acute kidney injury. Pediatrics 2015;136:e463-73.
10. Viswanathan S, Manyam B, Azhibekov T, Mhanna MJ. Risk factors associated with acute kidney injury in extremely low birth weight (ELBW) infants. Pediatr Nephrol 2012;27:303-11.
11. Bansal SC, Nimbalkar AS, Kungwani AR, Patel DV, Sethi AR, Nimbalkar SM, *et al.* Clinical profile and outcome of newborns with acute kidney injury in a level 3 neonatal unit in western india. J Clin Diagn Res 2017;11:SC01-SC04.
12. Bezerra CT, Vaz Cunha LC, Libório AB. Defining reduced urine output in neonatal ICU: Importance for mortality and acute kidney injury classification. Nephrol Dial Transplant 2013;28:901-9.
13. Jetton JG, Askenazi DJ. Update on acute kidney injury in the neonate. Curr Opin Pediatr 2012;24:191-6.
14. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005;16:3365-70.
15. Alaro D, Bashir A, Musoke R, Wanaiana L. Prevalence and outcomes of acute kidney injury in term neonates with perinatal asphyxia. Afr Health Sci 2014;14:682-8.
16. Koralkar R, Ambalavanan N, Levitan EB, McGwin G, Goldstein S, Askenazi D, *et al.* Acute kidney injury reduces survival in very low birth weight infants. Pediatr Res 2011;69:354-8.
17. Askenazi DJ, Morgan C, Goldstein SL, Selewski DT, Moxey-Mims MM, Kimmel PL, *et al.* Strategies to improve the understanding of long-term renal consequences after neonatal acute kidney injury. Pediatr Res 2016;79:502-8.

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Role of perfusion index in pulse oximetry screening for critical congenital heart disease in neonates

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ABSTRACT

Introduction: Screening for critical congenital heart diseases (CCHD) with oxygen saturation (SpO_2) by pulse oximeter often misses left-sided obstructive heart diseases. **Objective:** The role of perfusion index (PI) along with SpO_2 in CCHD screening was studied. **Methodology:** The Masimo, RADICAL-7 pulse oximeter was used to record the SpO_2 and PI in the right hand and left foot of asymptomatic babies at 24–72 h of life. Babies with SpO_2 <95% or PI <0.7 were rechecked at an hourly interval for three recordings. SpO_2 90–94% or PI <0.7 in all three recordings or SpO_2 <90% at any one recording were considered as screen positive. An echocardiogram was done for screen-positive cases. Screen negative cases were clinically followed for 6 weeks. **Result:** Of 1011 screened babies, four were screen positive. One baby had PI <0.7 and SpO_2 <90%. This baby had single ventricle, transposed great vessels, and interrupted aortic arch. Other three cases had SpO_2 between 90% and 94% in all three recordings. Echocardiogram showed severe right ventricle outflow obstruction in 2 cases and normal heart in one baby. At follow-up, no baby had CCHD. **Conclusion:** In this study with small sample size, only one baby had left-sided obstructive lesion but also had single ventricle physiology. Hence, there was no difference in the diagnostic accuracy between SpO_2 alone and SpO_2 with PI in screening for CCHD. Thus, combining PI with SpO_2 may improve CCHD screening using pulse oximeter, but large-scale study is needed.

Key words: *Critical congenital heart disease, Newborn screening, Oxygen saturation, Perfusion index, Pulse oximetry*

Congenital heart diseases (CHD) requiring intervention in the 1st month of life to ensure survival is considered as critical CHD (CCHD). CCHD has an incidence of about 170 in 100,000 live births. Early diagnosis and timely therapy are crucial to prevent acute deterioration of the affected children [1]. CHD with right-sided obstruction or well-mixing lesion present predominately with cyanosis. These cyanotic CHD initially present with faint cyanosis which may be indistinguishable clinically. Thus, the clinical examination (CE) fails to identify about 50% of CHD in the neonatal period [2]. However, these babies can be identified with low oxygen saturation (SpO_2) in the pulse oximeter. Hence, American Academy of Pediatrics (AAP) has recommended SpO_2 measurement by pulse oximeter as a screening strategy to identify cyanotic CHD [3].

However, left-sided obstructive lesions such as hypoplastic left heart syndrome, interrupted aortic arch, and coarctation of aorta present initially with poor peripheral perfusion. SpO_2 may remain within normal limits at an early stage of the disease and can be missed by pulse oximeter screening [1]. Studies have shown that more than 3% difference in saturation ($DSpO_2$) between pre-ductal and post-ductal regions may give a clue to these left-sided obstructive heart diseases [4]. Granelli and Ostman-Smith have found that peripheral perfusion index (PI) measured with new generation pulse oximeter can help in screening for left-sided obstructive lesions [5]. PI is a measure of the pulsatile blood flow in the underlying tissues and is decreased in babies with reduced peripheral tissue perfusion [6]. PI below 0.7 was suggested as a screening tool for identifying left-sided obstructive heart diseases [5].

This study was planned with a hypothesis that combining PI with SpO_2 may improve CCHD detection. The primary objective was to compare the diagnostic accuracy of SpO_2 alone, with SpO_2 and PI in screening for CCHD among asymptomatic newborn babies at 24–72 h of life. The secondary objective was to find out the diagnostic accuracy of CE in screening for CCHD either alone or in combination with SpO_2 and PI.

METHODOLOGY

This prospective study was done in a tertiary care hospital in the Tamil Nadu state of India. The study was carried out over a period of 4 months from October 2011 to January 2012. The Institutional Ethics Committee approved the study. All babies born during the study period and asymptomatic at 24–72 h of life were included in the study. Asymptomatic babies under evaluation for sepsis due to various perinatal risk factors were excluded from the study.

Parents of all babies who satisfied the inclusion criteria were approached for the study. Written informed consent was obtained from the parents and basic demographic details were collected. Babies were then clinically examined for any dysmorphic features, central cyanosis, respiratory distress, apical impulse location, femoral pulses, and grade ≥3/6 precordial murmur.

The new generation pulse oximeter with signal extraction technology (Masimo, RADICAL-7, Signal extraction pulse Co-Oximeter with rainbow technology) was used for recording functional hemoglobin SpO_2 percentage and PI. The instrument displays the pulse waveform, heart rate, SpO_2, and PI. The reusable neonatal probe was applied in the right hand (Pre-ductal area) and the left foot (Post-ductal area) serially when the baby was calm and quiet. The SpO_2 and PI were recorded, once the monitor's display panel showed regular pulse waves. The probe was cleaned with a compatible disinfectant solution between babies.

If PI was ≥0.7 with SpO_2 ≥95% in the both tested limbs and $DSpO_2$ between the two limbs was ≤3%, the screening was considered negative. If anyone of these readings was abnormal, the test was repeated after 1 h. If the second recording result remained abnormal, it was reconfirmed by repeating the test 3rd time after another hour. Babies who consistently had SpO_2 <95% or PI <0.7 or $DSpO_2$ >3% in all three recordings were declared screen positive. SpO_2 <90% in either limb at any time either in the initial recording or in the repeat recordings was immediately declared as screen positive without further testing. All babies with positive screening and those babies with abnormal clinical findings underwent echocardiography (Philips ER VISION-HD-7 with pediatric probe) by a pediatric cardiologist with 5-year experience in the field. Confirmed CCHD cases were referred to cardiothoracic surgeons for further management.

The screen negative cases were followed up clinically at 6 weeks of age when they attended the well-baby clinic for review and vaccination. Any suspicion of CHD was confirmed by echocardiography. The parents of babies who did not come for follow-up were tracked through phone about the health status of the baby. For those parents who could not be contacted by phone, a letter enquiring the health status of their baby was posted to their mailing address. The babies who could not be contacted by phone or post were considered as dropouts.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for SpO_2, PI, and CE were calculated individually and in combination using OpenEpi, Version 2, open source calculator for diagnostic tests. Categorical data were analyzed with two-tailed Fisher's exact test for small groups and Chi-square test for the large population using SPSS Version 16. $p<0.05$ was considered as significant.

RESULTS

A total of 1011 babies out of 1059 eligible babies were screened. 48 babies were discharged before 24 h of life due to various reasons and were not included in the study (Fig. 1).

Male to female ratio in the study population was 1.03:1. The babies were screened at a mean age of 34 h (±10.5), and their mean birth weight was 2850 g (±440) (Table 1).

Mean SpO_2 in the right hand and left foot was 97.42% (±1.35) and 97.58% (±1.44), respectively, with a mean $DSpO_2$ of 1.07% (±0.86). Mean PI in the right hand and left foot was 2.43 (±1.48) and 2.43 (±1.32), respectively (Table 2).

We had four screen positive cases: One at first recording and other three at the end of three recordings. The baby who was screen positive at first recording had SpO_2 <90% and PI< 0.7 in the right hand. This baby was antenatally diagnosed to have single ventricle. Postnatal echocardiogram of this baby showed transposition of great vessels (TGV), interrupted aortic arch, single ventricle, hypoplastic left atrioventricular valve, and patent ductus arteriosus. Three babies persistently had SpO_2 90–94%

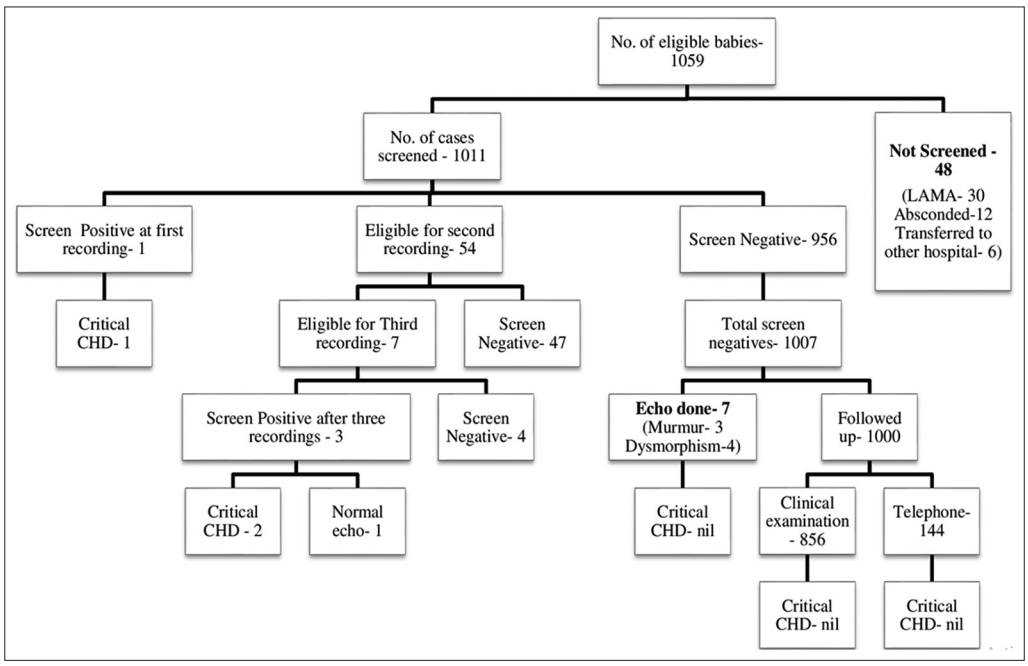


Figure 1: Flow of cases through the study

Table 1: Baseline characteristics of the study population

Characteristics	Observation
Male: female ratio	1.03:1 (p=0.687)
Mean birth weight	2850 (±440) g
Mean age at screening	34.03 (±10.5) h
Mode of delivery	Labor naturale-453; Cesarean section-532; Assisted breech-3; Outlet forceps-17; Vacuum-6

Table 2: Study results

Characteristics	Observation
Mean SpO_2 in right hand	97.42% (±1.35)
Mean SpO_2 in left foot	97.58% (±1.44)
Mean $DSpO_2$	1.07% (±0.86)
Mean PI in right hand	2.43 (±1.48)
Mean PI in left foot	2.43 (±1.32)
Number of babies with dysmorphism	4 (down syndrome-1, Preauricular skin tag-3)
Number of babies with cardiac murmur	4 (CCHD-1, VSD-3)
Number of screen positive cases	4 (Low SpO_2 and low PI-1, low SpO_2-3)
Number of confirmed CCHD	3 (Antenatally detected-2, detected by screening-1)

SpO_2: Pulse oximeter saturation, $DSpO_2$: Difference in pulse oximeter saturation between right upper limb and left lower limb, PI: Perfusion index, CCHD: Critical congenital heart disease, VSD: Ventricular septal defect

at all three recordings. Echocardiogram in these babies showed tetralogy of fallot (TOF) with severe right ventricular outlet obstruction in one baby, right ventricular dysplasia with severe right ventricular outlet obstruction in the second baby and structurally normal heart in another baby. The baby with TOF was antenatally diagnosed to have CHD and postnatally had systolic murmur by CE. The other two cases had normal antenatal scans and normal CE. The screen positive baby with normal echo subsequently had normal SpO_2 before discharge.

Thus, two babies antenatally detected to have CCHD had positive screen by pulse oximeter. Four babies had systolic murmur in CE, and this includes one screen positive baby with TOF. The other three babies with systolic murmur and negative pulse oximeter screening were found to have ventricular septal defect (VSD) by echocardiogram. In addition, four babies with dysmorphic features underwent echocardiography as a part of dysmorphology work up, and all were found to have a structurally normal heart (Table 2).

Other cases were followed up at 6 weeks of age. A total of 856 babies came for follow-up, and none had clinical features of CHD. 144 babies who did not turn for follow-up were contacted by phone and were reported to be healthy without any symptoms suggestive of CCHD. Thus, the incidence of CCHD in our population was 2.97/1000 babies, and the incidence of CHD was 5.93/1000 babies.

The diagnostic accuracy of SpO_2, PI, and CE was calculated individually and in combination. The sensitivity, specificity, PPV, and NPV of SpO_2 alone and SpO_2 with PI were similar. PI alone has low sensitivity but better PPV compared to SpO_2. If the role of PI alone in identifying left-sided obstructive lesions is considered then the sensitivity is 100% and specificity is 100%. CE has low sensitivity and PPV compared to SpO_2. When all three methods of screening are combined, i.e., SpO_2, PI, and CE the sensitivity is 100%, specificity is 99.7%, and PPV is 50% with a false positivity rate of 50% in identifying CCHD (Table 3).

DISCUSSION

In this study, an attempt was made to study the role of combined SpO_2 and PI screening in identifying the CCHD. The results are encouraging since PI has rightly identified one baby with the interrupted aortic arch. Interestingly, this baby also had SpO_2 <90% due to TGV and single ventricle. It can be assumed that even if it had been an isolated interrupted aortic arch, this case would have been picked up by pulse oximeter when both SpO_2 and PI were recorded in all babies. Thus, PI and SpO_2, each have a unique role as a screening tool for identifying left-sided obstructive heart diseases and cyanotic heart diseases, respectively.

de-Wahl Granelli *et al*. have suggested that incorporating cutoff values for PI into routine pulse oximetry screening would probably increase sensitivity for detection of the left heart obstructive disease [4]. In our study, the PI <0.7 has a sensitivity of 33.33%, specificity of 100%, and PPV of 100% in identifying all CCHD. However, it has a sensitivity of 100% and specificity of 100% in identifying CCHD with the left-sided obstructive lesion since there was only one case with the interrupted aortic arch in the study population which was identified by low PI.

de-Wahl Granelli *et al*. stated that in a complex heart disease with a combination of TGV, arch obstruction and duct dependent circulation, the post-ductal saturation may well be >95%, and hence, >3% $DSpO_2$ between pre-ductal and post-ductal regions was included as a screening tool in their study [4]. Although we had one baby with TGV and aortic arch obstruction, the $DSpO_2$ was not significant. Probably this was due to the complex nature of the defect with single ventricle physiology which resulted in SpO_2 <90% in both pre-ductal and post-ductal areas.

There were two babies in this study with antenatally detected CHD whose SpO_2 screening was positive and echo later confirmed the critical cardiac lesions. This supports Richmond *et al.*'s comment that even if the antenatal diagnosis was not made in these babies, the screening saturation measurement would have triggered the evaluation for CHD [7]. Riede *et al*. stated that in babies with the prenatal diagnosis of CCHD, if medical treatment is initiated soon after birth, SpO_2 will be spuriously high because of medical therapy [8]. Koppel *et al*. have noted that in a center where fetal echocardiography is readily accessible, many lesions will be diagnosed prenatally, and therefore, SpO_2 screening may be less useful in detecting new CCHD. However, centers, where fetal echocardiography is performed less frequently, are likely to demonstrate higher yields from pulse oximetry screening [9].

Although CE is said to miss about 50% of CHD in infants, many studies on CHD screening have included the CE in their

Table 3: Evaluation of the screening test

Variables	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
SpO_2	100 (43.85, 100)	99.9 (99.44, 99.98)	75 (30.06, 95.44)	100 (99.62, 100)
PI	33.33 (6.149, 79.23)	100 (99.62, 100)	100 (20.65, 100)	99.8 (99.28, 99.95)
CE	33.33 (6.149, 79.23)	99.8 (99.28, 99.95)	33.33 (6.149, 79.23)	99.8 (99.28, 99.95)
SpO_2 and PI	100 (43.85, 100)	99.9 (99.44, 99.98)	75 (30.06, 95.44)	100 (99.62, 100)
SpO_2 and CE	100 (43.85, 100)	99.7 (99.13, 99.9)	50 (18.76, 81.24)	100 (99.62,100)
SpO_2, PI and CE	100 (43.85, 100)	99.7 (99.13, 99.9)	50 (18.76, 81.24)	100 (99.62, 100)

SpO_2: Pulse oximeter saturation, PI: Perfusion index, CE: Clinical examination, CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value

protocol [7-10]. In this study, though CE failed to pick up two CCHD, it has identified three screen negative babies with VSD. This stresses the importance of clinical evaluation for CHD in all babies, including the screen negative babies, at least once before discharge. In developing countries, where delay in seeking medical care for sick babies is a common occurrence, early recognition of this non CCHD may also play a key role in influencing CHD related mortality.

Thangaratinam *et al*. on the basis of eight studies have found that in pulse oximeter screening, the summary estimates of sensitivity and specificity were 63% and 99.8%, respectively, yielding a false positive rate of 0.2% [2]. Valmari based on 10 studies found a high specificity (99.9–99.99%), and the overall rate of detection of 15 specified defects with pulse oximetry was 72%, which exceeds that of the CE (58%) [11]. In this study, the diagnostic value of SpO_2 in diagnosing CCHD has a sensitivity of 100%, specificity of 99.9%, and PPV of 75% with the false positivity rate of 25%.

Liske *et al*. have defined CCHD as heart diseases that require intervention or cause death within 1 month of age in contrast to AAP definition of up to 1 year of age [1,3]. The follow-up in this study was limited to 6 weeks of age, as patient compliance with long-term follow-up is poor in this study scenario. Ideally, pulse oximetry and echo have to be done for all babies [12]. However, this is not practically feasible in large community studies. Hence, in most of the studies, authors have done echo only for screen positive cases and have clinically followed the screen negative cases for manifestations of CCHD [4,8].

The study has following limitations: It is a hospital-based study done with small sample size, and clinical follow-up was not available for 15% of babies at 6 weeks of age. Hence, this may not reflect the true screening scenario at the community level. However, this study has tried to address the challenge in identifying left side obstructive heart diseases with pulse oximetry by including PI measurement in the screening protocol.

CONCLUSION

In our study, PI was low in one baby with left-sided obstructive lesion, but due to coexisting single ventricle physiology, SpO_2 was also low. Hence, in this study, there was no difference in the diagnostic accuracy between SpO_2 alone and SpO_2 with PI in screening for CCHD. However, the study was limited by small sample size. Thus, combining PI with SpO_2 may improve CCHD screening using pulse oximeter but large-scale study is needed.

REFERENCES

1. Liske MR, Greeley CS, Law DJ, Reiche JD, Morrow WR, Baldwin HS, *et al*. Report of the Tennesse task force on screening newborn infants for critical congenital heart diseases. Pediatrics 2006;118:e1250.
2. Thangaratinam S, Daniels J, Ewer AK, Zamora J, Khan KS. Accuracy of pulse oximetry in screening for congenital heart disease in asymptomatic newborns: A systematic review. Arch Dis Child Fetal Neonatal Ed 2007;92:F176-80.
3. Mahle WT, Martin GR, Beekman III[rd] RH, Morrow WR, Rosenthal GL, Snyder CS, *et al*. Endorsement of health and human service recommendation for pulse oximetry screening for critical congenital heart disease. Pediatrics 2012;129:190.
4. de-Wahl Granelli A, Wennergren M, Sandberg K, Mellander M, Bejlum C, Inganäs L, *et al.* Impact of pulse oximetry screening on the detection of duct dependent congenital heart disease: A Swedish prospective screening study in 39,821 newborns. BMJ 2009;338:a3037.
5. Granelli AD, Ostman-Smith I. Noninvasive peripheral perfusion index as a possible tool for screening for critical left heart obstruction. Acta Paediatr 2007;96:1455-9.
6. Perfusion Index, Clinical Applications of Perfusion index-Summary; 2009. Available from: http://www.masimo.co.uk/pdf/whitepaper/LAB3410F.pdf. [Last accessed on 2012 Jan 23].
7. Richmond S, Reay G, Harb MA. Routine pulse oximetry in the asymptomatic newborn. Arch Dis Child Fetal Neonatal Ed 2002;87:F83-8.
8. Riede FT, Worner C, Dahnert I, Mockel A, Kostelka M, Schneider P. Effectiveness of neonatal pulse oximetry screening for detection of critical congenital heart disease in daily clinical routine—results from a prospective multicenter study. Eur J Pediatr 2010;169:975-81.
9. Koppel RI, Druschel M, Carter T, Goldberg BE, Mehta NP, Talwar R, *et al*. Effectiveness of pulse oximetry screening for congenital heart disease in asymptomatic newborns. Pediatrics 2003;111:451.
10. Arlettas R, Bauschatz AS, Monkhoff M, Essers B, Bauersfeld U. The contribution of pulse oximetry to the early detection of congenital heart disease in newborns. Eur J Pediatr 2006;165:94-8.
11. Valmari P. Should pulse oximetry be used to screen for congenital heart disease? Arch Dis Child Fetal Neonatal Ed 2007;92:F219-24.
12. Vaidyanathan B, Sathish G, Mohanan ST, Sundaram KR, Warrier KK, Kumar RK. Clinical screening for congenital heart disease at birth: A prospective study in a community hospital in Kerala. Ind Ped 2011;48:25-30.

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[illegible]

[illegible] Coronavirus disease 2019 (COVID-19), in children has varied clinical presentations from asymptomatic infection to severe pneumonia, multi system inflammatory syndrome in children (MIS-C) and rarely death. To date, limited data has been published on the profile of COVID-19 infection among Indian children. The objective of this study was to analyze the clinical spectrum, laboratory parameters, treatment and it's outcome in children less than 15 years admitted with COVID-19 infection.

[illegible] This was a cross sectional study done in a tertiary care hospital. Children with COVID-19 infection detected by Reverse transcriptase polymerase chain reaction (RT-PCR) were included.

[illegible] Out of 167 children, 93 were males (55.6%) and median (Interquartile range- IQR) age of children admitted were 9 (4-13) years. Family cluster (71%) was the dominant source of infection. Majority were asymptomatic (60%, N=100). The predominant symptoms were fever (29%), cough (16%) and rhinorrhea (4.1%). Seven (4.1%) children had comorbidities and two (28%) among them had moderate to severe disease. Median (IQR) total leucocyte count was 7 (4-10) ×103 µl and platelet count was 390 (275-500) ×103 µl. Leukopenia was observed in 58% (N=81), lymphocytosis in 32.8% (N=46) and thrombocytopenia in 3.5% (N=5). Inflammatory markers were raised in 10-15% of children. Peri-bronchial cuffing was the common abnormality (N=14, 17.5%) in chest X-ray (CXR) and peripheral heterogeneous opacities were noted in severe disease. Children with moderate to severe symptoms were treated with remdesivir and steroids.

[illegible] COVID-19 infection in children was often benign and increased severity was noted among those with comorbidities. Leukopenia, lymphocytosis were commonly observed. High inflammatory markers were noted in severe disease. Nil fatality among our study group.

[illegible] COVID-19, Children, Chest X-ray, Leukopenia, Lymphocytosis, Laboratory profile

[illegible]

In the pre-coronavirus disease-19 (COVID-19) era human coronavirus (HCoV) was one among the causes of bronchiolitis in infants and upper respiratory infection in children.[1] Previous outbreaks of Severe acute respiratory syndrome (SARS) and Middle east respiratory syndrome (MERS) caused by coronavirus were associated with less mortality in children.[2] Similarly COVID-19 which causes severe acute respiratory syndrome (SARS-CoV2) in adults causes milder illness in children. The incidence in children is around 8.7% in below 19 years according to CDC.[3] The hypothesis behind the less severe disease in children being differences in innate and adaptive immunity, pre-existing antibodies to corona virus, more frequent respiratory infections, protective effects of other live vaccines, different expression of angiotensin converting enzyme-2 (ACE-2) receptors and less intense exposure.[4] Very rarely it may lead to Acute respiratory distress syndrome (ARDS), Multi organ dysfunction (MODS) and death. Multi system inflammatory syndrome in children (MIS-C), a post viral immune dysregulation following SARS-

CoV-2 infection is common among children and adolescents which may rarely lead to significant morbidity and mortality if not treated early.[5]

Hematological and biochemical markers of COVID-19 were well studied in adults. This cannot be extrapolated as such for children. The aim of the study was to present the detailed clinical, laboratory profile, treatment and outcome of 167 children below 15 years of age admitted with COVID-19 infection.

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This was a descriptive observational study and followed the Strengthening the reporting of observational studies in epidemiology (STROBE) guideline.

This study was done at PSG Institute of Medical Sciences and Research hospital which is one of the largest designated tertiary care hospitals in Coimbatore, India which caters to patients from around 250 km radius. Institutional ethics committee approval was obtained. Informed consent was taken from parents or patients included in prospective analysis (N=107) and waiver of consent was obtained for those taken retrospectively (N=60). All children less than 15 years admitted with COVID-19 infection between March 2020 to February 2021 were included in our study. Positive cases were confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) validated as per ICMR standards. Both nasopharyngeal and oropharyngeal swabs were taken for confirmation. The severity of illness were categorized into mild, moderate, severe and critical as per the COVID-19 clinical management-Interim guidance.[6] Mild illness is defined as symptomatic COVID-19 positive patients without evidence of pneumonia, moderate as with clinical signs of pneumonia and no signs of hypoxia and severe as clinical signs of pneumonia and SpO_2<90%. Critical disease is with ARDS, sepsis or septic shock and MODS.

Fever was defined when the temperature was >38°C, the source of infection was identified by recording which family member developed the symptoms first. Basic hematological and radiological investigations were taken for most of the patients admitted except in few because of logistical issues. Investigations were repeated in patients showing persistent symptoms.

All the necessary information was collected from electronic records on the hospital database. Chest X-ray findings were analyzed and reported by a single radiologist. Information on baseline demographics, clinical features, symptoms, signs, laboratory reports, imaging, treatment and outcome were recorded. Laboratory values were interpreted with age specific reference ranges. If the patient had a serial investigation for a particular parameter, the highest value was taken into consideration. Asymptomatic and mildly symptomatic children were given supportive care. Those presented with sore throat, fever beyond 3 days, lower respiratory symptoms, pneumonia (confirmed by clinical and radiological signs) were treated with antibiotics. Steroids were used only in severe cases.

Statistical analysis

Data was entered in Microsoft excel (Microsoft corporation, Redmond, USA) sheet and analyzed using SPSS software version 24. Descriptive statistics were used to summarize the data. Continuous variables were described as median and interquartile range. Categorical variables were described as frequencies and percentages. Chi square test was used to assess the relationship between gender and incidence of COVID-19. Mann-Whitney U-test was used to assess the correlation between severity of illness and inflammatory markers. A p value of <0.05 was considered significant.

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A total of 167 children admitted with COVID-19 positive reports were included in our analysis. The baseline demographic and clinical characteristics of these children are shown in Table 1.□

Demographic and clinical characteristics

The median age (IQR) of children admitted were 9 (4-13 years) and male children (N=93, 55.6%) outnumber females with a male: female ratio of 1.25:1. There was no relationship between gender and incidence of COVID infection in children (p=0.12, Chi square test). Family cluster as the source of infection was identified in 71% (N=119) (Table 1).

Fever was the predominant symptom in 29.3% (N=49) followed by cough (N=27, 16.1%) and rhinorrhea (N=7, 4.1%). Diarrhoea was noted in 3.5%, abdominal pain in 1.1% and loss of taste was present in 1.1%. Very few had fever beyond 24-48 hours after hospital admission. Among the admitted, 7 (4.1%) had preexisting comorbid conditions (Table 2). In them, a child with a germ cell tumor and a hemolytic anemia had moderate to severe illness. More than half were asymptomatic, 38% (N=64) had mild symptoms, 0.5% (N=1) had moderate symptoms and 1.1% (N=2) had severe symptoms. Out of 4 children admitted in ICU, 2 had comorbidities (hemolytic anemia and germ cell tumor) one among them required oxygen support by mask (germ cell tumor), a 2-months-old without any comorbidity had respiratory distress and hypoxemia supported by mechanical ventilation and a child with head injury was admitted for monitoring (Table 3). There was a significant association between comorbidity and severe illness (p=0.014, Fisher's exact test).

Radiological and laboratory finding

CXR was performed in 48% (N=80) of children and out of which 78% (N=63) were normal. Few had prominent

central peri bronchial cuffing (N=14, 17.5%) and peripheral heterogeneous opacities were noted in 3.7% (N=3). CT was done only in patients with moderate to severe illness which showed ground glass opacities (Table 4). On admission, the median (IQR) WBC count was 7 (4-10)×10^3/µl. Leukopenia, lymphopenia and lymphocytosis were noted in 58%, 11.4% and 32.8% respectively. Thrombocytopenia was seen only in 3.5%. Inflammatory markers IL-6, ferritin, D-dimer were raised in 10%, 6% and 13% respectively (Table 5). Moderate to severe group exhibited significantly higher IL-6, ferritin and D-dimer than milder group (p=0.04, p=0.04, p=0.06, Mann-Whitney U-test) (Figure 1-3). Hypoalbuminemia was noted in 3.5% and liver enzymes were elevated in 7-8%.

Treatment and outcome

Intravenous antibiotics and remdesivir were given in 3 children. Steroid was given only in a child with a Germ cell tumor. An infant with severe respiratory distress (0.5%) was treated with mechanical ventilatory support considering High frequency nasal cannula (HFNC) having high aerosolization risk during the initial phase of pandemic. In all others symptomatic treatment was given accordingly. Those with severe illness have recovered and discharged by 2nd to 3rd week of admission. There was nil mortality among our study group (Table 6).

Table 1: Demographic and clinical characteristics of children with COVID-19

Characteristics	n (%)
Median (IQR) age in years	9 (4-13)
Age group	
<1	19/167 (11)
1-5	39/167 (23.3)
6-10	47/167 (28.5)
10-15	62/167 (37)
Gender	
Male	93/167 (55.6)
Female	74/167 (44.3)
Exposure	
Family cluster	119/167 (71)
Other exposure	28/167 (17)
Unknown	20/167 (12)

Note: Data presented are in numbers (%) or median (interquartile range).

Table 2: Symptoms and signs in children with COVID-19

Symptoms	n (%)
Fever	49 (29.3)
Cough	27 (16.1)
Breathlessness	4 (2.3)
Sore throat	7 (4.1)
Headache	1 (0.5)
Vomiting	3 (1.7)
Abdominal pain	3 (1.7)
Diarrhea	6 (3.5)
Myalgia	2 (1.1)
Rash	2 (1.1)
Coryza	6 (3.5)
Asymptomatic	2 (1.1)

Table 3: Co-morbidities in children with COVID-19

Co-morbidities	n
Asthma	1
VSD	1
Hemolytic anemia	1
Seizure disorder	1
Germ cell tumor on chemotherapy	1
Congenital hypothyroid	1
Hirschsprung disease with dysmorphic facies	1
Severity	
Asymptomatic	100/167 (60)
Mild	64/167 (38)
Moderate	1/167 (0.5)
Severe	2/167 (1.1)

Table 4: Chest radiograph findings in children with COVID-19

Chest radiograph findings	n (%)
Normal	80/167 (48)
Normal	63/80 (78)
Peribronchial cuffing	14/80 (17.5)
Peripheral heterogeneous opacity	3/80 (3.7)
Ground glass opacity	3/167 (1.7)
Ground glass opacity	3/3 (100)

Note: CXR- Chest X-ray, CT-Computed tomography.

Table 5: Laboratory findings in children with COVID-19

Parameters	Median (IQR)	Normal range
Total leucocyte count (10^3/µl)	7 (4-10)	4-10×10^3 cells/µl
Leucocytosis	17/140 (12.1)	>10×10^3 cells/µl
Leucopenia	81/140 (58)	<4×10^3 cells/µl
Absolute neutrophil count (10^3/µl)	3.0 (0.8-5.2)	1.5-3 cells/µl
Lymphopenia	16/140 (11.4)	ALC <1.5×10^3 cells/µl
Lymphocytosis	46/140 (32.8)	>4×10^3 cells/µl
Platelet count (10^3/µl)	2.5(0.5-4.5)	1-8 ×10^3 cells/µl
Thrombocytosis (10^3/µl)	390 (275-500)	150-400×10^3 cells/µl
Thrombocytopenia	5/140 (3.5)	<150 ×10^3 cells/µl

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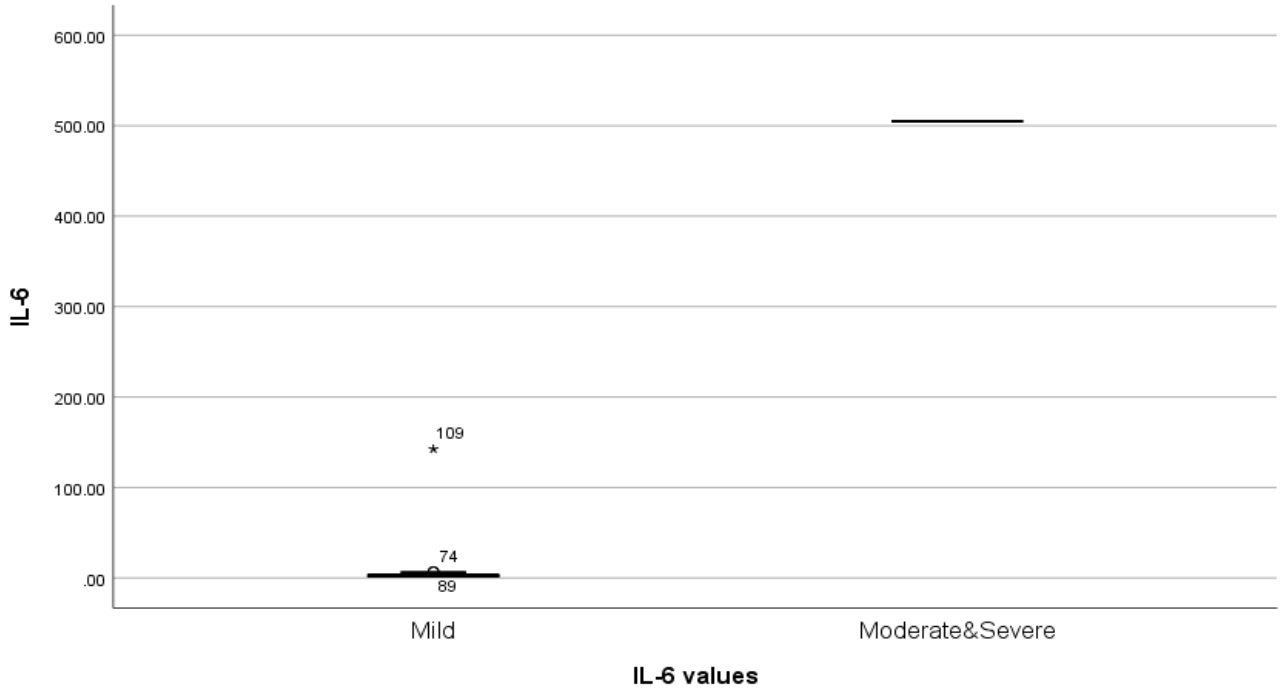
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□□□□□□□d□□ □ □□	4/38 (10.5)	>0.6 mg/dl
□□□□□□□d□□ □□ □	4/30 (10)	≥40 mm/hour
□□□□□□□d□□ □ □ □	1/40 (2.5)	>500 u/l
□ □□□□r□□□□□□□ □□□□□□□□	1/85 (1.2)	<4 mg/dl
□ □□□□□□□□ □□□□ □□□□□□□	3/85 (3.5)	<3.5 mg/dl
□□□□□□□d□□ □□ □	7/85 (8)	>50 u/l
□□□□□□□d□□ □ □ □	6/85 (7)	>45 u/l
□□□□□□□d□□r□□□□□□□□	3/106 (2.8)	As per age-based cut-off mg/dl
□□□□□□□d□□□6□	5/50 (10)	≥7 pg/ml
□□□□□□□d□□rr□□□□□□□□□	3/50 (6)	>500 ng/ml
□□□□□□□d□□ □d□□ □r□	4/31 (13)	≥0.5 mg/l FEU

Note: IQR- interquartilerange, CRP- C-reactiveprotein, ESR- Erythrocyte sedimentation rate, LDH-lactatedehydrogenase, AST- aspartate amino transferase, ALT- alanineaminotransferase, IL-6-interleukin 6.

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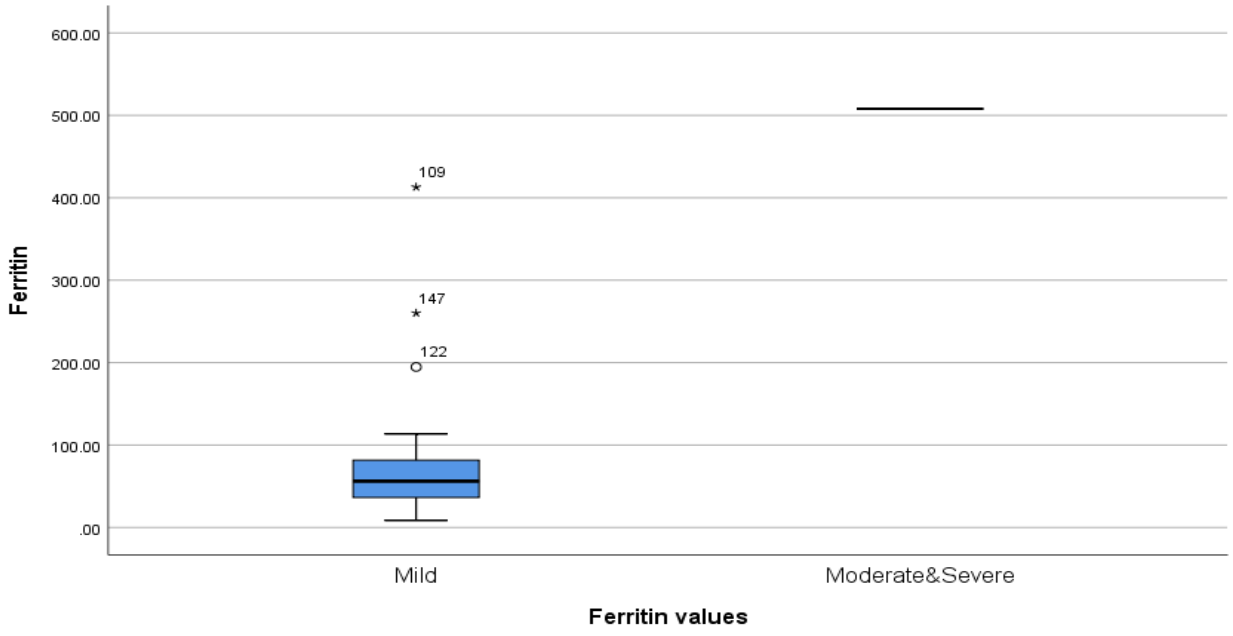
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Respiratory failure	1/167(0.5)
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IV antibiotics	4/167 (2.3)
Remdesivir	3/167 (1.8)
Oral azithromycin	10/167 (5.9)
Systemic glucocorticoid	1/167 (0.5)
Oxygen by mask	1/167 (0.5)
Mechanical ventilation	1/167 (0.5)
Admission to ICU	4/167 (2.3)
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Discharge	167/167 (100)
Mortality	Nil

Note: Reference ranges as per from Nelson textbook of paediatrics.



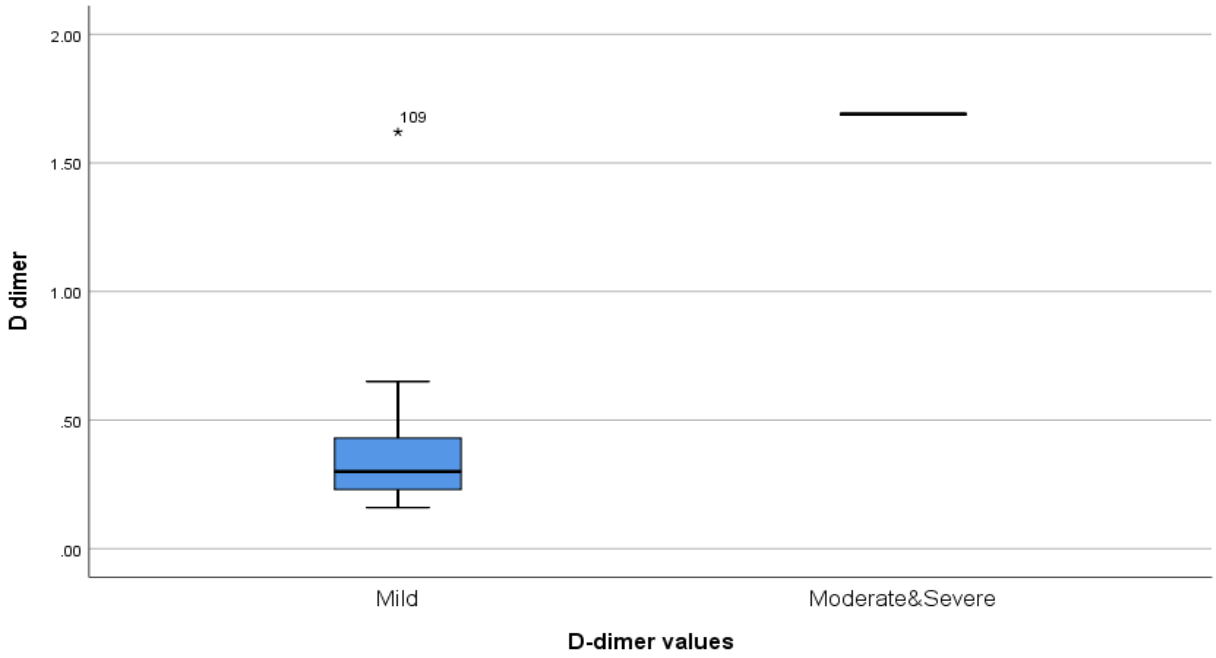
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Note: Data were expressed in median. P=0.04 by Mann-Whitney U test.□



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Note: Data were expressed in median. P=0.04 by Mann-Whitney U test. □



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Note: Data were expressed in median. P=0.06 by Mann-Whitney U test.

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The main focus of this study was to analyze the clinical, laboratory profile, treatment and it's outcome of 167 children admitted in the designated tertiary care COVID unit which admits children from asymptomatic infection to critical disease. When compared to adults, paediatric age groups were reported with less severe disease. As the schools were closed and outdoor activities were restricted the primary source of infection in children were household contacts. Majority of children in our cohort were asymptomatic (60%), and few had variable severity of symptoms from mild to severe. As per WHO-China joint mission on COVID-19, 2.5% of cases under 19 years had severe infection and 0.2% had critical disease.[7] A large

report from China stated that 10.6% ,7.3% and 4.2% children between 0-1, 1-5, 6-10 years had severe infection respectively.[8] High risk groups for severe infection are infants below 1 year and those with underlying medical conditions like neurological problems, metabolic disorders, congenital heart disease, diabetes, asthma, chronic lung disease, sickle cell disease and obesity.[9] Similarly, in this study a 2-months old infant and a child with comorbidity developed severe disease. Incidence of severe disease among comorbid children was high (14%) in this study. This is in accordance with a systematic review of 42 studies on severe COVID-19 infection where the clinical manifestations were severe in 5.1% of children with comorbidities and 0.2% without comorbidities.[10] Among children admitted in US and Canadian PICU with COVID-19, 83% had pre-existing comorbidities and out of which 73% had respiratory symptoms and 38% required mechanical ventilation.[11] Hence, children with comorbidities need careful monitoring during the first 2 weeks of illness.

Most common symptoms in our patients were fever, cough and rhinorrhoea followed by gastrointestinal symptoms which were consistent with previous reports.[6,12] Dermatological and neurological manifestations were not observed in our children.

Central peri bronchial cuffing was the common CXR abnormality noted in our study. 92% of them with the above finding neither had respiratory symptoms at admission nor during hospital stay. Hence, the central peri bronchial cuffing may be a nonspecific finding and further evaluation may be required only if there are worsening symptoms and signs. Another study from Spain had shown similar findings in CXR in 86% of it's patients.[13] In our study those with peripheral heterogeneous opacity in CXR had significant clinical symptoms and ground glass opacities in CT. This was identical to COVID pneumonia causing diffuse interstitial infiltrates involving the periphery of the lung.[14]

Similar to few meta-analysis and systematic review on COVID-19, leukopenia (58%) and lymphocytosis (32.8%) were most commonly observed in our study.[15] Lymphopenia (11.4%) and thrombocytopenia (3.5%) were comparable to that of study by Meena et al.[12] Though lymphopenia was frequently seen in adults with severe disease and high mortality, it was rarely observed in children. It may be because of milder illnesses in children.[16] Hence, these hematological findings along with clinical features similar to COVID should raise a strong suspicion of COVID-19 infection and it will be a useful predictor for planning treatment in children with moderate to severe illness.[12,16]

Inflammatory markers were raised in 15-25% of our children. Studies found that higher the CRP, IL-6 and ferritin more severe was the disease.[16] Liver enzymes were increased in 7-8% which is consistent with other studies and its prognostic significance is still uncertain.[17]

Children with severe infection can develop serious complications like acute respiratory distress syndrome, renal failure, myocarditis, shock, acute renal failure, coagulopathy, MODS and later MIS-C. In our study population only a single child developed respiratory failure and none had MODS. Antivirals and steroids were used in very few. Guidelines on COVID-19 treatment in children evolved only later and most were extrapolated from adult studies during our study period. Two children were readmitted with MIS-C manifestations after 3-4 weeks and both had very minimal symptoms during the 1st admission. More studies are needed to determine the children at risk for MIS-C. No mortality was encountered among the admissions in our study.

Hence our present study included the majority of asymptomatic children, these clinical and laboratory data may be helpful in supporting COVID-19 diagnosis in children when diagnostic tests are not available and it provides the true representation of disease spectrum of paediatric population. Limitations of this study was small sample size and single centre study.

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COVID-19 infection was milder among the majority. Infants and children with comorbidities had increased risk of severe disease. Inflammatory markers were helpful in predicting the severity of illness. Antivirals and steroids were needed in very few children. Since the disease has many new variants it might present differently in future. Hence, clinicians need to have a high index of suspicion for early diagnosis and management to have a good outcome and recovery.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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1. Varghese L, Zachariah P, Vargas C, LaRussa P, Demmer RT, Furuya YE, et al. Epidemiology and Clinical Features of Human Coronaviruses in the Pediatric Population. J Pediatr Infect Dis Soc. 2018;7(2):151-8.
2. Memish ZA, Tawfiq JA, Assiri A, Rabiah FA, Hajjar S, Albarrak A, et al. Middle East respiratory syndrome coronavirus disease in children. Pediatr Infect Dis J. 2014;33(9):904-6.

3. CDC. Healthcare Workers, 2020. Available at: https://www.cdc.gov/coronavirus/2019ncov/hcp/pediatric-hcp.html. Accessed on 25 May 2021.
4. Zimmermann P, Curtis N. Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. Arch Dis Child. 2020;320338.
5. CDC. Multisystem Inflammatory Syndrome (MIS), 2021. Available at: https://www.cdc.gov/mis/index.html. Accessed on 25 May 2021.
6. WHO. Clinical management of COVID-19: interim guidance, 2020. Available at: https://apps.who.int/iris/handle/10665/3. Accessed on 25 May 2021.
7. WHO. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), 2020. Available at: https://www.who.int/docs/default-source/coronaviruse/who-chinajointmissiononcovid-19-final-report.pdf. Accessed on 25 May 2021.
8. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiology of COVID-19 Among Children in China. Pediatrics. 2020;145(6):20200702.
9. CDC. COVID-19 and Your Health, 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/your-health/index.html. Accessed on 25 May 2021.
10. Tsankov BK, Allaire JM, Irvine MA, Lopez AA, Sauvé LJ, Vallance BA, et al. Severe COVID-19 Infection and Pediatric Comorbidities: A Systematic Review and Meta-Analysis. Int J Infect Dis. 2021;103:246-56.
11. Shekerdemian LS, Mahmood NR, Wolfe KK, Riggs BJ, Ross CE, McKiernan CA, et al. Characteristics and Outcomes of Children With Coronavirus Disease 2019 (COVID-19) Infection Admitted to US and Canadian Pediatric Intensive Care Units. JAMA Pediatr. 2020;174(9):868-73.
12. Meena J, Yadav J, Saini L, Yadav A, Kumar J. Clinical Features and Outcome of SARS-CoV-2 Infection in Children: A Systematic Review and Meta-analysis. Indian Pediatr. 2020;57(9):820-6.
13. Serrano C, Alonso E, Andrés M, Buitrago NM, Pérez VA, Parrón PM, et al. Pediatric chest x-ray in covid-19 infection. Eur J Radiol. 2020;131:109236.
14. Kumar J, Meena J, Yadav A, Yadav J. Radiological Findings of COVID-19 in Children: A Systematic Review and Meta-Analysis. J Trop Pediatr. 2021;67(3):45.
15. Ma X, Liu S, Chen L, Zhuang L, Zhang J, Xin Y. The clinical characteristics of pediatric inpatients with SARS-CoV-2 infection: A meta-analysis and systematic review. J Med Virol. 2021;93(1):234-40.
16. Terpos E, Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. Am J Hematol. 2020;95(7):834-47.
17. Moon AM, Barritt AS. Elevated Liver Enzymes in Patients with COVID-19: Look, but Not Too Hard. Dig Dis Sci. 2021;66(6):1767-9.

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Original article

Clinical spectrum and short-term outcomes of multisystem inflammatory syndrome in children in a south Indian hospital

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Background: Multisystem inflammatory syndrome in children (MIS-C) is a new hyperinflammatory variant that evolved during the coronavirus disease 2019 pandemic. Although the precise pathophysiology of MIS-C is uncertain, it is thought to be due to immune dysregulation occurring after recovery from acute infection.

Purpose: Our study aimed to analyze the clinical spectrum, laboratory parameters, imaging characteristics, treatment strategies, and short-term outcomes of children with a diagnosis of MIS-C.

Methods: This retrospective and prospective observational study included children less than 16 years of age who were admitted to the pediatric unit of a tertiary care teaching hospital in south India between August 2020 to January 2021 with a diagnosis of MIS-C according to World Health Organization criteria.

Results: Twenty-one children were included in the analysis; all had fever with variable combinations of other symptoms. The mean age was 6.9 years; 71.4% were male. Gastrointestinal (80.9%) and cardiovascular (80.9%) systems were the most commonly affected. The majority of children had elevated inflammatory markers, and 16 (76.2%) had echocardiographic abnormalities mimicking Kawasaki disease. Eleven children (52.4%) required intensive care admission, 3 (14.3%) required supplemental oxygen, and 4 (19%) required inotropes. Nine (42.9%) were treated with intravenous immunoglobulin alone, 6 (28.6%) with steroids alone, and 3 (14.3%) with steroids and immunoglobulin. The median hospital stay was 6 days; there were no fatalities. Overweight/obesity, elevated ferritin, and mucocutaneous involvement were significantly associated with a prolonged hospital stay (≥7 days). Sixteen children (76.2%) were followed up till now and all of them had no clinical concerns.

Conclusion: MIS-C is an emerging disease with variable presentation. A high index of suspicion is necessary for its early identification and appropriate management. Further research is essential for developing optimal treatment strategies.

Key words: Clinical spectrum, Coronavirus disease 2019, Kawasaki disease, Multisystem inflammatory syndrome in children

Key message

- **Question:** What are the clinical spectrum, course, and short-term outcomes of multisystem inflammatory syndrome in children (MIS-C)?
- **Finding:** MIS-C can have variable clinical manifestations. Fever is most common, followed by gastrointestinal and cardiovascular symptoms. Early identification and appropriate management lead to favorable outcomes.
- **Meaning:** MIS-C can present in a myriad of ways and severities. High suspicion is necessary to ensure its early identification and appropriate management and favorable patient outcomes.

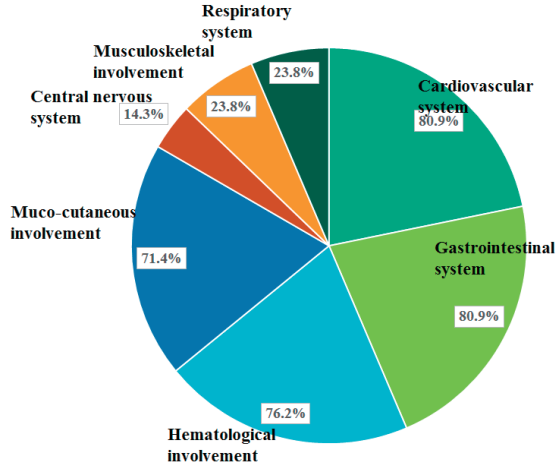
Introduction

The coronavirus disease 2019 (COVID-19) pandemic in India is a part of the global pandemic caused by severe acute respiratory syndrome corona virus-2 (SARS-CoV-2). India is one of the countries with the highest burden of confirmed COVID-19 cases in the world and ranks second in the list of countries with a maximum number of cases.[1] Earlier reports suggested that children infected with COVID-19 were highly resilient and could present with only a mild upper respiratory illness.[2-4] But in April 2020, clinicians in the United Kingdom reported a cluster of previously healthy children presenting with shock, fever, and hyper inflammation.[5] The Royal College of Paediatrics and Child Health referred to this acute condition as pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2.[6] As the pandemic progressed and the number of such cases increased globally the illness was labelled as multisystem inflammatory syndrome in children (MIS-C) by the Centers for Disease Control and Prevention and

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Clinical Spectrum of Multisystem Inflammatory Syndrome in Children



- Intensive care admission – 52.4 %
- Supplemental oxygen requirement – 14.3%
- Inotrope requirement – 19 %
- Abnormal ECHO – 76.2%
- Mortality - 0%
- Follow-up – 76.2%

Graphical abstract

the World Health Organization (WHO).[7,8] Although the exact pathophysiology of MIS-C is uncertain, it is thought to be due to immune dysregulation occurring after recovery from acute infection. As the burden of COVID-19 infection is increasing in India, more children are being recognized with this condition. Although many articles are reported from western countries there is a dearth of published reports from Asian countries.

Our study aimed at analyzing the clinical spectrum, laboratory parameters, and imaging characteristics of children less than 16 years admitted with a diagnosis of MIS-C. In addition, this study aimed to analyze the treatment strategies and short-term outcomes.

Methods

1. Study design and setting

This study was a partly retrospective and partly prospective observational study done in children admitted with a diagnosis of MIS-C in a tertiary care teaching hospital in south India from August 2020 to January 2021. The hospital is one of the largest COVID-19 care centers in south India and receives referrals from a radius of 200 kilometers. The study was approved by the Institutional Human Ethics Committee of PSGIMS&R (project No. 20/262 dated 27th November, 2020). Informed written consent was obtained from parents prior to the inclusion of children in the prospective part of the study. Waiver of consent was obtained for the retrospective collection of data.

2. Participants

Children less than 16 years of age diagnosed with MIS-C as per WHO criteria were included in the study. The following parameters were considered for diagnosis: (1) fever ≥3 days, (2) 2 of the following: a) rash or bilateral nonpurulent conjunctivitis or mucocutaneous inflammation signs (oral mucosa, hands, or feet), b) hypotension or shock, c) features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiography findings or elevated troponin/N-terminal pro-B-type natriuretic peptide [NT-proBNP]), d) evidence of coagulopathy (by prothrombin time, partial thromboplastin time, elevated D-dimers), e) Acute gastrointestinal problems [diarrhea, vomiting, or abdominal pain], (3) elevated markers of inflammation such as erythrocyte sedimentation rate, C-reactive protein, or procalcitonin, (4) no other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal toxic shock syndromes, (5) evidence of COVID-19 (real-time reverse transcription polymerase chain reaction [RT-PCR], antigen test or serology positive), or likely contact with patients with COVID-19.

3. Data collection

Data on demographics, clinical features, laboratory results, imaging studies, treatment details, outcomes, and follow-up were collected. All the investigations and treatment were carried out as per institutional protocol and treating physician's discretion.

COVID-19 infection was confirmed by testing for SARS-CoV-2 antibody by chemiluminescence immunoassay (VITROS 3600 immunodiagnostic system, Ortho Clinical Diagnostics, Raritan, NJ, USA) and/or RT-PCR as recommended by Indian Council of Medical Research. The laboratory values were interpreted as either increased or decreased based on the age-specific normal reference ranges. Organ system involvement was described based on clinical features, laboratory reports, and imaging studies. Echocardiography was performed in all children by the pediatric cardiologist to identify the following abnormalities; (a) left ventricular dysfunction (ejection fraction

less than 55%), (b) pericarditis or pericardial effusion, (c) coronary artery abnormalities (dilatation, aneurysm, or irregular nontapering coronary arteries), or (d) valvular regurgitation. The dimension of the coronary artery was measured as per standard criteria and a coronary z score above 2 was reported as dilated. Other imaging studies were done as clinically indicated.

4. Statistical analysis

Data analysis was done using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). Descriptive statistics were used to summarize population characteristics. Categorical variables were expressed as frequency or percentage and continuous variables were expressed as mean with standard deviation or median with interquartile range. IBM SPSS Statistics ver. 26.0 (IBM Co., Armonk, NY, USA) was used to perform univariate analysis and a *P* value of <0.05 was considered significant.

Results

From August 2020 to January 2021, twenty-one children met the WHO MIS-C criteria and were included in the analysis. Among them, only 2 children were admitted with active coronavirus-2 infection and were subsequently diagnosed as MIS-C. The demographic and clinical characteristics of the study population are mentioned in Table 1. The mean age of the study population was 6.9 years and 71.4% were male. All children were previously healthy and 9.5% were either obese or overweight.

All patients had fever with a variable combination of other symptoms. Gastrointestinal symptoms like abdominal pain, diarrhea, and vomiting were the most common presenting symptoms. The mean duration of symptoms prior to admission was 4.8 days and the mean total duration of fever was 7.9 days. The majority of children in this series had multiorgan involvement. Gastrointestinal (80.9%) and cardiovascular (80.9 %) systems were the most commonly affected organ systems. This was followed by hematological (76.2%), mucocutaneous (71.4%), and central nervous system (14.2%) involvement. None of the children had acute kidney injury in this series. Two children in this series had been treated for COVID-19 3 weeks prior to the diagnosis of MIS-C, and 5 children had history of confirmed exposure to COVID-19 patients in the preceding 3 to 4 weeks. In other children, no history associated with COVID-19 was revealed.

In the study population, 19 children (90.4%) had positive COVID-19 antibody tests, 1 (4.8%) had positive COVID-19 RT-PCR test, and another (4.8%) had both positive COVID-19 RT-PCR and antibody tests. The results of laboratory tests and imaging studies are summarized in Tables 2 and 3. Lymphopenia and thrombocytopenia were seen in 47.6% and 38.1% of cases, respectively. Inflammatory markers were elevated in the majority of cases. Specifically, D-dimer and interleukin 6 (IL-6) were elevated in 95.2% and 90.5% of cases, respectively. Children with cardiovascular system involvement showed elevation of

Table 1. Patients' demographic and clinical characteristics

Population characteristic	Value
Age (yr)	6.9±4
Male sex	15 (71.4)
Weight (kg), median (IQR)	20 (12.8–35.5)
Overweight/obesity	2 (9.5)
Duration of symptoms at admission (day)	4.8±2.1
Contact with COVID-19 patient	5 (23.8)
Past COVID-19 infection	2 (9.5)
Clinical features	
General	
Fever	21 (100)
Total duration of fever (day)	7.9±3.1
Highest temperature recorded in hospital admission (°F)	102.4±1.8
Giddiness	1 (4.8)
Fatigue±lethargy	13 (61.9)
Headache	5 (23.8)
Respiratory	
Cough	2 (9.5)
Sore throat/ throat pain	3 (14.3)
Runny nose±sneezing	1 (4.8)
Fast breathing	4 (19)
Gastrointestinal	
Nausea	3 (14.3)
Vomiting	12 (57.1)
Diarrhea	11 (52.4)
Abdominal pain	11 (52.4)
Abdominal distension	5 (23.8)
Musculoskeletal	
Joint pain	1 (4.8)
Myalgia	7 (33.3)
Mucocutaneous	
Rash	8 (38.1)
Bilateral non-exudative conjunctivitis	12 (57.1)
Lymphadenopathy	3 (14.3)
Strawberry tongue±cracked lips	7 (33.3)
Oedema/erythema of hands and feet	6 (28.6)
Cardiovascular	
Shock requiring inotropes	4 (19)
Neurological	
Seizures	1 (4.8)
Altered level of consciousness	3 (14.3)
Cerebellar signs	1 (4.8)
Focal neurological deficit	1 (4.8)
Organ system involvement[a)]	
One	1 (4.8)
Two	3 (14.3)
Three	4 (19)
Four or more	13 (61.9)

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
IQR, interquartile range; COVID-19, coronavirus disease 2019.
[a)]Organ system involvement was classified as cardiovascular, gastrointestinal, respiratory, nervous, musculoskeletal, hematological, or renal based on clinical features and investigations.

Table 2. Laboratory test results

Parameter	Number (%)	Definition
Blood counts		
Anemia	4/21 (19)	Hb <10 g/dL in <1 yr Hb <9 g/dL in ≥1 yr
Neutrophilia	16/21 (76.2)	ANC >7.7×10^3 cells/μL
Lymphopenia	10/21 (47.6)	ALC <1.5×10^3 cells/μL in child >8 mo ALC <4.5×10^3 cells/μL in child <8 mo
Thrombocytopenia	8/21 (38.1)	Platelet <150×10^3 cells/mm^3
Thrombocytosis	6/21 (28.6)	Platelet >500×10^3 cells/mm^3
Inflammatory markers		
Elevated ESR	17/20 (85)	≥40 mm/hr
Elevated CRP	20/20 (100)	≥0.6 mg/dL
Elevated procalcitonin	5/5 (100)	≥0.5 ng/mL
Elevated ferritin	9/21 (42.9)	>500 ng/mL
Elevated IL-6	19/21 (90.5)	≥7 pg/mL
Elevated LDH	11/16 (68.8)	LDH >300 U/L
Electrolytes		
Hyponatremia	12/17 (70.6)	Sodium <135 mEq/L
Hypokalemia	8/17 (47.1)	Potassium <3.5 mEq/L
Liver function test		
Elevated AST	6/17 (35.3)	AST >40 U/L
Elevated ALT	5/17 (29.4)	ALT >40 U/L
Hypoalbuminemia	9/17 (52.9)	Albumin <3 g/dL
Cardiac biomarkers		
Elevated troponin-T	4/13 (30.8)	≥14 pg/mL
Elevated NT-proBNP	12/15 (80)	>450 pg/mL
Coagulation profile		
Elevated D-dimer	20/21 (95.2)	≥0.5 mg/L FEU
Deranged coagulation	2/8 (25)	INR ≥1.2

Hb, hemoglobin; ANC, absolute neutrophil counts; ALC, absolute lymphocyte counts; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IL, interleukin; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; FEU, fibrinogen equivalent units; INR, international normalized ratio.

cardiac biomarkers, and NT-proBNP (80%) was found to be more commonly elevated than troponin-T (30.8%). Electrolyte imbalances like hyponatremia and hypokalemia were seen in 70.6% and 47.1% of cases, respectively, which were managed appropriately.

Echocardiography was performed in all the children, and abnormality was noted in 16 children (76.2%). A greater number of children had a combination of abnormalities rather than a single abnormal finding. Coronary artery abnormality (57.1%) was the most common finding, followed by pericardial effusion (52.4%), left ventricular dysfunction (23.8%), and mitral regurgitation (23.8%). Gastrointestinal imaging studies like x-ray, ultrasound, and computed tomography were done as indicated, and ascites (50%) was the most common abnormality, followed by dilated bowel loops (35.7%), and mesenteric lymphadenitis (35.7%). Of the 17 patients who underwent chest imaging (x-ray or computed tomography), pleural effusion and lung infiltrates were observed in 3 (17.6%) and 1 patient (5.9%),

Table 3. Results of COVID-19 tests and imaging studies

Variable	Number (%)
COVID-19 test results	
Positive serology alone	19 (90.4)
Positive RT-PCR alone	1 (4.8)
Both serology and RT-PCR positive	1 (4.8)
Chest imaging (n=17)	
Pleural effusion	3 (17.6)
Lung infiltrates	1 (5.9)
Gastrointestinal imaging (n=14)	
Ascites	7 (50)
Mesenteric lymphadenitis	5 (35.7)
Dilated bowel loops	5 (35.7)
Appendicular inflammation	3 (21.4)
Appendicular abscess	1 (7.1)
Hepatomegaly	3 (21.4)
Gallbladder wall thickening	1 (7.1)
Echocardiography findings (n=21)	
Abnormal ECHO	16 (76.2)
Coronary artery abnormality	12 (57.1)
Pericardial effusion	11 (52.4)
Left ventricular dysfunction	5 (23.8)
Mitral regurgitation	5 (23.8)

COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription polymerase chain reaction; ECHO,

Table 4. Treatment details and outcomes

Variable	Value
Highest level of care	
PICU admission	11 (52.4)
Duration of PICU stay (hr)	74.8±41.6
Respiratory support	
Supplemental oxygen requirement	3 (14.3)
Duration of oxygen requirement (hr)	39.6±28.6
High flow nasal cannula	1 (4.8)
Circulatory support	
Inotropic support	4 (19)
Duration of inotrope requirement (hr)	37±24.5
Pharmacotherapy	
Antibiotics	18 (85.7)
Intravenous immunoglobulin alone	9 (42.9)
Steroids (intravenous or oral) alone	6 (28.6)
Both intravenous immunoglobulin and steroids	3 (14.3)
Aspirin	14 (66.7)
Anticoagulant	3 (14.3)
Antiepileptics	1 (4.8)
Outcomes	
Length of hospital stay (day), median (IQR)	6 (5–8)
Mortality	0 (0)
Follow-up	16 (76.2)

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
PICU, pediatric intensive care unit; IQR, interquartile range.

respectively. Of the 3 children with central nervous system involvement, 1 had encephalopathy with features of cerebellar involvement, 1 had seizures, and another had headache with bilateral papilledema. All 3 children with central nervous system involvement showed normal neuroimaging. Two children underwent cerebrospinal fluid analysis, and findings of aseptic meningitis were seen in the patient who presented with encephalopathy.

Treatment details and outcomes are summarized in Table 4. Eleven children (52.4%) required pediatric intensive care unit (PICU) admission and the mean duration of PICU stay was 74.8 hours. Three children (14.3%) required supplemental oxygen, and none of the children required mechanical ventilation. Four children (19%) required inotropic support for hemodynamic instability, and the mean duration of inotrope requirement was 37 hours. Nine children (42.9%) were treated with intravenous immunoglobulin (IVIG) alone, 6 (28.6%) were treated with steroids alone and 3 (14.3%) were treated with both IVIG and steroids. None of the cases required other immunomodulators for treatment.

An 8-year-old girl presented with features of acute appendicitis, and an emergency surgery was done, which revealed a perforated appendix with abscess collection. She was subsequently diagnosed as MIS-C and was treated with IVIG.

The median length of hospitalization was 6 days in this series. Table 5 shows the results of univariate analysis for prolonged hospital stay (≥7 days). Children who were either overweight or obese (P=0.035), had elevated ferritin (P=0.019), or had mucocutaneous involvement (P=0.040) were found to be significantly associated with prolonged hospital stay. Further extensive analysis could not be performed due to small sample size. There was no mortality in patients of our study, and all patients were discharged home. At the time of writing this article, 16 patients (76.2%) had attended follow-up visits.

Table 5. Results of univariate analysis of factors associated with prolonged hospital stay (n=21)

Variable	Hospital stay<7 days (n=14), n (%)	Hospital stay≥7 days (n=7), n (%)	*P* value	OR (95% CI)
Age groups			0.376	-
Less than 5 yr (n=7)	6 (85.7)	1 (14.3)		
5 to 9 yr (n=6)	3 (50.0)	3 (50.0)		
10 to 15 yr (n=8)	5 (62.5)	3 (37.5)		
Sex			1.000	1.000 (0.134–7.451)
Male (n=15)	10 (66.7)	5 (33.3)		
Female (n=6)	4 (66.7)	2 (33.3)		
Overweight/obesity (n=2)	0 (0)	2 (100)	**0.035**	0.263 (0.124–0.558)
Temperature > 38.9℃ (n=11)	5 (45.5)	6 (54.5)	0.089	10.80 (0.997–116.998)
Anemia (n=4)	2 (50.0)	2 (50.0)	0.432	2.400 (0.261–22.105)
Lymphopenia (n=10)	5 (50.0)	5 (50.0)	0.122	4.500 (0.627–32.295)
Thrombocytopenia (n=8)	6 (75.0)	2 (25.0)	0.525	0.533 (0.076–3.755)
Elevated ESR (n=17)	12 (70.6)	5 (29.4)	0.212	0.208 (0.015–2.854)
Elevated ferritin (n=9)	3 (33.3)	6 (66.7)	**0.019**	22.0 (1.857–260.648)
Elevated IL-6 (n=19)	12 (63.2)	7 (33.3)	0.293	-
Elevated D-dimer (n=20)	13 (65.0)	7 (35.0)	0.469	-
Hyponatremia (n=12)	6 (50.0)	6 (50.0)	0.252	4.000 (0.340–47.112)
Hypokalemia (n=8)	4 (50.0)	4 (50.0)	0.486	2.000 (0.282–14.198)
Hypoalbuminemia (n=9)	5 (55.6)	4 (44.4)	0.772	1.333 (1.191–9.311)
Abnormal ECHO (n=16)	10 (62.5)	6 (37.5)	0.469	2.400 (0.215–26.822)
PICU admission (n= 12)	7 (58.3)	5 (41.7)	0.350	2.500 (0.357–17.500)
Supplemental oxygen requirement (n=3)	1 (33.3)	2(66.7)	0.186	5.200 (0.381–70.903)
Inotropic support (n=4)	2 (50.0)	2 (50.0)	0.432	2.400 (0.261–22.105)
Organ system involvement				
Cardiovascular system (n=17)	10 (58.8)	7 (41.2)	0.116	-
Respiratory system (n=5)	2 (40.0)	3 (60.0)	0.147	4.500 (0.542–37.378)
Gastrointestinal system (n=17)	11 (64.7)	6 (35.3)	0.694	1.636 (0.138–19.387)
Central nervous system (n=3)	2 (66.7)	1 (33.3)	1.000	1.000 (0.075–13.367)
Hematological system (n=16)	9 (56.3)	7 (43.8)	0.070	1.778 (1.154–2.379)
Mucocutaneous involvement (n=15)	12 (80.0)	3 (20.0)	**0.040**	0.125 (0.15–1.038)
Musculoskeletal system (n=5)	3 (60.0)	2 (40.0)	0.717	1.467 (0.184–11.718)
Multiorgan involvement (≥4 organs) (n=13)	8 (61.5)	5 (38.5)	0.525	1.875 (0.266–13.202)

OR, odds ratio; CI, confidence interval; ESR, erythrocyte sedimentation rate; IL, interleukin; ECHO, :PICU, pediatric intensive care unit.
Boldface indicates a statistically significant difference with P<0.05.

Table 6. Common clinical characteristics in our study versus those of other studies

Clinical feature	Our study (n=21)	Whittaker et al.[10] (n=58)	Dhanalakshmi et al.[11] (n=19)
Fever	21 (100)	58 (100)	19 (100)
Vomiting	12 (57.1)	26 (45)	6 (31.5)
Abdominal pain	11 (52.4)	31 (53)	8 (42)
Diarrhea	11 (52.4)	30 (52)	3 (15.8)
Rash	8 (38.1)	30 (52)	12 (63.2)
Bilateral conjunctival congestion	12 (57.1)	26 (45)	9 (47.4)
Cervical lymphadenopathy	3 (14.3)	9 (16)	6 (31.5)
Strawberry tongue±cracked lips	7 (33.3)	17 (29)	9 (47.4)
Oedema/erythema of hands and feet	6 (28.6)	9 (16)	10 (52.6)

Values are presented as number (%).

Among them, 11 children (52.4%) had attended one follow-up visit, and 5 (23.8%) had attended 2 follow-up visits, all of whom had no clinical concerns. Among 16 children with abnormal echocardiography, 12 (75%) underwent follow-up echocardiography, and their cardiac imaging showed partial or complete resolution of previous abnormalities. Further follow-up echocardiography was recommended to some of them.

Discussion

MIS-C is an evolving concept of disease and its exact incidences in different geographic areas are uncertain. The focus of this study was to analyze the clinical spectrum and short-term outcomes of children with MIS-C managed in a tertiary care teaching hospital in south India. We described 21 children under the age of 16 years with MIS-C associated with SARS-CoV-2 infection. All these children had evidences of previous or active COVID-19 infection. The spectrum of clinical presentations in our study was broadly consistent with available literatures. The mean age of children in this study was 6.9 years, and 61.9% of affected children were above 5 years, which was similar to studies by Kaushik et al.,[9] Whittaker et al.,[10] and Dhanalakshmi et al.[11] The male preponderance in this study was also identical to previous reports by Kaushik et al.,[9] Whittaker et al.,[10] and Gupta et al.[12] Table 6 shows the comparison between common clinical features of children in this study and 2 previous studies.[10,11] Concordant to previous reports, a greater number of children in our series had multiorgan involvement with gastrointestinal and cardiovascular systems being the most commonly affected systems.[11,13-15]

The incidence of hematological abnormalities like lymphopenia (47.6%) and thrombocytopenia (38.1%) in our study was in agreement with previous reports.[11,14,16] The majority of children in this study had raised inflammatory markers, which was similar to previous reports by Kaushik et al.,[9] Dhanalakshmi et al.,[11] Feldstein et al.,[13] and Mamishi et al.[16] In contrast to previous studies by Dhanalakshmi et al.,[11] Feldstein et al.,[13] Toubiana et al.,[15] and Miller et al.,[17] none of the children in this series had acute kidney injury. The incidence of hyponatremia (70.6%) in this study was higher than in previous reports by Dhanalakshmi et al.[1] and Mamishi et al.[16] Hypokalemia was found in 47.1% of children, and it was more common in children with gastrointestinal manifestations, which was not reported in any previous studies. Hypoalbuminemia was noted in 52.9% of children in this study, which was almost similar to previous reports by Dhanalakshmi et al.[1] and Mamishi et al.[16] The elevation of cardiac biomarkers was also concordant to previous reports by Whittaker et al.[10] and Mamishi et al.[16] The incidence of echocardiographic abnormalities in this study was comparable to previous reports by Ramcharan et al.[15] and Toubiana et al.[18] The abnormalities seen in gastrointestinal imaging studies in this study were comparable to the previous report by Miller et al.[17]

Since MIS-C is an emerging disease, guidelines regarding the management are still evolving and there is a lack of consistency in management. The majority of patients in our study were treated with either IVIG, steroids, or a combination of both and, none required other immunomodulators. The treatment decision for the patients in our study was made based on severity of the illness and the treating physician's discretion. Significant differences in treatment strategies were noted among studies from different parts of the world, which could be due to lack of standard treatment protocols in the initial phase of disease evolution.[9-11,14,16] However, a considerable number of studies would be required for developing standard treatment protocols. Concordant to previous reports by Torres et al.[14] and Dhanalakshmi et al.,[11] 52.4% of children in this series required PICU admission. The median length of hospital stay was 6 days which was comparable to previous reports.[9,14,15] Although previous studies showed variable rates of mortality, no death was reported in this study.[11,14,15,17] The follow-up rate in this study was 76.2%. All children had no health concerns at the time of follow-up, and most echocardiographic abnormalities had resolved. However, these children should be followed up at regular intervals to know the long-term consequences of the disease.

A major limitation of this study is that it was conducted in a single center over a limited period of time, and has a small sample size. Another limitation is that this study only included children under the age of 16 years, because our institutional policy defined children under 16 years to be admitted to the pediatric department. It might have missed MIS-C patients older than 16

years of age.

In conclusion, MIS-C is a novel disease that can present in a myriad of ways with variable severity, and a high index of suspicion is necessary in this era of COVID-19 pandemic for early identification, appropriate management, and favorable outcomes of patients. Further research is essential to develop optimal treatment strategies.

Footnotes

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

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References

1. WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. Geneva (Switzerland): World Health Organization; 2021 [cited 2021 Feb 24]. Available from: https://covid19.who.int/.
2. Castagnoli R, Votto M, Licari A, Brambilla I, Bruno R, Perlini S, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. JAMA Pediatr 2020;174:882-9.
3. Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis 2020;34:101623.
4. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr 2020;109:1088-95.
5. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet 2020;395:1607-8.
6. Paediatric multisystem inflammatory syndrome temporally associated with COVID-19 (PIMS) - guidance for clinicians [Internet]. London: Royal College of Paediatrics and Child Health; 2021 [cited 2021 Feb 24]. Available from: https://www.rcpch.ac.uk/resources/paediatric-multi-system-inflammatory-syndrome-temporally-associated-covid-19-pims-guidance.
7. Emergency preparedness and response. Multisystem inflammatory syndrome in children associated with coronavirus disease 2019 [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2020 [cited 2021 Feb 24]. Available from: https://emergency.cdc.gov/han/2020/han00432.asp.
8. Multisystem inflammatory syndrome in children and adolescents with COVID-19 [Internet]. Geneva (Switzerland): World Health Organization [cited 2021 Feb 24]. Available from: www.who.int/publicationsdetail/multisystem-inflammatory-syndrome-in-children-and-adolescents-withcovid-19.
9. Kaushik S, Aydin SI, Derespina KR, Bansal PB, Kowalsky S, Trachtman R, et al. Multisystem inflammatory syndrome in children associated with severe acute respiratory syndrome coronavirus 2 infection (MIS-C): a multi-institutional study from New York City. J Pediatr 2020;224:24-9.
10. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. JAMA 2020;324:259-69.
11. Dhanalakshmi K, Venkataraman A, Balasubramanian S, Madhusudan M, Amperayani S, Putilibai S, et al. Epidemiological and clinical profile of pediatric inflammatory multisystem syndrome - temporally associated with SARS-CoV-2 (PIMS-TS) in Indian children. Indian Pediatr 2020;57:1010-4.
12. Gupta Dch S, Chopra Md N, Singh Md A, Gera R, Chellani Md H, Pandey PhD R, et al. Unusual clinical manifestations and outcome of multisystem inflammatory syndrome in children (MIS-C) in a tertiary care hospital of North India. J Trop Pediatr 2021;67:fmaa127.
13. Feldstein LR, Rose EB, Horwitz SM, Collins JP, Newhams MM, Son MBF, et al. Multisystem inflammatory syndrome in U.S. children and adolescents. N Engl J Med 2020;383:334-6.
14. Torres JP, Izquierdo G, Acuña M, Pavez D, Reyes F, Fritis A, et al. Multisystem inflammatory syndrome in children (MIS-C): report of the clinical and epidemiological characteristics of cases in Santiago de Chile during the SARS-CoV-2 pandemic. Int J Infect Dis 2020;100:75-81.
15. Toubiana J, Poirault C, Corsia A, Bajolle F, Fourgeaud J, Angoulvant F, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. BMJ 2020;369:m2094.
16. Mamishi S, Movahedi Z, Mohammadi M, Ziaee V, Khodabandeh M, Abdolsalehi MR, et al. Multisystem inflammatory syndrome associated with SARS-CoV-2 infection in 45 children: a first report from Iran. Epidemiol Infect 2020;148:e196.
17. Miller J, Cantor A, Zachariah P, Ahn D, Martinez M, Margolis KG. Gastrointestinal symptoms as a major presentation component of a novel multisystem inflammatory syndrome in children that is related to coronavirus disease 2019: a single center experience of 44 cases. Gastroenterology 2020;159:1571-4.e2.
18. Ramcharan T, Nolan O, Lai CY, Prabhu N, Krishnamurthy R, Richter AG, et al. Paediatric inflammatory multisystem syndrome: temporally associated with SARS-CoV-2 (PIMS-TS): cardiac features, management and short-term outcomes at a UK tertiary paediatric hospital. Pediatr Cardiol 2020;41:1391-401.

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Sublingual Ulceration Due to Natal Tooth – Riga – Fedes Disease.

KEYWORDS: Riga – Fede's disease, natal tooth, sublingual ulceration

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INTRODUCTION :

Ulceration on ventral surface of the tongue in newborn is most commonly associated with trauma by Natal (or) Neonatal tooth.[1,2] It is due to forward and backward movement of the tongue over the primary teeth. In older children ulceration occurs after eruption of primary lower incisors with repetitive tongue thrusting habits.[3] Neurological disorders like familial dysautonomia (insensitive to pain)[4] will also be associated with above such lesions.

The lesion usually begins as ulceration on ventral surface of tongue with repeated trauma. It may progress to fibrous enlarged ulcerative granulomatous mass. But its nature is relatively benign and seldom requires histopathological examination. It will be brought to the Pediatrician's attention since it interferes with sucking,feeding leading on to nutritional deficiency risk for the neonate.

This case is reported to recognize this entity to prevent unnecessary workups and invasive procedures.

Case report:

A 22–days-old term neonate was brought with complaints of difficulty and excessive irritability while breast feeding. The mother noticed an ulcer beneath the tongue (figure -1) for the past 3 days before she came to us and can't able to feed her baby properly. On examination there was a sharp natal tooth in the place of mandibular central incisor ,whitish in colour causing a deep ulceration 20 x 10 mm in the ventral aspect of the tongue extending from anterior border to frenulum with white fibrinopurulent membrane and surrounding erythema(figure 2). Tenderness was noted on gentle digital palpation. There was no other intraoral mucosal lesions. There was no family history of such lesions and was negative of congenital syndromes, neurological disorders and disorders in development .

A diagnosis of Riga – Fedes disease (RFD) was made and the tooth was extracted under topical local anesthesia. The ulcer has healed remarkably well after 3 weeks of extraction and the feeding was reestablished.

Discussion :

Teeth eruption at birth was reported age old in many medical as well as dental literature.[5] These entities have been coined as 'Natal teeth', 'Neonatal teeth', 'congenital teeth' (or) Precocious dentition by Mayhall and Baderhoff.[6,7] The most accepted terminology in present literature has been defined by Massler and Savara.[8] Natal teeth indicates the teeth present in oral cavity at birth and 'neonatal teeth' those which erupt during the neonatal period ie from birth to thirtieth day of life.

RFD is observed in 6 to 10 percent of neonates with natal tooth and it is most commonly with its sharp nature. These ulcerations interfere with proper suckling and feeding and put the neonates at risk for nutritional deficiencies resulting in failure to thrive.

This lesion was first described by Antonio Riga, an Italian Physician in 1881 and Fede, an Italian Pediatrician published its histopathological studies in 1890 [3] and subsequently it has been known as " Riga-Fedes disease".

Another entity described by Elzay in 1983, ' traumatic ulcerative granuloma with stromal eosinophilia' (TUGSE) with similar histopathological features like RigaFedes disease[9].TUGSE is reported mostly in late adulthood and not restricted only to tongue also to buccal mucosa , vestibule, gingiva (or) palate. But Riga Fedes disease has been reported as exclusively restricted to the tongue. In our case, the lesion is restricted mainly to tongue and no other mucosal lesions anywhere.

Neurological disorder association has been described by Domingues - Cruz et al classifying it into " Precocious Riga Fede disease" in first 6 months with no neurological disorders and "late Riga Fede disease "after 6-8 months after primary dentition with possible relationship to neurologic disease.

Treatment modalities of Riga- Fedes has varied over years from extraction of the tooth and curettage of underlying Dental papilla which is the standard way of care to other conservative therapeutic options like Grinding (or) discing the sharp edges (or) coverage of the tooth with light-cured composite resin.[10,11]

In our case, since the ulcer area was large and denuded and interferes with suckling & feeding the tooth has been extracted.

On follow up the lesion has healed well and the feeding was normally established.



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REFERENCE

1.Goho C.Neonatal sublingual traumatic ulceration(Riga-Fede Disease):Reports of cases.J Dent Child 1996;63:362-4. | 2.Buchanan S,Jenkins CR.Riga-Fede's syndrome:Natal or Neonatal teeth associated with tongue ulceration,case report.Aust Dent J 1997;42;225-7. | 3.Slaryton R.Treatment alternatives for sublingual traumatic ulceration(Riga-Fede disease).Fed Dent 2000;22;413-4. | 4.Rakocz M,Frand M,Brand N.Familial dysautonomia with Riga-Fede's disease:report of case.ASDC J Dent Child 1987;54;57-9. | 5.Ruth AA.Natal and Neonatal teeth:Histologic investigation of two black females.ASDC J Dent Child 1982;49;300-3. | 6.Mayhall JT.Natal and Neonatal teeth among the thinget Indians.J Dent Res 1967;46:748-9. | 7.Badenhoff J.Natal and neonatal teeth.Dent Abstr 1960;5;485-6. | 8.Massler M,Savara BS.Natal and neonatal teeth.J Pediat 1950;36:349-59. | 9.Elzay RP;Traumatic ulcerative granuloma with stromal eosinophilia (Riga-Fede's disease and traumatic eosinophilic granuloma).Oral surg Oral Med Oral Pathol 1983,55;497-506. | 10.Allwright W.Natal and neonatal teeth.A study among Chinese in Hong Kong.Br Dent J 1958;105;163-72. | 11.Baghdadi ZD.Riga-Fede disease :Report of a case and review.J Cl Fed Dent 2001;25;209-13. |

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Antibiotic Prophylaxis and Surgical Site Infection

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Abstract

Background: Surgical Site Infection rate is an important health care index. Prophylactic antibiotics have been used to prevent surgical site infections. A continuous surveillance is called for to keep a check on the resistance pattern of microbes to the prophylactic antibiotic.

Objectives:

1. To estimate the Rate of Surgical Site Infections in the years 2014 and 2015 with reference to CDC guidelines at a tertiary care hospital (PSGIMS&R) located in Tamil Nadu, Southern India
2. To estimate the frequency of various organisms causing Surgical Site Infections
3. To study the antibiotic resistance pattern to the prophylactic antibiotic used.

Type of Study: Retrospective Observational study

Period of Study: January 2014 – December 2015

Methodology: The present study was conducted in PSG Institute of Medical Sciences & Research, Coimbatore. We did a thorough retrospective analysis of case files of 467patients reported to have culture positive SSI. SSI was established using clinical and Standard microbiological testing.

Results: The outcome of this study is that the SSI rate of 4.9% reported in our hospital is consistent with or less than the rates reported in other parts of the country. Diabetes is a very important comorbidity which makes the person significantly vulnerable to SSI. In culture positive cases 24.4% of SSI showed resistance to the prophylactic antibiotic used.

Conclusion: There is a significant rise in incidence of organisms exhibiting resistance to the prophylactic antibiotic and a constant surveillance with feedback to the surgeons and strict adherence to antibiotic policies is essential.

Keywords: Surgical Site Infection Rate; Prophylactic Antibiotics; Diabetes Mellitus; Surveillance.

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Introduction

Surgical Site Infections (SSI) are those which occur in the wound created by invasive surgical procedures. Historically they were called 'Irritative Fevers' [1] before the era of antisepsis brought about by Joseph Lister. These 'fevers' were uniformly lethal and so dreaded by all surgeons. They are an important cause of Healthcare associated infections. They continue to be an important health hazard even in hospitals with standard protocols of preoperative preparation and prophylactic antibiotic usage. Their prevalence is usually underestimated [2] owing to the occurrence of many of these infections after the patient has been discharged from the hospital. The spectrum of SSI can range from a simple wound discharge to life threatening sepsis. The incidence of SSI in India is estimated [3] to be around 11% at tertiary care centers.

The present study was aimed at obtaining theincidence of surgical site infections and determining the role of Diabetes Mellitus as an

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Original Research Article

Outcome of skin incision by cautery versus steel scalpel in hernia surgery: a prospective cohort study at a tertiary medical college hospital in South India

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ABSTRACT

Background: Since a long time, skin incisions have routinely been made with scalpels. Now a days there is a shift in trend from this method to electrosurgical skin incisions. However, fear of bad scars and improper wound healing has prevented its wide spread use. The aim of the study was to compare the Diathermy versus Scalpel skin incision in elective hernia surgeries with regards to post-operative pain, post-operative wound infection rate and wound healing.
Methods: A total of 200 patients were taken for this study. 100 patients underwent diathermy incision (group A) who were compared with 100 scalpel incision patients (group B). Variables used in this study were postoperative pain, wound infection and scar.
Results: Patients with diathermy skin incision were having less post-operative pain which was assessed by visual analogue scale when compared with scalpel incision since the p value was 0.01 which is significant (<0.05). The postoperative infection rate and scar were comparable in both the groups since the p-value is >0.05 in each which is insignificant.
Conclusions: Diathermy incisions are therefore less harmful to the skin. It has got more advantages than scalpel incisions like less postoperative pain.

Keywords: Electrosurgery, ESU, Monopolar, Surgical site infections, Visual analogue scale

INTRODUCTION

Surgical incisions are usually made with scalpel. Usage of scalpel, usually results in skin bleeding which obscure the operating field resulting in wastage of operating time. Other alternative in making surgical incision is diathermy. Diathermy is mainly used for tissue dissection and hemostasis.[1] Usually skin incision by diathermy is avoided due to fear of deeps burn, poor wound healing and excessive scarring. These presumptions stem from experimental and clinical studies that yielded varied reports.[2,3] Modern electrosurgical units capable of delivering pure sinusoidal currents have evolved a change in this concept. The advantages are rapid hemostasis, faster dissection, and a reduced overall operative blood loss.[4,5] Majority of studies had compared electrocautery and scalpel incision in terms of wound infection, postoperative pain, blood loss, duration of healing and postoperative wound complication in only selected groups of patients with the exclusion of patients with medical co-morbidities.

No single study to date has focused on diathermy incisions in elective surgical cases exclusively so as to figure out the post-operative wound infection rate. This study was conducted to compare diathermy and scalpel incisions in terms of postoperative pain, wound healing i.e. scar character and postoperative wound infection.

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Original Research Article

Lateral partial internal sphincterotomy-a complete relief in chronic fissure in ANO

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ABSTRACT

Background: Lateral internal sphincterotomy is the only choice in the treatment of the fissure in ano. Complete and partial sphincterotomy was followed in variable individuals. But post-operative complication like incontinence, early mobilization and early recovery is to be made into account for best results among these patients.
Methods: This study is made in 2015-2016 period among 52 patients with chronic fissure in ano, partial sphincterotomy i.e., division of internal sphincter below the dentate line was performed. Post-operative follows up and symptoms were recorded, in such cases treated adequately. The period of revisit was decided according to patient compliance 1 month, 3months, 6 months. The data was collected prospectively and recorded in view of change in the treatment.
Results: 47 of the patients were pain free within first 5 days of surgery. Remaining 5 patients required further division for pain relief due to persistent pain after 3 weeks. The associated post-operative complication among these patients was itching due to burning sensation in and around the anus which resolved later. None among these patients developed fecal incontinence.
Conclusions: Among all age group of the patients included in this study were subjected to same type of the procedure and results were recorded to give better prognosis with lowest degree of recurrence for which repeat of the same procedure gave the complete relief. Lateral internal partial sphincterotomy is an effective technique for complete relief of pain in chronic fissure in ano.

Keywords: Chronic fissure in ano, Earlymobilisation, Lateral internal partial sphincterotomy

INTRODUCTION

Chronic fissure in ano is very common in all age group of people due to present days hi-fi method of food habits, modified life style and inborn errors of health conscience. The present generation is more moribund on the development of technology and work styles of these techis are tedious. Requirement of the remedies are long standing, permanent and symptom or complication free. Early mobilization after any procedure is the only way out for earned population. In the development of the internet medicine access leads to the attitude of clearance of the physician doubts. Based on the above wide range of criteria among these lies the choice of the treatment to be very selective and preferably high in placement of the best treatment for them.

Lateral internal sphincterotomy is the only choice in the treatment of the fissure in ano. Complete Sphincterotomy and partial sphincterotomy was followed in variable individuals.[1,2] But post-operative complication like incontinence is to be made into account for the follow up

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Clinical Study and Management of Calculus in Upper 1/3rd of the Ureter

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Abstract
Aim: *To identify the various clinical presentations and the complications of upper 1/3rd Ureteric calculus, To study the most common type of stone in upper 1/3rd ureter(stone analysis). To study the difference and relative advantage of different treatment modalities so that an appropriate treatment regimen can be instituted for the patient and further complications may be prevented.*
Materials and Methods: *Prospective study of 100 patients diagnosed to have upper ureteric calculi at Kempegowda Institute Of Medical Sciences Hospital, Bangalore from October 2014 to August 2016 and of them 74 underwent operative procedure for upper ureteric calculi. Data was analysed using descriptive statistics and chi-square test.*
Results: *Results were analysed among who underwent operative procedure in different ascepts like, Age (mean age=43.59), Incidence (M>F), dietary habits (mixed diet> veg diet), site of pain (U/L>B/L), complications, need for ESWL(19), different operatives modalities followed by stone analysis.*
Conclusion: *the incidence of urolithiasis has increased drastically, probably due to modern lifestyle and dietary habits and shift in the age distribution of Urolithiasis in recent times. There is an increased number of patients suffering fromthis condition in younger age group. Shift in gender distribution of urolithiasis with age, especially in females in post-menopausal period.*

Introduction

Urolithiasis is one of the common cause for morbidity in modern society. The lifetime prevalence of kidney stone disease is estimated at 1% to 15%, varying according to age, gender, race, and geographic location with the most recent prevalence estimate of 8.8%.[1] It has been suggested that the rise in stone incidence and prevalence seen in the worldwide can be attributed in part to a rise in the detection of asymptomatic calculi through increased utilization of radiographic imaging, particularly computed tomography.[2,3] Edvardsson and colleagues (2013) found that the annual incidence of symptomatic stones did not increase significantly, despite significant increases in the incidence of asymptomatic stones in both genders (from 7 to 24 per 100,000 in men, P < .001, and from 7 to 21 per 100,000 in women< .001).

Original Research Article

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Diabetic Foot Ulcer and Multidrug-Resistant Organisms: Prevalence and Bacteriological Profile

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Abstract

Aim: To study the prevalence and bacteriological profile of multi-drug resistant organism (MDRO) infection in diabetic foot ulcers. *Methods:* 150 Diabetic patients with foot ulcer were prospectively studied. Detailed clinical history and clinical examination of the ulcer were done for all patients. The microbiological profile was analysed, in detail, for each patient. The multi-drug resistant organisms (MDRO) were identified, using internationally accepted criteria. *Results:* 153 MDROs were isolated, out of a total of 279 organisms (54.8%). These were isolated from 99 out of 150 patients (66%). The commonest MDRO isolated was *Escherechia coli*, followed by *Pseudomonas aeruginosa*. *Conclusion:* The prevalence of MDRO is alarmingly high in infected diabetic foot ulcers.

Keywords: Diabetic Foot Ulcer; Multi-Drug Resistant Organisms (MDRO).

Introduction

Foot ulceration is a major cause of morbidity in diabetes mellitus. It is said that a sixth of all patients with diabetes mellitus develop foot ulcers at some point in their lifetime [1]. Infections in diabetic foot ulcers have become increasingly difficult to treat, because of the emergence of multi drug resistant organisms (MDRO). In the past, the problem of MDRO was less well understood, because of a lack of uniform définition of MDRO. This study was done, as very few studies have been done in India to analyse the prevalence of MDRO in diabetic foot ulcers.

Patients and Methods

Conducted between January 2011 and July 2012 at PSG Institute of Medical Sciences & Research, Coimbatore, India, this is a prospective observational study to find the prevalence of MDRO in diabetic foot ulcers. Prior approval from the institution review board was obtained. 150 diabetic patients with foot lesions were included in the study. Written informed consents were obtained from the patients. Detailed clinical history of the patient and other relevant data were collected using structured case report forms.

Wound swabs were obtained from the floor of the ulcer, before starting on empirical antibiotic therapy. Direct microscopic examination and aerobic cultures were done by standard methods. The bacteriological spectrum and the sensitive antibiotics were noted for each patient. MDROs were identified using the ECDC and CDC criteria [2].

Results

Seventy Eight percent (78%) of the patients were 51 years or older, with the average age being 58.21. 74.6% of the patients were males. Almost all the patients had Type II diabetes, with only 4% of them having Type I. Only 19.33% of patients had a good glycaemic control, with HbA1c 6–7%. 40 % of patients with foot ulcer had diabetes for less than 5 yrs.

Sixty eight percent (68%) of patients had ulcers of less than one month duration. Most of the patients had Wagner's grade II, III, or IV ulcers. There were very few

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Original Research Article

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Analysis of Antibiotic Therapy in Acute Severe Pancreatitis

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Abstract

Background: Acute Pancreatitis is a common surgical entity that is increasing in incidence. Gallstones and alcohol consumption remain the major contributors of Acute pancreatitis. The disease usually follows a prolonged course with increased hospital stay, early and late complications contributing to increased patient morbidity and mortality. Traditionally the role of antibiotics is usually limited to severe necrotising pancreatitis. There is however increasing evidence to prove the role of antibiotic therapy in reducing mortality and long term morbidity in acute pancreatitis patients.

Objectives:

1. To retrospectively analyse antibiotic therapy in patients with Acute severe pancreatitis in the years 2014 - 2017 in the tertiary care hospital PSG IMSR located in Coimbatore, Tamilnadu, India
2. To analyse the role of antibiotics in patient prognosis and outcome

Type of study: Retrospective Observational study. *Duration of study*: January 2014-January 2017. *Methodology:* The present study was conducted in PSG Institute of Medical Sciences & Research, Coimbatore. We did a thorough retrospective analysis of case files of 900 patients with acute severe pancreatitis. Analysing the available data from case files. *Results*: Of 900 patients, 411satisfied the inclusion criteris, of these 63% (n=258) of patients were started antibiotics within 48 hrs of hospital admission. The first choice antibiotics were Piperacillin tazobactum and metronidazole in 45% of cases, Fluoroquinolones and metronidazole in 12% of cases and Cefaperazone sulbactum ± metronidazole in 14% of cases. In 26% of cases a combination of imipenem + Cilastatin was used. Antibiotic use did not improve survival, nor was there any observed survival benefit when the different antibiotic agents were compared (p = 0.7 and 0.4 respectively). The timing of antibiotic use also does not appear to confer a survival benefit (p = 0.5). All patients with proven pancreatic infection died, there was not a significant difference in survival in those with extra-pancreatic infections (p = 0.2).

Keywords: Acute Pancreatitis; Necrotising Pancreatitis; Severe Acute Pancreatitis; Antibiotic Prophylaxis; Retrospective Analysis.

Introduction

Acute pancreatitis is a common gastrointestinal emergency. Its incidence varies from 5 to 80 cases per 100,000 inhabitants per year, with an overall mortality rate of 10–15% [1]. More than two-thirds of patients will recover within 1 week, however the remaining one-third will experience multiple systemic and/or local complications, with a high mortality rate of 10–30%, 80% of deaths are due to infectious complications [2-3].

The use and efficacy of prophylactic antibiotic therapy in acute pancreatitis has long been debated. The role of prophylactic antibiotics to prevent infection and reduce mortality in pancreatitis was first evaluated in the 1970s, where several randomised controlled trials (RCTs) had been conducted and concluded that prophylactic antibiotics were effective in preventing secondary pancreatic infections and therefore in

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STUDY ON THE CONVERSION OF LAPAROSCOPIC CHOLECYSTECTOMY OWING TO PER OPERATIVE COMPLICATIONS

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ABSTRACT

BACKGROUND

In the modern medical era, laparoscopic approach to surgical conditions has reached to a height that it is performed even for malignant conditions. The minimal access surgery which has grown up from minimally invasive surgery has given us the faith that nearly all surgeries can be done by laparoscopy. The first laparoscopic cholecystectomy was done by Prof. Dr. Med Erich Muhe of Germany in the year 1985. About ten lakh cholecystectomies are performed ever year, of which 96% are done by laparoscopic method. Laparoscopic cholecystectomy is the most common laparoscopic procedure. By number of studies and research works, it is stated that laparoscopic cholecystectomy is superior to conventional open method because of its advantages like smaller incisions, early recovery, less post-operative pain and hospital stay with better cosmesis. However, laparoscopic cholecystectomy also has got its own disadvantages and complications. In 1992, an NIH consensus conference held in Bethesda approved laparoscopic cholecystectomy as the treatment of choice for symptomatic cholelithiasis. Conversion to open technique is a major morbidity of laparoscopy, as it loses its supremacy over open technique once the conversion takes place. With growing experience of laparoscopic cholecystectomy and completion of the learning curve, the indications for laparoscopic cholecystectomy have been extended approaching that of open cholecystectomy. Complications of laparoscopic cholecystectomy have been minimised to as low as 2 - 6%. However, a substantial proportion of patients had to be converted to open surgery because of technical difficulties or intraoperative complications. Conversion rates of 2.6% to 14% had been described in different studies. The factor to be considered with conversion is that it should never be considered a complication, but rather a correct judgement by the surgeon.

The aims of the present study are:

1. To study the incidence of conversion of laparoscopic cholecystectomy.
2. To analyse the reasons for the conversion of laparoscopic cholecystectomy owing to per operative complications like-
   a) Arterial injury,
   b) Hepatobiliary tract injury,
   c) Dense adhesions,
   d) Aberrant anatomy,
   e) Technical issues.

MATERIALS AND METHODS

This prospective observational study was conducted in patients who underwent laparoscopic cholecystectomy from November 2016 to November 2017 in the Department of General Surgery, Karpagam Faculty of Medical Sciences and Research, Coimbatore. There were totally 98 patients, of which 36 were males and 62 were females. The study has been done after the patient's informed consent. All these patients were evaluated in a proper manner as given in the proforma and had been assessed pre-operatively and operated under perfect anaesthetic fitness. This study mainly focussed on the patients who had been converted to open method. They were analysed further regarding the reasons for conversion to open method due to per-operative complications.

RESULTS

From November 2016 - November 2017, a total of 98 patients had undergone laparoscopic cholecystectomy in the Department of General Surgery, Karpagam Faculty of Medical Sciences and Research, Coimbatore. Out of 98 patients who underwent laparoscopic cholecystectomy, 13 patients (13.26%) were converted to open cholecystectomy owing to per-operative complications. All patients underwent cholecystectomy as an elective case. Though some morbidity was there in terms of post-operative complications, which we are not analysing, no mortality was reported.

CONCLUSION

After analysing the results of our study, we conclude that the incidence of conversion of laparoscopic cholecystectomy is 13.26%. The reasons for conversion in descending order of frequency are: 1. Dense adhesions (5.1%), 2. Difficult anatomy around Calot's triangle (3.06%), 3. Arterial injury (3.06%), 4. Hepatobiliary injury (1.02%) and 5. Technical issues (1.02%).

KEY WORDS

Laparoscopic Cholecystectomy, Cholelithiasis, Per-Operative Complications, Bile Duct, Cystic Artery, Adhesions, Calot's Triangle.

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BACKGROUND

In the modern medical era, laparoscopic approach to surgical conditions have reached to a height that it is performed even for malignant conditions. The minimal access surgery, which has grown up from minimal invasive surgery has given us the faith that nearly all surgeries can be done by laparoscopy. The first laparoscopic cholecystectomy was done by Prof. Dr. Med Erich Muhe of Germany in the year 1985. About ten lakh cholecystectomies are performed ever year, of which 96% are done by laparoscopic method. Laparoscopic cholecystectomy

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An Evaluation of serum IgE in Atopic patients -predictor for Post-Operative Mesh reaction

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Abstract

Background: *In the modern surgical practice, Hernioplasty remains the commonest surgical procedure in general surgery. Though most of the patient recovers completely without complications, certain number of patients experience a stormy and complicated post-operative period in terms of seroma, infection, pain and last but not the least mesh reaction which is a rare but serious complication. Current day practice involves hernioplasty with polypropylene mesh rather than herniorraphy in most surgical centres, hence every effort should be made to reduce mesh related complications. Mesh reactions are more common in patients with preexisting allergic diseases like atopic dermatitis, asthma, allergic rhinitis, seasonal urticarial and also in patients with elevated serum IgE levels. This retrospective observational study was conducted in patients who underwent open inguinal and ventral hernioplastyfrom March 2010 to Feb 2017 in the Department of General Surgery, Coimbatore medical college hospital, Coimbatore. This study focuses mainly on patients who developed mesh reaction in the post-operative period particularly in patients who have existing atopic manifestations. After analyzing the results of our study, we conclude that IgE antibodies estimation pre-operatively in patients with existing allergic diseases can be used as a predictor for assessing mesh related reactions in the post-operative period. In this continuation we are planning immuno-electrophoresis to pinpoint the gamma globulin associated with mesh reaction.*

Keywords: *Hernioplasty; Mesh reactions; IgE; Atopy.*

Introduction

In the modern surgical practice, hernioplasty remains the commonest surgical procedure in general surgery. Though most of the patient recovers completely without complications, certain number of patients experience a stormy and complicated post-operative period in terms of seroma, infection, pain and last but not the least mesh reaction which is a rare but serious complication. Hernia surgery, like many other operations, has undergone considerable transformation over the past decade with the

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ORIGINAL ARTICLE

CR-Possum—Can It Be Used to Predict Morbidity? A Single-Centre Retrospective Study

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Abstract
Preoperative prediction of morbidity in colorectal cancer (CRC) surgery helps to optimize the surgical outcome. In this study, we aim to develop a dedicated equation for predicting operative morbidity using colorectal possum scoring system and also to validate the predictive accuracy of CR-POSSUM scoring system in prognosticating actual complications. We did a retrospective analysis of 322 patients undergoing colorectal cancer surgery from a single centre in South India from 2004 to 2016. Mortality and morbidity risk factors as defined by CR POSSUM were collected from 322 patients who underwent CRC surgery and were used to derive equations to predict morbidity, and the results were compared with the observed morbidity. Logistic regression analysis was used to derive the equation. The model fit and model discrimination were analysed using the Hosmer-Lemeshow statistical test for goodness of fit, the Nagelkerke R^2 and area under the receiver operating characteristic (ROC) curve respectively. Out of 322 patients, 103 (32%) patients developed complications and 10 (3%) died due to complications. The regression equation we derived has an overall correct classification of about 70% ($P < 0.01$) with positive and negative predictive value of 60% and 73% respectively. The Hosmer-Lemeshow goodness of fit was 3.147 ($P = 0.829$), and the Nagelkerke R^2 was 17% and area under ROC as model discrimination was 71.6%. Hence, CR-POSSUM scoring which was originally used for predicting mortality risk can also be extrapolated to predict morbidity.

Keywords Colorectal POSSUM · POSSUM · Colorectal surgery · SSI · Morbidity and mortality · Prediction model

Introduction

Colorectal malignancy is the third most common malignancy among men and the second most common among women around the world [1]. Fortunately, in India, the incidence is not too high. However, the incidence has been found to be rising, with the AAR (adjusted annual incidence rate) for colon cancer in men and women being 4.4 and 3.9 per 100,000, respectively [2]. Being in a country, where people living with poor access to quality healthcare, extreme presentation like advanced stage and perforation are common in tertiary care centres [3, 4]. In such scenarios, the mortality and morbidity for the patients undergoing surgery are relatively high [5]. Complications following major surgeries will proportionately increase the hospital cost and duration of hospital stay [6]. Apart from such hardships, complications also had an adverse impact on long-term survival [7]. So in a country like India, where the spending on health by the government is low and health insurance is under-penetrated, morbidity following a major surgical procedure will further drain the already constrained resources [8]. The complication rates are not uniform and cannot be generalised, as there are certain factors, such as age, patients' co-morbidity and operative severity, influencing the outcome of surgery [9]. So, putting together these factors and assessing the postoperative outcome preoperatively will help in optimizing the available resources. In such cases, the predictive scoring has to be more accurate, and its predictive ability should

Key message: CR-Possum can be used to predict morbidity in patient undergoing colorectal cancer surgery.

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A CADAVERIC STUDY OF THE MYOCARDIAL BRIDGES

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ABSTRACT

BACKGROUND

Myocardial bridging is a congenital anomaly, where a segment of an epicardial coronary artery is undercover of myocardial fibres for a variable distance and the underlying arterial segment is described as a mural coronary artery or a tunnelled artery. The history of myocardial bridges dates back to 1737 when it was first recognized by Reyman and then by Black in 1805. The first detailed post mortem examination of myocardial bridges was done by Geiringer in 1951 and the first radiological description was given by Portmann and Iwig in 1960. The bridged segment of the coronary artery is susceptible to various pathophysiological mechanisms leading to myocardial ischemia, especially when associated with other cardiovascular diseases. The myocardial bridges were initially thought to be innocent bystanders in the development of acute coronary syndromes. But in the last two decades, the outlook has changed with the increasing incidence of sudden cardiac deaths in young athletes, where myocardial bridges were the only finding at autopsy. The mechanism by which the myocardial bridges induce clinical symptoms is uncertain, but among the proposed are vasospasm, thrombus formation, endothelial dysfunction and impaired coronary flow reserve. Most important of these is the dynamic systolic compression of the tunnelled artery with sustained early diastolic diameter reduction.

The aim of this study is to provide more information on the incidence of myocardial bridges, arterial preponderance for myocardial bridging, the length of the bridged segment and multiple bridging.

MATERIALS AND METHODS

A descriptive study of fifty formalin fixed hearts from the department of anatomy were dissected by conventional dissection methods as guided by Cunningham.

RESULTS

Myocardial bridges were found in 18 (36%) hearts with a total of about 28 bridges. Most common artery involved was the anterior inter ventricular artery (66.6%), which even had multiple bridges over it. The mean length of the bridges was 26.54 mm.

CONCLUSION

From the present study, we infer that the presence of myocardial bridges in an unsuspected population is relatively higher. The definitive role of these bridges in acute coronary syndromes is yet uncertain. But owing to its higher rate of detection in those with myocardial ischemia, it is better to screen for the bridges along with other aetiological factors and treat the patients prophylactically. Of the imaging modalities, CT Coronary Angiography is found to be the investigation of choice. In the near future, holistic studies involving cadaveric, radiologic and clinical data are mandatory in researching the myocardial bridges.

KEY WORDS

Myocardial Bridges, Myocardial Ischemia, Atherosclerosis, Tunnelled Artery, Systolic Compression.

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BACKGROUND

Myocardial bridging is a congenital anomaly, in which an epicardial coronary artery lies in the myocardium for part of its course. The bridged segment of the coronary artery is susceptible to various pathophysiological mechanisms leading to myocardial ischemia, especially when associated with other cardio vascular diseases. Recently, myocardial bridges have become a main topic of interest owing to its probable causation of sudden cardiac death in young athletes.

The aim of this study is to provide more information on the arteries with myocardial bridges. Of the fifty formalin fixed hearts dissected, myocardial bridges were found in 18 (36%) hearts with a total of about 28 bridges. Most common artery involved was the anterior inter ventricular artery, which even had multiple bridges over a single artery. The mean length of the bridges was 26.54 mm.

Myocardial bridging is a congenital anomaly, where a segment of an epicardial coronary artery is undercover of myocardial fibres for a variable distance and the underlying arterial segment is described as a mural coronary artery or a tunnelled artery.[1] (Fig. 1).

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Original Research Article

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Monitoring Postoperative Pain Management in General Surgical Wards: A Clinical Audit of Feedback Pain Protocol in Our Institution

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Abstract

"Pain" is defined by the International Association for the Study of Pain as an unpleasant sensory and emotional experience arising from actual or potential tissue damage or described in terms of such damage. There is both the physiologic and affective nature of the pain experience. Inadequate pain control, apart from being inhumane, may result in increased morbidity or mortality. Despite this overwhelming rationale for effective postoperative pain control, the clinical reality is unfortunately, still far from satisfactory. As the importance of postoperative pain relief is undebated, there is a need for an effective protocol in the care of all postoperative patients. In our hospital, we follow a "feedback" pain protocol, in which the VAS pain scale is used to record pain at least once a day, or more depending on the analgesia frequency, with a follow-up after 15 to 45 min to see the level of pain relief, and to receive drug orders for further analgesia if the pain relief is not adequate. The aim of this audit is to find the level of compliance to this protocol, and its effectiveness. The main reason being that there is no standard protocol for postoperative pain management widely being practiced. The surgeons are the ones who are managing the postoperative pain. This audit will determine the effectiveness, and the adherence to protocol, which will suggest whether there is a need for a change in the protocol, or adherence. Objectives being to ensure that the analgesia protocol is followed in all postoperative patients and to ensure that atleast 95% of those who receive the analgesia protocol, has a VAS score of less than 3 in their first two postoperative days.

Keywords: Postoperative pain; Pain protocol; Feedback protocol; Institution.

Introduction

"Pain" is defined by the International Association for the Study of Pain as an unpleasant sensory and emotional experience arising from actual or potential tissue damage or described in terms of such damage[1]. Although this is a technical description of pain, it recognizes both the physiologic and affective nature of the pain experience. Inadequate pain control, apart from being inhumane, may result in increased morbidity or mortality. Acute pain following surgery, can activate the sympathetic branch of the autonomic nervous system and produce such responses as hypertension, tachycardia, diaphoresis, shallow respiration, restlessness, facial grimacing, guarding behavior, pallor, and pupil dilation. Although pain in response to tissue damage is a normal phenomenon, it may be associated with significant, unnecessary physical, psychological, and emotional distress. Inadequate relief of acute pain can contribute to hypercoagulability and impaired immunity, leading to such complications as venous thromboembolic disease and infections.

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Original Research Article

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Acetic Acid Based Dressings on *Pseudomonas aeruginosa* Infected Diabetic Ulcers: A Randomized Controlled Study

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Abstract

Wound infections had increased the risk of amputations in Diabetic patients by 90 times. *Pseudomonas aeruginosa*, has been a local deterrant to wound healing by its various properties of biofilm formation, increased virulence and multi drug resistance by various methods. Unfortunately, due to its resistance to first generation spectrum of antibiotics, and its gradual increase in resistance to third generation cephalosporins, aminoglycosides etc, the need for alternative methodologies arise. Acetic acid helps in local control of the microbe by reducing the wound pH to acidic. This was an Open labelled prospective randomized control trial. Totally 80 patients with diabetic foot ulcers with culture proven *Pseudomonas aeruginosa* positive status were included, and non diabetic ulcers were excluded. Two groups based on simple randomization into test and control groups each containing 40 patients. The control group received traditional saline dressings. The test variables and the control variables were tabulated and compared using Chi square test. The efficacy of 3% Acetic acid in eradication of *Pseudomonas aeruginosa* infected diabetic ulcers was assessed. $p<0.01$ was considered to be statistically significant. At the end of the study, it was seen that there was complete eradication of *Pseudomonas aeruginosa* in 87.5% of the individuals in the test group and 62.5% of the individuals in the control group which was statistically significant ($p = 0.009$). Thus, in conclusion, Acetic-acid based dressings are effective for management of diabetic foot.

Keywords: Acetic acid; Pseudomonas; Dressing.

Introduction

Diabetes mellitus is a disorder characterized by uncontrolled sugar levels in the blood and its various manifestations to the human body. Diabetic foot and diabetic foot ulcers occurs as a complication of its sequelae. Foster et al discovered in 1997 that nearly 30 percent of patients with diabetes mellitus have increased risk of developing foot ulcers. 15% of diabetics developing foot ulcers, accounting for 30% of the hospital admissions with the hospital stay of those patients being 60% longer than the rest of the patients. These high risk patients had an increased risk of amputations[1]. The overall healing period of the diabetic ulcer was estimated to be 3 months with persistence of a small ulcer for more than a year[2]. Various management methodologies have been implemented in treating diabetic wounds including Debridement, Dressings, Infection control, Amputation, correcting vasculopathies, Off-loading. Wound infection deters the healing process of the diabetic ulcer and leads to amputations. It was seen wound infections had increased the risk of amputations by 90 times to that of a non-infected wound[4]. It has been seen that the following microbial organisms are more

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Case Series

A case series of adult intussusception diagnosed by ultrasound in South India: an uncommon notion

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ABSTRACT

Intussusception is the telescoping of one segment of the gastrointestinal tract into an adjacent one. Adult intussusception is less than 0.1% of all hospital admissions. Non-specific symptoms often delay the diagnosis with most cases diagnosed only after emergency laparotomy, hence the need for imaging as a screening investigation. The gold standard is computed tomography with the limitations of cost, radiation and contrast hazards. Our suggested initial screening tool is ultrasound. the aim of this study was to evaluate the sensitivity of ultrasound in diagnosing this rare entity. Thirteen patients (2013 to 2019) in a single tertiary care hospital diagnosed as "intussusception" were analysed. They were initially diagnosed by ultrasound and confirmed by CT or intra-op as needed. There were no exclusion criteria. The mean age was 50. There were 6 males and 7 females. Ultrasound sensitivity was 72%. Symptoms were variable. 100% had abdominal pain (1 day to 2 years duration). 69% had Intestinal obstruction. The most common type of adult intussusception observed was ileo-ileal (39%). The least common noted was gastro-jejunal (8%). The most common treatment was resection and anastomosis (77%). 3 cases were managed conservatively successfully. The biopsy was mostly benign (54%), 3 were malignant (23%) and 3 were unknown as they were not operated (23%). Our suggested initial screening is ultrasound with a fairly good sensitivity (72%). The upcoming improvement in technology can raise this sensitivity even further. The management protocols, biopsy findings, and clinical findings are however subjected to discretion.

Keywords: Adult, Intussusception, Ultrasound, Diagnosis

INTRODUCTION

Intussusception is the telescoping of one segment of the gastrointestinal tract into an adjacent one.[1] Any focus of an intraluminal irritant such as inflamed mucosa or a mass lesion may act as a lead point and the resulting hyperperistaltic activity causes a segment of bowel (intussusceptum) possibly along with its mesentery to telescope into the adjacent distal bowel lumen (intussuscipiens).[2] Adult intussusception is classified according the location of the lead point as entero-enteric, ileocolic, ileocecal, and colo-colic.

Paediatric intussusception is more common and they are easily identified because they present typically with intermittent colicky pain, abdominal mass and passage of dark clots mixed with mucus.[3] Intussusception, compared to the paediatric population, is an infrequent cause of bowel obstruction in adults, with only two to three cases occurring in a population of 1,000,000 per annum, accounting for less than 0.1% of all adult hospital admissions.[4-6] There is thus a low suspicion of intussusception due to vague range of acute to longstanding or intermittent non-specific symptoms which delays the diagnosis.[3,7] Most cases are diagnosed only after emergency laparotomy.[8] In non-emergency

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Comparison of CTSI and BISAP Scoring in the Assessment of Severity and Mortality in Cases of Acute Pancreatitis

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Abstract

Background: Acute pancreatitis is a disease where earlier initiation of treatment in case of complication gives a better prognosis to the patient. It has been firmly established that the BISAP scoring system is superior to the other clinical scoring system and the superiority of the CTSI scoring has been established for almost a decade. Yet, there are very few studies that directly compare these two scores directly. Hence, such a study is of utmost importance at this time as it can decrease the financial burden for the patient as well as provide a simple scoring system for the surgeon. This study compares CTSI and BISAP scoring in the assessment of severity and mortality of acute pancreatitis, and assess the validity, the specificity and the sensitivity of both the scoring systems.

Methodology: In 35 patients diagnosed with acute pancreatitis, the BISAP score was calculated, and after 48 hours of onset of symptoms, CTSI scoring done. The data was then analyzed with STATA statistical software.

Results: BISAP score showed a sensitivity of 100% and specificity of 36.8% in establishing the mortality of acute pancreatitis with cut-off value taken as > 1. If the cut-off value is taken as > 2, the sensitivity became 56.3% and specificity became 79%. CTSI score showed a sensitivity of 64% and specificity of 100% in establishing the onset of complications in cases of acute pancreatitis.

Conclusion: Our study showed that BISAP score is comparable to CTSI in predicting the prognosis and mortality of cases of acute pancreatitis but does not compare with CTSI in establishing degree of necrosis. Hence, BISAP score is a simple, cost-effective scoring system that can be implemented in tertiary and even most primary healthcare centres.

Keywords: BISAP; CTSI; Acute pancreatitis; Predicting morbidity; Complications of acute pancreatitis; Pancreatic necrosis BISAP; CTSI; Acute pancreatitis; Predicting morbidity; Complications of acute pancreatitis; Pancreatic necrosis.

Introduction

Acute inflammation of pancreas named acute pancreatitis, is becoming common abdominal disease posing some major challenge to general and intestinal surgeons around the world.[1,2] It is one of the most challenging disease that leads to various local and systemic complications. Acute pancreatitis presents with the hallmark features of acute pancreatic inflammation which is associated with little or no fibrosis. The presentation of acute pancreatitis may range from a pancreatic inflammation that is mild and self-limiting to life threatening pancreatic necrosis with secondary infection and multi-organ failure.[3] The acute pancreatitis incidence world wide varies from 5 to 80 per 1, 00,000 acute population. Finland and United States of America records the highest incidence of acute pancreatitis worldwide.[4] The incidence of acute pancreatitis varies among different racial and ethnic group. Alcohol consumption without moderation is an important cause for acute pancreatitis in India, with smoking being an independent risk for developing acute pancreatitis.[5] Acute pancreatitis can be classified,

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Retrospective Study on Patients Undergoing Laparotomy to Assess the Risk Factors of Re-Laparotomy

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Abstract

Introduction: Abdominal surgery that has to be redone in association with initial surgery is referred to as re laparotomy. Re-laparotomy is associated with 22 to 513% morbidity and mortality, therefore it's the final choice of surgery. The main aim of this study was to find out the incidence of re- laparotomy and to identify the risk factors i.e. predictors of re laparotomy in patients undergoing general surgery operations.

Materials and Methodology: This was a retrospective study involving 100 patients, done from 1st Jan 2016 till 1st June 2017 Patients with age more than 18 years, those requiring laparotomy for both general and trauma surgery were included. Those with initial laparostomy, with only flank drain placement, any Laparotomies during colostomy or ileostomy closure, those with Initial laparoscopic procedure, minimal invasive procedure like ultrasound guided drainage etc. Were excluded. Data was analyzed using SPSS Software. The Results were expressed in percentage. Associations were analyzed using chi-square or 't' test depending on outcome variables.

Results: The incidence of revision laparotomy in this study was 7% and the incidence of second revision laparotomy was 1%. The indications for relaparotomy were anastamotic leak 2/7 (20%), burst abdomen 2/7 (20%), pancreatic injury 1/7 (10%), bladder injury 1/7 (10%), negative laparotomy 1/7 (10%), anastamotic leak and burst abdomen. The variables with significant *p*-value are systemic hypertension, COPD, CAD, intra-op and post-op inotoropic support, wound infection, wound dehiscence and intra-abdominal abscess. These data can thus be used in future to formulate a prediction scoring system.

Keywords: Laparotomy; Re-lapaprotomy; Post-op peritonitis; Burst abdomen.

Introduction

Laparotomy is the surgical incision into the abdominal cavity, for diagnosis or in preparation for major surgery. From its origins in a private house in the backwoods of Kentucky in 1807, a large number of patients undergo various operative procedures every day, out of which laparotomy forms a major proportion.

Abdominal surgery that has to be re-done in association with initial surgery is referred to as re laparotomy. Laparotomy has to be re done due to complications like biliary peritonitis, faecal fistula, anastamotic leak, burst abdomen etc. Of these, post-operative peritonitis and intra abdominalsepsis[1] are the most common cause. Clinical and haematological parameters and radiological evidence form the basis of re laparotomy. Incidence of relaparotomy differs accordingto patient characteristics, initial surgery and post-op care. The surgeon factors include hesitation to decide on second surgery, focus more on conservative treatment. Need for supervision under a qualified surgeon.[2] Despite

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Case Series

Studying the impact of the cost-effective Suvidha off-loading dressing in healing neuropathic ulcers in diabetic foot: a case series of 83 cases from South India

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ABSTRACT

Neuropathic ulcers pose a global burden carrying a risk of amputation of 15-46 times especially in developing countries. These ulcers are mainly managed with offloading techniques. In this study we share our experience of using an easy to use, cost effective method the Suvidha offloading dressings in terms of its acceptability and effectiveness in managing these cases. A prospective case series of 83 patients with mean age 58 years, managed with Suvidha offloading dressing in our institution from 2016 till 2019, excluding patients with ABI less than 0.4 and Wagner 4 and 5. They were reviewed after 6 months/SOS. Ulcer measured 1×1 to 4×4 cm, was present most commonly in the mid foot and least commonly in the lateral aspect of the foot. 53 cases were Wagner grade 2 and 9 cases Wagner grade 3. Forty cases were initially infected, 29 cases had a deformed foot, 5 cases needed interval wound debridement. The duration of ulcer healing was 2 weeks for 1×1 cm great toe ulcer, to 12 weeks for the 4×4 cm mid foot ulcer. All 83 patients were followed up for 6 months. 5 ulcers recurred. The patient satisfaction was measured by a 5-points Likert scale with a mean value of 17.4 out of 20. The Suvidha offloading footwear is a cost effective, easily replicable and efficient dressing requiring only the readily available dressing materials, with good healing rates, good patient satisfaction and adapted for developing countries. The results are comparable with other methods of offloading practiced worldwide.

Keywords: Suvidha, Offloading, Diabetes, Ulcer, DFUs

INTRODUCTION

Ulceration of the lower limb affects 1% of the adult population and 3.6% of people older than 65 years. Leg ulcers are debilitating and painful. They greatly reduce patient's quality of life. Ulcer healing has been shown to restore quality of life. Lower limb ulceration tends to be recurrent and the total annual cost of treating leg ulcers to the NHS has been estimated at £400m.[1] Chronic ulcers ruin the life of patients and increases the burden on health care as a whole. Neuropathic ulcers are formed due to undue pressure leading to breakdown of skin. Various causes are implicated, but diabetes is the most common cause. The number of diabetics worldwide was estimated at 131 million in 2000. It is projected to increase to 366 million by 2030. Diabetic patients have up to 25% lifetime risk of developing foot ulcers. The annual incidence of diabetic foot ulcers is ~3%.[2] The worldwide prevalence rate of diabetic foot ulcer (DFU) was 6.3%. North America showed the highest prevalence rate of 13.0% compared with Oceania with prevalence rate of 3.0%. Africa showed a prevalence rate of 7.2% which is higher than Asia 5.5%. Europe showed a prevalence rate of 5.1%, Australia has prevalence rate of 1.5%.[3] Belgium

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Original Research Article

Routine baseline serum carcinoembryonic antigen as a negative predictor of peritoneal metastasis in colorectal malignancies: a cost-effective tool in South India

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ABSTRACT

Background: Colorectal cancer, a formidable health problem worldwide has upto 8% synchronous peritoneal carcinomatosis. As only diagnostic laparoscopy can identify them, in countries with economic burden, selection of patients for laparoscopy is ideal. Our aim is to evaluate whether the already done baseline carcinoembryonic antigen (CEA) is a good selection tool.

Methods: A retrospective study of 125 patients, who were diagnosed to have colorectal malignancy (any stage) and underwent elective surgery at our institution from 2012 till 2019 were included. The baseline serum CEA was compared with the intraoperative findings. The threshold levels of serum CEA compared were 6.5 and 100 ng/dl. The sensitivity, specificity, positive predictive value and negative predictive value for both thresholds were compared in 3 categories of patients, namely peritoneal metastasis (9 cases), metastasis in other organs (36 cases) and cases with no metastasis either in peritoneum or other organs (85 cases). The results were analysed using SPSS software.

Results: The mean age was 65, sex ratio was 72:53. The sensitivity, specificity, positive predictive value, negative predictive value (NPV) for CEA threshold of 6.5 ng/dL was 44.44%, 60.34%, 8% and 93.33% for category 1. For CEA threshold of 100 ng/dl, it was 33.33%, 97.41%, 50% and 94.95% for category 1. NPV was 96.55% for category 3 (the highest value).

Conclusions: We can be 96.55% sure that if baseline CEA is less than 100 ng/dl, we don't need a diagnostic laparoscopy. This hopefully will cut down the cost of unnecessary diagnostic laparoscopies, and reduce the morbidity of unnecessary laparotomies.

Keywords: Carcinoembryonic antigen, Peritoneal metastasis, Diagnostic laparoscopy, Colorectal malignancies, Cost, India

INTRODUCTION

Colorectal cancer (CRC) is a formidable health problem worldwide. It is the third most common cancer in men (663000 cases, 10.0% of all cancer cases) and the second most common in women (571000 cases, 9.4% of all cancer cases).[1] Almost 60% of cases are encountered in developed countries. The number of CRC-related deaths is estimated to be approximately 608,000 worldwide, accounting for 8% of all cancer deaths and making CRC the fourth most common cause of death due to cancer. In India, the annual incidence rates (AARs) or colon cancer and rectal cancer in men are 4.4 and 4.1 per 100000, respectively. The AAR for colon cancer in women is 3.9 per 100000. Colon cancer ranks 8th and rectal cancer ranks 9th among men. For women, rectal cancer does not figure in the top 10 cancers, whereas colon cancer ranks 9th.[2] Usually the patients have a favourable prognosis when diagnosed at an early stage: 70-80% are eligible for curative-intent surgery, with a 5 years survival of 72-93% for stages I-II.[3] Approximately 25% of the remaining

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A single-blind, randomized controlled study to compare Desarda technique with Lichtenstein technique by evaluating short- and long-term outcomes after 3 years of follow-up in primary inguinal hernias

Hemanth Vupputuri, Satish Kumar R, Priya Subramani, Venugopal K

Abstract:

BACKGROUND: Lichtenstein tension-free repair is associated with postoperative complications and dysfunctions; hence, there is a need to look for a new hernia repair techniques while retaining its advantages. Desarda technique is a physiologic repair and essentially restores physiology of the inguinal canal. This single-blind, randomized controlled study was conducted to compare Desarda with Lichtenstein technique evaluating short- and long-term outcomes after 3 years of follow-up in primary inguinal hernias.

MATERIALS AND METHODS: One hundred and twenty-three adult male patients with primary inguinal hernia (both direct and indirect) were randomly allocated intraoperatively to Lichtenstein repair, Mesh (M) Group or Desarda repair, nonmesh (NM) Group. Baseline characteristics were recorded before the surgery. Short- and long-term outcomes and patients responses on patient global impression of change (PGIC) and Prolo scale after surgery were recorded.

RESULTS: Sixty-two patients were assigned to NM and 61 to M group. Surgery time was significantly higher for M group ($P < 0.001$) in M than NM group whereas complications were comparable. The total mean duration of follow-up for M was 35.2 months while for NM was 35.7 months. The recurrence rate was not significantly different; however, chronic groin pain was significantly higher in M compared to NM ($P = 0.05$). After surgery, PGIC score was consistently higher in NM group with better functionality in NM group.

CONCLUSIONS: After 3 years of follow-up, Lichtenstein technique and Desarda technique results were similar. After considering the pros and cons of both the methods, a tailor-made approach is required while choosing a procedure for hernia repair.

Keywords:

Desarda technique, Lichtenstein technique, patient global impression of change scale, primary inguinal hernia, Prolo scale

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Introduction

Inguinal hernias comprise about 96% of all groin hernias.[1] About 27% of men and 3% of women ranged between 1 and 85 years are at risk of developing an inguinal hernia, and around 20 million hernias are estimated to be repaired every year. It ultimately raises the healthcare cost and resource

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Original Research Article

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A Comparative Study of Platelet-Rich Fibrin (PRF) in Treating Diabetic Foot Ulcers

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Abstract

Aim: To compare the efficacy of autologous platelet-rich fibrin (PRF) over moist sterile saline/povidone-iodine dressing in diabetic foot ulcers.

Objective: To compare the mean reduction in ulcer area at the end of 4 weeks of dressings.

Methodology: 60 diabetic patients with foot ulcers from the department of general surgery, general medicine, endocrinology, and cardiology were prospectively studied. Detailed clinical history was obtained, the ulcer was evaluated and the presence of wound infection was assessed for all the patients. Patients were randomised into two groups of 30 patients each. While one group received PRF dressings, the other received saline/povidone-iodine dressings. The wound healing was then compared in the two groups. The efficacy of platelet-rich fibrin dressing over moist saline/povidone-iodine dressings was assessed by comparing the percentage reduction of the ulcer area, at the end of four weeks, using chi-square test and A NOVA test.

Results: It was found that there was a better reduction in the area of the ulcer at the end of four weeks in patients who received platelet-rich fibrin dressings than in moist saline/povidone-iodine dressings.

Conclusion: PRF dressing was superior to moist saline/povidone-iodine dressings.

Keywords: Diabetes; Foot ulcer; PRF.

Introduction

Foot ulcer is a devastating complication of diabetes mellitus, particularly in the elderly. The majority of diabetic foot ulcers may heal, whereas a smaller percentage will remain active and finally lead to gangrene and amputation of the limb.[1,2] With increasing duration of the ulcer and the increasing age of the patient, the risk of amputation also rises. Thus early prevention plays a vital role.

Wound dressings constitute a major part of the management of diabetic foot ulceration. An ideal dressing will have to reduce the symptoms, give adequate protection of the wound and promote good healing. Platelet-rich fibrin (PRF) prepared from the patient's own blood is used in the fields of orthopedics and dentistry to promote wound healing. PRF transfers growth factors to the wound surface. The use of autologous preparation reduces the risk of allergic reactions. There are very few studies comparing the efficacy of PRF with other dressing materials. This study compare the efficacy of PRF dressing with moist saline/povidone-iodine dressing in diabetic foot ulcers.

Materials and Methods

The period of study was between February 2015 and February 2016. It was an open-labeled

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Aorto-oesophageal fistula: rarest cause of haematemesis - is it salvageable?

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SUMMARY

A 60-year-old man presented with severe abdominal pain, two episodes of massive haematemesis and chest discomfort. CT angiography showed a saccular aneurysm of the juxtaphrenic aorta with possible oesophageal erosion. Upper GI endoscopy revealed external compression of the lower oesophagus—near total luminal obstruction with impending rupture of the aortic aneurysm. Emergency aneurysmal repair by interposition grafting using 20 mm Dacron graft with oesophageal–gastric reconstruction done. Postoperative period was uneventful

BACKGROUND

This report describes the management of a life threatening rare vascular emergency of aneurysm of the juxtaphrenic aorta with oesophageal erosion leading to formation of aorto-oesophageal fistula complicated with acute dissection. The presentation was inevitable for cardiovascular collapse in the absence of timely surgical intervention. The aneurysm being located in the descending thoracic aorta, the repair requires a left atrium-to-femoral bypass to maintain perfusion to the abdominal viscera and lower extremities. During the repair, the brain and the spinal cord is protected by cerebrospinal fluid drainage.

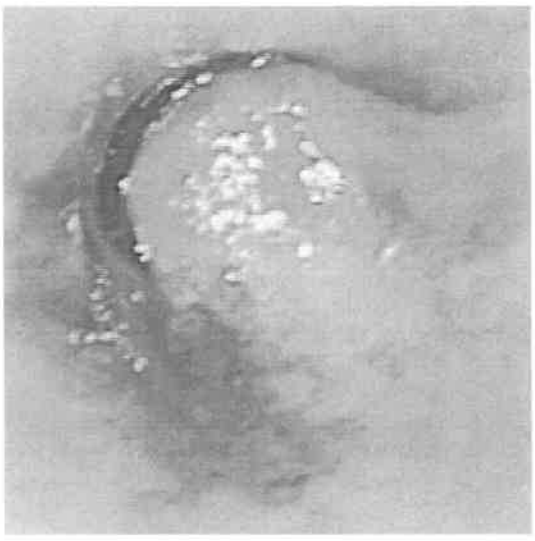


Figure 1 Upper gastrointestinal endoscopy – lower oesophageal external compression – near total luminal obstruction with impending rupture/ leaking nature of aortic aneurysm with mucosal ulceration of oesophagus covered with blood clot, eroding the distal oesophagus just above the oesophageal hiatus of the diaphragm.



Figure 2 CT angiogram.

CASE PRESENTATION

A moderately built 60-year-old man, habitual smoker for 40 years with recently diagnosed hypertension not on medications was admitted with severe abdominal pain, two episodes of massive haematemesis and chest discomfort for the last 2 days. The abdominal pain was sudden in onset, gradual in progression, localised to the epigastric region.

On examination, he was conscious, oriented, afebrile, pallor present with pulse rate of 86/min, blood pressure 100/80 mm Hg and respiratory rate of 30/min. On cardiovascular system examination, S1, S2 heard in the mitral, tricuspid, aortic and pulmonary area with no murmur. Respiratory system examination, normal vesicular breath sounds heard with equal bilateral air entry, no added sounds. On abdomen examination, soft, non tender with no organomegaly. On central nervous system examination, clinically normal with no focal neurological deficits.

INVESTIGATIONS

Routine investigations revealed haemoglobin of 90 g/L, blood group O negative, bleeding parameters



Figure 3 CT angiogram: large saccular aneurysm of juxtaphrenic aorta with large intramural thrombus and sign of impending rupture.

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Original Research Article

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Vacuum Assisted Closure Versus Conventional Wound Therapy in the Management of Diabetic Wounds

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Abstract

Background: Diabetic foot ulcers are a major cause of hospitalization which increases hospital stay because of multiple surgical procedures. The objective of this study is to compare Vacuum Assisted Closure and the conventional therapy for diabetic wounds with respect to time and wound healing and to prove the efficacy of one method over the other by appropriate statistical methods at the end of data collection.

Methods: A retrospective randomized hospital based observational study of 40 patients was conducted at PSGIMSR, Coimbatore, Tamilnadu, India for a period of 24 months (January 2017–December 2018). All these 40 patients were studied and the data was statistically evaluated to determine the efficacy of vacuum assisted closure in healing of diabetic wounds and to compare it with the conventional method of treatment of diabetic wounds. The patients were divided into 2 groups, Group-A which consisted of 20 patients who received vacuum therapy (VAC), Group-B which consisted of 20 patients who received conventional dressings.

Results: The patients on VAC had early appearance of granulation tissue (90% vs 60% at the end of Day 3), increased percentage of decrease in wound depth (53% vs 25% at the end of day 9) as compared to patients treated by conventional dressing. All patients developed granulation tissue by the end of Day 9.

Conclusion: We thus conclude that VAC appears to be superior in the treatment of diabetic foot ulcers in terms of early appearance of granulation tissue and decrease in wound depth when compared to conventional dressings.

Keywords: VAC; DFU; Wagners classification.

Introduction

Acute and chronic wounds are a major cause of morbidity and reduced quality of life, they affect atleast 1% of the population and present as a risk factor for hospitalization, amputation, sepsis and even death.[4]

Foot ulcers are a major cause of hospitalization in patients with diabetes mellitus which increases hospital stay because of multiple surgical procedures, prolonged length of stay. Diabetics have 25% risk of developing foot ulcers which preceeds amputation in about 85% of cases.[3]

A main stay of diabetic foot ulcer (DFU) therapy is debridement of all necrotic, callus and fibrous tissue with a primary goal to obtain wound closure. The management of DFU is determined by its severity (grade) vascularity of the limb and presence of infection.[3]

Wound healing is a complex and dynamic process that begins with removal of debris,

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control of infection, clearance of inflammation, angiogenesis, deposition of granulation tissue, contraction, remodeling of the connective tissue matrix and maturation.[2]

Saline moistened gauze has been the standard method, however, it has been difficult to continuously maintain a moist wound environment with these wound dressings. Subsequently various hydrocolloid wound gels, growth factors, hyperbaric oxygen therapy and other wound therapies have been advocated. All of these therapies are associated with increased cost and are being utilized without adequate scientific evidence favouring their efficacy.[1]

Vacuum assisted wound closure (VAC) is a wound management technique that exposes wound bed to negative pressure and provides a moist wound healing environment. VAC promotes wound healing by removing fluid from open wounds, preparing the wound bed for closure, reducing oedema and promoting formation and perfusion of granulation tissue.

This study is being done to compare the effectiveness of VAC with conventional dressings in the healing of DFU, in terms of healing rate: early appearance of granulation tissue and percentage decrease in wound depth.

Materials and Methods

A hospital based retrospective randomized control trial was conducted in the Department of Surgery, PSGIMSR, Coimbatore.

The study period was 2 years in total and both methods were tested out over a period of one and a half weeks.

All the eligible subjects fulfilling the inclusion and exclusion criteria were included in the study (sample size: 40).

Group A: Patients treated with VAC.

Group B: Patients treated with conventional dressings.

Inclusion criteria

- Above 18 years of age.
- Patients with diabetic foot ulcer more than 4 cm square.

Exclusion criteria

- Patients age less than 18 or more than 70 years
- Pregnant and nursing mothers.
- Patients on medications such as corticosteroids, immunosuppressive agents.
- Patients with severe wound ischemia and severe neuropathy.
- Deep infections such as osteomyelitis and septic arthritis.
- Patients were divided into Group-A (treated with VAC) and Group-B treated with conventional dressings, with an equal number of patients in each group. For each patient, ulcers were treated till wound closure, either spontaneously or surgically.

Patients were assessed by the following methods:

- % decrease in the wound depth.
- Early appearance of granulation tissue.

Treatment Schedule

Group A

Patients treated with vacuum assisted closure. Dressings were regularly changed every 3 days. Wound tracing and photos were taken every 3 days. Ulcers were followed up until wound closure, either spontaneously or surgically.

In patients undergoing VAC therapy, foam was autoclaved and was cut according to the shape of the wound and ryles tube placed between the two layers of the foam. Adhesive plaster applied around the foam airtight. Ryles tube connected to the wall suction using tubings. Negative pressure set to 125 mmHg. Negative pressure applied for 72 hours continuously, patient was taught to detach the tubing when ambulating. Dressing opened after 72 hours.

Group B

Patients treated with conventional dressings. Daily dressings were done. Wound tracing and photos were taken every 3 days. Ulcers were followed up until wound closure, either spontaneously or surgically.

In patients undergoing Conventional dressings, after wound wash, povidone soaked gauze pieces were used for initial 72 hours followed by dressings of normal saline soaked gauze pieces for twice daily.

Patients in both groups were insulin therapy according to blood levels and antibiotics started empirically initially and then according to culture sensitivity reports. Necessary debridement and wound toilet done before application of dressings.

Treatment outcome was assessed in terms of time taken for the appearance of granulation tissue and measurement of wound depth and area at subsequent follow up. Wound depth was measured using a sterile thread vertically in the deepest part of the wound crater to the skin surface level.

Results

Statistics

All the collected data was entered in Microsoft Excel Sheet. All the quantitative data was presented as Mean and Standard Deviation and compared using student's T-test.

Qualitative data was presented as Frequency and percentage and analysed using chi-square test. P-value of less than 0.05 was considered as significant.

Distribution of Patients based on age group

Mean age of study subjects was 51.2 and 50.4 years in conventional and VAC group respectively (Fig 1).

Distribution of Patients based on gender

Male preponderance was observed in both the groups (80% in conventional and 85% in VAC group respectively) (Fig. 2).

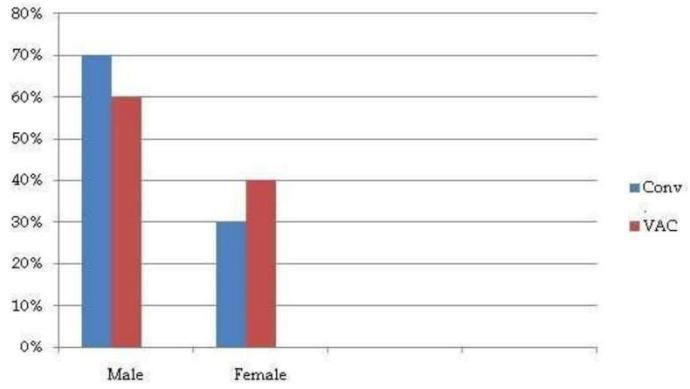


Fig. 1: Distribution graph of patients based on age.

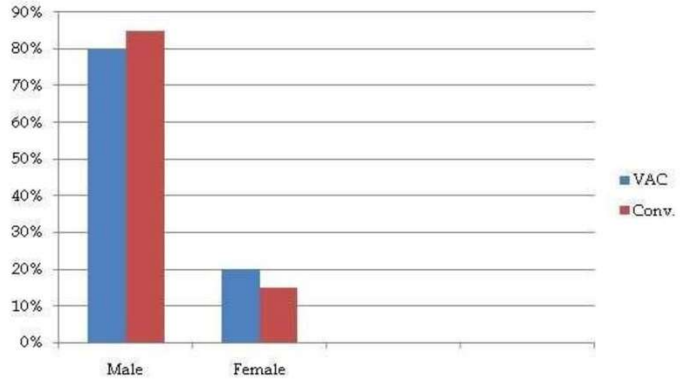


Fig. 2: Distribution graph of patients based on gender.

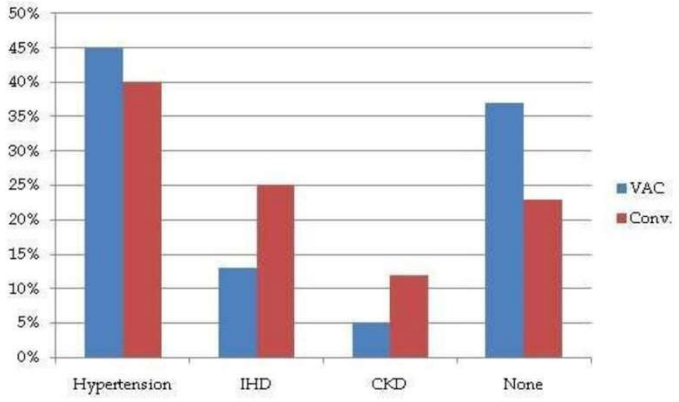


Fig. 3: Distribution of patients based on co-morbidities.

Distribution of patients based on co-morbidities

Hypertension was the most common co-morbidity observed in study subjects (40% and 45% in Conventional and VAC Groups respectively) followed by ischemic heart disease (25% vs. 13% in both the groups respectively) (Fig. 3).

Distribution of patients based on wound depth

The percentage decrease in the wound depth was more in the VAC group than conventional dressing at the end of Day 9 (53% vs 25% at the end of day 9) (Table 1).

Table 1: Distribution of patients based on wound depth.

Wound Depth	Group	N	Mean	SD	P-value
Before debridement	VAC	20	2.50	1.27	0.172
	Conv	20	2.00	0.97	
After debridement	VAC	20	4.30	1.17	0.023
	Conv	20	3.50	0.94	
Day 3	VAC	20	3.10	1.02	0.285
	Conv	20	2.75	1.01	
Day 6	VAC	20	2.38	0.86	0.812
	Conv	20	2.31	0.97	
Day 9	VAC	20	1.17	0.60	0.006
	Conv	20	1.90	0.92	

Distribution of patients based on granulation tissue

The patients on VAC therapy had early appearance of granulation tissue as compared to patients treated by conventional dressing (90% vs. 60% at the end of Day 3). All patients developed granulation tissue by the end of Day 9 (Table 2).

Table 2: Distribution of patients based on granulation tissue

Granulation tissue	Group		Total
	Conv	VAC	
Day 3	12	18	30
	60%	90%	75%
Day 6	19	18	37
	95%	90%	92.5%
Day 9	20	20	40
	100%	100%	100%

Discussion

Vacuum assisted closure has been recommended as a novel method in the healing of diabetic foot ulcers by stimulating the chronic wound environment in such a way that it reduces the bacterial burden and chronic interstitial wound fluid, increases vascularity and cytokine expression.

Application of negative pressure over the wound bec allows arterioles to dilate, increasing the effectiveness of local circulation, promoting angiogenesis which assists in the proliferation of the granulation tissue.

We observed that the patients on VAC therapy had an early appearance of granulation tissue as compared to patients treated by conventional

dressing (90% vs 60% at the end of Day 3). All patients developed granulation tissue by the end of Day 9.

The percentage decrease in wound depth was more in VAC group than conventional dressing at the end of Day 9 (53% vs 25% at the end of day 9).

In a study by Lone *et al.*, granulation tissue appeared in 26 (92.85%) patients by the end of week 2 in VAC group in contrast to 15 (53.57%) patients by that time in conventional group.

In a study by Eginton *et al.*, rate of wound healing with VAC was compared with conventional moist dressings in the treatment of large diabetic foot wounds. VAC dressings decreased the wound volume and depth significantly more than moist gauze dressings (59% vs. 0% and 49% vs. 8% respectively).

Priyatham K *et al.*, in a prospective study, assessed the efficacy of Vacuum assisted closure with conventional moist wound dressing in improving the healing process in chronic diabetic wounds. Shorter duration of hospital stay and better graft uptake was observed in the Vacuum dressing group.

Outcome

The primary end point in this study was a granulated wound or a wound ready for skin grafting or healing by secondary intention. Primary closure of the wound or split thickness skin grafting was done in patients of conventional and VAC group respectively.

Lone *et al.* observed that in 86.4% of patients, wounds were closed by a split thickness skin graft in VAC group as compared to 90.9% of patients in conventional.

Conclusion

Rate of granulation tissue formation, overall graft survival and patient compliance was better in VAC group as compared to conventional dressing group. It was also seen that the overall hospital stay and post operative complications were less in VAC group.

A retrospective randomized control trial was conducted for a period of 2 years in the Department of Surgery, PSGIMSR with the aim of studying the efficacy of VAC in healing of diabetic foot ulcers and to compare it with the conventional wound dressing.

A total of 40 patients with Grade 2 to 3 diabetic foot ulcers (Wagners classification) were randomly divided into 2 Groups. Group-A: Vacuum assisted closure and Group-B : Conventional dressing.

Following observations were made during the study:

- Hypertension was the most common co-morbidity observed in study subjects (40% and 45% in Conventional and VAC Groups respectively) affecting the wound healing time.
- The percentage decrease in wound depth was more in VAC group than conventional dressing at the end of day 9. (53% vs 25% at the end of day 9).
- The patients on VAC therapy had early appearance of granulation tissue as compared to patients on conventional dressing (90% vs 60% at the end of day 3). All patients developed granulation tissue at the end of day 9.

We thus conclude that VAC appears to be superior compared to conventional dressings in the treatment of diabetic foot ulcers in terms of early appearance of granulation tissue and decrease in wound depth.

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Conflict of interest: None

Ethical Approval: The study was approved by the Institutional Ethics Committee.

References

1. Ali M. Lone, Mohd I. Zaroo, Bashir A. Laway, *et al.* Vacuum-assisted closure versus conventional dressings in the management of diabetic foot ulcers: a prospective case–control study. Diabetic Foot Ankle. 2014;5:10.3402/dfa.v5.23345.
2. Hemant B. Janugade, Raunaq S. Chabbra, Ankur G. Das, *et al.* Outcomes of VAC versus conventional dressings in patients with lower limb ulcer. Int Surg J. 2018 May;5(5):1792-96.
3. Bagul A, Narwade N, Bhupatkar A, *et al.* Is vacuum assisted closure dressing better than conventional management of diabetic wounds? Int Surg J. 2018 Jun;5(6):2199-2204.

4. Nagaraj S, Hosmani R, Ravi Shankar JC. Negative Pressure Wound Therapy versus Conventional Wound Therapy in Large Wounds. International Journal of Scientific and Research Publications, 2015 May;5(5):1-10.
5. White R, McIntosh C. Topical therapies for diabetic foot ulcers: standard treatments. J Wound Care. 2008;17:426–32. [PubMed]
6. Mark TE, Kellie RB, Gary RS, *et al.* Prospective randomized evaluation of negative–pressure wound dressing for diabetic foot wounds. Ann Vasc Surg. 2003;17:645–9. [PubMed]
7. Apelqvist J, Armstrong DG, Lavery LA, *et al.* Resource utilization and economic costs of care based on a randomized trial of vacuum-assisted closure therapy in the treatment of diabetic foot wounds. Am J Surg. 2008;195:782–8. [PubMed]



Original Research Article

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Analysis of Antibiotic Therapy in Acute Severe Pancreatitis

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Abstract

Background: Acute Pancreatitis is a common surgical entity that is increasing in incidence. Gallstones and alcohol consumption remain the major contributors of Acute pancreatitis. The disease usually follows a prolonged course with increased hospital stay, early and late complications contributing to increased patient morbidity and mortality. Traditionally the role of antibiotics is usually limited to severe necrotising pancreatitis. There is however increasing evidence to prove the role of antibiotic therapy in reducing mortality and long term morbidity in acute pancreatitis patients.

Objectives:

1. To retrospectively analyse antibiotic therapy in patients with Acute severe pancreatitis in the years 2014 - 2017 in the tertiary care hospital PSG IMSR located in Coimbatore, Tamilnadu, India
2. To analyse the role of antibiotics in patient prognosis and outcome

Type of study: Retrospective Observational study. *Duration of study:* January 2014-January 2017. *Methodology:* The present study was conducted in PSG Institute of Medical Sciences & Research, Coimbatore. We did a thorough retrospective analysis of case files of 900 patients with acute severe pancreatitis. Analysing the available data from case files. *Results:* Of 900 patients, 411satisfied the inclusion criteris, of these 63% (n=258) of patients were started antibiotics within 48 hrs of hospital admission. The first choice antibiotics were Piperacillin tazobactum and metronidazole in 45% of cases, Fluoroquinolones and metronidazole in 12% of cases and Cefaperazone sulbactum ± metronidazole in 14% of cases. In 26% of cases a combination of imipenem + Cilastatin was used. Antibiotic use did not improve survival, nor was there any observed survival benefit when the different antibiotic agents were compared (p = 0.7 and 0.4 respectively). The timing of antibiotic use also does not appear to confer a survival benefit (p = 0.5). All patients with proven pancreatic infection died, there was not a significant difference in survival in those with extra-pancreatic infections (p = 0.2).

Keywords: Acute Pancreatitis; Necrotising Pancreatitis; Severe Acute Pancreatitis; Antibiotic Prophylaxis; Retrospective Analysis.

Introduction

Acute pancreatitis is a common gastrointestinal emergency. Its incidence varies from 5 to 80 cases per 100,000 inhabitants per year, with an overall mortality rate of 10–15% [1]. More than two-thirds of patients will recover within 1 week, however the remaining one-third will experience multiple systemic and/or local complications, with a high mortality rate of 10–30%, 80% of deaths are due to infectious complications [2-3].

The use and efficacy of prophylactic antibiotic therapy in acute pancreatitis has long been debated. The role of prophylactic antibiotics to prevent infection and reduce mortality in pancreatitis was first evaluated in the 1970s, where several randomised controlled trials (RCTs) had been conducted and concluded that prophylactic antibiotics were effective in preventing secondary pancreatic infections and therefore in

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reducing the related mortality[4-6]. However, in recent studies, there have been multiple large controlled trials, with conflicting results [7-9], different consensuses reached and differing guidelines for the use of prophylactic antibiotics [10].

Acute Pancreatitis and its Management

Acute pancreatitis is complex disease. It ranges from a mild degree inflammation that lasts for few days to severe pancreatitis, which can lead to mortality, especially in the presence of multiple organ failure or severe pancreatic infections.

Severe pancreatitis progresses in two phases [11-12]. The early stage – the first 14 days from the onset of the disease – is characterised by a systemic inflammatory response syndrome (SIRS), which may be complicated by multiple organ dysfunction syndrome (MODS). In 15–20% of cases, this may be followed by a stage of secondary bacterial infection within the inflamed pancreas, typically 2–3 weeks from the onset of pancreatitis [13].

Pathogenesis of secondary bacterial pancreatic infection is still debated. Pathogens can reach the pancreas through the haematogenous pathway, the biliary system, ascending from the duodenum via the main pancreatic duct, or through transmural colonic migration via translocation of the colonic bacteria to the lymphatics. Most pathogens in pancreatic infection are gastrointestinal Gram-negative bacteria (Escherichia coli, Pseudomonas, Proteus, Klebsiella), which occur via disruption of the intestinal flora and damage to the bowel mucosa. Impaired body defences predispose to translocation of the gastrointestinal organisms and toxins with subsequent secondary pancreatic infection. But Gram-positive bacteria (Staphylococcus aureus, Streptococcus faecalis, Enterococcus), anaerobes and, occasionally, fungi have also been found [14-16]. Infection of sterile necrosis is attributed to bacteria of gut origin in up to 70% of cases [17].

Assessment of Severity and Prognosis

Tools have been developed to predict the severity of pancreatitis and the likelihood of complications and mortality. They have been shown to be superior to clinical judgment alone, and should be used in conjunction with typical clinical criteria, such as presence of comorbid conditions, age, and first episode of pancreatitis.

The Atlanta criteria use early prognostic signs, organ failure, and local complications to define disease severity [18-19] (Figure 1). Early prognostic signs include a Ranson score of 3 or greater, or an acute physiology and chronic health evaluation (APACHE II) score of 8 or greater. Organ failure is defined as shock, hypoxemia (partial arterial oxygen tension of 60 mm Hg or less), creatinine level greater than 2 mg per dL (177 μmol per L), or gastrointestinal bleeding (greater than 500 mL per 24 hours). Local complications include necrosis, abscess, or pseudocyst [20].

The Ranson score evaluates 11 factors within 48 hours of hospital admission to predict severity of pancreatitis and risk of mortality. However, the sensitivity for predicting poor outcome is only 70% [21]. The APACHE II scoring system uses 12 criteria to predict the severity of pancreatitis, with the risk of death increasing as the score increases. The CT severity index is based on CT findings at admission and evaluates for the presence of peripancreatic inflammation , phlegmon, and, if present, the amount of pancreatic necrosis. A total score of 5 or greater is associated with a statistically significant increase in morbidity and mortality.

Severity	*Criteria*
Mild	No organ failure
	No local complications (e.g., peripancreatic fluid collections, pancreatic necrosis, peripancreatic necrosis)
	No systemic complications
	Typically resolves in first week
Moderate	Transient organ failure (≤ 48 hours)
	or
	Local complications
	or
	Exacerbation of comorbid disease
Severe	Persistent organ failure (> 48 hours)

Fig. 1: Modified Atlanta Classification for acute pancreatitis (Column width)

Antibiotic Therapy in Acute Pancreatitis

The ideal drug to use should:

1. Have specific activity against the bacteria responsible for pancreatic infections
2. Be able to penetrate the pancreatic tissue, pancreatic exocrine secretions, and peri-pancreatic fluid/ exudates at therapeutic mean inhibitory concentrations
3. Be able to penetrate the pancreas during acute pancreatitis; and
4. Have a clear-cut clinical capacity to reduce the development of infected necrosis [22].

There is no evidence to support the previous criteria of ideal antibiotics, and physicians should realise that pancreatic infection normally starts in necrotic tissue. No antibiotics effectively penetrate necrotic tissue without blood supply, which makes pancreatic infections sometimes very resistant to antibiotics.

Imipenem, clindamycin, piperacillin, fluoroquinolones and metronidazole are known to have adequate tissue penetration and bactericidal properties in infected pancreatic necrosis, in contrast to penicillins, first-generation cephalosporins, aminoglycosides and tetracyclines, which are ineffective in acute pancreatitis [22]. Meropenem is shown to have as wide a spectrum as imipenem in preventing septic complications in acute pancreatitis [23]. The use of systemic antibiotics in pancreatic infections must be accompanied with drainage, either surgical or percutaneous.

One of the main problems of prolonged administration of antibiotics in severe acute pancreatitis is the development of multidrug resistance bacterial and fungal infection, which is associated with long hospital stay and poor outcome [24]. Hence, each case should be individually evaluated, weighing the benefits of antibiotics against the significant adverse events associated with their use, including increased bacterial resistance and fungal infections. Microbiologists with a specific interest in pancreatitis should be involved in such decisions, and blood culture is highly suggested as this might detect bloodstream infections associated with pancreatitis [25-26].

Objectives

1. To retrospectively analyse antibiotic therapy in patients with Acute severe pancreatitis in the years 2014 - 2017 in the tertiary care hospital PSG IMSR located in Coimbatore, Tamilnadu, India.
2. To analyse the role of antibiotics in patient prognosis and outcome.

Type of Study

Retrospective Observational study

Duration of Study

January 2014 - January 2017

Inclusion Criteria

1. Patient admitted in PSG hospitals coimbatore with the diagnosis of Acute pancreatitis (alcoholic and biliary) graded as Severe by Modified Atlanta classification.
2. Antibiotic therapy for the patients instituted within 48 hours

Exclusion Criteria

1. Post ERCP/Post operative pancreatitis
2. Non alcoholic non biliary pancreatitis
3. Mild and moderate pancreatitis

Methodology

The study was conducted in PSG IMS & R a tertiary care centre in southern India. There have been 900 patients admitted with the diagnosis of Acute Pancreatitis in the four year period considered for this study.

Among this the total number of cases that had Acute severe pancreatitis and had been given antibiotics wishing 48 hours were 411. These fit into the inclusion and exclusion criteria. Their data was analysed with their age , sex, alcoholic and gall stone pancreatitis, antibiotic instituted, duration if antibiotic and mortality.

The data was analyzed using Microsoft Excel

Results

Of 900 patients surveyed, 500 fit the inclusion and exclusion criteria. Of which 411 received antibiotics. 63% (n = 259) of patients were started antibiotics within 48 hrs of hospital admission. 37% (n=152) did not receive any antibiotics (Figure 2).

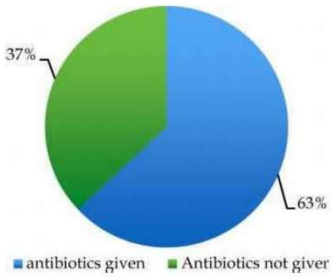


Fig. 2: Chart showing percentage of patients started on antibiotics vs Patients who did not receive antibiotics (Column width)

The first choice antibiotics were Piperacillin tazobactum and metronidazole in 45% of cases, Fluoroquinolones and metronidazole in 12% of cases and Cefaperazone sulbactum ± metronidazole in 14% of cases. In 26% of cases a combination of imipenem + Cilastatin was used (Figure 3).

Total mortality was 11 due to various complications negative bacilli as a causative organism.Antibiotic use did not improve survival, nor was there any observed survival benefit when the different antibiotic agents were compared (p = 0.7 and 0.4 respectively). The timing of antibiotic use also does not appear to confer a survival benefit (p = 0.5). It was, however, associated with a statistically significant reduction in hospital stay (p = 0.040). All patients with proven pancreatic infection died, there was not a significant difference in survival in those with extra-pancreatic infections (p = 0.2).

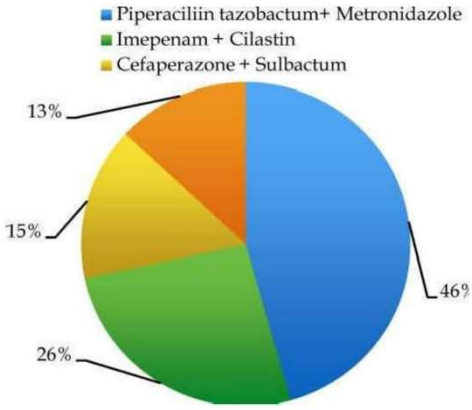


Fig. 3: Chart showing percentage of antibiotics used

Discussion

Antibiotic prophylaxis in the setting of pancreatic necrosis refers to the use of antibiotics to avoid infection in severe AP. This issue has remained controversial for the last four decades. The most important questions raise dare about antibiotic indications, antibiotic selection and length of treatment. Inappropriately selected or distributed over time antibiotics may carry complications such as anaphylaxis and selection of resistant bacteria [27-28]. The latter affects not only the patient, but also the hospital bacterial flora and the population around the hospital. The same subjects may be applied to the treatment of fungal infections. Available studies are not conclusive although some have shown benefit from antibiotic prophylaxis. These last studies used different antibiotic drugs, different selection criteria, and different length of treatment [29-31].

Also, definitions of severe disease varied between trials although in each the aim was to deliver antimicrobial prophylaxis to patients with severe AP and evidence of pancreatic necrosis. Duration of prophylaxis was relatively long (up to 14 days) [25-30].

The studies do not specify an appropriate antibiotic or duration protocol common to all. In our study we have found that while antibiotic use did not alter the mortality or survival benefit, it did alter the length of hospital stay and ICU admissions.

Conclusion

Evidence is accumulating to suggest that prophylactic antibiotics in patients with acute pancreatitis is not associated with a significant decrease in secondary pancreatic infection and mortality. We do not therefore recommend routine prophylactic antibiotic therapy for all patients with acute pancreatitis.

Table 1: Table showing patients surveyed in the year 2014 – 2017

Year	Total cases analysed (n=411)	Antibiotics given (n=259)	Antibiotics not given	Mortality
2014	126	80	46	3
2015	129	97	32	2
2016	130	71	59	4
2017	26	11	15	0

Table 2: Table showing sex, mean duration of antibiotics and mean duration of hospital stay in both groups and mortality in both groups

Patients surveyed (n=411)	Male (n=266)	Female (n=145)	Mean duration of antibiotics	Mortality
Patients who received antibiotics (n=259)	172	87	10	11
Patients who did not receive antibiotics (n=152)	94	58	Not applicable	0

Conversely, the prompt use of prophylactic antibiotics once a physician detects early markers associated with high risk of pancreatic infection is mandatory. These subset of patients with proven evidence of infection may benefit from easy antibiotic prophylaxis. Being able to identify biomarkers indicating pancreatic infection and whether they predict responsiveness to antibiotics would significantly enhance the clinical management of acute pancreatitis.

References

1. Yousaf M, McCallion K, Diamond T. Management of severe acute pancreatitis. Br J Surg 2003;90(4):407–20.
2. Toouli J, Brooke-Smith M, Bassi C et al Working Party of the Program Commitee of the Bangkok World Congress of Gastroenterology. Guidelines for the management of acute pancreatitis. J Gastroenterol Hepatol 2002;17(Suppl):S15–S39.
3. Heinrich S, Schafer M, Rousson V et al. Evidence-based treatment of acute pancreatitis: a look at established paradigms. Ann Surg 2006;243(2):154–168.
4. Howes R, Zuidema GD, Cameron JL. Evaluation of prophylactic antibiotics in acute pancreatitis. J Surg Res 1975;18(2):197–200.
5. Finch WT, Sawyers JL, Schenker S. A prospective study to determine the efficacy of antibiotics in acute pancreatitis. Ann Surg 1976;183(6):667–671.
6. Witt C, Brinkhaus B, Jena S et al. Acupuncture in patients with osteoarthritis of the knee: a randomised trial. Lancet 2005;366(9480):136–143.
7. Isenmann R, Runzi M, Kron M et al German Antibiotics in Severe Acute Pancreatitis Study G. Prophylactic antibiotic treatment in patients with predicted severe acute pancreatitis: a placebo-controlled, double-blind trial. Gastroenterology 2004;126(4):997–1004.
8. Xue P, Deng LH, Zhang ZD et al. Effect of antibiotic prophylaxis on acute necrotizing pancreatitis: results of a randomized controlled trial. J Gastroenterol Hepatol 2009;24(5):736–742.
9. Jiang K, Huang W, Yang XN et al. Present and future of prophylactic antibiotics for severe acute pancreatitis. World J Gastroenterol 2012;18(3):279–284.
10. Nathens AB, Curtis JR, Beale RJ et al. Management of the critically ill patient with severe acute pancreatitis. Crit Care Med 2004;32(12):2,524–2,536.
11. Takeda K, Takada T, Kawarada Y et al. JPN Guidelines for the management of acute pancreatitis: medical management of acute pancreatitis. J Hepatobiliary Pancreat Surg 2006; 13(1): 42–47.
12. Uhl W, Warshaw A, Imrie C et al, International Association of P. IAP Guidelines for the Surgical Management of Acute Pancreatitis. Pancreatology 2002; 2(6): 565–573.
13. Dupuis CS, Baptista V, Whalen G et al. Diagnosis and management of acute pancreatitis and its complications. Gastrointest Interv 2013;2(1):36–46.
14. Luiten EJ, Hop WC, Lange JF et al. Controlled clinical trial of selective decontamination for the treatment of severe acute pancreatitis. Ann Surg 1995;222(1):57–65. Crossref, Medline.
15. Sedman PC, Macfie J, Sagar P et al. The prevalence of gut translocation in humans. Gastroenterology 1994; 107(3):643–649.
16. Schmid SW, Uhl W, Friess H et al. The role of infection in acute pancreatitis. Gut 1999;45(2):311–16.
17. Dervenis C, Johnson CD, Bassi C et al. Diagnosis, objective assessment of severity, and management of acute pancreatitis. Santorini Consensus Conference. Int J Pancreatol 1999;25(3):195–210.
18. Dambrauskas Z, Gulbinas A, Pundzius J et al. Meta-analysis of prophylactic parenteral antibiotic use in acute necrotizing pancreatitis. Medicina (Kaunas) 2007;43(4): 291–300.
19. UK Working Party on Pancreatitis. UK guidelines for the management of acute pancreatitis. Gut 2005;54(Suppl III):iii1–iii9.
20. Ukai T, Shikata S, Inoue M et al. Early prophylactic antibiotics administration for acute necrotizing pancreatitis: a meta-analysis of randomized controlled trials. J Hepatobiliary Pancreat Sci 2015;22(4):316–21.
21. Banks PA, Bollen TL, Dervenis C, et al. Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102–111.
22. Banks PA; Freeman ML; Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol. 2006;101(10):2379–2400.
23. Ranson JH. Etiological and prognostic factors in human acute pancreatitis: a review. Am J Gastroenterol. 1982; 77(9): 633–38.
24. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13(10):818–29.
25. Wilson C, Heath DI, Imrie CW. Prediction of outcome in acute pancreatitis: a comparative study of APACHE II, clinical assessment and multiple factor scoring systems. Br J Surg. 1990;77(11):1260–64.
26. Knaus WA, Wagner DP, Draper EA, et al. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. Chest. 1991;100(6):1619–36.
27. Barreto SG, Rodrigues J. Comparison of APACHE II and Imrie Scoring Systems in predicting the severity of acute pancreatitis. World J Emerg Surg. 2007;2:33.
28. Delcenserie R, Yzet T, Ducroix J. Prophylactic antibioticin treatment of severe acute alcoholic pancreatitisPancreas 1996;13:198-201.
29. Mann D, Hershman M, Hittinger R et al. Multi centre audit of death from acute pancreatitis. Br J Surg 1994;81: 890-93.
30. Nordback I, Sand J, Saaristo R, et al. Early treatment with antibiotics reduces the need for surgeryin acute necrotizing pancreatitis - a single-centre randomized study. J Gastrointest Surg 2001;5:113-118.

Original Article

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The Characteristics of routine Histopathological Examination of Appendicectomy Specimen: A Study from a Tertiary Care Centre

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Abstract

Acute appendicitis is one of the most common diseases, needing surgical intervention at its time of presentation. A histologic basis on diagnosis is essential in further workup and management of the patient in view of prognosis of the patient. Resection of the appendix whether via an open or laparoscopic approach is a very common procedure. Patients who were clinically and sonologically diagnosed with acute appendicitis, were taken up for this study. The resected specimen is sent for routine Histopathological Examination(HPE). The Histopathologic data of such patients were studied in PSG hospitals from the period of January 2015 to December 2016. 158 appendicectomy specimens were recovered of the said duration and amongst which 85(53.79%) werefrom males and 73(46.2%) from females. Perforated appendix(0.6%), lymphoid hyperplastic changes (52.38%) was seen at the maximum in the age group of 20 and below and maximum features of acute appendicitis (53.12%) was seen in the age group of 21- 40 years.

Keywords: Acute Appendicitis; Histopathology; Lymphoid Hyperplasia.

Introduction

Acute appendicitis is the most common disease of surgical field in worldwide and affects about seven percent of the population [1]. Surgeons have many challenges in the diagnosis of this common condition. Diagnosis of acute appendicitis is made primarily on the basis of the history and the physical examination, with additional assistance from laboratory and radiographic findings [7-9]. However histopathologic studies are routinely done after surgery for acute appendicitis. This makes the appendix one of the more commonly received specimens at pathology department. Histopathologic features of appendicitis are myriad. A great number of patients are operated but the specimen may or may not portrayevidence of severe involvement [17-19]. This study aims to find the value of routine Histopathological Examination in post operative course of the patient, and estimates the frequency of various histopathological findings in patients diagnosed with acute appendicitis [24,25].

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Objectives

1. To find the value of routine Histopathological Examination of Appendicectomy specimen in the postoperative course of the patient.
2. To estimate the frequency of various Histopathological findings in the setting of appendicitis

Materials and Methodology

This is a retrospective analysis of appendectomies carried out in a single center (PSG IMSR) between January 2015 and December 2016. Specimens were collected from the patients, keeping in mind the inclusion and exclusion criteria mentioned below.

Inclusion Criteria

- Patient admitted in PSG Hospitals who have undergone appendicectomy (laparoscopic or open) diagnosed clinically and sonologically and confirmed with blood tests

Exclusion Criteria

- Incidental appendicectomies (Appendicectomies done during other procedures)

The Histopathology reports of appendectomy specimens were retrieved and all specimens had been Formalin-fixed, Paraffin-based and at least reviewed by two pathologists. A total of 158 appendicectomy specimens were received, and analysed based on the prevalence in different sexes, age groups andhistopathological feature of the specimens, namely:

- Acute appendicitis
- Perforated appendix
- Lymphoid hyperplasia
- Acute appendicitis with periappendicular inflammation
- Normal appendix

The various histopathological findings in the appendicectomy specimen will be studied and was correlated with the demographic data and the clinical benefit of doing routine Histopathological examination of appendicectomy specimen will be studied.

Results

Based on the Gender Predilection

85 males and 73 females underwent laparoscopic appendectomy with a prevalence of 53.79% and 46.2% respectively.

A total of 158 appendix specimens were retrieved from the patients operated from January 2015 to December 2016, amongst which, the following were the histopathological findings.

Table 1:

Histopathology Finding	No. of Specimens
Normal Appendix	3
Acute Appendicitis	69
Acute Appendicitis With Periappendicitis	32
Lymphoid Hyperplasia	53
Perforated Appendix	1
Total	158

Prevalence Between Sexes

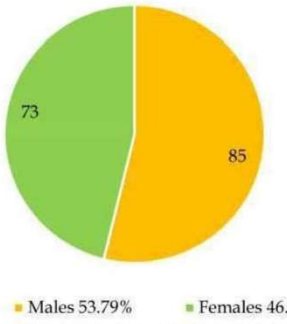


Fig. 1: Based on the gender predilection

Table 2: Histopathological findings in different age groups

	Normal Appendix	Acute Appendicitis	Acute Appendicitis with Periappendicitis	Lymphoid Hyperplasia	Perforated Appendix
20 yrs. and Below	0	25	5	33	0
21 – 40 yrs.					
More than 40 yrs.	2	34	12	16	0
	1	10	15	4	1

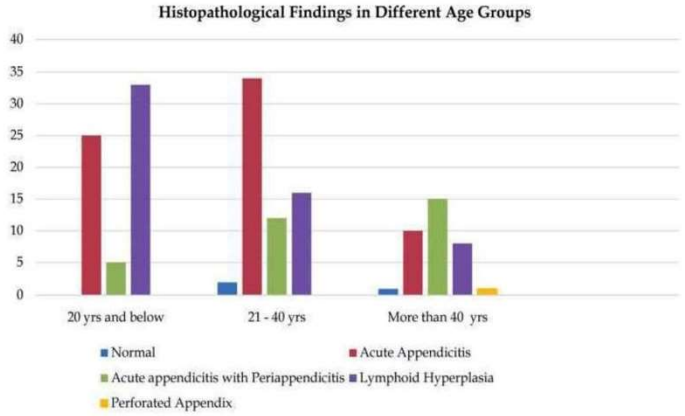


Fig. 2: Based on the histopathological findings in different age groups

Discussion

In the 1st group of patients aged 20 yrs and below, amongst the 63 patients, 25 patients (39.68%) had features suggestive of acute appendicitis, 5 patients (7.93%) had features suggestive of acute appendicitis with periappendicular inflammation and 33 patients (52.38%) had lymphoid hyperplasia. The second group of patients aged 21 to 40 years, amongst 64 patients, 2 specimens (3.12%) received were of normal histological findings, 34 patients (53.12%) had features suggestive of Acute Appendicitis, 12 patients (18.75%) had features suggestive of acute appendicitis with pariappendicular inflammation, 16 patients (25%) had features suggestive of lymphoid hyperplasia. In the third group of patients more than 40 yrs, amongst 31 patients, one specimen (3.2%) received had perforation at the tip, one specimen (3.2%) had features suggestive of normal Histology, 10 patients (32.26%) had features suggestive of acute appendicitis, 15 patients (48.39%) with features suggestive of acute appendicitis with periappendicular inflammation and 4 patients (12.9%) with feature of lymphoid hyperplasia

A perforated appendix of 0.6% was seen in the above study. Predominant lymphoid hyperplasia changes were seen in the age group of 20 years and below. Age specific incidence of acute appendicitis followed a similar pattern for males and females, with an increased predominance over the age groups of less than 40 years. Histopathological features of appendix are very difficult to diagnose the stage of appendicitis and may need new cuts and slides as needed for the patient's prognosis and outcome.

References

1. Hospital episodes Statistics. Admitted Patient Care, England. 2012-2013. Procedures and Interventions. Available online: http://www.hscic.gov.uk/catalogue/PUB12566/hosp-epis-stat-admi-proc-2012-13-tab.xlsx.
2. Addiss DG, Shaffer N, Fowler BS, et al. The epidemiology of appendicitis and appendectomy in the United States. Am J Epidemiol 1990;132:910-25.
3. Zoarets I, Poluksht N, Halevy A. Does selective use of computed tomography scan reduce the rate of "white" (negative) appendectomy? Isr Med Assoc J 2014;16:335.
4. Jones AE, Phillips AW, Jarvis JR, et al. The value of routine histopathological examination of appendicectomy specimens. BMC Surg 2007.
5. Charfi S, Sellami A, Affes A, et al. Histopathological findings in appendectomy specimens: a study of 24,697 cases. Int J Colorectal Dis 2014;29:1009-12.
6. Marudanayagam R, Williams GT, Rees BI.. Review of the pathological results of 2660 appendicectomy specimens. J Gastroenterol 2006;41:745-9.
7. Raja AS, Wright C, Sodickson AD, et al. Negative appendectomy rate in the era of CT: an 18-year perspective. Radiology 2010;256:460-5.
8. Webb EM, Nguyen A, Wang ZJ, et al. The negative appendectomy rate: who benefits from preoperative CT? AJR Am J Roentgenol 2011;197:861-6.

9. Slotboom T, Hamminga JT, Hofker HS, et al. Intraoperative motive for performing a laparoscopic appendectomy on a postoperative histological proven normal appendix. Scand J Surg 2014;103:245-8.
10. Panagiotopoulou IG, Parashar D, Lin R, et al. The diagnostic value of white cell count, C-reactive protein and bilirubin in acute appendicitis and its complications. Ann R CollSurgEngl 2013;95:215-21.
11. Mariadason JG, Wang WN, Wallack MK, et al. Negative appendicectomy rate as a quality metric in the management of appendicitis: impact of computed tomography, Alvarado score and the definition of negative appendicectomy. Ann R CollSurgEngl 2012;94:395-401.
12. Raman SS, Osuagwu FC, Kadell B, et al. Effect of CT on false positive diagnosis of appendicitis and perforation. N Engl J Med 2008;358:972-3.
13. Flum DR, McClure TD, Morris A, et al. Misdiagnosis of appendicitis and the use of diagnostic imaging.J Am CollSurg 2005;201:933-9.
14. Akbulut S, Tas M, Sogutcu N, et al. Unusual histopathological findings in appendectomy specimens: a retrospective analysis and literature review. World J Gastroenterol 2011;17:1961-70.
15. Emre A, Akbulut S, Bozdag Z, et al. Routine histopathologic examination of appendectomy specimens: retrospective analysis of 1255 patients. IntSurg 2013;98:354-62.
16. Yabanoglu H, Caliskan K, OzgurAytac H, et al. Unusual findings in appendectomy specimens of adults: retrospective analyses of 1466 patients and a review of literature. Iran Red Crescent Med J 2014;16:e12931.
17. Ojo OS, Udeh SC, Odesanmi WO.. Review of the histopathological findings in appendices removed for acute appendicitis in Nigerians. J R CollSurg Edinb 1991;36:245-8.
18. Akbulut S, Tas M, Sogutcu N, Arikanoglu Z, Basbug M, Ulku A. Unusual histopathological findings in appendectomy specimens: a retrospective analysis and literature review. World J Gastroenterol. 2011; 17(15):1961-1970.
19. Zdichavsky M, Gögele H, Blank G, Kraulich M, Meile T, von Feilitzsch M. Histological characterization of appendectomy specimens with intraoperative appearance of vascular injection. SurgEndosc. 2013;27(3):849-853.
20. Chandrasegaram MD, Rothwell LA, An EI, Miller RJ. Pathologies of the appendix: a 10-year review of 4670 appendicectomy specimens. ANZ J Surg. 2012;82(11): 844-847.
21. Marudanayagam R, Williams GT, Rees BI. Review of the pathological results of 2660 appendicectomy specimens. J Gastroenterol. 2006;41(8):745-749.
22. Swank HA, Eshuis EJ, Ubbink DT, Bemelman WA. Is routine histopathological examination of appendectomy specimens useful? A systematic review of the literature. Colorectal Dis. 2011;13(11):1214-1221.
23. Duzgun AP, Moran M, Uzun S, Ozmen MM, Ozer VM, Seckin S. Unusual findings in appendicectomy specimens: evaluation of 2458 cases and review of the literature. Indian J Surg. 2004;66(4):221-226.
24. Lally, K.P., C.S. Cox and R.J. Andrassy, 2004. Appendix, In: Townsend, C.M., R.D. Beauchamp, B.M. Evers and K.L. Mattox, Sabiston textbook of surgery, 17th Edn. Elsevier Sunders Co., Philadelphia, 2004.p. 1381-1400.
25. Rosai, J., Rosai and Akerman surgical pathology, 9th Edn., Mosby Co., China, 2004.p.757-61
26. Herd, M.E., P.A. Cross and D. Dutt. Histopathologic audit of acute appendicitis. J. Clin. Pathol., 1992;45:456-8.

Original Article

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Antibiotic Prophylaxis and Surgical Site Infection

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Abstract

Background: Surgical Site Infection rate is an important health care index. Prophylactic antibiotics have been used to prevent surgical site infections. A continuous surveillance is called for to keep a check on the resistance pattern of microbes to the prophylactic antibiotic.

Objectives:

1. To estimate the Rate of Surgical Site Infections in the years 2014 and 2015 with reference to CDC guidelines at a tertiary care hospital (PSGIMS&R) located in Tamil Nadu, Southern India
2. To estimate the frequency of various organisms causing Surgical Site Infections
3. To study the antibiotic resistance pattern to the prophylactic antibiotic used.

Type of Study: Retrospective Observational study

Period of Study: January 2014 - December 2015

Methodology: The present study was conducted in PSG Institute of Medical Sciences & Research, Coimbatore. We did a thorough retrospective analysis of case files of 467patients reported to have culture positive SSI. SSI was established using clinical and Standard microbiological testing.

Results: The outcome of this study is that the SSI rate of 4.9% reported in our hospital is consistent with or less than the rates reported in other parts of the country. Diabetes is a very important comorbidity which makes the person significantly vulnerable to SSI. In culture positive cases 24.4% of SSI showed resistance to the prophylactic antibiotic used.

Conclusion: There is a significant rise in incidence of organisms exhibiting resistance to the prophylactic antibiotic and a constant surveillance with feedback to the surgeons and strict adherence to antibiotic policies is essential.

Keywords: Surgical Site Infection Rate; Prophylactic Antibiotics; Diabetes Mellitus; Surveillance.

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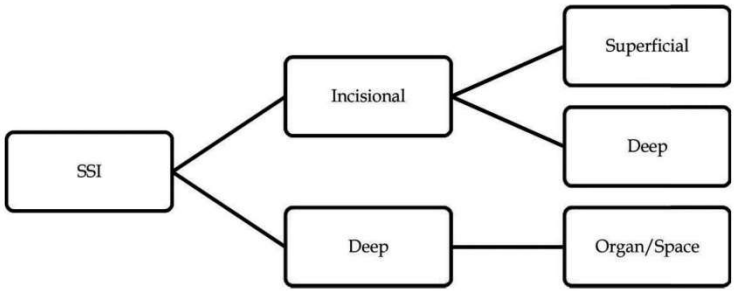
Introduction

Surgical Site Infections (SSI) are those which occur in the wound created by invasive surgical procedures. Historically they were called 'Irritative Fevers' [1] before the era of antisepsis brought about by Joseph Lister. These 'fevers' were uniformly lethal and so dreaded by all surgeons. They are an important cause of Healthcare associated infections. They continue to be an important health hazard even in hospitals with standard protocols of preoperative preparation and prophylactic antibiotic usage. Their prevalence is usually underestimated [2] owing to the occurrence of many of these infections after the patient has been discharged from the hospital. The spectrum of SSI can range from a simple wound discharge to life threatening sepsis. The incidence of SSI in India is estimated [3] to be around 11% at tertiary care centers.

The present study was aimed at obtaining theincidence of surgical site infections and determining the role of Diabetes Mellitus as an

important risk factor for the development of SSI. The Study also aims at determining the percentage of SSI that were resistant to the prophylactic antibiotic

Defining a SSI should involve : 1. Demonstration of signs & symptoms of Inflammation 2. Microbiological evidence of infection. The skin is colonized by a wide range of microbes and clinical evidence of inflammation is mandatory to establish a diagnosis if SSI.



The CDC definition [4] describes three levels of SSI:

superficial incisional, involves the skin and subcutaneous tissue. These infections may be indicated by Celsian signs of localized inflammation such as redness, pain, heat or swelling at the site of the incision or by the drainage of pus.

Deep Incisional involves the fascial and muscle layers. These infections may be indicated by the presence of pus or an abscess, fever with tenderness of the wound, or a separation of the edges of the incision exposing the deeper tissues.

Organ or Space Infection involves any part of the anatomy other than the incision that is opened or manipulated during the surgical procedure, for example joint or peritoneum. These infections may be indicated by the drainage of pus or the formation of an abscess detected by histopathological or radiological examination or during re-operation

Time period: The majority of SSIs become apparent within 30 days of an operative procedure and most often between the 5th and 10th postoperative days. However, where a prosthetic implant is used, SSIs affecting the deeper tissues may occur several months after the operation

Superficial Incisional SSI Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following:

1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative.
4. Diagnosis of superficial incisional SSI by the surgeon or attending physician.

Deep Incisional SSI Infection occurs within 30 days after the operation if no implant† is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves deep soft tissues (e.g., fascial and muscle layers) of the incision and at least one of the following:

1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site.
2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs orsymptoms: fever (>38°C), localized pain, or tenderness, unless site is culture-negative.
3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of a deep incisional SSI by a surgeon or attending physician.

Organ/Space SSI Infection occurs within 30 days after the operation if no implant† is left in place or within 1 year if implant is in place and the infection appears to be related to the operation and infection involves any part of the anatomy (e.g.,

organs or spaces), other than the incision, which was opened or manipulated during an operation and at least one of the following:

1. Purulent drainage from a drain that is placed through a stab wound‡ into the organ/space.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space.
3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of an organ/space SSI by a surgeon or physician

Objectives

1. To estimate the rate of Surgical Site Infections in our hospital separately for the years 2014 & 2015.
2. To estimate the frequency of various organisms causing SSI.
3. To study the antibiotic resistance pattern to the prophylactic antibiotic used.

Type of Study

Retrospective Observational Study

Period of Study

January 2014 - December 2015

Inclusion Criteria

1. Patient admitted in PSG Hospitals who have undergone surgeries here in and reported to have SSI. The definitions were based on CDC guidelines.
2. The patients should have microbial growth on wound swab culture.
3. Prophylactic Antibiotic should have been used in all cases.
4. The SSI should have been reported within 30 days of the Surgery.

Exclusion Criteria

1. Patients operated in other hospitals but presenting with SSI.
2. Swab cultures from other centers.
3. Preop antibiotics not used or not tested for sensitivity in microbiology.

Methodology

The study was conducted in PSG IMS&R a tertiary care center in southern India. There have been 9470 surgeries performed in the two year period considered for this study. These include surgeries performed in the departments of General surgery, Urology, Pediatric surgery, Cardiothoracic and Vascular surgery, Neuro surgery, HepatoPancreaticobiliary, Plastic surgery, Obstetrics & Gynecology and Orthopedics.

Among this the total number of cases that had SSI was 467. These cases met the CDC definition and fit into the inclusion and exclusion criteria. They were diagnosed using wound swabs that were processed using standard microbiological methods

The data was analyzed using Microsoft Excel

Results

Our SSI rate is estimated to be 4.92% for the year 2014 and 4.95% for the year 2015

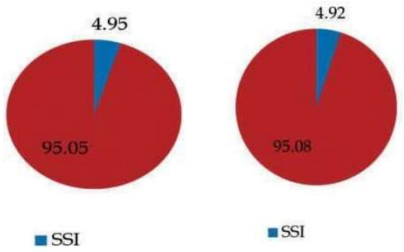


Fig. 2: SSI 2014

The most common antibiotic used preoperatively was IV Cefazolin and this was in accordace with our institution antibiotic policy

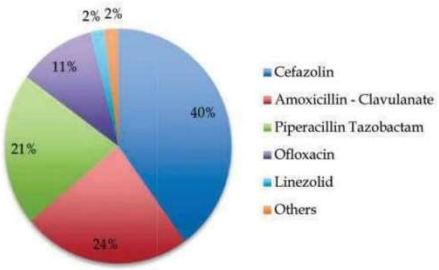


Fig. 3: Antibiotic usage

The commonest organism that showed up in wound swab culture was Escherichia Coli followed by Klebsiella pneumonia

Table 1: SSI rates for the years 2014 & 2015

Year	Total cases	SSI	SSI rate
2014	4719	232	4.92%
2015	4751	235	4.95%

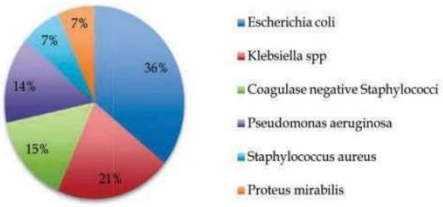


Fig. 4: Organisms grown in culture

Our study showed a predominance of SSI in Diabetics - 27.62%(n=129)

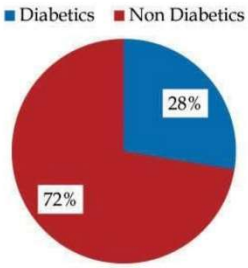


Fig. 5: Incidence of SSI in Diabetics

Resistance to the antibiotic used prophylactically was noticed in - 24.41%(n=114)

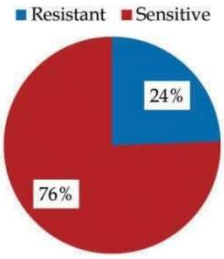


Fig. 6: Resistance to prophylactic antibiotic

Discussion

Surgical site infections are an important cause of Health Care Associated Infections (HCAI). They stand second only to Urinary Tract Infections as a significant cause of morbidity and occasional mortality in operated patients. This rate is widely variable among institutions. The rate of SSI is significantly higher in abdominal than extra abdominal procedures.

The rate of SSI varies greatly worldwide and from hospital to hospital. The rate of SSI varies from 2.5% to 41.9% as per different studies [13,14]. Thepresent study shows SSI rate 4.9% which isless than the rate of SSI reported by other authors in India.

National Academy of Science reported [15] higher rate of infection in patients with Diabetes Mellitus which is similar to our study Microbes that cause SSI are acquired either endogenously from the patient's own flora orexogenously from contact with the environment. However, theperiod of greatest risk remains the time betweenopening and closing the operating site [16].

Prophylactic antibiotics play a major role in the prevention of SSI. But a significant number of culture positive SSI show resistance to the prophylactic antibiotic.

Conclusion

All hospitals should aim at keeping a check on Surgical Site Infections. Special consideration should be given to Diabetics as this population is at a very great risk of developing SSI. Periodic surveillance and feedback to the surgeons is essential to monitor the rise of antibiotic resistant SSI.

References

1. Guideline for prevention of surgical site infection 1999 Alicia J. Mangram, MD; Teresa C. Horan, MPH, CIC; Michele L. Pearson, MD; Leah Christine Silver, BS; William R. Jar vis, MD; The Hospital Infection Control Practices Advisory Committee.

2. Surgical Site Infection - Prevention and Treatment of Surgical Site Infection NICE Clinical Guidelines, No. 74National Collaborating Centre for Women's and Children's Health (UK). London: RCOG Press; 2008 Oct. ISBN-13: 978-1-904752-69-1.
3. Akhter MS, Verma R, Madhukar KP, Vaishampayan AR[3], Unadkat PC. Incidence of surgical site infection in postoperative patients at a tertiary care centre in India.J Wound Care. 2016 Apr; 25(4):210-2,214-7.doi:10.12968/jowc.2016. 25.4.210.
4. Health Protection Agency. Surveillance of Surgical Site Infection in England: October 1997-September 2005. London: Health Protection Agency; 2006.
5. Agarwal SL. Study of postoperative wound infection. Indian J Surg 1972; 34:314-20.
6. Rao AS, Harsha M. Postoperative wound infections. J Indian Med Assoc 1975; 64:90-3.
7. Cruse Peter JE, Foord R. The epidemiology of wound infection. A 10-year prospective study of 62,939 wounds. SurgClin North Am 1980; 60:27-40.
8. Anvikar AR, Deshmukh AB, Karyakarte RP, Damle AS, Patwardhan NS, Malik AK, et al . A one year prospective study of 3,280 surgical wounds. Indian J Med Microbiol 1999; 17:129-32
9. Tripathy BS, Roy N.Post-operative wound sepsis. Indian J Surg 1984; 47:285-8.
10. Olson MM, Lee JT. Continuous, 10-year wound infection surveillance. Results, advantages, and unanswered questions.ArchSurg 1990; 125:794-803.
11. Altemeier WA, Culbertson WR, Hummel RP. Surgical considerations of endogenous infectious - sources, types and methods of control. SurgClin North Am 1968; 48:227-40.
12. Kowli SS, Nayak MH, Mehta AP, Bhalerao RA. Hospital infection. Indian J Surg 1985; 48:475-86.
13. Lilani SP, Jangale N, Chowdhary A, Daver GB. Surgicalsite infection in clean and clean-contaminated cases.Indian J MedicalMicrobiology 2005; 23(4):249-252.
14. Anvikar A.R., Deshmukh A.B., Karyakarte R.P., Damle A.S., Patwardhan N.S., Malik A.K., et al. A one year prospective study of 3280 surgical wounds. Indian J Medical Microbiology 1999; 17(3):129-132
15. National Academy of Science/ National Research Council. Post operative wound infections Influence ofultraviolet irradiation of the operating room & of various other factorAnnal of Surgery. 1964; 160 (supp2):1-132.
16. Edward S. Wong. Surgical site infections. In: C. Glen Mayhall, editors. Hospital Epidemiology and Infectioncontrol. 2nd ed. Philadelphia: Lippincott Williams &Wilkins; 1999.p.189-210.

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Predictors of Major Lower Limb Amputation among Type II Diabetic Patients

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Abstract

Diabetes mellitus (DM) is the most common cause of amputations. This study aimed toidentify the predictive factors for major lower limb amputation among patients with type 2 DM (T2DM) who were admitted to our hospital, in order to reduce its likelihood. *Methods:* This study involved 218 patients with T2DM who were admitted for diabetic foot problems from January 2015 to July 2016. A form was developed to document the patients' profiles, comorbidities, complications, investigations, treatment and clinical outcomes. The predictors for major lower limb amputations were determined using univariate and stepwise analysis. *Results:* A total of 31 patients underwent major lower limb amputations (25 transtibial, 6 transfemoral). The followingfactors were found to be associated with the incidence of major lower limb amputations: T2DM duration ≥ 10 years, diabetic neuropathy, diabetic nephropathy, presentation with gangrene, diabetic foot conditions of Wagner grade 4 or 5, and necrotising fasciitis. Patients who underwent major amputations had significantly lower haemoglobin and albumin levels, and higher total white blood cell counts, erythrocyte sedimentation rates, and C-reactive protein, urea and creatinine levels. However, only T2DM duration ≥ 10 years, positive bacterial culture and albumin levels were significant on stepwise analysis. *Conclusion:* T2DM duration ≥ 10 years, positive bacterial culture and low albumin levels were found to be significantpredictive factors for major lower limb amputation among patients with T2DM admitted for diabetic foot problems.

Keywords: Diabetes Mellitus; Amputation; T2DM; Diabetic Foot.

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Introduction

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. According to Wild et al, the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease.

The Average amputations performed were related to Patients with diabetic foot problems were also found to have a poorer health-related quality of life, especially in terms of physicality Given the abundant problems related to amputations and prostheses, it would be beneficial to reduce the amputation rate due to DM. The present study sought to determine predictive factors for major lower limb amputations

Methods

This study involving 218 patients who were

admitted PSH IMSR and Hospitals from January 2015 to 2016 with type 2 DM (T2DM) were included in the study. A case record form was developed for the documentation of data prior to the commencement of this study. The following predictive factors were analysed: age, gender, marital status, duration of T2DM, smoking habits, alcohol consumption, hypertension, ischaemic heart disease, neuropathy, peripheral vascular disease, hyperlipidaemia, glycated haemoglobin level, renal profile, total white blood cell count, and erythrocyte sedimentation rate (ESR).

Peripheral Vascular Assessment was Done Using a Doppler Ultrasound

Other information that was documented included the type of diabetic foot problems (i.e. abscess, osteomyelitis, gangrene, ulcers, cellulitis, necrotising fasciitis and/or charcotosteoarthropathy). Foot conditions were graded according to the Wagner classification (grade 0: high-risk foot; grade 1: superficial ulcer; grade 2: deep ulcer, penetrating tendon, bone or joint; grade 3: deep ulcer with abscess or osteomyelitis; grade 4: localised toe, forefoot or heel gangrene; grade 5: hindfoot gangrene). Patients who underwent below- or above-knee (i.e. transtibial or transfemoral, respectively) amputations were considered to have had a major lower limb amputation.

Minor lower limb amputation was defined as an amputation distal to the ankle joint. The decision to amputate was made by the general surgeons.

Duration of ten years and above was chosen as it yielded about 80% sensitivity and specificity for having a major lower limb amputation. Demographics as well as clinical and laboratory variables of the minor and major amputation groups were compared. Unless stated otherwise, numerical data was described using mean ± standard deviation, while categorical data was described using percentages. The statistical differences of the variables for minor and major amputations were tested using independent sample *t*-tests for numerical variables and chi-square tests for categorical variables. Important variables were modelled with logistic regression to determine significant predictors.

Results

Of the 218 patients included in the present study, 147 were male and 71 were female. Their mean age was 60.97 (range 36–98) years About half (n = 131, 60.1%) of the patients had hyperlipidaemia and 183 (83.9%) had hypertension; 79 (36.2%) patients were obese and 86 (39.4%) were smokers.

Nearly 60% of the patients (n = 130) were on oral hypoglycaemic agents and 88 (40.4%) patients were on insulin therapy. In terms of follow-up location, 143 (65.6%) patients were followed up at government health clinics, 57 (26.1%) at tertiary government hospitals and 18 (8.3%) at general practitioner. Retinopathy was observed in 62 (28.4%) patients, neuropathy in 94 (43.1%) patients, nephropathy in 65 (29.8%) patients, and peripheral vascular disease in 80 (36.7%) patients. 15 (6.9%) patients had a history of cerebrovascular accidents and 55 (25.2%) had underlying coronary heart disease. The most common presentation was abscesses (n = 68, 31.2%), followed by osteomyelitis (n = 56, 25.7%), cellulitis (n = 45, 20.6%), gangrene (n = 30, 13.8%), necrotising fasciitis (n = 19, 8.7%), charcotosteoarthropathy (n = 2, 0.9%) and ulcers (n = 1, 0.5%). When the foot conditions were categorised according to the Wagner classification (n = 203), 27 (13.3%) were grade 1, 19 (9.4%) were grade 2, 114 (56.2%) were grade 3, 19 (9.4%) were grade 4 and 24 (11.8%) were grade 5. Among the 218 patients, 31 (14.2%) underwent major lower limb amputation – 6 (2.8%) were transfemoral amputations, while 25 (11.5%) were transtibial amputations.

We found that age, gender, ethnicity, monthly income, dependency on family, smoking status, obesity, hyperlipidaemia, hypertension and history of ischaemic heart disease were not associated with major lower limb amputation (Table 1). The factors associated with major lower limb amputations included T2DM duration of ≥ 10 years, higher or tertiary education level, diabetic neuropathy, presentation with gangrene, diabetic foot condition of Wagner grade 4 or 5, and necrotising fasciitis (Table 2). Coronary heart disease, abscesses, osteomyelitis, ulcers and cellulitis were not significant factors for major lower limb amputation. Gangrene was a significant factor because 12 (40%) patients with gangrene underwent major lower limb amputation compared to 19 (10.1%) patients without gangrene.

Compared to those who underwent minor lower limb amputations, the patients who underwent major lower limb amputations were found to have significantly lower haemoglobin and albumin levels, higher white blood cell count, ESR, and, urea and creatinine levels. A significantly higher number of patients who underwent major amputations also had a positive bacterial culture result, compared to those who underwent minor amputations (Table 3).

To determine the significant predictors for major

lower limb amputation, we included those variables that we considered to be clinically important. The variables were duration of T2DM(< 10 years vs. ≥ 10 years), Wagner classification (grade 1–3 vs. grade 4–5), positive bacterial culture (regardless of type of bacterial specimen cultured) and plasma albumin level.

A stepwise logistic regression was performed and the final model consisted of: duration of T2DM, positive bacterial culture and. Based on a 95% confidence interval level, the independent significant predictive factors for major lower limb amputations among our patients were duration of T2DM of ≥10 years, positive bacterial culture and low albumin levels).

Table 1: Characteristics of the patients with type 2 diabetes mellitus

Gender	Minor lower limb amputation (n = 187)	No. (%)	Major lower limb amputation (n = 31)
Female (n = 71)	60 (84.5)	11	(15.5)
Male (n = 147)	127 (86.4)	20	(13.6)
Age (yr)			
< 60 (n = 96)	84 (87.5)	12	(12.5)
≥ 60 (n = 122)	103 (84.4)	19	(15.6)
Comorbidity			
Smoker			
Yes (n = 86)	76 (88.4)	10	(11.6)
No (n = 132)	111 (84.1)	21	(15.9)
Obesity			
Yes (n = 79)	67 (84.8)	12	(15.2)
No (n = 139)	120 (86.3)	19	(13.7)
Hyperlipidaemia			
Yes (n = 131)	109 (83.2)	22	(16.8)
No (n = 87)	78 (89.7)	9	(10.3)
Hypertension			
Yes (n = 183)	158 (86.3)	25	(13.7)
No (n = 35)	29 (82.9)	6	(17.1)
History of ischaemic heart disease			
Yes (n = 53)	49 (92.5)		4 (7.5)
No (n = 165)	138 (83.6)		27 (16.4)

Table 2: Characteristics of type 2 diabetes mellitus (T2DM) in the patients (n = 218), according to whether they underwent major or minor lower limb amputation

Characteristic	Minor lower limb amputation (n = 187)		No. (%)	Major lower limb amputation (n = 31)
Duration of T2DM (yr)				
< 10 (n = 145)	141	(97.2)	4	(2.8)
≥ 10 (n = 73)	46	(63.0)	27	(37.0)
Medication				
Oral hypoglycaemic agents (n = 130)	116	(89.2)	14	(10.8)
Insulin (n = 88)	71	(80.7)	17	(19.3)
Follow-up location				
Government health clinics (n = 143)	124	(86.7)	19	(13.3)
General practitioner clinics (n = 18)	17	(94.4)	1	(5.6)
Tertiary government hospitals (n = 57)	46	(80.7)	11	(19.3)
T2DM complication				
Diabetic retinopathy				
Yes (n = 62)	55	(88.7)	7	(11.3)
No (n = 156)	132	(84.6)	24	(15.4)
Diabetic neuropathy				
Yes (n = 94)	63	(67.0)	31	(33.0)
No (n = 124)	124	(100.0)		0
Diabetic nephropathy				
Yes (n = 65)	61	(93.8)	4	(6.2)
No (n = 153)	126	(82.4)	27	(17.6)
Cerebrovascular accident				
Yes (n = 15)	15	(100.0)		0

No (n = 203)	172	(84.7)	31	(15.3)
Coronary heart disease				
Yes (n = 55)	54	(98.2)	1	(1.8)
No (n = 163)	133	(81.6)	30	(18.4)
Peripheral vascular disease				
Yes (n = 80)	70	(87.5)	10	(12.5)
No (n = 138)	117	(84.8)	21	(15.2)
Type of diabetic foot problem				
Abscesses				
Yes (n = 68)	68	(100.0)		0
No (n = 150)	119	(79.3)	31	(20.7)
Osteomyelitis				
Yes (n = 56)	56	(100.0)		0
No (n = 162)	131	(80.9)	31	(19.1)
Gangrene				
Yes (n = 30)	18	(60.0)	12	(40.0)
No (n = 188)	169	(89.9)	19	(10.1)
Necrotising fasciitis				
Yes (n = 19)	1	(5.3)	18	(94.7)
No (n = 199)	186	(93.5)	13	(6.5)
Charcot osteoarthropathy				
Yes (n = 2)	1	(50.0)	1	(50.0)
No (n = 216)	186	(86.1)	30	(13.9)
Ulcers				
Yes (n = 1)	1	(100.0)		0
No (n = 217)	186	(85.7)	31	(14.3)
Cellulitis				
Yes (n = 45)	45	(100.0)		0
No (n = 173)	142	(82.1)	31	(17.9)
Wagner grade of diabetic foot†				
1–3 (n = 160)	154	(96.3)	6	(3.8%)
4–5 (n = 43)	19	(44.2)	24	(55.8)

Table 3: Vital signs and investigation results of the patients during admission (n = 218)

Vital sign/investigation result	Mean ± standard deviation			
	Minor lower limb amputation (n = 187)		Major lower limb amputation (n = 31)	
Systolic blood pressure (mmHg)	151	± 23	110	± 25
Diastolic blood pressure (mmHg)	91	± 13	62	± 13
Pulse (bpm)	84	± 13	112	± 13
Temperature (°C)	37.7	± 1.1	38.5	± 0.6
Haemoglobin level (g/dL)	10.7	± 1.7	9.3	± 1.7
Total white blood cell count (× 10^9/L)	13.4	± 3.1	18.4	± 4.3
Erythrocyte sedimentation rate (mm/hr)	68.9	± 25.8	84.8	± 21.0
C-reactive protein level (mg/dL)	35.8	± 36.2	105.5	± 23.4
Glycated haemoglobin level (%)	8.0	± 1.4	8.1	± 0.4
Urea level (mmol/L)	8.4	± 2.8	14.7	± 4.3
Creatinine level (mmol/L)	111.2	± 36.2	231.9	± 104.5
Albumin level (g/L)	35.3	± 5.7	23.2	± 4.4
Positive bacterial culture*	88 (80.7)		21 (19.3)	
Negative bacterial culture*	99 (90.8)		10 (9.2)	

Discussion & Conclusion

The rate of major lower limb amputations for diabetic foot problems in the present study (14.2%) is lower than the ratesreported by other studies.These differences in the rates of major lower limb amputation could be due to poor acceptance of amputations as a means of diabetic foot management among our study population. In the present study, duration of T2DM of ≥ 10 years was found to be a significant independent predictive factor for major lower limb amputation and age was not found to be a significant predictive factor for major lower limb amputation. While neuropathy was found to be associated with major lower limb amputation in the present study, it was not a significant independent predictive factor. Necrotising fasciitis was found to be associated with major lower limb amputation, following stepwise logistic regression, only a positive bacterial culture result was

found to be a significant independent predictive factor for major lower limb amputation. Abscess, osteomyelitis, ulcers and cellulitis were not significant factors for major lower limb amputation, suggesting that they could be treated with minor limb amputation. In the present study, total white blood cell count, ESR and Albumin were dependent factors for major lower limb amputation. While major lower limb amputation was not significantly associated with peripheral vascular disease. It was significantly associated with gangrene at presentation anddiabetic foot conditions. Albumin level was found to be an independent predictive factor for major lower limb amputation. Nephropathy and high levels of urea and creatinine were also found to be associated with major lower limb amputation. A low albumin level indicates poor nutritional and renal function. Low albumin levels were also found to be associated with poor wound healing after Syme amputations, leading to major lower limb amputation in patients with DM. In conclusion, the present study showed that a duration of T2DM ≥ 10 years, positive bacterial culture and low serum albumin levels were predictors for major lower limb amputation in patients admitted with diabetic foot problems. Hence, we recommend early aggressive treatment for infections in patients with a long history of T2DM who are admitted for diabetic foot problems, without neglecting their clinical management and nutritional support.

Conflict of Interest

None

Source of Funding

None

References

1. Sargen MR, Hoffstad O, Margolis DJ. Geographic variation in Medicare spending and mortality for dia-betic patients with foot ulcers and amputations. J Diabetes Complicat. 2013; 27: 128–133. doi: 10. 1016/j.jdiacomp.2012.09.003PMID: 23062327.
2. Gordois A, Scuffham P, Shearer A, Oglesby A, Tobian JA. The health care costs of diabetic peripheral neuropathy in the US. Diabetes Care. 2003; 26: 1790–1795. PMID: 12766111.
3. Gordois A, Scuffham P, Shearer A, Oglesby A. The healthcare costs of diabetic peripheral neuropathy in the UK. The Diabetic Foot. 2003; 6: 62–73.
4. Katsilambros N, Tentolouris N, Tsapogas P, Dounis E. Atlas of Diabetic Foot. Chichester, UK: Wiley-Blackwell; 2003.
5. International Working Group on the Diabetic Foot. Epidemiology of Diabetic Foot Infections in a Popula-tion Based Cohort. Noordwijkerhout, The Netherlands; 2003.
6. Lavery LA, Armstrong DG, Wunderlich RP, Tredwell J, Boulton AJM. Diabetic foot syndrome: evaluat-ing the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. Diabetes Care. 2003; 26: 1435–1438. PMID: 12716801.
7. Boulton AJM, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. Lancet. 2005; 366: 1719–1724. doi: 10.1016/S0140-6736(05)67698-2 PMID: 16291066.
8. Krishnan S, Nash F, Baker N, Fowler D, Rayman G. Reduction in diabetic amputations over 11 years in a defined U.K. population: benefits of multi-disciplinary team work and continuous prospective audit. Di-abetes Care. 2008; 31: 99–101. doi: 10.2337/dc07-1178 PMID: 17934144.
9. Carstensen B, Kristensen JK, Ottosen P, Borch-Johnsen K, Steering Group of the National Diabetes Register. The Danish National Diabetes Register: trends in incidence, prevalence and mortality. Diabetologia. 2008; 51: 2187–2196. doi: 10.1007/s00125-008-1156-z PMID: 18815769.
10. Gudbjörnsdottir S, Cederholm J, Nilsson PM, Eliasson B, Steering Committee of the Swedish National Diabetes Register. The National Diabetes Register in Sweden: an implementation of the St. Vincent Declaration for Quality Improvement in Diabetes Care. Diabetes Care. 2003; 26: 1270–1276. PMID: 12663609.

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To Operate or not to Operate, That is the Question! Review of Management of Splenic Trauma in our Institution over the Past 15 Years

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Abstract

Objectives: To review the management of Splenic trauma (operative vs conservative management) and our outcomes. *Type of Study:* Retrospective observational study. *Period of Study:* 2000-2015. *Methodology:* There have been 36 cases who were admitted with splenic trauma in our institution over the last 15 yrs. ICD-10 Code S36.0 was used to identify these patients. A thorough review of their files was undertaken. *Results:* AAST Grading for splenic injury was done for these cases and there were 3 cases of grade 1, 6 cases of grade 2, 13 cases of grade 3, 12 cases of grade 4 and 2 cases of grade 5.

Of these, both cases of Grade 5 injury were operated immediately, while 4 out of 12 cases of grade 4 injury were operated immediately. Of the 8 cases of grade 4 injury which were managed conservatively, One case needed to be operated at a later date, which is our only case of failure of conservative management.

Usion criteria. One case of grade 3 injury was operated, while all cases of grade 1 and 2 were managed conservatively.

We had 7 cases of Operative management (OM), 28 cases of Successful conservative management (CM) and 1 case of Failure of conservative management (FOC). *Conclusion:* The goal of this study was to evaluate the success of the various strategies (conservative vs surgical) and their outcome. Our study shows that conservative management has a major role to play in hemodynamically stable patients even if their AAST grading of Splenic injury was 4. But these patients who are managed conservatively, need to be monitored closely and the surgeon should be ready to take the patient up for emergency surgery if any signs of worsening are evident.

The failure of non-operative management can be life-threatening and even fatal if the deterioration is not picked up immediately.

Keywords: Splenic Trauma; Splenic Injury; Conservative Management; Non-Operative Management; Splenectomy; AAST Grading.

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Introduction

Historically, patients presenting with splenic trauma were taken up for emergency splenectomy. But due to advances in Imaging and ICU care, a conservative approach is presently in vogue. Patients who are managed conservatively tend to have shorter hospital stay, have lesser blood transfusions and avoid risk of long term infectious complications.

But patients who are managed conservatively require very close monitoring as failure of conservative management can be a life threatening event if the deterioration is not picked up early and the patient is not operated immediately.

Failure of conservative management can be due to missed injury, delayed splenic rupture, splenic artery pseudoaneurysm or spontaneous haemorrhage.

AAST Grading for splenic injury is a useful tool to assess necessity for surgery in these cases. Historically, Grade 4 and Grade 5 were taken up for immediate surgery. However, we are now attempting to manage Grade 4 splenic injury patients, who are hemodynamically stable, with conservative management.

Aast Grading for Splenic Injury

Grade*	Injury type	Description of injury
I	Hematoma	Subcapsular, <10% surface area
	Laceration	Capsular tear, <1cmparenchymal depth
II	Hematoma	Subcapsular, 10%-50% surface area intraparenchymal, <5 cm in diameter
	Laceration	Capsular tear, 1-3cm parenchymal depth that does not involve a trabecular vessel
III	Hematoma	Subcapsular, >50% surface area or expanding; ruptured subcapsular or parecymal hematoma; intraparenchymal hematoma ≥ 5 cm or expanding
	Laceration	>3 cm parenchymal depth or involving trabecular vessels
IV	Laceration	Laceration involving segmental or hilar vessels producingmajor devascularization (>25% of spleen)
V	Laceration	Completely shattered spleen
	Vascular	Hilar vascular injury with devascularizes spleen

*Advance one grade for multiple injuries up to grade III

Our institution, PSGIMSR, Coimbatore, is a multi-speciality tertiary care centre with advanced trauma care facilities.

We look at the incidence of Splenic trauma cases that were admitted and managed in our institution over the past 15 yrs.

Objectives

To review the management of Splenic trauma (operative vs conservative management) and our outcomes

Type of Study

Retrospective observational study

Period of Study

2000-2015

Inclusion Criteria

1. Patients who were admitted in PSG Hospital with Splenic trauma
2. They should have a CECT Abdomen done here.
3. They could have been treated elsewhere initially but definitive treatment should have been in PSG Hospital.

Exclusion Criteria

1. If the patient was operated elsewhere.

2. If the patient has undergone a laparotomy primarily for other abdominal injuries and not for splenic injury.

Methodology

There have been 36 cases who were admitted with splenic trauma in our institution over the last 15yrs who fit into our inclusion and exclusion criteria.

ICD-10 Code S36.0 was used to identify these patients

A thorough review of their files was undertaken.

Definitions

1. Operative Management (OM): Patient taken up for emergency surgery within 24 hrs of admission.

2. Conservative Management (CM): non operative management till discharge.

3. Failure of Conservative Management (FOC): initial Conservative Management but eventually required Surgery after 24hrs

Results

- We collected 36 cases over the last 15 years (2000 -2015) which met our inclusion criteria.
- Patients ranged in age from 12 to 70 years with an average of 34.5 (median -35).
- A male preponderance was noted with 31 of the 36 patients falling in this category.
- Of the 36 cases, 26 were road traffic accidents.
- As expected in RTA's, the majority of patients were male (22 patients out of 26).
- Time taken for the patient to reach the emergency department, PSG Hospital after the RTA ranged

from 1hr 15 mins to 8 days. Some cases were initially evaluated and investigated elsewhere and then referred here to a tertiary trauma care centre for further management.

- Average length of stay was 8.7 days ranging from 1 to 21 days with a median of 8 days. The patient who was our failure of conservative management was the one who was admitted for 21 days.
- Type of injury was blunt injury in 34 cases while it was penetrating injury in 2 cases.

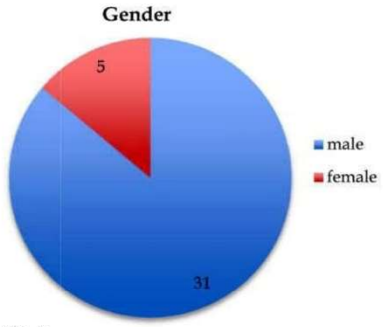


Fig. 1:

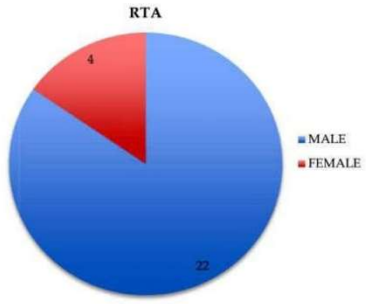


Fig. 2:

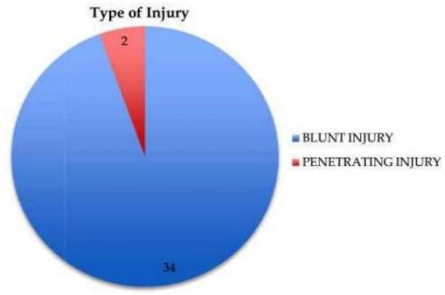


Fig. 3:

- AAST Grading for splenic injury was done for these cases and the results are as follows:

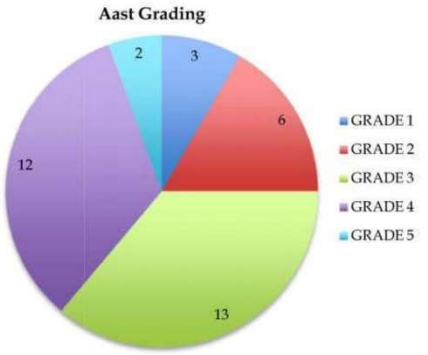
Gr 1 – 3 cases

Gr 2 – 6 cases

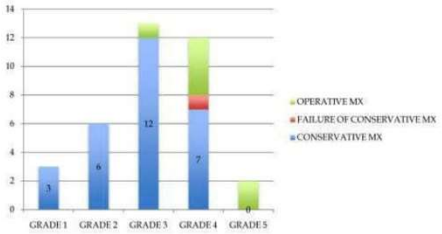
Gr 3 – 13 cases

Gr 4 – 12 cases

Gr 5 – 2 cases



Of these, both cases of Grade 5 injury were operated immediately, while 4 out of 12 cases of grade 4 injury were operated immediately. Of the 8 cases of grade 4 injury which were managed conservatively, One case needed to be operated at a later date, which is our only case of failure of conservative management.



- usion criteria. One case of grade 3 injury was operated, while all cases of grade 1 and 2 were managed conservatively.
- Haemoglobin at admission, which is a criteria to asses the necessity for emergency surgery, ranged from 15.2 g/dl to 3.2 g/dl with the average being 10.4g/dl.
- Maximum drop in Haemoglobin was noted to be 4.9 g/dl.
- Maximum units of blood transfusion required preoperatively to stabilize the patient were 5

packed RBCs.

- The cases with low haemoglobin at admission and with significant or persistant drop in haemoglobin and the one requiring large volume blood transfusion were all taken up for emergency surgery.

We had 7 cases of Operative management (OM), 28 cases of Successful conservative management (CM) and 1 case of failure of conservative management (FOC).

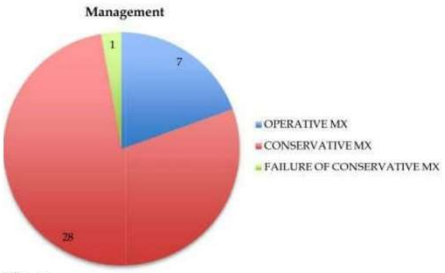
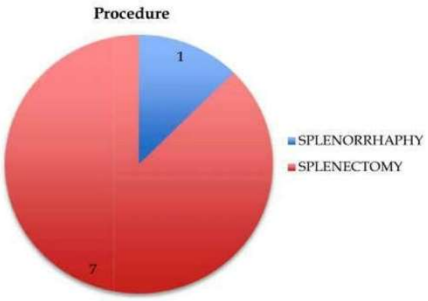


Fig. 6:

Splenoraphy was the procedure of choice for one patient, a 13 yr old girl, while the rest of the cases underwent splenectomy.



- As expected, those who were selected for emergency operative management were more severely injured and hence were more likely to have lower Blood pressure on admission, be hemodynamically unstable, have lower haemoglobin on admission and thus require more blood transfusions.

The only case of Failure of conservative management was a middle aged gentleman, who presented with H/O RTA. He was found to have a Grade 4 splenic laceration, but as he was hemodynamically stable and his baseline haemoglobin was within normal limits, the decision was made to manage him conservatively. His haemoglobin was checked twice daily and he was kept on strict bed rest and intensively monitored in Surgical ICU. On the seventh day post trauma, as he was showing some signs of deterioration, an urgent CECT abdomen was done which showed perisplenic contrast leak. His blood pressure dropped immediately after the CECT scan was done and the decision was taken to Operate immediately. Intra-operatively, he was found to have active oozing from the spleen and he underwent a splenectomy. He recovered well post operatively and was stable on discharge.

Conclusion

The goal of this study was to evaluate the success of the various strategies (conservative vs surgical) and their outcome. Our study shows that conservative management has a major role to play in hemodynamically stable patients even if their AAST grading of Splenic injury was 4. But these patients who are managed conservatively, need to be monitored closely and the surgeon should be ready to take the patient up for emergency surgery if any signs of worsening are evident.

The failure of non-operative management can be life-threatening and even fatal if the deterioration is not picked up immediately.

References

1. The song remains the same although the instruments are changing: complications following selective nonoperative management of blunt spleen trauma: a retrospective review of patients at a level I trauma centre from 1996 to 2007. Clancy AA, Tiruta C, Ashman D, Ball CG, Kirkpatrick AW. J Trauma Manag Outcomes. 2012 Mar 13.
2. Case series of nonoperative management vs. operative management of splenic injury after blunt trauma. Cirocchi R, Corsi A, Castellani E, Barberini F, Renzi C, Cagini L, Boselli C, Noya G. Ulus TravmaAcilCerrahiDerg. 2014 Mar.
3. Non operative management of blunt splenic trauma: a prospective evaluationof a standardized treatment protocol. Brillantino A, Iacobellis F, Robustelli U, Villamaina E, Maglione F, Colletti O, De Palma M, PaladinoF,Noschese G Eur J Trauma Emerg Surg. 2015 Sep 28.
4. Blunt splenic trauma: Assessment, management and outcomes. El-Matbouly M, Jabbour G, El-Menyar A, Peralta R, Abdelrahman H, Zarour A, Al-Hassani A, Al-Thani H. Surgeon. 2015 Aug 29.

5. Management of splenic trauma: a single institution's 8-year experience. Rosati C1, Ata A2, Siskin GP2, Megna D2, Bonville DJ2, Stain SC2 Am J Surg. 2015 Feb.
6. Is non-operative management safe and effective for all splenic blunt trauma? A systematic review. Cirocchi R, Boselli C, Corsi A, Farinella E, Listorti C, Trastulli S, Renzi C, Desiderio J, Santoro A, Cagini L, Parisi A, Redler A, Noya G, Fingerhut A. Crit Care. 2013 Sep.
7. Splenic trauma - our experience at a level I Trauma Center. Saurabh G1, Kumar S, Gupta A, Mishra B, Sagar S, Singhal M, Khan RN, Misra MC UlusTravmaAcilCerrahiDerg. 2011 May.

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Follow Up of Surgical Treatment for Varicose Veins in the Last Three Years in our Institution

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Abstract

Objectives: To assess the influence of venous reflux pattern and superficial venous surgery on ulcer healing and long-term recurrence rates and also to prove that surgery for varicose veins will help in resolution of venous ulcer, thereby improving their quality of life and improve their efficiency at work. *Design:* Retrospective observational study *Methods:* Data was collected from the inpatient and outpatient records at a tertiary care setup hospital. All operated cases of varicose veins with venous ulcers(CEAP C class 6) were followed up. A proforma was made highlighting all the salient findings as type of varicose surgery and choice of post operative compression and datas were collected. Study duration was from june 2012 to june 2015. *Results:* A total of 91 cases of operated varicose veins (11 GSV stripping, 72 GSV stripping + perforator ligation and 8 cases of perforator ligation alone) with venous ulcers were studied, out of which 27 patients were non compliant to postoperative compression and 11 cases had recurrent venous ulcers with 6 cases having recurrent varicose veins. Pattern of superficial venous reflux and risk factors associated with recurrent ulcers were also studied. *Conclusion:* Superficial venous surgery done in an effective manner, with proper postoperative compression of these patients definitely produces good results with less recurrence of both venous ulcers and varicose veins.

Keywords: Varicose; Venous; Ulcer; Veins; Trendlenbergs; Procedure; Stripping; Ligations; Compliance; Compression; Stockings; Crepebandage; Insufficiency; Duplex Imaging; Saphenous; Veinsurgery.

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Introduction

Varicose veins is a common disease condition we see in our daily practice and most of them present to us with non healing venous ulcers.

Chronic venous ulcers represent a major burden to health care services affecting at least 1-2 percent of our total population, often with a protracted course of delayed healing and multiple recurrences.Ulcer aetiology is often multifactorial and results from prolonged venous hypertension.

It has been suggested that surgery alone is sufficient to heal ulcers with isolated superficial reflux. Other consultants employ compression bandaging in addition to surgery. Patients are advised to wear elastic compression hosiery following ulcer healing, but compliance is often poor and difficult to assess.

The role of surgical correction of saphenous vein reflux as a therapeutic manoeuvre in the treatment of venous ulceration have demonstrated that saphenous vein ligation withstripping to the knee (combined with postoperative compression) results in ulcer healing ifthe deep veins are normal, with associated improvementin ambulatory venous pressure.

Because of the substantial morbidity, financial and psychosocial cost it is important to identify the most effective means of treatment for venous ulceration. The progressive development and modification of treatments are essential to contest the natural history of prolonged ulceration and recurrence that makes venous ulceration extremely difficult to cure.

Superficial or deep venous incompetence allows high- pressure venous blood to gravitate to the ankle, this venous hypertension is thought to be the principal cause of venous ulceration, secondary to superficial and deep venous in- sufficiency,obstruction to venous flow, and/or failure of the "venous calf pump". Other associated factors include elevated ambulatory

venous pressures, minor trauma, edema, obesity, arthritis and neuropathies.

Pathophysiological abnormalities contribute to changes at a cellular level that result in venous ulceration, for which a number of hypotheses have been put forward. Despite varying explanations for venous ulceration, it appears that correction of venous hypertension results in ulcer healing and lower recurrence.

Compression therapy and surgical correction of superficial venous incompetence are the main methods currently employed for the treatment for venous ulceration. Graduated external compression to the lower extremity has resulted in accelerated healing of venous ulcers and has become the mainstay of treatment. Compression bandaging therapy is thought to produce a decrease in ankle fluid, softening of the lipodermatosclerosis, decrease in venous volume, increase in deep venous velocity, blood shifts into central compartments, reduction in venous reflux, improvement of venous pumping, improvement in microcirculation and lymph drainage.

As Coimbatore district and surrounding areas is a highly industrialized region with thousands of manual laborers, We, the Department of General surgery, PSG IMS&R, tend to see a lot of patients with varicose veins. Most patients only come for treatment if they are symptomatic or if they have an ulcer. This study looks at the improvement in venous ulcers in patients undergoing varicose vein surgery in our institution.

Aim of Study

1. To assess the influence of venous reflux pattern and superficial venous surgery on ulcer healing and long-term recurrence rates.
2. To prove that surgery for varicose veins will help in resolution of venous ulcer, thereby improving their quality of life and improve their efficiency at work.

Materials and Methods

Study Type

Retrospective observational study

Study Population

91 patients with varicose veins and venous ulcers

Study Duration

3 years (June 2012 to June 2015)

Place of Study

Study was done at a tertiary care multispecialty hospitals (PSG hospitals , coimbatore).

Data Collection

Data was collected from both inpatient and outpatient files from PSG hospitals medical records department.

Inclusion Criteria

1. Patients with active venous ulceration (C-class 6 in the CEAP classification), that we have operated at our institution.

Exclusion Criteria

1. Patient numbers with healed venous ulceration (C-class 5 in the CEAP classification) that recurred after treatment and was operated.
2. All cases of varicose veins operated without a venous ulcer, (C-class 2-4 in the CEAP classification).

Data Analysis

The studies were analyzed and data abstracted with regard to the patient demographics; Number of cases operated; Type of surgery performed; Time of follow up; Size of ulcer; Time taken for ulcer healing; number of cases with recurrence to ulcer; time taken for ulcer recurrence; venous Doppler reports of those patients showing SFJ, SPJ and perforator incompetence; mean hospital stay and methods use postoperatively:comp. stockings/crepe bandage. We also collected data on risk factors associated with non-healing ulcers and ulcer recurrences.

Results

The study included a total of 91 cases of operated varicose veins at our hospital with active venous ulceration (C-class 6 in the CEAP classification).

Of these 11 cases had undergone only trendlenbergs procedure, 72 cases had trendlenberg with perforator ligation and 8 cases had only perforator ligation.

85 of these cases had ulcers lesser than 3 cms size and only 6 cases had ulcers > 3 cms and largest was a 6 cmssized ulcer. Size of ulcer was not associated with recurrence, as we also saw larger ulcers that healed well, post surgery.

All 91 cases had their first review within 10 – 15 days from the date of discharge and all 91 cases had good ulcer healing at their first review. 20 cases did not turn up for second review and all those cases were considered as not compliant to stockings/crepe bandage.

Some of the Risk factors associated with ulcer formation that we identified in our study was (i) Age of the patients usually fall greater than 40 years in more than 80% of cases; (ii) Sex of patient showed a female preponderance in more than 80% of cases; (iii) Occupation of patient is one of long standing professions in greater than 95% of cases.

Venous Doppler imaging studies were invariably done in all cases, which showed 90 % of cases to have SFJ incompetence, 50 % cases to have associated SPJ incompetence and 85% of cases to have associatedperforator incompetence. 100% of recurrent venous ulcer cases had multiple perforator incompetence and also had underwent surgeries for the same along with trendlenbergs procedure.

27 cases were not compliant to crepebandage / stockings application, of which 11 cases had recurrent venous ulcers and time period for recurrence ranged from 3 months to 6 months. 100% of these ulcers were healed well in 10 to 15 days post surgery, only to recur 3 months later.

Time taken for ulcer healing ranged from 10 days to 1 month in most of cases. Only 6 cases took a longer duration of 3 months to 6 months and all of them were positively associated with larger size of venous ulcer.

Post operatively, both compression stockings and crepe bandage were used. 27 cases were not compliant to these measures. Out of 11 recurrent cases, 100% of them were not compliant to stocking / crepe application. But it was not true otherwise, 27 cases were not compliant with these measures and not all of them were associated with recurrence.

It was also noted that 6 cases had recurrent varicose veins and 100% of them were associated with recurrent venous ulcers, non compliance to postoperative compression and also 100% of these had multiple perforator incompetence.

Fig. 1

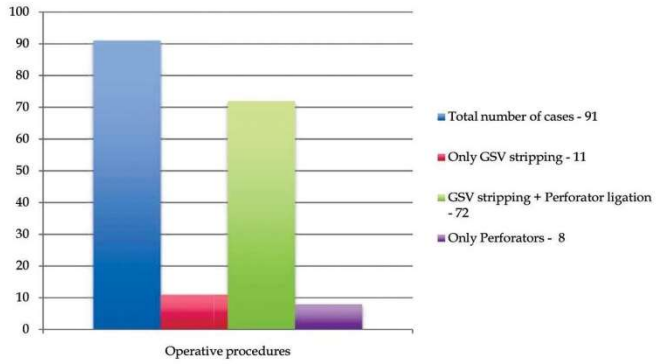


Fig. 2

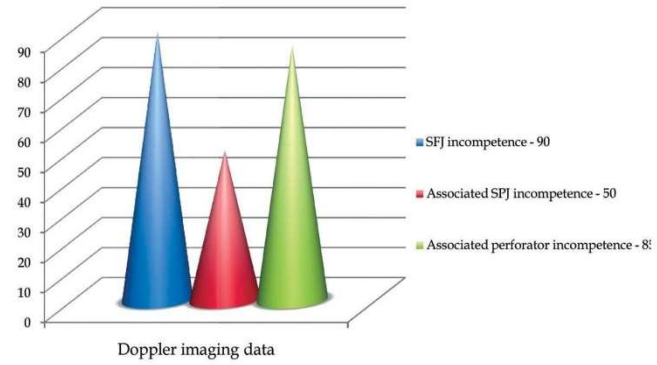
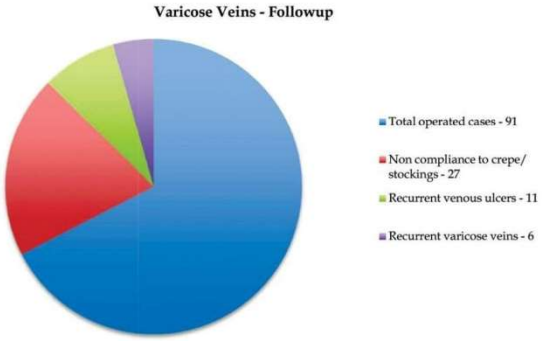


Fig. 3



Discussion

In this study a total of 91 operated cases of varicose veins were followed up and out of them 11 cases had recurrent venous ulcers and 6 had recurrence of varicosities .

Labropoulos *et al.* assessed the progression of 150 operated cases of varicose veins and venous ulcers in the New York region.The factors associated with nonhealing were advanced age, increased body mass index, history of DVT, non-compliance with compression therapy, and large ulcer area.

In our study, duration of active ulcer, pattern of venous incompetence, associated risk factors and poor compliance to postoperative compression, all correlated with a poor prognosis for healing. Butsize of ulcer was not associated with recurrence, as we also saw larger ulcers that healed well, post surgery.

Margolis *et al.*, in a U.S. study have shown that colonization and infection play a role in delaying the healing of chronic ulcers. In our sample, these factors (slough) were present in a substantial percentage of cases, demonstrating how often these conditions occur in patients with chronic leg ulcers. Infection was a major factor associated with worse prognosis during follow-up.

In our study, compliance with compression therapy had a major favorable effect on healing at 6 months. Other risk factors reported in the literature as having a negative influence on healing of chronic venous leg ulcers, such as, ulcer area at baseline, and post-thrombotic etiology, were not identified as such in our sample.

External compression is regarded as essential to the ulcer healing process. In our study 27 cases were not compliant to post operative compression and all 11 cases that had ulcer recurrence were positively associated with non compliance to postop compression. It shows Compliance with compression therapy has favorable effects on the healing process.

There is a consensus in the literature that the most effective intervention for treatment and prevention of recurrence in varicose is strong compression, as it minimizes the effects of venous hypertension on the affected leg. Compression acts on the macro circulation by increasing deep venous return, reducing pathological reflux during walking, and increasing the stroke volume during activation of the calf muscles. Limb compression increases tissue pressure, thus facilitating resorption of edema and improving lymphatic drainage. Furthermore, it acts on the microcirculation to decrease fluid and macromolecule outflow from the capillaries and venules to the interstitial space, and can also stimulate fibrinolytic activity.

It was also noted that 100% of recurrent venous ulcer cases had multiple perforator incompetence and also had underwent surgeries for the same along with trendlenbergs procedure, which may suggest left behind perforators may be a cause of recurrence in these patients.

Also the risk factors as increasing age, female preponderance and long standing occupation were all identified as additional factors that may have played a role in recurrence of varicosities and ulcers in those patients as suggested by various metastudies.

Conclusion

Evidence from the current literature, would suggest that superficial venous surgery done in an effective manner,with proper compression of these patients definitely produces good results with less recurrence of both venous ulcers and varicose veins.

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Ethical Approval

Obtained from Ethical committee of PSGIMS&R.

Competing Interests

None

References

1. The Role of Superficial Venous Surgery in the Management of Venous Ulcers: A Systematic Review; D.P.J. Howard a,*, A. Howard b, A. Kothari a, L. Wales C, M. Guest C, A.H. Davies; Eur J Vasc Endovasc Surg. 2008; 36: 458e465.
2. Surgical Correction of Isolated Superficial Venous Reflux Reduces Long-term Recurrence Rate in Chronic Venous Leg Ulcers; J. R. Barwell, M. Taylor, J. Deacon, A. S. K. Ghauri, C. Wakely, L. K. Phillips, M. R. Whyman and K. R. Poskitt"; Eur J Vasc Endovasc Surg. 2000; 20: 363-368. (doi:10.1053/ ejvs.2000.1196.
3. Total superficial vein reflux eradication in the treatment of venous ulcer.Kanchanabat B[1], Stapanavatr W, Kanchanasuttiruk P.Department of Surgery, Faculty of Medicine, Vajira Hospital, Navamindradhiraj University, 681 Samsain Rd. Dusit, Bangkok, 10300, Thailand.World J Surg. 2015 May; 39(5): 1301-5. doi: 10.1007/s00268-014-2935-y.
4. Surgical treatment of large vascular leg ulcers: a retrospective review evaluating risk factors forhealing and recurrence.Tzaneva S[1], Heere-Ress E, Kittler H, Böhler K. Department of Dermatology, Division of General Dermatology, Medical University Vienna, Vienna, Austria; †Private Practice in Dermatology and Angiology, Vienna, Austria.Dermatol Surg. 2014 Nov; 40(11): 1240-8. doi: 10.1097/DSS.0000000000000137.
5. Saphenofemoral ligation as a safe and effective alternative for the treatment of chronic venous leg ulcer.El-Hafez EA[1], Seleem MI.Department of General Surgery, Benha Faculty of Medicine, Zagazig University, Egypt. Saudi Med J. 2004 Feb; 25(2): 172.

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ORIGINAL ARTICLE

CR-Possum—Can It Be Used to Predict Morbidity? A Single-Centre Retrospective Study

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Abstract

Preoperative prediction of morbidity in colorectal cancer (CRC) surgery helps to optimize the surgical outcome. In this study, we aim to develop a dedicated equation for predicting operative morbidity using colorectal possum scoring system and also to validate the predictive accuracy of CR-POSSUM scoring system in prognosticating actual complications. We did a retrospective analysis of 322 patients undergoing colorectal cancer surgery from a single centre in South India from 2004 to 2016. Mortality and morbidity risk factors as defined by CR POSSUM were collected from 322 patients who underwent CRC surgery and were used to derive equations to predict morbidity, and the results were compared with the observed morbidity. Logistic regression analysis was used to derive the equation. The model fit and model discrimination were analysed using the Hosmer-Lemeshow statistical test for goodness of fit, the Nagelkerke R^2 and area under the receiver operating characteristic (ROC) curve respectively. Out of 322 patients, 103 (32%) patients developed complications and 10 (3%) died due to complications. The regression equation we derived has an overall correct classification of about 70% ($P < 0.01$) with positive and negative predictive value of 60% and 73% respectively. The Hosmer-Lemeshow goodness of fit was 3.147 ($P = 0.829$), and the Nagelkerke R^2 was 17% and area under ROC as model discrimination was 71.6%. Hence, CR-POSSUM scoring which was originally used for predicting mortality risk can also be extrapolated to predict morbidity.

Keywords Colorectal POSSUM · POSSUM · Colorectal surgery · SSI · Morbidity and mortality · Prediction model

Introduction

Colorectal malignancy is the third most common malignancy among men and the second most common among women around the world [1]. Fortunately, in India, the incidence is not too high. However, the incidence has been found to be rising, with the AAR (adjusted annual incidence rate) for colon cancer in men and women being 4.4 and 3.9 per 100,000, respectively [2]. Being in a country, where people living with poor access to quality healthcare, extreme presentation like advanced stage and perforation are common in tertiary care centres [3, 4]. In such scenarios, the mortality and morbidity for the patients undergoing surgery are relatively high [5]. Complications following major surgeries will proportionately increase the hospital cost and duration of hospital stay [6]. Apart from such hardships, complications also had an adverse impact on long-term survival [7]. So in a country like India, where the spending on health by the government is low and health insurance is under-penetrated, morbidity following a major surgical procedure will further drain the already constrained resources [8]. The complication rates are not uniform and cannot be generalised, as there are certain factors, such as age, patients' co-morbidity and operative severity, influencing the outcome of surgery [9]. So, putting together these factors and assessing the postoperative outcome preoperatively will help in optimizing the available resources. In such cases, the predictive scoring has to be more accurate, and its predictive ability should

Key message: CR-Possum can be used to predict morbidity in patient undergoing colorectal cancer surgery.

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[illegible]To analyse the risk-factors contributing to infection with multidrug-resistant organisms.

M[illegible] A 150 diabetic patients with foot ulcer were prospectively studied. Detailed clinical history and clinical examination of the ulcer were done for all patients. The microbiological profile was analyzed for each patient. Using internationally accepted criteria, the multidrug-resistant organisms were identified. Risk factors for acquiring MDRO infection were identified using appropriate statistical tools.

[illegible]MDRO were isolated from 99 patients of 150 (66%). A 54.8% (153 out of 279) of isolated organisms were multidrug-resistant organisms. By univariate analysis poor glycaemic control, previous hospitalisation, previous history of amputation, previous antibiotic usage, size of the ulcer, necrotic ulcer, recurrent ulcers, higher grade of ulcer, the presence of osteomyelitis, the presence of retinopathy, peripheral vascular disease, neuropathy and polymicrobial culture, were significantly associated with MDRO infected foot ulcers. Analysis by logistic regression indicated that only two factors significantly increased the risk of acquiring MDRO infection. They are recurrent ulcer (OR = 3.39, p <0.05, 95% CI = 1.081-10.664) and higher grade of ulcer (OR = 13.44, p <0.001, 95 % CI =3.595-50.278).

[illegible]The prevalence of MDRO is alarmingly high in infected diabetic foot ulcers. Recurrent ulcers and higher grade of ulcers are more prone to acquire MDROs.

[illegible]Diabetic foot, MDRO, Polymicrobial infection, Risk factors

[illegible]

India has the second largest number of patients living with diabetes world wide, with as many as 82 million adults suffering from this disease and its complications.[1] Development of foot ulcers is seen in 15% of all diabetic patients during their lifetime.[2] This is associated with a significant economic burden. The health expenditure is 5 times more in diabetics with foot ulcers than in those without foot ulcers.[1]

Infection of foot ulcers is often due to more than one organism and it is, therefore, imperative that appropriate antibiotic therapy is instituted.[3] Infection with multidrug resistance organisms will increase the morbidity and consequently, the finances involved, to a significant extent.[4] Emergence of multi-drug resistance organisms (MDRO) is the area of major concern in both developing and developed countries.[5] In India, a lack of adherence to protocol in prescribing antibiotics leads to the emergence of these super bugs.[6] Also patient related factors also play

an important role in the development of such organisms. Diabetes is one such factor and its complication such as foot ulcer harbors MDRO.

There are various other factors involved in the emergence of MDRO in diabetic foot ulers. Very few studies have been done in India, to elucidate the underlying risk factors for the development MDRO in diabetic foot ulcer. Present study aims to correlate the risk factors and their association with the development of MDRO in diabetic foot ulcers in South India.

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One hundred fifty diabetic patients with foot lesions were included in the study, conducted between January 2011 and July 2012, at PSG Institute of Medical Sciences and Research, Coimbatore, India, after obtaining approval from the institutional review board. Clinical history was collected using structured case report forms. Mode of presentation of foot ulcers were classified as grade I to V as per Meggit Wagner Classification System (Wagner, 1981). Ulcers were categorised into necrotic/non-necrotic ulcers based on signs of infection (swelling, exudates, surrounding cellulitis, odour, tissue necrosis and crepitation). Size was determined by multiplying the longest and widest diameters expressed in centimeters squared (cm^2), and the diagnosis of extension to the bone was made by plain radiographs. Presence of neuropathy was detected by assessing vibration sensation using a 128 HTZ tuning fork and a 10gm Semmes - Weinstein monofilament. Peripheral diabetic neuropathy was defined as an abnormal monofilament test, as described by the international consensus on the diabetic foot.[7] Presence of nephropathy was detected by screening the patient's urine for micro / macro-albuminuria after ruling out urinary tract infection. The fundus was examined by the ophthalmologist for evidence of retinopathy. Absence of both dorsalis-pedis pulsations and/or an Ankle Brachial Index (ABI) less than 0.9 was termed as peripheral vascular disease.

Wound swabs were obtained from the floor of the ulcer, before starting empirical antibiotic therapy. Direct microscopic examination and aerobic cultures were done by standard methods. The bacteriological spectrum and the sensitive antibiotics were noted for each patient. All patients were started on empirical antibiotics depending on the status of the wound. In mild infection amoxyclav (amoxicillin/clavulanic acid) was given by oral route. But in patients with necrotic wounds, an additional antibiotic, clindamycin or metronidazole, was added for anaerobic and gram-negative coverage and the intravenous route was preferred. In the presence of an unhealthy ulcer, surgical debridement / amputation was done immediately after admission. Later wounds were managed with regular dressings and antibiotics modified according to the culture report. All patients were re-inspected or enquired over phone after a period of 10 weeks to assess the status of wound. For each patient, the following details were entered: age, sex, duration of ulcer, duration of diabetes, glycaemic control, presence of retinopathy, presence of micro/macro-albuminuria, hypertension, history of smoking, history of previous amputation, duration of hospital stay, interventions (medical and surgical), organisms cultured from ulcer, antibiotic profile and status of ulcer after 10 weeks. Previous hospitalization was defined as any hospital stay, which was not necessarily for the management of ulcer, during the year preceding the current hospitalization. Previous antibiotic usage was defined in present study, as those who had received antibiotics in six months preceding current hospitalization.

The data was collected and entered in the SPSS data sheet. The data was analysed using SPSS 20 for descriptive statistics. To assess the risk factors for acquiring MDRO, the patients were grouped into MDRO and non-MDRO groups. All patients, who had at least one multidrug resistant organism, were grouped under MDRO group. The test variables were compared using Chi-square test for qualitative variables and Student's test for quantitative variables. The variables for which the association was statistically significant ($p<0.1$) were introduced in a logistic model. Univariate analysis was performed to compare infected ulcers according to the presence or absence of MDRO. Logistic regression was used to identify explanatory variables for the presence of MDRO.

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Seventy-eight percentage of the patients were 51 years or older. A 74.6% of the patients were males, showing a distinct male preponderance. Most of the patients (44%) belonged to class II socio-economic status followed by class III (26 %), as per Modified Prasad's classification (Table 1).[8]

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Age distribution		
<40	5	3.3%
41-50	16	10.7%
51-60	55	36.7%
61-70	62	41.3%
71-80	10	6.7%
81-90	2	1.3%
Sex distribution		
Male	112	74.6%
Female	38	25.33%
Socio-economic status		
Class I	39	26%
Class II	66	44%
Class III	39	26%
Class IV	6	4%
Class V	0	0%

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Duration of diabetes		
<5 yrs	60	40 %
5-10 yrs	51	34 %
10-15 yrs	26	17 %
15-20 yrs	11	7.3 %
>20 yrs	2	1.3 %
Glycaemic control		
6-7% (good)	29	19.33%
7-8% (fair)	54	36 %
>8 % (poor)	67	44.66 %

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Duration of ulcer□		
<1 month	102	68%
1-2 months	34	22.7%
2-3 months	9	6 %
>3 months	5	3.3%
Size of the ulcer□		
<4 cm^2	11	7.3%
4-8 cm^2	49	32.7%
8-16 cm^2	61	40.7%
16-24 cm^2	23	15.3%
>24 cm^2	6	4%
Depth of ulcer□		
Superficial	92	61.33%
Deep	58	38.66%
Grade of ulcer□		
Grade I	17	11.33%
Grade II	40	26.66%
Grade III	45	30%
Grade IV	35	23.33%
Grade V	13	8.66%
Nature of ulcer□		
Non-necrotic	78	52%
Necrotic	72	48%
Recurrence□		
Non-recurrent	79	52.66%
Recurrent	71	47.33%
Osteomyelitis □		
Absent	99	66%
Present□	51	34%
Site of ulcer□		
Plantar	16	10.66%
Margins	24	16%
Heel	42	28%
Digits	32	21.33%
Malleoli	18	12%
Leg	16	10.66%
Multiple areas□	2	1.33%

Almost all the patients had type II diabetes, with only 4% of them having type I. Only 19.33% of patients had a good glycemic control, with HbA1c 6-7%. 40% of patients with ulcer, had diabetes for less than five years (Table 2).

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Culture		
Mono-microbial	62	41.33%
Poly-microbial	88	58.66%
Drug resistance		
MDRO	99	66%
Non- MDRO	51	34%

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Arteriopathy		
Absent	79	52.66%
Present	71	47.33%
Retinopathy		
Absent	113	75.33%
Present	37	24.66%
Nephropathy		
Absent	77	51.33%
Present	73	48.66%
Neuropathy		
Absent	34	22.66%
Present	116	77.33%
Hypertension		
Absent	58	38.66%
Present	92	61.33%
Smoking		
Non-smoker	75	50%
Smoker	75	50%
Alcohol		
Non-alcoholic	88	58.66%
Alcoholic	62	41.33%
Previous hospitalization		
Not hospitalized	70	46.66%
Hospitalized	80	53.33%
H/o amputation		
Absent	118	78.6%
Present	32	21.33%
Previous antibiotic use		
Absent	86	57.33%
Present	64	42.66%

Sixty-eight percentage of patients had ulcers of less than one-month duration. Concerning the size of the ulcer, most were between 4 to 8cm^2 and 8 to 16cm^2. Most of the patients had Wagner's grade II, III, or IV ulcers. There were very few ulcers with Wagner's grade V. There was an almost equal distribution of necrotic and non-necrotic ulcers and also recurrent and non-recurrent ulcers.

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□□r□□□□□	□□□M□□□□	M□□□□	□□□□□□	□^{2}□	□□□□□□□
Age					
<40	3 (60%)	2 (40%)	5 (100%)	6.373	0.272
41 - 50	8 (50%)	8 (50%)	16 (100%)		
51 - 60	14 (25.5%)	41 (74.5%)	55 (100%)		
61 - 70	22 (35.5%)	40 (64.5%)	62 (100%)		
71 - 80	4 (40%)	6 (60%)	10 (100%)		
81 - 90	0 (0%)	2 (100%)	2 (100%)		
Sex					
Male	35 (31.2%)	77 (68.8%)	112 (100%)	1.490	0.222
Female	16 (42.1%)	22 (57.9%)	38 (100%)		
Socio eco- status					
Class I	14 (35.9%)	25 (64.1%)	39 (100%)	0.266	0.966
Class II	23 (34.8%)	43 (65.2%)	66 (100%)		
Class III	12 (30.8%)	27 (69.2%)	39 (100%)		
Class IV	2 (33.3%)	4 (66.7%)	6 (100%)		
Class V	0 (0%)	0 (0%)	0 (0%)		
Type of diabetes					
I	3 (50%)	3 (50%)	6 (100%)	0.713	0.398
II	48 (33.3%)	96 (66.7%)	144 (100%)		
Depth of ulcer					
Superficial	26 (28.3%)	66 (71.7%)	92 (100%)	3.492	0.062
Deep	25 (43.1%)	33 (56.9%)	58 (100%)		
Nature of ulcer					
Non-necrotic	42 (53.8%)	36 (46.2%)	78 (100%)	28.52	0.000
Necrotic	9 (12.5%)	63 (87.5%)	72 (100%)		
Recurrence					
Non-recurrent	42 (53.2%)	37 (46.8%)	79 (100%)	27.31	0.000
Recurrent	9 (12.7%)	62 (87.3%)	71 (100%)		
Grade of ulcer					
Class I	15 (88.2%)	2 (11.8%)	17 (100%)	62.83	0.000
Class II	26 (65%)	14 (35%)	40 (100%)		
Class III	3 (6.7%)	42 (93.3%)	45(100%)		
Class IV	6 (17.1%)	29 (82.9%)	35 (100%)		
Class V	1 (7.7%)	12 (92.3%)	13 (100%)		
Retinopathy					
Absent	44 (38.9%)	69 (61.1%)	113 (100%)	4.978	0.026
Present	7 (18.9%)	30 (81.1%)	37 (100%)		
Nephropathy					
Absent	29 (37.7%)	48 (62.3%)	77 (100%)	0.946	0.331
Present	22 (30.1%)	51 (69.9%)	73 (100%)		
Osteomyelitis					
Absent	43 (43.4%)	56 (56.6%)	99 (100%)	11.549	0.001
Present	8 (15.7%)	43 (84.3%)	51 (100%)		
Arteriopathy					
Absent	38 (48.1%)	41 (51.9%)	79 (100%)	14.789	0.000
Present	13 (18.3%)	58 (81.7%)	71 (100%)		
Neuropathy					
Absent	20 (58.8%)	14 (41.2%)	34 (100%)	12.0	0.001
Present	31 (26.7%)	85 (73.3%)	116 (100%)		
Hypertension					

□

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Absent	19 (32.8%)	39 (67.2%)	58 (100%)	0.065	0.799
Present	32 (34.8%)	60 (65.2%)	92 (100%)		
Glycaemic control (HBA1C)					
6-7 %(good)	17 (58.6%)	12 (41.4%)	29 (100%)	18.84	0.000
7-8 % (fair)	23 (42.6%)	31 (57.4%)	54 (100%)		
>8 % (poor)	11 (16.4%)	56 (83.6%)	67 (100%)		
Previous admission					
Not hospitalized	32 (45.7%)	38 (54.3%)	70 (100%)	8.026	0.005
Hospitalized	19 (23.8%)	61 (76.2%)	80 (100%)		
Smoking					
Non-smoker	30 (40%)	45 (60%)	75 (100%)	2.406	0.121
Smoker	21 (28%)	54 (72%)	75 (100%)		
Alcoholic					
Non- alcoholic	33 (37.5%)	55 (62.5%)	88 (100%)	1.162	0.281
Alcoholic	18 (29%)	44 (71%)	62 (100%)		
History of amputation					
Absent	45 (38.1%)	73 (61.9%)	118 (100%)	4.216	0.040
Present	6 (18.8%)	26 (81.2%)	32 (100%)		
Culture					
Mono microbial	31 (50%)	31 (50%)	62 (100%)	12.05	0.001
Poly microbial	20 (22.7%)	68 (77.3%)	88 (100%)		
Site					
Plantar	10 (62.5%)	6 (37.5%)	16 (100%)	7.831	0.251
Margins	8 (33.3%)	16 (66.7%)	24 (100%)		
Heel	14 (33.3%)	28 (66.7%)	42 (100%)		
Inter digital	10 (31.2%)	22 (68.8%)	32 (100%)		
Malleoli	5 (27.8%)	13 (72.2%)	18 (100%)		
Leg	4 (25%)	12 (75%)	16 (100%)		
Multiple areas	0 (0%)	2 (100%)	2 (100%)		
Duration of diabetes (years) mean (SD)	1.84 (0.94)	2.02 (1.02)		T= 1.032	0.30
Duration of ulcer (months mean) (SD)	1.49 (0.73)	1.42 (0.77)		T = 0.50	0.61
Size of the ulcer cm^2 mean (SD)	2.47 (0.80)	2.91 (0.97)		T = 2.76	0.006

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Nature	0.172	0.730	0.056	1	0.814	1.188	0.284	4.970
Recurrence	1.222	0.584	4.382	1	0.036	3.395	1.081	10.664
Grade	2.599	0.673	14.910	1	0.000	13.445	3.595	50.278
Retinopathy	0.221	0.631	0.123	1	0.726	1.247	0.362	4.292
Osteomyelitis	-0.260	0.672	0.150	1	0.699	0.771	0.206	2.880
PVD	0.540	0.537	1.013	1	0.314	1.716	0.600	4.911
Neuropathy	-0.275	0.675	0.166	1	0.684	0.760	0.202	2.854
HBA1C	1.105	0.631	3.062	1	0.080	3.019	0.876	10.403
Prev-hospital	0.161	0.608	0.070	1	0.791	1.175	0.357	3.870
H/o amputation	0.630	0.769	0.672	1	0.412	1.878	0.416	8.481
Pre-antibiotic	0.267	0.636	0.176	1	0.675	1.305	0.376	4.537
Culture	0.889	0.523	2.884	1	0.089	2.432	0.872	6.783
Size	0.293	0.285	1.053	1	0.305	1.340	0.766	2.345
Age	-0.005	0.029	0.029	1	0.865	0.995	0.939	1.054
Constant	-3.418	2.054	2.767	1	0.096	0.033		

Thirty-four percentage of ulcers had associated osteomyelitis. 28% of ulcers were seen in the heel, followed by 21.33% in digits/inter-digital areas (Table 3).

A total of 279 organisms were isolated from 150 patients. On an average 1.86 species were isolated from each patient. 58.66% of patients (88 of the 150 patients) had polymicrobial culture. Among the isolates, most were gram-negative rods (69.89%), and almost all the rest were gram-positive cocci. There was a solitary gram-negative coccus. Gram-positive to gram-negative ratio, among the isolates, was 1:2.3. Among the isolates, *Escherichia coli* was the most common one constituting 17.9%. MDROs were seen in 99 of the 150 patients (Tables 4).

Peripheral arterial disease was seen in 52.66%, retinopathy detected in 24.66% and albuminuria suggesting nephropathy was found in 48.66%. Majority of the patients had neuropathy. 61.33% were hypertensive.

Fifty percentage of the patients were smokers and 41.33% alcoholics. History of previous hospital admission in the last one year was seen in 53.33%. 21.33 % of patients had history of some form of amputation. 42.66% of the patients had a history of antibiotic use in the preceding six months before admission (Table 5).

Results of the univariate analysis showed poor glycemic control, previous hospitalization, previous history of amputation, previous antibiotic usage, size of ulcer, necrotic ulcer, recurrent ulcers, higher grade of ulcer, presence of osteomyelitis, presence of retinopathy, peripheral vascular disease, neuropathy and polymicrobial culture, were significantly associated with MDRO infected foot ulcers (Table: 6). However logistic regression results indicated that only two factors significantly increased the chances of acquiring MDRO infection; recurrent ulcer (OR=3.39, p <0.05, 95% CI=1.081-10.664), higher grade of ulcer (OR=13.44, p <0.001, 95% CI=3.595-50.278) (Table 7).

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Infection with MDRO in diabetic foot ulcers associated with inadequate glycemic control and increased requirement of surgical management.[9] In this study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers, especially about multidrug-resistant organisms. With a large number of patients being admitted with diabetic foot ulcers and with the growing global problem of multidrug-resistant organisms, this was a study to ascertain the role of multidrug-resistant organisms in relation to diabetic foot ulcers.

In the present study, the foot ulcers were more prevalent in the fifth and sixth decade of life. The average age of the patients with foot ulcer was 58.21±9.3 years, which is similar to the age prevalence described in another Indian study.[10] The foot ulcers were more common in male. Similar gender preponderance was observed in another study conducted in India.[11]

Most of the patients (68%) had ulcers of less than 1-month duration which is similar to the observations from a north Indian study.[11] An early presentation is often due to the fact that ulcers with acute onset often have systemic symptoms. Comparable with the literature, most of the patients in the present study had poor glycaemic control.[11] Poor glycaemic control is associated with higher degree of microvascular complications.

Majority of the patients in present study had a higher grade of ulcers (Wagner's grade III or worse) similar to the other north Indian studies.[11,12] The reason for presentation with higher grade could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers.[12]

In the present study, the neuropathy was seen in 77.33% of the diabetic foot ulcer patients. The other studies reported from India showed a similar high prevalence (86.2%, 66.6%, and 56.8% respectively).[9,11] A prevalence ranging from 32 to 33.5% have been reported in studies done in Europe.[13,14] Studies elsewhere have a shown a varied prevalence ranging from 12.7% to 77.8%. This marked variation in the prevalence may be due to different methods used for diagnosing neuropathy. A higher prevalence in Indian population, could be because of patient's ignorance and poor glycaemic control.

In the present study, the prevalence of retinopathy was 24.66%. Studies conducted in northern India showed a higher prevalence.[9,12]

Peripheral arterial disease was observed in 47.33% of present study group. A very high prevalence of 85% was seen in a north Indian study.[9] The prevalence of nephropathy was 48.66% in present study. The north Indian study showed a high prevalence (75%).[11]

The bacteriological evaluation of diabetic foot ulcer from present study showed that the gram-negative organisms were found to have a higher occurrence than gram-positive organisms in the ratio 2.3: 1. Some of the other Indian studies also showed a higher occurrence of gram-negative organisms.[9,12] However, most of the western literature showed a predominance of gram-positive organisms as supposed to gram-negative organisms.[15-17] This could be partly due to differences in the causative organisms occurring over time, geographical variations, or the types and severity of infection included in the studies.[18]

Diabetic foot infection is usually polymicrobial in nature which is well documented in the literature. In present study, 58.66% of ulcers had polymicrobial culture. Similar observations were found in other Indian studies and western studies.[9,12,18] There are, however, a few studies which showed more patients with monomicrobial culture.[19,20] Polymicrobial infection, to a certain extent, may be due to prior treatment history of the patients studied, as reported earlier.[21,22]

In the present study, the rate of isolation of organism per ulcer was 1.86 while two other Indian studies showed a rate of 1.25 and 2.3 organisms per ulcer.[9,11] A study from Malaysian reported 1.47 organisms per lesion.[20] A study from US showed a very high rate of 5.8 organisms recovered from the ulcer.[23]

The commonest organism isolated in present study was *Escherichia coli* followed by *Staphylococcus aureus*, *Pseudomonas* and *Klebsiella pneumoniae*. Most of the other studies from India and other countries showed Staphylococcus aureus as the commonest isolate from diabetic foot ulcers.[9,11,16,18,20]

In the present study, 66% of the ulcers grew multi-drug resistant organisms (MDRO) and 54.8% of all isolated organisms were multidrug resistant. Many different definitions for multi-drug resistant organisms were used in medical literature. Due to a lack of uniform definition for MDROs, the overall prevalence of MDRO, as seen in the literature, could not be studied. European centre for disease prevention and control has arrived at a definition for MDROs and has defined specific criteria for categorising an organism as MDRO.[24]

Apart from the multi-drug resistant organisms like MRSA, ESBL and VRE which were extensively studied in the literature, other groups of organisms like MDR *Pseudomonas*, *Acinetobacter*, *Enterococcus* and *Enterobactereciae* were also identified in present study. The higher prevalence of multidrug-resistant organisms was also observed in another north Indian study.[9] The higher antibiotic resistance in tertiary care hospitals is because, widespread use of broad-spectrum antibiotics results in selective survival of drug-resistant organisms. In contrast, a western study showed a lower prevalence of MDRO when compared to Indian literature, which is perhaps a reflection of higher antibiotic use and abuse.[16] The increasing occurrence of MDROs is disconcerting because infection with these organisms limits the choice of antibiotic treatment and may lead to a worse outcome.

In the present study, univariate analysis showed that, poor glycaemic control, previous hospitalisation, previous history of amputation, previous antibiotic usage, size of the ulcer, necrotic ulcer, recurrent ulcers, higher grade of ulcer, presence of osteomyelitis, presence of retinopathy, peripheral vascular disease, neuropathy and polymicrobial culture, were significantly associated with MDRO infected foot ulcers.

However, analysis by logistic regression revealed that only recurrent ulcers and higher grade of ulcers were significantly associated with multi-drug resistant organism infections. It is possible that patients with recurrent ulcers have had several courses of antibiotics, both during previous hospital admissions and from practitioners in the community, which led to resistance to multiple antibiotics. Higher grade of ulcers has an associated systemic sepsis and excessive local necrotic tissues.

Another study from India showed that presence of neuropathy and ulcer size >4cm^2 were significantly associated with multi-drug resistant organism infections.[9] The two significant factors associated with MDRO, in a study from France, were previous hospitalization and proliferative retinopathy.[16]

Factors like previous hospitalization, previous antibiotic usage, poor glycaemic control, ischemic ulcers have emerged as possible risk factors for MDRO in several other studies.[4,9,16] However, we have not found any significant association in present study.

Although author have identified a few factors associated with MDRO, the effect of diabetes-related immunopathology has not been studied. This and its possible impact on infection need a closer look.

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The prevalence of multi-drug resistant organisms is alarmingly high in infected diabetic foot ulcers. Recurrent ulcers are more prone to acquire multi-drug resistant organisms. Higher grade of ulcers is more prone to acquire multi-drug resistant organisms.

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1. International Diabetes Federation. IDF Diabetes Atlas, 8th Ed. Brussels, Belgium: International Diabetes Federation, 2017. http://www.diabetesatlas.org/resources/2017atlas.html. Accessed 26 December 2017.
2. Lipsky BA, Pecoraro RE, Larson SA, Hanley MA, Ahroni J. Outpatient management of uncomplicatedlower-extremity infections in diabetic patients. Arch Inter Med. 1990;150:790-7.

3. Viswanathan V, Jasmine JJ, Snehalatha C, Ramachandran A. Prevalence of pathogens in diabetic foot infection in South India type 2 diabetic patients. J Ass Physic India. 2002;50:1013-6.
4. Hartemann-Heurtier A, Robert J, Jacqueminet S, Ha Van G, Golmard JL, Jarlier V, et al. Diabetic foot ulcer and multidrug-resistantorganisms: risk factors and impact. Diabetic Med. 2004;21:710-5.
5. World Health Organization. The world health report 1996.
6. Chereau F. Risk assessment for antibiotic resistance in South East Asia, BMJ. 2017;358.
7. Practical guidelines on the management and prevention of the diabetic foot. Diabetes Metab Res Rev. 2008;24:S181-7.
8. Ministry of Statistics and Programme Implementation, Government of India, Central Statistical Organization, No. M-12011/2/2005-PCL, Release of linked CPI (UNME) for December 2009, dated 22nd February 2010. Available at: http://mospi.nic.in/Mospi_New/upload/t4_22feb10. html.
9. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry RA. Clinicomicrobiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabet Care. 2006;29:1727-73.
10. Manda V. Foot ulcers and risk factors among diabetic. Int J Med Public Health. 2012;2(3):34-8.
11. Zubair M, Malik A, Ahmad J. Clinico-bacteriology and risk factors for the diabetic foot infection with multi-drug resistant microorganisms in North India. Biol Med. 2010;2(4):22-34.
12. Boulton AJ, Vileikyte L. Diabetic foot problems and their management around the world, in Levin and O Neal's. The Diabetic Foot, 6th Ed. St. Louis MO: Mosby. 2001;6:261-71.
13. Barbosa AP, Medina JL, Ramos EP, Barros HP. Prevalence and risk factors of clinical diabetic polyneuropathy in a Portuguese primary health care population. Diabt Metab. 2001;27:496-502.
14. Manes CH, Papazoglou N, Sossidou E, Soulis K, Milarakis D, Satsoglou A, et al. Prevalence of diabetic neuropathy and foot ulceration: identification of potential risk factors-a population-based study. Wounds-a compendium of clinical research and practice. 2002;14(1):11-5.
15. Dang CN, Prasad YD, Boulton AJ, Jude EB. Methicillin-resistant *Staphylococcus aureus:* a worsening problem. Diabet Med. 2003;20:159-61.
16. Richard JL, Sotto A, Jourdan N. Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. Diabet Metab. 2008;34(4):363-9.
17. Mantey I, Hill RL, Foster AV, Welson S, Wade JJ, Edmonds ME. Infection with foot ulcers with *Staphylococcus aureus* associated with increase mortality in diabetic patients. Commun Dis Public Health. 2000;3(4):288-90.
18. Diane M, Citron, Ellie JC, Goldstein. Bacteriology of Moderate-to-severe diabetic foot infections and *in vitro* activity of antimicrobial agents. J Clin Microbiol. 2007;45(9) 2819-28.
19. Dhanasekaran G, Sastry G, Viswanathan M, Microbial pattern of soft-tissue infections in diabetic patients in South India. Asian J Diabet. 2003;5:8-10.
20. Raja NS. Microbiology of diabetic foot infection in a teaching hospital at Malaysia. A retrospective study of 194 cases. J micribiol immunol infect. 2007;40(1):39-44.
21. Hunt JA. Foot infections in diabetes are rarely due to a single microorganism. Diabet Med. 1992;9:749-52.
22. Urbančič-Rovan V, Gubina M. Infection in superficial diabetic foot ulcers. Clin Infect Dis. 1997;25:S184-5.
23. Gerding DN. Foot infections in diabetic patients: the role of anaerobes. Clin Infect Dis. 1995;20:S283-8.
24. Magiorakos AP, Srinivasan A, Carey RB. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Micro biol Infect. 2012;18(3):268-81.

Kathirvel M, Prabakaran V, Jayarajan J, Sivakumar A, Govindan V. Risk factors for the diabetic foot infection with multidrug-resistant microorganisms in South India. Int Surg J 2018;5:675-82.

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Proximal femoral nailing in unstable intertrochanteric fractures: a prospective study

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Abstract

Background: Intertrochanteric fractures occur mostly in elderly patients, and the outcome may be extremely poor if there is prolonged bed-rest. The best treatment for trochanteric fracture remains controversial. Proximal femoral nail antirotation (PFNA) II has excellent fit as the design is adapted to the anatomical situation of small statured patients also. It has a medial lateral angle of 5 degrees. It has several distal locking options viz static/dynamic. Objective of this study was to observe the operating time, blood loss and fracture union in intertrochanteric fractures treated with PFNA II from the data collected from patient's case sheets and post-operative X-rays.

Methods: 25 patients who were operated with PFNA II for unstable intertrochanteric fracture were selected using random selection and following parameters were noted from the records.

Results: The mean age of the participants is 70.72±8.55 years with range from 52 years to 89 years. The mean duration was 1.98 hours ±0.66. The median duration was 2 with interquartile range from 1.5 to 2.5 hours. Mean blood loss was estimated to be 390 ml±62.78. The median blood loss was 370 ml with interquartile range from 360 to 420 ml.

Conclusions: The PFNA II is an ideal implant for unstable intertrochanteric fracture since stable intramedullary fixation can be achieved with almost 100% union in the studied population.

Keywords: Intertrochanteric fractures, PFNA2, Proximal femoral nailing

Introduction

Intertrochanteric fractures occur mostly in elderly patients, and the outcome may be extremely poor if there is prolonged bed-rest. The best treatment for trochanteric fracture remains controversial.[1-3] Many methods have been recommended.[4-6] Stable fixation that allows early mobilization is the treatment of choice. Cephalomedullary devices are favored as the treatment for unstable A2 and A3 fractures, especially in the absence of medial buttressing.[2,3,6,7] While there are numerous operative devices for treatment of trochanteric fractures, none of them are totally free of complications. There is no advantage to an intramedullary nail versus a sliding compression hip screw for low-energy pertrochanteric fractures classified by arbeitsgemeinschaftfür osteosynthesefragen/orthopaedic Trauma Association (AO/OTA) 31-A1 and A2, specifically given its increased cost and the lack of evidence to show decreased complications or improved patient outcome.[4]

Proximal femoral nail antirotation (PFNA II) has excellent fit as the design is adapted to the anatomical situation of small statured patients also. It has a medial lateral angle of 5 degrees. It has several distal locking options viz static/dynamic.

Insertion of the blade compacts the cancellous bone. The inserted PFNA blade achieves an excellent fit through

bone compaction and requires less bone removal compared to a screw.[6,7]

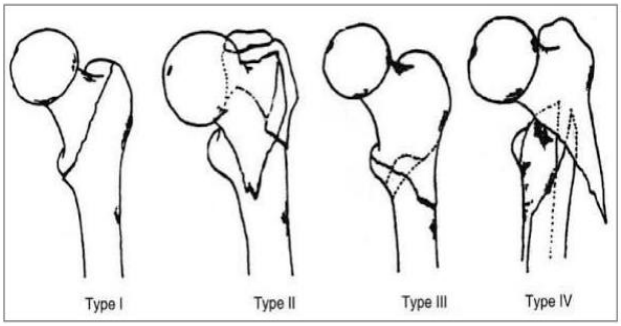
Boyd and Griffin (1949) classified fractures in the trochanteric area of femur into 4 types. Their classification is useful in planning treatment and estimating prognosis.[8,13]

Type 1 fractures that extend along the intertrochanteric line from the greater to the lesser trochanter. Reduction usually is simple and is maintained with little difficulty. Results generally are satisfactory.

Type 2 comminuted fractures, the main fracture being along the line, but with multiple fractures in the cortex. Reduction of these fractures is more difficult because the comminution can vary from slight to extreme.

Type 3 fractures that are basically subtrochanteric with at least one fracture passing across the proximal end of the shaft just distal to or at the lesser trochanter.

Type 4 fractures of the trochanteric region and the proximal shaft, with fracture in at least two planes, one of which usually is the sagittal plane and may be difficult to see on routine anteroposterior radiographs. If open reduction and internal fixation are used, two plane fixations are required because of the spiral, oblique, or butterfly fracture of the shaft.



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□□□□□	□□r □□□□□□□□r □□□
1□	2.5
2□	2
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□□	3
6□	1.5
□□	3
□□	2
□□	1.5
10□	1.5
11□	3
12□	2
1□□	2
1□□	1
1□□	1.5
16□	1
1□□	2
1□□	2.5
1□□	1.5
20□	3
21□	2.5
22□	1.5
2□□	2.5
2□□	2
2□□	1.5

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1□	480
2□	370
□□	400
□□	360
□□	520
6□	320
□□	550
□□	360
□□	450
10□	310
11□	360
12□	370
1□□	390
1□□	420
1□□	400
16□	380
1□□	360
1□□	310
1□□	360
20□	450
21□	330
22□	390
2□□	440
2□□	320
2□□	350

The mean duration was 1.98 hours (SD±0.66). The median duration was 2 with interquartile range from 1.5 to 2.5 hours. Mean blood loss was estimated to be 390 ml (SD±62.78). The median blood loss was 370 ml with interquartile range from 360 to 420 ml.

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PFNA II is a newer device used in fixation of intertrochanteric fracture. Previously, most surgeons used the dynamic hip screw as the choice of implant for intertrochanteric fracture. In this study the advantages of the device have been studied by means of operating time, blood loss and fracture union. Among the cases studied the mean blood loss was found to be very low when compared with that of dynamic hip screw (DHS) as quoted in the few studies. The operating time has been found to be reduced (statistically significant $p<0.001$) when this is compared with that of DHS.[10-14]

The blood loss in our study was lesser in this study when compared to most other studies that used DHS.[12-16] The difference in blood loss was statistically significant ($p<0.001$) with 95% C.I. Since this method of nail insertion uses small incisions and less soft tissue dissection, the mean blood loss is very less.

The simple operating steps reduces the duration of the surgery when compared to that of the DHS. The mean duration of surgery was less than 2 hours. This difference is statistically significant ($p<0.001$).[18-24] There was almost 100% union noted in this study than the previous studies, hence there is no worry for the operating surgeon about the fracture union.[13,27-31]

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The PFNA II is an ideal implant for unstable intertrochanteric fracture since stable intramedullary fixation can be achieved with almost 100% union in the studied population. The PFNA II was easy and quicker procedure with minimally invasive technique and minimal blood loss when compared with previous studies that used the dynamic hip screws.

Limitations

Small sample size and short duration of follow up.

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Ethical approval: The study was approved by the institutional ethics committee

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1. Babhulkar S. Management of trochanteric fractures. Indian J Orthopaedics. 2006;40(4):210.
2. Rockwood C, Green D, Bucholz R. Rockwood and Green' fractures in adults, Philadelphia: Lippincott Williams and Wilkins; 2006.

3. Singh A, Thong G, Laloo N, Singh A, Singh S. Management of trochanteric fractures. Indian J Orthopaedics. 2006;40(2):100.
4. Cooper A. Fractures and Dislocations of Joints.10th ed. London: 1839.
5. Bick E. Source Book of Orthopaedics. New York: Hafner; 1968.
6. Moore H. Treatment of intertrochanteric fractures of femur. JBJS. 1983;65:262-7.
7. Schumpelick W, Jantzen OM. A new principle in orthopaedic treatment of trochanteric fractures of femur. J Bone Joint Surg Am. 1955;37(4):693-8.
8. Whitmann R. A new method of treatment for fractures of the neck of the femur, together with remarks on coxavara. Ann Surg. 1902;36:746.
9. Massie WK. Extracapsular fractures of the hip treated by impaction using a sliding nail - plate fixation. Clin Orthop. 1962;22:180-202.
10. Jewet. One-piece angle nail for trochanteric fractures. JBJS. 1941;23:803.
11. Capener N. The treatment of pertrochanteric fractures. JBJS Br. 1957;39(3):436-7.
12. Taylor MN, Janzen J. Internal fixation for intertrochanteric fractures. JBJS Am. 1944;26(4):707-12.
13. Boyd HB, Griffin LL. Classification and treatment of trochanteric fractures. Ach Surg. 1949;58:853-66.
14. Boyd H, Anderson LD. Management of unstable trochanteric fractures. Surg Gynecol Obstrtrics. 1961;55:853-63.
15. Bendo JA, Weiner LS, Strauss E. Collapse of interochanteric hip fractures fixed with sliding screws. Orthop Rev. 1944;30-7.
16. Dimon JHJC. Unstable intertrochanteric fravtures. JBJS Am. 1967;49(3):440-50.
17. Harrington KJ. The management of communitaed unstable intertrochanteric fracture. JBJS Am. 1973;55(7):1367-76.
18. Sarmiento A. Intertrochanteric fractures of femur. JBJS. 1995;75(3):90-2.
19. Ara D, Newman NP. Unstable intertrochanteric fracture of femur. JBJS Br. 1993;75(3):445-7.
20. Gargan MGR, Simpson A. How effective are osteotomies for unstable intertrochanteric fractures. JBJS BR. 1994;76(5):789-92.
21. Parker MJ, Hando HH, Bhargara A. Conservative versus Operative treatment for hip fractures.
22. The use of ender's pin in extra capsular fractures of hip. JBJS. 1981;63;14-28.
23. Kosygan KP. The gotfried percutaneous compression plate compared with conventional classical hip screw fixation of intertrochanteric fractures of hip. JBJS. 2002;84(1):19-22.
24. Gotfried Y. Percutaneous compression plating of pertrochanteric hip fractures. J Orthop Trauma. 2000;14(7):490-5.
25. Medoff RM. A new device for fixation of unstable pertrochanteric fracture of the hip. JBJS Am. 1911;73(8);1192-9.
26. Lunsjo K, Ceder L, Stigsson L, Hauggaard A. One-way compression along the femoral shaft with the Medoff sliding plate: the first European experience of 104 intertrochanteric fractures with a 1 year follow-up. Acta Orthop Scand. 1995;66:343-6.
27. Watson JM. Comparison of the compression hip screw with the med off sliding plate for intertrochanteric fractures. Clin Orthop. 1998;(348):79-86.
28. Janzing HM. Percutaneous compression plate verses DHS in treatment of pertrochanteric hip fracture. J Trauma. 2002;52:293.
29. Hardy DC. Sloted ontramedullary hip screw nail reduces proximal mechanical unloading. Climorthprelat Res. 2003;406:176.
30. Karn NK, Singh GK, Singh MP. Comparison between external fixation and sliding hip screw in the management of trochanteric fracture of femur. JBJS Br. 2006;88:1347-50.
31. Yechielgotfried. Integrity of the lateral femoral wall in intertrochanteric hip fracture an important predictor of reoperation. IBJS. 2007;89:2552-3.

Kumar ASM, Kumar AB. Proximal femoral nail antirotation II in fixation of intertrochanteric fractures: retrospective and prospective observational study. Int J Res Orthop 2020;6:593-6.

International Journal of Research in Orthopaedics
Kumar NV et al. Int J Res Orthop. 2017 May;3(3):350-353
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Platelet rich plasma in treatment of plantar fascitis

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ABSTRACT

Background: Platelet rich plasma (PRP) has been a breakthrough in the field of medicine especially in the field of orthopaedics for enhancing bone and soft tissue healing. Plantar Fasciitis is a very common problem in the field of orthopaedics and are very difficult to treat and a challenge to orthopaedicians. The aim of our study was to find out the efficacy of autologous PRP in relieving pain in patients with plantar fasciitis.
Methods: In this study, 25 patients with plantar fasciitis (age above 18 years) were selected. The pain intensity was assessed with visual analogue score initially and during follow up. All subjects were given single autologous intralesional PRP injection and the results were assessed using difference in VAS.
Results: The mean pain score at presentation was 8.08. The mean pain scores at subsequent intervals of 1,2,4,6 months after injection were 4.32, 2.60, 1.88 & 2.00 respectively. ($P<0.05$ which is statistically significant). The maximum pain relief was seen at 4th month post PRP injection and the pain relief was sustained till the last follow up.
Conclusions: From our study we have found that intralesional autologous PRP is effective in relieving pain in patients with plantar fasciitis. PRP is biological and can be a safe adjuvant in the treatment of Plantar Fasciitis.

Keywords: Platelet rich plasma, Plantar fascitis, Visual analogue score

INTRODUCTION

Platelet rich plasma (PRP) has been a breakthrough in the field of medicine especially in the field of orthopaedics for enhancing bone and soft tissue healing. Plantar Fasciitis is a very common problem in the field of orthopaedics and are very difficult to treat and a challenge to orthopaedicians. Autologous PRP is a simple and cost effective means of treating plantar fasciitis by tissue regeneration.[1]

Plantar fasciitis is one of the most common causes of heel pain in adults. The cause of pain is due to collagen degeneration at the origin of plantar fascia. The plantar fascia is thickened fibrous fascia that originates from the medial tubercle of calcaneum and run forward to support the longitudinal foot arch.

The aim of our study was to find out the efficacy of autologous PRP in relieving pain in patients with plantar fasciitis.

METHODS

This is a prospective trial involving the patients in the department of orthopedics, PSGIMSR Coimbatore, Tamil Nadu, India from Jan 2015 to Jan 2016.

In this study, 25 patients with plantar fasciitis (age above 18 years) were selected. The pain intensity was assessed

with visual analogue score initially and during follow up. All subjects were given single autologous intralesional PRP injection and the results were assessed using difference in VAS.

Selection of patients

Patients with history of pain and localized tenderness on the medial calcaneal tubercle.[2] The classic sign is pain that occurs with few steps in the morning or at the start of walking which decreases as they warm up.[2]

Inclusion criteria

Patients clinically diagnosed to have plantar fasciitis, patients not responding to conservative treatment even after a minimum period of 3 months, both males and females of age 18 years and above.

Exclusion criteria

Less than 3 month duration of plantar fasciitis, patients without any trial of conservative treatment, recent local steroid injection (less than 2 months), infection or ulcer at the injection site, rheumatoid arthritis, seronegative spondyloarthritis, pregnant ladies, patients younger than 18 years, suspicion of diagnosis.

Methodology



Figure 1: Study design.

Method of preparation of PRP

PRP was prepared using double spin centrifugation method.

10 ml of venous blood is drawn from cubital vein. The blood is transferred into 2.7ml vaccutainer prefilled with acid citrate dextrose. Acid citrate dextrose will bind to calcium and prevents blood clotting with no known interference to platelet function and no contraindications for using in human beings. The blood is then transferred in the vaccutainer till the marking and centrifugation was done. Initially, with 1500 RPM for 3 minutes. After the initial rotations, blood was separated into plasma and RBC. The plasma will lie in the top with light yellow colour and was withdrawn and transferred into another container without mixing RBC. Later this container is again centrifuged with 2500 rpm FOR 3 MINUTES. After the 2nd centrifugation, the plasma is separated into 2 layers. The top half is platelet poor plasma and bottom half will be platelet rich plasma. The top half is discarded and bottom half is used for infiltration.

Technique of infiltration

Using a 21 Gauge, 1 and 1/2 inch needle, 1ml PRP is injected initially over the maximum tenderness point, and another 1 ml, was injected into the surrounding tissue with the same entry portal. This technique is known as peppering technique.

Pain scoring system

Visual analogue pain scoring system was used (Figure 2).

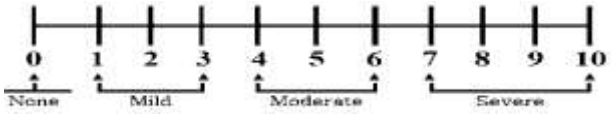


Figure 2: Visual analogue scoring system for assessment of pain.

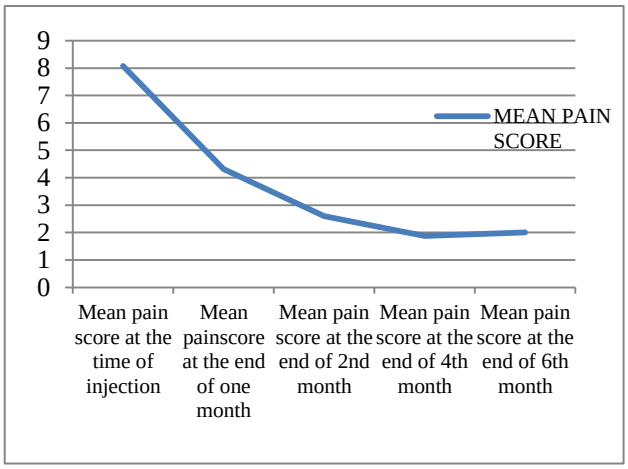


Figure 3: Mean pain score.

RESULTS

Patients were analyzed for pain relief subjectively at 0, 1, 2, 4 and 6 months. The results are given below.

Mean pain score

The mean pain score of patients with plantar fasciitis at 0, 1,2,4,6 months were 8.08, 4.32, 2.60, 1.88 & 2.00 respectively. While comparing the results at 0,1,2,4,6 months follow up, it was found that patients started getting pain relief at one month post injection ,which was statistically significant (p value <0.05). However the maximum pain relief was seen at 4th month post injection which was also statistically significant (p value <0.05).

Table 1: Showing mean pain scores at 0, 1, 2, 4 and 6 months intervals.

Patients receiving	Mean pain score at the time of injection	Mean pain score after	Mean pain score after	Mean pain score after	Mean pain score after
PRP		1 month	2 months	4 months	6 months
Plantar Fasciitis	8.08	4.32	2.6	1.88	2

Table 2: Showing Mean, T-Test and P-values at 0, 1, 2, 4 and 6 months intervals.

Pairs	Time interval	Mean	T-Test	P- Value
Pair 1	Pain at presentation & 1 month	4.32 ± 0.802	16.660; CI (3.29-4.22)	<0.00
Pair 2	Pain at presentation & 2 month	2.60 ± 0.645	33.309; CI (5.1-5.8)	<0.00
Pair 3	Pain at presentation & 4 month	1.88 ± 0.881	26.847; CI (5.72-6.67)	<0.00
Pair 4	Pain at presentation & 6 month	2.00 ± 0.957	24.877; CI (5.57-6.58)	<0.00

Statistical analysis

Statistical analyses were performed by SPSS for windows 19.0 software system version. Comparing the results at 0, 1, 2, 4, 6 months. P value for the test was taken as 0.05. Our study shows that the pain relief obtained following autologous PRP injection is statistically significant.

DISCUSSION

Platelet rich plasma (PRP) is also known as platelet rich concentrate, autologous platelet gel or platelet releasate. Platelet rich plasma is a portion of the plasma fraction of autologous blood with a concentration of platelets above the base line values.[3] In addition to platelet concentrate, autologous PRP contains complement of clotting factors and secretary proteins.[4] Normal platelet counts ranges between 150000/µL and 350000/µL, and average about 200000/µL in blood.[5] "PRP was introduced by M. Ferrari in 1987 as an autologous component in an open heart surgery to avoid homologous blood product transfusion".[6]

Platelets contain biologically active substance for blood clotting, such as coagulation factors, adhesive proteins and protease inhibitors. Platelets contain growth factors like TGF –beta 1, CGF, VEGF, and PDGF.[7,8] When activated these growth factors initiates the process of tissue healing by cellular proliferation and differentiation, chemo taxis, tissue debris removal, angiogenesis, and extra cellular matrix formation.[9,10] These properties of autologous PRP are used in the treatment of plantar fasciitis. An added advantage of autologous PRP is that it eliminates concerns about immunogenic reactions and disease transmission.[11]

Our study was a prospective study including 25 patients with plantar fasciitis (age above 18 years). The pain intensity was assessed with visual analogue score initially and during follow up. PRP was prepared by double spin centrifugation method and the platelet count in our samples ranged between three to six lakhs per cc. All subjects were given single intra-lesional PRP injection and the results were assessed using difference in VAS. In our study we did not use local anaesthetic agent which could bias our study by producing pain relief.

The results of our study matched with the results of the study by Ehab Mohamed Selem Ragab et al on 25 patients with plantar fasciitis, they concluded that average pain at the time of presentation was 9.1 and post autologous PRP injection was 1.6 at end of follow up (10.3 months).[1] Which was statistically significant (P <0.001).

In a comparative study by Ertugrul Aksahin et al they concluded that both PRP and corticosteroid treatment is equally effective in treating plantar fasciitis.[2]

The mean pain score at presentation was 8.08. The mean pain scores at subsequent intervals of 1,2,4,6 months after injection were 4.32, 2.60, 1.88 and 2.00 respectively. (P <0.05 which is statistically significant). The maximum pain relief was seen at 4th month post PRP injection and the pain relief was sustained till the last follow up.

The limitations of our study were that our study is not a randomized control double-blinded study, and the sample size was small and follow up was of shorter duration. The available data is insufficient to validate the efficacy of PRP in relieving pain in patients with Plantar Fasciitis. Further randomized control studies with large study population and the longer duration of follow up are needed to validate the efficacy of autologous PRP in the management of plantar fasciitis.

CONCLUSION

From our study we have found that intralesional autologous PRP is effective in relieving pain in patients with plantar fasciitis. PRP is biological and can be a safe adjuvant in the treatment of Plantar Fasciitis.

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Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Ragab EMS, Othman AMA. Platelet rich plasma for treatment of chronic plantar fasciitis. Arch Orthop Trauma Surg. 2012;132:1065-70.
2. Aksahin E, Dogruyol D, Yuksel HY, Hapa O, Dogan O, Celebi L, et al. The comparison of the effects of corticosteroids and platelet rich plasma for treatment of plantar fasciitis. Arch Orthop Trauma Surg. 2012;132:781-5.
3. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85:638-46.
4. Mehtra S, Watson JT. Platelet rich concentrate: basic science and current clinical applications. J Orthop Trauma. 2008;22(6):433-43.
5. Robert E Marx: platelet rich plasma what is prp and what is not PRP. Implant Dentistry. 2001;10(4):225-8.
6. Ferrari M, Zia S, Valbonesi M, Henriquet F, Venere G, Spagnolo S, et al. A new technique for hemodilution, preparation of autologous platelet-rich plasma and intraoperative blood salvage in cardiac surgery. Int J Artif Organs. 1987;10(1):47-50.
7. Maynard DM, Heijnen HF, Horne MK, White JG, Gahl WA. Proteomic analysis of platelet alpha-granules using mass spectrometry. J Thromb Haemost. 2007;5(9):1945-55.
8. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med. 2009;37(11):2259-72.
9. Sanchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? : a current review. Int J Oral Maxillofac Implants. 2003;18:93-103.
10. Einhorn TA. The science of fracture healing. J Orthop Trauma. 2005;19(10):4-6.
11. Alsousou J, Thompson M, Hulley P, Noble A, Willet K. The biology of platelet rich plasma and its application in trauma and orthopaedic surgery. J Bone Joint Surg Br. 2009;91(8):987-96.

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Functional outcome of Tibial condyle fractures treated by minimally invasive plate osteosynthesis

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Abstract
Introduction: Tibial plateau fractures with intra-articular extension are very difficult to manage. Age, skin conditions, compartment syndrome and osteoporosis further increase the obstacles in the healing process.
Complex biomechanics of its weight bearing position and complex ligamentous stability and articular congruency are the main reason why these fractures are of concern to surgeon.
In our study we have evaluated the functional outcome of locking plate fixation of tibial condyle fractures using minimally invasive technique, a minimum period of 6 months after plate fixation by Rasmussen score and Knee society score.
Materials and Methods: Tibial plateau fractures treated by minimally invasive plate osteosynthesis using locking compression plate from January 2010 to January 2015 were taken into the study. All the patients who had completed the inclusion criteria were called for assessment of functional outcome of knee using Rasmussen score and knee society score.
Results and Discussion: In our study, we were able to get away with unilateral plating for type V and type VI fractures without any late complications like loss of reduction and malalignment.
Choice of the procedure/implant should be based on the fracture pattern, bone quality and intraoperative reduction.
Conclusion: Treatment of intraarticular tibial plateau fractures is still unsolved.
Our results in minimally invasive percutaneous plate osteosynthesis (MIPPO) technique is in par with the literature.

Keywords: Tibial fracture, Schatzker, osteosynthesis, implants

Introduction
The knee joint is complex and most commonly injured joint now because of increased motor vehicle accidents and sports related injuries. As it is a superficial joint, it is more exposed to external forces and gets easily injured. Tibial plateau fractures with intra-articular extension are very difficult to manage. Age, skin conditions, compartment syndrome and osteoporosis further increase the obstacles in the healing process.
Complex biomechanics of its weight bearing position and complex ligamentous stability and articular congruency are the main reason why these fractures are of concern to surgeon. The ideal treatment of high-energy tibial plateau fractures is controversial. Open reduction and stable internal fixation helps in maintaining the articular surface and restoration of the mechanical alignment which allows early mobilization of knee. But, techniques of open reduction and internal fixation compromise the soft tissues and the rate of wound infection is relatively high.
Various other methods of treatment like hybrid fixation and now plate fixation using minimally invasive technique have been suggested. Each method has its own advantage and disadvantages.The development of locking implants has allowed the use of minimally invasive technique for unilateral plating [37-39] with improvement in handling the soft tissue [40-43].
There are lot of studies which assess the general outcome of these fractures but there are only few studies which assess the functional outcome of these fractures which is more important to the patient. In our study we have evaluated the functional outcome of locking plate fixation of tibial condyle fractures using minimally invasive technique, a minimum period of 6 months after plate fixation by Rasmussen score and Knee society score.

Materials and methods

Tibial plateau fractures treated by minimally invasive plate osteosynthesis using locking compression plate from January 2010 to January 2015 were taken into the study.

Inclusion criteria

Age group: 18 years to 60 years all tibial condyle fracture treated by minimally invasive plate osteosynthesis.

Exclusion criteria

1) Skeletally immature patients,
2) Neurovascular injuries,
3) Concomitant lowerlimb fractures like patella, femur, ankle and pelvic fractures.
4) Open fractures

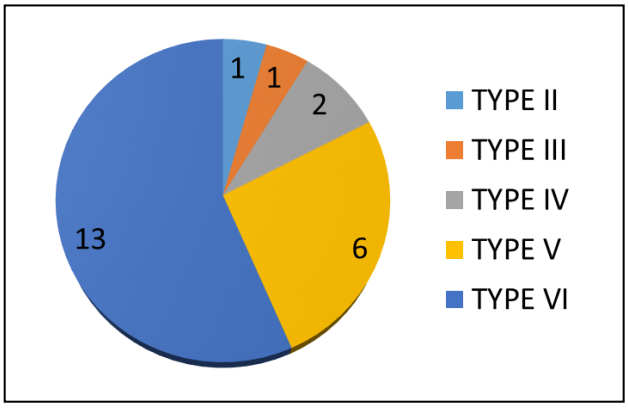
Methodology

This was both retrospective as well as a prospective study. For the retrospective study in-patients and out-patient records of the study population were collected from the medical records department and OT register. Age, gender of the patients, mode of injury, side of involvement, Associated injuries and medical comorbidities were documented. The Neurovascular status of the affected leg, compartment syndrome and any blisters or open wounds was noted. All Tibial condyles fractures were graded preoperatively using Schatzker classification. Patients were followed up postoperatively after a minimum period of six months after surgery. All the patients who had completed the inclusion criteria were called for assessment of functional outcome of knee using Rasmussen score and knee society score.

Results

n our study, 13 patients belonged to type VI, 6 patients belonged to type V, 2 patients belonged to type IV, 1 patient belonged to type III and type II respectively.

Total Number of Patients



Thirteen patients had right tibial plateau fractures and remaining ten patients had left tibial plateau fractures. Only fractures which were treated with MIPPO technique were taken for the study. RTA was the mode of injury in all the cases. In our study type VI Schatzker fractures was the commonest type. Functional assessment was assessed by Rasmussen score (subjective score) and knee society score (objective score). Average follow up period was 22.08 months.

Mean Follow Up

SL.NO	TYPE	AVERAGE FOLLOW UP (MONTHS)
1.	I	-
2.	II	17 (6-17)
3	III	18 (6-18)
4.	IV	32.5 (6-47)
5.	V	19.8 (6-27)
6.	VI	23.1 (6-53)

In type VI Schatzker there were thirteen patients of whom seven patients were treated by lateral plating and six patients were treated by medial plating. Mean Rasmussen score of type VI fractures was found to be 25.6, average knee society score was found to be 93.2 and mean range of knee flexion was found to be 118.8 degrees. All the patients were started on partial weight bearing from 8 weeks and full weight bearing by 12 weeks.

Average Scores by Knee Society and Rasmussen Score

Sl. No	Type	Knee Society Score	Rasmussen Score
1	1	-	-
2	2	98	27
3	3	96	29
4	4	94	26.4
5	5	81.3	22.8
6	6	93.2	25.6

Six patients belonged to type V Schatzker group. Mean follow up was 19.8 months (6-27). Lateral plating was done for 2 patients and medial plating was done for 4 patients. Average Rasmussen score was 22.8, mean knee society score was 81.3 and average range of knee flexion was found to be 118.3 degrees. Only one patient had flexion contracture of 10 degrees. All the patients were started on partial weight bearing at 8 weeks and full weight bearing was started at 12th week. Postoperatively there were no wound infections.

In type IV Schatzker fractures there were two patients. Mean follow up was found to be 32.5 months (6-47). All patients were treated by medial plating. Average range of flexion was found to be 122.5 degrees. Mean Rasmussen score was found to be 26.5. Mean knee society score was found to be 94. There was no wound infection and weight bearing was started at 12th week. In our study only one patient belonged to type III Schatzker fracture. Follow up was done at 18 months (6-18). He was treated by lateral plating and had Rasmussen score of 29. Range of flexion was found to be 120 degrees. Knee society score was found to be 96. No complications were noted.

In type II Schatzker there was only one case which met the criteria. Lateral plating was done. Follow up was done at 17 months (6-17). Rasmussen score was found to be 27, knee society score was 98 and range of knee motion was 125 degrees. No complications were noted. No bone grafts used in our study.

Discussion

Intraarticular tibial plateau fractures are complex fractures accounting for about 1.2% of all fractures. They affect knee function and stability which results in considerable morbidity. These fractures are caused by high velocity injuries and often associated with severe comminution and soft-tissue damage. The goals of treatment are to restore joint congruity, limb alignment and early mobilisation of joint. Stable internal plate fixation without damaging the soft-tissue envelope is very difficult to achieve, only fair results are seen in 20% to 50% in these fractures. Outcome after tibial plateau fracture fixation depends mainly on knee range of motion and strength of the quadriceps. Knee stiffness is more clinically relevant than instability in these fractures.

Open reduction and internal fixation (ORIF) with plates and screws enables direct fracture visualisation, reduction, and fixation, but there is high risk of soft tissue injury, stiffness and deep infection. The hybrid external fixator avoids soft tissue problems, but risks non-union, pin tract infections and poor patient compliance.

The concept of preserving the blood supply and atraumatic surgical technique led to the development of biological fixation techniques. Using this technique, soft tissue damage is reduced and shows higher union rate.

The development of locking implants has allowed the use of minimally invasive technique for unilateral plating [37-39] with improvement in handling the soft tissue [41-43].

Laterally placed locking plates provide better stability in the presence of complex proximal 1/3rd tibia fracture with metaphyseal comminution and serves as an alternative to medial plate or external fixator for additional support of the medial column when a non-locking plate is used for bicondylar fractures. This plate allows fixation through single incision which avoids wound dehiscence, infection and prolonged immobilisation associated with extensile approaches.

MIPPO enables indirect fracture reduction and percutaneous sub muscular implant placement. Favourable outcome is not due to MIPPO but due to less extensive dissection of soft-tissue envelope and devitalisation of fracture fragments.

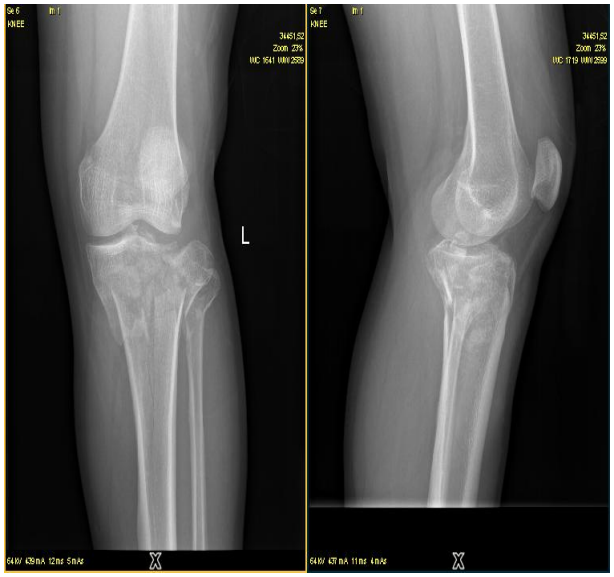
The aim of our study was to evaluate the functional outcome of tibial condyle fractures treated by minimally invasive percutaneous plate osteosynthesis. There is no universal scoring system for assessing the functional outcome for these fractures. Literature shows multiple scoring system like Rasmussen, knee society score and oxford knee score. In our study, we have evaluated the patients using Rasmussen score which is subjective score and knee society score which is an objective score. All these fractures were treated by a single plate either medial or lateral (11 lateral and 12 medial). In case of type V and type VI fractures, if needed the opposite condyle was fixed with percutaneous cancellous screws.

Mechanism of injury was road traffic accident for all these patients. The fractures were classified by Schatzker's classification. 13 patients belonged to type VI, 6 patients belonged to type V, 2 patients belonged to type IV, 1 patient belonged to type III and type II respectively. Even though, according to literature type II fractures were the most common, only one patient with type II fracture was included in our study as all other type II & type I fractures in the study period were fixed only by percutaneous screws hence excluded from the study. Type VI fracture with 13 patients (56.5%) were the most common in our study, next was type V (26.08) with 6 patients. Average followup period was 22 months (6-53). Hasnain Raza *et al*, in their study of assessing the functional outcome of tibial condyle fractures of 41 patients by minimally invasive plate osteosynthesis by Rasmussen functional score found excellent results in 18 patients, good in 19 patients and 4 patients had unacceptable results. The mean Rasmussen score was found to be 25.3 and range of knee flexion was 118 degrees. In our study mean Rasmussen score was found to be 26.1 and average range of knee flexion was found to be 120.9 degrees. Of which ten patients had excellent and good results each. Only 3 patients had fair results. This is comparable to the study done by Hasnain Raza. Mohammad Ali Tahririan, Seyyed Hamid Mousavitadi, and Mohsen Derakhshan [13] in their clinical study comparing the functional outcomes of tibial plateau fractures treated with nonlocking and locking plate fixation by knee society score, found a score of 80.2 for locking plate and 72.5 for non-locking plate. Average range of knee flexion was found to be 122.3 degrees for locking plate and 115.7 degrees for non-locking plate. In our study, locking plate was used for all the cases. Average knee society score was found to be 92.5 and average knee flexion was found to be 120.9 degrees. So, functional outcome in our study was marginally better than the locking plate group in that study and significantly better than the non-locking group. This shows the superiority of the locking plate in view of stable fixation and early range of motion when compared to non-locking plate. Chang-Wug Oh *et al* [18] in their study on double plating of (twenty three) type V and type VI proximal tibial fractures using minimally invasive percutaneous osteosynthesis found Eighteen patients with excellent, three patients with good and two patients with fair results. Average Rasmussen score was found to be 26 and average knee range of motion was found to be 123 degrees. In our study, nineteen patients belonged to type V and VI fractures. Average Rasmussen score of these type V and type VI fractures were found to be 24.2. Average range of flexion achieved by type V and type VI was found to be 118.5 degrees. Seven patients had excellent, ten patients with good and two patients had poor results. In their study of ten patients Kye-Youl Cho *et al* [5], used a single midline longitudinal incision and dual plating for the treatment of type V and type VI schatzker fractures. Their mean knee society score for the study group was 85 and the mean range of motion was 125 degrees. They had only one case with delayed wound healing as postoperative complication. In our study, average knee society score was found to be 87.25. One patient had 10 degrees of valgus malalignment in type VI fracture and one patient had flexion contracture of 5-10 degrees. But the functional outcome was not significantly altered when compared with others.

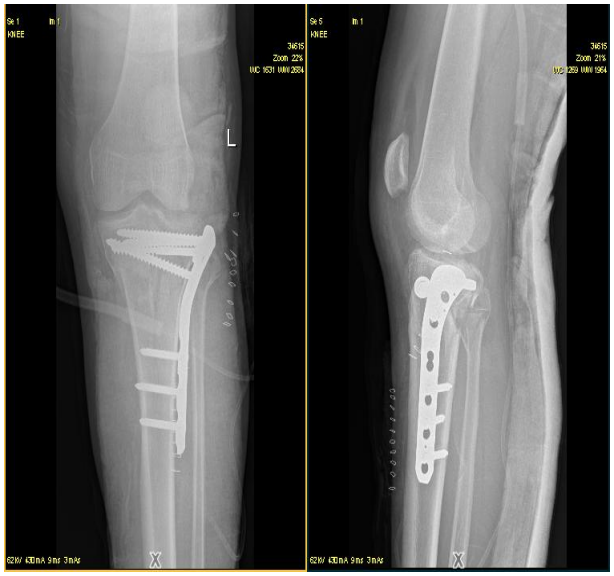
Dual plate gives better biomechanical strength and rigid construct thereby better control of both columns thus avoiding late collapse. There were no major wound problems in any of these studies. Weight bearing was started only at 8-12 weeks which was similar to our study. In our study, we were able to get away with unilateral plating for type V and type VI fractures without any late complications like loss of reduction and malalignment.

Choice of the procedure/implant should be based on the fracture pattern, bone quality and intraoperative reduction.

CASE 1:
Preop Xray



Immediate Postop Xray



After 6 Months



Clinical Picture



Conclusion

Treatment of intraarticular tibial plateau fractures is still unsolved. Our results in minimally invasive percutaneous plate osteosynthesis (MIPPO) technique is in par with the literature. There is no significant difference in the functional outcome between single plating in our study and dual plating of other studies at midterm followup. Choice of the procedure/implant should be based on the fracture pattern, bone quality and intraoperative fracture reduction.

Reference

1. Douglas R Dirschl, Daniel Del Gaizo. Staged Management of Tibial Plateau Fractures A Supplement to The American Journal of Orthopedics. 2007.
2. Comparison of outcome of unilateral locking plate and dual plating in the treatment of bicondylar tibial plateau fractures Lee *et al*. Journal of Orthopaedic Surgery and Research. 2014; 9:62.
3. Tibial plateau fractures treated with the less invasive stabilisation system. Jackson A. Lee & Stamatios A. Papadakis & Charles Moon & Charalampos G. Zalavras International Orthopaedics (SICOT) 2007; 31:415-418.
4. New trends and techniques in open reduction and internal fixation of fractures of the tibial plateau. Musah V, Tarkin I, Kobbe P, Tzioupis C, Siska PA, Pape HC. 2009 British Editorial Society of Bone and Joint Surgery 2009. 91-B, 4.
5. Treatment of Schatzker Type V and VI Tibial Plateau Fractures Using a Midline Longitudinal Incision and Dual Plating. Kye-Youl Cho, Hyun-Sup Oh, Jae-Ho Yoo, Duk-Hyun Kim, Young-Joo Cho, Kang-Il Kim. Knee Surg Relat Res, 2013; 25(2).
6. Traditional buttress plating v/s MIPO in management of proximal tibial fractures - A clinical study. Rakesh Sharma, Rajesh Kapila, Brahm Preet Singh, Yadwinder Singh Sohal. Pb Journal of Orthopaedics. 2013, 1.
7. Results of proximal tibial fractures managed with periarticular locking plates: A series of 34 cases. Jain D, Selhi HS, Mahindra P, Kohli S, Yamin M. IJRRMS 2012; 2(4).
8. Apley AG. fractures of the lateral tibia condyle treated by skeletal traction and early mobilization. A review of sixty cases with special reference to the long term result, J Bone Joint Surg 1956; 38-B:699,
9. Schatzker J, McBroom R, Bruce D. The tibial plateau fracture. The Toronto experience 1968-1975. Clin Orthop Relat Res. 1979; 138:94-104.

10. Diass JJ, Stirling AJ, Finlay DBL, Gregg PJ. Computerised axial tomography for tibial plateau fractures, J Bone Joint Surg 1987; 69-B:84.
11. Barei DP, Nork SE, Mils WJ. Functional outcome of severe bicondylar tibial plateau fractures treated with dual incision and medial and lateral plates. J Bone Joint Surg. 2006; 88-A:1713-1721.
12. Tscherne H, Lobenhoffer P. Tibial plateau fractures. Management and expected results. Clin Orthop Relat Res. 1993; 292:87-100.
13. Clinical Study Comparison of Functional Outcomes of Tibial Plateau Fractures Treated with Nonlocking and Locking Plate Fixations: A Nonrandomized Clinical Trial. ISRN Orthopedics Volume March Article ID324573 Mohammad Ali Tahririan, Seyyed Hamid Mousavitadi, Mohsen Derakhshan. 2014,
14. Functional and radiological evaluations of high energy tibial plateau fractures treated with double buttress plate fixation. Yu Z, Zheng L, Zhang Y, Li J, Ma B. Eur J Med Res. 2009; 14:200-205.
15. Recovery of knee function following fracture of the tibial plateau. Gaston P, Will EM, Keating JF. J Bone Joint Surg [Br] 2005; 87-B:1233-6.
16. Albuquerque RP, Hara R, Prado J, Schiavo L, Giordano V, Amaral NP. Epidemiological study on tibial plateau fractures at a level I trauma center. Acta Ortop Bras. [online]. 2013; 21(2):109-15.
17. Comparison of Outcomes of Operatively Treated Bicondylar Tibial Plateau Fractures by External Fixation and Internal Fixation. Malaysian orthopaedic Journal CC Chan, MRCS (Ed), J Keating, FRCS Orth (Ed). 2012; 6(1).
18. Double plating of unstable proximal tibial fractures using minimally invasive percutaneous osteosynthesis technique. Chang-Wug, Jong-Keon, Hee-Soo Kyung. In-Ho Jeon, Byung-Chul Park, Woo-Kie Min *et al*. Acta Orthopaedica. 2006; 77(3):524-530.
19. David Levay. History of Orthopaedics 1990, 625:592,
20. Sir Robert Jones. Orthopaedic surgery of injuries 1921; 1:86,
21. Wilson Wg, Jacobs JE. Patellar graft for severely depressed comminuted fractures of the lateral tibial condyle. J Bone Joint Surg 1952; 34A:436,
22. Rasmussen PS. Tibial condylar fractures. Impairment of knee joint stabilityas an indication for surgical treatment. J Bone Joint Surg Am. 1973; 55:1331-50.
23. Moore TM, Harvey JP, JR.: Roentogenographic measurement of tibial plateau depression due to fractures: J Bone Joint Surg 1974; 56A:155,
24. Drennen DB, Loucher FG, Maylahn DJ. Fractures of the tibial plateau treatment by closed reduction and spica cast, J Bone Joint Surg. 1979; 61-A:989,
25. Bowes DN, Hohl M. Tibial condyle fractures: evaluation of treatment and outcome, Clin Orthop 1982; 171:104.
26. Blokker CP, Rorabeck CH, Bourne RB. Tibial plateau fractures. An analysis of the results of treatment in 60 patients. Clin Orthop. 1984; 182:193,
27. Insall JN, Dorr LD, Scott RD, Scott WN. Rationale of the knee society clinical rating system. Clin Orthop Relat Res. 1989; (248):13-4.
28. Delamarter R, Hohl M. The cast brace and tibial plateau fractures, Clin Orthop 1987; 242:26.
29. Jensen DB, Rude C, Duus B, Bjerg-Nielsen A. Tibial plateau fractures. A comparison of conservative and surgical treatment. J Bone Joint Surg; 1990; 72B:49-52,
30. Honkonen SE, Jarvienen MJ. Classification of fractures of tibial condyles, J Bone Joint Surg 1992; 74B:840,
31. Marsh JL, Smith ST, Do TT. External fixation and limited internal fixation for complex fractures of the tibial plateau. J Bone Joint Surg 1995; 77A:661,
32. Cambell s operative orthopaedics; Fractures of lower extremity: Tibial plateau 3, 2094-2111
33. Weigel DP, Marsh JL. High energy fracture of the tibial plateau: knee function after longer follow-up. J Bone Joint Surg 2002; 84-A:1541-1551,
34. Ali AM, Bruton M, Hashmi M, Saleh M. outcome of complex fractures of the tibial plateau treated with a beam loading ring fixation system. J Bone Joint Surg. 2003; 85B:691-699,
35. Lubowitz JH, Elson WS, Guttmann D. Arthroscopic management of tibial plateau fracture part-I: Arthroscopy. 2004; 20:1063-1070.
36. Duwelius PJ, Connolly JF. Closed reduction of tibial plateau fractures a comparison of functional and roentogenographic and results, Clin Orthop. 1988; 230:116.
37. Farouk O, Krettek C, Miclau T. Minimally invasive plate osteosynthesis: does percutaneous plating disrupt femoral blood supply less than the traditional tech- nique? J Orthop Trauma. 1999; 13:401-6.
38. Farouk O, Krettek C, Miclau T, Schandelmaier P, Tscherne H. Effects of percu- taneous and conventional plating techniques on the blood supply to the femur. Arch Orthop Trauma Surg. 1998; 117:438-41.

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Acetabular erosion after hemiarthroplasty

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ABSTRACT

Background: Neck of femur fractures are one of the devastating injuries in the old age. Their incidence is on the rise causing immense stress on the society. One of the most commonly done procedure is bipolar hemiarthroplasty. In our study we have evaluated the acetabular erosion after hemiarthroplasty, in neck of femur fracture patients after a minimum period of 2 years and have tried to correlate it with activity level of the patient. The aim of the study were early detection of acetabular erosion; to assess the functional outcome after minimum of 2 years after hemiarthroplasty by modified UCLA score; to correlate the functional activity level and radiological acetabular erosion.
Methods: This is a retrospective radiological and clinical study. The post hemiarthroplasty plain radiographs, showing AP view of hip joint taken in the Department of Radiodiagnosis, PSGIMS&R will be studied along with activity level assessment. By Convenient sampling method, all the patients undergone cemented bipolar hemiarthroplasty, for fracture neck of femur after minimum of 2 years were assessed both radiologically and clinically. Functional activity level was assessed by Modified UCLA scoring system.
Results: By this study we suggest an easy and effective way of evaluating acetabular erosion and clinical activity. There is significant increase in acetabular erosion as the duration after surgery increases.
Conclusions: By this study we suggest an easy and effective way of evaluating acetabular erosion and clinical activity. There is significant increase in acetabular erosion as the duration after surgery increases. The clinical activity is by and large not significantly altered as the erosion progress at mid-term follow up.

Keywords: Neck of femur fractures, Bipolar hemiarthroplasty, Acetabular erosion

INTRODUCTION

Neck of femur fractures are one of the devastating injuries in the old age. It is well recognized even from the era of Hippocrates. The accurate number of hip fractures worldwide is impossible to determine, but the global incidence in the year 2000 has been estimated at 1.6 million and the projections for the future suggest further increasing numbers. In addition to the suffering of the individual the economic strain on society due to hip fracture is immense.

Management of displaced intracapsular hip fracture in elderly remains controversial. Options include hemiarthroplasty or total hip arthroplasty. Total hip arthroplasty has shown better clinical outcome and lesser reoperative rate, but in the elderly frail population who often suffer from fracture of the neck of the femur, morbidity rates are high.[1]

Hemiarthroplasty is one of the commonest procedures done for neck of femur fractures. It provides pain relief and early mobilization. The Moore and Thompson prostheses have been successful implants in treating

fracture neck of femur. Disabling pain and acetabular erosions are frequent complications after the use of Moore prosthesis.[1] So in an attempt to retard the acetabular wear, prolong the life of the implant and delay the need for revision surgery the bipolar prosthesis was developed by Bateman in Toronto in 1974, which had the advantage of hip motion occurring at 2 interfaces, primarily at the prosthetic interface and secondarily at the metal cartilage interface, thus minimizing the articular wear.

This prosthesis was found to be very useful and results were encouraging. However in long-term studies show that the bipolar prosthesis starts acting as unipolar prosthesis with time and hence leads to some erosion. However not all patients with acetabular erosions were symptomatic.

In our study we have evaluated the acetabular erosion after hemiarthroplasty, in neck of femur fracture patients after a minimum period of 2 years and have tried to correlate it with activity level of the patient.

Aim and objective

1. Early detection of acetabular erosion.
2. To assess the functional outcome after minimum of 2 years after hemiarthroplasty by modified UCLA score.
3. To correlate the functional activity level and radiological acetabular erosion.

METHODS

Source of data

This is a retrospective radiological and clinical study. The post hemiarthroplasty plain radiographs, showing AP view of hip joint taken in the Department of Radiodiagnosis, PSGIMS&R during the period between June 2015 to May 2016 will be studied along with activity level assessment. A total of 22 patients were evaluated.

Mode of data collection

By convenient sampling method, all the patients undergone cemented bipolar hemiarthroplasty, for fracture neck of femur after minimum of 2 years were assessed both radiologically and clinically.

Inclusion criteria

All patients operated for neck of femur fracture with cemented bipolar hemiarthroplasty after a minimum period of 2 years.

Exclusion criteria

Exclusion criteria were surgical site hip Infection; any preexisting pathologies around the hip; previous hip surgeries; post-operative periprosthetic fractures; neurological conditions like CVA, parkinsonism.

X-ray technique

A plain anteroposterior view of the operated hip joint is taken and assessed for acetabular erosion grading (Figure 1) following the technique of SakrMazen, MD, Girard Julien, MD and FakihRiad, MD. Patient positioned in supine, using digital X-ray, cassette tube distance is set to 100 cms and the beam is centered directly over the hip.

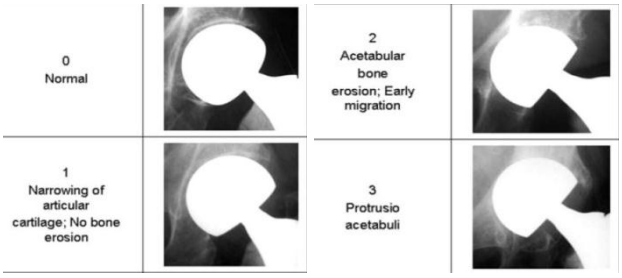


Figure 1: Grades of acetabular erosion.[2]

Activity level assessment[3]

Table 1: Functional activity level was assessed by modified UCLA scoring system.

Modified UCLA scoring system
1. Completely inactive, dependent on other and cannot leave the residence.
2. Mostly inactive or restricted to minimum activities of daily living
3. Sometimes going to social outdoor activities or visiting neighbours.
4. Regularly going to social outdoor activities or visiting neighbours.
5. Sometimes engaging in unlimited house works or shopping.
6. Regularly engaging in unlimited house work or shopping.
7. Regularly using staircase to climb.
8. Lifting or playing with small children.
9. Involving in heavy labour works.
10. Regularly using public transport.
Good --- 7 to 10 Fair-- 4 to 6 Poor--- below 4

Data analysis

Data collected were entered in Excel Spread sheet and analyzed using STATA statistical software package release 11. We used the two-sided independent-samples t test to compare means across dichotomous variables (i.e. men v. women); the one-way ANOVA test for comparison of means across multilevel variables. Simple calculations like Percentages, Proportions and Mean

values were derived. A type I error of 0.05 was considered in all analyses.

RESULTS

Table 2: Total number of participants according to gender.

Gender	Number of participants
Male	12
Female	10

Table 3: Total number of participants according to age.

Age in decades	Number of participants
60-69	9
70-79	11
80-89	1
90+	1

Total number of patient included in this study was 22. Total number of male patients 12, total number of female patients 10 and mean follow up age was 65 years. Right side hip involved was 11 and left side hip was 11. The mean follow up period was 3.54 years. The mean modified UCLA score was 6.22.

Table 4: Mean Modified UCLA score according to gender.

Gender	N	Modified UCLA score
Men	12	6.08±0.9
Women	10	6.4±0.7

The number of patients in Grades 0 and 1 acetabular erosion was 16 (72.70%) and in Grades 2 and 3 were 6 (27.30%).

Table 5: Mean acetabular erosion grade according to side affected.

Hip side	Number of participants
Left	11
Right	11

Table 6: Distribution of patients according to acetabular erosion grades.

Acetabular erosion grades	No. of patients	
Grade 0 and Grade 1	16	72.70
Grade 2 and Grade 3	6	27.30
Total	22	100%

The mean acetabular erosion when correlated with duration since surgery was statistically significant with p<0.001. Grade 0 & 1 has a mean value of 2.81 years and Grade 2 & 3 has a mean value of 5.75 years. This shows there was increased acetabular erosion as the duration post-surgery increases.

Table 7: Distribution of the study participants based on duration since surgery and grades of acetabular erosion.

Duration since surgery	Number of study participants	Erosion	No.	%
<4 years	18	0 and 1	16	88.9
		2 and 3	2	11.1
4 years	4	0 and 1	0	0
		2 and 3	4	100

Table 8: Mean duration since surgery (in months) according to acetabular erosion grades.

Acetabular erosion Grades	N	Duration since surgery
Grade 0 and Grade 1	16	2.81±0.32
Grade 2 and Grade 3	6	5.75±2.36

P<0.001.

The acetabular erosion grade when correlated with modified UCLA score was not clinically significant with p=0.71. In acetabular erosion Grade 0 and 1 the modified UCLA score was 6.33 and in Grade 2 and 3 the modified UCLA score was 6.18. Since the sample size was small the significance could not be correlated statistically. As the age of the patient increases there was a gradual reduction in activity.

Table 9: Mean Acetabular erosion grades according to duration since surgery (in months).

Duration since surgery	N	Acetabular erosion grades
<4 years	18	0.67±0.69
4 years	4	2.25±0.51

P<0.001

Table 10: Distribution of patients according to modified UCLA score.

Modified UCLA score	N	%
5	5	22.73
6	7	31.82
7	10	45.45
Total	22	

Table 11: Mean modified UCLA score according to acetabular erosion grades.

Acetabular erosion grades	Modified UCLA score
Grade 0 and Grade 1	6.33± 0.83
Grade 2 and Grade 3	6.18 ± 0.82

P=0.71(not significant).

Table 12: Mean modified UCLA score according to age in decades.

Age in decades	N	Modified UCLA Score
60-69	9	6.44±0.73
70-79	11	6.18±0.87
80-89	1	6±0
90+	1	5±0

Table 8 and shows description about Mean acetabular erosion grades according to duration since surgery (in months). This is statistically significant with P-value (<0.001) Erosion increases as the duration increases.

DISCUSSION

Incidence of fracture neck of femur is increasing gradually, probably due to increase in life expectancy of individuals.[4] The ideal treatment is still controversial[5]. Two common procedures done for elderly people are hemiarthroplasty and total hip arthroplasty. Some people advocate primary total hip arthroplasty for neck of femur fracture in elderly. Total hip arthroplasty results in more functional activity level and lesser reoperative rates but has more morbidity.

Hemiarthroplasty is more economical especially in developing countries like India and has lesser morbidity, but has a higher reoperation rate when compared to total hip arthroplasty.

The long term complications of bipolar hemiarthroplasty are acetabular erosion, aseptic loosening and infection.[6] Bipolar prosthesis is thought to have lesser incidence of acetabular erosion than unipolar prosthesis due to reduced movement occurring at acetabular prosthesis interface.

In this study we have tried to assess the acetabular erosion by radiological grading and functional outcome by modified UCLA scoring after a minimum period of 2 years after hemiarthroplasty.

Total numbers of patients assessed in this study were 22 and all underwent cemented bipolar hemiarthroplasty. The mean period of follow up was 42 months (range 2 years - 7 years). Six patients (27.30%) had a moderate to severe acetabular erosion and sixteen patients (72.70%) had minimal or no erosion. The grade of erosion was found to increase with follow up interval. With the follow up period of less than 4 years (18 patients) 16 patients (88.9%) had nil or minimal erosion and 2 patients (11.1%) had moderate to severe erosion. With follow up period of more than 4 years 4 patients had moderate to severe erosion. The mean follow up period in our study was 3.54 years and the significant acetabular erosion noted was 27.3% this could be compared with the study of Mazen et al, they had 33% erosion grade at the follow up period of 3 years.[7]

In this study all the patient's functional activity level were assessed by modified UCLA score, the mean modified UCLA score was found to decrease with increase in the age of the patients. Due to small study group the correlation of the modified UCLA score anti acetabular erosion was not statistically significant. One patient who had the longest period of follow up and maximum grade of acetabular erosion had a good level of functional outcome with modified UCLA score of 7. The pain did not restrict her functional activity.

Philips, London in his study found that the factors that had highest correlation were the level of physical activity and duration of follow up.[8]

Moon et al also found in their study that the progression of acetabular erosion correlated directly with the duration of articulation of the prosthesis within the acetabulum and this degeneration of articular cartilage becomes the cause of migration of bipolar cup, which is the major cause of failure of cemented bipolar arthroplasty. They also found the cause of degeneration to be multifactorial and it is accurately difficult to distinguish and measure the contributory level of each factor. They also found that all acetabular cartilage will show degeneration approximately 7 to 8 years after surgery.[9]

In our study all the 4 patients who had a follow up of more than 7 years had significant acetabular erosion. Hence the choice of total hip arthroplasty or a hemiarthroplasty in a fracture neck of femur may be determined by the life expectancy and the activity of the patient.

CONCLUSION

By this study we suggest an easy and effective way of evaluating acetabular erosion and clinical activity. There is significant increase in acetabular erosion as the duration after surgery increases. The clinical activity is by and large not significantly altered as the erosion progress at mid-term follow up.

Limitations of the study

Small study group, short period of follow up.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Sharma V, Awasthi B, Kumar K, Kohli N, Katoch P. Outcome analysis of hemiarthroplasty vs. Total hip replacement in displaced femoral neck fractures in the elderly. J Clin Diagn Res. 2016;10(5):11–3.
2. Mazen S, Julien G, Riad F. Retrospective evaluation of bipolar hip arthroplasty in fractures of the

proximal femur. N Am J Med Sci. 2010;2(9):409-15.
3. Zahiri CA, Schmalzried TP, Szuszczewicz ES, Amstutz HC. Assessing activity in joint replacement patients. J Arthroplasty. 1998;13(8):890-5.
4. Filipov O. Epidemiology and social burden of the femoral neck fractures. J IMAB- Annual Proceeding (Scientific Papers). 2014;20(4).
5. Bhandari M, Devereaux PJ, Tornetta P 3rd, Swiontkowski MF, Berry DJ, Haidukewych G, et al. Operative management of displaced femoral neck fractures in elderly patients: an international survey. J Bone Joint Surg Am. 2005;87(9):2122-30.
6. Haidukewych GJ, Israel TA, Berry DJ. Long-term survivorship of cemented bipolar hemiarthroplasty for fracture of the femoral neck. Clin Orthop Relat Res. 2002;403:118-26.
7. Mazen S, Julien G, Riad F. Retrospective evaluation of bipolar hip arthroplasty in fractures of the proximal femur. N Am J Med Sci. 2010;2(9):409–15.
8. Phillips TW. Thompson hemiarthroplasty and acetabular erosion. J Bone Joint Surg Am. 1989;71(6):913-7.
9. Moon KH, Kang JS, Lee TJ, Lee SH, Choi SW, Won MH. Degeneration of acetabular articular cartilage to bipolar hemiarthroplasty. Yonsei Med J. 2008;49(5):719–24.

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Issues in management of fractures complicated by compartment syndrome

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Abstract
Introduction: Compartment syndrome has been identified as an acute devastating orthopaedic emergency and early fasciotomy is the only way to prevent any complications This retrospective study proposes to analyse the issues in management of fractures complicated by compartment syndrome occurring pre-operatively and post-operatively.
Aim: To study the issues involved in the rationale for deciding the method of fracture stabilization following fasciotomy. To study the problems, complications and functional outcome of fracture stabilization. To analyze and establish methods to the optimally manage the issues involved.
Materials and Methods: A review of patients admitted in the orthopaedic department at our institution who presented with or developed compartment syndrome and associated with fractures were chosen. Passive stretch pain and serve pain out of proportion were the main clinical indicators considered. Decision to perform fasciotomy was carried out based on the differential P value and were treated with double incision fasciotomy Patients were followed up every month for the first 6 months and then every 6 months. All the patients, who were reviewed, were functionally assessed based on Upper Extremity Functional Index (UEFI) and Lower Extremity Functional Scale (LEFS).
Results: A high suspicion of compartment syndrome must be maintained for all cases. The early diagnosis and treatment within 12 hours is critical to reduce morbidity and prevent any long term sequale. Compartment syndrome causes delay in bone healing. Risk of infection is not increased.

Keywords: compartment syndrome, fracture healing, faciotomy

Introduction
Compartment syndrome has been identified as an acute devastating orthopaedic emergency. Till date, compartment syndrome is one of the major complications in an injured limb. And early fasciotomy is the only way to prevent any complications due to compartment syndrome. Delay in diagnosis had been identified as the only cause of failure of treatment.
This retrospective study proposes to analyse the issues in management of fractures complicated by compartment syndrome occurring pre-operatively and post-operatively.

Aim and Objectives
Aim
To retrospectively study the issues in management of fractures complicated by compartment syndrome occurring pre-operatively and post-operatively.

Objectives
- To study the issues involved in the rationale for deciding the method of fracture stabilization following fasciotomy.
- To study the problems, complications and functional outcome of fracture stabilization.
- To analyze and establish methods to the optimally manage the issues involved.

Materials and Methods
- A review of patients admitted in the orthopaedic department at our institution was carried out. Patients who presented with or developed compartment syndrome and associated with

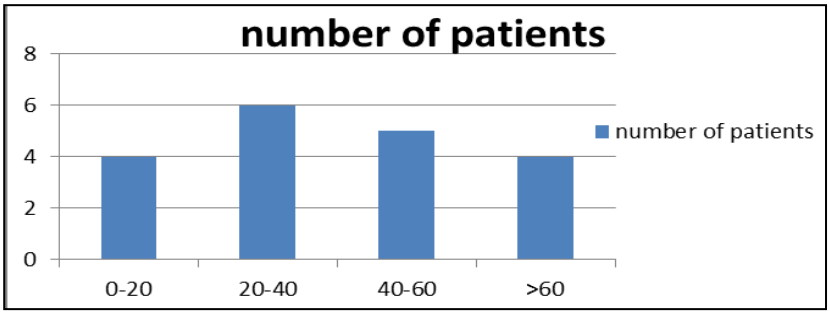
fractures were chosen.

- Compartment syndrome was diagnosed clinically in all the cases, except one. Passive stretch pain and serve pain out of proportion were the main clinical indicators considered. Paraesthesia, paralysis and pulselesness were considered to be supportive of the diagnosis. Comportment pressure was measured in one patient who had a spinal injury. Decision to perform fasciotomy was carried out based on the differential p value. Differential P was calculated by the difference between the patients diastolic pressure and compartment pressure. Fasciotomy is indicated if the value was less than 30mm Hg[24].
- All the patients who had developed compartment syndrome of the leg were treated with double incision fasciotomy, anterolateral and posteromedial. Compartment syndrome of forearm was treated with single volar incision fasciotomy, while that of the thigh also was treated with a single lateral incision.
- The fracture were treated with external, internal or hybrid fixation.
- The fasciotomy wounds were taken up for secondary closure from 4-7 days after fasciotomy with split skin grafting.
- Patients were followed up every month for the first 6 months and then every 6 months. All the patients, who were reviewed, were functionally assessed based on Upper Extremity Functional Index (UEFI) and Lower Extremity Functional Scale (LEFS).
- Painless unprotected weight-bearing and presence of bridging callus on X-ray were together considered as signs of bony union.

Results

There were 21 patients who were diagnosed with compartment syndrome and underwent fasciotomy for the same. But compartment syndrome in 2 of the patients were not associated with fractures and hence excluded from the study.

Incidence of compartment syndrome was found to be more common in physiologically young males, especially in the 3rd and 4th decades of life. This is probably due to the comparatively increased muscle mass in younger individuals.

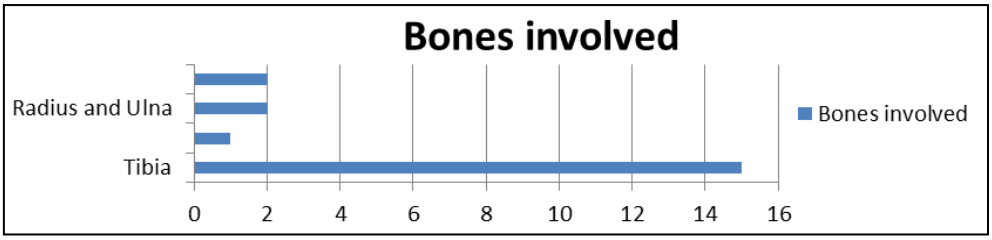


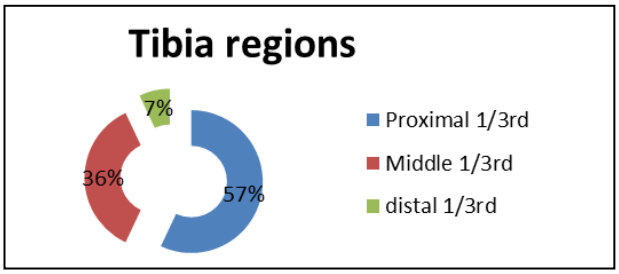
Compartment syndrome is more frequently seen in fractures due to high velocity injuries, mainly in road traffic accidents. (Table 1)

Table 1

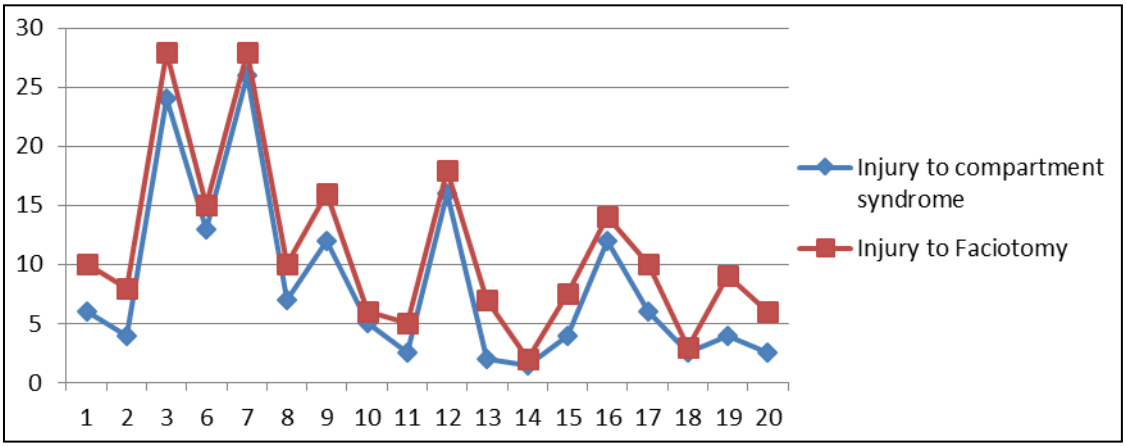
Mode of injury	No. of cases
Road Traffic Accident	16
Fall from height	2
Industrial injury	1
Trivial Fall	1

Fifteen of the fractures involved Tibia. Of the fifteen Tibia fractures, three involved the Tibial plateau and one involved a proximal Tibia epiphyseal injury and the rest involved tibioal diaphysis. Compartment syndrome frequently occurs in Tibia compared to all other sites. Occurance in Proximal one third Tibial is the commonest. (Graph 3, 4)





Of the remaining patients, one was a subtrochanteric fracture of the femur. The patient developed compartment syndrome of thigh probably as a consequence of the renal failure he was suffering from. Two were fractures of both bones forearm. One was a fracture around the elbow involving medial epicondyle and olecranon. One patient had additional injuries along with tibial fracture involving the ipsilateral femur and humerus and also compartment syndrome involving the forearm. One patient (Case 21) had sustained a D12 compression fracture with altered neurology of both lower limbs, along with a tibial plateau fracture (internally fixed in an institution outside). When he presented 10 days later, he had the altered neurology and also passive stretch pain with swelling of the affected leg.



The compartment syndromes were diagnosed by clinical monitoring. The delay in development of compartment syndrome following injury was 8.3 hours (1.5 hours to 26 hours, excluding case 21). The average delay in performing the fasciotomy after injury was 11.3 hours (2.5 hours to 28 hours).

There was delay in 2 of the cases (case 3 and 7) presenting to our institution as they received first aid in an outside institution primarily. Case 21, a 10 day old missed post-operative compartment syndrome, was not considered in the time of delay, because it was a case of established and missed compartment syndrome, on presentation from a different hospital. The patient's intra-compartmental pressure of all the compartments of the leg was measured using an electric arterial pressure monitor by an infusion technique. The differential P of the involved leg was found to be 10mm Hg, indicating the need for fasciotomy. Differential P of the other leg was found to be 40mm Hg.

All patients were clinically monitored for compartment syndrome from time of presentation, and also post-operatively. During the study, two distinct groups of patients were identified.

1. Patients who presented with compartment syndrome following a fracture.
2. Patients who developed compartment syndrome after surgical fixation of a fracture (cases 1, 2, 6, 8, 9 and 14)

Table 2: Grouping of patients with compartment syndrome

Compartment Syndrome Following	No. of Patients
Fracture	12
Surgical fixation of fracture (s0	6

Of the 12 patients who developed compartment syndrome after a fracture, 7 cases which were monitored from admission developed compartment syndrome. And they were taken up for fasciotomy with appropriate fixation.

Of the 18 cases considered, 6 of the fractures (cases 1, 2, 6, 8, 9 and 14), 5 tibial diaphyseal fractures and 1 ulna fracture, treated with intramedullary nailing developed compartment syndrome post-operatively. Pre-operatively, four of the fracture limbs were found to be edematous. All the 6 cases were diagnosed with compartment syndrome post-operatively after intramedullary nailing. The delay in establishing the diagnosis of compartment syndrome following intramedullary nailing was 8.5 hours (4 hours to 13.5 hours), caused probably by reduction of the fracture prior to intramedullary fixation. And the average delay in fasciotomy after intramedullary nailing was 11.8 hours (8hours to 16 hours).

Of the fifteen Tibial fractures, 6 were treated with intramedullary nailing (One-Unreamed nail), 4 were treated with Hybrid fixation, 3 were treated with external fixation and the Proximal Tibia epiphyseal injury (case 20) was treated with cancellous screw fixation. One patient with tibial plateau fracture was treated with hybrid fixator primarily. (case 17) the external fixation was converted to internal fixation primarily. (case 17) the external fixation was converted to internal fixation by means of intramedullary nailing after 3 weeks. One patient underwent internal fixation for tibial plateau fracture in an institution elsewhere before presenting to us. The subtrochanteric fracture was treated with Dynamic condylar screw and one of the forearm fractures was treated with K-wire fixation while the other was treated with Ulna square nail. And the elbow fracture was treated with internal fixation. The time lag in diagnosis, delay in fasciotomy and method of fixation of each case has been detailed in the master chart.

Except one, none of the cases had incidence infections or non-union. The patient who developed infection was a case of missed compartment syndrome.

All the patients were reviewed with an average follow-up of 33 months (6 months to 60 months). 2 patients (case 3 and 6) died due to unrelated causes. One patient (case 21) died due to sepsis leading to multi organ failure secondary to a 10 day old missed compartment syndrome.

The average time of healing in upper limb fractures was 11 weeks (9 to 15 weeks) and in lower limb fractures was 17 weeks (12 to 24 weeks). In two tibial fractures, the delay in healing needed bone grafting. Healing was relatively delayed in patients who had undergone fasciotomy 10 hours after the injury or internal fixation compared to those who had gone fasciotomy in less than 10 hours, in case of both upper and lower limb fractures.

3 patients (Case 1, 9 and 16) had neurological insult secondary to acute compartment syndrome. One patient (case 1) had weakness (Power 1/5) of the anterior compartment muscles of the leg at the time of diagnosis of compartment syndrome. He went on to recover partially (Power3/5) over a period of 3 months and then no further. Another patient had weakness (Power 3/5) of extensor hallucis longus at time of diagnosis of compartment syndrome and went on to recover in 3 weeks. One other patient (case 16) had developed severe volksmann ischemic contracture of the forearm due to delay in presentation to the hospital and hence delay in presentation to the hospital and hence delay in fasciotomy. The average in fasciotomy following injury in these cases was 13 hours (10 hours to 16 hours). Though the risk of infection is high, except one, none of our cases had any infection. Severe and extensive muscle necrosis of most of the muscles of the leg was found during fasciotomy of one of the patients (case 21), which ultimately led to sepsis and above knee amputation of the limb.

Two of the cases (case 7 and 9) had no signs of union at the end of 12 weeks both clinically and radiographically. The patients required bone grafting as a secondary procedure at 3 months to induce bony healing. The patient with volksmann ischemic contracture lost functionally of the upper limb, from elbow distally.

The functional outcome is decreased in patients who had undergone fasciotomy after 10 hours. However, younger patients showed better recovery even if they had undergone fasciotomy after 10 hours, compared to older patients.

Table 3

Patient Groups	Average functional outcome
Compartment syndrome after a fracture	85%
Compartment syndrome after fracture fixation	86%

The average functional outcome score of the cases, excluding those lost to follow-up is 68 and percentage of maximal function is 85%. The decrease in functional is 85%. The decrease in functional outcome score was due to the dysfunctional status of the affected limb in case 16. The percentage of maximal function increased to 91% on excluding case 16. The average percentage of maximal function of cases with post-operative compartment syndrome (cases 1, 2, 8, 9) was 86%, ompared to the 85% in cases primarily diagnosed with compartment syndrome (93% on excluding case 16). Mo significant difference was found in the functional outcome of both the group of patients.

Discussion

Compartment syndrome has been extensively studied. But difficulty and delay in diagnosis of the condition is the major problem encountered. The management of fractures associated with compartment syndrome also does not have fixed guidelines.

The study analysis nearly all the issues involved in management of the compartment syndrome and the associated fractures.

The study shows that compartment syndrome commonly occurred in physiologically young males, similar to all other studies [1, 21, 25, 37]. And high velocity injuries were also identified as the major cause of compartment syndrome as in most studies [1, 21, 25, 37]. Compartment syndrome of leg was the most common followed by that of forearm. And tibial fractures were the major cause of compartment syndrome of leg. McQueen et al showed that 36% of compartment syndromes follow tibial diaphyseal fractures.[22] Of all tibial fractures, proximal tibial fractures were most commonly involved. The higher incidence of compartment syndrome in proximal tibial fractures has been attributed to the vulnerable blood supply of the popliteal artery and the posterior tibial artery, due to the vessel bifurcation and enclosing tight osse of ascial canal.

Six of our cases developed compartment syndrome after the associated fracture was treated by surgical stabilization. McQueen et al found an incidence of 5.5% compartment syndrome in fractures treated by reamed nailing compared with 12.2 % in externally fixed fractures [22] The cause of compartment syndrome in post –operative cases has been postulated to be sudden decrease in compartment size following reduction of the fracture prior to intramedullary fixation

All diagnosis of compartment syndrome were made clinically. One patient was diagnosed with compartment syndrome 10 days after internal fixation of a tibial plateau fracture. The compartment syndrome was missed due to the spinal injury sustained by the patient and altered neurology below the thoracic level. All patients with tibial and forearm fractures were monitored clinically, for stretch pain and out of proportion pain, preoperatively from admission and also postoperatively.

Cases at risk of developing compartment syndrome were identified and monitored. Measure of intra-compartmental pressure was done only for patients under nerve blocks, with altered state of consciousness, with spinal cord injury and children who are not able to express themselves. In other patients, intra – compartmental pressure was measured only when clinical signs are inconclusive and to determine the need for fasciotomy. McQueen emphasized in numerous studies that diagnosis of compartment syndrome can be made only by continuous monitoring of the intra –compartmental pressure [4, 21, 22, 23, 24]. Continuous intra-compartmental monitoring is preferred to a single reading. However, Twaddle and Amendola stressed that continuous monitoring of all patients due to the need for equipment and manpower. They advised clinical monitoring to diagnose compartment syndrome and to measure intra compartmental pressure in a specified group of patients as mentioned above. And they observe that the judicious use of monitoring is required to prevent over diagnosing compartment syndrome.

Turen proposed that skeletal stabilization of fractures was needed immediately after fasciotomy to aid in the healing of soft tissues which was also concurred by Twassle and Amandola [36, 37]. Georgidas and Hake et al observed that closed fractures and uncontaminated open fractures, presenting with compartment syndrome, can be treated with primary internal fixation following fasciotomy, as there are low or negligible risks of infection and non-union.[9, 11] And this treatment protocol simplifies the bony and soft tissues cover must be obtained to cover the implant and fracture site.

Twaddle and Amendola observed that the stabilization technique used depends on the location and character of the fracture and the skill of the surgeon, but should minimize operative trauma to a limb that may already have had its

circulation compromised. Therefore, if possible, intramedullary nailing to stabilize the bone (and hence the soft tissues) is recommended. After the osteosynthesis has been completed, soft tissues coverage over the bone should be attempted [37].

All the fractures in our study were stabilized after fasciotomy by external fixation or internal fixation. Following fasciotomy, the fracture was considered to be an open one.

In metaphyseal and metaphyseal–diaphyseal fractures, we used external fixation as the method of choice for primary stabilization, We used external fixation for primary method of stabilization in 9 of the 15 tibial fractures. For juxta–articular fractures, we used external fixators or hybrid fixators. Hybrid fixators with inter fragmentary screw fixation were used in 4 cases of which 2 were tibial plateau fractures. The integrity of the articular surface was maintained by means of the hybrid fixators. The advantages of external fixation are the safety and ease of application, less devitalization of soft tissues and decreased operating time. Difficulties were faced early mobilization of the patient and wound care.

Of the 6 tibial diaphyseal fractures, 5 were treated primarily by means of external fixation. External fixation was used in diaphyseal fractures, when there was delay in fasciotomy and the viability of the muscles were in doubt and in cases without adequate soft tissues cover following fasciotomy. One of the cases treated with external fixator was taken up for intramedullary nailing at the earliest. The intramedullary conversion increased patient compliance. One tibial diaphyseal fracture was primarily treated with unreamed intramedullary nailing. Wound management was found to be easier after the internal fixation and the patient was also mobilizes early. Early mobilization, easy accessibility to the fasciotomy wounds for wound care, considerable decrease in the bulk of the implant and patient compliance are the advantage of intramedullary internal fixation.

Plate osteosynthesis was never considered in any of the tibial fractures, as the risk of devitalizing the already compromised soft tissues and infection were high.

Bony healing was delayed in all cases irrespective of method of stabilization. Time of bony healing did not differ much from that of post-operative compartment syndromes. In both the groups of patients, those who presented with compartment syndrome following an injury and those who developed compartment syndrome after internal fixation, the fracture was considered to be open. Both the groups had delayed bone healing. The average time of bone healing was nearly the same in bothgroups. And the functional outcome of both the groups of patients was nearly the same. Acute complications encountered in our study were neurological insult in three of the patients who underwent delayed fasciotomy of the three, one patient recovered completely, another patient had incomplete recovery, while the third patient had no neurological recovery. Incomplete recovery or total absence of recovery is due to the development of myonecrosis during the compartment syndrome and consecutive fibrosis of the muscles [27, 29].

Delay in diagnosis is primarily due to the delay in presentation to our centre.

Infection of the fasciotomy wounds and fracture site are expected. Fracture site is exposed during fasciotomy and the fasciotomy wound is left open for secondary closure increasing thechaces of infection. In our study, none of the cases developed infection at the fracture site of fasciotomy wound.

All the cases in our study, including the cases that developed compartment syndrome post-operatively, had delayed bone healing. This is probably due to loss of fracture haematoma during fasciotomies. But bone healing was further delayed I patients who underwent fasciotomy after 10 hours compared to those who underwent fasciotomy earlier. Similar results were also observed by Mullet [27]. Court-Brown and McQueen found that complication rates decreased to 4 % from 54 % with early fasciotomy [4].

Functional outcome was decreased I our patients who had undergone fasciotomy after 1 hours. Younger patients showed better functionality. The functional outcome of patients who had post-operative compartment syndrome was no different from those who had presented with compartment syndrome. The functional outcome of any case is determined mainly by the delay in fasciotomy followed by age than any other factor. Mullet et al found that younger patients had better functional outcome. This was attributed due to change in the muscle fiber composition and decreased muscle regeneration due to age [27].

Shortcomings of the study are the limited of number patients, the absence of a control group and also the analysis being done retrospectively. The chance of developing compartment syndrome has decreased considerably due to the early intervention following injury and advancement in monitoring patients early. In spite of these limitations zX, the factors influencing the outcome of the fractures associated with compartment syndrome were identified.

Orthopaedic surgeons must remain vigilant to identify compartment syndromes, both in in- patient and out- patient settings. Future development is likely to center around non-invasive methods of diagnosing acute compartment syndrome is being examined, like near infrared spectroscopy, which measures the amount of oxygenated hemoglobin in muscle tissues transcutaneously.

Conclusion

- A high suspicion of compartment syndrome must be maintained for all cases.
- The early diagnosis and treatment within 12 hours is critical to reduce morbidity and prevent any long term sequale.
- Compartment syndrome causes delay in bone healing.
- Risk of infection is not increased.
- External fixation is the method of choice in metaphyseal and metaphyseal-diaphyseal fractures and fractures without adequate soft tissue cover, when associated with compartment syndrome.
- Hybrid fixators with intra fragmentary screw fixation are used in juxta-articular fractures with communition.
- As bony healing is not compromised in any way, use of intramedulluary fixation is not contraindicated in diaphyseal fractures associated with compartment syndrome.

References

1. Azar FM. Compartment syndromes. In: Canale ST, Beaty JH, eds. Campbell's Operative Orthopaedics. 11th ed. Philadelphia, Pa: Mosby Elsevier, 2007, 46.
2. Berman SS, Schilling JD, McIntyre KE, *et al*. Shoelace technique for delayed primary closure of fasciotomies. Am J Surg. 1994; 169:435-436.
3. Blick SS, Brumback RJ, Poka A, *et al*. Compartment syndrome in open tibial fractures. J Bone Joint Surg Am. 1986; 68:1348.
4. Court-Brown CM, McQueen MM. Compartment

syndrome delays tibial union. Acta Orthop Scand. 1987; 58:249-252.
5. DeLee JC, Stiehl JB. Open tibia fracture with compartment syndrome. Clin Orthop. 1987; 160:175-84.
6. Fitzgerald AM, Gaston P, Wilson Y, *et al*. Long term sequelae of fasciotomy wounds. Br J Plast Surg. 2000; 53:690-693.
7. Galanakos S, Sakellariou VI, Kotoulas H, Sofianos IP. Acta Orthopaedica et Traumatologica Hellenica. 2009; 60(2):127-133.
8. Garfin S, Mubarak SJ, *et al*. Quantification of intercompartmental pressure and volume under plaster casts. J Bone Joint Surg. 1981; 63-A:449-53.
9. Georgiadis GM. Tibial shaft fractures complicated by compartment syndrome: treatment with immediate fasciotomy and locked unreamed nailing. J Trauma. 1995; 38(3):448-452.
10. Gershuni DH, Mubarak SJ, Yaru NC, Lee YF. Fracture of the tibia complicated by acute compartment syndrome. Clin Orthop. 1987; 217:221.
11. Hak. The use of the unreamed nail in tibial fractures with concomitant preoperative or intraoperative elevated compartment pressure or compartment syndrome. J Orthop Trauma. 1994; 8(3):203-11.
12. Heckman MM, Whitesides Jr TE, Grewe SR, *et al.* Histologic determination of the ischemic threshold in the canine compartment syndrome model. J Orthop Trauma. 1993; 7:199.
13. Heckman MM, Whitesides TE Jr, Grewe SR, *et al.* Compartment pressure in association with closed tibial fractures: Therelationship between tissue pressure, compartment, and the distance from the site of the fracture. J Bone Joint Surg Am. 1994; 76:1285-1292.
14. Janzing H, Broos P. Dermotraction: an effective technique for the closure of fasciotomy: A preliminary report of fifteen patients. J Orthop Trauma. 2001; 15:438-441.
15. Katz LM, Nauriyal V, Nagaraj S, Finch A, Pearlstein K, Szymanowski A. Infrared imaging of trauma patients for detection of acute compartment syndrome of the leg. Crit Care Med. 2008; 36(6):17.
16. Koval KJ, Clapper MF, Brumback RJ, *et al.* Complications of reamed intramedullary nailing of the tibia. J Orthop Trauma. 1991; 5:184-189.
17. Lindsay TF, Liauw S, Rouraschin AD, *et al*. The effect of ischaemia/reperfusion on adenosine nucleotide metabolism and xanthine oxidase production in skeletal muscle. J VascSurg. 1990; 12:8-15.
18. Matava MJ, Whitesides TE Jr, Seiler JG Ill, *et al.* Determination of the compartment pressure threshold of muscle ischemia in a canine model. J Trauma. 1994; 37:50-58.
19. Matsen III FA, Mayo KA, Sheridan GW, Krugmire Jr RB. Monitoring of intramuscular pressure. Surgery. 1976; 79:702.
20. Matsen FA III, Mayo KA, Krugmire RB, *et al*. A Model Compartmental Syndrome in Man with Particular Reference to the Quantification of Nerve Function. J Bone Joint Surg. 1977; 59-A:648.
21. McQueen MM. Acute Compartment syndrome. In: Bucholz RW, Heckman JD, Court-Brown CM, eds. Rockwood & Green's Fractures in Adults. 6th ed. Philadelphia, Pa: Lippincott Williams & Wilkins. 2006, 13.
22. McQueen MM, Christie J, Court-Brown CM. Compartment pressures after intramedullary nailing of the tibia. J Bone Joint surg Br. 1990; 72:95-98.
23. McQueen MM. The Effect of Acute Cornpartmcnt Syndrome on Bone Blood Flow and Bone Union. MD Thesis, University of Edinburgh, 1995.
24. McQueen MM, Court-Brown CM. Compartment monitoring in tibial fractures. J Bone Joint Surg Br. 1996; 78:99.
25. Meyer RS, Mubarak SJ. Compartment syndromes. In: Chapman MW, eds. Chapman's Orthopaedic Surgery. 3rd ed. Philadelphia, Pa: Lippincott Williams & Wilkins, 2001, 13.
26. Moed BR, Strom DE. Compartment syndrome after closed intramedullary nailing of the tibia: a canine model and report of two cases. J Orthop Trauma. 1991; 5:71-77.
27. Mullett H, Al-Abed K, Prasad CV, *et al*. Outcome of compartment syndrome following intramedullary nailing of tibialdiaphyseal fractures. Injury. 2001; 32(5):411-3.
28. Nario CV. La enfermedad de Volkman experimental. Ann Fac Med Montivideo. 1938; 10:87-128.
29. Nassif JM, Gorczyca JT, Cole JK, *et al*. Effect of acute reamed versus unreamed intramedullary nailing on compartment pressure when treating closed tibial shaft fractures: a randomised prospective study. J Orthop Trauma. 2000; 14:554-558.
30. Petrasek PF, Homer-Vanmasinkam S, Walker PM. Determinants of ischaemic injury to skeletal muscle. J Vasc Surg. 1994; 19:623-631.
31. Rang M. Injury. In: Rang M, eds. The story of Orthopaedics. 1 st ed. Philadelphia, Pa: W.B. Saunders. 2001, 16.
32. Rorabeck CH. The treatment of compartment syndromes of the leg. J Bone Joint Surg Br. 1984; 66:93.
33. Rorabeck CH, Macnab L. Anterior tibial compartment syndrome complicating fractures of the shaft of the tibia. J Bone Joint Surg Am. 1976; 58:549.
34. Shakespeare DT, Henderson NJ. Compartmental pressure changes during calcaneal traction in tibial fractures. J Bone Joint Surg Br. 1982; 64:498-499.
35. Tornetta P, French BG. Compartment pressures during nonreamedtibial nailing without traction. J Orthop Trauma. 1997; 11:24-27.
36. Turen CH. Skeletal stabilization for tibial fractures associated with acute compartment syndrome. Clin Orthop Relat Res. 1995; 315:163-168.
37. Twaddle BC, Amendola A. Compartment syndrome. In: Browner BD, Jupiter JB, Levine AM, Trafton PG, Krettek C, eds. Skeletal Trauma. 4th ed. Philadelphia, Pa: Saunders Elsevier, 2008, 13.
38. Webb LX. New techniques in wound management: vacuum assisted wound closure. J Am Acad Orthop Surg. 2002; 10:303-311.
39. Whitesides Jr. TE, Haney TC, Morimoto K, Hirada H. Tissue pressure measurements as a determinant for the need of fasciotomy. Clin Orthop. 1975; 113:43.
40. Whitesides Jr TE, Heckman MM. Acute Compartment Syndrome: Update on Diagnosis and Treatment. J Am Acad Orthop Surg. 1996; 4:209-218.
41. Vrahas M, Bhattacharyya T. The Medical-Legal Aspects of compartment Syndrome. J Bone Joint Surg Br. 2004; 86A:864-868.

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□□□r□d□□□□□□□Fracture neck of femur has been recognised since the time of Hippocrates and is a common orthopaedic problem in old age and they have a tremendous impact on both the health care system. Despite marked improvements in the implant design, surgical technique and patient care, they still remain an unsolved fracture as for as treatment and results are concerned among the orthopaedic fraternity around the world.
By this study we will be able to assess whether bipolar prosthesis really functions as its name suggests or vice versa as the literature suggests.
M□□r□□□□□□d□**M**□□□d□□Consist of 2 parts. Part I is a (prospective study) in which Patients who undergone Bipolar hemiarthroplasty for fracture neck of femur at PSG Hospitals were included in the study after obtaining their consent during the period of December 2011 to June 2013.
Part II was a retrospective study where we took a data of patients who were operated earlier in our institute and had completed atleast 2 years of follow-up.
□ □□□□□□□□□d□□ □□□□□□□□□□With this study we found that for effective functioning of bipolar prosthesis IPJ movement remains a vital cog in the success of the bipolar prosthesis. In this study we found that there is good amount Inter-prosthetic joint movement of bipolar prosthesis at short – term and mid - term follow-up. The functional outcome is also good when Inter-prosthetic joint movement is more than 25%.

□ □□□ □rd□□Neck of femur fracture, bipolar hemiarthroplasty, inter prosthesis joint

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Fracture neck of femur has been recognised since the time of Hippocrates and is a common orthopaedic problem in old age and they have a tremendous impact on both the health care system and society in general [1]. Despite marked improvements in the implant design, surgical technique and patient care, they still remain an unsolved fracture as for as treatment and results are concerned among the orthopaedic fraternity around the world [2]. Fractures of the neck of femur are on increase, which is not unexpected, as the general life expectancy has significantly increased during the few decades. These fractures may double within next 20 years and fracture rate doubles for each decade of life after 5th decade of life [□]. It's has been predicted that by the year of 2050, the number of hip fractures would triple.
This is the commonest fracture in old aged individuals because of osteoporosis and advancing age causing more brittleness of bone. So prolonged immobilization during such fractures in elderly will jeopardise the life span of the patient and further complicates the problem. This forces one to totally abandon complete immobilisation to achieve a bony union, or to resort early ambulatory procedures by surgery.
The blood supply to neck and head of femur is extensive, intricate and complicated. Healing process mainly depends on good blood supply. Non-union, avascular necrosis of femoral head and secondary degenerative arthritis are the principal complications of this fracture. The surgeon may have control over the problem of non-union, whereas he may have none over avascular necrosis and arthritis. This further handicaps the treatment of these fractures as the healing process is always in doubt. It's also a known fact that hip is a weight bearing joint and many performance based functions depend on hip joint. A successful operation at hip joint should provide painless, stable hip with wide range of movements.

Under these circumstances one has to decide whether the prolonged immobilization has to be employed to achieve bony union or quick ambulation by hemi-replacement arthroplasty, to achieve fair degree of function. Since early 1950's prosthetic replacement was introduced for solving the problems of Fracture neck of femur and vitallium intramedullary prosthesis received a hearty welcome, thus preventing Non-union and avascular necrosis. The Austin – Moore and Thompson prostheses have been successful implants in treating fracture neck of femur. Disabling pain and acetabular erosions are frequent complications after the use of Moore prosthesis [□]. So in an attempt to retard the acetabular wear, prolong the life of the implant and delay the need for revision surgery the bipolar prosthesis was developed by James E Bateman in Toronto in 1974, which had the great advantage of second joint, below the acetabulum. It was named bipolar prosthesis, since it had an outer head of metal which articulates with acetabulum and a second inner metallic head which articulates with High Density Poly-Ethylene (HDPE), lining the inner surface of outer head. So hip motion is to occur at 2 interfaces – primarily at the prosthetic interface and secondarily at the metal – cartilage interface, thus minimising the articular wear. This prosthesis was found to be very useful and results were encouraging [□].

But studies attempting to demonstrate the relative movements at the interfaces have yielded conflicting results. It's known that a friction produces particulate debris from the polyethylene liner and this was thought to be the cause of foreign body reaction causing stiffening up of the inter-prosthetic joint and also osteolysis and aseptic loosening of the implant. Recent studies have shown that over a variable period of time the bipolar prosthesis will become unipolar functionally due to stiffening up of the inter-prosthetic joint (IPJ).

This study represents the assessment of the inter-prosthetic joint movement in bipolar prosthesis done for fracture neck of femur at 6months and mid-term follow-up by radiological means. By this study we will be able to assess whether bipolar prosthesis really functions as it's name suggests or vice versa as the literature suggests.

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Consist of 2 parts. Part I is a (prospective study) in which Patient's who undergone Bipolar hemiarthroplasty for fracture neck of femur at PSG Hospitals were included in the study after obtaining their consent during the period of December 2011 to June 2013.

Part II was a retrospective study where we took a data of patients who were operated earlier in our institute and had completed atleast 2 years of follow-up.

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By Convenient sampling method, the patients undergoing bipolar hemiarthroplasty for fracture neck of femur were assessed radiologically for amount of inter-prosthetic joint motion during post-opeartive period.□

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- For all patients Life ortho care bipolar prosthesis was used (ISO – 13485 certified company). Implant made of 316 – Stainless steel. Femoral stem length is 150 mm, diameter – 8 mm. Outer head diameter varies from 37 to 53 mm with 2 mm increment. Inner prosthetic femoral head diameter was 26 mm and the lining between outer head and inner head is made of UHMWPE (Ultra High molecular Weight Polyethtylene). Neck shaft angle is 130 degrees. 2 types of prosthesis are available (Fixed only with collar, Modular with or without collar and Extra – long stem also available).
- No funding was received from the Implant Company for the purpose of this study.

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All patients who are undergoing bipolar hemiarthroplasty□for fracture neck of femur are considered for the study after getting a written consent from them. All Patients were adequately worked up before surgery and all patients were taken up for surgery within 72 hours. Certain therapeutic measures such as Deep breathing exercises, Static Quadriceps exercises, ankle pumps were taught to the patient pre-operatively. A detailed history about the mode of injury and type of fracture were noted.

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All surgeries were performed on an elective basis using standard□aseptic precautions, Surgery was performed under spinal or general anaesthesia. Patient was positioned laterally lying on the unaffected side. For all the patient's lateral approach (Hardinge approach) was used in our series.

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Throughout the post-operative period adequate care was taken□ to prevent abduction and external rotation of the limb. All patients received Quads strengthening X's and mechanical DVT prophylaxis during immediate post-operative period. All patient's were started on Full weight bearing ambulation as tolerated by the patient. Once initial pain subsided specific X-rays were done to assess the inter-prosthetic joint (IPJ) and outer bearing movement was assessed during immediate post-op (24 to 48 hours) , at 6 weeks and after 6 months with operated hip in neutral and maximum abducted positions.

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Two A.P x-rays of pelvis were taken. One with limbs in neutral position and neutral rotation and the other x-ray with affected limb in Maximum abduction are taken.

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We followed the method of p lain radiographs, as described by Bochner *et al*.

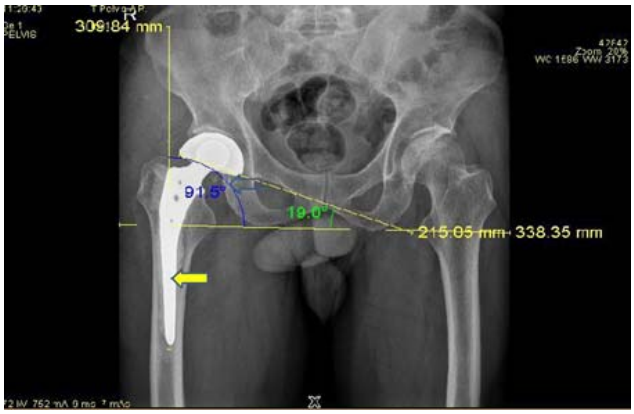
On the X-ray in the neutral position, 3 lines are drawn as follows:



□□□□**1** □a line drawn tangential to the most inferior aspects of the ischial tuberosities which is used as a reference line.

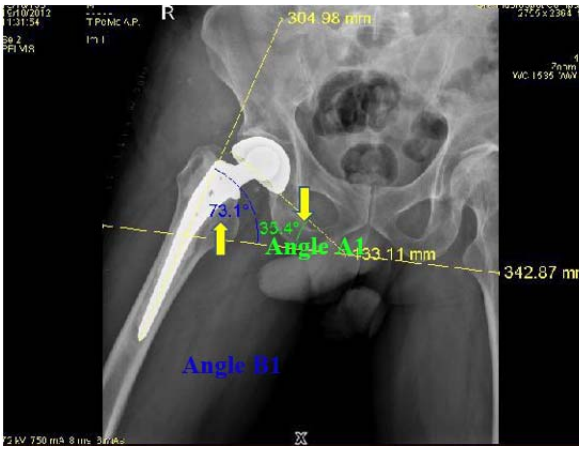


□□□□**2** □drawn along the the inferior margin of the acetabulum



□□□□□□Drawn along the centre of the long axis of femoral stem.

- Angle A was defined as the intersection of the line drawn from the inferior margin of acetabular component and the reference line (line of ischial tuberosities).
- Angle B was formed by the intersection of the ischial reference line with a line drawn along the center of the long axis of the femoral stem.
- The same exercise was repeated on the maximum abduction anteroposterior radiograph also and the angles are marked as A^1 and B^1.



□□□□□□Operated Limb in Maximum Abduction

- Now, B^2 is the difference between angle B and B^1 which represents the total amount of Hip abduction of the operated limb.
- A^2 is the difference between angle A and A^1 which represents the amount of motion taking place between the acetabular component and the acetabulum (Outer bearing interface).
- As we have 2 variables ie. (B2 – total amount of abduction, A2 – motion at the outer bearing interface), the difference between B^2 and A^2 represents the amount of abduction taking place at the inner bearing (Inter-Prosthetic joint movement).
- Thus the total amount of abduction, movement at outer bearing interface and movement at inter-prosthetic joint were calculated and tabulated for each patient at each follow-up.

X-ray of pelvis with both hips (Abduction of operated hip) with bipolar prosthesis on right side showing Angle A1 – 43.3 and Angle B1 – 74.4 degrees.
A = 45.1, B = 83.4, A1 = 43.3, B1 = 74.4 (Degrees)
B2= 9 Degrees (Total Abduction)
A2 = 1.8degrees (Motion @ Outer Cup)
B2-A2 = 7.2 Degrees (Inter-Prosthetic Joint Motion)
A2 = 20% (Motion @ Outer Cup)
B2 - A2 = 80% (Inter-Prosthetic Joint Motion).
At the end of 6 months follow-up, in addition to Radiological assessment Functional outcome was also assessed with Oxford hip score.
Part – 2 (Retrospective study):
Patients in this group who had already undergone Bipolar H.A and completed 2 years follow-up were reviewed. The x-rays were taken in the similar fashion as described above. Functional outcome assessment was done using Oxford hip score. The retrospective study helped us to assess IPJ motion at midterm follow-up.

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- It's useful to assess the outcome after surgical procedures by measuring 'patients' perceptions in adjunction to surgery. OHS assesses pain (6 items) and function (6 items) of the hip in relation to daily activities such as walking, dressing, sleeping etc.12 items with 5 categories of responses. Scores range from 0 to 4 (worst to best).
- Grading :
- 0-19 □ Worst, May indicate severe hip arthritis, requires some form of surgical intervention.
- 20-29 □ Fair, May indicate moderate to severe hip arthritis. Assessment by X-ray
- 30- 39 □ Good, May indicate mild to moderate hip arthritis. Non-surgical treatment, such as exercise, weight loss, and /or anti-inflammatory medication.
- 40-48 Excellent, May indicate satisfactory joint function. May not require any formal treatment.

Table 1: Radiologically Assessed Total Abduction, Outer Cup Motion And Inter – Prosthetic Joint Motion

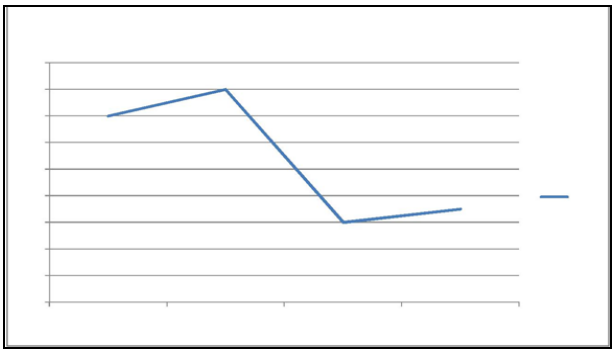
Follow up	Patient's	Mean B^2 (in degrees)	Mean A^2 (in degrees)	Mean $B^2 - A^2$ (in degrees)	Mean A^2 (in%)	Mean $B^2 - A^2$ (in%)
Immediate						
Post-op	30	18	8	10	45	55
6 weeks	28	23	13	10	57	43
6 months	30	25	18	7	72	28

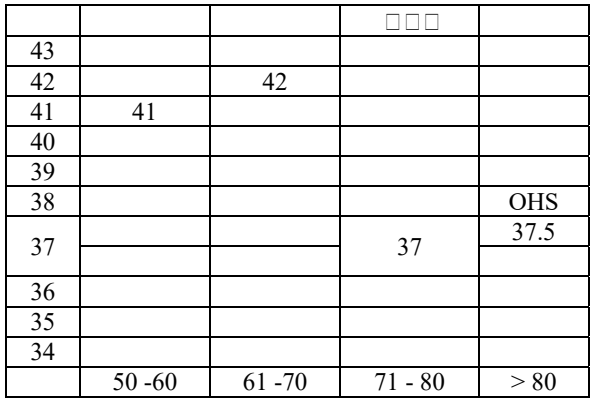
B^2 – represents Amount of Total abduction, A^2 – represents amount of movement between the outer cup and the acetabulum, $B^2 - A^2$ represents amount of IPJ motion.

Table 2: [illegible]

[illegible]	[illegible]	Mean [illegible]	[illegible]	Mean [illegible]	Mean [illegible]
50-60	7	41.5714	6.72947	32.00	48.00
60-70	9	42.0000	2.73861	39.00	48.00
70-80	12	37.0833	6.88157	26.00	46.00
ABOVE 80	2	37.5000	13.00000	36.00	39.00
TOTAL	30	38.9355	7.19692	26.00	48.00

Table depicts the Oxford Hip score of different age gropus in involving 30 patients of prospective study group, showing decrease in OHS as the age increases. But the decrease in OHS with increasing age group was not statistically significant (P = 0.062 ie > 0.05).





This graph predicts the decline in the OHS as the age progresses, except for the initial part.

Table 3: Mean [illegible] 6 Mean [illegible]

[illegible]	[illegible]	Mean $B^2 - A^2$	[illegible]	Mean [illegible]	Mean [illegible]
50-60	7	27.8571	8.31522	14.00	38.00
60-70	9	30.1111	5.30199	25.00	39.00
70-80	12	28.6667	6.31497	18.00	38.00
Above 80	2	35.5000	.70711	35.00	36.00
Total	30	29.3667	6.38146	14.00	39.00



Table 10 shows the IPJ motion at end of 6 months in various age groups in prospective study of 31 patients. On data analysis there was no statistical significance between the IPJ motion and various age groups (P = 0.495 > 0.05). Though the IPJ motion seems to be higher in patients above 80 years, the sample size for the particular age group was not significant.

This graph demonstrates the relation of $B^2 - A^2$ in various age groups

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Male	18	38.5263	7.99561	28.00	48.00
Female	12	39.5833	5.99179	26.00	48.00
Total	30	38.9355	7.19692	16.00	48.00

□

Table 12 depicts the comparison of B2 – A2 at 6 months against the gender in prospective study of 30 patients. The IPJ motion was found to be almost the same in both females and males. There was also a statistical difference between the 2 groups in relation to IPJ motion IPJ□□

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More than	□	□	□	□	□
25%	20	42.7000	3.21346	38.00	48.00
Less than 25%	10	33.7000	5.51866	26.00	41.00
Total	30	39.7000	5.90237	26.00	48.00

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Table 13 depicts the comparison of IPJ motion at 6 months with Oxford Hip Score in prospective study of 30 patients. IPJ motion of the patients was divided into 2 groups for the ease of data analysis (Group I – IPJ motion > 25%, Group II – IPJ motion < 25%). Based on our data analysis the mean OHS was statistically significant in patients with
IPJ motion > 25% (Mean OHS – 42.7), (P = 0.00 < 0.05).

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10	31.0000	10.36018	15	50

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Table shows the Mean IPJ motion of 31 degrees in the retrospective study.

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10	38.1	28	44

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Table shows Oxford hip score of retrospective study of 10 patients with mean OHS – 38.1.

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>25%	8	39.625	35	44
<25%	2	32	28	36
Total	10	38.1	28	44

Table shows comparison between the IPJ motion and OHS in Retrospective Study. Since the sample size is small in retrospective study this data was not compared with the data in the Prospective study. For the current retrospective data the analysis was not statistically significant.

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The basic advantage of the Bipolar prosthesis over conventional unipolar prosthesis is movement at the two interfaces ie. Between the prosthetic inner femoral head and polyethylene liner and between acetabulum and the outer head. It was proposed that the complications like acetabular erosions would be delayed or prevented by reducing wear due to sliding motion in the acetabular socket. The high molecular weight polyethylene (HMWPE) articulation also absorbs some of the impact forces during gait. This advantage of Bipolar over unipolar prosthesis has been accepted by many studies□but there have been some reports in the literature that cast doubt on the continuity of inter-prosthetic movement in a bipolar prosthesis over time. Various radiographic studies have been done in which the prosthesis was imaged in various non-weight-bearing and static weight-bearing positions to estimate the fraction of movement that is occurring at the inter-prosthetic joint in a bipolar prosthesis. Phillips[6]□ used radiograph in supine position, first in neutral and then in maximum abduction and adduction.

Verberne used image intensifier to do radiological examination. In this context we undertook the present study of evaluating the IPJ motion radiologically over a period of time at 6 months and at the end of 2 years. We had also tried to co-relate the amount of IPJ movement to Functional outcome to assess whether there's any improvement in the quality of life in patient's with good IPJ motion. We followed the method of plain radiographs, as described by Bochner *et al*. In our series the amount of IPJ movement was 55% during immediate post-op, 43% during 6weeks & 28% at the end of 6 months. The mean Inter – prosthetic joint movement at end of 2 years follow – up was 31% for patients in the retrospective group. So at the end of 6 months 66% of the patients had > 25% of Inter prosthetic joint movement in our prospective study and 80% had > 25% in the retrospective study. However we are not analyzing the same group of patients in the prospective and retrospective study & the sample size of retrospective study is small. This could have probably resulted in higher inter – prosthetic joint movement at the end of 2 years. Drinker and Murray reported that although some inner motion occurred in most implants, it was less than predicted. Philips TW (1987) had done a study on Fluroscopic movement in 100 patients who had undergone Bateman Bipolar arthroplasty. Out of these 100 patients Group I had 76 patients with arthritis of hip and Group II had 24 patients with neck of femur fractures. In 80% of group I patients, the prosthesis retained Bipolar function at the end of 4 years follow-up study as compared to only 25% of group II patients retained the bipolar functioning of the prosthesis□

Verbene G.H.M (1983), did a radiological study of movements of two components in Variokopf prosthesis in 20 patients with fracture neck of femur during Immediate, 1 month and 3 months post-operative period. He observed that the IPJ lost mobility and at 3 months it became almost completely stiff with inter-prosthetic joint motion of only 16.9% being retained.

Bochner RM, in a study 26 patients with Bateman's Prosthesis who were assessed radiologically for inter-prosthetic joint motion, concluded that bipolar function was retained at the end of 4 years and the motion was shared between both the joint interfaces [□]. In their study the inter-prosthetic joint motion at 6 months was 19%. In a study conducted Anil kumar rai, from Banaras hindu university, varanasi, india during the period march 2003 to january 2011 treated with BHU bicentric bipolar prosthesis, showed that in cases of fracture neck of femur, the percentage of total abduction occurring at the interprosthetic joint at 3 months was 33.74% (mean value of all the patients), which fell to 25.66% at 1.5 years and remained stationary till 6 years. So they concluded that some amount of IPJ motion is still preserved at the time of their mid-term follow-up of 6 years. Higher Inter-prosthetic joint motion was observed in the arthritic group as compared to fracture group. This could be explained possibly due to friction phenomenon.

The results of our study regarding the Inter – prosthetic joint movement was comaparable to other studies in literature. Verbene *et al*. showed only 16.9% inter-prosthetic Joint movement at the end of 3 months in 20 patients & Bochner *et al*. showed 19% movement at the end of 6 months in 18 patients. Our study had better inter-prosthetic joint movement of 28% at end 6 months. This result probably could have been due to lower sample size of the above 2 studies.

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1. Condition of the Acetabulum: In patients with normal articular cartilage of acetabulum, the outer cup probably slides more when compared patients with arthritis. This can probably due to friction at which the primary movement occurs in IPJ. Only at the terminal range of movements prosthetic femoral head impinges on the neck leading to movement of the outer cup.
2. The Inner femoral head size used by Verbene *et al* was 32 mm which could have probably decreased the IPJ motion, when compared to Bochner and Philips *et al* where they used 22 mm inner femoral head. In the study done by Philips TW they had suggested that lower the femoral prosthetic head size, then lower will be the friction between the Polyethylene liner & femoral head & decreased stress on outer metal cup, which will result in lower acetabular erosions.
3. In previous studies they had used prosthesis with High density polyethylene which is prone for formation of wear debris over time, which could have possibly played a role in decreasing the IPJ motion. The wear debris formed from UHMWPE is much lower than the HDPE used in those days which could have contributed to better IPJ movement.
4. Since in our study we had used Bipolar prosthesis consisting of UHMWPE liner & optimum size prosthetic Femoral Head, our patients had better inter-prosthetic joint movement when compared with Verberne *et al*. & Bochner *et al*. we also assessed the age of the patient co – relating with Inter –prosthetic joint movement, but we did not find any statistical signifance.

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Various criteria were used to assess the functional results following bipolar hemiarthroplasty. How best the patient is returned to the pre-fracture status has been the main criteria. In India our customs demand Squatting and sitting cross leged without difficulty. To achieve this patient should have good range of flexion, abduction, adduction and external roation at the hip and full flexion at the knee. Our final results at 6 months after bipolar hemiarthroplasty in our series were analyzed using Oxford Hip Score. The results were compared with the available western and Indian series where bipolar hemiarthroplasty was done for neck of femur patients.

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Hinchey (1976)	225	52.4	20.4	10.7	16.4
Saxena and saraf (1978)[76]	82	46.1	44.8	6.5	2.6
Mukeherjee and Puri (1986)	55	29	49	18	4
Bavadekar and Manelkar (1987)	328	60		30	10
Arvade (1987)	104	70		16	14
Our series (2012 -13)	40	56	32	10	2

His table depicts the percentage of functional results following bipolar arthroplasty done for fracture neck of femur.

The difference between excellent and good results are minimal and therefore they can be gropued together as satisfactory results. In the series above Hinchey had 72.8%, Saxena [76]90.9%, Mukerjee and Puri 78%, Bavedekar 60% satisfactory results were achieved. In out series satisfactory results were achieved in 88% of our cases. Only 2% of the patients had poor results due to moderate /marked pain.

We found that there was no stastistical significance in between the age, sex Vs OHS. However we were able to analyse the relation between the IPJ motion and OHS. We found that there was significant increase in OHS in patients with IPJ motion of more than 25%. So we conclude that more the amount of IPJ motion preserved better will be the functional outcome in patients with bipolar arthroplasty done for femoral neck fractures

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With this study we found that for effective functioning of bipolar prosthesis IPJ movement remains a vital cog in the success of the bipolar prosthesis. In this study we conclude that: There is good amount Inter-prosthetic joint movement of Bipolar prosthesis at short – term and mid - term follow-up. The functional outcome is also good when Inter-prosthetic joint movement is more than 25%.

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- In this study the patients in the prospective and retrospective are not the same. And hence the results show a trend rather than being specific.
- Sample size is small
- Long term studies are required

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1. Mark F, Smionthloski *et al.* Current concepts of Intra-capsular fracture of Hip. JBJS. 1994; 76A:129-135.
2. Terry Cannole, Ed: Campbell's Operative orthopaedics, 10th edition: Missourie; Mosby –year book. 1998, 339-40.
3. Rockwood, Charles A, *et al.* Rockwood and green's fracture in Adults 6th edition: Philadelphia, Lipincott – raven publishers. 1996, 1693.
4. Johnson CE, Ripley LP, Bray CB, *et al.* Primary endoprosthetic replacement for acute Neck of femur fractures: A review of 150 cases. Clin. Orthop. Of North America. 1982; 167:123-130.
5. Bateman JE. Single assembly THA, Prelimnary report. Orthop Digest. 1974; 15:35-43.
6. Badgley C. Treatment of displaced subcapital femoral neck fractures in aged with immediate replacement arthroplasty (discussion). JBJS. 1961; 43B:606.
7. Carnesale PG, Anderson LD. primary prosthetic replacement for femoral neck fractures. Arch Surg. 1975; 110:27-29.
8. Chandler SB, Kreuscher PM. A study of the blood supply of the Ligamentum teres and it's relation to circulation of head of femur. JBJS. 1932; 14:834-846.
9. Garden RS, Praston. The structure and function of the proximal end of femur. JBJS. 1961; 43B:577-582.
10. Schek M. the significance of posterior comminution in femoral neck fractures. Clin. Orhop. 1980; 152:138-142.
11. Cane JM, Sulco TP, Zolan S. Treatment of pathological fractures of hip by endoprosthetic replacement. JBJS. 1980; 62A:954-959.
12. Crack HV. An atlas of the arterial supply of the head and neck of femur in man. Clin. Orthop. 1980; 152:17-28.
13. Boyd HB, Salvatore JE. Acute fractures of the femoral neck: Internal fixation or prosthesis? JBJS. 1964; 46A:1066-1068.
14. Arvade DJ. A review of internal fixation and prosthetic replacement for fresh femoral neck fractures. Clin Orthop. India. 1987; 1:77-82.
15. King D. primary prosthetic replacement in frash femoral neck fractures. JBJS. 1964; 46A:260.
16. Hey-grooves EW. Treatment of the fractured neck of femur with special regard to results. JBJS. 1930; 12:1-14.
17. Johnson JTH, Gothers O. Nailing Vs Prosthesis of femoral neck fractures. JBJS. 1972; 57A:686-792.
18. Arnold WD, Lyden JP, Minkoff J. Treatement of intracapsular fractures of femoral neck. JBJS. 1974; 56A:254-262.
19. Arnold CC, Lemperg RK. Fractures of femoral neck. Clin Orthop. 1977; 129:217-222.
20. Brown JT, Alrami G. Transcervcial femoral fractures. JBJS. 1964; 46B:648-663.
21. Mohandas PVA, Anadhi CP. Prosthetic replacement in the treatment of femoral neck fractures. Indian Journal of Orthopaedics. 1977; 11(1):31-37.
22. Austin T Moore. Discussion on femoral head prosthesis. JBJS. 1965; 47A:43.
23. Austin T Moore. The self-locking metallic hip prosthesis. JBJS 1957; 39A: 811 -27.
24. Peter Cordasso. Evolution of treatment of femoral neck fractures. Arch Surg. 1972, 37.

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Assessment of proximal femur anthropometry in South Indian population through cadaveric bones and radiologically correlating difference if any between other ethnic groups

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ABSTRACT

Background: The morphology of the proximal femur is a topic of extensive research. The hip joint is one of the most commonly replaced joint. The era of replacement has given rise to various implants that can be used to replace the proximal femur. This present study addresses these issues involving ethnic differences in the geometry of the proximal femur in Indians and its differences between people of various ethnicity.
Methods: The total population that was radiographed was 178 (n=178). There were 78 males and 100 females. The age of the participants were spaced from 25-75 years age groups. The parameters were measured.
Results: The canal flare index in South-Indians was an average of 3.23 with 70% of the study population having normal CFI (3-4.5), 30% of the population having a stove pipe configuration CFI (<3). Majority of the Indian population favour a un-cemented fixation (70%).
Conclusions: In summary all current implants have to be revised on population basis to fit the changing anthropometry of our proximal femur.

Keywords: Femur canal, South Indian population, Canal flare index

INTRODUCTION

The morphology of the proximal femur is a topic of extensive research. The hip joint is one of the most commonly replaced joint. The era of replacement has given rise to various implants that can be used to replace the proximal femur. The integral part of any replacement is to reproduce the biomechanics of the original joint in the prosthetic components in order to achieve good clinical outcome both in terms of patient and implant. Many of the conventional implant systems manufactured by various conglomerate companies are made in correlation with the sizes of the femora of Europeans.[1] The use of these implants in the Indian population, owing to its small size of the femur has been plagued with numerous complications like intra-op splintering of the proximal end of femur. It is also the bane of the Asian-Indian Orthopaedic surgeons to work with such ill-fitting implants.

Most implant systems are usually designed on the basis of European femora, which are believed to be larger than Asian population. The implantation of these prosthesis often results in problems like fractures of the proximal femur or less confirming prosthesis leading to loosening.[2]

This present study addresses these issues involving ethnic differences in the geometry of the proximal femur in Indians and its differences between people of various ethnicity. It also evaluates the adequacy of fit of the conventional femoral stems in Indian population.

The aim and objective of the study was to determine the proximal femur morphology in South Indian population determined with radiographs and from cadaveric dry bones and to determine the differences between anthropometry of South Indian population and other ethnic groups.

METHODS

This is a prospective study consisting of two fundamental parts, involved in assessing the anthropometric dimensions of the proximal femur. In the first part of the study we have evaluated anthropometry of 178 volunteers radiographically. The second part of the assessment was done with cadaveric femora obtained from the Department of Anatomy, PSGIMSR. 50 cadaveric dry femora were obtained and direct measurement of measurable anthropometric data was done. The study was conducted in PSGIMSR Coimbatore, Tamil nadu, India from August 2013 to August 2014.

Inclusion criteria were South Indian population and people of age 25 years and above. Exclusion criteria were no prior pathology in the femur- infection (old/healed/active), congenital anomalies, contractures and deformities around the hip, previous hip surgeries and open epiphysis.

Parameters templated

- Femoral head diameter
- Horizontal offset
- Neck shaft angle
- Endosteal diameter at a level 20 mm above lesser trochanter (D)
- Endosteal diameter at the summit of the lesser trochanter (E)
- Endosteal diameter at a level 20 mm below the lesser trochanter (F)
- Endosteal diameter at the level of the Isthumus (G-10 cm below lesser trochanter)
- Canal flare index.

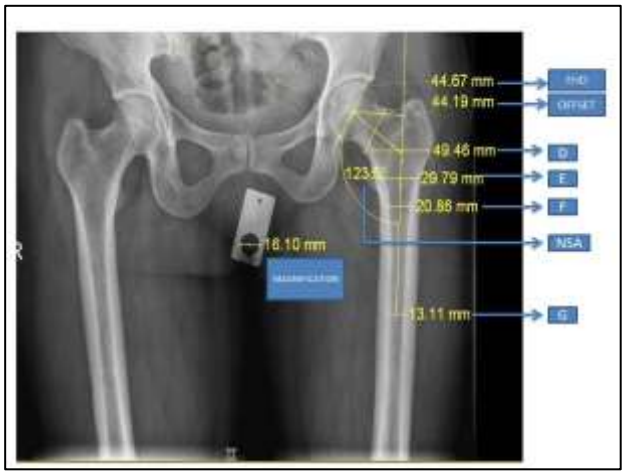


Figure 1: Radiograph templating.

Methodology of templating

Endosteal diameters

The endosteal width is measured at various locations based on reference lines which have been already defined as per Noble P C et al. The apex of the lesser trochanter is taken as the first landmark. The endosteal width at the level of the apex of lesser trochanter (E), endosteal width 20 mm above (D) and below (F) the lesser trochanter and 10 cm below the lesser trochanter (G) is measured. The mid-point of these endosteal width lines are connected to form the axis of the shaft of femur. One more point is taken measuring 10 cm distal to the apex of the lesser trochanter (G) which is considered as the isthumus. Endosteal width at that level is also measured.[3-5]

Femoral head diameter

The femoral head diameter is taken as the largest vertical diameter (superior-inferior) of head perpendicular to the axis of the neck of femur. The neck axis is drawn by drawing neck widths at 2 regions on the neck of femur, preferably in the trans-cervical and sub-capital region. The midpoints of these two lines are joined and extended further to form the axis of the neck. The femoral head diameter is measured perpendicular to this line taking the largest superior-inferior diameter of the femoral head.

Horizontal offset

The horizontal offset is also known as the actual femoral offset. The horizontal offset is measured as the distance between the centre of the femoral head to the axis of the shaft of femur. The x ray is taken in the said protocol mainly to reveal the proper and maximal offset of the femur.[6]

Neck shaft angle

The neck shaft angle is the angle subtended between the shaft axis and the axis of the neck of femur.[3,4,6]

Canal flare index

The ratio between the endosteal diameters 20 mm above the lesser trochanter (D) and at the level of isthumus (G) is called the canal flare index. Based on the values of canal flare index they were grouped in to normal (3-4.7) , champagne flute (high tapering in the proximal segment 4.7-6.5), stove pipe (a straight proximal femur relative to distal).[3]

The diameter of the magnification marker in the radiograph is determined for identification of the radiographic magnification error.

Cadaveric dry femora- study

The second part of the study involved with measuring the endosteal dimensions of cadaveric femur specimens. This was done to find out the true anthropometric parameters

of proximal femur in addition to knowing the fit of available femoral stems, So as to determine mismatch between implant and bone if any.

In our study we have taken 50 cadaveric femora. The cadaveric femora were cut at various positions so as to ascertain the endosteal dimensions at various regions of the cadaveric femur. The femora were cut using a motorized cadaveric cutting saw in the Department of Anatomy- PSGIMSR. The endosteal dimensions were measured using a Vernier calliper, at the regions mentioned below.



Figure 2: Cut sections of dry bones at various reference levels (left to right – D, E, F, G).



Figure 3: Method to measure neck shaft angle of cadaveric dry bone.

RESULTS

The mean femoral head diameter was 47.41 mm with a 95% confidence interval for the mean values, a standard deviation of ±4.5 mm. The mean offset in the study population was found to be 42.75±4.3 mm. The mean CFI was found to be 3.23±0.5.

Table 1: Results of radiographic study.

	Mean	SD	Minimum	Maximum
Age	47	10	25	75
Femoral head diameter	47.41	4.5	35	57
Medullary canal width				
D	43.79	5.2	33	61
E	27.35	4.3	17	43
F	19.92	3.5	12	31
G	13.88	2.8	9	23
Horizontal offset	42.75	4.3	34	53
Canal flare index	3.23	0.5	2	5
Neck shaft angle	126.03	4.6	114	137

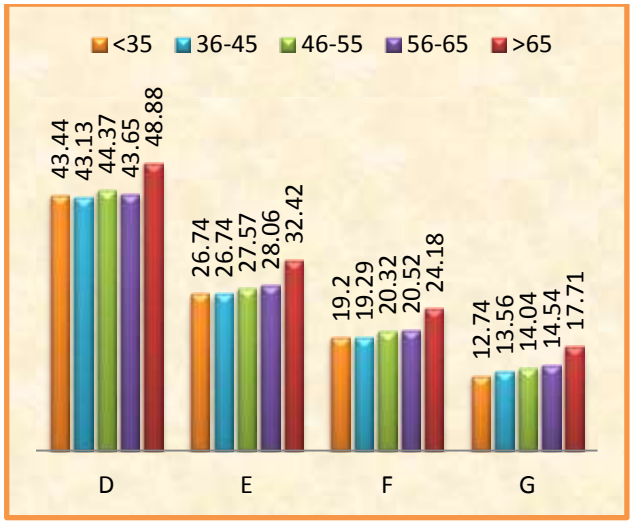


Figure 4: Mean medullary canal width at various levels matched with different age groups.

The above Figure 4 explains the difference in the medullary canal width as the age groups progressed there was a tendency towards increase in the mean width of the endosteal canal diameter.

Table 2: Age wise distribution of canal flare index.

Age group	Stove pipe(<3.0)	Normal (3.0-4.5)	Champagne	Total
<35	4	15	0	19
35-45	21	51	0	72
45-55	14	42	0	56
55-65	12	13	1	26
>65	2	3	0	5
Total	53	124	1	178
%	30%	70%	1%	100%

In the study population 30% had a stove pipe appearance of medullary canal with mean CFI <3. 70 % of the population had normal appearing femora with CFI ranging from 3-4.5 as shown in Table 2.

DISCUSSION

The aim of our study is to evaluate ethnic differences in the anthropometry of proximal femur of South Indians in comparison with other ethnic groups around the world.

The total population that was radiographed was 178 (n=178). There were 78 males and 100 females. The age of the participants were spaced from 25-75 years age groups. The following parameters were measured:

Femoral head diameter

The lowest femoral head diameter measured in our study was 35 and largest femoral head diameter in the study population was 57. The mean femoral head diameter was 47.41 mm with a 95% confidence interval for the mean values, a standard deviation of ±4.5 mm. The most prevalent head size in the given population was estimated in terms of mode for the given range, was found to be 49 mm.

Medullary canal diameters

Medullary canal widths were measured at various locations on the radiographs as described earlier in correlation to the prime seating points of the femoral stem. These levels are the standard reference points to address the changing geometry of the proximal femur. The mean and standard deviations were estimated for each parameter.

Mean canal widths at reference points-

- Endosteal canal width 20 mm above lesser trochanter (D)- 43.79±5.2 mm
- Endosteal canal width at the level of lesser trochanter (E)- 27.35±4.3 mm
- Endosteal canal width 20 mm below the lesser trochanter (F)- 19.92±3.5 mm
- Endosteal canal width at the level of isthumus (G)- 13.88±2.8 mm.

The distribution of endosteal canal widths at various levels were analysed with their age group wise distribution.

As the age groups progressed there was a tendency towards increase in the mean width of the endosteal canal diameter. This was most significant in the >65 years age groups. This was consistent with decreasing trend of canal flare index as age progressed.

Canal flare index

The mean and standard deviation for the same was calculated. The mean CFI was found to be 3.23±0.5. An age wise distribution of the canal flare index.

In the study population 30% had a stove pipe appearance of medullary canal with mean CFI <3.70% of the population had normal appearing femora with CFI ranging from 3-4.5.

There was progressive tendency towards stove pipe appearance of femoral endosteum as age progressed. Most strikingly noted in the >65 years age group with a mean canal flare index of 2.86. The younger age groups predominantly have a more normal type of femur. The distribution was found to be statistically significant in terms of distribution with progressive decline in the endosteal dimension as age progressed, and the femoral endosteum attaining a stove pipe like appearance ($p < 0.05$).

The gender wise distribution of the canal flare index was assessed, mean CFI in males was found to be 3.14±0.4, compared to the mean of 3.29±0.5 in the female pool. There were statistically significant differences in the gender wise distribution of the mean canal flare index ($P < 0.05$).

Horizontal offset

Horizontal offset calculated as the distance between the center of femoral head to the axis of the femoral shaft, which defines the abductor muscle tension was measured in all 178 subjects. The mean offset in the study population was found to be 42.75±4.3 mm.

Differences in anthropometric parameters of Indian femora vs. other ethnic population

The ethnic differences in the anthropometry of the proximal femur was assessed by matching the values of our present study with that of values previously measured in other ethnic populations. For the comparison we have taken the values of proximal femoral anthropometry of the Caucasian population according to the study by Noble P C et al (n-200), the importance of this study was its vitality in bringing up of the dimensions for creation of the various somatotypes for the cemented and un-cemented replacement armamentarium.[4] The study has allowed us to have sufficient arsenal for replacement at the same time allowing the implant to accommodate itself in to bulk of the femora. Anthropometric analysis of a Swiss population based on study by Rubin et al, French population by Massin et al, Malay population by Baharuddin et al, Thai population by Mahasavariya et al.[5,14-16]

Femoral head diameters

The difference in the femoral head diameters across various ethnic groups was assessed.

The present study showed mean femoral head diameters in the South Indian population to be 47.16±4.5 mm. The mean femoral head diameter of Indian population was compared with that of the Malay population 40.81±3.43 mm. The Malay population was found to have smallest femoral head diameters. The mean femoral head diameter of the present study was compared with the Thai population - 43.98±3.47, the Thai population had smaller femoral head dimensions. The mean femoral head diameter of the present study was compared with the Swiss population- 43.40±2.26 mm, the Swiss population had significantly smaller femoral head diameters. The mean femoral head diameters of the South Indian population was compared with the French population, they had lesser mean femoral head diameters- 45.60±4.20 mm compared to Indian population. There was no statistically significant difference in the mean femoral head diameters between Indian and Caucasian femora- 46.1±4.8 mm.

Canal diameters

The population mean endosteal dimensions were measured at four reference levels and assessed between various groups. The mean femoral head diameter at the four reference levels for the South Indian population was endosteal diameter 20 mm above lesser trochanter (D)- 43.79±5.2 mm, endosteal diameter at the level of lesser trochanter (E)- 27.35±4.3 mm, endosteal diameter 20 mm below lesser trochanter (F)- 19.92±3.5 mm Endosteal diameter at the isthumus (G)- 13.8±2.8 mm. There was no statistically significant difference between the endosteal diameters of South Indian and Malay population when matched against present study at the level of D (20 mm above lesser trochanter) and F (20 mm below the lesser trochanter). However at the level of G (isthumal diameter) it was found to be smaller in the Malay population (9.73±1.8 mm). These differences were statistically significant. There is no statistically significant difference in the mean canal diameters at the four reference levels when Indian femora were matched with the Swiss population indicating a close resemblance in terms of endosteal dimensions. There was no statistically significant difference between the endosteal diameters of the South Indian population (present study) and the French population. The endosteal widths of Indian femora was smaller by 2.0 mm in comparison to the Caucasian femora at all 4 reference levels which was statistically significant ($P < 0.05$). Even with a magnification error of 3 mm between present study and Caucasian study the endosteal dimensions are smaller by 2 mm in comparison to Caucasian femora. This suggests that the actual difference is much more.

Offset

The mean offset in the Indian population was 42.75±4.3 mm. The offset values of the South Indian population were compared with the Malay population- 31.50±5 mm. The offset of the Malay population was found to be significantly smaller than the South Indian population. The offset values of the South Indian population were compared with the Japanese population– 30.45±4.26 mm. The offset of the Japanese population was found to be significantly lower than the South Indian population. The offset of the South Indian population was compared with the French population who had a mean offset of 41±6.20 mm. The offset of the French population was close to that of the South Indian population and the difference was not statistically significant. The offset of the South Indian population was compared with the Swiss population who had a mean offset of 47±7.2 mm which was the highest among the ethnic groups in study. The mean offset of the South Indian population was 4.25mm lower than the Swiss population. This probably correlates with the bigger size of the femora among European population. There is no significant difference between the offset of South Indian population and Caucasian population- 43±6.8 mm.

Canal flare index

The mean canal flare index in the Indian population was found to be 3.23.The mean canal flare index of the South Indian population was compared to the Caucasian population which had a canal flare index of 3.8. The difference was statistically significant. The canal flare index of the South Indian population was compared with the French population who had a canal flare index of 3.6. The differences were found to be statistically significant between South Indian and French population, in terms of trending of the canal flare index. This probably suggests a better bone stock in the European population, with the European populations having more Champagne flute configuration of canal flare, therefore will favour more of an un-cemented fixation.

Neck shaft angle

The mean neck shaft angle was found to be 126 degrees in the present study of South Indian population. The verge neck shaft angle of the South Indian population was compared with the Japanese population. The Japanese population had an average valgus femoral angulation with mean neck shaft angle of 137 degrees in comparison to South Indian population.

The neck shaft angle of the South Indian population was compared with the Malay population. The mean neck shaft angle of the Malay population was 130 degrees. There was a slight inclination towards valgus mean femoral angulation in the Malay population when compared with the South Indian population in the present

study. There was no statistically significant difference between the neck shaft angles of present study and Caucasians -125 degrees. Analysis thus indicates more valgus femoral neck shaft angulations Asian population groups.

CONCLUSION

The canal flare index was found to be decreasing with age which is in correlation to the age related decrease in the femoral bone stock. The Asian and Indian femur bone is of much smaller sizes in comparison to European femurs.

Limitations of study

The radiographic study had a mean magnification of 3 mm. There was radiation exposure to normal subjects who have volunteered for the study. The age of the dry bones was not available.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Charnley J. Anchorage of the femoral head prosthesis to the shaft of the femur. J Bone Joint Surg Br. 1960;42:28-30.
2. Charnley J. The Lubrication of animal joints. In: Symposium on Biomechanics. Institution of Mechanical Engineers. 1959: 12-22.
3. Atilla B, Oznur A, Caglar O, Tokgozoglu M, Alpaslan M. Osteometry of the femora in Turkish individuals: a morphometric study in 114 cadaveric femora as an anatomic basis of femoral component design. Acta Orthop Traumatol Turc. 2007;41(1):64-8.
4. Noble PC, Alexander JW, Lindahl LJ, Yew DT, Granberry WM, Tullos HS. The anatomic basis of femoral component design. Clincial Orthopedics Rel Res. 1998:34(1)227-35.
5. Baharuddin. Morphology study of the proximal femur in Malay population. Int J Morphol. 2011;29(4):1321-5.
6. Ravichandran D. The proximal femoral geometry in Indians and its clincial applications. J Anat Soc Ind. 2011;60(1):6-12.
7. Singh, Bhasin. Anthropometry. 1st edition. Delhi: Educational Publishers; 1968: 142.
8. Parsons PG. The characteristics of the English thigh bone. J Anat Physiol. 1914;48:238-67.
9. Kate BR. The angle of the femoral neck in Indians. Eastern Anthropologists. 1967;20:54-60.
10. Humphrey. The angle of neck with the shaft of the femur at different periods of life and at different circumstances. J Anat Physiol. 1958;23:273.
11. Isaac B, Vettivel S, Prasad R, Jeyaseelan L, Chandi G. Prediction of the femoral neck-shaft angle from the length of the femoral neck. Clin Anat.1997;10(5):318-23..
12. Chauhan. The anatomic parameters of the North Indian hip joint- a cadaveric study. J Anat Soc India. 2002;51:39-42.
13. Siwach. Anthropometric study of the proximal femur geometry and its clinical applications. Indian J Orthopaedics. 2003;37(4):247-51.
14. Rubin. The morphology of proximal femur – a three dimensional radiographic analysis. J Bone Joint Surgery (Br). 1992;74:28-32.
15. Massin P, Geais L, Astoin E. The anatomic basis for the concept of lateralised femoral stems: frontal plane radiographic study of the proximal femur. J Arthroplasty. 2000;15(1):93-101.
16. Mahaisavariya B, Sitthiseripratip K, Tongdee T Bohez EU, Vander Sloten J. Morphological study of proximal femur: a new method of geometrical assessment using 3 dimensional reverse engineering. Med Eng Phys. 2002;24(9):617-22.

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Mechanical comparison of reconstruction nail and dynamic condylar screw in the treatment of unstable Subtrochanteric fracture *in vitro* study

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Abstract
Background: Subtrochanteric femur fractures are severe injuries that are often associated with high-energy trauma in young age and a trivial fall in osteoporotic patients. These fractures constitute a major bulk of hip fractures and are difficult to treat.
Objective of this study was to determine the rigidity and strength of fixation and ultimate load of failure of certain composite femur models designed to simulate unstable subtrochanteric femur fractures fixed with DCS and Reconstruction nail.
Methods: Paired femora were osteotomized to produce like models of an unstable subtrochanteric femur fracture in vitro. The femora were fixed with the Reconstruction nail and a 95 degree dynamic condylar screw plate (DCS). Materials testing machine was used to apply compression to the femoral head through an adapter plate. Stiffness values were calculated from the load-deformation curves obtained.
Results: The Reconstruction nail-femur model was stiffer than the 95 degree DCS. The Reconstruction nail also had the highest ultimate load-to-failure than the 95 degree DCS.
Conclusion: Our findings suggest that the Reconstruction nail has greater stiffness and strength than the 95 degree dynamic condylar screw when tested in this model of an unstable subtrochanteric femur fracture.

Keywords: Subtrochanteric fractures, dynamic condylar screws, reconstruction nail

Introduction
Subtrochanteric femur fractures are severe injuries that are often associated with high-energy trauma in young age and a trivial fall in osteoporotic patients. These fractures constitute a major bulk of hip fractures [1]. These fractures are known to be difficult to treat successfully [2]. Anatomic and biomechanical variation in this region makes this area peculiar for the treating surgeons. The compressive stress in this area of the medial femur is as high as 1,200 pounds per square inch [3], which cause enormous stress on the fracture fixation devices. No single implant is ideal for all the Subtrochanteric fractures. Various implants used to address these fractures are 95 degree blade plate, 95 degree dynamic condylar screw, reconstruction nails and proximal femoral locking plate.
Through the years, numerous classification systems have been proposed for subtrochanteric fracture which have a prognostic importance and are of benefit in planning treatment.
Russell and Taylor [4] classified these fractures based on the involvement of the lesser trochanter and the piriformis fossa (Fig. 1). Intramedullary nail is the choice of implant in type I fractures as the fracture does not extend into the piriformis fossa. Extramedullary fixation with various plates is indicated in type II fractures in view of involvement of piriformis fossa. Loss of medial cortical contact and increased stress on the implant causes difficulty in treatment of Type IIB fracture.
Good results have been achieved using Dynamic condylar screw and reconstruction nail in unstable subtrochanteric fractures. The purpose of the present study is to compare the biomechanics of 95 degree dynamic condylar screw and reconstruction nail in resisting compressive loads in an unstable subtrochanteric fracture.

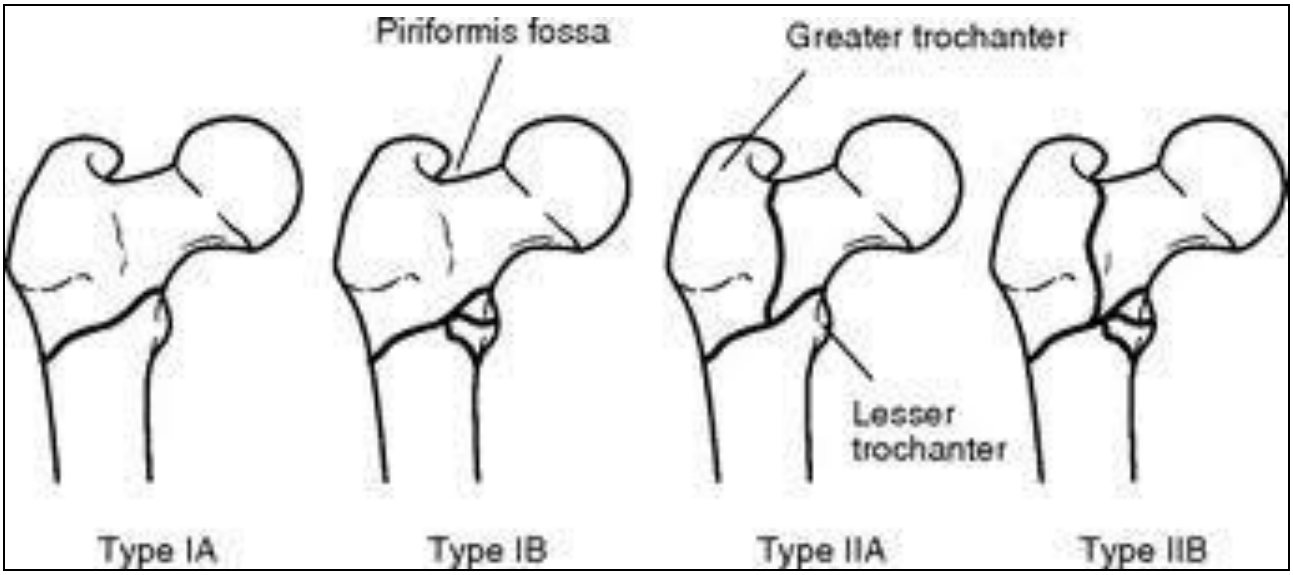


Fig 1: Russell Taylor classification of sub trochanteric fractures

Materials and Methods

Type of study: Observational study (in vitro)

Place: PSG IMS&R, Coimbatore

Selection: 3 Paired adult fresh cadaveric femora were used.

They were osteotomized to produce like models of an unstable subtrochanteric femur fracture. The femora were fixed with the Reconstruction nail and a 95 degree dynamic condylar screw plate (DCS).

The femora were placed into the materials testing machine with the femoral head placed in the metal mount over the base and the condyles superiorly held by the metal plate of the machine. The femora were placed vertically and loaded in compression to 500 newtons at a rate of one millimeter/second and a load displacement curve was obtained.

The Synthes 95 degree dynamic condylar screw plate (DCS) chosen (stock number S040-95-08) was a six-hole plate. A 75-millimeter lag screw was placed into the proximal fragment, and the plate was inserted over the lag screw and secured with the compressing screw. A 6.5-millimeter cancellous screw (70 millimeters long with a 32-millimeter thread) was placed into the most cephalad hole in
the plate. The next hole was left empty, and the last four holes were filled with bicortical 4.5-millimeter screws.

The Reconstruction nail used was 09x 380mm size (stock number SMPL, S182-09L-380) and the two proximal screws were of 6.4x95mm and 6.4x80mm size and locked distally with cortical screws.

The femora were prepared for the DCS and Reconstruction nail before the osteotomy was cut. The lag screw was placed over a guide wire in the specimen receiving the DCS. The osteotomies were cut in a similar manner in both the femora. A 30 degree osteotomy was cut, leaving a two centimeter defect on the medial side of the femur. The plates were then fixed to the distal fragment with 4.5-millimeter cortical screws. Failure was determined by a sudden change in the load deformation curve. Each of the femora was then radiographed and examined to determine the cause of failure.

The stiffness of each system was determined by measuring the slope of the load deformation curve. These stiffness values and the load-to-failure values were tabulated and averaged. The measurements were analyzed by analysis of variance to determine statistical significance.

Results

From Compression Testing of Simple Bone

At the peak load the bone suddenly fractured completely as shown in the figure 2

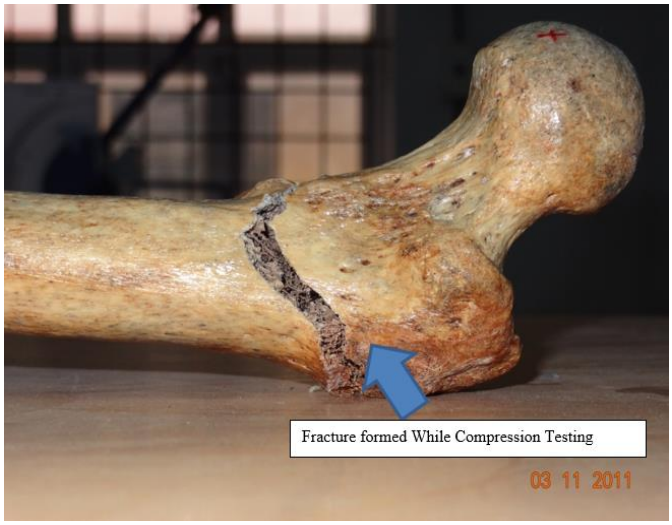


Fig 2: Fractured simple bone

The fracture is created due to the load application of load in the compression testing. When the bone is placed at a 6 degree angle as like in the human body

The fracture happened as shown in the fig 2. if the one is placed perpendicular loading arm the fracture is in the middle also breaking load also increases.

The peak load from this compression testing of the simple bone is given as he force value for Finite element analysis using ANSYS for study of the stress distribution over bone while compression loading.

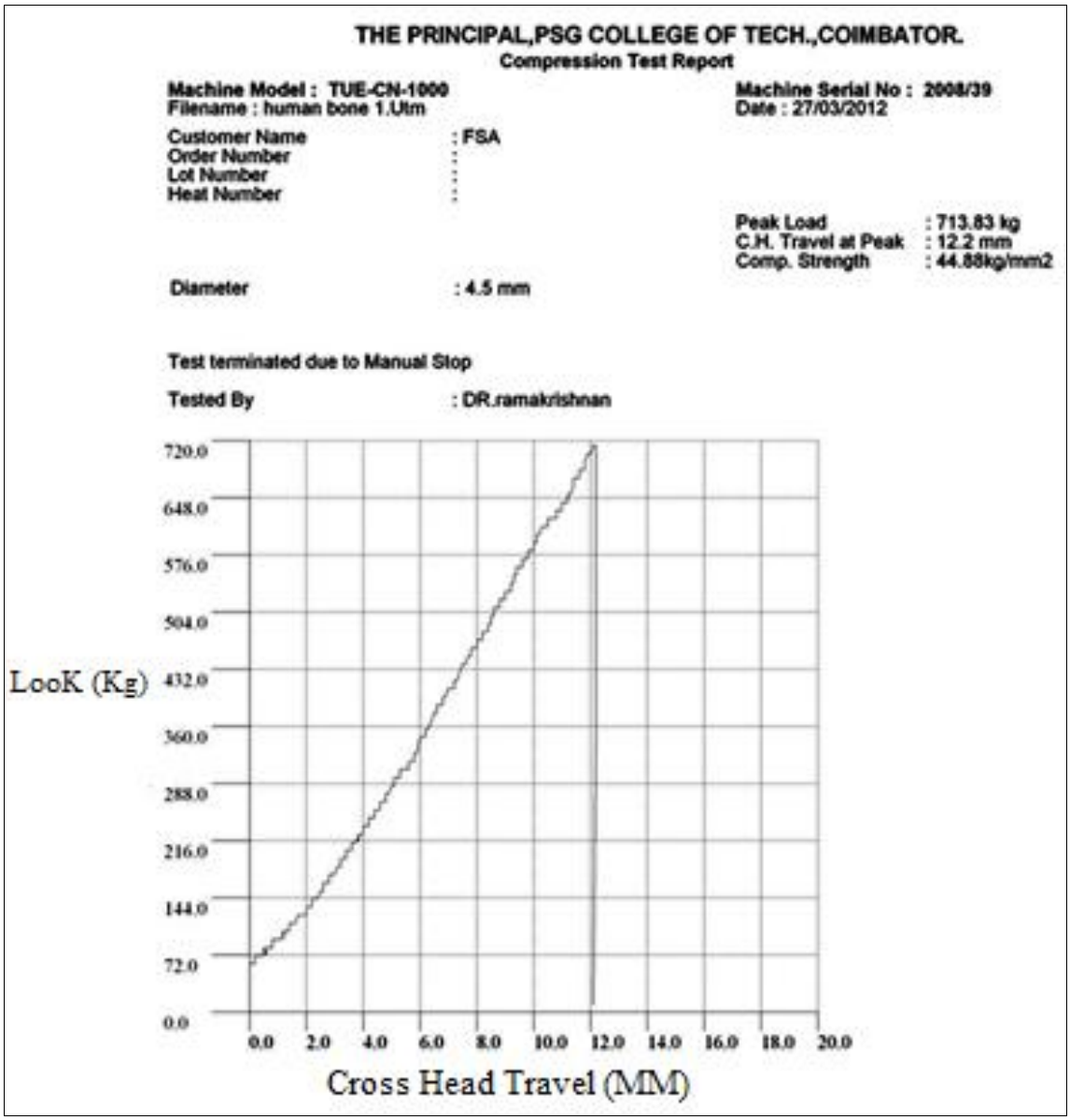


Fig 3: Load deformation curve for simple bone

The peak load at which bone Breaked is 713.83 kg and cross head travel that is deflection of the specimen is 12.2mm. The final result compressive strength is 44.88kg/mm2 was shown in the Fig 3

For testing dynamic condylar screw (DCS) Implant

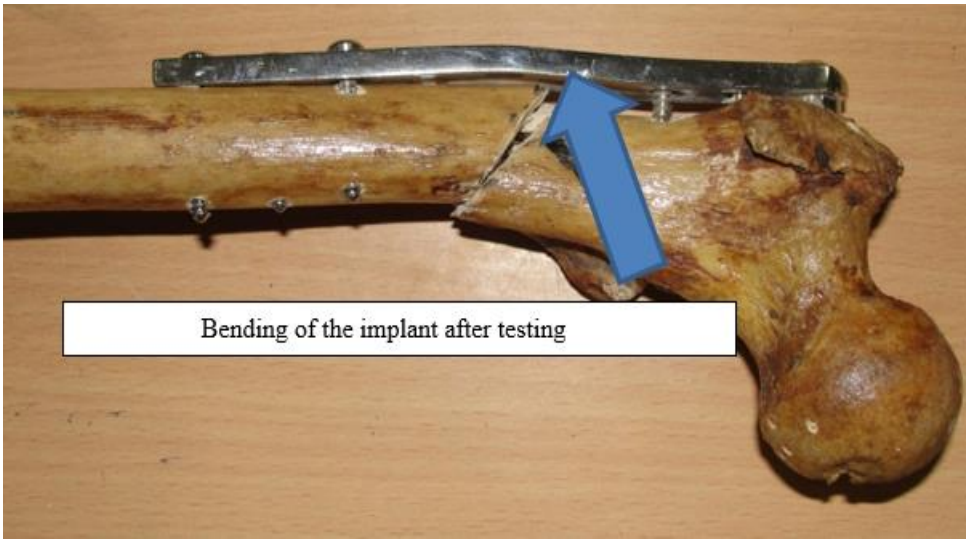


Fig 4: Failure of the DCS Fixed Bone

While testing, the bones shattered into pieces are brought together shown in fig 4 the fractured part i.e., artificial fracture created for testing the implant stability is the one first get affected and broken early while loading. At initial loading bone itself takes all the load so its obvious for fractured part to gets affected

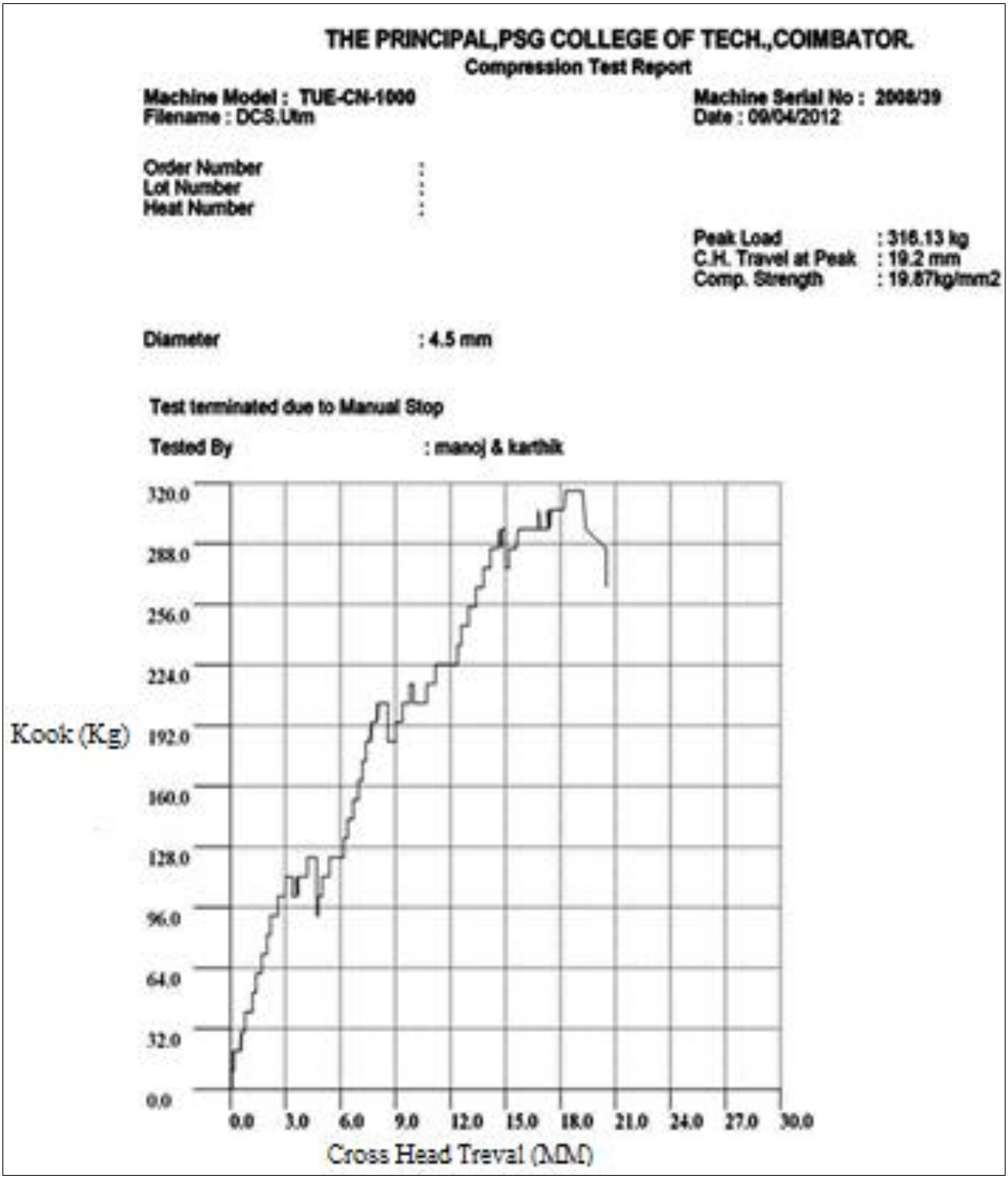


Fig 5: Load deformation curve obtained for DCS

The peak load at which implant failed is 316.13kg and cross head travel that is deflection of the specimen is 19.2mm. The final result compressive strength is 19.67kg/mm2 was shown in the fig 5.

For testing proximal femoral nail (PFN) implant

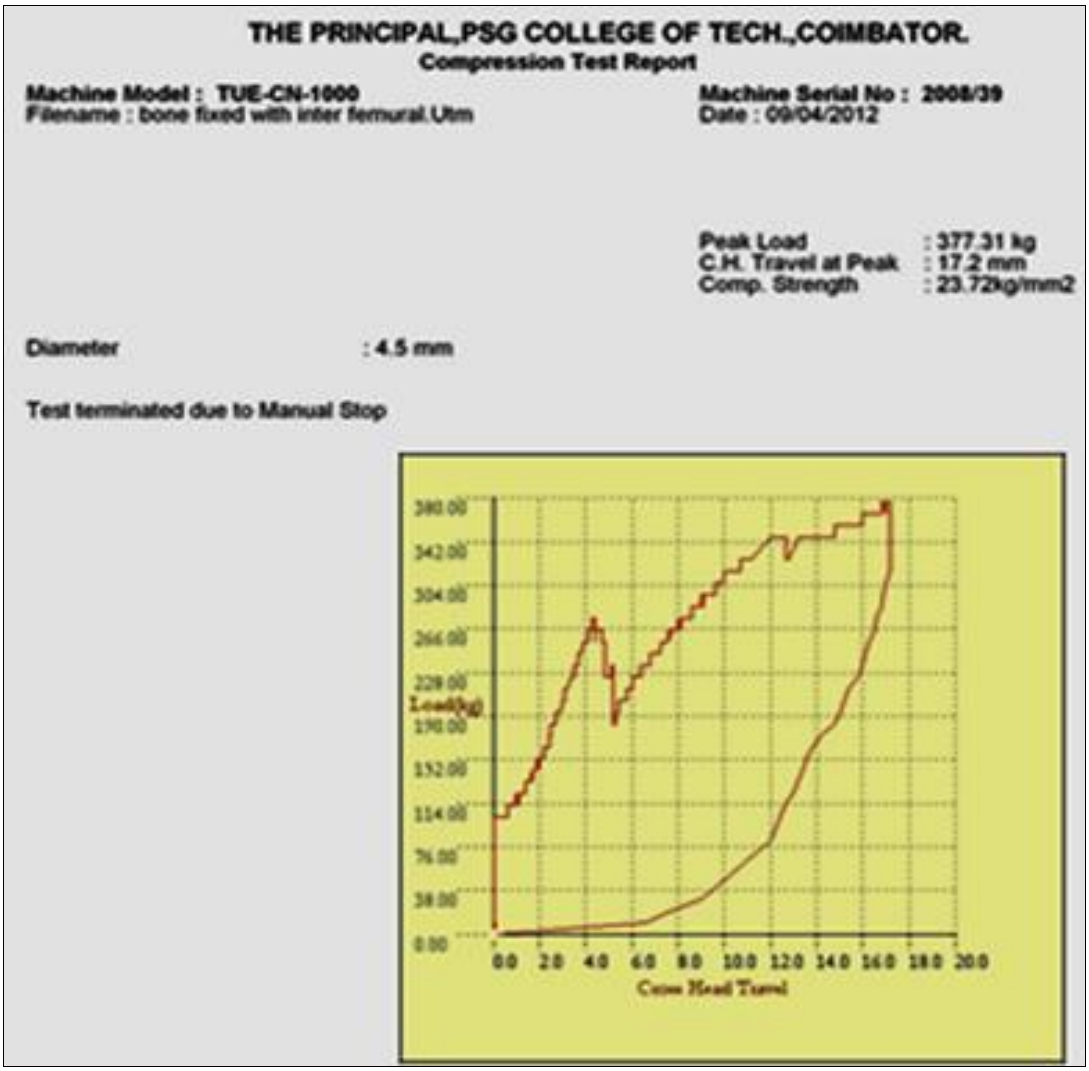


Fig 6: Load deformation curve obtained for PFN

The peak load at which implant failed is 377.31kg and cross head travel that is deflection of the specimen is 17.2mm. The final result compressive strength is 23.72kg/mm2 was shown in the fig 6

In both implant testing the loading is terminated manually at peak loads because of the significant deformation in the bone and implants is considered as failure of the implant. Compressive strength are generated based on the peak load. Simple bone's compressive strength is maximum because loading is done till the complete breaking of the bone



Fig 7: Failure of Proximal Femoral Nail fixed bone

The figure 7 & 8 shows the failure of the PFN fixed bone. PFN implant never showed any deformation while loading only the bone started to crack is considered as failure of the implant.

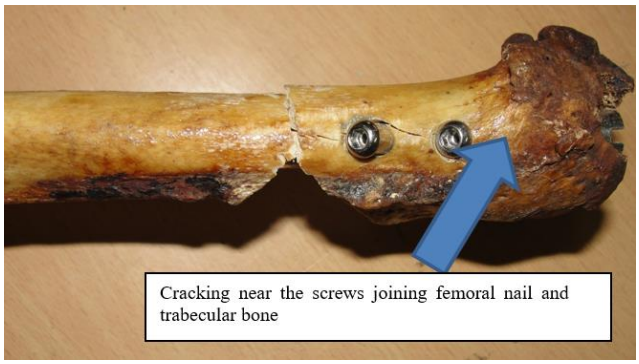


Fig 8: cracking of bone

Reconstruction nail femur composite demonstrated a stiffness of 665 newtons/millimeter and the DCS femur composite demonstrated a stiffness of 373 newtons/millimeter. Using ANOVA test the statistics were analsed. These differences were statistically significant ($p< 0.00001$).

The Reconstruction nail femur composite also had the highest load-to-failure (3773 Newton's), and for the DCS femur construct it is 3,161 newtons. These results were also statistically significant ($p< 0.00001$). The sudden change in the load-deformation curve coincided with an obvious deformation of the implant. Table 1

Discussion

Subtrochanteric fractures of the femur demands a vital place

in orthopedic traumatology, given the high rate of complications associated with their management due to the high loading forces and immense stresses in this area. An ideal implant should achieve stable fixation with no interference with the vascularity and hold the fracture till it unites. Fixation is a race between fracture healing and implant failure. Irrespective of the mode of fixation emphasis is laid on the medial cortex reconstitution as described in the study by Senter B *et al*. [5]. Intramedullary devices require less surgical exposure, enable early weight bearing and exert less biomechanical stresses (as the lever arm is moved medially) [6-8] However technical difficulties are observed in upto 63% of the cases [9, 10].

Comminuted subtrochanteric femoral fractures are often caused by high-energy trauma [12, 13] Fractures may extend into the greater and the inter-trochanteric regions [11]. Open reduction further devitalizes fragments, damages the vascular supply or soft tissues, and increases the risks of non-union, infection, and implant failure [12] whereas indirect reduction does not [19]. One case of implant failure (12.5%) is observed in fractures fixed with DCS by open reduction compared to failure rates of 20 to 23% in different studies [14, 15].

In this study the inference from the each load deformation curve is as follows.

Inference for DCS Curve

Graph indicates the increase in deformation as the load increases at the load of 122kg first failures takes place in the fractured section of the bone first drop in graph indicates this failure.

At the load of 204kg fracture gets more affected is noticed by propagation of crack all around the bone with crisping sound.

Implants start to bend at the load of 298kg that is considered as the failure of DCS fixed bone.

Inference for PFN Curve

As the load increases the deflection in the bone also increases. Drop in the deflection curve at 270 kg indicates the first failure at the fractured section of the bone after the first failure the continuous loading results in cracking of the bone at the fractured section at 350kg

Conclusion

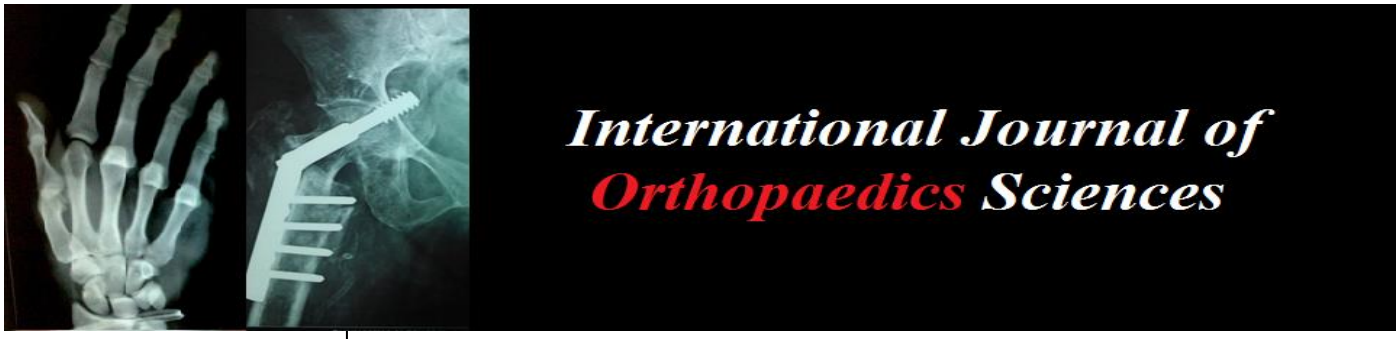
Taking into consideration that the femoral nail implant is way tougher than the DCS implant the failure can be verified with the fracture of the bone only.

Since the inter femoral nail fails at load higher than the DCS implant it can be inferred that:

- The inter femoral (PFN) is found as more stable implant followed by dynamic condylar screw (DCS) to treat unstable sub trochanteric fracture.
- The rate of displacement of the head also is less at a higher force when compared with that of the DCS and hence it can be inferred that PFN also resists displacements.
- In the failure pattern the implant did not break in PFN but only the fracture occurred between the two screws hence again confirming that PFN is a rigid implant.

References

1. David G. Lavelle. Fractures and dislocations of the hip. In: Canale ST, Beaty JH, eds. Campbell's operative orthopaedics, 11th edn. Philadelphia, Pa: Mosby Elsevier; 2007, 52.
2. Asher MA, Tippet JW, Rockwood CA, Zilber S. Compression Fixation of subtrochanteric fractures. CORR, 117
3. Koch JC. The laws of bone architecture. Am J Anat. 1917; 21:177-298.
4. Russell TA, Taylor JC. Subtrochanteric fractures of the femur. In: *Skeletal Trauma*, ed by Browner BD, Jupiter JB, Levine AM, Trafton PG. Philadelphia, WB Saunders Company, 1992, 1485-1524.
5. Senter B, Kendig R, Savoie FH. Operative stabilization of subtrochanteric fractures of the femur. J Orthop Trauma. 1990; 4(4):399-405.
6. Ramakrishnan M, Prasad SS, Parkinson RW, Kaye JC. Management of subtrochanteric femoral fractures and metastases using long proximal femoral nail. Injury. 2004; 35:184-90.
7. Curtis MJ, Jinnah RH, Wilson V, Cunningham BW. Proximal femoral fractures: A biomechanical study to compare intramedullary and extramedullary fixation. Injury. 1994; 25:99-104.
8. Charnley GJ, Ward AJ. Reconstruction femoral nailing for nonunion of Subtrochanteric fracture: A revision technique following dynamic condylar screw failure. Int Orthop. 1996; 20:55-7.
9. Boldin C, Seibert FJ, Fankhauser F, Peicha G, Grechenig W, Szyszkowitz R. The proximal femoral nail (PFN)-a minimal invasive treatment of unstable proximal femoral fractures: A prospective study of 55 patients with a follow up of 15 months. Acta Orthop Scand. 2003; 74:53-8.
10. Garnavos C, Peterman A, Howard PW. The treatment of difficult proximal femoral fractures with the Russell-Taylor reconstruction nail. Injury. 1999; 30:407-15.
11. Siebenrock KA, Muller U, Ganz R. Indirect reduction with a condylar blade plate for osteosynthesis of subtrochanteric femoral fractures. Injury 1998; 29(3):C7-15.
12. Kinast C, Bolhofner BR, Mast JW, Ganz R. Subtrochanteric fractures of the femur. Results of treatment with the 95 degrees condylar blade-plate. Clin Orthop Relat Res. 1989; 238:122-30.
13. Celebil, Can M, Muratli HH, Yagmurlu MF, Yuksel HY, Bicimoglu A. Indirect reduction and biological internal fixation of comminuted subtrochanteric fractures of the femur. Injury. 2006; 37:740-50.
14. Nungu KS, Olerud C, Rehnberg L. Treatment of subtrochanteric fractures with the AO dynamic condylar screw. Injury. 1993; 24:90-2.
15. Kulkarni SS, Moran CG. Results of dynamic condylar screw for subtrochanteric fractures. Injury. 2003; 34:117-22.



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A modified dynamic hip screw in unstable intertrochanteric fracture: A retro-prospective study

Sandeep MMR and Dinakar Rai

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Abstract
Background: Intertrochanteric fractures occur mostly in elderly patients, and the outcome may be extremely poor if there is prolonged bed-rest.
Many treatment modalities have come up in management of these unstable intertrochanteric fractures. Sliding Hip Screw Fixation is still the gold standard in treatment of stable intertrochanteric fracture. In unstable comminuted intertrochanteric fractures, there is high incidence of failure in view of excessive collapse seen with Dynamic hip screw.
In order to limit the collapse we have done a modification on Dynamic Hip Screw implant. The aim of this study is to assess fracture healing, collapse and implant failure, in unstable intertrochanteric fractures (Boyd and Griffin type-2) treated by modified DHS fixation.
Methods: 23 patients who were operated with modified DHS for unstable intertrochanteric fracture were selected using random selection and following parameters were noted from the records.
Results: Out of 23 patients in this study, 20 patient showed fracture healing (87%) with or without minimal collapse and 3 patient had non-union (13%) at the end of 5 months follow up. Ultimately all fractures united at the end of 1 year followup.
Conclusion: Modified Dynamic Hip Screw has showed improved results as compared to normal Dynamic Hip Screw in treating communited intertrochanteric fracture, which limits the collapse at fracture site.

Keywords: Intertrochanteric fractures, modified DHS, collapse

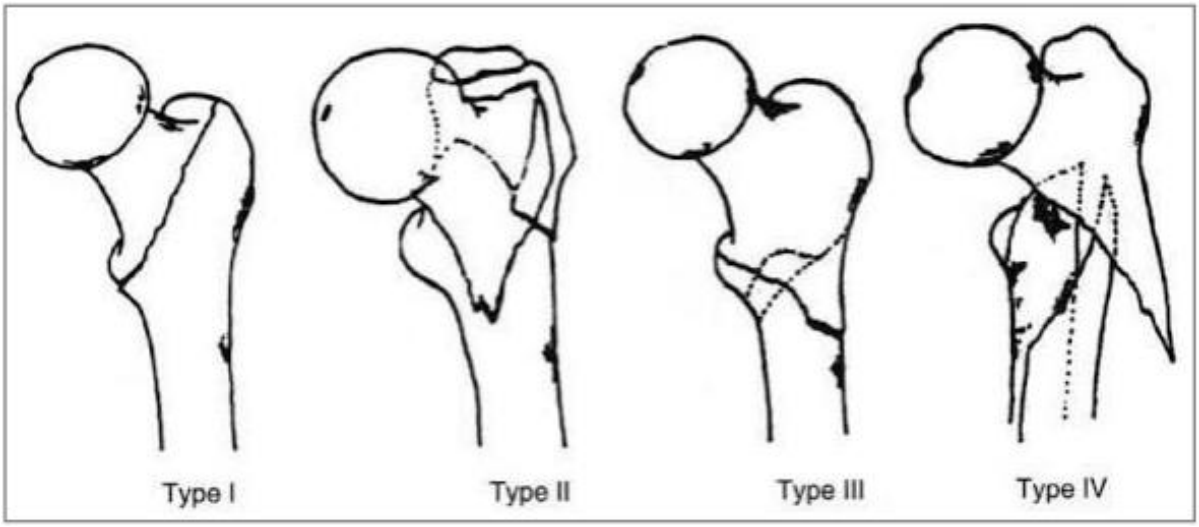
Introduction
Intertrochanteric fractures occur mostly in elderly patients, and the outcome may be extremely poor if there is prolonged bed-rest. The best treatment for trochanteric fracture remains controversial [1-3]. Many methods have been recommended [4-6]. Early mobilization of these patients reduces the morbidity and mortality rate.
Intertrochanteric fractures with severe communition and displacement are commonly seen in elderly patients. Most of the elderly patients have poor bone quality due to which fractures are often associated with complications like non-union, implant failure and femoral head perforation.
Management of unstable intertrochanteric fractures is challenging because of excessive collapse, loss of fixation, implant failure, lag screw cut out and in addition osteoporosis also adds to the complication resulting in unpredictable outcome.
In order to limit the collapse we have done a modification on Dynamic Hip Screw implant. In this study we are assessing the outcome of unstable intertrochanteric fractures treated with modified dynamic hip screw fixation designed by us.
Boyd and Griffin (1949) classified fractures in the trochanteric area of femur into 4 types. Their classification included all fractures from extra capsular part of neck to a point 5cm below the lesser trochanter. Their classification is useful in planning treatment and estimating prognosis [7].

Type 1: Fractures that extend along the intertrochanteric line from the greater to the lesser trochanter. Reduction usually is simple and is maintained with little difficulty. Results generally are satisfactory.

Type 2: Comminuted fractures, the main fracture being along the line, but with multiple fractures in the cortex. Reduction of these fractures is more difficult because the comminution can vary from slight to extreme. A particularly deceptive form is the fracture in which an anteroposterior linear fracture occurs, as in type 1, but with an additional fracture in the coronal plane, which can be seen on the lateral radiograph.

Type 3: Fractures that are basically subtrochanteric with at least one fracture passing across the proximal end of the shaft just distal to or at the lesser trochanter. Varying degrees of comminution are associated. These fractures usually are more difficult to reduce and result in more complications during surgery and convalescence period.

Type 4: Fractures of the trochanteric region and the proximal shaft, with fracture in at least two planes, one of which usually is the sagittal plane and may be difficult to see on routine anteroposterior radiographs. If open reduction and internal fixation are used, twoplane fixation is required because of the spiral, oblique, or butterfly fracture of the shaft.



Aim of the study

The aim of this study is to assess fracture healing, collapse and implant failure, in unstable intertrochanteric fractures (Boyd and Griffin type-2) treated by modified DHS fixation.

Objectives

1. To assess fracture healing, collapse in management of communited intertrochanteric fracture with modified Dynamic Hip Screw.
2. To look for implant failure- migration of implant, implant loosening, implant cutout or non-union.

Materials and Methods

Study Design: Retrospective observational study population: Patients diagnosed to have intertrochanteric fractures and treated with Modified DHS Study Area : PSG Hospitals.

Inclusion criteria

1. Boyd and griffin classification type-2 fractures only
2. Patients willing for treatment and given written informed consent.

Exclusion criteria

1. Boyd and griffin classification type-1, type-3 and type -4
2. Pathological fractures
3. Infection
4. Treated after 3 weeks of trauma.
5. Patients medically unfit for surgery.
6. Compound fractures associated with vascular injuries, ipsilateral femoral shaft fractures and pelvic fractures.
7. Patients not willing for treatment.

23 patients who were operated with modified DHS for unstable intertrochanteric fracture were selected using random selection and following parameters were noted from the records.

The criteria taken into consideration were fracture healing , collapse of fracture fragments, implant failure

About Modified Dynamic Hip Screw

One of the cause for failure of Dynamic Hip Screw is over collapse at fracture site [8].

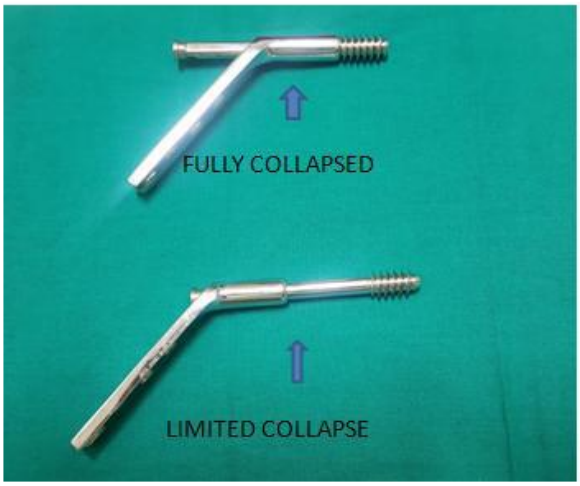
In this study, we have used modified dynamic hip screw to limit the over collapse which is undesired in unstable intertrochanteric fractures. In this implant we have done modification in the shaft of lag screw, we have reduced the length of keyed screw system, so that we can have a maximum of 1 cm collapse, hence there will be limitation in collapse unlike the original implant.



Modified lag screw versus Normal lag screw



Before collapse



After collapse

Results

In this retro-prospective study a total of 23 patients were assessed. Pre-operative

AP radiographs of pelvis was taken for all the patients who were treated by modified dynamic hip screw fixation. All patients who underwent treatment were of age from 38 years to 90 years. Out of 23 patients in this study, 13 patients were male (56.5%) and 10 patients were female (43.5%). Only boyd and griffin type-2 fractures were taken in view of standardisation. Post-operatively follow up xray were taken at 1st month, 3rd month and 5th month and the outcome was assessed.

Factors assessed were

1. Fracture healing
2. Fracture collapse
3. Implant failure

Fracture Healing

1st month 3rd month 5th month

	1st month	3rd month	5th month
Union	8(34.8%)	20(87%)	20(87%)
Non union	15(65.2%)	3(13%)	3(13%)
Total	23(100%)	23(100%)	23(100%)

Out of 23 patients in this study, at first month follow-up, 8 patients showed fracture healing (34.8%), at third month follow-up, 20 patients showed fracture healing (87%), at fifth month follow-up, 20 patient showed fracture healing (87%) and three patient had non-union (13%)

Fracture Collapse

	1st month
Minimal collapse	16(69.6%)
No collapse	7(30.4%)
Total	23(100%)

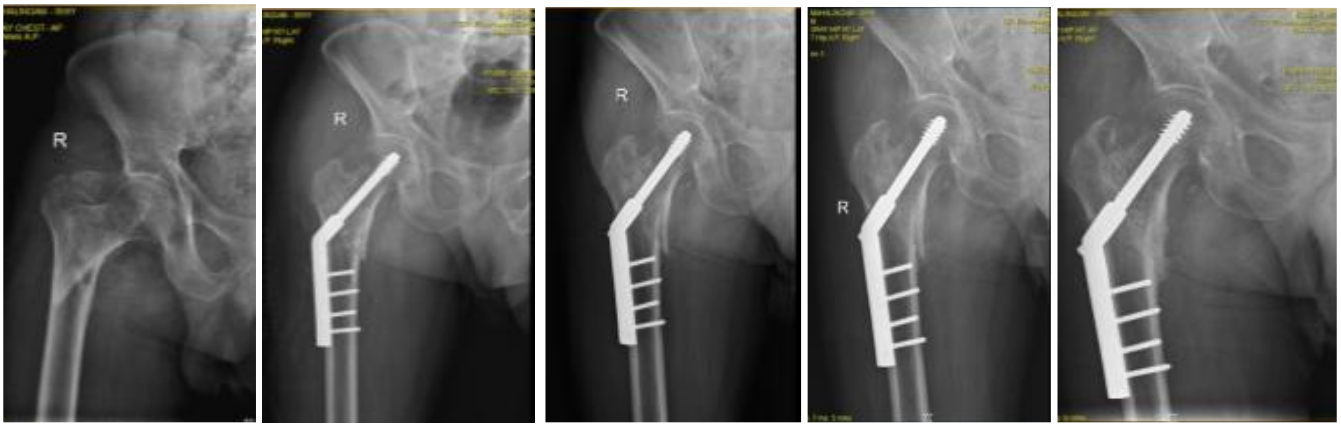
Out 23 patients in this study, at first month follow-up, 7 patients showed no Collapse (30.4%) and 16 patients showed minimal collapse (69.6%) [less than 1 cm], however the collapse rate remained same for all patients till end of this study.

Implant Failure

	1st month	3rd month	5th month
No implant failure	19(82.6%)	19(82.6%)	19(82.6%)
Lag screw migration not breaching cortex	4(17.4%)	4(17.4%)	4(17.4%)

At the end of 5 months followup out of 23 patients, 4 patient had implant migration not breaching cortex (17.3%).

Radiographs



Pre op Post op 1st month 3rd month 5th month

Discussion

Intertrochanteric fractures are mainly treated by surgical intervention. Despite longterm experience in many centres, there have been factors still contributing to pooroutcome of managing unstable intertrochanteric fractures.

Controversies persist because there is lack of proper per-

operative risk factors assessment that affect the outcome in these fractures treated by various methods. Failures of Dynamic hip screw in unstable intertrochanteric fracures are due toover collapse at fracture site [8].

In this study, we have used modified Dynamic Hip screw for treating comminuted intertrochanteric fractures, we have used this implant to limit the over collapse at fracture site.

Fracture united in 20 patients with minimal collapse and 3 patients had no signs of union at the end of 5 months. But all fractures united at the followup period of 1 year, lag screw migration occurred in 4 patients who started early weight bearing.

Nordin S in their study on treatment of intertrochanteric fractures with Dynamic Hip Screw, 83.3 percent of patients had fracture healing at one month [9]. In our seriesonly 34.8 percent of patients showed fracture healing at first month, however at the endof fifth month 87% fractures went on to heal. This delay in fracture healing relates to the modification in design of implant which limits the collapse at fracture site.

Sadowski cal *et al.* in their study on treatment of unstable intertrochanteric fractures with sliding hip screw, implant failure and non-union was noted in 7 of 19patients (38%) who had been treated with the sliding hip screw [10]. In our series, implant migration was noted in 3 out of 23 patients (13%) but no breaching of cortex occured. This results compare favourably and highlights the advantage of modified Dynamic Hip Screw in treatment of communited intertrochanteric fractures.

Conclusion

Modified Dynamic Hip Screw has showed improved results as compared to normal Dynamic Hip Screw in treating communited intertrochanteric fracture.

1. Fracture healing was slow in comparing with regular Dynamic hip screw.
2. Modified Dynamic hip screw significantly limits the collapse at fracture site.
3. When modified Dynamic Hip Screw is used in managed of communited intertrochanteric fracture, weight bearing mobilisation should be delayed to improve the outcome.

Limitations

Small sample size

Other forms of fixation of trochanteric fractures were not compared in this study.

References

1. Babhulkar S. Management of trochanteric fractures. Indian Journal of Orthopaedics. 2006; 40(4):210
2. Rockwood C, Green D, Bucholz R. Rockwood and Green' fractures in adults, Philadelphia: Lippincott Williams & Wilkins, 2006.
3. Singh A, Thong G, Laloo N, Singh A, Singh S. Management of trochanteric fractures. Indian Journal of Orthopaedics. 2006; 40(2):100.
4. Cooper A. Fractures and Dislocations of Joints.10th ed. London, 1839
5. Bick E. Source Book of Orthopaedics. Newyork: hafner, 1968.
6. Heyse-moore *et al*. treatment of intertrochanteric fractures of femur JBJS. 1983; 65-B:262-267
7. Whitmann R. A new method of treatment for fractures of the neck of the femur, together with remarks on coxavara. Ann Surg. 1902; 36:746.
8. Mohan N, Chandrashekar H. An Analysis of Failure of Dynamic Hip Screw with Plate in the Management of Intertrochanteric (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. 2014; 13(3, IV):54-57
9. Nordin S, Mechanical fixation of DHS in intertrochanteric fracture Med J Malaysia. 2001; 56(D):12-7.
10. Sadowski CAL. Treatment of reverse oblique and transverse interrochanteric fractures with use of an intramedullary nail or a 95 deg screw. JBJS Am. 2002; 84;372-381
11. Capener N. The treatment of pertrochanteric fractures. JBJS Br. 1957; 39-b(3):436-437.
12. Taylor MN, Janzen J. Internal fixation for intertrochanteric fractures JBJS Am. 1944; 26(4):707-712.
13. Boyd HGLL. Classification and treatment of trochanteric fractures. Ach Surg. 1949; 58:853-863.
14. Boyd H, Anderson LD. Management of unstable trochanteric fractures. Surggynecol Obstrtrics 1961; 55:853-863.
15. Bendo JA, Weiner LS, Strauss E *et al*. Collapse of interochanteric hip fractures fixed with sliding screws. orthop Rev, 1944, 30-37.
16. Dimon JHJC. Unstable intertrochanteric fravtures. JBJSAm. 1967; 49(3):440-450.
17. Harrington KJ. The management of communited unstable intertrochanteric fracture. JBJS Am. 1973; 55(7):1367-1376
18. Sarmiento A. Intertrochanteric fractures of femur. JBJS. 1995; 75(3):90-92
19. Desjardins Ara, Paiement G Newman N, *et al*. Unstable intertrochanteric fracture of femur. JBJS Br. 1993; 75(3):445-447.
20. Gargan MGR, Simpson Ah. How effective are osteotomies for unstable intertrochanteric fractures

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Original Research Article

Platelet rich plasma in treatment of plantar fascitis

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ABSTRACT

Background: Platelet rich plasma (PRP) has been a breakthrough in the field of medicine especially in the field of orthopaedics for enhancing bone and soft tissue healing. Plantar Fasciitis is a very common problem in the field of orthopaedics and are very difficult to treat and a challenge to orthopaedicians. The aim of our study was to find out the efficacy of autologous PRP in relieving pain in patients with plantar fasciitis.
Methods: In this study, 25 patients with plantar fasciitis (age above 18 years) were selected. The pain intensity was assessed with visual analogue score initially and during follow up. All subjects were given single autologous intra-lesional PRP injection and the results were assessed using difference in VAS.
Results: The mean pain score at presentation was 8.08. The mean pain scores at subsequent intervals of 1,2,4,6 months after injection were 4.32, 2.60, 1.88 & 2.00 respectively. ($P<0.05$ which is statistically significant). The maximum pain relief was seen at 4th month post PRP injection and the pain relief was sustained till the last follow up.
Conclusions: From our study we have found that Intra-lesional autologous PRP is effective in relieving pain in patients with plantar fasciitis. PRP is biological and can be a safe adjuvant in the treatment of Plantar Fasciitis.

Keywords: Platelet rich plasma, Plantar fascitis, Visual analogue score

INTRODUCTION

Platelet rich plasma (PRP) has been a breakthrough in the field of medicine especially in the field of orthopaedics for enhancing bone and soft tissue healing. Plantar Fasciitis is a very common problem in the field of orthopaedics and are very difficult to treat and a challenge to orthopaedicians. Autologous PRP is a simple and cost effective means of treating plantar fasciitis by tissue regeneration.[1]

Plantar fasciitis is one of the most common causes of heel pain in adults. The cause of pain is due to collagen degeneration at the origin of plantar fascia. The plantar fascia is thickened fibrous fascia that originates from the medial tubercle of calcaneum and run forward to support the longitudinal foot arch.

The aim of our study was to find out the efficacy of autologous PRP in relieving pain in patients with plantar fasciitis.

METHODS

This is a prospective trial involving the patients in the department of orthopedics, PSGIMSR Coimbatore, Tamilnadu, India from Jan 2015 to Jan 2016.

In this study, 25 patients with plantar fasciitis (age above 18 years) were selected. The pain intensity was assessed

with visual analogue score initially and during follow up. All subjects were given single autologous intra-lesional PRP injection and the results were assessed using difference in VAS.

Selection of patients

Patients with history of pain and localized tenderness on the medial calcaneal tubercle [2].The classic sign is pain that occurs with few steps in the morning or at the start of walking which decreases as they warm up.[2]

Inclusion criteria

Patients clinically diagnosed to have plantar fasciitis, patients not responding to conservative treatment even after a minimum period of 3 months, both males and females, age- 18 years and above.

Exclusion criteria

Less than 3 month duration of plantar fasciitis, patients without any trial of conservative treatment, recent local steroid injection (less than 2 months), infection or ulcer at the injection site, rheumatoid arthritis, sero-negative spondyloarthritis, pregnant ladies, patients younger than 18 years, suspicion of diagnosis.

Methodology



Figure 1: Study design.

Method of preparation of PRP

PRP was prepared using double spin centrifugation method.

10 ml of venous blood is drawn from cubital vein. The blood is transferred into 2.7ml vaccutainer prefilled with acid citrate dextrose. Acid citrate dextrose will bind to calcium and prevents blood clotting with no known interference to platelet function and no contraindications for using in human beings. The blood is then transferred in the vaccutainer till the marking and centrifugation was done. Initially, with 1500 RPM for 3 minutes. After the initial rotations, blood was separated into plasma and RBC. The plasma will lie in the top with light yellow colour and was withdrawn and transferred into another container without mixing RBC. Later this container is again centrifuged with 2500 rpm FOR 3 MINUTES. After the 2nd centrifugation, the plasma is separated into 2 layers. The top half is platelet poor plasma and bottom half will be platelet rich plasma. The top half is discarded and bottom half is used for infiltration.

Technique of infiltration

Using a 21 Gauge, 1 and 1/2 inch needle, 1ml PRP is injected initially over the maximum tenderness point, and another 1 ml, was injected into the surrounding tissue with the same entry portal. This technique is known as peppering technique.

Pain scoring system

Visual analogue pain scoring system was used (Figure 2).

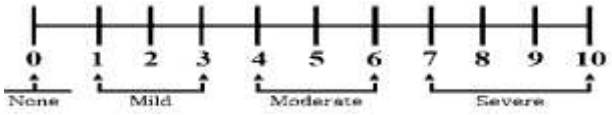


Figure 2: Visual analogue scoring system for assessment of pain.

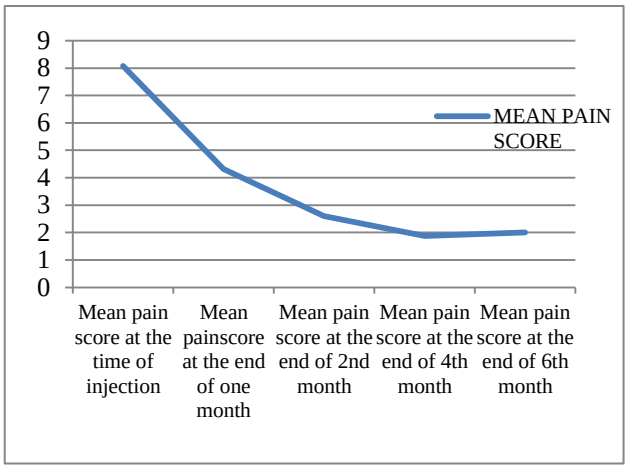


Figure 3: Mean pain score.

RESULTS

Patients were analyzed for pain relief subjectively at 0, 1, 2, 4 and 6 months. The results are given below.

Mean pain score

The mean pain score of patients with plantar fasciitis at 0, 1,2,4,6 months were 8.08, 4.32, 2.60, 1.88 & 2.00 respectively. While comparing the results at 0,1,2,4,6 months follow up, it was found that patients started getting pain relief at one month post injection ,which was statistically significant (p value <0.05). However the maximum pain relief was seen at 4th month post injection which was also statistically significant (p value <0.05).

Table 1: Showing mean pain scores at 0, 1, 2, 4 and 6 months intervals.

Patients receiving	Mean pain score at the time of injection	Mean pain score after	Mean pain score after	Mean pain score after	Mean pain score after
PRP		1 month	2 months	4 months	6 months
Plantar Fasciitis	8.08	4.32	2.6	1.88	2

Table 2: Showing Mean, T-Test & P-values at 0, 1, 2, 4 and 6 months intervals.

Pairs	Time interval	Mean	T-Test	P- Value
Pair 1	Pain at presentation & 1 month	4.32 ± 0.802	16.660; CI (3.29-4.22)	<0.00
Pair 2	Pain at presentation & 2 month	2.60 ± 0.645	33.309; CI (5.1-5.8)	<0.00
Pair 3	Pain at presentation & 4 month	1.88 ± 0.881	26.847; CI (5.72-6.67)	<0.00
Pair 4	Pain at presentation & 6 month	2.00 ± 0.957	24.877; CI (5.57-6.58)	<0.00

Statistical analysis

Statistical analyses were performed by SPSS for windows 19.0 software system version. Comparing the results at 0, 1, 2, 4, 6 months. P value for the test was taken as 0.05. Our study shows that the pain relief obtained following autologous PRP injection is statistically significant.

DISCUSSION

Platelet rich plasma (PRP) is also known as platelet rich concentrate, autologous platelet gel or platelet releasate. Platelet rich plasma is a portion of the plasma fraction of autologous blood with a concentration of platelets above the base line values.[3] In addition to platelet concentrate, autologous PRP contains complement of clotting factors and secretary proteins.[4] Normal platelet counts ranges between 150000/µL and 350000/µL, and average about 200000/µL in blood.[5] "PRP was introduced by M. Ferrari in 1987 as an autologous component in an open heart surgery to avoid homologous blood product transfusion".[6]

Platelets contain biologically active substance for blood clotting, such as coagulation factors, adhesive proteins and protease inhibitors. Platelets contain growth factors like TGF –beta 1, CGF, VEGF, and PDGF.[7,8] When activated these growth factors initiates the process of tissue healing by cellular proliferation and differentiation, chemo taxis, tissue debris removal, angiogenesis, and extra cellular matrix formation.[9,10] These properties of autologous PRP are used in the treatment of plantar fasciitis. An added advantage of autologous PRP is that it eliminates concerns about immunogenic reactions and disease transmission.[11]

Our study was a prospective study including 25 patients with plantar fasciitis (age above 18 years). The pain intensity was assessed with visual analogue score initially and during follow up. PRP was prepared by double spin centrifugation method and the platelet count in our samples ranged between three to six lakhs per cc. All subjects were given single intra-lesional PRP injection and the results were assessed using difference in VAS. In our study we did not use local anaesthetic agent which could bias our study by producing pain relief.

The results of our study matched with the results of the study by Ehab Mohamed Selem Ragab et al on 25 patients with plantar fasciitis, they concluded that average pain at the time of presentation was 9.1 and post autologous PRP injection was 1.6 at end of follow up (10.3 months).[1] Which was statistically significant ($p<0.001$).

In a comparative study by Ertugrul Aksahin et al they concluded that both PRP and corticosteroid treatment is equally effective in treating plantar fasciitis.[2]

The mean pain score at presentation was 8.08. The mean pain scores at subsequent intervals of 1,2,4,6 months after injection were 4.32, 2.60, 1.88 and 2.00 respectively. ($P<0.05$ which is statistically significant). The maximum pain relief was seen at 4th month post PRP injection and the pain relief was sustained till the last follow up.

The limitations of our study were that our study is not a randomized control double-blinded study, and the sample size was small and follow up was of shorter duration. The available data is insufficient to validate the efficacy of PRP in relieving pain in patients with Plantar Fasciitis. Further randomized control studies with large study population and the longer duration of follow up are needed to validate the efficacy of autologous PRP in the management of plantar fasciitis.

CONCLUSION

From our study we have found that Intra-lesional autologous PRP is effective in relieving pain in patients with plantar fasciitis. PRP is biological and can be a safe adjuvant in the treatment of Plantar Fasciitis.

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REFERENCES

1. Ragab EMS, Othman AMA. Platelet rich plasma for treatment of chronic plantar fasciitis. Arch Orthop Trauma Surg. 2012;132:1065-70.
2. Aksahin E, Dogruyol D, Yuksel HY, Hapa O, Dogan O, Celebi L, et al. The comparison of the effects of corticosteroids and platelet rich plasma for treatment of plantar fasciitis. Arch Orthop Trauma Surg. 2012;132:781-5.
3. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85:638-46.
4. Mehtra S, Watson JT. Platelet rich concentrate: basic science and current clinical applications. J Orthop Trauma. 2008;22(6)433-43.
5. Robert E Marx: platelet rich plasma what is prp and what is not PRP. Implant Dentistry. 2001;10;(4)225-8.
6. Ferrari M, Zia S, Valbonesi M, Henriquet F, Venere G, Spagnolo S, et al. A new technique for hemodilution, preparation of autologous platelet-rich plasma and intraoperative blood salvage in cardiac surgery. Int J Artif Organs. 1987;10(1):47-50.
7. Maynard DM, Heijnen HF, Horne MK, White JG, Gahl WA. Proteomic analysis of platelet alpha-granules using mass spectrometry. J Thromb Haemost. 2007;5(9):1945-55.
8. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med. 2009;37(11):2259-72.
9. Sanchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? : a current review. Int J Oral Maxillofac Implants. 2003;18:93-103.
10. Einhorn TA. The science of fracture healing. J Orthop Trauma. 2005;19(10):4-6.
11. Alsousou J, Thompson M, Hulley P,Noble A, Willet K. The biology of platelet rich plasma and its application in trauma and orthopaedic surgery. J Bone Joint Surg Br. 2009;91(8):987-96.

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Assessment of proximal femur anthropometry in South Indian population through cadaveric bones and radiologically correlating difference if any between other ethnic groups

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ABSTRACT

Background: The morphology of the proximal femur is a topic of extensive research. The hip joint is one of the most commonly replaced joint. The era of replacement has given rise to various implants that can be used to replace the proximal femur. This present study addresses these issues involving ethnic differences in the geometry of the proximal femur in Indians and its differences between people of various ethnicity.
Methods: The total population that was radiographed was 178 (n=178). There were 78 males and 100 females. The age of the participants were spaced from 25-75 years age groups. The parameters were measured.
Results: The canal flare index in South-Indians was an average of 3.23 with 70% of the study population having normal CFI (3-4.5), 30% of the population having a stove pipe configuration CFI (<3). Majority of the Indian population favour a un-cemented fixation (70%).
Conclusions: In summary all current implants have to be revised on population basis to fit the changing anthropometry of our proximal femur.

Keywords: Femur canal, South Indian population, Canal flare index

INTRODUCTION

The morphology of the proximal femur is a topic of extensive research. The hip joint is one of the most commonly replaced joint. The era of replacement has given rise to various implants that can be used to replace the proximal femur. The integral part of any replacement is to reproduce the biomechanics of the original joint in the prosthetic components in order to achieve good clinical outcome both in terms of patient and implant. Many of the conventional implant systems manufactured by various conglomerate companies are made in correlation with the sizes of the femora of Europeans.[1] The use of these implants in the Indian population, owing to its small size of the femur has been plagued with numerous complications like intra-op splintering of the proximal end of femur. It is also the bane of the Asian-Indian Orthopaedic surgeons to work with such ill-fitting implants.

Most implant systems are usually designed on the basis of European femora, which are believed to be larger than Asian population. The implantation of these prosthesis often results in problems like fractures of the proximal femur or less confirming prosthesis leading to loosening.[2]

This present study addresses these issues involving ethnic differences in the geometry of the proximal femur in Indians and its differences between people of various ethnicity. It also evaluates the adequacy of fit of the conventional femoral stems in Indian population.

The aim and objective of the study was to determine the proximal femur morphology in South Indian population determined with radiographs and from cadaveric dry bones and to determine the differences between anthropometry of South Indian population and other ethnic groups.

METHODS

This is a prospective study consisting of two fundamental parts, involved in assessing the anthropometric dimensions of the proximal femur. In the first part of the study we have evaluated anthropometry of 178 volunteers radiographically. The second part of the assessment was done with cadaveric femora obtained from the Department of Anatomy, PSGIMSR. 50 cadaveric dry femora were obtained and direct measurement of measurable anthropometric data was done. The study was conducted in PSGIMSR Coimbatore, Tamil nadu, India from August 2013 to August 2014.

Inclusion criteria were South Indian population and people of age 25 years and above. Exclusion criteria were no prior pathology in the femur- infection (old/healed/active), congenital anomalies, contractures and deformities around the hip, previous hip surgeries and open epiphysis.

Parameters templated

- Femoral head diameter
- Horizontal offset
- Neck shaft angle
- Endosteal diameter at a level 20 mm above lesser trochanter (D)
- Endosteal diameter at the summit of the lesser trochanter (E)
- Endosteal diameter at a level 20 mm below the lesser trochanter (F)
- Endosteal diameter at the level of the Isthumus (G-10 cm below lesser trochanter)
- Canal flare index.

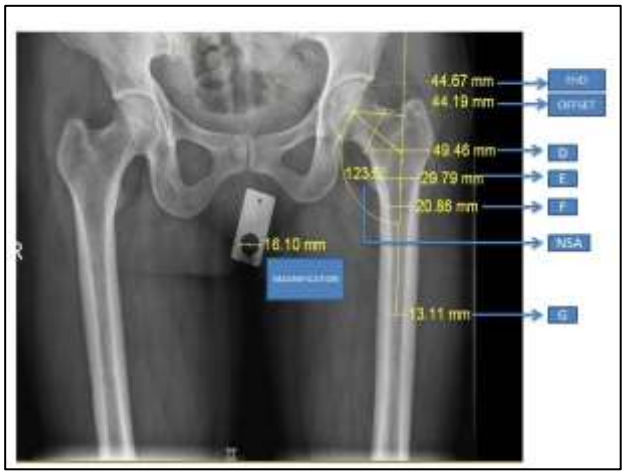


Figure 1: Radiograph templating.

Methodology of templating

Endosteal diameters

The endosteal width is measured at various locations based on reference lines which have been already defined as per Noble P C et al. The apex of the lesser trochanter is taken as the first landmark. The endosteal width at the level of the apex of lesser trochanter (E), endosteal width 20 mm above (D) and below (F) the lesser trochanter and 10 cm below the lesser trochanter (G) is measured. The mid-point of these endosteal width lines are connected to form the axis of the shaft of femur. One more point is taken measuring 10 cm distal to the apex of the lesser trochanter (G) which is considered as the isthumus. Endosteal width at that level is also measured.[3-5]

Femoral head diameter

The femoral head diameter is taken as the largest vertical diameter (superior-inferior) of head perpendicular to the axis of the neck of femur. The neck axis is drawn by drawing neck widths at 2 regions on the neck of femur, preferably in the trans-cervical and sub-capital region. The midpoints of these two lines are joined and extended further to form the axis of the neck. The femoral head diameter is measured perpendicular to this line taking the largest superior-inferior diameter of the femoral head.

Horizontal offset

The horizontal offset is also known as the actual femoral offset. The horizontal offset is measured as the distance between the centre of the femoral head to the axis of the shaft of femur. The x ray is taken in the said protocol mainly to reveal the proper and maximal offset of the femur.[6]

Neck shaft angle

The neck shaft angle is the angle subtended between the shaft axis and the axis of the neck of femur.[3,4,6]

Canal flare index

The ratio between the endosteal diameters 20 mm above the lesser trochanter (D) and at the level of isthumus (G) is called the canal flare index. Based on the values of canal flare index they were grouped in to normal (3-4.7) , champagne flute (high tapering in the proximal segment 4.7-6.5), stove pipe (a straight proximal femur relative to distal).[3]

The diameter of the magnification marker in the radiograph is determined for identification of the radiographic magnification error.

Cadaveric dry femora- study

The second part of the study involved with measuring the endosteal dimensions of cadaveric femur specimens. This was done to find out the true anthropometric parameters

of proximal femur in addition to knowing the fit of available femoral stems, So as to determine mismatch between implant and bone if any.

In our study we have taken 50 cadaveric femora. The cadaveric femora were cut at various positions so as to ascertain the endosteal dimensions at various regions of the cadaveric femur. The femora were cut using a motorized cadaveric cutting saw in the Department of Anatomy- PSGIMSR. The endosteal dimensions were measured using a Vernier calliper, at the regions mentioned below.



Figure 2: Cut sections of dry bones at various reference levels (left to right – D, E, F, G).



Figure 3: Method to measure neck shaft angle of cadaveric dry bone.

RESULTS

The mean femoral head diameter was 47.41 mm with a 95% confidence interval for the mean values, a standard deviation of ±4.5 mm. The mean offset in the study population was found to be 42.75±4.3 mm. The mean CFI was found to be 3.23±0.5.

Table 1: Results of radiographic study.

	Mean	SD	Minimum	Maximum
Age	47	10	25	75
Femoral head diameter	47.41	4.5	35	57
Medullary canal width				
D	43.79	5.2	33	61
E	27.35	4.3	17	43
F	19.92	3.5	12	31
G	13.88	2.8	9	23
Horizontal offset	42.75	4.3	34	53
Canal flare index	3.23	0.5	2	5
Neck shaft angle	126.03	4.6	114	137

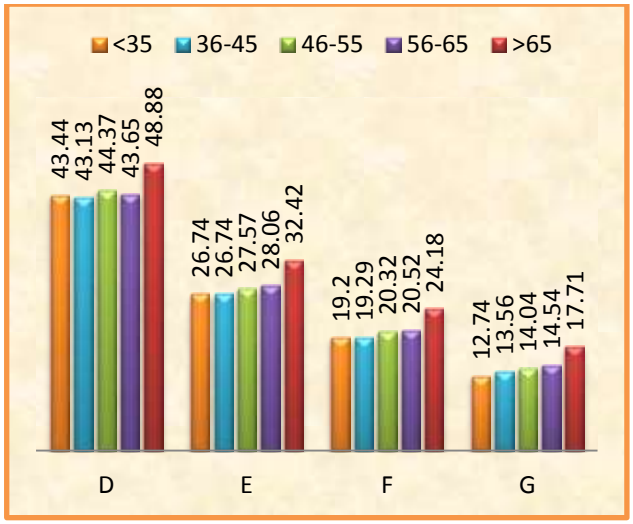


Figure 4: Mean medullary canal width at various levels matched with different age groups.

The above Figure 4 explains the difference in the medullary canal width as the age groups progressed there was a tendency towards increase in the mean width of the endosteal canal diameter.

Table 2: Age wise distribution of canal flare index.

Age group	Stove pipe(<3.0)	Normal (3.0-4.5)	Champagne	Total
<35	4	15	0	19
35-45	21	51	0	72
45-55	14	42	0	56
55-65	12	13	1	26
>65	2	3	0	5
Total	53	124	1	178
%	30%	70%	1%	100%

In the study population 30% had a stove pipe appearance of medullary canal with mean CFI <3. 70 % of the population had normal appearing femora with CFI ranging from 3-4.5 as shown in Table 2.

DISCUSSION

The aim of our study is to evaluate ethnic differences in the anthropometry of proximal femur of South Indians in comparison with other ethnic groups around the world.

The total population that was radiographed was 178 (n=178). There were 78 males and 100 females. The age of the participants were spaced from 25-75 years age groups. The following parameters were measured:

Femoral head diameter

The lowest femoral head diameter measured in our study was 35 and largest femoral head diameter in the study population was 57. The mean femoral head diameter was 47.41 mm with a 95% confidence interval for the mean values, a standard deviation of ±4.5 mm. The most prevalent head size in the given population was estimated in terms of mode for the given range, was found to be 49 mm.

Medullary canal diameters

Medullary canal widths were measured at various locations on the radiographs as described earlier in correlation to the prime seating points of the femoral stem. These levels are the standard reference points to address the changing geometry of the proximal femur. The mean and standard deviations were estimated for each parameter.

Mean canal widths at reference points-

- Endosteal canal width 20 mm above lesser trochanter (D)- 43.79±5.2 mm
- Endosteal canal width at the level of lesser trochanter (E)- 27.35±4.3 mm
- Endosteal canal width 20 mm below the lesser trochanter (F)- 19.92±3.5 mm
- Endosteal canal width at the level of isthumus (G)- 13.88±2.8 mm.

The distribution of endosteal canal widths at various levels were analysed with their age group wise distribution.

As the age groups progressed there was a tendency towards increase in the mean width of the endosteal canal diameter. This was most significant in the >65 years age groups. This was consistent with decreasing trend of canal flare index as age progressed.

Canal flare index

The mean and standard deviation for the same was calculated. The mean CFI was found to be 3.23±0.5. An age wise distribution of the canal flare index.

In the study population 30% had a stove pipe appearance of medullary canal with mean CFI <3.70% of the population had normal appearing femora with CFI ranging from 3-4.5.

There was progressive tendency towards stove pipe appearance of femoral endosteum as age progressed. Most strikingly noted in the >65 years age group with a mean canal flare index of 2.86. The younger age groups predominantly have a more normal type of femur. The distribution was found to be statistically significant in terms of distribution with progressive decline in the endosteal dimension as age progressed, and the femoral endosteum attaining a stove pipe like appearance ($p < 0.05$).

The gender wise distribution of the canal flare index was assessed, mean CFI in males was found to be 3.14±0.4, compared to the mean of 3.29±0.5 in the female pool. There were statistically significant differences in the gender wise distribution of the mean canal flare index ($P < 0.05$).

Horizontal offset

Horizontal offset calculated as the distance between the center of femoral head to the axis of the femoral shaft, which defines the abductor muscle tension was measured in all 178 subjects. The mean offset in the study population was found to be 42.75±4.3 mm.

Differences in anthropometric parameters of Indian femora vs. other ethnic population

The ethnic differences in the anthropometry of the proximal femur was assessed by matching the values of our present study with that of values previously measured in other ethnic populations. For the comparison we have taken the values of proximal femoral anthropometry of the Caucasian population according to the study by Noble P C et al (n-200), the importance of this study was its vitality in bringing up of the dimensions for creation of the various somatotypes for the cemented and un-cemented replacement armamentarium.[4] The study has allowed us to have sufficient arsenal for replacement at the same time allowing the implant to accommodate itself in to bulk of the femora. Anthropometric analysis of a Swiss population based on study by Rubin et al, French population by Massin et al, Malay population by Baharuddin et al, Thai population by Mahasavariya et al.[5,14-16]

Femoral head diameters

The difference in the femoral head diameters across various ethnic groups was assessed.

The present study showed mean femoral head diameters in the South Indian population to be 47.16±4.5 mm. The mean femoral head diameter of Indian population was compared with that of the Malay population 40.81±3.43 mm. The Malay population was found to have smallest femoral head diameters. The mean femoral head diameter of the present study was compared with the Thai population - 43.98±3.47, the Thai population had smaller femoral head dimensions. The mean femoral head diameter of the present study was compared with the Swiss population- 43.40±2.26 mm, the Swiss population had significantly smaller femoral head diameters. The mean femoral head diameters of the South Indian population was compared with the French population, they had lesser mean femoral head diameters- 45.60±4.20 mm compared to Indian population. There was no statistically significant difference in the mean femoral head diameters between Indian and Caucasian femora- 46.1±4.8 mm.

Canal diameters

The population mean endosteal dimensions were measured at four reference levels and assessed between various groups. The mean femoral head diameter at the four reference levels for the South Indian population was endosteal diameter 20 mm above lesser trochanter (D)- 43.79±5.2 mm, endosteal diameter at the level of lesser trochanter (E)- 27.35±4.3 mm, endosteal diameter 20 mm below lesser trochanter (F)- 19.92±3.5 mm Endosteal diameter at the isthumus (G)- 13.8±2.8 mm. There was no statistically significant difference between the endosteal diameters of South Indian and Malay population when matched against present study at the level of D (20 mm above lesser trochanter) and F (20 mm below the lesser trochanter). However at the level of G (isthumal diameter) it was found to be smaller in the Malay population (9.73±1.8 mm). These differences were statistically significant. There is no statistically significant difference in the mean canal diameters at the four reference levels when Indian femora were matched with the Swiss population indicating a close resemblance in terms of endosteal dimensions. There was no statistically significant difference between the endosteal diameters of the South Indian population (present study) and the French population. The endosteal widths of Indian femora was smaller by 2.0 mm in comparison to the Caucasian femora at all 4 reference levels which was statistically significant ($P < 0.05$). Even with a magnification error of 3 mm between present study and Caucasian study the endosteal dimensions are smaller by 2 mm in comparison to Caucasian femora. This suggests that the actual difference is much more.

Offset

The mean offset in the Indian population was 42.75±4.3 mm. The offset values of the South Indian population were compared with the Malay population- 31.50±5 mm. The offset of the Malay population was found to be significantly smaller than the South Indian population. The offset values of the South Indian population were compared with the Japanese population– 30.45±4.26 mm. The offset of the Japanese population was found to be significantly lower than the South Indian population. The offset of the South Indian population was compared with the French population who had a mean offset of 41±6.20 mm. The offset of the French population was close to that of the South Indian population and the difference was not statistically significant. The offset of the South Indian population was compared with the Swiss population who had a mean offset of 47±7.2 mm which was the highest among the ethnic groups in study. The mean offset of the South Indian population was 4.25mm lower than the Swiss population. This probably correlates with the bigger size of the femora among European population. There is no significant difference between the offset of South Indian population and Caucasian population- 43±6.8 mm.

Canal flare index

The mean canal flare index in the Indian population was found to be 3.23.The mean canal flare index of the South Indian population was compared to the Caucasian population which had a canal flare index of 3.8. The difference was statistically significant. The canal flare index of the South Indian population was compared with the French population who had a canal flare index of 3.6. The differences were found to be statistically significant between South Indian and French population, in terms of trending of the canal flare index. This probably suggests a better bone stock in the European population, with the European populations having more Champagne flute configuration of canal flare, therefore will favour more of an un-cemented fixation.

Neck shaft angle

The mean neck shaft angle was found to be 126 degrees in the present study of South Indian population. The verge neck shaft angle of the South Indian population was compared with the Japanese population. The Japanese population had an average valgus femoral angulation with mean neck shaft angle of 137 degrees in comparison to South Indian population.

The neck shaft angle of the South Indian population was compared with the Malay population. The mean neck shaft angle of the Malay population was 130 degrees. There was a slight inclination towards valgus mean femoral angulation in the Malay population when compared with the South Indian population in the present

study. There was no statistically significant difference between the neck shaft angles of present study and Caucasians -125 degrees. Analysis thus indicates more valgus femoral neck shaft angulations Asian population groups.

CONCLUSION

The canal flare index was found to be decreasing with age which is in correlation to the age related decrease in the femoral bone stock. The Asian and Indian femur bone is of much smaller sizes in comparison to European femurs.

Limitations of study

The radiographic study had a mean magnification of 3 mm. There was radiation exposure to normal subjects who have volunteered for the study. The age of the dry bones was not available.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Charnley J. Anchorage of the femoral head prosthesis to the shaft of the femur. J Bone Joint Surg Br. 1960;42:28-30.
2. Charnley J. The Lubrication of animal joints. In: Symposium on Biomechanics. Institution of Mechanical Engineers. 1959: 12-22.
3. Atilla B, Oznur A, Caglar O, Tokgozoglu M, Alpaslan M. Osteometry of the femora in Turkish individuals: a morphometric study in 114 cadaveric femora as an anatomic basis of femoral component design. Acta Orthop Traumatol Turc. 2007;41(1):64-8.
4. Noble PC, Alexander JW, Lindahl LJ, Yew DT, Granberry WM, Tullos HS. The anatomic basis of femoral component design. Clincial Orthopedics Rel Res. 1998:34(1)227-35.
5. Baharuddin. Morphology study of the proximal femur in Malay population. Int J Morphol. 2011;29(4):1321-5.
6. Ravichandran D. The proximal femoral geometry in Indians and its clincial applications. J Anat Soc Ind. 2011;60(1):6-12.
7. Singh, Bhasin. Anthropometry. 1st edition. Delhi: Educational Publishers; 1968: 142.
8. Parsons PG. The characteristics of the English thigh bone. J Anat Physiol. 1914;48:238-67.
9. Kate BR. The angle of the femoral neck in Indians. Eastern Anthropologists. 1967;20:54-60.
10. Humphrey. The angle of neck with the shaft of the femur at different periods of life and at different circumstances. J Anat Physiol. 1958;23:273.
11. Isaac B, Vettivel S, Prasad R, Jeyaseelan L, Chandi G. Prediction of the femoral neck-shaft angle from the length of the femoral neck. Clin Anat.1997;10(5):318-23..
12. Chauhan. The anatomic parameters of the North Indian hip joint- a cadaveric study. J Anat Soc India. 2002;51:39-42.
13. Siwach. Anthropometric study of the proximal femur geometry and its clinical applications. Indian J Orthopaedics. 2003;37(4):247-51.
14. Rubin. The morphology of proximal femur – a three dimensional radiographic analysis. J Bone Joint Surgery (Br). 1992;74:28-32.
15. Massin P, Geais L, Astoin E. The anatomic basis for the concept of lateralised femoral stems: frontal plane radiographic study of the proximal femur. J Arthroplasty. 2000;15(1):93-101.
16. Mahaisavariya B, Sitthiseripratip K, Tongdee T Bohez EU, Vander Sloten J. Morphological study of proximal femur: a new method of geometrical assessment using 3 dimensional reverse engineering. Med Eng Phys. 2002;24(9):617-22.

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□ □□□r□□□
□□□r□d□□□□□□□Fracture neck of femur has been recognised since the time of Hippocrates and is a common orthopaedic problem in old age and they have a tremendous impact on both the health care system. Despite marked improvements in the implant design, surgical technique and patient care, they still remain an unsolved fracture as for as treatment and results are concerned among the orthopaedic fraternity around the world.
By this study we will be able to assess whether bipolar prosthesis really functions as its name suggests or vice versa as the literature suggests.
M□□r□□□□□□d□**M**□□□d□□Consist of 2 parts. Part I is a (prospective study) in which Patients who undergone Bipolar hemiarthroplasty for fracture neck of femur at PSG Hospitals were included in the study after obtaining their consent during the period of December 2011 to June 2013.
Part II was a retrospective study where we took a data of patients who were operated earlier in our institute and had completed atleast 2 years of follow-up.
□ □□□□□□□□□d□□ □□□□□□□□□□With this study we found that for effective functioning of bipolar prosthesis IPJ movement remains a vital cog in the success of the bipolar prosthesis. In this study we found that there is good amount Inter-prosthetic joint movement of bipolar prosthesis at short – term and mid - term follow-up. The functional outcome is also good when Inter-prosthetic joint movement is more than 25%.

□ □□□ □rd□□Neck of femur fracture, bipolar hemiarthroplasty, inter prosthesis joint

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Fracture neck of femur has been recognised since the time of Hippocrates and is a common orthopaedic problem in old age and they have a tremendous impact on both the health care system and society in general [1]. Despite marked improvements in the implant design, surgical technique and patient care, they still remain an unsolved fracture as for as treatment and results are concerned among the orthopaedic fraternity around the world [2]. Fractures of the neck of femur are on increase, which is not unexpected, as the general life expectancy has significantly increased during the few decades. These fractures may double within next 20 years and fracture rate doubles for each decade of life after 5th decade of life [□]. It's has been predicted that by the year of 2050, the number of hip fractures would triple.
This is the commonest fracture in old aged individuals because of osteoporosis and advancing age causing more brittleness of bone. So prolonged immobilization during such fractures in elderly will jeopardise the life span of the patient and further complicates the problem. This forces one to totally abandon complete immobilisation to achieve a bony union, or to resort early ambulatory procedures by surgery.
The blood supply to neck and head of femur is extensive, intricate and complicated. Healing process mainly depends on good blood supply. Non-union, avascular necrosis of femoral head and secondary degenerative arthritis are the principal complications of this fracture. The surgeon may have control over the problem of non-union, whereas he may have none over avascular necrosis and arthritis. This further handicaps the treatment of these fractures as the healing process is always in doubt. It's also a known fact that hip is a weight bearing joint and many performance based functions depend on hip joint. A successful operation at hip joint should provide painless, stable hip with wide range of movements.

Under these circumstances one has to decide whether the prolonged immobilization has to be employed to achieve bony union or quick ambulation by hemi-replacement arthroplasty, to achieve fair degree of function. Since early 1950's prosthetic replacement was introduced for solving the problems of Fracture neck of femur and vitallium intramedullary prosthesis received a hearty welcome, thus preventing Non-union and avascular necrosis. The Austin – Moore and Thompson prostheses have been successful implants in treating fracture neck of femur. Disabling pain and acetabular erosions are frequent complications after the use of Moore prosthesis [□]. So in an attempt to retard the acetabular wear, prolong the life of the implant and delay the need for revision surgery the bipolar prosthesis was developed by James E Bateman in Toronto in 1974, which had the great advantage of second joint, below the acetabulum. It was named bipolar prosthesis, since it had an outer head of metal which articulates with acetabulum and a second inner metallic head which articulates with High Density Poly-Ethylene (HDPE), lining the inner surface of outer head. So hip motion is to occur at 2 interfaces – primarily at the prosthetic interface and secondarily at the metal – cartilage interface, thus minimising the articular wear. This prosthesis was found to be very useful and results were encouraging [□].

But studies attempting to demonstrate the relative movements at the interfaces have yielded conflicting results. It's known that a friction produces particulate debris from the polyethylene liner and this was thought to be the cause of foreign body reaction causing stiffening up of the inter-prosthetic joint and also osteolysis and aseptic loosening of the implant. Recent studies have shown that over a variable period of time the bipolar prosthesis will become unipolar functionally due to stiffening up of the inter-prosthetic joint (IPJ).

This study represents the assessment of the inter-prosthetic joint movement in bipolar prosthesis done for fracture neck of femur at 6months and mid-term follow-up by radiological means. By this study we will be able to assess whether bipolar prosthesis really functions as it's name suggests or vice versa as the literature suggests.

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Consist of 2 parts. Part I is a (prospective study) in which Patient's who undergone Bipolar hemiarthroplasty for fracture neck of femur at PSG Hospitals were included in the study after obtaining their consent during the period of December 2011 to June 2013.

Part II was a retrospective study where we took a data of patients who were operated earlier in our institute and had completed atleast 2 years of follow-up.

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By Convenient sampling method, the patients undergoing bipolar hemiarthroplasty for fracture neck of femur were assessed radiologically for amount of inter-prosthetic joint motion during post-opeartive period.□

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- For all patients Life ortho care bipolar prosthesis was used (ISO – 13485 certified company). Implant made of 316 – Stainless steel. Femoral stem length is 150 mm, diameter – 8 mm. Outer head diameter varies from 37 to 53 mm with 2 mm increment. Inner prosthetic femoral head diameter was 26 mm and the lining between outer head and inner head is made of UHMWPE (Ultra High molecular Weight Polyethtylene). Neck shaft angle is 130 degrees. 2 types of prosthesis are available (Fixed only with collar, Modular with or without collar and Extra – long stem also available).
- No funding was received from the Implant Company for the purpose of this study.

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All patients who are undergoing bipolar hemiarthroplasty□for fracture neck of femur are considered for the study after getting a written consent from them. All Patients were adequately worked up before surgery and all patients were taken up for surgery within 72 hours. Certain therapeutic measures such as Deep breathing exercises, Static Quadriceps exercises, ankle pumps were taught to the patient pre-operatively. A detailed history about the mode of injury and type of fracture were noted.

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All surgeries were performed on an elective basis using standard□aseptic precautions, Surgery was performed under spinal or general anaesthesia. Patient was positioned laterally lying on the unaffected side. For all the patient's lateral approach (Hardinge approach) was used in our series.

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Throughout the post-operative period adequate care was taken□ to prevent abduction and external rotation of the limb. All patients received Quads strengthening X's and mechanical DVT prophylaxis during immediate post-operative period. All patient's were started on Full weight bearing ambulation as tolerated by the patient. Once initial pain subsided specific X-rays were done to assess the inter-prosthetic joint (IPJ) and outer bearing movement was assessed during immediate post-op (24 to 48 hours) , at 6 weeks and after 6 months with operated hip in neutral and maximum abducted positions.

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Two A.P x-rays of pelvis were taken. One with limbs in neutral position and neutral rotation and the other x-ray with affected limb in Maximum abduction are taken.

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We followed the method of p lain radiographs, as described by Bochner *et al*.

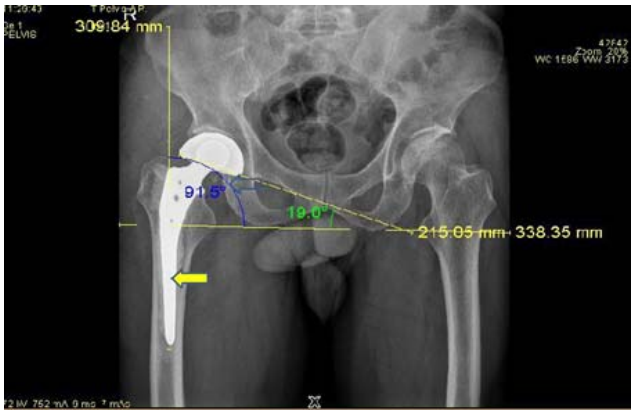
On the X-ray in the neutral position, 3 lines are drawn as follows:



□□□□**1** □a line drawn tangential to the most inferior aspects of the ischial tuberosities which is used as a reference line.

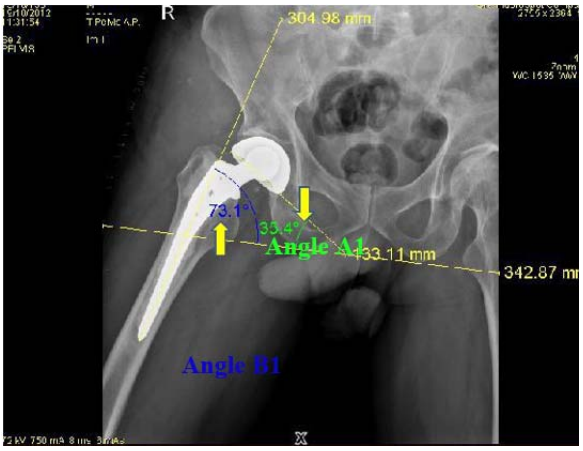


□□□□**2** □drawn along the the inferior margin of the acetabulum



□□□□□□Drawn along the centre of the long axis of femoral stem.

- Angle A was defined as the intersection of the line drawn from the inferior margin of acetabular component and the reference line (line of ischial tuberosities).
- Angle B was formed by the intersection of the ischial reference line with a line drawn along the center of the long axis of the femoral stem.
- The same exercise was repeated on the maximum abduction anteroposterior radiograph also and the angles are marked as A^1 and B^1.



□□□□□□Operated Limb in Maximum Abduction

- Now, B^2 is the difference between angle B and B^1 which represents the total amount of Hip abduction of the operated limb.
- A^2 is the difference between angle A and A^1 which represents the amount of motion taking place between the acetabular component and the acetabulum (Outer bearing interface).
- As we have 2 variables ie. (B2 – total amount of abduction, A2 – motion at the outer bearing interface), the difference between B^2 and A^2 represents the amount of abduction taking place at the inner bearing (Inter-Prosthetic joint movement).
- Thus the total amount of abduction, movement at outer bearing interface and movement at inter-prosthetic joint were calculated and tabulated for each patient at each follow-up.

X-ray of pelvis with both hips (Abduction of operated hip) with bipolar prosthesis on right side showing Angle A1 – 43.3 and Angle B1 – 74.4 degrees.
A = 45.1, B = 83.4, A1 = 43.3, B1 = 74.4 (Degrees)
B2= 9 Degrees (Total Abduction)
A2 = 1.8degrees (Motion @ Outer Cup)
B2-A2 = 7.2 Degrees (Inter-Prosthetic Joint Motion)
A2 = 20% (Motion @ Outer Cup)
B2 - A2 = 80% (Inter-Prosthetic Joint Motion).
At the end of 6 months follow-up, in addition to Radiological assessment Functional outcome was also assessed with Oxford hip score.
Part – 2 (Retrospective study):
Patients in this group who had already undergone Bipolar H.A and completed 2 years follow-up were reviewed. The x-rays were taken in the similar fashion as described above. Functional outcome assessment was done using Oxford hip score. The retrospective study helped us to assess IPJ motion at midterm follow-up.

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- It's useful to assess the outcome after surgical procedures by measuring 'patients' perceptions in adjunction to surgery. OHS assesses pain (6 items) and function (6 items) of the hip in relation to daily activities such as walking, dressing, sleeping etc.12 items with 5 categories of responses. Scores range from 0 to 4 (worst to best).
- Grading :
- 0-19 □ Worst, May indicate severe hip arthritis, requires some form of surgical intervention.
- 20-29 □ Fair, May indicate moderate to severe hip arthritis. Assessment by X-ray
- 30- 39 □ Good, May indicate mild to moderate hip arthritis. Non-surgical treatment, such as exercise, weight loss, and /or anti-inflammatory medication.
- 40-48 Excellent, May indicate satisfactory joint function. May not require any formal treatment.

Table 1: Radiologically Assessed Total Abduction, Outer Cup Motion And Inter – Prosthetic Joint Motion

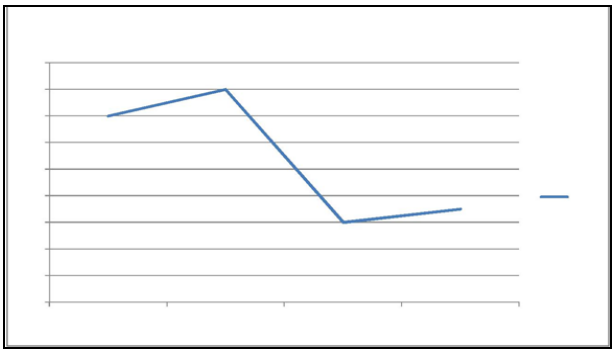
Follow up	Patient's	Mean B^2 (in degrees)	Mean A^2 (in degrees)	Mean $B^2 - A^2$ (in degrees)	Mean A^2 (in%)	Mean $B^2 - A^2$ (in%)
Immediate						
Post-op	30	18	8	10	45	55
6 weeks	28	23	13	10	57	43
6 months	30	25	18	7	72	28

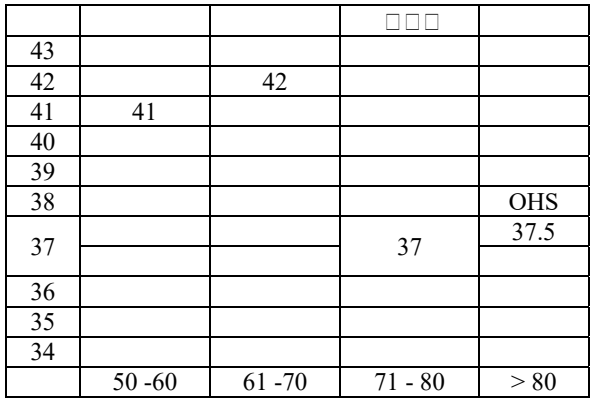
B^2 – represents Amount of Total abduction, A^2 – represents amount of movement between the outer cup and the acetabulum, $B^2 - A^2$ represents amount of IPJ motion.

Table 2: [illegible]

[illegible]	[illegible]	Mean [illegible]	[illegible]	Mean [illegible]	Mean [illegible]
50-60	7	41.5714	6.72947	32.00	48.00
60-70	9	42.0000	2.73861	39.00	48.00
70-80	12	37.0833	6.88157	26.00	46.00
ABOVE 80	2	37.5000	13.00000	36.00	39.00
TOTAL	30	38.9355	7.19692	26.00	48.00

Table depicts the Oxford Hip score of different age gropus in involving 30 patients of prospective study group, showing decrease in OHS as the age increases. But the decrease in OHS with increasing age group was not statistically significant (P = 0.062 ie > 0.05).





This graph predicts the decline in the OHS as the age progresses, except for the initial part.

Table 3: Mean [illegible] 6 Mean [illegible]

[illegible]	[illegible]	Mean $B^2 - A^2$	[illegible]	Mean [illegible]	Mean [illegible]
50-60	7	27.8571	8.31522	14.00	38.00
60-70	9	30.1111	5.30199	25.00	39.00
70-80	12	28.6667	6.31497	18.00	38.00
Above 80	2	35.5000	.70711	35.00	36.00
Total	30	29.3667	6.38146	14.00	39.00



Table 10 shows the IPJ motion at end of 6 months in various age groups in prospective study of 31 patients. On data analysis there was no statistical significance between the IPJ motion and various age groups (P = 0.495 > 0.05). Though the IPJ motion seems to be higher in patients above 80 years, the sample size for the particular age group was not significant.

This graph demonstrates the relation of $B^2 - A^2$ in various age groups

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Male	18	38.5263	7.99561	28.00	48.00
Female	12	39.5833	5.99179	26.00	48.00
Total	30	38.9355	7.19692	16.00	48.00

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Table 12 depicts the comparison of B2 – A2 at 6 months against the gender in prospective study of 30 patients. The IPJ motion was found to be almost the same in both females and males. There was also a statistical difference between the 2 groups in relation to IPJ motion IPJ□□

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More than	□	□	□	□	□
25%	20	42.7000	3.21346	38.00	48.00
Less than 25%	10	33.7000	5.51866	26.00	41.00
Total	30	39.7000	5.90237	26.00	48.00

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Table 13 depicts the comparison of IPJ motion at 6 months with Oxford Hip Score in prospective study of 30 patients. IPJ motion of the patients was divided into 2 groups for the ease of data analysis (Group I – IPJ motion > 25%, Group II – IPJ motion < 25%). Based on our data analysis the mean OHS was statistically significant in patients with
IPJ motion > 25% (Mean OHS – 42.7), (P = 0.00 < 0.05).

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10	31.0000	10.36018	15	50

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Table shows the Mean IPJ motion of 31 degrees in the retrospective study.

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10	38.1	28	44

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Table shows Oxford hip score of retrospective study of 10 patients with mean OHS – 38.1.

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>25%	8	39.625	35	44
<25%	2	32	28	36
Total	10	38.1	28	44

Table shows comparison between the IPJ motion and OHS in Retrospective Study. Since the sample size is small in retrospective study this data was not compared with the data in the Prospective study. For the current retrospective data the analysis was not statistically significant.

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The basic advantage of the Bipolar prosthesis over conventional unipolar prosthesis is movement at the two interfaces ie. Between the prosthetic inner femoral head and polyethylene liner and between acetabulum and the outer head. It was proposed that the complications like acetabular erosions would be delayed or prevented by reducing wear due to sliding motion in the acetabular socket. The high molecular weight polyethylene (HMWPE) articulation also absorbs some of the impact forces during gait. This advantage of Bipolar over unipolar prosthesis has been accepted by many studies□but there have been some reports in the literature that cast doubt on the continuity of inter-prosthetic movement in a bipolar prosthesis over time. Various radiographic studies have been done in which the prosthesis was imaged in various non-weight-bearing and static weight-bearing positions to estimate the fraction of movement that is occurring at the inter-prosthetic joint in a bipolar prosthesis. Phillips[6]□ used radiograph in supine position, first in neutral and then in maximum abduction and adduction.

Verberne used image intensifier to do radiological examination. In this context we undertook the present study of evaluating the IPJ motion radiologically over a period of time at 6 months and at the end of 2 years. We had also tried to co-relate the amount of IPJ movement to Functional outcome to assess whether there's any improvement in the quality of life in patient's with good IPJ motion. We followed the method of plain radiographs, as described by Bochner *et al*. In our series the amount of IPJ movement was 55% during immediate post-op, 43% during 6weeks & 28% at the end of 6 months. The mean Inter – prosthetic joint movement at end of 2 years follow – up was 31% for patients in the retrospective group. So at the end of 6 months 66% of the patients had > 25% of Inter prosthetic joint movement in our prospective study and 80% had > 25% in the retrospective study. However we are not analyzing the same group of patients in the prospective and retrospective study & the sample size of retrospective study is small. This could have probably resulted in higher inter – prosthetic joint movement at the end of 2 years. Drinker and Murray reported that although some inner motion occurred in most implants, it was less than predicted. Philips TW (1987) had done a study on Fluroscopic movement in 100 patients who had undergone Bateman Bipolar arthroplasty. Out of these 100 patients Group I had 76 patients with arthritis of hip and Group II had 24 patients with neck of femur fractures. In 80% of group I patients, the prosthesis retained Bipolar function at the end of 4 years follow-up study as compared to only 25% of group II patients retained the bipolar functioning of the prosthesis□

Verbene G.H.M (1983), did a radiological study of movements of two components in Variokopf prosthesis in 20 patients with fracture neck of femur during Immediate, 1 month and 3 months post-operative period. He observed that the IPJ lost mobility and at 3 months it became almost completely stiff with inter-prosthetic joint motion of only 16.9% being retained.

Bochner RM, in a study 26 patients with Bateman's Prosthesis who were assessed radiologically for inter-prosthetic joint motion, concluded that bipolar function was retained at the end of 4 years and the motion was shared between both the joint interfaces [□]. In their study the inter-prosthetic joint motion at 6 months was 19%. In a study conducted Anil kumar rai, from Banaras hindu university, varanasi, india during the period march 2003 to january 2011 treated with BHU bicentric bipolar prosthesis, showed that in cases of fracture neck of femur, the percentage of total abduction occurring at the interprosthetic joint at 3 months was 33.74% (mean value of all the patients), which fell to 25.66% at 1.5 years and remained stationary till 6 years. So they concluded that some amount of IPJ motion is still preserved at the time of their mid-term follow-up of 6 years. Higher Inter-prosthetic joint motion was observed in the arthritic group as compared to fracture group. This could be explained possibly due to friction phenomenon.

The results of our study regarding the Inter – prosthetic joint movement was comaparable to other studies in literature. Verbene *et al*. showed only 16.9% inter-prosthetic Joint movement at the end of 3 months in 20 patients & Bochner *et al*. showed 19% movement at the end of 6 months in 18 patients. Our study had better inter-prosthetic joint movement of 28% at end 6 months. This result probably could have been due to lower sample size of the above 2 studies.

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1. Condition of the Acetabulum: In patients with normal articular cartilage of acetabulum, the outer cup probably slides more when compared patients with arthritis. This can probably due to friction at which the primary movement occurs in IPJ. Only at the terminal range of movements prosthetic femoral head impinges on the neck leading to movement of the outer cup.
2. The Inner femoral head size used by Verbene *et al* was 32 mm which could have probably decreased the IPJ motion, when compared to Bochner and Philips *et al* where they used 22 mm inner femoral head. In the study done by Philips TW they had suggested that lower the femoral prosthetic head size, then lower will be the friction between the Polyethylene liner & femoral head & decreased stress on outer metal cup, which will result in lower acetabular erosions.
3. In previous studies they had used prosthesis with High density polyethylene which is prone for formation of wear debris over time, which could have possibly played a role in decreasing the IPJ motion. The wear debris formed from UHMWPE is much lower than the HDPE used in those days which could have contributed to better IPJ movement.
4. Since in our study we had used Bipolar prosthesis consisting of UHMWPE liner & optimum size prosthetic Femoral Head, our patients had better inter-prosthetic joint movement when compared with Verberne *et al*. & Bochner *et al*. we also assessed the age of the patient co – relating with Inter –prosthetic joint movement, but we did not find any statistical signifance.

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Various criteria were used to assess the functional results following bipolar hemiarthroplasty. How best the patient is returned to the pre-fracture status has been the main criteria. In India our customs demand Squatting and sitting cross leged without difficulty. To achieve this patient should have good range of flexion, abduction, adduction and external roation at the hip and full flexion at the knee. Our final results at 6 months after bipolar hemiarthroplasty in our series were analyzed using Oxford Hip Score. The results were compared with the available western and Indian series where bipolar hemiarthroplasty was done for neck of femur patients.

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Hinchey (1976)	225	52.4	20.4	10.7	16.4
Saxena and saraf (1978)[76]	82	46.1	44.8	6.5	2.6
Mukeherjee and Puri (1986)	55	29	49	18	4
Bavadekar and Manelkar (1987)	328	60		30	10
Arvade (1987)	104	70		16	14
Our series (2012 -13)	40	56	32	10	2

His table depicts the percentage of functional results following bipolar arthroplasty done for fracture neck of femur.

The difference between excellent and good results are minimal and therefore they can be gropued together as satisfactory results. In the series above Hinchey had 72.8%, Saxena [76]90.9%, Mukerjee and Puri 78%, Bavedekar 60% satisfactory results were achieved. In out series satisfactory results were achieved in 88% of our cases. Only 2% of the patients had poor results due to moderate /marked pain.

We found that there was no stastistical significance in between the age, sex Vs OHS. However we were able to analyse the relation between the IPJ motion and OHS. We found that there was significant increase in OHS in patients with IPJ motion of more than 25%. So we conclude that more the amount of IPJ motion preserved better will be the functional outcome in patients with bipolar arthroplasty done for femoral neck fractures

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With this study we found that for effective functioning of bipolar prosthesis IPJ movement remains a vital cog in the success of the bipolar prosthesis. In this study we conclude that: There is good amount Inter-prosthetic joint movement of Bipolar prosthesis at short – term and mid - term follow-up. The functional outcome is also good when Inter-prosthetic joint movement is more than 25%.

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- In this study the patients in the prospective and retrospective are not the same. And hence the results show a trend rather than being specific.
- Sample size is small
- Long term studies are required

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1. Mark F, Smionthloski *et al.* Current concepts of Intra-capsular fracture of Hip. JBJS. 1994; 76A:129-135.
2. Terry Cannole, Ed: Campbell's Operative orthopaedics, 10th edition: Missourie; Mosby –year book. 1998, 339-40.
3. Rockwood, Charles A, *et al.* Rockwood and green's fracture in Adults 6th edition: Philadelphia, Lipincott – raven publishers. 1996, 1693.
4. Johnson CE, Ripley LP, Bray CB, *et al.* Primary endoprosthetic replacement for acute Neck of femur fractures: A review of 150 cases. Clin. Orthop. Of North America. 1982; 167:123-130.
5. Bateman JE. Single assembly THA, Prelimnary report. Orthop Digest. 1974; 15:35-43.
6. Badgley C. Treatment of displaced subcapital femoral neck fractures in aged with immediate replacement arthroplasty (discussion). JBJS. 1961; 43B:606.
7. Carnesale PG, Anderson LD. primary prosthetic replacement for femoral neck fractures. Arch Surg. 1975; 110:27-29.
8. Chandler SB, Kreuscher PM. A study of the blood supply of the Ligamentum teres and it's relation to circulation of head of femur. JBJS. 1932; 14:834-846.
9. Garden RS, Praston. The structure and function of the proximal end of femur. JBJS. 1961; 43B:577-582.
10. Schek M. the significance of posterior comminution in femoral neck fractures. Clin. Orhop. 1980; 152:138-142.
11. Cane JM, Sulco TP, Zolan S. Treatment of pathological fractures of hip by endoprosthetic replacement. JBJS. 1980; 62A:954-959.
12. Crack HV. An atlas of the arterial supply of the head and neck of femur in man. Clin. Orthop. 1980; 152:17-28.
13. Boyd HB, Salvatore JE. Acute fractures of the femoral neck: Internal fixation or prosthesis? JBJS. 1964; 46A:1066-1068.
14. Arvade DJ. A review of internal fixation and prosthetic replacement for fresh femoral neck fractures. Clin Orthop. India. 1987; 1:77-82.
15. King D. primary prosthetic replacement in frash femoral neck fractures. JBJS. 1964; 46A:260.
16. Hey-grooves EW. Treatment of the fractured neck of femur with special regard to results. JBJS. 1930; 12:1-14.
17. Johnson JTH, Gothers O. Nailing Vs Prosthesis of femoral neck fractures. JBJS. 1972; 57A:686-792.
18. Arnold WD, Lyden JP, Minkoff J. Treatement of intracapsular fractures of femoral neck. JBJS. 1974; 56A:254-262.
19. Arnold CC, Lemperg RK. Fractures of femoral neck. Clin Orthop. 1977; 129:217-222.
20. Brown JT, Alrami G. Transcervcial femoral fractures. JBJS. 1964; 46B:648-663.
21. Mohandas PVA, Anadhi CP. Prosthetic replacement in the treatment of femoral neck fractures. Indian Journal of Orthopaedics. 1977; 11(1):31-37.
22. Austin T Moore. Discussion on femoral head prosthesis. JBJS. 1965; 47A:43.
23. Austin T Moore. The self-locking metallic hip prosthesis. JBJS 1957; 39A: 811 -27.
24. Peter Cordasso. Evolution of treatment of femoral neck fractures. Arch Surg. 1972, 37.

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Issues in management of fractures complicated by compartment syndrome

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Abstract
Introduction: Compartment syndrome has been identified as an acute devastating orthopaedic emergency and early fasciotomy is the only way to prevent any complications This retrospective study proposes to analyse the issues in management of fractures complicated by compartment syndrome occurring pre-operatively and post-operatively.
Aim: To study the issues involved in the rationale for deciding the method of fracture stabilization following fasciotomy. To study the problems, complications and functional outcome of fracture stabilization. To analyze and establish methods to the optimally manage the issues involved.
Materials and Methods: A review of patients admitted in the orthopaedic department at our institution who presented with or developed compartment syndrome and associated with fractures were chosen. Passive stretch pain and serve pain out of proportion were the main clinical indicators considered. Decision to perform fasciotomy was carried out based on the differential P value and were treated with double incision fasciotomy Patients were followed up every month for the first 6 months and then every 6 months. All the patients, who were reviewed, were functionally assessed based on Upper Extremity Functional Index (UEFI) and Lower Extremity Functional Scale (LEFS).
Results: A high suspicion of compartment syndrome must be maintained for all cases. The early diagnosis and treatment within 12 hours is critical to reduce morbidity and prevent any long term sequale. Compartment syndrome causes delay in bone healing. Risk of infection is not increased.

Keywords: compartment syndrome, fracture healing, faciotomy

Introduction
Compartment syndrome has been identified as an acute devastating orthopaedic emergency. Till date, compartment syndrome is one of the major complications in an injured limb. And early fasciotomy is the only way to prevent any complications due to compartment syndrome. Delay in diagnosis had been identified as the only cause of failure of treatment.
This retrospective study proposes to analyse the issues in management of fractures complicated by compartment syndrome occurring pre-operatively and post-operatively.

Aim and Objectives
Aim
To retrospectively study the issues in management of fractures complicated by compartment syndrome occurring pre-operatively and post-operatively.

Objectives
- To study the issues involved in the rationale for deciding the method of fracture stabilization following fasciotomy.
- To study the problems, complications and functional outcome of fracture stabilization.
- To analyze and establish methods to the optimally manage the issues involved.

Materials and Methods
- A review of patients admitted in the orthopaedic department at our institution was carried out. Patients who presented with or developed compartment syndrome and associated with

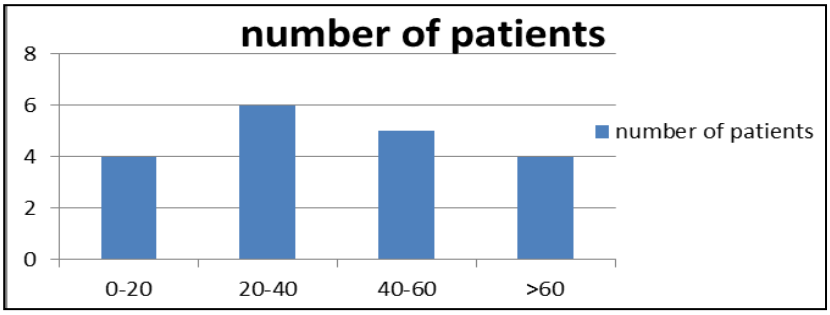
fractures were chosen.

- Compartment syndrome was diagnosed clinically in all the cases, except one. Passive stretch pain and serve pain out of proportion were the main clinical indicators considered. Paraesthesia, paralysis and pulselesness were considered to be supportive of the diagnosis. Comportment pressure was measured in one patient who had a spinal injury. Decision to perform fasciotomy was carried out based on the differential p value. Differential P was calculated by the difference between the patients diastolic pressure and compartment pressure. Fasciotomy is indicated if the value was less than 30mm Hg[24].
- All the patients who had developed compartment syndrome of the leg were treated with double incision fasciotomy, anterolateral and posteromedial. Compartment syndrome of forearm was treated with single volar incision fasciotomy, while that of the thigh also was treated with a single lateral incision.
- The fracture were treated with external, internal or hybrid fixation.
- The fasciotomy wounds were taken up for secondary closure from 4-7 days after fasciotomy with split skin grafting.
- Patients were followed up every month for the first 6 months and then every 6 months. All the patients, who were reviewed, were functionally assessed based on Upper Extremity Functional Index (UEFI) and Lower Extremity Functional Scale (LEFS).
- Painless unprotected weight-bearing and presence of bridging callus on X-ray were together considered as signs of bony union.

Results

There were 21 patients who were diagnosed with compartment syndrome and underwent fasciotomy for the same. But compartment syndrome in 2 of the patients were not associated with fractures and hence excluded from the study.

Incidence of compartment syndrome was found to be more common in physiologically young males, especially in the 3rd and 4th decades of life. This is probably due to the comparatively increased muscle mass in younger individuals.

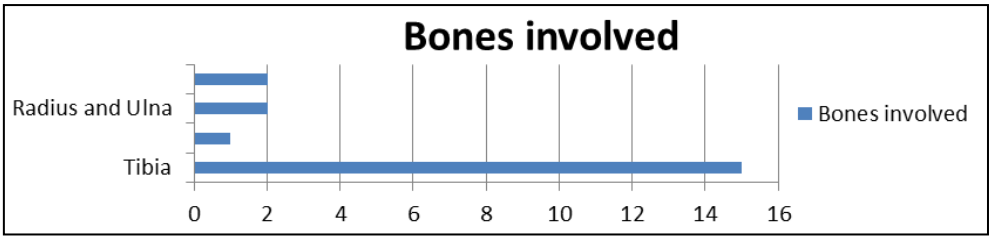


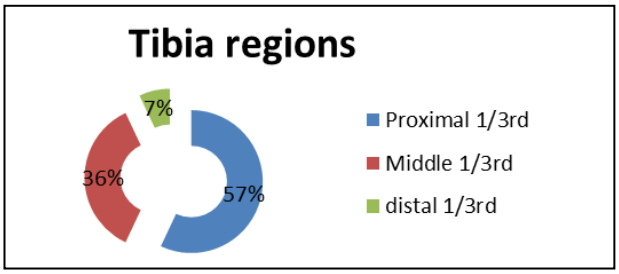
Compartment syndrome is more frequently seen in fractures due to high velocity injuries, mainly in road traffic accidents. (Table 1)

Table 1

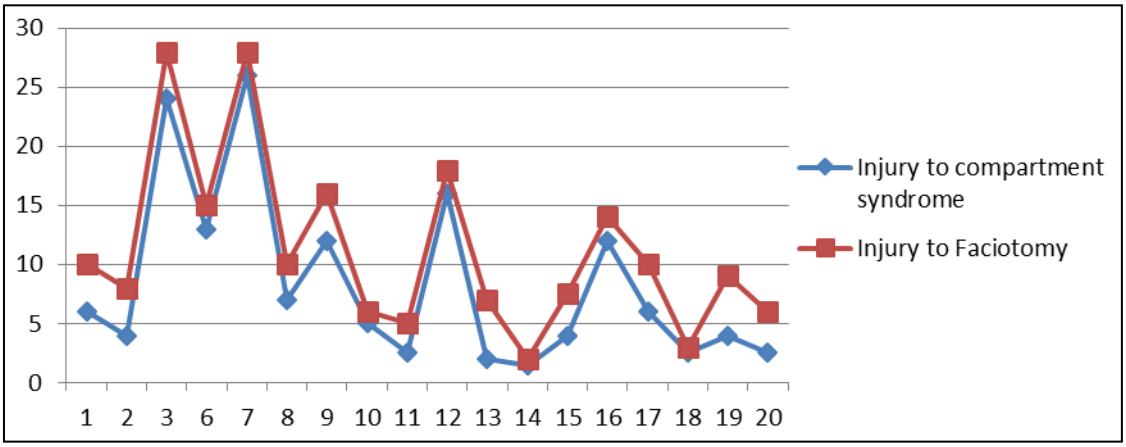
Mode of injury	No. of cases
Road Traffic Accident	16
Fall from height	2
Industrial injury	1
Trivial Fall	1

Fifteen of the fractures involved Tibia. Of the fifteen Tibia fractures, three involved the Tibial plateau and one involved a proximal Tibia epiphyseal injury and the rest involved tibioal diaphysis. Compartment syndrome frequently occurs in Tibia compared to all other sites. Occurance in Proximal one third Tibial is the commonest. (Graph 3, 4)





Of the remaining patients, one was a subtrochanteric fracture of the femur. The patient developed compartment syndrome of thigh probably as a consequence of the renal failure he was suffering from. Two were fractures of both bones forearm. One was a fracture around the elbow involving medial epicondyle and olecranon. One patient had additional injuries along with tibial fracture involving the ipsilateral femur and humerus and also compartment syndrome involving the forearm. One patient (Case 21) had sustained a D12 compression fracture with altered neurology of both lower limbs, along with a tibial plateau fracture (internally fixed in an institution outside). When he presented 10 days later, he had the altered neurology and also passive stretch pain with swelling of the affected leg.



The compartment syndromes were diagnosed by clinical monitoring. The delay in development of compartment syndrome following injury was 8.3 hours (1.5 hours to 26 hours, excluding case 21). The average delay in performing the fasciotomy after injury was 11.3 hours (2.5 hours to 28 hours).

There was delay in 2 of the cases (case 3 and 7) presenting to our institution as they received first aid in an outside institution primarily. Case 21, a 10 day old missed post-operative compartment syndrome, was not considered in the time of delay, because it was a case of established and missed compartment syndrome, on presentation from a different hospital. The patient's intra-compartmental pressure of all the compartments of the leg was measured using an electric arterial pressure monitor by an infusion technique. The differential P of the involved leg was found to be 10mm Hg, indicating the need for fasciotomy. Differential P of the other leg was found to be 40mm Hg.

All patients were clinically monitored for compartment syndrome from time of presentation, and also post-operatively. During the study, two distinct groups of patients were identified.

1. Patients who presented with compartment syndrome following a fracture.
2. Patients who developed compartment syndrome after surgical fixation of a fracture (cases 1, 2, 6, 8, 9 and 14)

Table 2: Grouping of patients with compartment syndrome

Compartment Syndrome Following	No. of Patients
Fracture	12
Surgical fixation of fracture (s0	6

Of the 12 patients who developed compartment syndrome after a fracture, 7 cases which were monitored from admission developed compartment syndrome. And they were taken up for fasciotomy with appropriate fixation.

Of the 18 cases considered, 6 of the fractures (cases 1, 2, 6, 8, 9 and 14), 5 tibial diaphyseal fractures and 1 ulna fracture, treated with intramedullary nailing developed compartment syndrome post-operatively. Pre-operatively, four of the fracture limbs were found to be edematous. All the 6 cases were diagnosed with compartment syndrome post-operatively after intramedullary nailing. The delay in establishing the diagnosis of compartment syndrome following intramedullary nailing was 8.5 hours (4 hours to 13.5 hours), caused probably by reduction of the fracture prior to intramedullary fixation. And the average delay in fasciotomy after intramedullary nailing was 11.8 hours (8hours to 16 hours).

Of the fifteen Tibial fractures, 6 were treated with intramedullary nailing (One-Unreamed nail), 4 were treated with Hybrid fixation, 3 were treated with external fixation and the Proximal Tibia epiphyseal injury (case 20) was treated with cancellous screw fixation. One patient with tibial plateau fracture was treated with hybrid fixator primarily. (case 17) the external fixation was converted to internal fixation primarily. (case 17) the external fixation was converted to internal fixation by means of intramedullary nailing after 3 weeks. One patient underwent internal fixation for tibial plateau fracture in an institution elsewhere before presenting to us. The subtrochanteric fracture was treated with Dynamic condylar screw and one of the forearm fractures was treated with K-wire fixation while the other was treated with Ulna square nail. And the elbow fracture was treated with internal fixation. The time lag in diagnosis, delay in fasciotomy and method of fixation of each case has been detailed in the master chart.

Except one, none of the cases had incidence infections or non-union. The patient who developed infection was a case of missed compartment syndrome.

All the patients were reviewed with an average follow-up of 33 months (6 months to 60 months). 2 patients (case 3 and 6) died due to unrelated causes. One patient (case 21) died due to sepsis leading to multi organ failure secondary to a 10 day old missed compartment syndrome.

The average time of healing in upper limb fractures was 11 weeks (9 to 15 weeks) and in lower limb fractures was 17 weeks (12 to 24 weeks). In two tibial fractures, the delay in healing needed bone grafting. Healing was relatively delayed in patients who had undergone fasciotomy 10 hours after the injury or internal fixation compared to those who had gone fasciotomy in less than 10 hours, in case of both upper and lower limb fractures.

3 patients (Case 1, 9 and 16) had neurological insult secondary to acute compartment syndrome. One patient (case 1) had weakness (Power 1/5) of the anterior compartment muscles of the leg at the time of diagnosis of compartment syndrome. He went on to recover partially (Power3/5) over a period of 3 months and then no further. Another patient had weakness (Power 3/5) of extensor hallucis longus at time of diagnosis of compartment syndrome and went on to recover in 3 weeks. One other patient (case 16) had developed severe volksmann ischemic contracture of the forearm due to delay in presentation to the hospital and hence delay in presentation to the hospital and hence delay in fasciotomy. The average in fasciotomy following injury in these cases was 13 hours (10 hours to 16 hours). Though the risk of infection is high, except one, none of our cases had any infection. Severe and extensive muscle necrosis of most of the muscles of the leg was found during fasciotomy of one of the patients (case 21), which ultimately led to sepsis and above knee amputation of the limb.

Two of the cases (case 7 and 9) had no signs of union at the end of 12 weeks both clinically and radiographically. The patients required bone grafting as a secondary procedure at 3 months to induce bony healing. The patient with volksmann ischemic contracture lost functionally of the upper limb, from elbow distally.

The functional outcome is decreased in patients who had undergone fasciotomy after 10 hours. However, younger patients showed better recovery even if they had undergone fasciotomy after 10 hours, compared to older patients.

Table 3

Patient Groups	Average functional outcome
Compartment syndrome after a fracture	85%
Compartment syndrome after fracture fixation	86%

The average functional outcome score of the cases, excluding those lost to follow-up is 68 and percentage of maximal function is 85%. The decrease in functional is 85%. The decrease in functional outcome score was due to the dysfunctional status of the affected limb in case 16. The percentage of maximal function increased to 91% on excluding case 16. The average percentage of maximal function of cases with post-operative compartment syndrome (cases 1, 2, 8, 9) was 86%, ompared to the 85% in cases primarily diagnosed with compartment syndrome (93% on excluding case 16). Mo significant difference was found in the functional outcome of both the group of patients.

Discussion

Compartment syndrome has been extensively studied. But difficulty and delay in diagnosis of the condition is the major problem encountered. The management of fractures associated with compartment syndrome also does not have fixed guidelines.

The study analysis nearly all the issues involved in management of the compartment syndrome and the associated fractures.

The study shows that compartment syndrome commonly occurred in physiologically young males, similar to all other studies [1, 21, 25, 37]. And high velocity injuries were also identified as the major cause of compartment syndrome as in most studies [1, 21, 25, 37]. Compartment syndrome of leg was the most common followed by that of forearm. And tibial fractures were the major cause of compartment syndrome of leg. McQueen et al showed that 36% of compartment syndromes follow tibial diaphyseal fractures.[22] Of all tibial fractures, proximal tibial fractures were most commonly involved. The higher incidence of compartment syndrome in proximal tibial fractures has been attributed to the vulnerable blood supply of the popliteal artery and the posterior tibial artery, due to the vessel bifurcation and enclosing tight osse of ascial canal.

Six of our cases developed compartment syndrome after the associated fracture was treated by surgical stabilization. McQueen et al found an incidence of 5.5% compartment syndrome in fractures treated by reamed nailing compared with 12.2 % in externally fixed fractures [22] The cause of compartment syndrome in post –operative cases has been postulated to be sudden decrease in compartment size following reduction of the fracture prior to intramedullary fixation

All diagnosis of compartment syndrome were made clinically. One patient was diagnosed with compartment syndrome 10 days after internal fixation of a tibial plateau fracture. The compartment syndrome was missed due to the spinal injury sustained by the patient and altered neurology below the thoracic level. All patients with tibial and forearm fractures were monitored clinically, for stretch pain and out of proportion pain, preoperatively from admission and also postoperatively.

Cases at risk of developing compartment syndrome were identified and monitored. Measure of intra-compartmental pressure was done only for patients under nerve blocks, with altered state of consciousness, with spinal cord injury and children who are not able to express themselves. In other patients, intra – compartmental pressure was measured only when clinical signs are inconclusive and to determine the need for fasciotomy. McQueen emphasized in numerous studies that diagnosis of compartment syndrome can be made only by continuous monitoring of the intra –compartmental pressure [4, 21, 22, 23, 24]. Continuous intra-compartmental monitoring is preferred to a single reading. However, Twaddle and Amendola stressed that continuous monitoring of all patients due to the need for equipment and manpower. They advised clinical monitoring to diagnose compartment syndrome and to measure intra compartmental pressure in a specified group of patients as mentioned above. And they observe that the judicious use of monitoring is required to prevent over diagnosing compartment syndrome.

Turen proposed that skeletal stabilization of fractures was needed immediately after fasciotomy to aid in the healing of soft tissues which was also concurred by Twassle and Amandola [36, 37]. Georgidas and Hake et al observed that closed fractures and uncontaminated open fractures, presenting with compartment syndrome, can be treated with primary internal fixation following fasciotomy, as there are low or negligible risks of infection and non-union.[9, 11] And this treatment protocol simplifies the bony and soft tissues cover must be obtained to cover the implant and fracture site.

Twaddle and Amendola observed that the stabilization technique used depends on the location and character of the fracture and the skill of the surgeon, but should minimize operative trauma to a limb that may already have had its

circulation compromised. Therefore, if possible, intramedullary nailing to stabilize the bone (and hence the soft tissues) is recommended. After the osteosynthesis has been completed, soft tissues coverage over the bone should be attempted [37].

All the fractures in our study were stabilized after fasciotomy by external fixation or internal fixation. Following fasciotomy, the fracture was considered to be an open one.

In metaphyseal and metaphyseal–diaphyseal fractures, we used external fixation as the method of choice for primary stabilization, We used external fixation for primary method of stabilization in 9 of the 15 tibial fractures. For juxta–articular fractures, we used external fixators or hybrid fixators. Hybrid fixators with inter fragmentary screw fixation were used in 4 cases of which 2 were tibial plateau fractures. The integrity of the articular surface was maintained by means of the hybrid fixators. The advantages of external fixation are the safety and ease of application, less devitalization of soft tissues and decreased operating time. Difficulties were faced early mobilization of the patient and wound care.

Of the 6 tibial diaphyseal fractures, 5 were treated primarily by means of external fixation. External fixation was used in diaphyseal fractures, when there was delay in fasciotomy and the viability of the muscles were in doubt and in cases without adequate soft tissues cover following fasciotomy. One of the cases treated with external fixator was taken up for intramedullary nailing at the earliest. The intramedullary conversion increased patient compliance. One tibial diaphyseal fracture was primarily treated with unreamed intramedullary nailing. Wound management was found to be easier after the internal fixation and the patient was also mobilizes early. Early mobilization, easy accessibility to the fasciotomy wounds for wound care, considerable decrease in the bulk of the implant and patient compliance are the advantage of intramedullary internal fixation.

Plate osteosynthesis was never considered in any of the tibial fractures, as the risk of devitalizing the already compromised soft tissues and infection were high.

Bony healing was delayed in all cases irrespective of method of stabilization. Time of bony healing did not differ much from that of post-operative compartment syndromes. In both the groups of patients, those who presented with compartment syndrome following an injury and those who developed compartment syndrome after internal fixation, the fracture was considered to be open. Both the groups had delayed bone healing. The average time of bone healing was nearly the same in bothgroups. And the functional outcome of both the groups of patients was nearly the same. Acute complications encountered in our study were neurological insult in three of the patients who underwent delayed fasciotomy of the three, one patient recovered completely, another patient had incomplete recovery, while the third patient had no neurological recovery. Incomplete recovery or total absence of recovery is due to the development of myonecrosis during the compartment syndrome and consecutive fibrosis of the muscles [27, 29].

Delay in diagnosis is primarily due to the delay in presentation to our centre.

Infection of the fasciotomy wounds and fracture site are expected. Fracture site is exposed during fasciotomy and the fasciotomy wound is left open for secondary closure increasing thechaces of infection. In our study, none of the cases developed infection at the fracture site of fasciotomy wound.

All the cases in our study, including the cases that developed compartment syndrome post-operatively, had delayed bone healing. This is probably due to loss of fracture haematoma during fasciotomies. But bone healing was further delayed I patients who underwent fasciotomy after 10 hours compared to those who underwent fasciotomy earlier. Similar results were also observed by Mullet [27]. Court-Brown and McQueen found that complication rates decreased to 4 % from 54 % with early fasciotomy [4].

Functional outcome was decreased I our patients who had undergone fasciotomy after 1 hours. Younger patients showed better functionality. The functional outcome of patients who had post-operative compartment syndrome was no different from those who had presented with compartment syndrome. The functional outcome of any case is determined mainly by the delay in fasciotomy followed by age than any other factor. Mullet et al found that younger patients had better functional outcome. This was attributed due to change in the muscle fiber composition and decreased muscle regeneration due to age [27].

Shortcomings of the study are the limited of number patients, the absence of a control group and also the analysis being done retrospectively. The chance of developing compartment syndrome has decreased considerably due to the early intervention following injury and advancement in monitoring patients early. In spite of these limitations zX, the factors influencing the outcome of the fractures associated with compartment syndrome were identified.

Orthopaedic surgeons must remain vigilant to identify compartment syndromes, both in in- patient and out- patient settings. Future development is likely to center around non-invasive methods of diagnosing acute compartment syndrome is being examined, like near infrared spectroscopy, which measures the amount of oxygenated hemoglobin in muscle tissues transcutaneously.

Conclusion

- A high suspicion of compartment syndrome must be maintained for all cases.
- The early diagnosis and treatment within 12 hours is critical to reduce morbidity and prevent any long term sequale.
- Compartment syndrome causes delay in bone healing.
- Risk of infection is not increased.
- External fixation is the method of choice in metaphyseal and metaphyseal-diaphyseal fractures and fractures without adequate soft tissue cover, when associated with compartment syndrome.
- Hybrid fixators with intra fragmentary screw fixation are used in juxta-articular fractures with communition.
- As bony healing is not compromised in any way, use of intramedulluary fixation is not contraindicated in diaphyseal fractures associated with compartment syndrome.

References

1. Azar FM. Compartment syndromes. In: Canale ST, Beaty JH, eds. Campbell's Operative Orthopaedics. 11th ed. Philadelphia, Pa: Mosby Elsevier, 2007, 46.
2. Berman SS, Schilling JD, McIntyre KE, *et al*. Shoelace technique for delayed primary closure of fasciotomies. Am J Surg. 1994; 169:435-436.
3. Blick SS, Brumback RJ, Poka A, *et al*. Compartment syndrome in open tibial fractures. J Bone Joint Surg Am. 1986; 68:1348.
4. Court-Brown CM, McQueen MM. Compartment

syndrome delays tibial union. Acta Orthop Scand. 1987; 58:249-252.
5. DeLee JC, Stiehl JB. Open tibia fracture with compartment syndrome. Clin Orthop. 1987; 160:175-84.
6. Fitzgerald AM, Gaston P, Wilson Y, *et al*. Long term sequelae of fasciotomy wounds. Br J Plast Surg. 2000; 53:690-693.
7. Galanakos S, Sakellariou VI, Kotoulas H, Sofianos IP. Acta Orthopaedica et Traumatologica Hellenica. 2009; 60(2):127-133.
8. Garfin S, Mubarak SJ, *et al*. Quantification of intercompartmental pressure and volume under plaster casts. J Bone Joint Surg. 1981; 63-A:449-53.
9. Georgiadis GM. Tibial shaft fractures complicated by compartment syndrome: treatment with immediate fasciotomy and locked unreamed nailing. J Trauma. 1995; 38(3):448-452.
10. Gershuni DH, Mubarak SJ, Yaru NC, Lee YF. Fracture of the tibia complicated by acute compartment syndrome. Clin Orthop. 1987; 217:221.
11. Hak. The use of the unreamed nail in tibial fractures with concomitant preoperative or intraoperative elevated compartment pressure or compartment syndrome. J Orthop Trauma. 1994; 8(3):203-11.
12. Heckman MM, Whitesides Jr TE, Grewe SR, *et al.* Histologic determination of the ischemic threshold in the canine compartment syndrome model. J Orthop Trauma. 1993; 7:199.
13. Heckman MM, Whitesides TE Jr, Grewe SR, *et al.* Compartment pressure in association with closed tibial fractures: Therelationship between tissue pressure, compartment, and the distance from the site of the fracture. J Bone Joint Surg Am. 1994; 76:1285-1292.
14. Janzing H, Broos P. Dermotraction: an effective technique for the closure of fasciotomy: A preliminary report of fifteen patients. J Orthop Trauma. 2001; 15:438-441.
15. Katz LM, Nauriyal V, Nagaraj S, Finch A, Pearlstein K, Szymanowski A. Infrared imaging of trauma patients for detection of acute compartment syndrome of the leg. Crit Care Med. 2008; 36(6):17.
16. Koval KJ, Clapper MF, Brumback RJ, *et al.* Complications of reamed intramedullary nailing of the tibia. J Orthop Trauma. 1991; 5:184-189.
17. Lindsay TF, Liauw S, Rouraschin AD, *et al*. The effect of ischaemia/reperfusion on adenosine nucleotide metabolism and xanthine oxidase production in skeletal muscle. J VascSurg. 1990; 12:8-15.
18. Matava MJ, Whitesides TE Jr, Seiler JG Ill, *et al.* Determination of the compartment pressure threshold of muscle ischemia in a canine model. J Trauma. 1994; 37:50-58.
19. Matsen III FA, Mayo KA, Sheridan GW, Krugmire Jr RB. Monitoring of intramuscular pressure. Surgery. 1976; 79:702.
20. Matsen FA III, Mayo KA, Krugmire RB, *et al*. A Model Compartmental Syndrome in Man with Particular Reference to the Quantification of Nerve Function. J Bone Joint Surg. 1977; 59-A:648.
21. McQueen MM. Acute Compartment syndrome. In: Bucholz RW, Heckman JD, Court-Brown CM, eds. Rockwood & Green's Fractures in Adults. 6th ed. Philadelphia, Pa: Lippincott Williams & Wilkins. 2006, 13.
22. McQueen MM, Christie J, Court-Brown CM. Compartment pressures after intramedullary nailing of the tibia. J Bone Joint surg Br. 1990; 72:95-98.
23. McQueen MM. The Effect of Acute Cornpartmcnt Syndrome on Bone Blood Flow and Bone Union. MD Thesis, University of Edinburgh, 1995.
24. McQueen MM, Court-Brown CM. Compartment monitoring in tibial fractures. J Bone Joint Surg Br. 1996; 78:99.
25. Meyer RS, Mubarak SJ. Compartment syndromes. In: Chapman MW, eds. Chapman's Orthopaedic Surgery. 3rd ed. Philadelphia, Pa: Lippincott Williams & Wilkins, 2001, 13.
26. Moed BR, Strom DE. Compartment syndrome after closed intramedullary nailing of the tibia: a canine model and report of two cases. J Orthop Trauma. 1991; 5:71-77.
27. Mullett H, Al-Abed K, Prasad CV, *et al*. Outcome of compartment syndrome following intramedullary nailing of tibialdiaphyseal fractures. Injury. 2001; 32(5):411-3.
28. Nario CV. La enfermedad de Volkman experimental. Ann Fac Med Montivideo. 1938; 10:87-128.
29. Nassif JM, Gorczyca JT, Cole JK, *et al*. Effect of acute reamed versus unreamed intramedullary nailing on compartment pressure when treating closed tibial shaft fractures: a randomised prospective study. J Orthop Trauma. 2000; 14:554-558.
30. Petrasek PF, Homer-Vanmasinkam S, Walker PM. Determinants of ischaemic injury to skeletal muscle. J Vasc Surg. 1994; 19:623-631.
31. Rang M. Injury. In: Rang M, eds. The story of Orthopaedics. 1 st ed. Philadelphia, Pa: W.B. Saunders. 2001, 16.
32. Rorabeck CH. The treatment of compartment syndromes of the leg. J Bone Joint Surg Br. 1984; 66:93.
33. Rorabeck CH, Macnab L. Anterior tibial compartment syndrome complicating fractures of the shaft of the tibia. J Bone Joint Surg Am. 1976; 58:549.
34. Shakespeare DT, Henderson NJ. Compartmental pressure changes during calcaneal traction in tibial fractures. J Bone Joint Surg Br. 1982; 64:498-499.
35. Tornetta P, French BG. Compartment pressures during nonreamedtibial nailing without traction. J Orthop Trauma. 1997; 11:24-27.
36. Turen CH. Skeletal stabilization for tibial fractures associated with acute compartment syndrome. Clin Orthop Relat Res. 1995; 315:163-168.
37. Twaddle BC, Amendola A. Compartment syndrome. In: Browner BD, Jupiter JB, Levine AM, Trafton PG, Krettek C, eds. Skeletal Trauma. 4th ed. Philadelphia, Pa: Saunders Elsevier, 2008, 13.
38. Webb LX. New techniques in wound management: vacuum assisted wound closure. J Am Acad Orthop Surg. 2002; 10:303-311.
39. Whitesides Jr. TE, Haney TC, Morimoto K, Hirada H. Tissue pressure measurements as a determinant for the need of fasciotomy. Clin Orthop. 1975; 113:43.
40. Whitesides Jr TE, Heckman MM. Acute Compartment Syndrome: Update on Diagnosis and Treatment. J Am Acad Orthop Surg. 1996; 4:209-218.
41. Vrahas M, Bhattacharyya T. The Medical-Legal Aspects of compartment Syndrome. J Bone Joint Surg Br. 2004; 86A:864-868.

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Acetabular erosion after hemiarthroplasty

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ABSTRACT

Background: Neck of femur fractures are one of the devastating injuries in the old age. Their incidence is on the rise causing immense stress on the society. One of the most commonly done procedure is bipolar hemiarthroplasty. In our study we have evaluated the acetabular erosion after hemiarthroplasty, in neck of femur fracture patients after a minimum period of 2 years and have tried to correlate it with activity level of the patient. The aim of the study were early detection of acetabular erosion; to assess the functional outcome after minimum of 2 years after hemiarthroplasty by modified UCLA score; to correlate the functional activity level and radiological acetabular erosion.
Methods: This is a retrospective radiological and clinical study. The post hemiarthroplasty plain radiographs, showing AP view of hip joint taken in the Department of Radiodiagnosis, PSGIMS&R will be studied along with activity level assessment. By Convenient sampling method, all the patients undergone cemented bipolar hemiarthroplasty, for fracture neck of femur after minimum of 2 years were assessed both radiologically and clinically. Functional activity level was assessed by Modified UCLA scoring system.
Results: By this study we suggest an easy and effective way of evaluating acetabular erosion and clinical activity. There is significant increase in acetabular erosion as the duration after surgery increases.
Conclusions: By this study we suggest an easy and effective way of evaluating acetabular erosion and clinical activity. There is significant increase in acetabular erosion as the duration after surgery increases. The clinical activity is by and large not significantly altered as the erosion progress at mid-term follow up.

Keywords: Neck of femur fractures, Bipolar hemiarthroplasty, Acetabular erosion

INTRODUCTION

Neck of femur fractures are one of the devastating injuries in the old age. It is well recognized even from the era of Hippocrates. The accurate number of hip fractures worldwide is impossible to determine, but the global incidence in the year 2000 has been estimated at 1.6 million and the projections for the future suggest further increasing numbers. In addition to the suffering of the individual the economic strain on society due to hip fracture is immense.

Management of displaced intracapsular hip fracture in elderly remains controversial. Options include hemiarthroplasty or total hip arthroplasty. Total hip arthroplasty has shown better clinical outcome and lesser reoperative rate, but in the elderly frail population who often suffer from fracture of the neck of the femur, morbidity rates are high.[1]

Hemiarthroplasty is one of the commonest procedures done for neck of femur fractures. It provides pain relief and early mobilization. The Moore and Thompson prostheses have been successful implants in treating

fracture neck of femur. Disabling pain and acetabular erosions are frequent complications after the use of Moore prosthesis.[1] So in an attempt to retard the acetabular wear, prolong the life of the implant and delay the need for revision surgery the bipolar prosthesis was developed by Bateman in Toronto in 1974, which had the advantage of hip motion occurring at 2 interfaces, primarily at the prosthetic interface and secondarily at the metal cartilage interface, thus minimizing the articular wear.

This prosthesis was found to be very useful and results were encouraging. However in long-term studies show that the bipolar prosthesis starts acting as unipolar prosthesis with time and hence leads to some erosion. However not all patients with acetabular erosions were symptomatic.

In our study we have evaluated the acetabular erosion after hemiarthroplasty, in neck of femur fracture patients after a minimum period of 2 years and have tried to correlate it with activity level of the patient.

Aim and objective

1. Early detection of acetabular erosion.
2. To assess the functional outcome after minimum of 2 years after hemiarthroplasty by modified UCLA score.
3. To correlate the functional activity level and radiological acetabular erosion.

METHODS

Source of data

This is a retrospective radiological and clinical study. The post hemiarthroplasty plain radiographs, showing AP view of hip joint taken in the Department of Radiodiagnosis, PSGIMS&R during the period between June 2015 to May 2016 will be studied along with activity level assessment. A total of 22 patients were evaluated.

Mode of data collection

By convenient sampling method, all the patients undergone cemented bipolar hemiarthroplasty, for fracture neck of femur after minimum of 2 years were assessed both radiologically and clinically.

Inclusion criteria

All patients operated for neck of femur fracture with cemented bipolar hemiarthroplasty after a minimum period of 2 years.

Exclusion criteria

Exclusion criteria were surgical site hip Infection; any preexisting pathologies around the hip; previous hip surgeries; post-operative periprosthetic fractures; neurological conditions like CVA, parkinsonism.

X-ray technique

A plain anteroposterior view of the operated hip joint is taken and assessed for acetabular erosion grading (Figure 1) following the technique of SakrMazen, MD, Girard Julien, MD and FakihRiad, MD. Patient positioned in supine, using digital X-ray, cassette tube distance is set to 100 cms and the beam is centered directly over the hip.

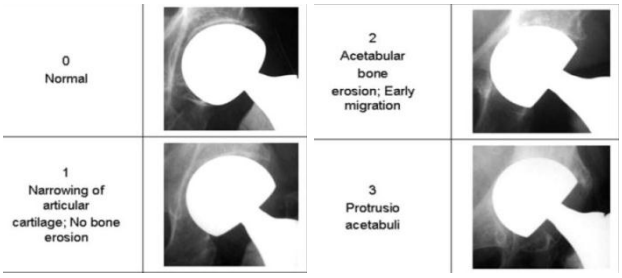


Figure 1: Grades of acetabular erosion.[2]

Activity level assessment[3]

Table 1: Functional activity level was assessed by modified UCLA scoring system.

Modified UCLA scoring system
1. Completely inactive, dependent on other and cannot leave the residence.
2. Mostly inactive or restricted to minimum activities of daily living
3. Sometimes going to social outdoor activities or visiting neighbours.
4. Regularly going to social outdoor activities or visiting neighbours.
5. Sometimes engaging in unlimited house works or shopping.
6. Regularly engaging in unlimited house work or shopping.
7. Regularly using staircase to climb.
8. Lifting or playing with small children.
9. Involving in heavy labour works.
10. Regularly using public transport.
Good --- 7 to 10 Fair-- 4 to 6 Poor--- below 4

Data analysis

Data collected were entered in Excel Spread sheet and analyzed using STATA statistical software package release 11. We used the two-sided independent-samples t test to compare means across dichotomous variables (i.e. men v. women); the one-way ANOVA test for comparison of means across multilevel variables. Simple calculations like Percentages, Proportions and Mean

values were derived. A type I error of 0.05 was considered in all analyses.

RESULTS

Table 2: Total number of participants according to gender.

Gender	Number of participants
Male	12
Female	10

Table 3: Total number of participants according to age.

Age in decades	Number of participants
60-69	9
70-79	11
80-89	1
90+	1

Total number of patient included in this study was 22. Total number of male patients 12, total number of female patients 10 and mean follow up age was 65 years. Right side hip involved was 11 and left side hip was 11. The mean follow up period was 3.54 years. The mean modified UCLA score was 6.22.

Table 4: Mean Modified UCLA score according to gender.

Gender	N	Modified UCLA score
Men	12	6.08±0.9
Women	10	6.4±0.7

The number of patients in Grades 0 and 1 acetabular erosion was 16 (72.70%) and in Grades 2 and 3 were 6 (27.30%).

Table 5: Mean acetabular erosion grade according to side affected.

Hip side	Number of participants
Left	11
Right	11

Table 6: Distribution of patients according to acetabular erosion grades.

Acetabular erosion grades	No. of patients	
Grade 0 and Grade 1	16	72.70
Grade 2 and Grade 3	6	27.30
Total	22	100%

The mean acetabular erosion when correlated with duration since surgery was statistically significant with p<0.001. Grade 0 & 1 has a mean value of 2.81 years and Grade 2 & 3 has a mean value of 5.75 years. This shows there was increased acetabular erosion as the duration post-surgery increases.

Table 7: Distribution of the study participants based on duration since surgery and grades of acetabular erosion.

Duration since surgery	Number of study participants	Erosion	No.	%
<4 years	18	0 and 1	16	88.9
		2 and 3	2	11.1
4 years	4	0 and 1	0	0
		2 and 3	4	100

Table 8: Mean duration since surgery (in months) according to acetabular erosion grades.

Acetabular erosion Grades	N	Duration since surgery
Grade 0 and Grade 1	16	2.81±0.32
Grade 2 and Grade 3	6	5.75±2.36

P<0.001.

The acetabular erosion grade when correlated with modified UCLA score was not clinically significant with p=0.71. In acetabular erosion Grade 0 and 1 the modified UCLA score was 6.33 and in Grade 2 and 3 the modified UCLA score was 6.18. Since the sample size was small the significance could not be correlated statistically. As the age of the patient increases there was a gradual reduction in activity.

Table 9: Mean Acetabular erosion grades according to duration since surgery (in months).

Duration since surgery	N	Acetabular erosion grades
<4 years	18	0.67±0.69
4 years	4	2.25±0.51

P<0.001

Table 10: Distribution of patients according to modified UCLA score.

Modified UCLA score	N	%
5	5	22.73
6	7	31.82
7	10	45.45
Total	22	

Table 11: Mean modified UCLA score according to acetabular erosion grades.

Acetabular erosion grades	Modified UCLA score
Grade 0 and Grade 1	6.33± 0.83
Grade 2 and Grade 3	6.18 ± 0.82

P=0.71(not significant).

Table 12: Mean modified UCLA score according to age in decades.

Age in decades	N	Modified UCLA Score
60-69	9	6.44±0.73
70-79	11	6.18±0.87
80-89	1	6±0
90+	1	5±0

Table 8 and shows description about Mean acetabular erosion grades according to duration since surgery (in months). This is statistically significant with P-value (<0.001) Erosion increases as the duration increases.

DISCUSSION

Incidence of fracture neck of femur is increasing gradually, probably due to increase in life expectancy of individuals.[4] The ideal treatment is still controversial[5]. Two common procedures done for elderly people are hemiarthroplasty and total hip arthroplasty. Some people advocate primary total hip arthroplasty for neck of femur fracture in elderly. Total hip arthroplasty results in more functional activity level and lesser reoperative rates but has more morbidity.

Hemiarthroplasty is more economical especially in developing countries like India and has lesser morbidity, but has a higher reoperation rate when compared to total hip arthroplasty.

The long term complications of bipolar hemiarthroplasty are acetabular erosion, aseptic loosening and infection.[6] Bipolar prosthesis is thought to have lesser incidence of acetabular erosion than unipolar prosthesis due to reduced movement occurring at acetabular prosthesis interface.

In this study we have tried to assess the acetabular erosion by radiological grading and functional outcome by modified UCLA scoring after a minimum period of 2 years after hemiarthroplasty.

Total numbers of patients assessed in this study were 22 and all underwent cemented bipolar hemiarthroplasty. The mean period of follow up was 42 months (range 2 years - 7 years). Six patients (27.30%) had a moderate to severe acetabular erosion and sixteen patients (72.70%) had minimal or no erosion. The grade of erosion was found to increase with follow up interval. With the follow up period of less than 4 years (18 patients) 16 patients (88.9%) had nil or minimal erosion and 2 patients (11.1%) had moderate to severe erosion. With follow up period of more than 4 years 4 patients had moderate to severe erosion. The mean follow up period in our study was 3.54 years and the significant acetabular erosion noted was 27.3% this could be compared with the study of Mazen et al, they had 33% erosion grade at the follow up period of 3 years.[7]

In this study all the patient's functional activity level were assessed by modified UCLA score, the mean modified UCLA score was found to decrease with increase in the age of the patients. Due to small study group the correlation of the modified UCLA score anti acetabular erosion was not statistically significant. One patient who had the longest period of follow up and maximum grade of acetabular erosion had a good level of functional outcome with modified UCLA score of 7. The pain did not restrict her functional activity.

Philips, London in his study found that the factors that had highest correlation were the level of physical activity and duration of follow up.[8]

Moon et al also found in their study that the progression of acetabular erosion correlated directly with the duration of articulation of the prosthesis within the acetabulum and this degeneration of articular cartilage becomes the cause of migration of bipolar cup, which is the major cause of failure of cemented bipolar arthroplasty. They also found the cause of degeneration to be multifactorial and it is accurately difficult to distinguish and measure the contributory level of each factor. They also found that all acetabular cartilage will show degeneration approximately 7 to 8 years after surgery.[9]

In our study all the 4 patients who had a follow up of more than 7 years had significant acetabular erosion. Hence the choice of total hip arthroplasty or a hemiarthroplasty in a fracture neck of femur may be determined by the life expectancy and the activity of the patient.

CONCLUSION

By this study we suggest an easy and effective way of evaluating acetabular erosion and clinical activity. There is significant increase in acetabular erosion as the duration after surgery increases. The clinical activity is by and large not significantly altered as the erosion progress at mid-term follow up.

Limitations of the study

Small study group, short period of follow up.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Sharma V, Awasthi B, Kumar K, Kohli N, Katoch P. Outcome analysis of hemiarthroplasty vs. Total hip replacement in displaced femoral neck fractures in the elderly. J Clin Diagn Res. 2016;10(5):11–3.
2. Mazen S, Julien G, Riad F. Retrospective evaluation of bipolar hip arthroplasty in fractures of the

proximal femur. N Am J Med Sci. 2010;2(9):409-15.
3. Zahiri CA, Schmalzried TP, Szuszczewicz ES, Amstutz HC. Assessing activity in joint replacement patients. J Arthroplasty. 1998;13(8):890-5.
4. Filipov O. Epidemiology and social burden of the femoral neck fractures. J IMAB- Annual Proceeding (Scientific Papers). 2014;20(4).
5. Bhandari M, Devereaux PJ, Tornetta P 3rd, Swiontkowski MF, Berry DJ, Haidukewych G, et al. Operative management of displaced femoral neck fractures in elderly patients: an international survey. J Bone Joint Surg Am. 2005;87(9):2122-30.
6. Haidukewych GJ, Israel TA, Berry DJ. Long-term survivorship of cemented bipolar hemiarthroplasty for fracture of the femoral neck. Clin Orthop Relat Res. 2002;403:118-26.
7. Mazen S, Julien G, Riad F. Retrospective evaluation of bipolar hip arthroplasty in fractures of the proximal femur. N Am J Med Sci. 2010;2(9):409–15.
8. Phillips TW. Thompson hemiarthroplasty and acetabular erosion. J Bone Joint Surg Am. 1989;71(6):913-7.
9. Moon KH, Kang JS, Lee TJ, Lee SH, Choi SW, Won MH. Degeneration of acetabular articular cartilage to bipolar hemiarthroplasty. Yonsei Med J. 2008;49(5):719–24.

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A Mechanical Device for the Reduction of Distal Radius Fracture

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Abstract: ***Distal radius fracture is one of the most common fractures that occur to a person or a child who undergoes any accidents or has any bone diseases. There are several ways of treating this fracture. It involves relocating the fractured bone by a process called reduction. A novel method of reducing the radial bone using a mechanical device has been proposed where it decreases the number of surgeons, time required to reduce the distal radius fracture and seeks to improve the accuracy of reduction. This paper describes the mechanism of reduction, design considerations and the disease conditions for which the device could be used.***

Keywords: Distal radius fracture; reduction; internal fixation; external fixation; K-wires; Aluminium; C-arm; Fluoroscopy.

I. INTRODUCTION

A distal radius fracture is a common bone fracture of the radial bone in the forearm. Because of its proximity to the wrist joint, this injury is often called a wrist fracture. The fractured hand is immobilised during treatment. Sometimes surgery is needed for complex fractures. Specific types of distal radius fractures include Colles' fracture, Smith's fracture, Barton's fracture and Chauffeur's fracture. As a generic term for distal radius fracture "Colles' fracture" is used to avoid confusion among the names applied to specific patterns of fracture.

The most common cause of this type of fracture is a fall on an outstretched hand. In adults this fracture is the consequence of moderate to severe force such as a fall from a substantial height or a motor vehicle accident. The risk of trauma is raised in patients with Osteoporosis and other metabolic bone diseases[1]-[7].

The distal radius fracture is treated by a procedure called Reduction. Reduction is a surgical procedure to restore the fracture or realigning to the right position by displacing the bone with the application of certain amount of force. Reduction could be classified as open reduction and closed reduction. Closed reduction is a procedure to reduce a broken bone without surgery. It allows a bone to grow back together. When this procedure is done as soon as possible it works well. The fractured area of the patient is first anesthetized with hematoma block, local anaesthesia, sedation or a general anaesthesia. Manipulation are done by placing the arm under traction and unlocking the fragments [8],[9].

Various surgical tools and implants are used in orthopaedic applications. Few such tools are K-wires, internal and external fixators, nails, plates and rods. The K-wires or Kirschner wires or pins are sharpened, smooth stainless-steel pins. They are used to hold the bone fragments at the injury site. In order to keep the reduced bones and soft tissues at a distance from the injured place a surgical treatment called External fixation is done with the use of K-wires and fixators. This treatment provides unimpeded access to the skeletal and soft tissue structures for the initial assessment and also for secondary treatments that are required to fix the bone continuity and a functional soft cover [10]-[15].

Depending on the type of deformity the deformed bone is then reduced with appropriate closed manipulations. A splint or a cast is applied to immobilize and protect the broken bone. A C-arm or any other imaging medical equipment is used tocheck the fracture site and to ensure the reduction was successful.

In Open reduction the fractured fragments are seen directly by dissecting the tissues. And the same procedure done for closed reduction is made.The Fig.2 shows K-wires made of stainless steel. The Fig.1 and Fig.3 shows the X-ray images of a normal hand and a hand with distal radius fracture respectively.The Fig.4 and Fig.5 shows the X-ray images of hand post internal fixation external fixation respectively.



Fig.1 X-ray image of a normal hand



Fig.2 Kirschner wires

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Fig.3 X-ray image of a hand with distal radius fracture



Fig.4 X-ray image of hand post internal fixation



Fig.5 X-ray image of hand post external fixation

Treatment of distal radius fracture using casts is usually successful with healing and return to the normal function as expected. Some residual deformity is common but this often reforms as the child grows. In the elderly, distal radius fractures heal and may result in adequate function after reduction. In younger patients the injury requires greater force and results in more displacement particularly to the articular surface. Unless an accurate reduction of the joint surface is obtained, these patients are very likely to have long term symptoms of pain, arthritis and stiffness.

The surgical tools and implants used for medical applications must be biocompatible and should not induce any ill effects to the patients. High corrosion resistance, durability, lightweight, radiolucent are the most important properties the surgical tools must possess. Materials like titanium alloys, stainless steel alloys and aluminium alloys are the most widely used alloys. Depending upon the degree of severity and cost of treatment different alloys are chosen. Aluminium is a low-cost biocompatible metal and its being used in hospitals widely [16]-[18].

II. MATERIALS

The designed mechanical device for the reduction distal radius fracture was made of 1060 grade aluminium. This alloy has high corrosion resistance, light weight and radiolucent properties that are needed for the surgical procedure. Also, this material can be sterilized as many times as needed. The properties of the material don't change in time. The composition of 1060 grade aluminium is shown in the Table 1.

Straps made of Velcro are wrapped around the palmar region and the radio-ulnar region to keep the hand fixed in position. Except for the small finger, the thumb and the index, middle and ring fingers are wrapped using Chinese finger straps to keep it intact.

The Chinese finger straps are made of nylon material which is used conventionally in surgeries. These straps attached to the fingers are connected to the T shaped screws to provide traction.

Table 1: Material Composition of 1060 grade Aluminium

Metal name	Composition (%)
Aluminium	99.6
Copper	0.05
Iron	0.13
Magnesium	0.03
Manganese	0.03
Silicon	0.03
Titanium	0.03
Vanadium	0.05
Zinc	0.05

III. METHODOLOGY

The hand length and hand width combined constitutes the hand size. The average hand length and width for male are nearly 190mm and 85mm respectively. The average hand length and width for female are nearly 170mm and 75mm respectively. Keeping these values into consideration the Wrist reduction device was made.

A. Design and Implementation of Wrist Reduction Device

The designing and implementation of the Wrist reduction involved several steps. Initially the model of the device was designed using PTC Creo, CAD software. Then a prototype of the model was done using 3D printer. The prototype was made of polylactic acid. The functionalities of the device were tested to know if it suits the required constraints. After few modifications the final Wrist Reduction Device was made using Aluminium alloy.The Fig.6 explains the designing and implementation of Wrist Reduction Device in the form of flowchart. The designed Wrist Reduction Device is shown in the Fig.7. The Wrist Reduction Device is made to produce load by adjusting the center radial adjuster. It can also withstand high loads as much the hand could handle.The dimensions of various components in Wrist Reduction Device are tabulated in Table 2.

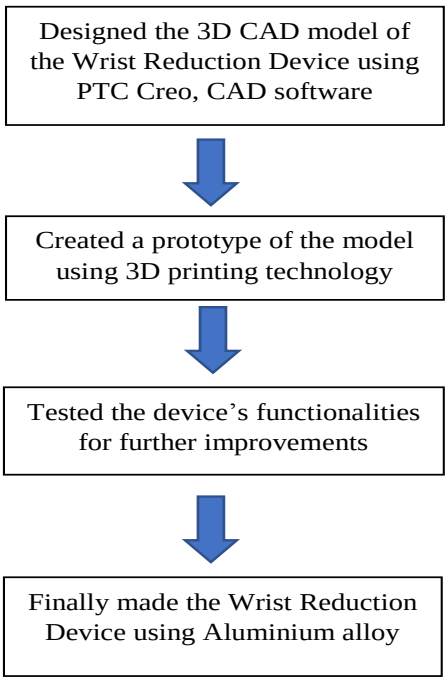


Fig.6 Flowchart of designing and implementation of Wrist Reduction Device

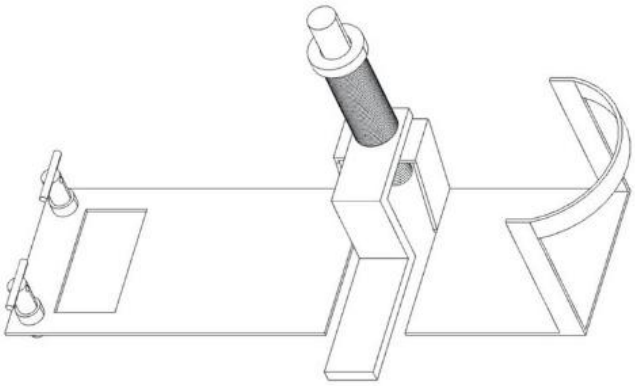


Fig.7 The 3D CAD model of the designed Wrist Reduction Device

Table 2: Dimension of various components

Parameter	Dimension (mm)
Pitch of the external thread	1.5 mm
Pitch of the internal thread	1.0 mm
Thicknesss of the Wrist and Base plates	3.0 mm
Length of the Wrist plate	150 mm
Length of the Base plate	140 mm
Width of the Wrist plate	65 mm
Width of the Base plate	65 mm

B. Mechanism of Reduction by the Mechanical Device

The mechanical Wrist Reduction Device has 2 plates. One for keeping the palmar region and other for placing the radio-ulnar region. These two plates are called Wrist plate and Base plate. These two regions are wrapped using straps or crepe bandages to avoid movements and keep the hand tight to the device.

In the first step, the thumb and the index finger, middle finger and ring finger are inserted into the Chinese finger straps. The fingers are fastened. The Wrist plate has two T shaped screws. After fastening, one nylon rope strapped to the thumb is attached to T shaped screw in front of it. Another nylon rope strapped to the three fingers is attached to T screw in front of it. The two T shaped screws are rotated to provide traction as required. The Wrist plate and Base plate are immovable and kept in horizontal direction while providing traction. The shaft with threads and a small circular nut present in the middle portion is interlocked. It keeps the plates fixed.

In the second step, the shaft is rotated to keep the Wrist plate in the required angle with respect to the shaft. The shaft is again interlocked with the nut. The Base plate and Wrist plate are made immovable again. As a result, ulnar deviation is done.

In the third step, the middle L-shaped part is raised and lowered by rotating the radial adjuster. As a result, the proximal fragments or the broken bone gets elevated. The broken bone gets aligned in the right position.

Either local anesthesia or general anesthesia is given to the patient prior to the reduction procedure. The bones of the hand are drilled to make bores of required dimensions. Then K-wires and fixators are inserted into the bones through the bores to keep the reduced bones in the fixed position. The position of the K-wires is monitored using a C-arm or fluoroscopy to guide it through the bones. After that a plaster of paris cast is made over the hand and the arm is allowed to heal.

This procedure can be done by a single surgeon alone.

The chances of occurrence of fracture due to the usage of the device in older patients and in patients with Osteoporosis are very low since the device itself doesn't produce any force or amplify the applied on it. But it only translates the force applied by the doctor on the wrist. The Fig.8 shows a surgeon operating a C-arm equipment to view the fractured hand while doing Wrist reduction.



Fig.8 A surgeon operating C-arm equipment

C. Mechanism of Reduction by the Conventional Method

In the conventional method, more than two surgeons are required. Anaesthesia is provided to the patient prior to surgery. The patients hand is placed over a radiolucent table. Then traction is provided to the hand by a surgeon while another surgeon holds the radio-ulnar bones. Then ulnar deviation is done by one surgeon. Then one or two surgeons realigns the fractured bone to the original position by the reduction procedure. Then K-wires and fixators are inserted to the bone depending on the severity. These are guided by X-ray based imaging machines like C-arm or fluoroscopy. A C-arm is widely used to guide the K-wires. Then a palter of paris cast is made over the hand to heal.

IV. RESULTS AND DISCUSSIONS

Thus, a low cost mechanical device is a viable inclusion in the orthopaedics for reduction of broken wrist. By using this device, the human effort needed to reduce the broken wrist is reduced. The Front view of the Wrist Reduction Device is shown in Fig.9.The Top view of the Wrist Reduction Device is shown in Fig.10.The Front view of the Wrist Reduction Device with a patient's hand placed in the device is shown in Fig.11.



Fig.9 The front view of the Wrist Reduction Device

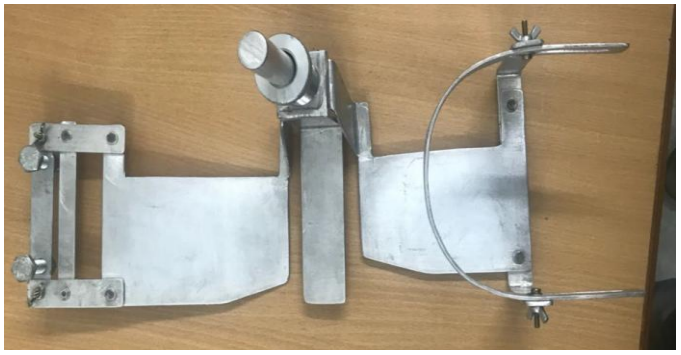


Fig.10 The top view of the Wrist Reduction Device

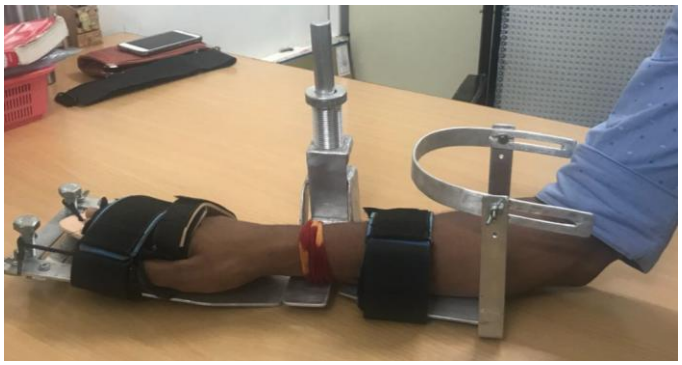


Fig.11 The front view of the patient's hand placed on the Wrist Reduction Device

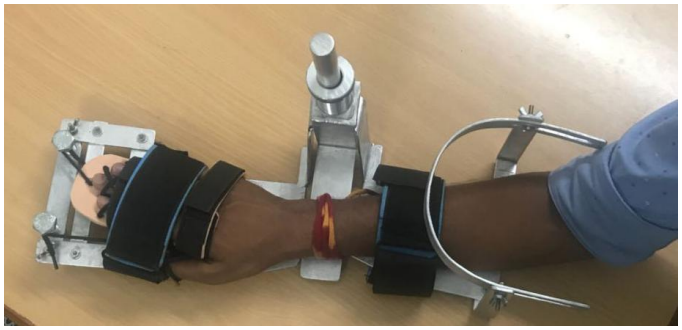


Fig.12 The top view of the patient's hand placed on the Wrist Reduction Device

The Top view of the Wrist Reduction Device with a patient's hand placed in the device is shown in Fig.12 shows the Wrist Reduction Device made of Aluminium. Comparison of conventional method and the mechanical based Wrist Reduction Device is to be done on the following aspects; Time taken for reducing the distal radius fracture, percentage of cases on which successful

reduction is achieved, accuracy of reduction achieved and feedback from doctors is to be used for the purpose of concluding whether the proposed method is better than conventional method. This device could be used for both invasive and non-invasive reduction of fractures bone.

V. READINGS

Department of orthopaedics, PSGIMSR were evaluated and accepted the research study entitled "*Wrist Reduction device*" and Clinical trial is held for a period of six months at PSG Hospitals, Coimbatore.

- **Objectives:** To design a mechanical device to reduce the human effort needed to fix a broken wrist.
- **Background:** medical practice in PSG Institute of Medical Sciences and Research.
- **Participants:** 20 patients- with broken wrist.

This device is operated by the physicians and they have rated the device based on its performance, stability and device longevity. Table 3 shows the rating values evaluated by the medical staffs in the scale of 5.

Table 3:Physician's outcome towards the device

Number of Physicians	Performance	Stability	Longevity
Physicians 1	3.5	4	4.5
Physicians 2	4	5	4.5
Physicians 3	3	3.5	5
Physicians 4	5	4.5	4.5
Physicians 5	4.5	4	5

VI. CONCLUSION

The Wrist Reduction Device can be used as an alternative method to reduce the distal radius fractures which relies upon manual reduction procedures for reducing it. By using the wrist reduction device, the human resource and human effort required to reduce the distal radius fracture can be dramatically reduced and by doing so the time consumed to reduce the most commonly occurring fracture is reduced thus making the medical personal available to treat the patients when they are most needed. An additional axial pull adjustment can be added. The device can be adjusted to fit all lengths of hand. The pitch could be optimised. More clinical trials should be done for improving the device's functionality. The device costs around Rs.5500 (INR). It could be made available in the market and used by many doctors for the reduction procedure. It might decrease the medical expenses of the patient if used.

REFERENCES

1. R. Arora, M. Gabl, S. Erhart, G. Schmidle, C. Dallapozza, M. Lutz, "Aspects of current management of distal radius fractures in the elderly individuals", *Geriatric orthopaedic surgery & rehabilitation*, vol.23, no.6, 2011, pp. 187-194.
2. P.L. Broos, I.A. Fourneau, D.V. Stoffelen, "Fractures of the distal radius", *Current concepts for treatment. Acta Orthopaedica Belgica*, vol. 67, no.3, 2001, pp. 211-218.
3. N.C. Chen, J.B. Jupiter, "Management of distal radial fractures", *Journal of Bone and Joint Surgery*, vol.89, no.9, 2007,pp. 2051-2062.
4. M.H. Henry, "Distal radius fractures: current concepts", *The Journal of Hand Surgery*, vol. 33, no.7, 2008, pp.1215-1227
5. J.A. PorrinoJr, E. Maloney, K. Scherer, H. Mulcahy, A.S. Ha, C. Allan, "Fracture of the distal radius: epidemiology and premanagement radiographic characterization", *American Journal of Roentgenology*, vol. 203, no.3, 2014, pp. 551-559.
6. T. Lindau, P. Aspenberg, "The radio-ulnar joint in distal radial fractures: A review", *Acta Orthopaedica Scandinavica*, vol. 73, no.5, 2002, pp. 579-588.
7. R.J. Diaz-Garcia, T. Oda, M.J. Shauver, K.C. Chung, "A systematic review of outcomes and complications of treating unstable distal radius fractures in the elderly", *Journal of Hand Surgery*, vol.36, no.5, 2011,pp. 824-835.
8. D.M. Lichtman, R.R. Bindra, M.I. Boyer, M.D. Putnam, D. Ring, D.J. Slutsky, J.S. Taras, W.C. Watters III, M.J. Goldberg, M. Keith, C.M. Turkelson, "Treatment of distal radius fractures", *JAAOS-Journal of the American Academy of Orthopaedic Surgeons*, vol.18, no.3, 2010, pp. 180-189.
9. A. Abramo, P. Kopylov, M. Geijer, M. Tägil, "Open reduction and internal fixation compared to closed reduction and external fixation in distal radial fractures: a randomized study of 50 patients", *Actaorthopaedica*, vol.80, no.4, 2009, pp. 478-485.
10. L. Obert, P.B. Rey, J. Uhring, N. Gasse, S. Rochet, D. Lepage, A. Serre, P. Garbuio, "Fixation of distal radius fractures in adults: a review", *Orthopaedics& Traumatology: Surgery & Research*, vol.99, no.2, 2013, pp. 216-234.
11. K.A. Egol, M. Walsh, S. Romo-Cardoso, S. Dorsky, N. Paksima, "Distal radial fractures in the elderly: operative compared with nonoperative treatment", *Journal of Bone and Joint Surgery*, vol.92, no.9, 2010, pp. 1851-1857.
12. L. Obert, F. Loisel, N. Gasse, D. Lepage, "Distal radius anatomy applied to the treatment of wrist fractures by plate: a review of recent literature", *International Society of Orthopaedic Surgery and Traumatology-Journal*, 2015, pp. 1-14.
13. J.L. Orbay, *DePuy Products Inc*, "Anatomical distal radius fracture fixation plate with fixed-angle K-wire holes defining a three-dimensional surface", U.S. Patent 7 635 381, 2009.
14. D.H. Wei, N.M. Raizman, C.J. Bottino, C.M. Jobin, R.J. Strauch, M.P. Rosenwasser, "Unstable distal radial fractures treated with external fixation, a radial column plate, or a volar plate: a prospective randomized trial", *Journal of Bone and Joint Surgery*, vol.91, no.7, 2009, pp. 1568-1577.
15. C. Steiner, P. Lehmann, B. Knuchel, V. Burgherr, *Stryker Trauma SA*, "Radiolucent orthopedic fixation plate", U.S. Patent 9 011 438, April 21, 2015.
16. M. Geetha, A.K. Singh, R. Asokamani, A.K. Gogia, "Ti based biomaterials, the ultimate choice for orthopaedic implants–a review", *Progress in materials science*, vol.54, no.3, 2009, pp. 397-425.
17. D. Chandramohan, K. Marimuthu, "Contribution of Biomaterials to Orthopaedics as Bone Implants - A review", *International Journal of Materials Science*, vol. 5, no.3, 2010, pp.309-409.
18. S. Flynn, "History of traction", *International journal of orthopaedic and trauma nursing*, vol.28, 2018, pp.4-7.

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ORIGINAL ARTICLE

OUTCOME ANALYSIS OF PROXIMAL HUMERUS FRACTURES TREATED BY MINIMAL INTERNAL FIXATION

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ABSTRACT: The aim of the study was to evaluate the functional outcome in patients treated with proximal humeral fracture who were treated with minimally internal fixations like K-wires, osteosutures etc.

KEYWORDS: Proximal humerus fractures, minimal internal fixation, k-wires, osteosutures, osteoporosis, functional outcome.

INTRODUCTION: Treatment of shoulder fractures has been the subject of much controversy and confusion. This is because of the complex nature of injury which makes the treating surgeon difficult to comment about displacement without proper x – rays, associated soft tissue injury (rotator cuff) and such injuries needs meticulous post-op rehabilitation[1].

Accurate definition of the type of fracture, patient compliance problems that limit rehabilitation medical co-morbidities affecting prognosis, and time from injury to treatment are critical factors affecting outcomes of these injuries. Additionally, technical factors in the surgical reconstruction of these fractures require surgical experience that few surgeons have the opportunity to develop[2].

About 2 to 3 % of upper extremity fractures occur in proximal humerus and 3/4th of these fractures occur after 60 years of age and commonly seen in women. More than 85% of proximal humerus fractures are minimally displaced. In the remaining 15% of fractures, only a few are severe fracture dislocations, about which much debate is centered [3, 4].

The aim of the study was to evaluate the functional outcome in patients treated with proximal humeral fracture who were treated with minimally internal fixations like K-wires, osteosutures etc.

MATERIALS AND METHODS: This study was conducted at PSG Institute of Medical Science and Research, Coimbatore. Our study was a retrospective study and involved 21 patients with proximal humerus fractures that were treated by minimal internal fixation. The age of our patients ranged from 21 to 70 years. Fractures were classified according to Neer's classification system. Patients were initially assessed for airway, breathing and circulation. Initial immobilization was done for the affected arm with a sling. Assessment was also done for associated injuries. Routine antero posterior and lateral radiographs were taken and CT-Scan was done if needed.

Inclusion Criteria:

- All two-part to four-part fractures according to Neer's classification[3].
- Those fractures treated by closed reduction and percutaneous pin fixation, osteosutures and cancellous screw fixation.

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Exclusion Criteria:

- Those fractures treated by conservative methods, plate osteosynthesis and nailing.
- Head splitting fractures.
- Compound fractures.

Operative Technique: General/Regional anesthesia was administered and the patient was placed in dental chair position with a sand bag underneath the scapula.

Parts were prepared and draped and closed reduction with arm abducted to about 70 degrees to 80 degrees was performed with an assistant holding at the elbow and progressive controlled longitudinal fraction, while posterior pressure is applied to humeral shaft to reduce it underneath the humeral head.

The accuracy of reduction was checked under image intensifier and if satisfactory, the same was fixed using either threaded or smooth 'K'- wires. Two 'K' wires were passed through the lateral aspect of humerus shaft just above the deltoid insertion and one through the anterior cortex. If greater tuberosity was displaced, then two more pins were inserted in retrograde for reduction and fixation.

If after closed reduction, the alignment was found not to be in satisfactory position or if the fragments were badly displaced, then open reduction with minimal soft tissue dissection was performed through deltopectoral approach. The fragments were fixed either with osteosutures using non-absorbable materials preferably ethibond, 'K' wires or cancellous screws carefully avoiding anterior humeral circumflex artery.

Post operatively, the arm was immobilized in a sling. Antibiotic coverage was given routinely (3 doses). Immediate post-operative X-Rays were done. Passive and pendulum exercises were started as soon as pain and swelling subsided. Due attention was given to pin tracts. Arm sling was discontinued by the third week and full range of movements was started.

Follow-up radiographs were taken at four weeks to six weeks-time. Pins were removed when radiological evidence of union was seen usually at five weeks (range four to ten weeks). Patients were then reviewed once in a month for initial two months. Then once in every three months for nine months.

FOLLOW UP ASSESSMENT:

POST OPERATIVELY:

- Adequacy of fixation, Re reduction/secondary procedure.
- Joint penetration, Axillary nerve, Pin infection.

FUNCTIONAL ASSESSMENT:

- ROM by goniometer, Neer's scoring

RADIOLOGICAL ASSESSMENT:

- Pin migration, Neck shaft angle, Union of fracture, Metal failure, Myositis, Avascular necrosis

OBSERVATIONS AND ANALYSIS: Out of 21 patients treated, 13 were male and 8 were female, with highest incidence at 4th decade. Right side being the dominant side was more affected, which

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accounted to 57 % and most of the injuries were due to the two-wheeler accidents.3-part fractures constituted to be the common type.

Common mode of fixation was by using K-wires.

Results are depicted in tabular columns.

DISCUSSION: The shoulder is the link of the thorax to the upper extremity. The function of the upper extremity is to provide mobility and power that allows a range of function, from powerful explosive movements seen in athlete actions (e.g., throwing a baseball 100 mph) to very accurate fine movements (e.g., placing the arm in a position to perform microscopic surgery).

Daily independence requires the ability to position the hand within the range of an imaginary sphere and to provide access to both sides of the alimentary system. Disorders of the shoulder, in addition to limiting function, also can produce pain, which can affect the ability to sleep comfortably and the ability to easily obtain gainful employment. Therefore, fractures that involve the proximal humerus (that portion involved with the glenohumeral articulation) can be devastating to quality of life. These fractures also can cost society a significant loss of productivity from otherwise viable members in the workforce.[2]

In this era of biological osteosynthesis, the concept of biological fixation offers many advantages in managing not only complex and high energy fractures but also in Juxta articular fractures as well as in fractures of non- weight bearing bones. In regard to proximal humerus fractures, this concept can be applied by means of closed reduction and percutaneous pinning or a minimal open reduction and internal fixation using Osteosutures or cancellous screws.

Although accurate anatomic reduction cannot be achieved at times, a good functional outcome can be attained which is mandatory for our activities of daily living. The advantages of such fractures being stabilized by minimal techniques are, minimal fixation modes but maintaining the cuff integrity, minimal scar, maximum anatomical restoration, better enhancement of fracture healing, early post-operative rehabilitation and easy implant removal.

Literature review[5-25] has shown that in managing the fractures of proximal humerus, although various options like closed reduction and percutaneous pin fixation, open reduction and internal fixation or Humeral head replacement are available, closed reduction and percutaneous pinning should be reserved for physiologically young and motivated individual with two part fractures and also in minimally displaced three part fractures.

In our study, 85.7% of the patients had a good functional outcome, which is comparable or even better with that of the literature. The average age of our patient was 56 range being 20 years to 80 years, which is little older when compared to most of the studies where patients had a mean age of 43 years but this has not influenced the outcome. The most common fracture pattern in our study was three part which accounted for 57.1%.

This is little contrary to that of literature where in all studies two part fractures were predominant. The common mode of fixation that was used in our study was by using closed reduction and percutaneous pin fixation with K – wires in 17 patients, which accounted for 81%. Of the remaining 4 patients, open reduction with minimal soft tissue handling was done and the fractures were fixed by Osteosutures and cancellous screws in 2 patients each. The functional outcomes of these 4 patients were good and comparable to that of Percutaneous pinning of fractures.

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This may have been due to the fact, that all these 4 patients had 2 part fractures (but displaced widely) which normally has a better outcome. But fallacy is that number of patients treated with this technique is small. So statistically they are not significant. Sex distribution, side of injury and mode of injury did not have any influence on the results.

The average time of pin removal in our study was 4 weeks and the time of union being 8 weeks range (4 weeks to 10 weeks). In a study conducted by Chow et al the average time of union was 10 weeks. In another study by Hans Jaberg et al (JBJS 1992) the union rate was between 6 weeks to 8 weeks almost similar to our study. In respect to complications, one patient had superficial pin tract infection and one had minimal pin loosening which subsequently was managed by antibiotics. We did not encounter any other complications like pin breakage, pin migration, joint infection or avascular necrosis of Humeral head.

The functional outcome that was evaluated according to Neer's criteria showed 85.7% good to excellent results in our study, which in chow et al study was 84%.However this may be attributed to the occurrence of these fractures in comparatively younger middle age group of patients in our study, whose compliance for physiotherapy and ability for motivation is better whereas in most of the study these fractures are seen to occur in older age groups.

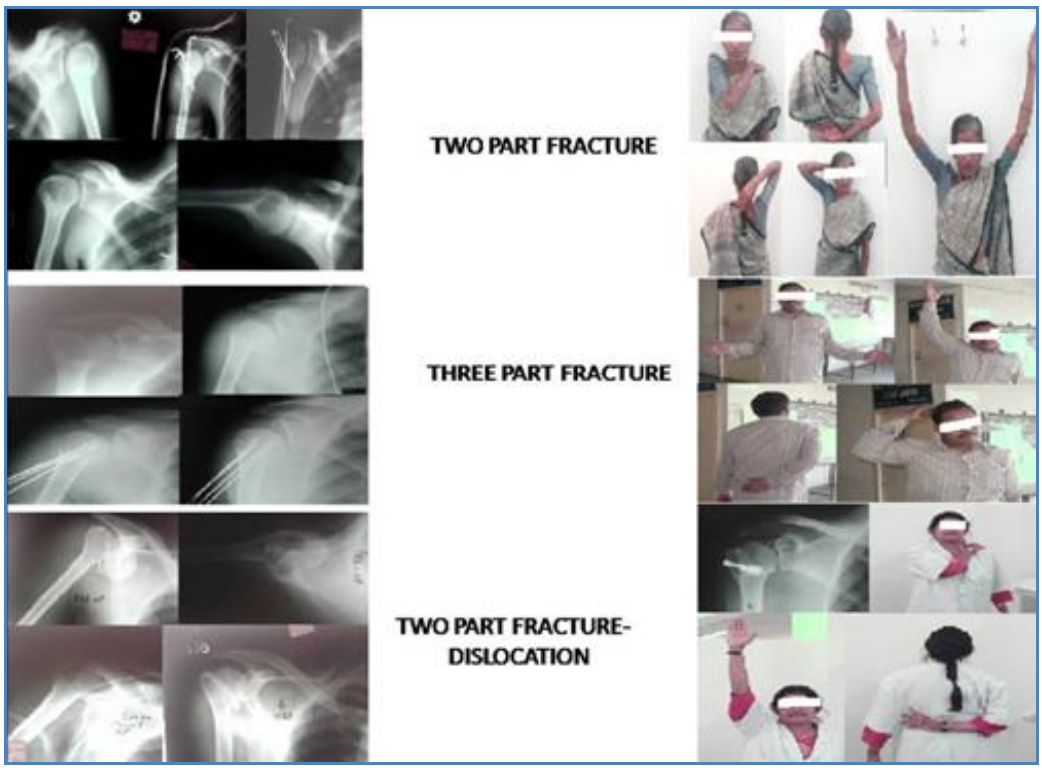
CONCLUSIONS: 2 part proximal humerus fractures yields 100% excellent/good functional outcome when treated with closed reduction and percutaneous fixation.19% fair/poor results in 3 part fractures were due to improper patient selection. Functional outcome is poor in patients > 60 years and this should be considered in patient selection along with communition and bone quality. There is always mild restriction (100) of abduction and forward flexion movement in 3 part fractures without significantly affecting functional outcome. Though technically demanding, with indirect reduction and adequate fixation, the results are good (83%).

BIBLIOGRAPHY:

1. Evan L Flatow, Department of orthopaedic surgery, New York Hospitals, New York. Evaluation and Classification of Proximal Humeral Fractures, Sophia cariati Medical WriterHost Orthopedic surgeon: **EVAN L.FLATOW**, MD chief of Shoulder surgery Department of orthopedics Mt.Sinai Medical centre Newyork, NY guest speaker: Frances Cuomo, MD Assistant Professor of clinical Orthopedic surgery New York University School of Medicine New York, NY Host Orthopedic Fellow: Yassamin Hazrati, MD Fellow, Shoulder surgery, Mt.Sinai Medical centre New York, Medscape Orthopedics,2001;5(3).
2. Proximal humerus fractures, Mark Frankle, Department of surgery, Division of Orthopedics, University of South Florida, Medscape Reference
3. Rockwood and Green, fractures in adults, 5th edition.
4. Kirk L Wong et al, UPOJ,Volume 11 Spring 1998, Pages 1-11,Proximal Humeral Fractures: Diagnosis and Management, From the Shoulder and Elbow Service, Department of Orthopedic surgery, University of Pennsylvania School of Medicine, PA
5. Resch H, PovaczH, Frohlich R, Department of traumatology, General Hospital of Salzburg, Austria, JBJS British 1997, March (79)2, 295 – 300.
6. Journal of shoulder Elbow surgery 1999 (8), 569 – 573.
7. S.Kamineni, H.Ankem and S.Sanghavi, Journal of injury, Jan 2004.
8. Henshaw, D.Ross and Murthi et al, current opinion in orthopedics, 12(4) 2001, 307 – 314.

DOI: 10.14260/jemds/2014/2633

9. U.Zings, D.Brunnschweiler, H.Keller et al, Swiss surgery seite 11 – 14 Band 8, 2002.
10. Cornel C N, Orthop rev, 1994 May, 19-23.
11. Hawkins R J, Bell R H and Gurr K, JBJS Amer, 1986 Dec, 68 (9): 1410-1414.
12. Park Maxwell and Murthi et al, Journal of orthopaedic trauma, may 2003 17(5):319-329.
13. Wheeler et al, journal of orthopaedic trauma, July 1997, 11(5): 363-367.
14. Chen, Chau-yu and Chao, journal of trauma, injury, infection and critical care, Dec 1998, 45(6): 1039-1045.
15. Williams and Wong, OCNA Amer, Jan 2000, 31(1): 1-21.
16. Szyszkowitz and Schippinger G, Unfallchirurg June 1999, 102(6): 422-428.
17. Povacz P and Resch H, TherUmsch, Mar 1998, 55(3): 192-196.
18. Szyszkowitz R et al, clinical Orthop, July 1993(292): 13-25.
19. Elkowitz, Stuart and Koval et al, Technique in Shoulder and Elbow surgery, Dec 2002, 3(4): 234-250.
20. JBJS 2001, 83 A: 1695-1699.
21. Hans Jaberg, Jon and Ronald et al, JBJS 1992, 74 A.
22. Jaberg et al, JBJS 74 A, 508-515.
23. Hawkins et al, OCNA 1987, 18: 421-431.
24. Hescovicci D, Saunders and Johnson et al, Clinical orthop, Jan 2000, 97-104.
25. Unfalchirug, 1997 (5): 349-353.



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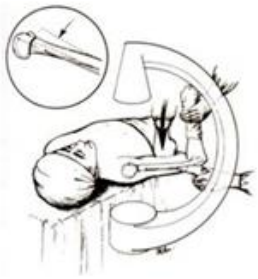
OPERATING TABLE

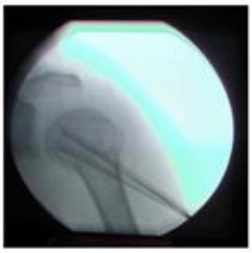


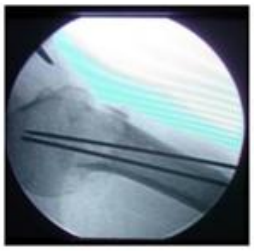
POSITION OF PT



C-ARM VISUALS POST-REDUCTION







NEERS SCORE

Parameters	Points Given
Pain	35
Function	30
Range of Motion	25
Anatomy	10
Total	100

Implant	Nos	Percentage
K-wire	17	81%
Osteosutures	2	9.5%
Cancellous Screws	2	9.5%

RESULTS

	AGE IN YEARS				
	21-30	31-40	41-50	51-60	61-70
PATIENTS	1	5	10	3	2
PAIN (35)	30	31	31	30	29
FUNCTION (30)	29	28	25	26	21
ROM (25)	25	22	16	20	16
ANATOMY (10)	9	8	9	8	6
NEERS AVERAGE (100)	92	92	80	84	72

NEERS FUNCTIONAL OUTCOME

	EXCELLENT	GOOD	FAIR	POOR	TOTAL
2-part	5	3	-	-	8
3-part	4	6	1	1	12
4-part	-	-	-	1	1
Total	9	9	1	2	18
	18		3		
%	(85.7)		(14.3)		

	RESTRICTION OF MOVEMENTS (In degrees)				
NEER	ABDUCTION	INTERNAL ROTATION	EXTERNAL ROTATION	FORWARD FLEXION	EXTENSION
2-part	10	Nil	Nil	17	Nil
3-part	20	Nil	13	20	Nil
4-part	175	50	-10	175	50

	MOVEMENTS IN DEGREES (Average / Range)				
NEER	ABDUCTION	INTERNAL ROTATION	EXTERNAL ROTATION	FORWARD FLEXION	EXTENSION
2-part	170	70	100	163	60
Range	55-180	MAX	MAX	110-180	MAX
3-part	161	70	87	160	45
Range	20-170	MAX	0-100	30-170	10-60
4-part	5	20	0	5	10

POINTS	REMARKS
>89	EXCELLENT
80-89	GOOD
70-79	FAIR
<70	POOR

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Original Article

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High Heels Footwear Causes Heel Pain and Back Pain: Myth or Reality?

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Abstract

Introduction: Heeled footwear has been in use by women for centuries and has now become an integral part of the wardrobe not only in the west but also in India. Recent research suggests that up to one third of women suffer from permanent problems as a result of their prolonged wearing of heels. One in 10 women wear it at least for 3 days a week, and a recent survey found that one-third of them had permanent problems.

Purpose: Whether the high heel wear is the actual cause of back pain is not yet clear. Literatures vary in their results regarding the same. Hence, the present study was done to assess this cause and effect.

Methods: The study was conducted at the orthopedic outpatient department of a tertiary care hospital, from July 2014 to December 2014.100 female patients who were using heeled shoes and who volunteered to participate in the study were included. The heel height was measured using measuring tape and were classified into three groups (<2.5 cm, 2.5-5 cm, >5 cm). A Proforma with a questionnaire was given to all the participants and their response were noted.

Results: Out of the 100 female volunteers, who participated in the study, 44 had complaints of heel pain regardless of the heel height, 56 had back pain, and many had both heel pain and back pain complaints, based on the questionnaire evaluation. We were further able to distinguish both heel pain and back pain, based on the heel height worn, duration of wear (in years) and duration worn/day.

Conclusion: There was a positive correlation between duration of wear and height of footwear with regards to both heel and back pain. However, a definitive cause and effect could not be established because of smaller sample size.

Key words: Back pain, Heel pain, Shoes, Statistical analysis, Women

INTRODUCTION

Walking is the most common form of human locomotion from a motor control perspective, human bipedalism makes the task of walking extremely complex. One condition known to compound the difficulty of walking is the use of heeled shoes, which alters the natural position of the foot-ankle complex.[1] This thereby produces a chain of reaction effects that travels up to the lumbar vertebrae. High-heeled footwear is defined as the footwear having a heel that is higher than the toe. Throughout the history, this footwear is considered as a symbol of sexuality, class and gender. When the foot slants forward, a much greater weight is transferred to the ball of the foot and toes, which increases the likelihood of damage to the underlying soft tissues that supports the foot. When the wearer tips the foot forward, this in turn puts pressure on the lower back and hence causes back pain. This particular study on post-adolescent volunteers was aimed to study the correlation between heeled footwear and heel and back pain.

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MATERIALS AND METHODS

The present study was conducted on 100 female patients who attended orthopedic outpatient department at institution during the period from July 2014 to December

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2014. Patients were drafted into the study based on inclusion criteria which included:

a. Age >15 years and <55 years
b. Without any foot and spine deformities. Patients were explained about the study and only those who volunteered and gave their consent were included in this study. The procedures followed were in accordance with the ethical standards.

The evaluation was based on questionnaire which was given to all the participating volunteers and heel height was measured with the help of inch tape as illustrated in Figures 1-3. Appropriate information collected in the questionnaire was entered in data collecting sheet for the purpose of statistical analysis.

Patients were categorized based on their age group (18-25, 25-45, 45-55), occupation (student, staff, housewife), heel height (normal [<2.5 cm], low heel [2.5-5 cm] and high heel [>5 cm]), duration of wear in years (0-5 and 5-10 years) and in hours/day (0-5 and 5-10), type of sole (leather, wooden, rubber, plastic). The data collected were statistically analyzed by Chi-square test.

Observation

A total of 100 female patients were included in the study after obtaining their informed consent. There were 63 students, 28 staff, 9 housewives who participated as volunteers. There were 12 people wearing heels with height 2.5-5 cm, 4 people wearing heels with height >5 cm and 74 people wearing heels with height 0-2.5 cm. In our study, 67 people were using soft sole type and 33 people were wearing hard sole type. There were 36 people wearing heeled footwear for more than 5 years, and 54 people were using heeled footwear for 0-5 years. 15 people were wearing heeled footwear for 0-5 h/day and 85 people were wearing heeled footwear for more than 5 h a day. Regardless of heel height worn, the duration of footwear had a significant influence on heel and back pain (Table1) and similarly prolonged wear in a day had a significant influence on heel pain (Table 2).



Figure 1: Heel height being measured



Figure 2: Method of measuring heel height using measuring tape



Figure 3: Volunteers who participated in the study

DISCUSSION

Heeled footwear has been in use by women for centuries but not all individuals wearing heels suffer from clinical problems. In ancient Egypt, wearing shoes may have served to set apart lower classes from the nobility as normal people walked around bare foot while the rich wore flat leather shoes. Moreover in ancient times, height would vary so that the higher the soles, higher the social status.

Research suggests that long-term heel use can both "compromise muscle efficiency and increase the risk of injuries." Heeled shoes slant the foot forward and down while bending the toes up, more the feet is forced into this position, more it may cause the gastrocnemius muscle to shorten.

Table 1: Relationship between heel height with back and heel pain (duration of wear in years)

Heel height	Heel pain			Back pain		
	0-5 years	>5 years	Significance	0-5 years	>5 years	Significance
0-2.5 cm (74[n])	14[n]	28[n]	0.017*	32[n]	-	0.016*
2.5-5 cm (12[n])	3[n]	6[n]		3[n]	Nil	
>5 cm (4[n])	1[n]	1[n]		1[n]	1[n]	

[n]Number of patients, *As analyzed by Chi-square test

Table 2: Relationship between heel height with back and heel pain (duration of wear in hours)

Heel height	Heel pain			Back pain		
	0-5 h	>5 h	Significance	0-5 h	>5 h	Significance
0-2.5 cm	6	36	0.053*	4	28	0.456
2.5-5 cm	3	6		3	-	
>5 cm	-	2		1	1	

*As analyzed by Chi-square test

When walking in high heeled shoes, a significant reduction in ankle plantar flexor muscle movement occurs, whereas increased work is performed by the hip flexor muscle during transition from stance to swing phase. Reduced effectiveness of ankle plantar flexors during late stance results in a compensatory enhanced hip flexor pull off. Large muscle movements and increased work at hip and knee occur while wearing high heels.[2]

Wearing high heels is thought to increase an individual's likelihood of experiencing a lateral Ankle sprain. The flexed and inverted posture of the plantar while wearing high heels may increase an individual's risk of experiencing a lateral ankle sprain.[3]

Foster *et al.*,[3] found signs of increase in ankle sprains in patients wearing high heels for a prolonged duration. Esenyel *et al.*,[2] described changes in the biomechanics of walking like increase in plantar flexion in people using high heels. In the particular study, we werenot able to assess the changes in biomechanics of foot while wearing high heels as it is a purely observational study.

There is also a definitive change in subtalar joint axis while walking on a high heeled shoe in comparison to a normal shoe, hence wearing of high heel may cause foot and heel pain.[4]

High heeled shoes cause an increase in the lordotic curve of the lumbar spine.[5] Increased ankle plantar flexion causes a kinetic chain of compensation up the lower extremity that ends with hypertonic psoas muscle, producing a lumbar hyperlordosis. A hyperlordotic lumbar spine in turn will lead to back pain.

Brent *et al.*,[9] found alteration in biomechanics of the lumbar spine. In prolonged high heels wear, chain of events around the ankle were observed, like increased ankle plantar flexion which in turn led to an increase in a lordotic curve.[6]

Eisenhardt *et al.*,[5] measured pressure distribution under foot for bare feet versus heel height and found increase in distribution related to heel height. Opila-Correia *et al.*,[7] studied the kinematics of high heeled walking and found that high heeled walking was associated with increased knee flexion in the stance phase. Kerrigan *et al.*,[6] used biomechanical gait analysis and inverse dynamics to evaluate joint loadings during high heel walking and found that even moderate high heels would contribute to osteoarthritis of the knee.[8]

Electromyography analysis of the lower limb muscles in high heel users and regular footwear users showed significantly increased leg muscle activity.[9]

Based on the observations from our study done on 100 volunteers wearing heels who came to the orthopedic outpatient department for other problems, prolonged duration of wear in years/hours of wear per day resulted in heel pain that significantly affected their daily activities. Furthermore from this study, it was observed that prolonged wear of heels in due course, also resulted in back pain which again was found to be directly proportional to duration of wear in years.[10]

Even though we have derived certain conclusive results from the study, it does have few pitfalls, for instance its an observational study and results obtained were purely based on statistical analysis of the questionnaire reports. Most of the studies done on heeled footwear considered heel height more than 9 cm as high heel, whereas in our study low heel is considered <2.5 cm while heels between 2.5 and 5 cm are considered moderate and more than which is considered as high heels. Moreover, we couldnot have a definite discrimination of the type of sole worn and no changes in the final outcome with respect to the sole type worn.[11,12]

The present study did prove it right that prolonged heels wear resulted in heel pain and back pain, but concrete justification couldn't be provided as the study was done on a small group of 100 volunteers.

CONCLUSION

This study was done with an intention to break the myth or confirm the reality that high heels footwear causes heel pain and back pain. We did succeed in obtaining a stastically significant correlation between the duration of heels wear in both hours/day and a number of year worn with heel and back pain. The pitfall of this study being the minimal sample size, and it was done as an observational study based on a questionnaire evaluation.

REFERENCES

1. Cronin NJ. The effects of high heeled shoes on female gait: a review. J Electromyogr Kinesiol 2014;24:258-63.
2. Esenyel M, Walsh K, Walden JG, Gitter A. Kinetics of high-heeled gait. J Am Podiatr Med Assoc 2003;93:27-32.
3. Foster A, Blanchette MG, Chou YC, Powers CM. The influence of heel height on frontal plane ankle biomechanics: Implications for lateral ankle sprains. Foot Ankle Int 2012;33:64-9.
4. Henderson PD. A biomechanical evaluation of standing in high - Heeled shoes. Pennsylvania State McNair J 2004;11:25.
5. Eisenhardt JR, Cook D, Pregler I, Foehl HC. Changes in temporal gait characteristics and pressure distribution for bare feet versus various heel heights. J Gait Posture 1996;4:280-6.
6. Kerrigan DC, Todd MK, Riley PO. Knee osteoarthritis and high-heeled shoes. Lancet 1998;351:1399-401.
7. Opila KA, Wagner SS, Schiowitz S, Chen J. Postural alignment in barefoot and high-heeled stance. Spine (Phila Pa 1976) 1988;13:542-7.
8. Simonsen EB, Svendsen MB, Nørreslet A, Baldvinsson HK, Heilskov-Hansen T, Larsen PK, *et al*. Walking on high heels changes muscle activity and the dynamics of human walking significantly. J Appl Biomech 2012;28:20-8.
9. Russell BS. The effect of high-heeled shoes on lumbar lordosis: a narrative review and discussion of the disconnect between Internet content and peer-reviewed literature. J Chiropr Med 2010;9:166-73.
10. Mika A, Oleksy L, Mika P, Marchewka A, Clark BC. The influence of heel height on lower extremity kinematics and leg muscle activity during gait in young and middle-aged women. Gait Posture 2012;35:677-80.
11. de Oliveira Pezzan PA, João SM, Ribeiro AP, Manfio EF. Postural assessment of lumbar lordosis and pelvic alignment angles in adolescent users and nonusers of high-heeled shoes. J Manipulative Physiol Ther 2011;34:614-21.
12. Franklin ME, Chenier TC, Brauninger L, Cook H, Harris S. Effect of positive heel inclination on posture. J Orthop Sports Phys Ther 1995;21:94-9.

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Assessment of Bone Mineral Health of Humans Based on X-Ray Images Using Inference

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Abstract. Bones that provide the structural support of the body, are composed of many inorganic compounds and organic materials that all together can be used to determine the mineral density of the bone. The bone mineral density (BMD) is an index measure that is widely used as an indicator of the health of the bone. A densitometry study from dual X-ray absorptiometry (DEXA) system is a popularly used method to assess BMD. BMD values vary depending on race, age, gender and other health conditions. As DEXA is quite an expensive method and requires frequent calibration process to work properly, in this paper, we explore the possibility of developing an affordable and reliable system depending on single X-ray absorptiometry with the use of supervised learning methods. The methodology based on inference is tested on a data set consisting of spine and pelvis X-ray images of patients of varying ages between 10 and 90 years of PSG Hospitals, Coimbatore, India and the results proved to be an indicator of density of the bone.

Keywords: BMD · Classification · Supervised learning · Support vector machines · Generalized boosted regression model · Extreme gradient boosting model

1 Introduction

Bones are dynamic connective tissue that constitute the endoskeleton of vertebrates. They support and protect many organs of the body, store minerals, produce red and white blood cells. Bones have complex internal and external structure, come in different shapes, hard and strong in nature but lightweight and serve multiple functions. Bones together with muscles, ligaments and joints help in movement of various body parts. Bone is made up of bone matrix. It is composed primarily of inorganic hydroxyapatite and organic collagen. Bone is formed by the process of mineralization and calcification of this matrix around entrapped cells. Remodelling is the process of resorption followed by replacement with little change in shape and occurs throughout a person's life which tends to

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phase down with advancement of age. The purpose of remodelling is to regulate and maintain calcium homeostasis, repair micro-damaged bones and to shape the skeleton during growth.

Bone mineral density (BMD) is a measure of the amount of hydroxyapatite (Hap) and mineral salts per unit area in the bone that is used as an indicator of the bone's health. The mineral content in a bone sample is evaluated by direct evaluation through the atomic absorption spectroscopy and inductively coupled plasma method or by indirect method, which consists of single or dual X-ray diffraction and computer tomography to obtain the bone mineral density. The indirect method of measurement is painless, non-invasive and involves low radiation exposure over the spine or hip region. By using direct methods, it is possible to obtain quantitative information regarding the bone mineral components, whereas in indirect methods, the information is closely related to the optical absorption of the bone tissues. Nowadays, BMD is measured by dual-energy X-ray absorptiometry (DEXA). The DEXA system uses double X-ray intensities in order to obtain two images, one of which is taken at high energy, whereas the second one is taken at low energy. The difference between the two is normally used to calculate the BMD by means of a calibration function of the system itself. WHO defines BMD to be 2.5 standard deviations (SD) or more below the peak bone mass as a normal T score (above −1 SD); between −1 and −2.5 SD defined as mildly reduced bone mineral density and below −2.5 SD as osteoporosis.

The average density of the bone is around 1000 to 1200 mg/cm^2. The density varies with age, race and gender. Bone density tests measure two conditions, osteopenia and osteoporosis. BMD would be greater than or equal to 833 mg/cm^2 for normal people. Osteopenia is a condition where BMD is lower than normal, like between 648 mg/cm^2 and 833 mg/cm^2 [2]. With further bone loss, osteopenia leads to Osteoporosis, a disease of the bones, where BMD is still reduced (less than 648 mg/cm^2), bone micro-architecture deteriorates and the amount and variety of proteins in bone are altered. Poor bone density relates to higher probability of fracture. Bones become weak and can break from a minor fall or even from simple actions like sneezing and twisting. People over the age of 50, people with smoking habits, drinking habits, long term use of steroid drugs, Vitamin D deficiency and women with early menopause are more susceptible for having less bone density. Women are more susceptible to get Osteoporosis due to the reasons: (1) Women tend to have thinner, smaller bones than men (2) Estrogen, a hormone that protects bones, decreases sharply when women reach menopause, which can cause bone loss. Fractures are the most dangerous aspect of osteoporosis. Acute and chronic pain is often attributed to fractures from osteoporosis and can lead to further disability and early morbidity and functional impairment. In essence, the daily activities of a person become a painful ordeal and overall quality of life declines. Reduced bone mass is therefore a useful predictor of increased fracture risk. Normal and Osteoporotic conditions of a bone are shown in Fig. 1.

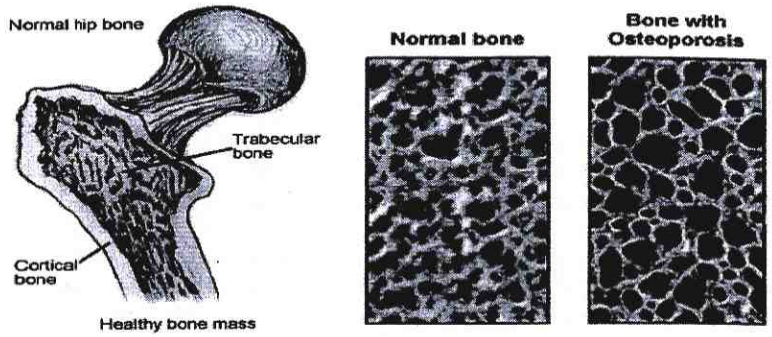


Fig. 1. Normal bone image: a. Trabecular bone b. Micro-architecture of normal and Osteoporosis affected bone

DEXA is the most widely used and most thoroughly studied bone density measurement technology. But it is quite an expensive method in developing countries like India. Moreover BMD values calculated depend on race, age and other conditions and not a constant for all people throughout the world. Hence, this paper explores the possibility of developing an affordable and reliable system to study bone mineral density using X-rays which is a very basic and conventional screening morality.

Section 2 discusses the related work. Section 3 discusses the proposed methodology used in this paper. Section 4 elaborates on image enhancement, feature extraction and bone mineral assessment using different classification methods. Section 5 reports on the experiments conducted and the results obtained. Finally, Sect. 6 concludes the work.

2 Related Work

DEXA is recognized as the acceptable method to measure BMD. DEXA scans can have errors between 5 and 8% due to calibration, positioning and other factors. X-rays are more cheaper and many researchers have shown that there exists relationship between plain radiographic patterns and three-dimensional trabecular architecture.

Luo et al. [12] have shown that the plain radiograph contains architectural information directly related to the underlying 3D structure based on the study of human calcaneal trabecular bone. Veenland [19] has assessed the suitability of different texture analysis methods for use in radiographs and also to select texture features that are able to quantify the changes in the radiographic trabecular pattern occurring in osteoporosis. Chappard et al. [3] showed that texture analysis by Euclidean and fractal methods provided significant differences in the two bones of a rat model submitted for disuse induced by Clostridium botulinum Toxin and found that such analysis on radiographs appears able to detect architectural differences in the trabecular architecture even when bone loss has not

reached a value sufficient enough to induce changes appreciable by DEXA. Again, Chappard et al. [4] have shown that the trabecular characteristics were found to be highly correlated with texture parameters describing the X-ray image and suggested that the texture analysis of X-ray films might be a suitable approach to investigate the disorganization of bone in osteoporosis. X-ray films constitute a 2D projection of the trabecular architecture, the resulting image is texture and this may be an indicator of disease etiology.

Guggenbuhl et al. [9] in their study found a good correlation between texture analysis of X-ray radiographs and 3-D bone microarchitecture assessed by micro-CT of human iliac bone. Jimenez-Mendoza and Espinosa-Arbelaez [11] have determined the parameter equation that defines the ratio of the pixels in radiographs and BMD and demonstrated the methodology on Wistar rats' femur bones on a calibrated system.

All this suggest that the study of bone mineral health by direct means is tedious and harmful to the human body and so an indirect measure by the DEXA is an accepted methodology. With the technology yet to reach the common masses being hindered by cost and the BMD values available are only for the white race, the study of BMD using X-ray images is a boon for the developing nations like India.

3 Proposed Methodology for Indication of Bone Mineral Density

Researchers have investigated the relationship between bone health and bone X-ray images through various experiments. This study is based on the concept of transitive inference. Suppose R is a relation on set A. Relation R is transitive if $\forall x, y, z \in A, ((xRy) \bigwedge (yRz)) \Rightarrow (xRz)$. A transitive relation between premises is said to occur when the conclusion a R c (read as 'a related to c') follows logically from the premise relations a R b and b R c(a related to b and b related to c). Humans are capable of making transitive inferences and do so routinely. Transitive inference is a form of deductive reasoning. This work is based on the transitive inference: If bone mineral density is related to the DEXA scan of the person, and the DEXA scan can be related to the single X-ray scan, then bone mineral density can be related to the single X-ray scan.

We propose the following methodology: (1) In preprocessing, image filters and H-domes image slicing is applied to enhance contrast in X-ray images, (2) Textural features, statistical features and fractal dimension are computed in feature extraction and (3) three supervised algorithms namely, support vector machines, generalized boosted regression model and extreme gradient boosting are applied to classify patients to have bone mineral density to be high (Normal), or Abnormal (medium and low). The three classification algorithms are also applied to discriminate between Normal, Medium (osteopenia condition) and Low (osteoporosis condition). The three algorithms are applied on female patients alone to infer if distinction is more apparent in females.

Raw X-ray images possess low frequency noise due to X-ray diffusion in soft tissues and high frequency noise due to X-ray acquisition and Imaging characteristics. Chappard et al. [3,4] have applied a median filter to eliminate low frequency noise and a "top hat" filter to eliminate high frequency noise to increase the contrast of the bony structures within the image. Guggenbuhl et al. [9] binarized the images by applying automatic thresholding based on the histogram frequency distribution of grey levels. Abidi et al. [1] applied a series of common enhancement algorithms like linear regression, gamma intensity adjustment, logarithmic intensity adjustment, histogram equalization, edge and morphological operations etc. on X-ray images of luggage scenes and compared the results; They also proposed a new method to determine the optimal number of clusters or thresholds when segmenting X-ray images consisting of low density threat weapons. They used image hashing via intensity slicing such that objects of different intensity values are more visible. Intensity slicing can be done by equal interval image slicing, cumulative image slicing and H-domes image slicing. In this paper, we propose to use median filter to eliminate low frequency noise and "top hat" filter to eliminate high frequency noise and H-domes image slicing for intensity slicing of image hashing for the bone X-ray images.

Materka and Strzlecki [13] have discussed that image texture is a rich source of visual information and that feature extraction, texture discrimination, texture classification and shape reconstruction are the major issues in texture analysis; Results from the feature extraction stage are used for texture discrimination, texture classification or object shape determination. Also, to be noted is that analysis of fractal dimension can additionally enrich diagnostic knowledge about bone microarchitecture. Chappard et al. [3,4], Guggenbuhl et al. [9] have used run-length distribution, "skyscraper" fractal analysis, "blanket" fractal analysis and statistical analysis in their experiments to establish the correlation between texture analysis and bone mineral content, between texture analysis and histomorphometry and between texture analysis and bone micro-CT, respectively. In this paper, we propose to use the texture features, run-length distribution, fractal dimension, and statistical parameters as features. Normally, bone density accumulates during childhood and reaches a peak by around 25 years of age. Bone density is then maintained for about 10 years. After the age of 35, both men and women will normally lose 0.3–0.5% of their bone density per year as part of the aging process. Also women are more susceptible to low bone density. Hence, age and gender are also added into the feature set.

Learning methods can be classified into supervised, unsupervised and reinforcement. Supervised learning methods (or Classifiers) exhibit good mapping capabilities between the input patterns and their associated outputs(classes). They learn by examples, can predict new outcomes from past trends and can process information in parallel at high speed and in a distributed manner. Data are partitioned into training and test sets. Classifiers are continuously made to learn the instances in the training set to map to their classes, upto an allowed tolerance and this is the training phase. In the testing phase, the test data are input to verify the class of the instances by the stabilized weights in the training

phase. Cross-validation is to split the training set into training set and validation set. A k-fold cross-validation partitions the training set into k sets. For each model complexity, the learner learns k times, each time using one of the sets as the validation set and the other sets as the training set. It selects the model that has the smallest average error on the validation set.

The features so extracted are applied to three classification algorithms Support Vector Machines (SVM), Generalized Boosted Regression Model (GBM) and Extreme Gradient Boosting (XGB). A 10-fold cross-validation was applied to all the algorithms. Support vector machines (SVM) [18] are supervised learning models with associated learning algorithms that analyze data for classification and regression. Boosting refers to a general and provably effective method of producing a very accurate prediction rule by combining rough and moderately inaccurate rules. It has good generalization properties and seems not to overfit in practice. The most straight forward generalization to a multiclass case, called AdaBoost is adequate when the weak learner is strong enough to achieve reasonable high accuracy [20]. Boosting has a sound theoretical base and also has the advantage that the extra computation time that it requires is known in advance. Moreover, in some applications where the dataset is large, the improvement is dramatic, due to the reduction of classification errors and well worth the computational cost [16]. The family of boosting methods is based on a different, constructive strategy of ensemble information. To establish a connection with the statistical framework, a gradient-descent based formulation of boosting methods was derived by Freund and Schapire [6] and Friedman et al. [7,8]. This formulation of boosting methods and the corresponding models were called the gradient boosting machines. This framework also provided the essential justifications of the model hyper parameters. The ensemble models are a useful practical tool for different predictive tasks, as they can consistently provide higher accuracy results compared to conventional single strong machine learning models [14]. Xgboost is an efficient and scalable implementation of gradient boosting framework [5].

This paper proposes to use the above set of algorithms in R [17] for the classification of bone mineral density and glean sufficient knowledge in order to deduce the transitive inference.

4 Classification of Bone Mineral Density in X-Ray Images

The raw X-ray images are impregnant with noise due to diffusion in soft tissues and acquisition and imaging characteristics. So a variety of preprocessing techniques need to be applied to each X-ray image before features are extracted. Extraction of essential features is a necessary step in a supervised learning process so as to differentiate the classes appropriately. The X-ray bone image undergoes preprocessing, feature extraction and classification phases.

4.1 Preprocessing of X-Ray Images

The raw X-ray image is subjected to Weiner filter to reduce noise. The background effects are removed by top hat and bottom hat filters which also have the benefit of adding a significant degree of smoothing to the spectrum. H-domes image slicing is further applied to remove small grains and this slicing method was chosen over equal interval image slicing and cumulative image slicing based on the peak signal to noise ratio (PSNR) on a subset of 25 bone images. The PSNR value is a quantitative measure that is used to compare filter performance between original and the reconstructed image. Figure 2a represents a sample original pelvis image of a patient and 2b represents the enhanced image.



Fig. 2. Pelvis X-ray image: a. original b. H-domes enhanced

4.2 Feature Extraction

Feature extraction is a process by which the X-ray image is broken down into components of essential information that are more useful for describing its structure. The histogram h(i) contains the first order statistical information about the image, i = $0, 1, .. G-1$ where G is the total number of intensity levels in the image. h(i) contains the number of pixels in the whole image, which has this intensity. The approximate probability density of occurrence of the intensity levels is given by

$$p(i) = h(i)/M * N \tag{1}$$

where the size of the image is M × N. First-order statistical properties or central moments are derived from it to characterize the texture. The mean takes the average level of intensity and is defined as

$$mean = \mu = \Sigma_{i=o}^{G-1}(i * p(i)) \tag{2}$$

The variance describes the variation of intensity around the mean and is defined as

$$variance = \sigma^2 = \Sigma_{i=o}^{G-1}(i-\mu)^2 * p(i)) \tag{3}$$

Energy is defined as

$$Energy(E) = \Sigma_{i=o}^{G-1}(p(i))^2 \tag{4}$$

Entropy is a measure of histogram uniformity and is defined as

$$Entropy(H) = -\Sigma_{i=o}^{G-1}(p(i) * log_2(p(i)) \tag{5}$$

These first order measures cannot completely describe texture. The second order histogram is defined as the gray level co-occurrence matrix (GLCM) (joint probability distributions of pair of pixels) [10]. Contrast (or inertia) returns a measure of the intensity contrast between a pixel and its neighbor over the whole image and is defined as

$$Contrast = \Sigma_{i=o}^{G-1}\Sigma_{i=o}^{G-1}(i-j)^2 * p(i,j) \tag{6}$$

Correlation returns a measure of how correlated a pixel is to its neighbor over the whole image and is defined as

$$Correlation = \frac{\Sigma_{i=o}^{G-1}\Sigma_{i=o}^{G-1}(i-\mu_i)(j-\mu_j)p(i,j)}{\sigma_i\sigma_j} \tag{7}$$

where μ_i, μ_j, σ_i and σ_j denote the mean and standard deviations of the row and column sums of the matrix. Energy is calculated from GLCM as

$$Energy = \Sigma_{i=o}^{G-1}\Sigma_{i=o}^{G-1}p(i,j)^2 \tag{8}$$

Homogeneity returns a value that measures the closeness of the distribution of elements in the GLCM to the GLCM diagonal and is defined as

$$Homogeneity = \Sigma_{i=o}^{G-1}\Sigma_{i=o}^{G-1}\frac{p(i,j)}{1+(i-j)^2} \tag{9}$$

A feature vector is created for each X-ray image based on global qualities like average gray level, average contrast, smoothness, moments, uniformity and

Table 1. Feature extraction

Algorithm I Feature Extraction
1: for each image in the X-ray set do
2: Apply Weiner filter
3: Apply top and bottom hat filtering
4: Apply hDome Image enhancement
5: Get texture features of the image
6: Get properties from gray level co-occurrence matrix
7: Get runlength features and fractal dimension
8: Include age and gender as features
10: end for
The feature values are stored

entropy (Eqs. 1 to 5), gray level run length statistics like Short Run Emphasis (SRE), Gray Level Run Emphasis (GRE), Gray Level Non-uniformity (GLN), Run Percentage (RP), Run length non-uniformity (RLN), and fractal dimension, gray level co-occurrence matrix (GLCM) characteristics like contrast, correlation, energy and homogeneity (Eqs. 6 to 9). Age and gender are also added into the feature vector. Algorithm I in Table 1 states the procedure for feature extraction.

4.3 Classification

R [17] is a language and environment for statistical computing and graphics. It is a GNU project developed at Bell laboratories by John Chambers and colleagues. R provides a wide variety of statistical (linear and nonlinear modelling, classical statistical tests, time-series analysis, classification, clustering) and graphical techniques, and is highly extensible. R is designed around a true computer language and it allows users to add additional functionality by defining new functions. As specified above the supervised learning algorithms used in this paper are SVM, GBM and XGB. The results are tabulated in Table 2 for a 2 class problem. The ROC curves are specified in Figs. 3, 4 and 5 for SVM, GBM abd XGB respectively.

Table 2. Results for normal/abnormal classification (all patients)

Classifier	Accuracy %	Sensitivity	Specivicity	AUC
SVM	82.00	0.92	0.68	0.85
GBM	82.00	0.90	0.68	0.88
XGB	89.00	0.92	0.65	0.88

5 Results and Discussion

PSG Hospitals is a 810 bedded, tertiary care hospital with qualified and experienced faculty and state of the art infrastructure, affiliated to PSG Institute of Medical Sciences & Research, Coimbatore, India [15]. The hospital houses various specialties like Medicine, Surgery, Obstetrics & Gynecology, Paediatrics, Orthopaedics, Radiology and Emergency Medicine. The hospital has been recognized for implementation of various social welfare schemes and is an internationally acclaimed centre to conduct various clinical trials.

Experiments are conducted on 311 pelvis and 160 spine images of the patients screened over a three month period at PSG Hospitals. The X-ray images are 760×580 pixels in size. The age of the patients varied between 10 and 90 and the average age was 46.37. There are 253 males and 218 females. Three experienced doctors identified the class attribute seperately based on the X-ray image and the

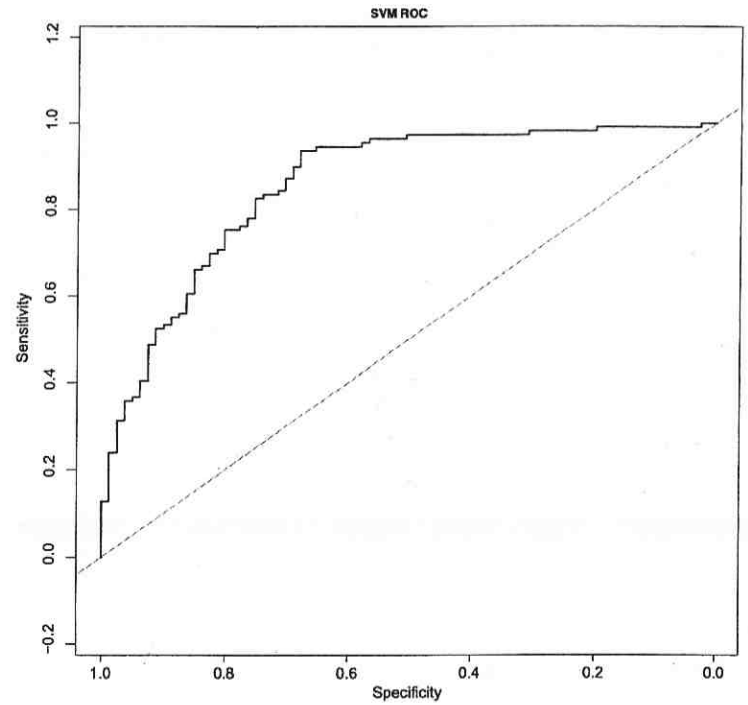


Fig. 3. ROC curve for SVM

class type (Normal/Osteopenia/Osteoporosis) was decided based on the majority voting by them. There was 25% of tie in the class attribute in such a process and lower the class attribute was decided with high density being normal, Osteopenia being medium and Osteoporosis being low.

After the images were pre-processed for enhancement, global, local statistical features, GLCM features, fractal dimension were extracted, age and gender were added and a total of 19 features are finally decided. The feature values are normalized. The 471 images are divided into training and testing at a 60:40 ratio at random and a 10-fold cross validation is used. For comparison purposes, the same set of data are classified by SVM, GBM and XGB. The results are tabulated in Table 2 for the two class problem (Normal/Abnormal) and in Table 3 for a three class problem (Normal/Osteopenia/Osteoporosis). It can be seen that the methodology identifies between normal and abnormal with an accuracy of 89% but when it is to distinguish between osteopenia and osteoporosis away from normal, the accuracy has reduced to 62%. Since, women were more susceptible to low bone density, the methodology was applied only to X-rays of female patients and found to have 82% accuracy. The results are tabulated in Table 4.

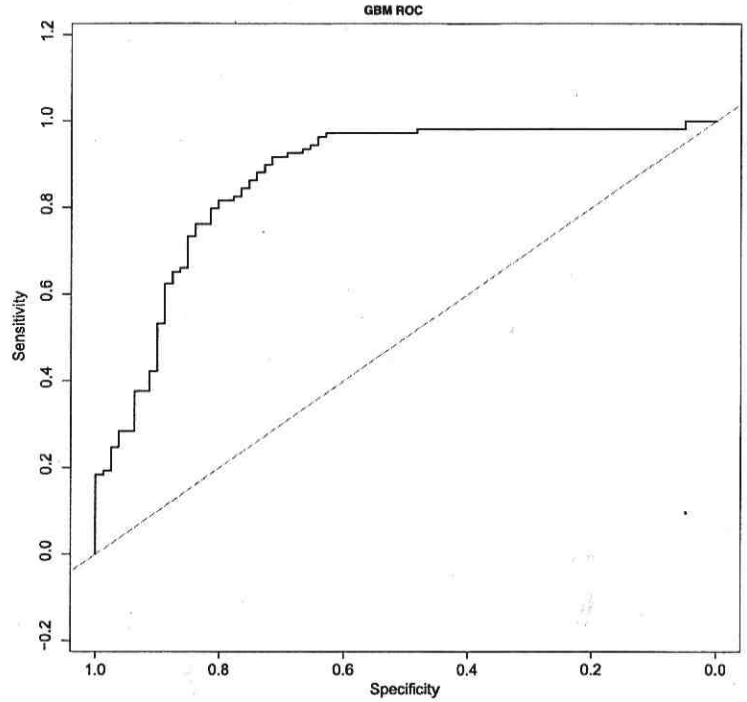


Fig. 4. ROC curve for GBM

Table 3. Results for normal/osteopenia/osteoporosis (all patients)

Classifier	Accuracy %	Sensitivity	Specivicity	AUC
SVM	57.00	0.55	0.78	0.55
GBM	62.00	0.60	0.82	0.55
XGB	59.00	0.55	0.76	0.57

Table 4. Results for normal/abnormal classification (only females)

Classifier	Accuracy %	Sensitivity	Specivicity	AUC
SVM	74.00	0.80	0.61	0.83
GBM	62.00	0.87	0.10	0.51
XGB	82.00	0.95	0.57	0.86

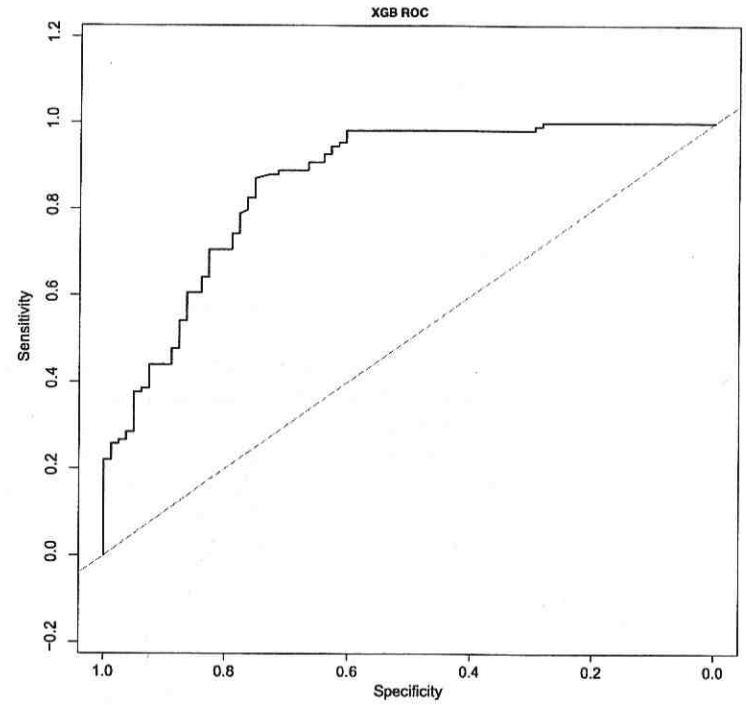


Fig. 5. ROC curve for XGB

6 Conclusion

The methodology described in this paper has extended the idea and relationship established by various researchers between bone mineral health and X-ray radiography images. DEXA equipment from different manufacturers might not give identical results, because of differences in calibration and bone edge detection algorithms. Moreover, the BMD values specified by WHO is based on white women [2] and as a result, the BMD values calculated for other countries or on other race for the same age cannot have this as the reference.

The proposed system is based on the strong correlation between the X-ray images and their underlying microarchitecture and the health of the bones. The system weighs on the transitive dependency and explores through inferential component, the bone mineral density by classification methods. The proposed system could identify abnormality with an accuracy of 89% for all patients and with an accuracy of 82% for only females, though the accuracy reduced to 62% for the three class problem. The proposed system is cheaper, portable, versatile and dependable to study changes in the density of minerals in X-ray images of bones. It is meant to be a guidance to the doctors to prescribe further medication

if required. Similar to DEXA, it is a non-contact and non-invasive method. As more radiography images are added to the data set, the classification algorithms would be able to learn the characteristics of the images pertaining to a particular demography, age and race and able to classify much better. Additional features or a better image enhancement algorithm on the X-ray images may indeed be the future scope of work.

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References

1. Abidi, B., Mitckes, M., Abidi, M., Liang, J.: Grayscale enhancement techniques of x-ray images of carry-on luggage. In: Proceedings of SPIE 6th International Conference on Quality Control by Artificial Vision, vol. 5132, pp. 579–591 (2003)
2. Bone Densitometry. http://courses.washington.edu/bonephys/opbmd.html. Accessed 26 Aug 2016
3. Chappard, D., Chennebault, A., Moreau, M., Legrand, E., Audran, M., Basle, M.F.: Texture analysis of x-ray radiographs is a more reliable descriptor of bone loss than mineral content in a Rat model of localized disuse induced by the Clostridium botulinum toxin. Bone **28**(1), 72–79 (2001)
4. Chappard, D., Guggenbuhl, P., Legrand, E., Basle, M.F., Audran, M.: Texture analysis of x-ray radiographs is correlated with bone histomorphometry. J. Bone Miner. Metab. **23**, 24–29 (2005)
5. Chen, T., He, T.: xgboost: eXtreme Gradient Boosting. R package version 0.4-2 (2015)
6. Freund, Y., Schapire, R.: A decision-theoretic generalization of on-line learning and an application to boosting. J. Comput. Syst. Sci. **55**, 119–139 (1997)
7. Friedman, J.: Greedy boosting approximation: a gradient boosting machine. Ann. Stat. **29**, 1189–1232 (2001). doi:10.1214/aos/1013203451
8. Friedman, J., Hastie, T., Tibshirani, R.: Additive logistic regression: a statistical view of boosting. Ann. Stat. **28**, 337–407 (2000). doi:10.1214/aos/1016218222
9. Guggenbuhl, P., Bodic, F., Hamel, L., Basle, M.F., Chappard, D.: Texture analysis of x-ray radiographs of iliac bone is correlated with bone micro-CT. Osteoporos. Int. **17**, 447–454 (2006)
10. Haralick, R.M., Shanmugam, K., Dinstein, I.H.: Textural features for image classification. IEEE Trans. Syst. Man Cybern. **6**, 610–621 (1973)
11. Jimenez-Mendoza, D., Espinosa-Arbelaez, D.G., Giraldo-Betancur, A.L., Hernandez-Urbiola, M.I., Vargas-vazquez, D., Mario, E.: Single x-ray transmission system for bone mineral density determination. Rev. Sci. Instrum. **82**, 125105 (2011)
12. Luo, G., Kinney, J.H., Kaufman, J.J., Haupt, D., Chiabrera, A., Siffert, R.S.: Relationship between plain radiographic patterns and three-dimensional trabecular architecture in the human calcaneus. Osteoporos. Int. **9**, 339–345 (1999)

13. Materka A., Strzlecki M.: Texture analysis methods a review. COST B11 report, Institute of Electronics, Technical University of Lodz, Brussels (1998)
14. Natekin, A., Knoll, A.: Gradient boosting machines, a tutorial. Front. Neurorobotics **7**, 21 (2013). http://doi.org/10.3389/fnbot.2013.00021
15. PSG Institute of Medical Sciences & Research, Coimbatore, India. http://psgimsr.ac.in/hospitals.html. Accessed 12 Aug 2016
16. Ross, Q.J.: Bagging, boosting, and C4. 5. In: AAAI/IAAI, vol. 1 (1996)
17. R Project and Foundation. https://www.r-project.org/about.html. Accessed 12 Aug 2016
18. Vapnik, V.N.: The Nature of Statistical Learning Theory. Springer, Berlin (1995)
19. Veenland J.F.: Texture analysis of the radiographic trabecular bone pattern in osteoporosis. Ph.D. thesis, Reasmus University, Rotterdam (1999). ISBN 90-75655-04-5
20. Freund, Y., Scaphire, R.E.: Experiments with a new boosting algorithm. In: Proceedings of 13th International Conference in Machine Learning, pp. 148–156 (1996)

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Functional outcome of Tibial condyle fractures treated by minimally invasive plate osteosynthesis

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Abstract
Introduction: Tibial plateau fractures with intra-articular extension are very difficult to manage. Age, skin conditions, compartment syndrome and osteoporosis further increase the obstacles in the healing process.
Complex biomechanics of its weight bearing position and complex ligamentous stability and articular congruency are the main reason why these fractures are of concern to surgeon.
In our study we have evaluated the functional outcome of locking plate fixation of tibial condyle fractures using minimally invasive technique, a minimum period of 6 months after plate fixation by Rasmussen score and Knee society score.
Materials and Methods: Tibial plateau fractures treated by minimally invasive plate osteosynthesis using locking compression plate from January 2010 to January 2015 were taken into the study. All the patients who had completed the inclusion criteria were called for assessment of functional outcome of knee using Rasmussen score and knee society score.
Results and Discussion: In our study, we were able to get away with unilateral plating for type V and type VI fractures without any late complications like loss of reduction and malalignment.
Choice of the procedure/implant should be based on the fracture pattern, bone quality and intraoperative reduction.
Conclusion: Treatment of intraarticular tibial plateau fractures is still unsolved.
Our results in minimally invasive percutaneous plate osteosynthesis (MIPPO) technique is in par with the literature.

Keywords: Tibial fracture, Schatzker, osteosynthesis, implants

Introduction
The knee joint is complex and most commonly injured joint now because of increased motor vehicle accidents and sports related injuries. As it is a superficial joint, it is more exposed to external forces and gets easily injured. Tibial plateau fractures with intra-articular extension are very difficult to manage. Age, skin conditions, compartment syndrome and osteoporosis further increase the obstacles in the healing process.
Complex biomechanics of its weight bearing position and complex ligamentous stability and articular congruency are the main reason why these fractures are of concern to surgeon. The ideal treatment of high-energy tibial plateau fractures is controversial. Open reduction and stable internal fixation helps in maintaining the articular surface and restoration of the mechanical alignment which allows early mobilization of knee. But, techniques of open reduction and internal fixation compromise the soft tissues and the rate of wound infection is relatively high.
Various other methods of treatment like hybrid fixation and now plate fixation using minimally invasive technique have been suggested. Each method has its own advantage and disadvantages.The development of locking implants has allowed the use of minimally invasive technique for unilateral plating [37-39] with improvement in handling the soft tissue [40-43].
There are lot of studies which assess the general outcome of these fractures but there are only few studies which assess the functional outcome of these fractures which is more important to the patient. In our study we have evaluated the functional outcome of locking plate fixation of tibial condyle fractures using minimally invasive technique, a minimum period of 6 months after plate fixation by Rasmussen score and Knee society score.

Materials and methods

Tibial plateau fractures treated by minimally invasive plate osteosynthesis using locking compression plate from January 2010 to January 2015 were taken into the study.

Inclusion criteria

Age group: 18 years to 60 years all tibial condyle fracture treated by minimally invasive plate osteosynthesis.

Exclusion criteria

1) Skeletally immature patients,
2) Neurovascular injuries,
3) Concomitant lowerlimb fractures like patella, femur, ankle and pelvic fractures.
4) Open fractures

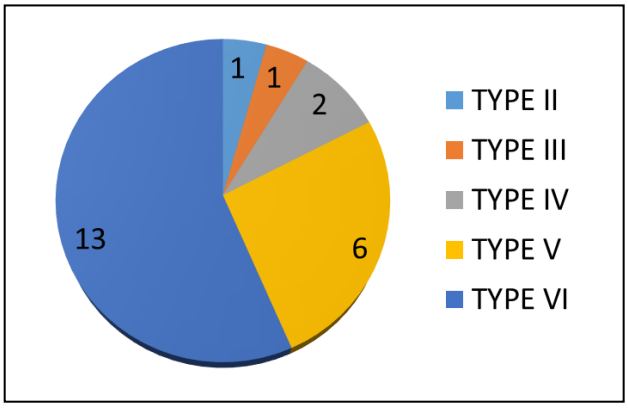
Methodology

This was both retrospective as well as a prospective study. For the retrospective study in-patients and out-patient records of the study population were collected from the medical records department and OT register. Age, gender of the patients, mode of injury, side of involvement, Associated injuries and medical comorbidities were documented. The Neurovascular status of the affected leg, compartment syndrome and any blisters or open wounds was noted. All Tibial condyles fractures were graded preoperatively using Schatzker classification. Patients were followed up postoperatively after a minimum period of six months after surgery. All the patients who had completed the inclusion criteria were called for assessment of functional outcome of knee using Rasmussen score and knee society score.

Results

n our study, 13 patients belonged to type VI, 6 patients belonged to type V, 2 patients belonged to type IV, 1 patient belonged to type III and type II respectively.

Total Number of Patients



Thirteen patients had right tibial plateau fractures and remaining ten patients had left tibial plateau fractures. Only fractures which were treated with MIPPO technique were taken for the study. RTA was the mode of injury in all the cases. In our study type VI Schatzker fractures was the commonest type. Functional assessment was assessed by Rasmussen score (subjective score) and knee society score (objective score). Average follow up period was 22.08 months.

Mean Follow Up

SL.NO	TYPE	AVERAGE FOLLOW UP (MONTHS)
1.	I	-
2.	II	17 (6-17)
3	III	18 (6-18)
4.	IV	32.5 (6-47)
5.	V	19.8 (6-27)
6.	VI	23.1 (6-53)

In type VI Schatzker there were thirteen patients of whom seven patients were treated by lateral plating and six patients were treated by medial plating. Mean Rasmussen score of type VI fractures was found to be 25.6, average knee society score was found to be 93.2 and mean range of knee flexion was found to be 118.8 degrees. All the patients were started on partial weight bearing from 8 weeks and full weight bearing by 12 weeks.

Average Scores by Knee Society and Rasmussen Score

Sl. No	Type	Knee Society Score	Rasmussen Score
1	1	-	-
2	2	98	27
3	3	96	29
4	4	94	26.4
5	5	81.3	22.8
6	6	93.2	25.6

Six patients belonged to type V Schatzker group. Mean follow up was 19.8 months (6-27). Lateral plating was done for 2 patients and medial plating was done for 4 patients. Average Rasmussen score was 22.8, mean knee society score was 81.3 and average range of knee flexion was found to be 118.3 degrees. Only one patient had flexion contracture of 10 degrees. All the patients were started on partial weight bearing at 8 weeks and full weight bearing was started at 12th week. Postoperatively there were no wound infections.

In type IV Schatzker fractures there were two patients. Mean follow up was found to be 32.5 months (6-47). All patients were treated by medial plating. Average range of flexion was found to be 122.5 degrees. Mean Rasmussen score was found to be 26.5. Mean knee society score was found to be 94. There was no wound infection and weight bearing was started at 12th week. In our study only one patient belonged to type III Schatzker fracture. Follow up was done at 18 months (6-18). He was treated by lateral plating and had Rasmussen score of 29. Range of flexion was found to be 120 degrees. Knee society score was found to be 96. No complications were noted.

In type II Schatzker there was only one case which met the criteria. Lateral plating was done. Follow up was done at 17 months (6-17). Rasmussen score was found to be 27, knee society score was 98 and range of knee motion was 125 degrees. No complications were noted. No bone grafts used in our study.

Discussion

Intraarticular tibial plateau fractures are complex fractures accounting for about 1.2% of all fractures. They affect knee function and stability which results in considerable morbidity. These fractures are caused by high velocity injuries and often associated with severe comminution and soft-tissue damage. The goals of treatment are to restore joint congruity, limb alignment and early mobilisation of joint. Stable internal plate fixation without damaging the soft-tissue envelope is very difficult to achieve, only fair results are seen in 20% to 50% in these fractures. Outcome after tibial plateau fracture fixation depends mainly on knee range of motion and strength of the quadriceps. Knee stiffness is more clinically relevant than instability in these fractures.

Open reduction and internal fixation (ORIF) with plates and screws enables direct fracture visualisation, reduction, and fixation, but there is high risk of soft tissue injury, stiffness and deep infection. The hybrid external fixator avoids soft tissue problems, but risks non-union, pin tract infections and poor patient compliance.

The concept of preserving the blood supply and atraumatic surgical technique led to the development of biological fixation techniques. Using this technique, soft tissue damage is reduced and shows higher union rate.

The development of locking implants has allowed the use of minimally invasive technique for unilateral plating [37-39] with improvement in handling the soft tissue [41-43].

Laterally placed locking plates provide better stability in the presence of complex proximal 1/3rd tibia fracture with metaphyseal comminution and serves as an alternative to medial plate or external fixator for additional support of the medial column when a non-locking plate is used for bicondylar fractures. This plate allows fixation through single incision which avoids wound dehiscence, infection and prolonged immobilisation associated with extensile approaches.

MIPPO enables indirect fracture reduction and percutaneous sub muscular implant placement. Favourable outcome is not due to MIPPO but due to less extensive dissection of soft-tissue envelope and devitalisation of fracture fragments.

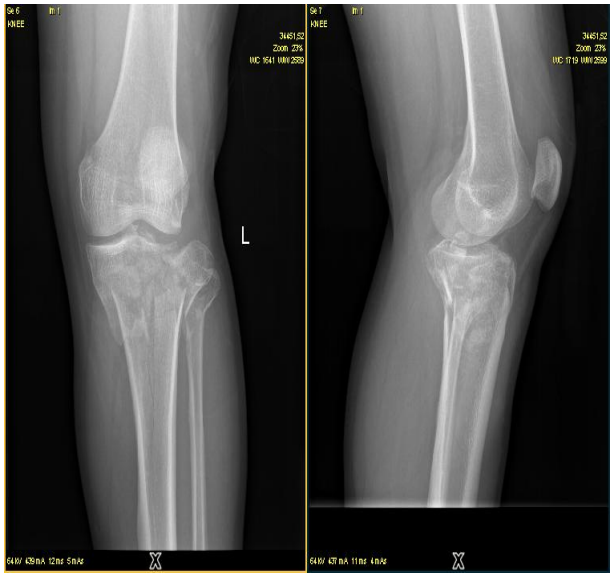
The aim of our study was to evaluate the functional outcome of tibial condyle fractures treated by minimally invasive percutaneous plate osteosynthesis. There is no universal scoring system for assessing the functional outcome for these fractures. Literature shows multiple scoring system like Rasmussen, knee society score and oxford knee score. In our study, we have evaluated the patients using Rasmussen score which is subjective score and knee society score which is an objective score. All these fractures were treated by a single plate either medial or lateral (11 lateral and 12 medial). In case of type V and type VI fractures, if needed the opposite condyle was fixed with percutaneous cancellous screws.

Mechanism of injury was road traffic accident for all these patients. The fractures were classified by Schatzker's classification. 13 patients belonged to type VI, 6 patients belonged to type V, 2 patients belonged to type IV, 1 patient belonged to type III and type II respectively. Even though, according to literature type II fractures were the most common, only one patient with type II fracture was included in our study as all other type II & type I fractures in the study period were fixed only by percutaneous screws hence excluded from the study. Type VI fracture with 13 patients (56.5%) were the most common in our study, next was type V (26.08) with 6 patients. Average followup period was 22 months (6-53). Hasnain Raza *et al*, in their study of assessing the functional outcome of tibial condyle fractures of 41 patients by minimally invasive plate osteosynthesis by Rasmussen functional score found excellent results in 18 patients, good in 19 patients and 4 patients had unacceptable results. The mean Rasmussen score was found to be 25.3 and range of knee flexion was 118 degrees. In our study mean Rasmussen score was found to be 26.1 and average range of knee flexion was found to be 120.9 degrees. Of which ten patients had excellent and good results each. Only 3 patients had fair results. This is comparable to the study done by Hasnain Raza. Mohammad Ali Tahririan, Seyyed Hamid Mousavitadi, and Mohsen Derakhshan [13] in their clinical study comparing the functional outcomes of tibial plateau fractures treated with nonlocking and locking plate fixation by knee society score, found a score of 80.2 for locking plate and 72.5 for non-locking plate. Average range of knee flexion was found to be 122.3 degrees for locking plate and 115.7 degrees for non-locking plate. In our study, locking plate was used for all the cases. Average knee society score was found to be 92.5 and average knee flexion was found to be 120.9 degrees. So, functional outcome in our study was marginally better than the locking plate group in that study and significantly better than the non-locking group. This shows the superiority of the locking plate in view of stable fixation and early range of motion when compared to non-locking plate. Chang-Wug Oh *et al* [18] in their study on double plating of (twenty three) type V and type VI proximal tibial fractures using minimally invasive percutaneous osteosynthesis found Eighteen patients with excellent, three patients with good and two patients with fair results. Average Rasmussen score was found to be 26 and average knee range of motion was found to be 123 degrees. In our study, nineteen patients belonged to type V and VI fractures. Average Rasmussen score of these type V and type VI fractures were found to be 24.2. Average range of flexion achieved by type V and type VI was found to be 118.5 degrees. Seven patients had excellent, ten patients with good and two patients had poor results. In their study of ten patients Kye-Youl Cho *et al* [5], used a single midline longitudinal incision and dual plating for the treatment of type V and type VI schatzker fractures. Their mean knee society score for the study group was 85 and the mean range of motion was 125 degrees. They had only one case with delayed wound healing as postoperative complication. In our study, average knee society score was found to be 87.25. One patient had 10 degrees of valgus malalignment in type VI fracture and one patient had flexion contracture of 5-10 degrees. But the functional outcome was not significantly altered when compared with others.

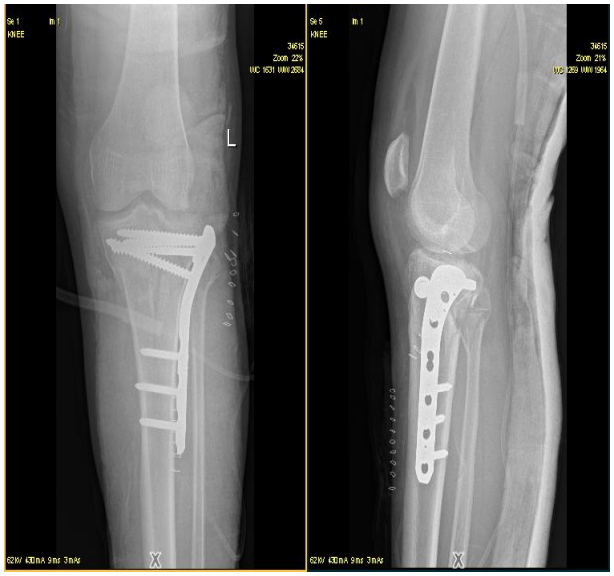
Dual plate gives better biomechanical strength and rigid construct thereby better control of both columns thus avoiding late collapse. There were no major wound problems in any of these studies. Weight bearing was started only at 8-12 weeks which was similar to our study. In our study, we were able to get away with unilateral plating for type V and type VI fractures without any late complications like loss of reduction and malalignment.

Choice of the procedure/implant should be based on the fracture pattern, bone quality and intraoperative reduction.

CASE 1:
Preop Xray



Immediate Postop Xray



After 6 Months



Clinical Picture



Conclusion

Treatment of intraarticular tibial plateau fractures is still unsolved. Our results in minimally invasive percutaneous plate osteosynthesis (MIPPO) technique is in par with the literature. There is no significant difference in the functional outcome between single plating in our study and dual plating of other studies at midterm followup. Choice of the procedure/implant should be based on the fracture pattern, bone quality and intraoperative fracture reduction.

Reference

1. Douglas R Dirschl, Daniel Del Gaizo. Staged Management of Tibial Plateau Fractures A Supplement to The American Journal of Orthopedics. 2007.
2. Comparison of outcome of unilateral locking plate and dual plating in the treatment of bicondylar tibial plateau fractures Lee *et al*. Journal of Orthopaedic Surgery and Research. 2014; 9:62.
3. Tibial plateau fractures treated with the less invasive stabilisation system. Jackson A. Lee & Stamatios A. Papadakis & Charles Moon & Charalampos G. Zalavras International Orthopaedics (SICOT) 2007; 31:415-418.
4. New trends and techniques in open reduction and internal fixation of fractures of the tibial plateau. Musah V, Tarkin I, Kobbe P, Tzioupis C, Siska PA, Pape HC. 2009 British Editorial Society of Bone and Joint Surgery 2009. 91-B, 4.
5. Treatment of Schatzker Type V and VI Tibial Plateau Fractures Using a Midline Longitudinal Incision and Dual Plating. Kye-Youl Cho, Hyun-Sup Oh, Jae-Ho Yoo, Duk-Hyun Kim, Young-Joo Cho, Kang-Il Kim. Knee Surg Relat Res, 2013; 25(2).
6. Traditional buttress plating v/s MIPO in management of proximal tibial fractures - A clinical study. Rakesh Sharma, Rajesh Kapila, Brahm Preet Singh, Yadwinder Singh Sohal. Pb Journal of Orthopaedics. 2013, 1.
7. Results of proximal tibial fractures managed with periarticular locking plates: A series of 34 cases. Jain D, Selhi HS, Mahindra P, Kohli S, Yamin M. IJRRMS 2012; 2(4).
8. Apley AG. fractures of the lateral tibia condyle treated by skeletal traction and early mobilization. A review of sixty cases with special reference to the long term result, J Bone Joint Surg 1956; 38-B:699,
9. Schatzker J, McBroom R, Bruce D. The tibial plateau fracture. The Toronto experience 1968-1975. Clin Orthop Relat Res. 1979; 138:94-104.

10. Diass JJ, Stirling AJ, Finlay DBL, Gregg PJ. Computerised axial tomography for tibial plateau fractures, J Bone Joint Surg 1987; 69-B:84.
11. Barei DP, Nork SE, Mils WJ. Functional outcome of severe bicondylar tibial plateau fractures treated with dual incision and medial and lateral plates. J Bone Joint Surg. 2006; 88-A:1713-1721.
12. Tscherne H, Lobenhoffer P. Tibial plateau fractures. Management and expected results. Clin Orthop Relat Res. 1993; 292:87-100.
13. Clinical Study Comparison of Functional Outcomes of Tibial Plateau Fractures Treated with Nonlocking and Locking Plate Fixations: A Nonrandomized Clinical Trial. ISRN Orthopedics Volume March Article ID324573 Mohammad Ali Tahririan, Seyyed Hamid Mousavitadi, Mohsen Derakhshan. 2014,
14. Functional and radiological evaluations of high energy tibial plateau fractures treated with double buttress plate fixation. Yu Z, Zheng L, Zhang Y, Li J, Ma B. Eur J Med Res. 2009; 14:200-205.
15. Recovery of knee function following fracture of the tibial plateau. Gaston P, Will EM, Keating JF. J Bone Joint Surg [Br] 2005; 87-B:1233-6.
16. Albuquerque RP, Hara R, Prado J, Schiavo L, Giordano V, Amaral NP. Epidemiological study on tibial plateau fractures at a level I trauma center. Acta Ortop Bras. [online]. 2013; 21(2):109-15.
17. Comparison of Outcomes of Operatively Treated Bicondylar Tibial Plateau Fractures by External Fixation and Internal Fixation. Malaysian orthopaedic Journal CC Chan, MRCS (Ed), J Keating, FRCS Orth (Ed). 2012; 6(1).
18. Double plating of unstable proximal tibial fractures using minimally invasive percutaneous osteosynthesis technique. Chang-Wug, Jong-Keon, Hee-Soo Kyung. In-Ho Jeon, Byung-Chul Park, Woo-Kie Min *et al*. Acta Orthopaedica. 2006; 77(3):524-530.
19. David Levay. History of Orthopaedics 1990, 625:592,
20. Sir Robert Jones. Orthopaedic surgery of injuries 1921; 1:86,
21. Wilson Wg, Jacobs JE. Patellar graft for severely depressed comminuted fractures of the lateral tibial condyle. J Bone Joint Surg 1952; 34A:436,
22. Rasmussen PS. Tibial condylar fractures. Impairment of knee joint stabilityas an indication for surgical treatment. J Bone Joint Surg Am. 1973; 55:1331-50.
23. Moore TM, Harvey JP, JR.: Roentogenographic measurement of tibial plateau depression due to fractures: J Bone Joint Surg 1974; 56A:155,
24. Drennen DB, Loucher FG, Maylahn DJ. Fractures of the tibial plateau treatment by closed reduction and spica cast, J Bone Joint Surg. 1979; 61-A:989,
25. Bowes DN, Hohl M. Tibial condyle fractures: evaluation of treatment and outcome, Clin Orthop 1982; 171:104.
26. Blokker CP, Rorabeck CH, Bourne RB. Tibial plateau fractures. An analysis of the results of treatment in 60 patients. Clin Orthop. 1984; 182:193,
27. Insall JN, Dorr LD, Scott RD, Scott WN. Rationale of the knee society clinical rating system. Clin Orthop Relat Res. 1989; (248):13-4.
28. Delamarter R, Hohl M. The cast brace and tibial plateau fractures, Clin Orthop 1987; 242:26.
29. Jensen DB, Rude C, Duus B, Bjerg-Nielsen A. Tibial plateau fractures. A comparison of conservative and surgical treatment. J Bone Joint Surg; 1990; 72B:49-52,
30. Honkonen SE, Jarvienen MJ. Classification of fractures of tibial condyles, J Bone Joint Surg 1992; 74B:840,
31. Marsh JL, Smith ST, Do TT. External fixation and limited internal fixation for complex fractures of the tibial plateau. J Bone Joint Surg 1995; 77A:661,
32. Cambell s operative orthopaedics; Fractures of lower extremity: Tibial plateau 3, 2094-2111
33. Weigel DP, Marsh JL. High energy fracture of the tibial plateau: knee function after longer follow-up. J Bone Joint Surg 2002; 84-A:1541-1551,
34. Ali AM, Bruton M, Hashmi M, Saleh M. outcome of complex fractures of the tibial plateau treated with a beam loading ring fixation system. J Bone Joint Surg. 2003; 85B:691-699,
35. Lubowitz JH, Elson WS, Guttmann D. Arthroscopic management of tibial plateau fracture part-I: Arthroscopy. 2004; 20:1063-1070.
36. Duwelius PJ, Connolly JF. Closed reduction of tibial plateau fractures a comparison of functional and roentogenographic and results, Clin Orthop. 1988; 230:116.
37. Farouk O, Krettek C, Miclau T. Minimally invasive plate osteosynthesis: does percutaneous plating disrupt femoral blood supply less than the traditional tech- nique? J Orthop Trauma. 1999; 13:401-6.
38. Farouk O, Krettek C, Miclau T, Schandelmaier P, Tscherne H. Effects of percu- taneous and conventional plating techniques on the blood supply to the femur. Arch Orthop Trauma Surg. 1998; 117:438-41.

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Original Article

The Charcot foot: Is it a commonly overlooked entity in diabetic population

Prasanna Chandiralingam and Vijay Baba N

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Abstract
Charcot Osteoarthropathy of the foot and ankle is one of a complication of diabetic neuropathy. The identification and diagnosis of these cases pose a challenging task to the general care physicians. Equally a challenging condition to treat, Charcot foot management can pose significant difficulties unless the treatment is initiated in early stages. The present study aimed at finding out the prevalence rate of Charcot foot among the diabetic patients presenting to a tertiary care centre.
We had a prevalence rate of 0.01% for Charcot foot among the study population. We could identify that the diagnosis was made late in all our cases and it was difficult to differentiate between infection and Charcot foot. The prevalence rate doubled to 0.02% when we take into account the cases with no clear cut evidence of infection.
Charcot foot is a debilitating complication of diabetes mellitus. Though not a common one we encounter, this complication needs an early diagnosis for prompt treatment. Hence any diabetic foot complications may be ideally referred for specialist opinion at the earliest.

Keywords: Charcot foot, diabetes mellitus, debilitating, early referral

Introduction
Charcot foot is a type of arthropathy due to peripheral neuropathy. It is named after Jean-Martin Charcot, who first recognised that peripheral neuropathy could lead to neuropathic joints [1]. Apart from diabetes mellitus, Charcot foot may present as a complication of various other peripheral neuropathic conditions like Neurosyphilis, syringomyelia, leprosy, poliomyelitis, and/or congenital neuropathy [2]. The Charcot foot disease, though an uncommon entity of diabetes it is a serious and potentially limb threatening complication.
The prevalence rate of Charcot foot in diabetes is not clearly known, but now it is appreciated that it is not as infrequent as it was generally thought[2]. Often it may be easily overlooked by the general practioners, especially in its earlier stages leading to underestimation of its frequency [2, 3]. In the present study we evaluated the prevalence rate of this complication among the diabetic population presenting to a tertiary care hospital.

Materials and Methods
The aim of this study was to determine the prevalence of Charcot foot among the patients presenting to the diabetic clinic of a tertiary care centre. All the patients who presented to the clinic from January 2018 to June 2019 were retrospectively analysed. The diabetic clinic of the hospital was managed by the general medicine department. Hence all the diabetic patients presenting to the hospital were initially managed by the general physicians.
The necessary data were collected with the help of out-patient register and in-patient case records. The total number of patients who were referred to the diabetic foot clinic, managed by the general surgery department for expert management of complications was noted down. The reasons for referral, if any mentioned in the register were also noted. The number of patients referred from the foot clinic to the orthopaedic department for the management of bone related complications was noted.

The patient details who were admitted for the management of foot complications as in-patients were taken from the case records.

Results and Discussion

A total of 2750 cases were enrolled in the diabetic clinic during the study period. 2175 of them were male and the remaining 575 female patients. The average age was 58 years (42 – 86 years). No specific data was available with regard to the types (type I or II) of diabetes. Neither the data regarding the duration of the disease were available.

365 cases were referred to the diabetic foot clinic for management of complications. Pain was the predominant reason for referral in 250 of the cases. The other reasons for the referral were swelling in the foot and leg in 65 cases and ulcerations in the foot and ankle region in 50 cases. A majority of these 365 cases were managed as out-patients with 88 of them being admitted for the management of severe complications. 37 of them were admitted for the management of diabetic foot ulcer. The remaining 51 cases were diagnosed as cellulitis and were admitted for intravenous antibiotics and further appropriate management.

An orthopaedic referral was given for 35 of the ulcer case after the X- rays taken had shown bone abnormalities. The referrals were given with the suspicion of osteomyelitis, but were proven to be a case of Charcot foot after the orthopaedic referral. This resulted in a prevalence rate of 0.01 percent (35/2750) of Charcot foot in the present study.

Charcot Osteoarthropathy is a degenerative, progressive and a relatively painless arthropathy of single or multiple joints caused by underlying neuropathy, with peripheral joints most commonly affected. The commonest cause of Charcot disease nowadays is diabetes mellitus. It presents as an erythematous, warm, swollen foot and ankle, thereby making it difficult to distinguish from infection clinically [4].

In the present study, when we evaluated the case records of the admitted patients diagnosed as cellulitis there was no conclusive evidence of cellulitis in more than half of the cases. 27 of the 51 cases were having an erythematous, warm and swollen foot, which were managed as cellulitis. These cases could probably represent an earlier stage of Charcot foot.

Eichenholtz [5] has defined 3 stages of Charcot arthropathy based on the natural history of the condition. (Table 1)

Table 1: The modified Eichenholtz [5] classification.

Stage	Radiographic findings	Clinical findings
0 (prodromal)	**Normal radiographs**	**Erythema, warmth, swelling**
I (development)	Osteopenia, fragmentation, joint subluxation or dislocation.	Erythema, warmth, swelling, ligamentous laxity.
II (coalescence)	Absorption of debris, sclerosis, fusion of larger fragments.	Decreased erythema, warmth and swelling.
III (reconstruction)	Consolidation of deformity, joint arthrosis, fibrous ankyloses, smoothening and rounding of fragments	Absence of erythema, warmth and swelling with stable joint +/- fixed deformity.

Note: Stages I – III were originally described by Eichenholtz, and stage 0 was later added by Shibata *et al*. [6], since the clinical signs in Charcot foot were found to precede the radiological changes.

Accordingly if the 27 cases diagnosed as cellulitis were countes as stage 1 Charcot foot, it would bring the total number of cases as 62. Then the prevalence of Charcot arthropathy would double to 0.02% (62/2750). Literature estimates of Charcot Arthropathy prevalence ranges from 0.08% in the general diabetic population to 13% in high-risk diabetic patients [7].

An anatomical classification depending on the pattern of involvement of Charcot foot was proposed by Brodsky *et al*. [8]. (Table 2)

Table 2: The anatomical classification of Charcot foot (Brodsky).

Type	Lesion location	Joints involved
1	Midfoot	Naviculocuneiform, tarsometatarsal.
2	Hind foot	Subtalar, calcaneocuboid, talonavicular.
3A	Ankle	Tibiotalar.
3B	Calcaneus	Tuberosity fracture.

In our study the majority (10) of the cases were of type 3A (Fig. 1) and the remaining (6) were of type 1 (Fig. 2). Charcot foot is most common in people with type 1 diabetes in their 5th and 6th decade of life but can also occur in younger patients with type 2 diabetes mellitus as well [9]. In our study the average age at presentation was 58 years. The patients could not be categorised into type 1 or 2 as the details were not available. None of our patients had bilateral involvement, whereas Charcot foot can be bilateral in about 25% of cases [10]. Usually the duration of diabetes is more than 12 years [11] and men are commonly affected as compared to women.



Fig 1: a, b: Clinical picture of a diabetic foot ulcer over the ball of great toe. c,d: X-rays the same patient reveal severe destruction of 1st MTP joint, suggestive of Charcot arthropathy.



Fig 2: note the grossly deformed ankle joint (a), with ulceration over the lateral malleolus (b), and a gross destruction of the ankle in X-rays (c).

Conclusion

The diagnosis of Charcot foot in its initial stages is still a challenging task for the general physicians. The best treatment for the Charcot foot however has to be initiated in its early phase for better outcomes. Though uncommon, Charcot foot is a debilitating complication of diabetes especially at later stages. Hence all diabetic patients with foot and ankle complications may be referred earlier to the concerned specialist for appropriate management.

References

1. Sanders LJ. The Charcot foot: historical perspective 1827_2003. Diabetes Metab Res Rev. 2004; 20:S4-8.
2. Gouveri E, Papanas N. Charcot Osteoarthropathy in diabetes: A brief review with an emphasis on clinical practice. World J Diabetes 2011; 2:59-65.
3. Rajbhandari SM, Jenkins RC, Davies C, Tesfaye S. Charcot neuropathy in diabetes mellitus. Diabetologia. 2002; 45:1085-96.
4. Ryan Donegan, Bauer Sumpio, Peter A. Blume. Diabetic foot and ankle. 2013; 4:21361.
5. Eichenholtz SN. Charcot Joints. Springfield, IL, USA Charles C. Thomas, 1966.
6. Shibata T, Tada K, Hashizume C. The results of arthrodesis of the ankle for leprotic Neuro arthropathy. J Bone Joint Surg Am. 1990; 72:749-756.
7. Jeffcoate W, Lima J, Nobrega L. The Charcot foot. Diabet Med. 2000; 17:253-58.
8. Brodsky JW. The diabetic foot. In: Coughlin MJ, Mann RA, Saltzman CL, eds. Surgery of the Foot and Ankle. 8th ed. St Louis, MO, USA: Mosby. 2006, 1281-1368.
9. Gupta PPK, Mohan V. Charcot foot - an update. Journal of the Association of Physicians of India. 2003; 51:367-372.
10. Frykberg RG. Charcot foot. In: Boulton AJM, Connor H, Cavanagh PR. Eds. The foot in diabetes. John Wiley Sons. Chichester, 2000, 235-60.
11. Laing PW. The foot in diabetes. In: Klerman L, Ed. The foot and its disorders. Blackwell Scientific, London, 1991, 139-52.

Original Article

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High Heels Footwear Causes Heel Pain and Back Pain: Myth or Reality?

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Abstract

Introduction: Heeled footwear has been in use by women for centuries and has now become an integral part of the wardrobe not only in the west but also in India. Recent research suggests that up to one third of women suffer from permanent problems as a result of their prolonged wearing of heels. One in 10 women wear it at least for 3 days a week, and a recent survey found that one-third of them had permanent problems.

Purpose: Whether the high heel wear is the actual cause of back pain is not yet clear. Literatures vary in their results regarding the same. Hence, the present study was done to assess this cause and effect.

Methods: The study was conducted at the orthopedic outpatient department of a tertiary care hospital, from July 2014 to December 2014.100 female patients who were using heeled shoes and who volunteered to participate in the study were included. The heel height was measured using measuring tape and were classified into three groups (<2.5 cm, 2.5-5 cm, >5 cm). A Proforma with a questionnaire was given to all the participants and their response were noted.

Results: Out of the 100 female volunteers, who participated in the study, 44 had complaints of heel pain regardless of the heel height, 56 had back pain, and many had both heel pain and back pain complaints, based on the questionnaire evaluation. We were further able to distinguish both heel pain and back pain, based on the heel height worn, duration of wear (in years) and duration worn/day.

Conclusion: There was a positive correlation between duration of wear and height of footwear with regards to both heel and back pain. However, a definitive cause and effect could not be established because of smaller sample size.

Key words: Back pain, Heel pain, Shoes, Statistical analysis, Women

INTRODUCTION

Walking is the most common form of human locomotion from a motor control perspective, human bipedalism makes the task of walking extremely complex. One condition known to compound the difficulty of walking is the use of heeled shoes, which alters the natural position of the foot-ankle complex.[1] This thereby produces a chain of reaction effects that travels up to the lumbar vertebrae. High-heeled footwear is defined as the footwear having a heel that is higher than the toe. Throughout the history, this footwear is considered as a symbol of sexuality, class and gender. When the foot slants forward, a much greater weight is transferred to the ball of the foot and toes, which increases the likelihood of damage to the underlying soft tissues that supports the foot. When the wearer tips the foot forward, this in turn puts pressure on the lower back and hence causes back pain. This particular study on post-adolescent volunteers was aimed to study the correlation between heeled footwear and heel and back pain.

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MATERIALS AND METHODS

The present study was conducted on 100 female patients who attended orthopedic outpatient department at institution during the period from July 2014 to December

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2014. Patients were drafted into the study based on inclusion criteria which included:

a. Age >15 years and <55 years
b. Without any foot and spine deformities. Patients were explained about the study and only those who volunteered and gave their consent were included in this study. The procedures followed were in accordance with the ethical standards.

The evaluation was based on questionnaire which was given to all the participating volunteers and heel height was measured with the help of inch tape as illustrated in Figures 1-3. Appropriate information collected in the questionnaire was entered in data collecting sheet for the purpose of statistical analysis.

Patients were categorized based on their age group (18-25, 25-45, 45-55), occupation (student, staff, housewife), heel height (normal [<2.5 cm], low heel [2.5-5 cm] and high heel [>5 cm]), duration of wear in years (0-5 and 5-10 years) and in hours/day (0-5 and 5-10), type of sole (leather, wooden, rubber, plastic). The data collected were statistically analyzed by Chi-square test.

Observation

A total of 100 female patients were included in the study after obtaining their informed consent. There were 63 students, 28 staff, 9 housewives who participated as volunteers. There were 12 people wearing heels with height 2.5-5 cm, 4 people wearing heels with height >5 cm and 74 people wearing heels with height 0-2.5 cm. In our study, 67 people were using soft sole type and 33 people were wearing hard sole type. There were 36 people wearing heeled footwear for more than 5 years, and 54 people were using heeled footwear for 0-5 years. 15 people were wearing heeled footwear for 0-5 h/day and 85 people were wearing heeled footwear for more than 5 h a day. Regardless of heel height worn, the duration of footwear had a significant influence on heel and back pain (Table1) and similarly prolonged wear in a day had a significant influence on heel pain (Table 2).



Figure 1: Heel height being measured



Figure 2: Method of measuring heel height using measuring tape



Figure 3: Volunteers who participated in the study

DISCUSSION

Heeled footwear has been in use by women for centuries but not all individuals wearing heels suffer from clinical problems. In ancient Egypt, wearing shoes may have served to set apart lower classes from the nobility as normal people walked around bare foot while the rich wore flat leather shoes. Moreover in ancient times, height would vary so that the higher the soles, higher the social status.

Research suggests that long-term heel use can both "compromise muscle efficiency and increase the risk of injuries." Heeled shoes slant the foot forward and down while bending the toes up, more the feet is forced into this position, more it may cause the gastrocnemius muscle to shorten.

Table 1: Relationship between heel height with back and heel pain (duration of wear in years)

Heel height	Heel pain			Back pain		
	0-5 years	>5 years	Significance	0-5 years	>5 years	Significance
0-2.5 cm (74[n])	14[n]	28[n]	0.017*	32[n]	-	0.016*
2.5-5 cm (12[n])	3[n]	6[n]		3[n]	Nil	
>5 cm (4[n])	1[n]	1[n]		1[n]	1[n]	

[n]Number of patients, *As analyzed by Chi-square test

Table 2: Relationship between heel height with back and heel pain (duration of wear in hours)

Heel height	Heel pain			Back pain		
	0-5 h	>5 h	Significance	0-5 h	>5 h	Significance
0-2.5 cm	6	36	0.053*	4	28	0.456
2.5-5 cm	3	6		3	-	
>5 cm	-	2		1	1	

*As analyzed by Chi-square test

When walking in high heeled shoes, a significant reduction in ankle plantar flexor muscle movement occurs, whereas increased work is performed by the hip flexor muscle during transition from stance to swing phase. Reduced effectiveness of ankle plantar flexors during late stance results in a compensatory enhanced hip flexor pull off. Large muscle movements and increased work at hip and knee occur while wearing high heels.[2]

Wearing high heels is thought to increase an individual's likelihood of experiencing a lateral Ankle sprain. The flexed and inverted posture of the plantar while wearing high heels may increase an individual's risk of experiencing a lateral ankle sprain.[3]

Foster *et al.*,[3] found signs of increase in ankle sprains in patients wearing high heels for a prolonged duration. Esenyel *et al.*,[2] described changes in the biomechanics of walking like increase in plantar flexion in people using high heels. In the particular study, we werenot able to assess the changes in biomechanics of foot while wearing high heels as it is a purely observational study.

There is also a definitive change in subtalar joint axis while walking on a high heeled shoe in comparison to a normal shoe, hence wearing of high heel may cause foot and heel pain.[4]

High heeled shoes cause an increase in the lordotic curve of the lumbar spine.[5] Increased ankle plantar flexion causes a kinetic chain of compensation up the lower extremity that ends with hypertonic psoas muscle, producing a lumbar hyperlordosis. A hyperlordotic lumbar spine in turn will lead to back pain.

Brent *et al.*,[9] found alteration in biomechanics of the lumbar spine. In prolonged high heels wear, chain of events around the ankle were observed, like increased ankle plantar flexion which in turn led to an increase in a lordotic curve.[6]

Eisenhardt *et al.*,[5] measured pressure distribution under foot for bare feet versus heel height and found increase in distribution related to heel height. Opila-Correia *et al.*,[7] studied the kinematics of high heeled walking and found that high heeled walking was associated with increased knee flexion in the stance phase. Kerrigan *et al.*,[6] used biomechanical gait analysis and inverse dynamics to evaluate joint loadings during high heel walking and found that even moderate high heels would contribute to osteoarthritis of the knee.[8]

Electromyography analysis of the lower limb muscles in high heel users and regular footwear users showed significantly increased leg muscle activity.[9]

Based on the observations from our study done on 100 volunteers wearing heels who came to the orthopedic outpatient department for other problems, prolonged duration of wear in years/hours of wear per day resulted in heel pain that significantly affected their daily activities. Furthermore from this study, it was observed that prolonged wear of heels in due course, also resulted in back pain which again was found to be directly proportional to duration of wear in years.[10]

Even though we have derived certain conclusive results from the study, it does have few pitfalls, for instance its an observational study and results obtained were purely based on statistical analysis of the questionnaire reports. Most of the studies done on heeled footwear considered heel height more than 9 cm as high heel, whereas in our study low heel is considered <2.5 cm while heels between 2.5 and 5 cm are considered moderate and more than which is considered as high heels. Moreover, we couldnot have a definite discrimination of the type of sole worn and no changes in the final outcome with respect to the sole type worn.[11,12]

The present study did prove it right that prolonged heels wear resulted in heel pain and back pain, but concrete justification couldn't be provided as the study was done on a small group of 100 volunteers.

CONCLUSION

This study was done with an intention to break the myth or confirm the reality that high heels footwear causes heel pain and back pain. We did succeed in obtaining a stastically significant correlation between the duration of heels wear in both hours/day and a number of year worn with heel and back pain. The pitfall of this study being the minimal sample size, and it was done as an observational study based on a questionnaire evaluation.

REFERENCES

1. Cronin NJ. The effects of high heeled shoes on female gait: a review. J Electromyogr Kinesiol 2014;24:258-63.
2. Esenyel M, Walsh K, Walden JG, Gitter A. Kinetics of high-heeled gait. J Am Podiatr Med Assoc 2003;93:27-32.
3. Foster A, Blanchette MG, Chou YC, Powers CM. The influence of heel height on frontal plane ankle biomechanics: Implications for lateral ankle sprains. Foot Ankle Int 2012;33:64-9.
4. Henderson PD. A biomechanical evaluation of standing in high - Heeled shoes. Pennsylvania State McNair J 2004;11:25.
5. Eisenhardt JR, Cook D, Pregler I, Foehl HC. Changes in temporal gait characteristics and pressure distribution for bare feet versus various heel heights. J Gait Posture 1996;4:280-6.
6. Kerrigan DC, Todd MK, Riley PO. Knee osteoarthritis and high-heeled shoes. Lancet 1998;351:1399-401.
7. Opila KA, Wagner SS, Schiowitz S, Chen J. Postural alignment in barefoot and high-heeled stance. Spine (Phila Pa 1976) 1988;13:542-7.
8. Simonsen EB, Svendsen MB, Nørreslet A, Baldvinsson HK, Heilskov-Hansen T, Larsen PK, *et al*. Walking on high heels changes muscle activity and the dynamics of human walking significantly. J Appl Biomech 2012;28:20-8.
9. Russell BS. The effect of high-heeled shoes on lumbar lordosis: a narrative review and discussion of the disconnect between Internet content and peer-reviewed literature. J Chiropr Med 2010;9:166-73.
10. Mika A, Oleksy L, Mika P, Marchewka A, Clark BC. The influence of heel height on lower extremity kinematics and leg muscle activity during gait in young and middle-aged women. Gait Posture 2012;35:677-80.
11. de Oliveira Pezzan PA, João SM, Ribeiro AP, Manfio EF. Postural assessment of lumbar lordosis and pelvic alignment angles in adolescent users and nonusers of high-heeled shoes. J Manipulative Physiol Ther 2011;34:614-21.
12. Franklin ME, Chenier TC, Brauninger L, Cook H, Harris S. Effect of positive heel inclination on posture. J Orthop Sports Phys Ther 1995;21:94-9.

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Original Article:

Low back pain in pregnancy: Prevalence among south Indian population

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Abstract
Females during pregnancy commonly encounter back pain at some point of time. Unfortunately, this symptom is quiet carried away and often neglected by the health care providers. Low back pain in pregnancy can have a significant impact both psychologically and physiologically as well. In the present study we intended to find out the prevalence rate for this symptom among the South Indian population by formulating a questionnaire proforma.
There was a 27.82 % prevalence rate for low back pain in our study. Though we did not differentiate the back pain as lumbar pain and pelvic girdle pain, quiet a majority of the patients (40%) were facing mild to moderate disabilities because of the pain. The mean duration at the onset of the symptoms was 26 weeks of pregnancy. The mean age of the patients with low back pain was 26 years.
Identification of the back pain symptoms during pregnancy is needed so as to assess and provide proper management to such patients.

Keywords: Low back pain, pregnancy, prevalence, disability

Introduction
Low back pain (LBP) is a commonly encountered symptom from pregnant females. Studies have estimated that LBP is prevalent in about 50 % of females at some point of time during pregnancy [1, 2]. Although it is confined to the pelvic area and not so harmful to the females or their foetuses, it is quiet a disabling symptom [3, 4]. LBP is said to cause moderate disability and about 10 % of patients will take off time from work [5, 6]. Back pain in pregnancy is due to various factors, such as mechanical, hormonal and others [1, 2, 7-9].
In the present study we tried to figure out the prevalence of LBP among the south Indian population, attending a primary health care.

Materials and Methods
The aim of this study was to determine the prevalence of LBP among the pregnant females. The study was conducted at a government primary health centre, Southern India, from January 2018 to December 2018. A proforma (fig.1) was created and data collected accordingly among all patients attending the antenatal clinic. A total of 488 patients data were collected during this period.
All the patients who were attending the antenatal clinic for the first time at the time of registration of their pregnancy were included in the study. Those patients who were in their subsequent visits at the time of initiation of this study were excluded because of the chances of error in calculations, as majority of the patients were not able to recollect an episode of LBP at their previous visits.
Apart from the patient's demographic profile, the first leading question was 'whether you have any type of back pain?' If the answer was 'No', further data's were skipped and if the answer was 'yes', the proforma was completed. The patients were subsequently followed-up until the time of delivery and if the patient had a symptom of LBP at any point of time, the proforma was completed at that time.
Patients who had LBP prior to the present pregnancy were excluded from the study. Similarly patients who had pre-existing organic pathology (intervertebral disc prolapse, spondylolisthesis, etc.,) that may contribute to LBP and patients who had a recent history of trauma to the back were also excluded from the study.

S. No.	Patient's Demographic Profile		
1.	Name		
2.	Age		
3.	GPLA		
4.	Weight and height		
5.	Address		
	LBP specific Questionnaire		
S.No.	Questions	Yes	No
1.	Whether you have LBP?		
2.	Any previous history of (H/0) LBP?		
3.	H/O any recent trauma?		
4.	Is the present pregnancy a multiple pregnancy (Twins or Triplets)?		
5.	Does the patient suffering from polyhydramnios?		
6.	H/O any spinal anaesthesia in the past?		
7.	Whether the weight gain is appropriate for the gestational age?		
8.	Duration of pregnancy at the onset of present symptoms(in weeks)		

G: gravida; P: parity; L: no. Of living children; A: no. of abortions

Fig 1: The questionnaire proforma used in the study

Results and Discussion

Data was collected for a total of 478 patients during the study period. 16 patients had a pre-existing history of back pain prior to the present pregnancy. The LBP was either a pain during menstruation or an on and off back pain of undiagnosed causes. All of these 16 cases were excluded from the study. One patient with complaint of LBP at 8 weeks of pregnancy had a history of trivial fall prior to the presentation and hence was excluded from the study. Another patient who was a multiparous woman in her third pregnancy had spondylolisthesis at L5-S1 level. Though she had no complaints of LBP during the previous two pregnancies, she was also excluded from the study. Hence a total of 460 patient's data was used to analyse the results.

A total of 128 patients were having LBP out of the 460 cases. That worked out a prevalence rate of 27.82 %. This prevalence was quiet low as compared to other studies from African [10] and Western [11] population who had prevalence of more than 50 %. LBP prevalence among Nepalese women was 34 % in a study [12] and was bit closer to that of our study. LBP in pregnancy has been predominantly classified [13, 14] into 2 types: lumbar pain (LP) and pelvic girdle pain (PGP) or posterior pelvic pain (PPP). LP is defined as pain between the twelfth rib and the gluteal fold, while PGP is defined as pain experienced between the sacroiliac joint and the gluteal fold, or in the pubic symphysis [15]. We in the present study did not differentiate between the two types and all patients were classified to be suffering from LBP.

About 40 % of our patients (52/128) who had LBP were facing mild to moderate amount of disabilities. The natures of disabilities were mainly in the form of handling the first child and in doing household chores like fetching water. Though there was some sort of disability only 2 out of these 52 cases had severe enough pain so as to have consulted a specialist for treatment. These 2 were working women and they never took any leave from their work for the LBP. In a study by Wang *et al* [5]. their disability rate was around 60 %.

Of the 460 patients in our study, a majority of them (320) were housewives and the remaining 140 were working women. 92 of them were doing constructional or household works in other homes and 48 were office going. 104 cases among the housewives had LBP, whereas 24 among the working group had LBP. The results are summarised in the table 1.

Table 1: Prevalence of LBP with regard to occupation

Patients		Number	With complaints of LBP (No.)	Prevalance (%)
Total cases		460		
Housewives		320	104	32.5
Working women	Manual work	92	22	23.91
	Office work	48	2	4.16

A total of 184 cases were in their first pregnancy, out of which 32 of them complained of LBP (17.39 %). The remaining 276 cases were either 2nd or 3rd time pregnant. 92 of them had LBP (34.78%). This trend was similar to literature which states that the prevalence of LBP increases with parity [16].

There was one case of twin pregnancy and 3 cases of polyhydramnios, but none of the 4 patients had LBP. The mean age of the patients with LBP was 26 years. The mean duration at the onset of symptoms was 26 weeks (8 to 34 weeks) towards the later part of second trimester. The mean duration at onset of pain was 24 weeks in Nepalese women [12].

LBP typically tends to begin as early as 18 weeks of pregnancy and may persist in the postpartum period for as long as 15 months [17, 18]. We in our study did not study the pattern of LBP and patients were not followed up in the postpartum period.

LBP in pregnancy is often regarded as harmless and quiet commonly ignored by the health care professionals [19]. But they have been reported to have some serious negative psychological effects in various studies [19-21]. Therefore a lack of awareness of this predominant complaint from pregnant women can lead to poor management of such cases. The commonly reported risk factors for pregnancy related back pain are high strenuous workload, frequent or prolonged torso flexion, previous history of LBP, previous history of any trauma to the pelvis [15, 22, 23], body mass index (BMI), and parity. Though, in the present study we did not try to find out the risk factors or their causal relationship. We just tried to make out the magnitude of the problem.

The prevalence rates are influenced by the perception of pain among the various group of population. Also we did not consider the various risk factors involved like BMI. A

detailed questioner involving all the risk factors and other indicators of pain like the visual analog scale (VAS) would have been a good indicator of actual prevalence. The socio-economic status of the patient was also not included in the present study, which was another drawback of the study.

Conclusion

'Back pain' as a complaint from a pregnant woman is often regarded as harmless and a common complaint. But the high prevalence of this complaint and the implications it have in a pregnant females necessitates a proper diagnosis and management.

References

1. Vleeming A, Albert HB, Ostgaard HC, Sturesson B, Stuge B. European guidelines for the diagnosis and treatment of pelvic girdle pain. Eur Spine J. 2008; 17:794-819.
2. Perkins J, Hammer RL, Loubert PV. Identification and management of pregnancy related low back pain. J Nurse Midwifery. 1998; 43:331-340.
3. Kanakaris NK, Roberts CS, Giannoudis PV. Pregnancy-related pelvic girdle pain: an update. BMC Med. 2011; 9:15.
4. Elden H, Lundgren I, Robertson E. Life's pregnant pause of pain: pregnant women's experiences of pelvic girdle pain related to daily life: a Swedish interview study. Sex Reprod Healthc. 2013; 4(1):29-34.
5. Wang SM, Dezinno P, Maranets I, Berman MR, Caldwell-Andrews AA, Kain ZN. Low back pain during pregnancy: prevalence, risk factors, and outcomes. Obstet Gynecol. 2004; 104(1):65-70.
6. Kristiansson P, Svardsudd K, von Schoultz B. Back pain during pregnancy: a prospective study. *Spine* (Phila Pa 1976). 1996; 21(6):702-709.
7. Van Dongen PW, de Boer M, Lemmens WA, Theron GB. Hypermobility and peripartum pelvic pain syndrome in pregnant South African women. Eur J Obstet Gynecol Reprod Biol. 1999; 84:77-82.
8. Stuge B, Hilde G, Vollestad N. Physical therapy for pregnancyrelated low back and pelvic pain: a systematic review. Acta Obstet Gynecol Scand. 2003; 82: 983-990.
9. Sabino J, Grauer JN. Pregnancy and low back pain. Curr Rev Musculoskelet Med. 2008; 1:137-141.
10. Ayanniyi O, Sanya AO, Ogunlade SO, Oni-Orisan MO. Prevalence and pattern of back pain among pregnant women attending ante-natal clinics in selected health care facilities. African Journal of Biomedical Research. 2006; 9:149-56.
11. Mantle MJ, Greewood RM, Currey HLF. Backache in pregnancy. Rheumatism and Rehabilitation, 1997, 95-101.
12. Ranjeeta Shijagurumayum Acharya *et al*. Prevalence and severity of low back- and pelvic girdle pain in pregnant Nepalese women. BMC Pregnancy and childbirth. 2019; 19:247.
13. Kristiansson P, Svardsudd K, Von Schooltz B. Back pain during pregnancy. A prospective study. Spine. 1996; 21:702-709.
14. Noren L, Ostgaard S, Nielsen TF, Ostgaard NC. Reduction of Sickleave for Lumber back and posterior pelvic pain in pregnancy. Spine. 1997; 22:2157-60.
15. Vleeming A, Albert HB, Östgaard HC, Sturesson B, Stuge B. European guidelines for the diagnosis and treatment of pelvic girdle pain. Eur Spine J. 2008; 17(6):794-819.
16. Mogren IM, Pohjanen AI. Low back pain and pelvic pain during pregnancy: prevalence and risk factors. Spine. 2005; 30:983-91.
17. Wu WH, Meijer OG, Uegaki K, Mens JM, van Dieen JH, Wuisman PI *et al*. Pregnancy related pelvic girdle pain (PPP), I: Terminology, clinical presentation, and prevalence Eur Spine J. 2004; 13(7):575-89.
18. Sjodahl J, Gutke A, Oberg B. Predictors for long-term disability in women with persistent postpartum pelvic girdle pain. Eur Spine J. 2013; 22(7):1665-73.
19. Gutke A, Boissonnault J, Brook G, Stuge B. The severity and impact of pelvic girdle pain and low-back pain in pregnancy: a multinational study. J Women's Health. 2018; 27(4):510-7.
20. Mackenzie J, Murray E, Lusher J. Women's experiences of pregnancy related pelvic girdle pain: a systematic review. Midwifery. 2018; 56:102-11.
21. Mens JMA, Huis in't Veld YH, Pool-Goudzwaard A. Severity of signs and symptoms in lumbopelvic pain during pregnancy. Mant Ther. 2012; 17:175-79.
22. Wu WH, Meijer OG, Uegaki K, Mens JM, van Dieen JH, Wuisman PI, Ostgaard HC. Pregnancy-related pelvic girdle pain (PPP), I: terminology, clinical presentation, and prevalence. Eur Spine J. 2004; 13(7):575-89.
23. Juhl M, Andersen PK, Olsen J, Andersen A-MN. Psychosocial and physical work environment and risk of pelvic pain in pregnancy. A study within the Danish national birth cohort. J Epidemiol Community Health. 2005; 59(7):580-5.

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An absolute indication of Manipulation under Anesthesia (MUA) for frozen shoulder is not extracted from literature. Though advanced medical sciences now have an option for arthroscopic release of soft tissue of shoulder, the results are variable in literature and recurrence recorded in literature varies up 30 to 40 percent. We adopted a non invasive approach for 40 cases of frozen shoulder. A functional outcome of patients treated with MUA and intra-articular injection with Methylprednisolone with Lignocaine (MP) was evaluated. Patients with frozen shoulder including those with diabetes were analyzed clinically, radio logically and SPADI score prior and post procedure done. The percentage of recovery noted. We noticed favorable and positive outcome in 67% despite strictly restricting our follow up for 3 months. A reminder here is the natural course of history which is generally self limiting and varies from 16 to 24 months. In view of possible recovery time, range of motions would further improve with recovery from pain. More than 4 studies were comparable, in this study we found external and internal rotation of shoulder joint not fully recovering, but reaching a range of motion for activities of daily living. Our results were comparable to other studies and conclude that MUA with injection of steroid speeds up recovery in range of movements and provides relief of pain.

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□ □□□ □rd□□□Frozen shoulder, Manipulation under Anaesthesia, Intra Articular, Methylprednisolone, Lignocaine 2%, SPADI SCORE.

1 □□□□r □d □□□□□□□□

Peri Arthritescpulaheumarale was the term used by Duplay in 1872. For painful shoulder with restriction of movements. Over the years aeitipathogenesis evolved with newer terms. 1934 Codman coined the condition Frozen shoulder, and Neviaser 1945 coined the term Adhesive Capsulitis [1]. And other surgeons brought light and charted theoretically the stages of frozen shoulder currently also known as Adhesive Capsulitis. The incidence varies from 2-4% and in some general population 3-7% [1, 2, 3].

Clinically diagnosed as Adhesive Capsulitis, this is a condition where spontaneous adhesion of ligaments, capsule and fibrosis occurs. There are two claims, one suggest synovial inflammation and other a condition similar to Dupuytren's Contracture [2, 4, 11]. Contractures along the long head of Biceps, Infraspinatus, and Supraspinatus.

Treatment varies from NSAIDs, moist heat, physical therapy, oral steroids,

IA Injections, hydro dilation, nerve blocks, Manipulation, open and arthroscopic soft tissue release. Literature supports more incidences in Diabetic patients, females, non dominant hand with bilateral involvement in 12-16% in same individual [1, 2, 3, 4]. Our study was to do noninvasive therapy that is Manipulation under Anaesthesia (MUA) with intra articular injection of 80 mg of Methylprednisolone with 1ml of Lignocaine; which is economical, needs no highly skilled expertise and can be done with standard aseptic procedure even in a District hospital.

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To determine the outcome of patients, after we assessed patients for pain, disability and Range of Movements (ROM). The study prospectively recorded the functional outcome of treatment after MUA. We analyzed 40 consecutive patients and outcomes were assessed clinically by SPADI Score.

The Shoulder Pain and Disability Index is the sum total of pain scale and Disability scale and total score is divided by 130 and multiplied by 100 to calculate percentage. Pain is measured by 0-10 with 5 questions. Disability is assessed by 8 questions, score 0-10. Where 0 means no pain and 10 means worst unbearable pain.

Activities of daily living were checked were 0 means no difficulty and 10 means severe difficulty. Higher scales on the subscale will indicate greater pain and disability. Prospective study of 40 patients was conducted in tertiary care Institution of Medical sciences. The study was between May 2019 and May 2020. 40 consecutive patients with painful loss of shoulder movements with limitations in their activities of daily living.

2.1 we included all patients, who have painful and stiff shoulders, with restricted active and passive motion, with normal glenohumeral joint radiologically.

2.2 Any calcific tendinitis, arthritis, traumatic conditions or any other surgeries around the shoulder.

Our study included 40 patients including those with diabetes. All patients gave detail history and evaluation of pain scale and disability done using SPADI score. Clinical Examination and their record of shoulder movements and strength of muscle power were recorded. Only a single X ray in Anteroposterior (AP) view was taken. No patient underwent ultrasound or MRI.

40 patients were hospitalized, and physiotherapy started and all patients were educated for self-mobilization of shoulder. With strict emphasis on avoiding shrugging.

Wall climbing exercise, Pendulum, and other exercises were taught to the patients.

All patients underwent MUA within 5 days and at the same sitting injection 80 mg of Methylprednisolone (MP) mixed with 1 ml of Xyclocaine given anteriorly into gleno humeral joint.

Patient was supine, scapula fixed and pressed with thoracic cage and border of scapula. With help of an assistant. Holding the short lever arm gradual abduction, external rotation adduction and lastly internally rotation movement were done sequentially until audible click was heard.

We had no prior knowledge of pathological thickness of Corocohumeral ligaments, thickness of the capsule, and extent of pathological contractures. Irrespective of the duration we treated all patients with a standard protocol, so intervention rate could be accessed based on outcome at end of 3 months. Out of 40 patients, there were 18 male and 22 females. Out of

40 patients 27 patients were diabetic. 19 patients had right shoulder involvement and 21 left.

The average duration of symptoms like pain and disability was 2.5 months; least duration was 1 month and maximum 7 months. 31 patients after MUA and Inj MP of 80 mg underwent physiotherapy which was supervised for 1 week. 9 patients did not attend supervised physiotherapy at the hospital.

All patients underwent clinical examination at time of MUA, immediately after MUA, after 1st month, 2th month and 3rd month. SPADI score was calculated before procedure immediately after procedure and for each month. The percentage of recovery calculated to merely reflect functional outcome.

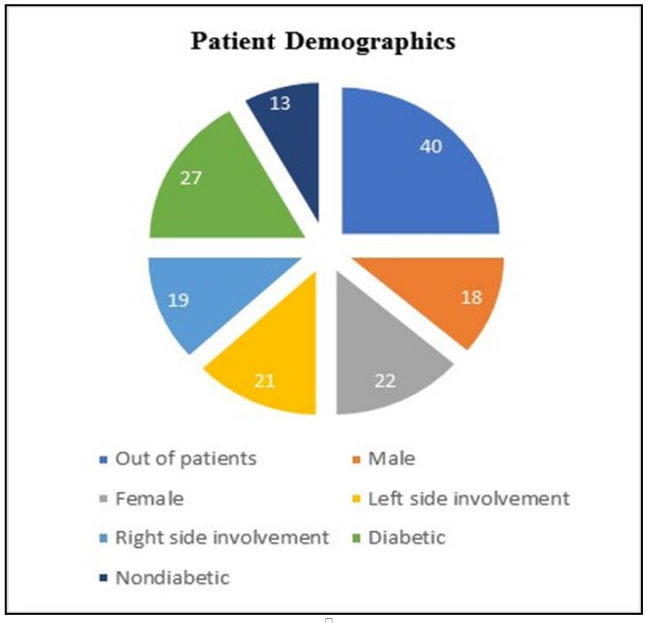


Table 1: Patient Demographics

Male	18
Female	22
Left side involvement	21
Right side involvement	19
Diabetic	27
Nondiabetic	13

Our work sheet tabulated the results in our study with average range of shoulder motions as follows: Abduction 60*, External Rotation (ER) 31°, Internal Rotation 30 (IR), and Flexion 66* at first examination.

We had one patient 55/F who had stiffness and pain who did not respond to treatment, not diabetic, her duration of symptoms was only for 3 months and was of left side. Our hypothesis that it would be like pathogenesis like Duptruyen Contracture in this particular case [4]. Immediately after MUA and injection MP Abduction (Abd) improved to 109.5 EXT rotation (ER) 59.6 Internal rotation (IR) 51° and flexion (FI) 106*

Table 2: Range of motion of shoulder at 3 months

1st month	Abd 125.2°	ER 65.25°	IR 56.5°	FL 123°
2nd month	Abd 132.7°	ER 67.5°	IR 60.2°	FL 130°
3rd month	Abd 135°	ER 68.2°	IR 61°	FL 134°

In our study by using SPADI score for pain and disability we calculated prior to SPADI, Post MUA, and at end of 3 months. In span of 3 months post MUA with injection the range of movements doubled.

The percentage of recovery calculated and averages taken, Preop SPADI Score was

70.7 and Post op 21.45 and the percentage of 67.1%. The predominant range of motions of external and internal rotations were quite resistant to MUA and the underlying pathology of shoulder rotators, their anatomy and pathological changes around them, probably is resistant to MUA.

Statistical Analysis with paired groups were done with comparison before and after MUA of shoulder.

Statistical Analysis paired test of Range of Movements

Abd	Pair 1 Before	VAR00001	110.7692	39	28.50527	7.332	0.000
	After MUA	VAR00005	135.1282	39	31.10862		
ER	Pair 2 Before	VAR00002	59.6250	40	19.36119	4.573	0.000
	After MUA	VAR00006	68.2500	40	20.4923		
IR	Pair 3 Before	VAR00003	51.2500	40	19.50509	6.811	0.000
	After MUA	VAR00007	60.5000	40	18.80398		
FL	Pair 4 Before	VAR00004	106.2500	40	29.23709	7.890	0.000
	After MUA	VAR00008	134.0000	40	28.62669		

Statistical Analysis with 95% confidence of Pre and Post MUA outcome measures, based on means, standard deviation, t-score and P-value.

Discussion

The Frozen shoulder is a condition where in, there is slowly progressive loss of movements of shoulder both active and passive, associated with pain and restriction of activities of daily living. The Natural History of Adhesive Capsulitis is that it is self-limiting. Recovery can take from 24-26 months [1, 2, 3, 4].

However Literature quotes various Surgeons who currently contradict this statement [6], several authors claim pain and loss of range of movements with disability of shoulder. Treatment varies from simple NSAID with moist heat, physiotherapy, oral steroids, intrarticular injections with steroid, hydrodialation, nerve blocks, manipulation, open surgical release and arthroscopic release. Contractures in shoulder may be responsible for ineffectiveness of any treatment. We don't know whether synovial inflammation is the cause or 'Duptruyens type' of capsule of shoulder.

We compared our results with Heidi Vastamaki, and Marti Vasatamaki' study [7].

In their study prior to MUA, Abduction was 75° (ABD), Eternal Rotation 21° (ER), Internal Rotation level of buttock (IR), and Flexion 99° (FL), in our result prior to MUA Abduction was 60°, External was 31°, IR 30° and FL 66°.

End of 8.8 months Vastamaki's study [7] showed in their patients Abduction 167°,

ER 51°, IR level L5 and Flexion 156°. In Our study we obtained at 3rd month on follow up ABD 135°, ER 68°, IR 61° and FI 134°.

Comparing with Thomas[8] et al ABD was 62°, ER 17° IR 17°, FI 165° (pre MUA)

Post MUA at one year follow up was ABD 168°, ER 65°, IR 65°, FL 169°. In our study

ABD 135°, ER 68°, IR 61° and FI 134° at end of 3rd month.

Studies by various authors showed good recovery but they assumed that elderly patients have restricted movements, we stand to contradict to their statements that 90% improvement in pain and range of movements.

Jenkins *et al.*, in their retrospectively had analyzed that 36% had unsatisfactory outcomes [8], in our study it was 33%. The natural history claimed to be self-limiting, can take prolonged duration of time between 14-32 months. Adhesive capsulitis of shoulder with pain and disability would require medication and pose risk of drug induced Nephropathy.

Though optimal time suggested by Vastamaki, and Vajonen is between 6-9 months for MUA, clinical outcome was questionable by many authors. Dodenoff, Levy, Wilson and Copeland in one series, and Janada and Hawins [9] proved that pain and disability can be reduced after MUA. This shortens the time interval of patients to resume work, disease itself cannot be evaluated merely on duration of months and prevailing changes for staging purpose cannot be guessed. Clinical importance of outcome can be questioned and hence its variable. To achieve necessary functional activities there has to be range of motion which is free.

1. Early Recovery

Hence irrespective of duration of symptoms all patients were hospitalized and for 5 days physiotherapy given and then taken up for MUA and Injection MP 80 mg with 1ml of 2% Xyclocaine into the joint.

To enable eating approximately 60-100 of abduction, horizontal abduction about

40-70 degrees. Rotations about lessor range. For reaching perineum 40-60 of abduction and 90 of Internal rotation. Similarly, for combing hair, tucking shirt, or putting on pants, etc.

A range of movements which are not full and free but can able patients do activities of daily living which are pain free [8, 9, 17].

Pain and Disability prior to MUA on Average score was 70.7 (SPADI) after MUA at 12 weeks the score was 21.45. Post SPADI score of 21.45 denotes some residual pain and disability, but with useful functional outcome.

There was no complication in our cases. Grant *et al.* [10] compared MUA with arthroscopically done surgical release and reported that there was not much difference [10]. Arthroscopically done release has risks like chondrolysis due to thermal heat, risk of infection, risk of accidental axillary nerve damage. Open Release with manipulation offers no advantage on the contrary it reduces the speed of recovery. Also risk of infection introduced into the joint.

Rotator Cuff was analysed by Atoun et al with ultrasound before and after MUA and found cuff undamaged [12].

Sasanuma *et al.* [13] found no rotator cuff tears on MRI after Manipulation.

2. Cuff Tear Arthropathy

In a Retrospective study of 51 patients' frozen shoulders done by Wang *et al.* [14] after MUA reported 89% of cases had good results. In our series 67% had good results.

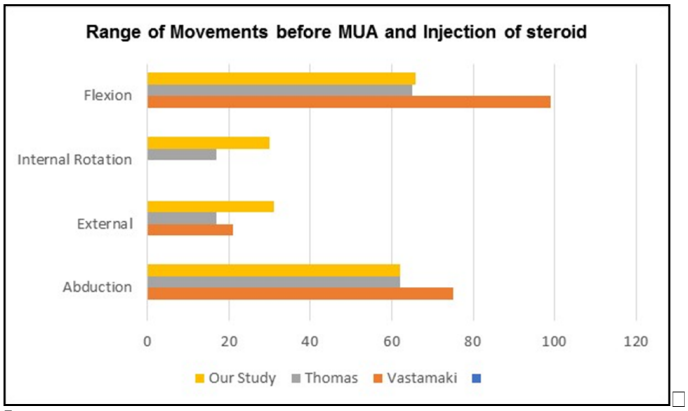
The difference of 22% in the outcome was because all patients were followed up to 3 months strictly. Whereas Wang followed up to 1 year. In their study patients were immediately mobilized, in our study all patients were given rest post of period for 24 hours, to enable them recover from pain.

Another probable cause is 9 patients dropping out of supervised physiotherapy at OPD for 5 days. One patient had

absolutely no recovery, few others had a slow start, our follow up was limited strictly till 12 weeks. We assume out of 22% of cases if given more time recovery rate would improve.

showing Range of Movements before MUA and Injection of steroid

Vastamaki	75	21	Buttock	99
Thomas	62	17	17	65
Our Study	62	31	30	66

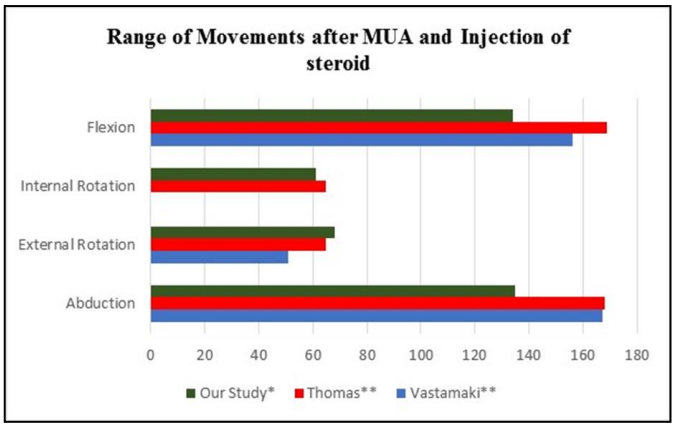


2 showing Range of Movements before MUA and Injection of steroid

showing Range of Movements after MUA and Injection of steroid

Vastamaki**	167	51	L 5 vertbrae	156
Thomas**	168	65	65	169
Our Study*	135	68	61	134

** follow up 1yr or more.



ROM after MUA and Injection of steroid

The study shows functional outcomes is improved after MUA and after Intra articular Inj of MP. Recovery of External rotation and Internal Rotation takes a longer time and their range of movements is again dependent on retraction of capsule and narrowing of clear space around ligaments. Non reversible pathological changes like fibrosis and capsular thickening with adhesions of glenohumeral ligaments.

Farrell *et al.* [15] their study of 19 shoulders after MUA, followed up for 7 years. They obtained Abduction 155°, External Rotation 76°, Internal Rotation 73° and Flexion 159°.

In our study we obtained 135° of abduction, 68° of External Rotation, 61° of Internal Rotation and 134° of Flexion at 3rd month which is beneficial to be patient.

Our study follows up limited up to 3 months. It is evident by MUA and inj of steroid the pain and stiffness improves, the shoulder recovers functional range of movements. However the limitations of this study: 40 patients and follow up to 3 months. No control group of patients, Non divided into 2 groups that is diabetic and non diabetic. Standard and same protocol of treatment followed. No 2 different methods of treatment. Our prospective study included both diabetic and non diabetic patients.

Manipulation of shoulder with injection of Steroid is a good method of treatment.

It's cost effective and speeds up recovery from pain and disability. It can be done safely as a day care procedure, and even in district Hospital. MUA carries little risk, without any need for expert technical skill. Majority of patients are happy for the range of movements recovered.

It is unnecessary to wait and observe for prolonged time or do prolonged follow up. Recurrence rate is low compared to arthroscopic surgical release and is subject to further trials and comparative studies.

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1. Jo A. Hannafin, Theresa A, Chiaia PT. Clinical Orthopaedic and Related Research, Adhesive Capsulitis, No. 372, 95-109.
2. Andrew S, Neviaser, Jo A. Hannafin. The American Journal of Sports Medicine, Adhesive Capsulitis: A Review of Current Treatment, 2010, 38.
3. Vander Windt DA, Koesw, de Jona, Boute LM. Shoulder disorder in general Practice: Incidence, patient characteristics Management Ann. Rheum. DIS 1995; 54:959-964.
4. Bo J. Lundber, Acta Orthopaedica Scandinavica. The Frozen Shoulder: Clinical and Radiographical Observations the Effect of Manipulation under General Anesthesia Structure and Glycosaminoglycan Content of the Joint Capsule Local Bone Metabolism, 2014, 1-59.
5. Hannafin JA, Dicarlo EF, Wickiewicz TL, *et al.* Adhesive capsulitis: capsular fibroplasia of the glenohumeral joint. J Shoulder Elbow Surg. 1994; 3(Suppl 5):435.
6. Shaffer DM, Tibone JE, Keralan RK. Frozen shoulder a long term follow up. Journal of Bone and Joint Surgery, AM. 1992; 74:738-46.
7. Vastamaki H, Varjonen L, Vastamaki M, Scandinavian. Journal of Surgery, Optimal Time for manipulation of frozen shoulder, 2015, 1-7.
8. Thomas WJ, Jenkins EF., Owen JM et al., Treatment of Frozen shoulder by MUA & Inj. Does the timing of treatment effect outcome? Journal of Bone & surgery Br 2011; 93:1377-1381.

9. Janada and Hawkin. Shoulder Adhesive Capsulitis: Manipulation and Arthroscopic Arthrolysis or Intra-Articular steroid Injections? Int. Orthopedics, 2012; 36(1):101-106.
10. Grant JA, Schroeder N, Miller BS, Carpenter JE. Comparison of manipulation and arthroscopic capsular release for adhesive capsulitis: a systematic review. J Shoulder Elbow Surg. 2013; 22:1135-1145.
11. Bunker TD, Anthony PP. The Pathology of frozen shoulder. A. Dupuyten-like disease. *J Bone Joint Surg* [Br]. 1995; 77-B677-683.
12. Atoun E, Funk L, Copland SA, *et al.* The effect of shoulder manipulation on rotator cuff integrity. Acta Orthop Belg. 2013; 79:255-259.
13. Sasanuma H, Sugimoto H, Kanaya Y *et al.* Magnetic resonance imaging and short-term clinical results of severe frozen shoulder treated with manipulation under ultrasound guided cervical nerve root block. J shoulder Elbow Surg. 2016; 25:e13-e20.
14. Jung-Pan Wang, tung-Fu Huang, Shih-Chieh Hung, Hsiao-Li Ma, Jiunn-Ger Wu, Tai-Hsiung Chen, International Orthopaedics (SICOT). 2017; 31:333-337.
15. Farrell Cm, Sperling JW, Cofield RH. Manipulation for frozen shoulder: long term results. J Shoulder Elb Surg. 2005; 14(5):480-4.
16. Dodenhoff RM, Levy O, Wilson A, Copeland SA. Manipulation under anesthesia for primary frozen shoulder: effect on early recovery and return to activity. J Shoulder Elbow Surg. 2000; 9:23-26.
17. Dutton's Orthopedic Examination, Evaluation Intervention, Third Edition, 2009.
18. Tarek Boutefnouchet, Robert Jordan, GevBhabra, Chetan Modi, Adnan Saithna, Orthopaedics & Traumatology: Surgery & Research, Comparison of outcomes following arthroscopic capsular release for idiopathic, diabetic and secondary shoulder adhesive capulitis: A Systematic Review, Birmingham, 2019, 839-846.
19. Tim Kraal, Lijkele Beimers, Bertram The, Inger Sierevelt, Michel van den Bekerom, Denise Eygendaal, Shoulder & Elbow, Manipulation under anaesthesia for frozen shoulder, 2019, 4.
20. Ibrahim T, Rahbi H, Beiri A, Jeyapalan K, Taylor, GJS. Original Article, Adhesive capsulitis of the shoulder: the rate of manipulation following distension arthrogram. 2006, 27:7-9.
21. Jae Hoon Jung, Du Hwan Kim, Jaehyuck Yi, Du-Han Kim, Chul-Hyun Cho, Rheumatology International, Determination of magnetic resonance imaging criteria for diagnosis of adhesive capsulitis. 2019; 39:453-460.
22. NiluferBalci, Mustafa Kemal Balci, Serdar Tuzuner, Elsevier, Shoulder Adhesive Cpsulitis and Shoulder Range of Motion in Type II Diabetes Mellitus: Association with Diabetic Complications.
23. Jo A. Hannafin, Theresa A, Chiaia, PT, Clinical Orthopaedic and Related Research, Adhesive Capsulitis, 372, 95-109.
24. Sean M, Griggs MD, Anthony Ahn MD, Andrew Green MD, Journal of Bone and Joint Surgery, Idiopathic Adhesive Capsulitis, 2000, 82, 10.
25. Ide J, Takagi K. Early and long-term results of arthroscopic treatment for shoulder stiffness. J Shoulder Elbow Surg. 2004; 13(2):174-179.
26. Iannotti JP, Manipulation under anesthesia did not enhance the benefit conferred by home exercises for frozen shoulder. J Bone Joint Surg Am. 2008; 90(8):1792.
27. Holloway GB, Schenk T, Williams GR, Ramsey ML, Iannotti JP. Arthroscopic capsular release for the treatment of refractory postoperative or post-fracture shoulder stiffness. J Bone Joint Surg Am. 2001; 83(11):1682-1687.
28. Hazleman BL. The painful stiff shoulder. Rheumatol Phys Med. 1972; 11(8):413-421.
29. Hand C, Clipsham K. Rees JL, Carr AJ. Long-term outcome of frozen shoulder. J Shoulder Elbow Surg. 2008; 17(23):1-6.
30. Lundberg BJ. The frozen shoulder Clinical and radiographical observations. The effect of manipulation under general anesthesia. Structure and glycosaminoglycan content of the joint capsule. Local bone metabolism. Acta Orthop Scand Suppl 1969; 119:51-9.
31. Ogilvie-Harris DJ, Biggs DJ, Fitsialos SP, Mackay M. The resistant frozen shoulder: Manipulation versus arthroscopic release. Clin Orthop. 1995; 319:238-48.
32. Le Lievre HMJ, Murrell GAC. Long-term outcomes after arthoscopic capsular release for idiopathic adhesive capsulitis. J Bone Joint Surg Am. 2012; 94:1208-16.

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Original Article

Analysis of surgical management of proximal humeral fractures

Vijay Baba N and Prasanna C

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Abstract
Aim: To assess the outcome of fractures of proximal humerus managed surgically.
Materials and Methods: This prospective clinical study is an analysis of 22 cases of surgically managed proximal humeral fractures, undertaken at the Department of Orthopaedics & Traumatology, Govt. General Hospital, Chennai. Standard anteroposterior radiographs of the affected shoulder were taken in all patients and most of them were further evaluated with Neer's three view trauma series. CT scan was done in patients with complex fracture dislocations, to delineate the fracture pattern and the direction of dislocation. The indications for surgery were displacement more than 1cm and angulation > 45^0. Patients not satisfying these criteria were treated conservatively and not included in this study. All the patients were operated by the standard anterior deltopectoral approach. All the patients were followed up monthly for the first 3 months and later every 3 months. During follow-up patients were clinically evaluated for pain, function and rotation. Radiological evidence of fracture union was observed by serial x-rays.
Result: Majority of the injured patients were males (72%). Mean age of the study population was 36.75 years. RTA was the most common mode of injury (36.3%). There were no bilateral fractures; all were right handed persons and the dominant arm was involved in 16 (72.7%) patients. Early complications were encountered in 6 (25%) patients and late complications were encountered in 8 (30%) patients
Conclusion: This study concluded that functional outcome of two part fractures is better than three part and four part fractures and the results are better with fractures than with fracture dislocations.

Keywords: proximal humerus, fracture, management, surgery

Introduction
Fractures of the proximal humerus are challenging to diagnose and treat. They are not uncommon, accounting for 4% to 5% of all fractures [1, 2]. Emphasis is placed on complete and accurate diagnosis and formulation of safe and simple techniques for restoration of stability, fracture healing, cuff integrity, motion and function.
Diagnosis has been facilitated with adoption of three-right angled trauma series [3-5] supplemented with CT or MRI. With more standard use of four-segment classification system for fracture and fracture dislocation [6-8] a protocol for management and comparison of long term outcome of similar injuries had been made possible.
There have been improvements in fixation techniques during open reduction and in the understanding of the role of prosthetic replacement [9-12] to maximise anatomic restoration while minimising immobilisation time during which stiffness develops.
With the advent of improved fixation techniques the elderly no longer need be denied effective surgical treatment, especially at a time in life, when the shoulders are often needed for ambulation with canes and crutches. Maintenance of good shoulder function may make a good difference for their independent life style. Hence the present prospective clinical study was conducted to assess the outcome of fractures of proximal humerus managed surgically.

Materials and Methods
This prospective clinical study is an analysis of 22 cases of surgically managed proximal humeral fractures, undertaken at the Department of Orthopaedics & Traumatology, Govt. General Hospital, Chennai.

Methodology

Standard anteroposterior radiographs of the affected shoulder were taken in all patients and most of them were further evaluated with Neer's three view trauma series which involves the AP view in the plane of scapula, lateral view in the plane of scapula and axillary lateral view. CT scan was done in patients with complex fracture dislocations, to delineate the fracture pattern and the direction of dislocation.

The indications for surgery were displacement more than 1cm and angulation > 45^0. Patients not satisfying these criteria were treated conservatively and not included in this study. All the patients were operated by the standard anterior deltopectoral approach.

Surgical procedure (Figure 1.1)

Under general anaesthesia, patients were positioned supine on the operating table. A sand bag was wedged under the medial border of scapula to push the affected shoulder forward while allowing the arm to fall backward, thus opening up the front of the joint. Elevation of the head end of the table considerably reduced the bleeding. An incision was made from the coracoid and carried distally in the line of deltopectoral groove to the deltoid insertion. The internervous plane was dissected between deltoid, which is supplied by axillary nerve and pectoralis major which is supplied by pectoral nerves. In most cases, cephalic vein was preserved with either the deltoid or pectoralis major. In some it was ligated. The deltoid was partially erased from the clavicle for better exposure and retracted laterally and superiorly. The long head of biceps found under the insertion of pectoralis major was used as a guide to the interval between greater and lesser tuberosities. It also helped expose the rotator interval between the anterior part of supraspinatus and superior edge of subscapsularis. With the arm abducted 70-90 degrees the tuberosities, humeral head were exposed. Fracture fragments were reduced with bone holding forceps and reduction confirmed with image intensifier. Implants were selected according to the geometry of the fracture.

Cancellous screw fixation

For cancellous screw fixation the displaced greater tuberosity fragment was reduced with bone holding forceps and drill holes made with 3.2mm dill bit and 6.5mm cancellous screws inserted after tapping the near cortex.

Buttress plating

For AO "T" buttress plating, the fracture fragments were reduced. 'T' plate was placed on the lateral aspect and stabilised with cancellous screws for the tuberosity fragments and cortical screws for the shaft.

Tension band wiring

For Tension band wiring, 'k' wires were passed from the greater tuberosity into the shaft. The `k' wires were cut close to the tuberosity and bent. Drill-hole was made in the shaft and a 18G SS wire passed in the form of 'figure of 8' from the `k' wires to the drill hole, stabilising the fracture fragments.

Minimal Fixation with 'k' wire

For minimal fixation with `k' wires, the `k' wires were passed from the shaft to the head of humerus and another `k' wire from the greater tuberosity to the medial cortex of humeral shaft.

Hemiarthroplasty

For hemiarthroplasty the standard anterior deltopectoral incision was extended down to expose the shaft. Subscapsularis was retracted medially with lesser tuberosity to expose the interior of joint. The articular head fragment was removed and the joint cleared of blood clots and bone debris. The intra articular portion of biceps was identified as it attaches to the supraglenoid tubercle of scapula. If it were not injured, it was preserved by retracting it laterally. The stem diameter and length required in the proximal 12 to 15cm of the medullary canal were estimated. Once the stem size and neck length were determined the prosthesis was inserted by hand in 35-40 degrees of retroversion. The amount of retroversion was determined by flexing the elbow to 90 degrees and palpating the epicondyles. Suture passed through tuberosities or tendon bone junction was passed through holes in the neck of prosthesis. Anatomic reduction of tuberosities was done beneath the collar or head of prosthesis and sutures tightened.

Polymethyl methacrylate cement was used to secure the prosthesis in bone, if fixation was not adequately stable.

Fracture dislocation

In irreducible fracture dislocations and head splitting fractures the coracoid was predrilled and osteotomised and retracted with the tendon. Arm was externally rotated and blunt instrument passed between subscapsularis and capsule and stay sutures applied. It was divided one inch from its insertion and retracted. Capsule was incised longitudinally to open the joint and reduce the articular fragment.

In all patients, the rotator interval between anterior edge of supraspinatus and superior edge of subscapsularis was closed with multiple interrupted sutures. The deltoid was reattached to the clavicle and wound irrigated and closed over suction drain.

Post-Op Care and Rehabilitation

Prophylactic antibiotics which were started before surgery were continued for 48 and 72 hours postoperatively.

In a few ice packs were used to decrease the swelling. Passive elbow flexion and extension were started by 24 to 48 hours.

Sutures were removed by 10th postoperative day.

Phase I: Exercises consisting of pendulum exercises were encouraged from the first week. Gentle passive forward flexion and internal and external rotation were started by third or fourth week.

Phase II: Exercises consisting of active range of motion exercises and resistive exercises were started by 4 to 6 weeks.

Phase III: Exercises consisting of advanced stretching and strengthening exercises were started by 3 months. Light weight lifting was started after 3 months.

Follow-up

All the patients were followed up monthly for the first 3 months and later every 3 months. During follow-up patients were clinically evaluated for pain, function and rotation. Radiological evidence of fracture union was observed by serial x-rays. (Figure 1.2)

Statistical Analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2010) and then exported to data editor page of SPSS version 19 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of percentages.

Results

Table 1: age and gender wise distribution of study subjects

Age group	No. of patients	Percentage	Males	Females
0-10	0	0	0	0
11-20	4	18	3	1
21-30	4	18	4	0
31-40	6	27	4	2
41-50	3	13	3	0
51-60	4	18	2	2
61-70	1	6	0	1
Total	22	100.0	16	6

Table 2: distribution of study subjects according to mode of injury

variables	No. of patients	Percentage
Mode of injury		
RTA	8	36.3
FFH	4	18.18
Epilepsy	2	9.09
Fall at ground level	6	27.27
Electrocution	2	9.09
Side Involved		
Dominant (right)	16	72.7
Non dominant (left)	6	27.3

Table 3: distribution of study subjects according to type of fracture and dislocation type

Variables	No. of patients
Fracture type	
Closed Fracture	21
Open Fracture	1
Neer's Type	
2 part	11
3 part	8
4 part	3
Dislocation	
No dislocation	12
Dislocation	10
2 Part	3
3 Part	4
4 Part	3

Table 4: distribution of study subjects according to type of Implant placed

Implants	No. of patients	2 part	3 part	4 part
T Buttress plate	7	3	3	1
TBW	3	0	3	0
K wire	7	4	2	1
Hemiarthroplasty	1	0	0	1
Cancellous Screws	4	4	0	0

Table 5: distribution of early and late complications

Complications	No. of patients
Early	
Skin necrosis	2
Wound gaping	1
Axillary nerve damage	2
Deltoid atony	1
Late	
Non union	0
Mal union	2
Joint Stiffness	3
Heterotopic ossification	1
Instability	1
Infection	1

Discussion

In this study, we have analyzed 22 cases of surgically managed proximal humeral fractures in our hospital. There was male preponderance in our study 16 (72%) which was the same as the study by Flatow *et al.* [13] In a study conducted by Hawkins *et al.* [14] involving 15 patients of proximal humeral fracture, there was female preponderance. This is thought to be a result of advanced osteoporosis.

In our study the average age of the patients was 36.75 years which was much lower than reports by Hawkins *et al.* [14] and Flatow *et al.* [13] and Cornell *et al.* [15]

RTA was the most common mode of injury in our study, probably because majority of the patients were in the younger age group (average 36.75). In a study by Flatow *et al.* [13] fall on the arm was the predominant mode of injury probably reflecting the increase in average age of the patients (53 mean) in their study.

In our study, unusual mode of injury like seizures was present in two patients and electrocution in two patients. There was a predominance of two part fracture in our study (50%) of which greater tuberosity fractures were the most common. Associated dislocations were present in 45% of the patients. With reduction of glenohumeral dislocation if tuberosity fragment remained displaced >1cm or angulated > 45 degrees, ORIF was done. Repair in such patients restored the dynamic stability by reattachment of the muscles of the rotator cuff. Flatow *et al.* [13] in a series of 12 patients reported 50% excellent results and 50% good results in patients treated by ORIF for two part greater tuberosity fracture.

Closed treatment of three part fracture is associated with moderate pain, poor motion and disability. ORIF was associated with good to excellent results in more than 80% of patients in a report by Hawkins *et al.* [14] and recommend operative treatment for healthy active individuals who have a three part fracture of the proximal humerus. Cornell *et al.* [15] reported good results in 10 of 13 patients treated with screw-tension band technique for 3 part fracture. Prosthetic replacement for 3 part fracture has been used by several authors but we have not done prosthetic replacement for three part fracture in our study.

In the treatment of four part fracture and fracture dislocations, less than 10% good or excellent results are obtained with either closed or open reduction or internal fixation. Isolated reports of revascularisation of humeral head following open reduction and internal fixation indicate satisfactory healing. Neer and Rockwood have reported nearly 90% excellent results with an improved technique utilizing long deltopectoral approach and better rehabilitation. [16] This has become the preferred technique of most surgeons and we have also used the same technique. From the data presented in this study, we have demonstrated that majority of the patients had no pain or only mild pain (80%) which is comparable to the study conducted by Hawkins *et al.*15 and Flatow *et al.* [13]

The average active elevation in our study in two part fractures was 149 degrees and average external rotation was 62.5 degrees which are comparable to the study by Flatow *et al.* [13] in a study of 12 patients of two part fractures treated surgically.

The average elevation in our study with three part fracture was 112.85 degrees and external rotation was 45 degrees which are also comparable to the study by Hawkins *et al.* [14] of 15 cases of 3 part proximal humerus fractures treated surgically.

We also have seen few complications in our study. Malunion of greater tuberosity fragment in a patient with 3 part fracture treated with buttress plating resulted in restriction of abduction and impingement. Another patient had malunion of

surgical neck, but had excellent functional result reflecting the fact that radiological outcome may not imply functional outcome.

Heterotopic ossification occurred in one patient with 2 part fracture dislocation, probably because the patient had received native treatment in the form of many attempted reductions and massage. Several authors have reported an incidence of upto 10% of heterotopic ossification in proximal humeral fractures. There were no non-unions or radiographic evidence of avascular necrosis or deep infection in our study.

Finally a prolonged, closely monitored and well defined program of rehabilitation was necessary to obtain the best functional results. We have followed the three phase rehabilitation protocol of Hughes and Neer [17] in all our patients and this has provided good results. For some patients this had taken as long as a year to achieve nearly full range of motion and function.

Conclusion

The present study concluded that: functional outcome of two part fractures is better than three part and four part fractures; the results are better with fractures than with fracture dislocations; in severely comminuted fractures, where anatomy cannot be restored without extensive soft tissue dissection, fixation with 'k' wires and screws gave better functional results and results are best when operative method results in stable fixation that allows early passive mobilization.

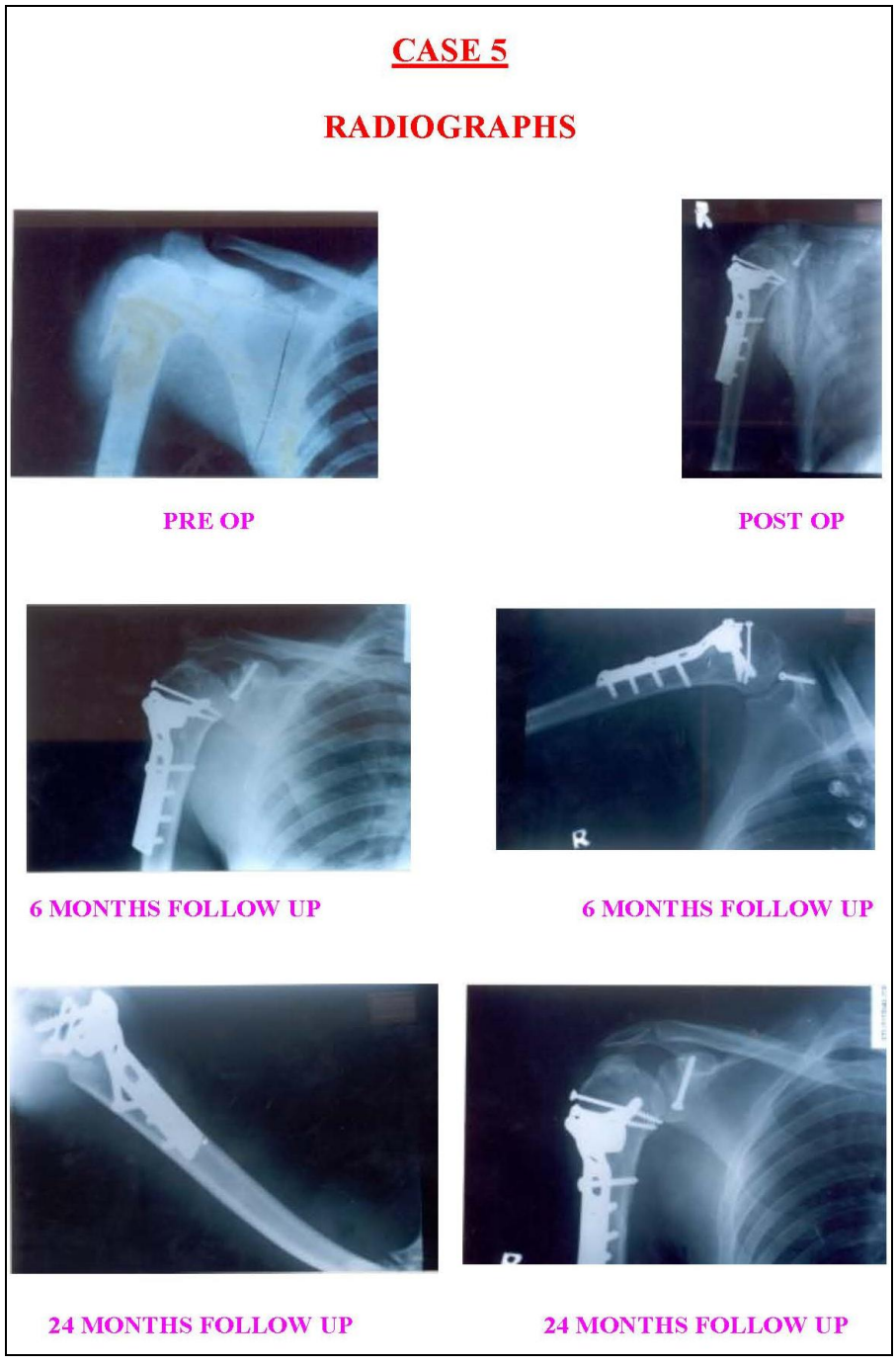


Fig 1. Radiographs

References

1. Tom R. Norris, Fractures and fracture dislocations of the glenohumeral complex, J.B. Lippin-Cott Company, Philadelphia, 1993;29:405-421.
2. Tom R Norris, Skeletal trauma. Fracture of the proximal humerus and dislocations of the shoulder. 1st Edition.

2(39):1201-1279.
3. Post M. Fractures of the upper humerus. Orthop Clin North Am. 1980;11(2):239-52
4. Hawkins RJ, Angelo RL. Selecting treatment, avoiding pitfalls; displaced proximal humeral fracture. Management of shoulder problems, Orthop Clin North Am 1987;18(3):421-31.
5. Robert J Neviaser. Radiologic assessment of the shoulder, plain & arthrographic. Orthopaedic Clinics of North America 1987;18(3):343-462.
6. Bernstein J, Adler LM, Blank JE, Dalsey RM, Williams GR, Iannotti JP. Evaluation of the Neer system of classification of proximal humeral fractures with computerized tomographic scans and plain radiographs. J Bone Joint Surg Am. 1996;78(9):1371-5.
7. Sidor ML, Zuckerman JD, Lyon T, Koval K, Cuomo F, Schoenberg N. The Neer classification system for proximal humeral fractures. An assessment of interobserver reliability and intraobserver reproducibility. J Bone Joint Surg Am. 1993;75(12):1745-50.
8. Daughtery TS, Linda Jones KL. Campbell's operative Orthopaedics, 9th Edition, 3, 2286-96.
9. Iannotti JP, Williams GR. Total shoulder arthroplasty. Factors influencing prosthetic design. Orthop Clin North Am. 1998;29(3):377-91.
10. Post M. Constrained Arthroplasty of the Shoulder. Orthop Clin North Am 1987;18(3):455-62.
11. Hattrup SJ. Indications, technique and results of shoulder arthroplasty. Orthop Clin North Am. 1998;29(3):445-66.
12. Brown TD, Bigliani LU. Complications with humeral head replacement. Orthop Clin North Am. 2000;31(1):77-90.
13. Flatow EL, Cuomo F, Maday MG, Miller SR, McIlveen SJ, Bigliani LU. Open reduction and internal fixation of two-part displaced fractures of the greater tuberosity of the proximal part of the humerus. J Bone Joint Surg Am. 1991;73(8):1213-8.
14. Hawkins RJ, Bell RH, Gurr K. The three-part fracture of the proximal part of the humerus. Operative treatment. J Bone Joint Surg Am. 1986;68(9):1410-4.
15. Cornell CN, Levine D, Pagnani MJ, Internal fixation of proximal humerus fracture using the screw-tension band technique. J Ortho trauma 1994;8(1):23-7.
16. Neer CS, Rockwood CA. Fractures and dislocation of the shoulder, in fractures (Eds. CA Rockwood and DP Green). JB Lippincott, Philadelphia, 1984, 675- 707.
17. Hughes M, Neer CS. 2nd. Glenohumeral joint replacement and postoperative rehabilitation. Phys Ther. 1975;55(8):850-8.

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ORIGINAL ARTICLE

A PRELIMINARY STUDY OF STRESS FRACTURES AMONG PARAMILITARY TRAINEES

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Prasanna C, Vijay Baba N, Rajinikanth S. "A Preliminary Study of Stress Fractures among Paramilitary Trainees". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 10, March 10; Page: 2565-2569, DOI: 10.14260/jemds/2014/2174

ABSTRACT: Stress fractures are common overuse injuries. The pathogenesis is multifactorial and usually involves repetitive sub-maximal stresses. Stress fractures have been frequently reported among young military recruits and in sports persons, but to our knowledge they have not been studied among the paramilitary trainees. The present study was carried out at the Central Industrial Security Force training center at Arakkonam, Tamilnadu from March 2010 to May 2012 to find the incidence of stress fractures among fresh trainees. All patients presenting with symptoms suggestive of stress fractures were evaluated. A total of 5 cases out of a total of 2000 trainees (0.25 %) were confirmed as stress fractures based on clinical and radiological findings.
KEYWORDS: Stress fractures, Paramilitary Trainees.

INTRODUCTION: A stress fracture is the response of bone to repeated stress, none of which by itself is sufficient to cause a fracture. Stress fractures represent one of the most common and potentially serious injuries.[1-3] Stress fractures occur among persons with normal bones and no acute injury who are undergoing physical activity to which they are unaccustomed.[4-6] The underlying pathophysiology is believed to relate to repetitive mechanical loading of bone secondary to physical activity that stimulates an incomplete remodeling response.[7-9]

Stress fractures are traditionally been described in military recruits, but are also seen in sports persons. However they are known to occur in anyone being exposed to a level of physical stress or activity to which he or she is not adapted.[10] A literature search revealed that stress fractures are extensively studied in army recruits and sports persons, but not in paramilitary personnel. Even though the amount of training varies between military and paramilitary trainees, they are also exposed to a high level of physical training to which they are unaccustomed. Hence the present study was initiated with an aim to find out the incidence of stress fractures among the paramilitary trainees

MATERIALS AND METHODS: The study was conducted among the paramilitary trainees at central industrial security forces (CISF) training centre, Arakkonam, Tamilnadu, India. The study period was from March 2010 to May 2012 (27 Months). Approximately 2, 000 trainees were trained at the centre during the study period. All the trainees were males and were in the age group of 21 – 25 years. Fresh trainees from the training centre who reported to our institute with symptoms suggestive of stress fractures were evaluated clinically and radiologically.

Clinical evaluation included a thorough history regarding the activities involved and interpretation of point tenderness at specific regions. All the trainees were particularly enquired about the habit of smoking and alcohol intake. Radiography included antero- posterior and lateral X-ray projections of the involved anatomical region.

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The training schedule included various forms of physical activities such as drill, short distance sprinting, long distance running, high – jump, obstacles, work- up and firing. All cases diagnosed as stress fractures were given splinting in the form of Plaster of Paris (POP) slab, except for one case of fibula stress fracture. All were refrained from physical activities and were kept on non-weight bearing mobilization with the help of a quadruple walker support for a period of 6 weeks. The patient with fibula fracture was put on a crepe bandage and was advised non-weight bearing. The POP slab was removed at 6 weeks and patients assessed clinically for point tenderness at the fracture site. Activities were gradually increased over a period of 4 weeks.

OBSERVATIONS: A total of five cases were diagnosed as stress fractures based on the clinico-radiological evaluation. The mean age was 23 years (range 22- 25). The fracture distribution was as follows: tibia- 3 cases, femur and fibula- one case each. The stress fracture in the tibia was located in the lower one third in two cases and upper one third region in one case. The femoral stress fracture was at the junction of middle and distal one third. The fibula fracture was located in the mid- shaft region.

All the cases presented with the symptom of pain in the involved extremity. The pain was localized to the fracture site. The patient with femoral stress fracture also had a limp at presentation. The mean duration at presentation was 3 weeks. Pain was typically aggravated with physical activities and progressively worsened with continuation of activities. The pain was relieved with rest in all the cases. None of the trainees were smokers or alcoholics. Radiologically a faint fracture line (lucency) was visible in the case of fibular stress fracture (Fig. 1). Three cases showed heaped up callus at the site of stress fracture without clearly showing a fracture line. Periosteal reaction in the form of sclerosis was seen in one case. Table1 summarizes the observations of this study.

DISCUSSION: Stress fractures are common injuries that begin with repetitive and excessive stress on the bone. This leads to the acceleration of normal bone remodeling, the production of micro-fractures (caused by insufficient time for the bone to repair), the creation of a bone stress injury (i.e., stress reaction), and eventually, a stress fracture.[11, 12] Persons who participate in repetitive, high intensity training, such as athletes and military recruits, are at increased risk of developing stress fractures. Stress fractures, classically been described in military recruits and in sports persons, hence, studied and reported extensively in such populations.

The Central Industrial Security Force (CISF) came into existence in 1969, to guard the industrial establishment and protect them from crime. CISF is just a unique organization in paramilitary forces for India, which works for sea ways, airways and some of the major installations in India. A rapidly grown organization over the past four decades, the trainees receive a highly demanding physical training at the training centers. Therefore, they are equally prone for stress injuries. To the best of our knowledge no studies of stress fractures in paramilitary persons have been reported in literature so far. Hence, the present study was initiated.

We found an incidence of stress fractures as 0.25 per cent in the present study, which was similar to the incidence reported by Das and Goel in trained soldiers.[13] We calculated the incidence taking into account the total number of trainees (5) who had stress fractures to the total number of trainees (2, 000) trained at the centre during the study period. Various studies of stress fractures in

military recruits have found an incidence ranging from less than 2 per cent[14] to an unusually high incidence of 31 per cent.[15]

Several risk factors have been identified for stress fractures. Persons who participate in repetitive, high-intensity training such as athletes and military recruits [16-19], recreational runners who average more than 25 miles per week[17, 20] are at increased risk of stress fractures. Poor nutrition and lifestyle habits may increase the risk of stress fracture. One study found lower 25-hydroxyvitamin D levels in Finnish male military recruits with stress fractures.[21] With the rising need for well-trained paramilitary forces in the country, we believe, they are equally prone for stress fractures as other persons.

In a study of 330 stress fractures in Indian recruit at a military training centre, it was found that; tibia is the commonest bone involved mostly in its upper third.[22] We also found the tibia as the commonest bone involved, but the lower third involvement was more (2 cases) as compared to the upper third (1 case).

Plain radiography should be the first imaging modality considered because of its availability and low cost.[23] Plain radiography is usually negative initially but is more likely to become positive over time.[24] The value of computed tomography (CT) is limited because of lower sensitivity and higher radiation exposure than other imaging modalities.[25] Magnetic resonance imaging (MRI) is superior for early detection of stress injury. However, MRI may also identify reactive bone remodeling (interpreted as early stress injuries) and, therefore, should be clinically correlated for stress fracture.[26, 27]

With plain radiography, a faint lucency may be seen at first, although stress fractures are usually identified by subsequent indirect findings: periosteal thickening or sclerosis, cortical changes with initial decreased density (“gray cortex”), and, more commonly, later callus formation, or endosteal thickening and sclerosis.[18, 24] We were able to identify the stress fracture in plain radiography on all 5 cases probably because, they presented to us at a mean period of 3 weeks. Callus was well seen in majority of our cases (Fig. 2).

Management in all the cases is essentially rest, support to the involved part and abstinence from the strenuous physical activity that caused the stress fracture.

REFERENCES:

1. Hulkko A, Orava S. Stress fractures in athletes. Int J Sports Med 1987; 8:221–6.
2. Jones BH, Harris JM, Vinh TN, et al. Exercise-induced stress fractures and stress reactions of bone: epidemiology, etiology and classification. Exerc Sports Sci Rev 1989; 17:379–422.
3. Matheson GO, Clement DB, McKenzie DC, et al. Stress fractures in athletes: a study of 320 cases. Am J Sports Med 1987; 15:46-58.
4. Belkin SC. Stress fractures in athletes. Orthop Clin North Am 1980; 11:735-42.
5. Devas MB. Stress fractures in athletes. Proc R Soc Med1969; 62:933-7.
6. Devas MB. Stress fractures in athletes. J R Coll Gen Pract 1970; 19:34-8.
7. Markey KL. Stress fractures. Clin Sports Med 1987; 6:405-26.
8. Hershman E, Mailly T. Stress fractures. Clin Sports Med 1990; 9:183-214.
9. Nattiv A, Armsey TD Jr. Stress injury to bone in the female athlete. Clin Sports Med1997; 16:197-224.

10. Bruce H. Jones, Stephen B. Thacker, Julie Gilchrist, C. Dexter Kimsey, Jr., and Daniel M. Sosin. Prevention of lower extremity stress fractures in athletes and soldiers: A systematic review. Epidemiol Rev 2002; 24:228-47.
11. Fayad LM, Kamel IR, Kawamoto S, Bluemke DA, Frassica FJ, Fishman EK. Distinguishing stress fractures from pathologic fractures: a multimodality approach. Skeletal Radiol. 2005; 34(5):245-259.
12. Niva MH, Mattila VM, Kiuru MJ, Pihlajamäki HK. Bone stress injuries are common in female military trainees: a preliminary study. Clin Orthop Relat Res. 2009; 467(11):2962-2969.
13. Das B and Goel KK. Stress fractures in trained soldiers. Indian J Orthop. 1987; 21:162-66.
14. Brudvig TJS, Gudger TD, Obermeyer L. Stress fractures in 295 trainees: a one year study of incidence as related to age, sex and race. Milit Med 1983; 148:666-7.
15. C.Milgrom, M. Giladi, M. Stein, H. Kashtan, J.Y. Margulies, R. Chisin, R. Chisin, R. Steinberg, Z. Aharonson. Stress fractures in military recruits: A prospective study showing an unusually high incidence. J Bone Joint Surg 1985; 67(B):732-35.
16. Brukner P, Bradshaw C, Khan KM, White S, Crossley K. Stress fractures: a review of 180 cases. Clin J Sport Med. 1996; 6(2):85-89.
17. Matheson GO, Clement DB, McKenzie DC, Taunton JE, Lloyd-Smith DR, MacIntyre JG. Stress fractures in athletes. A study of 320 cases. Am J Sports Med. 1987; 15(1):46-58.
18. Ohta-Fukushima M, Mutoh Y, Takasugi S, Iwata H, Ishii S. Characteristics of stress fractures in young athletes under 20 years. J Sports Med Phys Fitness. 2002; 42(2):198-206.
19. Lappe J, Davies K, Recker R, Heaney R. Quantitative ultrasound: use in screening for susceptibility to stress fractures in female army recruits. J Bone Miner Res. 2005; 20(4):571-578.
20. Clement DB, Ammann W, Taunton JE et al. Exercise-induced stress injuries to the femur. Int J Sports Med. 1993; 14(6):347-352.
21. Ruohola JP, Laaksi I, Ylikomi T et al. Association between serum 25 (OH) D concentrations and bone stress fractures in Finnish young men. J Bone Miner Res. 2006; 21(9):1483-1488.
22. Khanna SK and Rao RP. Stress fractures among recruits. Med J Armed Forces 1982; 38:5-8.
23. Daffner RH, Weissman BN, Bennett DL et al. Expert Panel on Musculoskeletal Imaging. ACR Appropriateness Criteria stress/insufficiency fracture, including sacrum, excluding other vertebrae. Reston, Va.: American College of Radiology; 2008. http://www.guideline.gov/content.aspx?id=13668. Accessed March 10, 2010.
24. Ishibashi Y, Okamura Y, Otsuka H, Nishizawa K, Sasaki T, Toh S. Comparison of scintigraphy and magnetic resonance imaging for stress injuries of bone. Clin J Sport Med. 2002; 12(2):79-84.
25. Gaeta M, Minutoli F, Scribano E et al. CT and MR imaging findings in athletes with early tibial stress injuries: comparison with bone scintigraphy findings and emphasis on cortical abnormalities. Radiology. 2005; 235(2):553-561.
26. Batt ME, Ugalde V, Anderson MW, Shelton DK. A prospective controlled study of diagnostic imaging for acute shin splints. Med Sci Sports Exerc. 1998; 30 (11):1564-1571.
27. Bergman AG, Fredericson M, Ho C, Matheson GO. Asymptomatic tibial stress reactions: MRI detection and clinical follow-up in distance runners. AJR Am J Roentgenol. 2004; 183(3):635-638.

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Case No.	Age (Years)	Time at Presentation (Weeks)	Anatomical site of Involvement	Symptoms	Radiological Features
1	22	3	Upper third tibia	Pain	Callus
2	22	2	Lower third tibia	Pain	Periosteal sclerosis
3	23	3	Lower third tibia	Pain	Callus
4	23	3	Fibula mid-shaft	Pain	Lucent fracture line
5	24	3	Femur lower third	Pain, limp	Callus

TABLE 1: Summary of the Observations



Fig. 1: Case of stress fracture of fibula showing a lucent incomplete fracture line (Arrow).



Fig. 2: Stress fracture at the junction of middle and lower third of femur showing heaped up callus (Arrow) without a fracture line.

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Functional and radiological outcome of lateral approach for hemiarthroplasty for hip

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Abstract
With increasing geriatric population, more number of femoral neck and trochanteric fractures are noted. Surgery being the gold standard for early mobilisation, less evidence exists on surgical approach. The aim of this study was to assess the functional and radiological outcome of lateral approach in patients of more than 55 years of age. 30 patients above 55 years of age with an acute fracture of the femoral neck, who underwent hemiarthroplasty through lateral approach, in the Department of Orthopaedics, MGMC&RI, SBV university were followed up for 6 months and their functional and radiological outcomes assessed. The patients were of a mean age of 69.2. The mean Harris hip score was 77.5. There was no incidence of prosthetic dislocation, infection. Complications like Limb length discrepancy, myositis ossificans were noted. Abductor lurch were present which recovered with regular physiotherapy and ambulation. The use of Lateral approach to the hip in hemiarthroplasty showed excellent outcomes and no dislocation rates and hence the preferred approach for hemiarthroplasty.

Keywords: Harding's approach, hemiarthroplasty, neck of femur fracture

1. Introduction
Proximal femur fractures including intra and extra capsular fractures are expected to increase in incidence with increasing average life expectancy of individuals throughout the world. These fractures constitute the higher quotient in all osteoporotic fractures that occur in elderly age group [1] It has been proved time and again that proximal fractures pose a great morbidity and mortality to the patient and economic burden to the society.
Conservative management of intracapsular fractures (neck of femur fracture, femoral head fractures) is associated with higher mortality in the first 30 days post trauma [2]. The surgical options available in 50 and below age group involve closed or open reduction and internal fixation. The options in elderly include hemi arthroplasty (monopolar or bipolar) or total hip replacement based on patient profiles, patients economic condition, personal preferences and training of the surgeon. Complications of anaesthesia and surgery far outweigh the complications of restricted mobility associated with non operative management, except in a very few who are unfit for surgery. The goal of this operation is to provide a stable, pain-free, mobile joint and making the patient mobile and non-dependent [3].



Fig 1: Intracapsular neck of femur fracture



Fig 2: Austin moore prosthesis in situ

Numerous new approaches to the hip have been described since the 1990s; most of the approaches are based on older approaches and are modified for a specific surgical procedure. Mainly two surgical approaches are performed while treating the fracture neck of femur with hemiarthroplasty. Posterior Approach is associated with an increased dislocation rate and revision rate due to dysfunction of the posterior soft tissue structures [4]. The lateral approach is associated with limping of the patient post surgery due to the damage to the abductor muscles [5].

McFarland and Osborne described a lateral approach to the hip that preserves the integrity of the gluteus medius muscle. They noted that gluteus medius and vastus lateralis muscles can be regarded as being in direct functional continuity through the thick periosteum covering the greater trochanter and can be erased as a single unit from posterior to trochanter. Harding described a useful modification of the McFarland and Osborne direct lateral approach by reflecting the anterior half of this bundle. [6] This approach can be easily made with the patient in supine position.

Frndak *et al*. modified the Harding's direct lateral approach by placing the abductor split more anterior, directly over femoral head and neck must not extend more than 2 cm above the lateral lip of the acetabulum to avoid damage to the gluteal neurovascular bundle [7]. This offers exposure of the femoral head and neck requires less retraction. McLauchlin described a direct lateral approach to the hip through the gluteus medius used for many years by Hay at the Stracathro Hospital. Omega incision was described by I.D. Learmonth, wherin a omega shaped incision is made in the Gluteus medius and vatus lateralis, retracting the gluteus medius, minimus along with few bone shingles from the aponeurotic junction of the greater trochanter [8] So we have decided to study a short term follow up of functional outcome hemiarthroplasty done for neck of femur fractures through lateral approach using hardinge's approach.

2. Materials

The study was conducted in MGMC&RI over a period of one and a half years following the ethical committee's approval.Thirty consecutive patients with fracture neck of femur admitted in the department of Orthopaedics through casualty/OPD who required Hemiarthroplasty were selected for the study.

3. Methodology

Patient with a suspected neck of femur was admitted in the casualty ward, he/she was clinically examined and analgesics were given to relieve pain. Traction was maintained during radiological examination to minimize the pain and the diagnosis was confirmed. X-ray of pelvis with both hip joints-anterior-posterior view, X-ray of involved side hip with femur full length-AP and lateral view were taken. This was followed by routine work up for surgery. Patients were detailed regarding the purpose of the study and surgery, all consents obtained. Garden's Classification of Fracture Neck of Femur. Skin traction was applied for the affected limb to reduce the pain. Pre-operative training was given on -Deep breathing exercises, Static quadriceps and calf muscle pumping exercises, strengthening exercises to upper limbs. We used Austin moore's prosthesis /bipolar prosthesis based on canal diameter and social background of the patient.

Lateral (Harding) approach under combined spinal +Epidural /spinal anaesthesia, patient was put in a lateral position with the affected hip facing upwards. After painting and draping the affected lower limb and hip a lazy J- incision centring over greater trochanter is made.

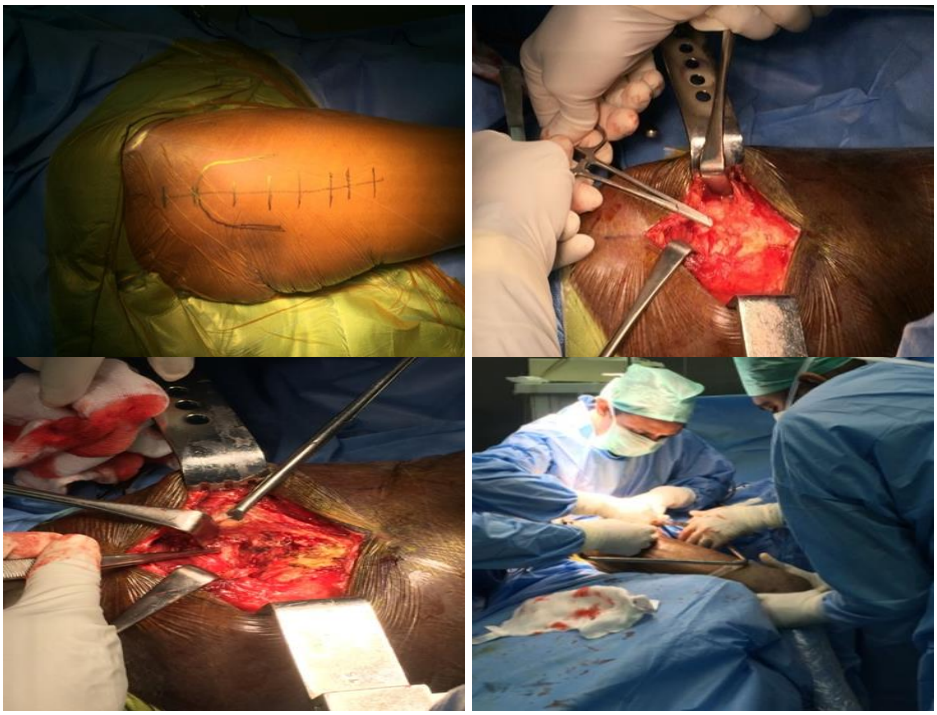


Fig 3-6: Shows marking of incision line with respect to greater trochanter, tendinous junction of gluteus medius and vastus lateralis and its incision ,dislocation of hip joint by flexion, adduction and internal rotation

Fascia lata is divided along skin incision. Tensor fascia lata is retracted anteriorly and gluteus maximus posteriorly. Tendinous junction of gluteus medius and vastuslateralis is exposed. An oblique incision is made over gluteus medius along the line of muscle fibres, across the greater trochanter leaving posterior two third still attached to the trochanter. This incision is carried proximally over the gluteus medius not more than 3 cms and distally in line with vastuslateralis.

The tendinous insertion of gluteus medius and vastuslateralis is elevated anteriorly. The capsule is visualised and 'T 'incision was made.

The hip and knee was flexed and the limb was externally rotated and adducted. Femoral head is extracted with a cork-screw extractor, taking care not to damage the articular surface of acetabulum. Acetabulum is inspected and all loose debris and bone fragments were removed, along with the remnants of the ligamentum teres. The entry in the proximal femur is made with a box cut and then the femoral canal is prepared taking care to give adequate lateralisation of rasp. The femoral canal is broached by hand with a rasp in 5 – 10° ante version and valgus position, During rasping decision is taken on use of cement depending on the size of the femoral canal. Head size of prosthesis was determined by measuring the head of femur, which was removed and measured with a measuring template. A trial reduction with the selected prosthesis was done. Range of movement, mobility and stability of the joint assessed. An appropriate size AMP/Bipolar Prosthesis is impacted with or without Bone cement in to the femoral canal. Care is taken while inserting the prosthesis to maintain adequate anteversion. Once the cement sets, prosthesis is then reduced by traction and internal rotation. The hip was tested for full range of movement and stability intra-operatively. Drill holes are made in the greater trochanter and 1 vicryl was used to anchor the abductors to the bone. The wound was closed meticulously in layers over suction drain maintaining haemostasis throughout the procedure and dressing was applied.

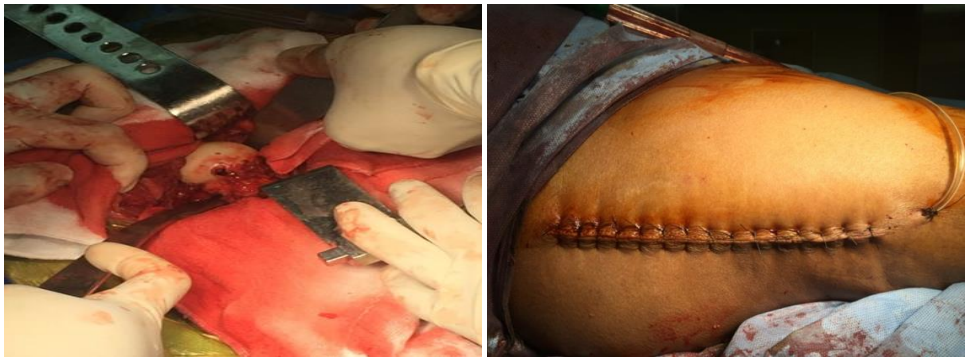


Fig 7 and 8: Shows exraction of femoral head and sutured surgical site respectively

3 doses of antibiotic were given post operatively. Analgesics were administered according to patient's analgesic requirements. The drain was removed on 2nd Postoperative day. Static quadriceps exercises were advised to all the patients as soon as they recovered from the anaesthesia. All the patients were advised to sit up with back rest from the 1st postoperative day. Deep breathing exercises were advised. A post-operative check x-ray was taken and the valgus seating with 10^0 anteversion was confirmed. Any limb length discrepancy was noted. Knee flexion and isometric quadriceps exercises were started on 2nd postoperative day and patients were mobilized with walkers with full weight bearing. They were discharged on post op Day 5.

The patient's were advised not to adduct or flex the hips beyond 90° or involve in activities that place heavy loads or stresses on the hip for the next 3 weeks. They are advised to carry out exercises as advised pre op and advised to come for follow up at the end of second week of post-operative period for suture removal. For further follow up at 1month, 3months and 6 months.At follow up, detailed clinical examination was done systematically. They were examined for gait, pain, tenderness, movements, deformities and length discrepancies. The distance the patient cover following surgery was questioned and compared to pre fracture state. The x-ray of the operated hip was taken at 1st, 3rd and 6th month.

Data Collection

All data was entered into a Data Collection Performa Sheet and were entered into Excel (MS Excel 2011). Other biographical details were also collected including date of birth, weight and height.

Statistical Methods

The data were analyzed in the study using proportion and Chi-Square test as the results were of categorical values. Values of $p<0.05$ was considered statistically significant.

Results and discussion

A total number of 30 patients were selected on the basis of pre-defined inclusion and exclusion criteria after informed consent. All patients were recruited through the out-patient and the emergency services department of Orthopaedics, MGMC &RI, SBV University, Pondicherry, INDIA.Majority of patients were between 61-70 years old (46.7%). and 30% of patients were between 71-80 years old and 13.3% of patients were between 50-60 years old. Females constituted 66.7% and Males 33.3%.Majority of patients had domestic fall (70%) and while 26.7% by RTA and 3.3% of patients by fall from height.

Table 1: Distribution by Mode of Injury

Mode of Injury	Frequency	Percentage
Domestic Fall	21	70%
Fall from Height	1	3.3%
RTA	8	26.7%
Total	30	100%

Left side constituted (60%) and 40% of the patients had injured their right side. Gardens type III fracture constituted (43.4%) and 30% of the patients had gardens type IV and 23.3% were under Garden's Type II and 3.3% under Type I fractures

Table 2: Distribution by Gardens Type

Gardens Type	Frequency	Percentage
I	1	3.3%
II	7	23.3%
III	13	43.4%
IV	9	30%
Total	30	100%

Uncemented hemiarthroplasty was done in 60%. While 40% required cement due to wide canal.

Outcome was evaluated by clinical and radiological means. Clinical assessment was done by using the Harris Hip Score and surgical site infection. Radiological assessment was done based on the presence or absence of myositis ossificans, Prosthesis alignment, peri-prosthetic fracture and loosening. 30 cases of intra-capsular fracture neck of femur were managed by hemiarthroplasty, through hardinge lateral approach. We had excellent results in 12 cases (42.9%), good in 12 cases (42.9%), fair in 3 cases (10.5%) and 1(3.5%) poor case, with an average score of 77 points according to the Harris hip rating system.

Table 3: Distribution by Harris Hip Score at 6 Months post op

HHS on 6th Month	Frequency	Percentage
<70	1	3.5%
70-79	4	10.5%
80-89	12	42.9%
90-100	12	42.9%
Total	28	100%

Among the 3 patients with fair results, one was a chronic alcoholic and did not fare better due to bad health and associated medical problems. The other was a 70 yrs old female, who was a housewife didn't fare well due to severe osteoarthritis knee which limited her mobility. Another patient developed myositis ossificans, which limited her hip abduction. Among the poor outcome group, patient had a proximal femur blowout due to domestic fall during her 1st month of post operative period.

We have got good (42.9%) to excellent(42.9%) results in 85.8% of the cases. In a series of 20 patients done by Dr.Sivakumar using lateral approach 20% of patients had excellent outcome,60% of patients had good outcome,20% had fair outcome.[12] We did total 30 cases of hemiarthroplasty in Hardinge's approach, at 3 months follow up we found excellent abductor muscle function in 20 patients, good in 5 patients and poor in 5 patients. After good physiotherapy, at 6 months' postoperative period we found excellent abductor muscle function in 26(85.7%) patients and poor in 2 (14.3%) patients. Picado had 7.5% abductor lurch at 6 months post op.[9]

Table 4: Distribution by Abductor Lurch at 6 months

Abductor Lurch	Frequency	Percentage
Yes	2	14.3%
No	26	85.7%
Total	28	100%

23 (76.6%) had no surgery related complications during the follow up period. 2 (6.7%) of patients had lengthening and 2 (6.7%) shortening and 1 had Myositis Ossificans which was detected at 3rd month and was nonprogressive at the end of six months.2 Patients lost their lives 3 months post surgery due to non surgery related health issues

Table 5: Distribution by Complications

Complications	Frequency	Percentage
Lengthening	2	6.7%
Shortening	2	6.7%
Myositis	1	3.3%
Expired	2	6.7%
Nil	23	76.6%
Total	30	100%

In the reported series by Mulliken *et al*, in 770 patients operated using the above approach, they encountered 0.3% of dislocation rates, limp in 10%,myositis in 3%. [10] No Infection was encountered in our patients. In this study, there was no incidence of dislocation. We had limb length discrepancy in 4 (13.4%) patients which was due to excessive or inadequate neck resection this was not due to the surgical approach.A recent meta analysis on the anterolateral and posterior approach over a period of five years shows no significant differences in dislocation rate [11]

Conclusion

After treating 30 patients with intracapsular fracture neck of femur, by Hemiarthroplasty through lateral approach, it has better advantage in terms of stability and functional results. There is reduced morbidity, with excellent to good results in 85.8% and mean Harris hip score of 77 points. We did not encounter any dislocation in our series probably due to modified Hardinge Approach, better soft tissue balancing and as incision at non dependent part of body, better wound care and hence less chance of infection. Modified Hardinges approach is a excellent surgical approach if proper re-suturing of the abductor band and capsule closure is done. It requires a clear understanding of not to injure the superior gluteal nerve by limiting the proximal dissection of gluteus medius within 3 cm from tip of greater trochanter thus avoiding lurch in post operative period – the commonest complication of hardinge approach in early learners.

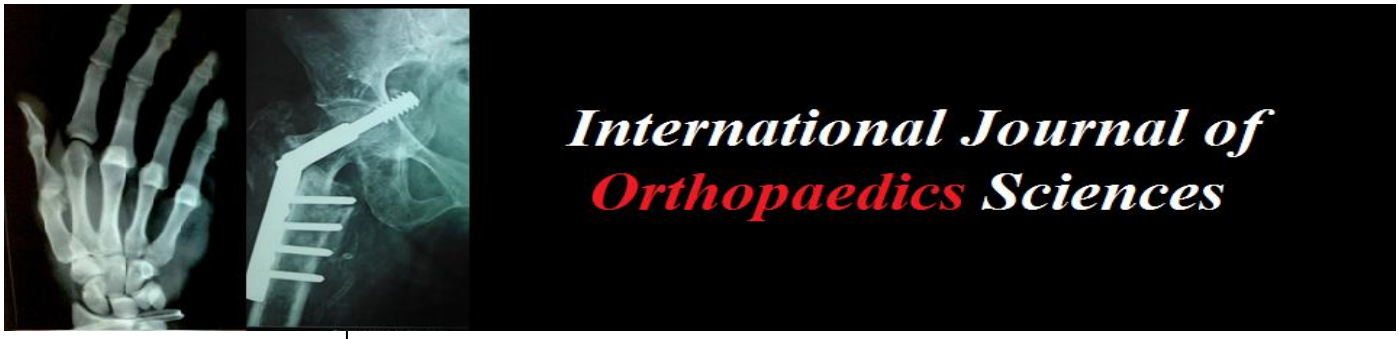
Limitations

We do not have a long term follow up to comment about the longevity of the prosthesis and the evidence on acetabular erosions. The study population of 30 patients is too small a number to give a statistically significant opinion and the study period was only for 6 months. Our early and short term results are encouraging and promising and long term results are awaited.

References

1. Shreyasee Amin, Osteoporosis in Men (second Edition), The effects of gender on skeletal health, 2010, 351-360
2. One-year outcome for elderly patients with displaced intracapsular fractures of the femoral neck managed non-operatively.*Gregory* JJ, Kostakopoulou K, Cool WP, Ford DJ.Injury. 2010; 41(12):1273-6.
3. Simon P, Gouin F, Veillard D, Laffargue P, Ehlinger M, Bel JC, *et al*. Rev Chir Orthop Reparatrice Appar Mot. 2008; 94(6):S108-32. Epub Sep 19
4. Bush JB, Wilson MR. Orthopedics. 2007; 30(2):138-44
5. Damage to the superior gluteal nerve following hardinge approach, M. Ramesh, J.M.O'Byrne, the bane and joint journal. 1996.
6. A modified direct lateral approach in total hip arthroplasty,V.S.Pai, journal of orthopaedic surgery. 2002; 10(1):35-39
7. Frndak PA, Mallory TH, Lombardi AV. Translateral surgical approach to the hip: the abductor muscle "split". Clin Orthop Relat Res. 1993; 295:135
8. Learmonth D, Allen I. Patricia. The omega lateral approach to the hip. The Journal of bone and joint surgery. British. 1996; 78:559-61. 10.1302/0301-620X.78B4.0780559.
9. Damage to the superior gluteal nerve after direct lateral approach to the hip. Picado CH, Garcia FL, Marques W Jr. Clin Orthop Relat Res. 2007; 455:209-11.

10. A modified direct lateral approach in total hip arthroplasty: a comprehensive review, Mulliken BD, Rorabeck CH, Bourne RB, Nayak N. J Arthroplasty. 1998; 13(7):737-47.
11. Does Surgical Approach Affect Outcomes in Total Hip Arthroplasty Through 90 Days of Follow-Up? A Systematic Review with Meta-Analysis, Larry E.MillerPhD, Joseph S.GonduskyMD, The Journal of arthroplasty. 2018; 33(4):1296-1302
12. z. A Prospective Study Comparing The Functional Outcome, Radiological Outcome and Gait Analysis between Lateral Surgical Approach and Posterior Surgical Approach in The Total Hip Replacement Dr.P.Sivakumar & Prof. E.S.Arivazhagan, IOSR Journal of Dental and Medical Sciences. 2016; 15(9,V):20-44.



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A modified dynamic hip screw in unstable intertrochanteric fracture: A retro-prospective study

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Abstract
Background: Intertrochanteric fractures occur mostly in elderly patients, and the outcome may be extremely poor if there is prolonged bed-rest.
Many treatment modalities have come up in management of these unstable intertrochanteric fractures. Sliding Hip Screw Fixation is still the gold standard in treatment of stable intertrochanteric fracture. In unstable comminuted intertrochanteric fractures, there is high incidence of failure in view of excessive collapse seen with Dynamic hip screw.
In order to limit the collapse we have done a modification on Dynamic Hip Screw implant. The aim of this study is to assess fracture healing, collapse and implant failure, in unstable intertrochanteric fractures (Boyd and Griffin type-2) treated by modified DHS fixation.
Methods: 23 patients who were operated with modified DHS for unstable intertrochanteric fracture were selected using random selection and following parameters were noted from the records.
Results: Out of 23 patients in this study, 20 patient showed fracture healing (87%) with or without minimal collapse and 3 patient had non-union (13%) at the end of 5 months follow up. Ultimately all fractures united at the end of 1 year followup.
Conclusion: Modified Dynamic Hip Screw has showed improved results as compared to normal Dynamic Hip Screw in treating communited intertrochanteric fracture, which limits the collapse at fracture site.

Keywords: Intertrochanteric fractures, modified DHS, collapse

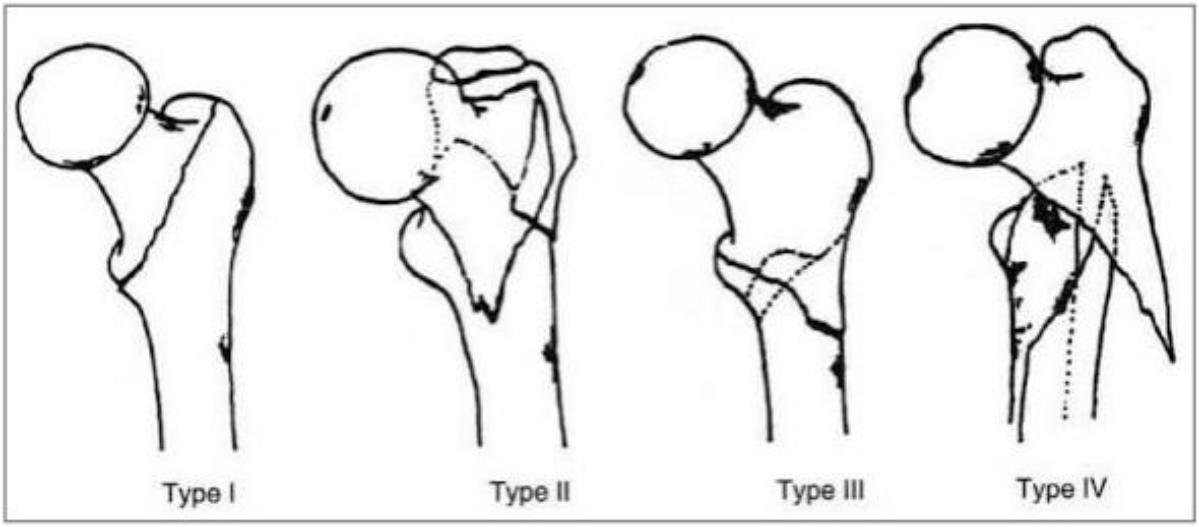
Introduction
Intertrochanteric fractures occur mostly in elderly patients, and the outcome may be extremely poor if there is prolonged bed-rest. The best treatment for trochanteric fracture remains controversial [1-3]. Many methods have been recommended [4-6]. Early mobilization of these patients reduces the morbidity and mortality rate.
Intertrochanteric fractures with severe communition and displacement are commonly seen in elderly patients. Most of the elderly patients have poor bone quality due to which fractures are often associated with complications like non-union, implant failure and femoral head perforation.
Management of unstable intertrochanteric fractures is challenging because of excessive collapse, loss of fixation, implant failure, lag screw cut out and in addition osteoporosis also adds to the complication resulting in unpredictable outcome.
In order to limit the collapse we have done a modification on Dynamic Hip Screw implant. In this study we are assessing the outcome of unstable intertrochanteric fractures treated with modified dynamic hip screw fixation designed by us.
Boyd and Griffin (1949) classified fractures in the trochanteric area of femur into 4 types. Their classification included all fractures from extra capsular part of neck to a point 5cm below the lesser trochanter. Their classification is useful in planning treatment and estimating prognosis [7].

Type 1: Fractures that extend along the intertrochanteric line from the greater to the lesser trochanter. Reduction usually is simple and is maintained with little difficulty. Results generally are satisfactory.

Type 2: Comminuted fractures, the main fracture being along the line, but with multiple fractures in the cortex. Reduction of these fractures is more difficult because the comminution can vary from slight to extreme. A particularly deceptive form is the fracture in which an anteroposterior linear fracture occurs, as in type 1, but with an additional fracture in the coronal plane, which can be seen on the lateral radiograph.

Type 3: Fractures that are basically subtrochanteric with at least one fracture passing across the proximal end of the shaft just distal to or at the lesser trochanter. Varying degrees of comminution are associated. These fractures usually are more difficult to reduce and result in more complications during surgery and convalescence period.

Type 4: Fractures of the trochanteric region and the proximal shaft, with fracture in at least two planes, one of which usually is the sagittal plane and may be difficult to see on routine anteroposterior radiographs. If open reduction and internal fixation are used, twoplane fixation is required because of the spiral, oblique, or butterfly fracture of the shaft.



Aim of the study

The aim of this study is to assess fracture healing, collapse and implant failure, in unstable intertrochanteric fractures (Boyd and Griffin type-2) treated by modified DHS fixation.

Objectives

1. To assess fracture healing, collapse in management of communited intertrochanteric fracture with modified Dynamic Hip Screw.
2. To look for implant failure- migration of implant, implant loosening, implant cutout or non-union.

Materials and Methods

Study Design: Retrospective observational study population: Patients diagnosed to have intertrochanteric fractures and treated with Modified DHS Study Area : PSG Hospitals.

Inclusion criteria

1. Boyd and griffin classification type-2 fractures only
2. Patients willing for treatment and given written informed consent.

Exclusion criteria

1. Boyd and griffin classification type-1, type-3 and type -4
2. Pathological fractures
3. Infection
4. Treated after 3 weeks of trauma.
5. Patients medically unfit for surgery.
6. Compound fractures associated with vascular injuries, ipsilateral femoral shaft fractures and pelvic fractures.
7. Patients not willing for treatment.

23 patients who were operated with modified DHS for unstable intertrochanteric fracture were selected using random selection and following parameters were noted from the records.

The criteria taken into consideration were fracture healing , collapse of fracture fragments, implant failure

About Modified Dynamic Hip Screw

One of the cause for failure of Dynamic Hip Screw is over collapse at fracture site [8].

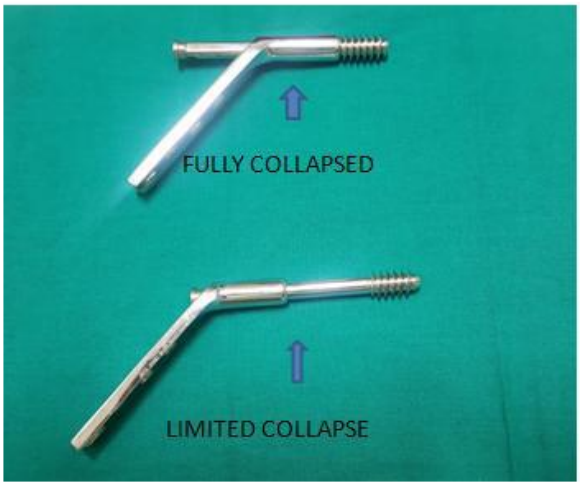
In this study, we have used modified dynamic hip screw to limit the over collapse which is undesired in unstable intertrochanteric fractures. In this implant we have done modification in the shaft of lag screw, we have reduced the length of keyed screw system, so that we can have a maximum of 1 cm collapse, hence there will be limitation in collapse unlike the original implant.



Modified lag screw versus Normal lag screw



Before collapse



After collapse

Results

In this retro-prospective study a total of 23 patients were assessed. Pre-operative

AP radiographs of pelvis was taken for all the patients who were treated by modified dynamic hip screw fixation. All patients who underwent treatment were of age from 38 years to 90 years. Out of 23 patients in this study, 13 patients were male (56.5%) and 10 patients were female (43.5%). Only boyd and griffin type-2 fractures were taken in view of standardisation. Post-operatively follow up xray were taken at 1st month, 3rd month and 5th month and the outcome was assessed.

Factors assessed were

1. Fracture healing
2. Fracture collapse
3. Implant failure

Fracture Healing

1st month 3rd month 5th month

	1st month	3rd month	5th month
Union	8(34.8%)	20(87%)	20(87%)
Non union	15(65.2%)	3(13%)	3(13%)
Total	23(100%)	23(100%)	23(100%)

Out of 23 patients in this study, at first month follow-up, 8 patients showed fracture healing (34.8%), at third month follow-up, 20 patients showed fracture healing (87%), at fifth month follow-up, 20 patient showed fracture healing (87%) and three patient had non-union (13%)

Fracture Collapse

	1st month
Minimal collapse	16(69.6%)
No collapse	7(30.4%)
Total	23(100%)

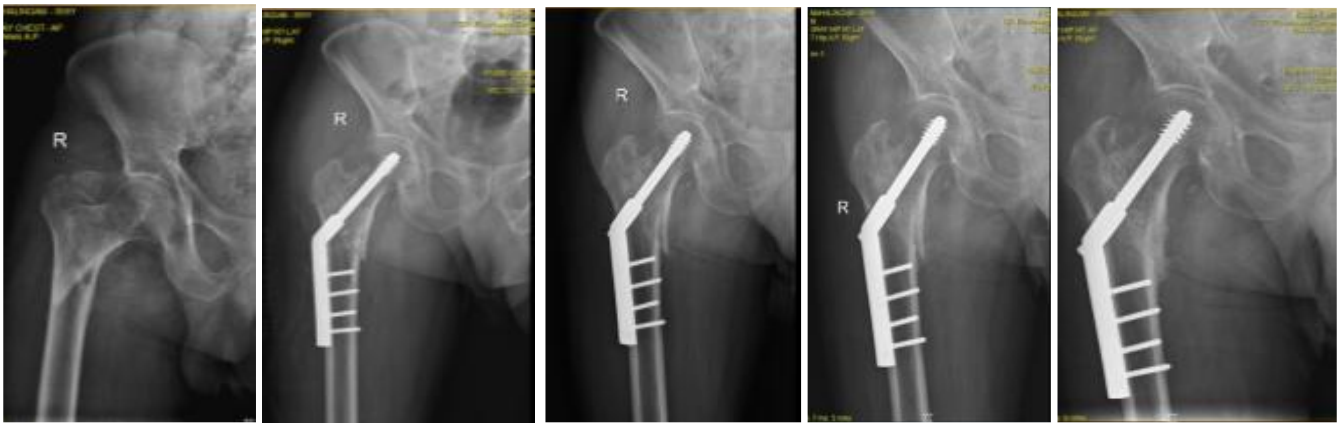
Out 23 patients in this study, at first month follow-up, 7 patients showed no Collapse (30.4%) and 16 patients showed minimal collapse (69.6%) [less than 1 cm], however the collapse rate remained same for all patients till end of this study.

Implant Failure

	1st month	3rd month	5th month
No implant failure	19(82.6%)	19(82.6%)	19(82.6%)
Lag screw migration not breaching cortex	4(17.4%)	4(17.4%)	4(17.4%)

At the end of 5 months followup out of 23 patients, 4 patient had implant migration not breaching cortex (17.3%).

Radiographs



Pre op Post op 1st month 3rd month 5th month

Discussion

Intertrochanteric fractures are mainly treated by surgical intervention. Despite longterm experience in many centres, there have been factors still contributing to pooroutcome of managing unstable intertrochanteric fractures.

Controversies persist because there is lack of proper per-

operative risk factors assessment that affect the outcome in these fractures treated by various methods. Failures of Dynamic hip screw in unstable intertrochanteric fracures are due toover collapse at fracture site [8].

In this study, we have used modified Dynamic Hip screw for treating comminuted intertrochanteric fractures, we have used this implant to limit the over collapse at fracture site.

Fracture united in 20 patients with minimal collapse and 3 patients had no signs of union at the end of 5 months. But all fractures united at the followup period of 1 year, lag screw migration occurred in 4 patients who started early weight bearing.

Nordin S in their study on treatment of intertrochanteric fractures with Dynamic Hip Screw, 83.3 percent of patients had fracture healing at one month [9]. In our seriesonly 34.8 percent of patients showed fracture healing at first month, however at the endof fifth month 87% fractures went on to heal. This delay in fracture healing relates to the modification in design of implant which limits the collapse at fracture site.

Sadowski cal *et al.* in their study on treatment of unstable intertrochanteric fractures with sliding hip screw, implant failure and non-union was noted in 7 of 19patients (38%) who had been treated with the sliding hip screw [10]. In our series, implant migration was noted in 3 out of 23 patients (13%) but no breaching of cortex occured. This results compare favourably and highlights the advantage of modified Dynamic Hip Screw in treatment of communited intertrochanteric fractures.

Conclusion

Modified Dynamic Hip Screw has showed improved results as compared to normal Dynamic Hip Screw in treating communited intertrochanteric fracture.

1. Fracture healing was slow in comparing with regular Dynamic hip screw.
2. Modified Dynamic hip screw significantly limits the collapse at fracture site.
3. When modified Dynamic Hip Screw is used in managed of communited intertrochanteric fracture, weight bearing mobilisation should be delayed to improve the outcome.

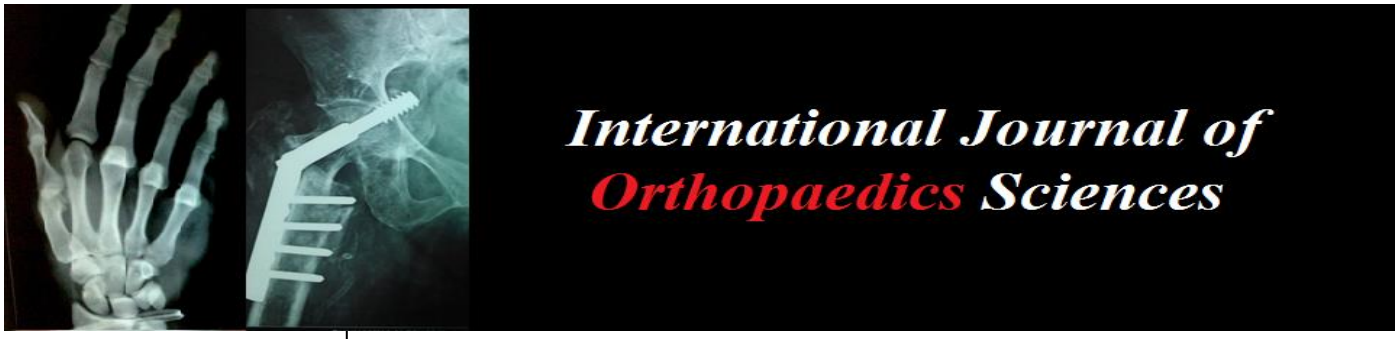
Limitations

Small sample size

Other forms of fixation of trochanteric fractures were not compared in this study.

References

1. Babhulkar S. Management of trochanteric fractures. Indian Journal of Orthopaedics. 2006; 40(4):210
2. Rockwood C, Green D, Bucholz R. Rockwood and Green' fractures in adults, Philadelphia: Lippincott Williams & Wilkins, 2006.
3. Singh A, Thong G, Laloo N, Singh A, Singh S. Management of trochanteric fractures. Indian Journal of Orthopaedics. 2006; 40(2):100.
4. Cooper A. Fractures and Dislocations of Joints.10th ed. London, 1839
5. Bick E. Source Book of Orthopaedics. Newyork: hafner, 1968.
6. Heyse-moore *et al*. treatment of intertrochanteric fractures of femur JBJS. 1983; 65-B:262-267
7. Whitmann R. A new method of treatment for fractures of the neck of the femur, together with remarks on coxavara. Ann Surg. 1902; 36:746.
8. Mohan N, Chandrashekar H. An Analysis of Failure of Dynamic Hip Screw with Plate in the Management of Intertrochanteric (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. 2014; 13(3, IV):54-57
9. Nordin S, Mechanical fixation of DHS in intertrochanteric fracture Med J Malaysia. 2001; 56(D):12-7.
10. Sadowski CAL. Treatment of reverse oblique and transverse interrochanteric fractures with use of an intramedullary nail or a 95 deg screw. JBJS Am. 2002; 84;372-381
11. Capener N. The treatment of pertrochanteric fractures. JBJS Br. 1957; 39-b(3):436-437.
12. Taylor MN, Janzen J. Internal fixation for intertrochanteric fractures JBJS Am. 1944; 26(4):707-712.
13. Boyd HGLL. Classification and treatment of trochanteric fractures. Ach Surg. 1949; 58:853-863.
14. Boyd H, Anderson LD. Management of unstable trochanteric fractures. Surggynecol Obstrtrics 1961; 55:853-863.
15. Bendo JA, Weiner LS, Strauss E *et al*. Collapse of interochanteric hip fractures fixed with sliding screws. orthop Rev, 1944, 30-37.
16. Dimon JHJC. Unstable intertrochanteric fravtures. JBJSAm. 1967; 49(3):440-450.
17. Harrington KJ. The management of communited unstable intertrochanteric fracture. JBJS Am. 1973; 55(7):1367-1376
18. Sarmiento A. Intertrochanteric fractures of femur. JBJS. 1995; 75(3):90-92
19. Desjardins Ara, Paiement G Newman N, *et al*. Unstable intertrochanteric fracture of femur. JBJS Br. 1993; 75(3):445-447.
20. Gargan MGR, Simpson Ah. How effective are osteotomies for unstable intertrochanteric fractures



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□□r □d □□□□□□□Osteoporosis is a major health problem which has devastating health consequences through its association with osteoporotic fractures. Prevention of osteoporosis by identifying the risk factors is a major challenge in the field of medical science. Elevated homocysteine level in blood can be a potential risk factor for the development of osteoporosis. We aim to study if a person with high circulating level of homocysteine has a decreased Bone Mineral Density (BMD), thus establishing an association between homocysteine and the risk of developing osteoporosis.

M□□□□d□Patients between the age group of 40-70 years attending BMD camps between July 2019 and December 2019 were included in the study. All of them underwent BMD test and blood samples were sent to the laboratory for estimation of serum homocysteine levels. The results were collected and analyzed to see if there was any association between serum homocysteine levels and osteoporosis.

□ □□□□□□□Out of the 58 males and 20 females with normal BMD, none had elevated serum homocysteine. 21 out of the 58 males and 47 out of the 82 females with osteopenia had elevated serum homocysteine. Of the 27 males with osteoporosis, 25 had elevated serum homocysteine while among the 125 females with osteoporosis, all 125 had elevated serum homocysteine levels.

□ □□□□□□□□□□From our study we concluded that people with high circulating level of homocysteine had a decreased Bone Mineral Density (BMD), thus establishing an association between homocysteine and the risk of developing osteoporosis.

□ □□□ □rd □□Homocysteine, osteoporosis, BMD, serum homocysteine levels

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Osteoporosis is a major health problem characterized by low bone mineraldensity (BMD) and increased risk of fractures [1]. Osteoporotic fractures are associated with increased morbidity and mortality & cause substantial financial loss to the patients and their families [2, 3, 4].

Elevated levels of homocysteine in the blood is associated with an early onset of osteoporosis [5, 6]. An elevated plasma homocysteine level (>15 mmol/l) is prevalent in 30-50% population above 60 years [5, 6]. High homocysteine levels have been associated with an increased risk of fractures, although the factors that contribute to this fracture risk are not fully elucidated.

Homocysteine is an intermediary amino acid formed by the conversion of methionine to cysteine. Elevated serum homocysteine levels show clinical manifestations involving the eyes, blood vessels, nervous system and skeleton including the development of early osteoporotic fractures. Vitamin B12 and folic acid play an important role in homocysteine metabolism [7]. They are cofactors in homocysteine metabolism and their intake can help in reducing serum homocysteine levels [8, 9, 10].

We aim to study if a person with high circulating level of homocysteine has a decreased Bone Mineral Density (BMD), thus establishing an association between homocysteine and the risk of developing osteoporosis.

M□□□□d □□

The study population included patients between the age group of 40-70 years attending BMD camps organized by the department of Orthopaedics, PSG institute of medical science and research, coimbatore between July 2019 and December 2019. Both male and female patients were included in the study.

Patients with previous fractures and those on long term steroids were excluded from the study.All of them underwent BMD test by DEXA scan. Blood samples were collected and sent to the laboratory for estimation of serum homocysteine levels.

M r r **d**
Normal: 1 to -1
Osteopenia: -1 to -2.5
Osteoporosis: Below -2.5
The normal serum homocysteine level: 15 mmol/L
The results of both BMD scan and serum homocysteine levels were collected and analyzed to see if there was
any association between serum homocysteine levels and osteoporosis.

During the study period of 6 months, 370 patients attending the BMD camps fell into our inclusion criteria and wereincluded in our study. Of them 143 were males and 227 were females (Table 1).

1 Age Distribution

r	**M**	
40 – 50	43	61
51 – 60	73	112
61 – 70	27	54
Total	143	227

The average BMD in the age group of 40-50 years was -0.72 in males and -1.6 in females. In the 51-60 years age group it was -1.47 in males and -2.53 in females. The average in 61-70 years age group was -2.61 in males and -3.4 in females (Table 2).

2 Average BMD levels

r	**M**	
40 – 50	-0.72	-1.6
51 – 60	-1.47	-2.53
61 – 70	-2.61	-3.4

In the patients with normal BMD the average serum homocysteine level was 5.4 mmol/L in males and 7.2 mmol/L in females. Osteopenic individuals showed an average serum homocysteine level of 14.1 mmol/L in males and 16.2 mmol/L in females. Those with osteoporosis showed average serum homocysteine level of 38.7 mmol/L in males and 46.1 mmol/L in females (Table 3).

Average serum homocysteine levels

M	**M**	
Normal	5.4	7.2
Osteopenia	14.1	16.2
Osteoporosis	38.7	46.1

Of the 43 males in the 40-50 years age group, 7 were osteopenic and the rest had normal BMD, while among the 61 females in the same age group, 35 were osteopenic and 6 were osteoporotic. Among the 73 males in the 51-60 years age group, 46 were osteopenic and 5 were osteoporotic, while among the 112 females in the same age group, 47 were osteopenic and 65 were osteoporotic. Out of the 27 males in the 61-70 years age group, 5 were osteopenic and 22 were osteoporotic, while among the 54 females in the same age group all 54 were found to have osteoporosis (Table 4).

Age and sex wise distribution of BMD results.

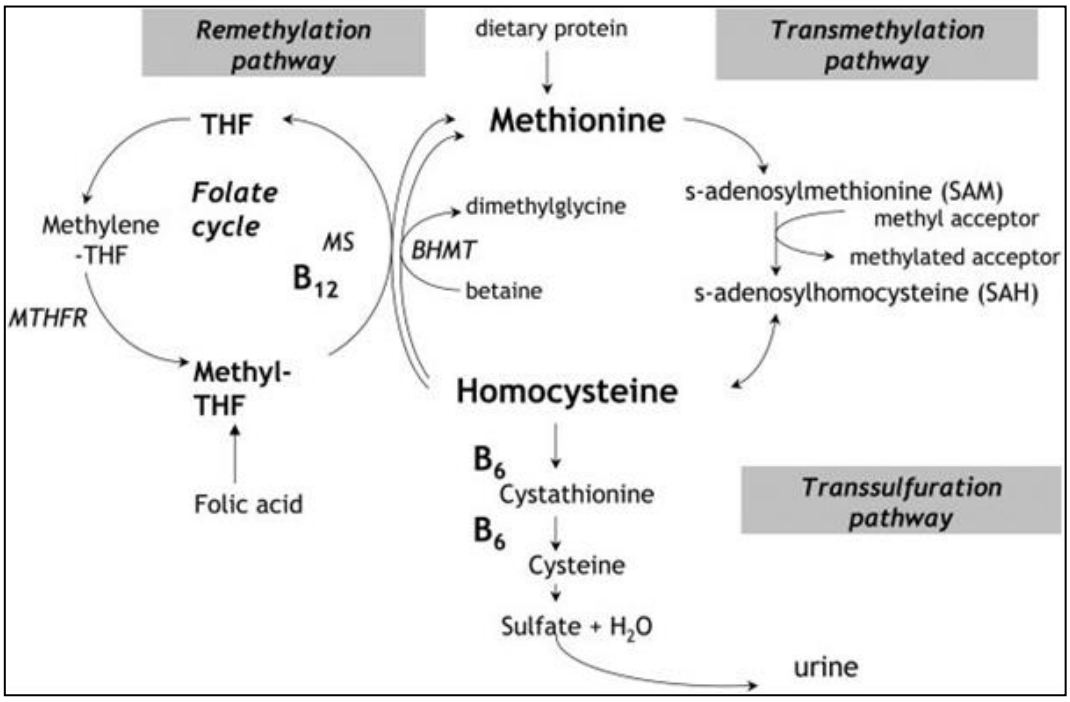
	0 – 0 r		1 60 r		61 0 r	
M	**M**		**M**		**M**	
Normal	36	20	22	Nil	Nil	Nil
Osteopenia	7	35	46	47	5	Nil
Osteoporosis	Nil	6	5	65	22	54
Total	43	61	73	112	27	54

Out of the 58 males and 20 females with normal BMD, none had elevated serum homocysteine. 21 out of the 58 males and 47 out of the 82 females with osteopenia had elevated serum homocysteine. Of the 27 males with osteoporosis, 25 had elevated serum homocysteine while among the 125 females with osteoporosis, all 125 had elevated serum homocysteine levels (Table 5).

Relation between BMD and elevated serum homocysteine.

	M			
M	**M**	d	**M**	d
Normal	58	Nil	20	Nil
Osteopenia	58	21	82	47
Osteoporosis	27	25	125	125
Total	143	46	227	172

Osteoporosis is a metabolic bone disease. It is the leading cause of fractures with advancing age leading to pain, hospitalization and increased financial burden to the patient and his family. Even though it is considered as a normalageing process, osteoporosis can be prevented and treated if detected early. Elevated serum homocysteine level or hyperhomocysteinemia is considered to be one of the possible modifiable risk factors of osteoporosis.
Homocysteine is a sulfur containing amino acid that is formed by methionine metabolism. It is metabolized in the body by remethylation or trans-sulfuration pathway (Fig.1).
The normal serum homocysteine level is considered to be below 15mmol/L. A raised serum homocysteine level can bedue to multiple factors – lifestyle, diet, hormonal [11]. Hyperhomocysteinemia is considered to be a potential risk factorfor the development of osteoporosis [12, 13]. Vitamin B12 acts as a co-factor in the remethylation of homocysteine to methylene [14]. Hence vitamin B12 deficiency can lead to elevated serum homocysteine levels.
A study done by van Meurs *et al.* concluded that elevated serum homocysteine levels lead to a 2 fold increase in the incidence of fractures [15]. Another study by Bucciarelli *et al.* showed inverse association of serum homocysteine and BMD in post- menopausal women [16]. Tyagi *et al.* concluded that hyperhomocysteinemia can lead to reduced blood flowin the bones compromising bone quality [17]. A study done by Enneman *et al.* in 2014 showed that elevated serum homocysteine was associated with a decreased BMD [18].
Similarly in our study we found an inverse correlation between serum homocysteine levels and BMD. Out of the 58 malesand 20 females with normal BMD, none had elevated serum homocysteine. 36% males (21 out of 58) and 57% females (47 out of 82) with osteopenia had elevated serum homocysteine levels. However when it came to people with osteoporosis on BMD, 93% males (25 out of 27) and 100% of females (125 out of 125) had elevated serum homocysteine levels. The average serum homocysteine levels in osteoporotic individuals were 38.7 mmol/L in males and46.1 mmol/L in females. Hence we see a definite relation between elevated serum homocysteine level and osteoporosis



□□□1 □□Homocysteine metabolism pathway□

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Osteoporosis is a major health problem which has devastating health consequences through its associationwith osteoporotic fractures. Prevention of osteoporosis by identifying the risk factors is a major challenge in the field of medicine. Bone metabolism is affected by homocysteine. Elevated homocysteine level in blood isa potential risk factor for the development of osteoporosis.

From our study we concluded that people with high circulating level of homocysteine had a decreased Bone Mineral Density (BMD), thus establishing an association between homocysteine and the risk of developing osteoporosis. Vitamin B12 and folic acid play an important role in homocysteine metabolism [7].

Supplementation with vitamin B12 and folic acid has been shown to normalize plasma homocysteine levels. This could reverse the problem of impaired bone health
and osteoporosis and help in preventing osteoporotic

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1. Consensus Development Conference on Osteoporosis. Hong Kong, April 1–2, 1993. Am J Med. 1993; 95(5A):1S-78S.
2. Ray NF, Chan JK, Thamer M, Melton LJ. Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995: report from the National Osteoporosis Foundation. J Bone Miner Res. 1997; 12:24-35.
3. Melton LJ. Adverse outcomes of osteoporotic fractures in the general population. J Bone Miner Res. 2003; 18(6):1139-41.
4. Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA. Mortality after all major types ofosteoporotic fracture in men and women: an observational study. Lancet. 1999; 353:878-82.
5. Harpey JP, Rosenblatt DS, Cooper BA, Le Moel G, Roy C, Lafourcade J *et al.* Homocystinuria caused by5,10-methylenetetrahydrofolate reductase deficiency: a case in an infant responding to methionine, folinic acid, pyridoxine, and vitamin B12 therapy. J Pediatr. 1981; 98:275-8.
6. Mudd SH, Skovby F, Levy HL *et al.* The natural history of homocystinuria due to cystathionine betasynthase deficiency. Am J Hum Genet. 1985; 37:1-31.
7. A de Bree, NM van der Put, LI Mennen *et al.* Prevalences of Hyperhomocysteinemia, unfavourable cholesterol profile and hypertension in European populations, European Journal of Clinical Nutrition. 2005; 59(4):480-488.
8. W Wouters-Wesseling, AEJ Wouters, CN Kleijer, JG Bindels, CPGM de Groot, WA van Staveren, Study of the effect of a liquid nutrition supplement on the nutritional status of psycho-geriatric nursing home patients," European Journal of Clinical Nutrition. 2002; 56(3):245-251.
9. R. Clarke, Lowering blood homocysteine with folicacid based supplements: meta-analysis ofrandomised trials," British Medical Journal. 1998; 316(7135):894-898.
10. AM Kuzminski, EJ Del Giacco, RH Allen, SP Stabler, J Lindenbaum. Effective treatment of cobalamin deficiency with oralcobalamin, Blood. 92(4):1191-1198,
11. Eussen SMP, De Groot PMG, Clarke R *et al.* Oral cyanocobalamin supplementation in older people with vitamin B 12 deficiency: a dose-finding trial. Arch Int Med. 2005; 165(10):1167-1172.
12. McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE *et al.* Homocysteine as a predictive factor for hip fracture in older persons. N Engl J Med. 2004; 351:2042–2049.
13. Hermann M, Schmidt PJ, Umanskaya N, wagner A, Taban-Shomal, widmann T *et al.* The role of Hyperhomocysteinemia as well as folate, vitamin B(6) and B(12) deficiencies in osteoporosis: a systematic review. Clin Chem. Lab Med. 2007; 45:1621-1632.
14. Clark R, Refsum H, Birks J *et al.* Screening for vitamin B 12 and folate deficiency in older persons.Am J ClinNutr. 2003; 77:1241-1247.
15. VanMeurs JBJ, Dhonukshe-Rutten RAM, Pluijm SM, van der Klift M, de Jonge R, Lindemans J *et al.* Homocysteine levels and the risk of osteoporotic fracture.

N Engl J Med. 2004; 350:2033-2041.
16. Bucciarelli P, Martini G, Martinelli I *et al.* The relationship between plasma homocysteine levels and bone mineral density in post-menopausal women.Eur J Int Med. 2010; 21(4):301-30.
17. Tyagi N, Vacek TP, Fleming JT, Vacek JC, Tyagi SC. Hyperhomocysteinemia decreases bone blood flow. Vasc Health Risk Manag. 2011; 7:31-35.
18. Enneman WA, Swart MK, Zillikens CM *et al.* The association between plasma homocysteine levels and bone quality and bone mineral density parameters in older persons. Bone. 2014; 63:141-146.

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Correlation between magnetic resonance imaging and arthroscopy in internal derangement of knee

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ABSTRACT

Background: Ligament injuries apart from fractures are more common in the knee joint owing to its complex anatomy. They account for a large number of referrals to our hospital, both from the general practitioner and from the accident and emergency department. Although there have been studies in literature comparing MRI with arthroscopy, the continuing improvement in diagnostic methods now available makes it especially important to compare the results and recommendations offered in the literature.
Methods: The aim of this study was to find out the diagnostic accuracy of MRI scans and to examine the value of MRI as a standard preoperative examination correlating them with the gold standard of arthroscopy. This is a prospective study involving 57 patients who were admitted in Department of Orthopaedics, Mahatma Gandhi Medical College and Research Institute, with the history of injury to the knee and diagnosed to have internal derangement of the knee clinically, using standardized clinical tests. MRI of the affected knee joint was done for all these patients either before or after admission. The patients were then subjected to diagnostic and therapeutic arthroscopy.
Results: The sensitivity of MRI in comparison with Arthroscopy was 100% in all studied lesions. The specificity of MRI in comparison with Arthroscopy was 94.1%, 98.1%, 100% and 97.6% for ACL, PCL, Medial meniscus and Lateral meniscus respectively.
Conclusions: According to our study MRI is a very good at determining the normal anatomy of the intra articular structures of the knee joint and is highly reliable in excluding pathology, in our case tear in ligaments. Hence we recommend MRI in doubtful cases of internal derangement of the knee joint whereby unnecessary diagnostic arthroscopy can be avoided which can significantly bring down the economic burden among rural population.

Keywords: MRI, Arthroscopy, IDK

INTRODUCTION

The dramatic increase in road traffic accidents and the highly demanding sporting life style makes the knee joint one of the most commonly injured joints in the body, either as a frequent component in a polytrauma patient or as isolated injury. Ligament injuries apart from fractures are more common in the knee joint owing to its complex anatomy. They account for a large number of referrals to our hospital, both from the general practitioner and from the accident and emergency department.

The knee is a complex joint, consisting of two condylar joints between the corresponding condyles of femur and tibia and a sellar joint between the patella and femur.[1] The principal intraarticular structures in knee are the two menisci, the two cruciate ligaments, and the two collateral ligaments. The menisci serve to distribute joint fluid, cartilage nutrition, mechanical shock absorption, increasing the surface area of the joint and therefore the stresses, serve to stabilize the joint, and a weight bearing function. The cruciate ligaments function as stabilizers of the knee in both forward and backward motions of the

tibia on the femur and provide an axis around which both medial and lateral rotary movements are assisted.[2] The injury to these intraarticular structures is generally termed as "Internal derangement of knee" which was first coined by William Hey in 1784.[3]

The clinical evaluation of knee injuries remains a difficult problem even today. The accuracy of a clinical diagnosis, reported in various series, varies between 64-85 percent, which suggests that even in the most experienced hands, a clinical diagnosis cannot be ascertained in about 20 percent of cases.[4]

Magnetic resonance imaging (MRI) is a diagnostic method most often used in diagnosis of internal derangements of the knee, because it is non-invasive, painless and has no risk of radiation. However it's an expensive investigation and it has a tendency to be misused and overused, to confirm diagnosis before proceeding with surgical intervention. The accuracy rate of MRI scans also varies. As knee injuries are on the rise and with varied accuracy rates of scans, it would be useful to know the accuracy of the MRI findings and correlate this with arthroscopy findings.

Arthroscopy has been used for many years as a diagnostic and therapeutic tool in knee disorders. It is considered a gold standard for the same as it allows direct visualization of the interior of the knee. Although there have been studies in literature comparing MRI with arthroscopy, the continuing improvement in diagnostic methods now available makes it especially important to compare the results and recommendations offered in the literature.

The aim of this study was to find out the diagnostic accuracy of MRI scans and to examine the value of MRI as a standard preoperative examination correlating them with the gold standard of arthroscopy.

METHODS

The aim of this study was to study the efficacy of Arthroscopy over MRI in diagnosing meniscal and ligament injuries of the knee joint; to compare the sensitivity and specificity of MRI and knee arthroscopy & to emphasize the accuracy of diagnosis in knee arthroscopy.

Inclusion criteria

Patients between 18-45 years of age with knee pain with or without instability and patients with symptoms of locking of knee were included in the study.

Exclusion criteria

Patients with open fractures of the knee, patients with bony injuries of the affected limb, those below the age of 18yrs & above the age of 45 years, patients with previous surgeries to the knee, patients with signs of infection, patients with ankylosed knee joint and those unfit for MRI study were excluded from the study.

This is a prospective study involving 57 patients who were admitted in Department of Orthopaedics, Mahatma Gandhi Medical College and Research Institute, with the history of injury to the knee and was diagnosed to have internal derangement of the knee clinically, using standardized clinical tests. MRI of the affected knee joint was done for all these patients either before or after admission. The patients were then subjected to diagnostic and therapeutic arthroscopy. MRI was done in 1.5T field strength in our institution and was reported on an objective proforma by a single senior consultant radiologist. All the arthroscopies were performed by a single orthopaedic surgeon.

The findings of MRI and arthroscopy were compared and analyzed in detail. It is a prospective comparative study done in the Department of Orthopaedics, Mahatma Gandhi Medical College & Research Institute, Pondicherry from May 2010 to September 2012.

Methods of assessment

Prior to surgery

1. Presenting complaints, history of presenting complaints, past history, personal history, general physical examination and complete local examination of affected knee were done that included complete inspection, medial joint line tenderness, McMurrays test, Apleys grinding test, Lachmann's test, anterior and posterior drawer test, pivot shift & Mcintosh test.

2. Radiographs of the involved knee anteroposterior and lateral views to rule out any bony injury.

MRI of the affected knee with the following sequences

- Localizer sequences in sagittal, coronal and axial planes
- Fat suppressed T2 axial turbo spin echo
- T1 Spin echo Sagittal.

Preoperative workup

- Routine hemogram, urine routine, biochemical parameters of blood, ECG & chest radiographs.

Preanaesthetic check-up and ASA grading for fitness for surgery

Surgery

All the arthroscopic procedures were performed under spinal anaesthesia after applying pneumatic tourniquet with patient in supine position and knee in 90 degrees

flexion. 30° arthroscope was used in all cases and the operative findings were documented and recorded simultaneously by the floor assistant: anatomical structure viewed and the presence or absence of tears, its location and additional details wherever possible.

The composite data was tabulated and studied for correlation with MRI findings and grouped into four categories.

- True-Positive: If the MRI diagnosis was confirmed by arthroscopic evaluation.
- True-Negative: When MRI negative for lesion but arthroscopy was negative.
- False-Positive: When MRI shows lesion but arthroscopy was negative.
- False-Negative: When arthroscopy showed lesion but MRI was negative.

Statistical analysis was used to calculate the sensitivity, specificity, positive predictive value (PPV) and the negative predictive value (NPV), in order to assess the reliability of MRI results. Based on the above categories, five parameters were calculated to assess the reliability of the MRI results.

Sensitivity

Sensitivity of MRI is the ability of the MRI to detect an abnormality. It is determined by the equation:

True-positive/ (true positive + false negative) × 100 per cent.

Specificity

Specificity of MRI is the ability of MRI to give how many detected tears are usually accurate. It is determined by the equation:

True-negative/ (true negative + false positive) × 100 per cent.

Positive predictive value

It correlates a positive result of MRI with findings at arthroscopy. It is calculated by the equation:

True-positive/ (true positive + false positive) × 100 per cent.

Negative predictive value

It correlates a negative result on MRI with the findings at arthroscopy. It is calculated by the equation:

True-negative/ (true negative + false negative) × 100 per cent.

Accuracy

It is given by the equation:

True positive + true negative/ total number of patients in the study.

Statistical analysis

Collected data was presented in the form of tables. Data was analyzed for the significant correlation between MRI knee and arthroscopic findings. Pearson correlation coefficient and T test used as statistical tool to analyze the data in our study.

Interpretation of sensitivity

Table 1: Interpretation of sensitivity.

Percentage	Grade
90% - 100%	Excellent
80% - 90%	Very Good
70% - 80%	Good
70% - 60%	Average
< 60%	Poor

RESULT

Sex distribution

The study had 57 patients of which 15 were females and 42 males which accounts to about 26.3% females and 73.7% males respectively as given in Table 2.

Table 2: Sex distribution.

Sex	No of cases	Percentage
Male	42	73.7
Female	15	26.3

Age distribution

The patients who suffered injury were in the age group ranging from 18 to 45years. The mean age was around 33.58. Some of the other interesting observations noted in our study are, as age increases right side injuries are more compared to that of the left side and frequency of road traffic accidents are more.

There was an increase in frequency of knee injuries as age advanced in our study, 25 cases were in the age group of 18-27 years, 18 cases were in the age group of 28-37 years and 14 cases were in the age group of 18-27 years.

Side involved

The right knee joint was found to be more commonly involved 35 cases (61.4%), than the left knee joint, 22

cases (38.6%) and there were no cases with bilateral knee involvement in our series as shown in Table 3.

Table 3: Side involved.

	Frequency	Percentage
Left	22	38.6
Right	35	61.4
Total	57	100

Mode of injury

Road traffic accident was the most common mode of injury involving 47 cases (82.46%) followed by sport injury involving 10 cases (17.54%) as given in Table 4.

Table 4: Mode of injury.

	Frequency	Percentage
Sports injury	10	17.5
RTA	47	82.5
Total	57	100

Structures injured

There are several explanations for the misleading results of MRI regarding the menisci and cruciate ligaments. Firstly, meniscal tears and meniscus degenerative changes have the same appearance in MRI, by giving high signal within the meniscus. Diagnosis then depends on the expansion of the high signal line towards meniscus articular surface.

Table 5: Structures injured.

Structure injured	MRI	Arthroscopy
ACL	41	40
PCL	6	5
Medial meniscus	32	32
Lateral meniscus	16	15

Moreover, one of the most frequent causes for false positive MRI regarding the lateral meniscus is the misinterpretation of the signal coming from the inferior knee artery. McKenzie et al summarized the four most common reasons for false positive diagnosis; wrong diagnosis due to variable anatomic structures, overestimation of pathology countered as meniscus tear (for example chondral injuries that mimic meniscus tears) false negative arthroscopic findings and tears within the meniscus without expansion to the articular surface.

Statistical analysis of tear of individual structures

Anterior cruciate ligament

Observing the pattern of ACL tears revealed a total of 40 cases with torn ACL shown in Table 6.

Table 6: Comparing ACL in MRI vs. Arthroscopy.

MRI ACL	Scopy ACL		
	Tear	Normal	Total
Tear	40	1	41
Normal	0	16	16
Total	40	17	57

In our study there was one false positive result in MRI while diagnosing anterior cruciate ligament tear.

The sensitivity and specificity of MRI with respect to Arthroscopy is 100% and 94.1%.

The positive predictive value and negative predictive value is 97.6% and 100% respectively.

Posterior cruciate ligament

Total number of PCL tears accounted to about 5 cases out of the 57 studied as shown in Table 7.

Table 7: Comparing PCL in MRI vs. arthroscopy.

MRI PCL	Scopy PCL		
	Tear	Normal	Total
Tear	5	1	6
Normal	0	51	51
Total	5	52	57

In our study there was one false positive result in MRI while diagnosing posterior cruciate ligament tear, the loss of signal intensity near the femoral attachment was considered as partial tear of PCL but on arthroscopic evaluation there was no evidence of tear in PCL.

The sensitivity and specificity of MRI with respect to Arthroscopy is 100% and 98.1%.

The positive predictive value and negative predictive value is 83.3% and 100% respectively.

Medial meniscus

Out of the 57, 32 cases had torn medial meniscus and 25 normal medial meniscus as shown in Table 8.

Table 8: Comparing medial meniscus in MRI vs. arthroscopy.

MRI M.MEN	Scopy M. MEN		
	Tear	Normal	Total
Tear	32	0	32
Normal	0	25	25
Total	32	25	57

In our study there were no discrepancies in the diagnosis of tears in medial meniscus between MRI and

Arthroscopy. Both these modalities correlated well in the diagnosis of medial meniscal tears.

The sensitivity and specificity of MRI with respect to Arthroscopy is 100% and 100%.

The positive predictive value and negative predictive value is 100% and 100% respectively.

Lateral meniscus

Total number of lateral meniscal tears reported is 15 as shown in Table 9.

Table 9: Comparing lateral meniscus in MRI vs. arthroscopy.

MRI L.MEN	Scopy L. MEN		
	Tear	Normal	Total
Tear	15	1	16
Normal	0	41	41
Total	15	42	57

In our study there was 1 false positive result in the diagnosis of tears in lateral meniscus between MRI and Arthroscopy. The sensitivity and specificity of MRI with respect to Arthroscopy is 100% and 97.6% respectively.

The positive predictive value and negative predictive value is 97.6% and 100% respectively.

DISCUSSION

This study was a prospective study done among 57 patients who were admitted with provisional diagnosis of Internal Derangement of Knee in the Department of Orthopaedics, Mahatma Gandhi Medical College and Research Institute, Pondicherry

The current study was done to determine the efficacy of Arthroscopy over MRI in diagnosing meniscus and ligament injuries of the knee joint.

MRI of the knee joint was done in all these patients and then these patients underwent diagnostic and therapeutic arthroscopy whenever necessary in the same institution.

The main strength of the study is the use of only one MRI machine Philips 1.5 Tesla and interpretation of examination by a single radiologist, thus making the results more reproducible. This is in congruence with the analysis done by Runkel et al which showed arthroscopies done could be reduced if the MRI was reported by an experienced radiologist .[5]

MRI images were studied for evidence of injuries to menisci and cruciate ligaments and other associated structures and soft tissues around the knee joint. Diagnostic arthroscopy was performed on all these patients to confirm the MRI findings and results were documented.

In the present study, of the 57 patients 42 were male and 15 were female patients. The age groups were ranging from 18 to 45 years with mean age of 33.58. The youngest patient was 18 years and the oldest was 45 years of age. The youngest male patient was aged 18 years and the oldest male patient was 45 years old, likewise the youngest female patient was aged 18 years and the oldest female patient was 45 years old. This showed that the tendency of being injured and getting operated at an earlier age was common in both male and female patients.

A study by Munk et al showed males are most likely to suffer knee injuries since they are active in sports and the right knee was more frequently injured than the left knee.[6] As mentioned earlier in our study also males comprise the predominant number of patients who suffered knee injuries owing to their highly active social and sporting life style.

Sports injuries were more common in male patients than in females, out of the 10 cases who suffered injuries in this mode there was only 1 female patient in this category. The overall percentage of sports injuries were 17.5% (10 cases) when compared to 82.5% (47 cases) who sustained injury through road traffic accident.

Meniscal injuries, anterior cruciate ligament and posterior cruciate ligaments injuries were classed as either torn or not torn.

Anterior cruciate ligament injures occurred in about 40 patients (70.2%). Posterior cruciate ligament injuries occurred in 5 patients (8.8%). The frequencies of injuries to medial meniscus (56.1%), in 32 patients were almost one fold higher than that of injuries to lateral meniscus (26.3%), in 15 patients.

False positive and false negative results

MRI studies have higher false positives than false negative results confirmed by literature and it was the same finding in our study too, even though the false positive results were very minimal which accounts to only 3 cases out of the 57 cases evaluated. Each of the false positive results was encountered in ACL, PCL and Lateral meniscus respectively whereas there were no discrepancies in Medial meniscal lesions.

The false positive results in our study are described below:

- The reported degenerative tear of a lateral meniscus was not visualized in arthroscopic examination.
- A reported right side ACL tear in MRI showed mild laxity and increased signal intensity of ACL near its tibial attachment which was interpreted as partial ACL tear, but

in arthroscopic examination there was no evidence of tear at the given location.

- Posterior Cruciate ligament of the right knee in a patient showed an intrasubstance cyst / tear in MRI, was not revealed in arthroscopic examination.

Sensitivity and specificity

The sensitivity of MRI in comparison with Arthroscopy was 100% in all studied lesions.

The specificity of MRI in comparison with Arthroscopy was 94.1%, 98.1%, 100% and 97.6% for ACL, PCL, Medial meniscus and Lateral meniscus respectively.

Positive and negative predictive value

Statistics revealed MRI has 100% negative predictive value with a variable positive predictive value of 97.6%, 83.3%, 100%, and 97.6% for ACL, PCL, medial meniscus and lateral meniscus respectively.

Thus according to our study MRI is a very good at determining the normal anatomy of the intra articular structures of the knee joint and is highly reliable in excluding pathology, in our case tear in ligaments. Hence we recommend MRI in doubtful cases of internal derangement of the knee joint whereby unnecessary diagnostic arthroscopy can be avoided which can significantly bring down the economic burden among rural population.

CONCLUSION

The use of MRI and arthroscopy of the knee has evolved substantially over the last several decades and the advancement in surgical treatment of traumatic ligament injuries of the knee has been improved because of both technologies. The astute orthopaedic surgeon must be able to associate the findings on MRI in the decision making before and during arthroscopy. Moreover for a better correlation of findings the surgeon has to go through the complete set of images available in all possible views to come to a definitive conclusion on the pathology. An accurate understanding of the surgical anatomy and pathology found on both clinical examination and preoperative imaging will help the surgeon to improve the surgical technique at the time of arthroscopy and ultimately improve patient outcomes.

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Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Williams PL, Warwick R. Arthrology. In: Gray's anatomy. 36th ed. Edinburgh, New York: Churchill Livingstone; 1980: 482.
2. Robert H, Miller III, Azar FM. Knee injuries. In: Canale ST, Beaty JH, editors. Campbell's Operative Orthopaedics, 11th edition. Mosby Elsevier; 2008: 2395-2600.
3. Hughston JC. Acute knee injuries in athletes, Clin Orthop. 1962;23:114-33.
4. Kumar V, Hui AC. A Comparative Study of MRI and Clinical Findings in Diagnosis of Internal Derangements of the Knee. J Orthopaedics. 2005;2(4):5.
5. Runkel M, Kreitner KF, Regentrop HJ, Kersjes W. Sensitivity of magnetic resonance tomography in detecting meniscus tears. Unfallchirurg. 2000;103:1079-85.
6. Munk B, Madsen F, Lundorf E, Staunstrup H, Schmidt SA, Bolvig L, et al. Clinical magnetic resonance imaging and arthroscopic findings in knees: a comparative prospective study of meniscus anterior cruciate ligament and cartilage lesions. Arthroscopy. 1998;14:171–5.

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Research Article
Orthopaedics

A STUDY OF CLINICAL AND RADIOLOGICAL OUTCOME OF TIBIAL PLATEAU FRACTURES TREATED WITH MINIMALLY INVASIVE PERCUTANEOUS PLATE OSTEOSYNTHESIS (MIPPO)

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Article submitted on: ****
Article Accepted on: ****

Abstract:

Background: Proximal tibial fractures involve a major weight-bearing joint and are serious injuries that frequently result in functional impairment. Treatment of proximal tibial fractures remains a challenge (intra articular and metaphyseal fractures) because the tibia is more superficial and has very little soft tissue cover. Minimally invasive percutaneous plate osteosynthesis (MIPPO) has been widely used in the treatment of long bone fractures in the recent years because of its technical advantages and satisfactory clinical outcome.

Methods: This study is conducted to evaluate the clinical and radiological outcome of proximal tibial fractures treated with minimally invasive percutaneous plate osteosynthesis (MIPPO). The plate is inserted by a percutaneous approach hence minimising the soft tissue disruption, periosteal injury, also preserves the fracture integrity and vascular integrity of bony fragments. MIPPO is a method that combines both principles of the biomechanical properties of fixation and an optimum bone-to-implant contact. Despite a plethora of articles, written in the past 50 years that have addressed the problems of classification and results of various treatments the optimal method of management remains controversial. It is a prospective study of 26 patients admitted in Mahatma Gandhi Medical College and Research Institute,Pondicherry between May 2010 and March 2012 with the diagnosis of tibial plateau fractures treated with MIPPO technique.

Results : 84% of our patients had excellent and good results in the functional outcome. These results were attributed to patients who underwent early surgery and early mobilisation. The average time taken for fracture healing was 15.34 weeks.

Conclusion: MIPPO technique gives good to excellent results even in high energy tibial condyle fractures (84% cases in our study) .Our patients were able to achieve a good functional range of movement, averaging 120 degrees. Those who were treated with early fixation and early mobilization were found to have a better functional outcome irrespective of the fracture type.

Key words: *MIPPO, Proximal Tibia Fracture, Tibial Plateau Fracture.*

Introduction

Proximal tibial fractures involve a major weight-bearing joint and are serious injuries that frequently result in functional impairment. They were originally described as "the fender fracture" because they resulted from low-energy pedestrian versus car accidents. In recent literature, majority are reported from high energy motor vehicle accidents, fall from height and industrial accidents. They can also be caused by sports injuries in younger individuals whereas less violent trauma produce them in elderly osteopenic/ osteoporotic patients. Proximaltibial fractures result from indirect coronal or direct axial compressive forces and comprises of 1% of all fractures and 8% of fractures in the elderly.[1,2] Treatment of proximal tibial fractures remains a challenge (intra articular and metaphyseal fractures) because the tibia is more superficial and has very little soft tissue cover.

Proximal tibial fractures are difficult to treat because of the involvement of articular surface, the often occurring comminution, and the precarious condition of the soft tissues, especially following high-energy trauma. Conservative treatment at any age may be complicated by knee stiffness, malunion,etc., whereas conventional operative treatment (open reduction and internal fixation) can lead to high rate of complications like, compartment syndrome,skindehi scence, infection and septic arthritis.

Aim of the treatment is to restore the congruence of the articular surface supporting the tibial plateau cartilage which is usually depressed; to fix the fracture with a stable device; to allow early rehabilitation. Conventional plating techniques remain the mainstay for managing proximal tibial fractures. However, moving towards more biologically appropriate plating techniques is occurring in an attempt to minimize soft-tissue stripping, decrease the need for bone grafting and improve union rates. Present concept of preservation of the blood supply and atraumatic surgical technique led to the biological fixation techniques.

Minimally invasive percutaneous plate osteosynthesis (MIPPO) has been widely used in the treatment of long bone fractures in the recent years because of its technical advantages and satisfactory clinical outcome.

The plate is inserted by a percutaneous approach hence minimising the soft tissue disruption, periosteal injury, also preserves the fracture integrity and vascular integrity of bony fragments. MIPPO is a new method that combines both principles of the biomechanical properties of fixation and an optimum bone-to-implant contact.[3] Despite a plethora of articles, written in the past 50 years that have addressed the problems of classification and results of various treatments the optimal method of management remains controversial.[4]

This study is conducted to evaluate the clinical and radiological outcome of proximal tibial fractures treated with minimally invasive percutaneous plate osteosynthesis (MIPPO).

Methods

Aims and objectives

To assess the clinical and radiological outcome following minimally invasive percutaneous plate osteosynthesis (MIPPO) for proximal tibial fractures and to evaluate the advantages, disadvantages in terms of success rates of fracture union,patient compliance and complications.

- Prospective study of 26 patients.
- All consecutive patients admitted in Mahatma Gandhi Medical College and Research Institute, Pondicherry between May 2010 and March 2012 with the diagnosis of tibial plateau fractures were included in the study.
- History and detailed physical examination were carried out to determine any associated ligamentous injuries,neurovascular injuries and compartment syndrome.
- Antero-posterior and lateral radiographs were taken to assess the type of tibial plateau fracture.
- All types of tibial plateau fractures(According to Schatzker classification type I to type VI of tibial plateau fractures) were taken into the study.
- All the patients diagnosed to have tibial plateau fractures were treated with Minimally invasive percutaneous plate osteosynthesis (MIPPO).
- Patients were followed up for a minimum period of 6 months with both clinical and radiological parameters according to Rasmussen's score.
- This study has been approved by the IHEC (Institutional human ethical committee) of Mahatma Gandhi medical college.

Inclusion criteria

- Age: Above 18 years (Skeletally matured).
- All types of tibial plateau fractures

Exclusion criteria:

- Age: Less than 18 years

(Skeletally immature).

- Other fractures in the ipsilateral limb.
- Compound fractures.

Initial Treatment

- All our patients underwent initial stabilization as per the ATLS guidelines.
- Patients with closed tibial plateau fractures associated with a tense haemarthrosis underwent aspiration of the joint under aseptic precautions.
- The limb was immobilised either in an above knee slab or through skeletal traction using a distal tibial or calcaneal pin traction on a Bohler Braun splint until definitive fixation was carried out.
- In patients complicated with excessive swelling and blistering, definitive fixation was delayed until the swelling/ blistering subsided.
- The patients were thoroughly evaluated from the systemic point of view and received appropriate treatment for existing co-morbidities like hypertension and diabetes prior to and after surgery.

Definitive management

In our study, all 30 patients with proximal tibial fractures underwent definitive fixation by Minimally Invasive Percutaneous Plate Osteosynthesis technique.

Operative technique

- The surgery was performed with the patient under spinal/ epidural anaesthesia.
- The patient was placed supine on a radiolucent operating table with the affected extremity in longitudinal manual traction.
- The affected limb prepared, painted and draped sterile.
- With the help of an image intensifier closed reduction was achieved by longitudinal traction. In certain cases percutaneous k-wires were introduced to aid in fracture alignment.
- A proximal tibial locking plate / L – Buttress locking plate or a T – Buttress locking plate of suitable length was used.
- An incision was made as large as necessary either medially or laterally, to help insert the plate, which is carefully slid in the submuscular plane, extraperiosteally and checked using image intensifier.
- Varus / valgus and rotational alignment were corrected.
- Cancellous and cortical screws were appropriately used to secure the plate adequately proximally and distally. These were introduced percutaneously through small stab incisions centered over the plate holes.
- A thorough wash with saline and betadine was given and the wounds closed. A sterile dressing was applied.
- Primary bone grafting was not done in any of the cases in this study.

Post operative protocol

- The patient received intravenous antibiotics for a minimum of 5 days. They also received analgesics / anti-inflammatory drugs.
- The limb was elevated on a Bohler Braun splint until edema subsided
- Knee mobilization was started once the acute pain subsided (ranged 3-7 days)
- Non weight bearing walking was permitted from 3rd post operative day with the help of a walker.
- Sutures were removed on the 12th post operative day.
- The patient was kept non weight bearing until radiological signs of union were evident, after which weight bearing was commenced initially from partial to full weight bearing in gradual progression depending on fracture consolidation.

Follow up

- Our patients were reviewed periodically after discharge at 6 weekly intervals for a minimum of 6 months or until complete radiological union.
- Knee movements were measured using a goniometer and the amount of extensor lag and flexion were recorded.
- Weight bearing was permitted only after radiological evidence of fracture union was present.
- At the time of follow up a functional assessment was done according to Rasmussen's clinical and radiological scoring system.
- Of the 30 patients operated 4 patients were lost to follow up.

We have followed the RASMUSSEN'S SCORING SYSTEM for our study.[5]

The criteria assessed in this scoring system include subjective complaints and clinical signs.

- The Subjective complaints assessed were Pain and Walking capacity
- The Clinical signs assessed were Extensor Lag, Range of Movement, Stability and Deformity.

Rasmussen's Score

Parameters	Points
Subjective complaints	
Pain [0-6]	
No pain	6
Occasional pain, bad weather pain	5
Stabbing pain in certain positions	4
Afternoon pain, intense, constant around the knee after activity	2
Night pain / rest pain	0
Walking capacity [0-6]	
Normal walking capacity [in relation to age]	6
Walking outdoors for at least 1 hour	4
Short walks outdoors >15 minutes	2
Walking indoors only	1
Wheelchair bound / Bed ridden	0
Clinical signs	
Extension [2-6]	
Normal / Full extension	6
Lack of extension [0 – 10 degrees]	4
Lack of extension > 10 degrees	2
Total Range of Motion [0-6]	
At least 140 degrees	6
At least 120 degrees	5
At least 90 degrees	4
At least 60 degrees	2
At least 30 degrees	1
No movement	0
Stability [2-6]	
Normal stability in extension and 20 degree flexion	6
Abnormal instability in 20 degree flexion	5
Instability in extension > 10 degrees	4
Instability in extension < 10 degrees	2

The resulting score obtained in each patient was graded into the following groups to categorise the functional outcome of the procedure.

- A score of 27 – 30 : Excellent
- A score of 20 – 26 : Good
- A score of 10 – 19 : Fair
- A score of <10 : Poor

Rasmussen's Radiological Score
Subjective Points

Articular Depression

None	6
< 5 mm	4
6 - 10 m	2
>10 mm	0

Condylar Widening

None	6
< 5 mm	4
6 - 10 mm	2
> 10 mm	0

Varus And Valgus Angulation

None	6
< 10°	4
10° - 20°	2
> 20°	0

Result

Maximum	18
Excellent	18
Good	12-17
Fair	6-11
Poor	< 6

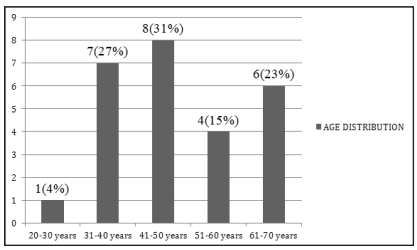
Observations

Patients Data

- Total number of patients : 26

Age

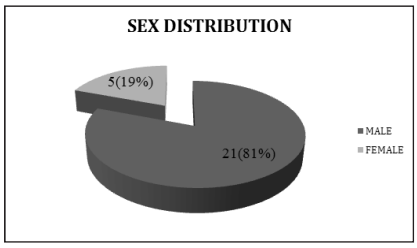
We had 1 patient within the age group of 20 to 30 years, 7 patients between the age 31 to 40 years, 8 patients between the age 41 to 50 years, 4 patients between the age 51 to 60 years, 6 patients between the age 61 to 70 years. Patients were in the age group 23 to 69 years with average being 48.23 years (Graph 1).



Graph 1 (Age Distribution)

Sex Distribution :

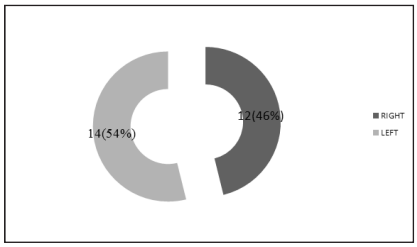
In our study there were 21 male patients and 5 female patients (Graph 2).



Graph 2 (Sex Distribution)

Side Involved

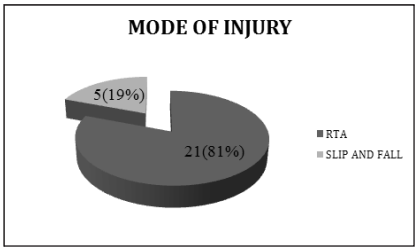
12 patients injured their right limb and 14 patients injured their right limb (Graph 3)



Graph - 3

Mode Of Injury

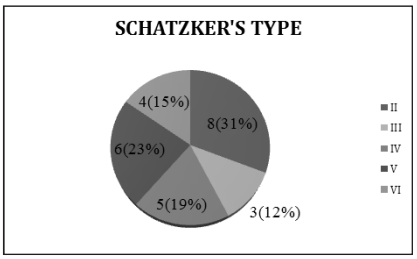
In our study the mode of injury of 21 patients was RTA, only 5 patients had slip and fall (Graph 4).



Graph- 4

Schatzker Classification

Based on Schatzker classification the maximum number of patients (31%) had type II fracture as shown in Graph 5.



Graph 5 :Schatzker classification

Duration of Follow Up

All our patients were followed up periodically with a minimum follow up duration of 6 months and a maximum follow up duration of 12 months (Fig 1,2,3).

Results

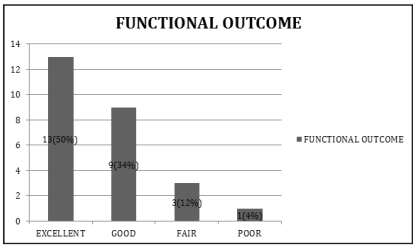
The post operative results were designated as excellent, good, fair and poor according to pain, walking capacity, range of motion and stability of the knee using Rasmussen's grading system. Similarly, Rasmussen's radiological scoring done by assessing articular congruity, condylar widening, Valgus/Varus angulation. In our study the average functional knee score was 25.61. Rasmussen's average radiological score was 15.76 .

Functional Outcome

In our study comprising of 26 patients, 13 patients [50%] had excellent results, 9 patients [35%] had good results, 3 patients [12%] had fair result and 1 patient [4%] had poor result as shown in the graph and Table 1 and Graph 6 below.

Results	No. of patients	Percentage (%)
Excellent	13	50
Good	9	34
Fair	3	12
Poor	1	4
Total	26	100

Table 1

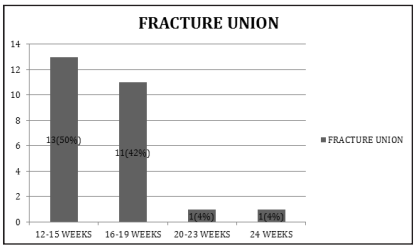


Graph 6

Fracture Union

All fractures in this study united without requiring any additional procedures. The average time taken for fracture union was 15.34 weeks (Graph 7).

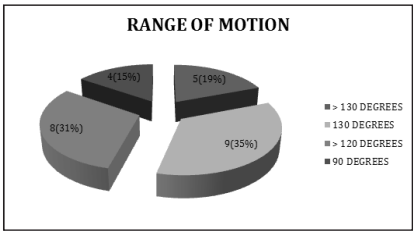
12-15 weeks : 13 patients
16-19 weeks : 11 patients
20-23 weeks : 1 patient
24 weeks : 1 patient



Graph 7

Range Of Motion [ROM]

The average range of movement achieved was 120 degrees. 5 patients achieved slightly more than 130 degrees of flexion, 9 achieved almost 130 degrees, 8 achieved almost 120 degrees, 4 patients had movement of only 90 degrees of flexion.



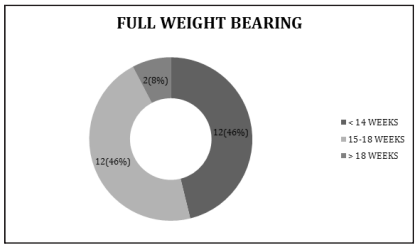
Graph 8

Full Weight Bearing Walking

In our study 24 patients walked full weight by eighteen weeks and 2 patients later than 18 weeks (20 and 24 weeks respectively) as shown in Table 2 and Graph 9.

Full Weight Bearing Walking	No. Of Patients
< 14 weeks	12
15 – 18 weeks	12
> 18 weeks	02

Table 2



Graph 9

Complications

The various complications encountered in our study as listed in the table 3 below

Type of complication	No. of patients	Case no.
Pulmonary embolism	1	3
Restricted knee ROM	4	2 , 5 , 12, 19
Plate loosening	1	2
Valgus deformity	1	12

Table 3

Pre Operative Radiographs (AP & LAT)

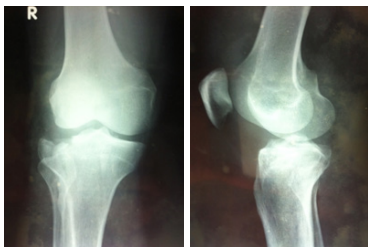


Figure 1

Postoperativeradiographs(Immediate) (AP&LAT)

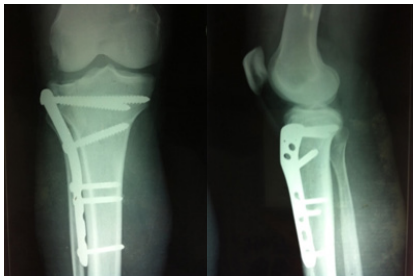


Figure 2

Post Operative Radiographs (6 Months) (AP&LAT)

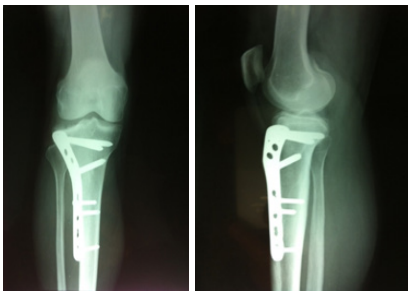


Figure 3

Discussion

Proximal tibial fractures due to high energy mechanisms (Schatzker type IV,V,VI) are inherently unstable injuries associated with fracture comminution, displacement, severe soft tissue injury and a higher incidence of complications.

Closed treatment of these complex fractures have resulted in a high percentage of complications (upto 70%) as seen in long term follow up in other studies.[6] The most widely used method for treatment of displaced tibial plateau fractures has been Open Reduction and Internal Fixation. The main aim is restoration of the articular surface to as near anatomical position as possible and to maintain the reduction while early knee joint mobilization is commenced which is however possible only after extensive soft tissue dissection. Due to the association of soft tissue injuries with these fractures, additional soft tissue stripping involved in open reduction and internal fixation often render bony fragments avascular and also significantly increase the chance of infection.

Moore et al, in his series reported a 23% incidence of infection after ORIF in these complex fractures.[7] Mallik et al encountered 4 of his 5 cases being infected after ORIF for such fractures.[8] Barrak reported of deep infection in seven of eight fractures that were treated with a medial plate.[9]

To avoid soft tissue damage in addition to the pre existing soft tissue injury associated with these complex fractures, minimally invasive biological fixation procedures have evolved over the last couple of decades. Among these procedures, **Minimally Invasive Percutaneous Plate Osteosynthesis (MIPPO)** potentially has become a good option in the treatment of these complex fractures.

In our study comprising 26 patients treated by Minimally Invasive Percutaneous Plate Osteosynthesis we had 84.61% of excellent and good results in the functional outcome.

Analysis Of Results With Classification

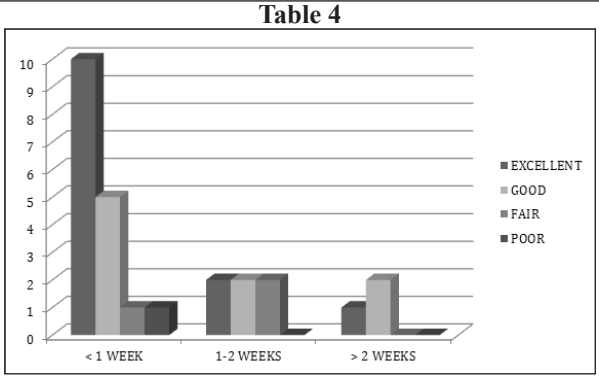
- 1 poor and 1 fair result were seen in type 6 fracture.
- 2 fair results were seen in type 5 fracture.
- All other types results were excellent and good.
- No other fracture type had fair or poor result.

Analysis Of Result - Injury And Surgery Time Interval

- In our study, patients who were mobilized earlier had a better functional outcome irrespective of the fracture type and surgery interval as shown in the table &graph below.

Time Between Injury And Surgery	Result	Number	Percent
< 1Week	Excellent	10	65%
	Good	5	
	Fair Poor	1 1	
1 – 2 Weeks	Excellent	2	23%
	Good	2	
	Fair	2	
2 - 3 Weeks	Excellent	1	12%
	Good	2	
	Fair	0	

Table 4



Graph.10

Age

- In our study average age of the patient was 48 years.
- Age in our study is compared with other studies.

Study	
Christian Boldin,Florian Fankhauser[10]	Average Age 62 Years
Lee JA ,Papadakis SA[11]	Average Age 42 Years
ours	Average Age 48 Years

Table 5

We observed that all the fractures united without any need for secondaryprocedure. It has been compared with other studies.

Study	Result
Stannard JP, Wilson TC[12]	87%
Christian Boldin, Florian Fankhauser[10]	96%
Lee JA ,Papadakis SA[11]	100%
ours	100%

Table 6

Follow Up

Study	Average Follow Up
Stannard JP, Wilson TC[12]	26 Months
Christian Boldin, Florian Fankhauser[10]	38 Months
ours	8 Months

Table 7

- Average follow up in our study is 8 Months (6 - 12 Months).
- Follow up in other studies are compared.

Range Of Movement

Average range of movement in our study is 120^0, it has been compared with other following studies,

Study	Average Range Of Movement
Stannard JP, Wilson TC[12]	Average Rom 110^0
Christian Boldin, Florian Fankhauser[10]	Average Rom 117^0
Ours	Average Rom 120^0

Table 8

Union Time

Average union time in our study is 15.3 weeks

Study	Result
Stannard JP, Wilson TC[12]	15.6 weeks
Christian Boldin, Florian Fankhauser[10]	11 weeks
Lee JA, Papadakis SA[11]	18 weeks
ours	15.3 weeks

Table 9

Restricted Knee Range Of Movement :

There was restricted knee range of movementin four of our patients and all four were schatzker's type V and type VI (Type V-2 and Type VI-2).All the patients who developed restricted knee range of movement had high energy proximal tibialfractures.Because these patients were immobilized for longer period of time which contributed for the restricted knee ROM.All patients who developed restricted ROM were treated appropriately with regular physiotherapy.

Loss Of Reduction

- There was implant loosening in one case schatzker type V fracture following which patient was immobilised for longer period of time hence outcome was fair.
- There was no loss of reduction in our study in comparison with Stannard JP study.[12]
- But in Christian boldin et al study there was one loss of reduction , because patient started full weight bearing earlier against advice of the surgeon.[10]

Malunion

- Our study has 1malunion, one valgus angulation.
- Occurred in type V fractures and due to comminution.
- In our study no intervention for the patient was done and outcome was fair because patient was immobilized for a longer period of time.
- Our results were comparable with other studies.

Study	Complication
Stannard JP, Wilson TC[71]	1 Valgus 1 Varus
Christian Boldin, Florian Fankhauser[10]	1 Varus
Lee JA, Papadakis SA[11]	1 Varus
Ours	1 Valgus

Table 10

Infection

- Infection factor has been compared with the following studies.

Study	Result
Stannard JP, Wilson TC[12]	2 Superficial infection
Christian Boldin, Florian Fankhauser[10]	No Infection
Lee JA, Papadakis SA[11]	2 Deep Infection
ours	No Infection

Table 11

Non Union

- No Non- Union In Our Study.
- All Fractures United With Out Any Intervention

Study	Non-Union
Stannard JP, Wilson TC[12]	Nil
Christian Boldin, Florian Fankhauser[10]	1 Non Union
Lee JA, Papadakis SA[11]	Nil
ours	Nil

Table 12

Conclusion

MIPPO technique gives good to excellent results even in high energytibial condyle fractures (84% cases in our study).Our patients were able to achieve a good functional range of movement, averaging 120 degrees (Krettek et al – 124 degrees[3]. Those who were treated with early fixation and early mobilization were found to have a better functional outcome irrespective of the fracture type. Patient compliance was good with MIPPO technique .No secondary bone grafting was required. The average time taken for fracture union was 15.3 weeks. [Krettek et al – 16 weeks][3]

From our study of 26 patients, we conclude MIPPO **(Minimally Invasive Percutaneous Plate Osteosynthesis)** technique is an effective method of treating proximal tibial fractures due to its overall good functional outcome. Average follow up in our study was only 8 months and long term follow up is needed to give a definitive opinion.

References

1. David J Jacofsky, George J Haidukewych:Tibial plateau fractures : *Insall Scott Surgery of the knee*;ch-66;1133-1146;4th edition;2006
2. WissDA,WatsonJT,JohnsonEE,Fractures of the knee : Rockwood CA, Green DP, BucholzRW,etal:ed*4 Philadelphia:Lipincott-Raven 1996.p.1999*
3. Chr. Krettek MD, FRAC t. Gerich MD, Th. Miclau, MD. A minimally invasive medial approach for proximal tibial fractures. Injury 2001, Vol. 32 Suppl 1:4-12,13.
4. Newell R LM and Davies.MS: Susanstandring. knee; Gray's Anatomy 29th ed. Spain Elsevier Churchill Livingstone. 2005;1471-86.
5. Rasmussen P: Tibial condyle fractures, impairment of knee joint stability as an indicator of surgical treatment. JBJS 55A pp: 1331-1350, 1973.
6. Sarmiento A. Kinnman KB, LattaLL : Fractures of proximal tibia and tabial condyle: A clinical and laboratory comparative study ClinOrthop 145 : 136-149, 1979.
7. Moore TM, Patzakis MJ, Harvey JP. Tibial plateau fractures: definition, demographics, treatment rationale and long term results of closed traction management or operative reduction: JOrthop Trauma 2 : 97-117, 1987.
8. Watson JT: high energy fractures of the tibial plateau. OrthopClin North Am: 25: 728,754.1994.
9. Young MJ, Barrack RL. Complications of Internal fixation of Tibial plateau fractures. Orthop Rev. 1994: 23 : 149-154.
10. Christian Boldin, Florian Fankshuser MB, Hervig peter Hofer: Three year results in proximal Tibia fractures treated with the LISS: CORR 2006, APRIL;445;222-9.
11. Lee JA ,Papadakis SA, Moon c ,Zalavras CG: Tibial plateau fractures treated with the LISS , IntOrthop , 2007 Jun ; 31 (3); 415 -8.
12. StannardJP,WilsonTC,Volgas DA,Alanso JE: LISS in treatment of complex fractures of tibial plateau; Short term results . JOT;2004 (SEP) ;18(8) ;552-558.

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An absolute indication of Manipulation under Anesthesia (MUA) for frozen shoulder is not extracted from literature. Though advanced medical sciences now have an option for arthroscopic release of soft tissue of shoulder, the results are variable in literature and recurrence recorded in literature varies up 30 to 40 percent. We adopted a non invasive approach for 40 cases of frozen shoulder. A functional outcome of patients treated with MUA and intra-articular injection with Methylprednisolone with Lignocaine (MP) was evaluated. Patients with frozen shoulder including those with diabetes were analyzed clinically, radio logically and SPADI score prior and post procedure done. The percentage of recovery noted. We noticed favorable and positive outcome in 67% despite strictly restricting our follow up for 3 months. A reminder here is the natural course of history which is generally self limiting and varies from 16 to 24 months. In view of possible recovery time, range of motions would further improve with recovery from pain. More than 4 studies were comparable, in this study we found external and internal rotation of shoulder joint not fully recovering, but reaching a range of motion for activities of daily living. Our results were comparable to other studies and conclude that MUA with injection of steroid speeds up recovery in range of movements and provides relief of pain.

□

□ □□□ □rd□□□Frozen shoulder, Manipulation under Anaesthesia, Intra Articular, Methylprednisolone, Lignocaine 2%, SPADI SCORE.

1 □□□□r □d □□□□□□□□

Peri Arthritescpulaheumarale was the term used by Duplay in 1872. For painful shoulder with restriction of movements. Over the years aeitipathogenesis evolved with newer terms. 1934 Codman coined the condition Frozen shoulder, and Neviaser 1945 coined the term Adhesive Capsulitis [1]. And other surgeons brought light and charted theoretically the stages of frozen shoulder currently also known as Adhesive Capsulitis. The incidence varies from 2-4% and in some general population 3-7% [1, 2, 3].

Clinically diagnosed as Adhesive Capsulitis, this is a condition where spontaneous adhesion of ligaments, capsule and fibrosis occurs. There are two claims, one suggest synovial inflammation and other a condition similar to Dupuytren's Contracture [2, 4, 11]. Contractures along the long head of Biceps, Infraspinatus, and Supraspinatus.

Treatment varies from NSAIDs, moist heat, physical therapy, oral steroids,

IA Injections, hydro dilation, nerve blocks, Manipulation, open and arthroscopic soft tissue release. Literature supports more incidences in Diabetic patients, females, non dominant hand with bilateral involvement in 12-16% in same individual [1, 2, 3, 4]. Our study was to do noninvasive therapy that is Manipulation under Anaesthesia (MUA) with intra articular injection of 80 mg of Methylprednisolone with 1ml of Lignocaine; which is economical, needs no highly skilled expertise and can be done with standard aseptic procedure even in a District hospital.

□

2 □M □□r □□□□□□□d □□ □□□□d □□

To determine the outcome of patients, after we assessed patients for pain, disability and Range of Movements (ROM). The study prospectively recorded the functional outcome of treatment after MUA. We analyzed 40 consecutive patients and outcomes were assessed clinically by SPADI Score.

The Shoulder Pain and Disability Index is the sum total of pain scale and Disability scale and total score is divided by 130 and multiplied by 100 to calculate percentage. Pain is measured by 0-10 with 5 questions. Disability is assessed by 8 questions, score 0-10. Where 0 means no pain and 10 means worst unbearable pain.

Activities of daily living were checked were 0 means no difficulty and 10 means severe difficulty. Higher scales on the subscale will indicate greater pain and disability. Prospective study of 40 patients was conducted in tertiary care Institution of Medical sciences. The study was between May 2019 and May 2020. 40 consecutive patients with painful loss of shoulder movements with limitations in their activities of daily living.

2.1 we included all patients, who have painful and stiff shoulders, with restricted active and passive motion, with normal glenohumeral joint radiologically.

2.2 Any calcific tendinitis, arthritis, traumatic conditions or any other surgeries around the shoulder.

Our study included 40 patients including those with diabetes. All patients gave detail history and evaluation of pain scale and disability done using SPADI score. Clinical Examination and their record of shoulder movements and strength of muscle power were recorded. Only a single X ray in Anteroposterior (AP) view was taken. No patient underwent ultrasound or MRI.

40 patients were hospitalized, and physiotherapy started and all patients were educated for self-mobilization of shoulder. With strict emphasis on avoiding shrugging.

Wall climbing exercise, Pendulum, and other exercises were taught to the patients.

All patients underwent MUA within 5 days and at the same sitting injection 80 mg of Methylprednisolone (MP) mixed with 1 ml of Xyclocaine given anteriorly into gleno humeral joint.

Patient was supine, scapula fixed and pressed with thoracic cage and border of scapula. With help of an assistant. Holding the short lever arm gradual abduction, external rotation adduction and lastly internally rotation movement were done sequentially until audible click was heard.

We had no prior knowledge of pathological thickness of Corocohumeral ligaments, thickness of the capsule, and extent of pathological contractures. Irrespective of the duration we treated all patients with a standard protocol, so intervention rate could be accessed based on outcome at end of 3 months. Out of 40 patients, there were 18 male and 22 females. Out of

40 patients 27 patients were diabetic. 19 patients had right shoulder involvement and 21 left.

The average duration of symptoms like pain and disability was 2.5 months; least duration was 1 month and maximum 7 months. 31 patients after MUA and Inj MP of 80 mg underwent physiotherapy which was supervised for 1 week. 9 patients did not attend supervised physiotherapy at the hospital.

All patients underwent clinical examination at time of MUA, immediately after MUA, after 1st month, 2th month and 3rd month. SPADI score was calculated before procedure immediately after procedure and for each month. The percentage of recovery calculated to merely reflect functional outcome.

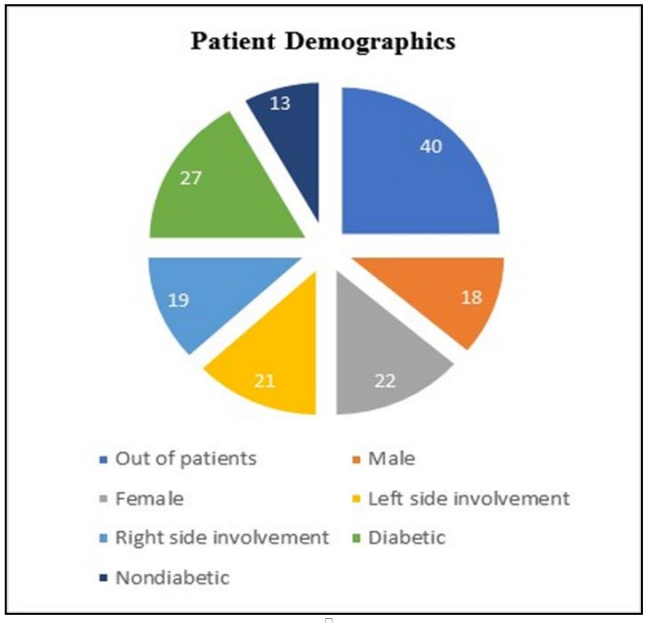


Table 1: Patient Demographics

Male	18
Female	22
Left side involvement	21
Right side involvement	19
Diabetic	27
Nondiabetic	13

Our work sheet tabulated the results in our study with average range of shoulder motions as follows: Abduction 60*, External Rotation (ER) 31°, Internal Rotation 30 (IR), and Flexion 66* at first examination.

We had one patient 55/F who had stiffness and pain who did not respond to treatment, not diabetic, her duration of symptoms was only for 3 months and was of left side. Our hypothesis that it would be like pathogenesis like Duptruyen Contracture in this particular case [4]. Immediately after MUA and injection MP Abduction (Abd) improved to 109.5 EXT rotation (ER) 59.6 Internal rotation (IR) 51° and flexion (FI) 106*

Table 2: Range of motion of shoulder at 3 months

1st month	Abd 125.2°	ER 65.25°	IR 56.5°	FL 123°
2nd month	Abd 132.7°	ER 67.5°	IR 60.2°	FL 130°
3rd month	Abd 135°	ER 68.2°	IR 61°	FL 134°

In our study by using SPADI score for pain and disability we calculated prior to SPADI, Post MUA, and at end of 3 months. In span of 3 months post MUA with injection the range of movements doubled.

The percentage of recovery calculated and averages taken, Preop SPADI Score was

70.7 and Post op 21.45 and the percentage of 67.1%. The predominant range of motions of external and internal rotations were quite resistant to MUA and the underlying pathology of shoulder rotators, their anatomy and pathological changes around them, probably is resistant to MUA.

Statistical Analysis with paired groups were done with comparison before and after MUA of shoulder.

Statistical Analysis paired test of Range of Movements

Abd	Pair 1 Before	VAR00001	110.7692	39	28.50527	7.332	0.000
	After MUA	VAR00005	135.1282	39	31.10862		
ER	Pair 2 Before	VAR00002	59.6250	40	19.36119	4.573	0.000
	After MUA	VAR00006	68.2500	40	20.4923		
IR	Pair 3 Before	VAR00003	51.2500	40	19.50509	6.811	0.000
	After MUA	VAR00007	60.5000	40	18.80398		
FL	Pair 4 Before	VAR00004	106.2500	40	29.23709	7.890	0.000
	After MUA	VAR00008	134.0000	40	28.62669		

Statistical Analysis with 95% confidence of Pre and Post MUA outcome measures, based on means, standard deviation, t-score and P-value.

Discussion

The Frozen shoulder is a condition where in, there is slowly progressive loss of movements of shoulder both active and passive, associated with pain and restriction of activities of daily living. The Natural History of Adhesive Capsulitis is that it is self-limiting. Recovery can take from 24-26 months [1, 2, 3, 4].

However Literature quotes various Surgeons who currently contradict this statement [6], several authors claim pain and loss of range of movements with disability of shoulder. Treatment varies from simple NSAID with moist heat, physiotherapy, oral steroids, intrarticular injections with steroid, hydrodialation, nerve blocks, manipulation, open surgical release and arthroscopic release. Contractures in shoulder may be responsible for ineffectiveness of any treatment. We don't know whether synovial inflammation is the cause or 'Duptruyens type' of capsule of shoulder.

We compared our results with Heidi Vastamaki, and Marti Vasatamaki' study [7].

In their study prior to MUA, Abduction was 75° (ABD), Eternal Rotation 21° (ER), Internal Rotation level of buttock (IR), and Flexion 99° (FL), in our result prior to MUA Abduction was 60°, External was 31°, IR 30° and FL 66°.

End of 8.8 months Vastamaki's study [7] showed in their patients Abduction 167°,

ER 51°, IR level L5 and Flexion 156°. In Our study we obtained at 3rd month on follow up ABD 135°, ER 68°, IR 61° and FI 134°.

Comparing with Thomas[8] et al ABD was 62°, ER 17° IR 17°, FI 165° (pre MUA)

Post MUA at one year follow up was ABD 168°, ER 65°, IR 65°, FL 169°. In our study

ABD 135°, ER 68°, IR 61° and FI 134° at end of 3rd month.

Studies by various authors showed good recovery but they assumed that elderly patients have restricted movements, we stand to contradict to their statements that 90% improvement in pain and range of movements.

Jenkins *et al.*, in their retrospectively had analyzed that 36% had unsatisfactory outcomes [8], in our study it was 33%. The natural history claimed to be self-limiting, can take prolonged duration of time between 14-32 months. Adhesive capsulitis of shoulder with pain and disability would require medication and pose risk of drug induced Nephropathy.

Though optimal time suggested by Vastamaki, and Vajonen is between 6-9 months for MUA, clinical outcome was questionable by many authors. Dodenoff, Levy, Wilson and Copeland in one series, and Janada and Hawins [9] proved that pain and disability can be reduced after MUA. This shortens the time interval of patients to resume work, disease itself cannot be evaluated merely on duration of months and prevailing changes for staging purpose cannot be guessed. Clinical importance of outcome can be questioned and hence its variable. To achieve necessary functional activities there has to be range of motion which is free.

1. Early Recovery

Hence irrespective of duration of symptoms all patients were hospitalized and for 5 days physiotherapy given and then taken up for MUA and Injection MP 80 mg with 1ml of 2% Xyclocaine into the joint.

To enable eating approximately 60-100 of abduction, horizontal abduction about

40-70 degrees. Rotations about lessor range. For reaching perineum 40-60 of abduction and 90 of Internal rotation. Similarly, for combing hair, tucking shirt, or putting on pants, etc.

A range of movements which are not full and free but can able patients do activities of daily living which are pain free [8, 9, 17].

Pain and Disability prior to MUA on Average score was 70.7 (SPADI) after MUA at 12 weeks the score was 21.45. Post SPADI score of 21.45 denotes some residual pain and disability, but with useful functional outcome.

There was no complication in our cases. Grant *et al.* [10] compared MUA with arthroscopically done surgical release and reported that there was not much difference [10]. Arthroscopically done release has risks like chondrolysis due to thermal heat, risk of infection, risk of accidental axillary nerve damage. Open Release with manipulation offers no advantage on the contrary it reduces the speed of recovery. Also risk of infection introduced into the joint.

Rotator Cuff was analysed by Atoun et al with ultrasound before and after MUA and found cuff undamaged [12].

Sasanuma *et al.* [13] found no rotator cuff tears on MRI after Manipulation.

2. Cuff Tear Arthropathy

In a Retrospective study of 51 patients' frozen shoulders done by Wang *et al.* [14] after MUA reported 89% of cases had good results. In our series 67% had good results.

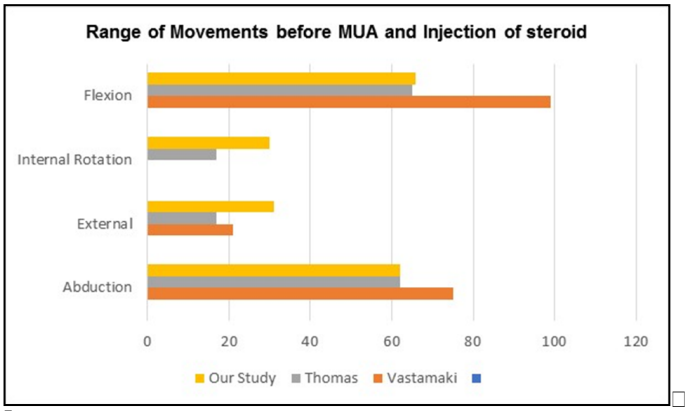
The difference of 22% in the outcome was because all patients were followed up to 3 months strictly. Whereas Wang followed up to 1 year. In their study patients were immediately mobilized, in our study all patients were given rest post of period for 24 hours, to enable them recover from pain.

Another probable cause is 9 patients dropping out of supervised physiotherapy at OPD for 5 days. One patient had

absolutely no recovery, few others had a slow start, our follow up was limited strictly till 12 weeks. We assume out of 22% of cases if given more time recovery rate would improve.

showing Range of Movements before MUA and Injection of steroid

Vastamaki	75	21	Buttock	99
Thomas	62	17	17	65
Our Study	62	31	30	66

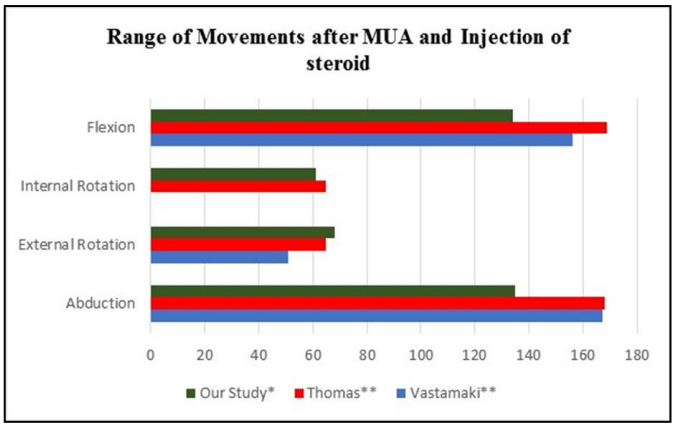


2 showing Range of Movements before MUA and Injection of steroid

showing Range of Movements after MUA and Injection of steroid

Vastamaki**	167	51	L 5 vertbrae	156
Thomas**	168	65	65	169
Our Study*	135	68	61	134

** follow up 1yr or more.



ROM after MUA and Injection of steroid

The study shows functional outcomes is improved after MUA and after Intra articular Inj of MP. Recovery of External rotation and Internal Rotation takes a longer time and their range of movements is again dependent on retraction of capsule and narrowing of clear space around ligaments. Non reversible pathological changes like fibrosis and capsular thickening with adhesions of glenohumeral ligaments.

Farrell *et al.* [15] their study of 19 shoulders after MUA, followed up for 7 years. They obtained Abduction 155°, External Rotation 76°, Internal Rotation 73° and Flexion 159°.

In our study we obtained 135° of abduction, 68° of External Rotation, 61° of Internal Rotation and 134° of Flexion at 3rd month which is beneficial to be patient.

Our study follows up limited up to 3 months. It is evident by MUA and inj of steroid the pain and stiffness improves, the shoulder recovers functional range of movements. However the limitations of this study: 40 patients and follow up to 3 months. No control group of patients, Non divided into 2 groups that is diabetic and non diabetic. Standard and same protocol of treatment followed. No 2 different methods of treatment. Our prospective study included both diabetic and non diabetic patients.

Manipulation of shoulder with injection of Steroid is a good method of treatment.

It's cost effective and speeds up recovery from pain and disability. It can be done safely as a day care procedure, and even in district Hospital. MUA carries little risk, without any need for expert technical skill. Majority of patients are happy for the range of movements recovered.

It is unnecessary to wait and observe for prolonged time or do prolonged follow up. Recurrence rate is low compared to arthroscopic surgical release and is subject to further trials and comparative studies.

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1. Jo A. Hannafin, Theresa A, Chiaia PT. Clinical Orthopaedic and Related Research, Adhesive Capsulitis, No. 372, 95-109.
2. Andrew S, Neviaser, Jo A. Hannafin. The American Journal of Sports Medicine, Adhesive Capsulitis: A Review of Current Treatment, 2010, 38.
3. Vander Windt DA, Koesw, de Jona, Boute LM. Shoulder disorder in general Practice: Incidence, patient characteristics Management Ann. Rheum. DIS 1995; 54:959-964.
4. Bo J. Lundber, Acta Orthopaedica Scandinavica. The Frozen Shoulder: Clinical and Radiographical Observations the Effect of Manipulation under General Anesthesia Structure and Glycosaminoglycan Content of the Joint Capsule Local Bone Metabolism, 2014, 1-59.
5. Hannafin JA, Dicarlo EF, Wickiewicz TL, *et al.* Adhesive capsulitis: capsular fibroplasia of the glenohumeral joint. J Shoulder Elbow Surg. 1994; 3(Suppl 5):435.
6. Shaffer DM, Tibone JE, Keralan RK. Frozen shoulder a long term follow up. Journal of Bone and Joint Surgery, AM. 1992; 74:738-46.
7. Vastamaki H, Varjonen L, Vastamaki M, Scandinavian. Journal of Surgery, Optimal Time for manipulation of frozen shoulder, 2015, 1-7.
8. Thomas WJ, Jenkins EF., Owen JM et al., Treatment of Frozen shoulder by MUA & Inj. Does the timing of treatment effect outcome? Journal of Bone & surgery Br 2011; 93:1377-1381.

9. Janada and Hawkin. Shoulder Adhesive Capsulitis: Manipulation and Arthroscopic Arthrolysis or Intra-Articular steroid Injections? Int. Orthopedics, 2012; 36(1):101-106.
10. Grant JA, Schroeder N, Miller BS, Carpenter JE. Comparison of manipulation and arthroscopic capsular release for adhesive capsulitis: a systematic review. J Shoulder Elbow Surg. 2013; 22:1135-1145.
11. Bunker TD, Anthony PP. The Pathology of frozen shoulder. A. Dupuyten-like disease. *J Bone Joint Surg* [Br]. 1995; 77-B677-683.
12. Atoun E, Funk L, Copland SA, *et al.* The effect of shoulder manipulation on rotator cuff integrity. Acta Orthop Belg. 2013; 79:255-259.
13. Sasanuma H, Sugimoto H, Kanaya Y *et al.* Magnetic resonance imaging and short-term clinical results of severe frozen shoulder treated with manipulation under ultrasound guided cervical nerve root block. J shoulder Elbow Surg. 2016; 25:e13-e20.
14. Jung-Pan Wang, tung-Fu Huang, Shih-Chieh Hung, Hsiao-Li Ma, Jiunn-Ger Wu, Tai-Hsiung Chen, International Orthopaedics (SICOT). 2017; 31:333-337.
15. Farrell Cm, Sperling JW, Cofield RH. Manipulation for frozen shoulder: long term results. J Shoulder Elb Surg. 2005; 14(5):480-4.
16. Dodenhoff RM, Levy O, Wilson A, Copeland SA. Manipulation under anesthesia for primary frozen shoulder: effect on early recovery and return to activity. J Shoulder Elbow Surg. 2000; 9:23-26.
17. Dutton's Orthopedic Examination, Evaluation Intervention, Third Edition, 2009.
18. Tarek Boutefnouchet, Robert Jordan, GevBhabra, Chetan Modi, Adnan Saithna, Orthopaedics & Traumatology: Surgery & Research, Comparison of outcomes following arthroscopic capsular release for idiopathic, diabetic and secondary shoulder adhesive capulitis: A Systematic Review, Birmingham, 2019, 839-846.
19. Tim Kraal, Lijkele Beimers, Bertram The, Inger Sierevelt, Michel van den Bekerom, Denise Eygendaal, Shoulder & Elbow, Manipulation under anaesthesia for frozen shoulder, 2019, 4.
20. Ibrahim T, Rahbi H, Beiri A, Jeyapalan K, Taylor, GJS. Original Article, Adhesive capsulitis of the shoulder: the rate of manipulation following distension arthrogram. 2006, 27:7-9.
21. Jae Hoon Jung, Du Hwan Kim, Jaehyuck Yi, Du-Han Kim, Chul-Hyun Cho, Rheumatology International, Determination of magnetic resonance imaging criteria for diagnosis of adhesive capsulitis. 2019; 39:453-460.
22. NiluferBalci, Mustafa Kemal Balci, Serdar Tuzuner, Elsevier, Shoulder Adhesive Cpsulitis and Shoulder Range of Motion in Type II Diabetes Mellitus: Association with Diabetic Complications.
23. Jo A. Hannafin, Theresa A, Chiaia, PT, Clinical Orthopaedic and Related Research, Adhesive Capsulitis, 372, 95-109.
24. Sean M, Griggs MD, Anthony Ahn MD, Andrew Green MD, Journal of Bone and Joint Surgery, Idiopathic Adhesive Capsulitis, 2000, 82, 10.
25. Ide J, Takagi K. Early and long-term results of arthroscopic treatment for shoulder stiffness. J Shoulder Elbow Surg. 2004; 13(2):174-179.
26. Iannotti JP, Manipulation under anesthesia did not enhance the benefit conferred by home exercises for frozen shoulder. J Bone Joint Surg Am. 2008; 90(8):1792.
27. Holloway GB, Schenk T, Williams GR, Ramsey ML, Iannotti JP. Arthroscopic capsular release for the treatment of refractory postoperative or post-fracture shoulder stiffness. J Bone Joint Surg Am. 2001; 83(11):1682-1687.
28. Hazleman BL. The painful stiff shoulder. Rheumatol Phys Med. 1972; 11(8):413-421.
29. Hand C, Clipsham K. Rees JL, Carr AJ. Long-term outcome of frozen shoulder. J Shoulder Elbow Surg. 2008; 17(23):1-6.
30. Lundberg BJ. The frozen shoulder Clinical and radiographical observations. The effect of manipulation under general anesthesia. Structure and glycosaminoglycan content of the joint capsule. Local bone metabolism. Acta Orthop Scand Suppl 1969; 119:51-9.
31. Ogilvie-Harris DJ, Biggs DJ, Fitsialos SP, Mackay M. The resistant frozen shoulder: Manipulation versus arthroscopic release. Clin Orthop. 1995; 319:238-48.
32. Le Lievre HMJ, Murrell GAC. Long-term outcomes after arthoscopic capsular release for idiopathic adhesive capsulitis. J Bone Joint Surg Am. 2012; 94:1208-16.

Original Research Article

A clinical study on etiopathogenesis of hoarseness of voice

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Abstract

Introduction: Voice is a natural medium adapted to communicate emotional contact. Hoarseness is a common symptom that otolaryngologist come across. **Aims and Objectives:** This study aims at studying the etiology predisposing factors and clinical profile of patients presenting with hoarseness. **Materials and Methods:** This study was carried out in department of ENT, PSG medical college, Coimabatore in the period of July 2015 to July 2017. A total of 100 cases presenting with hoarseness were studied. **Results:** The most common etiology was found to be malignancy of larynx with incidence of 33% among the hoarseness of voice patients. The incidence of hoarseness was 0.27% among the total ENT cases. **Conclusion:** The symptom of hoarseness of voice should not be ignored as it might be an indication of laryngeal malignancy. Any patient with hoarseness should be thoroughly investigated to rule out laryngeal malignancy, that might cause respiratory distress leading to life threatening complications.

Keywords: Hoarseness of voice, Laryngeal malignancy, indirect laryngoscopy, direct laryngoscopy, Video laryngoscopy

Introduction

Voice is an integral part of human attribute known as speech. A person with voice problem may present with hoarseness, voice fatigue, breathy voice, reduced phonational range, pitch breaks etc. Hoarseness is a coarse, scratchy sound most often associated with abnormalities of the vibratory margins of the vocal cords. Hoarseness of voice describes the voice quality that is noticeably evident in its lack of clarity and discordance. In Otorhinolaryngological practice one of the commonest symptoms encountered is hoarseness of voice.

It is more common in certain groups like teachers and voice professionals. Both males and females are affected, and it can be a presenting symptom in any age group. In addition to impact of health, it has an effect on quality of life. Hoarseness can be due to both benign and malignant conditions which requires promt diagnosis and appropriate management. If hoarseness of the voice persists for more than two weeks, then it should be investigated properly to find the cause [1]. The etiology of hoarseness is very diverse, and it varies greatly. Hoarseness can be divided into acute and chronic onset [2]. The acute onset of hoarseness may be secondary to viral infection, voice abuse, trauma to the larynx and thyroid surgery [3]. Chronic onset may be due to vocal polyps, vocal cord nodules, laryngeal papillomatosis, laryngeal neoplasms, tumors of the vocal cords, functional dysphonia, smoking, gastroesophageal reflux, post nasal drip, malignant neoplasms of the thyroid, oesophagus, lungs and neurological involvement by systemic diseases like diabetes mellitus and chronic granulomatous diseases like tuberculosis [4]. Hoarseness can be identified just by hearing to the spoken voice. It is a common symptom which warrants investigation to rule out many local and systemic causes [5]. The present study is an attempt to analyze the clinical profile, incidence of common etiological factors and the association of common predisposing factors for hoarseness of voice.

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Aims and Objective

1.To analyze clinical profile of hoarseness of voice
2.To find out incidence of common etiological factors of hoarseness
3.To find out the association of common predisposing factors leading to hoarseness

Materials and Methods

This was a prospective observational study conducted during July 2015 to July 2017 in department of Otorhinolaryngology, PSG institute of medical sciences and research. This study includes 100 patients with various laryngeal pathologies on the basis of inclusion and exclusion criteria. All cases presented with change in voice.

Inclusion criteria for cases considered in the study

1. Patients presenting with hoarseness of voice.
2. Both genders

Exclusion Criteria for cases considered in the study

1. Age group below 5 years.
2. Voice disorders other than hoarseness like rhinolaliaaperta, rhinolaliaclausa, articulation disorders and central nervous system like bulbar palsy, multiple sclerosis, stroke and Parkinson's disease.

Methods of data collection: Proforma was designed based on the objective of the study and it was pretested and used after modification. As per the proforma detailed clinical history was taken followed by thorough ENT examination and systemic examination and clinical diagnosis was arrived in support with the relevant investigations.

Videolaryngoscopy under local anaesthesia was carried out in all 100 patients as a part of local examination. Out of which many patients required surgical intervention for both diagnostic and therapeutic purposes.

When the findings were inadequate to arrive at a clinical diagnosis, direct laryngoscopic examination with or without biopsy, microlaryngoscopy with or without biopsy, flexible endoscopy with or without biopsy was performed. The biopsy specimen was sent for histopathological examination.

Results

In our present study incidence of hoarseness voice was 0.27% of total ENT cases, with incidence of laryngeal malignancy in 33% of cases.

Cases: Maximum number of cases (31%) were between the age group of 41-50 years. Among males 41-50 years and females 31-40 years were the common age group of presentation. Hoarseness was commonly found in labourer class (48%). Both among males and females this was commonest group.

Lower socio-economic group was commonly noted among patients (40.00%) both in males and females. Majority patients were from rural area (59%). Smoking was commonly encountered habit among males (30.00%) and no habits among females.

Along with hoarseness (100%) other symptom with which patient presented were dysphagia (25%), cough (41%), breathlessness (14%), foreign body sensation in throat (10%), neck mass (5%) and Hemoptysis (5%). Maximum number of patients presented with hoarseness of voice with duration of 1-3 months. On video laryngoscopic examination commonest finding was laryngeal growth (33.33%).

Among histopathological (47pts) studies, commonest finding was squamous cell carcinoma in 70%.

The present study showed following aetiological factors for hoarseness of voice.

Laryngeal malignancy	:	33%
Chronic laryngitis	:	22%
Vocal cord paralysis	:	15%
Vocal cord nodules	:	10%
Tuberculosis of larynx	:	8%
Vocal cord papilloma	:	5%
Vocal cord polyp	:	3%
Vocal cord cyst	:	2%
Laryngeal trauma	:	2%

Laryngeal malignancy was the commonest cause of hoarseness of voice (33%) and males were commonly affected. Smoking was noted in all male patients with malignancy (80%), along with alcohol consumption in (70%) and chewing tobacco preparation in (65%) of cases.

Chronic laryngitis was next common cause for hoarseness of voice (22%) and was fond in 23% among males and 18% among females, with male to female ratio 1:1.

Vocal cord palsy was found in 15% of cases and was common in females (29%). Vocal cord nodules were found in 10% of patient and was common among female patients (24%) and with male to female ratio 1:2.

Tuberculosis was found in 8% all cases were males, with pulmonary tuberculosis and showed AFB on sputum examination.

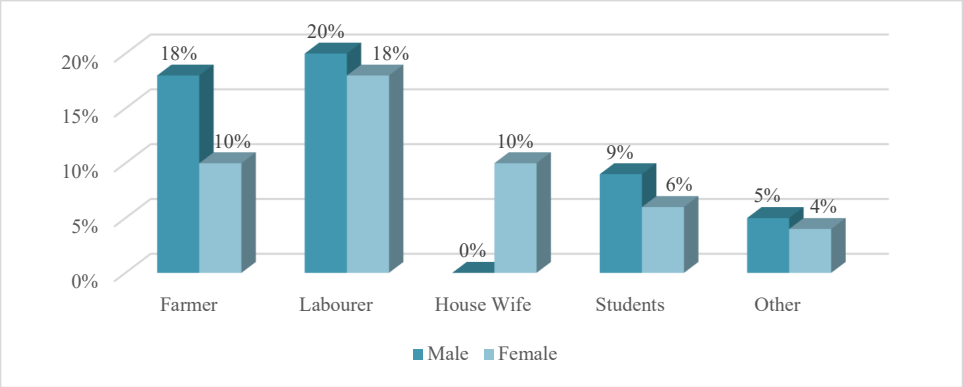


Figure 1: Occupation and sex wise distribution of hoarseness of voice.

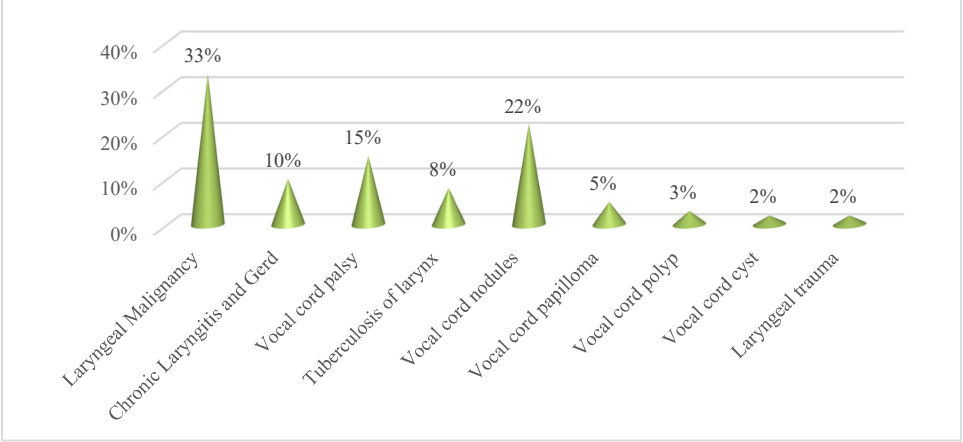


Figure 2: Aetiology of hoarseness of voice.

Table 1: Histopathology.

Histopathology	No. of cases (73pts)	Percentage
Squamous cell carcinoma (keratin pearls)	33	45.2
caseating granuloma and acid-fast bacilli	8	10.96
Hyperplastic stratified squamous epithelium with pigment laden macrophags	24	32.88
Squamous papilloma	5	6.85
Dense fibrin deposition with stellate cells with hemorrhages	3	4.11

Vocal cord papilloma presented in 5% of cases and females were commonly affected (66%) with male to female ration 1:2. Vocal fold polyp was found in 3% of cases, each one in male (33%) and two in female (67%). Vocal cord cyst was presented in 2% of cases. Two cases (2%) presented with laryngeal trauma.

Discussion

Hoarseness is considered a presenting symptom of a disease and not a disease by itself. It is one of the most common symptoms in conditions affecting laryngeal apparatus. Usually Benign pathologies are more common than malignant pathologies in patients with hoarseness and early diagnosis and treatment aids in good voice quality. Hoarseness lasting longer than two weeks needs complete evaluation to rule out malignancy as a cause.

A total 100 cases of hoarseness of voice were studied during study period. In the present study period incidence of hoarseness voice was 0.27% with laryngeal malignancy being the most common cause. while in a study by Shambhu Baitha (6) incidence of hoarseness was 0.32%, which nearly coincides with the present study.

Age and Sex: In the present study age of patients with hoarseness of voice ranged from 8 years to 78 years majority of patients i.e. 31 cases were in the group of 41-50 years and minimum number of patients i.e. 2 case was in the age of group of <10 years. In a study by Shambhu Baitha [6] majority of patients i.e. 31 cases (28.18%) were in the age group of 31-40 years. In a study by Swapan Ghosh [7] maximum patients i.e. 28 cases (28%) were in the age group of 21-30 years.

In our present study 66 patients were males and 34 patients were females, thus male to female ratio of approximately 2:1 was observed. This finding was similar to the study done by Shambhu Baitha [8] where males 74 cases (67.27%) and female 36 cases (32.72%) were noted. But in a study done by Parikh and Sudhir babu where the male to female ratio was 1:1. and 1.89:1, which was found to be little bit lower than the present study [8, 9].

Occupation: In the present study majority of patients of about 48 cases were of labourer class and least i.e. 5 cases were in the others group. In a study by Shambhu Baitha al the same observation was made i.e. majority of patients were of labourer class (36.36%). In the present study group among males and females labour classes, male was found to be predominated i.e. male 52.63% and female 48.30%. In females next common group was housewife (27.28%) least which was contrast to the study done by Swapan Ghosh where majority of patients were housewife (29%).

Socio economic status: In the present study, majority of patients (40.00%) belonged to low socio-economic status and also majority of patients among males (36.84%) and females (45.45%) belong to lower socio-economic status. Upper lower group was next common (30.00%), other groups with decreasing frequency were lower middle (18%), upper middle (8%) and upper (4%).

Residence: In the present study about 59 patients were from rural area and minimum cases of 41patients were from urban area. This was similar and coincides with the study done by Shambhu Baita [6] were predominantly patients were from rural areas comprising of 83 cases (75.5%).

Habits: Brock has mentioned inhaled irritants especially cigarette smoke as most important predisposing factors for hoarseness (10). In our present study commonest habit noted was smoking which was seen in 30 patients and the alcohol was the lowest habit seen in 8% of patients. The present study was similar to the study done Sambhu Baitha [6]. In the study done by Swapna Ghosh [7] vocal abuse was noted in 72% of cases which was contrast to the present study. In the study done by Shambhu Baitha [6] smoking was noted in 25.45% of cases, chewing tobacco preparation was noted in 17.27% and alcohol in 12.72% similar to the present study. Parik [3] has found that smoking was associated with hoarseness in about 20% of cases only and vocal abuse was found in 56% which was contrast to ours.

Videolaryngoscopic examination: On videolaryngoscopic examination commonest finding was – Ulcero-proliferative growth involving larynx and laryngo-pharynx which was seen in 41% of cases. And the rare finding is the vocal folds cyst and submucosal haemorrhage of vocal folds and false cords were noted in 2%. In a study by Shambhu Baitha [6], congestion of vocal cords noted in 34.54%, growth in only 9% of cases on IDL examination. This was contrast to the present study. This may be due to patients' habits.

Etiology of hoarseness of voice: In the present study, commonest etiology observed was malignancy of larynx and laryngopharynx in about 33% 0f patients. Among males commonest etiology was malignancy of larynx and laryngopharynx 29 cases (41.86%) and among

females' malignancy of larynx and laryngopharynx was only 11.77% (4 cases).Among patients with malignancy males were common 29 cases (92.86%) and females 4 cases (7.14%) with male to female ratio as 13:1. In study by Shambhu Bhaita [6] Incidence of malignancy was 14.54% with male to female ratio as 15:1. In a study done by Kadambari [11] incidence of malignancy was 18% and in study by Swapan Ghosh [7] incidence of malignancy was only 8% and in Parikh [8] incidence of malignancy was 12%. When compared to the other study, incidence of malignancy in patients presenting with hoarseness was found to be high this may be due to their socioeconomic status and habit related.

In the present study next common etiology was chronic laryngitis 22 cases. Among females it was common etiology 11cases (17.65%). Male to female ratio was found to be 1:1 as 11 cases of males had chronic laryngitis. In both studies of Parikh [8] and Shambhu Baitha [6] chronic laryngitis was commonest aetiology comprising of 48% in each, whereas in study by Swapan Ghosh [7], it was only 6% and in study by Kadambari [11] it was 8%. The present study was similar to the study done by Swapna Ghosh [7] and Kadambari [11] but contrast to the other studies like Parikh [8] and Shambhu Baitha [6].

Third common etiology was vocal cord paralysis noted in 15 cases. Among males the incidence was 9.30% (7 cases) and among female 29.41% (8 cases), with male to female ratio as 0.8:1. In study by Parikh [8], Kadambari [11] and Shambhu Baitha [6], It was only 3%, 9% and 9% respectively, with male to female ratio in Shambhu Baitha [6] study was 9:1. The present study had a very lower male female ratio. The present study showed clearly that females have a higher vocal cord palsy rate compared to male.

The fourth common etiology was vocal cord nodules, 10 cases with male to female ratio 1:2. Among males 4.65% (3 cases) were affected and among females 23.53% (7 cases) patients had vocal nodules. In all cases vocal nodules were bilateral.

Vocal nodules were the commonest aetiology in the studies done by Parikh [8] (50%) with males 43.3% and females 56.7% and also in Swapna Ghosh [8] with incidence of 30% with male to female ratio 1:1.5.

In study by Shambhu Baitha [6] incidence was only 12.72% with male to female ratio 1:1.3. in the present study also, it was clearly defined that vocal nodule is more common in female than male but the ratio is found to be higher when compared to other studies.

The fifth common etiology was tuberculosis of larynx 8 cases. All the patients were males with pulmonary tuberculosis. In study by Parik [8]· Shambhu Baitha [6] and Kadambari [11] incidence of tuberculosis was 23%, 5.45% and 1% respectively.

Vocal cord papilloma was found in 5 cases with male to female ratio as 1:2. Vocal cord polyp was presented in 3 patients with male to female ratio as 1:2 as one male and two female had vocal cord polyp. In the study done by Swapan Ghosh [7], Parikh [8] and Shambhu Baitha [6] incidence of vocal cord polyp were 23%, 15%, and 4.54% with male to female ratio as 3.6:1, in Swapan Ghosh Study [7] and 1:1.5 in Parikh et al study. Only Parikh [8] study was similar to the present study whereas in other studies the vocal cord polyp had a higher incidence than others. Other cases, which presented with hoarseness, were vocal cord cyst in 2 patients and laryngeal trauma in 2 patients which was similar to above study [8].

Conclusion

Thus, from this study it was found that the incidence of hoarseness of voice in the present study is 0.27%. Laryngeal malignancy (squamous cell carcinoma) was found to be the most common cause of hoarseness of voice (33%). Smoking was found to be the most common etiological factor for squamous cell carcinoma of Larynx (30%). In the present study, male patients were found to be affected more in laryngeal carcinoma than females with male female ratio of 13: 1. Rural population and patients in low socio-economic status were the most affected. Finally, it can be concluded that, the symptom of hoarseness of voice should not be ignored as it might be an indication of laryngeal malignancy. Any patient with hoarseness should be thoroughly investigated to rule out laryngeal malignancy, that might cause respiratory distress leading to life threatening complications.

What the study adds to the existing knowledge?

The importance of the present study is to highlight the importance of not neglecting hoarseness of voice lasting for more than a week.

Author's contribution

Dr. A. Dayanand: Concept, study design

Dr. Minu Madeswaran: Data analysis, manuscript preparation

Dr. S. Palaninathan: Guidance

Funding: Nil, **Conflict of interest**: Nil
Permission from IRB: Yes

References

1. Rosen CA, Anderson D, Murry T. Evaluating hoarseness: keeping your patient's voice healthy. Am Fam Physician. 1998;57(11):2775-2782.

2. Dettelbach M, Eibling DE, Johnson JT. Hoarseness. From viral laryngitis to glottic cancer. Postgrad Med. 1994; 95(5):143-6, 151, 154 passim.

3. Chagnon FP, Mulder DS. Laryngotracheal trauma. Chest Surg Clin N Am. 1996;6(4):733-748.

4. Smit CF, van Leeuwen JA, Mathus-Vliegen LM, Devriese PP, Semin A, Tan J, et al. Gastropharyngeal and gastroesophageal reflux in globus and hoarseness. Arch Otolaryngol Head Neck Surg. 2000;126(7):827-830. doi: 10.1001/archotol.126.7.827

5. von LEDEN. The electronic synchron-stroboscope: its value for the practicing laryngologist. Ann Otol Rhinol Laryngol. 1961;70:881-93. doi:10.1177/000348946107000319

6. Baitha S, Raizada RM, Singh AK, Puttewar MP, Chaturvedi VN. Predisposing factors and aetiology of hoarseness of voice. Indian J Otolaryngol Head Neck Surg. 2004;56(3):186-190.

7. Ghosh SK, Chattopadhyay S, Bora H, Mukherjee PB. Microlaryngoscopic study of 100 cases of hoarseness of voice. Ind J Otolaryngol Head Neck Surg. 2001;53(4): 270-272. doi: 10.1007/BF02991545.

8. Parikh NP. Aetiological study of 100 cases of hoarseness of voice. Ind J Otolaryngol. 1991;43(2):71-73. doi: https://doi.org/10.1007/BF02992547.

9. Sudhir babu and Shamsheer Shaik. Hoarsness of voice: A retrospective study of 251 cases: Int Jor Adv R. 2016;4(2):227-234.

10. Vinod Shah. Probodh Karnik. Otolaryngology Review 2000;160-165.

11. Batra K, Motwani G, Sagar PC. Functional voice disorders and their occurrence in 100 patients of hoarseness as seen on fibreoptic laryngoscopy. Ind J Otolaryngol Head Neck Surg. 2004;56(2):91-95. doi: 10. 1007/BF02974305.

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A Study on Prevalence of Hearing Loss as a Complication of Diabetes

A. Dayanand, J. Dheebika, S. Prathula and S. Palaninathan

***Abstract* —**

***Index Terms* —**

I. Introduction

Hearing impairment is the most frequent sensory deficit in human populations, affecting more than 250 million people in the world. Diabetes Mellitus is a metabolic disorder, due to relative or absolute lack of insulin resulting in elevated blood glucose levels associated with long term vascular and neurological complications. Among glucose metabolism disorders, diabetes mellitus is the one most commonly related with auditory disorders. The complex arrangement of inner ear makes it potential target of hyperglycemic damage. Diabetic patients are more prone to complications of hyperglycemic, as all body cells are exposed to high levels of plasma glucose. The organ of corti cells are important structures for hearing mechanism and turn out to be the potential target for damage, due to high glycemic levels, micro vascular compromise, their complex structure and arrangement. Thus, screening of these patients at a high risk of developing sensory neural hearing loss, will aid in early diagnosis and management.

II. Aim Of The Study

- To Study the prevalence of Hearing loss its association with Diabetes.
- To assess the hearing loss in diabetics and correlate it with age and duration of diabetes.

III. Materials

This is a cross-sectional study in which evaluation of study population who were diagnosed with diabetes mellitus study method undergoing treatment on Out-Patient or In-Patient basis in department of ENT and Endocrinology. In PSG Institute of Medical Science & Research. Coimbatore, Tamilnadu from July 2012 to July 2014 were considered.

- History of every patient was taken in detail and importance was given on their presenting complaints. Any significant past history of ear discharge. Hearing loss or previous ear surgeries were elicited. History of duration of diabetes and mode of treatment were given importance.
- All the patients were taken up for Otoscopic examination, in which the ear canal and status of tympanic membrane were assessed.

Inclusion Criteria for cases considered in the study

- Known cases of Diabetes mellitus
- Age-group > 30 years to < 60 years
- Both genders

Exclusion Criteria for cases

- Individuals involved in occupations exposing to loud noise.
- Individuals with previous history of ear discharge/hearing loss/ tinnitus/ear surgery
- Otoscopic examination showing any ear pathology like wax, discharge, retracted tympanic membrane, perforated tympanic membrane or tympanosclerosis.
- Patients unwilling to comply with the study.

IV. Methodology

All 200 patients were worked up with a pure tone audiometry. The audiogram was analyzed for hearing loss and if present it will be classified according to WHO classification. Pure Tone Audiometry is a routine audiometric evaluation and the resulting pure tone audiogram is used as a basic description of the degree of hearing loss. Audiological examination was performed using a Pure Tone Audiometer model GS1 61, in a soundproof room in the ENT department, PSG Institute of Medical Sciences & research. Earphones were used to test hearing by air conduction and a small vibrator, which was placed over the mastoid was used to test hearing by bone conduction. All audiometers are incorporated with a calibration circuit, which allows the output sound level to be set at particular frequency. The signals presented to the subject by the audiometer were all characterized by its sound pressure level, frequency and wave form which were all delivered at

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controlled rates.

The entire procedure and purpose of the test was explained in detail to the patients. Biological calibration of the system was done on daily basis before starting the procedure. Both air and bone conduction were tested for each ear.

V. RESULT

In this study, analysis of 200 patients diagnosed mellitus was done in the Department of E.N.T, PSG Institute of Medical Sciences& Research from July 2012 to July 2014 to determine the incidence of hearing loss as a complication of diabetes mellitus. Detailed evaluation of each case was done comprising of the history, clinical examination including otoscopic examination with otoscopic and pure tone audiometry. The clinical data was collected by means of a proforma and the observations from the audiogram were analyzed with the Master chart as shown in the Annexure. In our study of 200 diabetic patients, 110 (55%) cases out to have sensorineural hearing loss.

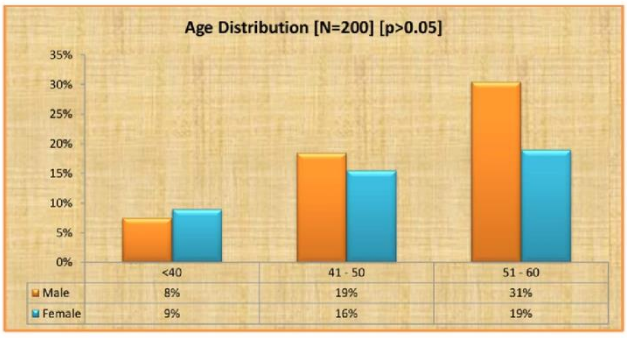


Fig.1. Age distribution of hearing loss.

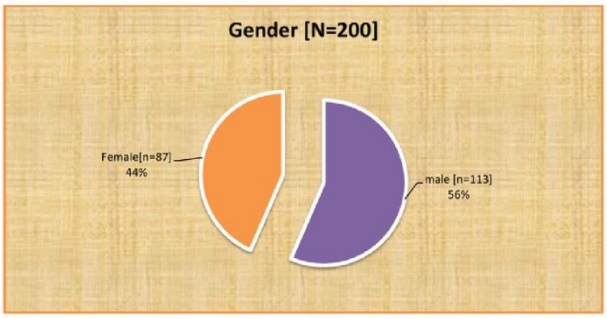


Fig. 2. Gender distribution.

TABLE I: OVERALL DISTRIBUTION OF HEARING LOSS

Hearing Loss	n	(%)
Bilateral – Normal hearing	90	45
Bilateral –hearing loss	95	47
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Left	11	6
Right	4	2
Total	200	100

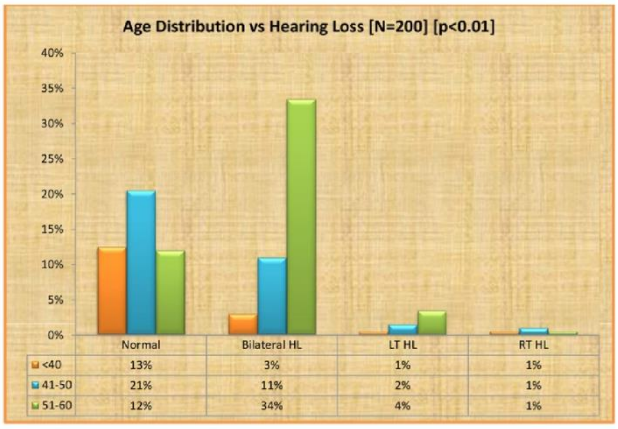


Fig. 3. Hearing loss in various Age groups.

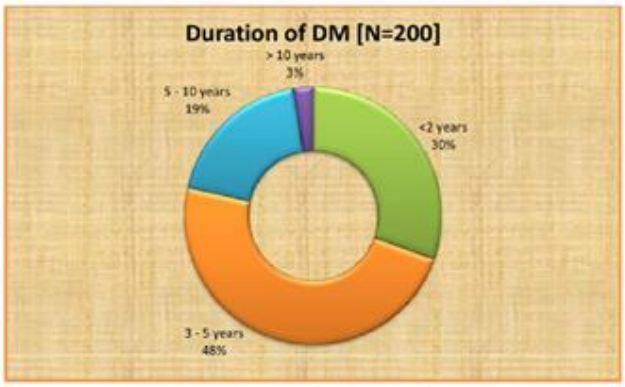


Fig. 4. Duration of DM in study population.

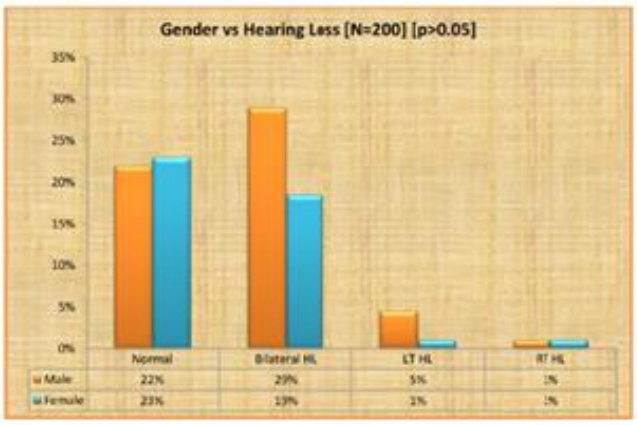


Fig. 5. Gender vs Hearing loss.

TABLE II: DISTRIBUTION OF HEARING LOSS WITH DURATION OF DM

	Mean DM Duration of Hearing Loss						
				95% CI or Mean			
	N	Mean	SD	Lower Bound	Upper Bound	Minimum	Maximum
Normal	90	3	3	2	4	1	20
Bilateral SNHL	95	5	3	5	6	1	25
LT HL	11	5	4	3	7	2	15
RT HL	4	5	3	1	9	1	6
Total	200	4	3	4	5	1	25

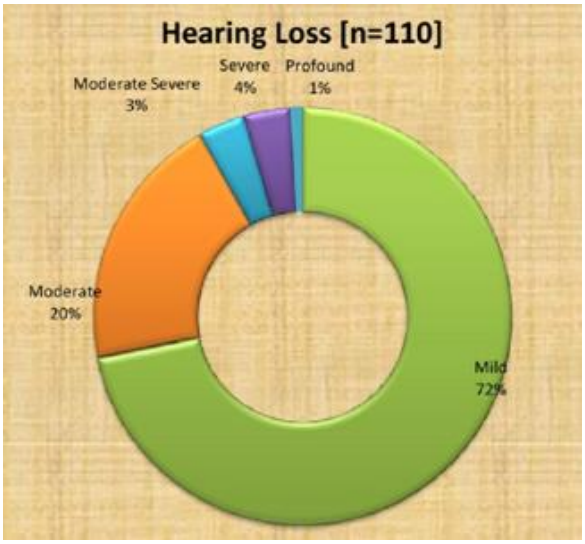


Fig. 6. Degree of Hearing Loss.

VI. Discussion

Hearing loss is important of hearing and its severity varies from mild to severe or profound and in general hearing loss may be conductive, sensorineural, or mixed. The typical hearing impairment described in diabetic patients is a bilateral sensorineural hearing loss. Diabetes mellitus has been implicated as independent causative factor of sensorineural hearing loss [14].

A. Age

Our study has evaluated the hearing loss in the patients with diabetes and the influence of hyperglycemia on hearing and it shows 55% incidence of deafness. And the peak age group being affected with hearing loss in our study was between 50-53 years of age.

In contrast a study conducted by Rajendran S. Anandhalakshmi et a1 [16] showed maximum incidence between 40-50 years of age. On further review, we realize that acquired on set sensorineural hearing loss can occur early in diabetic patients. Usually age-related sensorineural deafness is more common in people more than 60 years of age. But here it is seen that diabetic patients are higher risk of developing this sensory loss at a much younger age group.

It is also that in our study there were more people with diabetes among the age group 51-60 years of age (50%). The p value is 0.05, which makes it statistically significant.

B. Sex

In our study it was noted that a greater number of diabetic males were affected with hearing loss, than females. In contrast Taylor and Irwin (1978) observed that female patients with diabetes had significantly greater hearing loss when compared with male patients with diabetes.

But according to Cullen and Cinnamond [17], Male patients with diabetes had worse hearing than female patients with diabetes.

From our study, we conclude that sex of an individual is not statistically significant ($p>0.05$) parameter in considering the males are more prone to develop Diabetes mellitus.

C. Overall incidence of hearing loss in diabetics

In our study, we evaluated the hearing loss in the patients and the influence of hyperglycemia on hearing and it shows a 55% incidence of deafness. Friedman at al [18] showed a 55% incidence of hearing loss in diabetic patients. Which is the same incidence found in our study.

Kakarlapudi et a1 [19] found that hearing loss was more common in diabetic patients (13.1%) than the control nondiabetic healthy subjects.

Wenget a1 [20] noted that among the 67 diabetic subjects examined, 44.8% of them had profound hearing loss. This is much lesser comparing to our study.

D. Duration of diabetes

In our study a greater number of patients with diabetes were found 3-5 years group, about 48%, followed by less than 2 years duration 31%.

Comparing with, the duration of diabetes and hearing loss, more patients in the group 3-5 years had hearing loss, around 49%, followed by less than 2 years, around 32 years. Whereas only 20% patients were found to have hearing loss in 5-10 years group.

In study it is seen that the duration of diabetes is not a significant criteria for developing sensoineural hearing loss. The p value is <0.05, making it statically significant.

A study by Celik et al [21] observed that as the duration of diabetes increased to 15 years, the incidence of hearing loss also increased. After 15 years of diabetes, the influence on hearing loss was not significant. This change was not observed in our study as cases in our study were all mostly found to be less than 15 years.

E. Overall severity of hearing in individual ear

It is noted that in the right ear 95 cases had normal hearing, 84 cases had mild hearing loss, 15 had moderate and 3 cases had moderately severe and severe hearing loss respectively.

It is noted that in the left ear, 92 cases had normal hearing, 81 had minimal hearing loss, 16 had moderate loss 8 moderately severe, 2 with severe and 1 with profound hearing loss. Thus, making it statically not significant ($p>0.05$). The above data was calculated taking the gender as consideration and compared the hearing. But no significant changes were noted in severity of hearing.

Taking into consideration the duration of diabetes, it is noted that in right ear maximum of 58 (30%) cases had hearing loss among the group 3-5 years, among which 49 (25%) cases had mild hearing loss; 6 (3%) cases had moderate hearing loss and 3 cases (2%) had moderately severe loss, followed by 5-10 years group where 31 cases (16%) had hearing loss

And in left ear it is noted that , maximum of 59(30%) cases had hearing loss among the group 3-5 years, among which 44(22%) cases had mild hearing loss; 8 (4%) cases had moderate hearing loss and 5 cases (3%) had moderately serve loss, followed by 5-10 years group where 32 cases (17%) had hearing loss.

Thus, we conclude that predominantly mild sensorineural hearing loss in both ears. The p value is <0.01, making it statistically significant.

F. Overall severity of hearing loss

In our study, 72% of individuals with diabetes were noted to have mild sensorineural hearing loss, followed by 20% with moderate sensorineural hearing loss. Only 1% had profound hearing loss.

A study conducted by Rajendran at al showed a result similar to our study. The diabetic individuals showed significant high frequency, bilateral, mild to moderate sensorineural hearing loss (73.3%) as compared to controls of similar age.

Whereas Weng at al [14] observed that among the 67 diabetic subjects examined, 44.8% of them had profound hearing loss.

Many studies were conducted to observe the pathogenesis of this sensorineural loss observed in diabetics. During this course, many studies suggested that diabetes is a cause of hearing loss. The probable mechanisms suggested were microangiopathy in the inner ear, neuropathy of cochlear nerve, or even a combination of both. Outer hair cell dysfunction and disruption of endolymphatic potential were noted in some noted in some studies. Some of the tissue effects of diabetes are considered to be related to the polyol pathway, in which glucose is reduced to sorbital.

Generally, the accumulation of Sorbitol is implicated in neuropathy as it causes a decrease in myo inositol content and further leads to decrease in Na+K+ATPase activity [22].

Makishima and Tanaka [23] observed that there was serere atrophy of the spiral ganglion in the basal lamina and the middle turns of cochlea in diabetic individuals with sensorineural hearing loss. It was also observed that 8th nerve reviled changes in the myelin sheath, which showed degeneration and fibrosis of perineurium

Jorgensen [24] in his story noted thickening in the walls of the vasa nervorum in the 8th nerve which lead to the development of acoustic neuropathy.

In the study by Wackym and Linthicum [25] noted small microanogopathic changes in the regions of endolymphatic sac, the stria vascularis and basilar membrance.

Van den Ouweland et al [26] observed mutational changes in the mitochondrial RNA. It was also observed that in a small subset of patients, with maternally inherited diabetes showed some degree of sensorineural hearing loss.

Lisowska et al [27] in their study noted that in diabetic patients, there was abnormality in the function of hair cells in auditory brain stem responses.

Fukushima et al [28] concluded that Type 2 Diabetes lead to changes in cochlea, like significant atrophy of stria vascularies and basal turn, which was the likelihood of hearing loss in their patients.

In our study, we conclude that after analyzing the audiograms of the diabetic patients, it is noted that they are more prone to develop mild to moderate sensorineural hearing loss. The duration of diabetes as well as sex of the individual were found to have no effect on the incidence of hearing loss.

REFERENCES

[1] Dhingra P.L. Diseases of Ear, Nose and Throat, Elsevier edition, 2009.
[2] Colin Mathersl. Andrew Smith2, Marisol Concha3; Global burden of hearing loss in the year 2000.
[3] World Health Organization. World Health Report 2001. Mental health; New Understanding, New Hope, Geneva, World Health Organization 2001.
[4] WHO Study Group. Diabetes mellitus. Technical Report Series 727. Geneva; World Health Organiztion, 1985.
[5] Savage PJ. Bennion LJ. Bennett PH. Normalization of insulin and glucagon secreation in ktosis-resistant diabetes mellitus with prolonged diet therapy. J Clin Endocrinol Metab 1979;49;830 (1)-833.
[6] Agner T. Damm p.Binder C. Remission in IDDM; prospective study of basal C-peptide and insulin dose in 268 consecutive patients. Diabetes Care 1987.
[7] Gauin JR III, Alberti KGMM, Davidson MB, et al. Report of the Expert Committee on the Diagnosis Mellitus Diabetes care l997;20.1183-1197.
[8] Tanks S. Kobayashi T. Momostu T. A novel subtype 1 diabetes mellitus. NEng J Med 2000;342;1835-1837.
[9] 9) Tuner Re. Cull CA, Frighi V, et al. Glycemic control with diet, suphonyl urea. Metformin, or insulin in patients with type 2 DM; Progressive requirement for multiple therapies. UK Prospective diabetes study group. JAMA 1999;281;2005-2012.
[10] King H. Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. Diabetes care 1993;16;617-621.
[11] Boyyko EJ, de courten M, Zimmet PZ, et al, Features of the metabolic syndrome predit higher risk of the diabetes and impresseded glucose tolerance; a prospective study in Maurtitus, Diabet Med 2003;20;915-920.
[12] Gareth Williams John C.Pickup. Text book of diabetes mellitus. Blackwell publishing Ltd, Oxford, Third edition, 2003.
[13] Fukushima H, Cureoglu S.Schachern PA , Paparella MM, Harada T. Oktay MF, Effects of type 2 diabetes mellitus on cochlear structure in humans, Arch Otolaryngol Head Neck Surg.2006;132(9);934-8.
[14] Maia CA, Campos Ca Diabetes mellitus as etiological factor of hearing loss. Rev Bras Otorrinolaringol. 2005;71;208-214.
[15] Taylor,I,G,Irwin,J. Some audiological aspects of diabetes mellitus, J.Laryngol Otol.1978;92;99-113.
[16] Rajendran s, Anandhalakshmi, Mythili B, ViswanTHA Rao Evaluation of the Incidence of Sensorineural hearing loss in Patients with Type 2 Diabetes Millitus, Int J Biol Med Res.2011;2(4); 982-987.
[17] Cullen JR. Cinnamond MJ. Hearing loss in diabetes. Int J Laryngol Otol.1993;107(3);179-82.
[18] Friedman SA, Schulman RH, Weiss S. Hearing and diabetic neuropathy, Arch Intern Med. 1975;135(4);573-6.
[19] KAkarlapudii V. Sawyer R, Staeeker H. The effect of diabetes on sensorineural hearing loss Otol Neurotol. 200;24(3); 382-6.
[20] Weng SF, Chan YS , Hus CJ, Tseng FY, Clinical features of sudden sensorineural heraring loss in diabetic patients Laryngoscope 2005;115(9); 1676-80.
[21] Celik O, Yalcin S, Celebi H, Ozturk A, Hearing loss in insulin dependent diabetes mellitus. Auris Nasus Larynx 1996;23;127-32.
[22] Dennis Kasper, Anthony S, Fauci, Dan L.Longo, Eugene Braunwald, Stephen L. Hauser, J. Larry Jameson and Joseph Losecalzo, Harrison's Principles of Internal Medicine, MeGraw-Hill Companies Inc. United States of America 17 th Ed.2008.
[23] Makashima, K., Tanaka, K. Pathological changes of the inner ear and central auditorypathways in diabetics. Ann Otol Rhinol Laryngol.1971;80;218-288.
[24] Jorgensen,M.B>.The inner ear in diabetes mellitus. Arch Otolaryngol Head Neck Surg;1961;74;31-39.
[25] Wackym. P.A. Linthicum F.H. (1986) Diabetes mellitus and hearing loss, Clinical and histopathologic relationship. Am. J. Otolaryngol. 1986;7;176-1823.
[26] Van den Ouweland JM, Lemakes HH Gerbitz KD, maassen JA, Maternally inherited diabetes and deafness (MIDD); A distinet subtype of diabetes associated with a mitochondrial t RNALeu (urr) gene point mutation. Muscle Nerve. 1995;3;s124-30.
[27] Lisowska G.Namyslowski G. Morawski K, Strojek K. Otoacoustic emissions and auditory brain stem responses in insulin dep[endent diabetic patients. Otolaryngol Pol.2002;56(2);217-25.
[28] Fukushima H.Cureoglu S.Schachern PA, Paparella MM, Harada T,Oktay MF. Effects of type 2 diabetes mellitus on cocochlear structure in humans, Arch Otolaryngol Had Neck Surg. 2006; 132(9).

[29] Fangchao Ma, Orlando Gomez – Marin., David J.Lee, Thomas Balkany . Diabetes and hearing impairment in Mexican American adults; a population – head study, J.Laryngol Otol. 1998;112'835-839.
[30] Gibbin KP, Davis CG, A hearing survey in diabetes mellitus ClinOtaryngol Allied Sei. 1981;6(5);345-50.
[31] Austin DF, Konrad- Martin D, Griest S, Memillan GP, MeDermott D, Fausti S. Diabetes related changes in hearing. Laryngoscope.2009;119(9);1788-96.
[32] Sakuta H, Suzuki T. Yasuda H, Ito T.Type 2 Diabetes and hearing losss in personnel of the Self-Defense Forces. Diabetes Res Clin Pract, 2007;75(2); 229-34.

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Original Research Article

Study on temporalis fascia graft versus temporalis fascia with cartilage graft

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Abstract

Background: The aim of this analytical study is to compare the hearing improvement in type I Tympanoplasty between Temporalis Fascia used alone and temporalis Fascia with cartilage as graft material. **Material and Methods:** The study was done in ENT department at PSGIMSR, from October 2016 to July 2018. A total of 21,453 cases attended the ENT Outpatient department during the study period. Out of these, 75 cases with dry perforation of the tympanic membrane were chosen for the study. Patients were selected in random, excluding ear discharge, Attico-antral disease, and complications of chronic otitis media. **Results:** In the study population, disease afflicting the nose and throat were also ruled out. Detailed evaluation of each case was done comprising of history taking, clinical examination, investigations including pure tone audiometry. Patients were divided into 2 groups. Group A consist of 50 patients underwent type I Tympanoplasty with temporalis fascia alone. Group B consist of 25 patients underwent type I Tympanoplasty with combined Temporalis Fascia & Conchal Cartilage. Post-operative hearing improvement measured by Audiometry is compared among the two groups and found that small perforations had best improvement with Temporalis Fascia graft, large and subtotal perforation had good improvement in postoperative hearing with combined Temporalis Fascia and Conchal Cartilage graft. **Conclusion:** Mean hearing improvement in Group A with temporalis graft was 12.98 dB (better) than Group B, with Temporalis Fascia & Cochal Cartilage graft mean hearing improvement was 8.96 dB.

Keywords: Temporalis Fascia, Conchal Cartilage, Type I Tympanoplasty.

Introduction

Tympanoplasty is done primarily to achieve a dry ear by eradicating middle ear disease and hearing improvement by closure of any Tympanic Membrane perforation by grafting with/without Ossicular reconstruction. The result of Tympanoplasty is measured by the success or failure of the graft uptake and hearing improvement. Various graft materials had been used by trial and error. These materials ranged from skin grafted from other areas of the body such as Thiersch graft, Split-skin graft, Pedicle graft from canal skin, Vein graft, Temporalis fascia graft, Scleral and Corneal graft, Perichondrial tissue, Cartilage [1-5].. Cartilage graft also provides firm support to prevent retraction formation. The greatest advantage of the Cartilage graft is its very low metabolic rate.

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Cartilage receives its nutrients by diffusion, is easy to work with because of its pliability, and its resistance to deformation from pressure variations.

Perichondrium and Cartilage [2] share with fascia the quality of being mesenchymal tissue. Cartilage are inherently thicker and stiffer compared to temporalis fascia. They mechanically reduce the vibratory pattern of the tympanic membrane in response to sound waves, contributing to some impairment in functional results.

This phenomenon is seen more in relation to higher tones. The mass effect of the cartilage over the prosthesis is always a concern. Cartilage has comparatively lower compliance than fascia. Cartilage is preferred in case of large or anteriorly placed perforations or associated eustachian tube dysfunction but at the cost of delayed hearing restoration for six months.

Temporalis fascia is generally considered to be superior with respect to the take-up rate.

Materials and Methods

Objective

1. To assess the hearing outcome after Type-1 Tympanoplasty.
2. To compare the hearing improvement by the type of material used for graft
3. To evaluate the results of this study and compare it with similar studies

Study design: Analytical and follow up study.
Period of study: October 2016 to July 2018
Study population: Patients of 20-60 years age group.
Study area: Department of Otorhinolaryngology, PSGIMS&R
Sample size: 5 Individuals.

Justification : This sample size correlates with the amount of patients with similar disease profile consulting at the ENT OPD

Subjects and selection method: 75 patients with perforated tympanic membrane due to Chronic Otitis Media, Trauma, recurrent middle ear infection presenting to ENT outpatient department at PSG IMS&R will be taken up for study.

As soon as the patient presents to the hospital, detailed clinical history and examination will be carried out as per the proforma prepared. Laboratory investigations will be done. All patients will be subjected to pre-operative Audiometric evaluation (PTA).

Type 1 Tympanoplasty will be done using different graft materials once the pre-operative investigations are noted and extent of disease established.

Follow up with audiometric evaluations will be conducted at the end of 3rd month following surgery and results will be compared with pre-operative Audiogram results

Inclusion criteria

Patients of age between 20 to 60 years presenting with

1. Perforated tympanic membrane due to Chronic Otitis media, Trauma, Recurrent middle ear infection
2. Intact ossicular chain
3. Dry ear
4. Intact Eustachian tube function

Exclusion criteria

1. Patients with Sensory neural hearing loss (SNHL)
2. CSOM with attico-antral type of disease.
3.Tympanosclerosis, middle ear Atelectasis, Middle ear tumours, Congenital Cholesteatoma.

Potential benefits

1. Improvement in hearing outcome of patients, will be helpful in proper diagnosis and better treatment of patients having conductive hearing loss.
2. Knowledge of the level of improvement of hearing and the rate of healing using different graft materials will be known.

Risks

1) Graft Failure
2) Minimal discomfort to the patient at the time of audiological testing performed post surgery.

Method of collection of data

Group A – 50 patients in which Temporalis Fascia was used as graft material
Group B– 25 patients in which Conchal Cartilage with fascia used as graft material

Results

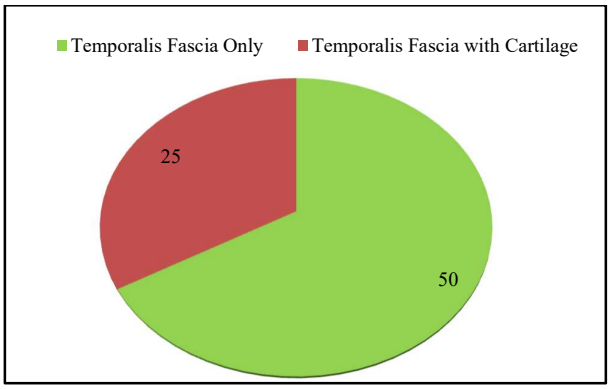


Figure 1: Distribution of graft material.

In a total of 75 patients, temporalis fascia was used as graft material in 50 patients (66.7%) and Conchal cartilage with fascia was used as graft in 25 patients (33.3%) (Figure 1).

Table 1: Surgical Approach

			Surgical approach		Total
			Endaural	Retroauricular	
Type of Perforation	Small	No	12	17	29
		%	41.4%	58.6%	100.0%
	Medium	No	2	24	26
		%	7.7%	92.3%	100.0%
	Large	No	0	16	16
		%	.0%	100.0%	100.0%
	Sub-total	No	0	4	4
		%	.0%	100.0%	100.0%
Total		No	14	61	75
		%	18.7%	81.3%	100.0%

Retroauricular approach was used in 24 cases (92.3%) with medium perforation of the tympanic membrane, which was the most. Endaural approach for surgery was used in 12 patients (41.4%) with small perforations of the tympanic membrane, which was the most (Table 2).

Table-2: Type of perforation.

	Frequency	Percent
Small	29	38.7
Medium	26	34.7
Large	16	21.3
Sub-total	4	5.3
Total	75	100.0

In a total of 75 cases, small perforation of the tympanic membrane was present in 29 individuals (38.7%) which was the highest. Subtotal perforation was present in 4 individuals (5.3%) which was the low (Table 3).

Table-3: Graft material

			Surgical approach		Total
			Endaural	Retroauricular	
Type of Perforation	Small	No	12	17	29
		%	41.4%	58.6%	100.0%
	Medium	No	2	24	26
		%	7.7%	92.3%	100.0%
	Large	No	0	16	16
		%	.0%	100.0%	100.0%
	Sub-total	No	0	4	4
		%	.0%	100.0%	100.0%
Total		No	14	61	75
		%	18.7%	81.3%	100.0%

Graft material used based on type of perforation- 29 Individuals had a small perforation of the tympanic membrane. Temporalis fascia was used in all 29 cases (100%). In cases with medium perforation, 19 cases (73.1%) had temporalis fascia as graft and 7 cases (26.9%) had fascia with cartilage as graft.

In cases with large perforations, 2 cases (12.5%) had temporalis fascia and 14 cases (87.5%) had fascia with cartilage as graft. All 4 individuals (100%) had cartilage with fascia as graft material in subtotal perforation.

Table-4: Graft material used based on surgical approach.

			Surgical approach		Total
			Endaural	Retroauricular	
Type of Perforation	Small	No	12	17	29
		%	41.4%	58.6%	100.0%
	Medium	No	2	24	26
		%	7.7%	92.3%	100.0%
	Large	No	0	16	16
		%	.0%	100.0%	100.0%
	Sub-total	No	0	4	4
		%	.0%	100.0%	100.0%
Total		No	14	61	75
		%	18.7%	81.3%	100.0%

Out of the total 75 cases, both endaural and retroauricular approach for surgery was used. Temporalis fascia was used in 50 cases (66.7%), out of which endaural approach and retroauricular approach was used in 14 cases and 36 cases respectively (Table 4).

Temporalis fascia with cartilage graft was used in 25 cases (33.3%) in which all cases were operated using retroauricular approach.

Table-5: Correlation between post-operative hearing and graft material.

Post-operative hearing (db) * Graft Material					
			Graft Material		Total
			Temporalis Fascia Only	Temporalis Fascia with Cartilage	
Post-operative hearing (db)	0-10	No	16	0	16
		%	100.0%	.0%	100.0%
	11-20	No	28	0	28
		%	100.0%	.0%	100.0%
	21-30	No	5	9	14
		%	35.7%	64.3%	100.0%
	31-40	No	1	13	14
		%	7.1%	92.9%	100.0%
	41-50	No	0	3	3
		%	.0%	100.0%	100.0%
Total		**No**	**50**	**25**	**75**
		%	**66.7%**	**33.3%**	**100.0%**

Post-operative hearing when temporalis fascia was used as graft material was 11-20 dB in 28 individuals which was the highest and was lowest in 1 individual (7.1%) with post-operative hearing of 31-40 dB (Table 5).

In the group in which cartilage with fascia was used, highest individuals was 13 (92.9%), where the post-operative hearing was 31-40 dB. The post-operative hearing was 41-50 dB in 3 individuals which was the lowest.

Table 5: Hearing improvement based on graft material.

Post-operative hearing (db) * Graft Material					
			Graft Material		Total
			Temporalis Fascia Only	Temporalis Fascia with Cartilage	
Improvement (db)	0-5	No	4	5	9
		%	44.4%	55.6%	100.0%
	6-10	No	7	11	18
		%	38.9%	61.1%	100.0%
	11-15	No	26	9	35
		%	74.3%	25.7%	100.0%
	16-20	No	11	0	11
		%	100.0%	.0%	100.0%
	21-25	No	2	0	2
		%	100.0%	.0%	100.0%
Total		**No**	**50**	**25**	**75**
		%	**66.7%**	**33.3%**	**100.0%**

When temporalis fascia alone was used as graft, Post-operative hearing improvement was observed in 26 individuals (74.3%) with an improvement of 11-15 dB. In the group with fascia and cartilage graft, the highest individuals was 11 (61.1%) with an improvement of 6-10 dB (Table 6).

The most improvement in hearing when temporalis fascia is used, was observed in 2 cases (100%) with 21-25 dB of hearing improvement. 11-15 dB was the maximum improvement observed when fascia with cartilage was used in 9 cases (25.7%).

Table 7: Post-operative hearing improvement.

Improvement (dB)			
Graft Material	Mean	N	Std. Deviation
Temporalis Fascia Only	12.9800	50	4.20636
Temporalis Fascia with Cartilage	8.9600	25	3.24654
Total	**11.6400**	**75**	**4.33290**

Mean hearing improvement in Group A was 12.98 dB and in Group B, mean hearing improvement was 8.96 Db (Table 7).

Discussion

A total of 21,453 cases attended the ENT Outpatient department during the study period. Out of these, 75 cases with dry perforation of the tympanic membrane were chosen for the study. Patients were selected in random, excluding patients with active ear discharge, attico-antral disease, and complications of chronic otitis media. In the study population, disease afflicting the nose and throat were also ruled out.

Detailed evaluation of each case was done comprising of history taking, clinical examination, investigations. Pre-operative as well as hearing assessment after the patients underwent type-1 tympanoplasty was performed using underlay technique.

Tympanic membrane perforation: Tympanic membrane perforation was present in all 75 patients in the study. Ear discharge was not seen in any of the patients. Based on the quadrant of tympanic membrane involved, perforation was graded into small, medium, large and subtotal perforation. Perforation of the Pars Flaccida was ruled out in all 75 of the participants in this study [6-10].

In the present study of 75 participants, small perforation was seen in 29 cases (38.6%), medium perforation was present in 26 cases (34.6%), large perforation was present in 16 cases (21.3%) and subtotal perforation was present in 4 cases (5.3%).

In a study done by Kshitij Patil [1], maximum number of cases had subtotal perforation, i.e., 59 cases (49.17%), followed by large central perforation in 51 cases (42.5%), while 10 cases (8.33%) had total perforation.

Surgical approach: In the present study, both endaural and retroauricular approach were used for different patients. 61 patients (81.3%) underwent surgery through retroauricular approach and 14 patients (18.7%) underwent surgery by endaural approach.

In the study done by Ramagiri Vijay Kumar [11], in patients of group A 14 (70%) were operated by postaural route, 5 patients (25%) by permeatal route and 1 patient (5%) by endaural route. 13 patients (65%) of group B were operated using endaural route and 7 patients (35%) by using postaural route. All the patients of group C were taken by permeatal route. So permeatal route was used in 63.3% of all cases.

Graft Material: The study participants of the present study were divided into two groups based on usage of graft material namely temporalis fascia alone and temporalis fascia used along with conchal cartilage. In the present study, 50 individuals (60.66%) underwent type-1 tympanoplasty using temporalis fascia alone as graft material. 25 individuals (39.44%) underwent surgery by using temporalis fascia along with conchal cartilage.

The usage of graft material and size of perforation had a correlation in the present study [12]. Temporalis fascia was used to close small and medium sized perforations. Small perforations of the tympanic membrane was present in 29 cases (100%), temporalis fascia alone was used in all of those cases.

In patients with medium perforation of the tympanic membrane, out of a total 26 cases 19 individuals (73.1%) underwent closure of perforation using temporalis fascia alone and in 7 individuals (26.9%) temporalis fascia with conchal cartilage was used as graft material.

In a study done by Ramagiri Vijay Kumar [11] participants were split into 3 groups of 20 patients in each group. Grouping of patients were based on graft material used, i.e. temporalis fascia, tragal perichondrium and ear lobule fat graft into group A, B and C respectively

Pre-operative hearing: In a total study population of 75 individuals, the participants were divided into group A and group B based on usage of graft material to repair the tympanic membrane perforation. Patients in Group A underwent type-I tympanoplasty with temporalis fascia used as graft material, in Group B patients were operated with conchal cartilage and fascia used as graft.

The pre-operative hearing in dB was noted for all participants before surgery. The maximum number of participants i.e. 27 patients (36%) had a pre-operative hearing loss of 21-30 dB. The least number was 5 patients (6.66%), who had a pre-operative hearing loss of 51-60 dB.

In group A, the total number of participants were 50 individuals (66.7%) who underwent surgery using temporalis fascia as graft. In that group, the mean pre-operative hearing was 26.9 dB. In group B, where conchal cartilage with fascia was used as graft material, the mean pre-operative hearing was 42.7 dB.

In a study done by Kshitij Patil [1], the mean pre-operative hearing loss was found to be maximum for cases with total perforations which were 49.15±7.34 dB, followed by subtotal perforations of 35.38±10.10 dB and for large perforations it was 31.73±9.84 dB. The overall mean pre-operative hearing loss was 34.72±10.4 dB.

Post-operative hearing: In the present study, group A participants who underwent type-1 tympanoplasty using temporalis fascia as graft, the highest improvement in post-operative hearing was 21-25 dB observed in 2 patients. In the same group, the greatest number of participants with hearing improvement was 26 patients (74.3%), with a hearing improvement of 11-15 dB.

In group B conchal cartilage with fascia was used as graft material. Here, the highest improvement in post-operative hearing was observed in 9 patients (25.7%) with hearing improvement of 11-15 dB. The most number of participants with hearing improvement was 11 patients (61.1%) with a hearing improvement of 6-10 dB.

In a study done by Gaurav Batni [9], in which type-1 tympanoplasty was performed on 100 patients. The mean pre-operative air conduction was 38.47±11.25 dB. The mean post-operative air conduction was 23.92±13.80 dB. Mean air bone gap pre-operatively was 23.55±7.56 dB and post operatively was 11.60±7.70 dB.

In a study done by Sharan kumar Shetty [10], 50 cases ofchronic suppurative otitis media with tubotympanic disease were used as participants.

The mean pre-operative hearing loss was 42.50 dB and mean post-operative hearing was 20.41 dB. In a study done by Mallikarjun S. Tegnoor [13], 50 patients underwent type-1 tympanoplasty. The mean pre-operative hearing was 33.34 dB and mean post-operative hearing was 20.20 dB with a mean audiological improvement of around 13 dB. The best mean hearing gain was 14.64 dB in age group of 24-34 years, while lesser gains are noted in 38-50 years age group.

Conclusion

1. Small perforation of the tympanic membrane is more common (38.6%)
2. Small and medium perforations can be repaired better using temporalis fascia alone than conchal cartilage with fascia
3. Post-operative Hearing improvement is comparatively better when temporalis fascia is used alone (14.0 dB)
4. Conchal cartilage is better in repair of large and subtotal perforations but hearing improvement post-operatively is comparatively less (33.7 dB)

Thus, from this study it can be concluded that repair of tympanic membrane perforations should be decided on size of perforation and appropriate grafting material. Small and medium perforations are best repaired using temporalis fascia alone which gives the best hearing improvement and large, subtotal perforations can be repaired using conchal cartilage and fascia but hearing improvement would be comparatively low.

What the study adds to the existing knowledge?

This study adds knowledge of graft-based hearing improvement apart from graft takeup and healing.

Author's contribution

Dr. A. Dayananad: Concept, monitoring, publication

Dr. B. Harshavardhan: Principle investigator-collect data and interpretation, statistical analysis.

Dr. J. Dheebika: Co-investigator - examination and investigation of patients, editing, IHEC submission.

Dr. S. Palaninathan: Concept, design.

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Reference

1. Patil K, Baisakhiya N, Deshmukh PT. Evaluation of different graft material in type 1 tympanoplasty. Indian J Otol. 2014;20 (3):106-114. doi: 10.4103/0971-7749. 136844.

2. Sarkar S. A review on the history of tympanoplasty. Indian J Otolaryngol Head Neck Surg. 2013;65(Suppl 3):455-60. doi: 10.1007/s12070-012-0534-5. Epub 2012 Mar 22.

3. Harris JP, Wong YT, Yang TH, Miller M, How I do it: Anterior pull-through tympanoplasty for anterior eardrum perforations. Acta Otolaryngol. 2016;136 (4):414-419. doi: 10.3109/00016489. 2016. 1139744. Epub 2016 Mar 18.

4. Mathai J. Myringoplasty with temporalis fascia: Analysis of 200 cases. Indian J Otolaryngol Head Neck Surg. 1999;51(2):9-13. doi: 10.1007/BF02997983.

5. Heermann J, Heermann H, Kopstein E. Fascia and cartilage palisade tympanoplasty: Nine years' experience. Arch Otolaryngol. 1970; 91(3):228-241. doi:10.1001/archotol.1970.00770040334004.

6. Gerber MJ, Mason JC, Lambert PR. Hearing results after primary cartilage tympanoplasty. Laryngoscope. 2000;110 (12):1994-1999. doi: 10.1097/00005537-200012000-00002

7. Yung M. Cartilage tympanoplasty: literature review. J Laryngol Otol. 2008;122(7):663-672. doi: 10.1017 /S0022215108001813. Epub 2008 Mar 3.

8. Nemade SV, Dabholkar JP. Healing and hearing results of temporalis fascia graft Vs cartilage graft (Full thickness and half thickness) in type I tympanoplasty. Online J Otolaryngol. 2014;4(3):1-20.

9. Batni G, Goyal R. Hearing outcome after type I tympanoplasty: a retrospective study. Indian J Otolaryngol Head Neck Sur. 2015;67(1):39-42. doi: 10.1007/s12070-014-0749-8.

10. Shetty S. Pre-operative and post-operative assessment of hearing following tympanoplasty. Indian J Otolaryngol Head Neck Surg. 2012;64(4):377-381. doi: 10.1007/s12070-011-0331-6.

11. Kumar RV, Ramakrishnaiah P, Radha Krishna N, Indira S. Outcome of different graft materials used in Myringoplasty. IAIM. 2016;3(2):119-128.

12. PIMS I, Awan Z. Myringoplasty: a comparative study of different graft materials and various surgical techniques. Ann. Pak. Inst. Med. Sci. 2008;4(4):209-211.

13. Tegnoor MS, Ali K, Mithun S. Audiometric evaluation of type 1 tympanoplasty for hearing results. Int J Otorhinolar

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Original Article

Ophthalmology Section

Inverse Association between Serum Bilirubin Levels and Retinopathy in Patients with Type 2 Diabetes Mellitus.

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ABSTRACT

Introduction: Oxidative stress plays a central role in the pathogenesis of Diabetic Retinopathy (DR) and serum bilirubin has been shown to have antioxidant properties.

Aim: To investigate the association between serum bilirubin concentration and DR in patients with Type 2 diabetes Mellitus (DM).

Materials and Methods: This was a hospital based, cross-sectional study where in 86 patients with Type 2 DM and 30 controls were recruited. The study was conducted at a tertiary care centre in South India between January 2014 and December 2014. The presence and the severity of DR were determined by fundus examination and grading of colour fundus photographs using the international clinical disease severity scale for diabetic retinopathy. Serum total, direct and indirect bilirubin levels were determined in all subjects and the association between bilirubin levels and severity of DR was studied.

Results: Among the 86 diabetics, 24 had no retinopathy and 62 had diabetic retinopathy of varying grades. The mean total bilirubin level among diabetic subjects (0.52±0.17) and controls (0.51±0.19) were found to be similar. The mean total as well as direct bilirubin levels were found to be lower in patients with retinopathy as compared to no retinopathy group ($p \leq 0.001$). The severity of DR was inversely proportional to the serum bilirubin levels ($p=0.010$). Serum total bilirubin was found to have a negative association with glycosylated haemoglobin and served as an independent determinant of diabetic retinopathy even after adjusting for risk factors known to be associated with DR ($p=0.001$).

Conclusion: Low serum bilirubin levels are significantly associated with increased risk of diabetic retinopathy independent of classic risk factors. Serum bilirubin can serve as a useful biomarker in identifying patients at risk for developing proliferative diabetic retinopathy.

Keywords: Chronic kidney disease, Heme oxygenase, Peripheral arterial disease

INTRODUCTION

Diabetes Mellitus (DM) has assumed the proportions of a global epidemic and the number of diabetics in the world today is 415 million. This number is being projected to increase to 642 million by 2040 [1]. India leads the world in terms of its diabetic population and estimates have revealed that there are 62.4 million people with diabetes, and 77.2 million with prediabetes in India [2]. It is predicted that by 2030, DM may affect up to 79.4 million in India [3]. Due to these sheer numbers, the socioeconomic burden as a result of diabetes in India is among the highest in the world [4].

Along with this rapidly increasing disease, there is an increase in the associated microvascular complication DR, which is said to affect more than 93 million people worldwide [5]. Almost all patients with Type 1 DM and over 60% of the patients with Type 2 DM will develop DR in 20 years. DR is the most frequent cause of preventable blindness in middle aged population and therefore,represents a major public health issue [6].

Oxidative stress is considered to be one of the key factors in the pathogenesis of DR [7]. The retina is especially more susceptible to oxidative damage because of its high energy demands and exposure to light [8]. Various mechanisms contributing to the pathogenesis of DR such as inflammation, polyol pathway, accumulation of Advanced Glycation End Products (AGEs), flux of hexosamine Pathway and Protein Kinase C (PKC) activation are associated with overproduction of Reactive Oxygen Species (ROS) by the mitochondria [9].

Although, bilirubin has long been considered as a toxic waste product of heme catabolism, its role as a potential endogenous anti-oxidant under physiological conditions is being increasingly recognized [10]. Apart from its ability to scavenge over produced ROS, bilirubin is also said to have anti inflammatory effects [11]. Based on these physiological effects of bilirubin, several population-based studies have reported a negative association between serum bilirubin concentrations and the risk of diabetes [12], coronary artery disease [13], chronic kidney disease [14], peripheral arterial disease [15] and diabetic peripheral neuropathy [16].

Few studies in literature have shown, the association between serum bilirubin and DR [17-20]. Apart from age, gender, smoking and intake of drugs serum bilirubin concentrations are also affected by factors such as altitude and ethnicity and these are likely to influence the biological effects of bilirubin on human body.

Considering the potential role of oxidative stress in the pathogenesis of DR and the antioxidant and cytoprotective effects of bilirubin, this cross-sectional study aimed to evaluate the association between serum bilirubin and severity of retinopathy in patients with Type 2 DM from South India.

MATERIALS AND METHODS

This was a hospital-based cross-sectional study which included patients with Type 2 DM who were referred to the Ophthalmology Department of a tertiary care centre in South India between January 2014 and December 2014 for screening of DR. Exclusion criteria included history of chronic alcohol intake, smoking, intake of hepatotoxic medications within past six months, preexisting hepatobiliary abnormalities or chronic liver disease. Patients with preexisting ocular diseases like glaucoma, high myopia, previous ocular surgery or photocoagulation were excluded from the study. Patients with hypertension (>160/90 mmHg), anaemia or other systemic diseases like nephropathy which can accelerate the progression of DR were also excluded from the study. The study protocol was approved by the Institutional Ethics Committee and this study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Information on age, gender, duration of diabetes, blood pressure and body mass index was obtained from all participants.

All participants underwent a complete ocular examination including fundus examination with slit lamp biomicroscope using a 90 diopter lens after dilatation of pupils using 1% tropicamide eyedrops. This was followed by 30 degree colour stereoscopic fundus photography of 7 standard Early Treatment Diabetic Retinopathy Study (ETDRS) fields using a digital fundus camera (TRC-50 DX Mydriatic retinal camera; Topcon Medical Systems, Oakland, NJ).The severity of DR was determined by grading of colour fundus photographs following the international clinical disease severity scale for diabetic retinopathy which is based upon the findings of WESDR (Wisconsin Epidemiological Study of Diabetic Retinopathy) and ETDRS [21].

The diabetic patients were assigned to one of the following groups based on presence and severity of retinopathy.

1. Diabetic patients with no retinopathy.
2. Mild Non-Proliferative Diabetic Retinopathy (NPDR)
3. Moderate NPDR.
4. Severe NPDR and
5. Proliferative Diabetic Retinopathy (PDR)

The eye with worse retinopathy was chosen for inclusion in the study examinations. If both eyes had equal retinopathy, the right eye was assigned to the study.

Fasting venous blood samples were collected for the determination of serum total, direct and indirect bilirubin, blood glucose and Glycosylated Haemoglobin (HbA1c). The normal range of total serum bilirubin levels used in the study was 0.2 to 1.2 mg/dl.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 19. The baseline characteristics of patients were presented as mean±standard deviation and n (%). Data on the patient's clinical characteristics were compared using One-way Analysis of Variance (ANOVA) as well as an independent sample Student's t-test. The serum bilirubin levels were divided into quartiles and Chi-square test was used to see the comparison between the proportions. Multiple logistic regression analysis was performed to assess independent associations between the presence of key risk factors of DR including the statistically significant factors in univariate analysis. All tests for statistical significance were two-tailed, and performed assuming a Type I error probability of <0.05.

RESULTS

A total of 116 subjects were included in the study out of whom 86 were patients with Type 2 diabetes and 30 were controls.

The mean total bilirubin level among diabetic subjects (0.52±0.17) and controls (0.51±0.19) were found to be similar. The direct bilirubin level was found to be higher in diabetics (0.29±0.13) as compared to controls (0.18±0.06, p=0.000). The indirect bilirubin level was lower in diabetics (0.23±0.07) as compared to healthy controls (0.33±0.13, p=0.000).

[Table/Fig-1] shows the clinical characteristics of the study population.

The mean duration of diabetes and HbA1c were significantly higher among patients with DR compared to those without retinopathy (p<0.001). We compared the serum total, direct and indirect bilirubin levels for diabetics without retinopathy (n=24) and those with DR (n=62). The total bilirubin was 0.65±0.16 in patients with no retinopathy and 0.47±0.16 in patients with DR. The mean total as well as direct bilirubin levels were found to be lower in patients with retinopathy as compared to no retinopathy group (p=0.000). There was no statistically significant difference in the levels of indirect bilirubin among both groups (p=0.100).

Characteristic	Controls (n=30)	Diabetics with no retinopathy (n=24)	Diabetics with retinopathy (n=62)
Age (yr)	52.97±8.95	57.63±10.54	57.29±8.5
Females, no. (%)	18 (60%)	9 (37.5%)	23 (37.1%)
Duration of diabetes (yr)	-	3.67±1.20	6.74±3.51*
Body mass Index (Kg/m^2)	28.1±0.7	27.8±0.4	28±0.7
Systolic blood pressure (mm Hg)	120.1±2.7	117.1±1.6	122.6±2.9
Diastolic blood pressure (mm Hg)	79.7±2.1	74.1±1.2	73.3±2.4
HbA1c (%)	5.72±0.35	8.09±0.78	9.75±1.74*
Total bilirubin (mg/dl)	0.51±0.19	0.65±0.16	0.47±0.16*
Direct bilirubin (mg/dl)	0.18±0.06	0.39±0.13	0.25±0.10*
Indirect bilirubin (mg/dl)	0.33±0.13	0.25±0.05	0.22±0.07

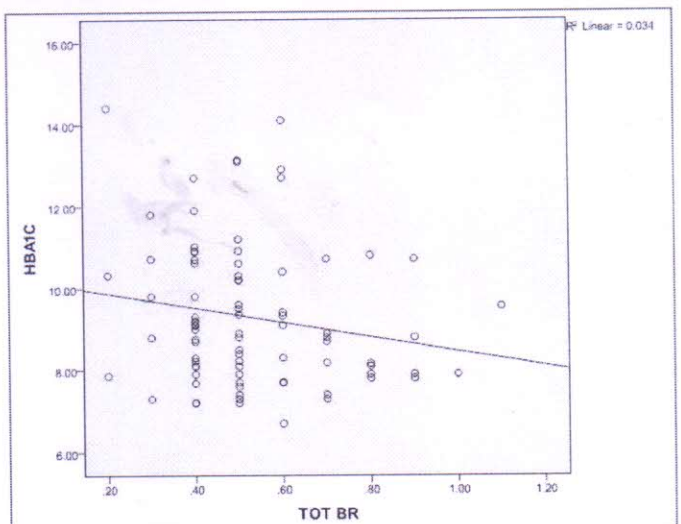
[Table/Fig-1]: Showing the clinical characteristics of study population.
* p-value < 0.05; HbA1c- Haemoglobin A1c

	Quartiles of serum total bilirubin (mg/dl)				p-value
	<0.40	0.41-.50	0.51-.60	>0.61	
Controls	11	10	3	6	0.010
NO DR	2	6	5	11	
Mild NPDR	5	4	3	3	
Moderate NPDR	8	8	3	3	
Severe NPDR	7	1	0	1	
PDR	11	4	1	0	

[Table/Fig-2]: Showing the correlation between serum total bilirubin and severity of diabetic retinopathy.
NPDR-Non proliferative diabetic retinopathy; PDR-Proliferative diabetic retinopathy

Risk factor	OR	95% CI	p-value
Age (years)	1.03	0.960-1.111	0.386
Duration of diabetes (years)	2.05	1.172-3.592	0.012*
HbA1c (%)	2.42	1.177-4.984	0.016*
Serum total bilirubin (mg/dl)	0.001	0.000-0.054	0.001*

[Table/Fig-3]: Showing the odds ratio for association between key risk factors and occurrence of diabetic retinopathy using logistic regression model.
* p-value < 0.05; OR-Odds ratio; CI-Confidence Interval; HbA1c- Haemoglobin A1c



[Table/Fig-4]: Scatter diagram showing negative association between serum total bilirubin and HbA1c (glycosylated haemoglobin) in diabetics.
TOT BR-Total bilirubin; HBA1C-glycosylated haemoglobin

Of the 62 diabetic patients with retinopathy, 15 patients had mild NPDR, 22 had moderate NPDR and 9 had severe NPDR. PDR was noted in 16 patients. We divided the study participants into quartiles on the basis of serum total bilirubin concentration (quartile 1=<0.40 mg/dl, quartile 2= 0.41-0.50 mg/dl, quartile 3= 0.51-0.60 mg/dl, quartile 4=>0.61 mg/dl).

[Table/Fig-2] shows the correlation between serum total bilirubin and severity of DR.

The percentage of DR in persons with lowest bilirubin quartile was 36.04%, compared with the percentage of 19.8%, 8.1% and 8.1% respectively in the second, third and fourth quartiles. Thus, the proportion of patients with DR was significantly higher in quartile 1 with lower levels of bilirubin as compared to the other three quartiles [Table/Fig-2]. The severity of DR was inversely proportional to the serum bilirubin levels (p=0.010) and none of the patients in the last quartile (>0.61) had proliferative retinopathy.

On multiple logistic regression analysis the duration of diabetes, HbA1c and total serum bilirubin were found to be independently associated with DR [Table/Fig-3]

Also, serum total bilirubin in diabetic subjects was found to have negative association with HbA1c [Table/Fig-4].

DISCUSSION

In the present, study serum bilirubin concentration correlated negatively with the severity of retinopathy in patients with Type 2 DM. Multiple regression analysis also revealed serum total bilirubin as an independent risk factor associated with diabetic retinopathy.

Bilirubin is generated when the enzyme Heme Oxygenase (HO) catalyzes the degradation of heme. This leads to the formation of biliverdin which is converted to bilirubin by biliverdin reductase. In the hepatocytes, unconjugated (lipid-soluble) bilirubin is conjugated by uridine diphosphate glucuronyl transferase (UDP-GT) to a water soluble form for excretion. Total bilirubin is the sum of unconjugated (indirect) and conjugated (direct) bilirubin and generally ranges from 0.2 to 1.2 mg/dl in healthy individuals.

Ever since the description of beneficial role of bilirubin as a physiologic antioxidant, several clinical studies evaluating, the possible associations between serum bilirubin and the risk of diabetes (and its complications) have been published [22-24].

In this observational study, we noted that Type 2 diabetics with retinopathy had significantly lower serum bilirubin concentrations than those without retinopathy. Our observations are in line with epidemiologic and hospital based studies elsewhere which have suggested an inverse relation between serum total bilirubin and DR [17-20]. There has been only one study on the association between serum bilirubin and DR from Indian population by Dave et al., [25]. They noted higher levels of total, direct and indirect bilirubin in patients without DR as compared to patients with retinopathy. However, in our study only total and direct bilirubin concentrations were found to be higher in patients without DR. Serum indirect bilirubin concentration was similar in both groups. Most studies on serum bilirubin and DR have focused on only total bilirubin without separating bilirubin types. Limited studies have shown a differentially protective effect of direct bilirubin on metabolic syndrome [26] and chronic kidney disease [27]. Further studies on the relation between bilirubin subtypes and DR are clearly needed.

In this study, we divided bilirubin levels into quartiles (<0.40, 0.41-0.50, 0.51-0.60 and >0.61 mg/dl and considered the lowest quartile as reference. This approach was to maintain adequate numbers within each quartile. The absolute values of serum bilirubin were low in our study compared to studies by Yasuda et al., and Najam et al., where the value of serum bilirubin in the lowest and highest quartile observed were<0.60 and >0.90 mg/dl respectively [17,18]. There are several potential reasons for the observed findings. Apart from differences in ethnicity, our hospital based study population was very much different from the above said epidemiologic studies in age distribution, anthropometry, comorbidities such as hypertension, cardiovascular disease and smoking habits, all factors that may affect the bilirubin levels and potentially the association between bilirubin and DR. Our study participants were non smokers and had no history of major systemic diseases.

Several potential mechanisms have been proposed for the observed relationship between serum bilirubin concentration and DR. Excessive production of free radicals and resultant oxidative stress has been said to contribute significantly to worsening of DR [28,29]. Bilirubin has been postulated as a potent antioxidant and cytoprotective agent that can protect cells from a 10,000 fold excess of hydrogen peroxide [30]. Therefore, lower serum bilirubin concentration would be expected to be associated with increased oxidative stress and thereby, accelerated progression of DR. Additionally clinical studies have demonstrated elevated levels of inflammatory markers like C-reactive protein and vascular cell adhesion molecules in patients with DR suggesting that inflammation is a critical factor leading to endothelial dysfunction in diabetic retinopathy [31,32]. Bilirubin has been proven to have anti-inflammatory properties and in-vitro studies have shown that bilirubin inhibited tumour necrosis factor α –induced upregulation of vascular cell adhesion molecules [33]. Hence, it could be hypothesized that higher serum bilirubin concentration is associated with a lower risk of development and progression of DR through its anti-inflammatory effects. Further, recent animal studies have shown that, increasing serum bilirubin levels either through endogenous synthesis or exogenous administration improves endothelial dysfunction in diabetes by activation of protein kinase B [34].

Considering these physiologic effects of bilirubin it can be postulated that bilirubin can effectively retard or interrupt the pathways to development of DR and therefore serve as a potential biomarker for risk of diabetic retinopathy.

We observed an inverse and graded association between serum total bilirubin and severity of DR in this study and the strength of serum total bilirubin concentration as an independent determinant of DR was similar to those of conventional risk factors such as HbA1c and duration of diabetes in multiple regression analysis. Serum total bilirubin concentration was an independent determinant of DR even after adjustment for other risk factors (p=0.001).

We also observed a negative association between serum total bilirubin and HbA1c among diabetic subjects. Our data are consistent with observations of Choi et al., who have also noted a significant inverse association between serum total bilirubin and HbA1c in their study on 690 Korean patients with Type 2 DM [35].

This can be partly explained by the fact that, increased serum bilirubin reduces the oxidative stress and thereby, inhibits the glycosylation of haemoglobin. Also, upregulation of the heme oxygenase system and increased generation of bilirubin/biliverdin have been proven to enhance insulin production and sensitivity in diabetic rats [36]. Therefore, it can be suggested that bilirubin improves glycaemic control through its effects on insulin. Further studies are needed to precisely elucidate the mechanism behind this association.

The strength of our study is based on the fact that our study participants were non-smokers and did not have other systemic diseases. We excluded these patients to focus on the association between serum bilirubin and retinopathy in patients with Type 2 diabetes.

LIMITATION

Our study has some limitations. First, our sample size was limited and the cross-sectional design of our study prevents determination of a temporal association or the cause-effect relationship between serum total bilirubin and the development of retinopathy. Only prospective studies can reveal whether serum bilirubin predicts the development of DR. Second, we performed only a single measurement of serum bilirubin which might have within subject variation. However, all subjects including controls were examined after overnight fast.

CONCLUSION

In conclusion, our cross-sectional study suggested that low total serum bilirubin level was independently associated with higher risk of DR in patients with type 2 diabetes, regardless of other conventional risk factors. Further, prospective studies on large samples are required to better assess the effects of bilirubin on diabetic retinopathy.

REFERENCES

[1] International Diabetes Federation. IDF Diabetes Atlas, 7th ed. Brussels, Belgium: International Diabetes Federation, 2015.

[2] Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: Phase I results of the Indian Council of Medical Research India Diabetes (ICMR INDIAB) study. Diabetologia. 2011;54:3022 27.

[3] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.

[4] Joshi SR, Das AK, Vijay VJ, Mohan V. Challenges in diabetes care in India: sheer numbers, lack of awareness and inadequate control. J Assoc Physicians India. 2008;56:443–50.

[5] Yau JWY, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35:556–64.

[6] Cheung N, Mitchell P, Wong TY. Diabetic retinopathy Lancet. 2010;376:124–36.

[7] Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord. 2008;9:315-27.

[8] Kumari S, Panda S, Mangaraj M, Mandal MK, Mahapatra PC. Plasma MDA and antioxidant vitamins in diabetic retinopathy.Indian J Clin Biochem. 2008;23:158-62.

[9] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615-25.

[10] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science. 1987;235:1043–46.

[11] Vítek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. Adv Clin Chem. 2007;43:1-57.

[12] Cheriyath P, Gorrepati VS, Peters I, Nookala V, Murphy ME, Srouji N, et al. High total bilirubin as a protective factor for Diabetes Mellitus: An analysis of NHANES Data From 1999 - 2006. J Clin Med Res. 2010;2:201-06.

[13] Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem. 1994;40:18-23.

[14] Tanaka M, Fukui M, Okada H, Senmaru T, Asano M, Akabame S, et al. Low serum bilirubin concentration is a predictor of chronic kidney disease. Atherosclerosis. 2014;234:421-25.

[15] Perlstein TS, Pande RL, Beckman JA, Creager MA. Serum total bilirubin level and prevalent lower extremity peripheral arterial disease: National health and nutrition examination survey (NHANES) 1999 to 2004. Arterioscler Thromb Vasc Biol. 2008;28:166-72.

[16] Kim ES, Lee SW, Mo EY, Moon SD, Han JH. Inverse association between serum total bilirubin levels and diabetic peripheral neuropathy in patients with type 2 diabetes. Endocrine. 2015;50:405-12.

[17] Yasuda M, Kiyohara Y, Wang JJ, Arakawa S, Yonemoto K, Doi Y, et al. High serum bilirubin levels and diabetic retinopathy: the Hisayama Study. Ophthalmology. 2011;118:1423-28.

[18] Najam SS, Sun J, Zhang J,Xu M, Lu J, Sun K, et al. Serum total bilirubin levels and prevalence of diabetic retinopathy in a Chinese population. J Diabetes. 2014;6:221–27.

[19] Cho HC. The relationship among Homocysteine, Bilirubin, and diabetic retinopathy. Diabetes Metab J. 2011;35:595-601.

[20] Sekioka R, Tanaka M, Nishimura T, Itoh H. Serum total bilirubin concentration is negatively associated with increasing severity of retinopathy in patients with type 2 diabetes mellitus. J Diabetes Complications. 2015;29:218-21.

[21] Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales.Global Diabetic Retinopathy Project Group. Ophthalmology. 2003;110:1677-82.

[22] Han SS, Na KY, Chae DW, Kim YS, Kim S, Chin HJ, et al. High serum bilirubin is associated with the reduced risk of diabetes mellitus and diabetic nephropathy. Tohoku J Exp Med. 2010;221:133-40.

[23] Jung CH, Lee MJ, Kang YM, Hwang JY, Jang JE, Leem J, et al. Higher serum bilirubin level as a protective factor for the development of diabetes in healthy Korean men: A 4 year retrospective longitudinal study. Metabolism. 2014;63:87-93.

[24] Kawamoto R, Ninomiya D, Hasegawa Y, Kasai Y, Kusunoki T, Ohtsuka N, et al. Mildly elevated serum total bilirubin levels are negatively associated with carotid atherosclerosis among elderly persons with type 2 diabetes. Clin Exp Hypertens. 2016;38:107-12.

[25] Dave A, Kalra P, Gowda BH, Krishnaswamy M. Association of bilirubin and malondialdehyde levels with retinopathy in type 2 diabetes mellitus. Indian J Endocrinol Metab. 2015;19:373–77.

[26] Hwang HJ, Kim SH. Inverse relationship between fasting direct bilirubin and metabolic syndrome in Korean adults. Clin Chim Acta. 2010;411:1496-501.

[27] Ryu S, Chang Y, Zhang Y, Woo HY, Kwon MJ, Park H, et al. Higher serum direct bilirubin Levels were associated with a lower risk of incident chronic kidney disease in middle aged Korean Men. PLoS One. 2014;9(2):e75178.

[28] Obrosova IG, Minchenko AG, Marinescu V, et al. Anti-oxidants attenuate early up regulation of retinal vascular endothelial growth factor in streptozotocin-diabetic rats. Diabetologia. 2001;44:1102–10.

[29] Kowluru RA, Atasi L, Ho YS. Role of mitochondrial superoxide dismutase in the development of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2006;47:1594–99.

[30] Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: A major physiologic cytoprotectant. Proc Natl Acad Sci U S A. 2002;9:16093–98.

[31] Schram MT, Chaturvedi N, Schalkwijk C, Giorgino F, Ebeling P, Fuller JH, et al. Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes: the EURODIAB Prospective Complications Study. Diabetes Care. 2003;26:2165-73.

[32] Olson JA, Whitelaw CM, McHardy KC, Pearson DW, Forrester JV. Soluble leucocyte adhesion molecules in diabetic retinopathy stimulate retinal capillary endothelial cell migration. Diabetologia. 1997;40:1166-71.

[33] Mazzone GL, Rigato I, Ostrow JD, Bossi F, Bortoluzzi A, Sukowati CH, et al. Bilirubin inhibits the TNF alpha-related induction of three endothelial adhesion molecules. Biochem Biophys Res Commun. 2009;386:338-44.

[34] Liu J, Wang L, Tian XY, Liu L, Wong WT, Zhang Y, et al. Unconjugated bilirubin mediates heme oxygenase-1-induced vascular benefits in diabetic mice. Diabetes. 2015;64:1564-75.

[35] Choi SW, Lee YH, Kweon SS, Song HR, Ahn HR, Rhee JA, et al. Association between total bilirubin and haemoglobin A1c in Korean type 2 diabetic patients. J Korean MedSci. 2012;27:1196–201.

[36] Ndisang JF, Lane N, Jadhav A. Upregulation of the heme oxygenase system ameliorates postprandial and fasting hyperglycemia in type 2 diabetes. Am J Physiol Endocrinol Metab. 2009;296(5):E1029-41.

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Brief Communication

Needs Assessment of Ophthalmology Education for Undergraduate Medical Students – A Study from a Medical College in South India

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ABSTRACT

Background: Adequate ophthalmic diagnostic and clinical skills are essential for practitioners in primary care settings as well as specialty care physicians. The objectives of this study were to assess the adequacy of ophthalmology teaching in undergraduate medical education and to evaluate the comfort of medical students in diagnosing common eye problems and performing ophthalmic skills. **Methods:** A questionnaire based, cross-sectional survey was conducted among third-year undergraduate students from a medical college in South India at the end of ophthalmology training from February 2014 to December 2014. The main outcome measures were hours of classroom-based instruction and clinical exposure to ophthalmology received by the students and their comfort level in diagnosing common eye problems and performing ophthalmic skills. **Results:** 134 students participated in the study. They had received an average of 96.2 ± 5.9 and 112.5 ± 11.3 hours of classroom and clinic-based instruction, respectively. The participants' comfort in diagnosing eye problems was satisfactory for cataract and eyelid disorders but not for ophthalmic emergencies. Only 45.5% had satisfactory knowledge in community ophthalmology. Respondents were more proficient in visual acuity testing (93.3%) and assessment of pupillary reaction (80.6%) than direct ophthalmoscopy (41%). **Discussion:** Undergraduate medical students from India received a greater amount of ophthalmology instruction compared to the International Council of Ophthalmology task force recommendations. Gaps in community ophthalmology and knowledge-skills discrepancies were noted. Review of curriculum, appropriate training resources, and effective teaching methods tailored towards primary care may be useful to improve the training.

Keywords: Comfort, knowledge, ophthalmology, undergraduate medical education

Background

Ophthalmology makes up a significant proportion of primary care consultations.[1] A basic knowledge of ophthalmology is essential in the management of several neurologic, endocrine, rheumatic, and infectious conditions. Despite the relevance of ophthalmology to medical practice, little time is allocated to teaching of ophthalmology in undergraduate medical education and the majority of primary care physicians do not feel that they have received adequate undergraduate ophthalmic education.[2] Also several studies have noted that medical students receive too little ophthalmic training.[3-5]

In India, the five and one-half year undergraduate medical training consists of four and one-half years of teaching of subjects comprising the medical curriculum and one year of internship. Ophthalmology is being taught in the third year after preclinical and paraclinical subjects.

The state of undergraduate ophthalmology training from medical colleges in India has not been studied so far. Evaluation

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of ophthalmic knowledge and skills of undergraduates at the end of ophthalmology training would help in generating critical feedback on undergraduate ophthalmic curriculum planning. The objectives of this study were:

1. To assess the adequacy of ophthalmology teaching in undergraduate medical education
2. To assess the comfort level of medical students in diagnosing and treating common eye problems and performing ophthalmic skills.

Methods

After approval from the institutional review board, undergraduate medical students from a medical college in South India were included in the study at the end of their ophthalmology training (February 2014 to December 2014). The adequacy of ophthalmology education was quantified by determining the hours of classroom-based instruction and clinical exposure to ophthalmology received by the students and comparing these with the recommendations of the Medical Council of India (MCI) as well as the International Council of Ophthalmology (ICO).

A validated questionnaire [Appendix 1] was given to the students and their responses collected anonymously. The questionnaire consisted of three parts. Parts 1 and 3 assessed the students' comfort with respect to diagnosing specific ophthalmology conditions and performing ophthalmology-related skills on a Likert scale from 1 to 5 (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, and 5 = very comfortable). In terms of comfort level, we examined the ease with which study participants reported they were able to diagnose common eye problems and perform ophthalmic skills.

The second part assessed knowledge on certain topics on a Likert scale, also from 1 to 5 (1 = very poor, 2 = poor, 3 = average, 4 = good, and 5 = very good). The conditions and skills covered in the questionnaire were based on the topics covered in the undergraduate ophthalmology curriculum recommended by the MCI.[6]

Participants were also asked about the number of hours spent in self-directed learning and how comfortable they felt in managing ophthalmology-associated issues overall.

Likert scale-based responses were subsequently dichotomized and reduced to proportions by categorizing scores 1–2 as unsatisfactory or inadequate and 3–5 as satisfactory or adequate. Data were entered using the Statistical Package for the Social Sciences (version 19 (SPSS, Inc., Chicago, IL, USA) and examined using descriptive and analytical statistics to yield frequencies, percentages, and proportions.

Results

Of the 150 undergraduate medical students, 134 responded to the questionnaire (response rate = 89.6%).

The main modes of ophthalmology exposure were didactic lectures (35 hours), tutorials (37.5 ± 3.8 hours), seminars (23.7 ± 2.2 hours), and clinic-based learning. Our study participants had received an average of 96.2 ± 5.9 hours of classroom-based instruction. They also had received 112.5 ± 11.3 hours of clinic-based instruction in the eight weeks of clinical posting. 121 participants (90.3%) had spent up to 10 hours in self-directed learning while 13 participants (9.7%) had spent more than 10 hours in self-directed learning.

The results of the participants' comfort in diagnosing ophthalmic conditions are presented in Figure 1. Figure 2 shows the students' adequacy of knowledge in community and systemic ophthalmology as well as indications for ophthalmic referral. Figure 3 shows the study participants' comfort in performing MCI recommended clinical skills. Tables 1-3 show the numerical data for the corresponding figures. Finally, 101 participants (75.4%) felt comfortable in managing ophthalmology associated issues overall.

Discussion

This cross-sectional observational study attempted to quantify the adequacy of undergraduate ophthalmic education and students' comfort with regard to the management of ocular conditions.

In comparison with MCI recommendations, our study participants received a satisfactory number of hours of both classroom and clinic-based instruction. The MCI recommends clinical training of 8 weeks and a total of 100 teaching hours in ophthalmology, which includes 30 h of didactic lectures,

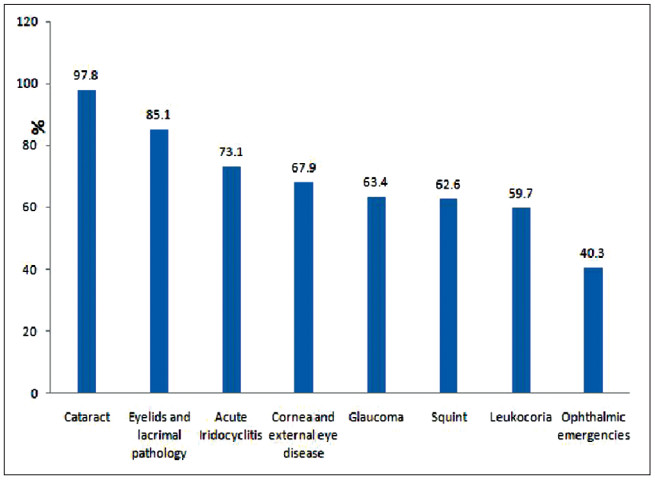


Figure 1: Medical students' comfort in diagnosing common ophthalmic conditions

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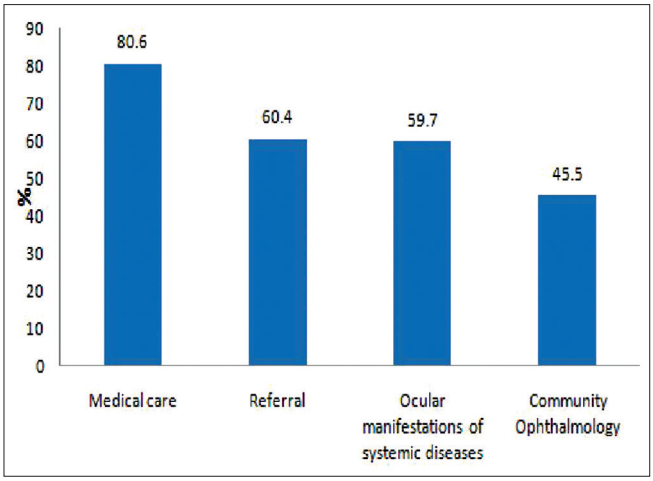


Figure 2: Students' adequacy of ophthalmic knowledge

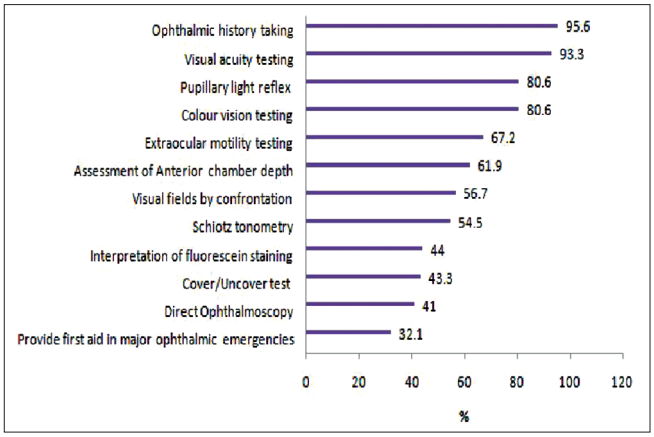


Figure 3: Students' self-reported proficiency in performance of ophthalmic skills

Table 1: Students' comfort in diagnosing common ophthalmic conditions

Ophthalmic conditions	Participants with adequate comfort in diagnosing common ophthalmic conditions (n=134), n (%)
Cataract	131 (97.8)
Eyelids and lacrimal pathology	114 (85.1)
Acute iridocyclitis	98 (73.1)
Cornea and external eye disease	91 (67.9)
Glaucoma	85 (63.4)
Squint	84 (62.6)
Leukocoria	80 (59.7)
Ophthalmic emergencies	54 (40.3)

60 h of tutorials and seminars (including integrated learning), and 10 h of self-directed learning.[7] These guidelines suggest a greater amount of ophthalmology exposure in undergraduate medical education in India when compared with ICO guidelines. The ICO recommends 40–60 h of ophthalmology exposure during undergraduate training.[8]

According to the curriculum recommended by the MCI, the goal of teaching ophthalmology to undergraduate medical students is to provide such knowledge and skills to the students that shall enable them to practice as a clinical and primary eye care physician.[6] The curriculum provides a list of competencies which need to be demonstrated by the student at the end of ophthalmology training. These include knowledge of common eye problems in the community; ability to manage common eye problems and identify indications for referral; recognize visual impairment and blindness in the community; and implement the National programme as applicable in the primary care setting.[7]

Most of the participants in the study reported a satisfactory level of comfort in diagnosing common ophthalmic problems. The fact that 97.8% reported a satisfactory level of comfort in diagnosing cataract is encouraging since cataract is the leading cause of preventable blindness in the developing world. Yet, despite this satisfactory level of comfort in managing common eye problems, most study participants' comfort in diagnosing ophthalmic emergencies such as endophthalmitis, rupture globe, and chemical injuries was found to be inadequate.

Nearly 60% reported to have adequate knowledge in ocular manifestations of systemic diseases. This could be because of better teaching resources as well as integration of ophthalmology topics into other subjects of the medical curriculum horizontally and longitudinally. The self-rated knowledge of more than one-half of the study participants was found to be unsatisfactory in community ophthalmology. With greater emphasis placed on teaching disorders of the eye, in theory lectures as well as in hospital settings, community ophthalmology is often neglected. A study on the status of ophthalmic medical education in the South-East Asia Region of World Health Organization has also highlighted this concern, where students have little exposure to community ophthalmology.[9]

The knowledge of most study participants on the indications of medical treatment and referral to eye clinics was adequate. With a significant shortage of ophthalmologists in developing countries, an adequate level of comfort of primary care physicians in providing medical care and accuracy in referral is essential. A high rate of referral places pressure on the limited resources of ophthalmic casualty departments and outpatient clinics.

More than 80% reported competency in ophthalmic history taking, visual acuity and color vision testing, and pupil examination. These are relevant not only in ophthalmology but also in general medicine, neurology, and trauma care. However, only 44% reported an adequate level of comfort in interpretation of fluorescein staining and 54.5% in Schiotz tonometry. This is an area of concern because corneal blindness

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Table 2: Students' adequacy of knowledge

Ophthalmic knowledge	Participants with adequate knowledge (*n*=134), *n* (%)
Medical care	108 (80.6)
Referral	81 (60.4)
Ocular manifestations of systemic diseases	80 (59.7)
Community ophthalmology	61 (45.5)

Table 3: Students' proficiency in performance of ophthalmic skills

Ophthalmic skills	Participants with adequate comfort in performing ophthalmic skills (*n*=134), *n* (%)
Ophthalmic history taking	128 (95.6)
Visual acuity testing	125 (93.3)
Pupillary light reflex	108 (80.6)
Color vision testing	108 (80.6)
Extraocular motility testing	90 (67.2)
Assessment of anterior chamber depth	83 (61.9)
Visual fields by confrontation	76 (56.7)
Schiotz tonometry	73 (54.5)
Interpretation of fluorescein staining	59 (44)
Cover/uncover test	58 (43.3)
Direct ophthalmoscopy	55 (41)
First aid in major ophthalmic emergencies	43 (32.1)

and glaucoma contribute to a major extent to the burden of blindness in India; adequate proficiency of the primary care physician in these skills will help in early diagnosis and treatment or referral, thereby reducing the risk of visual loss.

Only 41% of respondents indicated that they had adequate proficiency in performing direct ophthalmoscopy which is consistent with studies elsewhere.[2,10] This could be because of the limited time devoted to teaching ophthalmoscopy in the medical school curriculum as well as the technical challenges involved.

Although most students were comfortable in managing ophthalmology associated issues overall, gaps were noted in certain key areas. Review of the curriculum with greater emphasis on community eye care and blindness control strategies, adequate training resources, and effective teaching methods tailored toward primary care would help to increase the medical students' comfort in managing common eye problems.

Since our observations are from a single center, generalization of results is limited. Subjective bias due to the candidates varying ability to accurately self-evaluate is also a limitation. The choice of the study population was medical students at the end of ophthalmology training rather than interns because of the possibility of recollection bias. A similar study on interns or junior medical officers would provide more insight into the knowledge and gaps in training.

In spite of these limitations, this needs assessment study is the first of its kind in India to assess the adequacy of ophthalmic teaching and the knowledge and clinical skills of undergraduate medical students. Ophthalmology in India is being taught as part of the essential undergraduate medical curriculum and not exclusively as an elective. Students receive adequate exposure to ophthalmology, and the undergraduate curricular hours are greater than the guidelines published by the ICO. Since ophthalmology related topics and examination skills are included in most of the standardized medical licensing examinations and internal medicine boards, medical graduates from India are at a distinct advantage.

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Conflicts of interest

There are no conflicts of interest.

References

1. Dart JK. Eye disease at a community health centre. Br Med J (Clin Res Ed) 1986;293:1477-80.
2. Shuttleworth GN, Marsh GW. How effective is undergraduate and postgraduate teaching in ophthalmology? Eye (Lond) 1997;11(Pt 5):744-50.
3. Fan JC, Sherwin T, McGhee CN. Teaching of ophthalmology in undergraduate curricula: A survey of Australasian and Asian medical schools. Clin Exp Ophthalmol 2007;35:310-7.
4. Bellan L. Ophthalmology undergraduate education in Canada. Can J Ophthalmol 1998;33:3-7.
5. Noble J, Somal K, Gill HS, Lam WC. An analysis of undergraduate ophthalmology training in Canada. Can J Ophthalmol 2009;44:513-8.
6. Medical Council of India Regulations on Graduate Medical Education; 1997. Available from: http://www.mciindia.org/Rules-and-Regulation/GME_REGULATIONS.pdf. [Last accessed on 2016 Oct 11].
7. Medical Council of India Regulations on Graduate Medical Education; 2012. Available from: http://www.psgimsr.ac.in/wp-content/uploads/2012/pdf/others/mci-regulations-graduate-medical-education-12.pdf. [Last accessed on 2016 Oct 11].
8. International Task Force on Opthalmic Education of Medical Students, International Council of Opthalmology. Principles and guidelines of a curriculum for ophthalmic education of medical students. Klin Monbl Augenheilkd 2006;223 Suppl 5:S1-19.
9. World Health Organization. Study on the Status of Ophthalmic Medical Education in the South-East Asia Region - An Overview. Available from: http://www.v2020eresource.org/content/files/SEA_OPHTHAL_121.pdf. [Last accessed on 2017 Jun 03].
10. Gupta RR, Lam WC. Medical students' self-confidence in performing direct ophthalmoscopy in clinical training. Can J Ophthalmol 2006;41:169-74.

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Appendix

Appendix 1: Questionnaire

1. Using the table below, please indicate how comfortable You feel diagnosing the following issues based on your present knowledge:
   (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, 5 = very comfortable)
   Conjunctiva/cornea 1 2 3 4 5
   Viral conjunctivitis
   Allergic conjunctivitis
   Subconjunctival hemorrhage
   Pterygium
   Corneal abrasion/ulcer
   Keratomalacia
   Corneal blindness
   Eyelid/lacrimal sac 1 2 3 4 5
   Blepharitis
   Chalazion
   Stye
   Dacryocystitis
   Lens/Uvea 1 2 3 4 5
   Cataract
   Acute iridocyclitis
   Glaucoma 1 2 3 4 5
   Primary open-angle glaucoma
   Acute angle-closure glaucoma
   Ophthalmic emergencies 1 2 3 4 5
   Globe rupture
   Chemical injuries
   Endophthalmitis
   Pediatric ophthalmology 1 2 3 4 5
   Strabismus
   Leukocoria

2. Using the table below, please indicate your knowledge on the following issues:
   (1 = very poor, 2 = poor, 3 = Average, 4 = good, 5 = Excellent)
   Ocular manifestations of systemic diseases 12345
   Diabetic retinopathy
   Hypertensive retinopathy
   Tuberculosis
   Toxemia of pregnancy
   HIV
   Community ophthalmology 1 2 3 4 5
   Magnitude of blindness in India
   NPCB and implementation at various levels
   Functions of ophthalmic assistant
   Organization of eye camp
   Medical care/referral 1 2 3 4 5
   Indications of topical antibiotics
   Indications of topical steroids
   Indications of antiglaucoma drugs
   Indications of ophthalmology referral

3. Using the table below, please indicate how comfortable You feel performing the following ophthalmology-related skills:
   (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, 5 = very comfortable)
   Clinical assessment 1 2 3 4 5
   Ophthalmic history taking
   Visual acuity testing
   Test color vision
   Cover/uncover test
   Assessment of anterior chamber depth
   Pupil examination/swinging flashlight test
   Visual field testing by confrontation
   Extraocular movements
   Interpretation of fluorescein staining
   Schiotz tonometry
   Direct ophthalmoscopy
   Provide first aid in major ophthalmic emergencies

4. How many hours would you have spent in self-directed learning in ophthalmology?
   ("self-directed learning" describes a process by which individuals take the initiative, with our without the assistance of others, in diagnosing their learning needs, formulating learning goals, identify human and material resources for learning, choosing and implement appropriate learning strategies, and evaluating learning outcomes)
   1. 0–2 h 2. 2–5 h 3. 5–7 h
   4. 7–9 h 5. 0 > 10 h
5. How comfortable do you feel managing ophthalmology associated issues overall?
   1. Not comfortable at all 2. Somewhat comfortable
   3. Moderately comfortable 4. Comfortable 5. Very comfortable

THE END

Thank you for taking time for our survey.

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Case Report

Periorbital and Mediastinal Emphysema after Upper Gastrointestinal Endoscopy: Case Report of a Rare Complication

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Abstract

Purpose: To report a rare case of periorbital emphysema concurrent with cervicofacial and mediastinal emphysema in an elderly woman who underwent upper gastrointestinal endoscopy for chronic liver disease.
Case Report: An elderly woman with decompensated chronic liver disease presented with features of periorbital, facial, and mediastinal emphysema, characterized with crepitant swelling over the right periorbital area, face, neck, and mediastinum after undergoing upper gastrointestinal endoscopy. There was no history of trauma or Valsalva maneuver. Ocular findings were stable with no evidence of orbital compartmental syndrome. Urgent computed tomography scans of the orbit and chest were performed, and emergency systemic treatment with nasogastric decompression and antibiotics was initiated. However, she suddenly collapsed and succumbed despite all resuscitative efforts.
Conclusion: Our case demonstrates that periorbital emphysema can occur following procedures such as upper gastrointestinal endoscopy, in the eyes without history of local trauma. This complication should be suspected especially if there is associated cervicofacial and mediastinal emphysema. Subcutaneous emphysema is usually self-resolving; however, extension of air into deeper planes can cause dangerous complications such as blindness due to orbital emphysema or mortality due to mediastinal emphysema. Hence, prompt diagnosis and urgent intervention are crucial to avoid vision and life threatening complications.

Keywords: Mediastinal Emphysema; Periorbital Emphysema; Upper Gastrointestinal Endoscopy

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INTRODUCTION

Periorbital emphysema or abnormal presence of air within the soft tissues of the orbit most commonly results from orbital fracture with a paranasal sinus communication.[1,2] It presents as a transient, crepitant swelling of the lids and periorbital areas. Rarely, trapped intraorbital air can lead to compressive orbital emphysema, which can threaten vision due to central retinal artery occlusion or ischemic optic neuropathy. Mediastinal emphysema is the presence of extra-alveolar air in the mediastinum and can be spontaneous or due to severe thoraco-abdominal injury.[3] The patient may be asymptomatic or develop

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chest pain, cough, vomiting, and even cardiopulmonary distress, which can be fatal. Prompt diagnosis using Computed Tomography (CT) and urgent intervention of these conditions by decompression is paramount to prevent blindness due to orbital emphysema or fatality due to mediastinal emphysema. We describe a case of an elderly woman who developed simultaneous periorbital and mediastinal emphysema following upper gastrointestinal endoscopy with a fatal outcome. To the best of our knowledge, this is the first report of such a rare complication.

CASE REPORT

Our patient was a 74-year-old woman, who underwent treatment in the gastroenterology department of our hospital for decompensated chronic liver disease, cirrhosis of liver with portal hypertension, volume overload status, and dysphagia. An ophthalmology consultation was arranged when she complained of swelling of the right upper and lower eyelids following a routine upper gastrointestinal endoscopy. It was not associated with defective vision, diplopia, pain, or redness. She also complained of hoarseness of voice without breathing difficulties. There was no history of trauma, forceful nose blowing, or Valsalva maneuver. Past medical history revealed cataract surgery in both eyes four years ago.

On examination, she was conscious, oriented, and afebrile with no respiratory distress. She was obese with ascites, pedal edema, and puffiness of face. There was swelling of the right upper and lower lids and periorbital area with mechanical ptosis. Palpation revealed crepitus suggestive of emphysema. Generalized swelling and crepitus were also noted over the face, neck, and front of the chest [Figures 1 and 2]. On ocular examination, vision was 6/9, N.6 in both eyes. Pupils were briskly reacting, and the extraocular movements were full. There was no proptosis, conjunctival congestion, chemosis, or discharge. Both eyes were pseudophakic with normal intraocular pressure and fundus. As there was no pressure effect on the globe, she was treated conservatively with antibiotics and decongestants. She was advised to avoid nose blowing or straining and to inform immediately if any visual change is noticed. Nevertheless, considering the features of periorbital and mediastinal emphysema, she was advised to undergo emergency CT scan of the orbit and chest, and immediate gastric aspiration and antibiotic therapy were initiated by the physician. However, the patient developed sudden seizures followed by collapse, and, could not be revived despite aggressive resuscitative efforts.



Figure 1. External photograph of the face showing corrugated appearance of the right upper eyelid due to the presence of subcutaneous air, suggesting emphysema.

DISCUSSION

Subcutaneous emphysema in the head and neck region occurs when air is introduced into the fascial planes of the connective tissue through a site of trauma.[4,5] The trapped air may sometimes extend deep along the facial planes of the neck causing para- and retropharyngeal emphysema with possible extension into the thorax and mediastinum. This complication, although rare, can be life threatening due to close proximity to vital structures in the neck and mediastinum. Orbital and cervicofacial emphysema extending into the mediastinum have been reported following orofacial trauma including fractures, compressed air injuries, and dental procedures involving the use of high speed air-driven hand pieces.[3,5–7] Similarly pneumomediastinum, pneumoperitoneum, and subcutaneous emphysema are known complications following procedures such as upper endoscopy, endoscopic retrograde cholangiopancreatography (ERCP), and colonoscopy, and can occur with or without perforation.[8–10] Pathophysiology of these complications is explained by the anatomical connections between the deep neck fascia, mediastinum, and retroperitoneum, and continuous air insufflations during procedures such as ERCP. When a perforation occurs, free air flows from the duodenum to the retroperitoneal space



Figure 2. External photograph of the face and upper part of chest showing emphysema involving the right periorbital area, face, and neck.

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and then extends to diaphragmatic hiatus causing pneumomediastinum, pneumothorax, and cervical subcutaneous emphysema.[8,11] In those cases without overt perforation, it is postulated that trauma to the duodenal wall by the endoscope causes the insufflated air under pressure to enter the mucosa and track along the perineural and perivascular sheaths to enter the mediastinum.[12] However, pneumomediastinum with associated orbital emphysema without any cervicofacial trauma is extremely rare. Aydin et al reported the case of a 63-year-old woman who developed bilateral periorbital emphysema associated with diffuse mediastinal and subcutaneous emphysema following ERCP.[11] The findings were confirmed using imaging, and she recovered following nasogastric decompression and antibiotic therapy.

We report a case of right-sided periorbital emphysema with cervicofacial and mediastinal emphysema following routine upper gastrointestinal endoscopy, which has not been reported so far. In our patient with chronic liver disease and volume overload status, lid swelling and facial puffiness could have been mistaken as passive edema due to the systemic comorbidities; however, the characteristic crepitus felt on palpation was pathognomonic of emphysema. It also ruled out any allergic causes and angioedema. Negative history of trauma or straining and lack of inflammation on ocular examination excluded local causes of orbital emphysema. A CT scan of the orbit and thorax would have been informative but was precluded by her sudden death. High index of suspicion and prompt and aggressive management is required to prevent vision threatening as well as life threatening sequelae in such scenarios. Ocular management includes close observation for pressure effect on the orbit manifested by increasing proptosis, intraocular pressure, or worsening of vision.[1] If any of these symptoms develop, emergency orbital decompression should be performed by lateral canthotomy/cantholysis, needle aspiration, or bony decompression depending on the severity. Similarly, those with pneumomediastinum should be evaluated for any aero-digestive tract injury and monitored for potential airway obstruction, air embolism, cardiovascular, or infectious processes.[5]

Identification of emphysema should prompt the search for associated injury, and ophthalmologists should be aware that periorbital emphysema can develop due to remote causes without direct or indirect periorbital trauma. Fatal outcome in our patient emphasizes the need for prompt recognition with urgent intervention to prevent disastrous consequences.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest

REFERENCES

1. Zimmer-Galler IE, Bartley GB. Orbital emphysema: Case reports and review of the literature. *Mayo Clin Proc* 1994;69:115-121.
2. Hunts JH, Patrinely JR, Holds JB, Anderson RL. Orbital emphysema. Staging and acute management. *Ophthalmology* 1994;101:960-966.
3. Abdelrahman H, Shunni A, El-Menyar A, Ajaj A, Afifi I, Zarour A, et al. Mediastinal emphysema following fracture of the orbital floor. *J Surg Case Rep* 2014:rju032.
4. Saleem A, Chaudhary A, Ali MU, Iqbal ZH. Cervicofacial emphysema. *J Coll Physicians Surg Pak* 2010;20:556-557.
5. Mather AJ, Stoykewych AA, Curran JB. Cervicofacial and mediastinal emphysema complicating a dental procedure. *J Can Dent Assoc* 2006;72:565-568.
6. Ong WC, Lim TC, Lim J, Sundar G. Cervicofacial, retropharyngeal and mediastinal emphysema: A complication of orbital fracture. *Asian J Surg* 2005;28:305-308.
7. Hiraoka T, Ogami T, Okamoto F, Oshika T. Compressed air blast injury with palpebral, orbital, facial, cervical, and mediastinal emphysema through an eyelid laceration: A case report and review of literature. *BMC Ophthalmol* 2013;13:68.
8. Schiavon LL, Rodrigues RA, Nakao FS, Sena VOD, Ferrari AP, Jr EDL. Subcutaneous Emphysema, Pneumothorax and Pneumomediastinum Following Endoscopic Sphincterotomy. *Gastroenterol Res* 2010;3:216-218.
9. Yen HH, Chen YY. Pneumomediastinum, pneumoperitoneum, pneumoretroperitoneum, and subcutaneous emphysema after ultrathin endoscopy. *Endoscopy* 2011;43:E49-E50.
10. Minocha A, Richards RJ. Pneumomediastinum as a complication of upper gastrointestinal endoscopy. *J Emerg Med* 1991;9:325-329.
11. Aydin M, Kisli E, Ozgoren E, Soylemez O, Baser M, Aslaner A. Mediastinal and retro-orbital emphysema following an uncomplicated endoscopic sphincterotomy. *Indian J Surg* 2005;67:147-149.
12. Jaiswal SK, Sreevastava DK, Datta R, Lamba NS. Unusual occurrence of massive subcutaneous emphysema during ERCP under general anaesthesia. *Indian J Anaesth* 2013;57:615-617.

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Clinicopathological Conference

An unusual cause of breathlessness and profuse micronodules

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ABSTRACT

We describe a 21-year-old male with a history of smoking and subacute onset of breathlessness with normal cardiorespiratory examination. The presence of "track marks" and digital infarcts prompted evaluation for infective endocarditis and confrontational history taking revealed anorexia, weight loss over 3 months along with intravenous drug abuse of reconstituted tablets of tapentadol. Echocardiography was normal and blood cultures were sterile; computed tomography showed bilateral, diffuse, small centrilobular nodules with "tree-in-bud" appearance. In this clinicopathologic conference, we discuss the clinical and radiological differential diagnosis of centrilobular nodules, lung biopsy findings, and management options for patients with such a presentation.

KEY WORDS: Excipient lung disease, intravascular talcosis, pulmonary foreign body angiogranulomatosis, talc granulomatosis

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PRESENTATION OF THE CASE

A 21-year-old college student presented with breathlessness and left-sided chest pain for a week. Breathlessness was insidious in onset and persisted on walking at own speed at level. Chest pain was left-sided, intermittent, pricking and non-exertional. There was no history of fever, rhinitis, headache, hemoptysis, cough, or joint pains. He did not raise pets but admitted to smoking 7–8 cigarettes/day and consuming whisky (192 g of alcohol weekly). He denied any recent worsening of educational performance. He did not report any significant prior medical illness or pertinent illness in family or occupational dust exposure. He was initially seen in cardiology and evaluation showed normal cardiac and respiratory examination without any pallor, clubbing, or organomegaly. Body mass index was 18.6 kg/m^2. The examination also showed extensive track marks over both arms with evidence of multiple peripheral digital infarcts [Figure 1], raising suspicion for infective endocarditis (IE). On confronting, he disclosed daily intravenous self-injections of three–four powdered and reconstituted 50 mg tablets of Tapentadol (Tydol, Sun Pharma) in sterile distilled water two–three times a day over the last 2 years. He also admitted to using inhaled cannabis twice weekly over the last 2 years. There was associated anorexia and unquantified weight loss over the last 3 months. Three sets of blood cultures were sterile. Electrocardiography showed T-wave inversions in leads V3–V6 and II, III, and aVF. Transthoracic echocardiography (TTE) showed normal valves without any evidence of vegetations or pulmonary hypertension and normal left ventricular function. Serum creatinine

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Figure 1: Composite image of the clinical photographs (left) of the right forearm showing "track marks," indicating healed superficial septic thrombophlebitis and (right) both hands showing multiple bilateral distal digital pulp infarcts. Similar findings were also seen in both plantar surfaces (not shown)

was 0.6 mg/dL; urine microscopy was normal without any albuminuria. Hemoglobin was 14.2 g/dL with normal leukocyte and platelet counts. He remained afebrile. Transesophageal echocardiography (TEE) was planned; in the meanwhile, review of his chest radiographs showed diffusely scattered fine nodules bilaterally [Figure 2, left]. Spirometry showed mild restriction with reduced diffusion capacity for carbon monoxide (forced expiratory volumes at 1 s 2.25 L [65%]%, forced vital capacity 2.87 L [71%], DLco 62%). There was desaturation to 92% while walking 6 minutes, with preserved walk distance (545 meters, 89% predicted). Human immunodeficiency virus (HIV), anti-nuclear antibodies, hepatitis B, and hepatitis C antibodies were negative by enzyme-linked immunosorbent assay. Immunoglobulin E levels were 9 IU/mL (normal <100 IU/mL). High-resolution computed tomography (HRCT) of the thorax showed bilateral profuse evenly distributed micronodules with "tree-in-bud" [Figure 2, right]. There was no sputum production on induction with 3% saline. A procedure was planned for diagnosis.

DIFFERENTIAL DIAGNOSIS

Important features of this case

This HIV-negative young adult with significant smoking history, cannabis use, and intravenous drug abuse with reconstituted tablets over 2 years has breathlessness, anorexia, and weight loss with radiological findings of micronodules. There is evidence of distal embolic infarcts, but initial evaluation for endocarditis was negative. The reduced DLco, restriction on spirometry and unexpectedly low walk distance in a young individual with desaturation on walking implies extensive parenchymal and/or pulmonary vascular disease. The differential diagnosis of profuse bilateral micronodules is extensive [Table 1] but can be narrowed by careful history and analysis of HRCT findings in conjunction with the expected pulmonary complications of intravenous drug abuse [Table 2].[1] The first step is to characterize the

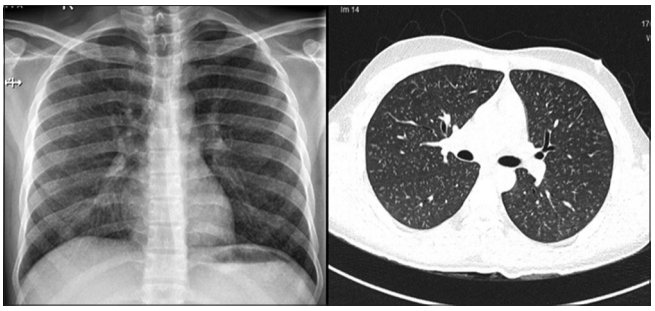


Figure 2: Composite image of the chest radiograph (left) showing small nodular opacities and high-resolution computed tomography of the thorax (right) showed bilateral profuse evenly distributed micronodules with tree-in-budding; pulmonary artery was normal in size, and there were no associated lymphadenopathy. Expiratory computed tomography did not show any air trapping (not shown)

Table 1: Causes of diffuse micronodular disease on computed tomography

With tree-in-budding
Infectious causes
Tuberculosis, nontubercular mycobacteria (typically MAC)
Bronchopneumonia: *Haemophilus influenzae*, *Mycoplasma pneumoniae*, Chlamydia
Viral bronchiolitis
Airway-invasive aspergillosis
Aspiration
Bronchiectasis: Cystic fibrosis, ciliary disorders, immunodeficiency
Immunologic
Allergic bronchopulmonary aspergillosis
Diffuse panbronchiolitis
Hematogenous: intravascular metastases (rare)
Without tree-in-budding
Infective causes
Tuberculosis
Fungi: *Histoplasma capsulatum*, blastomycosis, candidemia
Varicella zoster
Immunologic causes
Tropical pulmonary eosinophilia
Hypersensitivity pneumonitis (subacute)
Follicular bronchiolitis
Respiratory bronchiolitis
Sarcoidosis
Diffuse pulmonary amyloidosis
Allergic bronchopulmonary aspergillosis (rarely)
Occupational lung disease
Berylliosis, silicosis, coal worker's pneumoconiosis
Idiopathic
Pulmonary alveolar microlithiasis
Idiopathic pulmonary hemosiderosis
Malignancy
Metastasizing leiomyoma
Pulmonary Langerhans cell histiocytosis (early)
Metastatic adenocarcinoma, renal cell carcinoma, papillary thyroid carcinoma
Others
Metastatic calcification (renal failure)
Pulmonary arteriolar causes
Pulmonary edema and hemorrhage, excipient lung disease, plexogenic arteriopathy, veno-occlusive disease, pulmonary capillary hemangiomatosis

MAC: *Mycobacterium avium* intracellulare, *Wuchereria bancrofti*

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Table 2: Pulmonary problems in intravenous drug abusers

Mechanism of Injury	Observed side-effects
Pharmacodynamic properties of injected drug	Noncardiogenic pulmonary edema (cocaine, opioids) Cocaine intoxication: pulmonary edema, pulmonary hemorrhage, cardiogenic pulmonary edema (coronary spasm) Methylphenidate intoxication: hemoptysis
Complications of unsafe intravenous injections	Lung abscess (with loss of consciousness, seizures, and aspiration) Infective endocarditis Septic pulmonary embolism Infections acquired from needle sharing (HIV, hepatitis B, hepatitis C)
Effects of contaminants	Excipient lung disease* Emphysema (lower lobe predominant "Ritalin" lung with methylphenidate) and Bullous lung disease Pulmonary vascular disease Group 1: Pulmonary hypertension (cocaine and methamphetamine) Group 3: Pulmonary hypertension (emphysema, foreign body granulomatosis, and interstitial lung disease) Interstitial lung disease Pneumothorax (subclavian injections, cocaine, and bullous lung disease), septic emboli
Related to associated abused substances, poverty, malnutrition	Pneumonia Tuberculosis More frequent exacerbations of asthma (fatal asthma)

*Talc, starch, crospovidone, and microcrystalline cellulose are used as fillers. HIV: Human Immunodeficiency Virus

distribution of the micronodules spatially within the lung and also the secondary pulmonary lobule. These nodules were uniform in size and were distributed evenly in a cephalocaudal direction, without any preferential lobar involvement. None of the nodules abut the pleural surface or the fissures [Figure 3]; there is clear evidence of extensive bilateral branching nodules in centrilobular distribution. There is no evidence of bronchiectasis; while no contrast was administered, in conjunction with echocardiography, there is no dilated pulmonary artery or lymphadenopathy. These findings limit the radiological differentials to causes of micronodules in a centrilobular distribution and extensive uniform "tree-in-bud" appearance. "Tree-in-bud" indicates the presence of dilated centrilobular bronchioles with lumen impacted by mucus, fluid, or pus and associated with peribronchiolar inflammation. "Tree-in-bud" indicates small airway disease and is associated with airway infection in the large majority but can also be rarely caused by arteriolar disease.[2] Its presence makes three common causes of centrilobular nodules unlikely: hypersensitivity pneumonitis, follicular bronchiolitis, and respiratory bronchiolitis.

Endobronchial tuberculosis

Mycobacterium tuberculosis is endemic in India, with more than 2.2 million new cases each year. Delayed recognition

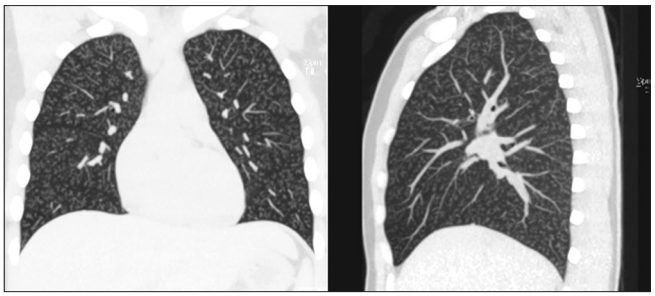


Figure 3: Coronal and sagittal reconstruction of the maximum intensity projection formatting of the high-resolution computed tomography images of the thorax showing diffuse, extensive, bilateral centrilobular nodules with branching and without any lobar predominance

and extensive disease are related to associated substance abuse, poverty, and malnutrition, all of which were present in this patient. Extensive endobronchial spread of tuberculosis can cause extensive tree-in-bud opacities although asymmetry is usually seen.[3] Tuberculosis is also the most common cause of micronodules with a random pattern (miliary). Even though the "pretest probability" of tuberculosis is high in this patient, the marked symmetry and the absence of any cavitation or consolidation is odd; digital infarcts are unusual but could be caused by an associated immune complex phenomenon that can occur with tuberculosis. *Mycobacterium avium*-intracellulare infection can also cause centrilobular nodules with "tree-in-bud," bronchiectasis, and consolidation. While it can be seen in immunocompetent patients, the classic phenotype is a middle-aged woman with slender build and without any constitutional symptoms.

Bronchopneumonia and viral bronchiolitis

Infectious bronchiolitis due to viruses, *Mycoplasma*, and chlamydia is a common cause of profuse and symmetric centrilobular nodules with "tree-in-bud;"[3] however, the prolonged illness and associated embolic phenomenon are unusual.

Allergic bronchopulmonary aspergillosis

Centrilobular nodules with "tree-in-bud" or miliary nodules can be caused by allergic bronchopulmonary aspergillosis but the absence of asthma, central bronchiectasis, and normal IgE levels rule out this diagnosis.[2,4]

Diffuse panbronchiolitis

Diffuse panbronchiolitis (DPB) is a rare disease of unknown cause presenting in males between the second and fifth decade with productive cough, diffuse bilateral centrilobular nodules with "tree-in-bud" and sinusitis. While reported in Indians,[5] it is extremely rare in our population but can explain all the clinical and radiological features.[6] Cold agglutinins are frequent in DPB and pulp infarcts may be due to an associated connective tissue disease. The absence of productive sputum, obstruction on spirometry, sinusitis, and air trapping on expiratory CT are unusual in DPB and necessitate further evaluation with surgical lung biopsy to establish the diagnosis.[6]

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Excipient lung disease

Centrilobular nodules are usually caused by bronchiolar disease but can rarely be caused by arteriolar disease. Arteriolar causes include pulmonary edema and hemorrhage, excipient lung disease (ELD), granulomatosis with polyangiitis (GPA), plexogenic arteriopathy (PH), veno-occlusive disease (VOD), pulmonary capillary hemangiomatosis (PCH), and metastatic calcification.[2] There was no evidence of heart failure, renal failure or anemia and hemoptysis to suggest alveolar hemorrhage. The absence of pulmonary hypertension makes centrilobular nodules due to PH, VOD, and PCH very unlikely. Nodules due to GPA are larger, fewer, and associated with cavitation. Excipients are insoluble inert particulate filler materials that bind and protect the active drug during production as well as shape and lubricate tablets for easy swallowing. Excipients include talc (hydrated magnesium silicate), microcrystalline cellulose, crospovidone, and starch. Typically, excipients constitute 5% of a tablet's mass. On intravenous injection, excipient particles lodge in pulmonary arterioles and trigger an angiogranulomatous reaction; progressive disease is associated with dyspnea and pulmonary hypertension.[7] However, the presence of finger pulp infarcts, "tree-in-bud" appearance and absence of pulmonary hypertension are unusual in ELD.

CLINICAL DIAGNOSIS

In summary, the differential diagnosis of extensive centrilobular nodules with "tree-in-bud" without bronchiectasis or pulmonary hypertension in this young adult with smoking, cannabis use, and intravenous drug abuse of reconstituted tablets can be narrowed down to extensive endobronchial tuberculosis, diffuse panbronchiolitis, and ELD.

Dr. RM. PL. Ramanthan: With these clinicoradiological possibilities in mind, this patient underwent fundus examination and bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial lung biopsy, with a plan for surgical biopsy if evaluation was inconclusive. Can we now have the findings?

Dr. K. Divya: Dilated fundus examination confirmed refractile particles scattered along both the retinal arteries and retinal veins [Figure 4], confirming systemic embolization of small (<5 μm) talc particles, consistent with intravenous talcosis. Inhalational talc exposure does not cause such marked retinal deposition as the inhaled particles are larger and are restricted by bronchioles and pulmonary circulation.[8,9]

Dr. Nidhya Ganesan: BAL was negative for acid-fast bacilli and for *M. tuberculosis* by Xpert MTB/RIF; occasional needle-shaped crystals were seen on cytology. The transbronchial lung biopsy specimen confirmed perivascular granulomatous reaction to talc particles [Figure 5, left] that appear as birefringent needle-shaped crystals within the granulomas under polarized light [Figure 5, right]. Review of the composition of Tydol confirmed the presence of talc as excipient. Microcrystalline cellulose appears rod like while starch has a maltese cross pattern and crospovidone is nonbirefringent on polarization.[7]

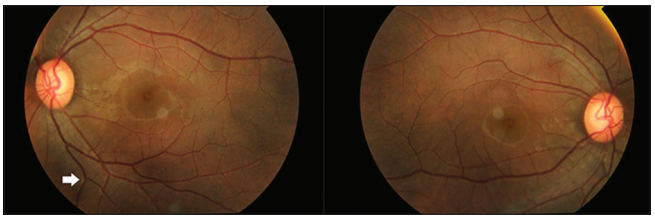


Figure 4: Fundus photograph of both eyes showing refractile particles scattered along both the retinal arteries and retinal veins, confirming the diagnosis of intravenous talcosis

Dr. Srinivas Rajagopala: A diagnosis of ELD (talc granulomatosis) was made. Psychiatry services were involved, and the patient underwent counseling, arrangement for family supervision, and daily-observed methadone replacement. He has successfully discontinued intravenous drug abuse and remains stable at 3 months of follow-up. Serial spirometry, DLco, and radiographs have been advised at 6 monthly intervals. Three unusual findings need an explantation; 1) The presence of "tree-in-bud" in a process that is hematogenous in origin 2)The absence of pulmonary hypertension in ELD and 3)The cause of the bilateral digital infarcts observed.

Dr. B. Devanand: Diseases involving pulmonary arterioles can cause centrilobular nodules as arterioles run in the peribronchovascular interstitium adjacent to bronchioles. Talcosis associated with intravenous abuse is most commonly associated with diffuse fine centrilobular nodules, ground-glass abnormalities or a combination of nodules and lower-lobe emphysema (Ritalin lung with methylphenidate).[9,10] The "tree-in-bud" pattern is unusual for arteriolar disease; it can be caused by intra-arteriolar accumulation of talc with associated adjacent perivascular granulomatous reaction giving the appearance of branching centrilobular nodules and has been described in a few cases of ELD earlier.[11]

Dr. Srinivas Rajagopala: What is the course of ELD? Can the disease progress despite cessation of abuse?

Dr. Kancherla Roopa: Lung fibrosis and pulmonary hypertension are related to the extent of vascular obstruction and granulomatous inflammation and are usually irreversible with talc, cellulose, and crospovidone as these cannot be mobilized; starch can be removed by metabolism and pulmonary hypertension can be reversible to a large degree. Disease progression can occur despite cessation due to ongoing granulomatous foreign-body reaction to the deposited, unmobilizable talc. The initial series of patients described all had progressive symptoms leading to death over 9 months to 5 years.[10,12] The index

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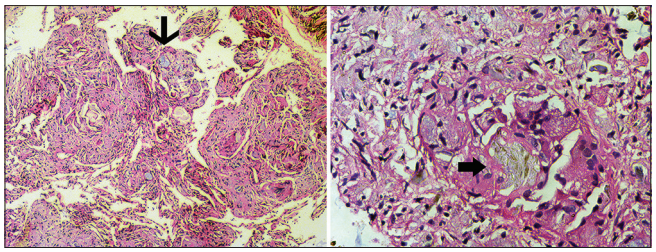


Figure 5: Photomicrographs of the transbronchial lung tissue biopsy specimen (left) demonstrating perivascular foreign body granulomatous reaction to the talc particles (thin arrow, H and E, ×100). Under polarized light, birefringent 20–95 μm needle-shaped crystals were seen in the granuloma, consistent with talc (block arrow, H and E, ×400)

patient has a large load of perivascular disease and is at risk for pulmonary hypertension and disease progression despite cessation of further abuse.

Dr. Srinivas Rajagopala: How common is systemic embolization of talc given that the lung acts as an effective filter?

Dr. K. Divya: The talc that is trapped in the pulmonary arterioles is typically around 10 μm, but smaller particles can escape to the systemic circulation and be detected in the retina, spleen, and virtually every organ on autopsy.[12] Finding excipient crystals in fundoscopy is a rapid noninvasive method to confirm intravenous talcosis.

Dr. Srinivas Rajagopala: Given the presence of digital infarcts, is TEE needed to exclude IE in this patient?

Dr. G. Rajendiran: The first consideration in this patient with possible unsafe needle practices, no murmurs, weight loss, breathlessness, and digital embolic manifestations was subacute native valve IE. TTE has low sensitivity compared to TEE (46% vs. 93%) although both are highly specific (96%).[13] Embolic manifestations are seen in about half of patients with IE.[14] He was not administered any antibiotics, three sets of blood cultures were sterile, and TTE showed all valves clearly without any vegetations. TEE is restricted in our practice to high probability for IE or in whom TTE is rendered less sensitive due to obesity, lung hyperinflation, or prosthetic valves. The index patient met only 2 minor Modified Duke's criteria and was considered low risk for IE after the initial evaluation and TEE deferred.[15] Furthermore, a clear alternative explanation for the observed distal infarcts in the form of talc deposition in the distal microcirculation with thrombosis is available and corroborated by fundoscopy. While this physical finding has not been described in ELD previously, it is consistent with the distal digital embolization and subsequent microthrombosis caused by talc.

Dr. Srinivas Rajagopala: What are the options for those with progressive breathlessness despite cessation of intravenous drug abuse?

Dr. RM. PL. Ramanthan: There are previous small series of lung allografts after a careful selection process in ELD with excellent outcomes.[16] Cadaveric bilateral sequential lung transplantation is a reality in our country today, and he can be listed for the same at our center if he meets criteria and remains free of substance abuse for at least 6 months with adequate psychiatry counseling.

SUMMARY

Clinical examination findings continue to have relevance in this era of investigation-driven medicine. Excipient lung disease can result from intravenous injection of reconstituted tablets and can present with ground glass opacities, small nodules, and emphysema. Pulmonary arteriolar diseases must be considered in the differential diagnosis of centrilobular nodules on CT.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that names, identifying marks, and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Marchiori E, Souza AS Jr., Franquet T, Müller NL. Diffuse high-attenuation pulmonary abnormalities: A pattern-oriented diagnostic approach on high-resolution CT. AJR Am J Roentgenol 2005;184:273-82.
2. Webb R, Muller NL, Naidich DP. HRCT Findings: Multiple nodules and nodular opacities. In: High-Resolution CT of the Lung. 5th ed. Philadelphia: LWW Wolters Kluwer; 2014. p. 125-9.
3. Walker CM, Abbott GF, Greene RE, Shepard JA, Vummidi D, Digumarthy SR, *et al*. Imaging pulmonary infection: Classic signs and patterns. AJR Am J Roentgenol 2014;202:479-92.
4. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, *et al*. Allergic bronchopulmonary aspergillosis: Review of literature and proposal of new diagnostic and classification criteria. Clin Exp Allergy 2013;43:850-73.
5. Nath A, Aggarwal AN, Gupta R. Diffuse panbronchiolitis: Report of a rare disease from India. Indian J Chest Dis Allied Sci 2010;52:43-5.
6. Poletti V, Casoni G, Chilosi M, Zompatori M. Diffuse panbronchiolitis. Eur Respir J 2006;28:862-71.
7. Nguyen VT, Chan ES, Chou SH, Godwin JD, Fligner CL, Schmidt RA, *et al*. Pulmonary effects of i.v. Injection of crushed oral tablets: "Excipient lung disease". AJR Am J Roentgenol 2014;203:W506-15.
8. Akira M, Kozuka T, Yamamoto S, Sakatani M, Morinaga K. Inhalational talc pneumoconiosis: Radiographic and CT findings in 14 patients. AJR

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Am J Roentgenol 2007;188:326-33.
9. Ward S, Heyneman LE, Reittner P, Kazerooni EA, Godwin JD, Müller NL, *et al*. Talcosis associated with IV abuse of oral medications: CT findings. AJR Am J Roentgenol 2000;174:789-93.
10. Feigin DS. Talc: Understanding its manifestations in the chest. AJR Am J Roentgenol 1986;146:295-301.
11. Bendeck SE, Leung AN, Berry GJ, Daniel D, Ruoss SJ. Cellulose granulomatosis presenting as centrilobular nodules: CT and histologic findings. AJR Am J Roentgenol 2001;177:1151-3.
12. Sieniewicz DJ, Nidecker AC. Conglomerate pulmonary disease: A form of talcosis in intravenous methadone abusers. AJR Am J Roentgenol 1980;135:697-702.
13. Bashore TM, Cabell C, Fowler V Jr. Update on infective endocarditis. Curr Probl Cardiol 2006;31:274-352.
14. McDonald JR. Acute infective endocarditis. Infect Dis Clin North Am 2009;23:643-64.
15. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr., Ryan T, *et al*. Proposed modifications to the duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;30:633-8.
16. Weinkauf JG, Puttagunta L, Nador R, Jackson K, LaBranche K, Kapasi A, *et al*. Long-term outcome of lung transplantation in previous intravenous drug users with talc lung granulomatosis. Transplant Proc 2013;45:2375-7.

Case Report

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DRESS Syndrome Secondary to Carbamazepine Therapy Presenting with Bilateral Acute Anterior Uveitis and Angle Closure Glaucoma

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Abstract

Purpose: Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a rare, life-threatening multi-system adverse drug reaction characterized by febrile skin rash, hematologic abnormalities, and involvement of internal organs. We report a case of DRESS syndrome in a child presenting with primary ophthalmic manifestations.
Case Report: An 11-year-old boy presented with severe pain and diminished vision in both eyes six weeks after starting carbamazepine therapy for seizure disorder. Ocular examination revealed features of bilateral acute anterior uveitis, acute onset myopia, and angle closure glaucoma secondary to uveal effusion. Additionally, the patient was febrile with a generalized maculopapular rash, and blood investigations revealed eosinophilic leukocytosis. A diagnosis of carbamazepine-induced DRESS syndrome was made, and carbamazepine therapy was discontinued. Treatment with cycloplegics, topical, and systemic steroids resulted in prompt clinical recovery.
Conclusion: Ophthalmologists should be aware that hypersensitivity to anticonvulsants, such as carbamazepine, can present with bilateral uveitis and uveal effusion along with systemic symptoms. Prompt diagnosis and treatment can prevent vision loss and life-threatening complications. Patients should be counselled about potential adverse effects of anticonvulsants before therapy.

Keywords: Anterior Uveitis; Carbamazepine; DRESS Syndrome

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INTRODUCTION

Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a rare, potentially fatal idiosyncratic reaction characterized by cutaneous manifestations, hematological changes, and involvement of internal organs.[1] The incidence of this syndrome ranges from 1 in 1000 to 1 in 10,000 drug exposures. The liver is the most commonly affected visceral organ.[2] Primary ocular involvement is uncommon, and only a few cases have been reported in adults.[3–7] Here, we report the occurrence of bilateral acute anterior uveitis and angle closure glaucoma associated with carbamazepine-induced DRESS syndrome in a child.

CASE REPORT

An 11-year-old boy was admitted at the ophthalmology department with complaints of severe pain and diminished vision in both eyes for two days. He had never worn glasses, and his ocular history was unremarkable. Six weeks earlier, he had been diagnosed with a seizure disorder by a pediatrician; oral carbamazepine therapy (200 mg twice daily) was started. Three weeks before the onset of ocular symptoms, he had developed fever and generalized skin rash, which was diagnosed as a viral exanthem and managed with supportive therapy.

Ophthalmic examination revealed a best corrected visual acuity of 20/120 in the right eye and 20/60 in the left eye with −9.0 diopters spherical (DSph) and −1.0 diopters cylinder (DCyl) at 90° in the right eye and −10.0 DSph and −1.0 DCyl at 90° in the left eye. The eyelids were swollen, and chemosis was noted in both eyes. Anterior segment examination revealed corneal epithelial edema, shallow anterior chamber with 3 plus cells, sluggishly reacting pupil, and clear crystalline lens in both eyes. Intraocular pressure by Goldmann applanation tonometry was 45 and 40 mmHg in the right and left eyes, respectively. Gonioscopy revealed bilateral 360° appositional angle closure, and no angle structures were visible. Fundus examination revealed normal optic disc and vessels with a cup-to-disc ratio of 0.3. The axial length of the right eye was 21.91 mm and that of the left eye was 22.05 mm. Considering the acute onset of myopia, bilateral shallow anterior chambers, and angle-closure glaucoma, ciliochoroidal effusion, probably due to carbamazepine, was diagnosed.



Figure 1. Clinical photograph showing swollen eyelids and crusted lesions on lips.

In addition to the ocular findings, the child was febrile with a temperature of 39.8°C and had a generalized maculopapular rash. The lips were swollen with crusted lesions [Figure 1]. Systemic examination revealed no abnormalities. Investigations revealed leukocytosis ($18.9\times10^3/\mu$L) with increased neutrophils ($14.0 \times 10^3/\mu$L) and eosinophils ($1.8 \times 10^3/\mu$L). Peripheral smear showed a few atypical lymphocytes and no blast cells. Hemoglobin level was 12.9 g/dL, and platelet count was $358 \times 10^3/\mu$L. Blood culture tests were negative, and liver function tests were within normal limits. A dermatologist was consulted for the skin lesions, and DRESS syndrome was diagnosed.

Carbamazepine was discontinued and substituted with levetiracetam. Treatment was initiated with homatropine, timolol, and topical steroids, along with intravenous mannitol (1 g/kg body weight). His symptoms improved dramatically after

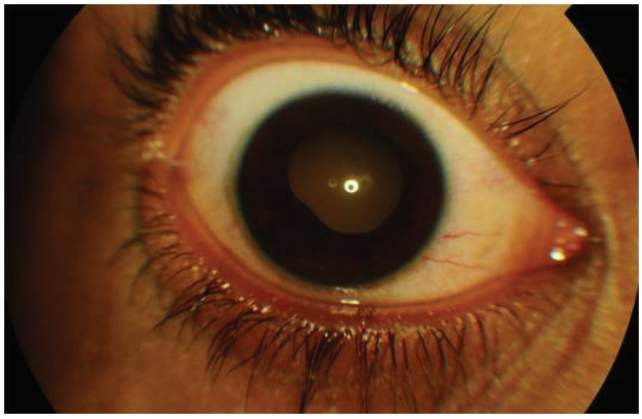


Figure 2. Anterior segment photograph of the right eye showing posterior synechiae.

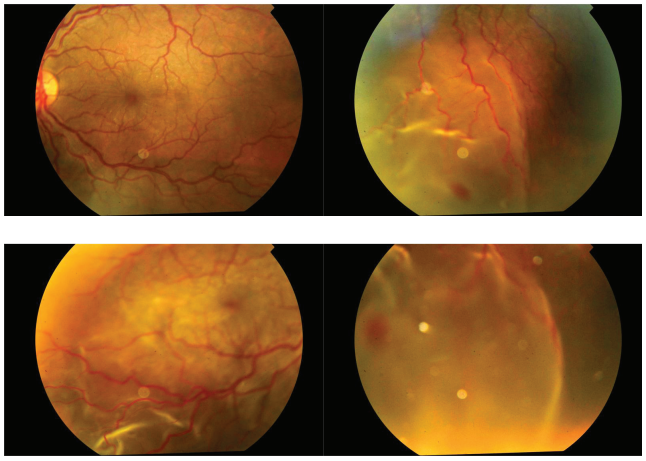


Figure 3. Fundus photograph of both eyes showing choroidal folds at the macula, retinal hemorrhages, and serous retinal detachment.

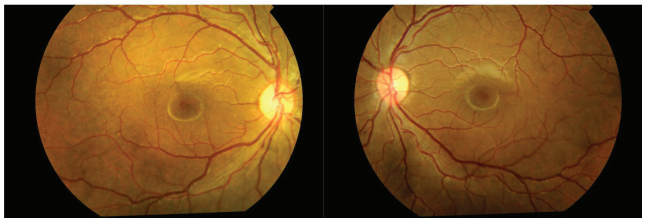


Figure 4. Fundus photograph at the three-week follow-up showing normal optic disc and macula.

two doses of intravenous dexamethasone (4 mg). Ocular examination on the third day revealed a clear cornea, deep anterior chamber with over three cells in both eyes, and posterior synechiae in the right eye [Figure 2]. Intraocular pressure in the right and left eyes was 6 and 5 mmHg, respectively. Fundus examination revealed choroidal folds in the macula and serous elevation of the retina along with scattered retinal hemorrhages [Figure 3].

Along with topical steroids and homatropine, oral prednisolone therapy was started (initial dose, 1 mg/kg body weight) and subsequently tapered. At the three-week follow-up, uveitis and retinal hemorrhages had resolved [Figure 4]. Unaided visual acuity was 20/20, and intraocular pressure was 16 mm Hg in both eyes.

DISCUSSION

Carbamazepine is not included among drugs causing uveitis or angle closure glaucoma. A literature search revealed one case of elevated intraocular pressure associated with carbamazepine in a patient with pseudoexfoliation glaucoma.[8]

Aromatic anticonvulsants, such as carbamazepine, phenytoin, and phenobarbitone, are the most common causes of DRESS; it usually occurs three weeks to three months after starting the drug therapy.[9] Although many children receive anticonvulsant therapy, DRESS is rare in childhood, with fewer cases reported in children compared to adults.[10] The exact pathogenesis of this syndrome is poorly understood. The implicated mechanisms include abnormalities in drug metabolism with accumulation of reactive metabolites, genetic predisposition of individuals to certain human leukocyte antigen (HLA) haplotypes, and interactions between herpes viruses and antiviral and drug-specific immune responses.[11]

Currently, there is no consensus on the best diagnostic criteria for DRESS syndrome. The original criteria were proposed by Bocquet et al.[1] Two separate scoring systems based on the diagnostic criteria have been developed by the Japanese Research Committee on Severe Cutaneous Adverse Reaction (J-SCAR) and the European Registry of Severe Cutaneous Adverse Reaction (RegiSCAR).[11, 12] The RegiSCAR, which outlines seven inclusion criteria, is the most widely used [Table 1].

Our patient fulfilled three of the four main criteria for the diagnosis of DRESS syndrome (as proposed by the European RegiSCAR), namely, acute rash, fever > 38°C, and abnormal eosinophil and lymphocyte counts. No internal organs were involved. This can be attributed to the timely discontinuation of carbamazepine and prompt initiation

Table 1. Diagnostic criteria for DRESS syndrome

Bocquet et al[1]	J-SCAR	RegiSCAR
All the three criteria are required	Typical DRESS: all the seven criteria are required Atypical DIHS: only the first five are required	Presence of more than three of the following criteria
1. Cutaneous drug eruption	1. Maculopapular rash developing three weeks after the treatment with the offending drug	1. Hospitalization
2. Hematologic abnormalities: Eosinophils $\geq 1.5 \times 10^9$/L and the presence of atypical lymphocytes	2. Prolonged clinical symptoms after discontinuing the causative drug	2. Acute rash
3. Systemic involvement: lymphadenopathy > 2 cm, hepatitis with transaminase levels twice the normal, interstitial nephritis, pneumonitis, and carditis	3. Fever > 38°C	3. Reaction suspected to be drug-related
	4. Hepatic abnormalities (ALT >100 U/L) or involvement of another organ	4. Fever > 38°C
	5. Leukocytosis, eosinophils $\geq 1.5 \times 10^9$/L, and the presence of atypical lymphocytes (> 5%)	5. Lymphadenopathy involving at least two sites
	6. Lymphadenopathy	6. Involvement of one internal organ (liver, kidney, or others)
	7. Reactivation of HHV-6	7. Hematologic abnormalities: lymphocyte count above or below normal limits, eosinophil count above laboratory limits, platelet count below laboratory limits.

ALT, Alanine transaminase; DIHS, drug-induced hypersensitivity syndrome; HHV, human herpes virus

of steroid therapy. Differential diagnosis in our patient includes viral infections, such as dengue, chikungunya, and hematological disorders, such as leukemia, anemia, and thrombocytopenia, all of which can present with retinal hemorrhages, serous retinal detachment, and uveitis.[13–15]

A clear temporal relationship between the administration of carbamazepine and onset of symptoms (three weeks), peripheral blood picture, an absence of findings in the clinical history and examination of the child suggestive of infection or neoplasia, and bilateral acute onset myopia with angle closure prompted us to suspect a drug-induced adverse reaction. The assessment of causality using the Naranjo Adverse Drug Reaction Probability Scale established a "probable" relationship (score of 6) with carbamazepine in the patient.[16]

Ocular involvement is a rare feature of DRESS syndrome and to the best of our knowledge, only five cases have been reported in literature. Uveitis (anterior, intermediate, and panuveitis) has been noted in all the cases. Reactivation of herpes viruses (human herpesvirus 6 and Epstein-Barr virus) were noted in two patients who had multi-organ involvement.[3–7]

We assume that the inflammation of the uveal tract and accumulation of exudate had resulted in uveal effusion. This led to the anterior displacement of the iris and lens diaphragm, eventually resulting in acute onset myopia, shallow anterior chamber, appositional angle closure, and elevation of intra ocular pressure in our patient. Cycloplegics promoted posterior rotation of the iris-lens diaphragm, and steroid therapy attenuated the allergic-type drug reaction, thereby stabilizing the blood-aqueous barrier and ensuring prompt recovery.

In contrast to other adverse drug reactions, dynamic changes in immune response have been reported in DRESS syndrome.[17] Hence, the uveal tract with its rich network of macrophages and

major histocompatibility complex (MHC) class II dendritic cells is most likely to be affected in patients with ocular involvement.

Ophthalmologists should be aware that hypersensitivity to anticonvulsants, such as carbamazepine, can present with bilateral uveitis and uveal effusion along with systemic symptoms. Given the delayed onset after the initiation of treatment with the offending drug, systemic symptoms, and potentially fatal multi-organ involvement, a high index of suspicion is necessary to prevent complications. Additionally, it is necessary to inform the patient about the adverse effects of carbamazepine before prescribing it.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

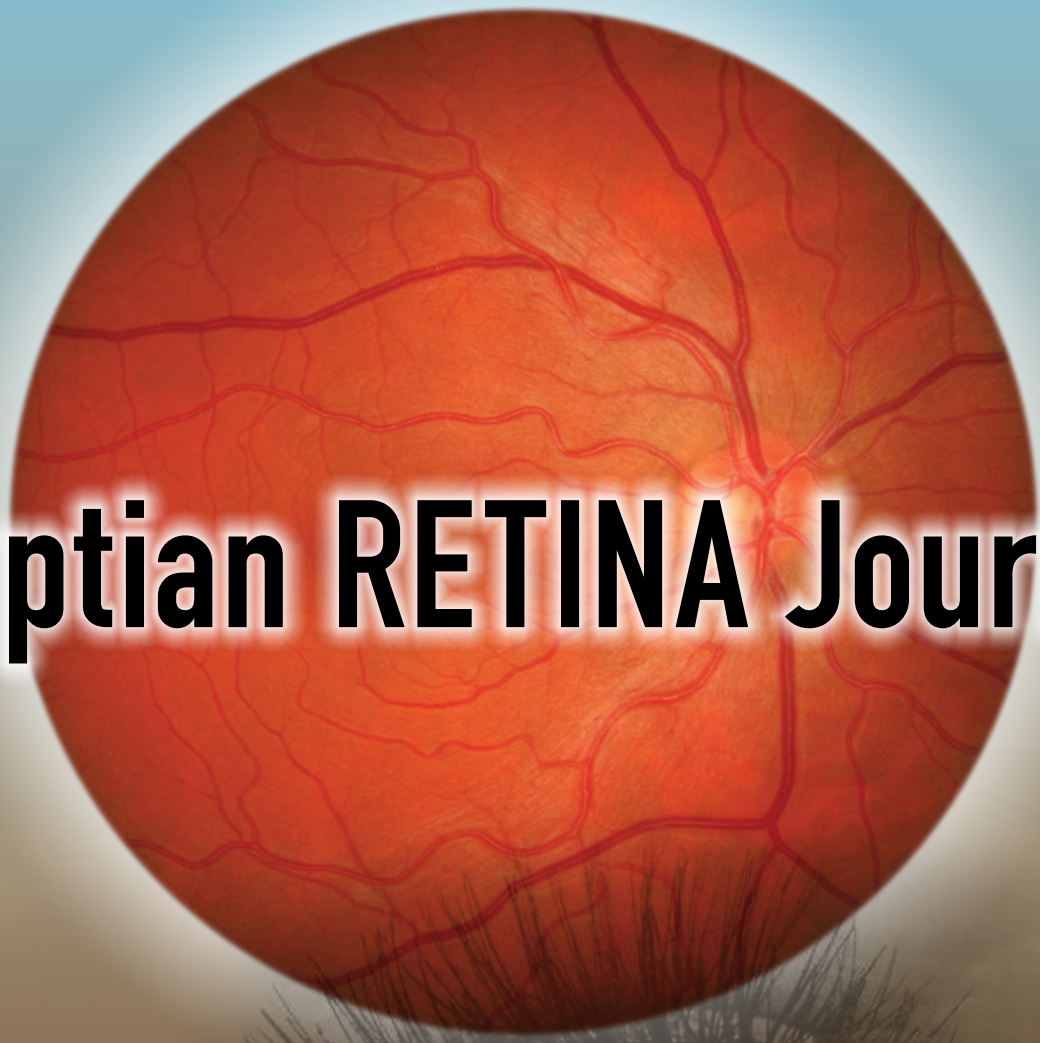
There are no conflicts of interest.

REFERENCES

1. Bocquet H, Boagot M, Roujeau JC. Drug-induced pseudolymphoma and drug hypersensitivity (Drug rash with eosinophilia and systemic symptoms: DRESS). *Semin Cutan Med Surg* 1996;15:250–257.
2. Kano Y, Ishida T, Hirahara K, Shiohara T. Visceral involvements and long-term sequelae in drug-induced hypersensitivity syndrome. *Med Clin North Am* 2010;94:743–759.
3. Schauer P, Salaun N, Bazin S, Labrouze JM, Bourguignon G. [DRESS syndrome with bilateral panuveitis, elevated intraocular pressure, and HHV-6 reactivation: a case report]. *J Fr Ophtalmol* 2006;29:659–664.
4. Laban E, Hainaut-Wierzbicka E, Pourreau F, Yacoub M, Sztermer E, Guillet G, et al. Cyclophosphamide therapy for corticoresistant drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome in a patient with severe kidney and eye involvement and Epstein-Barr virus reactivation. *Am J Kidney Dis* 2010;55:e11–e14.
5. Colon B, Horta JM, Casillas S, Bertoli M, Gonzalez-Keelan C, Oliver AL. Retinal hemorrhages and intermediate uveitis in a patient with drug reaction with eosinophilia and systemic symptoms syndrome: a case report. *Retina Cases Brief Rep* 2014;8:150–152.
6. Gros-Otero J, Gorrono-Echebarria M, Benitez-Herreros J. [Bilateral acute anterior uveitis and anticonvulsant hypersensitivity syndrome]. *Arch Soc Esp Oftalmol* 2010;85:376-377.
7. Ciernik IF, Thiel M, Widmer U. Anterior uveitis and the anticonvulsant hypersensitivity syndrome. *Arch Intern Med* 1998;158:192.
8. Atalay E, Tamçelik N, Capar O. High intraocular pressure after carbamazepine and gabapentin intake in a pseudoexfoliative patient. *J Glaucoma* 2014;23:574–576.
9. Cacoub P, Musette M, Descamps V, Meyer O, Speirs C, Finzi L, et al. The DRESS syndrome: a literature review. *Am J Med* 2011;124:588–597.
10. Verrotti A, Trotta D, Salladini C, Chiarelli F. Anticonvulsant hypersensitivity syndrome in children: incidence, prevention and management. *CNS Drugs* 2002;16:197–205.
11. Shiohara T, Inaoka M, Kano Y. Drug-induced hypersensitivity syndrome (DIHS): a reaction induced by a complex interplay among herpesviruses and antiviral and antidrug immune responses. *Allergol Int* 2006;55:1–8.
12. Kardaun SH, Sidoroff A, Valeyrie-Allanore L, Halevy S, Davidovici BB, Mockenhaupt M, et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? *Br J Dermatol* 2007;156:609–611.
13. Chan DP, Teoh SC, Tan CS, Nah GK, Rajagopalan R, Prabhakaragupta MK, et al. Ophthalmic complications of dengue. *Emerg Infect Dis* 2006;12:285–289.
14. Mahendradas P, Avadhani K, Shetty R. Chikungunya and the eye: a review. *J Ophthalmic Inflamm Infect* 2013;3:35.
15. Dhasmana R, Prakash A, Gupta N, Verma SK. Ocular manifestations in leukemia and myeloproliferative disorders and their association with hematological parameters. *Ann Afr Med* 2016;15:97–103.
16. Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, et al. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981;30:239–245.
17. Criado PR, Criado RF, Avancini JM, Santi CG. Drug reaction with Eosinophilia and Systemic Symptoms (DRESS) /Drug-induced Hypersensitivity Syndrome (DIHS): a review of current concepts. *An Bras Dermatol* 2012;87:435–449.

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Original Article

Needle Subretinal Fluid Drainage Assisted Pneumatic Retinopexy for Primary Rhegmatogenous Retinal Detachment

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Abstract

Purpose: Pneumatic retinopexy (PR) continues to remain an important technique in the management of rhegmatogenous retinal detachment (RRD). We describe the results of a novel surgical technique of PR with subretinal fluid (SRF) drainage in this retrospective, nonrandomized case series. **Subjects and Methods:** Medical records of patients with primary RRD who underwent PR with SRF drainage and had been followed up for 6 months or more were reviewed. The procedure involved transconjunctival cryotherapy followed by drainage of SRF using a 26G needle and intravitreal injection of perfluoropropane. Outcome data measured included final visual acuity, anatomical success with single surgical procedure, surgical complications, and need for subsequent procedures. **Results:** Out of the 12 patients, there were 7 males and 5 females. The 12 patients aged in range from 24 to 64 years (average - 43 ± 15.55 years). Average duration of follow-up was 10 ± 3.56 months (range: 6–14 months). Eleven eyes achieved anatomical reattachment of the retina with a single procedure (91.6%). Redetachment was noted in one patient who was successfully treated with pars plana vitrectomy and scleral buckle.The final anatomical success rate was 100%. Visual acuity improved in all the eyes treated, and no major complications related to SRF drainage were encountered. **Conclusion:** PR with needle-assisted SRF drainage is a safe and useful technique for selected cases of primary RRDs with good anatomical and functional outcomes.

Keywords: Pneumatic retinopexy, retinal detachment, subretinal fluid

Introduction

Pneumatic retinopexy (PR) is a minimally invasive surgical procedure useful in selected cases of primary rhegmatogenous retinal detachments (RRDs). Ever since its introduction by Hilton and Grizzard, PR has become a widely accepted alternative surgical technique to scleral buckle and pars plana vitrectomy for specific types of retinal detachments with comparable final anatomical results, less morbidity, and potential cost savings.[1-3] The procedure involves transconjunctival cryopexy or laser photocoagulation of retinal breaks along with intravitreal injection of gas and appropriate patient positioning.[4] Subretinal fluid (SRF) drainage is not usually performed in PR. The surface tension of the bubble closes the retinal break and prevents the bubble from passing into the subretinal space. With the retinal break closed, the retinal pigment epithelial pump removes the SRF. However, delayed resorption of SRF after PR has been reported by several authors.[5,6] Loculated pockets of SRF in the macula can delay visual recovery and may affect the final visual outcome.[7-9]

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In this article, we present the results of a novel technique combining needle-assisted SRF drainage with PR for primary RRD. This approach combines the advantages of both PR and SRF drainage in conventional retinal reattachment procedures.

Subjects and Methods

This study was a retrospective, nonrandomized case analysis of patients with primary RRD who underwent PR with SRF drainage at a tertiary care center in South India. The medical records of these patients were reviewed. All patients had been followed up for a minimum duration of 6 months after the surgical procedure. Six months limit was chosen because the

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risk of redetachment is reported to be highest within the first 6 months.[10]

The procedure was performed for new-onset primary RRD with breaks in the superior 8 o' clock hours (between 8 o' clock and 4'o clock) in patients older than 18 years of age after informed consent. The procedure was not performed in eyes with hazy media, glaucoma, proliferative vitreoretinopathy (PVR) Grade C, previous retinal surgery, or poor patient compliance. The Institutional review board approved the study protocol and the protocol complied with the tenets of the Declaration of Helsinki.

All patients had undergone complete ophthalmic evaluation including a detailed history, preoperative visual acuity, refraction, intraocular pressure measurement, and slit-lamp biomicroscopy. Indirect ophthalmoscopy with scleral indentation was performed to assess the number, size, and location of retinal breaks and macular status. Detailed retinal drawings had been drawn on standard retinal charts. All patients had undergone PR using the standard technique described below. Postoperatively, the patients had been followed at day 1, 1 week, 1 month, and 6 months. Wherever applicable, additional cryo/laser retinopexy was performed at the surgeon's discretion. New/missed peripheral breaks were treated by cryotherapy, while laser was performed for reopened breaks and persistent SRF. Additional procedures were performed around 1–2 weeks after the surgery.

Outcome data measured included final visual acuity, anatomical success with single surgical procedure, surgical complications, and need for subsequent procedures.

Technique

All procedures were performed under retrobulbar anesthesia. The retinal breaks were visualized and surrounded by transconjunctival cryotherapy. Localized conjunctival peritomy was performed in the quadrant where drainage was planned. After peritomy, one rectus muscle was bridled. The drainage site was marked with the help of bipolar cautery. The sclera was punctured with a ½-inch 26G needle, and the SRF was drained under the area of highest retinal elevation [Figure 1]. As the eye became quite soft after SRF drainage a pull on the bridled muscle helped in stabilizing the globe. This also helped in avoiding rotation of the eye and/or possible damage to the crystalline lens during the introduction of needle for gas injection. Around 0.6 cc of 100% perfluoropropane gas was injected via the superior pars plana 3–4 mm posterior to the limbus with the help of 26G needle in a brisk manner to achieve a single large bubble. With the injection site, uppermost and the needle vertical, moderately brisk injection of gas results in one single bubble at the needle tip. Conjunctiva was sutured with 8-0 vicryl. At the end of the procedure, indirect ophthalmoscopy was performed and perfusion of the optic nerve head was ensured in all patients. The patients were positioned such that the retinal breaks were uppermost to ensure tamponade by the injected gas bubble. Patients were asked to maintain the head position for 5–7 days.

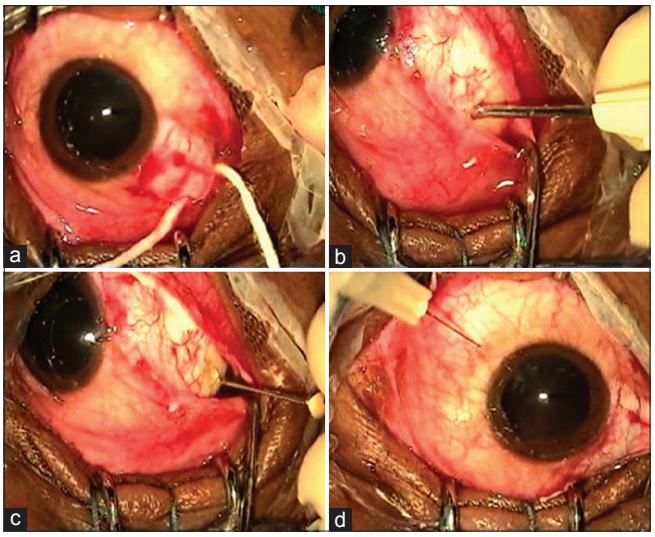


Figure 1: (a) Localized conjunctival peritomy with bridled rectus muscle, (b) subretinal fluid drainage site marked with cautery, (c) drainage of subretinal fluid with 26G needle, (d) intravitreal injection of gas via superior pars plana

Results

Out of the 12 patients, there were 7 males and 5 females. The 12 patients aged in range from 24 to 64 years (average - 43 ± 15.55 years). Average duration of follow-up was 10 ± 3.56 months (range - 6–14 months). None of the patients had phakic refraction >6 D. Nine had superior breaks (10:00–2:00) and three had temporal breaks (8:00–10:00). In patients with multiple breaks (5/12), all the breaks were clustered within the same quadrant. One patient (patient 12) had an additional inferior break in the attached retina. Table 1 shows the summary of patient data and Table 2 shows preoperative characteristics of the patients studied.

Out of the 12 eyes, 11 eyes achieved anatomical reattachment of the retina with a single procedure. Postoperative barrage LASER was done in five cases. Additional cryoretinopexy was done in one patient with peripheral new/missed break at follow-up. Resurgery was done in one patient (patient 11) who had severe vitreous condensation and a missed break. This patient underwent a scleral buckle along with pars plana vitrectomy and silicone oil infusion for persistent vitreoretinal traction.

Considering two Snellen lines of visual acuity change significant, vision improved in all the 12 patients studied. There were 10 patients with visual acuity of 20/200 or less preoperatively, while none of the patients had this acuity postoperatively [Table 3]. No major complications such as endophthalmitis, persistent elevated intraocular pressure, or cataract were encountered in our series. One patient had minimal choroidal hemorrhage at the drainage site in the inferotemporal periphery which resolved spontaneously. Patient 6 had inferior lattice with atrophic holes and a persistent shallow inferior RD postoperatively. She underwent additional laser retinopexy in the postoperative period and the detachment slowly settled.

Table 1: Summary of patient data

Patient number	Age/sex	Preoperative VA	Lens status	Macula on/off	Site of primary break	Additional treatment	Follow-up (months)	Postoperative BCVA	Complications/ secondary procedure
1	25/female	20/200	Phakic	On	Superior	Barrage laser	14	20/80	Nil
2	38/female	20/80	Phakic	On	Temporal	Nil	7	20/40	Nil
3	31/female	20/400	Phakic	Off	Superior	Cryotherapy	6	20/60	Nil
4	55/male	HM	Phakic	Off	Superior	Barrage laser	12	20/60	Nil
5	49/male	HM	Phakic	Off	Superior	Nil	13	20/30	Nil
6	24/female	CFCF	Phakic	Off	Temporal	Barrage laser	8	20/30	Nil
7	64/male	CFCF	Pseudophakic	Off	Superior	Nil	6	20/120	Nil
8	59/male	CFCF	Phakic	Off	Temporal	Barrage laser	7	20/120	Nil
9	51/male	20/400	Pseudophakic	On	Superior	Barrage laser	14	20/20	Nil
10	64/male	20/40	Phakic	On	Superior	Nil	13	20/20	Nil
11	30/female	HM	Phakic	Off	Superior	Nil	6	20/120	PVR/PPV + SB
12	27/male	20/400	Phakic	Off	Superior	Nil	14	20/20	Nil

VA: Visual acuity, BCVA: Best-corrected VA, HM: Hand movements, CFCF: Counting fingers close to face, PVR: Proliferative vitreoretinopathy, PPV: Pars plana vitrectomy, SB: Scleral buckle

Table 2: Preoperative characteristics of the patients studied

Preoperative features	No. of patients
Pseudophakia	2/12
Macula off	8/12
Extent of detachment	
1 quadrant	3/12
2 quadrants	7/12
3 quadrants	1/12
4 quadrants	1/12
>1 retinal break in detached retina	5/12
Lattice degeneration in detached retina	6/12
Lattice degeneration in attached retina	3/12
Breaks in attached retina	1/12

Table 3: Comparison of preoperative and postoperative visual acuity

BCVA	Preoperative	Postoperative
CFCF and less	6	0
≤20/200	4	0
20/120-20/80	1	4
20/60-20/20	1	8

CFCF: Counting fingers close to face, BCVA: Best-corrected visual acuity

Discussion

In this retrospective analysis, we found the rate of single surgery anatomical success after PR using the new technique to be 91.6%. This group included patients who received additional cryotherapy or laser in the postoperative period and maintained retinal reattachment throughout 6 months of follow-up. One patient who developed redetachment 1 month after surgery underwent additional reattachment procedure and had attached retina at the final follow-up visit. Hence, the final anatomical success rate was 100%.

Success rates following PR reported in the literature are found to vary in a wide range. Hilton and Tornambe reviewed the outcome of 1274 eyes in 26 published reports on PR performed between 1986 and 1989.[11] The average single operation success rate was found to be 80% (range: 53%–100%) and average final success rate was 98% (range: 92%–100%). A comprehensive review on the surgical outcome of 4138 eyes that underwent PR over a 21-year period (1986–2007) has reported a single operation success rate of 74.4% and the final success rate of 96.1%.[1] Few recent studies have reported lower rates of single operation success ranging from 59.5% to 69.6%.[12-14] This has been attributed to the wider inclusion criteria including RRDs that generally have lower PR success rates such as multiple breaks in multiple quadrants and retinal breaks in the inferior clock hours. Pseudophakia, large retinal break, and/or PVR are reported to be significant predictors of treatment failure.[14]

Cochrane Database Systematic Review 2015 comparing the relative efficacy of PR versus scleral buckle (with or without vitrectomy) for RRD found slightly less reattachment rate with PR (relative rate 0.89; 95% confidence interval: 0.77–1.02) and higher chances of recurrence (1.80; 95% confidence interval 1.0–3.24) but does not rule out no difference between the procedures.[15] Eyes in the scleral buckle group have been reported to be more susceptible to choroidal detachment and myopic shift than eyes in the PR group. However, the authors have concluded that there is the absence of sufficient high-quality evidence from randomized controlled trials comparing the risks and benefits of PR versus scleral buckle. The choice of procedure depends on the clinical judgment, patient preferences and the surgeon's skill, and experience with each procedure. In spite of reports of variable success rates, PR has been shown to be a very useful, cost-effective method of RD repair with the potential to significantly reduce health-care burden. This is of utmost importance for a developing nation like ours.[16]

Our high success rate can be attributed to the smaller sample size, meticulous preoperative examination, appropriate postoperative retinopexy, and the fact that most of our patients

had classic indications for PR such as retinal breaks in the superior retina and absence of significant PVR.

Literature search on PR with drainage of SRF revealed only one similar study by Gündüz and Günalp in 1994.[17] The authors have reported a series of thirty bullous retinal detachments treated with PR and SRF drainage using 25G diathermy needle. After 150°–180° limbal peritomy, drainage of SRF was performed under the area of highest retinal elevation first, and subsequently, cryotherapy and perfluoroethane injection were done. All the eyes were reattached after the first operation. In three (10%) eyes, new breaks with RD developed 2 weeks – 2 months after the initial procedure and were treated successfully with scleral buckling. The single operation success rate was 90% and eventual reattachment rate was 96.7%. Visual acuity improved in 80% of the eyes treated. They have concluded that anatomic and visual success rates after this procedure are comparable to conventional PR and that SRF drainage did not result in additional complications.

The authors have suggested that cryotherapy after drainage reduces risk of pigment epithelial dispersion into the vitreous and therefore lessens risk of PVR as well as avoids the need for steamroller technique. However, in our study, cryopexy was done before drainage of SRF. Adequate indentation of the sclera was achieved to produce enough cryopexy before SRF drainage in our series. In cases where the cryopexy was considered inadequate, it was repeated in the postoperative period. We observed no increased risk of PVR after this technique in our limited sample.

There are several advantages of PR with needle drainage of SRF over conventional PR. If bullous SRF extends almost to an attached macula, placement of bubble against the bullous detachment may push fluid into the macula and cause macular detachment. Steamroller technique has been suggested to prevent an iatrogenic detachment of macula, as well as iatrogenic detachment of an attached retinal break.[2] However, with drainage of SRF before gas injection, the steamroller technique can be avoided and the theoretical possibility of PVR secondary to movement of SRF into vitreous is prevented.

One of the major reasons for failure of conventional PR is the presence of peripheral or missed retinal breaks which lead to reaccumulation of SRF and persistent retinal detachment.[1] Drainage of SRF before gas injection allows better visualization of the peripheral fundus. This is useful in aphakic and pseudophakic eyes with multiple far peripheral breaks which have been reported to carry lower success rate after PR.[18,19] However, recent studies have noted similar anatomical outcome in phakic as well as pseudophakic eyes.[12,20] This has been attributed to an in-depth examination of the peripheral retina and treatment of breaks in all eyes, especially the nonphakic eyes.

A recent study comparing the outcome of PR as a primary procedure for RRD versus secondary procedure for recurrent RRD found a single operation success rate of 60% for primary intervention and 65.5% for secondary intervention.[21] Age and preoperative and postoperative visual acuity were found to have significant association with single operation success rate. Progression of PVR was found to be the most common cause of failure, followed by nonresolving SRF, reopening of the original break, and formation of new breaks. Although the technique described in this article looks little invasive compared to conventional PR, it hastens resolution of SRF, is highly cost-effective, and obviates the need for a major surgical procedure.

This procedure can also be selected intraoperatively when scleral buckling techniques are likely to be hazardous or likely to have significant postoperative complications such as eyes with marked scleral thinning or the need for a bulky scleral buckle beneath the superior rectus muscle.

The rates of delayed SRF resorption after PR have been reported to vary between 4% and 12%.[5,13,22] Although the SRF does get absorbed spontaneously over several months, persistent SRF can delay visual recovery and affect the final visual outcome. Drainage of SRF using a 26G needle as described in this article ensures rapid resolution of the detachment and visual recovery.

Patients with extensive lattice degeneration have been reported to be unsuitable for PR because of vitreoretinal adhesions and traction resulting in new breaks. However, in our study, six patients had lattice in the detached retina and three patients had lattice degeneration in the attached retina. Our results are consistent with the previous studies, in which lattice degeneration did not increase rate of surgical failure.[16,23]

Poor preoperative visual acuity has been reported to be associated with PR failure and poor final visual outcome by several authors and preoperative visual acuity has been suggested as one of the factors which predict visual recovery.[13,19] In contrast to these studies, worse initial visual acuity was not associated with adverse visual outcome in our series.

Our choice of long-acting perfluoropropane for intravitreal injection was based on its availability. The bubble expands slowly that allows for equilibration of intraocular pressure during the first few days following injection. Perfluoropropane also eliminated the need to reinject gas if a new break develops.

We did not encounter complications such as intraocular hemorrhage or vitreoretinal incarceration related to SRF drainage in our series. It has been argued that cryotherapy may cause vascular congestion of the choriocapillaris and that SRF drainage should not follow cryotherapy. However, we found that SRF drainage after cryotherapy was not associated with increased risk of intraocular hemorrhage or adverse visual outcome. Our observations are in line with other published studies.[24] The 26G needle used for SRF drainage results in a very narrow transscleral tract and therefore the risk of vitreoretinal incarceration is minimal. Furthermore, localized

conjunctival peritomy minimizes the conjunctival damage as compared to scleral buckling.

The limitations of our study include its limited sample size and retrospective design. Subgroup analysis could not be performed owing to the small sample size. Furthermore, we have not examined the relative efficacy of primary vitrectomy and/or scleral buckle versus PR with SRF drainage which would be useful, but beyond the scope of this article.

Conclusion

PR with needle-assisted drainage of SRF is a safe and useful technique for selected cases of primary RRD. Conjunctival damage is less and minimal additional instrumentation is required. This technique has many advantages over conventional PR and good anatomic and visual outcomes can be achieved with less morbidity.

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Conflicts of interest

There are no conflicts of interest.

References

1. Chan CK, Lin SG, Nuthi AS, Salib DM. Pneumatic retinopexy for the repair of retinal detachments: A comprehensive review (1986-2007). Surv Ophthalmol 2008;53:443-78.
2. Hilton GF, Das T, Majji AB, Jalali S. Pneumatic retinopexy: Principles and practice. Indian J Ophthalmol 1996;44:131-43.
3. Goldman DR, Shah CP, Heier JS. Expanded criteria for pneumatic retinopexy and potential cost savings. Ophthalmology 2014;121:318-26.
4. Hilton GF, Grizzard WS. Pneumatic retinopexy. A two-step outpatient operation without conjunctival incision. Ophthalmology 1986;93:626-41.
5. Desatnik H, Alhalel A, Treister G, Moisseiev J. Management of persistent loculated subretinal fluid after pneumatic retinopexy. Br J Ophthalmol 2001;85:189-92.
6. Atmaca-Sonmez P, Johnson MW, Zacks DN. Persistent detachment of the fovea after non-buckling repair of rhegmatogenous retinal detachment. Br J Ophthalmol 2006;90:920-1.
7. Chan CK, Wessels IF. Delayed subretinal fluid absorption after pneumatic retinopexy. Ophthalmology 1989;96:1691-700.
8. Veckeneer M, Derycke L, Lindstedt EW, van Meurs J, Cornelissen M, Bracke M, *et al.* Persistent subretinal fluid after surgery for rhegmatogenous retinal detachment: Hypothesis and review. Graefes Arch Clin Exp Ophthalmol 2012;250:795-802.
9. Ricker LJ, Noordzij LJ, Goezinne F, Cals DW, Berendschot TT, Liem AT, *et al.* Persistent subfoveal fluid and increased preoperative foveal thickness impair visual outcome after macula-off retinal detachment repair. Retina 2011;31:1505-12.
10. Ellakwa AF. Long term results of pneumatic retinopexy. Clin Ophthalmol 2012;6:55-9.
11. Hilton GF, Tornambe PE. Pneumatic retinopexy. An analysis of intraoperative and postoperative complications. The retinal detachment study group. Retina 1991;11:285-94.
12. Cohen E, Zerach A, Mimouni M, Barak A. Reassessment of pneumatic retinopexy for primary treatment of rhegmatogenous retinal detachment. Clin Ophthalmol 2015;9:2033-7.
13. Gilca M, Duval R, Goodyear E, Olivier S, Cordahi G. Factors associated with outcomes of pneumatic retinopexy for rhegmatogenous retinal detachments: A retrospective review of 422 cases. Retina 2014;34:693-9.
14. Rootman DB, Luu S, M Conti S, Mandell M, Devenyi R, Lam WC, *et al.* Predictors of treatment failure for pneumatic retinopexy. Can J Ophthalmol 2013;48:549-52.
15. Hatef E, Sena DF, Fallano KA, Crews J, Do DV. Pneumatic retinopexy versus scleral buckle for repairing simple rhegmatogenous retinal detachments. Cochrane Database Syst Rev 2015;5:CD008350.
16. Narula R. Pneumatic retinopexy: A cost-effective alternative. Indian J Ophthalmol 2018;66:426-7.
17. Gündüz K, Günalp I. Pneumatic retinopexy with drainage of subretinal fluid. Int Ophthalmol 1994;18:143-7.
18. Abecia E, Pinilla I, Olivan JM, Larrosa JM, Polo V, Honrubia FM, *et al.* Anatomic results and complications in a long-term follow-up of pneumatic retinopexy cases. Retina 2000;20:156-61.
19. Grizzard WS, Hilton GF, Hammer ME, Taren D, Brinton DA. Pneumatic retinopexy failures. Cause, prevention, timing, and management. Ophthalmology 1995;102:929-36.
20. Fabian ID, Kinori M, Efrati M, Alhalel A, Desatnik H, Hai OV, *et al.* Pneumatic retinopexy for the repair of primary rhegmatogenous retinal detachment: A 10-year retrospective analysis. JAMA Ophthalmol 2013;131:166-71.
21. Dhami A, Shah KK, Ratra D. Pneumatic retinopexy outcomes as primary or secondary surgical option for treating rhegmatogenous retinal detachment. Indian J Ophthalmol 2018;66:420-5.
22. Ambler JS, Zegarra H, Meyers SM. Chronic macular detachment following pneumatic retinopexy. Retina 1990;10:125-30.
23. Kulkarni KM, Roth DB, Prenner JL. Current visual and anatomic outcomes of pneumatic retinopexy. Retina 2007;27:1065-70.
24. Pearce IA, Wong D, McGalliard J, Groenewald C. Does cryotherapy before drainage increase the risk of intraocular haemorrhage and affect outcome? A prospective, randomised, controlled study using a needle drainage technique and sustained ocular compression. Br J Ophthalmol 1997;81:563-7.

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Original Article

Dry Eye Disease and Tear Dysfunction in Patients with Type 2 Diabetes: A Hospital-Based Study from South India

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Abstract

Purpose: Diabetics are more prone to dry eye disease (DED) and tear film dysfunction. The aim of this study was to assess the frequency of DED and tear film abnormalities in patients with type 2 diabetes compared to healthy controls. **Materials and Methods:** This was a hospital-based prospective case-control study. One hundred patients with type 2 diabetes and 100 healthy controls were enrolled by consecutive sampling. All study participants completed the Ocular Surface Disease Index (OSDI) questionnaire to score dry eye symptoms. Tear film breakup time (TFBUT), ocular surface staining with fluorescein and Schirmer's test were performed, and results were analyzed. Student's *t*-test was applied for comparisons between groups. Correlation between variables was detected using Pearson's correlation coefficient. Statistical significance was set at $P < 0.05$. **Results:** Dry eye was encountered in 54 among the 100 diabetic patients studied. Age and sex of diabetic patients were not found to affect the dry eye status. Longer duration of diabetes was significantly associated with DED ($P = 0.03$). Significant differences in OSDI scores, TFBUT, fluorescein staining, and Schirmer's test values were observed between the study groups ($P < 0.001$). An increased level of glycosylated hemoglobin was significantly associated with abnormal tear function tests. **Conclusion:** Patients with longer duration of diabetes and poor glycemic control are more predisposed to have abnormalities of the tear film and DED. Apart from conventional therapies, optimal glycemic control should be an important consideration in the management of tear dysfunction in diabetic patients.

Keywords: *Diabetes, dry eye, tear film*

Introduction

The prevalence of diabetes mellitus (DM) has increased significantly in the past several decades and the disease continues to remain a global epidemic. Estimates suggest that 439 million people in the world are likely to have DM by 2030 and this increase in number of diabetics is predicted to be disproportionately more in developing countries.[1] The two major potentially blinding complications of diabetes, namely cataract and retinopathy, have been extensively researched in literature. Apart from these, people with diabetes are also prone for ocular surface disease that includes dry eye, persistent epithelial defects, decreased corneal sensation, and tear film dysfunction. Studies have shown an intimate, complex relationship between diabetes, dry eye, and ocular surface abnormalities.[2,3]

The reported prevalence rates of dry eye in diabetics have been found to vary widely between 27% and 54%.[4,5] A recent study from a cornea specialty clinic found the overall presence and the severity of dry eye to be similar in the diabetic and nondiabetic groups. However, significant differences were observed between groups with regard to individual diagnostic assessments.[6] Studies on changes in tear function parameters in diabetes have also shown conflicting results.[7,8] These can be attributed to major differences in the study characteristics of the population, methodologies, and wide array of diagnostic methods used for assessment. Limited data exist on the occurrence of dry eye and ocular surface disease among diabetics in the Indian population.[9]

This prospective study from India sought to study the frequency of dry eye disease (DED) and tear film changes in patients with type 2 diabetes compared to healthy controls.

Materials and Methods

This was a hospital-based cross-sectional study which included 100 patients

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with type 2 DM. Consecutive patients attending the Ophthalmology Department of a tertiary care center in South India between June 2016 and 2017 were invited to participate. Exclusion criteria included the history of smoking, contact lens wear, past ocular surgery, intake of systemic medications such as antihistamines, tricyclic antidepressants, and other drugs known to cause dry eye. Patients with known ocular or systemic disease that might affect the ocular surface were also excluded from the study. One hundred individuals matched by age were enrolled as a control group. The controls were recruited from individuals attending the department of ophthalmology for routine eye examination and did not have any ocular or systemic disease other than refractive errors. The study protocol was approved by the Institutional Ethics Committee, and the study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

For each participant, a detailed ocular and general medical history, including duration of diabetes, medications, and glycosylated hemoglobin (HbA1c) value within the past 3 months was obtained. All study participants completed the Ocular Surface Disease Index (OSDI; Allergan, Inc., Irvine, CA, USA) questionnaire to score dry eye symptoms. The OSDI consists of 12 questions based on symptoms within the past 1 week. The frequency of ocular subjective symptoms (soreness, blurred vision), difficulty with vision-related function (television, visual display unit, driving, and reading) and discomfort due to environmental triggers (low humidity, high wind) are assessed on a Likert scale. The scores range from 0 (least severe) to 100 (most severe).[10]

All participants underwent a general physical and comprehensive ophthalmic examination with slit lamp, including best-corrected visual acuity. Evaluation of the ocular surface and tear film was done by performing the following tests described below in a sequential manner.[11]

Tear film breakup time (TFBUT) was performed by applying a fluorescein strip (Fluostrip/India/Contacareophthalmics and diagnostics) moistening it with a drop of sterile saline, to the lower tarsal conjunctiva without the use of topical anesthesia. The participants were asked to blink several times and then cease blinking until instructed. The tear film was observed under slit lamp with cobalt blue filter. The time interval between the last blink to the appearance of the first random dry spot in the cornea was taken as the TFBUT. The average of three consecutive breakup times was calculated and recorded.

The ocular surface was evaluated by staining the cornea with fluorescein and Oxford grading scheme was used for the recording of staining severity. This scheme has five panels, labeled A to E, with staining represented by punctate dots that increase logarithmically between panels.[12] The appearance of staining on the exposed interpalpebral conjunctiva and cornea is compared with each panel, and the closest match gives the grade. Based on the number of dots seen a grade between 0 and 3 was given for staining on the cornea, nasal, and temporal conjunctiva. The individual scores were added giving a maximum possible total of 9 points. A score of 3 and above was considered to be abnormal.[13]

Schirmer's test was performed without topical anesthesia using standardized Whatman filter paper 41(Tearstrip/India/Contacareophthalmics and diagnostics). The strip was folded at the notch and placed at the junction of the middle and lateral thirds of the lower eyelids and left in place for 5 min. A value of <10 mm wetting in 5 min was considered abnormal.

Due to lack of consensus among the diagnostic criteria for dry eye described in different clinical studies we adopted the following criteria used by Alves *et al.*: OSDI score >20 and/or Schirmer test <10 mm or TFBUT <6 s and/or ocular surface staining score >3.[14] A diagnosis of DED was made if the participant had at least one positive test according to these criteria. In various clinical studies, authors have adopted different approaches for the diagnosis of dry eye on an *ad hoc* basis. Several studies have used the positivity of one or more of the tests (tear breakup time [TBUT] or Schirmer or OSDI) for the diagnosis of dry eye.[5,15,16] Since there is no uniform set of diagnostic criteria for DED, we considered a diagnosis of dry eye if the patient presented at least one positive test according to these preestablished criteria.

All measurements were performed by the same investigators on the same day under similar testing conditions. Clinical evaluations were done on both eyes, and the mean of the two eyes was used for analysis. To eliminate diurnal variation and possible diagnostic error, all tests were performed in the morning.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 24. Descriptive statistics was presented as a mean ± standard deviation. The Shapiro–Wilk test was used to test the normality of data distribution. Student's *t*-test was applied for comparisons between two groups. Correlation between variables was detected using Pearson's correlation coefficient. Statistical significance was set at $P < 0.05$.

Results

The mean age of controls and diabetic patients was 54.7 ± 10.2 and 55.7 ± 9.5 years, respectively. There were 53 females among 100 controls and 45 females among 100 diabetics ($P = 0.25$). The baseline characteristics of the study participants are shown in Table 1. The mean duration of diabetes among diabetics with the dry

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eye was significantly higher compared to those without dry eye (P = 0.03).

Table 2 shows the comparison of OSDI, Schirmer test, fluorescein staining, and TFBUT between groups using the Student's *t*-test. Significant differences in OSDI scores, Schirmer test values, fluorescein staining, and TFBUT were observed between the study groups. OSDI Score was >20 in 32 participants (32%) in the diabetic group. Nineteen percent had OSDI scores between 12 and 20. In the diabetic group, 7 participants (7%) had Schirmer scores <10 mm and 30 (30%) had TBUT <10 s. Ocular surface staining score of >3 was encountered in 51% of the diabetic participants. None of the participants in the control group had abnormal OSDI, Schirmer test, fluorescein staining, or TFBUT.

We evaluated the relationship between HbA1c, duration of diabetes, and ocular surface parameters [Table 3]. The analysis showed significant positive correlation between the duration of diabetes and OSDI scores as well as fluorescein staining of the ocular surface. Although negative, the correlation between duration of diabetes and Schirmer/TFBUT was not statistically significant.

Table 1: Baseline characteristics of study participants

Characteristic	Controls	Diabetics without dry eye	Diabetics with dry eye
Number (%)	100	46	54
Age (years)	54.7±10.2	54.6±9.5	56.7±9.6
Females (%)	53 (53)	22 (47.8)	23 (42.6)
Duration of diabetes (years)	-	5.6±6.1	8.05±5.1*
HbA1c	5.3±0.3	8.5±2.2	9.4±2.3

*P=0.03. HbA1c: Glycosylated haemoglobin

Table 2: Comparison of ocular surface disease index scores, Schirmer test, fluorescein staining and tear film breakup time between groups

	Controls	Diabetics	*P*
OSDI	6.9±6.5	15.1±12.4	<0.001
Schirmer test	24.1±5.1	20.8±7.3	<0.001
Fluorescein staining	1.85±0.24	3.8±2.1	<0.001
TFBUT	16.7±3.6	12.6±4.5	<0.001

OSDI: Ocular surface disease index, TFBUT: Tear film breakup time

Table 3: Correlation coefficients between duration of diabetes, glycosylated haemoglobin and ocular surface parameters

Variable	Duration of diabetes (*P*)	HbA1c (*P*)
OSDI	0.342 (<0.001)	0.340 (<0.001)
Schirmer test	−0.07 (0.47)	−0.23 (0.01)
Fluorescein staining	0.3 (0.003)	0.3 (0.001)
TFBUT	−0.1 (0.27)	−0.21 (0.03)

HbA1c: Glycosylated haemoglobin, OSDI: Ocular surface disease index, TFBUT: Tear film breakup time

The HbA1c level was found to correlate positively with OSDI scores and ocular surface staining. HbA1c levels correlated negatively with Schirmer values and TFBUT.

Discussion

The purpose of this clinical study was to assess the frequency of DED and tear film abnormalities in people with diabetes and compare with that of healthy controls. The OSDI scores were higher, and tear film parameters were significantly altered in diabetic patients.

Out of the 100 diabetics studied, 54 were found to have dry eye. Mean age of diabetic individuals with and without dry eye were found to be similar (P = 0.262). Among the 100 patients with diabetes 45 (45%) were females, of which 23 (42.6%) had dry eye and 22 (47.8%) did not have evidence of dry eye (P = 0.600). Hence, there was no statistically significant association between dry eye and age or gender of the diabetic patients. The frequency of dry eye was significantly higher in patients with longer duration of diabetes.

Studies on dry eye in diabetics have revealed wide variation in the prevalence rates due to different diagnostic criteria as well as patient characteristics. Our observations are similar to that of Fuerst *et al*. who have noted a dry eye prevalence of 54% based on tear osmolarity.[16] Manaviat *et al*. performed Schirmer and TBUT tests and utilized the criterion of one positive test to diagnose dry eye. Out of the 199 participants studied, 108 (54.3%) were found to have dry eye.[5]

In contrast to these hospital-based studies, a community-based study from Shanghai has reported 17.5% prevalence of definitive DED.[17] Apart from methods used for diagnosis, several other factors such as age, gender, duration of diabetes, and metabolic control have also been reported to affect the prevalence of DED.[18,19]

Duration of diabetes was found to be significantly associated with dry eye in our study similar to observations of Manaviat *et al*.[5] Chronic hyperglycemia, diabetic neuropathy, decreased insulin levels, microvasculopathy, and systemic hyperosmotic disturbances in long-standing diabetes lead to dysfunction of the lacrimal functional unit as well as abnormal tear dynamics and DED.[20] However, some studies have found no correlation between DED and duration of diabetes.[4]

Diabetics in this study had significantly higher OSDI scores as compared to the control group. In contrast to our observations, Beckman found the mean OSDI scores to be higher in nondiabetic patients as compared to diabetic patients.[6] This could be attributed to the fact that the mean duration of diabetes in this study was much less (6.9 ± 5.7 years) compared to their study where the mean diabetic duration was 11.1 ± 9 years (range 1–41 years). Long-standing diabetes has been reported to

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be associated with reduced perception of dry eye symptoms due to impaired corneal sensation and therefore decreased OSDI scores.[16]

We found a significant positive correlation between OSDI scores and duration of diabetes in our study. One possibility for the observed positive correlation is that, as mentioned previously participants with shorter duration of diabetes have normal corneal sensation and therefore, will be aware of dry eye symptoms. Our findings are in agreement with the natural history of DED proposed by Bron *et al.* This model comprises three stages, namely initiation of DED, reflex compensation, and loss of compensatory response due to sensory nerve impairment.[21]

OSDI scores were found to correlate positively with HbA1c levels in our study. Our observations are in line with previous studies elsewhere, which have shown a positive correlation between OSDI scores and glycemic control.[22,23]

Although mean Schirmer test scores were in the normal range for both study groups, statistically significant differences were found between the Schirmer values in control and diabetic groups. Our observations are similar to that of Beckman and Yoon *et al*. who found the values of the Schirmer's test to be >10 mm in both diabetic and nondiabetic patients.[6,24] In contrast to these findings, the Schirmer values averaged 7.4 ± 0.38 mm in patients with diabetes versus 13.53 ± 0.50 mm in the controls in a series by Dogru *et al*.[7] Twenty eyes (22.7%) of the diabetic patients in their series had Schirmer test values <5 mm. In our study, only 3% had Schirmer scores <5 mm. Reduced Schirmer scores in people with diabetes compared with nondiabetic participants have been documented in several studies. Some studies have noted a reduction in basal tear secretion, whereas some authors have reported only the reflex tear secretion to be significantly reduced in diabetics.[8,19,25]

We found a significant negative correlation between Schirmer test scores and HbA1c levels. Our observations are in line with that of Ozdemir *et al*. who also found the Schirmer test and TBUT results to be worse in poorly controlled diabetics compared to those with good metabolic control. Kaiserman *et al*. have reported increasing consumption of ocular lubrication with increased mean annual levels of HbA1c.[18] Poor Schirmer test scores were not related to the duration of diabetes in our study which is in agreement with previous studies.[19]

Significant staining of the ocular surface with fluorescein was noted in diabetic patients in our study. Impaired corneal epithelial barrier function in diabetic patients has been reported by several studies and poor metabolic control, diabetic neuropathy, and advanced retinopathy have been found to correlate with epitheliopathy.[3,24] Ocular surface staining scores were found to correlate positively with duration of diabetes as well as HbA1c levels in this study. Accumulation of advanced glycation end products (AGE) on the basement membrane of the corneal epithelium has been shown to play a causative role in corneal epithelial disorders of diabetic patients.[26] Since HbA1c is a product of early stage glycation, it correlates well with AGE formation and hence impaired barrier function resulting in staining of the ocular surface.

Significant differences in TFBUT values were noted between diabetic and nondiabetic participants, and lower TFBUT scores were noted in patients with poor glycemic control. These results are comparable to that of Yoon *et al*. who had evaluated the tear film and ocular surface changes among diabetics from Korea.[24] Ma *et al*. have reported a significant inverse correlation between serum HbA1c levels and noninvasive TBUT in their study on 80 Chinese participants with type 2 diabetes.[23] These results suggest that poor glycemic control is associated with an unstable tearfilm and ocular surface disease in diabetic patients.

The study has few limitations. We had not performed tear film osmolarity assessment which is an objective test for the diagnosis and follow-up of DED. However, test combination of OSDI/Schirmer test/TFBUT has been shown to have an accuracy of 99.3% for the diagnosis of DED.[14] Sample size and conduct of the study at a single site are other potential limitations of the study. Further studies on large samples with the inclusion of tests such as corneal esthesiometry, tear osmolarity, and other markers of DED will provide valuable insight into the potential mechanisms and treatment options for diabetic DED.

Conclusion

There are limited data available in the literature about the frequency of dry eye and tear film dysfunction in diabetics from India. With the increasing prevalence of diabetes and its associated complications in India, there is need for more studies from this part of the world. Nearly half of the people with diabetes in this study had features consistent with DED. Patients with longer duration of diabetes and higher HbA1c levels are more predisposed to have abnormalities of tear film and DED. Examination of the ocular surface and testing for DED should be added to the routine screening of diabetes. Apart from conventional therapies such as lubricants, anti-inflammatory agents, and antioxidants optimal glycemic control should be an important consideration in the management of tear dysfunction in diabetic patients.

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Conflicts of interest

There are no conflicts of interest.

References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
2. Achtsidis V, Eleftheriadou I, Kozanidou E, Voumvourakis KI, Stamboulis E, Theodosiadis PG, *et al.* Dry eye syndrome in subjects with diabetes and association with neuropathy. Diabetes Care 2014;37:e210-1.
3. Gekka M, Miyata K, Nagai Y, Nemoto S, Sameshima T, Tanabe T, *et al.* Corneal epithelial barrier function in diabetic patients. Cornea 2004;23:35-7.
4. Najafi L, Malek M, Valojerdi AE, Aghili R, Khamseh ME, Fallah AE, *et al.* Dry eye and its correlation to diabetes microvascular complications in people with type 2 diabetes mellitus. J Diabetes Complications 2013;27:459-62.
5. Manaviat MR, Rashidi M, Afkhami-Ardekani M, Shoja MR. Prevalence of dry eye syndrome and diabetic retinopathy in type 2 diabetic patients. BMC Ophthalmol 2008;8:10.
6. Beckman KA. Characterization of dry eye disease in diabetic patients versus nondiabetic patients. Cornea 2014;33:851-4.
7. Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. Ophthalmology 2001;108:586-92.
8. Saito J, Enoki M, Hara M, Morishige N, Chikama T, Nishida T. Correlation of corneal sensation, but not of basal or reflex tear secretion, with the stage of diabetic retinopathy. Cornea 2003;22:15-8.
9. Kesarwani D, Rizvi SW, Khan AA, Amitava AK, Vasenwala SM, Siddiqui Z. Tear film and ocular surface dysfunction in diabetes mellitus in an Indian population. Indian J Ophthalmol 2017;65:301-4.
10. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the ocular surface disease index. Arch Ophthalmol 2000;118:615-21.
11. Methodologies to diagnose and monitor dry eye disease: Report of the diagnostic methodology subcommittee of the international dry eye workShop (2007). Ocul Surf 2007;5:108-52.
12. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea 2003;22:640-50.
13. McGinnigle S, Naroo SA, Eperjesi F. Evaluation of dry eye. Surv Ophthalmol 2012;57:293-316.
14. Alves M, Reinach PS, Paula JS, Vellasco e Cruz AA, Bachette L, Faustino J, *et al.* Comparison of diagnostic tests in distinct well-defined conditions related to dry eye disease. PLoS One 2014;9:e97921.
15. Aljarousha M, Badarudin NE, Che Azemin MZ. Comparison of dry eye parameters between diabetics and non-diabetics in district of Kuantan, Pahang. Malays J Med Sci 2016;23:72-7.
16. Fuerst N, Langelier N, Massaro-Giordano M, Pistilli M, Stasi K, Burns C, *et al.* Tear osmolarity and dry eye symptoms in diabetics. Clin Ophthalmol 2014;8:507-15.
17. Zou X, Lu L, Xu Y, Zhu J, He J, Zhang B, *et al.* Prevalence and clinical characteristics of dry eye disease in community-based type 2 diabetic patients: The beixinjing eye study. BMC Ophthalmol 2018;18:117.
18. Kaiserman I, Kaiserman N, Nakar S, Vinker S. Dry eye in diabetic patients. Am J Ophthalmol 2005;139:498-503.
19. Ozdemir M, Buyukbese MA, Cetinkaya A, Ozdemir G. Risk factors for ocular surface disorders in patients with diabetes mellitus. Diabetes Res Clin Pract 2003;59:195-9.
20. Zhang X, Zhao L, Deng S, Sun X, Wang N. Dry eye syndrome in patients with diabetes mellitus: Prevalence, etiology, and clinical characteristics. J Ophthalmol 2016;2016:8201053.
21. Bron AJ, Yokoi N, Gafney E, Tiffany JM. Predicted phenotypes of dry eye: Proposed consequences of its natural history. Ocul Surf 2009;7:78-92.
22. Kan S, Acar U, Kizilgul M, Beyazyildiz E, Cankaya AB, Ozcelik O, *et al.* The effects of blood glucose regulation on tear function tests in diabetic patients. J Fr Ophtalmol 2017;40:499-504.
23. Ma A, Mak MS, Shih KC, Tsui CK, Cheung RK, Lee SH, *et al.* Association of long-term glycaemic control on tear break-up times and dry eye symptoms in Chinese patients with type 2 diabetes. Clin Exp Ophthalmol 2018;46:608-15.
24. Yoon KC, Im SK, Seo MS. Changes of tear film and ocular surface in diabetes mellitus. Korean J Ophthalmol 2004;18:168-74.
25. Lv H, Li A, Zhang X, Xu M, Qiao Y, Zhang J, *et al.* Meta-analysis and review on the changes of tear function and corneal sensitivity in diabetic patients. Acta Ophthalmol 2014;92:e96-104.
26. Kaji Y, Usui T, Oshika T, Matsubara M, Yamashita H, Araie M, *et al.* Advanced glycation end products in diabetic corneas. Invest Ophthalmol Vis Sci 2000;41:362-8.

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Original Article

Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging

K Divya, Vikrant Kanagaraju[1], B Devanand[1], C Jeevamala, A Raghuram, D Sundar

Purpose: To investigate the retrobulbar circulatory parameters in type 2 diabetes mellitus patients with color Doppler imaging (CDI) and compare the results with nondiabetic controls. **Methods:** This prospective study included 50 type 2 diabetic patients and 50 age-matched controls. Seven field stereo fundus photography was used to diagnose and classify diabetic retinopathy (DR). Diabetic patients were further divided into two: Group 1, consisted of patients with no DR, mild and moderate non-proliferative DR (n = 36); Group 2, severe nonproliferative and proliferative DR (n = 14). CDI was performed using Philips iU22 xMATRIX ultrasound. The peak systolic velocity (PSV), end-diastolic velocity (EDV), resistivity index (RI) and pulsatile index (PI) of ophthalmic (OA), posterior ciliary artery (PCA), and central retinal artery (CRA) along with central retinal vein (CRV) were recorded. **Results:** RI in the ophthalmic artery was significantly higher in both DR groups than the control group (P = 0.000). Diabetic Group 1 had decreased blood flow velocity (PSV and EDV) in PCA compared to controls (P = 0.046 and P = 0.010, respectively). Group 2 DR had significantly reduced EDV and increased RI in CRA compared to Group 1 (P = 0.015). Binary logistic regression analysis revealed glycosylated hemoglobin and RI of OA to be independent risk factors of DR. **Conclusion:** Significant changes in resistivity index and flow velocities were observed in the retrobulbar vessels, especially in ophthalmic artery in diabetics compared to controls. CDI with results of increased resistance or decreased flow could be useful to predict individuals at higher risk for developing severe DR.

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Ocular manifestations of diabetes are numerous and among them, diabetic retinopathy (DR) is a major clinical issue for ophthalmologists. DR is the most frequent cause of preventable blindness in working-age adults (20–74 years) and is therefore considered as a major public health issue.[1] Though treatable in appropriate stages, the prevention of retinal complications of diabetes and consequent visual impairment is vital to reduce the burden of DR. There is a felt need for better diagnostic modalities and surrogate markers in the evaluation and management of DR.

In spite of decades of research, the mechanisms behind the onset and progression of DR still remain unclear. From a hemodynamic point of view, evidence has shown that there is an early reduction in blood flow to retina before the onset of DR followed by a gradual increase as the retinopathy progresses.[2] Color Doppler imaging (CDI) is an established, safe, and noninvasive ultrasonic method useful for qualitatively and quantitatively assessing the blood flow velocities and indirect blood flow.[3] CDI has been suggested to be valuable in the diagnosis and management of several ocular diseases with altered hemodynamics such as glaucoma, retinal vascular diseases, ischemic optic neuropathy, and ocular ischemic syndrome.[4] An analysis of retrobulbar hemodynamics in diabetes using CDI can provide insight into the pathogenesis and prospective treatment for DR as well as predict those at higher risk of developing vision threatening DR. Several authors have evaluated the circulatory changes in diabetics using CDI and reported conflicting results. While few studies have reported a reduction in the retrobulbar blood flow velocities,[5-7] others have noted an increase[8] or no change.[9] Studies on the resistive index (RI) of orbital vessels which is a measure of peripheral vascular resistance have also shown significant discrepancies.[10,11] Factors such as demographic variables, duration of diabetes, methods of DR assessment, positions of CDI, and sample sizes account for the observed discrepancies. Moreover, most studies have not excluded patients with hypertension and those on medications affecting systemic blood flow which can influence the results. Studies on orbital hemodynamic alterations among type 2 diabetics from India are relatively few. The diabetic population in India is close to hitting the alarming mark of 69.9 million by 2025 and 80 million by 2030, denoting an expected increase of 266%. Hence, India is deemed as the world's capital of diabetes.[12] With increasing prevalence of diabetes and DR, it is important to know the hemodynamic alterations among diabetics from the Indian population.

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The objective of this study was to evaluate the flow velocities and vascular resistance of orbital blood vessels using CDI in a pure cohort of type 2 diabetic patients from India and compare the results with healthy controls.

Methods

This prospective hospital-based study was carried out at a tertiary care institution from south India between March 2017 and April 2019. The study included consecutive patients of type 2 DM (as per definition of the American Diabetes Association) attending the ophthalmology outpatient clinic for the screening of retinopathy. Exclusion criteria were history of any ocular disease that might affect ocular blood flow such as glaucoma, nondiabetic retinal vascular occlusion, ocular inflammation, high myopia, previous ocular surgery, or panretinal photocoagulation. Patients with systemic hypertension, dyslipidemia, nephropathy, and cardiovascular diseases were excluded from the study. Pregnant and breastfeeding women were not included. Patients taking medications known to affect systemic hemodynamics such as Angiotensin-converting enzyme inhibitors, calcium channel blockers, and anti-migraine drugs were also excluded.

50 age-matched healthy subjects were enrolled as a control group. The control subjects were recruited from individuals attending the department of ophthalmology for a routine eye examination. They were not on any medication and did not have ocular or systemic disease. The study was conducted according to the principles of the Declaration of Helsinki and the study protocol was approved by the institutional ethics committee. Informed consent was obtained from all participants.

Information regarding age, gender, body mass index, duration of diabetes, antidiabetic medications, and blood pressure were obtained from all participants. Serum glycosylated hemoglobin (HbA1c) was determined from fasting venous blood sample.

All study participants underwent a comprehensive ophthalmic examination including dilated fundus with slit-lamp biomicroscope using a 90 diopter lens and indirect ophthalmoscopy. A 30° stereoscopic fundus photograph of 7 standard early treatment diabetic retinopathy study (ETDRS) fields was taken from both eyes of diabetic patients using a digital fundus camera (TRC-50 DX Mydriatic retinal camera; Topcon Medical Systems, Oakland, NJ).

The presence, as well as the severity of diabetic retinopathy, was determined by grading of color fundus photographs following the international clinical disease severity scale for diabetic retinopathy which is based on the findings of WESDR (Wisconsin Epidemiological Study of Diabetic Retinopathy) and ETDRS.[13] The diabetic patients were assigned to one of the following groups namely no retinopathy, mild nonproliferative diabetic retinopathy (NPDR), moderate NPDR, severe NPDR, and proliferative diabetic retinopathy (PDR). The

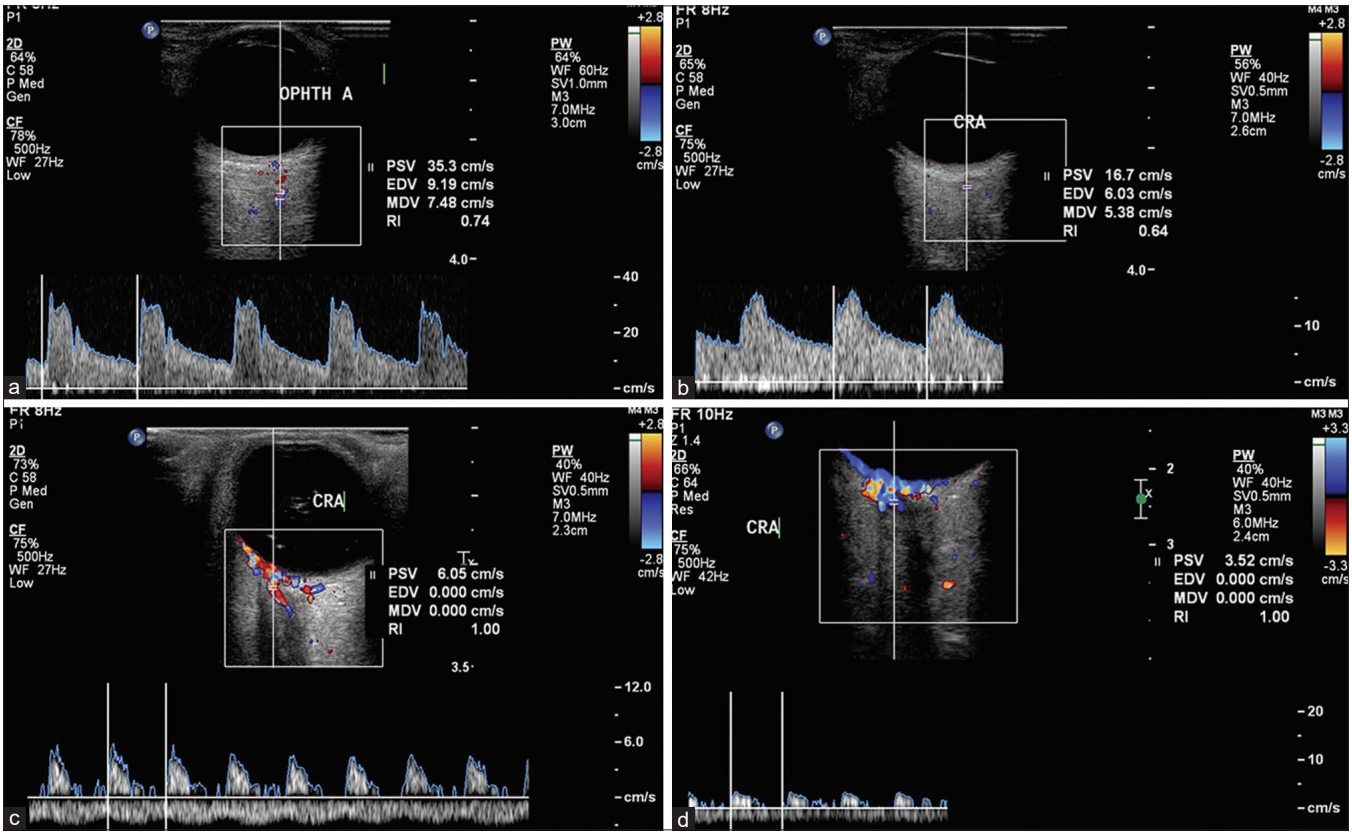


Figure 1: (a) Color Doppler image (CDI) showing normal color flow and spectral waveform in ophthalmic artery with preserved M wave pattern; (b) CDI showing normal color flow and spectral waveform in central retinal artery; (c) CDI showing low-velocity monophasic flow in central retinal artery in a patient with moderate NPDR; (d) CDI showing monophasic spectral waveform with absent diastolic flow in central retinal artery in a patient with severe NPDR

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eye with worse retinopathy was included in the study. If both eyes had equal retinopathy, the right eye was assigned to the study.

Color Doppler sonography of the eye was performed for cases and controls by a masked expert Radiologist using a color Doppler unit and a 12 to 5 MHZ linear-array transducer (Philips iU22 xMATRIX ultrasound system). The patients were examined in the supine position and care was taken to avoid any undue pressure on the eye. Examiner's hand was rested on the orbital margin and sterile coupling gel was applied over closed eyelids. The Doppler signal was registered online after the cursor was positioned on the region of interest. To reduce errors in measurement of velocities, angle correction was applied to the pulsed Doppler recordings.

The peak systolic velocity (PSV) and end-diastolic velocity (EDV) readings were obtained from the ophthalmic artery (OA), central retinal artery (CRA), and the posterior ciliary artery (PCA). Since the velocity wave in a central retinal vein (CRV) is not in direct relation to the cardiac cycle its hemodynamic parameters were expressed as maximum (V max) and minimum (V min) blood velocity. Signals from the ophthalmic artery were obtained from the medial aspect of the eyeball, superior to the optic nerve. The central retinal artery and vein were located within the optic nerve, around 10 mm behind the eyeball. [Fig. 1] Signals from the temporal branch of the short posterior ciliary artery were located lateral to the optic nerve.

With the help of PSV and EDV, we calculated the vascular resistance expressed by the resistivity index and pulsatile index using Pourcelot's formula.

Resistive index (RI) = (PSV-EDV)/PSV; Pulsatile index (PI) = (PSV-EDV)/Vmean

Statistical analysis

For statistical analysis diabetic patients were further divided into two groups; Group 1 included patients with no retinopathy, mild NPDR, and moderate NPDR while Group 2 consisted of patients with severe NPDR and PDR.

Statistical analysis was performed using statistical package for social sciences (SPSS, SPSS Inc, Chicago, IL, USA) version 24. Data were expressed as mean ± standard deviation. The normality of data distribution was tested by the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) with a *post hoc* Bonferroni was used for the analysis of normally distributed continuous data. Kruskal-Wallis analysis was used for analyzing non-normally distributed data. A comparison between hemodynamic parameters was calculated by ANOVA followed by Tukey-Kramer *post hoc* comparison. Pearson's coefficient was used to study the correlation between variables.

Table 1: Demographic and clinical characteristics of controls and diabetic subjects

Characteristic	Controls (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
Age (years)	56.5±9.00	55.5±8.60	51.9±5.64	0.209
Body mass index (kg/m^2)	23.34±3.62	24.81±3.15	24.53±3.88	0.141
Median Systolic BP (mmHg)	110 (20)	120 (18.75)	125 (12.5)	0.076
Median Diastolic BP (mmHg)	70 (30)	76.5 (10)	70 (10)	0.259
Median duration of diabetes (years)	-	3 (8)	10 (11)	0.115
Glycosylated hemoglobin (%)	5.81±0.29	9.09±2.13	10.86±2.33	0.014*

BP=Blood pressure; *P<0.05

Table 2: Hemodynamic parameters of retrobulbar blood vessels among controls and patient groups

Hemodynamic parameters (cm/s)	Control (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
OA PSV	29.72±8.10	30.48±9.78	28.76±12.84	0.838
OA EDV	6.87±2.69	6.34±3.41	5.09±3.50	0.166
OA RI	0.75±0.06	0.79±0.06	0.83±0.05	0.000*
OA PI	1.58±0.27	1.74±0.30	1.86±0.25	0.002*
CRA PSV	12.42±3.85	10.40±4.87	8.42±2.92	0.004*
CRA EDV	2.73±1.17	2.33±1.27	1.27±0.94	0.000*
CRA RI	0.77±0.06	0.74±0.16	0.85±0.11	0.019*
CRA PI	1.63±0.30	1.65±0.50	2.08±0.65	0.004*
PCA PSV	15.44±5.61	12.81±3.83	14.95±5.15	0.054
PCA EDV	4.19±2.23	2.97±1.29	3.33±1.63	0.011*
PCA RI	0.73±0.06	0.75±0.08	0.78±0.08	0.061
PCA PI	1.45±0.24	1.58±0.37	1.68±0.45	0.044*
CRV V max	6.14±1.63	6.12±1.66	5.85±2.07	0.853
CRV V min	3.51±0.97	3.34±0.89	3.02±1.05	0.243
CRV RI	0.41±0.11	0.44±0.11	0.46±0.08	0.329
CRV PI	0.60±0.24	0.65±0.25	0.70±0.17	0.350

OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=Maximum blood velocity; V min=Minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

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Binary logistic regression analysis was performed to determine independent risk factors for retinopathy. All tests for statistical significance were two-tailed and performed assuming a type I error probability of <0.05.

Results

Demographic and clinical parameters

The study included 52 males and 48 females. The age of patients ranged from 41 to 76 years. The duration of diabetes ranged from 6 months to 20 years. Among the diabetic patients, 29 were on oral hypoglycemic agents, 9 were on insulin, and 12 were on combination therapy. Group 1 (n = 36) consisted of 16 patients with no DR, 13 with mild NPDR, and 7 with moderate NPDR. Group 2 (n = 14) consisted of 6 patients with severe NPDR and 8 with PDR. There were no statistically significant differences in the age, body mass index and blood pressure between controls and diabetic groups. Glycosylated hemoglobin (HbA1c) levels were found to be significantly higher in Group 2 diabetic patients (P = 0.014). No significant difference was noted in the duration of diabetes among diabetic groups. The demographic and clinical characteristics of controls and diabetic subjects are presented in Table 1.

Hemodynamic parameters

Table 2 shows the hemodynamic parameters of retrobulbar blood vessels among controls and patient groups.

Ophthalmic artery

Significant differences were noted in the RI and PI values of the ophthalmic artery between controls and diabetic patients with various DR grades. OA flow velocities did not vary between groups.

Table 3: Pearson correlation coefficients between the duration of diabetes, HbA1c, and hemodynamic parameters

Variable	Duration of diabetes	HbA1c
OA PSV	0.021 (0.885)	-0.120 (0.407)
OA EDV	-0.141 (0.328)	-0.055 (0.706)
OA RI	0.314* (0.026)	0.097 (0.504)
OA PI	0.299* (0.035)	0.078 (0.588)
CRA PSV	-0.135 (0.349)	-0.269 (0.059)
CRA EDV	-0.464* (0.001)	-0.299*(0.035)
CRA RI	0.375* (0.007)	-0.046 (0.753)
CRA PI	0.487* (0.000)	0.131 (0.370)
PCA PSV	-0.052 (0.722)	0.170 (0.239)
PCA EDV	-0.234 (0.102)	0.196 (0.172)
PCA RI	0.332* (0.019)	-0.075 (0.606)
PCA PI	0.321*(0.023)	-0.085 (0.556)
CRV V max	0.134 (0.354)	0.128 (0.375)
CRV V min	-0.143 (0.322)	-0.058 (0.687)
CRV RI	0.390* (0.005)	0.172 (0.233)
CRV PI	0.415*(0.003)	0.210 (0.144)

Corresponding P values are shown in brackets. HbA1c=Glycosylated hemoglobin; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=maximum blood velocity; V min=minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

Central retinal artery and vein

The hemodynamic parameters of CRA were significantly different between controls, DR Group 1, and Group 2 whereas no such difference was seen in the CRV. *Post hoc* comparison revealed a significantly reduced PSV and EDV of CRA (P = 0.005 and 0.000, respectively) along with increased PI (P = 0.003) in DR group 2 compared to controls. Patients with group 2 DR also demonstrated a significant decrease of EDV and increased RI of CRA compared to group 1 (P = 0.015). PSV, EDV, and RI of CRA did not differ significantly between controls and DR group 1.

Posterior ciliary artery

The EDV and PI of PCA were found to vary significantly between controls and diabetic groups. Significant reduction in PSV and EDV of PCA was noted in DR group 1 compared to controls (P = 0.046 and P = 0.010, respectively after *post hoc* comparison).

Relation between duration of diabetes, HbA1c, and hemodynamic parameters

Duration of diabetes correlated positively with the resistive and pulsatile indices of all retrobulbar vessels studied namely ophthalmic artery, CRA, PCA, and CRV. HbA1c levels, as well as the duration of diabetes, were found to correlate negatively with EDV of CRA. No correlation was found between duration of diabetes and HbA1c levels (R = 0.183, P = 0.203). Table 3 shows the relationship between duration of diabetes, HbA1c levels, and hemodynamic parameters.

Binary logistic regression analysis revealed that HbA1c levels and RI of OA were independent risk factors of retinopathy.

Discussion

The main observations of our study are as follows: (1) RI in the ophthalmic artery was significantly higher in both DR groups than the control group and was an independent risk factor of retinopathy, (2) Patients with Group 1 DR had decreased blood flow velocity (PSV and EDV) in the posterior ciliary artery compared to control group, (3) Patients with Group 2 DR had significantly reduced EDV and increased RI in the CRA compared to Group 1, (4) Compared to the control group, patients with Group 2 DR had slower flow velocities and increased PI in CRA but RI values did not vary significantly.

Our finding of decreased EDV in the posterior ciliary artery in patients with Group 1 DR supports the hypothesis that choroidal blood flow is affected in the early stages of DR. Laser Doppler flowmetry has shown decreased choroidal blood flow in the foveal region in early DR.[14] Choroidal vascularity index (CVI) analysis by swept-source optical coherence tomography (OCT) has also shown reduced CVI in patients with mild/moderate NPDR.[15] Cao *et al.* have reported a 4 times more choriocapillary loss in diabetic eyes compared to non-diabetic eyes in histopathologic studies.[16] Ferrara *et al.* have demonstrated the loss of intermediate and large blood vessels in the Sattler's layer and Haller's layers in diabetic eyes in choroidal OCT.[17] Our observation of decreased EDV in the PCA suggests increased peripheral vascular resistance of the choroidal circulation in early DR and is in concordance with these studies. Dimitrova *et al.* reported decreased EDV and increased RI in PCA in patients

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with background DR compared to controls in CDI of diabetic patients performed in sitting posture.[6] The authors have suggested that erect posture during CDI measurement is essential to appreciate significant alterations in posterior ciliary circulation. Supine posture has been suggested to increase EDV and decrease the RI.[18] As autoregulation and autonomic nervous responses are altered in diabetics, the correlation of change in circulatory parameters between normal individuals and diabetics associated with postural variation may not be expected to be equal. However, CDI was performed in supine posture in our patients. Our observations could be attributed to the shorter duration of diabetes in our patients than theirs.

RI in OA was higher in both DR groups compared with controls in our study. These findings are similar to those of previous studies.[5,19] Basturk *et al.* evaluated the association between the resistivity index of orbital arteries and DR in 91 types 2 diabetics with microalbuminuria using CDI. The authors have demonstrated increased RI values of all orbital arteries in patients with DR compared to those without DR and controls. RI value of ≥ 0.72 in OA indicated the presence of DR with 78.4% sensitivity and 70% specificity.[10] Our finding of increased RI in the OA in both DR groups can be attributed to the statistically nonsignificant reduction in EDV observed in diabetic subjects because of downstream vascular changes in the retina and choroid. We did not observe significant differences in CRA parameters between controls and DR Group 1. Hence, we believe that the OA hemodynamic parameters in our study probably represent alterations in choroidal blood flow. It has been suggested that of the total ocular blood supply from OA, less than 10% flows to the retina while the remainder is directed to choroid via the ciliary circulation due to the high metabolic demand of photoreceptors.[20] Moreover, the diastolic blood flow of PCA is higher than CRA since these vascular channels of choroid have low resistance.[21]

Table 4: Various published studies on ocular circulation in diabetic patients using color Doppler imaging

Study	Country	Sample size	Duration of diabetes (years)	Findings
Arai *et al.*, 1998[9]	Japan	74 Controls: 22 NDR: 27 DR: 25	NA	Significant increase in RI of CRA in patients with DR compared to those without DR and controls (P<0.01)
MacKinnon *et al.*, 2000[5]	Scotland	62 Controls: 17 NDR/BDR: 24 PPDR/PDR: 21	NDR/BDR: 10 PPDR/PDR: 12	Significant decrease in both PSV and EDV of CRA in the PPDR/PDR group compared to NDR/BDR and control group (P<0.05) while RI showed no significant change. RI of OA was increased in diabetics compared to controls.
Dimitrova *et al.* , 2001[6]	Japan	95 Controls: 22 NDR: 38 BDR: 35	NDR: 9.0±7.2 BDR: 18. 9±11.2	Significant decrease in EDV and increase in RI of PCA in BDR group compared to controls (P=0.01 and 0.0003, respectively)
Gracner *et al.*, 2004[8]	Slovenia	66 Controls: 22 NPDR: 22 SNPDR/PDR: 22	13.5±9.31	Significant increase in PSV of OA in the SNPDR/PDR group compared to controls. Significant increase in RI of PCA in SNPDR/PDR group compared to NPDR and controls
Sullu *et al.*, 2005[7]	Turkey	34 Controls: 20 PDR: 14	13.5±6.1	Decrease in mean EDV and increase in RI of both OA and CRA in PDR compared to controls (P<0.05)
Baydar *et al.*, 2007[11]	Australia	44 (65 eyes) Controls: 11 Diabetics: 33	4.94±3.90	The RI of CRA was significantly higher in the control group than in the NDR group. RI of OA was significantly higher in NDR than in the moderate NPDR group.
Basturk *et al.*, 2012[10]	Turkey	118 Controls: 27 NDR: 40 DR: 51	NDR: 6.87±4.37 DR: 8.23±2.55	Patients with DR had significantly higher mean RIs of OA, CRA and PCA compared to those without DR and controls (P<0.001)
Karami *et al.*, 2012[30]	Iran	123 Controls: 25 BDR: 74 PPDR: 24	BDR: 5.6±0.7 PPDR: 12.6±1.1	RI of OA and CRV were significantly higher in patients with DR compared to controls (P<0.005)
Our study	India	100 Controls: 50 NDR/mild/ moderate NPDR: 36 SNPDR/PDR: 14	NDR/mild/moderate NPDR: 8 SNPDR/PDR: 11	RI of OA was significantly increased in diabetics compared to controls. Significant decrease in both PSV and EDV of PCA in mild/ moderate NPDR compared to controls (P<0.05) Significantly reduced EDV and increased RI of CRA in SNPDR/PDR compared to mild/moderate NPDR group (P=0.015)

NDR=No diabetic retinopathy; BDR=Background diabetic retinopathy; PPDR=Preproliferative diabetic retinopathy; NPDR=Nonproliferative diabetic retinopathy; SNPDR=Severe nonproliferative diabetic retinopathy; PDR=Proliferative diabetic retinopathy; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; RI=Resistivity index; NA=Not available

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Khatri *et al.* assessed the correlation between RI of OA and topographic alterations of retinal pigment epithelium (RPE) on spectral-domain optical coherence tomography (SD-OCT).[22] In their study involving 75 patients with type 2 diabetes and 24 controls, a significant increase in OA RI was noted with the severity of DR ($F = 14.23$, $P < 0.001$). A positive correlation was noted between RI of OA and grades of RPE alterations on SD-OCT suggesting that decreased blood flow contributes to RPE dysfunction. Studies demonstrating early retinal damage in experimental diabetes have reported morphological changes in RPE and animal studies have also shown reduced choroidal blood flow in young diabetic animals suggesting that blood flow deficit could be an early pathological change in DR.[23,24] Although it is difficult to propose a causal association it can be postulated that changes in choroidal circulation (as reflected by increased RI of OA) lead to downregulation of antiangiogenic PEDF (pigment epithelial-derived factor) and upregulation of VEGF (vascular endothelial growth factor) thereby contributing to progression of DR. Differential expression and secretion of these growth factors derived from the retinal pigment epithelium have been suggested to lead to nonproliferative and proliferative DR.[25]

Patients with Group 2 DR had significantly reduced EDV in the CRA compared to Group 1 DR as well as healthy controls. Our observations are in line with previous studies.[5,6,8] These can be attributed to the increased peripheral vascular resistivity with the progression of DR. Pathological findings in DR such as capillary basement membrane thickening, retinal capillary leukostasis, loss of retinal capillaries, and altered blood rheology support this circulatory feature.[2,26,27]

Another observation of our study was that DR Group 2 had significantly reduced flow velocity in the CRA but an increase in RI was not statistically significant compared to the control group. This can be due to the concomitant decrease of both PSV and EDV in patients with Group 2 DR. Neudorfer *et al.* evaluated the long-term changes in the retrobulbar circulation of 138 eyes of diabetic subjects at a 10-year follow-up.[28] Patients were divided into four groups, namely, nondiabetic controls, diabetics without DR, nonproliferative DR, and proliferative DR. RI values of CRA and PCA had increased in the two no retinopathy groups and in the nonproliferative DR group, with a surprising nonsignificant decrease in the proliferative DR group. The authors have concluded that an increase of resistance in the retrobulbar blood vessels due to DR can decrease over time and may even be reversed. It has been suggested that the decrease in resistance reflects the beginning of an increase in retinal blood flow with the progression of DR due to the short-circuiting of the obstructed retinal capillary network.[28] This is supported by histopathologic studies, which have shown areas of capillary closure being traversed by preferential dilated anastomotic channels.[29]

Our finding of a positive correlation between duration of diabetes and RI of all retrobulbar vessels was similar to the observations of Karami *et al.*[30] We found negative correlation between EDV of CRA and duration of diabetes as well as HbA1c levels ($R = -0.464$, $P = 0.001$; $R = -0.299$, $P = 0.035$, respectively). Poor glycemic control (as reflected by increased HbA1c) and longer duration of diabetes can be associated with increased peripheral vascular resistance and thus reduced EDV in CRA. Our findings of HbA1c and RI of OA as independent risk factors of DR are similar to that of Basturk *et al.*[10]

Table 4 shows the various published studies on ocular circulation in diabetic patients using CDI.

Our study has several strengths. None of the diabetic patients had associated vascular diseases. Hypertension as well as antihypertensive medications significantly alter the resistive indices of blood vessels and therefore confound the results. We intentionally excluded these patients to study the influence of diabetes alone on ocular hemodynamics. A single masked, experienced radiologist performed all the Doppler examinations gently with the least pressure on the globe thus eliminating interobserver variability. The presence and severity of DR were graded based on seven field stereo fundus photography for accuracy.

To our knowledge, this study is the first of its kind in a pure cohort of diabetics from India.

There are a few limitations to our study. The sample size was small which accounts for the limited subgroup analysis. CDI provides information on blood flow velocity only and absolute volumetric blood flow cannot be quantified since it is not possible to study the diameter of retrobulbar vessels with CDI accurately. In spite of this limitation, blood velocity is probably a good indicator of blood flow within a given vessel.[31,32] Moreover, measurement of retinal blood flow is difficult and no accurate method to quantitatively assess perfusion is currently used clinically.

Conclusion

In conclusion, significant changes in the blood flow velocities and RI were observed in the retrobulbar vessels, especially in an ophthalmic artery in diabetics compared to controls. RI could be potentially useful for early diagnosis and follow-up of DR. CDI with results of increased resistance or decreased flow can be of help to identify diabetic individuals at higher risk for developing severe DR. This is especially useful in patients with opaque media where conventional methods are of little use.

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Conflicts of interest

There are no conflicts of interest.

References

1. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.

[Downloaded free from http://www.ijo.in on Thursday, June 4, 2020, IP: 192.168.10.239]

2. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye (Lond) 2009;23:1496-508.
3. Aburn NS, Sergott RC. Orbital colour Doppler imaging. Eye (Lond) 1993;7:639-47.
4. Dimitrova G, Kato S. Color Doppler imaging of retinal diseases. Surv Ophthalmol 2010;55:193-214.
5. MacKinnon JR, McKillop G, O'Brien C, Swa K, Butt Z, Nelson P. Colour Doppler imaging of the ocular circulation in diabetic retinopathy. Acta Ophthalmol Scand 2000;78:386-9.
6. Dimitrova G, Kato S, Tamaki Y, Yamashita H, Nagahara M, Sakurai M, *et al.* Choroidal circulation in diabetic patients. Eye (Lond) 2001;15:602-7.
7. Sullu Y, Hamidova R, Beden U, Yakupov K, Canbaz S, Danaci M. Effects of pars plana vitrectomy on retrobulbar haemodynamics in diabetic retinopathy. Clin Exp Ophthalmol 2005;33:246-51.
8. Gracner T. Ocular blood flow velocity determined by color Doppler imaging in diabetic retinopathy. Ophthalmologica 2004;218:237-42.
9. Arai T, Numata K, Tanaka K, Kiba T, Kawasaki S, Saito T, *et al.* Ocular arterial flow hemodynamics in patients with diabetes mellitus. J Ultrasound Med 1998;17:675-81.
10. Basturk T, Albayrak R, Ulas T, Akcay M, Unsal A, Toksoy M, *et al.* Evaluation of resistive index by color Doppler imaging of orbital arteries in type II diabetes mellitus patients with microalbuminuria. Ren Fail 2012;34:708-12.
11. Baydar S, Adapinar B, Kebapci N, Bal C, Topbas S. Colour Doppler ultrasound evaluation of orbital vessels in diabetic retinopathy. Australas Radiol 2007;51:230-5.
12. Pandey SK, Sharma V. World diabetes day: Battling the emerging epidemic of diabetic retinopathy. Indian J Ophthalmol 2018;66:1652-3.
13. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, *et al.* Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Global Diabetic Retinopathy Project Group. Ophthalmology 2003;110:1677-82.
14. Nagaoka T, Kitaya N, Sugawara R, Yokota H, Mori F, Hikichi T, *et al.* Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. Br J Ophthalmol 2004;88:1060-3.
15. Kim M, Ha MJ, Choi SY, Park YH. Choroidal vascularity index in type-2 diabetes analyzed by swept-source optical coherence tomography. Sci Rep 2018;8:70.
16. Cao J, McLeod S, Merges CA, Lutty GA. Choriocapillaris degeneration and related pathologic changes in human diabetic eyes. Arch Ophthalmol 1998;116:589-97.
17. Ferrara D, Waheed NK, Duker JS. Investigating the choriocapillaris and choroidal vasculature with new optical coherence tomography technologies. Prog Retin Eye Res 2016;52:130-55.
18. Evans DW, Harris A, Garrett M, Chung HS, Kagemann L. Glaucoma patients demonstrate faulty autoregulation of ocular blood flow during posture change. Br J Ophthalmol 1999;83:809-13.
19. Tamaki Y, Nagahara M, Yamashita H, Kikuchi M. Blood velocity in the ophthalmic artery determined by color Doppler imaging in normal subjects and diabetics. Jpn J Ophthalmol 1993;37:385-92.
20. Cioffi GA, Granstam E, Alm A. Ocular circulation. In: Kaufman PL, Alm A, editors. Adler's Physiology of the Eye. 10th ed. St. Louis, MO: Mosby; 2003. p. 747-73.
21. Güven D, Ozdemir H, Hasanreisoglu B. Hemodynamic alterations in diabetic retinopathy. Ophthalmology 1996;103:1245-9.
22. Khatri M, Saxena S, Kaur A, Bhasker SK, Kumar M, Meyer CH. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retina Vitreous 2018;4:12.
23. Decanini A, Karunadharma PR, Nordgaard CL, Feng X, Olsen TW, Ferrington DA. Human retinal pigment epithelium proteome changes in early diabetes. Diabetologia 2008;51:1051-61.
24. Muir ER, Rentería RC, Duong TQ. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. Invest Ophthalmol Vis Sci 2012;53:6488-94.
25. Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO. Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 2003;22:1-29.
26. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. Microcirculation 2007;14:25-38.
27. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008;2:1130-8.
28. Neudorfer M, Kessner R, Goldenberg D, Lavie A, Kessler A. Retrobulbar blood flow changes in eyes with diabetic retinopathy: A 10-year follow-up study. Clin Ophthalmol 2014;8:2325-32.
29. Garner A. Histopathology of diabetic retinopathy in man. Eye (Lond) 1993;7:250-3.
30. Karami M, Janghorbani M, Dehghani A, Khaksar K, Kaviani A. Orbital Doppler evaluation of blood flow velocities in patients with diabetic retinopathy. Rev Diabet Stud 2012;9:104-11.
31. Hansen NB, Stonestreet BS, Rosenkrantz TS, Oh W. Validity of Doppler measurements of anterior cerebral artery blood flow velocity: Correlation with brain blood flow in piglets. Pediatrics 1983;72:526-31.
32. Taylor GA, Short BL, Walker LK, Traystman RJ. Intracranial blood flow: Quantification with duplex Doppler and color Doppler flow US. Radiology 1990;176:231-6.

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Original Article

Relationship between myopia and central corneal thickness – A hospital based study from South India

ABSTRACT

Background and Purpose: The prevalence of myopia has been increasing steadily, and studies on the relationship between myopia and central corneal thickness (CCT) have shown conflicting results. The purpose of this study was to investigate the relationship between myopia and CCT in a hospital based population from South India. **Materials and Methods:** This prospective, cross-sectional study was carried out from a tertiary care institution in South India between January 2017 and June 2018. One hundred and ninety-four myopic individuals between 11 and 40 years of age with spherical equivalent (SE) ranging from -0.5 to -6 Diopters after cycloplegic refraction were enrolled in the study. CCT was measured in both eyes of each patient using Tomey EM-3000 noncontact specular microscope (Tomey Corporation, Nagoya, Japan). The relation between CCT and myopia was analyzed using Pearson's correlation coefficient. Statistical significance was set at $P < 0.05$. **Results:** A total of 388 eyes were analyzed. 50% (n=97) of study participants were females. The mean age of the study participants was 25.47 ± 7.25 years (range: 12–40 years). The mean SE was -2.79±1.34 D (range: -0.5 to -6.0 D). The mean CCT was 540.53 ± 56.16 μm (range: 441–664 μm). No significant association was observed between CCT and age or gender of the study participants ($P = 0.706$ and 0.550, respectively). No correlation was observed between mean CCT and SE (Pearson, $r = 0.095$, $P = 0.062$). **Conclusion:** There was no correlation between CCT and degree of myopia. These data contribute to the ongoing discussion on the structural alterations in ocular coats and susceptibility to glaucoma in myopia.

Keywords: Central corneal thickness measurement, myopia, noncontact specular microscope, spherical equivalent

INTRODUCTION

Myopia, referred to as "short sight," is increasing in prevalence at an alarming rate and has emerged as a major cause for visual impairment globally. Estimates suggest that around 1.5 billion people or 22% of the present world population is myopic.[1] Apart from the ethnic and genetic factors, environmental and lifestyle changes have contributed significantly to the increasing prevalence of myopia. A recent meta-analysis on the prevalence of myopia in South-east Asia has shown an estimated pool prevalence of 4.9% (95% confidence interval [CI]: 1.6–8.1) in children and 32.9% (95% CI: 25.1–40.7) in adults.[2] The recent shift to younger age at the onset of myopia is even more worrisome considering its impact on the economic burden and quality of life.

With an increasing prevalence of myopia, there has also been a tremendous rise in the number of laser refractive procedures for the correction of myopia. Central corneal thickness (CCT) is a major factor determining the choice of refractive surgery for myopia, and a thin cornea is a recognized risk factor for corneal ectasia postrefractive surgery.[3] Corneal refractive surgery for myopia results in reduced corneal thickness and thereby leads to the underestimation of intraocular pressure.[4,5] This is relevant in the diagnosis and treatment of any glaucoma later in life,

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since both myopia and reduced CCT are well-known risk factors for glaucoma.[6-8]

Studies evaluating the relationship between myopia and CCT have revealed conflicting results. While few studies have found a correlation between CCT and myopia,[9-11] some have reported no significant correlation.[12-15] This can be attributed to the differences in ethnicity, characteristics of the population studied as well as varied methods of the measurement of corneal thickness. Two population-based studies using ultrasonic pachymetry (UP) from India have reported thinner corneas in Indians than that of other populations.[12,16] Relatively fewer studies have evaluated CCT using noncontact specular microscopy, which is a noninvasive and accurate method with good reproducibility of CCT measurements.[17-19]

Given the increasing prevalence of myopia from India and conflicting evidence on the association between CCT and myopia, we attempted to study the relationship between myopia and CCT among patients attending the refraction clinic of a tertiary care institute using a noncontact specular microscope (NCSM).

MATERIALS AND METHODS

This prospective, hospital-based, cross-sectional study was carried out from a tertiary care institution in South India between January 2017 and June 2018. Individuals between 11 and 40 years of age who presented with visual impairment and found to have myopic refractive error (spherical equivalent [SE] more than −0.5 D) on cycloplegic refraction were included in the study. The exclusion criteria were myopia >−6 D, prior refractive or other ocular surgery, contact lens wear, trauma, corneal diseases, cataract, glaucoma, and ocular hypertension. Pregnant and lactating females and individuals with systemic diseases such as diabetes mellitus and collagen vascular disease were also excluded from the study. The study was performed according to the principles of the Declaration of Helsinki after prior approval from the Institutional Human Ethics Committee. Informed consent was obtained from all the participants.

All the study participants underwent a comprehensive ophthalmic examination, including slit-lamp biomicroscopy and fundus evaluation using +90 D lens and indirect ophthalmoscopy. Cycloplegia was achieved with three topical applications of 1% cyclopentolate at 15-min intervals. Cycloplegic refraction was performed 30–60 min after the first instillation of drug and spherical equivalent (SE, sphere + half-cylindrical component) was calculated.

CCT was measured by a single experienced ophthalmologist using Tomey EM-3000 NCSM (Tomey Corporation, Nagoya, Japan). The participants were instructed to fixate on the central target with chin and forehead positioned appropriately. Then, the participants were asked to blink completely once before measurements to spread an optically smooth tear film over the corneal surface. The measurements were taken using the auto-alignment and capture mode, thereby eliminating observer bias. Three consecutive valid measurements were taken, and the average of the three readings was used for the analysis. The CCT values were considered valid when the endothelial cells were focused clearly and bright corneal reflections were centered on the monitor. All measurements were performed in midday (between 12 and 4 PM) to eliminate diurnal variation.[20]

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, LA, USA) software version 24. Descriptive statistics were presented as mean ± standard deviation. Discrete variables were presented as numbers (n) and percentages (%). Patients were classified into the following three groups according to SE: Group 1 included patients with SE -0.5 to <-2 D; Group 2 consisted of patients with SE -2 to < -4 D; and Group 3 individuals with SE -4 to < -6 D. The normality of CCT distribution was determined by the Shapiro–Wilk test. One-way analysis of variance followed by *post-hoc* Bonferroni was used for the analysis between the groups. The student's t-test was used to analyze the mean difference between two independent samples. Pearson's coefficient was used to study the correlation between variables. $P < 0.05$ was considered to be statistically significant.

RESULTS

This study included 388 eyes of 194 individuals with varying grades of myopia. 50% ($n = 97$) of the study participants were females. The mean age of the study participants was 25.47 ± 7.25 years (range: 12–40 years). The mean SE was − 2.79 ± 1.34 D (range: -0.5 to -6.0 D). The mean CCT was 540.53 ± 56.16 µm (range: 441–664 µm).

The CCT measurements were found to be nonnormally distributed. Figure 1 shows the distribution of CCT readings among the study participants. Figure 2 shows the distribution of SE among study participants.

Table 1 shows the association of gender of study participants with CCT. No significant differences were noted in the mean CCT between males and females ($P = 0.550$, Student's t-test).

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Table 2 shows the association between mean CCT and age of study participants. No significant differences were noted in mean CCT values between various age groups ($P = 0.706$).

Table 3 shows the mean CCT values among the SE groups. Figure 3 shows the correlation between mean CCT and SE. No correlation was observed between mean CCT and SE (Pearson, $r = 0.095$, $P = 0.062$).

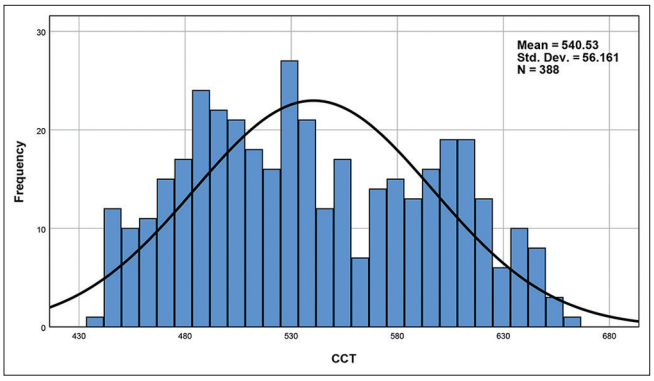


Figure 1: Distribution of central corneal thickness readings among the study participants

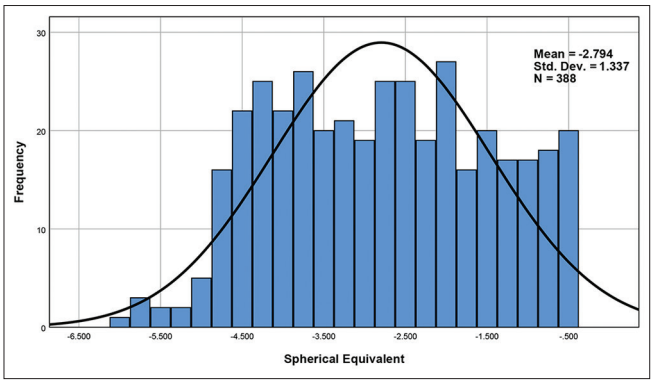


Figure 2: Distribution of spherical equivalent among the study participants

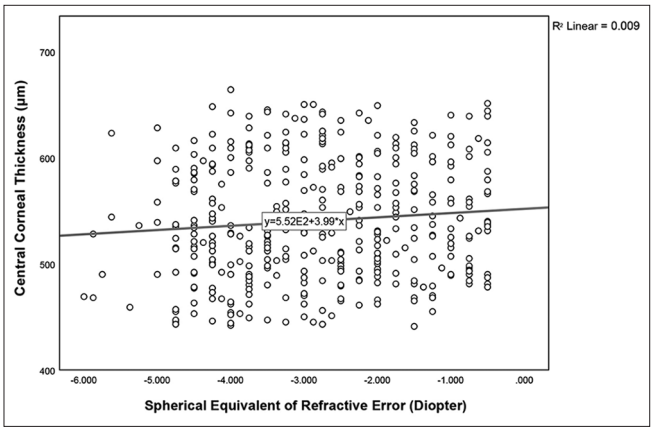


Figure 3: Scatter plot showing the correlation between mean central corneal thickness and spherical equivalent; no correlation was observed between mean central corneal thickness and spherical equivalent (Pearson, $r = 0.095$, $P = 0.062$)

DISCUSSION

In this hospital-based study from South India, we evaluated the CCT measurements of young myopic individuals with mild-to-moderate myopia using NCSM and found no correlation between mean CCT and degree of myopia.

Population-based studies from India using UP for CCT measurements have reported significantly thinner corneas among Indians compared to Africans (530 µm),[21] Nepalese (539 µm),[22] Caucasians (540 µm),[23] and Chinese (542 µm).[24] Nangia *et al*. evaluated the distribution of CCT among adults aged 30+ from rural Central India using UP. Mean CCT among 9370 eyes studied was 514 ± 33 µm (range: 290–696 µm). CCT was associated significantly with younger age, male gender, and lower corneal refractive power ($P < 0.001$). However, no statistically significant association was found between CCT and refractive error ($P = 0.54$).[12] Vijaya *et al*. evaluated 6754 adults aged 40+ from South India using UP and found the mean CCT to be 511.4 ± 33.5 µm.[16]

A hospital-based, prospective study involving 156 participants from South India, with a mean age of 29.27 years and mean refractive error of −3.10 D revealed a mean CCT value of 533.87 µm using UP. CCT had a positive correlation with axial length and negative correlation with SE, suggesting that axial

Table 1: Association between the gender of study participants and central corneal thickness

Gender	Number of patients, *n* (%)	CCT (µm), mean±SD	*P**
Males	97 (50)	538.82±56.33	0.550
Females	97 (50)	542.24±56.08	

*Student's *t*-test. CCT – Central corneal thickness, SD – Standard deviation

Table 2: Association between mean central corneal thickness and age of study participants

Age of participants (years)	Number of patients, *n* (%)	CCT (µm), mean±SD	*P**
11-20	73 (37.6)	539.35±49.11	0.706
21-30	63 (32.5)	544.03±58.34	
31-40	58 (29.9)	538.22±62.08	

*ANOVA. ANOVA – Analysis of variance, SD – Standard deviation, CCT – Central corneal thickness

Table 3: The mean central corneal thickness values among spherical equivalent groups

Spherical equivalent	Number of eyes, *n* (%)	CCT (µm), mean±SD	*P**
Group 1	108 (27.8)	546.07±52.29	0.144
Group 2	182 (46.9)	542.23±58.05	
Group 3	98 (25.3)	531.28±56.16	

*ANOVA. SD – Standard deviation, CCT – Central corneal thickness, ANOVA – Analysis of variance

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myopes tend to have thicker corneas.[10] Kunert *et al*. measured the CCT of 615 myopic patients of Indian origin (aged 18+) before laser *in situ* keratomileusis with UP as well as Orbscan scanning-slit topography/pachymetry.[25] Mean CCT reported was 519.92 ± 33.36 µm (range: 420–640 µm) using ultrasound method and 518.23 ± 31.03 µm (range: 428–607 µm) using Orbscan. No significant differences were noted in the CCT values measured using these two methods. Lower CCT readings were observed in individuals with myopia −0.5 to −4.9 D compared to those with myopia ranging from −5 to −9.9 D.

Mean CCT measurements obtained with EM-3000 (NCSM) in our study were 540.53 ± 56.16 µm (range: 441–664 µm). Módis *et al*. reported that the CCT values obtained using EM-3000 NCSM were higher than that found using ultrasound pachymetry ($P < 0.0001$).[26]

It would not be appropriate to compare our CCT measurements with other studies from India because we measured CCT using noncontact specular microscopy EM-3000 while others have utilized ultrasound pachymetry. The operating principles behind these two instruments are entirely different which explains the variation in measured values. While UP measurements depend on the reflection of ultrasonic sound waves from the anterior and posterior corneal surfaces, NCSM measurements rely on the reflection of light. CCT may be under or overestimated due to high inter-device variations, and studies have shown poor inter-device agreement among CCT measurements taken with different instruments.[27,28]

Although ultrasound pachymetry is the gold standard for CCT measurements, it is a contact method that needs topical anesthesia and user skill with regard to the placement of the probe. Furthermore, studies have reported alteration in corneal thickness values after the application of local anesthetic eye drops.[29,30] Unlike ultrasound pachymetry, NCSM is noninvasive and provides reproducible measurements of CCT.

There was no significant association between CCT and gender or age in our study. The role of gender on CCT has been reported to be variable, with few studies showing thicker CCT in males,[18,31] while others have reported no significant differences between males and females.[32] Few studies in patients older than 40 years of age have reported a negative association between CCT and age.[16,21,33]

The effect of refractive error on CCT has been analyzed by several investigators with contrasting results. Our study results demonstrate that CCT does not correlate with the myopia of different grades. Our observations are in agreement with the majority of studies elsewhere.[12-15] Chen *et al*. correlated the CCT measurements and degree of myopia among 528 Taiwanese adults enrolled for myopic laser refractive surgery.[13] No statistically significant association was found between CCT and refractive error ($P = 0.49$). The authors also studied the relation between CCT and myopia by intraindividual comparison in myopic anisometropia. The mean myopic anisometropia was 3.09 ± 1.06 D in their series, and the CCT in more myopic eye was not significantly different from the other less myopic eye. The authors have concluded that the cornea does not thin in the same way as the sclera in myopic eyes. Similarly, Fam *et al*. evaluated 714 consecutive Chinese patients with mean SE of − 5.3 D (range: −17.5 to −0.625).[15] No correlation was found between CCT and the degree of myopia ($r = -0.13$, $P = 0.719$). Ortiz *et al*. studied the relationship between CCT as well as mid-peripheral corneal thickness (PCT) in 175 myopic participants using Orbscan II.[34] Participants were divided into three groups, namely myopia <−6 D ($n = 76$), -6 to -12 D ($n = 72$), and more than −12 D ($n = 27$). No statistically significant differences were found in both CCT and PCT between the groups ($P > 0.05$).

A recent study comparing the thickness of preocular tear film, corneal layers (epithelium, Bowman layer, stroma and Descemet's membrane-endothelium complex in the central cornea), and anterior sclera using anterior segment spectral-domain optical coherence tomography found no statistically significant differences between emmetropia and those with moderate-to-high myopia ($P > 0.05$).[35]

However, few authors have found a significant correlation between CCT and myopic refractive error. Kadhim and Farhood evaluated CCT measurements of 418 eyes of 209 healthy individuals using UP.[9] The mean CCT was 543.95 ± 32.58 µm. CCT measurements significantly negatively correlated with age and myopes were noted to have significantly thinner corneas than emmetropic individuals ($P = 0.019$). Chang *et al*. evaluated a total of 216 participants, with a mean age of 22.2 years and mean refractive error of −4.17 D using UP.[36] A significant correlation was observed between CCT and SE ($r = 0.16$, $P = 0.021$). Corneas tended to be thinner in more myopic eyes. The authors have concluded that a decrease in corneal thickness is one among the changes in the anterior segment of the eye as myopia progresses. In contrast, Pedersen *et al*. measured CCT in 57 emmetropes and 48 high myopes using a high-precision Haag-Streit optical low coherence reflectometry pachymeter.[37] No statistically significant differences were noted between myopic participants and emmetropes (527.7 ± 35 µm versus

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538.6 ± 32.1 μm), suggesting that the process by which myopia progresses does not influence the central cornea to a measurable degree.

The wide discrepancy in the observed results can be attributed to the varied age groups of patients, smaller sample sizes, nonelimination of contact lens wearers as well as the influence of diurnal variation, and use of pachymeters with variable reproducibility. The relatively new NCSM EM-3000 (Tomey, Japan) used in this study has been suggested to have high intraoperator repeatability in normal corneas.[27]

Our study has a few limitations. Observations are from a single-center, and axial length was not measured to differentiate axial myopia from other causes for myopia. However, we intentionally excluded patients older than 40 years to eliminate index myopia. To the best of our knowledge, this is the first study from India using NCSM to evaluate CCT measurements among myopic individuals.

CONCLUSION

In summary, we evaluated CCT measurements using noncontact specular microscopy in a cohort of young individuals with mild-to-moderate myopia. The mean CCT values did not correlate with myopic SE. Further studies with a larger sample size and different grades of myopia are needed to better understand the relation between myopia and CCT.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Holden B, Sankaridurg P, Smith E, Aller T, Jong M, He M. Myopia, an underrated global challenge to vision: Where the current data takes us on myopia control. Eye (Lond) 2014;28:142-6.
2. Hashemi H, Fotouhi A, Yekta A, Pakzad R, Ostadimoghaddam H, Khabazkhoob M. Global and regional estimates of prevalence of refractive errors: Systematic review and meta-analysis. J Curr Ophthalmol 2018;30:3-22.
3. Santhiago MR, Giacomin NT, Smadja D, Bechara SJ. Ectasia risk factors in refractive surgery. Clin Ophthalmol 2016;10:713-20.
4. Ajazaj V, Kaçaniku G, Asani M, Shabani A, Dida E. Intraocular pressure after corneal refractive surgery. Med Arch 2018;72:341-3.
5. Duch S, Serra A, Castanera J, Abos R, Quintana M. Tonometry after laser *in situ* keratomileusis treatment. J Glaucoma 2001;10:261-5.
6. Marcus MW, de Vries MM, Junoy Montolio FG, Jansonius NM. Myopia as a risk factor for open-angle glaucoma: A systematic review and meta-analysis. Ophthalmology 2011;118:1989-9400.
7. Mitchell P, Hourihan F, Sandbach J, Wang JJ. The relationship between glaucoma and myopia: The Blue Mountains Eye Study. Ophthalmology 1999;106:2010-5.
8. Dimasi DP, Burdon KP, Craig JE. The genetics of central corneal thickness. Br J Ophthalmol 2010;94:971-6.
9. Kadhim YJ, Farhood QK. Central corneal thickness of Iraqi population in relation to age, gender, refractive errors, and corneal curvature: A hospital-based cross-sectional study. Clin Ophthalmol 2016;10:2369-76.
10. Muthu Krishnan V, Jayalatha K, Vijayakumar C. Correlation of central corneal thickness and keratometry with refraction and axial length: A prospective analytic study. Cureus 2019;11:e3917.
11. Mimouni M, Flores V, Shapira Y, Graffi S, Levartovsky S, Sela T, *et al*. Correlation between central corneal thickness and myopia. Int Ophthalmol 2018;38:2547-51.
12. Nangia V, Jonas JB, Sinha A, Matin A, Kulkarni M. Central corneal thickness and its association with ocular and general parameters in Indians: The Central India Eye and Medical Study. Ophthalmology 2010;117:705-10.
13. Chen YC, Kasuga T, Lee HJ, Lee SH, Lin SY. Correlation between central corneal thickness and myopia in Taiwan. Kaohsiung J Med Sci 2014;30:20-4.
14. Al-Mezaine HS, Al-Obeidan S, Kangave D, Sadaawy A, Wehaib TA, Al-Amro SA. The relationship between central corneal thickness and degree of myopia among Saudi adults. Int Ophthalmol 2009;29:373-8.
15. Fam HB, How AC, Baskaran M, Lim KL, Chan YH, Aung T. Central corneal thickness and its relationship to myopia in Chinese adults. Br J Ophthalmol 2006;90:1451-3.
16. Vijaya L, George R, Arvind H, Ve Ramesh S, Baskaran M, Raju P, *et al*. Central corneal thickness in adult South Indians: The Chennai Glaucoma Study. Ophthalmology 2010;117:700-4.
17. Tomidokoro A, Araie M, Iwase A; Tajimi Study Group. Corneal thickness and relating factors in a population-based study in Japan: The Tajimi study. Am J Ophthalmol 2007;144:152-4.
18. Suzuki S, Suzuki Y, Iwase A, Araie M. Corneal thickness in an ophthalmologically normal Japanese population. Ophthalmology 2005;112:1327-36.
19. Almubrad TM, Osuagwu UL, Alabbadi I, Ogbuehi KC. Comparison of the precision of the Topcon SP-3000P specular microscope and an ultrasound pachymeter. Clin Ophthalmol 2011;5:871-6.
20. Lattimore MR Jr, Kaupp S, Schallhorn S, Lewis R 4th. Orbscan pachymetry: Implications of a repeated measures and diurnal variation analysis. Ophthalmology 1999;106:977-81.
21. Nemesure B, Wu SY, Hennis A, Leske MC; Barbados Eye Study Group. Corneal thickness and intraocular pressure in the Barbados eye studies. Arch Ophthalmol 2003;121:240-4.
22. Thapa SS, Paudyal I, Khanal S, Paudel N, Mansberger SL, van Rens GH. Central corneal thickness and intraocular pressure in a Nepalese population: The Bhaktapur Glaucoma Study. J Glaucoma 2012;21:481-5.
23. Rahman ML, Bunce C, Healey PR, Mitchell P, Sham PC, McGuffin P, *et al*. Commingling analyses of central corneal thickness and adjusted intraocular pressure in an older Australian population. Invest Ophthalmol Vis Sci 2010;51:2512-8.
24. Wang D, Huang W, Li Y, Zheng Y, Foster PJ, Congdon N, *et al*. Intraocular pressure, central corneal thickness, and glaucoma in Chinese adults: The Liwan eye study. Am J Ophthalmol 2011;152:454-620.
25. Kunert KS, Bhartiya P, Tandon R, Dada T, Christian H, Vajpayee RB. Central corneal thickness in Indian patients undergoing LASIK for myopia. J Refract Surg 2003;19:378-9.
26. Módis L Jr., Szalai E, Németh G, Berta A. Evaluation of a recently developed noncontact specular microscope in comparison with conventional pachymetry devices. Eur J Ophthalmol 2010;20:831-8.
27. Bao F, Wang Q, Cheng S, Savini G, Lu W, Feng Y, *et al*. Comparison and evaluation of central corneal thickness using 2 new noncontact specular microscopes and conventional pachymetry devices. Cornea 2014;33:576-81.

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28. Maloca PM, Studer HP, Ambrósio R Jr., Goldblum D, Rothenbuehler S, Barthelmes D, *et al*. Interdevice variability of central corneal thickness measurement. PLoS One 2018;13:e0203884.
29. Asensio I, Rahhal SM, Alonso L, Palanca-Sanfrancisco JM, Sanchis-Gimeno JA. Corneal thickness values before and after oxybuprocaine 0.4% eye drops. Cornea 2003;22:527-32.
30. Herse P, Siu A. Short-term effects of proparacaine on human corneal thickness. Acta Ophthalmol (Copenh) 1992;70:740-4.
31. Sng C, Barton K, Kim H, Yuan S, Budenz DL. Central corneal thickness and its associations with ocular and systemic factors in an urban West African Population. Am J Ophthalmol 2016;169:268-75.
32. Soleimanizad R, Nowroozzadeh MH, Ziaei H, Pakravan M, Yaseri M, Katibeh M. The association of central corneal thickness with ocular and general parameters in a community setting: The Yazd eye study. J Ophthalmic Vis Res 2017;12:141-50.
33. Hahn S, Azen S, Ying-Lai M, Varma R; Los Angeles Latino Eye Study Group. Central corneal thickness in Latinos. Invest Ophthalmol Vis Sci 2003;44:1508-12.
34. Ortiz S, Mena L, Rio-San Cristobal A, Martin R. Relationships between central and peripheral corneal thickness in different degrees of myopia. J Optom 2014;7:44-50.
35. Pekel G, Yağcı R, Acer S, Ongun GT, Çetin EN, Simavlı H. Comparison of corneal layers and anterior sclera in emmetropic and myopic eyes. Cornea 2015;34:786-90.
36. Chang SW, Tsai IL, Hu FR, Lin LL, Shih YF. The cornea in young myopic adults. Br J Ophthalmol 2001;85:916-20.
37. Pedersen L, Hjortdal J, Ehlers N. Central corneal thickness in high myopia. Acta Ophthalmol Scand 2005;83:539-42.

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Original Article

Evaluation of Resistive Index of Orbital Vessels Using Color Doppler Imaging in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Resistive index (RI), derived from color Doppler imaging (CDI), is a marker of vascular resistance used widely in varied clinical settings. The aim of this study was to analyze the association between RIs of the orbital vessels in a pure cohort of type 2 diabetic patients with or without retinopathy using CDI. **Methods:** Fifty patients having type 2 diabetes and 50 age-matched controls were evaluated in this prospective study. Diabetic retinopathy (DR) was diagnosed based on seven-field stereo fundus photography and diabetic patients were divided into two. Patients with no DR ($n = 26$) were taken as Group 1, while patients with DR ($n = 24$) were taken as Group 2. CDI was performed and the RIs of the ophthalmic artery (OA), posterior ciliary artery (PCA), central retinal artery (CRA), and central retinal vein (CRV) were measured. **Results:** Significant differences were observed in the mean RI values of all orbital arteries between controls and patients with DR ($P < 0.05$). Comparison of RI values between controls and Group 1 showed no significant differences. Mean RI values of the PCA and CRA were found to be significantly higher in the patients in Group 2 than in Group 1 ($P = 0.03$ and $P < 0.001$, respectively). The duration of diabetes correlated with the mean RI of all the orbital vessels. RI of the CRA was a reliable predictive indicator for DR ($P = 0.001$). **Conclusion:** RIs of the orbital arteries are significantly higher in patients with DR. RI of the orbital vessels can be a potentially useful biomarker in the early diagnosis and follow-up of patients with DR.

Keywords: Color Doppler ultrasonography, diabetic retinopathy, vascular resistance

Introduction

Color Doppler imaging (CDI) is a convenient noninvasive method that gives information about the velocity of blood and vascular resistance at various sites of complex vasculature using simultaneous B mode ultrasound and Doppler images. The resistive index (RI), calculated from CDI, is a universally used measure of resistance to the arterial blood flow. In the recent past, RIs of the orbital arteries have been studied extensively to gain diagnostic and prognostic insight into a variety of clinical conditions, such as chronic obstructive pulmonary disease, hypertension, chronic kidney disease, and diabetes-related micro and macrovasculopathies.[1-5]

Usefulness of RI of the retrobulbar vessels in risk stratification and management of ophthalmic diseases such as diabetic retinopathy (DR), age-related macular degeneration, glaucoma, retinal vein occlusion, and thyroid-associated orbitopathy has been documented in the literature.[6,7]

Several studies have evaluated the RI of the retrobulbar vessels in diabetic patients with or without retinopathy and have shown inconsistent results. While few studies have shown significant correlation between RI of the orbital vessels and severity of DR,[8,9] few others suggest that the hemodynamic alterations observed in the orbital arteries reflect macrovascular changes and are not related to DR.[5,10] The observed inconsistencies can be attributed partly to the differences in sample sizes, patient characteristics, duration of diabetes, methods of assessment of DR, and probe positions used for color Doppler

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measurement. Further, most of these studies included patients with comorbidities such as hypertension and coronary artery disease as well as those patients on cardiac or antihypertensive drugs, which might alter the vascular resistance.

The aim of this study was to analyze the association between RIs of the orbital vessels in a pure cohort of type 2 diabetic patients with or without retinopathy using CDI.

Materials and Methods

This prospective study was conducted between March 2017 and May 2019 from a tertiary care institution in South India. The institutional human ethical committee approval was obtained before the initiation of the study (IRB approval number: 16/325), and the principles of Declaration of Helsinki were adhered to. Informed, written consent was obtained from all the study participants. Fifty consecutive patients of type 2 diabetes (as per definition of the American Diabetes Association) were recruited for the study. Exclusion criteria were the presence of associated systemic hypertension, nephropathy, dyslipidemia, and cardiovascular diseases. Patients on medications known to affect systemic hemodynamics such as angiotensin-converting enzyme inhibitors, calcium channel blockers, and antimigraine drugs were excluded. Patients with a history of any ocular disease that might affect ocular blood flow such as retinal diseases, high myopia, glaucoma, previous ocular surgery, or panretinal photocoagulation were also excluded from the study. A control group of 50 age-matched subjects were included. None of the controls had any ocular or systemic disease.

Data regarding age, gender, body mass index (BMI), duration of diabetes and antidiabetic medications were obtained from all the study participants. Blood pressure and serum glycosylated hemoglobin (HbA1c) levels were determined.

All participants underwent a comprehensive eye examination and the presence/absence of retinopathy was diagnosed through dilated fundus examination and 30° stereoscopic fundus photograph of seven standard early treatment DR study fields using a digital fundus camera (TRC-50 DX Mydriatic Retinal Camera; Topcon Medical Systems, Oakland, NJ, USA). Diabetic patients were divided based on retinopathy into two groups. Group 1 contained patients with no DR; Group 2 included patients with DR. One eye (right eye, unless another ocular pathology was identified) was included from each study participant.

A masked expert radiologist performed CDI of the eye for all study participants using a color Doppler unit and a 12–5 MHZ linear-array transducer (Philips iU22 xMATRIX ultrasound system). The CDI was performed with patients lying in the supine position with a 30° head tilt and any undue pressure on the eye was avoided. Examiner's hand was rested on the orbital margin, and sterile coupling gel was applied over closed eyelids. Angle of the transducer was taken as 30°–60° during the examination. CDI was performed after exclusion of any other ocular or orbital pathologies by B mode, and optic nerve was considered as a reference for all further measurements. The velocity of blood flow in the ophthalmic artery (OA) was measured 20–30 mm behind the globe after crossing of the optic nerve in the nasal aspect. Blood flow velocity in the central retinal vein (CRV) and central retinal artery (CRA) was measured within the optic nerve around 5 mm behind the posterior margin of the eyeball. Blood flow velocity from the temporal branch of the short posterior ciliary artery (PCA) was determined approximately 5–10 mm posterior to the globe. The peak systolic velocity (PSV) and end diastolic velocity (EDV) measurements were taken for OA, CRA, PCA, and CRV. With the help of PSV and EDV, we calculated the vascular resistance based on the RI using Pourcelot's formula:

$$RI = (PSV - EDV)/PSV.$$

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) Version 24. Data were reported as mean ± standard deviation. The normality of data distribution was assessed by Kolmogorov–Smirnov test. One-way analysis of variance with a *post hoc* Bonferroni was used for analysis of normally distributed continuous data. Kruskal–Wallis analysis was used for analyzing nonnormally distributed data. Bivariate analysis using Pearson's correlation coefficient was used to study the relationship of RIs with biochemical and demographic variables of patients with diabetes. Receiver operating characteristic (ROC) curve analysis was used to explore the predictive indicators of DR. A $P < 0.05$ (two-tailed) was considered statistically significant.

Results

The study consisted of 51 males and 49 females. The age of the participants ranged from 41 to 76 years. Duration of diabetes ranged between 6 months and 20 years. Among the diabetic patients studied, 26 had no DR and constituted Group 1. Group 2 consisted of 24 patients with DR. In Group 2, three patients had mild nonproliferative DR (NPDR) characterized by only few microaneurysms, seven had moderate NPDR (presence of more than just microaneurysms but less than severe NPDR), six had severe NPDR (presence of more than 20 intraretinal hemorrhages in each of four quadrants or definite venous beading in 2+ quadrants or prominent intraretinal microvascular abnormalities in 1+ quadrant), and eight had proliferative DR (presence of neovascularization of disc or elsewhere in the retina).

There were no statistically significant differences in the age, BMI, and blood pressure between controls and diabetic groups. Duration of diabetes and HbA1c levels were found to be significantly increased in Group 2 patients with DR ($P < 0.001$). Significant differences were observed in the RI values of all orbital arteries between controls and diabetic groups. Table 1 shows the demographic data and clinical attributes of controls and diabetic subjects.

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Subgroup analysis with *post hoc* comparison revealed significantly higher RI values in OA, PCA, and CRA in Group 2 compared to controls ($P < 0.001$, $P < 0.001$, and $P = 0.03$, respectively). Comparison of RI values between Group 1 and controls showed no significant differences. When diabetic Groups 1 and 2 were compared, statistically significant differences were observed in the mean RI values of CRA and PCA ($P < 0.001$ and $P = 0.03$, respectively).

In bivariate analysis, the mean RI values of all orbital vessels, namely OA, CRA, PCA, and CRV, had a significant correlation with the duration of diabetes ($R = 0.396$, $P < 0.001$; $R = 0.282$, $P = 0.005$; $R = 0.334$, $P = 0.001$; and $R = 0.290$, $P = 0.003$, respectively) [Figure 1]. In addition, the mean RI of the OA correlated significantly with HbA1c levels ($R = 0.321$, $P = 0.001$) [Figure 2].

ROC analysis showed that the RI of CRA was a definitive predictive indicator for DR. RI of the CRA ≥ 0.79 indicated the existence of DR with a sensitivity of 75% and a specificity of 73% (area under the curve = 0.780 [95% confidence interval: 0.650–0.909]) [Figure 3].

Discussion

DR, a common microvascular complication of diabetes, is one of the leading causes for preventable blindness in people of the working age.[11] There has been conflicting evidence on the ocular hemodynamics as to whether blood flow is increased or decreased in diabetics. Curtis *et al*. have proposed a hemodynamic framework which states that there is an initial reduction in the perfusion of retina before the onset of DR which later increases as DR progresses.[12]

The various hematological changes observed in diabetes such as increased aggregation of erythrocytes and platelets, increased blood viscosity, and reduced deformability of

Table 1: Demographic and clinical characteristics of controls and diabetic subjects

Characteristic	Controls ($n=50$)	Group 1 ($n=26$)	Group 2 ($n=24$)	*P*
Mean age (years)	56.58±8.81	54.85±8.52	54.42±7.84	0.52
Mean body mass index (kg/m^2)	23.35±3.62	25.33±2.69	24.09±3.88	0.07
Median systolic BP (mmHg)	110 (110-130)	120 (110-126.25)	120 (110-130)	0.16
Median diastolic BP (mmHg)	70 (70-80)	76.5 (70-80)	70 (70-80)	0.27
Median duration of diabetes (months)	-	24 (6-72)	120 (48-144)	<0.001
Mean glycosylated hemoglobin (%)	5.81±0.30	8.62±1.91	10.64±2.29	<0.001
Mean RI of OA	0.76±0.06	0.79±0.06	0.82±0.06	<0.001
Mean RI of CRA	0.77±0.07	0.72±0.17	0.84±0.11	0.001*
Mean RI of PCA	0.73±0.06	0.74±0.08	0.79±0.08	0.004*
Mean RI of CRV	0.42±0.12	0.44±0.12	0.46±0.11	0.29

*P<0.05. BP: Blood pressure, OA: Ophthalmic artery, CRA: Central retinal artery, PCA: Posterior ciliary artery, CRV: Central retinal vein, RI: Resistivity index

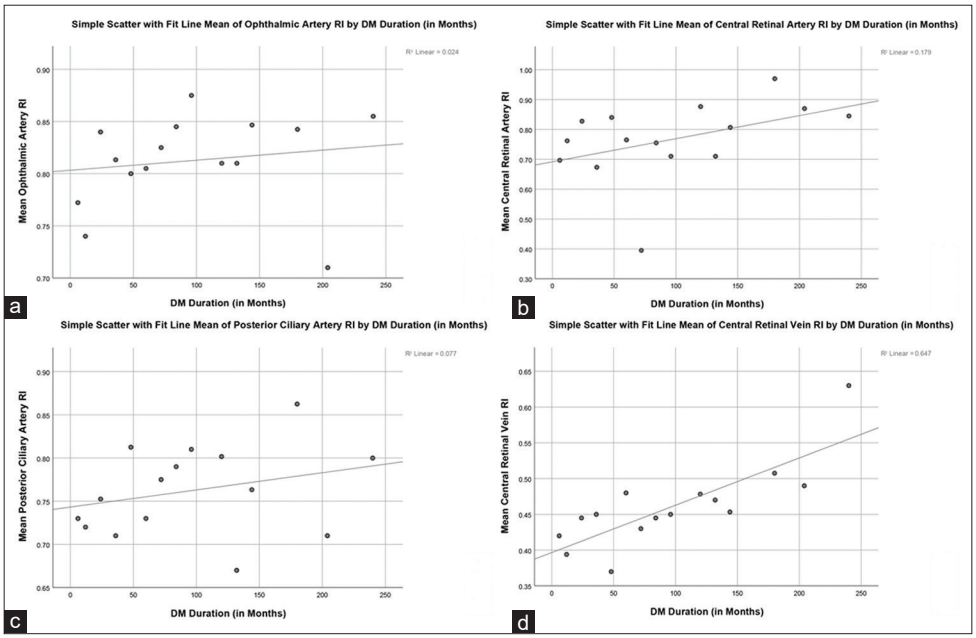


Figure 1: Scatter diagram showing correlation between duration of diabetes and resistive index of (a) ophthalmic artery, (b) central retinal artery, (c) posterior ciliary artery, and (d) central retinal vein

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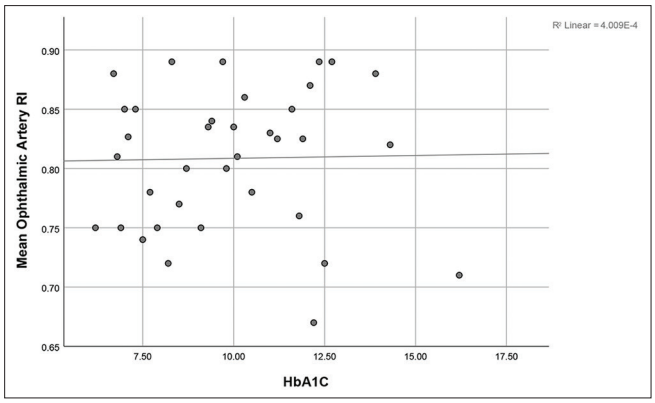


Figure 2: Scatter diagram showing correlation between mean resistive index of ophthalmic artery and glycosylated hemoglobin

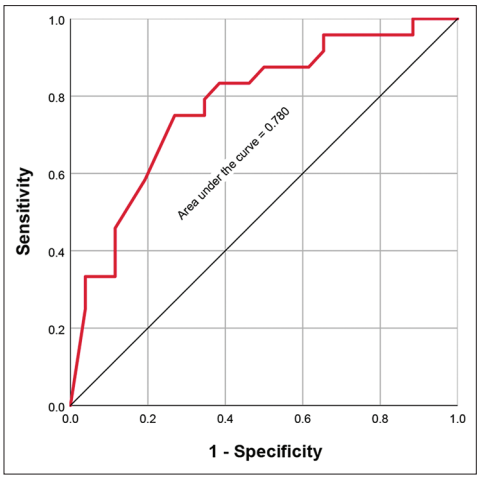


Figure 3: Receiver operating characteristic curve analysis of resistive index of central retinal artery and diabetic retinopathy (area under the curve was 0.780)

erythrocytes contribute to increased peripheral vascular resistance.[13] RI, an indirect measure of vascular resistance, can be useful to identify individuals at higher risk for developing DR. Further, RI, being independent of Doppler angle, has been shown to be reliable and reproducible with least coefficient of variation.[14]

Our study results demonstrate that significant changes occur in the RIs of the orbital vessels in patients with diabetes. The orbital vascular resistance (as reflected by RI) was found to be significantly increased in patients with DR as compared to controls and those in Group 1. Our observations are in agreement with certain other published studies.[15,16]

We observed no significant differences in the mean RI values between controls and those without DR. Our findings are similar to that of Basturk *et al*., who evaluated the RI of the orbital arteries in type 2 diabetics with microalbuminuria. No significant differences were observed in the mean RIs of OA, CRA, and PCA between controls and no DR group.[9]

In contrast, Dimitrova *et al*. have noted higher RI in the PCA in patients with no DR than controls.[17] However, their study included patients with hypertension and longer duration of diabetes than ours. In patients with hypertension, the RI of the PCA is reported to be high and correlates with duration of hypertension.[18] Moreover, CDI was performed in erect posture, which might have influenced the results. Few other investigators have noted increased RI values in the OA and CRA in patients with diabetes but without DR compared to that of controls, suggesting that even before the appearance of overt DR, there is increased resistance to blood flow in the peripheral vascular bed.[19,20]

We found significantly greater RI in the PCA and CRA ($P = 0.03$ and $P < 0.001$, respectively) in patients with DR compared to those without DR. While the inner layers of the retina are perfused by the CRA, the choroidal vasculature (derived from PCA) is responsible for the nourishment of outer retina. The various pathological changes observed in DR, such as loss of pericytes, thickening of the capillary basement membrane, and vascular smooth muscle cells, lead to compromised arteriolar integrity and autoregulation of blood flow. Loss of capillaries is critical to the pathogenesis of progressive retinal ischemia in DR and is a ubiquitous retinal finding from histopathological specimens.[21] Dropout of capillaries could be responsible for the increased vascular resistance observed in the RI of CRA and PCA in patients with DR. Further, the RI of the CRA was found to be a reliable predictive indicator of DR in our study. In this context, our findings are partly in agreement with that of Krasnicki *et al*., who have noted significant differences in the RI of CRA in subjects with DR and concomitant coronary artery disease.[5] The CRA, one of the major feeding vessels of the retina, reflects the identified vascular changes that have been associated with DR more sensitively. It has been suggested that hemodynamics in OA, a medium-sized muscular artery with many branches, is unlikely to be influenced by isolated alterations in blood flow in one of the branches.[22]

However, with progression of DR, the RI of the OA can eventually increase due to ensuing vascular changes related to diabetes in both retinal and choroidal vasculature.

Our finding of positive correlation between RI of all orbital vessels and the duration of diabetes is in concordance with that of Karami *et al*.[23] With prolonged duration of diabetes, alterations in blood vessel diameter and perfusion pressure occur due to disturbed autoregulatory mechanisms, subsequently leading to changes in the resistance of all retrobulbar vessels. We identified a statistically significant correlation between RI of the OA and the levels of HbA1c ($P = 0.001$). Clermont *et al*. have demonstrated reduction in the blood flow to retina with increasing HbA1c in subjects with type 1 diabetes without DR.[24]

Nagaoka *et al*. evaluated subjects with type 2 diabetes for changes in the retinal circulatory parameters using laser Doppler velocimetry and found significantly higher HbA1c in patients with the least retinal blood flow quartile, suggesting that poor glycemic control is associated with lowering of the retinal blood flow.[25]

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Our study has several strengths. None of the patients had any associated vascular diseases such as hypertension. The presence of DR was assessed through seven-field stereo fundus photography for accuracy. A single experienced radiologist performed the CDI studies of the entire study participants, thereby eliminating interobserver variability. All examinations were performed with least pressure on the globe.

Limitations of our study include cross-sectional design and a small sample size. However, we intentionally excluded patients with other comorbid conditions to focus the effects of diabetes on retrobulbar circulation. Subgroup analysis of patients with different stages of retinopathy could not be performed because of limited sample size.

Conclusion

RIs of the orbital arteries are significantly higher in subjects with DR. RI of the orbital vessels can be a potentially useful biomarker in the early diagnosis and follow-up of patients with DR. This may be particularly helpful in patients with opacities in the ocular media.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ozer T, Altin R, Ugurbas SH, Ozer Y, Mahmutyazicioglu K, Kart L. Color Doppler evaluation of the ocular arterial flow changes in chronic obstructive pulmonary disease. Eur J Radiol 2006;57:63-8.
2. Reddy R. Usefulness of color Doppler imaging of orbital arteries in young hypertensive patients. Proc (Bayl Univ Med Cent) 2019;32:514-9.
3. Rokni Yazdi H, Faraji S, Ahmadi F, Shahmirzae R. Color Doppler indices of orbital arterial flow in end-stage renal disease patients; are the changes related to chronic hemodialysis or chronic renal failure? Iran J Radiol 2012;9:12-6.
4. Basturk T, Akcay M, Albayrak R, Unsal A, Ulas T, Koc Y. Correlation between the resistive index values of renal and orbital arteries. Kidney Blood Press Res 2012;35:332-9.
5. Krasnicki P, Dmuchowska DA, Proniewska-Skretek E, Dobrzycki S, Mariak Z. Ocular haemodynamics in patients with type 2 diabetes and coronary artery disease. Br J Ophthalmol 2014;98:675-8.
6. Dimitrova G, Kato S. Color Doppler imaging of retinal diseases. Surv Ophthalmol 2010;55:193-214.
7. Walasik-Szemplińska D, Pauk-Domańska M, Sanocka U, Sudoł-Szopińska I. Doppler imaging of orbital vessels in the assessment of the activity and severity of thyroid-associated orbitopathy. J Ultrason 2015;15:388-97.
8. Khatri M, Saxena S, Kaur A, Bhasker SK, Kumar M, Meyer CH. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retina Vitreous 2018;4:12.
9. Basturk T, Albayrak R, Ulas T, Akcay M, Unsal A, Toksoy M, *et al*. Evaluation of resistive index by color Doppler imaging of orbital arteries in type II diabetes mellitus patients with microalbuminuria. Ren Fail 2012;34:708-12.
10. Ino-ue M, Azumi A, Yamamoto M. Ophthalmic artery blood flow velocity changes in diabetic patients as a manifestation of macroangiopathy. Acta Ophthalmol Scand 2000;78:173-6.
11. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.
12. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye (Lond) 2009;23:1496-508.
13. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008;2:1130-8.
14. Neudorfer M, Kessner R, Goldenberg D, Lavie A, Kessler A. Retrobulbar blood flow changes in eyes with diabetic retinopathy: A 10-year follow-up study. Clin Ophthalmol 2014;8:2325-32.
15. MacKinnon JR, McKillop G, O'Brien C, Swa K, Butt Z, Nelson P. Colour Doppler imaging of the ocular circulation in diabetic retinopathy. Acta Ophthalmol Scand 2000;78:386-9.
16. Gracner T. Ocular blood flow velocity determined by color Doppler imaging in diabetic retinopathy. Ophthalmologica 2004;218:237-42.
17. Dimitrova G, Kato S, Tamaki Y, Yamashita H, Nagahara M, Sakurai M, *et al*. Choroidal circulation in diabetic patients. Eye (Lond) 2001;15:602-7.
18. Akal A, Ulas T, Goncu T, Karakas E, Karakas O, Kurnaz F, *et al*. Evaluation of resistive index using color Doppler imaging of orbital arteries in geriatric patients with hypertension. Indian J Ophthalmol 2014;62:671-4.
19. Arai T, Numata K, Tanaka K, Kiba T, Kawasaki S, Saito T, *et al*. Ocular arterial flow hemodynamics in patients with diabetes mellitus. J Ultrasound Med 1998;17:675-81.
20. Tamaki Y, Nagahara M, Yamashita H, Kikuchi M. Blood velocity in the ophthalmic artery determined by color Doppler imaging in normal subjects and diabetics. Jpn J Ophthalmol 1993;37:385-92.
21. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. Microcirculation 2007;14:25-38.
22. Harris A, Joos K, Kay M, Evans D, Shetty R, Sponsel WE, *et al*. Acute IOP elevation with scleral suction: Effects on retrobulbar haemodynamics. Br J Ophthalmol 1996;80:1055-9.
23. Karami M, Janghorbani M, Dehghani A, Khaksar K, Kaviani A. Orbital Doppler evaluation of blood flow velocities in patients with diabetic retinopathy. Rev Diabet Stud 2012;9:104-11.
24. Clermont AC, Aiello LP, Mori F, Aiello LM, Bursell SE. Vascular endothelial growth factor and severity of nonproliferative diabetic retinopathy mediate retinal hemodynamics *in vivo*: A potential role for vascular endothelial growth factor in the progression of nonproliferative diabetic retinopathy. Am J Ophthalmol 1997;124:433-46.
25. Nagaoka T, Sato E, Takahashi A, Yokota H, Sogawa K, Yoshida A. Impaired retinal circulation in patients with type 2 diabetes mellitus: Retinal laser Doppler velocimetry study. Invest Ophthalmol Vis Sci 2010;51:6729-34.

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Case Report

Pseudotumor Cerebri Complicating Multisystem Inflammatory Syndrome in a Child

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Abstract

Purpose: To report a case of pseudotumor cerebri (PTC) in a child associated with multisystem inflammatory syndrome in children (MIS-C), associated with presumed coronavirus disease 2019.

Methods: A previously healthy 11-year-old female child presented with a 4-day history of fever, headache, vomiting, and loose stools. Laboratory investigations revealed neutrophilic leukocytosis, and markers of inflammation (C-reactive protein, ferritin, and interleukin-6) were significantly elevated. Pharyngeal swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by polymerase chain reaction was negative while anti-SARS-CoV-2 antibody was highly reactive. Ophthalmic evaluation for transient visual obscurations during hospital course revealed swelling of the optic disc in both eyes. Spectral-domain optical coherence tomography and ultrasonography confirmed the ophthalmoscopic findings. There was no neurologic deficit. Magnetic resonance imaging of the brain and magnetic resonance venogram revealed no structural lesion. The opening pressure of cerebrospinal fluid (CSF) was 336 mm of water, and CSF composition was normal.

Results: A diagnosis of PTC associated with MIS-C was made, and the child was treated with oral acetazolamide. Edema of the optic disc regressed following therapy, and the child is under follow-up.

Conclusions: PTC can occur in association with MIS-C. Clinicians need to be aware of this potential neuro-ophthalmic complication in MIS-C. Prompt diagnosis and treatment can prevent visual loss.

Keywords: COVID-19, Multisystem inflammatory syndrome in children, Pseudotumor cerebri

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Introduction

Pseudotumor cerebri syndrome (PTCS) is a disorder characterized by increased intracranial pressure of unclear etiology that carries significant morbidity and limited therapeutic options. PTCS can be primary otherwise called idiopathic intracranial hypertension (IIH) or secondary, attributable to a number of medical conditions and drugs. This neurologic syndrome predominantly affects obese women of reproductive age group, and clinical features include symptoms and signs of raised intracranial pressure with normal cerebrospinal fluid (CSF) composition and no structural abnormality on brain imaging.[1] PTCS is relatively uncommon in children and is associated with variable risk factors and clinical presentation.[2] We report a case of PTCS in a child associated with multisystem inflammatory syndrome in children (MIS-C), temporally associated with coronavirus disease 2019 (COVID-19).

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Case Report

An 11-year-old girl with no significant past medical history was brought to the emergency department with complaints of fever over the past 4 days associated with headache, vomiting,

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abdominal pain, and loose stools. There was associated lethargy but no history of seizures, altered sensorium, or respiratory symptoms. There were no sick contacts. At admission, the child had a temperature of 39°C, heart rate of 112 beats/min, and oxygen saturation of 100% on room air. Body mass index was 16 kg/m^2. Physical examination was unremarkable except for bilateral conjunctival congestion. Investigations revealed decreased hemoglobin, leukocytosis with neutrophilia, and elevated inflammatory markers including ferritin, interleukin-6 (IL-6), and C-reactive protein. Blood and urine cultures were negative, and liver function tests were found to be normal. Nasopharyngeal and oropharyngeal swabs for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time polymerase chain reaction (RT-PCR) were negative. Serologic testing for SARS-CoV-2 antibodies by chemiluminescence immunoassay was highly reactive (observed titer – 212, ≥1 being labeled reactive) suggestive of SARS-CoV-2 infection. Table 1 shows the laboratory parameters. Echocardiogram was normal. On day 3 of admission, the child complained of transient obscurations of vision lasting for a few seconds associated with change in posture, and was referred for ophthalmic consultation.

On examination, uncorrected visual acuity was 20/20, and color vision was normal in both eyes. Anterior segment examination was unremarkable, and pupillary responses were normal. Extraocular movements were full. Fundus examination revealed optic disc swelling in both eyes [Figure 1a]. Neurologic examination was otherwise normal. Visual field evaluation by automated perimetry (Humphrey field analyzer) was found to be unreliable due to extensive fixation losses. Spectral-domain optical coherence tomography (OCT) showed increased thickness of peripapillary retinal nerve fiber layer (RNFL) in both eyes in all the four quadrants confirming edema of the optic disc [Figure 2a]. Magnetic resonance imaging of the brain and magnetic resonance venography (MRV) revealed normal ventricles, brain parenchyma, and venous sinuses. Transorbital ultrasound revealed bulging optic disc in both eyes. The optic nerve sheath diameter (ONSD) measurements obtained 3 mm posterior to the globe were found to be 5.6 mm in the right eye and 6 mm in the left eye [Figure 3a]. Lumbar puncture revealed a CSF opening pressure of 336 mm of water. CSF cultures were negative, and biochemistry was normal.

Since the child was hemodynamically stable and not hypoxic (SpO_2 100% on room air) at admission as well as during hospital course, she was managed with empiric broad-spectrum antibiotic therapy (intravenous ceftriaxone 2 g for 7 days) along with supportive care for fever (paracetamol) and loose stools (intravenous fluid replacement). As serial IL-6 and serum ferritin showed declining trend, the child was treated only with supportive measures. A diagnosis of pseudotumor cerebri (PTC) associated with MIS-C was made, and the child was started on oral acetazolamide 500 mg twice daily. By 4 weeks, headache and transient visual obscurations had subsided. Visual acuity remained 20/20, and fundus examination revealed resolving disc edema. The dose of oral acetazolamide was reduced to 250 mg thrice daily. At her last visit, 7 weeks since her initial presentation, she was found to have visual acuity of 20/20 and resolved disc edema in both eyes [Figure 1b]. Follow-up OCT revealed normalization of peripapillary RNFL thickness [Figure 2b]. The ONSD measurements were found to be 4 mm in both eyes at follow-up [Figure 3b]. The dose of acetazolamide has been tapered further, and she is under follow-up.

Table 1: Laboratory parameters of the patient and reference range

Parameter	Value	Reference range
Hemoglobin (g/dL)	10.5	11.5-15.5
White blood cell count (10^3/μL)	17	5-13
Neutrophil (%)	91.1	45-65
Lymphocyte (%)	7.5	32-52
Erythrocyte sedimentation rate (mm/h)	31	4-12
C-reactive protein (mg/dL)	39	<0.6
Serum ferritin (ng/mL)	352.4	7-140
IL-6 (pg/mL)	197.8	<7
NT-proBNP (pg/mL)	1163	<125
D-dimer (mg/L FEU)	1.48	<0.5
Anti-SARS-CoV-2 titer	212	<1

IL-6: Interleukin-6, NT-proBNP: N-terminal-pro-brain natriuretic peptide, Anti-SARS-CoV-2: Antibody against severe acute respiratory syndrome coronavirus 2

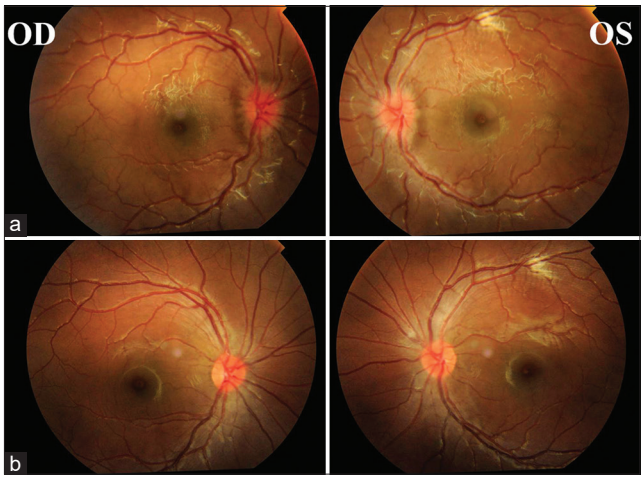


Figure 1: (a) Fundus photograph both eyes showing edema of optic disc. (b) Fundus photograph both eyes showing resolved disc edema at follow-up

Discussion

MIS-C, also called pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2, is a life-threatening condition that can lead to multi-organ dysfunction and long-term sequelae. The clinical features of MIS-C include severe illness necessitating hospitalization, fever, and involvement of two or more organ systems, in combination with laboratory evidence of inflammation and laboratory or epidemiologic evidence of SARS-CoV-2 infection.[3] Our

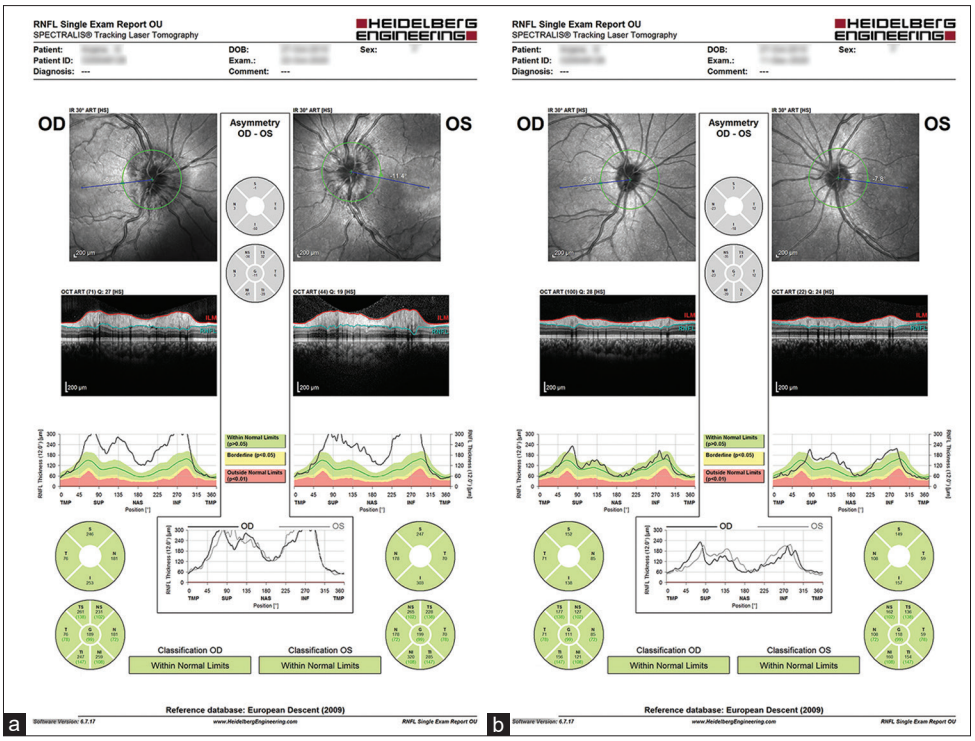


Figure 2: (a) Spectral-domain optical coherence tomography (SD-OCT) showing increased thickness of peripapillary retinal nerve fiber layer (RNFL) in both eyes. (b) SD-OCT both eyes at follow-up showing normalization of peripapillary RNFL thickness

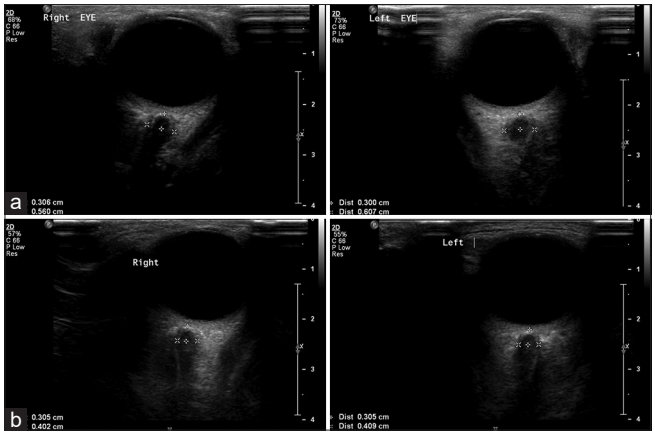


Figure 3: (a) Transorbital ultrasound showing increased optic nerve sheath diameter (ONSD) 3 mm posterior to inner scleral surface; 5.6 mm in the right eye and 6 mm in the left eye. (b) Transorbital ultrasound at follow-up showing normalization of ONSD; 4 mm in both eyes

patient was found to meet all the criteria specified in the Centers for Disease Control and Prevention and World Health Organization case definitions of MIS-C.[4,5]

The pathophysiology of MIS-C is unclear, and possible mechanisms include T-cell or antibody recognition of self-antigens resulting in autoantibodies, immune response against viral antigens expressed on infected cells and formation of immune complexes activating inflammation as well as viral superantigen sequences which activate host immune cells.[6] Dysregulated hyperinflammation is a critical event in the pathogenesis of MIS-C, and this condition is frequently associated with gastrointestinal symptoms and cardiovascular dysfunction.[7]

Although neurologic manifestations have been documented during acute phase of infection with SARS-CoV-2,[8,9] involvement of the central nervous system is uncommon in MIS-C. The neurologic features of MIS-C have been strongly suggested to represent a postinfectious immune response, and manifestations reported include headache, encephalopathy, and aseptic meningitis.[10]

We had not performed RT-PCR for SARS-CoV-2 on CSF since our patient showed evidence of previous infection with SARS-CoV-2 rather than current infection (pharyngeal swab was negative while antibodies to SARS-CoV-2 were present). Only one-third of cases of MIS-C have been reported to be positive by RT-PCR for SARS-CoV-2. The delayed presentation of MIS-C relative to the pandemic curve, a low proportion of cases who were SARS-CoV-2 positive by RT-PCR, and a high proportion who were antibody positive suggest that this inflammatory syndrome is not mediated by direct viral invasion but coincides with the development of acquired immune responses to SARS-CoV-2.[6] Previous studies have noted the absence of SARS-CoV-2 RNA in CSF analysis of COVID-19 patients with neurological symptoms suggesting that indirect mechanisms are responsible for neurologic manifestations in these patients.[11,12]

Although the symptoms of PTCS in children are similar to that of adults, the demographics vary, and children are reported to

have a higher incidence of secondary PTC. A distinct seasonal variation has been reported in the clinical presentation of IIH in prepubertal children, suggesting a possible association between IIH and concurrent infections in this age group.[13] Agraz *et al*. have noted a high incidence of atopy in their study cohort of children with PTC, suggesting that autoimmune component may play a role in pediatric population.[2]

To the best of our knowledge, four similar cases of PTCS associated with MIS-C (two case reports and a case series of two patients) have been reported in literature.[14-16] Although two patients had received therapy with doxycycline and steroids, the short course and recent initiation preclude the possibility of these medications contributing to increased intracranial pressure.

The mechanism behind the occurrence of PTCS in association with MIS-C is unclear. It has been postulated that increased intracranial pressure reflects systemic inflammation related to SARS-CoV-2 infection, resulting in central nervous system effects.[16] It is of interest to note that obesity, the most striking risk factor for PTCS, is associated with dysregulation of several inflammatory cytokines and aberrant glucocorticoid metabolism through manipulation of the enzyme 11-beta-hydroxysteroid dehydrogenase (11β-HSD).[17] 11β-HSD is a bidirectional enzyme that regulates prereceptor corticosteroid availability and glucocorticoid availability in the central nervous system that is one of the factors pivotal to intracranial pressure homeostasis. Among the two isoforms, 11β-HSD1 activates cortisol from cortisone while the isoform 11β-HSD2 inactivates cortisol. It is well established that several inflammatory cytokines such as tumor necrosis factor-α and ILs 1 and 6 are potent activators of 11β-HSD1.[18]

We speculate that PTC associated with MIS-C can be multifactorial, and one another possibility could be aberrant glucocorticoid metabolism as a result of cytokine storm and 11β-HSD1 activity. Increased 11β-HSD1 activity has been shown to influence the pathogenesis of PTC through manipulation of CSF dynamics at the level of choroidal plexus as well as arachnoidal granulations.[19] Further studies are warranted in this regard.

Few cases of isolated intracranial hypertension in association with active SARS-CoV-2 infection have been reported in adults. The proposed pathophysiologic mechanisms for intracranial hypertension include acute encephalitis, venous sinus thrombosis, intracranial venous congestion due to inflammation as a result of SARS-CoV-2 infection, and the associated coagulopathy as well as hyperviscosity state leading to impaired absorption of CSF.[20,21] The absence of clinical features such as altered sensorium/seizures and normal brain parenchyma evident on neuroimaging in our patient ruled out encephalitis. Normal MRV ruled out venous sinus thrombosis in our patient.

The other possible etiologies of swelling of optic disc in the setting of SARS-CoV-2 infection include optic neuritis, ischemic optic neuropathy, papillophlebitis, and retinal vein occlusion. The presence of normal visual acuity and color vision along with normal pupillary responses and the absence of associated retinal hemorrhages or venous tortuosity in our patient made us exclude these causes.

Prompt diagnosis and treatment of PTCS is essential since it can lead to irreversible visual loss. Although the pathophysiology remains unclear, clinicians should be aware of this potential complication with MIS-C. Fundus examination is valuable as part of a multidisciplinary approach toward management of MIS-C.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Friedman DI, Liu GT, Digre KB. Revised diagnostic criteria for the pseudotumor cerebri syndrome in adults and children. Neurology 2013;81:1159-65.
2. Agraz D, Morgan LA, Fouzdar Jain S, Suh DW. Clinical features of pediatric idiopathic intracranial hypertension. Clin Ophthalmol 2019;13:881-6.
3. Nakra NA, Blumberg DA, Herrera-Guerra A, Lakshminrusimha S. Multi-System Inflammatory Syndrome in Children (MIS-C) following SARS-CoV-2 infection: Review of clinical presentation, hypothetical pathogenesis, and proposed management. Children (Basel) 2020;7:69.
4. Centers for Disease Control and Prevention. Emergency Preparedness and Response: Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19). Health Advisory. Available from: https://emergency.cdc.gov/han/2020/han00432.asp. [Last accessed on 2020 Dec 26].
5. World Health Organization. Multisystem Inflammatory Syndrome in Children and Adolescents with COVID-19: Scientific Brief; 2020. Available from: https://www.who.int/publications-detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19. [Last accessed on 2020 Dec 26].
6. Jiang L, Tang K, Levin M, Irfan O, Morris SK, Wilson K, *et al.* COVID-19 and multisystem inflammatory syndrome in children and adolescents. Lancet Infect Dis 2020;20:e276-88.
7. Radia T, Williams N, Agrawal P, Harman K, Weale J, Cook J, *et al.* Multi-system inflammatory syndrome in children & adolescents (MIS-C): A systematic review of clinical features and presentation. Paediatr Respir Rev. 2021;38:51-57.
8. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, *et al.* Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020;77:683-90.
9. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, *et al.* Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18-22.
10. Chen TH. Neurological involvement associated with COVID-19 infection in children. J Neurol Sci 2020;418:117096.
11. Bellon M, Schweblin C, Lambeng N, Cherpillod P, Vazquez J,

Lalive PH, *et al.* Cerebrospinal fluid features in SARS-CoV-2 RT-PCR positive patients. Clin Infect Dis 2020;ciaa1165.
12. Neumann B, Schmidbauer ML, Dimitriadis K, Otto S, Knier B, Niesen WD, *et al.* Cerebrospinal fluid findings in COVID-19 patients with neurological symptoms. J Neurol Sci 2020;418:117090.
13. Distelmaier F, Tibussek D, Schneider DT, Mayatepek E. Seasonal variation and atypical presentation of idiopathic intracranial hypertension in pre-pubertal children. Cephalalgia 2007;27:1261-4.
14. Verkuil LD, Liu GT, Brahma VL, Avery RA. Pseudotumor cerebri syndrome associated with MIS-C: A case report. Lancet 2020;396:532.
15. Parsons E, Timlin M, Starr C, Fries A, Wells R, Studer M, *et al.* MIS-C in February 2020 and implications of genomic sequencing for SARS-CoV-2. J Pediatric Infect Dis Soc 2020;10:695-97.
16. Baccarella A, Linder A, Spencer R, Jonokuchi AJ, King PB, Maldonado-Soto A, *et al.* Increased intracranial pressure in the setting of multisystem inflammatory syndrome in children, associated with COVID-19. Pediatr Neurol 2021;115:48-9.
17. Sinclair AJ, Ball AK, Burdon MA, Clarke CE, Stewart PM, Curnow SJ, *et al.* Exploring the pathogenesis of IIH: An inflammatory perspective. J Neuroimmunol 2008;201-202:212-20.
18. Chapman KE, Coutinho AE, Zhang Z, Kipari T, Savill JS, Seckl JR. Changing glucocorticoid action: 11β-hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. J Steroid Biochem Mol Biol 2013;137:82-92.
19. Sinclair AJ, Walker EA, Burdon MA, van Beek AP, Kema IP, Hughes BA, *et al.* Cerebrospinal fluid corticosteroid levels and cortisol metabolism in patients with idiopathic intracranial hypertension: A link between 11beta-HSD1 and intracranial pressure regulation? J Clin Endocrinol Metab 2010;95:5348-56.
20. Svedung Wettervik T, Kumlien E, Rostami E, Howells T, von Seth M, Velickaite V, *et al.* Intracranial pressure dynamics and cerebral vasomotor reactivity in coronavirus disease 2019 patient with acute encephalitis. Crit Care Explor 2020;2:e0197.
21. Silva MT, Lima MA, Torezani G, Soares CN, Dantas C, Brandão CO, *et al.* Isolated intracranial hypertension associated with COVID-19. Cephalalgia 2020;40:1452-8.

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Astudy ofPrognostic Factors Influencing Visual Outcome inPatients ofIndirect Traumatic Optic Neuropathy

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Abstract:*Aim: To Analyse The Various Prognostic Factors Which Will Influence The Visual Outcome In Patients With Indirect Traumatic Optic Neuropathy. Design: Hospital Based Prospective Study. Materials And Methods: Patients More Than 20 Years Of Age, Admitted In Coimbatore Medical College Hospital With Head Injury And Diagnosed As Indirect Traumatic Optic Neuropathy Underwent Detailed Ophthalmic Evaluation Including Visual Acuity, Anterior Segment Examination, Colour Vision Testing, Visual Field Charting And Fundus Examination. Results And Conclusion: Visual Improvement Was Found To Be Good When Steroids Were Started Within 48 Hours Of Injury. It Was Also Observed That If Visual Improvement Did Not Occur Within 48 Hours Of Initiation Of Steroids, Further Visual Improvement Was Unlikely.An Initial Visual Acuity Of More Than Counting Fingers Was Associated With A Good Visual Prognosis.*
Key Words: Indirect Traumatic Optic Neuropathy, Initial Visual Acuity, Steroids

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I. Introduction

Blindness Due To Trauma Is An Important Menace In The Industrialised World. Trauma Causes Various Manifestations In Eyes. It Can Be A Direct Mechanical Injury To The Eyes Or An Indirect Effect Due To The Transmission Of Forces Following Concussive Blow To Head Or Orbit. Optic Nerve Is An Important Structure Involved In Trauma And The Sequelae Of Optic Nerve Injury Is Severe As It Can Cause Permanent Visual Impairment[1]. Injury To The Optic Nerve Can Be Direct Or Indirect. Direct Injuries To The Optic Nerve Are Caused By Penetrating Trauma And Orbital Fractures.Indirect Injury, Being The More Common Type Is Due To The Transmission Of Force From The Blow To Head Or Orbit[1]. The Rise Of Road Traffic Accidents In The Industrialized World Has Led To An Increase In The Incidence Of Optic Nerve Injuries. There Have Been Various Studies Analysing The Causes Of Optic Nerve Injury And Most Of Them Have Concluded Motor Vehicle Accidents To Be The Most Common Cause Of Optic Nerve Injury[2,3]. The Initial Visual Acuity Is An Important Parameter To Be Assessed As It Influences The Improvement With Treatment. Results From Various Studies Show That The Improvement In Visual Acuity Following Treatment Was Better In Patients With An Initial Visual Acuity Of Perception Of Light Than In Patients With Visual Acuity Of No Perception Of Light.TheDiagnosis And Treatment Of Optic Nerve Injuries Can Be Challenging Especially In Unconscious Or Comatose Patients. Numerous Studies Have Been Published Comparing The Efficacy Of Various Modalities Of Management Of Optic Neuropathy I.E Conservative Management, Steroids Alone, Combination Of Steroids And Surgery, Surgery Alone. This Study Aims To Analyse The Prognostic Factors Influencing Visual Outcome In Patients With Indirect Traumatic Optic Neuropathy.

II. Materials And Methods

This Prospective Study Was Conducted In Patients More Than 20 Years Of Age, Admitted In Coimbatore Medical College Hospital With Head Injury And Diagnosed As Indirect Traumatic Optic Neuropathy During The Period Of August 2014-July 2015. A Total Of 50 Patients Were Included In The Study.
Study Design: HospitalBased Prospective Study

Study Location: Coimbatore Medical College Hospital, Coimbatore, Tamil Nadu, India, Which Is A Tertiary Care Centre And Regional Referral Centre.
Study Duration: August 2014 – July 2015
Sample Size: 50
Sample Selection: Patients To Be Included In The Study Were Selected Among Those Admitted In Trauma Ward With Head Injury. Ophthalmic Examination Was Done And Those Patients Diagnosed As Indirect Traumatic Optic Neuropathy Were Selected For The Study, Based On Inclusion And Exclusion Criteria.

Inclusion Criteria:
1. Patients With Head Injury
2. Age More Than 20 Years
3. Normal Anterior Segment Anatomy
4. Diagnosis Of Indirect Traumatic Optic Neuropathy

Exclusion Criteria:
1. Penetrating Eye Injury And Globe Rupture
2. Blunt Trauma To The Globe
3. Patients With Optic Canal Fracture
4. Past History Of Eye Diseases, Surgery
5. History Of Loss Of Consciousness For More Than 72 Hours
6. History Of Diabetes Mellitus, Hypertension, Cardiac Disorders, Neurological Disorders
7. Patients Not Willing For Regular Follow Up
8. Comatose Patients

III. Procedure Methodology:

After Getting Informed Consent From The Patients In Their Own Language, Detailed History Was Elicited From The Patients Or Their Relatives In Case Of Comatose Patients, Using A Questionnaire Which Included Characteristics Such As Age And Sex Of The Patient; Time And Cause Of Injury; Loss Of Consciousness And Its Duration If Present, With Associated Seizures, ENT Bleed; Immediate Amaurosis Following Injury; Previous Eye Disorders, Refractive Error Or Surgery; Previous Head Injury Or Neurological Disorders; Past History Of Diabetes Mellitus, Systemic Hypertension, Any Other Systemic Disorders.

General Examination Of The Patient Was Done Including Assessment Of Cardiovascular Status And Respiratory Status, BP Measurement, Detailed Neurological Evaluation. Injuries Over The Forehead And Temple Were Noted.

Detailed Ophthalmic Examination Was Done Which Included Uncorrected And Best Corrected Visual Acuity In Both Eyes, Anterior Segment Examination Using Slit Lamp, Detailed Pupillary Examination, Extraocular Movements, Detailed Fundus Examination Using Direct And Indirect Ophthalmoscope, Colour Vision Testing Using Ishihara Chart, And Visual Field Assessment Using Automated Perimetry Or Confrontation Method.

Investigations Done Included Blood Investigations Such As Blood Count, Blood Sugar And Renal Function Tests; Electrocardiogram; X Ray Orbit; CT, MRI Scan Of The Orbit.

After Detailed Evaluation, Patients With Indirect Traumatic Optic Neuropathy Were Started On Treatment With High Dose On Injection IV Methyl Prednisolone, After Ruling Out Contraindications For The Same, And With The Consent Of Neurosurgeon.

The Treatment Regimen Of Steroids Is As Follows:

Injection Methyl Prednisolone 1gm IV OD X 3 Days Followed By Tab. Prednisolone 1mg/Kg/Day Per Oral X 11days. After 11 Days, The Steroids Were Tapered And Stopped.

The Patients Were Monitored By Testing Visual Acuity Using Snellen's Visual Acuity Chart At 24, 48 And 72 Hours After Starting High Dose Methyl Prednisolone Therapy. Patients Were Examined Daily During The First Week With Recording Of Visual Acuity. The Patients Were Followed Up Weekly During The First Month And Monthly Once For Three Months. During Follow Up, Visual Acuity, Anterior Segment Examination, Pupillary Assessment, Fundus Examination Was Done Along With Colour Vision And Visual Field Charting.

Improvement In Best Corrected Visual Acuity By One Line After Treatment, From The Baseline Visual Acuity Was Considered As Improvement In Vision.

Statistical Analysis:

At The End Of The Study Period, The Data Were Subjected To Statistical Analysis. The Differences In Quantitative Variables Between Groups Were Assessed By Unpaired Test. Comparison Between Groups Was

Made By Non-Parametric Mann Whitney Test. A Chi-Square Test Was Used To Assess Differences In Categoric Variables Between Groups.

III. Results

Age Wise Distribution Of The 50 Patients Showed That Majority Of Patients Were In The 20 – 40 Years Age Group, With 30% In 20 – 30 Years Age Group And 24 Patients (48%) Were In 31 – 40 Years Age Group. Patients In 41 – 50 Years Age Group Constituted 14% Of Total Cases, Whereas Only 4 Patients (8%) Were More Than 50 Years Of Age. The Mean Age Of Patients Studied Was 35 Years With Minimum Age Of 20 Years And Maximum Age Of 58 Years.

Table 1: Age Wise Distribution

Age Group	Number Of Patients	Percentage
20 – 30	15	30%
31 – 40	24	48%
41 – 50	7	14%
>50 Years	4	8%
Total	**50**	**100%**

Sex Distribution Showed All Patients In The Study To Be Males, With No Female Patients Being Diagnosed With Indirect Traumatic Optic Neuropathy During The Study Period.

Table 2: Sex Distribution

Gender	Number Of Patients	Percentage
Male	50	100%
Female	0	0%
Total	**50**	**100%**

There Was An Evidence Of Injury Over Forehead In 92% Patients. Among Them Abrasion Was The Most Common Constituting 48% Of The Total. Laceration Was Seen In 16 (32%) Patients And 8% Patients Had No Injury.

Table3: Injury Over Forehead

Injury	Number Of Patients	Percentage
Abrasion	24	48%
Laceration	16	32%
Contusion	6	12%
No Injury	4	8%
Total	**50**	**100%**

Best Corrected Visual Acuity Of Patients Was Assessed Using Snellen's Chart Immediately Following Injury. It Was Found That BCVA Was <6/60 In 38 (76%) Patients. In 17 (34%) Patients, Vision Was In The Range Of 6/60 – CFCF, Whereas 12 (24%) Patients Had Vision Of Hand Movements To Perception Of Light. 9 (18%) Out Of 50 Patients Had No Perception Of Light. Only 4(8%) Patients Had Vision Of 6/36, Whereas 8 (16%) Had Visual Acuity Of 6/60. No Patients Had Visual Acuity Better Than 6/36.

Table 4:BCVA At Presentation

Vision	Number Of Patients	Percentage
6/36	4	8%
6/60	8	16%
<6/60 – CFCF	17	34%
HM – PL	12	24%
No PL	9	18%
Total	**50**	**100%**

All Patients Diagnosed As Indirect Traumatic Optic Neuropathy Were Started On Steroids After Ruling Out Contraindications For The Same. Steroids Were Started In 8(16%) Patients Within A Period Of 12 Hours. In 18(36%) Patients It Was Started Between 12 – 24 Hours Whereas In 15 (30%) Patients It Was Started Between 25 – 48 Hours. Due To Delay In Admission, Steroids Could Be Started Between 49 – 72 Hours In 5(10%) And After 72 Hours In 4 (8%).

Table 5: Interval Between Injury And Starting Treatment

Duration	Number Of Patients	Percentage
<12 Hours	8	16%
12-24 Hours	18	36%

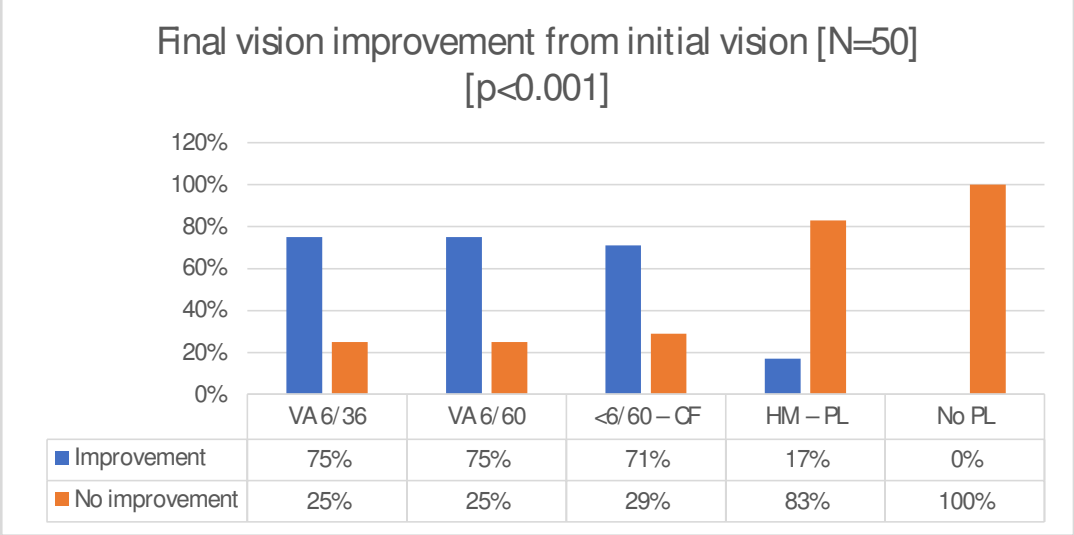
25-48 Hours	15	30%
49-72 Hours	5	10%
>72 Hours	4	8%
Total	**50**	**100%**

Analysis Of Improvement Of Visual Acuity In Patients In Our Study Showed That There Was No Visual Improvement In Patients Who Presented With Immediate Amaurosis Following Injury With No Perception Of Light. Only 2 Among The 12 Patients With Slightly Better Vision Ranging From HM To PL Showed Improvement In Vision Which Is Only 17% Of Patients In This Group. Among Patients With Initial BCVA Of 6/36 And 6/60 There Was Improvement In 75% Of Patients In Each Of These Two Group Of Patients. In The Group Of Patients With Initial BCVA Of 6/60 – CF There Was Improvement In 12 Among 17 Patients. There Was Significant Statistical Analysis Between Initial Visual Acuity And Visual Improvement At 3 Months With P Value Less Than 0.001.

Table6: Association Of Final Visual Improvement (At 3 Months Follow Up) With Initial BCVA

	Visual Acuity At Three Months			
Initial Vision	**Improvement**	**No Improvement**	**Total**	**Percentage**
6/36	3 (75%)	1 (25%)	4	8%
6/60	6 (75%)	2 (25%)	8	16%
<6/60 – CF	12 (71%)	5 (29%)	17	34%
HM – PL	2 (17%)	10 (83%)	12	24%
No PL	0 (0%)	9 (100%)	9	18%
Total	**23**	**27**	**50**	**100%**

Figure1:



Visual Improvement At 3 Months In All Patients Were Compared With Initial Visual Acuity. It Showed That Patients With Initial BCVA Of 6/36 And 6/60 Had Good Improvement Of BCVA Compared With Patients With Initial BCVA Of Less Than 6/60. All Patients With Initial BCVA Of 6/36 Who Improved With Therapy Showed Final BCVA Of 6/6 – 6/18. Final BCVA Improved To 6/6 – 6/36 In All Patients Who Had Initial BCVA Of 6/60 And Showed Improvement With Treatment. Among The 12 Patients Who Had Initial BCVA Ranging From HM – PL There Was Visual Improvement In Only Two Patients In Which One Showed Final BCVA Of 6/36 And The Other Patient Showed 6/60.

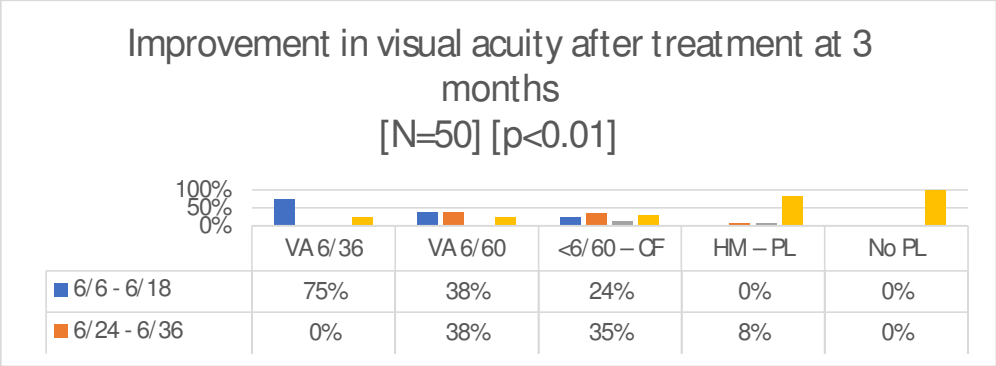
There Was No Visual Improvement In All Patients Without Initial Perception Of Light. The Analysis Showed Very Strong Statistical Correlation Between Baseline Visual Acuity And Final BCVA Achieved At 3 Months With P Value Less Than 0.001.

Table 7: Association Of Final BCVA With Initial Vision

	Visual Acuity At Three Months Follow Up					
Initial Vision	**6/6 – 6/18**	**6/24 – 6/36**	**6/60**	**No Improvement**	**Total**	**Percentage**
VA 6/36	3 (75%)	0	0	1 (25%)	4	8%
VA 6/60	3 (38%)	3 (38%)	0	2 (25%)	8	16%
<6/60 – CF	4 (24%)	6 (35%)	2 (12%)	5 (29%)	17	34%

HM – PL	0	1 (8%)	1 (8%)	10 (83%)	12	24%
No PL	0	0	0	9 (100%)	9	18%
Total	**10**	**10**	**3**	**27**	**50**	**100%**

Figure2:



Patients With Indirect Traumatic Optic Neuropathy Were Treatedwith Full Course Of High Dose Steroids As Per Our Treatment Regimen.PatientsWere Regularly Followed Up And Best Corrected Visual Acuity Was Assessed Regularly. Improvement Of One Line In Snellen's Chart Wasconsidered As Improvement In Visual Acuity. According To That Criteria23 Patients Showed Improvement In BCVA At 3 Months, Constituting46% Of Study Population. But There Was No Visual Improvement In 27(57%) Of Patients In Spite Of Steroid Treatment**.**

Table 8: Improvement Of BCVA With Steroid Treatment

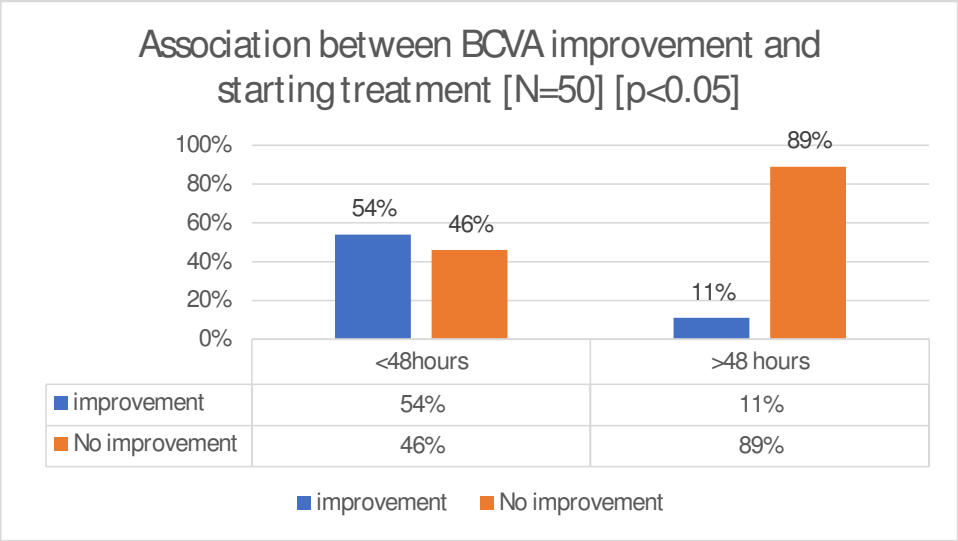
Status	**Number Of Patients**	**Percentage**
Improvement	23	46%
No Improvement	27	54%
Total	**50**	**100%**

There Was A Strong Statistical Association Between Improvement Of Visual Acuity At 3months And Duration Of Starting Steroid Treatment After Injury With P Value Of Less Than 0.05. Among The 41 Patients In Whom Steroid Treatment Was Started Before 48hours, There Was Improvement In 22 Patients Which Account For 54%. But Among The 9 Patients In Whom Steroids Was Started After 48hours There Was Improvement In Only 1 Patient.

Table 9: Association Between Final BCVA Improvement And Duration Of Starting Treatment

	Visual Acuity			
Duration	**Improvement**	**No Improvement**	**TOTAL**	**Percentage**
<48 Hrs	22	19	41	82%
>48 Hrs	1	8	9	18%
TOTAL	**23**	**27**	**50**	**100%**

Figure3:

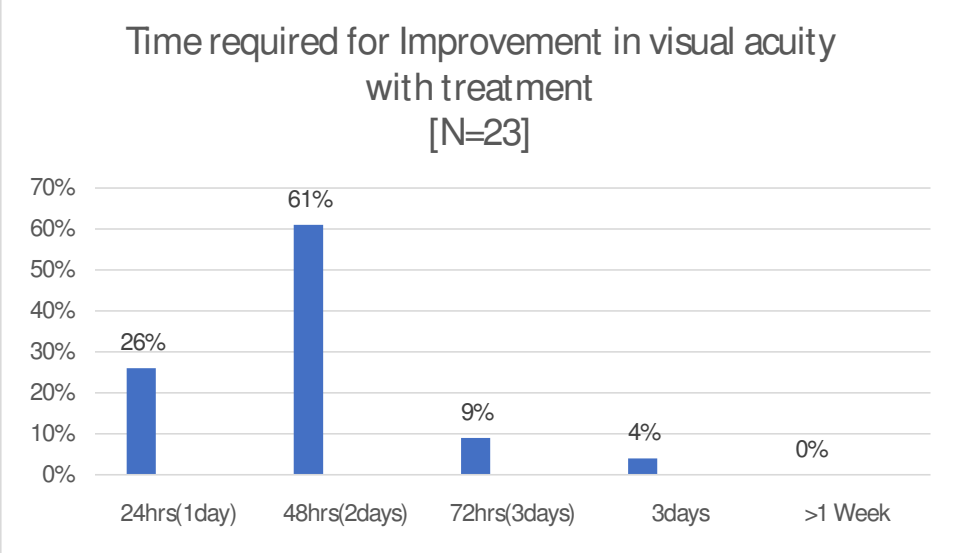


Visual Acuity Improvement Was Assessed Every 24 Hours Following Initiation Of Steroid Treatment In All Patients. Analysis Showed That 6 Among The 23 Patients (26%) Showed Improvement Within First 24 Hours. Majority Of Patients (71%) Among Those With Improved Vision Showed Improvement On The Second Day Of Starting Treatment. Only 2 Patients Showed Improvement On The 3rd Day, While There Was Improvement In Vision In Only One Patient From 3 To 7 Days. Those Patients Who Did Not Show Improvement Of Vision In The First Week Of Starting Treatment Never Showed Improvement After First Week. The Chi Square Test Of This Analysis Showed A P Value Of More Than 0.05 Which Was Statistically Significant.

Table 10: Time Required For Initial BCVA Improvement After Initiation Of Treatment

Duration	**No. Of Patients**	**Percentage**	**Cumulative (%)**
24hrs(1day)	6	26%	26%
48hrs(2days)	14	61%	87%
72hrs(3days)	2	9%	96%
3days	1	4%	100%
>1 Week	0	0%	0%
Total	**23**	**100%**	

Figure 4:



IV. Discussion

Indirect Traumatic Optic Neuropathy Is Due To Closed Head Trauma Which Results In Transmission Of Forces From Forehead To The Optic Nerve In The Optic Canal Or In The Intracranial Portion Of The Optic Nerve. It Causes Severe Visual Morbidity In The Form Of Vision Loss, Field Defects, Colour Vision Defects.

The Diagnosis Of Indirect Traumatic Optic Neuropathy Is Based On The Clinical Finding Of Reduced Visual Acuity With Relative Afferent Pupillary Defect. The Diagnosis Is Mainly Clinical. The Role Of Imaging Is To Rule Out Causes Of Direct Injury To The Optic Nerve Such As Optic Canal Fracture Or Avulsion Of The Optic Nerve.

Treatment Options Of Traumatic Optic Neuropathy Include Conservative Management, Medical Management With High Or Mega Dose Steroids And Surgery. Various Studies Have Shown Variable Results With Different Treatment Options.

A Hospital Based Prospective Study Was Conducted To Analyse The Various Prognostic Factors Affecting The Final Visual Outcome In Patients With Indirect Traumatic Optic Neuropathy. Demographic Profile Showed That All The Patients In Our Study Were Males And The Most Of The Patients Belonged To The Second And Third Decade Of Life. Among The 50 Patients Studied, 78% Belonged To The Age Group Of 20 – 40 Years. Mean Age Of The Patients In Our Study Was 38. This Was Consistent With Other Studies Which Showed A Similar Age And Sex Distribution[4,5]. There Was A Slight Preponderance In The Involvement Of Right Eye, Which Accounted For 56% Of Cases With No Bilateral Involvement.

The Most Common Cause Of Indirect Traumatic Optic Neuropathy In Our Study Was Motor Vehicle Accidents Which Constituted 92% Of Patients. Among Motor Vehicle Accidents, Two-Wheeler Accidents Were The Most Common Constituting 84%. These Findings Were Consistent With The Studies Done By MatsuzakiEt Al[6] And Lee Et Al[5].

Best Corrected Visual Acuity Of All Patients Was Evaluated Following Injury. 76% Of Patients Had BCVA Less Than 6/60 While Only 8% Patients Had BCVA Of 6/36 And 16% Had BCVA Of 6/60. This Observation Was Similar To Other Studies[1].

High Dose Steroid Therapy Was Started In All Patients After Ruling Out Contraindications For The Same. In Conscious Patients, Steroid Therapy Was Started Soon As Possible Following Admission. In 16% Of Patients It Was Started Within 12 Hours. The Delay In Starting Steroid Therapy In Other Patients Was Either Due To Delay In Admission Or Delay In Recovery Of Consciousness. In 8% Of Patients, Steroid Was Started After 72 Hours. All Patients Were Followed Up Daily During The First Week And Periodically Thereafter.

Assessment Of BCVA At 3 Months Showed Visual Improvement In 46% Patients Who Were Started On Steroids. Improvement Of One Line Of Vision In Snellen's Chart Was Considered As Improvement In Visual Acuity. A Study By FujitamiEt Al[7], Levin Et Al[8], And International Optic Nerve Trauma Study[5]also Showed Improvement In Visual Acuity In 44%, 52% And 52% Patients Respectively.

There Was No Statistically Significant Association Between The Age Of The Patient And Final Improvement In Visual Acuity (P=0.563). This Implies That Age Does Not Influence The Final Visual Outcome. This Inference Was Similar To The Study By Wang Et Al[9].

There Was Found To Be No Statistical Significance Between Loss Of Consciousness Following Injury And Improvement In Visual Acuity. Studies Have Shown That Irrespective Of Level Of Consciousness, Patients Showed Improvement In Vision.

The Timing Of Initiation Of Steroids Following Injury Had An Influence On The Visual Outcome In Patients With Indirect Traumatic Optic Neuropathy. Among The 41 Patients Who Were Started On Steroids Within 48 Hours Of Injury, There Was Improvement In BCVA In 54% Of Them As Against 11% Who Were Started On Steroids After 48 Hours On Injury. The Association Between Time Of Initiation Of Steroids And Final Visual Improvement As Statistically Significant With P Value Less Than 0.05. These Results Were Consistent With Studies Done By SteiffEt Al[10] And MaurielloEt Al[11]. This Shows The Importance Of Starting Steroids As Soon As Possible And Possibly Within 48 Hours Of Injury.

Among The 23 Patients Who Improved With Steroids, 20 (40%) Of Them Improved Within 48 Hours Of Starting Steroid Treatment. After 48 Hours There Was Improvement In Only 6% And No Patients Showed Improvement After One Week Of Starting Steroids. There Was A Statistically Significant Association Of Poor Visual Improvement After 48 Hours Of Starting Steroid Therapy ($P<0.05$)[12]. This Result Suggests That Patients With No Improvement Within 48 Hours Of Starting Treatment Need Not Be Treated With Further Steroids. Surgical Decompression Can Also Be Considered In Patients With Indirect Traumatic Optic Neuropathy Who Do Not Show Improvement With Medical Management Within 48 Hours Of Initiation.

There Was A Strong Statistical Association Between Immediate Amaurosis Following Trauma And Absence Of Final Visual Improvement ($P<0.001$)[12]. It Was Also Evident That Patients With Initial Good Vision Had Better Final Visual Recovery Than In Patients With Poor Initial Visual Acuity. The Correlation Of Final Vision Achieved With Initial Visual Acuity Was Highly Statistically Significant ($P<0.001$)[4]. This Result Showed That There Will Be No Improvement In Visual Acuity In Patients With Initial Vision Of No Perception Of Light And The Visual Acuity Improvement Will Be Very Poor In Patients With Initial Vision Of Less Than

Counting Fingers. This Implies That There Is Not Much Use In Treating Patients With Very Poor Initial Vision With High Dose Steroids.

V. Conclusion

ThusitIs Concluded That Indirect Traumatic Optic Neuropathy Has A Better Visual Outcome When Treated With High Dose Systemic Steroid Therapy. Visual Outcome Was Better When The Treatment Was Initiated Within 48 Hours Of Injury. If Response To Treatment Was Not Apparent Within 48 Hours Of Starting Treatment, Improvement Is Unlikely To Occur. An Initial Visual Acuity Of More Than Counting Fingers Was Associated With A Better Visual Prognosis.

References:

[1]. Walsh FB, Hoyt WF. Clinical Neuro-Ophthalmology. (Ed 3, Vol. 3. Baltimore, Williams &Wilkins, 1969)
[2]. Wolin MJ, Lavin PJM: Spontaneous Visual Recovery From Traumatic Optic Neuropathy After Blunt Head Injury. Am J Ophthalmol1990; 109: 430 – 435.
[3]. Edwards P, Arango M, Balica L, Et Al: Final Results Of MRC CRASH, A Trial Of Intravenous Corticosteroid In Adults With Head Injury Outcomes At 6 Months. Lancet 2005; 365: 1957 – 1959
[4]. Sundeep, H Niveditha, Nivedhitha Nikhil, B V Vinutha. "Visual Outcome Of Traumatic Optic Neuropathy In Patients Treated With Intravenous Methylprednisolone". Int J Sci Stud; 2(3) 2014;67-70.
[5]. Lee KF, Muhd Nor NI, Yaakub A, Wan Hitam WH. Traumatic Optic Neuropathy: A Reviewof 24 Patients.Int J Ophthalmol3(2)2010: 175-178
[6]. Matsuzaki H, Kunita M, Kawai K: Optic Nerve Damage In Head Trauma: Clinical And Experimental Studies. Jpn J OphthalmolVol: 26, 1982; 447 – 461
[7]. Fujitami T, Inoue K, Takahashi T, Et Al: Indirect Traumatic Optic Neuropathy –Visual Outcome Of Operative And Nonoperative Cases, Jpn J Ophthalmol Vol:30, 1986;125 – 134
[8]. Levin,L.A.,BeckRW,JosephMP,Et Al., The Treatment Of Optic Neuropathy: The International Optic Nerve Trauma Study. Ophthalmology,106(7):1999 P.1268-77.
[9]. Wang BH, Robertson BC, Girotto JA Et Al: Traumatic Optic Neuropathy: A Review Of 61 Patients. PlastReconstrSurg 1072001:1655 - 1664
[10]. SeiffSR.HighDose Corticosteroids For Treatment Of Vision Loss Due To Indirect Injury To The Optic Nerve.OphthalmicSurg211990:389–395.
[11]. Mauriello JA, Deluca J, Krieger A Et Al. Management Of Traumatic Optic Neuropathy: A Study Of 23 Patients. Br J Ophthalmol76; 1992;349–352.
[12]. Carta A, Ferrigno L, Salvo M, Bianchi-Marzoli S, Boschi A, Carta F.Visual Prognosis After Indirect Traumatic Optic Neuropathy. J.NeuralNeurosurg Psychiatry74; 2003:246-248.

An analytical study on the epidemiology of pterygium in a tertiary care centre

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Abstract

Aim: To study and analyse the epidemiology of pterygium in a tertiary care centre. **Design:** Cross sectional observational study. **Materials and methods:** The patients more than 20 years of age, with true pterygium attending the outpatient clinic of Department of Ophthalmology of Coimbatore Medical College Hospital were included in the study. Data collection was done using a structured questionnaire which comprises of socio-demographic characteristics including age, sex, occupation and detailed history. The exact hours of sun exposure per day and the years of sun exposure were noted as well as any other ocular or systemic co morbid conditions like diabetes and hypertension. A complete ocular examination was done and recording of the uncorrected and best corrected visual acuity along with the degree of astigmatism was done with the help of automated keratometry. Tear film was assessed with Schirmer's test and tear film break-up time. **Results and conclusion:** Middle aged to elderly people were found to be more affected due to their prolonged exposure to inciting agents in the environment. There was no significant gender predilection, if the environmental factors are the same. Sunlight exposure was found to be a significant risk factor and dry eye played a contributory role. No co relation was found between development of pterygium and systemic diseases like diabetes and hypertension.

Key Word: true pterygium, sunlight, dry eye, astigmatism.

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INTRODUCTION

Pterygium is one of the common ocular conditions found in middle aged and elderly individuals. It is a benign, degenerative and proliferative condition of conjunctiva and subconjunctival tissue, with potential to cause visual disturbances[10] It can be easily diagnosed and staged by clinical examination. The most important factor related to the development of pterygium is thought to be sunlight exposure[2,5]. Other contributing factors include dry eyes, smoking, prolonged outdoor activities, low socioeconomic status and high altitude[7]. Major complication is the obscuration of visual axis if it grows into the centre of the cornea. The development of irregular astigmatism due to flattening of the cornea in the horizontal meridian is another cause of decrease in vision[9]. Proper understanding of the epidemiology and risk factors of this condition is essential for planning appropriate measures to prevent its occurrence and thereby reducing the physical and financial burden to the society. This study aims to identify the risk factors of pterygium and specific associations and correlations if any.

MATERIALS AND METHODS

This cross-sectional observational study was done in patients more than 20 years of age, with true pterygium presenting to the outpatient clinic of Department of Ophthalmology, Coimbatore Medical College Hospital

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during the period of one year. A total of 104 patients were included in the study.

Criteria for patient selection were as follows:

Inclusion criteria

- People of age group >20 years
- True pterygium
- Patients with other comorbid conditions like diabetes and hypertension
- Patients with known dry eye syndrome

Exclusion criteria

- Pediatric patients
- Patients with fundus pathology
- Glaucoma patient
- Pseudopterygium
- Injuries of eye
- Suspected ocular surface squamous neoplasia
- The patients who had asymmetrical pterygium in both eyes

METHODOLOGY

After getting informed consent, data collection was done using a structured questionnaire which comprises of socio-demographic characteristics including age, sex, occupation and detailed history. The exact hours of sun exposure per day and the years of sun exposure were noted as well as any other ocular or systemic co morbid conditions like diabetes and hypertension. Patients were grouped into outdoor and indoor workers according to the nature of their occupation. They were categorized as rural and urban population depending on their hometown. Previous history of trauma, smoking habits and alcohol intake were also documented. For the purpose of analysis, the patients coming from areas which were higher than 1000 meters from the sea level were considered as high altitude and less than 1000 considered as low altitude. During symptoms assessment, care was taken to find out if patient had any dry eye symptoms like irritation and burning sensation. A complete ocular examination was done and recording of the uncorrected and best corrected visual acuity along with the degree of astigmatism was done with the help of automated keratometry. Astigmatism was recorded as with the rule, against the rule or oblique astigmatism. For the purpose of analysis pterygium was considered in one eye of bilateral cases. The asymmetric bilateral cases were excluded from the study. Detailed slit lamp examination to find the stage and measurement of the pterygium was done. It was classified as stage I- restricted to limbus, stage 2- marginally invade the cornea, stage 3- between limbus and pupillary margin and stage 4- central to the pupillary margin. Pterygium was classified as regressing, intermediate or fleshy pterygium according to Tan *et al* classification [11] according to the visibility of episcleral vessels. The recording of the position of pterygium was also done as nasal, temporal or double pterygium as well as whether the pterygium was unilateral or bilateral. The tear film assessment was done with the help of Schirmer's test and tear film break up time (TBUT). Data analysis was performed using statistical software package SPSS version 22.0. Both descriptive and inferential statistics were used. The comparison of continuous variable across different subgroups was done using ONE WAY ANOVA. The comparison of categorical and ordinal variables across different subgroups were done using chi square test. Correlation analysis was done by estimating Pearson estimation co-efficient. P value of less than 0.05 was considered significant.

RESULTS

Age of the patients ranged from 27 years to 78 years. The mean age of the study population is 51.5 years, the standard deviation being 13.5 years (Table 1). Majority of the patients belonged to the age group of 46- 55 (n=28, 26.92%). None of the patients were below 25 years of age. Females were more in the study group (n=70, 67%) (Table 2).Majority of patients were engaged in outdoor works (n=82, 9%) (Table 3).Most of the subjects were from low altitude areas (n=73, 70%) (Table 4).Pterygium was unilateral in 54 patients and bilateral in 50 (Table 5).In 43.27% of patients, pterygium was of the regressive type, which was the most common. The next common type was intermediate followed by fleshy type (Table 6). Mean sun exposure hours was 5.7 hours per day (SD.2.7hours), Mean years of sun exposure was 27.7 years (SD 11.7) (Table 8).The patients engaged in indoor working had less than 4 hours of sun xposure per day and those working outdoor had 4-9 hours of exposure per day.77.9 % (n=81) had significant sun exposure per day (>4 hours).63.5% (n=66) were exposed to long duration of sunlight for more than 20 years. Duration of sun exposure is not significantly different across types (Kruskal Wallis test, $P = 0.087$). But it is close to the significance (Table 9). Years of sun exposure is significantly higher in the fleshy type in comparison to regressive ($p<0.01$) and intermediate types ($P<0.01$) (One-way ANOVA overall, $P = 0.0008$) (Table 10) Proportion of the patients with dry eyes as a risk factor was not significantly different across the various types (Chi square test for trend $P = 0.79$) (Table 11 and Table 12) More number of patients were found to have with the rule astigmatism (Table 13).

Table 1: Age distribution of study participants

Sl No	Age group	Frequency	Percentage
1	26-35	15	14.42
2	36-45	22	21.15
3	46-55	28	26.92
4	56-65	21	20.19
5	>65	18	17.31
	Total	104	100.00

Figure 1: Age distribution

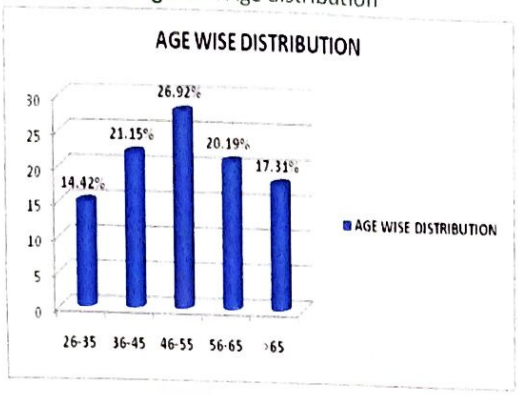


Table 2: Gender Distribution

Sl no	Sex	Frequency (n)	Percentage (%)
1	Male	34	33
2	Female	70	67
3	Total	104	100

Table 3: Occupation

Sl no	Occupation	Frequency (n)	Percentage (%)
1	Indoor	22	21
2	Outdoor	82	79
3	Total	104	100

Table 4: altitude

Sr no	Altitude	Frequency(n)	Percentage(%)
1	Low(1000 Meters)	73	70
2	High(>1000 meters)	31	30
3	Total	104	100

Table 5: Laterality of pterygium

Sl no	Laterality	Frequency	Percentage
1	Unilateral	54	51.9
2	Bilateral	50	48.1
3	Total	104	100

Table 6: Type of pterygium

Type of pterygium	Frequency	Percentage
Regressive	45	43.27
Intermediate	37	35.58
Fleshy	22	21.15
Total	104	100

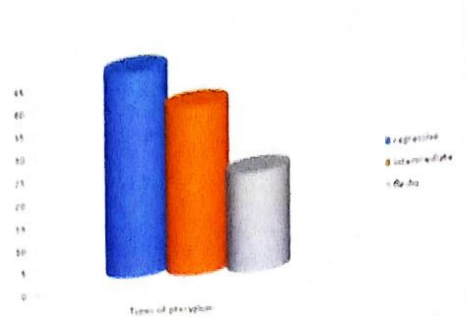


Figure 2: Type of pterygium

Table 7: Stage of pterygium

Stage	Frequency	Percentage
1	4	3.85
2	37	35.58
3	60	57.69
4	3	2.88
Total	104	100

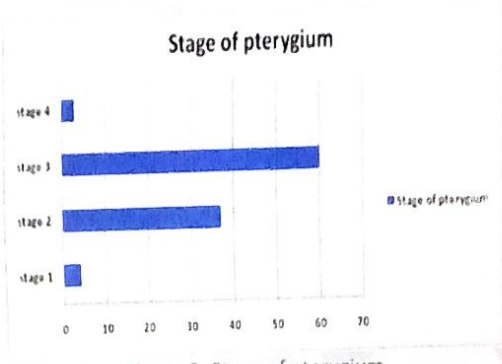


Figure 3: Stage of pterygium

Table 8: Duration of sun exposure

		Frequency	Percentage
Hours of sun exposure	<=4 hours per day	23	22.1
	>4 hours per day	81	77.9
Years of sun exposure	<=20 years	38	36.5
	>20 years	66	63.5

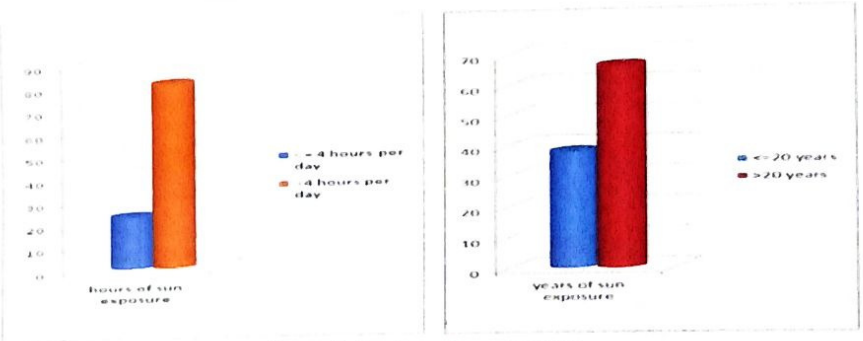


Figure 4: Duration of sun exposure

Table 9: Duration of sun exposure and type of pterygium

Type of Pterygium	Regressive	Intermediate	Fleshy
Sun exposure in hours (Median – IQR)	6 (6-7)	6 (2.5-7.5)	7 (6-8)

Table 10: Years of sun exposure and type of pterygium

Type of Pterygium	Regressive	Intermediate	Fleshy
Sun exposure in years (Mean ± SD)	24.38 ± 12.20	24.68 ± 8.94	34.77 ± 11.28**

Table 11: Dry eyes and type of Pterygium

Type of Pterygium	Regressive	Intermediate	Fleshy
Proportion of patients with dry eyes	7/45	10/37	2/22

Table 12: Dry eye

Sl no	Dry eye	Frequency	Percentage
1	No dry eye	85	81.73
2	Dry eye	19	18.27
3	Total	104	100

Table 13: Type of astigmatism

Sl no	Astigmatism type	Frequency	Percentage
1	No astigmatism	27	25.96
2	With the rule	40	38.46
3	Against the rule	11	10.58
4	Oblique	26	25.00

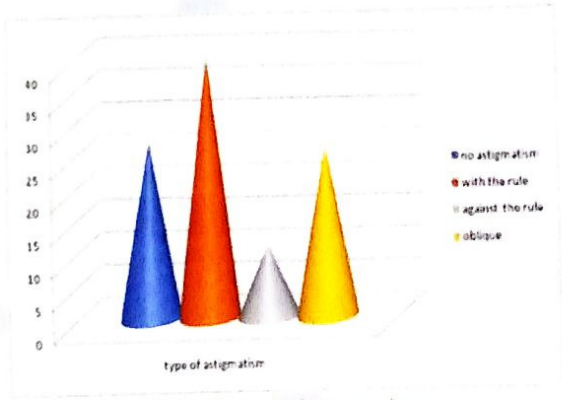


Figure 9: Type of astigmatism

DISCUSSION

Our study included 104 subjects who were diagnosed to have true pterygium in either or both of the eyes. The mean age of the patients in our study was 51.5 years with a standard deviation of 13.5 years, which is comparable to previous studies. Youngest patient was 27 years and the oldest 78 years. 15 patients (14.42%) were in the age group 26-35, 22 (21.15%) in the age group 36-45, 28(26.92%) in the age group 46-55, 21(20.19%) in the age group 56-65 and 18 (17.31%) in the age group >65 years, in our study. Majority of the patients presented in the age group of 45 to 55 years (n=28, 26.92%) Females were predominant in our population (n=70, 67%). Most of the previous studies have shown an increased incidence of pterygium in males, as men were commonly involved in outdoor works.[2] Our study population consisted of the people coming from low socioeconomic conditions who were ladies involved in outdoor occupation. This may account for the increased number of female pterygium cases in our study. The association of UV light with occurrence of pterygium is well documented in previous studies.[3,4,5]. Mean sun exposed hours were 5.7 hours per day with a standard deviation of 2.7 hours and mean year of sun exposure was 27.7 years with a standard deviation of 11.7 years. The average daily duration of sun exposure was not significantly different across types of pterygium (p=0.087). However, the years of sun exposure (p=0.008) and cumulative hours of sun exposure (p=0.002) were significantly higher in the fleshy type. Most of the patients in our study were from the rural area (n=74, 70.2%). Rural area and low socioeconomic status are associated with more amount of outdoor work and more UV exposure. These people will have low level of education and may not be aware of the harmful effects of the UV light and thus don't adopt protective measures such as hats and glasses [6] It is well documented that people living in high altitude have higher chance developing pterygium because of the higher UV light

exposure along with dry eye.[7,8] In our study no significant association between high altitude and pterygium were found probably because of the low sample size. We could not find any significant association between diabetes (p=0.52) and hypertension (p=0.75) with the type of the pterygium, in our study. No such correlation was documented in other studies also. The diabetic patients have more chance of developing tear film instability thereby making the eye more vulnerable to UV damage. Dry eye was found to be present in 19 patients (18.27%). Even though dry eye may be a risk factor for pterygium, statistical correlation could not be established in our study due to the small sample size (p=0.79). Out of 104 patients 77 patients (74.04%) had astigmatism among which maximum were having with the rule astigmatism (n=40, 38.46%). This is in accordance with the other studies[9].

CONCLUSION

The objective of our study was to document the demography and clinical assessment of pterygium and to identify risk factors or associations, if any. The following conclusions were made. Middle aged to elderly people are more affected due to their prolonged exposure to inciting agents in the environment. There is no significant gender predilection, if the environmental factors are the same. UV exposure was a significant risk factor in the development of pterygium. Patients working outdoors should be counselled about the importance of wearing protective eye gears. It should be emphasized that such protective measures can not only delay the onset but also the progression of pterygium to some extent. Adequate management of asymptomatic dry eye is mandatory especially in patients working outdoors, as it can play a contributory role and hence the importance of dry eye evaluation in all cases of pterygium. Though co morbid conditions like hypertension and diabetes do not play any role in the initiation and progression of pterygium, they can still contribute further to the visual morbidity. Proper understanding of the epidemiology and risk factors of this condition is essential for planning appropriate measures to prevent its occurrence and thereby reduce the physical and financial burden to the society.

REFERENCES

1. Tan DT, Chee SP, Dear KB, Lim AS. Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. Arch Ophthalmol Chic Ill 1960. 1997 Oct;115(10):1235–40
2. Hilgers JH. Pterygium: its incidence, heredity and etiology. Am J Ophthalmol. 1960 Oct; 50: 635–44.
3. M Cameron. pterygium throughout the world.
4. Mackenzie FD, Hirst LW, Battistutta D, Green A. Risk analysis in the development of pterygia. Ophthalmology. 1992 Jul; 99(7):1056–61.
5. Moran DJ, Hollows FC. Pterygium and ultraviolet radiation: a positive correlation. Br J Ophthalmol. 1984 May;68(5):343–6
6. Lee YB, Kim SY, Park YG, Han KD, Kim J-W, Chae HS, *et al*. Evaluation of socioeconomic status as a risk factor of pterygium using the Korean National Health and Nutrition Examination Survey 2010 to 2011. Medicine (Baltimore) [Internet]. 2017 Mar 24; 96(11). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5369921/
7. Saw SM, Banerjee K, Tan D. Risk factors for the development of pterygium in Singapore: a hospital-based case-control study. ActaOphthalmol Scand. 2000 Apr; 78(2):216–20.
8. Gupta N, Prasad I, Himashree G, D'Souza P. Prevalence of dry eye at high altitude: a case controlled comparative study. High Alt Med Biol. 2008; 9(4):327–34.
9. Maheshwari S. Pterygium-induced corneal refractive changes. Indian J Ophthalmol. 2007;55(5):383–6
10. Duke-Elder-Diseases of outer eye195, 8: 573-585.
11. Chui J, Di Girolamo N, Wakefield D, Coroneo MT. The pathogenesis of pterygium: current concepts and their therapeutic implications. Ocul Surf. 2008 Jan; 6(1):24–43.

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Original research article

Sight Threatening Ocular Complications in Dengue fever -A Prospective Study

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Abstract:

Aim: To report cases of sight threatening ophthalmic complications observed during dengue epidemic in 2017 at a tertiary health center in Tamil Nadu.

Settings and Design: Tertiary Care Referral Center in Tamil Nadu. Prospective, single institution, Observational case-series.

Methods: Five patients with dengue fever with grave ophthalmic complications seen in fever intensive care ward in our center during the period of dengue epidemic in 2017 were included.

Results: Out of 3338 dengue positive cases reported in our center from June-2017 to December-2017, five cases presented with grave eye findings with loss of vision / loss of the eye in due course.All presented with a short history of fever with thrombocytopenia, with ocular manifestations namely 1.Retro-bulbar hemorrhage with hyphema-ended with globe luxation, 2.Suprachoroidal hemorrhage with globe rupture, 3.Endogenous Endophthalmitis with ring abscess going for Panophthalmitis, 4.Premacular hemorrhage resolved with some improvement in vision after hyaloidotomy, 5.Massive suprachoroidal hemorrhage.

Conclusion: Dengue hemorrhagic fever itself is a dreadful and sometimes fatal disease, in which we encountered few patients with very rare and severe ocular manifestations during the dengue epidemic in our hospital in 2017.Some of these complications have not been reported so far.

Keywords: Dengue epidemic,dengue eye complications, dengue fever, globe rupture, luxated globe, thrombocytopenia.

INTRODUCTION

Dengue fever is a mosquito-borne tropical disease caused by the dengue virus[1]. It is an RNA virus of the family Flaviviridae, genus Flavivirus. The virus has four different types, where infection with one type usually gives lifelong immunity to that type, but a short-term immunity to the other types. Subsequently infection with a different type increases the risk of severe complications. Dengue is spread by the bite of several fresh water breeding species of mosquito of the Aedes type, principally A. Aegypti [1].

Dengue fever usually presents with an acute onset of fever associated with malaise, rhinitis, sore throat and cough, headache, muscle ache, retro-bulbar pain, joint pain, abdominal discomfort and rash[1,3]. Other clinical features of dengue are bleeding manifestations attributed to thrombocytopenia. Dengue fever is confirmed with laboratory tests based on the duration of presentation; polymerase chain reaction

(PCR) is the frequently used test during the early phase of the infection, when fever is within 5 days. If fever exceeds 5 days, the preferred tests are dengue IgM and IgG tests [2].

Dengue is a self-limiting infection. But sequential infections with other serotypes may increase the risk of serious systemic disease, such as dengue hemorrhagic fever or dengue shock syndrome, which are life-threatening[3,4]. Herein we report few grave ocular manifestations associated with dengue infection. The precise pathophysiologic mechanism of dengue ocular complications is not yet well understood[5]; however, many studies have alluded to the possibility of an immune-mediated process as a likely mechanism[6].

Materials and methods

This study is a prospective review of five consecutive sight threatening ocular complications observed during the dengue epidemic period, June-2017 to December-2017 in a tertiary institute in Tamil nadu. Out of 5652 blood samples collected from patients with fever with thrombocytopenia, 3338 were diagnosed with dengue infection by dengue IgM ELISA and NS1 antigen tests. Among them five patients presented with serious ocular manifestations which are reported here.

Case 2.1: Retrobulbar hemorrhage with globe luxation:A 23 years old male presented with fever with cough and loose stools for 5 days and was found to have thrombocytopenia. His dengue IgM was positive. He developed pain Left eye on 6th day, with severe deterioration of vision. On examination patient's general condition was found to be very poor and ocular examination showed vision in Right eye-6/6, Left eye-no perception of light. Right eye-anterior and posterior segment were normal, Left eye was proptosed and the globe luxated outside the palpebral aperture, lids were edematous and retracted,hemorrhagic chemosis present with dry and lustreless cornea. Further details not visualized due to hyphema in anterior chamber. All extraocular movements were restricted [Fig.1]. Intra-ocular pressure in left eye was digitally elevated. Day-by-day his symptoms worsened. MRI brain and orbit was reported as vitreous hemorrhage and subretinal hemorrhage and retinal detachment with Retrobulbar hemorrhage and temporal fossa hematoma [Fig.2].



Figures 1 and 2: Patient with luxated globe Left eye with temporal fossa hematoma.

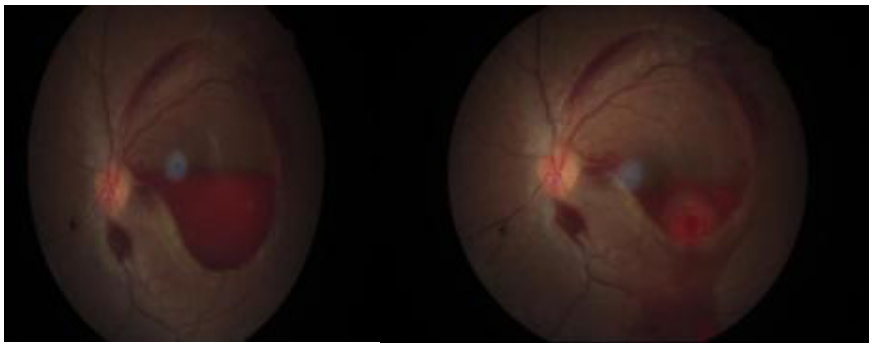
Case 2.2: Suprachoroidal hemorrhage with globe rupture:A 75 years old female patient with history of fever for 2 days with thrombocytopenia was admitted in fever intensive care ward. Dengue NS1Ag

was positive. Patient had complaints of defective vision and pain in Left eye. On examination of Left eye vision was perception of light, mild lid edema, chemosis, mild shallow anterior chamber, pupil-3 mm, sluggishly reacting to light with hazy fundus view. Intra-ocular tension raised digitally [Fig.3]. Next day patient's vision in Left eye further deteriorated to no perception of light and anterior chamber was very shallow.Clinically diagnosed as suprachoroidal hemorrhage, which was confirmed by B-scan. During the early hours of next morning,patient had excruciating pain with excessive bleeding from the Left eye. Immediate examination revealed rupture of the globe Left eye [Fig.4).



Figures 3 and 4:(3) Left eye with chemosis on day 1 (4) Left eye globe rupture at the limbus {white arrow} with prolapse of uveal tissue and vitreous on day 2.

Case 2.3: Sub-hyaloid premacular hemorrhage: A 23 years old male presented with fever for 10 days with thrombocytopenia and dengue IgM was positive. Patient developed painless defective vision in both eyes over the last 5 days. On examination,vision in Right eye was 6/36, Left eye was 3/60 with mild conjunctival congestion, anterior chamber was mild shallow with 3 mm pupil, reacting to light. Fundus of both eye showed sub-hyaloid premacular hemorrhage [Fig.5]. Nd:Yag laser hyaloidotomy was performed in each eye[Fig.6]. Later patient's vision improved to 6/9 in each eye.



Figures 5 and 6:(5) Left eye premacular subhyaloid hemorrhage (6) Left eye hemorrhage draining after hyaloidotomy.

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Case 2.4: Endogenous Endophthalmitis: A 25 years old male admitted in fever ward with complaints of fever for 6 days with thrombocytopenia. Dengue IgM was positive. Patient developed painful loss of vision in Left eye with history of vomiting. On examination patient was toxic, vision in Left eye was no projection of light, mild lid edema with hemorrhagic chemosis and ring abscess in the cornea with keratic precipitates over back of the cornea, anterior chamber showing flare and cells and other details could not be made out [Fig.7]. Extra-ocular movements were painful and restricted. B-scan Left eye revealed thickening of sclero-choroidoretinal complex and few echoes in the vitreous cavity suggestive of Endogenous Endophthalmitis going to Panophthalmitis [Fig.8]. Grave visual prognosis was explained to the patient and patient was lost for further follow-up.



Figures 7 and 8: (7) Left eye with hemorrhagic chemosis with corneal ring abscess (8) B-scan of Left eye showing sclero-choroidoretinal complex thickening with echoes in vitreous.

Case 2.5 : Massive suprachoroidal hemorrhage : A 23 years old male referred from secondary center as fever for 5 days with thrombocytopenia admitted in intensive care unit. Dengue NS1Ag was positive. Patient's general condition was very poor. On examination patient was unconscious. Right eye showed mild hazy cornea and shallow anterior chamber with relative affarent pupillary defect and intra-ocular pressure was raised (27.3 mmHg by Schiotz tonometer) [Fig.9]. Left eye anterior segment was normal with fundus showing a Roth spot. When we reviewed the case 6 hours later, Right eye showed worsening of all signs viz. lid edema with ecchymosis, hemorrhagic chemosis, cornea became very hazy with Descemet's folds, more shallowing of anterior chamber and pupil now 3 mm and non-reacting to light [Fig.10]. A clinical diagnosis of massive suprachoroidal hemorrhage was made. CT-Brain showed right intracerebral hemorrhage with fronto-parietal edema. Patient deteriorated and declared dead after 1 hour the same day.

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Figures 9 and 10: (9) Right eye with mild hazy cornea and shallow anterior chamber (10) Right eye with hemorrhagic chemosis.

Results

Out of five patients observed 4 were male and 1 was a female patient. Left eye alone was affected in 3 patients and both eyes were affected in 2 patients with left eye affected more than right eye. All had a short history of fever with thrombocytopenia, ranging from 3 to 10 days duration and got treated in fever intensive care unit specifically created for treatment of fever cases during the dengue epidemic. The diagnosis of dengue fever was confirmed by dengue IgM and NS1Ag. Predominant eye symptom was defective vision with or without eye pain presented after 5 days of onset of dengue hemorrhagic fever. All five Ophthalmic manifestations were sight threatening. These ocular presentations often correspond to the nadir of thrombocytopenia. Two patients developed suprachoroidal hemorrhage,resulted with rupture globe in the one who survived the course of the disease. One patient developed Retrobulbar hemorrhage with luxated globe. Another patient developed Endogenous Endophthalmitis going to Panophthalmitis. The last patient had premacular subhyaloid hemorrhage which was intervened at right time and the vision was restored.

Discussion

Dengue fever is endemic in tropical countries which is classified by WHO as neglected tropical disease (NTDs)[7]. It can manifest as simple **dengue fever** which is self-limiting and recovers in two-seven days. In a small proportion of cases, due to subsequent infection by other subtypes, the disease develops into a life-threatening **dengue hemorrhagic fever** with low blood platelets levels with bleeding manifestations and blood plasma leakage or into **dengue shock syndrome** where dangerously low blood pressure occurs[4]. The infection typically affects young immunocompetent adults, who often present at the nadir of thrombocytopenia with a myriad of ocular complications relate to dengue infection like sub-conjunctival hemorrhage, uveitis, shallow anterior chamber due to choroidal effusion or angle closure glaucoma, intra-retinal hemorrhages, dengue-related maculopathy, optic neuropathy, vitreous hemorrhage, vascular occlusions[8]. Endogenous Endophthalmitis[9] and Panophthalmitis[10,11]rarely documented. The mechanism behind dengue infection and ocular involvement is unknown, but related to an immune-mediated process and possibly associated with dengue serotyping. Till now the prognosis for the Ophthalmic complications in documented literature is reported to be good with resolution of ocular signs and improved visual acuity in most patients without treatment following dengue infection[5] with

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occasional reports of Endogenous Endophthalmitis[5,9]. So the authors here propose to create awareness regarding the possibility of few rare sight threatening complications in patients with dengue fever with thrombocytopenia.

Conclusion

Physician are aware that dengue fever with thrombocytopenia may be fatal in some patients and the risk is explained to the patient and the attendant. Rarely as shown in few of the above instances there may be loss of vision or the eye.In some cases visual recovery may occur. Ophthalmologist and physician should be aware and vigilant towards the patient with dengue-related ophthalmic symptoms as a small proportion of patients of dengue fever with thrombocytopenia may have poor visual outcome refractory to the treatment, the possibility of which should be informed to the patient and their attendant during the course of the treatment. Moreover the study also highlights occurence of globe luxation and ruptured globe in dengue fever and thrombocytopenia which have not been documented so far for the best of our knowledge.

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References

[1]. World Health Organization. Dengue and dengue hemorrhagic fever-Fact sheet.2009. Available at http://www.who.int/csr/disease/dengue/en. Accessed 19 May 2011.

[2]. Barkham TM, Chung YK, Tang KF, Ooi EE. The performance of RT-PCR compared with a rapid serological assay for acute dengue fever in a diagnostic laboratory. Trans R Soc Trop Med Hyg. 2006;100:142-148. doi:10.1016/jtrstmh.2005.05.015.

[3]. Kularatne SA (September 2015). “Dengue fever”. BMJ.351:h4661.

[4]. Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev.1998;11:480-496.

[5]. Haritoglou C, Dotse SD, Rudolph G, Stephan CM, Thurau SR, Klauss V. A tourist with dengue fever and visual loss. Lanncet 2002;360:1070.

[6]. Bascal KE, Chee SP, Cheng CL, Flores JV. Dengue-associated maculopathy. Arch Ophthalmol 2007;125:501-10.

[7]. “CDC - Neglected Tropical Diseases - diseases”.www.cdc.gov. Retrieved 2016-10-30.

[8]. Vivien Cherng-Hui Yip, Srinivasan Sanjay and Tan Tong Koh. Ophthalmic Complication of dengue fever: a systemic review. Ophthalmol Ther. 2012 Dec;1(1):2

[9]. Dr.Shivanand Bubanale, B11613, Dr.Bubanale SC, Dr.Shreya Raikar, Dr.A.Vidhyadhar, Dr.M.Bharadwaj. FP110: Endophthalmitis in dengue fever: a case report. AIOC 2017 Jaipur.

[10]. Saranappa SB, Sowbhagya HN. Panophthalmitis in dengue fever. Indian Pediatr 2012;49:760.

[11] Sriram S, Kavalakatt JA, Pereira Ad, Murty S. Bilateral panophthalmitis in dengue fever.Ann Trop Med Public Health 2015;8:217-8.

Original Research Article

The prevalence of dry eye and diabetic retinopathy in diabetes mellitus and comparing with duration and urea, creatinine level

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Abstract

Currently, an estimated 347 million people in the world have diabetes, making it one of the most common non-communicable medical conditions worldwide. India, considered as the "diabetes capital" of the world, is home to 74 million diabetics with a prevalence of ~8.7% among the adult population. Ocular manifestations of diabetes and its complications are well known over year's. In recent years, attention has been drawn to ocular surface disorders, especially dry eye in diabetic patients. Our purpose was to correlate the dry eye and diabetic retinopathy with duration of diabetes and blood urea and serum creatinine level. Ours was a hospital based cross sectional study done from August 2013 to July 2014. Thorough fundus examination was done using indirect ophthalmoscopy, tear film and ocular surface were evaluated using Schirmer test and tear film break-up time (TBUT) and renal parameters analysed in our hospital lab. Out of 150 patients studied 61.3% of patients had diabetes of 1 to 5 years duration. 35.3% of diabetic patients had dry eye symptoms. 75% of them had poor glycemic control. 72 of them had some degrees of diabetic retinopathy with altered renal parameters. P value of the above correlation was significant. Dry eye is the most common problem encountered in patients with diabetes mellitus. Hence, all the diabetic patients should be evaluated and screened at the earliest for retinopathy changes and presence of ocular surface disorders and treated accordingly. Early treatment would prevent complications associated with ocular surface disorders and diabetic retinopathy. They should be emphasized upon on need for regular follow up and maintaining a good glycemic control.

Key Word: diabetes mellitus, dry eye, diabetic retinopathy

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia associated with disturbances of carbohydrate, protein and fat metabolism due to absolute or relative deficiency in insulin action and or secretion. Diabetes is the leading cause of blindness in twenty to seventy four year age group[1]. Exact etiology of diabetic retinopathy is not known. It is believed that exposure to hyperglycemia over an extended period results in specific changes in retinal capillaries such as loss of pericytes and thickening of the basement membrane resulting in occlusion of capillaries and non-perfusion of retina and decompensation in retinal barrier function. The prevalence of diabetic retinopathy increases with the duration of diabetes and age of the patient. Dry eye is the most common problem encountered in patients with diabetes mellitus[3]. The exact mechanism for cause of dry eye in diabetes is not known[2]. Autonomic dysfunction plays a role in causing dry eye. Aldose reductase enzyme which converts glucose to sorbitol also plays a role[4]. Jin *et al* found that diabetes mellitus type 2 patients are prone to develop dysfunction of tear film. Cousen *et al* studied

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production of tears and sensation of cornea in diabetes. In addition to dry eye, tear film instability, metaplasia of conjunctiva, decreased goblet cells, decreased sensation of cornea and decreased tear film lipid layer are encountered in diabetes[5].Chronic hyperglycemia impairs inflammatory process and invasion of lids by bacteria leads on to blepharitis in diabetes patients[6]. This is cause for evaporative dry eye. The etio-pathological factors for dry eye in diabetic patients includes autonomic neuropathy, chronic hyperglycemia, insulin deficiency and chronic inflammation. Autonomic neuropathy is the key result of hyperglycemia in diabetes[7].Nerve signal pathways are damaged in autonomic neuropathy. Microvascular abnormalities and high blood sugar damage the corneal nerves and block the nerve feedback mechanism that controls secretion of tears. So lacrimal gland does not secrete tears[7]. Benetis-Del-Castillo *et al* studied relation between innervation of cornea with confocal microscopy and corneal sensations using noncontact esthesiometry in dry eye patients with diabetes[8]. Dogru stated that diabetes mellitus patients with autonomic neuropathy and uncontrolled diabetes patients exhibit abnormal Schirmer and TBUT test[9]. In diabetic patients, Schirmer test exhibits lower than normal values[10]. 55% of patients with diabetes had dry eyes symptoms and signs[11]. The Beaver Dam eye study suggested that twenty percent of diabetes mellitus patients aged between forty three and eighty six years diagnosed to have dry eyes[12]. Kaiserman and associates have told that good control of blood sugar is important for the control and prevention of dry eye syndrome among diabetic patients. Nepp and associates stated that severity of dry eye correlates with diabetic retinopathy severity[13]. In diabetes goblet cell density is reduced in patients with neuropathy[14].Schultz *et al* reported forty seven to sixty four percentage of diabetic patients having corneal abnormalities as epithelial defects, delayed healing of epithelium, corneal ulcer and kerato conjunctivitis sicca[15]. Increased duration influence the occurrence of DR and its severity was due to prolonged exposure to hyperglycemia. Duration of diabetes is an independent risk factor. Ten percent of the newly diagnosed diabetes shows DR, suggesting that these patients were not diagnosed earlier. The duration of diabetes is an important predictor of DR and its severity[16].

AIMS AND OBJECTIVES

To correlate the dry eye and diabetic retinopathy with duration of diabetes and blood urea and serum creatinine level and to evaluate the risk factors attributed to dry eye and diabetic retinopathy in diabetes mellitus patients and also to study the prevalence of dry eye and diabetic retinopathy in diabetes mellitus patients.

MATERIALS AND METHODS

It is a hospital based cross sectional study. This study was done in the period between August 2013 to July 2014. The study was done on all diabetic patients attending ophthalmology outpatient clinic. Before commencing the study Ethics committee approval was obtained from the Coimbatore medical college and government hospital. Diabetic patients attending outpatient clinic were screened for dry eye and diabetic retinopathy after obtaining consent. Detailed history was obtained including name, age, sex, presenting symptoms, duration and associated systemic disease and type of diabetes. The blood investigations such as fasting and post prandial blood sugar, urea, creatinine level were done. All patients of either sex in all age groups diagnosed to have diabetes mellitus of any duration were included. Patients with known ocular surface disorder, contact lenses usage, history of ocular surgeries in past and patients on local or systemic medications which cause dry eye are excluded. Detailed ophthalmic examination was done including best corrected visual acuity using Snellen chart and anterior segment examination using slit lamp. Symptoms of the dry eye such as redness, burning sensation, grittiness were evaluated using the questionnaire given to the patients. Condition of lid, meibomian gland and corneal surface were noted. Tear film evaluation was done by Schirmer test and tear film break up time. Intra ocular pressure measurement done using applanation tonometer. Detailed fundus examination was done and Diabetic retinopathy graded according to ETDRS criteria.

OBSERVATION AND RESULTS

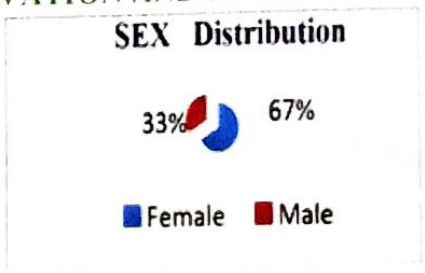


Figure 1: Sex Distribution

Among 150 patients studied, 100 patients were females and 50 patients were males.

Table 1: Duration Range

Duration	Frequency	Percent
Less than 1 year	6	4.0
1 to 5 years	92	61.3
6 to 10 years	38	25.3
11 to 20 years	14	9.3

Table 1 shows, patients diagnosed with diabetes less than 1 year duration were 4 %. Majority of population were between 1 to 5 years duration i.e., 61.3 %

Table 2: prevalence of dry eye symptoms

Dry eye Symptoms	No of patients	Percent
Absent	97	64.7
Present	53	35.3
Total	150	100.0

Table 2 shows 64.7% had no dry eye symptoms. 35.3% had dry eye symptoms.

Table 3 A and 3 B: fasting and post prandial blood sugar range

FBS(3A)	Frequency	Percent
FBS < 110	38	25.3
FBS >110	112	74.7
Total	150	100.0

PPBS (3B)	Frequency	Percent
Up to 160	37	24.7
Above 160	113	75.3
Total	150	100.0

Table 3A shows 25.3% of patients had FBS less than 110mg dl. 74.7% had FBS more than 110mg dl. Table 3B shows 37 patients had PPBS less than 160 mg dl. 113 patients had PPBS more than 160 mg dl.

Table 4A and 4B: Blood Urea And Serum Creatinine Rang

Urea(4A)	Frequency	Percent
Upto 40	127	84.7
>40	23	15.3
Total	150	100.0

Creatinine(4B)	Frequency	Percent
Upto 1	133	88.7
> 1	17	11.3
Total	150	100.0

Table 4A shows 127 patients had urea level less than 40 mg dl. 23 patients had urea level more than 40 mg. Table 4B shows 133 patients had Creatinine level less than 1 mg dl.17 patients had Creatinine more than 1 mg/dl.

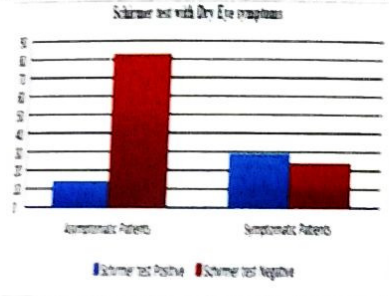


Figure 2: Schirmer Test

Among 150 patients. 43 patients were Schirmer test positive. 107 patients were negative for Schirmer test. Figure 2 shows Among 53 symptom positive patients, 29 of them Schirmer test positive and 24 of them Schirmer test negative.

Table 5: Schirmer Test and Duration Of Diabetes

Schirmer test	Less than 1 year	1 to 5 years	6 to 10 years	11 to 20 years
Positive	0	10	22	11
Negative	6	82	16	3

Table 5 shows among 43 Schirmer test positive patients, 10 patients had 1 to 5 years of diabetes. 22 of them were 6 to 10 years of diabetes. 11 of them were 10 to 20 years of diabetes. The P value of above comparison was significant.

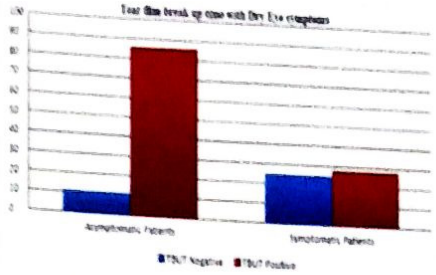


Figure 3: Tear Film Breakup Time

Among 53 dry eye symptom positive patients, Only 25 of them were TBUT positive in symptomatic patients. 28 of them TBUT test negative. The p value of the below chart is < 0.01.

Table 6: Tbut And Duration Of Diabetes Mellitus

Duration	Less than 1 year	1 to 5 years	6 to 10 years	11 to 20 years
positive	0	5	20	11
negative	6	87	18	3

Table 6 shows among 36 TBUT test positive patients, 5 of them were in 1 to 5 years duration of diabetes.20 of the were in 6 to 10 years duration and 11 of them were in 11 to 20 years duration of diabetes

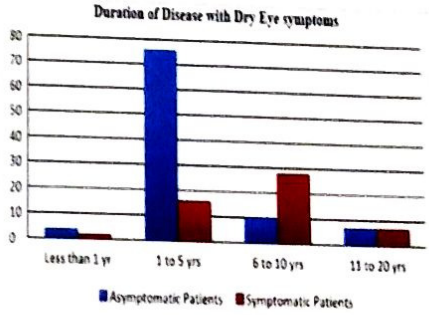


Figure 4: Duration Range and Dry Eye

Among 53 dry eye symptom positive patients.2 of them had diabetes of less than 1 year duration,.16 of them of 1 to 5 years duration, 28 of them of 5 to 10 years duration and 7 of them of 10 to 20 years duration. The p value of the above chart is < 0.01which is highly significant.

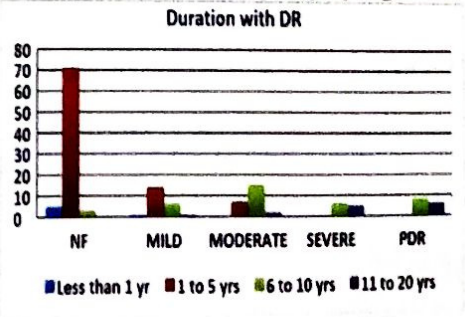


Figure 5: Duration Range And DR

92 patients were in 1 to 5 years duration. Among 92 patients,71 of them had normal fundus, 14 of them mild NPDR and 7 of them had moderate NPDR. The p value of the above chart is significant

Table 7: Schirmer Test And Dr

Schirmers Test	Normal Fundus	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
Positive	3	8	14	7	11
Negative	76	14	10	4	3

The p value of the above table is <0.01, significant. 43 patients are Schirmers test positive. Among 43 patients 3 of them normal fundus, 8 of them mild NPDR, 14 of them moderate NPDR, 7 of them severe NPDR, 11 of them had PDR.

Table 8: Tbut And Dr

Tbut	Normal Fundus	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
Positive	0	4	12	7	13
Negative	79	18	12	4	1

The p value of above table is <0.01 which is significant. 36patients were TBUT positive. Among 36 patients ,4 patients had mild NPDR, 12 patients had moderate NPDR, 7 patients had severe NPDR and 13 of them had PDR

Table 9: Fbs Range With Dr

FBS	Normal Fundus	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
Upto 110	29	3	4	0	2
Above 110	50	19	20	11	12

The p value of the above table is <0.01. significant.112patients had FBS more than 110 mg/dl. Among 112 patients 50 of them had normal fundus , 19 of them had mild NPDR, 20 of them had moderate NPDR, 11 of them had severe NPDR and 12 0f them had PDR

Table 10: Ppbs Range With Dr

PPBS	NORMAL FUNDUS	MILD NPDR	MODERATE NPDR	SEVERE NPDR	PDR
upto 160	33	1	2	1	0
above 160	46	21	22	10	14

The p value of the above table is <0.01, significant.113 patients had PPBS more than 160 mg/dl.46 of them had normal fundus, 21 of them had mild NPDR, 22 of them moderate NPDR, 10 of them had severe NPDR and 14 of them had PD

Table 11: Dry Eye and Sex Predilection

dry eye symptoms	female	male
Negative	58	39
Positive	42	11

The p value of table no. 11 is <0.01, significant. Among 53 symptomatic patients 42 were females.

DISCUSSION

In this study 150 patients with type 2 diabetes mellitus were examined for dry eye and diabetic retinopathy manifestations and above manifestations are correlated with age, duration, control of diabetes and urea, creatinine level. Among 150 patients ,100 patients were females and 50 were males. 61.3% of patients diabetes of between 1 to 5 years duration.35.3% of diabetic patients had dry eye symptoms. Our study showed that 75% of them were not in good glycemic control. Binder A *et al* study showed that 55% of patients with diabetes had dry eye signs and symptoms at least at some of the time. Among 53 symptomatic patients, 29 were Schirmer test positive and 24 of them were Schirmer negative. 14 of the asymptomatic patients showed Schirmer test positive. Schirmer test is useful screening test for diagnosing hyposecretion, but not for an adequate determination of tear production. Its sensitivity is between 10% and 30%. Among symptom positive patients (53), TBUT is positive in 25 patients. The p value of above comparison is significant. Mannavit published prevalence of dry eye in diabetic patients utilising either Schirmers test or TBUT. In our study 42 females are dry eye symptom positive and only 11 males are symptom positive. p value is significant. Prevalence of dry eye is higher among advancing age, female sex, poor glycemic control, patients with diabetic retinopathy and high urea, creatinine level. In our study higher grades of diabetic retinopathy is associated with higher incidence of dry eye symptoms and the p value of the test is significant. Among 150 patients,23 patients had urea level more than 40 mg/dl. In 23 patients only 2 of them had normal fundus. 1 patient had mild NPDR. 7 of them had moderate NPDR. 5 of them had severe NPDR. 8 of them had PDR.P value of the above test is significant. With regard to serum Creatinine level, 17 of them had more than 1mg/dl. Only one patient had normal fundus. Remaining patients with elevated creatinine level had some degree of DR. p value of the above correlation is significant. 38 patients among 150 patients had fasting blood sugar less than 110mg/dl. Remaining patients(112) had more than 110 mg/dl. 50 patients had normal fundus in more than 110 FBS group. 72 of them had some degrees of diabetic retinopathy. Microvascular complications develop in nearly all patients with type 1 diabetes and in more than 77% of those with type 2 diabetes who survives for over 20 years with the disease. Vision threatening retinopathy is rare in type 1 diabetes in the first 3 to 5 years of diabetes or before puberty. After 2 decades nearly all patients with type 1 DM develop retinopathy. 21% of type 2 diabetes patients have retinopathy at the time of first diagnosis of diabetes. UKPDS demonstrated that improved blood glucose control reduced the risk of developing retinopathy, nephropathy and possibly reducing neuropathy. Moss and associates also reported a high incidence of dry eye

among females 16 .7% compared to males 11.4%, but in our study p value of gender correlation with diabetic retinopathy is not significant.

CONCLUSION

Dry eyes are more prevalent in diabetic individuals. The ocular surface disease in diabetic individuals was confirmed by Schirmer test and tear film breakup time. Early detection of dry eye and diabetic retinopathy prevents vision threatening complications like formation of corneal opacity, diabetic macular edema, tractional retinal detachment and vitreous haemorrhage. Dry eye syndrome is a definite entity, that occurs in diabetic patients, more so, when the duration of diabetes is longer, poor glycemic control, high urea, creatinine level are associated with DR, irrespective of severity. Hence it is mandatory to look for ocular surface abnormality in diabetic patients, as it can interfere with vision further. Schirmer test and TBUT test are easy to perform and easily available. They can be routinely done in all diabetic patients, in order to diagnose dry eye in early stage, so that prompt treatment can be initiated. As dry eye symptoms and DR have a correlation, it is important to rule out DR in symptomatic dry eye patients and vice versa. Since poor glycemic control and elevated urea, Creatinine levels have been associated with increased incidence of dry eye, it is important to look for the same, even in asymptomatic patients with altered biochemical parameters. Apart from ophthalmoscopy, Schirmer test and TBUT should be compulsorily done in all diabetic patients.

REFERENCES

1. Harrison TR: Diabetes Mellitus. In Harrison Principle of Internal Medicine. 15th edition.
2. Scultz RO, Horn DLV, Peters MA, Klewin KM, Schutten WH: Diabetic keratopathy.Trans A OphthalmolSoc 1981, 79:180
3. Cousen P, Cackett P, Bennett H, et al. Tear production and corneal sensitivity in diabetes. J Diabetes Complications. 2007 Nov-Dec; 21(6):371-3.
4. Fujishima H, Shimazaki J, and Yagi Y, Tsubota K: Improvement of corneal sensation and tear dynamics in diabetic patients by oral aldose reductase inhibitor, ONO-2235: a preliminary study.
5. Horn M, De I and P. Self-reported dry eyes and diabetic history. Optometry. 2006 Nov; 77(11):554-
6. Ghasemi H, Gharebaghi R, Heidary F. Diabetes as a possible predisposer for blepharitis. Can J Ophthalmol. 2008 Aug; 43(4):485.
7. Chous P. Dry eyes and diabetes often go hand in hand. Available at: www.dlife.com/dl_ife/do/ShowContent/inspiration_exper t_advi/ROEsams/expert_columns/chous_sept2006.html (Accessed September 7, 2010).
8. Benitez-Del-Castillo JM, Acosta MC, Wassfi MA, et al. Relation between corneal innervation with confocal microscopy and corneal sensitivity with noncontact esthesiometry in patients with dry eye. Invest Ophthalmol Vis Sci. 2007 Jan; 48(1):173-81
9. DogruM,KatakamiC,Inoue M .Tear fuction and ocular surface changes in noninsulin dependent diabetes mellitus.Ophthalmology 2001
10. YoonK,ImS,SeoM.Changes of tear film and ocular surface in diabetes mellitus.Korean J Ophthalmol. 2004
11. Lambert DW, Foster CS,PerryHD.Schirmer test after tropical anaesthesia and tear menincus height in normal eyes.ArchOphthalmol. 1979
12. NelsonJD,HavenerVR,CameronJD.Cellouse acetate impressions of the ocular surface.Dryeyesstates.ArchOphthalmol. 1983
13. Nepp J, Abela C, Polzer I, Derbolav A. Is there a correlation between the severities of diabetic retinopahyaandKeratoconjunctivitisSicca? Cornea 2000
14. Amin K ,Nagra M,H, Javed M, Hussain I, Alam Z .Diabetes Mellitus: incidence of retinopathy. Professional Med J 2003
15. K huwaja A.K, Rafique G ,White F,Azam S,I .Macrovascular complications and their associated factors in type 2 diabetes in Karachi,Pakistan –a multi – centre study. J Pak Med Assoc 2004
16. Bamashmus MA, Gunaid AA, Khandekar RB: Diabetic retinopathy, visual impairment and ocular status among patients with diabetes mellitus in Yemen: a hospital-based study. Indian J Ophthalmol 2009, 57:293-298.

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Congenital capillary hemangioma arising from palpebral conjunctiva of a neonate

S Padmanaban, P Sumathi, Prayagi Kandoth, R P Dharmendra[1]

Capillary hemangioma is the most common benign vascular eyelid tumor in childhood. The periocular lesion appears within the first few weeks after birth and usually has superficial or deep components. Primary conjunctival capillary hemangiomas are rarely reported. We present the case of a 2-day-old child with a pedunculated capillary hemangioma arising from superior palpebral conjunctiva. A complete surgical excision was performed under general anesthesia, and the child was asymptomatic at follow-up of two months.

Key words: Congenital capillary hemangioma, conjunctival capillary hemangioma, management, pedunculated capillary hemangioma

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Capillary hemangioma is the most common benign vascular eyelid tumor in childhood.[1] It is present in 1%–4% of all births[2] and is more common in premature infants and often following chorionic villus sampling.[3] It is usually a cutaneous, subcutaneous, or deep orbital lesion and commonly presents a few weeks after birth. The usual clinical course of infantile cutaneous or subcutaneous hemangiomas includes an initial engorgement (age 6–12 months) followed by regression

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(age 1–7 years). It usually regresses spontaneously and hence is kept under close observation. Active intervention is performed only if the lesion is very extensive and causes amblyopia, mechanical ptosis, exposure keratopathy, or optic neuropathy. Treatment modalities for cutaneous or subcutaneous hemangiomas include topical[4] and oral beta blockers,[5-8] local and oral steroids,[9,10] surgical excision, immunotherapy,[11] laser photocoagulation with pulsed dye laser,[3] and embolization.[12] Although conjunctiva may be involved as a part of capillary hemangioma of the lid, isolated conjunctival lesion is rare and its congenital presence is rarer. In a series of 1463 cases of conjunctival tumors studied, only 10 were capillary hemangiomas, forming 16% of conjunctival vascular tumors, only 1% of all conjunctival tumors.[13] Usually, hemangiomas of conjunctiva are small and require only observation. Chang and Estes, in a recent paper, report usage of topical timolol in the management of small bulbar conjunctival hemangioma.[14] Oral propranolol was also recently reported to be in use in the treatment of a case of bilateral sessile infantile hemangioma of posterior lamella of upper lid by Syed Ali Raza Rizvi *et al*., with clearing of visual axis within 12 weeks of treatment.

We report a case of capillary hemangioma in a newborn, which could be treated completely by surgical excision due to its unique pedunculated morphology. Such a case of congenital pedunculated conjunctival capillary hemangioma has not been reported so far to the best of our knowledge.

Case Report

A 2-day-old female baby presented to our pediatric surgery department with a red mass arising from left upper eyelid since birth [Fig. 1]. In the antenatal history elicited, mother was found to be a primipara who had regular antenatal checkups, with no history of any invasive interventions, and the baby was born out of full-term normal vaginal delivery with no perinatal complications.

On examination, the right eye appeared to be normal. Examination of the left eye showed a red, pedunculated, highly vascular, nontender mass of 2.5 cm × 1.5 cm arising from the palpebral conjunctiva of upper lid. The mass had a flattened stalk with terminal lobulations, at the tip of which, there was dried up mixture of blood and discharge. On upper eyelid eversion, the base of peduncle was found to be at 4 mm from lid margin. The lesion appeared to cause mechanical ptosis and covered the visual axis. The eyelid opening appeared inadequate [Fig. 2]. There was minor bleeding from the surface of the swelling on manipulation. The rest of the conjunctiva was normal except for mild congestion. The cornea of the left eye was clear. There was no evidence of similar cutaneous lesions on the rest of the body.

A provisional diagnosis of capillary hemangioma was made. There was also the possibility of pyogenic granuloma and rare possibility of rhabdomyosarcoma. Complete hemogram of the baby showed normal hemoglobin (17.3 g%), white blood cell (11,000/μL), and platelet (2.5 lakhs/μL) levels. Ultrasonogram (USG) showed high-internal reflectivity with irregular acoustic structures suggestive of capillary hemangioma. USG of cranium and abdomen were done to rule out internal hemangiomas but were found to be normal.

Taking into consideration the pedunculated nature, unsightly appearance, possibility of developing amblyopia due to the central location and also to obtain a histopathological diagnosis (to rule out any other tumor), surgical removal was finalized as the treatment. On postnatal day 6, the baby was prepared for general anesthesia. Under anesthesia, detailed examination of the conjunctiva of both eyes was done to rule out deeper lesions and bilaterality. Excision of the mass was done with bipolar electrocautery device by a team consisting of ophthalmologist and pediatric surgeon. It was cut close to tarsal plate, and adequate hemostasis was obtained [Fig. 3].

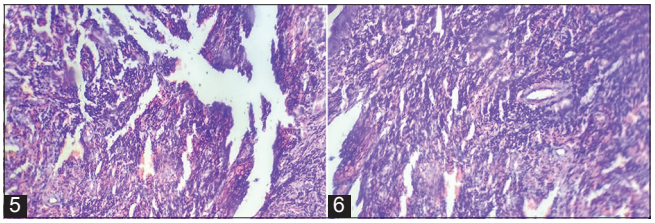
On the 1st postoperative day, the eye was quiet with adequate eyelid opening, clear cornea, and normal fundus [Fig. 4]. On follow-up visits, there was no conjunctival scarring and cornea appeared clear. Multiple sections studied showed a cellular neoplasm composed of plump endothelial cells with well-canalized and poorly canalized vessels, confirming the diagnosis of cellular infantile hemangioma [Figs. 5 and 6]. The



Figures 1 and 2: (1) Baby with left eye upper lid pedunculated swelling arising from palpebral conjunctiva (2) Upon lifting and resting the swelling on upper lid, thin broad pedicle appreciated



Figures 3 and 4: (3) On table image of baby after excision of lesion (4) Baby on first postoperative day



Figures 5 and 6: (5) HPE-Multiple sections studied showed a cellular neoplasm composed of plump endothelial cells with well-canalized and poorly canalized vessels, confirming the diagnosis of cellular infantile hemangioma (6) Multiple sections studied showed a cellular neoplasm composed of plump endothelial cells with well-canalized and poorly canalized vessels, confirming the diagnosis of cellular infantile hemangioma

baby was followed up for 2 months postsurgery and showed no deformities and had adequate lid movements.

Discussion

Capillary hemangioma is common in eyelids but uncommon in the conjunctiva. These are benign vascular tumors which consist of endothelial cell proliferation. It usually presents a few weeks after birth, sometimes at birth, grows for several months, and later regresses spontaneously over years. These occur singly or as part of syndromes (PHACES). Capillary hemangiomas of conjunctiva are rare, and mostly, the lesions are small, demanding only close watch. Usually, cutaneous and orbital hemangiomas are treated with oral steroids or propranolol. Recently, cases of effective management of small conjunctival hemangiomas with topical timolol have been reported. The current literature also shows management of sessile palpebral conjunctival capillary hemangiomas with oral beta blockers. In our case, the baby was brought to us with a red, lobulated mass with stalk present since birth and arising exclusively from the upper eyelid conjunctiva. In order to prevent amblyopia,the lesion was surgically excised,which was possible due to its pedunculated nature.

Conclusion

Conjunctival capillary hemangioma of pedunculated nature has not been reported in literature so far to the best of our knowledge.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Albert DM, Jakobiec FA. Principles and Practice of Opthalmology. 2nd ed., Vol. 5. Philadelphia: W. B. Saunders; 2000. p. 4436.
2. Sethuraman G, Yenamandra VK, Gupta V. Management of infantile hemangiomas: Current trends. J Cutan Aesthet Surg 2014;7:75-85.
3. Drolet BA, Esterly NB, Frieden IJ. Hemangiomas in children. N Engl J Med 1999;341:173-81.
4. Chambers CB, Katowitz WR, Katowitz JA, Binenbaum G. A controlled study of topical 0.25% timolol maleate gel for the treatment of cutaneous infantile capillary hemangiomas. Ophthal Plast Reconstr Surg 2012;28:103-6.
5. Vassallo P, Forte R, Di Mezza A, Magli A. Treatment of infantile capillary hemangioma of the eyelid with systemic propranolol. Am J Ophthalmol 2013;155:165-7000.
6. Thoumazet F, Léauté-Labrèze C, Colin J, Mortemousque B. Efficacy of systemic propranolol for severe infantile haemangioma of the orbit and eyelid: A case study of eight patients. Br J Ophthalmol 2012;96:370-4.
7. Missoi TG, Lueder GT, Gilbertson K, Bayliss SJ. Oral propranolol for treatment of periocular infantile hemangiomas. Arch Ophthalmol 2011;129:899-903.
8. Brad B. Kanski's Clinical Ophthalmology. 8th ed. China: Elsevier; 2016. p. 101.
9. Schwartz SR, Kodsi SR, Blei F, Ceisler E, Steele M, Furlan L, *et al.* Treatment of capillary hemangiomas causing refractive and occlusional amblyopia. J AAPOS 2007;11:577-83.
10. Coats DK, O'Neil JW, D'Elia VJ, Brady-McCreery KM, Paysse EA. SubTenon's infusion of steroids for treatment of orbital hemangiomas. Ophthalmology 2003;110:1255-9.
11. Wilson MW, Hoehn ME, Haik BG, Rieman M, Reiss U. Low-dose cyclophosphamide and interferon alfa 2a for the treatment of capillary hemangioma of the orbit. Ophthalmology 2007;114:1007-11.
12. Ooi KG, Wenderoth JD, Francis IC, Wilcsek GA. Selective embolization and resection of a large noninvoluting congenital hemangioma of the lower eyelid. Ophthal Plast Reconstr Surg 2009;25:111-4.
13. Shields JA, Shields CL. Eyelid, Conjunctival and Ocular Tumors: An Atlas and Textbook. 2nd ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2008. p. 250-445.
14. Chang TC, Estes R. Beta blocker treatment of infantile conjunctival hemangiomas – Observations from 2 cases. J AAPOS 2014;18:80-2.

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Case Report

Pseudotumor Cerebri Complicating Multisystem Inflammatory Syndrome in a Child

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Abstract

Purpose: To report a case of pseudotumor cerebri (PTC) in a child associated with multisystem inflammatory syndrome in children (MIS-C), associated with presumed coronavirus disease 2019.

Methods: A previously healthy 11-year-old female child presented with a 4-day history of fever, headache, vomiting, and loose stools. Laboratory investigations revealed neutrophilic leukocytosis, and markers of inflammation (C-reactive protein, ferritin, and interleukin-6) were significantly elevated. Pharyngeal swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by polymerase chain reaction was negative while anti-SARS-CoV-2 antibody was highly reactive. Ophthalmic evaluation for transient visual obscurations during hospital course revealed swelling of the optic disc in both eyes. Spectral-domain optical coherence tomography and ultrasonography confirmed the ophthalmoscopic findings. There was no neurologic deficit. Magnetic resonance imaging of the brain and magnetic resonance venogram revealed no structural lesion. The opening pressure of cerebrospinal fluid (CSF) was 336 mm of water, and CSF composition was normal.

Results: A diagnosis of PTC associated with MIS-C was made, and the child was treated with oral acetazolamide. Edema of the optic disc regressed following therapy, and the child is under follow-up.

Conclusions: PTC can occur in association with MIS-C. Clinicians need to be aware of this potential neuro-ophthalmic complication in MIS-C. Prompt diagnosis and treatment can prevent visual loss.

Keywords: COVID-19, Multisystem inflammatory syndrome in children, Pseudotumor cerebri

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Introduction

Pseudotumor cerebri syndrome (PTCS) is a disorder characterized by increased intracranial pressure of unclear etiology that carries significant morbidity and limited therapeutic options. PTCS can be primary otherwise called idiopathic intracranial hypertension (IIH) or secondary, attributable to a number of medical conditions and drugs. This neurologic syndrome predominantly affects obese women of reproductive age group, and clinical features include symptoms and signs of raised intracranial pressure with normal cerebrospinal fluid (CSF) composition and no structural abnormality on brain imaging.[1] PTCS is relatively uncommon in children and is associated with variable risk factors and clinical presentation.[2] We report a case of PTCS in a child associated with multisystem inflammatory syndrome in children (MIS-C), temporally associated with coronavirus disease 2019 (COVID-19).

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Case Report

An 11-year-old girl with no significant past medical history was brought to the emergency department with complaints of fever over the past 4 days associated with headache, vomiting,

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abdominal pain, and loose stools. There was associated lethargy but no history of seizures, altered sensorium, or respiratory symptoms. There were no sick contacts. At admission, the child had a temperature of 39°C, heart rate of 112 beats/min, and oxygen saturation of 100% on room air. Body mass index was 16 kg/m^2. Physical examination was unremarkable except for bilateral conjunctival congestion. Investigations revealed decreased hemoglobin, leukocytosis with neutrophilia, and elevated inflammatory markers including ferritin, interleukin-6 (IL-6), and C-reactive protein. Blood and urine cultures were negative, and liver function tests were found to be normal. Nasopharyngeal and oropharyngeal swabs for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time polymerase chain reaction (RT-PCR) were negative. Serologic testing for SARS-CoV-2 antibodies by chemiluminescence immunoassay was highly reactive (observed titer – 212, ≥1 being labeled reactive) suggestive of SARS-CoV-2 infection. Table 1 shows the laboratory parameters. Echocardiogram was normal. On day 3 of admission, the child complained of transient obscurations of vision lasting for a few seconds associated with change in posture, and was referred for ophthalmic consultation.

On examination, uncorrected visual acuity was 20/20, and color vision was normal in both eyes. Anterior segment examination was unremarkable, and pupillary responses were normal. Extraocular movements were full. Fundus examination revealed optic disc swelling in both eyes [Figure 1a]. Neurologic examination was otherwise normal. Visual field evaluation by automated perimetry (Humphrey field analyzer) was found to be unreliable due to extensive fixation losses. Spectral-domain optical coherence tomography (OCT) showed increased thickness of peripapillary retinal nerve fiber layer (RNFL) in both eyes in all the four quadrants confirming edema of the optic disc [Figure 2a]. Magnetic resonance imaging of the brain and magnetic resonance venography (MRV) revealed normal ventricles, brain parenchyma, and venous sinuses. Transorbital ultrasound revealed bulging optic disc in both eyes. The optic nerve sheath diameter (ONSD) measurements obtained 3 mm posterior to the globe were found to be 5.6 mm in the right eye and 6 mm in the left eye [Figure 3a]. Lumbar puncture revealed a CSF opening pressure of 336 mm of water. CSF cultures were negative, and biochemistry was normal.

Since the child was hemodynamically stable and not hypoxic (SpO_2 100% on room air) at admission as well as during hospital course, she was managed with empiric broad-spectrum antibiotic therapy (intravenous ceftriaxone 2 g for 7 days) along with supportive care for fever (paracetamol) and loose stools (intravenous fluid replacement). As serial IL-6 and serum ferritin showed declining trend, the child was treated only with supportive measures. A diagnosis of pseudotumor cerebri (PTC) associated with MIS-C was made, and the child was started on oral acetazolamide 500 mg twice daily. By 4 weeks, headache and transient visual obscurations had subsided. Visual acuity remained 20/20, and fundus examination revealed resolving disc edema. The dose of oral acetazolamide was reduced to 250 mg thrice daily. At her last visit, 7 weeks since her initial presentation, she was found to have visual acuity of 20/20 and resolved disc edema in both eyes [Figure 1b]. Follow-up OCT revealed normalization of peripapillary RNFL thickness [Figure 2b]. The ONSD measurements were found to be 4 mm in both eyes at follow-up [Figure 3b]. The dose of acetazolamide has been tapered further, and she is under follow-up.

Table 1: Laboratory parameters of the patient and reference range

Parameter	Value	Reference range
Hemoglobin (g/dL)	10.5	11.5-15.5
White blood cell count (10^3/μL)	17	5-13
Neutrophil (%)	91.1	45-65
Lymphocyte (%)	7.5	32-52
Erythrocyte sedimentation rate (mm/h)	31	4-12
C-reactive protein (mg/dL)	39	<0.6
Serum ferritin (ng/mL)	352.4	7-140
IL-6 (pg/mL)	197.8	<7
NT-proBNP (pg/mL)	1163	<125
D-dimer (mg/L FEU)	1.48	<0.5
Anti-SARS-CoV-2 titer	212	<1

IL-6: Interleukin-6, NT-proBNP: N-terminal-pro-brain natriuretic peptide, Anti-SARS-CoV-2: Antibody against severe acute respiratory syndrome coronavirus 2

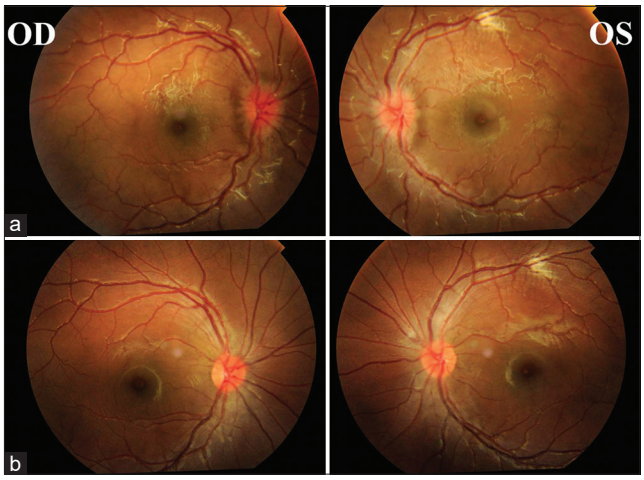


Figure 1: (a) Fundus photograph both eyes showing edema of optic disc. (b) Fundus photograph both eyes showing resolved disc edema at follow-up

Discussion

MIS-C, also called pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2, is a life-threatening condition that can lead to multi-organ dysfunction and long-term sequelae. The clinical features of MIS-C include severe illness necessitating hospitalization, fever, and involvement of two or more organ systems, in combination with laboratory evidence of inflammation and laboratory or epidemiologic evidence of SARS-CoV-2 infection.[3] Our

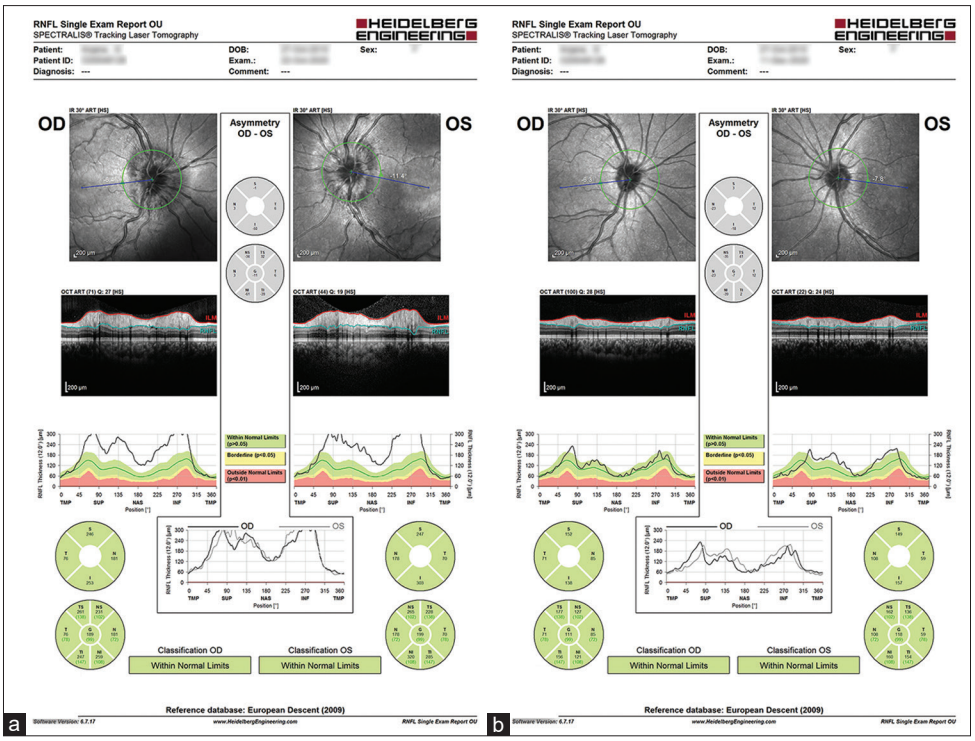


Figure 2: (a) Spectral-domain optical coherence tomography (SD-OCT) showing increased thickness of peripapillary retinal nerve fiber layer (RNFL) in both eyes. (b) SD-OCT both eyes at follow-up showing normalization of peripapillary RNFL thickness

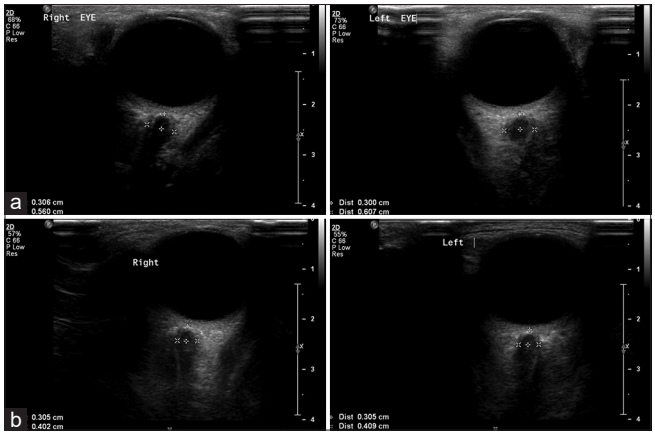


Figure 3: (a) Transorbital ultrasound showing increased optic nerve sheath diameter (ONSD) 3 mm posterior to inner scleral surface; 5.6 mm in the right eye and 6 mm in the left eye. (b) Transorbital ultrasound at follow-up showing normalization of ONSD; 4 mm in both eyes

patient was found to meet all the criteria specified in the Centers for Disease Control and Prevention and World Health Organization case definitions of MIS-C.[4,5]

The pathophysiology of MIS-C is unclear, and possible mechanisms include T-cell or antibody recognition of self-antigens resulting in autoantibodies, immune response against viral antigens expressed on infected cells and formation of immune complexes activating inflammation as well as viral superantigen sequences which activate host immune cells.[6] Dysregulated hyperinflammation is a critical event in the pathogenesis of MIS-C, and this condition is frequently associated with gastrointestinal symptoms and cardiovascular dysfunction.[7]

Although neurologic manifestations have been documented during acute phase of infection with SARS-CoV-2,[8,9] involvement of the central nervous system is uncommon in MIS-C. The neurologic features of MIS-C have been strongly suggested to represent a postinfectious immune response, and manifestations reported include headache, encephalopathy, and aseptic meningitis.[10]

We had not performed RT-PCR for SARS-CoV-2 on CSF since our patient showed evidence of previous infection with SARS-CoV-2 rather than current infection (pharyngeal swab was negative while antibodies to SARS-CoV-2 were present). Only one-third of cases of MIS-C have been reported to be positive by RT-PCR for SARS-CoV-2. The delayed presentation of MIS-C relative to the pandemic curve, a low proportion of cases who were SARS-CoV-2 positive by RT-PCR, and a high proportion who were antibody positive suggest that this inflammatory syndrome is not mediated by direct viral invasion but coincides with the development of acquired immune responses to SARS-CoV-2.[6] Previous studies have noted the absence of SARS-CoV-2 RNA in CSF analysis of COVID-19 patients with neurological symptoms suggesting that indirect mechanisms are responsible for neurologic manifestations in these patients.[11,12]

Although the symptoms of PTCS in children are similar to that of adults, the demographics vary, and children are reported to

have a higher incidence of secondary PTC. A distinct seasonal variation has been reported in the clinical presentation of IIH in prepubertal children, suggesting a possible association between IIH and concurrent infections in this age group.[13] Agraz *et al*. have noted a high incidence of atopy in their study cohort of children with PTC, suggesting that autoimmune component may play a role in pediatric population.[2]

To the best of our knowledge, four similar cases of PTCS associated with MIS-C (two case reports and a case series of two patients) have been reported in literature.[14-16] Although two patients had received therapy with doxycycline and steroids, the short course and recent initiation preclude the possibility of these medications contributing to increased intracranial pressure.

The mechanism behind the occurrence of PTCS in association with MIS-C is unclear. It has been postulated that increased intracranial pressure reflects systemic inflammation related to SARS-CoV-2 infection, resulting in central nervous system effects.[16] It is of interest to note that obesity, the most striking risk factor for PTCS, is associated with dysregulation of several inflammatory cytokines and aberrant glucocorticoid metabolism through manipulation of the enzyme 11-beta-hydroxysteroid dehydrogenase (11β-HSD).[17] 11β-HSD is a bidirectional enzyme that regulates prereceptor corticosteroid availability and glucocorticoid availability in the central nervous system that is one of the factors pivotal to intracranial pressure homeostasis. Among the two isoforms, 11β-HSD1 activates cortisol from cortisone while the isoform 11β-HSD2 inactivates cortisol. It is well established that several inflammatory cytokines such as tumor necrosis factor-α and ILs 1 and 6 are potent activators of 11β-HSD1.[18]

We speculate that PTC associated with MIS-C can be multifactorial, and one another possibility could be aberrant glucocorticoid metabolism as a result of cytokine storm and 11β-HSD1 activity. Increased 11β-HSD1 activity has been shown to influence the pathogenesis of PTC through manipulation of CSF dynamics at the level of choroidal plexus as well as arachnoidal granulations.[19] Further studies are warranted in this regard.

Few cases of isolated intracranial hypertension in association with active SARS-CoV-2 infection have been reported in adults. The proposed pathophysiologic mechanisms for intracranial hypertension include acute encephalitis, venous sinus thrombosis, intracranial venous congestion due to inflammation as a result of SARS-CoV-2 infection, and the associated coagulopathy as well as hyperviscosity state leading to impaired absorption of CSF.[20,21] The absence of clinical features such as altered sensorium/seizures and normal brain parenchyma evident on neuroimaging in our patient ruled out encephalitis. Normal MRV ruled out venous sinus thrombosis in our patient.

The other possible etiologies of swelling of optic disc in the setting of SARS-CoV-2 infection include optic neuritis, ischemic optic neuropathy, papillophlebitis, and retinal vein occlusion. The presence of normal visual acuity and color vision along with normal pupillary responses and the absence of associated retinal hemorrhages or venous tortuosity in our patient made us exclude these causes.

Prompt diagnosis and treatment of PTCS is essential since it can lead to irreversible visual loss. Although the pathophysiology remains unclear, clinicians should be aware of this potential complication with MIS-C. Fundus examination is valuable as part of a multidisciplinary approach toward management of MIS-C.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Friedman DI, Liu GT, Digre KB. Revised diagnostic criteria for the pseudotumor cerebri syndrome in adults and children. Neurology 2013;81:1159-65.
2. Agraz D, Morgan LA, Fouzdar Jain S, Suh DW. Clinical features of pediatric idiopathic intracranial hypertension. Clin Ophthalmol 2019;13:881-6.
3. Nakra NA, Blumberg DA, Herrera-Guerra A, Lakshminrusimha S. Multi-System Inflammatory Syndrome in Children (MIS-C) following SARS-CoV-2 infection: Review of clinical presentation, hypothetical pathogenesis, and proposed management. Children (Basel) 2020;7:69.
4. Centers for Disease Control and Prevention. Emergency Preparedness and Response: Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19). Health Advisory. Available from: https://emergency.cdc.gov/han/2020/han00432.asp. [Last accessed on 2020 Dec 26].
5. World Health Organization. Multisystem Inflammatory Syndrome in Children and Adolescents with COVID-19: Scientific Brief; 2020. Available from: https://www.who.int/publications-detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19. [Last accessed on 2020 Dec 26].
6. Jiang L, Tang K, Levin M, Irfan O, Morris SK, Wilson K, *et al.* COVID-19 and multisystem inflammatory syndrome in children and adolescents. Lancet Infect Dis 2020;20:e276-88.
7. Radia T, Williams N, Agrawal P, Harman K, Weale J, Cook J, *et al.* Multi-system inflammatory syndrome in children & adolescents (MIS-C): A systematic review of clinical features and presentation. Paediatr Respir Rev. 2021;38:51-57.
8. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, *et al.* Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020;77:683-90.
9. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, *et al.* Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18-22.
10. Chen TH. Neurological involvement associated with COVID-19 infection in children. J Neurol Sci 2020;418:117096.
11. Bellon M, Schweblin C, Lambeng N, Cherpillod P, Vazquez J,

Lalive PH, *et al.* Cerebrospinal fluid features in SARS-CoV-2 RT-PCR positive patients. Clin Infect Dis 2020;ciaa1165.
12. Neumann B, Schmidbauer ML, Dimitriadis K, Otto S, Knier B, Niesen WD, *et al.* Cerebrospinal fluid findings in COVID-19 patients with neurological symptoms. J Neurol Sci 2020;418:117090.
13. Distelmaier F, Tibussek D, Schneider DT, Mayatepek E. Seasonal variation and atypical presentation of idiopathic intracranial hypertension in pre-pubertal children. Cephalalgia 2007;27:1261-4.
14. Verkuil LD, Liu GT, Brahma VL, Avery RA. Pseudotumor cerebri syndrome associated with MIS-C: A case report. Lancet 2020;396:532.
15. Parsons E, Timlin M, Starr C, Fries A, Wells R, Studer M, *et al.* MIS-C in February 2020 and implications of genomic sequencing for SARS-CoV-2. J Pediatric Infect Dis Soc 2020;10:695-97.
16. Baccarella A, Linder A, Spencer R, Jonokuchi AJ, King PB, Maldonado-Soto A, *et al.* Increased intracranial pressure in the setting of multisystem inflammatory syndrome in children, associated with COVID-19. Pediatr Neurol 2021;115:48-9.
17. Sinclair AJ, Ball AK, Burdon MA, Clarke CE, Stewart PM, Curnow SJ, *et al.* Exploring the pathogenesis of IIH: An inflammatory perspective. J Neuroimmunol 2008;201-202:212-20.
18. Chapman KE, Coutinho AE, Zhang Z, Kipari T, Savill JS, Seckl JR. Changing glucocorticoid action: 11β-hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. J Steroid Biochem Mol Biol 2013;137:82-92.
19. Sinclair AJ, Walker EA, Burdon MA, van Beek AP, Kema IP, Hughes BA, *et al.* Cerebrospinal fluid corticosteroid levels and cortisol metabolism in patients with idiopathic intracranial hypertension: A link between 11beta-HSD1 and intracranial pressure regulation? J Clin Endocrinol Metab 2010;95:5348-56.
20. Svedung Wettervik T, Kumlien E, Rostami E, Howells T, von Seth M, Velickaite V, *et al.* Intracranial pressure dynamics and cerebral vasomotor reactivity in coronavirus disease 2019 patient with acute encephalitis. Crit Care Explor 2020;2:e0197.
21. Silva MT, Lima MA, Torezani G, Soares CN, Dantas C, Brandão CO, *et al.* Isolated intracranial hypertension associated with COVID-19. Cephalalgia 2020;40:1452-8.

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Original Article

Ophthalmology Section

Inverse Association between Serum Bilirubin Levels and Retinopathy in Patients with Type 2 Diabetes Mellitus.

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ABSTRACT

Introduction: Oxidative stress plays a central role in the pathogenesis of Diabetic Retinopathy (DR) and serum bilirubin has been shown to have antioxidant properties.

Aim: To investigate the association between serum bilirubin concentration and DR in patients with Type 2 diabetes Mellitus (DM).

Materials and Methods: This was a hospital based, cross-sectional study where in 86 patients with Type 2 DM and 30 controls were recruited. The study was conducted at a tertiary care centre in South India between January 2014 and December 2014. The presence and the severity of DR were determined by fundus examination and grading of colour fundus photographs using the international clinical disease severity scale for diabetic retinopathy. Serum total, direct and indirect bilirubin levels were determined in all subjects and the association between bilirubin levels and severity of DR was studied.

Results: Among the 86 diabetics, 24 had no retinopathy and 62 had diabetic retinopathy of varying grades. The mean total bilirubin level among diabetic subjects (0.52±0.17) and controls (0.51±0.19) were found to be similar. The mean total as well as direct bilirubin levels were found to be lower in patients with retinopathy as compared to no retinopathy group ($p \leq 0.001$). The severity of DR was inversely proportional to the serum bilirubin levels ($p=0.010$). Serum total bilirubin was found to have a negative association with glycosylated haemoglobin and served as an independent determinant of diabetic retinopathy even after adjusting for risk factors known to be associated with DR ($p=0.001$).

Conclusion: Low serum bilirubin levels are significantly associated with increased risk of diabetic retinopathy independent of classic risk factors. Serum bilirubin can serve as a useful biomarker in identifying patients at risk for developing proliferative diabetic retinopathy.

Keywords: Chronic kidney disease, Heme oxygenase, Peripheral arterial disease

INTRODUCTION

Diabetes Mellitus (DM) has assumed the proportions of a global epidemic and the number of diabetics in the world today is 415 million. This number is being projected to increase to 642 million by 2040 [1]. India leads the world in terms of its diabetic population and estimates have revealed that there are 62.4 million people with diabetes, and 77.2 million with prediabetes in India [2]. It is predicted that by 2030, DM may affect up to 79.4 million in India [3]. Due to these sheer numbers, the socioeconomic burden as a result of diabetes in India is among the highest in the world [4].

Along with this rapidly increasing disease, there is an increase in the associated microvascular complication DR, which is said to affect more than 93 million people worldwide [5]. Almost all patients with Type 1 DM and over 60% of the patients with Type 2 DM will develop DR in 20 years. DR is the most frequent cause of preventable blindness in middle aged population and therefore,represents a major public health issue [6].

Oxidative stress is considered to be one of the key factors in the pathogenesis of DR [7]. The retina is especially more susceptible to oxidative damage because of its high energy demands and exposure to light [8]. Various mechanisms contributing to the pathogenesis of DR such as inflammation, polyol pathway, accumulation of Advanced Glycation End Products (AGEs), flux of hexosamine Pathway and Protein Kinase C (PKC) activation are associated with overproduction of Reactive Oxygen Species (ROS) by the mitochondria [9].

Although, bilirubin has long been considered as a toxic waste product of heme catabolism, its role as a potential endogenous anti-oxidant under physiological conditions is being increasingly recognized [10]. Apart from its ability to scavenge over produced ROS, bilirubin is also said to have anti inflammatory effects [11]. Based on these physiological effects of bilirubin, several population-based studies have reported a negative association between serum bilirubin concentrations and the risk of diabetes [12], coronary artery disease [13], chronic kidney disease [14], peripheral arterial disease [15] and diabetic peripheral neuropathy [16].

Few studies in literature have shown, the association between serum bilirubin and DR [17-20]. Apart from age, gender, smoking and intake of drugs serum bilirubin concentrations are also affected by factors such as altitude and ethnicity and these are likely to influence the biological effects of bilirubin on human body.

Considering the potential role of oxidative stress in the pathogenesis of DR and the antioxidant and cytoprotective effects of bilirubin, this cross-sectional study aimed to evaluate the association between serum bilirubin and severity of retinopathy in patients with Type 2 DM from South India.

MATERIALS AND METHODS

This was a hospital-based cross-sectional study which included patients with Type 2 DM who were referred to the Ophthalmology Department of a tertiary care centre in South India between January 2014 and December 2014 for screening of DR. Exclusion criteria included history of chronic alcohol intake, smoking, intake of hepatotoxic medications within past six months, preexisting hepatobiliary abnormalities or chronic liver disease. Patients with preexisting ocular diseases like glaucoma, high myopia, previous ocular surgery or photocoagulation were excluded from the study. Patients with hypertension (>160/90 mmHg), anaemia or other systemic diseases like nephropathy which can accelerate the progression of DR were also excluded from the study. The study protocol was approved by the Institutional Ethics Committee and this study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Information on age, gender, duration of diabetes, blood pressure and body mass index was obtained from all participants.

All participants underwent a complete ocular examination including fundus examination with slit lamp biomicroscope using a 90 diopter lens after dilatation of pupils using 1% tropicamide eyedrops. This was followed by 30 degree colour stereoscopic fundus photography of 7 standard Early Treatment Diabetic Retinopathy Study (ETDRS) fields using a digital fundus camera (TRC-50 DX Mydriatic retinal camera; Topcon Medical Systems, Oakland, NJ).The severity of DR was determined by grading of colour fundus photographs following the international clinical disease severity scale for diabetic retinopathy which is based upon the findings of WESDR (Wisconsin Epidemiological Study of Diabetic Retinopathy) and ETDRS [21].

The diabetic patients were assigned to one of the following groups based on presence and severity of retinopathy.

1. Diabetic patients with no retinopathy.
2. Mild Non-Proliferative Diabetic Retinopathy (NPDR)
3. Moderate NPDR.
4. Severe NPDR and
5. Proliferative Diabetic Retinopathy (PDR)

The eye with worse retinopathy was chosen for inclusion in the study examinations. If both eyes had equal retinopathy, the right eye was assigned to the study.

Fasting venous blood samples were collected for the determination of serum total, direct and indirect bilirubin, blood glucose and Glycosylated Haemoglobin (HbA1c). The normal range of total serum bilirubin levels used in the study was 0.2 to 1.2 mg/dl.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 19. The baseline characteristics of patients were presented as mean±standard deviation and n (%). Data on the patient's clinical characteristics were compared using One-way Analysis of Variance (ANOVA) as well as an independent sample Student's t-test. The serum bilirubin levels were divided into quartiles and Chi-square test was used to see the comparison between the proportions. Multiple logistic regression analysis was performed to assess independent associations between the presence of key risk factors of DR including the statistically significant factors in univariate analysis. All tests for statistical significance were two-tailed, and performed assuming a Type I error probability of <0.05.

RESULTS

A total of 116 subjects were included in the study out of whom 86 were patients with Type 2 diabetes and 30 were controls.

The mean total bilirubin level among diabetic subjects (0.52±0.17) and controls (0.51±0.19) were found to be similar. The direct bilirubin level was found to be higher in diabetics (0.29±0.13) as compared to controls (0.18±0.06, p=0.000). The indirect bilirubin level was lower in diabetics (0.23±0.07) as compared to healthy controls (0.33±0.13, p=0.000).

[Table/Fig-1] shows the clinical characteristics of the study population.

The mean duration of diabetes and HbA1c were significantly higher among patients with DR compared to those without retinopathy (p<0.001). We compared the serum total, direct and indirect bilirubin levels for diabetics without retinopathy (n=24) and those with DR (n=62). The total bilirubin was 0.65±0.16 in patients with no retinopathy and 0.47±0.16 in patients with DR. The mean total as well as direct bilirubin levels were found to be lower in patients with retinopathy as compared to no retinopathy group (p=0.000). There was no statistically significant difference in the levels of indirect bilirubin among both groups (p=0.100).

Characteristic	Controls (n=30)	Diabetics with no retinopathy (n=24)	Diabetics with retinopathy (n=62)
Age (yr)	52.97±8.95	57.63±10.54	57.29±8.5
Females, no. (%)	18 (60%)	9 (37.5%)	23 (37.1%)
Duration of diabetes (yr)	-	3.67±1.20	6.74±3.51*
Body mass Index (Kg/m^2)	28.1±0.7	27.8±0.4	28±0.7
Systolic blood pressure (mm Hg)	120.1±2.7	117.1±1.6	122.6±2.9
Diastolic blood pressure (mm Hg)	79.7±2.1	74.1±1.2	73.3±2.4
HbA1c (%)	5.72±0.35	8.09±0.78	9.75±1.74*
Total bilirubin (mg/dl)	0.51±0.19	0.65±0.16	0.47±0.16*
Direct bilirubin (mg/dl)	0.18±0.06	0.39±0.13	0.25±0.10*
Indirect bilirubin (mg/dl)	0.33±0.13	0.25±0.05	0.22±0.07

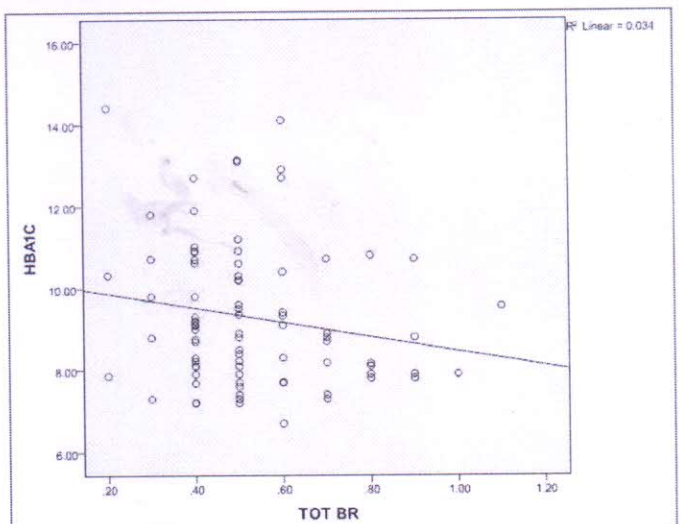
[Table/Fig-1]: Showing the clinical characteristics of study population.
* p-value < 0.05; HbA1c- Haemoglobin A1c

	Quartiles of serum total bilirubin (mg/dl)				p-value
	<0.40	0.41-.50	0.51-.60	>0.61	
Controls	11	10	3	6	0.010
NO DR	2	6	5	11	
Mild NPDR	5	4	3	3	
Moderate NPDR	8	8	3	3	
Severe NPDR	7	1	0	1	
PDR	11	4	1	0	

[Table/Fig-2]: Showing the correlation between serum total bilirubin and severity of diabetic retinopathy.
NPDR-Non proliferative diabetic retinopathy; PDR-Proliferative diabetic retinopathy

Risk factor	OR	95% CI	p-value
Age (years)	1.03	0.960-1.111	0.386
Duration of diabetes (years)	2.05	1.172-3.592	0.012*
HbA1c (%)	2.42	1.177-4.984	0.016*
Serum total bilirubin (mg/dl)	0.001	0.000-0.054	0.001*

[Table/Fig-3]: Showing the odds ratio for association between key risk factors and occurrence of diabetic retinopathy using logistic regression model.
* p-value < 0.05; OR-Odds ratio; CI-Confidence Interval; HbA1c- Haemoglobin A1c



[Table/Fig-4]: Scatter diagram showing negative association between serum total bilirubin and HbA1c (glycosylated haemoglobin) in diabetics.
TOT BR-Total bilirubin; HBA1C-glycosylated haemoglobin

Of the 62 diabetic patients with retinopathy, 15 patients had mild NPDR, 22 had moderate NPDR and 9 had severe NPDR. PDR was noted in 16 patients. We divided the study participants into quartiles on the basis of serum total bilirubin concentration (quartile 1=<0.40 mg/dl, quartile 2= 0.41-0.50 mg/dl, quartile 3= 0.51-0.60 mg/dl, quartile 4=>0.61 mg/dl).

[Table/Fig-2] shows the correlation between serum total bilirubin and severity of DR.

The percentage of DR in persons with lowest bilirubin quartile was 36.04%, compared with the percentage of 19.8%, 8.1% and 8.1% respectively in the second, third and fourth quartiles. Thus, the proportion of patients with DR was significantly higher in quartile 1 with lower levels of bilirubin as compared to the other three quartiles [Table/Fig-2]. The severity of DR was inversely proportional to the serum bilirubin levels (p=0.010) and none of the patients in the last quartile (>0.61) had proliferative retinopathy.

On multiple logistic regression analysis the duration of diabetes, HbA1c and total serum bilirubin were found to be independently associated with DR [Table/Fig-3]

Also, serum total bilirubin in diabetic subjects was found to have negative association with HbA1c [Table/Fig-4].

DISCUSSION

In the present, study serum bilirubin concentration correlated negatively with the severity of retinopathy in patients with Type 2 DM. Multiple regression analysis also revealed serum total bilirubin as an independent risk factor associated with diabetic retinopathy.

Bilirubin is generated when the enzyme Heme Oxygenase (HO) catalyzes the degradation of heme. This leads to the formation of biliverdin which is converted to bilirubin by biliverdin reductase. In the hepatocytes, unconjugated (lipid-soluble) bilirubin is conjugated by uridine diphosphate glucuronyl transferase (UDP-GT) to a water soluble form for excretion. Total bilirubin is the sum of unconjugated (indirect) and conjugated (direct) bilirubin and generally ranges from 0.2 to 1.2 mg/dl in healthy individuals.

Ever since the description of beneficial role of bilirubin as a physiologic antioxidant, several clinical studies evaluating, the possible associations between serum bilirubin and the risk of diabetes (and its complications) have been published [22-24].

In this observational study, we noted that Type 2 diabetics with retinopathy had significantly lower serum bilirubin concentrations than those without retinopathy. Our observations are in line with epidemiologic and hospital based studies elsewhere which have suggested an inverse relation between serum total bilirubin and DR [17-20]. There has been only one study on the association between serum bilirubin and DR from Indian population by Dave et al., [25]. They noted higher levels of total, direct and indirect bilirubin in patients without DR as compared to patients with retinopathy. However, in our study only total and direct bilirubin concentrations were found to be higher in patients without DR. Serum indirect bilirubin concentration was similar in both groups. Most studies on serum bilirubin and DR have focused on only total bilirubin without separating bilirubin types. Limited studies have shown a differentially protective effect of direct bilirubin on metabolic syndrome [26] and chronic kidney disease [27]. Further studies on the relation between bilirubin subtypes and DR are clearly needed.

In this study, we divided bilirubin levels into quartiles (<0.40, 0.41-0.50, 0.51-0.60 and >0.61 mg/dl and considered the lowest quartile as reference. This approach was to maintain adequate numbers within each quartile. The absolute values of serum bilirubin were low in our study compared to studies by Yasuda et al., and Najam et al., where the value of serum bilirubin in the lowest and highest quartile observed were<0.60 and >0.90 mg/dl respectively [17,18]. There are several potential reasons for the observed findings. Apart from differences in ethnicity, our hospital based study population was very much different from the above said epidemiologic studies in age distribution, anthropometry, comorbidities such as hypertension, cardiovascular disease and smoking habits, all factors that may affect the bilirubin levels and potentially the association between bilirubin and DR. Our study participants were non smokers and had no history of major systemic diseases.

Several potential mechanisms have been proposed for the observed relationship between serum bilirubin concentration and DR. Excessive production of free radicals and resultant oxidative stress has been said to contribute significantly to worsening of DR [28,29]. Bilirubin has been postulated as a potent antioxidant and cytoprotective agent that can protect cells from a 10,000 fold excess of hydrogen peroxide [30]. Therefore, lower serum bilirubin concentration would be expected to be associated with increased oxidative stress and thereby, accelerated progression of DR. Additionally clinical studies have demonstrated elevated levels of inflammatory markers like C-reactive protein and vascular cell adhesion molecules in patients with DR suggesting that inflammation is a critical factor leading to endothelial dysfunction in diabetic retinopathy [31,32]. Bilirubin has been proven to have anti-inflammatory properties and in-vitro studies have shown that bilirubin inhibited tumour necrosis factor α –induced upregulation of vascular cell adhesion molecules [33]. Hence, it could be hypothesized that higher serum bilirubin concentration is associated with a lower risk of development and progression of DR through its anti-inflammatory effects. Further, recent animal studies have shown that, increasing serum bilirubin levels either through endogenous synthesis or exogenous administration improves endothelial dysfunction in diabetes by activation of protein kinase B [34].

Considering these physiologic effects of bilirubin it can be postulated that bilirubin can effectively retard or interrupt the pathways to development of DR and therefore serve as a potential biomarker for risk of diabetic retinopathy.

We observed an inverse and graded association between serum total bilirubin and severity of DR in this study and the strength of serum total bilirubin concentration as an independent determinant of DR was similar to those of conventional risk factors such as HbA1c and duration of diabetes in multiple regression analysis. Serum total bilirubin concentration was an independent determinant of DR even after adjustment for other risk factors (p=0.001).

We also observed a negative association between serum total bilirubin and HbA1c among diabetic subjects. Our data are consistent with observations of Choi et al., who have also noted a significant inverse association between serum total bilirubin and HbA1c in their study on 690 Korean patients with Type 2 DM [35].

This can be partly explained by the fact that, increased serum bilirubin reduces the oxidative stress and thereby, inhibits the glycosylation of haemoglobin. Also, upregulation of the heme oxygenase system and increased generation of bilirubin/biliverdin have been proven to enhance insulin production and sensitivity in diabetic rats [36]. Therefore, it can be suggested that bilirubin improves glycaemic control through its effects on insulin. Further studies are needed to precisely elucidate the mechanism behind this association.

The strength of our study is based on the fact that our study participants were non-smokers and did not have other systemic diseases. We excluded these patients to focus on the association between serum bilirubin and retinopathy in patients with Type 2 diabetes.

LIMITATION

Our study has some limitations. First, our sample size was limited and the cross-sectional design of our study prevents determination of a temporal association or the cause-effect relationship between serum total bilirubin and the development of retinopathy. Only prospective studies can reveal whether serum bilirubin predicts the development of DR. Second, we performed only a single measurement of serum bilirubin which might have within subject variation. However, all subjects including controls were examined after overnight fast.

CONCLUSION

In conclusion, our cross-sectional study suggested that low total serum bilirubin level was independently associated with higher risk of DR in patients with type 2 diabetes, regardless of other conventional risk factors. Further, prospective studies on large samples are required to better assess the effects of bilirubin on diabetic retinopathy.

REFERENCES

[1] International Diabetes Federation. IDF Diabetes Atlas, 7th ed. Brussels, Belgium: International Diabetes Federation, 2015.

[2] Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: Phase I results of the Indian Council of Medical Research India Diabetes (ICMR INDIAB) study. Diabetologia. 2011;54:3022 27.

[3] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.

[4] Joshi SR, Das AK, Vijay VJ, Mohan V. Challenges in diabetes care in India: sheer numbers, lack of awareness and inadequate control. J Assoc Physicians India. 2008;56:443–50.

[5] Yau JWY, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35:556–64.

[6] Cheung N, Mitchell P, Wong TY. Diabetic retinopathy Lancet. 2010;376:124–36.

[7] Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord. 2008;9:315-27.

[8] Kumari S, Panda S, Mangaraj M, Mandal MK, Mahapatra PC. Plasma MDA and antioxidant vitamins in diabetic retinopathy.Indian J Clin Biochem. 2008;23:158-62.

[9] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615-25.

[10] Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science. 1987;235:1043–46.

[11] Vítek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. Adv Clin Chem. 2007;43:1-57.

[12] Cheriyath P, Gorrepati VS, Peters I, Nookala V, Murphy ME, Srouji N, et al. High total bilirubin as a protective factor for Diabetes Mellitus: An analysis of NHANES Data From 1999 - 2006. J Clin Med Res. 2010;2:201-06.

[13] Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem. 1994;40:18-23.

[14] Tanaka M, Fukui M, Okada H, Senmaru T, Asano M, Akabame S, et al. Low serum bilirubin concentration is a predictor of chronic kidney disease. Atherosclerosis. 2014;234:421-25.

[15] Perlstein TS, Pande RL, Beckman JA, Creager MA. Serum total bilirubin level and prevalent lower extremity peripheral arterial disease: National health and nutrition examination survey (NHANES) 1999 to 2004. Arterioscler Thromb Vasc Biol. 2008;28:166-72.

[16] Kim ES, Lee SW, Mo EY, Moon SD, Han JH. Inverse association between serum total bilirubin levels and diabetic peripheral neuropathy in patients with type 2 diabetes. Endocrine. 2015;50:405-12.

[17] Yasuda M, Kiyohara Y, Wang JJ, Arakawa S, Yonemoto K, Doi Y, et al. High serum bilirubin levels and diabetic retinopathy: the Hisayama Study. Ophthalmology. 2011;118:1423-28.

[18] Najam SS, Sun J, Zhang J,Xu M, Lu J, Sun K, et al. Serum total bilirubin levels and prevalence of diabetic retinopathy in a Chinese population. J Diabetes. 2014;6:221–27.

[19] Cho HC. The relationship among Homocysteine, Bilirubin, and diabetic retinopathy. Diabetes Metab J. 2011;35:595-601.

[20] Sekioka R, Tanaka M, Nishimura T, Itoh H. Serum total bilirubin concentration is negatively associated with increasing severity of retinopathy in patients with type 2 diabetes mellitus. J Diabetes Complications. 2015;29:218-21.

[21] Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales.Global Diabetic Retinopathy Project Group. Ophthalmology. 2003;110:1677-82.

[22] Han SS, Na KY, Chae DW, Kim YS, Kim S, Chin HJ, et al. High serum bilirubin is associated with the reduced risk of diabetes mellitus and diabetic nephropathy. Tohoku J Exp Med. 2010;221:133-40.

[23] Jung CH, Lee MJ, Kang YM, Hwang JY, Jang JE, Leem J, et al. Higher serum bilirubin level as a protective factor for the development of diabetes in healthy Korean men: A 4 year retrospective longitudinal study. Metabolism. 2014;63:87-93.

[24] Kawamoto R, Ninomiya D, Hasegawa Y, Kasai Y, Kusunoki T, Ohtsuka N, et al. Mildly elevated serum total bilirubin levels are negatively associated with carotid atherosclerosis among elderly persons with type 2 diabetes. Clin Exp Hypertens. 2016;38:107-12.

[25] Dave A, Kalra P, Gowda BH, Krishnaswamy M. Association of bilirubin and malondialdehyde levels with retinopathy in type 2 diabetes mellitus. Indian J Endocrinol Metab. 2015;19:373–77.

[26] Hwang HJ, Kim SH. Inverse relationship between fasting direct bilirubin and metabolic syndrome in Korean adults. Clin Chim Acta. 2010;411:1496-501.

[27] Ryu S, Chang Y, Zhang Y, Woo HY, Kwon MJ, Park H, et al. Higher serum direct bilirubin Levels were associated with a lower risk of incident chronic kidney disease in middle aged Korean Men. PLoS One. 2014;9(2):e75178.

[28] Obrosova IG, Minchenko AG, Marinescu V, et al. Anti-oxidants attenuate early up regulation of retinal vascular endothelial growth factor in streptozotocin-diabetic rats. Diabetologia. 2001;44:1102–10.

[29] Kowluru RA, Atasi L, Ho YS. Role of mitochondrial superoxide dismutase in the development of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2006;47:1594–99.

[30] Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: A major physiologic cytoprotectant. Proc Natl Acad Sci U S A. 2002;9:16093–98.

[31] Schram MT, Chaturvedi N, Schalkwijk C, Giorgino F, Ebeling P, Fuller JH, et al. Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes: the EURODIAB Prospective Complications Study. Diabetes Care. 2003;26:2165-73.

[32] Olson JA, Whitelaw CM, McHardy KC, Pearson DW, Forrester JV. Soluble leucocyte adhesion molecules in diabetic retinopathy stimulate retinal capillary endothelial cell migration. Diabetologia. 1997;40:1166-71.

[33] Mazzone GL, Rigato I, Ostrow JD, Bossi F, Bortoluzzi A, Sukowati CH, et al. Bilirubin inhibits the TNF alpha-related induction of three endothelial adhesion molecules. Biochem Biophys Res Commun. 2009;386:338-44.

[34] Liu J, Wang L, Tian XY, Liu L, Wong WT, Zhang Y, et al. Unconjugated bilirubin mediates heme oxygenase-1-induced vascular benefits in diabetic mice. Diabetes. 2015;64:1564-75.

[35] Choi SW, Lee YH, Kweon SS, Song HR, Ahn HR, Rhee JA, et al. Association between total bilirubin and haemoglobin A1c in Korean type 2 diabetic patients. J Korean MedSci. 2012;27:1196–201.

[36] Ndisang JF, Lane N, Jadhav A. Upregulation of the heme oxygenase system ameliorates postprandial and fasting hyperglycemia in type 2 diabetes. Am J Physiol Endocrinol Metab. 2009;296(5):E1029-41.

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Brief Communication

Needs Assessment of Ophthalmology Education for Undergraduate Medical Students – A Study from a Medical College in South India

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ABSTRACT

Background: Adequate ophthalmic diagnostic and clinical skills are essential for practitioners in primary care settings as well as specialty care physicians. The objectives of this study were to assess the adequacy of ophthalmology teaching in undergraduate medical education and to evaluate the comfort of medical students in diagnosing common eye problems and performing ophthalmic skills. **Methods:** A questionnaire based, cross-sectional survey was conducted among third-year undergraduate students from a medical college in South India at the end of ophthalmology training from February 2014 to December 2014. The main outcome measures were hours of classroom-based instruction and clinical exposure to ophthalmology received by the students and their comfort level in diagnosing common eye problems and performing ophthalmic skills. **Results:** 134 students participated in the study. They had received an average of 96.2 ± 5.9 and 112.5 ± 11.3 hours of classroom and clinic-based instruction, respectively. The participants' comfort in diagnosing eye problems was satisfactory for cataract and eyelid disorders but not for ophthalmic emergencies. Only 45.5% had satisfactory knowledge in community ophthalmology. Respondents were more proficient in visual acuity testing (93.3%) and assessment of pupillary reaction (80.6%) than direct ophthalmoscopy (41%). **Discussion:** Undergraduate medical students from India received a greater amount of ophthalmology instruction compared to the International Council of Ophthalmology task force recommendations. Gaps in community ophthalmology and knowledge-skills discrepancies were noted. Review of curriculum, appropriate training resources, and effective teaching methods tailored towards primary care may be useful to improve the training.

Keywords: Comfort, knowledge, ophthalmology, undergraduate medical education

Background

Ophthalmology makes up a significant proportion of primary care consultations.[1] A basic knowledge of ophthalmology is essential in the management of several neurologic, endocrine, rheumatic, and infectious conditions. Despite the relevance of ophthalmology to medical practice, little time is allocated to teaching of ophthalmology in undergraduate medical education and the majority of primary care physicians do not feel that they have received adequate undergraduate ophthalmic education.[2] Also several studies have noted that medical students receive too little ophthalmic training.[3-5]

In India, the five and one-half year undergraduate medical training consists of four and one-half years of teaching of subjects comprising the medical curriculum and one year of internship. Ophthalmology is being taught in the third year after preclinical and paraclinical subjects.

The state of undergraduate ophthalmology training from medical colleges in India has not been studied so far. Evaluation

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of ophthalmic knowledge and skills of undergraduates at the end of ophthalmology training would help in generating critical feedback on undergraduate ophthalmic curriculum planning. The objectives of this study were:

1. To assess the adequacy of ophthalmology teaching in undergraduate medical education
2. To assess the comfort level of medical students in diagnosing and treating common eye problems and performing ophthalmic skills.

Methods

After approval from the institutional review board, undergraduate medical students from a medical college in South India were included in the study at the end of their ophthalmology training (February 2014 to December 2014). The adequacy of ophthalmology education was quantified by determining the hours of classroom-based instruction and clinical exposure to ophthalmology received by the students and comparing these with the recommendations of the Medical Council of India (MCI) as well as the International Council of Ophthalmology (ICO).

A validated questionnaire [Appendix 1] was given to the students and their responses collected anonymously. The questionnaire consisted of three parts. Parts 1 and 3 assessed the students' comfort with respect to diagnosing specific ophthalmology conditions and performing ophthalmology-related skills on a Likert scale from 1 to 5 (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, and 5 = very comfortable). In terms of comfort level, we examined the ease with which study participants reported they were able to diagnose common eye problems and perform ophthalmic skills.

The second part assessed knowledge on certain topics on a Likert scale, also from 1 to 5 (1 = very poor, 2 = poor, 3 = average, 4 = good, and 5 = very good). The conditions and skills covered in the questionnaire were based on the topics covered in the undergraduate ophthalmology curriculum recommended by the MCI.[6]

Participants were also asked about the number of hours spent in self-directed learning and how comfortable they felt in managing ophthalmology-associated issues overall.

Likert scale-based responses were subsequently dichotomized and reduced to proportions by categorizing scores 1–2 as unsatisfactory or inadequate and 3–5 as satisfactory or adequate. Data were entered using the Statistical Package for the Social Sciences (version 19 (SPSS, Inc., Chicago, IL, USA) and examined using descriptive and analytical statistics to yield frequencies, percentages, and proportions.

Results

Of the 150 undergraduate medical students, 134 responded to the questionnaire (response rate = 89.6%).

The main modes of ophthalmology exposure were didactic lectures (35 hours), tutorials (37.5 ± 3.8 hours), seminars (23.7 ± 2.2 hours), and clinic-based learning. Our study participants had received an average of 96.2 ± 5.9 hours of classroom-based instruction. They also had received 112.5 ± 11.3 hours of clinic-based instruction in the eight weeks of clinical posting. 121 participants (90.3%) had spent up to 10 hours in self-directed learning while 13 participants (9.7%) had spent more than 10 hours in self-directed learning.

The results of the participants' comfort in diagnosing ophthalmic conditions are presented in Figure 1. Figure 2 shows the students' adequacy of knowledge in community and systemic ophthalmology as well as indications for ophthalmic referral. Figure 3 shows the study participants' comfort in performing MCI recommended clinical skills. Tables 1-3 show the numerical data for the corresponding figures. Finally, 101 participants (75.4%) felt comfortable in managing ophthalmology associated issues overall.

Discussion

This cross-sectional observational study attempted to quantify the adequacy of undergraduate ophthalmic education and students' comfort with regard to the management of ocular conditions.

In comparison with MCI recommendations, our study participants received a satisfactory number of hours of both classroom and clinic-based instruction. The MCI recommends clinical training of 8 weeks and a total of 100 teaching hours in ophthalmology, which includes 30 h of didactic lectures,

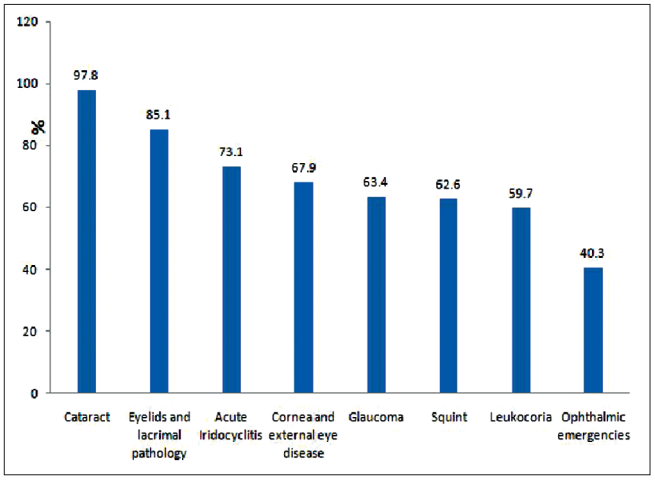


Figure 1: Medical students' comfort in diagnosing common ophthalmic conditions

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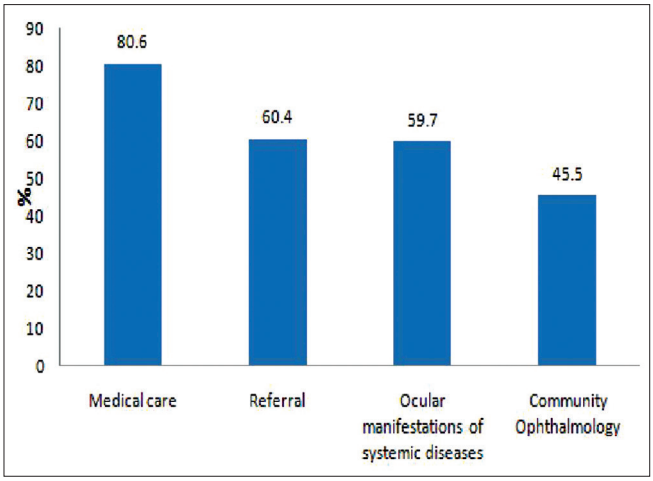


Figure 2: Students' adequacy of ophthalmic knowledge

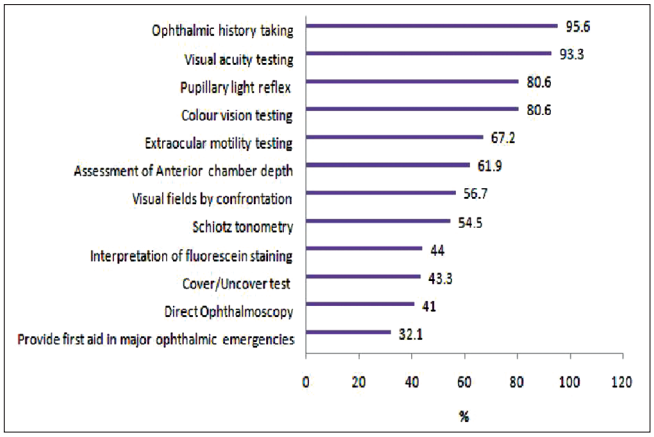


Figure 3: Students' self-reported proficiency in performance of ophthalmic skills

Table 1: Students' comfort in diagnosing common ophthalmic conditions

Ophthalmic conditions	Participants with adequate comfort in diagnosing common ophthalmic conditions (n=134), n (%)
Cataract	131 (97.8)
Eyelids and lacrimal pathology	114 (85.1)
Acute iridocyclitis	98 (73.1)
Cornea and external eye disease	91 (67.9)
Glaucoma	85 (63.4)
Squint	84 (62.6)
Leukocoria	80 (59.7)
Ophthalmic emergencies	54 (40.3)

60 h of tutorials and seminars (including integrated learning), and 10 h of self-directed learning.[7] These guidelines suggest a greater amount of ophthalmology exposure in undergraduate medical education in India when compared with ICO guidelines. The ICO recommends 40–60 h of ophthalmology exposure during undergraduate training.[8]

According to the curriculum recommended by the MCI, the goal of teaching ophthalmology to undergraduate medical students is to provide such knowledge and skills to the students that shall enable them to practice as a clinical and primary eye care physician.[6] The curriculum provides a list of competencies which need to be demonstrated by the student at the end of ophthalmology training. These include knowledge of common eye problems in the community; ability to manage common eye problems and identify indications for referral; recognize visual impairment and blindness in the community; and implement the National programme as applicable in the primary care setting.[7]

Most of the participants in the study reported a satisfactory level of comfort in diagnosing common ophthalmic problems. The fact that 97.8% reported a satisfactory level of comfort in diagnosing cataract is encouraging since cataract is the leading cause of preventable blindness in the developing world. Yet, despite this satisfactory level of comfort in managing common eye problems, most study participants' comfort in diagnosing ophthalmic emergencies such as endophthalmitis, rupture globe, and chemical injuries was found to be inadequate.

Nearly 60% reported to have adequate knowledge in ocular manifestations of systemic diseases. This could be because of better teaching resources as well as integration of ophthalmology topics into other subjects of the medical curriculum horizontally and longitudinally. The self-rated knowledge of more than one-half of the study participants was found to be unsatisfactory in community ophthalmology. With greater emphasis placed on teaching disorders of the eye, in theory lectures as well as in hospital settings, community ophthalmology is often neglected. A study on the status of ophthalmic medical education in the South-East Asia Region of World Health Organization has also highlighted this concern, where students have little exposure to community ophthalmology.[9]

The knowledge of most study participants on the indications of medical treatment and referral to eye clinics was adequate. With a significant shortage of ophthalmologists in developing countries, an adequate level of comfort of primary care physicians in providing medical care and accuracy in referral is essential. A high rate of referral places pressure on the limited resources of ophthalmic casualty departments and outpatient clinics.

More than 80% reported competency in ophthalmic history taking, visual acuity and color vision testing, and pupil examination. These are relevant not only in ophthalmology but also in general medicine, neurology, and trauma care. However, only 44% reported an adequate level of comfort in interpretation of fluorescein staining and 54.5% in Schiotz tonometry. This is an area of concern because corneal blindness

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Table 2: Students' adequacy of knowledge

Ophthalmic knowledge	Participants with adequate knowledge (*n*=134), *n* (%)
Medical care	108 (80.6)
Referral	81 (60.4)
Ocular manifestations of systemic diseases	80 (59.7)
Community ophthalmology	61 (45.5)

Table 3: Students' proficiency in performance of ophthalmic skills

Ophthalmic skills	Participants with adequate comfort in performing ophthalmic skills (*n*=134), *n* (%)
Ophthalmic history taking	128 (95.6)
Visual acuity testing	125 (93.3)
Pupillary light reflex	108 (80.6)
Color vision testing	108 (80.6)
Extraocular motility testing	90 (67.2)
Assessment of anterior chamber depth	83 (61.9)
Visual fields by confrontation	76 (56.7)
Schiotz tonometry	73 (54.5)
Interpretation of fluorescein staining	59 (44)
Cover/uncover test	58 (43.3)
Direct ophthalmoscopy	55 (41)
First aid in major ophthalmic emergencies	43 (32.1)

and glaucoma contribute to a major extent to the burden of blindness in India; adequate proficiency of the primary care physician in these skills will help in early diagnosis and treatment or referral, thereby reducing the risk of visual loss.

Only 41% of respondents indicated that they had adequate proficiency in performing direct ophthalmoscopy which is consistent with studies elsewhere.[2,10] This could be because of the limited time devoted to teaching ophthalmoscopy in the medical school curriculum as well as the technical challenges involved.

Although most students were comfortable in managing ophthalmology associated issues overall, gaps were noted in certain key areas. Review of the curriculum with greater emphasis on community eye care and blindness control strategies, adequate training resources, and effective teaching methods tailored toward primary care would help to increase the medical students' comfort in managing common eye problems.

Since our observations are from a single center, generalization of results is limited. Subjective bias due to the candidates varying ability to accurately self-evaluate is also a limitation. The choice of the study population was medical students at the end of ophthalmology training rather than interns because of the possibility of recollection bias. A similar study on interns or junior medical officers would provide more insight into the knowledge and gaps in training.

In spite of these limitations, this needs assessment study is the first of its kind in India to assess the adequacy of ophthalmic teaching and the knowledge and clinical skills of undergraduate medical students. Ophthalmology in India is being taught as part of the essential undergraduate medical curriculum and not exclusively as an elective. Students receive adequate exposure to ophthalmology, and the undergraduate curricular hours are greater than the guidelines published by the ICO. Since ophthalmology related topics and examination skills are included in most of the standardized medical licensing examinations and internal medicine boards, medical graduates from India are at a distinct advantage.

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Conflicts of interest

There are no conflicts of interest.

References

1. Dart JK. Eye disease at a community health centre. Br Med J (Clin Res Ed) 1986;293:1477-80.
2. Shuttleworth GN, Marsh GW. How effective is undergraduate and postgraduate teaching in ophthalmology? Eye (Lond) 1997;11(Pt 5):744-50.
3. Fan JC, Sherwin T, McGhee CN. Teaching of ophthalmology in undergraduate curricula: A survey of Australasian and Asian medical schools. Clin Exp Ophthalmol 2007;35:310-7.
4. Bellan L. Ophthalmology undergraduate education in Canada. Can J Ophthalmol 1998;33:3-7.
5. Noble J, Somal K, Gill HS, Lam WC. An analysis of undergraduate ophthalmology training in Canada. Can J Ophthalmol 2009;44:513-8.
6. Medical Council of India Regulations on Graduate Medical Education; 1997. Available from: http://www.mciindia.org/Rules-and-Regulation/GME_REGULATIONS.pdf. [Last accessed on 2016 Oct 11].
7. Medical Council of India Regulations on Graduate Medical Education; 2012. Available from: http://www.psgimsr.ac.in/wp-content/uploads/2012/pdf/others/mci-regulations-graduate-medical-education-12.pdf. [Last accessed on 2016 Oct 11].
8. International Task Force on Opthalmic Education of Medical Students, International Council of Opthalmology. Principles and guidelines of a curriculum for ophthalmic education of medical students. Klin Monbl Augenheilkd 2006;223 Suppl 5:S1-19.
9. World Health Organization. Study on the Status of Ophthalmic Medical Education in the South-East Asia Region - An Overview. Available from: http://www.v2020eresource.org/content/files/SEA_OPHTHAL_121.pdf. [Last accessed on 2017 Jun 03].
10. Gupta RR, Lam WC. Medical students' self-confidence in performing direct ophthalmoscopy in clinical training. Can J Ophthalmol 2006;41:169-74.

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Appendix

Appendix 1: Questionnaire

1. Using the table below, please indicate how comfortable You feel diagnosing the following issues based on your present knowledge:
   (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, 5 = very comfortable)
   Conjunctiva/cornea 1 2 3 4 5
   Viral conjunctivitis
   Allergic conjunctivitis
   Subconjunctival hemorrhage
   Pterygium
   Corneal abrasion/ulcer
   Keratomalacia
   Corneal blindness
   Eyelid/lacrimal sac 1 2 3 4 5
   Blepharitis
   Chalazion
   Stye
   Dacryocystitis
   Lens/Uvea 1 2 3 4 5
   Cataract
   Acute iridocyclitis
   Glaucoma 1 2 3 4 5
   Primary open-angle glaucoma
   Acute angle-closure glaucoma
   Ophthalmic emergencies 1 2 3 4 5
   Globe rupture
   Chemical injuries
   Endophthalmitis
   Pediatric ophthalmology 1 2 3 4 5
   Strabismus
   Leukocoria

2. Using the table below, please indicate your knowledge on the following issues:
   (1 = very poor, 2 = poor, 3 = Average, 4 = good, 5 = Excellent)
   Ocular manifestations of systemic diseases 12345
   Diabetic retinopathy
   Hypertensive retinopathy
   Tuberculosis
   Toxemia of pregnancy
   HIV
   Community ophthalmology 1 2 3 4 5
   Magnitude of blindness in India
   NPCB and implementation at various levels
   Functions of ophthalmic assistant
   Organization of eye camp
   Medical care/referral 1 2 3 4 5
   Indications of topical antibiotics
   Indications of topical steroids
   Indications of antiglaucoma drugs
   Indications of ophthalmology referral

3. Using the table below, please indicate how comfortable You feel performing the following ophthalmology-related skills:
   (1 = not at all comfortable, 2 = somewhat comfortable, 3 = moderately comfortable, 4 = comfortable, 5 = very comfortable)
   Clinical assessment 1 2 3 4 5
   Ophthalmic history taking
   Visual acuity testing
   Test color vision
   Cover/uncover test
   Assessment of anterior chamber depth
   Pupil examination/swinging flashlight test
   Visual field testing by confrontation
   Extraocular movements
   Interpretation of fluorescein staining
   Schiotz tonometry
   Direct ophthalmoscopy
   Provide first aid in major ophthalmic emergencies

4. How many hours would you have spent in self-directed learning in ophthalmology?
   ("self-directed learning" describes a process by which individuals take the initiative, with our without the assistance of others, in diagnosing their learning needs, formulating learning goals, identify human and material resources for learning, choosing and implement appropriate learning strategies, and evaluating learning outcomes)
   1. 0–2 h 2. 2–5 h 3. 5–7 h
   4. 7–9 h 5. 0 > 10 h
5. How comfortable do you feel managing ophthalmology associated issues overall?
   1. Not comfortable at all 2. Somewhat comfortable
   3. Moderately comfortable 4. Comfortable 5. Very comfortable

THE END

Thank you for taking time for our survey.

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Original Article

Dry Eye Disease and Tear Dysfunction in Patients with Type 2 Diabetes: A Hospital-Based Study from South India

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Abstract

Purpose: Diabetics are more prone to dry eye disease (DED) and tear film dysfunction. The aim of this study was to assess the frequency of DED and tear film abnormalities in patients with type 2 diabetes compared to healthy controls. **Materials and Methods:** This was a hospital-based prospective case-control study. One hundred patients with type 2 diabetes and 100 healthy controls were enrolled by consecutive sampling. All study participants completed the Ocular Surface Disease Index (OSDI) questionnaire to score dry eye symptoms. Tear film breakup time (TFBUT), ocular surface staining with fluorescein and Schirmer's test were performed, and results were analyzed. Student's *t*-test was applied for comparisons between groups. Correlation between variables was detected using Pearson's correlation coefficient. Statistical significance was set at $P < 0.05$. **Results:** Dry eye was encountered in 54 among the 100 diabetic patients studied. Age and sex of diabetic patients were not found to affect the dry eye status. Longer duration of diabetes was significantly associated with DED ($P = 0.03$). Significant differences in OSDI scores, TFBUT, fluorescein staining, and Schirmer's test values were observed between the study groups ($P < 0.001$). An increased level of glycosylated hemoglobin was significantly associated with abnormal tear function tests. **Conclusion:** Patients with longer duration of diabetes and poor glycemic control are more predisposed to have abnormalities of the tear film and DED. Apart from conventional therapies, optimal glycemic control should be an important consideration in the management of tear dysfunction in diabetic patients.

Keywords: *Diabetes, dry eye, tear film*

Introduction

The prevalence of diabetes mellitus (DM) has increased significantly in the past several decades and the disease continues to remain a global epidemic. Estimates suggest that 439 million people in the world are likely to have DM by 2030 and this increase in number of diabetics is predicted to be disproportionately more in developing countries.[1] The two major potentially blinding complications of diabetes, namely cataract and retinopathy, have been extensively researched in literature. Apart from these, people with diabetes are also prone for ocular surface disease that includes dry eye, persistent epithelial defects, decreased corneal sensation, and tear film dysfunction. Studies have shown an intimate, complex relationship between diabetes, dry eye, and ocular surface abnormalities.[2,3]

The reported prevalence rates of dry eye in diabetics have been found to vary widely between 27% and 54%.[4,5] A recent study from a cornea specialty clinic found the overall presence and the severity of dry eye to be similar in the diabetic and nondiabetic groups. However, significant differences were observed between groups with regard to individual diagnostic assessments.[6] Studies on changes in tear function parameters in diabetes have also shown conflicting results.[7,8] These can be attributed to major differences in the study characteristics of the population, methodologies, and wide array of diagnostic methods used for assessment. Limited data exist on the occurrence of dry eye and ocular surface disease among diabetics in the Indian population.[9]

This prospective study from India sought to study the frequency of dry eye disease (DED) and tear film changes in patients with type 2 diabetes compared to healthy controls.

Materials and Methods

This was a hospital-based cross-sectional study which included 100 patients

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with type 2 DM. Consecutive patients attending the Ophthalmology Department of a tertiary care center in South India between June 2016 and 2017 were invited to participate. Exclusion criteria included the history of smoking, contact lens wear, past ocular surgery, intake of systemic medications such as antihistamines, tricyclic antidepressants, and other drugs known to cause dry eye. Patients with known ocular or systemic disease that might affect the ocular surface were also excluded from the study. One hundred individuals matched by age were enrolled as a control group. The controls were recruited from individuals attending the department of ophthalmology for routine eye examination and did not have any ocular or systemic disease other than refractive errors. The study protocol was approved by the Institutional Ethics Committee, and the study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

For each participant, a detailed ocular and general medical history, including duration of diabetes, medications, and glycosylated hemoglobin (HbA1c) value within the past 3 months was obtained. All study participants completed the Ocular Surface Disease Index (OSDI; Allergan, Inc., Irvine, CA, USA) questionnaire to score dry eye symptoms. The OSDI consists of 12 questions based on symptoms within the past 1 week. The frequency of ocular subjective symptoms (soreness, blurred vision), difficulty with vision-related function (television, visual display unit, driving, and reading) and discomfort due to environmental triggers (low humidity, high wind) are assessed on a Likert scale. The scores range from 0 (least severe) to 100 (most severe).[10]

All participants underwent a general physical and comprehensive ophthalmic examination with slit lamp, including best-corrected visual acuity. Evaluation of the ocular surface and tear film was done by performing the following tests described below in a sequential manner.[11]

Tear film breakup time (TFBUT) was performed by applying a fluorescein strip (Fluostrip/India/Contacareophthalmics and diagnostics) moistening it with a drop of sterile saline, to the lower tarsal conjunctiva without the use of topical anesthesia. The participants were asked to blink several times and then cease blinking until instructed. The tear film was observed under slit lamp with cobalt blue filter. The time interval between the last blink to the appearance of the first random dry spot in the cornea was taken as the TFBUT. The average of three consecutive breakup times was calculated and recorded.

The ocular surface was evaluated by staining the cornea with fluorescein and Oxford grading scheme was used for the recording of staining severity. This scheme has five panels, labeled A to E, with staining represented by punctate dots that increase logarithmically between panels.[12] The appearance of staining on the exposed interpalpebral conjunctiva and cornea is compared with each panel, and the closest match gives the grade. Based on the number of dots seen a grade between 0 and 3 was given for staining on the cornea, nasal, and temporal conjunctiva. The individual scores were added giving a maximum possible total of 9 points. A score of 3 and above was considered to be abnormal.[13]

Schirmer's test was performed without topical anesthesia using standardized Whatman filter paper 41(Tearstrip/India/Contacareophthalmics and diagnostics). The strip was folded at the notch and placed at the junction of the middle and lateral thirds of the lower eyelids and left in place for 5 min. A value of <10 mm wetting in 5 min was considered abnormal.

Due to lack of consensus among the diagnostic criteria for dry eye described in different clinical studies we adopted the following criteria used by Alves *et al.*: OSDI score >20 and/or Schirmer test <10 mm or TFBUT <6 s and/or ocular surface staining score >3.[14] A diagnosis of DED was made if the participant had at least one positive test according to these criteria. In various clinical studies, authors have adopted different approaches for the diagnosis of dry eye on an *ad hoc* basis. Several studies have used the positivity of one or more of the tests (tear breakup time [TBUT] or Schirmer or OSDI) for the diagnosis of dry eye.[5,15,16] Since there is no uniform set of diagnostic criteria for DED, we considered a diagnosis of dry eye if the patient presented at least one positive test according to these preestablished criteria.

All measurements were performed by the same investigators on the same day under similar testing conditions. Clinical evaluations were done on both eyes, and the mean of the two eyes was used for analysis. To eliminate diurnal variation and possible diagnostic error, all tests were performed in the morning.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 24. Descriptive statistics was presented as a mean ± standard deviation. The Shapiro–Wilk test was used to test the normality of data distribution. Student's *t*-test was applied for comparisons between two groups. Correlation between variables was detected using Pearson's correlation coefficient. Statistical significance was set at $P < 0.05$.

Results

The mean age of controls and diabetic patients was 54.7 ± 10.2 and 55.7 ± 9.5 years, respectively. There were 53 females among 100 controls and 45 females among 100 diabetics ($P = 0.25$). The baseline characteristics of the study participants are shown in Table 1. The mean duration of diabetes among diabetics with the dry

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eye was significantly higher compared to those without dry eye (P = 0.03).

Table 2 shows the comparison of OSDI, Schirmer test, fluorescein staining, and TFBUT between groups using the Student's *t*-test. Significant differences in OSDI scores, Schirmer test values, fluorescein staining, and TFBUT were observed between the study groups. OSDI Score was >20 in 32 participants (32%) in the diabetic group. Nineteen percent had OSDI scores between 12 and 20. In the diabetic group, 7 participants (7%) had Schirmer scores <10 mm and 30 (30%) had TBUT <10 s. Ocular surface staining score of >3 was encountered in 51% of the diabetic participants. None of the participants in the control group had abnormal OSDI, Schirmer test, fluorescein staining, or TFBUT.

We evaluated the relationship between HbA1c, duration of diabetes, and ocular surface parameters [Table 3]. The analysis showed significant positive correlation between the duration of diabetes and OSDI scores as well as fluorescein staining of the ocular surface. Although negative, the correlation between duration of diabetes and Schirmer/TFBUT was not statistically significant.

Table 1: Baseline characteristics of study participants

Characteristic	Controls	Diabetics without dry eye	Diabetics with dry eye
Number (%)	100	46	54
Age (years)	54.7±10.2	54.6±9.5	56.7±9.6
Females (%)	53 (53)	22 (47.8)	23 (42.6)
Duration of diabetes (years)	-	5.6±6.1	8.05±5.1*
HbA1c	5.3±0.3	8.5±2.2	9.4±2.3

*P=0.03. HbA1c: Glycosylated haemoglobin

Table 2: Comparison of ocular surface disease index scores, Schirmer test, fluorescein staining and tear film breakup time between groups

	Controls	Diabetics	*P*
OSDI	6.9±6.5	15.1±12.4	<0.001
Schirmer test	24.1±5.1	20.8±7.3	<0.001
Fluorescein staining	1.85±0.24	3.8±2.1	<0.001
TFBUT	16.7±3.6	12.6±4.5	<0.001

OSDI: Ocular surface disease index, TFBUT: Tear film breakup time

Table 3: Correlation coefficients between duration of diabetes, glycosylated haemoglobin and ocular surface parameters

Variable	Duration of diabetes (*P*)	HbA1c (*P*)
OSDI	0.342 (<0.001)	0.340 (<0.001)
Schirmer test	−0.07 (0.47)	−0.23 (0.01)
Fluorescein staining	0.3 (0.003)	0.3 (0.001)
TFBUT	−0.1 (0.27)	−0.21 (0.03)

HbA1c: Glycosylated haemoglobin, OSDI: Ocular surface disease index, TFBUT: Tear film breakup time

The HbA1c level was found to correlate positively with OSDI scores and ocular surface staining. HbA1c levels correlated negatively with Schirmer values and TFBUT.

Discussion

The purpose of this clinical study was to assess the frequency of DED and tear film abnormalities in people with diabetes and compare with that of healthy controls. The OSDI scores were higher, and tear film parameters were significantly altered in diabetic patients.

Out of the 100 diabetics studied, 54 were found to have dry eye. Mean age of diabetic individuals with and without dry eye were found to be similar (P = 0.262). Among the 100 patients with diabetes 45 (45%) were females, of which 23 (42.6%) had dry eye and 22 (47.8%) did not have evidence of dry eye (P = 0.600). Hence, there was no statistically significant association between dry eye and age or gender of the diabetic patients. The frequency of dry eye was significantly higher in patients with longer duration of diabetes.

Studies on dry eye in diabetics have revealed wide variation in the prevalence rates due to different diagnostic criteria as well as patient characteristics. Our observations are similar to that of Fuerst *et al*. who have noted a dry eye prevalence of 54% based on tear osmolarity.[16] Manaviat *et al*. performed Schirmer and TBUT tests and utilized the criterion of one positive test to diagnose dry eye. Out of the 199 participants studied, 108 (54.3%) were found to have dry eye.[5]

In contrast to these hospital-based studies, a community-based study from Shanghai has reported 17.5% prevalence of definitive DED.[17] Apart from methods used for diagnosis, several other factors such as age, gender, duration of diabetes, and metabolic control have also been reported to affect the prevalence of DED.[18,19]

Duration of diabetes was found to be significantly associated with dry eye in our study similar to observations of Manaviat *et al*.[5] Chronic hyperglycemia, diabetic neuropathy, decreased insulin levels, microvasculopathy, and systemic hyperosmotic disturbances in long-standing diabetes lead to dysfunction of the lacrimal functional unit as well as abnormal tear dynamics and DED.[20] However, some studies have found no correlation between DED and duration of diabetes.[4]

Diabetics in this study had significantly higher OSDI scores as compared to the control group. In contrast to our observations, Beckman found the mean OSDI scores to be higher in nondiabetic patients as compared to diabetic patients.[6] This could be attributed to the fact that the mean duration of diabetes in this study was much less (6.9 ± 5.7 years) compared to their study where the mean diabetic duration was 11.1 ± 9 years (range 1–41 years). Long-standing diabetes has been reported to

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be associated with reduced perception of dry eye symptoms due to impaired corneal sensation and therefore decreased OSDI scores.[16]

We found a significant positive correlation between OSDI scores and duration of diabetes in our study. One possibility for the observed positive correlation is that, as mentioned previously participants with shorter duration of diabetes have normal corneal sensation and therefore, will be aware of dry eye symptoms. Our findings are in agreement with the natural history of DED proposed by Bron *et al.* This model comprises three stages, namely initiation of DED, reflex compensation, and loss of compensatory response due to sensory nerve impairment.[21]

OSDI scores were found to correlate positively with HbA1c levels in our study. Our observations are in line with previous studies elsewhere, which have shown a positive correlation between OSDI scores and glycemic control.[22,23]

Although mean Schirmer test scores were in the normal range for both study groups, statistically significant differences were found between the Schirmer values in control and diabetic groups. Our observations are similar to that of Beckman and Yoon *et al*. who found the values of the Schirmer's test to be >10 mm in both diabetic and nondiabetic patients.[6,24] In contrast to these findings, the Schirmer values averaged 7.4 ± 0.38 mm in patients with diabetes versus 13.53 ± 0.50 mm in the controls in a series by Dogru *et al*.[7] Twenty eyes (22.7%) of the diabetic patients in their series had Schirmer test values <5 mm. In our study, only 3% had Schirmer scores <5 mm. Reduced Schirmer scores in people with diabetes compared with nondiabetic participants have been documented in several studies. Some studies have noted a reduction in basal tear secretion, whereas some authors have reported only the reflex tear secretion to be significantly reduced in diabetics.[8,19,25]

We found a significant negative correlation between Schirmer test scores and HbA1c levels. Our observations are in line with that of Ozdemir *et al*. who also found the Schirmer test and TBUT results to be worse in poorly controlled diabetics compared to those with good metabolic control. Kaiserman *et al*. have reported increasing consumption of ocular lubrication with increased mean annual levels of HbA1c.[18] Poor Schirmer test scores were not related to the duration of diabetes in our study which is in agreement with previous studies.[19]

Significant staining of the ocular surface with fluorescein was noted in diabetic patients in our study. Impaired corneal epithelial barrier function in diabetic patients has been reported by several studies and poor metabolic control, diabetic neuropathy, and advanced retinopathy have been found to correlate with epitheliopathy.[3,24] Ocular surface staining scores were found to correlate positively with duration of diabetes as well as HbA1c levels in this study. Accumulation of advanced glycation end products (AGE) on the basement membrane of the corneal epithelium has been shown to play a causative role in corneal epithelial disorders of diabetic patients.[26] Since HbA1c is a product of early stage glycation, it correlates well with AGE formation and hence impaired barrier function resulting in staining of the ocular surface.

Significant differences in TFBUT values were noted between diabetic and nondiabetic participants, and lower TFBUT scores were noted in patients with poor glycemic control. These results are comparable to that of Yoon *et al*. who had evaluated the tear film and ocular surface changes among diabetics from Korea.[24] Ma *et al*. have reported a significant inverse correlation between serum HbA1c levels and noninvasive TBUT in their study on 80 Chinese participants with type 2 diabetes.[23] These results suggest that poor glycemic control is associated with an unstable tearfilm and ocular surface disease in diabetic patients.

The study has few limitations. We had not performed tear film osmolarity assessment which is an objective test for the diagnosis and follow-up of DED. However, test combination of OSDI/Schirmer test/TFBUT has been shown to have an accuracy of 99.3% for the diagnosis of DED.[14] Sample size and conduct of the study at a single site are other potential limitations of the study. Further studies on large samples with the inclusion of tests such as corneal esthesiometry, tear osmolarity, and other markers of DED will provide valuable insight into the potential mechanisms and treatment options for diabetic DED.

Conclusion

There are limited data available in the literature about the frequency of dry eye and tear film dysfunction in diabetics from India. With the increasing prevalence of diabetes and its associated complications in India, there is need for more studies from this part of the world. Nearly half of the people with diabetes in this study had features consistent with DED. Patients with longer duration of diabetes and higher HbA1c levels are more predisposed to have abnormalities of tear film and DED. Examination of the ocular surface and testing for DED should be added to the routine screening of diabetes. Apart from conventional therapies such as lubricants, anti-inflammatory agents, and antioxidants optimal glycemic control should be an important consideration in the management of tear dysfunction in diabetic patients.

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Conflicts of interest

There are no conflicts of interest.

References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
2. Achtsidis V, Eleftheriadou I, Kozanidou E, Voumvourakis KI, Stamboulis E, Theodosiadis PG, *et al.* Dry eye syndrome in subjects with diabetes and association with neuropathy. Diabetes Care 2014;37:e210-1.
3. Gekka M, Miyata K, Nagai Y, Nemoto S, Sameshima T, Tanabe T, *et al.* Corneal epithelial barrier function in diabetic patients. Cornea 2004;23:35-7.
4. Najafi L, Malek M, Valojerdi AE, Aghili R, Khamseh ME, Fallah AE, *et al.* Dry eye and its correlation to diabetes microvascular complications in people with type 2 diabetes mellitus. J Diabetes Complications 2013;27:459-62.
5. Manaviat MR, Rashidi M, Afkhami-Ardekani M, Shoja MR. Prevalence of dry eye syndrome and diabetic retinopathy in type 2 diabetic patients. BMC Ophthalmol 2008;8:10.
6. Beckman KA. Characterization of dry eye disease in diabetic patients versus nondiabetic patients. Cornea 2014;33:851-4.
7. Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. Ophthalmology 2001;108:586-92.
8. Saito J, Enoki M, Hara M, Morishige N, Chikama T, Nishida T. Correlation of corneal sensation, but not of basal or reflex tear secretion, with the stage of diabetic retinopathy. Cornea 2003;22:15-8.
9. Kesarwani D, Rizvi SW, Khan AA, Amitava AK, Vasenwala SM, Siddiqui Z. Tear film and ocular surface dysfunction in diabetes mellitus in an Indian population. Indian J Ophthalmol 2017;65:301-4.
10. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the ocular surface disease index. Arch Ophthalmol 2000;118:615-21.
11. Methodologies to diagnose and monitor dry eye disease: Report of the diagnostic methodology subcommittee of the international dry eye workShop (2007). Ocul Surf 2007;5:108-52.
12. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea 2003;22:640-50.
13. McGinnigle S, Naroo SA, Eperjesi F. Evaluation of dry eye. Surv Ophthalmol 2012;57:293-316.
14. Alves M, Reinach PS, Paula JS, Vellasco e Cruz AA, Bachette L, Faustino J, *et al.* Comparison of diagnostic tests in distinct well-defined conditions related to dry eye disease. PLoS One 2014;9:e97921.
15. Aljarousha M, Badarudin NE, Che Azemin MZ. Comparison of dry eye parameters between diabetics and non-diabetics in district of Kuantan, Pahang. Malays J Med Sci 2016;23:72-7.
16. Fuerst N, Langelier N, Massaro-Giordano M, Pistilli M, Stasi K, Burns C, *et al.* Tear osmolarity and dry eye symptoms in diabetics. Clin Ophthalmol 2014;8:507-15.
17. Zou X, Lu L, Xu Y, Zhu J, He J, Zhang B, *et al.* Prevalence and clinical characteristics of dry eye disease in community-based type 2 diabetic patients: The beixinjing eye study. BMC Ophthalmol 2018;18:117.
18. Kaiserman I, Kaiserman N, Nakar S, Vinker S. Dry eye in diabetic patients. Am J Ophthalmol 2005;139:498-503.
19. Ozdemir M, Buyukbese MA, Cetinkaya A, Ozdemir G. Risk factors for ocular surface disorders in patients with diabetes mellitus. Diabetes Res Clin Pract 2003;59:195-9.
20. Zhang X, Zhao L, Deng S, Sun X, Wang N. Dry eye syndrome in patients with diabetes mellitus: Prevalence, etiology, and clinical characteristics. J Ophthalmol 2016;2016:8201053.
21. Bron AJ, Yokoi N, Gafney E, Tiffany JM. Predicted phenotypes of dry eye: Proposed consequences of its natural history. Ocul Surf 2009;7:78-92.
22. Kan S, Acar U, Kizilgul M, Beyazyildiz E, Cankaya AB, Ozcelik O, *et al.* The effects of blood glucose regulation on tear function tests in diabetic patients. J Fr Ophtalmol 2017;40:499-504.
23. Ma A, Mak MS, Shih KC, Tsui CK, Cheung RK, Lee SH, *et al.* Association of long-term glycaemic control on tear break-up times and dry eye symptoms in Chinese patients with type 2 diabetes. Clin Exp Ophthalmol 2018;46:608-15.
24. Yoon KC, Im SK, Seo MS. Changes of tear film and ocular surface in diabetes mellitus. Korean J Ophthalmol 2004;18:168-74.
25. Lv H, Li A, Zhang X, Xu M, Qiao Y, Zhang J, *et al.* Meta-analysis and review on the changes of tear function and corneal sensitivity in diabetic patients. Acta Ophthalmol 2014;92:e96-104.
26. Kaji Y, Usui T, Oshika T, Matsubara M, Yamashita H, Araie M, *et al.* Advanced glycation end products in diabetic corneas. Invest Ophthalmol Vis Sci 2000;41:362-8.

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Original Article

Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging

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Purpose: To investigate the retrobulbar circulatory parameters in type 2 diabetes mellitus patients with color Doppler imaging (CDI) and compare the results with nondiabetic controls. **Methods:** This prospective study included 50 type 2 diabetic patients and 50 age-matched controls. Seven field stereo fundus photography was used to diagnose and classify diabetic retinopathy (DR). Diabetic patients were further divided into two: Group 1, consisted of patients with no DR, mild and moderate non-proliferative DR (n = 36); Group 2, severe nonproliferative and proliferative DR (n = 14). CDI was performed using Philips iU22 xMATRIX ultrasound. The peak systolic velocity (PSV), end-diastolic velocity (EDV), resistivity index (RI) and pulsatile index (PI) of ophthalmic (OA), posterior ciliary artery (PCA), and central retinal artery (CRA) along with central retinal vein (CRV) were recorded. **Results:** RI in the ophthalmic artery was significantly higher in both DR groups than the control group (P = 0.000). Diabetic Group 1 had decreased blood flow velocity (PSV and EDV) in PCA compared to controls (P = 0.046 and P = 0.010, respectively). Group 2 DR had significantly reduced EDV and increased RI in CRA compared to Group 1 (P = 0.015). Binary logistic regression analysis revealed glycosylated hemoglobin and RI of OA to be independent risk factors of DR. **Conclusion:** Significant changes in resistivity index and flow velocities were observed in the retrobulbar vessels, especially in ophthalmic artery in diabetics compared to controls. CDI with results of increased resistance or decreased flow could be useful to predict individuals at higher risk for developing severe DR.

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Ocular manifestations of diabetes are numerous and among them, diabetic retinopathy (DR) is a major clinical issue for ophthalmologists. DR is the most frequent cause of preventable blindness in working-age adults (20–74 years) and is therefore considered as a major public health issue.[1] Though treatable in appropriate stages, the prevention of retinal complications of diabetes and consequent visual impairment is vital to reduce the burden of DR. There is a felt need for better diagnostic modalities and surrogate markers in the evaluation and management of DR.

In spite of decades of research, the mechanisms behind the onset and progression of DR still remain unclear. From a hemodynamic point of view, evidence has shown that there is an early reduction in blood flow to retina before the onset of DR followed by a gradual increase as the retinopathy progresses.[2] Color Doppler imaging (CDI) is an established, safe, and noninvasive ultrasonic method useful for qualitatively and quantitatively assessing the blood flow velocities and indirect blood flow.[3] CDI has been suggested to be valuable in the diagnosis and management of several ocular diseases with altered hemodynamics such as glaucoma, retinal vascular diseases, ischemic optic neuropathy, and ocular ischemic syndrome.[4] An analysis of retrobulbar hemodynamics in diabetes using CDI can provide insight into the pathogenesis and prospective treatment for DR as well as predict those at higher risk of developing vision threatening DR. Several authors have evaluated the circulatory changes in diabetics using CDI and reported conflicting results. While few studies have reported a reduction in the retrobulbar blood flow velocities,[5-7] others have noted an increase[8] or no change.[9] Studies on the resistive index (RI) of orbital vessels which is a measure of peripheral vascular resistance have also shown significant discrepancies.[10,11] Factors such as demographic variables, duration of diabetes, methods of DR assessment, positions of CDI, and sample sizes account for the observed discrepancies. Moreover, most studies have not excluded patients with hypertension and those on medications affecting systemic blood flow which can influence the results. Studies on orbital hemodynamic alterations among type 2 diabetics from India are relatively few. The diabetic population in India is close to hitting the alarming mark of 69.9 million by 2025 and 80 million by 2030, denoting an expected increase of 266%. Hence, India is deemed as the world's capital of diabetes.[12] With increasing prevalence of diabetes and DR, it is important to know the hemodynamic alterations among diabetics from the Indian population.

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The objective of this study was to evaluate the flow velocities and vascular resistance of orbital blood vessels using CDI in a pure cohort of type 2 diabetic patients from India and compare the results with healthy controls.

Methods

This prospective hospital-based study was carried out at a tertiary care institution from south India between March 2017 and April 2019. The study included consecutive patients of type 2 DM (as per definition of the American Diabetes Association) attending the ophthalmology outpatient clinic for the screening of retinopathy. Exclusion criteria were history of any ocular disease that might affect ocular blood flow such as glaucoma, nondiabetic retinal vascular occlusion, ocular inflammation, high myopia, previous ocular surgery, or panretinal photocoagulation. Patients with systemic hypertension, dyslipidemia, nephropathy, and cardiovascular diseases were excluded from the study. Pregnant and breastfeeding women were not included. Patients taking medications known to affect systemic hemodynamics such as Angiotensin-converting enzyme inhibitors, calcium channel blockers, and anti-migraine drugs were also excluded.

50 age-matched healthy subjects were enrolled as a control group. The control subjects were recruited from individuals attending the department of ophthalmology for a routine eye examination. They were not on any medication and did not have ocular or systemic disease. The study was conducted according to the principles of the Declaration of Helsinki and the study protocol was approved by the institutional ethics committee. Informed consent was obtained from all participants.

Information regarding age, gender, body mass index, duration of diabetes, antidiabetic medications, and blood pressure were obtained from all participants. Serum glycosylated hemoglobin (HbA1c) was determined from fasting venous blood sample.

All study participants underwent a comprehensive ophthalmic examination including dilated fundus with slit-lamp biomicroscope using a 90 diopter lens and indirect ophthalmoscopy. A 30° stereoscopic fundus photograph of 7 standard early treatment diabetic retinopathy study (ETDRS) fields was taken from both eyes of diabetic patients using a digital fundus camera (TRC-50 DX Mydriatic retinal camera; Topcon Medical Systems, Oakland, NJ).

The presence, as well as the severity of diabetic retinopathy, was determined by grading of color fundus photographs following the international clinical disease severity scale for diabetic retinopathy which is based on the findings of WESDR (Wisconsin Epidemiological Study of Diabetic Retinopathy) and ETDRS.[13] The diabetic patients were assigned to one of the following groups namely no retinopathy, mild nonproliferative diabetic retinopathy (NPDR), moderate NPDR, severe NPDR, and proliferative diabetic retinopathy (PDR). The

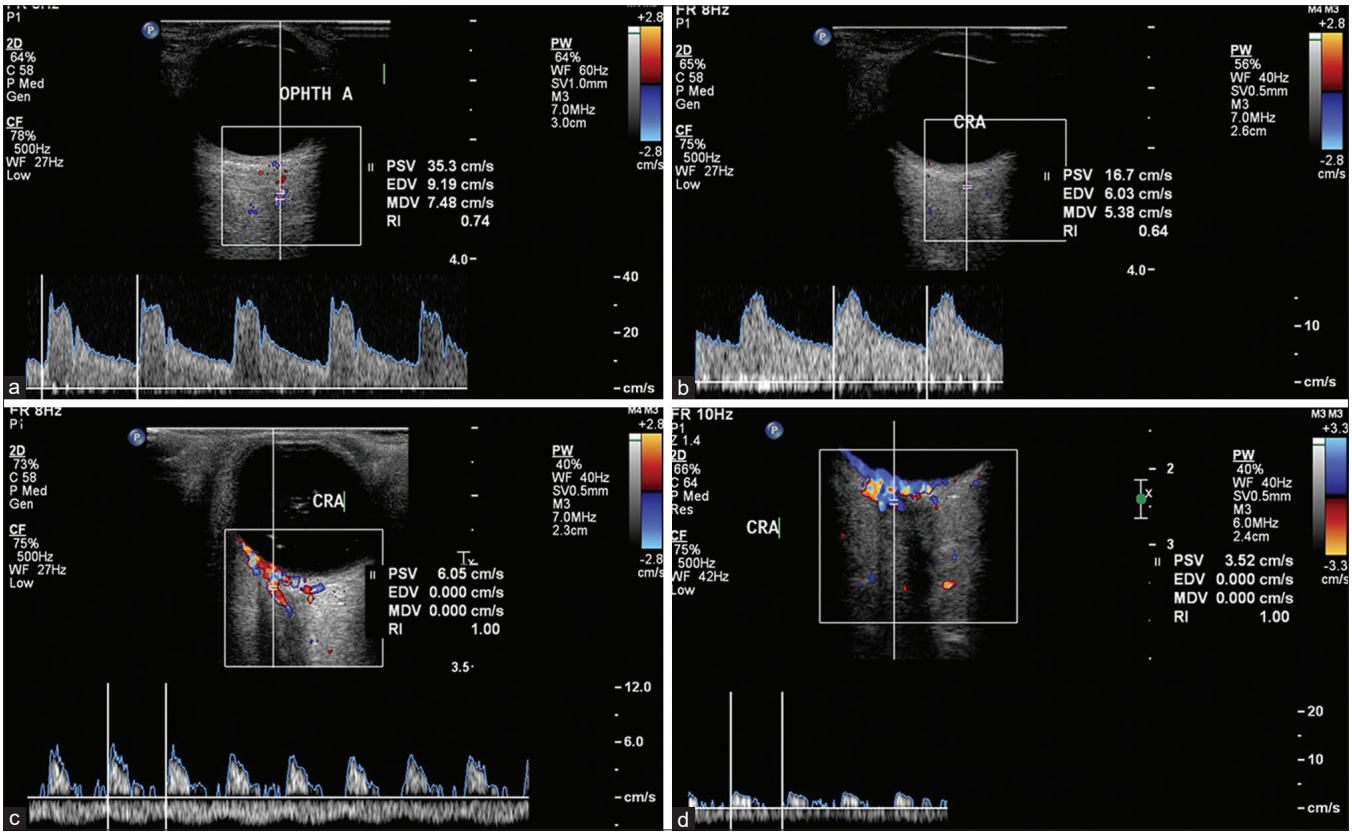


Figure 1: (a) Color Doppler image (CDI) showing normal color flow and spectral waveform in ophthalmic artery with preserved M wave pattern; (b) CDI showing normal color flow and spectral waveform in central retinal artery; (c) CDI showing low-velocity monophasic flow in central retinal artery in a patient with moderate NPDR; (d) CDI showing monophasic spectral waveform with absent diastolic flow in central retinal artery in a patient with severe NPDR

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eye with worse retinopathy was included in the study. If both eyes had equal retinopathy, the right eye was assigned to the study.

Color Doppler sonography of the eye was performed for cases and controls by a masked expert Radiologist using a color Doppler unit and a 12 to 5 MHZ linear-array transducer (Philips iU22 xMATRIX ultrasound system). The patients were examined in the supine position and care was taken to avoid any undue pressure on the eye. Examiner's hand was rested on the orbital margin and sterile coupling gel was applied over closed eyelids. The Doppler signal was registered online after the cursor was positioned on the region of interest. To reduce errors in measurement of velocities, angle correction was applied to the pulsed Doppler recordings.

The peak systolic velocity (PSV) and end-diastolic velocity (EDV) readings were obtained from the ophthalmic artery (OA), central retinal artery (CRA), and the posterior ciliary artery (PCA). Since the velocity wave in a central retinal vein (CRV) is not in direct relation to the cardiac cycle its hemodynamic parameters were expressed as maximum (V max) and minimum (V min) blood velocity. Signals from the ophthalmic artery were obtained from the medial aspect of the eyeball, superior to the optic nerve. The central retinal artery and vein were located within the optic nerve, around 10 mm behind the eyeball. [Fig. 1] Signals from the temporal branch of the short posterior ciliary artery were located lateral to the optic nerve.

With the help of PSV and EDV, we calculated the vascular resistance expressed by the resistivity index and pulsatile index using Pourcelot's formula.

Resistive index (RI) = (PSV-EDV)/PSV; Pulsatile index (PI) = (PSV-EDV)/Vmean

Statistical analysis

For statistical analysis diabetic patients were further divided into two groups; Group 1 included patients with no retinopathy, mild NPDR, and moderate NPDR while Group 2 consisted of patients with severe NPDR and PDR.

Statistical analysis was performed using statistical package for social sciences (SPSS, SPSS Inc, Chicago, IL, USA) version 24. Data were expressed as mean ± standard deviation. The normality of data distribution was tested by the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) with a *post hoc* Bonferroni was used for the analysis of normally distributed continuous data. Kruskal-Wallis analysis was used for analyzing non-normally distributed data. A comparison between hemodynamic parameters was calculated by ANOVA followed by Tukey-Kramer *post hoc* comparison. Pearson's coefficient was used to study the correlation between variables.

Table 1: Demographic and clinical characteristics of controls and diabetic subjects

Characteristic	Controls (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
Age (years)	56.5±9.00	55.5±8.60	51.9±5.64	0.209
Body mass index (kg/m^2)	23.34±3.62	24.81±3.15	24.53±3.88	0.141
Median Systolic BP (mmHg)	110 (20)	120 (18.75)	125 (12.5)	0.076
Median Diastolic BP (mmHg)	70 (30)	76.5 (10)	70 (10)	0.259
Median duration of diabetes (years)	-	3 (8)	10 (11)	0.115
Glycosylated hemoglobin (%)	5.81±0.29	9.09±2.13	10.86±2.33	0.014*

BP=Blood pressure; *P<0.05

Table 2: Hemodynamic parameters of retrobulbar blood vessels among controls and patient groups

Hemodynamic parameters (cm/s)	Control (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
OA PSV	29.72±8.10	30.48±9.78	28.76±12.84	0.838
OA EDV	6.87±2.69	6.34±3.41	5.09±3.50	0.166
OA RI	0.75±0.06	0.79±0.06	0.83±0.05	0.000*
OA PI	1.58±0.27	1.74±0.30	1.86±0.25	0.002*
CRA PSV	12.42±3.85	10.40±4.87	8.42±2.92	0.004*
CRA EDV	2.73±1.17	2.33±1.27	1.27±0.94	0.000*
CRA RI	0.77±0.06	0.74±0.16	0.85±0.11	0.019*
CRA PI	1.63±0.30	1.65±0.50	2.08±0.65	0.004*
PCA PSV	15.44±5.61	12.81±3.83	14.95±5.15	0.054
PCA EDV	4.19±2.23	2.97±1.29	3.33±1.63	0.011*
PCA RI	0.73±0.06	0.75±0.08	0.78±0.08	0.061
PCA PI	1.45±0.24	1.58±0.37	1.68±0.45	0.044*
CRV V max	6.14±1.63	6.12±1.66	5.85±2.07	0.853
CRV V min	3.51±0.97	3.34±0.89	3.02±1.05	0.243
CRV RI	0.41±0.11	0.44±0.11	0.46±0.08	0.329
CRV PI	0.60±0.24	0.65±0.25	0.70±0.17	0.350

OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=Maximum blood velocity; V min=Minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

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Binary logistic regression analysis was performed to determine independent risk factors for retinopathy. All tests for statistical significance were two-tailed and performed assuming a type I error probability of <0.05.

Results

Demographic and clinical parameters

The study included 52 males and 48 females. The age of patients ranged from 41 to 76 years. The duration of diabetes ranged from 6 months to 20 years. Among the diabetic patients, 29 were on oral hypoglycemic agents, 9 were on insulin, and 12 were on combination therapy. Group 1 (n = 36) consisted of 16 patients with no DR, 13 with mild NPDR, and 7 with moderate NPDR. Group 2 (n = 14) consisted of 6 patients with severe NPDR and 8 with PDR. There were no statistically significant differences in the age, body mass index and blood pressure between controls and diabetic groups. Glycosylated hemoglobin (HbA1c) levels were found to be significantly higher in Group 2 diabetic patients (P = 0.014). No significant difference was noted in the duration of diabetes among diabetic groups. The demographic and clinical characteristics of controls and diabetic subjects are presented in Table 1.

Hemodynamic parameters

Table 2 shows the hemodynamic parameters of retrobulbar blood vessels among controls and patient groups.

Ophthalmic artery

Significant differences were noted in the RI and PI values of the ophthalmic artery between controls and diabetic patients with various DR grades. OA flow velocities did not vary between groups.

Table 3: Pearson correlation coefficients between the duration of diabetes, HbA1c, and hemodynamic parameters

Variable	Duration of diabetes	HbA1c
OA PSV	0.021 (0.885)	-0.120 (0.407)
OA EDV	-0.141 (0.328)	-0.055 (0.706)
OA RI	0.314* (0.026)	0.097 (0.504)
OA PI	0.299* (0.035)	0.078 (0.588)
CRA PSV	-0.135 (0.349)	-0.269 (0.059)
CRA EDV	-0.464* (0.001)	-0.299*(0.035)
CRA RI	0.375* (0.007)	-0.046 (0.753)
CRA PI	0.487* (0.000)	0.131 (0.370)
PCA PSV	-0.052 (0.722)	0.170 (0.239)
PCA EDV	-0.234 (0.102)	0.196 (0.172)
PCA RI	0.332* (0.019)	-0.075 (0.606)
PCA PI	0.321*(0.023)	-0.085 (0.556)
CRV V max	0.134 (0.354)	0.128 (0.375)
CRV V min	-0.143 (0.322)	-0.058 (0.687)
CRV RI	0.390* (0.005)	0.172 (0.233)
CRV PI	0.415*(0.003)	0.210 (0.144)

Corresponding P values are shown in brackets. HbA1c=Glycosylated hemoglobin; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=maximum blood velocity; V min=minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

Central retinal artery and vein

The hemodynamic parameters of CRA were significantly different between controls, DR Group 1, and Group 2 whereas no such difference was seen in the CRV. *Post hoc* comparison revealed a significantly reduced PSV and EDV of CRA (P = 0.005 and 0.000, respectively) along with increased PI (P = 0.003) in DR group 2 compared to controls. Patients with group 2 DR also demonstrated a significant decrease of EDV and increased RI of CRA compared to group 1 (P = 0.015). PSV, EDV, and RI of CRA did not differ significantly between controls and DR group 1.

Posterior ciliary artery

The EDV and PI of PCA were found to vary significantly between controls and diabetic groups. Significant reduction in PSV and EDV of PCA was noted in DR group 1 compared to controls (P = 0.046 and P = 0.010, respectively after *post hoc* comparison).

Relation between duration of diabetes, HbA1c, and hemodynamic parameters

Duration of diabetes correlated positively with the resistive and pulsatile indices of all retrobulbar vessels studied namely ophthalmic artery, CRA, PCA, and CRV. HbA1c levels, as well as the duration of diabetes, were found to correlate negatively with EDV of CRA. No correlation was found between duration of diabetes and HbA1c levels (R = 0.183, P = 0.203). Table 3 shows the relationship between duration of diabetes, HbA1c levels, and hemodynamic parameters.

Binary logistic regression analysis revealed that HbA1c levels and RI of OA were independent risk factors of retinopathy.

Discussion

The main observations of our study are as follows: (1) RI in the ophthalmic artery was significantly higher in both DR groups than the control group and was an independent risk factor of retinopathy, (2) Patients with Group 1 DR had decreased blood flow velocity (PSV and EDV) in the posterior ciliary artery compared to control group, (3) Patients with Group 2 DR had significantly reduced EDV and increased RI in the CRA compared to Group 1, (4) Compared to the control group, patients with Group 2 DR had slower flow velocities and increased PI in CRA but RI values did not vary significantly.

Our finding of decreased EDV in the posterior ciliary artery in patients with Group 1 DR supports the hypothesis that choroidal blood flow is affected in the early stages of DR. Laser Doppler flowmetry has shown decreased choroidal blood flow in the foveal region in early DR.[14] Choroidal vascularity index (CVI) analysis by swept-source optical coherence tomography (OCT) has also shown reduced CVI in patients with mild/moderate NPDR.[15] Cao *et al.* have reported a 4 times more choriocapillary loss in diabetic eyes compared to non-diabetic eyes in histopathologic studies.[16] Ferrara *et al.* have demonstrated the loss of intermediate and large blood vessels in the Sattler's layer and Haller's layers in diabetic eyes in choroidal OCT.[17] Our observation of decreased EDV in the PCA suggests increased peripheral vascular resistance of the choroidal circulation in early DR and is in concordance with these studies. Dimitrova *et al.* reported decreased EDV and increased RI in PCA in patients

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with background DR compared to controls in CDI of diabetic patients performed in sitting posture.[6] The authors have suggested that erect posture during CDI measurement is essential to appreciate significant alterations in posterior ciliary circulation. Supine posture has been suggested to increase EDV and decrease the RI.[18] As autoregulation and autonomic nervous responses are altered in diabetics, the correlation of change in circulatory parameters between normal individuals and diabetics associated with postural variation may not be expected to be equal. However, CDI was performed in supine posture in our patients. Our observations could be attributed to the shorter duration of diabetes in our patients than theirs.

RI in OA was higher in both DR groups compared with controls in our study. These findings are similar to those of previous studies.[5,19] Basturk *et al.* evaluated the association between the resistivity index of orbital arteries and DR in 91 types 2 diabetics with microalbuminuria using CDI. The authors have demonstrated increased RI values of all orbital arteries in patients with DR compared to those without DR and controls. RI value of ≥ 0.72 in OA indicated the presence of DR with 78.4% sensitivity and 70% specificity.[10] Our finding of increased RI in the OA in both DR groups can be attributed to the statistically nonsignificant reduction in EDV observed in diabetic subjects because of downstream vascular changes in the retina and choroid. We did not observe significant differences in CRA parameters between controls and DR Group 1. Hence, we believe that the OA hemodynamic parameters in our study probably represent alterations in choroidal blood flow. It has been suggested that of the total ocular blood supply from OA, less than 10% flows to the retina while the remainder is directed to choroid via the ciliary circulation due to the high metabolic demand of photoreceptors.[20] Moreover, the diastolic blood flow of PCA is higher than CRA since these vascular channels of choroid have low resistance.[21]

Table 4: Various published studies on ocular circulation in diabetic patients using color Doppler imaging

Study	Country	Sample size	Duration of diabetes (years)	Findings
Arai *et al.*, 1998[9]	Japan	74 Controls: 22 NDR: 27 DR: 25	NA	Significant increase in RI of CRA in patients with DR compared to those without DR and controls (P<0.01)
MacKinnon *et al.*, 2000[5]	Scotland	62 Controls: 17 NDR/BDR: 24 PPDR/PDR: 21	NDR/BDR: 10 PPDR/PDR: 12	Significant decrease in both PSV and EDV of CRA in the PPDR/PDR group compared to NDR/BDR and control group (P<0.05) while RI showed no significant change. RI of OA was increased in diabetics compared to controls.
Dimitrova *et al.* , 2001[6]	Japan	95 Controls: 22 NDR: 38 BDR: 35	NDR: 9.0±7.2 BDR: 18. 9±11.2	Significant decrease in EDV and increase in RI of PCA in BDR group compared to controls (P=0.01 and 0.0003, respectively)
Gracner *et al.*, 2004[8]	Slovenia	66 Controls: 22 NPDR: 22 SNPDR/PDR: 22	13.5±9.31	Significant increase in PSV of OA in the SNPDR/PDR group compared to controls. Significant increase in RI of PCA in SNPDR/PDR group compared to NPDR and controls
Sullu *et al.*, 2005[7]	Turkey	34 Controls: 20 PDR: 14	13.5±6.1	Decrease in mean EDV and increase in RI of both OA and CRA in PDR compared to controls (P<0.05)
Baydar *et al.*, 2007[11]	Australia	44 (65 eyes) Controls: 11 Diabetics: 33	4.94±3.90	The RI of CRA was significantly higher in the control group than in the NDR group. RI of OA was significantly higher in NDR than in the moderate NPDR group.
Basturk *et al.*, 2012[10]	Turkey	118 Controls: 27 NDR: 40 DR: 51	NDR: 6.87±4.37 DR: 8.23±2.55	Patients with DR had significantly higher mean RIs of OA, CRA and PCA compared to those without DR and controls (P<0.001)
Karami *et al.*, 2012[30]	Iran	123 Controls: 25 BDR: 74 PPDR: 24	BDR: 5.6±0.7 PPDR: 12.6±1.1	RI of OA and CRV were significantly higher in patients with DR compared to controls (P<0.005)
Our study	India	100 Controls: 50 NDR/mild/ moderate NPDR: 36 SNPDR/PDR: 14	NDR/mild/moderate NPDR: 8 SNPDR/PDR: 11	RI of OA was significantly increased in diabetics compared to controls. Significant decrease in both PSV and EDV of PCA in mild/ moderate NPDR compared to controls (P<0.05) Significantly reduced EDV and increased RI of CRA in SNPDR/PDR compared to mild/moderate NPDR group (P=0.015)

NDR=No diabetic retinopathy; BDR=Background diabetic retinopathy; PPDR=Preproliferative diabetic retinopathy; NPDR=Nonproliferative diabetic retinopathy; SNPDR=Severe nonproliferative diabetic retinopathy; PDR=Proliferative diabetic retinopathy; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; RI=Resistivity index; NA=Not available

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Khatri *et al.* assessed the correlation between RI of OA and topographic alterations of retinal pigment epithelium (RPE) on spectral-domain optical coherence tomography (SD-OCT).[22] In their study involving 75 patients with type 2 diabetes and 24 controls, a significant increase in OA RI was noted with the severity of DR ($F = 14.23$, $P < 0.001$). A positive correlation was noted between RI of OA and grades of RPE alterations on SD-OCT suggesting that decreased blood flow contributes to RPE dysfunction. Studies demonstrating early retinal damage in experimental diabetes have reported morphological changes in RPE and animal studies have also shown reduced choroidal blood flow in young diabetic animals suggesting that blood flow deficit could be an early pathological change in DR.[23,24] Although it is difficult to propose a causal association it can be postulated that changes in choroidal circulation (as reflected by increased RI of OA) lead to downregulation of antiangiogenic PEDF (pigment epithelial-derived factor) and upregulation of VEGF (vascular endothelial growth factor) thereby contributing to progression of DR. Differential expression and secretion of these growth factors derived from the retinal pigment epithelium have been suggested to lead to nonproliferative and proliferative DR.[25]

Patients with Group 2 DR had significantly reduced EDV in the CRA compared to Group 1 DR as well as healthy controls. Our observations are in line with previous studies.[5,6,8] These can be attributed to the increased peripheral vascular resistivity with the progression of DR. Pathological findings in DR such as capillary basement membrane thickening, retinal capillary leukostasis, loss of retinal capillaries, and altered blood rheology support this circulatory feature.[2,26,27]

Another observation of our study was that DR Group 2 had significantly reduced flow velocity in the CRA but an increase in RI was not statistically significant compared to the control group. This can be due to the concomitant decrease of both PSV and EDV in patients with Group 2 DR. Neudorfer *et al.* evaluated the long-term changes in the retrobulbar circulation of 138 eyes of diabetic subjects at a 10-year follow-up.[28] Patients were divided into four groups, namely, nondiabetic controls, diabetics without DR, nonproliferative DR, and proliferative DR. RI values of CRA and PCA had increased in the two no retinopathy groups and in the nonproliferative DR group, with a surprising nonsignificant decrease in the proliferative DR group. The authors have concluded that an increase of resistance in the retrobulbar blood vessels due to DR can decrease over time and may even be reversed. It has been suggested that the decrease in resistance reflects the beginning of an increase in retinal blood flow with the progression of DR due to the short-circuiting of the obstructed retinal capillary network.[28] This is supported by histopathologic studies, which have shown areas of capillary closure being traversed by preferential dilated anastomotic channels.[29]

Our finding of a positive correlation between duration of diabetes and RI of all retrobulbar vessels was similar to the observations of Karami *et al.*[30] We found negative correlation between EDV of CRA and duration of diabetes as well as HbA1c levels ($R = -0.464$, $P = 0.001$; $R = -0.299$, $P = 0.035$, respectively). Poor glycemic control (as reflected by increased HbA1c) and longer duration of diabetes can be associated with increased peripheral vascular resistance and thus reduced EDV in CRA. Our findings of HbA1c and RI of OA as independent risk factors of DR are similar to that of Basturk *et al.*[10]

Table 4 shows the various published studies on ocular circulation in diabetic patients using CDI.

Our study has several strengths. None of the diabetic patients had associated vascular diseases. Hypertension as well as antihypertensive medications significantly alter the resistive indices of blood vessels and therefore confound the results. We intentionally excluded these patients to study the influence of diabetes alone on ocular hemodynamics. A single masked, experienced radiologist performed all the Doppler examinations gently with the least pressure on the globe thus eliminating interobserver variability. The presence and severity of DR were graded based on seven field stereo fundus photography for accuracy.

To our knowledge, this study is the first of its kind in a pure cohort of diabetics from India.

There are a few limitations to our study. The sample size was small which accounts for the limited subgroup analysis. CDI provides information on blood flow velocity only and absolute volumetric blood flow cannot be quantified since it is not possible to study the diameter of retrobulbar vessels with CDI accurately. In spite of this limitation, blood velocity is probably a good indicator of blood flow within a given vessel.[31,32] Moreover, measurement of retinal blood flow is difficult and no accurate method to quantitatively assess perfusion is currently used clinically.

Conclusion

In conclusion, significant changes in the blood flow velocities and RI were observed in the retrobulbar vessels, especially in an ophthalmic artery in diabetics compared to controls. RI could be potentially useful for early diagnosis and follow-up of DR. CDI with results of increased resistance or decreased flow can be of help to identify diabetic individuals at higher risk for developing severe DR. This is especially useful in patients with opaque media where conventional methods are of little use.

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Conflicts of interest

There are no conflicts of interest.

References

1. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.

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2. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye (Lond) 2009;23:1496-508.
3. Aburn NS, Sergott RC. Orbital colour Doppler imaging. Eye (Lond) 1993;7:639-47.
4. Dimitrova G, Kato S. Color Doppler imaging of retinal diseases. Surv Ophthalmol 2010;55:193-214.
5. MacKinnon JR, McKillop G, O'Brien C, Swa K, Butt Z, Nelson P. Colour Doppler imaging of the ocular circulation in diabetic retinopathy. Acta Ophthalmol Scand 2000;78:386-9.
6. Dimitrova G, Kato S, Tamaki Y, Yamashita H, Nagahara M, Sakurai M, *et al.* Choroidal circulation in diabetic patients. Eye (Lond) 2001;15:602-7.
7. Sullu Y, Hamidova R, Beden U, Yakupov K, Canbaz S, Danaci M. Effects of pars plana vitrectomy on retrobulbar haemodynamics in diabetic retinopathy. Clin Exp Ophthalmol 2005;33:246-51.
8. Gracner T. Ocular blood flow velocity determined by color Doppler imaging in diabetic retinopathy. Ophthalmologica 2004;218:237-42.
9. Arai T, Numata K, Tanaka K, Kiba T, Kawasaki S, Saito T, *et al.* Ocular arterial flow hemodynamics in patients with diabetes mellitus. J Ultrasound Med 1998;17:675-81.
10. Basturk T, Albayrak R, Ulas T, Akcay M, Unsal A, Toksoy M, *et al.* Evaluation of resistive index by color Doppler imaging of orbital arteries in type II diabetes mellitus patients with microalbuminuria. Ren Fail 2012;34:708-12.
11. Baydar S, Adapinar B, Kebapci N, Bal C, Topbas S. Colour Doppler ultrasound evaluation of orbital vessels in diabetic retinopathy. Australas Radiol 2007;51:230-5.
12. Pandey SK, Sharma V. World diabetes day: Battling the emerging epidemic of diabetic retinopathy. Indian J Ophthalmol 2018;66:1652-3.
13. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, *et al.* Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Global Diabetic Retinopathy Project Group. Ophthalmology 2003;110:1677-82.
14. Nagaoka T, Kitaya N, Sugawara R, Yokota H, Mori F, Hikichi T, *et al.* Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. Br J Ophthalmol 2004;88:1060-3.
15. Kim M, Ha MJ, Choi SY, Park YH. Choroidal vascularity index in type-2 diabetes analyzed by swept-source optical coherence tomography. Sci Rep 2018;8:70.
16. Cao J, McLeod S, Merges CA, Lutty GA. Choriocapillaris degeneration and related pathologic changes in human diabetic eyes. Arch Ophthalmol 1998;116:589-97.
17. Ferrara D, Waheed NK, Duker JS. Investigating the choriocapillaris and choroidal vasculature with new optical coherence tomography technologies. Prog Retin Eye Res 2016;52:130-55.
18. Evans DW, Harris A, Garrett M, Chung HS, Kagemann L. Glaucoma patients demonstrate faulty autoregulation of ocular blood flow during posture change. Br J Ophthalmol 1999;83:809-13.
19. Tamaki Y, Nagahara M, Yamashita H, Kikuchi M. Blood velocity in the ophthalmic artery determined by color Doppler imaging in normal subjects and diabetics. Jpn J Ophthalmol 1993;37:385-92.
20. Cioffi GA, Granstam E, Alm A. Ocular circulation. In: Kaufman PL, Alm A, editors. Adler's Physiology of the Eye. 10th ed. St. Louis, MO: Mosby; 2003. p. 747-73.
21. Güven D, Ozdemir H, Hasanreisoglu B. Hemodynamic alterations in diabetic retinopathy. Ophthalmology 1996;103:1245-9.
22. Khatri M, Saxena S, Kaur A, Bhasker SK, Kumar M, Meyer CH. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retina Vitreous 2018;4:12.
23. Decanini A, Karunadharma PR, Nordgaard CL, Feng X, Olsen TW, Ferrington DA. Human retinal pigment epithelium proteome changes in early diabetes. Diabetologia 2008;51:1051-61.
24. Muir ER, Rentería RC, Duong TQ. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. Invest Ophthalmol Vis Sci 2012;53:6488-94.
25. Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO. Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 2003;22:1-29.
26. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. Microcirculation 2007;14:25-38.
27. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008;2:1130-8.
28. Neudorfer M, Kessner R, Goldenberg D, Lavie A, Kessler A. Retrobulbar blood flow changes in eyes with diabetic retinopathy: A 10-year follow-up study. Clin Ophthalmol 2014;8:2325-32.
29. Garner A. Histopathology of diabetic retinopathy in man. Eye (Lond) 1993;7:250-3.
30. Karami M, Janghorbani M, Dehghani A, Khaksar K, Kaviani A. Orbital Doppler evaluation of blood flow velocities in patients with diabetic retinopathy. Rev Diabet Stud 2012;9:104-11.
31. Hansen NB, Stonestreet BS, Rosenkrantz TS, Oh W. Validity of Doppler measurements of anterior cerebral artery blood flow velocity: Correlation with brain blood flow in piglets. Pediatrics 1983;72:526-31.
32. Taylor GA, Short BL, Walker LK, Traystman RJ. Intracranial blood flow: Quantification with duplex Doppler and color Doppler flow US. Radiology 1990;176:231-6.

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Original Article

Relationship between myopia and central corneal thickness – A hospital based study from South India

ABSTRACT

Background and Purpose: The prevalence of myopia has been increasing steadily, and studies on the relationship between myopia and central corneal thickness (CCT) have shown conflicting results. The purpose of this study was to investigate the relationship between myopia and CCT in a hospital based population from South India. **Materials and Methods:** This prospective, cross-sectional study was carried out from a tertiary care institution in South India between January 2017 and June 2018. One hundred and ninety-four myopic individuals between 11 and 40 years of age with spherical equivalent (SE) ranging from -0.5 to -6 Diopters after cycloplegic refraction were enrolled in the study. CCT was measured in both eyes of each patient using Tomey EM-3000 noncontact specular microscope (Tomey Corporation, Nagoya, Japan). The relation between CCT and myopia was analyzed using Pearson's correlation coefficient. Statistical significance was set at $P < 0.05$. **Results:** A total of 388 eyes were analyzed. 50% (n=97) of study participants were females. The mean age of the study participants was 25.47 ± 7.25 years (range: 12–40 years). The mean SE was -2.79±1.34 D (range: -0.5 to -6.0 D). The mean CCT was 540.53 ± 56.16 μm (range: 441–664 μm). No significant association was observed between CCT and age or gender of the study participants ($P = 0.706$ and 0.550, respectively). No correlation was observed between mean CCT and SE (Pearson, $r = 0.095$, $P = 0.062$). **Conclusion:** There was no correlation between CCT and degree of myopia. These data contribute to the ongoing discussion on the structural alterations in ocular coats and susceptibility to glaucoma in myopia.

Keywords: Central corneal thickness measurement, myopia, noncontact specular microscope, spherical equivalent

INTRODUCTION

Myopia, referred to as "short sight," is increasing in prevalence at an alarming rate and has emerged as a major cause for visual impairment globally. Estimates suggest that around 1.5 billion people or 22% of the present world population is myopic.[1] Apart from the ethnic and genetic factors, environmental and lifestyle changes have contributed significantly to the increasing prevalence of myopia. A recent meta-analysis on the prevalence of myopia in South-east Asia has shown an estimated pool prevalence of 4.9% (95% confidence interval [CI]: 1.6–8.1) in children and 32.9% (95% CI: 25.1–40.7) in adults.[2] The recent shift to younger age at the onset of myopia is even more worrisome considering its impact on the economic burden and quality of life.

With an increasing prevalence of myopia, there has also been a tremendous rise in the number of laser refractive procedures for the correction of myopia. Central corneal thickness (CCT) is a major factor determining the choice of refractive surgery for myopia, and a thin cornea is a recognized risk factor for corneal ectasia postrefractive surgery.[3] Corneal refractive surgery for myopia results in reduced corneal thickness and thereby leads to the underestimation of intraocular pressure.[4,5] This is relevant in the diagnosis and treatment of any glaucoma later in life,

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since both myopia and reduced CCT are well-known risk factors for glaucoma.[6-8]

Studies evaluating the relationship between myopia and CCT have revealed conflicting results. While few studies have found a correlation between CCT and myopia,[9-11] some have reported no significant correlation.[12-15] This can be attributed to the differences in ethnicity, characteristics of the population studied as well as varied methods of the measurement of corneal thickness. Two population-based studies using ultrasonic pachymetry (UP) from India have reported thinner corneas in Indians than that of other populations.[12,16] Relatively fewer studies have evaluated CCT using noncontact specular microscopy, which is a noninvasive and accurate method with good reproducibility of CCT measurements.[17-19]

Given the increasing prevalence of myopia from India and conflicting evidence on the association between CCT and myopia, we attempted to study the relationship between myopia and CCT among patients attending the refraction clinic of a tertiary care institute using a noncontact specular microscope (NCSM).

MATERIALS AND METHODS

This prospective, hospital-based, cross-sectional study was carried out from a tertiary care institution in South India between January 2017 and June 2018. Individuals between 11 and 40 years of age who presented with visual impairment and found to have myopic refractive error (spherical equivalent [SE] more than −0.5 D) on cycloplegic refraction were included in the study. The exclusion criteria were myopia >−6 D, prior refractive or other ocular surgery, contact lens wear, trauma, corneal diseases, cataract, glaucoma, and ocular hypertension. Pregnant and lactating females and individuals with systemic diseases such as diabetes mellitus and collagen vascular disease were also excluded from the study. The study was performed according to the principles of the Declaration of Helsinki after prior approval from the Institutional Human Ethics Committee. Informed consent was obtained from all the participants.

All the study participants underwent a comprehensive ophthalmic examination, including slit-lamp biomicroscopy and fundus evaluation using +90 D lens and indirect ophthalmoscopy. Cycloplegia was achieved with three topical applications of 1% cyclopentolate at 15-min intervals. Cycloplegic refraction was performed 30–60 min after the first instillation of drug and spherical equivalent (SE, sphere + half-cylindrical component) was calculated.

CCT was measured by a single experienced ophthalmologist using Tomey EM-3000 NCSM (Tomey Corporation, Nagoya, Japan). The participants were instructed to fixate on the central target with chin and forehead positioned appropriately. Then, the participants were asked to blink completely once before measurements to spread an optically smooth tear film over the corneal surface. The measurements were taken using the auto-alignment and capture mode, thereby eliminating observer bias. Three consecutive valid measurements were taken, and the average of the three readings was used for the analysis. The CCT values were considered valid when the endothelial cells were focused clearly and bright corneal reflections were centered on the monitor. All measurements were performed in midday (between 12 and 4 PM) to eliminate diurnal variation.[20]

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, LA, USA) software version 24. Descriptive statistics were presented as mean ± standard deviation. Discrete variables were presented as numbers (n) and percentages (%). Patients were classified into the following three groups according to SE: Group 1 included patients with SE -0.5 to <-2 D; Group 2 consisted of patients with SE -2 to < -4 D; and Group 3 individuals with SE -4 to < -6 D. The normality of CCT distribution was determined by the Shapiro–Wilk test. One-way analysis of variance followed by *post-hoc* Bonferroni was used for the analysis between the groups. The student's t-test was used to analyze the mean difference between two independent samples. Pearson's coefficient was used to study the correlation between variables. $P < 0.05$ was considered to be statistically significant.

RESULTS

This study included 388 eyes of 194 individuals with varying grades of myopia. 50% ($n = 97$) of the study participants were females. The mean age of the study participants was 25.47 ± 7.25 years (range: 12–40 years). The mean SE was − 2.79 ± 1.34 D (range: -0.5 to -6.0 D). The mean CCT was 540.53 ± 56.16 µm (range: 441–664 µm).

The CCT measurements were found to be nonnormally distributed. Figure 1 shows the distribution of CCT readings among the study participants. Figure 2 shows the distribution of SE among study participants.

Table 1 shows the association of gender of study participants with CCT. No significant differences were noted in the mean CCT between males and females ($P = 0.550$, Student's t-test).

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Table 2 shows the association between mean CCT and age of study participants. No significant differences were noted in mean CCT values between various age groups ($P = 0.706$).

Table 3 shows the mean CCT values among the SE groups. Figure 3 shows the correlation between mean CCT and SE. No correlation was observed between mean CCT and SE (Pearson, $r = 0.095$, $P = 0.062$).

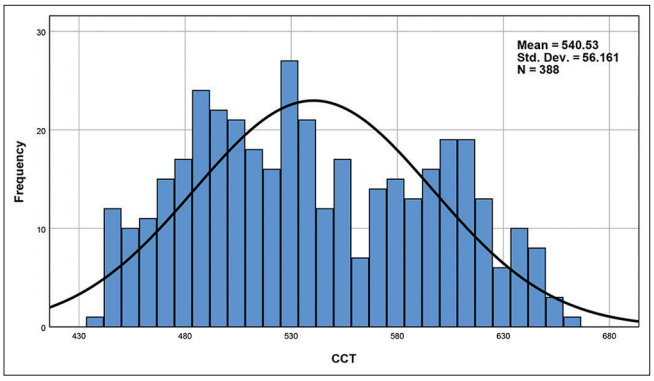


Figure 1: Distribution of central corneal thickness readings among the study participants

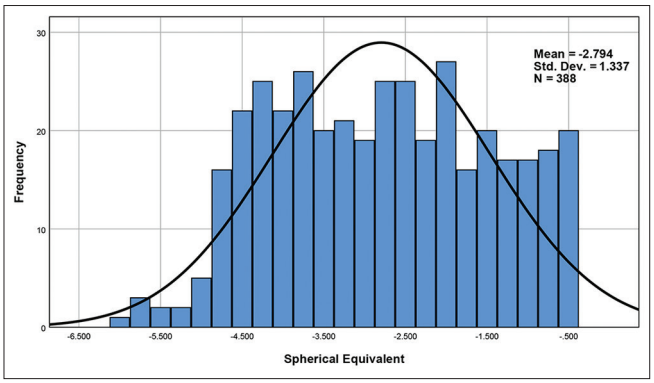


Figure 2: Distribution of spherical equivalent among the study participants

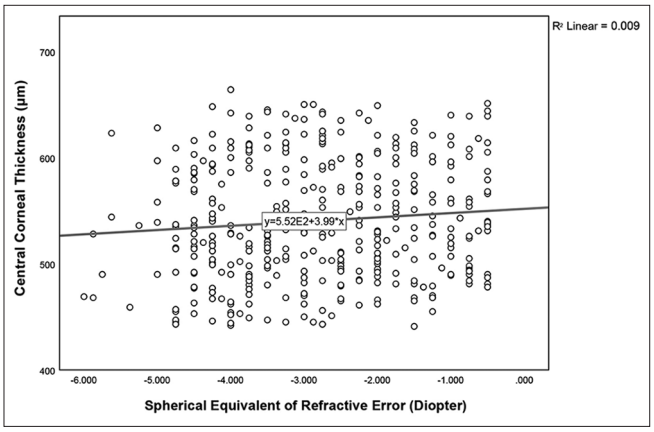


Figure 3: Scatter plot showing the correlation between mean central corneal thickness and spherical equivalent; no correlation was observed between mean central corneal thickness and spherical equivalent (Pearson, $r = 0.095$, $P = 0.062$)

DISCUSSION

In this hospital-based study from South India, we evaluated the CCT measurements of young myopic individuals with mild-to-moderate myopia using NCSM and found no correlation between mean CCT and degree of myopia.

Population-based studies from India using UP for CCT measurements have reported significantly thinner corneas among Indians compared to Africans (530 µm),[21] Nepalese (539 µm),[22] Caucasians (540 µm),[23] and Chinese (542 µm).[24] Nangia *et al*. evaluated the distribution of CCT among adults aged 30+ from rural Central India using UP. Mean CCT among 9370 eyes studied was 514 ± 33 µm (range: 290–696 µm). CCT was associated significantly with younger age, male gender, and lower corneal refractive power ($P < 0.001$). However, no statistically significant association was found between CCT and refractive error ($P = 0.54$).[12] Vijaya *et al*. evaluated 6754 adults aged 40+ from South India using UP and found the mean CCT to be 511.4 ± 33.5 µm.[16]

A hospital-based, prospective study involving 156 participants from South India, with a mean age of 29.27 years and mean refractive error of −3.10 D revealed a mean CCT value of 533.87 µm using UP. CCT had a positive correlation with axial length and negative correlation with SE, suggesting that axial

Table 1: Association between the gender of study participants and central corneal thickness

Gender	Number of patients, *n* (%)	CCT (µm), mean±SD	*P**
Males	97 (50)	538.82±56.33	0.550
Females	97 (50)	542.24±56.08	

*Student's *t*-test. CCT – Central corneal thickness, SD – Standard deviation

Table 2: Association between mean central corneal thickness and age of study participants

Age of participants (years)	Number of patients, *n* (%)	CCT (µm), mean±SD	*P**
11-20	73 (37.6)	539.35±49.11	0.706
21-30	63 (32.5)	544.03±58.34	
31-40	58 (29.9)	538.22±62.08	

*ANOVA. ANOVA – Analysis of variance, SD – Standard deviation, CCT – Central corneal thickness

Table 3: The mean central corneal thickness values among spherical equivalent groups

Spherical equivalent	Number of eyes, *n* (%)	CCT (µm), mean±SD	*P**
Group 1	108 (27.8)	546.07±52.29	0.144
Group 2	182 (46.9)	542.23±58.05	
Group 3	98 (25.3)	531.28±56.16	

*ANOVA. SD – Standard deviation, CCT – Central corneal thickness, ANOVA – Analysis of variance

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myopes tend to have thicker corneas.[10] Kunert *et al*. measured the CCT of 615 myopic patients of Indian origin (aged 18+) before laser *in situ* keratomileusis with UP as well as Orbscan scanning-slit topography/pachymetry.[25] Mean CCT reported was 519.92 ± 33.36 µm (range: 420–640 µm) using ultrasound method and 518.23 ± 31.03 µm (range: 428–607 µm) using Orbscan. No significant differences were noted in the CCT values measured using these two methods. Lower CCT readings were observed in individuals with myopia −0.5 to −4.9 D compared to those with myopia ranging from −5 to −9.9 D.

Mean CCT measurements obtained with EM-3000 (NCSM) in our study were 540.53 ± 56.16 µm (range: 441–664 µm). Módis *et al*. reported that the CCT values obtained using EM-3000 NCSM were higher than that found using ultrasound pachymetry ($P < 0.0001$).[26]

It would not be appropriate to compare our CCT measurements with other studies from India because we measured CCT using noncontact specular microscopy EM-3000 while others have utilized ultrasound pachymetry. The operating principles behind these two instruments are entirely different which explains the variation in measured values. While UP measurements depend on the reflection of ultrasonic sound waves from the anterior and posterior corneal surfaces, NCSM measurements rely on the reflection of light. CCT may be under or overestimated due to high inter-device variations, and studies have shown poor inter-device agreement among CCT measurements taken with different instruments.[27,28]

Although ultrasound pachymetry is the gold standard for CCT measurements, it is a contact method that needs topical anesthesia and user skill with regard to the placement of the probe. Furthermore, studies have reported alteration in corneal thickness values after the application of local anesthetic eye drops.[29,30] Unlike ultrasound pachymetry, NCSM is noninvasive and provides reproducible measurements of CCT.

There was no significant association between CCT and gender or age in our study. The role of gender on CCT has been reported to be variable, with few studies showing thicker CCT in males,[18,31] while others have reported no significant differences between males and females.[32] Few studies in patients older than 40 years of age have reported a negative association between CCT and age.[16,21,33]

The effect of refractive error on CCT has been analyzed by several investigators with contrasting results. Our study results demonstrate that CCT does not correlate with the myopia of different grades. Our observations are in agreement with the majority of studies elsewhere.[12-15] Chen *et al*. correlated the CCT measurements and degree of myopia among 528 Taiwanese adults enrolled for myopic laser refractive surgery.[13] No statistically significant association was found between CCT and refractive error ($P = 0.49$). The authors also studied the relation between CCT and myopia by intraindividual comparison in myopic anisometropia. The mean myopic anisometropia was 3.09 ± 1.06 D in their series, and the CCT in more myopic eye was not significantly different from the other less myopic eye. The authors have concluded that the cornea does not thin in the same way as the sclera in myopic eyes. Similarly, Fam *et al*. evaluated 714 consecutive Chinese patients with mean SE of − 5.3 D (range: −17.5 to −0.625).[15] No correlation was found between CCT and the degree of myopia ($r = -0.13$, $P = 0.719$). Ortiz *et al*. studied the relationship between CCT as well as mid-peripheral corneal thickness (PCT) in 175 myopic participants using Orbscan II.[34] Participants were divided into three groups, namely myopia <−6 D ($n = 76$), -6 to -12 D ($n = 72$), and more than −12 D ($n = 27$). No statistically significant differences were found in both CCT and PCT between the groups ($P > 0.05$).

A recent study comparing the thickness of preocular tear film, corneal layers (epithelium, Bowman layer, stroma and Descemet's membrane-endothelium complex in the central cornea), and anterior sclera using anterior segment spectral-domain optical coherence tomography found no statistically significant differences between emmetropia and those with moderate-to-high myopia ($P > 0.05$).[35]

However, few authors have found a significant correlation between CCT and myopic refractive error. Kadhim and Farhood evaluated CCT measurements of 418 eyes of 209 healthy individuals using UP.[9] The mean CCT was 543.95 ± 32.58 µm. CCT measurements significantly negatively correlated with age and myopes were noted to have significantly thinner corneas than emmetropic individuals ($P = 0.019$). Chang *et al*. evaluated a total of 216 participants, with a mean age of 22.2 years and mean refractive error of −4.17 D using UP.[36] A significant correlation was observed between CCT and SE ($r = 0.16$, $P = 0.021$). Corneas tended to be thinner in more myopic eyes. The authors have concluded that a decrease in corneal thickness is one among the changes in the anterior segment of the eye as myopia progresses. In contrast, Pedersen *et al*. measured CCT in 57 emmetropes and 48 high myopes using a high-precision Haag-Streit optical low coherence reflectometry pachymeter.[37] No statistically significant differences were noted between myopic participants and emmetropes (527.7 ± 35 µm versus

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538.6 ± 32.1 μm), suggesting that the process by which myopia progresses does not influence the central cornea to a measurable degree.

The wide discrepancy in the observed results can be attributed to the varied age groups of patients, smaller sample sizes, nonelimination of contact lens wearers as well as the influence of diurnal variation, and use of pachymeters with variable reproducibility. The relatively new NCSM EM-3000 (Tomey, Japan) used in this study has been suggested to have high intraoperator repeatability in normal corneas.[27]

Our study has a few limitations. Observations are from a single-center, and axial length was not measured to differentiate axial myopia from other causes for myopia. However, we intentionally excluded patients older than 40 years to eliminate index myopia. To the best of our knowledge, this is the first study from India using NCSM to evaluate CCT measurements among myopic individuals.

CONCLUSION

In summary, we evaluated CCT measurements using noncontact specular microscopy in a cohort of young individuals with mild-to-moderate myopia. The mean CCT values did not correlate with myopic SE. Further studies with a larger sample size and different grades of myopia are needed to better understand the relation between myopia and CCT.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Holden B, Sankaridurg P, Smith E, Aller T, Jong M, He M. Myopia, an underrated global challenge to vision: Where the current data takes us on myopia control. Eye (Lond) 2014;28:142-6.
2. Hashemi H, Fotouhi A, Yekta A, Pakzad R, Ostadimoghaddam H, Khabazkhoob M. Global and regional estimates of prevalence of refractive errors: Systematic review and meta-analysis. J Curr Ophthalmol 2018;30:3-22.
3. Santhiago MR, Giacomin NT, Smadja D, Bechara SJ. Ectasia risk factors in refractive surgery. Clin Ophthalmol 2016;10:713-20.
4. Ajazaj V, Kaçaniku G, Asani M, Shabani A, Dida E. Intraocular pressure after corneal refractive surgery. Med Arch 2018;72:341-3.
5. Duch S, Serra A, Castanera J, Abos R, Quintana M. Tonometry after laser *in situ* keratomileusis treatment. J Glaucoma 2001;10:261-5.
6. Marcus MW, de Vries MM, Junoy Montolio FG, Jansonius NM. Myopia as a risk factor for open-angle glaucoma: A systematic review and meta-analysis. Ophthalmology 2011;118:1989-9400.
7. Mitchell P, Hourihan F, Sandbach J, Wang JJ. The relationship between glaucoma and myopia: The Blue Mountains Eye Study. Ophthalmology 1999;106:2010-5.
8. Dimasi DP, Burdon KP, Craig JE. The genetics of central corneal thickness. Br J Ophthalmol 2010;94:971-6.
9. Kadhim YJ, Farhood QK. Central corneal thickness of Iraqi population in relation to age, gender, refractive errors, and corneal curvature: A hospital-based cross-sectional study. Clin Ophthalmol 2016;10:2369-76.
10. Muthu Krishnan V, Jayalatha K, Vijayakumar C. Correlation of central corneal thickness and keratometry with refraction and axial length: A prospective analytic study. Cureus 2019;11:e3917.
11. Mimouni M, Flores V, Shapira Y, Graffi S, Levartovsky S, Sela T, *et al*. Correlation between central corneal thickness and myopia. Int Ophthalmol 2018;38:2547-51.
12. Nangia V, Jonas JB, Sinha A, Matin A, Kulkarni M. Central corneal thickness and its association with ocular and general parameters in Indians: The Central India Eye and Medical Study. Ophthalmology 2010;117:705-10.
13. Chen YC, Kasuga T, Lee HJ, Lee SH, Lin SY. Correlation between central corneal thickness and myopia in Taiwan. Kaohsiung J Med Sci 2014;30:20-4.
14. Al-Mezaine HS, Al-Obeidan S, Kangave D, Sadaawy A, Wehaib TA, Al-Amro SA. The relationship between central corneal thickness and degree of myopia among Saudi adults. Int Ophthalmol 2009;29:373-8.
15. Fam HB, How AC, Baskaran M, Lim KL, Chan YH, Aung T. Central corneal thickness and its relationship to myopia in Chinese adults. Br J Ophthalmol 2006;90:1451-3.
16. Vijaya L, George R, Arvind H, Ve Ramesh S, Baskaran M, Raju P, *et al*. Central corneal thickness in adult South Indians: The Chennai Glaucoma Study. Ophthalmology 2010;117:700-4.
17. Tomidokoro A, Araie M, Iwase A; Tajimi Study Group. Corneal thickness and relating factors in a population-based study in Japan: The Tajimi study. Am J Ophthalmol 2007;144:152-4.
18. Suzuki S, Suzuki Y, Iwase A, Araie M. Corneal thickness in an ophthalmologically normal Japanese population. Ophthalmology 2005;112:1327-36.
19. Almubrad TM, Osuagwu UL, Alabbadi I, Ogbuehi KC. Comparison of the precision of the Topcon SP-3000P specular microscope and an ultrasound pachymeter. Clin Ophthalmol 2011;5:871-6.
20. Lattimore MR Jr, Kaupp S, Schallhorn S, Lewis R 4th. Orbscan pachymetry: Implications of a repeated measures and diurnal variation analysis. Ophthalmology 1999;106:977-81.
21. Nemesure B, Wu SY, Hennis A, Leske MC; Barbados Eye Study Group. Corneal thickness and intraocular pressure in the Barbados eye studies. Arch Ophthalmol 2003;121:240-4.
22. Thapa SS, Paudyal I, Khanal S, Paudel N, Mansberger SL, van Rens GH. Central corneal thickness and intraocular pressure in a Nepalese population: The Bhaktapur Glaucoma Study. J Glaucoma 2012;21:481-5.
23. Rahman ML, Bunce C, Healey PR, Mitchell P, Sham PC, McGuffin P, *et al*. Commingling analyses of central corneal thickness and adjusted intraocular pressure in an older Australian population. Invest Ophthalmol Vis Sci 2010;51:2512-8.
24. Wang D, Huang W, Li Y, Zheng Y, Foster PJ, Congdon N, *et al*. Intraocular pressure, central corneal thickness, and glaucoma in Chinese adults: The Liwan eye study. Am J Ophthalmol 2011;152:454-620.
25. Kunert KS, Bhartiya P, Tandon R, Dada T, Christian H, Vajpayee RB. Central corneal thickness in Indian patients undergoing LASIK for myopia. J Refract Surg 2003;19:378-9.
26. Módis L Jr., Szalai E, Németh G, Berta A. Evaluation of a recently developed noncontact specular microscope in comparison with conventional pachymetry devices. Eur J Ophthalmol 2010;20:831-8.
27. Bao F, Wang Q, Cheng S, Savini G, Lu W, Feng Y, *et al*. Comparison and evaluation of central corneal thickness using 2 new noncontact specular microscopes and conventional pachymetry devices. Cornea 2014;33:576-81.

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28. Maloca PM, Studer HP, Ambrósio R Jr., Goldblum D, Rothenbuehler S, Barthelmes D, *et al*. Interdevice variability of central corneal thickness measurement. PLoS One 2018;13:e0203884.
29. Asensio I, Rahhal SM, Alonso L, Palanca-Sanfrancisco JM, Sanchis-Gimeno JA. Corneal thickness values before and after oxybuprocaine 0.4% eye drops. Cornea 2003;22:527-32.
30. Herse P, Siu A. Short-term effects of proparacaine on human corneal thickness. Acta Ophthalmol (Copenh) 1992;70:740-4.
31. Sng C, Barton K, Kim H, Yuan S, Budenz DL. Central corneal thickness and its associations with ocular and systemic factors in an urban West African Population. Am J Ophthalmol 2016;169:268-75.
32. Soleimanizad R, Nowroozzadeh MH, Ziaei H, Pakravan M, Yaseri M, Katibeh M. The association of central corneal thickness with ocular and general parameters in a community setting: The Yazd eye study. J Ophthalmic Vis Res 2017;12:141-50.
33. Hahn S, Azen S, Ying-Lai M, Varma R; Los Angeles Latino Eye Study Group. Central corneal thickness in Latinos. Invest Ophthalmol Vis Sci 2003;44:1508-12.
34. Ortiz S, Mena L, Rio-San Cristobal A, Martin R. Relationships between central and peripheral corneal thickness in different degrees of myopia. J Optom 2014;7:44-50.
35. Pekel G, Yağcı R, Acer S, Ongun GT, Çetin EN, Simavlı H. Comparison of corneal layers and anterior sclera in emmetropic and myopic eyes. Cornea 2015;34:786-90.
36. Chang SW, Tsai IL, Hu FR, Lin LL, Shih YF. The cornea in young myopic adults. Br J Ophthalmol 2001;85:916-20.
37. Pedersen L, Hjortdal J, Ehlers N. Central corneal thickness in high myopia. Acta Ophthalmol Scand 2005;83:539-42.

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Original Article

Relationship between Levels of Vascular Endothelial Growth Factor in Blood and Severity of Diabetic Retinopathy

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Abstract

Aim: The aim of the study was to establish the relationship between levels of vascular endothelial growth factor (VEGF) in blood and severity of diabetic retinopathy (DR). **Background:** The importance of VEGF in the pathogenesis of DR is evident from numerous studies demonstrating a significant increase in VEGF levels in samples obtained from the eye. However, a correlation between blood levels of VEGF and DR has not been conclusively proven or disproven. In this article, we demonstrate the relationship between blood levels of VEGF and the severity of DR. **Materials and Methods:** This is a hospital-based descriptive analytic study. The study population consisted of 75 Type 2 diabetic patients attending outpatient department for routine DR screening. After obtaining informed consent, 5 ml of blood was drawn from each patient and estimated for the levels of serum VEGF. The data thus obtained were correlated with the grade of DR. Additional parameters studied were duration of diabetes mellitus (DM), hemoglobin levels, blood urea, serum creatinine, fasting blood sugar (FBS) and random blood sugar (RBS), and hemoglobin A1c (HBA1c) levels. Statistical analysis used in this study was Chi-square test, Student's *t*-test, independent one sample *t*-test, and analysis of variance using SPSS software version. 19. **Results:** Overall mean VEGF levels for the study population of 75 diabetic patients were 577.01 ± 291.13 pg/ml. The average duration of DM in the study population was 7.2 years. The average hemoglobin level in the study population was 11.53 ± 2.00 g/dl. The mean urea levels in mg/dl of the whole population were 35.47 ± 25.80 mg/dl. The mean creatinine levels for the population were 1.42 ± 1.55 mg/dL. The mean RBS levels for the whole population were 228.95 ± 74.499 mg/dL. The mean FBS levels for the 75 patients were 165.87 ± 59.04 mg/dl. The mean HBA1c levels were 9.49 ± 2.37%. **Conclusion:** Levels of blood VEGF are elevated in diabetic patients regardless of whether they have DR or not. There is no statistically significant relationship between blood levels of VEGF and the severity of DR. Even though severe grades of DR showed more anemia, there is no statistically significant relationship between anemia and DR. The levels of urea and creatinine were elevated in the more severe grade of DR. VEGF, therefore, could present a potential treatment and preventive strategy for not only DR but also DM and its complications in general.

Keywords: Diabetes mellitus, diabetic retinopathy, ELISA, ETDRS, vascular endothelial growth factor

Introduction

Diabetic retinopathy (DR) is one of the most debilitating complications of diabetes mellitus (DM). It can either be a nonproliferative or proliferative pathology of the retina. It is probably the most significant complication of a systemic disease in the eye. Over a period of half a century, the world has witnessed remarkable progress in the field of DR and its management. From the early days of the Arlie house classification for staging of DR, we have now entered firmly into the realm of antivascular endothelial growth factor (anti-VEGF) therapy. Literature review shows five major points of change in the field of DR. First, diabetes and its complications have become a global problem; to the extent that DR has assumed the status of a global epidemic. DR has thus become the leading cause of blindness in the middle-aged population.[1] Second, a number of randomized control trials, strict control of blood sugars, and additional systemic comorbidities play a major role in the disease progression.[2,3] Third, optical coherence

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tomography has become a major component in DR staging and management. With the improved resolution of images, ophthalmologists can cut down on the use of fundus fluorescein angiography. Fourth, timely retinal photocoagulation can protect the retina from developing end-stage disease. And Finally, the role of VEGF appears to be central to the pathogenesis of DR. Thus, anti-VEGF therapy has become one of the first lines of treatment, especially of diabetic macular edema.

The importance of VEGF in the disease pathogenesis was realized when the vitreous cavity of patients with DR had higher levels of VEGF than those of controls. To the best of our knowledge, previous studies were mainly based on measurements of VEGF in the vitreous cavity.[4-8] We aim to study the serum levels of VEGF in diabetic patients with DR and also the correlation, if any, between serum VEGF and the grading of DR.

Materials and Methods

This was a hospital-based descriptive analytic study performed at a tertiary health-care institution and hospital in the department of ophthalmology over a period of 12 months after obtaining institutional ethical committee approval. A total of 75 Type 2 DM patients with or without DR were included in the study. Patients with Type 1 DM, with other retinal pathologies, terminally ill and those who have previously received anti-VEGF therapy were excluded from the study.

A written informed consent in patient's own language was obtained. A detailed history and a thorough ocular examination were performed. The 75 patients were divided into five groups consisting of 15 patients each according to the ETDR classification of DR – no DR, mild nonproliferative DR (mild NPDR), moderate nonproliferative DR (moderate NPDR), severe nonproliferative DR (severe NPDR), and proliferative DR (PDR). Fundus examination through 90D slit-lamp biomicroscopy and 20D indirect ophthalmoscopy was done followed by fundus photographs with a field of 50°. 5 ml blood samples were then withdrawn to measure the concentration of serum VEGF levels quantitatively using ELISA kit for human VEGF (Neogen USA Human VEGF Product #452610).

Statistical analysis of data was done using SPSS (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). Correlation of data between the study variables were done by Chi-square test, Student's *t*-test, independent one sample *t*-test, and analysis of variance.

Results

In this study, of 75 patients, 15 patients were grouped each on the basic of the ETDRS classification of DR into no DR, mild NPDR, moderate NPDR, severe NPDR, and PDR. The age of the patients ranged from 41 to 82 years, and the mean age of the patients was 58.16 ± 8.6 years and the mean age in each group is as listed in Table 1. The gender distribution of population was 56 males and 19 females. The average male-to-female ratio was 2.75:1 and for each group is listed in Table 1.

Primary outcome measures

Vascular endothelial growth factor levels

Overall mean VEGF levels for the study population of 75 diabetic patients were 577.01 ± 291.13 pg/ml. The lowest value of VEGF was seen in the no DR group (34 pg/ml). The highest value was seen in the mild DR group (1296 pg/ml). Overall, there was no statistical significance in the variation of VEGF and the grade of DR ($P = 0.544$) [Table 2].

Secondary outcome measures

Duration of diabetes mellitus

The average duration of DM in the study population was 7.2 years. The subpopulation without any DR had the minimum duration of 6.53 years. The population with mild NPDR had the maximum duration of 12.80 years. There was no statistically significant difference in the duration of DM and the grade of DR ($P = 0.137$) [Table 2].

Hemoglobin levels

The average hemoglobin level in the study population was 11.53 ± 2.00 g/dl. There was no significant difference in the hemoglobin levels and the severity of DR ($P = 0.791$) [Table 2].

Urea levels

The mean urea levels in mg/dl of the whole population were 35.47 ± 25.80 mg/dl. There was no significant difference in the levels of urea and the severity of DR ($P = 0.138$) [Table 2].

Serum creatinine levels

The mean creatinine levels for the population were 1.42 mg/dL ± 1.55. The minimum value was seen in the patients with no DR (0.91 mg/dL). The highest values were seen in the group with PDR (2.26 mg/dL). However, any intergroup variation of creatinine was not statistically significant ($P = 0.11$) [Table 2].

Random blood sugar levels

The mean random blood sugar (RBS) levels for the whole population were 228.95 ± 74.499 mg/dL. The normal RBS was taken to be 70–140 mg/dL. After statistical analysis, the rise in the mean RBS was found to be significant. However, there was no statistically significant change in the RBS levels and the severity of DR ($P = 0.430$) [Table 2].

Table 1: Demographic characteristics of the population under study

	Average in study population	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	*P*
Age (years)	58.16±8.6	58.67±10.4	58.53±8.4	59.07±7.3	56.00±7.8	58.53±9.1	0.880
Sex ratio (male: female)	2.75:1	1.5:1	2.7:1	2.7:1	6.5:1	2.7:1	

DR: Diabetic retinopathy, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy

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Table 2: Relationship of grades of diabetic retinopathy and mean vascular endothelial growth factor, haemoglobin, urea, creatinine, random blood sugar, fasting blood sugar, and hemoglobin A1c

	Duration of diabetes in years	Mean VEGF (pg/ml)	Mean haemoglobin in (g/dl)	Mean urea levels in (mg/dl)	Mean creatinine values (mg/dL)	Mean RBS (mg/dL)	Mean fasting sugars (mg/dL)	Mean HBA1c (%)
No DR	6.53±6.73	620.73±293.50	12.11±1.86	24.73±19.36	0.91±0.76	222.07±73.22	162.87±55.56	8.45±1.57
Mild NPDR	12.80±9.38	666.15±314.68	11.31±2.32	33.90±17.95	1.32±1.03	249.07±86.63	183.00±82.53	9.72±2.06
Moderate NPDR	9.33±5.62	545.06±264.13	11.57±2.61	35.45±28.97	1.25±1.09	216.00±71.76	156.67±40.33	9.47±1.65
Severe NPDR	11.93±6.66	556.06±293.53	11.36±1.69	34.10±29.43	1.28±1.16	249.00±85.67	179.13±54.53	10.46±3.45
PDR	10.27±6.23	496.48±296.43	11.29±1.47	49.17±28.07	2.36±2.69	208.60±49.40	145.67±54.05	9.32±1.90

VEGF: Vascular endothelial growth factor, HBA1c: Haemoglobin A1c, DR: Diabetic retinopathy, NPDR: Nonproliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy, RBS: Random blood sugar

Fasting blood sugar levels

The mean fasting blood sugar (FBS) levels for the 75 patients were 165.87 ± 59.04 mg/dl. However, there was no significant difference in the FBS levels and the severity of DR ($P = 0.408$) [Table 2].

Haemoglobin A1c levels

The mean hemoglobin A1c (HBA1c) levels were 9.49% ± 2.37%. The lowest HBA1c levels were seen in the group with no DR (8.45%) and the highest was seen with the group with severe NPDR (10.46%). However, the difference was not statistically different ($P = -0.230$) [Table 2].

Discussion

DR is without doubt one of the most significant ocular complications of a systemic disease. Several studies have showed that the VEGF plays a prime role in the pathogenesis of DR. The vitreous levels of VEGF are elevated in patients with DR in particular active, advanced stages of DR is an undisputable fact. However, previous studies show conflicting data with the association of blood VEGF and DR.

The aim was to study the comparison between the serum VEGF levels of DM patients with and without DR, and if there was any association between serum VEGF levels with the severity of DR.

Demography

The average age of the patients in the study population was 58.16 years, of which only Type 2 diabetics was included in the study. Any patient with Type 1 diabetes was excluded from the study.

Earlier studies done in similar populations from South India report age and longer duration of DM as risk factors for DR.[9-11] However, in our study, further analysis of the age and the severity of diabetes showed no statistical significance. Regarding the association with gender, majority of the patients were male, with an overall male-to-female ratio of 2.75:1. A similar result was reported by Raman *et al*., where they report an increased risk of DR among male patients (odds ratio = 1.41).[11]

Primary outcome measures

The serum VEGF level in the 75 patients was taken as our primary outcome. The average serum VEGF levels were 577.01 g/ml. On comparing this value with the known normal serum VEGF levels, our result was proven to be very significant. This result has been reported in a number of other studies as well.[12,13]

We then analyzed the elevation between the group with no DR and the normal population values. This too was significantly elevated ($P = 0.0076$). Therefore, it appears that VEGF is elevated in diabetics *per se* and not specifically in patients with DR. This can be explained by the fact that VEGF is a systemic factor and not confined to the eye. VEGF has been implicated in the pathogenesis of not only DR but also in diabetic

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nephropathy, neuropathy, and even diabetic macrovascular disease.[14-16]

Paradoxically, the group with PDR had the minimum mean value of VEGF. The group with mild NPDR had the maximum mean VEGF value. On testing the relation between VEGF and the severity of DR, there was no statistical significance detected. Similar results have been obtained in other studies.[17] Hence, serum VEGF levels are elevated in diabetics when compared with the normal population. However, there is no relationship with the severity of DR.

Secondary outcome measures

Duration of diabetes mellitus

In our study, the average duration of DM was 7.2 years. There was no statistically significant relationship between the duration of DM and severity of DR. However, the group with the minimum duration of DM was the group with no DR. This is in keeping with the established fact that the duration of DM is an important risk factor for the development of DR.

Hemoglobin level

The mean hemoglobin level was 11.53 g/dL. Taking the normal range of hemoglobin to be 12–15 g/dL, our population appears to be on the anemic side. In addition, the group with PDR had the lowest mean hemoglobin of 11.29; the group with the highest hemoglobin was the group with no DR. Although this is not statistically significant, this trend is in keeping with the fact that anemia worsens the retinopathy status. In addition, treating anemia has been proven to improve retinopathy and delay its progression.[18]

Urea and creatinine levels

The average urea level for our population was 35.47 mg/dL. Taking the normal range of serum urea to be from 14 to 40 mg/dL, this value falls into the high normal range. The mean creatinine levels were 1.42 mg/dL. As the normal range of creatinine is 0.66–1.09 mg/dL, this definitely falls into the high range. There was no statistically significant relationship between the creatinine levels or urea levels and the severity of diabetes. Even though the group with PDR had the highest urea levels and creatinine levels, this is very much in keeping with the fact that both nephropathy and retinopathy are microvascular disease and often the presence of one indicates the presence of the other. However, there is also a good chance of this renal disease being nondiabetic in origin. As highlighted in an earlier study by Prakash *et al*., nondiabetic renal disease versus diabetic disease was 22.6% versus 3.2% when the duration of DM was between 5 and 10 years. It is only after 10 years of DM that diabetic renal disease jumped ahead to 32.2% versus 6.5% for nondiabetic renal disease.[19] As our population, mean duration of diabetes was 7.2 years, it is likely that any renal impairment is more of a nondiabetic origin.

Random blood sugar and fasting blood sugar levels

The average RBS levels for the whole population were 228.95 mg/dL and the mean FBS was 165.87 mg/dL. Both these values are elevated when compared with normal values. This fact has important long-term prognostic value as it has been proved in numerous studies that control of blood sugar and other comorbidities play a major role in the control of diabetic complications. There was no statistically significant association between the levels of RBS and FBS and the severity of diabetes.

Hemoglobin A1c levels

The mean HBA1c level for our study population was 9.49%. The normal upper limit is taken to be 6.3%. As the HBA1c level indicates the state of blood sugar control for a prior 3 months, this indicates a very poor control. However, there was no significant relationship between HBA1c levels and the severity of DR. Moreover, no general trends were observed.

Limitation of this study

Many of the parameters studied showed wide standard deviations. This could be because of a small sample size. Possibly due to this same reason, many of the outcome measures did not show a statistically significant relationship with the severity of DR. Therefore, by increasing the sample size, it is possible that we could have obtained more significant results.

We did not have a population of healthy controls. Hence, we could only compare our study population with known normal ranges. Having a group of age- and sex-matched healthy control could have eliminated any confounders which might have arisen.

We did not study other comorbid conditions such as blood pressure, obesity, and hypercholesterolemia. All these factors have been proven to affect the complications of diabetes and they would have been useful in this study.

Conclusion

Serum VEGF levels were found to be significantly elevated in our study population when compared with the normal VEGF levels. This elevation of VEGF was observed in the entire diabetic population irrespective of whether they had retinopathy or not. There was no significant relationship between the serum VEGF levels and the severity of DR. Anemia was associated with severe grades of retinopathy. There was an elevation of urea and creatinine levels in the study population; this elevation was higher in the more severe grades of DR. RBS and FBS levels were high in the study population indicating a state of chronic hyperglycemia and also suboptimal control of blood sugars. The trend in HbA1c levels mirrored that of RBS and FBS again highlighting a poor control of sugars. In conclusion, an elevated serum VEGF level is significantly associated with DM and is linked to many of its complications. Although we are yet to discover about VEGF and its exact role in the pathogenesis of DR, one thing is certain, levels of VEGF lie a potential treatment and preventive strategy of not only DR but also of many of the complications of DM.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Das A. Diabetic retinopathy: A global epidemic. Middle East Afr J Ophthalmol 2015;22:133-4.
2. Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, *et al*. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
3. Klein BE, Moss SE, Klein R, Surawicz TS. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XIII. Relationship of serum cholesterol to retinopathy and hard exudate. Ophthalmology 1991;98:1261-5.
4. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, *et al*. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994;331:1480-7.
5. Wang X, Wang G, Wang Y. Intravitreous vascular endothelial growth factor and hypoxia-inducible factor 1a in patients with proliferative diabetic retinopathy. Am J Ophthalmol 2009;148:883-9.
6. Matsuoka M, Ogata N, Minamino K, Matsumura M. Expression of pigment epithelium-derived factor and vascular endothelial growth factor in fibrovascular membranes from patients with proliferative diabetic retinopathy. Jpn J Ophthalmol 2006;50:116-20.
7. Abu El-Asrar AM, Missotten L, Geboes K. Expression of hypoxia-inducible factor-1alpha and the protein products of its target genes in diabetic fibrovascular epiretinal membranes. Br J Ophthalmol 2007;91:822-6.
8. Lim JI, Spee C, Hinton DR. A comparison of hypoxia-inducible factor-α in surgically excised neovascular membranes of patients with diabetes compared with idiopathic epiretinal membranes in nondiabetic patients. Retina Phila Pa 2010;30:1472-8.
9. Rema M, Premkumar S, Anitha B, Deepa R, Pradeepa R, Mohan V. Prevalence of diabetic retinopathy in urban India: The Chennai Urban Rural Epidemiology Study (CURES) eye study, I. Invest Ophthalmol Vis Sci 2005;46:2328-33.
10. Namperumalsamy P, Kim R, Vignesh TP, Nithya N, Royes J, Gijo T, *et al*. Prevalence and risk factors for diabetic retinopathy: A population-based assessment from Theni District, south India. Postgrad Med J 2009;85:643-8.
11. Raman R, Rani PK, Reddi Rachepalle S, Gnanamoorthy P, Uthra S, Kumaramanickavel G, *et al*. Prevalence of diabetic retinopathy in India: Sankara Nethralaya diabetic retinopathy epidemiology and molecular genetics study report 2. Ophthalmology 2009;116:311-8.
12. Fan X, Wu Q, Li Y, Hao Y, Ning N, Kang Z, *et al*. Association of polymorphisms in the vascular endothelial growth factor gene and its serum levels with diabetic retinopathy in Chinese patients with type 2 diabetes: A cross-sectional study. Chin Med J (Engl) 2014;127:651-7.
13. Koleva-Georgieva DN, Sivkova NP, Terzieva D. Serum inflammatory cytokines IL-1beta, IL-6, TNF-alpha and VEGF have influence on the development of diabetic retinopathy. Folia Med (Plovdiv) 2011;53:44-50.
14. Khamaisi M, Schrijvers BF, De Vriese AS, Raz I, Flyvbjerg A. The emerging role of VEGF in diabetic kidney disease. Nephrol Dial Transplant 2003;18:1427-30.
15. Zakareia FA. Electrophysiological changes, plasma vascular endothelial growth factor, fatty acid synthase, and adhesion molecules in diabetic neuropathy. Neurosciences (Riyadh) 2008;13:374-9.
16. Wirostko B, Wong TY, Simó R. Vascular endothelial growth factor and diabetic complications. Prog Retin Eye Res 2008;27:608-21.
17. Praidou A, Papakonstantinou E, Androudi S, Georgiadis N, Karakiulakis G, Dimitrakos S. Vitreous and serum levels of vascular endothelial growth factor and platelet-derived growth factor and their correlation in patients with non-proliferative diabetic retinopathy and clinically significant macula oedema. Acta Ophthalmol 2011;89:248-54.
18. Qiao Q, Keinänen-Kiukaanniemi S, Läärä E. The relationship between hemoglobin levels and diabetic retinopathy. J Clin Epidemiol 1997;50:153-8.
19. Prakash J, Gupta T, Prakash S, Bhushan P, Usha, Sivasankar M, *et al*. Non-diabetic renal disease in type 2 diabetes mellitus: Study of renal – Retinal relationship. Indian J Nephrol 2015;25:222-8.

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Research Article

Effect of Antenatal Yoga on Labour Outcome- A Non-Randomized Control Study from a Tertiary Hospital in South India

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INTRODUCTION: Giving birth to a healthy baby is one of the happiest moments in a woman's life. Yoga is a 'body-mind' practice. The prospective of yoga to create balance along emotional, mental, physical, and spiritual dimensions is immense. It uses physical positions (asana), meditation (dharana and dhyana), concentration, breathing exercises (pranayama) and contemplative practice. OBJECTIVE: To study the effect of yoga on antenatal outcome. MATERIALS AND METHODS: It is a Prospective study done during the time period July 2013 to July 2014.In a series of 50 women who fit in the inclusion criteria, attending the antenatal clinic of a private medical college in Tamilnadu, training to practice yoga as relaxation therapy during pregnancy and labour was given and it was compared with 50 antenatal women who obtained regular care. The various outcomes like Mode of delivery, Bishop's score, Duration of active labour; Administration of analgesics and Weight of infant were studied. RESULTS: The major advantage noted in this study was reduced duration of active phase of labour. 70% of the antenatal women doing yoga went into spontaneous labour. 34% of induced labour in the control group were noted. All the patients in the yoga arm delivered at term. Whereas 4% of the patients in control arm had preterm delivery. CONCLUSION: Practicing yoga in the antenatal period decreases induced labour and shortens the duration of active phase of labour. The mode of delivery has a multifactorial contribution and cannot be compared in this study.

Keywords: Yoga, Pregnancy, Labour, Active Phase

INTRODUCTION

Motherhood transforms a woman forever. It extracts out a life lying dormant for months deep within her. Remaining physically active during pregnancy is the best a woman can do. At least thirty minutes of moderate exercise each day is recommended for improving the health and wellbeing of non pregnant women according to Len Kravitz (1998). In accordance with the American College Of Obstetrics and Gynaecology (2002), exercise during the period of pregnancy is very good and appropriate in pregnant women without any obstetric and medical complications. Yoga may be able to provide a gentle alternative to physical activity.Yoga is a 'body - mind' practice .It is a combination of various physical positions, breathing and relaxation exercises according to Smith C.A (2011). The word 'Yoga' comes from the Sanskrit word which means "to join" and can be understood as unity or oneness It uses physical positions (asana), meditation (dharanaanddhyana), concentration, breathing exercises (pranayama) and contemplative practice. Yoga mends psychological wellbeing. Labour is the active process of delivering a foetus and is characterised by regular, painful uterine contractions which increase in frequency and intensity. It is difficult to predict the length of labour. Duration of labour was positively corelated with the weight of the infant, duration of pregnancy, weight gain and prepregnant weight. It was negatively corelated with mother's height. Mother's age did not influence duration of labor according to Britt-Ingjerd Nesheim (1988). According to Kelly Riley (2017) Malpositions of the head prolonged labor in nulliparas, but not in multiparas, while breech presentation had no influence. 35 trials lecturing the outcomes of yoga on anxiety and stress were reviewed by LiAW et al in (2012) and the results were indicative of the role of yoga in alleviating stress and concern· Randomized controlled study on 74 women by Pamela Jones (2008) who

reported that in yoga group there was improved comfort during labour and after labour. The interval of first stage of labour was also shorter as well as the total labour duration according to a study done in Prince of Songkla university in Thailand by Chuntharapat S(2008) .So ours is an attempt to evaluate the effect of yoga on antenatal outcome in our study population.

METHODOLOGY

Study Design: Prospective non randomized control study
Time period: July 2013 to July 2014
Ethical approval: OBTAINED FROM ETHICS COMMITTEE OF PSGIMSR AND INFORMED CONSENT TAKEN.

A series of 50 women attending the antenatal clinic of a private medical college in Tamilnadu were given training to practice yoga as relaxation therapy during pregnancy and the labour outcome was compared with 50 antenatal women who obtained regular care.

Pregnant women of gestational age> 12 weeks are given training in 6 separate sessions by specially trained people in yoga through training sessions. They are encouraged to practice this on a regular basis. The regularity is assessed through personal interview. They are advised to do yoga minimum of 5 times a week.

Inclusion criteria
- Singleton pregnancy
- Primi less than 35 years
- Spontaneous conception
- No antenatal complications
- Co-operative and compliant patients

Exclusion criteria
- Multiple pregnancy
- Threatened abortion or previous preterm delivery
- Elderly Primi
- Multigravida
- Patients not willing to participate in the study

Types of outcome measured
- Mode of delivery
- Bishop's score
- Duration of active labour
- Administration of analgesics
- Weight of infant
- Type of delivery was assessed as normal vaginal delivery, assisted vaginal delivery (instrumental) or caesarean section.

➢ BISHOP'S SCORE (TABLE-1)

Table 1: BISHOP'S SCORE

Sign	Points				Score
	0	1	2	3	
Position	Posterior	Intermed.	Anterior		
Consistency	Firm	Intermed.	Soft		
Effacement	0-30%	31-50%	51-80%	>80%	
Dilation	0 cm	1-2 cm	3-4 cm	>5 cm	
Fetal station	-3	-2	-1, 0	+1, +2	
Results	The Bishop Score is: Calculate Bishop Score Reset				

The Bishop Score scores patients who would be most likely to accomplish a successful induction. It is a pre induction record. A score that go beyond 8 defines the patient most likely to accomplish a fruitful vaginal birth. A low Bishop's score frequently designates that induction is unlikely to be fruitful. Kelly C (2019) specify that only a score of 8 or more is reliably prognostic of a positive induction

➢ Active phase duration

Cervical dilatation of 4cm or more with uterine contractions can be taken to dependably signify the onset of active labour .According to Friedman the mean duration of active phase of labour in nullipara was 4 .9 hours .As the standard deviation of 3 .4 hours is large ,the active phase was testified to have a numerical maximum of 11 .7 hours .In all patients in labour we plot a partogram and the active phase duration was calculated.

➢ Analgesia

The various analgesia options in labour are epidural, sedation and inhalational analgesia with entonox. The use of epidural analgesia was examined in our study.

THE VARIOUS POSTURES OF YOGA PRACTICED IN PREGNANCY ARE AS FOLLOWS: TABLE 2

Table 2: THE VARIOUS POSTURES OF YOGA PRACTICED IN PREGNANCY ARE AS FOLLOWS

Day1(2ND TM)	Day2 (2ND TM)	Day 3 (2NDTM)	DAY 4 (3RD TM)	Day 5 ,6(3RD TM)
1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Suryanadipranayama	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Chopping wood pose 7) Pawanamukthasana part 1 8) Suryanadipranayama 9) Chandranadi	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Hands in and out breathing 7) Hands up and down breathing 8) Tadasana 9) Butterfly 10) Kadichakrasana 11) Pawanamukthasana 12) Warrior pose 13) Vajrasana 14) Suryanadipranayama 15) Chandranadi 16) Suryabhedana 17) Veerabadrasana 18) Cat stretch	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Vajrasana 7) Pawanamukthasana 8) Warrior pose 9) Chopping wood pose 10) Namaskarasana 11) Seated side stretch 12) Gentle twists 13) Suryanadipranayama 14) Chandranadi 15) Suryabhedana	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Pawanamukthasana 7) Warrior pose 8) Chopping wood pose 9) Sleeping abdominal stretch 10) Namaskaarasana 11) Seatedsidestretch 12) Gentle twist 13) Suryanadi 14) Chandranadi 15) Suryabhedana 16) Chandrabhedana 17) Mukthadhouti

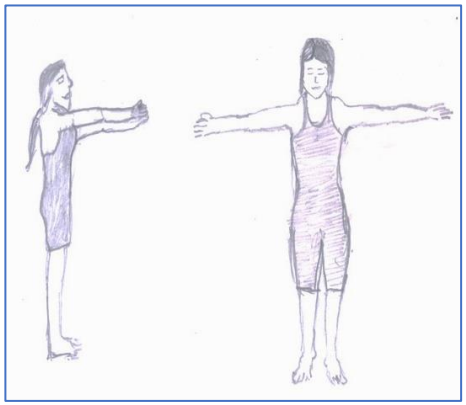


Figure 1: Hands in and out breathing



Figure2: Tadasana

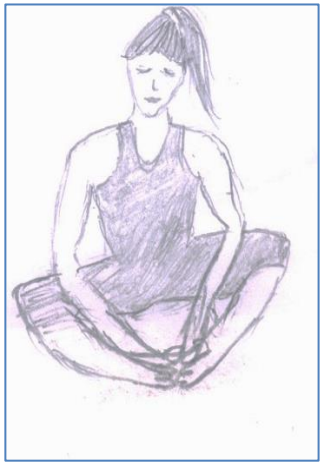


Figure 3: Butterfly posture



Figure 4: Suryanadi Pranayama

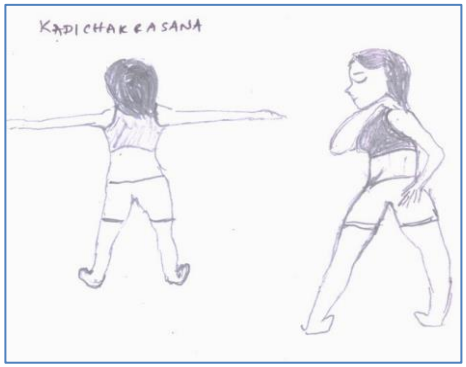


Figure 5: Kadichakrasana

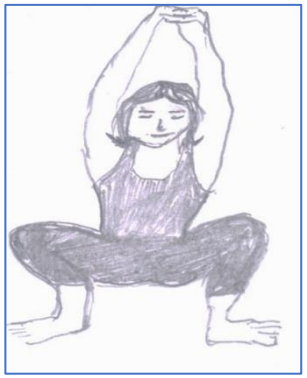


Figure 6: Chopping Wood Pose



Figure 7: Vajrasana



Figure 8: Warrior Pose

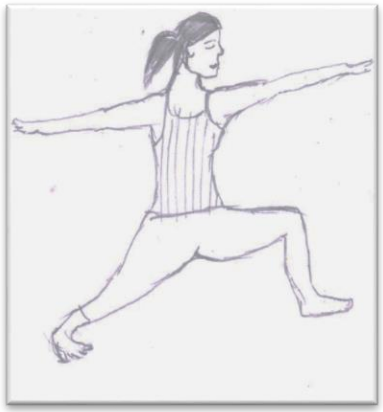


Figure 9: Veerabhadrasana



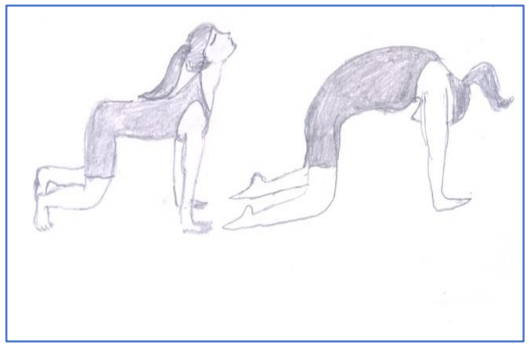


Figure 10: Cat Stretch



Figure 11: Seated Side Stretch



Figure 12: MukhaDhauti

Inhale. exhale and press the thighs and knees downward towards the floor. Make a gentle effort to keep pressing them forward. Start flapping both the legs up and down.

SURYA NADI PRANAYAMA - FIGURE 4

This refers to the Breathing Exercise. Inhale through the right nostril, Exhale through the left, press the index and middle finger of the right hand against the palm of that hand. Use thumb to occlude the right nostril. Ring and little fingers to occlude the left nostril.

KADICHAKRASANA - FIGURE 5:

Exhale and twist from the waist to the right and look back over the right shoulder. Keep the distance between your palms constant. Inhale comeback to the center, Exhale and turn to left and repeat the yoga posture on your left side.

CHOPPING WOOD POSE – FIGURE 6

Sit in a squatting position with your back straight .Clasp the hands in front and with inhalation raise the hands gently up in the air and come down with an exhale .

PAWANAMUKTHASANA PART 1

Sit outstretched, keep both the knees together and the heel on floor spine and the neck in the same level. put the palms of the hands on the floor. Keep the fingers outstretched. Apply pressure on the palm. Close eyes and breath. Begin the exercise with open eyes

VAJRASANA - FIGURE 7

The name comes from the Sanskrit words *vajra* - "thunderbolt". Sit on heels with the calves beneath thighs. There should be four finger gap between the knee-caps., sit straight.

WARRIOR POSE - FIGURE 8

VEERABHADRASANA – FIGURE 9

Breathe in as you come up and exhale as you go down with your hands from side. Redo the same from left side

CAT STRETCH FIGURE:10

STEP 1: Position your palm directly below your shoulders. Place knees directly below your hips . Spread your fingers with the middle fingers pointed straight.

STEP 2: Press towards the floor using your hands and then lift yourself up out of your shoulders. Go back and forth several times. Exhale and sag shoulders.

SEATED SIDE STRETCH - FIGURE 11

Rotate your head to look beyond your left elbow. Retain this position for 5 seconds. Now inhale and lift trunk and move left upper limb upward. Breathe out by bringing down left upper limb.

MUKHA DHAUTI- FIGURE 12

Lean forward and rest the arms on the respective thighs keeping the arms straight. Inhale through the nose and exhale forcefully through the mouth. Stand in Tadasana and relax.

Ethical approval: OBTAINED FROM ETHICS COMMITTEE OF PSGIMSR WITH IHEC APPROVAL NUMBER-13/121
SPSS SOFTWARE USED FOR DATE MANAGEMENT AND CALCULATION

RESULTS

In the study of antenatal yoga on labour outcome 100 antenatal women who were not age matched were included. 50 women were doing yoga and 50 received regular antenatal care.

TABLE 3: The age distribution is as follows

Age Distribution			
Age	Group		Total
	Yoga	Control	
<20	0	4	4
21-25	25	24	49
26-30	21	20	41
31-35	4	2	6
Total	50	50	100

The result shows that there is no significant age difference among the antenatal in group who were doing yoga and in routine care category

NO OF YOGA CLASSES ATTENDED BY THE ANTENATAL WOMEN IN YOGA GROUP.

46% of the antenatal women in yoga group had attended 4 classes. Only 10% of the women attended 5 yoga classes. 44% of the antenatal women who were practicing yoga attended only 3 yoga classes.

Table 4: Distribution of preterm or term deliveries

Term/ Preterm			
Out come	Group		Total
	Yoga	Control	
Term	50	48	98
Pre Term	0	2	2
Total	50	50	100

All the patients in the yoga arm delivered at term. Whereas 96% in the control arm delivered at term and 4% of the patients had preterm delivery, but this was not statistically significant as the p value was >0.5.

Table 5: The bishop's score among the antenatal women in yoga group and control group

Bishop Score	Group		Total
	Yoga	Control	
< 5	28	28	56
> 5	19	20	39
Total	47	48	95

As per the result there is no significant difference in Bishop's scoring among the two groups.

Table 6: The onset of labour among the yoga group and the control

Labour	Group		Total
	Yoga	Control	
Spontaneous	33	33	66
Induced	14	17	31
Total	47	50	97

The onset of labour is comparable in both the groups. 70% of the antenatal women doing yoga went into spontaneous labour. 34% of induced labour in the control group were noted. In the yoga group only 30% were induced labour.

Table 7: Distribution of mode of delivery

Mode of Delivery			
Delivery	Group		Total
	Yoga	Control	
NVD	20	36	56
LSCS	17	10	27
Instrumental	13	4	17
Total	50	50	100

The percentage of patients went in for lscs was 34 % in the yoga group where as it was 20% in the control group. Only 66% of women doing yoga had a vaginal delivery out of them 26% was instrumental delivery. Whereas in the control group 80% had a vaginal delivery. This finding was also not statistically significant,

Table 8: Distribution of Instrumental Delivery

Delivery Instrumental			
	Group	Total	
Instrumental	Yoga	Control	
VACUUM	12	3	15
FORCEPS	1	1	2
Total	13	4	17

Vacuum assisted delivery was significantly high among the yoga patients.

Table 9: Indications for lscs

LSCS	Group		Total
	Yoga	Control	
CPD	1	2	3
NPL	1	1	2
ARREST	2	2	4
FETAL DISTRESS	3	5	8
MSL	4	0	4
CONDYLOMA	1	0	1
DTA	1	0	1
FAILED	1	0	1
MATERNAL WISH	1	0	1
TRANSVERSE LIE	1	0	1
MOBILE HEAD	1	0	1
Total	17	10	27

The common Indication for LSCS in yoga patients were meconium stained liquor in early labour and fetal distress.

Table 10: Duration of active phase of labour

Duration	Group		Total
	Yoga	Control	
<= 2 HRS	3	0	3
2 - 3 HRS	16	7	23
3 - 4 HRS	10	16	26
> 4 HRS	4	17	21
Total	33	40	73

There is significant difference in the duration of active phase among the women practicing yoga and the control group. Majority had an active phase duration of< 2-3 hours in the yoga group when compared to the controls who had an average duration more than 4 hours which was statistically significant.

THE USE OF ANALGESICS

There is no significant difference among both the groups in usage of analgesics .

Table 11: Effect on birthweight

Birth Weight [kgs]	Group		Total
	Yoga	Control	
< 2 .5	2	5	7
2 .5 - 3 .5	43	42	85
> 3 .5	5	3	8
Total	50	50	100

The mean birth weight of the babies born to women practicing yoga was 3.10kg .

MEAN OF CLINICAL PARAMETERS – TABLE 12

Mean duration of active labour , in the yoga group came about 190 minutes ,whereas the mean duration of active phase of labour for the control group was 244 minutes which was statistically significant.

Table 12: Mean of clinical parameters								
		Mean	SD	95% CI for Mean		Minimum	Maximum	p value
	Group			Lower Bound	Upper Bound			
Age	Yoga	26	3	25	27	21	35	
	Control	25	3	24	26	19	32	>0 .05
	Total	26	3	25	26	19	35	
Bishopscore	Yoga	4 .45	1 .63	3 .97	4 .92	1	9	
	Control	4 .46	1 .68	3 .97	4 .94	1	9	>0 .05
	Total	4 .45	1 .64	4 .12	4 .79	1	9	
Birthweight [kgs]	Yoga	3 .10	0 .3	3 .0	3 .2	2	4	
	Control	2 .99	0 .4	2 .9	3 .1	2	4	>0 .05
	Total	3 .04	0 .3	3 .0	3 .1	2	4	
Duration [Hrs]	Yoga	190	60	168	211	40	330	
	Control	244	64	223	264	120	360	<0 .01
	Total	219	67	203	235	40	360	

Table 13: Data summary

Group		YOGA	CONTROL	p value		p value
Clinical characteristics						
AGE						
<20		0%	8%			
21-25		50%	48%	>0 .05		0 .194
26-30		42%	40%			
31-35		8%	4%			
Outcome						
Term		100%	96%			
Pre Term		0%	4%	>0 .05		0 .495
Bishop score						
< 5		60%	58%			
> 5		40%	42%	>0 .05		0 .902
Labour						
Spontaneous		70%	66%			**0 .67**
Induced		30%	34%	>0 .05		
Mode of Delivery						
NVD		40%	72%			
LSCS		34%	20%	>0 .05		0 .004
Instrumental		26%	8%			
Delivery Instrumental						
VACUUM		92%	75%			0 .426
FORCEPS		8%	25%	>0 .05		
Duration of active Labour						
<= 2 HRS		9%	0%			
2 - 3 HRS		48%	18%			0 .001
3 - 4 HRS		30%	40%	<0 .01		
> 4 HRS		12%	43%			
Analgesics						
EPIDURAL		8%	10%			
IM		92%	90%	>0 .05		0 .822
Birth Weight [kgs]						
< 2 .5		4%	10%			
2 .5 - 3 .5		86%	84%	>0 .05		0 .407
> 3 .5		10%	6%			
Mean Baseline characteristics						
Age		26	25	>0 .05		0 .015
Bishop score		4 .45	4 .46	>0 .05		0 .973
Birth weight [kgs]		3 .10	2 .99	>0 .05	0 .048	**0 .081**
Duration [min]		190	244	<0 .01		0 .001

DISCUSSION

This study which was conducted in a Tertiary hospital in Tamilnadu was done with the objective to analyse the effect of yoga on antenatal outcome. We enrolled 100 antenatal patients-of which fifty underwent yoga classes and fifty had routine antenatal care and the outcomes were analysed and the results were given above.

The age group of the antenatal patients who did yoga and the control group was similar in our study. All the patients practicing yoga had a term delivery which is similar to the study by Narendran et al (2005) where preterm deliveries were significantly low. In our study there was no significant difference in the Bishop's score at admission in the yoga and control group. There was no data to compare this.

There was also no difference in the onset of labour for both the groups.28% had induced labour in the yoga group and 34% in the control group in our study. This is similar to the study by Jahdi et al (2017) where there was a decreased percentage of labour induction in the yoga group.

In our study 34% of the yoga group patients had caesarean delivery while only 20% of the control group had caesarean delivery. This is in contrast to the study by Jahdi et al (2017) and Satyapriya Maharana et al in 2013 who showed reduced incidence of caesarean delivery after yoga therapy i.e.15% versus 40% in the control group.Since the mode of delivery has got multifactorial contribution ,it is difficult to compare it in these two groups. Even though the mode of delivery is not significantly altered, larger sample size is required to conclude this fact.

The major advantage noted in our study was the reduced duration of active phase of labour.This is very much significant as the duration of labour is an important factor in patient comfort,experience with childbirth and satisfaction.The reduction in active phase duration is consistent with the studies conducted by Songporn chuntharapat (2008), Sathyapriya M et al (2013) where there was significant reduction in labour duration.

The mean birth weight of the babies born to women practicing yoga was 3.10kg and 2.99kg in the control group which was not statistically significant. This is similar to the study by Maria Pais in 2016 where the mean weight of the yoga group was 3kg and the control group was 2. 7kg.This may be due to the increase gestational age of delivery in the yoga group. This was also consistent with the study by Shamanthakamani Narendran et al. in (2005) where there was significant higher babies with birth weight more than 2.5kg in the yoga group.

CONCLUSION

As per my study antenatal women doing yoga had significant reduction in the duration of active phase of labour than who receive regular care. Although not statistically significant, all of the patients in yoga group had term deliveries and good mean weight of babies. May be a larger study group in the future can give more definitive results.

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INFORMED CONSENT - Taken

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REFERENCES

ACOG Committee opinion. Number 267, January 2002: exercise during pregnancy and the postpartum period.Obstet Gynecol. 2002 Jan; 99(1):171-3.

Britt-IngjerdNesheim.Duration of Labor: An analysis of influencing factorsActaobstetricia et gynecologicascandinavica 67:121-124https://obgyn.onlinelibrary.wiley.com/journal/16000412

Chuntharapat S, Petpichetchian W, Hatthakit U. Yoga during pregnancy: effects on maternal comfort, labor pain and birth outcomes. Complement 2008 May;14(2):105-15. doi: 10.1016/j.ctcp.2007.12.007. Epub 2008 Mar 4.

Jahdi F, Sheikhan F, Haghani H, Sharifi B, Ghaseminejad A, Khodarahmian M, Rouhana N. Yoga during pregnancy: The effects on labor pain and delivery outcomes (A randomized controlled trial).Complement Ther Clin Pract. 2017 May; 27:1-4. Doi: 10.1016/j.ctcp.2016.12.002. Epub 2016 Dec 23

Kelly C. Wormer; Ann E. Williford. Bishop Score. https://www.ncbi.nlm.nih.gov/books/NBK470368/

Kelly Riley, Emily Drake .The Effects of Prenatal Yoga on Birth Outcomes: A Systematic Review of the Literature .https://birthpsychology.com/journal/issue/volume-28-issue-1

Len Kravitz, Ph.D. From 1998 to 2011: ACSM Publishes Updated Exercise Guidelines https://www.unm.edu/~lkravitz/Article%20folder/ACSMGuidelinesUNM.pdf

Li AW, Goldsmith CA.The effects of yoga on anxiety and stress. Altern Med Rev. 2012 Mar; 17(1):21-35

Maria Pais,PaiMuralidhar. Effect of yoga therapy on antenatal stress and pregnancy outcome. http://hdl.handle.net/10603/169974shodhganga: a reservoir of Indian theses @ inflibnet.

Narendran S[1], Nagarathna R, Narendran V, Gunasheela S, Nagendra HR Efficacy of yoga on pregnancy outcome.J Altern Complement Med. 2005 Apr; 11(2):237-44.

Pamela Jones, MA Yoga in Pregnancy May Improve Labor Discomfort https://www.cancercarewny.com/content.aspx?chunkiid=345901

Satyapriya M, Nagendra HR, *et al.* Effect of integrated yoga on stress and heart rate variability in pregnant women. Int J Gynaecol Obstet. 2009; 104(3):218-22.

Shamanthakamani Narendran, Raghuram Nagarathna, Vivek Narendran, Sulochana Gunasheela, Hongasandra Rama Rao Nagendra .Efficacy of Yoga on Pregnancy Outcome. Journal of Alternative and Complementary Medicine Apr 2005, Vol. 11, No. 2: 237-244

Smith CA[1], Levett KM, Collins CT, Crowther CA. Relaxation techniques for pain management in labour.Cochrane Database Syst Rev. 2011 Dec 7 ;(12):CD009514. doi: 10.1002/14651858.CD00951

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One year retrospective analysis of PAP smears-from a tertiary hospital in Tamil Nadu

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Abstract
Introduction: Cervical cancer is one of the leading causes of maternal mortality in India and worldwide, it usually takes 3–7 years for high-grade changes in cervical cells to become cancer. There are many effective screening tests for cervical cancer. PAP smear is one such test
Aim: To analyse the pap smears done in our hospital for a period of one year
Objectives: 1). To determine the percentage of abnormal smears detected 2). To analyse the myriad of findings in a PAP smear.
Materials and methods: It is a Retrospective Observational study, Data was collected from the PAP smears taken in the Gynaecology department outpatients and master health check-up of our hospital. Data was collected for a period of one year from January 2017 to December 2017.
Results: Around 2333 PAP smears were analyzed over a period of one year, of it 885 was from our OPD and 1448 was from the master health check-up, the detection of precancerous lesion was 1.2%, in addition to detecting pre-cancerous lesions, additional information showed a variety of infections like a Candida, Trichomonas, Actinomyces, etc.
Conclusion: Cytology based screening is one of the most cost effective and easy method of cervical cancer screening.

Keywords: Ca cervix, Pap smear, screening, pre malignant lesions

Introduction
Cervical cancer is one of the leading causes of morbidity and mortality in women worldwide. Cervical cancer is the fourth most frequent cancer in women with an estimated 570,000 new cases in 2018 representing 6.6% of all female cancers. The high mortality rate from cervical cancer globally could be reduced through a comprehensive approach that includes prevention, early diagnosis, effective screening and treatment programmes [1]. The screening methods range from VIA (visual inspection with acetic acid) and VILI (visual inspection with Lugol's Iodine in low resource setup where pathologist is not available, to the PAP smear and liquid base cytology with or without HPV testing and sophisticated colposcopy in high resource centres.

Materials and Methods
This is a retrospective study conducted in PSGIMSR. Data was collected from the PAP smears taken in the Gynaecology department outpatients and master health check-up.
Of our hospital. Data was collected for a period of one year from January 2017 to December 2017

Inclusion criteria
1) All PAP smears taken in our OP
2) All PAP smears taken as a part of master health check-up in our hospital

Exclusion criteria
1) Women without sexual exposure

Procedure
Cells from ectocervix and endocervix are collected simultaneously with the broom like spatula. The central bristles of the broom are inserted into the endocervical canal until the lateral bristles bend fully against the ectocervix. The sampling device is rotated 360º in the same direction five

times while maintaining gentle pressure. The broom is removed and with a single paint stroke motion the cellular sample is transferred along the long axis of the labelled surface of the slide. Minimum of two slides are taken except for vault smears where a single slide is taken. The slide is rapidly fixed by immersion in 95%ethanol and ether mix in a Koplik's jar and transferred to the cytology lab [2].

Slides are analysed by the pathologists and reported based on Bethesda classification.

Bethesda classification (2014) [3].

Specimen Type
Indicate conventional Pap smear vs. liquidbasedpreparation vs. other

Specimen Adequacy
Satisfactory fore valuation /Unsatisfactory for evaluation

Interpretation/Result
Negative for intraepithelial lesion or malignancy Non-neoplastic findings (optional to report*)*

Organisms
Trichomonas vaginalis/ *Candida* spp/ Bacterial vaginosis/ *Actinomyces* spp. /Herpes simplex virus/Cytomegalovirus

Other Endometrial cells

Epithelial Cell Abnormalities
Squamous Cell
Atypical squamous cells of undetermined significance (ASC-US) cannot exclude HSIL (ASC-H) low grade squamous intraepithelial lesion (LSIL) High-grade squamous intraepithelial lesion (HSIL) Squamous cell carcinoma

Glandular Cell
Atypical
- Endocervical cells NOS /favour neoplastic
- Endometrial cells NOS *or specify in comments*
- Glandular cells (NOS /*favour neoplastic*)

Endocervical adenocarcinoma in situ
Adenocarcinoma
Endocervical/endometrial /extra uterine/not otherwise specified

Other Malignant Neoplasms: *(specify)*
Adjunctive Testing
Computer-Assisted Interpretation of Cervical Cytology

Results
Around 2333 PAP smears were analysed over a period of one year from Jan 2017 to Dec 2017, of it 885 was from our OPD and 1448 was from the master health check-up. The age distribution, the indications for taking a smear and the cytology report was analysed and is put up in the tabular column.

Table 1: Age distribution of Pap smear

Age of the patient in years	Number of patients in MHC	Number of patients in OPD	Total	Percentage
20-29	37	68	105	4.50
30-39	221	274	495	21.22
40-49	398	340	738	31.63
50-59	493	143	636	27.26
60-69	256	45	301	12.90
70-79	37	15	52	2.23
80-89	6	0	6	0.26
Total	1448	885	2333	100%

Table 2: Number of vault smear

Number of vault smears in master health checkup	215
Number of vault smears in OPD	25
Total	240
Percentage of vault smears	10.29%

Table 3: Indications for Pap smear

Indication	No of patients in MHC	No of patients in OPD	Total	Percentage
AUB		102	102	4.37%
Leucorrhoea		107	107	4.59%
Itching		6	6	0.26%
Mass P/V		1	1	0.04%
Pain Abdomen		36	36	1.54%
Post-Menopausal Bleeding		17	17	0.73%
Screening	1448	604	2052	87.96%
Strawberry vagna		1	1	0.04%
Post Ca Follow Up			11	0.47%
Post Ca Ovary		1		
Post Ca endometrium		5		
Post ca fallopian tube		1		
Post Lletz		2		
Post Ca cervix		2		
Total	1448	885	2333	100%

Table 4: Analysis of cytology report

Report	Number in MHC	Number in OPD	Total	Percentage	Total
Negative for IEL/Malignancy	849	413	1262	54.10%	Niel 2280 (97.73%)
Inflammation	406	319	725	31.07%	
Organisms					
Candida	93	62	155	6.64%	
BV	71	52	123	5.27%	
TV	4	9	13	0.56%	
Actinomycosis	0	2	2	0.09%	
Epithelial cell abnormalities			24	1.03%	Abnormal 24 (1.03%)
Squamous cells					
ASC-US	4	4	8		
ASC-H	2	2	4		
LSIL	2	2	4		
HSIL	0	4	4		
Glandular Cells					
AGUS	0	4	4		
Other Malignant Cells	0	0	0	0	
Unsatisfactory	17	12	29	1.24%	29 (1.24%)
Total	1448	885	2333	100%	2333(100%)

Table 5: Agewise Analysis of Abnormal Smears

AGE	Ascus	ASC-H	AGUS	LSIL	HSIL	SCC	Total Smears	
21-30					1		1	O.04%
31-40	1		2		1		4	O.17%
41-50	4	3	1		1		9	O.39%
51-60	1		1	4	1		7	0.30%
61-70	1	1					2	O.O9%
71-80	1						1	O.O4%
Total	8	4	4	4	4		24	1.03%
	O.34%	O.17%	O.17%	O.17%	O.17%	0	1.03%	

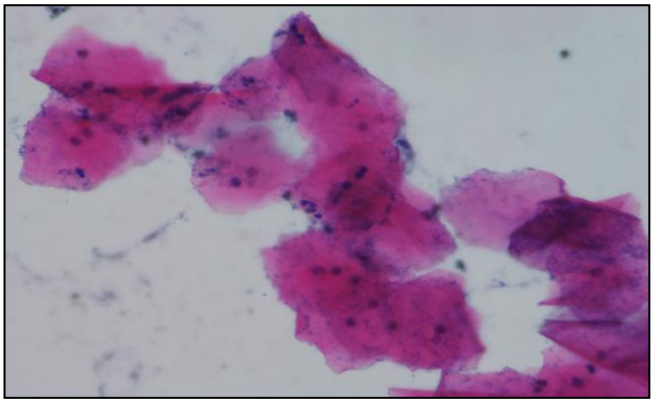


Fig 1: Bacterial Vaginosis

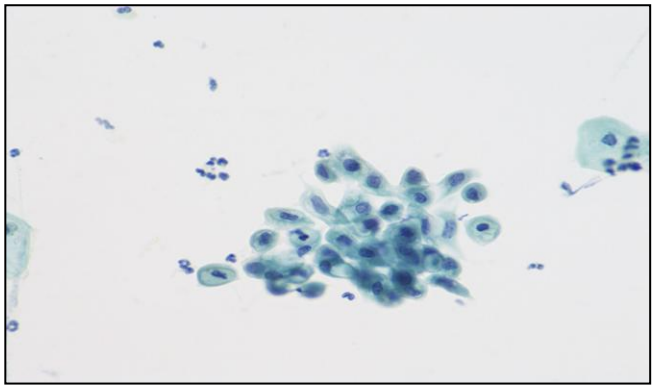


Fig 3: LSIL

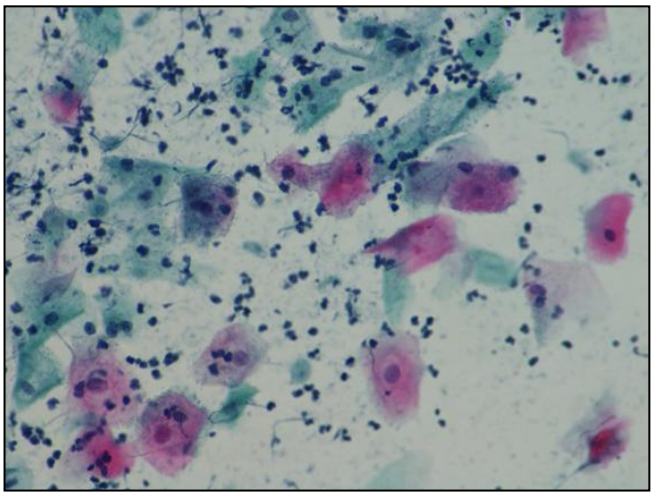


Fig 2: Trichomonas Vaginalis

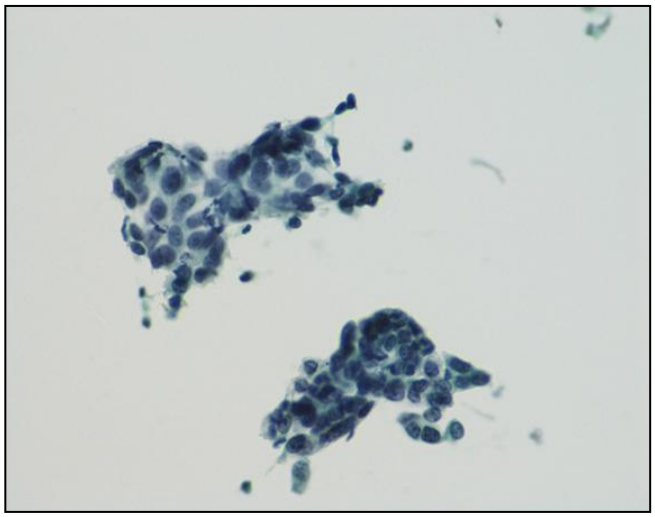


Fig 4: HSIL

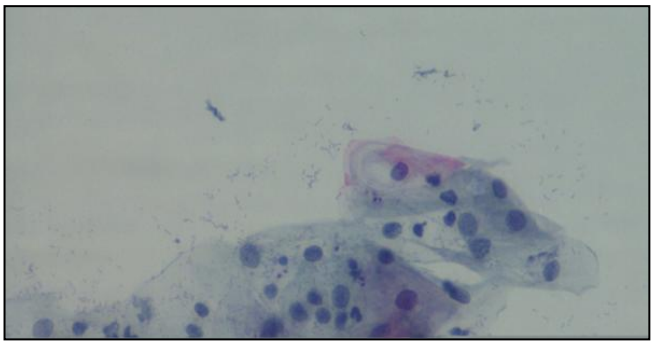


FIG 5: Candida

Discussion

PAP smears are analytical tools for screening of cervical cancer. It is a cost effective way for detecting the pre-invasive cervical lesions. In addition it also gives supplementary data on a variety of infections like Candidiasis, Bacterial vaginosis etc.

Of the 2333 pap smears taken over a period of one year, the maximum number of patients was of the age group 40-49 years accounting for 31.63%. The lowest age of the patient was 20 years –a married woman with severe itching and who's Pap smear showed inflammation. The oldest lady was 84 years old who came for a routine screening and the report was negative for IEL. According to a study by Kalyani *et al*. [4] the rate was 28.7%in the same age group and 32.5% in the 30-39year group which was maximum. According to Vijayalakshmi *et al.* [5], the maximum number of patients was in the age45-55 age group which was 34% The most common indication for Pap smear was for routine screening, accounting for 87.96% of the smears, Leucorrhea was the next common indication in those patients in the OPD- 4.59% with AUB being close behind - 4.37%. There were 0.4% of post Malignancy surgery follow up. Post ca endometrium being the commonest. The most common indication was leukorrhea accounting for 30% in a study by Vijayalakshmi *et al.* [5] while the routine screening was around 23%. The high rate of screening in our study might be due to increased awareness among the city population. Around 10.29% where vault smears.

Of the 2333 smears analyzed 2280 were negative for malignancy though they detected inflammation and infections. We had a detection rate of 1.03% (24 cases) of abnormal PAP smears. of the 24 cases there were 4 each of HSIL, LSIL, ASC-H AND AGUS and 8 of ASC-US, only 50% of these patients underwent colposcopy and directed biopsies and except two LSIL all the biopsy reports confirmed the lesions. This abnormal reports-1.03% in our study is close to the study conducted by Tamrakar *et al*. [6] which was 1.7%, but was less than the international and Indian studies done by Sherpa *et al.* [7]. (3.6%) and Sankaranarayanan *et al.* [8] (3.4%) respectively. There were around 1.24% of unsatisfactory smears were opinion was not possible which iscomparable to the study by Crasta *et al.* [5] which is 1.36%, The rate of unsatisfactory smear was high in the study by Kalyani *et al.*, [5] i.e., 17.8%.

Among the negative smears -Inflammmatory smears were found in 54.1% which was comparable to the study by Patel *et al.* [10] (51.5%).In our study, the various organisms detected where candida in 6.44% followed by bacterial vaginosis in 5.27%.in the study by Kalyani *et al.* [8] the inflammatory smears where 83.1%.. candida was found in 2.03% and bacterial vaginosis in 9.8%.while Candida was 3.71% in the study by Patel *et al.* [10]. In our analysis there were two cases of Actinomycosis also and 13cases of Trichomonas Vaginalis of which one patient had strawberry vagina. The comparison of the unsatisfactory and the pathological reports in the various studies are put in a tabular column below:

Table 6: The comparison of the unsatisfactory and the pathological reports in the various studies

Diagnosis	Kalyani *et al.* [5]	Crasta *et al.* [9]	Narasimha *et al.* [3]	Sankaranarayanan *et al.* [8]	Our study
Unsatisfactory	17.80%	1.36%	24.42%	4.10%	1.24%
ASCUS	1.46%	0.37%	4.14%	8.80%	0.34%
LSIL	0.24%	0.19%	2.70%	6.20%	0.17%
HSIL	0.41%	0.61%	2.50%	1.60%	0.17%

Conclusion

PAP smear examination is an economic method for screening forcervical pre-malignant lesions and help us to deal with the global scare of cervical cancer. Mass educational and awareness programs should be conducted to increase the detection rate and

Reference

1. https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/1https://www.cytopathology.org/specimen-collection adequacy-requisition/shaws textbook
2. The Pap test and Bethesda 2014. FullText-Acta Cytologica 2015. https://www.karger.com/article/fulltext/381842
3. Solomon D. Foreword; in Nayar R, Wilbur DC (EDS): The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes, ed 3. New York, Springer 2015. National Cancer Institute Workshop: The 1988 Bethesda System for reporting cervical/ vaginal cytologic diagnoses, 2015.
4. Kalyani R, Sharief N, Shariff S. A Study of Pap Smear in a Tertiary Hospital in South India. J Cancer Biol Res. 2016; 4(3):1084.
5. P Vijaya Lakshmi, SR Sree Gouri. Study and analysis of two hundred cervical PAP smears in our hospital. International Journal of Contemporary Medical Research. 2016; 3(9):2787-2789.
6. SR Tamrakar. Kathmandu University School of Medical Sciences, Nepal, Charul Chawla. A Clinical Audit of Pap Smear Test for Screening of Cervical Cancer Article in Nepal Journal of Obstetrics and Gynaecology· 2014
7. Sherpa AT, Clifford GM, Vaccarella S, Shrestha S, Nygård M, Karki BS *et al*. Human papillomavirus infection in women with and without cervical cancer in Nepal. Cancer Causes Control. 2010; 21:323-330.
8. Sankaranarayanan R, Thara S, Sharma A, Roy C, Shastri S, Mahé C *et al*. Accuracy of conventional cytology: results from a multicentre screening study in India. J Med Screen. 2004; 11:77-84.
9. Crasta JA, Chaitra V, Simi C, Correa M. An audit of cervicovaginal cytology in a teaching hospital: Are atypical glandular cells under-recognised on cytological screening? J Cytol. 2009; 26:69-73.
10. Hemali J Tailor, Patel RD, Prashant R Patel, Vasudha M. Bhagat Study of cervical pap smears in a tertiary care hospital of south Gujarat, India, international journal of research in medical sciences. 2016; 4(1):286-288.
11. Aparna Narasimha *et al*. An audit of Pap smear cytology Journal of south Asian federation of obstetrics and gynecology sep-dec. 2011; 3(3):121-124.

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Comparison of serum neutrophil gelatinase associated lipocalin (NGAL) in preeclamptic and normotensive pregnant women

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d 29 December 2018

rr d
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r d Preeclampsia is a systemic disorder that affects multiple organs and is characterized by the new onset of hypertension and proteinuria or end-organ dysfunction or both in the second half of pregnancy. NGAL is a 25-KDa protein of the lipocalin family and is considered to be a novel biomarker for ischemic injury. The objective of this study is to compare the levels of serum NGAL in preeclamptic patients and gestational age matched normotensive controls.

M d The study design is case control study in which pregnant women with preeclampsia (n=40) are selected as cases. Cases were selected from pregnant women attending OG-OPD and IP satisfying the inclusion criteria and not coming under exclusion criteria. 0.5ml of blood was collected in vacutainers and was centrifuged at 3500rpm for 10 minutes. The serum thus separated was aliquoted into smaller plain containers and stored at -20 degree Celsius for analysis. The urine sample was also collected. Controls were also selected from the OP patients.

In present study, the serum NGAL ranged from 40-900ng/ml in cases and from 110-795ng/ml in controls. There is no difference in NGAL between cases and control. The correlation coefficients between the NGAL levels and other parameters like maternal age, gestational age, systolic Blood pressure, diastolic Blood pressure, uric acid levels, urine PCR are also not statistically significant.

Serum NGAL levels are not significantly elevated in patients with preeclampsia when compared with the normotensive controls and also there is no significant correlation between serum NGAL levels and other assessed parameters.

rd Neutrophil gelatinase associated lipocalin (NGAL), Preeclampsia, Urine PCR

Introduction

Hypertensive disorders of pregnancy are a group of complications encountered in up to 10% of all the pregnancies worldwide. This is one of the leading causes of maternal and perinatal morbidity and mortality. Hypertensive disorders during pregnancy are of four categories: Gestational hypertension, preeclampsia-eclampsia, chronic hypertension (of any cause), chronic hypertension with superimposed preeclampsia. Of this preeclampsia is the most prevalent and most serious form of hypertensive disorder that complicates pregnancy.[1,2] With mortality due to preeclampsia and its complications estimated to be 50,000-60,000, it is one of the leading causes of maternal morbidity and mortality.[3,4] The pathogenesis of preeclampsia is due to an imbalance between the angiogenic and anti-angiogenic factors that leads to the release of inflammatory cytokines. As kidney

is a highly vascular organ, this endothelial dysfunction results in kidney injury. The development of AKI in pregnancy is termed as pregnancy-related AKI (PRAKI). Studies revealed that preeclampsia and eclampsia accounted for most of PRAKI cases.[5] There are many biomarker studies for AKI prediction. One of them is serum neutrophil gelatinase-associated lipocalin (NGAL). NGAL is a 25-KDa protein of the lipocalin family. NGAL reduces renal damage by inhibiting cell death and escalating the usual proliferation of kidney tubule cells. So, this protein is up regulated in conditions of kidney injury. Rise of NGAL levels has been recognized in the plasma and urine of animal models of ischemic and nephrotoxic acute kidney injury. Hence, NGAL is considered to be a novel biomarker for ischemic injury. In the study by Patel et al, the study population included the entire spectrum of hypertensive disorders of pregnancy and it was done in North Indian population.[6] In present study authors have measured the serum NGAL levels in preeclamptic patients in south Indian population and have compared them with control population. If the association between preeclampsia and NGAL levels could be proved in south Indian population also, further studies could be done to use it as a biomarker for preeclampsia thus reducing the morbidity and mortality associated with it.

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The study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore. Ethical clearance was obtained from the Institutional Human Ethics Committee.

An informed consent was taken from the patients before sample collection. The study design is case control study in which pregnant women with preeclampsia (n=40) are selected as cases.

The diagnosis of preeclampsia is based on case record description which is based on blood pressure measurements and urine protein creatinine ratio at the time of diagnosis. Cases were selected from pregnant women attending OG-OPD and IP.

Patients satisfying the diagnosis, inclusion criteria and not coming under exclusion criteria were given explanation about this study. If they were willing to participate, the consent forms were filled, and the samples were collected. 5ml of blood was collected in red topped vacutainers and was centrifuged at 3500rpm for 10 minutes. The serum thus separated was aliquoted into smaller plain containers and stored at-20degree Celsius for analysis. The urine sample was also collected.

Control individuals were also selected from the OG-OPD. Pregnant women fulfilling the control group criteria were requested to participate in the study. If they were willing, the consent forms were filled, and samples were collected from them. Their samples were also processed and stored for analysis.

Inclusion criteria

Cases

- All pregnant women more than 20 weeks of gestation diagnosed with pre-eclampsia with blood pressure ≥140/90mm of Hg or urine PCR ≥0.3.

Controls

- Normal pregnant women more than 20 weeks of gestation without preeclampsia matched for gestational age.

Exclusion criteria

Subjects with:

- Known chronic hypertension
- Known diabetes mellitus
- Multiple pregnancy
- Any other known medical disorder.

Collection of blood samples: for both cases and controls, blood was collected in red topped serum tube for the estimation of uric acid and urine was also collected for estimation of spot urine protein creatinine ratio. The left-over serum after the estimation of uric acid was transferred to secondary labelled plain tube. This sample was stored at-20-degree Celsius for NGAL assay.

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Serum NGAL	ELISA
Serum uric acid	Enzymatic colorimetric test
Urine protein creatinine ratio	Turbidimetric method
Urine protein: urine creatinine	Enzymatic colorimetric method
The ratio of urine protein to urine creatinine is calculated.	

Statistical analysis

The data obtained were statistically analyzed using IBM SPSS software version19. The data distribution was displayed by box and whisker plot and bar diagram. To find any statistically significant difference in the distribution of the demographic characteristics and measured parameters independent t-test was used. The dependence of categorical variable was tested with Chi Square test.

The correlation between quantitative parameters was done using Pearson correlation coefficient and the parameters with statistically significant correlations were further checked by scatter plot analysis. The correlation between quantitative parameters was done using Pearson correlation coefficient and the parameters with statistically significant correlations were further checked by scatter plot analysis.

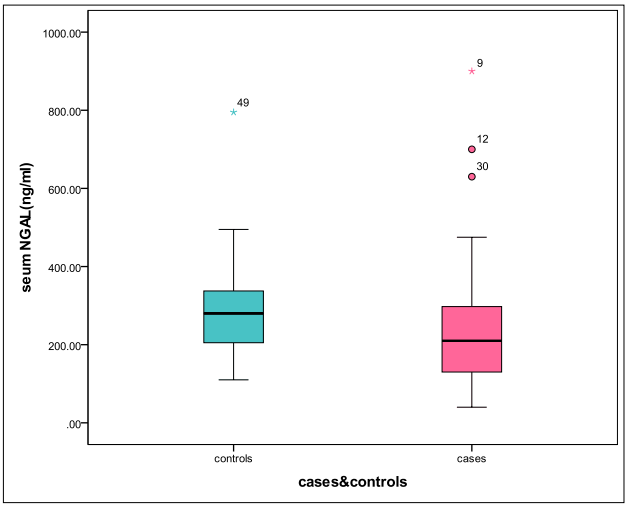
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The characteristics like maternal age and gestational age are equally distributed in both the cases and controls showing that the two study groups are well matched for these parameters. The socioeconomic status of the two groups is also similar as shown by their distribution.

The distribution of various other parameters including systolic blood pressure, diastolic blood pressure, serum uric acid, urine protein creatinine ratio is also studied. As expected, systolic Blood pressure, diastolic blood pressure, serum uric acid level, urine protein creatinine ratios are higher in cases when compared with the control population.

The values of the t-test done to compare the means of these two groups' maternal age, gestational age, body mass index, socioeconomic status, serum uric acid, urine-PCR is shown in Table 3.

This also shows that there is a significant difference between the two groups with respect to parameters like systolic and diastolic blood pressure, body mass index, serum uric acid, urine PCR. It also shows that there is no significant difference between the two groups with regard to maternal age, gestational age of the mother at the time of sampling and socioeconomic status. This implies that the two groups are well matched in these parameters. The serum NGAL level appears to be equally distributed in both the cases and controls as shown in Figure 1.



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The t-test also does not show any significant difference in NGAL levels in these two groups as shown in Table 2.

There is no significant difference in NGAL level between the two groups. The correlation coefficients between various parameters are shown in Table 3.

The r values are significant for gestational age and urine PCR, BMI and urine PCR, Uric acid and urine PCR, diastolic Blood pressure and urine PCR, BMI and systolic Blood pressure, BMI and diastolic Blood pressure, diastolic blood pressure and uric acid, systolic Blood pressure and uric acid, but only diastolic Blood pressure and uric acid, systolic Blood pressure and uric acid, uric acid and urine PCR showed positive linear relation.

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Maternal age		1	0.172	0.130	0.065	-0.071	-0.060	0.0002	-0.073
			0.127	0.250	0.568	0.534	0.597	0.987	0.522
		80	80	80	80	80	80	80	80
BMI		0.172	1	0.362**	0.339**	- 0.031	0.189	0.268*	-0.076
		0.127		0.001	0.002	0.787	0.093	0.016	0.502
		80	80	80	80	80	80	80	80
Systolic BP		0.130	0.362**	1	0.902**	-0.084	0.549**	0.539**	-0.176
		0.250	0.001		0.000	0.460	0.000	0.000	0.119
		80	80	80	80	80	80	80	80
Diastolic BP		0.065	0.339**	0.902**	1	- 0.127	0.628**	0.575**	-0.066
	Sig. (2-tailed)	0.568	0.002	0.000		0.263	0.000	0.000	0.563
	N	80	80	80	80	80	80	80	80
Gest. age	Pearson correlation	-0.071	-0.031	-0.084	-0.127	1	-0.085	-0.274*	-0.117
	Sig. (2-tailed)	0.534	0.787	0.460	0.263		0.453	0.014	0.300
	N	80	80	80	80	80	80	80	80
Serum uric acid	Pearson correlation	-0.060	0.189	0.549**	0.628**	-0.085	1	0.626**	0.129
	Sig. (2-tailed)	0.597	0.093	0.000	0.000	0.453		0.000	0.254
	N	80	80	80	80	80	80	80	80
Urine PCR	Pearson Correlation	0.002	0.268*	0.539**	0.575**	-0.274*	0.626**	1	0.107
	Sig. (2-tailed)	0.987	0.016	0.000	0.000	0.014	0.000		0.343
	N	80	80	80	80	80	80	80	80
Seum NGAL levels	Pearson correlation	-0.073	-0.076	-0.176	-0.066	-0.117	0.129	0.107	1
	Sig. (2-tailed)	0.522	0.502	0.119	0.563	0.300	0.254	0.343	
	N	80	80	80	80	80	80	80	80

** Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

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Preeclampsia is a systemic disorder that affects multiple organs and is characterized by the new onset of hypertension and proteinuria or end-organ dysfunction or both in the second half of pregnancy. The acute complications associated with preeclampsia include eclampsia, renal failure, stroke, permanent neurologic impairment, cardiac dysfunction or arrest, respiratory compromise, coagulopathy and liver failure.[7] The diagnosis of preeclampsia is based on the increase in blood pressure in a previously normal patient on two occasions 4hours apart after 20 weeks of gestation or the presence of proteinuria (>300mg/day). Preeclampsia can be mild/severe. Severe preeclampsia is characterized by more substantial blood pressure elevations (systolic ≥160mm Hg, diastolic ≥110mm Hg), a greater degree of proteinuria (>5g/24 hours) and the presence of symptoms associated with target organ involvement.[8] Xiao et al, evaluated a combination of biomarkers for diagnosing AKI with considerable accuracy in preeclampsia.[9] Serum cystatin C, urine retinol-binding protein, urine NGAL and urine kidney injury molecule-1 levels in the preeclampsia group were higher than the normal pregnancy group. When these markers were combined, the sensitivity and specificity for diagnosing AKI were almost 100%. Patel et al, reported that NGAL level was significantly associated with serum creatinine in the Indian pregnant population with hypertension.[6] Serum NGAL level in oliguric patients was significantly higher compared with non-oliguric patients. Serum NGAL level also had a positive correlation with disease severity including blood pressure level, blood urea nitrogen (Bun), serum creatinine, and serum uric acid in hypertensive pregnant patients. The distribution of the maternal age and gestational age are similar in the cases and the control population and their t-values are also not significant. This shows that the two groups are well matched in these characteristics. The two groups showed different distributions in case of parameters like systolic Blood pressure, diastolic Blood pressure, serum uric acid levels, urine protein creatinine ratio. The t-values are also

significant in these parameters among the two groups. This is expected because the diagnosis of preeclampsia is based on the systolic Blood pressure, diastolic Blood pressure, serum uric acid levels, urine protein creatinine ratio and the division of the study population into two groups is based on these parameters.

When the correlation between various parameters are studied using Pearson correlation coefficient, the resulting R-values are significant for gestational age and urine PCR, BMI and urine PCR, uric acid and urine PCR, diastolic Blood pressure and urine PCR, BMI and systolic Blood pressure, BMI and diastolic blood pressure, diastolic Blood pressure and uric acid, systolic blood pressure and uric acid. In consistent with the studies done by Aliyu et al, Bodnar et al, in this study, the distribution of body mass index is in the higher range in women with preeclampsia when compared with the normotensive controls. The t-test value (p=0.001) obtained by comparing the means of the two groups is also significant. This finding suggests that increased body mass index poses a threat for developing preeclampsia. Pandinis D et al, and Lou et al, have shown in their study that there is a significant correlation between serum NGAL levels and body mass index.[10,11] But, in present study no significant correlation is found between serum NGAL levels and body mass index

In the studies done by Anna et al, Simonazzi et al, Patel et al, to find out the correlation between serum NGAL levels and preeclampsia, the two study groups, cases and controls were matched for body mass index as elevated BMI was considered as a confounding factor.[6,12,13] But in present study, the cases and controls are not matched for BMI. Since NGAL is also produced by adipocytes, a positive correlation was expected between serum NGAL levels and the body mass index. But the Pearson correlation coefficient between NGAL and BMI is not significant to prove any correlation (r=0.076, p=0.502). This suggests that obesity and preeclampsia are correlated but not obesity and NGAL. So, it can be said that obesity may not be a confounding factor in estimation of serum NGAL levels and the pre-pregnancy BMI rather than the BMI at the time of sampling could be considered. In present study, serum uric acid level was also significantly higher in the preeclamptic women than in the normotensive controls as indicated by t-test value (p=0.00).Since estrogen is a uricosuric agent serum uric acid levels fall by 25-35% in pregnant women.[14] Apart from estrogen, expanded plasma volume, elevated GFR also leads to decrease in uric acid levels in pregnancy.[14] In women with preeclampsia, hyperuricemia is a regular finding. Impaired renal function has been considered the causative factor for hyperuricemia in preeclampsia.

In the study done by Pereira et al, to evaluate the use of uric acid as a marker of severity of pre-eclampsia, they were able to find significant correlations between uric acid and systolic blood pressure.[15] In a similar study done by Pramanik et al, to assess whether serum uric acid can be used as a biochemical indicator or not in preeclamptic patients, they were able to demonstrate a positive correlation between serum uric acid levels and elevated systolic pressure and diastolic blood pressure.[16] Consistent with these findings, in this study, a significant positive correlation is found between serum uric acid levels and systolic BP (r=0.549,p=0.00). A linear relation is found between these two parameters by scatter plot analysis.

Nischinta et al, have done a study to find out the correlation between 24-hour urine protein, spot urine protein creatinine ratio and uric acid.[17] They were able to demonstrate a positive and statistically significant correlation between serum uric acid level and urine protein creatinine ratio. Similarly, in this study, a statistically significant positive correlation is found between serum uric acid level and spot urine protein creatinine ratio (r=0.626, p=0.00).

In the study done by Anna et al, they have concluded that serum NGAL levels were higher in the preeclamptic women in all three trimesters when compared with the control population.[12] Although Kim et al, have also demonstrated a statistically significant difference in NGAL level between the two groups, the range of NGAL levels in the two groups were similar (66.1-575.4 ng/ml in cases and 7.0-669.7 ng/ml in controls).[18]

Simonazzi et al, have done a cross sectional case control study to compare the serum and urinary NGAL levels in preeclampsia and in normal pregnancies and also to find out the correlation between NGAL and blood pressure, creatinine, uric acid,24-hour proteinuria.[13] They could not demonstrate a statistically significant difference in the NGAL levels between the two populations. They were not able to demonstrate a positive correlation between NGAL and uric acid, creatinine, blood pressure. In a study done by Dogan et al, to evaluate the serum NGAL levels and plasma Nitric oxide in preeclamptic women and normal controls, the investigators were not able to find a statistically significant difference in the NGAL levels between the two groups.[19] But they were able to find a statistically significant correlation between NGAL levels and BMI.

In present study, the serum NGAL ranged from 40-900ng/ml in cases and from 110-795ng/ml in controls. The distribution range of NGAL in cases covers the distribution range of controls. This shows that there is no difference in NGAL between cases and control. The correlation coefficients between the NGAL levels and other parameters like maternal age, gestational age, systolic Blood pressure, diastolic Blood pressure, uric acid levels, urine PCR are also not statistically significant.

These findings of this study are consistent with those of Simonazi et al, and Dogen et al, and contrast from the findings of Anna et al, Kim et al.[12,13,17,19]

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Serum NGAL levels are not significantly elevated in patients with preeclampsia when compared with the normotensive controls. There is no significant correlation between serum NGAL levels and systolic Blood pressure, diastolic Blood pressure, serum uric acid, urine PCR, maternal age and gestational age.

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1. Lindheimer, Taler SJ, Cunningham. Hypertension in pregnancy. J Am Soc Hypertension. 2010;4:68-78.
2. American College of Obstetricians and Gynecologists. Diagnosis and management of preeclampsia and eclampsia: ACOG practice bulletin, No. 33. Int J Gynaecol Obstet. 2002;77:67-5.
3. World Health organization. The World Health report: 2005: make every mother and child count. Geneva: WHO;2005.
4. Daley L. Maternal mortality associated with hypertensive disorders of pregnancy in Africa, Asia, Latin America and the Carribean. Br J Obstet Gynecol. 1992;99:547-3.
5. Siribamrungwong M, Chinudomwong P. Relation between acute kidney injury and pregnancy-related factors. J Acute Dis. 2016;5(1):22-8.
6. Patel ML, Sachan R, Gangwar R, Sachan P, Natu SM. Correlation of serum neutrophil gelatinase-associated lipocalin with acute kidney injury in hypertensive disorders of pregnancy. Int J Nephrol Renov Dis. 2013;6:181-6.
7. Wallis AB, Saftlas AF, Hsia J, Atrash HK. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987-2004. Am J Hypertens. 2008;21(5):521-6.
8. Machado S, Figueiredo N, Borges A, Pais MS, Freitas L, Moura P, et al. Acute kidney injury in pregnancy: a clinical challenge. J Nephrol. 2012;25(01):19-30.
9. Xiao J, Niu J, Ye X, Yu Q, Gu Y. Combined biomarkers evaluation for diagnosing kidney injury in preeclampsia. Hypertension pregnancy. 2013;32(4):439-9.
10. Panidis D, Tziomalos K. The effects of obesity and polycystic ovary syndrome on serum lipocalin-2 levels: a cross-sectional study. Reprod Biol Endocrinol. 2010;8:151.
11. Lou Y1, Wu C1, Wu M1, Xie C, Ren L. The changes of neutrophil gelatinase-associated lipocalin in plasma and its expression in adipose tissue in pregnant women with gestational diabetes. Diab Res Clin Pract. 2014;104(1):136-42.
12. D'anna R, Baviera G, Giordano D, Todarello G, Russo S, Recupero S, et al. Neutrophil gelatinase-associated lipocalin serum evaluation through normal pregnancy and in pregnancies complicated by preeclampsia. Acta obstet Gynecol Scandinav. 2010;89(2):275-8.
13. Simonazzi G, Capelli I, Curti A, Comai G, Rizzo N, La Manna G. Serum and urinary neutrophil gelatinase-associated lipocalin monitoring in normal pregnancy versus pregnancies complicated by pre-eclampsia. In Vivo. 2015;29(1):117-21.
14. Quinones Galvan A, Natali A, Baldi SI, Frascerra SI, Sanna GI, Ciociaro DE, et al. Effect of insulin on uric acid excretion in humans. Am J Physiol Endocrinol Metabol. 1995;268(1):E1-5.
15. Pereira KN, Knoppka CK, da Silva JE. Association between uric acid and severity of pre-eclampsia. Clin Lab. 2014;60(2):309-14.
16. Pramanik T, Khatiwada B, Pradhan P. Serum uric acid level in normal pregnant and preeclamptic ladies: a comparative study. Nepal Med Coll J 2014; 16(1):30-2.
17. Nischintha S, Pallavee P, Ghose S. Correlation between 24-h urine protein, spot urine protein/creatinine ratio, and serum uric acid and their association with fetomaternal outcomes in preeclamptic women. J Natural Sci Biol Med. 2014;5(2):255.
18. Kim SM, Park JS, Norwitz ER, Jung HJ, Kim BJ, Park CW, et al. Circulating levels of neutrophil gelatinase-associated lipocalin (NGAL) correlate with the presence and severity of preeclampsia. Reprod Sci. 2013;20(9):1083-9.
19. Dogan N1, Yildirmaki S, Mihmanli V, Vardar M, Ozbanazi YG, Cakmak M, et al. Serum neutrophil gelatinase associated lipocalin and plasma nitric oxide levels in healthy and preeclamptic pregnants. Clin Exp Obstet Gynecol. 2014;41(6):700-3.

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Original Research Article

Co-relation of endometrial thickness by transvaginal sonogram with histopathology pattern in abnormal uterine bleeding: a study from South India

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ABSTRACT

Background: Under normal circumstances, a woman's uterus sheds a limited amount of blood during each menstrual period (around 80 ml). Bleeding that occurs erratically or excessive menstrual bleeding is called abnormal uterine bleeding (AUB). The causes of AUB are many and varied. Initial investigations include transvaginal ultrasound and histopathologic assessment of the endometrium. Objective of this study was to evaluation of endometrial thickness with trans-vaginal ultrasound and its correlation with histopathology by dilatation and curettage in abnormal uterine bleeding. To determine the efficacy of transvaginal ultrasound in evaluating the endometrial thickness. To correlate the endometrial thickness by transvaginal ultrasound with endometrial histopathology in women with AUB.
Methods: It is a retrospective observational study. All reproductive and perimenopausal age group women who underwent dilatation and curettage for abnormal uterine bleeding during the period June 2014-June 2016 was taken and analyzed and correlated with their endometrial thickness measured with Transvaginal ultrasound.
Results: Around 478 patients who underwent endometrial sampling over a period of two years were analyzed. Maximum number of patients were in the fourth decade and the overweight category 36.6%. Proliferative endometrium was the most common histopathologic picture (44.76%). Detection of precancerous lesions were-5.87% and endometrial cancer was 1.05%.
Conclusions: An ET of 8 mm and above gave 100% sensitivity and negative predictive value for precancerous and cancerous lesions.

Keywords: Abnormal uterine bleeding, Dilatation and curettage, Endometrial hyperplasia and cancer, Endometrial thickness, Transvaginal ultrasound

NTRODUCTION

Abnormal uterine bleeding is one of the most common gynecological problems in patients approaching the outpatient clinics in the country. These complaints definitely affect the quality of life of the patient. The etiology of AUB varies with the age group of the patient. There is the PALM COEIN classification for AUB by FIGO.[1] There are many diagnostic modalities for evaluating endometrial pathologies in women who have AUB. Transvaginal sonogram (TVS) has permitted the use of higher frequency ultrasound waves at greater proximity of the uterus. It is a relatively cheap and non-invasive diagnostic modality for studying the endometrial pattern and its thickness and at the same time to exclude organic pathology in case of AUB while dilatation and curettage gives us a definitive histopathologic picture. This study attempts to correlate the USG findings and histopathology in patients with AUB. The gold standard

for diagnosing AUB is hysteroscopy and directed biopsy but it is expensive when compared to the simple endometrial sampling. According to the PALM COIEN classification the causes of AUB can be divided into structural and non-structural cause which is given in detail in Table 1.

Table 1: PALM COEIN classification for AUB.[1]

Structural	Non structural
Polyps	Coagulopathy
Adenomyosis	Ovulatory dysfunction
Leiomyomas	Endometrial hyperplasia
Malignancy	Iatrogenic
	Not yet specified

Aims and objectives of this study were

- Evaluation of endometrial thickness with trans-vaginal ultrasound and its correlation with histopathology by dilatation and curettage in abnormal uterine bleeding.
- To determine the efficacy of Transvaginal ultrasound in evaluating the endometrial thickness.
- To correlate the endometrial thickness by Transvaginal ultrasound with endometrial histopathology in women with AUB.

METHODS

Retrospective observational study included all reproductive and perimenopausal age group women who underwent dilatation and curettage for abnormal uterine bleeding during the period June 2014 to June 2016.

Inclusion criteria

- AUB in all reproductive and perimenopausal women
- Only gynecological indications for dilatation and curettage.

Exclusion criteria

- Unmarried women
- Pregnancy and related causes of bleeding
- Postmenopausal bleeding
- Puberty menorrhagia.

From minor and major OT registers, list of all D and Cs which were done during 2014 June to 2016 June was taken. Cases which satisfied the inclusion and exclusion criteria was taken and analyzed

Statistical analysis

Chi square test was used for the statistical analysis.

RESULTS

All the patients with abnormal uterine bleeding, fitting the inclusion criteria, who underwent endometrial sampling was taken for analysis. There were around 478 patients. All of them had a premenstrual ultrasound before the procedure. The age distribution, parity, BMI, presenting complaints, the histopathology and the endometrial thickness by TVS was evaluated. The results are given below:

Of the 478 patients, the youngest patient was 22 years old and the oldest was 55 years, the mean age was 38.5 years. Maximum number of patients were in the 41-50 years age group which was 60.25% and the least in the 20-30 years group and was 4.6% (Table 2).

Table 2: Age distribution of patients with AUB.

Age	No. of cases	%
20-30	22	4.60%
31-40	117	24.48%
41-50	288	60.25%
51-60	51	10.67%
Total	478	100%

Majority of the patients were multiparious i.e. 79.71% and 2.93% was nulliparous as seen in (Table 3).

Table 3: Parity of the patients with AUB.

Parity	No. of cases	%
Nullipara	14	2.93%
Primipara	83	17.36%
Multipara	381	79.71%
Total	478	100%

A total 35.8% of the patients under analysis were of the normal BMI and maximum numbers of patients were overweight-36.6% and the least number of patients were underweighted i.e. 3.3% as given in Table 4.

Table 4: BMI of patients with AUB.

BMI	No. of cases	%
Underweight	16	3.3%
Normal	171	35.8%
Overweight	175	36.6%
Obese	116	24.3%
Total	478	100%

A total 98.3% had heavy menstrual bleeding among the 478 patients and 72.4% had undergone tubal ligation. Among the other USG findings, adenomyotic changes were found in 11.1%, fibroids seen in 18.6%, while simple ovarian cysts were found in 11.7% and PCO in 4.2%.

There were 42 cases of hormone induced changes, 25 menstrul endometium, 17 were inadequate and 2 were TB endometritis which were all entered under others.44.7%

were having proliferative phases (Figure 1), of which 29.7% was disordered proliferative (Figure 2). 30.33% showed secretory changes (Figure 3) endometrial adenocarcinoma (Figure 4) was found in 1.05% of the cases and hyperplasia was seen in 5.86% (Table 5).

Table 5: Histopathology findings in study population.

HPR	No. of cases	Percentage	
Disordered proliferative	142	29.7%	44.76%
Proliferative	72	15.06%	
Secretory	145	30.33%	
Endometrial hyperplasia	28	5.86%	
Endometrial carcinoma	5	1.05%	
Others	86	17.99%	
Total	478	100%	

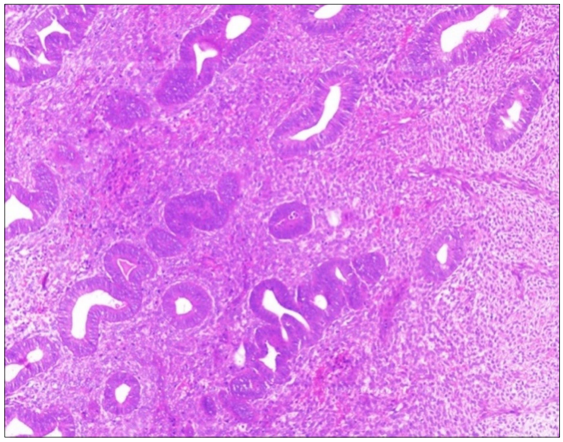


Figure 1: Proliferative endometrium.

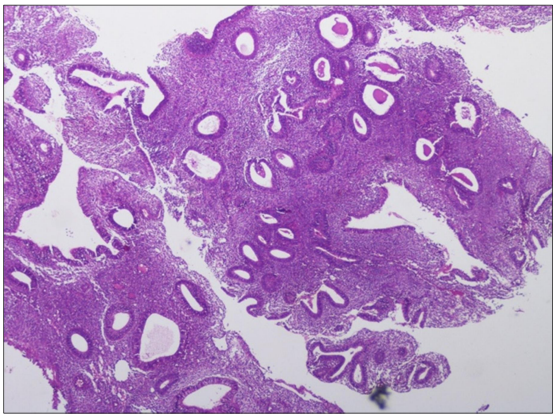


Figure 2: Disordered proliferative.

Among the hyperplasia, 50% was simple hyperplasia without atypia. Atypical hyperplasia (Figure 5) was found in 12 cases. Complex hyperplasia was found in 13 out of the 28 cases of hyperplasia of which complex atypical was 11 cases (Table 6).

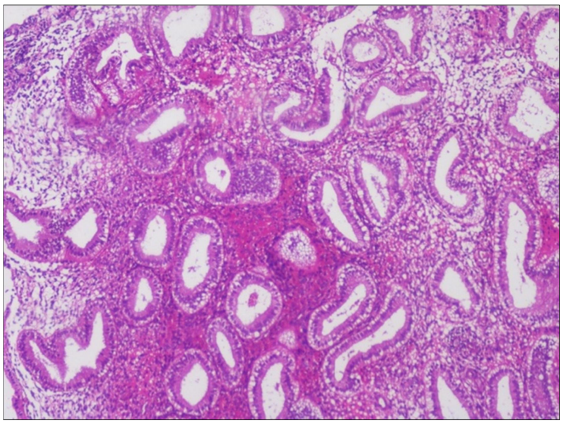


Figure 3: Secretory endometrium.

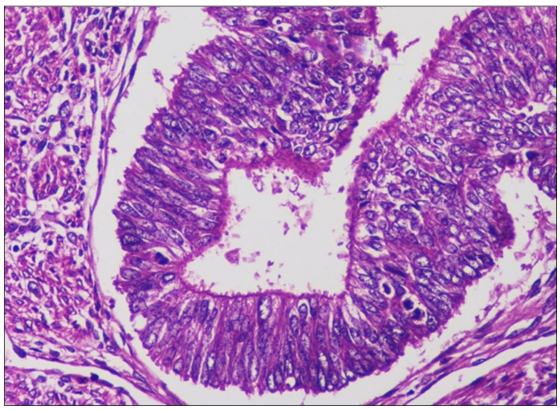


Figure 4: Adenocarcinoma.

Table 6: Cases of endometrial hyperplasia.

Type of hyperplasia	Number	%
Simple hyperplasia without atypia	14	50%
Simple hyperplasia with atypia	1	3.57%
Complex hyperplasia without atypia	2	7.14%
Complex hyperplasia with atypia	11	39.29%
Total	28	100%

Endometrial thickness was 8mm and above up to 15 mm in majority of cases of AUB-54.81%. ET less than 8mm was found only in 19.3% of the study population.ET 15 mm and above was also less which was 25.5% (Table 7). ET less than 4mm in AUB was very less-only 1.67%. One ET was not measured due to diffuse adenomyosis. Out of the 477 patients whose endometrial thickness were measured, all the five cases of endometrial carcinoma was found with an ET ≥15 mm among the 28 cases of endometrial hyperplasia 12 cases of hyperplasia were also found with ET ≥15 mm and the rest 16 cases had ET ≥8-15 (Table 8). Surprisingly all the five cases of carcinoma was endometrioid adenocarcinoma.

It was found in this study that proliferative and secretory endometrium was maximum when the endometrial

thickness was ≥8-15 mm and least with an ET less than 4 mm (Table 9).

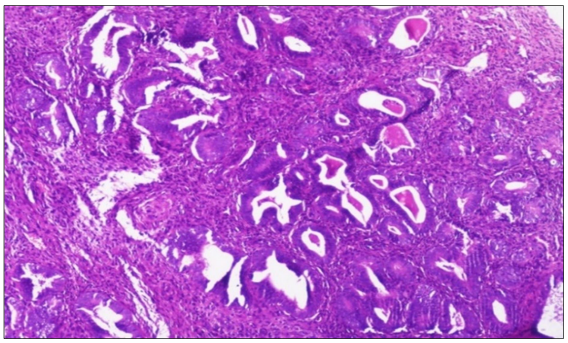


Figure 5: Atypical hyperplasia.

Table 7: Endometrial thicknesses by transvaginal ultrasound.

ET IN mm	Number	%
<4	8	1.67%
4-8	85	17.78%
≥8-15	262	54.81%
≥15	122	25.52%
Not measured	1	0.22%
Total	478	100%

If we take 8 mm as the cut off, we can see that in this study population all the cases of hyperplasia and malignancy had an ET ≥ 8 mm. Out of the 384 cases with ET ≥ 8 mm 28 cases had hyperplasia and five patients had endometrial carcinoma i.e. 8.59% (Table 10).

Table 8: Co-relation of et with endometrial hyperplasia and carcinoma.

ET in mm	Number	Endometrial hyperplasia	%	Endometrioid carcinoma	%
≥15	122	12	9.84%	5	4.46%
≥8-15	262	16	6.11%	0	0%
4-8	85	0	0%	0	0%
<4	8	0	0%	0	0%
Total	477	28	5.87%	5	4.46%

Table 9: Co-relation of et by TVS and histopathology.

ET	Number	Proliferative	Secretory	EH	Carcinoma	Others
<4 mm	8	3	3	0	0	2
4 - <8	85	34	29	0	0	22
≥8-15	262	121	86	16	0	39
≥15	122	56	27	12	5	22
Total	477	214	145	28	5	85

Table 10: Co-relation of endometrial thickness with hyperplasia and malignancy.

ET in mm	Number	Hyperplasia and carcinoma	%
≥8	384	33	8.59%
<8	94	0	0%

Table 11: Co-relation of endometrial thickness with HPR.

ET in mm	Number	Hyperplasia and carcinoma	Not having carcinoma
≥8	384	33 (a)	351 (b)
<8	94	0 (c)	94 (d)

Sensitivity, specificity, the positive and negative predictive value for an ET of ≥8 mm was calculated. 94 patients with ET less than 8 and 351 patients with ET ≥8 mm did not have carcinoma (Table 11).

- Sensitivity = a/a + c =33/33 = 100%
- Specificity= d/b + d = 94/445 = 21.12%
- Positive predictive value = a/a + b = 33/384 = 8.59%
- Negative predictive value = d/c + d = 94/94 = 100%

DISCUSSION

Abnormal uterine bleeding is a very common diagnosis in the op population. According to this study the maximum age groups of the patients were in the perimenopausal age of 41-50 years-around 60.25%. This is similar to the study by Shobitha et al -68.1% in the same age group while it was 38% in a study by Mahapatra M et al where the study group was less.[2,3]

The youngest patient was a 22-year-old primary infertility patient with heavy menstrual bleeding for which diagnostic curettage was done and had an ET of 6 mm and secretory endometrium.

In this study, 79.7% were multiparious while 2.93% were nulli, in almost all the studies this is similar. This is in accordance to the study by Nidhi et al, where 88% were multipara and 1.2% were nulliparous.[4] In a study by Betha K et al 81% were multipara.[5]

BMI was in the overweight category in 36.6% and obese in 24.3% in this study which is similar to the study in Iran by Kazamijalezah et al, where it was 33% and also with the study by Sahai A et al, where 56.2% were in overweight category and obese was 25%.[6,7]

There was heavy menstrual bleeding in 98% of patients.

Of these 478 patients only 39 had diabetes as risk factor and out of 39 one had endometrial carcinoma.

When we analyze the histopathologic findings of the 478 cases 44.5% was proliferative endometrium, of this disordered proliferative endometrium seems to be the commonest, in a study by Elavarasan et al, where disordered proliferative endometrium seems to be the commonest pattern observed i.e. 31.1%.[8] The term disordered proliferative endometrium refers to proliferative phase that does not seems to be appropriate for any one time in the menstrual cycle and mimics simple hyperplasia, but the process is focal rather than diffuse. Histologically there shows mild disorganization of architecture, with focal dilated glands, with normal gland to stromal ratio. In this study 30% was secretory endometrium; endometrial hyperplasia was found in 5.8% and endometrial cancer in 1%.

Transvaginal ultrasound was done to assess the endometrial thickness and it was done in the premenstrual phase, of the 478 cases 54.8% had an ET of ≥8-15, 25.5% had ET more than 15 mm. In a study by Jikki et al, 57% had et ≥8 mm which is similar to this study of 54%.[9]

In the study by Jikki et al, the endometrial hyperplasia was seen in 96.4% cases when ET was more than 8 mm and in this study 100% of the patients with endometrial hyperplasia had ET ≥8 mm.[9] In this study 100% cases of endometrial carcinoma occurred with ET ≥15 and all cases of hyperplasia had ET ≥8 mm.

Simple hyperplasia without atypia was 50% in this study which was comparable to 62% in a study by Tabata et al.[10]

Complex endometrial hyperplasia was 14.8% in a study conducted by Idris et al, while their atypical hyperplasia was only 2% whereas atypical hyperplasia was 42.4% in this study.[11] This may be because of the fact that most of the patients in the study group were in the fourth decade (60%) and in their study was in the third decade (80%).

Of the 384 cases with ET ≥8 mm, 33 had endometrial hyperplasia and carcinoma, and 351 did not have e/o hyperplasia and carcinoma, none of the cases below 8mm had any e/o hyperplasia or carcinoma. Sensitivity and negative predictive value was 100% for an ET of ≥8 mm. This is similar to studies by Chatpavit Getpook an ET of 8 mm showed optimal sensitivity and specificity -83.9% and 58.8% respectively and 90.4% negative predictive value for an abnormal endometrium.[12] They concluded that an ET of 8mm or less is less likely to be associated with malignant pathologies. In this study the negative predictive value was 100% and sensitivity was 100% but the specificity was only 21.1%. According to a study by Ozdemir S et al, there was negative predictive value of 95.6%, sensitivity and specificity of 83.6 and 56.4% respectively for an ET of 8 mm.[13]

Kurman et al, classified hyperplasia in to simple and complex and subcategory as atypical.[14] The 2014 revised WHO classification simply separates endometrial hyperplasia into 2 groups-hyperplasia without atypia and atypical hyperplasia.[15] According to the EIN classification all atypical endometrial hyperplasia is classified as endometrial intraepithelial neoplasia.[15] Progesterone was found to cause regression in 90% cases with hyperplasia.

The RCOG cutoff of ET is less than 7 mm below which there is no endometrial hyperplasia in a prospective study of 56 women with pcos.[11,16] In studies by Gianella et al, 11 mm was taken as the cut off.

The most common presenting symptoms of endometrial hyperplasia is abnormal uterine bleeding. According to studies, the risk of progression of simple hyperplasia to carcinoma is 1% and complex hyperplasia with atypia is 29%.[18]

CONCLUSION

The clinical importance of endometrial hyperplasia is because of its progression to endometrial carcinoma. An ET of 8 mm in this study gives a 100% negative predictive value for hyperplasia and carcinoma and can be taken as a cut off for deciding the patients for dilatation and curettage especially in low risk patients.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of PSGIMSR

REFERENCES

1. Munro MG, Critchley HO, Broder MS, Fraser IS. FIGO classification system (PALM COIEN) for causes of abnormal uterine bleeding in non-gravid women of reproductive age. FIGO Working Group on Menstrual Disorders. Int J Gynaecol Obstet. 2011;113:3-13.
2. Shobhitha GL, Kumari VI, Priya PL, Sundari BT. Endometrial study by TVS and It's correlation with

histopathology in abnormal uterine bleeding. J Dental Med Sci. 2015;14(4):21-32.
3. Mahapatra M, Mishra P. Clinicopathological evaluation of abnormal uterine bleeding. J Health Res Rev. 2015;2:45-9
4. Meena N, Meena S. Correlation between histology and bleeding pattern in a case of AUB and risk factors analysis of AUB in reproductive age group. JMSCR. 2017;5(7):24629-34.
5. Betha K, Malvatu L, Talasani S Distribution of causes of abnormal uterine bleeding using new FIGO classification system-PALM COEIN: a rural tertiary-based study. Int J Reprod Contracept Obstet Gynecol. 2017;6(8):3523-7.
6. Kazemijaliseh H, Ramezani Tehrani F, Behboudi-Gandevani S, Khalili D, Hosseinpanah F, Azizi F. A Population-based study of the prevalence of abnormal uterine bleeding and its related factors among Iranian reproductive-age women: An updated data. Arch Iran Med. 2017;20(9):558-63.
7. Sharma AS, Gupta S. Analysis of body mass index in patients with abnormal uterine bleeding. Inter J Clin Obstet Gynaecol. 2019;3(1):92-5
8. Puvitha RD, Elavarasan T, Shruthi MS, Shylaja S. Histopathological study of endometrium in abnormal uterine bleeding an experience in a tertiary care centre of rural south India. Nat J Basic Med Sci. 2017;8:1.
9. Kalaiselvi PSJ. Thenmozhi G. A study on endometrial thickness by endovaginal sonography with histopathology in perimenopausal women in a tertiary care centre. Indian J Res. 2017;6(10):33-6.
10. Tabata T, Yamawaki T, Yabana T, Ida M, Nishimura K, Nose Y. Natural history of endometrial hyperplasia Arch Gynecol Obstet. 2001;265(2):85-8.
11. Takai IU, Bukar M, Mayun AA, Ugwa EA, Audu BM. Aisha abdurrahman endometrial hyperplasia: a 2 decade retrospective analysis of histopathological pattern at a university teaching hospital in Northern Nigeria Sub-Saharan. Afr J Med. 2016;3:171-5.
12. Getpook C, Wattanakumtornkul S. Endometrial thickness screening in premenopausal women with abnormal uterine bleeding. J Obstet Gynaecol Res. 2006;32(6):588-92 .
13. Özdemir S, Çelik Ç, Gezginç K, Kıreşi D, Esen H. Evaluation of endometrial thickness with transvaginal ultrasonography and histopathology in premenopausal women with abnormal vaginal bleeding. Arch Gynecol Obstet. 2010;282(4):395-9.
14. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia: a long-term study of "untreated" hyperplasia in 170 patients. Cancer. 1985;56:403-12.
15. Palmer JE, Perunovic B, Tidy JA. Endometrial hyperplasia. The Obstet Gynaecol. 2008;10(4):211-6.
16. Cheung AP. Ultrasound and menstrual history in predicting endometrial hyperplasia in polycystic ovary syndrome. Obstet Gynecol. 2001;98:325-31.
17. Giannella L, Cerami LB, Setti T, Bergamini E, Boselli F. Prediction of endometrial hyperplasia and cancer among premenopausal women with abnormal uterine bleeding. BioMed Res Inter. 2019;2019.
18. No GT. Management of Endometrial Hyperplasia. 2016. Available at: ttps://www.rcog.org.uk/globalassets/documents/guidelines/green-top-guidelines/gtg_67_endometrial_hyperplasia.pdf. Accessed on 01st February 2020.

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ORIGINAL ARTICLE

Cesarean Myomectomy: An Experience from a Tertiary Care Teaching Hospital

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Abstract

Background Recent literature supports the removal of myomas during cesarean section, which traditionally was considered a relative contraindication, given a higher complication rate. This study is to share our experience of cesarean myomectomy in the last decade.

Methods This study is a retrospective review of our prospectively maintained database, from January 2008 to December 2017, at a tertiary care level teaching institution. All patients who underwent myomectomy during cesarean section were included. There were no exclusions.

Results A total of twenty patients underwent myoma removal along with the cesarean operation during this period with a mean age of 30 years. Majority of patients were nulliparous (70%). Common comorbidities were diabetes mellitus (40%) and hypothyroidism (20%). Mean size of myomas were 5.33 cm (± 2.08), and the number varied from one to three. The most common location was the posterior surface of the uterus with the commonest variety being subserous. Most patients were discharged on the fifth postoperative day.

Conclusion This study demonstrates that cesarean myomectomy to be a safe and feasible procedure in experienced hands. It offers the advantage of avoiding a second surgery in selected patients.

Keywords Cesarean myomectomy · Myoma uterus · Cesarean delivery · Myomectomy

Introduction

The myomas are the most common benign tumors of the uterus. The highest reported incidence of myomas during pregnancy is 10.7% [1]. The myomas are common during the reproductive period due to an influence of higher estrogen levels. Their increased incidence during pregnancy can be partly explained by the late age of conception [2]. Myomas in pregnancy are associated with a significantly higher risk for cesarean section (CS).

Conventionally, concomitant myomectomy during CS was considered a relative contraindication, given perceived higher complication rate. Last decade has witnessed emerging literature evidence supporting myoma excision along with CS, with acceptable results [3]. This study aims to share our experience of cesarean myomectomy during the last decade.

Materials and Methods

This study is a retrospective review of our prospectively maintained database of the last decade, from January 2008 to December 2017, at a tertiary care teaching hospital. All consecutive patients who underwent myomectomy during cesarean section, in this mentioned duration, were included.

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This formed a small subset, among the patients, having uterine myomas and pregnancy, a majority of those underwent vaginal delivery or had LSCS, without myomas removal. Those not matching the inclusion were not considered. There were no exclusions. The institutional ethical committee approved the study, and waiver of consent was obtained, in view of retrospective nature.

Data were collected from outpatient and inpatient records, operative and discharge notes. Data included maternal demographics such as age, parity, medical diseases, prior surgery, gestational age at the time of CS, type of anesthesia, indication for CS, elective/emergency and number of previous operations including CS. Myoma characteristics such as size, number, location and variety were noted. Other parameters like an indication for myomectomy, the number of incisions made and the presence of adhesions were observed. The total duration of surgery was taken as the time from the initial skin incision to skin closure. Approximate intraoperative blood loss was measured, and record of transfusion if any was noted. Postoperative drop in hemoglobin was checked at 48 h after surgery, and blood transfusion was given if the fall was more than 3 gm/dl. Postoperative complications like pyrexia (> 38-degree C) and wound infection were analyzed. Duration of hospital stay was noted, and patients were followed up after 2 weeks in the outpatient department.

Patients were counseled during the early third trimester regarding the need for myomectomy depending on the mode of delivery and consent obtained for the same.

Experienced gynecologists performed surgery with minimum of 10 years of experience. Preoperatively, half an hour before skin incision Inj. Tranexamic acid 1 gm in 100 ml isotonic saline was infused over 20 min. Except in four cases, myomas were situated at the incision site, and in other instances, CS preceded myomectomy. When myomas were located at the lower uterine segment, myomectomy was performed before delivery of the fetus. During CS, myomas were inspected for their size, number, type and location after the closure of the uterine incision (Fig. 1).

Though myomectomy was tried with a single incision, in few cases where multiple fibroids were present, more than one incision over uterus was needed. An incision was made over myoma by using monopolar cautery, and the tumor was enucleated. To achieve hemostasis, bipolar cautery was used. The defect in the myometrium was closed with 1-0 vicryl suture (Fig. 2). High-dose oxytocin infusion was continued postoperatively for 24 h to prevent bleeding.

Results

A total of twenty patients underwent myoma removal along with the cesarean operation during this period with a mean age of 30 years. Majority of patients were nulliparous (70%).



Fig. 1 Multiple fibroids over the anterior surface of uterus postdelivery of the baby



Fig. 2 Postmyomectomy, closure by baseball sutures

Common comorbidities were diabetes mellitus (40%) and hypothyroidism (20%). One except all patients received regional anesthesia (spinal/epidural); the only case needed general anesthesia due to the presence of idiopathic intracranial hypertension. Sixty-five percent of surgeries were elective operations. The details of various preoperative characteristics are shown in Table 1.

Intraoperatively, two patients had small bowel and omental adhesions at the operative site. Two patients (10%) received a blood transfusion during the procedure. The mean hemoglobin drop reported 24 h postoperative was 1.23 (± 1.16) grams. No patient warranted blood transfusion in the postoperative period (Table 2).

Majority of patients got the discharge on the fifth day of surgery. There were no incidences of postpartum pyrexia or

Table 1 Demographic and preoperative characteristic of patients ($n=20$)

Sr. No.	Variable	Value (mean ± SD)
1.	Age (in years)	30.85 ± 3.84
2.	Parity	
	(a) Nulliparity	14
	(b) Multiparity	06
3.	Gestational Age	37.3 ± 1.08
4.	Number of prior surgeries	
	(a) Nil	11
	(b) LSCS	06
	(c) Myomectomy	03

SD standard deviation, *LSCS* lower segment cesarean section

Table 2 Intraoperative and follow-up variables ($n=20$)

Sr. No.	Variable	Value (mean ± SD)
1.	Size of myoma (cm)	5.33 ± 2.08
2.	Number of myoma (mean and range)	1 (1–3)
3.	Location of tumor	
	(a) Posterior	07
	(b) Anterior	06
	(c) Right lateral wall	04
	(d) Fundal	04
	(e) Left lateral wall	03
	(f) Lower uterine segment	04
4.	Type of Myoma	
	(a) Subserous	11
	(b) Intramural	09
	(c) Submucosal	02
5.	Operating time (minutes)	79 ± 35.35
6.	Blood loss (mL)	876 ± 386.9
7.	Hospital stay (days)	5.5 ± 1.43

SD standard deviation

surgical site infections. The patients were regularly followed in the outpatient department.

Discussion

Fibroid excision along with caesarian section was considered difficult until recently. Fibroids with pregnancy might cause miscarriage, preterm delivery, premature rupture of membranes, early separation of the placenta, labor dystocia, postpartum hemorrhage and increased rate of operative deliveries. Fibroids might also undergo red degeneration and associated complications. Myomectomy during CS has not been done routinely due to the risk of uncontrolled bleeding and the possibility of hysterectomy [4]. If myomectomy is not performed along with CS patient might need another surgery to remove it, which amounts to two operations, two anesthetic exposures and increasing cost, and there is also an enhanced risk of complications caused by fibroids in a subsequent pregnancy.

The average age of mothers in our study was 30 years. Most studies have shown myomas to be more common in nulliparous women [5]. We too observed a higher incidence of myomas among nulliparous women (70%) [6]. This could be explained due to hormonal changes and reduced sensitivity of leiomyoma to estrogen receptor [7].

Similar to many other studies, the average gestational age at the time of CS was 37 to 38 weeks in our study [4, 6, 8]. We had four patients delivering at 35 to 36 weeks of gestation. Among these four patients who had delivered prematurely, three deliveries can be attributed to the presence of fibroids. Two out of these patients have already undergone fibroid removal before becoming pregnant. The possible explanation for preterm labor could be decreased distensibility of uterine myometrium due to the presence of fibroids as the pregnancy advances [9].

Though CM is to be avoided in cases of the previous myomectomy due to more chances of complications [10], we had the opportunity to do repeat CS for one of our patient who underwent CS myomectomy 3 years ago. The indication for primary CS was a transverse lie. The malpresentation was suspected to be due to a single large (8 cm), intramural fibroid located in the lower uterine segment. Incidentally, this was the largest myoma we had come across in our study. As the fibroid was situated in the incision site, myomectomy was followed by CS. During repeat CS, which was done 3 years later, there was no fibroid detected intraoperatively. There were three more cases of lower uterine segment (LUS) fibroids; if they were less than 5 cm, then myomectomy were done following the delivery of the baby. All fibroids present in the LUS were removed through the same CS incision, without any additional incision on the uterus. The same technique was suggested in several other studies [11, 12].

Three of our patients had undergone myomectomy previously in the non-pregnant state. One of the women had undergone myomectomy twice before becoming pregnant. This confirms the well-established fact that fibroids are recurrent [13]. Among the study group, the patients who had undergone previous myomectomies had a high incidence of intraoperative adhesions as expected. However, in all three patients, the surgery was uneventful with minimal blood loss and no added morbidity.

Eight of our study subjects had multiple fibroids. Six patients had fibroids of size 8 cm (largest dimension). 55% of the myomas had size > 5 cm. The smallest dimension encountered was 2.5 cm. We had more anterior wall fibroids than posterior [4]. Traditionally myomectomy is avoided in cases of fundal and cornual sites because of excessive

bleeding and possible obstruction of fallopian tubes [14, 15], but in our study, myomectomy was done regardless of the location of the myomas.

Except for a single instance of the submucosal fibroid, there was an even distribution of intramural and subserosal fibroids. Removal of submucosal fibroids during cesarean section is usually avoided as it involves resection of the full thickness of myometrium and reduced contractility might lead on to increased hemorrhage [16]. Secondary changes due to red degeneration were found in a case with single sizeable fundal fibroid. Histopathological reports of all myomectomy specimens were closely followed up, and none showed malignant change.

The same incision removed fibroids in the anterior wall and close to CS incision. Whenever possible, a minimal number of incisions were made, and adjacent ones removed through the same incision. This was done to reduce blood loss and future adhesions and improve reproductive outcome. While the operating time was more or less uniform in most of our cases, two of the cases exceeded the average operating time significantly. In these two instances, the increased operating time could be explained by multiple fibroids needing more than a single incision for removal. Other studies have also observed more than average operating time in cases of multiple fibroids [17].

Intraoperative blood transfusion was done in two cases only as they met the criteria for blood loss of more than one liter. One case had a fibroid at the incision site. The second patient who had a loss of more than two liters which was the largest in our series had three fibroids at different locations and they were removed by as many incisions. Despite intraoperative transfusion and other pharmacological measures to attain hemostasis, she had a significant drop in hemoglobin at 48 h postoperatively and needed another transfusion. She had no other postoperative morbidity like pyrexia or ileus and was discharged on the fourth day after surgery.

CM is not done routinely, because of the associated hemorrhage and the need for the hysterectomy [15]. Specific hemostatic techniques have been advocated by various authors [18, 19], but we have followed tranexamic acid infusion, vasopressin instillation, uterine artery ligation, electrocautery and high-dose oxytocin. There was not a single case which needed a hysterectomy in our study of twenty cases, though patients were counseled regarding the possibility in the prenatal period due to intractable hemorrhage. We also found no increased postoperative morbidity or expanded hospital stay in any of our patients who underwent CM as was observed by Dam Hye Kwon et al. [8].

There are a few limitations to our study. Its retrospective design coupled with proportionally small sample size limits generalization of results. This study also lacks long-term follow-up of these patients and thus to highlight effect on future pregnancies. Additionally, as this study was conducted in a tertiary level medical college setup with available expertise, the results cannot be generalized, especially in comparison with smaller installations or in the absence of an expert.

We author would like to stress this to the readers that routine use of myomectomy during CS is neither advisable nor been followed in the current study. The CM should be carefully planned for selected patients in an appropriate setup and expertise. A similar conclusion is drawn by a recent review on this topic [20], recommending standardization of best practices for CM, with the availability of more robust data and in the presence of necessary facilities.

Conclusion

Overall, our series has shown CM to be a safe and feasible procedure in well-selected patients in a tertiary care center with experienced hands. It can offer an advantage of avoiding a second intervention in selected patients. Appropriate hemostatic techniques can curtail hemorrhage which is the most severe complication. Long-term morbidity concerning future reproductive outcome should be addressed in a study with large participants and reliable design.

Authors' Contributions All authors have a significant contribution for preparation of this manuscript, following ICMJE Guidelines. First and second authors share the first position.

Compliance with Ethical Standards

Conflict of interest No author has any potential conflict of interest.

Ethical approval The current study is approved by the responsible institutional ethics committee for human research and fully comply with the declaration of Helsinki for guideline on human research.

References

1. Laughlin SK, Baird DD, Savitz DA, et al. Prevalence of uterine leiomyomas in the first trimester of pregnancy. Obstet Gynecol. 2009;113(3):630–5.
2. Tian J, Hu W. Cervical leiomyomas in pregnancy: report of 17 cases. Aust N Z J Obstet Gynaecol. 2012;52(3):258–61. https://doi.org/10.1111/j.1479-828X.2012.01414.x.
3. Sparić R, Kadija S, Stefanović A, et al. Cesarean myomectomy in modern obstetrics: more light and fewer shadows. J Obstet Gynaecol Res. 2017;43(5):798–804.
4. Incebiyik A, Hilali NG, Camuzcuoglu A, et al. Myomectomy during caesarean: a retrospective evaluation of 16 cases. Arch Gynecol Obstet. 2014;289(3):569–73.
5. Sparic R, Mirkovic L, Malvasi A, et al. Epidemiology of uterine myomas: a review. Int J Fertil Steril. 2016;9(4):424–35.
6. Kanthi JM, Sumathy S, Sreedhar S, et al. Comparative study of cesarean myomectomy with abdominal myomectomy in

terms of blood loss in single fibroid. J Obstet Gynecol India. 2016;66(4):287–91. https://doi.org/10.1007/s13224-015-0685-x.
7. Wise LA, Palmer JR, Harlow BL, et al. Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African–American women: a prospective study. Am J Epidemiol. 2004;159(2):113–23.
8. Kwon DH, Song JE, Yoon KR, et al. The safety of cesarean myomectomy in women with large myomas. Obstet Gynecol Sci. 2014;57(5):367. https://doi.org/10.5468/ogs.2014.57.5.367.
9. Chen Y-H, Lin H-C, Chen S-F, et al. Increased risk of preterm births among women with uterine leiomyoma: a nationwide population-based study. Hum Reprod. 2009;24(12):3049–56.
10. Sparić R, Berisavac M, Buzadzić S, et al. Complications during cesarean delivery in a patient with two previous myomectomies. Acta Chir Iugosl. 2013;60(1):99–100.
11. Yellamareddygari S, Chakrabarti M, Ravuri S, et al. Leaving fibroids at caesarean section, is it safe? Gynecol Surg. 2010;7(2):173–5. https://doi.org/10.1007/s10397-008-0441-7.
12. Mahendru R, Sekhon P, Gaba G, et al. At times, myomectomy is mandatory to effect delivery. Ann Surg Innov Res. 2011;5(1):9.
13. Ande ABA, Ehigiegba AE, Umeora OUJ. Repeat myomectomy at caesarean section. Arch Gynecol Obstet. 2004;270(4):296–8.
14. Celal K, Hülya Ç. The evaluation of myomectomies performed during cesarean section in our clinic. Niger Med J. 2011;52(3):186.
15. Song D, Zhang W, Chames MC, Guo J. Myomectomy during cesarean delivery. Int J Gynecol Obstet. 2013;121(3):208–13. https://doi.org/10.1016/j.ijgo.2013.01.021.
16. Gbadebo AA, Charles AA, Austin O. Myomectomy at caesarean section: descriptive study of clinical outcome in a tropical setting. J Ayub Med Coll Abbottabad. 2009;21(4):7–9.
17. Ramesh Kumar R, Patil M, Sa S. The utility of caesarean myomectomy as a safe procedure: a retrospective analysis of 21 cases with review of literature. J Clin Diagn Res. 2014;8(9):OC05–8.
18. Shavell VI, Thakur M, Sawant A, et al. Adverse obstetric outcomes associated with sonographically identified large uterine fibroids. Fertil Steril. 2012;97(1):107–10.
19. Lee J-H, Cho D-H. Myomectomy using purse-string suture during cesarean section. Arch Gynecol Obstet. 2011;283(S1):35–7.
20. Chauhan AR. Cesarean myomectomy: necessity or opportunity? J Obstet Gynecol India. 2018;68(6):432–6. https://doi.org/10.1007/s13224-018-1114-8.

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Surgical site infections in a tertiary care hospital: incidence, risk factors and microbiological spectrum

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Abstract

Background: Surgical site infections (SSI) are one of the major health problems throughout the world with an incidence of 3%-16%. Hospital acquired surgical site infection is further complicated by the emergence of multi drug resistant strains. SSI surveillance is an established monitoring tool and has been shown to reduce infection rates. The importance of preventing surgical site infections is well recognized since they lead to increased morbidity, prolonged hospital stay, need for readmission, high end antibiotic treatment and re-surgery. This study was undertaken to determine the incidence, risk factors, and microbiological spectrum of surgical site infections and to identify the multidrug resistant strains. Analysis of the effectiveness of the existing surveillance methods was also done.

Methods: This retrospective study was done for a period of 1-year form Jan 2016 to Dec 2016. All patients with infection following caesarean section and abdominal and vaginal hysterectomy were included. Laparoscopic surgery and patients with preexisting infection were excluded.

Results: Incidence of SSI in present study is 5.27%. The major risk factors identified were obesity, diabetes and prolonged operating time. The commonest infective organism was Klebsiella Pneumoniae in 37% of which 19% were ESBL producing and 3.8% were carbapenemase producing. E. coli was identified in 20% of isolates. The incidence of ESBL in both isolates was higher.

Conclusions: Regular audit of SSIs is a very effective tool to analyse risk factors, identify causes and plan strategies to prevent infection.

Keywords: Drug resistance, Hospital acquired, Surgical site infection, Surveillance

Introduction

Surgical site infections (SSI) are significant source of morbidity, prolonged hospital stay and recovery and rarely mortality. They complicate 3 to 16%.[1-4] of all surgeries but even these estimates are likely to be low as some infections occur after the patient's discharge from the hospital or they report to other hospitals with infection. Hospital acquired SSIs are further complicated by the emergence of multidrug resistance strains. The importance of preventing surgical site infections is well recognized since they lead to increased morbidity, prolonged hospital stay, need for readmission, high end antibiotic treatment and resurgery.[1,3]

The Center for Disease Control (CDC) defines SSI as an infection related to an operative procedure that occurs at or near the surgical incision within 30 days. This time frame is extended to 12 months if a surgical implant is used. Infections are further divided into:

- Superficial involving skin and subcutaneous tissue
- Deep involving deeper soft tissue of the incision like fascia or muscle
- Organ or space involving any part other than the incised body layers.

Surgical wounds are further classified as clean, clean contaminated, contaminated and dirty.

Most obstetric and gynaecological procedures are classified as clean contaminated as the genital tract is entered into controlled fashion and there is no unusual contamination. Some surgeries like pelvis abscess, perforated bowel or positive culture pre-op are classified as dirty. SSI surveillance is an established monitoring tool and has been shown to reduce infection rates.

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This retrospective study was conducted in the department of Obstetrics and Gynaecology at a teaching hospital in South India from the period of January 2016 to December 2016.

Inclusion criteria

- All emergency and elective obstetric and gynaec procedures like caesarean section, abdominal and vaginal hysterectomies and laparotomies were included.

Exclusion criteria

- Laparoscopic surgeries and patients who had pre-op infection.

The departmental protocol includes two visits after surgery: the first visit a week after discharge and second visit a month later. The charts were analysed at the end of a month after surgery to identify patients with infection. Their case records were analyzed for pre-op risk factors, intra-operative factors like timing of prophylactic antibiotics and duration of surgery and post-operative course. All patients with wound infection had a culture and sensitivity done. The culture report was analysed for the infecting organism and sensitivity pattern. A monthly audit of all SSIs in the department was done to determine the incidence and microbiological spectrum of the infecting organisms. Infection control practices currently in place were also reviewed and analysed to determine if there was a scope for improvement.

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The numbers of surgeries in present study period who fulfilled in present inclusion criteria were 982 caesarean sections and 497 gynaec surgeries. The results are shown in Table 1.

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C section	982	55	5.6%
Gynaec surgeries	497	23	4.62%
Total	1479	78	5.27%

The patients who had wound infection were analysed for pre-operative risk factors like obesity, diabetes, other medical disorders and intra operative factors like prolonged operating time, previous surgeries and presence of malignancy.

As shown in Table 2, obesity was the major risk factor as 48.7% of patients had BMI of >25. Other risk factors included diabetes and prior surgery with adhesions in 19.2%, anemia and hypertension was seen in 12% of patients. 9% of patients had prolonged operating time of >3 hours.

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BMI > 25	25	13	38	48.7
Diabetes	7	8	15	19.2
Prior surgery with adhesions	7	8	15	19.2
Anemia	3	7	10	12.8
Hypertension	4	6	10	12.18
Prolonged operative time (>3 hours)	1	6	7	9.0
Bronchial asthma	3	3	6	7.7
Cardiac disease	2	2	4	5.1
Malignancy	0	3	3	3.8
PROM >12 hours	2	NA	2	3.6
Prophylactic antibiotics given	Given in all patients			

All patients who had discharge from the wound had a culture taken prior to starting antibiotics. The culture report was analysed for infecting organism and sensitivity pattern. The pathogens isolated is shown in Table 3.

Figure 1 shows the month wise analysis of the organisms which were MRSA (Methicillin Resistant Staph Aureus), ESBL (Extended Spectrum Beta Lactamase) and Carbapenemase producers.

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giving an incidence of 5.6 %. 23 of patient who underwent gynaec surgeries had SSI giving an incidence of 4.62%. The total incidence was 5.27%.

This compares favorably with the reported rate of 11.8 % in the WHO review of SSIs in low and middle-income countries.[5] 93 % of these were superficial infections. Three patients had wound debridement and re-suturing under anaesthesia. One patient had a pelvic collection that got infected. However, it drained spontaneously through the vagina.

SSIs are hospital acquired infections and their occurrence is dependent on many factors. The profile of patients undergoing surgery is also changing. There is increase in the number of patients with multiple co-morbidities and in the older age group. Preoperative factors include patient characteristics like obesity, diabetes, other medical disorders, prior surgery and steroid use. Other pre-operative factors include skin cleaning and hair removal techniques.

Intra operative factors include timing and dose of prophylactic antibiotics, abdominal and vaginal preparation, duration of surgery and technique of wound closure. Post-operative factors include glycemic control in diabetic patients and presence of anemia and blood transfusion.

Table 2 shows the pre-op and intra-op risk factors identified in present study. A systematic review of 57 studies by the WHO from both high income and low and middle income countries identified high body mass index (BMI) , diabetes and prolongation of duration of surgery as risk factors associated with increased risk of SSIs .In patients with high BMI, various factors like increase in adipose tissue, need for larger incision, decreased circulation in fat tissue and increase in local tissue trauma related to retraction contribute to the increased SSIs in these patients. Obese patients also have an altered hemostatic balance and decreased immune function.

Hyperglycemia in patients with diabetes is a well-known risk factor for infections including SSIs. A study by Al-Niaimi et al compared 2 groups of post-operative patients after gynaecological oncology surgery. Patients whose blood sugar > 150mg/dl were managed either with intermittent subcutaneous insulin injection or insulin infusion. Patients who were on insulin infusion had significantly lower infection rates (19% versus 29% P=.001).[6] Hyperglycemia can have several deleterious effects on host immune functions, stimulation of inflammatory markers, increased thrombotic activity and endothelial cell dysfunction. This is of importance even in non-diabetics as stress hyperglycemia can have the same effects.

Prolonged duration of surgery was associated with increased incidence of SSIs in the majority of studies. There was also a linear correlation between operative time and the likelihood of SSIs. The reasons postulated for this are:

1. With increased operative time the patient's open incision is exposed to the environment for a longer time.
2. Tissue desiccation is more.
3. Tissue concentration of antibiotics will decrease as the procedure continues.

However, it is important to remember prolonged surgery may also mean more complex procedures and other intra operative factors like adhesions which by themselves can increase the likelihood of infection.

Other risk factors included anemia which diminishes the resistance to infection in 12.8% prolonged PROM >24 hours in 3.6% of caesarean sections and malignancy in 3.8%.

Other risk factors considered important in SSI in a study by Devjani et al included pre-operative hospital stay with exposure to procedures, therapies and hospital micro flora and administration of blood products. Allogenic blood products have immunomodulatory effects that may increase the risk of post-op infections.[7]

The prophylactic antibiotic policy at present in the hospital is administration of Injection Cephazolin 1 gm given within one hour of skin incision and the second dose 6 hours later. All the patients received the prophylaxis however the time was >1 hour in few patients. David Classen et al reported that administration of antibiotics within a 2-hour period before an operation was associated with the lowest infection rate.[8]

On analyzing the culture report, Table 3 shows the organisms isolated in present patients. *Klebsiella pneumonia* was seen in 29 patients (37.2%) of these 51% were ESBL producers and 10% were carbapenamase producers. The second commonest organism was E coli in 16 patients (20.5%) of these 43% were ESBL producing. Pseudomonas was isolated in 10%. Routine anaerobic cultures were not performed. More than one organism was isolated in 31 out of 78 patients (39.7%).

In a study conducted by Devjani De et al the most frequently isolated organism was Acinetobacter species in 32% followed by *Staphylococcus aureus* in 22.3%.[9] In mild infections, patients were started on Levofloxacin and Metrogyl pending the culture report. In more severe infections Cefotaxime and Metrogyl or Piperacillin/ Tazobactam was started.

The resistance pattern of the infecting organism was also analysed. Figure 1 and Table 4 shows the number and percentage of drug resistant organisms. MRSA infections are of concern and there is need to screen both the patients and the health care workers involved for asymptomatic nasal carriers. In 51 out of 78 patients

(65%) infection occurred after discharge from the hospital. This may be due to the policy of early discharge from the hospital. So, post discharge surveillance is important to arrive at a correct estimate of the magnitude of the problem. A WHO review on Global guidelines for SSI prevention reported that 13% to 71% of infections occurred after discharge from the hospital. Rosenthal et al determined that 63.8% of SSIs were detected in hospital and 36.2% after discharge.[10]

Surveillance of SSIs also includes a review and analysis of the preventive strategies in place in the hospital. Prevention of SSIs requires a multifaceted approach including proper air conditioning and ventilation engineering, appropriate cleaning and disinfection of the operating theatres and proper sterilization of instruments. Proper safeguards for checking sterilization of instruments, maintenance of OR discipline and sustained education of all OR personnel regarding safe practices are no less important.[11] Hence every hospital should have protocols for prevention of SSIs and follow continuous surveillance of infections.

As part of this, the hospital infection control committee met on monthly basis. The rates of various hospital acquired infections were discussed in the monthly hospital infection control committee meetings. SSI surveillance by surgical staff can also be cross checked by the infection control team.[10] In addition to the SSI rates in various surgical specialties, ventilator associated pneumonia and catheter associated urinary tract infection and blood stream infection rates were also discussed. Environmental surveillance included air sampling done by settle plate in all OTs and the results were analyzed. The incidence of multi drug resistant organisms in all areas in the hospital was analysed and compared to the previous months. To audit the usage of antibiotics, patients on high end antibiotics was analysed department wise and feedback was disseminated to the concerned department. Hand hygiene compliance by health care workers was also analysed in all acute care areas and feedback disseminated.

The information on Hospital Acquired Infection in developing countries is limited because of the lack of national surveillance systems.[12] Most of the reported studies are from single hospital and therefore are not representative of the entire country. Prevention of hospital acquired infection is one of the key agendas of various international bodies like the WHO, CDC and others.

Both the WHO and Center for Disease Control and Prevention guidelines for prevention of surgical site infection (2017) included the following strong recommendations[5,13]:

- Patients are advised to bathe with soap or antiseptic agents at least the night before surgery.
- Skin preparation: Hair removal if required is done only by clipping Shaving is strongly discouraged at all times Intra op skin preparation with alcohol-based chlorhexidine gluconate is recommended.
- Prophylactic antibiotics should be administered 1 hour before skin incision so that serum and tissue concentration are established before the incision is made. For clean and clean contaminated procedures additional prophylactic antimicrobial agents should not be administered after the surgical incision is closed in the operating room even in a presence of a drain. Topical antimicrobial agents should not be applied on the surgical incision.
- Protocols for perioperative glycemic control to be implemented using blood glucose target level of <200mg/dl in patients with or without diabetes.
- Perioperative normothermia should be maintained in all patients. Increased fraction of inspired oxygen should be administered during surgery and after extubation in the immediate postoperative period for patients with normal pulmonary function undergoing general anesthesia.

Transfusion of blood products should not be withheld from surgical patients as a means to prevent SSIs.

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Prevention of SSI is an important part of providing optimum care for patients. Throughout the world there is a growing concern about patient safety. This prompted the WHO to launch the World Alliance for patient safety. Prevention of health care acquired infection is the one of the first targets of the Alliance. “Clean care is safer care” was launched in October 2005. A key objective of the programme is to launch a feasible practical approach to improve hand hygiene in healthcare globally. Continuous audit and implementation of infection prevention strategies should part of the standard protocols of every hospital. It is challenging and requires dedication of both time and resources. This includes simple things like hand hygiene, pre-operative bathing and skin preparation that are no less important than tracking of multi drug resistant organisms and usage of high end antibiotics.

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1. Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F, D'errico MM, et al. Epidemiology

and microbiology of surgical wound infections. J Clinical Microbiol. 2000;38(2):918-22.
2. Mulu W, Kibru G, Beyene G, Damtie M. Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. Ethiopian J Health Sci. 2012;22(1):7-18.
3. Owens CD1, Stoessel K. Surgical site infections: epidemiology, microbiology and prevention. J Hosp Infect. 2008;70 Suppl2:3-10.
4. Saeed KB, Greene RA, Corcoran P, O'Neill SM. Incidence of surgical site infection following caesarean section: a systematic review and meta-analysis protocol. BMJ Open. 2017;7(1):e013037.
5. WHO-Global Guidelines for the Prevention of Surgical Site Infection. World Health Organization. ISBN 978 92 4 154988 2. Available at: http://www.who.int/infection-prevention/publications/ssi-prevention-guidelines/en/
6. Steiner HL, Strand EA. Surgical-site infection in gynecologic surgery: pathophysiology and prevention. Am J Obstet Gynecol. 2017;217(2):121-8.
7. De D, Saxena S, Mehta G, Yadav R, Dutta R. Risk factor analysis and microbial etiology of surgical site infections following lower segment caesarean section. Int J Antibiot. 2013;2013.
8. Classen DC, Evans RS, Pestotnik SL, Horn SD, Menlove RL, Burke JP. The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection. New England J Med. 1992;326(5):281-6.
9. De D, Saxena S, Mehta G, Yadav R, Dutta R. Risk factor analysis and microbial etiology of surgical site infections following lower segment caesarean section. Int J Antibiotics. 2013;2013.
10. Rosenthal R, Weber WP, Marti WR, Misteli H, Reck S, Dangel M, et al. Surveillance of surgical site infections by surgeons: biased underreporting or useful epidemiological data?. J Hospital Infect. 2010;75(3):178-82.
11. Woodhead K, Taylor EW, Bannister G, Chesworth T, Hoffman P, Humphreys H. Behaviours and rituals in the operating theatre: a report from the Hospital Infection Society Working Party on Infection Control in Operating Theatres. J Hospital Infect. 2002;51(4):241-55.
12. Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011;377(9761):228-41.
13. Berríos-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, et al. Centers for disease control and prevention guideline for the prevention of surgical site infection, 2017. JAMA Surg. 2017;152(8):784-91.

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ORIGINAL RESEARCH ARTICLE

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MODERATE TO SEVERE THROMBOCYTOPENIA IN THE PARTURIENT

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ABSTRACT

Objectives: To analyse patients with moderate to severe thrombocytopenia in labour, to identify the etiology, assess severity, correlate with the maternal disease and management.
Methods: A retrospective study of all pregnant women admitted in labour or in obstetric critical area and found to have a low platelet count were included in a study period of 18 months from July 2013 to Dec 2014.
Results: 25 women out of 2738 deliveries were identified and commonest cause was severe pre-eclampsia with or without HELLP. Coincidental dengue with super added pre-eclampsia was found in 3 women. Severe thrombocytopenia was found in haematological conditions like ITP and aplastic anemia.
Conclusion: Low platelets or fall in platelets showed a worsening of disease condition. Platelet transfusion needs to be done if the cut off is below the desired target level. Timely administration of platelets and discrete use of SDP especially when massive platelet transfusion is required required as mandatory. Delivery should be as atraumatic as possible, avoidance of episiotomy to be practiced. Sometimes treatment can be challenging and involve multi-disciplinary approach.

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INTRODUCTION

Thrombocytopenia is the most common hemostatic abnormality in pregnancy occurring in approximately 10% of all pregnant women. Abnormalities of platelet count may either be mild when the platelet count is between 150,000 and 100,000/mm^3, moderate when the count is between 100,000 and 50,000/mm^{3}or severe when the count is < 50,000/mm^3 (1). Gestational thrombocytopenia is the commonest cause of low platelets in pregnancy, however the count rarely falls below 70,000/mm^3 and is not of much clinical significance. Moderate to severe thrombocytopenia may occur due to either pre-existing disease like immuno thrombocytopenia (ITP), Systemic lupus erythematosus (SLE), anti-phospholipid syndrome (APLA) or pregnancy related conditions like pregnancy induced hypertension (PIH), HELLP, acute fatty liver of pregnancy (AFLP), ect (Kam, 2004). In labour low platelet count is a risk not only to the mother but also to the fetus. Most patients with mild thrombocytopenia do not have any hemostatic risk and do not require treatment unless the degree of thrombocytopenia worsens.

However moderate to severe thrombocytopenia is associated with adverse effects. The threshold for significant spontaneous bleeding is a count of 10,000/mm^3 and in the case of surgical interventions or delivery the threshold is higher >50,000/mm^3. The underlying disorder may manifest as thrombocytopenia and warrant immediate termination. The obstetrician concern is how to obtain hemostasis in the event of delivery and may involve multidisciplinary approach. Many articles describe thrombocytopenia in pregnancy however in labour only few references could be found. The aim of this article is to analyse the etiology of thrombocytopenia in labour, clinical features, management, platelet transfusions and fetal outcome.

MATERIALS AND METHODS

A retrospective study of all pregnant women admitted in labour or in obstetric critical area and found to have a low platelet count were included in a study period of 18 months. Only women with counts less than 1,00,000/mm^3 were included. The cases were analysed for the following:

- Etiology
- Clinical features
- Platelet count on admission
- Correlation with platelet count and maternal disorder.
- Need for platelet transfusion
- Gestational age at presentation.
- Outcome of pregnancy.

The condition of the baby in terms of gestational age, live birth or stillbirth were also assessed. Problems during transfusion were also assessed. The criteria for severe pre-eclampsia were blood pressure > 160/110mmhg, proteinuria +++ or more, oliguria, visual disturbance, epigastric pain, thrombocytopenia or impaired liver function.

Criteria for HELLP were

- Hemolysis as shown by peripheral smear or elevated billirubin > 1.2mg/dl
- Elevated liver enzymes : AST > 72IU/L, LDH > 600 IU/L
- Low platelets : count < 100,000/mm^3.

Management of these women, when and how they were terminated, type of transfusions and problems encountered were also analysed.

RESULTS

Table 1. Analysis of etiology

Cause	Number of cases
Severe pre-eclampsia with and without HELLP	18
PIH with Dengue	3
Megaloblastic anemia	1
ITP	1
Aplastic anemia	1
DIC	1
Total cases	25

Table 2 : Clinical features – combination of clinical features were seen

Clinical feature	Number of cases
Elevated BP	21
HELLP	7
Severe anemia	2
Eclampsia	4
Abruption	2
Renal failure	4
Bleeding episodes	6
Fever	3
Abdominal distention	1

Table 3. Correlation between platelet count on admission and diagnosis

Platelet Count	PIH	PIH with Dengue	Megaloblastic anemia	ITP	Aplastic anemia	DIC
100,000-50,000	10		1			1
50,000-20,000	6					
20,000-10,000	2	1		1		
<10,000		2			1	

The total number of deliveries were 2738 in the study period. The number patients with severe to moderate thrombocytopenia were 25.

The etiology was predominantly PIH seen in 18 women. PIH with super added dengue was seen in 3 women. Hematological disorders like ITP, aplastic anemia and megaloblastic anemia were seen in one each. One patient was found to have hemoperitoneum after caesarean and was found to have DIC. The clinical features at presentation were predominantly those of severe pre-eclampsia as shown in table 2. Combination of features like elevated blood pressure, HELLP, eclampsia, abruption and renal failure were seen. Bleeding episodes like haematuria, malena, bleeding from IV sites, bleeding gum, purpuric spots were seen in 6 women. Fever with elevated BP, abdominal distention following caesarean were also seen.

Table 3 shows the correlation between the platelet count on admission and diagnosis. The platelet count was moderate thrombocytopenia in 12 women, severe thrombocytopenia in 13 women. In the severe thrombocytopenic group 3 women had counts <10,000 cells/mm^3 and 4 women had counts between 10,000 to 20,000 cells/mm^3. In the PIH group platelet count was moderate thrombocytopenia in 10 out of 18 women. 6 women had counts between 50,000 to 20,000 cells/mm^3 and only 2 women had counts between 20,000 to 10,000 cells/mm^3. In patients with dengue and superadded PIH (3 women) the platelet count was <20,000 cells/mm^3 in all. The woman with megaloblastic anemia and dilutional coagulopathy had counts of 60,000/mm^3. Woman with ITP and aplastic anemia had very low counts <20,000cells/mm^3 and <10,000cells/mm^3 respectively. On analysis of platelet transfused in PIH women only 8 received transfusion and 10 did not. All women with dengue received multiple transfusions of 20 to 24 units. The woman with ITP received 44 units of platelet transfusion and the women with aplastic anemia received 40 units of platelets and 10 units of single donor platelets (SDP) as shown in table 4. There was one spontaneous expulsion at 26 weeks of pregnancy, live births were seen in 19 women and 5 women had IUD or stillbirth. 8 women delivered before 34 weeks and 16 women delivered 34 to 38 weeks of pregnancy. Table 5 shows the gestational age at presentation and fetal outcome.

DISCUSSION

Pregnancy is a hypercoagulable state with increased level of clotting factors. However platelet count declines as pregnancy advances. A platelet count < 150,000/mm^3 occurs in 1% of population but occurs in 6 to 12% of pregnancy women at term (McCrae, 2003; Boehlen, 2000). Approximately 1% of all pregnancy women have a count <100,000/mm^3 and about 1 in 1000 to 2000 have a count <50,000/mm^3. In our series also the incidence was similar around 1% of all patients had counts <100,000/mm^3 and severe thrombocytopenia was seen in 4-5/1000 women. In general, patients with a platelet count < 20,000//mm^3 are at risk of spontaneous bleeding. Platelet counts between 20,000 to 50,000/mm^3 have a risk of bleeding during procedures or delivery (Andra, 2013). Patients with counts >50,000/mm^3 generally have minimal risk of bleeding. PPH is the leading cause of maternal mortality (Lale, 2014) and when a patient enters the labour room with a low platelet there is great fear as to whether she would bleed during delivery, how much and how to prevent PPH. Therefore there is great concern to ascertain the diagnosis and immediate treatment. In our study pre-eclampsia was the commonest cause of severe and moderate thrombocytopenia.

Table 4: Transfusion chart

Platelet Count	PIH		Dengue		Megaloblastic anemia		ITP		Aplastic anemia		DIC	
	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
100,000-50,000	2	8			1						1	
50,000-20,000	4	2										
20,000-10,000	2		1				1					
<10,000			2						1			

R – Received, NR – Not Received

Table 5. Gestational age at presentation & fetal outcome

Gestational age	PIH	Others	IUD/Still birth
< 28 weeks	1	0	1
28 to 34 weeks	7	1	3
34 to 38 weeks	13	3	2
>38 weeks	0	0	0

Pre-eclampsia is said to be present in 21% of maternal thrombocytopenia[6]. Thrombocytopenia occurs in 50% of pre-eclampsia and occasionally precedes other manifestation of the disease. A decrease in platelet count is considered as an early sign of worsening of pre-eclampsia and may occur even before other clinical manifestation of disease are apparent (McCrae, 2003). The severity of thrombocytopenia directly correlates with the severity of the lesion. HELLP syndrome is often considered to be a variant of pre-eclampsia and in 1982 Weinstein coined the term HELLP and also stated that its presence is an indication for delivery as there is increased risk of maternal and fetal mortality (Weinstein, 1982). The hallmark of HELLP is microangiopathic hemolytic anemia, elevated liver enzymes and low platelet count. The reported cutoff value have ranged from 75,000/mm^3 to 229,000/mm^3 with a level of <100,000/mm^3 most often cited. HELLP may be complete with all the elements or partial with one or two components. HELLP occurs in 0.5 to 0.9 of all pregnancies and in 20% of all cases with severe pre-eclampsia.

The Mississippi Triple class system classifies this syndrome based on platelet counts (Joshi, 2010).

- Class I< 50,000/mm^3
- Class II50,000 to 1,00,000/mm^3
- Class III1,00,000 to 1,50,000/mm^3

In our study of 21 women with PIH, 7 women had HELLP, 5 belonging Mississippi Class I and 2 to Class II. Abruption was found in 2 and eclampsia was found in 4. HELLP, abruption and eclampsia require immediate termination irrespective of the gestational age. All our patients were terminated before 38 weeks of pregnancy, most of them were early or late preterm. Only one patient expelled at 26 weeks. M Sibai et al also states the same in his analysis of 442 pregnancies with HELLP syndrome [9]. In a study of 437 women who had 442 pregnancy with HELLP syndrome, serious maternal morbidity including DIC (21%), abruption 16%, ARF 8%, Pulmonary edema 6%, liver haematoma 1%, retinal detachment 1%. 55% of patients required blood component therapy and 2% required laparotomy for bleeding. Maternal mortality was 1%. Of 21 patients with low platelets with PIH, transfusion was done only in 11 patients, 9 of them had counts <50,000/mm^3 and only 2 had counts >50,000/mm^3 - one had 55,000/mm^3 and other had 62,000/mm^3, since LSCS had to be performed platelet transfusion was done as per British guidelines. Platelet count of > 80,000/mm^3 is recommended for epidural anesthesia by the British committee for the standards in hematology [10]. The count that was aimed in our patients was 50,000/mm^3 for vaginal delivery and 80,000/mm^3 for LSCS.

Dengue with super added PIH was seen in 3 patients. 2 of these patients in addition had HELLP-one complete and another partial. These patients had extremely low platelet counts (7,000/mm^3). The counts were much lower than those seen in severe pre-eclamsia. All of them had bleeding tendencies and all required transfusions. 24 units were given for the patient with 9000/mm^3, 22 units for a patient with twins and a count of 7,000 cells and 7 units for a patient with partial HELLP and previous caesarean. The rise in platelet count was very slow in this group, it took 10 days for platelet count to stablise. Dengue is a common tropical disease in our country. All our 3 patient had secondary dengue with super added pre-eclampsia and presented with bleeding tendencies. No reports of dengue with pre-eclampsia have been reported so far. The compounded effect of pre-eclampsia and dengue lead to very low platelet count and massive platelet transfusion was required in these women. The interval between the diagnosis and delivery was within 2 to 3 days. Termination was done immediately for all case of abruption, eclampsia, renal failure and HELLP after platelet correction to the desire level. In preterm mothers steroid administration was done for lung maturity and subsequently induction or LSCS was resorted to based on other parameters.

Haematological disorders must always be borne in mind especially when the clinical situation rules out pregnancy related conditions like PIH. In cases of severe anemia, megaloblastic anemia must always be thought of as was done in our case. In case of megaloblastic anemia the patient was referred as severe anemia with hemoglobin of 4.3gm/dl, platelet count showed 60,000cells/mm^3 . Anemia was more profound in this case and peripheral smear and bone marrow confirmed the diagnosis. A patient with known ITP presented at 28 weeks with PROM. She was on steroids. After delivery she developed hemothorax. She was treated as per ASH guidelines (The American Society of Hematology, 2011) Since she did not respond to steroids, Anti Rh immunoglobulin was given. She received a total of 44 units of platelets. Splenectomy was done as a last resort. A normotensive woman was referred as severe anemia with low platelet count. Dengue and drug history were ruled out. ITP was initially thought of, but inspite of transfusion the platelet count never raised. The expected rise was 5000cell/mm^3 after the single unit of transfusion but in this patient even after 4-5 units the counts did not raise. Further evaluation was done and ultimately a bone marrow confirmed aplastic anemia. We had to delivery her for fear of abruption or bleeding elsewhere and planned to refer her for bone marrow transplant. The patient was delivered under cover of single donor platelet.

However following delivery the counts fell to <2000/mm^3, she developed huge vulval haematoma, multiple bleeds, cerebral bleed and could not be resuscitated. Sometimes massive bleeding as in PPH may result in dilution coagulopathy as seen in our patient. This patient was referred from outside with abdominal distention after caesarean section, was found to have hemoperitoneum with platelet count of 60,000cells/mm^3, hemoglobin of 5gm/dl, fibrinogen of 150mg/dl, INR 1.6, she was diagnosed as DIC. NIH consensus conference report noted that pathological haemorrhage in a patient receiving massive transfusion is caused more frequently by thrombocytopenia than by depletion of coagulation factors. This may be due to hemodilution using crystalloids or using only packed cells. In such a situation it is always worthwhile to serially check for platelet count, PT & INR to see if dilution coagulopathy has occurred, since unless it is corrected bleeding would not stop (Platelet Transfusion Therapy, 1986). Still birth and IUD were seen in 6 women all in the PIH group. The high perinatal mortality was due to prematurity and IUGR. Similar reports are quoted by Kubilay Ertan (A Kubilay Ertan, 2002). However thrombocytopenia was not related to the preterm birth.

Factors to be considered in thrombocytopenic patients in the last trimester are

- When to terminate?
- What is the mode of termination?
- What is the ideal count to be obtained?
- What is the type of transfusion to be given?

Termination needs to be thought of only for maternal conditions like PIH, HELLP or abruption. The severity of thrombocytopenia directly correlates with the severity of the lesion[(14)]. Patient with severe PIH need to be serially monitored and a drop in platelet count may heralds the onset of HELLP. Careful surveillance is necessary. Vaginal birth is the preferred mode delivery. The chance of bleeding is less when trauma is minimal. Even episiotomy need to be given only where necessary because 1 patient with aplastic anemia had huge vulval haematoma after delivery. Both British and American guidelines suggest a platelet count >50,000/mm^3 is safe for vaginal delivery. British guidelines suggest aiming at a platelet count >80,000/mm^3 for a caesarean section. The count that was aimed in our patients was 50,000/mm^3 for vaginal delivery and 80,000/mm^3 for LSCS.

A single unit of platelet transfusion rises the platelet count by 5,000/mm^3. A unit obtained by apheresis of a single donor (SDP) contains an equivalent of 5 to 6 units raising the platelet count by around 30,000 to 60,000/mm^3. Platelet should be ABO and Rh compatible. Since stored platelets have a very short shelf- life, procuring many units may be a problem as the blood bank may not have that many bags. When requirement of platelet is large SDP is preferred as the risk of blood born pathogenesis is low, as also alloimmunisation. Where aphaeresis unit is not available, obtaining one required a lot of effort and co-ordination. Since the presentation varied interdepartmental assistance was often required. Good co-ordination with blood bank was required for procuring blood and blood products. In addition consultation with intensivist, haematologist, nephrologist, anesthetists and pediatrician was required. Team approach was the secret of success of survival of these patients.

Conclusion

Unlike in the antenatal period, moderate to severe thrombocytopenic patients near term are in a grave situation – one because the diagnoses has still to be made and secondly due to limited time constraints as delivery is imminent. A detailed history & physical examination is necessary in evaluating a patient with thrombocytopenia. Timely administration of platelets and discrete use of SDP especially when massive platelet transfusion is required helps prevent risk of infection and alloimmunisation. A platelet count of above 10,000cells/mm^3 must be maintained even after delivery to prevent spontaneous bleeding. Delivery should be as atraumatic as possible, avoidance of episiotomy to be practiced. Any pregnancy with a low platelet count less than 50,000cells/ml requires to be shifted to a tertiary centre, since etiology is varied and treatment is multidisciplinary.

REFERENCES

Kam PC, Thompson SA, Liew AC. 2004. Thrombocytopenia in the parturient. Anaesthesia. Mar;59(3):255-64. Review. PubMed PMID: 14984524.

McCrae KR. 2003. Thrombocytopenia in pregnancy: differential diagnosis, pathogenesis and management. Blood Rev., 17:7–14

Boehlen F(1), Hohlfeld P, Extermann P, Perneger TV . *2000.* Platelet count at term pregnancy: a reappraisal of the threshold. *Obstet Gynecol. 2000* Jan;95(1):29-33.

Andra H, James. Bleeding and the management of haemorrhagic disease in pregnancy. Text book of Consultative hemostasis and thrombosis. 3rd Ed. Chapter 34. Pg: 616-626.

Lale Say et al. Global causes of maternal death: a WHO systematic analysis. Lancet Global Health 2014.2:e323-333.

Burrows RF(1), Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. *N Engl J Med. 1993* Nov 11;329(20):1463-6.

Weinstein.L. Syndrome of hemolysis, elevated liver enzymes and low platelet count: A severe consequence of hypertension in pregnancy. Am J obstet gynecol 1982;142:159-167

Joshi D(1), James A, Quaglia A, Westbrook RH. Liver disease in pregnancy. *Lancet. 2010*;375:594-605. doi: 10.1016/S0140-6736(09)61495-1.

Sibai BM, Ramadan MK, Usta I, et al. Maternal morbidity and mortality in 442 pregnancies with hemolysis, elevated liver enzymes and low platelets (HELLP syndrome). Am J Obstet Gynecol. 1993;169:1000-1006.

Gernsheimer T, McCrae KR. Immune thrombocytopenic purpura in pregnancy. *Curr Opin Hematol. 2007*;*14*:*574-580*

The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia

Platelet Transfusion Therapy. National Institutes of Health Consensus Development Conference Statement October 6-8, 1986

A Kubilay Ertan, Steffen wagner, H.Joachim Hendrik et al . Clinical and biophysical aspects of HELLP syndrome. J Perinat Med. 2002;30:483–489.

Michal Parnas, Eyal Sheiner, Ilana Shoham-Vardi et al. Moderate to severe thrombocytopenia during pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology (2006) EURO-5684.

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RESEARCH ARTICLE

Labor Pattern in South Indian Population

Chitra Bhat[1], Seetha Panicker[2]

Abstract

Aim: Labor dystocia is one of the commonest indications of lower segment cesarean section (LSCS). Recent studies have shown a dramatic change in the rate of cervical dilatation and labor progress. There are no large studies to assess labor in this ethnic group and hence this study has been done to find out labor behavior in women with spontaneous labor in South India.

Materials and methods: A retrospective study was done in our teaching hospital. Labor was managed as per protocol and a partogram was maintained. Data were extracted from case-records and analyzed.

Results: Labor progress was much slower than what was thought of. There was wide variability in the duration of labor.

Conclusion: Many women took time up to the 95th percentile and some even crossed three times the median time to deliver.

Clinical significance: Though many women will deliver in the expected time, it is important to know the maximum time or the 95th percentile for each dilatation so that sufficient time can be allowed before labeling a case as prolonged labor.

Keywords: Labor curve, Labor duration, Rate of cervical dilatation, Slowest rate of dilatation.

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Introduction

Cervical dilatation is one of the key determinants of the progress of labor and may be dependent on many factors. There have been many attempts to define the norms for the progress of labor that have yielded variable results.

This is not an easy task as there are many challenges to accurately measure the duration of labor. The labor norms are also dependent on a woman's ethnicity, age, parity, BMI, use of epidural analgesia, and may vary among population groups. Despite all these difficulties, it is important to arrive at a proper understanding of labor norms and identify the slowest acceptable rates of cervical dilatation.

The rates of cervical dilatation in the active phase of labor largely stem from the data published by Friedman in 1950.[1] The Friedman's labor curve and the active management of labor pioneered by O'Driscoll et al.[2] where the lowest acceptable rate of dilatation is 1 cm/hr has influenced obstetric practice worldwide. WHO partogram[3] is practiced all over the world and the expected rate of dilatation is 1 cm/hr in the active phase. Any shift to the right and if the curve crosses the action line, labor is labeled as prolonged labor or non-progression of labor, and lower segment cesarean section (LSCS) is performed.

Dystocia characterized by a delay in progression of cervical dilatation is one of the most common indications for primary cesarean sections, ACOG Bulletin states it is the leading cause contributing to around 50% of all primary LSCS being performed.[4]

Zhang et al. reassessed Friedman's curves.[5] They studied the electronic records of 62,416 parturients and found the rates of cervical dilatation to be much slower. Data from this study formed the basis of the new guidelines for the active phase of labor. Labor may take even 6 hours to progress from 4–5 cm and more than 3 hours to progress from 5–6 cm. Only after 6 cm was the progress faster.

Due to ethnic differences, it is important to have insight regarding labor in our population that is critical in diagnosing labor dystocia and making a decision regarding the mode of delivery

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Aims and Objectives

- To determine the duration of labor and the rates of cervical dilatation in low-risk primigravida and multigravida women with spontaneous onset of labor.
- To determine the slowest acceptable rates of cervical dilatation.
- To create a labor curve for this population.

Materials and Methods

This was a prospective observational study done in a teaching hospital in South India between 2017 and 2018.

The study population included both primigravida and multigravida who were admitted with spontaneous labor.

The inclusion criteria were term gestation, singleton pregnancy, and vertex presentation with spontaneous onset of labor who delivered either normally or by assisted vaginal delivery. The exclusion criteria were induced labor, pre-term labor, mal-presentations, and patients who were delivered by cesarean section.

The institutional protocols for monitoring of labor were followed. Maternal and fetal monitoring was done in the usual way. The progress of labor was assessed by monitoring uterine contractions per 10 minutes for every half an hour, cervical

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dilatation, and descent of the head was assessed by vaginal examination every 3–4 hours in the first stage of labor.

The rate of cervical dilatation was plotted on a partogram with time in hours on the x-axis and cervical dilatation on the y-axis.

Ethical clearance was obtained from our institutional human ethical committee.

Statistical Analysis

The data was entered on an Excel spreadsheet and analysis was done using SPSS STATA13.1 software.

The data were analyzed concerning maternal demographic characteristics. The cumulative labor duration from cervical dilatation on admission (i.e., at 1, 2, 3, 4, 6, 7) to reach 10 cm was assessed. Data were extracted according to two parity groups nulliparous and parous women.

With each starting dilatation, the mean rate of dilatation was calculated for primigravida and multigravida. Taking the value of 2SD, the slowest rate of dilatation was also calculated.

A non-regression model was used to plot the labor curve with cervical dilatation against time in hours.

Results

The total number of deliveries during the study period was 4150.

A total of 2558 women were delivered by cesarean section. The number of women with spontaneous onset of labor who delivered vaginally was 1196. A total of 152 patients who had incomplete data and 21 patients who were admitted at more than 8 cm were excluded. So, only 1023 deliveries were analyzed.

Primigravida were 556 in number and multigravida were 467 in number. The age, cervical findings on admission, and mode of delivery are shown in Table 1.

The cumulative time taken for each dilatation starting from 1 to reach 10 cm was analyzed in both primigravida and multigravida.

In our study, the median duration of labor was chosen, as the mean duration may be skewed by a few laborers of very short or long duration. If the labor duration is positively skewed, the mean duration will be longer than the median duration as shown in this study thus at 2 cm the mean duration was 7.7 hours and the median duration was 7 hours.

Table 2 shows the cumulative mean time taken for each dilatation to reach 10 cm in primigravida and multigravida, respectively. It shows that multigravidae took a shorter time to progress to 10 cm when compared to primigravida. Primigravida took a median time of 9 hours to reach 10 cm from 1 cm, whereas it was 8 hours in multiparous women. At 2 cm it was 7 and 6 hours. At 3, 4, 5, 6–10 cm it was 5, 3, 3, and 2 hours in primigravida and 4, 3, 2, and 2 hours in multigravida.

Table 2 also shows the 5th and the 95th percentile, the number of women who crossed the 95th percentile, and the maximum time allowed for labor to continue that resulted in normal vaginal delivery.

At 2 cm though the meantime was 7.7 hour in primigravida, 16 women crossed the 95th percentile and 10 patients had a vaginal delivery after almost three times this mean-ranging from 18–26 hours. In six multiparous women at the same dilatation, labor was prolonged to 16–27 hours and 15 women crossed the 95th percentile.

At 3 cm dilatation, although the mean duration of labor was 5.5 hours, four primigravida women had a vaginal delivery after 19, 17, 16, and 13 hours, respectively. Correspondingly three multigravidas had taken 21, 15, and 13 hours to deliver from 3 cm dilatation for a mean of 4.7 hours.

At 4 cm against a mean of 3.8 hours, five primigravidas had taken between 8 and 11 hours to deliver, and in multigravida for a mean of 2.9 hours four had taken between 6 and 9 hours. At 7 cm a multiparous woman took 6 hours to reach full dilatation.

With each starting dilatation, the mean rate of dilatation was calculated for primis and multis. Taking the value of 2SD, the slowest rate of dilatation was also calculated as shown in Table 3.

The labor curve obtained was hyperbolic and not a sigmoid curve. There was no definite point of acceleration seen (Figs 1 and 2).

Table 1: Demographics

Details	*Primigravida*	*Multigravida*
Number of cases	556	467
Age	24.2 years	27.4 years
% of normal delivery	74.3%	87.9%
Instrumental	25.7%	12.1%
Mean dilatation on admission	2.85 cm	3.2 cm

Table 2: Cumulative duration of labor (dilatation on admission to 10 cm)

Dilatation on admission (cm)	*Obs score*	*Numbers*	*Mean*	*Median*	*SD*	*P 5*	*P 95*	*No > 95*	*Max time taken*
1–2	Primi	121	9.36	9	5.05	2.5	19	17	30
	Multi	84	9.39	8	5	2.5	19	16	27
2–3	Primi	201	7.7	7	4.5	2	16	10	26
	Multi	158	6.8	6	4.2	2	15	6	27
3–4	Primi	112	5.5	5	3.2	2	10	5	19
	Multi	122	4.7	4	3.1	1	10	5	21
4–5	Primi	69	3.8	3	2.2	1	8	3	11
	Multi	74	2.9	3	1.6	1	6	2	9
5–6	Primi	29	3	3	1.8	1	6	1	10
	Multi	30	2.2	2.2	1.7	1	6	1	8
6–7	Primi	12	1.8	2	0.68	1	3	–	–
	Multi	6	1.8	2	0.68	1	3	–	–
7–8	Primi	12	1.4	1	0.64	–	3	–	–
	Multi	21	1.4	1	1.13	–	3	1	6

Table 3: Rate of cervical dilatation

Dilatation (cm)	*Obs score*	*Mean rate of dilatation (cm)*	*Slowest rate of dilatation (cm)*
2–3	Primi	1.03	0.65
	Multi	1.17	0.72
3–4	Primi	1.27	0.8
	Multi	1.48	0.89
4–5	Primi	1.57	1
	Multi	2.06	1.33
5–6	Primi	1.66	1.04
	Multi	2.27	1.28
6–7	Primi	2.2	1.61
	Multi	2.2	1.61

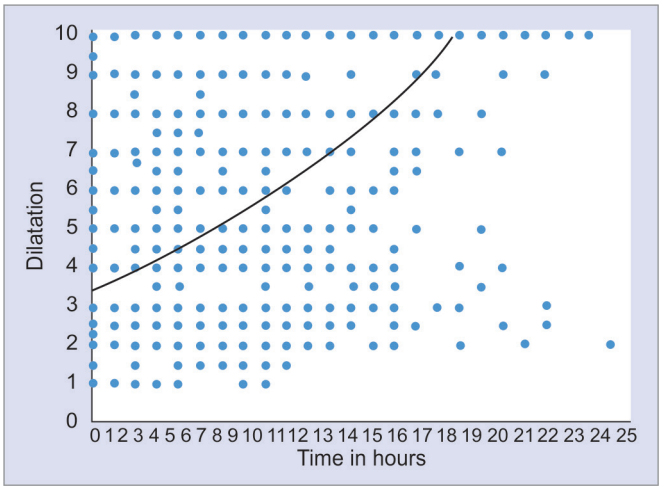


Fig. 1: Labor curve—primigravida

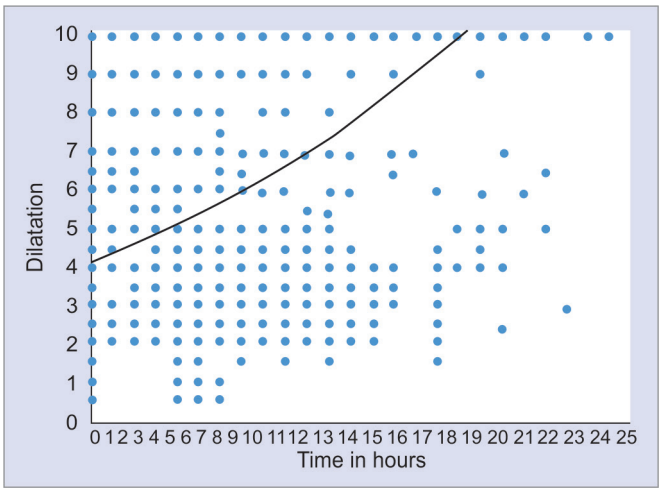


Fig. 2: Labor curve—multigravida

Discussion

A total of 1023 patients were analyzed, out of which 556 were primigravida and 467 were multigravida. We found the following in our study:

- In the active phase, the average time taken was more than that described by Friedmann.[6,7] In his study, he has stated that from 2.5 cm to reach 10 cm the average time is 4.6–4.9 hours, and once they reach 4 cm half of them deliver in 2.6 hours. Our corresponding figures were 7.7 hours to reach 10 cm from 2.5 cm and the mean duration at 4 cm was 3.8 hours against 2.6 hours. Peisner and Rosen[8] found that 75% of women at 3 cm, 50% at 4 cm, and 25% at 5 cm do not dilate at the indicated rates associated with active labor.

However, recent studies have shown a longer first stage of labor with slower acceptable rates of dilatation. Zhang et al. derived the average labor curves from 27,170 nulliparous women in the US population with spontaneous onset of labor. The curve had a slower progression of 1.1 cm/hr with no definite inflection point. The slowest acceptable rate was 0.6 cm/hr.

Albers et al.,[9] Albers,[10] and Jones and Larson[11] found that labor progression takes a longer time than the thought of earlier. These investigators have reported that in the active phase of labor from 4 cm to reach 10 cm the time ranges from 6.2–7.7 hours on average. In Albers's study, the time taken was 7.7 hours since there was no use of oxytocin and labor was spontaneous.

- The second derivative was that there was a wide difference in time taken between individuals. When women were admitted at 2 cm, the median time taken to reach 10 cm was 7 hours in primigravida and 6 hours in multigravida. However, 2SD at this dilatation was 4.5 hours extending labor to 11.5 hours. Many women crossed the 95th percentile and also, ten primigravidas delivered normally after a duration that was almost three times the median ranging from 18–26 hours indicating wide variability in the duration of labor are still associated with normal outcomes. The same was seen at all dilatations. Our findings were similar to that quoted by Oladapo et al.[12] as part of WHO BOLD PROJECT (better outcome in labor difficulty) where he says median cumulative duration of labor indicates that nulliparous women admitted at 4, 5, and 6 cm reached 10 cm within an expected time, but their corresponding 95th percentile shows that labor could last up to 14, 11, and 10 hours.
- It was found that the rates of cervical dilatation accelerate as labor progresses and for each dilatation, an acceptable slow rate of progress exists. This is comparable to the study by Zhang et al. who found that the slopes of cervical dilatation progressively steepen with each passing centimeter. In his study, he also found that the median rates of dilatation between 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, and 9–10 cm were 0.4, 0.6, 1.2, 1.7, 2.2, 2.4, and 2.4 cm, respectively. At the 5th percentile that is used to define the slowest normal dilatation rates the rates were 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, and 0.7 cm/hour, respectively.
- Finally, the labor curve that was obtained showed a smooth hyperbolic curve, there was no point of acceleration. A similar labor curve pattern is seen in other studies. Suzuki et al.[13] studied the labor patterns in 2369 low-risk nulliparous Japanese women with spontaneous onset of labor and derived the labor curve for that population. It showed that the curve had a smooth shape with an active phase duration of 5.1 hours with slower progression than that of Friedman's curve. The deceleration phase was not observed and the rate of dilatation started to double from 5–6 cm onwards. A study done in South India by Pitchaimuthu and Bhaskaran[14] showed the same pattern

of slower dilatation, a smooth labor curve, and no point of inflection.

The limitations of this study include interpersonal variations in the assessment of cervical dilatation. The effect of ARM and oxytocin were not analyzed since many patients were subjected to either both or one of them.

Conclusion

In low-risk women, both primigravida and multigravida with spontaneous onset of labor, there is wide variability in the duration of labor with normal maternal and fetal outcomes. Our study showed that in low-risk women admitted in early spontaneous labor, half of them took more than the meantime and a significant number of women took even three times the mean. The rate gradually increased with dilatation and in nulliparous women at 6 cm of dilatation the rate was much higher. It is worthwhile to note that many women despite crossing the 95th percentile delivered normally, hence, revision of the expectations of the rate of cervical dilatation would reduce both unnecessary interventions in labor and cesarean sections for slow or abnormal progress of labor. Labor should be more patient-centered and the same pattern will not fit all.

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References

1. Friedman EA. Primigravid labour: a graphicostatical analysis. Obstet Gynecol 1955;6(6):567–589. DOI: 10.1097/00006250-195512000-00001. Pubmed:13272981.
2. O'Driscoll K, Stronge JM, Minogue M. Active management of labour. Br Med J 1973;3(5872):135–137. DOI: 10.1136/bmj.3.5872.135. Pubmed: 4720762.
3. World Health Organizaton. Division of family health. Maternal health and safe motherhood programme. The partograph; the application of the WHO partograph in the management of labour. Report of a WHO multicentre study 1990–1991. 1994. WHO/FHE/MSM/94.4.
4. American College of Obstetrics and Gynecology Committee on Practice Bulletins-Obstetrics. ACOG Practice Bulletin Number 49, December 2003; dystocia and augmentation of labour. Obstet Gynecol 2003;102(6):1445–1454. DOI: 10.1016/j.obstetgynecol.2003.10.011 [PubMed:14662243].
5. Zhang J, Landy HJ, Branch DW, et al. Contemporary patterns of spontaneous labour with normal neonatal outcomes. Obstet Gynecol 2010;116(6):1281–1287. DOI: 10.1097/AOG.0b013e3181fdef6e.
6. Friedman EA, Kroll BH. Computer analysis of labour progression. III. Pattern variations by parity. J Reprod Med 1971;6(4):179–183. PubMed: 4938465.
7. Friedman EA, editor. Labor: clinical evaluation and management. 2nd ed. New York: Appleton century-Crofts; 1978.
8. Peisner DB, Rosen MG. Transition from latent to active labor. Obstet Gynecol 1986;68(4);448–451. Pubmed: 3748488.
9. Albers LL, Schiff M, Gorwoda JG. The length of active labor in normal pregnancies. Obstet Gynecol 1996;87(3);355–359. DOI: 10.1016/0029-7844(95)00423-8. Pubmed: 8598954.
10. Albers LL. The duration of labor in healthy women. J Perinatol 1999;19(2):114–119. DOI: 10.1038/sj.jp.7200100. PubMed:10642971.
11. Jones M, Larson E. Length of normal labor in women of Hispanic origin. J Midwifery Womens Health 2003;48(1);2–9. DOI: 10.1016/s1526-9523(02)00367-7. Pubmed:12589300.
12. Oladapo OT, Souza JP, Fawole B, et al. Progression of the first stage of spontaneous labour: a prospective cohort study in two sub-Saharan African countries. PLoS Med 2018;15(1): e1002492. DOI: 10.1371/journal.pmed.1002492.
13. Suzuki R, Horiuchi S, Ohtsu H. Evaluation of the labor curve in nulliparous Japanese women. Am J Obstet Gynecol 2010;203(3):226.e1–6. DOI: 10.1016/j.ajog.2010.04.014.
14. Pitchaimuthu N, Bhaskaran S. Labor patter among primigravida in local population. J Obstet Gynaecol India 2018;68(6);482–486. DOI: 10.1007/s13224-017-1083-3.

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Efficacy of intermittent iron versus daily iron in antenatal women

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Abstract
Aim: This study was done to determine if alternate day oral iron as effective as daily oral iron in antenatal women including those with mild anemia.
Methods & Material: A prospective randomised trial was done in 215 antenatal women attending a tertiary centre in South India. Group 1 received 100mg of elemental iron daily and group 2 received 100mgs of elemental iron on alternate days. Haemoglobin was estimated in all the trimesters and change in Haemoglobin from baseline and at 32-34 weeks were estimated. Side effects and neonatal outcomes were analysed. Further analysis were done within groups depending upon the Haemoglobin levels.
Results: There was no difference in Haemoglobin levels between the two groups. The response was better in patients with mild anemia in both groups, however when iron was given daily in mildly anemia women the increase in Haemoglobin was better than alternate iron therapy.
Conclusion: Intermittent iron is as effective as daily iron.

Keywords: Haemoglobin, daily iron, intermittent iron, iron therapy in pregnancy, anemia in pregnancy

Introduction

Anaemia complicating pregnancy is one of the most common medical disorders of pregnancy. The WHO [1] has defined anaemia as a Haemoglobin less than 11gms/dl in the first and third trimester and a Haemoglobin of less than 10.5gms/dl in the second trimester. Any Haemoglobin less than the 5th percentile for a given gestation is defined as anaemia by CDC [2]. Globally about 58.25 million women are found to be anaemic during pregnancy and 95% of them belong to the developing world [3]. In India as per the population based survey in 2016 it was found that about 50% of pregnant women were anaemic [4]. Anaemia is a direct and an indirect cause of maternal mortality and causes around 13% of all maternal deaths and about 20% in South East Asia [5].

As part of a global strategy to prevent anaemia, WHO has recommended that all pregnant women be given 60mgs of elemental iron with 400mgs of folic acid every day prophylactically[6]. However in the event of iron deficiency anaemia, higher doses are prescribed. Oral iron is associated with many undesirable side effects causing poor compliance. An ideal dosing schedule is yet to be found, which patients will not default and yet give the desired results.

There has been lot of research in iron metabolism over the past two decades.

First is the presence of protein Hepcidin which regulates iron absorption [7], second is the mucosal turn over time which is said to occur once in 3 to 5 days. Thus after a given dose, further supplementation with iron until turn overtime is completed might lead to impaired absorption since the cells are already saturated [8]. Excess iron may cause oxidative stress and other side effects [9]. This has changed the thinking that intermittent iron might work as well. Subsequent to this there are many studies which state that iron two or three times per week or even once a week might be all that is required. Most of these studies have been done in pregnant women who had normal haemoglobin levels.

The WHO in a recent guideline has suggested administration of weekly iron of 120mgs with 2.8mgs of folic acid in pregnant women in areas where the prevalence of anaemia is less than 20% [10].

In India, the practise is still to administer daily iron during pregnancy. Being an area where the prevalence of anaemia is high, it would be worthwhile to know the efficiency and advantages of intermittent iron and hence this study was conducted to determine the usefulness of intermittent iron in both mildly anaemic and non anaemic pregnant women.

Materials and Methods

This was a prospective observational study conducted at a teaching hospital in South India. 215 pregnant women attending the antenatal OPD were randomly recruited for this study. 105 pregnant women from Unit 1 were assigned to daily iron and formed group 1 and 110 pregnant women from Unit 2 were given alternate day iron and were assigned as group 2. During the first visit between 10 to 14 weeks investigations like CBC, Peripheral smear and serum ferritin were done. This helped to rule out thalassemia which is not very uncommon in this area. Ferrous tablets containing 100mgs of elemental iron and 500mgs of folic acid was used after completion of 13 weeks and continued till delivery.

Exclusion criteria: Were Haemoglobin less than 9gms in the first trimester, history of haemtological disorders like sickle cell or thalassemia and history of autoimmune disorders.

Inclusion criteria: All patients attending OPD from at least 12 weeks of pregnancy. The patients were asked to take iron at night before food or at least one hour after food. They were asked to report if they had any side effects like vomiting, diarrhoea, heart burn or constipation. Repeat Haemoglobin was done in the second trimester and around 32 to 34 weeks to assess the Haemoglobin status of the individual. If the Haemoglobin fell to less than 8gms they were advised parenteral iron. Haemoglobin was done using Coulter LH 780 Haematology Analyser method. The maternal and fetal outcomes were noted.
Patients who did not have their delivery in our institution and if the Haemoglobin levels were not done in the third trimester, were excluded from the final analysis and two patients who were given parenteral iron were also excluded from final analysis.

Statistical Analysis

The categorical variables were analysed using chi square test and the continuous variables were analysed using test and ANOVA. P value of .005 was taken as significant. Both groups were compared in regard to mean change from baseline Haemoglobin to Haemoglobin at the 2nd and 3rdtrimester.
The groups were further subdivided into three groups based on Haemoglobin levels. Group A had Haemoglobin level between 9 to 11gms, Group B had Haemoglobin levels between 11.1 to 13gms and Group C had Haemoglobin levels more than 13gms and analysis done within the subgroup with regards to the change in Haemoglobin levels in the first and last trimester.

Results

Total of 215 pregnant women were included in this study 105 in the daily group and 110 in the alternate group. A total of 173 women, 80 in group 1 (daily Iron) and 93 in group 2 (alternate day Iron) were analysed and the attrition was mainly due to change of place for delivery, or incomplete data and investigations. Table 1 shows the age distribution in both groups and Table 2 shows the parity.

Table 1: Age

Age Yrs	Daily Regimen	Alternate Iron
<20	1	3
	1.3%	3.2%
21-25	34	31
	42.5%	33.3%
26-30	36	48
	45.0%	51.6%
>30	9	11
	11.3%	11.8%
Total	80	93

Table 2: Parity

	Daily Regimen	Alternate Iron
Primi	53	62
	66.25%	66.66%
Multi	27	31
	33.75%	33.33%
Total	80	93

The distribution of age and parity were similar in both groups. Table 3 shows the mean Haemoglobin levels in all trimesters in both groups which showed that the mean Haemoglobin levels were almost identical in both groups. The levels were higher in the first trimester and third trimester and slightly lower in the second trimester as expected due to haemodilution.

Table 3: Mean Hemoglobin in all trimester

Trimester		Daily regimen (N=80)	Alternate regimen (N=93)
1st trimester	Mean Hb	11.553	11.983
	SD	(1.2447)	(1.1462)
2nd trimester	Mean Hb	11.138	11.271
	SD	(0.9611)	(0.8489)
3rd trimester	Mean Hb	11.3065	11.382
	SD	1.1487	1.1218

The relationship between the Haemoglobin level and the ferritin level is shown in Fig1.

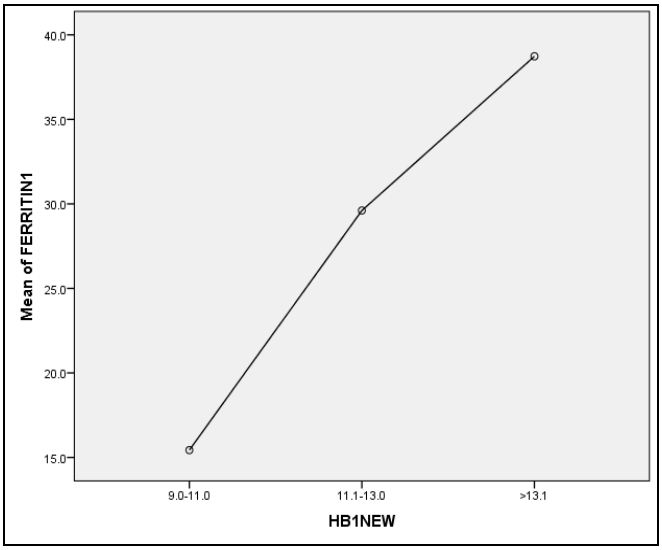


Fig 1: Relationship between the Haemoglobin level and the Ferritin level

38 women had ferritin levels less than 12ngm/dl. 24 in the daily group and 14 in the alternate group had ferritin less than 12 ngm/dl.
Table 4 shows the side effects of oral iron in both groups. Side effects were much lesser in the alternate iron group and so also was the compliance, which was found to be significant statistically with a P value of <001.

Table 4: Side effects

Side effects	Daily regimen (N=80)	Alternate regimen (N=93)
Vomiting	38	5
Constipation	30	8
Diarrhoea	2	1
Metallic taste	5	2
Bloatedness	10	2

Both groups were analysed as subgroups with the Haemoglobin levels in each trimester as shown in Table 5. This table shows that the response and Haemoglobin rise was better in women with mild anemia in both groups and the rise in Haemoglobin was better in the daily group.

Table 5: Analysis of haemoglobin in different sub groups in all trimesters

Hb gm/dl		1st trimester	2nd trimester	3rd trimester
9.0-11.0	Daily regimen N=26	10.085	10.872	11.1960
	Alternate regimen N=22 cases	10.395	10.629	10.9719
11.1-13.0	Daily regimen N=45 cases	11.996	11.107	11.2500
	Alternate regimen N=55 cases	12.169	11.375	11.3611
>13.1	Daily regimen N=9 cases	13.578	12.033	11.9625
	Alternate regimen N=16 cases	13.525	11.756	12.0333
Total	Daily regimen N=80 cases	11.553	11.138	11.3065
	Alternate regimen N=93 cases	11.983	11.271	11.3823

There were no preterm deliveries and the birth weight of babies was similar as shown in Table 6.

Table 6: Birth weight

	Hb	Number	Mean birth weight
Daily regimen (N=80)	9.0 – 11.0	25	3.0548
	11.1 - 13.0	45	2.9983
	>13.1	9	3.2938
	Total	80	3.0487
Alternate regimen (N=93)	9.0 – 11.0	22	3.0514
	11.1 - 13.0	55	2.9898
	>13.1	16	2.9106
	Total	93	2.9908

Discussion

In our study 80 women with a mean Haemoglobin 11.5gms/dl were given daily iron and 93 women with a mean Haemoglobin of 11.93gms/dl were given alternate iron. The third trimester Haemoglobin was 11.30gms/dl in the daily group and 11.38gms/dl in the alternate group showing that in both the treatment groups the Haemoglobin was almost the same.

WHO recommends 30-60mgs of elemental iron for all pregnant women depending on the prevalence of anaemia. This lower dose of 30mgs by WHO supersedes the previous recommendation of 60mgs taking into account the side effects and iron pharmokinetics [11].

The National iron plus initiative of India recommends iron folic acid [IFA] supplementation of 100 mg elemental iron and 500 mgs of folic acid every day for at least 100 days starting after the first trimester at 14–16 weeks of gestation for all non-anemic pregnant women followed by the same dose for 100 days postpartum [12].

Currently intermittent iron supplementation has been accepted for prophylaxis except in antenatal programmes for which debate is still ongoing.

In the past few years many studies have proven the hypothesis that intermittent iron is as effective as daily iron in terms of Haemoglobin change with the added advantage of having less side effects and being cost-effective. In spite of this information the change to intermittent therapy has not yet been implemented, maybe, since the prevalence is so high and further studies are warranted.

Cochrane in 2015 reviewed 21 trials of daily iron versus intermittent iron and stated that while there was no difference between groups for any maternal or fetal outcomes, the intermittent groups had less side effects [13].

Pena-rosa [14] suggested that women receiving intermittent iron had similar maternal and fetal outcomes. Study by Mukhopadhyay [15], Goonewardene [16] and ZInatossadaat [17] in have all suggested that intermittent iron is as effective as daily dosing.

Our study also proved that intermittent iron was as good as daily iron.

The second observation was that in patients who were found to have Haemoglobin between 9 to 11gms/dl, the rise in Haemoglobin was better in these patients with mild anaemia than non-anaemia and a mean increase of 1.1gm/dl was noted in daily group and 0.60gms/dl in the alternate group. The patients in the group with Haemoglobin between 11.1 to 13gms/dl and more than 13gms/dl did not show an increase, but showed a slight fall in Haemoglobin level from baseline.

There were two observations different in our study when compared to many studies. The first observation was that post treatment Haemoglobin did not show an increase from baseline as many studies have shown. However Adaji [18] in his Nigerian study between once and twice daily dosing has shown a fall in baseline Haemoglobin from 11.1gms/dl and 11gms/dl to 10.2gms/dl and 10.5gms/dl respectively in both arms. Similar fall in Haemoglobin has been noted by Okwara [19] who showed a fall from 11.2gms/dl in the first trimester to 10.4 gms/dl in the third trimester. In our study too, in the daily group the fall in Haemoglobin level was 0.2gms/dl and was 0 .6gms/dl in the alternate day group which was not statistically significant.

The second observation was that Haemoglobin improvement was better in women with anaemia in both groups. Although the final Haemoglobin was not very different in both groups, daily iron proved to be slightly better than alternate iron. The maximum oral dose per day is only 160mgs/day. Hence doubling the dose to twice daily is not beneficial, anaemic women would do well by just once daily dosing

Intake of iron is generally associated with untoward side effects like vomiting, constipation, dark stools, diarrhoea and a metallic taste in the tongue. A few rare myths discovered during the study was the belief that babies might be darker or be heavier which lead to women refraining from taking the medications. Side effects were less when given on alternate days, making women more compliant. According to a study by Khalafalloh [20] the side effects of oral iron include gastro intestinal disturbances characterised by colicky pain, vomiting, diarrhoea and constipation which occurred in about 50% of patients. Many patients taking more than 30mgs of iron per day have side effects causing them to discontinue the medication. In our study two women had to be given parenteral iron both belonging to the daily iron group since they experienced severe nausea and vomiting with oral iron.

Slon [21] *et al.*, Shatrugnaan [22] and Beard [23] and Souxa [24] have all corroborated the fact that increasing dose frequency also increases the side effects.

Preterm labour and low birth weight are common when Haemoglobin is less than 9gms or more than 13gms. Haemoglobin above the upper limit can give rise to decreased plasma expansion and is associated with poor pregnancy outcomes. In our study there was no preterm babies and no difference in birth weight in both groups, neither was there any growth restricted babies.

Conclusion

Intermittent dose seems convenient compared with daily dosing. There was no difference in Haemoglobin levels when administered daily or on alternate days. This regime was more cost effective and had less side effects and better compliance. Intermittent iron can also be practised for anaemic pregnant women since the absorption is better, however the improvement is better with daily iron. There is no benefit in increasing the dose to twice or thrice daily since required benefit was seen even with a daily dosing

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References

1. World Health Organization. Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity. Vitamin and Mineral Nutrition Information System. Geneva: WHO, 2011.
2. CDC CDC criteria for anemia in children and childbearing-aged women. MMWR Morbidity and mortality weekly report. 1989; 138(22):400-404.
3. World Health Organization (WHO). The prevalence of Anemia in women: A tabulation of available information. Geneva, Switerlans: WHO, 1992. WHO/MCH/MSM/92.2.
4. Ministry of Health and Family Welfare, Government of India. India National Family Health Survey (NFHS4) 2015–16. Mumbai: International Institute for Population Sciences; 2017 (http://rchiips.org/nfhs/NFHS-4Reports/India.pdf, accessed 15 January 2018).
5. Rahman MM, Abe SK, Rahman MS *et al*. Maternal anemia and risk of adverse birth and health outcomes in low- and middleincome countries: systematic review and meta-analysis. Am J Clin Nutr. 2016; 103(2):495-50458.
6. Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization; 2012 http://www.who.int/nutrition/publications/micronutrients/guidelines/daily_ifa_supp_pregnant_women/en/
7. Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011; 117(17):4425-33.
8. Anderson GJ, Frazer DM, McKie AT, Vulpe CD, Smith A. Mechanisms of haem and non-haem iron absorption: lessons from inherited disorders of iron metabolism. Biometals. 2005;18(4):339-48
9. Srigiridhar K, Nair KM, Subramanian R, Singotamu L. Oral repletion of iron induces free radical mediated alterations in the gastrointestinal tract of rat. Molecular and Cellular Biochemistry. 2001; 219(1-2):91-8
10. WHO Guideline: Intermittent iron and folic acid supplementation in non-anemic pregnant women. Geneva: World Health Organization. 2012 http://www.who.int/nutrition/publications/micronutrients/guidelines/guideline_intermittent_ifa_non_anaemic_pregnancy/en/).
11. WHO Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization; 2012 http://www.who.int/nutrition/publications/micronutrients/guidelines/daily_ifa_supp_pregnant_women/en/
12. Umesh Kapil, Ajeet Singh Bhadoria. National Iron-plus Initiative guidelines for control of iron deficiency anaemia in India, 2013. The National Medical Journal of India. 2013; 27:27-9
13. Pena-Rosas JP, De-Regil LM, Dowswell T, Viteri FE. Daily oral iron supplementation during pregnancy. Cochrane Systematic Review 2012; 12:CD004736. https://doi.org/10.1002/14651858.CD004736.pub4
14. Pena-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. Cochrane Database of Systematic Reviews 2015, 7. [DOI: 10.1002/14651858.CD004736.pub5
15. Asima Mukhopadhyay, Neerja Bhatla, Alka Kriplani, Ravindra M Pandey, Renu Saxena. Daily versus intermittent iron supplementation in pregnant women: Hematological and pregnancy outcome. J Obstet gynaecol Res. 2004; 30(6):409-417. https://doi.org/10.1111/j.1447-0756.2004.00223.x
16. Indra Malik R, Goonewardene, Diluk I, Senadheera. Randomized control trial comparing effectiveness of weekly versus daily antenatal oral iron supplementation in preventing anemia during pregnancy. J Obstet gynaecol Res. 2018; 44(3):417-424. https://doi.org/10.1111/jog.13546
17. Zinatossadat Bouzari, Zahra Basirat, Mahtab Zeinal Zadeh *et al*. Daily versus intermittent iron supplementation in pregnant women. BMC Res Notes 2011; 4:444 https://doi.org/10.1186/1756-0500-4-444
18. Adaji JA, Isah AY, Agida ET, Otu T, HI Abdullahi. Daily versus twice daily dose of ferrous sulphate supplementation in pregnant women: A randomized clinical trial. Niger J clin pract. 2019; 22:1132-9.
19. Okwara JE, Nnabuo LC, Nwosu DC, Ahaneku JE, Anolue F, Okwara NA *et al*. Iron Status of Some Pregnant Women in Orlu Town--Eastern Nigeria. Niger J Med. 2013; 22(1):15-8.
20. Alhossain A Khalafallah, Amanda Dennis E. Iron Deficiency Anaemia in Pregnancy and Postpartum:

Pathophysiology and Effect of Oral versus Intravenous Iron Therapy. J Pregnancy. 2012; 2012:630519. doi: 10.1155/2012/630519. Epub 2012 Jun 26.
21. Sloan NL, Jordan E, Winikoff B. Effects of iron supplementation on maternal hematologic status in pregnancy. Am J Public Health. 2002; 92:288-93.
22. Shatrugna V, Raman L, Kailash U, Balakrishna N, Rao KV. Effect of dose and formulation on iron tolerance in pregnancy. Natl Med J India. 1999; 12:18-20.
23. Beard JL. Effectiveness and strategies of iron supplementation during pregnancy. Am J Clin Nutr. 2000; 71(5):1288S-94S.
24. Souza AI, Batista Filho M, Bresani CC, Ferreira LO, Figueiroa JN. Adherence and side effects of three ferrous sulfate treatment regimens on anemic pregnant women in clinical trials. Cad Saude Publica. 2009; 25:1225-33.

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[illegible]r[illegible]d It is essential to intervene pregnancy for safety of mother, fetus or both. Successful labor induction is clearly related to the state of the cervix. Women with an unfavorable cervix who have not experienced cervical ripening phase before labor present the greatest challenge with regard to labor induction. Therefore, it is necessary to use optimal technique for cervical ripening and safe confinement. One of the common methods includes use of PGE2 gel for cervical ripening. The rationale of this study is to compare the safety and efficacy of Iso-sorbide mononitrate as pre-induction cervical ripening with PGE2 gel induction.

M[illegible]d After attaining ethics approval [PSG IHEC], a prospective randomised, case-controlled study was conducted on 182 women undergoing elective induction of labour. They were allocated to either Study or Control group by computer generated random number table method. ISMN was used vaginally prior to labour induction in the study group whereas PGE2 gel induction was used in the control group. Change in bishop score and Induction to delivery interval was assessed in both the groups.

[illegible]There was a significant reduction in induction to delivery interval in the study group (ISMN) 15.2 hours when compared to 23.2 hours in the control group (PGE2 gel) with p=0.000. Need for augmentation of labour was reduced in the study group significantly with p=0.003. Cost of induction was less when compared to the control group. ISMN had less side effects hence can be used as OP basis.

[illegible]Vaginal Isosorbide mononitrate when used as a cervical ripening agent significantly reduces induction to delivery interval. Use of Isosorbide mononitrate was associated with very less side effects and it is a cost-effective drug. Thus, ISMN can be used safely and effectively in term patients for pre-induction cervical ripening when compared to PGE2 gel.

[illegible]rd Cervi prime gel, safety, Induction delivery interval, Induction of labour, Iso-sorbide mononitrate

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Induction of labor is indicated when the risks to either the mother or the fetus of continuing the pregnancy outweigh the benefits. The conditions which commonly necessitate induction of labor include preeclampsia, postdatism, prelabor rupture of membranes, maternal medical disorders, fetal growth restriction and intrauterine fetal demise (Hibbard et al). The success of labor induction largely depends on the `favorability' of cervix.[1] Various methods like insertion of extra amniotic self-retaining catheter, hygroscopic cervical dilators and prostaglandins have been tried in an effort to ripen the cervix prior to induction (Hibbard, Vengalil, Owen).

Prostaglandin E2 or dinoprostone administered intracervically, has been shown to improve bishop score and induction to delivery times in comparison to untreated controls (Owen). However, use of prostaglandins is associated with complications like uterine tachysystole and rupture of uterus and fetal cardiac rhythm abnormalities (Keller, Vaisanen).[2] Besides, more caution is advocated when these agents are used in women with a scarred uterus.[3]

Nitric oxide (NO) has been recently postulated to have a regulatory role in the myometrium and cervix during pregnancy and parturition, and an endogenous NO system is documented in the human cervix.[4] The present study was planned to evaluate the effects of NO donor isosorbide mononitrate (ISMN) on cervical ripening at term, to compare its efficacy and safety with that of dinoprostone gel and to evaluate the maternal and fetal outcome after use of these agents for cervical priming.

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This prospective study was carried out on 182women with singleton live pregnancy (between 37 to 42 weeks of gestation) and an unfavorable cervix (modified Bishop score of 0-6), posted for induction of labour during the period between June 2014 to May 2015, in the Department of Obstetrics and Gynaecology at PSG, Coimbatore. Women with malpresentations, multiple pregnancy and those with history of epilepsy, hypertension or bronchial asthma were excluded from the study.

After a thorough history, general physical and obstetric examination (including assessment of Bishop Score) all women underwent admission NST to assess the fetal condition. Randomization was done, Women of group I were administered 40 mg ISMN vaginally 2 doses 12 hours apart while those of group II received dinoprostone gel (0.5 mg in 3 gm base) intracervically which was repeated after 6 hours, if the bishop score remained below 6. Bishop score was reassessed after 24 and 6 hours in group I and II respectively. EFM was used in labour.

All women were monitored for pulse rate, blood pressure, fetal heart rate, uterine contractions, any adverse effects and maternal and neonatal outcome.

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The majority of women (39 in group I and 44 in group II) in the study were in the age group of 20-24 years. A total of 63 (78%) women in group I and 43 (57%) in group II were between 40-42 weeks of gestation, the remaining being at 37 weeks. Indications of induction in the study were postdated fetal growth restriction, decreased fetal movements, oligohydramnios or maternal medical disorders. Table 1 shows the change in bishop score in the study. The change in bishop score after 6 hours of instillation of the ripening agent in both groups was not significant. In group II, 80% (24 out of 30) women with an initial score of 0-3 and 20% (4 out of 20) of those with initial score of 4-5 needed a repeat dose of dinoprostone.

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Pre-score	1.84±0.87	2.16±1.1	0.151
Post score	4.1±1.91	3.6±2.1	0.134
P value	0.08	0.144	

In group I- as ISMN was used as a pre-induction cervical ripening agent, induction of labour was done with PGE2 gel after 2 doses of ISMN. In group II- PGE2 gel not only ripens the cervix but also initiates labour, hence maximum of 3 doses PGE2 gel were instilled 6 hrs apart depending upon the bishop score. Further augmentation of labour was done with misoprostol, ARM and oxytocin as indicated. Induction to delivery interval was calculated from time of first PGE2 gel dose to delivery in both the groups. 24 hours of ISMN was excluded from group I as it was used only as a pre-induction ripening agent.

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<12 hours	36	39	10	11.1
12-24 hours	44	47.8	38	42.2
24-36 hours	9	9.7	35	38.8
36-48 hours	3	3.2	7	7.7
>48 hours	-	-	-	-
Mean±SD	15.2±9.08		23.2±9.14	
Inference	The difference between both is statistically significant			

The mean duration of induction to delivery is more with PGE2 gel 23.2 hours and mean duration in ISMN group was 15.2 hrs. In PGE2 gel group 11% had induction delivery interval <12 hrs whereas 39% had less than 12 hours in ISMN group. This was statistically significant. Similarly, duration of labour from 36-48 hrs was 7.7% in PGE2 gel and 3.2% in ISMN group this was not statistically significant. Overall, In ISMN group 86.8% had delivered within 24hrs whereas in PGE2 gel group only 53.3% delivered within 24hrs.

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Nil	30	32.6	14	15.5
Required	62	67.3	76	87.4
Total	92		90	
Inference	Cases which required acceleration were significantly reduced in ISMN group p=0.003			

None of the patients went into labour after administration of ISMN, as it had no action on myometrium. In PGE2 gel group apart from ripening of cervix patients also had onset of uterine contractions even with a single dose. In group I, requirement of drugs such as misoprostol, PGE2 gel and oxytocin for acceleration is less (67%) when compared to group II (87.4%).32.6% of the patients did not require acceleration of labor in the study group when compared to 15.5% of the patients in PGE2 gel group did not require acceleration. Patients with spontaneous rupture of membranes were augmented with oxytocin and others had ARM and oxytocin.

[illegible]

[illegible]	[illegible]		[illegible]	
	[illegible]	[illegible]	[illegible]	[illegible]
<200	28	30.4	-	0
200-500	57	61.9	22	24.9
>500	7	7.6	68	75.5
Mean±SD	285.3±123.9		463.3±256.8	
Inference	There was a significant reduction in cost of induction when ISMN was used as number of PGE2 gel used was less.			

The mean cost of ISMN used is Rs. 12, each ISMN 20mg tablet cost Rs. 3 each PGE2 gel cost Rs. 375. Average cost of induction in PGE2 gel was Rs. 463, and this was reduced by 50% when ISMN was used prior to induction as number of PGE2 gel used was significantly reduced.

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NO is a common product of arginine metabolism in many tissues. It appears to be an important paracrine vasodilator and may be involved in cell death and nerve transfer.[5] NO is also released from several vasodilator drug molecules. It is released from important medications such as nitroprusside, nitrates, and nitrites.[6] This substance is a potent vasodilator in all vascular substrates and a powerful relaxant in most smooth muscle cells. Its performance mechanism in this case is activation of guanylyl cyclase and production of cyclic guanosine monophosphate (cGMP) which causes dephosphorylation and disables the light chains of myosin and causes relaxation of smooth muscle. It also has a physiological role in erectile tissue function.[7]

As mentioned earlier, several studies have tried to show the effect of NO on cervical pre-induction ripening.[8,9] Findings of previous studies indicated that delivery length from the onset of intervention in the misoprostol group was shorter and the need for induction in this group was lower.[10,11]

The results were comparable to other studies like Bollapragada et al who concluded that clinical utility of IMN as a pre-induction cervical ripening agent was minimal.[12] Though in their study, IMN had a significant effect on the change in cervical Bishop score (mean difference of 0.65, p=0.013), they could not demonstrate a significant change in their primary outcome measure which was admission to delivery interval (mean difference-1.03 hours, p=0.68).

Bishop score appeared to be an important predictor for induction to delivery interval in the study. Of the women with an initial score of 0-3, only 3.71% (1 patient) from group I and 16.67% (5 patients) from group I delivered vaginally within 12 hours. While 66.67% (18 patients) of women with an initial bishop score of 0-3 delivered between 12-24 hours in group I, only 40% (12 women) did so in group II. Of the women with bishop score of 4-5, 34.78% (8 women) women of group I and 40% (8 women) of group II delivered in the first 12 hours and another 47.83% (11 women) and 30% (6 patients) woman delivered between 12-24 hours in group I and II, respectively. The mean induction to delivery interval was 15.2±9.08 in group I and 23.2±9.14 hours in group II (p=0.00). Oxytocin was required in (38 of group I and 33 of group II) women in the study.

The mean difference in cervical scores at 24 hours was 0.01 with a p = 0.983 and there was no significant change in drug insertion to delivery time. Osmanet al also concluded in their study that isosorbide mononitrate was less effective as a cervical ripening agent, though they used prostaglandins (PGE2) as the comparator. In contrast, Agarwal et al attested to the effectiveness of IMN as cervical ripening agent by showing a significant change in Bishop scores after 2 doses of 40 mg IMN (3.17±2.02; p < 0.001) and a reduction in admission to delivery interval (p < 0.001).[13,14]

There was no case of hyper stimulation in the IMN group in the study done by Habib et al, but a higher incidence of tachysystole (1.96%) was observed in the IMN group.[15] Most of the other studies have reported headache as the most common side effect of IMN, although it was treatable with simple analgesics like paracetamol. Maternal hemodynamics, like blood pressure or pulse rate was also not affected. In a study conducted by Ekerhovd et al, significant changes were recorded in maternal hemodynamics which however did not prove to be of any clinical importance (at 180mins, change in maternal PR, p < 0.01, SBP, p < 0.001, DBP, p < 0.0001).[16]

Fetal distress formed the indication of CS for 8 (out of 36) patients of group II and 7 (out of 27) patients of group I (p<0.05). 4 patients of group I and 6 of group II underwent abdominal delivery for failed induction. Bollapragada et al did not report any differences in the number of women going into labour within the first 24 hrs (9% in IMN; 4% in placebo, p = 0.09).[12] However, in the study conducted by Bullarbo et al more women went into labour within 24 hours in the IMN group than in the placebo group.[16]

Most of the studies such as those conducted by Agarwal et al and Habib et al have shown lower incidence of

caesarean deliveries in IMN group albeit statistically insignificant.[14,15] Though the number of caesarean deliveries were more in IMN group than placebo in present study, it was not statistically significant. Most of these were done for fetal distress following PGE2 and failed induction of labour after 2 doses of PGE2 and maximal doses of oxytocin infusion.

Headache was the most common adverse effect observed in group I 31 women (33%). 9 women of group II had uterine hyperstimulation, in comparison to none from group I. Two women of group I had atonic postpartum hemorrhage (PPH). One woman of group I had cervical tears. NICU admissions and APGAR scores were not significantly different in both the groups. There was a significant reduction in cost of induction when ISMN was used as number of PGE2 gel used was less.

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Induction of labour is a challenge to all obstetricians. Various drugs were used for labour induction, among which PGE2 gel was most widely accepted. ISMN has been studied as a pre-induction cervical ripening agent in recent times.

Advantage in this group was that cervix became soft and responded well to induction with other drugs. Side effect was only headache which subsided with analgesics. There was no evident change in bishop score following ISMN induction. Whereas induction to delivery interval in this group was significantly reduced. There was no case of hyper stimulation recorded in any of the studies as it does not cause myometrial contractions.

It is a cost-effective method of cervical ripening does not require monitoring during the period of ISMN induction. Hence patients do not require admission. It can be safely used at home.

Hereby authors conclude that ISMN is an effective drug when used as a pre-induction cervical ripening agent.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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1. Tschugguel W, Schneeberger C, Lass H, Stonek F, Zaghlula MB, Czerwenka K, et al. Human cervical ripening is associated with an increase in cervical inducible nitric oxide synthase expression. Biol Reprod. 1999;60(6):1367-72.
2. Keirse MJ, de Koning Gans HJ. Randomized comparison of the effects of endocervical and vaginal prostaglandin E2 gel in women with various degrees of cervical ripeness. Am J Obstet Gynecol. 1995;173(6):1859-64.
3. Ledingham MA, Thomson AJ, Young A, Macara LM, Greer IA, Norman JE. Changes in the expression of nitric oxide synthase in the human uterine cervix during pregnancy and parturition. Mol Human Reprod. 2000;6(11):1041-8.
4. Chwalisz K, Shao-Qing S, Garfield RE, Beier HM. Cervical ripening in guinea-pigs after a local application of nitric oxide. Human Reprod (Oxford, England). 1997;12(10):2093-101.
5. Väisänen-Tommiska M, Nuutila M, Aittomäki K, Hiilesmaa V, Ylikorkala O. Nitric oxide metabolites in cervical fluid during pregnancy: further evidence for the role of cervical nitric oxide in cervical ripening. Am J Obstet Gynecol. 2003;188(3):779-85.
6. Chanrachakul B, Herabutya Y, Punyavachira P. Potential efficacy of nitric oxide for cervical ripening in pregnancy at term. Int J Gynecol Obstet. 2000;71(3):217-9.
7. Yazdizadeh H, Abedi P, Najar S, Angali KA. The impact of isosorbide mononitrate on cervical ripening and labor induction in primiparous women with term pregnancy: A double-blind, randomized, controlled trial. Iranian J Nursing Midwifery Res. 2013;18(3):246-50.
8. Kelly AJ, Munson C, Minden L. Nitric oxide donors for cervical ripening and induction of labour. Cochrane Database Systematic Reviews. 2011(6).
9. Krishnamurthy R, Pallavee P, Ghose S. Evaluation of Isosorbide Mononitrate for Preinduction of Cervical Ripening: A Randomized Placebo-Controlled Trial. J Fam Reprod Health. 2015;9(2):75-81.
10. Radulovic N, Norström A, Ekerhovd E. Outpatient cervical ripening before first-trimester surgical abortion: a comparison between misoprostol and isosorbide mononitrate. Acta Obstetricia et Gynecologica Scandinavica. 2007;86(3):344-8.
11. Sharma Y, Kumar S, Mittal S, Misra R, Dadhwal V. Evaluation of glyceryl trinitrate, misoprostol, and prostaglandin E2 gel for preinduction cervical ripening in term pregnancy. J Obstet Gynaecol Res. 2005;31(3):210-5.
12. Bollapragada SS, MacKenzie F, Norrie JD, Eddama O, Petrou S, Reid M, et al. Randomised placebo-controlled trial of outpatient (at home) cervical ripening with isosorbide mononitrate (IMN) prior to induction of labour–clinical trial with analyses of efficacy and acceptability. The IMOP Study. BJOG: Int J Obstet Gynaecol. 2009;116(9):1185-95.
13. Osman I, MacKenzie F, Norrie J, Murray HM, Greer IA, Norman JE. The "PRIM" study: a randomized comparison of prostaglandin E2 gel with the nitric oxide donor isosorbide mononitrate for cervical ripening before the induction of labor at term. Am J Obstet Gynecol. 2006;194(4):1012-21.
14. Agarwal K, Batra A, Batra A, Dabral A, Aggarwal A. Evaluation of isosorbide mononitrate for cervical ripening prior to induction of labor for postdated pregnancy in an outpatient setting. Int J Gynecol Obstet. 2012;118(3):205-9.

15. Habib SM, Emam SS, Saber AS. Outpatient cervical ripening with nitric oxide donor isosorbide mononitrate prior to induction of labor. Int J Gynecol Obstet. 2008;101(1):57-61.
16. Bullarbo M, Orrskog ME, Andersch B, Granström L, Norström A, Ekerhovd E, et al. Outpatient vaginal administration of the nitric oxide donor isosorbide mononitrate for cervical ripening and labor induction postterm: a randomized controlled study. Am J Obstet Gynecol. 2007;196(1):50e1-5.

Malathi TM, Kanchanamalai K. A comparative study of safety and efficacy of vaginal isosorbide mononitrate (40mg) with dinoprostone gel (0.5mg). Int J Reprod Contracept Obstet Gynecol 2018;7:4197-201.

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CASE REPORT

Supraventricular tachycardia in one of the twins: The ethical dilemmas involved in treatment

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ABSTRACT

The pediatric cardiologist now has an important role to play in fetal medicine. They are often called upon to manage fetal cardiac problems such as arrhythmias or perform fetal cardiac interventions such as balloon valvuloplasty or atrial septostomy. In these scenarios, it becomes very important for the pediatric cardiologist to understand the concepts of "fetus as a patient," "viability," etc., and their implications in management. We try to shed light on these principles through our case scenario of managing supraventricular tachycardia in one of the fetuses of a twin pregnancy.

Keywords: Beneficence, fetal arrhythmia, fetus as a patient

INTRODUCTION

Fetal arrhythmias are detected in 2% of unsuspected pregnancies during routine obstetric ultrasound. Majority of them are benign. However, serious arrhythmias such as supraventricular tachycardia (SVT) and atrial flutter are common and can lead to fetal hydrops, premature delivery, and death if not aggressively treated.[1] SVT in one of the fetuses of a multiple gestation is uncommon.[2-4] In the treatment of fetal arrhythmia, there is no consensus on the regimen of drugs, and the protocol varies from institution to institution.[1,5] We describe such an unusual case and the ethical dilemmas involved.

CASE REPORT

A 23-year-old primigravida with 25 weeks' gestation presented to us with a dichorionic-diamniotic twin pregnancy. She conceived by *in vitro* fertilization resulting in a triplet pregnancy. Triplet reduction was done at 16 weeks' gestation. Her obstetric ultrasound showed twin 1 having intermittent atrial ectopics and persistent SVT [Figure 1a and b]. Twin 2 was normal. After obtaining informed consent, the mother was loaded with oral digoxin (1 mg/24 h) followed by 500 μg/day in two divided doses. After 36 h, twin 1 developed pericardial effusion [Figure 1c] and cardiomegaly. In view of impending fetal hydrops, oral sotalol was started at 160 mg/day in two divided doses. It was increased to 240 mg/day in three divided doses due to persistent SVT. Twin 1 reverted to sinus rhythm on day 4 of admission [Figure 1d] and twin 2 was healthy. The patient was discharged on day 7 of admission.

On follow-up after 2 weeks, both twins and mother were healthy, and pericardial effusion resolved in twin 1. Digoxin was stopped and the dosage of sotalol was tapered gradually to 80 mg/day and continued till delivery. She was delivered by elective cesarean section at 36 weeks' gestation, twin 1 and 2 weighing 2.16 kg and 2.1 kg, respectively. At present, they are 3 months of age with no recurrence of arrhythmia.

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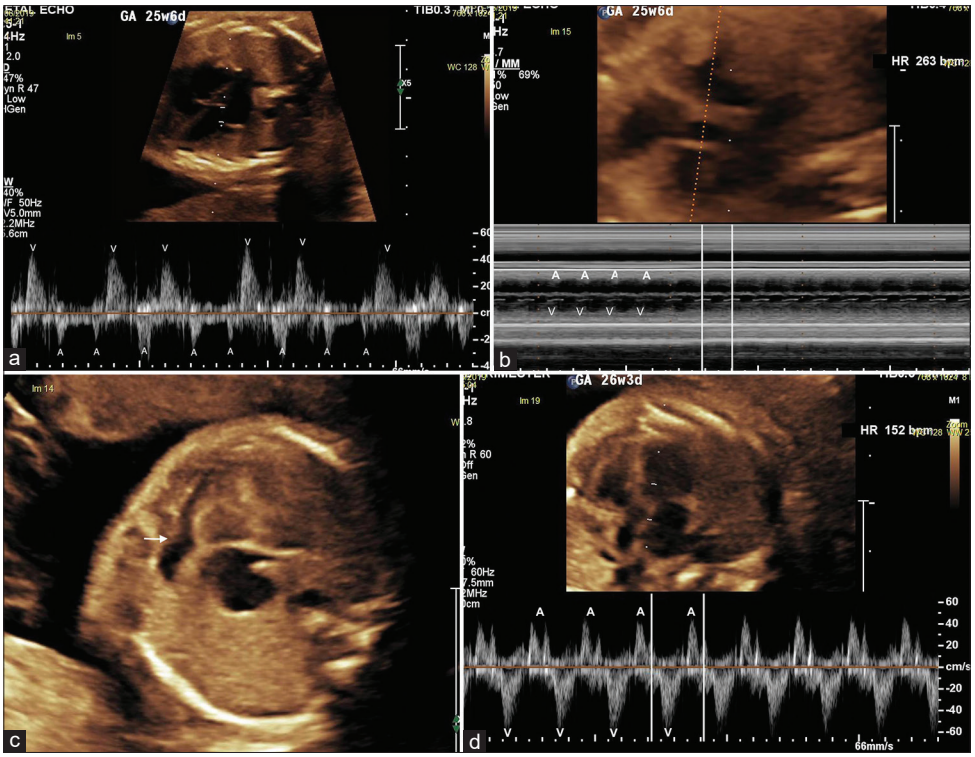


Figure 1: (a) Pulsed-wave Doppler of left atrial inflow and outflow tracts showing multiple nonconducted atrial ectopic beats. A: Atrial wave, V: Ventricular wave, (b) M mode tracing showing supraventricular tachycardia with 1:1 conduction and short ventriculoatrial interval. Heart rate is 263 beats/min. A: Atrial contraction, V: ventricular contraction, (c) two-dimensional fetal echocardiography showing pericardial effusion. White arrow – Pericardial effusion. (d) Pulsed-wave Doppler of left atrial inflow and outflow tracts showing sinus rhythm. Heart rate is 152 beats/min. A: Atrial wave, V: Ventricular wave

DISCUSSION

The concept of "fetus as a patient" was mainly the work of McCullough and Chervenak who opposed the word "unborn child." "Unborn child" was used by opponents of abortion to confer independent moral status to the fetus, meaning that parents and practitioners had a moral obligation to protect and promote the interests of the fetuses above everything.[6] The authors, however, emphasized more on the dependent moral status where the fetus could not be seen as a separate entity.[6] Hence, the physician had beneficence-based obligations to the mother and fetus which had to be balanced against autonomy-based obligations to the mother. The fetus, of course, being neurologically immature, there was no question of fetal autonomy.[7,8]

"Beneficence" simply means that clinical benefit should outweigh harm, based on sound evidence-based treatment. It would be common for the physician's recommendations to sometimes go against the mother's autonomy.[7,8]

In our situation, we had three patients and three possible treatment options. They included giving transplacental antiarrhythmics, no treatment, or urgent delivery. Our team opted for therapy. Here, we could justify our beneficence-based obligations to twin 1, but twin 2 and mother were exposed to side effects of the drugs with unknown consequences and harm.

The mother initially opted for no treatment. Her reasoning being, she could at least save twin 2. This resulted in conflict with our recommendations. Due to the scarcity of similar case reports, lack of treatment guidelines,[2-4] and unpredictable side effects, sound evidence-based recommendations were difficult. Based on the limited literature available, our experience, and constant counseling, the mother eventually made an informed decision to treat. If conflict still persisted, would legal methods have to be sought? If the court ruled in our favor, wouldn't the decision be in conflict with the mother's autonomy?

The third option involved urgent delivery. Here lies the importance of the concept of "viability." A fetus is said to be viable if it is of sufficient maturity to survive in the neonatal period and attain independent moral status given the available technological and professional support.[7,8] In high-income countries, this is generally between 24 and 25 weeks.[9] In India, it has been fixed administratively at 28 weeks,[10] but there is a wide interregional variation of technological support available. Hence, the issue of viability becomes

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many times institution based.[11] If urgent delivery was resorted to, both the twins of questionable viability would be exposed to the problems of extreme prematurity.

The role of pediatric cardiologists is expanding in the field of fetal cardiac interventions (FCIs). The FCI of early years was primarily for pharmacological therapy of arrhythmias, heart block, and heart failure. Then came the era of direct FCI with the advent of valvuloplasty and balloon atrial septostomy.[12] All the above interventions were aimed toward improving fetal cardiac function, survival, and/or achieving a biventricular circulation to improve postnatal outcome. It is difficult to put forth guidelines in the above scenarios due to inability to conduct randomized control trials, uncertainty of the natural history of the disease, risk/benefit ratio of the intervention to both the mother and fetuses, etc. Especially in hypoplastic hearts, many fall in the gray zone between univentricular and biventricular circulations.[12] Decision-making becomes even more difficult when you have a multiple gestation where the fetuses are discordant for the problem, as the nonaffected fetus in addition to the mother becomes an innocent bystander.

The management of such patients should always involve a multidisciplinary team. When it comes to counseling for/against an FCI, the team should understand the concepts of beneficence, autonomy, viability, and their ethical implications. In any event, should there arise a conflict between the mother and healthcare giver, it should be resolved through constant knowledge sharing and counseling, respecting the mother's autonomy and beliefs.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bravo-Valenzuela NJ, Rocha LA, Machado Nardozza LM, Araujo Júnior E. Fetal cardiac arrhythmias: Current evidence. Ann Pediatr Cardiol 2018;11:148-63.
2. Czeszyńska MB, Wegrzynowski J, Czajkowski Z, Dawid G. Fetal and neonatal arrhythmia in one of the twins – A case history. Acta Genet Med Gemellol (Roma) 1998;47:197-200.
3. Tanawattanacharoen S, Uerpairojkit B, Prechawat S, Manotaya S, Charoenvidhya D. Intrauterine therapy for fetal supraventricular tachycardia in a twin pregnancy. J Obstet Gynaecol Res 2005;31:94-7.
4. Jones LM, Garmel SH. Successful digoxin therapy of fetal supraventricular tachycardia in a triplet pregnancy. Obstet Gynecol 2001;98:921-3.
5. Zoeller BB. Treatment of fetal supraventricular tachycardia. Curr Treat Options Cardiovasc Med 2017;19:7.
6. McCullough LB, Chervenak FA. A critical analysis of the concept and discourse of 'unborn child'. Am J Bioeth 2008;8:34-9.
7. Kurjak A, Carrera JM, Mccullough LB, Chervenak FA. The ethical concept of the fetus as a patient and the beginning of human life. Period Biol 2009;111:341-8.
8. Chervenak FA, McCullough LB. Ethical dimensions of the fetus as a patient. Best Pract Res Clin Obstet Gynaecol 2017;43:2-9.
9. American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine. Obstetric care consensus No. 6: Periviable Birth. Obstet Gynecol 2017;130:e187-99.
10. Park K, editor. Demography and family planning. Park's Textbook of Preventive and Social Medicine. 25th ed. Jabalpur, Pune: M/s Banarsidas Bhanot; 2019. p. 530-71.
11. Nimbalkar SM, Bansal SC. Periviable birth – The ethical conundrum. Indian Pediatr 2019;56:13-7.
12. Yuan SM. Fetal cardiac interventions. Pediatr Neonatol 2015;56:81-7.

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Maternal and perinatal outcomes of twin pregnancies conceived through assisted reproductive technology: a retrospective cohort study

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□□□r□d□□□□□□□Selective fetoscopic laser photocoagulation (SFLP) for twin-to-twin transfusion syndrome (TTTS) is challenging for new surgeons at the start of their learning curve. We described an approach utilising telementoring and team-based training to facilitate rapid attainment of the skills required for safe and efficient practice with a limited caseload.□

M□□□□d□□□We conducted a prospective observational study of SFLP performed by the novice primary surgical team in three stages: under direct on-site supervision from an expert mentor (Group 1), with remote tele-guidance from that mentor (Group 2) and independently (Group 3), at an academic tertiary hospital in Singapore. The primary team undertook regular training on high-fidelity tissue models to accelerate skills acquisition and complement the surgical performance. □

□□□□□□□□Nine patients diagnosed with Stage 2 TTTS were assessed for procedural characteristics, surgical outcomes and perinatal survival following SFLP. There were no significant differences in operative duration, anastomoses ablated, gestational age or birth weight at delivery. The complications observed were: recurrent TTTS (22.2% of pregnancies), twin anaemia polycythaemia sequence (33.3%), preterm prelabour membrane rupture (22.2%) and delivery at < 32 weeks (44.4%). At least one twin was live-born in 88.9% of cases, while postnatal survival to six months of at least one twin occurred in 77.8%.

□□□□□□□□□□Systematic mentoring and specialised skills training are useful in aiding new surgeons to negotiate the steep learning curve and achieve good outcomes at the start of a new practice, particularly in the setting of low patient numbers. This is best paired with dedicated model training to achieve and maintain surgical dexterity for this complex procedure.

Keywords: fetoscopic laser photocoagulation, fetoscopy training, learning curve, surgical mentoring, twin-twin transfusion syndrome

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Monochorionic twinning affects 30% of multiple pregnancies, while twin-to-twin transfusion syndrome (TTTS) occurs in 10%–15% of monochorionic diamniotic (MCDA) pregnancies with an untreated mortality of 70%–100%.[1,2] TTTS results in unilateral blood transfusion from the donor to the recipient twin, mostly via arteriovenous anastomoses (AVAs), causing oligohydramnios from the hypovolaemic and oliguric donor, and polyhydramnios from the hypervolaemic recipient. This can progress eventually to cardiac failure and the eventual death of one or both twins.[2] Of the various treatments offered for TTTS, only selective fetoscopic laser photocoagulation (SFLP) of causative AVAs at the inter-twin vascular equator is potentially curative and permits the rescue of both twins (compared to selective reductive techniques which cause the demise of one twin), and in experienced centres, is associated with 75% survival of both twins.[3,4] Twin anaemia polycythaemia sequence (TAPS), caused by slow blood transfusion through a few small-calibre (< 1 mm) placental AVAs, may complicate SFLP and result in anaemia and polycythaemia of the donor and the recipient twins, respectively.[5] SFLP has been available in Singapore at two tertiary maternity units since 2011 and 2015; both have published perinatal outcomes from their initial experiences that were comparable to international reports.[6,7] Prior to this, patients with affected pregnancies were offered palliative therapies or referral to a regional fetal therapy centre for SFLP, options that both delay the institution of definitive treatment and substantially inflate expenses. The initiation of this advanced fetal therapy service at two Singapore hospitals has ensured that SFLP is accessible to local and foreign affected patients for whom it is the best therapeutic option.

SFLP is a complex procedure that has been associated with a steep learning curve and significant maternal and fetal morbidity.[8-10] As with the majority of complex surgeries, the

acquisition of skills in SFLP usually follows the traditional path of surgical apprenticeship vis-a-vis close observation followed by progressive hands-on performance under direct supervision.[11,12] This classic training model is less time- and cost-efficient for new fetal therapy centres, and may limit patient access to SFLP.[13] We have experienced similar challenges as other low-volume fetal therapy centres in terms of negotiating the long surgical learning curve while achieving procedural aptitude, maintaining competent and safe practice with a limited number of patients (not meeting the suggested minimum of 25 annual procedures)[10] and providing rapid access to SFLP to an underserved population.[7,13] This is a common experience, given that 48% of fetal laser surgery centres, mostly situated in Asia, Australia and South America, perform under 20 procedures annually.[14]

To overcome these limitations, a 'collaborative surgical services' model has been proposed, in which personnel and resources are pooled and shared to improve timely access to surgery and quality care in low-volume centres.[13] New models of surgical training are designed to emulate the benefits of traditional surgical apprenticeship, especially the skills-focused training supervised by a surgeon-mentor, while minimising the cost of specialist surgeons spending time away from their primary clinical practice, including expenditures incurred in temporary relocation and lost manpower. These models utilise teleconferencing advances to bring the expert to the surgical team across geographical barriers, employing the mentoring process in the primary clinical setting to assist the surgical team in adopting new procedures successfully.[15-18] Continued off-site mentoring after the initial training phase, combined with skills practice on high-fidelity models and competence-based assessments, can be an effective strategy to overcome the aforementioned situational limitations and ensure that procedural competence is maintained beyond the period of formal training.[19-22]

We have previously described our team-based approach to implementing SFLP, which relied on model practice and stepwise skills learning with an on-site ('hands-off') proctor for the first three cases performed at our institution.[7] We herein discuss a natural extension of this arrangement to include continued off-site mentoring for the second phase of cases, leading to independent surgery without a stand-by mentor, updating the 'see one, do one, teach one' approach.[23-25] We compared the outcomes and complications of our first nine cases to determine the utility of this collaborative approach as an alternative to the lengthy and costly surgical apprenticeship model.

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Patients diagnosed with MCDA twin pregnancies at booking visits were monitored fortnightly from 16 weeks' gestation for TTTS, which was diagnosed and staged using the Quintero criteria (Fig. 1).[1,26-29] All patients were offered combined first trimester screening at 11.0–13.9 weeks or non-invasive prenatal testing according to clinical protocol, followed by early anatomic survey at 18 weeks. Inter-twin discordance in nuchal translucency of > 20%, crown-rump length > 10%, nuchal translucency > 95th centile, or cardiac dysfunction in one twin were taken as early indicators of TTTS based on meta-analyses of MCDA biomarkers.[30,31] Patients with Stage 1 TTTS were monitored at least twice weekly for clinical progression, including cardiac failure in the recipient twin,[29] and were offered SFLP based on the following criteria: (a) at least Stage 2 TTTS; (b) gestational age < 26 weeks; and (c) the absence of perinatally lethal structural or genetic anomalies in either fetus. Patients were extensively counselled regarding the diagnosis, alternative therapeutic options and potential complications of SFLP.[3] The multidisciplinary team consisted of fetal therapists, maternal-fetal medicine specialists, obstetric anaesthetists, nurse specialists and

administrative support staff, who had prepared for this clinical service together with workflow drills and training on high-fidelity models.[7,19] Patients were informed upfront about the relative inexperience of the surgical team and the on-site or telepresence of the overseas-based surgeon-mentors for support and supervision. All patients provided written informed consent for the SFLP, video recording, photography and placental dye studies, and agreed to the mentoring arrangement. Preprocedure planning involved placental and vascular equator mapping to determine the point of fetoscope entry.[7,19] Surgery was scheduled as soon as the mentor could travel to our centre; when this was not possible, real-time telementoring was arranged via multiple-user internet teleconferencing applications Skype (Skype Technologies SARL, Palo Alto, CA, USA) and WhatsApp (WhatsApp Inc, Mountain View, CA, USA) over fourth-generation (4G) broadband cellular networks, as the operating room was not equipped for formal videoconferencing. Fetoscopic images were captured directly from the screen and transmitted to the mentor, who was able to follow the entire surgery and advise on the proceedings. Patients diagnosed with TTTS who underwent SFLP were assigned into Groups 1–3 chronologically. The first cases were performed with a mentor on-site (Group 1) and the last cases were performed by the primary surgical team independently (Group 3). Cases 5 and 6 (Group 2) were performed by the team with the mentor observing remotely (i.e. telementoring) to assist with technical difficulties.

All procedures were performed under general anaesthesia in anticipation of a potentially lengthy surgery. Patient position and choice of fetoscope (curved or straight; Karl Storz Endoskope Berlin GmbH, Berlin, Germany) were determined by placental location, and surgical team members performed specific roles, as previously described.[7,19] Selective ablation of all identified AVAs along the vascular equator was achieved using a 400-μm or 600-μm diode laser at 15–30W (Medilas D Multibeam; Dornier MedTech Asia, Singapore), followed by ablation of residual

superficial anastomoses across the vascular equator from one placental margin to the other (Solomon technique), if technically feasible, to minimise the risk of post-SFLP TAPS.[32] Bleeding from ablated AVAs was handled using coagulation of feeder vessels and by applying transabdominal pressure to achieve a tamponade effect. Amnioreduction was performed at the end of the procedure from the recipient twin sac in all cases until the deepest vertical pocket reached ~4 cm. Cervical length and dilatation were assessed using transperineal ultrasonography (US). After the procedure, patients were observed in the ward for 48 hours, followed by repeat US before discharge. All patients were placed on a short course of prophylactic oral antibiotics and tocolytics (nifedipine and indomethacin) to maintain uterine quiescence.

Patients were followed up with weekly US monitoring for resolution of TTTS features such as re-accumulation of donor twin amniotic fluid and improved recipient twin cardiac function. Umbilical artery and middle cerebral artery Doppler studies were performed to assess placental function, developing anaemia and cardiac dysfunction. US surveillance for procedure- and monochorionicity-related complications continued once to twice weekly, particularly for persistent TTTS, TAPS and selective fetal growth restriction. Placental dye injection was performed, as previously described, to assess completeness of laser ablation.[33]

Ethical approval for collection and use of human data was granted by the National Healthcare Group Domain Specific Review Board (reference no. DSRB/2017/01128). Statistical analysis was performed using GraphPad Prism version 6.04 (GraphPad Software Inc, La Jolla, CA, USA). Data was expressed as median and range. Groups were compared using two-way analysis of variance with Tukey correction for multiple comparisons. Statistical significance was set at $p < 0.05$.

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Between 2015 and 2017, nine pregnancies that were complicated by Stage 2 TTTS were treated with SFLP (Table I). Cases 1–4 were performed under on-site supervision by the surgeon-mentor (Group 1), Cases 5 and 6 were performed under off-site supervision via real-time videoconferencing (Group 2) and Cases 7–9 were performed independently with the expert proctor available by telephone for troubleshooting (Group 3). There were no demographic differences between the groups. The median gestational age (GA) at TTTS diagnosis was 20.1 (range 19.1–20.7) weeks. SFLP was performed at a median GA of 20.7 (19.4–21.6) weeks (Table II). The median diagnosis-to-procedure time was 4.0 (2.0–7.0) days with lower intervals in Group 2 (2.0 [range 1.0–3.0] days) and Group 3 (5.0 [range 1.0–6.0] days) compared to Group 1 (7.0 [range 5.0–12.0] days).

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Group 1 had on-site mentoring, Group 2 had remote mentoring, and Group 3 performed surgery independently. Cases were numbered in chronological order of treatment. *Data presented in median (range). †Measurement available in only one case. cFTS: combined first trimester screening; EFW: estimated fetal weight; TTTS: twin-to-twin transfusion syndrome

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GA at procedure* (wk)	20.7 (19.4–21.6)	21.6 (18.6–26.3)	19.4 (18.9–20.0)	20.3 (18.6–23.1)
Time from diagnosis to procedure* (day)	4.0 (2.0–7.0)	7.0 (5.0–12.0)	2.0 (1.0–3.0)	5.0 (1.0–6.0)
Duration of procedure* (min)	96.5 (67.0–145.5)	101.0 (75.0–109.0)	145.5 (99.0–192.0)	67.0 (58.0–117.0)
No. of anastomoses ablated*	7.5 (6.0–8.0)	7.0 (6.0–10.0)	6.0 (5.0–7.0)	7.0 (6.0–11.0)
Solomon technique	55.6	1	1	3
Amnioreduction volume* (L)	1.2 (1.1–2.2)	2.2 (1.5–3.0)	1.2 (0.9–1.5)	1.1 (0.9–1.2)
Anterior placenta	55.6	3	2	0
Posterior placenta	44.4	1	0	3
Straight fetoscope	66.7	3	0	3
Curved fetoscope	33.3	1	2	0
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Placenta	0	0	0	0
Uterine entry site	22.2	0	2	0
Blood-stained fluid	33.3	1	2	0
Washout required	11.11	0	0	1
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Length* (cm)	3.3 (2.9–3.6)	2.9†	3.3 (3.2–3.3)	3.6 (3.1–3.9)
Cerclage	0	0	0	0
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Donor hydrops	11.1	0	0	1
Recurrent TTTS	22.2	1	0	1
Repeat SFLP	11.1	0	0	1
TAPS	33.3	1	0	2
sIUGR	55.6	2	1	2
Discordant for fetal anomaly	11.1	0	0	1‡
Amnioinfusion	11.1	1	0	0
Amnioreduction	22.2	1	0	1
PPROM	22.2	1	0	1
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Delivery > 32 wk	44.4	3	1	0
Delivery < 32 wk	44.4	1	0	3
PPROM	22.2	1	0	1

Group 1 had on-site mentoring, Group 2 had remote mentoring, and Group 3 had independently performed surgery. *Data presented in median (range). †Measurement available in only one case. ‡Selective feticide by radiofrequency ablation for cerebellar aplasia in ex-donor twin. AF: amniotic fluid; GA: gestational age; PPROM: preterm prelabour rupture of membranes; SFLP: selective fetoscopic laser photocoagulation; sIUGR: selective intrauterine growth restriction; TAPS: twin anaemia polycythaemia sequence; TTTS: twin-to-twin transfusion syndrome

The mentor's advice on team and equipment organisation (Fig. 2a), fetoscope entry, identification and photocoagulation of individual AVAs at the vascular equator (Fig. 2b) assisted the primary surgical team in completing the surgery and troubleshooting complications (e.g. placental-site bleeding). Residents recorded the locations of AVAs within the recipient twin amniotic sac during the initial fetoscopic end-to-end examination of the vascular equator (Fig. 2c). Detailed evaluation was performed after each surgery with critical feedback on the team's performance and improvements required. When telementoring arrangements were required on challenging cases, real-time images were continuously provided to the remote mentor using a mobile smartphone and 4G Internet connection (Figs. 2d & e). The image quality provided was generally satisfactory for the mentor to confirm the correct anastomoses (if the appearance raised doubts) and to advise a suitable approach for large vessels at risk of rupture. The mentor stayed online throughout the surgery.

The median duration of surgery was 96.5 (67.0–145.5) minutes (Table II). 5–11 AVAs were ablated in each patient. Large vessels were carefully ablated by 'shaving' the girth of the vessels from the periphery inwards and by coagulating the smaller feeder vessels to achieve satisfactory cessation of blood flow.[19] The main complications were brisk bleeding from uterine vessels that were lacerated upon entry; haemostasis was achieved by applying pressure with the fetoscope under direct vision. The Solomon technique was performed under direct supervision in

Cases 3 and 6, and in all cases in Group 3 to minimise the risks of TTTS recurrence and TAPS. It was not performed in Cases 1, 2, 4 or 5 because the primary team decided it would have been too technically demanding. No other adverse outcomes (e.g. placental abruption or hysterectomy) were encountered. Transient donor hydrops was observed in Case 8 within the first week after SFLP and resolved spontaneously.[34]

Cases 5 and 9 had persistent oligohydramnios and absence of the urinary bladder in the donor twin following satisfactory AVA ablation, with eventual re-accumulation of polyhydramnios in the recipient twin, and were classified under recurrent TTTS (incidence 22.2%, Table II). Both cases required amnioreduction from the recipient sac within a week of SFLP, which in Case 5 preceded preterm membrane rupture and emergency Caesarean delivery at 27.1 weeks, approximately 1.9 weeks post SFLP. In Case 9, post-SFLP sonographical surveillance showed rapidly progressing Stage 3 TTTS, with abnormal placental blood flows in the donor fetus and evidence of cardiac strain in the recipient fetus, requiring repeat SFLP. This was performed at another regional fetal centre that was better equipped to perform the complex surgery. Two large central anastomoses that had originally been ablated had recannulated causing recurrence and were successfully re-ablated. The patient was eventually delivered for progressively abnormal placental blood flows. Three cases of TAPS that were diagnosed sonographically (Cases 2, 7 and 8, incidence 33.3%) were managed expectantly.[5]

Selective intrauterine growth restriction (sIUGR) was diagnosed in Cases 2, 4, 5, 7 and 9 based on > 25% intertwin discordance from the estimated fetal weight (range 25.3%–46.8%).[35] Case 2 was complicated by TAPS, while in Case 4, intrauterine demise of the growth-restricted twin occurred at approximately 32 weeks. The incidence of sIUGR was 55.6% (n = 5) out of nine patients. The incidence of preterm prelabour membrane rupture (PPROM) was 22.2%. One sibling

twin (Case 7) was selectively terminated post SFLP upon diagnosis of a serious structural anomaly by radiofrequency ablation.[36] The prenatal loss rate of at least one fetus was 33.3% (n = 3) and the loss rate of both fetuses was 11.1% (n = 1; Table III).

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EFW discordance (at last US)*	18.0 (9.6–33.0)	17.0 (5.7–32.4)	33.0†	9.6 (1.7–17.5)
GA at delivery* (wk)	32.7 (28.6–36.2)	36.2 (27.1–36.9)	32.7†	28.6 (27.3–29.4)
Procedure-to-delivery interval* (wk)	8.9 (8.9–14.7)	14.7 (0.9–18.1)	8.9 (5.0–12.7)	9.1 (4.1–10.0)
Caesarean section	55.6	3	1	1
Vaginal	33.3	1	0	2
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Ex-donor* (g)	1,402 (985–1,982)	1,982 (860–2,765)	1,402†	985 (830–1,138)
Ex-recipient* (g)	1,888 (877–2,200)	2,200 (1,330–3,155)	1,888†	877 (530–1,224)
Weight discordance* (%)	25.7 (21.6–31.8)	31.8 (12.4–35.3)	25.7†	21.6 (7.0–36.1)
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≥ 1 twin	77.8	4	1	2
Both twins	44.4	3	0	1
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≥ 1 twin	77.8	4	1	2
Both twins	33.3	2	0	1
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Ex-donor* (g/dL)	14.7 (13.0–23.2)	16.7 (13.0–23.2)	NA	11.9 (9.4–14.4)
Ex-recipient* (g/dL)	13.9 (11.3–18.2)	11.3 (8.6–21.7)	13.5	18.2
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Ex-donor§	33.3	1	1	1
Ex-recipient§	33.3	2	0	1
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Intracranial haemorrhage	7.7	1	0	0
Minor non-specific	38.4	4	1	4
Neurological deficits	7.7	0	1	0
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Group 1 had on-site mentoring, Group 2 had remote mentoring, and Group 3 performed surgery independently. *Data presented in median (range). †Stillbirth of ex-donor twin diagnosed just before delivery. ‡Does not include Case 7, in which selective feticide of the ex-donor was performed for inferior vermian agenesis by radiofrequency ablation. §For anaemia of prematurity and twin anaemia-polycythaemia syndrome. EFW: estimated fetal weight; GA: gestational age; ICH: intracranial haemorrhage; NA: not available; US: ultrasonography

There were no significant differences in postnatal outcomes between groups. There were trends towards lower GA at delivery, shorter procedure-to-delivery intervals and lower birth weight in both twins in Group 3 compared to Group 1 (Table III). Survival of at least one twin to birth was 88.9% (n = 8), with double survival in 55.6% (n = 5) of all pregnancies treated. All were born preterm at < 37 weeks, with 44.4% delivered at < 32 weeks. Median ages at delivery were similar: 36.2 (range 27.1–36.9) weeks (Group 1), 32.7 weeks (Group 2, single survivor) and 28.6 (range 27.3–29.4) weeks (Group 3). Median procedure-to-delivery interval was 8.9 (8.9–14.7) weeks with a trend towards shorter intervals in Groups 2 and 3 (Table III).

Placental dye injections were performed in every case in which patients gave consent to identify arteries and veins from donors and recipients. Six placentas were examined after birth for

residual anastomoses (Figs. 3a–d), excluding Case 7 (declined consent), and Cases 6 and 9 (delivered overseas). A partially recannulated AVA between the proximately sited cords was observed in Case 5 (recurrent TTTS, white star in Fig. 3a & b), while the remaining AVAs were successfully coagulated (yellow stars in Figs. 3a & c). The other placentas showed no obvious residual anastomoses (yellow stars in Fig. 3d) and satisfactory Solomon ablation (broken line in Fig. 3d).

Infant survival of at least one twin was observed in 77.8% of pregnancies at both 28 days and six months, and 33.3% of pregnancies had both twins survive at six months (Table III). One twin pair and one ex-recipient died at seven days and 49 days respectively from prematurity-related respiratory distress syndrome. Neonatal complications included spastic cerebral palsy (7.7%, one out of 13 infants) and benign intraventricular changes (66.7%) in six infants who had normal neurological outcomes at 8–35 months of age.□

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This report illustrates the usefulness of a collaborative effort to introduce a new complex surgery while maintaining safety and producing satisfactory outcomes. Prior to establishing SFLP locally, patients with treatable TTTS were referred to regional centres; these arrangements, commonly self-funded, result in delayed treatment and represent an unsustainable solution, particularly when surgery is needed urgently. The conventional apprenticeship model requires resources for surgical training and a minimal case-load (25–75 procedures) to attain proficiency.[8,10] Outcomes reflect individual and institutional learning curves that are influenced by the clinical team working collectively to manage the patient.[37] While achievable in high-volume centres, successful adoption of SFLP is a real challenge in a low-volume clinic servicing a much smaller

population.[13] We thus adapted a collaborative model utilising on-site mentoring and telementoring with model practice to achieve these goals. This review of our initial outcomes in the early part of the learning curve is important to validate the safety and efficacy of this approach.

We worked closely with two regionally based fetal surgeon mentors who were able to travel quickly and teleconference easily across negligible time differences. The primary team acquired basic fetoscopy skills through model training, as no local surgeon had undergone a full fetoscopy apprenticeship.[19] These arrangements proved particularly helpful in the first cases that presented technical challenges (i.e. anterior placentas, large vessels), which were overcome with much greater efficiency under direct supervision of the mentor,[7] and in post SFLP management for recurrent TTTS and TAPS.[19] There were greater procedural delays for Group 1 as travel arrangements had to be made for the overseas mentor, and these first cases predictably took the primary team longer to organise. Duration of surgery was the longest for Group 2, as these cases were complicated by anterior placentae and significant intrauterine bleeding. Placental dye injections enabled reflection and continuous learning from the unsuccessful cases.

Survival rates in our preliminary series are comparable to those in the published literature, including survival rates for pregnancies with at least one live birth (88.9% vs. 70%–88%),[4,14] two survivors (55.6% vs. 35%–74%) and no survivors (11.1% vs. 9.5%–27%),[1,38] respectively. Rates of PPROM, perinatal survival and neonatal death in our cohort were similar to published outcomes.[1,38] Incidences of recurrent TTTS (22.2%) and TAPS (33.3%) were higher than the reported rates of 0%–16% and 2%–13%, respectively.[5,39] This data reflects the primary team's inexperience in identifying and ablating smaller or more peripheral anastomoses, and in dealing with large AVAs, as well as the small patient numbers in this series.

The approach we used illustrates the prerequisites for a mentoring system to work effectively: the primary team operating efficiently as a single unit (performing all cases together to improve operating efficacy) with each member fulfilling specified functions,[7,16,19] immediate feedback from the mentor, and specific skills reinforced by model practice.[19,40] The proximity of regional centres allowed us to coordinate the entire team, including the overseas mentor, within days. The main limitation to the timely growth of a new fetal therapy centre is a caseload that is insufficient to attain and maintain proficiency in the traditional sense.[41] As our centre services a small population with a low birth-rate, it will not easily meet the conventional numeric requirements for institutional competence in uncommon procedures.[42] This solution gives the primary team the confidence to proceed with service provision without compromising patients' rapid access to SFLP within the existing healthcare system, and can be adapted to provide accessible advanced therapies at similar low-volume centres.

While surgical apprenticeship is the accepted practice,[11] it is inadequate in itself to meet the increasing demand for SFLP at low-volume centres.[43,44] Skills acquisition as a surgical team and effective mentor-mentee communication were the critical factors in the success of the collaborative model driven by patient and institutional needs and can be applied to other complex surgical therapies.[13,45] Mentoring beyond the apprenticeship period provides continued benefit,[18,46] resulting in fewer clinical errors, complications and mortality.[47,48] We acknowledge that although this is a promising approach to skills acquisition for new techniques, our numbers are small and hence further studies with larger numbers are required to reinforce our conclusions.

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1. Society for Maternal-Fetal Medicine , Simpson LL. Twin-twin transfusion syndrome. Am J Obstet Gynecol 2013; 208:3-18.
2. WAPM Consensus Group on Twin-to-Twin Transfusion, Baschat A, Chmait RH, et al. Twin-to-twin transfusion syndrome (TTTS). J Perinat Med 2011; 39:107-12.
3. Ropacka M, Markwitz W, Blickstein I. Treatment options for the twin-twin transfusion syndrome: a review. Twin Res 2002; 5:507-14.
4. Quintero RA, Kontopoulos E, Chmait RH. Laser treatment of twin-to-twin transfusion syndrome. Twin Res Hum Genet 2016; 19:197-206.
5. Donepudi R, Akkermans J, Mann L, et al. Impact of cannula size on recurrent twin-twin transfusion syndrome and twin anemia-polycythemia sequence after fetoscopic laser surgery. Ultrasound Obstet Gynecol 2018; 52:744-9.
6. Thia E, Thain S, Yeo GS. Fetoscopic laser photocoagulation in twin-to-twin transfusion syndrome: experience from a single institution. Singapore Med J 2017; 58:321-6.
7. Gosavi A, Vijayakumar PD, Ng BS, et al. Rapid initiation of fetal therapy services with a system of learner-centred training under proctorship: the National University Hospital (Singapore) experience. Singapore Med J 2017; 58:311-20.

8. Morris RK, Selman TJ, Harbidge A, Martin WI, Kilby MD. Fetoscopic laser coagulation for severe twin-to-twin transfusion syndrome: factors influencing perinatal outcome, learning curve of the procedure and lessons for new centres. BJOG 2010; 117:1350-7.

9. Morris RK, Selman TJ, Kilby MD. Influences of experience, case load and stage distribution on outcome of endoscopic laser surgery for TTTS--a review. Ahmed S et al. Prenatal Diagnosis 2010. Prenat Diagn 2010; 30:808-9; author reply 810.

10. Peeters SH, Van Zwet EW, Oepkes D, et al. Learning curve for fetoscopic laser surgery using cumulative sum analysis. Acta Obstet Gynecol Scand 2014; 93:705-11.

11. Lubowitz JH, Provencher MT, Brand JC, Rossi MJ. The apprenticeship model for surgical training is inferior. Arthroscopy 2015; 31:1847-8.

12. Goff BA. Changing the paradigm in surgical education. Obstet Gynecol 2008; 112(2 Pt 1):328-32.

13. Edwards AG, Teoh M, Hodges RJ, et al. Balancing patient access to fetoscopic laser photocoagulation for twin-to-twin transfusion syndrome with maintaining procedural competence: are collaborative services part of the solution? Twin Res Hum Genet 2016; 19:276-84.

14. Akkermans J, Peeters SH, Klumper FJ, et al. Twenty-five years of fetoscopic laser coagulation in twin-twin transfusion syndrome: a systematic review. Fetal Diagn Ther 2015; 38:241-53.

15. Agha RA, Fowler AJ. The role and validity of surgical simulation. Int Surg 2015; 100:350-7.

16. Stepaniak PS, Vrijland WW, de Quelerij M, de Vries G, Heij C. Working with a fixed operating room team on consecutive similar cases and the effect on case duration and turnover time. Arch Surg 2010; 145:1165-70.

17. Snyderman CH, Gardner PA, Lanisnik B, Ravnik J. Surgical telementoring: a new model for surgical training. Laryngoscope 2016; 126:1334-8.

18. Schubert A. Teleconferencing, distance learning, and telementoring: new technology harnessed for perioperative education. J Clin Anesth 2000; 12:250-1.

19. Wataganara T, Gosavi A, Nawapun K, et al. Model surgical training: skills acquisition in fetoscopic laser photocoagulation of monochorionic diamniotic twin placenta using realistic simulators. J Vis Exp 2018; (133).

20. Peeters SH, Akkermans J, Bustraan J, et al. Operator competence in fetoscopic laser surgery for twin-twin transfusion syndrome: validation of a procedure-specific evaluation tool. Ultrasound Obstet Gynecol 2016; 47:350-5.

21. Peeters SH, Akkermans J, Westra M, et al. Identification of essential steps in laser procedure for twin-twin transfusion syndrome using the Delphi methodology: SILICONE study. Ultrasound Obstet Gynecol 2015; 45:439-46.

22. Peeters SH, Akkermans J, Slaghekke F, et al. Simulator training in fetoscopic laser surgery for twin-twin transfusion syndrome: a pilot randomized controlled trial. Ultrasound Obstet Gynecol 2015; 46:319-26.

23. Lenchus JD. End of the "see one, do one, teach one" era: the next generation of invasive bedside procedural instruction. J Am Osteopath Assoc 2010; 110:340-6.

24. Curry JI. 'See one, practise on a simulator, do one' - the mantra of the modern surgeon. S Afr J Surg 2011; 49:4-6.

25. Vozenilek J, Huff JS, Reznek M, Gordon JA. See one, do one, teach one: advanced technology in medical education. Acad Emerg Med 2004; 11:1149-54.

26. Sueters M, Middeldorp JM, Lopriore E, et al. Timely diagnosis of twin-to-twin transfusion syndrome in monochorionic twin pregnancies by biweekly sonography combined with patient instruction to report onset of symptoms. Ultrasound Obstet Gynecol 2006; 28:659-64.

27. Linskens IH, de Mooij YM, Twisk JW, et al. Discordance in nuchal translucency measurements in monochorionic diamniotic twins as predictor of twin-to-twin transfusion syndrome. Twin Res Hum Genet 2012; 12:605-10.

28. Kontopoulos E, Chmait RH, Quintero RA. Twin-to-twin transfusion syndrome: definition, staging, and ultrasound assessment. Twin Res Hum Genet 2016; 19:175-83.

29. Van Mieghem T, Lewi L, Gucciardo L, et al. The fetal heart in twin-to-twin transfusion syndrome. Int J Pediatr 2010; 2010. pii: 379792.

30. Mackie FL, Hall MJ, Morris RK, Kilby MD. Early prognostic factors of outcomes in monochorionic twin pregnancy: systematic review and meta-analysis. Am J Obstet Gynecol 2018; 219:436-46.

31. Stagnati V, Zanardini C, Fichera A, et al. Early prediction of twin-to-twin transfusion syndrome: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2017; 49:573-82.

32. Slaghekke F, Lewi L, Middeldorp JM, et al. Residual anastomoses in twin-twin transfusion syndrome after laser: the Solomon randomized trial. Am J Obstet Gynecol 2014; 211:285.e1-7.

33. Lopriore E, Slaghekke F, Middeldorp JM, et al. Accurate and simple evaluation of vascular anastomoses in monochorionic placenta using colored dye. J Vis Exp 2011; (55):e3208.

34. Gratacós E, Van Schoubroeck D, Carreras E, et al. Transient hydropic signs in the donor fetus after fetoscopic laser coagulation in severe twin-twin transfusion syndrome: incidence and clinical relevance. Ultrasound Obstet Gynecol 2002; 19:449-53.

35. Gratacós E, Lewi L, Muñoz B, et al. A classification system for selective intrauterine growth restriction in monochorionic pregnancies according to umbilical artery Doppler flow in the smaller twin. Ultrasound Obstet Gynecol 2007; 30:28-34.

36. Bebbington MW, Danzer E, Moldenhauer J, Khalek N, Johnson MP. Radiofrequency ablation vs bipolar umbilical cord coagulation in the management of complicated monochorionic pregnancies. Ultrasound Obstet Gynecol 2012; 40:319-24.

37. Ramsay CR, Grant AM, Wallace SA, et al. Assessment of the learning curve in health technologies. A systematic review. Int J Technol Assess Health Care 2000; 16:1095-108.

38. Chmait RH, Kontopoulos EV, Korst LM, et al. Stage-based outcomes of 682 consecutive cases of twin-twin transfusion syndrome treated with laser surgery: the USFetus experience. Am J Obstet Gynecol2011;204:393.e1-6.

39. Slaghekke F, Oepkes D. Solomon technique versus selective coagulation for twin-twin transfusion syndrome. Twin Res Hum Genet 2016; 19:217-21.

40. Peyre SE, Ashley SW. Teaching uncommon and highly complex operations: maximizing the teaching and learning. J Gastrointest Surg 2011; 15:1724-5.

41. The Royal Australian and New Zealand College of Obstetricians and Gynaecologists. Management of monochorionic twin pregnancy. C-Obs 42. 2014. Available at: https://www.ranzcog.edu.au/RANZCOG_SITE/media/RANZCOG-MEDIA/Women%27s%20Health/Statement%20and%20guidelines/Clinical-Obstetrics/Management-of-Monochorionic-Twins-(C-Obs-42)-review-July-2017.pdf?ext=.pdf. Accessed November 12, 2018.

42. The World Bank. Fertility rate, total (births per woman). Available at: https://data.worldbank.org/indicator/SP.DYN.TFRT.IN. Accessed December 16, 2018.

43. Population Division, Department of Economic and Social Affairs, United Nations. World Population Prospects: The 2017 Revision. Methodology of the United Nations Population Estimates and Projections, Working Paper No. ESA/P/WP.250. New York, NY: United Nations, 2017. Available at:

https://population.un.org/wpp/Publications/Files/WPP2017_Methodology.pdf. Accessed December 16, 2018.

44. El-Sabawi B, Magee W 3rd. The evolution of surgical telementoring: current applications and future directions. Ann Transl Med 2016; 4:391.

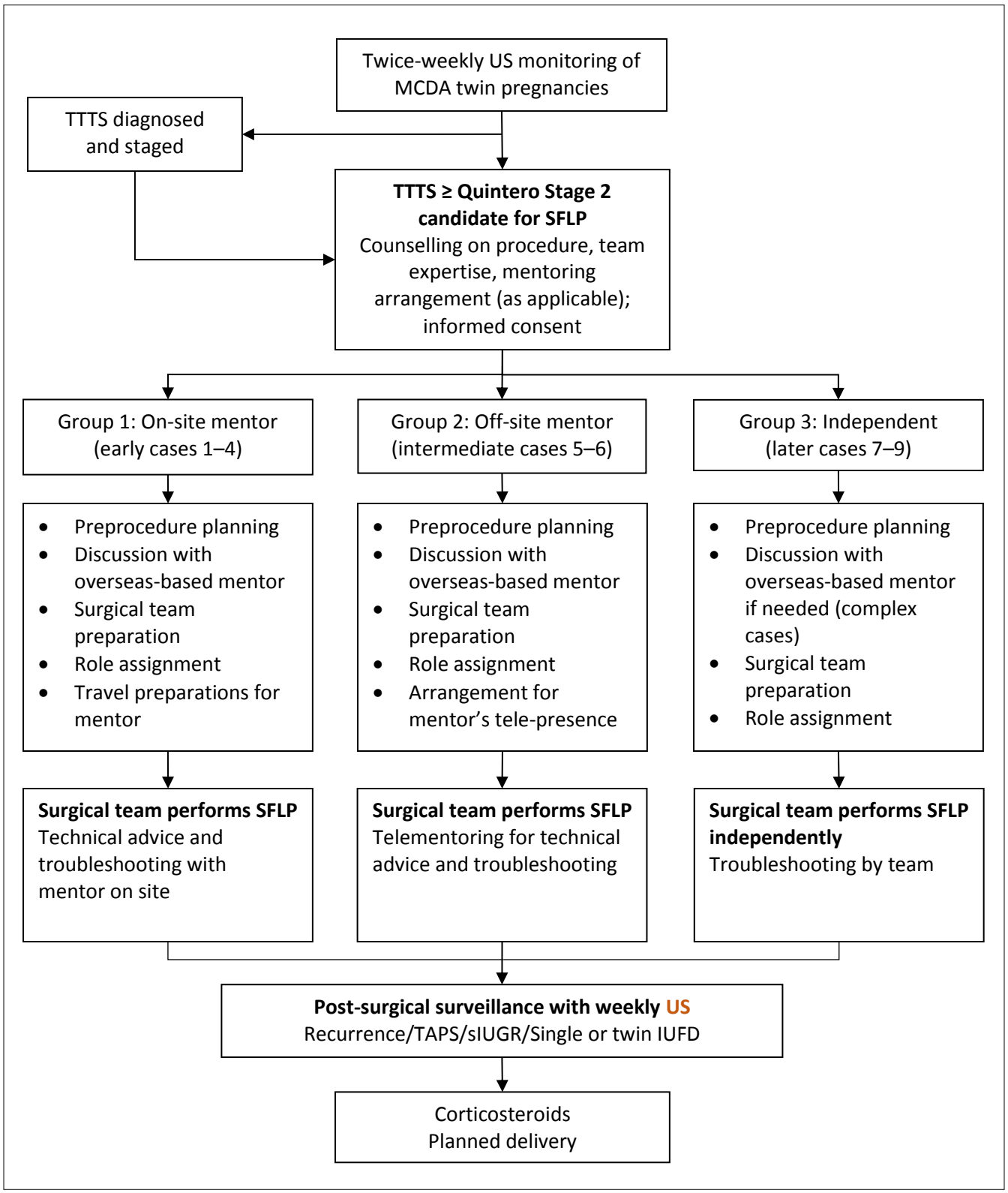
45. Chan FY. Fetal tele-ultrasound and tele-therapy. J Telemed Telecare 2007; 13:167-71.

46. Bruns NE, Irtan S, Rothenberg SS, et al. Trans-atlantic telementoring with pediatric surgeons: technical considerations and lessons learned. J Laparoendosc Adv Surg Tech A 2016; 26:75-8.

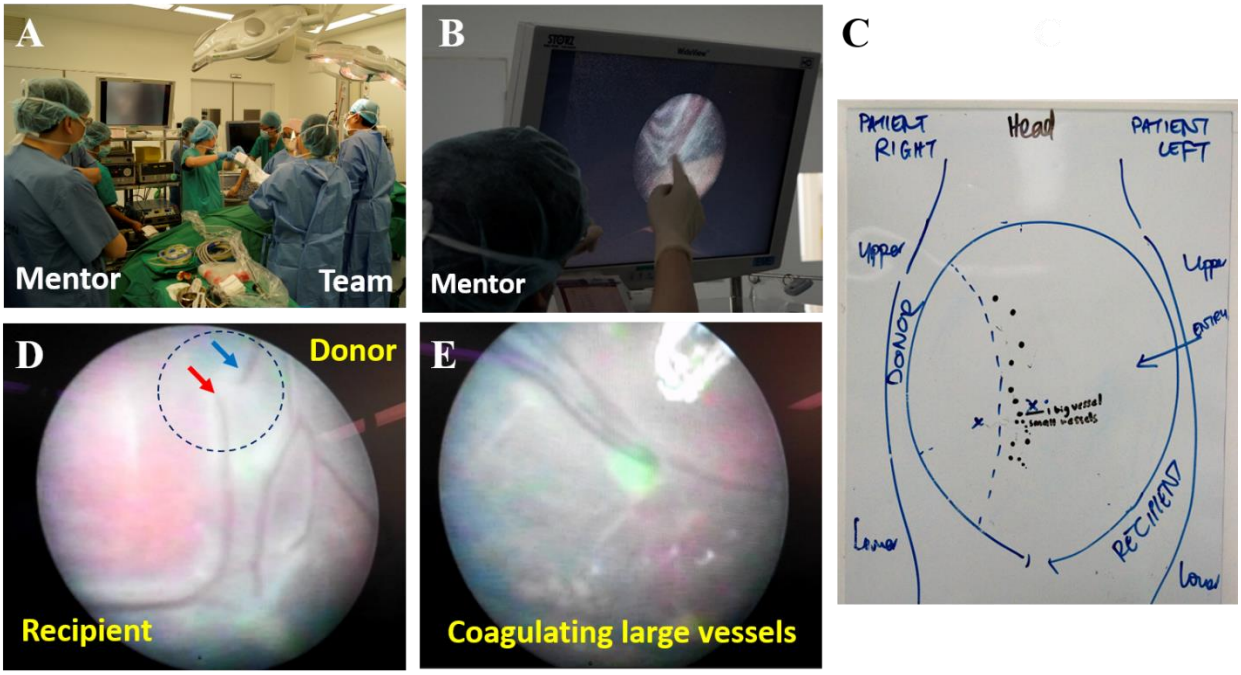
47. Gilbert JH, Yan J, Hoffman SJ. A WHO report: framework for action on interprofessional education and collaborative practice. J Allied Health 2010; 39 Suppl 1:196-7.

48. Schmitt MH. Collaboration improves the quality of care: methodological challenges and evidence from US health care research. J Interprof Care 2001; 15:47-66.

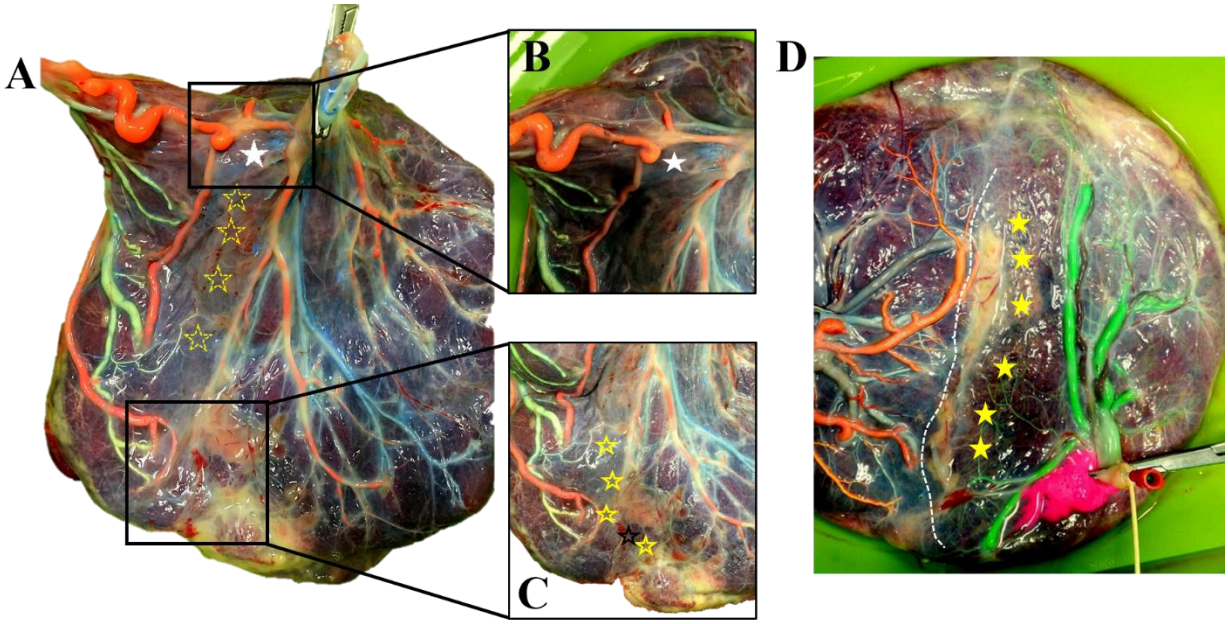
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Twice-weekly US monitoring of MCDA twin pregnancies
TTTS diagnosed and staged
TTTS ≥ Quintero Stage 2 candidate for SFLP
Counselling on procedure, team expertise, mentoring arrangement (as applicable); informed consent
Group 1: On-site mentor (early cases 1–4)
Group 2: Off-site mentor (intermediate cases 5–6)
Group 3: Independent (later cases 7–9)
Preprocedure planning
Discussion with overseas-based mentor
Surgical team preparation
Role assignment
Travel preparations for mentor
Preprocedure planning
Discussion with overseas-based mentor
Surgical team preparation
Role assignment
Arrangement for mentor's tele-presence
Preprocedure planning
Discussion with overseas-based mentor if needed (complex cases)
Surgical team preparation
Role assignment
Surgical team performs SFLP
Technical advice and troubleshooting with mentor on site
Surgical team performs SFLP
Telementoring for technical advice and troubleshooting
Surgical team performs SFLP independently
Troubleshooting by team
Post-surgical surveillance with weekly US
Recurrence/TAPS/sIUGR/Single or twin IUFD
Corticosteroids
Planned delivery

□□□□□**1** □Procedural flowchart shows the diagnosis and staging of patients with twin-to-twin transfusion syndrome (TTTS) who underwent selective fetoscopic laser photocoagulation (SFLP).IUFD: intrauterine fetal death; MCDA: monochorionic diamniotic; sIUGR: selective intrauterine growth restriction; TAPS: twin anaemia polycythaemia sequence



□□□□**2** □Photographs show on-site mentoring and telementoring during the first cases of selective fetoscopic laser photocoagulation (SFLP). (a) The primary surgical team was mentored in a hands-off manner, with a focus on team organisation and (b) completing SFLP efficiently and safely, especially in technically demanding situations (e.g. on large vessels). (c) Residents recorded the locations of arteriovenous anastomoses (black circles) within the recipient twin amniotic sac during the initial fetoscopic end-to-end examination of the vascular equator. (d) For telementoring, fetoscopic images were filmed directly from the screen using a mobile device and sent to the mentor via the Internet through the course of the surgery. Good image quality was obtained, allowing the telementor and team to identify outlying anastomoses with the donor or recipient origins of vessels (arrows) and (e) carefully ‘shave’ larger vessels with photocoagulation to prevent rupture or bleeding.



□□□□□□Photographs show the results of placental dye injections performed to find the accuracy of selective fetoscopic laser photocoagulation, identifying (a) AVAs (yellow stars); (b) recannulated AVAs, which in this case were the cause of recurrent twin-to-twin transfusion syndrome (white star in a & b); and in optimally completed procedures, (c & d) individually ablated AVAs (yellow stars) followed by superficial ablation of residual anastomoses (broken line in d). AVA: ablated arteriovenous anastomosis

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Placental morphology and perinatal outcome in gestational hyperglycemia on diet and medication

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Abstract

Background: To evaluate the placental morphology and perinatal outcome in patients with gestational hyperglycemia on diet and medication.

Methods: Placental examinations performed at the Department of Pathology between August 2016 to August 2018 were retrospectively reviewed. Of the received 140 placentas, 35 of gestational diabetes (GDM) and pre gestational diabetes were identified and segregated into hyperglycemia on diet and on medication. The clinical details, placental findings and perinatal outcome of patients in both the groups (gestational hyperglycemia on diet and medication) were collected and analyzed.

Results: Among the 35 cases, there were 24 cases of mild gestational hyperglycemia controlled with diet and 11 cases of hyperglycemia on medication (oral hypoglycemic drugs ± insulin).

Most of the placentae in both the groups weighed less than tenth centile. The cord abnormalities such as hyper coiling, velamentous /marginal insertion and furcate cord were observed more in women with GDM on diet. There was no significant gross placental lesion in those on medication. Placental histological features most consistently associated with both the groups include, disturbances of villous maturation (DVM), Derangements in uteroplacental / foetoplacental circulation and villous capillary lesions. Small for gestational age and intrauterine foetal death were found in both the groups, but more commonly in patients with hyperglycemia on medication.

Conclusions: Villous maturation defects, uteroplacental / foetoplacental malperfusion are the essential placental changes which can result in adverse perinatal outcomes in women with hyperglycemia irrespective of the diabetic control.

Keywords: Gestational diabetes, Gestational hyperglycemia, Perinatal outcome, Placental malperfusion, Placental pathology

Introduction

India has the second largest diabetic population in the world after China,with 8.7% of the total population in the age group 20-70 years being diabetic.[1] A cross sectional study among 15 states showed an estimated prevalence of 7.3%.[2] Diabetes in pregnancy is a growing health concern among the antenatal mothers in India. A recent meta-analysis shows the pooled estimate for mean prevalence of gestational diabetes to be 10.1% with a rising trend from 0.53% to 27.3%.[3-5]

Gestational diabetes is defined as glucose intolerance with onset or first recognition during pregnancy and is associated with a multitude of maternal and foetal complications.[6] There is a higher incidence of spontaneous miscarriages, congenital malformations, macrosomia, intrauterine death, operative deliveries, birth injuries, neonatal respiratory distress, neonatal hypoglycaemia in diabetic pregnancies and increased incidence of type 2 diabetes in offspring. Maternal complications include a higher incidence of preeclampsia, polyhydramnios, obstructed labour, caesarean sections, postpartum haemorrhage and infections.

Placenta is the organ which serves as a means of communication at the maternal foetal interface, meanwhile acting as a selective barrier between both the circulations. Changes in the glycemic status are known to affect the placenta and the resulting histopathological findings are categorised based on the time of onset of hyperglycemia.[7,8] Earlier onset of altered glycemic profile results in structural alterations whereas later onset causes functional impairment. Common changes observed include villous immaturity, villous fibrinoid necrosis, chorangiosis, and increased angiogenesis.

Hence the placenta may be regarded as a route map guiding us in our understanding of the pathological basis of the foetal complications encountered in diabetic pregnancies.

We sought to determine the placental morphological changes in diabetic pregnancies and to correlate them with neonatal outcome.

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In this retrospective study, 35 singleton placentas from mothers with altered/abnormal glucose profile were studied. Institutional ethical committee approval was obtained. The study materials were archived from the departments of Pathology, Obstetrics and gynaecology and from medical record department. The study period was between August 2016 to August 2018 (2 years).

Inclusion criteria

- Placentas from patients with the clinical history of gestational diabetes / hyperglycemia on diet and medication irrespective of associated comorbidities

Exclusion criteria

- Unfixed/poorly fixed placental specimen and Placentas with no clinical details will be excluded

Clinical data collected were patient's age, gestational age, obstetric score, co-morbidities and types of glucose intolerance. All the antenatal women were routinely screened for gestational diabetes during 24 to 28 weeks of gestation by the DIPSI OGCT (75 grams glucose load in non-fasting state followed by a 2-hour plasma glucose).[9] Women with 2 hour plasma glucose more than 140mg/dl were categorized as gestational diabetes. They were categorized into 2 groups: Mild gestational hyperglycemia (on diet), diabetes on medication (pregestational /gestational).

Perinatal factors observed were mode of delivery, pregnancy outcome [intrauterine foetal death (IUD), neonatal death], birth weight, Apgar scores and NICU (neonatal intensive care unit) admission. According to the birth weight, the babies are classified as small for gestational age/ foetal growth restriction (SGA/FGR), appropriate for gestational age (AGA) and large for gestational age (LGA).

The placental tissues were fixed in 10% buffered formalin. The gross parameters observed were trimmed disc weight, measurements, cord (color, length, diameter, number of vessels, twists, insertion site, true/false knots), membranes (color, insertion and rupture site), foetal surface, maternal surface and parenchyma (color, sponginess, location & size of any lesion, calcification, etc.).[10]

After taking photographs of the foetal and maternal surfaces, 2 bits were taken each from the membrane roll and umbilical cord. The placental parenchyma was then cut into thin slices with the interval of 1.0cm (bread loaf method). Picture of the parenchyma was taken for all the placentas. 3 bits were taken from the normal looking central parenchyma and few more from the lesional area (if present grossly). Haematoxylin and eosin stained slides were reviewed, and significant changes were noted.

Since it was a descriptive study with limited numbers, statistical analysis for comparison was not attempted.

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The 35 cases were identified with hyperglycemia in pregnancy. There were 24 cases of mild gestational hyperglycemia controlled with diet and 11 cases of hyperglycemia on medication, which included gestational and pre gestational diabetes.

Mild gestational hyperglycemia on diet (n=24)

Clinical characteristics

The age distribution of the patients whose placentae were examined ranged from 23 to 40 years with the mean age of 30.83 years. The earliest gestational age was 25 weeks and the maximum was 40 weeks. Fourteen patients were primigravida and ten were multigravida. Half of the placentas were from preterm pregnancies and half from term pregnancies. Associated maternal conditions were analysed with few cases showing comorbidities such as oligohydramnios (n=3),hypertension /preeclampsia (n=2),

antiphospholipid antibody (n=2), anemia (n=2), abruption (n=2) and one each of familial hypercholesterolemia, placenta previa, polyhydramnios and hypothyroidism.

Foetal outcome

The birth weights of nine (37.5%) of the neonates were appropriate for gestational age (AGA). Among the remaining, 8 (33%) were small for gestational age (SGA), 3 (12.5%) were large for gestational age (LGA) and 4 (16.6%) of the pregnancies resulted in intrauterine foetal demise (IUFD). Table 1 highlights the perinatal outcome in both the groups.

Placental findings

Weight: Of the 24 studied placentas, 11 weighed less than the 10th centile (11/24). This is in correlation with the growth restriction in 6 foetuses (6/11) and IUD in 1 case (1/11). The remaining small placentas (4/11) were of foetuses with normal weights.

The weights of nine of the placentas(9/24) were in the normal range

Gestational and pre gestational diabetes on medication (n=11)

Clinical characteristics

The age distribution of the patients whose placentae were examined ranged from 25 to 42 years with the mean age of 32.9 years.

The earliest gestational age was 30 weeks and the maximum was 39 weeks. Four patients were primigravida and seven were multigravida. Three of the placentas were from term pregnancies and eight from preterm pregnancies. Associated maternal conditions included were three cases each of gestational hypertension/preeclampsia and hypothyroidism and one each of anemia and immune thrombocytopenic purpura.

Foetal outcome

Five (45%) of the neonates were SGA, 2(18%) were AGA and 1(9%) was LGA. The remaining 3 (27%) were IUFD (Table 1).

Table 1: Perinatal outcome in GDM on medication and GDM on diet

Perinatal outcome	SGA	AGA	LGA	IUFD
GDM on diet (n=24)	8	3	9	4
GDM on medication (n=11)	5	1	2	3

Placental findings

Weight: 7/11 placentas weighed less than 10th centile. Four of them were associated with small foetuses and 2 each were associated with AGA and IUD respectively.

Gross features

Table 2 highlights the gross placental findings including umbilical cord.

Microscopic findings

Table 3 displays the microscopic features of placenta in both the groups.

Apart from the tabulated findings, calcification, perivillous fibrin deposition, increased NRBCs, subchorionic cysts and mild focal villitis were also observed.

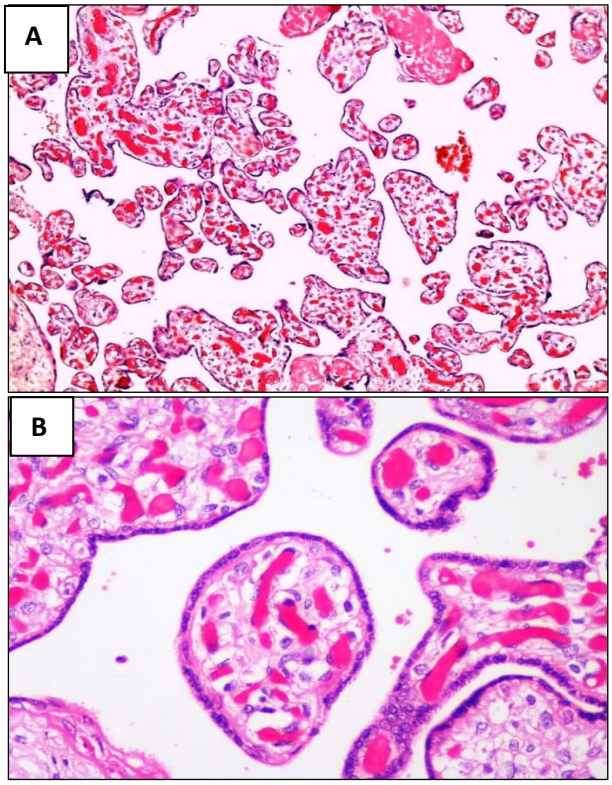
Table 2: Gross placental findings in GDM on medication and GDM on diet

Gross placental findings	GDM on diet (n=24)				GDM on medication (n=11)			
	SGA (n=8)	AGA (n=3)	LGA (n=9)	IUFD (n=4)	SGA (n=5)	AGA (n=1)	LGA (n=2)	IUFD (n=3)
Succenturiate lobe	1	-	-	1	-	-	-	-
Meconium staining	-	1	-	-	-	-	-	1
Subchorionic hemorrhage	-	-	-	-	-	-	-	1
Retroplacental hematoma	-	2	-	-	-	-	-	-
Marginal atrophy	1	3	-	1	-	1	1	2
Cord: 1) Hypercoiled	2	1	3	-	1	-	-	-
2) Hypocoiled	1	-	-	1	-	1	-	-
3) Velamentous insertion	1	-	-	1	-	-	-	-
4) Marginal insertion	2	-	-	-	-	-	-	-
5) Furcate cord	1	-	-	1	-	-	-	--
6) True knot	-	-	1	-	-	-	-	-

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	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
DVI (Fig .1)	5	4	2	2	4	2	0	1
Vascular ectasia	2	2	2	1	3	0	0	0
Villous capillary lesions (chorangiosis)	3	5	2	1	4	1	1	2
Chorangioma	0	1	1	0	0	0	0	0
MVM (infarcts+IVT)	4	6	0	0	5	0	0	2
FVM (Fig .2)	3	2	0	1	3	0	0	1
Laminar necrosis	2	4	1	1	0	0	1	0
A/C CAM (Fig.3)	1	1	1	1	0	1	0	0
Increased syncytial knotting	2	6	0	0	3	1	1	3
Increased cell islands	3	4	1	1	2	2	1	2
CHIV	0	0	0	1	0	0	0	0

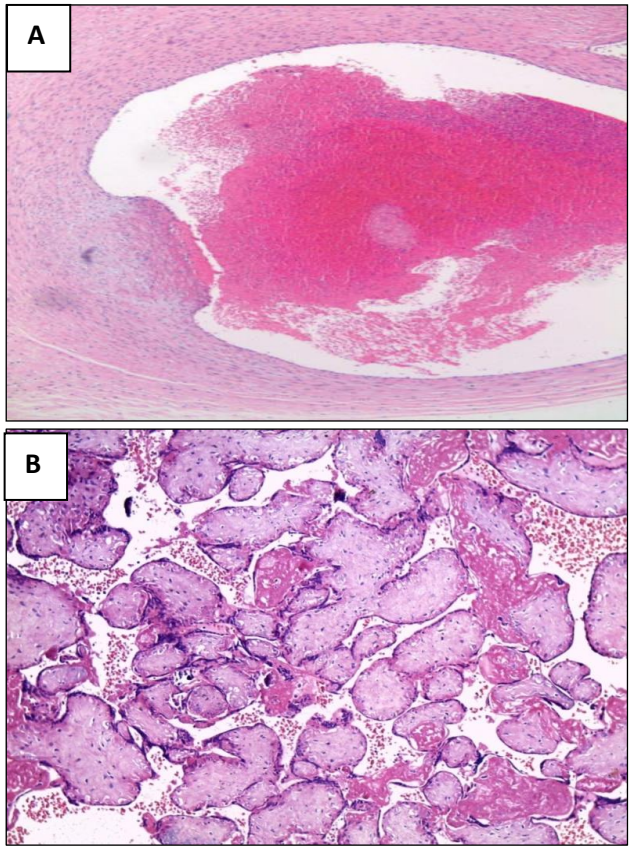
DVI, Distal villous immaturity; MVM,maternal vascular malperfusion; IVT, intervillousthrombus; FVM, foetal vascular malperfusion; A/C CAM, acutechorioamnionitis; CHIV, chronic histiocytic intervillositis



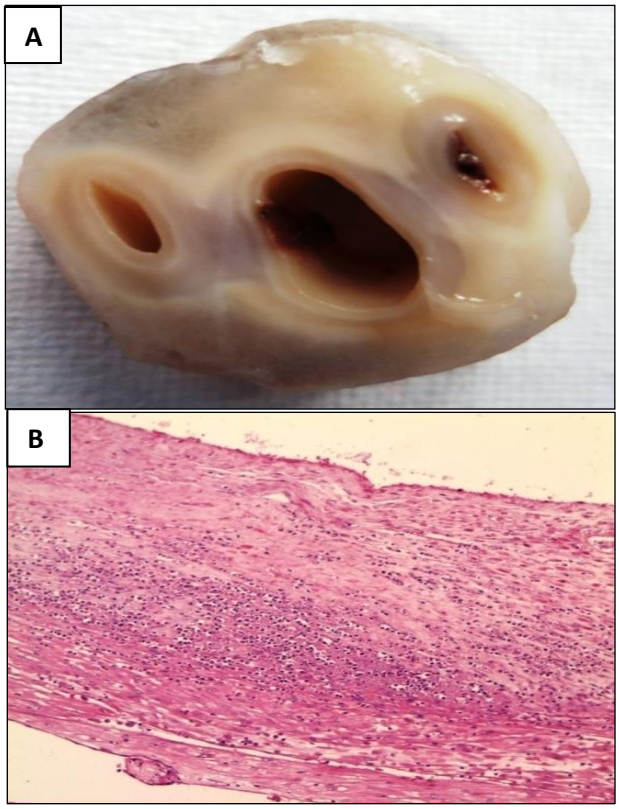
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In the present study, we analyzed the placental morphology and clinical outcome in pregnant women with mild hyperglycemia on diet and Gestational and pre-gestational diabetes on medication.



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There have been studies on placentas of diabetic women which have reported large babies and higher placental weights.[11-15] In contrast to that, we encountered more number of small placentas which belonged to small to normally grown babies and was comparable with Edu et al.[16]

Risk of foetal loss is increased in diabetic pregnancy irrespective of glycemic control.[17] Similarly, incidence of intrauterine death was higher in women in both the groups.

There was no significant increase in gross placental lesions among GDM on medication.[18] The cord abnormalities such as hypercoiling, velamentous/marginal insertion and furcate cord were observed more in women with GDM on diet.[19] Single umbilical artery is generally associated with gestational diabetes, but there were no such instances in our study.

Placental histologic changes

Disturbances of villous maturation (DVM)

DVM also known as distal villous immaturity were found in more than half of the cases in both groups. In contrast to the findings of Huynh et al, we did not find any differences between the two groups.[20]

Generally, in the last trimester of pregnancy, there is accelerated differentiation into terminal villi with increase in vasculosyncytial membrane formation. However, in pregnancies complicated by diabetes, this terminal differentiation is inhibited due to growth factor like activity of foetal insulin. Additionally, this is amplified by coexistent hypercoiling of the cord as seen in our study.[21]

As a result of defective villous maturation, there is reduced placental reserve leading to poor tolerance to acute hypoxia resulting in late intra uterine foetal loss. Since, this entity has a 10% risk of recurrence in subsequent pregnancies, it has been recommended to rescue the foetus by early delivery.[22]

Derangements in uteroplacental circulation/Maternal vascular malperfusion (MVM)

In our study, we observed greater incidence of features of MVM such as, infarcts, intervillous thrombus and increased syncytial knotting in both the groups. As described in literature, decidual vasculopathy was the prominent finding in women with coexistent preeclampsia/APLA as compared to isolated GDM.[23] Retroplacental hematoma with blood dissecting into the intraparenchymal villous space and subsequent haemorrhagic necrosis was also noted in few cases of GDM on diet.

Derangements in foetoplacental circulation/foetal vascular malperfusion (FVM)

FVM frequently goes undetected antenatally and is commonly diagnosed with pathologic examination of placenta. Among the multitude of factors associated with FVM, maternal hyperglycemia plays a significant role.[24]

Pregnancy per se is a thrombogenic state. When it is combined with maternal hyperglycemia (prothrombotic tendency) and hypercoiling of the cord (thereby causing vascular stasis and ischemia), it results in thrombosis in the foetal vascular tree, leading to foetal growth restriction and increased perinatal mortality and morbidity.[25,26]

The following findings were seen as part of FVM in our study population: chorionic plate/ stem villous vessel thrombi, vascular ectasia, groups of avascular, fibrotic to hyalinized villi and villous stromal karyorrhexis.

Villous capillary lesions

A gamut of villous capillary lesions encompassing chorangiosis, chorangiomatosis and chorangiomas believed to be caused by preplacental hypoxia.[27]

GDM in pregnancy is commonly associated with chorangiosis, which is hypothesized to be caused by insulin/IGF-1 receptor mediated angiogenesis.[25]

In our series, we predominantly encountered chorangiosis and an occasional case of microscopic chorangioma, but none of the cases showed chorangiomatosis.

Inflammatory lesions

Acute chorioamnionitis was detected in a few cases of maternal hyperglycemia, of which more than half were complicated by preterm labour /preterm rupture of membranes. Severe maternal inflammatory response and early foetal inflammatory response were noted.[28]

Mild chronic villitis and chronic histiocytic intervillositis (CHIV) were also observed in some cases. Both the lesions have strong association with maternal autoimmune diseases.

Chronic histiocytic intervillositis is an uncommon placental lesion, characterized by a dense lymphohistiocytic infiltrate confined to the intervillous space. CHIV has a tendency for causing recurrent pregnancy loss due to the presence of persistent increase in memory T cells in subsequent pregnancies.[29,30]

Subchorionic pseudocysts, increased cell islands, foetal nucleated red blood cells and vascular ectasia are a known histologic sign of hypoxic lesions and found more in cases of GDM on diet.[31]

The strengths of our study include that all the women were booked at our hospital, the investigations, follow up and delivery was in the same institution and there was no loss to follow up and hence no attrition of data.

The limitations of our study include, smaller sample size and retrospective nature. Not all placentas with GDM were sent for pathological examination. Only those cases with maternal clinical conditions warranting special attention and those with adverse perinatal outcomes were sent for examination.

To summarize, Villous maturation defects, uteroplacental / fetoplacental malperfusion and villous capillary lesions are the essential placental changes which can result in adverse perinatal outcomes in women with hyperglycemia irrespective of the diabetic control.Further prospective studies with large sample size including all GDM placentas with exclusion of confounding factors would help in better characterization of placental lesions in different levels of glycemic control. Understanding the placental pathogenesis will help to develop protocols for prevention and treatment strategies to improve perinatal outcomes.

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1. World Health Organization – Diabetes country profiles, 2016. Available at: https://www.who.int/diabetes/country-profiles/ind_en.pdf
2. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK, et al. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR–INDIAB population-based cross-sectional study. lancet Diabe Endocrinol. 2017;5(8):585-96.
3. Nguyen CL, Pham NM, Binns CW, Duong DV, Lee AH. Prevalence of gestational diabetes mellitus in eastern and southeastern Asia: a systematic review and meta-analysis. J Diabet Res. 2018;6536974:1-10.
4. Ramachandran A, Snehalatha C, Shyamala P, Vijay V, Viswanathan M. Prevalence of diabetes in pregnant women- a study from southern India. Diabet Res Clinic Pract. 1994;25(1):71-4.
5. Nallaperumal S, Bhavadharini B, Mahalakshmi MM, Maheswari K, Jalaja R, Moses A, et al. Comparison of the world health organization and the International association of diabetes and pregnancy study groups criteria in diagnosing gestational diabetes mellitus in South Indians. Ind J Endocrinol Metabol. 2013;17(5):906.
6. Government of India. Maternal and Health Division, Diagnosis and Management of Gestational Diabetes Mellitus: Technical and Operational Guidelines. New Delhi, India: Ministry of Health & Family Welfare, New Concept Inform Syst; 2018.
7. Jarmuzek P, Wielgos M, Bomba-Opon D. Placental pathologic changes in gestational diabetes mellitus. Neuro Endocrinol Lett. 2015;36(2):101-5.
8. Gabbay-Benziv R, Baschat AA. Gestational diabetes as one of the great obstetrical syndromes–the maternal, placental, and fetal dialog. Best Pract Res Clinic Obstetr Gynaecol. 2015;29(2):150-5.
9. Seshiah V, Das AK, Balaji V, Joshi SR, Parikh MN, Gupta S. Gestational diabetes mellitus-guidelines. JAPI. 2006;54:622.
10. Benirschke K, Burton GJ, Baergen RN. Early development of the human placenta. In Pathology of the human placenta 2012:41-53. Springer, Berlin, Heidelberg.
11. Singer DB. The placenta in pregnancies complicated by diabetes mellitus. Perspect Pediatr Pathol. 1984 Fall;8(3):199-212.
12. Ramos A, Caimari F, Pujol IM, García-Patterson A, Ginovart G, Adelantado JM, Corcoy R. In women with gestational diabetes mellitus factors influencing growth have a larger effect on placental weight than on birth weight. Euro J Obstetr Gynecol Reproduc Biol. 2016;202:60-5.
13. Kucuk M, Doymaz F. Placental weight and placental weight-to-birth weight ratio are increased in diet-and exercise-treated gestational diabetes mellitus subjects

but not in subjects with one abnormal value on 100-g oral glucose tolerance test. J Diabet its Complicat. 2009;23(1):25-31.
14. Taricco E, Radaelli T, De Santis MN, Cetin I. Foetal and placental weights in relation to maternal characteristics in gestational diabetes. Placenta. 2003;24(4):343-7.
15. Baptiste-Roberts K, Salafia CM, Nicholson WK, Duggan A, Wang NY, Brancati FL. Maternal risk factors for abnormal placental growth: the national collaborative perinatal project. BMC pregnancy and childbirth. 2008;8(1):44.
16. Edu A, Teodorescu C, Dobjanschi CG, Socol ZZ, Teodorescu V, Matei A, Albu DF, Radulian G. Placenta changes in pregnancy with gestational diabetes. Rom J Morphol Embryol. 2016;57(2):507-12.
17. Stillbirth Collaborative Research Network Writing Group. Association between stillbirth and risk factors known at pregnancy confirmation. JAMA: J Am Med Assoc. 2011;306(22).
18. Fox H. Pathology of the placenta in maternal diabetes mellitus. Obstetr Gynecol. 1969;34(6):792-8.
19. Ezimokhai M, Rizk DE, Thomas L. Maternal risk factors for abnormal vascular coiling of the umbilical cord. Am J perinatol. 2000;17(08):441-6.
20. Huynh J, Yamada J, Beauharnais C, Wenger JB, Thadhani RI, Wexler D, Roberts DJ, Bentley-Lewis R. Type 1, type 2 and gestational diabetes mellitus differentially impact placental pathologic characteristics of uteroplacental malperfusion. Placenta. 2015;36(10):1161-6.
21. Redline RW. Distal villous immaturity. MiniSymposium: Placental and Trophoblastic pathology. Diagnost Histopathol. 2012;18(5):189-94.
22. Paciencia M, Dolley P, Jeanne-Pasquier C, Jacob B, Sadfi A, Leseigneur P, et al. Acute-placental dysfunction by villous-maturation defect and late-fetal mortality. J Gynecol Obstet Biol Reprod. 2008;37(6):602-7.
23. Kovo M, Granot Y, Schreiber L, Divon M, Ben-Haroush A, Bar J. Pregnancy outcome and placental pathology differences in term gestational diabetes with and without hypertensive disorders. J Maternal-Fetal Neonat Med. 2016;29(9):1462-7.
24. Redline RW, Ravishankar S. Fetal vascular malperfusion, an update. Apmis. 2018;126(7):561-9.
25. Raymond W. Redline, Theonia K. Boyd, Drucilla J. Roberts. Diagnostic Pediatric Pathology: Placental and Gestational Pathol. Cambridge Uni Press; 2018:93-102.
26. Gorar S, Alioglu B, Ademoglu E, Uyar S, Bekdemir H, Candan Z, et al. Is there a tendency for thrombosis in gestational diabetes mellitus?. J Lab Physici. 2016;8(2):101.
27. Ogino S, Redline RW. Villous capillary lesions of the placenta: distinctions between chorangioma, chorangiomatosis, and Chorangiosis. Human Pathol. 2000;31(8):945-54.
28. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Development Pathol. 2003;6(5):435-48.
29. Boyd TK, Redline RW. Chronic histiocytic intervillositis: a placental lesion associated with recurrent reproductive loss. Human pathol. 2000;31(11):1389-96.
30. Ramya T, Chaitra V, Umamaheswari G. Chronic histiocytic intervillositis a rare placental cause of poor obstetric outcome: a clinicopathological study and literature review. Int J Reprod Contracept Obstet Gynecol. 2014 Dec;3(4):1146-1149.
31. Stanek J, Weng E. Microscopic chorionic pseudocysts in placental membranes: a histologic lesion of in utero hypoxia. Pediatr Development Pathol. 2007;10(3):192-8.

Natarajan L, Maheswari GU. Gestational hyperglycemia on diet and medication: impact on placental pathology and pregnancy outcomes. Int J Reprod Contracept Obstet Gynecol 2019;8:3350-6.

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A retrospective study of maternal mortality in a tertiary private medical college hospital in South India

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Abstract

Background: This study was designed to evaluate the institutional Maternal Mortality Ratio (iMMR) in our institution, a tertiary private medical college hospital and to suggest recommendations and possible interventions to reduce it.

Methods: This retrospective descriptive study was conducted by reviewing the hospital records over a period of ten years from January 2009-December 2018. The case records were reviewed for maternal demographic characteristics and complications.

Results: The total number of deaths during the study period was 21, giving an iMMR of 85.268 per 100000 live births. Most of the maternal deaths (>80%) occurred postpartum. Obstetric causes contributed to 57% of the deaths with hypertension and hemorrhage topping the list. Other causes were sepsis and non obstetric causes including one case of maternal suicide. 52.38% of the women died more than 48 hours after admission to the hospital, while 28.57% succumbed in less than six hours. Secondary complications noted were ICU admission, extended intubation, massive transfusion, operative intervention and multi organ dysfunction.

Conclusions: The classical triad of Hypertension, Hemorrhage and Sepsis continues to be the major determinant of maternal mortality and are potentially preventable by promoting universal access to quality health care, strengthening of health services and ensuring accountability.

Keywords: Hemorrhage, Hypertension, Maternal mortality, MMR, Multi organ dysfunction, Suicide

INTRODUCTION

In the year 2000, the United Nations adopted the Millennium Development Goals (MDG) out of which MDG 5 was targeted on reducing the Maternal Mortality Ratio (MMR) by three fourths between 1999-2015.[1] By the year 2015, Sustainable Development Goals (SDG) were introduced and SDG 3 aimed at Good Health and Well-Being.[2] Under this, the aim is to bring down the global maternal mortality to below 70 per 100000 by the year 2030.

At an MMR of 130 per 100,000 live births in 2016,India is ranked 128 in the world.[3,4] WHO commended India for its groundbreaking progress in reducing maternal mortality by 77% to the present rate.[3] Strategies responsible for this achievement include (i) Increased coverage of maternal health services and promoting institutional deliveries (ii) Incentives and financial support for women delivering in the public health system (iii) Improving literacy among women and increasing the average age at marriage (iv) promotion of public-private partnerships in the care of pregnant women.[3]

To put the country on track to achieve the SDG targets, Government of India established the NITI (National Institution for Transforming India) in the year 2015 to monitor, coordinate and ensure implementation of the SDGs.[2]

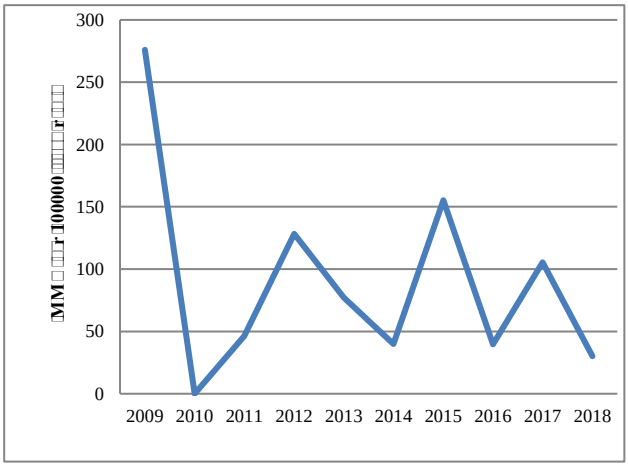
This study was designed to evaluate the MMR in our institution, a tertiary private medical college hospital and to suggest recommendations and possible interventions to reduce the institutional Maternal mortality ratio (iMMR). Continued and focussed efforts to end preventable deaths due to pregnancy and childbirth will help our country reach the SDG.

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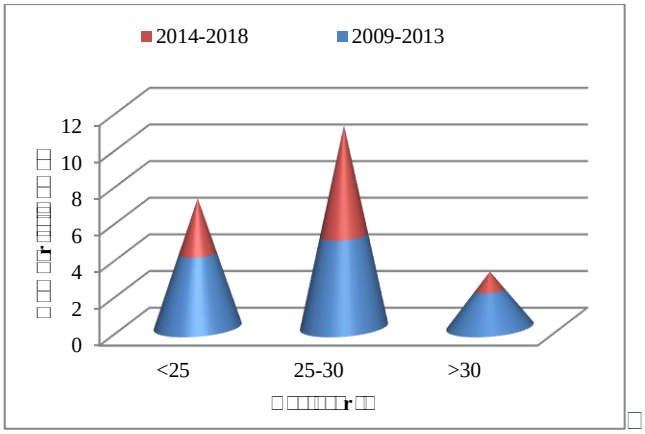
This retrospective descriptive study was undertaken in the Department of Obstetrics and Gynecology at the PSG Institute of Medical Sciences and Research, Coimbatore, India during the period from January 2009- December 2018. The approval of the Institutional Human Ethics Committee was obtained. Informed consent was not required since it was a retrospective study and there was no direct patient contact. The case records of all the women who died during pregnancy or within 42 days of delivery due to complications of pregnancy or other causes were reviewed for the following factors: age, obstetric history, antenatal complications and co morbidities, gestational age at onset of symptoms (if any), ICU admission, the need for ventilator support, blood transfusion, multidisciplinary management, cause of death and admission death interval. The number of live births during the study period was obtained from the parturition register. MMR was calculated as number of maternal deaths per 100000 live births.

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There were a total of 24628 live births in our institution during the study period. Data regarding the socioeconomic status, urban/rural divide were difficult to ascertain from the case records.



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The total number of deaths during the study period was 21, giving an iMMR of 85.268 per 100000 live births. The annual trend in iMMR has been pictorially represented in Figure 1. The age of the women ranged from 20-32 years (mean-25.9±3.6 years). The age distribution of the women is graphically represented in Figure 2.

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Hypertensive disorders	3	2	23.8
Haemorrhage	3	2	23.8
Sepsis	1	1	9.52
Anaemia	1	1	9.52
Viral infection	2	0	9.52
Peripartum Cardiomyopathy	0	2	9.52
Pulmonary embolism	0	1	4.76
Jaundice	1	0	4.76
Suicide	0	1	4.76

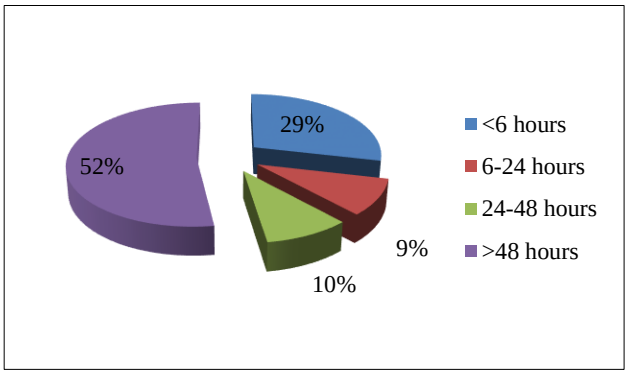
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ICU admission	8	7	71.42
Extended intubation>12 hours	4	5	42.87
Multiorgan dysfunction	4	5	42.87
Blood transfusion>5 units	6	3	42.87
Operative intervention	1	2	14.28

Majority of the women died postpartum, except for three. Two of the women who died antenatally were referred to our facility in a moribund state and died within two to three hours of admission despite intensive resuscitative measures. Ten of the women were multiparous and eleven of them were primiparous. Referrals from outside facilities were predominant in the first five years. Obstetric causes dominated the scenario in the first five years (6/11), whereas deaths during the next five years were mainly due to medical disorders (Table 1).

52.38% of the women died more than 48 hours after admission to the hospital, while 28.57% succumbed in less than six hours of admission (Figure 3).

Secondary complications noted were ICU admission, extended intubation, massive transfusion, operative intervention and multi organ dysfunction. Commonest among the above was ICU admission (71%) and the least common was operative intervention (14.28 %) (Table 2).



Maternal mortality is considered an index of the quality of healthcare services in a society. MMR of Tamil Nadu (a state in South India) at 66 per 100,000 live births is low as compared to most of the states in India.[4]

The lower rates are due to a higher proportion of institutional deliveries, effective healthcare delivery system and high quality emergency obstetric care provided by CEmONC (Comprehensive Emergency Obstetric and Newborn Care) centres.

In the present study, the highest mortality rates were in the age group 25-30 years, which is comparable to those of similar studies from South India.[5,6] This may be explained by the fact that the highest proportion of the pregnant population belongs to this age group.71.43% of the women were primiparous as against other studies where multiparous women formed the majority.[7] Both vaginal deliveries and cesarean sections were encountered equally in our series.

A fluctuating trend in iMMR was observed during the study period with peaks during the years 2009, 2012 and 2015. This was a chance observation since the women were referred from maternity units from the surrounding areas and there was a modest but steady increase in the number of deliveries over the years.

Major proportion of the deaths occurred in the postpartum period (80.95%).[6,8] One perimortem caesarean section was done on a patient with peripartum cardiomyopathy (PPCM) who had a cardiac arrest on table.

Two thirds of the women who died were referred from other hospitals.[5] Increased mortality was seen in patients who were referred from other hospitals, due to the bad general condition in which they were received and the time delay. The type of delay could not be assessed in the referral cases as the primary care was given elsewhere.

Obstetric causes of maternal mortality include those resulting from obstetric complications of the pregnant state (pregnancy, labour and puerperium). Non obstetric causes result from pre existing diseases or those that develop during pregnancy and which is not due to direct obstetric cause, but was aggravated by the physiological effects of pregnancy.[9]

Interestingly, 57% of the deaths were due to direct obstetric causes in contrast to those by Sowjanya kumari et al (85%), B. Paul et al (76.7) %, Murthy et al (72.5%) and similar to Khandale SN et al (61%).[6,10-12]

The leading causes of death were hypertensive disorders (23.8%) and haemorrhage (23.8%) comparable to the study by Paily et al. The major cause of death in women with hypertension in our series was hepatic dysfunction (60%).[13]

Severe preeclampsia is associated with high rates of maternal mortality and morbidity. The major contributors to maternal mortality in preeclampsia are eclampsia, HELLP, cerebral haemorrhage, renal dysfunction and coagulopathy. The two pronged approach to management of preeclampsia, i.e., control of hypertension with Labetalol and seizure prophylaxis with Magnesium sulphate has to be adopted to minimise complications.[14] Standard protocols have to be in place for early detection, referral, appropriate treatment and timely delivery.[15]

80% of the women who succumbed to obstetric haemorrhage were referred from other hospitals in a critical condition and died within few hours of admission despite intensive resuscitative measures including obstetric hysterectomy. Atonic postpartum haemorrhage (PPH) was the cause of death in four cases while one patient died due to rupture uterus. Rupture uterus followed a second trimester termination in a woman with two previous cesarean sections with an inappropriate dose of misoprostol.

Active management of third stage of labour (AMTSL) and oxytocin as the first line uterotonic has been advocated as the gold standard in preventing complications due to PPH.[16] In health care settings without facilities like access to blood and blood products and specialist care, early referral will play a pivotal role in preventing mortality. Women at high risk for PPH could be appropriately managed antenatally and delivered in a tertiary centre so that complications arising henceforth could be anticipated and prevented. Staff in the tertiary centres have to be trained in protocols for management of PPH with frequent mock drills.

Since our institution is an urban centre, the prevalence of cases of severe anaemia was less.[13] This could be attributed to early identification of women with anaemia and institution of appropriate treatment. Still anaemia was a contributory factor in five women in our series. Blood transfusion for moderate anaemia close to delivery was the trigger for deterioration of the maternal condition in three instances. Two of the three women were diagnosed with transfusion reaction which led to acute renal failure requiring dialysis.[6,8] The importance of early detection of anaemia, its correction and the need for raising the threshold for transfusion is reiterated by these cases.

Two women in our study had a diagnosis of PPCM (9.52%) as compared to Creanga et al (10-15%).[17] One of them was diagnosed antenatally at 29 weeks and the other followed a normal vaginal delivery.

Four women had hepatic dysfunction and jaundice, out of which three were due to preeclampsia and one was due to viral hepatitis. All patients with hepatic dysfunction were referred from other hospitals due to availability of good intensive care facilities and were directly referred to the gastroenterology unit. The incidence of pulmonary embolism, hepatitis and sepsis in our series were similar to that of Singh et al.[8]

One death in our study was due to suicide. According to the ICD-MM guidelines published by the WHO all antepartum and post partum suicide deaths should be included as direct obstetric deaths even if it is not possible to establish the diagnosis of depression or puerperal psychosis.[18] This is a major deviation in maternal mortality reporting and has the potential to increase the MMR since hitherto unreported deaths will be accounted for henceforth.[13,19]

The commonest complications encountered by these women were prolonged ICU stay and ventilator support. Multidisciplinary care was required in more than 50% of our cases, commonest being the involvement of nephrologist, gastroenterologist and cardiologist. 29% of the women died within six hours of admission, whereas 52% succumbed to complications after 48 hours, the longest being 33 days.[5,7,8,12]

The only operative intervention performed was obstetric hysterectomy which was required in three women-two following failed medical management of atonic PPH and one following rupture uterus. 50% of our patients required multiple transfusions since obstetric haemorrhage was a leading cause of mortality, and coagulopathy due to hepatic dysfunction was also seen in a few cases.

More than 50% of deaths in our study were due to obstetric causes, which are largely preventable. This could be achieved by intensive monitoring with incorporation of early obstetric warning tools.[20] Early warning tools include monitoring of maternal vital parameters such as blood pressure, heart rate, temperature, oxygen saturation, respiratory rate and altered sensorium; and notification to the physician in case of any abnormality.

As with all retrospective studies, our study also has limitations. Our institution being a private medical college in a city with many other well equipped hospitals, we reported a lesser number of maternal deaths. Fewer numbers preclude adequate statistical analysis and hence reduce the power of the study. Higher proportions of cases were referred from far-off areas and hence details regarding the initial management could not be accessed. In addition, autopsy was not performed routinely and therefore, the exact cause of death could not be verified in many of the cases.

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Though the iMMR has shown a fluctuating trend, it has remained at acceptable levels in comparison to the state and national averages. The classical triad of Hypertension, Haemorrhage and Sepsis remains the major determinant of maternal mortality in our institution and most of them are preventable. By promoting universal health coverage, access to quality health care, strengthening of health services and ensuring accountability, we can strive to reduce deaths due to pregnancy and childbirth.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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1. World Health Organisation. Millennium Development Goals, 2000. Available at: https://www.who.int/topics/millennium_development_goals/about/en/. Accessed 10 April 2019.
2. United Nations. Sustainable Development Goals, 2016. Available at: https://www.un.org/sustainabledevelopment/blog/2015/12/sustainable-development-goals-kick-off-with-start-of-new-year/. Accessed 10 April 2019.
3. WHO Regional Office for South East Asia. June 2018. Available at: http://www.searo.who.int/mediacentre/features/2018/india-groundbreaking-sucess-reducing-maternal-mortality-rate/en/. Accessed 20 March, 2019.
4. National Institution for Transforming India. Available at: https://niti.gov.in/content/maternal-mortality-ratio-mmr-100000-live-births. Accessed on 16 January, 2019.
5. Rajeswari, Shobha. Maternal mortality in an urban tertiary care hospital of South India. Indian J Obstet Gynaecol Res. 2016;3(1):32-7.
6. Kumari JS, Revathi V, Reddy KS. A study of maternal mortality in Government Maternity

Hospital, Sri Venkateswara Medical College, Tirupati. Indian J Obstet Gynaecol Res. 2018;5(4):525:9.
7. Pipal VR, Chouhan B, Pipal DK, Chouhan M, Yaduvanshi N, Mahavar N. Study of maternal mortality at Umaid Hospital, Jodhpur: a review of five years. Indian J Obstet Gynaecol. 2018;6:6.
8. Singh S, Singh N, Nagar JK, Jain S. Maternal mortality at a tertiary health care: a retrospective study. Int J Reprod Contracept Obstet Gynecol. 2018;7(5):1773-7.
9. Park K, Park's textbook of Preventive and Social Medicine, 24th edition. Page 593. India. Banarsidas Bhanot Publications. 2017.
10. Paul B, Mohapatra B, Kar K. Maternal deaths in a tertiary health care centre of Odisha: an in-depth study supplemented by verbal autopsy. Indian J Community Med. 2011;36(3):213-6.
11. Murthy BK, Murthy MB. Maternal mortality in tertiary care hospital: A 10 years review. Int J Prevent Med. 2013;4(1):105-9.
12. Khandale SN, Kedar K. Analysis of maternal mortality: a retrospective study at tertiary care centre. Int J Reprod Contracept Obstet Gynecol. 2017;6:1610-3.
13. Paily VP, Ambujam K, Rajasekharan Nair V, Thomas B. Confidential review of maternal deaths in Kerala: a country case study. BJOG. 2014;121:(Suppl. 4):61-6.
14. Lo JO, Mission JF, Caughey AB. Hypertensive disease of pregnancy and maternal mortality. Curr Opin Obstet Gynecol. 2013;25:124-32.
15. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. Semin Perinatol. 2012;36:56-9.
16. WHO. Available at: www.who.int/reproductive health/publications/maternal_perinatal_health/9789241548502/en/WHO recommendations for the prevention and treatment of postpartum haemorrhage-2018 update. Accessed 16 January 2019.
17. Creanga AA, Berg CJ, Jean Y. Maternal mortality and morbidity in the United States: Where are we now? J Women's Health. 2014;23:3-9.
18. WHO. The WHO Application of ICD-10 to deaths during pregnancy, childbirth and the puerperium: ICD-MM. Geneva: World Health Organization, 2012. Accessed on 20 March, 2019.
19. Fuhr DC, Calvert C, Ronsmans C. Contribution of suicide and injuries to pregnancy-related mortality in low-income and middle-income countries:a systematic review and meta-analysis. Lancet Psychiatry. 2014;1:213-25.
20. Shields LE, Wiesner S, Klein C. Use of maternal early warning trigger tool reduces maternal morbidity. Am J Obstet Gynecol. 2016;214:527.e1-6.

Thangavelu R, Natarajan L. Towards safe motherhood to - are we moving in the right direction?: experience from an urban health facility in South India. Int J Reprod Contracept Obstet Gynecol 2019;8:2851-5.

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A retrospective analysis of ectopic pregnancy in a tertiary care hospital

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Abstract

Background: Ectopic pregnancy (EP) is a common, life threatening emergency during the first trimester and a significant cause for maternal morbidity and mortality. In any woman of reproductive age presenting with abdominal pain and vaginal bleeding, ectopic pregnancy should be considered. The objectives of the present study is to determine the incidence, risk factors, clinical presentation, type of ectopic pregnancy, treatment, morbidity and mortality.

Methods: Retrospective analysis of case sheets of patients admitted with ectopic pregnancy at PSGIMSR, Coimbatore during the period July 2011 to June 2016 was done. The details noted were age, parity, risk factors {previous abortion, previous EP, previous pelvic surgery, intrauterine contraceptive device, tubectomy, history of pelvic inflammatory disease}, treatment for infertility, clinical presentation, ultrasound findings, investigations including beta HCG value and hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity.

Results: During the five year, there were 12,407 deliveries at our hospitals and 88 cases of ectopic pregnancies were managed. The incidence is 6.6/1000 deliveries. Among them 44% belonged to the age group of 25-30 years and multi gravida were 73.8%. Most common risk factors were previous abortion (23.8%) and previous surgeries including caesarean section, tubectomy, appendicectomy, tubal microsurgery (23.8%). Most common presentation was pain abdomen (85.2%). Out of the 88 cases, 73 patients were managed surgically (82.9%) and 11 cases were managed successfully with methotrexate (12.5%). Three of them underwent conservative management and one had Uterine Artery Embolization.

Conclusions: Early diagnosis based on risk factors and timely intervention plays a main role in reducing morbidity and mortality associated with ectopic pregnancy.

Keywords: Beta HCG, Ectopic pregnancy, Methotrexate, Risk factors, Salphingectomy Transvaginal USG

Introduction

An ectopic pregnancy is implantation of fertilized ovum outside the normal endometrial cavity i.e, fallopian tube, ovary, caesarean scar, cervix and peritoneal cavity.[1] In the first trimester, ectopic pregnancy is the most common cause for maternal morbidity and mortality.[2] It has also been shown that EP reduces future fertility and increases the chance of subsequent EP.[3] The incidence of EP is increasing since the last decade, but the incidence of complications like rupture and morbidity are declining due to early diagnosis with serial Beta-HCG(human chorionic gonadotrophin) monitoring, transvaginal USG(ultrasonogram) and minimally invasive surgery.[4] Early diagnosis not only reduces the risk of tubal rupture but also allows for conservative and medical management.[5] Prior damage to the fallopian tube due to PID(pelvic inflammatory disease), tubal surgeries, pelvic adhesions are the major risk factors for ectopic pregnancy.[6] The other identified risk factors are,

advanced maternal age, previous EP, previous pelvic surgeries, IUCD (intra uterine contraceptive device) insertion and infertility treatment.[7-12] Identifying these risk factors among high risk mothers helps in early diagnosis and reduces the morbidity related to this condition.[13] Unfortunately, most of the patients presenting with EP do not have risk factors.

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This was a retrospective analysis of case records of patients admitted with ectopic pregnancy, done at PSG IMSR Hospital, Coimbatore from July 2011- June 2016.

Inclusion criteria

We included all the patients with suspected ectopic pregnancy who were confirmed by βHCG and transvaginal ultrasound.

Exclusion criteria

None

Methodology

The case sheets were retrieved from medical record department and the details such as age, parity, risk factors like previous abortion, previous EP, previous pelvic surgery, contraception in the form of IUCD and tubectomy, history of PID, infertility treatment, clinical presentation, investigations including beta HCG value, hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity were collected and analyzed.

Statistical analysis

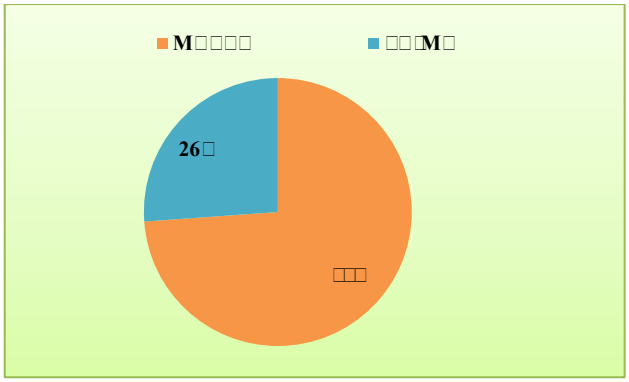
No statistical tool used.

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During the 5-year period, there were 12,407 deliveries at our institution and 88 cases of ectopic pregnancies were managed. The incidence was 6.6/1000 deliveries. Among the patients most of them belonged to the age group of 25-30 years (Table-1). 26% of patients were primigravida, 73.8% of patients were multigravida (Figure -1).

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< 25	22	25
25-30	39	44
31-35	18	20
36-40	5	5.6
>41	4	4.5



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As shown in (Table 2) 23.8% of patients had one or more spontaneous or induced abortions.23.8% of patients had undergone pelvic surgery (Caesarean section, tubectomy appendicectomy, tubal recanalistion). PID and previous ectopic pregnancy accounted for 4.5% each.

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Previous abortion	21	23.8
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Caesarean section	21	23.8
Tubectomy	14	15.9
Appendicectomy	3	3.4
Tubal recanalistion	2	2.2
Ovulation induction	7	7.9
□ □□□r □□□□□□□□		
IUCD insertion	6	6.8
PID	4	4.5
Previous ectopic	4	4.5

Commonest presenting complaint was pain abdomen in 85.2% of patients. Other presenting complaints were Amenorrhoea in 68% and vaginal bleeding in 59% (Table-3).

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Pain	75	85.2
Amenorrhea	60	68.1
Bleeding PV	52	59

Most of them presented between 5-8 weeks of gestational age (61.3%).27.2% of patients were diagnosed before 4 weeks of amenorrhea and 11.3% were above 9 weeks. All the cases had been confirmed by USG.17 of them had Beta-HCG less than 1000 m I U /ml. A patient with heterotopic pregnancy had Beta -HCG of more than 1 lakh m I U /ml.

Commonest site of ectopic pregnancy was fallopian tube in 93% of patients (Table 4).

[illegible]r [illegible]

[illegible]	[illegible] r [illegible]	[illegible]
Tube	82	93
Caesarean scar	3	3.4
Ovary	2	2.4
Heterotopic	1	1

The commonest site of tubal involvement was in ampulla (Table 5).

[illegible] [illegible]

[illegible]	[illegible] r [illegible]	[illegible]
Ampulla	60	73.1
Cornua	10	12.1
Isthmus	6	7.3
Fimbria	4	4.8
Tubal abortion	2	2.4

On receiving, 86% of patients were hemodynamically stable, 13.6% of patients were in various stages of shock. 53.6% of patients presented with ruptured ectopic, unruptured ectopic was noted in 43.9% of patients (Table 6).

[illegible] 6 [illegible] d [illegible]

[illegible] d [illegible]	[illegible] r [illegible]	[illegible]
Ruptured	44	53.6
Unruptured	36	43.9
Tubal abortion	2	2.5

Right sided tubal ectopic was noted in 65.8%, left side tube involved in 34.1% of patients. Tubal abortion was observed in 2.5% of patients. Intra-operatively hemoperitoneum was noted in 64% of patients. Most common treatment offered for tubal ectopic was surgical, i.e. salpingectomy (Table 7).

[illegible] r [illegible]

r [illegible]	[illegible] r [illegible]	[illegible]
Medical	11	12.5
[illegible] r [illegible]		
Laparotomy	49	59.7
Laparoscopy	9	10.9
M d [illegible] d		
Laparotomy	8	9.7
Laparoscopy	2	2.4
Conservative	3	3.6

Twenty-one patients were fit into medical management, among them 12.5% of patients were managed medically with methotrexate successfully.

10 cases failed to medical management which were subsequently managed with either laparoscopic or laparotomy and salphingectomy.

3 cases were managed conservatively by observing the declining trend of Beta-HCG.

Other types of ectopic

Caesarean scar pregnancy was diagnosed in 3 patients, among them 2 patients underwent surgical excision. One patient was managed with UAE (uterine artery embolisation) followed by methotrexate. Heterotopic pregnancy following ovulation induction which resulted in twin intra uterine gestation and tubal ectopic was noted in one patient and she underwent emergency laparotomy and salpingectomy. Two patients were diagnosed as having ovarian ectopic and surgical excision was offered to both of them. Most common morbidity noted was anemia in 42%, out of which severe anemia (4-7gm) was seen in 5.6% (Table 8). Among them 28.4% of patients received blood and blood products. No surgical site infection or mortality was noted.

[illegible] [illegible] [illegible] d [illegible]

[illegible] [illegible]	[illegible] r [illegible]	[illegible]
4-7 gm/dl	5	5.6
7-10 gm/dl	32	36.3
>10 gm/dl	51	58.1

[illegible]

The incidence of ectopic pregnancy among women attending emergency department with first trimester abdominal pain, bleeding or both is between 6-16 %(14). A study conducted by Shraddha Shetty et al had incidence of 5.6/1000 deliveries.[15] In our study the incidence is 6.6%/1000 deliveries which is comparable. Majority of patients were between 25-30years which is similar to the above study and Khaleeqe et al.[15,16]

As in Karki et al (17), we had similar higher incidence of ectopic pregnancies (73.8%) in multigravida. In our study, the commonest predisposing factors were previous abortion either spontaneous or induced, previous pelvic surgeries including caesarean section, history of IUCD insertion, tubectomy, history of PID and infertility treatment, which are similar to various other studies.[17,18] As in the studies by Porwal Sanjay et al, Shraddha Shetty et al, the most common symptom in our series was pain abdomen which is comparable.[15,18]

Urinary beta HCG, serum beta HCG, transvaginal USG are the diagnostic tools for diagnosing EP. USG should be the investigation of choice for symptomatic women. In asymptomatic women, serial monitoring of serum beta HCG should be done. As in the study by Shraddha Shetty et al, we also had more number of right sided EP and involvement of the ampullary portion of the tube.[15]

Heterotopic pregnancy is rare in natural conception which is around 1 in 30,000 pregnancies. But the incidence is more in case of ovulation induction or with

assisted reproduction techniques. In our study, we had a case of triplet heterotopic pregnancy following ovulation induction with clomiphene citrate. It is recommended that even patients shown on ultrasound to have intrauterine pregnancy should be given comprehensive pelvic ultrasound so that possibility of synchronous heterotopic pregnancy can be excluded, particularly in pregnancies following fertility treatment.[19]

In our study, ruptured EP was noted in 53% of cases, 43.9% of cases had an unruptured ectopic gestation. In Latchaw G et al study, the same were 59% and 41% respectively.[20]

Laparoscopy and medical therapy have now emerged as the widely used therapeutic modalities with great success in terms of reduced morbidity, shorter hospital stay and conservation of fertility.[21] However choice depends upon early identification of ectopic pregnancy and hemodynamic status of the patients.[22] In our study, 11% of patients responded well to single dose of methotrexate.

Surgery should be the treatment of choice in unstable patient.[19] In our study 13.6% were unstable and all of them were managed surgically. Commonest surgery performed was partial/total salpingectomy. We did bilateral total salpingectomy for patients who had undergone tubectomy previously and were not desirous of future childbearing. It has been extensively studied and reported that conservative management does not increase the risk of future ectopic.[15] In our study, three patients were managed conservatively by observing the declining trend of Beta HCG. Morbidity included anemia, blood transfusion and wound infection. In our study, we had 42% of patients with hemoglobin level of less than 10grams. Among them 28.4% of patients received blood and blood products. No surgical site wound infection was noted.

No maternal mortality was noted in our study which is consistent with the study by A Abbas et al.[23] Early identification of the patients with EP by a multipronged approach including high index of suspicion, risk prediction, serum beta HCG and transvaginal ultrasound helps in improving the prognosis as far as morbidity, mortality and future fertility are concerned.[24]

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Identification of underlying risk factors, early diagnosis with essential investigating modalities like beta-HCG, transvaginal USG, timely intervention with medical or surgical method will definitely reduce morbidity and mortality and improve the future reproductive outcome.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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1. Walker JJ. Ectopic pregnancy. Clin Obstet Gynecol. 2007;50:89-99.
2. Mahboob U, Mazhar SB. Management of ectopic pregnancy: a two-year study. J Ayub Med Coll Abbottabad. 2006;18:34-7
3. Chow WH, Daling JR, Cates WJ, Greenberg RS. Epidemiology of ectopic pregnancy. Epidemiol Rev. 1987;9:70-94.
4. Karaer A, Avsar FA, Batioglu S. Risk factors for ectopic pregnancy: a case-control study. Aust N Z J Obstet Gynaecol. 2006;46:521-7.
5. Barnhart KT. Clinical practice. Ectopic pregnancy. N Engl J Med. 2009;361:379-87.
6. Akande V, Turner C, Horner P, Horne A, Pacey A, British Fertility Society. Impact of Chlamydia trachomatis in the reproductive setting: British Fertility Society Guidelines for practice. Human Fertil. 2010;13:115-25.
7. Job-Spira N, Collet P, Coste J, Bremond A, Laumon B. Risk factors for ectopic pregnancy. Results of a case control study in the Rhone-Alpes region. Contracept Fertil Sex. 1993;21:307-12.
8. Bouyer J, Coste J, Shojaei T, Pouly JL, Fernandez H, Gerbaud L, et al. Risk factors for ectopic pregnancy: a comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol. 2003;157:185-94.
9. Parashi S, Moukhah S, Ashrafi M. Main risk factors for ectopic pregnancy: a case-control study in a sample of Iranian women. Int J Fertil Steril. 2014; 8:147-54.
10. Moini A, Hosseini R, Jahangiri N, Shiva M, Akhoond MR. Risk factors for ectopic pregnancy: a case-control study. J Res Med Sci. 2014; 19:844-9.
11. Ankum WM, Mol BW, Van der Veen F, Bossuyt PM. Risk factors for ectopic pregnancy: a meta-analysis. Fertil Steril. 1996;65:1093-9.
12. Barnhart KT, Sammel MD, Gracia CR, Chittams J, Hummel AC, Shaunik A. Risk factors for ectopic pregnancy in women with symptomatic first-trimester pregnancies. Fertil Steril. 2006;86:36-43.
13. Timmerman D. Predictive models for the early diagnosis of ectopic pregnancy.Verh K Acad Geneeskd Belg. 2004;66:155-71.
14. Murray H, Baakdah H, Bardell T, Tulandi T. Diagnosis and treatment of ectopic pregnancy. CMAJ. 2005;173:905.
15. Shetty KS, Shetty KA. A clinical study of ectopic pregnancies in atertiary care hospital of Mangalore, India. Innovat J Med H Sci. 2014;4:305-9.
16. Khaleeque F, Siddiqui RI, Jafarey SN. Ectopic pregnancies: A Three-year study. J Pak Med Assoc 2001;51:240-3.
17. Karki RCL, Pradhan B, Duwa S. Annual Analysis of Ectopic Pregnancy in Tertiary Care Hospital. PMJN. 2011;11:5-8
18. Gupta R, Porwal S, Swarnkar M, Sharma N, Maheshwari P. Incidence, trends and risk factors for

Ectopic Pregnancies in a tertiary care hospital of Rajasthan. JPBMS. 2012;16:1-3.
19. Maheswari S, Panicker S. Triplet heterotopic pregnancy following ovulation induction with clomiphene citrate: a case report and review of literature Int J Rerod Contracept Obstet Gynecol. 2013;2:743-5.
20. Latchaw G, Takacs P, Gaitan L, Geren S, Burzawa J. Risk factors associated with the rupture of tubal ectopic pregnancy. GynecolObstet Invest. 2005;60:177-80.
21. Jurkovie D. Ectopic pregnancy. In: Edmonds DK, editor. Dew Hurst's textbook of Obstetrics and Gynecology. 7 ed. USA; Blackwell Publishers;2007
22. Shah N, Khan NH. Ectopic pregnancy: Presentation and risk factors. J Coll Physicians Surg Pak. 2005;15:535-8.
23. Abbas A, Akram H. Ectopic Pregnancy; Audit at Maula Bakhsh Teaching Hospital Sargodha.Prof Med J. 2011;18:24-7.
24. Lozeau AM, Potter B. Diagnosis and management of ectopic pregnancy. Am Fam Physician. 2005; 72:1707-14.
25. Majhi AK, Roy N, Karmakar KS, Banerjee PK. Ectopic pregnancy--an analysis of 180 cases. J Indian Med Assoc. 2007;105:308-10.

Maheswari S, Poornima C, Lalitha N, Panicker S. Analysis of various types of ectopic pregnancies: a five-year review. Int J Reprod Contracept Obstet Gynecol 2018;7:2410-4.

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Fetal medicine as a part of early clinical exposure

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Abstract:

CONTEXT: Clinical relevance of basic sciences is not well appreciated by the preclinical students. In an effort to remedy this, Medical Council of India has introduced the program of early clinical exposure. Although broad guidelines are provided, institutions have been bestowed with the responsibility of conducting this important program depending on the available resources and local logistics.

AIMS: The aim of the study is to create an awareness of correlation of fetal medicine with the participants taught during preclinical year, particularly embryology.

SETTINGS AND DESIGN: Preclinical students were posted in the Division of Fetal Medicine in the Department of Obstetrics and Gynecology, as observers. The program enabled the students to appreciate the procedures performed in the clinics and to interact with the consultants and health-care staff during their posting. They interacted with the patients as well.

SUBJECTS AND METHODS: Preclinical students observed the clinical procedures performed in the clinics. Tests were conducted before and after the intervention, and their results were compared to evaluate the effectiveness of the program. Open-ended feedback was also taken to assess the impact of this program on the students.

STATISTICAL ANALYSIS USED: Fisher's exact test was used to evaluate the test scores.

RESULTS: Test scores revealed that this program improved the general awareness about the scope of fetal medicine and its direct relevance to the fetal developmental events; they learn in embryology. Feedback comments reiterated this fact.

CONCLUSIONS: Posting of preclinical students in fetal medicine during ECE program has observed the desired effect of them realizing the clinical significance of subjects; they are learning during their preclinical year of study. It has also impressed upon them the scope of fetal medicine as a subject.

Keywords:

Affective domain, early clinical exposure, fetal medicine

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Introduction

Medical Council of India had mandated a program of "Early clinical exposure" (ECE) to be implemented during the preclinical training period of undergraduate training, which has now been endorsed by National Medical Council as well.[1] This program aims at bringing out clinical relevance of preclinical participants and link it to patient care. Implementation of this program was made easy in our institution, since we had initiated a unique program of "Clinical Observership" earlier on 2001 itself, the learning objectives of which matched very much with that of ECE.[2] During the 1st year of the MBBS course, students were posted in selected clinical departments, wherein they had the opportunity of being in the hospital surroundings, observing a few clinical procedures such as blood transfusion, cardiac catheterization, and dialysis. They also had the opportunity of interacting with the patients as well as the clinical consultants. At present, ECE is being held in

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two rotations of five clinical departments each. Clinical departments for this important program have been identified, based on their direct clinical relevance and the willingness of the faculty to be part of this program. Section of fetal medicine, within the Department of Obstetrics and Gynecology, was one such department, where students got posted as part of ECE, during the past academic year.

Significance of introducing preclinical students to live clinical scenarios in the hospital environment has been reported from quite a few European countries and also from South Africa, as well.[3-5] There seems to be a paucity of information regarding the conduct and content of ECE from our country. Students of our institution have reported their experience of exposure to ECE in the departments of cardiology, neurology, and nephrology and the clinical simulation laboratory.[6-9] To our knowledge, there has been no report of fetal medicine, as a component of ECE and hence this report.

Subjects and Methods

First-year medical students divided into groups of ten were posted in the Fetal Medicine Unit of the Department of Obstetrics and Gynecology as observers for half a day, as part of the ECE program. This study was approved by the Institutional Human Ethics Committee at our institution (No: 17/335).

Design of the program

The program was designed such that it enabled the students to observe, assimilate all the proceedings in the clinic during their postings, and to interact with the consultants and health-care staff during their posting. Students could interact with the patients as well. A questionnaire related to the core content of the program was administered as a pretest and posttest to assess the impact of the program on the students.

The students got the opportunity to observe ultrasound examinations of antenatal cases during 11–14 weeks of pregnancy, which included nuchal translucency scan and the midtrimester (18–22 weeks) target scans. They were briefed on the normal appearance of each organ system in the first and second trimester, in correlation with the embryology that is being taught to them as part of their 1st year curriculum. In addition to observation of the ultrasound examinations, students had the opportunity to go through one of the following four basic modules, prepared for this program: central nervous system, gastrointestinal system, urogenital system, and the face and limbs. Two short videos on the development of the fetal CNS and the fetal circulation were added to integrate the knowledge of embryology into the program. The modules contained the normal appearance and a few common major anomalies of all the organ systems that are commonly encountered in current practice [Table 1].

Selection of the video graphs and modules depended on the clinical case, available for the program on that particular day. Anomalies, if any, encountered in the patient were discussed with reference to the underlying fetal pathology.

Results

Program evaluation

The program was evaluated quantitatively based on the pre and posttest scores. The test covered questions to assess their awareness of events during normal development, particularly of cardiovascular, pulmonary, and nervous systems and the possible congenital

Table 1: Content of the early clinical exposure course in fetal medicine

System	Topics included
CNS module	Normal appearance of:
	The spine, lateral ventricles, corpus callosum
	Cerebellum and vermis in the first and second trimesters
	These were compared to encephalocele, meningomyelocele/spina bifida, lateral ventriculomegaly, agenesis of corpus callosum
	Arnold Chiari malformation and vermian agenesis
	Dandy-Walker malformation
Gastrointestinal system	Normal appearance of the fetal stomach and bowel in comparison to diaphragmatic hernia, bowel dilatation in intestinal atresia/obstruction
	Physiological bowel herniation of the first trimester was shown as against omphalocele and gastroschisis
Urogenital system	Fetal kidneys and bladder
	Abnormalities such as renal agenesis, hydronephrosis, enlarged echogenic kidneys of infantile polycystic kidneys, cystic dysplastic kidneys, and bladder outlet obstruction as in posterior urethral valve were also brought out in this module
Face and limbs module	Normal appearance of the fetal face in three cardinal planes and the abnormalities encountered in each plane - absent nasal bone, cleft lip and palate, and frontal bossing
	Hands and feet were compared to anomalies such as club foot, syndactyly, polydactyly, and abnormally shaped long bones

CNS=Central nervous system

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anomalies. Qualitative assessment was performed based on their feedback comments.

Test scores

The test scores revealed that the students have improved their comprehension regarding the content of the program. Fisher's exact tests on the test scores proved that the postactivity responses have registered significant improvement regarding their awareness of fetal anatomy and physiology. The program has helped them to understand the general aspects of fetal medicine and the relevance of embryology to this clinical participant.

Feedback

Students have expressed their appreciation of the content of the modules presented to them. It was, in general, amazing experience for them to have visualized the events during embryonic development in a pregnant lady, who underwent the regular mandatory antenatal checkup. A few of their remarks have been appended to illustrate the students' response [Table 2].

Discussion

ECE as an effective and interesting learning program has been brought out in earlier observations.[6-12] ECE posting in fetal medicine becomes important, since the undergraduate medical student does not have the opportunity of getting exposed to the practice of fetal medicine, as a specialty in spite of their rather long postings in the Department of Obstetrics and Gynecology. The departments, selected for ECE, are the ones where an intern/compulsory rotatory residential intern does not get posted. Feedback analysis of the present batch of students reveals that the program in fetal medicine has provided them the rich opportunity of monitoring fetal growth and intrauterine events. Even though basic details of human development are expected to be learned in their embryology course, during the 1st year of MBBS, they do not have access to witness the intrauterine events in the conventional academic schedule. Embryology remains as a standalone purely theoretical. They tend to memorize the facts, without appreciating the intricacies of amazing embryological events. Exposure of the students to the field of fetal medicine has given them the opportunity to connect the abstract science with clinical medicine and reality. Analyzing their experience in fetal medicine, the observations indicate that they had gained practical insight into the human fetal development and some of its complications. A 1st year medical student finds embryology a tough subject, mainly because the events that happen *in utero* are not easy to imagine and comprehend. It is true that embryological events are depicted with varying clarity in three dimensions, in video graphs and youtube presentations. Yet, very many intricacies are left to the sheer imagination of the students. In the department of fetal medicine, students got to observe the routine ultrasound scans of pregnant ladies and to interact with the clinicians. They visualize position of the fetus during different trimesters and the ultrasound images of the developing organ systems. They also got the first-hand opportunity to appreciate that embryological events happen simultaneously with events interacting with parallel developments. This program has provided them the unique opportunity of getting an overall idea of the general behavior of the fetus in *utero* and eventually to consider the fetus as a patient.

Table 2: Selected comments about the course from the student feedback

The whole idea of early clinical exposure fascinated us, and in the department of fetal medicine, being able to see the ultrasonography appliances understand how the imaging correlates with the embryology we learn in anatomy was a fantastic experience
Fetal medicine and the idea of ultrasounds did not sound very appealing at the first. ECE in fetal medicine made all the difference. It was a very interesting session that made us want to learn more of diagnostic imaging and maternal-fetal health
Made us realize the significance of the intrinsic process of embryological events, which we had found tough to imagine, during our embryology classes. This session made the essential connect of the facts

ECE=Early clinical exposure

Students realized that fetal medicine is a specialty which calls for maternal-fetal interventions, which basically involve two patients – the mother and the fetus, demanding extremely thoughtful and careful approach. They witnessed the routinely conducted anomalies scans and appreciated the significance of identifying congenital anomalies, early during pregnancy.

Fetal medicine makes use of ultrasonography for routine screening procedures. It has been well documented that ultrasonography training equips a student for diagnostic skills. Students had the opportunity of realizing the value of this very important diagnostic modality, right during the preclinical year of their training itself.

Conclusion

Posting in fetal medicine has remarkably helped them to realize the role of ultrasound examination in patient care.[13,14] The experience has enhanced their awareness of contemporary medical practice, which happens to be one of the goals of ECE. New specialties are evolving in the practice of medicine, and this program has helped the students to realize one such specialty, during the very 1st year of their career in medicine itself. This exposure to the specialty could develop an interest in fetal medicine in the young medico's mind, early in his career. ECE provides an avenue to know recent advances and emerging specialties. It does not involve extra resources

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to implement an interesting and beneficial session of ECE in fetal medicine. The routine clinical material coming up in the clinic could provide the core content of such sessions. Our experience of posting the students for ECE in fetal medicine has been very encouraging and enriching, since it has contributed immensely to achieving the goals of successful ECE.

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Conflicts of interest

There are no conflicts of interest.

References

1. Medical Council of India. Vision 2015. New Delhi: Medical Council of India. Available from: https://old.mciindia.org/tools/announcement/MCI_booklet.pdf. [Last accessed on 2021 Sep 19].
2. Kumar PA, Kumar PN. Clinical observership: A desirable adjunct to preclinical training. Australas Med J 2011;4:294-9.
3. Tayade MC, Latti RG. Need of early clinical exposure in medical curriculum: Review. Int J Healthcare Biomed Res 2017;5:120-7.
4. Başak O, Yaphe J, Spiegel W, Wilm S, Carelli F, Metsemakers JF. Early clinical exposure in medical curricula across Europe: An overview. Eur J Gen Pract 2009;15:4-10.
5. Wenrich MD, Jackson MB, Wolfhagen I, Ramsey PG, Scherpbier AJ. What are the benefits of early patient contact? A comparison of three preclinical patient contact settings. BMC Med Educ 2013;13:80.
6. Devi V, Suriya N, Sundaram RS, Sumitra G, Kumar PA. Students' Perspective of their Postings in Cardiology during early clinical exposure. Indian J Clin Anat Physiol (IJCAP) 2017;4:389-91.
7. Thirumoorthy H, Nair SS, Azmi S, Aishwarya D, Catherine P, Sumitra G, Kumar PA. Students' perspective of their rotation in nephrology during Early clinical exposure. J Res Med Educ Ethics 2017;7:43-6.
8. Deepalakshmi K, Sathyavathi P, Kumar PA, Mambatta AK, Narasimhan D. Students and faculty perspectives towards early clinical experience in learning neurosciences. Natl J Res Community Med 2017;6:2277-3517.
9. Govindarajan S, Vasanth G, Kumar PA, Priyadarshini C, Radhakrishnan SS, Kanagaraj V, *et al*. Impact of a comprehensive early clinical exposure Program for preclinical year medical students. Health Prof Educ 2018;4:133-8.
10. Athipathy V, Kumar PA, Tolstoy R, Jeyakrishnan P, Mohanasundaram P, Reddy A. Basic life support through early clinical exposure: Students' perspective. Australas Med J 2016;9:319-23.
11. Rawekar A, Jagzape A, Srivastava T, Gotarkar S. Skill learning through ECE. J Clin Diagn Res 2016;10:JC01-4.
12. Verma M. Early clinical exposure: New paradigm in medical and dental education. Contemp Clin Dent 2016;7:287-8.
13. Kondrashova T, Kondrashov P. Integration of ultrasonography into the undergraduate medical curriculum: Seven years of experience. Mo Med 2018;115:38-43.
14. Chiem AT, Soucy Z, Dinh VA, Chilstrom M, Gharahbaghian L, Shah V, *et al*. Christian fox. Integration of ultrasound in undergraduate medical education at the California medical schools. J Ultrasound Med 2016;35:221-33.

Hyperreactio luteinalis following frozen embryo transfer managed by fetal reduction

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SUMMARY

We report a rare case of hyperreactio luteinalis (HL) which developed in association with triplet pregnancy in a 27-year-old woman who underwent assisted reproductive technology treatment for primary infertility. She had undergone frozen embryo transfer 2 months after ovarian retrieval. She presented in the first trimester with abdominal pain and distension associated with ovarian enlargement and did not respond to conservative management. Fetal reduction was done from triplets to twins which resulted in good symptomatic relief for the patient. Fetal reduction may be an effective approach in such situations when conservative strategies fail in HL.

BACKGROUND

Hyperreactio luteinalis (HL) is a rare condition occurring during pregnancy, associated with multiple theca lutein cysts causing bilateral ovarian enlargement. It is caused by elevated β-human chorionic gonadotropin (β-hCG) levels or an abnormal response to normal levels due to heightened sensitivity of the ovarian tissue to β-hCG receptors.[1] HL is commonly seen in association with gestational trophoblastic disease, multiple pregnancies, fetal hydrops, fertility treatment and isoimmunisation.[1] Generally, HL is benign, typically resolving postpartum and hence managed conservatively. Rarely HL can present as acute abdomen due to ovarian torsion or haemorrhage necessitating intervention.[2]

We describe a rare occurrence of HL following frozen embryo transfer (FET) which required intervention in the form of fetal reduction for symptomatic relief of the patient.

CASE PRESENTATION

A 27-year-old woman presented with primary infertility after 6 years of marriage. She had regular menstrual cycles, normal ovaries and uterus and normal hormonal profile (Follicle stimulating hormone (FSH): 5.4mIU/mL, LH: 4mIU/mL, Anti Mullerian hormone (AMH): 4.3mIU/mL). Her partner had severe oligoasthenozoospermia with sperm count of 2.5 million/mL and a total motility of 15%. Considering the severe male factor, they were advised artificial reproduction with intracytoplasmic sperm injection (ICSI). She reported on the second day of menstrual cycle with normal hormonal levels (E2: 2:5 pg/mL, Luteinising hormone (LH): 1.7mIU/mL; FSH: 5.4mIU/mL) and was started on 150 IU of recombinant FSH and 75 IU of human menopausal gonadotropin for ovarian stimulation. Follicle growth was monitored. She was started on a gonadotropin-releasing hormone antagonist 0.25 mg s.c from the sixth day of stimulation. She was maintained on the same dose of gonadotropins for 9 days as growth rates were normal. On the 10th day, she was triggered with recombinant human chorionic gonadotropin (Ovitrelle) 250 μg given subcutaneously. Estradiol levels on the day of trigger was 4200 pg/mL. Twenty oocytes were retrieved followed by ICSI which was done on 19 mature oocytes. This resulted in fertilisation of 15 embryos which were cryopreserved. Embryo transfer was deferred for fear of ovarian hyperstimulation. Post retrieval, she was monitored for signs of ovarian hyperstimulation and was asked to review after 2 months for FET.

Two months later, she was prepared for FET. Her hormonal assay on the second day of menstrual cycle and pelvic ultrasound was normal. She was started on oral estradiol valerate 2 mg two times a day for 14 days. Endometrial assessment on day 14 showed a triple line endometrium measuring 9 mm. Both ovaries appeared normal with follicles less than 5 mm in size. She was started on intramuscular progesterone 100 mg daily for 3 days followed by embryo transfer. Oral estradiol was maintained at 2 mg two times a day. Three embryos were transferred. After 14 days of transfer, her β-hCG was 2228 mIU/mL. Ultrasound done at 6 weeks of gestation showed triplet pregnancy with cardiac activity in all the three sacs. Both ovaries were normal in size. She was continued on intramuscular progesterone for luteal support. At 8 weeks of gestation, ultrasound showed trichorionic triamniotic pregnancy with normal cardiac activity in all sacs (figure 1A). Both ovaries were enlarged with right ovary measuring 7.4×4×4 cm and left ovary measuring 6.4×4.1×4.8 cm with multiple cysts in both ovaries (figure 1B,C).

INVESTIGATIONS

At 9 weeks, the patient developed nausea and vomiting with abdominal distension and pain. Ultrasound showed normally developing triplet gestation with bilateral enlarged ovaries (right: 15.8 cm × 7.7cm × 12.2 cm; left: 12 cm × 5.8 cm × 7.3 cm) with a 'spoke-wheel' appearance and minimal free fluid in the pelvis and pleural space bilaterally (figure 1D–F). At 10 weeks, her symptoms became worse and she could not sit or walk. She was admitted with a diagnosis of hyperreactio luteinalis. She had mild hypoproteinaemia and hypoalbuminaemia with no haemoconcentration (haemoglobin: 109 g/L; haematocrit: 33.7%). Her β-hCG was 200 000 mIU/mL.

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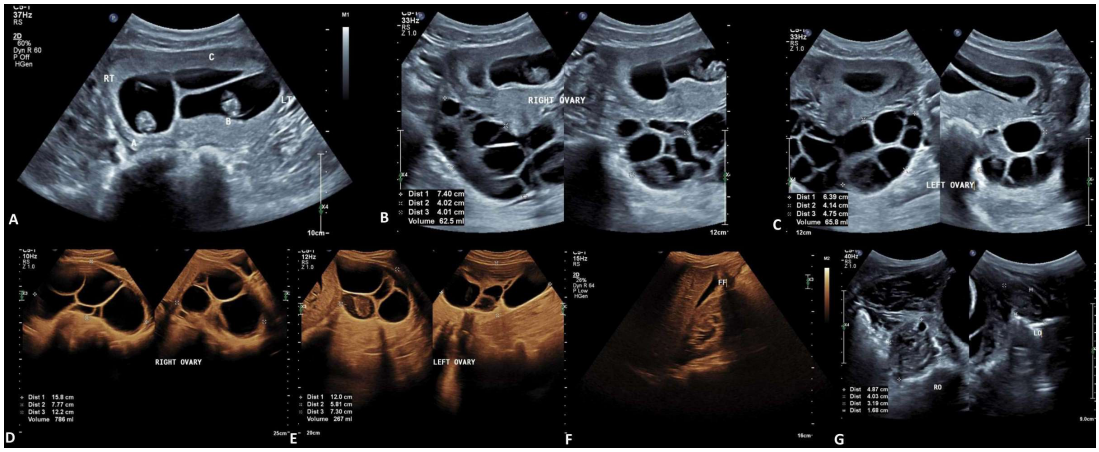


Figure 1 Ultrasound at 8 weeks of gestation showing triplets (A) and enlarged ovaries with multiple cysts (B and C). Ultrasound around 10 weeks showing increased enlargement of ovaries with a 'spoke-wheel' appearance and minimal free fluid in the pelvis (D, E and F). Both ovaries gradually decreased in size and returned back to normal size by 4 weeks of gestation (G).

TREATMENT

She was administered 20% 100 mL albumin every 4 hours and initiated on prophylactic heparin. The patient was offered medical termination of pregnancy or fetal reduction to singleton so as to decrease the β-hCG and give her symptomatic relief. After detailed counselling regarding the procedure, she opted for fetal reduction to twin gestation. Fetal reduction is beneficial in higher order multiples since it provides a reduction in the rates of miscarriages and preterm births. Reduction from triplet to a twin pregnancy in our patient would result in a reduction of pregnancy loss from 15% to 3.5%.[3] All the three fetuses were screened for aneuploidy with Nuchal translucency (NT) and markers such as nasal bone, tricuspid and ductus venosus flow and structural abnormalities were ruled out. The fetus with the highest NT and the one that is farthest from the cervix was chosen for reduction. The procedure was performed at 11 weeks of gestation under local anaesthesia with the transabdominal insertion of a 20 G × 15 cm needle. Under continuous ultrasound guidance, potassium chloride was instilled into the fetal thoracic cavity and the needle withdrawn after cardiac asystole was confirmed.

OUTCOME AND FOLLOW-UP

Post reduction, she improved symptomatically and the β-hCG level dropped to less than a lakh mIU/mL in 3 days. She could sit and walk now and was discharged to home with twin pregnancy. Pregnancy was closely monitored as per protocol for twin gestation. Both ovaries gradually decreased in size and returned back to normal size by 4 weeks of gestation (figure 1G). There were no signs of virilisation. At 34 weeks of gestation, one of the fetuses had abnormal Doppler findings showing low resistance flow at middle cerebral artery with reversal of cerebroplacental ratio. In view of the abnormal Doppler, the patient underwent caesarean section and delivered a healthy baby boy and a baby girl of 2 kg weight each with Apgar scores of 9 at 1 min and 5 min.

DISCUSSION

Our case of HL is unique in many ways. First, there are only two such reported instances in the literature of this condition developing after FET.[4 5] Second, HL in the first trimester is very rare in contrast to its more common occurrence in the third trimester and postpartum. This makes it difficult to differentiate it from spontaneous ovarian hyperstimulation syndrome (OHSS).[6] Third, the deviation from the more commonly advocated strategy of conservative management for HL in our patient. Progressive ovarian enlargement resulted in the patient becoming bedridden at 10 weeks. In such a scenario, conservative approach is not an option and we proceeded with fetal reduction. Ours is only second such instance in the literature of fetal reduction being employed for the management of HL.[7]

The ovarian enlargement occurring in the first trimester of pregnancy following assisted reproductive technology (ART)

Table 1 Outline of differences between hyperreactio luteinalis (HL) and ovarian hyperstimulation syndrome (OHSS)

Characteristics	OHSS	HL
Pathophysiology	1. Defect of the aromatase gene and FSH receptor 2. Abnormal sensitivity to vascular endothelial growth factor	1. Constantly elevated hCG levels 2. Increased β-hCG receptor sensitivity of the ovaries
Common association	Fertility treatment, commonly ovarian stimulation with exogenous gonadotrophins	Nulligravida and multigravida
Relationship to pregnancy	With or without pregnancy	Only with pregnancy
Time of occurrence	First trimester	Late trimesters and post partum
Main clinical feature	Ascites	Ovarian enlargement
Extra ovarian symptoms	Virilisation is rare	Virilisation in 25% of patients
Clinical course	More acute	More indolent
Life-threatening complications	More frequent—includes fluid shifts resulting in hypovolemia and thrombosis	Less frequent—misdiagnosis as ovarian tumour is possible
Pregnancy-related complications	Preterm labour	Pre-eclampsia and fetal growth restriction

hCG, human chorionic gonadotropin.

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would make one consider OHSS as the closest differential diagnosis. The differences between the two entities are subtle rather than distinct and are detailed in table 1. Since there are many things in common between them including aetiology, clinical features and the predominantly conservative management, they may be considered as entities in continuum.[8] More important is the other differential of HL, which is ovarian malignancy. In a review of 96 cases of HL, malignancy was suspected in 38% of patients with many inadvertently undergoing oopherectomy.[9] The bilaterality of involvement along with morphological regularity suggests more of functional abnormality rather than malignancy. The spoke-wheel appearance suggests theca lutein cysts without solid component. The rapid resolution of ovarian enlargement and free fluid along with reduction in the β-hCG levels following fetal reduction seen in our case suggests HL due to raised β-hCG.

Following ART, HL has been reported both with ovulation induction with gonadotrphins as well as FET.[4 5 10] Cases of HL following fresh cycles of IVF have not been reported. Increased β-hCG receptor sensitivity of the ovarian tissue may be more important than simple raise in β-hCG, since multiple pregnancies following fertility treatment, though common do not seem to predispose the individual to HL. Though majority of cases of HL are managed conservatively, more aggressive approach is suggested when the condition becomes life threatening as happened in our case. Fetal reduction may be one such approach which by decreasing the placental production of β-hCG reduces the ovarian enlargement bringing symptomatic relief to the patient. Also, it is a safe procedure since it neither interferes with the maternal health nor affects the prospects of future fertility.

Learning points

- Hyperreactio luteinalis (HL) is a rare condition in pregnancy associated with bilateral ovarian enlargement caused by elevated β-human chorionic gonadotropin (β-hCG) levels or increased sensitivity of the ovarian tissue to β-hCG receptors and commonly seen in association with gestational trophoblastic disease, multiple pregnancies and following fertility treatment.
- HL can also be seen in association with assisted reproductive technology procedures including frozen embryo transfer.
- Ovarian hyperstimulation syndrome and ovarian malignancies are the important differential diagnoses for HL and conservative strategies are the mainstay of management.
- In selected cases of multiple pregnancies presenting with HL in the first trimester, fetal reduction can bring symptomatic relief by decreasing β-hCG levels.
- The rare self-limiting entity of HL should be remembered while encountering ovarian enlargement in pregnancy so that unnecessary surgical procedures including oopherectomy are avoided.

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REFERENCES

1 Taylor HS, Pal L, Seli E. Normal and Abnormal Growth and Pubertal Development. In: *Speroff's clinical gynecologic endocrinology and infertility, 9e*. , 2000: 9, 363–4.
2 Li Q, Li X, Zhang P. Ovarian torsion caused by hyperreactio luteinalis in the third trimester of pregnancy: a case report. *Int J Clin Exp Med* 2015;8:19612–5.
3 Evans MI, Andriole S, Britt DW. Fetal reduction: 25 years' experience. *Fetal Diagn Ther* 2014;35:69–82.
4 Saisto T, Tiitinen A, Ulander V-M, *et al*. Clinical cure of severe, early onset preeclampsia with low molecular weight heparin therapy in primigravida with hyperreactio luteinalis and thrombophilia. *Hum Reprod* 2004;19:725–8.
5 Check JH, Choe JK, Nazari A. Hyperreactio luteinalis despite the absence of a corpus luteum and suppressed serum follicle stimulating concentrations in a triplet pregnancy. *Hum Reprod* 2000;15:1043–5.
6 Foulk RA, Martin MC, Jerkins GL, *et al*. Hyperreactio luteinalis differentiated from severe ovarian hyperstimulation syndrome in a spontaneously conceived pregnancy. *Am J Obstet Gynecol* 1997;176:1300–4.
7 Detti L, Phillips OP, Schneider M, *et al*. Fetal reduction for hyperreactio luteinalis. *Fertil Steril* 2011;96:934–8.
8 Suzuki S. Comparison between spontaneous ovarian hyperstimulation syndrome and hyperreactio luteinalis. *Arch Gynecol Obstet* 2004;269:227–9.
9 Cavoretto P, Giorgione V, Sigismondi C, *et al*. Hyperreactio luteinalis: timely diagnosis minimizes the risk of oophorectomy and alerts clinicians to the associated risk of placental insufficiency. *Eur J Obstet Gynecol Reprod Biol* 2014;176:10–16.
10 Yapar EG, Vural T, Ekici E, *et al*. Hyperreactio luteinalis masquerading as an ovarian neoplasm in a triplet pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1996;65:177–80.

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Maternal and perinatal outcome in pregnant women presenting with first trimester vaginal bleeding

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Background: First trimester bleeding is one of the common complications during pregnancy which affects almost 16-25% of all pregnancies. To evaluate and ascertain the adverse maternal and perinatal outcomes in pregnant women presenting with first trimester vaginal bleeding.

Methods: Prospective case-control study. A case control study involving 60 pregnant women with vaginal bleeding in the first 13 weeks + 6 days of gestational age with 60 matched controls. The study period was from March 2015 to March 2016 and conducted at PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu.

Results: The complications seen in the study group were: first trimester abortion (16.7%), second trimester abortion (6.7%), preterm labour (25%), abruption (6.7%), neonatal intensive care admission (25%), ectopic (6.7%), IUGR (10%), IUD (1.7%) and PROM (8.3%). When compared with the parity matched controls there was statistically significant increase in first and second trimester abortions, preterm labour, abruption, NICU admission and ectopic pregnancy whereas there was no significant difference between the two groups with regard to intrauterine growth restriction (IUGR) and intrauterine death (IUD).

Conclusions: Women with first trimester vaginal bleeding had several adverse outcomes in both the mother and the fetus, and it is very important to explain about the possibility of these outcomes and ensure proper follow up with close antenatal surveillance.

Keywords: Adverse maternal outcome, Adverse fetal outcome, First trimester threatened abortion, First trimester vaginal bleeding, Sub chorionic hematoma, Threatened miscarriage

Introduction

First trimester bleeding is one of the common complications during pregnancy which affects almost 16-25% of all pregnancies.[1,2]

The presumptive symptom of threatened miscarriage is the history of vaginal bleeding in early pregnancy with closed cervix. Meta-analysis indicates that vaginal bleeding increases the risk of pregnancy complications by twofold.[3] Vaginal bleeding may herald the initiation of spontaneous abortion, or it can be a sign of implantation of pregnancy, or it can be a pathological sign indicating gestational trophoblastic disease or ectopic pregnancy.

Vaginal bleeding during early pregnancy needs further evaluation to identify normal or abnormal pregnancy or any pathological condition which requires immediate intervention.[4,5] It is hypothesized that vaginal bleeding in first trimester may be an indication of an underlying placental dysfunction, which may manifest later on with adverse outcomes like first or second trimester abortion,

pre-eclampsia, preterm delivery, premature rupture of membranes, intrauterine growth restrictions and intra uterine death.[6]

This study aims to evaluate maternal and perinatal outcomes in women with first trimester vaginal bleeding.

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This study was approved by the institutional Human Ethics Committee (IHEC), PSG Institute of Medical Sciences and Research, which has been recognized by the Strategic Initiative for Developing Capacity in Ethical Review (SIDNER). This study was conducted among 120 subjects in PSG Hospitals. Among the 120 patients studied, 60 pregnant women who had first trimester vaginal bleeding were compared with 60 matched controls, over one year from March 2015 to March 2016.

All pregnant women who attended antenatal clinic with first trimester vaginal bleeding (<12 weeks) were included in this study. Women with multiple pregnancies, bad obstetric history, congenital uterine abnormality, known case of any bleeding disorders, chronic medical complications including diabetes and hypertension were excluded from this study. Informed consent was obtained and they were put under surveillance till delivery. Ultrasonography was performed for all these women as routine and serum beta-HCG was done in indicated cases. They were followed up once a month for the first six months, once a fortnight during seventh and eighth month and weekly during the last month of pregnancy. The data collected was analyzed and significant outcomes were reported.

Statistical analysis

Data collected from the patients were initially entered into Microsoft Excel-sheets analyzed using SPSS. Pearson's chi-square was used to compare the association between the first trimester vaginal bleeding and the adverse maternal and fetal outcomes. A predictive value of less than 0.05 was taken as statistically significant.

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In this study 120 women were included (those who met the inclusion criteria) among which 60 women were in control group and 60 in study group. The study and control group were selected based on the following demographic characteristics. The demographic data in each of these groups are represented in Table 1. There was no statistically significant difference between the two groups with respect to the baseline characteristics as age, height, weight, BMI and parity.

The maternal and perinatal outcomes of the women with first trimester vaginal bleeding were compared among the study and the control groups and represented in Table 2. There was significant difference between two groups in the following primary outcomes such as first and second trimester abortions, pre-term labor, abruption, NICU admission, ectopic pregnancy and nil complications. The results were statistically significant.

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IUGR	10% (6/60)	3.3% (2/60)	0.143
IUD	1.7% (1/60)	NIL	0.315
APGAR<7	10% (6/60)	3.3% (2/60)	0.143
PROM	8.3% (5/60)	5% (3/60)	0.464

□

The secondary outcomes such as intra-uterine growth restriction (IUGR), intrauterine fetal demise (IUD), premature rupture of membranes (PROM) and neonatal APGAR score of <7 were not statistically significant (Table 3).

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Vaginal bleeding is one of the most common and alarming early pregnancy symptoms. The aim of this study was to evaluate the risk factors associated with first trimester bleeding and the various adverse outcomes. Several studies had reported the association between vaginal bleeding in first trimester and the adverse maternal and perinatal outcomes which include abortion, preterm labor, low birth weight, intra uterine growth restriction. Most of these studies were retrospective.

This study was a randomized, prospective case - control study.

In this study, the amount of blood loss and its effects in pregnancy outcomes was also evaluated as it is important in predicting the risk of miscarriage. In the study group 8 among 10 women who had a first trimester miscarriage had heavy bleeding (quantified by the pictorial charts).

From this study it is clear that more than 80% of the women with first trimester miscarriage had heavy bleeding. Further analysis also suggests that pain associated with this heavy bleeding is also significant.

Whereas women with spotting per vaginum had comparatively good outcome. This result is similar to other studies which also indicate greater risk with heavy bleeding.[7]

This study showed that there was statistically significant difference in the first and second trimester miscarriage between the study and control groups. Many other studies have also documented that the rate of first and second trimester abortions in women with first trimester vaginal bleeding were approximately 20% and 18% respectively.[8-10]

Preterm labor and delivery are one of the most leading cause of neonatal death. This study showed that women with first trimester bleeding have significantly increased risk of having preterm labor in study group (25%) when compared to control group (6.7%) and premature rupture of membranes in study group (8.3%) when compared to control group (5%). This result is similar to the previous studies.[10,11]

A study by Saraswat et al which was a meta-analysis also suggested a higher incidence of preterm labor and premature rupture of membranes, but a similar study done by Strobino et al did not show any significant association between first trimester bleeding and preterm labour.[12,13] Placental abruption occurs in approximately 2% of all the pregnancies and the most common cause is first trimester bleeding. The hematoma that collects in between the chorionic plate and the uterus gradually increases in size and separates the placenta and causes heavy bleeding and eventually leads to abruption.

In this study the abruption rate in the study group was 6.7% whereas there was no abruption in control group. Hence this study suggests that women with threatened miscarriage have a very high risk of abruption hence close monitoring in very essential. The results of this study are similar with the findings of Weiss et al and Ananth et al, which also showed a significant incidence in abruption in women associated with first trimester bleeding, but on the contrary studies done by Wijesiriwardana and Jihn's et al did not show any significance in the relationship between the two.[14-16]

The incidence of IUGR (10%) and IUD (1.7%) in the study group was not statistically different from the control group (3.3% and 0% respectively). A similar outcome was seen in a study done by Amirkahani Z which also had an incidence of 1.7% (IUD) and 12% (IUGR) in women with first trimester vaginal bleeding.[8]

To our surprise some women also had a positive outcome by delivering a live baby without any complications such as preterm labour, placental abnormalities, PROM, IUD, NICU admissions or intra uterine growth restriction.

In this study about 16.7% of women in the study group had a good outcome with none of the above-mentioned complications when compared with the control group where 83.3% women had no complications. This result was statistically significant.

This result suggests that women who had first trimester threatened miscarriage, if given proper antenatal care and by routine ultrasound and regular follow up and specific

management, could deliver a live term baby without any complications.

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Considering the outcome of this study, women with first trimester vaginal bleeding had several adverse outcomes in both the mother and the fetus, and it is very important to explain about these outcomes and ensure regular close care antenatal surveillance.

Further, intervention by the clinicians has an important role in not only helping the women to continue the pregnancy but also help in reducing the maternal and fetal complications in high risk pregnancies. Hence precise treatment and planning is essential.

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1. Farrell T, Owen P. The significance of extra chorionic membrane separation in threatened miscarriage. Br J Obstet Gynaecol. 1996;103:926-8.
2. Bowe P, Murphy H. Complications of pregnancy following threatened abortion. Irish J Med Sci. 1987;156:328-9.
3. Ananth C, Savitz D. Vaginal bleeding and adverse reproductive outcomes: a meta-analysis. Paediatric Perinatal Epidemiol. 1994;8:62-78.
4. Snell BJ. Assessment and management of bleeding in the first trimester of pregnancy. J Midwifery Women's Health. 2009;54:483-91.
5. Dogra V, Paspulati RM, Bhatt S. First trimester bleeding evaluation. Ultrasound Q. 2005;21:69-85.
6. Weiss JL, Malone ED, Vidaver J. Threatened miscarriage: a risk factor for poor pregnancy outcome, a population-based screening study. Am J Obstet Gynecol. 2004;190:745-50.
7. Yang J, Savitz DA, Dole N. Predictors of vaginal bleeding during the first two trimesters of pregnancy. Paediatr Peri Epidemiol. 2005;19(4):276-83.
8. Amirkhani Z, Akhlaghdoust M, Abedian M, Salehi GR, Zarbati N, Mogharehabed M, et al. Maternal and perinatal outcomes in pregnant women with first trimester vaginal bleeding. J Family Reprod Health. 2013;7(2):57.
9. Agrawal S, Khoiwal S, Kumar J, Agarwa R. Predicting adverse maternal and perinatal outcomes after threatened miscarriage. Open J Obstet Gynaecol. 2013;2014.
10. Ishtiaq S, Qadir T, Ali HS. Maternal and perinatal outcome in threatened miscarriage in first trimester. ISRA Med J. 2014;6(3):169-72.
11. Bennett G, Bromley B, Lieberman E, Benacerraf BR. Sub chorionic hemorrhage in first-trimester pregnancies: prediction of pregnancy outcome with sonography. 1996;200(3):803-6.
12. Saraswat l, Bhattacharya S, Maheshwari A, Bhattacharya S. Maternal and perinatal outcome in women with threatened miscarriage in the first trimester: a systematic review. BJOG. 2010;117:245-57.
13. Strobino B, Pantel-Silverman J. Gestational vaginal bleeding and pregnancy outcome. Am J Epidemiol. 1989;129(4):806-15.
14. Weiss JL, Malone FD, Vidaver J. Threatened abortion: a risk factor for poor pregnancy outcome, a population-based screening study. Am J Obstet Gynecol. 2004;190(3):745-50.
15. Ananth CV, Oyelese Y, Prasad V. Evidence of placental abruption as a chronic process: associations with vaginal bleeding early in pregnancy and placental lesions. Eur J Obstet Gynecol Reprod Biol. 2006;128(1-2):15-21.
16. Wijesiriwardana A, Bhattacharya S, Shetty A. Obstetric outcome in women with threatened miscarriage in the first trimester. Obstet Gynecol. 2006;107(3):557-62.

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Adnexal torsion in reproductive age group: a retrospective study

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Abstract

Background: Adnexal torsion is one of the gynecologic emergency conditions requiring prompt diagnosis and surgical treatment to save the ovarian function. This retrospective study is intended to analyze the clinical manifestations and treatment in a tertiary care center.

Methods: This is a retrospective study Case records during five-year period of February 2012-2017 in PSG Institute of Medical Sciences and research, Coimbatore, India.

Results: Adnexal torsion was seen in all age groups and commonly in reproductive age. It was seen in few pregnant patients also. Lower abdominal pain of acute onset associated with nausea and vomiting was the commonest clinical feature. Ovarian cyst and PCOD were the common risk factors predisposing torsion. Though ultra sound was the common modality used, the diagnosis was done based on strong clinical suspicion with surgical confirmation. The ovaries were preserved in some patients of reproductive age group.

Conclusions: Adnexal torsion is one of the gynecologic emergencies. The symptoms and signs are nonspecific and may not have a confirmatory lab or imaging study. Hence a strong clinical suspicion followed by prompt surgical management in both pregnant and non -pregnant patients of any age group saves the ovary and further complications.

Keywords: Abdominal pain, Ovary, Torsion, Ultrasound

Introduction

Adnexal torsion is one of the gynecological emergencies affecting females of all ages. Ovarian torsion is defined as rotation of ovary or adnexa with the vascular pedicle on its axis resulting in arterial, venous,or lymphatic obstruction.[1]

It often presents as intermittent acute abdominal pain exacerbated by positional shifts.[2] Approximately 70% have symptoms of nausea and vomiting mimicking other surgical causes of pain like acute appendicitis or renal colic. So, the clinical scenario can lead to misdiagnosis.[3]

In addition, the conventional ultrasound may not be conclusive and the diagnosis can become difficult. Delay in diagnosis and treatment leads to necrosis of the affected ovary.

The aim of the study was to analyze the clinical manifestations and management done during the past five years.

Methods

A Retrospective analysis of 47 medical records with surgically confirmed cases of adnexal torsion was done. The clinical parameters taken for analyses taken were age, medical history, clinical manifestations and examination findings. In clinical presentation the type of abdominal pain with associated clinical findings were noted.

Additionally, whether the condition was associated with fever and leukocytosis was also noted. The imaging performed with its findings, operative details and histopathological reports of the specimens were also analyzed.

[illegible]

Clinical findings

The total number of patients with ovarian torsion were 47 in number. The median age of our patients was 22 years (18days-66years). There were 5 (10.6%) patients in premenarchal age group, 41 patients (87.2%) in reproductive age group and 1 (2.1%) in postmenopausal group. Acute onset of pain was the common symptom present in all patients (100%). The abdominal pain was diffuse and nonspecific in 21patients (44.6%), localized to right side in 19 patients (40.4%), and on the left side in 7 patients (14.8%). The next common symptom was nausea and vomiting seen in 37 (78.7%) patients followed by fever in 5 (10.6%) patients. A mass was palpable in 14 (29.7%) and tenderness elicited in 17 (36.17%) of the cases.

There were 3 (6.3 %) patients in early pregnancy with torsion.

Lab investigations revealed leukocytosis in 18 (38.2%) and anemia in 4 (8.5%) cases.

In all cases ultrasound along with Doppler was performed and torsion was reported in 25 patients (53.19%). 11 cases (23.4%) had minimal free fluid in pouch of Douglas. Ovarian cyst was reported in 31 cases (65.9%) in ultrasound. Of that 16 patients (51.6%) had cyst size of 5- 10cms, 11 patients (35.4%) had cyst size of more than 10cm and 4 patients (12.1%) had cyst size of less than 5cms. The contralateral ovary showed features of PCOD in 5 (10.6%) patients.

Operative findings

Of 47 patients with adnexal torsion, right adnexa was involved in 27 (57.4%) cases followed by 20 (42.5%) cases on left side. The risk factors like tubal ligation was noted in 3 (6.3%) cases, previous surgery adhesions in 1 (2.1%) patient.

Isolated fallopian torsion was seen in 3 (6.3%) in patients.

Detorsion with oophoropexy was done in 8 (17%) patients, ovarian cystectomy was done in 20 (42.5%) patients, unilateral salpingectomy in 3 (6.3%) patients, unilateral salphingo oophorectomy in 11 (23.4%) cases and total hysterectomy with bilateral salphingo oophorectomy in 5 (10.6%) patients. The patients with conserved ovaries were found to have normal ovarian function and asymptomatic on follow-up.

[illegible]

[illegible]	[illegible]	[illegible]
Serous cyst adenoma	6	12.77
Serous cyst adenofibroma	1	2.13
Serous cyst adeno carcinoma	1	2.13
Mucinous cyst adenoma	2	4.26
Teratoma	2	4.26
Follicular cyst	1	2.13
Corpus luteal cyst	4	8.51
Paraovarian cyst	5	10.63
Endometriotic cyst	1	2.13
Total specimens with haemorrhagic necrosis	17	36.17

Histopathological examination was done in all excised specimens and results are shown in Table 1. The common finding seen in specimens was hemorrhagic necrosis (36.1%) and the common benign tumor noted was serous cyst adenoma in 6 patients (12.7%).

[illegible]

Adnexal torsion is the reported to be the fifth most common gynecological emergency condition encountered with a prevalence of 2.7%.[4] Though it is fifth most common emergency it poses difficulty to diagnose clinically due to nonspecific presentation. The lower quadrant abdominal pain described in adnexal torsion is of sudden onset usually following exercise or jerky movement. It is described as sharp, stabbing or less frequently crampy.[5] The ultrasound findings of ovarian torsion may change depending on the duration and degree of torsion (Complete or incomplete) and the presence or absence of ovarian mass.[6,7] The other adnexal pathologies like endometriosis, hemorrhagic cysts, ectopic pregnancy, pelvic inflammatory disease form complex adnexal mass and make it difficult to differentiate sonographically.[4,8,9]

All age groups are susceptible to develop adnexal torsion though it is commonly seen in reproductive women.[3,10-12] Majority of our cases in this study also were in younger reproductive age group (Table 2). It commonly occurs in normal ovaries in young children.[13] Torsion was seen in 5 (10.6%) of our cases in premenarchal age group. Our youngest patient was 18 days neonate, prenatally diagnosed to have an ovarian cyst. Oltmann SC et al reports occurrence of 16% in infant population.[14] It was less common in post-menopausal women in this study.

The most common symptom seen in all our cases (100%) was acute onset of abdominal pain. Other studies also reported the increased incidence of acute abdominal pain.[12,15] In respect to site of pain 21 (44.6%) cases had diffuse pelvic pain, 19 (40.4%) cases had pain on right flank and 7 (14.8%) cases on the left flank. The site of pain may not always denote the site of torsion because twisted adnexal masses are often midline, positioned cranial to the uterine fundus of abdominal pain.[1] The next

common symptom in this study was nausea and vomiting seen in 37 (78.7%) cases similar to other studies.[11,14] In this study palpable mass was seen in 14 (29.7%) cases and tenderness was demonstrated only in 17 (36.1%) cases and was similar to another study.[12] Other symptoms like vaginal bleeding and urinary symptoms reported in other studies were not noted in this study.[11,16]

Table 2: Comparison of clinical presentation and management in various studies

Parameters	Present study	Comparison study	Comparison study
Number of patients	47	70	30
Age	18 days-66y	11y-91y	2 months-18 y
Premenarchal age	10.60%	2.90%	
Reproductive age <45yrs	87.20%	82.60%	
Parity	31.90%	50.00%	
Pregnancy	6.30%	2.90%	
Menopause	2.10%	17.00%	
Pain abdomen	100.00%	95.70%	100.00%
Nausea/Vomiting	78.70%	65.70%	56.70%
Fever	10.60%	12.90%	20.00%
leukocytosis	38.20%	44.00%	63.30%
Palpable mass	29.70%	42.90%	
Tenderness	17.00%	25.70%	
USG diagnosed torsion	53.19%	25.70%	63.00%
USG Free fluid POD	23.40%	23.80%	26.70%
Right side	57.40%	55.70%	70.00%
Left side	42.50%	42.90%	30.00%
Size 5-10cm	51.60%	71.40%	
Size > 10cm	35.40%	11.40%	
Serouscystadenoma	12.70%	15.70%	
Mucinouscystadenoma	4.20%	7.10%	3.30%
Teratoma	4.20%	22.80%	16.70%
Hemorrhagic necrosis	36.10%	30.40%	
Conservative surgery	59.50%	54.30%	46.70%
Radical surgery	40.30%	45.70%	53.30%

Adnexal torsion can occur rarely in pregnancy. The incidence reported is 1 in 5000 and occurs more frequently in the first trimester in patients that underwent treatment for infertility with in vitro fertilization or ovarian stimulation.[17] Present study had 2 cases of adnexal torsion in first trimester and one case in second trimester of pregnancy. Similar case series also reports torsion common in first trimester and less common in second and third trimesters.[18] The diagnosis of torsion in pregnancy becomes more challenging and also there is tendency to go for conservative approach. Adnexal torsion requires surgical intervention in pregnancy with preservation of corpus luteum. Organ preservation is important for the continuity of the pregnancy and a study by Oelsener et al reports performing detorsion in all 40 cases of torsion regardless of the macroscopic appearance.[19]

Fever and leukocytosis was seen in 5 (10.6%) and 18 (38.2%) cases respectively. Nurith et al reports incidence of fever in 20% and leukocytosis in 43 % of cases.[15] Fever and leukocytosis occurs due to adnexal mass necrosis. Four patients had anemia, and of that one had 6gm%, 2 cases had 7gm% and one had 8gm% which is similar to another study.[11] Anemia is usually rare in cases with ovarian torsion and may be seen in few patients due to occurrence of hemorrhagic necrosis.

Ultrasound as a modality to diagnose torsion is found to have a senstivity of 63%-95% due to difficulty in interpretation.[1,14,20] Normal adnexal appearance is common in 69% of premenarchal patients.[21] Some studies[9,20] show enlarged ovary with multiple peripheral follicles as sign of torsion with a sensitivity of 64-74%. So, to improve the detection rate further studies have focused on blood flow using doppler.[22] Since there is possibility of arterial flow in cases of partial type or early adnexal torsion it led to further studies to improve detection rate to 88-95% by focusing on twisted pedicle described commonly as whirlpool sign.[1,23] In present study torsion was diagnosed using ultrasound with doppler in 53.19% of our cases. The ultrasound finding showing presence of ovarian cyst in 31 (65.9%) cases suggests that it is the common risk factor in most of our

cases, especially if the size was 5-10cm in 16 (51.6%) followed by size >10cms in 11 cases (35.4%) and size of <5cm in 4 (12.9%) cases. Ovarian cyst as common risk factor was reported by Oltmann SC et al.[24] It was noticed polycystic ovary in 5(10.6%) cases as the next common risk factor. In young women polycystic ovary is reported to be a common risk factor for adnexal torsion.[11]

Regarding the side of torsion our study showed right side was involved in 27(57.4%) cases and on left side in 20 (42.5%) cases. Other studies also similar findings. This could be because of long right ovarian ligament or presence of sigmoid on the left side.[12,14] Isolated tube torsion was seen in three (6.3%) cases. No specific risk factors like hydrosalpinx was seen in our cases.

Previously oophorectomy or salphingo oophorectomy was the preferred procedure fearing the possibility of gangrenous transformation and sepsis. Now recently the studies show preserved ovaries maintains its function on follow up.[25] In present study 8 (17%) cases had undergone detorsion and oopheropexy, 20 (42.5%) had ovarian cystectomy, 3 cases (6.3%) had unilateral salphingectomy, 5 cases (10.6%) had hysterectomy with bilateralsalphingo oophorectomy, and 11(23.4%) cases underwent unilateral salphingo oophorectomy. In total, conservative surgery was done in 59.5% of patients and radical surgery in 40.3% of patients (Table 2). Removal of adnexa was performed in patients having a gangrenous mass and ovarian preservation was done in cases having edematous or bluish black ovaries. On follow up patients with preserved ovaries were comfortable, was asymptomatic and showed evidence of ovarian function.

Histopathology examination (Table 1) showed benign conditions in most specimens and were associated with hemorrhagic necrosis commonly. The common benign tumor was serous cyst adenoma followed by mucinous cyst adenoma and teratoma. One of patients diagnosed as malignancy had torsion following the completion of third cycle of presurgical chemotherapy. But the pain was misdiagnosed as ureteric colic and was treated conservatively before taking up for surgery. There are other case series reporting the rarity of malignant tumour undergoing torsion.[24]

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Diagnosis of adnexal torsion is a challenge to both clinician and radiologist due to its nonspecific clinical and radiological findings. So, it requires strong clinical suspicion and radiological expertise. Early diagnosis and surgical management saves the ovary, thereby preserving its function.

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1. Vijayaraghavan SB. Sonographic whirlpool sign in ovarian torsion. J Ultrasound Medic. 2004;23(12):1643-9.
2. Mc Williams GD, Hill MJ, Dietrich CS. Gynaecologic emergencies. Surg Clin North Am. 2008;88:265.
3. Niafar F, Mirfendereski S, Rostamzadeh A. Diagnostic efficacy of sonography for diagnosis of ovarian torsion. Zahedan J Res Med Sci. 2014;16(11):37-9.
4. Hibbard LT. Adenxal torsion. Am J Obstet Gynecol. 1985;152:456-61.
5. Schraga ED. Ovarian torsion: Practice Essentials. Available at https://emedicine.medscape.com/article/2026938-overview
6. Rosado WM Jr, Trambert MA, Gosink BB, Pretorius DH. Adnexal torsion;diagnosis using Doppler sonography. AJR Am J Roentgenol. 1992;159:1251-3.
7. Fleischer AC, Stein SM, Cullianan JA, Warner MA. Colour Doppler sonography of adnexal torsion. J Ultrasound Med. 1995;14;523-8.
8. Warner MA, Fleischer AC, Edell SL, Thieme GA, Bundy AL, Kurtz AB, et al. Uterine adnexal torsion: sonographic findings. Radiology 1985;154:773-5.
9. Graif M, ItzchacY. Sonographic evaluation of ovarian torsion in childhood and adolescence. AJR AmJ Roentgenol. 1988;150:647-9.
10. Tsafrir Z, Hasson J, Levin I, Solomon E, Lessing JB et al. Adnexal torsion: cystectomy and ovarian fixation are equally important in preventing recurrence. Eur J Obstet Gynecol Reprod Biol. 2012;162:203.
11. Nair S, Joy S, Nayar J. Five year retrospective case series of adnexal torsion. JCDR. 2014 Dec;8(12):OC09.
12. Vijayalakshmi K, Reddy GM, Subbiah VN, Sathiya S, Arjun B. Clinico-pathological profile of adnexal torsion cases: a retrospective analysis from a tertiary care teaching hospital. JCDR. 2014 Jun;8(6):OC04.
13. Karman E, Beger B, Cetin O, Melek M, Karaman Y. Ovarian Torsion in the normal ovary. A Diagnostic Challenge in adolescent girls in Emergency Department. Med Sci Monit. 2017;23:1312-6.
14. Spinelli C, Buti I, Pucci V, Liserre J, Alberti E, Nencini L et al. Adnexal torsion in children and adolescents: new trends to conservative surgical approach-our experience and review of literature. Gynaecol Endocrinol. 2013:29(1):54-8.
15. Hiller N, Appelbaum L, Simanovsky N, Lev-Sagi A, Aharoni D, Sella T. CT features of adnexal torsion. Am J Roentgenol. 2007 Jul;189(1):124-9.
16. Lo LM, Chang SD, Horng SG, Yang TY, Lee CL, Liang CC. Laparoscopy versus laparotomy surgical intervention of ovarian torsion. J Obstet Gynaecol Res. 2008;34(6)1020-5.

17. Mancusco A, Broccio G, Angio LG, Pirri V. Adnexal torsion in pregnancy. Acta Obstet Gynecol Scand. 1997;76(1);83-4.
18. Ventolini G, Hunter L, Drollinger D, Hurd WW. Ovarian Torsion during pregnancy. Resident Staff Physician. 2007;12(1):1-4.
19. Oelsner G, Shashar D. Adnexal torsion. Clin Obstet Gynecol. 2006;49(3):459-63.
20. Graif M, Shalev J, Strauss S, Engelberg S, Mashiach S, Itzchak Y. Torsion of the ovary: sonographic features. Am J Roentgenol. 1984;143(6):1331-4.
21. Ganer Herman H, Shalev A, Ginat S, Kerner R, Keidar R, Bar J et al. Clinical characteristics of adnexal torsion in premenarchal patients. Arch Gynecol Obstet. 2016 Mar 1;293(3):603-8.
22. Fleischer AC, Brader KR. Sonographic depiction of ovarian vascularity and flow. Current improvements and future applications. J Ultrsound Med. 2001;20(3):241-50.
23. Lee EJ, Kwon HC, Joo HJ, Suh JH, Fleischer AC. Diagnosis of ovarian torsion with color Doppler sonography; depiction of twisted vascular pedicle. J Ultrasound Med .1998;17(2):83-9.
24. Oltmann SC, Fischer A, Barber R, Huang R, Hicks B, Garcia N. Cannot exclude torsion: a 15year review. Pediatric Surg. 2009;44:1212-6.
25. Lasso BC, Garrido PJ, Murcia PF, Granero CR, Vargas CV, Paredes ER. Ovarian torsion long term follow up of the black- bluish ovary after laparoscopic detorsion. Cir Pediatr. 2014;27(1):26-30.

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Evaluation of first trimester fasting blood glucose as a predictor of gestational diabetes mellitus

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Abstract

Background: Our goal was to find association between first trimester fasting blood sugar value compared with the second trimester oral glucose tolerance test value (75gm DIPSI criteria) for diagnosis of GDM. To find the efficiency of FBS and BMI as a screening test for GDM.

Material and Method: 270 antenatal patients having antenatal follow up from 1st trimester of pregnancy between June 2014 – May 2015. The study period was 12 months. Their fasting blood glucose levels was measured in first trimester. The patients were followed up during second trimester and 75 grams OGTT was done. Correlation between first trimester FBS, BMI versus second trimester OGTT were done. Screening property of both fasting blood sugar and BMI were calculated and compared using receiver operating characteristic (ROC) curves.

Result: Out of 270 patients, 14 patients developed GDM, 1 patient developed overt diabetes. With threshold of FBS>90 yielded sensitivity of 86%, specificity of 52%. FBS >90mg/dl can be considered as a threshold value for predicting GDM. With a threshold of BMI>24 yielded sensitivity of 60%, specificity of 67.5%, hence BMI >24 is a good predictor of GDM. Receiver operating characteristic curve shows FBS(AUC-0.69) has little more discriminatory power in identifying GDM than BMI(AUC=0.63**)**. P value <0.05 was considered statistically significant.

Conclusion: In conclusion, FBS measurement at first prenatal visit or at first booking will be useful to screen for previously undiagnosed pre-existing diabetes mellites and also help to predict the development of GDM earlier.

Keywords: Gestational, Diabetes mellites, Fasting, Blood, Sugar, BMI

Introduction

GDM is defined as ―glucose intolerance of variable degree with onset or first recognised during pregnancy. GDM has been recently reported to affect approximately 18% of pregnancies. According to World Health Organisation India has the largest number of cases of Diabetes in the world. As estimated 31.7 million people with diabetes in 2000 in India are projected to increase to 79.4 million in 2030.[(1)] Women who are diagnosed with gestational diabetes have an increased chance (35% to 60%) of developing diabetes in the next 1-2 decades and the predicted health care expenses are definitely going to be high.

Many of these women were amenorrhoeic and only about 2% of diabetic patients conceived. The diabetic patients who conceived had an increased risk of morbidity and mortality.

Poor sugar control in mother lead to poor maternal and perinatal outcomes. There is approximately 42.9% chances of perinatal mortality. With proper diagnosis and treatment of GDM, the perinatal and maternal outcome can be increased.

Intrauterine exposure of increased sugar levels has a negative impact on the pregnancy and also lead to glucose intolerance in the offspring latter period of life.

No uniform guidelines for the management of GDM exist on a local, national or global level. Given the high rate of hyperglycaemia in pregnancy, selective testing based on known risk factors has poor sensitivity for detecting GDM, universal rather than risk factor-based testing seem to be more practical. Universal testing is recommended by several organizations including International Association of Diabetes and Pregnancy study Group (IADPSG), Diabetes In Pregnancy Study group India (DIPSI). Asian Indian women are considered to be at the highest risk of GDM and therefore require universal testing.

In India, approximately 27 million births occur annually requiring at least 27 million OGTTs annually; considering a 10% average prevalence of GDM, the number of GDM pregnancies would be around 2.7 million, a huge burden to deal with for any health system. Any recommendation for testing women for hyperglycaemia during pregnancy must, therefore, be pragmatic, feasible, convenient and cost-effective. FBS in first trimester will help in establishing this.

Our aim is to find association between first trimester fasting blood sugar value compared with the second trimester oral glucose tolerance test value (75gm DIPSI criteria) for diagnosis of GDM. To find the efficiency of FBS and BMI as a screening test for GDM.

Early diagnosis and early intervention of diabetes is useful for improving pregnancy outcomes. FBS measurement at first prenatal visit or at the time of first booking will be useful to screen for previously undiagnosed pre-existing diabetes and also help to predict the development of GDM earlier.

Materials and Methods

The study was conducted in the department of Obstetrics and Gynaecology, PSG Hospitals, Coimbatore from June 2014 – May 2015. The study period was 12 months.

Study Design: Prospective Observational Study

Study Population: 270 antenatal patients having antenatal follow up from 1st trimester in the department of Obstetrics & Gynaecology, PSG IMSR & Hospitals, Coimbatore.

Inclusion Criteria: All antenatal patients from first trimester of pregnancy, Singleton pregnancy

Exclusion Criteria: Pre gestational diabetes mellitus

Patient who lost follow up for OGTT testing during 2nd trimester.

Patients with first trimester FBS more than 105mg/dl.

Antenatal patients on long term steroids for medical disorder.

Methodology

The study was initiated after obtaining approval from the ethics committee in PSG IMSR.

The patients selected were according to the inclusion criteria- antenatal patients from first trimester without having pre-existing diabetes mellitus and oral and written consent were obtained.

Patients who had not turned up for OGTT during the second trimester or who were not willing to participate in the study were excluded.

Basic assessment of their risk factors was already done in the first antenatal visit along with detailed family history. Their height and weight was measured. Weight was noted at the time of first visit. BMI was calculated form the first visits data. Gestational age was noted for both the tests during first and second trimester.

About 270 antenatal mothers were selected during their first trimester from Obstetrics & Gynaecology department OPD during June 2014-May 2015 were explained about the study after excluding other women who were not eligible for the study and fasting blood glucose levels were measured and documented. The patients were followed up during the second trimester and a 75 grams OGTT was done and the levels were noted.

All the patients were asked to follow unrestricted carbohydrate diet and not to change the diet pattern and fasting blood glucose was tested during the first trimester with overnight fast of atleast 8 hours.

During second trimester, when the patients entered the hospital for second trimester OGTT irrespective of the last meal given, 75gms oral glucose mixed in 150 ml of water and blood test taken 2 hrs later according to the DIPSI criteria. All the patients were instructed not to have meals after the 75gm glucose ingestion.

If the patient experienced nausea during the drinking procedure a pinch of fresh lime was added. If she had vomited after glucose ingestion then the testing is done at the further time of the day or asked to come on the following day for re-testing and the same procedure is followed. The patient is requested not to have meals in between and venous blood was collected 2 hrs later.

Two ml of venous blood was collected in sterile fluride vial. These samples were centrifuged at 3500 rotation per minute for 10 minutes. Plasma was used for estimation. Blood test should be done within 3 hours of collection using autoanalyser, Cobas Integra 400 plus-Roche diagnostics by glucose hexokinase method using spectrometric assay. It has analytical sensitivity of 0.59mg/dl and test range of upto 720mg/dl.

Test Principle:

Hexokinase catalyses the phosphorylation of glucose by ATP to form glucose 6 phosphate and ADP. To follow the reaction, a second enzyme glucose 6 phosphate dehydrogenase is used to catalyse oxidation of glucose 6 phosphate by NAD+ to form NADH

D Glucose+ ATP $\xrightarrow{HK}$ Glucose 6 phosphate + ADP

D Glucose 6 phosphate + NAD $\xrightarrow{G6PD}$ D-6-Phosphogluconate + NADH + H+

The concentration of NADH formed is directly proportional to glucose concentration. It is determined by increase in absorption at 340nm.

Patients with first trimester fasting blood glucose levels were categorised as <92 mg/dl, 92-105 mg/dl were included, >105 mg/dl were excluded from the study. Patients with FBS <92 mg/dl were subjected to second trimester 75 gms OGTT. Patients with FBS between 92-105 mg/dl were subjected to diabetic diet, FBS and PPBS was done after 2 weeks of diabetic diet and if it was found to be normal, they were subjected to second trimester 75 OGTT DIPSI criteria. If FBS, PPBS after 2 weeks of diabetic diet were high they were not subjected to second trimester OGTT and were excluded from the study. Patients with FBS of >105 mg/dl were excluded from the study.

First trimester FBS value and second trimester 75 gms GTT values were analysed and the results were tabulated. Correlation between first trimester FBS, BMI versus second trimester OGTT were done. Women diagnosed as GDM were managed appropriately.

Screening property of both fasting blood sugar and BMI were calculated and compared using receiver operating characteristic (ROC) curves.

Results and Analysis

Statistical Analysis:

1. First trimester FBS as a screening test for GDM
2. BMI as a screening test for GDM
3. BMI as a comparison for GDM
4. FBS as a comparison for GDM

Table 1: First trimester fasting blood glucose as a screening test for gestational diabetes mellitus (statistical consolidated data)

FBS Level		70	75	80	85	90	95	100
Patients >	Number of case	270	268	257	223	135	67	22
Threshold Value	%	100	99.2	95.1	83	50	24	8.14
Patients with GDM > Threshold Value	Number of case	15	15	15	15	13	5	1
False Positive Rate	%	100	99	91.7	81.6	47.9	24.4	8.3
Sensitivity	%	100	100	100	100	86.6	33.3	6.6
Specificity	%	0	0.78	8.3	18.9	52.1	75.6	91.7
PPV	%	5.5	5.6	5.8	6.7	9.6	7.5	4.5
NNP	%	100	100	100	100	98.5	95	94.4

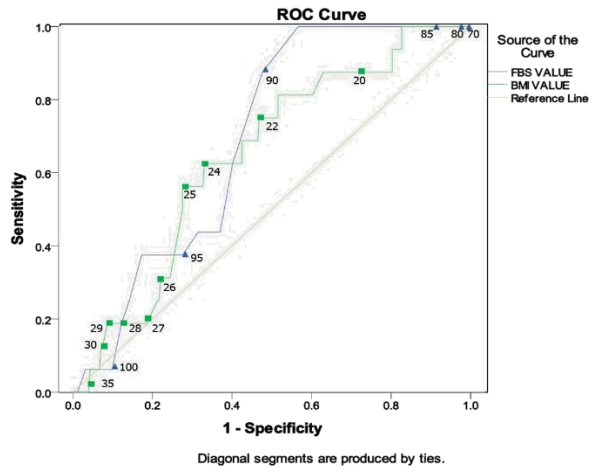
Patients with FBS>90 had sensitivity of 86.6%, specificity of 52.1%, Positive predictive value of 9.6%, negative predictive value of 98.5%.

Table 2: I Trimester body mass index as a screening test for gestational diabetes mellitus (statistical consolidated data)

BMI		20	22	24	25	26	27	28	29	30	35
Patients Threshold >	Numbers	199	129	92	78	58	40	30	25	20	3
	%	73.7	47.7	34	28.9	21.5	14.8	11.1	9.25	7.4	1.1
Patients with GDM > Threshold	Numbers	14	11	9	8	4	3	3	3	2	0
False Positive Rate	%	72.5	46.3	32.5	27.5	21.2	21.2	10.6	8.6	7.1	1.2
Sensitivity	%	93.3	73.3	60	53.3	26.7	20	20	20	13.3	0
Specificity	%	27.5	53.7	67.5	72.5	78.8	78.8	89.9	91.4	92.9	98.9
PPV	%	7	8.5	9.8	10.2	6.9	7.5	10	12	10	0
NNP	%	98.6	97	96.6	96	94.8	94.7	95	95.1	94.8	94.3

Patients with BMI >24 had sensitivity of 60%, specificity of 67.5%, positive predictive value of 9.8%, negative predictive value of 96.6%.

Receiver operative characteristic curve



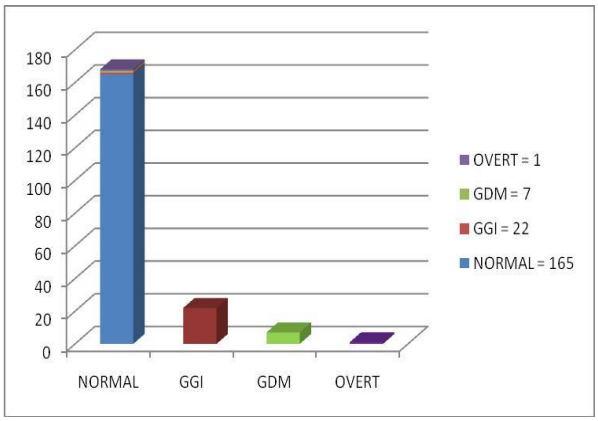
Area under curve for FBS=0.694
Area under curve for BMI=0.63

Table 3: Body mass index as a comparison for gestational diabetes mellites (cross tab)

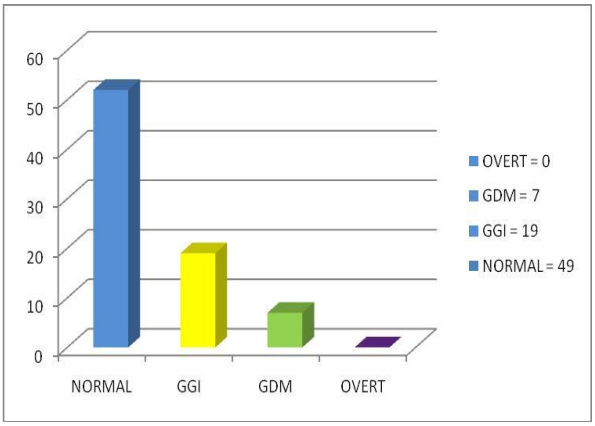
BMI VS GDM	OGTT				
	Normal	GGI	GDM	OVERT	Total
BMI <25	165	22	7	1	195
BMI >=25	49	19	7	0	75
Total	214	41	14	1	270

P value <0.01

BMI <25



BMI>= 25



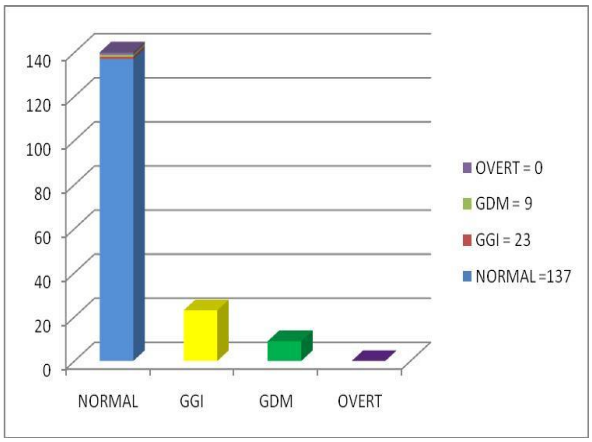
Out of 270 patients, patients who had BMI<25 had more normal values. Patients who had BMI >=25 had increased GCT values. This data was found to be statistically significant.

Table 4: Fasting blood sugar as a comparison for gestational diabetes mellites (cross tab)

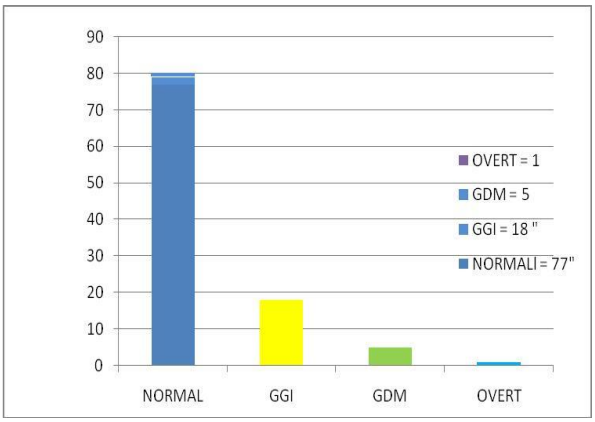
FBS VS GDM	OGTT				Total
	Normal	GGI	GDM	OVERT	
FBS <92	137	23	9	0	169
FBS >=92	77	18	5	1	101
Total	214	41	14	1	270

P value =0.455

FBS < 92



FBS >=92



In group I patients with FBS <92 were 169 patients, of which 23 developed GGI, 9 developed GDM In group II FBS>=92-105 were 101 patients out of which 18 patients developed GGI, 5 patients developed GDM, 1 patient developed overt diabetes respectively. Group II patients were subjected to diabetic diet earlier in view of initial high sugar values. So analysing the outcomes, we inferred that starting diabetic diet earlier has a significant role in decreasing the risk of developing GDM in advance pregnancy.

Result

Mean calculated continuous variable. Percentage calculated for categorical variable (sensitivity). Pearson chi –square test was used to find association between categorical variable.

In addition sensitivity, specificity, false positive, false negative were calculated. Receiver operating characteristic (ROC) curve was plotted to find cut off value for FBS & BMI for GDM.

Further Area under Curve (AUC) was calculated to observe discriminatory power between FBS & BMI. FBS(AUC=0.69) has little more discriminatory power in identifying GDM than BMI(AUC-0.63). P value <0.05 was considered statistically significant

All statistical analysis was done using SPSS Software (statistical package for social sciences) With a threshold of FBS>90, sensitivity of 86%, specificity of 52%, hence FBS >90mg/dl can be considered as a threshold value for predicting GDM which is lesser than the threshold 92 mg/dl which is already considered as a cut off for prediction of GDM. With a threshold of BMI>24, sensitivity of 60%, specificity of 67.5%, hence BMI >24 is a good predictor of GDM.

Discussion

Our aim was to find out the correlation between FBS and OGTT and to find the threshold value of FBS, BMI for developing GDM.

Out of 270 patients, 14 patients developed GDM which is 5.2% of the total study population. 1 patient developed overt diabetes which is 0.3% of study population.

Our study shows that pregnant women with FBS >90 mg/dl are more likely to develop GDM later in pregnancy and more likely to develop adverse pregnancy outcome if no intervention is done for these patients. Therefore, we suggest that women with Fasting blood glucose >90mg/dl who are more prone to develop GDM hence should be subjected to medical nutritional therapy. FBS (AUC=0.69) is a better than BMI (AUC=0.63) as a screening test for GDM.

Early diagnosis of gestational diabetes mellitus (GDM) is important to improve for both maternal and fetal outcomes. The burden of diabetes in India is very high. It is an urgent need to establish screening and diagnostic procedure which is easy, understandable and simple. FBS measurement is a well-tolerated and inexpensive routine examination.[2] FBS measurement has better test accuracy throughout the pregnancy.[3,4] Riskin-Mashiah et al.[2] study had high predictive value of first trimester FBS, and consider FBS as a screening test and not as diagnostic test with a suggested cut off value. This study concluded that, The FBS value lower than what is be considered as impaired fasting glucose, is associated with development of GDM. There is no clear cut off above which the risk of GDM is substantially increased. So for every 5 mg/dl increase in FBS or 3.5kg/m^2 increase in BMI there was 1.5 fold increased risk. First trimester fasting blood glucose value is an excellent test for determining the need to continue with the oral glucose tolerance test in the second trimester.[4,5]

The hyperglycaemia and adverse pregnancy outcome study estimated that Fasting blood glucose measurement identifies about 50% of all affected women without an additional 1 and 2 hour OGTT values.[6] Though previously FBS was neglected as a screening test for GDM, in high risk population it provides simple, practical algorithm to screen for GDM.[7] Riskin-Mashiah et al,[8] has already reported that mild hyperglycemia in early pregnancy will lead to adverse pregnancy outcomes.

So instead of subjecting all patient to a glucose load to do OGTT in first trimester, which may aggravate nausea, vomiting which is more prevalent in first trimester. If patient vomits during the test, then OGTT has to be repeated again on another day which is time consuming. So, it is better to perform an easy, less time consuming, cost effective test that is fasting blood glucose.

Our study shows that pregnant women with FBS >90 mg/dl are more likely to develop GDM later in pregnancy and more likely to develop adverse pregnancy outcome if no intervention is done for these patients. Therefore, we suggest that these women should be subjected to medical nutritional therapy. FBS as a screening test has little more discriminatory power in identifying GDM than BMI.

Conclusion

Our study shows that FBS at first trimester will be helpful in the early prediction of gestational diabetes mellitus. Early diagnosis and early intervention of diabetes is useful for improving pregnancy outcomes. In conclusion, FBS measurement at first prenatal visit or at the time of first booking will be useful to screen for previously undiagnosed pre-existing diabetes and also help to predict the development of GDM earlier.

References

1. Cousins L, Rigg L, Hollingsworth D, et al. "The 24-hour excursion and diurnal rhythm of glucose, insulin and C peptide in normal pregnancy" Am J Obstet Gynecol (1980);136:483.
2. Riskin-Mashiah S, Damti A, Auslender R "First trimester fasting hyperglycemia as a predictor for the development of gestational diabetes mellitus" Eur J Obstet Gynecol Reprod Biol (2010) 152:163–167.
3. Farrar D, Duley L, Lawlor DA "Different strategies for diagnosing gestational diabetes to improve maternal and infant health". Cochrane Database Syst Rev, (2011) Issue 10.
4. Agarwal MM, Dhatt GS, Punnose J "Gestational diabetes: utility of fasting plasma glucose as a screening test depends upon the diagnostic criteria" Diabet Med (2006);23:1319–1326.
5. Agarwal MM, Hughes PF, Punnose J, Ezimokhai M "Fasting plasma glucose as a screening test for gestational diabetes in a multi-ethnic, high-risk population". Diabet Med (2000);17:720–726.
6. Coustan DR, Lowe LP, Metzger BE, Dyer AR "The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: paving the way for new diagnostic criteria for gestational diabetes mellitus" (2010). Am J Obstet Gynecol 202:654.e.16.
7. Maria I. Schmidt, Bruce B. Duncan, Angela J. Reichelt et al. Gestational Diabetes Mellitus Diagnosed With a 2-h 75-g Oral Glucose Tolerance Test and Adverse Pregnancy Outcomes. Diabetes Care July (2001) vol. 24no.7 1151-1155.
8. Riskin-Mashiah S, Younes G, Damti A, Auslender R. "First-trimester fasting hyperglycemia and adverse pregnancy outcomes. Diabetes Care". 2009;32(9):1639–1643.
9. Riskin-Mashiah S, Damti A, Auslender R "First trimester fasting hyperglycemia as a predictor for the development of gestational diabetes mellitus". Eur J Obstet Gynecol Reprod Biol (2010) 152:163–167.

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Pregnancy outcome in autoimmune disorders

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Abstract

Background: Autoimmune disorders are chronic multisystem disorders affecting women of their reproductive age. Pregnancy among these women is complicated by the disease itself. Flaring up of disease, uteroplacental insufficiency due to vasculitis and drugs used for treatment are main reason for unfavourable pregnancy outcomes. The objective of this study was to analyze the pregnancy complications and outcome among 113 cases of autoimmune disorders.

Methods: This study is a retrospective analysis of case records of pregnant women with autoimmune disorders. This study was conducted at PSG IMSR and Hospital, Coimbatore, and Tamil Nadu from July 2012 to June 2018. The case sheets were retrieved from medical record department and the details such as type of disease, maternal age, parity, status of disease at conception, treatment taken during pregnancy, maternal complications and fetal complications were collected and analyzed.

Results: During 6-year period there were 113 pregnant women with autoimmune disorders, and they were studied of their previous and present pregnancy outcome. The disease incidence was 7.01% per 1000 deliveries. 40.7% were less than 25 years age group and 71.6% were multigravidae. We had forty-one patients (36%) who were positive for APLA syndrome which were the maximum number of patients with auto immune disorder in pregnancy and 37 patients (32.7) with SLE. We had significant numbers of maternal and fetal complications for common disorders. Most of the rare auto immune disorders were diagnosed before pregnancy and these pregnancies were managed by multi-disciplinary approach, and continued on immunomodulators throughout pregnancy, hence maternal and fetal complications were less for them.

Conclusions: Adequate pre-conceptional counseling, vigilant monitoring during pregnancy and post-partum will avoid pregnancy related complications and have favorable outcomes.

Keywords: Autoimmune disorder, Fetal complications, Immunomodulators, Maternal complications, Pregnancy outcome, Pre-conceptional counseling

Introduction

Autoimmune disorders are chronic multisystem disorders which are characterized by tissue damage and inflammation due to alteration of adaptive immune response in the absence of trigger.[1] Its prevalence is between 7.6%-9.4%.[2] Since most of the autoimmune disorders are diagnosed frequently during reproductive age in females, these women are at high risk not only during pregnancy but also during postpartum.[3] When compared to females without autoimmune disorders, these women carry more incidence of miscarriages in all trimesters, preeclampsia, intrauterine growth restriction, premature rupture of membranes, placental insufficiency, preterm labour, low birth weight and have increased caesarean section rates.[4] Though these pregnancies carry

high incidence of adverse effects, improved care in terms of pre-conceptional counselling and continuation of treatment throughout the pregnancy and post-partum have improved the outcome of pregnancies.[5]

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This study is a retrospective analysis of a total 113 case records of pregnant women with autoimmune disorders. This study was conducted at PSG IMSR and Hospital, Coimbatore, Tamil Nadu from July 2012 to June 2018. The case sheets were retrieved from medical record department and the details such as type of autoimmune disorder, maternal age, parity, status of disease at conception, treatment taken during pregnancy, maternal complications like pregnancy induced hypertension, gestational diabetes mellitus, thrombotic events, anemia, renal flares, thrombocytopenia, preterm labour, mode of delivery, indications for caesarian section and fetal complications like abortion, anomalies, intra uterine fetal demise, low birth weight, intra uterine growth restriction, oligohydramnios, need for admission to neonatal intensive care unit, low Apgar score were collected and analyzed.

Inclusion criteria

- All primigravidae and multigravidae who are known case of autoimmune disorder and all primigravidae and multigravidae who are diagnosed with autoimmune disorder during present pregnancy.

Exclusion criteria

- Pregnant mothers without autoimmune disorders.

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During this period of 6 years (2012-2018), there were 113 pregnant women with autoimmune disorders and study was carried out based on their previous and present pregnancy outcomes. The incidence of autoimmune disorders in pregnancy was 7.01% per 1000 deliveries.

Table 1 shows the number of pregnancies with autoimmune disorders in our study.

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Antiphospholipid antibody syndrome	41	36.2
Systemic lupus erythematoses	37	32.7
Idiopathic thrombocytopenic purpura	11	9.7
Takayasu syndrome	5	4.4
Sjögren syndrome	4	3.5
Hashimoto's thyroiditis	3	2.6
Multiple sclerosis	3	2.6
Rheumatoid arthritis	2	1.7
Schönlein-henoch purpura	2	1.7
GBS	1	0.8
Von Williebrand disease	1	0.8
Multisystem connective tissue disorder	1	0.8
Medial vessel vasculitis	1	0.8
Ulcerative colitis	1	0.8

In our study, we had forty-one patients (36%) with APLA syndrome including primary and secondary APLA and 37 patients (32.7%) with SLE.

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<25	46	40.7
25-30	45	39.8
31-35	21	18.5
>35	1	0.8

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APLA	2 (4.8%)	13 (31%)	1 (2%)	3 (7.3%)	-	-	7 (17%)	1 (2.4)
SLE	4 (10.8%)	6 (16%)	3 (8%)	2 (5.4%)	-	1 (2.7%)	16 (43%)	8 (21%)
ITP	1 (9%)	6 (54%)	-	1 (9%)	2 (18%)	-	7 (63%)	1 (9%)
Takayasu	3 (60%)	-	1 (20%)	-	-	-	4 (80%)	-
Sjögren syndrome	-	1 (25%)	-	-	-	-	3 (75%)	□□

In our study, most of the patients were below 30 years of age. Table 2 shows the age group distribution. Multigravida (71.6%) were more in number than primigravida (28.3%) in our study.

Parity distribution is shown in Figure 1.

Table 3 shows the most common autoimmune disorders in our study and their maternal complications.

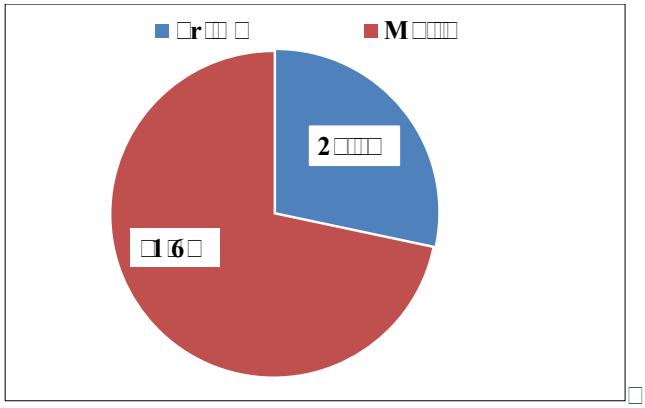


Table 3: Maternal complications in previous pregnancy.

Disorder	Number	Percentage
APLA	26 (63%)	9 (21.9%)
SLE	22 (59%)	5 (13.5%)
ITP	4 (36%)	-
Takayasu	-	1 (20%)
Sjögren syndrome	3 (75%)	-

Two patients diagnosed with SLE had abnormal doppler changes at 32 weeks and 34 weeks respectively and underwent caesarean section. The most common indication for caesarean section was non-progression of labour followed by previous section.

Table 4 shows fetal complications in previous pregnancy for more common disorders in our study.

Table 4: Fetal complications in previous pregnancy.

Disorder	IUGR	Abortion	Stillbirth
APLA	7 (17%)	2 (4.8%)	1 (2.4%)
SLE	13 (35%)	6 (16%)	5 (13.5%)
ITP	2 (18%)	-	1 (9%)
Takayasu	3 (60%)	-	-
Sjögren syndrome	-	2 (50%)	-

Table 5 shows the fetal complications in the current pregnancy for same disorders.

Table 5: Maternal complications in current pregnancy.

Disorder	Current	Previous
Pre-eclampsia	10.8%	13%
GDM	16%	NA
Renal flare	8%	NA
Anemia	7.3%	21%
C-section	43%	53%
Preterm labour	21%	21%

Table 6: Fetal complications in current pregnancy.

Disorder	Current	Previous
Abortion in previous conception	59%	46%
IUD	13.5%	8%
IUGR	35%	32%
LBW	24.3%	22%
NICU admission	13.5%	15%

APLA

Obstetric antiphospholipid antibody syndrome (OB-APS) is defined according to the updated Sydney classification criteria developed by expert consensus agreement in 2006.[6] APLs include the lupus anticoagulant, anticardiolipin IgG and IgM and anti-β2-glycoprotein I antibody. In addition to the detection of aPLs on at least two occasions more than 12 weeks apart, OB-APS is defined by any one of the following adverse pregnancy outcomes: 1) otherwise unexplained recurrent pregnancy loss before the 10th week of gestation, 2) otherwise unexplained fetal death ≥10 weeks of gestation, 3) preterm birth before 34 weeks of gestation due to preeclampsia or placental insufficiency.[7] Other OB-APS complications include catastrophic APS, thrombosis, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), IUGR, fetal distress, premature rupture of membranes, and thrombocytopenia.[8] It has been recognized that among the three aPLs, lupus anticoagulant (LAC) is the primary predictor of poor pregnancy outcome in women both with or without SLE, and with or without other aPLs.[9] Among the 41 patients with APLA, 20 patients were positive for lupus anticoagulant antibody, 11 were positive for anticardiolipin antibody and 10 had anti-β2-glycoprotein I antibody.

In our study, 31.8% of patients with primary APLA and 4.4% of patients with secondary APLA with associated SLE were diagnosed. The incidence of pre-eclampsia among APLA positive patients in our study was 4.8% whereas in Wijeyaratne study, it was 10.9%.[10] Similarly the incidence of GDM in our study was 31% as compared to 19.2% in Wijeyaratne study. In our study 2.4 % had preterm labour and 17% of patients underwent cesarean section. In regard to fetal complications, 63% of patients in our study had previous one or more abortions which was comparable to Wijeyratne study (59.8%). The incidence of IUD in previous pregnancy was 21.9.%. The incidence of low birth weight babies was 12% and babies requiring NICU admission was 2.4% respectively.

SLE

Systemic lupus erythematosus (SLE, or “lupus”), affects females in a 9:1 ratio to males.[11] The adverse pregnancy

outcomes observed with lupus are well known, and include increased rates of intrauterine growth restriction (IUGR), preterm birth and fetal loss, as well as the syndrome of neonatal lupus (NL), associated with trans placental passage of auto antibodies. The risks for thrombosis, infection, thrombocytopenia, and transfusion were each 3- to 7-fold higher for women with systemic lupus erythematosus. Lupus patients also had a higher risk for cesarean sections (odds ratio: 1.7), preterm labor (odds ratio: 2.4), and preeclampsia (odds ratio: 3.0) than other women. Women with systemic lupus erythematosus are more likely to have other medical conditions, including diabetes, hypertension, and thrombophilia, that are associated with adverse pregnancy outcomes.[12]

High disease activity at the time of conception or in the months preceding conception is an independent predictor for increased risk of adverse pregnancy outcomes including prematurity, IUGR and fetal demise. This has led to the current recommendations that lupus patients achieve 6 months of disease quiescence prior to attempting conception.[13] Maternal and fetal complications associated with SLE were compared with Eman et al study.[14] These are shown in table 6 and 7 respectively.

Six patients had first trimester abortions in the current pregnancy.

Takayasu syndrome

In our study, 60% patients had hypertension complicating pregnancy whereas in Mandal et al study had 92.8%.[15] Caesarean section rate in our study was 60%, whereas in Mandal study it was 71.4%. Intrauterine growth restriction was 60% in our study, which was comparable with Mandal et al study (51.7%).

Schönlein-henoch purpura

Schönlein-henoch purpura (SHP) is a systemic vasculitis that affects vessels of a small caliber.[16] The onset of SHP during pregnancy is very rare. Treatment with orally administered corticosteroids may lead to a beneficial outcome for both mother and newborn.[17] In our study, we had 2 patients diagnosed with Schönlein-Henoch purpura, of which one developed GDM, other had uneventful pregnancy.

Medium vessel vasculitis

Most of the women with medium vessel vasculitis fared well during pregnancy without significant disease progression, but instances of aneurysm rupture, renal insufficiency, pulmonary embolism, and myocardial infarction were reported in Gatto et al study.[18] In our study, one patient diagnosed with medium vessel vasculitis developed myocarditis during pregnancy and she was managed with steroids.

ITP

Nine percent of chronic ITP patients in our study had preeclampsia, whereas Diego et al, study had 8.3%.[19] In our study 9% had preterm labour which was comparable to Diego et al (9.5%) study. Diego et al showed 8.5% of gestational diabetes in the study whereas in our study we had 54% of gestational diabetes which could be explained by ethnicity and usage of steroids.

In Yassaee et al, study occurrence of IUGR was 10.5% whereas in our study it was 18%, among which one baby was admitted in NICU.[20] In Yassaee et al study 57% of patients had moderate thrombocytopenia (more than 50,000) whereas 18% of thrombocytopenia and 9% of anemia were diagnosed in our study.

When comparing the caesarean section rate, Yassaee et al had 81% whereas we had 63%.

Rheumatoid arthritis

In women with well-controlled RA, pregnancy outcomes are comparable to the general obstetric population, whereas in women with higher RA disease activity, there is more chances of less favorable pregnancy outcomes.[21,22]

Akasbi et al, study showed improved maternal and fetal outcome in patients with RA in pregnancy. Similarly, in our study two patients diagnosed with RA had uneventful pregnancy.

Guillain-barré syndrome

Guillain-barré syndrome (GBS) represents a heterogeneous group of immune mediated peripheral neuropathies. A feature common to all GBS variants is a rapidly evolving polyradiculoneuropathy preceded by a triggering event, most often an infection. GBS generally manifests as a symmetric motor paralysis with or without sensory and autonomic disturbances. Delayed diagnosis is common in pregnancy or immediate post-partum period because the initial non-specific symptoms may mimic changes in pregnancy. GBS should be considered in any pregnant patient complaining of muscle weakness, general malaise, tingling of the fingers, and respiratory difficulty.[23,24]

We had one patient diagnosed with GBS who had an uneventful course during pregnancy.

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms.[25] Pregnancy is typically a stabilizing period in the clinical course of MS. During the

third trimester the MS relapse rate can be 70% lower when compared with the time prior to pregnancy, but exaggeration of the disease is commonly seen postpartum. Presently, there is no evidence that MS patients are more susceptible to pregnancy or delivery related complications, such as ectopic pregnancy, pre-eclampsia, gestational diabetes mellitus, prolonged labour or miscarriage than women in general, nor is their infants more likely to be delivered preterm or to be of low birth weight, have malformations or experiences an early death.[26,27] Mothers with MS required operative delivery more often than healthy mothers, which might be attributed to MS-related symptoms such as neuromuscular perineal weakness and spasticity in addition to fatigue and exhaustion.[28] In our study, we had 3 mothers diagnosed with multiple scelerosis, of them one developed PIH, one had GDM.

Sjögren syndrome

Sjögren syndrome is known to occur predominantly in women. Affected women are likely to experience more complicated pregnancies than are women without the disease.[29] Its effect on pregnancy differs according to the maternal disease, disease activity, and severity of organ damage, antibody profile, and drug treatment.6.

Several studies have reported an increased rate of spontaneous abortion and fetal loss associated with Sjögren syndrome.[30] This may be explained by, first, the age of the patients at the time of concep-tion which usually belongs to an elderly age group and, second, a possible immunologic factor involved in the mechanisms of miscarriage.[31]

In our study, out of four mothers diagnosed with Sjögren syndrome, two had spontaneous abortions.

The outcome of pregnancies in women with Sjögren syndrome can be improved with use of a multidisciplinary approach for management involving an obstetrician who has specialized in high-risk pregnancies, a rheumatologist, and a pediatrician.[32]

Hashimotos thyroiditis

Hashimotos thyroiditis is associated with an increase rate of spontaneous.

Abortions, preterm delivery and increased use of cesarean section.[33] All the three patients diagnosed with Hashimotos thyroiditis underwent cesarean section for non-progression of labour.

Ulcerative colitis

Stephansson et al study concluded that pregnant women with ulcerative colitis had a greater number of preterm births, babies with low birth weight and cesarean section deliveries than those without ulcerative colitis.[34] In our study we had one patient who delivered a low birth weight baby of 2.2 kg. One patient diagnosed with multisystem connective tissue disorder had an intra uterine growth restriction and the same baby had low birth weight.

Von Willebrand's disease

Pregnancy and delivery are considered events predisposing to a high risk of bleeding in women with von Willebrand's disease.[35]

Our patient did not have any complication. All the patients diagnosed with GDM were found to be on steroids.

Among the 113 patients, 3 underwent caesarean section in view of abnormal umbilical artery doppler findings.

Patients previously diagnosed were continued on disease modifying agents and this could be the reason for their successful pregnancy outcome.

Rauto immune disorders were diagnosed before pregnancy and these pregnancies were managed by multi-disciplinary approach, and continued on immune modulators throughout the pregnancy, hence minimizing maternal and fetal complications.

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In our study, most of the patients were either continued on the same treatment they were taking before pregnancy or were started on treatment once they were diagnosed with autoimmune disorders, and hence they were found to have successful pregnancy outcomes. Therefore, appropriate pre-conceptional counselling, continuation of treatment, intense maternal and fetal monitoring and timely delivery plays an important role in improving the pregnancy outcome.

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1. Sarah M, Mark Q, Paul E, Chapter 32 – Rheumatoid arthritis Editors: Noel R. Rose, Ian R. Mackay, The Autoimmune Diseases. 4th Ed, Academic press; 2006:417-436.
2. Cooper G, Bynum M, Somers E. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. J Autoimmun. 2009;33(3-4):197-207.
3. Perricone C, Carolis C, Perricone R. Pregnancy and autoimmunity: a common problem. Best Pract Res Clin Rheumatol. 2012;26(1):47-60.

4. Andreoli L, Fredi M, Nalli C, Reggia R, Lojacono A, Motta M et al. Pregnancy implications for systemic lupus erythematosus and the antiphospholipid syndrome. J Autoimmun. 2012;38(2-3):197-208.
5. Mecacci F, Pieralli A, Bianchi B, Paidas MJ. The impact of autoimmune disorders and adverse pregnancy outcome. Semin Perinatol. 2007;31(4):223-6.
6. Wilson WA, Gharavi AE, Koike T. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arth Rhe. 1999;42:1309-11.
7. Miyakis S, Lockshin MD, Atsumi T. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295-306.
8. Hughes GR. The antiphospholipid syndrome: ten years on. Lancet (London, England). 1993;342:341-4.
9. Lockshin MD, Kim M, Laskin Ca. Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies. Arth Rh. 2012;64:2311-8.
10. Wijeyaratne CN, Galappaththi SLA, Palipane E, Jayawardane DBIA, Dodampahala SH, Tudawe MN, et al. Pregnancy outcomes of antiphospholipid syndrome: In a low resource South Asian setting. Med. 2016;9(2):83-9.
11. Cooper GS, Gilbert KM, Greidinger EL. Recent advances and opportunities in research on lupus: environmental influences and mechanisms of disease. Environ Health Perspect. 2008;116:695-702.
12. Clowse MEB, Jamison M, Myers E. A national study of the complications of lupus in pregnancy. Am J Obstet Gynecol. 2008;199:1-12.
13. Jakobsen IM, Helmig RB, Stengaard-Pedersen K. Maternal and foetal outcomes in pregnant systemic lupus erythematosus patients: an incident cohort from a stable referral population followed during 1990-2010. Scand J Rheumatol. 2015;44:377-84.
14. Eman Aly, Hussein Aly, Riyad RM, Mokbel AN. Pregnancy outcome in patients with systemic lupus erythematosus: a single center study in the high-risk Pregnancy unit. Middle East Fert Society J. 2016;21:168-74.
15. Mandal D, Mandal S, Dattaray C, Banrrjee D, Ghosh P, Ghosh A, et al. Takayasu arteritis in pregnancy: an analysis from eastern India. Arch Gynecol Obstet. 2012;285(3):567-71.
16. Jennette CJ, Milling DM, Falk RJ. Vasculitis affecting the skin. Arch Dermatol. 1994;130:899-906.
17. Robert F, Walter R, Sator PG, Gschnait F, Breier F. Schönlein-Henoch purpura during pregnancy with successful outcome for mother and newborn BMC Dermatol. 2002;2:1.
18. Gatto M, Iaccaino L, Canova M, Nalotto L, Ramonda R. Pregnancy and vasculitis; a systematic review of the literature. Autoimmun Rev. 2012;11:A447-59.
19. Wyszynski DF, Carman WJ, Cantor AB, Graham JM, Kunz LH, Slavotinek AM, et al. Pregnancy and birth outcomes among women with idiopathic thrombocytopenic purpura. J Preg. 2016:Article id 8297407.
20. Yassaee F, Eskandari R, Amiri Z. Pregnancy outcomes in women with idiopathic thrombocytopenic purpura. Iran J Reprod Med. 2012;10(5):489-92.
21. De Man YA, Hazes JMW, Van Der Heide H. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: Results of a national prospective study. Arth Rhe. 2009;60:3196-206.
22. Bharti B, Lee SJ, Lindsay SP. Disease severity and pregnancy outcomes in women with rheumatoid arthritis: Results from the organization of teratology information specialists autoimmune diseases in pregnancy project. J Rheumatol. 2015;42:1376-82.
23. Hughes RA, Cornblath DR. GuillainBarre' syndrome. Lancet. 2005;366:1653-66.
24. Vijayaraghavan J, Vasudevan D, Sadique N, Rajeswari KS, Pondurangi M, Jayshree A. Rare case of Guillain-Barre' syndrome with pregnancy. J Indian Med Assoc. 2006;104:269-70.
25. Coles A. Multiple sclerosis. Lancet. 2008;372:1502-17.
26. Jalkanen A, Alanen A, Airas L. The finnish multiple sclerosis and pregnancy study group. Pregnancy outcome in women with multiple sclerosis – results from a prospective nationwide study in Finland. Mult Scler. 2010;16:950-5.
27. Mueller BA, Zhang J, Critchlow CW. Birth outcomes and need for hospitalization after delivery among women with multiple sclerosis. Am J Obstet Gynecol. 2002;186:446-52.
28. Dahl J, Myhr KM, Daltveit AK, Hoff JM, Gilhus NE. Pregnancy, delivery, and birth outcome in women with multiple sclerosis. Neurol. 2005;65:1961-3.
29. Siamopoulou-Mavridou A, Manoussakis MN, Mavridis AK, Moutsopoulos HM. Outcome of pregnancy in patients with autoimmune rheumatic disease before the disease onset. Ann Rheum Dis. 1988;47(12):982-7.
30. Carvalheiras G, Faria R, Braga J, Vasconcelos C. Fetal outcome in autoimmune diseases. Autoimmun Rev. 2012;11(6-7):A520-30.
31. Sandhya P, Jeyaseelan L, Scofield RH, Danda D. Clinical characteristics and outcome of primary Sjogren's syndrome: a large Asian Indian cohort. Open Rheumatol J. 2015;9:36-45.
32. Brucato A, Cimaz R, Caporali R, Ramoni V, Buyon J. Pregnancy outcomes in patients with autoimmune diseases and anti-Ro/SSA antibodies. Clin Rev Allergy Immunol. 2011;40(1):27-41.
33. Lao TT. Thyroid disorders in pregnancy. Curr Opin Obstet Gynecol. 2005;17:123-7.

34. Stephansson O, Larsson H, Pedersen L. Congenital abnormalities and other birth outcomes in children born to women with ulcerative colitis in Denmark and Sweden. Inflamm Bowel Dis. 2011;17(3):795-801.
35. Kujovich JL. Von Willebrand disease and pregnancy. J Thromb Haemost. 2005;3(2):246-53.

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A retrospective analysis of ectopic pregnancy in a tertiary care hospital

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Abstract

Background: Ectopic pregnancy (EP) is a common, life threatening emergency during the first trimester and a significant cause for maternal morbidity and mortality. In any woman of reproductive age presenting with abdominal pain and vaginal bleeding, ectopic pregnancy should be considered. The objectives of the present study is to determine the incidence, risk factors, clinical presentation, type of ectopic pregnancy, treatment, morbidity and mortality.

Methods: Retrospective analysis of case sheets of patients admitted with ectopic pregnancy at PSGIMSR, Coimbatore during the period July 2011 to June 2016 was done. The details noted were age, parity, risk factors {previous abortion, previous EP, previous pelvic surgery, intrauterine contraceptive device, tubectomy, history of pelvic inflammatory disease}, treatment for infertility, clinical presentation, ultrasound findings, investigations including beta HCG value and hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity.

Results: During the five year, there were 12,407 deliveries at our hospitals and 88 cases of ectopic pregnancies were managed. The incidence is 6.6/1000 deliveries. Among them 44% belonged to the age group of 25-30 years and multi gravida were 73.8%. Most common risk factors were previous abortion (23.8%) and previous surgeries including caesarean section, tubectomy, appendicectomy, tubal microsurgery (23.8%). Most common presentation was pain abdomen (85.2%). Out of the 88 cases, 73 patients were managed surgically (82.9%) and 11 cases were managed successfully with methotrexate (12.5%). Three of them underwent conservative management and one had Uterine Artery Embolization.

Conclusions: Early diagnosis based on risk factors and timely intervention plays a main role in reducing morbidity and mortality associated with ectopic pregnancy.

Keywords: Beta HCG, Ectopic pregnancy, Methotrexate, Risk factors, Salphingectomy Transvaginal USG

Introduction

An ectopic pregnancy is implantation of fertilized ovum outside the normal endometrial cavity i.e, fallopian tube, ovary, caesarean scar, cervix and peritoneal cavity.[1] In the first trimester, ectopic pregnancy is the most common cause for maternal morbidity and mortality.[2] It has also been shown that EP reduces future fertility and increases the chance of subsequent EP.[3] The incidence of EP is increasing since the last decade, but the incidence of complications like rupture and morbidity are declining due to early diagnosis with serial Beta-HCG(human chorionic gonadotrophin) monitoring, transvaginal USG(ultrasonogram) and minimally invasive surgery.[4] Early diagnosis not only reduces the risk of tubal rupture but also allows for conservative and medical management.[5] Prior damage to the fallopian tube due to PID(pelvic inflammatory disease), tubal surgeries, pelvic adhesions are the major risk factors for ectopic pregnancy.[6] The other identified risk factors are,

advanced maternal age, previous EP, previous pelvic surgeries, IUCD (intra uterine contraceptive device) insertion and infertility treatment.[7-12] Identifying these risk factors among high risk mothers helps in early diagnosis and reduces the morbidity related to this condition.[13] Unfortunately, most of the patients presenting with EP do not have risk factors.

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This was a retrospective analysis of case records of patients admitted with ectopic pregnancy, done at PSG IMSR Hospital, Coimbatore from July 2011- June 2016.

Inclusion criteria

We included all the patients with suspected ectopic pregnancy who were confirmed by βHCG and transvaginal ultrasound.

Exclusion criteria

None

Methodology

The case sheets were retrieved from medical record department and the details such as age, parity, risk factors like previous abortion, previous EP, previous pelvic surgery, contraception in the form of IUCD and tubectomy, history of PID, infertility treatment, clinical presentation, investigations including beta HCG value, hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity were collected and analyzed.

Statistical analysis

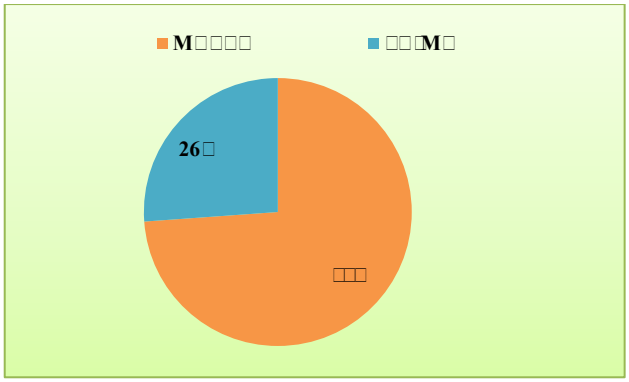
No statistical tool used.

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During the 5-year period, there were 12,407 deliveries at our institution and 88 cases of ectopic pregnancies were managed. The incidence was 6.6/1000 deliveries. Among the patients most of them belonged to the age group of 25-30 years (Table-1). 26% of patients were primigravida, 73.8% of patients were multigravida (Figure -1).

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< 25	22	25
25-30	39	44
31-35	18	20
36-40	5	5.6
>41	4	4.5



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As shown in (Table 2) 23.8% of patients had one or more spontaneous or induced abortions.23.8% of patients had undergone pelvic surgery (Caesarean section, tubectomy appendicectomy, tubal recanalistion). PID and previous ectopic pregnancy accounted for 4.5% each.

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Previous abortion	21	23.8
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Caesarean section	21	23.8
Tubectomy	14	15.9
Appendicectomy	3	3.4
Tubal recanalistion	2	2.2
Ovulation induction	7	7.9
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IUCD insertion	6	6.8
PID	4	4.5
Previous ectopic	4	4.5

Commonest presenting complaint was pain abdomen in 85.2% of patients. Other presenting complaints were Amenorrhoea in 68% and vaginal bleeding in 59% (Table-3).

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Pain	75	85.2
Amenorrhea	60	68.1
Bleeding PV	52	59

Most of them presented between 5-8 weeks of gestational age (61.3%).27.2% of patients were diagnosed before 4 weeks of amenorrhea and 11.3% were above 9 weeks. All the cases had been confirmed by USG.17 of them had Beta-HCG less than 1000 m I U /ml. A patient with heterotopic pregnancy had Beta -HCG of more than 1 lakh m I U /ml.

Commonest site of ectopic pregnancy was fallopian tube in 93% of patients (Table 4).

[illegible]r [illegible]

[illegible]	[illegible] r [illegible]	[illegible]
Tube	82	93
Caesarean scar	3	3.4
Ovary	2	2.4
Heterotopic	1	1

The commonest site of tubal involvement was in ampulla (Table 5).

[illegible] [illegible]

[illegible]	[illegible] r [illegible]	[illegible]
Ampulla	60	73.1
Cornua	10	12.1
Isthmus	6	7.3
Fimbria	4	4.8
Tubal abortion	2	2.4

On receiving, 86% of patients were hemodynamically stable, 13.6% of patients were in various stages of shock. 53.6% of patients presented with ruptured ectopic, unruptured ectopic was noted in 43.9% of patients (Table 6).

[illegible] 6 [illegible] d [illegible]

[illegible] d [illegible]	[illegible] r [illegible]	[illegible]
Ruptured	44	53.6
Unruptured	36	43.9
Tubal abortion	2	2.5

Right sided tubal ectopic was noted in 65.8%, left side tube involved in 34.1% of patients. Tubal abortion was observed in 2.5% of patients. Intra-operatively hemoperitoneum was noted in 64% of patients. Most common treatment offered for tubal ectopic was surgical, i.e. salpingectomy (Table 7).

[illegible] r [illegible]

r [illegible]	[illegible] r [illegible]	[illegible]
Medical	11	12.5
[illegible] r [illegible]		
Laparotomy	49	59.7
Laparoscopy	9	10.9
M d [illegible] d		
Laparotomy	8	9.7
Laparoscopy	2	2.4
Conservative	3	3.6

Twenty-one patients were fit into medical management, among them 12.5% of patients were managed medically with methotrexate successfully.

10 cases failed to medical management which were subsequently managed with either laparoscopic or laparotomy and salphingectomy.

3 cases were managed conservatively by observing the declining trend of Beta-HCG.

Other types of ectopic

Caesarean scar pregnancy was diagnosed in 3 patients, among them 2 patients underwent surgical excision. One patient was managed with UAE (uterine artery embolisation) followed by methotrexate. Heterotopic pregnancy following ovulation induction which resulted in twin intra uterine gestation and tubal ectopic was noted in one patient and she underwent emergency laparotomy and salpingectomy. Two patients were diagnosed as having ovarian ectopic and surgical excision was offered to both of them. Most common morbidity noted was anemia in 42%, out of which severe anemia (4-7gm) was seen in 5.6% (Table 8). Among them 28.4% of patients received blood and blood products. No surgical site infection or mortality was noted.

[illegible] [illegible] [illegible] d [illegible]

[illegible] [illegible]	[illegible] r [illegible]	[illegible]
4-7 gm/dl	5	5.6
7-10 gm/dl	32	36.3
>10 gm/dl	51	58.1

[illegible]

The incidence of ectopic pregnancy among women attending emergency department with first trimester abdominal pain, bleeding or both is between 6-16 %(14). A study conducted by Shraddha Shetty et al had incidence of 5.6/1000 deliveries.[15] In our study the incidence is 6.6%/1000 deliveries which is comparable. Majority of patients were between 25-30years which is similar to the above study and Khaleeqe et al.[15,16]

As in Karki et al (17), we had similar higher incidence of ectopic pregnancies (73.8%) in multigravida. In our study, the commonest predisposing factors were previous abortion either spontaneous or induced, previous pelvic surgeries including caesarean section, history of IUCD insertion, tubectomy, history of PID and infertility treatment, which are similar to various other studies.[17,18] As in the studies by Porwal Sanjay et al, Shraddha Shetty et al, the most common symptom in our series was pain abdomen which is comparable.[15,18]

Urinary beta HCG, serum beta HCG, transvaginal USG are the diagnostic tools for diagnosing EP. USG should be the investigation of choice for symptomatic women. In asymptomatic women, serial monitoring of serum beta HCG should be done. As in the study by Shraddha Shetty et al, we also had more number of right sided EP and involvement of the ampullary portion of the tube.[15]

Heterotopic pregnancy is rare in natural conception which is around 1 in 30,000 pregnancies. But the incidence is more in case of ovulation induction or with

assisted reproduction techniques. In our study, we had a case of triplet heterotopic pregnancy following ovulation induction with clomiphene citrate. It is recommended that even patients shown on ultrasound to have intrauterine pregnancy should be given comprehensive pelvic ultrasound so that possibility of synchronous heterotopic pregnancy can be excluded, particularly in pregnancies following fertility treatment.[19]

In our study, ruptured EP was noted in 53% of cases, 43.9% of cases had an unruptured ectopic gestation. In Latchaw G et al study, the same were 59% and 41% respectively.[20]

Laparoscopy and medical therapy have now emerged as the widely used therapeutic modalities with great success in terms of reduced morbidity, shorter hospital stay and conservation of fertility.[21] However choice depends upon early identification of ectopic pregnancy and hemodynamic status of the patients.[22] In our study, 11% of patients responded well to single dose of methotrexate.

Surgery should be the treatment of choice in unstable patient.[19] In our study 13.6% were unstable and all of them were managed surgically. Commonest surgery performed was partial/total salpingectomy. We did bilateral total salpingectomy for patients who had undergone tubectomy previously and were not desirous of future childbearing. It has been extensively studied and reported that conservative management does not increase the risk of future ectopic.[15] In our study, three patients were managed conservatively by observing the declining trend of Beta HCG. Morbidity included anemia, blood transfusion and wound infection. In our study, we had 42% of patients with hemoglobin level of less than 10grams. Among them 28.4% of patients received blood and blood products. No surgical site wound infection was noted.

No maternal mortality was noted in our study which is consistent with the study by A Abbas et al.[23] Early identification of the patients with EP by a multipronged approach including high index of suspicion, risk prediction, serum beta HCG and transvaginal ultrasound helps in improving the prognosis as far as morbidity, mortality and future fertility are concerned.[24]

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Identification of underlying risk factors, early diagnosis with essential investigating modalities like beta-HCG, transvaginal USG, timely intervention with medical or surgical method will definitely reduce morbidity and mortality and improve the future reproductive outcome.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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1. Walker JJ. Ectopic pregnancy. Clin Obstet Gynecol. 2007;50:89-99.
2. Mahboob U, Mazhar SB. Management of ectopic pregnancy: a two-year study. J Ayub Med Coll Abbottabad. 2006;18:34-7
3. Chow WH, Daling JR, Cates WJ, Greenberg RS. Epidemiology of ectopic pregnancy. Epidemiol Rev. 1987;9:70-94.
4. Karaer A, Avsar FA, Batioglu S. Risk factors for ectopic pregnancy: a case-control study. Aust N Z J Obstet Gynaecol. 2006;46:521-7.
5. Barnhart KT. Clinical practice. Ectopic pregnancy. N Engl J Med. 2009;361:379-87.
6. Akande V, Turner C, Horner P, Horne A, Pacey A, British Fertility Society. Impact of Chlamydia trachomatis in the reproductive setting: British Fertility Society Guidelines for practice. Human Fertil. 2010;13:115-25.
7. Job-Spira N, Collet P, Coste J, Bremond A, Laumon B. Risk factors for ectopic pregnancy. Results of a case control study in the Rhone-Alpes region. Contracept Fertil Sex. 1993;21:307-12.
8. Bouyer J, Coste J, Shojaei T, Pouly JL, Fernandez H, Gerbaud L, et al. Risk factors for ectopic pregnancy: a comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol. 2003;157:185-94.
9. Parashi S, Moukhah S, Ashrafi M. Main risk factors for ectopic pregnancy: a case-control study in a sample of Iranian women. Int J Fertil Steril. 2014; 8:147-54.
10. Moini A, Hosseini R, Jahangiri N, Shiva M, Akhoond MR. Risk factors for ectopic pregnancy: a case-control study. J Res Med Sci. 2014; 19:844-9.
11. Ankum WM, Mol BW, Van der Veen F, Bossuyt PM. Risk factors for ectopic pregnancy: a meta-analysis. Fertil Steril. 1996;65:1093-9.
12. Barnhart KT, Sammel MD, Gracia CR, Chittams J, Hummel AC, Shaunik A. Risk factors for ectopic pregnancy in women with symptomatic first-trimester pregnancies. Fertil Steril. 2006;86:36-43.
13. Timmerman D. Predictive models for the early diagnosis of ectopic pregnancy.Verh K Acad Geneeskd Belg. 2004;66:155-71.
14. Murray H, Baakdah H, Bardell T, Tulandi T. Diagnosis and treatment of ectopic pregnancy. CMAJ. 2005;173:905.
15. Shetty KS, Shetty KA. A clinical study of ectopic pregnancies in atertiary care hospital of Mangalore, India. Innovat J Med H Sci. 2014;4:305-9.
16. Khaleeque F, Siddiqui RI, Jafarey SN. Ectopic pregnancies: A Three-year study. J Pak Med Assoc 2001;51:240-3.
17. Karki RCL, Pradhan B, Duwa S. Annual Analysis of Ectopic Pregnancy in Tertiary Care Hospital. PMJN. 2011;11:5-8
18. Gupta R, Porwal S, Swarnkar M, Sharma N, Maheshwari P. Incidence, trends and risk factors for

Ectopic Pregnancies in a tertiary care hospital of Rajasthan. JPBMS. 2012;16:1-3.
19. Maheswari S, Panicker S. Triplet heterotopic pregnancy following ovulation induction with clomiphene citrate: a case report and review of literature Int J Rerod Contracept Obstet Gynecol. 2013;2:743-5.
20. Latchaw G, Takacs P, Gaitan L, Geren S, Burzawa J. Risk factors associated with the rupture of tubal ectopic pregnancy. GynecolObstet Invest. 2005;60:177-80.
21. Jurkovie D. Ectopic pregnancy. In: Edmonds DK, editor. Dew Hurst's textbook of Obstetrics and Gynecology. 7 ed. USA; Blackwell Publishers;2007
22. Shah N, Khan NH. Ectopic pregnancy: Presentation and risk factors. J Coll Physicians Surg Pak. 2005;15:535-8.
23. Abbas A, Akram H. Ectopic Pregnancy; Audit at Maula Bakhsh Teaching Hospital Sargodha.Prof Med J. 2011;18:24-7.
24. Lozeau AM, Potter B. Diagnosis and management of ectopic pregnancy. Am Fam Physician. 2005; 72:1707-14.
25. Majhi AK, Roy N, Karmakar KS, Banerjee PK. Ectopic pregnancy--an analysis of 180 cases. J Indian Med Assoc. 2007;105:308-10.

Maheswari S, Poornima C, Lalitha N, Panicker S. Analysis of various types of ectopic pregnancies: a five-year review. Int J Reprod Contracept Obstet Gynecol 2018;7:2410-4.

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Original Research Article

Comparative study on gynaecological issues in adolescence among private and government school girls

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ABSTRACT

Background: Gynaecological problems of adolescents occupy a special spectrum of gynaecological disorder of all ages. This is because of the transitional phase of the physical nature of the problems which are so unique, special and specific for the age group and also because of the associated physiological factors which are very important in the growth and psychological remodelling of someone in the transition between childhood and womanhood. The study conducted with the objective to assess the extent of awareness regarding adolescent changes and problems among school going adolescents and to compare the same between government and private school adolescent girls.
Methods: We used a preformed questionnaire as our study material to assess the knowledge and attitude regarding nutritional requirement, BMI, obesity, puberty changes, menstrual hygiene and irregularities, anaemia, vaginal discharge, gynaecological tumours and HPV vaccine among the adolescent girls in both government and private schools and thereby, to compare the same between them.
Results: Awareness regarding nutritional requirement (45%, 98%), BMI (66%, 15.5%), obesity related gynaecological problems (9%, 81%) and causes for anaemia (28%, 93%) were less in government school girls when compared private school girls. Awareness on menstrual hygiene was satisfactory in both the groups. Awareness on HPV vaccination was unsatisfactory in both the groups.
Conclusions: Government school girls need better health education regarding adolescent related gynaecological issues. There is need to educate both the groups on HPV vaccination.

Keywords: Adolescence, Government school girls, Menstruation, Private school girls, Puberty

INTRODUCTION

Adolescence is a period of biological, cognitive and social transition of such magnitude and rapidity, that it is no surprise to find that it is associated with the onset or exacerbation of a number of health related problems. India has the largest population of adolescents in the world, accounts for a quarter of the country's population (243 million estimated).[1] WHO identifies adolescence as the period in woman growth and development that occurs after childhood and before adulthood, from age 10 to 19 years. It represents one of the critical transitions in the life span and is characterized by a tremendous pace in growth and change that is second only to that of infancy.[2] It is the level of self-awareness among adolescents, which enables them to see where their thoughts and emotions take them. Adolescents greatly lack correct information related to their bodies' physiological, psychological and sexual changes. There is an urgent need for regular adolescent friendly information, education and communication activities covering different aspects of adolescent knowledge, needs/problems. The study was conducted with the objective to assess the extent of awareness regarding adolescent changes and problems

among school going adolescents and to compare the same between government and private school adolescent girls.

The objective of the study was to know the attitude of the adolescent girls in the awareness regarding the gynaecological problems and to compare the awareness level of government school girls to that of private/management school girls.

METHODS

A observational study on Gynaecological issues in adolescence among private and government school girls was conducted after approval from the Institutional ethics committee PSGIMSR Coimbatore in the year 2015 (3 months).

Study material: Predesigned questionnaire

Sample size: 100 in government school and 100 in private school

Inclusion criteria

All adolescent girls who attained puberty/maturity

Exclusion criteria

Those who does not belong to adolescent age group, unwillingness to participate in the study.

We used a preformed questionnaire as our study material to assess the knowledge and attitude regarding nutrition, BMI, obesity, puberty changes, menstrual hygiene and irregularities, anemia, vaginal discharge, gynaecological tumors and HPV vaccine among the adolescent girls in both government and private schools and thereby, to compare the awareness level of government school girls to that of the private school girls. After getting permission from the school authorities, we explained the aim of the study one by one of our research. Informed written consent was obtained from the students and their parents. Data was collected; statistical analysis was done by percentage and chi-square test. Statistical significance of differences between groups was tested. P value <0.05 was taken as statistically significant.

RESULTS

Table 1: Awareness of nutritional requirements.

School	Nutrition		Chi-square	P value
	Yes	No		
Private	45%	55%	51.42	0.00*
Government	2%	98%		

Table 1 showed that the government school girls had almost no awareness regarding the nutritional requirements and the additional calories needed in this age group. In the private school, about 45 girls were aware about the importance of nutrition and also the additional calorie requirement in adolescence. We observed a statistical significance among the private and the government school girls.

Table 2 showed that in Government school, about 15.5% were aware about BMI and in private school, 66% were aware. Statistical significance was found among the private and government school adolescents.

Table 3 showed that only 9% of the Government school girls and 81% of private school girls were aware of obesity and its consequences. Statistical significance has been found among the private and government school girls.

Table 4 shows that the correct response was about 85% and 89% from government and private schools respectively. No statistically significant value has been found out regarding the awareness about puberty age.

Table 2: Awareness of BMI.

School	Awareness of BMI		Chi-square	P value
	Yes	No		
Private	66%	44%	35.16	0.00*
Government	15.5%	84.5%		

Table 3: Awareness of obesity.

School	Awareness of obesity		Chi-square	P value
	Yes	No		
Private	81%	19%	104.72	0.00*
Government	9%	91%		

Table 4: Awareness regarding puberty age.

School	Awareness of puberty age		Chi-square	P value
	Yes	No		
Private	89%	11%	0.707	0.400
Government	85%	15%		

Table 5: Awareness of physiological body changes in puberty.

School	Puberty changes		Chi-square	P value
	Yes	No		
Private	82%	18%	2.51	0.113
Government	89%	11%		

Table 5 evidences that 89% of the government school girls and 82% of the private school girls showed positive response. The awareness mindset of both the private and the government school girls remained almost same,

which thereby denotes no statistical significance regarding the physiological body changes in puberty.

Menstrual hygiene and irregularities

In our study, we had asked about the awareness of menstrual hygiene and how do they maintain it and the various menstrual irregularities.

Table 6 showed in our study that equal number of girls in both schools was aware of the necessity of hygiene during menstruation. Towards maintenance of hygienic practices during menstruation 88% and 78% of private and government school girls were aware. Regarding normal duration of periods (p=0.106) and factors affecting periods(p=0.00) ,there was a statistical significance found among the government and private school.93% and 86% of private and government school girls were aware of the menstrual irregularities. We found that majority of private school girls were less aware than government school girls regarding the consultation of doctor during menstrual problems.

Table 6: Awareness about menstrual hygiene.

School	Menstrual hygiene		Chi-square	P value
	Yes	No		
Private	100%	0%		
Government	98%	2%	2.02	0.115
	Practices of menstrual hygiene		**Chi-square**	**P value**
	Yes	No		
Private	88%	12%		
Government	78%	22%	1.87	0.171
	Duration of periods		**Chi-square**	**P value**
	Yes	No		
Private	88%	12%		
Government	47%	53%	2.60	0.106
	Factors affecting periods		**Chi-square**	**P value**
	Yes	No		
Private	46%	54%		
Government	58%	42%	38.31	0.00*
School	**Menstrual irregularities**		**Chi-square**	**P value**
	Yes	No		
Private	93%	7%		
Government	86%	14%	1.62	0.203
	Consultation of doctor in case of menstrual irregularities		**Chi-square**	**P value**
	Yes	No		
Private	74%	26%		
Government	96%	4%	18.98	0.00*

Table 7: Awareness of anaemia.

School	Anaemia		Chi-square	p value
	Yes	No		
Private	64%	36%	1.45	0.228
Government	88%	22%		
School	Causes of anaemia		chi-square	P value
	Yes	No		
Private	82%	18%		
Government	93%	7%	8.21	0.004*

Table 7 shows that about 88% and 64% were aware about adolescent prone anaemia and the causes in government school respectively. About 93%, 82% were aware in the private school. No statistical significance was found among them regarding anaemia, but the causes of anaemia had a significant value towards the government school girls.

Vaginal discharge

In our study, two questions were asked about vaginal discharge; one about normal vaginal discharge and the second question about when to consult a doctor in case of vaginal discharge, being excessive, foul smelling or associated with itching. About 57% and 76% were aware about the normal vaginal discharge and 88% and 89% were aware about the abnormal vaginal discharge and the need to consult a doctor.

Table 8: Awareness of vaginal discharge.

School	Normal vaginal discharge		Chi-square	P value
	Yes	No		
Private	76%	24%		
Government	57%	43%	8.10	0.004*
School	**Abnormal vaginal discharge**		**Chi-square**	**P value**
	Yes	No		
Private	89%	11%		
Government	88%	12%	0.49	0.825

Table 8 shows that about 57% and 76% adolescent girls were aware about the normal vaginal discharge and about 88% and 89% adolescent girls were aware about the abnormal vagina discharge from the government and the private school respectively. We found that there was a statistical significance among government and private school girls regarding the normal vaginal discharge.

Table 9: Awareness of gynaecological malignancies.

School	Gynaecological malignancies		Chi-square	P value
	YES	**NO**		
Private	62%	38%		
Government	71%	29%	2.67	0.102

Table 9 shows that about 71% and about 63% adolescents were aware about the gynaecological malignancies from government and private school. There was no statistical difference among them.

HPV vaccination

In our study, there were two questions regarding HPV vaccination; one about whether they were aware of the vaccine and its role and the second about whether they had done the vaccination or not. The results obtained were about 5% and 42% girls from government and private school were aware of the vaccine. Even the percentage of girls, who were aware of the vaccination, did not take the vaccine. None of the girls from government school has done the vaccination and only 8% girls from the private school have done their vaccination.

Table 10: Awareness and attitude regarding HPV vaccination.

School	HPV vaccination		Chi-square	P value
	Yes	No		
Private	42%	58%		
Government	5%	95%	39.58	0.00*
School	**Practice of HPV vaccination**		**Chi-square**	**P value**
	Yes	No		
Private	8%	92%		
Government	0%	100%	8.33	0.004*

DISCUSSION

Nutritionally, adolescent period is the most vulnerable period because of increase demand and requirement for rapid growth and development. Nutrition and physical growth are integrally related. A healthy diet throughout puberty and adolescence is important to provide nutrients that support optimal physical growth and cognitive development. Gurpreet et al showed that adolescent subjects belonging to urban area had better knowledge than subjects belonging to rural area. Regarding height and weight and body mass index, measurement of height and weight and calculation of BMI showed that about 81% were underweight and 1.8% was approaching towards obesity.[8] In our study, government school girls had almost no awareness on nutritional requirement.

Adolescent obesity has been identified as a significant and growing health problem leading to type-2 Diabetes, metabolic syndrome, and other health problems (Thomas and walker, 2012). Rising obesity rates around the world have had a profound impact on female reproductive health. Childhood obesity is associated with early onset of puberty, menstrual irregularities and polycystic ovarian syndrome during adolescence.[9]

The World Health Organization (WHO) describes overweight and obesity as one of the today's most important public health problem, which is being escalated as a global epidemic.[10] It is also increasingly recognized as a significant problem in developing countries and countries undergoing economic transition.[11] The problem of being overweight and obesity is confined not only to adults but also being reported among the children and adolescents in the developed countries during the past two decades as well as similar trends are being observed even in the developing world, though less rapidly.[12-14] In

our study, the awareness on BMI and obesity was about 66% and 81% among the private school girls and 81% and 9% from the government school girls respectively.

Adolescence is a time for growth spurt and puberty changes. It is a stage in which adolescents cross the line between childhood and adulthood.[15] Puberty is a period of several years in which rapid physical growth and psychological changes occur, culminating in sexual maturity. The average onset of puberty is at 10 or 11 for girls and 11 or 12 for boys. Every person's individual timetable for puberty is influenced primarily by heredity, although environmental factors, such as diet and exercise also exert some influences. A cross sectional study was conducted with a sample of 320 adolescent students of ninth to twelfth classes (80 from each class). Data were collected on predesigned, pretested and semi structural schedules by conducting in-depth interviews of selected study adolescents by the investigator. Mean age of female respondents was 15.02 years. 212 study adolescents were aware of at least one adolescent change. 248/272 adolescents perceived those changes as 'normal' and on the contrary, 24/272 adolescents either did not consider these changes as normal or they did not know whether the changes were normal or abnormal.[16] Our results showed that both government and Private School girls had almost same knowledge on pubertal body changes.

Menstruation is a milestone event in a girls' life and the beginning of reproductive life so all aspects of menstruation need to be understood by adolescent girls.[17] Lack of knowledge and poor personal hygiene practices can lead to various gynaecological problems including reproductive tract infections.

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They found that awareness regarding menstruation was more in urban adolescent girls (63.38%) as compared to rural (47.57%).Many studies revealed that most of the adolescent girls had incomplete and inaccurate information about the menstrual physiology and hygiene.[18] Our study showed that, total awareness regarding need of menstrual hygiene is 100% and 98% among the private and government school girls, which was satisfactory.

The awareness about gynaecological malignancies is important in this age group as the lifestyle and attitude can prevent the occurrence of the tumours. Though gynaecological malignancies are rare among adolescents, awareness about gynaecological malignancies is important in this age group as treatment of cancers may affect the future reproductive health. In our study, 62% and 71% awareness was present among the private and the government school girls, respectively.

Recently two HPV vaccines quadrivalent Gardasil and bivalent Cervarix have been shown to be highly effective in preventing infection with high-risk type HPV16 and HPV18, the two most oncogenic types. As these vaccines are highly effective before exposure to HPV, current guidelines priorities adolescent girls as primary group for HPV vaccination.[19] HPV vaccine implementation is still facing challenges even though it seems to be a definitive intervention to control mortality from cervical cancer and has the potential to address challenges of screening in India. Implementation faces barriers of cost, acceptance and lack of awareness.[20]

Our study showed almost none of the girls from the government school had done the vaccination. Only 8% of the private school girls are vaccinated.

CONCLUSION

In our study, we found that private school girls were more aware in the aspects of nutritional requirement, BMI, obesity related gynaecological problems and vaginal discharge. HPV vaccine awareness was unsatisfactory in both the respondents. Thus, there is a need to educate and aware the adolescent girls regarding HPV vaccination. Awareness regarding the prevalence of HPV infection and the cancer prevention benefits of the vaccine has to be made at the earliest to prevent the tomorrow's mothers being affected by cervical cancer.

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REFERENCES

1. Jain RB, Kumar A, Khanna P. Assessment of self-awareness among rural adolescents: A cross-sectional study. Indian Journal of Endocrinology and metabolism. 2013;17(7):367-72.
2. WHO: Adolescent development assessed 1/6/2015)
3. Dars S, Sayed K. Relationship of menstrual irregularities to BMI and nutritional status in adolescent girls. Pak J Med Sci. 2014;30(1):141-4.
4. Rogers J, Mitchell GW Jr. The relation of obesity to menstrual disturbances. N Engl J Med. 1952;247(2):53-5.
5. Ray S, Ghosh T. Knowledge and information on Psychological, physiological and gynaecological problems among adolescent school girls of Eastern India. Ethiop J Health Sci. 2011;21(3):183-9.
6. Adikari P, Kadel B. Knowledge and practice regarding menstrual hygiene in rural adolescent girls of Nepal. Kathmandu University. 2006;5(3):382-6.
7. Ramavath KK, Olyai R. Knowledge and awareness of HPV infection and vaccination among urban adolescents in India. J Obstet Gynaecol India. 2013;63(6):399-404.
8. Arora G. Nutritional Awareness and status of Adolescent Girls studying in schools of Urban and Rural Areas of District Kurukshatra. Journal of food and nutrition sciences. 2015;3(3):126-30.

9. Thomas J, Walker DK. A practical guide to metabolic syndrome. The clinical adviser. 2012.
10. World Health Organization controlling the global obesity pandemic (document on the internet) [Last accessed on 2014, Jul 17]. Available from: http://www.who.int/nutrition/topics/obesity/en/.
11. Popkin BM. The nutrition transition and obesity in the developing world. J Nutr. 2001;131:871-3.
12. Lobstein T, Baur L, Uauy R. Report to the WHO. London: IASO International Obesity Task Force; 2004. Obesity in children and young people: A crisis in public health. 2004.
13. Martorell R, KettelKhan L, Hughes ML, Grummer-Strawn LM. Overweight and obesity in preschool children from developing countries. Int J Obes Relat Metab Disord. 2000; 24:959-67.
14. Pouliot MC, Després JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, et al. Waist circumference and abdominal sagittal diameter: Best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. Am J Cardiol. 1994;73:460-8.
15. Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, et al. Relation of body fat distribution to metabolic complications of obesity. J Clin EndocrinolMetab. 1982;54:254-60.
16. Jain RB, Kumar A, Khanna P. Assessment of self-awareness among rural adolescents: A cross-sectional study. Indian Journal of Endocrinology and metabolism. 2013;17(7):367-72.
17. Paul D. A report of an ICMR funded research project: knowledge and practices of adolescent girls regarding reproductive with special emphasis on hygiene during menstruation. New Delhi: national Institute of Public Cooperation and child Development (NIPCCD); 2007. P. 1-98.
18. Patlel R, Kubde S. comparative study on menstrual hygiene in rural and urban adolescent. International Journal of medical science and Public Health. 2014;3(2).
19. Kim JJ, Ortendahl J, Goldie SJ. Cost-effectiveness of human papilloma-virus vaccination and cervical screening in women older than 30 years in the United States. Ann Intern Med. 2009;151:538-45.
20. Kollar Linda M, Kahn Jessica A. Education about human papillomavirus and human papillomavirus vaccines in adolescents. Curr Opin Obstet.Gynecolo. 2008;20:479-83.

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Pregnancy outcome in autoimmune disorders

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Abstract

Background: Autoimmune disorders are chronic multisystem disorders affecting women of their reproductive age. Pregnancy among these women is complicated by the disease itself. Flaring up of disease, uteroplacental insufficiency due to vasculitis and drugs used for treatment are main reason for unfavourable pregnancy outcomes. The objective of this study was to analyze the pregnancy complications and outcome among 113 cases of autoimmune disorders.

Methods: This study is a retrospective analysis of case records of pregnant women with autoimmune disorders. This study was conducted at PSG IMSR and Hospital, Coimbatore, and Tamil Nadu from July 2012 to June 2018. The case sheets were retrieved from medical record department and the details such as type of disease, maternal age, parity, status of disease at conception, treatment taken during pregnancy, maternal complications and fetal complications were collected and analyzed.

Results: During 6-year period there were 113 pregnant women with autoimmune disorders, and they were studied of their previous and present pregnancy outcome. The disease incidence was 7.01% per 1000 deliveries. 40.7% were less than 25 years age group and 71.6% were multigravidae. We had forty-one patients (36%) who were positive for APLA syndrome which were the maximum number of patients with auto immune disorder in pregnancy and 37 patients (32.7) with SLE. We had significant numbers of maternal and fetal complications for common disorders. Most of the rare auto immune disorders were diagnosed before pregnancy and these pregnancies were managed by multi-disciplinary approach, and continued on immunomodulators throughout pregnancy, hence maternal and fetal complications were less for them.

Conclusions: Adequate pre-conceptional counseling, vigilant monitoring during pregnancy and post-partum will avoid pregnancy related complications and have favorable outcomes.

Keywords: Autoimmune disorder, Fetal complications, Immunomodulators, Maternal complications, Pregnancy outcome, Pre-conceptional counseling

Introduction

Autoimmune disorders are chronic multisystem disorders which are characterized by tissue damage and inflammation due to alteration of adaptive immune response in the absence of trigger.[1] Its prevalence is between 7.6%-9.4%.[2] Since most of the autoimmune disorders are diagnosed frequently during reproductive age in females, these women are at high risk not only during pregnancy but also during postpartum.[3] When compared to females without autoimmune disorders, these women carry more incidence of miscarriages in all trimesters, preeclampsia, intrauterine growth restriction, premature rupture of membranes, placental insufficiency, preterm labour, low birth weight and have increased caesarean section rates.[4] Though these pregnancies carry

high incidence of adverse effects, improved care in terms of pre-conceptional counselling and continuation of treatment throughout the pregnancy and post-partum have improved the outcome of pregnancies.[5]

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This study is a retrospective analysis of a total 113 case records of pregnant women with autoimmune disorders. This study was conducted at PSG IMSR and Hospital, Coimbatore, Tamil Nadu from July 2012 to June 2018. The case sheets were retrieved from medical record department and the details such as type of autoimmune disorder, maternal age, parity, status of disease at conception, treatment taken during pregnancy, maternal complications like pregnancy induced hypertension, gestational diabetes mellitus, thrombotic events, anemia, renal flares, thrombocytopenia, preterm labour, mode of delivery, indications for caesarian section and fetal complications like abortion, anomalies, intra uterine fetal demise, low birth weight, intra uterine growth restriction, oligohydramnios, need for admission to neonatal intensive care unit, low Apgar score were collected and analyzed.

Inclusion criteria

- All primigravidae and multigravidae who are known case of autoimmune disorder and all primigravidae and multigravidae who are diagnosed with autoimmune disorder during present pregnancy.

Exclusion criteria

- Pregnant mothers without autoimmune disorders.

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During this period of 6 years (2012-2018), there were 113 pregnant women with autoimmune disorders and study was carried out based on their previous and present pregnancy outcomes. The incidence of autoimmune disorders in pregnancy was 7.01% per 1000 deliveries.

Table 1 shows the number of pregnancies with autoimmune disorders in our study.

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Antiphospholipid antibody syndrome	41	36.2
Systemic lupus erythematoses	37	32.7
Idiopathic thrombocytopenic purpura	11	9.7
Takayasu syndrome	5	4.4
Sjögren syndrome	4	3.5
Hashimoto's thyroiditis	3	2.6
Multiple sclerosis	3	2.6
Rheumatoid arthritis	2	1.7
Schönlein-henoch purpura	2	1.7
GBS	1	0.8
Von Williebrand disease	1	0.8
Multisystem connective tissue disorder	1	0.8
Medial vessel vasculitis	1	0.8
Ulcerative colitis	1	0.8

In our study, we had forty-one patients (36%) with APLA syndrome including primary and secondary APLA and 37 patients (32.7%) with SLE.

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<25	46	40.7
25-30	45	39.8
31-35	21	18.5
>35	1	0.8

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APLA	2 (4.8%)	13 (31%)	1 (2%)	3 (7.3%)	-	-	7 (17%)	1 (2.4)
SLE	4 (10.8%)	6 (16%)	3 (8%)	2 (5.4%)	-	1 (2.7%)	16 (43%)	8 (21%)
ITP	1 (9%)	6 (54%)	-	1 (9%)	2 (18%)	-	7 (63%)	1 (9%)
Takayasu	3 (60%)	-	1 (20%)	-	-	-	4 (80%)	-
Sjögren syndrome	-	1 (25%)	-	-	-	-	3 (75%)	□□

In our study, most of the patients were below 30 years of age. Table 2 shows the age group distribution. Multigravida (71.6%) were more in number than primigravida (28.3%) in our study.

Parity distribution is shown in Figure 1.

Table 3 shows the most common autoimmune disorders in our study and their maternal complications.

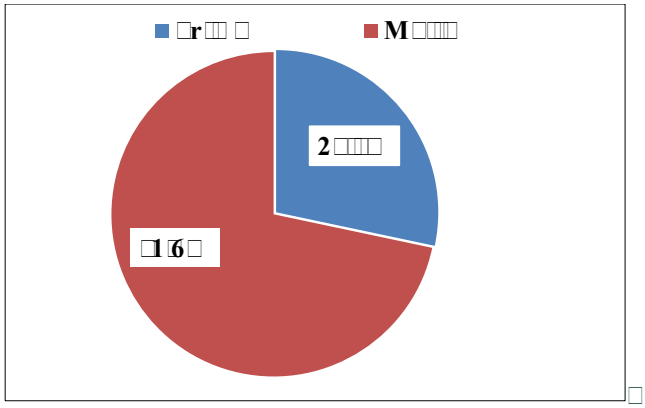


Table 3: Maternal complications in previous pregnancy.

Disorder	Number	Percentage
APLA	26 (63%)	9 (21.9%)
SLE	22 (59%)	5 (13.5%)
ITP	4 (36%)	-
Takayasu	-	1 (20%)
Sjögren syndrome	3 (75%)	-

Two patients diagnosed with SLE had abnormal doppler changes at 32 weeks and 34 weeks respectively and underwent caesarean section. The most common indication for caesarean section was non-progression of labour followed by previous section.

Table 4 shows fetal complications in previous pregnancy for more common disorders in our study.

Table 4: Fetal complications in previous pregnancy.

Disorder	IUGR	Abortion	Stillbirth
APLA	7 (17%)	2 (4.8%)	1 (2.4%)
SLE	13 (35%)	6 (16%)	5 (13.5%)
ITP	2 (18%)	-	1 (9%)
Takayasu	3 (60%)	-	-
Sjögren syndrome	-	2 (50%)	-

Table 5 shows the fetal complications in the current pregnancy for same disorders.

Table 5: Maternal complications in current pregnancy.

Disorder	Current	Previous
Pre-eclampsia	10.8%	13%
GDM	16%	NA
Renal flare	8%	NA
Anemia	7.3%	21%
C-section	43%	53%
Preterm labour	21%	21%

Table 6: Fetal complications in current pregnancy.

Disorder	Current	Previous
Abortion in previous conception	59%	46%
IUD	13.5%	8%
IUGR	35%	32%
LBW	24.3%	22%
NICU admission	13.5%	15%

APLA

Obstetric antiphospholipid antibody syndrome (OB-APS) is defined according to the updated Sydney classification criteria developed by expert consensus agreement in 2006.[6] APLs include the lupus anticoagulant, anticardiolipin IgG and IgM and anti-β2-glycoprotein I antibody. In addition to the detection of aPLs on at least two occasions more than 12 weeks apart, OB-APS is defined by any one of the following adverse pregnancy outcomes: 1) otherwise unexplained recurrent pregnancy loss before the 10th week of gestation, 2) otherwise unexplained fetal death ≥10 weeks of gestation, 3) preterm birth before 34 weeks of gestation due to preeclampsia or placental insufficiency.[7] Other OB-APS complications include catastrophic APS, thrombosis, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), IUGR, fetal distress, premature rupture of membranes, and thrombocytopenia.[8] It has been recognized that among the three aPLs, lupus anticoagulant (LAC) is the primary predictor of poor pregnancy outcome in women both with or without SLE, and with or without other aPLs.[9] Among the 41 patients with APLA, 20 patients were positive for lupus anticoagulant antibody, 11 were positive for anticardiolipin antibody and 10 had anti-β2-glycoprotein I antibody.

In our study, 31.8% of patients with primary APLA and 4.4% of patients with secondary APLA with associated SLE were diagnosed. The incidence of pre-eclampsia among APLA positive patients in our study was 4.8% whereas in Wijeyaratne study, it was 10.9%.[10] Similarly the incidence of GDM in our study was 31% as compared to 19.2% in Wijeyaratne study. In our study 2.4 % had preterm labour and 17% of patients underwent cesarean section. In regard to fetal complications, 63% of patients in our study had previous one or more abortions which was comparable to Wijeyratne study (59.8%). The incidence of IUD in previous pregnancy was 21.9.%. The incidence of low birth weight babies was 12% and babies requiring NICU admission was 2.4% respectively.

SLE

Systemic lupus erythematosus (SLE, or “lupus”), affects females in a 9:1 ratio to males.[11] The adverse pregnancy

outcomes observed with lupus are well known, and include increased rates of intrauterine growth restriction (IUGR), preterm birth and fetal loss, as well as the syndrome of neonatal lupus (NL), associated with trans placental passage of auto antibodies. The risks for thrombosis, infection, thrombocytopenia, and transfusion were each 3- to 7-fold higher for women with systemic lupus erythematosus. Lupus patients also had a higher risk for cesarean sections (odds ratio: 1.7), preterm labor (odds ratio: 2.4), and preeclampsia (odds ratio: 3.0) than other women. Women with systemic lupus erythematosus are more likely to have other medical conditions, including diabetes, hypertension, and thrombophilia, that are associated with adverse pregnancy outcomes.[12]

High disease activity at the time of conception or in the months preceding conception is an independent predictor for increased risk of adverse pregnancy outcomes including prematurity, IUGR and fetal demise. This has led to the current recommendations that lupus patients achieve 6 months of disease quiescence prior to attempting conception.[13] Maternal and fetal complications associated with SLE were compared with Eman et al study.[14] These are shown in table 6 and 7 respectively.

Six patients had first trimester abortions in the current pregnancy.

Takayasu syndrome

In our study, 60% patients had hypertension complicating pregnancy whereas in Mandal et al study had 92.8%.[15] Caesarean section rate in our study was 60%, whereas in Mandal study it was 71.4%. Intrauterine growth restriction was 60% in our study, which was comparable with Mandal et al study (51.7%).

Schönlein-henoch purpura

Schönlein-henoch purpura (SHP) is a systemic vasculitis that affects vessels of a small caliber.[16] The onset of SHP during pregnancy is very rare. Treatment with orally administered corticosteroids may lead to a beneficial outcome for both mother and newborn.[17] In our study, we had 2 patients diagnosed with Schönlein-Henoch purpura, of which one developed GDM, other had uneventful pregnancy.

Medium vessel vasculitis

Most of the women with medium vessel vasculitis fared well during pregnancy without significant disease progression, but instances of aneurysm rupture, renal insufficiency, pulmonary embolism, and myocardial infarction were reported in Gatto et al study.[18] In our study, one patient diagnosed with medium vessel vasculitis developed myocarditis during pregnancy and she was managed with steroids.

ITP

Nine percent of chronic ITP patients in our study had preeclampsia, whereas Diego et al, study had 8.3%.[19] In our study 9% had preterm labour which was comparable to Diego et al (9.5%) study. Diego et al showed 8.5% of gestational diabetes in the study whereas in our study we had 54% of gestational diabetes which could be explained by ethnicity and usage of steroids.

In Yassaee et al, study occurrence of IUGR was 10.5% whereas in our study it was 18%, among which one baby was admitted in NICU.[20] In Yassaee et al study 57% of patients had moderate thrombocytopenia (more than 50,000) whereas 18% of thrombocytopenia and 9% of anemia were diagnosed in our study.

When comparing the caesarean section rate, Yassaee et al had 81% whereas we had 63%.

Rheumatoid arthritis

In women with well-controlled RA, pregnancy outcomes are comparable to the general obstetric population, whereas in women with higher RA disease activity, there is more chances of less favorable pregnancy outcomes.[21,22]

Akasbi et al, study showed improved maternal and fetal outcome in patients with RA in pregnancy. Similarly, in our study two patients diagnosed with RA had uneventful pregnancy.

Guillain-barré syndrome

Guillain-barré syndrome (GBS) represents a heterogeneous group of immune mediated peripheral neuropathies. A feature common to all GBS variants is a rapidly evolving polyradiculoneuropathy preceded by a triggering event, most often an infection. GBS generally manifests as a symmetric motor paralysis with or without sensory and autonomic disturbances. Delayed diagnosis is common in pregnancy or immediate post-partum period because the initial non-specific symptoms may mimic changes in pregnancy. GBS should be considered in any pregnant patient complaining of muscle weakness, general malaise, tingling of the fingers, and respiratory difficulty.[23,24]

We had one patient diagnosed with GBS who had an uneventful course during pregnancy.

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms.[25] Pregnancy is typically a stabilizing period in the clinical course of MS. During the

third trimester the MS relapse rate can be 70% lower when compared with the time prior to pregnancy, but exaggeration of the disease is commonly seen postpartum. Presently, there is no evidence that MS patients are more susceptible to pregnancy or delivery related complications, such as ectopic pregnancy, pre-eclampsia, gestational diabetes mellitus, prolonged labour or miscarriage than women in general, nor is their infants more likely to be delivered preterm or to be of low birth weight, have malformations or experiences an early death.[26,27] Mothers with MS required operative delivery more often than healthy mothers, which might be attributed to MS-related symptoms such as neuromuscular perineal weakness and spasticity in addition to fatigue and exhaustion.[28] In our study, we had 3 mothers diagnosed with multiple scelerosis, of them one developed PIH, one had GDM.

Sjögren syndrome

Sjögren syndrome is known to occur predominantly in women. Affected women are likely to experience more complicated pregnancies than are women without the disease.[29] Its effect on pregnancy differs according to the maternal disease, disease activity, and severity of organ damage, antibody profile, and drug treatment.6.

Several studies have reported an increased rate of spontaneous abortion and fetal loss associated with Sjögren syndrome.[30] This may be explained by, first, the age of the patients at the time of concep-tion which usually belongs to an elderly age group and, second, a possible immunologic factor involved in the mechanisms of miscarriage.[31]

In our study, out of four mothers diagnosed with Sjögren syndrome, two had spontaneous abortions.

The outcome of pregnancies in women with Sjögren syndrome can be improved with use of a multidisciplinary approach for management involving an obstetrician who has specialized in high-risk pregnancies, a rheumatologist, and a pediatrician.[32]

Hashimotos thyroiditis

Hashimotos thyroiditis is associated with an increase rate of spontaneous.

Abortions, preterm delivery and increased use of cesarean section.[33] All the three patients diagnosed with Hashimotos thyroiditis underwent cesarean section for non-progression of labour.

Ulcerative colitis

Stephansson et al study concluded that pregnant women with ulcerative colitis had a greater number of preterm births, babies with low birth weight and cesarean section deliveries than those without ulcerative colitis.[34] In our study we had one patient who delivered a low birth weight baby of 2.2 kg. One patient diagnosed with multisystem connective tissue disorder had an intra uterine growth restriction and the same baby had low birth weight.

Von Willebrand's disease

Pregnancy and delivery are considered events predisposing to a high risk of bleeding in women with von Willebrand's disease.[35]

Our patient did not have any complication. All the patients diagnosed with GDM were found to be on steroids.

Among the 113 patients, 3 underwent caesarean section in view of abnormal umbilical artery doppler findings.

Patients previously diagnosed were continued on disease modifying agents and this could be the reason for their successful pregnancy outcome.

Rauto immune disorders were diagnosed before pregnancy and these pregnancies were managed by multi-disciplinary approach, and continued on immune modulators throughout the pregnancy, hence minimizing maternal and fetal complications.

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In our study, most of the patients were either continued on the same treatment they were taking before pregnancy or were started on treatment once they were diagnosed with autoimmune disorders, and hence they were found to have successful pregnancy outcomes. Therefore, appropriate pre-conceptional counselling, continuation of treatment, intense maternal and fetal monitoring and timely delivery plays an important role in improving the pregnancy outcome.

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1. Sarah M, Mark Q, Paul E, Chapter 32 – Rheumatoid arthritis Editors: Noel R. Rose, Ian R. Mackay, The Autoimmune Diseases. 4th Ed, Academic press; 2006:417-436.
2. Cooper G, Bynum M, Somers E. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. J Autoimmun. 2009;33(3-4):197-207.
3. Perricone C, Carolis C, Perricone R. Pregnancy and autoimmunity: a common problem. Best Pract Res Clin Rheumatol. 2012;26(1):47-60.

4. Andreoli L, Fredi M, Nalli C, Reggia R, Lojacono A, Motta M et al. Pregnancy implications for systemic lupus erythematosus and the antiphospholipid syndrome. J Autoimmun. 2012;38(2-3):197-208.
5. Mecacci F, Pieralli A, Bianchi B, Paidas MJ. The impact of autoimmune disorders and adverse pregnancy outcome. Semin Perinatol. 2007;31(4):223-6.
6. Wilson WA, Gharavi AE, Koike T. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arth Rhe. 1999;42:1309-11.
7. Miyakis S, Lockshin MD, Atsumi T. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295-306.
8. Hughes GR. The antiphospholipid syndrome: ten years on. Lancet (London, England). 1993;342:341-4.
9. Lockshin MD, Kim M, Laskin Ca. Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies. Arth Rh. 2012;64:2311-8.
10. Wijeyaratne CN, Galappaththi SLA, Palipane E, Jayawardane DBIA, Dodampahala SH, Tudawe MN, et al. Pregnancy outcomes of antiphospholipid syndrome: In a low resource South Asian setting. Med. 2016;9(2):83-9.
11. Cooper GS, Gilbert KM, Greidinger EL. Recent advances and opportunities in research on lupus: environmental influences and mechanisms of disease. Environ Health Perspect. 2008;116:695-702.
12. Clowse MEB, Jamison M, Myers E. A national study of the complications of lupus in pregnancy. Am J Obstet Gynecol. 2008;199:1-12.
13. Jakobsen IM, Helmig RB, Stengaard-Pedersen K. Maternal and foetal outcomes in pregnant systemic lupus erythematosus patients: an incident cohort from a stable referral population followed during 1990-2010. Scand J Rheumatol. 2015;44:377-84.
14. Eman Aly, Hussein Aly, Riyad RM, Mokbel AN. Pregnancy outcome in patients with systemic lupus erythematosus: a single center study in the high-risk Pregnancy unit. Middle East Fert Society J. 2016;21:168-74.
15. Mandal D, Mandal S, Dattaray C, Banrrjee D, Ghosh P, Ghosh A, et al. Takayasu arteritis in pregnancy: an analysis from eastern India. Arch Gynecol Obstet. 2012;285(3):567-71.
16. Jennette CJ, Milling DM, Falk RJ. Vasculitis affecting the skin. Arch Dermatol. 1994;130:899-906.
17. Robert F, Walter R, Sator PG, Gschnait F, Breier F. Schönlein-Henoch purpura during pregnancy with successful outcome for mother and newborn BMC Dermatol. 2002;2:1.
18. Gatto M, Iaccaino L, Canova M, Nalotto L, Ramonda R. Pregnancy and vasculitis; a systematic review of the literature. Autoimmun Rev. 2012;11:A447-59.
19. Wyszynski DF, Carman WJ, Cantor AB, Graham JM, Kunz LH, Slavotinek AM, et al. Pregnancy and birth outcomes among women with idiopathic thrombocytopenic purpura. J Preg. 2016:Article id 8297407.
20. Yassaee F, Eskandari R, Amiri Z. Pregnancy outcomes in women with idiopathic thrombocytopenic purpura. Iran J Reprod Med. 2012;10(5):489-92.
21. De Man YA, Hazes JMW, Van Der Heide H. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: Results of a national prospective study. Arth Rhe. 2009;60:3196-206.
22. Bharti B, Lee SJ, Lindsay SP. Disease severity and pregnancy outcomes in women with rheumatoid arthritis: Results from the organization of teratology information specialists autoimmune diseases in pregnancy project. J Rheumatol. 2015;42:1376-82.
23. Hughes RA, Cornblath DR. GuillainBarre' syndrome. Lancet. 2005;366:1653-66.
24. Vijayaraghavan J, Vasudevan D, Sadique N, Rajeswari KS, Pondurangi M, Jayshree A. Rare case of Guillain-Barre' syndrome with pregnancy. J Indian Med Assoc. 2006;104:269-70.
25. Coles A. Multiple sclerosis. Lancet. 2008;372:1502-17.
26. Jalkanen A, Alanen A, Airas L. The finnish multiple sclerosis and pregnancy study group. Pregnancy outcome in women with multiple sclerosis – results from a prospective nationwide study in Finland. Mult Scler. 2010;16:950-5.
27. Mueller BA, Zhang J, Critchlow CW. Birth outcomes and need for hospitalization after delivery among women with multiple sclerosis. Am J Obstet Gynecol. 2002;186:446-52.
28. Dahl J, Myhr KM, Daltveit AK, Hoff JM, Gilhus NE. Pregnancy, delivery, and birth outcome in women with multiple sclerosis. Neurol. 2005;65:1961-3.
29. Siamopoulou-Mavridou A, Manoussakis MN, Mavridis AK, Moutsopoulos HM. Outcome of pregnancy in patients with autoimmune rheumatic disease before the disease onset. Ann Rheum Dis. 1988;47(12):982-7.
30. Carvalheiras G, Faria R, Braga J, Vasconcelos C. Fetal outcome in autoimmune diseases. Autoimmun Rev. 2012;11(6-7):A520-30.
31. Sandhya P, Jeyaseelan L, Scofield RH, Danda D. Clinical characteristics and outcome of primary Sjogren's syndrome: a large Asian Indian cohort. Open Rheumatol J. 2015;9:36-45.
32. Brucato A, Cimaz R, Caporali R, Ramoni V, Buyon J. Pregnancy outcomes in patients with autoimmune diseases and anti-Ro/SSA antibodies. Clin Rev Allergy Immunol. 2011;40(1):27-41.
33. Lao TT. Thyroid disorders in pregnancy. Curr Opin Obstet Gynecol. 2005;17:123-7.

34. Stephansson O, Larsson H, Pedersen L. Congenital abnormalities and other birth outcomes in children born to women with ulcerative colitis in Denmark and Sweden. Inflamm Bowel Dis. 2011;17(3):795-801.
35. Kujovich JL. Von Willebrand disease and pregnancy. J Thromb Haemost. 2005;3(2):246-53.

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A retrospective analysis of ectopic pregnancy in a tertiary care hospital

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Abstract

Background: Ectopic pregnancy (EP) is a common, life threatening emergency during the first trimester and a significant cause for maternal morbidity and mortality. In any woman of reproductive age presenting with abdominal pain and vaginal bleeding, ectopic pregnancy should be considered. The objectives of the present study is to determine the incidence, risk factors, clinical presentation, type of ectopic pregnancy, treatment, morbidity and mortality.

Methods: Retrospective analysis of case sheets of patients admitted with ectopic pregnancy at PSGIMSR, Coimbatore during the period July 2011 to June 2016 was done. The details noted were age, parity, risk factors {previous abortion, previous EP, previous pelvic surgery, intrauterine contraceptive device, tubectomy, history of pelvic inflammatory disease}, treatment for infertility, clinical presentation, ultrasound findings, investigations including beta HCG value and hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity.

Results: During the five year, there were 12,407 deliveries at our hospitals and 88 cases of ectopic pregnancies were managed. The incidence is 6.6/1000 deliveries. Among them 44% belonged to the age group of 25-30 years and multi gravida were 73.8%. Most common risk factors were previous abortion (23.8%) and previous surgeries including caesarean section, tubectomy, appendicectomy, tubal microsurgery (23.8%). Most common presentation was pain abdomen (85.2%). Out of the 88 cases, 73 patients were managed surgically (82.9%) and 11 cases were managed successfully with methotrexate (12.5%). Three of them underwent conservative management and one had Uterine Artery Embolization.

Conclusions: Early diagnosis based on risk factors and timely intervention plays a main role in reducing morbidity and mortality associated with ectopic pregnancy.

Keywords: Beta HCG, Ectopic pregnancy, Methotrexate, Risk factors, Salphingectomy Transvaginal USG

Introduction

An ectopic pregnancy is implantation of fertilized ovum outside the normal endometrial cavity i.e, fallopian tube, ovary, caesarean scar, cervix and peritoneal cavity.[1] In the first trimester, ectopic pregnancy is the most common cause for maternal morbidity and mortality.[2] It has also been shown that EP reduces future fertility and increases the chance of subsequent EP.[3] The incidence of EP is increasing since the last decade, but the incidence of complications like rupture and morbidity are declining due to early diagnosis with serial Beta-HCG(human chorionic gonadotrophin) monitoring, transvaginal USG(ultrasonogram) and minimally invasive surgery.[4] Early diagnosis not only reduces the risk of tubal rupture but also allows for conservative and medical management.[5] Prior damage to the fallopian tube due to PID(pelvic inflammatory disease), tubal surgeries, pelvic adhesions are the major risk factors for ectopic pregnancy.[6] The other identified risk factors are,

advanced maternal age, previous EP, previous pelvic surgeries, IUCD (intra uterine contraceptive device) insertion and infertility treatment.[7-12] Identifying these risk factors among high risk mothers helps in early diagnosis and reduces the morbidity related to this condition.[13] Unfortunately, most of the patients presenting with EP do not have risk factors.

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This was a retrospective analysis of case records of patients admitted with ectopic pregnancy, done at PSG IMSR Hospital, Coimbatore from July 2011- June 2016.

Inclusion criteria

We included all the patients with suspected ectopic pregnancy who were confirmed by βHCG and transvaginal ultrasound.

Exclusion criteria

None

Methodology

The case sheets were retrieved from medical record department and the details such as age, parity, risk factors like previous abortion, previous EP, previous pelvic surgery, contraception in the form of IUCD and tubectomy, history of PID, infertility treatment, clinical presentation, investigations including beta HCG value, hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity were collected and analyzed.

Statistical analysis

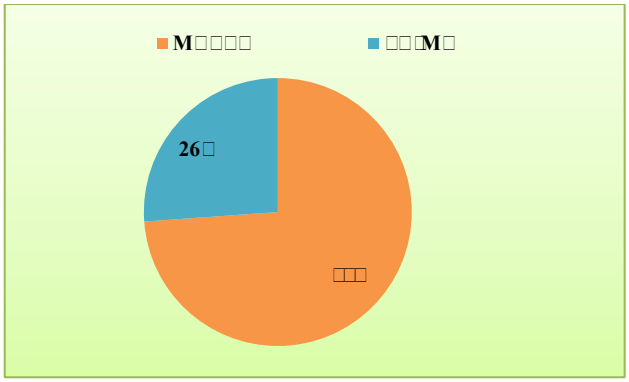
No statistical tool used.

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During the 5-year period, there were 12,407 deliveries at our institution and 88 cases of ectopic pregnancies were managed. The incidence was 6.6/1000 deliveries. Among the patients most of them belonged to the age group of 25-30 years (Table-1). 26% of patients were primigravida, 73.8% of patients were multigravida (Figure -1).

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< 25	22	25
25-30	39	44
31-35	18	20
36-40	5	5.6
>41	4	4.5



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As shown in (Table 2) 23.8% of patients had one or more spontaneous or induced abortions.23.8% of patients had undergone pelvic surgery (Caesarean section, tubectomy appendicectomy, tubal recanalistion). PID and previous ectopic pregnancy accounted for 4.5% each.

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Previous abortion	21	23.8
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Caesarean section	21	23.8
Tubectomy	14	15.9
Appendicectomy	3	3.4
Tubal recanalistion	2	2.2
Ovulation induction	7	7.9
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IUCD insertion	6	6.8
PID	4	4.5
Previous ectopic	4	4.5

Commonest presenting complaint was pain abdomen in 85.2% of patients. Other presenting complaints were Amenorrhoea in 68% and vaginal bleeding in 59% (Table-3).

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Pain	75	85.2
Amenorrhea	60	68.1
Bleeding PV	52	59

Most of them presented between 5-8 weeks of gestational age (61.3%).27.2% of patients were diagnosed before 4 weeks of amenorrhea and 11.3% were above 9 weeks. All the cases had been confirmed by USG.17 of them had Beta-HCG less than 1000 m I U /ml. A patient with heterotopic pregnancy had Beta -HCG of more than 1 lakh m I U /ml.

Commonest site of ectopic pregnancy was fallopian tube in 93% of patients (Table 4).

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[illegible]	[illegible] r [illegible]	[illegible]
Tube	82	93
Caesarean scar	3	3.4
Ovary	2	2.4
Heterotopic	1	1

The commonest site of tubal involvement was in ampulla (Table 5).

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[illegible]	[illegible] r [illegible]	[illegible]
Ampulla	60	73.1
Cornua	10	12.1
Isthmus	6	7.3
Fimbria	4	4.8
Tubal abortion	2	2.4

On receiving, 86% of patients were hemodynamically stable, 13.6% of patients were in various stages of shock. 53.6% of patients presented with ruptured ectopic, unruptured ectopic was noted in 43.9% of patients (Table 6).

[illegible] 6 [illegible] d [illegible]

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Ruptured	44	53.6
Unruptured	36	43.9
Tubal abortion	2	2.5

Right sided tubal ectopic was noted in 65.8%, left side tube involved in 34.1% of patients. Tubal abortion was observed in 2.5% of patients. Intra-operatively hemoperitoneum was noted in 64% of patients. Most common treatment offered for tubal ectopic was surgical, i.e. salpingectomy (Table 7).

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r [illegible]	[illegible] r [illegible]	[illegible]
Medical	11	12.5
[illegible] r [illegible]		
Laparotomy	49	59.7
Laparoscopy	9	10.9
M d [illegible] d		
Laparotomy	8	9.7
Laparoscopy	2	2.4
Conservative	3	3.6

Twenty-one patients were fit into medical management, among them 12.5% of patients were managed medically with methotrexate successfully.

10 cases failed to medical management which were subsequently managed with either laparoscopic or laparotomy and salphingectomy.

3 cases were managed conservatively by observing the declining trend of Beta-HCG.

Other types of ectopic

Caesarean scar pregnancy was diagnosed in 3 patients, among them 2 patients underwent surgical excision. One patient was managed with UAE (uterine artery embolisation) followed by methotrexate. Heterotopic pregnancy following ovulation induction which resulted in twin intra uterine gestation and tubal ectopic was noted in one patient and she underwent emergency laparotomy and salpingectomy. Two patients were diagnosed as having ovarian ectopic and surgical excision was offered to both of them. Most common morbidity noted was anemia in 42%, out of which severe anemia (4-7gm) was seen in 5.6% (Table 8). Among them 28.4% of patients received blood and blood products. No surgical site infection or mortality was noted.

[illegible] [illegible] [illegible] d [illegible]

[illegible] [illegible]	[illegible] r [illegible]	[illegible]
4-7 gm/dl	5	5.6
7-10 gm/dl	32	36.3
>10 gm/dl	51	58.1

[illegible]

The incidence of ectopic pregnancy among women attending emergency department with first trimester abdominal pain, bleeding or both is between 6-16 %(14). A study conducted by Shraddha Shetty et al had incidence of 5.6/1000 deliveries.[15] In our study the incidence is 6.6%/1000 deliveries which is comparable. Majority of patients were between 25-30years which is similar to the above study and Khaleeqe et al.[15,16]

As in Karki et al (17), we had similar higher incidence of ectopic pregnancies (73.8%) in multigravida. In our study, the commonest predisposing factors were previous abortion either spontaneous or induced, previous pelvic surgeries including caesarean section, history of IUCD insertion, tubectomy, history of PID and infertility treatment, which are similar to various other studies.[17,18] As in the studies by Porwal Sanjay et al, Shraddha Shetty et al, the most common symptom in our series was pain abdomen which is comparable.[15,18]

Urinary beta HCG, serum beta HCG, transvaginal USG are the diagnostic tools for diagnosing EP. USG should be the investigation of choice for symptomatic women. In asymptomatic women, serial monitoring of serum beta HCG should be done. As in the study by Shraddha Shetty et al, we also had more number of right sided EP and involvement of the ampullary portion of the tube.[15]

Heterotopic pregnancy is rare in natural conception which is around 1 in 30,000 pregnancies. But the incidence is more in case of ovulation induction or with

assisted reproduction techniques. In our study, we had a case of triplet heterotopic pregnancy following ovulation induction with clomiphene citrate. It is recommended that even patients shown on ultrasound to have intrauterine pregnancy should be given comprehensive pelvic ultrasound so that possibility of synchronous heterotopic pregnancy can be excluded, particularly in pregnancies following fertility treatment.[19]

In our study, ruptured EP was noted in 53% of cases, 43.9% of cases had an unruptured ectopic gestation. In Latchaw G et al study, the same were 59% and 41% respectively.[20]

Laparoscopy and medical therapy have now emerged as the widely used therapeutic modalities with great success in terms of reduced morbidity, shorter hospital stay and conservation of fertility.[21] However choice depends upon early identification of ectopic pregnancy and hemodynamic status of the patients.[22] In our study, 11% of patients responded well to single dose of methotrexate.

Surgery should be the treatment of choice in unstable patient.[19] In our study 13.6% were unstable and all of them were managed surgically. Commonest surgery performed was partial/total salpingectomy. We did bilateral total salpingectomy for patients who had undergone tubectomy previously and were not desirous of future childbearing. It has been extensively studied and reported that conservative management does not increase the risk of future ectopic.[15] In our study, three patients were managed conservatively by observing the declining trend of Beta HCG. Morbidity included anemia, blood transfusion and wound infection. In our study, we had 42% of patients with hemoglobin level of less than 10grams. Among them 28.4% of patients received blood and blood products. No surgical site wound infection was noted.

No maternal mortality was noted in our study which is consistent with the study by A Abbas et al.[23] Early identification of the patients with EP by a multipronged approach including high index of suspicion, risk prediction, serum beta HCG and transvaginal ultrasound helps in improving the prognosis as far as morbidity, mortality and future fertility are concerned.[24]

□□□□□□□□□□

Identification of underlying risk factors, early diagnosis with essential investigating modalities like beta-HCG, transvaginal USG, timely intervention with medical or surgical method will definitely reduce morbidity and mortality and improve the future reproductive outcome.

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1. Walker JJ. Ectopic pregnancy. Clin Obstet Gynecol. 2007;50:89-99.
2. Mahboob U, Mazhar SB. Management of ectopic pregnancy: a two-year study. J Ayub Med Coll Abbottabad. 2006;18:34-7
3. Chow WH, Daling JR, Cates WJ, Greenberg RS. Epidemiology of ectopic pregnancy. Epidemiol Rev. 1987;9:70-94.
4. Karaer A, Avsar FA, Batioglu S. Risk factors for ectopic pregnancy: a case-control study. Aust N Z J Obstet Gynaecol. 2006;46:521-7.
5. Barnhart KT. Clinical practice. Ectopic pregnancy. N Engl J Med. 2009;361:379-87.
6. Akande V, Turner C, Horner P, Horne A, Pacey A, British Fertility Society. Impact of Chlamydia trachomatis in the reproductive setting: British Fertility Society Guidelines for practice. Human Fertil. 2010;13:115-25.
7. Job-Spira N, Collet P, Coste J, Bremond A, Laumon B. Risk factors for ectopic pregnancy. Results of a case control study in the Rhone-Alpes region. Contracept Fertil Sex. 1993;21:307-12.
8. Bouyer J, Coste J, Shojaei T, Pouly JL, Fernandez H, Gerbaud L, et al. Risk factors for ectopic pregnancy: a comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol. 2003;157:185-94.
9. Parashi S, Moukhah S, Ashrafi M. Main risk factors for ectopic pregnancy: a case-control study in a sample of Iranian women. Int J Fertil Steril. 2014; 8:147-54.
10. Moini A, Hosseini R, Jahangiri N, Shiva M, Akhoond MR. Risk factors for ectopic pregnancy: a case-control study. J Res Med Sci. 2014; 19:844-9.
11. Ankum WM, Mol BW, Van der Veen F, Bossuyt PM. Risk factors for ectopic pregnancy: a meta-analysis. Fertil Steril. 1996;65:1093-9.
12. Barnhart KT, Sammel MD, Gracia CR, Chittams J, Hummel AC, Shaunik A. Risk factors for ectopic pregnancy in women with symptomatic first-trimester pregnancies. Fertil Steril. 2006;86:36-43.
13. Timmerman D. Predictive models for the early diagnosis of ectopic pregnancy.Verh K Acad Geneeskd Belg. 2004;66:155-71.
14. Murray H, Baakdah H, Bardell T, Tulandi T. Diagnosis and treatment of ectopic pregnancy. CMAJ. 2005;173:905.
15. Shetty KS, Shetty KA. A clinical study of ectopic pregnancies in atertiary care hospital of Mangalore, India. Innovat J Med H Sci. 2014;4:305-9.
16. Khaleeque F, Siddiqui RI, Jafarey SN. Ectopic pregnancies: A Three-year study. J Pak Med Assoc 2001;51:240-3.
17. Karki RCL, Pradhan B, Duwa S. Annual Analysis of Ectopic Pregnancy in Tertiary Care Hospital. PMJN. 2011;11:5-8
18. Gupta R, Porwal S, Swarnkar M, Sharma N, Maheshwari P. Incidence, trends and risk factors for

Ectopic Pregnancies in a tertiary care hospital of Rajasthan. JPBMS. 2012;16:1-3.
19. Maheswari S, Panicker S. Triplet heterotopic pregnancy following ovulation induction with clomiphene citrate: a case report and review of literature Int J Rerod Contracept Obstet Gynecol. 2013;2:743-5.
20. Latchaw G, Takacs P, Gaitan L, Geren S, Burzawa J. Risk factors associated with the rupture of tubal ectopic pregnancy. GynecolObstet Invest. 2005;60:177-80.
21. Jurkovie D. Ectopic pregnancy. In: Edmonds DK, editor. Dew Hurst's textbook of Obstetrics and Gynecology. 7 ed. USA; Blackwell Publishers;2007
22. Shah N, Khan NH. Ectopic pregnancy: Presentation and risk factors. J Coll Physicians Surg Pak. 2005;15:535-8.
23. Abbas A, Akram H. Ectopic Pregnancy; Audit at Maula Bakhsh Teaching Hospital Sargodha.Prof Med J. 2011;18:24-7.
24. Lozeau AM, Potter B. Diagnosis and management of ectopic pregnancy. Am Fam Physician. 2005; 72:1707-14.
25. Majhi AK, Roy N, Karmakar KS, Banerjee PK. Ectopic pregnancy--an analysis of 180 cases. J Indian Med Assoc. 2007;105:308-10.

Maheswari S, Poornima C, Lalitha N, Panicker S. Analysis of various types of ectopic pregnancies: a five-year review. Int J Reprod Contracept Obstet Gynecol 2018;7:2410-4.

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ORIGINAL ARTICLE

Cesarean Myomectomy: An Experience from a Tertiary Care Teaching Hospital

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Abstract

Background Recent literature supports the removal of myomas during cesarean section, which traditionally was considered a relative contraindication, given a higher complication rate. This study is to share our experience of cesarean myomectomy in the last decade.

Methods This study is a retrospective review of our prospectively maintained database, from January 2008 to December 2017, at a tertiary care level teaching institution. All patients who underwent myomectomy during cesarean section were included. There were no exclusions.

Results A total of twenty patients underwent myoma removal along with the cesarean operation during this period with a mean age of 30 years. Majority of patients were nulliparous (70%). Common comorbidities were diabetes mellitus (40%) and hypothyroidism (20%). Mean size of myomas were 5.33 cm (± 2.08), and the number varied from one to three. The most common location was the posterior surface of the uterus with the commonest variety being subserous. Most patients were discharged on the fifth postoperative day.

Conclusion This study demonstrates that cesarean myomectomy to be a safe and feasible procedure in experienced hands. It offers the advantage of avoiding a second surgery in selected patients.

Keywords Cesarean myomectomy · Myoma uterus · Cesarean delivery · Myomectomy

Introduction

The myomas are the most common benign tumors of the uterus. The highest reported incidence of myomas during pregnancy is 10.7% [1]. The myomas are common during the reproductive period due to an influence of higher estrogen levels. Their increased incidence during pregnancy can be partly explained by the late age of conception [2]. Myomas in pregnancy are associated with a significantly higher risk for cesarean section (CS).

Conventionally, concomitant myomectomy during CS was considered a relative contraindication, given perceived higher complication rate. Last decade has witnessed emerging literature evidence supporting myoma excision along with CS, with acceptable results [3]. This study aims to share our experience of cesarean myomectomy during the last decade.

Materials and Methods

This study is a retrospective review of our prospectively maintained database of the last decade, from January 2008 to December 2017, at a tertiary care teaching hospital. All consecutive patients who underwent myomectomy during cesarean section, in this mentioned duration, were included.

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This formed a small subset, among the patients, having uterine myomas and pregnancy, a majority of those underwent vaginal delivery or had LSCS, without myomas removal. Those not matching the inclusion were not considered. There were no exclusions. The institutional ethical committee approved the study, and waiver of consent was obtained, in view of retrospective nature.

Data were collected from outpatient and inpatient records, operative and discharge notes. Data included maternal demographics such as age, parity, medical diseases, prior surgery, gestational age at the time of CS, type of anesthesia, indication for CS, elective/emergency and number of previous operations including CS. Myoma characteristics such as size, number, location and variety were noted. Other parameters like an indication for myomectomy, the number of incisions made and the presence of adhesions were observed. The total duration of surgery was taken as the time from the initial skin incision to skin closure. Approximate intraoperative blood loss was measured, and record of transfusion if any was noted. Postoperative drop in hemoglobin was checked at 48 h after surgery, and blood transfusion was given if the fall was more than 3 gm/dl. Postoperative complications like pyrexia (> 38-degree C) and wound infection were analyzed. Duration of hospital stay was noted, and patients were followed up after 2 weeks in the outpatient department.

Patients were counseled during the early third trimester regarding the need for myomectomy depending on the mode of delivery and consent obtained for the same.

Experienced gynecologists performed surgery with minimum of 10 years of experience. Preoperatively, half an hour before skin incision Inj. Tranexamic acid 1 gm in 100 ml isotonic saline was infused over 20 min. Except in four cases, myomas were situated at the incision site, and in other instances, CS preceded myomectomy. When myomas were located at the lower uterine segment, myomectomy was performed before delivery of the fetus. During CS, myomas were inspected for their size, number, type and location after the closure of the uterine incision (Fig. 1).

Though myomectomy was tried with a single incision, in few cases where multiple fibroids were present, more than one incision over uterus was needed. An incision was made over myoma by using monopolar cautery, and the tumor was enucleated. To achieve hemostasis, bipolar cautery was used. The defect in the myometrium was closed with 1-0 vicryl suture (Fig. 2). High-dose oxytocin infusion was continued postoperatively for 24 h to prevent bleeding.

Results

A total of twenty patients underwent myoma removal along with the cesarean operation during this period with a mean age of 30 years. Majority of patients were nulliparous (70%).



Fig. 1 Multiple fibroids over the anterior surface of uterus postdelivery of the baby



Fig. 2 Postmyomectomy, closure by baseball sutures

Common comorbidities were diabetes mellitus (40%) and hypothyroidism (20%). One except all patients received regional anesthesia (spinal/epidural); the only case needed general anesthesia due to the presence of idiopathic intracranial hypertension. Sixty-five percent of surgeries were elective operations. The details of various preoperative characteristics are shown in Table 1.

Intraoperatively, two patients had small bowel and omental adhesions at the operative site. Two patients (10%) received a blood transfusion during the procedure. The mean hemoglobin drop reported 24 h postoperative was 1.23 (± 1.16) grams. No patient warranted blood transfusion in the postoperative period (Table 2).

Majority of patients got the discharge on the fifth day of surgery. There were no incidences of postpartum pyrexia or

Table 1 Demographic and preoperative characteristic of patients ($n=20$)

Sr. No.	Variable	Value (mean ± SD)
1.	Age (in years)	30.85 ± 3.84
2.	Parity	
	(a) Nulliparity	14
	(b) Multiparity	06
3.	Gestational Age	37.3 ± 1.08
4.	Number of prior surgeries	
	(a) Nil	11
	(b) LSCS	06
	(c) Myomectomy	03

SD standard deviation, *LSCS* lower segment cesarean section

Table 2 Intraoperative and follow-up variables ($n=20$)

Sr. No.	Variable	Value (mean ± SD)
1.	Size of myoma (cm)	5.33 ± 2.08
2.	Number of myoma (mean and range)	1 (1–3)
3.	Location of tumor	
	(a) Posterior	07
	(b) Anterior	06
	(c) Right lateral wall	04
	(d) Fundal	04
	(e) Left lateral wall	03
	(f) Lower uterine segment	04
4.	Type of Myoma	
	(a) Subserous	11
	(b) Intramural	09
	(c) Submucosal	02
5.	Operating time (minutes)	79 ± 35.35
6.	Blood loss (mL)	876 ± 386.9
7.	Hospital stay (days)	5.5 ± 1.43

SD standard deviation

surgical site infections. The patients were regularly followed in the outpatient department.

Discussion

Fibroid excision along with caesarian section was considered difficult until recently. Fibroids with pregnancy might cause miscarriage, preterm delivery, premature rupture of membranes, early separation of the placenta, labor dystocia, postpartum hemorrhage and increased rate of operative deliveries. Fibroids might also undergo red degeneration and associated complications. Myomectomy during CS has not been done routinely due to the risk of uncontrolled bleeding and the possibility of hysterectomy [4]. If myomectomy is not performed along with CS patient might need another surgery to remove it, which amounts to two operations, two anesthetic exposures and increasing cost, and there is also an enhanced risk of complications caused by fibroids in a subsequent pregnancy.

The average age of mothers in our study was 30 years. Most studies have shown myomas to be more common in nulliparous women [5]. We too observed a higher incidence of myomas among nulliparous women (70%) [6]. This could be explained due to hormonal changes and reduced sensitivity of leiomyoma to estrogen receptor [7].

Similar to many other studies, the average gestational age at the time of CS was 37 to 38 weeks in our study [4, 6, 8]. We had four patients delivering at 35 to 36 weeks of gestation. Among these four patients who had delivered prematurely, three deliveries can be attributed to the presence of fibroids. Two out of these patients have already undergone fibroid removal before becoming pregnant. The possible explanation for preterm labor could be decreased distensibility of uterine myometrium due to the presence of fibroids as the pregnancy advances [9].

Though CM is to be avoided in cases of the previous myomectomy due to more chances of complications [10], we had the opportunity to do repeat CS for one of our patient who underwent CS myomectomy 3 years ago. The indication for primary CS was a transverse lie. The malpresentation was suspected to be due to a single large (8 cm), intramural fibroid located in the lower uterine segment. Incidentally, this was the largest myoma we had come across in our study. As the fibroid was situated in the incision site, myomectomy was followed by CS. During repeat CS, which was done 3 years later, there was no fibroid detected intraoperatively. There were three more cases of lower uterine segment (LUS) fibroids; if they were less than 5 cm, then myomectomy were done following the delivery of the baby. All fibroids present in the LUS were removed through the same CS incision, without any additional incision on the uterus. The same technique was suggested in several other studies [11, 12].

Three of our patients had undergone myomectomy previously in the non-pregnant state. One of the women had undergone myomectomy twice before becoming pregnant. This confirms the well-established fact that fibroids are recurrent [13]. Among the study group, the patients who had undergone previous myomectomies had a high incidence of intraoperative adhesions as expected. However, in all three patients, the surgery was uneventful with minimal blood loss and no added morbidity.

Eight of our study subjects had multiple fibroids. Six patients had fibroids of size 8 cm (largest dimension). 55% of the myomas had size > 5 cm. The smallest dimension encountered was 2.5 cm. We had more anterior wall fibroids than posterior [4]. Traditionally myomectomy is avoided in cases of fundal and cornual sites because of excessive

bleeding and possible obstruction of fallopian tubes [14, 15], but in our study, myomectomy was done regardless of the location of the myomas.

Except for a single instance of the submucosal fibroid, there was an even distribution of intramural and subserosal fibroids. Removal of submucosal fibroids during cesarean section is usually avoided as it involves resection of the full thickness of myometrium and reduced contractility might lead on to increased hemorrhage [16]. Secondary changes due to red degeneration were found in a case with single sizeable fundal fibroid. Histopathological reports of all myomectomy specimens were closely followed up, and none showed malignant change.

The same incision removed fibroids in the anterior wall and close to CS incision. Whenever possible, a minimal number of incisions were made, and adjacent ones removed through the same incision. This was done to reduce blood loss and future adhesions and improve reproductive outcome. While the operating time was more or less uniform in most of our cases, two of the cases exceeded the average operating time significantly. In these two instances, the increased operating time could be explained by multiple fibroids needing more than a single incision for removal. Other studies have also observed more than average operating time in cases of multiple fibroids [17].

Intraoperative blood transfusion was done in two cases only as they met the criteria for blood loss of more than one liter. One case had a fibroid at the incision site. The second patient who had a loss of more than two liters which was the largest in our series had three fibroids at different locations and they were removed by as many incisions. Despite intraoperative transfusion and other pharmacological measures to attain hemostasis, she had a significant drop in hemoglobin at 48 h postoperatively and needed another transfusion. She had no other postoperative morbidity like pyrexia or ileus and was discharged on the fourth day after surgery.

CM is not done routinely, because of the associated hemorrhage and the need for the hysterectomy [15]. Specific hemostatic techniques have been advocated by various authors [18, 19], but we have followed tranexamic acid infusion, vasopressin instillation, uterine artery ligation, electrocautery and high-dose oxytocin. There was not a single case which needed a hysterectomy in our study of twenty cases, though patients were counseled regarding the possibility in the prenatal period due to intractable hemorrhage. We also found no increased postoperative morbidity or expanded hospital stay in any of our patients who underwent CM as was observed by Dam Hye Kwon et al. [8].

There are a few limitations to our study. Its retrospective design coupled with proportionally small sample size limits generalization of results. This study also lacks long-term follow-up of these patients and thus to highlight effect on future pregnancies. Additionally, as this study was conducted in a tertiary level medical college setup with available expertise, the results cannot be generalized, especially in comparison with smaller installations or in the absence of an expert.

We author would like to stress this to the readers that routine use of myomectomy during CS is neither advisable nor been followed in the current study. The CM should be carefully planned for selected patients in an appropriate setup and expertise. A similar conclusion is drawn by a recent review on this topic [20], recommending standardization of best practices for CM, with the availability of more robust data and in the presence of necessary facilities.

Conclusion

Overall, our series has shown CM to be a safe and feasible procedure in well-selected patients in a tertiary care center with experienced hands. It can offer an advantage of avoiding a second intervention in selected patients. Appropriate hemostatic techniques can curtail hemorrhage which is the most severe complication. Long-term morbidity concerning future reproductive outcome should be addressed in a study with large participants and reliable design.

Authors' Contributions All authors have a significant contribution for preparation of this manuscript, following ICMJE Guidelines. First and second authors share the first position.

Compliance with Ethical Standards

Conflict of interest No author has any potential conflict of interest.

Ethical approval The current study is approved by the responsible institutional ethics committee for human research and fully comply with the declaration of Helsinki for guideline on human research.

References

1. Laughlin SK, Baird DD, Savitz DA, et al. Prevalence of uterine leiomyomas in the first trimester of pregnancy. Obstet Gynecol. 2009;113(3):630–5.
2. Tian J, Hu W. Cervical leiomyomas in pregnancy: report of 17 cases. Aust N Z J Obstet Gynaecol. 2012;52(3):258–61. https://doi.org/10.1111/j.1479-828X.2012.01414.x.
3. Sparić R, Kadija S, Stefanović A, et al. Cesarean myomectomy in modern obstetrics: more light and fewer shadows. J Obstet Gynaecol Res. 2017;43(5):798–804.
4. Incebiyik A, Hilali NG, Camuzcuoglu A, et al. Myomectomy during caesarean: a retrospective evaluation of 16 cases. Arch Gynecol Obstet. 2014;289(3):569–73.
5. Sparic R, Mirkovic L, Malvasi A, et al. Epidemiology of uterine myomas: a review. Int J Fertil Steril. 2016;9(4):424–35.
6. Kanthi JM, Sumathy S, Sreedhar S, et al. Comparative study of cesarean myomectomy with abdominal myomectomy in

terms of blood loss in single fibroid. J Obstet Gynecol India. 2016;66(4):287–91. https://doi.org/10.1007/s13224-015-0685-x.
7. Wise LA, Palmer JR, Harlow BL, et al. Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African–American women: a prospective study. Am J Epidemiol. 2004;159(2):113–23.
8. Kwon DH, Song JE, Yoon KR, et al. The safety of cesarean myomectomy in women with large myomas. Obstet Gynecol Sci. 2014;57(5):367. https://doi.org/10.5468/ogs.2014.57.5.367.
9. Chen Y-H, Lin H-C, Chen S-F, et al. Increased risk of preterm births among women with uterine leiomyoma: a nationwide population-based study. Hum Reprod. 2009;24(12):3049–56.
10. Sparić R, Berisavac M, Buzadzić S, et al. Complications during cesarean delivery in a patient with two previous myomectomies. Acta Chir Iugosl. 2013;60(1):99–100.
11. Yellamareddygari S, Chakrabarti M, Ravuri S, et al. Leaving fibroids at caesarean section, is it safe? Gynecol Surg. 2010;7(2):173–5. https://doi.org/10.1007/s10397-008-0441-7.
12. Mahendru R, Sekhon P, Gaba G, et al. At times, myomectomy is mandatory to effect delivery. Ann Surg Innov Res. 2011;5(1):9.
13. Ande ABA, Ehigiegba AE, Umeora OUJ. Repeat myomectomy at caesarean section. Arch Gynecol Obstet. 2004;270(4):296–8.
14. Celal K, Hülya Ç. The evaluation of myomectomies performed during cesarean section in our clinic. Niger Med J. 2011;52(3):186.
15. Song D, Zhang W, Chames MC, Guo J. Myomectomy during cesarean delivery. Int J Gynecol Obstet. 2013;121(3):208–13. https://doi.org/10.1016/j.ijgo.2013.01.021.
16. Gbadebo AA, Charles AA, Austin O. Myomectomy at caesarean section: descriptive study of clinical outcome in a tropical setting. J Ayub Med Coll Abbottabad. 2009;21(4):7–9.
17. Ramesh Kumar R, Patil M, Sa S. The utility of caesarean myomectomy as a safe procedure: a retrospective analysis of 21 cases with review of literature. J Clin Diagn Res. 2014;8(9):OC05–8.
18. Shavell VI, Thakur M, Sawant A, et al. Adverse obstetric outcomes associated with sonographically identified large uterine fibroids. Fertil Steril. 2012;97(1):107–10.
19. Lee J-H, Cho D-H. Myomectomy using purse-string suture during cesarean section. Arch Gynecol Obstet. 2011;283(S1):35–7.
20. Chauhan AR. Cesarean myomectomy: necessity or opportunity? J Obstet Gynecol India. 2018;68(6):432–6. https://doi.org/10.1007/s13224-018-1114-8.

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Non immune hydrops foetalis: a perinatal autopsy study

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Background: Authors sought to determine the possible factors in the causation of nonimmune hydrops foetalis by perinatal autopsy with placental examination and to reduce the number of cases in which the cause remains elusive.

Methods: Twenty five cases of nonimmune hydrops foetalis were identified in about 200 consecutive perinatal autopsies (including placental examination) performed during a 11 year period. The results were correlated with clinical, laboratory and imaging characteristics in an attempt to establish the aetiology.

Results: Perinatal autopsy and placental examination confirmed the following aetiologies: cardiovascular causes (8) [isolated (4), syndromic (3) and associated chromosomal (1)], placental causes (5), chromosomal (4) [isolated(3) and associated cardiovascular disease (1)], intrathoracic (3), genitourinary causes (3), infections(1),gastrointestinal lesions (1) and idiopathic causes (1). Placental mesenchymal dysplasia was a unique pathology identified among the placental lesions, which constituted the second most common cause of nonimmune hydrops foetalis. Despite careful examination no cause was identified in one case. In more than 50% of studied cases, autopsy examination either refuted or altered the ultrasound diagnosis completely.

Conclusions: The perinatal autopsy in combination with placental study and prenatal imaging represents the most promising tool in the evaluation of aetiology of nonimmune hydrops foetalis. The identification of a cause for nonimmune hydrops foetalis will provide a better correlation with recurrence risk and parental counselling.

Keywords: Aetiology, Nonimmune hydrops foetalis, Perinatal/foetal autopsy, Placental mesenchymal dysplasia, Prenatal imaging

Introduction

Foetal hydrops is a syndrome characterized by generalized skin oedema and abnormal accumulation of fluid in one or more serous cavities of foetuses. It has been broadly classified into immune mediated (materno - foetal blood group incompatibility) and non immune types. There is a steady fall in the incidence of immunologic hydrops foetalis due to the prevention of maternal isoimmunisation and treatment of affected foetuses in utero.[1] This has shifted the focus towards the non immunologic causes of hydrops which now constitutes up to 90% of cases of foetal hydrops.[2]

There are many impressive and ever growing causes of non immune hydrops foetalis (NIHF) and its development is known to be associated with maternal, foetal and placental diseases.[3] The foetal hydrops can be identified anytime throughout the pregnancy. The perinatal mortality rate is generally high. The spectrum of possible causative factors of NIHF include cardiovascular diseases, chromosomal aberrations, haematologic

abnormalities, infections, intrathoracic lesions, lymphatic dysplasias and placental causes including twin-twin transfusion syndrome. The other causes are syndromes, urinary tract malformations, inborn errors of metabolism, extra thoracic tumours, gastrointestinal disorders, miscellaneous and idiopathic.[4]

Improvements in obstetric ultrasound (USG) and foetal echocardiography enable the early and frequent detection of hydrops, resulting in foetal interventions / termination of pregnancy and have altered the incidence of hydrops.[5]

Given the multiple aetiologies of NIHF, we sought to determine the presence of possible factors in its causation by analysing the autopsy with placental material and to reduce the number of cases in which the cause remains elusive.

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Twenty five cases of NIHF were identified in about 200 consecutive perinatal autopsies (including placental examination) performed during a 11 year period from January 2007 to December 2017 at the department of pathology, PSG Institute of Medical Sciences and Research. The institutional ethical review board approval was obtained for the study.

Exclusion criteria

Hydrops foetalis of immunologic origin were excluded from the study on the basis of blood typing, direct and indirect coomb's tests.

The detailed maternal history, antenatal ultrasounds and genetic tests if any were collected. The autopsy was performed after getting informed consent. Photograph of the foetus was routinely taken with special attention to sites where abnormalities were suspected. A thorough systematic examination of foetuses and placenta was done, which included foetogram, foetal weight, anthropometric measurements (head circumference, chest circumference, abdominal circumference, crown rump length, crown heel length, foot length), external and internal examination.

After opening the body cavities, serous fluid collections were measured, and the thoracic and abdominal organs were removed en bloc. All the visceral organs were examined, and the bits were given for microscopic analysis. The brain was removed, inspected, weighed and suspended in a formalin fixative. After fixation, the brain was sliced; examined and relevant bits were taken. Examination of bone, including marrow and spinal cord were performed in selected cases. The placenta and cord were also examined thoroughly, photographed (maternal / foetal surfaces, parenchyma, umbilical cord), cut at 1cm intervals and the parenchyma was assessed for specific lesions. Relevant lesional sections were taken along with 2 sections of membrane roll, 2 sections from the umbilical cord and 3 full thickness sections of the normal parenchyma.

All the histologic sections were stained with haematoxylin and eosin. Special stains such as Grocott-Gomori's methenamine silver (GMS), Giemsa stains for toxoplasma gondii, warthin starry stain for spirochetes and reticulin stain for vascular network were done, when required. Immunohistochemical marker for cytomegalovirus was available, hence done for suspected infectious cases. After the microscopic study, the results were correlated with clinical, laboratory and imaging characteristics and the final results were drawn.

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Out of 200 perinatal autopsies performed during the 10-year period, 25 cases met the criteria for NIHF, representing 12.5%. The gestational age ranged from 13 weeks to 35 weeks. All were dead-born foetuses except two. The two liveborn foetuses were delivered at 35 weeks and survived only a few hours postnatally. There were no cases of multiple gestation.

Clinical findings

Maternal age ranged from 19 to 35 years, of whom 9 were primigravidas. Five cases each of polyhydramnios and oligohydramnios were present. Maternal medical diseases included 2 cases each of preeclampsia and autoimmune diseases and 1 case each of hypothyroidism and rheumatic heart disease. None of the mothers had diabetes. Recurrence of hydrops was found on 5 occasions. Previous history of hydrops was documented in 2 cases of cardiac (complete heart block and transposition of great arteries with ventricular septal defect), one case each of intra thoracic lesion (Congenital pulmonary airway malformation), chromosomal (Down syndrome) and placental mesenchymal dysplasia.

Ultrasound findings

Hydrops foetalis is diagnosed with typical findings during prenatal imaging. The sonographic features include subcutaneous / scalp oedema, fluid accumulation in different serous cavities (Figure 1), polyhydramnios and placentomegaly. Correlation between prenatal USG and autopsy findings in elucidating the causes of hydrops were studied (Table 1). Complete agreement between prenatal USG and autopsy findings were seen in 11 cases (44%), while in 6 cases autopsy examination revealed additional findings (24%). In 8 cases, autopsy findings changed the diagnosis (32%).

Autopsy findings

Foetus

There were 12 males and 13 females with an almost equal gender distribution. Hydrops was noted in <20 weeks (10

cases), 21-28 weeks (10 cases) and >28 weeks (5 cases) of gestation. Aetiological distribution of NIHF is listed in Table 2.



[illegible]

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
<20 weeks (10)	4	3	3
21-28 weeks (10)	3	3	4
>28 weeks (5)	4	0	1

[illegible]

[illegible]	[illegible]
Intrathoracic lesions	3
[illegible]	
Hypertrophic pyloric stenosis	1
Cardiac defects	4
Genitourinary lesions	3
[illegible]	
Down syndrome	3 (1*)
Turner syndrome	1
[illegible]	
Parvo virus	1
Placental causes	5
[illegible]	
Ellis van creveld syndrome	1
Zellweger syndrome	1
Vacterl	1
Idiopathic	1

*Coexistent with congenital heart disease

Cardiovascular diseases (n=8)

Congenital cardiac defects represented the largest aetiological group and were seen in 8 foetuses (32.0%). Among these 8 cases, 7 foetuses had structural malformations and 1 had functional defect. 2 cases of isolated cardiac defects, 3 cases of syndromic association and one case of chromosomal abnormalities were identified (Table 3). Cystic hygroma was noted in 2 instances.

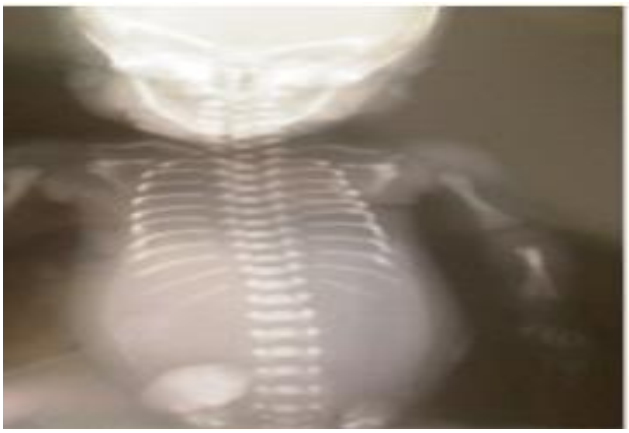
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Premature closure of ductus	
Complete heart block secondary to fibrosis of cardiac conduction system, immune mediated	
TGA with hypoplastic pulmonary arterial trunk	
Subaortic VSD	
TOF with right aortic arch and left sided PDA	VACTERL association
Secundum ASD	Multiple congenital anomaly syndrome -? Zellweger syndrome-? Smith Lemli Opitz syndrome
Common atrium with complete common AVSD	Ellis- Van Creveld syndrome
Common AVSD with persistent left superior vena cava and mild tubular hypoplasia of aortic arch	Down syndrome
TGA and VSD	Partial urorectal septum malformation sequence (Cloacal dysgenesis sequence)

TGA-transposition of great arteries; VSD-ventricular septal defect; TOF-tetralogy of fallot; PDA-patent ductus arteriosus; VACTERL-vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities; ASD-atrial septal defect; AVSD- atrioventricular septal defect.

The most common causes of NIHF in our series were cardiovascular diseases, of which structural anomalies predominate. The most frequently found structural heart lesion with hydrops is atrio ventricular septal defects, and transposition of great vessels. Less common structural cardiac abnormalities observed were premature closure of ductus, tetrology of Fallot and secundum atrial septal defect. There were 2 cases of isolated heart defects. One foetus had premature closure of ductus and the other was a neonate with complete heart block. In the second instance, an association with maternal connective tissue disease was identified. This newborn female with complete heart block also had a sibling delivered at same gestational age with similar hydropic features and died within a few hours of birth. Autopsy was not performed

for the sibling. Our newborn was delivered preterm at 34 weeks of gestational age due to severe bradyarrhythmia. The neonate was hydropic, hypertonic and had intractable bradycardia at birth. Pace maker insertion was not acceptable to the parents. Child expired within 3 hours of birth. Autopsy examination revealed biventricular hypertrophy, dysplasia of atrioventricular valves, fibrosis of sinoatrial and atrioventricular nodes and patchy endocardial fibroelastosis.





In present series, cardiac defects were also seen in association with Ellis-Van Creveld Syndrome (Figure 2), Multiple congenital anomaly syndrome and VACTERL association.

There were 3 cases of Down syndrome, all with typical phenotypic manifestations and one with atrioventricular septal defect (Figure 5). The other significant associated anomaly noted was partial urorectal septum malformation sequence (Cloacal dysgenesis sequence).



Other disorders associated with NIHF

Placental, intrathoracic and genitourinary lesions are summarized in (Table 4).

Placental lesions (n=5)

The placenta was examined in all the cases and showed villous edema, prominent Hofbauer cells and villous capillary erythropoiesis.

Placental causes of hydrops were found in 5 cases. Two cases each of maternal vascular malperfusion and placental mesenchymal dysplasia. The underlying pathology in the fifth case was foetal vascular malperfusion (organising and recanalizing thrombi with avascular villi), villitis of unknown aetiology and acute chorioamnionitis.

Histologically, the cases with maternal vascular malperfusion had multiple parenchymal infarcts, increased syncytial knotting and distal villous hypoplasia. Preeclampsia was noted in both the mothers.

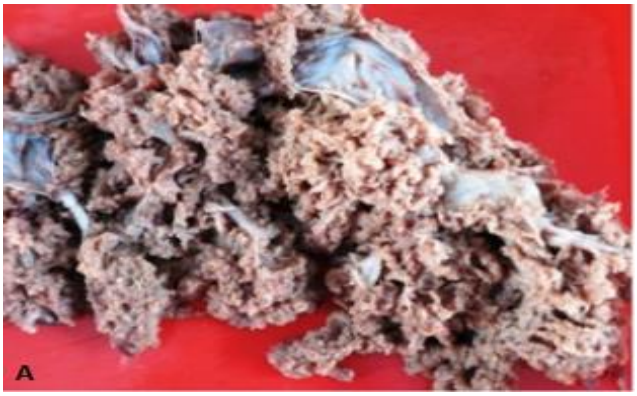


Figure 6: Gross photograph of placenta showing hypercoiled cord, parenchymal disruption with thick, oedematous villi and small vesicles

The placentas with mesenchymal dysplasia grossly showed hypercoiled cord, parenchymal disruption with thick, oedematous villi and small vesicles (Figure 6). Histologically, all villi showed collapsed vesicle with central cystic degeneration with absent trophoblastic proliferation and markedly dilated vascular channels (Figure 7).

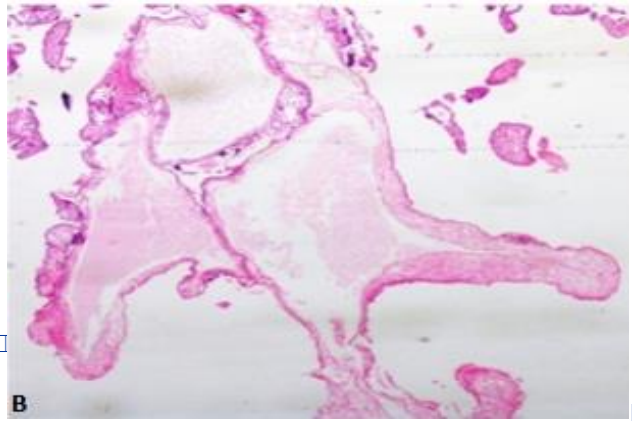


Figure 7: Histology showing collapsed vesicle with central cystic degeneration with absent trophoblastic proliferation and markedly dilated vascular channels

Table 2: Distribution of cases with non-immune hydrops fetalis and associated malformations

Placental causes (N=5)	Case 1: Preeclampsia, Multiple parenchymal infarcts, increased syncytial knotting: MVM
	Case 2: Preeclampsia, infarcts, Distal villous hypoplasia: MVM
	Case 3: The Placental parenchyma is disrupted, fleshy, pulled apart and the villi appear thick. The stem villi oedematous, hypercoiled cord : Placental mesenchymal dysplasia
	Case 4: Small vesicles in the parenchyma, thick stem villi: Placental mesenchymal dysplasia
	Case 5: placenta with FVM.VUE and A/C CAM
Intra thoracic lesions (n=3)	Case 1: Both lungs: dilated airways and lymphatics : CPAM
	Case 2: The right lung appears large and occupies almost the entire thoracic cavity: CPAM
	Case 3: Laryngeal (Glottic) atresia : CHAOS
Genitourinary abnormalities (n=3)	Case 1: BOO due to posterior urethral valve causing dilated posterior urethra, bladder, ureters and bilateral hydronephrosis. Ventriculomegaly, absent corpus callosum
	Case 2: Distal urethral stenosis with dilatation and hypertrophy of the urinary bladder: BOO. Pulmonary hypoplasia
	Case 3: BOO due to posterior urethral valves. Mild dilatation of both ureters and pelvis

CPAM- congenital pulmonary airway malformation; CHAOS- congenital high airway obstruction syndrome; BOO- bladder outlet obstruction; MVM-maternal vascular malperfusion; FVM- foetal vascular malperfusion; VUE- villitis of unknown aetiology; A/C CAM- acute chorioamnionitis

Authors did not find any chorangioma as a cause of NIHF.

Intrathoracic lesions (n=3)

There were 3 instances of intrathoracic lesions causing NIHF. Two cases of congenital pulmonary airway malformation (CPAM) (Figure 8) and one case of congenital high airway obstruction Syndrome (CHAOS) were identified in our series. Mediastinal deviation was noted in all foetuses.

Genitourinary abnormalities (n=3)

Three foetuses with NIHF were complicated by genitourinary causes. Two of them had bladder outlet obstruction (BOO) due to posterior urethral valves, and the third one had distal urethral stenosis with dilatation and hypertrophy of the urinary bladder. However, one

subject with posterior urethral valve also had ventriculomegaly, absent corpus callosum, deep posterior fossa, hypoplastic ductus and pulmonary arteries.



[illegible]

Chromosomal abnormalities (n=4)

Among cases with chromosomal abnormalities, we found one foetus with Turner syndrome, in which cytogenetic study revealed partial deletion of short arm of X chromosome. There were 3 cases of Down syndrome, all with typical phenotypic features and one foetus additionally had cardiac defect.

Miscellaneous causes

Congenital parvovirus B 19 infection was suspected in one instance. The foetus had marked subcutaneous oedema, serous effusions, enlarged liver and spleen. Lymphocytic infiltration of all viscera, predominantly myocardium with no identifiable inclusions were seen. The placenta had no lesion.

One of the foetus had hypertrophic pyloric stenosis which is a relatively uncommon cause of NIHF. The mother had polyhydramnios and autopsy revealed thickened and narrowed pylorus.

The cause of the hydrops foetalis could not be determined in only one case (4%) and it was classified as idiopathic. Subcutaneous oedema, serous effusions, micrognathia, high arched palate and dysmorphic villi were identified, and chromosomal abnormality was suspected. However, the karyotype was normal.

[illegible]

Nonimmune hydrops foetalis is a clinical sequel of wide range of pathology and is not a diagnosis in itself. It is known to be associated with maternal, foetal and placental diseases. In the past, the causes of hydrops were not identified in significant number of cases, even after extensive investigations. Postmortem examination including clinical assessments and targeted investigations can identify the underlying cause in majority of cases.

Cardiovascular diseases

Cardiovascular causes of NIHF accounts for about 20% of cases and includes both structural and functional defects.[6] In the present study cardiac defects represented the largest group and were found in 32% of foetuses.

Association of cardiac lesions with well-defined syndromes, chromosomal aberrations such as Down syndrome, cystic hygroma and additional major congenital anomalies were also noted in some of our cases.

The lone functional cardiac cause for hydrops identified in our study was complete heart block, which had serological evidence of maternal connective tissue disease. Even though sectioning is difficult, and time consuming, microscopic examination is critical in identifying fibrosis of the conduction system.

The cardiogenic hydrops would result from increase in right atrial pressure, reduced preload reserve and congestive cardiac failure.[7]

The immaturity of foetal myocardium (both structural and functional) with reduced relaxation is more vulnerable to any in utero insult leading to decrease in cardiac output.[1]

Hydrops associated with cardiac malformations generally have poor prognosis with high mortality. Recent developments in diagnostic capabilities of prenatal ultrasound along with in utero interventions will probably improve the outcome of foetuses with congenital cardiac lesions.[8]

Placental lesions

Interestingly, in present study group, placental lesions constituted the second most common cause of NIHF. Five of our patients demonstrated exclusive placental lesions significant enough to explain the aetiology of hydrops. Rodriguez et al, demonstrated placental pathology as the cause of hydrops in 10 patients (19.6%). In agreement with their study we too observed a similar incidence.[9]

Notwithstanding its rarity, we found placental mesenchymal dysplasia (PMD) as a unique pathology in two of our cases. PMD is an underreported and under recognized placental vascular anomaly causing hydrops.

It is characterized by placentomegaly (>90th percentile) with hydropic villi and absent trophoblastic proliferation.

The vascular changes observed in PMD might have led to heart failure with hydrops and ultimately resulting in intrauterine foetal demise.[10]

The other causes identified were maternal vascular malperfusion, foetal vascular malperfusion, villitis of unknown aetiology and acute chorioamnionitis.

Intrathoracic lesions

Among the intrathoracic causes (CPAM and CHAOS), an increase in intrathoracic pressure by enlarged lungs leading to impaired venous return could have contributed to foetal hydrops.[11]

Genitourinary abnormalities

Bladder outlet obstruction causing hydrops foetalis have been well documented in the literature. Mechanical obstruction of great vessels due to enlarged urinary bladder with resultant reduced venous return to heart is the underlying pathology.[12] Early onset oligohydramnios secondary to BOO leads to pulmonary hypoplasia, further reducing the survival chances.

Chromosomal abnormalities

In previously reported series of NIHF, chromosomal abnormalities were found in 10% of cases.[4,6,13] In the present study, there was 1 case of Turner syndrome and 3 instances of Down syndrome accounting for 16% of cases. Except for the Turner syndrome, none were confirmed by karyotyping. Among the 4 foetuses, 3 were identified in early gestation and one was associated with cardiac defect and cystic hygroma. Chromosomal analysis should be performed in all cases of NIHF, more so when identified in earlier gestation and when associated with structural anomalies.[14]

Miscellaneous causes

Intrauterine infections can cause foetal hydrops. There was one case of Parvovirus B19 infection in our study which showed hepatosplenomegaly with dense lymphocytic infiltration of myocardium. The underlying mechanisms include foetal anaemia, viral myocarditis and impaired hepatic function.[14,15] High index of suspicion and prenatal screening for intrauterine infections will make the interpretation easier. Incidence of "idiopathic" category varies among studies.[9,16,17] After detailed and systematic examination of foetuses including placenta, we found only one case (4%) of NIHF in which the cause was not identified. This highlights the value of autopsy with accompanying placental examination as the most complete procedure to establish the cause of NIHF.

Ultrasound correlation

Studies comparing the utilisation of foetal autopsy with prenatal USG findings have shown correlation in 98% of cases.[18,19] But these studies did not involve cases with hydrops foetalis. In more than 50% of studied cases, autopsy examination either refuted or altered the USG diagnosis completely. Though it is known that USG might miss some findings due to many reasons like equipment quality, sonographer expertise, maternal body habitus and liquor volume, this gross discrepancy is speculative. This might emphasis the need for autopsy, especially in cases of hydrops to arrive at a final aetiological diagnosis.

Also worthy of mention are the absence / rare diagnosis of inborn errors of metabolism and infective diseases in our series of cases. A better and complete laboratory work up with utilization of immunohistochemistry for lymphatic dysplasia/viral inclusions may be helpful to overcome this low diagnostic yield.

The causes of NIHF is heterogenous and the survival of the foetus depends on the underlying aetiology. The most promising tool in understanding the aetiology of NIHF is systematic evaluation of the foetuses including placenta. Multidisciplinary approach including foetal imaging, laboratory work up, postmortem bacterial / viral culture, genetic study, complete autopsy with utilization of immunohistochemistry (when necessary) will identify the potential causes in foetuses with suspected NIHF. This will determine new strategies for in utero therapy, and thus make a positive impact on perinatal outcome. Knowledge about the aetiology and pathogenesis will provide a better correlation with risk of recurrence. It may also improve parental counselling and prevent similar mishaps in subsequent pregnancies.

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In conclusion, the autopsy provides the final diagnosis and enables us to understand the disease process and its natural history. The decline in clinical autopsies is a matter of great concern to the clinicians and pathologists alike. State of the art imaging devices and molecular biology techniques can complement, but not replace a comprehensive autopsy.

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1. Nagy A, Malcomson RD. Fetal hydrops. In Khong, T. Yee, Malcomson, Roger D. G. (Editors), Keeling's Fetal and Neonatal Pathology, 5th Ed. Springer-Verlag, London, 2015:299-328.

2. Bellini C, Hennekam RC, Fulcheri E, Rutigliani M, Morcaldi G, Boccardo F, et al. Etiology of nonimmune hydrops fetalis: a systematic review. Am J Med Genetics Part A. 2009 May;149(5):844-51.
3. Villaespesa AR, Mier MPS, Ferrer PL, Baleriola IA, Gonzalez JIR. Nonimmunologic hydrops fetalis: an etiopathogenetic approach through the postmortem study of 59 patients. Am J Med Genet. 1990;35:274-9.
4. Bellini C, Hennekam RC. Non-immune hydrops fetalis: a short review of etiology and pathophysiology. Am J Med Genet A. 2012 Mar;158A (3):597-605.
5. Moreno CA, Kanazawa T, Barini R, Nomura ML, Andrade KC, Gomes CP, et al. Non-immune hydrops fetalis: A prospective study of 53 cases. Am J Med Genet Part A. 2013 Dec;161(12):3078-86.
6. Bellini C, Donarini G, Paladini D, Calevo MG, Bellini T, Ramenghi LA, et al. Etiology of non-immune hydrops fetalis: an update. Am J Med Genet. Part A. 2015 May;167(5):1082-8.
7. Yuan SM. Cardiac etiologies of hydrops fetalis. Z Geburtshilfe Neonatol. 2017 Apr 221(2):67-72.
8. Knilans TK. Cardiac abnormalities associated with hydrops fetalis. Semin Perinatol. 1995 Dec;19(6):483-92.
9. Rodríguez MM, Chaves F, Romaguera RL, Ferrer PL, de la Guardia C, Bruce JH. Value of autopsy in nonimmune hydrops fetalis: series of 51 stillborn fetuses. Pediatr Dev Pathol. 2002 Jul-Aug;5(4):365-74.
10. Akbarzadeh-Jahromi M, Sari Aslani F, Parvari S. Placental mesenchymal dysplasia complicated by hydrops fetalis and fetal death: a case report. Arch Iran Med. 2013 Sep; 16(9):551-4.
11. Rodríguez MM, Bruce JH, Jiménez XF, Romaguera RL, Bancalari E, García OL, et al. Nonimmune hydrops fetalis in the liveborn: series of 32 autopsies. Pediatr Developmental Pathol. 2005 Jun 1;8(3):369-78.
12. Faye-Petersen OM, Heller DS. Pathology of the Stillborn Infant for the General Pathologist: Part 2. Adv Anat Pathol. 2015;22:71-93.
13. Machin GA. Hydrops revisited: literature review of 1,414 cases published in the 1980s. Am J Med Genet. 1989 Nov;34(3):366-90.
14. Society of Obstetricians and Gynaecologists of Canada (SOGC) clinical practice guidelines. Investigation and Management of Non-immune Fetal Hydrops J Obstet Gynaecol. 2013;35:e1-e14.
15. Society of Obstetricians and Gynaecologists of Canada (SOGC) clinical practice guidelines. Parvovirus B19 Infection in Pregnancy J Obstet Gynaecol. 2014;36 (12):1107-16
16. Lallemand AV, Doco-Fenzy M, Gaillard DA. Investigation of nonimmune hydrops fetalis: multidisciplinary studies are necessary for diagnosis-review of 94 cases. Pediatr Dev Pathol. 1999 Sep-Oct; 2 (5):432-9.
17. Randenberg AL. Nonimmune hydrops fetalis part II: does etiology influence mortality?. Neonatal Netw. 2010 Nov-Dec;29 (6):367-80.
18. Sankar VH, Phadke SR. Clinical utility of fetal autopsy and comparison with prenatal ultrasound findings. J Perinatol. 2006 Apr;26(4):224.
19. Johns N, Al-salti W, Cox P, Kilby MD. A comparative study of prenatal ultrasound findings and post-mortem examination in a tertiary referral center. Prenat Diag. 2004;24:339-46.

r Ramya T, Umamaheswari G, Chaitra V. Nonimmune hydrops foetalis: value of perinatal autopsy and placental examination in determining aetiology. Int J Res Med Sci 2018;6:3327-34.

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Significance of foetal autopsy in cases of nonimmune hydrops foetalis

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Abstract

Background: Nonimmune hydrops foetalis (NIHF) is a terminal catastrophic event of pregnancy caused by numerous diverse etiology. The aim of this study was to assess the significance of foetal autopsy and to compare the prenatal ultrasound (USG) and foetal autopsy findings in cases of NIHF.

Methods: All perinatal autopsies performed at the department of pathology between March 2011-February 2018 were retrospectively reviewed. Of the received 130 autopsies, twenty cases of NIHF were identified, records of which were collected and correlated with maternal medical history, prenatal imaging and autopsy findings.

Results: The malformations with hydrops foetalis were classified according to the involved organ system. They were cardiothoracic (7/20 cases), genitourinary (3/20 cases), gastrointestinal lesions (1/20 cases), chromosomal (4/20 cases) and multisystem anomaly/syndromic association (5/20 cases). Complete agreement between USG and autopsy was seen in 8 (40%) cases. In 5 (25%) cases autopsy findings were in total disagreement with USG diagnosis. The rest of the 7 (35%) cases, autopsy revealed additional information and changed the recurrence risk in two cases.

Conclusions: Present study demonstrates the high rate of discordancy between USG and autopsy examination in cases complicated by NIHF. Foetal autopsy confirms the USG findings (quality control/audit), adds additional findings or changes the final diagnosis, which helps in redefining the recurrence risk and plausible genetic counselling for future pregnancies. Hence present study underscores the need for autopsy in all cases of NIHF.

Keywords: Nonimmune hydrops foetalis, Perinatal/ foetal autopsy, Prenatal imaging, Ultrasound

Introduction

Hydrops foetalis remains a distressing pregnancy complication with high perinatal mortality. It is characterized by generalized oedema and presence of fluid in pleural, peritoneal and pericardial cavities. Polyhydramnios and abnormally thickened placenta are often associated with hydrops.[1]

Traditionally, hydrops is classified into immune and nonimmune types. There is marked reduction in the incidence of immune hydrops due to effective immunologic prophylaxis for maternal isoimmunisation. Currently, the focus is on nonimmune hydrops foetalis (NIHF), which accounts for >90% of cases of hydrops.[2]

NIHF is associated with multiple aetiology. The various causes include cardiovascular, genitourinary, thoracic lesions, chromosomal aberrations, infections, haematological, genetic/metabolic, placental and idiopathic.[3]

Initial diagnosis of hydrops foetalis is usually made by ultrasound. In most of the cases, an accurate and complete antenatal imaging also points to the cause behind the development of hydrops. Despite having a

high diagnostic accuracy, foetal ultrasonography (USG) has some limitations, especially in cases with hydrops. Foetal autopsy in such instances can help to overcome the challenges.

The goals of this retrospective study were to compare the accuracy and completeness of prenatal ultrasonography with perinatal autopsy in cases of NIHF.

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All perinatal autopsies performed at the department of pathology between March 2011-February 2018, were retrospectively reviewed. It included foetuses of all gestational ages. Causes of deaths were termination of pregnancy for foetal malformations, spontaneous abortions, stillbirths and early neonatal deaths. Of the 130 autopsies, twenty cases of NIHF were identified, records of which were collected and correlated with maternal medical history and prenatal imaging studies.

Immune hydrops foetalis were excluded from the study. Institutional ethical committee approval was obtained. Maternal data collection included maternal age, obstetric history, maternal medical diseases, gestational age of the foetus, and prenatal ultrasound findings.

Sonograms were predominantly performed by radiologists and some by obstetricians trained in obstetric ultrasound examination. The diagnostic criteria for hydrops foetalis were foetal skin oedema >5.0mm with one serous cavity effusion or fluid collection in at least two serous cavities (peritoneal, pleural or pericardial cavities).

The autopsies were performed by pathologists with perinatal experience. Informed written consent was obtained for all cases. A standard autopsy protocol was followed which included foetogram, photography, biometric measurements, external and internal examination. Microscopic examination of relevant organs such as thyroid, thymus, lungs, heart, liver, spleen, pancreas, adrenals, kidneys and ovary (in case of female), testis (in case of male) were done.

The brain was removed and fixed in formalin solution with the help of a gauze in a hanging position, then relevant sections were studied. Spinal cord, bone, muscle were also studied when necessary.

Special stains such as Periodic acid–Schiff (PAS) and Perls' Prussian blue were done if needed. Placental examination was done in all cases, but findings were not considered for the present study.

Placental causes of hydrops were also excluded from the study. Results of karyotype, if known was collected. The final autopsy diagnosis was correlated with prenatal ultrasound findings and results were drawn.

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The maternal age ranged from 19 to 35 years with the mean age of 25.55years. Gestational age ranged from 13 to 35 weeks, with the mean gestational age of 21.45 weeks. There were 18 stillborn foetuses and 2 neonates with the Male: Female ratio of 3:2. Authors did not come across any multiple gestation. Four cases had recurrent hydrops.

Prenatal diagnosis of hydrops was noted in 14 cases and in the remaining 6, diagnosis was made at post-mortem. present data showed prenatal diagnosis of the presence of hydrops in only two-third of the cases.

Subcutaneous oedema and serous cavity effusions were identified in the rest one-third only during autopsy. All of these were second trimester foetuses. Cystic hygroma was identified in five cases.

The malformations with hydrops foetalis were classified according to the involved organ system. They were cardiothoracic (7/20 cases), genitourinary (3/20 cases), gastrointestinal lesions (1/20 cases), chromosomal (4/20 cases) and multisystem anomaly/syndromic association (5/20 cases).

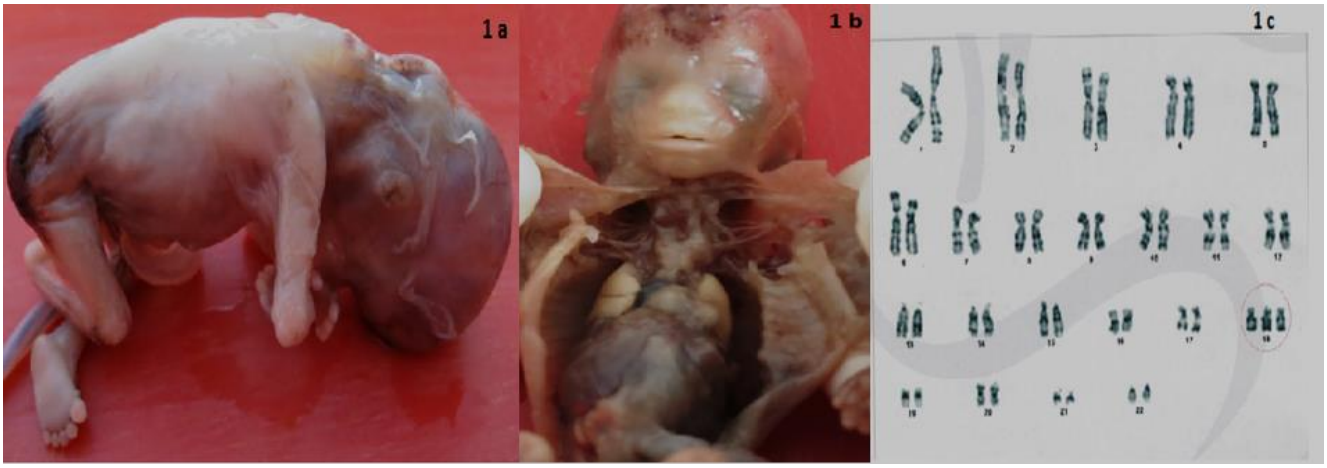
Cardiac lesions (n=4): The cardiovascular malformations included premature closure of ductus (1case), complete heart block(1case), atrioventricular septal defect (AVSD) with persistent left superior vena cava (SVC) with other findings suggestive of Down syndrome(1case) and 1 case of transposition of great arteries (TGA), which also had an associated partial urorectal septum malformations.

Thoracic lesions (n=3): The thoracic lesions were congenital pulmonary adenomatoid malformations (CPAM, 2 cases) and congenital high airway obstruction syndrome (CHAOS, 1 case).

Genitourinary lesions (n=3): Three cases of bladder outlet obstruction (BOO) were included in the group of urogenital malformations, of which one had an associated ventriculomegaly, absent corpus callosum (ACC) with hypoplasia of ductus, right and left pulmonary arteries, distal aortic arch and left subclavian artery

Gastrointestinal lesions (n=1): The gastrointestinal lesions included, a 28weeks foetus with hypertrophic pyloric stenosis.

Chromosomal abnormalities (n=4): Of the 4 cases, two showed phenotypic features of Down syndrome, but karyotype confirmation was not available. Karyotyping was possible in the remaining 2 cases of which one showed partial deletion of short arm of X chromosome (Turner syndrome) and the other showed trisomy 18 (Edwards syndrome) (Figure 1).



[illegible] (Edward's [illegible]

Multisystem anomalies/ syndromic association (n=5): The autopsy findings suggested syndromic associations in five instances. They were one case each of Ellis- Van Creveld syndrome, Multiple congenital anomaly syndrome, VACTERL (vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies and limb abnormalities) association, FRYNS Syndrome and Cerebro-oculo-facio-skeletal syndrome. Karyotype analysis was done for the last two cases and were found to be normal.

Correlation between prenatal ultrasound and autopsy findings: Complete agreement between USG and autopsy was seen in 8 (40%) cases. In 5 (25%) cases, autopsy findings were in total disagreement with USG diagnosis. The rest of the 7 (35%) cases, autopsy revealed additional information.

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
1.	Cardiac	HF	Premature ductus closure.
2.	Thoracic	Large cystic hygroma	CPAM
3.	Chromosomal	HF, oesophageal atresia, absence of stomach	Phenotypic features of foetal trisomy No oesophageal atresia Karyotype-not done
4.	Urogenital	No anomaly	BOO-distal urethral stenosis
5.		HF	BOO-distal urethral stenosis Ventriculomegaly, ACC

HF- hydrops foetalis; BOO- bladder outlet obstruction; CPAM- congenital pulmonary airway malformation; ACC –absent corpus callosum.

Cases with complete disagreement has been shown in Table 1.

Two had bladder outlet obstruction, one each had CPAM and premature closure of ductus.

In all the four cases, there was a complete change in the diagnosis after the autopsy.

The fifth case showed no oesophageal atresia, in contrast to prenatal imaging.

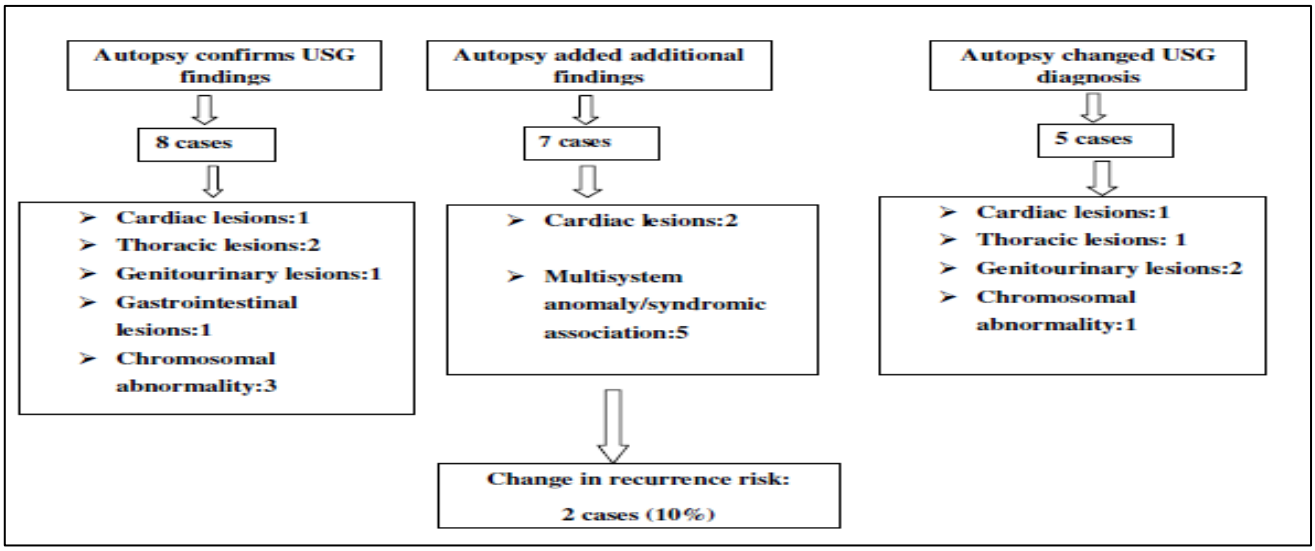
Though this was a significant disagreement, there was no major shift in the final diagnosis as both modalities suggested chromosomal aetiology.

[illegible]

[illegible]	[illegible]	[illegible]
1.	Short long bones, polydactyly, tricuspid atresia with VSD-probably Ellis Van Creveld syndrome	Common atrium with complete AVSD- Ellis-Van Creveld syndrome
2.	Polyhydramnios, HF, micrognathia, hypospadias, cleft soft palate, ACC, cardiomegaly, tricuspid regurgitation	Hypotelorism, depressed nasal bridge, low set ears, Heart – secundum ASD, Liver – haemosiderosis Pancreas – giant islets and nesidioblastosis Multiple congenital anomaly syndrome, Zellweger syndrome, Smith Lemli Opitz syndrome.
3.	SUA, TOF, ectopic hydronephrotic Left kidney, Left club foot with absent 2nd to 5th digits	Hemivertebra, fusion of ribs on the left side, Atresia of distal sigmoid, rectum with absent anal opening VACTERL association
4.	Left diaphragmatic hernia, hypoplastic left heart, short humerus on left side, absent forearm with claw hand on left side; dilated jugulolymphatic sacs: multiple anomalies and with SUA, could be VACTERL association.	Partial ACC, and hydrocephalus Karyotype was normal -Suggestive of FRYNS Syndrome
5.	HF, Foetal akinesia deformation sequence, sloppy forehead, micrognathia, gross bilateral lateral ventriculomegaly	Cerebro-oculo-facio-skeletal syndrome with persistent urogenital sinus
6.	Increased nuchal translucency, cystic hygroma, absent nasal bone	AVSD with persistent left SVC -Suggestive of Down syndrome
7.	Cystic hygroma with TGA and VSD	Partial urorectal septum malformation

VSD-ventricular septal defect; AVSD- atrioventricular septal defect; HF- hydrops foetalis; ACC-agenesis of corpus callosum; ASD-atrial septal defect; SUA-single umbilical artery; TOF-tetralogy of Fallot; VACTERL-vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities; SVC-superior vena cava; TGA-transposition of great arteries

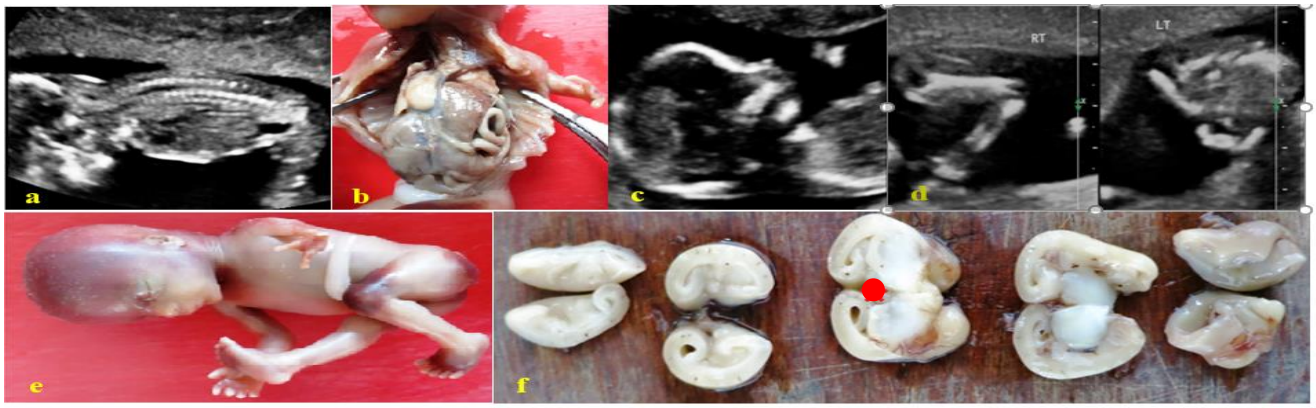
There were seven cases with additional autopsy findings which has been summarized in Table 2. Authors had two cases with cardiac lesions and the rest of the cases showed multisystem involvement. After autopsy, two out of seven cases had a change in recurrence risk (Figure 2).



[illegible]

In cases of multi system involvement, the autopsy finding of hemi vertebra, atresia of distal sigmoid, rectum, anal canal with absent anal opening, completed the diagnosis of VACTERL association (case 3, table 2).

In the same group (case 4, table 2), prenatal diagnosis of VACTERL association was changed to FRYNS syndrome after the identification of central nervous system anomaly (Figure 3).



[illegible]

Table 3 highlights the cases that showed full agreement between USG and autopsy findings. This included three cases of chromosomal abnormality, two cases of thoracic lesions (CPAM and CHAOS) and one case each from cardiovascular system (complete heart block), genitourinary (BOO) and gastrointestinal group (hypertrophic pyloric stenosis). There were none from the multiple anomaly group.

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
1.	Cardiac	Polyhydramnios, HF, complete heart block	Complete heart block secondary to fibrosis of cardiac conduction system
2.	Thoracic	CHAOS with HF.	Laryngeal atresia (CHAOS)
3.		Polyhydramnios, HF, CPAM with short long bones	CPAM, type 3
4.	Urogenital	Oligohydramnios, bilateral hydronephrosis, distended bladder	BOO - posterior urethral valves.
5.	Gastrointestinal	Polyhydramnios, HF. Hepatomegaly. Stomach visualized	Hypertrophic pyloric stenosis. Hirsutism.
6.	Chromosomal	NT: 3mm. Combined test – increased risk of trisomy 21	Phenotypic features of foetal trisomy Karyotype-not done
7.		NT: 1.75mm Combined test – increased risk of trisomy 21	Phenotypic features consistent with Turners syndrome Karyotyping shows partial deletion of short arm of X chromosome
8.		Cystic hygroma, HF Single outflow with peri membranous VSD, pulmonary atresia	TGA with hypoplastic pulmonary arterial trunk subaortic VSD Cystic hygroma Anteriorly placed anus Hypoplasia of thymus Karyotyping shows trisomy 18.

After extensive search of literature authors came across many studies comparing prenatal USG findings with post-mortem examination. Most of these studies dealt with cases of foetal malformations, stillbirth and neonatal death.[4-8] But, there were only few studies comparing the same in cases of NIHF.[9,10] Hence authors chose to address the accuracy of prenatal USG findings and compare it with perinatal autopsy in cases of NIHF.

Prenatal diagnosis of hydrops was missed in one third of present cases. Fluid collection is initially diagnosed by USG during the first or mid trimester. Significant fluid accumulation is recognized easily, but sometimes the effusions are limited and can escape diagnosis during imaging.

Present data showed complete agreement between USG and autopsy findings in 40% of cases. This was similar to a study by Antonella et al done in 2012.[11] In their study they have compared the USG and autopsy findings of 144 second trimester foetuses. In 49% of cases there were total agreement between the two. Additional information was provided by post-mortem in 34% of cases, while in present study it was 35%. Astonishingly in 13% of their cases, functional anomalies detected by ultrasound could not be confirmed by autopsy. This is due to the fact that ultrasound examination is a dynamic examination, optimum to reveal functional changes like valve insufficiencies, flow reversals and sometimes even cavity effusions. This should not be considered as false positive USG findings, but as an advantage of the imaging modality.

In 7 cases (35%), there were additional findings in autopsy not detected by USG. In two of these cases, the additional findings provided by autopsy redefined the recurrence risk. It seems that the higher proportion of additional findings in the present study might be due to critical identification of minor anomalies at autopsy such as anal atresia, partial agenesis of corpus callosum and urorectal septum malformation.[10]

The identification of these minor anomalies is significant as they might change the diagnosis from an isolated lesion to complex malformation.[4]

All cases in the multiple anomaly had additional findings in autopsy. The identification of partial agenesis of corpus callosum with hydrocephalus in a case with prenatal imaging diagnosis of VACTERL association changed the final diagnosis to be Fryns syndrome. The other anomalies were concordant. The foetus was 16 weeks at the time of diagnosis, and identification of the cranial anomaly at this gestational age is challenging.[12] Fryns syndrome (FS) is the commonest autosomal recessive syndrome in which congenital diaphragmatic hernia (CDH) is a cardinal feature.[13]The autosomal recessive inheritance in FS contrasts with the sporadic inheritance seen in cases of VACTERL anomaly.[14] This underscores the importance of the correct diagnosis for accurate genetic counselling.

Among the cases with cardiovascular lesions, prenatal ultrasound showed increased nuchal translucency, cystic hygroma and absent nasal bone which was all suggestive of trisomy 21. The presence of AVSD, the characteristic heart lesion in these foetuses was made out by post-mortem examination. This more or less confirmed the diagnosis of Down syndrome.

In addition to multiple anomaly, the histological presence of haemosiderosis of the liver with giant islets and nesidioblastosis of the pancreas led to the suggestive diagnosis of multiple congenital anomaly syndrome, Zellweger syndrome, Smith Lemli Opitz syndrome.

One of the study case showed USG findings of Foetal akinesia deformation sequence, with gross bilateral lateral ventriculomegaly. But post-mortem examination also revealed hydranencephaly, lens opacity along with facial and skeletal lesions which led to the diagnosis of cerebro-oculo-facio-skeletal syndrome. Persistent urogenital sinus was noted additionally.

There was total discordance between USG and autopsy in 5 cases of 20(25%). In all 5 cases, though hydrops was diagnosed prenatally, the causative lesion could not be detected by imaging. But autopsy revealed structural anomaly in 4/5 cases. Though all these four lesions (premature closure of ductus, CPAM, BOO) could be identified by antenatal imaging, they were missed. As hydrops foetalis carries a bad prognosis and once it is evident by ultrasound, further careful and complete imaging is overlooked. This could have led to the non-identification of structural abnormality by USG and the discordance with post-mortem examination.

USG as well as autopsy have unavoidable limitations in analysing cases of NIHF. So, they are considered complementary to one another. Both of these modalities depend on the expertise of the personnel, the technological advancement of the ultrasound equipment and the autopsy technique.

The causes for differences in prenatal USG include early gestational age (structures are too tiny to evaluate, respective organs not completed development structurally or functionally), abnormal amount of liquor (oligo/polyhydramnios) which makes visualization of organs difficult, altered maternal habitus (obese, thick abdominal wall) and foetal position. Authors found that additional findings were high in cases of multi system involvement, which was similar to the observation by Rossi et al.[5] This is attributed to the increased focus of the sonographer on severe and more interesting abnormalities, overlooking minor or less lethal abnormalities or not completing the examination once a major/lethal anomaly is detected .[4]

The main limitations of post-mortem examination are longer duration of reporting and the need for appropriate training and expertise in the field of perinatal pathology. There are a few other factors which may hinder the accurate post-mortem examination:

- intrauterine foetal death with long period of foetal retention and superimposed maceration may distort the foetal anatomy
- in foetuses with suspected CNS abnormality, the autopsy findings may be inconclusive due to extreme autolysis
- satisfactory dissection may not be possible in foetuses of very early gestation with small organs (can be overcome by dissecting microscope) and presence of post-mortem artefacts
- functional anomalies can be missed.[10,15-18]

Although present study is a retrospective analysis involving less number of cases, it is the only study to specifically correlate the prenatal USG findings and post-mortem examination of foetuses with nonimmune hydrops. This knowledge and further studies involving large number of cases of NIHF will contribute to improve parental counselling.

NIHF is a terminal catastrophic event of pregnancy caused by numerous diverse aetiology and is known for its low diagnostic yield. Initial recognition of hydrops is usually made by ultrasonographic examination. But the aetiology in many cases remains elusive even after a carefully performed imaging.

In such instances post-mortem study of the foetus including placental examination and other ancillary work up like genetic study, testing for infections and metabolic derangements help in identifying the cause. present study demonstrates a high rate of discordancy between USG and autopsy examination in cases complicated by NIHF.

Foetal autopsy confirms the USG findings (quality control/audit), adds additional findings or changes the final diagnosis. Although, foetuses with nonimmune hydrops carry worse prognosis, it is indispensable to identify the genesis of the hydrops to counsel the couple regarding prognosis, recurrence risk and future pregnancy outcomes. Hence present study underscores the need for autopsy in all cases of NIHF.

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1. Randenberg AL. Nonimmune hydrops fetalis part II: does etiology influence mortality?. Neonatal Netw. 2010 Nov-Dec;29(6):367-80.
2. Bellini C, Hennekam RC. Non-immune hydrops fetalis: a short review of etiology and pathophysiology. Am J Med Genet A. 2012;158A:597-605.
3. Nagy A, Malcomson RDG. Fetal hydrops. In Yee KT, Malcomson RDG, eds. Keeling's Fetal and Neonatal Patholog, 5th ed. London: Springer-Verlag; 2015:299-328.
4. Chescheir NC, Reitnauer PJ. A comparative study of prenatal diagnosis and perinatal autopsy. J Ultrasound Med. 1994 Jun;13(6):451-6.
5. Rossi AC, Prefumo F. Correlation between fetal autopsy and prenatal diagnosis by ultrasound: A systematic review. Eur J Obstet Gynecol Reprod Biol. 2017 Mar;210:201-6.
6. Antonsson P, Sundberg A, Kublickas M, Pilo C, Ghazi S, Westgren M, et al. Correlation between ultrasound and autopsy findings after 2nd trimester terminations of pregnancy. J Perinat Med. 2008;36(1):59-69.
7. Vogt C, Blaas H-GK, Salvesen KA, Eik-Nes SH. Comparison between prenatal ultrasound and postmortem findings in fetuses and infants with developmental anomalies. Ultrasound Obstet Gynecol. 2012;39(6):666-72.
8. Boecking CA, Drey EA, Kerns JL, Finkbeiner WE. Correlation of prenatal diagnosis and pathology findings following dilation and evacuation for fetal anomalies. Arch Pathol Lab Med. 2016 Oct 20;141(2):267-73.
9. Sankar VH, Phadke SR. Clinical utility of fetal autopsy and comparison with prenatal ultrasound findings. J Perinatol. 2006 Apr;26(4):224.
10. Struksnæs C, Blaas HG, Eik-Nes SH, Vogt C. Correlation between prenatal ultrasound and postmortem findings in 1029 fetuses following termination of pregnancy. Ultrasound Obstet Gynecol. 2016 Aug 1;48(2):232-8.
11. Vimercati A, Grasso S, Abruzzese M, Chincoli A, de Gennaro A, Miccolis A, et al. Correlation between ultrasound diagnosis and autopsy findings of fetal malformations. J Prenatal Med. 2012 Apr;6(2):13.
12. Guibert-Trainer F, Piton J, Billerey J. Agenesis of the corpus callosum. J Neuroradiol. 1982;9:135.
13. Slavotinek AM. Fryns syndrome-a review of the phenotype and diagnostic guidelines. Am J Med Genet. 2004;124A:427-33.
14. Solomon BD, Pineda-Alvarez DE, Raam MS, Cummings DA. Evidence for inheritance in patients with VACTERL association. Hum Genet. 2010;127:731-3.
15. Rodriguez MA, Prats P, Rodriguez I, Cusi V, Comas C. Concordance between prenatal ultrasound and autopsy findings in a tertiary center. Prenat Diagn. 2014;34:784-9.

16. Gilbert-Barness E, Debich-Spicer D. Embryo and fetal pathology: color atlas with ultrasound correlation. Cambridge: Cambridge University Press; 2004.
17. Kaasen A, Tuveng J, Heiberg A, Scott H, Haugen G. Correlation between prenatal ultrasound and autopsy findings: A study of second-trimester abortions. Ultrasound Obstet Gynecol. 2006;28:925-33.
18. Amini H, Antonsson P, Papadogiannakis N, Ericson K, Pilo C, Eriksson L, et al. Comparison of ultrasound and autopsy findings in pregnancies terminated due to fetal anomalies. Acta Obstet Gynecol Scand. 2006;85(10):1208-16.

Umamaheswari G, Ramya T, Chaitra V. Nonimmune hydrops foetalis (NIHF): value of fetal autopsy and comparison with ultrasound findings. Int J Reprod Contracept Obstet Gynecol 2018;7:3330-7.

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[illegible] The purpose of this study was to evaluate gross umbilical cord abnormalities and to examine its relationship with placental pathological features and perinatal outcome.

M [illegible] We retrospectively collected 101 singleton placentas with gross umbilical cord abnormalities over 3-year period (delivered on or after 20 weeks of gestational age). Association between gross umbilical cord abnormalities such as hypercoiled cords, hypocoiled cords, thin cords, true knot, strictures, excessively long cord, and abnormal cord insertions were reviewed and evaluated for placental histology, clinical parameters and perinatal outcome.

[illegible] The most common umbilical cord abnormality (UCA) observed was coiling abnormality (hypercoiling, hypocoiling and strictures). Gross UCAs were associated with maternal factors (preeclampsia, oligohydramnios and gestational diabetes), foetal factors (intrauterine foetal death, SGA and intrapartum complications such as NRFHR tracing and low Apgar scores) and histologic placental features (maternal vascular malperfusion, foetal vascular malperfusion, villous capillary lesions and inflammation). Cords with multiple abnormalities were significantly correlated with histologic evidence of foetal vascular obstruction and intrauterine foetal death.

[illegible] Gross UCAs, especially when multiple were associated with clinically significant placental findings and adverse perinatal outcome. Therefore, our study reinforces that all placentas with gross UCAs should be submitted for examination with complete (full length of the) umbilical cord.

[illegible] Coiling abnormality perinatal outcome, Foetal vascular malperfusion, Maternal vascular malperfusion, Placental histology, Umbilical cord abnormalities

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Umbilical cord (UC) is a vital structure which acts as a conduit between the developing foetus and placenta. It carries nutrients, oxygen and fluids necessary for the intrauterine life. This unique lifeline needs optimal protection which is provided by Wharton's jelly, amniotic fluid and coiling of the umbilical vessels.[1] The fully developed umbilical cord is pearly white, 50-60cms long with an average diameter of 1-2cm. It contains 2 arteries and a vein which course through the Wharton's jelly in a helical fashion with one coil/twist every 5 cms of cord. The cord normally inserts on the placental surface, more often near or at the center.[2]

Conditions such as abnormal cord length, abnormal coiling, knots, entanglements, constrictions, velamentous vessels, single umbilical artery (SUA) and thrombosis

may lead to cord compression and consequent reduction of blood flow in the umbilical vessels and have been associated with adverse foetal outcome and intrauterine foetal demise (IUFD).[3]

The purpose of this study was to evaluate gross patterns of umbilical cord abnormalities and determine correlations with placental histology and perinatal outcome.

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This retrospective study was carried out from January 2014 to December 2016 in the Departments of Obstetrics and Gynaecology and Department of Pathology.

101 Placentas with gross umbilical cord abnormalities delivered on or after 20 weeks of gestational age were taken for study consideration. Clinical indications for histological examination of the placenta were stillbirth, foetal growth restriction, preterm premature rupture of membranes, severe pre- eclampsia, diabetes, gross abnormalities of the placenta, clinical suspicion of infection or abruption and compromised neonatal outcome.

Umbilical cord abnormalities (UCA) included in the study were hypercoiled cord (>3 coils/10cm segment of umbilical cord), hypocoiled cord (< 1 coil per 10 cm), thin /narrow cord due to diminished Wharton's jelly (maximum cord diameter <0.8 cm), strictures (focal narrowing/thinning in relation to the remaining UC), true knot(TK), SUA, abnormal cord insertion-marginal (< 1cm from the true disc margin-MUC) / velamentous (membranous insertion-VEL) / furcate (umbilical vessels splitting and leaving Wharton's jelly prior to reaching the chorionic plate surface).[4] Excessively long umbilical cord (ELUC) was defined as those UCs measuring approximately 2 standard deviations (SD) above the mean or ≥ 70cms in length.[4,5]

Clinical details obtained along with the placental specimens were maternal age, gravidity and parity, gestational age, medical conditions complicating pregnancies, presence of IUFD, foetal growth restriction (FGR), non-reassuring foetal heart rate (NRFHR), meconium stained liquor, Apgar scores at 1 and 5 minutes and birth weight as recorded by the obstetricians. Infants with birth weight <10th percentile and >90th percentile of the gestational age were termed as small for gestational age (SGA) and large for gestational age (LGA) respectively. The rest were considered as appropriate for gestational age (AGA).[6]

Umbilical cord and placental assessment were based on gross and microscopic examinations. An entire placenta was available from all 101 patients and were fixed in 10% formalin. Placentas were weighed after the membranes and umbilical cord were trimmed off and compared with the reference weights for trimmed singleton placentas.[7]

Umbilical cord was examined for length, diameter, colour, insertion site, number of twists and vessels. Photography of the placentas were routinely taken after examining foetal, maternal surfaces and parenchyma. Relevant lesional sections were taken along with 2 sections of membrane roll, 2 or 3 sections from the UC at the stricture and non-strictured area and 3 full thickness sections of the normal parenchyma. All the histologic sections were stained with haematoxylin and eosin.

Umbilical cord abnormalities were correlated with gross, histologic placental characteristics and foetal outcome.

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Hundred and one cases were identified with gross UCAs. There were 48 cases of hypercoiling (Figure 1), 31 cases of hypocoiling (Figure 2) and 11 cases each of thin cords and strictures (Figure 3). Abnormal cord insertions were seen in 7 cases each of MUC, VEL (Figure 4) and 2 cases of furcate. Other less commonly seen abnormalities were 2 cases each of TK (Figure 5), ELUC and SUA. Of the 101 cases of analysed placentas 20 had more than one umbilical cord abnormality.

Isolated UCAs were each analyzed for association with clinical and histologic findings (Tables 1 and Table 2).

Clinical characteristics

The most common UCA identified was hypercoiling, followed by hypocoiling. Stillbirth was observed in 42 cases across all cord abnormalities but was by far most frequently associated with abnormal coiling.

Preeclampsia, oligohydramnios and gestational diabetes were the commonest maternal characteristics observed in UCAs. Infections, PPROM, polyhydramnios and autoimmune diseases were other maternal features identified in altered structure of UCs.

NRFHR, low Apgar and Meconium stained liquor were common in coiling abnormalities, more so in the group with hypercoiling. Fifty percent of hypercoiled and one third of hypocoiled cords had birth weights of < 10th percentile.

Placental findings

More than 50% of the placentas with UCAs were small for gestational age. Significant histologic features identified were maternal vascular malperfusion (MVM-35/101), foetal vascular malperfusion (FVM-26/101) and villous capillary lesions (19/101). The other microscopic features noted were distal villous immaturity (DVI), massive perivillous fibrin deposition (MPVFD), and inflammatory lesions.

Table 3: Clinical and histologic findings in cases with multiple UCAs

Parameter	Cases with multiple UCAs (n= 31)	IUFD (n=11)	Live born (n=20)	SGA (n=11)	AGA (n=20)	Hyper coiling (n=20)	Hypo coiling (n=20)	Strictures (n=20)	Thin cord (n=20)
Placental weight									
SGA	31	19	10	0	4	1	2	4	5	1
MVM	21	8	4	0	1	0	1	3	2	1
Infarcts	13	10	4	0	1	0	0	1	2	0
Retroplacental haematoma	3	5	1	0	0	0	0	0	1	0
Intervillous thrombus	9	8	2	0	0	0	0	1	1	1
MPVFD	1	1	0	0	2	1	0	0	0	0
FVM	8	10	5	0	6	0	2	3	1	0
Chorionic plate/stem villous vessel thrombus	5	7	2	-	6	0	2	3	1	0
Avascular villi	8	8	5	-	6	0	2	3	0	0
Villous Stromal Karyorrhexis	4	5	4	-	2	0	1	0	0	0
Nucleated RBCs	2	3	-	-	1	0	1	1	0	0
Inflammation	8	9	3	1	2	0	1	1	4	1
Acute chorioamnionitis	2	5	0	1	1	0	0	0	2	0
VUE	6	4	3	0	1	0	1	1	2	1
Villous capillary/vascular lesions	14	3	3	2	2	1	0	0	0	0
Chorangiosis	9	1	1	1	1	0	0	0	0	0
Chorangiomatosis	3	2	2	0	1	1	0	0	0	0
Villous congestion	2	0	0	1	0	0	0	0	0	0
DVI	5	4	1	0	0	0	0	2	1	0

Infarcts, intervillous thrombus, retroplacental haematoma and MPVFD (histologic markers of MVM) were observed in cases with gross UCAs. The other features noted were decidual vasculopathy, distal villous hypoplasia, increased syncytial knotting, cell islands, villous agglutination, and multinucleate trophoblastic giant cells.

The histologic features of chronic FVM such as chorionic plate /stem villous vessel thrombus, avascular villi, villous stromal karyorrhexis and nucleated red blood cells were significantly more common in cords with hyper, hypo coiling and strictures. Ectatic vessels were also noted in many of the chorionic plate /stem villous vessels of FVM.

Villous vascular lesions such as chorangiosis (increased number of capillaries - more than 10 capillaries/terminal villi, in 10 villi) in many areas of the placental parenchyma, patchy and occasional multifocal chorangiomatosis (excessive capillaries with surrounding pericytes in immature intermediate / stem villi) and villous congestion were found to be associated with hypercoiled cords.

Multiple UCAs

Placentas with multiple UCAs and their associations with clinical and histologic findings were also analysed. IUFD was significantly associated with the presence of multiple UCAs (13/20). In the remaining live born cases the clinical outcome was SGA (6/20) and AGA (1/20). The most common maternal obstetric complication was hypertension and the commonest placental histology was foetal vascular malperfusion (FVM) (Figure 6). These findings are summarized in (Table 3).

Discussion

The coiling pattern of the UC largely affects umbilical blood flow that is vital for sustaining foetal growth and development and its pathophysiological significance has been studied by many authors. UCAs often result in mechanical compression leading to venous stasis, endothelial damage and loss of perfusion to a large area of placental gas exchanging villi and ultimately resulting in foetal distress and hypoxia.[8-10] This study analyzed the UCAs such as hypercoiling, hypocoiling, strictures, thin cord, true knot, SUA, ELUC and abnormal insertions in

relation to gross, histologic placental changes as well as clinical parameters and foetal outcome.

Table 2: Correlation between umbilical cord coiling abnormalities and clinical parameters and foetal outcome

Parameters	Total number of cases	Total number of hypercoiled cords	Total number of hypocoiled cords	Total number of normal coiled cords	Total number of IUFD	Total number of FGR (<10th percentile)	Total number of NRFHR tracing	Total number of low Apgar score (<7 at 1 min)	Meconium stained liquor	Total number of preterm births (<37 weeks)
Gestational age										
<28 weeks	8	6	5	0	4	0	0	1	3	1
>28 weeks	40	25	6	2	7	2	2	6	4	1
Maternal factors										
HT	15	6	2	0	1	0	0	2	3	0
GDM	8	0	0	1	1	0	0	1	0	1
Rh Negative	1	0	0	0	0	0	0	1	0	0
Oligo	10	6	2	0	0	0	0	1	3	0
PPROM	3	6	2	0	0	0	0	0	1	0
Polyhydramnios	1	1	0	0	0	0	1	0	0	0
Auto immune	1	1	2	0	1	0	0	1	1	0
Infections	4	1	1	0	1	0	0	0	0	0
Foetal factors										
SGA	21	9	5	0	1	0	0	5	2	1
AGA	10	8	1	0	1	0	0	0	0	0
LGA	3	0	0	1	0	0	0	0	0	0
IUFD	14	14	5	1	9	2	2	2	5	1
NRFHR	15	2	1	0	1	0	0	2	1	1
Liquor										
<4 @1 mt	6	1	0	0	0	0	0	1	0	0
<7 @ 1 mt	7	2	1	0	1	0	0	1	0	0
Thick meconium stained liquor	4	1	0	0	0	0	0	0	0	0

Consistent with prior studies, our investigation showed that UCA has significant association with IUFD, SGA and intrapartum complications such as NRFHR tracing and low Apgar scores.[8-10] Meconium stained liquor was not a consistent finding in our study.

Umbilical cord coiling abnormality

Little is known about factors determining cord coiling. It is generally believed that coiling is established very early in gestation and is thought to be the result of foetal activity. Lack of coiling may then reflect foetal inactivity, which is supported by the fact that coiling is reduced in cases of restricted foetal movements due to various causes.1 The prenatal ultrasonographic evaluation of umbilical cord is usually limited to vessel number and umbilical artery Doppler. Predanic et al demonstrated a strong correlation between mid-trimester umbilical coiling index (UCI) and post-natal coiling index.[11]

Among 101 studied placentas, abnormal coiling was the commonest UCA, which accounted for 80%. The frequency and clinical correlations of hypercoiled cords were FGR (43%), IUFD (29%), NRFHR (31%). For hypocoiled cords, the frequencies of adverse outcomes were IUFD (45%) and FGR (29%). Our results were in contrast to the study by Machin et al who observed more IUFDs among hypercoiled cords. Our data support the finding that abnormal coiling may cause chronic umbilical cord obstruction and unfavourable foetal outcome.[12]

Among hypercoiled umbilical cords, specific gross patterns of coiling were recognized with histologic evidence of foetal vascular malperfusion and stillbirth.[8]

The placental weight was small for gestational age in more than two thirds of the studied cases. Placental histologic findings associated with UC coiling abnormalities include villous capillary congestion, chorangiosis, chorionic plate / stem villous vessel thrombi, avascular villi, MVM, inflammatory lesions and DVI. Placental infarcts and intervillous thrombus are the conspicuous lesions observed among coiling abnormality cases in our series.

They represent deficient maternal circulation (maternal vascular malperfusion) leading to parts of placental atrophy or infarction in areas of poor nutrition. However,

the association between MVM and cord coiling abnormalities appear speculative.

Hypercoiling+ Stricture	4	SGA	A) congestion around cord insertion	A) FVM	A) NIL	IUFD
		SGA	B) NIL	B) vascular involution, patchy mild villitis	B) post varicella	IUFD
		SGA	C) marginal atrophy and patchy	C) FVM	C)NIL	IUFD
		SGA	firm pale brown areas D) NIL	D) FVM	D)NIL	IUFD
Hypocoiling+ Thin cord	4	SGA	NIL	DVI, decidual arteriopathy, laminar necrosis	A) PPROM	AGA
		SGA	Uneven thickness of	FVM	B) Oligo,FGR	SGA
		SGA	parenchyma Intervillous thrombus, infarcts	MVM, FVM	C)preeclampsia	IUFD
		SGA	NIL	Villous hydrops,FVM,mild intervillositis,viral/thrombophilia possibilities	D)Fever	IUFD
Hypercoiling+ Thin cord	3	A)SGA	Infarcts	FVM, MVM, mild chronic villitis, obliterative vasculopathy	a)Systemic hypertenion, GDM	SGA
		B)SGA	Marginal thinning,uneven thick, yellow brown lesion(infarcts)	extensive multifocal Chorangiomatosis, MVM	b)FGR	SGA
		C)SGA	C subchorionic dark brown congested area	C MVM, Multifocal chorangiomatosis-treated SLE	c)autoimmune disease, PE, FGR, PPROM	SGA
Hypercoiling + Marginal Insertion	2	A)SGA	A) infarcts, intervillous thrombus	MVM	A)PE	IUFD
		B)SGA	B) Basal yellow brown nodule	Infarcts, laminar necrosis, DVI	B) Autoimmune disease, PPROM	SGA
Hypocoiling+ Marginal	1	SGA	Focal Atrophy, Subchorionic Hematoma	Large transmural hematoma occupying >50% parenchyma, recanalizing thrombi (stem vill)-FVM, MVM	Systemic hypertension	IUFD
Hypocoiling+ Stricture	1	SGA	Wedge, pale yellow INFARCT	SMALL, hypocoiled cord with a stricture, MVM	FGR	SGA
Hypocoiling+ SUA	1	SGA	Edematous, congested cord, parenchymal marginal atrophy	FVM	Polyhydramnios	IUFD
VEL + stricture	1	SGA	Uneven thickness of parenchyma	Small placenta with umbilical cord stricture and a velamentous vessel with thrombus FVM vascular involution following foetal death with ? Temporal heterogeneity	Autoimmune disease	IUFD
VEL+Furcate	1	SGA	succenturiate, furcate cord	Chronic histiocytic intervillositis	Fever with rash	IUFD
ELUC+Nuchal+Stricture	1	SGA	maternal surface-plaque like thickening >25%,6 firm pale-yellow areas in parenchyma	MPVFD, focal chorangiomatosis	NIL	IUFD
ELUC+Nuchal+True knot	1	AGA	NIL	DVI, FVM	NIL	Term IUFD

We wondered whether the less placental reserve due to deficient intervillous circulation can affect foetal outcome if cord coiling abnormalities arise additionally.

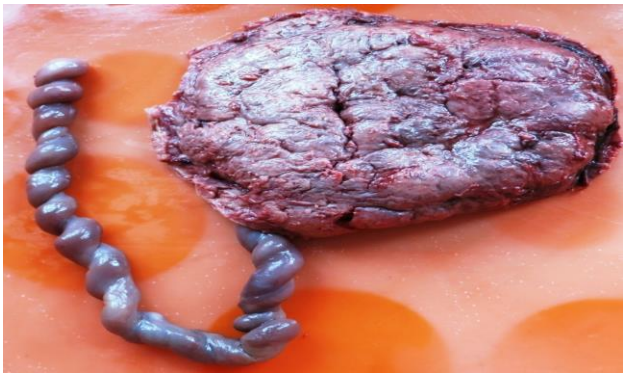


Figure 1: Hypercoiled cord

Presence of avascular villi, thrombosis of foetal vasculature, VSK and NRBCs reflect foetal blood flow obstruction and hypoxia. In our study 25% of the abnormal coiling cases tended to have FVM suggesting that these lesions would have caused chronic vascular obstruction.[10]

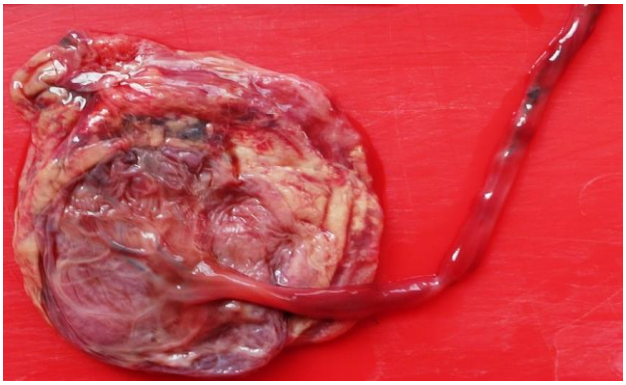


Figure 2: Hypocoiled cord



Figure 3: Hypocoiled cord

Chorangiosis, multifocal chorangiomatosis are overlapping villous capillary lesions believed to be related to hypoxia. Benirschke observed an association between chorangiosis and cord problems.[13] It is an adaptive mechanism to overcome hypoxia occurring in coiling abnormalities. Our observations were also similar.[14] Some of the cases also showed distal villous immaturity, one other lesion known to be associated with coiling abnormalities.



Figure 4: Hypercoiled cord with avascular villi and thrombosis

There was some correlation between inflammatory lesions such as VUE and, acute chorioamnionitis with individual UCA.



Figure 5: Hypercoiled cord with avascular villi and thrombosis

Cord strictures

Most investigators considered strictures and hypercoiling to be similar or related entities. In our study we observed a total of 11 cases with cord strictures. Four among the cases were associated with hypercoiling; one each with hypocoiling, velamentous cord insertion and ELUC. 4 cases had isolated stricture. This data suggested that cord stricture is a distinctive entity as agreed upon by Hong Qi Peng et al in their study.[15] We have also noted a high incidence (more than 80%) of foetal demise associated with cord stricture, coiling abnormalities or a

combination of both. Foetal vascular malperfusion was common when strictures and hypercoiling were coexistent. This implies that UC strictures and hypercoiling could decrease the blood flow with possible predisposition to development of thrombosis.

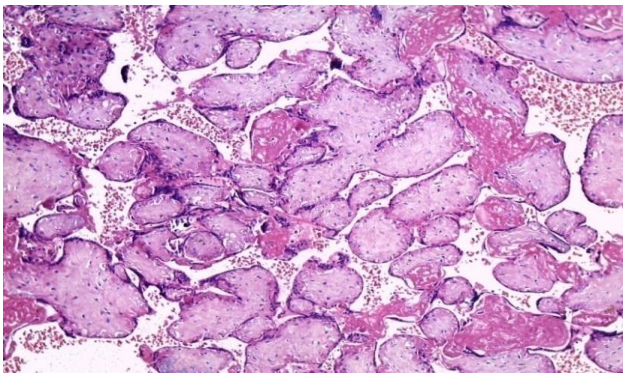


Figure 6: Photomicrograph of placental villi showing avascular hyalinised villi, villous stromal karyorrhexis and nucleated RBCs suggesting that these lesions would have caused prolonged obstruction (H&E, 10×)

Thin cord, SUA

It has been suggested that diameter of the umbilical cord is determined by the water content of Wharton's jelly. Previous studies have found an association of thin cord with FGR and other poor foetal outcome.[16,17]

A Wharton's jelly reductions have also been recognized as a possible cause of foetal death in the presence of single umbilical artery, which is the most common abnormality of the umbilical cord. These associations were confirmed in our study. According to Heifetz SA, non-malformed SUA infants can have an increased mortality rate, although SUA with associated malformations are the common cause of the high perinatal mortality.[18]

True knot, ELUC

Interestingly, both cases of true knot were associated with LGA. The case with a tight TK ended up in IUFD. Common associations like multi gravida and ELUC were also observed in our study. Like earlier studies we also found an association between ELUC and other multiple UCAs.[5] Both cases of ELUC were associated with multiple UCAs such as nuchal cord, TK and stricture and expectantly both resulted in IUFD.

Abnormal cord insertions

Marginal and velamentous insertions are suggested to result from disturbances of implantation and considered a marker of placental insufficiency.[19] We found that non-reassuring foetal status, SGA and IUFD were significantly associated with abnormal insertion which may reflect intrauterine hypoxia.

Multiple UCAs

Any UCA can sufficiently reduce placental function, when multiple predispose the foetus to placental circulatory stasis lesions and thrombosis, thus leading to vascular obstruction and unfavourable clinical sequelae.[3]

In our study the majority of multiple UCA cases tended to have extensive rather than focal FVM with admixture of avascular hyalinised villi, villous stromal karyorrhexis and nucleated RBCs suggesting that these lesions would have caused prolonged obstruction. Infants with multiple UCAs where FVM is extensive were found to have brain imaging abnormalities and/or abnormal neurological follow-up.

There were some limitations in our data. First, as a retrospective study, the clinical information was available only for the immediate neonatal period and further follow up could not be ascertained. Second, not all placentas and complete length of the UC (that remained attached to the neonate) were available for examination. Finally, different patterns of hypercoiling were not studied.

Conclusions

Gross UCAs, especially when multiple was associated with clinically significant placental findings and adverse perinatal outcome. Therefore, our study reinforces that all placentas with gross UCAs should be submitted for examination with complete (full length of the) umbilical cord. Coiling index with patterns of coiling should be a part of routine examination. A prospective study that also includes the follow up of these neonates could address the clinical effects of UCAs.

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References

1. De Laat MW. The umbilical coiling index in complicated pregnancy. Eur J Obstet Gynecol. Reprod Biol. 2007;130(1):66-72.
2. Rebecca N. Baergen. Umbilical Cord Pathology. Surgical pathol. 2013;6:61-85.
3. Chan JS, Baergen RN. Gross umbilical cord complications are associated with placental lesions of circulatory stasis and fetal hypoxia. Pediatr Dev Pathol. 2012;15(6):487-94.
4. T. Yee Khong. Sampling and Definitions of Placental Lesions. Amsterdam Placental Workshop

Group Consensus Statement. Arch Pathol Lab Med. 2016;140:698-713.
5. Benirschke K, Burton GJ, Baergen RN. Pathology of the Human Placenta. Sixth edition. New York, NY: Springer; 2012.
6. Amy Heerema-McKenney, MD, Monique E De Paepe, MD, MSc and Edwina J Popek, DO. Diagnostic Pathology: Placenta. Published by AMIRSYS; 2015.
7. Almog B. Placenta weight percentile curves for singleton and twins deliveries. Placenta. 2011;32:58e62.
8. Ernst LM, Minturn L, Huang MH, Curry E, Su EJ. Gross patterns of umbilical cord coiling: correlations with placental histology and stillbirth. Placenta. 2013;34(7):583-8.
9. Baergen RN. Cord abnormalities, structural lesions, and cord "accidents". Semin Diagn Pathol. 2007;24 (1):23-32.
10. Tantbirojn P, Saleemuddin A. Gross Abnormalities of the Umbilical Cord: Related Placental Histology and Clinical Significance. Placenta. 2009;30:1083-8.
11. Verkleij CPM. Evaluation of antenatal umbilical coiling index at 16–21 weeks of gestation as a predictor of trisomy 21and other chromosomal defects. Ultrasound Obstet Gynecol. 2013;42:545–52.
12. Diwakar RK, Naik MM, Jindal MM. Umbilical cord coiling: case report and review of literature. BJR Case Rep. 2017;2:20150152.
13. Ryan WD, Trivedi N, Benirschke K. Placental Histologic Criteria for Diagnosis of Cord Accident: Sensitivity and Specificity. Pediatr Dev Pathol. 2012;15:275-80.
14. Tihana Rumboldt, Paul T Eberts. Disorders of Fetal Vascular Development Chorangioma, Localised Chorangiomatosis, Chorangiosis, and Diffuse Multifocal Chorangiomatosis, Pathology Case Reviews. 2008;13:236-40.
15. Shun-Jen Tan. Fetal demise by umbilical cord around abdomen and stricture. Archives Gynecol bstetr. 2010;281:137.
16. Waldo Sepulveda, MD et al. Improving Sonographic Evaluation of the Umbilical Cord at the Second-Trimester Anatomy Scan. J Ultrasound Med. 2009;28:831-5.
17. Kulkarni ML. Absence of Wharton's jelly around the umbilical arteries. Indian J Pediatr. 2007;74(8):787-9.
18. Hyeong Ju Kim. Association of isolated single umbilical artery with perinatal outcomes: Systemic review and meta-analysis. Obstet Gynecol Sci. 2017;60(3):266-73.
19. Pathak S, Sebire NJ, Hook L, Hackett G, Murdoch E, Jessop F, et al. Relationship between placental morphology and histological findings in an unselected population near term. Virchows Arch. 2011;459(1):11-20.

Gurusamy U, Thangavelu R, Venkataswamy C. Abnormalities of the umbilical cord: correlation with placental histology and perinatal outcome. Int J Res Med Sci 2017;5:5009-16.

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Placental lesions in autoimmune disorders in pregnancy and its clinical correlation

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Abstract

Background: Pregnancy in women with autoimmune diseases is frequently associated with placental insufficiency leading to adverse perinatal outcome. Aim of the study was to investigate the presence and possible clinical significance of placental lesions in mothers with different autoimmune disorders.

Methods: In this retrospective study, 11 placentas from 10 mothers with diverse autoimmune diseases including systemic lupus Erythematosus (SLE), antiphospholipid antibodies (APLA), idiopathic thrombocytopenic purpura (ITP) and antinuclear antibodies (ANA) were studied.

Results: Placental correlates were reduced placental weight, maternal vascular under perfusion, abruption, villitis of unknown etiology, multifocal chorangiomatosis, distal villous immaturity, massive perivillous fibrin deposition/maternal floor infarction and foetal thrombotic vasculopathy. Of the 11 pregnancies 3 were untreated (1 SLE, 2 APLA) and resulted in intrauterine foetal demise. The lesions were more severe in these cases. All the treated pregnancies resulted in live born babies (8), of which 3 were growth restricted, 2 were complicated with oligohydramnios and 3 were delivered preterm.

Conclusions: In this group of diverse autoimmune disorders, placental lesions were not specific for each of them. Apart from maternal vascular under perfusion, lesions like villitis of unknown etiology, distal villous immaturity and massive perivillous fibrin deposition were identified and may recur in subsequent pregnancies and treatment should be directed towards modifying it. The placental examination should be mandatory in all cases of maternal autoimmunity and pregnancies with poor foetal outcome.

Keywords: Maternal autoimmune diseases, Maternal vascular under perfusion, Placental lesions

Introduction

Placenta plays a major role for a pregnancy to proceed normally, ensuring an adequate blood supply to support and promote the growth of the developing foetus. Autoimmune disease, both organ specific and non-organ specific is associated with intrauterine growth retardation (IUGR), preterm delivery and intrauterine foetal demise (IUFD) and appears to be a consequence of failure of the normal uterine physiologic changes to occur with resultant placental insufficiency and the features that accompany a failing placenta.[1] Despite being an important etiology of poor perinatal outcome, placental lesions and its clinical correlation of autoimmune diseases lack a unified diagnostic criterion.

These clinical associations as well as foetal outcome suggest that there may be multiple pathologies that

complicate and disrupt pregnancy in patients with autoimmune disorders.[2]

Given these uncertainties surrounding the placental pathology of autoimmune diseases, we sought to determine the presence and possible clinical significance of maternal vascular lesions, chronic villitis of unknown etiology (VUE), distal villous immaturity (DVI), massive perivillous fibrin deposition/maternal floor infarction (MPVFD/MFI) in placentas of mothers with different autoimmune disorders.

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This retrospective study was carried out from January 2014 to April 2017 in the Departments of Obstetrics and Gynecology and Department of Pathology at PSG IMS and R.

A total of 11 placentas were studied from ten mothers with maternal immunological disorders which included systemic lupus erythematosus (SLE) with secondary anti phospholipid antibodies (APLA), primary anti phospholipid antibodies (APLA), idiopathic thrombocytopenic purpura (ITP) and one with antinuclear antibody (ANA).

All patients had singleton pregnancies without any foetal malformations. Infants below tenth percentile were considered as having intrauterine growth restriction.

According to the revised sydney classification criteria, apla is defined by

- Presence of lupus anticoagulant (LA) in plasma on two or more occasions at least 12 weeks apart
- Presence of moderate to high levels of anticardiolipin (aCL) (IgG or IgM) in serum or plasma (i.e., >40 IgG phospholipid units (GPL)/mL or IgM phospholipid units (MPL)/mL or >99th percentile) on two or more occasions at least 12 weeks apart
- Presence of moderate to high levels of anti–beta-2 glycoprotein I antibodies (IgG or IgM) in serum or plasma (> 99th percentile) on two or more occasions at least 12 weeks apart.[2]

Patients with SLE were diagnosed according to the American Rheumatism Association Diagnostic Criteria.[2] Both patients with ITP were diagnosed during the pre-pregnancy period.[4]

An entire placenta was available from all 10 patients and were fixed in 10% formalin. Placentas were weighed after the membranes and umbilical cord were trimmed off and compared with the reference weights for trimmed singleton placentas. Two rolls of membranes were taken and umbilical cord was examined for length, diameter, colour, insertion site and number of twists.[5] Photography of the placentas were routinely taken after examining foetal and maternal surfaces.

The placentas were cut at 1cm intervals and the parenchyma was assessed for colour, sponginess and specific lesions. Relevant lesional sections were taken along with membrane roll, cord and normal parenchyma. All the histologic sections were stained with hematoxylin and eosin.

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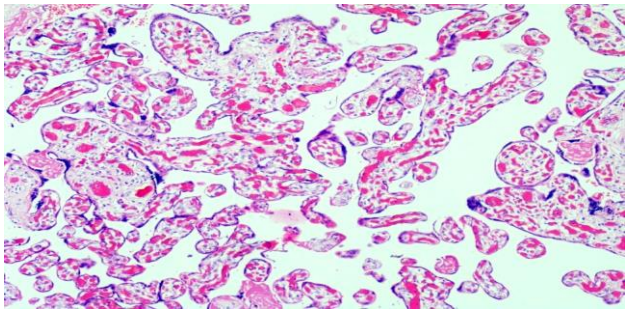
Over a period of 3-years 11 placentas from 10 mothers with the clinical and laboratory evidence of maternal autoimmune diseases were included in the study.

Two out of ten studied patients had SLE with secondary APLA, five had primary APLA, two had ITP and one with ANA positivity. One patient with ITP had two consecutive pregnancies and both placentas were studied

Patients with SLE

Clinical data and placental findings from the two studied patients with SLE are shown in Table 1. Both of our patients had SLE with secondary APLA.

Patient 1 was diagnosed 9 years before the index pregnancy. Her first pregnancy was an induced abortion owing to SLE flare, while the second was a spontaneous abortion. She had active renal involvement and superimposed preeclampsia in the present pregnancy. She was treated with low molecular weight heparin (LMWH), immunomodulators, anti-hypertensives and aspirin. She delivered a preterm small for gestational age (SGA) baby at 34 weeks of gestation due to preterm premature rupture of membranes (PPROM). Her placenta was small with thin hypercoiled cord (8 twists for 11.0cms of cord length-). Microscopically it showed maternal vascular underperfusion which included distal villous hypoplasia, increased syncytial knots, abundant perivillous fibrin deposition and calcification. Multifocal chorangiomatosis (Figure 1) was also noted.



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Patient 2 was a second gravida with previous spontaneous abortion at 8 weeks. Now she was referred with an intrauterine foetal demise at 24 weeks of gestation. On evaluation she was found to have malar rash,

thrombocytopenia and presence of anti Sm antibodies. On investigating further, she also had an abnormal lupus anticoagulant (LA), with elevated titres of anticardiolipin (aCL) antibody IgG and beta 2 glycoprotein 1 IgM.

The placenta was very small (less than 10th percentile) and had thin, flat umbilical cord. The parenchyma grossly showed marginal atrophy and a basal firm yellow nodule. Microscopically, some of the chorionic plate and stem villous vessels showed recanalizing thrombi and haemorrhagic endovasculitis (foetal thrombotic vasculopathy). There was laminar necrosis of the decidua, patchy lymphohistiocytic villitis, intervillositis (Figure 2), microinfarcts and foci of avascular, hyalinized villi. Basal haematoma with indentation of parenchyma was also noted.

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Maternal age	27	21
autoimmune etiology	SLE (Class 4 lupus nephritis), secondary APLA, hypothyroidism	SLE with secondary APLA
Duration since diagnosis	9 years	Post -partum
Laboratory data	aCL IgM, IgG positive, anti-ds DNA positive	ANA positive (Sm positive, nucleosome positive), Abnormal LA, aCL IgG positive, anti-beta 2 glycoprotein 1 IgM positive, Thrombocytopenia
Previous obstetric history	G1-MTP at 8weeks due to SLE flare G2-spontaneous abortion at 18 weeks G3-PP	G1-Spontaneous abortion at 8weeks G2-PP
Gestational age at delivery	34W+1D	24W+1D
Course during pregnancy/complications	Superimposed preeclampsia, IUGR, PPROM	Malar rash
Treatment during pregnancy	LMWH, antihypertensives, Aspirin, Steroids, immunomodulators and eltroxin	None
Foetal outcome	SGA, Live birth	IUFD
Foetal weight	1.4 kg	150gms
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Placental weight	*194.0gms (<10th percentile)	*19.0gms (<10th percentile) *Thin, flat cord
Cord	*Thin, hypercoiled cord	Basal firm yellow nodule, at the level of cord insertion
Any specific lesions	*Small subchorionic haemorrhage	Marginal atrophy and basal haematoma
Microscopy	Maternal vascular underperfusion (Distal villous hypoplasia, Increased syncytial knots, Abundant perivillous fibrin deposition and calcification)	Thrombi, infarcts Recanalizing thrombi and haemorrhagic endovasculitis of stem villous vessels, avascular hyalinized villi (Foetal thrombotic vasculopathy)
	Multifocal chorangiomatosis	Retroplacental haematoma Villitis and perivillositis Laminar necrosis of the decidua

Patients with APLA

Clinical data and placental findings from the five studied patients with primary antiphospholipid antibodies are shown in Table 2. In patients 4 and 6, presence of APLA were diagnosed postpartum and the pregnancies resulted in intrauterine foetal demise. In the remaining three patients two were diagnosed even before attempting the studied pregnancies and were treated optimally which resulted in live births. Patient 3 was diagnosed to have APLA at the end of first trimester. She was tested during the ongoing pregnancy as she had previously delivered a premature, growth restricted baby with absent diastolic flow in umbilical artery doppler. Two out of the 3 live births were appropriate for gestational age (AGA). Of the three treated patients two were delivered near term, while the third (patient 3) was preterm.

In four out of 5 patients, the placental weight was small for gestational age. The placenta of the three live born

fetuses showed maternal vascular under perfusion (infarcts, intervillous thrombi and decidual vasculopathy) and vascular ectasia with hypercapillarization.

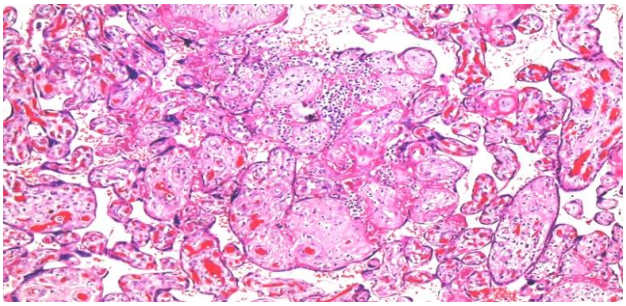


Figure 2. Placenta of patient 6 (IUFD) showed retroplacental haematoma with parenchymal indentation, subchorionic haematoma and intervillous thrombus. Interestingly the other IUFD (patient 4) had a AGA stillbirth and her placenta showed distal villous immaturity (Figure 3).

There were patchy, multifocal chorangiomatosis and villous stromal karyorrhexis. Lowgrade, focal villitis of unknown etiology was also noted.

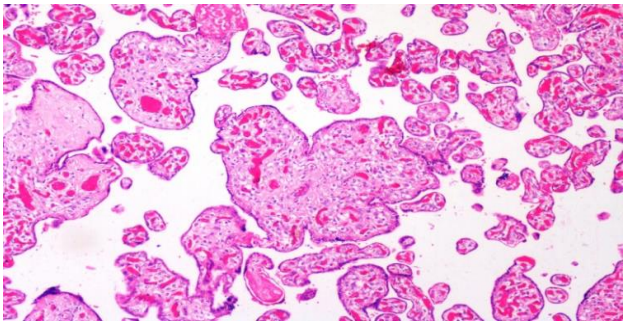


Figure 3. Placenta of patient 4 (IUFD) showed distal villous immaturity. Placenta of patient 8 showed chorangiomatosis and villous stromal karyorrhexis. Lowgrade, focal villitis of unknown etiology was also noted.

Patients with ITP

Clinical data and placental findings from the 3 studied pregnancies with idiopathic thrombocytopenic purpura are shown in Table 3.

Patient 9 had two successive pregnancies that were studied. Both the pregnancies were complicated by oligohydramnios. She had active disease during her first pregnancy and was treated with immunoglobulins, steroids and multiple transfusions. None of the babies were SGA or stillborn.

Almost all the placentas were ≤ 10th percentile. Maternal vascular underperfusion was found in 2 placentas of the three studied pregnancies (patient 8 and 9B) with ITP. Hypercoiling of the cord and haemorrhage around the umbilical vessels were present in patient 9B.

Transmural massive perivillous fibrin deposition (maternal floor infarction) was noted in patient 9A (Figure 4). Intervillous thrombi and lymphocytic deciduitis were also identified.

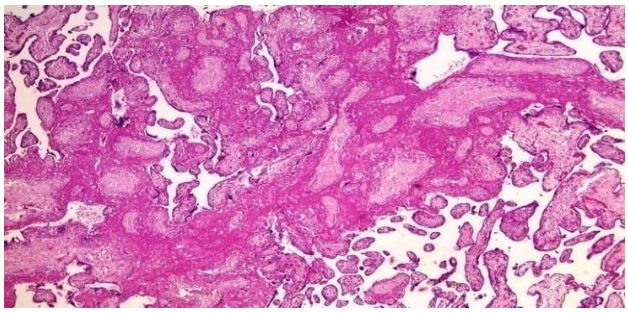


Figure 4. Placenta of Patient 9A showed massive perivillous fibrin deposition (maternal floor infarction) and intervillous thrombi. Placenta of patient 10 showed hypercapillarization of villi.

Patient with ANA

Clinical data and placental findings of the patient with antinuclear antibody is shown in Table 3.

Patient 10 was a low risk primigravida and on routine screening was found to have high resistance flow in uterine artery at mid trimester. On screening for auto-immunity she tested positive for antinuclear antibodies. She was treated with LMWH from 20 weeks and delivered a SGA baby at near term.

Placenta showed both maternal vascular under perfusion and foetal thrombotic vasculopathy which was evident by infarcts, distal villous hypoplasia, hypercapillarization, increased syncytial knots and fibrin thrombi of chorionic plate and stem villous vessels with large foci of avascular hyalinized villi (more than two foci/average of 15 or more affected villi/slide/ foetal vessel lesions) (Figure 5).[6,7]

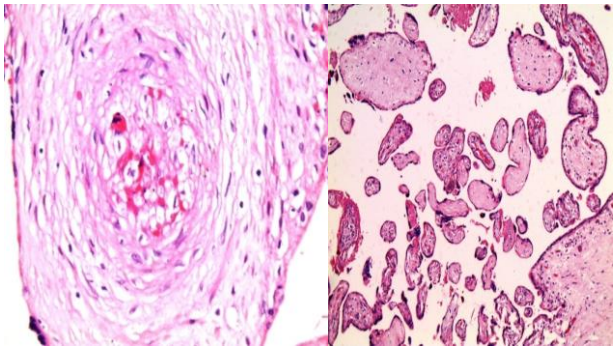


Figure 5. Placenta of patient 10 showed hypercapillarization of villi. Placenta of patient 1 showed

Maternal age	24 Years	23 years	19years	22years	28 years
Autoimmune etiology	APLA	APLA, Overt hypothyroidism	APLA	APLA	APLA, old cerebral venous thrombosis
Duration since diagnosis	First trimester of PP	Post-partum of present pregnancy	1 year (post abortal)	Post-partum (present pregnancy)	9 years
Laboratory data	aCL IgM positive	Anti-beta 2 glycoprotein 1 IgG positive	aCL IgM positive	Abnormal LA, aCL IgG Positive, anti beta 2 glycoprotein1 IgG and IgM positive	Abnormal LA, aCL IgG positive
Previous obstetric history	G1-emergency LSCS at 35 weeks (IUGR, AEDF) G2-MTP at 8 weeks G3-PP	G1-Spontaneous abortion at 20weeks G2-PP	G1-missed abortion G2-missed abortion G3-PP	Primi	G1-normal vaginal delivery(treated)
Gestational age at delivery	33W+2D	38 W+5D	36weeks	32W+6D	37 W+3D
Course during pregnancy/ complications	Subclinical hypothyroidism, GGI	Cervical encirclage done at 13 weeks (history indicated)	GGI	Grade 3 abruption	None
Treatment during pregnancy	Aspirin 75mg from 10 weeks UFH from 12 weeks, steroids for lung maturity	Eltroxin	LMWH from 12 weeks	None	LMWH from 12 weeks
Foetal outcome	AGA, Live birth	IUFD	AGA, Live birth	IUFD	SGA, Live birth
Foetal weight	1.8 kg	3.4KG	2.75 kg	1.7 kg	2.44kg
Placental weight	307.0gms	389.0 gms (<10th percentile	445.0gms	271.0gms (<10th percentile)	345.0gms (<10th percentile)
Cord	Marginally inserted hypercoiled cord	Edematous hypocoiled cord	Eccentrically placed	Hypocoiled cord	Eccentrically placed
Any specific lesions	Basal yellow brown nodule, 1.0cm from cord insertion	None	None	Retroplacental haematoma with parenchymal indentation	None
				Subchorionic haematoma	
				Intervillous thrombus	
Microscopy	Intervillous thrombi	Distal villous immaturity	Vascular ectasia of chorionic plate, stem villous vessels and hypercapillarisation of distal villi	Retroplacental and subchorionic haematoma, intervillos thrombus, C/W abruption	Fibrin thrombi in the decidual Vessels and focal laminar necrosis
	Infarct	Patchy chorangiomatosis	Distal villous immaturity		Distal villous immaturity
	Laminar necrosis, decidua	Lowgrade, focal villitis of unknown aetiology			Maternal vascular underperfusion
	Maternal vascular underperfusion				

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]10[illegible]
Maternal age	26 years	29 years	31 years	27 years
autoimmune etiology	ITP	ITP	ITP	ANA positive
Duration since diagnosis	2 years	14 years	16 years	20 weeks of gestation
Laboratory data	Severe Thrombocytopenia	Severe Thrombocytopenia, ANA-Anticytoplasmic antibodies positive	Severe Thrombocytopenia, ANA-Anticytoplasmic antibodies positive	ANA-Anticytoplasmic antibodies positive
Previous obstetric history	G1-normal vaginal delivery G2-PP	Primi	G1- normal vaginal delivery, postpartum splenectomy G2-PP	Primi
Gestational age at delivery	38W+6D	36W+6D	38W+4D	37W+4D
Course during pregnancy/complications	-	PPROM, Oligohydramnio, Postpartum splenectomy	Oligohydramnios	Subclinical hypothyroidism
Treatment during pregnancy	Platelet transfusions	IV Ig, steroids, platelet transfusions	Steroids, platelet transfusions	LMWH from 20 weeks
Foetal outcome	AGA, live born	AGA, liveborn	AGA, liveborn	SGA, liveborn
Foetal weight	2.6kg	2.33kg	3.05 kg	2.23kg
[illegible]				
Placental weight	312.0gms (<10th percentile)	358.0gms (<10th percentile)	410.0gms (=10th percentile)	386.0gms (<10th percentile)
Cord	Eccentrically placed, edemato us cord	Eccentrically placed, flat, hypocoiled cord	Hypercoiled, edematous cord	Hypocoiled cord
Any specific lesions	2 full thickness firm yellow lesions with central haemorrhagic area	Three basal wedge shaped, pale brown, firm areas	None	Three basal to full thickness, firm yellow brown lesions
Microscopy	Intervillous thrombi	Massive perivillous fibrin deposition(transmural)	Haemorrhage around the three umbilical vessels	Decidual laminar necrosis
	Infarcts	Intervillous thrombi	Decidual laminar necrosis	Infarcts
	DVH, hypercapillarization, increased syncytial knots, C/W maternal vascular underperfusion	Lymphocytic deciduitis	Incipient chorangiosis	Chorionic plate and stem villous vessels show fibrin thrombi
			Maternal vascular underperfusion	Large Foci of avascular hyalinized villi
				DVH, hypercapillarization, increased syncytial knots, C/W maternal vascular underperfusion and foetal vascular obstruction

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Many physiologiocal changes must occur to allow the foetus to persist and grow. Part of the implantation process involves syncytiotrophoblast invasion and erosion of maternal endometrial blood vessels resulting in the development of a utero-placental circulation. Ultimately, the trophoblast becomes incorporated into the wall of the vessel which has now become a thin walled, dilated vascular channel with lack of muscle, thereby ensuring an adequate blood supply to the developing foetus. These vascular changes have been termed physiological change and lack of physiological change or decidual vasculopathy has been found to be a significant placental feature in certain diseases, for example preeclampsia alone, chronic hypertension with

superimposed preeclampsia, autoimmune diseases and idiopathic SGA infants.[8-10]

In this report, we describe clinical and pathologic findings of 11 placentas from 10 patients with autoimmune diseases. We prefer to specify the lesions that are present rather than to embrace them all in the imprecise terminology of placental insufficiency. The primary pathology observed in the present study was maternal vascular underperfusion (fibrin thrombi in the decidual vessels, intervillous thrombi, infarcts, distal villous hypoplasia and increased syncytial knotting).[8-10] Interestingly, the other pathological features observed were chorangiomatosis, villitis, massive perivillous fibrin deposition, distal villous immaturity, abruption and foetal thrombotic vasculopathy.

Distal villous immaturity (DVI) is a maturation defect of the terminal villi.[13] Microscopically the terminal villi are enlarged with excessive stroma, increased vascularity, with a continuous layer of syncytiotrophoblast, paucity of vasculosyncytial membranes and lack peripheral polarization of capillaries. This in turn decreases the efficiency of maternal-foetal exchange leading to late gestational hypoxia and IUFD. This finding is most often associated with maternal diabetes. DVI was apparent in the only term IUFD of our study. Contrary to the observations made so far in the literature, there was no evidence of glucose intolerance in the mother, but she had primary APLA. Presence of DVI in autoimmune diseases remains speculative and needs further characterization.

Chorangiomatosis involves immature intermediate villi or stem villi and the vessels are surrounded by pericytes with increased stromal cellularity and collagen. Diffuse multifocal chorangiomatosis is associated with extreme prematurity, IUGR, preeclampsia and it has been suggested that it may be a developmental abnormality of the villi.[14] This was the major lesion along with maternal vascular underperfusion in one of the studied SLE patients with secondary APLA. The pregnancy was complicated by preeclampsia, prematurity and intrauterine growth restriction.

The other patient who had SLE with secondary APLA was diagnosed postpartum and was not treated antenatally. She had a severe growth retarded foetus which resulted in stillbirth. The predominant lesion in the placenta was villitis of unknown etiology (VUE), perivillositis and retroplacental haematoma.[15] The villi were invaded and expanded by lymphohistiocytic infiltrate and showed secondary changes of ischemia,infarction,recanalizing thrombi in the stem villous vessels with resultant groups of avascular villi (foetal thrombotic vasculopathy).

VUE is a well-recognized entity, but remains a significant challenge because of its frequency, high recurrence rate and associated poor outcome. The most accepted theory is that VUE is an immune reaction akin to placental "rejection".[16]

Thrombi in the foetal circulation of the placenta cause a pattern of clustered avascular fibrotic villi called foetal thrombotic vasculopathy (FTV). Causal factors of FTV include stasis, maternal autoimmune diseases and vascular injury. The most serious outcomes include growth restriction, neurologic injury and perinatal death.[6,7]

Our patient with ANA positivity also had foetal thrombotic vasculopathy with superimposed maternal vascular under perfusion and resultant SGA baby.[11,12]

Apart from maternal vascular underperfusion, one of the placenta of ITP patient also showed massive perivillous fibrin deposition/maternal floor infarction (MPVFD/MFI). This particular patient had active disease during pregnancy and was treated with immunoglobulins, steroids and had improved pregnancy outcome. MPVFD/MFI has a recurrence rate of 30% or more.[17] We had an opportunity to study the subsequent pregnancy of the same patient where the lesion did not recur. There were no associations found between placental lesions and foetal outcome in all three studied ITP cases.

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Placenta remains an undervalued and underutilized surgical specimen in the evaluation of maternal autoimmune disorders. Studies have clearly demonstrated that there are a multitude of mechanisms where by autoimmunity can prevent normal placental development and can cause significant pathology. In this group of diverse autoimmune diseases placental lesions were not specific for any of them.

But it appears that diseases that are clinically more severe have more severe placental vascular changes, related to foetal outcome.

Therefore, although the histopathologic lesions are similar, there appears to be a "grading" in severity. Placental thrombosis and infarction are just one part of the placental lesions. Analyzing previous placentas has been helpful as patients appear to repeat the recurrent lesions such as VUE, MPVFD/MFI and DVI. Pathologists should be aware of these changes and alert the clinicians to the possibility of recurrences in subsequent pregnancies.

Given the heterogeneity of histologic findings, it would be unlikely that a single treatment would be effective in all cases. Perhaps placental pathology should be used to guide therapy; however, it needs to be validated by a study on a larger sample. Placental examination should be indicated in defined subset of patients which includes maternal autoimmune disorders.

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1. Park AL. Placental pathology in antiphospholipid syndrome, In: Hughes' Syndrome, Khamashta MA. Springer-Verlag, London. 2006;362-74.
2. Gómez-Puerta JA. Diagnosis and classification of the antiphospholipid syndrome. J Autoimmunity. 2014;48-49:20-25.
3. Aringer M, Dorner T. Toward new criteria for systemic lupus erythematosus-a standpoint. Lupus. 2016;25:805-11.
4. Perepu U, Rosenstein L. Maternal thrombocytopenia in pregnancy. Obstetr Gynecol. 2013;3(1):6.
5. Rebecca N. Baergen. Umbilical Cord Pathology. Surg pathol. 2013;6:61-85.
6. Raymond W. Redline. Fetal Thrombotic Vasculopathy. Pathology Case Reviews. 2010;15: 37-9.
7. Saleemuddin A, Tanbirojn P, Sirios K. Obstetric and Perinatal Complications in Placentas with Fetal Thrombotic Vasculopathy. Pediatric and Developmental Pathology. 2010;13:459-64.
8. Baergen RN. Manual of Pathology of the Human Placenta, New York, Springer; 2011.
9. Benirschke K, Burton GJ, Baergen RN. Pathology of the Human Placenta, Sixth Edition, New York, Springer-Verlag; 2012.
10. Redline RW. The Placenta. Aliya N. Husain, J. Thomas Stocker, and Louis P. Dehner. Stocker and Dehner's: Pediatric Pathology, 4 th Edition. Wolters Kluwer;2016:325-350.
11. Rodger MA, Walker MC. Is thrombophilia associated with placenta-mediated pregnancy complications? A prospective cohort study. Journal of thrombosis and Haemostasis. 2014;12:469-78.
12. Vijayalakshmi B, Kittali S. Morphological Changes of Placenta in Cases of Pre-eclampsia and Perinatal Outcome. Internat J Scient Study. 2015;3(5):137-42.
13. Redline RW. Distal villous immaturity. Mini-Symposium: Placental and Trophoblastic pathology. Diagnost Histopathol. 2012;18(5):189-94.
14. Bagby C, Raymond W. Redline. Multifocal Chorangiomatosis. Pediatr Development Pathol. 2011;14:38-44.
15. McKay EM. Placental diagnoses-placental abruption. In: Amy Heerema-McKenney, Edwina J. Popek and Monique E. De Paepe. eds. Diagnostic Pathology: Placenta, first edition. AMIRSYS publishing, part II. 2015;7-2.
16. Chan JS. Villitis of unknown etiology and massive chronic intervillositis. Surgical pathology clinics. 2013;6(1):115-26.
17. Romero R, Whitten A. Maternal Floor Infarction/Massive Perivillous Fibrin Deposition: A Manifestation of Maternal Antifetal Rejection. Am J Reprod Immunol. 2013;70(4):285-98.

Ramya T, Umamaheswari G, Chaitra V. Placental pathology in maternal autoimmune diseases-new insights and clinical implications. Int J Reprod Contracept Obstet Gynecol 2017;6:4090-7.

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A retrospective study of maternal mortality in a tertiary private medical college hospital in South India

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Abstract

Background: This study was designed to evaluate the institutional Maternal Mortality Ratio (iMMR) in our institution, a tertiary private medical college hospital and to suggest recommendations and possible interventions to reduce it.

Methods: This retrospective descriptive study was conducted by reviewing the hospital records over a period of ten years from January 2009-December 2018. The case records were reviewed for maternal demographic characteristics and complications.

Results: The total number of deaths during the study period was 21, giving an iMMR of 85.268 per 100000 live births. Most of the maternal deaths (>80%) occurred postpartum. Obstetric causes contributed to 57% of the deaths with hypertension and hemorrhage topping the list. Other causes were sepsis and non obstetric causes including one case of maternal suicide. 52.38% of the women died more than 48 hours after admission to the hospital, while 28.57% succumbed in less than six hours. Secondary complications noted were ICU admission, extended intubation, massive transfusion, operative intervention and multi organ dysfunction.

Conclusions: The classical triad of Hypertension, Hemorrhage and Sepsis continues to be the major determinant of maternal mortality and are potentially preventable by promoting universal access to quality health care, strengthening of health services and ensuring accountability.

Keywords: Hemorrhage, Hypertension, Maternal mortality, MMR, Multi organ dysfunction, Suicide

INTRODUCTION

In the year 2000, the United Nations adopted the Millennium Development Goals (MDG) out of which MDG 5 was targeted on reducing the Maternal Mortality Ratio (MMR) by three fourths between 1999-2015.[1] By the year 2015, Sustainable Development Goals (SDG) were introduced and SDG 3 aimed at Good Health and Well-Being.[2] Under this, the aim is to bring down the global maternal mortality to below 70 per 100000 by the year 2030.

At an MMR of 130 per 100,000 live births in 2016,India is ranked 128 in the world.[3,4] WHO commended India for its groundbreaking progress in reducing maternal mortality by 77% to the present rate.[3] Strategies responsible for this achievement include (i) Increased coverage of maternal health services and promoting institutional deliveries (ii) Incentives and financial support for women delivering in the public health system (iii) Improving literacy among women and increasing the average age at marriage (iv) promotion of public-private partnerships in the care of pregnant women.[3]

To put the country on track to achieve the SDG targets, Government of India established the NITI (National Institution for Transforming India) in the year 2015 to monitor, coordinate and ensure implementation of the SDGs.[2]

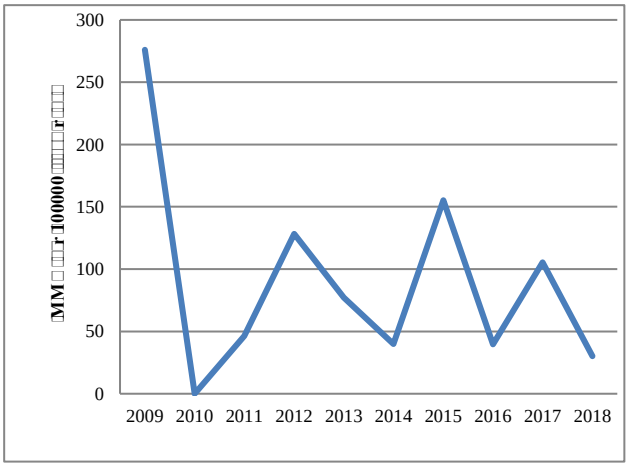
This study was designed to evaluate the MMR in our institution, a tertiary private medical college hospital and to suggest recommendations and possible interventions to reduce the institutional Maternal mortality ratio (iMMR). Continued and focussed efforts to end preventable deaths due to pregnancy and childbirth will help our country reach the SDG.

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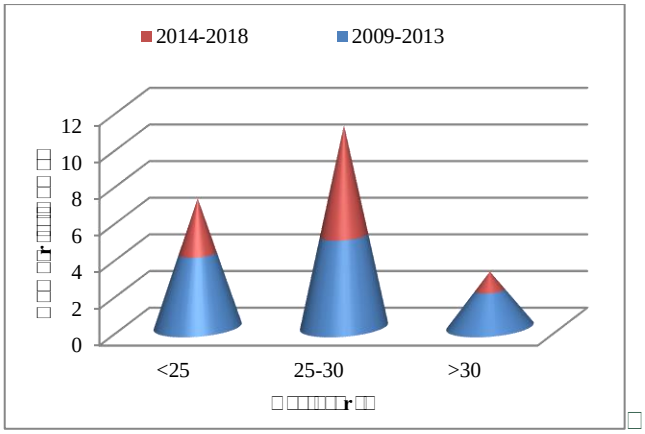
This retrospective descriptive study was undertaken in the Department of Obstetrics and Gynecology at the PSG Institute of Medical Sciences and Research, Coimbatore, India during the period from January 2009- December 2018. The approval of the Institutional Human Ethics Committee was obtained. Informed consent was not required since it was a retrospective study and there was no direct patient contact. The case records of all the women who died during pregnancy or within 42 days of delivery due to complications of pregnancy or other causes were reviewed for the following factors: age, obstetric history, antenatal complications and co morbidities, gestational age at onset of symptoms (if any), ICU admission, the need for ventilator support, blood transfusion, multidisciplinary management, cause of death and admission death interval. The number of live births during the study period was obtained from the parturition register. MMR was calculated as number of maternal deaths per 100000 live births.

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There were a total of 24628 live births in our institution during the study period. Data regarding the socioeconomic status, urban/rural divide were difficult to ascertain from the case records.



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The total number of deaths during the study period was 21, giving an iMMR of 85.268 per 100000 live births. The annual trend in iMMR has been pictorially represented in Figure 1. The age of the women ranged from 20-32 years (mean-25.9±3.6 years). The age distribution of the women is graphically represented in Figure 2.

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Hypertensive disorders	3	2	23.8
Haemorrhage	3	2	23.8
Sepsis	1	1	9.52
Anaemia	1	1	9.52
Viral infection	2	0	9.52
Peripartum Cardiomyopathy	0	2	9.52
Pulmonary embolism	0	1	4.76
Jaundice	1	0	4.76
Suicide	0	1	4.76

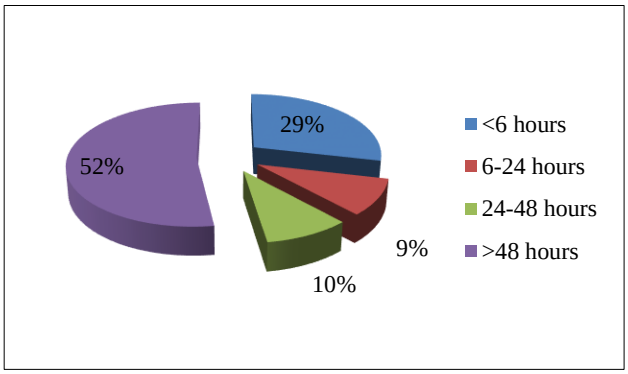
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ICU admission	8	7	71.42
Extended intubation>12 hours	4	5	42.87
Multiorgan dysfunction	4	5	42.87
Blood transfusion>5 units	6	3	42.87
Operative intervention	1	2	14.28

Majority of the women died postpartum, except for three. Two of the women who died antenatally were referred to our facility in a moribund state and died within two to three hours of admission despite intensive resuscitative measures. Ten of the women were multiparous and eleven of them were primiparous. Referrals from outside facilities were predominant in the first five years. Obstetric causes dominated the scenario in the first five years (6/11), whereas deaths during the next five years were mainly due to medical disorders (Table 1).

52.38% of the women died more than 48 hours after admission to the hospital, while 28.57% succumbed in less than six hours of admission (Figure 3).

Secondary complications noted were ICU admission, extended intubation, massive transfusion, operative intervention and multi organ dysfunction. Commonest among the above was ICU admission (71%) and the least common was operative intervention (14.28 %) (Table 2).



Maternal mortality is considered an index of the quality of healthcare services in a society. MMR of Tamil Nadu (a state in South India) at 66 per 100,000 live births is low as compared to most of the states in India.[4]

The lower rates are due to a higher proportion of institutional deliveries, effective healthcare delivery system and high quality emergency obstetric care provided by CEmONC (Comprehensive Emergency Obstetric and Newborn Care) centres.

In the present study, the highest mortality rates were in the age group 25-30 years, which is comparable to those of similar studies from South India.[5,6] This may be explained by the fact that the highest proportion of the pregnant population belongs to this age group.71.43% of the women were primiparous as against other studies where multiparous women formed the majority.[7] Both vaginal deliveries and cesarean sections were encountered equally in our series.

A fluctuating trend in iMMR was observed during the study period with peaks during the years 2009, 2012 and 2015. This was a chance observation since the women were referred from maternity units from the surrounding areas and there was a modest but steady increase in the number of deliveries over the years.

Major proportion of the deaths occurred in the postpartum period (80.95%).[6,8] One perimortem caesarean section was done on a patient with peripartum cardiomyopathy (PPCM) who had a cardiac arrest on table.

Two thirds of the women who died were referred from other hospitals.[5] Increased mortality was seen in patients who were referred from other hospitals, due to the bad general condition in which they were received and the time delay. The type of delay could not be assessed in the referral cases as the primary care was given elsewhere.

Obstetric causes of maternal mortality include those resulting from obstetric complications of the pregnant state (pregnancy, labour and puerperium). Non obstetric causes result from pre existing diseases or those that develop during pregnancy and which is not due to direct obstetric cause, but was aggravated by the physiological effects of pregnancy.[9]

Interestingly, 57% of the deaths were due to direct obstetric causes in contrast to those by Sowjanya kumari et al (85%), B. Paul et al (76.7) %, Murthy et al (72.5%) and similar to Khandale SN et al (61%).[6,10-12]

The leading causes of death were hypertensive disorders (23.8%) and haemorrhage (23.8%) comparable to the study by Paily et al. The major cause of death in women with hypertension in our series was hepatic dysfunction (60%).[13]

Severe preeclampsia is associated with high rates of maternal mortality and morbidity. The major contributors to maternal mortality in preeclampsia are eclampsia, HELLP, cerebral haemorrhage, renal dysfunction and coagulopathy. The two pronged approach to management of preeclampsia, i.e., control of hypertension with Labetalol and seizure prophylaxis with Magnesium sulphate has to be adopted to minimise complications.[14] Standard protocols have to be in place for early detection, referral, appropriate treatment and timely delivery.[15]

80% of the women who succumbed to obstetric haemorrhage were referred from other hospitals in a critical condition and died within few hours of admission despite intensive resuscitative measures including obstetric hysterectomy. Atonic postpartum haemorrhage (PPH) was the cause of death in four cases while one patient died due to rupture uterus. Rupture uterus followed a second trimester termination in a woman with two previous cesarean sections with an inappropriate dose of misoprostol.

Active management of third stage of labour (AMTSL) and oxytocin as the first line uterotonic has been advocated as the gold standard in preventing complications due to PPH.[16] In health care settings without facilities like access to blood and blood products and specialist care, early referral will play a pivotal role in preventing mortality. Women at high risk for PPH could be appropriately managed antenatally and delivered in a tertiary centre so that complications arising henceforth could be anticipated and prevented. Staff in the tertiary centres have to be trained in protocols for management of PPH with frequent mock drills.

Since our institution is an urban centre, the prevalence of cases of severe anaemia was less.[13] This could be attributed to early identification of women with anaemia and institution of appropriate treatment. Still anaemia was a contributory factor in five women in our series. Blood transfusion for moderate anaemia close to delivery was the trigger for deterioration of the maternal condition in three instances. Two of the three women were diagnosed with transfusion reaction which led to acute renal failure requiring dialysis.[6,8] The importance of early detection of anaemia, its correction and the need for raising the threshold for transfusion is reiterated by these cases.

Two women in our study had a diagnosis of PPCM (9.52%) as compared to Creanga et al (10-15%).[17] One of them was diagnosed antenatally at 29 weeks and the other followed a normal vaginal delivery.

Four women had hepatic dysfunction and jaundice, out of which three were due to preeclampsia and one was due to viral hepatitis. All patients with hepatic dysfunction were referred from other hospitals due to availability of good intensive care facilities and were directly referred to the gastroenterology unit. The incidence of pulmonary embolism, hepatitis and sepsis in our series were similar to that of Singh et al.[8]

One death in our study was due to suicide. According to the ICD-MM guidelines published by the WHO all antepartum and post partum suicide deaths should be included as direct obstetric deaths even if it is not possible to establish the diagnosis of depression or puerperal psychosis.[18] This is a major deviation in maternal mortality reporting and has the potential to increase the MMR since hitherto unreported deaths will be accounted for henceforth.[13,19]

The commonest complications encountered by these women were prolonged ICU stay and ventilator support. Multidisciplinary care was required in more than 50% of our cases, commonest being the involvement of nephrologist, gastroenterologist and cardiologist. 29% of the women died within six hours of admission, whereas 52% succumbed to complications after 48 hours, the longest being 33 days.[5,7,8,12]

The only operative intervention performed was obstetric hysterectomy which was required in three women-two following failed medical management of atonic PPH and one following rupture uterus. 50% of our patients required multiple transfusions since obstetric haemorrhage was a leading cause of mortality, and coagulopathy due to hepatic dysfunction was also seen in a few cases.

More than 50% of deaths in our study were due to obstetric causes, which are largely preventable. This could be achieved by intensive monitoring with incorporation of early obstetric warning tools.[20] Early warning tools include monitoring of maternal vital parameters such as blood pressure, heart rate, temperature, oxygen saturation, respiratory rate and altered sensorium; and notification to the physician in case of any abnormality.

As with all retrospective studies, our study also has limitations. Our institution being a private medical college in a city with many other well equipped hospitals, we reported a lesser number of maternal deaths. Fewer numbers preclude adequate statistical analysis and hence reduce the power of the study. Higher proportions of cases were referred from far-off areas and hence details regarding the initial management could not be accessed. In addition, autopsy was not performed routinely and therefore, the exact cause of death could not be verified in many of the cases.

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Though the iMMR has shown a fluctuating trend, it has remained at acceptable levels in comparison to the state and national averages. The classical triad of Hypertension, Haemorrhage and Sepsis remains the major determinant of maternal mortality in our institution and most of them are preventable. By promoting universal health coverage, access to quality health care, strengthening of health services and ensuring accountability, we can strive to reduce deaths due to pregnancy and childbirth.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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1. World Health Organisation. Millennium Development Goals, 2000. Available at: https://www.who.int/topics/millennium_development_goals/about/en/. Accessed 10 April 2019.
2. United Nations. Sustainable Development Goals, 2016. Available at: https://www.un.org/sustainabledevelopment/blog/2015/12/sustainable-development-goals-kick-off-with-start-of-new-year/. Accessed 10 April 2019.
3. WHO Regional Office for South East Asia. June 2018. Available at: http://www.searo.who.int/mediacentre/features/2018/india-groundbreaking-sucess-reducing-maternal-mortality-rate/en/. Accessed 20 March, 2019.
4. National Institution for Transforming India. Available at: https://niti.gov.in/content/maternal-mortality-ratio-mmr-100000-live-births. Accessed on 16 January, 2019.
5. Rajeswari, Shobha. Maternal mortality in an urban tertiary care hospital of South India. Indian J Obstet Gynaecol Res. 2016;3(1):32-7.
6. Kumari JS, Revathi V, Reddy KS. A study of maternal mortality in Government Maternity

Hospital, Sri Venkateswara Medical College, Tirupati. Indian J Obstet Gynaecol Res. 2018;5(4):525:9.
7. Pipal VR, Chouhan B, Pipal DK, Chouhan M, Yaduvanshi N, Mahavar N. Study of maternal mortality at Umaid Hospital, Jodhpur: a review of five years. Indian J Obstet Gynaecol. 2018;6:6.
8. Singh S, Singh N, Nagar JK, Jain S. Maternal mortality at a tertiary health care: a retrospective study. Int J Reprod Contracept Obstet Gynecol. 2018;7(5):1773-7.
9. Park K, Park's textbook of Preventive and Social Medicine, 24th edition. Page 593. India. Banarsidas Bhanot Publications. 2017.
10. Paul B, Mohapatra B, Kar K. Maternal deaths in a tertiary health care centre of Odisha: an in-depth study supplemented by verbal autopsy. Indian J Community Med. 2011;36(3):213-6.
11. Murthy BK, Murthy MB. Maternal mortality in tertiary care hospital: A 10 years review. Int J Prevent Med. 2013;4(1):105-9.
12. Khandale SN, Kedar K. Analysis of maternal mortality: a retrospective study at tertiary care centre. Int J Reprod Contracept Obstet Gynecol. 2017;6:1610-3.
13. Paily VP, Ambujam K, Rajasekharan Nair V, Thomas B. Confidential review of maternal deaths in Kerala: a country case study. BJOG. 2014;121:(Suppl. 4):61-6.
14. Lo JO, Mission JF, Caughey AB. Hypertensive disease of pregnancy and maternal mortality. Curr Opin Obstet Gynecol. 2013;25:124-32.
15. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. Semin Perinatol. 2012;36:56-9.
16. WHO. Available at: www.who.int/reproductive health/publications/maternal_perinatal_health/9789241548502/en/WHO recommendations for the prevention and treatment of postpartum haemorrhage-2018 update. Accessed 16 January 2019.
17. Creanga AA, Berg CJ, Jean Y. Maternal mortality and morbidity in the United States: Where are we now? J Women's Health. 2014;23:3-9.
18. WHO. The WHO Application of ICD-10 to deaths during pregnancy, childbirth and the puerperium: ICD-MM. Geneva: World Health Organization, 2012. Accessed on 20 March, 2019.
19. Fuhr DC, Calvert C, Ronsmans C. Contribution of suicide and injuries to pregnancy-related mortality in low-income and middle-income countries:a systematic review and meta-analysis. Lancet Psychiatry. 2014;1:213-25.
20. Shields LE, Wiesner S, Klein C. Use of maternal early warning trigger tool reduces maternal morbidity. Am J Obstet Gynecol. 2016;214:527.e1-6.

Thangavelu R, Natarajan L. Towards safe motherhood to - are we moving in the right direction?: experience from an urban health facility in South India. Int J Reprod Contracept Obstet Gynecol 2019;8:2851-5.

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Research Article

Effect of Antenatal Yoga on Labour Outcome- A Non-Randomized Control Study from a Tertiary Hospital in South India

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INTRODUCTION: Giving birth to a healthy baby is one of the happiest moments in a woman's life. Yoga is a 'body-mind' practice. The prospective of yoga to create balance along emotional, mental, physical, and spiritual dimensions is immense. It uses physical positions (asana), meditation (dharana and dhyana), concentration, breathing exercises (pranayama) and contemplative practice. OBJECTIVE: To study the effect of yoga on antenatal outcome. MATERIALS AND METHODS: It is a Prospective study done during the time period July 2013 to July 2014.In a series of 50 women who fit in the inclusion criteria, attending the antenatal clinic of a private medical college in Tamilnadu, training to practice yoga as relaxation therapy during pregnancy and labour was given and it was compared with 50 antenatal women who obtained regular care. The various outcomes like Mode of delivery, Bishop's score, Duration of active labour; Administration of analgesics and Weight of infant were studied. RESULTS: The major advantage noted in this study was reduced duration of active phase of labour. 70% of the antenatal women doing yoga went into spontaneous labour. 34% of induced labour in the control group were noted. All the patients in the yoga arm delivered at term. Whereas 4% of the patients in control arm had preterm delivery. CONCLUSION: Practicing yoga in the antenatal period decreases induced labour and shortens the duration of active phase of labour. The mode of delivery has a multifactorial contribution and cannot be compared in this study.

Keywords: Yoga, Pregnancy, Labour, Active Phase

INTRODUCTION

Motherhood transforms a woman forever. It extracts out a life lying dormant for months deep within her. Remaining physically active during pregnancy is the best a woman can do. At least thirty minutes of moderate exercise each day is recommended for improving the health and wellbeing of non pregnant women according to Len Kravitz (1998). In accordance with the American College Of Obstetrics and Gynaecology (2002), exercise during the period of pregnancy is very good and appropriate in pregnant women without any obstetric and medical complications. Yoga may be able to provide a gentle alternative to physical activity.Yoga is a 'body - mind' practice .It is a combination of various physical positions, breathing and relaxation exercises according to Smith C.A (2011). The word 'Yoga' comes from the Sanskrit word which means "to join" and can be understood as unity or oneness It uses physical positions (asana), meditation (dharanaanddhyana), concentration, breathing exercises (pranayama) and contemplative practice. Yoga mends psychological wellbeing. Labour is the active process of delivering a foetus and is characterised by regular, painful uterine contractions which increase in frequency and intensity. It is difficult to predict the length of labour. Duration of labour was positively corelated with the weight of the infant, duration of pregnancy, weight gain and prepregnant weight. It was negatively corelated with mother's height. Mother's age did not influence duration of labor according to Britt-Ingjerd Nesheim (1988). According to Kelly Riley (2017) Malpositions of the head prolonged labor in nulliparas, but not in multiparas, while breech presentation had no influence. 35 trials lecturing the outcomes of yoga on anxiety and stress were reviewed by LiAW et al in (2012) and the results were indicative of the role of yoga in alleviating stress and concern· Randomized controlled study on 74 women by Pamela Jones (2008) who

reported that in yoga group there was improved comfort during labour and after labour. The interval of first stage of labour was also shorter as well as the total labour duration according to a study done in Prince of Songkla university in Thailand by Chuntharapat S(2008) .So ours is an attempt to evaluate the effect of yoga on antenatal outcome in our study population.

METHODOLOGY

Study Design: Prospective non randomized control study
Time period: July 2013 to July 2014
Ethical approval: OBTAINED FROM ETHICS COMMITTEE OF PSGIMSR AND INFORMED CONSENT TAKEN.

A series of 50 women attending the antenatal clinic of a private medical college in Tamilnadu were given training to practice yoga as relaxation therapy during pregnancy and the labour outcome was compared with 50 antenatal women who obtained regular care.

Pregnant women of gestational age> 12 weeks are given training in 6 separate sessions by specially trained people in yoga through training sessions. They are encouraged to practice this on a regular basis. The regularity is assessed through personal interview. They are advised to do yoga minimum of 5 times a week.

Inclusion criteria
- Singleton pregnancy
- Primi less than 35 years
- Spontaneous conception
- No antenatal complications
- Co-operative and compliant patients

Exclusion criteria
- Multiple pregnancy
- Threatened abortion or previous preterm delivery
- Elderly Primi
- Multigravida
- Patients not willing to participate in the study

Types of outcome measured
- Mode of delivery
- Bishop's score
- Duration of active labour
- Administration of analgesics
- Weight of infant
- Type of delivery was assessed as normal vaginal delivery, assisted vaginal delivery (instrumental) or caesarean section.

➢ BISHOP'S SCORE (TABLE-1)

Table 1: BISHOP'S SCORE

Sign	Points				Score
	0	1	2	3	
Position	Posterior	Intermed.	Anterior		
Consistency	Firm	Intermed.	Soft		
Effacement	0-30%	31-50%	51-80%	>80%	
Dilation	0 cm	1-2 cm	3-4 cm	>5 cm	
Fetal station	-3	-2	-1, 0	+1, +2	
Results	The Bishop Score is: Calculate Bishop Score Reset				

The Bishop Score scores patients who would be most likely to accomplish a successful induction. It is a pre induction record. A score that go beyond 8 defines the patient most likely to accomplish a fruitful vaginal birth. A low Bishop's score frequently designates that induction is unlikely to be fruitful. Kelly C (2019) specify that only a score of 8 or more is reliably prognostic of a positive induction

➢ Active phase duration

Cervical dilatation of 4cm or more with uterine contractions can be taken to dependably signify the onset of active labour .According to Friedman the mean duration of active phase of labour in nullipara was 4 .9 hours .As the standard deviation of 3 .4 hours is large ,the active phase was testified to have a numerical maximum of 11 .7 hours .In all patients in labour we plot a partogram and the active phase duration was calculated.

➢ Analgesia

The various analgesia options in labour are epidural, sedation and inhalational analgesia with entonox. The use of epidural analgesia was examined in our study.

THE VARIOUS POSTURES OF YOGA PRACTICED IN PREGNANCY ARE AS FOLLOWS: TABLE 2

Table 2: THE VARIOUS POSTURES OF YOGA PRACTICED IN PREGNANCY ARE AS FOLLOWS

Day1(2ND TM)	Day2 (2ND TM)	Day 3 (2NDTM)	DAY 4 (3RD TM)	Day 5 ,6(3RD TM)
1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Suryanadipranayama	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Chopping wood pose 7) Pawanamukthasana part 1 8) Suryanadipranayama 9) Chandranadi	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Hands in and out breathing 7) Hands up and down breathing 8) Tadasana 9) Butterfly 10) Kadichakrasana 11) Pawanamukthasana 12) Warrior pose 13) Vajrasana 14) Suryanadipranayama 15) Chandranadi 16) Suryabhedana 17) Veerabadrasana 18) Cat stretch	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Vajrasana 7) Pawanamukthasana 8) Warrior pose 9) Chopping wood pose 10) Namaskarasana 11) Seated side stretch 12) Gentle twists 13) Suryanadipranayama 14) Chandranadi 15) Suryabhedana	1) Hands in and out breathing 2) Hands up and down breathing 3) Tadasana 4) Butterfly 5) Kadichakrasana 6) Pawanamukthasana 7) Warrior pose 8) Chopping wood pose 9) Sleeping abdominal stretch 10) Namaskaarasana 11) Seatedsidestretch 12) Gentle twist 13) Suryanadi 14) Chandranadi 15) Suryabhedana 16) Chandrabhedana 17) Mukthadhouti

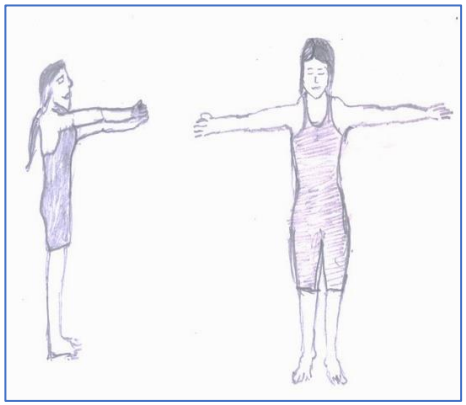


Figure 1: Hands in and out breathing



Figure2: Tadasana

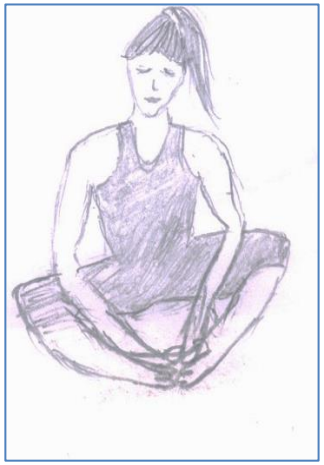


Figure 3: Butterfly posture



Figure 4: Suryanadi Pranayama

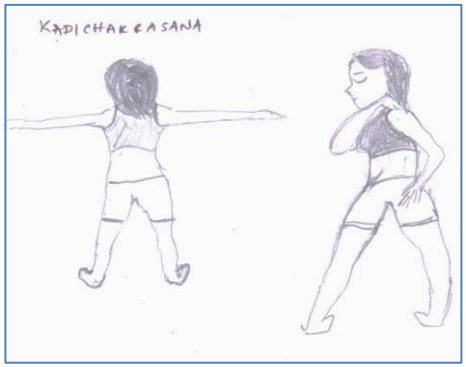


Figure 5: Kadichakrasana

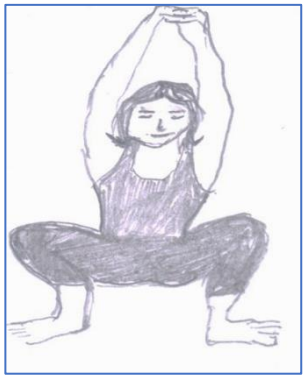


Figure 6: Chopping Wood Pose



Figure 7: Vajrasana



Figure 8: Warrior Pose

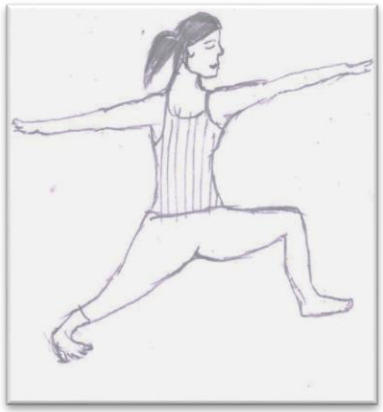


Figure 9: Veerabhadrasana



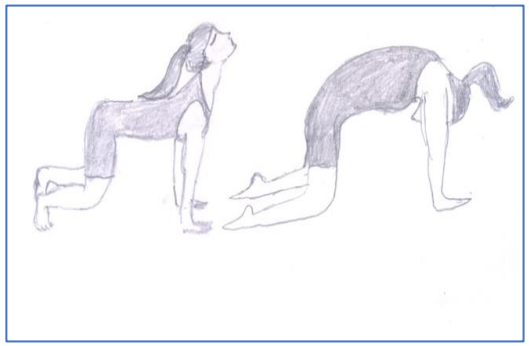


Figure 10: Cat Stretch



Figure 11: Seated Side Stretch



Figure 12: MukhaDhauti

Inhale. exhale and press the thighs and knees downward towards the floor. Make a gentle effort to keep pressing them forward. Start flapping both the legs up and down.

SURYA NADI PRANAYAMA - FIGURE 4

This refers to the Breathing Exercise. Inhale through the right nostril, Exhale through the left, press the index and middle finger of the right hand against the palm of that hand. Use thumb to occlude the right nostril. Ring and little fingers to occlude the left nostril.

KADICHAKRASANA - FIGURE 5:

Exhale and twist from the waist to the right and look back over the right shoulder. Keep the distance between your palms constant. Inhale comeback to the center, Exhale and turn to left and repeat the yoga posture on your left side.

CHOPPING WOOD POSE – FIGURE 6

Sit in a squatting position with your back straight .Clasp the hands in front and with inhalation raise the hands gently up in the air and come down with an exhale .

PAWANAMUKTHASANA PART 1

Sit outstretched, keep both the knees together and the heel on floor spine and the neck in the same level. put the palms of the hands on the floor. Keep the fingers outstretched. Apply pressure on the palm. Close eyes and breath. Begin the exercise with open eyes

VAJRASANA - FIGURE 7

The name comes from the Sanskrit words *vajra* - "thunderbolt". Sit on heels with the calves beneath thighs. There should be four finger gap between the knee-caps., sit straight.

WARRIOR POSE - FIGURE 8

VEERABHADRASANA – FIGURE 9

Breathe in as you come up and exhale as you go down with your hands from side. Redo the same from left side

CAT STRETCH FIGURE:10

STEP 1: Position your palm directly below your shoulders. Place knees directly below your hips . Spread your fingers with the middle fingers pointed straight.

STEP 2: Press towards the floor using your hands and then lift yourself up out of your shoulders. Go back and forth several times. Exhale and sag shoulders.

SEATED SIDE STRETCH - FIGURE 11

Rotate your head to look beyond your left elbow. Retain this position for 5 seconds. Now inhale and lift trunk and move left upper limb upward. Breathe out by bringing down left upper limb.

MUKHA DHAUTI- FIGURE 12

Lean forward and rest the arms on the respective thighs keeping the arms straight. Inhale through the nose and exhale forcefully through the mouth. Stand in Tadasana and relax.

Ethical approval: OBTAINED FROM ETHICS COMMITTEE OF PSGIMSR WITH IHEC APPROVAL NUMBER-13/121
SPSS SOFTWARE USED FOR DATE MANAGEMENT AND CALCULATION

RESULTS

In the study of antenatal yoga on labour outcome 100 antenatal women who were not age matched were included. 50 women were doing yoga and 50 received regular antenatal care.

TABLE 3: The age distribution is as follows

Age Distribution			
Age	Group		Total
	Yoga	Control	
<20	0	4	4
21-25	25	24	49
26-30	21	20	41
31-35	4	2	6
Total	50	50	100

The result shows that there is no significant age difference among the antenatal in group who were doing yoga and in routine care category

NO OF YOGA CLASSES ATTENDED BY THE ANTENATAL WOMEN IN YOGA GROUP.

46% of the antenatal women in yoga group had attended 4 classes. Only 10% of the women attended 5 yoga classes. 44% of the antenatal women who were practicing yoga attended only 3 yoga classes.

Table 4: Distribution of preterm or term deliveries

Term/ Preterm			
Out come	Group		Total
	Yoga	Control	
Term	50	48	98
Pre Term	0	2	2
Total	50	50	100

All the patients in the yoga arm delivered at term. Whereas 96% in the control arm delivered at term and 4% of the patients had preterm delivery, but this was not statistically significant as the p value was >0.5.

Table 5: The bishop's score among the antenatal women in yoga group and control group

Bishop Score	Group		Total
	Yoga	Control	
< 5	28	28	56
> 5	19	20	39
Total	47	48	95

As per the result there is no significant difference in Bishop's scoring among the two groups.

Table 6: The onset of labour among the yoga group and the control

Labour	Group		Total
	Yoga	Control	
Spontaneous	33	33	66
Induced	14	17	31
Total	47	50	97

The onset of labour is comparable in both the groups. 70% of the antenatal women doing yoga went into spontaneous labour. 34% of induced labour in the control group were noted. In the yoga group only 30% were induced labour.

Table 7: Distribution of mode of delivery

Mode of Delivery			
Delivery	Group		Total
	Yoga	Control	
NVD	20	36	56
LSCS	17	10	27
Instrumental	13	4	17
Total	50	50	100

The percentage of patients went in for lscs was 34 % in the yoga group where as it was 20% in the control group. Only 66% of women doing yoga had a vaginal delivery out of them 26% was instrumental delivery. Whereas in the control group 80% had a vaginal delivery. This finding was also not statistically significant,

Table 8: Distribution of Instrumental Delivery

Delivery Instrumental			
	Group	Total	
Instrumental	Yoga	Control	
VACUUM	12	3	15
FORCEPS	1	1	2
Total	13	4	17

Vacuum assisted delivery was significantly high among the yoga patients.

Table 9: Indications for lscs

LSCS	Group		Total
	Yoga	Control	
CPD	1	2	3
NPL	1	1	2
ARREST	2	2	4
FETAL DISTRESS	3	5	8
MSL	4	0	4
CONDYLOMA	1	0	1
DTA	1	0	1
FAILED	1	0	1
MATERNAL WISH	1	0	1
TRANSVERSE LIE	1	0	1
MOBILE HEAD	1	0	1
Total	17	10	27

The common Indication for LSCS in yoga patients were meconium stained liquor in early labour and fetal distress.

Table 10: Duration of active phase of labour

Duration	Group		Total
	Yoga	Control	
<= 2 HRS	3	0	3
2 - 3 HRS	16	7	23
3 - 4 HRS	10	16	26
> 4 HRS	4	17	21
Total	33	40	73

There is significant difference in the duration of active phase among the women practicing yoga and the control group. Majority had an active phase duration of< 2-3 hours in the yoga group when compared to the controls who had an average duration more than 4 hours which was statistically significant.

THE USE OF ANALGESICS

There is no significant difference among both the groups in usage of analgesics .

Table 11: Effect on birthweight

Birth Weight [kgs]	Group		Total
	Yoga	Control	
< 2 .5	2	5	7
2 .5 - 3 .5	43	42	85
> 3 .5	5	3	8
Total	50	50	100

The mean birth weight of the babies born to women practicing yoga was 3.10kg .

MEAN OF CLINICAL PARAMETERS – TABLE 12

Mean duration of active labour , in the yoga group came about 190 minutes ,whereas the mean duration of active phase of labour for the control group was 244 minutes which was statistically significant.

Table 12: Mean of clinical parameters								
		Mean	SD	95% CI for Mean		Minimum	Maximum	p value
	Group			Lower Bound	Upper Bound			
Age	Yoga	26	3	25	27	21	35	
	Control	25	3	24	26	19	32	>0 .05
	Total	26	3	25	26	19	35	
Bishopscore	Yoga	4 .45	1 .63	3 .97	4 .92	1	9	
	Control	4 .46	1 .68	3 .97	4 .94	1	9	>0 .05
	Total	4 .45	1 .64	4 .12	4 .79	1	9	
Birthweight [kgs]	Yoga	3 .10	0 .3	3 .0	3 .2	2	4	
	Control	2 .99	0 .4	2 .9	3 .1	2	4	>0 .05
	Total	3 .04	0 .3	3 .0	3 .1	2	4	
Duration [Hrs]	Yoga	190	60	168	211	40	330	
	Control	244	64	223	264	120	360	<0 .01
	Total	219	67	203	235	40	360	

Table 13: Data summary

Group		YOGA	CONTROL	p value		p value
Clinical characteristics						
AGE						
<20		0%	8%			
21-25		50%	48%	>0 .05		0 .194
26-30		42%	40%			
31-35		8%	4%			
Outcome						
Term		100%	96%			
Pre Term		0%	4%	>0 .05		0 .495
Bishop score						
< 5		60%	58%			
> 5		40%	42%	>0 .05		0 .902
Labour						
Spontaneous		70%	66%			**0 .67**
Induced		30%	34%	>0 .05		
Mode of Delivery						
NVD		40%	72%			
LSCS		34%	20%	>0 .05		0 .004
Instrumental		26%	8%			
Delivery Instrumental						
VACUUM		92%	75%			0 .426
FORCEPS		8%	25%	>0 .05		
Duration of active Labour						
<= 2 HRS		9%	0%			
2 - 3 HRS		48%	18%			0 .001
3 - 4 HRS		30%	40%	<0 .01		
> 4 HRS		12%	43%			
Analgesics						
EPIDURAL		8%	10%			
IM		92%	90%	>0 .05		0 .822
Birth Weight [kgs]						
< 2 .5		4%	10%			
2 .5 - 3 .5		86%	84%	>0 .05		0 .407
> 3 .5		10%	6%			
Mean Baseline characteristics						
Age		26	25	>0 .05		0 .015
Bishop score		4 .45	4 .46	>0 .05		0 .973
Birth weight [kgs]		3 .10	2 .99	>0 .05	0 .048	**0 .081**
Duration [min]		190	244	<0 .01		0 .001

DISCUSSION

This study which was conducted in a Tertiary hospital in Tamilnadu was done with the objective to analyse the effect of yoga on antenatal outcome. We enrolled 100 antenatal patients-of which fifty underwent yoga classes and fifty had routine antenatal care and the outcomes were analysed and the results were given above.

The age group of the antenatal patients who did yoga and the control group was similar in our study. All the patients practicing yoga had a term delivery which is similar to the study by Narendran et al (2005) where preterm deliveries were significantly low. In our study there was no significant difference in the Bishop's score at admission in the yoga and control group. There was no data to compare this.

There was also no difference in the onset of labour for both the groups.28% had induced labour in the yoga group and 34% in the control group in our study. This is similar to the study by Jahdi et al (2017) where there was a decreased percentage of labour induction in the yoga group.

In our study 34% of the yoga group patients had caesarean delivery while only 20% of the control group had caesarean delivery. This is in contrast to the study by Jahdi et al (2017) and Satyapriya Maharana et al in 2013 who showed reduced incidence of caesarean delivery after yoga therapy i.e.15% versus 40% in the control group.Since the mode of delivery has got multifactorial contribution ,it is difficult to compare it in these two groups. Even though the mode of delivery is not significantly altered, larger sample size is required to conclude this fact.

The major advantage noted in our study was the reduced duration of active phase of labour.This is very much significant as the duration of labour is an important factor in patient comfort,experience with childbirth and satisfaction.The reduction in active phase duration is consistent with the studies conducted by Songporn chuntharapat (2008), Sathyapriya M et al (2013) where there was significant reduction in labour duration.

The mean birth weight of the babies born to women practicing yoga was 3.10kg and 2.99kg in the control group which was not statistically significant. This is similar to the study by Maria Pais in 2016 where the mean weight of the yoga group was 3kg and the control group was 2. 7kg.This may be due to the increase gestational age of delivery in the yoga group. This was also consistent with the study by Shamanthakamani Narendran et al. in (2005) where there was significant higher babies with birth weight more than 2.5kg in the yoga group.

CONCLUSION

As per my study antenatal women doing yoga had significant reduction in the duration of active phase of labour than who receive regular care. Although not statistically significant, all of the patients in yoga group had term deliveries and good mean weight of babies. May be a larger study group in the future can give more definitive results.

FINANCIAL DISCLOSURE OR FUNDING – None

CONFLICT OF INTEREST - Nil

INFORMED CONSENT - Taken

AUTHOR CONTRIBUTIONS - First author contributed to the design and implementation of the research, first, second and third author contributed to the analysis of the results third author is the corresponding author and helped in the writing of the manuscript.

REFERENCES

ACOG Committee opinion. Number 267, January 2002: exercise during pregnancy and the postpartum period.Obstet Gynecol. 2002 Jan; 99(1):171-3.

Britt-IngjerdNesheim.Duration of Labor: An analysis of influencing factorsActaobstetricia et gynecologicascandinavica 67:121-124https://obgyn.onlinelibrary.wiley.com/journal/16000412

Chuntharapat S, Petpichetchian W, Hatthakit U. Yoga during pregnancy: effects on maternal comfort, labor pain and birth outcomes. Complement 2008 May;14(2):105-15. doi: 10.1016/j.ctcp.2007.12.007. Epub 2008 Mar 4.

Jahdi F, Sheikhan F, Haghani H, Sharifi B, Ghaseminejad A, Khodarahmian M, Rouhana N. Yoga during pregnancy: The effects on labor pain and delivery outcomes (A randomized controlled trial).Complement Ther Clin Pract. 2017 May; 27:1-4. Doi: 10.1016/j.ctcp.2016.12.002. Epub 2016 Dec 23

Kelly C. Wormer; Ann E. Williford. Bishop Score. https://www.ncbi.nlm.nih.gov/books/NBK470368/

Kelly Riley, Emily Drake .The Effects of Prenatal Yoga on Birth Outcomes: A Systematic Review of the Literature .https://birthpsychology.com/journal/issue/volume-28-issue-1

Len Kravitz, Ph.D. From 1998 to 2011: ACSM Publishes Updated Exercise Guidelines https://www.unm.edu/~lkravitz/Article%20folder/ACSMGuidelinesUNM.pdf

Li AW, Goldsmith CA.The effects of yoga on anxiety and stress. Altern Med Rev. 2012 Mar; 17(1):21-35

Maria Pais,PaiMuralidhar. Effect of yoga therapy on antenatal stress and pregnancy outcome. http://hdl.handle.net/10603/169974shodhganga: a reservoir of Indian theses @ inflibnet.

Narendran S[1], Nagarathna R, Narendran V, Gunasheela S, Nagendra HR Efficacy of yoga on pregnancy outcome.J Altern Complement Med. 2005 Apr; 11(2):237-44.

Pamela Jones, MA Yoga in Pregnancy May Improve Labor Discomfort https://www.cancercarewny.com/content.aspx?chunkiid=345901

Satyapriya M, Nagendra HR, *et al.* Effect of integrated yoga on stress and heart rate variability in pregnant women. Int J Gynaecol Obstet. 2009; 104(3):218-22.

Shamanthakamani Narendran, Raghuram Nagarathna, Vivek Narendran, Sulochana Gunasheela, Hongasandra Rama Rao Nagendra .Efficacy of Yoga on Pregnancy Outcome. Journal of Alternative and Complementary Medicine Apr 2005, Vol. 11, No. 2: 237-244

Smith CA[1], Levett KM, Collins CT, Crowther CA. Relaxation techniques for pain management in labour.Cochrane Database Syst Rev. 2011 Dec 7 ;(12):CD009514. doi: 10.1002/14651858.CD00951

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Adnexal torsion in reproductive age group: a retrospective study

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Abstract

Background: Adnexal torsion is one of the gynecologic emergency conditions requiring prompt diagnosis and surgical treatment to save the ovarian function. This retrospective study is intended to analyze the clinical manifestations and treatment in a tertiary care center.

Methods: This is a retrospective study Case records during five-year period of February 2012-2017 in PSG Institute of Medical Sciences and research, Coimbatore, India.

Results: Adnexal torsion was seen in all age groups and commonly in reproductive age. It was seen in few pregnant patients also. Lower abdominal pain of acute onset associated with nausea and vomiting was the commonest clinical feature. Ovarian cyst and PCOD were the common risk factors predisposing torsion. Though ultra sound was the common modality used, the diagnosis was done based on strong clinical suspicion with surgical confirmation. The ovaries were preserved in some patients of reproductive age group.

Conclusions: Adnexal torsion is one of the gynecologic emergencies. The symptoms and signs are nonspecific and may not have a confirmatory lab or imaging study. Hence a strong clinical suspicion followed by prompt surgical management in both pregnant and non -pregnant patients of any age group saves the ovary and further complications.

Keywords: Abdominal pain, Ovary, Torsion, Ultrasound

Introduction

Adnexal torsion is one of the gynecological emergencies affecting females of all ages. Ovarian torsion is defined as rotation of ovary or adnexa with the vascular pedicle on its axis resulting in arterial, venous,or lymphatic obstruction.[1]

It often presents as intermittent acute abdominal pain exacerbated by positional shifts.[2] Approximately 70% have symptoms of nausea and vomiting mimicking other surgical causes of pain like acute appendicitis or renal colic. So, the clinical scenario can lead to misdiagnosis.[3]

In addition, the conventional ultrasound may not be conclusive and the diagnosis can become difficult. Delay in diagnosis and treatment leads to necrosis of the affected ovary.

The aim of the study was to analyze the clinical manifestations and management done during the past five years.

Methods

A Retrospective analysis of 47 medical records with surgically confirmed cases of adnexal torsion was done. The clinical parameters taken for analyses taken were age, medical history, clinical manifestations and examination findings. In clinical presentation the type of abdominal pain with associated clinical findings were noted.

Additionally, whether the condition was associated with fever and leukocytosis was also noted. The imaging performed with its findings, operative details and histopathological reports of the specimens were also analyzed.

[illegible]

Clinical findings

The total number of patients with ovarian torsion were 47 in number. The median age of our patients was 22 years (18days-66years). There were 5 (10.6%) patients in premenarchal age group, 41 patients (87.2%) in reproductive age group and 1 (2.1%) in postmenopausal group. Acute onset of pain was the common symptom present in all patients (100%). The abdominal pain was diffuse and nonspecific in 21patients (44.6%), localized to right side in 19 patients (40.4%), and on the left side in 7 patients (14.8%). The next common symptom was nausea and vomiting seen in 37 (78.7%) patients followed by fever in 5 (10.6%) patients. A mass was palpable in 14 (29.7%) and tenderness elicited in 17 (36.17%) of the cases.

There were 3 (6.3 %) patients in early pregnancy with torsion.

Lab investigations revealed leukocytosis in 18 (38.2%) and anemia in 4 (8.5%) cases.

In all cases ultrasound along with Doppler was performed and torsion was reported in 25 patients (53.19%). 11 cases (23.4%) had minimal free fluid in pouch of Douglas. Ovarian cyst was reported in 31 cases (65.9%) in ultrasound. Of that 16 patients (51.6%) had cyst size of 5- 10cms, 11 patients (35.4%) had cyst size of more than 10cm and 4 patients (12.1%) had cyst size of less than 5cms. The contralateral ovary showed features of PCOD in 5 (10.6%) patients.

Operative findings

Of 47 patients with adnexal torsion, right adnexa was involved in 27 (57.4%) cases followed by 20 (42.5%) cases on left side. The risk factors like tubal ligation was noted in 3 (6.3%) cases, previous surgery adhesions in 1 (2.1%) patient.

Isolated fallopian torsion was seen in 3 (6.3%) in patients.

Detorsion with oophoropexy was done in 8 (17%) patients, ovarian cystectomy was done in 20 (42.5%) patients, unilateral salpingectomy in 3 (6.3%) patients, unilateral salphingo oophorectomy in 11 (23.4%) cases and total hysterectomy with bilateral salphingo oophorectomy in 5 (10.6%) patients. The patients with conserved ovaries were found to have normal ovarian function and asymptomatic on follow-up.

[illegible]

[illegible]	[illegible]	[illegible]
Serous cyst adenoma	6	12.77
Serous cyst adenofibroma	1	2.13
Serous cyst adeno carcinoma	1	2.13
Mucinous cyst adenoma	2	4.26
Teratoma	2	4.26
Follicular cyst	1	2.13
Corpus luteal cyst	4	8.51
Paraovarian cyst	5	10.63
Endometriotic cyst	1	2.13
Total specimens with haemorrhagic necrosis	17	36.17

Histopathological examination was done in all excised specimens and results are shown in Table 1. The common finding seen in specimens was hemorrhagic necrosis (36.1%) and the common benign tumor noted was serous cyst adenoma in 6 patients (12.7%).

[illegible]

Adnexal torsion is the reported to be the fifth most common gynecological emergency condition encountered with a prevalence of 2.7%.[4] Though it is fifth most common emergency it poses difficulty to diagnose clinically due to nonspecific presentation. The lower quadrant abdominal pain described in adnexal torsion is of sudden onset usually following exercise or jerky movement. It is described as sharp, stabbing or less frequently crampy.[5] The ultrasound findings of ovarian torsion may change depending on the duration and degree of torsion (Complete or incomplete) and the presence or absence of ovarian mass.[6,7] The other adnexal pathologies like endometriosis, hemorrhagic cysts, ectopic pregnancy, pelvic inflammatory disease form complex adnexal mass and make it difficult to differentiate sonographically.[4,8,9]

All age groups are susceptible to develop adnexal torsion though it is commonly seen in reproductive women.[3,10-12] Majority of our cases in this study also were in younger reproductive age group (Table 2). It commonly occurs in normal ovaries in young children.[13] Torsion was seen in 5 (10.6%) of our cases in premenarchal age group. Our youngest patient was 18 days neonate, prenatally diagnosed to have an ovarian cyst. Oltmann SC et al reports occurrence of 16% in infant population.[14] It was less common in post-menopausal women in this study.

The most common symptom seen in all our cases (100%) was acute onset of abdominal pain. Other studies also reported the increased incidence of acute abdominal pain.[12,15] In respect to site of pain 21 (44.6%) cases had diffuse pelvic pain, 19 (40.4%) cases had pain on right flank and 7 (14.8%) cases on the left flank. The site of pain may not always denote the site of torsion because twisted adnexal masses are often midline, positioned cranial to the uterine fundus of abdominal pain.[1] The next

common symptom in this study was nausea and vomiting seen in 37 (78.7%) cases similar to other studies.[11,14] In this study palpable mass was seen in 14 (29.7%) cases and tenderness was demonstrated only in 17 (36.1%) cases and was similar to another study.[12] Other symptoms like vaginal bleeding and urinary symptoms reported in other studies were not noted in this study.[11,16]

Table 2: Comparison of clinical presentation and management in various studies

Parameters	Present study	Comparison study	Comparison study
Number of patients	47	70	30
Age	18 days-66y	11y-91y	2 months-18 y
Premenarchal age	10.60%	2.90%	
Reproductive age <45yrs	87.20%	82.60%	
Parity	31.90%	50.00%	
Pregnancy	6.30%	2.90%	
Menopause	2.10%	17.00%	
Pain abdomen	100.00%	95.70%	100.00%
Nausea/Vomiting	78.70%	65.70%	56.70%
Fever	10.60%	12.90%	20.00%
leukocytosis	38.20%	44.00%	63.30%
Palpable mass	29.70%	42.90%	
Tenderness	17.00%	25.70%	
USG diagnosed torsion	53.19%	25.70%	63.00%
USG Free fluid POD	23.40%	23.80%	26.70%
Right side	57.40%	55.70%	70.00%
Left side	42.50%	42.90%	30.00%
Size 5-10cm	51.60%	71.40%	
Size > 10cm	35.40%	11.40%	
Serouscystadenoma	12.70%	15.70%	
Mucinouscystadenoma	4.20%	7.10%	3.30%
Teratoma	4.20%	22.80%	16.70%
Hemorrhagic necrosis	36.10%	30.40%	
Conservative surgery	59.50%	54.30%	46.70%
Radical surgery	40.30%	45.70%	53.30%

Adnexal torsion can occur rarely in pregnancy. The incidence reported is 1 in 5000 and occurs more frequently in the first trimester in patients that underwent treatment for infertility with in vitro fertilization or ovarian stimulation.[17] Present study had 2 cases of adnexal torsion in first trimester and one case in second trimester of pregnancy. Similar case series also reports torsion common in first trimester and less common in second and third trimesters.[18] The diagnosis of torsion in pregnancy becomes more challenging and also there is tendency to go for conservative approach. Adnexal torsion requires surgical intervention in pregnancy with preservation of corpus luteum. Organ preservation is important for the continuity of the pregnancy and a study by Oelsener et al reports performing detorsion in all 40 cases of torsion regardless of the macroscopic appearance.[19]

Fever and leukocytosis was seen in 5 (10.6%) and 18 (38.2%) cases respectively. Nurith et al reports incidence of fever in 20% and leukocytosis in 43 % of cases.[15] Fever and leukocytosis occurs due to adnexal mass necrosis. Four patients had anemia, and of that one had 6gm%, 2 cases had 7gm% and one had 8gm% which is similar to another study.[11] Anemia is usually rare in cases with ovarian torsion and may be seen in few patients due to occurrence of hemorrhagic necrosis.

Ultrasound as a modality to diagnose torsion is found to have a senstivity of 63%-95% due to difficulty in interpretation.[1,14,20] Normal adnexal appearance is common in 69% of premenarchal patients.[21] Some studies[9,20] show enlarged ovary with multiple peripheral follicles as sign of torsion with a sensitivity of 64-74%. So, to improve the detection rate further studies have focused on blood flow using doppler.[22] Since there is possibility of arterial flow in cases of partial type or early adnexal torsion it led to further studies to improve detection rate to 88-95% by focusing on twisted pedicle described commonly as whirlpool sign.[1,23] In present study torsion was diagnosed using ultrasound with doppler in 53.19% of our cases. The ultrasound finding showing presence of ovarian cyst in 31 (65.9%) cases suggests that it is the common risk factor in most of our

cases, especially if the size was 5-10cm in 16 (51.6%) followed by size >10cms in 11 cases (35.4%) and size of <5cm in 4 (12.9%) cases. Ovarian cyst as common risk factor was reported by Oltmann SC et al.[24] It was noticed polycystic ovary in 5(10.6%) cases as the next common risk factor. In young women polycystic ovary is reported to be a common risk factor for adnexal torsion.[11]

Regarding the side of torsion our study showed right side was involved in 27(57.4%) cases and on left side in 20 (42.5%) cases. Other studies also similar findings. This could be because of long right ovarian ligament or presence of sigmoid on the left side.[12,14] Isolated tube torsion was seen in three (6.3%) cases. No specific risk factors like hydrosalpinx was seen in our cases.

Previously oophorectomy or salphingo oophorectomy was the preferred procedure fearing the possibility of gangrenous transformation and sepsis. Now recently the studies show preserved ovaries maintains its function on follow up.[25] In present study 8 (17%) cases had undergone detorsion and oopheropexy, 20 (42.5%) had ovarian cystectomy, 3 cases (6.3%) had unilateral salphingectomy, 5 cases (10.6%) had hysterectomy with bilateralsalphingo oophorectomy, and 11(23.4%) cases underwent unilateral salphingo oophorectomy. In total, conservative surgery was done in 59.5% of patients and radical surgery in 40.3% of patients (Table 2). Removal of adnexa was performed in patients having a gangrenous mass and ovarian preservation was done in cases having edematous or bluish black ovaries. On follow up patients with preserved ovaries were comfortable, was asymptomatic and showed evidence of ovarian function.

Histopathology examination (Table 1) showed benign conditions in most specimens and were associated with hemorrhagic necrosis commonly. The common benign tumor was serous cyst adenoma followed by mucinous cyst adenoma and teratoma. One of patients diagnosed as malignancy had torsion following the completion of third cycle of presurgical chemotherapy. But the pain was misdiagnosed as ureteric colic and was treated conservatively before taking up for surgery. There are other case series reporting the rarity of malignant tumour undergoing torsion.[24]

□□□□□□□□□□

Diagnosis of adnexal torsion is a challenge to both clinician and radiologist due to its nonspecific clinical and radiological findings. So, it requires strong clinical suspicion and radiological expertise. Early diagnosis and surgical management saves the ovary, thereby preserving its function.

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1. Vijayaraghavan SB. Sonographic whirlpool sign in ovarian torsion. J Ultrasound Medic. 2004;23(12):1643-9.
2. Mc Williams GD, Hill MJ, Dietrich CS. Gynaecologic emergencies. Surg Clin North Am. 2008;88:265.
3. Niafar F, Mirfendereski S, Rostamzadeh A. Diagnostic efficacy of sonography for diagnosis of ovarian torsion. Zahedan J Res Med Sci. 2014;16(11):37-9.
4. Hibbard LT. Adenxal torsion. Am J Obstet Gynecol. 1985;152:456-61.
5. Schraga ED. Ovarian torsion: Practice Essentials. Available at https://emedicine.medscape.com/article/2026938-overview
6. Rosado WM Jr, Trambert MA, Gosink BB, Pretorius DH. Adnexal torsion;diagnosis using Doppler sonography. AJR Am J Roentgenol. 1992;159:1251-3.
7. Fleischer AC, Stein SM, Cullianan JA, Warner MA. Colour Doppler sonography of adnexal torsion. J Ultrasound Med. 1995;14;523-8.
8. Warner MA, Fleischer AC, Edell SL, Thieme GA, Bundy AL, Kurtz AB, et al. Uterine adnexal torsion: sonographic findings. Radiology 1985;154:773-5.
9. Graif M, ItzchacY. Sonographic evaluation of ovarian torsion in childhood and adolescence. AJR AmJ Roentgenol. 1988;150:647-9.
10. Tsafrir Z, Hasson J, Levin I, Solomon E, Lessing JB et al. Adnexal torsion: cystectomy and ovarian fixation are equally important in preventing recurrence. Eur J Obstet Gynecol Reprod Biol. 2012;162:203.
11. Nair S, Joy S, Nayar J. Five year retrospective case series of adnexal torsion. JCDR. 2014 Dec;8(12):OC09.
12. Vijayalakshmi K, Reddy GM, Subbiah VN, Sathiya S, Arjun B. Clinico-pathological profile of adnexal torsion cases: a retrospective analysis from a tertiary care teaching hospital. JCDR. 2014 Jun;8(6):OC04.
13. Karman E, Beger B, Cetin O, Melek M, Karaman Y. Ovarian Torsion in the normal ovary. A Diagnostic Challenge in adolescent girls in Emergency Department. Med Sci Monit. 2017;23:1312-6.
14. Spinelli C, Buti I, Pucci V, Liserre J, Alberti E, Nencini L et al. Adnexal torsion in children and adolescents: new trends to conservative surgical approach-our experience and review of literature. Gynaecol Endocrinol. 2013:29(1):54-8.
15. Hiller N, Appelbaum L, Simanovsky N, Lev-Sagi A, Aharoni D, Sella T. CT features of adnexal torsion. Am J Roentgenol. 2007 Jul;189(1):124-9.
16. Lo LM, Chang SD, Horng SG, Yang TY, Lee CL, Liang CC. Laparoscopy versus laparotomy surgical intervention of ovarian torsion. J Obstet Gynaecol Res. 2008;34(6)1020-5.

17. Mancusco A, Broccio G, Angio LG, Pirri V. Adnexal torsion in pregnancy. Acta Obstet Gynecol Scand. 1997;76(1);83-4.
18. Ventolini G, Hunter L, Drollinger D, Hurd WW. Ovarian Torsion during pregnancy. Resident Staff Physician. 2007;12(1):1-4.
19. Oelsner G, Shashar D. Adnexal torsion. Clin Obstet Gynecol. 2006;49(3):459-63.
20. Graif M, Shalev J, Strauss S, Engelberg S, Mashiach S, Itzchak Y. Torsion of the ovary: sonographic features. Am J Roentgenol. 1984;143(6):1331-4.
21. Ganer Herman H, Shalev A, Ginat S, Kerner R, Keidar R, Bar J et al. Clinical characteristics of adnexal torsion in premenarchal patients. Arch Gynecol Obstet. 2016 Mar 1;293(3):603-8.
22. Fleischer AC, Brader KR. Sonographic depiction of ovarian vascularity and flow. Current improvements and future applications. J Ultrsound Med. 2001;20(3):241-50.
23. Lee EJ, Kwon HC, Joo HJ, Suh JH, Fleischer AC. Diagnosis of ovarian torsion with color Doppler sonography; depiction of twisted vascular pedicle. J Ultrasound Med .1998;17(2):83-9.
24. Oltmann SC, Fischer A, Barber R, Huang R, Hicks B, Garcia N. Cannot exclude torsion: a 15year review. Pediatric Surg. 2009;44:1212-6.
25. Lasso BC, Garrido PJ, Murcia PF, Granero CR, Vargas CV, Paredes ER. Ovarian torsion long term follow up of the black- bluish ovary after laparoscopic detorsion. Cir Pediatr. 2014;27(1):26-30.

Maheswari LS, Abraham R, Arunachalam P. Adnexal torsion-five-year retrospective study. Int J Reprod Contracept Obstet Gynecol 2018;7:508-12.

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Efficacy of intermittent iron versus daily iron in antenatal women

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Abstract
Aim: This study was done to determine if alternate day oral iron as effective as daily oral iron in antenatal women including those with mild anemia.
Methods & Material: A prospective randomised trial was done in 215 antenatal women attending a tertiary centre in South India. Group 1 received 100mg of elemental iron daily and group 2 received 100mgs of elemental iron on alternate days. Haemoglobin was estimated in all the trimesters and change in Haemoglobin from baseline and at 32-34 weeks were estimated. Side effects and neonatal outcomes were analysed. Further analysis were done within groups depending upon the Haemoglobin levels.
Results: There was no difference in Haemoglobin levels between the two groups. The response was better in patients with mild anemia in both groups, however when iron was given daily in mildly anemia women the increase in Haemoglobin was better than alternate iron therapy.
Conclusion: Intermittent iron is as effective as daily iron.

Keywords: Haemoglobin, daily iron, intermittent iron, iron therapy in pregnancy, anemia in pregnancy

Introduction

Anaemia complicating pregnancy is one of the most common medical disorders of pregnancy. The WHO [1] has defined anaemia as a Haemoglobin less than 11gms/dl in the first and third trimester and a Haemoglobin of less than 10.5gms/dl in the second trimester. Any Haemoglobin less than the 5th percentile for a given gestation is defined as anaemia by CDC [2]. Globally about 58.25 million women are found to be anaemic during pregnancy and 95% of them belong to the developing world [3]. In India as per the population based survey in 2016 it was found that about 50% of pregnant women were anaemic [4]. Anaemia is a direct and an indirect cause of maternal mortality and causes around 13% of all maternal deaths and about 20% in South East Asia [5].

As part of a global strategy to prevent anaemia, WHO has recommended that all pregnant women be given 60mgs of elemental iron with 400mgs of folic acid every day prophylactically[6]. However in the event of iron deficiency anaemia, higher doses are prescribed. Oral iron is associated with many undesirable side effects causing poor compliance. An ideal dosing schedule is yet to be found, which patients will not default and yet give the desired results.

There has been lot of research in iron metabolism over the past two decades.

First is the presence of protein Hepcidin which regulates iron absorption [7], second is the mucosal turn over time which is said to occur once in 3 to 5 days. Thus after a given dose, further supplementation with iron until turn overtime is completed might lead to impaired absorption since the cells are already saturated [8]. Excess iron may cause oxidative stress and other side effects [9]. This has changed the thinking that intermittent iron might work as well. Subsequent to this there are many studies which state that iron two or three times per week or even once a week might be all that is required. Most of these studies have been done in pregnant women who had normal haemoglobin levels.

The WHO in a recent guideline has suggested administration of weekly iron of 120mgs with 2.8mgs of folic acid in pregnant women in areas where the prevalence of anaemia is less than 20% [10].

In India, the practise is still to administer daily iron during pregnancy. Being an area where the prevalence of anaemia is high, it would be worthwhile to know the efficiency and advantages of intermittent iron and hence this study was conducted to determine the usefulness of intermittent iron in both mildly anaemic and non anaemic pregnant women.

Materials and Methods

This was a prospective observational study conducted at a teaching hospital in South India. 215 pregnant women attending the antenatal OPD were randomly recruited for this study. 105 pregnant women from Unit 1 were assigned to daily iron and formed group 1 and 110 pregnant women from Unit 2 were given alternate day iron and were assigned as group 2. During the first visit between 10 to 14 weeks investigations like CBC, Peripheral smear and serum ferritin were done. This helped to rule out thalassemia which is not very uncommon in this area. Ferrous tablets containing 100mgs of elemental iron and 500mgs of folic acid was used after completion of 13 weeks and continued till delivery.

Exclusion criteria: Were Haemoglobin less than 9gms in the first trimester, history of haemtological disorders like sickle cell or thalassemia and history of autoimmune disorders.

Inclusion criteria: All patients attending OPD from at least 12 weeks of pregnancy. The patients were asked to take iron at night before food or at least one hour after food. They were asked to report if they had any side effects like vomiting, diarrhoea, heart burn or constipation. Repeat Haemoglobin was done in the second trimester and around 32 to 34 weeks to assess the Haemoglobin status of the individual. If the Haemoglobin fell to less than 8gms they were advised parenteral iron. Haemoglobin was done using Coulter LH 780 Haematology Analyser method. The maternal and fetal outcomes were noted.
Patients who did not have their delivery in our institution and if the Haemoglobin levels were not done in the third trimester, were excluded from the final analysis and two patients who were given parenteral iron were also excluded from final analysis.

Statistical Analysis

The categorical variables were analysed using chi square test and the continuous variables were analysed using test and ANOVA. P value of .005 was taken as significant. Both groups were compared in regard to mean change from baseline Haemoglobin to Haemoglobin at the 2nd and 3rdtrimester.
The groups were further subdivided into three groups based on Haemoglobin levels. Group A had Haemoglobin level between 9 to 11gms, Group B had Haemoglobin levels between 11.1 to 13gms and Group C had Haemoglobin levels more than 13gms and analysis done within the subgroup with regards to the change in Haemoglobin levels in the first and last trimester.

Results

Total of 215 pregnant women were included in this study 105 in the daily group and 110 in the alternate group. A total of 173 women, 80 in group 1 (daily Iron) and 93 in group 2 (alternate day Iron) were analysed and the attrition was mainly due to change of place for delivery, or incomplete data and investigations. Table 1 shows the age distribution in both groups and Table 2 shows the parity.

Table 1: Age

Age Yrs	Daily Regimen	Alternate Iron
<20	1	3
	1.3%	3.2%
21-25	34	31
	42.5%	33.3%
26-30	36	48
	45.0%	51.6%
>30	9	11
	11.3%	11.8%
Total	80	93

Table 2: Parity

	Daily Regimen	Alternate Iron
Primi	53	62
	66.25%	66.66%
Multi	27	31
	33.75%	33.33%
Total	80	93

The distribution of age and parity were similar in both groups. Table 3 shows the mean Haemoglobin levels in all trimesters in both groups which showed that the mean Haemoglobin levels were almost identical in both groups. The levels were higher in the first trimester and third trimester and slightly lower in the second trimester as expected due to haemodilution.

Table 3: Mean Hemoglobin in all trimester

Trimester		Daily regimen (N=80)	Alternate regimen (N=93)
1st trimester	Mean Hb	11.553	11.983
	SD	(1.2447)	(1.1462)
2nd trimester	Mean Hb	11.138	11.271
	SD	(0.9611)	(0.8489)
3rd trimester	Mean Hb	11.3065	11.382
	SD	1.1487	1.1218

The relationship between the Haemoglobin level and the ferritin level is shown in Fig1.

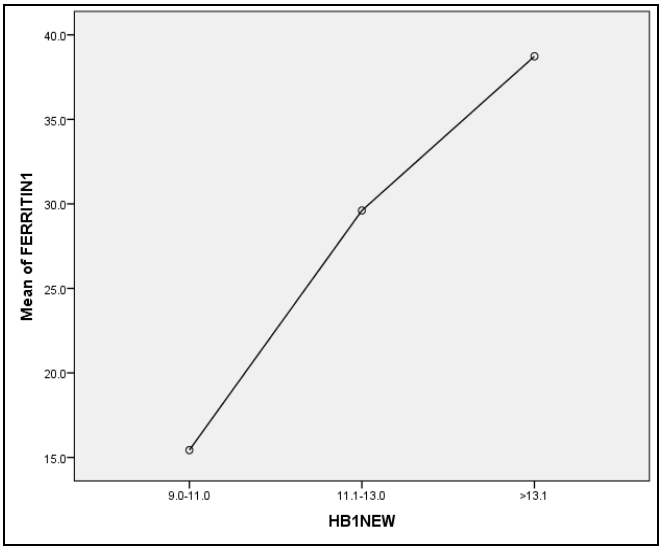


Fig 1: Relationship between the Haemoglobin level and the Ferritin level

38 women had ferritin levels less than 12ngm/dl. 24 in the daily group and 14 in the alternate group had ferritin less than 12 ngm/dl.
Table 4 shows the side effects of oral iron in both groups. Side effects were much lesser in the alternate iron group and so also was the compliance, which was found to be significant statistically with a P value of <001.

Table 4: Side effects

Side effects	Daily regimen (N=80)	Alternate regimen (N=93)
Vomiting	38	5
Constipation	30	8
Diarrhoea	2	1
Metallic taste	5	2
Bloatedness	10	2

Both groups were analysed as subgroups with the Haemoglobin levels in each trimester as shown in Table 5. This table shows that the response and Haemoglobin rise was better in women with mild anemia in both groups and the rise in Haemoglobin was better in the daily group.

Table 5: Analysis of haemoglobin in different sub groups in all trimesters

Hb gm/dl		1st trimester	2nd trimester	3rd trimester
9.0-11.0	Daily regimen N=26	10.085	10.872	11.1960
	Alternate regimen N=22 cases	10.395	10.629	10.9719
11.1-13.0	Daily regimen N=45 cases	11.996	11.107	11.2500
	Alternate regimen N=55 cases	12.169	11.375	11.3611
>13.1	Daily regimen N=9 cases	13.578	12.033	11.9625
	Alternate regimen N=16 cases	13.525	11.756	12.0333
Total	Daily regimen N=80 cases	11.553	11.138	11.3065
	Alternate regimen N=93 cases	11.983	11.271	11.3823

There were no preterm deliveries and the birth weight of babies was similar as shown in Table 6.

Table 6: Birth weight

	Hb	Number	Mean birth weight
Daily regimen (N=80)	9.0 – 11.0	25	3.0548
	11.1 - 13.0	45	2.9983
	>13.1	9	3.2938
	Total	80	3.0487
Alternate regimen (N=93)	9.0 – 11.0	22	3.0514
	11.1 - 13.0	55	2.9898
	>13.1	16	2.9106
	Total	93	2.9908

Discussion

In our study 80 women with a mean Haemoglobin 11.5gms/dl were given daily iron and 93 women with a mean Haemoglobin of 11.93gms/dl were given alternate iron. The third trimester Haemoglobin was 11.30gms/dl in the daily group and 11.38gms/dl in the alternate group showing that in both the treatment groups the Haemoglobin was almost the same.

WHO recommends 30-60mgs of elemental iron for all pregnant women depending on the prevalence of anaemia. This lower dose of 30mgs by WHO supersedes the previous recommendation of 60mgs taking into account the side effects and iron pharmokinetics [11].

The National iron plus initiative of India recommends iron folic acid [IFA] supplementation of 100 mg elemental iron and 500 mgs of folic acid every day for at least 100 days starting after the first trimester at 14–16 weeks of gestation for all non-anemic pregnant women followed by the same dose for 100 days postpartum [12].

Currently intermittent iron supplementation has been accepted for prophylaxis except in antenatal programmes for which debate is still ongoing.

In the past few years many studies have proven the hypothesis that intermittent iron is as effective as daily iron in terms of Haemoglobin change with the added advantage of having less side effects and being cost-effective. In spite of this information the change to intermittent therapy has not yet been implemented, maybe, since the prevalence is so high and further studies are warranted.

Cochrane in 2015 reviewed 21 trials of daily iron versus intermittent iron and stated that while there was no difference between groups for any maternal or fetal outcomes, the intermittent groups had less side effects [13].

Pena-rosa [14] suggested that women receiving intermittent iron had similar maternal and fetal outcomes. Study by Mukhopadhyay [15], Goonewardene [16] and ZInatossadaat [17] in have all suggested that intermittent iron is as effective as daily dosing.

Our study also proved that intermittent iron was as good as daily iron.

The second observation was that in patients who were found to have Haemoglobin between 9 to 11gms/dl, the rise in Haemoglobin was better in these patients with mild anaemia than non-anaemia and a mean increase of 1.1gm/dl was noted in daily group and 0.60gms/dl in the alternate group. The patients in the group with Haemoglobin between 11.1 to 13gms/dl and more than 13gms/dl did not show an increase, but showed a slight fall in Haemoglobin level from baseline.

There were two observations different in our study when compared to many studies. The first observation was that post treatment Haemoglobin did not show an increase from baseline as many studies have shown. However Adaji [18] in his Nigerian study between once and twice daily dosing has shown a fall in baseline Haemoglobin from 11.1gms/dl and 11gms/dl to 10.2gms/dl and 10.5gms/dl respectively in both arms. Similar fall in Haemoglobin has been noted by Okwara [19] who showed a fall from 11.2gms/dl in the first trimester to 10.4 gms/dl in the third trimester. In our study too, in the daily group the fall in Haemoglobin level was 0.2gms/dl and was 0 .6gms/dl in the alternate day group which was not statistically significant.

The second observation was that Haemoglobin improvement was better in women with anaemia in both groups. Although the final Haemoglobin was not very different in both groups, daily iron proved to be slightly better than alternate iron. The maximum oral dose per day is only 160mgs/day. Hence doubling the dose to twice daily is not beneficial, anaemic women would do well by just once daily dosing

Intake of iron is generally associated with untoward side effects like vomiting, constipation, dark stools, diarrhoea and a metallic taste in the tongue. A few rare myths discovered during the study was the belief that babies might be darker or be heavier which lead to women refraining from taking the medications. Side effects were less when given on alternate days, making women more compliant. According to a study by Khalafalloh [20] the side effects of oral iron include gastro intestinal disturbances characterised by colicky pain, vomiting, diarrhoea and constipation which occurred in about 50% of patients. Many patients taking more than 30mgs of iron per day have side effects causing them to discontinue the medication. In our study two women had to be given parenteral iron both belonging to the daily iron group since they experienced severe nausea and vomiting with oral iron.

Slon [21] *et al.*, Shatrugnaan [22] and Beard [23] and Souxa [24] have all corroborated the fact that increasing dose frequency also increases the side effects.

Preterm labour and low birth weight are common when Haemoglobin is less than 9gms or more than 13gms. Haemoglobin above the upper limit can give rise to decreased plasma expansion and is associated with poor pregnancy outcomes. In our study there was no preterm babies and no difference in birth weight in both groups, neither was there any growth restricted babies.

Conclusion

Intermittent dose seems convenient compared with daily dosing. There was no difference in Haemoglobin levels when administered daily or on alternate days. This regime was more cost effective and had less side effects and better compliance. Intermittent iron can also be practised for anaemic pregnant women since the absorption is better, however the improvement is better with daily iron. There is no benefit in increasing the dose to twice or thrice daily since required benefit was seen even with a daily dosing

Acknowledgement

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References

1. World Health Organization. Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity. Vitamin and Mineral Nutrition Information System. Geneva: WHO, 2011.
2. CDC CDC criteria for anemia in children and childbearing-aged women. MMWR Morbidity and mortality weekly report. 1989; 138(22):400-404.
3. World Health Organization (WHO). The prevalence of Anemia in women: A tabulation of available information. Geneva, Switerlans: WHO, 1992. WHO/MCH/MSM/92.2.
4. Ministry of Health and Family Welfare, Government of India. India National Family Health Survey (NFHS4) 2015–16. Mumbai: International Institute for Population Sciences; 2017 (http://rchiips.org/nfhs/NFHS-4Reports/India.pdf, accessed 15 January 2018).
5. Rahman MM, Abe SK, Rahman MS *et al*. Maternal anemia and risk of adverse birth and health outcomes in low- and middleincome countries: systematic review and meta-analysis. Am J Clin Nutr. 2016; 103(2):495-50458.
6. Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization; 2012 http://www.who.int/nutrition/publications/micronutrients/guidelines/daily_ifa_supp_pregnant_women/en/
7. Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011; 117(17):4425-33.
8. Anderson GJ, Frazer DM, McKie AT, Vulpe CD, Smith A. Mechanisms of haem and non-haem iron absorption: lessons from inherited disorders of iron metabolism. Biometals. 2005;18(4):339-48
9. Srigiridhar K, Nair KM, Subramanian R, Singotamu L. Oral repletion of iron induces free radical mediated alterations in the gastrointestinal tract of rat. Molecular and Cellular Biochemistry. 2001; 219(1-2):91-8
10. WHO Guideline: Intermittent iron and folic acid supplementation in non-anemic pregnant women. Geneva: World Health Organization. 2012 http://www.who.int/nutrition/publications/micronutrients/guidelines/guideline_intermittent_ifa_non_anaemic_pregnancy/en/).
11. WHO Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva: World Health Organization; 2012 http://www.who.int/nutrition/publications/micronutrients/guidelines/daily_ifa_supp_pregnant_women/en/
12. Umesh Kapil, Ajeet Singh Bhadoria. National Iron-plus Initiative guidelines for control of iron deficiency anaemia in India, 2013. The National Medical Journal of India. 2013; 27:27-9
13. Pena-Rosas JP, De-Regil LM, Dowswell T, Viteri FE. Daily oral iron supplementation during pregnancy. Cochrane Systematic Review 2012; 12:CD004736. https://doi.org/10.1002/14651858.CD004736.pub4
14. Pena-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. Cochrane Database of Systematic Reviews 2015, 7. [DOI: 10.1002/14651858.CD004736.pub5
15. Asima Mukhopadhyay, Neerja Bhatla, Alka Kriplani, Ravindra M Pandey, Renu Saxena. Daily versus intermittent iron supplementation in pregnant women: Hematological and pregnancy outcome. J Obstet gynaecol Res. 2004; 30(6):409-417. https://doi.org/10.1111/j.1447-0756.2004.00223.x
16. Indra Malik R, Goonewardene, Diluk I, Senadheera. Randomized control trial comparing effectiveness of weekly versus daily antenatal oral iron supplementation in preventing anemia during pregnancy. J Obstet gynaecol Res. 2018; 44(3):417-424. https://doi.org/10.1111/jog.13546
17. Zinatossadat Bouzari, Zahra Basirat, Mahtab Zeinal Zadeh *et al*. Daily versus intermittent iron supplementation in pregnant women. BMC Res Notes 2011; 4:444 https://doi.org/10.1186/1756-0500-4-444
18. Adaji JA, Isah AY, Agida ET, Otu T, HI Abdullahi. Daily versus twice daily dose of ferrous sulphate supplementation in pregnant women: A randomized clinical trial. Niger J clin pract. 2019; 22:1132-9.
19. Okwara JE, Nnabuo LC, Nwosu DC, Ahaneku JE, Anolue F, Okwara NA *et al*. Iron Status of Some Pregnant Women in Orlu Town--Eastern Nigeria. Niger J Med. 2013; 22(1):15-8.
20. Alhossain A Khalafallah, Amanda Dennis E. Iron Deficiency Anaemia in Pregnancy and Postpartum:

Pathophysiology and Effect of Oral versus Intravenous Iron Therapy. J Pregnancy. 2012; 2012:630519. doi: 10.1155/2012/630519. Epub 2012 Jun 26.
21. Sloan NL, Jordan E, Winikoff B. Effects of iron supplementation on maternal hematologic status in pregnancy. Am J Public Health. 2002; 92:288-93.
22. Shatrugna V, Raman L, Kailash U, Balakrishna N, Rao KV. Effect of dose and formulation on iron tolerance in pregnancy. Natl Med J India. 1999; 12:18-20.
23. Beard JL. Effectiveness and strategies of iron supplementation during pregnancy. Am J Clin Nutr. 2000; 71(5):1288S-94S.
24. Souza AI, Batista Filho M, Bresani CC, Ferreira LO, Figueiroa JN. Adherence and side effects of three ferrous sulfate treatment regimens on anemic pregnant women in clinical trials. Cad Saude Publica. 2009; 25:1225-33.

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Pregnancy outcome in autoimmune disorders

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Abstract

Background: Autoimmune disorders are chronic multisystem disorders affecting women of their reproductive age. Pregnancy among these women is complicated by the disease itself. Flaring up of disease, uteroplacental insufficiency due to vasculitis and drugs used for treatment are main reason for unfavourable pregnancy outcomes. The objective of this study was to analyze the pregnancy complications and outcome among 113 cases of autoimmune disorders.

Methods: This study is a retrospective analysis of case records of pregnant women with autoimmune disorders. This study was conducted at PSG IMSR and Hospital, Coimbatore, and Tamil Nadu from July 2012 to June 2018. The case sheets were retrieved from medical record department and the details such as type of disease, maternal age, parity, status of disease at conception, treatment taken during pregnancy, maternal complications and fetal complications were collected and analyzed.

Results: During 6-year period there were 113 pregnant women with autoimmune disorders, and they were studied of their previous and present pregnancy outcome. The disease incidence was 7.01% per 1000 deliveries. 40.7% were less than 25 years age group and 71.6% were multigravidae. We had forty-one patients (36%) who were positive for APLA syndrome which were the maximum number of patients with auto immune disorder in pregnancy and 37 patients (32.7) with SLE. We had significant numbers of maternal and fetal complications for common disorders. Most of the rare auto immune disorders were diagnosed before pregnancy and these pregnancies were managed by multi-disciplinary approach, and continued on immunomodulators throughout pregnancy, hence maternal and fetal complications were less for them.

Conclusions: Adequate pre-conceptional counseling, vigilant monitoring during pregnancy and post-partum will avoid pregnancy related complications and have favorable outcomes.

Keywords: Autoimmune disorder, Fetal complications, Immunomodulators, Maternal complications, Pregnancy outcome, Pre-conceptional counseling

Introduction

Autoimmune disorders are chronic multisystem disorders which are characterized by tissue damage and inflammation due to alteration of adaptive immune response in the absence of trigger.[1] Its prevalence is between 7.6%-9.4%.[2] Since most of the autoimmune disorders are diagnosed frequently during reproductive age in females, these women are at high risk not only during pregnancy but also during postpartum.[3] When compared to females without autoimmune disorders, these women carry more incidence of miscarriages in all trimesters, preeclampsia, intrauterine growth restriction, premature rupture of membranes, placental insufficiency, preterm labour, low birth weight and have increased caesarean section rates.[4] Though these pregnancies carry

high incidence of adverse effects, improved care in terms of pre-conceptional counselling and continuation of treatment throughout the pregnancy and post-partum have improved the outcome of pregnancies.[5]

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This study is a retrospective analysis of a total 113 case records of pregnant women with autoimmune disorders. This study was conducted at PSG IMSR and Hospital, Coimbatore, Tamil Nadu from July 2012 to June 2018. The case sheets were retrieved from medical record department and the details such as type of autoimmune disorder, maternal age, parity, status of disease at conception, treatment taken during pregnancy, maternal complications like pregnancy induced hypertension, gestational diabetes mellitus, thrombotic events, anemia, renal flares, thrombocytopenia, preterm labour, mode of delivery, indications for caesarian section and fetal complications like abortion, anomalies, intra uterine fetal demise, low birth weight, intra uterine growth restriction, oligohydramnios, need for admission to neonatal intensive care unit, low Apgar score were collected and analyzed.

Inclusion criteria

- All primigravidae and multigravidae who are known case of autoimmune disorder and all primigravidae and multigravidae who are diagnosed with autoimmune disorder during present pregnancy.

Exclusion criteria

- Pregnant mothers without autoimmune disorders.

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During this period of 6 years (2012-2018), there were 113 pregnant women with autoimmune disorders and study was carried out based on their previous and present pregnancy outcomes. The incidence of autoimmune disorders in pregnancy was 7.01% per 1000 deliveries.

Table 1 shows the number of pregnancies with autoimmune disorders in our study.

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Antiphospholipid antibody syndrome	41	36.2
Systemic lupus erythematoses	37	32.7
Idiopathic thrombocytopenic purpura	11	9.7
Takayasu syndrome	5	4.4
Sjögren syndrome	4	3.5
Hashimoto's thyroiditis	3	2.6
Multiple sclerosis	3	2.6
Rheumatoid arthritis	2	1.7
Schönlein-henoch purpura	2	1.7
GBS	1	0.8
Von Williebrand disease	1	0.8
Multisystem connective tissue disorder	1	0.8
Medial vessel vasculitis	1	0.8
Ulcerative colitis	1	0.8

In our study, we had forty-one patients (36%) with APLA syndrome including primary and secondary APLA and 37 patients (32.7%) with SLE.

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<25	46	40.7
25-30	45	39.8
31-35	21	18.5
>35	1	0.8

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APLA	2 (4.8%)	13 (31%)	1 (2%)	3 (7.3%)	-	-	7 (17%)	1 (2.4)
SLE	4 (10.8%)	6 (16%)	3 (8%)	2 (5.4%)	-	1 (2.7%)	16 (43%)	8 (21%)
ITP	1 (9%)	6 (54%)	-	1 (9%)	2 (18%)	-	7 (63%)	1 (9%)
Takayasu	3 (60%)	-	1 (20%)	-	-	-	4 (80%)	-
Sjögren syndrome	-	1 (25%)	-	-	-	-	3 (75%)	□□

In our study, most of the patients were below 30 years of age. Table 2 shows the age group distribution. Multigravida (71.6%) were more in number than primigravida (28.3%) in our study.

Parity distribution is shown in Figure 1.

Table 3 shows the most common autoimmune disorders in our study and their maternal complications.

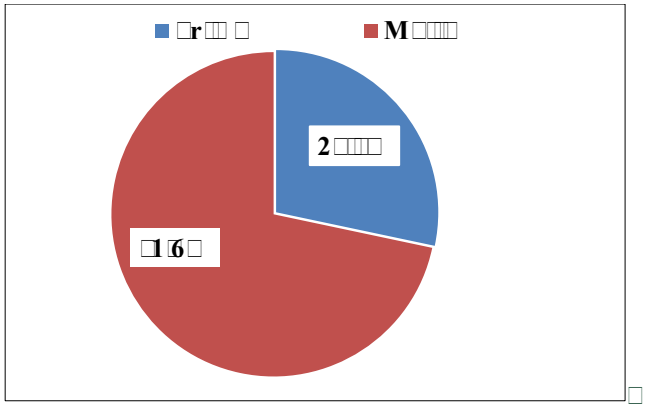


Table 3: Maternal complications in previous pregnancy.

Disorder	Number	Percentage
APLA	26 (63%)	9 (21.9%)
SLE	22 (59%)	5 (13.5%)
ITP	4 (36%)	-
Takayasu	-	1 (20%)
Sjögren syndrome	3 (75%)	-

Two patients diagnosed with SLE had abnormal doppler changes at 32 weeks and 34 weeks respectively and underwent caesarean section. The most common indication for caesarean section was non-progression of labour followed by previous section.

Table 4 shows fetal complications in previous pregnancy for more common disorders in our study.

Table 4: Fetal complications in previous pregnancy.

Disorder	IUGR	Abortion	Stillbirth
APLA	7 (17%)	2 (4.8%)	1 (2.4%)
SLE	13 (35%)	6 (16%)	5 (13.5%)
ITP	2 (18%)	-	1 (9%)
Takayasu	3 (60%)	-	-
Sjögren syndrome	-	2 (50%)	-

Table 5 shows the fetal complications in the current pregnancy for same disorders.

Table 5: Maternal complications in current pregnancy.

Disorder	Current	Previous
Pre-eclampsia	10.8%	13%
GDM	16%	NA
Renal flare	8%	NA
Anemia	7.3%	21%
C-section	43%	53%
Preterm labour	21%	21%

Table 6: Fetal complications in current pregnancy.

Disorder	Current	Previous
Abortion in previous conception	59%	46%
IUD	13.5%	8%
IUGR	35%	32%
LBW	24.3%	22%
NICU admission	13.5%	15%

APLA

Obstetric antiphospholipid antibody syndrome (OB-APS) is defined according to the updated Sydney classification criteria developed by expert consensus agreement in 2006.[6] APLs include the lupus anticoagulant, anticardiolipin IgG and IgM and anti-β2-glycoprotein I antibody. In addition to the detection of aPLs on at least two occasions more than 12 weeks apart, OB-APS is defined by any one of the following adverse pregnancy outcomes: 1) otherwise unexplained recurrent pregnancy loss before the 10th week of gestation, 2) otherwise unexplained fetal death ≥10 weeks of gestation, 3) preterm birth before 34 weeks of gestation due to preeclampsia or placental insufficiency.[7] Other OB-APS complications include catastrophic APS, thrombosis, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), IUGR, fetal distress, premature rupture of membranes, and thrombocytopenia.[8] It has been recognized that among the three aPLs, lupus anticoagulant (LAC) is the primary predictor of poor pregnancy outcome in women both with or without SLE, and with or without other aPLs.[9] Among the 41 patients with APLA, 20 patients were positive for lupus anticoagulant antibody, 11 were positive for anticardiolipin antibody and 10 had anti-β2-glycoprotein I antibody.

In our study, 31.8% of patients with primary APLA and 4.4% of patients with secondary APLA with associated SLE were diagnosed. The incidence of pre-eclampsia among APLA positive patients in our study was 4.8% whereas in Wijeyaratne study, it was 10.9%.[10] Similarly the incidence of GDM in our study was 31% as compared to 19.2% in Wijeyaratne study. In our study 2.4 % had preterm labour and 17% of patients underwent cesarean section. In regard to fetal complications, 63% of patients in our study had previous one or more abortions which was comparable to Wijeyratne study (59.8%). The incidence of IUD in previous pregnancy was 21.9.%. The incidence of low birth weight babies was 12% and babies requiring NICU admission was 2.4% respectively.

SLE

Systemic lupus erythematosus (SLE, or “lupus”), affects females in a 9:1 ratio to males.[11] The adverse pregnancy

outcomes observed with lupus are well known, and include increased rates of intrauterine growth restriction (IUGR), preterm birth and fetal loss, as well as the syndrome of neonatal lupus (NL), associated with trans placental passage of auto antibodies. The risks for thrombosis, infection, thrombocytopenia, and transfusion were each 3- to 7-fold higher for women with systemic lupus erythematosus. Lupus patients also had a higher risk for cesarean sections (odds ratio: 1.7), preterm labor (odds ratio: 2.4), and preeclampsia (odds ratio: 3.0) than other women. Women with systemic lupus erythematosus are more likely to have other medical conditions, including diabetes, hypertension, and thrombophilia, that are associated with adverse pregnancy outcomes.[12]

High disease activity at the time of conception or in the months preceding conception is an independent predictor for increased risk of adverse pregnancy outcomes including prematurity, IUGR and fetal demise. This has led to the current recommendations that lupus patients achieve 6 months of disease quiescence prior to attempting conception.[13] Maternal and fetal complications associated with SLE were compared with Eman et al study.[14] These are shown in table 6 and 7 respectively.

Six patients had first trimester abortions in the current pregnancy.

Takayasu syndrome

In our study, 60% patients had hypertension complicating pregnancy whereas in Mandal et al study had 92.8%.[15] Caesarean section rate in our study was 60%, whereas in Mandal study it was 71.4%. Intrauterine growth restriction was 60% in our study, which was comparable with Mandal et al study (51.7%).

Schönlein-henoch purpura

Schönlein-henoch purpura (SHP) is a systemic vasculitis that affects vessels of a small caliber.[16] The onset of SHP during pregnancy is very rare. Treatment with orally administered corticosteroids may lead to a beneficial outcome for both mother and newborn.[17] In our study, we had 2 patients diagnosed with Schönlein-Henoch purpura, of which one developed GDM, other had uneventful pregnancy.

Medium vessel vasculitis

Most of the women with medium vessel vasculitis fared well during pregnancy without significant disease progression, but instances of aneurysm rupture, renal insufficiency, pulmonary embolism, and myocardial infarction were reported in Gatto et al study.[18] In our study, one patient diagnosed with medium vessel vasculitis developed myocarditis during pregnancy and she was managed with steroids.

ITP

Nine percent of chronic ITP patients in our study had preeclampsia, whereas Diego et al, study had 8.3%.[19] In our study 9% had preterm labour which was comparable to Diego et al (9.5%) study. Diego et al showed 8.5% of gestational diabetes in the study whereas in our study we had 54% of gestational diabetes which could be explained by ethnicity and usage of steroids.

In Yassaee et al, study occurrence of IUGR was 10.5% whereas in our study it was 18%, among which one baby was admitted in NICU.[20] In Yassaee et al study 57% of patients had moderate thrombocytopenia (more than 50,000) whereas 18% of thrombocytopenia and 9% of anemia were diagnosed in our study.

When comparing the caesarean section rate, Yassaee et al had 81% whereas we had 63%.

Rheumatoid arthritis

In women with well-controlled RA, pregnancy outcomes are comparable to the general obstetric population, whereas in women with higher RA disease activity, there is more chances of less favorable pregnancy outcomes.[21,22]

Akasbi et al, study showed improved maternal and fetal outcome in patients with RA in pregnancy. Similarly, in our study two patients diagnosed with RA had uneventful pregnancy.

Guillain-barré syndrome

Guillain-barré syndrome (GBS) represents a heterogeneous group of immune mediated peripheral neuropathies. A feature common to all GBS variants is a rapidly evolving polyradiculoneuropathy preceded by a triggering event, most often an infection. GBS generally manifests as a symmetric motor paralysis with or without sensory and autonomic disturbances. Delayed diagnosis is common in pregnancy or immediate post-partum period because the initial non-specific symptoms may mimic changes in pregnancy. GBS should be considered in any pregnant patient complaining of muscle weakness, general malaise, tingling of the fingers, and respiratory difficulty.[23,24]

We had one patient diagnosed with GBS who had an uneventful course during pregnancy.

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms.[25] Pregnancy is typically a stabilizing period in the clinical course of MS. During the

third trimester the MS relapse rate can be 70% lower when compared with the time prior to pregnancy, but exaggeration of the disease is commonly seen postpartum. Presently, there is no evidence that MS patients are more susceptible to pregnancy or delivery related complications, such as ectopic pregnancy, pre-eclampsia, gestational diabetes mellitus, prolonged labour or miscarriage than women in general, nor is their infants more likely to be delivered preterm or to be of low birth weight, have malformations or experiences an early death.[26,27] Mothers with MS required operative delivery more often than healthy mothers, which might be attributed to MS-related symptoms such as neuromuscular perineal weakness and spasticity in addition to fatigue and exhaustion.[28] In our study, we had 3 mothers diagnosed with multiple scelerosis, of them one developed PIH, one had GDM.

Sjögren syndrome

Sjögren syndrome is known to occur predominantly in women. Affected women are likely to experience more complicated pregnancies than are women without the disease.[29] Its effect on pregnancy differs according to the maternal disease, disease activity, and severity of organ damage, antibody profile, and drug treatment.6.

Several studies have reported an increased rate of spontaneous abortion and fetal loss associated with Sjögren syndrome.[30] This may be explained by, first, the age of the patients at the time of concep-tion which usually belongs to an elderly age group and, second, a possible immunologic factor involved in the mechanisms of miscarriage.[31]

In our study, out of four mothers diagnosed with Sjögren syndrome, two had spontaneous abortions.

The outcome of pregnancies in women with Sjögren syndrome can be improved with use of a multidisciplinary approach for management involving an obstetrician who has specialized in high-risk pregnancies, a rheumatologist, and a pediatrician.[32]

Hashimotos thyroiditis

Hashimotos thyroiditis is associated with an increase rate of spontaneous.

Abortions, preterm delivery and increased use of cesarean section.[33] All the three patients diagnosed with Hashimotos thyroiditis underwent cesarean section for non-progression of labour.

Ulcerative colitis

Stephansson et al study concluded that pregnant women with ulcerative colitis had a greater number of preterm births, babies with low birth weight and cesarean section deliveries than those without ulcerative colitis.[34] In our study we had one patient who delivered a low birth weight baby of 2.2 kg. One patient diagnosed with multisystem connective tissue disorder had an intra uterine growth restriction and the same baby had low birth weight.

Von Willebrand's disease

Pregnancy and delivery are considered events predisposing to a high risk of bleeding in women with von Willebrand's disease.[35]

Our patient did not have any complication. All the patients diagnosed with GDM were found to be on steroids.

Among the 113 patients, 3 underwent caesarean section in view of abnormal umbilical artery doppler findings.

Patients previously diagnosed were continued on disease modifying agents and this could be the reason for their successful pregnancy outcome.

Rauto immune disorders were diagnosed before pregnancy and these pregnancies were managed by multi-disciplinary approach, and continued on immune modulators throughout the pregnancy, hence minimizing maternal and fetal complications.

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In our study, most of the patients were either continued on the same treatment they were taking before pregnancy or were started on treatment once they were diagnosed with autoimmune disorders, and hence they were found to have successful pregnancy outcomes. Therefore, appropriate pre-conceptional counselling, continuation of treatment, intense maternal and fetal monitoring and timely delivery plays an important role in improving the pregnancy outcome.

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1. Sarah M, Mark Q, Paul E, Chapter 32 – Rheumatoid arthritis Editors: Noel R. Rose, Ian R. Mackay, The Autoimmune Diseases. 4th Ed, Academic press; 2006:417-436.
2. Cooper G, Bynum M, Somers E. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. J Autoimmun. 2009;33(3-4):197-207.
3. Perricone C, Carolis C, Perricone R. Pregnancy and autoimmunity: a common problem. Best Pract Res Clin Rheumatol. 2012;26(1):47-60.

4. Andreoli L, Fredi M, Nalli C, Reggia R, Lojacono A, Motta M et al. Pregnancy implications for systemic lupus erythematosus and the antiphospholipid syndrome. J Autoimmun. 2012;38(2-3):197-208.
5. Mecacci F, Pieralli A, Bianchi B, Paidas MJ. The impact of autoimmune disorders and adverse pregnancy outcome. Semin Perinatol. 2007;31(4):223-6.
6. Wilson WA, Gharavi AE, Koike T. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arth Rhe. 1999;42:1309-11.
7. Miyakis S, Lockshin MD, Atsumi T. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295-306.
8. Hughes GR. The antiphospholipid syndrome: ten years on. Lancet (London, England). 1993;342:341-4.
9. Lockshin MD, Kim M, Laskin Ca. Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies. Arth Rh. 2012;64:2311-8.
10. Wijeyaratne CN, Galappaththi SLA, Palipane E, Jayawardane DBIA, Dodampahala SH, Tudawe MN, et al. Pregnancy outcomes of antiphospholipid syndrome: In a low resource South Asian setting. Med. 2016;9(2):83-9.
11. Cooper GS, Gilbert KM, Greidinger EL. Recent advances and opportunities in research on lupus: environmental influences and mechanisms of disease. Environ Health Perspect. 2008;116:695-702.
12. Clowse MEB, Jamison M, Myers E. A national study of the complications of lupus in pregnancy. Am J Obstet Gynecol. 2008;199:1-12.
13. Jakobsen IM, Helmig RB, Stengaard-Pedersen K. Maternal and foetal outcomes in pregnant systemic lupus erythematosus patients: an incident cohort from a stable referral population followed during 1990-2010. Scand J Rheumatol. 2015;44:377-84.
14. Eman Aly, Hussein Aly, Riyad RM, Mokbel AN. Pregnancy outcome in patients with systemic lupus erythematosus: a single center study in the high-risk Pregnancy unit. Middle East Fert Society J. 2016;21:168-74.
15. Mandal D, Mandal S, Dattaray C, Banrrjee D, Ghosh P, Ghosh A, et al. Takayasu arteritis in pregnancy: an analysis from eastern India. Arch Gynecol Obstet. 2012;285(3):567-71.
16. Jennette CJ, Milling DM, Falk RJ. Vasculitis affecting the skin. Arch Dermatol. 1994;130:899-906.
17. Robert F, Walter R, Sator PG, Gschnait F, Breier F. Schönlein-Henoch purpura during pregnancy with successful outcome for mother and newborn BMC Dermatol. 2002;2:1.
18. Gatto M, Iaccaino L, Canova M, Nalotto L, Ramonda R. Pregnancy and vasculitis; a systematic review of the literature. Autoimmun Rev. 2012;11:A447-59.
19. Wyszynski DF, Carman WJ, Cantor AB, Graham JM, Kunz LH, Slavotinek AM, et al. Pregnancy and birth outcomes among women with idiopathic thrombocytopenic purpura. J Preg. 2016:Article id 8297407.
20. Yassaee F, Eskandari R, Amiri Z. Pregnancy outcomes in women with idiopathic thrombocytopenic purpura. Iran J Reprod Med. 2012;10(5):489-92.
21. De Man YA, Hazes JMW, Van Der Heide H. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: Results of a national prospective study. Arth Rhe. 2009;60:3196-206.
22. Bharti B, Lee SJ, Lindsay SP. Disease severity and pregnancy outcomes in women with rheumatoid arthritis: Results from the organization of teratology information specialists autoimmune diseases in pregnancy project. J Rheumatol. 2015;42:1376-82.
23. Hughes RA, Cornblath DR. GuillainBarre' syndrome. Lancet. 2005;366:1653-66.
24. Vijayaraghavan J, Vasudevan D, Sadique N, Rajeswari KS, Pondurangi M, Jayshree A. Rare case of Guillain-Barre' syndrome with pregnancy. J Indian Med Assoc. 2006;104:269-70.
25. Coles A. Multiple sclerosis. Lancet. 2008;372:1502-17.
26. Jalkanen A, Alanen A, Airas L. The finnish multiple sclerosis and pregnancy study group. Pregnancy outcome in women with multiple sclerosis – results from a prospective nationwide study in Finland. Mult Scler. 2010;16:950-5.
27. Mueller BA, Zhang J, Critchlow CW. Birth outcomes and need for hospitalization after delivery among women with multiple sclerosis. Am J Obstet Gynecol. 2002;186:446-52.
28. Dahl J, Myhr KM, Daltveit AK, Hoff JM, Gilhus NE. Pregnancy, delivery, and birth outcome in women with multiple sclerosis. Neurol. 2005;65:1961-3.
29. Siamopoulou-Mavridou A, Manoussakis MN, Mavridis AK, Moutsopoulos HM. Outcome of pregnancy in patients with autoimmune rheumatic disease before the disease onset. Ann Rheum Dis. 1988;47(12):982-7.
30. Carvalheiras G, Faria R, Braga J, Vasconcelos C. Fetal outcome in autoimmune diseases. Autoimmun Rev. 2012;11(6-7):A520-30.
31. Sandhya P, Jeyaseelan L, Scofield RH, Danda D. Clinical characteristics and outcome of primary Sjogren's syndrome: a large Asian Indian cohort. Open Rheumatol J. 2015;9:36-45.
32. Brucato A, Cimaz R, Caporali R, Ramoni V, Buyon J. Pregnancy outcomes in patients with autoimmune diseases and anti-Ro/SSA antibodies. Clin Rev Allergy Immunol. 2011;40(1):27-41.
33. Lao TT. Thyroid disorders in pregnancy. Curr Opin Obstet Gynecol. 2005;17:123-7.

34. Stephansson O, Larsson H, Pedersen L. Congenital abnormalities and other birth outcomes in children born to women with ulcerative colitis in Denmark and Sweden. Inflamm Bowel Dis. 2011;17(3):795-801.
35. Kujovich JL. Von Willebrand disease and pregnancy. J Thromb Haemost. 2005;3(2):246-53.

Maheswari S, Poornima C, Panicker S. Review of 113 cases of autoimmune disorders in pregnancy. Int J Reprod Contracept Obstet Gynecol 2019;8:4786-92.

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Universal screening for hypothyroidism in pregnancy: is it mandatory?

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Abstract

Background: Thyroid diseases are one of the commonest endocrine disorders affecting women of reproductive age group, and hence constitute one important disorders complicating pregnancy. The objective of this study was to determine the importance of universal screening for hypothyroidism in pregnancy at the first antenatal visit and to formulate whether this routine screening is mandatory in our country.

Methods: This retrospective study was conducted in the year 2018 at PSG IMSR Hospital for all pregnant women who attended the first antenatal visit between Jan 2012 to Dec 2012 after obtaining ethical clearance. Pregnant women who were already taking treatment for hypothyroidism, diabetes mellitus, hypertension and those pregnant women who lost their follow up were excluded from the study.

Results: The incidence of subclinical hypothyroidism among antenatal women were 7.06%. In our study the maternal complications like anemia 12 (8%), preeclampsia 26 (17.3%), gestational diabetes 25 (16.7%), fetal growth restriction 8 (5.3%), Oligohydramnios 13 (8.7%), pre mature rupture of membranes 25 (16.7%), placental abruption in 2 (1.33%), APLA syndrome 2 (1.33%), low birth weight 26 (17.3%) were observed.

Conclusions: Universal screening for hypothyroidism is recommended for all antenatal women especially in iodine depleted country like India.

Keywords: Anemia, Gestational diabetes, Pre-eclampsia, Subclinical hypothyroidism, Thyroxine, Thyroid stimulating hormone

Introduction

Thyroid diseases are among the commonest endocrine disorders affecting women of reproductive age group, and hence constitutes one of the important medical disorders complicating pregnancy.[1] About 2-5% of all pregnant women are reported to have some degree of hypothyroidism.[2] Subclinical hypothyroidism is a prevalent condition which may go unrecognised and has the potential to cause adverse maternal and fetal outcome.[3] Sub clinical hypothyroidism defined as elevated serum thyrotropin level accompanied by normal serum thyroxin concentration. TSH plays a central role in screening and diagnosis of hypothyroidism.

Pregnancy complications with hypothyroidism include increased incidence of preeclampsia, abruptio placentae, anemia, and preterm labour. Unexplained still birth, fetal distress, low birth weight and neonatal hypothyroidism are perinatal complications.[4]

There has been a wide geographic variation in prevalence of hypothyroidism during pregnancy. Reports indicate it varies from 2.5% from the West to 11% from India also it is more in Asian countries compared to the West.[5,6] In a recent study by Dhanwal et al, a high prevalence of hypothyroidism (14.3%) was noted.[7] Looking at these data the purpose of the study was to determine whether

universal screening for hypothyroidism in pregnancy would prove to be beneficial in our country.

The objective of this study was to determine the incidence of hypothyroidism in pregnancy at the first antenatal visit and complications associated to formulate whether routine screening can be recommended in our country.

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This retrospective study was conducted in the year 2018 at PSG IMSR Hospital for all pregnant women attending the first antenatal visit between January 2012 to December 2012 after obtaining ethical clearance.

Inclusion criteria

- All pregnant women attending the antenatal OPD at the first antenatal visit and who delivered in our hospital.

Exclusion criteria

- Pregnant women who were already taking treatment for hypothyroidism, diabetes mellitus, hypertension and those who lost their follow up in our hospital.

The Sr.TSH is done as a screening tool for hypothyroidism for all antenatal women. The Sr.TSH reference range as per Indian thyroid society guideline 2015 was applied to diagnose hypothyroidism in pregnancy. Sr.TSH upper limit value is in the following range in each of the trimesters:

- First trimester <2.5 mIU/L
- Second trimester <3.0 mIU/L
- Third trimester <3.5 mIU/L

Sr.TSH > 2.5mIU/L in the first trimester were treated with thyroxine supplementation after checking FT3 and FT4.

These patients were subjected to repeat Sr.TSH in second and third trimester and analysed whether they are treated adequately or inadequately based upon the trimester specific upper reference limit. The dose of drug was adjusted if required. More over all the subclinical hypothyroid and low risk (euthyroid) study participants were followed up till delivery and analysed for any maternal complications like preeclampsia, anemia, preterm labour, gestational diabetes, fetal growth restrictions, low birth weight in both the groups.

Statistical analysis

The data was entered in Microsoft excel spread sheet and analysed by Statistical Package for Social science (SPSS version 21) and chi square test.

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In the present study total number of antenatal women who booked and delivered at PSG Hospital was 2124 of which the total number women with subclinical hypothyroidism were 150 (7.06%). The total number of low risk deliveries (euthyroid) excluding chronic hypertension (10), overt diabetes (19) and overt hypothyroidism (25) were 1920 and these patients were also analysed for maternal complications.

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Age	<20	3	2%
	20-24	49	33%
	25-29	57	38%
	30-34	32	21%
	>35	9	6%
Parity	Primi	85	57%
	Multi	65	43%
Gestational age at diagnosis	<14 weeks	38	25%
	15-28 weeks	87	58%
	>28 weeks	25	17%

The above table shows distribution of subclinical hypothyroidism among different age groups. The gestational age at which the hypothyroidism was diagnosed were more between 15 to 28 weeks (58%). The total number of subclinical hypothyroidism diagnosed in the >15 weeks of gestational age was 75%.

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Anemia	12 (8.0%)
Pregnancy induced hypertension	26 (17.3%)
Gestational diabetes	25 (16.7%)
Fetal growth restriction	8 (5.3%)
Oligohydramnios	13 (8.7%)
Previous caesarean section	20 (13.3%)
Rh negative	15 (10%)
Pre mature rupture of membranes	25 (16.7%)
Low birth weight	26 (17.3%)

Table 2 shows the most common maternal complications among the subclinical hypothyroidism were PIH 17.3%, low birth weight 17.3%, PROM 16.7% and GDM 16.7%.

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The thyroid gland size increases by 10% in size during pregnancy in iodine-deplete countries and to greater

extent by 10-20% in areas of iodine deficiency. Hence the thyroid hormone production, thyroxin (T4) and tri-iodothyronine (T3) increases by 50%, along with a 50% increase in daily iodine requirement during pregnancy. These physiological changes may result in hypothyroidism during pregnancy in iodine-deficient women.

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Anemia	Yes	12	48	.000*
	No	138	1872	
Pregnancy Induced Hypertension	Yes	26	70	.000*
	No	124	1850	
Gestational diabetes	Yes	25	84	.000*
	No	125	1836	
Fetal growth restriction	Yes	8	50	.561
	No	142	1870	
Oligohydramnios	Yes	13	85	.000*
	No	137	1835	
Pre mature rupture of membranes	Yes	25	110	.210
	No	125	1810	
Low birth weight	Yes	26	36	.000*
	No	124	1884	

The prevalence of subclinical hypothyroidism in our study group was 7.06%. A study conducted by Sapna C. Shah et al, in Karnataka shows a prevalence of sub clinical hypothyroidism of 9%.[8] Nambiar et al, conducted a study in Europe among Asian Indian pregnant women and said that the prevalence of hypothyroidism is about 7.2%.[9] In contrast to our study Rajput et al, conducted a study and said that the prevalence of hypothyroidism in pregnancy in northern India is 14.3%.[10]

Table 1 shows the demographic distribution of age, parity and gestational age at the diagnosis of subclinical hypothyroidism. Among the age distribution most of the women were below 30 years of age 109 patients (73%). Among 150 subclinical hypothyroidism pregnant women 85 of them were primi gravid and 65 of them were multigravida. The gestational age at which subclinical thyroidism was diagnosed based upon the TSH value were more in the second and third trimester.

In our study Table 2 shows the maternal complications like anemia 12 (8%), preeclampsia 26 (17.3%), gestational diabetes 25 (16.7%), fetal growth restriction 8 (5.3%), Oligohydramnios 13 (8.7%), pre mature rupture of membranes 25 (16.7%), placental abruption in 2 (1.33%), APLA syndrome 2 (1.33%), low birth weight 26 (17.3%), papillary carcinoma thyroid 1 (0.66%) treated with neck radiation were observed. In our study there were 20 (13.3%) patients who underwent caesarean section. Radha et al, in her study observed the maternal complications in subclinical hypothyroidism like anemia in 18 (36%), preeclampsia in 13 (26%), placental abruption in 2 (4%), caesarean section in 20 (44%), low birth weight baby 28%.[11] Anupama et al, in her study observed that 0.65% women had APLA syndrome, 0.3% had history of neck irradiation.[12]

Table 3 shows the maternal complications correlation between subclinical hypothyroidism and low risk (euthyroid) antenatal women.

In the present study among 150 subclinical hypothyroidism patients who were treated with thyroxine supplementation, 60 patients were adequately treated and 90 were inadequately treated. The adequately treated patients were diagnosed in the first trimester and the inadequately treated patients were in the late second and third trimester. Maternal complications like anemia, pregnancy induced hypertension, gestational diabetes, oligohydramnios, low birth weight were statistically significant (P value <0.05) in subclinical hypothyroidism group comparing to euthyroid. Leung et al, in his study found that there was a significant increased incidence of pre-eclampsia (15%) among inadequately treated subclinical hypothyroidism.[13] Ajmani et al, also observed 22.3% of pre-eclampsia and 12.11% of low birth weight in his study.[14] Fetal growth restriction and premarture rupture of membrane were not statistically significant (P value >0.05) in the subclinical hypothyroidism group compared to the euthyroid group.

[illegible]

The prevalence of subclinical hypothyroidism among the pregnant women in the present study is 7.06%, most of the adverse maternal and fetal complications are observed

in subclinical hypothyroidism. Since hypothyroid is easily treated, hence universal screening for hypothyroidism is recommended for all antenatal women especially in the first trimester to reduce the burden of adverse maternal and fetal outcome.

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1. Studd J. Progress in Obstetrics and Gynecology, Volume 15. London. Churchill Livingstone: 2003:75-101.
2. Casey BM, Dashe JS, Wells CE, McIntire DD, Byrd W, Leveno KJ, et al. Subclinical hypothyroidism and pregnancy outcomes. Obst Gynec. 2005;105(2):239-45.
3. Dhanwal DK, Prasad S, Agarwal AK, Dixit V, Banerjee AK. High prevalence of subclinical hypothyroidism during first trimester of pregnancy in North India. Indian J Endocrinol Metabol. 2013;17(2):281.
4. Sahu MT, Das V, Mittal S, Agarwal A, Sahu M. Overt and subclinical thyroid dysfunction among Indian pregnant women and its effect on maternal and fetal outcome. Archives Gynec Obst. 2010;281(2):215-20.
5. Männistö T, Vääräsmäki M, Pouta A. Perinatal outcome of children born to mothers with thyroid dysfunction or antibodies: A prospective population-based cohort study. J Clin Endocrinol Metab. 2009;94:772-9.
6. Wang W, Teng W, Shan Z, Wang S, Li J, Zhu L, et al. The prevalence of thyroid disorders during early pregnancy in China: The benefits of universal screening in the first trimester of pregnancy. Eur J Endocrinol. 2011;164:263-8.
7. Dhanwal DK, Prasad S, Agarwal AK, Dixit V, Banerjee AK. High prevalence of subclinical hypothyroidism during first trimester of pregnancy in North India. Indian J Endocrinol Metab. 2013;17:281-4.
8. Sapna IS. Prevalence and outcome of hypothyroidism in women attending antenatal clinic at J. J. M Medical College. Int J Reprod Contracept Obstet Gynecol. 2017;6(8):3537-9.
9. Nambiar V Jagtap VS, Sarathi V, Lila AR, Kamalanath S, Bandgar TR, et al. Prevalence and impact of thyroid disorders on maternal outcome in Asian-Indian pregnant women. J Thyroid Res. 2011;2011:429097.
10. Rajput R, Goel V, Nanda S, Rajput M, Seth S. Original article prevalence of thyroid dysfunction among women during the first trimester of pregnancy at a tertiary care hospital in Haryana. 2015;19:5-8.
11. Radha KR, Sugunan N, Resmy CR. Do we need to screen and treat pregnant women for subclinical hypothyroidism? A cross sectional study in a rural tertiary centre in Kerala, India. Int J Reprod Contracept Obstet Gynecol. 2017;6(3):781-6.
12. Dave A, Maru L, Tripathi M. Importance of Universal screening for thyroid disorders in first trimester of pregnancy. Indian J Endocrinol Metabol. 2014;18(5):735-8.
13. Leung AS, Millar LK, Kooning PP, Montorom, Mestman J. Perinatal outcome in hypothyroid pregnancies. Obstetric Gynaecol. 1993;81(3):349-53.
14. Nangia AS. Prevalence of overt and subclinical thyroid dysfunction among pregnant women and its effect on maternal and fetal outcome. J Obstet Gynaecol India. 2014;64(2):105-10.

Priyadharshini VM, Panicker S. Universal screening for hypothyroidism in pregnancy: is it necessary?. Int J Reprod Contracept Obstet Gynecol 2019;8:2683-6.

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ORIGINAL ARTICLE

Cesarean Myomectomy: An Experience from a Tertiary Care Teaching Hospital

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Abstract

Background Recent literature supports the removal of myomas during cesarean section, which traditionally was considered a relative contraindication, given a higher complication rate. This study is to share our experience of cesarean myomectomy in the last decade.

Methods This study is a retrospective review of our prospectively maintained database, from January 2008 to December 2017, at a tertiary care level teaching institution. All patients who underwent myomectomy during cesarean section were included. There were no exclusions.

Results A total of twenty patients underwent myoma removal along with the cesarean operation during this period with a mean age of 30 years. Majority of patients were nulliparous (70%). Common comorbidities were diabetes mellitus (40%) and hypothyroidism (20%). Mean size of myomas were 5.33 cm (± 2.08), and the number varied from one to three. The most common location was the posterior surface of the uterus with the commonest variety being subserous. Most patients were discharged on the fifth postoperative day.

Conclusion This study demonstrates that cesarean myomectomy to be a safe and feasible procedure in experienced hands. It offers the advantage of avoiding a second surgery in selected patients.

Keywords Cesarean myomectomy · Myoma uterus · Cesarean delivery · Myomectomy

Introduction

The myomas are the most common benign tumors of the uterus. The highest reported incidence of myomas during pregnancy is 10.7% [1]. The myomas are common during the reproductive period due to an influence of higher estrogen levels. Their increased incidence during pregnancy can be partly explained by the late age of conception [2]. Myomas in pregnancy are associated with a significantly higher risk for cesarean section (CS).

Conventionally, concomitant myomectomy during CS was considered a relative contraindication, given perceived higher complication rate. Last decade has witnessed emerging literature evidence supporting myoma excision along with CS, with acceptable results [3]. This study aims to share our experience of cesarean myomectomy during the last decade.

Materials and Methods

This study is a retrospective review of our prospectively maintained database of the last decade, from January 2008 to December 2017, at a tertiary care teaching hospital. All consecutive patients who underwent myomectomy during cesarean section, in this mentioned duration, were included.

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This formed a small subset, among the patients, having uterine myomas and pregnancy, a majority of those underwent vaginal delivery or had LSCS, without myomas removal. Those not matching the inclusion were not considered. There were no exclusions. The institutional ethical committee approved the study, and waiver of consent was obtained, in view of retrospective nature.

Data were collected from outpatient and inpatient records, operative and discharge notes. Data included maternal demographics such as age, parity, medical diseases, prior surgery, gestational age at the time of CS, type of anesthesia, indication for CS, elective/emergency and number of previous operations including CS. Myoma characteristics such as size, number, location and variety were noted. Other parameters like an indication for myomectomy, the number of incisions made and the presence of adhesions were observed. The total duration of surgery was taken as the time from the initial skin incision to skin closure. Approximate intraoperative blood loss was measured, and record of transfusion if any was noted. Postoperative drop in hemoglobin was checked at 48 h after surgery, and blood transfusion was given if the fall was more than 3 gm/dl. Postoperative complications like pyrexia (> 38-degree C) and wound infection were analyzed. Duration of hospital stay was noted, and patients were followed up after 2 weeks in the outpatient department.

Patients were counseled during the early third trimester regarding the need for myomectomy depending on the mode of delivery and consent obtained for the same.

Experienced gynecologists performed surgery with minimum of 10 years of experience. Preoperatively, half an hour before skin incision Inj. Tranexamic acid 1 gm in 100 ml isotonic saline was infused over 20 min. Except in four cases, myomas were situated at the incision site, and in other instances, CS preceded myomectomy. When myomas were located at the lower uterine segment, myomectomy was performed before delivery of the fetus. During CS, myomas were inspected for their size, number, type and location after the closure of the uterine incision (Fig. 1).

Though myomectomy was tried with a single incision, in few cases where multiple fibroids were present, more than one incision over uterus was needed. An incision was made over myoma by using monopolar cautery, and the tumor was enucleated. To achieve hemostasis, bipolar cautery was used. The defect in the myometrium was closed with 1-0 vicryl suture (Fig. 2). High-dose oxytocin infusion was continued postoperatively for 24 h to prevent bleeding.

Results

A total of twenty patients underwent myoma removal along with the cesarean operation during this period with a mean age of 30 years. Majority of patients were nulliparous (70%).



Fig. 1 Multiple fibroids over the anterior surface of uterus postdelivery of the baby



Fig. 2 Postmyomectomy, closure by baseball sutures

Common comorbidities were diabetes mellitus (40%) and hypothyroidism (20%). One except all patients received regional anesthesia (spinal/epidural); the only case needed general anesthesia due to the presence of idiopathic intracranial hypertension. Sixty-five percent of surgeries were elective operations. The details of various preoperative characteristics are shown in Table 1.

Intraoperatively, two patients had small bowel and omental adhesions at the operative site. Two patients (10%) received a blood transfusion during the procedure. The mean hemoglobin drop reported 24 h postoperative was 1.23 (± 1.16) grams. No patient warranted blood transfusion in the postoperative period (Table 2).

Majority of patients got the discharge on the fifth day of surgery. There were no incidences of postpartum pyrexia or

Table 1 Demographic and preoperative characteristic of patients ($n=20$)

Sr. No.	Variable	Value (mean ± SD)
1.	Age (in years)	30.85 ± 3.84
2.	Parity	
	(a) Nulliparity	14
	(b) Multiparity	06
3.	Gestational Age	37.3 ± 1.08
4.	Number of prior surgeries	
	(a) Nil	11
	(b) LSCS	06
	(c) Myomectomy	03

SD standard deviation, *LSCS* lower segment cesarean section

Table 2 Intraoperative and follow-up variables ($n=20$)

Sr. No.	Variable	Value (mean ± SD)
1.	Size of myoma (cm)	5.33 ± 2.08
2.	Number of myoma (mean and range)	1 (1–3)
3.	Location of tumor	
	(a) Posterior	07
	(b) Anterior	06
	(c) Right lateral wall	04
	(d) Fundal	04
	(e) Left lateral wall	03
	(f) Lower uterine segment	04
4.	Type of Myoma	
	(a) Subserous	11
	(b) Intramural	09
	(c) Submucosal	02
5.	Operating time (minutes)	79 ± 35.35
6.	Blood loss (mL)	876 ± 386.9
7.	Hospital stay (days)	5.5 ± 1.43

SD standard deviation

surgical site infections. The patients were regularly followed in the outpatient department.

Discussion

Fibroid excision along with caesarian section was considered difficult until recently. Fibroids with pregnancy might cause miscarriage, preterm delivery, premature rupture of membranes, early separation of the placenta, labor dystocia, postpartum hemorrhage and increased rate of operative deliveries. Fibroids might also undergo red degeneration and associated complications. Myomectomy during CS has not been done routinely due to the risk of uncontrolled bleeding and the possibility of hysterectomy [4]. If myomectomy is not performed along with CS patient might need another surgery to remove it, which amounts to two operations, two anesthetic exposures and increasing cost, and there is also an enhanced risk of complications caused by fibroids in a subsequent pregnancy.

The average age of mothers in our study was 30 years. Most studies have shown myomas to be more common in nulliparous women [5]. We too observed a higher incidence of myomas among nulliparous women (70%) [6]. This could be explained due to hormonal changes and reduced sensitivity of leiomyoma to estrogen receptor [7].

Similar to many other studies, the average gestational age at the time of CS was 37 to 38 weeks in our study [4, 6, 8]. We had four patients delivering at 35 to 36 weeks of gestation. Among these four patients who had delivered prematurely, three deliveries can be attributed to the presence of fibroids. Two out of these patients have already undergone fibroid removal before becoming pregnant. The possible explanation for preterm labor could be decreased distensibility of uterine myometrium due to the presence of fibroids as the pregnancy advances [9].

Though CM is to be avoided in cases of the previous myomectomy due to more chances of complications [10], we had the opportunity to do repeat CS for one of our patient who underwent CS myomectomy 3 years ago. The indication for primary CS was a transverse lie. The malpresentation was suspected to be due to a single large (8 cm), intramural fibroid located in the lower uterine segment. Incidentally, this was the largest myoma we had come across in our study. As the fibroid was situated in the incision site, myomectomy was followed by CS. During repeat CS, which was done 3 years later, there was no fibroid detected intraoperatively. There were three more cases of lower uterine segment (LUS) fibroids; if they were less than 5 cm, then myomectomy were done following the delivery of the baby. All fibroids present in the LUS were removed through the same CS incision, without any additional incision on the uterus. The same technique was suggested in several other studies [11, 12].

Three of our patients had undergone myomectomy previously in the non-pregnant state. One of the women had undergone myomectomy twice before becoming pregnant. This confirms the well-established fact that fibroids are recurrent [13]. Among the study group, the patients who had undergone previous myomectomies had a high incidence of intraoperative adhesions as expected. However, in all three patients, the surgery was uneventful with minimal blood loss and no added morbidity.

Eight of our study subjects had multiple fibroids. Six patients had fibroids of size 8 cm (largest dimension). 55% of the myomas had size > 5 cm. The smallest dimension encountered was 2.5 cm. We had more anterior wall fibroids than posterior [4]. Traditionally myomectomy is avoided in cases of fundal and cornual sites because of excessive

bleeding and possible obstruction of fallopian tubes [14, 15], but in our study, myomectomy was done regardless of the location of the myomas.

Except for a single instance of the submucosal fibroid, there was an even distribution of intramural and subserosal fibroids. Removal of submucosal fibroids during cesarean section is usually avoided as it involves resection of the full thickness of myometrium and reduced contractility might lead on to increased hemorrhage [16]. Secondary changes due to red degeneration were found in a case with single sizeable fundal fibroid. Histopathological reports of all myomectomy specimens were closely followed up, and none showed malignant change.

The same incision removed fibroids in the anterior wall and close to CS incision. Whenever possible, a minimal number of incisions were made, and adjacent ones removed through the same incision. This was done to reduce blood loss and future adhesions and improve reproductive outcome. While the operating time was more or less uniform in most of our cases, two of the cases exceeded the average operating time significantly. In these two instances, the increased operating time could be explained by multiple fibroids needing more than a single incision for removal. Other studies have also observed more than average operating time in cases of multiple fibroids [17].

Intraoperative blood transfusion was done in two cases only as they met the criteria for blood loss of more than one liter. One case had a fibroid at the incision site. The second patient who had a loss of more than two liters which was the largest in our series had three fibroids at different locations and they were removed by as many incisions. Despite intraoperative transfusion and other pharmacological measures to attain hemostasis, she had a significant drop in hemoglobin at 48 h postoperatively and needed another transfusion. She had no other postoperative morbidity like pyrexia or ileus and was discharged on the fourth day after surgery.

CM is not done routinely, because of the associated hemorrhage and the need for the hysterectomy [15]. Specific hemostatic techniques have been advocated by various authors [18, 19], but we have followed tranexamic acid infusion, vasopressin instillation, uterine artery ligation, electrocautery and high-dose oxytocin. There was not a single case which needed a hysterectomy in our study of twenty cases, though patients were counseled regarding the possibility in the prenatal period due to intractable hemorrhage. We also found no increased postoperative morbidity or expanded hospital stay in any of our patients who underwent CM as was observed by Dam Hye Kwon et al. [8].

There are a few limitations to our study. Its retrospective design coupled with proportionally small sample size limits generalization of results. This study also lacks long-term follow-up of these patients and thus to highlight effect on future pregnancies. Additionally, as this study was conducted in a tertiary level medical college setup with available expertise, the results cannot be generalized, especially in comparison with smaller installations or in the absence of an expert.

We author would like to stress this to the readers that routine use of myomectomy during CS is neither advisable nor been followed in the current study. The CM should be carefully planned for selected patients in an appropriate setup and expertise. A similar conclusion is drawn by a recent review on this topic [20], recommending standardization of best practices for CM, with the availability of more robust data and in the presence of necessary facilities.

Conclusion

Overall, our series has shown CM to be a safe and feasible procedure in well-selected patients in a tertiary care center with experienced hands. It can offer an advantage of avoiding a second intervention in selected patients. Appropriate hemostatic techniques can curtail hemorrhage which is the most severe complication. Long-term morbidity concerning future reproductive outcome should be addressed in a study with large participants and reliable design.

Authors' Contributions All authors have a significant contribution for preparation of this manuscript, following ICMJE Guidelines. First and second authors share the first position.

Compliance with Ethical Standards

Conflict of interest No author has any potential conflict of interest.

Ethical approval The current study is approved by the responsible institutional ethics committee for human research and fully comply with the declaration of Helsinki for guideline on human research.

References

1. Laughlin SK, Baird DD, Savitz DA, et al. Prevalence of uterine leiomyomas in the first trimester of pregnancy. Obstet Gynecol. 2009;113(3):630–5.
2. Tian J, Hu W. Cervical leiomyomas in pregnancy: report of 17 cases. Aust N Z J Obstet Gynaecol. 2012;52(3):258–61. https://doi.org/10.1111/j.1479-828X.2012.01414.x.
3. Sparić R, Kadija S, Stefanović A, et al. Cesarean myomectomy in modern obstetrics: more light and fewer shadows. J Obstet Gynaecol Res. 2017;43(5):798–804.
4. Incebiyik A, Hilali NG, Camuzcuoglu A, et al. Myomectomy during caesarean: a retrospective evaluation of 16 cases. Arch Gynecol Obstet. 2014;289(3):569–73.
5. Sparic R, Mirkovic L, Malvasi A, et al. Epidemiology of uterine myomas: a review. Int J Fertil Steril. 2016;9(4):424–35.
6. Kanthi JM, Sumathy S, Sreedhar S, et al. Comparative study of cesarean myomectomy with abdominal myomectomy in

terms of blood loss in single fibroid. J Obstet Gynecol India. 2016;66(4):287–91. https://doi.org/10.1007/s13224-015-0685-x.
7. Wise LA, Palmer JR, Harlow BL, et al. Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African–American women: a prospective study. Am J Epidemiol. 2004;159(2):113–23.
8. Kwon DH, Song JE, Yoon KR, et al. The safety of cesarean myomectomy in women with large myomas. Obstet Gynecol Sci. 2014;57(5):367. https://doi.org/10.5468/ogs.2014.57.5.367.
9. Chen Y-H, Lin H-C, Chen S-F, et al. Increased risk of preterm births among women with uterine leiomyoma: a nationwide population-based study. Hum Reprod. 2009;24(12):3049–56.
10. Sparić R, Berisavac M, Buzadzić S, et al. Complications during cesarean delivery in a patient with two previous myomectomies. Acta Chir Iugosl. 2013;60(1):99–100.
11. Yellamareddygari S, Chakrabarti M, Ravuri S, et al. Leaving fibroids at caesarean section, is it safe? Gynecol Surg. 2010;7(2):173–5. https://doi.org/10.1007/s10397-008-0441-7.
12. Mahendru R, Sekhon P, Gaba G, et al. At times, myomectomy is mandatory to effect delivery. Ann Surg Innov Res. 2011;5(1):9.
13. Ande ABA, Ehigiegba AE, Umeora OUJ. Repeat myomectomy at caesarean section. Arch Gynecol Obstet. 2004;270(4):296–8.
14. Celal K, Hülya Ç. The evaluation of myomectomies performed during cesarean section in our clinic. Niger Med J. 2011;52(3):186.
15. Song D, Zhang W, Chames MC, Guo J. Myomectomy during cesarean delivery. Int J Gynecol Obstet. 2013;121(3):208–13. https://doi.org/10.1016/j.ijgo.2013.01.021.
16. Gbadebo AA, Charles AA, Austin O. Myomectomy at caesarean section: descriptive study of clinical outcome in a tropical setting. J Ayub Med Coll Abbottabad. 2009;21(4):7–9.
17. Ramesh Kumar R, Patil M, Sa S. The utility of caesarean myomectomy as a safe procedure: a retrospective analysis of 21 cases with review of literature. J Clin Diagn Res. 2014;8(9):OC05–8.
18. Shavell VI, Thakur M, Sawant A, et al. Adverse obstetric outcomes associated with sonographically identified large uterine fibroids. Fertil Steril. 2012;97(1):107–10.
19. Lee J-H, Cho D-H. Myomectomy using purse-string suture during cesarean section. Arch Gynecol Obstet. 2011;283(S1):35–7.
20. Chauhan AR. Cesarean myomectomy: necessity or opportunity? J Obstet Gynecol India. 2018;68(6):432–6. https://doi.org/10.1007/s13224-018-1114-8.

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First Trimester Cervical Length And Pre Term Birth

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Abstract: ***AIMS & OBJECTIVE:*** *To assess the 1st trimester cervical length by Transvaginal USG as a predictor for spontaneous preterm birth.* ***MATERIALS & METHODS:*** *This cross sectional study was conducted from July 2012 to December 2012 in the antenatal clinic at PSGIMSR Hospitals. About 100 antenatal women who met the inclusion and exclusion criteria were enrolled in the study, the first trimester transvaginal ultrasonography was taken. These patients were followed up to determine gestational age at delivery.* ***RESULTS:*** *The data was entered into MS-Excel sheet and statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 19. All reported p-values are two-tailed, and confidence intervals were calculated at the 95% level. The data was presented using frequencies, percentages, descriptive statistics followed by charts, histogram and graphs.*

CONCLUSION: *Cervical length by first trimester transvaginal ultrasonography is not a predictor for spontaneous preterm birth.*

KEYWORDS: *first trimester ultrasound, cervical length, preterm birth*

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I. Introduction

Preterm delivery (PTD) defined by World Health Organization as birth before the completion of 37 weeks' of gestation, is the leading cause of perinatal mortality and long-term morbidity [1-3]. The incidence of PTD in ranges from 5% to 18% in developing countries and 5 to 10 % in developed countries [4-8].

Preterm birth (PTB) is the main cause of neonatal death and neurological handicap in children [9-11] . Consequently prediction and prevention of this complication is a major challenge in pregnancy care. Evaluation of the cervix has been used as a tool to predict Preterm birth based on the concept that the cervix acts as an anatomic marker of the underlying pathologic process leading to preterm delivery. The cervical length has been measured using a digital examination in the past. Investigations using transvaginal ultrasound measurement as the standard confirmed that digital examination underestimates cervical length and the majority of studies found that ultrasound assessment of cervical length is superior to clinical examination for the prediction of PTB.[6]

Transabdominal ultrasonography has limitations for the measurement of the cervix because the fetal presenting part may obscure the cervix and the full bladder may cause a lengthened measurement of the cervical canal. Transvaginal sonography (TVS) is the imaging modality of choice for measuring length of the uterine cervix. TVS is simple, reproducible acceptable to patients and is inexpensive. It also provides high-quality images of the cervix, enables the assessment of the cervical length and funneling as defined as dilatation of the internal cervical os, when present, and has little or no risk of carrying vaginal micro organisms into the cervical canal as the probe is placed in the vaginal fornix.

In the literature there is evidence that the detection of a short cervix in the late second trimester by means of TVS is a strong predictor of PTD [12,13]. Nevertheless, the role of this method as a screening tool to predict PTD before 16 weeks' gestation is still controversial [4].

The purpose of our study was to determine whether there is a relationship between the first trimester cervical length which was measured between 11-14 weeks in a low risk patients with singleton pregnancies and the risk of spontaneous preterm birth.

II. Aim

To assess the 1st trimester cervical length by Transvaginal USG as a predictor for spontaneous preterm birth.

III. Inclusion And Exclusion Criteria

The inclusion criteria is all antenatal patients who attended the antenatal clinic between 11 to 14 weeks gestational age and the exclusion criteria is with previous history of preterm delivery, multiple pregnancy, history of cervical conisation and patients who underwent cervical encerclage.

IV. Materials & Methods

This cross sectional study was executed between the period of July 2012 – Dec 2012 at PSG IMSR Hospitals after obtaining ethical approval, who met the inclusion and exclusion criteria in about 100 patients.
The transvaginal ultrasonographic examinations were performed between 11 to 14 weeks of gestation using Philips USG system with a 5MHz TVS probe. The antenatal women were asked to empty the bladder just before transvaginal USG examination and the transducer was placed in the anterior fornix without pressure to avoid any deformation or enlongation of the cervical canal. The entire length of the cervical canal is identified by the sonolucent endocervical mucosa and the callipers were used to measure the distance between internal and external os. These patients were followed up to determine the gestational age at delivery.

V. Results And Observations

The data was entered into MS-Excel sheet and statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 19. All reported p-values are two-tailed, and confidence intervals were calculated at the 95% level. The data was presented using frequencies, percentages, descriptive statistics followed by charts, histogram and graphs. Total number of antenatal women who underwent transvaginal ultrasonography between 11-14 weeks were 100.

TABLE 1 : MATERNAL DEMOGRAPHIC DATA AND PREGNANCY OUTCOME

CHARACTERISTICS	PRE TERM DELIVERY < 37 WEEKS (N=34)	DELIVERY ≥ 37 WEEKS (N=66)
Maternal age in years	25.00 (18-37)	26 (18-40)
Maternal weight in kilogram	57.15 (35-86.2)	52.35 (36.5-80)
Maternal height in centimeter	155 (138-169)	155.5 (143-172)
Gestational age	37 (28-37)	39 (38-40)

Table 1 describes the maternal demographic data and pregnancy outcome among the studied population into two groups < 37 weeks (34%) and term delivery ≥ 37 weeks (66%).
The mean ± SD GA at transvaginal ultrasound was 12 weeks ± 2 days (range, 11 +0 weeks to 14 + 1 week). The mean ± SD maternal age of the patients who underwent transvaginal ultrasound was 26.19 ± 4.3 years among which who delivered < 37 weeks is 26.29 ± 4.5 years (range 18 to 37 years), > 37 weeks is 26.14 ± 4.3 years, (range 18 to 40 years)

Figure 1 : Histogram

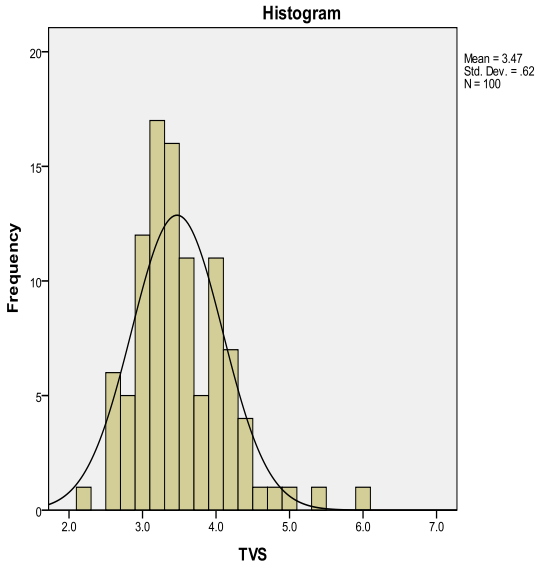


Figure 1 – Histogram shows normal distribution (solid line) and frequency distribution (bars) of cervical length at 11-14 weeks gestation [Mean ±SD,3.47±0.62; n=100]

TABLE 2 : TRANSVAGINAL CERVICAL LENGTH AND GESTATIONAL AGE AT DELIVERY

TVS CERVICAL LENGTH	GESTATIONAL AGE		Total
	≤ 37 WEEKS	≥ 37 WEEKS	
2.5 - 3.0 cm	12	12	24
	50.0%	50.0%	100.0%
>3 cm	22	54	76
	28.9%	71.1%	100.0%
Total	34	66	100
	34.0%	66.0%	100.0%

Table 2 shows the pregnancy outcome between the transvaginal cervical length and the gestational age at delivery which was statistically insignificant with P value of > 0.05
CHI-SQUARE TEST USING SPSS 19 ; P - >0.05, NOT SIGNIFICANT.

TABLE 3 : PARITY AND TRANS VAGINAL CERVICAL LENGTH

TVS CERVICAL LENGTH	PARITY		
	PRIMI GARVIDA	MULTI GARVIDA	PARITY
2.5 - 3.0 cm	17	7	24
	70.8%	29.2%	100.0%
>3 cm	54	22	76
	71.1%	28.9%	100.0%
Total	71	29	100
	71.0%	29.0%	100.0%

Table 3 shows the comparison between the parity and pregnancy outcome which is again statistically insignificant of P value 0.98.
CHI-SQUARE TEST USING SPSS 19 ; P - 0.98, NOT SIGNIFICANT.

VI. Discussion

Pre term birth prediction and management is one of the most challenging problems in modern obstetrics. Cervical incompetence accounts for not more than 2% of miscarriages or pre-term delivery. Many studies have shown that cervical changes seen by TVS are associated with increased risk of preterm birth and that the length of the cervix is directly correlated to the duration of pregnancy, the shorter the cervix, greater the likelyhood of preterm delivery [14]. The mid trimester cervical length cutoff of 25mm is a good predictor of preterm delivery [4,14,15]. Moreover, patients with midtrimester cervical length of less than or equal to 15mm had 48% (19) and 52% (40) risk of early preterm birth (less than or equal to 32 weeks of gestation).

Naim et al in his study said TVS cervical length <3 cm before 16 weeks concluded that short cervix was associated with 23% risk of preterm delivery [16]. In our study although the risk for preterm delivery TVS cervical length <3cm was 35% (12), only 5.9% (2) of patients went into spontaneous preterm delivery, the remaining 23.5%(8) had premature rupture of membranes and 5.9%(2) had severe preeclampsia.

Hassan et al demonstrated that a mean cervical length of 37.5mm at 14 weeks of gestation was a weak predictor of preterm delivery than seen later in the midtrimester [17]. In our study the mean cervical length is 34.7mm between 11 to 14 weeks as shown in figure 1 histogram and found to be a weak predictor of preterm birth.

Hasegawa et al and Zorzoli et al found that there is no correlation between first trimester transvaginal cervical length and gestational age at delivery [18,19]. In our study out of 24 patient transvaginal cervical length between 2.5 to 3 cm and more than 3cm were 12 each who delivered at less than 37 weeks and more than 37 weeks GA, which is statistically insignificant, and we found that there is weak correlation between first trimester cervical length and gestational age at delivery.

Conoscenti et al in his prospective study performed TVS cervical assessment at 14 weeks of gestation as a screening test and concluded that it is not a predictor for pre-term delivery [20]. In our study also we found that first trimester cervical length by transvaginal ultrasound is not a predictor for preterm delivery.

VII. Conclusion

The first trimester cervical length by transvaginal ultrasonography is not a good predictor for spontaneous preterm birth.

ETHICAL CLEARANCE OBTAINED.
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References

[1]. International classification of diseases and related health problems.10th revision. Geneva: World Health Organization; 1992
[2]. Government Statistical Service for the department of Health. NHS Maternity Statistics. England. 2002-2003
[3]. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Kirmeyer S, Munson ML; Centers for Disease Control and Prevention National Center for Health Statistics National Vital Statistics System. Births: final data for 2005. Natl Vital Stat Rep. 2007 Dec 5;56(6):1-103 http://www.cdc.gov/nchs/data/nvsr/nvsr56/nvsr56_06.pdf
[4]. Haas DM. Preterm birth.ClinEvid (Online). 2008 Jun 2;2008. pii: 1404
[5]. Creasy RK. Preterm birth prevention: where are we? Am J Obstet Gynecol. 1993 Apr;168(4):1223-30
[6]. Copper RL, Goldenberg RL, Creasy RK, DuBard MB, Davis RO, Entman SS, Iams JD, Cliver SP.A multicenter study of preterm birth weight and gestational age-specific neonatal mortality. Am J Obstet Gynecol. 1993 Jan;168(1 Pt 1):78-84
[7]. Steer P, Flint C. ABC of labour care: preterm labour and premature rupture of membranes. BMJ. 1999 Apr 17;318(7190):1059-62
[8]. Chalmers JW, Shanks E, Stott A, Paton A. Preterm birth in Scotland. 22 April 2006. http://bmj.bmjjournals.com/cgi/eletters/332/7547/937#132366 Last accessed 10th February 2012
[9]. Centre for Maternal and Child Enquiries (CMACE): Perinatal Mortality 2008: United Kingdom. London, CMACE, 2010.
[10]. Goldenberg RL, Culhane JF, Iams JD, Romero R: Epidemiology and causes of preterm birth. Lancet 2008; 371: 75–84.
[11]. McCormick MC: The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985; 312: 82–
[12]. Iams JD, Goldenberg RL, Meis PJ et al : The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med 1996; 334: 567–572.
[13]. Heath VC, Southall TR, Souka AP, Elisseou A, Nicolaides KH: Cervical length at 23 weeks of gestation: prediction of spontaneous preterm delivery. Ultrasound ObstetGynecol 1998; 12: 312–317.
[14]. Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, Thom E, McNellis D, Copper RL, Johnson F, Roberts JM. The length of the cervix and the risk of spontaneous premature delivery.N EnglJMed1996; 334: 567–572.
[15]. Andrews WW, Copper R, Hauth JC, Goldenberg RL, Neely C,Dubard M. Second-trimester cervical ultrasound: associationswith increased risk for recurrent early spontaneous delivery.ObstetGynecol2000; 95: 222–226.
[16]. Naim A, Haberman S, Burgess T, Navizedeh N, Minkoff H.Changes in cervical length and the risk of preterm labor. Am JObstetGynecol2002; 186: 887–889.
[17]. Hassan SS, Romero R, Berry SM,Dang K, Blackwell SC, Treadwell MC,Wolfe HM. Patients with an ultrasonographic cervical length of ≤15 mm have nearly a 50% risk of early spontaneous preterm delivery. Am J ObstetGynecol2000; 182: 1458–1467.
[18]. Hasegawa I, Tanaka K, Takahashi K, Tanaka T, Aoki K,Torii Y, Kanayama N, Okai T, Saji F, Takahashi T, Sato K,Fujimura M, Ogawa Y. A prospective clinical study for theprediction of preterm delivery in a low-risk population. JMaternFetal Invest 1996; 6: 148–151.
[19]. Zorzoli
[20]. Conoscenti G, Meir YJ, Dottavio G et al. Does cervical length at 13-15 weeks gestation predict preterm delivery in an unselected population?. Ultrasound Obstet Gynecol 2003; 21:128-134.

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Surgical site infections in a tertiary care hospital: incidence, risk factors and microbiological spectrum

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Abstract

Background: Surgical site infections (SSI) are one of the major health problems throughout the world with an incidence of 3%-16%. Hospital acquired surgical site infection is further complicated by the emergence of multi drug resistant strains. SSI surveillance is an established monitoring tool and has been shown to reduce infection rates. The importance of preventing surgical site infections is well recognized since they lead to increased morbidity, prolonged hospital stay, need for readmission, high end antibiotic treatment and re-surgery. This study was undertaken to determine the incidence, risk factors, and microbiological spectrum of surgical site infections and to identify the multidrug resistant strains. Analysis of the effectiveness of the existing surveillance methods was also done.

Methods: This retrospective study was done for a period of 1-year form Jan 2016 to Dec 2016. All patients with infection following caesarean section and abdominal and vaginal hysterectomy were included. Laparoscopic surgery and patients with preexisting infection were excluded.

Results: Incidence of SSI in present study is 5.27%. The major risk factors identified were obesity, diabetes and prolonged operating time. The commonest infective organism was Klebsiella Pneumoniae in 37% of which 19% were ESBL producing and 3.8% were carbapenemase producing. E. coli was identified in 20% of isolates. The incidence of ESBL in both isolates was higher.

Conclusions: Regular audit of SSIs is a very effective tool to analyse risk factors, identify causes and plan strategies to prevent infection.

Keywords: Drug resistance, Hospital acquired, Surgical site infection, Surveillance

Introduction

Surgical site infections (SSI) are significant source of morbidity, prolonged hospital stay and recovery and rarely mortality. They complicate 3 to 16%.[1-4] of all surgeries but even these estimates are likely to be low as some infections occur after the patient's discharge from the hospital or they report to other hospitals with infection. Hospital acquired SSIs are further complicated by the emergence of multidrug resistance strains. The importance of preventing surgical site infections is well recognized since they lead to increased morbidity, prolonged hospital stay, need for readmission, high end antibiotic treatment and resurgery.[1,3]

The Center for Disease Control (CDC) defines SSI as an infection related to an operative procedure that occurs at or near the surgical incision within 30 days. This time frame is extended to 12 months if a surgical implant is used. Infections are further divided into:

- Superficial involving skin and subcutaneous tissue
- Deep involving deeper soft tissue of the incision like fascia or muscle
- Organ or space involving any part other than the incised body layers.

Surgical wounds are further classified as clean, clean contaminated, contaminated and dirty.

Most obstetric and gynaecological procedures are classified as clean contaminated as the genital tract is entered into controlled fashion and there is no unusual contamination. Some surgeries like pelvis abscess, perforated bowel or positive culture pre-op are classified as dirty. SSI surveillance is an established monitoring tool and has been shown to reduce infection rates.

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This retrospective study was conducted in the department of Obstetrics and Gynaecology at a teaching hospital in South India from the period of January 2016 to December 2016.

Inclusion criteria

- All emergency and elective obstetric and gynaec procedures like caesarean section, abdominal and vaginal hysterectomies and laparotomies were included.

Exclusion criteria

- Laparoscopic surgeries and patients who had pre-op infection.

The departmental protocol includes two visits after surgery: the first visit a week after discharge and second visit a month later. The charts were analysed at the end of a month after surgery to identify patients with infection. Their case records were analyzed for pre-op risk factors, intra-operative factors like timing of prophylactic antibiotics and duration of surgery and post-operative course. All patients with wound infection had a culture and sensitivity done. The culture report was analysed for the infecting organism and sensitivity pattern. A monthly audit of all SSIs in the department was done to determine the incidence and microbiological spectrum of the infecting organisms. Infection control practices currently in place were also reviewed and analysed to determine if there was a scope for improvement.

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The numbers of surgeries in present study period who fulfilled in present inclusion criteria were 982 caesarean sections and 497 gynaec surgeries. The results are shown in Table 1.

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C section	982	55	5.6%
Gynaec surgeries	497	23	4.62%
Total	1479	78	5.27%

The patients who had wound infection were analysed for pre-operative risk factors like obesity, diabetes, other medical disorders and intra operative factors like prolonged operating time, previous surgeries and presence of malignancy.

As shown in Table 2, obesity was the major risk factor as 48.7% of patients had BMI of >25. Other risk factors included diabetes and prior surgery with adhesions in 19.2%, anemia and hypertension was seen in 12% of patients. 9% of patients had prolonged operating time of >3 hours.

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BMI > 25	25	13	38	48.7
Diabetes	7	8	15	19.2
Prior surgery with adhesions	7	8	15	19.2
Anemia	3	7	10	12.8
Hypertension	4	6	10	12.18
Prolonged operative time (>3 hours)	1	6	7	9.0
Bronchial asthma	3	3	6	7.7
Cardiac disease	2	2	4	5.1
Malignancy	0	3	3	3.8
PROM >12 hours	2	NA	2	3.6
Prophylactic antibiotics given	Given in all patients			

All patients who had discharge from the wound had a culture taken prior to starting antibiotics. The culture report was analysed for infecting organism and sensitivity pattern. The pathogens isolated is shown in Table 3.

Figure 1 shows the month wise analysis of the organisms which were MRSA (Methicillin Resistant Staph Aureus), ESBL (Extended Spectrum Beta Lactamase) and Carbapenemase producers.

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giving an incidence of 5.6 %. 23 of patient who underwent gynaec surgeries had SSI giving an incidence of 4.62%. The total incidence was 5.27%.

This compares favorably with the reported rate of 11.8 % in the WHO review of SSIs in low and middle-income countries.[5] 93 % of these were superficial infections. Three patients had wound debridement and re-suturing under anaesthesia. One patient had a pelvic collection that got infected. However, it drained spontaneously through the vagina.

SSIs are hospital acquired infections and their occurrence is dependent on many factors. The profile of patients undergoing surgery is also changing. There is increase in the number of patients with multiple co-morbidities and in the older age group. Preoperative factors include patient characteristics like obesity, diabetes, other medical disorders, prior surgery and steroid use. Other pre-operative factors include skin cleaning and hair removal techniques.

Intra operative factors include timing and dose of prophylactic antibiotics, abdominal and vaginal preparation, duration of surgery and technique of wound closure. Post-operative factors include glycemic control in diabetic patients and presence of anemia and blood transfusion.

Table 2 shows the pre-op and intra-op risk factors identified in present study. A systematic review of 57 studies by the WHO from both high income and low and middle income countries identified high body mass index (BMI) , diabetes and prolongation of duration of surgery as risk factors associated with increased risk of SSIs .In patients with high BMI, various factors like increase in adipose tissue, need for larger incision, decreased circulation in fat tissue and increase in local tissue trauma related to retraction contribute to the increased SSIs in these patients. Obese patients also have an altered hemostatic balance and decreased immune function.

Hyperglycemia in patients with diabetes is a well-known risk factor for infections including SSIs. A study by Al-Niaimi et al compared 2 groups of post-operative patients after gynaecological oncology surgery. Patients whose blood sugar > 150mg/dl were managed either with intermittent subcutaneous insulin injection or insulin infusion. Patients who were on insulin infusion had significantly lower infection rates (19% versus 29% P=.001).[6] Hyperglycemia can have several deleterious effects on host immune functions, stimulation of inflammatory markers, increased thrombotic activity and endothelial cell dysfunction. This is of importance even in non-diabetics as stress hyperglycemia can have the same effects.

Prolonged duration of surgery was associated with increased incidence of SSIs in the majority of studies. There was also a linear correlation between operative time and the likelihood of SSIs. The reasons postulated for this are:

1. With increased operative time the patient's open incision is exposed to the environment for a longer time.
2. Tissue desiccation is more.
3. Tissue concentration of antibiotics will decrease as the procedure continues.

However, it is important to remember prolonged surgery may also mean more complex procedures and other intra operative factors like adhesions which by themselves can increase the likelihood of infection.

Other risk factors included anemia which diminishes the resistance to infection in 12.8% prolonged PROM >24 hours in 3.6% of caesarean sections and malignancy in 3.8%.

Other risk factors considered important in SSI in a study by Devjani et al included pre-operative hospital stay with exposure to procedures, therapies and hospital micro flora and administration of blood products. Allogenic blood products have immunomodulatory effects that may increase the risk of post-op infections.[7]

The prophylactic antibiotic policy at present in the hospital is administration of Injection Cephazolin 1 gm given within one hour of skin incision and the second dose 6 hours later. All the patients received the prophylaxis however the time was >1 hour in few patients. David Classen et al reported that administration of antibiotics within a 2-hour period before an operation was associated with the lowest infection rate.[8]

On analyzing the culture report, Table 3 shows the organisms isolated in present patients. *Klebsiella pneumonia* was seen in 29 patients (37.2%) of these 51% were ESBL producers and 10% were carbapenamase producers. The second commonest organism was E coli in 16 patients (20.5%) of these 43% were ESBL producing. Pseudomonas was isolated in 10%. Routine anaerobic cultures were not performed. More than one organism was isolated in 31 out of 78 patients (39.7%).

In a study conducted by Devjani De et al the most frequently isolated organism was Acinetobacter species in 32% followed by *Staphylococcus aureus* in 22.3%.[9] In mild infections, patients were started on Levofloxacin and Metrogyl pending the culture report. In more severe infections Cefotaxime and Metrogyl or Piperacillin/ Tazobactam was started.

The resistance pattern of the infecting organism was also analysed. Figure 1 and Table 4 shows the number and percentage of drug resistant organisms. MRSA infections are of concern and there is need to screen both the patients and the health care workers involved for asymptomatic nasal carriers. In 51 out of 78 patients

(65%) infection occurred after discharge from the hospital. This may be due to the policy of early discharge from the hospital. So, post discharge surveillance is important to arrive at a correct estimate of the magnitude of the problem. A WHO review on Global guidelines for SSI prevention reported that 13% to 71% of infections occurred after discharge from the hospital. Rosenthal et al determined that 63.8% of SSIs were detected in hospital and 36.2% after discharge.[10]

Surveillance of SSIs also includes a review and analysis of the preventive strategies in place in the hospital. Prevention of SSIs requires a multifaceted approach including proper air conditioning and ventilation engineering, appropriate cleaning and disinfection of the operating theatres and proper sterilization of instruments. Proper safeguards for checking sterilization of instruments, maintenance of OR discipline and sustained education of all OR personnel regarding safe practices are no less important.[11] Hence every hospital should have protocols for prevention of SSIs and follow continuous surveillance of infections.

As part of this, the hospital infection control committee met on monthly basis. The rates of various hospital acquired infections were discussed in the monthly hospital infection control committee meetings. SSI surveillance by surgical staff can also be cross checked by the infection control team.[10] In addition to the SSI rates in various surgical specialties, ventilator associated pneumonia and catheter associated urinary tract infection and blood stream infection rates were also discussed. Environmental surveillance included air sampling done by settle plate in all OTs and the results were analyzed. The incidence of multi drug resistant organisms in all areas in the hospital was analysed and compared to the previous months. To audit the usage of antibiotics, patients on high end antibiotics was analysed department wise and feedback was disseminated to the concerned department. Hand hygiene compliance by health care workers was also analysed in all acute care areas and feedback disseminated.

The information on Hospital Acquired Infection in developing countries is limited because of the lack of national surveillance systems.[12] Most of the reported studies are from single hospital and therefore are not representative of the entire country. Prevention of hospital acquired infection is one of the key agendas of various international bodies like the WHO, CDC and others.

Both the WHO and Center for Disease Control and Prevention guidelines for prevention of surgical site infection (2017) included the following strong recommendations[5,13]:

- Patients are advised to bathe with soap or antiseptic agents at least the night before surgery.
- Skin preparation: Hair removal if required is done only by clipping Shaving is strongly discouraged at all times Intra op skin preparation with alcohol-based chlorhexidine gluconate is recommended.
- Prophylactic antibiotics should be administered 1 hour before skin incision so that serum and tissue concentration are established before the incision is made. For clean and clean contaminated procedures additional prophylactic antimicrobial agents should not be administered after the surgical incision is closed in the operating room even in a presence of a drain. Topical antimicrobial agents should not be applied on the surgical incision.
- Protocols for perioperative glycemic control to be implemented using blood glucose target level of <200mg/dl in patients with or without diabetes.
- Perioperative normothermia should be maintained in all patients. Increased fraction of inspired oxygen should be administered during surgery and after extubation in the immediate postoperative period for patients with normal pulmonary function undergoing general anesthesia.

Transfusion of blood products should not be withheld from surgical patients as a means to prevent SSIs.

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Prevention of SSI is an important part of providing optimum care for patients. Throughout the world there is a growing concern about patient safety. This prompted the WHO to launch the World Alliance for patient safety. Prevention of health care acquired infection is the one of the first targets of the Alliance. “Clean care is safer care” was launched in October 2005. A key objective of the programme is to launch a feasible practical approach to improve hand hygiene in healthcare globally. Continuous audit and implementation of infection prevention strategies should part of the standard protocols of every hospital. It is challenging and requires dedication of both time and resources. This includes simple things like hand hygiene, pre-operative bathing and skin preparation that are no less important than tracking of multi drug resistant organisms and usage of high end antibiotics.

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1. Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F, D'errico MM, et al. Epidemiology

and microbiology of surgical wound infections. J Clinical Microbiol. 2000;38(2):918-22.
2. Mulu W, Kibru G, Beyene G, Damtie M. Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. Ethiopian J Health Sci. 2012;22(1):7-18.
3. Owens CD1, Stoessel K. Surgical site infections: epidemiology, microbiology and prevention. J Hosp Infect. 2008;70 Suppl2:3-10.
4. Saeed KB, Greene RA, Corcoran P, O'Neill SM. Incidence of surgical site infection following caesarean section: a systematic review and meta-analysis protocol. BMJ Open. 2017;7(1):e013037.
5. WHO-Global Guidelines for the Prevention of Surgical Site Infection. World Health Organization. ISBN 978 92 4 154988 2. Available at: http://www.who.int/infection-prevention/publications/ssi-prevention-guidelines/en/
6. Steiner HL, Strand EA. Surgical-site infection in gynecologic surgery: pathophysiology and prevention. Am J Obstet Gynecol. 2017;217(2):121-8.
7. De D, Saxena S, Mehta G, Yadav R, Dutta R. Risk factor analysis and microbial etiology of surgical site infections following lower segment caesarean section. Int J Antibiot. 2013;2013.
8. Classen DC, Evans RS, Pestotnik SL, Horn SD, Menlove RL, Burke JP. The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection. New England J Med. 1992;326(5):281-6.
9. De D, Saxena S, Mehta G, Yadav R, Dutta R. Risk factor analysis and microbial etiology of surgical site infections following lower segment caesarean section. Int J Antibiotics. 2013;2013.
10. Rosenthal R, Weber WP, Marti WR, Misteli H, Reck S, Dangel M, et al. Surveillance of surgical site infections by surgeons: biased underreporting or useful epidemiological data?. J Hospital Infect. 2010;75(3):178-82.
11. Woodhead K, Taylor EW, Bannister G, Chesworth T, Hoffman P, Humphreys H. Behaviours and rituals in the operating theatre: a report from the Hospital Infection Society Working Party on Infection Control in Operating Theatres. J Hospital Infect. 2002;51(4):241-55.
12. Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011;377(9761):228-41.
13. Berríos-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, et al. Centers for disease control and prevention guideline for the prevention of surgical site infection, 2017. JAMA Surg. 2017;152(8):784-91.

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A retrospective analysis of ectopic pregnancy in a tertiary care hospital

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Abstract

Background: Ectopic pregnancy (EP) is a common, life threatening emergency during the first trimester and a significant cause for maternal morbidity and mortality. In any woman of reproductive age presenting with abdominal pain and vaginal bleeding, ectopic pregnancy should be considered. The objectives of the present study is to determine the incidence, risk factors, clinical presentation, type of ectopic pregnancy, treatment, morbidity and mortality.

Methods: Retrospective analysis of case sheets of patients admitted with ectopic pregnancy at PSGIMSR, Coimbatore during the period July 2011 to June 2016 was done. The details noted were age, parity, risk factors {previous abortion, previous EP, previous pelvic surgery, intrauterine contraceptive device, tubectomy, history of pelvic inflammatory disease}, treatment for infertility, clinical presentation, ultrasound findings, investigations including beta HCG value and hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity.

Results: During the five year, there were 12,407 deliveries at our hospitals and 88 cases of ectopic pregnancies were managed. The incidence is 6.6/1000 deliveries. Among them 44% belonged to the age group of 25-30 years and multi gravida were 73.8%. Most common risk factors were previous abortion (23.8%) and previous surgeries including caesarean section, tubectomy, appendicectomy, tubal microsurgery (23.8%). Most common presentation was pain abdomen (85.2%). Out of the 88 cases, 73 patients were managed surgically (82.9%) and 11 cases were managed successfully with methotrexate (12.5%). Three of them underwent conservative management and one had Uterine Artery Embolization.

Conclusions: Early diagnosis based on risk factors and timely intervention plays a main role in reducing morbidity and mortality associated with ectopic pregnancy.

Keywords: Beta HCG, Ectopic pregnancy, Methotrexate, Risk factors, Salphingectomy Transvaginal USG

Introduction

An ectopic pregnancy is implantation of fertilized ovum outside the normal endometrial cavity i.e, fallopian tube, ovary, caesarean scar, cervix and peritoneal cavity.[1] In the first trimester, ectopic pregnancy is the most common cause for maternal morbidity and mortality.[2] It has also been shown that EP reduces future fertility and increases the chance of subsequent EP.[3] The incidence of EP is increasing since the last decade, but the incidence of complications like rupture and morbidity are declining due to early diagnosis with serial Beta-HCG(human chorionic gonadotrophin) monitoring, transvaginal USG(ultrasonogram) and minimally invasive surgery.[4] Early diagnosis not only reduces the risk of tubal rupture but also allows for conservative and medical management.[5] Prior damage to the fallopian tube due to PID(pelvic inflammatory disease), tubal surgeries, pelvic adhesions are the major risk factors for ectopic pregnancy.[6] The other identified risk factors are,

advanced maternal age, previous EP, previous pelvic surgeries, IUCD (intra uterine contraceptive device) insertion and infertility treatment.[7-12] Identifying these risk factors among high risk mothers helps in early diagnosis and reduces the morbidity related to this condition.[13] Unfortunately, most of the patients presenting with EP do not have risk factors.

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This was a retrospective analysis of case records of patients admitted with ectopic pregnancy, done at PSG IMSR Hospital, Coimbatore from July 2011- June 2016.

Inclusion criteria

We included all the patients with suspected ectopic pregnancy who were confirmed by βHCG and transvaginal ultrasound.

Exclusion criteria

None

Methodology

The case sheets were retrieved from medical record department and the details such as age, parity, risk factors like previous abortion, previous EP, previous pelvic surgery, contraception in the form of IUCD and tubectomy, history of PID, infertility treatment, clinical presentation, investigations including beta HCG value, hemoglobin level, treatment offered, need for blood transfusion, operative findings and morbidity were collected and analyzed.

Statistical analysis

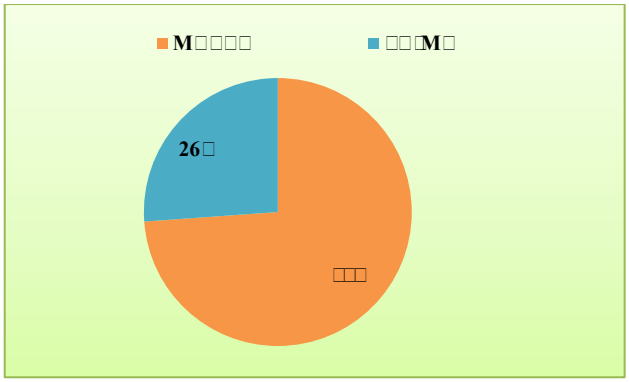
No statistical tool used.

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During the 5-year period, there were 12,407 deliveries at our institution and 88 cases of ectopic pregnancies were managed. The incidence was 6.6/1000 deliveries. Among the patients most of them belonged to the age group of 25-30 years (Table-1). 26% of patients were primigravida, 73.8% of patients were multigravida (Figure -1).

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< 25	22	25
25-30	39	44
31-35	18	20
36-40	5	5.6
>41	4	4.5



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As shown in (Table 2) 23.8% of patients had one or more spontaneous or induced abortions.23.8% of patients had undergone pelvic surgery (Caesarean section, tubectomy appendicectomy, tubal recanalistion). PID and previous ectopic pregnancy accounted for 4.5% each.

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Previous abortion	21	23.8
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Caesarean section	21	23.8
Tubectomy	14	15.9
Appendicectomy	3	3.4
Tubal recanalistion	2	2.2
Ovulation induction	7	7.9
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IUCD insertion	6	6.8
PID	4	4.5
Previous ectopic	4	4.5

Commonest presenting complaint was pain abdomen in 85.2% of patients. Other presenting complaints were Amenorrhoea in 68% and vaginal bleeding in 59% (Table-3).

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Pain	75	85.2
Amenorrhea	60	68.1
Bleeding PV	52	59

Most of them presented between 5-8 weeks of gestational age (61.3%).27.2% of patients were diagnosed before 4 weeks of amenorrhea and 11.3% were above 9 weeks. All the cases had been confirmed by USG.17 of them had Beta-HCG less than 1000 m I U /ml. A patient with heterotopic pregnancy had Beta -HCG of more than 1 lakh m I U /ml.

Commonest site of ectopic pregnancy was fallopian tube in 93% of patients (Table 4).

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[illegible]	[illegible] r [illegible]	[illegible]
Tube	82	93
Caesarean scar	3	3.4
Ovary	2	2.4
Heterotopic	1	1

The commonest site of tubal involvement was in ampulla (Table 5).

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Ampulla	60	73.1
Cornua	10	12.1
Isthmus	6	7.3
Fimbria	4	4.8
Tubal abortion	2	2.4

On receiving, 86% of patients were hemodynamically stable, 13.6% of patients were in various stages of shock. 53.6% of patients presented with ruptured ectopic, unruptured ectopic was noted in 43.9% of patients (Table 6).

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[illegible] d [illegible]	[illegible] r [illegible]	[illegible]
Ruptured	44	53.6
Unruptured	36	43.9
Tubal abortion	2	2.5

Right sided tubal ectopic was noted in 65.8%, left side tube involved in 34.1% of patients. Tubal abortion was observed in 2.5% of patients. Intra-operatively hemoperitoneum was noted in 64% of patients. Most common treatment offered for tubal ectopic was surgical, i.e. salpingectomy (Table 7).

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r [illegible]	[illegible] r [illegible]	[illegible]
Medical	11	12.5
[illegible] r [illegible]		
Laparotomy	49	59.7
Laparoscopy	9	10.9
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Laparotomy	8	9.7
Laparoscopy	2	2.4
Conservative	3	3.6

Twenty-one patients were fit into medical management, among them 12.5% of patients were managed medically with methotrexate successfully.

10 cases failed to medical management which were subsequently managed with either laparoscopic or laparotomy and salphingectomy.

3 cases were managed conservatively by observing the declining trend of Beta-HCG.

Other types of ectopic

Caesarean scar pregnancy was diagnosed in 3 patients, among them 2 patients underwent surgical excision. One patient was managed with UAE (uterine artery embolisation) followed by methotrexate. Heterotopic pregnancy following ovulation induction which resulted in twin intra uterine gestation and tubal ectopic was noted in one patient and she underwent emergency laparotomy and salpingectomy. Two patients were diagnosed as having ovarian ectopic and surgical excision was offered to both of them. Most common morbidity noted was anemia in 42%, out of which severe anemia (4-7gm) was seen in 5.6% (Table 8). Among them 28.4% of patients received blood and blood products. No surgical site infection or mortality was noted.

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[illegible] [illegible]	[illegible] r [illegible]	[illegible]
4-7 gm/dl	5	5.6
7-10 gm/dl	32	36.3
>10 gm/dl	51	58.1

[illegible]

The incidence of ectopic pregnancy among women attending emergency department with first trimester abdominal pain, bleeding or both is between 6-16 %(14). A study conducted by Shraddha Shetty et al had incidence of 5.6/1000 deliveries.[15] In our study the incidence is 6.6%/1000 deliveries which is comparable. Majority of patients were between 25-30years which is similar to the above study and Khaleeqe et al.[15,16]

As in Karki et al (17), we had similar higher incidence of ectopic pregnancies (73.8%) in multigravida. In our study, the commonest predisposing factors were previous abortion either spontaneous or induced, previous pelvic surgeries including caesarean section, history of IUCD insertion, tubectomy, history of PID and infertility treatment, which are similar to various other studies.[17,18] As in the studies by Porwal Sanjay et al, Shraddha Shetty et al, the most common symptom in our series was pain abdomen which is comparable.[15,18]

Urinary beta HCG, serum beta HCG, transvaginal USG are the diagnostic tools for diagnosing EP. USG should be the investigation of choice for symptomatic women. In asymptomatic women, serial monitoring of serum beta HCG should be done. As in the study by Shraddha Shetty et al, we also had more number of right sided EP and involvement of the ampullary portion of the tube.[15]

Heterotopic pregnancy is rare in natural conception which is around 1 in 30,000 pregnancies. But the incidence is more in case of ovulation induction or with

assisted reproduction techniques. In our study, we had a case of triplet heterotopic pregnancy following ovulation induction with clomiphene citrate. It is recommended that even patients shown on ultrasound to have intrauterine pregnancy should be given comprehensive pelvic ultrasound so that possibility of synchronous heterotopic pregnancy can be excluded, particularly in pregnancies following fertility treatment.[19]

In our study, ruptured EP was noted in 53% of cases, 43.9% of cases had an unruptured ectopic gestation. In Latchaw G et al study, the same were 59% and 41% respectively.[20]

Laparoscopy and medical therapy have now emerged as the widely used therapeutic modalities with great success in terms of reduced morbidity, shorter hospital stay and conservation of fertility.[21] However choice depends upon early identification of ectopic pregnancy and hemodynamic status of the patients.[22] In our study, 11% of patients responded well to single dose of methotrexate.

Surgery should be the treatment of choice in unstable patient.[19] In our study 13.6% were unstable and all of them were managed surgically. Commonest surgery performed was partial/total salpingectomy. We did bilateral total salpingectomy for patients who had undergone tubectomy previously and were not desirous of future childbearing. It has been extensively studied and reported that conservative management does not increase the risk of future ectopic.[15] In our study, three patients were managed conservatively by observing the declining trend of Beta HCG. Morbidity included anemia, blood transfusion and wound infection. In our study, we had 42% of patients with hemoglobin level of less than 10grams. Among them 28.4% of patients received blood and blood products. No surgical site wound infection was noted.

No maternal mortality was noted in our study which is consistent with the study by A Abbas et al.[23] Early identification of the patients with EP by a multipronged approach including high index of suspicion, risk prediction, serum beta HCG and transvaginal ultrasound helps in improving the prognosis as far as morbidity, mortality and future fertility are concerned.[24]

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Identification of underlying risk factors, early diagnosis with essential investigating modalities like beta-HCG, transvaginal USG, timely intervention with medical or surgical method will definitely reduce morbidity and mortality and improve the future reproductive outcome.

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1. Walker JJ. Ectopic pregnancy. Clin Obstet Gynecol. 2007;50:89-99.
2. Mahboob U, Mazhar SB. Management of ectopic pregnancy: a two-year study. J Ayub Med Coll Abbottabad. 2006;18:34-7
3. Chow WH, Daling JR, Cates WJ, Greenberg RS. Epidemiology of ectopic pregnancy. Epidemiol Rev. 1987;9:70-94.
4. Karaer A, Avsar FA, Batioglu S. Risk factors for ectopic pregnancy: a case-control study. Aust N Z J Obstet Gynaecol. 2006;46:521-7.
5. Barnhart KT. Clinical practice. Ectopic pregnancy. N Engl J Med. 2009;361:379-87.
6. Akande V, Turner C, Horner P, Horne A, Pacey A, British Fertility Society. Impact of Chlamydia trachomatis in the reproductive setting: British Fertility Society Guidelines for practice. Human Fertil. 2010;13:115-25.
7. Job-Spira N, Collet P, Coste J, Bremond A, Laumon B. Risk factors for ectopic pregnancy. Results of a case control study in the Rhone-Alpes region. Contracept Fertil Sex. 1993;21:307-12.
8. Bouyer J, Coste J, Shojaei T, Pouly JL, Fernandez H, Gerbaud L, et al. Risk factors for ectopic pregnancy: a comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol. 2003;157:185-94.
9. Parashi S, Moukhah S, Ashrafi M. Main risk factors for ectopic pregnancy: a case-control study in a sample of Iranian women. Int J Fertil Steril. 2014; 8:147-54.
10. Moini A, Hosseini R, Jahangiri N, Shiva M, Akhoond MR. Risk factors for ectopic pregnancy: a case-control study. J Res Med Sci. 2014; 19:844-9.
11. Ankum WM, Mol BW, Van der Veen F, Bossuyt PM. Risk factors for ectopic pregnancy: a meta-analysis. Fertil Steril. 1996;65:1093-9.
12. Barnhart KT, Sammel MD, Gracia CR, Chittams J, Hummel AC, Shaunik A. Risk factors for ectopic pregnancy in women with symptomatic first-trimester pregnancies. Fertil Steril. 2006;86:36-43.
13. Timmerman D. Predictive models for the early diagnosis of ectopic pregnancy.Verh K Acad Geneeskd Belg. 2004;66:155-71.
14. Murray H, Baakdah H, Bardell T, Tulandi T. Diagnosis and treatment of ectopic pregnancy. CMAJ. 2005;173:905.
15. Shetty KS, Shetty KA. A clinical study of ectopic pregnancies in atertiary care hospital of Mangalore, India. Innovat J Med H Sci. 2014;4:305-9.
16. Khaleeque F, Siddiqui RI, Jafarey SN. Ectopic pregnancies: A Three-year study. J Pak Med Assoc 2001;51:240-3.
17. Karki RCL, Pradhan B, Duwa S. Annual Analysis of Ectopic Pregnancy in Tertiary Care Hospital. PMJN. 2011;11:5-8
18. Gupta R, Porwal S, Swarnkar M, Sharma N, Maheshwari P. Incidence, trends and risk factors for

Ectopic Pregnancies in a tertiary care hospital of Rajasthan. JPBMS. 2012;16:1-3.
19. Maheswari S, Panicker S. Triplet heterotopic pregnancy following ovulation induction with clomiphene citrate: a case report and review of literature Int J Rerod Contracept Obstet Gynecol. 2013;2:743-5.
20. Latchaw G, Takacs P, Gaitan L, Geren S, Burzawa J. Risk factors associated with the rupture of tubal ectopic pregnancy. GynecolObstet Invest. 2005;60:177-80.
21. Jurkovie D. Ectopic pregnancy. In: Edmonds DK, editor. Dew Hurst's textbook of Obstetrics and Gynecology. 7 ed. USA; Blackwell Publishers;2007
22. Shah N, Khan NH. Ectopic pregnancy: Presentation and risk factors. J Coll Physicians Surg Pak. 2005;15:535-8.
23. Abbas A, Akram H. Ectopic Pregnancy; Audit at Maula Bakhsh Teaching Hospital Sargodha.Prof Med J. 2011;18:24-7.
24. Lozeau AM, Potter B. Diagnosis and management of ectopic pregnancy. Am Fam Physician. 2005; 72:1707-14.
25. Majhi AK, Roy N, Karmakar KS, Banerjee PK. Ectopic pregnancy--an analysis of 180 cases. J Indian Med Assoc. 2007;105:308-10.

Maheswari S, Poornima C, Lalitha N, Panicker S. Analysis of various types of ectopic pregnancies: a five-year review. Int J Reprod Contracept Obstet Gynecol 2018;7:2410-4.

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Amniotic fluid lactate levels in labour and its correlation with duration of labour, mode of delivery and Apgar score

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Abstract

Background: Inspite of good monitoring in labour, there are still adverse labour outcomes. There is a need for supplementary test for early prediction of prolonged labour and fetal hypoxia. This study was undertaken to find to find the association between intrapartum amniotic fluid lactate level and labour outcome.

Methods: 103 antenatal patients low risk at term, who entered into active labour either spontaneously or induced between September 2012 to August 2013 were included. Amniotic fluid was collected after doing ARM and lactate level was measured. Chi square test was used to find correlation between lactate level and duration of labour, mode of delivery and Apgar score.

Results: In our study, patients with duration of labour between 8-<20hours 88 % had lactate level <10mmol/l, thus intra partum amniotic fluid lactate levels had a good negative predictive value when it was compared with the duration of labour. When the mode of delivery was analysed using Chi square test there was no statistically significant correlation ($p<0.825$) with amniotic fluid lactate. Spearman Rank correlation was done to observe correlation between lactate levels and Apgar which showed a correlation of 0.20 with $p<0.05$.

Conclusions: Amniotic fluid lactate levels had better correlation with duration of labour and Apgar score. The lower the amniotic fluid lactate level, the better is the Apgar at birth. Better correlation could have been observed if amniotic fluid lactate levels were repeated every 3-4 hours during active labor. However, this was not possible due to practical difficulty in collection of sample.

Keywords: Apgar score, Duration of labour, Lactate, Mode of delivery

Introduction

Inspite of close monitoring in labour according to established guidelines, there are still adverse labour outcomes.[1,2] One of the most important causes is dysfunctional labour.

The major cause for perinatal mortality and morbidity is birth asphyxia secondary to abnormalities occurring during labor. Many trials have been done in the past for detection of the abnormalities in labor, so that timely intervention can be done to improve perinatal outcome without increasing the caesarean section rate. Maintaining a partogram is very useful for assessing the dysfunctional labor.

However this alone was not sufficient in some patients. Many labors which were shown as abnormal had normal parturition without any adverse maternal or perinatal outcome, so there is a need for supplementary tests for early prediction of adverse labor outcomes.[3,4] So research are being carried out in this direction.

Studies have shown that if there is increased muscle activity there is accumulation of lactate in response to it. In cases of prolonged labour due to exhaustion of uterine musculature and hypoxia, there is to accumulation of lactate. Hypoxia is known to reduce the force and co-ordination of smooth muscle contraction[5]. Inefficient uterine action is one of the most common causes of poor progress in labor.[6] So based on this principle assessment of lactate in the amniotic fluid will help in the diagnosis of dysfunctional labor, This method has the advantage of being more precise and objective in diagnosis of prolonged labor which can cause adverse maternal and perinatal outcome.

So, the resulting increase in the acidity in the myometrium is the cause for the decreased force of further contractions which will lead to dysfunctional labour. The increased level of lactate in the uterus is measured in the amniotic fluid sample. Therefore it is believed that the raised amniotic fluid lactate levels are mostly associated with dysfunctional labour. Partogram along with the amniotic fluid lactate levels is better than using partogram alone in predicting the need for operative intervention in dysfunctional labor.[7] Level of amniotic fluid lactate may provide useful information while assessing progress of labour. Trials have shown that the lactate levels are many times lesser in the maternal and fetal blood when compared to amniotic fluid.

According to earlier studies when the level of lactate in the amniotic fluid is normal, it indicates that the likelihood of adverse neonatal outcome is very low [8], so that patient can be allowed for normal delivery if all other parameters are normal. A higher level of amniotic fluid lactate showed that the probability for normal delivery is less, if at all present it is almost always associated with instrumental delivery, and may have abnormal CTG findings. This study was conducted to find the association between intrapartum amniotic fluid lactate levels and labour outcome. The parameters studied were Duration of labour, Mode of delivery and APGAR score at birth.

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A prospective study was conducted in the Department of Obstetrics and Gynaecology, PSG Hospitals, Coimbatore from September 2012 to August 2013.

Study population

Study group consisted of low risk pregnant women at term admitted in PSG Hospitals, who entered into active labour either spontaneously or by induction

Inclusion criteria

- Women in active labour without any obstetrical complications
- Vertex, presentation
- Term, Post-dated pregnancy

Exclusion criteria

- <37 Weeks not in active labour
- Obstetrical complication
- Biohazard
- Multiple gestation
- Absent/Ruptured Membranes

Methodology

All patients who entered into labour during the study period and who were eligible for the study were included. They were given information about the study and collection of amniotic fluid. After obtaining informed written consent regarding the study, these patients were followed up accordingly.

When the patients entered into labour either spontaneously or by induction, initial per vaginal examination was done and Bishop Score was assessed. The fetal wellbeing and the pelvis were assessed. Based on the findings patient were allowed to progress spontaneously or further augmentation was done. In induced labour per vaginal examination was repeated after 6 or 12 hrs if PgE2 gel 0.5 mg kept intracervically, 24 hours later if the labour was induced by Foleys induction with or without extra amniotic saline infusion, 6 hours later if induced with oral misoprostol 25mcg, 4 hours if augumented with vaginal misoprostol 25 mcg. Once the patient entered active labour, vaginal examination was done every 4 hours.

ARM was done when cervix is at least 3 cm dilated. With the bladder empty the patient is put in supine position with legs dorsiflexed and under aseptic precautions speculum examination is done. ARM is done under vision with good light and the amniotic fluid is collected.

Once the patient enters active labour, labour progress is monitored with partogram. If a repeat PV examination finding crosses the alert line, labour was augmented with oxytocin or in patients who were in induced labour with oxytocin; the dose used was 2.5 Units in 500 ml of Ringer Lactate infused with infusion pump. Depending upon the uterine contractions infusion was increased by 4 drops per minute every half an hour. Choice of labour analgesia was selected according to the patient's wish, either with pethidine 50 mg and inj phenergan 12.5 mg or by epidural analgesia. Entonox gas inhalation also used if needed.

The amniotic fluid of at least 2 ml was collected in a syringe after doing ARM. Amniotic fluid was transferred into a biochemistry laboratory container and it was labelled with the patient's details (name and medical record number). The amniotic fluid used in the study only if it was clear and not blood or meconium stained, as this

might alter the value of lactate. The sample reached the laboratory within 5 minutes through the pneumatic system in the hospital. The amniotic fluid sample is centrifuged in the laboratory and analysed immediately by automated lactate analyser, and the reporting was done immediately. So the delay in reporting was prevented. The result was viewed from the labour ward using hospital information system.

Once the patient entered the second stage of labour, she was encouraged to bear down. When the fetal head was seen, the obstetric and neonatology team were ready for delivery and neonatal resuscitation. Normal delivery was done either with or without episiotomy. Instrumental delivery was done either with vacuum or forceps depending upon the obstetric indication. The duration of the first and second stages of labour were noted. The baby's weight, initial, 5 minute and 10 minute APGAR scores were noted. If the patient was taken up for emergency LSCS, the same details of the baby were noted. The indication for LSCS and instrumental delivery were also noted down. All the details were entered in the study proforma and the results were analysed finally through statistical analysis.

The objectives are to correlate:

- Amniotic fluid lactate levels with duration of labour
- Amniotic fluid lactate levels with mode of delivery
- Amniotic fluid lactate levels with APGAR score

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Around 70 % of the study group whose lactate levels was 5-<8mmol/l had duration of labour between 8-<20 hours. Of the patients whose duration of labour was in 8-<20 hours, 88 % had lactate levels less than 10. The intra partum amniotic fluid lactate levels had a good negative predictive value when it was compared with the duration of labour.

In the group of 14 patients whose duration of labour >20 hours, 11 patients had normal lactate levels.

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Lactate	5-<8	Count	9	31	4	44
		% within lactate	20.5%	70.5%	9.1%	100.0%
		% within duration of labour	52.9%	43.1%	28.6%	42.7%
	8-<10	Count	6	32	7	45
		% within lactate	13.3%	71.1%	15.6%	100.0%
		% within duration of labour	35.3%	44.4%	50.0%	43.7%
	≥10	Count	2	9	3	14
		% within lactate	14.3%	64.3%	21.4%	100.0%
		% within duration labour		12.5%	21.4%	13.6%
		Count	17	72	14	103
		% within lactate	16.5%	69.9%	13.6%	100.0%
Total	% within duration of labour		100.0%	100.0%	100.0%	100.0%
	Pearson Chi-square		2.222		P=0.695	

When the duration of labour was analysed there was no statistically significant correlation between amniotic fluid lactate and duration of labour. But it had good negative predictive value. When lactate level was >10 mmol/l, 10 patients had normal vaginal delivery.

When the mode of delivery were analysed there was no statistically significant correlation between amniotic fluid lactate and mode of delivery.

When we analysed amniotic fluid lactate and Apgar score:

- In lactate levels 5-<8 mmol/l-9.1% had Apgar <7
- In lactate levels 8-<10 mmol/l-15.6% had Apgar score <7.
- But in lactate levels mmol/l >10-35.7% had low Apgar score.

However spearman rank correlation showed a correlation of 0.20 with P < 0.05. When the Apgar score was analysed there was statistically significant correlation between amniotic fluid lactate and Apgar score.

Pearson chi –square test was used to find association between categorical variables. All statistical analysis was done using SPSS Software (statistical package for social sciences).

Chi square were used to find correlation between lactate

level and duration of labour, mode of delivery and apgar score. In addition Spearman Rank correlations were also done to observe correlation between lactate levels and apgar score.

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			Normal	Vacuum	Forceps	LSCS	Total	
Lactate	5-<8	Count	30	7	1	6	44	
		% within lactate	68.2%	15.9%	2.3%	13.6%	100.0%	
		% within duration of labour	42.3%	36.8%	33.3%	60.0%	42.7%	
	8-<10	Count	31	10	1	3	45	
		% within lactate	68.9%	22.2%	2.2%	6.7%	100.0%	
		% within duration of labour	43.7%	52.6%	33.3%	30.0%	43.7%	
	≥10	Count	10	2	1	1	14	
		% within lactate	71.4%	14.3%	7.1%	7.1%	100.0%	
		% within duration labour	14.1%	10.5%	33.3%	10.0%	13.6%	
		Count	71	19	3	10	103	
		% within lactate	68.9%	18.4%	2.9%	9.7%	100.0%	
Total		% within duration of labour	100.0%	100.0%	100.0%	100.0%	100.0%	
		Pearson Chi-square	2.868[a]	P=0.825				

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Lactate	5-<8	Count	1	3	40	44	
		% within lactate	2.3%	6.8%	90.9%	100.0%	
		% within duration of labour	100.0%	20.0%	46.0%	42.7%	
	8-<10	Count	0	7	38	45	
		% within lactate	0%	15.6%	84.4%	100.0%	
		% within duration of labour	0%	46.7%	43.7%	43.7%	
	>=10	Count	0	5	9	14	
		% within lactate	0%	35.7%	64.3%	100.0%	
		% within duration labour	0%	33.3%	10.3%	13.6%	
		Count	1	15	87	103	
		% within lactate	1.0%	14.6%	84.5%	100.0%	
Total		% within duration of labour	100.0%	100.0%	100.0%	100.0%	
		Pearson Chi-square	8.376[a]	P=0.079			

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Spearman's rho	lactate	Correlation coefficient	1.000	-0.200*
		Sig. (2-tailed)	-	.043
		N	103	103
	Apgar score	Correlation coefficient	-0.200*	1.000
		Sig. (2-tailed)	0.043	-
		N	103	103
*Correlation is significant at the 0.05 level (2-tailed)				

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Amniotic fluid lactate levels were measured during active phase of labour, and the mode of delivery, duration of labour and the Apgar score were noted down and correlation was analysed with these three. Results

correlating amniotic fluid lactate levels and duration of labour: Around 70 % of the study group who had lactate level between 5-<8 mmol/l had duration of labour between 8-<20 hours. Of the patients whose duration of labour were between 8-<20 hours, 88 % had lactate levels <10 mmol/l.

The intra partum amniotic fluid lactate levels had a good negative predictive value when it was compared with the duration of labour. Results correlating amniotic fluid lactate level and mode of delivery:

When lactate levels were more than 10, only 14% had normal vaginal delivery. When the mode of delivery were analysed there was no statistically significant correlation between amniotic fluid lactate and mode of delivery (p value of 0.825). Result correlating amniotic fluid lactate level and Apgar score:

- In lactate levels 5-<8 mmol/l – 9.1% had Apgar <7
- In lactate levels 8-<10 mmol/l - 15.6 % had Apgar score <7.
- But in lactate levels > 10 mmol/l - 35.7 % had low Apgar score.

Chi square test for Apgar score was analysed P value=0.079, there was no statistically significant correlation between amniotic fluid lactate and Apgar score.

Spearman Rank correlation was also done to observe correlation between lactate levels and Apgar score and showed correlation of 0.20 ($P < 0.05$) and was found to be statically significant. Thus high lactate concentration in amniotic fluid before delivery indicated an increased risk of adverse neonatal outcome at delivery.[8]

In normal labour during contraction there is decrease in the uteroplacental blood flow which gets restored immediately after the contraction is over when the uterus gets relaxed. But when the uterine contractions become irregular, the uteroplacental blood flow gets reduced for longer period as there is lesser duration for uterine muscles to relax.

As a result feto-maternal unit gas exchange is reduced and this leads to fetal hypoxic-ischaemia and lactic acidosis. There is a hypothesis that when there is hypoxia of myometrium there is increased production of lactate which is reflected as increased concentration of lactate in the amniotic fluid. This explains the adverse neonatal outcome in patients having increased lactate level in amniotic fluid. So the labour duration by itself does not affect the neonatal outcome[1].

The neonatal outcome may not be affected if the myometrial tissue is oxygenated well. We were not able to repeat the lactate levels just before the delivery due to difficulty in collection. But if the lactate levels were repeated just before delivery then further correlation could have been found out.

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The myometrial capillary blood lactate increases in women following prolonged labour. The amniotic fluid lactate is 4-6 times higher when compared with fetal and maternal blood, which occurs mainly during dysfunctional labour. The traditional partogram is a good tool used for diagnosing dysfunctional labour. The combination of intrapartum amniotic fluid lactate levels along with WHO partogram could be used as a better tool in diagnosing dysfunctional labour.

According to our study, the intra partum amniotic fluid lactate levels had a good negative predictive value for normal labour outcome. Amniotic fluid lactate levels had better correlation with duration of labour and fetal scalp lactate has been demonstrated to have good correlation with APGAR score. The lower the amniotic fluid lactate levels, the better is the APGAR score.

Better correlation could have been observed if amniotic fluid lactate levels were analysed 4-6 hours after the initial assessment. However, this was not possible due to practical difficulty in collection of sample. Lactate analysis is a simple easy method of analyzing the labour outcome and the results of this test can be obtained very fast since its analyzed with auto analyzer. The laboratory results are also standardised since its done with the auto analyzer. The intrapartum amniotic fluid lactate levels can be correlated with umbilical cord blood lactate and fetal scalp blood lactate for further analysis.

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1. Hellman LM, Prystowsky H. The duration of the second stage of labor. Am J Obstet Gynecol. 1952;63:1223-33.
2. Altman MR, Lydon-Rochelle MT. Prolonged second stage of labor and risk of adverse maternal and perinatal outcomes: a systematic review. Birth. 2006;33:315-22.
3. Hon EH, Hess OW. Instrumentation of fetal electrocardiography. Science. 1957;125:553-4.
4. Hon EH, Petrie RH. Clinical value of fetal heart rate monitoring. Clin Obstet Gynecol. 1975;18:1-23.
5. Taggart MJ, Wray S. Hypoxia and smooth muscle function: key regulatory events during metabolic

stress. J Physiol Lond. 1998;509:315-25.
6. Steer PJ, Carter MC, Beard RW. The effect of oxytocin infusion on uterine activity levels in slow labour. Br J Obstet Gynecol. 1985;92:1120-6.
7. Wiberg-Itzel. Lactate concentration in amniotic fluid: a good predictor of labor outcome. European Journal of Obstetrics, Gynecology and Reproductive Biology. 2010;152(1):34-8.
8. Wiberg-Itzel E. Association between Adverse Neonatal Outcome and Lactate Concentration in Amniotic Fluid. Obstet Gynecol. 2011;118(1):135-42.

Chander A, Panicker S, Shri S. To find the association between intrapartum amniotic fluid lactate level and labour outcome. Int J Reprod Contracept Obstet Gynecol 2017;6:620-5.

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ORIGINAL RESEARCH ARTICLE

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MODERATE TO SEVERE THROMBOCYTOPENIA IN THE PARTURIENT

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ABSTRACT

Objectives: To analyse patients with moderate to severe thrombocytopenia in labour, to identify the etiology, assess severity, correlate with the maternal disease and management.
Methods: A retrospective study of all pregnant women admitted in labour or in obstetric critical area and found to have a low platelet count were included in a study period of 18 months from July 2013 to Dec 2014.
Results: 25 women out of 2738 deliveries were identified and commonest cause was severe pre-eclampsia with or without HELLP. Coincidental dengue with super added pre-eclampsia was found in 3 women. Severe thrombocytopenia was found in haematological conditions like ITP and aplastic anemia.
Conclusion: Low platelets or fall in platelets showed a worsening of disease condition. Platelet transfusion needs to be done if the cut off is below the desired target level. Timely administration of platelets and discrete use of SDP especially when massive platelet transfusion is required required as mandatory. Delivery should be as atraumatic as possible, avoidance of episiotomy to be practiced. Sometimes treatment can be challenging and involve multi-disciplinary approach.

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INTRODUCTION

Thrombocytopenia is the most common hemostatic abnormality in pregnancy occurring in approximately 10% of all pregnant women. Abnormalities of platelet count may either be mild when the platelet count is between 150,000 and 100,000/mm^3, moderate when the count is between 100,000 and 50,000/mm^{3}or severe when the count is < 50,000/mm^3 (1). Gestational thrombocytopenia is the commonest cause of low platelets in pregnancy, however the count rarely falls below 70,000/mm^3 and is not of much clinical significance. Moderate to severe thrombocytopenia may occur due to either pre-existing disease like immuno thrombocytopenia (ITP), Systemic lupus erythematosus (SLE), anti-phospholipid syndrome (APLA) or pregnancy related conditions like pregnancy induced hypertension (PIH), HELLP, acute fatty liver of pregnancy (AFLP), ect (Kam, 2004). In labour low platelet count is a risk not only to the mother but also to the fetus. Most patients with mild thrombocytopenia do not have any hemostatic risk and do not require treatment unless the degree of thrombocytopenia worsens.

However moderate to severe thrombocytopenia is associated with adverse effects. The threshold for significant spontaneous bleeding is a count of 10,000/mm^3 and in the case of surgical interventions or delivery the threshold is higher >50,000/mm^3. The underlying disorder may manifest as thrombocytopenia and warrant immediate termination. The obstetrician concern is how to obtain hemostasis in the event of delivery and may involve multidisciplinary approach. Many articles describe thrombocytopenia in pregnancy however in labour only few references could be found. The aim of this article is to analyse the etiology of thrombocytopenia in labour, clinical features, management, platelet transfusions and fetal outcome.

MATERIALS AND METHODS

A retrospective study of all pregnant women admitted in labour or in obstetric critical area and found to have a low platelet count were included in a study period of 18 months. Only women with counts less than 1,00,000/mm^3 were included. The cases were analysed for the following:

- Etiology
- Clinical features
- Platelet count on admission
- Correlation with platelet count and maternal disorder.
- Need for platelet transfusion
- Gestational age at presentation.
- Outcome of pregnancy.

The condition of the baby in terms of gestational age, live birth or stillbirth were also assessed. Problems during transfusion were also assessed. The criteria for severe pre-eclampsia were blood pressure > 160/110mmhg, proteinuria +++ or more, oliguria, visual disturbance, epigastric pain, thrombocytopenia or impaired liver function.

Criteria for HELLP were

- Hemolysis as shown by peripheral smear or elevated billirubin > 1.2mg/dl
- Elevated liver enzymes : AST > 72IU/L, LDH > 600 IU/L
- Low platelets : count < 100,000/mm^3.

Management of these women, when and how they were terminated, type of transfusions and problems encountered were also analysed.

RESULTS

Table 1. Analysis of etiology

Cause	Number of cases
Severe pre-eclampsia with and without HELLP	18
PIH with Dengue	3
Megaloblastic anemia	1
ITP	1
Aplastic anemia	1
DIC	1
Total cases	25

Table 2 : Clinical features – combination of clinical features were seen

Clinical feature	Number of cases
Elevated BP	21
HELLP	7
Severe anemia	2
Eclampsia	4
Abruption	2
Renal failure	4
Bleeding episodes	6
Fever	3
Abdominal distention	1

Table 3. Correlation between platelet count on admission and diagnosis

Platelet Count	PIH	PIH with Dengue	Megaloblastic anemia	ITP	Aplastic anemia	DIC
100,000-50,000	10		1			1
50,000-20,000	6					
20,000-10,000	2	1		1		
<10,000		2			1	

The total number of deliveries were 2738 in the study period. The number patients with severe to moderate thrombocytopenia were 25.

The etiology was predominantly PIH seen in 18 women. PIH with super added dengue was seen in 3 women. Hematological disorders like ITP, aplastic anemia and megaloblastic anemia were seen in one each. One patient was found to have hemoperitoneum after caesarean and was found to have DIC. The clinical features at presentation were predominantly those of severe pre-eclampsia as shown in table 2. Combination of features like elevated blood pressure, HELLP, eclampsia, abruption and renal failure were seen. Bleeding episodes like haematuria, malena, bleeding from IV sites, bleeding gum, purpuric spots were seen in 6 women. Fever with elevated BP, abdominal distention following caesarean were also seen.

Table 3 shows the correlation between the platelet count on admission and diagnosis. The platelet count was moderate thrombocytopenia in 12 women, severe thrombocytopenia in 13 women. In the severe thrombocytopenic group 3 women had counts <10,000 cells/mm^3 and 4 women had counts between 10,000 to 20,000 cells/mm^3. In the PIH group platelet count was moderate thrombocytopenia in 10 out of 18 women. 6 women had counts between 50,000 to 20,000 cells/mm^3 and only 2 women had counts between 20,000 to 10,000 cells/mm^3. In patients with dengue and superadded PIH (3 women) the platelet count was <20,000 cells/mm^3 in all. The woman with megaloblastic anemia and dilutional coagulopathy had counts of 60,000/mm^3. Woman with ITP and aplastic anemia had very low counts <20,000cells/mm^3 and <10,000cells/mm^3 respectively. On analysis of platelet transfused in PIH women only 8 received transfusion and 10 did not. All women with dengue received multiple transfusions of 20 to 24 units. The woman with ITP received 44 units of platelet transfusion and the women with aplastic anemia received 40 units of platelets and 10 units of single donor platelets (SDP) as shown in table 4. There was one spontaneous expulsion at 26 weeks of pregnancy, live births were seen in 19 women and 5 women had IUD or stillbirth. 8 women delivered before 34 weeks and 16 women delivered 34 to 38 weeks of pregnancy. Table 5 shows the gestational age at presentation and fetal outcome.

DISCUSSION

Pregnancy is a hypercoagulable state with increased level of clotting factors. However platelet count declines as pregnancy advances. A platelet count < 150,000/mm^3 occurs in 1% of population but occurs in 6 to 12% of pregnancy women at term (McCrae, 2003; Boehlen, 2000). Approximately 1% of all pregnancy women have a count <100,000/mm^3 and about 1 in 1000 to 2000 have a count <50,000/mm^3. In our series also the incidence was similar around 1% of all patients had counts <100,000/mm^3 and severe thrombocytopenia was seen in 4-5/1000 women. In general, patients with a platelet count < 20,000//mm^3 are at risk of spontaneous bleeding. Platelet counts between 20,000 to 50,000/mm^3 have a risk of bleeding during procedures or delivery (Andra, 2013). Patients with counts >50,000/mm^3 generally have minimal risk of bleeding. PPH is the leading cause of maternal mortality (Lale, 2014) and when a patient enters the labour room with a low platelet there is great fear as to whether she would bleed during delivery, how much and how to prevent PPH. Therefore there is great concern to ascertain the diagnosis and immediate treatment. In our study pre-eclampsia was the commonest cause of severe and moderate thrombocytopenia.

Table 4: Transfusion chart

Platelet Count	PIH		Dengue		Megaloblastic anemia		ITP		Aplastic anemia		DIC	
	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
100,000-50,000	2	8			1						1	
50,000-20,000	4	2										
20,000-10,000	2		1				1					
<10,000			2						1			

R – Received, NR – Not Received

Table 5. Gestational age at presentation & fetal outcome

Gestational age	PIH	Others	IUD/Still birth
< 28 weeks	1	0	1
28 to 34 weeks	7	1	3
34 to 38 weeks	13	3	2
>38 weeks	0	0	0

Pre-eclampsia is said to be present in 21% of maternal thrombocytopenia[6]. Thrombocytopenia occurs in 50% of pre-eclampsia and occasionally precedes other manifestation of the disease. A decrease in platelet count is considered as an early sign of worsening of pre-eclampsia and may occur even before other clinical manifestation of disease are apparent (McCrae, 2003). The severity of thrombocytopenia directly correlates with the severity of the lesion. HELLP syndrome is often considered to be a variant of pre-eclampsia and in 1982 Weinstein coined the term HELLP and also stated that its presence is an indication for delivery as there is increased risk of maternal and fetal mortality (Weinstein, 1982). The hallmark of HELLP is microangiopathic hemolytic anemia, elevated liver enzymes and low platelet count. The reported cutoff value have ranged from 75,000/mm^3 to 229,000/mm^3 with a level of <100,000/mm^3 most often cited. HELLP may be complete with all the elements or partial with one or two components. HELLP occurs in 0.5 to 0.9 of all pregnancies and in 20% of all cases with severe pre-eclampsia.

The Mississippi Triple class system classifies this syndrome based on platelet counts (Joshi, 2010).

- Class I< 50,000/mm^3
- Class II50,000 to 1,00,000/mm^3
- Class III1,00,000 to 1,50,000/mm^3

In our study of 21 women with PIH, 7 women had HELLP, 5 belonging Mississippi Class I and 2 to Class II. Abruption was found in 2 and eclampsia was found in 4. HELLP, abruption and eclampsia require immediate termination irrespective of the gestational age. All our patients were terminated before 38 weeks of pregnancy, most of them were early or late preterm. Only one patient expelled at 26 weeks. M Sibai et al also states the same in his analysis of 442 pregnancies with HELLP syndrome [9]. In a study of 437 women who had 442 pregnancy with HELLP syndrome, serious maternal morbidity including DIC (21%), abruption 16%, ARF 8%, Pulmonary edema 6%, liver haematoma 1%, retinal detachment 1%. 55% of patients required blood component therapy and 2% required laparotomy for bleeding. Maternal mortality was 1%. Of 21 patients with low platelets with PIH, transfusion was done only in 11 patients, 9 of them had counts <50,000/mm^3 and only 2 had counts >50,000/mm^3 - one had 55,000/mm^3 and other had 62,000/mm^3, since LSCS had to be performed platelet transfusion was done as per British guidelines. Platelet count of > 80,000/mm^3 is recommended for epidural anesthesia by the British committee for the standards in hematology [10]. The count that was aimed in our patients was 50,000/mm^3 for vaginal delivery and 80,000/mm^3 for LSCS.

Dengue with super added PIH was seen in 3 patients. 2 of these patients in addition had HELLP-one complete and another partial. These patients had extremely low platelet counts (7,000/mm^3). The counts were much lower than those seen in severe pre-eclamsia. All of them had bleeding tendencies and all required transfusions. 24 units were given for the patient with 9000/mm^3, 22 units for a patient with twins and a count of 7,000 cells and 7 units for a patient with partial HELLP and previous caesarean. The rise in platelet count was very slow in this group, it took 10 days for platelet count to stablise. Dengue is a common tropical disease in our country. All our 3 patient had secondary dengue with super added pre-eclampsia and presented with bleeding tendencies. No reports of dengue with pre-eclampsia have been reported so far. The compounded effect of pre-eclampsia and dengue lead to very low platelet count and massive platelet transfusion was required in these women. The interval between the diagnosis and delivery was within 2 to 3 days. Termination was done immediately for all case of abruption, eclampsia, renal failure and HELLP after platelet correction to the desire level. In preterm mothers steroid administration was done for lung maturity and subsequently induction or LSCS was resorted to based on other parameters.

Haematological disorders must always be borne in mind especially when the clinical situation rules out pregnancy related conditions like PIH. In cases of severe anemia, megaloblastic anemia must always be thought of as was done in our case. In case of megaloblastic anemia the patient was referred as severe anemia with hemoglobin of 4.3gm/dl, platelet count showed 60,000cells/mm^3 . Anemia was more profound in this case and peripheral smear and bone marrow confirmed the diagnosis. A patient with known ITP presented at 28 weeks with PROM. She was on steroids. After delivery she developed hemothorax. She was treated as per ASH guidelines (The American Society of Hematology, 2011) Since she did not respond to steroids, Anti Rh immunoglobulin was given. She received a total of 44 units of platelets. Splenectomy was done as a last resort. A normotensive woman was referred as severe anemia with low platelet count. Dengue and drug history were ruled out. ITP was initially thought of, but inspite of transfusion the platelet count never raised. The expected rise was 5000cell/mm^3 after the single unit of transfusion but in this patient even after 4-5 units the counts did not raise. Further evaluation was done and ultimately a bone marrow confirmed aplastic anemia. We had to delivery her for fear of abruption or bleeding elsewhere and planned to refer her for bone marrow transplant. The patient was delivered under cover of single donor platelet.

However following delivery the counts fell to <2000/mm^3, she developed huge vulval haematoma, multiple bleeds, cerebral bleed and could not be resuscitated. Sometimes massive bleeding as in PPH may result in dilution coagulopathy as seen in our patient. This patient was referred from outside with abdominal distention after caesarean section, was found to have hemoperitoneum with platelet count of 60,000cells/mm^3, hemoglobin of 5gm/dl, fibrinogen of 150mg/dl, INR 1.6, she was diagnosed as DIC. NIH consensus conference report noted that pathological haemorrhage in a patient receiving massive transfusion is caused more frequently by thrombocytopenia than by depletion of coagulation factors. This may be due to hemodilution using crystalloids or using only packed cells. In such a situation it is always worthwhile to serially check for platelet count, PT & INR to see if dilution coagulopathy has occurred, since unless it is corrected bleeding would not stop (Platelet Transfusion Therapy, 1986). Still birth and IUD were seen in 6 women all in the PIH group. The high perinatal mortality was due to prematurity and IUGR. Similar reports are quoted by Kubilay Ertan (A Kubilay Ertan, 2002). However thrombocytopenia was not related to the preterm birth.

Factors to be considered in thrombocytopenic patients in the last trimester are

- When to terminate?
- What is the mode of termination?
- What is the ideal count to be obtained?
- What is the type of transfusion to be given?

Termination needs to be thought of only for maternal conditions like PIH, HELLP or abruption. The severity of thrombocytopenia directly correlates with the severity of the lesion[(14)]. Patient with severe PIH need to be serially monitored and a drop in platelet count may heralds the onset of HELLP. Careful surveillance is necessary. Vaginal birth is the preferred mode delivery. The chance of bleeding is less when trauma is minimal. Even episiotomy need to be given only where necessary because 1 patient with aplastic anemia had huge vulval haematoma after delivery. Both British and American guidelines suggest a platelet count >50,000/mm^3 is safe for vaginal delivery. British guidelines suggest aiming at a platelet count >80,000/mm^3 for a caesarean section. The count that was aimed in our patients was 50,000/mm^3 for vaginal delivery and 80,000/mm^3 for LSCS.

A single unit of platelet transfusion rises the platelet count by 5,000/mm^3. A unit obtained by apheresis of a single donor (SDP) contains an equivalent of 5 to 6 units raising the platelet count by around 30,000 to 60,000/mm^3. Platelet should be ABO and Rh compatible. Since stored platelets have a very short shelf- life, procuring many units may be a problem as the blood bank may not have that many bags. When requirement of platelet is large SDP is preferred as the risk of blood born pathogenesis is low, as also alloimmunisation. Where aphaeresis unit is not available, obtaining one required a lot of effort and co-ordination. Since the presentation varied interdepartmental assistance was often required. Good co-ordination with blood bank was required for procuring blood and blood products. In addition consultation with intensivist, haematologist, nephrologist, anesthetists and pediatrician was required. Team approach was the secret of success of survival of these patients.

Conclusion

Unlike in the antenatal period, moderate to severe thrombocytopenic patients near term are in a grave situation – one because the diagnoses has still to be made and secondly due to limited time constraints as delivery is imminent. A detailed history & physical examination is necessary in evaluating a patient with thrombocytopenia. Timely administration of platelets and discrete use of SDP especially when massive platelet transfusion is required helps prevent risk of infection and alloimmunisation. A platelet count of above 10,000cells/mm^3 must be maintained even after delivery to prevent spontaneous bleeding. Delivery should be as atraumatic as possible, avoidance of episiotomy to be practiced. Any pregnancy with a low platelet count less than 50,000cells/ml requires to be shifted to a tertiary centre, since etiology is varied and treatment is multidisciplinary.

REFERENCES

Kam PC, Thompson SA, Liew AC. 2004. Thrombocytopenia in the parturient. Anaesthesia. Mar;59(3):255-64. Review. PubMed PMID: 14984524.

McCrae KR. 2003. Thrombocytopenia in pregnancy: differential diagnosis, pathogenesis and management. Blood Rev., 17:7–14

Boehlen F(1), Hohlfeld P, Extermann P, Perneger TV . *2000.* Platelet count at term pregnancy: a reappraisal of the threshold. *Obstet Gynecol. 2000* Jan;95(1):29-33.

Andra H, James. Bleeding and the management of haemorrhagic disease in pregnancy. Text book of Consultative hemostasis and thrombosis. 3rd Ed. Chapter 34. Pg: 616-626.

Lale Say et al. Global causes of maternal death: a WHO systematic analysis. Lancet Global Health 2014.2:e323-333.

Burrows RF(1), Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. *N Engl J Med. 1993* Nov 11;329(20):1463-6.

Weinstein.L. Syndrome of hemolysis, elevated liver enzymes and low platelet count: A severe consequence of hypertension in pregnancy. Am J obstet gynecol 1982;142:159-167

Joshi D(1), James A, Quaglia A, Westbrook RH. Liver disease in pregnancy. *Lancet. 2010*;375:594-605. doi: 10.1016/S0140-6736(09)61495-1.

Sibai BM, Ramadan MK, Usta I, et al. Maternal morbidity and mortality in 442 pregnancies with hemolysis, elevated liver enzymes and low platelets (HELLP syndrome). Am J Obstet Gynecol. 1993;169:1000-1006.

Gernsheimer T, McCrae KR. Immune thrombocytopenic purpura in pregnancy. *Curr Opin Hematol. 2007*;*14*:*574-580*

The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia

Platelet Transfusion Therapy. National Institutes of Health Consensus Development Conference Statement October 6-8, 1986

A Kubilay Ertan, Steffen wagner, H.Joachim Hendrik et al . Clinical and biophysical aspects of HELLP syndrome. J Perinat Med. 2002;30:483–489.

Michal Parnas, Eyal Sheiner, Ilana Shoham-Vardi et al. Moderate to severe thrombocytopenia during pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology (2006) EURO-5684.

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RESEARCH ARTICLE

Labor Pattern in South Indian Population

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Abstract

Aim: Labor dystocia is one of the commonest indications of lower segment cesarean section (LSCS). Recent studies have shown a dramatic change in the rate of cervical dilatation and labor progress. There are no large studies to assess labor in this ethnic group and hence this study has been done to find out labor behavior in women with spontaneous labor in South India.

Materials and methods: A retrospective study was done in our teaching hospital. Labor was managed as per protocol and a partogram was maintained. Data were extracted from case-records and analyzed.

Results: Labor progress was much slower than what was thought of. There was wide variability in the duration of labor.

Conclusion: Many women took time up to the 95th percentile and some even crossed three times the median time to deliver.

Clinical significance: Though many women will deliver in the expected time, it is important to know the maximum time or the 95th percentile for each dilatation so that sufficient time can be allowed before labeling a case as prolonged labor.

Keywords: Labor curve, Labor duration, Rate of cervical dilatation, Slowest rate of dilatation.

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Introduction

Cervical dilatation is one of the key determinants of the progress of labor and may be dependent on many factors. There have been many attempts to define the norms for the progress of labor that have yielded variable results.

This is not an easy task as there are many challenges to accurately measure the duration of labor. The labor norms are also dependent on a woman's ethnicity, age, parity, BMI, use of epidural analgesia, and may vary among population groups. Despite all these difficulties, it is important to arrive at a proper understanding of labor norms and identify the slowest acceptable rates of cervical dilatation.

The rates of cervical dilatation in the active phase of labor largely stem from the data published by Friedman in 1950.[1] The Friedman's labor curve and the active management of labor pioneered by O'Driscoll et al.[2] where the lowest acceptable rate of dilatation is 1 cm/hr has influenced obstetric practice worldwide. WHO partogram[3] is practiced all over the world and the expected rate of dilatation is 1 cm/hr in the active phase. Any shift to the right and if the curve crosses the action line, labor is labeled as prolonged labor or non-progression of labor, and lower segment cesarean section (LSCS) is performed.

Dystocia characterized by a delay in progression of cervical dilatation is one of the most common indications for primary cesarean sections, ACOG Bulletin states it is the leading cause contributing to around 50% of all primary LSCS being performed.[4]

Zhang et al. reassessed Friedman's curves.[5] They studied the electronic records of 62,416 parturients and found the rates of cervical dilatation to be much slower. Data from this study formed the basis of the new guidelines for the active phase of labor. Labor may take even 6 hours to progress from 4–5 cm and more than 3 hours to progress from 5–6 cm. Only after 6 cm was the progress faster.

Due to ethnic differences, it is important to have insight regarding labor in our population that is critical in diagnosing labor dystocia and making a decision regarding the mode of delivery

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Aims and Objectives

- To determine the duration of labor and the rates of cervical dilatation in low-risk primigravida and multigravida women with spontaneous onset of labor.
- To determine the slowest acceptable rates of cervical dilatation.
- To create a labor curve for this population.

Materials and Methods

This was a prospective observational study done in a teaching hospital in South India between 2017 and 2018.

The study population included both primigravida and multigravida who were admitted with spontaneous labor.

The inclusion criteria were term gestation, singleton pregnancy, and vertex presentation with spontaneous onset of labor who delivered either normally or by assisted vaginal delivery. The exclusion criteria were induced labor, pre-term labor, mal-presentations, and patients who were delivered by cesarean section.

The institutional protocols for monitoring of labor were followed. Maternal and fetal monitoring was done in the usual way. The progress of labor was assessed by monitoring uterine contractions per 10 minutes for every half an hour, cervical

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dilatation, and descent of the head was assessed by vaginal examination every 3–4 hours in the first stage of labor.

The rate of cervical dilatation was plotted on a partogram with time in hours on the x-axis and cervical dilatation on the y-axis.

Ethical clearance was obtained from our institutional human ethical committee.

Statistical Analysis

The data was entered on an Excel spreadsheet and analysis was done using SPSS STATA13.1 software.

The data were analyzed concerning maternal demographic characteristics. The cumulative labor duration from cervical dilatation on admission (i.e., at 1, 2, 3, 4, 6, 7) to reach 10 cm was assessed. Data were extracted according to two parity groups nulliparous and parous women.

With each starting dilatation, the mean rate of dilatation was calculated for primigravida and multigravida. Taking the value of 2SD, the slowest rate of dilatation was also calculated.

A non-regression model was used to plot the labor curve with cervical dilatation against time in hours.

Results

The total number of deliveries during the study period was 4150.

A total of 2558 women were delivered by cesarean section. The number of women with spontaneous onset of labor who delivered vaginally was 1196. A total of 152 patients who had incomplete data and 21 patients who were admitted at more than 8 cm were excluded. So, only 1023 deliveries were analyzed.

Primigravida were 556 in number and multigravida were 467 in number. The age, cervical findings on admission, and mode of delivery are shown in Table 1.

The cumulative time taken for each dilatation starting from 1 to reach 10 cm was analyzed in both primigravida and multigravida.

In our study, the median duration of labor was chosen, as the mean duration may be skewed by a few laborers of very short or long duration. If the labor duration is positively skewed, the mean duration will be longer than the median duration as shown in this study thus at 2 cm the mean duration was 7.7 hours and the median duration was 7 hours.

Table 2 shows the cumulative mean time taken for each dilatation to reach 10 cm in primigravida and multigravida, respectively. It shows that multigravidae took a shorter time to progress to 10 cm when compared to primigravida. Primigravida took a median time of 9 hours to reach 10 cm from 1 cm, whereas it was 8 hours in multiparous women. At 2 cm it was 7 and 6 hours. At 3, 4, 5, 6–10 cm it was 5, 3, 3, and 2 hours in primigravida and 4, 3, 2, and 2 hours in multigravida.

Table 2 also shows the 5th and the 95th percentile, the number of women who crossed the 95th percentile, and the maximum time allowed for labor to continue that resulted in normal vaginal delivery.

At 2 cm though the meantime was 7.7 hour in primigravida, 16 women crossed the 95th percentile and 10 patients had a vaginal delivery after almost three times this mean-ranging from 18–26 hours. In six multiparous women at the same dilatation, labor was prolonged to 16–27 hours and 15 women crossed the 95th percentile.

At 3 cm dilatation, although the mean duration of labor was 5.5 hours, four primigravida women had a vaginal delivery after 19, 17, 16, and 13 hours, respectively. Correspondingly three multigravidas had taken 21, 15, and 13 hours to deliver from 3 cm dilatation for a mean of 4.7 hours.

At 4 cm against a mean of 3.8 hours, five primigravidas had taken between 8 and 11 hours to deliver, and in multigravida for a mean of 2.9 hours four had taken between 6 and 9 hours. At 7 cm a multiparous woman took 6 hours to reach full dilatation.

With each starting dilatation, the mean rate of dilatation was calculated for primis and multis. Taking the value of 2SD, the slowest rate of dilatation was also calculated as shown in Table 3.

The labor curve obtained was hyperbolic and not a sigmoid curve. There was no definite point of acceleration seen (Figs 1 and 2).

Table 1: Demographics

Details	*Primigravida*	*Multigravida*
Number of cases	556	467
Age	24.2 years	27.4 years
% of normal delivery	74.3%	87.9%
Instrumental	25.7%	12.1%
Mean dilatation on admission	2.85 cm	3.2 cm

Table 2: Cumulative duration of labor (dilatation on admission to 10 cm)

Dilatation on admission (cm)	*Obs score*	*Numbers*	*Mean*	*Median*	*SD*	*P 5*	*P 95*	*No > 95*	*Max time taken*
1–2	Primi	121	9.36	9	5.05	2.5	19	17	30
	Multi	84	9.39	8	5	2.5	19	16	27
2–3	Primi	201	7.7	7	4.5	2	16	10	26
	Multi	158	6.8	6	4.2	2	15	6	27
3–4	Primi	112	5.5	5	3.2	2	10	5	19
	Multi	122	4.7	4	3.1	1	10	5	21
4–5	Primi	69	3.8	3	2.2	1	8	3	11
	Multi	74	2.9	3	1.6	1	6	2	9
5–6	Primi	29	3	3	1.8	1	6	1	10
	Multi	30	2.2	2.2	1.7	1	6	1	8
6–7	Primi	12	1.8	2	0.68	1	3	–	–
	Multi	6	1.8	2	0.68	1	3	–	–
7–8	Primi	12	1.4	1	0.64	–	3	–	–
	Multi	21	1.4	1	1.13	–	3	1	6

Table 3: Rate of cervical dilatation

Dilatation (cm)	*Obs score*	*Mean rate of dilatation (cm)*	*Slowest rate of dilatation (cm)*
2–3	Primi	1.03	0.65
	Multi	1.17	0.72
3–4	Primi	1.27	0.8
	Multi	1.48	0.89
4–5	Primi	1.57	1
	Multi	2.06	1.33
5–6	Primi	1.66	1.04
	Multi	2.27	1.28
6–7	Primi	2.2	1.61
	Multi	2.2	1.61

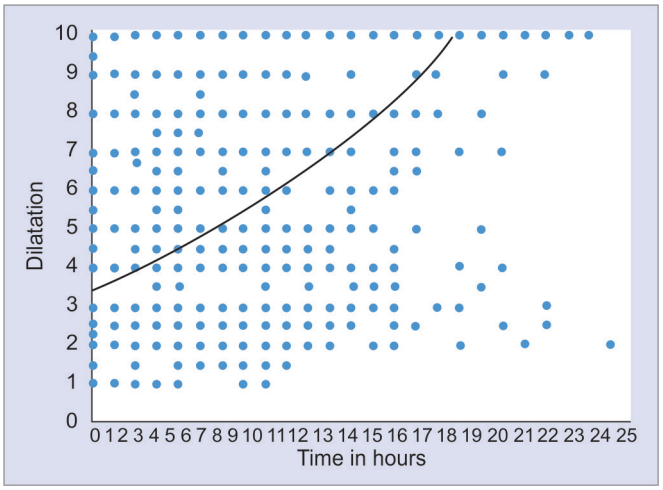


Fig. 1: Labor curve—primigravida

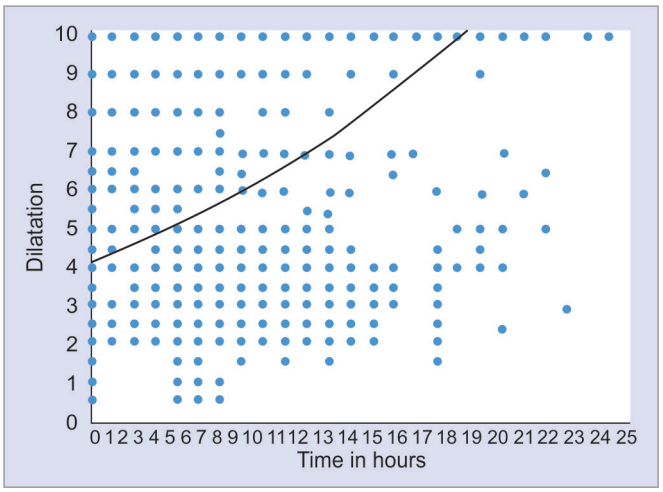


Fig. 2: Labor curve—multigravida

Discussion

A total of 1023 patients were analyzed, out of which 556 were primigravida and 467 were multigravida. We found the following in our study:

- In the active phase, the average time taken was more than that described by Friedmann.[6,7] In his study, he has stated that from 2.5 cm to reach 10 cm the average time is 4.6–4.9 hours, and once they reach 4 cm half of them deliver in 2.6 hours. Our corresponding figures were 7.7 hours to reach 10 cm from 2.5 cm and the mean duration at 4 cm was 3.8 hours against 2.6 hours. Peisner and Rosen[8] found that 75% of women at 3 cm, 50% at 4 cm, and 25% at 5 cm do not dilate at the indicated rates associated with active labor.

However, recent studies have shown a longer first stage of labor with slower acceptable rates of dilatation. Zhang et al. derived the average labor curves from 27,170 nulliparous women in the US population with spontaneous onset of labor. The curve had a slower progression of 1.1 cm/hr with no definite inflection point. The slowest acceptable rate was 0.6 cm/hr.

Albers et al.,[9] Albers,[10] and Jones and Larson[11] found that labor progression takes a longer time than the thought of earlier. These investigators have reported that in the active phase of labor from 4 cm to reach 10 cm the time ranges from 6.2–7.7 hours on average. In Albers's study, the time taken was 7.7 hours since there was no use of oxytocin and labor was spontaneous.

- The second derivative was that there was a wide difference in time taken between individuals. When women were admitted at 2 cm, the median time taken to reach 10 cm was 7 hours in primigravida and 6 hours in multigravida. However, 2SD at this dilatation was 4.5 hours extending labor to 11.5 hours. Many women crossed the 95th percentile and also, ten primigravidas delivered normally after a duration that was almost three times the median ranging from 18–26 hours indicating wide variability in the duration of labor are still associated with normal outcomes. The same was seen at all dilatations. Our findings were similar to that quoted by Oladapo et al.[12] as part of WHO BOLD PROJECT (better outcome in labor difficulty) where he says median cumulative duration of labor indicates that nulliparous women admitted at 4, 5, and 6 cm reached 10 cm within an expected time, but their corresponding 95th percentile shows that labor could last up to 14, 11, and 10 hours.
- It was found that the rates of cervical dilatation accelerate as labor progresses and for each dilatation, an acceptable slow rate of progress exists. This is comparable to the study by Zhang et al. who found that the slopes of cervical dilatation progressively steepen with each passing centimeter. In his study, he also found that the median rates of dilatation between 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, and 9–10 cm were 0.4, 0.6, 1.2, 1.7, 2.2, 2.4, and 2.4 cm, respectively. At the 5th percentile that is used to define the slowest normal dilatation rates the rates were 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, and 0.7 cm/hour, respectively.
- Finally, the labor curve that was obtained showed a smooth hyperbolic curve, there was no point of acceleration. A similar labor curve pattern is seen in other studies. Suzuki et al.[13] studied the labor patterns in 2369 low-risk nulliparous Japanese women with spontaneous onset of labor and derived the labor curve for that population. It showed that the curve had a smooth shape with an active phase duration of 5.1 hours with slower progression than that of Friedman's curve. The deceleration phase was not observed and the rate of dilatation started to double from 5–6 cm onwards. A study done in South India by Pitchaimuthu and Bhaskaran[14] showed the same pattern

of slower dilatation, a smooth labor curve, and no point of inflection.

The limitations of this study include interpersonal variations in the assessment of cervical dilatation. The effect of ARM and oxytocin were not analyzed since many patients were subjected to either both or one of them.

Conclusion

In low-risk women, both primigravida and multigravida with spontaneous onset of labor, there is wide variability in the duration of labor with normal maternal and fetal outcomes. Our study showed that in low-risk women admitted in early spontaneous labor, half of them took more than the meantime and a significant number of women took even three times the mean. The rate gradually increased with dilatation and in nulliparous women at 6 cm of dilatation the rate was much higher. It is worthwhile to note that many women despite crossing the 95th percentile delivered normally, hence, revision of the expectations of the rate of cervical dilatation would reduce both unnecessary interventions in labor and cesarean sections for slow or abnormal progress of labor. Labor should be more patient-centered and the same pattern will not fit all.

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References

1. Friedman EA. Primigravid labour: a graphicostatical analysis. Obstet Gynecol 1955;6(6):567–589. DOI: 10.1097/00006250-195512000-00001. Pubmed:13272981.
2. O'Driscoll K, Stronge JM, Minogue M. Active management of labour. Br Med J 1973;3(5872):135–137. DOI: 10.1136/bmj.3.5872.135. Pubmed: 4720762.
3. World Health Organizaton. Division of family health. Maternal health and safe motherhood programme. The partograph; the application of the WHO partograph in the management of labour. Report of a WHO multicentre study 1990–1991. 1994. WHO/FHE/MSM/94.4.
4. American College of Obstetrics and Gynecology Committee on Practice Bulletins-Obstetrics. ACOG Practice Bulletin Number 49, December 2003; dystocia and augmentation of labour. Obstet Gynecol 2003;102(6):1445–1454. DOI: 10.1016/j.obstetgynecol.2003.10.011 [PubMed:14662243].
5. Zhang J, Landy HJ, Branch DW, et al. Contemporary patterns of spontaneous labour with normal neonatal outcomes. Obstet Gynecol 2010;116(6):1281–1287. DOI: 10.1097/AOG.0b013e3181fdef6e.
6. Friedman EA, Kroll BH. Computer analysis of labour progression. III. Pattern variations by parity. J Reprod Med 1971;6(4):179–183. PubMed: 4938465.
7. Friedman EA, editor. Labor: clinical evaluation and management. 2nd ed. New York: Appleton century-Crofts; 1978.
8. Peisner DB, Rosen MG. Transition from latent to active labor. Obstet Gynecol 1986;68(4);448–451. Pubmed: 3748488.
9. Albers LL, Schiff M, Gorwoda JG. The length of active labor in normal pregnancies. Obstet Gynecol 1996;87(3);355–359. DOI: 10.1016/0029-7844(95)00423-8. Pubmed: 8598954.
10. Albers LL. The duration of labor in healthy women. J Perinatol 1999;19(2):114–119. DOI: 10.1038/sj.jp.7200100. PubMed:10642971.
11. Jones M, Larson E. Length of normal labor in women of Hispanic origin. J Midwifery Womens Health 2003;48(1);2–9. DOI: 10.1016/s1526-9523(02)00367-7. Pubmed:12589300.
12. Oladapo OT, Souza JP, Fawole B, et al. Progression of the first stage of spontaneous labour: a prospective cohort study in two sub-Saharan African countries. PLoS Med 2018;15(1): e1002492. DOI: 10.1371/journal.pmed.1002492.
13. Suzuki R, Horiuchi S, Ohtsu H. Evaluation of the labor curve in nulliparous Japanese women. Am J Obstet Gynecol 2010;203(3):226.e1–6. DOI: 10.1016/j.ajog.2010.04.014.
14. Pitchaimuthu N, Bhaskaran S. Labor patter among primigravida in local population. J Obstet Gynaecol India 2018;68(6);482–486. DOI: 10.1007/s13224-017-1083-3.

Original Article

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A Comparative Study of Right and Left IJV Cannulation Using Surface Anatomy or USG Guidance: A Prospective Randomized Study

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Abstract

Context: Internal jugular vein (IJV) is cannulated in a variety of patients. Left IJV cannulation sometimes becomes necessary. *Aim:* This study was done to evaluate the usefulness of ultrasonography (USG) in performing left and right IJV cannulation compared with use of surface anatomy landmarks. *Setting and Design:* Prospective randomized study in a tertiary care teaching hospital. *Methods:* Ninety one American Society of Anesthesiologists (ASA) class I, II or III adult patients scheduled for elective cardiothoracic, vascular, neurosurgical or major abdominal surgeries requiring IJV cannulation were randomized to undergo either right IJV cannulation using surface landmarks (Group 1) or USG (Group 2) or left IJV cannulation using surface landmarks (Group 3) or USG (Group 4). The number of attempts, time taken, the success rate, any change in technique or side of cannulation and any complications were recorded. *Results:* There was a significant difference in the success rate and number of complication between group 3 and group 4 ($p<0.05$). There was a statistically significant difference in the time taken for cannulation between Group 3 and Group 4 (273.9±127.6 Vs 135.2±105.1 sec, $p<0.001$). There was a significant difference in the number of attempts between Group 1 and Group 2 (2.05±1.2 Vs 1.46±0.64, $p<0.05$) and between Groups 3 and Group 4 (2.78±1.41 Vs 1.69±0.18, $p<0.01$). Complications (carotid puncture) were significantly more common on left side using surface landmarks ($p<0.05$). *Conclusion:* Ultrasound significantly improves the success rate of left and right IJV cannulation when compared to use of surface landmarks. It also reduces the number of attempts, the time required for cannulation and incidence of complications.

Keywords: Cannulation; Central Venous; Jugular; Ultrasonography; Vein.

Introduction

Internal jugular vein (IJV) is cannulated for a variety of indications in perioperative period and in the critically ill. This includes both diagnostic and therapeutic indications. The right IJV is usually chosen because it has a relatively straight course to the heart, has a lesser chance of pneumothorax and avoids the risk of injury to thoracic duct altogether [1,2]. The clinician's experience with right IJV is also commonly greater than with left IJV. However, left IJV cannulation frequently becomes necessary when the right IJV cannulation is unsuccessful or the right IJV is thrombosed or has a catheter in-situ or the right carotid artery is significantly stenosed.

Ultrasonography (USG) greatly improves the success rate and reduces the risks of IJV cannulation, particularly for inexperienced clinicians [3]. And the NICE guidance suggests the two-dimensional (2D) imaging USG as the preferred method for cannulation of the IJV in adults and children in elective situations [4]. However, in majority of studies on use of 2D-USG for IJV cannulation the right IJV was used. Since the left IJV is longer, more tortuous and smaller in calibre than the right IJV, it is more likely to be difficult to cannulate [5]. To our knowledge, there is only one randomized study that compares right and left IJV

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cannulations using surface landmarks or USG [6]. Unlike these authors, in patients scheduled for major elective surgeries we perform IJV cannulations before induction of anesthesia which could affect the results of the study. We performed a randomized study to evaluate the usefulness of real-time USG in cannulation of left or right IJV compared to use of surface landmarks.

The primary objective of our study was to compare the success rate and complications of left and right IJV cannulation using real-time USG versus use of surface landmarks. The secondary objectives was to compare the number of attempts and access times for left and right IJV cannulation performed with or without USG.

Materials and Methods

After Institutional ethics committee approval and written informed consent from each patient a total of 106 ASA class I, II &III patients scheduled for elective cardiothoracic, vascular, neurosurgical and major abdominal surgeries requiring IJV cannulation were enrolled into the study. With the exclusion of 15 patients who did not meet the inclusion criteria, the final study population was 91 patients and was randomly allocated into 4 groups using a computer-generated table of random numbers (Figure 1).

Patients were excluded from randomization if they have had a previous IJV cannulation, neck dissection, burns or radiotherapy to the neck, have a mass or infection at the cannulation site or refuse to participate. Patients underwent either right IJV (RIJV) cannulation using surface landmarks (group 1) or using USG (group 2) or underwent left IJV (LIJV) cannulation using surface landmarks (group 3) or using USG (group 4).

All cannulation were done as per the recommendations and practice guidelines suggested by the ASA Task force on central venous access, under strict aseptic precautions and sterile draping with a slight Trendelenburg positioning and head turned by about 30° to contralateral side, using Seldinger technique. All cannulations were performed by staff anesthesiologists experienced in IJV cannulations and expertise in USG-guided technique.

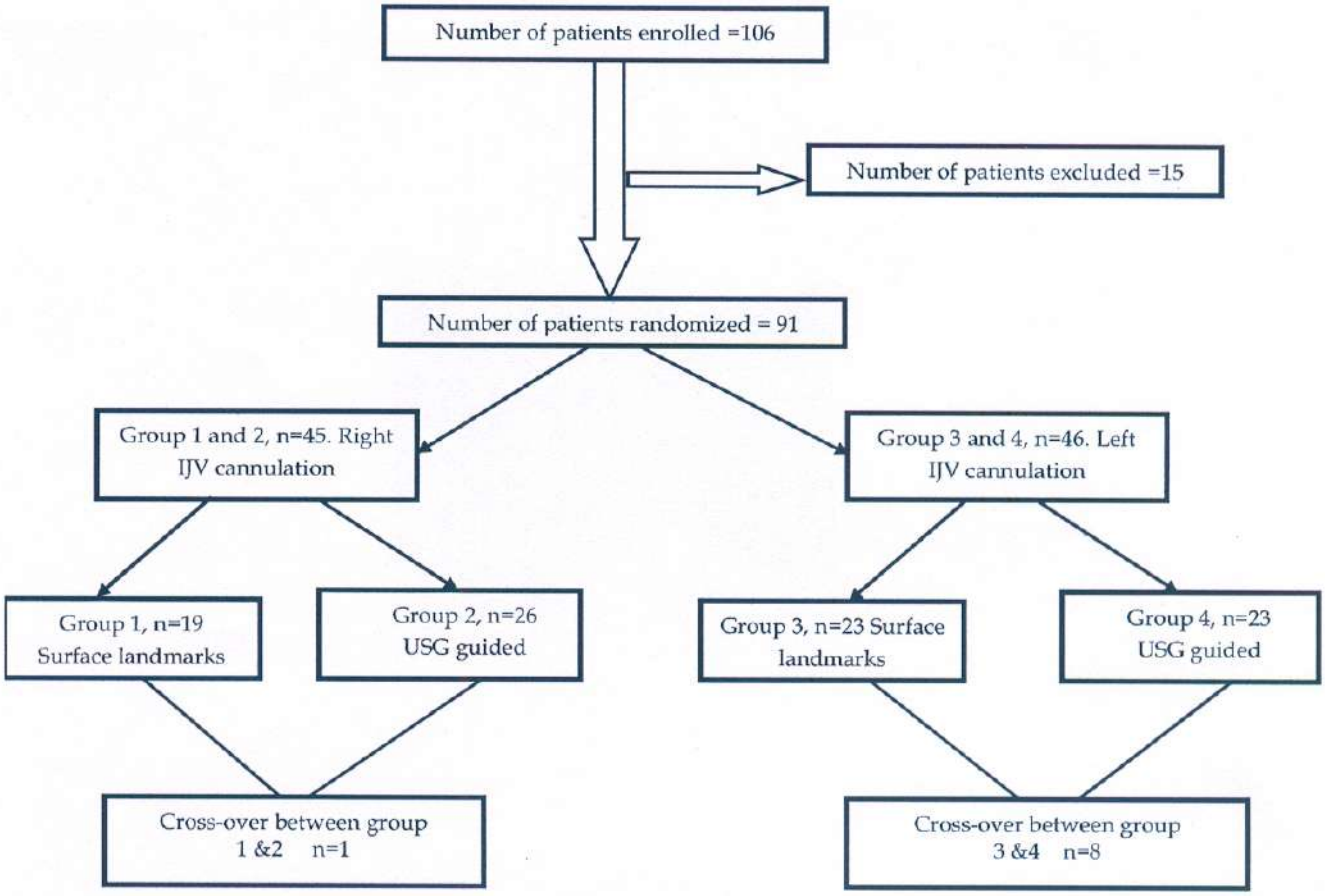


Fig. 1: Study design

Surface Landmark Approach

The site of puncture was the apex of the triangle formed by the clavicle and the two heads of sternomastoid muscle. A 23G locator needle was inserted at an angle of approximately 45° and directed towards the ipsilateral nipple. Aspiration of dark, non-pulsatile blood guided a subsequent 16G cannulating needle in the same direction. A further aspiration of dark, non-pulsatile blood was followed by guidewire insertion through the cannulation needle. And a 7 Fr triple- or double-lumen catheter was inserted over the guidewire (Cetrofix, B. Braun, Melsungen AG, Germany).

An attempt at cannulation was defined as the number of skin punctures and the technique was considered successful if the guidewire was successfully inserted on the ipsilateral side using the technique the patient was randomly assigned to. Whenever the IJV was not located by more than six attempts or there was difficulty in passage of guidewire or there was arterial puncture with the 16G cannulation needle, USG was used to guide ipsilateral IJV cannulation. In the event of guidewire placement using USG being unsuccessful, the contralateral IJV was cannulated under ultrasound guidance.

Ultrasound-Guided Approach

A 7.5MHZ linear array ultrasound transducer probe (Vivid-i) covered in ultrasonic gel was wrapped with a sterile plastic bag and connected to a USG machine (vivid-i, GE Medical Systems, Milwaukee, USA). The probe was placed on the neck at the level of cricoid cartilage and the IJV was then identified by its shape, absent pulsations, probe compressibility and by colour flow Doppler. A 16G cannulating needle was then used to puncture the IJV visualized in short axis in real-time USG. After aspiration of blood, guidewire was inserted and its position confirmed by USG of IJV in long axis.

Data Collection and Analysis

The data collected included the number of successful cannulations in each group, number of attempts, time taken from initial skin puncture to successful guide wire placement, incidence of complications such as arterial puncture, hematoma and pneumothorax, and the number of patients in whom cross-over of technique was used. Post-procedure chest X-ray films were taken routinely. Data analysis was done using STATA statistical software version 19.0., independent sample T-test for continuous variables and chi- square test for categorical variables.

Results

There was no statistically significant difference among the groups with respect to age, gender distribution, weight or height (Table 1). There was a significant difference in the success rate and number of complication between group 3 and group 4 ($p<0.05$). However, there was no significant difference in the success rate or complications between group 1 and group 2 (Table 2). The only complication observed in our study was carotid artery puncture. One patient in group 1 and 8 patients in group 3 had carotid puncture. Cannulation in these patients was accomplished using USG on the same side. None of the patients developed hematoma. Also the success rate was significantly higher in group 1 compared to group 3.

There was a significant difference in the number of attempts between group 1 and group 2 and between group 3 and group 4 (Table 3). There was no significant difference in the number of attempts between group 2 and group 4. Using surface landmarks, cannulation of the LIJV took significantly more time compared to RIJV. Ultrasonography greatly reduced the time taken for cannulation on left side compared to surface landmark (Table 3, Figure 2).

Table 1: Patient demographics

	IJV		LIJV	
	Group 1 (SL, n=19)	Group 2 (USG, n=26)	Group 3 (SL, n=23)	Group 4 (USG, n=23)
Age (yrs)	51.4±12.0	56.9±13.0	49.8±10.2	56.56±10.0
Gender				
Male	14	19	17	17
Female	5	7	6	6
Weight (kg)	63.6±7.0	64.3±5.1	65.2±6.2	64.4±7.3
Height (cm)	171.3±3.0	172.6±2.3	173.0±2.1	171.8±2.6

RIJV: right internal jugular vein, LIJV: left internal jugular vein, SL: surface landmark, USG: ultrasonography

Table 2: Success rate and complications

Group	Success rate (%)	Complication (%)
Group 1	94.7#	5.3
Group 2	100	0.0
Group 3	65.2	34.8
Group 4	100#	0.0#

*p<0.05 compared to group 3, #p<0.05 compared to group 3

Table 3: Number of attempts and access times

Group	Number of attempts (mean ± SD)	Access time (sec, mean ± SD)
1	2.05 ± 1.22	172.4 ± 213.6
2	1.46 ± 0.64*	136.5 ± 122.9
3	2.78 ± 1.41	273.9 ± 127.6†
4	1.69 ± 0.18#	135.2 ± 105.1#

*p<0.05 compared to group 1, †p<0.05 compared to group 1, #p<0.01 compared to group 3

Table 4: Success in multiple attempts among groups

Group	Success in 1st attempt, n (%)	Success in two attempts, n (%)	Success in three attempts, n (%)
1	9 (47.4%)	12 (63.1%)	17 (89.4%)
2	16 (61.5%)	24 (92.3%)*	26 (100%)
3	6 (26.1%)	9 (39.1%)	16 (69.7%)
4	13 (56.5%)#	17 (73.9%)#	23 (100%)#

*p<0.05 compared to group 1, #p<0.05 compared to group 3 at respective number of attempts

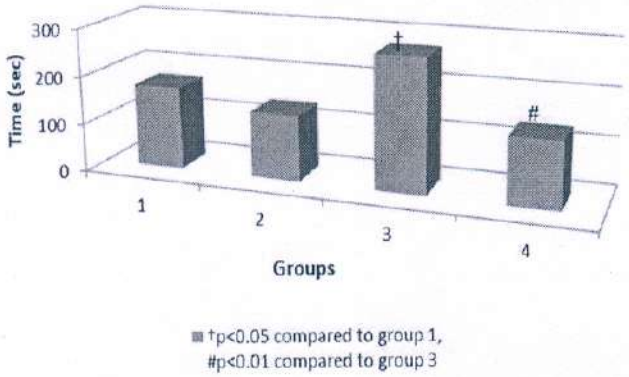


Fig. 2: Access time

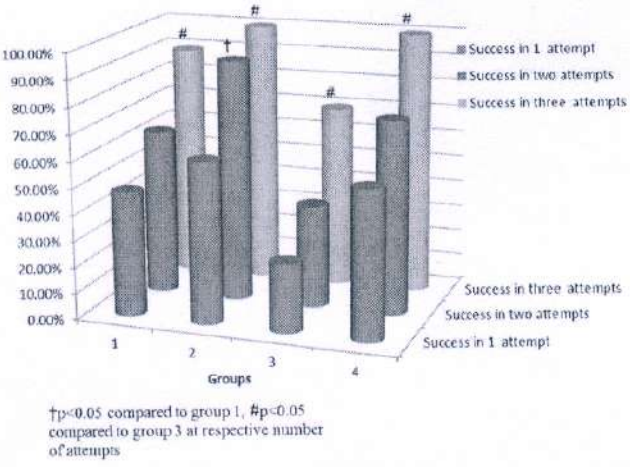


Fig. 3: Cumulative success in multiple attempts

We also evaluated the usefulness of ultrasonography in improving the success rate with every attempt at cannulation after a failed first attempt. Cumulative success in one, two and three attempts is shown in Table 4, and Figure 3. There was a significant improvement in the success rate in upto 3 attempts in group 4 compared to group 3. On the right side, USG improved the success rate in two attempts at cannulation (Table 4, Figure 3).

Discussion

Our study showed that ultrasonography significantly improves the success rate and reduces the complications of left IJV cannulation. On the right side however, the success rate and complications were not different statistically with or without USG, probably because all the cannulations were done by faculty with vast experience in IJV cannulations. Our success rate of right IJV cannulations is comparable with previous studies [7,-9]. Denys and Uretsky have shown that IJV is located anterolateral to the carotid artery (CA) in 92%, >1 cm lateral to the carotid in 1%, medial to the carotid in 2%, and outside of the path predicted by surface landmarks in 5.5% of patients [10]. A failure rate of 7.0% to 19.4% is due partly to the inability of external landmarks to precisely correlate with the location of the vessel [11,12]. The

failure rate of 5.3% on right side using surface landmarks in our study is well within this range.

Using surface landmarks, the left IJV was more difficult to cannulate compared to the right IJV. It took more time and attempts to cannulate the left IJV than the right IJV using surface landmarks. This may be due to its longer and more tortuous course compared to right IJV. In addition, the vertical and horizontal diameter of left IJV is smaller than the right IJV as measured by preoperative CT scans [13]. Left IJV also lies deeper compared to right IJV. On the right side USG did reduce the number of attempts compared to surface landmarks.

Our study also showed that when multiple attempts are made at cannulation of left IJV, a USG-guided technique is more likely to succeed than a surface landmark guided technique. Since there is a strong direct correlation between the number of attempts and the incidence of complications, increasing patient anxiety and discomfort, it may be prudent to use USG for all left IJV cannulations. Patient head position also has important implications for the relation between CA and IJV. Sulek CA *et al* have shown that the percentage of overlap between the IJV and the CA increased as the head was rotated contralaterally from neutral (0°) to 40° to 80° [14]. When overlap occurred, it was always more on the left than the right side, and it was significant at 80 degrees head rotation ($P < 0.05$). In our study patient head was rotated by about 30°. The concern with overlap of IJV and CA is that it increases the likelihood of unintentional CA puncture by a through-and-through puncture of the IJV. The accidental puncture of the posterior wall of IJV can occur even with the use of USG when short axis view is used for the cannulation [15]. As the advancing needle indents the anterior wall of the low-pressure IJV, it collapses and in the absence of blood aspired from the needle the advancing needle could puncture the posterior wall of IJV and then the CA. Vessel overlap increasing with head rotation is most apparent among patients with increased body surface areas (>1.87 m^2) and increased body mass indexes (>25 kg/m^2) [16]. The 5.2% right CA puncture incidence in our study is much less compared to those reported in previous studies [7-9]. This may be because of several factors including experienced operators, low mean body mass index (BMI) among our study groups of 21.7kg/m^2 and head turned to contralateral side by about 30°. The incidence of CA puncture on the left side using surface landmarks was 34.8 % which is high compared to a previous study [6], probably because of a smaller sample size and our lack of familiarity with the left IJV cannulations using surface landmarks. Ultrasound can be used to alter the approach angle to avoid CA puncture by directing the advancing needle away from the CA [17]. In our study, there was no incidence of CA puncture using USG on right or left side.

Our study showed that USG-guided cannulation provides advantage of increased success rate with decreased number of attempts and complications compared to the use of surface landmark as shown in previous studies [18-19]. Ultrasound has a definite role in achieving vascular access for difficult-to-cannulate patients with increased success rate and decreased complications. However, USG is a specialized technique and requires advanced training and knowledge [20]. Continued training of physicians in USG techniques ensures that it can be used in various clinical applications to improve the patient outcome.

Conclusion

Ultrasound significantly improves the success rate of left and right IJV cannulation when compared to use of surface landmarks. It also reduces the number of attempts, the time required for cannulation and incidence of complications. Since the left IJV is more difficult to cannulate and has a higher potential for complications than the right IJV, it may be prudent to use USG for all left IJV cannulations.

Key Message

The left IJV is particularly difficult to cannulate using surface landmarks and has higher potential for complications. Hence, USG should be used in all left IJV cannulations

References

1. Khalil KG, Parker FB Jr., Mukherjee N, Webb WR. Thoracic duct injury. A complication of jugular vein catheterization. JAMA, 1972; 221:908-9.
2. Kwon SS, Falk A, Mitty HA. Thoracic duct injury associated with left internal jugular vein catheterization: anatomic considerations. J Vasc Interv Radiol. 2002; 13:337-9.
3. Shekelle PG, Dallas P. Use of real-Time ultrasound guidance during central line insertion: Brief Update Review. In: Making Health Care Safer II: An Updated Critical Analysis of the Evidence for Patient Safety Practices. Executive Summary (Publication No. 13-E001-1-EF). Rockville, MD: Agency for Healthcare

Research and Quality; 2013.p.172-77.

4. National Institute for Clinical Excellence. London. Guidance on the use of ultrasound locating devices for placing central venous catheters. Technology appraisal guidance [TA49] (Cited-2002 October 04). Available from: https://www.nice.org.uk/guidance/ta49.
5. Tartière D, Seguin P, Juhel C, Laviolle B, Mallédant Y. Estimation of the diameter and cross-sectional area of the internal jugular veins in adult patients. Critical Care, 2009, 13:R197.
6. Sulek CA, Blas ML, Lobato EB. A Randomized study of left versus right internal jugular vein cannulation in adults. Journal of Clinical Anesthesia, 2000; 12: 142–145.
7. Ovezov A, Zakirov I, Vishnyakova M: Effectiveness and safety of the internal jugular vein catheterization in pediatrics: Ultrasound navigation vs anatomical landmarks (A prospective, randomized, double-blind study). Intens Care Med, 2010; 36:S275.
8. Shrestha BR, Gautam B: Ultrasound versus the landmark technique: A prospective randomized comparative study of internal jugular vein cannulation in an intensive care unit. JNMA J Nepal Med Assoc, 2011; 51:56–61.
9. Zhang YL, Mi WD, Yu DJ, Fu Q, Feng XX: [Application of ultrasonic surface location for internal jugular vein catheterization via central approach]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao, 2011; 33:479–84.
10. Denys BG, Uretsky BF. Anatomical variations of internal jugular vein location: impact on central venous access. Crit Care Med, 1991; 19:1516-9.
11. Sznajder JI, Zveibil FR, Bitterman H, Weiner P, Bursztein S. Central vein catheterization: failure and complication rates by three percutaneous approaches. Arch Intern Med, 1986; 146:259-61.
12. Gallieni M.Central venous catheterization of dialysis patients.J Vasc Access, 2000; 1:10-4.
13. Ishizuka M, Nagata H, Takagi K, Kubota K. Right internal jugular vein is recommended for central venous catheterization. Journal of Investigative Surgery. 2010; 23:110–114.
14. Sulek CA, Gravenstein N, Blackshear RH, Weiss L. Head rotation during internal jugular vein cannulation and the risk of CA puncture. Anesth Analg, 1996; 82:125-8.
15. Blaivas M, Adhikari S. An unseen danger: frequency of posterior vessel wall penetration by needles during attempts to place internal jugular vein central catheters during ultrasound guidance. Crit Care Med, 2009; 37:2345-9.
16. Lieberman JA, Williams KA, Rosenberg AL. Optimal head rotation for internal jugular vein cannulation when relying on external landmarks. Anesth Analg, 2004; 99:982-8.
17. Turba UC, Uflacker R, Hannegan C, Selby JB. Anatomic relationship of the internal jugular vein and the common carotid artery applied to percutaneous transjugular procedures. Cardiovasc Intervent Radiol, 2005; 28:303-6.
18. Wu S, Ling Q , Cao L, Wang J, Xu M, Zeng W. Real - time two- dimentional ultrasound guidance for central venous cannulation. Anesthesiology, 2013; 118:361-75.
19. Shah H, Bhavsar M . Ultrasound-guided internal jugular venous cannulation comparison with palpation technique: Benefits and drawbacks. IJRRMS, 2013; 3(4):14-17.
20. Reusz G and Csomos A. The role of ultrasound guidance for vascular access. Curr Opin Anesthesiol, 2015, 28:710–716.

Original Research Article

A clinical audit on cancellation of elective surgery on a scheduled day in a teaching institution

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Abstract

Background: Cancellation of surgery on the scheduled date poses physical, psychological and emotional disturbances not only to the operating surgeon, but it also includes the perioperative physician as well as the patients. The most common cause of cancellation of elective surgery on a scheduled day in a teaching institution includes lack of operating time in theatre. The reasons to quantify the cancellation list is usually vivid and difficult. **Aim:** The objective of this audit was to find out the prevalence and the reasons for cancellation of elective surgery on the scheduled day and to find out the clinical quality indicator with regard to effective theatre utilization time on a scheduled day. **Materials and Methods:** This retrospective analysis was included for one year duration in a tertiary teaching hospital. We coined the cancellation of the elective surgery as any proposed surgery which was listed and planned the previous day; and subsequently not done on the scheduled day of surgery. The source of audit was the documentation maintained in cancellation records in the anaesthesia department and these data were analyzed. The most common cause for cancellation of surgery was categorized as Patient related, Pre operative (or Surgical) preparation related and Facility related factors. Observations and **Results:** All the data were entered in the Microsoft excel spreadsheet and a Pareto chart was constructed to prioritize the reasons and analyzed using Epi info software version. Out of 10,306 patients during the scheduled period in our audit, 532 (5.16%) patients had cancellation on the elective list. **Conclusion:** Appropriate pre operative assessment, better inter departmental coordination, adequate optimization prior to surgery, improving man power availability and increasing the operation theatre utilization time help to avoid cancellation of surgery on the scheduled day.

Key Words: Cancellation, elective surgeries, scheduled day, teaching institution

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INTRODUCTION

Surgery is the primary modality of treatment in specified diseases and it requires a multi disciplinary team work approach in the operating room. Pre anaesthetic assessment, adequate preparation and optimization of the surgical patient prior to the elective surgery, avoids the major catastrophic events perioperatively and also decreases the morbidity and mortality of the surgical patients. Cancellation of surgery on the scheduled date poses physical, psychological and emotional disturbances not only to the operating surgeon, but it also includes the perioperative physician as well as the patients.[19] The prevalence and the cause for cancellation of elective surgery on a scheduled day is diverse and vary from one institution to another. The incidence of cancellation is between 10% and 40% among the booked elective list in various institutions[1,6,15,16,17]. The most common cause of cancellation of elective surgery on a scheduled day in a

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teaching institution includes lack of operating time in theatre[1,2,16,17,18] (running beyond the scheduled hours), poorly optimized surgical patients due to varied medical conditions or the administrative reasons[1,7,16,17,18]. Delayed start of elective list, unanticipated anaesthetic or surgical problems or shortages of the operating staff also contribute an pivotal role in cancellation[10]. The reasons to quantify the cancellation list is usually vivid and difficult. Hence the simplest approach is to evaluate the cause for cancellation of surgery and the specified issues needs to be focused preventing the postponement of the elective surgery.

AIM

The objective of this audit was

1. To find out the prevalence and the reasons for cancellation of elective surgery on the scheduled day and to focus on improving the quality of health care
2. To find out the clinical quality indicator with regard to effective theatre utilization time on a scheduled day and thereby offering better utilization of theatre services and resources

MATERIALS AND METHODS

This audit was a retrospective analysis and an observational study conducted in a NABH accredited, ISO certified 1300 bedded tertiary teaching hospital PSG Institute of Medical Sciences and Research, Coimbatore. The audit study period was included for one year duration from 1st October 2017 to 30th September 2018. The elective operating list scheduled for the next working day was planned by the concerned operating surgeon by 3 pm the day prior to operation and the list is printed and displayed in the department. The operating list included the complete elective surgery list on the scheduled day and the cancellation list were maintained in a separate registers along with a detailed note mentioning the details of cancellation. Our hospital comprises of 10 operating room in the main Operation Theatre block involving surgeries with various specialities like General surgery, laparoscopic surgery, obstetrics and gynecology, orthopedic surgery and neuro surgery on all days; with a separate theatre in labor ward and 2 cardiothoracic theatre functioning exclusively separately. Speciality surgeries like pediatric surgery, ENT, plastic surgery, hepato-biliary surgery, surgical gastroenterology, urology and transplant surgery with allocation for three days in a week for elective surgery. The scheduled elective surgery operating duration list was included between 8 am and 5 pm in our institution, with exceptions working on Sundays and public holidays. We coined the cancellation of the elective surgery as any proposed surgery which was listed and planned the previous day; and subsequently not done on the scheduled day of surgery. The source of audit was the documentation maintained in cancellation records in the anaesthesia department and these data were analyzed. The most common cause for cancellation of surgery was categorized as Patient related, Pre operative (or Surgical) preparation related and Facility related factors. All the data were entered in the Microsoft excel spreadsheet and a Pareto chart was constructed to prioritize the reasons and analyzed using Epi info software version. Although the audit was done for improving departmental work under quality assurance, this study received ethical committee approval (Ref No: PSG/IHEC/2018/Appr/Exp/207/ Proposal No.18/202 dated 13.08.2018).

OBSERVATIONS AND RESULTS

Table 1: Cancellation of cases Month wise from October 2017 to September 2018

Month	Number Of Cases (n)	Cancelled Cases (n)	Case Cancelled (%)
October 2017	775	52	6.70
November 2017	900	70	7.77
December 2017	851	50	5.87
January 2018	754	38	5.03
February 2018	740	35	4.72
March 2018	858	46	5.36
April 2018	829	37	4.46
May 2018	1,008	56	5.55
June 2018	911	28	3.07
July 2018	971	40	4.11
August 2018	829	33	3.98
September 2018	880	47	5.34
Total cases	**10,306**	**532**	**5.16**

Table 2 – Factors determining the cancellation of cases

Factors for cancellation of surgery	Cancelled cases (n)	Cancellation (%)
Patient related factors	125	23.49
Pre operative preparation related factors	265	49.81
Facility related factors	142	26.69

Table 3: Patient related factors for cancellation of surgery

S.No.	Patient related factors	Number of cancelled cases
1	Financial constraint	11
2	Unwillingness for surgery	66
3	AMA discharge	18
4	Patient not admitted to hospital	6
5	Wants surgery in future date	14
6	Unexplained death	1
7	Patient had food	3
8	Non availability of attendars	4
9	Turned pregnant	2

Table 4: Pre operative preparation related factors for cancellation of surgery

S.No.	Pre operative preparation related factors	Number of cancelled cases (n)
1	Pending specialised opinion	49
2	Abnormal laboratory reports	57
3	Inadequate optimisation	10
4	Active respiratory infection	20
5	Needed further pre operative evaluation	27
6	Baby fitness – Sterilisation procedure	23
7	Late laboratory reporting time	7
8	Wound infection	16
9	Improving on conservative management	16
10	Sudden detoriation of clinical condition with new onset signs and symptoms	40

Table 5: Facility related factors for cancellation of surgery

S.No.	Facility related factors	Number of cancelled cases
1	Pending insurance / scheme	77
2	Equipment / Implant unavailability	12
3	Non availability of OT	19
4	Incorrect / wrong list booking	2
5	Inadequate OT staff	2
6	Lack of time for parent unit cases	17
7	Academic purpose	2
8	Operating surgeon on leave	11

Table 6: Depicts the Operation Theatre Utilization Time during the working hours

Month	Sample Size	*OT Utilization Rate (%)											
		OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	Labour OT	CT OT
October 2017	775	55.5	65.6	18	43.2	49.5	45.7	30.6	53.8	47.3	64.2	11	44.2
November 2017	900	52.8	71.2	18	45.5	55.9	63.0	33.9	60.6	54.9	65.8	8	47
December 2017	851	50.6	68.3	35.5	42.4	51.3	66.3	20.4	56.7	46.8	65.3	9.4	37.8
January 2018	754	49.6	64.7	37	41.8	47.7	50	18.9	55.5	56.9	50.5	11.8	39.6
February 2018	740	64.6	70.6	17.8	51.5	51	60.8	24.4	50.6	43.8	59.4	8.9	48.8
March 2018	858	73.4	80	21.5	36.5	39.5	56.8	36	60.8	44.5	58.5	22.6	50
April 2018	829	65.5	82.8	17.6	37.1	46.4	55.6	28.6	60.7	46.8	51.9	16	42
May 2018	1,008	98.1	94.8	22.5	43.8	63.5	75.9	37.5	67.8	58.6	56.3	12.9	41.7
June 2018	911	97.1	98.6	17.7	56.2	46.5	61.9	29.8	66.8	53.9	63.1	14.7	49.5
July 2018	971	59.7	73.5	16.6	49.7	57.1	71.3	36.6	60.4	56.5	67.8	23.3	47.3
August 2018	829	58.2	60.6	21.6	55.4	50.0	62.7	25.9	53.2	48.2	54.5	14	41.3
September 2018	880	68.4	81.6	9.8	60.9	50.6	64.8	28.2	60.8	49.2	64.5	24.2	46

*OT Utilization Rate = OT Utilization Time in hours X 24 / Resource Hours X 100

DISCUSSION

The National Health Service (NHS), UK through its Modernization Agency Theater Program has defined case cancellation as those that occur after the patient has been notified of operation date[8]. Few define it as the procedures that were cancelled either on the day on which the surgery was scheduled, or cases which appeared in the definitive scheduled list but ultimately was not performed. Out of 10,306 patients during the scheduled period in our audit, 532 (5.16%) patients had cancellation on the elective list. Rakesh Garg *et al*[5,17] in a north Indian government hospital reported 30.3 % cancellation list followed by, Ezike *et al*[7] stated 25.3 % cancellations, Rajendra Kumar *et al*[16] in an government hospital showed 17.6 % cancellation, Abderrazak Sahraoui and Mohammed Elarref *et al*[2] observed 13.4 % and JimEnez *et al*[9] noted with 3.9 % as cancellation comparable to a study in a Jordanian hospital[11] with 3.6% cancellation on the elective list respectively. The cancellations at a teaching hospital in south india was found to be 4.4 % which was in accordance with our study (5.16%). In our audit, the frequency of cancellations was pronounced more in pre operative or surgical preparation related factors accounting for 265 patients (49.81%), followed by facility related factors in 142 patients (26.69%) and 125 patients (23.49%) in patient related factors respectively. On categorizing Surgical (or pre operative) preparation related factors which includes patients presenting with active respiratory infections, acute exacerbation of any medical conditions, electrolyte disturbances, uncontrolled blood sugar or blood pressure; the overall cancellation was maximum with abnormal laboratory values (21%) on the day of surgery. URI-related cancellations was reported by Palomero Rodriguez *et al*[13], however this decreased from 12.9% in 2001 to 7.2% in 2003 through enhanced communication of simple and appropriate information to parents resembling with 7.54% in our audit. Knox *et al* reported a significant reduction in medical-related surgical cancellations after the establishment of a preoperative assessment clinic (P = 0.013).[12] Patients unwillingness for surgery accounted for 53% with regard to patient related factors for cancellation of surgery. Patients not willing for surgery at the last moment prior to surgery were due to fear, unfavorable conditions existing in the family or unawareness about the importance of surgeries. Female patients who were menstruating on the day of surgery were also considered in our study. Study by Fayed *et al* showed that 9% of patients decided not to undergo elective surgery on the day of surgery[4]. Being a teaching medical institution, few reasons for lack of operating time such as teaching and training the junior residents and interns could not be avoided. There was no issues with administrative or logistic causes such as power failure in operating room, failure of air conditioners, availability of oxygen source, usage of anaesthetic drugs, availability of blood and blood products, suture materials, linen, supply of water, bed availability and hospital workers strike in our audit. Cancellation by Insurance sanctioning availed by government scheme and private insurance peaked maximum cause due to delayed clearance or failure of obtaining the insurance. In general, surgeons took longer than estimated time to finish the procedure and was especially true for less-experienced surgeons and trainees. Surgeons are sometimes too optimistic and aware of the long waiting lists, but they want to operate as many patients as possible. This reason can be avoided by a better internal organization of work as well as planned use of staff breaks and the adequate preoperative preparation of the patients. Patient non compliance regarding medication regimens. Schofield *et al*[18] reported bed availability and theatre time management as a main reason for cancellation; which was not an issue in our audit. Pandit JJ, Carry A[14] estimated that over-booking of operation list is the common cause for postponement due to waiting list pressures. Windokun *et al*[20] reported surgeon's un-availability as main reason. Dufek *et al*[3] recommended the improvement in protocol for pre-op patients' evaluation to address these problems. Most of the reasons for cancellation were debatable as an issue of health care quality and was avoidable by judicious scheduling of lists and careful planning by the team involved in surgeries. We justified with improving following recommendations in our institution :

- Calm, smooth, pleasant and noiseless pre operating assessment room can avoid missing major clinical findings
- Closed loop communication and good rapport with the patient helps to instill confidence preventing the patient's bias regarding the anesthesia and surgery
- Follow appropriate pre operative instructions to improve the health care system avoiding major issues on the day of surgery
- Alleviation of the psychological distress of cancelling the surgery
- Early start of the first case in the operating room
- Surgical patients were adequately counseled, optimized their medical condition, appropriate continuation of medications and obtained informed consent regarding all relevant procedures
- Inter departmental coordination in managing complicated and high risk cases
- Increased utility of operating time in the theatre complex during the working hours

- Following the medical ethics and to practice in accordance with the law

LIMITATIONS

Our study was related to inherent bias in categorizing the cancellation of cases due to varied reasons on behalf of multi factorial components leading to cancellation. The study was confined only to our institution and may not be standardized since the hospital policies varies in other teaching institutions.

CONCLUSION

Although cancellations were only 532 (5.16%) out of 10,306 patients of total elective surgeries, this can even be reduced by implementing and following the recommendations as per guidelines proposed, so that the potential causes can be avoided. To improve the better quality of life we recommend that the cancellations of surgeries can be reduced by improving pre operative assessment, requesting patient information on inter current illnesses between preadmission and surgery, better inter departmental coordination and adequate optimization prior to surgery, improving man power availability, availability of adequate resource sources and increasing the operation theatre utilization time to avoid cancellation of surgery on the scheduled day. Frequent conduct of audits, rectifying the pitfalls in the institution will definitely improve the better healthcare facility in the teaching hospital.

REFERENCES

1. Abderrazak Sahraoui and Mohamed Elarref. Bed crisis and elective surgery late cancellations: An approach using the theory of constraints. Qatar Med J.2014; 2014(1):1–11.
2. A. Gonzalez-Arevalo1, J. I. Gomez-Arnau, F. J. DelaCruz, J. M. Marzal, S. Ramirez, E. M. Corral and S. Garcia-del-Valle·Causes for cancellation of elective surgical procedures in a Spanish general hospital.Anaesthesia. 2009 May; 64(5): 487–493.
3. Dufek S, Gaucher E, Gialanella. The total quality process applied to the operating rooms and other clinical processes. Surgery. 1993; 113: 255-259.
4. Fayed A, Elkouny A, Zoughaibi N, Wahabi HA. Elective surgery cancellation on day of surgery: An endless dilemma. Saudi J Anestth. 2016 Jan-Mar; 68-73.
5. Garg R, Bhalotra AR, Bhadoria P, Gupta N, Anand R. Reasons for cancellation of cases on the day of surgery: A prospective study. Indian J Anaesth 2009; 53:35–9.
6. George Stavrou, Stavros Panidis, John Tsouskas, Georgia Tsaousi, and Katerina Kotzampassi Audit of Operating Room Time Utilization in a Teaching Hospital: Is There a Place for Improvement? ISRN Surg. 2014; 2014: 431740.
7. HA Ezike, VO Ajuzieogu, and AO Amucheazi Reasons for Elective Surgery Cancellation in a Referral Hospital.Ann Med Health Sci Res. 2011 Jul-Dec; 1(2): 197–202.
8. Ilango Ganesan, R. Anuradha, P. Ravindrakumar. "Audit of Cancellation of Elective Surgeries in a Teaching Hospital in South India". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 31, April 16; Page: 5322-5328, DOI:10.14260/jemds/2015/778
9. JimEnez A, Artigas C, Elia M, Casamayor C, Gracia J, Martinez M. Cancellation in ambulatory day surgery: An observational study. Ambul Surg. 2006; 12: 119-123.
10. J. J. Pandit, A. Carey. Estimating the duration of common elective operations: implications for operating list management. Anaesthesia. Aug 2006; 61(8): 768-776.
11. Kaddoum *et al*. Causes of cancellations on the day of surgery at a Tertiary Teaching Hospital. BMC Health Services Research (2016) 16:259 DOI 10.1186/s12913-016-1475-6
12. Knox M, Myers E, Hurley M. The impact of pre-operative assessment clinics on elective surgical case cancellations. Surgeon 2009; 7:76–8. doi: 10.1016/S1479-666X(09)80019-X.
13. Palomero Rodriguez MA, Sanabria Carretero P, Rodriguez Perez E, Goldman Tarlousky L, Nodal Ramos A, Diaz Gonzalez C. [Upper respiratory tract infections in children: Impact on surgical scheduling at a pediatric hospital]. Rev Esp Anestesiol Reanim 2005; 52:597–602.
14. Pandit JJ, Carey A. Estimating the duration of common elective operations: implications for operating list management. Anesthesia. 2006; 61: 768-776
15. PL Chalya, JM Gilyoma, JB Mabula, S Simbila, IH Ngayomela, AB Chandika, and W Mahalu. Incidence, causes and pattern of cancellation of Elective surgical operations in a University Teaching Hospital in the Lake Zone, Tanzania. Afr Health Sci. 2011 Sep; 11(3): 438–443.
16. Rajender Kumar and Ritika Gandhi. Reasons for cancellation of operation on the day of intended surgery in a multidisciplinary 500 bedded hospital. J Anaesthesiol Clin Pharmacol. 2012 Jan-Mar; 28(1): 66–69.
17. Rakesh Garg, Anju R Bhalotra, Poonam Bhadoria, Nishkarsh Gupta, and Raktima Anand. Reasons for Cancellation of Cases on the Day of Surgery–A Prospective Study. Indian J Anaesth. 2009 Feb; 53(1): 35–39.
18. Schofield WN, Rubin GL, Piza M, Lai YY, Sindhusake D, Fearnside MR, Klineberg PL. Cancellation of operations on the day of intended surgery at a major Australian referral hospital. Medical Journal of Australia 2005; 182(12):612–615.
19. Tait AR, Voepel-Lewis T, Munro HM, Gutstein HB, Reynolds PI. Cancellation of pediatric outpatient surgery: Economic and emotional implications for patients and their families. J Clin Anesth 1997; 9:213–19. doi: 10.1016/S0952-8180(97)00032-9.
20. Windokun A, Obideyi A. Audit of emergency theatre utilization. African Journal of Medicine and Medical Sciences2002; 31: 59-62.

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Aorto-oesophageal fistula: rarest cause of haematemesis - is it salvageable?

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SUMMARY

A 60-year-old man presented with severe abdominal pain, two episodes of massive haematemesis and chest discomfort. CT angiography showed a saccular aneurysm of the juxtaphrenic aorta with possible oesophageal erosion. Upper GI endoscopy revealed external compression of the lower oesophagus—near total luminal obstruction with impending rupture of the aortic aneurysm. Emergency aneurysmal repair by interposition grafting using 20 mm Dacron graft with oesophageal–gastric reconstruction done. Postoperative period was uneventful

BACKGROUND

This report describes the management of a life-threatening rare vascular emergency of aneurysm of the juxtaphrenic aorta with oesophageal erosion leading to formation of aorto-oesophageal fistula complicated with acute dissection. The presentation was inevitable for cardiovascular collapse in the absence of timely surgical intervention. The aneurysm being located in the descending thoracic aorta, the repair requires a left atrium-to-femoral bypass to maintain perfusion to the abdominal viscera and lower extremities. During the repair, the brain and the spinal cord is protected by cerebrospinal fluid drainage.

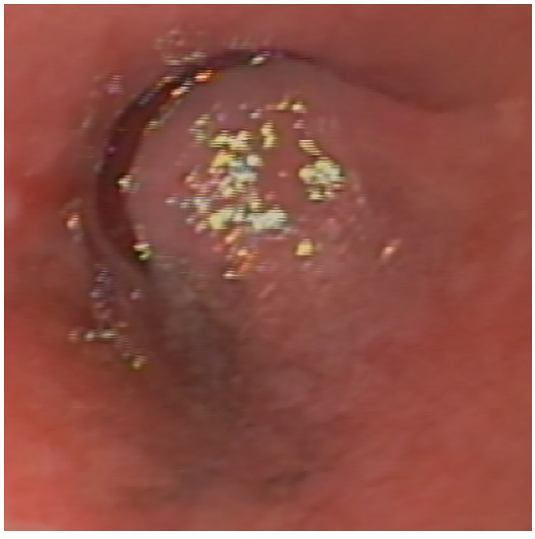


Figure 1 Upper gastrointestinal endoscopy—lower oesophageal external compression—near total luminal obstruction with impending rupture/ leaking nature of aortic aneurysm with mucosal ulceration of oesophagus covered with blood clot, eroding the distal oesophagus just above the oesophageal hiatus of the diaphragm.



Figure 2 CT angiogram.

CASE PRESENTATION

A moderately built 60-year-old man, habitual smoker for 40 years with recently diagnosed hypertension not on medications was admitted with severe abdominal pain, two episodes of massive haematemesis and chest discomfort for the last 2 days. The abdominal pain was sudden in onset, gradual in progression, localised to the epigastric region.

On examination, he was conscious, oriented, afebrile, pallor present with pulse rate of 86/min, blood pressure 100/80 mm Hg and respiratory rate of 30/min. On cardiovascular system examination, S1, S2 heard in the mitral, tricuspid, aortic and pulmonary area with no murmur. Respiratory system examination, normal vesicular breath sounds heard with equal bilateral air entry, no added sounds. On abdomen examination, soft, non-tender with no organomegaly. On central nervous system examination, clinically normal with no focal neurological deficits.

INVESTIGATIONS

Routine investigations revealed haemoglobin of 90 g/L, blood group O negative, bleeding parameters

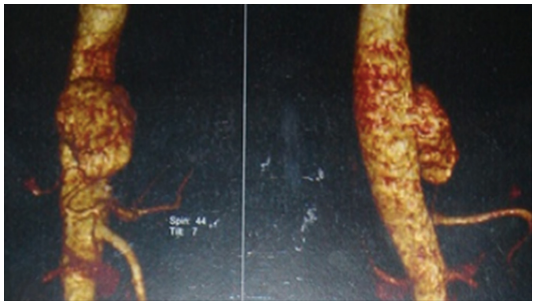


Figure 3 CT angiogram: large saccular aneurysm of juxtaphrenic aorta with large intramural thrombus and sign of impending rupture.

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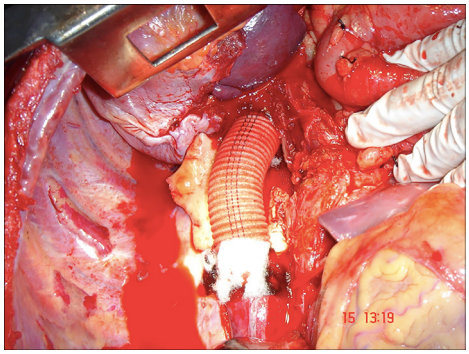


Figure 4 Interposition grafting—20 mm tubular Dacron graft from mid thoracic aorta to supra coeliac abdominal aorta by an thoracolaparotomy approach at eighth intercostal space under left atrium-femoral bypass.

were normal, renal and liver function test was normal, serology for HIV and hepatitis B was negative

Lower oesophageal external compression—near total luminal obstruction with impending rupture/leaking nature of aortic aneurysm with mucosal ulceration of oesophagus covered with blood clot (figure 1).

Large saccular aneurysm of juxtaphrenic aorta with oesophageal erosion (Figures 2 and 3).[1]

TREATMENT

Management strategies

The patient was taken up for emergency aneurysm repair with interposition grafting and oesophago–gastric anastomosis. By thoracolaparotomy—left atrium femoral bypass was done, following which oesophageal repair by excision of the perforated segment with gastric anastomosis was done. After intubation, while positioning, the patient developed massive amount of bleeding evidenced through mouth with cardiovascular collapse as a result of aneurysm rupture into the oesophagus.

Emergency aneurysmal crash care

Step 1

- Emergency thoracotomy performed.
- In view of massive ongoing bleeding, manual compression was given to the descending thoracic aorta proximal to the aneurysm.
- Fluids and blood transfusion given for resuscitation.
- Extension of thoracotomy to thoracolaparotomy.

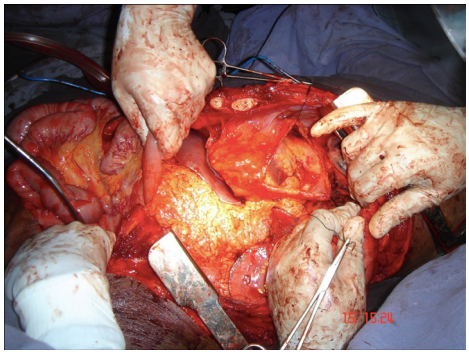


Figure 5 Oesophagectomy and stomach pull through with oesophago gastric anastomosis.

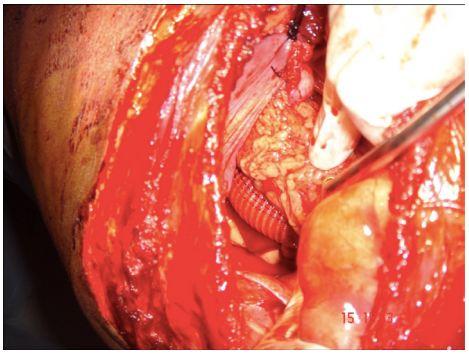


Figure 6 Lie of the Dacron graft and the oesophago gastric anastomosis covered by pedicled omentum.

- Loop control of supradiaphragmatic thoracic aorta and cross clamping above the aneurysm.
- Loop control of suprarenal abdominal aorta below the aneurysm and cross clamping.

Step 2

Left atrium femoral bypass done.

Step 3

Aneurysm repair—the aneurysmal segment reconstructed with 20 mm interposition Dacron graft (figure 4).

Step 4

Off left atrium femoral bypass.

Step 5

Oesophagus continuity established by gastro-oesophageal anastomosis after excising the distal perforated segment of the oesophagus, oesophageal gastric anastomosis wrapped with pedicled omentum (figures 5 and 6).

Step 6

Thoracolaparotomy closed with apical and basal pleural drain.

OUTCOME AND FOLLOW-UP

- The postoperative period was uneventful.

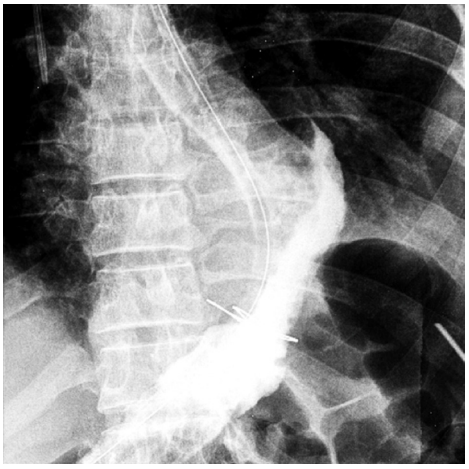


Figure 7 Immediate postoperative period—barium swallow using gastrografin dye performed—oesophagogastric anastomosis integrity is good, no leaks.

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- Postoperatively routine complete blood count, liver function test and renal function test was done and was normal.
- Chest X-ray was taken—lung expansion is good, no pleural effusion.
- Barium swallow using gastrografin dye performed—oesophagogastric anastomosis integrity is good, no leaks (figure 7).
- The patient was followed continuously for 15 days with daily haemoglobin monitoring, the haemoglobin value before discharge was 114 g/L.
- The patient was subsequently followed for 6 years, Echocardiogram (ECHO), gastrografin study, routine lab investigations like complete blood count was done.

DISCUSSION

Aorto-oesophageal fistula is a potentially a fatal condition, characterised classically by Chiari triad,[2] that is, midthoracic pain, sentinel arterial haemorrhage and exsanguination after a symptom free period. The causes of this fistula could be a primary aortic aneurysm or secondary causes like foreign body ingestion, oesophageal malignancy or an endoaortic stent causing erosion of oesophagus leading to fistula formation.[1]

The fistula formed between the descending thoracic aorta and oesophagus causes high pressure blood jetting into the oesophagus,[3] causing massive haematemesis and the patient can exsanguinate before they reach the hospital for further care.

The diagnosis is based on specific investigations like CT angiogram and upper GI endoscopy.

The volume of blood that flows through thoracic aorta is at higher pressure, greater than any other vascular structure, any condition that disrupts the integrity of the thoracic aorta such as aneurysm can lead to catastrophic consequences, therefore, emergency surgical repair is required and protecting the brain and spinal cord during such repairs is critical.[4 5] Slash thoracotomy, aortic loop control, volume resuscitation and appropriate circulatory support saved this patient.

The patient was taken up for emergency aneurysm repair with interposition grafting. By thoracolaparotomy—left atrium femoral bypass was done, following which oesophageal repair by excision of the perforated segment with gastric anastomosis was done. The oesophago–gastric anastomosis was covered with pedicled omentum to safe guard the anastomotic line from the synthetic graft.

Patient's perspective

I take this opportunity to thank all the doctors to identify my disease and treat it promptly on time.

Learning points

- Emergency surgical repair with simultaneous resuscitation is mandatory.
- Adequate amount of blood products needs to be kept ready.
- Postoperative follow-up with gastrografin study is done to check for anastomotic leaks.

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REFERENCES

1 Eggebrecht H, Mehta RH, Dechene A, *et al*. Aortoesophageal fistula after thoracic aortic stent-graft placement: a rare but catastrophic complication of a novel emerging technique. *JACC Cardiovasc Interv* 2009;2:570–6.
2 Carter R, Mulder GA, Snyder EN, *et al*. Aortoesophageal fistula. *Am J Surg* 1978;136:26–30.
3 Lee RY, Flaherty L, Khushalani NI, *et al*. Aorto-esophageal fistula: a rare fatal case caused by esophageal malignancy. *J Gastrointest Oncol* 2010;1:64–5.
4 Heckstall RL, Hollander JE. Aortoesophageal fistula: recognition and diagnosis in the emergency department. *Ann Emerg Med* 1998;32:502–5.
5 da Silva ES, Tozzi FL, Otochi JP, *et al*. Aortoesophageal fistula caused by aneurysm of the thoracic aorta: successful surgical treatment, case report, and literature review. *J Vasc Surg* 1999;30:1150–7.

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Original Research Article

Comparison of C-MAC blade and McCoy blade for laryngoscopy in adult patients undergoing tracheal intubation for elective surgeries with simulated cervical spine injury using manual in line stabilization

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Abstract

Background: The gold standard and ideal position for laryngoscopy is sniffing position. Trauma life support guidelines recommend the use of Manual In Line Stabilization or a rigid collar to stabilize the spine in suspected cervical spine injury patients. The gold standard for intubation in patients with cervical spine injury is fibre-optic bronchoscopy. But its use is restricted by lack of expertise, availability and time requirement. C-MAC blade and McCoy blade have been independently compared with Macintosh laryngoscope in simulated difficult airway. Aim: In our study, we compare C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury using Manual In Line Stabilisation (MILS). **Materials and methodology:** 100 patients of age group between 18 – 65 years belonging to ASA 1 & 2 posted for elective surgeries under general anesthesia with endotracheal intubation participated in this study. Patients were randomly allocated to one of the two groups by computer generated random table number. Group C - Endotracheal intubation done using C-MAC D blade video laryngoscope and Group M - Endotracheal intubation done using McCoy blade laryngoscope. Observation and results: Baseline characteristics were presented as mean ± S.D. Two-sided unpaired t-test and chi-square test was applied to analyze the data and p value less than 0.05 were considered as significant. **Conclusion:** C-MAC video laryngoscope requires less time for laryngoscopy, provides better visualisation of glottis, lower IDS score with similar duration of intubation and haemodynamic responses when compared to McCoy laryngoscope in patients with simulated cervical spine injury.

Key Word: C-MAC blade, McCoy blade, laryngoscopy, simulated cervical spine injury, manual in line stabilization

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INTRODUCTION

The gold standard and ideal position for laryngoscopy is sniffing position. The sniffing position aligns the oral, pharyngeal and laryngeal axes and provides better glottic visualization.[2] The sniffing position produces flexion of the lower cervical spine, extension of the upper cervical spine and extension of the atlanto–occipital joint.[3] Trauma life support guidelines recommend the use of Manual In Line Stabilization (MILS) or a rigid collar to stabilize the spine in suspected cervical spine injury patients.[4] MILS prevents head extension and neck flexion which is necessary for optimal alignment of three airway axes.[5]

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The presence of collar can worsen the laryngoscopic view and makes the intubation difficult with conventional laryngoscope.[4] To overcome this various devices and options like direct laryngoscope with the aid of gum elastic bougie, fibre-optic bronchoscopy, airway scope, McCoy laryngoscope, Intubating laryngeal mask airway, C-Trach and Bullard laryngoscope has been recommended by many authors.[6] The gold standard for intubation in patients with cervical spine injury is fibre-optic bronchoscopy. But its use is restricted by lack of expertise, availability and time requirement.[4] The McCoy levering laryngoscope is a modification of the standard Macintosh blade.[6] It has a hinged tip and the angle of the hinged portion can be altered by a lever attached to the handle.[7,8] Depression of the lever towards the handle elevates the tip.[8] The hinged tip aids in improving the Cormack and Lehane laryngoscopic view by 1 grade in comparison to Macintosh blade in patient with cervical spine injury. The blade is available in size 3 and 4.[4] The latest generation C-MAC video laryngoscope has several distinct improvements.[8] It has external light source and small digital camera at the distal third of the blade, which extends to a video display monitor.[9] It provides optimum view of the glottis by direct and indirect view.[9] C-MAC laryngoscope can accommodate Macintosh blade 2, 3 & 4. A special D blade with greater curvature is designed to facilitate intubation of the difficult airways.[8] Both the devices have been independently compared with Macintosh laryngoscope in simulated difficult airway. Only a few studies are available comparing C-MAC and McCoy laryngoscope.[4] This study was carried out to compare the efficacy of C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury.

AIM

To compare C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury using Manual In Line Stabilisation (MILS) with the following parameters.

Primary Aim:

- Duration of laryngoscopy
- Duration of intubation
- Total duration of intubation
- Ease of intubation - Intubation Difficulty Scale (IDS score)

Secondary Aim:

- Haemodynamic responses

MATERIALS AND METHODOLOGY

A study titled "Comparison of C-MAC D blade and McCoy blade for laryngoscopy in patients with simulated cervical spine injury" was done in PSG Institute of Medical Sciences & Research, Coimbatore. This study was done after obtaining ethical committee clearance and informed written consent from all the patients participated in this study. It was a prospective and randomised study. 100 patients of age group between 18 – 65 years belonging to ASA 1 & 2 posted for elective surgeries under general anesthesia with endotracheal intubation participated in this study.

Inclusion criteria

- Patient's acceptance
- Patients aged between 18 – 65 years
- ASA 1 and ASA 2 patients
- Patients undergoing elective surgery under GA with endotracheal intubation

Exclusion criteria

- Anticipated difficult airway
- Risk of aspiration
- Hypertensive patients
- Patients on β-blockers, Clonidine.
- Duration of intubation exceeding 120 sec
- Change in laryngoscopy during intubation
- Emergency surgeries

Routine pre-operative assessment was done and the patients were kept nil per oral from 10 pm the day prior to surgery. Informed written consent was obtained. All the patients were pre-medicated with tablet Ranitidine 150mg orally at night the day prior to surgery and at 6 am on the morning of surgery. Patients were randomly allocated to one of the two groups by computer generated random table number.

Group C: Endotracheal intubation done using C-MAC D blade video laryngoscope

Group M: Endotracheal intubation done using McCoy blade laryngoscope

Parameters recorded

Duration of laryngoscopy - defined as the time taken from insertion of the blade between the teeth until the anaesthetist obtained the best possible view of the vocal cords

Duration of intubation - defined as the time taken from when the anaesthetist indicate the best view at laryngoscopy until the tracheal tube was placed through the vocal cords, as evidenced by visual confirmation by the anaesthetist.

Total duration of intubation - It is the sum of the duration of laryngoscopy and the duration of intubation

Ease of intubation - Intubation Difficulty Scale (IDS score). It includes the following:

1. Number of intubation attempts
2. Number of operators
3. Number of alternative technique used
4. Glottis exposure – CL gradin
5. Lifting force required

6. Necessity for laryngeal pressure
7. Vocal cord mobility

Haemodynamic responses - Heart rate, systolic BP, diastolic BP, mean arterial BP after 1st min, 3rd min and 5th min of intubation. The intubation difficulty scale (IDS)[37] : In 1997 Frederic Adnet *et al* evaluated and proposed an Intubation Difficulty Scale (IDS) score characterising the complexity of endotracheal intubation in both the prehospital and operating room settings to assess the degree of difficulty. The Intubation Difficulty Scale (IDS) score is a function of seven parameters, resulting in a progressive, quantitative determination of intubation complexity and they are as follows:

Parameters	Definition		
N^1-The number of intubation attempts	An attempt is defined as one advancement of the tube in the direction of the glottis during direct laryngoscopy	N^1 = 0 if only one attempt	Every additional attempt adds one point
N^2-The number of supplementary operators	It represents the number of additional persons directly attempting intubation	N^2 = 0 if only one operator	Every additional operator adds one point
N^3-The number of alternative techniques used	Change in approach - oral intubation to blind nasotracheal intubation, change in material – blade, endotracheal tube, addition of stylette	Change in approach	Each alternative technique adds one point
N^4-Glottic exposure	defined by the Cormack grade minus one	GradeI (N^4 = 0) GradeII(N^4 = 1) GradeIII(N^4= 2) GradeIV(N^4=3)	Glottic exposure is evaluated during the first attempt by the first operator
N^5- Lifting force applied during laryngoscopy	If little effort is necessary If subjectively increased lifting force is necessary	N^5 = 0 N^5= 1	
N^6- Necessity for external laryngeal pressure for optimizedglottic exposure	If no external pressure is applied If external laryngeal pressure is necessary	N^6= 0 N^6= 1	
N^7- Position of vocal cords during intubation	if vocal cords are in abduction if the vocal cords are in adduction	N^7= 0 N^7= 1	

The degree of difficulty is described according to IDS score as follows

IDS Score	Degree of difficulty
0	Easy
1 – 5	Slightly difficult
>5	Moderate to major difficulty
Infinity	Impossible intubation

Patients were connected to ASA standard monitors – ECG, Non invasive blood pressure(NIBP) and pulse oximeter (SpO_2) and baseline readings recorded. Intravenous line started and patency of the vessel checked. Patients were pre oxygenated with 100% Oxygen for 3 minutes and Induced with Fentanyl 2mcg/kg, Lignocaine (preservative free) 20mg and Propofol 2mg/kg intravenously. After ensuring adequate mask ventilation, patients were paralysed with Inj. Succinylcholine 1.5mg/kg. Pre intubation heart rate, systolic BP, diastolic BP, mean arterial BP were recorded. After 1 minute MILS was achieved by an anaesthetist, standing to the side of the patient, using fingers and palms of both hands to stabilize the patient's occiput and mastoid process and gently counteract the forces created by the intubator. Laryngoscopy was done by another anaesthetist who is experienced with both the techniques. Laryngoscopy was done with C-MAC D blade laryngoscope in group C and McCoy blade laryngoscope in group M. After visualization of the cords, patients were intubated with appropriately sized endotracheal tube with stylet bent to hockey stick shape. During intubation the duration of laryngoscopy, the duration of intubation and the ease of intubation (IDS scoring) were recorded. After securing the endotracheal tube, the tube position was confirmed with stethoscope and $ETCO_2$ monitoring, anaesthesia was maintained with Oxygen & Nitrous oxide 40:60 with 6 litres fresh gas flow, Sevoflurane 2% and Inj.Vecuronium (loading dose 0.1mg/kg). Heart rate, systolic BP, diastolic BP, mean arterial BP were recorded after 1st min, 3rd min and 5th min of intubation.

Statistical analysis: Data were statistically analysed with the SPSS version 17.0 software. Baseline characteristics were presented as mean ± S.D. Two-sided unpaired t-test and chi-square test was applied to analyze the data and p value less than 0.05 were considered as significant. A repeated measure of ANOVA was applied for the two groups to know the with-in subject variability in Ease of intubation and $p < 0.05$ was considered to be significant.

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OBSERVATION AND RESULTS

Table 1: Demographic details - Group C and Group M

1.Age distribution	Age in years	Group C		Group M		p- value	Results
		No of patient	Percentage	No of patients	Percentage		
	20 – 29	11	22	12	24	0.808	Similar in both the groups
	30 – 39	16	32	14	28		
	40 – 49	11	22	10	20		
	≥ 50	12	24	14	28		
	Mean (in yrs) ± S.D	40 ± 11.9		40.6 ± 12.601			
2.Sex distribution	**Sex**	**Group C**		**Group M**		**p- value**	**Results**
		No of patient	Percentage	No of patients	Percentage		
	Female	24	48	25	50	0.843	Compar-able between the two groups
	Male	26	52	25	50		
3.BMI distribution	**BMI Range**	**Group C**		**Group M**		**p- value**	**Results**
		No of patient	Percentage	No of patients	Percentage		
	≥30	0	0	1	2	0.337	Compar-able between the two groups
	25 -29.9	26	52	28	56		
	18 -24.9	21	42	19	38		
	≤18	3	6	2	4		
	Mean (in yrs) ± S.D	24.36 ± 3.26		24.9 ± 3.12			

Table 2: Laryngoscopy and Intubation Duration for Group C and Group M

Parameters (seconds)	Group C (Mean±SD) N=50	Group M (Mean±SD) N=50	Mean Difference	p- value	Results
1.Duration of Laryngoscopy	12.15± 3.11	14.47± 3.55	-02.32	0.0001	Statistically significant
2.Duration of Intubation	13.85± 3.51	12.07± 3.23	1.78	0.01	Statistically significant
3.Total duration of Intubation	26 ± 5.65	26.55± 5.99	-0.55	0.639	Comparable between the groups

Table 3: Intubation Difficulty Scale (IDS) score for Group C and Group M

IDS score	Group C n (%)	Group M n (%)	p value	Results
N_1 (Attempts)				
1st - score 0	48(96)	44(88)	0.143	Not significant
2nd - score 1	2(4)	6(12)		
N_2 (operators)				
1 – score 0	50(100)	47(94)	0.080	Not significant
2 – score 1	0(0)	3(6)		
N_3 (alternative techniques)				
Not used – score 0	50(100)	50(100)		
N_4 (CL grade)				
1 – score 0	47(94)	34(68)	0.001	Significant
2 - score 1	3(6)	16(32)		
N_5(lifting force)				
Normal – score 0	45(90)	32(64)	0.002	Significant
Increased– score 1	5(10)	18(36)		
N_6(laryngeal pressure)				
Notapplied–score0	36(72)	42(84)	0.150	Not significant
Applied – score 1	14(28)	8(16)		
N_7 (vocal cord position)				
Abducted – score 0	50(100)	50(100)		

Table 4: Ease of Intubation (IDS score) for Group C and Group M

IDS	No of patients (%)		P value	Results
	Group C	Group M		
0 (easy)	35 (70)	27 (54)	0.009	Statistically significant
0-5 (Slight difficulty)	15 (30)	22 (44)		
> 5 (moderate to major difficulty)	0	1 (2)		

Table 5: Intergroup comparison of mean Heart rate between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	87.5 ± 14.5	84.7 ± 12.1	0.30
Before Intubation	87.9 ± 11.6	87.7 ± 10.6	0.93
1st min	99.9 ± 15.3	102.3 ± 16.6	0.45
3rd min	88.2 ± 13.0	89.9 ± 13.4	0.50
5th min	80.2 ± 10.9	79.9 ± 11.4	0.90

Table 6: Intergroup comparison of Mean Systolic BP between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	126.5 ±13.8	122.1 ±10.8	0.078
Before Intubation	106.6 ± 13.2	104.3 ± 8.8	0.309
1st min	124.6 ± 15.8	128.8 ± 15.3	0.179
3rd min	107.8 ± 11.7	110.3 ± 14.2	0.340
5th min	100.16 ± 11.2	102.6 ± 14.2	0.335

Table 7: Intergroup comparison of mean Diastolic BP between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	77.12 ± 7.2	75.3 ± 8.4	0.256
Before Intubation	65.7 ± 8.1	75.3 ± 8.4	0.457
1st min	78.4 ± 12.8	82.7 ± 12.9	0.098
3rd min	68.1 ± 9.2	70.8 ± 12.6	0.210
5th min	63.2 ± 8.1	65.5 ± 10.5	0.210

Table 8: Intergroup comparison of mean MAP between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	93.9 ± 8.3	91.3 ± 8.6	0.112
Before Intubation	79.7 ± 8.6	79.7 ± 6.2	0.963
1st min	94.1 ± 13.3	98.3 ± 13.5	0.112
3rd min	81.6 ± 9.2	84.3 ± 12.9	0.222
5th min	75.8 ± 8.3	78.2 ± 11.4	0.231

DISCUSSION

Approximately 2-5% of trauma patients will have cervical spine injury. Patient with cervical spine injury may require airway management for airway protection, to prevent hypoxia and hypoventilation.[1] In our study, we have compared the efficacy of C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury by comparing duration of laryngoscopy, duration of intubation, total duration of intubation, ease of intubation (IDS score) and haemodynamic response. In our study the demographic variables like age, gender and BMI were comparable between both the groups. The mean duration of laryngoscopy when compared between two groups, it was shorter in C-MAC group (12.5 seconds) than McCoy group (14.47 seconds) and it was statistically significant. The mean duration of intubation when compared between two groups, it was longer in C-MAC group (13.85 seconds) than McCoy group (12.07 seconds) and it was statistically significant. The mean total duration of intubation was comparable between C-MAC group (26 sec) and McCoy group (26.55 sec) and statistically not significant. Even though the time taken for laryngoscopy was shorter in C-MAC group, the time taken for successful intubation was prolonged. So the

mean total duration of intubation was comparable and statistically insignificant between the groups. Our results of duration of laryngoscopy, duration of intubation and total duration of intubation were similar with the study done by Jain *et al*.[4] comparing McCoy and C-MAC video laryngoscope in simulated cervical spine injury. The ease of intubation was observed in our study using IDS scoring. In our study 48 patients in C-MAC group and 44 patients in McCoy group were intubated in the first attempt and were comparable. This results were similar to study by Jain *et al*.[10,11] who compared the conventional C-MAC and the C-MAC D blade with direct laryngoscopes in simulated cervical spine injury (manikin study) and observed that out of 33 patients, 30 patients were intubated in first attempt using C-MAC D blade and 24 patients using McCoy and was not statistically significant. 50 patients in group C and 47 patients in group M required single operator and all the patients were intubated using single technique in our study. In C-MAC group 47 patients had CL grade 1 and 3 patients had CL grade 2. In McCoy group 34 patients had CL grade 1 and 16 patients had CL grade 2. Hence the glottis exposure was better with C-MAC group than McCoy group and it was statistically significant which was similar in studies by Jain *et al*.[4] and Sabry *et al*.[8] Jain *et al*.[4] compared McCoy laryngoscope and C-MAC video laryngoscope in simulated cervical spine injury and observed that out of 30 patients, 29 patients in C-MAC group and 16 patients in McCoy group had CL grade 1 and was statistically significant. Sabry*et al*.[8,12] compared C-MAC D blade and McCoy laryngoscopes during cervical immobilization and observed that out of 30 patients, 16 patients in C-MAC group and 4 patients in McCoy group had CL grade 1 and was statistically significant. The McCoy group (18 patients) needed more lifting force for visualisation of glottis than C-MAC group (5 patients) and it was statistically significant. 14 patients in C-MAC group needed external laryngeal pressure during intubation compared to 8 patients in McCoy group, but it was not statistically significant. The IDS score between both the groups was found to be statistically significant. The use of C-MAC resulted in more number of easy intubation when compared to McCoy[13]. This was similar to a study by Jain *et al*.[4] comparing C-MAC and McCoy laryngoscopes where C-MAC resulted in lower IDS than McCoy group. In our study the heart rate, systolic BP, diastolic BP and mean BP recorded at all times were comparable between C-MAC, McCoy group and was statistically insignificant. This was similar to study by Jain *et al*.

CONCLUSION

In our study though there was a statistical significance in duration of laryngoscopy and duration of intubation, the total duration of intubation was comparable and insignificant. Because with C-MAC the time taken for successful intubation was prolonged though the duration of laryngoscopy was shorter. The C-MAC group had better glottic visualisation, needed optimal lifting force and clinically insignificant external laryngeal pressure with lower IDS score when compared to McCoy group. To conclude C-MAC video laryngoscope requires less time for laryngoscopy, provides better visualisation of glottis, lower IDS score with similar duration of intubation and haemodynamic responses when compared to McCoy laryngoscope in patients with simulated cervical spine injury.

REFERENCES

1. Austin N, Krishnamoorthy V, Dagal A. Airway management in cervical spine injury. International Journal of Critical Illness and Injury Science. 2014;4(1):50-56. doi:10.4103/2229-5151.128013.
2. Pal R, Chauhan S, Ved BK, Lad SR. Evaluation of laryngoscopic view, intubation difficulty and sympathetic response during direct laryngoscopy in sniffing position and simple head extension: A prospective and randomized comparative study. Int J Res Med Sci 2015;3: 1895-901.
3. Magill IW. Endotracheal anaesthesia. American Journal of Surgery 1936; 34:450 – 5.
4. Jain D, Bala I, Gandhi K. Comparative effectiveness of McCoy laryngoscope and CMAC(®) videolaryngoscope in simulated cervical spine injuries. J AnaesthesiolClinPharmacol. 2016 Jan; 32(1):59-64.
5. Joseph J, Sequeira T, Upadya M. Comparison of the use of McCoy and TruView EVO2 laryngoscopes in patients with cervical spine immobilization. Saudi J Anaesth. 2012; 6: 248–53.
6. Bharti N, Arora S, Panda NB. A comparison of McCoy, TruView, and Macintosh laryngoscopes for tracheal intubation in patients with immobilized cervical spine. Saudi Journal of Anaesthesia. 2014;8(2):188-192. doi:10.4103/1658-354X.130705.
7. Laurent SC, de Melo AE, Alexander-Williams JM. The use of the McCoy laryngoscope in patients with simulated cervical spine injuries. Anaesthesia. 1996; 51: 74–5.
8. Sabry LA, Shaarawy SS, Ellakany MH, Elmasry AA. Comparison between C-MAC D-blade and McCoy laryngoscopes in intubating patients during cervical immobilization. Res OpinAnesth Intensive Care 2016; 3: 122-8.
9. Kilicaslan, A. Topal, A. Tavlan, A. Erol, and S. Otelcioglu, "Effectiveness of the C-MAC video laryngoscope in the management of unexpected failed intubations," Brazilian Journal of Anesthesiology, vol. 64, no. 1, pp. 62–65, 2014.
10. Jain D, Dhankar M, Wig J, Jain A. Comparison of the conventional CMAC and the D-blade CMAC with the

direct laryngoscopes in simulated cervical spine injury-a manikin study. Braz J Anesthesiol. 2014 Aug 31;64(4):269-274.
11. Kılıçaslan A, Topal A, Erol A, Uzun ST. Comparison of the C-MAC D-Blade,
Conventional C-MAC, and Macintosh Laryngoscopes in Simulated Easy and Difficult Airways. Turk J AnaesthesiolReanim. 2014; 42: 182–9.
12. Bharti N, Arora S, Panda BP. A comparison of McCoy, TruWiew and Macintosh laryngoscopes for tracheal intubation in patients with immobilized cervical spine. Saudi J Anesth. 2014; 8(2):188-92. doi: 10.4103/1658-354X.130705.
13. Adnet F, Borron SW, Racine SX, *et al*. The intubation difficulty scale (IDA). Anesthesiology 1997; 87: 1290–7.

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Original Research Article

Comparison of Intrathecal bupivacaine with fentanyl to intrathecal bupivacaine for elective lower abdominal and lower Limb surgeries

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Abstract

Background:: Pain is defined according to the International Association for the study of pain, as"An unpleasant, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage"**Aim:** The aim of the study is to compare the hyperbaric bupivacaine 0.5% and hyperbaric 0.5%+Fentanyl 20 micrograms in spinal anesthesia for elective lower abdominal and lower limb surgery. **Methods**: This prospective comparative study was conducted in patients of grade I and II physical status, undergoing elective surgeries of lower abdomen and lower extremities aged 18 to65 years. In Group A 30 patients received 2.5ml of hyperbaric bupivacaine 0.5% with 0.4ml of Normal saline and in Group B 30 patients received 2.5ml of hyperbaric bupivacaine 0.5% with 0.4ml of (20ug) of fentanyl citrate and normal saline was added to 5ml syringe containing 2.5ml of bupivacaine using a 1ml syringe for accuracy. **Results**: Out of 60 patients, 30 patients included in group A and 30 patients included in group B.In group A meantime for two-segment regression was 83.0 minutes and in group B was 114.5 minutes, time for full motor recovery in group A was 192.7minutes and in group B was 206.6 minutes, the meantime for complete sensory recovery in group A was 214 minutes and in group B it was 242 minutes, the time for the first request of analgesics postoperatively in group A 246.7 minutes and in group B it was 333minutes. The visual analogue score in group A at 3 hours, 6hours postoperatively and 24 hours was 0.6,4 and 4.0 and, in the group, B was 0.1, 1.7, 2.6. **Conclusion:** From this study, we concluded that the addition of 20ug of fentanyl to 0.5%hyperbaric bupivacaine in spinal anaesthesia, prolongs the duration of sensory and motor blockade, increase the duration and quality of postoperative analgesia 12 – 24 hours without causing any gross haemodynamic disturbances or adverse effects.

Keywords: Fentanyl, Bupivacaine, Hyperbaric, Anaesthesia.

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INTRODUCTION

Pain is defined according to the International Association for the study of pain, as "An unpleasant, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage".[1] Spinal anaesthesia consists of the temporary interruption of nerve transmission within the subarachnoid space produced by injection of a local anaesthetic solution into cerebrospinal fluid**.** SA is a routinely used anaesthetic technique for operations involving the lower limbs, lower abdomen, pelvis and perineal surgeries.[2,3,4] An increasing proportion of the patients undergoing these surgical procedures are the elderly.[5] Age-related changes in physiology and pharmacology can affect every aspect of pre-operative care.[6] The use of spinal anaesthesia is increasing in popularity compared to general anaesthesia.[2,3,7] Used widely, safely and successfully for

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almost 100 years spinal anaesthesia has many potential advantages over general anaesthesia, especially for operations involving the lower abdomen, the perineum and the lower extremities.[2,3,4] The role of an anaesthesiologist is to render pain free during surgical procedures. However, the patient's problem with pain does not end with surgical procedures. Pain during the postoperative period is a cause of concern not only for the patient but also for the surgeon and the anaesthesiologist. Postoperative pain control is generally best managed by an anesthesiologist because they offer regional techniques of anaesthesia as well as pharmacological expertise in analgesics. Spinal anesthesia is advantageous in that it uses a small dose of anaesthetic, is simple to perform and offers a rapid onset of action, gives reliable surgical and good muscle relaxation. These advantages are sometimes offset by a relatively short duration of action and complaining of postoperative pain when it wears off. Spinal anesthesia with hyperbaric bupivacaine 0.5% is a popular method, but there is a need for increasing the duration of analgesia without increasing the duration of motor blockade and thus prolong the duration of postoperative analgesia. The addition of fentanyl has been suggested as a method to accomplish these goals. This study is designed to quantitatively examine the effects of adding fentanyl to hyperbaric bupivacaine on the duration and recovery of sensory and motor block.

AIM

The aim of the study is to compare the hyperbaric bupivacaine 0.5% and hyperbaric 0.5%+ Fentanyl 20 micrograms in spinal anesthesia for elective lower abdominal and lower limb surgery.

MATERIALS AND METHODS

A prospective comparative study was conducted in the PSG hospitals, Coimbatore over a periodof 18monthstocompare the Hyperbaric bupivacaine 0.5% and hyperbaric 0.5%+ Fentanyl 20micrograms in spinal anesthesia for elective lower abdominal and lower limb surgery under different study parameters like onset and duration of analgesia, duration of sensory and motor blockade, quality of analgesia and side effects or complications. Inclusion criteria include ASA grade I and II physical status, undergoing elective surgeries of lower abdomen and lower extremities aged 18 to 65 years and exclusion criteria include the history of allergy or sensitivity to local anaesthetic or opioids, any contraindication to regional anaesthesia and ASA grading IIIand IV.A detailed pre-anesthetic examination including history, clinical examinations, the systemic examination of the cardiovascular, respiratory, central nervous system and examination of spine for deformity was performed. Routine investigations like haemogram, bleeding time, clotting time, blood sugar, blood urea, serum creatinine, ECG, Chest X-Ray were done wherever necessary. Patients' weight and height were also recorded prior to surgery. Premedication was standardized with Tab diazepam 0.2mg/kg preoperative on the night before surgery. All patients were instructed about the visual analogue scale(VAS).Patients were allocated into two groups viz., groups A, group B. In Group A 30 patients received2.5ml of hyperbaric bupivacaine 0.5% with 0.4ml of Normal saline and in Group B 30 patientsreceived 2.5ml of hyperbaric bupivacaine 0.5% with 0.4ml of (20ug) of fentanyl citrate andnormal saline was added to 5ml syringe containing 2.5ml of bupivacaine using a 1ml syringe foraccuracy.Before the start of the procedure, patients' pulse rate, blood pressure, respiratory rate, oxygensaturation was recorded. An intravenous line was secured using an 18G intravenous cannula.All patients were preloaded with 500mlof Ringer's lactate prior to spinal anaesthesia. Thepatients were kept nil per orally for 8 – 10 hours beforesurgery. The side effects of intrathecalfentanyl-like nausea, vomiting, pruritis, shivering respiratory depression (respiratory rate<10min), drowsiness, hypotension euphoria, chest tightness and urinary retention were noted.Hypotension was defined as a decrease in systolic blood pressure more than 20% of the baselinevalues and was treated with injection mephentermine 5mg intravenous increments andbradycardia (pulse rate <60/min) was treated by atropine 0.6mg intravenous stat.Under aseptic precautions lumbar puncture was performed in the left lateral position or sittingposition by midline approach by using disposable quincke spinal needle (23-26G) at L3-L4,intervertebral space. Patients were monitored continuously using sphygmomanometer, pulseoximeter and electrocardiogram, patients pulse rate, blood pressure, oxygen saturation wererecorded at 0,5,10,20,30,60,120 and 180 minutes. The sensory level was tested by pinprick using a hypodermic needle and the motor level was assessed by the Bromage scale. A pretested proforma will be used for collecting relevant data. Quantitative data will be analysed by students'"t" test and qualitative data will be analyzed by the chi-square test.

RESULTS

The mean age group A was 39.3 years and group B 42.6 years. The two groups did not differ significantly (P=0.28) with respect to their age. In Group A There were 10 male and 20 female patients. In Group B there were 12 male and 18 female patients. The height of patients in group A ranged from 4 feet (ft) 1 inch to 5 feet 9 inch with a mean height of 5.33 ft. In group B also theheight ranged from 4 feet 1 inch to 5 feet 9 inches but the mean height was 5.24ft. The height of patients in group A

ranged from 4 feet (ft) 1 inch to 5 feet 9 inch with a mean height of 5.33 ft. In group B also the height ranged from 4 feet 1 inch to 5 feet 9 inches but the meanheight was 5.24ft.

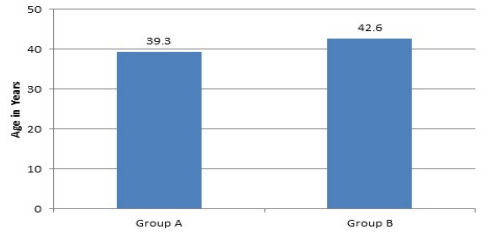


Figure 1: Age wise distribution

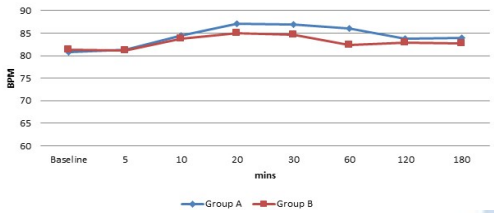


Figure 2: Distribution of Heart rate

In Group A the mean baseline heart rate was 80.8bts/min. In Group B the mean baseline heart rate was 81.33 beats/min.

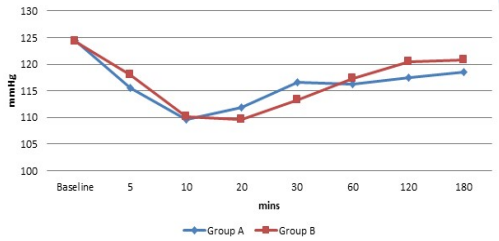


Figure 3: Distribution of Systolic BP

In Group A the mean systolic BP was 116.3mmHg. In Group B the mean systolic BP 116.75mmHg.

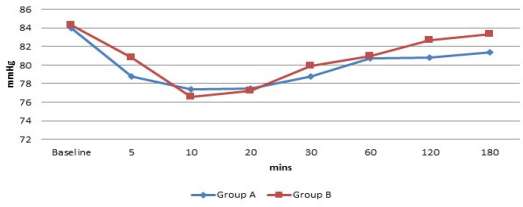


Figure 4: Distribution of Diastolic BP

The preoperative mean diastolic blood pressure in group A was 84.0mmhg, In Group B the preoperative mean diastolic blood pressure was 84.3mmhg. The mean time of onset of sensory blockade in Group A was 134.4 ±14.9 seconds and group B was 137.5 ± 13.5 seconds.

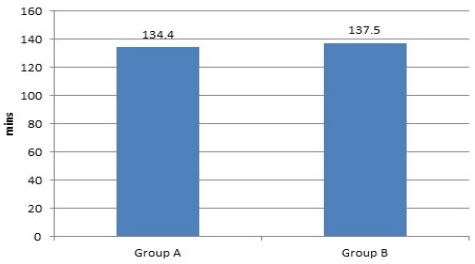


Figure 5: Onset of sensory blockade

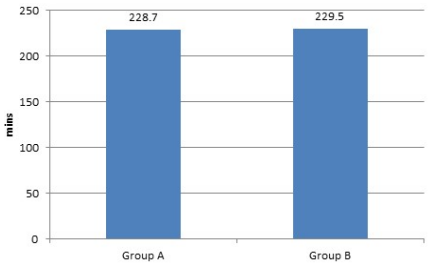


Figure 6: Onset of motor blockade

The mean time of onset of motor blockade group A was 228.7 ± 20.5 seconds and the meantime of the onset of the motor blockade in Group B was 229.5 seconds.In this study out of 60 patients the meantime for two-segment regression in groupA was 83.0 Minutes and in group B was 114.5 minutes.

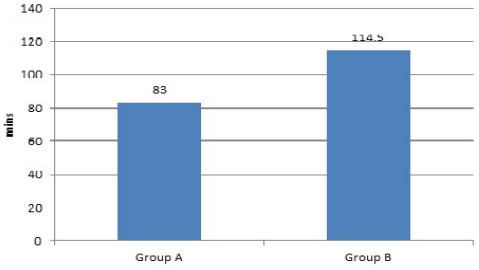


Figure 7: Time for two segment regression of sensory level

In this study out of 60 patients, the time for full motor recovery in group A was 192.7 minutesand in group B was 206.6 minutes.

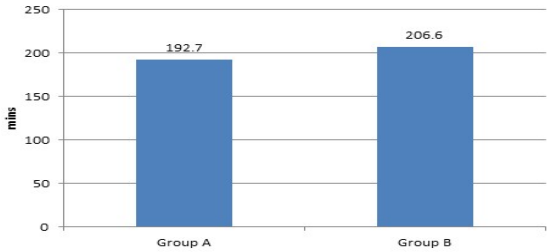


Figure 8: Time to full motor recovery

In this study out of 60 patients the meantime for complete sensory recovery in group A was 214minutes and in group B it was 242 minutes.

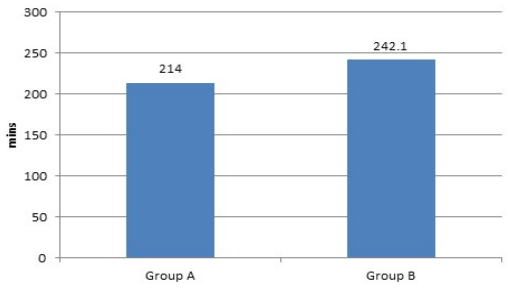


Figure 9: Time for complete sensory recovery

In this study out of 60 patients the time for the first request of analgesics postoperatively in group 246.7 minutes and in group B it was 333 minutes.

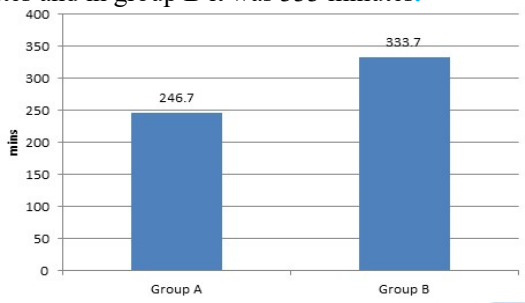


Figure 10: Time for the first request of analgesics

In this study out of 60 patients the visual analogue score at 3 hours was 0.6 in group A and 0.1 in Group B. At 6 hours postoperatively VAS was 4 in group A and 1.7 in group B. At24 hours VAS `was 4.0 in group A and 2.6 in Group B.

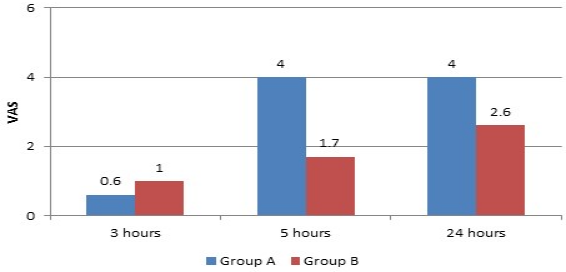


Figure 11: Visual analogue scores (vas) at different time

DISCUSSION

The subarachnoid block is one of the commonest local anaesthetic technique and would probablymaintain its place in the developing countries, because of simplicity minimal skill requirement,rapidity of onset, economy and minimum postoperative complications. It has the advantage thatit is easy to perform, requires a small dose of the drug, making systemic absorption unimportant. The disadvantage is the limited duration of action and lacks postoperative pain relief.The drugs used for the spinal subarachnoid block are lignocaine and bupivacaine. The disadvantage of spinal anaesthesia with local anaesthetic is that analgesia ends with regression of the block, which means there is an early postoperative need for analgesia.The use of neuraxial opioids has increased dramatically in recent years, augmenting the analgesiaproduced by local anaesthetic by binding directly to opiate receptors. The synergistic action of local anaesthetic and morphine is well known, but Fentanyl may have an advantage over morphine because of its rapid onset of action and superior intraoperative conditions.In the present study the patients studied in both the groups did not vary much with respect to age,sex, weight or height. Majority of the patients were middle-aged in both groups between 26-55years, with mean age of 39.3 years in group A and 42.4 years in group B. In both the groups'females were more, 66.66% of females were studied in group A and 60% of females in group B. The mean height and weight in either group were also identical. The type of surgeries performed was also identical in both groups. These parameters were kept identical in both groups to avoidthe intraoperative and postoperative outcomes of patients.In the present study all the patients weremonitored clinically in the intraoperative period. Pulserate, systolic blood pressure and diastolic blood pressure was monitored at regular intervals. Theincidence of hypotension was noticed in four patients of group A and in only two patients ofgroup B. Hypotension was corrected by administration of injection mephentermine 5 mg IV inincremental doses, giving IV fluids and foot end elevation. Systolic diastolic blood pressure inboth groups did not vary significantly in other patients. The incidence of bradycardia wasnot seen in any of the patients of either group and there was no significant difference in theonset of sensory and motor blockade and respiratory rate.In our study we found that time for two-segment regression was significantly prolonged inFentanyl group. Liu S *et al.*[8] in 1995, Harbhej Singh *et al.*[9] in 1998, Techanivate A *et al.*[10]in2004 found that two-segment regression was significantly prolonged in patients who receivedintrathecal Fentanyl with bupivacaine and was similar to our studies. Hunt CO *et al.*[11] in1989 conducted a study in 50 patients who received either 0, 2.5, 5,6.25, 25, 27.5 or 50ug offentanyl along with bupivacaine. He noticed that at 60 minutes the number of segmentsregressed was prolonged in 50ug fentanyl groups. But by 120 minutes there were no differences= between groups in the number of segments regressed. In the study, the duration of motor and the sensory block was significantly prolonged in the fentanyl group. Techanivate A *et al.*[10] in 2004 concluded that fentanyl increases the duration andthe intensity of bupivacaine in spinal anaesthesia, whereas Liu S*et al.*[8]in 1995, Harbhej Singh etal[9] in 1998found that addition fentanyl to 0.5% bupivacaine does not prolong the duration ofmotor

blockade but it only increases the time of sensory blockade without prolonging recovery tomicturition or street fitness. In a study conducted by Kuusniemi KS *et al*[12] in 2000 he foundthere was no difference in the duration of motor block among patients who received bupivacaine10mgonly and bupivacaine 7.5ug and fentanyl 25ug, but the duration were prolonged in thethe group which received 10mg bupivacaine and 25ug fentanyl. The duration was shortestamong the group which received 5mg bupivacaine + 25ug fentanyl. Similarly, the duration ofsensory block was maximum in the group which received 10mg bupivacaine and fentanyl 25ugand least in the group which received 5mg bupivacaine 25ug of fentanyl.In our study the meantime for the first request of analgesics in group A was 246 minutes and group B was 333 minutes. This was statistically highly significant. Hunt CO *et al.*[13] in 1987 found in their studies that fentanyl increases the duration of analgesia. Harbhej Singh *et al.*[9] in 1998concluded that intrathecal fentanyl 25 micrograms with 0.5% bupivacaine reduced analgesicthe requirement in the early postoperative period.The visual analogue pain score (VAS) at 3 hours was 0.6 ± 0.8 in group A and 0.1 ± 0.3 ingroup B. At 6 hours it was 4 ± 0.9 in group A and 1.7 ± 0.9 in group B. At 24 hours it was 4.0± 1.3 and 2.6 ± 1.3 in groups A and B respectively. The VAS was lower in group B at all timeintervals, which indicates that the quality of analgesia was better in group B patients even upon24 hours postoperatively. Hunt Co *et al.*[13] in 1987, Techanivate A *et al.*[10] in 2004 found that the use of intrathecal Fentanyl significantly reduces pain scores in the early postoperative period extending upon 12 hours.Hunt CO *et al.*[11] in 1989 found that fentanyl added to bupivacaine increased the quality of perioperative analgesia.

CONCLUSION

The addition of 20ug of fentanyl to 0.5% hyperbaric bupivacaine in spinal anaesthesia, prolongsthe duration of sensory and motor blockade, increase the duration and quality of postoperativeanalgesia 12 – 24 hours without causing any gross haemodynamic disturbances or adverseeffects. Based on the above facts we conclude the addition of fentanyl has many advantages and can be recommended with all spinal anaesthesia techniques.

REFERENCES

1. Merskey H, AlbeFessard D, Bonica JJ, Carmon A, Dubner R, Kerr FWL, Lindblom U, Mumford JM, Nathan PW, Noordenbos W, Pagni CA, Renaer MJ, Sternbach RA, Sunderland
2. S. Pain terms: a list with definitions and notes on usage. Recommended by the IASP subcommittee on taxonomy. PAIN 1979;6:249–52.
3. Me K. of the practice of regional anaesthesia. Journal of the Royal Society of Medicine. 1990 Nov; 83:709–712.
4. Sabaté S, Anesthesiologist S. Anesthesia for urological surgery in a European region with. Journal of Clinical Anesthesia [Internet] 2009;21(1):30–37. Rodgers A, Walker N, Schug S, Mckee A, Kehlet H, Zundert AV, *et al.*. Reduction in postoperative mortality and, morbidity with epidural and spinal anaesthesia: results from anoverview of randomized trials. BMJ. 2000;321:1–12.
5. Klopfenstein CE, Herrmann FR, Clergue F, Forster A, Michel JP. The Influence of an AgingSurgical Population Anesthesia Workload: A Ten-Year Survey. Anesthesia Analgesia. 1998;86:1165–1170.
6. Cook DJ, Rooke GA. Priorities in Perioperative Geriatrics. Anesthesia and Analgesia 2003 Jun:1823–1836.
7. Covert CR, Fox GS. Anaesthesia for hip surgery in the elderly. Canadian journal of anaesthesia = Journal canadiend'anesthésie 1989 May;36(3 Pt 1):311–319.
8. Chu CC, Shy S, Lin SM, Chu NW, Lee YK, Tsai SK, Lee TV. The effect of intrathecalbupivacaine combined with fentanyl in caesarean section. ActaAnaesthesiol Sin 1995: 33(3):– 54.,
9. Harbhej Singh, Intrathecal fentanyl with small – dose dilute bupivacaine: better anaesthesiawithout prolonging recovery. Anaesthesia and Analgesia 1998: 86 (4) 917 – 18.
10. Techanivate A, Urosopone P, KiatGungunanglia P, Kosawiboonpal R, Intrathecal fentanyl inspinal anaesthesia for appendectomy, J Med Assoc Thai 2004; 87 (5); 525 – 30.
11. Hunt CO, nautly JS, Bader AM, Haush MA, Vartikar JV, Dutta S, Hertwig LM, QstheimerGM, Perioperative analgesia with subarachnoid fentanyl bupivacaine for caesarean delivery.Anesthesiology 1989: 71: 535 – 40.
12. Kuusineni KS, pihlajamaki KK, Pitkanen MT, HelenivsHy, KIrvela OA. The use ofbupivacaine and fentanyl for spinal anaesthesia for urologic surgery. Anaesthesiaand analgesia 2000; 91; 1452 – 56.
13. Hunt CO, Dutta S. Hauch M, Osthmer GW, Hertwig L, Nautkly JS, Perioperative analgesiawith subarachnoid fentanyl – bupivacaine. Anesthesiology 1987: 67; 3A.

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Original Research Article

Efficacy of paracetamol when added as an adjunct to lignocaine in intravenous regional anaesthesia- A prospective randomised double blinded study

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Abstract

Background: Intravenous regional anaesthesia (IVRA) using Lignocaine is a safe, reliable, and cost-effective technique for providing anaesthesia as well as a bloodless field during upper limb surgery. It has been postulated that the site of action in IVRA is probably by blockade of small nerves or possibly nerve endings and not the major nerve trunks. **Aim:** In this study we evaluated the effects of Paracetamol on the onset of sensory and motor block time, sensory and motor recovery time and the requirement of postoperative analgesia. **Methods:** Sixty patients undergoing upper limb extremity surgeries were randomized into two groups .IVRA was achieved by injecting 10 ml of 2% Lignocaine with 30 ml of Paracetamol to a total of 40 ml in Group 1(n=30) and 10 ml of 2% Lignocaine with 30 ml of Normal saline to a total of 40 ml in Group 2(n=30). **Results:** Onset of sensory and motor block was shorter and sensory recovery time was longer in Group 1 ($p < 0.001$). Intraoperative VAS scores were significantly lower in Group 1 ($p< 0.05$). Intraoperative Fentanyl consumption was 8.33 ± 21.82 mcg in Group 1 and 38 ± 37.82 mcg in Group 2, the number of patients requiring Fentanyl was 4 and 17 respectively ($p< 0.001$). The postoperative analgesic requirement was lower in Group 1 with 9 patients and was 25 patients in Group 2 ($p< 0.001$). **Conclusion:** The administration of Paracetamol as an adjunct to Lignocaine in IVRA was found to be efficacious and it provided significant shortening in the onset of sensory block, a decrease in the intraoperative analgesic requirement and an improvement in the postoperative analgesia with a reduced need for analgesics in the postoperative period.

Key Words: Intravenous regional anesthesia, Lignocaine, Paracetamol, Postoperative Pain

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INTRODUCTION

Intra Venous Regional Anaesthesia (IVRA) is a regional anaesthetic technique commonly used in forearm surgeries. It was first introduced by a German surgeon August Gustav Bier in 1908 by injecting Procaine intravenously between twotourniquets[1]. He found that there was a rapid onset of anaesthesia between the tourniquets and a slower onset beyond the distal tourniquet. This technique gained more importance in the late 1960s after its reintroduction by Holmes[2]. There have been many modifications to this technique over time, presently the use of double tourniquet with an injection of drugs distal to the cuffs. Its use has been proved to have an advantage of the faster recovery,

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shorter hospital stays, cost-effectiveness and reduced nursing care requirements making it an ideal choice for Daycare surgeries[3].When IVRA is appropriately performed there is a 96 - 100% success rate[4-6].This technique has the advantage of the rapid return of sensory and motor power at the end of surgical procedure allowing normal functioning of the operated limb and the surgeons are able to assess neurological status after surgery. This rapid recovery facilitates early discharge of patients. IVRA is advantageous being reliable, easy to administer and cost-effective for short operative procedures of the extremities performed on an ambulatory basis3.There are some disadvantages like the delayed onset of action, poor muscle relaxation and rapid onset of pain at the operative site after the tourniquet is deflated[7]. Various additives like Opioids, Muscle relaxants, NSAIDs such as Ketorolac.[8] Tenoxicam[9] and Aspirin[10] have been used to overcome these disadvantages to improve analgesia. There is a risk of local anaesthetic toxicity due to the sudden release of large amounts of local anaesthetic as a result of leakage due to high venous pressure or accidental tourniquet failure past the inflated tourniquet. Due to the above-mentioned effects it is desirable to limit the amount of local anaesthetic to a minimum[11].In previous studies, IVRA with Paracetamol was shown to improve the overall quality of the block, early onset of motor block, reduced tourniquet pain, delayed recovery of motor and sensory block, low intraoperative pain scores and decreased analgesic requirements both during the intraoperative and postoperative period. The ideal IVRA solution should have a rapid onset of action, require less amount of local anaesthetic, reduce tourniquet pain and prolong post-deflation analgesia. This may be achieved by the addition of adjuncts to the localanaesthetic. Paracetamol added as an adjunct to Lignocaine has been shown to provide decreased tourniquet pain, increased anaesthesia quality and decreased postoperative analgesic consumption.

AIM

To determine the efficacy of Paracetamol when added as an adjuvant to Lignocaine for IVRA.

MATERIALS AND METHODS

This randomized double-blinded study was conducted in the Department of Anaesthesiology, PSG Institute of Medical Sciences, Coimbatore in ASA I and II patients posted for upper limb surgeries from August 2013 – March 2015.The study group patients were assigned into two groups as Group 1 and Group2, comprising of 30 patients each. IVRA was performed using 10 ml of 2%Lignocaine with 30 ml of Paracetamol in Group 1 patients and 10 ml of 2%Lignocaine with 30 ml of Normal saline in Group 2.

Inclusion Criteria:

ASA I and II patients, aged between 20-60 yrs, Upper limb extremity surgeries of duration not exceeding 60 minutes. Exclusion Criteria: a history of allergy to local anaesthetics, expected duration of surgery more than 60 minutes, Raynaud's disease, Sickle cell anaemia, Coagulation disorders. Simple randomization was done and assigned into two groups (with 30 patients in each group as Group -1 and Group - 2). According to ASA starvation guidelines patients were kept nil per oral status overnight and pre-medicated with Tablet Ranitidine 150 mg and Tablet Diazepam 5 mg the night prior to surgery and on the morning of surgery. Anaesthesia machine safety checklist including resuscitation equipment, emergency drugs and tourniquet equipment were kept ready before the patient arrived in the operating room (OR). Starvation status and surgical consent confirmed in the preoperative room before the patient was shifted inside the operating room. Preinduction monitors like ECG leads, Noninvasive blood pressure (NIBP)cuff and pulseoximeter were connected and baseline readings noted. All the patients were explained about the Visual Analogue Scale (VAS) prior to the start of the procedure. Vital signs like MAP, HR, and SpO2 were recorded before and after application of a tourniquet and monitored continuously during the procedure at 5, 10, 15, 20, 30,40, 50 minutes interval till the release of a tourniquet. The pain was assessed using a 10cm visual analogue scale. If the patient reported a VAS > 4, rescue analgesic of 1 mcg/kg Fentanyl was intravenously given and the requirement for analgesics was recorded.

RESULTS

In this study, 60 patients were selected after considering the inclusion and exclusion criteria. Consent was obtained from all the 60 patients and was divided into2 groups with 30 patients in each group. Group 1 received 30 ml of Paracetamol and 10 ml of 2% Lignocaine (preservative-free (PF)). Group 2 received 30 ml of Normal Saline and 10 ml of 2% Lignocaine (preservative-free (PF)). In our study, after reviewing the literature we selected the study group in the age between 20 – 60 years. The mean age distribution in our study was 42.87±11.57 in Group 1 and 39.37±13.04 in Group 2. Out of 60 patients in our study, the sex distribution was 18:12 (Male: Female)in Group 1 and 16:14 (Male: Female) in Group 2.In our study, we used a double pneumatic tourniquet placing the first tourniquet on the proximal portion of the extremity to be operated and the second tourniquet distal to the proximal one. Group 1 patients in our study had very negligible intraoperative pain / discomfort during the procedure, but

Group 2 patients reported with pain intraoperatively, presumably tourniquet pain. In our study, the meantime of onset of sensory block was 5.60± 1.58 minutes in Group 1 and 7.13± 1.59 minutes in Group 2 patients. The p-value<0.001 shows there is a statistically highly significant difference between the two groups in terms of the time of onset of sensory block. In our study amongst patients in Group 1, the average onset of motor block time was 8.70± 2.52 minutes and in Group 2 it was 12.27± 2.79 minutes. The p-value<0.001 shows that there is a statistically significant difference among the two groups in terms of the time of onset of motor block. In our study, the sensory recovery time was 7.60± 1.102 minutes in Group 1and 5.60± 1.610 minutes in Group 2. The p-value<0.001 showed that there is a statistically highly significant difference with regard to the onset of the sensory recovery time between the two groups. In our study, the motor recovery time was 8.90± 2.13 minutes in Group 1 and10.37 ± 2.498 minutes in Group 2. The p-value 0.018 showed that there is a statistically significant difference between the two groups in terms of motor recovery time. In our study, 4 patients out of 30 required Fentanyl consumption intraoperatively in Group 1 and 17 patients out of 30 in Group 2 and proved to be statistically significant. In our study, the mean Fentanyl consumed was 8.33±21.82 micrograms in Group 1 and 38±37.820 micrograms in Group 2, p-value<0.001 showed that there is a statistically significant difference between the two groups in terms of Fentanyl consumption as rescue analgesic intraoperatively. The mean VAS pain score intraoperatively was 2.17±1.62 in Group 1 and3.80±2.36 in Group 2. The p-value 0.003 showed that there is a statistically significant difference in the intraoperative VAS score between the two groups. Out of 30 patients in each group in our study, 9 patients required analgesia following tourniquet deflation in Group 1 and 5 patients in Group 2; which was statistically significant between the two groups with p-value< 0.05.Out of 30 patients in each group in our study, surgeon satisfaction grade was excellent in 14 patients in Group 1 and 6 patients in Group 2. The grade was good in16 patients of Group 1 and 24 patients of Group 2. There was a statistically significant difference between the two groups as the p-value is 0.028 (which is < 0.05) with regard to surgeon satisfaction in our study. Out of 60 patients in our study, 7 patients from Group 1 and 2 patients from Group 2 gave excellent grading (Grade 1), 18 patients from Group 1 and 14 patients from Group 2 gave good grading (Grade 2) and 5 patients from Group 1 and 14patients from Group 2 gave moderate grade (Grade 3).There was a statistically significant difference between the two groups as p is0.023 (<0.05) with regard to patient satisfaction in our study.

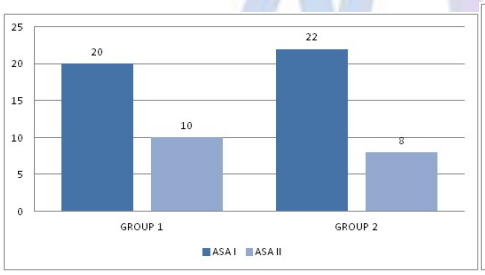


Figure 1

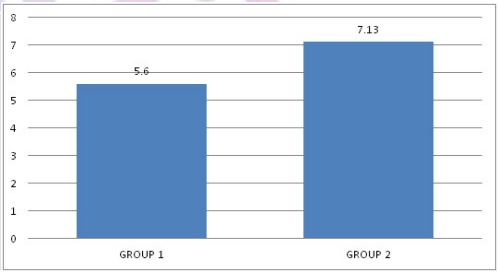


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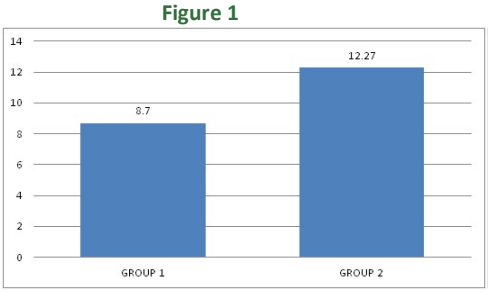


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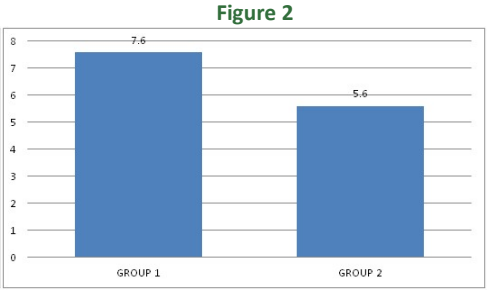


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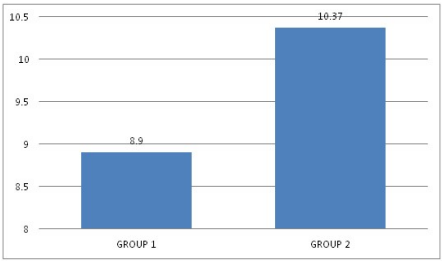


Figure 1

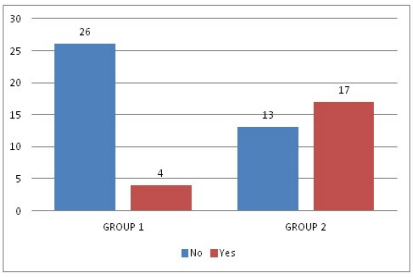


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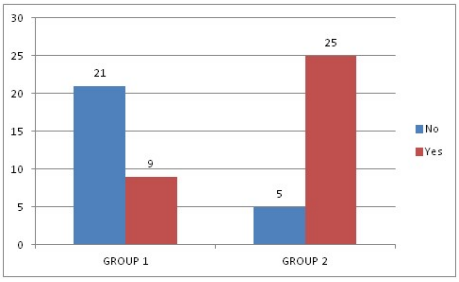


Figure 7

Figure 1: ASA grading distribution **Figure 2:** Onset of the sensory block (mins)
Figure 3: Onset of the Motor block (mins) **Figure 4:**Sensory recovery time
Figure 5: Motor recovery time; **Figure 6:** Fentanyl Consumption; **Figure 7:** Postoperative analgesic requirement

DISCUSSION

In our study, we investigated whether the addition of Paracetamol to IVRA solution decreased tourniquet pain, intraoperative use (Fentanyl) and enhanced the sensory and motor block duration by increasing the quality of IVRA. However, in our study we also demonstrated a decrease in postoperative pain score and analgesic requirement. Elhakim M and Sadek RA[12] carried out IVRA on patients between 25 – 55years. Palecha S *et al*[13] used IVRA on patients above 20 years of age. Sanjay Kherde *et al*[14] used the age group of 15 – 55 years in his study. Tourniquets are used in IVRA to restrict the drugs from entering beyond the cuff into the systemic circulation causing untoward consequences. It is also used to provide analgesia and motor blockade distal to the tourniquet. Chandrashekara PM *et al*[15] used single rubber latex bandage as a tourniquet above the site of surgery. He noted the tourniquet pain and discomfort in surgeries that prolonged for more than 40-50 minutes. Holmes C *et al*[1], Sorbie C and Chancha P[16] and Janardhan *et al*[17] used double tourniquet with the second tourniquet placed on the anaesthetized portion of the extremity distal to the proximal one to prevent tourniquet pain and discomfort. Intravenous regional anaesthesia technique is successful when there are proper tourniquet placement and adequate exsanguination1.Holmes C *et al*[1] and Chandrashekara PM *et al*[15] achieved exsanguination either by simple gravity draining method or by using Esmarch bandage and combined both in some cases. Blond L *et al*[18] showed there was a 69% reduction in blood volume in the limb after exsanguinations using Esmarch bandage. The observation of the present study is in accordance with regards to the meantime of onset of the sensory block by Myoung Jin Ko and Jeong Han Lee *et al*[19]. He reported a similar statistically significant difference in his IVRA study, in patients who received 40 ml of 0.5% Lignocaine in one group and 0.5% Lignocaine diluted with intravenous Acetaminophen 300 mg to a total volume of 40ml. The observation of the present study is in accordance with the observations of Sen H, Kulahci Y *et al*[20] who stated the similar statistically significant difference in theirs tudy population with the meantime motor block onset, mean time of sensory block recovery time, mean time of motor block recovery time. This observation was in accordance with observation obtained in Sen H *et al*[20]where intraoperative VAS scores were seen at 20 and 30 minutes were significantly lower in Group 2 which was 10 ml 3mg/kg Lignocaine with 300mg Paracetamol similar to the present study. Post deflation analgesic requirement in a study done by Sen H *et al*[20] and Myoung Jin Ko *et al*[19] where they showed a statistically significant difference between the two groups with a p value <0.005 using Diclofenac and Tramadol respectively.

CONCLUSION

To conclude from our study, that the administration of Paracetamol as an adjunct to Lignocaine in IVRA, was found to be efficacious and it provided significant shortening in the onset of sensory block, a decrease in the intraoperative analgesic requirement and an improvement in the postoperative analgesia with a reduced needf or analgesics in the postoperative period.

REFERENCES

1. Holmes C. Intravenous regional analgesia: A useful method of producing analgesia of the limb. Lancet 1963; 1:245-247.
2. Bartholomew A, Sloan J P. Prilocaine for Bier's Block: how safe is safe? Archives of Emergency medicine, 1990; 7: 189-195.
3. Chan VW, Peng PW, Kaszas Z, Middleton WJ, Muni R, Anastakis DG, et al A comparative study of general anesthesia, intravenous regional anesthesia, and axillary block for outpatient hand surgery: clinical outcome and cost analysis. AnesthAnalg 2001; 93:1181–4.

4. Brill S, Middleton W, Brill G, Fisher A. Bier's block; 100 years old and still going strong! Acta Anaesthesiol Scand. 2004; 48: 117-22.
5. Brown EM, Mc Griff JT, Malinowski RW. Intravenous regional anaesthesia (Bier's block): review of 20 years experience. Can J Anaesth. 1989; 36: 307-10.
6. Hollingworth A, Wallace WA, Dabir R. Comparison of Bupivacaine and Prilocaine used in Bier's block: a double blind trial . Injury. 1982; 13:331.
7. Perlas A, Peng PW, Plaza MB, Middleton WJ, Chan VW, Sanandaji K. Forearm rescue cuff improves tourniquet tolerance during intravenous regional anesthesia. Reg Anesth Pain Med 2003;28:98–102.
8. Reuben SS, Steinberg RB, Kreitzer JM, Duprat KM. Intravenous regional anesthesia using lidocaine and ketorolac. AnesthAnalg 1995; 81:110–30.
9. Jones NC, Pugh SC. The addition of tenoxicam to prilocaine for intravenous regional anaesthesia. Anaesthesia 1996; 51:446–8.
10. Corpataux JB, Van Gessel EF, Donald FA, Forster A, Gamulin Z. Effect on postoperative analgesia of small-dose lysine acetylsalicylate added to prilocaine during intravenous regional anesthesia. AnesthAnalg 1997; 84:1081–5.
11. Vishala G, Ram Siva Naik DV, Balaji T. Comparative study of analgesic effects in Intravenous regional anaesthesia with Lidocaine vs Lidocaine with Paracetamol IV. Indian journal of applied research 2015; 5:19-30.
12. Elhakim M, Sadek RA. Addition of Atracurium to lidocaine for intravenous regional anesthesia. Acta AnaesthesiolSc and 1994; 38: 542-44.
13. Palecha S, Gupta HK, Thind SS, Sharma S. The use of 0.5% and 0.25% Lignocaine with Pentazocine and Pancuronium for intravenous regional anesthesia - a comparative study. Indian J Anaesth 2001; 45(2): 133-35.
14. Sanjay K, Vaishali S, Akulwar VV, Ghosh AA. A comparison of Buprenorphine and Pethidine with lignocaine for intravenous regional analgesia. Indian J Anaesth 2000; 44: 31-33.
15. Chandrashekara PM, Deshpande SS, Ramchandra V. Modified method of intravenous regional analgesia for surgery on distal part of limbs. Indian J Anaesth 1972; 20: 375.
16. Sorbie C, Chancha P. Regional anaesthesia by the intravenous route. Br Med J 1965; 1: 957-960.
17. Janardhan TN, Venkata Rao M. Intravenous regional analgesia- a clinical study. Indian J Anaesth 1969; 17: 230.
18. Blond L, Madsen JL. Exsanguinations of the upper limb in healthy young volunteers. J Bone Joint Surg 2002; 84B:489-491.
19. Myoung JK, Jeong HL, Soon HC, Chee MS, Young JA, Young KC *et al*. Comparison of the effects of acetaminophen to ketorolac when added to lidocaine for intravenous regional anesthesia. Korean J Anesthesiol 2010; 58(4): 357-361.
20. Sen H, Kulahci Y, Bicerer E, Ozkan S, Dagli G, Turan A. The analgesic Effect of paracetamol when added to lidocaine for intravenous regional anesthesia. AnesthAnalg 2009; 109: 1327–30.

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Original Research Article

Comparison of two doses of intravenous Dexmedetomidine 0.6mcg/kg and 1mcg/kg given prior to induction in attenuation of hemodynamic stress response of laryngoscopy and endotracheal intubation

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Abstract

Laryngoscopy and endotracheal intubation is accompanied by sympathomimetic stimulation resulting in increase in Heart Rate and Arterial blood pressure. These hemodynamic adverse effects are minimized by multiple non pharmacological methods and pharmacological agents including alpha 2 agonists such as Dexmedetomidine. Dexmedetomidine has a shorter duration of action and effectively attenuates the hemodynamic response of laryngoscopy and endotracheal intubation during the peri operative period. The aim of our study is to compare two doses (0.6mcg/kg and 1mcg/kg) of the drug in effective attenuation of the sympathetic stimulation. **M**ethod 80 patients assessed under ASA 1 – 2 between the age group 18-60 years of both genders were included in this study and randomly divided into two groups namely GROUP A – 0.6mcg/kg Dexmedetomidine and GROUP B – 1mcg/kg of the same drug 10mins before induction. Heart rate, Systolic Blood pressure, Diastolic blood pressure, Mean arterial pressure and SpO_2 were recorded at various intervals. Result In both groups, HR, SBP, DBP and MAP reduced significantly. During intubation, 1 and 3 minutes following intubation there was minimal rise in blood pressure in Group A while in Group B the decrease in pressure was marked and continuous. Conclusion Both doses of Dexmedetomidine (0.6mcg/kg and 1mcg/kg) attenuated the stress response of laryngoscopy and intubation effectively. The reduction in heart rate, Systolic blood pressure, Diastolic blood pressure and Mean arterial pressure was more in 1mcg/kg group. Hence Dexmedetomidine at a dose of 0.6mcg/kg itself is adequate to control the stress response of laryngoscopy and intubation.

Key words: Attenuation; Laryngoscopy; Intubation; Dexmedetomidine.

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Introduction

Laryngoscopy and endotracheal intubation is accompanied by intense sympathomimetic stimulation resulting in increase in Heart Rate (HR) and arterial Blood Pressure (BP). This response is very hazardous in patients with reduced myocardial reserve / hypertension / cerebro-vascular insufficiency / raised intracranial or intraocular pressure and may predispose to pulmonary edema / myocardial infarction / cerebro-vascular accident[1,2,3]/ dysrhythmias. To minimize these hemodynamic adverse effects, multiple non pharmacological methods (minimizing mechanical stimuli of laryngoscopy by blind nasal intubation, reducing time of laryngoscopy to < 15 seconds[6]) and various pharmacological agents have been tried which act at different levels of reflex arc[4]. Dexmedetomidine is a lipophilic α methylol derivative which has sedative, anxiolytic and analgesic effects[7]. It is a highly selectiv

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alpha 2 agonist (1620 times highly selective to α_2 receptor) and has a shorter duration of action. Dexmedetomidine administered at a dose of 1 mcg/kg IV over 10 minutes, 15 minutes prior to the procedure, effectively attenuates the hemodynamic response during the peri-operative period[8,9,10,11] but it is associated with side effects like bradycardia and hypotension. The aim of our study was to compare the efficacy of Dexmedetomidine in reducing the hemodynamic stress response in two different doses (0.6 mcg/Kg and 1 mcg/Kg body weight) which was given prior to laryngoscopy and endotracheal intubation.

Materials and Methods

After getting the institutional ethical committee clearance, the study was conducted in PSG institute of medical sciences and research, Coimbatore. Patients aged 18-60years belonging to both genders, assessed under ASA 1 – 2 with Mallampatti class I- II were included in the study. Patients with hypertension, bradycardia, cardiac disease, coronary artery disease, cerebro vascular disease, difficult airway, BMI > 30 Kg/ m^2, on β blocker therapy or known hypersensitivity to the study drug were excluded. 80 patients who fulfilled the inclusion criteria were included in the study and informed consent was obtained. They were premedicated with Tab. Alprazolam 0.5 mg and Tab. Ranitidine 150 mg on the night before surgery. Patients were randomly allocated to one of the following two groups by random table number generated by computer. The procedure was double blinded wherein the consultant anaesthesiologist prepared the infusion of the study drug in 100ml normal saline and handed over to the resident. Hence, the resident conducting the study and the patient both were blinded to the group of drug. The two study groups were labelled as follows -

Group A: 0.6 mcg/kg Dexmedetomidine

Group B: 1 mcg/kg Dexmedetomidine

After preoxygenation, Inj.Glycopyrrolate 0.2 mg i.v, Inj.Fentanyl 2 mcg /kg i.v was given and induced with Inj.Thiopentone sodium till the abolition of the eyelash reflex. After confirming the adequacy of mask ventilation, inj. Succinylcholine 1.5 mg/kg was given and intubated after a minute. Anesthesia was maintained with Oxygen, Nitrous oxide, Isoflurane 1% and inj. Vecuronium bromide. The duration for laryngoscopy and intubation was recorded. Heart rate, systolic BP, diastolic BP, mean arterial BP and SpO_2 were recorded before and after induction, at the time of intubation (0 minute), 1st min, 3rd min, 5th min and 10th min after intubation. Surgical stimulus was avoided for 10 minutes after intubation. Adverse effects were noted and treated accordingly as follows

- Hypotension – systolic blood pressure < 20% from baseline value, Inj Ephedrine 6mg iv bolus was given
- Bradycardia – heart rate < 50 beats per minute, Inj Atropine 0.6mg iv was given.

Statistical analysis was performed using the statistical software (SPSS) 19.0. Data was expressed as percentages and mean values (with standard deviations). Differences between groups were analyzed with the Independent Sample t-test for continuous variables and Pearson's Chi-square test for categorical variables. Paired sample t-test was used to compare the difference between pre and post values. Results were defined as statistically significant when the *P* value was less than 0.05.

Table 1: Age distribution between two groups

Age in years	Group A		Group B	
	No of patients	Percentage	No of patients	Percentage
< 20	1	2.5	2	5.0
20 – 29	17	42.5	10	25.0
30 – 39	12	30.0	16	40.0
40 – 49	7	17.5	9	22.5
> = 50	3	7.5	3	7.5
Total	40	100	40	100
Mean age in years ± SD	32.65 ± 8.891		34.70 ± 10.16	
p value	0.563			

Table 2: Sex distribution between two groups

SEX	Group A		Group B	
	No of patients	Percentage	No of patients	Percentage
Male	7	17.5	12	30.0
Female	33	82.5	28	70.0
Total	40	100	40	100
p value	0.189			

There was no significant statistical difference in the sex and age distribution between the two groups.

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Table 3: Side effects in two groups

Side effects	Group A	Group B
No side effects	36	37
Bradycardia	1	0
Hypotension	3	3
Bradycardia and Hypotension	0	0
p Value	0.602	

There was no significant statistical difference between the two groups with regard to side effects (p value 0.602).

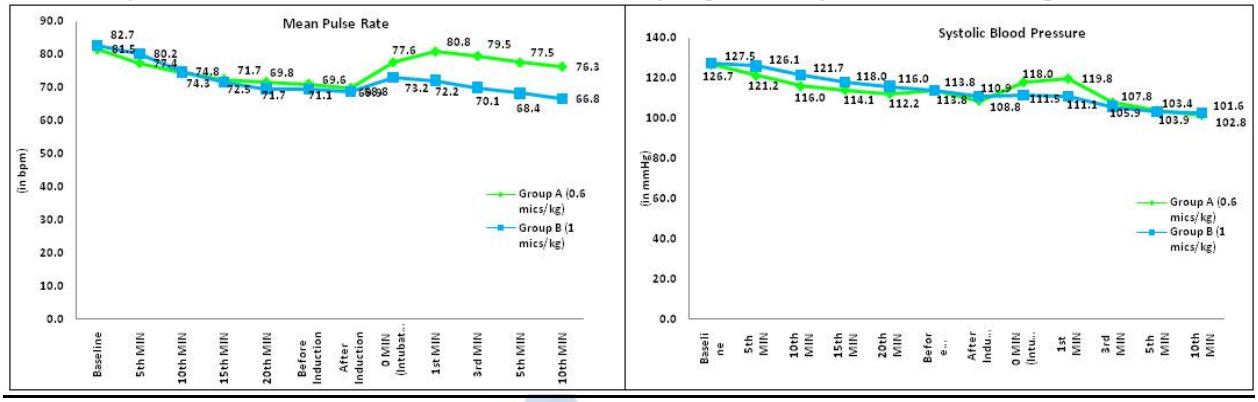


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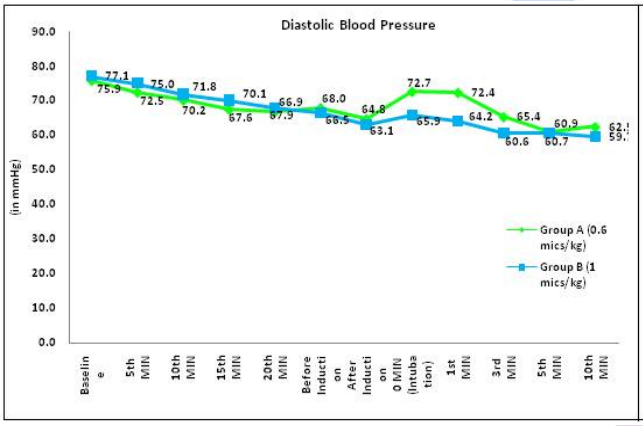


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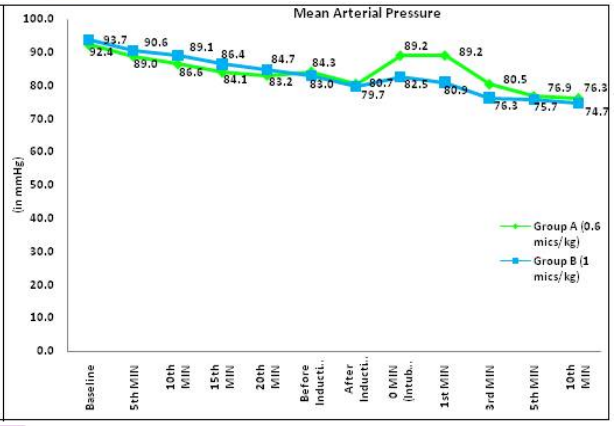


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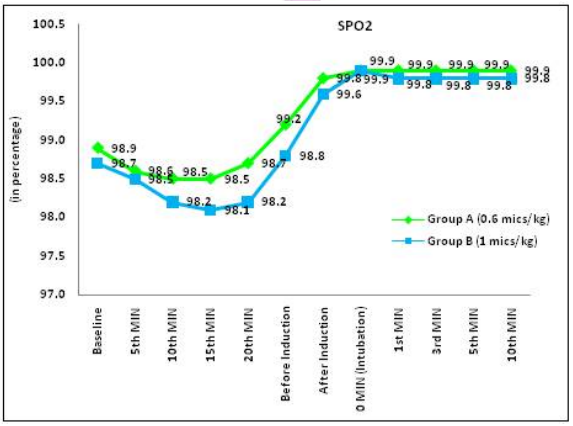


Figure 5

Figure 1: Intergroup comparison for heart rate between Group A and Group B; **Figure 2:** Intergroup comparison for SBP between Group A and Group B; **Figure 3:** Intergroup comparison for DBP between Group A and Group B; **Figure 4:** Intergroup comparison for MAP between Group A and Group B; **Figure 5:** Intergroup comparison for SpO_2 between Group A and Group B

Figure 1, In group A, the basal mean heart rate was 81.58 ± 8.86 bpm. The mean heart rate after administration of the study drug at 5 th, 10 th, 15 th, 20 th min, before induction, after induction, at the time of intubation was 77.40 ± 11.06, 74.30 ± 11.31, 72.58 ± 10.69, 71.72 ± 10.41, 71.10 ± 9.76, 69.90 ± 11.65, 77.63 ± 10.89 respectively and showed a significant reduction from mean heart rate (p value <0.05). No change was noted in the 1st and 3rd minute following intubation. The reduction in heart rate started from the 5th minute following intubation onwards (p value < 0.05). In group B, the basal mean heart rate was 82.70 ± 4.48 bpm. The mean heart rate reduced after administration of the study drug at the 5th, 10th, 15th, 20 th min, before induction and after induction. Further fall in the heart rate was noted during intubation and 1st, 3rd, 5 th, 10 th min following intubation (p value <0.05), The baseline heart rate was comparable in both the groups as p value of 0.476. However 5th, 10th, 15th, 20th min, just before and post induction there was no statistical significance as the p value was > 0.05. There was a significant difference in the mean heart rate of the two groups at the time of intubation, 1st, 3rd, 5th, 10th min after intubation (p value < 0.05). Figure 2, In group A, the basal mean SBP was 126.70 ± 12.07 mmHg. The mean SBP after administration of the study drug at the 5 th, 10 th, 15 th, 20 th min, before induction and after induction were 121.20 ± 10.92, 116.05 ± 12.53, 114.13 ± 11.90, 112.20 ± 11.26, 113.83 ± 14.24 and 108.83 ± 14.75 mmHg respectively. There was a subsequent fall in mean SBP following intubation also. (p <0.05) In group B, the basal mean SBP was 127.58 ± 6.33 mmHg. Significant reduction in the mean SBP was seen constantly thereafter till the measurements were noted (p <0.05). The baseline mean SBP was comparable in both the groups (p value - 0.686). There was a significant reduction in the 5th and 10th min following administration of the study drug and at the 1st min after intubation (p value < 0.05). Figure 3, In group A, the basal mean DBP was 75.97 ± 5.96 mmHg. The mean DBP after the administration of study drug at 5th, 10th, 15th, 20th min, before induction and after induction was 72.58 ± 6.79, 70.20 ± 7.30, 67.68 ± 8.31, 66.95 ± 7.17, 68.00 ± 10.44, 64.80 ± 12.36 mmHg respectively. This showed highly significant reduction of mean DBP from baseline (p value 0.000). But at the time of intubation and 1st min after intubation the mean DBP showed no change. However, Statistical significance was noted during 3rd, 5th and 10th min after intubation with p value < 0.05. In group B, the basal mean DBP was 77.18 ± 5.28 mmHg. The mean DBP after the administration of the study drug was constantly less than the baseline (p value 0.000). There was a significant difference in the mean DBP between the two groups at the time of intubation, 1st and 3rd min after intubation (p value less than 0.05). Figure 4, In group A, the basal mean MAP was 92.48 ± 6.83 mmHg. The mean MAP after the administration of study drug at 5th, 10th, 15th, 20th min, before induction, after induction were 89.00 ± 7.68, 86.68 ± 8.81, 84.18 ± 9.47, 83.23 ± 7.73, 84.35 ± 10.25, 80.70 ± 12.94 mmHg respectively and showed a significant reduction of mean MAP (p value < 0.05). There was no statistical significance at the time of intubation and 1st min after intubation, but the mean MAP at the 3rd, 5th and 10th min after intubation were 80.53 ± 11.65, 76.90 ± 10.34, 76.35 ± 9.28 mmHg respectively, suggestive of a significant reduction from baseline (p value 0.000). In group B, the basal mean MAP was 93.78 ± 4.33 mmHg. There was a significant reduction of the mean MAP from the 10th minute onwards following administration of the drug from the baseline value (p value was 0.000). There was a significant difference in mean MAP at the time of intubation and 1st min after intubation in both the groups (p value of less than 0.05). Figure 5, In group A, the basal mean SpO_2 was 98.93 ± 0.69. The mean SpO_2 after the administration of study drug at 5th, 10th min were 98.63 ± 1.33, 98.50 ± 1.21 respectively and showed a significant reduction from baseline value (p value < 0.05). The mean SpO_2 after induction till 10th min was comparable to baseline value with (p value 0.000). In group B, the basal mean SpO_2 was 98.78 ± 1.00. The mean SpO_2 after the administration of study drug at 5th, 10th, 15th, 20th min from baseline and till 10th min post induction showed significant change from baseline value (p value < 0.05). There was no significant difference in mean SpO_2 between two groups (p value >0.05).

[illegible]

Multiple non pharmacological methods and pharmacological agents have been tried before intubation to attenuate the hemodynamic adverse effects of laryngoscopy and intubation that act at different levels of the reflex arc. The commonly used pharmacological agents are iv Fentanyl, Morphine, Lignocaine, Beta Blockers, Calcium channel blockers, Magnesium Sulphate, Alpha 2 Agonists such as Clonidine and Dexmedetomidine. Dexmeditomidine is a highly selective alpha 2 agonist (1620 times highly selective to α_2 receptor when compared to clonidine). In our study we compared the efficacy of Demeditomidine in attenuating the sympathetic stress response to laryngoscopy and intubation in two different doses (0.6 mcg/Kg and 1 mcg/Kg body weight) when given prior to intubation. After administering the study drug, both groups showed a statistically significant fall in heart rate from the baseline till induction. In Group A, the post intubation heart rate increased but not more than the baseline value. In group B, there was a minimal increase in the heart rate during intubation and one minute post intubation while it

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decreased after that. To conclude the fall in heart rate was comparatively more in Group B than group A[12]. The fall in the systolic blood pressure from the baseline (126.70 ± 12.07 mm Hg) following administration of the drug upto 10 minutes post intubation in Group A was associated with a slight increase during intubation (118.03 ± 16.65 mm Hg)[13].While in group B, a significant decrease in the systolic blood pressure (111.58 ± 15.30 mm Hg) from the baseline (127.58 ± 6.33 mm Hg) was noted after administrating the study drug with no increase during intubation (111.13 ± 10.06 mm Hg)[11] {Menda *et al*}. Changes in Diastolic BP following administration of drug in Group A was similar to Systolic BP [14]. The range of increase was 8 mmHg from induction value but not more than baseline value with a significant difference in the diastolic blood pressure from baseline till 10 minutes post intubation. In group B the fall in Diastolic BP was constant[11], statistically significant and more when compared to Group A. A significant fall in the mean arterial pressure from the baseline till 10minutes post induction was recorded in Group A with a mild increase of 9mmHg above the induction value at the time of intubation and 1 minute post intubation. This finding was similar to the study done by Mowafi *et al*[15] who observed that there was an increase in MAP of 5 mmHg from post induction value but less than basal value. The decrease in MAP was continous in Group B[11]. here was significant fall in SpO_2 in both groups during the infusion of the study drug but post induction the SpO_2 increased significantly probably due to pre oxygenation. In both groups, there was a marked reduction in dose requirement of Thiopentone for induction (Group A- 160.00 ± 15.19, Group B- 166.75 ± 23.73) than the usual dose required to anaesthetise patients[16]. In our study, 3 patients in each group developed hypotension and 1 patient in group A developed bradycardia which required pharmacological management.

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Both doses of Dexmedetomidine (0.6mcg/kg and 1mcg/kg) attenuated the stress response of laryngoscopy and intubation effectively. The reduction in heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure was more in 1mcg/kg group. Hence Dexmedetomidine at a dose of 0.6mcg/kg itself is adequate to control the stress response of laryngoscopy and intubation.□

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1. Prys-Roberts C, Green LT, Meloche R, Foex P. Studies of Anaesthesia in relation to hypertension. Hemodynamic consequenses of intubation. Br J Anaes 1971; 43:531-47.
2. Dalton B, Guiney T. Myocardial ischaemia from tachycardia and hypertension in coronary heart disease – Patients undergoing Anaesthesia. Boston Ann, MTG: American society of Anaesthesiologists; 1972 pp 201-202.
3. Fox EJ, Sklar GS, Hill CH, Villanue var, King BD. Complications related to pressor response to endotracheal intubation. Anaesthesiology 1997; 47:524-25.
4. King BD et al. Reflex circulatory responses to direct laryngoscopy and tracheal intubation performed during general anaesthesia. Anesthesiology, 1951; 12:556-566.
5. Meiklejohn BH, Coley S. Pressor and catecholamine response to nasal intubation of the trachea. BJA 1989; 63(3):283-286.
6. Stoelting RK et al. Circulatory changes during direct laryngoscopy and tracheal intubation: Influence of duration of laryngoscopy with or without prior lidocaine Anaesthesiology, 1977; 47:381–84.
7. Stoelting RK, Hiller SC. Textbook of pharmacology and physiology in anesthetic practice. Philadelphia, Lippincott Williams and Wilkins, 4th edition; 2006:340-45.
8. Bloor BC, Ward DS, Belleville JP, Maze M. Effects of intravenous dexmedetomidine in humans. II Haemodynamic changes. Anaesthesiology 1992; 77:1134-1142.
9. Yildiz M, Tavlon A, Tunsler S, Reisley R, Yosunkaya A, Otulcioglu. Effect of Dexmedetomidine on Hemodynamic response during Laryngoscopy and Intubation: Perioperative hemodynamics and Anaesthetic requirements: Drugs RD, 2006:7(1):43-52.
10. Kunisawa T, Nagata O, Nagashima M. Dexmedetomidine suppresses the Blood pressure during anaesthetic induction and blunts the cardiovascular response to Tracheal intubation. Jornal of clinical Anaesthesia. 2009:21:194-9.
11. Menda F, Koner O, Sayin M, Ture H, Imer P, Aykac B. Dexmedetomidine as an adjunct to anaesthetic induction to attenuate hemodynamic response to endotracheal intubation in patients undergoing fast track CABG. Ann Card Anaesth 2010; 13:16-21.
12. Basar H, Akpinar S, Doganci N, Buyukkocak U, Kaymak C, Sert O, Apan A. The effects of preanesthetic, single dose dexmedetomidine on induction, hemodynamic, and cardiovascular parameters. J Clin Anesth.2008 Sep; 20(6):431-6.
13. Scheinin B, Lindgren L, Randell T, Scheinin H, Scheinin M. Dexmedetomidine attenuates sympathoadrenal response to Tracheal intubation and reduces the need for Thiopentone and perioperative Fentanyl. Br J of Anaes..1992; 68:128-32.Jaakola MF, Salonen M, Lentinen R, Scheinein H. The analgesic action of Dexmedetomidine, a novel alpha 2 agonist in healthy volunteer. Pain 1991:46:281-85.
14. Mowafi HA, Aldossary N, Ismail SA, Alqahtani J. Effect of dexmedetomidine premedication on the intraocular pressure changes after succinylcholine and intubation. Br J of Anaes.2008; 100(4): 485-89.
15. Buhrer M, Mappes A, Lauber R, Stanski DR, Maitre PO. Demedetomidine decreases thiopental dose requirement and alters distribution pharmacokinetics. Anesthesiology. 1994 Jun; 80(6):1216-27.

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Original Research Article

Comparison of perineural and intravenous dexamethasone on duration of analgesia in supraclavicular brachial plexus block

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Abstract Pain in the postoperative period is a major concern for anaesthesiologists, which is usually managed by administration of opioids or analgesics. Single shot nerve blocks with additives such as alpha 2 agonists, opioids and glucocorticoids provide adequate analgesia for a longer duration postoperatively when compared to intravenous opioids or analgesics thereby reducing polypharmacy. The addition of glucocorticoids such as Dexamethasone perinerually increases the duration of analgesia but there are concerns regarding potential neural toxicity. Hence, we compared the analgesic duration, onset of sensory block and motor block in patients receiving Dexamethasone 8 milligram perineurally vs intravenously in supraclavicular brachial plexus block to avoid the potential neural toxicity due to perineural injection of the same. **M** d 60 patients assessed under ASA 1 and 2 belonging to both genders aged between 20- 70years posted for upper limb surgeries under Supraclavicular brachial plexus block were randomly allocated into two groups: Group 1 – patients received 8mg Dexamethasone perinerually, Group 2 – patients received intravenous 8mg dexamethasone The onset of sensory blockade, onset of motor blockade, duration of analgesia, requirement of rescue analgesia postoperatively and severity of pain at the time of administration of analgesia was noted. The onset of sensory blockade, motor blockade, duration of analgesia in both the groups were comparable with no statistical significance. Both IV and perineural Dexamethasone similarly prolonged the duration of analgesia in Supraclavicular Brachial Plexus block. Due to the existence of some controversies over perineural use of Dexamethasone, IV Dexamethasone can be used as a safer alternative to prolong duration of analgesia in patients undergoing upper limb surgeries under Supraclavicular Brachial plexus block.

rd perineural, intravenous, dexamethasone, analgesia, brachial plexus

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Introduction

Pain is the most common complaint of patients in the post-operative period and its management forms an important component of peri-operative care1.Supplementation of excessive opioids and other analgesics such as Non steroidal anti-inflammatory drugs (NSAID'S) for pain management often becomes necessary in most of the cases. Opioids have their own side effects such as nausea, vomiting, respiratory depression and dependence when used in large doses while NSAID's are contraindicated in patients with renal and liver dysfunction2. Brachial plexus nerve blocks with additives for upper limb surgeries provide superior analgesia, avoid side effects of general anaesthesia and also minimise use of analgesics in the post-operative period[3,4]. Single shot Brachial Plexus blocks provide analgesia in the immediate post-operative period whereas continuous peripheral nerve blocks with the use of catheters provide extended analgesia as post-operative pain can exist for several days[1].Catheter migration, leakage of the local anaesthetic, infection[5,6,7,] lack of expertise to perform continuous peripheral nerve blockade and lack of continuous monitoring are some of the major problems with continuous nerve block catheters. Hence, various additives have been studied in an attempt to enhance the

effect of single shot nerve blockade. These additives prolong the duration of blockade either by producing local vasoconstriction or delaying diffusion of local anaesthetic from the site of injection. Commonly used additives include alpha 2 agonists, NSAID's, opioids, and glucocorticoids[8]. Addition of Dexamethasone with local anaesthetics in peripheral nerve blocks to prolong duration of analgesia is being extensively studied. Although many studies have shown that the addition of Dexamethasone perineurally increases the duration of analgesia, there are concerns regarding potential neural toxicity but the evidences remain inconclusive [9,10]. The use of intravenous Dexamethasone as a safe alternative to perineural Dexamethasone in prolonging the analgesia duration is being studied and investigated recently. In our study we have compared the analgesic duration between patients receiving Dexamethasone 8 milligram perineurally and by the intravenous (IV) route in supraclavicular brachial plexus block. We have also compared the difference, if any between the onset of sensory and motor blockade in both the groups.

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□r□□ □r□□□ □□ □□The aim of the study is to compare the effects of perineural and IV Dexamethasone in the duration of analgesia in adult patients undergoing elective upper limb surgeries under Supraclavicular Brachial Plexus block.

□□□□d□r□□□ □□ □□To find out the difference if any, between the onset of sensory and motor blockade in patients receiving perineural and IV Dexamethasone.

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The study was done in PSG Institute of Medical sciences and Research, Coimbatore after obtaining clearance from the Institutional Human Ethics Committee and informed written consent from the patients.

Sample size of 30 patients per group was calculated using duration of analgesia as the primary outcome based on previous studies.

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- Patient acceptance
- ASA 1 and 2 patients
- Patients aged between 20-70 years
- All patients posted for elective upper limb surgeries under Supraclavicular Brachial Plexus block

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- Patients with history of allergy to local anaesthetics
- Patients on steroid therapy
- Patient with Diabetes and Coagulopathies
- Infection at the site of proposed puncture for block
- Neuropathies

Routine pre-operative assessment of the patients was done prior to the surgery and tablet Ranitidine 150 mg was prescribed the night before surgery and on the day of surgery. Starvation guidelines were followed. The patients were randomly allocated into two groups (group 1 and group 2) using the random table method. After shifting the patient to the operating room, monitors were connected and baseline parameters recorded. The consultant in the operating room who was not involved in the study prepared the study drug according to the group in which the patient belonged to. All vital parameters were recorded every 5 minutes. Under aseptic precautions Supraclavicular Brachial plexus block was performed using a nerve stimulator.

□r□□□1 □Patients received 3mg/kg of Bupivacaine and 8 mg (2ml) of Dexamethasone perineurally and 2ml normal saline intravenously.

□r□□□2 □Patients received 3mg/kg of Bupivacaine and 2 ml of normal saline
perineurally and 8mg (2ml) of Dexamethasone intravenously.

The time of injection of the local anaesthetic, onset of complete sensory and motor blockade was noted and recorded. Patients were followed up post-operatively and the time of administration of first analgesic was recorded. The severity of pain at the time of administration of analgesic was graded with the verbal analogue scale.

□ □□□□□□□□□□□Onset of sensory blockade was defined as the time interval between the end of local anaesthetic administration to loss of sensation to needle prick. In our study this was tested using needle prick method. Onset of motor blockade was defined as the time interval between the end of administration of the local anaesthetic to inability to move the fingers. In our study it was tested using the modified Bromage score. Duration of sensory blockade was defined as the time interval between the completion of injection of local anaesthetic to the administration of first analgesic to the patient.

Modified Bromage Scale:

GRADE 0	Normal motor function with flexion/ extension of elbow wrist and fingers
GRADE 1	Decreased motor function with ability to move fingers or wrist only
GRADE 2	Complete motor blockade with inability to move fingers

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Type Of Pain	Score
No pain	0
Mild pain	1
Moderate pain	2
Severe pain	3

The data was entered in Microsoft excel 2010 and statistical analysis was performed using the software SPSS version 23.0. Data was expressed as percentages and mean values with standard deviations. Differences between both the groups were analysed using the independent sample 't' test and the Pearson's chi square test. Results were defined as statistically significant if the 'p' value was less than 0.05.

Demographic Distribution Of The Two Groups

AGE IN YEARS	GROUP 1		GROUP 2	
	No. of Patients	Percentage	No. of patients	Percentage
30 and below	5	16.6	9	30
31-40	5	16.6	3	10
41-50	9	30	8	26.6
51-60	7	23.3	7	23.3
61-70	3	10	1	3.3
>70	1	3.3	2	6.6
TOTAL	30	100	30	100
Mean ± SD	45.7± 7.87		43 ± 9.07	
'p' VALUE	0.685			

Table 2:

GENDER	GROUP 1		GROUP 2	
	No. of patients	Percentage	No. of patients	Percentage
MALE	19	63.33	20	67.3
FEMALE	11	36.66	10	33.3
TOTAL	30	100	30	100
MALE	0.861			

For statistical purpose, the onset of sensory blockade was segmented into two categories according to the mean and median values. According to the statistical analysis mean sensory onset in both groups was 14.47 with a median value of 15. Hence the grouping was done as sensory onset less than 14 minutes and more than 15 minutes. In group 1, 33% of patients had a sensory onset below 14 minutes and 66% of patients had sensory onset more than 15 minutes. The mean sensory onset in group 1 was found to be 15.23 In group 2, 36.6 % of patients had sensory onset less than 14 minutes and 63.3%of patients had a sensory onset of more than 15 minutes. The mean sensory onset in group 2 was found to be 14.45. When the sensory onset in both the groups were compared, there was no significant statistical difference according to the 'p' value of 0.861.

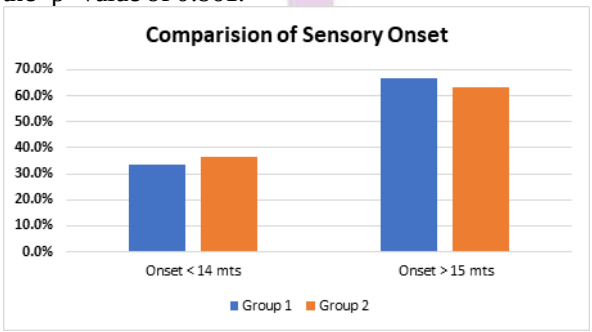


Figure 1: Comparision Of Sensory Onset In Both Groups

For statistical purpose, the onset of motor blockade was segmented into two categories according to the mean and median values. According to the statistical analysis mean sensory onset in both groups was 17.14 with a median value of 17. Hence the grouping was done as motor onset less than 17 minutes and more than 18 minutes. In group 1, 58.62% of patients had a motor onset of less than 17 minutes and 43.33% had a motor onset of more than 18 minutes. In group 2, 48.3% of patients had a motor onset of less than 17 minutes and 56.6% of patients had a motor onset of more than 18 minutes. The mean motor onset was 17.21 in group 1 and 17.34 in group 2. According to the 'p' value of 0.240 there was no significant statistical difference in onset of motor blockade between both the groups.

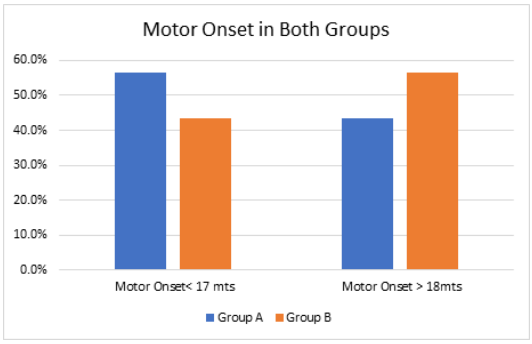


Figure2 : Comparision Of Motor Onset In Both Groups

□□ □□r□□□□□□ □□ □r □□□□□ □□ □□□□□□□□Duration of analgesia was segmented into two, based on the mean and median values. According to statistical analysis the mean duration of analgesia was 35 hrs and the median was 36 hrs. In group 1, 36.6 % of patients had analgesia for less than 36hours and 63.3 % of patients had analgesia for more than 37 hours. The mean analgesic duration in group 1 being 38.89 hours. In group 2, 60% of patients had analgesia duration of less than 36 hours and 40% of patients had analgesia more than 37 hours. The mean analgesia duration in group 2 was 34.39 hours. When duration of analgesia was compared between the two groups, there was no significant statistical difference according to the 'p' value of 0.240.

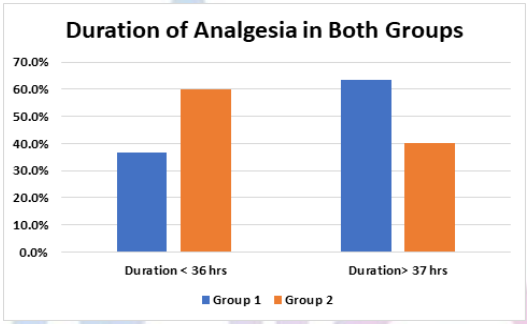


Figure 3: Comparision Of Duration Of Analgesia

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Post- operative pain is the commonest complaint in patients undergoing upper limb surgeries. Administration of multiple analgesics in the post-operative period often may result in a number of adverse effects. Single shot brachial plexus nerve blocks often fail to provide extended analgesia in the post-operative period, hence the use of additives with local anaesthetics to prolong the duration of sensory blockade is practiced[11]. Many additives have been used with local anaesthetics to prolong analgesic duration and improve the quality of the regional block[5,10]. The addition of glucocorticoids with local anaesthetics in brachial plexus supraclavicular blocks significantly prolongs analgesia without much side effects[12,13]. The analgesic effect of Dexamethasone added to local anaesthetics in the perineural route has been studied. The mechanism of action of locally administered Dexamethasone is probably by increasing the activity of inhibitory potassium channels on nociceptive 'C' fibres. It also causes some amount of vasoconstrictor effect[14,15]. The route of administration of Dexamethasone for prolonged analgesia in Brachial plexus Supraclavicular block remains a controversy. It is shown that perineural Dexamethasone may cause potential neurotoxicity but is not proved and evidences are also inconclusive [10]. The use of IV Dexamethasone for prolonged post-operative analgesia is being studied recently. Analgesic effect of systemically administered Dexamethasone could be due to decreased production of inflammatory mediators and increased production of anti-inflammatory mediators as a result of binding of cytoplasmic glucocorticoid receptors to glucocorticoid response elements in the DNA[10,17]. Very few studies have been done comparing the analgesic effect of Dexamethasone administered IV vs perineurally in Brachial Plexus block. In our study we have compared the duration of analgesia with 8 milligram of Dexamethasone administered IV and perineurally in 60 patients undergoing elective upper limb surgeries under supraclavicular Brachial plexus block. In our study the demographic variables like, age, gender and weight were comparable between both the groups and no significant difference occurred between them in both the groups.

The onset of sensory blockade was comparable between both the groups. The mean sensory onset in group 1 was found to be 15.23 minutes and that in group 2 was 14.45

minutes. Our results matched with the results of the study done by Parveen *et al*[2] in 2015 who also inferred that no significant statistical difference occurred between the onset of sensory blockade in the IV and the perineural group. The studies conducted by Desmet *et al*[1] and Rosenfeld *et al*[10] also did not show any significant difference in the onset of sensory blockade between the two groups. The onset of motor block was compared in both the groups. The mean motor onset in group 1 was found to be 17.21 minutes and the mean motor onset in group 2 was 17.34 minutes with no statistical significance similar to the findings of Abdallah *et al*[16]. Alarasan *et al*[17] compared the effects of perineural Dexamethasone in supraclavicular block and showed that the Dexamethasone group had a shorter onset of motor blockade than the control group. The mean duration of analgesia in group 1 was found to be 38.89 hours and that in group 2 was found to be 34.39 hours. Though the mean analgesia duration in group 2 was lower than the mean analgesia duration in group 1 there was no significant statistical difference in the analgesia duration between the two groups. Our study results matched with the results of the study conducted by Abdallah *et al*[16] who also inferred that both IV and perineural Dexamethasone had the same duration of analgesia when added in Supraclavicular brachial plexus block. Kawanishi *et al*[18] compared the effects of Dexamethasone 4mg with interscalene brachial plexus block using Ropivacaine and concluded that only perineural Dexamethasone prolonged analgesia duration when compared to the control group.

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A study titled "Comparison of IV and perineural Dexamethasone in prolonging duration of analgesia in Supraclavicular Brachial plexus block" was carried out in PSG Institute of Medical Sciences and Research. The study included 60 patients randomly divided into two groups of 30 patients in each group. One group received Dexamethasone 8 mg perineurally with supraclavicular block and the other group received Dexamethasone 8mg IV along with supraclavicular block. Parameters such as onset of sensory and motor blockade and duration of analgesia was noted. All data were entered in Microsoft excel 2010 and were analysed in SPSS software version 23.0. Demographic variables such as age, gender and weight were comparable between both the groups and no significant difference existed between both the groups. The onset of sensory and motor blockade was similar in both the groups and showed no significant statistical difference. Theprimary outcome of the study was duration of analgesia. The mean duration of analgesia was less in the IV group as compared with the perineural group but the results were not statistically significant.

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Both IV and perineural Dexamethasone similarly prolonged the duration of analgesia in Supraclavicular Brachial Plexus block. The onset of sensory and motor blockade showed no difference between the two groups. The mean duration of analgesia in the perineural group was slightly higher as compared to the IV group but the results were not statistically significant. Due to the existence of some controversies over perineural use of Dexamethasone, IV Dexamethasone can be used as a safer alternative to prolong duration of analgesia in patients undergoing upper limb surgeries under Supraclavicular Brachial plexus block.

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1□ Desmet M, Braems H, Reynvoet M, Plasschaert S, Van Cauwelaert J, Pottel H, *et al.* I.V. and perineural dexamethasone are equivalent in increasing the analgesic duration of a single-shot interscalene block with ropivacaine for shoulder surgery: A prospective, randomized, placebo-controlled study. Br J Anaesth2013;111: 445-52.

2□ Parveen S, Athaluri VV, Lakshmi BS. Effect of Intravenous Dexamethasone in Prolonging the Duration of Supraclavicular Brachial Plexus Block with 0.5% Ropivacaine: A Prospective, Randomized, Placebo Controlled Study. Int J Sci Stud 2015;2(10):56-60.

□□ Biradar PA, Kaimar P, Gopalakrishna K. Effect of dexamethasone added to lidocaine in supraclavicular brachial plexus block: A prospective, randomised, double-blind study. Indian J Anaesth2013; 57: 180-4

□□ Movafegh, A., Mehran, R., Hajimaohamadi, F., andMeysamie, A. (2006). Dexamethasone added to lidocaine prolongs axillary brachial plexus blockade. *Anesthesia and Analgesia*, doi: 10.1213/01.ane.

□□ Albrecht E, Kern C, Kirkham KR. A systematic review and meta-analysis of perineural dexamethasone for peripheral nerve blocks. Anaesthesia 2015; 70: 71-83

6□ Cummings KC III, Napierkowski DE, Parra-Sanchez I, *et al.* Effect of dexamethasone on the duration of interscalene nerve blocks with ropivacaine or bupivacaine. Br J Anaesth 2011; 107: 446–53

□□ Karakaya D. Addition of fentanyl to bupivacaine prolongs anesthesia and analgesia in axillary brachial plexus block. Regional Anesthesia and Pain Medicine. 2001;26(5):434-438.

□□ Soaida S, Abdel Naim H, Elshafaie K, Abdel-Haq M, Nawar K. Systemic versus perineural dexamethasone as an adjuvant to bupivacaine in combined femoral and sciatic nerve blocks in lower-limb vascular surgeries: a prospective randomized study. Ain-Shams Journal of Anaesthesiology. 2016;9(4):569.

□□ Golwala MP, Swadia VN, Dhimar AA, Sridhar NV. Pain relief by dexamethasone as an adjunct to local anaesthetics in supraclavicular brachial plexus block. J AnaesthesiolClinPharmacol2009; 25: 285-8.

10□ Rosenfeld D, Ivancic M, Hattrup S, Renfree K, Watkins A, Hentz J *et al.* Perineural versus intravenous dexamethasone as adjuncts to local anaesthetic brachial

plexus block for shoulder surgery. Anaesthesia. 2016;71(4):380-388

11. McCartney CJ, Brull R, Chan VW, Katz J, Abbas S, Graham B, *et al.* Early but no long-term benefit of regional compared with general anesthesia for ambulatory hand surgery. Anesthesiology2004 ;101: 461-7.
12. Marks R, Barlow JW, Funder JW. Steroid-induced vasoconstriction: Glucocorticoid antagonist studies. J Clin Endocrinol Metab1982; 54:1075-7.
13. rickson M.D., M., Danesh-Clough M.D., T., and White, R. (2013). Adjuvant dexamethasone for bupivacaine sciatic and ankle blocks: results from two randomized placebo-controlled trials. *Regional Anesthesia and Pain Medicine*, doi:10.1097/AAP.0b)13e318292c121.
14. Movafegh A, Razazian M, Hajimaohamadi F, Meysamie A. Dexamethasone added to lidocaine prolongs axillary brachial plexus blockade. AnesthAnalg 2006; 102: 263–7
15. Tandoc MN, FanL, KolesnikovS, KruglovA, Nader ND. Adjuvant dexamethasone with bupivacaine prolongsthe duration of interscalene block: a prospective randomizedtrial. J Anesth2011; 25: 704–9
16. Abdallah FW, Johnson J, Chan V, Murgatroyd H, Ghafari M, Ami N, JiN R *et al.* Intravenous dexamethasone and perineural dexamethasone similarly prolong the duration of analgesia after supraclavicular brachial plexus block. Reg Anesth Pain Med 2015; 40:125–132.
17. Alarasan AK, Agrawal J, Choudhary B, Melhotra A, Uike S, Mukherji A. Effect of dexamethasone in low volume supraclavicular brachial plexus block: A double-blinded randomized clinical study. J AnaesthesiolClinPharmacol2016; 32: 234-9.
18. Kawanishi, R., Yamamoto, K., Tobetto, Y., Nomura, K., Kato, M., Go, R., Tsutsumi, Y., Tanaka, K., Takeda, Y. (2014). Perineural but not low-dose dexamethasone prolongs the duration of interscalene block with ropivacaine: a prospective randomized trial. *Local and Regional Anesthesia*, 7: 5-9

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Research Article

INCIDENCE OF POST DURAL PUNCTURE HEADACHE WITH DIFFERENT SIZE QUINCKE SPINAL NEEDLES IN PARTURIENTS UNDERGOING CAESEREAN SECTION

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A R T I C L E I N F O

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Key words:

Post dural puncture headache, Quincke spinal needles, Lower Segment Caeserean Section, Spinal anesthesia

A B S T R A C T

Background: The most common, nagging and inconveniencing complication of spinal anesthesia is Post Dural Puncture Headache, which is very distressing and debilitating for the patient. Onset is usually within three days and worsens when the patient assumes erect posture. Pain is usually occipital or frontal, occasionally nuchal, radiating to neck and shoulders and dragging in nature. Headache is usually self limiting and disappears within a week. The aim of this study was to compare the incidence, intensity and severity of PDPH with different size Quincke spinal needles (25 G, 26 G and 27 G), in parturients undergoing elective Lower Segment Caesarean Section under Spinal anesthesia. **Methodology:** 150 patients belonging to ASA I and II in the age group 20 to 35 years, undergoing elective LSCS were included in the study. They were randomly allocated into 3 groups, depending upon the size of the spinal needle used - Group I-25G, Group II-26G, Group III-27G. The number of attempts made to locate the subarachnoid space was noted. The patients were followed up in the ward till discharge and the incidence, intensity and severity of PDPH was assessed. **Results:** The intensity of pain was assessed by Visual Analogue Scale in patients with symptoms suggestive of PDPH. The incidence, severity and intensity of PDPH were recorded and statistically analysed using 't' test and Chi square test. From our study, we find that in young obstetric patients, the incidence of PDPH is more with large bore spinal needles and multiple dural punctures. **Conclusion:** We conclude from our study the incidence, intensity and severity of Post dural Puncture Headache are more with large bore Quincke spinal needles. Hence we recommend the use of fine bore needles of 27 G or less for caesarean sections to minimize the incidence of PDPH.

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INTRODUCTION

Spinal anesthesia was developed in the late 1800's with the work of Wynter, Quincke and Corning. In 1898, it was the German Surgeon Karl August Bier who introduced spinal anesthesia into clinical practice. Bier also gained first hand experience of the disabling headache related to dural puncture by trying spinal on himself.

The most common, nagging and inconveniencing complication of spinal anesthesia is Post Dural Puncture Headache (PDPH), which is very distressing and debilitating for the patient. This untoward complication is a challenging and frustrating task for the anaesthesiologist.

In 1902 it was Sicard, who suggested the leakage of Cerebrospinal fluid (CSF) into the extradural space might be the cause of Post Dural Puncture Headache. PDPH is used conventionally to denote "after" puncture[1], but the term "Post dural" mimics the word "peridural" and should mean "behind the dura[10]. The synonyms for PDPH are

1. Post spinal Headache
2. Post spinal Cephalagia

PDPH may occur after deliberate or accidental dural puncture with epidural needle. Onset is usually within three days and worsens when the patient assumes erect posture i.e. sits or stands. Pain is usually occipital or frontal, occasionally nuchal, radiating to neck and shoulders - dragging in nature and associated with stiffness of the neck[8]. The temporal and vertex areas are reported less commonly as a site of discomfort.

The headache has been described in various forms such as a feeling of heaviness, constricting band, vacuum like and lead weight, searing and spreading like hot metal. Cranial nerve palsies are not uncommon. The causative leakage of CSF into epidural space is 10 ml/hr and healing may take place up to 3 to 4 weeks. 90 % of headaches will occur within three days of the procedure and 66 % start within the first 48 hours. Rarely the headache develops between 5 and 14 days after the procedure. Headache is usually self limiting and disappears within a week.

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Aim

The aim of this study was to compare the incidence, intensity and severity of PDPH with different size Quincke spinal needles (25 G, 26 G and 27 G), in parturients undergoing elective Lower Segment Caesarean Section (LSCS) under Spinal anesthesia.

MATERIALS AND METHODS

After obtaining ethical committee clearance from the institution and informed consent from the patient the study was done. 150 patients belonging to ASA I and II in the age group 20 to 35 years, undergoing elective LSCS were included in the study. They were randomly allocated into 3 groups, depending upon the size of the spinal needle used - Group I -25 G, Group II - 26G, Group - III - 27G.

Inclusion criteria

1. All parturients belonging to ASA I and ASA II
2. Parturients with no systemic diseases like hypertension, diabetes, thyroid

Exclusion criteria

1. All patients who had general contraindications for spinal anesthesia
2. Patients with previous history of headache of any etiology were excluded

All parturients undergoing elective LSCS were premedicated with Tablet Ranitidine 150 mg and Tablet Metoclopromide 10 mg orally the previous night and 2 hours before surgery. An intravenous access was established with 18 G cannula in the preoperative room and the patients were preloaded with 10 ml/kg body weight Ringer's lactate solution over 20 to 30 minutes prior to spinal blockade. The patients were monitored with baseline readings of ECG, non invasive blood pressure, pule rate, oxygen saturation and urine output when shifted inside the operating room and continuous monitoring was done.

The parturient was positioned in right lateral position; sterile draping was done after cleaning back with 10% povidone iodine and 70% alcohol and Subarachnoid blockade was attempted. The bevel of the Quincke spinal needle was kept facing the ceiling. After establishing the free flow of CSF, 0.5 % Bupivacaine Heavy 2 ml was injected intrathecally. Patients were turned supine and oxygen supplementation given with Hudson's mask till the delivery of the baby. Systemic hypotension was treated with intravenous fluids and vasopressors when indicated.

The number of attempts made to locate the subarachnoid space was noted. Any attempt to redirect or reposition the needle after one or more attempts to locate the subarachnoid space with the spinal needle was considered as multiple attempts. Intra operative vital signs were recorded and the patients were shifted to the recovery room after the completion of the procedure. The hydration status was maintained in all the patients and they received Inj. Ketorolac sodium eighth hourly and Inj.Pethidine hydrochloride 10 mg when needed for post operative pain relief.

The patients were followed up in the ward till discharge. At each visit the following questions were asked:

1. Do you have headache, if yes where is it located - occipital or frontal?
2. What is the nature of the headache /
3. Is it relieved on lying down?
4. Any posture like sitting, standing or erect posture aggravating the headache?
5. Does it worsen on coughing, sneezing or straining?
6. Is it accompanied by any other symptoms?
7. When did you first sit up after surgery and when was the ambulation started?

Patients were diagnosed to have PDPH when the following criteria are present,

1. Onset within 3 days
2. Site-Occipital or Frontal
3. Dragging type pain
4. Aggravating factor-sitting, standing, erect posture, coughing, straining or sneezing
5. Relieving factor-lying down or supine posture
6. Associated symptoms-nausea, dizziness, neck stiffness, tinnitus, auditory and visual hallucinations

For the patients who complained of symptoms suggestive of PDPH, the intensity of pain was assessed by Visual Analogue Scale (VAS) as mild, moderate and severe. The scale was graded from 0 to 10, which measures the subjective intensity of pain the patient experiences. The incidence, severity and intensity of PDPH were recorded and statistically analysed using 't' test and Chi square test.

Score	Intensity of pain	Features of PDPH
0 -3	Mild	• Slight restriction of physical activity • Patient not confined to bed • Occipital or frontal headache with postural variation • No associated symptoms*
4 - 6	Moderate	• Restricted physical activities which forces the patient to be confined to the bed for part of the day • Associated symptoms not usually present*
7 - 10	Severe	• Patient bedridden for the whole day and makes no attempt to raise her head or is unable to stand • Associated symptoms present*

*Associated symptoms include:

- Nausea, vomiting, nuchal pain, neck stiffness and paraesthesia
- Ocular disturbances - double vision, blurring of vision, difficulty in focusing and photophobia
- Auditory disturbances - dizziness, tinnitus and reduced hearing

OBSERVATIONS AND RESULTS

The study was undertaken on 150 ASA Grade I and II parturients undergoing elective LSCS with different size Quincke spinal needles and the incidence, intensity and severity of PDPH were noted.

Table 1 Age Distribution

Age	Group -I		Group -II		Group - III	
	N	%	N	%	n	%
21 - 25	16	32	20	40	13	26
26 - 30	21	42	23	46	25	50
31 - 35	13	26	7	14	12	24

n-Number of patients

Table 2 Number of punctures attempted during spinal blockade

Number of Punctures	Group - I (25G)	Group - II (26G)	Group - III (27G)	X^2	Level of Significance
1	30	28	41		
2	14	18	9		
3	5	4	0	12.49	0.05 (Sig.)*
4	1	0	0		

*Statistically significant

Table 3 Incidence of PDPH

Gauge	Incidence	X^2	Level of significance
Group - I	8		
Group - II	6	9.49	**0.01 (Sig.)**
Group - III	3		

Table 4 Intensity of pain using 't' test

Needle	N	Mean	S.D	T	Level of significance
Group - I	50	0.6000	1.442	0.274	0.78
Group - II	50	0.5200	1.474		(N.S)
Group - I	50	0.6000	1.442	1.999	**0.05**
Group - III	50	0.1600	0.584		**(Sig.)**
Group - II	50	0.5200	1.474	1.605	0.11
Group - III	50	0.6000	0.584		(N.S)

Table 5 Onset of PDPH

Onset	Spinal needle	N	X^2	Level of significance
<24 hrs	Group - I	1	4.01	0.40 (N.S)
	Group - II	2		
	Group - III	1		
24-48 hrs	Group - I	6	8.85	0.35 (N.S)
	Group - II	4		
	Group - III	3		
48-72 hrs	Group - I	8	13.46	0.09 (N.S)
	Group - II	6		
	Group - III	3		

n-Number of patients X^2-Chi square N.S-Not significant

Table 6 Duration of PDPH

Duration (Days)	Gauges			X^2	Level of significance
	Group -I	Group - II	Group - III		
1	1	0	0		
2	4	0	1		
3	0	2	2		
4	2	1	0		0.14
5	1	1	0	14.600	(N.S)
6	0	1	0		

Table 7 Severity of PDPH by VAS

Severity	Group - I	Group - II	Group - III	X^2	Level of significance
Mild	5	0	3		
Moderate	2	5	0		
Severe	1	1	0	13.28	**0.01 (Sig)**
Total	8	6	3		

Table 8 Severity of pain duration

Needle Gauge	< 24 hrs				X^2	Level of significance
	No pain	Mild	Moderate	Severe		
Group - I	49	0	0	0	5.014	0.54 (N.S)
Group - II	48	1	1	1		
Group - III	49	1	0	0		
	24 - 48 hrs				X^2	Level of significance
Group - I	44	3	2	1	6.902	0.33 (N.S)
Group - II	46	0	3	1		
Group - III	47	3	0	0		
	48 - 72 hrs				X^2	Level of significance
Group - I	42	6	2	0	9.065	0.05 (Sig.)
Group - II	44	1	5	0		
Group - III	46	4	0	0		

DISCUSSION

The etiology of PDPH is still unclear, but is attributable to the loss of CSF through the hole in the duramater made by the spinal needle leading to lowering of CSF pressure and CSF hypotension[6]. PDPH could be due to the downward traction of the structures of CNS and blood vessels that are attached to the pain sensitive structures in the duramater and cranium or Loss of cushioning effect of the brain[6]. Recent evidences indicates that arachnoiditis or a local inflammatory process might be the etiology for these symptoms and may be associated with urinary and faecal incontinence.

Occurrence of PDPH seems to be related to several factors such as younger patients, females, large gauge spinal needle, design of needle tip, multiple punctures made through the duramater, pregnancy and early ambulation[17]. Atypical presentations of PDPH have been reported. Spielman (1982) mentioned, the stress of labour, changing hormonal level and dehydration to be the factors responsible for an increased incidence of PDPH in obstetric patients.

Ocular symptoms include double vision, blurring of vision, photophobia and difficulty in focusing. The reason being the sixth cranial nerve abducens has a long intracranial course and is more susceptible to low pressure paralysis. The auditory symptoms include decreased hearing and tinnitus, due to endolymphatic expansion secondary to perilymphatic hypotension. The CSF closely resembles perilymph and the pressure changes in the perilymph.

From our study, we find that in young obstetric patients, the incidence of PDPH is more with large bore spinal needles and multiple dural punctures. Choi *et al*[3] reported PDPH 1 in 67 parturients. Statistically there was no significance in the age groups of the parturients as similar with Rasmussen *et al*[14] studies.

From table 2, we infer that successful subarachnoid puncture made was 66% in first attempt (99 out of 150), 27.3 % in second (41 out of 150), 6 % in third (9 out of 150) and 0.66 % in fourth attempt (1 in 150). In all the three groups, no one developed PDPH with single puncture, but the incidence was 14.28 %, 16.6 % and 33.3 % in Group I, II and III respectively with second attempt and successive attempts and was statistically significant at p 0.05 level with close association between multiple punctures and the incidence of PDPH. Seeberger *et al*[19] also found that repeated puncture significantly increases the incidence of PDPH. Costigan *et al*[4] reported similar results in his study.

The overall incidence of PDPH was 11.32 % which was similar to studies from choi *et al*[3] with incidence ranged from 1.5 % to 11.2 %. In our study, 16 % (8 out of 50) in Group I, 12 % (6 out of 50) in Group II and 6 % (3 out of 50) in Group III developed PDPH. The probable reason could be majority of the case was done during summer season and the other one could be the young parturient. Statistical analysis showed a close association between the gauge of the needle and the PDPH incidence by Chi square test significant at p value 0.01. Choi *et al*[3] found that once dural puncture headache occurred, the risk of PDPH was 52 %; but the size of the needle was not mentioned. Flatten *et al*[5] stated 6.7 % developed PDPH with usage of 26 G fine needle, but no PDPH with 29 G. Schultz *et al*[18] concluded that the incidence was less with fine bore

needles and it was not affected by the type of the needle chosen.

The intensity of headache experienced by the patients on different days was measured by VAS and was found to be not statistically significant. The duration of headache was for a maximum period of 6 days in our study. None of our patients had the headache beyond the first week. The largest follow up of PDPH is still that of Vandam and Dripps[20] who reported that 72 % headaches resolved within 7 days and 87 % had resolved in 6 months.

In our study, majority of patients had the onset of PDPH within 24 - 48 hours which was similar to Reynolds *et al*[16] with 90 % onset of headache within three days of the procedure. Leibold[9] *et al* in their study showed 66 % headache started within first 48 hours.

The severity of PDPH was graded as mild, moderate and severe according to our study and the reason for increased severity in Group II (26 G) as compared to Group I (25 G) may be due to patient factors and multiple punctures attempted in this group[12]. Pan *et al*[13] reported an incidence of 4% and 3.9% of PDPH respectively after spinal anesthesia for postpartum tubal ligation with 26-G Atraucan. With 27 G needles in Group III patients all patients had only mild headache. From the statistical analysis, p value is significant at 0.01 level and we infer that there was a strong association between the severity of PDPH and the use of large bore needles.

The intensity and severity of the PDPH was statistically analysed using 't' test and chi square test respectively and was concluded that the intensity of pain experienced between25 G and 27 G was much significant. The severity of pain was much significant at 48 - 72 hrs using chi square test and this indicates that there is an association between the large bore needles and severity of pain.

Mihic DN[11] in his review on PDPH in relation to the direction of the needle bevel hypothesized that splitting rather than cutting the longitudinally directed duramater fibres resulted in lower incidence of PDPH. Recent electron microscopic studies by Reina *et al*[15] showed that the dural fibres doesn't run longitudinally but they are criss-cross; hence the direction of the bevel of the needle does not really matter at all while making the lumbar puncture.

In our study, the patients having PDPH were treated with simple conservative measures like strict bed rest, supine or comfortable position , liberal intake of fluids, intravenous fluids and analgesics. None of the patients with PDPH required epidural blood patch. Jones RJ[7] states that there is no clinical evidence to support the maintainence of supine position before or after the onset of the headache as a means of treatment.[2] Though prone position has been advocated, it is not a comfortable position to lie down by the patient. The prone position raises the intra abdominal pressure which is transmitted to the epidural space and this is said to reduce the CSF leak from spinal space into epidural space, thereby reducing the headache.

CONCLUSION

We conclude from our study the incidence, intensity and severity of Post dural Puncture Headache are more with large bore Quincke spinal needles. Hence we recommend the use of fine bore needles of 27 G or less for caesarean sections to minimize the incidence of PDPH.

References

1. Bridenbaugh PO, "Post Dural Puncture Headache": Regional Anesthesia 1978; 3: 5-8.
2. Buettner J & Wresh KP, "Post Dural Puncture Headache-Comparison of 25G Quincke and Whitacre needles" : Regional Anesthesia 1993; 18: 166-168.
3. Choi PT, Galinski SE, Lucas S, Takeuchi L & Jadad AR, " Examining the evidence in Anesthesia Literature : A survey and evaluation of Obstetric Post Dural Puncture Headache reports". *Canadian Journal of Anesthesia* 2002; 49: 49-56.
4. Costigan SN & Sprigged JS, "Dural puncture: the patient's perspective. A patient survey of cases at a DGH maternity unit 1983-1993". Acta Anaesthesiol Scand 1996; 40; 710-714.
5. Flattern H, Rodt SA, Rosland J, "Post Operative Headache in Young Patients after Spinal Anesthesia". Anesthesia 1987; 42: 202-205.
6. Hammad T, DeDent A, Algahtani R, Alastal Y, Elmer L, Medhkour A, *et al*. Posterior Reversible Encephalopathy Syndrome Secondary to CSF Leak and Intracranial Hypotension: A Case Report and Literature Review. Case Rep Neurol Med. 2015; 2015: 538523.
7. Jones RJ, "The Role of Recumbancy in the prevention and treatment of post spinal headache". Anesthesia Analgesia, 1974; 53: 788-95.
8. Kyung-Hwa Kwak Postdural puncture headache Korean J Anesthesiol. 2017 Apr; 70(2): 136-143.
9. Leibold RA, Yealy DM, Coppola M & Cantees KK, "Post Dural Puncture Headache. Characteristics, management and prevention". Annual Emergency Medicine 1993; 22: 1863-70.
10. Lund PC, "Principles and Practice of Spinal Anesthesia", Springfield, IL: Charles Thomas; 1971.
11. Mihic DN, "Post Spinal Headache and Relationship of needle Bevel to Longitudinal Dural Fibres". Regional Anesthesia, 1985; 10: 76-81.
12. Nafiu OO, Salam RA, Elegbe EO. Post dural puncture headache in obstetric patients: Experience from a West African teaching hospital. *Int J Obstet Anesth* 2007; 16: 4-7.
13. Pan PH, Fragneto R, Moore C, Ross V. Incidence of Post dural puncture headache and backache, and success rate of dural puncture: Comparison of two spinal needle designs. *South Med J* 2004; 97: 359-63.
14. Rausmeussen BS, Blom L. Hausen P & Mikkelsen SS. "Post Spinal Headache in young and elderly patients". Anesthesia, 1989; 44: 571-573.
15. Reina MA, de heon-casasola OA, Lopiz A, De Andres J, Martin S & Mora M. "An in-vitro study of dural lesions produced by 25G Quincke and Whitacre needles evaluated by scanning electron microscopy". Regional Anesthesia of Pain Medicine 2000, 25: 393-400.
16. Reynolds F "Dural Puncture and Headache". British Journal of Anesthesia, 1993; 306 : 874-876.
17. Ruslan Abdullayev, Omer Burak Kucukebe, Bulent Celik Sinan Hatipoglu, Filiz Hatipoglu. "Incidence of postdural puncture headache: Two different fine gauge spinal needles of the same diameter". *Journal of Obstetric Anesthesia And Critical Care* 2014; 4 : 64-68.

18. Schultz AM, Ulbing S, Kaider A & Lehofer. "Post Dural Puncture Headache and back pain after spinal anesthesia with 27G Quincke and 26G Atraucan needles. "Regional Anesthesia 1996; 21: 461-464.
19. Seeberger MD, Kaufmann M, Staender S, Schneider M & Scheidegger D. "Repeated Dural Punctures Increases the Incidence of Post Dural Puncture Headache". Anesthesia Analgesia 1996; 82: 302 - 304.
20. Vandam LD & Dripps RD. "Long term follow up patients who received 10098 Spinal Anesthesia". JAMA 1956; 161 : 586 - 591.

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Original Research Article

Comparison of C-MAC blade and McCoy blade for laryngoscopy in adult patients undergoing tracheal intubation for elective surgeries with simulated cervical spine injury using manual in line stabilization

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Abstract

Background: The gold standard and ideal position for laryngoscopy is sniffing position. Trauma life support guidelines recommend the use of Manual In Line Stabilization or a rigid collar to stabilize the spine in suspected cervical spine injury patients. The gold standard for intubation in patients with cervical spine injury is fibre-optic bronchoscopy. But its use is restricted by lack of expertise, availability and time requirement. C-MAC blade and McCoy blade have been independently compared with Macintosh laryngoscope in simulated difficult airway. Aim: In our study, we compare C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury using Manual In Line Stabilisation (MILS). **Materials and methodology:** 100 patients of age group between 18 – 65 years belonging to ASA 1 & 2 posted for elective surgeries under general anesthesia with endotracheal intubation participated in this study. Patients were randomly allocated to one of the two groups by computer generated random table number. Group C - Endotracheal intubation done using C-MAC D blade video laryngoscope and Group M - Endotracheal intubation done using McCoy blade laryngoscope. Observation and results: Baseline characteristics were presented as mean ± S.D. Two-sided unpaired t-test and chi-square test was applied to analyze the data and p value less than 0.05 were considered as significant. **Conclusion:** C-MAC video laryngoscope requires less time for laryngoscopy, provides better visualisation of glottis, lower IDS score with similar duration of intubation and haemodynamic responses when compared to McCoy laryngoscope in patients with simulated cervical spine injury.

Key Word: C-MAC blade, McCoy blade, laryngoscopy, simulated cervical spine injury, manual in line stabilization

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INTRODUCTION

The gold standard and ideal position for laryngoscopy is sniffing position. The sniffing position aligns the oral, pharyngeal and laryngeal axes and provides better glottic visualization.[2] The sniffing position produces flexion of the lower cervical spine, extension of the upper cervical spine and extension of the atlanto–occipital joint.[3] Trauma life support guidelines recommend the use of Manual In Line Stabilization (MILS) or a rigid collar to stabilize the spine in suspected cervical spine injury patients.[4] MILS prevents head extension and neck flexion which is necessary for optimal alignment of three airway axes.[5]

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The presence of collar can worsen the laryngoscopic view and makes the intubation difficult with conventional laryngoscope.[4] To overcome this various devices and options like direct laryngoscope with the aid of gum elastic bougie, fibre-optic bronchoscopy, airway scope, McCoy laryngoscope, Intubating laryngeal mask airway, C-Trach and Bullard laryngoscope has been recommended by many authors.[6] The gold standard for intubation in patients with cervical spine injury is fibre-optic bronchoscopy. But its use is restricted by lack of expertise, availability and time requirement.[4] The McCoy levering laryngoscope is a modification of the standard Macintosh blade.[6] It has a hinged tip and the angle of the hinged portion can be altered by a lever attached to the handle.[7,8] Depression of the lever towards the handle elevates the tip.[8] The hinged tip aids in improving the Cormack and Lehane laryngoscopic view by 1 grade in comparison to Macintosh blade in patient with cervical spine injury. The blade is available in size 3 and 4.[4] The latest generation C-MAC video laryngoscope has several distinct improvements.[8] It has external light source and small digital camera at the distal third of the blade, which extends to a video display monitor.[9] It provides optimum view of the glottis by direct and indirect view.[9] C-MAC laryngoscope can accommodate Macintosh blade 2, 3 & 4. A special D blade with greater curvature is designed to facilitate intubation of the difficult airways.[8] Both the devices have been independently compared with Macintosh laryngoscope in simulated difficult airway. Only a few studies are available comparing C-MAC and McCoy laryngoscope.[4] This study was carried out to compare the efficacy of C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury.

AIM

To compare C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury using Manual In Line Stabilisation (MILS) with the following parameters.

Primary Aim:

- Duration of laryngoscopy
- Duration of intubation
- Total duration of intubation
- Ease of intubation - Intubation Difficulty Scale (IDS score)

Secondary Aim:

- Haemodynamic responses

MATERIALS AND METHODOLOGY

A study titled "Comparison of C-MAC D blade and McCoy blade for laryngoscopy in patients with simulated cervical spine injury" was done in PSG Institute of Medical Sciences & Research, Coimbatore. This study was done after obtaining ethical committee clearance and informed written consent from all the patients participated in this study. It was a prospective and randomised study. 100 patients of age group between 18 – 65 years belonging to ASA 1 & 2 posted for elective surgeries under general anesthesia with endotracheal intubation participated in this study.

Inclusion criteria

- Patient's acceptance
- Patients aged between 18 – 65 years
- ASA 1 and ASA 2 patients
- Patients undergoing elective surgery under GA with endotracheal intubation

Exclusion criteria

- Anticipated difficult airway
- Risk of aspiration
- Hypertensive patients
- Patients on β-blockers, Clonidine.
- Duration of intubation exceeding 120 sec
- Change in laryngoscopy during intubation
- Emergency surgeries

Routine pre-operative assessment was done and the patients were kept nil per oral from 10 pm the day prior to surgery. Informed written consent was obtained. All the patients were pre-medicated with tablet Ranitidine 150mg orally at night the day prior to surgery and at 6 am on the morning of surgery. Patients were randomly allocated to one of the two groups by computer generated random table number.

Group C: Endotracheal intubation done using C-MAC D blade video laryngoscope

Group M: Endotracheal intubation done using McCoy blade laryngoscope

Parameters recorded

Duration of laryngoscopy - defined as the time taken from insertion of the blade between the teeth until the anaesthetist obtained the best possible view of the vocal cords

Duration of intubation - defined as the time taken from when the anaesthetist indicate the best view at laryngoscopy until the tracheal tube was placed through the vocal cords, as evidenced by visual confirmation by the anaesthetist.

Total duration of intubation - It is the sum of the duration of laryngoscopy and the duration of intubation

Ease of intubation - Intubation Difficulty Scale (IDS score). It includes the following:

1. Number of intubation attempts
2. Number of operators
3. Number of alternative technique used
4. Glottis exposure – CL gradin
5. Lifting force required

6. Necessity for laryngeal pressure
7. Vocal cord mobility

Haemodynamic responses - Heart rate, systolic BP, diastolic BP, mean arterial BP after 1st min, 3rd min and 5th min of intubation. The intubation difficulty scale (IDS)[37] : In 1997 Frederic Adnet *et al* evaluated and proposed an Intubation Difficulty Scale (IDS) score characterising the complexity of endotracheal intubation in both the prehospital and operating room settings to assess the degree of difficulty. The Intubation Difficulty Scale (IDS) score is a function of seven parameters, resulting in a progressive, quantitative determination of intubation complexity and they are as follows:

Parameters	Definition		
N^1-The number of intubation attempts	An attempt is defined as one advancement of the tube in the direction of the glottis during direct laryngoscopy	N^1 = 0 if only one attempt	Every additional attempt adds one point
N^2-The number of supplementary operators	It represents the number of additional persons directly attempting intubation	N^2 = 0 if only one operator	Every additional operator adds one point
N^3-The number of alternative techniques used	Change in approach - oral intubation to blind nasotracheal intubation, change in material – blade, endotracheal tube, addition of stylette	Change in approach	Each alternative technique adds one point
N^4-Glottic exposure	defined by the Cormack grade minus one	GradeI (N^4 = 0) GradeII(N^4 = 1) GradeIII(N^4= 2) GradeIV(N^4=3)	Glottic exposure is evaluated during the first attempt by the first operator
N^5- Lifting force applied during laryngoscopy	If little effort is necessary If subjectively increased lifting force is necessary	N^5 = 0 N^5= 1	
N^6- Necessity for external laryngeal pressure for optimizedglottic exposure	If no external pressure is applied If external laryngeal pressure is necessary	N^6= 0 N^6= 1	
N^7- Position of vocal cords during intubation	if vocal cords are in abduction if the vocal cords are in adduction	N^7= 0 N^7= 1	

The degree of difficulty is described according to IDS score as follows

IDS Score	Degree of difficulty
0	Easy
1 – 5	Slightly difficult
>5	Moderate to major difficulty
Infinity	Impossible intubation

Patients were connected to ASA standard monitors – ECG, Non invasive blood pressure(NIBP) and pulse oximeter (SpO_2) and baseline readings recorded. Intravenous line started and patency of the vessel checked. Patients were pre oxygenated with 100% Oxygen for 3 minutes and Induced with Fentanyl 2mcg/kg, Lignocaine (preservative free) 20mg and Propofol 2mg/kg intravenously. After ensuring adequate mask ventilation, patients were paralysed with Inj. Succinylcholine 1.5mg/kg. Pre intubation heart rate, systolic BP, diastolic BP, mean arterial BP were recorded. After 1 minute MILS was achieved by an anaesthetist, standing to the side of the patient, using fingers and palms of both hands to stabilize the patient's occiput and mastoid process and gently counteract the forces created by the intubator. Laryngoscopy was done by another anaesthetist who is experienced with both the techniques. Laryngoscopy was done with C-MAC D blade laryngoscope in group C and McCoy blade laryngoscope in group M. After visualization of the cords, patients were intubated with appropriately sized endotracheal tube with stylet bent to hockey stick shape. During intubation the duration of laryngoscopy, the duration of intubation and the ease of intubation (IDS scoring) were recorded. After securing the endotracheal tube, the tube position was confirmed with stethoscope and $ETCO_2$ monitoring, anaesthesia was maintained with Oxygen & Nitrous oxide 40:60 with 6 litres fresh gas flow, Sevoflurane 2% and Inj.Vecuronium (loading dose 0.1mg/kg). Heart rate, systolic BP, diastolic BP, mean arterial BP were recorded after 1st min, 3rd min and 5th min of intubation.

Statistical analysis: Data were statistically analysed with the SPSS version 17.0 software. Baseline characteristics were presented as mean ± S.D. Two-sided unpaired t-test and chi-square test was applied to analyze the data and p value less than 0.05 were considered as significant. A repeated measure of ANOVA was applied for the two groups to know the with-in subject variability in Ease of intubation and $p < 0.05$ was considered to be significant.

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OBSERVATION AND RESULTS

Table 1: Demographic details - Group C and Group M

1.Age distribution	Age in years	Group C		Group M		p- value	Results
		No of patient	Percentage	No of patients	Percentage		
	20 – 29	11	22	12	24	0.808	Similar in both the groups
	30 – 39	16	32	14	28		
	40 – 49	11	22	10	20		
	≥ 50	12	24	14	28		
	Mean (in yrs) ± S.D	40 ± 11.9		40.6 ± 12.601			
2.Sex distribution	**Sex**	**Group C**		**Group M**		**p- value**	**Results**
		No of patient	Percentage	No of patients	Percentage		
	Female	24	48	25	50	0.843	Compar-able between the two groups
	Male	26	52	25	50		
3.BMI distribution	**BMI Range**	**Group C**		**Group M**		**p- value**	**Results**
		No of patient	Percentage	No of patients	Percentage		
	≥30	0	0	1	2	0.337	Compar-able between the two groups
	25 -29.9	26	52	28	56		
	18 -24.9	21	42	19	38		
	≤18	3	6	2	4		
	Mean (in yrs) ± S.D	24.36 ± 3.26		24.9 ± 3.12			

Table 2: Laryngoscopy and Intubation Duration for Group C and Group M

Parameters (seconds)	Group C (Mean±SD) N=50	Group M (Mean±SD) N=50	Mean Difference	p- value	Results
1.Duration of Laryngoscopy	12.15± 3.11	14.47± 3.55	-02.32	0.0001	Statistically significant
2.Duration of Intubation	13.85± 3.51	12.07± 3.23	1.78	0.01	Statistically significant
3.Total duration of Intubation	26 ± 5.65	26.55± 5.99	-0.55	0.639	Comparable between the groups

Table 3: Intubation Difficulty Scale (IDS) score for Group C and Group M

IDS score	Group C n (%)	Group M n (%)	p value	Results
N_1 (Attempts)				
1st - score 0	48(96)	44(88)	0.143	Not significant
2nd - score 1	2(4)	6(12)		
N_2 (operators)				
1 – score 0	50(100)	47(94)	0.080	Not significant
2 – score 1	0(0)	3(6)		
N_3 (alternative techniques)				
Not used – score 0	50(100)	50(100)		
N_4 (CL grade)				
1 – score 0	47(94)	34(68)	0.001	Significant
2 - score 1	3(6)	16(32)		
N_5(lifting force)				
Normal – score 0	45(90)	32(64)	0.002	Significant
Increased– score 1	5(10)	18(36)		
N_6(laryngeal pressure)				
Notapplied–score0	36(72)	42(84)	0.150	Not significant
Applied – score 1	14(28)	8(16)		
N_7 (vocal cord position)				
Abducted – score 0	50(100)	50(100)		

Table 4: Ease of Intubation (IDS score) for Group C and Group M

IDS	No of patients (%)		P value	Results
	Group C	Group M		
0 (easy)	35 (70)	27 (54)	0.009	Statistically significant
0-5 (Slight difficulty)	15 (30)	22 (44)		
> 5 (moderate to major difficulty)	0	1 (2)		

Table 5: Intergroup comparison of mean Heart rate between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	87.5 ± 14.5	84.7 ± 12.1	0.30
Before Intubation	87.9 ± 11.6	87.7 ± 10.6	0.93
1st min	99.9 ± 15.3	102.3 ± 16.6	0.45
3rd min	88.2 ± 13.0	89.9 ± 13.4	0.50
5th min	80.2 ± 10.9	79.9 ± 11.4	0.90

Table 6: Intergroup comparison of Mean Systolic BP between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	126.5 ±13.8	122.1 ±10.8	0.078
Before Intubation	106.6 ± 13.2	104.3 ± 8.8	0.309
1st min	124.6 ± 15.8	128.8 ± 15.3	0.179
3rd min	107.8 ± 11.7	110.3 ± 14.2	0.340
5th min	100.16 ± 11.2	102.6 ± 14.2	0.335

Table 7: Intergroup comparison of mean Diastolic BP between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	77.12 ± 7.2	75.3 ± 8.4	0.256
Before Intubation	65.7 ± 8.1	75.3 ± 8.4	0.457
1st min	78.4 ± 12.8	82.7 ± 12.9	0.098
3rd min	68.1 ± 9.2	70.8 ± 12.6	0.210
5th min	63.2 ± 8.1	65.5 ± 10.5	0.210

Table 8: Intergroup comparison of mean MAP between Group C & Group M

Time	Group C N=50	Group M N=50	p value
Baseline	93.9 ± 8.3	91.3 ± 8.6	0.112
Before Intubation	79.7 ± 8.6	79.7 ± 6.2	0.963
1st min	94.1 ± 13.3	98.3 ± 13.5	0.112
3rd min	81.6 ± 9.2	84.3 ± 12.9	0.222
5th min	75.8 ± 8.3	78.2 ± 11.4	0.231

DISCUSSION

Approximately 2-5% of trauma patients will have cervical spine injury. Patient with cervical spine injury may require airway management for airway protection, to prevent hypoxia and hypoventilation.[1] In our study, we have compared the efficacy of C-MAC D blade and McCoy blade laryngoscope in simulated cervical spine injury by comparing duration of laryngoscopy, duration of intubation, total duration of intubation, ease of intubation (IDS score) and haemodynamic response. In our study the demographic variables like age, gender and BMI were comparable between both the groups. The mean duration of laryngoscopy when compared between two groups, it was shorter in C-MAC group (12.5 seconds) than McCoy group (14.47 seconds) and it was statistically significant. The mean duration of intubation when compared between two groups, it was longer in C-MAC group (13.85 seconds) than McCoy group (12.07 seconds) and it was statistically significant. The mean total duration of intubation was comparable between C-MAC group (26 sec) and McCoy group (26.55 sec) and statistically not significant. Even though the time taken for laryngoscopy was shorter in C-MAC group, the time taken for successful intubation was prolonged. So the

mean total duration of intubation was comparable and statistically insignificant between the groups. Our results of duration of laryngoscopy, duration of intubation and total duration of intubation were similar with the study done by Jain *et al*.[4] comparing McCoy and C-MAC video laryngoscope in simulated cervical spine injury. The ease of intubation was observed in our study using IDS scoring. In our study 48 patients in C-MAC group and 44 patients in McCoy group were intubated in the first attempt and were comparable. This results were similar to study by Jain *et al*.[10,11] who compared the conventional C-MAC and the C-MAC D blade with direct laryngoscopes in simulated cervical spine injury (manikin study) and observed that out of 33 patients, 30 patients were intubated in first attempt using C-MAC D blade and 24 patients using McCoy and was not statistically significant. 50 patients in group C and 47 patients in group M required single operator and all the patients were intubated using single technique in our study. In C-MAC group 47 patients had CL grade 1 and 3 patients had CL grade 2. In McCoy group 34 patients had CL grade 1 and 16 patients had CL grade 2. Hence the glottis exposure was better with C-MAC group than McCoy group and it was statistically significant which was similar in studies by Jain *et al*.[4] and Sabry *et al*.[8] Jain *et al*.[4] compared McCoy laryngoscope and C-MAC video laryngoscope in simulated cervical spine injury and observed that out of 30 patients, 29 patients in C-MAC group and 16 patients in McCoy group had CL grade 1 and was statistically significant. Sabry*et al*.[8,12] compared C-MAC D blade and McCoy laryngoscopes during cervical immobilization and observed that out of 30 patients, 16 patients in C-MAC group and 4 patients in McCoy group had CL grade 1 and was statistically significant. The McCoy group (18 patients) needed more lifting force for visualisation of glottis than C-MAC group (5 patients) and it was statistically significant. 14 patients in C-MAC group needed external laryngeal pressure during intubation compared to 8 patients in McCoy group, but it was not statistically significant. The IDS score between both the groups was found to be statistically significant. The use of C-MAC resulted in more number of easy intubation when compared to McCoy[13]. This was similar to a study by Jain *et al*.[4] comparing C-MAC and McCoy laryngoscopes where C-MAC resulted in lower IDS than McCoy group. In our study the heart rate, systolic BP, diastolic BP and mean BP recorded at all times were comparable between C-MAC, McCoy group and was statistically insignificant. This was similar to study by Jain *et al*.

CONCLUSION

In our study though there was a statistical significance in duration of laryngoscopy and duration of intubation, the total duration of intubation was comparable and insignificant. Because with C-MAC the time taken for successful intubation was prolonged though the duration of laryngoscopy was shorter. The C-MAC group had better glottic visualisation, needed optimal lifting force and clinically insignificant external laryngeal pressure with lower IDS score when compared to McCoy group. To conclude C-MAC video laryngoscope requires less time for laryngoscopy, provides better visualisation of glottis, lower IDS score with similar duration of intubation and haemodynamic responses when compared to McCoy laryngoscope in patients with simulated cervical spine injury.

REFERENCES

1. Austin N, Krishnamoorthy V, Dagal A. Airway management in cervical spine injury. International Journal of Critical Illness and Injury Science. 2014;4(1):50-56. doi:10.4103/2229-5151.128013.
2. Pal R, Chauhan S, Ved BK, Lad SR. Evaluation of laryngoscopic view, intubation difficulty and sympathetic response during direct laryngoscopy in sniffing position and simple head extension: A prospective and randomized comparative study. Int J Res Med Sci 2015;3: 1895-901.
3. Magill IW. Endotracheal anaesthesia. American Journal of Surgery 1936; 34:450 – 5.
4. Jain D, Bala I, Gandhi K. Comparative effectiveness of McCoy laryngoscope and CMAC(®) videolaryngoscope in simulated cervical spine injuries. J AnaesthesiolClinPharmacol. 2016 Jan; 32(1):59-64.
5. Joseph J, Sequeira T, Upadya M. Comparison of the use of McCoy and TruView EVO2 laryngoscopes in patients with cervical spine immobilization. Saudi J Anaesth. 2012; 6: 248–53.
6. Bharti N, Arora S, Panda NB. A comparison of McCoy, TruView, and Macintosh laryngoscopes for tracheal intubation in patients with immobilized cervical spine. Saudi Journal of Anaesthesia. 2014;8(2):188-192. doi:10.4103/1658-354X.130705.
7. Laurent SC, de Melo AE, Alexander-Williams JM. The use of the McCoy laryngoscope in patients with simulated cervical spine injuries. Anaesthesia. 1996; 51: 74–5.
8. Sabry LA, Shaarawy SS, Ellakany MH, Elmasry AA. Comparison between C-MAC D-blade and McCoy laryngoscopes in intubating patients during cervical immobilization. Res OpinAnesth Intensive Care 2016; 3: 122-8.
9. Kilicaslan, A. Topal, A. Tavlan, A. Erol, and S. Otelcioglu, "Effectiveness of the C-MAC video laryngoscope in the management of unexpected failed intubations," Brazilian Journal of Anesthesiology, vol. 64, no. 1, pp. 62–65, 2014.
10. Jain D, Dhankar M, Wig J, Jain A. Comparison of the conventional CMAC and the D-blade CMAC with the

direct laryngoscopes in simulated cervical spine injury-a manikin study. Braz J Anesthesiol. 2014 Aug 31;64(4):269-274.
11. Kılıçaslan A, Topal A, Erol A, Uzun ST. Comparison of the C-MAC D-Blade,
Conventional C-MAC, and Macintosh Laryngoscopes in Simulated Easy and Difficult Airways. Turk J AnaesthesiolReanim. 2014; 42: 182–9.
12. Bharti N, Arora S, Panda BP. A comparison of McCoy, TruWiew and Macintosh laryngoscopes for tracheal intubation in patients with immobilized cervical spine. Saudi J Anesth. 2014; 8(2):188-92. doi: 10.4103/1658-354X.130705.
13. Adnet F, Borron SW, Racine SX, *et al*. The intubation difficulty scale (IDA). Anesthesiology 1997; 87: 1290–7.

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Original Research Article

Efficacy of dexamethasone in reducing the severity of postoperative sorethroat after endotracheal intubation

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Abstract

Background: Airway management with cuffed endotracheal intubation is an integral part of an anaesthesiologist's responsibilities. Local irritation and inflammation caused by prolonged inflation of the cuff can result in minor sorethroat to more serious transglottic complications inherent to the usage of cuffed endotracheal tube. Many studies have been performed and published exploring the measures to minimize or eliminate post-intubation morbidities. **Aim:** The aim of our study was to evaluate the efficacy of intravenous administration of Dexamethasone in reducing the incidence and severity of postoperative sore throat in patients receiving general anaesthesia with cuffed endotracheal intubation. **Materials and methodology:** 100 patients of age group between 18 – 65 years belonging to ASA 1 and 2 of both the sexes posted for elective surgeries under general anaesthesia with endotracheal intubation participated in this study. Patients were randomly allocated to one of the two groups, 50 in each by using envelope method. **Group C:** Control group was given 2 ml Normal Saline Intravenously and **Group D:** Dexamethasone group was given 2 ml Dexamethasone 8mg Intravenously. **Observation and Results:** Statistical analysis was done using SPSS PC (11.5) version. Student's t-test was used to compare the mean difference in quantitative variables and Chi-square test was used to study the association among qualitative variables. p value < 0.05 was considered statistically significant. **Conclusion:** The conclusion of our study is that single dose of 8 mg prophylactic Dexamethasone given intravenously reduces effectively the incidence and severity of sore throat after general anaesthesia with cuffed endotracheal intubation.

Key Word: Dexamethasone, Post operative sorethroat, Endotracheal intubation

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INTRODUCTION

Airway management with cuffed endotracheal intubation is an integral part of an anaesthesiologist's responsibilities ensuring adequate ventilation and preventing aspiration of secretions. In addition, the wastage of anaesthetic gases and pollution of the operation theatres are also reduced substantially. However, local irritation and inflammation caused by prolonged inflation of the cuff can result in minor sorethroat to more serious transglottic complications (like vocal cord problems and subglottic stenosis), inherent to the usage of cuffed endotracheal tube. The minor sequelae of sore throat from the use of cuffed endotracheal tube results in up to 90% incidence in the postoperative period. Several factors like trauma to laryngopharyngeal mucosa during laryngoscopy, contact of tracheal tube with the vocal cords and pharyngeal wall resulting in oedema, pressure affecting the tracheal mucosal capillary perfusion, cuff design, oral suctioning, placement of nasogastric tube, mobility of the tube

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inwards, outwards or sideways can contribute to sorethroat. After general anaesthetic one may develop a sore throat which can range between minor discomfort to a more severe continuous pain. One may also have a very dry throat or feel pain on speaking or swallowing and the symptoms may disappear after a few hours but may take a two days or more to settle down completely. Many studies have been performed and published exploring the measures to minimize or eliminate post-intubation morbidities, viz,

- Use of high volume-low pressure cuffed endotracheal tubes[6]
- Use of smaller sized endotracheal tubes[1]
- Inhalation of steroids[4]
- Topical application of lubricant jellies[20], Local anaesthetic (2% Lignocaine) jellies[8] and Steroids like Betamethasone gel[1]

Dexamethasone is a potent corticosteroid with anti-inflammatory, analgesic and antiemetic properties and has been reported to reduce the postoperative pain and swelling following oral surgeries.[2,3,13]

AIM

The aim of our study was to evaluate the efficacy of intravenous administration of Dexamethasone in reducing the incidence and severity of postoperative sore throat in patients receiving general anaesthesia with cuffed endotracheal intubation.

MATERIALS AND METHODS

A study titled "Efficacy of Dexamethasone in reducing the severity of postoperative sore throat after endotracheal intubation" was done after obtaining ethical committee clearance from the institution and informed written consent were obtained from all the patients who participated in this prospective and randomised study.

100 patients of age group between 18 – 65 years belonging to ASA 1 and 2 of both the sexes posted for elective surgeries under general anaesthesia with endotracheal intubation participated in this study.

Inclusion criteria

- Patients belonging to ASA grade 1 and 2
- Age group between 18-66 yrs
- Body weight between 40-75 kg
- Surgery duration lasting for more than 1 hr

Exclusion criteria

- History of recent respiratory infection
- Risk factors for post operative aspiration
- H/o smoking
- Obesity
- Pregnancy
- Prior treatment with analgesics
- Use of corticosteroids
- Neuromuscular diseases
- Those requiring more than two attempts
- Presence of blood on endotracheal tube or on oral suction after extubation

Routine pre-operative assessment was done and the patients were kept nil per oral from 10 pm the day prior to surgery. Informed written consent was obtained. All the patients were pre-medicated with Tab. Pantoprazole 40 mg and Tab. Alprazolam 0.25mg at night the day prior to surgery and at 6 am on the day of surgery. Patients were randomly allocated to one of the two groups, 50 in each by using envelope method.

Group C: Control group was given 2 ml Normal Saline iv

Group D: Dexamethasone group was given 2 ml Dexamethasone 8mg iv

Patients were connected to ASA standard monitors – ECG, Non invasive blood pressure (NIBP), pulse oximeter (SpO_2) and baseline readings recorded. An 18 G Intravenous catheter was inserted and intravenous fluids started. Preoxygenation was done with 100 % Oxygen for 3 minutes and general anaesthesia was instituted with intravenous Fentanyl 2mcg/kg, Lignocaine (preservative free) 20mg and Propofol 2mg/kg intravenously for induction. Mask ventilation was done for 3 mins and Endotracheal intubation was facilitated with 0.5 mg/kg Atracurium besylate. High volume low pressure endotracheal tube from the same manufacturer (Rusch Inc.) were used for all patients from both groups. Male patients received 8 and 8.5 mm and female patients received 7 and 7.5 mm inner diameter endotracheal tubes respectively. Intubation was performed with a standard MacIntosh curved blade size 3 or 4. Application of external laryngeal pressure to aid endotracheal intubation and number of attempts at laryngoscopy were recorded. The cuff was inflated with air just to achieve an airtight seal in the presence of intermittent positive airway pressure ventilation. Intra cuff pressure was adjusted as required every 30 minutes to 25 – 40 cm H_20 (18 – 30 mm Hg) using a cuff pressure monitor to limit nitrous oxide related intracuff pressure increase. Anaesthesia was maintained with oxygen and nitrous oxide in a ratio of 33:66 in isoflurane. Muscle relaxants and analgesia were repeated when required at regular intervals. Ventilation was adjusted to maintain normocarbia (35 – 40 mmHg). Oral or nasal airways were not placed in any patient. Coughing or bucking on the ETT if any, were recorded. At the end of the surgery, Ondansetron 0.1mg/kg i.v was administered and muscle relaxation was reversed with a combination of Neostigmine 0.05 mg/kg and Glycopyrrolate 0.01 mg/kg. Oropharyngeal suction before extubation was done under direct vision to avoid trauma

to pharyngeal mucosal tissues as well as to confirm that secretions were completely removed. Presence of blood in oropharyngeal suction or on the endotracheal tube after extubation excluded these patients from the study. In the Post anaesthesia care unit, presence of soreness of throat was evaluated by a trained observer who, along with the patient, was blinded to the study group assignment. The assessment was done using visual analogue scale (linear 10 cm, starting from 0 = no pain to 10 = worst imaginable pain) at rest and with effort (swallowing movement) at one hour, three hour, six hour, 12 hour and 24 hour periods.

Statistical analysis: Our randomized double blind placebo controlled prospective study comprised of 100 patients, 50 in each group.

Group C-Control Group (n=50)

Group D- Dexamethasone (n=50)

Statistical analysis was done using SPSS PC (11.5) version. Student's t-test was used to compare the mean difference in quantitative variables and Chi-square test was used to study the association among qualitative variables. p value < 0.05was considered statistically significant.

OBSERVATION AND RESULTS

Table 1: Demographic details - Group C and Group D

Parameters		Group C		Group D		p- value	Results
1.Age distribution	Age in years Mean (in yrs)±S.D	38.62±11.54		39.56±9.97		0.66	NoStatistical Significance
2.Sex distribution	**Sex**	**No of patient**	**Percentage**	**No of patients**	**Percentage**		No Statistical significant
	Female	25	50	22	44	0.54	difference in
	Male	25	50	28	56		boththe groups
3.Weight distribution	BMI Range Mean(in kg) ±S.D	57.50±7.47		59.84±7.86		0.13	No Statistical Significance
4. ASA	I	39		36		0.493	Both the groups
	II	11		14			were unique
5.Duration of surgery	In minutes	103.80±30.43		100.20±36.16		0.59	Statistically there was no difference

Table 2: Intra Cuff pressure (cm of H20) at various periods

Period	Group C	Group D	p value	Significance
Baseline	26.32±1.07	26.76±1.23	0.061	not significant
30 min	28.50±1.47	28.38±1.00	0.636	not significant
60 min	29.68±1.37	29.20±1.08	0.056	not significant
90 min	30.35±1.54	30.03±0.89	0.304	not significant
120 min	**31.00±1.41**	**29.72±1.03**	**0.001**	**Significant**
150 min	31.11±0.92	28.23±8.52	0.328	not significant

Table 3: Incidence of sorethroat between two groups

	Incidence	
Group	Number of patients	%
Group C(n=50)	27	54%
Group D(n=50)	9	18%

Table 4: VAS scores at rest

Time period	Group C	Group D	t value	p value	Statistical Significance
1 hr	2.30± 2.25	0.36± 0.80	5.738	0.000	Significant
3 hr	1.98± 1.98	0.26± 0.59	5.867	0.000	Significant
6 hr	1.52± 1.59	0.18± 0.48	5.733	0.000	Significant
12hr	1.00± 1.24	0.04± 0.19	5.383	0.000	Significant
24hr	0.30± 0.70	0.00± 0.00	3.000	0.003	Significant

Table 5: VAS scores at effort

Time period	Group C	Group D	t value	p value	Statistical Significance
1 hr	2.96± 3.00	0.56± 1.24	5.218	0.000	Significant
3 hr	2.40± 2.37	0.48± 1.07	5.212	0.000	Significant
6 hr	2.08± 2.09	0.36± 0.82	5.393	0.000	Significant
12 hr	1.66± 1.75	0.16± 0.46	5.834	0.000	Significant
24 hr	0.97± 1.52	0.06± 0.31	4.187	0.000	Significant

Table 6 : Parameters observed intraoperatively

Parameters	Group C n = 50 (%)	Group D n = 50 (%)
Second laryngoscopy attempt	5 (10%)	6 (12%)
External laryngealpressure	8 (16%)	10 (20%)
Bucking/coughing during intubation	5 (10%)	4 (8%)
Nasogastric tube placement	7 (14%)	8 (16%)
Blood on endotracheal tube after extubation or on oropharyngeal suction	Nil	Nil

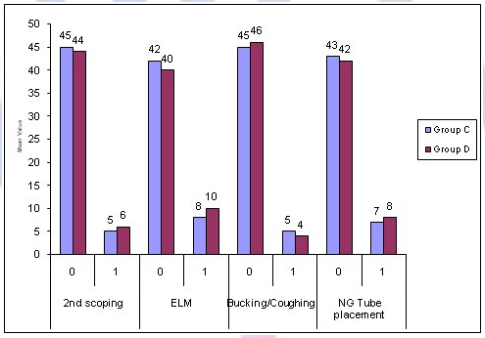


Figure 1: Incidence of 2nd attempt, External laryngeal pressure, Bucking/Coughing on ETT between groups

DISCUSSION

Sorethroat though a minor complaint following endotracheal intubation, is one of the most common and irritating complaints similar to Post operative nausea and vomiting (PONV); and if associated with cough, it increases the pain and oozing when there is abdominal or thoracic wounds. Postoperative sore throat is a symptom caused by many factors, such as the intubation procedure, cuffed endotracheal tube, intracuff pressure and the use of stylets or lubricants. Stenqvist O *et al*[16]in his studies showed the incidence of postoperative sore throat does not necessarily reflect damage caused by the endotracheal cuff only. In our study, Dexamethasone group had a statistically significant reduction in the incidence (18% Vs 54%) and severity of sore throat after general anaesthesia with endotracheal intubation compared to the placebo group. Contrarily the occurrence of sore throat was similar between the two groups, in respect to the duration of surgery, the number of laryngoscopic attempts and intracuff pressure. In our study, patients from both groups had their intracuff pressure checked every half hourly and maintained within acceptable limits. Jenson PJ *et al*[6], El Hakim *et al*[4] and Mc Hardy FC *et al*[9] in three different studies concluded that the incidence of sorethroat ranges from 6.6 – 90%, a wide spectrum. 54% with sorethroat in our study is indicating that every alternative patient had postoperative sore throat. Stout DM *et al*[17] showed a higher incidence of sore throat caused by larger size endotracheal tube compared with smaller size tube. The size of the endotracheal tube in

both age groups was similar in both groups, not letting the size affect the incidence of sore throat. The duration of surgery did not alter the incidence of sore throat in our study, in contrast Jensen PJ *et al*[6] where they found a direct relation with the length of cuff – tracheal contact. In respect to the cuff design and lubrication, Loeser EA *et al*[7] in their study demonstrated high volume low pressure cuffs were associated with higher incidence of sore throat due to the larger area of contact with the tracheal mucosa but we did not have any such increase in the incidence of sore throat in spite of using high volume low pressure cuffs (Rusch AVT Ind. Ltd.,) in our study, which is possibly explained by the improvement in the endotracheal tubes and cuff designs over the last three decades.Stanley TH *et al*[15]showed a significant increase in cuff volume and cuff pressure after use of nitrous oxide intraoperatively and this has been blamed for increase in the incidence of sorethroat. But in our study, though the cuff pressure and cuff volume increased due to the use of nitrous oxide, it was monitored every 30 minutes by a cuff pressure monitor and brought back to acceptable limits, thus not allowing it to affect the incidence of sore throat. 64.5% of postoperative sore throat was reported by Monroe MC *et al*[11] in patients with blood tinge of airway instruments like endotracheal tube tip, compared to those 30.9% without blood tinge, due to improper suctioning which was wrongly attributed to the increased sore throat incidence. None of our patients from either group had any blood staining. Sang-Hyan Park *et al*[12]concluded prophylactic administration of 0.2 mg/kg body weight Dexamethasone significantly decreased the incidence and severity of sore throat and hoarseness 1 hour and 24 hours after tracheal extubation of a double lumen tube. We have used Dexamethasone 8 mg IV, as the exact dose is not known and the results of our study agree with the above study. Hamelberg W *et al*[5] found insignificant decrease in the incidence of sore throat when 1% hydrocortisone ointment was applied to the endotracheal tube. Stride[18] used the same method together with standardized anaesthesia and direct questioning technique and concluded that hydrocortisone ointment was ineffective in the prevention of postoperative sore throat due to the presence of additive substances which are irritating to the tracheal mucosa. Dexamethasone being better anti-inflammatory agent compared to hydrocortisone probably explains better results in reducing sorethroat.Dexamethasone being a steroid may produce complications like gastritis, avascular necrosis, delayed wound healing, glucose intolerance and susceptibility to infection, but a single bolus dose of intravenous dexamethasone does not produce these complicationsas evidenced by Melby JC *et al*.[10] The results of our study very well correlates with the findings of Wang *et al*[19] which primarily studied the effect of dexamethasone on postoperative nausea and vomiting (PONV) and these results were consistent with the study by Siji Thomas *et al*.[14] Postoperative sore throat is a symptom caused by many factors, such as the intubation procedure, cuffed endotracheal tube, intracuff pressure and the use of stylets or lubricants. Methods to minimize sore throat will bring patient satisfaction, less pain and less oozing, thereby promoting early discharge from the recovery room and hospital. One such method is using intravenous Dexamethasone.

Draw backs of this study

- This study was conducted only in ASA I and II patients and not in other groups
- We did not use humidity moisture exchanger in gas delivery circuit, and dry airway gases have been implicated in the development of sore throat Loeser*et al*[42]
- Our study was not conducted in patients with difficult airway
- The dose of Dexamethasone required for the prevention of postoperative sore throat and exact time of administration is not known

CONCLUSION

The conclusion of our study is that single dose of 8 mg prophylactic Dexamethasone given intravenously reduces effectively the incidence and severity of sore throat after general anaesthesia with cuffed endotracheal intubation. Dexamethasone minimizes two of the minor, but irritating postoperative problems of general anaesthesia with endotracheal intubation i.e. PONV and postoperative sore throat and hence it may be recommended in patients undergoing surgery under general anesthesia.

REFERENCES

1. Ayoub M C, Ghobashy A, Mc Grimday L, Koch M E, Qadir S, Silverman D G. Wide spread application of topical steroids to decrease sore throat, hoarseness and cough after tracheal intubation. Anesthesia Analgesia 1998; 87: 714-716
2. Baxendale BR, Vater M, Lavery KM.Dexamethasone reduces pain and swelling following extraction of third molar teeth.Anaesthesia 1993;48: 961-4
3. Eihakim M, Ali NM, Rashed I, Riad MK, Refat M. Dexamethasone reduces pain and vomiting after pediatric tonsillectomy.Can J Anesth 2003;50: 392-7
4. El Hakim M. Beclomethasone prevents postoperative sore throat. Acta AnaesthesiologicaScandinavica 1993; 37: 250-2.
5. Hamelberg W, Welch CM, Siddall J, Jacoby J. Complication of endotracheal intubation.J Am Med Assoc 1958;168:1959-62.
6. Jenson P J, Hommelgaard P, Sondergaard S. Sore throat after operation: Influence of tracheal intubation, intracuff

pressure and type of cuff. British Journal of Anaesthesia 1982; 54:453-7.
7. Loeser E A, Machin R, Colleg J, Orr D L, Benneth G M, Stanley T H. Postoperative sore throat: Importance of endotracheal tube conformity versus cuff design. Anesthesiology 1978; 49: 430-32.
8. Loeser E A, Stanley T H, Jordan W, Machin R. Postoperative sore throat: Influence of tracheal tube lubrication versus cuff design. Canadian Anesthetist's Society Journal 1980; 27: 156-8.
9. McHardy FC, Chang F. Postoperative sore throat : Cause, prevention and treatment. Anaesthesia 1999;54: 444-453.
10. Melby JC.Drug spotlight program:systemic corticosteroid therapy-pharmacology and endocrinologic considerations.Ann Intern Med 1974;81: 505-12.
11. Monroe MC, Gravenstein N, Saga-Rumbley S. Postoperative sore throat effect of orophrayngeal airway in orotracheally intubated patients. AnaesthAnalg 1990;70: 512-516.
12. Sang-Hyun Park, sung-Hee Han, Sang-Hwan Do, Jung-Won Kim, Ka-Young Rhee, Jin-Hee Kim.Prophylactic dexamethsone decreases the incidence of sore throat and hoarseness after tracheal extubation with a double lumen endobronchialtube.AnesthAnalg 2008;107: 1814
13. Schmelzeisen R, Frolich JC. Prevention of postoperative swelling and pain by dexamethasone after removal of impacted third molar teeth.Eur J Clin Pharmacol 1993;44: 275-7.
14. Siji Thomas, SuharaBeevi.Dexamethasone reduces severity of postoperative sore throat.Can J Anaesth 2007;54:11: 897-901.
15. Stanley T H, Loeser E A. Minimizing sore throat. Anesthesiology 1979; 51: 488-9.
16. Stenqvist O, Nilsson K. Postoperative sorethroat related to tracheal tube cuff design.CanAnaesth Soc J 1982;29: 384-6.
17. Stout D M, Bishop M J, Dwersteg J K, Cullen B F. Correlation of endotracheal tube size with sore throat and hoarseness following general anaesthesia. Anesthesiology 1987; 67: 419-21.
18. Stride P C. Postoperative sore throat: Topical hydrocortisone. Anaesthesia 1990; 45: 968-71
19. Wang JJ, Ho ST, Lee SC, Liu YH, Liao YC.Theprophylactic of dexamethasone on postoperative nausea and vomiting in women undergoing thyroidectomy: a comparison of droperidal with saline. Anesth Analg1999;89: 200-
20. Winkel E, Knudsen J. Effects on the incidence of postoperative sore throat of 1 percent cinchocaine jelly for endotracheal intubation. Anesthesia Analgesia 1971; 50: 92-4.

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Original Research Article

A clinical audit on cancellation of elective surgery on a scheduled day in a teaching institution

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Abstract

Background: Cancellation of surgery on the scheduled date poses physical, psychological and emotional disturbances not only to the operating surgeon, but it also includes the perioperative physician as well as the patients. The most common cause of cancellation of elective surgery on a scheduled day in a teaching institution includes lack of operating time in theatre. The reasons to quantify the cancellation list is usually vivid and difficult. **Aim:** The objective of this audit was to find out the prevalence and the reasons for cancellation of elective surgery on the scheduled day and to find out the clinical quality indicator with regard to effective theatre utilization time on a scheduled day. **Materials and Methods:** This retrospective analysis was included for one year duration in a tertiary teaching hospital. We coined the cancellation of the elective surgery as any proposed surgery which was listed and planned the previous day; and subsequently not done on the scheduled day of surgery. The source of audit was the documentation maintained in cancellation records in the anaesthesia department and these data were analyzed. The most common cause for cancellation of surgery was categorized as Patient related, Pre operative (or Surgical) preparation related and Facility related factors. Observations and **Results:** All the data were entered in the Microsoft excel spreadsheet and a Pareto chart was constructed to prioritize the reasons and analyzed using Epi info software version. Out of 10,306 patients during the scheduled period in our audit, 532 (5.16%) patients had cancellation on the elective list. **Conclusion:** Appropriate pre operative assessment, better inter departmental coordination, adequate optimization prior to surgery, improving man power availability and increasing the operation theatre utilization time help to avoid cancellation of surgery on the scheduled day.

Key Words: Cancellation, elective surgeries, scheduled day, teaching institution

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INTRODUCTION

Surgery is the primary modality of treatment in specified diseases and it requires a multi disciplinary team work approach in the operating room. Pre anaesthetic assessment, adequate preparation and optimization of the surgical patient prior to the elective surgery, avoids the major catastrophic events perioperatively and also decreases the morbidity and mortality of the surgical patients. Cancellation of surgery on the scheduled date poses physical, psychological and emotional disturbances not only to the operating surgeon, but it also includes the perioperative physician as well as the patients.[19] The prevalence and the cause for cancellation of elective surgery on a scheduled day is diverse and vary from one institution to another. The incidence of cancellation is between 10% and 40% among the booked elective list in various institutions[1,6,15,16,17]. The most common cause of cancellation of elective surgery on a scheduled day in a

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teaching institution includes lack of operating time in theatre[1,2,16,17,18] (running beyond the scheduled hours), poorly optimized surgical patients due to varied medical conditions or the administrative reasons[1,7,16,17,18]. Delayed start of elective list, unanticipated anaesthetic or surgical problems or shortages of the operating staff also contribute an pivotal role in cancellation[10]. The reasons to quantify the cancellation list is usually vivid and difficult. Hence the simplest approach is to evaluate the cause for cancellation of surgery and the specified issues needs to be focused preventing the postponement of the elective surgery.

AIM

The objective of this audit was

1. To find out the prevalence and the reasons for cancellation of elective surgery on the scheduled day and to focus on improving the quality of health care
2. To find out the clinical quality indicator with regard to effective theatre utilization time on a scheduled day and thereby offering better utilization of theatre services and resources

MATERIALS AND METHODS

This audit was a retrospective analysis and an observational study conducted in a NABH accredited, ISO certified 1300 bedded tertiary teaching hospital PSG Institute of Medical Sciences and Research, Coimbatore. The audit study period was included for one year duration from 1st October 2017 to 30th September 2018. The elective operating list scheduled for the next working day was planned by the concerned operating surgeon by 3 pm the day prior to operation and the list is printed and displayed in the department. The operating list included the complete elective surgery list on the scheduled day and the cancellation list were maintained in a separate registers along with a detailed note mentioning the details of cancellation. Our hospital comprises of 10 operating room in the main Operation Theatre block involving surgeries with various specialities like General surgery, laparoscopic surgery, obstetrics and gynecology, orthopedic surgery and neuro surgery on all days; with a separate theatre in labor ward and 2 cardiothoracic theatre functioning exclusively separately. Speciality surgeries like pediatric surgery, ENT, plastic surgery, hepato-biliary surgery, surgical gastroenterology, urology and transplant surgery with allocation for three days in a week for elective surgery. The scheduled elective surgery operating duration list was included between 8 am and 5 pm in our institution, with exceptions working on Sundays and public holidays. We coined the cancellation of the elective surgery as any proposed surgery which was listed and planned the previous day; and subsequently not done on the scheduled day of surgery. The source of audit was the documentation maintained in cancellation records in the anaesthesia department and these data were analyzed. The most common cause for cancellation of surgery was categorized as Patient related, Pre operative (or Surgical) preparation related and Facility related factors. All the data were entered in the Microsoft excel spreadsheet and a Pareto chart was constructed to prioritize the reasons and analyzed using Epi info software version. Although the audit was done for improving departmental work under quality assurance, this study received ethical committee approval (Ref No: PSG/IHEC/2018/Appr/Exp/207/ Proposal No.18/202 dated 13.08.2018).

OBSERVATIONS AND RESULTS

Table 1: Cancellation of cases Month wise from October 2017 to September 2018

Month	Number Of Cases (n)	Cancelled Cases (n)	Case Cancelled (%)
October 2017	775	52	6.70
November 2017	900	70	7.77
December 2017	851	50	5.87
January 2018	754	38	5.03
February 2018	740	35	4.72
March 2018	858	46	5.36
April 2018	829	37	4.46
May 2018	1,008	56	5.55
June 2018	911	28	3.07
July 2018	971	40	4.11
August 2018	829	33	3.98
September 2018	880	47	5.34
Total cases	**10,306**	**532**	**5.16**

Table 2 – Factors determining the cancellation of cases

Factors for cancellation of surgery	Cancelled cases (n)	Cancellation (%)
Patient related factors	125	23.49
Pre operative preparation related factors	265	49.81
Facility related factors	142	26.69

Table 3: Patient related factors for cancellation of surgery

S.No.	Patient related factors	Number of cancelled cases
1	Financial constraint	11
2	Unwillingness for surgery	66
3	AMA discharge	18
4	Patient not admitted to hospital	6
5	Wants surgery in future date	14
6	Unexplained death	1
7	Patient had food	3
8	Non availability of attendars	4
9	Turned pregnant	2

Table 4: Pre operative preparation related factors for cancellation of surgery

S.No.	Pre operative preparation related factors	Number of cancelled cases (n)
1	Pending specialised opinion	49
2	Abnormal laboratory reports	57
3	Inadequate optimisation	10
4	Active respiratory infection	20
5	Needed further pre operative evaluation	27
6	Baby fitness – Sterilisation procedure	23
7	Late laboratory reporting time	7
8	Wound infection	16
9	Improving on conservative management	16
10	Sudden detoriation of clinical condition with new onset signs and symptoms	40

Table 5: Facility related factors for cancellation of surgery

S.No.	Facility related factors	Number of cancelled cases
1	Pending insurance / scheme	77
2	Equipment / Implant unavailability	12
3	Non availability of OT	19
4	Incorrect / wrong list booking	2
5	Inadequate OT staff	2
6	Lack of time for parent unit cases	17
7	Academic purpose	2
8	Operating surgeon on leave	11

Table 6: Depicts the Operation Theatre Utilization Time during the working hours

Month	Sample Size	*OT Utilization Rate (%)											
		OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	Labour OT	CT OT
October 2017	775	55.5	65.6	18	43.2	49.5	45.7	30.6	53.8	47.3	64.2	11	44.2
November 2017	900	52.8	71.2	18	45.5	55.9	63.0	33.9	60.6	54.9	65.8	8	47
December 2017	851	50.6	68.3	35.5	42.4	51.3	66.3	20.4	56.7	46.8	65.3	9.4	37.8
January 2018	754	49.6	64.7	37	41.8	47.7	50	18.9	55.5	56.9	50.5	11.8	39.6
February 2018	740	64.6	70.6	17.8	51.5	51	60.8	24.4	50.6	43.8	59.4	8.9	48.8
March 2018	858	73.4	80	21.5	36.5	39.5	56.8	36	60.8	44.5	58.5	22.6	50
April 2018	829	65.5	82.8	17.6	37.1	46.4	55.6	28.6	60.7	46.8	51.9	16	42
May 2018	1,008	98.1	94.8	22.5	43.8	63.5	75.9	37.5	67.8	58.6	56.3	12.9	41.7
June 2018	911	97.1	98.6	17.7	56.2	46.5	61.9	29.8	66.8	53.9	63.1	14.7	49.5
July 2018	971	59.7	73.5	16.6	49.7	57.1	71.3	36.6	60.4	56.5	67.8	23.3	47.3
August 2018	829	58.2	60.6	21.6	55.4	50.0	62.7	25.9	53.2	48.2	54.5	14	41.3
September 2018	880	68.4	81.6	9.8	60.9	50.6	64.8	28.2	60.8	49.2	64.5	24.2	46

*OT Utilization Rate = OT Utilization Time in hours X 24 / Resource Hours X 100

DISCUSSION

The National Health Service (NHS), UK through its Modernization Agency Theater Program has defined case cancellation as those that occur after the patient has been notified of operation date[8]. Few define it as the procedures that were cancelled either on the day on which the surgery was scheduled, or cases which appeared in the definitive scheduled list but ultimately was not performed. Out of 10,306 patients during the scheduled period in our audit, 532 (5.16%) patients had cancellation on the elective list. Rakesh Garg *et al*[5,17] in a north Indian government hospital reported 30.3 % cancellation list followed by, Ezike *et al*[7] stated 25.3 % cancellations, Rajendra Kumar *et al*[16] in an government hospital showed 17.6 % cancellation, Abderrazak Sahraoui and Mohammed Elarref *et al*[2] observed 13.4 % and JimEnez *et al*[9] noted with 3.9 % as cancellation comparable to a study in a Jordanian hospital[11] with 3.6% cancellation on the elective list respectively. The cancellations at a teaching hospital in south india was found to be 4.4 % which was in accordance with our study (5.16%). In our audit, the frequency of cancellations was pronounced more in pre operative or surgical preparation related factors accounting for 265 patients (49.81%), followed by facility related factors in 142 patients (26.69%) and 125 patients (23.49%) in patient related factors respectively. On categorizing Surgical (or pre operative) preparation related factors which includes patients presenting with active respiratory infections, acute exacerbation of any medical conditions, electrolyte disturbances, uncontrolled blood sugar or blood pressure; the overall cancellation was maximum with abnormal laboratory values (21%) on the day of surgery. URI-related cancellations was reported by Palomero Rodriguez *et al*[13], however this decreased from 12.9% in 2001 to 7.2% in 2003 through enhanced communication of simple and appropriate information to parents resembling with 7.54% in our audit. Knox *et al* reported a significant reduction in medical-related surgical cancellations after the establishment of a preoperative assessment clinic (P = 0.013).[12] Patients unwillingness for surgery accounted for 53% with regard to patient related factors for cancellation of surgery. Patients not willing for surgery at the last moment prior to surgery were due to fear, unfavorable conditions existing in the family or unawareness about the importance of surgeries. Female patients who were menstruating on the day of surgery were also considered in our study. Study by Fayed *et al* showed that 9% of patients decided not to undergo elective surgery on the day of surgery[4]. Being a teaching medical institution, few reasons for lack of operating time such as teaching and training the junior residents and interns could not be avoided. There was no issues with administrative or logistic causes such as power failure in operating room, failure of air conditioners, availability of oxygen source, usage of anaesthetic drugs, availability of blood and blood products, suture materials, linen, supply of water, bed availability and hospital workers strike in our audit. Cancellation by Insurance sanctioning availed by government scheme and private insurance peaked maximum cause due to delayed clearance or failure of obtaining the insurance. In general, surgeons took longer than estimated time to finish the procedure and was especially true for less-experienced surgeons and trainees. Surgeons are sometimes too optimistic and aware of the long waiting lists, but they want to operate as many patients as possible. This reason can be avoided by a better internal organization of work as well as planned use of staff breaks and the adequate preoperative preparation of the patients. Patient non compliance regarding medication regimens. Schofield *et al*[18] reported bed availability and theatre time management as a main reason for cancellation; which was not an issue in our audit. Pandit JJ, Carry A[14] estimated that over-booking of operation list is the common cause for postponement due to waiting list pressures. Windokun *et al*[20] reported surgeon's un-availability as main reason. Dufek *et al*[3] recommended the improvement in protocol for pre-op patients' evaluation to address these problems. Most of the reasons for cancellation were debatable as an issue of health care quality and was avoidable by judicious scheduling of lists and careful planning by the team involved in surgeries. We justified with improving following recommendations in our institution :

- Calm, smooth, pleasant and noiseless pre operating assessment room can avoid missing major clinical findings
- Closed loop communication and good rapport with the patient helps to instill confidence preventing the patient's bias regarding the anesthesia and surgery
- Follow appropriate pre operative instructions to improve the health care system avoiding major issues on the day of surgery
- Alleviation of the psychological distress of cancelling the surgery
- Early start of the first case in the operating room
- Surgical patients were adequately counseled, optimized their medical condition, appropriate continuation of medications and obtained informed consent regarding all relevant procedures
- Inter departmental coordination in managing complicated and high risk cases
- Increased utility of operating time in the theatre complex during the working hours

- Following the medical ethics and to practice in accordance with the law

LIMITATIONS

Our study was related to inherent bias in categorizing the cancellation of cases due to varied reasons on behalf of multi factorial components leading to cancellation. The study was confined only to our institution and may not be standardized since the hospital policies varies in other teaching institutions.

CONCLUSION

Although cancellations were only 532 (5.16%) out of 10,306 patients of total elective surgeries, this can even be reduced by implementing and following the recommendations as per guidelines proposed, so that the potential causes can be avoided. To improve the better quality of life we recommend that the cancellations of surgeries can be reduced by improving pre operative assessment, requesting patient information on inter current illnesses between preadmission and surgery, better inter departmental coordination and adequate optimization prior to surgery, improving man power availability, availability of adequate resource sources and increasing the operation theatre utilization time to avoid cancellation of surgery on the scheduled day. Frequent conduct of audits, rectifying the pitfalls in the institution will definitely improve the better healthcare facility in the teaching hospital.

REFERENCES

1. Abderrazak Sahraoui and Mohamed Elarref. Bed crisis and elective surgery late cancellations: An approach using the theory of constraints. Qatar Med J.2014; 2014(1):1–11.
2. A. Gonzalez-Arevalo1, J. I. Gomez-Arnau, F. J. DelaCruz, J. M. Marzal, S. Ramirez, E. M. Corral and S. Garcia-del-Valle·Causes for cancellation of elective surgical procedures in a Spanish general hospital.Anaesthesia. 2009 May; 64(5): 487–493.
3. Dufek S, Gaucher E, Gialanella. The total quality process applied to the operating rooms and other clinical processes. Surgery. 1993; 113: 255-259.
4. Fayed A, Elkouny A, Zoughaibi N, Wahabi HA. Elective surgery cancellation on day of surgery: An endless dilemma. Saudi J Anestth. 2016 Jan-Mar; 68-73.
5. Garg R, Bhalotra AR, Bhadoria P, Gupta N, Anand R. Reasons for cancellation of cases on the day of surgery: A prospective study. Indian J Anaesth 2009; 53:35–9.
6. George Stavrou, Stavros Panidis, John Tsouskas, Georgia Tsaousi, and Katerina Kotzampassi Audit of Operating Room Time Utilization in a Teaching Hospital: Is There a Place for Improvement? ISRN Surg. 2014; 2014: 431740.
7. HA Ezike, VO Ajuzieogu, and AO Amucheazi Reasons for Elective Surgery Cancellation in a Referral Hospital.Ann Med Health Sci Res. 2011 Jul-Dec; 1(2): 197–202.
8. Ilango Ganesan, R. Anuradha, P. Ravindrakumar. "Audit of Cancellation of Elective Surgeries in a Teaching Hospital in South India". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 31, April 16; Page: 5322-5328, DOI:10.14260/jemds/2015/778
9. JimEnez A, Artigas C, Elia M, Casamayor C, Gracia J, Martinez M. Cancellation in ambulatory day surgery: An observational study. Ambul Surg. 2006; 12: 119-123.
10. J. J. Pandit, A. Carey. Estimating the duration of common elective operations: implications for operating list management. Anaesthesia. Aug 2006; 61(8): 768-776.
11. Kaddoum *et al*. Causes of cancellations on the day of surgery at a Tertiary Teaching Hospital. BMC Health Services Research (2016) 16:259 DOI 10.1186/s12913-016-1475-6
12. Knox M, Myers E, Hurley M. The impact of pre-operative assessment clinics on elective surgical case cancellations. Surgeon 2009; 7:76–8. doi: 10.1016/S1479-666X(09)80019-X.
13. Palomero Rodriguez MA, Sanabria Carretero P, Rodriguez Perez E, Goldman Tarlousky L, Nodal Ramos A, Diaz Gonzalez C. [Upper respiratory tract infections in children: Impact on surgical scheduling at a pediatric hospital]. Rev Esp Anestesiol Reanim 2005; 52:597–602.
14. Pandit JJ, Carey A. Estimating the duration of common elective operations: implications for operating list management. Anesthesia. 2006; 61: 768-776
15. PL Chalya, JM Gilyoma, JB Mabula, S Simbila, IH Ngayomela, AB Chandika, and W Mahalu. Incidence, causes and pattern of cancellation of Elective surgical operations in a University Teaching Hospital in the Lake Zone, Tanzania. Afr Health Sci. 2011 Sep; 11(3): 438–443.
16. Rajender Kumar and Ritika Gandhi. Reasons for cancellation of operation on the day of intended surgery in a multidisciplinary 500 bedded hospital. J Anaesthesiol Clin Pharmacol. 2012 Jan-Mar; 28(1): 66–69.
17. Rakesh Garg, Anju R Bhalotra, Poonam Bhadoria, Nishkarsh Gupta, and Raktima Anand. Reasons for Cancellation of Cases on the Day of Surgery–A Prospective Study. Indian J Anaesth. 2009 Feb; 53(1): 35–39.
18. Schofield WN, Rubin GL, Piza M, Lai YY, Sindhusake D, Fearnside MR, Klineberg PL. Cancellation of operations on the day of intended surgery at a major Australian referral hospital. Medical Journal of Australia 2005; 182(12):612–615.
19. Tait AR, Voepel-Lewis T, Munro HM, Gutstein HB, Reynolds PI. Cancellation of pediatric outpatient surgery: Economic and emotional implications for patients and their families. J Clin Anesth 1997; 9:213–19. doi: 10.1016/S0952-8180(97)00032-9.
20. Windokun A, Obideyi A. Audit of emergency theatre utilization. African Journal of Medicine and Medical Sciences2002; 31: 59-62.

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Original Research Article

Comparing the efficacy and haemodynamic response between intrathecal hyperbaric 0.5% bupivacaine with isobaric 0.75% ropivacaine using fentanyl as adjuvant in patients undergoing infra umbilical surgeries

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ABSTRACT

Background: Central neuraxial blockade is the most widely used form of regional anesthesia in surgeries involving abdominal, urological, obstetric, gynaecological and lower limb. The nerve blocking properties of the R and S-enantiomers were similar but that the S-enantiomer was less cardiotoxic. The aim of our study is to compare the efficacy and haemodynamic response between intrathecal hyperbaric 0.5% Bupivacaine with isobaric 0.75% Ropivacaine using Fentanyl as adjuvant in patients undergoing infra umbilical surgeries.

Materials and Methods: 60 patients of age group between 25 – 65 years belonging to American Society of Anaesthesiologist (ASA) 1 and 2 posted for general surgical, urological, orthopaedic and gynaecological procedures involving lower abdominal surgeries under spinal anaesthesia divided into two groups of 30 each and randomly allocated to one of the two below mentioned groups:
Bupivacaine group (Group B) n=30; Ropivacaine group (Group R) n=30

Observation and Results: Chi-square test and Student's 't' test were used to analyse the results. The sensory block in Bupivacaine group B was significantly higher compared to Ropivacaine group R attaining statistical significance. The 3 minutes motor block, incidence of hypotension was denser and higher in Bupivacaine group compared with Ropivacaine group. Incidence of bradycardia was not significantly different between the two groups. Although the onset of motor blockade was denser in Bupivacaine group, the total duration of motor blockade was similar between the groups.

Conclusion: We conclude from our study that 0.75% isobaric Ropivacaine produces similar duration of efficacy with stable haemodynamics, as compared with 0.5% hyperbaric Bupivacaine.

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1. Introduction

Central neuraxial blockade is the most widely used form of regional anesthesia in surgeries involving abdominal, urological, obstetric, gynaecological and lower limb. The advantages being well established, widely accepted, ease of subarachnoid puncture and studies suggest that spinal anaesthesia may be superior to general anesthesia. The endocrine - metabolic response to surgery is blunted when spinal anaesthesia is employed compared to the response during general anaesthesia (GA).[1]

Various local anaesthetic agents such as cocaine, procaine, etidocaine, tetracaine, lignocaine, bupivacaine and ropivacaine were tried for sub arachnoid blockade. Bupivacaine was marketed as a long acting local anaesthetic, its advantages compared to Lignocaine being long duration of action and differential sensory-motor block; but untoward adverse effects like arrhythmias, prolonged duration of sensory and motor blockade require a need to overcome these problems. Hyperbaric 5% Lidocaine has been reported to be associated with transient radicular irritation following single-dose of spinal anaesthesia and is not being used much now-a-days.

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In 1977, the propyl derivative of the pipecoloxylidides was less toxic than the butyl derivative (Bupivacaine). The nerve blocking properties of the R and S-enantiomers were similar but that the S-enantiomer was less cardiotoxic. Thus Ropivacaine a single (S) stereoisomer was chosen for further development.[2] Ropivacaine, structurally resembling Bupivacaine, is a relatively new amino-amide local anaesthetic agent, similar in chemical structure to Bupivacaine, having various advantages like early onset and shorter duration of action and having lesser cardio toxicity. Ropivacaine relieves the psychological distress of being immobile for a longer period of time after lower abdominal surgeries. In view of the above context, the present study was undertaken to compare these two drugs.

2. Aim

The aim of our study is to compare the efficacy and haemodynamic response between intrathecal hyperbaric 0.5% Bupivacaine with isobaric 0.75% Ropivacaine using Fentanyl as adjuvant in patients undergoing infra umbilical surgeries.

3. Materials and Methods

A study titled "Comparing the efficacy and haemodynamic response between intrathecal hyperbaric 0.5% Bupivacaine with isobaric 0.75% Ropivacaine using Fentanyl as adjuvant in patients undergoing infra umbilical surgeries" was done in PSG Institute of Medical Sciences and Research, Coimbatore, after obtaining institutional human ethical committee clearance and informed written consent from all the patients who participated in this study.

This is a randomized, single blind study where the person assessing the response (observer) was blinded to the group the patient belonged to and the person administering the spinal was aware of the drug he is administering. 60 patients of age group between 25 – 65 years belonging to ASA 1 & 2 posted for general surgical, urological, orthopaedic and gynaecological procedures involving lower abdominal surgeries under spinal anaesthesia were included in the study.

60 patients were divided into two groups of 30 each and randomly allocated to one of the two below mentioned groups as Bupivacaine group (Group B) n=30 and Ropivacaine group (Group R) n=30. The Inclusion criteria in our study includes ASA Grade 1 and 2 patients, age ranging from 25 to 65 years, procedures done under spinal anesthesia and patients posted for general surgery, urological, orthopaedic and gynaecological procedures expected to last not more than 3 hours. Exclusion criteria includes patient denial, absolute contraindication for regional anesthesia, inability to communicate with the patient, combined spinal and general anesthesia, pregnancy, patchy or failed spinal.

All patients were premedicated orally with Tablet Pantoprazole 40 mg and Tablet Alprazolam 0.25 mg the previous night and 2 hours prior to surgery. In the receiving room an 18G intravenous cannula was inserted and an infusion of Ringer's lactate solution started at 2ml/ kg/ hr. On arrival in the operation theatre Electocardiogram, Non invasive blood pressure and pulsoxymeter were connected as pre-induction monitors and recording of these parameters started after noting the baseline values. The patient was placed in lateral position, sterile painting and draping done and spinal puncture was made in L_3L_4 interspace with 26G Quinke spinal needle. After establishment of free flow of Cerebrospinal fluid, intrathecal administration of 2.6ml 0.75% Ropivacaine with Fentanyl 25 μg or 2.6ml 0.5% Bupivacaine with Fentanyl 25 μg was given over a period of 30 seconds as per the random allocation and patient was turned supine post spinal injection. No tilt was given to any patients.

The haemodynamic parameters were monitored. Hypotension when Systolic Blood Pressure recorded < 90mmHg was treated with Intravenous (IV) crystalloids and Vasopressors Injection Ephedrine or Mephentremine 6 mg IV bolus, as and when needed if there was persistent hypotension. Bradycardia was noted when the Heart rate was <50 beats per minute and treated with Injection Glycopyrrolate 0.02mg per kilogram IV bolus, as and when needed. The density and recovery of the motor blockade were monitored using Bromage scale and the height of sensory level was determined bilaterally using loss of sensation to pin prick.

Description of the bromage scale

Grade	Criteria	Degree of block
I	Free movement of legs and feet	Nil (0%)
II	Just able to flex knees with free movement of feet	Partial (33%)
III	Unable to flex knees, but with free movement of feet	Almost complete (66%)
IV	Unable to move legs or feet	Complete (100%)

Assessment of motor blockade was done three minutes after intrathecal injection, after completion of surgery, every 15 minutes till the patient completely recovers, during discharge in recovery room.

3.1. Statistical analysis

The observations and results were compiled and analysed by Chi-square test and Student's 't' test to see if there was any significant difference in the onset and density of motor blockade between the two groups. Haemodynamic parameters like hypotension, bradycardia, nausea, vomiting and ventricular arrhythmias (ectopics) were also compared and analysed statistically.

Qualitative variables like sex, ASA gradings, incidence of hypotension, bradycardia, arrhythmia, nausea and vomiting were compared between the two groups using Chi-square test and Quantitative variables like age, weight, height, duration of surgery, Bromage score and total duration of motor blockade between the two groups were compared by using Student's 't'test.

4. Observation and Results

Table 1 to Table 5.

5. Discussion

The first clinical report of spinal anaesthesia was made in the year 1899 by Dr August Bier, who described the intrathecal administration of cocaine.[3] The greatest challenge of the technique is to control the spread of the local anaesthetic through the cerebrospinal fluid (CSF) in order to produce a block that is adequate for the proposed surgery without producing a needless extensive spread.

Subarachnoid block is a commonly employed anaesthetic technique for performing lower abdominal and lower limb surgeries. It is a safe, inexpensive and easy-to-administer technique which also offers a high level of post–operative satisfaction with good pain relief to the patients. The technique is simple, has rapid onset and the risk of general anaesthesia including mishaps due to airway management are avoided by this technique.

The selection of the local anaesthetic to be used for spinal anaesthesia is usually based on the expected duration of surgery and need for early patient discharge. Though cardiotoxicity is not a concern in subarachnoid block, the quality of sensory blockade, motor blockade, hemodynamic changes and side effect profile are some considerations in selecting a drug for spinal anaesthesia.

The use of long acting agents is associated with a lower risk for transient neurologic dysfunction.[4–7] Bupivacaine is the local anaesthetic used routinely for surgeries because of its high potency and minimal neurological symptoms. The efficacy and safety of intrathecal administration of both plain and hyperbaric solutions of Ropivacaine have been evaluated in different clinical settings including orthopaedic,[8] urological surgery,[9] caesarean section and labour pain.[10,11]

Ropivacaine, a S-enantiomer of Bupivacaine is being increasingly used for spinal anaesthesia and the advantages claimed are shorter duration of motor block with similar sensory block properties and lesser cardiotoxic property compared to Bupivacaine; thus it minimizes the psychological discomfort of being immobile for a long time.

In our study, a single blinded clinical trial was undertaken to compare the duration and density of motor blockade and haemodynamic instability between intrathecal hyperbaric 0.5% Bupivacaine and isobaric 0.75% Ropivacaine using Fentanyl as adjuvant in 60 patients between the age group of 25 to 65 years of age either gender, belonging to ASA Grade I and II scheduled for general surgical, gynaecological and orthopaedic procedures (Infra umbilical surgeries).

Demographic data such as age, sex, weight and height between the two groups were comparable and there was no statistical significance since p value was > 0.05 between the two groups. The duration of surgery was 57.67±34.16 mins in Ropivacaine group and 48.17±19.49 mins in Bupivacaine group and p value 0.191 was found to be statistically insignificant.

In our study, maximum cephalad spread is assessed after 3 minutes of injection of local anaesthetic by using loss of sensation to pinprick T10 level. In Bupivacaine group (B) the mean sensory level was T4 to T6 (4.93± 0.828) which is higher when compared to Ropivacaine group (R) where the mean level was T5 to T8 (5.70± 1.055). This is in contrast with the study conducted by Jean-Marc Malinovsky et al[12] (Mean level for Bupivacaine: T7 & Ropivacaine: T9), Whiteside et al[13] (Mean level for Bupivacaine: T5 & Ropivacaine: T7). A study by Koltka k et al[14] reported spread of sensory block was higher in Bupivacaine than Ropivacaine. we infer that the maximum cephalad spread or height of sensory level was significantly lower in Ropivacaine group in out study.

The 3 minutes motor block was denser in Bupivacaine group than Ropivacaine group with statistical significance. The difference in motor blockade between both the group was statistically significant since p < 0.05 and this score was comparable with the study by Mantouvalou et al[15] & Erturk E et al[8] who reported onset of motor block was significantly faster in Bupivacaine group. The total duration of motor blockade was similar in both the groups and there was no statistical significant difference in the discharge time of the patient.

Miller C G et al[16] determined level of sensory (cold sensation) and motor blockade (Bromage) and measured heart rate, blood pressure and oxygen saturation. With the exception of maximum spread of sensory blockade, there was no difference with regard to demographics, sensory or motor blockade and hemodynamic variability.

Mc Namee DA et al[17] recorded the onset and duration of sensory block at dermatome level T10, maximum upper and lower spread of sensory block and the onset, intensity and duration of motor block. Onset of motor and sensory block was rapid with no significant differences between the two groups. But the median duration of complete motor block (modified Bromage Scale 3) was significantly shorter in the Ropivacaine group compared with the Bupivacaine group (220±22.67 versus 228±32.09 mins) in our study.

Y.Y.Lee et al[18] used Fentanyl 15 μg as an adjuvant in both Ropivacaine and Bupivacaine group and achieved sensory block to the T10 dermatome or higher at 15 min after intrathecal injection revealing the duration of complete

Table 1: Demographic details - Group B and Group R

Parameters	Group B	Group R	t- value	p value	Significance
Age					
Mean age in yrs ± S.D	38.37±10.50	37.7±11.91	0.230	0.819	Not significant
Sex distribution					
Male	18	17	0.069	1	Not significant
Female	12	13			
Weight	66.13±9.06	64.33±10.26	0.720	0.474	Not significant
Height	164.27±6.96	163.07±7.85	0.627	0.533	Not significant
ASA Grading					
I	21	24	0.800	0.0552	Not significant
II	9	6			

Table 2: Duration of surgery and height of sensory level between two groups

Parameters	Group	Values	t value	p value	Significance
Duration of Surgery Mean(mins)	B	48.17±19.49	1.323	0.191	Not significant
	R	57.67±34.16			
Height of sensory level (Mean thoracic level)	B	4.93±0.828	3.131	0.003	Statistically Significant
	R	5.70±1.055			

Table 3: Bromage score after 3 minutes in Group B and Group R

Bromage Score	Grade III	Grade IV	X^2	p value	Significance
Group B	6 (20%)	24 (80%)	26.786	0.000	Statistically Significant
Group R	26 (86.7%)	4 (13.3%)			

Table 4: Hemodynamic parameters – Group B and Group R

Parameters	Group B	Group R	X^2	p value	Significance
1. Incidence of hypotension					
Yes	17	7	6.994	0.017	Statistically Significant
No	13	23			
2. Bradycardia					
Yes	9	9	0.00	1	Not significant
No	21	21			
3. Nausea/Vomiting					
Yes	8	1	6.405	0.026	Statistically Significant
No	22	29			
4. Ventricular arrthymia					
Yes	2	2	2.069	0.492	Not significant
No	28	30			

Table 5: Total duration of motor blockade

Group	Mean (mins)	t value	p value	Significance
Group B	228±32.09	1.034	0.305	Not significant
Group R	220±27.67			

motor block shorter in the Ropivacaine group compared with the Bupivacaine group. But in our study we used 25 μg as an adjuvant and had similar results, which was also similar to Erturk et al[8] studies who had used 20 μg fentanyl with Ropivacaine or Bupivacaine.

Koltka K et al[14] performed his study in Ropivacaine and Bupivacaine with Fentanyl 50 μg to make 3 ml total spinal fluid volume. The primary outcome, the duration of motor block, was significantly shorter in the Ropivacaine group as compared with our study. No patient had pruritus, eczema, shivering, respiratory depression intraoperativelyNausea and Vomiting

5.1. Definition

Nausea is the sensation of being about to vomit. Vomiting, or emesis, is the expelling of undigested food through the mouth. ..... Click the link for more information. In our study, none of the patient had residual neurological deficit, post-dural puncture headache or transient neurological symptoms at the postoperative follow-up.

In our study the hypotension was a significant statistically in consistent with the study conducted by Lopez-soriano F et al,[5] Whiteside et al,[19] Mantouvalou et al[6] & Erturk E et al.[8] In their studies it is shown that there is increased incidence of hypotension and higher requirements of vasoactive drugs in Bupivacaine group than in Ropivacaine group of patients. This can be attributed to the higher cephalad spread of hyperbaric Bupivacaine causing more sympathetic blockade than isobaric Ropivacaine. Incidence of hypotension is higher in Bupivacaine group compared to Ropivacaine group.

The increased incidence of nausea and vomiting in group B can be explained due to higher incidence of hypotension and maximum cephalad spread. The study by Mantouvalou et al[6] showed occurrence of nausea and vomiting is equally distributed between the two groups, thereby not agreeing with our study.

In our study, the incidence of bradycardia is similar between two groups. 9 patients in each group had bradycardia and were treated with anti-cholinergics. This is in contrast with the study done by Kessler P et al,[12] Boztug N et al[2] and Koltka k et al[10] which reported no significant bradycardia between the two groups (Bupivacaine and Ropivacaine). Regarding intra-op arrhythmias only 2 patients in Bupivacaine group had ectopics and was not statistically significant.

In our study, total duration refers to the time after intra-thecal injection to the complete recovery of the patients from motor block. In Bupivacaine group, the mean duration was 228 minutes whereas in ropivacaine group it was 220 minutes. P value is 0.305 and is statistically insignificant. This is in complete agreement with the studies done by Gautier PE et al,[19] Liu et al,[20] Jean-Marc Malinovsky et al[7] andKessler P et al[12] compared isobaric Ropivacaine and Bupivacaine, which showed similar motor blockade between the two groups.

In our study, there was no failed spinal cases and there was no need for supplementation with general anesthesia.

6. Conclusion

We conclude from our study that 0.75% isobaric Ropivacaine produces similar duration of motor blockade with stable haemodynamics, as compared to 0.5% hyperbaric Bupivacaine producing denser motor blockade with hypotension when Fentanyl is used as adjuvant in both the groups.

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8. Conflict of Interest

None.

References

1. Urwin SC, Parker MJ, Griffiths R. General versus regional anaesthesia for hip fracture surgery: a meta-analysis of randomized trials. *Br J Anaesth*. 2000;84(4):450–5.
2. Boztuğ N, Bigat Z, Karsli B, Saykal N, Ertok E. Comparison of ropivacaine and bupivacaine for intrathecal anesthesia during outpatient arthroscopic surgery. *J Clin Anesth*. 2006;18(7):521–5.
3. Chung CJ, Choi SR, Yeo KH, Park HS, Lee SI, Chin YJ. Hyperbaric spinal ropivacaine for cesarean delivery: a comparison to hyperbaric bupivacaine. *Anesth Analg*. 2001;93:157–61.
4. Gonter AF, Kopacz DJ. Spinal 2-Chloroprocaine: A Comparison with Procaine in Volunteers. *Anesth Analg*. 2005;100(2):573–9.
5. Soriano FL, Lajarín B, Rivas F, Verdú JM, López-Robles J. Hyperbaric subarachnoid ropivacaine in ambulatory surgery: Comparative study with hyperbaric bupivacaine. *Rev Esp Anestesiol Reanim*. 2002;49(2):71–5.
6. Mantouvalou M, Ralli S, Arnaoutoglou H, Tziris G, Papadopoulos G. Spinal anesthesia: Comparison of plain ropivacaine, bupivacaine and levobupivacaine for lower abdominal surgery. *Acta Anaesth Belg*. 2008;59:65–71.
7. Malinovsky JM, Charles F, Kick O, Lepage JY, Malinge M, Cozian A, et al. Intrathecal Anesthesia: Ropivacaine Versus Bupivacaine. *Anesth Analg*. 2000;91(6):1457–60.
8. Soni AK, Erturk E. Clinical comparison of 12 mg ropivacaine and 8 mg bupivacaine, both with 20 microg fentanyl, in spinal anaesthesia for major orthopaedic surgery in geriatric patients. *Med Princ Pract*. 2010;19(2):142–7.
9. Khaw KS, Kee WDN, Wong M, Ng F, Lee A. Spinal Ropivacaine for Cesarean Delivery: A Comparison of Hyperbaric and Plain Solutions. *Anesth Analg*. 2002;94(3):680–5.
10. Koltka K, Uludag E, Senturk M, Yavru A, Karadeniz M, Sengul T, et al. Comparison of Equipotent Doses of Ropivacaine-fentanyl and Bupivacaine-fentanyl in Spinal Anaesthesia for Lower Abdominal Surgery. *Anaesth Intensive Care*. 2009;37(6):923–8.
11. Salazar F, Bogdanovich A, Adalia R, Chabás E, Gomar C. Transient neurologic symptoms after spinal anaesthesia using isobaric 2% mepivacaine and isobaric 2% lidocaine. *Acta Anaesthesiol Scand*. 2001;45(2):240–5.
12. Kessler P, Eichler A, Wilke HJ, Strouhal U, Bremerich D. Intrathecal ropivacaine vs. bupivacaine in lower abdominal gynaecological procedures. *Eur J Anaesthesio*. 2001;18(21):86.

13. Whiteside JB, Burke D, Wildsmith JAW. Comparison of ropivacaine 0.5% (in glucose 5%) with bupivacaine 0.5% (in glucose 8%) for spinal anaesthesia for elective surgery †. *Br J Anaesth*. 2003;90(3):304–8.
14. Truong HHL, Girard M, Drolet P, Grenier Y, Boucher C, Bergeron L. Spinal anesthesia: a comparison of procaine and lidocaine. *Can J Anaesth*. 2001;48(5):470–3.
15. Polley LS, Columb MO, Naughton NN, Wagner DS, van de Ven CJM. Relative Analgesic Potencies of Ropivacaine and Bupivacaine for Epidural Analgesia in Labor. *Anesthesiol*. 1999;90(4):944–50.
16. Soni AK, Miller CG, Pratt SD, Hess PE, Oriol NE, Sarna MC. Low dose intrathecal ropivacaine with or without sufentanil provides effective analgesia and does not impair motor strength during labour: a pilot study. *Can J Anaesth*. 2001;48(7):677–80.
17. Namee DAM, McClelland AM, Scott S, Milligan KR, Westman L, Gustafsson U. Spinal anaesthesia: Comparison of plain ropivacaine 5 mg ml(-1) with bupivacaine 5 mg ml(-1) for major orthopaedic surgery. *Br J Anaesth*. 2003;91(1):155–6.
18. Lee YY, Kee WDN, Muchhal K, Chan CK. Randomized double-blind comparison of ropivacaine-fentanyl and bupivacaine-fentanyl for spinal anaesthesia for urological surgery. *Acta Anaesthesiol Scand*. 2005;49(10):1477–82.
19. Gautier PE, Kock MD, Steenberge AV, Poth N, Lahaye-Goffart B, Fanard L, et al. Intrathecal Ropivacaine for Ambulatory Surgery. *Anesthesiol*. 1999;91(5):1239–45.
20. Liu SS, McDonald SB. Current Issues in Spinal Anesthesia. *Anesthesiol*. 2001;94(5):888–906.

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Original Research Article

Comparing the efficacy of tramadol, ketamine and dexmedetomidine in the prevention of intraoperative shivering in patients undergoing surgery under subarachnoid blockade

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ABSTRACT

Background: The most common nagging and inconveniencing complication of spinal anesthesia is intra operative shivering. Central neuraxial blockade blunts the thermoregulatory mechanism by restricting vasoconstriction, resulting in shifting of central heat to the periphery from the trunk. Various pharmacological and non pharmacological methods are used for the control of intraoperative shivering.
Aim: The aim of our study was to compare the efficacy of Tramadol, Ketamine and Dexmedetomidine in the prevention of intra operative shivering in patients undergoing surgeries under subarachnoid blockade.
Materials and Methods: 90 patients of age group between 20 to 65 years belonging to ASA 1 and 2 posted for elective surgery under spinal anaesthesia will participate in this study. The patients were randomly allocated into 3 groups of 30 each and were named as by computer generated random table number. Group T received Tramadol 0.5 mg/kg in 100 ml NS over 10 – 15 mins**;** Group K Ketamine 0.25 mg/kg in 100 ml NS over 10 – 15 mins and Group D Dexmedetomidine 0.5 μg/kg in 100 ml NS over 10 – 15 mins.
Observation and Results: Data were statistically analysed with the SPSS version 22.0 software using two-sided unpaired t-test and Chi-square test. A repeated measure of ANOVA was applied for the three groups to know the with-in subject variability in prevention of shivering following subarachnoid blockade and p value < 0.05 was considered to be significant.
Conclusion: The conclusion of our study is that Dexmedetomidine at the dose of 0.5 μg/kg decreases effectively in the prevention of intra operative shivering in patients undergoing surgery under subarachnoid blockade when compared with Tramadol and Ketamine.

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1. Introduction

Subarachnoid blockade or spinal anesthesia is the commonest regional anesthesia technique performed for surgeries involving lower abdomen, pelvis and lower limb extremities. The most common nagging and inconveniencing complication of spinal anesthesia is intra operative shivering which is very distressing experience causing physiological stress both to the patients as well as the perioperative physicians and operating surgeon.

Shivering is the involuntary recurrent muscle action of skeletal muscles, is a normal thermo regulatory mechanism and the reported occurence is wide varied ranging from 40 – 70 % from the literature.[1,2] Central neuraxial blockade blunts the thermoregulatory mechanism by restricting vasoconstriction, resulting in shifting of central heat to the periphery from the trunk (below the level of subarachnoid block).[3,4]

Hypothermia worsens in perioperative period insulting in major adverse cardiac events and it doubles to triples the requirement of oxygen consumption, rising the metabolic

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rate by 100 to 600%, thereby increasing the catecholamine production resulting in lactic acidosis, which is very detrimental in poor cardio respiratory reserve patients.[5] Shivering can produce motion artifacts in anaesthesia monitors showing erroneous values.[2]

Various pharmacological and non-pharmacological methods are dealt for the management of intraoperative shivering.[5] Pethidine have been used for many years as the treatment modality for shivering.[6] In our study, we have used Tramadol, Ketamine and Dexmedetomidine to study the effect of preventing the intra operative shivering in patients undergoing surgery under spinal anesthesia.

2. Aim and Objectives of the Study

The main aim is to compare the efficacy of Tramadol, Ketamine and Dexmedetomidine in the prevention of intra operative shivering in patients undergoing surgeries under subarachnoid blockade. This study also focuses on effects of drugs on intra operative haemodynamics, monitoring the temperature, comparing the level of sedation and other undesirable effects.

3. Materials and Methods

A study titled "Comparing the efficacy of Tramadol, Ketamine and Dexmedetomidine in the prevention of intraoperative shivering in patients undergoing surgery under subarachnoid blockade" was done in PSG Institute of Medical Sciences & Research, Coimbatore. This study was done after obtaining Institutional Human ethics committee clearance from the institution and informed written consent from all the patients who participated in the study. It is a prospective randomised control study and the sample size was calculated using 95% confidence interval and power of the study being 80%. The sample size was 30 in each group with allocation ratio being 1:1:1.

90 patients of age group between 20 to 65 years corresponding to ASA 1 and 2 posted for elective surgery under subarachnoid blockade will participate in this study. The patients were assigned at random with 30 participants in each group and were called as Group T receiving (Tramadol 0.5 mg/kg), Group K (Ketamine 0.25 mg/kg) and Group D (Dexmedetomidine 0.5μg/kg). Patients were randomly allocated to one of the three groups by computer generated random table number (numbered from 1 to 90) and the corresponding number was represented with the concerned group of the study drug. It is a double blinded study and the performer is also unaware of the group of the drug till the end of the trial.

Patients aged between 20 – 65 years belonging to ASA 1 and ASA 2 undergoing elective surgery under spinal anaesthesia < 150 minutes duration with surgeries involving lower abdomen, urological, gynaecological, orthopaedic and lower limb extremity was considered the inclusion criteria for our study. Patient denial, absolute contraindications for subarachnoid blockade, inability to communicate with the patient, combined spinal and general anaesthesia, known drug intolerance or proven allergic reactions to study drugs, baseline core temperature >37.5ºC or < 35.5ºC, blood transfusion during surgery, pregnancy and lactating mothers were excluded from the study.

Routine pre-operative assessment was done and the patients were kept nil per oral from 10 pm the day prior to surgery. Informed written consent was obtained. All the patients were pre-medicated with tablet Pantoprazole 40 mg and Alprazolam 0.25 mg orally the day prior to surgery at night and Pantoprazole 40 mg at 6 am on the morning of day of surgery. Patients were assigned individually to one among the three groups by computer generated random table number.

Group T: Tramadol (n=30) 0.5 mg/kg in 100 ml NS over 10 – 15 mins

Group K: Ketamine (n= 30) 0.25 mg/kg in 100 ml NS over 10 – 15 mins

Group D: Dexmedetomidine (n=30) 0.5 μg/kg in 100 ml NS over 10 – 15 mins

In the pre operative room, an 18G intravenous cannula was inserted and an infusion of Plasmalyte solution started at 2ml/ kg/ hr. On arrival in the operation theatre, pre induction monitors like ECG, NIBP and SpO_2 were connected and monitoring of these parameters started after noting the baseline values. The operating room ambient was continued at 25ºC. The patient was placed in right lateral posture and under standard universal precautions, sterile painting draping done. After skin infilteration with 2 ml of 2% Lignocaine, spinal puncture was attempted in L_3L_4 or L_4 L_5 interspace with 26G Quincke spinal needle. After establishment of free flow of CSF, intrathecal administration of 15mg 0.5% Bupivacaine Heavy was given over a period of 30 seconds as per the random allocation and patient was turned supine post spinal injection. No tilt was given to any patients. The study drug was infused by the blind observer. Oxygen delivered at a rate of 5L/min using Hudsons mask and injection Ondansetron 4 mg was supplemented to all patients. Surgery was commenced when the spinal blockade level reached T7/T8 and Patients were observed for 120 minutes or until the end of the surgery; considering the longer duration among them.

The haemodynamic parameters were monitored. Hypotension (Systolic BP <90mmHg and Diastolic BP < 50 mm Hg was treated with intravenous fluids ± vasopressors – Injection Ephedrine or Mephentremine 6 mg IV bolus SOS. Bradycardia (Heart rate <50/min) was treated with injection Glycopyrrolate 0.004 mg/kg IV bolus SOS. Intra operative temperature monitoring and level of sedation was also assessed. The patients who developed shivering intra operatively were supplemented with injection Pethidine 0.25 mg/kg as rescue drug.

Shivering was observed by a grading system as described by Wrench

Grade 0 : No shivering

Grade 1 : One or more of the following – Piloerection peripheral vasoconstriction peripheral cyanosis but without visible muscle activity.

Grade 2 : Visible muscle activity confined to one muscle group.

Grade 3 : Visible muscle activity in more than 1 muscle group.

Grade 4 : Gross muscle activity involving the whole body Sedation was assessed by a four point scale as per Filos et al.

Grade 1 : Awake and alert.

Grade 2 : Drowsy, responsive to verbal stimuli.

Grade 3 : Drowsy, arousable to physical stimuli.

Grade 4 : Unarousable.

Data were statistically analysed with the SPSS version 22.0 software using Chisquare and ANOVA. Baseline characteristics were presented as mean ± S.D. Two-sided unpaired t-test and Chi-square test was applied to analyze the data and p value less than 0.05 were considered as significant. A repeated measure of ANOVA was applied for the three groups to know the with-in subject variability in prevention of shivering following subarachnoid blockade and $p < 0.05$ was considered to be significant.

4. Result and Discussion

Homeothermic mammals maintain a uniform isothermal characteristics internally and the central temperature varies between 36.5℃ and 37.5℃ in humans. Anterior hypothalamus incorporates the thermal inputs from various tissues of the body and differentiates peripheral information with a set point to reach or approach the temperature lower than this set point; resulting in responses to warm the body, while higher temperatures will initiate reflexes to cool the body.[2]

Autonomic thermoregulation is attenuated during central neuraxial blockade resulting in decrease in central or core temperature. Central neuraxial blockade decrease the shivering and vasoconstriction thresholds cephalad to the level of the neuraxial block by 0.6^0C. The concept of postanaesthesia shivering resembling like seizures or tremors remains still unproven. The postulates suggest that the decrease in reflex response in the descending spinal reflexes due to central neuraxial blockade could be the cause for tremors.[3] The two variants of tremor patterns noted could be either a tonic pattern resembling normal shivering with 4 – 8 cycle/min waxing and waning model or a phasic pattern with 5 to 7 Hz shattering pattern similar to pathologic clonus response. The net effect of this tonic and phasic patterns are due to thermoregulatory responses superseding with drop in temperature and arteriovenous shunt vasoconstriction.[7]

The consequences of hypothermia and shivering includes reversible coagulopathy, increased blood loss necessating blood transfusion, impaired wound healing, delayed drug metabolism, altered mental status, cardiac arrhythmias and ischaemia, increased risk of infection, myocardial oxygen consumption and basal metabolic rate.[4]

Treatment of shivering includes both non pharmacological methods like use of forced air warmers, warmed blankets, warmed intravenous fluids, use of plastic sheets and surgical drapes and maintaining warmed humidified operating room; and pharmacological methods that includes usage of drugs like pethidine, tramadol, meperidine, clonidine, ketamine, magnesium sulphate, dexmedetomidine, alfentanyl and nefopam.

In our study, extremities of population like paediatric, pregnancy and geriatric patients were not chosen due to variable confounding factors in relation to their age. Demographic profile in all the groups are comparable but statistically insignificant.

The primary objective in our study was about the drugs in causing decreased incidence of shivering in the desired study group population.[8] Dexmedetomidine had better prevelance in decreased incidence of intra operative shivering compared to Tramadol and Ketamine as only one patient had shivering at 30 minutes following subarachnoid blockade in close equivalent with Lim fern et al[9] studies with similar outcome. 6 patients at 15 minutes and 11 patients at 30 minutes in Tramadol group had the highest incidence of shivering with shivering grade more than two and showed statistical significance with p value 0.045 and 0.003 respectively. Only one patient in Ketamine group had shivering at 30 mins. Mittal et al[10] in his study compared dexmedetomidine with tramadol supplementing the drug after the onset of shivering and concluded that both drugs were equally efficacious and dexmedetomidine had a faster onset of action. Bozgeyik et al[11] used dexmedetomidine and tramadol compared with a placebo and commented both drugs were equally similar in the prevention of shivering. Dhimar et al.[12] in his study used Tramadol 100 mg in all patients which showed variation in their results while comparing with our study. Though the shivering was seen in all the groups at 45 and 60 minutes, with varying grades statistically it was insignificant. Statistical insignificance was noted at 90 and 105 mins since none of the patient had shivering. Prophylactic low dose Ketamine was found to be effective in preventing postoperative shivering in Gecaj-Gashi et al[13] and similar results were seen in our study also. Yong shin et al[14] in their study used 0.75 with 1 μg/kg and found to be effective against 0.5 μg/kg in our study. Genopadhyay et al.[15] concluded that pethidine, tramadol and ketamine effectively prevent shivering following spinal anaesthesia, prove ketamine was smart and effective alternative over two drugs due to the better haemodynamic stability and less adverse effect.

Table 1: Demographic data of all three groups

Parameters	Group T	Group K	Group D	p value	Significance
Age					
Mean age in yrs ± S.D	39.97±9.84	40.80±9.81	38.70±9.64	0.715	Statistically insignificant
Sex distribution					
Male	21 (23.3%)	27 (30%)	25 (27.8%)	0.131	Statistically insignificant
Female	9 (10%)	3 (3.3%)	5 (5.5%)		
Weight (kg)	65.24±9.86	66.13±9.06	64.33±10.26	0.720	Statistically insignificant
Height (cm)	162.8±7.04	167.57±6.59	163.33±6.99	0.533	Statistically insignificant
ASA Grading					
I	23(76.7%)	23(76.7%)	24(80%)	0.938	Statistically insignificant
II	7 (23.3%)	7 (23.3%)	6 (20%)		

Table 2: Incidence of shivering between three groups

Time (mins)	Group T	Group K	Group D	p value	Significance
Baseline	0	0	0	0	N.S*
5	0	0	0	0	N.S
10	0	0	0	0	N.S
15	6	0	0	0.045	H.S£
30	11	2	1	0.003	H.S
45	3	0	1	0.384	N.S
60	1	0	0	0.364	N.S
75	1	1	0	0.403	N.S
90	0	0	0	0	N.S
105	0	0	0	0	N.S
120	0	1	0	0.364	N.S

* - N.S - Nil significant
£- H.S - Highly significant

Table 3: Incidence of sedation between three groups

Time (mins)	Group T	Group K	Group D	p value	Significance
Baseline	0	0	0	0	N.S
5	0	0	25	0.000	H.S
10	0	0	27	0.000	H.S
15	5	17	28	0.000	H.S
30	13	22	29	0.004	H.S
45	14	21	29	0.008	H.S
60	15	15	26	0.000	H.S
75	11	11	24	0.000	H.S
90	7	6	22	0.000	H.S
105	0	1	13	0.000	H.S
120	0	0	0	0	N.S

Dal et al,[16] in their study found that the number of patients shivering on arrival in the recovery room, and at 10 and 20 min after operation were significantly less in Groups P (pethidine 20 mg) and K (ketamine 0.5 mg kg) than in Group S (normal saline) and concluded that use of Prophylactic low-dose ketamine was very useful in preventing postoperative shivering.

The secondary objective of the study was sedation[17] and all our used study group drugs can produce drowsiness to a certain extent; hence none of the study population were supplemented with sedatives, hypnotics or anxiolytics. Dexmedetomidine showed good sedation score of 3 within 5 minutes in majority of the population comfortably lasting for about 90 minutes without producing haemodynamic disturbances comparing with other two drugs. Initiation of sedation was slow in onset, peaking around 15 minutes for a sedation score of 2 in 40% in Tramadol group. Ketamine had a sedation score superior to tramadol, almost similar

Table 4: Mean axillary temperature (ºC) between groups

Time (mins)	Group T	Group K	Group D	p value	Significance
Baseline	36.21	36.29	36.29	0.130	N.S
5	36.18	36.15	36.15	0.671	N.S
10	36.21	36.22	36.19	0.759	N.S
15	36.03	36.10	35.98	0.560	N.S
30	36.09	36.03	36.03	0.637	N.S
45	36.13	36.15	36.18	0.727	N.S
60	36.21	36.13	36.19	0.367	N.S
75	36.12	36.22	36.28	0.098	N.S
90	36.20	36.34	36.34	0.092	N.S
105	36.31	36.28	36.28	0.856	N.S
120	36.40	36.39	36.34	0.516	N.S

Table 5: Rescue drug usage between groups

Rescue	Group T	Group K	Group D	p value	Significance
Yes	12	3	1	0.001	Highly significant
No	18	27	29		

to dexmedetomidine with peak effect within 15 minutes producing good sedation score but lacked in awakening state. Mittal et al[10] in his studies concluded sedation due to dexmedetomidine provides additional comfort to the patient. Bozgeyik et al[11] also reported similar results of sedation within 5 minutes about dexmedetomidine in his study. Usta B et al[18] showed higher sedation scores in dexmedetomidine group providing conveniencing sedation for the patient. Dexmedetomidine sedated population followed oral commands and maintained in a tranquil state as seen in Elvan et al[19] studies. Bradycardia was reported in 3 patients and hypotension in 2 patients only in dexmedetomidine group necessating conservative treatment but proved statistically insignificant similar to Usta B et al.[17] There was no incidence of respiratory depression, nausea, vomiting and headache in all the three groups. No statistical significance was noted in the axillary temperatures measurement in all the 3 groups.

The limitations noted in our study are ideal Dexmedetomidine dose needs to be evaluated to produce negligible haemodynamic instability.[20] Tramadol used at 0.5mg/kg seemed to be inconclusive necessating the work up of optimal dose to prevent shivering.[21] Dosage of 0.25mg/kg Ketamine as studied by Kose et al.[22] was used in our study and needs reconsideration.

To summarise Dexmedetomidine was more effective in the prevention of shivering when compared with Ketamine and Tramadol with an added advantage of adequate reliable sedation and stable haemodynamics without any detrimental adverse effects.

5. Conclusion

The conclusion of our study is that Dexmedetomidine at the dose of 0.5 μg/kg decreases effectively in the prevention of intra operative shivering in patients undergoing surgery under subarachnoid blockade when compared with Tramadol and Ketamine.

6. Source of Funding

None.

7. Conflict of Interest

The author declares no conflict of interest.

References

1. Koay CK, Chan WY, Chin MK. Shivering during regional anaesthesia and its control with pethidine. *Singapore Med J*. 1991;32(3):160–2.
2. Buggy DJ, Crossley AW. Thermoregulation, mild perioperative hypothermia and postanaesthetic shivering. *Br J Anaesth*. 2000;84(5):615–28.
3. Azhagappan C. A study of Dexmedetomidine, Tramadol and Pethidine in the prevention of intraoperative shivering in patients undergoing surgery under spinal anaesthesia. Chennai; 2016.
4. Giesbrecht GG, Sessler DI, Mekjavic IB, Schroeder M, Bristow GW. Treatment of immersion hypothermia by direct body-tobody contact. *J Appl Physiol*. 1994;76:2373–9.
5. Shreyavathi R, Kavitha AK, Raghavendra RSR, Bhaskara B. Comparison of Intravenous Ketamine, Clonidine or Dexmedetomidine Prior to Subarachnoid Blockade for Control of Shivering. *J Evol Med Dent Sci*. 2014;3(45):11021–7.
6. Mohan M, Kumari N, Tyagi A, Sethi AK, Agarwal D, Singh M, et al. Tramadol for prevention of postanaesthetic shivering: A randomised double-blind comparison with pethidine. *Anaesthesia*. 2009;64:141–6.
7. Rosenberg H, Clofine R, Bialik O. Neurologic changes during awakening from anesthesia. *Anesthesiology*. 1981;54(2):125–30.
8. Azam M, Asad N, Butt TA, Ahmad W. Efficacy of Prophylactic Intravenous Ketamine Vs Tramadol for Prevention of Intraoperative Shivering in Spinal Anesthesia for Patient undergoing cesarean section. *Pak J Med Health Sci*. 2018;12:455–8.
9. Fern L, Misiran K. Comparison of dexmedetomidine, pethidine and tramadol in the treatment of post-neuraxial anaesthesia shivering. *South Afr J Anaesth Analg*. 2015;21(1):21–6.

10. Mittal G, Gupta K, Katyal S, Kaushal S. Randomised double-blind comparative study of dexmedetomidine and tramadolfor post-spinal anaesthesia shivering. *Indian J Anaesth*. 2014;58:257–62.
11. Bozgeyik S, Mizrak A, Kilic E, Yendi F, Ugur B. The Effects of Preemptive Tramadol and Dexmedetomidine on Shivering During Arthroscopy. *Saudi J Anaesth*. 2014;8(2):238–43.
12. Dhimar A, Patel M, Swadia V. Tramadol for control of shivering (Comparison with pethidine). *Indian J Anaesth*. 2007;51(1):28–31.
13. Gecaj-Gashi A, Hashimi M, Sada F, Salihu S, Terziqi H. Prophylactic ketamine reduces incidence of postanaesthetic shivering. *Niger J Med*. 2010;19(3):267–70.
14. Kim YS, Kim Y, Seo KH, Kang HR. Optimal Dose of Prophylactic Dexmedetomidine for Preventing Postoperative Shivering. *Int J Med Sci*. 2013;10(10):1327–32.
15. Genopadhyay S, Gupta K, Acharjee S, Nayak SK, Dawn S, Piplai G. Ketamine, tramadol and pethidine in prophylaxis of shivering during spinal anaesthesia. *J Anaesthesiol Clin Pharmacol*. 2010;26:59–63.
16. Dal D, Kose A, Honca M, Akinci SB, Basgul E, Aypar U. Efficacy of prophylactic ketamine in preventing postoperative shivering. *Br J Anaesth*. 2005;95:189–92.
17. Bajwa SJ, Gupta S, Kaur J, Singh A, Parmar S. Reduction in the incidence of shivering with perioperative dexmedetomidine: A randomized prospective study. *J Anaesthesiol Clin Pharmacol*. 2012;28:86–91.
18. Usta B, Gozdemir M, Demircioglu RI, Muslu B, Sert H, Yaldiz A. Dexmedetomidine for the prevention of shiveringduring spinal anesthesia. *Clinics (Sao Paulo)*. 2011;66(7):1187–91.
19. Elvan EG, Qc B, Uzun S. Dexmedetomidine and postoperative shivering in patients undergoing elective abdominal hysterectomy. *Eur JAnaesthesiol*. 2008;25:357–64.
20. Mathews S, Mulla A, Varghese A, Radim PK, Mumtaz K, S. Postanaesthetic shivering-a new look at tramadol. *Anaesth*. 2002;57(4):387–403.
21. Chaturvedi S, Domkondwar G. Control of shivering under regional anaesthesia using Tramadol. *Asian Arch Anaesthesiol Resusc*. 2002;57:491–6.
22. Kose EA, Dal D, Akinci SB, Saricaoglu F, Aypar U. The efficacy of ketamine for the treatment of postoperative shivering. *Anesth Analg*. 2008;106:120–2.

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Comparative evaluation of single level paravertebral block versus caudal block for postoperative analgesia in pediatric inguinal surgery

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ABSTRACT

Background and Aims: To compare and evaluate the efficacy of Single Level Paravertebral Block (SLPVB) with Caudal Block (CB) using 0.2% ropivacaine. **Materials and Methods:** A prospective study was performed for 40 children aged 2-8 years, undergoing paediatric inguinal hernia repair. They were divided into 2 groups, of 20 patients each, and SLPVB or CB was administered. They were followed up for 24hours and assessed for efficacy of the block in terms of duration of analgesia and quality of block as assessed by FLACC score. The amount of rescue analgesia used, parent satisfaction, and complications if any were also noted. The data collected was analyzed using SPSS software version 17 and a P value of <0.05 was considered significant and appropriate tests were applied. **Results:** The mean duration of action was found to be 1001.25±564.34 minutes (Median 1440 and IQR 975 minutes) in CB and 1440 minutes (Median 1440 IQR 0) in the PVB. This was statistically significant (P value = 0.03). The efficacy of the block as assessed by the FLACC score was comparable in the two groups. Rescue analgesia was required in 8 patients in the CB as compared to none in the PVB. The parent satisfaction levels were also significantly higher with PVB as compared to CB. No complications were noted with PVB. Failure of block occurred in 1 patient with CB, and there was complaint of urinary retention in 3 patients in the same group. **Conclusion:** SLPVB has been demonstrated to be superior to CB to provide postoperative analgesia children undergoing inguinal hernia surgery.

Key words: Analgesia, Caudal, Inguinal, Lumbar, Paravertebral, Ropivacaine

INTRODUCTION

Introduction of regional anaesthetic techniques into modern pediatric anaesthesia has revolutionized pain management. Regional anaesthesia also reduces general anaesthesia requirement, thereby reducing the potential risks associated with deeper planes of anaesthesia[1].

Surgeries through inguinal approach like orchidopexy, orchidectomy and especially inguinal hernia repair are fairly common surgeries in paediatric population. This type of surgery can cause significant discomfort postoperatively and CB is often used to provide pain relief[2]. However single shot CB has the disadvantage of short duration of action with more children needing supplementary analgesia in the immediate postoperative period.

Paravertebral block has been used for postoperative analgesia in children since1992. It provides excellent pain relief following both continuous and multi-level nerve stimulator – guided techniques[4]. Through this study we evaluated if a single level, single injection paravertebral block can provide longer duration of analgesia with lesser requirement to supplement analgesia than single shot caudal block for children undergoing inguinal surgery.

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This study compared the effect of caudal block versus SLPVB using 0.2% Ropivacaine following inguinal surgery in children in terms of postoperative analgesia and complications encountered during the study.

MATERIALS AND METHODS

This prospective randomized study was conducted in a tertiary care Centre and Institutional ethics committee clearance was obtained.

Forty Children of the age group of 2 years to 8 years, posted for inguinal hernia repair surgery were enrolled for the study over a period of 1 year. Written informed consent was taken from the parents of the children.

Inclusion Criterion included children who were American Society of Anaesthesiologists (ASA) grade 1 and posted for inguinal hernia surgery. Children with history of allergy to local anaesthetics, coagulation abnormality or bleeding disorders or with infection at the site of the block or any abnormality or disease of the spine were excluded from the study.

A detailed pre anaesthetic check up was carried out in all the cases.

Patients were randomly allocated by computer generated random tables into two groups of 20 patients each namely, group P, where SLPVB was administered and group C, where caudal block was administered.

Before the surgery, premedication was done with oral midazolam (0.5mg/kg). Patient was taken up in the operative room and standard monitoring devices (electrocardiogram, pulse oximetry and non invasive arterial blood pressure) were attached to the patient. Anaesthesia was induced with 6-8 % sevoflurane by facemask and maintained with N2O:O2 50:50 in 0.5 to 2.5% sevoflurane. After securing intravenous access injection fentanyl (2mcg/kg) was administered and appropriate size Proseal LMA was inserted according to the age of the patient and connected to Mapleson F modification of Ayre's T piece. No neuromuscular blocking drugs were given and the spontaneous ventilation was assisted as required. Fluid administration was as per standard protocol.

All patients in group C received CB. As per the technique mentioned by Suresh et. al[5], with the child in lateral position, flexion of hips was done. Sacral cornua was located either by palpating near the cephalad margin of the gluteal crease or by palpating the posterior superior iliac spine and taking the line between them as one side of an equilateral triangle. The depression inferior to the cornua, that is, the sacral hiatus was then identified and caudal needle inserted at an angle of 45 degrees. While advancing the needle a decrease in resistance to needle insertion was felt as it pierced the sacrococcygeal membrane. At this point 0.2% ropivacaine (1ml/kg) was given after aspiration.

Patients in-group P were given PVB as per the technique described by Berta et al[6]. With the patient in lateral decubitus position, and under all aseptic precautions, needle insertion was made at an easily palpable second lumbar vertebrae (L2) lateral to the spinal process with the distance from the midline being about 1-2cm as per the formula given by Lonnqvist et. al[7],. A 19 gauge epidural needle was inserted perpendicular to the skin in all planes until contact with the transverse process was made. The needle was then withdrawn to the subcutaneous tissue and angled to walk off the cranial edge of the transverse process. The needle was then advanced under the transverse process and the paravertebral space identified by loss of resistance to air. After aspiration, a bolus of 0.2ml/kg ofropivacaine (0.2%) was injected.

After completion of the surgery, 100% oxygen was administered and proseal removed. In both the groups, assessment was done at 30mins, 1hour, 1hour 30mins, 2, 4, 8, 12 and 24 hours in postoperative ward by an independent observer who was blinded to which group the patient belonged.

Complications were noted and compared in the above two groups.

Efficacy of the block: Assessed by duration of analgesia and FLACC SCORE Face, Legs, Activity, Cry and Consolability (FLACC) score[8] was used to evaluate the effectiveness of the block (supplementary Table 1).

Table 1: Age and weight distribution in the two groups (C = caudal group, P = paravertebral group)

Groups	Mean Age (years)	Mean Weight(kg)
Group C	5.60 ±2.46	17.15±7.15
Group P	4.90± 2.13	15.50±4.84
C VS P (P value)	0.171(NS)	0.199(NS)

Rescue Analgesia: The time and required dose of rescue analgesia was noted and compared. If a FLACC score >2 were recorded, the child was first managed by non-pharmacological means (tactile stimulation, change of position, warming etc). If no response was noted within 5-10 minutes, intravenous tramadol (1mg/kg) was administered. Patients were also attended whenever the child experienced pain even if that was not the assessment time of the study and analgesia was provided as per the protocol. If rescue opioid was needed within the first two postoperative hours, the block was considered a failure.

Parental satisfaction to treatment given was evaluated based on the child's comfort and activity level. Parents were asked to rate their degree of satisfaction and scored as very satisfied (3), acceptably satisfied (2), somewhat satisfied (1) or dissatisfied (score 0).

Any events like bladder hesitancy, postural hypotension, prolonged motor blockade leading to delayed ambulation, epidural spread or any other of the rare complications like nerve injury, intravascular injectionand pneumothorax were noted.

Researchers have recorded the mean duration of analgesia in C group as 4.52±2.02 hours as compared to 21.67±5.61 hours in the P group[3,9,10]. Assuming these as reference values, the minimum required sample size at 5% level of significance and 95% power is 3 patients in each group. We have taken 20 patients in each group considering the influx of patients in our hospital and the duration of study.

For quantitative data (pain score, parent satisfaction score), for comparison b/w the two groups, difference between the two means was observed by t-test for normally distributed data and Mann Whitney test (non parametric) for non normal distributed data.

Chi square or Fischer test was used for computing consumption of analgesics and other categorical variables.

RESULTS

A total of 40 patients were enrolled, 20 in each group and all the patients were included and analyzed.

The data collected was analyzed using SPSS statistical software version 17 and p-value<0.05was considered statistically significant. A total of 40 patients were studied with 20 patients in each group. The incidence of inguinal hernia was found to be more in males as compared to females and more common on the right side as compared to the left.

All the subjects were ASA grade 1. The two groups were comparable in terms of age and weight (Table 1).

The mean duration of action, as determined by the time to rescue analgesia was found to be 1001.25 ±564.34 minutes (Median 1440 and IQR 975 minutes) and1440 minutes (Median 1440) in the paravertebral group (Patients were followed up only for 24hrs) and this difference was found to be statistically significant (P value = 0.03) by Mann Whitney U test.

The efficacy of the block as assessed by the FLACC score was comparable in the two groups (Table 2).

Rescue analgesia was required in 8 patients in the caudal group as compared to none in the paravertebral group. This was statistically significant (P = 0.003). Rescue was provided with inj. Tramadol 1mg/kg i.v. None of the patients required rescue analgesia for more than once in 24 hrs.

In PVB group, 90% parents were completely satisfied with the postoperative comfort of their children compared to

Table 2: Comparison of FLACC score between the two groups at different time intervals

FLACC score median (IQR)	30min	1hr	1.5hr	2hr	4hr	8hr	12hr	24hr
C	0 (1.5)	0 (0)	0 (0)	1 (1)	1 (1)	1 (1)	0 (0)	0 (0)
P	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0.5 (0.5)	0 (0)
C vs. P (p-value)	0.18	0.41	0.50	0.20	0.44	0.09	0.49	0.99

Group C: caudal epidural block group; Group P: paravertebral block group

only 45% in the CB group (Table 3). This was statistically significant (P = 0.011)

Table 3: Comparison of parental satisfaction score between the two groups

Parent satisfaction score	C		P	
	Frequency	%	Frequency	%
0 (dissatisfied)	0	0	0	0
1 (somewhat satisfied)	4	20	0	0
2 (acceptably satisfied)	7	35	2	10
3 (very satisfied)	9	45	18	90
Total	20	100	20	100
Mean±S.D	2.25±0.79		2.90±0.31	
p-value	0.01			

No complications were noted in the paravertebral group. Failure of block occurred in 1 patient in the caudal group, and there was complaint of urinary retention in 3 patients in the same group. No other complications with the local anaesthetics or blocks were noted.

DISCUSSION

Inguinal hernia repair is the most common surgery performed worldwide and has the potential of being performed on an outpatient basis. The spectrum of regional anaesthetic techniques extend from CB and lumbar epidurals[1] to various other techniques like combined ilioinguinaliliohypogastric[4] nerve block, field blocks to wound infiltration with local anaesthetic agents in children.

CB is an established technique for providing postoperative analgesia. The technique of CB is easy to perform, however large volumes of drug are required and there is always a risk motor paralysis, subarachnoid block, urinary retention and failure of block even in experienced hands.

This prospective study was undertaken to compare CB and PVB with respect to the effectiveness and duration of block and the complications of the two techniques. The results shows that caudal as well as PVB are effective in decreasing postoperative pain. We found that there was a significant increase in the duration of postoperative analgesia in the group treated with PVB compared to those undergoing caudal. All this was achieved with a dose of local anaesthetic (0.2ml/kg) that was one fifth of the dose of the local anaesthetic used in the caudal group (1ml/kg). Needless to say this would also translate into a lesser incidence of side effects and risk of toxicity associated with local anaesthetics.

Though the technique of PVB requires some expertise it was noted that the location of the transverse process very well corroborated with the formula given by Berta and Lonnqvist et al6, thus making the technique easiest to master. Besides none of the 20 patients had a block failure. In our study none of the patients in the paravertebral group required supplemental analgesia. The mean duration of analgesia (as described by the time to rescue analgesia) was noted to be 1440 minutes i.e., 24 hours in the paravertebral group as compared to 1001.25±564.34 minutes in the caudal group. We found that majority of the children had maximum pain in the first 24hrs following which they were discharged with oral analgesic medication. Therefore, a technique, which can provide analgesia for 24hours without any need for supplemental analgesia, is certainly more desirable.

This finding has been corroborated by previous studies by Tug et. al[3], who gave PVB in children and found the mean duration of action in the paravertebral group to be 1300 minutes. Akcaboy et al reported that PVB obtained very long and effective analgesia compared with spinal block in adult patients[10]. A comparison study between PVB (using 2%lidocaine) and field block (using 0.5% bupivacaine and 1%lidocaine) by Wassef et al in children also showed that the PVB had a higher success rate in terms of frequency of pain relative to surgical manipulation of spermatic cord, hernia sac and also in terms of supplementing analgesia using local anaesthetics[2]. Various other studies by Naja et al and Berta et al in children have also reported the effect of PVB to last from 12-48 hours[4,5]. These results support our findings. It was claimed that this results from the relative a vascularity of the paravertebral space and hence the slow uptake of local anaesthetics. Division of the spinal nerves into smaller branches in the paravertebral space also enables better penetration of the local anaesthetic[5].

We did not encounter any complications with PVB in our study. However, we had failure of block in one patient in the CB group and 3 patients also complained of urinary retention. Because of the inherent nature of the PVB and the lesser amount of drug required, there is negligible risk of damage to the spinal cord, lower limb weakness, urinary retention and widespread sympathetic blockade.

Previously, multiple level paravertebral blocks had been used to provide adequate blockade[5]. Since the psoas muscle enveloped each lumbar nerve within the paravertebral space, it was considered essential to block each lumbar segment individually to achieve sufficient blockade. However, cadaveric studies have reported that if the dye was injected at L1 level, it was observed that the genitofemoral, iioinguinal, iliohypogastric and lateral cutaneous nerves were stained with dye[3]. This may explain

why the PVB obtained in our study was very effective. Besides, multiple segments PVB also increase the risk of pleural puncture and pneumothorax.

Further research can compare the effectiveness of the block using Ultrasound guidance and the inability to do that is also one of the limitations of our study.

To conclude this study has demonstrated that a single level, single shot PVB has been found to be superior in providing postoperative analgesia in terms of duration of analgesia, quality of analgesia and need for rescue analgesics as compared to CB in children undergoing inguinal hernia repair. It has also been noted to have lesser complications and higher parent satisfaction.

REFERENCES

1. Yaster M, Maxwell LG. Paediatric regional anaesthesia. Anesthesiology. 1989; 70:324–38. crossref PMid:2643896
2. Wassef MR, Randazzo T, Ward W. The paravertebral nerve root block for inguinal herniorraphy- A comparison with field block approach. Reg Anesth Pain Med. 1998; 23:451–6. crossref
3. Tug R, Ozcengiz D, Gunes Y. Single level paravertebral versus caudal block in paediatric inguinal surgery. Anaesth Intensive Care. 2011; 39:909–13. PMid:21970138
4. Naja ZM, Raf M , El Rajab M, Ziade FM, Al-Tannir MA, Lonnqvist PA. A comparison of nerve stimulator guided paravertebral block and ilio inguinal nerve block for analgesia after inguinal herniorrhaphy in children. Anaesthesia. 2006; 61:1064–68. crossref PMid:17042844
5. Suresh S, Wheeler M. Practical paediatric regional anaesthesia. Anesthesiol Clin North America. 2002; 20:83–113. crossref
6. Berta E, Spanhel J ,Gabrhelik T, Lonnqvist PA. Paravertebral block in children. Tech Reg Anesth Pain Manag. 2007; 11:247–54. crossref
7. Lönnqvist PA, Hesser U. Location of the paravertebral space in children and adolescents in relation to surface anatomy assessed by computed tomography. Pediatr Anaesth. 1992; 285–9.
8. Merkel SI, Voepel-Lewis T, Shayevitz JR, Malviya S. The FLACC: A behavioural scale for scoring post operative pain in younger children. Pediatr Nurs. 1997; 23:293–7 PMid:9220806
9. Ivani G, Lampugnani E, Torre M, Calevo MG, DeNegri P, Borrometi F, et al. Comparison of ropivacaine with bupivacaine for paediatric caudal block. Br J Anaesth. 1998; 81:247–8. crossref PMid:9813532
10. Akcaboy EY, Akcaboy ZN, Gougus N. Ambulatory inguinal herniorraphy: Paravertebral block versus spinal anaesthesia. Minerva Anestesiol. 2009; 75:684–91. PMid:19940820

Safety and efficacy of multiple site thoracic paravertebral nerve block vs. modified pectoral nerve block for postoperative analgesia after modified radical mastectomies-a randomised controlled trial

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[illegible]d 12th March, 2018 [illegible]d 1st May, 2018

Abstract

This study compares the safety and efficacy of multiple level thoracic paravertebral block and modified Pecs block for postoperative pain relief after Modified Radical Mastectomies.

After Institutional Review Board [IRB] and Human Ethics Committee [HEC approval, the study was conducted on 41 patients who were scheduled to undergo modified radical mastectomy. Written informed consent was taken. Female patients in the age group of 18-75 years who were ASA grade I and II were randomly allocated to two groups to either receive Ultrasound guided Thoracic paravertebral block TPVB at T2 and T4 levels (Group 2) or an ultrasound guided modified PECS block (Group 1) preoperatively in the block room.

Paravertebral block was given at two vertebral levels T2 (10 ml) and T4 level (10 ml) under ultrasound guidance utilising the parasagital in-plane technique. Modified PEC block was administered to patients in the PEC group.10 ml of 0.5% Ropivacaine for Pec1 and 20ml for Pec 2. In all patients surgery was done under GA. Pain was assessed with Numerical Rating Scale (NRS) on a 0 to 10 scale, for 24 hours after surgery, at 0,0.5,1,1.5,2,4,6,12 and 24 hours after surgery by a blinded observer.

Rescue opioid and Paracetamol requirement was recorded. The patients were also monitored for any specific block related complications.

Primary outcome measures were the numerical rating scores for pain and rescue analgesic requirement. Secondary outcome measures was looking for any block related complications. Demographic categorical variables were expressed in frequency and percentage, and the continuous variables were reported in terms of mean and standard deviation. The comparison of pain scores between the thoracic paravertebral nerve block and modified pectoral nerve block was done using Mann-Whitney U test. Mean duration of analgesia and mean 24 hour Morphine, Paracetamol consumption was compared using Student's t test.

There was no statistically significant difference between the two groups with respect to the pain scores at 0,0.5,1,1.5,2,4,6,12,and 24 hours at rest or with movement of the ipsilateral arm. The 24 hour Morphine consumption was not also statistically significant. [pec gp,mean 2.17, SD 1.09, tpvb gp mean 2 SD 0.77, p value 0.752.] Paracetamol consumption in the initial 24 hours after surgery was also not statistically significant between the two groups (pec gp. 36.8% and tpvb gp. 50% patients received paracetamol with a p value of 0.523). We found both the blocks, modified Pecs and multiple level paravertebral block were equally effective in treating postoperative pain.

[illegible]rd Modified pectoral nerve block, Multiple level thoracic paravertebral block, Postoperative analgesia, Modified radical mastectomy.

Introduction

The age adjusted rate and mortality of breast cancer among Indian females is 25.8 and 12.7 per 100,000 women respectively.[11] Modified radical mastectomy, can cause considerable pain in the immediate postoperative period and also has the potential to cause chronic pain[1]. Adequate pain control in the acute postoperative period is of paramount importance for patients' wellbeing, shorter hospital stay and also to prevent chronic pain due to the fact that inadequately treated postoperative pain is an important predictor of chronic pain.[2,3] Many regional anaesthesia options are now available for the anaesthesiologist to choose from for breast surgeries. A proper understanding of the nerve supply and analgesic requirements for various breast surgical procedures is necessary to choose the correct regional technique. In our institution, a tertiary care cancer hospital in South India an average of six breast surgeries are done daily of which 3 to 4 will be modified radical mastectomies. These patients are offered General Anaesthesia with a peripheral nerve block, either paravertebral block or PEC block or local infiltration.

Thoracic paravertebral block (TPVB) has been well researched and has been found to provide not only excellent analgesia in breast surgeries but also has less severe chronic pain and thereby good physical and mental health related quality of life after surgery.[4-7] Pectoral nerve block is an ultrasound guided interfascial plane block as described by Blanco in 2011 for postoperative pain relief in breast surgeries.[8-10] Recently a few studies have compared the safety and efficacy of the two blocks. It was observed that modified Pecs block provides superior postoperative analgesia than

TPVB in radical mastectomies with axillary clearance. (Wahba and Kamal 2013; Kulhari S et al 2016). Our study compares the safety and efficacy of multiple level thoracic paravertebral block and modified Pecs block for postoperative pain relief after Modified Radical Mastectomies.

Materials and Methods

After Institutional Review Board (IRB.No.06/2017/03) and Human Ethics committee (HEC No.14/2017) approval the trial was registered at clinical trials registry of India (CTRI/2018/02/011757 Principal investigator: Dr. Viji S. Pillai Date of registration: 07/02/2018.).The study was conducted on 41 patients who were scheduled to undergo modified radical mastectomy after obtaining written informed consent. Female patients in the age group of 18-75 years who were ASA grade I and II were randomly allocated to two groups to either receive or an ultrasound guided modified PECS block (Group 1) or Ultrasound guided multiple level Thoracic paravertebral block at T2 and T4 levels (Group 2) preoperatively in the block room. Patients were randomised into two groups with computer generated random numbers in sealed opaque envelopes which were opened before administering the blocks. Patients with preexisting local site infection, coagulopathy, morbid obesity (BMI>35), allergy to local anaesthetic were excluded from the study. Patients with other major organ system dysfunction were also excluded from the study. Patients were reviewed in the preoperative day in the ward by one of the investigators. Procedure and Numerical Rating Score is then explained to the patient and informed consent is obtained. All the patients were kept nil per oral for 6 hours before procedure. They received Alprazolam 0.25-0.5mg on the night before and Pantoprazole 40mg on the morning of surgery. Prior to the block patient was monitored using standard ECG, SpO2 and NIBP measurements. After securing an intravenous line they received premedication with 1mg of Midazolam and Fentanyl 30 microgram.

Modified PEC block was administered to patients in the group 1. Under strict aseptic precautions, patient in supine position the probe is placed below the lateral part of the clavicle, similar to the probe position for infraclavicular brachial plexus block and then moving inferolaterally. After recognition of the appropriate anatomical structures, the skin puncture point is infiltrated with 2% lignocaine, then the block is performed by using a 22-gauge Quinke's needle. The needle is advanced to the tissue plane between the pectoralis major and pectoralis minor muscle at the vicinity of the pectoral branch of the acromiothoracic artery, and 10 mL of 0.5% ropivacaine deposited. In a similar manner, 20 mL is deposited at the level of the third rib between the pectoralis minor and Serratus Anterior muscle. Thoracic Paravertebral block with Ropivacaine (0.5%) was given to patients in group 1. Procedure was done with patient in sitting position under strict aseptic precautions. Paravertebral block was given at two vertebral levels T2 (10 ml) and T4 level (10 ml) under ultrasound guidance utilising the parasagital in plane technique. Pleural displacement on injection of the drug is taken as confirmation for correct drug deposition.

After administering the blocks the patient's vitals, heart rate, blood pressure, SpO2 will be monitored continuously. Response to pinprick sensations in the thoracic dermatomes T1 – T6 at 20 minutes and 30 minutes after the block was noted.

Patients in both the groups underwent surgery with general anaesthesia standardized according to our centre's current clinical practice. General Anaesthesia was induced with Fentanyl 2microgm/kg, Xylocard 1.5mg/kg IV, Propofol (titrated to verbal response), Vecuronium 0.1mg/kg for muscle relaxation and trachea intubated with appropriate sized ETT. Anaesthesia maintained with Sevoflorane and Oxygen, Nitrous oxide mixture with 1 MAC., FiO2 0.4 and ventilation titrated to maintain ETCO2 35 - 40mmHg, with low fresh gas flows. Fentanyl 0.5 microgram/kg bolus will be given if the mean blood pressure or heart rate exceeded 20% of the baseline recordings, repeated once more if it does not return to baseline values after 10 minutes. After surgery patients were reversed with Neostigmine 2.5mg and Glycopyrrolate 0.4mg and extubated after good respiratory efforts and stable vitals. Patients were then shifted to the recovery room.

Post-operative pain was assessed in the recovery room and periodically after discharge to the ward until 24 hours postoperatively by another observer who was blinded regarding the block performed. Pain was assessed with Numerical Rating Scale (NRS) on a 0 to 10 scale, where 0 means no pain and 10stands for the maximum pain ever experienced for 24 hours after surgery, at 0,0.5,1,1.5,2,6,12 and 24 hours after surgery.

Morphine 1.5mg bolus was given to treat postoperative pain. Paracetamol 1gm IV was given if pain did not subside after two boluses of Morphine. Rescue opioid and Paracetamol requirement was recorded. The patients were also monitored for any specific block related complications like local anaesthetic toxicity, pleural puncture, vascular puncture, pneumothorax and intrathecal spread of the drug.

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Sample size was calculated with Master 2.0 sample size software. Sample size calculation was done based on the 24 hour postoperative Morphine consumption from a previous study by Kulhari et al.

Data was entered in Microsoft Excel sheet. Normality of data tested with P-P probability plot and histogram. Demographic categorical variables were expressed in frequency and percentage, and the continuous variables were reported in terms of mean

and standard deviation. The comparison of pain scores between the thoracic paravertebral nerve block and modified pectoral nerve block was done using Mann-Whitney U test. Mean duration of analgesia and mean 24 hour Morphine, Paracetamol consumption was compared using Student's t test.

Results

Both groups were comparable with respect to age, body mass index, ASA status and the duration of surgery.The post block haemodynamic status remained stable throughout the period of monitoring. (Table 1)

There was no statistically significant difference between the two groups with respect to the pain scores at 0,0.5,1,1.5,2,4,6,12, and 24 hours at rest or with movement of the ipsilateral arm. (Table 2, 3) The 24 hour Morphine consumption was not also statistically significant. [pec gp, mean 2.17, SD 1.09, tpvb gp mean 2 SD 0.77, p value 0.752.] Paracetamol consumption in the initial 24 hours after surgery was also not statistically significant between the two groups (pec gp. 36.8% and tpvb gp. 50% patients received paracetamol with a p value of 0.523). (Table 4)

Time (in hours) to first analgesic was 5.667 (8.84) in the Pecs group and 2.985 (3.55) in the paravertebral group with a p value of 0.335 which was not significant. (Table 5)

There was a significant difference between two groups at the level of T2 dermatome block with 31.6% blocked in the PEC gp. and 80%% in Tpvb with a p value of 0.006.

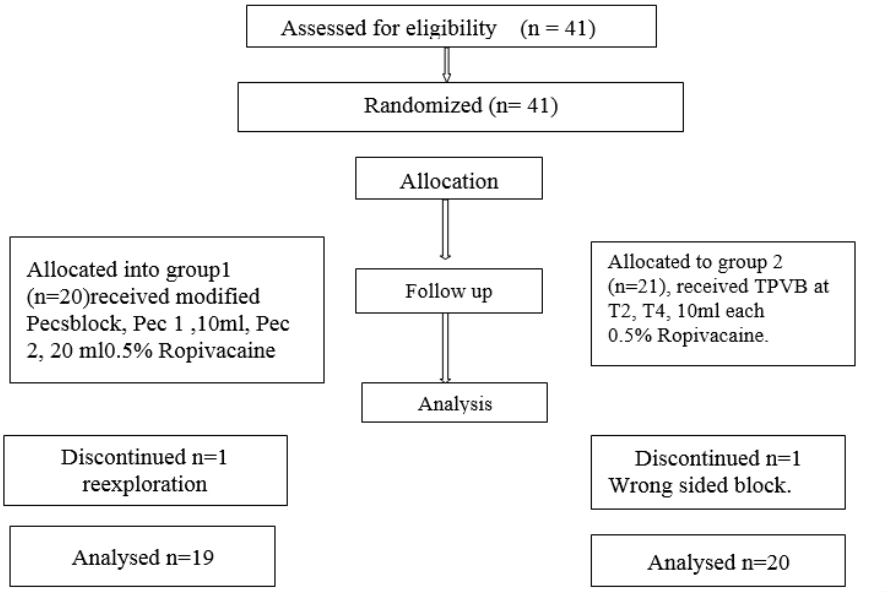


Table 1: Demographic variables comparison Student's t test

Age	1	19	49.42	10.09	0.347
	2	20	52.40	9.46	
BMI	1	19	24.37	3.43	0.425
	2	20	25.19	2.92	

Table 2: Mean VAS scores at rest in the two groups

	0	0.5	1	1.5	2	4	6	12	24
	0.58	1	1.11	1.42	1.42	1.89	1.89	1.68	1.68
	0.9	1.15	1.3	1.4	1.55	1.55	1.95	1.65	1.85

Table 3: Mean VAS scores on movement in the two groups

	0	0.5	1	1.5	2	4	6	12	24
	1	1.74	1.53	2.26	2.11	2.58	2.68	2.37	2.47
	0.9	1.35	1.65	2.05	1.85	2	2.3	2.2	2.4

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			□□□□□□□□□□	□ □□□□□		
Block	1	Count	12	7	19	
		% within Block	63.2%	36.8%	100.0%	
	2	Count	10	10	20	0.523
		% within Block	50.0%	50.0%	100.0%	
Total		Count	22	17	39	
		% within Block	56.4%	43.6%	100.0%	

□

□□□□□□: **Student's t** □□□□□□□r□□□ □□□□1□□□□□□□□□r□□□□□□□2 □□□□r□□ □r□□□□□□□□□□□□ □□□□□□

□r□□□□		□□	M□□□□	□d□□ □□□□□□□□□	□□□□□□□□
Time to first analgesic_hrs	1	12	5.667	8.84	0.335
	2	12	2.958	3.55	
Mor _mg	1	9	2.17	1.09	0.752
	2	6	2.00	.77	

□

Forty one patients were recruited into the study of which one patient in the Pecs group had reexploration and was later excluded from the study. One patient in the Tpvb group had wrong sided block who underwent surgery under general anaesthesia, postoperative pain managed with, Morphine and NSAIDS. Thus there were twenty patients in the paravertebral group and 19 in the PEC group.

Discussion

Axillary dissection and nerve injury may play a major role in causing chronic pain after breast cancer surgery.[12-15] Nerve injury can cause central sensitization and trigger the development of chronic pain. Since nerve blocks are capable of preventing central sensitisation we feel the blockade of intercostobrachial nerve, the main nerve supplying the axilla plays a major role in decreasing the incidence of chronic pain after axillary dissection. ICBN is almost always handled by the surgeon and suffers varying degrees of damage.[38] Hence we designed our technique of paravertebral block at two levels T2 and T4 with 10 ml of Ropivacaine at each level to assure adequate block of the intercostobrachial nerve.

The breast receives its innervation from the lateral and anterior cutaneous branches of the second to the sixth intercostal nerves and from the supraclavicular nerves. Axilla is mainly supplied by the intercostobrachial nerve which in turn communicates with other intercostal nerves T1, T3 and T4, and also with nerves from brachial plexus.[20-22] The pectoral muscle forms the anterior axillary wall which is supplied by the medial and the lateral pectoral nerves, serratus anterior muscle supplied by long thoracic nerve forms part of the medial wall and lattissimusdorsi supplied by thoracodorsal forms part of the posterior wall.

The fascia covering the pectoralis major muscle is removed during modified radical mastectomy.[1,40] This contributes to the postoperative pain which can be alleviated by the pectoral nerve blocks. In reconstructive breast surgeries with subpectoral prosthesis, there can be considerable postoperative pain due to pectoralis muscle stretching, for which the Pecs 1 block was originally described.[8,9,25] The pectoral muscles, serratus anterior and lattisimusdorsi are retracted during axillary dissection which can cause intraoperative pain.[1] The long thoracic nerve and thoracodorsal nerve may suffer injury during axillary dissection which can cause motor weakness of the muscles supplied by them.[40] The contribution of these nerves to postoperative pain after axillary dissection has yet to be understood. Sacrificing the intercostobrachial nerve may be required for oncological clearance during surgery which in turn can cause varying degrees of sensory loss in the axilla and medial side of arm.[16,40]

A few previous studies have evaluated the efficacy of PEC and TPVB for surgeries of breast including axillary dissection. Kulhari S[11]et al in their study comparing PEC and paravertebral block found that the PEC block was superior to paravertebral block with respect to the duration of analgesia, 24 hour morphine consumption and postoperative pain scores in the initial two hours after surgery. Wahba et al[12] in a similar study comparing these two blocks had less opioid consumption at 24 hours, longer time to first analgesic with the Pecs group. They had lower pain scores on movement at initial 12 hours and higher pain scores at 18 and 24 hours with Pecsblock. Another randomised double blind trial comparing Pecsblock, TPVB, and local infiltration by KarthikSyal et al had lower pain scores and longer duration of analgesia with the paravertebral block group.[23] Here the blocks were given after surgery under GA before extubation.

It is quite interesting to note that in all these studies the paravertebral block was given at T4 level as a single site injection without a catheter. The authors point out this single site injection of thoracic paravertebral block as a drawback for adequate spread of the local

anaesthetic to the higher dermatomal levels owing to the fact that spread of drug can be variable with this technique. Richardson et al[30] in their study found that 1.5 mg/kg bupivacaine 0.5% produced sensory loss at the level of injection with a mean superior spread of 1.4 (range, 0–4) dermatomes and a mean inferior spread of 2.8 (range, 0–7) dermatomes. Thoracic paravertebral anesthesia does not appear to be gravity-dependent, but there is a tendency for preferential caudal spread of somatic and sympathetic blockade.[26,31-34] Eason and Wyatt[27] found that at least four intercostal spaces could be covered by a single 15-ml injection of 0.375% bupivacaine. Single site injection at the T4 level may not always anaesthetise the cephalad dermatomes, especially at the T2 level. This is more important when there is axillary dissection since it involves much of tissue handling and has major contribution to postoperative pain. The ICBN is a thick nerve compared to the other intercostals and needs to be blocked separately when axillary clearance is done.

Hetta DF[35] et al, in their study on Pectoralis-serratusinterfascial plane block vs thoracic paravertebral block for unilateral radical mastectomy with axillary evacuation concluded that Pectoralis-serratus interfascial plane block was safe and easy to perform and decreased intensity of postmastectomy pain, but it was inferior to thoracic paravertebral block. Kasimahanti R.[36] et al in their study on Ultrasound-guided single vs double-level thoracic paravertebral block for postoperative analgesia in total mastectomy with axillary clearance concludes that patients receiving double-level TPVB had significantly less 24-hour analgesic consumption in the postoperative period than those in the single-level TPVB group. They attribute this could be due to significantly greater number of segments blocked in the double-level TPVB group. This is similar to our study outcome.

The results of the study by Sopena-Zubiria LA[37] et al showed that the combination of the two techniques was associated with a better post-operative analgesic control immediately after surgery: pain level assessed with VAS was significantly lower in the group treated with the combination of pectoral and thoracic paravertebral block 8 hours but not 24 hours after surgery. Combination of the two techniques reported a reduced need for intra-operative sedation.

We had a wrong sided block in one patient in the paravertebral block group who was excluded from the study. Surgery was done under general anaesthesia ad postoperative pain managed with Morphine and Paracetamol. Being a para median block extra precautions has to be exercised to prevent this.

We had similar experience as that of Bakshi et al,[39] with surgeons having difficulty to use electrocautery during axillary dissection in the Modified Pecs group. This was due to the presence of local anaesthetic drug in the tissue planes and they had to use higher currents or resort to bipolar cautery.

Modified Pecs block has the advantage of simple and safe technique with ultrasound whereas paravertebral block is an advanced level block and demands more procedural expertise. Single level paravertebral blocks may not adequately cover all dermatomes always as the spread of local anaesthetic is variable. So in our study we compared paravertebral block at two levels with modified Pecs block and found both techniques had comparable outcomes with respect to numerical rating scales, time to first analgesic request and rescue analgesic consumption.

Conclusion

We found both the blocks, modified Pecs and multiple level paravertebral block equally effective in treating postoperative pain without any statistically significant difference between the two groups. Trials with large sample sizes may bring out differences. We feel that blocking the intercostobrachial nerve with a dedicated T2 level paravertebral block has an important role in perioperative analgesia in breast surgeries with axillary dissection since it is the major nerve supply to axilla and also because it is implicated in chronic pain.

References

1. Woodworth GE, Ivie RMJ, Nelson SM, Walker CM, Maniker RB. Perioperative Breast Analgesia: A Qualitative Review of Anatomy and Regional Techniques. *Reg Anesth Pain Med.* 2017;42(5):609-631.
2. Fassoulaki A, Melemeni A, Staikou C, Triga A, Sarantopoulos C. Acute postoperative pain predicts chronic pain and long-term analgesic requirements after breast surgery for cancer. *Acta Anaesthesiol Belg*. 2008;59:241–248.
3. Bokhari FN, McMillan DE, McClement S, Daeninck PJ. Pilot study of a survey to identify the prevalence of and risk factors for chronic neuropathic pain following breast cancer surgery. *Oncol Nurs Forum*. 2012;39:E141–E149.
4. Karmakar MK, Samy W, Li JW, Lee A, Chan WC, Chen PP, Ho AM. Thoracic paravertebral block and its effects on chronic pain and health-related quality of life after modified radical mastectomy. *Reg Anesth Pain Med.* 2014;39(4):289-98.
5. Wu J, Buggy D, Fleischmann E, Parra-Sanchez I, Treschan T, Kurz A, Mascha EJ, Sessler DI. Thoracic paravertebral regional anesthesia improves analgesia after breast cancer surgery: a randomized controlled multicentre clinical trial. *Can J Anaesth.* 2015;62(3):241-51.
6. Pérez-González O, Cuéllar-Guzmán LF, Soliz J, Cata JP. Impact of Regional Anesthesia on Recurrence, Metastasis, and Immune Response in Breast Cancer Surgery: A Systematic Review of the Literature. *Reg Anesth Pain Med.* 2017;42(6):751-756.
7. Klein SM, Bergh A, Steele SM, Georgiade GS, GreengrassRA.Thoracic paravertebral block for breast surgery. *Anesth Analg.* 2000;90:1402-5.
8. Blanco R. The 'pecs block': a novel technique for providing analgesia after breast surgery. *Anaesthesia.* 2011;66(9):847-8.
9. Blanco R, Fajardo M, Parras Maldonado T. Ultrasound description of Pecs II (modified Pecs I): a novel approach to breast surgery. *Rev Esp Anestesiol Reanim.* 2012;59(9):470-5.

10. Chakraborty A, Khemka R, Datta T. Ultrasound-guided truncal blocks: A new frontier in regional anaesthesia. *Indian J Anaesth.* 2016;60:703-11.
11. Malvia, S., Bagadi, S. A., Dubey, U. S. and Saxena, S. Epidemiology of breast cancer in Indian women. *Asia-Pac J Clin Oncol.* 2017;13:289–295.
12. Andersen KG, Duriaud HM, Jensen HE, Kroman N, Kehlet H. Predictive factors for the development of persistent pain after breast cancer surgery. *Pain*. 2015;156:2413–2422.
13. Wallace MS, Wallace AM, Lee J, Dobke MK. Pain after breast surgery: a survey of 282 women. *Pain*. 1996;66:195–205.
14. E. L. Poleshuck, J. Katz, C. H. Andrus. Risk factors for chronic pain following breast cancer surgery: a prospective study. *Journal of Pain*, 2006;7(9):626–634.
15. R. Gärtner, M. Jensen, J. Nielsen, M. Ewertz, N. Kroman, and H. Kehlet. Prevalence of and factors associated with persistent pain following breast cancer surgery. *JAMA—Journal of the American Medical Association*. 2009;302(18):1985–1992.
16. Torresan RZ, Cabello C, Conde DM, Brenelli HB. Impact of the preservation of the intercostobrachial nerve in axillary lymphadenectomy due to breast cancer. *Breast J.* 2003;9(5):389-92.
17. 17.Vadivelu N, Schreck M, Lopez J, Kodumudi G, Narayan D. Pain after mastectomy and breast reconstruction. *Am Surg*. 2008;74:285-296.
18. Sarhadi NS, Shaw Dunn J, Lee FD, Soutar DS. An anatomical study of the nerve supply of the breast, including the nipple and areola. *Br J Plast Surg.* 1996;49(3):156-64.
19. Jaspars JJ, Posma AN, van Immerseel AA, Gittenberger-de Groot AC. The cutaneous innervation of the female breast and nipple-areola complex: implications for surgery. *Br J Plast Surg*. 1997;50:249–259.
20. Davies F, Gladstone RJ, Stibbe EP. The anatomy of the intercostal nerves. *J Anat*. 1932;66:323–333.
21. Kulhari S, Bharti N, Bala I, Arora S, Singh G. Efficacy of pectoral nerve block versus thoracic paravertebral block for postoperative analgesia after radical mastectomy: A randomized controlled trial. *Br J Anaesth.* 2016;117:382-6.
22. Wahba SS, Kamal SM. Thoracic paravertebral block versus pectoral nerve block for analgesia after breast surgery. *Egypt J Anaesth*. 2014;30:129-35.
23. Kartik Syal, Ankita Chandel. Comparison of the post-operative analgesic effect of paravertebral block, pectoral nerve block and local infiltration in patients undergoing modified radical mastectomy: A randomised double-blind trial. *Indian.* 2017;61(8):643–648.
24. Greengrass R. Is PECS superior to PVB? Methodological flaws with study ESRA Academy. Mar 7, 2017;170348.
25. Desroches J1, Grabs U, Grabs D. Selective ultrasound guided pectoral nerve targeting in breast augmentation: How to spare the brachial plexus cords? *Clin Anat.* 2013;26:49–55.
26. Cheema SP, Ilsley D, Richardson J, Sabanathan S: A thermographic study of paravertebral analgesia. *Anaesthesia.* 1995;50:118–21.
27. Eason MJ, Wyatt R: Paravertebral thoracic block-a reappraisal. *Anaesthesia.* 1979;34: 638–42.
28. A. Schnabel, S. U. Reichl, P. Kranke, E. M. Pogatzki-Zahn, and P. K. Zahn. Efficacy and safety of paravertebral blocks in breast surgery: a meta-analysis of randomized controlled trials. *British Journal of Anaesthesia.* 2010;105(6):842–852.
29. Terkawi AS, Tsang S, Sessler DI, Terkawi RS, Nunemaker MS, Durieux ME, Shilling A, Improving Analgesic Efficacy and Safety of Thoracic Paravertebral Block for Breast Surgery: A Mixed-Effects Meta-Analysis, Pain Physician, 2015;18(5):E757-80.
30. Richardson J, Jones J, Atkinson R: The effect of thoracic paravertebral blockade on intercostal somatosensory evoked potentials. *Anesth Analg.* 1998;87:373–6.
31. Saito T, Den S, Cheema SP. A single-injection, multi-segmental paravertebral block-extension of somatosensory and sympathetic blockinvolunteers. *Acta Anaesthesiol Scand.* 2001;45:30–33.
32. Albokrinov AA, Fesenko UA. Spread of dye after single thoracolumbar paravertebral injection in infants. A cadaveric study. *Eur J Anaesthesiol.* 2014;31:305–309.
33. Marhofer D, Marhofer P, Kettner SC. Magnetic resonance imaging analysis of the spread of local anesthetic solution after ultrasound-guided lateral thoracic paravertebral blockade: a volunteer study. *Anesthesiology.* 2013;118:1106–1112.
34. Cowie B, McGlade D, Ivanusic J, Barrington MJ. Ultrasound-guided thoracic paravertebral blockade: A cadaveric study. *Anesth Analg.* 2010;110:1735-9.
35. Hetta DF, Rezk KM. Pectoralis-serratusinterfascial plane block vs thoracic paravertebral block for unilateral radical mastectomy with axillary evacuation. Randomized controlled trial. *J ClinAnesth.* 2016;34:91-7.
36. Bhatia N, Singh G Ultrasound-guided single- vs double-level thoracic paravertebral block for postoperative analgesia in total mastectomy with axillary clearance.Randomized controlled trial. *J Clin Anesth.* 2016;33:414-21.
37. Sopena-Zubiria LA, Fernández-Meré LA, Valdés Arias C, Muñoz González F, Sánchez Asheras J, Ibáñez Ernández C. Thoracic paravertebral block compared to thoracic paravertebral block plus pectoral nerve block in reconstructive breast surgery. *Rev Esp Anestesiol Reanim.* 2012;59:12-17.
38. Monique A. Steegers, Bas Wolters, Andrea W, Evers Luc Strobbe, Oliver H, Wilder-Smith. Effect of Axillary Lymph Node Dissection on Prevalence and Intensity of Chronic and Phantom Pain after Breast Cancer Surgery. *The Journal of Pain.* 2008;9(9):813-822.
39. Bakshi SG, Karan N, Parmar V. Pectoralis block for breast surgery: A surgical concern?. *Indian J Anaesth.* 2017;61:851-2.
40. S.D. Nathanson, K. Rosso, D. Chitale, M. Burke. Lymph Node Metastasis. *Editedby: Aamir Ahmad* Introduction to Cancer Metastasis *P.* 235-261. ISBN: 978-0-12-804003-4

r Pillai VS, Ramesh B, Varughese SA. Safety and efficacy of multiple site thoracic paravertebral nerve block vs. modified pectoral nerve block for postoperative analgesia after modified radical mastectomies-a randomised controlled trial. Indian J Clin Anaesth. 2018;5(3):445-450.

Comparison of vital capacity rapid inhalation and tidal ventilation induction with sevoflurane in adults: a prospective cohort study

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Abstract

Vital capacity rapid inhalation induction (VCRII) results in faster achievement of desired minimum alveolar concentration while reducing the incidence of excitatory phenomenon compared to conventional incremental technique. This study aimed to determine whether the VCRII can achieve faster induction of anesthesia in adults compared to the traditional tidal ventilation (TV) technique. Following the approval from the Institutional Ethics Committee, Amala Institute of Medical Sciences, with an approval No. AIMSIEC/07/2017, on July 1, 2017, 51 adults belonging to American Society of Anesthesiologists physical status I–II, undergoing elective surgery at a tertiary care teaching hospital were prospectively assigned to two groups: 25 in VCRII (38.3 ± 13.3 years old, 20 (80%) females) and 26 in TV inhalation induction (35.2 ± 11.9 years old, 17 (65%) females) using 8% sevoflurane in 66% nitrous oxide. The induction time, such as time (in seconds) to the cessation of voluntary finger tapping, time to loss of eyelash reflex, time to return of regular breathing, the return of conjugate gaze, was measured. The primary outcome was time to induction as defined by time to loss of eyelash reflex. Hemodynamic effects of both methods were compared at baseline and 1, 3, 5, 10, 15-minute intervals from induction. Induction was significantly faster in the VCRII group compared with the TV group in all the measured parameters. Hemodynamic parameters were comparable in both the groups. VCRII resulted in a faster induction time compared to the TV technique in adults.

Key words: adults; excitatory phenomenon; inhalational agent; inhalational induction; minimum alveolar concentration; prospective study; sevoflurane; tidal ventilation; vital capacity

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Introduction

With the advent of newer agents, inhalational induction is becoming an attractive alternative to intravenous induction. Because of its non-irritating nature and higher efficacy, sevoflurane is the preferred agent. There is evidence that inhalational induction of anesthesia is increasingly preferred by subjects over intravenous induction.[1] In a meta-analysis, the time to induction with sevoflurane was found to be comparable with propofol while sevoflurane was associated with higher first-time success with laryngeal mask airway insertion.[2] High inspired concentrations of sevoflurane are safe and do not provoke airway reflex responses as shown in a multiple adult and pediatric study.[3]

As required by vital capacity rapid inhalation induction (VCRII), the subject is asked to first take a deep breath followed by maximum exhalation. Afterwards, the anesthetic circuit with facemask is applied, the subject is asked to take a deep breath, hold his/her breaths for as long as possible and then breathe out to residual volume.[4] VCRII technique requires patient education and cooperation for a vital capacity breath and an adequate breath-hold following it. The other technique is tidal ventilation (TV) induction, in which the patient breathes normal tidal volume breaths. TV induction does not require any patient training as it involves the patient taking normal breaths. A comparison of VCRII and TV techniques performed in the pediatric age group has shown that VCRII achieved shorter time to induction and was preferred by the subjects.[5] This study aimed to ascertain whether the VCRII technique can result in faster induction in Asian adults compared to the traditional TV technique and to compare the incidence of adverse events using both the techniques.

Subjects and Methods

An approval from the Institutional Ethics Committee, Amala Institute of Medical Sciences with an approval No. AIMSIEC/07/2017 on July 11, 2017 was obtained. Written informed consent was obtained from all subjects. We prospectively enrolled subjects aged 18 years and above scheduled to undergo elective urological, head and neck, orthopedic, or visceral surgeries at our tertiary care teaching hospital from June 2018 to December 2019 and belonging to the American Society of Anesthesiologists (ASA) physical status I–II. Adults with contra-indications to inhalational induction (gastro-esophageal reflux, myopathy, or familial history of malignant hyperthermia) and those with a history of unstable cardiac disease, neurological disease, obstructive airway disease, and recent acute respiratory illness/infection were excluded. The circuit was primed with 8% sevoflurane (Sevotroy 250, Troikaa Pharmaceuticals Ltd., Ahmedabad, India) in nitrous oxide and oxygen before the application of a mask to the patient's face. Before anesthesia, subjects in the VCRII group were instructed on the vital capacity breath.[4] The concentration of gases used was 8% sevoflurane in nitrous oxide (66%) and oxygen (33%) at a flow rate of 8 L/min in both the groups.

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In the VCRII group, the subject was asked to exhale forcefully, followed by a maximal inspiration via a face mask connected to the anesthetic circuit. The subject was then asked to hold his/her breath for the maximum time possible and then exhale residual volume. In the TV group, the subject was asked to continue quiet breathing through the facemask. The onset of induction of anesthesia was assessed as the time to the cessation of voluntary finger tapping, time to loss of eyelash reflex, time to return of regular breathing, and time to return of conjugate gaze.[5] Heart rate, peripheral oxygen saturation, systolic, and diastolic blood pressures were compared at baseline and 1-, 3-, 5-minute intervals from induction. The occurrence of stridor, cough, laryngospasm, hypoxemia, and excitatory movements was recorded. All patients received fentanyl 2 μg/kg (Verfen, Verve Pharma, India) 3 minutes before sevoflurane induction. Rescue remedy of intravenous propofol (Troypofol, Troikaa Pharmaceuticals Ltd.) at 2 mg/kg and succinylcholine (Sucol, Neon Pharma, Mumbai, India) 100 mg was prepared for laryngospasm or desaturation. The trial was non-randomized. A trained nurse who was blinded to the group allocation was recording the outcome endpoints. Acceptance of anesthesia induction technique was assessed by the willingness to undergo a repeat procedure using the same method of induction. The trial flow chart showing the distribution of patients is given in **Figure 1**.

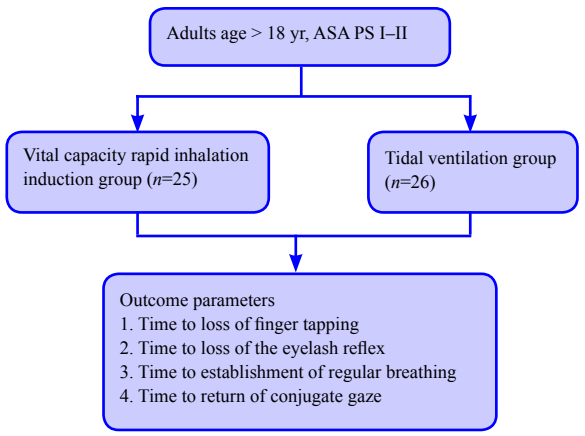


Figure 1: The flow chart.
Note: ASA PS: American Society of Anesthesiologists physical status.

The sample size was calculated according to the results of Yurino et al.[6] A sample size of 23 in each group was calculated for a difference in onset of induction of 11 seconds for a power of 80% and an alpha of 0.01 using SPSS 23 software (IBM, Armonk, NY, USA). All results are expressed as the mean ± standard deviation (SD) or median (range; continuous variables) or as percentages of the group from which they were derived (categorical variables). Continuous variables were compared with the Student's *t*-test for normally distributed variables and the Mann-Whitney *U* test for non-normally distributed variables. Categorical variables were evaluated with the Chi-square analysis or two-tailed Fisher exact test. A value of *P* less than 0.05 was considered significant.

Results

Baseline characters of patients under vital capacity rapid inhalation and tidal ventilation induction with sevoflurane

A total of 51 patients were included in the study, 25 in the VCRII group and 26 in the TV group. The baseline parameters of both groups are depicted in **Table 1**. Age, sex, weight, and ASA status distributions, baseline heart rate, percutaneous oxygen saturation, systolic, and diastolic blood pressures were comparable between the two groups ($P > 0.05$).

Hemodynamic and respiratory outcomes of patients under vital capacity rapid inhalation and tidal ventilation induction

Induction was significantly faster in the VCRII group compared with the TV group. The time to loss of voluntary finger tapping ($P < 0.001$), time to loss of eyelash reflex ($P < 0.001$), time for the establishment of regular breathing ($P < 0.001$) and time to return of conjugate gaze ($P = 0.046$) were all significantly less in the VCRII group than those in the TV group.

To evaluate the cardiovascular effects of methods of induction, a comparison of vital signs was done between two groups before induction, and upon loss of consciousness. Heart rate in the VCRII group was significantly lower compared to the TV group at 3 minutes ($P = 0.015$) and 5 minutes post-induction ($P = 0.012$). Systolic and diastolic blood pressures were comparable between the two groups at all time points ($P > 0.05$; **Table 2**).

No adverse event (cough, laryngospasm, excitatory movements, hypoxemia) occurred in both groups. Patient willingness to return for repeat procedure using the same method of induction of anesthesia was 100% in both the groups.

Discussion

In this study, the VCRII technique using 8% sevoflurane resulted in a faster induction of anesthesia, as estimated by time to loss of finger tapping, loss of the eyelash reflex, establishment of regular breathing, and return of conjugate gaze, compared with the TV technique.

The time for induction of anesthesia was taken as the time from the beginning of the administration of the agent to the loss of finger tapping which is an acceptable outcome in similar studies.[7-9] Though bispectral index monitoring was not done, clinical endpoints for recognition of induction like loss of finger tapping to verbal command are valid surrogates for bispectral index monitoring.[10]

The roadblock to wider acceptance of inhalational anesthesia is the longer time of induction compared to intravenous agents.[11,12] Modification of administration techniques facilitates the speed of induction. VCRII achieves a high alveolar concentration of sevoflurane in a shorter time resulting in smoother induction. Typically, this single-breath technique achieves the 2% alveolar sevoflurane concentration required to tolerate a surgical incision.[2,13] In a study by Lin et al.[14] the use of VCRII inhalational induction in 36 ASA physical status I–II patients resulted in an induction time of 60.6 ± 19.2 seconds which could be shortened to 48.3 ± 17.9 seconds with the use of fentanyl before induction. By contrast, our study employed fentanyl pre-induction in both the groups along with nitrous oxide and hence demonstrated faster induction times. In the

Table 1: Baseline characteristics of patients under VCRII and TV induction

Parameter	VCRII group (*n*=25)	TV group (*n*=26)	*P* value
Age (yr)	38.3±13.3	35.2±11.9	0.39
Female	20 (80)	17 (65)	0.24
American Society of Anesthesiologists physical status (I/II)	2/23	2/24	0.96
Weight (kg)	61.8±8.8	59.8±7.3	0.38
Heart rate (beats/min)	78.8±7.7	80.5±6.5	0.39
Percutaneous oxygen saturation (%)	99.9±0.2	100.0±0	0.313
Systolic blood pressure (mmHg)	126.7±9.3	124.2±8.0	0.302
Diastolic blood pressure (mmHg)	78.3±7.4	78.0±6.4	0.902
Anesthesia induction			
Loss of finger tapping (s)	24.4±4.7	31.7±4.3	0.0001
Loss of eyelash reflex (s)	43.8±8.3	65.0±8.3	0.0001
Establishment of regular breathing (s)	73.7±12.7	86.9±7.5	0.0001
Return of conjugate gaze (s)	108.8±18.2	117.7±12.8	0.046

Note: Data are expressed as the mean ± SD, except female [number (percentage)] and American Society of Anesthesiologists physical status (number). Continuous variables were compared with the Student's *t*-test (for normally distributed variables) or the Mann-Whitney *U* test (for non-normally distributed variables). Categorical variables were evaluated with the Chi-square analysis or two-tailed Fisher exact test. TV: Tidal ventilation; VCRII: vital capacity rapid inhalation induction.

Table 2: Hemodynamic and respiratory parameters after induction of patients under VCRII and TV induction with sevoflurane

Parameter	VCRII group (*n*=25)	TV group (*n*=26)	*P* value
Heart rate (beats/min)			
1 min	74.3±9.4	75.8±15.2	0.66
3 min	69.2±8.0	74.6±7.1	0.015
5 min	67.1±7.4	72.1±6.3	0.012
Systolic blood pressure (mmHg)			
1 min	119.8±8.8	114.4±23.0	0.277
3 min	114.4±9.2	115.5±9.1	0.66
5 min	113.3±10.6	114.9±10.7	0.598
Diastolic blood pressure (mmHg)			
1 min	73.3±6.8	73.5±6.7	0.924
3 min	70.1±7.9	71.2±5.4	0.572
5 min	66.8±6.9	69.3±6.3	0.188
Percutaneous oxygen saturation (%)			
1 min	99.5±0.8	99.8±0.4	0.058
3 min	99.2±0.7	99.8±0.5	0.001
5 min	98.9±0.9	99.6±0.6	0.001

Note: TV: Tidal ventilation; VCRII: vital capacity rapid inhalation induction.

pediatric population, the VCRII technique can produce faster induction as measured by loss of eyelash reflex, which has been preferred over tidal ventilation technique in 73 subjects over the age of 5 years in a randomized controlled trial comparing the two techniques.[5] This pediatric study used sevoflurane 7% in 50% nitrous oxide with oxygen at 6 L/min fresh gas flow along with midazolam premedication, while our study used sevoflurane 8% in 66% nitrous oxide with oxygen at 8 L/min fresh gas flow with fentanyl premedication, which may result in a comparable induction time in both the studies. Another pediatric study showed similar results with VCRII producing fewer adverse events, faster induction and better patient satisfaction compared with TV using 8% sevoflurane.[15] A similar study in adults using sevoflurane and nitrous oxide showed that VCRII, in comparison with stepwise tidal ventilation induction, resulted in an earlier loss of consciousness (54 ± 10 seconds *vs*. 108 ± 19 seconds) but was associated with a higher incidence of coughing.[16] This study used a lower concentration of sevoflurane at 4.5% which explains the longer induction times compared to our study.

The present study shows that inhalational induction is a feasible and effective alternative to intravenous induction in Asian adults. There is emerging evidence that inhalational induction using sevoflurane has better hemodynamic stability, equal patient acceptance, and is cheaper than intravenous induction using propofol.[7] Usage of a single inhalational agent for induction and maintenance reduces adverse events accompanying the use of multiple intravenous agents, such as hemodynamic compromise.[17]

None of the patients in our study had coughing or laryngospasm. This indicates an adequate depth of anesthesia and suppression of airway reflexes. The VCRII technique requires some training but this is easily achieved in all our subjects. The feasibility of achieving adequate VCRII is 95% by 14 years of age as described in a previous study.[18] Besides, there was no difference in patient willingness to return for a repeat procedure under both the techniques, indicating that the VCRII technique was acceptable for the subjects.

The use of inhalational agents especially as maintenance of anesthesia is found to be feasible and cost-effective in ambulatory surgeries.[19,20] Our study also demonstrates the utility and safety of a faster induction technique using the same inhalational agent.

Our study has some limitations. This non-randomized study may be subject to bias. Although the VCRII technique produces a faster induction, this advantage remains small in clinical practice. In this study, intubation times or emergence times

were incomparable between the two groups as we designed primarily for the comparison of induction characteristics of the two techniques.

VCRII rapid inhalational induction technique using 8% sevoflurane with nitrous oxide can result in a faster induction compared to the TV induction technique in adults.

Author contributions
Study design: JJC; patient selection: RV, JJC, RP, KM; data acquisition, statistical analysis and manuscript preparation: RV; data interpretation: RP, KM; manuscript revision: JJC, RP, KM. All authors have read and approved the final version of the manuscript.

Conflicts of interest
None of the authors have any conflicts of interest.

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Nil.

Institutional review board statement
The study was approved by Institutional Ethics Committee, Amala Institute of Medical Sciences (approval No. AIMSIEC/07/2017) on July 1, 2017.

Declaration of patient consent
The authors certify that they have obtained patients' consent forms. In the form, patients have given their consent for their clinical information to be reported in the journal. The patients understand that their names and initials not be published and due efforts will be made to conceal their identity.

Reporting statement
The writing and editing of the study report were performed in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.

Biostatistics statement
The statistical methods of this study were conducted and reviewed by the biostatistician of Amala Institute of Medical Sciences, India.

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The Copyright License Agreement has been signed by all authors before publication.

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Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices) and anonymized trial data will be available on request.

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References

1. van den Berg AA, Chitty DA, Jones RD, Sohel MS, Shahen A. Intravenous or inhaled induction of anesthesia in adults? An audit of preoperative patient preferences. *Anesth Analg*. 2005;100:1422-1424, table of contents.
2. Joo HS, Perks WJ. Sevoflurane versus propofol for anesthetic induction: a meta-analysis. *Anesth Analg.* 2000;91:213-219.
3. Michel F, Constantin JM. Sevoflurane inside and outside the operating room. *Expert Opin Pharmacother*. 2009;10:861-873.
4. Agnor RC, Sikich N, Lerman J. Single-breath vital capacity rapid inhalation induction in children: 8% sevoflurane versus 5% halothane. *Anesthesiology.* 1998;89:379-384.
5. Lejus C, Bazin V, Fernandez M, et al. Inhalation induction using sevoflurane in children: the single-breath vital capacity technique compared to the tidal volume technique. *Anaesthesia*. 2006;61:535-540.
6. Yurino M, Kimura H. A comparison of vital capacity breath and tidal breathing techniques for induction of anaesthesia with high sevoflurane concentrations in nitrous oxide and oxygen. *Anaesthesia*. 1995;50:308-311.
7. Dhande K, Kshirsagar J, Dhande A, Patil N, V P Jr. Hemodynamic stability, patient acceptance and cost of intravenous propofol and inhalational sevoflurane for induction of anaesthesia: a prospective, randomized comparative study. *Cureus.* 2020;12:e7687.
8. Yogendran S, Prabhu A, Hendy A, et al. Vital capacity and patient controlled sevoflurane inhalation result in similar induction characteristics. *Can J Anaesth.* 2005;52:45-49.
9. Reshef ER, Schiff ND, Brown EN. A neurologic examination for anesthesiologists: assessing arousal level during induction, maintenance, and emergence. *Anesthesiology*. 2019;130:462-471.
10. Kim DW, Kil HY, White PF. Relationship between clinical endpoints for induction of anesthesia and bispectral index and effect-site concentration values. *J Clin Anesth.* 2002;14:241-245.
11. Thwaites A, Edmends S, Smith I. Inhalation induction with sevoflurane: a double-blind comparison with propofol. *Br J Anaesth.* 1997;78:356-361.
12. Dongare DH, Kale JV, Naphade RW. Comparison of vital capacity induction with sevoflurane to intravenous induction with propofol in adult patients. *Anesth Essays Res*. 2014;8:319-323.
13. Aranake A, Mashour GA, Avidan MS. Minimum alveolar concentration: ongoing relevance and clinical utility. *Anaesthesia.* 2013;68:512-522.
14. Lin TC, Lu CC, Kuo CK, et al. Single vital-capacity and successive tidal-volume breathing of sevoflurane in induction of anesthesia for tracheal intubation in gynecologic patients. *Acta Anaesthesiol Taiwan*. 2008;46:66-70.
15. Singh J, Tandukar A, Kharbuja K. Comparison of the single breath vital capacity technique with the tidal volume technique. *J Nepal Paediatr Soc.* 2018;38:84-89.
16. Yurino M, Kimura H. Induction of anesthesia with sevoflurane, nitrous oxide, and oxygen: a comparison of spontaneous ventilation and vital capacity rapid inhalation induction (VCRII) techniques. *Anesth Analg.* 1993;76:598-601.
17. Brioni JD, Varughese S, Ahmed R, Bein B. A clinical review of inhalation anesthesia with sevoflurane: from early research to emerging topics. *J Anesth.* 2017;31:764-778.
18. Fernandez M, Lejus C, Rivault O, et al. Single-breath vital capacity rapid inhalation induction with sevoflurane: feasibility in children. *Paediatr Anaesth*. 2005;15:307-313.
19. Smith I, Nathanson MH, White PF. The role of sevoflurane in outpatient anesthesia. *Anesth Analg.* 1995;81:S67-72.
20. Gupta A, Stierer T, Zuckerman R, Sakima N, Parker SD, Fleisher LA. Comparison of recovery profile after ambulatory anesthesia with propofol, isoflurane, sevoflurane and desflurane: a systematic review. *Anesth Analg.* 2004;98:632-641, table of contents.

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Clinicoepidemiological Features and Mortality Analysis of Deceased Patients with COVID-19 in a Tertiary Care Center

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Abstract

Background and objective

A large number of studies describing the clinicoepidemiological features of coronavirus disease-2019 (COVID-19) patients are available but very few studies have documented similar features of the deceased. This study was aimed to describe the clinicoepidemiological features and the causes of mortality of COVID-19 deceased patients admitted in a dedicated COVID center in India.

Methodology

This was a retrospective study done in adult deceased patients admitted in COVID ICU from April 4 to July 24, 2020. The clinical features, comorbidities, complications, and causes of mortality in these patients were analyzed. Pediatric deceased were analyzed

separately.

Results

A total of 654 adult patients were admitted in the ICU during the study period and ICU mortality was 37.7% (247/654). Among the adult deceased, 65.9% were males with a median age of 56 years [interquartile range (IQR), 41.5–65] and 94.74% had one or more comorbidities, most common being hypertension (43.3%), diabetes mellitus (34.8%), and chronic kidney disease (20.6%). The most common presenting features in these deceased were fever (75.7%), cough (68.8%), and shortness of breath (67.6%). The mean initial sequential organ failure assessment score was 9.3 ± 4.7 and 24.2% were already intubated at the time of admission. The median duration of hospital stay was 6 days (IQR, 3–11). The most common cause of death was sepsis with multi-organ failure (55.1%) followed by severe acute respiratory distress syndrome (ARDS) (25.5%). All pediatric deceased had comorbid conditions and the most common cause of death in this group was severe ARDS.

Conclusion

In this cohort of adult deceased, most were young males with age less than 65 years with one or more comorbidities, hypertension being the most common. Only 5% of the deceased had no comorbidities. Sepsis with multi-organ dysfunction syndrome was the most common cause of death.

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Keywords: Clinicoepidemiological features, Comorbidities, COVID-19 deceased, ICU, Mortality analysis, Multi-organ dysfunction syndrome

Introduction

Coronavirus disease-2019 (COVID-19) emerged in China in December 2019 and engulfed the whole world in a very short period of time. Worldwide till now around 95 million patients have been infected and there have been more than 2 million deaths. India is also severely affected. It has infected more than 10 million people in India and has led to more than 1.5 lakh deaths till now.[1]

The clinical spectrum of COVID-19 is varied, ranging from asymptomatic carriers to severe pneumonia, acute respiratory distress syndrome (ARDS) profiles, multi-organ involvement, and death.[2] Nearly 14–15% of patients develop severe disease and 5% become critically ill.[3] Earlier thought to be primarily affecting the pulmonary system, a number of symptoms related to other organ systems have been widely recognized as initial presenting features of COVID-19 infection.[4,5] These extrapulmonary manifestations can lead to complications and increase morbidity. The prevalence of mortality among hospitalized patients with COVID-19 disease varies from 6.3 to 26.85% in different parts of the world.[6] Various studies have looked into and analyzed the risk factors for severe disease and predictors of mortality.[7,8] However, studies regarding the clinicoepidemiological features of the deceased and causes of mortality are sparse. Some

studies of COVID-19 related mortalities from US, China, Italy, and Spain are available;[9–17] however, such descriptive study has not been done from Indian subcontinent so far. This study was aimed to describe the clinicoepidemiological features and the causes of mortality of COVID-19 deceased patients admitted in our hospital during the initial period of the pandemic spread in the country from April to July.

Materials and Methods

This was a retrospective study conducted in the COVID center of AIIMS, New Delhi, after approval from the institutional ethics committee. This study analyzed data of patients who died in the course of treatment in the ICU of COVID-19 center during the study period between April 4 and July 24, 2020. All the patients admitted to the center were moderate to severe confirmed COVID-19 patients whose real-time reverse-transcription polymerase chain reaction assay findings for nasal or pharyngeal swab specimens were positive. Mild cases were admitted in different centers. Criteria for ICU admission were patients with severe disease i.e., tachypnea with respiratory rate greater than 30/minute or SpO_2 less than 90% on room air, patient with hemodynamic instability, encephalopathy or patients with moderate disease who had risk factors for severe disease.

Data Collection

Data related to demography and clinical course of all the patients who died in the ICU during the study period was collected from their electronic medical records, patients' daily progress charts, and nursing notes. Patients' age, sex, comorbidities, complaints at the time of presentation, complications during treatment, duration of hospital stay, and cause of death were noted and analyzed. Data regarding adult and pediatric deceased were analyzed separately. Adult patients were further divided into age-groups of 18 to 50, 51 to 65, and more than 65 years and their clinical characteristics and outcomes were compared.

Statistical Analysis

The data were summarized and analyzed using STATA (Version 14.0) software and expressed as mean ± SD/Median (min, max) and numbers and percentages as appropriate to describe the patient's demographic and clinical characteristics. Wilcoxon–Ranksum test was used to compare the non-normal quantitative values while chi-square/Fisher's exact test was applied for association between categorical variables and for comparison between stratified age-groups. Kruskal–Wallis was used to see the difference in age-groups for the days of stay followed by multiple comparison tests between the groups. A value of p less than 0.05 was considered as statistically significant in the study.

Results

During the study period, a total of 1,420 patients (adult and pediatric) were admitted in the center. The deaths of adults and pediatric age-groups were analyzed separately.

Adult Fatalities

There were 654 adult admissions in the ICU during the study period out of which 247 died. The ICU mortality for adults was 37.7% (247/654).

Among the adult patients who did not survive, the youngest was 18 years old and the eldest was 97 years old. The median age was 56 years [interquartile range (IQR), 41.5–65] and most of them were males (65.9%). Age distribution of adults in various age-groups is given in Table 1.

Most of the adult patients who died (94.74%) had 1 or more comorbidities. Preexisting hypertension was the most frequently seen comorbidity in 107 (43.32%) patients followed by diabetes mellitus in 86 (34.82%) patients and chronic kidney disease in 51 (20.6%) patients. Other comorbidities are tabulated in Table 1. Among the patients with comorbidities, 39% of patients had one comorbidity, 27.5% had two comorbidities, 21% had three, and 6% had more than three comorbidities. The frequency of patients with different numbers of comorbidities is depicted in Figure 1.

Twenty-nine patients had some kind of malignancy, out of which 14 suffered from hematological malignancies and remaining 15 had solid organ tumors. Besides these, other infrequently encountered comorbidities were postrenal transplant, cardiomyopathy, cholelithiasis, complete heart block with pacemaker *in situ*, HIV, ulcerative colitis, sarcoidosis, interstitial lung disease, liver abscess, aspergilloma, shoulder abscess, peripheral vascular disease, parkinsonism, corrosive ingestion, mesenteric ischemia, dementia, depression, aplastic anemia, cervical spondylosis, systemic sclerosis, ulnar, and subclavian artery thrombosis.

The most common presenting symptom among deceased was fever (75.7%) followed by cough (68.8%), shortness of breath (67.6%), and altered sensorium (19.8%) as shown in Table 2. Besides these, other patients presented with chest pain, abdominal pain or distension, generalized weakness, diarrhea, and headache. Approximately 20% of patients presented with nonspecific complaints including myalgia, decreased urine output, jaundice, upper limb pain, generalized edema, epistaxis, and joint pain.

The patients had varying degrees of severity of illness. The initial sequential organ failure assessment (SOFA) score was calculated for 232 deceased, the data were missing for 15 patients. The mean SOFA score was 9.3 with a standard deviation of 4.07. The initial SOFA score was significantly higher in males as compared to females (p = 0.03). Out of 247 deceased, 60 patients were received intubated in our center from the screening area. Patients were given treatment according to the institutional protocol. The patients were initially managed on oxygen by facemask and then as the oxygen requirement increased, they were managed on high flow nasal cannula (HFNC) and noninvasive ventilation (NIV) before invasive ventilation as per SCCM guidelines. Seventy-five patients were intubated in the first 24 hours of admission. The number of patients who received HFNC and NIV are mentioned in Table 3. As the disease advanced, many patients developed multisystem involvement. However, the predominant system involved due to COVID was the respiratory system in 66% of the patients followed by the neurological system and cardiovascular system. One twenty one patients developed acute kidney injury (AKI) and 39 received RRT (Table 3). Repeat SOFA score was calculated on day 4 of admission after 72 hours. Repeat SOFA was available for 133 patients as 99 patients died within 3 days of admission to ICU.

Duration of Stay and Cause of Death

Among the deceased adults, the median duration of hospital stay was 6 days (IQR, 3–11) with maximum stay of 37 days and minimum of 1 day.

The cause of death was analyzed in all the deceased (Table 4). Sepsis with multi-organ dysfunction remained the leading cause of death (55.1%) followed by severe ARDS (25.5%), cardiogenic shock, intracranial hypertension, and suspected pulmonary embolism.

There were 41 patients who died within 24 hours of admission. Among these, 90.24% of them reported a history of one or more comorbidities and majority had respiratory distress at presentation. Most of them were shifted intubated to our center. Four patients received cardiopulmonary resuscitation in the emergency room and were postarrest patients. The cause of death of these patients is depicted in Figure 2.

Age-related Analysis

The study patients were stratified into three age-groups (18–50, 51–65, and >65 years) to find out age-related differences in the number of comorbidities, presenting symptoms, duration of illness, and cause of death (Table 5).

In the study cohort, 42.1% of the deceased were in age-group between 18 years and 50 years, 34.8% in age-group 51–65 years, and 23.1% in the group above the age of 65 years. There was no difference in the male-to-female ratio between the three groups. The percentage of deceased having multiple comorbidities (two or more) was significantly higher in the age-group above 65 years as compared to younger age-groups. Multiple comorbidities were seen in 68.4% of patients above the age of 65 years, as compared to 59.3% in age-group 51–65 years and 44.2% in patients in 18–50 years of age.

The most frequent presenting complaints were similar in deceased of all age-groups with no significant difference. Initial SOFA score and percentage of patients given HFNC and NIV, and patients developing AKI were also similar in all the three groups. However, the duration of stay in the hospital was higher in the elderly deceased population. The percentage mortality was significantly higher in the age-group of more than 65 years as compared to other groups. The percentage mortality in different age-groups was 30.2% in age-group between 18 and 50 years, 43.2% in age-group between 51 years and 65 years, and 51.3% in age-group more than 65 years.

Pediatric Fatalities

There were 46 pediatric admissions during the study period and six COVID-19 related deaths, the ICU mortality among the pediatric age-group being 13% (6/46). The age-group of the deceased varied from 6 months to 16 years as depicted in Table 6. All of these patients had comorbidities including congenital heart disease, tubercular meningitis, endocrine disorders, and hematologic malignancies. Mean hospital duration of stay was 4.33 days. Most common cause of death was ARDS followed by sepsis with multi-organ dysfunction.

Discussion

This study retrospectively analyzed clinicoepidemiological characteristics, course of the disease, and cause of mortality of COVID-19 patients who died in the ICU. Total of 1,420 confirmed COVID-19 patients of all ages were admitted in the COVID center in the study

period. Almost half (49.3%) of the hospitalized patients received ICU care during the course of illness. This figure varies from 5 to 46.8% of hospitalized patients in different studies[9–11] and is related to the availability of ICU resources in that region.

The ICU mortality of COVID-19 patients varies from 8.0 to 66.7% in different studies.[18] In our center, the hospital mortality was 18.2% and ICU mortality was 36.1%. Similar mortality rates have been reported in other studies from US, Spain, Italy, and few studies from China.[9,10,16] However, there are other studies from China[2] which depict lower overall mortality rates. As the pandemic is evolving and our understanding of the disease is increasing, there has been a steady decline in the mortality rate.[19]

Approximately 60% of all admitted patients were males in our center. An almost alike proportion of male patients has been documented in all other studies suggesting male predisposition for this disease across various ethnic and racial groups.[9,10,15] Similarly, among the deceased adults, 66% were males and among pediatric fatalities, all were males except one.

In our study population of adult deceased, 42.1% were of age-group of 18–50 years, 35% in the age-group 51–64 years, and only about 23% were above 65 years age-group. This was a deviation from the studies from other parts of the world where most of the nonsurvivors were from a much older age-group[10,15,20] and could be explained by the majority of the young population in India, the percentage of the elderly population (>60 years) being 6.8%.[21] In our study, 95% of the adults deceased and all of the pediatric ones had at least one comorbid condition. Mortality studies from Spain and Italy[10,15] have reported a similar incidence of comorbidities in nonsurvivors. However, few other studies[11,16] report lesser incidence of comorbidities than ours. In our cohort, hypertension was the most common comorbidity seen followed by DM and CKD. Similarly, worldwide studies have shown hypertension followed by diabetes mellitus and cardiovascular comorbid conditions to be associated with the highest mortality.[12,14,22] Role of ACE-2 receptor in entry of SARS-CoV-2 virus into a host cell is one of the proposed reasons for the highest incidence of COVID-19 in hypertensive patients.[23]

In the present study, the elderly (>65 years old) deceased had more comorbidities than younger deceased suggesting that elderly population with higher number of comorbidities are more susceptible to adverse outcomes following COVID-19 infection, while factors other than number of comorbidities could be a significant variable in younger population.[15] Malignancy was seen in 11.74% of patients in our study which was higher than other reported incidences.[12,13,16]

The most common presenting complaints in our deceased population were fever, cough, and breathlessness similar to the findings of earlier studies.[3,24] Few patients initially presented for unrelated diagnoses and then later tested positive for COVID-19. Nonspecific symptoms were significantly more common in younger deceased.

The median initial SOFA score was 9.3 in our study. This was higher as compared to studies from US and China[11,25,26] reflecting increased severity of COVID-19 at ICU admission in our cohort. High SOFA scores at ICU admission or their progression over 48 hours have been associated with poor outcome of COVID-19.[25,27] Around 24% of our deceased were received intubated from the screening area and another 30% got intubated within the first 24 hours. HFNC was used in 45% of deceased in our study similar to

studies from China where HFNC use among nonsurvivors was 21.1 to 41%.[11,14] Only 18.2% of patients received NIV in our cohort and even in the published literature, proportion of nonsurvivors receiving noninvasive ventilatory support has been variable.[11,14,17] Use of NIV was greater in elderly subgroup of nonsurvivors in other studies[16] unlike our study.

The median duration of ICU stay among deceased was 6 days in our study. There was significant variation in median duration of ICU stay reported in other studies ranging from 6 days to 11.5 days.[12,16] Elderly deceased (>65 years) had significantly longer average duration of hospital stay of 9 days while younger had average 5 days of stay. However, a study in China on deceased patients[28] reported a longer average duration of hospital stay in younger patients. The reason for shorter stay in the younger age-group could be either the patients presented to the hospital late in the disease process or had severe cytokine storm-related multi-organ failure. Ours being a tertiary care center, patients were shifted after deterioration from other hospitals. The incidence of AKI in our study was 48.9 and 15.8% received RRT similar to the study by Ahmed et al. in which AKI incidence was around 10 to 50% in critically ill nonsurvivors and around 10–18.5% received RRT.[29]

Forty-one patients died within 24 hours of admission to ICU. These patients either had severe illness, coexisting decompensated comorbid illness, or were referred from other hospitals. Some were postarrest patients who collapsed in the emergency room, received CPR, and were revived. These patients were more likely to have a poor prognosis and shorter clinical course in the hospital.

Sepsis with multi-organ dysfunction syndrome (MODS) was the most common cause of death in our adult patients followed by severe ARDS and cardiogenic shock. Earlier studies[11,24] have also described multi-organ failure as the most common cause of death in the Chinese population while many other studies reported respiratory failure as the leading cause of death.[12,13,30] The reduced incidence of respiratory failure among the deceased in our study can be explained on the basis that 95% of our study cohort had one or more comorbidities that already compromised other organs. Moreover, all our severely affected patients were given ICU admissions as there were enough ICU beds available and we were able to give rescue therapies like prone ventilation for severe ARDS patients. Our scenario was different from other studies where many patients were not able to get supportive treatment as the number of patients requiring ICU care overwhelmed their medical system. Other rare causes of death were raised intracranial pressure and suspected pulmonary embolism.

Of all the admitted patients, COVID-19 related mortality was much lower (5.6%) in pediatric age-group compared to adults (18.8%). This correlated with the acknowledged low pediatric mortality rates by other authors.[31–33] In pediatric patients, respiratory failure secondary to ARDS was the most common cause of death. Only a few cases of pediatric COVID-19 mortality have been reported in the literature, wherein comorbidities and multi-organ dysfunction are the most common cause.[33]

Limitations

Ours was a retrospective study done in patients who died in ICU. As only data related to the deceased was studied, it is not possible for us to comment on risk factors for more severe infection or death due to COVID-19. Only deaths till July 24 were included in the study. Patients admitted to the hospital during the study duration could have died later during the illness, which has not been included.

Conclusion

In this single-center retrospective study of the deceased, 65.9% of the deceased were males. Most of them (76.8%) were of the age less than 65 years indicative of the majority of young population of India. Nearly all patients had single or multiple comorbidities, most common being hypertension followed by diabetes mellitus and chronic kidney disease. The most common cause was of death was sepsis with MODS followed by ARDS. There were a significant number of patients (16.6%) who presented late in the disease process and died within 24 hours.

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Footnotes

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References

1. Worldometer info. World/Countries/India; https://www.worldometers.info/coronavirus/country/india. [[Accessed on January 17, 2020]];2020 Available from:

2. Bal A, Agrawal R, Vaideeswar P, Arava S, Jain A. COVID-19: an up-to-date review – from morphology to pathogenesis. *Indian J Pathol Microbiol.* 2020;63(3):358–366. doi: 10.4103/IJPM.IJPM_779_20. DOI: [PubMed: 32769322] [CrossRef: 10.4103/IJPM.IJPM_779_20]

3. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA.* 2020;323(13):1239–1242. doi: 10.1001/jama.2020.2648. DOI: [PubMed: 32091533] [CrossRef: 10.1001/jama.2020.2648]

4. Behzad S, Aghaghazvini L, Radmard AR, Gholamrezanezhad A. Extrapulmonary manifestations of COVID-19: radiologic and clinical overview. *Clin Imaging.* 2020;66:35–41. doi: 10.1016/j.clinimag.2020.05.013. DOI: [PMCID: PMC7233216] [PubMed: 32425338] [CrossRef: 10.1016/j.clinimag.2020.05.013]

5. Zheng KI, Feng G, Liu WY, Targher G, Byrne CD, Zheng MH. Extrapulmonary complications of COVID-19: a multisystem disease? *J Med Virol.* 2020 doi: 10.1002/jmv.26294. doi: 10.1002/jmv.26294. DOI: [PMCID: PMC7405144] [PubMed: 32648973] [CrossRef: 10.1002/jmv.26294] [CrossRef: 10.1002/jmv.26294]

6. Noor FM, Islam MM. Prevalence and associated risk factors of mortality among COVID-19 patients: a meta-analysis. *J Community Health.* 2020;45(6):1270–1282. doi: 10.1007/s10900-020-00920-x. DOI: [PMCID: PMC7486583] [PubMed: 32918645] [CrossRef: 10.1007/s10900-020-00920-x]

7. Gupta S, Hayek SS, Wang W, Chan L, Mathews KS, Melamed ML, et al. Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. *JAMA Intern Med.* 2020;180(11):1–12. doi: 10.1001/jamainternmed.2020.3596. DOI: [PMCID: PMC7364338] [PubMed: 32667668] [CrossRef: 10.1001/jamainternmed.2020.3596]

8. Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G. Risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. *JAMA Intern Med.* 2020;180(10):1345–1355. doi: 10.1001/jamainternmed.2020.3539. DOI: [PMCID: PMC7364371] [PubMed: 32667669] [CrossRef: 10.1001/jamainternmed.2020.3539]

9. Cummings MJ, Baldwin MR, Abrams D, Jacobson SD, Meyer BJ, Balough EM, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet.* 2020;395(10239):1763–1770. doi: 10.1016/S0140-6736(20)31189-2. DOI: [PMCID: PMC7237188] [PubMed: 32442528] [CrossRef: 10.1016/S0140-6736(20)31189-2]

10. Rivera-Izquierdo M, Del Carmen Valero-Ubierna M, R-delAmo JL, Fernández-García MÁ, Martínez-Diz S, Tahery-Mahmoud A, et al. Sociodemographic, clinical and laboratory factors on admission associated with COVID-19 mortality in hospitalized patients: a retrospective observational study. *PLoS One.* 2020;15(6):e0235107. doi: 10.1371/journal.pone.0235107. DOI: [PMCID: PMC7316360] [PubMed: 32584868] [CrossRef: 10.1371/journal.pone.0235107]

11. Du RH, Liu LM, Yin W, Wang W, Guan LL, Yuan ML, et al. Hospitalization and critical care of 109 decedents with COVID-19 pneumonia in Wuhan, China. *Ann Am Thorac Soc.* 2020;17(7):839–846. doi: 10.1513/AnnalsATS.202003-225OC. DOI: [PMCID: PMC7328178] [PubMed: 32255382] [CrossRef: 10.1513/AnnalsATS.202003-225OC]

12. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, et al. Clinical features of 85 fatal cases of COVID-19 from Wuhan. A retrospective observational study. *Am J Respir Crit Care Med.* 2020;201(11):1372–1379. doi: 10.1164/rccm.202003-0543OC. DOI: [PMCID: PMC7258652] [PubMed: 32242738] [CrossRef: 10.1164/rccm.202003-0543OC]

13. Yang F, Shi S, Zhu J, Shi J, Dai K, Chen X. Analysis of 92 deceased patients with COVID-19. *J Med Virol.* 2020;92(11):2511–2515. doi: 10.1002/jmv.25891. DOI: [PMCID: PMC7262332] [PubMed: 32293741] [CrossRef: 10.1002/jmv.25891]

14. Wang Y, Lu X, Li Y, Chen H, Chen T, Su N, et al. Clinical course and outcomes of 344 intensive care patients with COVID-19. *Am J Respir Crit Care Med.* 2020;201(11):1430–1434. doi: 10.1164/rccm.202003-0736LE. DOI: [PMCID: PMC7258632] [PubMed: 32267160] [CrossRef: 10.1164/rccm.202003-0736LE]

15. Palmieri L, Vanacore N, Donfrancesco C, Lo Noce C, Canevelli M, Punzo O, et al. Clinical characteristics of hospitalized individuals dying with COVID-19 by age group in Italy. *J Gerontol A Biol Sci Med Sci.* 2020;75(9):1796–1800. doi: 10.1093/gerona/glaa146. DOI: [PMCID: PMC7314182] [PubMed: 32506122] [CrossRef: 10.1093/gerona/glaa146]

16. Biagi A, Rossi L, Malagoli A, Zanni A, Sticozzi C, Comastri G, et al. Clinical and epidemiological characteristics of 320 deceased patients with COVID-19 in an Italian Province: a retrospective observational study. *J Med Virol.* 2020;92(11):2718–2724. doi: 10.1002/jmv.26147. DOI: [PMCID: PMC7300458] [PubMed: 32515500] [CrossRef: 10.1002/jmv.26147]

17. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. *JAMA.* 2020;323(16):1574–1581.

doi: 10.1001/jama.2020.5394. DOI: [PMCID: PMC7136855] [PubMed: 32250385] [CrossRef: 10.1001/jama.2020.5394]

18. Quah P, Li A, Phua J. Mortality rates of patients with COVID-19 in the intensive care unit: a systematic review of the emerging literature. *Crit Care.* 2020;24(1):285. doi: 10.1186/s13054-020-03006-1. DOI: [PMCID: PMC7271132] [PubMed: 32498689] [CrossRef: 10.1186/s13054-020-03006-1]

19. Rajgor DD, Lee MH, Archuleta S, Bagdasarian N, Quek SC. The many estimates of the COVID-19 case fatality rate. *Lancet Infect Dis.* 2020;20(7):776–777. doi: 10.1016/S1473-3099(20)30244-9. DOI: [PMCID: PMC7270047] [PubMed: 32224313] [CrossRef: 10.1016/S1473-3099(20)30244-9]

20. Pellaud C, Grandmaison G, Pham Huu Thien HP, Hoa Phong PH, Baumberger M, Carrel G, et al. Characteristics, comorbidities, 30-day outcome and in-hospital mortality of patients hospitalised with COVID-19 in a Swiss area – a retrospective cohort study. *Swiss Med Wkly.* 2020;150:w20314. doi: 10.4414/smw.2020.20314. DOI: [PubMed: 32662869] [CrossRef: 10.4414/smw.2020.20314]

21. United Nations, Department of Economic and Social Affairs. World population ageing 2019 highlights; https://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2019-Highlights.pdf 2019 Available from:

22. Singh AK, Gillies CL, Singh R, Singh A, Chudasama Y, Coles B, et al. Prevalence of co-morbidities and their association with mortality in patients with COVID-19: a systematic review and meta-analysis. *Diabetes Obes Metab.* 2020;22(10):1915–1924. doi: 10.1111/dom.14124. DOI: [PMCID: PMC7361304] [PubMed: 32573903] [CrossRef: 10.1111/dom.14124]

23. Singh AK, Gupta R, Misra A. Comorbidities in COVID-19: outcomes in hypertensive cohort and controversies with renin angiotensin system blockers. *Diabetes Metab Syndr.* 2020;14(4):283–287. doi: 10.1016/j.dsx.2020.03.016. DOI: [PMCID: PMC7144598] [PubMed: 32283499] [CrossRef: 10.1016/j.dsx.2020.03.016]

24. Sun YJ, Feng YJ, Chen J, Li B, Luo ZC, Wang PX. Clinical features of fatalities in patients with COVID-19. *Disaster Med Public Health Prep.* 2020:1–3. doi: 10.1017/dmp.2020.235. DOI: [PMCID: PMC7443557] [PubMed: 32665052] [CrossRef: 10.1017/dmp.2020.235]

25. Liu S, Yao N, Qiu Y, He C. Predictive performance of SOFA and qSOFA for in-hospital mortality in severe novel coronavirus disease. *Am J Emerg Med.* 2020;38(10):2074–2080. doi: 10.1016/j.ajem.2020.07.019. DOI: [PMCID: PMC7354270] [PubMed: 33142178] [CrossRef: 10.1016/j.ajem.2020.07.019]

26. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395(10229):1054–1062. doi: 10.1016/S0140-6736(20)30566-3. DOI: [PMCID: PMC7270627] [PubMed: 32171076] [CrossRef: 10.1016/S0140-6736(20)30566-3]

27. Martinez AC, Dewaswala N, Tuarez FR, Pino J, Chait R, Chen K, et al. Validation of SOFA score in critically ill patients with COVID-19. *Chest.* 2020;158(4):A613. doi: 10.1016/j.chest.2020.08.577. DOI: [CrossRef: 10.1016/j.chest.2020.08.577]

28. Chen Y, Li T, Ye Y, Chen Y, Pan J. Impact of fundamental diseases on patients with COVID-19. *Disaster Med Public Health Prep.* 2020:1–6. doi: 10.1017/dmp.2020.139. DOI: [PMCID: PMC7240136] [PubMed: 32375909] [CrossRef: 10.1017/dmp.2020.139]

29. Ahmed AR, Ebad CA, Stoneman S, Satti MM, Conlon PJ. Kidney injury in COVID-19. *World J Nephrol.* 2020;9(2):18. doi: 10.5527/wjn.v9.i2.18. DOI: [PMCID: PMC7701935] [PubMed: 33312899] [CrossRef: 10.5527/wjn.v9.i2.18]

30. Keeley P, Buchanan D, Carolan C, Pivodic L, Tavabie S, Noble S. Symptom burden and clinical profile of COVID-19 deaths: a rapid systematic review and evidence summary. *BMJ Support Palliat Care.* 2020;10(4):381–384. doi: 10.1136/bmjspcare-2020-002368. DOI: [PubMed: 32467101] [CrossRef: 10.1136/bmjspcare-2020-002368]

31. Chang TH, Wu JL, Chang LY. Clinical characteristics and diagnostic challenges of pediatric COVID-19: a systematic review and meta-analysis. *J Formos Med Assoc.* 2020;119(5):982–989. doi: 10.1016/j.jfma.2020.04.007. DOI: [PMCID: PMC7161491] [PubMed: 32307322] [CrossRef: 10.1016/j.jfma.2020.04.007]

32. Patel NA. Pediatric COVID-19: systematic review of the literature. *Am J Otolaryngol.* 2020;41(5):102573. doi: 10.1016/j.amjoto.2020.102573. DOI: [PMCID: PMC7833675] [PubMed: 32531620] [CrossRef: 10.1016/j.amjoto.2020.102573]

33. Jeng MJ. Coronavirus disease 2019 in children: current status. *J Chin Med Assoc.* 2020;83(6):527–533. doi: 10.1097/JCMA.0000000000000323. DOI: [PMCID: PMC7199766] [PubMed: 32502117] [CrossRef: 10.1097/JCMA.0000000000000323]

Figures and Tables

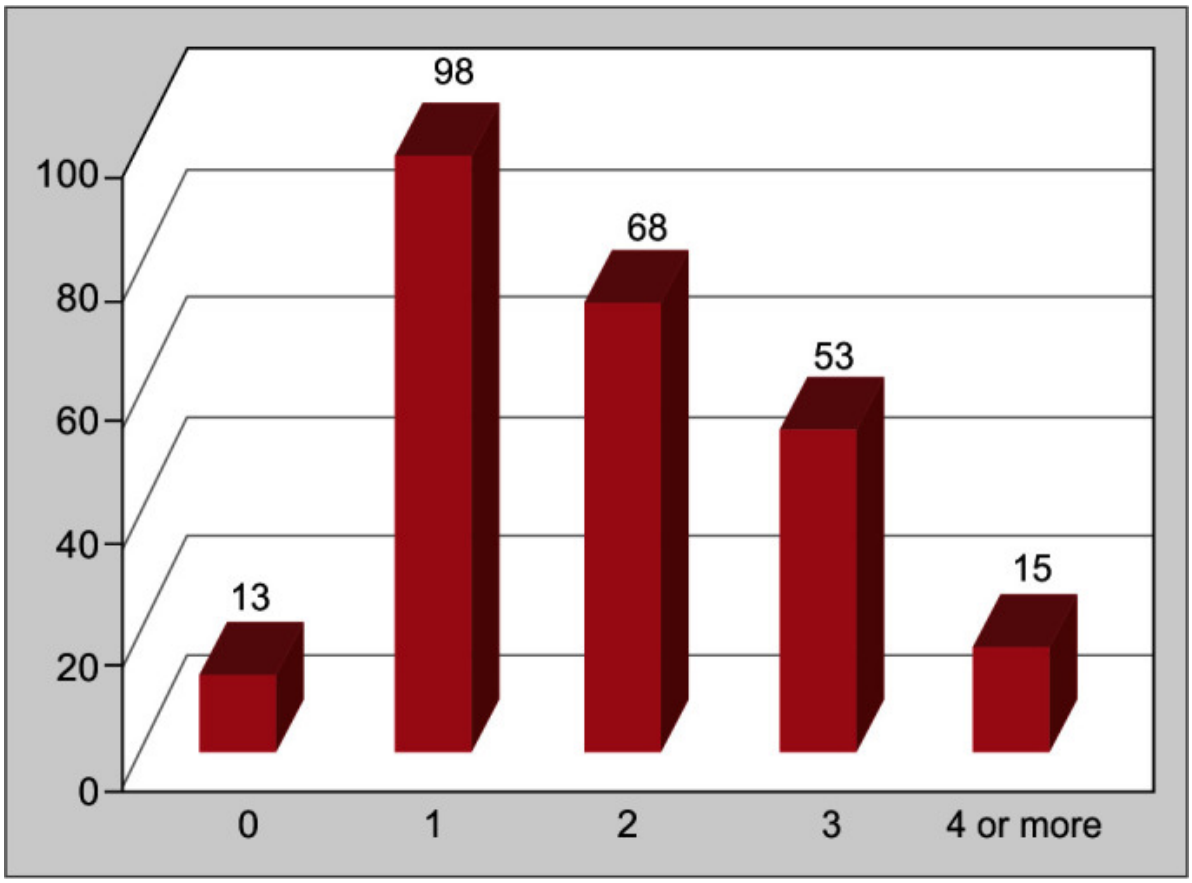
Table 1

Clinical characteristics of study patients

Characteristics	***Patients***
Age median (IQR) (years)	56 (41.5–65)
Age distribution (years)	**No. (%)**
18–50	104/247 (42.1)
51–65	86/247 (34.8)
More than 65	57/247 (23.1)
Male sex (%)	163/247 (65.9)
Comorbidities	**No. of patients (%)**
Hypertension	107 (43.3)
Diabetes mellitus	86 (34.8)
Chronic kidney disease	51 (20.6)
Coronary artery disease	40 (16.2)
Malignancy	29 (11.7)
Cerebrovascular event	29 (11.7)
Tuberculosis	21 (8.5)
Chronic liver disease	19 (7.7)
Hypothyroidism	13 (5.3)
Chronic obstructive airway disease	11 (4.5)
Trauma	7 (2.8)
Rheumatic heart disease	5 (2.0)
Others	55 (22.3)

IQR, interquartile range

Fig. 1



Frequency of patients with different number of comorbidities

Table 2

Distribution of presenting complaints

Presenting complaints	*No. (%)*
Fever	187 (75.7)
Cough	170 (68.8)
Shortness of breath	167 (67.6)
Altered sensorium	49 (19.8)
Chest pain	13 (5.3)
Abdominal pain/distension	12 (4.9)
Weakness	6 (2.4)
Diarrhea	6 (2.4)
Headache	4 (1.6)
Others	50 (20.2)

Table 3

SOFA scoring, modes of oxygen therapy given, and renal complications in deceased

ICU parameters	*Mean (SD) or No. of Patients (%)*
Initial SOFA (232 patients)	9.3 (4.07)
SOFA at 72 hrs (133 patients)	10.2 (3.9)
Patients received intubated	60 (24.2)
Patients intubated within first 24 hrs	75 (30.3)
Patients received HFNC	112 (45.3)
Patients received NIV	45 (18.2)
Patients developed AKI	121 (48.9)
Patients received RRT	39 (15.8)

SOFA, sequential organ failure assessment; HFNC, high-flow nasal cannula; NIV, noninvasive ventilation; AKI, acute kidney injury; RRT, renal replacement therapy

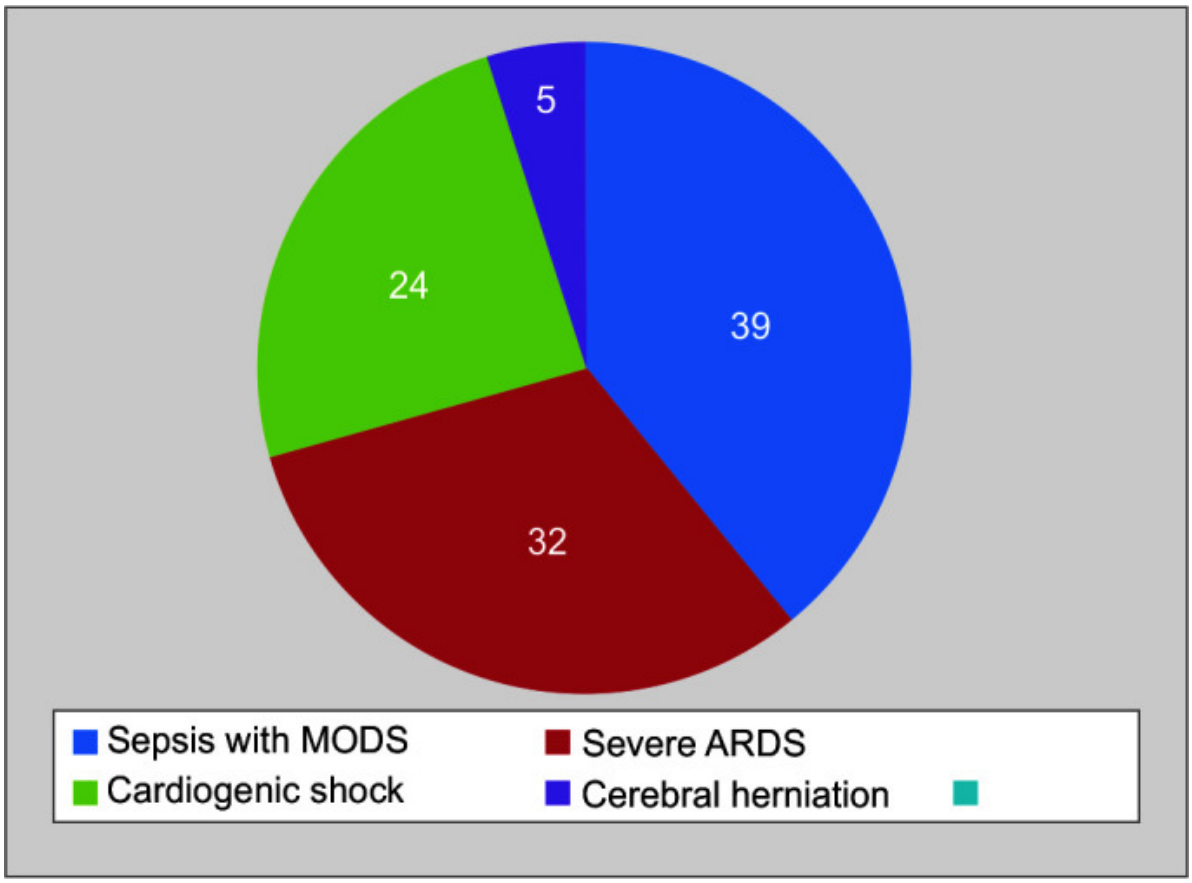
Table 4

Cause of death

Sl. No.	*Cause of death*	*Frequency (% of patients)*
1	Sepsis with multi organ dysfunction	136 (55.1)
2	Severe ARDS	63 (25.5)
3	Cardiogenic shock	23 (9.3)
4	Stroke/cerebral bleed	17 (6.9)
5	Pulmonary embolism	7 (2.8)
6	Hemorrhage	1 (0.40)

MODS, multi-organ dysfunction syndrome; ARDS, acute respiratory distress syndrome

Fig. 2



Cause of death in patients who died within 24 hours of admission to COVID-19 center. MODS, multi-organ dysfunction; ARDS, acute respiratory distress syndrome

Table 5

Characteristics of the patients according to different age-groups

Total admissions in COVID-19 center	*Age-group (years)*			*p value*
	Age-group (years)			*p value*
	18–50 n (%)	*51–65 n (%)*	*>65 n (%)*	
ICU admissions (% of total ICU admissions)	344 (48.6)	199 (28.1)	111 (15.7)	<0.001
ICU mortality (%)	104 (30.2)	86 (43.2)	57 (51.3)	<0.001
Sex (male:female)	65:39	57:29	41:16	0.48
Number of comorbidities (% of patients)				
0	9 (8.6)	3 (3.5)	1 (1.7)	
1	49 (47.1)	32 (37.2)	17 (29.8)	
≥ 2	46 (44.2)	51 (59.3)	39 (68.4)	0.024
Presenting complaints (% of patients)				
Fever	77 (74.0)	65 (75.6)	45 (78.9)	0.78
Cough	71 (68.3)	56 (65.1)	43 (75.4)	0.42
Breathlessness	67 (64.4)	58 (67.4)	42 (73.7)	0.48
Altered sensorium	21 (20.2)	15 (17.4)	13 (22.8)	0.72
Abdominal pain/distension	6 (5.8)	6 (6.9)	0 (0)	0.11
Chest pain	3 (2.9)	8 (9.3)	2 (3.5)	0.14
Nonspecific symptoms	28 (26.9)	16 (18.6)	6 (10.5)	0.04
Initial SOFA mean (SD)	9.8 (3.9)	9.2 (4.1)	8.4(3.9)	0.86
Patients ventilated within first 24 hrs including patients received intubated	57 (54.8)	51(59.3)	27 (47.3)	0.37
Patients given HFNC	47 (45.1)	35 (40.7)	30 (52.6)	0.37
Patients given NIV	21(20.1)	11 (12.7)	13 (22.8)	0.24
Patients developing AKI	51 (49.0)	45 (52.3)	25 (43.8)	0.61
Patients received RRT	20	12	7	0.43

SOFA, sequential organ failure assessment; HFNC, high flow nasal cannula; NIV, noninvasive ventilation; AKI, acute kidney injury; RRT, renal replacement therapy

Table 6

Pediatric patients

Sl. No.	*Age (years)/sex*	*Presenting symptoms*	*Length of stay in hospital*	*Comorbidities*	*Cause of death*
1	0.5/M	Fever	2	Cyanotic congenital heart disease	ARDS
2	3/M	Fever, shortness of breath	7	ALL with CNS metastasis	Sepsis
3	13/M	Fever, vomiting, hematemesis	1	Aplastic anemia	ARDS
4	12/M	Shortness of breath	7	DM, Hypothyroid, nephrotic syndrome	ARDS
5	15/M	Fever, altered sensorium	1	TB meningitis with hydrocephalus	Cerebral herniation
6	16/F	Fever, cough	8	AML with fungal pneumonia	Acute pulmonary hemorrhage

MODS, multi-organ dysfunction syndrome; ARDS, acute respiratory distress syndrome; ALL, acute lymphoblastic leukemia; AML, acute myeloid leu-kemia; CNS, central nervous system; DM, diabetes mellitus; TB, tuberculosis

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Original Article

Effect of proning in patients with COVID-19 acute hypoxemic respiratory failure receiving noninvasive oxygen therapy

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ABSTRACT

Background: Novel coronavirus (SARS-CoV-2) infection is associated with hypoxemic respiratory failure. Mechanical ventilation (MV) is reported to have high mortality in SARS-CoV-2 acute respiratory distress syndrome. We aimed to investigate whether awake prone positioning (PP) can improve oxygenation and prevent intubation when employed early. **Methods:** This prospective interventional study included proven coronavirus disease 2019 (COVID-19) patients with room air saturation 93% or less. The primary outcome was the rate of intubation between the two groups. The secondary outcomes included ROX index (SpO_2/FiO_2%/respiratory rate, breaths/min) at 30 min following the intervention, ROX index at 12 h, time to recovery of hypoxemia, and mortality. **Results:** A total of 45 subjects were included (30 cases and 15 controls) with a mean (standard deviation [SD]) age of 53.1 (11.0) years. The age, comorbidities, and baseline ROX index were similar between the two groups. The median duration of PP achieved was 7.5 h on the 1st day. The need for MV was higher in the control group (5/15; 33.3%) versus prone group (2/30; 6.7%). At 30 min, there was a statistically significant improvement in the mean (SD) ROX index of cases compared with that of the controls (10.7 [3.8] vs. 6.7 [2.6], $P < 0.001$). No significant adverse effects related to intervention were noted. **Conclusion:** Awake PP is associated with significant improvement in oxygenation and may reduce the need for MV in subjects with COVID-19.

KEY WORDS: Awake proning, COVID-19, prone positioning, SARS-CoV-2

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) has resulted in a significant number of critically ill patients requiring intensive unit care admissions. A systematic review of intensive care unit mortality of COVID-19 reported mortality of 31.1%–79.0% among patients on invasive mechanical ventilation (MV).[1] Among intubated patients

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with severe acute respiratory distress syndrome (ARDS), prolonged prone positioning (PP) for at least 12–16 h/day, is associated with significant improvement in oxygenation and mortality.[2] During this pandemic, there is a pressing need to conserve essential resources, and awake PP may be useful to improve oxygenation and reduce the need for MV.[3] There is emerging data on the same, but most studies lack a control group to provide information about the definite utility of this intervention. This study aimed to determine whether the early use of PP combined with noninvasive modalities of oxygen therapy can improve oxygenation and avoid the need for intubation in patients with hypoxemic respiratory failure due to COVID-19.

METHODS

This prospective, interventional study was conducted in Delhi, India, after obtaining approval from the institutional ethics board (IEC-308/27.04.2020, AA-4/08.05.2020). Subjects with nasopharyngeal swab RT-PCR-confirmed COVID-19, having room air pulse oxygen saturation (SpO_2) <94%, were included in the study. We excluded subjects with hypercapnic respiratory failure, hemodynamic instability, altered sensorium; those requiring immediate tracheal intubation; those with duration of hypoxia or hospitalization for more than 12 h; those with obesity with body mass index (BMI) >30 kg/m^2; those with PaO_2/FiO_2 <100 on noninvasive ventilation (NIV)/high-flow nasal cannula (HFNC); and having an intolerance to PP. All consecutive subjects admitted with hypoxemic respiratory failure were screened. The treating team decided the mode of oxygen delivery among conventional oxygen therapy, NIV, or HFNC as per the availability. Subjects who did not allow for PP underwent standard treatment and were included as controls for study purpose. Baseline variables, including age, comorbidities, radiological involvement, symptom duration, and severity of illness, were recorded. The severity of pneumonia was assessed by chest X-ray severity scoring.[4] On chest X-ray, divided into three zones in each lung, a severity score was assigned based on the presence or absence of opacity in each zone (maximum score 6, minimum 0).

The protocol followed for proning has been previously published.[5] Patients undergoing PP were assisted in changing positions. Proning was made comfortable by the use of multiple pillows over pressure points to avoid pain. Prone position was maintained for a minimum of 2 h per session and with a target duration of 8 h/day. A reverse Trendelenburg position of the bed was used to help increase comfort. The PP was stopped if there were patient intolerance, worsening of hypoxia, or the patient has recovered from respiratory failure. In case of any worsening hypoxia with respiratory distress, technical problems were checked and corrected, and prone position was withdrawn. Any respiratory distress with increased work of breathing was monitored, and MV was employed as per the discretion of the treating team. There was no protocolized transition to other noninvasive methods of oxygen delivery so as to avoid any delay in instituting MV. Recovery was defined as sustained improvement in oxygenation defined as more than 93% saturation in room air for at least 2 h following supination. Patients were followed up until hospital discharge or death.

ROX index (SpO_2/FiO_2%/respiratory rate, breaths/min) was monitored for improvement in oxygenation at baseline, 30 min, and 12 h in both the groups. ROX index is simple to calculate at the bedside and gives a summary of the patient's degree of hypoxemic respiratory failure.[6]

The primary outcome was the rate of intubation between the two groups. The secondary outcomes included ROX index at 30 min from the start of the intervention, ROX index at 12 h, days to the recovery of hypoxia (defined as room air SpO_2 >93%), and mortality.

Statistical analysis was done using Stata version 14.0 (StataCorp, Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). Continuous variables were compared with *t*-tests and categorical variables with a Chi-square test. The paired *t*-test was used to compare pre- and post-PP change in oxygenation parameters of cases if normally distributed.

RESULTS

Among 78 subjects with COVID-19-related hypoxemic respiratory failure, 45 were enrolled following the inclusion and exclusion criteria. Among the 45 subjects included in the study, 30 patients received PP, while 15 were in the control group [Figure 1]. The mean (SD) age was 53.1 (11.0) years, with the majority being males. Age, comorbidities, and radiological severity were similar between the two groups [Table 1]. Medical management of both the groups included hydroxychloroquine (83% in cases and 73% in controls, $P = 0.45$) and corticosteroids (methylprednisolone 40 mg twice daily, 86.7% of both cases and controls) for 5 days. Baseline oxygenation, as measured by the ROX index, was similar in both the groups. The median duration of PP on the 1st day was 7.5 h (range, 4–12 h). The need for MV was higher in the control group (5/15; 33.3%) versus prone group (2/30; 6.7%). At 30 min from the initiation of proning, there was a statistically significant difference in the ROX index between cases and controls (10.7 ± 3.8 in cases vs. 6.7 ± 2.6 in controls, $P < 0.001$). At 12 h, both respiratory rate per minute (23.8 ± 3.4 among cases vs. 27.5 ± 4.6 among controls, $P = 0.004$) and ROX index (12.4 ± 4.5 among cases vs. 6.4 ± 3.0 among controls, $P < 0.001$) were significantly different between the two groups. The time to resolution of hypoxia as well as the mortality rate was not different. Adverse events associated with proning were mild and included backache (6.6%)

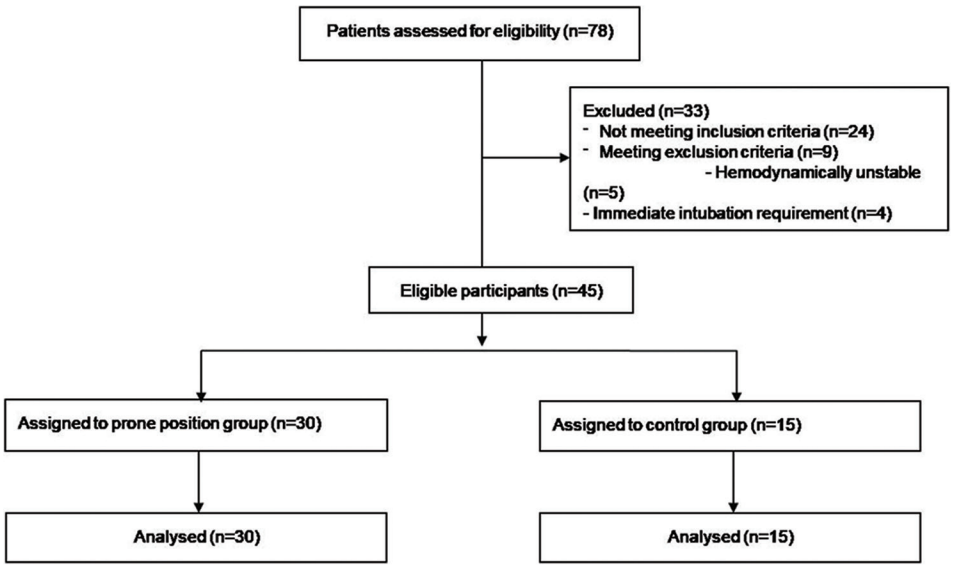


Figure 1: Participant flow in the study

Table 1: Baseline characteristics as well as outcome measures in the two groups

Characteristics	Total (*n*=45)	Cases (*n*=30)	Controls (*n*=15)	*P*
Demographic characteristics				
Age (year), mean±SD	53.1±11.0	50.9±10.1	57.5±12.2	0.06
Male, *n* (%)	38 (84.4)	29 (96.7)	9 (60)	0.001
Comorbidities				
Any comorbidity, *n* (%)	34 (75.6)	22 (73.3)	12 (80)	0.62
Hypertension, *n* (%)	19 (42.2)	12 (40)	7 (46.7)	0.67
Diabetes mellitus, *n* (%)	20 (44.4)	11 (36.7)	9 (60)	0.13
Symptom duration, days, median (minimum–maximum)	7 (2-16)	7 (3-16)	7 (2-15)	0.49*
Chest X-ray severity score 3 or more	42 (93.3)	29 (96.7)	13 (86.7)	0.2
Mode of oxygen supplementation *n* (%)				
NIV	2 (4.4)	1	1	NA
HFNC	1	1	0	NA
Conventional oxygen therapy	42 (93.3)	28	14	NA
Baseline vital parameters				
Respiratory rate (breaths/min), mean±SD	28.3±3.6	28.9±3.6	27±4.1	0.09
SpO_2 (%), mean±SD	92.9±3.4	92.4±2.8	94.1±4.3	0.11
ROX index, mean±SD	8.1±2.4	8.5±2.3	7.3±2.6	0.12
Duration of proning on the first day (h), median (minimum-maximum)	-	7.5 (4-12)	-	-
Primary outcome measure *n* (%)				
Requirement of mechanical ventilation	7 (15.6)	2 (6.7)	5 (33.3)	0.02
Secondary outcome measures				
Vital parameters at 30 min of prone positioning				
Respiratory rate (breaths/min), mean±SD	27.3±3.6	27.1±3.4	27.6±4.1	0.67
SpO_2 (%), mean±SD	95.1±1.9	94.8±1.7	95.5±2.5	0.26
ROX index	9.4±3.9	10.7±3.8	6.7±2.6	<0.001
Vital parameters at 12 h after the initiation of prone positioning				
Respiratory rate (breaths/min), mean±SD	25±4.2	23.8±3.4	27.5±4.6	0.004
SpO_2 (%), mean±SD	94.8±4.9	95.3±2.3	93.9±8.1	0.40
ROX index	10.4 (4.9)	12.4 (4.5)	6.4 (3.0)	< 0.001
Days to recovery of hypoxia, median (minimum–maximum) (among patients survived)	7 (3-20) (*n*=39)	6.5 (3-16) (*n*=28)	8 (3-20) (*n*=11)	0.14*
Death, *n* (%)	6 (13.3)	2 (6.7)	4 (26.7)	0.06

*Wilcoxon rank sum test. SD: Standard deviation, SpO_2: Oxygen saturation, NA: Not available, HFNC: High-flow nasal cannula, NIV: Noninvasive ventilation

and bloating sensation (6.6%). There were no episodes of desaturation or hemodynamic worsening. Among cases, the ROX index improved statistically significantly from 8.5 ± 2.3 at baseline to 10.7 ± 3.8 at 30 min ($P < 0.001$). There was also an improvement in heart rate, respiratory rate, and saturation at 30 min and 12 h [Table 2].

DISCUSSION

In this study of COVID-19 patients with hypoxic respiratory failure, there was a lower rate of need for MV as well as a significant improvement in the oxygenation parameters with awake PP as compared to that of the control group.

Table 2: Baseline, 30 min, and 12 h postintervention parameters among cases (*n*=30)

Parameter	Baseline	30 min postprone	*P**	12 h postprone	*P**
Heart rate (beats/min), mean±SD	105.9±11.6	102±12.3	0.03	92.8±11.7	<0.001
Respiratory rate (breaths/min), mean±SD	28.9±3.6	27.1±3.4	<0.001	23.8±3.4	<0.001
Pulse oxygen saturation (%)	92.4 (2.8)	94.8 (1.7)	<0.001	95.3 (2.3)	<0.001
ROX index	8.5 (2.3)	10.7 (3.8)	<0.001	12.4 (4.5)	<0.001

*Compared to baseline. SD: Standard deviation, SpO_2: Oxygen saturation

Proning in COVID-19 hypoxemic respiratory failure has been shown to result in improved oxygenation in multiple small studies.[7-11] PP can improve oxygenation by multiple mechanisms, resulting in improved ventilation–perfusion matching. Most studies have demonstrated an improvement in PaO_2/FiO_2 ratio and the respiratory rate among patients who tolerated a session of PP. The prone sessions reported in these studies were short, partly because of limited patient tolerance. The effects were transient, and respiratory rates and oxygenation parameters often returned to baseline after supination.[9] Most of these studies were limited by their retrospective design and lacked a control group. Our study stands apart as we included a comparison group, though in a nonrandomized fashion. We were able to achieve a longer duration of PP, with a median of 7 h/day.

The ROX index was used as it reflects an objective measure of work of breathing. ROX index was validated for identifying patients requiring intubation in hypoxic respiratory failure treated with HFNC.[12] ROX at 4 h of starting noninvasive oxygen therapy ≥5.37 was significantly associated with a lower risk for intubation in COVID-19 hypoxemic respiratory failure in intensive care admitted patients from a retrospective single-center study.[13]

Caution against early intubation has been raised in COVID-19 ARDS, and assessment of work of breathing is an essential determinant of the same.[14] We included patients early during the respiratory failure as this subgroup of patients are likely to benefit most from PP. Due to the limited sample size, we cannot conclude on the minimum duration of proning, which may be beneficial for crucial clinical outcomes. We also did not find any harm associated with the intervention in terms of mortality or recovery of hypoxia. The reported mortality rate in another prospective study of awake proning in COVID-19 was 43.5% in the prone position group, compared with 28 (75.7%) COVID-19 patients in the nonprone position group.[15]

There are several limitations to our study. It was a nonrandomized study and is subjected to bias. The tolerance of patients to PP was not measured as we excluded patients, not tolerating PP. We did not perform regular ABG, which is considered best to assess oxygenation and used noninvasive surrogates. All patients included were hemodynamically stable and had a similar baseline oxygenation index (ROX), which suggests homogeneity between cases and controls with respect to the severity of hypoxia. The mean ROX index in our study was 8.1, which suggests a milder form of hypoxemic respiratory failure in the included population. Computed tomography depiction of lung involvement has been used in some studies to predict the underlying physiology and determine the utility of PP; however, this was not done in our study due to feasibility issues.[15,16]

CONCLUSION

We conclude that awake PP, when employed early, for patients with COVID-19-associated hypoxemic respiratory failure, improves oxygenation, and may obviate the need for MV. Further randomized controlled trials are needed to assess its effect on mortality, although it may be difficult to perform in such a pandemic situation.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Quah P, Li A, Phua J. Mortality rates of patients with COVID-19 in the intensive care unit: A systematic review of the emerging literature. Crit Care 2020;24:285.
2. Munshi L, Del Sorbo L, Adhikari NKJ, Hodgson CL, Wunsch H, Meade MO, *et al*. Prone position for acute respiratory distress syndrome. A systematic review and meta-analysis. Ann Am Thorac Soc 2017;14:S280-8.
3. Sun Q, Qiu H, Huang M, Yang Y. Lower mortality of COVID-19 by early recognition and intervention: Experience from Jiangsu Province. Ann Intensive Care 2020;10:33.
4. Toussie D, Voutsinas N, Finkelstein M, Cedillo MA, Manna S, Maron SZ, *et al*. Clinical and chest radiography features determine patient outcomes in young and middle age adults with COVID-19. Radiology 2020;29:201754.
5. Sryma PB, Mittal S, Madan K, Mohan A, Hadda V, Tiwari P, *et al*. Reinventing the wheel in ARDS: Awake proning in COVID-19. Arch Bronconeumol 2020;56:747-9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332953/. [Last acessed on 2020 Jul 22].
6. Hill NS, Ruthazer R. Predicting outcomes of high-flow nasal cannula for acute respiratory distress syndrome. An index that ROX. Am J Respir Crit Care Med 2019;199:1300-2.
7. Despres C, Brunin Y, Berthier F, Pili-Floury S, Besch G. Prone positioning combined with high-flow nasal or conventional oxygen therapy in severe Covid-19 patients. Critical Care 2020;24:256.
8. Coppo A, Bellani G, Winterton D, Di Pierro M, Soria A, Faverio P, *et al*. Feasibility and physiological effects of prone positioning in non-intubated patients with acute respiratory failure due to COVID-19 (PRON-COVID): A prospective cohort study. Lancet Respir Med 2020;8:765-74.
9. Elharrar X, Trigui Y, Dols A-M, Touchon F, Martinez S, Prud'homme E, *et al*. Use of prone positioning in nonintubated patients with COVID-19 and hypoxemic acute respiratory failure. JAMA 2020;323:2336-8. Available from: https://jamanetwork.com/journals/jama/fullarticle/2766292. [Last accessed on 2020 May 22].

10. Sartini C, Tresoldi M, Scarpellini P, Tettamanti A, Carcò F, Landoni G, *et al*. Respiratory parameters in patients with COVID-19 after using noninvasive ventilation in the prone position outside the intensive care unit. JAMA 2020;323:2338-40. Available from: https://jamanetwork.com/journals/jama/fullarticle/2766291. [Last accessed on 2020 May 22].
11. Caputo ND, Strayer RJ, Levitan R. Early self-proning in awake, non-intubated patients in the emergency department: A single ED's experience during the COVID-19 pandemic. Acad Emerg Med 2020;27:375-8. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/acem.13994. [Last accessed on 2020 Apr 30].
12. Roca O, Caralt B, Messika J, Samper M, Sztrymf B, Hernández G, *et al*. An index combining respiratory rate and oxygenation to predict outcome of nasal high-flow therapy. Am J Respir Crit Care Med 2019;199:1368-76.
13. Zucman N, Mullaert J, Roux D, Roca O, Ricard JD, Contributors. Prediction of outcome of nasal high flow use during COVID-19-related acute hypoxemic respiratory failure. Intensive Care Med 2020;46:1924-6.
14. Tobin MJ, Laghi F, Jubran A. Caution about early intubation and mechanical ventilation in COVID-19. Ann Intensive Care 2020;10:78.
15. COVID-19 Early Prone Position Study Group, Zang X, Wang Q, Zhou H, Liu S, Xue X. Efficacy of early prone position for COVID-19 patients with severe hypoxia: A single-center prospective cohort study. Intensive Care Med 2020;46:1927-9. Available From: http://link.springer.com/10.1007/s00134-020-06182-4. [Last acessed on 2020 Aug 01].
16. Gattinoni L, Coppola S, Cressoni M, Busana M, Rossi S, Chiumello D. Covid-19 does not lead to a "Typical" acute respiratory distress syndrome. Am J Respir Crit Care Med 2020;201:1299-300.

Comparative study between insulin bolus regimen and glucose insulin infusion regimen on effectiveness of intraoperative blood glucose control in patients with type 2 diabetes mellitus undergoing non-cardiac surgery

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Background: The surgical rates are comparatively greater among diabetic patients. Surgery, a period of stress, mandates fasting and anti-diabetic medications are skipped on the morning of the procedure. High blood glucose levels in the peri-operative period can lead to ischaemia, infection, acute kidney injury and electrolyte disturbances. Hence, control of blood glucose levels during the peri-operative period becomes important and there are various regimens described. We compared two of the existing regimens, intravenous insulin bolus and glucose insulin infusion regimen, for their effectiveness of intraoperative blood glucose control.

Method: In this randomized, prospective controlled study, 120 patients were enrolled with 60 patients in bolus group and 60 in infusion group. Capillary blood glucose was measured every hour during the intra-operative period. The primary outcome measure was proportion of patients in the target glucose levels between 100 mg/dL and 180 mg/dL and proportion of intra-operative surgery time (in minutes) during which patient was in the target range. Secondary outcome measure was serum potassium levels pre-operatively and post-operatively.

Results: The proportion of patients in the target range in the bolus group was 39 out of 60 as compared to 31 out of 60 in the infusion group (P=0.13). Also, proportion of intraoperative surgery time (in minutes) during which patient was in the target glycaemic range was 41.57% in bolus group when compared to 27.22% in the infusion group (P < 0.05). No hypoglycaemic episodes were seen in any of the patients in the two groups.

Conclusion: As compared with infusion regimen, bolus regimen provides better glycaemic control measured in terms of target blood glucose levels and duration for which the patient remained in the target range.

Keywords: Diabetes mellitus; intraoperative glucose management

Introduction

The rate of surgeries in diabetic patients are more when compared to non-diabetic patients.[1,2] Surgery under general or regional anaesthesia requires patient to be nil by mouth and glucose control medications omitted during this period. Glucose balance in the body can change due to metabolic disturbances occurring as a result of surgical stress.

Continuous high blood glucose levels can predispose to infection post-surgery, ischaemia of brain and impaired wound healing.[3] It is also a risk factor for diabetic emergencies perioperatively, which can lead to negative prognostic consequences. Gastrointestinal derangements induced by drugs, stress and anaesthesia can cause nausea, vomiting and dehydration. Also, osmotic diuresis induced by hyperglycaemia leads to volume contraction. These place the patients at risk for acute kidney injury. Electrolyte imbalance because of altered glucose levels may increase the risk for arrhythmias. Hence, the metabolic profile of these patients who undergo surgery must be attended to perioperatively.[3-6] The focus of these diabetic patients during surgery is prevention of either severely low or high blood sugar levels.[5]

There are many regimens that are described for perioperative blood glucose control each having its own advantages and disadvantages.

Intravenous insulin bolus is simple and reliable method for intra-operative glycaemic control and has been described by Poldermann et al.[7] Intravenous insulin infusion along with glucose is another popular regimen for intra-operative blood glucose management.[8] We compared the intra-operative glycaemic levels between insulin

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bolus regimen and glucose insulin infusion regimen. The aim was to compare the intraoperative capillary blood glucose levels between intravenous insulin bolus regimen and glucose insulin infusion regimen in diabetic patients. The primary objective was to compare the intra-operative capillary blood glucose levels in patients managed using insulin bolus and glucose insulin infusion regimen. The secondary objective was to compare the preoperative and postoperative serum potassium levels.

Methods

Trial design

This randomized, prospective study was a single centred trial conducted at Kasturba Hospital, Manipal, Karnataka, India, after obtaining approval of the Institutional Ethics Committee. The study was registered with Clinical Trials Registry India (CTRI/2017/12/011037).

Eligibility criteria

Patients with type 2 diabetes mellitus (diagnosed from pat history or medicine consultation) between 18 and 70 years with preoperative fasting blood glucose value > 150 mg/dL undergoing elective and emergency non-cardiac surgery of at least one hour were eligible for inclusion. Patients scheduled under local anaesthesia were not included. Patients with known history of cardiac, respiratory, renal, hepatic failure (either compensated or decompensated, as indicated by physician's evaluation and laboratory investigations), those having active infection or taking steroids (irrespective of whether on insulin therapy or not) were excluded. Also, pregnant females and those with serum potassium below the reference range (<3.5 mmol/L) were excluded.

Randomization

Consecutive patients scheduled for surgery were considered for enrolment. If eligible, the participants were randomized to one of the two groups, group bolus or group infusion in the study.

We used simple randomization method. Randomization was done with the help of a computer-generated table. The principal investigator generated the random allocated sequence, enrolled participants and assigned them to interventions. Patients and investigators were not blinded for the treatment.

Interventions

During preanaesthetic evaluation the patients were explained about the study methodology and written informed consent for participation in the study was obtained. Preoperative fasting instruction was nil per orally 8 hours for solids and 2 hours for clear fluids (based on local practice guidelines). All patients received acid aspiration prophylaxis with ranitidine 150mg and metoclopramide 10mg orally, on the previous night and on the morning of the surgery. All patients were advised to skip the oral hypoglycaemic agents or insulin injections on the day of surgery. Patients who had received long acting insulin on the night before the surgery were started on 5% dextrose in 0.9% saline infusion with insulin added based on morning value of fasting blood glucose. During fasting period, decision to start on 5% dextrose in 0.9% saline with neutralizing dose of insulin was as per the concerned anaesthesiology consultant in charge of the operating room. This fluid was discontinued in the operating room. The technique of anaesthesia depended upon the requirement of the patient as per the concerned anaesthesiology consultant in charge of the operating room. After arrival of the patient in the operating room and before induction of anaesthesia, random capillary blood glucose was measured (zero hour) and patients with values of >150 mg/dL were included in the study.[15] Subsequently capillary blood glucose was measured every hourly using glucometer (Accu-Chek Performa and Accu-Chek Performa glucose strips; error of measurement is ±15% of the measured glucose values when compared with standard laboratory values) until the end of the surgery.

For the patients in *group bolus*, intravenous insulin - Monocomponent Human Insulin, biosynthetic r-DNA origin – Human Actrapid® (manufactured by Torrent Pharmaceuticals Ltd, Indrad 382 721, Dist: Mehsana, India, under license from Novo Nordisk India Private Ltd. Whitefield, Bangalore) was administered as given in Table 1.[7]

This regimen was adapted from a trial published by Poldermann et al, converting mmol/L to mg/dL using multiplication factor 18 for conversion of mmol/L to mg/dL (1mmol/L = 18 mg/dL), since the common mode of blood glucose measurement in our practice is in mg/dL.[7]

Table 1: Bolus insulin regimen

Glucose measurement (mmol/L)	Glucose Measurement (mg/dL)	Insulin bolus (IU)
4-8	72-144	-
8-9	145-162	2
9-10	163-180	3
10-11	181-198	4
11-12	199-216	5
12-13	217-234	6
13-14	235-252	7
14-15	253-270	8
15-16	271-288	9
>16	>288	10

In an event of hypoglycaemia (blood glucose level < 70 mg/dL), treatment was provided as bolus 100ml 5% dextrose in water (D5W) rapidly followed by repeat blood sugar level after 15mins. For the patients in *group infusion*, insulin was administered as infusion in 100ml of D5W taken in a burette set as given in Table 2.[8] Before the start of infusion, 10ml of 5% D5W + 1 IU insulin was added and mixed well in the burette, flushed through the tubings and discarded. After measuring the blood glucose value, burette was filled with the 100ml of infusing solution and according to Table 2 insulin was added to the burette through the side arm using an insulin syringe. The blood glucose measurements were performed when the burette got empty at the end of one hour, using a glucometer.

Patients in both the groups received 0.9% saline to meet their fluid requirements. In both the groups, preoperative serum potassium value was compared with the postoperative serum potassium level measured after 1 hour of surgery.

Outcome measures

The primary outcome measures were (a) The proportion of patients in the target glucose levels between 100mg/dl and 180mg/dl and (b) proportion of intraoperative surgery time (in minutes) during which patient was in the target glucose range. The secondary outcome measure was serum potassium level.

Sample size

With 80% power and a difference of 10% of patients who could be maintained in the target glucose level (100-180mg/dl) between the two studied regimens considered clinically significant, the sample size was estimated to be 360. Since the estimated sample size was relatively large with 360 patients, provision was made during the study design phase for two interim analyses i.e. when 120 patients and then 240 patients were enrolled. Interim analysis was done when two arms had a sample size of 60 each. It was found that the desired power and effect was achieved.

Table 2: Insulin infusion regimen

Blood glucose (mg/dL)	Treatment
<70	Stop insulin infusion if any. Rapidly infuse 100ml of D5W. Measure blood sugar after 15 min.
71 to 100	Stop insulin if any. Infuse 100ml/h of D5W
101 to 150	1 U of insulin in100ml of D5W/h
151 to 200	2 U of insulin in100ml of D5W/h
201 to 250	3 U of insulin in100ml of D5W/h
251 to 300	4 U of insulin in100ml of D5W/h
More than 300	1 U of insulin for every 1–50mg >100mg/dl in 100ml of NS/h

D5W - 5% dextrose in water; NS - normal saline

Statistical methods

The proportion of patients in the target glucose levels between 100mg/dl and 180mg/dl and the proportion of intra-operative surgery time (in minutes) during which the patient was in the target range were analysed with Chi square test. The secondary outcome measure (serum potassium level compared between preoperative value and postoperative value within the group) was tested with t test. Other statistical tests were used appropriately as required. SPSS 19 for Windows was used for statistical analysis.

Results

Patient characteristics and duration of surgery are given in Table 3. CONSORT flow chart is given in Figure 1. Comparison of proportion of patients in the target glycaemic range showed that 39 patients (65%) in bolus group and 31 patients (51.66%) in infusion group remained in the target glycaemic range in the intraoperative period. This was comparable (P=0.138, Chi square test).

Table 3: Patient characteristics and duration of surgery

	Bolus group (n = 60)	Infusion group (n=60)	P value
Age	53.68 (9.20)	54.76 (8.49)	0.51
Gender (Male/Female)	32/28	37/23	0.36
Duration of surgery (min)	99.58 (35.50)	88.50 (25.75)	0.14

Data are mean (standard deviation) for age and median (interquartile range) for duration of surgery Patients remained in the target glycaemic range for 4440 minutes out of 10680 minutes of surgery in the bolus group and 2940 minutes out of 10800 minutes of surgery in the infusion group. Patients were in the target glycaemic range for a longer period of time (41.57% of the intraoperative period) in bolus group when compared to infusion group (27.22% of the intraoperative period). This difference was significant (P <0.001; Chi square test).

To see if preoperative glucose levels confounded the results, we compared the preoperative blood glucose levels between the two groups (Table 4). It is evident from the table that a greater number of patients in the bolus group were outside the target range before the start of surgery.

Table 4: Blood glucose range in patients before the start of surgery

Blood glucose range (mg/dL)	Bolus group (number of patients)	Infusion group (number of patients)
150-180	10	17
181-210	14	35
211-240	11	6
241-270	15	1
271-300	4	0
>300	6	1

Postoperative serum potassium level decreased in both the groups [from 4.34 (0.55) mmol/l to 4.13 (0.48) mmol/l in bolus group, P<0.001 and from 4.29 (0.48) mmol/l to 4.26 (0.36) mmol/l in infusion group, P=0.54; Data are mean (standard deviation), t test)] but was clinically and statistically not significant.

Discussion

An ideal blood glucose control regimen should give good glycaemic control with least incidence of hypoglycaemia. Optimal blood sugar control is essential not only in preventing untoward complications, but also reflects the quality of health care. In this regard, a simple, easy to use regimen will help in working towards these goals.

In this study, we chose to compare the proportion of patients in the target glucose levels between 100mg/dl and 180mg/dl and the proportion of intra-operative surgery time (in minutes) during which patient was in the target range as primary outcomes rather than comparing the absolute values of blood sugar at different time intervals to be more meaningful clinically. Insulin bolus regimen maintained the patients for longer duration of time in target glycaemic range (41.57% of intraoperative period) as compared to infusion group (27.22%), though there was no significant difference in the proportion of patients in the target range between both the groups. Also, we noted that though a greater number of patients were outside the target blood glucose range preoperatively in bolus group when compared to infusion group, we had better control of blood glucose with the former. This confirms the effectiveness of insulin bolus regimen in management of blood sugars during the intraoperative period, especially in patients with poor preoperative glycaemic control.

The major concern with intravenous insulin boluses is the fear of hypoglycaemia. In the group of patients that we studied, there was no hypoglycaemia with both the regimens. We also compared to see if there was any significant difference in the serum potassium levels between both the studied groups as insulin is known to cause decrease in serum potassium levels. We found that there was no significant difference between both the groups clinically, although it was statistically significant in bolus group.

Prior studies have shown varying results while comparing insulin bolus regimen with infusion regimen.[9,14] But there are no studies to compare between intravenous bolus and infusion regimens in the intraoperative period.

Alberti et al and AAGBI guidelines in 2015 compared glucose control in two sets of diabetic patients and concluded that insulin infusion is a better choice in terms of glycaemic control over subcutaneous regimen.[9,13] ADVANCE trial,[10] study by Berghe et al[11] and ACCORD study[12] were conducted comparing two intravenous insulin regimens with different target ranges of blood glucose levels for the regimens. The above studies thus emphasize the effectiveness of intravenous insulin regimens with additional emphasis on the target blood glucose levels (between 100mg/dl and 180mg/dl).

Our study had some limitations. Blood glucose levels were monitored only once an hour. The fluctuations within this hour could not be captured since there is no monitor for continuous glucose monitoring. The onset time of intravenous human actrapid is immediate and

the peak effect is at 10-15 minutes. So, it is unlikely that these patients could have experienced hypoglycaemia and recovered from the same as well within one hour of monitoring. Markers of stress levels were not monitored in the study. So, despite the absence of baseline dextrose infusion in the bolus group blood glucose levels were maintained. Whether this happened at the expense of catabolism is not clear from this study. Preoperative glycaemic control was not compared between the groups using glycosylated haemoglobin levels.

Conclusion

Intravenous insulin bolus regimen as compared to insulin infusion regimen for intraoperative blood glucose management provides better glycaemic control measured in terms of proportion of intraoperative duration during which the patient remained in the target blood glucose levels. However, the proportion of patients in the target blood glucose levels was similar with both the regimens.

References

1. Galloway JA, Shuman CR: Diabetes and surgery. Am J Med 1963;**34**: 177-191. https://doi.org/10.1016/0002-9343(63)90052-4
2. Goldmann DR: Surgery in patients with endocrine dysfunction. Med Clin North Am 1987;**71**: 499-509. https://doi.org/10.1016/S0025-7125(16)30854-9
3. Jack SD, Alberti KGMM. Management of Diabetes Mellitus in Surgical Patients. Diabetes Spectrum 2002; **15**: 44 -48. https://doi.org/10.2337/diaspect.15.1.44
4. Sudhakaran S, Surani SR. Guidelines for Perioperative Management of the Diabetic Patient. Surgery Research and Practice 2015; 2015: Article ID 284063. https://doi.org/10.1155/2015/284063 PMid:26078998 PMCid:PMC4452499
5. Duggan EW, Carlson K, Umpierrez GE. Perioperative Hyperglycemia Management: An Update. Anesthesiology2017;**126**(3):547-560. https://doi.org/10.1097/ALN.0000000000001515 PMid:28121636 PMCid:PMC5309204
6. Simha V, Shah P. Perioperative Glucose Control in Patients With Diabetes Undergoing Elective Surgery. JAMA. 2019;**321**(4):399-400. https://doi.org/10.1001/jama.2018.20922 PMid:30615031
7. Polderman JAW, Houweling LP, et al. Study protocol of a randomised controlled trial comparing perioperative intravenous insulin, GIK or GLP-1 treatment in diabetes-PILGRIM trial. BMC Anesthesiology 2014, **14**:91. https://doi.org/10.1186/1471-2253-14-91 PMid:25419179 PMCid:PMC4240889
8. Miriam A, Korula G. A simple glucose insulin regimen for perioperative blood glucose control: The Vellore regimen. Anesth Analg 2004**; 99**: 598 -602. https://doi.org/10.1213/01.ANE.0000122824.21065.CA PMid:15271748
9. Alberti KGMM, Thomas DJB. The management of diabetes during surgery. Br J Anaesth 1979;**51**:693-710. https://doi.org/10.1093/bja/51.7.693 PMid:399195
10. The ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008; **358**:2560-2572. https://doi.org/10.1056/NEJMoa0802987 PMid:18539916
11. The ACCORD study group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008; **358**: NO. 24. https://doi.org/10.1056/NEJMoa0802743 PMid:18539917 PMCid:PMC4551392.
12. Association of Anaesthetists of Great Britain and Ireland. Peri-operative management of the surgical patient with diabetes 2015. Anaesthesia 2015;**70**: 1427-1440. https://doi.org/10.1111/anae.13233 PMid:26417892 PMCid:PMC5054917
13. Hemmerling TM, Schmid MC, Kern S, Jacobi KE. Comparison of a continuous glucose-insulin-potassium infusion versus intermittent bolus application of insulin on perioperative glucose control and hormone status in insulin-treated type 2 diabetics. J Clin Anesth 2001;**13**: 293-300. https://doi.org/10.1016/S0952-8180(01)00276-8
14. Jacobi J, Bircher N, Krinsley J, Agus M, Braithwaite SS, Deutschman C, Freire AX, Geehan D, Kohl B, Nasraway SA, Rigby M, Sands K, Schallom L, Taylor B, Umpierrez G, Mazuski J, Schunemann H: Guidelines for the use of an insulin infusion for the management of hyperglycemia in critically ill patients. Crit Care Med 2012; **40**:3251-76 https://doi.org/10.1097/CCM.0b013e3182653269 PMid:23164767

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the older CT scanner are not always optimized to suit children. The result is that pediatric patients are being given considerably larger radiation doses than required for an optimum image quality.[13,14]

As children are intrinsically more sensitive to the effects of X-rays than adults, there is an urgent need to optimize CT exposure protocols for pediatric patients. The objective of optimization in CT exposures is to obtain acceptable image quality with minimum dose to the patients; reduction of dose in itself is not the objective of medical exposures. From this point of view, in recent times, much work has been done on optimization of scanning parameters in routine clinical conditions.[15-18] The foundation of optimization is the establishment of diagnostic reference levels (DRLs), first proposed by the ICRP in 1996[19] and later introduced into European[20] and Irish legislation.[21]

ICRP defines DRLs as "a form of investigation level applied to an easily measured quantity, usually the absorbed dose in air or tissue-equivalent material at the surface of (or inside) a simple standard phantom or a representative patient." This definition strongly emphasizes that DRLs are not the dose limits and do not help distinguish between good and poor medical practice. Although dose limits for occupational exposures must not be exceeded, patient DRLs may be exceeded if clinically necessary. DRLs also differ from dose limits for occupational exposure because they are not used to constrain individual patient exposures; this is because a dose higher than the standard dose may be required depending on the patient's body size and weight. DRLs are a tool for identifying facilities with unusually high doses and for promoting the optimization process.

Separate DRLs have been established for each country and/or region because equipment and procedure protocols can vary between different facilities in countries or regions, mainly due to different patient sizes and their weights. It is usually defined for a large collection of data at the 75th percentile. It can be defined at local level for a minimum number of 10–20 patients and preferably for a much larger number. By averaging such data from a large number of hospitals, the national DRLs can be estimated.[22,23] Hence, establishing national DRL would definitely ensure a safer pediatric CT diagnosis from patient's perspective. The purpose of DRLs is optimization of the imaging technique rather than radiation dose reduction to patients. If a justified examination does not provide the necessary clinical information because of too low dose resulting in an inadequate image quality, then the patient has been exposed needlessly to radiation. In clinical practice, it is assumed that the necessary dosage, within stipulated margins, will be used. In this line, it is essential to initially establish zonal DRL, namely, South, North, East, West and central and finally consolidate them to arrive at the pediatric DRL for the country. Thus, the objective of our study was to measure radiation dose for most commonly performed pediatric head, chest, and abdomen procedures in the radiology department in Tamil Nadu hospitals and suggest/derive pediatric DRLs, compare them with the internationally recommended DRLs and to suggest dose reduction methods without disturbing image quality.

Materials and Methods

Selection of computed tomography scanners

When attempting to establish regional CT DRLs which are applicable to all hospitals in Tamil Nadu, it is essential to sample as many hospitals as possible. The hospitals incorporated were equally spread over in the state of Tamil Nadu. This work was performed in thirty CT scanners which include both conventional and multidetector CT (MDCT) types installed at various major cities (Chennai, Coimbatore, Madurai, Salem, Erode, Tirupur, Namakkal, Trichy, Vellore, Dindigul, Dharmapuri, Hosur, Thiruvarur). The selection of the scanners was based on the number of pediatric patients investigated. The average number of pediatric patients scanned each day in the Tamil Nadu region was 3000: From this 1100 for head, 900 for chest and 1000 for abdomen CT procedures were performed. The total number of patients examined each day in the region was approximately 9000. Table 1 summarizes the make and model of the CT scanners involved in this study.

Radiation dose calibration

Before collecting the patient dose data, CT dose index (CTDI) measurements were carried out on all CT scanners by using calibrated 100 mm pencil ionization chamber (DCT10 RS, S/N 1636) and solidose electrometer 400 (S/N 4253) of RTI Electronics, Sweden. For this purpose, polymethyl methacrylate head (10 cm diameter) and body (16 cm diameter) phantoms were used.[24] The dosimetry methods recommended in the European guidelines[20] were followed. The individual approximate patient dose data were estimated from the phantom $CTDI_v$ and DLP measurements. It should be noted that phantom measurements are only a measure of the actual patient dose. Furthermore, it requires correction for different pediatric patient diameter.

Computed tomography dose measurements

Before initiating measurements in hospitals, a questionnaire was prepared to collect data regarding the pediatric CT protocols and clinical practices adopted by the hospitals in Tamil Nadu. These data helped to record the pediatric CTDI values for different scanning protocols adopted by the various departments. Participants were asked to extract from the scanner library, data for twenty patients belonging to each examination type and each age group in thirty CT departments (a total of 120 [20 (patients) × 3 (sites) × 2 (age group)] × 30 = 3600 procedures). In each category of machine, the lowest, highest, and mean physical parameters and operating parameters of both age group of pediatric patients are presented in Tables 2 and 3. Each row in this table refers to the parameters of the scanners mentioned in Table 1 in the same order.

This data abstraction has been done as per "Nationwide Evaluation of X-ray Trends" protocol.[25] The questionnaire contains a number of parameters including (i) make and model of the CT scanner, (ii) patient physical parameters

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Table 1: Make and model of the computed tomography scanners involved in this study

Number of slice	Make	Models	Tamil Nadu (number of units)
Single	Hitachi	Pratico	1
	Philips	Secura	1
	Siemens	Emotion	1
Dual/4 slice	Hitachi	CXR4	1
	Philips	Brilliance Big Bore	1
	Siemens	Emotion Duo	2
	Toshiba	Asteion	2
	GE	High speed dual	3
	Siemens	Somatom	2
	Toshiba	Asteion	1
	GE	Lightspeed QX-I Quad CT	2
16	Philips	Brilliance	1
	Siemens	Somatom Emotion	1
	Toshiba	Aquilion	2
	GE	Brivo and LightSpeed	2
64	Siemens	Somatom Sensation	2
	Toshiba	Aquilion	1
	GE	High-speed VCT	1
128	Siemens	Somatom Definition AS and Edge	2
	GE	Optima 660	1
	Total		30

GE: General electric

such as height, weight, lateral diameter and anteroposterior diameter, (iii) indication, (iv) interested organ, (v) phase such as pre- and post-contrast, arterial phase, venous phase, full bladder, and delay phase, (vi) routine scan parameters such as tube potential, tube current, scan time, rotation time, slice thickness, slice beam collimation, pitch, total slices, field of view, start couch level and end couch level, (vii) dose-related data such as displayed volumetric CTDI and DLP.

The majority of the hospitals have followed almost similar exposure parameters for chest and abdomen procedures. However, the newer scanner systems like 64 slice, 128 slice CT machines use AEC/care kV technique for pediatric patients. Some of the CT centers have used larger scan length for routine brain and abdomen procedures. Further, it was noted that individual CT radiographers selected different scan parameters for the same type of pediatric patients.

CT dose experiments were carried out by placing the standard head and body phantom at the isocenter of the CT scanner and applying one axial slice of a clinical head protocol in sequential scan mode. The dose received by the phantom at the five positions for a set of scan parameters was measured by placing the ion chamber in one hole at a time while plugging the rest of holes with acrylic rods.

The CTDI is defined as:[26]

$$CTDI = (1/nT) \int D_z \, dz \text{ (integration limits from } -50 \text{ mm to } +50 \text{ mm)} \quad (1)$$

where, n is the number of data channels in the multiscan CT scanner, T is the slice thickness corresponding to one channel and the integration is done over the length of the pencil chamber (100 mm). The CTDI was measured as per the above definition by the pencil chamber-electrometer system and displayed on the dosimeter unit. CTDI is defined for a single complete rotation of the CT scanner. Using these dose values, the other CT dose indices, namely, weighed CTDI ($CTDI_w$), $CTDI_v$, and DLP were calculated using the following relations:

$$\text{Weighted } CTDI_w = 1/3\ (CTDI_{100,c}) + 2/3\ (CTDI_{100,p}) \quad (2)$$

$$\text{Volumetric } CTDI_v = CTDI_w/\text{pitch} \quad (3)$$

$$DLP = CTDI_v \times \text{scan length} \quad (4)$$

Results and Discussion

Before carrying out the regional dose estimation, complete QA (electrical, mechanical, and radiation checks) was performed for all the machines involved in this work. One among these tests was the measurement of $CTDI_w$ for standard protocol involving tube potential of 80 kV, 100 kV, and 120 kV, tube current-time product of 100 mAs and 5 mm slice thickness. These values were compared with the $CTDI_w$ displayed on the console to ensure that the measured and displayed values agreed as per Atomic Energy Regulatory Board standards (maximum ± 18%).[27]

The CT dose indices were measured based on the five point method proposed by European guidelines using standard CTDI phantoms and suitable detector. The pediatric CTDI phantom was placed on the couch and aligning the central axis of the phantom with isocentre point of the gantry. As per FDA's recommendation the dose is measured at the four peripheral holes as well as the central hole. The measured $CTDI_v$ and DLP of pediatric head, chest and abdomen procedures for

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Table 2: Lowest, highest and mean of the operating parameters

Type of machine	Head				Chest				Abdomen			
	Tube potential (kV)	Tube current time product (mAs)	Scan length (cm)	Pitch	Tube potential (kV)	Tube current time product (mAs)	Scan length (cm)	Pitch	Tube potential (kV)	Tube current time product (mAs)	Scan length (cm)	Pitch
Single slice	100, 120	100, 180	10, 18	Axial	100, 120	100, 160	15, 25	1.0	100, 120	100, 180	15, 25	1.0
	110	160	16		105	120	20		105	125	22	
Dual slice	100, 120	110, 200	10, 20	Axial	100, 110	100, 180	18, 25	0.9, 1.2	100, 110	100, 180	15, 30	0.9, 1.1
	110	175	15		105	130	22	1.0	105	124	25	1.0
4 slice	100, 120	100, 250	10, 20	0.8, 1.1	100, 110	90, 150	16, 26	0.8, 1.3	100, 110	100, 180	15, 30	0.9, 1.4
	115	150	15	0.9	105	100	24	1.0	100	120	26	1.2
16 slice	100, 110	80, 200	10, 23	0.9, 1.0	100, 120	80, 140	15, 25	1.0, 1.1	100, 120	80, 160	15, 30	1.0, 1.6
	105	110	20	1.0	110	109	23	1.5	110	140	25	1.2
64 slice	100, 110	100, 200	10, 25	0.6, 1.1	80, 110	90, 160	15, 28	1.0, 1.4	80, 110	90, 180	15, 30	1.0, 1.2
	108	128	19	1.0	96	110	22	1.1	98	135	26	1.1
128 slice	80, 100	80, 160	10, 25	0.5, 1.0	80, 100	90, 140	15, 22	1.0, 1.6	80, 100	60, 180	15, 30	1.0, 1.4
	90	135	18	0.9	90	100	20	1.1	90	115	25	1.2

Table 3: Pediatric patient physical parameters in Tamil Nadu region

Pediatric patient physical parameters	Age group (years)	
	0-1	1-5
Weight (kg)	2.5-4.5	4-15
Height (cm)	40-80	80-130
Antero-posterior diameter (cm)		
Head	5-10	8-14
Chest	5-10	5-13
Abdomen	5-13	5-15
Lateral diameter (cm)		
Head	4-11	5-14
Chest	5-13	8-15
Abdomen	5-15	6-18
Abdominal circumference (cm)		
Head	4-12	5-14
Chest	4-13	5-15
Abdomen	5-14	5-18

both age groups at different CT departments are presented in Figures 1 and 2.

Figures 1 and 2 show that the large variations in CT radiation doses for pediatric head, chest, and abdomen procedures were noted for both age groups. This may be attributed to scans conducted without optimization of tube voltage and tube current-time product (mAs) with respect to the patient anatomy and organ of interest. Out of thirty CT departments, twenty CT departments have followed larger scan length for routine procedures; this will also give rise to an increase in the patient dose. Further, in this work, it was observed that based on the experience and knowledge of the radiographers, the selection of exposure parameters was altered from the default values. Those who have experience, have selected appropriate scan parameters when compared with newer radiographers; this also contributes to minimize radiation dose. Further, in this study, latest advancements and concepts in CT technology and selection of techniques such as detector arrangements, detector size, number of detectors, scan speed, tube rotation time, and type of detector (e.g., stellar detector in Siemens CT) available in recent MDCT scanners have been employed with reduction of radiation dose when compared with older CT scanners for pediatric patients.

Further, using overall calculated $CTDI_v$ and DLP value for both age groups of pediatric patients, the 75th percentile of $CTDI_v$ and DLP for head, chest and abdomen procedures thus calculated was taken as their respective third quartile values for each group of pediatric patients. The pediatric CT DRL proposed for Tamil Nadu region in south India has been compared with DRL recently proposed by EC[28] in Tables 4 and 5 [extracted from Figures 1 and 2].

From Tables 4 and 5, it was observed that the 75th percentile of $CTDI_v$ of pediatric head is lower than European DRL for both age groups. For chest and abdomen procedures, the 75th percentile of $CTDI_v$ is higher than European DRL

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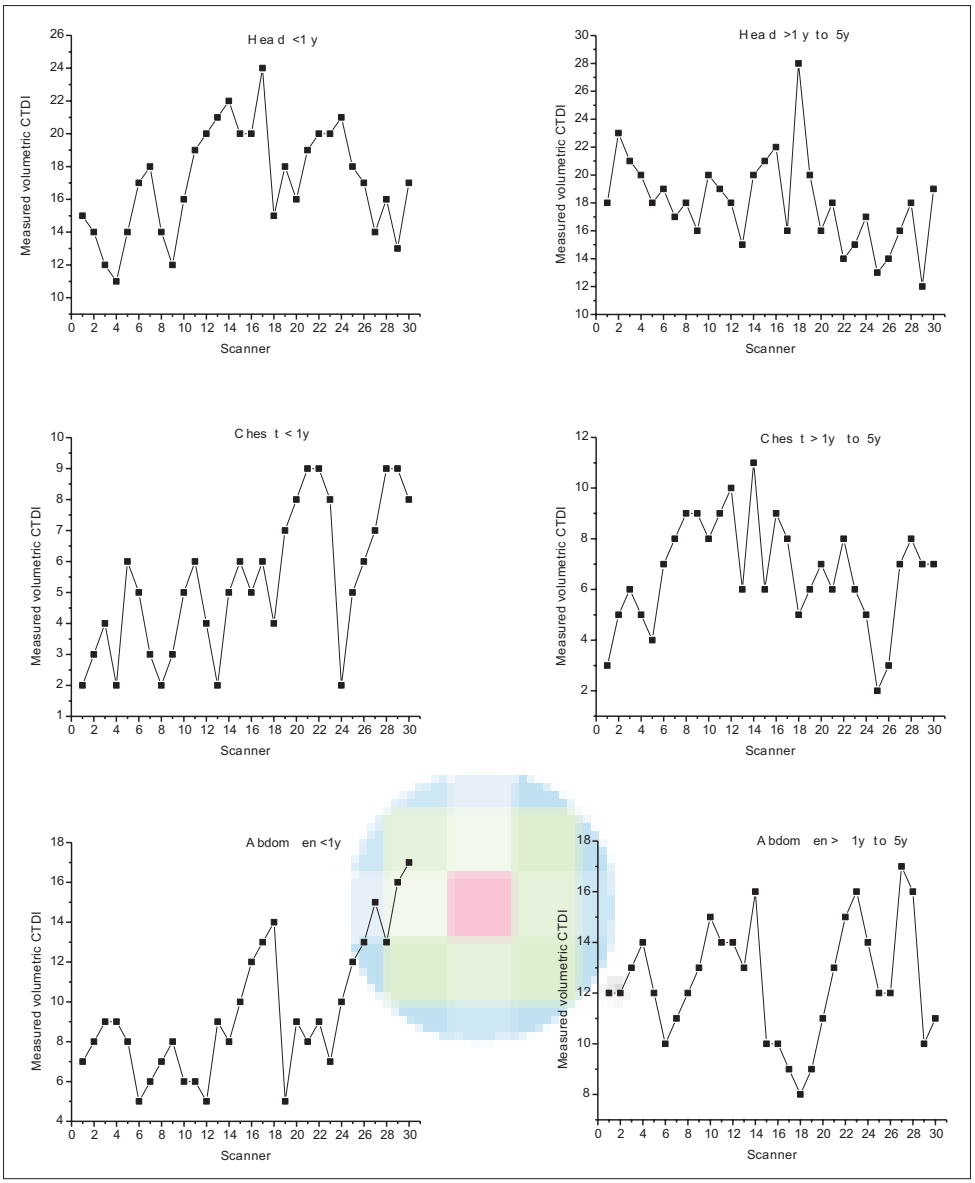


Figure 1: Calculated volumetric computed tomography dose index for <1 year and 1–5 years age group of pediatric patients for select procedures.

Table 4: Mean, range and third quartile values for volumetric computed tomography dose index for select procedures

Study region	Age group in year	Volumetric CTDI (mGy)			
		Mean	Range	Third quartile value of Tamil Nadu 2015	Pediatric European DRL 2015
Head	<1	17	11-24	20	25
	1-5	18	12-28	20	38
Chest	<1	5	2-9	7	3.3
	1-5	7	2-11	8	5.6
Abdomen	<1	9	5-17	12	5.7
	1-5	12	8-17	14	5.7

CTDI: Computed tomography dose index, DRL: Diagnostic reference level

for both age groups. This may be attributed to selection of greater exposure parameters for pediatric chest and abdomen procedures as compared to European practice. Following the European regulations, it becomes necessary to take into account the body size and habitus for selection of optimum machine parameters (e.g., tube voltage and mAs) for the

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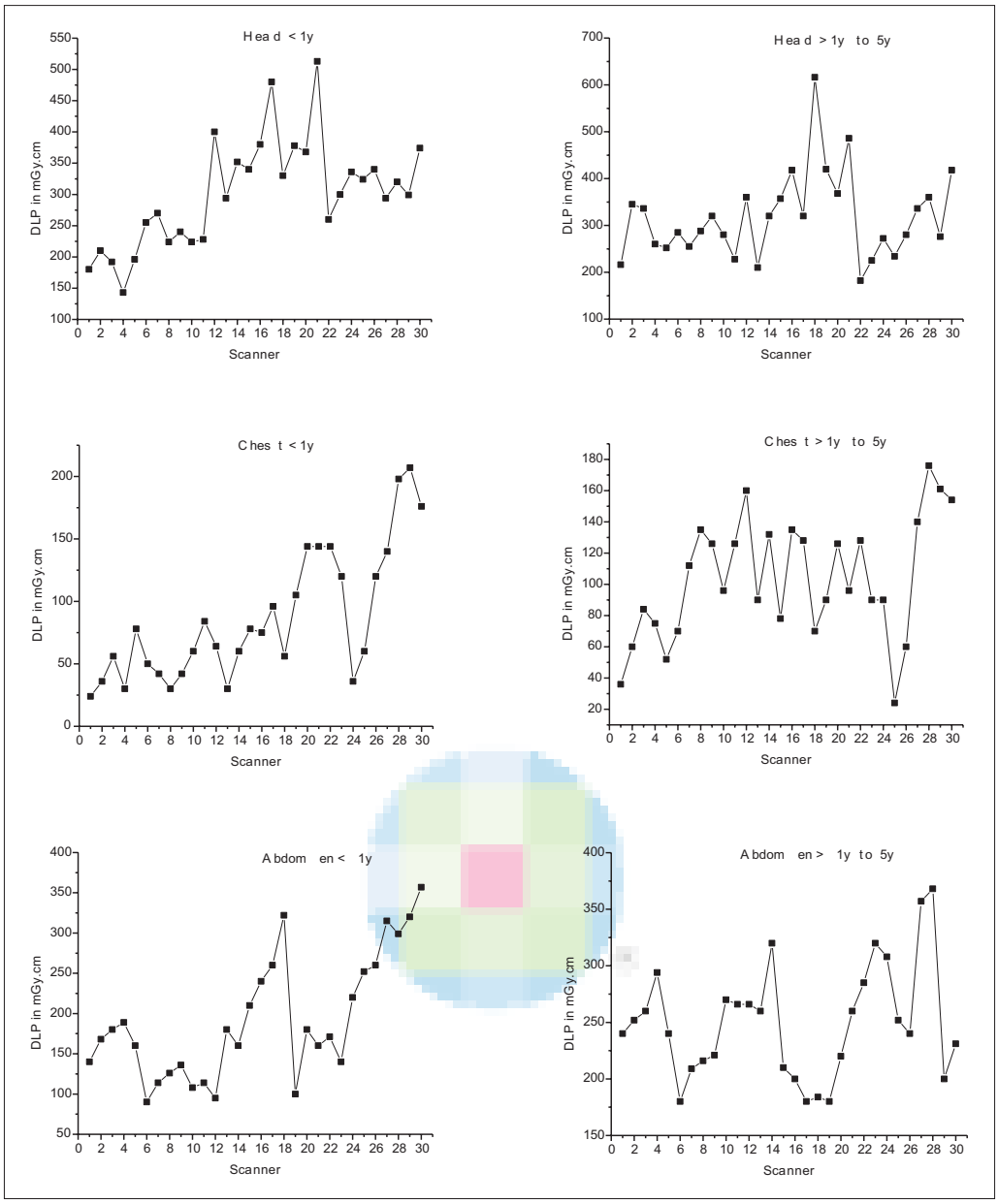


Figure 2: Calculated dose length product for <1 year and 1–5 years age group of pediatric patients for select procedures.

Table 5: Mean, range and third quartile values for dose length product for select procedures

Study region	Age group in year	DLP (mGy.cm)			
		Mean	Range	Third quartile value of Tamil Nadu 2015	Pediatric European DRL 2015
Head	<1	300	143-513	352	300
	1-5	304	182-616	360	505
Chest	<1	69	24-207	120	80
	1-5	96	24-176	132	115
Abdomen	<1	175	90-357	252	160
	1-5	246	180-368	270	170

DLP: Dose length product

required diagnostic information with minimum dose to the patient. In addition, it was observed that the 75th percentile of DLP of pediatric head for 0–1 year old patients is higher and for 1–5 years old patients is lower than European PiDRL, and for chest and abdomen procedures was higher than European pediatric DRL for both age groups. This is mainly

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because of choosing higher scan length for both procedures and both age groups.

From this study, it is suggested that it is very important to justify CT examinations in advance and once the decision for CT scan is taken, it is mandatory to adopt the ALARA principle strictly. In this line, radiologists should play essential advisory role with referring clinicians. When equal or greater diagnostic yields are expected, CT should be replaced by alternative imaging modalities such as sonography, magnetic resonance imaging, or radionuclide voiding cystography. On the other hand, radiologists should make every effort to reduce the pediatric radiation dose of CT examinations while maintaining diagnostic quality when CT is indicated. Furthermore, minimizing the scan range of CT examinations as required is a straightforward way to achieve this goal. CT radiographer should follow the strategies for pediatric CT dose optimization methods, namely, body size adopted CT protocols, tube current modulation, optimal tube voltage, scan modes, appropriate pitch, appropriate scan length, and field of view to reduce the pediatric CT radiation dose for selected procedures.

Conclusion

This study reports the regional pediatric CT DRLs for head, chest and abdomen procedures for the first time in Tamil Nadu region. More than 3600 individual patients' data were recorded and studied. The experimentally measured $CTDI_v$ and DLP values for all procedures were compared with displayed control console values (maximum ± 18%). Tamil Nadu pediatric CT DRL values have been proposed for $CTDI_v$ and DLP of head, chest, and abdomen procedures for <1 year and 1–5 years age group of patients. These proposed DRL values were compared with the pediatric European DRL values and strategies for pediatric CT dose reduction have been suggested. In this line, all clinicians who request pediatric CT should frequently assess its suitability on a case-by-case basis. When used carefully, CT is a valuable imaging modality for both age groups of children.

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Conflicts of interest

There are no conflicts of interest.

References

1. Donnelly LF, Emery KH, Brody AS, Laor T, Gylys-Morin VM, Anton CG, *et al.* Minimizing radiation dose for pediatric body applications of single-detector helical CT: Strategies at a large Children's Hospital. AJR Am J Roentgenol 2001;176:303-6.
2. Lam W, Wendy WM Lam. Pediatric CT radiation risks: What you should know. Med Bull 2006;11:5-7.
3. Brenner David J. One size does not fit all: Reducing risks from pediatric CT. ACR Bull 2001;57:20-3.
4. Frush DP, Donnelly LF, Rosen NS. Computed tomography and radiation risks: What pediatric health care providers should know. Pediatrics 2003;112:951-7.
5. Wakeford R. The cancer epidemiology of radiation. Oncogene 2004;23:6404-28.
6. 1990 recommendations of the international commission on radiological protection. Ann ICRP 1991;21:1-201.
7. UNSCEAR. Sources and Effects of Ionizing Radiation. New York: UNSCEAR; 2000.
8. Pierce DA, Shimizu Y, Preston DL, Vaeth M, Mabuchi K. Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer: 1950-1990. Radiat Res 1996;146:1-27.
9. Preston DL, Ron E, Tokuoka S, Funamoto S, Nishi N, Soda M, *et al.* Solid cancer incidence in atomic bomb survivors: 1958-1998. Radiat Res 2007;168:1-64.
10. Chodick G, Kim KP, Shwarz M, Horev G, Shalev V, Ron E. Radiation risks from pediatric computed tomography scanning. Pediatr Endocrinol Rev 2009;7:29-36.
11. Bongartz G, Golding S, Jurik A, Leonardi M. European Commission. European Guidelines on Quality Criteria for Computed Tomography (Report EUR 16262). Luxemburg: European Commission; 1999.
12. Feigal DW Jr. FDA public health notification: Reducing radiation risk from computed tomography for pediatric and small adult patients. Int J Trauma Nurs 2002;8:1-2.
13. Brenner D, Elliston C, Hall E, Berdon W. Estimated risks of radiation-induced fatal cancer from pediatric CT. AJR Am J Roentgenol 2001;176:289-96.
14. Sternberg S. CT Scans in Children Linked to Cancer Later. USA Today, 01 April, 2001.
15. Hamberg LM, Rhea JT, Hunter GJ, Thrall JH. Multidetector row CT: Radiation dose characteristics. Radiology 2003;226:762-72.
16. Jangland L, Sanner E, Persliden J. Dose reduction in computed tomography by individualized scan protocols. Acta Radiol 2004;45:301-7.
17. Boone JM, Geraghty EM, Seibert JA, Wootton-Gorges SL. Dose reduction in pediatric CT: A rational approach. Radiology 2003;228:352-60.
18. Radiological protection and safety in medicine. A report of the International Commission on Radiological Protection. Ann ICRP 1996;26:1-47.
19. European Community. On health protection of individuals against the dangers of ionizing radiation in relation to medical exposure. Council directive 97/43 (Euratom). J Radiol Prot 1998;18:133-7.
20. European Guidelines on Quality Criteria for Computed Tomography. Report EUR 16262. Brussels, Belgium: European Commission; 2000.
21. Staniszewska MA. Evaluation of patient exposure in computerised tomogram in Poland. Radiat Prot Dosimetry 2002;98:437-40.
22. McCollough CH, Rochester MN. Diagnostic Reference Levels, American College of Radiology; 2010. Available from: http://WWW.IMAGEWISELY.ORG. [Last accessed on 2016 Nov 10].
23. CT Dose Profiler, Probe for Evaluation of CT Systems Manual-Version 5.2A, RTI, Sweden. Available from: http://www.rti.se/. [Last accessed on 2013 May 20].
24. Saravanakumar A, Vaideki K, Govindarajan KN, Jayakumar S, Devanand B. Cost-effective pediatric head and body phantoms for computed tomography dosimetry and its evaluation using pencil ion chamber and CT dose profiler. J Med Phys 2015;40:170-5.
25. Nationwide Evaluation of X-ray Trends (N.E.X.T). Tabulation and Graphical Summary of 2000 Survey of Computed Tomography; 2007. Available from: http://www.crcpd.org/PDF/NEXT2000CTProl.pdf. [Last accessed on 2016 Nov 14].
26. Saravanakumar A, Vaideki K, Govindarajan KN, Jayakumar S Establishment of diagnostic reference levels in computed tomography for select procedures in Pudhuchery, India. J Med Phys 2014;39:50-5.
27. AERB/RSD/MDX-CT/QAR/2010, Acceptance/Performance Test for Computed Tomography (CT) Scanner; 2010. Available from: http://www.aerb.gov.in/cgi-bin/X-Ray/XRAYuserform.asp. [Last accessed on 2016 Nov 14].
28. European Guidelines on DRLs for Pediatric Imaging, Final Complete Draft for PiDRL Workshop; 30 September, 2015. p. 38. Available from: http://www.eurosafeimaging.org. [Last accessed on 2015 Nov 05].

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Original Article

Radiology Section

Qualitative and Quantitative Assessment of Enhancement and Washout Characteristics of HCC and non HCC Hypervascular Liver Tumours

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Introduction: The image based diagnosis of Hepatocellular Carcinoma (HCC) relies on assessment of arterial enhancement and venous or delayed phase washout. This assessment is based on visual analysis; however, studies quantifying such enhancement and washout are lacking.

Aim: To qualitative assessment of the imaging characteristics of HCC and non HCC hypervascular liver tumours followed by quantitative assessment of the enhancement and washout kinetics, and also to derive objective values that may help in differentiating HCC from non HCC on Computed Tomography (CT).

Materials and Methods: The present study was carried out in PSG hospitals, Coimbatore, Tamil Nadu, India. Retrospective analysis of hypervascular liver lesions was performed over a study period of four years (January 2012 to February 2016). These lesions were divided into two groups namely; HCC and non HCC. All non HCCs were pathologically proven, while HCC cases included those diagnosed on histopathology or typical imaging findings based on American Association for the Study of Liver Diseases (AASLD) (2010) and Liver Imaging Reporting and Data Systems (LIRADS) (2014) diagnostic criteria along with an elevated Alpha Fetoprotein (AFP) of more than 400 IU/mL.

A qualitative analysis of the enhancement and washout patterns was done followed by quantitative analysis using Regions of Interest (ROI) measurement of attenuation in Hounsfield units (HU). The quantitative parameters assessed were; Mean Attenuation (MA) of lesions in different phases, Attenuation Gain Percentage (AGP) on arterial phase, Arterial Phase Attenuation Change (AAC), Attenuation Loss Percentage in venous and delayed phases (ALP-V, ALP-D), Percentage Attenuation Ratio in venous and delayed phases (PAR-V, PAR-D) and Relative Washout Ratios from arterial to venous/delayed (RWR A-V, RWR A-D) and from venous to delayed phases (RWR V-D). Sensitivity and specificity were calculated for qualitative washout.

For quantitative variables comparative analysis between HCCs and non HCCs was done using Student's t-test. ANOVA was used to compare different categories. ROC curves were drawn for values that were significantly different to establish a cutoff value.

Results: A total of 120 lesions were evaluated consisting of HCCs (n=88) and non HCCs (n=32). Qualitatively majority (81) of HCCs demonstrated arterial enhancement and washout with eight cases of these demonstrating subtle washout requiring settlement by consensus. Washout were absent in seven cases. True mimics of HCC on qualitative analysis were hepatic adenomas, few cases of metastasis and hepatoblastoma were also there. The overall sensitivity of the washout appearance for predicting HCC was 92% while specificity was 78%. On quantitative analysis a PAR-V≥106 has sensitivity of 70% and specificity of 75%, RWR-V-D≥41.79 has sensitivity of 75% and specificity of 70% for HCC detection. Additionally significant difference was seen between RWR V-D of HCC when compared to cholangiocarcinomas (p-value 0.035) and metastasis (p-value 0.026) and also between PAR-V of HCC and metastasis (p-value 0.033).

Conclusion: The qualitative analysis combined with quantitative assessment of washout using percentage attenuation ratio in venous phase and relative washout ratio from venous to delayed phase offers good sensitivity and specificity in image based diagnosis of HCC.

Keywords: Hepatocellular carcinoma, Percentage attenuation ratio, Relative washout ratio

INTRODUCTION

The diagnosis of HCC on imaging relies on subjective assessment of arterial enhancement and venous or delayed phase washout. The arterial phase enhancement is attributed to an increase in intralesional flow with relative reduction in portal flow which occurs as the lesion progresses from dysplastic nodule to HCC [1,2]. Venous or delayed phase washout is decrease in attenuation of the lesion relative to background liver. Factors attributed to washout include early drainage of contrast material as compared to parenchyma and retention of contrast by surrounding fibrotic parenchyma [1-3]. There is ample literature on arterial enhancing mimics of HCC [1,3-7]. Arterial enhancing metastases, cholangiocarcinomas are known, similarly washout, a hallmark of HCC has been described in other tumours [5-7]. According to some studies Intrahepatic Mass-forming Cholangiocarcinoma (IMCC) showing arterial enhancement, was the most common lesion misinterpreted as HCC in preliver transplant imaging work up in recipients [5,9]. In cirrhotic livers lesions like regenerative nodules, dysplastic nodules, arterioportal or venous shunts and confluent fibrosis may also mimic this appearance [2,5-9]. Owing to typical enhancement characteristics of HCC, radiological imaging plays a crucial role in diagnosis and this combined with certain clinical and biochemical characteristics have been adopted as practice guideline for diagnosing HCC over the world. Differentiating HCC from other hypervascular tumours is important since HCC has therapeutic implications and prognostic factors which are very different from other lesions [2,8,10].

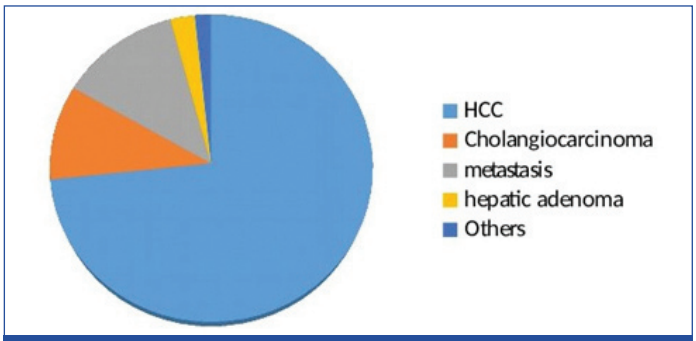
The assessment for hypervascularity of tumours and washout or persistent enhancement relies heavily on radiologist's subjective visual assessment. Only few studies have attempted to quantitate these variables and define the washout phenomenon [2,10]. In one

study, parameters such as relative contrast washout ratio between the arterial phase and delayed phase and attenuation ratio of the lesion to the background liver were studied and the attenuation ratio of the lesion to the background liver in the delayed phase was found to have excellent sensitivity and specificity for HCC detection and good correlation with subjective assessment [2]. In yet another study, an attenuation difference of greater than 10 HU between the arterial and delayed phase yielded optimal sensitivity and specificity for detecting HCC [10]. Certain studies have also quantitatively assessed the washout pattern on Magnetic Resonance Imaging (MRI), with a study evaluating the signal to noise ratio and liver to lesion contrast [11]. Overall the available literature is limited in this aspect with variable results. In present study, we analysed a large retrospective cohort of hypervascular liver tumours which were consecutively diagnosed over a period of four years in our hospital. We performed a qualitative assessment of the imaging characteristics of HCC and non HCC hypervascular liver tumours followed by quantitative assessment of the enhancement, washout kinetics and derived objective values that help in differentiating HCC from non HCC on CT. To the best of our knowledge this is the first such Indian cohort of hypervascular liver tumours being subjected to such qualitative and quantitative assessment.

MATERIALS AND METHODS

Institutional Ethics Committee approval was obtained and requirement for informed consent was waived after considering the retrospective nature of study. An extensive search of our Hospital Information System/Picture Archiving And Communication System (HIS/PACS-Vepro Medical Systems) for liver lesions on CT using keywords Hepatic, liver lesion, hepatic lesion, space occupying lesion, mass, HCC, Hepatocellular carcinoma and each of the individual common malignant and benign liver lesions revealed a total of 389 patients from January 2012 to February 2016 (four years duration). All Multidetector Computed Tomography (MDCT) examinations were performed on 64 slice MDCT (Somatom 64, Seimens healthcare) or 128 slice (Somatom Definition edge, Siemens healthcare) helical scanners. All studies were carried out in craniocaudal direction with patient supine with average 120 KV and 250-450 mA, values varying depending on patients build. Unenhanced images were obtained followed by administration of IV contrast (Iopromide 350-370 mg/mL), power injector was used for injection of contrast at a rate of 4 mL/sec. Automated bolus tracking was used. Images were obtained in arterial, venous and delayed phases, typically at 18-28 seconds, 55-65 seconds and 300 seconds after initiation of contrast administration.

Hypervascular tumours on arterial phase were studied; thus, all hypo or avascular lesions were excluded. Hypodense lesions with thin peripheral enhancement, the pattern which is most common in metastatic lesions, were also excluded. The other exclusion criteria were: i) HCCs without histopathology and not meeting the criteria mentioned below in confirmation of tumor subtype, ii) Diffusely infiltrative HCCs as enhancement pattern and washout characteristics are difficult to assess in them, iii) HCCs with changes related to treatments like thermal ablation or transarterial chemoembolisation, iv) Non HCCs without definitive histological diagnosis, v) Very small tumours less than one cm in size as placing ROI for measurements is difficult in them, vi) Benign lesions like hemangiomas, abscess and cysts were also excluded as they show fairly typical enhancement patterns and in most no histopathological confirmation is done. Histopathological confirmation was available for all non HCCs and HCC (35 cases). 53 HCCs, all in at risk population having underlying cirrhosis were diagnosed based on AASLD guidelines (2010) and LI-RADS (version 2014). First AASLD criteria was applied (a lesion of more than 2 cm showing arterial phase hyperenhancement and subsequent washout, in presence of cirrhosis and elevated AFP levels, we used AFP cutoff of >400 IU/mL to enhance specificity), tumours meeting these criteria are virtually diagnostic of HCC [13]. All of these 53 cases were LI-RADS 5 category (indicating 100% certainty of HCC), with 34 cases being LR-5, 17 cases LR-5V with macrovascular invasion and two cases being LR-5us (being 1-2 cm in size and being visible as discrete nodules on ultrasound) [14]. Two HCCs were histological variants, one a fibrolamellar HCC and another cholangiocellular variant. After application of exclusion and inclusion criteria we arrived at a study population of 120 patients (86 males and 34 females) mean age 58.5 years, range (3-78 years). There were 88 (73%) HCCs, 12 (10%) mass forming cholangiocarcinomas, 15 (12.5%) metastasis, 3 (2%) adenomas, 1 (0.08%) hepatoblastoma and 1 (0.08%) angiosarcoma [Table/Fig-1]. For purpose of comparability the tumours were divided in two broad groups of HCC and non HCC.

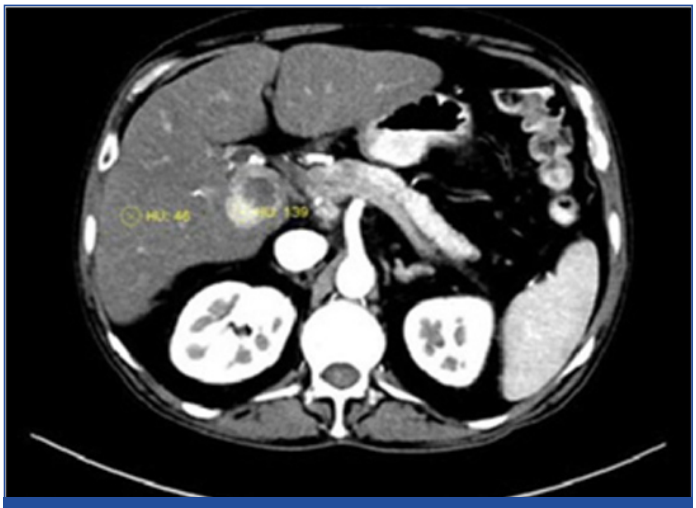


[Table/Fig-1]: Distribution of tumours.

A subjective or qualitative assessment was performed by two radiologists; having nine and five years of experience in abdominal imaging. After confirming the hypervascular nature of the tumour on the arterial phase assessment of subjective washout was done and presence or absence of washout on venous or delayed phases was noted. Presence of any delayed hyperenhancing central stroma was also noted. The examining radiologists were blinded to the results of histopathology and final diagnosis. Discrepancies if any were settled by consensus.

The quantitative analysis was done by one radiologist. The attenuation were measured in HU by drawing circular or oval ROI over the lesion. The ROIs were drawn to include maximally enhancing areas on arterial phase and ranged from 10 to 20 mm with similar ROI drawn on corresponding venous and delayed phase images. The necrotic areas of the tumour, major intratumoral feeding vessels or intratumoral arterioportal shunts were excluded [Table/Fig-2]. In case of large tumours with variable areas of enhancement, multiple ROIs were drawn in the enhancing regions of the tumour and averaged. Two ROIs were also obtained in the adjacent normal background liver in all the phases, taking care to avoid blood vessels. In case of multiple lesions, largest lesion was selected.

The following quantitative indices were calculated 1) Mean attenuation(MA) of lesions in different phases 2) Attenuation gain



[Table/Fig-2]: Hepatocellular carcinoma in caudate lobe with an eccentric necrotic component, ROI placement was done in the enhancing tumour component .

percentage on arterial phase (AGP): SOL a-BL a / BL a x100), Arterial phase attenuation change (AAC): SOL a –BL a (these indices assess the arterial enhancement of the lesions). 3) Attenuation loss percentage in venous and delayed phases (ALP-V, ALP-D): BL v/d-SOL v/d/ BL v/d x100; Percentage attenuation ratio in venous and delayed phases (PAR-V, PAR-D): 100x BL v/d/SOL v/d; Relative wash out ratio (RWR A-V, RWR A-D) from arterial to venous and delayed phases: 100x (SOL a-SOL v/d)/SOL a and from venous to delayed phases(RWR V-D): 100 x (SOLv-SOL d)/ SOL v.(these variables define the washout characteristics). (SOL a and BL a were attenuation of space occupying lesion and background liver attenuation on arterial phase respectively, SOL v/d and BL v/d were lesion and background liver attenuation on venous and delayed phases).

STATISTICAL ANALYSIS

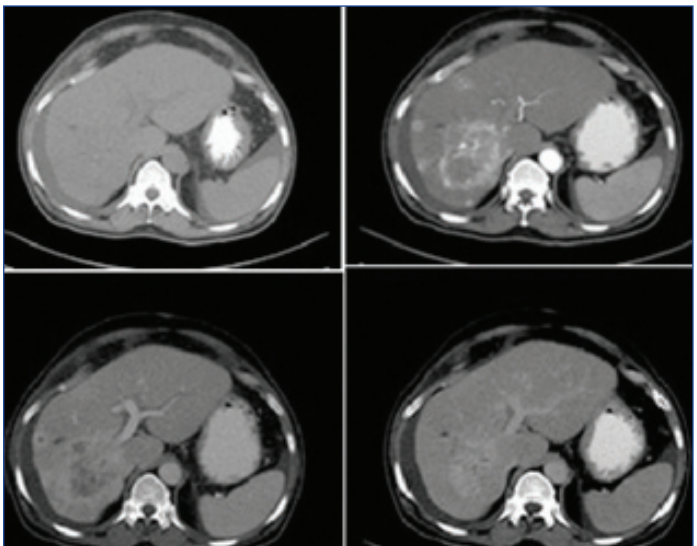
Statistical analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). Sensitivity and specificity for washout appearance were calculated. For quantitative variables comparative analysis between HCCs and non HCCs was done using Student's t-test. ANOVA analysis was used for comparison between HCC and individual categories in Non HCC (cholangiocarcinomas, metastasis and others). ROC curves were drawn for values that were significantly different to establish a cutoff value.

RESULTS

The demographic and qualitative characteristics of the HCC and non HCC are as in [Table/Fig-3]. On qualitative analysis all HCCs showed homogenous or patchy enhancement on arterial phase. There was excellent interobserver agreement between the two radiologists for hypervascularity (Kappa value of 0.91) and presence of washout (Kappa value of 0.86) and discrepancies if any, were settled by consensus. Washout was noted on venous phase in 62/88 (69.3%) patients while on delayed phase additional 19 patients showed washout (81/88,92%). Overall 92% HCCs demonstrated washout in either venous or delayed phase. There was lack of subjective washout as perceived by both radiologists in 7/88 (8%) tumours [Table/Fig-4]. Of these however, two tumours did demonstrate a difference in quantitative washout if absolute values of HU obtained from ROI between the lesion and background liver were considered (though the difference was less than 10 HU). In addition in 8 (9%) tumours the washout was perceived as subtle and required either extensive window setting adjustments (in five cases) or settlement by consensus (three cases). The confounding factors encountered affecting visual assessment were presence of 1) steatosis in the background liver with lesion appearing hyperdense relative to the surrounding fatty liver in two cases, 2) presence of enhancing peripheral pseudocapusle in five cases and a delayed enhancing central fibrous stroma (noted in one case of fibrolamellar HCC). The metastases were the commonest non HCC lesion. Most of the hypervascular metastases were from adenocarcinomas with known or unknown primary sites. A qualitative washout appearance similar to HCC was noted in three cases (20%). Amongst the carcinoid metastasis or neuroendocrine tumours in present study sparing one which washed out the other two were hyper or isodense to parenchyma. Both cases of sarcoma metastasis (n=2) had peripheral enhancement, while washout was seen in one, the other was seen filling in from periphery to the centre. Thus, most of metastatic lesions showed no washout in the venous or delayed phase except one case of transitional cell carcinoma, one of neuroendocrine tumour and one sarcoma. Most of the adenocarcinoma metastases were isodense to parenchyma on delayed phase. The metastasis from gastrointestinal stromal tumour had peripheral fill in pattern.

	HCC	Non HCC	p-value
Females	21	13	0.107
Mean age (years)	63.8	54.03	0.000
Size <3 cm	18 (20.45%)	3 (9.37%)	0.1868
Mean size (cm)	6.55±4.17	7.00±3.7	0.5916
Washout present (%)	81 (92%)	7 (21.8%)	0.001

[Table/Fig-3]: Demographic and qualitative parameters of HCC and Non HCC. The difference is significant at p<0.05
HCC*- Hepatocellular carcinoma



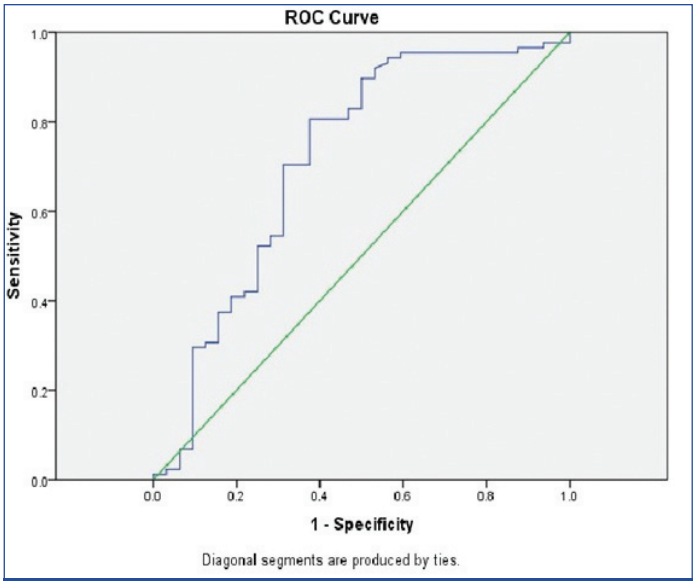
[Table/Fig-4]: Histopathologically proven hepatocelluar carcinoma, multinodular in alcohol related cirrhosis, showing progressive enhancement pattern with lack of subjective or quantitative washout.

Amongst cholangiocarcinomas, none demonstrated washout on delayed phase, all the lesions appeared isodense, hyperdense or showed central enhancing stroma in the delayed phase. The true HCC mimics on qualitative analysis were the three cases of hepatic adenomas and one case of hepatoblastoma which had an enhancement and washout appearance similar to HCC. The only case of angiosarcoma showed hyperdense appearance on delayed phase. Thus, hepatic adenomas, few hypervascular non adenocarcinoma metastasis and hepatoblastoma showed washout appearance in venous or delayed phase; thus, mimicking HCC. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%.

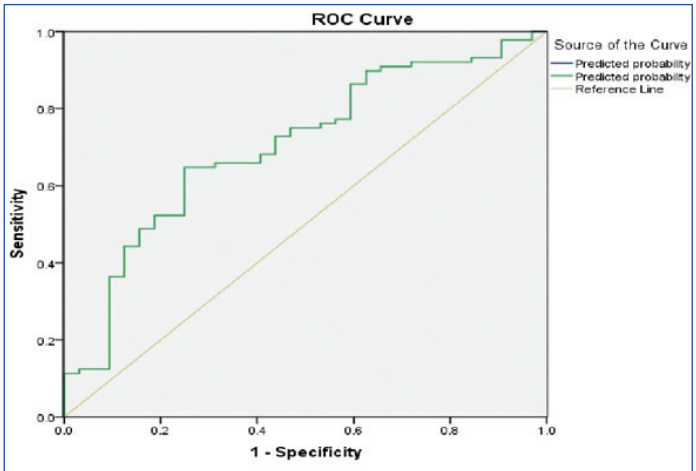
The difference between the mean attenuation of the HCCs and non HCCs was significant in noncontrast (p-value 0.01), arterial (p-value 0.002) and delayed phases (p-value 0.013), while the difference was not significant in the venous phase (p-value 0.614). The quantitative indices are as listed in [Table/Fig-5]. There was no significant difference between the two groups in attenuation change in arterial phase (AAC), while AGP was significant (p-value 0.018). Of the washout parameters, in venous phase significant difference (p-value 0.002) was found in PAR-V in venous phases with no significant difference seen between the two groups in PAR-D. The washout parameters assessed were relative washout from arterial to venous or delayed phase (RWR A-V, A-D) and from venous to delayed phase (RWR-V-D). The relative washout from arterial to venous or delayed phases was (RWR A-V,A-D) was found to be significant (p-value 0.022, 0.004). Washout from venous to delayed phase (RWR-V-D) was also significant between the two groups (p-value 0.000). ALP-V that is attenuation loss percentage in venous phase was also significant (p-value 0.001). However, area under curve was significant for RWR V-D and PAR-V only (r-value >0.7) and analysis of ROC curves for PAR-V and RWR-V-D revealed good sensitivity and specificity for detection of HCC [Table/Fig-6,7].

	HCC (Mean)	Non HCC (Mean)	p-value
MA-NCCT	66.31	52.71	0.01
MA-Arterial	105.96	89.8	0.002
MA-Venous	89.34	91.73	0.614
MA-Delayed	51.75	63.09	0.013
AAC	13.5	2.46	0.070
AGP	27.9	7.17	0.018
PAR-V	116.29	101.89	0.002
PAR-D	117.33	116.09	0.810
RWR A-V	9.3	-6.6	0.022
RWR A-D	45.05	25.6	0.004
RWR V-D	43.27	31.38	<0.001
ALP-V	10.83	-1.16	0.001
ALP-D	12.01	9.73	0.487

[Table/Fig-5]: Details of quantitative variables assessed.
MA-NCCT*-Mean Attenuation Non Contrast CT
AAC*- Arterial phase attenuation change, AGP*- Attenuation gain percentage, PAR-V*- Percentage attenuation ratio-venous phase, PAR-D*- Percentage attenuation ratio-delayed phase, RWR A-V*- Relative washout ratios arterial-ratio, RWR A-D*- Relative washout ratios arterial-delayed, RWR V-D*- Relative washout ratios venous-phase, PLP-V*- Attenuation loss percentage-venous phase, ALP-D*- Attenuation loss percentage-delayed phase



[Table/Fig-6]: Receiver operating curve (ROC) for RWR-V-D, area under curve >0.7



[Table/Fig-7]: Receiver operating curve (ROC) for PAR-V, area under curve >0.7

On individual comparison of HCC with metastasis, cholangiocarcinoma and others category, significant difference was seen between RWR V-D of HCC (mean 43.27) when compared to cholangiocarcinomas (mean 31.2, p value 0.035) and metastasis (mean 33.63, p value 0.026).

DISCUSSION

Studies show that combination of arterial hypervascularity and washout appearance of HCC on MDCT offers a specificity approaching 100% for HCC >20 mm in at risk populations. According to the AASLD guidelines (2010) a lesion of more than 10 mm showing arterial phase hyperenhancement and subsequent washout, in presence of cirrhosis can be considered as HCC and treated as such. Similar guidelines have been proposed by European Association for the Study of the Liver (EASL) in 2012 [13-15]. More recently LIRADS (2014) has been developed for categorising liver lesions in at risk population; this classification aims to facilitate clear communication between different personnel involved in care of these patients. The lesions falling in LR-5 category are nearly certain to be HCCs and consists of hypervascular lesions of more than 2 cm with one or more combinations of washout, capsule, threshold growth. In addition macrovascular mainly portal vein invasion constitutes LR-5V category [14].

A comprehensive quantitative analysis may help in providing further parameters that assists differentiation between HCC and non HCC. A quantitative assessment may also reduce interobserver variability and lay foundation for development of diagnostic algorithms and computed aided detection parameters. To the best of our knowledge, this is also the first study of this kind in India with no previous Indian cohort of hypervascular liver tumours having being subjected to this kind of qualitative and quantitative assessment.

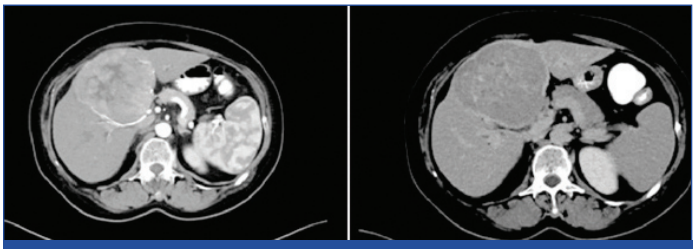
HCCs showed uniform or nonuniform enhancement with most showing washout appearance. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%. The venous phase washout was seen in 69.3% patients while in delayed phase this number increased to 92%. About 8% of HCC did not washout at all while another 9% had a subtle washout. Latter could be resolved subjectively itself and washout even though subtle could be resolved qualitatively with a consensus agreement of two experienced radiologists. Thus, a qualitative washout assessment in a tertiary level setup with high volumes and experienced radiological staff does hold an excellent sensitivity and a reasonable specificity for HCC detection. The specificity may approach 100% if additionally presence of imaging features of cirrhosis is taken into account since none of the non HCCs had underlying cirrhosis. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%.

On quantitative evaluation there was significant difference in the attenuation between the two groups on noncontrast, arterial and delayed phases. Of the arterial parameters the AAC was found to be significantly different between the HCCs and non HCCs. However, when comparing the HCCs with individual subgroups of non HCCs the significance could not be elicited. The AGP was found to be significantly different between the two groups however, it merely represents arterial hypervascularity and doesn't quantitate washout. The PAR as suggested by Liu Y et al., is a measurement of washout appearance of HCC, measured as a ratio of liver and lesion attenuation [2]. The values for PAR in venous phase showed significant difference between the HCC and non HCC while in delayed phase the difference was not statistically significant. The mean PAR-V of HCC was higher than Non HCC. The absence of difference of PAR in delayed phase is probably because there were lesions like adenoma and certain metastases that washed out significantly more than HCC from venous to delayed phase as compared to HCC resulting in almost similar values of PAR-D. A cutoff of PAR-V of 106 provided 70% sensitivity in diagnosing HCC. Relative Washout Ratio (RWR) from arterial to delayed and venous phase was significantly different between the two groups but seems irrelevant as many of the values were negative, since maximum enhancement was obtained in venous phase in many tumours. Considering the fact that attenuation of the lesion was maximum in

venous phase, the calculated RWR from venous to delayed phase (RWR-V-D) showed that the HCC washout significantly more than non HCC and this temporal parameter of washout from venous to delayed phase seems more relevant than ascertaining washout from arterial to venous or arterial to delayed phase. As discussed earlier the washout phenomena is an appearance based on a lot of factors and RWR from venous to delayed phase seems to be one of the parameters that may differentiate the HCC and non HCC negating the effect of liver parenchymal attenuation. It is likely that gradient of loss of attenuation of lesion from venous to delayed phase is much more significant. The mean values of RWR-V-D of HCC (43.27) and non HCC (31.38) were significantly different and a cutoff of 41.79 offered a sensitivity of 75%.

When comparing individual groups there was a significant difference between the mean RWR-V-D values of HCC (mean 43.27) versus hypervascular IMCC (mean 31.2) and metastasis (mean 33.63). Loyer EM et al., compared the kinetics of HCC versus IMCC and found that HCC loose enhancement from venous to delayed phase while IMCC gain enhancement from the corresponding phases, however they included all cases of cholangiocarcinomas and not just the hypervascular variants [16]. Liu Y et al., used PAR ratio as a simple tool to measure the washout appearance [2]. In present study it is apparent that there are some arterial phase enhancing mimics showing the washout appearance complicating the oversimplified guidelines. We also had a much larger study population. In present study PAR-V was significant offering good sensitivity and specificity. This is in contradistinction to the study by Liu Y et al., which found PAR-D to be significant [2]. RWR-V-D was also significantly different between two groups and this gradient may actually help in cases that are not so straight forward.

In present study we found some true HCC mimics; however, which have similar qualitative appearance and quantitative indices like HCC, something which has not been highlighted in the other studies. These mimics were hepatocellular adenomas, hepatoblastoma and certain hypervascular metastasis [Table/Fig-8]. Hypervascular liver metastases from neuroendocrine tumours mimicking hepatocellular carcinoma in enhancement and washout pattern has been described in literature [16,17]. These mimics should be considered as differentials for HCC arising in noncirrhotic liver. The value of quantitative indices also appears to be doubtful for these mimics and appeared to be similar in both subgroups. Although, proving this statistically was difficult in present study due to the small sample size of the mimics. Thus, while a qualitative assessment combined with some useful quantitative parameters, can provide a noninvasive image based diagnosis in majority of cases in the settings of cirrhosis, the same may not hold true for HCC in noncirrhotic livers. A thorough clinicoradiological assessment can however, point to correct diagnosis with hepatocellular adenoma having distinct age group and predispositions, hepatoblastoma usually arising in pediatric age groups and metastatic lesions usually being accompanied by their primary known tumours, though biopsy may be needed for confirmation. Nevertheless a thorough qualitative assessment combined with assessment of certain useful quantitative indices mentioned above, can permit confident noninvasive image based diagnosis for a majority of HCCs in most radiological setup.



[Table/Fig-8]: Neuroendocrine tumour metastatic to liver, showing arterial phase enhancement and delayed phase washout, a pattern indistinguishable from HCC.

LIMITATION

There were some limitations of present study, firstly the lack of histopathology for all HCCs and the use of AASLD guidelines and LIRADS classification with AFP levels for identifying the same (partly because obtaining a biopsy is now almost unethical in patients with cirrhosis meeting the classic imaging diagnostic criteria), but we were stringent in including patients with image based diagnosis, only cirrhotic patients with AFP of more than 400 IU/mL with washout were included. The incidence of cholangiocarcinomas which is known to be the most common mimic would be very low in this group and is unlikely to alter the results. The other limitations included, HCCs less than 1 cm which were not included in our data set. Thus, their behavior was not assessed, heterogeneity within the non HCC group and the sample size of the non HCC group was small compared to HCC. Retrospective nature of analysis also limits the evaluation.

CONCLUSION

While there are number of guidelines which include the more popular likes of AASLD and LI-RADS for diagnosis of HCCs and categorising liver lesions; yet, most of these continue to be subjective in nature with lack of quantification of hypervascularity or washout. A quantitative analysis defining the washout is useful additional determinant adding objectivity to image based diagnosis. Quantitative variables like PAR-V (threshold value 106), RWR-V-D (threshold of 41.79) complement the qualitative analysis, for diagnosis of HCC and have future implications like incorporation into guidelines and development of diagnostic algorithms for computed aided detection software packages.

REFERENCES

[1] Kamaya A, Maturen KE, Tye GA, Liu Y, Parti N, Desser T. Hypervascular liver lesions; Semin Ultrasound CT MR. 2009;30(5):387-407.
[2] Liu Y, Shin L, Brooke J, Kamaya A. Quantitatively defining washout in hepatocellular carcinoma. AJR Am J Roentgenol. 2013;200(1):84-89.
[3] Khosa F, Khan AN, Eisenberg RL. Hypervascular liver lesions on MRI. AJR Am J Roentgenol. 2011;197(2):W204-20.
[4] Murakami T, Tsurusaki M. Hypervascular benign and malignant liver tumors that require differentiation from hepatocellular carcinoma: Key points of imaging diagnosis. Liver Cancer. 2014;3(2):85-96.
[5] Kim SA, Lee JM, Lee KB, Kim SH, Yoon SH, Han JK, et al. Intrahepatic mass-forming cholangiocarcinomas: enhancement patterns at multiphasic CT, with special emphasis on arterial enhancement pattern-correlation with clinicopathologic findings. Radiology. 2011;260(1):148-57.
[6] Oliver J, Baron R, Federle M, Jones B, Sheng R. Hypervascular liver metastases: Do unenhanced and hepatic arterial phase CT images affect tumor detection ?. Radiology. 1997;205(3):709-15.
[7] Kim TK, Lee E, Jang HJ. Imaging findings of mimickers of hepatocellular carcinoma. Clin Mo Hepatol. 2015;21(4):326-43.
[8] Kang Y, Lee JM, Kim SH, Han JK, Choi BI. Intrahepatic Mass-forming cholangiocarcinoma: Enhancement patterns on gadoxetic acid-enhanced MR images. Radiology. 2012;264(3):751-60.
[9] Luca A, Caruso S, Milazzo M, Mamone G, Marrone G, Miraglia R, et al. Multidetector-row computed tomography (MDCT) for the diagnosis of hepatocellular carcinoma in cirrhotic candidates for liver transplantation: prevalence of radiological vascular patterns and histological correlation with liver explants. Eur Radiol. 2010;20(4):898-907.
[10] Tan CH, Thng CH, Low AS, Tan VK, Hartono S, Koh TS, et al. Washout of hepatocellular carcinoma carcinoma; quantitative region of interest analysis on CT. Ann Acady Med, Singapore. 2011;40(6):269-75.
[11] Frericks BB, Loddenkemper C, Huppertz A, Valdeig S, Stroux A, Seja M, et al. Qualitative and quantitative evaluation of hepatocellular carcinoma and cirrhotic liver enhancement using Gd-EOB-DTPA. AJR Am J Roentgenol. 2009;193(4):1053-60.
[12] McEvoy SH, McCarthy CJ, Lavelle LP, Moran DE, Cantwell CP, Skehan SJ, et al. Hepatocellular carcinoma: illustrated guide to systematic radiologic diagnosis and staging according to guidelines of the american association for the study of liver diseases. RadioGraphics. 201333(6):1653-68.
[13] Song DS, Bae SH. Changes of guidelines diagnosing hepatocellular carcinoma during the last ten-year period. Clin Mol hepatol. 2012;18(3):258-67.
[14] Rakhmonova G, Choi J, Kim M. Liver imaging reporting and data system (LI-RADS) version 2014: understanding and application of the diagnostic algorithm. Clin Mole Hepatol. 2016;22(2):296-307.
[15] Cruite I, Tang A, Sirlin C. Imaging-based diagnostic systems for hepatocellular carcinoma. AJR Am J Roentgenol. 2013;201(1):41-55.

[16] Loyer EM, Chin H, Dubrow RA, David CL, Eftekhari F, Charnsangavej C. Hepatocellular carcinoma and intrahepatic peripheral cholangiocarcinoma: enhancement patterns with quadruple phase helical CT-A comparative study. Radiology. 1999;212(3):866-75.

[17] Laroia ST, Sasturkar S, Rastogi A, Pamecha V. Solitary hypervascular liver metastasis from neuroendocrine tumor mimicking hepatocellular cancer: All that glitters is not gold. Indian J Nucl Med. 2015:30(1):42-46 .

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Case Report

An unusual cause of breathlessness and profuse micronodules

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ABSTRACT

We describe a 21-year-old male with a history of smoking and subacute onset of breathlessness with normal cardiorespiratory examination. The presence of "track marks" and digital infarcts prompted evaluation for infective endocarditis and confrontational history taking revealed anorexia, weight loss over 3 months along with intravenous drug abuse of reconstituted tablets of tapentadol. Echocardiography was normal and blood cultures were sterile; computed tomography showed bilateral, diffuse, small centrilobular nodules with "tree-in-bud" appearance. In this clinicopathologic conference, we discuss the clinical and radiological differential diagnosis of centrilobular nodules, lung biopsy findings, and management options for patients with such a presentation.

KEY WORDS: Excipient lung disease, intravascular talcosis, pulmonary foreign body angiogranulomatosis, talc granulomatosis

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PRESENTATION OF THE CASE

A 21-year-old college student presented with breathlessness and left-sided chest pain for a week. Breathlessness was insidious in onset and persisted on walking at own speed at level. Chest pain was left-sided, intermittent, pricking and non-exertional. There was no history of fever, rhinitis, headache, hemoptysis, cough, or joint pains. He did not raise pets but admitted to smoking 7–8 cigarettes/day and consuming whisky (192 g of alcohol weekly). He denied any recent worsening of educational performance. He did not report any significant prior medical illness or pertinent illness in family or occupational dust exposure. He was initially seen in cardiology and evaluation showed normal cardiac and respiratory examination without any pallor, clubbing, or organomegaly. Body mass index was 18.6 kg/m^2. The examination also showed extensive track marks over both arms with evidence of multiple peripheral digital infarcts [Figure 1], raising suspicion for infective endocarditis (IE). On confronting, he disclosed daily intravenous self-injections of three–four powdered and reconstituted 50 mg tablets of Tapentadol (Tydol, Sun Pharma) in sterile distilled water two–three times a day over the last 2 years. He also admitted to using inhaled cannabis twice weekly over the last 2 years. There was associated anorexia and unquantified weight loss over the last 3 months. Three sets of blood cultures were sterile. Electrocardiography showed T-wave inversions in leads V3–V6 and II, III, and aVF. Transthoracic echocardiography (TTE) showed normal valves without any evidence of vegetations or pulmonary hypertension and normal left ventricular function. Serum creatinine

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Figure 1: Composite image of the clinical photographs (left) of the right forearm showing "track marks," indicating healed superficial septic thrombophlebitis and (right) both hands showing multiple bilateral distal digital pulp infarcts. Similar findings were also seen in both plantar surfaces (not shown)

was 0.6 mg/dL; urine microscopy was normal without any albuminuria. Hemoglobin was 14.2 g/dL with normal leukocyte and platelet counts. He remained afebrile. Transesophageal echocardiography (TEE) was planned; in the meanwhile, review of his chest radiographs showed diffusely scattered fine nodules bilaterally [Figure 2, left]. Spirometry showed mild restriction with reduced diffusion capacity for carbon monoxide (forced expiratory volumes at 1 s 2.25 L [65%]%, forced vital capacity 2.87 L [71%], DLco 62%). There was desaturation to 92% while walking 6 minutes, with preserved walk distance (545 meters, 89% predicted). Human immunodeficiency virus (HIV), anti-nuclear antibodies, hepatitis B, and hepatitis C antibodies were negative by enzyme-linked immunosorbent assay. Immunoglobulin E levels were 9 IU/mL (normal <100 IU/mL). High-resolution computed tomography (HRCT) of the thorax showed bilateral profuse evenly distributed micronodules with "tree-in-bud" [Figure 2, right]. There was no sputum production on induction with 3% saline. A procedure was planned for diagnosis.

DIFFERENTIAL DIAGNOSIS

Important features of this case

This HIV-negative young adult with significant smoking history, cannabis use, and intravenous drug abuse with reconstituted tablets over 2 years has breathlessness, anorexia, and weight loss with radiological findings of micronodules. There is evidence of distal embolic infarcts, but initial evaluation for endocarditis was negative. The reduced DLco, restriction on spirometry and unexpectedly low walk distance in a young individual with desaturation on walking implies extensive parenchymal and/or pulmonary vascular disease. The differential diagnosis of profuse bilateral micronodules is extensive [Table 1] but can be narrowed by careful history and analysis of HRCT findings in conjunction

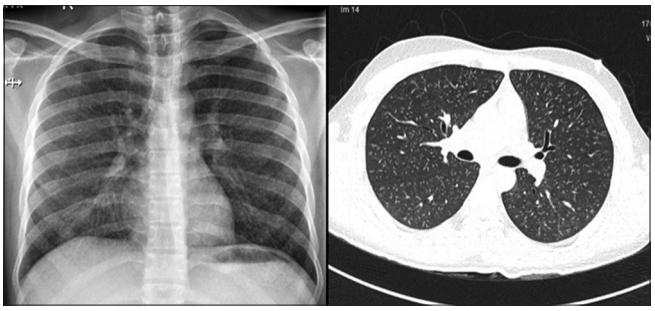


Figure 2: Composite image of the chest radiograph (left) showing small nodular opacities and high-resolution computed tomography of the thorax (right) showed bilateral profuse evenly distributed micronodules with tree-in-budding; pulmonary artery was normal in size, and there were no associated lymphadenopathy. Expiratory computed tomography did not show any air trapping (not shown)

Table 1: Causes of diffuse micronodular disease on computed tomography

With tree-in-budding
Infectious causes
Tuberculosis, nontubercular mycobacteria (typically MAC)
Bronchopneumonia: *Haemophilus influenzae*, *Mycoplasma pneumoniae*, Chlamydia
Viral bronchiolitis
Airway-invasive aspergillosis
Aspiration
Bronchiectasis: Cystic fibrosis, ciliary disorders, immunodeficiency
Immunologic
Allergic bronchopulmonary aspergillosis
Diffuse panbronchiolitis
Hematogenous: intravascular metastases (rare)
Without tree-in-budding
Infective causes
Tuberculosis
Fungi: *Histoplasma capsulatum*, blastomycosis, candidemia
Varicella zoster
Immunologic causes
Tropical pulmonary eosinophilia
Hypersensitivity pneumonitis (subacute)
Follicular bronchiolitis
Respiratory bronchiolitis
Sarcoidosis
Diffuse pulmonary amyloidosis
Allergic bronchopulmonary aspergillosis (rarely)
Occupational lung disease
Berylliosis, silicosis, coal worker's pneumoconiosis
Idiopathic
Pulmonary alveolar microlithiasis
Idiopathic pulmonary hemosiderosis
Malignancy
Metastasizing leiomyoma
Pulmonary Langerhans cell histiocytosis (early)
Metastatic adenocarcinoma, renal cell carcinoma, papillary thyroid carcinoma
Others
Metastatic calcification (renal failure)
Pulmonary arteriolar causes
Pulmonary edema and hemorrhage, excipient lung disease, plexogenic arteriopathy, veno-occlusive disease, pulmonary capillary hemangiomatosis

MAC: *Mycobacterium avium* intracellulare, *Wuchereria bancrofti*

with the expected pulmonary complications of intravenous drug abuse [Table 2].[1] The first step is to characterize the

Table 2: Pulmonary problems in intravenous drug abusers

Mechanism of Injury	Observed side-effects
Pharmacodynamic properties of injected drug	Noncardiogenic pulmonary edema (cocaine, opioids) Cocaine intoxication: pulmonary edema, pulmonary hemorrhage, cardiogenic pulmonary edema (coronary spasm) Methylphenidate intoxication: hemoptysis
Complications of unsafe intravenous injections	Lung abscess (with loss of consciousness, seizures, and aspiration) Infective endocarditis Septic pulmonary embolism Infections acquired from needle sharing (HIV, hepatitis B, hepatitis C)
Effects of contaminants	Excipient lung disease* Emphysema (lower lobe predominant "Ritalin" lung with methylphenidate) and Bullous lung disease Pulmonary vascular disease Group 1: Pulmonary hypertension (cocaine and methamphetamine) Group 3: Pulmonary hypertension (emphysema, foreign body granulomatosis, and interstitial lung disease) Interstitial lung disease Pneumothorax (subclavian injections, cocaine, and bullous lung disease), septic emboli
Related to associated abused substances, poverty, malnutrition	Pneumonia Tuberculosis More frequent exacerbations of asthma (fatal asthma)

*Talc, starch, crospovidone, and microcrystalline cellulose are used as fillers. HIV: Human Immunodeficiency Virus

distribution of the micronodules spatially within the lung and also the secondary pulmonary lobule. These nodules were uniform in size and were distributed evenly in a cephalocaudal direction, without any preferential lobar involvement. None of the nodules abut the pleural surface or the fissures [Figure 3]; there is clear evidence of extensive bilateral branching nodules in centrilobular distribution. There is no evidence of bronchiectasis; while no contrast was administered, in conjunction with echocardiography, there is no dilated pulmonary artery or lymphadenopathy. These findings limit the radiological differentials to causes of micronodules in a centrilobular distribution and extensive uniform "tree-in-bud" appearance. "Tree-in-bud" indicates the presence of dilated centrilobular bronchioles with lumen impacted by mucus, fluid, or pus and associated with peribronchiolar inflammation. "Tree-in-bud" indicates small airway disease and is associated with airway infection in the large majority but can also be rarely caused by arteriolar disease.[2] Its presence makes three common causes of centrilobular nodules unlikely: hypersensitivity pneumonitis, follicular bronchiolitis, and respiratory bronchiolitis.

Endobronchial tuberculosis

Mycobacterium tuberculosis is endemic in India, with more than 2.2 million new cases each year. Delayed recognition

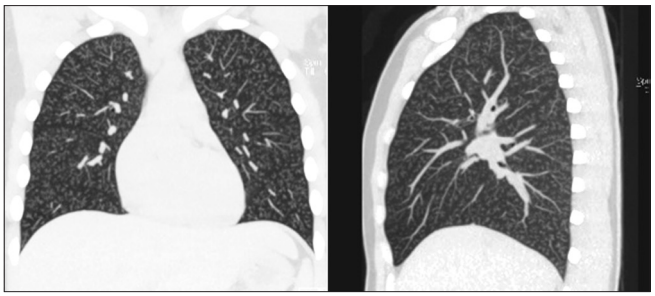


Figure 3: Coronal and sagittal reconstruction of the maximum intensity projection formatting of the high-resolution computed tomography images of the thorax showing diffuse, extensive, bilateral centrilobular nodules with branching and without any lobar predominance

and extensive disease are related to associated substance abuse, poverty, and malnutrition, all of which were present in this patient. Extensive endobronchial spread of tuberculosis can cause extensive tree-in-bud opacities although asymmetry is usually seen.[3] Tuberculosis is also the most common cause of micronodules with a random pattern (miliary). Even though the "pretest probability" of tuberculosis is high in this patient, the marked symmetry and the absence of any cavitation or consolidation is odd; digital infarcts are unusual but could be caused by an associated immune complex phenomenon that can occur with tuberculosis. *Mycobacterium avium*-intracellulare infection can also cause centrilobular nodules with "tree-in-bud," bronchiectasis, and consolidation. While it can be seen in immunocompetent patients, the classic phenotype is a middle-aged woman with slender build and without any constitutional symptoms.

Bronchopneumonia and viral bronchiolitis

Infectious bronchiolitis due to viruses, *Mycoplasma*, and chlamydia is a common cause of profuse and symmetric centrilobular nodules with "tree-in-bud;"[3] however, the prolonged illness and associated embolic phenomenon are unusual.

Allergic bronchopulmonary aspergillosis

Centrilobular nodules with "tree-in-bud" or miliary nodules can be caused by allergic bronchopulmonary aspergillosis but the absence of asthma, central bronchiectasis, and normal IgE levels rule out this diagnosis.[2,4]

Diffuse panbronchiolitis

Diffuse panbronchiolitis (DPB) is a rare disease of unknown cause presenting in males between the second and fifth decade with productive cough, diffuse bilateral centrilobular nodules with "tree-in-bud" and sinusitis. While reported in Indians,[5] it is extremely rare in our population but can explain all the clinical and radiological features.[6] Cold agglutinins are frequent in DPB and pulp infarcts may be due to an associated connective tissue disease. The absence of productive sputum, obstruction on spirometry, sinusitis, and air trapping on expiratory CT are unusual in DPB and necessitate further evaluation with surgical lung biopsy to establish the diagnosis.[6]

Excipient lung disease

Centrilobular nodules are usually caused by bronchiolar disease but can rarely be caused by arteriolar disease. Arteriolar causes include pulmonary edema and hemorrhage, excipient lung disease (ELD), granulomatosis with polyangiitis (GPA), plexogenic arteriopathy (PH), veno-occlusive disease (VOD), pulmonary capillary hemangiomatosis (PCH), and metastatic calcification.[2] There was no evidence of heart failure, renal failure or anemia and hemoptysis to suggest alveolar hemorrhage. The absence of pulmonary hypertension makes centrilobular nodules due to PH, VOD, and PCH very unlikely. Nodules due to GPA are larger, fewer, and associated with cavitation. Excipients are insoluble inert particulate filler materials that bind and protect the active drug during production as well as shape and lubricate tablets for easy swallowing. Excipients include talc (hydrated magnesium silicate), microcrystalline cellulose, crospovidone, and starch. Typically, excipients constitute 5% of a tablet's mass. On intravenous injection, excipient particles lodge in pulmonary arterioles and trigger an angiogranulomatous reaction; progressive disease is associated with dyspnea and pulmonary hypertension.[7] However, the presence of finger pulp infarcts, "tree-in-bud" appearance and absence of pulmonary hypertension are unusual in ELD.

CLINICAL DIAGNOSIS

In summary, the differential diagnosis of extensive centrilobular nodules with "tree-in-bud" without bronchiectasis or pulmonary hypertension in this young adult with smoking, cannabis use, and intravenous drug abuse of reconstituted tablets can be narrowed down to extensive endobronchial tuberculosis, diffuse panbronchiolitis, and ELD.

Dr. RM. PL. Ramanthan: With these clinicoradiological possibilities in mind, this patient underwent fundus examination and bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial lung biopsy, with a plan for surgical biopsy if evaluation was inconclusive. Can we now have the findings?

Dr. K. Divya: Dilated fundus examination confirmed refractile particles scattered along both the retinal arteries and retinal veins [Figure 4], confirming systemic embolization of small (<5 μm) talc particles, consistent with intravenous talcosis. Inhalational talc exposure does not cause such marked retinal deposition as the inhaled particles are larger and are restricted by bronchioles and pulmonary circulation.[8,9]

Dr. Nidhya Ganesan: BAL was negative for acid-fast bacilli and for *M. tuberculosis* by Xpert MTB/RIF; occasional needle-shaped crystals were seen on cytology. The transbronchial lung biopsy specimen confirmed perivascular granulomatous reaction to talc particles [Figure 5, left] that appear as birefringent

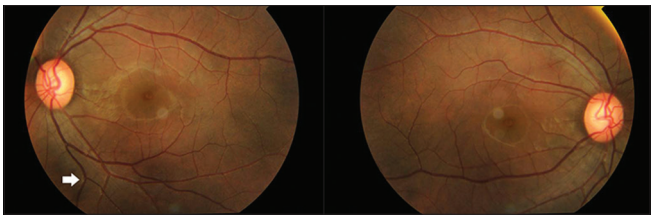


Figure 4: Fundus photograph of both eyes showing refractile particles scattered along both the retinal arteries and retinal veins, confirming the diagnosis of intravenous talcosis

needle-shaped crystals within the granulomas under polarized light [Figure 5, right]. Review of the composition of Tydol confirmed the presence of talc as excipient. Microcrystalline cellulose appears rod like while starch has a maltese cross pattern and crospovidone is nonbirefringent on polarization.[7]

Dr. Srinivas Rajagopala: A diagnosis of ELD (talc granulomatosis) was made. Psychiatry services were involved, and the patient underwent counseling, arrangement for family supervision, and daily-observed methadone replacement. He has successfully discontinued intravenous drug abuse and remains stable at 3 months of follow-up. Serial spirometry, DLco, and radiographs have been advised at 6 monthly intervals. Three unusual findings need an explantation; 1) The presence of "tree-in-bud" in a process that is hematogenous in origin 2)The absence of pulmonary hypertension in ELD and 3)The cause of the bilateral digital infarcts observed.

Dr. B. Devanand: Diseases involving pulmonary arterioles can cause centrilobular nodules as arterioles run in the peribronchovascular interstitium adjacent to bronchioles. Talcosis associated with intravenous abuse is most commonly associated with diffuse fine centrilobular nodules, ground-glass abnormalities or a combination of nodules and lower-lobe emphysema (Ritalin lung with methylphenidate).[9,10] The "tree-in-bud" pattern is unusual for arteriolar disease; it can be caused by intra-arteriolar accumulation of talc with associated adjacent perivascular granulomatous reaction giving the appearance of branching centrilobular nodules and has been described in a few cases of ELD earlier.[11]

Dr. Srinivas Rajagopala: What is the course of ELD? Can the disease progress despite cessation of abuse?

Dr. Kancherla Roopa: Lung fibrosis and pulmonary hypertension are related to the extent of vascular obstruction and granulomatous inflammation and are usually irreversible with talc, cellulose, and crospovidone as these cannot be mobilized; starch can be removed by metabolism and pulmonary hypertension can be reversible to a large degree. Disease progression can occur despite cessation due to ongoing granulomatous foreign-body reaction to the deposited, unmobilizable talc. The initial series of patients described all had progressive symptoms leading to death over 9 months to 5 years.[10,12] The index

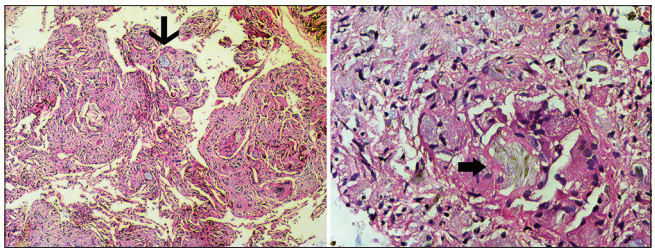


Figure 5: Photomicrographs of the transbronchial lung tissue biopsy specimen (left) demonstrating perivascular foreign body granulomatous reaction to the talc particles (thin arrow, H and E, ×100). Under polarized light, birefringent 20–95 μm needle-shaped crystals were seen in the granuloma, consistent with talc (block arrow, H and E, ×400)

patient has a large load of perivascular disease and is at risk for pulmonary hypertension and disease progression despite cessation of further abuse.

Dr. Srinivas Rajagopala: How common is systemic embolization of talc given that the lung acts as an effective filter?

Dr. K. Divya: The talc that is trapped in the pulmonary arterioles is typically around 10 μm, but smaller particles can escape to the systemic circulation and be detected in the retina, spleen, and virtually every organ on autopsy.[12] Finding excipient crystals in fundoscopy is a rapid noninvasive method to confirm intravenous talcosis.

Dr. Srinivas Rajagopala: Given the presence of digital infarcts, is TEE needed to exclude IE in this patient?

Dr. G. Rajendiran: The first consideration in this patient with possible unsafe needle practices, no murmurs, weight loss, breathlessness, and digital embolic manifestations was subacute native valve IE. TTE has low sensitivity compared to TEE (46% vs. 93%) although both are highly specific (96%).[13] Embolic manifestations are seen in about half of patients with IE.[14] He was not administered any antibiotics, three sets of blood cultures were sterile, and TTE showed all valves clearly without any vegetations. TEE is restricted in our practice to high probability for IE or in whom TTE is rendered less sensitive due to obesity, lung hyperinflation, or prosthetic valves. The index patient met only 2 minor Modified Duke's criteria and was considered low risk for IE after the initial evaluation and TEE deferred.[15] Furthermore, a clear alternative explanation for the observed distal infarcts in the form of talc deposition in the distal microcirculation with thrombosis is available and corroborated by fundoscopy. While this physical finding has not been described in ELD previously, it is consistent with the distal digital embolization and subsequent microthrombosis caused by talc.

Dr. Srinivas Rajagopala: What are the options for those with progressive breathlessness despite cessation of intravenous drug abuse?

Dr. RM. PL. Ramanthan: There are previous small series of lung allografts after a careful selection process in ELD with excellent outcomes.[16] Cadaveric bilateral sequential lung transplantation is a reality in our country today, and he can be listed for the same at our center if he meets criteria and remains free of substance abuse for at least 6 months with adequate psychiatry counseling.

SUMMARY

Clinical examination findings continue to have relevance in this era of investigation-driven medicine. Excipient lung disease can result from intravenous injection of reconstituted tablets and can present with ground glass opacities, small nodules, and emphysema. Pulmonary arteriolar diseases must be considered in the differential diagnosis of centrilobular nodules on CT.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that names, identifying marks, and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Marchiori E, Souza AS Jr., Franquet T, Müller NL. Diffuse high-attenuation pulmonary abnormalities: A pattern-oriented diagnostic approach on high-resolution CT. AJR Am J Roentgenol 2005;184:273-82.
2. Webb R, Muller NL, Naidich DP. HRCT Findings: Multiple nodules and nodular opacities. In: High-Resolution CT of the Lung. 5th ed. Philadelphia: LWW Wolters Kluwer; 2014. p. 125-9.
3. Walker CM, Abbott GF, Greene RE, Shepard JA, Vummidi D, Digumarthy SR, *et al*. Imaging pulmonary infection: Classic signs and patterns. AJR Am J Roentgenol 2014;202:479-92.
4. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, *et al*. Allergic bronchopulmonary aspergillosis: Review of literature and proposal of new diagnostic and classification criteria. Clin Exp Allergy 2013;43:850-73.
5. Nath A, Aggarwal AN, Gupta R. Diffuse panbronchiolitis: Report of a rare disease from India. Indian J Chest Dis Allied Sci 2010;52:43-5.
6. Poletti V, Casoni G, Chilosi M, Zompatori M. Diffuse panbronchiolitis. Eur Respir J 2006;28:862-71.
7. Nguyen VT, Chan ES, Chou SH, Godwin JD, Fligner CL, Schmidt RA, *et al*. Pulmonary effects of i.v. Injection of crushed oral tablets: "Excipient lung disease". AJR Am J Roentgenol 2014;203:W506-15.
8. Akira M, Kozuka T, Yamamoto S, Sakatani M, Morinaga K. Inhalational talc pneumoconiosis: Radiographic and CT findings in 14 patients. AJR

Am J Roentgenol 2007;188:326-33.
9. Ward S, Heyneman LE, Reittner P, Kazerooni EA, Godwin JD, Müller NL, *et al*. Talcosis associated with IV abuse of oral medications: CT findings. AJR Am J Roentgenol 2000;174:789-93.
10. Feigin DS. Talc: Understanding its manifestations in the chest. AJR Am J Roentgenol 1986;146:295-301.
11. Bendeck SE, Leung AN, Berry GJ, Daniel D, Ruoss SJ. Cellulose granulomatosis presenting as centrilobular nodules: CT and histologic findings. AJR Am J Roentgenol 2001;177:1151-3.
12. Sieniewicz DJ, Nidecker AC. Conglomerate pulmonary disease: A form of talcosis in intravenous methadone abusers. AJR Am J Roentgenol 1980;135:697-702.
13. Bashore TM, Cabell C, Fowler V Jr. Update on infective endocarditis. Curr Probl Cardiol 2006;31:274-352.
14. McDonald JR. Acute infective endocarditis. Infect Dis Clin North Am 2009;23:643-64.
15. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr., Ryan T, *et al*. Proposed modifications to the duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;30:633-8.
16. Weinkauf JG, Puttagunta L, Nador R, Jackson K, LaBranche K, Kapasi A, *et al*. Long-term outcome of lung transplantation in previous intravenous drug users with talc lung granulomatosis. Transplant Proc 2013;45:2375-7.

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Congenital Biotinidase Deficiency-Clinching the Diagnosis with Classic Imaging Features

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Abstract: *Congenital biotinidase deficiency (CBD) is a rare inborn error of metabolism, resulting in biotin deficiency. The recognition of clinical, laboratory and magnetic resonance imaging findings can help us to arrive at a correct diagnosis at the earliest which can enable institution of appropriate early treatment and prevention of late complications. We present the typical MRI findings in a 3 ½ month old baby who presented with multiple episodes of seizures which finally turned out to be profound biotinidase deficiency.*

Key Words*: Congenital biotinidase deficiency, recurrent seizures, skin manifestations, bilateral symmetrical diffusion restriction, cerebral atrophy.*

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I. Introduction

Congenital biotinidase deficiency (CBD) is a rare autosomal recessive congenital neuro- metabolic disorder,[1] which leads to profound deficiency of biotin which is an essential cofactor for many enzymes[2] Biotin deficiency can lead to neurological, cutaneous and visual problems.-4 Making a diagnosis based on clinical manifestations alone is difficult. Brain imaging especially Magnetic Resonance Imaging (MRI) with advanced MR techniques like diffusion weighted imaging and MR spectroscopy (MRS) has typical features which can clinch the diagnosis. As MRI plays a key role in making the diagnosis at an early stage of the disease it should be considered in all patients who present with clinical features suggestive of metabolic disorders.

II. Case Report

A 3 ½ months old baby girl presented with complaints of recent onset of multiple episodes of seizures, lethargy, hypotonia, skin peeling and alopecia. The antenatal and birth history were unremarkable. The baby was born of a non-consanguineous marriage. Baby was immunized up to the age. The baby was developmentally normaltill 3 months of age and attained social smile at 2 months. Head control has not yet been attained. The baby started to develop recurrent episodes of seizure for 15 days and regression of previously attained social smile. At presentation the child weighted 5.1kg. Head circumference measured about 40cm.In view of multiple episodes of seizures and lethargy, baby was admitted in neonatal intensive care ward and started on empirical antibiotic therapy. Routine blood investigations revealed markedly elevated lactate (11.6mmol/L), reduced bicarbonate (2.7mmol/L) and pH of 6.9. Further blood investigations showed elevated ammonia (200microgram/dl.) and urine ketones(3+). Blood culture and cerebrospinal fluid (CSF) culture were negative. In view of multiple episodes of seizures, skin manifestations, alopecia, severe metabolic acidosis and hyperammonemia, the diagnostic possibility of inborn errors of metabolism was suspected and referred to department of radiology for further evaluation with MRI of the brain.



Figure 1: Clinical photograph of the child showing severe alopecia and skin peeling.

MRI of the brainrevealed prominent cortical sulci and fissures, mildly dilated ventricles– suggesting loss of brain volume. In addition there wereconfluent areas of T2/ fluid attenuated inversion recovery (FLAIR) sequence hyperintensities with T1 hypointensities involving bilateral fronto-parietal white matter, bilateral cerebellar white matter, and cerebellar vermis. There were also bilateral symmetrical areas of diffusion restriction seen in corona radiata, posterior limb of internal capsule, cerebellar white matter, cerebellar vermis, midbrain and left medial temporal gyri. In addition to the above findings, subdural hygroma noted along bilateral frontal convexities (more on the left side). No evidence of mass lesion / hemorrhage seen within brain parenchyma. MRS showed elevated lactate and glycine. Based on the above MRI findings, the diagnostic possibility of inborn errors of metabolism such as CBD, Maple syrup urine disease (MSD)andLeigh's syndrome (LS)were suggested.

With the combination of clinical and radiological findings, biotinidase deficiency was considered as most probablediagnosisand baby was started on oral biotin.Subsequently the diagnosis was confirmed with tandem mass spectroscopy which revealed profound biotinidase deficiency. The baby responded well to oral biotin with reduction of lactate and ammonia and rise in bicarbonate and pH levels.

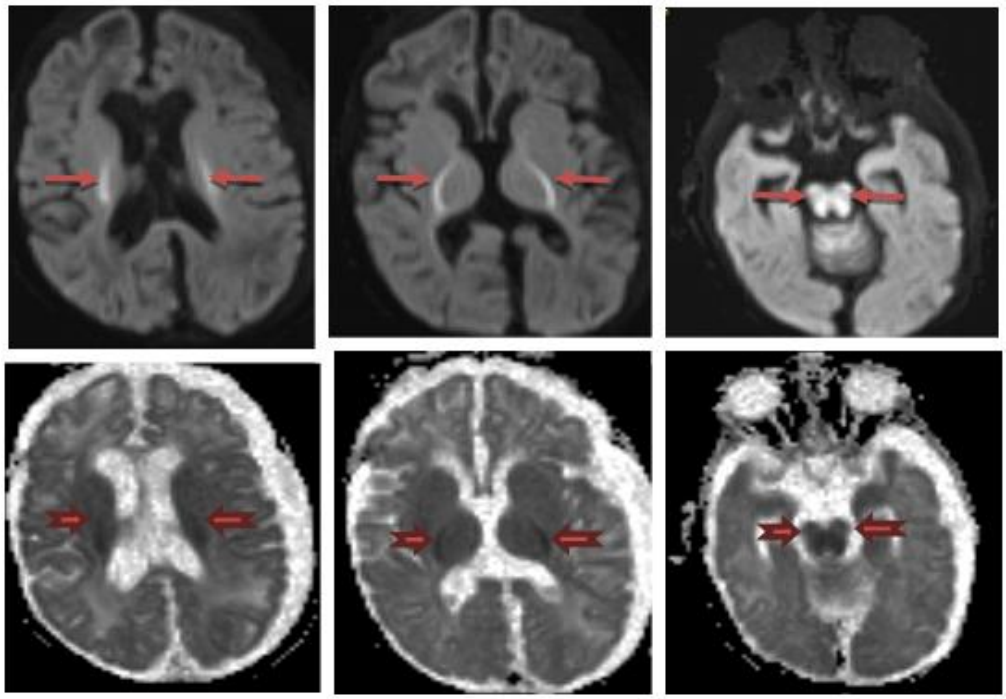


Figure 2: Diffusion weighted images (top row) and Apparent Diffusion Co-efficient (ADC) maps (bottom row) showing bilaterally symmetrical areas of diffusion restriction in the corona radiata, posterior limbs of internal capsule and midbrain which appear hyperintense in DWI (horizontal regular arrows in top row) and hypointense (horizontal notched arrows in bottom row) in ADC.

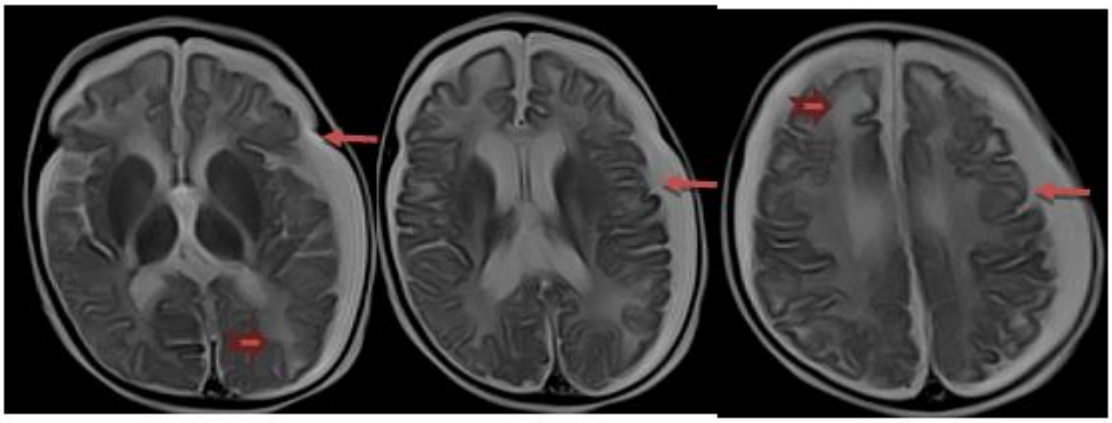


Figure 3: T2Weighted images showing bilateral prominent subdural hygroma (horizontal regular arrows) and abnormal hyperintense areas in the frontal and parieto-occipital white matter (Notched arrow)

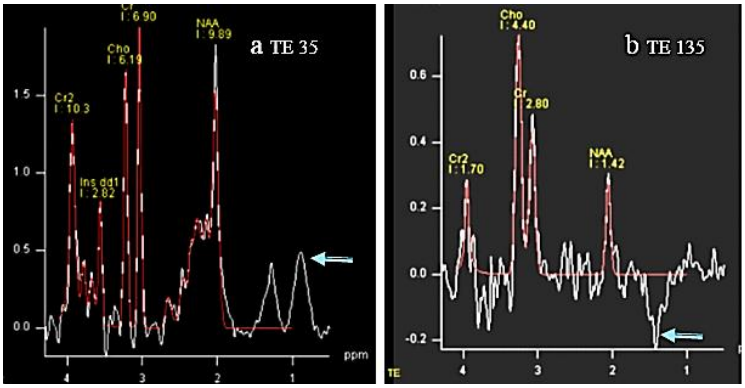


Figure 4: Magnetic resonance imaging H1 spectroscopy images (a, b) acquired with short time to echo (TE 35ms) and intermediate time to echo (TE 135ms). With short TE there is doublet peak at 1.3ppm (Horizontal arrow in a) and reversal of doublet in intermediate TE (horizontal arrow in b), consistent with lactate peak.

III. Discussion

CBD a neurocutaneous disorder,is a rare inborn error of metabolism with autosomal recessiveinheritancein which the body is unable to breakdown the conjugated form of biotin (vitaminB7)and clinical biotin deficiency[4]. This condition manifests due to mutation in biotinidase(BTD) gene at chromosome 3q25. The estimated prevalence of profound biotinidase deficiency (<10% of normal level) is approximately 1: 137000 newborns. The partial biotinidasedeficiency(<30%of normal) is estimated at 1:60,000 newborns[1]. The symptoms manifest in early to mid-infancy (mean age of onset – 3.5 months) [5].

Biotinidase deficiency causes biotin deficiency through multiple mechanisms such as, prevention of release of biotin bound to protein in food particles thus preventing absorption of biotin from intestine, impaired recycle of biotin from carboxylases and histones. The biotin serves as a co-factor for many enzymes such as pyruvate carboxylase, acetyl co-A carboxylase, propionyl Co-A carboxylase, beta methylcrotonyl-coA carboxylase and hence the enzyme biotinidase plays a role in fatty acid synthesis, amino acid catabolism and gluconeogenesis. The substrates accumulated in case of biotinidase deficiency are 3-hydroxy isovaleric acid, 3-methyl crotonylglycine, methylcitrate and 3-hydroxy propionate[6].When biotin is deficient,the activity of pyruvatecarboxylase in CNS falls, leading to localized lactic acidosis. The alopecia and dermatitis in these patients are possibly due to depletion of fatty acids as a result of deficient acetyl –CoA carboxylase.

Clinically, the babies present with multiple episodes of seizures, lethargy, hypotonia, ataxia, developmental delays, coma, alopecia, skin rash (mucocutaneous candidiasis) [2]. Hearing loss and decreased visual acuity are the late complications and are seen less frequently. Biochemically, there will be severe metabolic acidosis, ketosis, hyperammonemia, lactic acidosis and abnormal organic acids in urine. Ketosis and skin manifestations are the atypical features.

Radiologically, MRI will reveal, features of delayed myelination likeabnormalT2/FLAIR hyperintensities with T1 hypointensities in bilateral fronto-parietal white matter, bilateral cerebellar white matter, and cerebellar vermis. There can be bilateral symmetrical areas of diffusion restriction involving corona radiata, posterior limb of internal capsule, cerebellar white matter, cerebellar vermis, anterior midbrain, medial

temporal gyri, hippocampi and parahippocampal gyrus[1].The changes of restricted diffusion will be seen more in the myelinated white matter fibers. MR spectroscopy will show elevated lactateandglycine and decreased N-Acetylaspartate and choline. Some studies have shown involvement of the corpus callosum as well, which was not seen in our study[4].

There are few other conditions in which the MRI findings can closely mimic CBD, which should be considered in the differential diagnoses. They are 1.Maple syrup urine disease[7] (MSUD)–here the onset of clinical signs and symptoms is relatively early, during first week of life with poor feeding, vomiting, dystonia and seizures. Skin manifestations will be absent. Biochemically – ketoacidosis and characteristic maple syrup odor in urine. In MRI, diffusion restriction is seen in deep cerebellar white matter, dorsal brain stem, cerebralpeduncles, and posterior limb of internal capsule, basal ganglia and thalami. Basal ganglia and thalami are typically spared in CBD. 2.Leigh'ssyndrome (LS) – mitochondrial disorder which presents later, towards the end of first year with hypotonia, psychomotor deterioration, ataxia, ptosis and swallowing difficulties without any skin manifestations[8]. In MRI grey matter is more frequently involved, diffusion restriction can be less pronounced and seen only in acute phase in corpus striatum, sub thalamic nucleus, deep cerebellar nuclei, pons, medulla and periaqueductal grey matter. In MRS there will be large lactate peakand small NAA peak.

IV. Conclusion

When a baby presents in early infancy with multiple episodes of seizures, skin manifestations, elevated lactate, metabolic acidosis, ketosis and hyperammonemia with typical MRI findings of bilateral symmetrical areas of diffusion restriction and brain atrophy, the likely diagnosis ofCBD has to be considered which will help to institute proper and timely treatment and thereby avoiding complications and long term sequeale of permanent neuronal damage. Since the structural changes are reversible, it is of paramount importance to make correct and prompt diagnosis of CBD.

Conflicts of interest: Nil
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Bibliography

[1]. Ranjan RS, Taneja S, Singh A, Gupta V. Congenital biotinidase deficiency – MRI findings in two cases. Indian J Radiol Imaging, 2019;29:99-103.
[2]. Matthias R. Baumgartner, Pediatric Neurology Part III, Biotinidase deficiency Handbook of Clinical Neurology 2013.
[3]. Shamina Rahman, Susan standing, R Neil Dalton, Michael G Pike, Late presentation of biotinidase deficiency with acute visual loss and gait disturbance: Developmental medicine and child neurology, 1997, 39: 830-831.
[4]. Karimzadeh P, Ahmadabadi F, Jafari N, Jabbehdari S, Alaee MR, Ghofrani M, Taghdiri MM, Tonekaboni SH. Biotinidase Deficiency: A Reversible Neurometabolic Disorder (An Iranian Pediatric Case Series). Iran J Child Neurol. 2013 Autumn; 7(4):47-52.
[5]. AmitavaDasgupta,, Biotin and other interferences in immunoassay, Chapter 2 - Biotin: Pharmacology, Pathophysiology, and Assessment of Biotin Status, , Pages 17-35, Elsevier 2019
[6]. Jeffrey M. Chinsky, Robert D. Steiner, Developmental-Behavioral Pediatrics (Fourth Edition), Elsevier 2009.
[7]. Aditi Jain, K. Jagdeesh, Ranoji Mane, and SaurabhSingla, Imaging in Classic Form of Maple Syrup Urine Disease: A Rare Metabolic Central Nervous System, J ClinNeonatol. 2013 Apr-Jun; 2(2): 98–100. PMID: 24049754.
[8]. AL Kartikasalwah et al. Leigh syndrome: MRI findings in two children Biomed Imaging Interv J 2010; 6(1):e6.

Original article

Correlation of splenic ARFI with endoscopic findings of esophageal varices in chronic liver parenchymal disease

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Abstract:

Background: Variceal bleed the major cause of mortality in cirrhosis. Upper gastrointestinal (GI) endoscopy remains the main stay of diagnosis and treatment in variceal bleed. Splenic elastography using Acoustic Radiation Force Impulse (ARFI) technique can be used to non-invasively predict presence and severity of esophageal varices.

Material and methods: It is a hospital based prospective case control study, done in 100 patients with chronic liver parenchymal disease, who had undergone upper GI endoscopy. Fifty consecutive patients with esophageal varies and 50 consecutive patients without esophageal varices were included in the study and subjected to splenic ARFI. Data was analyzed with SPSS version 20.0 software using chi-square test or Fischer exact test, independent sample-t test. The receiver operating characteristics (ROC) curve was plotted and the area under the curve was used to determine the cut off points for statistically significant variables.

Results: Spleen ARFI showed significant correlation between cirrhotics with esophageal varices and without varices groups. The cut off of ≥ 3.16 m/s (AUROC: 0.906) precisely detects varices with sensitivity of 94% and specificity of 92%. Spleen ARFI also correlates with severity of varices. Cut off of ≥ 3.29 m/s (AUROC: 0.874) can confidentially differentiate low grade from high grade varices in cirrhotics with sensitivity of 85% and specificity of 100%.

Conclusions: Amongst the noninvasive parameters analyzed in this study, spleenic ARFI exclusively showed highest sensitivity and specificity in the detection of varices and differentiation of high grade varices from low grade varices.

Key words: Splenic ARFI, cirrhosis, Endoscopy, Esophageal varices.

Introduction:

Diverse insults on the liver lead to a common endpoint as cirrhosis. Cirrhosis is pathologically characterized by extensive fibrosis, regenerative nodules and distortion of hepatic parenchymal architecture [1]. Alcoholism and viral hepatitis have been the most common etiologies leading to cirrhosis, however nonalcoholic fatty liver disease is emerging as an important underlying pathology.

Complications of cirrhosis can be categorized as portal hypertension related and portal hypertension unrelated complications. Most common portal hypertension related complications is development of esophageal varices and variceal bleeding [2]. Till date, invasive upper gastrointestinal endoscopy (UGI scopy) remains the mainstay

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for the detection and grading of esophageal varices [3]. In addition to diagnostic advantage, UGI scopy plays a major role in treatment such as band ligation and sclerotherapy. Despite its diagnostic and therapeutic utility, UGI scopy is an invasive procedure, has its inherent complications such as bleeding, perforation, infection and cardio-respiratory complications related to sedation [4].

With current advances in the field of Ultrasonography, estimation of elasticity of the tissue has been made possible with Elastography. It is analogous to manual palpation and in fact offers better information, based on the principle that pathology changes the stiffness of the tissue. Shear Wave Elastography (SWE) is a technique of elastography that uses "shear wave" generated within tissue secondary to the acoustic push, the so called Acoustic Radiation Force Impulse (ARFI). In this technique as no external compression is required and a push impulse can be directed anywhere, it is possible to attain a fine elastogram of entire organ. Additionally, quantification of elasticity of particular region of interest based on shear wave velocity (SWV) is possible [5].

Elasticity of the spleen will be decreased in cirrhosis due to portal venous congestion and hyperplasia of splenic tissue; hence the splenic stiffness will be increased. Portal venous congestion results in esophageal varices. Screening UGI scopy is recommended in all cirrhotic patients in accordance with recent guidelines as primary prophylaxis against variceal hemorrhage[6]. Implementing these guidelines is confronted with cost, complications and invasiveness of UGI scopy. Hence, it necessitates accurate and noninvasive methods for detecting and grading the severity of esophageal varices.

Aim:

To evaluate splenic ARFI as a noninvasive tool for detection of esophageal varices and grading of varices as to high and low grades in chronic liver parenchymal disease.

Materials and methods:

It is a prospective case control study done on 100 patients over a period of 14 months, who were referred to the department of radiology with a diagnosis of chronic liver parenchymal disease. Subsequently all these patients underwent UGI scopy and were divided into two groups. Consecutive 50 patients without esophageal varices comprised the first group and 50 patients with esophageal varices comprised second group. Study was undertaken after obtaining necessary clearance from the institutional human ethics committee. Patients who are of less than 18 years of age and who did not undergo UGI scopy were excluded from the study. All the patients were subjected to splenic ARFI and UGI scopy.

Splenic ARFI protocol:

Splenic ARFI was performed with Siemens Acuson S2000^{TM} / Siemens Acuson S3000 ^{TM} ultrasound systems with ARFI enabled 6C1 / 4C1 curvilinear transducers. Patient was made to lie in right lateral decubitus position and assessment was done by intercostal approach. In order to cover uniformly the spleen, we adopted the stratified random sampling method used in the previous studies [7] (Figure 1). Spleen will be subdivided into cranial external(1), cranial central(2), cranial internal(1), intermediate external(1), intermediate central(1), intermediate internal(1), caudal external(1), caudal central(1) and caudal internal(1) segments. Numbers in the parenthesis represents the number of ARFI values obtained in each segment. In breath hold position, region of interest measuring 10mm in depth and 5mm in width was placed in parenchyma devoid of visible blood vessels and >1 cm below the level of spleen capsule and ARFI evaluation was done (Figure 2). Total of 10 successful measurements were obtained for each patient. Mean ARFI values were automatically calculated and expressed

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in meters per second (m/s).

The data obtained was coded and entered in Microsoft Excel Spreadsheet. Data was analyzed using SPSS version 20.0 statistical software. Categorical variables were expressed as percentages and the comparative analysis was done using chisquare test or Fischer exact test. Continuous variables were expressed as mean ±standard deviation (SD) and the comparative analysis was done by independentsample 't' test. The receiver operating characteristics (ROC) curve was plotted and the area under the curve was determined to the cut off points for statistically significant variables. A probability value (p value) of less than or equal to 0.05 at 95% confidence interval was considered as statistically significant.

Various radilogical parameters like liver size, portal vein diamater, portal vein flow velocity, spleen size, liver ARFI and splenic ARFI were analysed for their value as predictors of presence or absence of esophageal varices, if present, to differentiate high grade varices from low grade varices using various statistical methods as mentioned above.

Results:

TABLE 1: SEX DISTRIBUTION

SEX	WITH EVs		WITHOUT EVs	
	NUMBER	%	NUMBER	%
MALE	36	72	38	76
FEMALE	14	28	12	24
TOTAL	50	100	50	100

TABLE 2: ETIOLOGY DISTRIBUTION IN CIRRHOTICS WITH EVs

ETIOLOGY	WITH EVs		WITHOUT EVs	
	NUMBER	%	NUMBER	%
ALCOHOL	37	74	35	70
NAFLD	6	12	6	12
AUTOIMMUNE	6	12	9	18
METABOLIC	1	2	0	0
TOTAL	50	100	50	100

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TABLE 3: SIGNIFICANCE OF RADIOLOGICAL PREDICTORS BETWEEN CIRRHOTICS WITH EVs AND WITHOUT EVs

PREDICTOR	INDEPENDENT 't' TEST		
	WITH EVs MEAN ± SD	WITHOUT EVs MEAN ± SD	P value
LIVER SIZE	140.50 ± 20.97	138.36 ± 19.62	0.599
PORTAL VEIN DIAMETER	12.54 ± 2.46	12.28 ± 2.06	0.569
PORTAL VEIN FLOW VELOCITY	18.91 ± 6.23	20.28 ± 4.56	0.216
SPLEEN SIZE	**147.54 ± 26.5**	**138.66 ± 16.90**	**0.049***
PLATELET COUNT SPLEEN SIZE RATIO	**588.52 ± 274.58**	**972.86 ± 177.99**	**0.000***
LIVER ARFI	3.03 ± 0.78	2.92 ± 0.69	0.458
SPLEEN ARFI	**3.33 ± 0.23**	**2.99 ± 0.17**	**0.000***

*** P= < 0.05 is statistically significant**

Spleen size, PSR and spleen ARFI showed statistical significance between cirrhotics with EVs and cirrhotics without EVs groups.

TABLE 4: SIGNIFICANCE OF RADIOLOGICAL PREDICTORS BETWEEN LOW GRADE EVs AND HIGH GRADE EVs

PREDICTORS			
	LOW GRADE MEAN ± SD	HIGH GRADE MEAN ± SD	P value
LIVER SIZE	144.91 ± 23.39	136.74 ± 18.27	0.172
PORTAL VEIN DIAMETER	12.22 ± 2.33	12.81 ± 2.58	0.399
PV FLOW VELOCITY	18.69 ± 7.09	19.10 ± 5.52	0.819
SPLEEN SIZE	140.70 ± 31.17	153.37 ± 20.60	0.092
PLATELET SPLEEN SIZE RATIO	651.63 ± 223.87	534.75 ± 305.22	0.135
LIVER ARFI	2.99 ± 0.65	3.07 ± 0.90	0.698
SPLEEN ARFI	**3.22 ± 0.36**	**3.42 ± 0.28**	**0.001***

*** P= < 0.05 IS STATISTICALLY SIGNIFICANT**

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Spleen ARFI solely independently shows statistical significance between low grade EVs and high grade EVs subgroups.

TABLE 5: ROC ANALYSIS: SPLEEN ARFI IN EVs DETECTION

PREDICTOR	CUT OFF	AUROC	SENSITIVITY	SPECIFICITY
SPLEEN ARFI	≥ 3.16	0.906	94%	92%

ROC analysis of spleen ARFI (Figure 3) in detecting EVs in this study showed maximum area under the curve of 0.906 at a cutoff of ≥ 3.16 with maximum sensitivity of 94% and specificity of 92%.

TABLE 6: ROC ANALYSIS: SPLENIC ARFI IN HIGH GRADE AND LOW GRADE EVs

PREDICTOR	CUT OFF	AUROC	SENSITIVITY	SPECIFICITY
SPLEEN ARFI	≥ 3.29	0.874	85%	100%

ROC analysis of spleen ARFI (Figure 4) in delineating high grade and low grade EVs depicted a cutoff of ≥ 3.29 with maximum area under the curve of 0.874 at cutoff of with sensitivity of 85% and specificity of 100%.



Figure: 1. Stratified random sampling method. Spleen will be subdivided into cranial external(1), cranial central(2), cranial internal(1), intermediate external(1), intermediate central(1), intermediate internal(1), caudal external(1), caudal central(1) and caudal internal (1) segments. Numbers in the parenthesis represents the number of ARFI values obtained in each segment.



Figure: 2. In breath hold position, region of interest measuring 10mm in depth and 5mm in width was placed in parenchyma devoid of visible blood vessels and >1 cm below the level of spleen capsule and ARFI evaluation was done.

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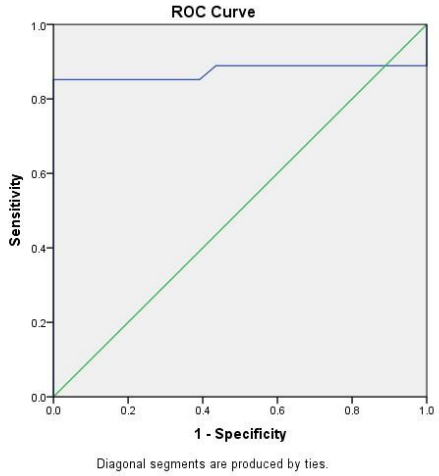


Figure: 3. ROC analysis of spleen ARFI in detecting EVs showed maximum area under the curve of 0.906 at a cutoff of ≥ 3.16 with maximum sensitivity of 94% and specificity of 92%.

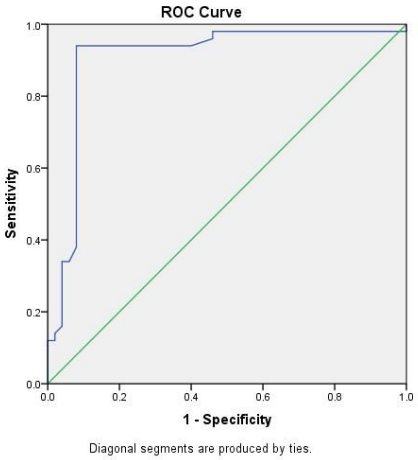


Figure: 4. ROC analysis of spleen ARFI in delineating high grade and low grade EVs depicted a cutoff of ≥ 3.29 with maximum area under the curve of 0.874 at cutoff of with sensitivity of 85% and specificity of 100%

Discussion:

Recent guidelines endorse that endoscopic screening should be done for all cirrhotic patients at the time of diagnosis to recognize those at high risk of bleeding and possibly to benefit from primary prophylaxis [6]. Implementation of these guidelines imposes huge burden on UGI scopy units and frequent testing over time can decrease the compliance of patient. By noninvasive prediction of the presence of EVs, one can recommend the appropriate category of patients to undergo invasive UGI scopy, thereby reducing unnecessary endoscopic examinations and burden on endoscopy units.

Previous studies have correlated various radiological and laboratory parameters either alone or in combination with the presence or absence of EVs in cirrhotics. Although, the efficiency of parameters or combination of parameters reported in each study differ amongst them, due to the heterogeneous nature of the studies. Good predictive value of several non-endoscopic variables for the presence or absence of EVs have been documented

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by many previous studies, but the data is limited from our part of the country. This study was performed to assess the effectiveness of various clinical, laboratory and radiological parameters, in reliably detecting the existence of EVs and high grade EVs in cirrhotic patients.

In the present study, there is no significant association of demographic parameters between the two study groups. Previous studies by Zaman et al [8] and Cherian et al [9], showed that Child Pugh class B or C cirrhotic patients, as compared to class A cirrhotics, have high propensity towards the detection of EVs on UGI scopy. In contrary, our study inferred no significant association between Child Pugh class and the presence of EVs (p = 0.799). In addition, we assessed MELD scores and found significant affiliation with presence of EVs, in congruence to the results of previous studies by De Mattos et al [10] , Tafarel et al [11] and Chandail VS et al [12].

Our study showed no significant variation in either portal vein diameter or portal vein flow velocity between cirrhotic patients with EVs and without EVs. Our study in contradiction to previous studies inferred that there is no significant difference in liver stiffness measured by elastography between cirrhotics with EVs and cirrhotics without EVs (p= 0.458).

In agreement with previous studies, our study also showed significant difference in the values of spleen stiffness measured by ARFI between cirrhotics with EVs and without EVs (p= 0.000). We acquired the optimal cutoff of ≥ 3.16m/s (AUROC: 0.906) with sensitivity of 94% and specificity of 92%, thereby validating spleen ARFI as a non-invasive radiological marker for the presence of EVs in cirrhotics.

In the present study, we furthermore evaluated the utility of the above discussed noninvasive clinical, laboratory and radiological parameters in the detection of high grade EVs in cirrhotics. Of all the noninvasive parameters analyzed, spleen ARFI exclusively showed significant difference between the two subgroups (p= 0.001). We derived the optimal cutoff of ≥ 3.29m/s (AUROC: 0.874) with sensitivity of 85% and specificity of 100%, which is in very close approximation with spleen ARFI cutoff of ≥ 3.30m/s obtained by Takuma et al[13]. Thereby substantiating spleen ARFI as a non-invasive radiological marker for the presence of high grade EVs in cirrhotics. In other words, the presence of high grade EVs can be reliably ruled out with the spleen ARFI cutoff of < 3.29m/s in patients with chronic liver parenchymal disease. Our study was limited by only few patients with grade IV EVs being included in this study. It is due to the lower prevalence of grade IV EVs, as in part by the practice of generous prophylactic and therapeutic measures which delays or eliminates progression of low grade EVs to high grade EVs. Inter observer variability is not assessed in this study. Future studies on this context should focus on multicenter design with evaluation of equipment of various manufacturers and assessment of inter observer variability.

Conclusions:

Amongst the noninvasive parameters analyzed in this study, spleenic ARFI exclusively showed highest sensitivity and specificity in the detection of varices and differentiation of high grade varices from low grade varices.

References :

1. Sangster GP, Previgliano CH, Nader M, Chwoschtschinsky E, and Heldmann MG. MDCT Imaging Findings of Liver Cirrhosis: Spectrum of Hepatic and Extrahepatic Abdominal Complications. HPB Surg 2013;129396:1-12.

2. Schuppan D, Afdhal NH. Liver Cirrhosis. Lancet 2008;371(9615):838-851.

3. Singh SP, Panigrahi MK. Spectrum of Upper Gastrointestinal Hemorrhage in Coastal Odisha. Trop Gastroenterol

2013;34(1):14-17.

4. Riley S, Alderson SD, Birmingham. Complications of upper gastrointestinal endoscopy. BSG Guidelines in Gastroenterology 2006 Nov:7-13.

5. Taljanovic MS, Gimber LH, Becker GW, Latt LD, Klauser AS, Melville DM et al. Shear-Wave Elastography: Basic Physics and Musculoskeletal Applications. Radiographics 2017 May-Jun;37(3):855-870.

6. Kim HY, Jin EH, Kim W, Lee JY, Woo H , Oh S et al. The Role of Spleen Stiffness in Determining the Severity and Bleeding Risk of Esophageal Varices in Cirrhotic Patients. Medicine (Baltimore) 2015 Jun;94(24):e1031.

7. Rizzo L, Attanasio M, Pinzone MR et al. A New Sampling Method for Spleen Stiffness Measurement Based on Quantitative Acoustic Radiation Force Impulse Elastography for Noninvasive Assessment of EsophagealVarices in Newly Diagnosed HCV-Related Cirrhosis. BioMed Research International 2014;Article ID 365982:1-8.

8. Zaman A, Hapke R, Flora K, Rosen HR, Benner K. Factors predicting the presence of esophageal or gastric varices in patients with advanced liverdisease. Am J Gastroenterol 1999 Nov;94(11):3292-3296

9. Cherian JV, Deepak N, Ponnusamy RP, Somasundaram A, Jayanthi V. Non-invasive predictors of esophageal varices. Saudi J Gastroenterol 2011 Jan-Feb;17(1):64-8.

10. de Mattos AZ, de Mattos AA, Daros LF, Musskopf MI. Aspartate aminotransferase-to-platelet ratio index (APRI) for the non-invasive prediction of esophageal varices. Ann Hepatol 2013 Sep-Oct;12:910-4.

11. Tafarel JR, Tolentino LH, Correa LM, Bonilha DR, Piauilino P, Martins FP et al. Prediction of esophageal varices in hepatic cirrhosis by noninvasive markers. Eur J Gastroenterol Hepatol 2011;23:754-58.

12. Chandail VS, Kotwal SK, Koul S, Gupta R, Mahajan A. Non-invasive markers for prediction of varices in patients with portal hypertension. Int J Res Med Sci 2017;5:1007-10.

13. Takuma Y, Nouso K, Morimoto Y, Tomokuni J, Sahara A, Toshikuni N et al. Measurement of spleen stiffness by acoustic radiation force impulse imaging identifies cirrhotic patients with esophageal varices. Gastroenterology 2013 Jan;144(1):92-101.

Original article

Correlation of diffusion tensor imaging with clinical and neurological findings in patients with cervical spondylotic myelopathy

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ABSTRACT:

Background: Cervical spondylosis is a common degenerative disease of spine generally seen in old age, frequently causing compression of spinal cord and /or nerve roots by protruding disc or osteophytes. Magnetic Resonance Imaging (MRI) is the modality of choice for evaluation of patients with features of cervical spondylotic myelopathy. In addition to routine MRI sequences, advanced MR techniques like diffusion tensor imaging (DTI) can be used to obtain specific information about the spinal cord.

Materials and methods: It is a prospective observational study conducted in 30 patients who presented with clinical and neurological features of cervical spondylotic myelopathy, who had undergone MRI.

Result: All the patients had undergone routine MRI along with diffusion tensor imaging. The Apparent Diffusion Coefficient (ADC) and Fractional Anisotropy (FA) values of the spinal cord were measured at the level of normal spinal canal dimension and at the level where there is narrowing of the spinal canal. The ADC values were found to be high and the FA values were low at the level of the spinal canal stenosis in comparison with values measured at the level of normal spinal canal dimension, which were statistically significant.

Conclusion: DTI parameters showed significant correlation with the clinical parameters, suggesting that DTI can be used to detect microscopic changes related to compressive myelopathy, before changes are apparent in the routine MR sequences. This can enable us to intervene early to avoid irreversible damage to the spinal cord.

Key words: Cervical spondylotic myelopathy, Diffusion tensor imaging, Fractional anisotropy.

Introduction

Cervical spondylosis is a common degenerative disease of spine generally seen in old age, frequently causing compression of spinal cord and /or nerve roots by various pathological entities like protruding disc, osteophytes and thickening of ligamentous structures around the spinal cord.[1] Spondylotic myelopathy refers to compression of the spinal cord by the above mentioned structures leading to impairment of spinal cord function.[2] Cervical spondylotic myelopathy (CSM) is usually seen after the 5th decade. Patients with CSM usually present with symptoms of numbness and weakness in limbs, pain over shoulder and neck, incoordination. Magnetic Resonance Imaging (MRI) of cervical spine is non-invasive and accurate imaging tool in assessing the structural changes of spinal cord due to its high contrast and soft tissue resolution[3]. It helps to ascertain the site and the

severity of degenerative compressive myelopathy. It is also useful to exclude any other pathology, like neoplasm which can cause compressive myelopathy and mimic degenerative disc disease.

Classical MRI finding seen in compressive myelopathy is focal T2 hyperintensity in spinal cord at the level of a compressive lesion. This signal change is suggestive of either spinal cord oedema in acute cases, or gliosis and myelomalacia in chronic cases[4,5]. Oedema is associated with swelling of the spinal cord and chronic changes on the other hand show thinning of the spinal cord. Other imaging modalities like Computed Tomography (CT) and radiography play a complementary role in the evaluation of CSM. Due to its inherent high spatial resolution CT is better than MRI in the assessment of cortical bone, osteophytes and other calcified ligamentous structures. These structures can also cause spinal canal compromise. Plain radiography is still being used as an initial tool for evaluating degenerative disc changes[6]. The ultimate aim of doing imaging studies is to identify the spinal cord abnormalities early and take remedial measures to prevent further damage[7].

Aims:

1. To compare Fractional Anisotropy (FA) and Apparent Diffusion Coefficient (ADC) values of the spinal cord obtained at the level with above and below the level of spinal canal stenosis.
2. To compare the Diffusion Tensor Imaging (DTI) parameters with Nurick score and neurological examination findings.
3. To detect the early microscopic level structural abnormalities of spinal cord using DTI before overt, irreversible changes manifest in routine MR sequences.

Materials and methods:

It is a prospective observational study performed in 30 patients who were referred by the department of neurology. These patients presented with clinical symptoms and neurological findings suggestive of cervical myelopathy. The study period was from January 2018 to March 2019 at the department of Radiology and imaging sciences PSG Institute of Medical Sciences & Research. Prior to commencement of the study, clearance has been obtained from Institutional Human Ethical Committee and informed written consent has been taken from the participants. Patients with clinical and neurological features of CSM were included in the study. Patients with following abnormalities were excluded from the study: 1.focal or diffuse signal abnormality in the spinal cord. 2. History of cervical spine injury / surgery. 3. Focal lesions in the brain which may be the underlying pathology causing the neurological abnormality.

Prior to undergoing MRI all these patients were assessed symptom wise for the severity of CSM using Nurick score and neurological examination was performed to assess the power of lower and upper limbs. In Nurick score patients were categorized from grade 0 to 3, based on clinical history alone, where grade 0 represented mild form of disease with only symptoms suggestive of nerve root involvement (radiculopathy), grade 3 represents more severe neurological deficit where the patients are wheel chair bound with no independent mobility (myelopathy). On clinical examination muscle power of the limbs was assessed using standard grade 1 to grade 5.

All the patients underwent MRI in Siemens 1.5 Tesla 16 channel machine.

MRI protocol:

1. Routine non contrast T1 and T2 weighted images to look for overt signal changes in T2 Weighted Images.
2. DTI acquisition using EPI yielding the following set of images:

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1. Diffusion weighted imaging, 2.Trace weighted, 3.FA and ADC mapping 4.Tensor imaging.

DTI parameters:

Diffusion directions measured – 20.

The b factors – 0, 100 and 1000

TR/ TE - 3600 /94ms

Field of view - 230mm

Slice thickness - 5mm.

Images were analysed using Siemens Syngo via work station. Initially presence of any focal or diffuse spinal cord lesions was excluded. After that dimension of the spinal canal was measured at all the vertebral level. Subsequently DTI analysis was performed by drawing region of interest at the level of maximum spinal canal stenosis, one vertebral level above the spinal canal stenosis and one level below (Figure 1a). DTI Parameters assessed were FA and ADC, the values of which were displayed as a table in the system (Figure 1b) along with images of visual maps of ADC (Figure 2a) and FA (Figure 2b).

Statistical analysis:

The data has been analysed using SPSS 23.0 version. To describe the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean & standard deviation (SD) were used for continuous variables. To find the significant difference in the multivariate analysis for repeated measures the repeated measures of ANOVA was used with Bonferroni correction to control the type I error on multiple comparison. To assess the relationship between the variables Pearson's Correlation was used. In all the above statistical tools the probability value .05 is considered as statistically significant.

Results:

A total of 30 patients were enrolled in our study of which 20 were men and 10 were women (Figure 3). Nurick Score distribution is given in the table 1.

All the patients had muscle power grade between III and V. (Figure 4). No patient in our study had muscle power less than 3.

Diffusion tensor parameters (ADC and FA) were measured at 3 levels of the spinal cord (Figure 1a). One measurement at the level of the spinal canal stenosis and 2 measurements (above and below the level of stenosis) at the level of normal canal dimension. The values of ADC and FA measured at different levels of the spinal cord are given in table 2.

Pairwise comparison of ADC and FA was performed and results are given in table 3 and table 4. Patients were found to have high ADC and low FA values at the level of stenosis when compared with ADC and FA values at the level of no stenosis. The differences were found to be statistically highly significant with a p value of 0.0005 (ANOVA test was used). There was negative correlation between the mean FA and ADC at the level of stenosis with r value of -0.424 and p value of 0.019. There was negative correlation between the mean FA and ADC at the level of no stenosis with r value of -0.0545 and p value of 0.002. There is negative correlation between Nurick score (clinical grading) and muscle power (neurological examination) r value of -0.07 and P value of 0.001.

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Correlation of FA and ADC with muscle power:

At the level of stenosis:

There was a positive correlation between muscle power and mean FA value with r value of 0.2 and p value 0.1. There is a negative correlation between muscle power and mean ADC value at the level of stenosis with r value of -0.1 and p value 0.1 and p value of 0.3.

At the level of no stenosis:

There is a positive correlation between muscle power grade and mean FA value at the non- stenosis level with r value of 0.18 and p value 0.32 and p value of 0.4. There is a negative correlation between muscle power grade and mean ADC value at the non-stenosis level upper with r value of -0.25 and p value 0.17.

Correlation of FA and ADC with Nurick score:

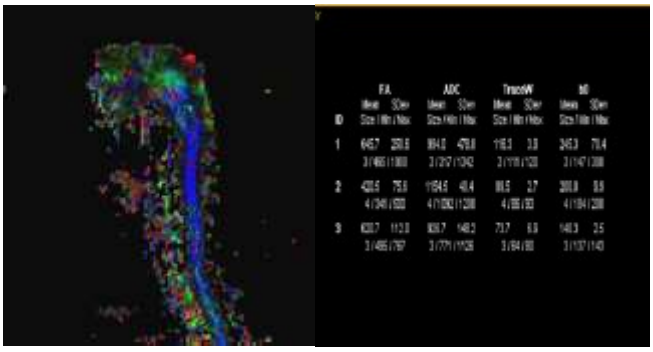
At the level of stenosis:

FA and Nurick score has negative correlation with r value of -0.4 and p value of .0005. ADC and Nurick score has a positive correlation with r value of -0.1 and p value of – 0.2

At the level of no stenosis:

FA and Nurick score had positive correlation with r value of 0.1 and p value of –0.4. ADC and Nurick score had negative correlation r value of -0.05 and p value of– 0.7

ROC analysis of FA in predicting microscopic level structural cord abnormality in the spinal cord at the level of the spinal canal stenosis. (Figure 5) was done. Subsequently test result variables (table 5) and sensitivity and specificity (table 6) were calculated. Likewise ROC analysis of ADC in predicting microscopic level structural cord abnormality was done (Figure 6). Test result variables (table 7) and sensitivity and specificity (table 8) were also calculated for ADC. Both FA and ADC showed good sensitivity and specificity in predicting the early microscopic level changes in the spinal cord caused by degenerative disease, ADC was slightly more specific than FA.

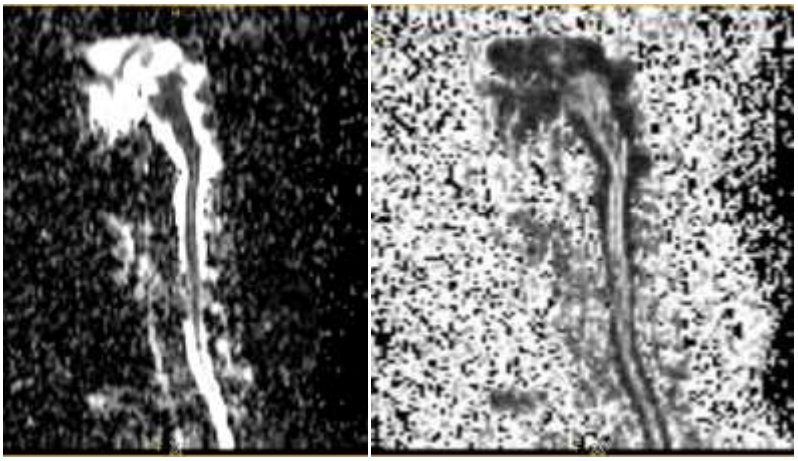


a **b**

Figure 1. Method of FA and ADC measurement.

Three circular Region of Interest (ROI) are drawn “at”, “above” and “below” the level of stenosis (a) yielding the numeric values of each level in a table (b).

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a b

Figure 2. ADC (a) and FA (b) maps obtained with DTI.

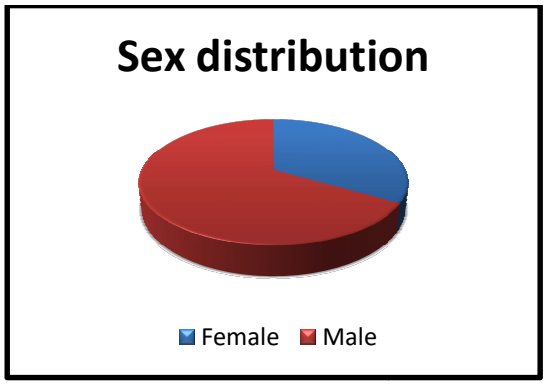


Figure 3. Sex distribution in the study population.

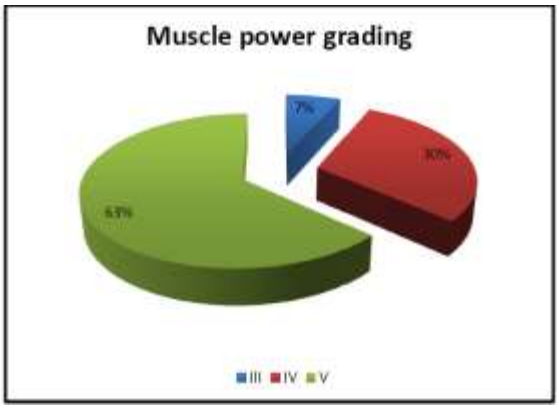


Figure 4. Muscle power distribution in the study population.

Majority of the patients had a muscle power of 5. Remaining patients had muscle power of 4 and 3.

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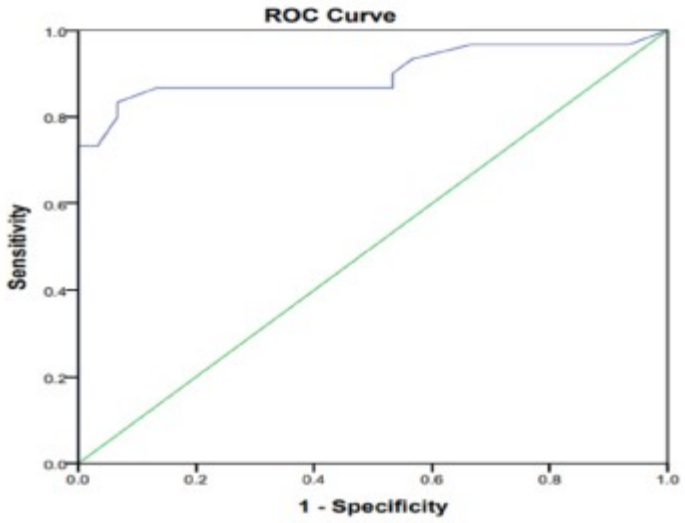


Figure 5: ROC analysis of FA in predicting the microscopic structural abnormality in the spinal cord.

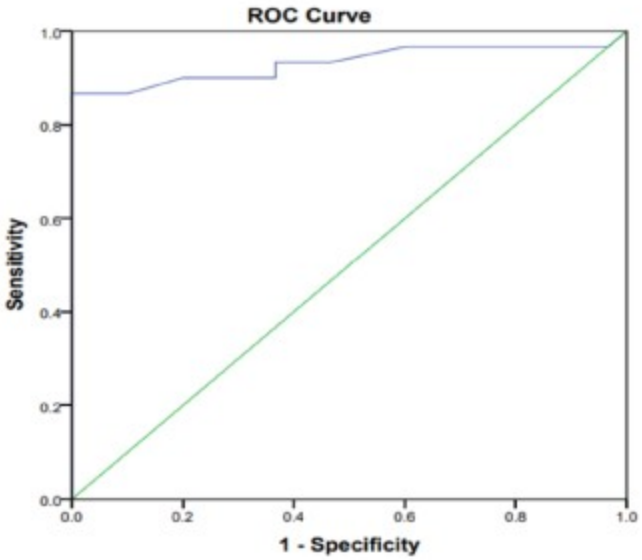


Figure 6: ROC analysis of ADC in predicting the microscopic structural abnormality in the spinal cord.

Table1: Nurick Score distribution in the study population:

Nurick Score	Number	Percent
0	2	6.7
1	9	30.0
2	9	30.0
3	10	33.3
Total	30	100.0

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Table 2: Values of ADC and FA measured at different levels of the spinal cord.

	ADC		FA	
Level of spinal cord	Mean	SD	Mean	SD
Stenotic Level	1.3470	.23997	.5067	.09484
Non-stenotic level upper	1.0141	.09272	.6670	.07957
Non-stenotic level lower	1.0120	.08919	.63443	.07566

Table 3: Pairwise comparison of ADC

(I) ADC		Mean Difference (I-J)	Std. Error	Sig.[b]
Mean ADC stenotic level	Mean ADC non-stenotic level upper	.333*	.045	.0005
	Mean ADC non-stenotic level lower	.335*	.043	.0005
Mean ADC non-stenotic level upper	Mean ADC stenotic level	-.333*	.045	.000
	Mean ADC non-stenotic level lower	.002	.021	1.000
Mean ADC non-stenotic level lower	Mean ADC stenotic level	-.335*	.043	.000
	Mean ADC non-stenotic level upper	-.002	.021	1.000

Based on estimated marginal means.

*The mean difference is significant at the .05 level.

b. Adjustments for multiple comparisons: Bonferroni.

Table 4: Pairwise comparison of FA:

(I) FA		Mean Difference (I-J)	Std. Error	Sig.[b]
Mean FA stenotic level	Mean FA non-stenotic level upper	.160*	.019	.0005
	Mean FA non-stenotic level lower	.128*	.023	.0005
Mean FA non-stenotic level upper	Mean FA stenotic level	-.160*	.019	.000
	Mean FA non-stenotic level lower	.033	.017	.219
Mean FA non-stenotic level lower	Mean FA stenotic level	-.128*	.023	.000
	Mean FA non-stenotic level upper	-.033	.017	.219

Based on estimated marginal means.

*The mean difference is significant at the .05 level.

b. Adjustments for multiple comparisons: Bonferroni.

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Table 5: Test result variables of FA

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.902	.045	.0005	.815	.990

Table 6: Sensitivity and specificity calculation of FA.

Predictor	Cut off	AUROC	Sensitivity	Specificity
FA	0.57	0.902	86.7%	86.7%

Table 7: Test result variables of ADC.

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.932	.039	.0005	.857	1.000

Table 8: Sensitivity and specificity calculation of ADC.

Predictor	Cut off	AUROC	Sensitivity	Specificity
ADC	1.1	0.932	86.7%	90.0%

Discussion:

MRI has revolutionized the evaluation of pathology related to spinal cord. Exceptionally high contrast resolution and soft tissue resolution place MRI way above other imaging modalities in the evaluation of spinal cord.[2] Recent advances in respect to high field strength and fast and robust sequences have greatly contributed to the superior performance of MRI[8]. Very small yet important structures like the nerve roots and the thin ligaments need to be assessed in almost all patients which can harbour the pathology. However significant degree of anatomical and structures abnormalities need to be present before they are visible in routine MRI sequences, primarily in the spinal cord[6]. Hence the need for imaging technique which is similar to a biomarker to pick up early microscopic level changes in the neural structures, specially the spinal cord. Role of DTI in the evaluation of the brain pathologies has been proved and it is being used routinely, however its usage in the evaluation of spinal cord is still evolving.

The application of DTI in the evaluation of spinal cord is hindered by various factors. Spinal cord is a very narrow structure with a high elasticity and situated in anatomically complex location, which are the factors

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making evaluation difficult. Other factors like CSF pulsation, motion artefacts and breathing related artefacts make usage of DTI difficult as a routine sequence. Newer techniques like reduced field of view with oblique spin echo and OVS (outer volume suppression) have been used to overcome the limitations and better images can be obtained. All these improved techniques provide high resolution images not only in the cervical cord but also the entire spinal cord.

Budzik et al[9] studied the feasibility of DTI and fiber tracking in CSM. FA values showed correlation with the patients' clinical scores. High signal intensity of the spinal cord on T2WI did not correlated either with the DTI parameters or with the clinical symptoms, suggesting that FA is better in depicting the early structural abnormality of the spinal cord when compared with T2W imaging[10, 11]. But they did not study the relationship of DTI scalars with cervical canal width. In our study all the patients had spinal canal stenosis, in that about 73% of the patients had relative narrowing and 27% has absolute narrowing.

In our study degenerative changes like disc bulge, protrusion, osteophytes and ligament thickening led to spinal canal narrowing. These pathological changes caused disruption of the white matter tracts and other secondary changes in cervical cord. Secondary changes like cord edema and macrophage proliferation lead to increased diffusivity of water molecules with in the spinal cord parenchyma, especially in the white matter which can alter the FA and ADC values[12]. These changes were apparent much before macroscopic changes appear in routine T1 and T2W images.**UzeyirAhmadli et al**[13] investigated the utility of DTI sequence in patients with CSM symptoms for early detection of microscopic changes in the spinal cord at the level of narrowed spinal canal without any T2 signal alterations. FA values with in the cord were reduced and ADC increased at site of canal stenosis when compared with non-stenotic segments above the level of canal stenosis. However no significant difference was noted in FA and ADC values between stenotic segment and non-stenotic segment distal to the level of stenosis.

In our study there was statistically significant reduction in the FA values and increase in the ADC values at the site of stenosis when compared to the non-stenotic levels (upper and lower). There was no statistically significant reduction in FA value noted at the non-stenotic levels (upper and lower). The reduction in FA value at the stenotic level is presumably due to reduced neuronal fibers with increase in the extracellular space or due to extracellular edema which can result in increased diffusivity of water molecules and consequently facilitated diffusion.

Jones et al[14] reported a significant correlation between FA and modified Japanese orthopedic association score and Nurick score, and reported that patients who had a higher FA at the compressed level of spinal cord tended to have a better recovery of functionality after surgery when compared to patients undergoing surgery who had a lower FA value pre operatively. In our study there was significant correlation between the patient’s symptoms and the DTI parameters. We graded clinical symptoms using only Nurick score, modified Japanese orthopedic association score was not used.

FA and Nurick score had a negative correlation with r value of -0.4 and p value –0.0005, which implies that the FA values tend to be less when the Nurick score is on the higher side. ADC and Nurick score had a positive correlation with r value of-0.1 and p value of – 0.2, which is equal to higher ADC means higher Nurick score.There was also a significant correlation between the DTI parameters and muscle power. There was positive correlation between muscle power and mean FA value at the level of stenosis with r value of 0.2 and p

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value of 0.1. There is a negative correlation between muscle power and mean ADC value at the level of stenosis with r value of -0.1 and p value of 0.3. ROC was calculated between the stenotic and non stenotic level. The mean was calculated between the upper and lower non-stenotic level and it was compared with the stenotic level. FA showed a sensitivity of 86.7% and specificity was 86.7% when the cut was kept at 0.57 (Table 6). ADC showed a sensitivity of 86.7% and specificity was 90.0%, when the cut off is kept at 1.1. (Table 8).

Conclusion:

- Our study showed significant correlation between the patients' clinical symptoms and DTI parameters.
- DTI has the potential to detect microscopic structural changes in the spinal cord, before the T2 signal changes appear on conventional MRI.
- DTI can be used as a tool for detecting early changes in cervical spondylotic myelopathy, which would prognosticate the clinical and surgical outcome[14, 15].
- DTI technique should be routinely used on patients with clinical symptoms of cervical spondylotic myelopathy.

Limitations:

- The sample size of the study population was relatively small
- The long-term follow-up of the patients were not done
- It was impossible to determine the percentage of patients with early cervical spondylotic myelopathy who would have advanced to T2 hyperintensity changes in spinal cord, when left untreated.
- Cost and the availability of MRI.

Further studies have to be done on large group with long term follow up of those patients to determine the potential utility of quantitative diffusion tensor imaging.

Conflicts of interest:

There are no conflicts of interest to declare.

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References:

1. Crandall, Paul H., and Ulrich Batzdorf. "Cervical spondylotic myelopathy."Journal of neurosurgery 25.1 (1966): 57
2. Montgomery, D. M., and R. S. Brower. "Cervical spondylotic myelopathy. Clinical syndrome and natural history." The Orthopedic clinics of North America23.3 (1992): 487-493.
3. Al-Mefty, Ossama, et al. "Myelopathic cervical spondylotic lesions demonstrated by magnetic resonance imaging." Journal of neurosurgery 68.2 (1988): 217-222.

4. Chen, Chi-Jen, et al. "Intramedullary High Signal Intensity on T2-Weighted MR Images in Cervical Spondylotic Myelopathy: Prediction of Prognosis with Type of Intensity 1." Radiology 221.3 (2001): 789-794.
5. Fernández de Rota, Juan Jose, et al. "Cervical spondylotic myelopathy due to chronic compression: the role of signal intensity changes in magnetic resonance images." Journal of Neurosurgery: Spine 6.1 (2007): 17-22.
6. Freeman, T. B., and C. R. Martinez. "Radiological evaluation of cervical spondylotic disease: Limitation of magnetic resonance imaging for diagnosis and preoperative assessment." Perspect Neurol Surg 3.91 (1992): 34-54.
7. Suri, Ashish, et al. "Effect of intramedullary signal changes on the surgical outcome of patients with cervical spondylotic myelopathy." The Spine Journal3.1 (2003): 33-45.
8. Fulong Dong, Wu Y, Song P "A preliminary study of 3.0-T magnetic resonance diffusion tensor imaging in cervical spondylotic myelopathy".Eur Spine J. 2018 Aug;27(8):1839-1845.
9. Budzik JF, Balbi V, Le Thuc V, Duhamel A, Assaker R, Cotten A "Diffusion tensor imaging and fibre tracking in cervical spondylotic myelopathy" Eur Radiol.2011 Feb;21(2):426-33.
10. Kara B, Celik A, Karadereler S, Ulusoy L, "The role of DTI in early detection of cervical spondylotic myelopathy: a preliminary study with 3-T MRI" Neuroradiology. 2011 Aug;53(8):609-16.
11. Mori S, Zhang JPrinciples of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 2006 Sep 7;51(5):527-39
12. Toktas ZO, Tanrıkulu B, Koban O, Kilic T, Konya D "Diffusion tensor imaging of cervical spinal cord: A quantitative diagnostic tool in cervical spondylotic myelopathy". J Craniovertebr Junction Spine. 2016 Jan-Mar;7(1):26-30
13. Uzeyir Ahmadli, Nils H Ulrich ,Yao Yuqiang, Daniel Nanz Early detection of cervical spondylotic myelopathy using diffusion tensor imaging: Experiences in 1.5-tesla magnetic resonance imaging. Neuroradiol J. 2015 Oct; 28(5): 508–514.
14. Jones JG, Cen SY, Lebel RM, Hsieh PC, Law M "Diffusion tensor imaging correlates with the clinical assessment of disease severity in cervical spondylotic myelopathy and predicts outcome following surgery". AJNR Am J Neuroradiol. 2013 Feb;34(2):471-8
15. Zheng W, Chen H, Wang N, Liang Y, Xiao W "Application of Diffusion Tensor Imaging Cutoff Value to Evaluate the Severity and Postoperative Neurologic Recovery of Cervical Spondylotic Myelopathy". World Neurosur. 2018 Oct;118: e849-e855.

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Original Article

Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging

K Divya, Vikrant Kanagaraju[1], B Devanand[1], C Jeevamala, A Raghuram, D Sundar

Purpose: To investigate the retrobulbar circulatory parameters in type 2 diabetes mellitus patients with color Doppler imaging (CDI) and compare the results with nondiabetic controls. **Methods:** This prospective study included 50 type 2 diabetic patients and 50 age-matched controls. Seven field stereo fundus photography was used to diagnose and classify diabetic retinopathy (DR). Diabetic patients were further divided into two: Group 1, consisted of patients with no DR, mild and moderate non-proliferative DR (n = 36); Group 2, severe nonproliferative and proliferative DR (n = 14). CDI was performed using Philips iU22 xMATRIX ultrasound. The peak systolic velocity (PSV), end-diastolic velocity (EDV), resistivity index (RI) and pulsatile index (PI) of ophthalmic (OA), posterior ciliary artery (PCA), and central retinal artery (CRA) along with central retinal vein (CRV) were recorded. **Results:** RI in the ophthalmic artery was significantly higher in both DR groups than the control group (P = 0.000). Diabetic Group 1 had decreased blood flow velocity (PSV and EDV) in PCA compared to controls (P = 0.046 and P = 0.010, respectively). Group 2 DR had significantly reduced EDV and increased RI in CRA compared to Group 1 (P = 0.015). Binary logistic regression analysis revealed glycosylated hemoglobin and RI of OA to be independent risk factors of DR. **Conclusion:** Significant changes in resistivity index and flow velocities were observed in the retrobulbar vessels, especially in ophthalmic artery in diabetics compared to controls. CDI with results of increased resistance or decreased flow could be useful to predict individuals at higher risk for developing severe DR.

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Key words: Blood flow velocities, color Doppler ultrasonography, diabetes mellitus, retrobulbar

Ocular manifestations of diabetes are numerous and among them, diabetic retinopathy (DR) is a major clinical issue for ophthalmologists. DR is the most frequent cause of preventable blindness in working-age adults (20–74 years) and is therefore considered as a major public health issue.[1] Though treatable in appropriate stages, the prevention of retinal complications of diabetes and consequent visual impairment is vital to reduce the burden of DR. There is a felt need for better diagnostic modalities and surrogate markers in the evaluation and management of DR.

In spite of decades of research, the mechanisms behind the onset and progression of DR still remain unclear. From a hemodynamic point of view, evidence has shown that there is an early reduction in blood flow to retina before the onset of DR followed by a gradual increase as the retinopathy progresses.[2] Color Doppler imaging (CDI) is an established, safe, and noninvasive ultrasonic method useful for qualitatively and quantitatively assessing the blood flow velocities and indirect blood flow.[3] CDI has been suggested to be valuable in the diagnosis and management of several ocular diseases with altered hemodynamics such as glaucoma, retinal vascular diseases, ischemic optic neuropathy, and ocular ischemic syndrome.[4] An analysis of retrobulbar hemodynamics in diabetes using CDI can provide insight into the pathogenesis and prospective treatment for DR as well as predict those at higher risk of developing vision threatening DR. Several authors have evaluated the circulatory changes in diabetics using CDI and reported conflicting results. While few studies have reported a reduction in the retrobulbar blood flow velocities,[5-7] others have noted an increase[8] or no change.[9] Studies on the resistive index (RI) of orbital vessels which is a measure of peripheral vascular resistance have also shown significant discrepancies.[10,11] Factors such as demographic variables, duration of diabetes, methods of DR assessment, positions of CDI, and sample sizes account for the observed discrepancies. Moreover, most studies have not excluded patients with hypertension and those on medications affecting systemic blood flow which can influence the results. Studies on orbital hemodynamic alterations among type 2 diabetics from India are relatively few. The diabetic population in India is close to hitting the alarming mark of 69.9 million by 2025 and 80 million by 2030, denoting an expected increase of 266%. Hence, India is deemed as the world's capital of diabetes.[12] With increasing prevalence of diabetes and DR, it is important to know the hemodynamic alterations among diabetics from the Indian population.

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The objective of this study was to evaluate the flow velocities and vascular resistance of orbital blood vessels using CDI in a pure cohort of type 2 diabetic patients from India and compare the results with healthy controls.

Methods

This prospective hospital-based study was carried out at a tertiary care institution from south India between March 2017 and April 2019. The study included consecutive patients of type 2 DM (as per definition of the American Diabetes Association) attending the ophthalmology outpatient clinic for the screening of retinopathy. Exclusion criteria were history of any ocular disease that might affect ocular blood flow such as glaucoma, nondiabetic retinal vascular occlusion, ocular inflammation, high myopia, previous ocular surgery, or panretinal photocoagulation. Patients with systemic hypertension, dyslipidemia, nephropathy, and cardiovascular diseases were excluded from the study. Pregnant and breastfeeding women were not included. Patients taking medications known to affect systemic hemodynamics such as Angiotensin-converting enzyme inhibitors, calcium channel blockers, and anti-migraine drugs were also excluded.

50 age-matched healthy subjects were enrolled as a control group. The control subjects were recruited from individuals attending the department of ophthalmology for a routine eye examination. They were not on any medication and did not have ocular or systemic disease. The study was conducted according to the principles of the Declaration of Helsinki and the study protocol was approved by the institutional ethics committee. Informed consent was obtained from all participants.

Information regarding age, gender, body mass index, duration of diabetes, antidiabetic medications, and blood pressure were obtained from all participants. Serum glycosylated hemoglobin (HbA1c) was determined from fasting venous blood sample.

All study participants underwent a comprehensive ophthalmic examination including dilated fundus with slit-lamp biomicroscope using a 90 diopter lens and indirect ophthalmoscopy. A 30° stereoscopic fundus photograph of 7 standard early treatment diabetic retinopathy study (ETDRS) fields was taken from both eyes of diabetic patients using a digital fundus camera (TRC-50 DX Mydriatic retinal camera; Topcon Medical Systems, Oakland, NJ).

The presence, as well as the severity of diabetic retinopathy, was determined by grading of color fundus photographs following the international clinical disease severity scale for diabetic retinopathy which is based on the findings of WESDR (Wisconsin Epidemiological Study of Diabetic Retinopathy) and ETDRS.[13] The diabetic patients were assigned to one of the following groups namely no retinopathy, mild nonproliferative diabetic retinopathy (NPDR), moderate NPDR, severe NPDR, and proliferative diabetic retinopathy (PDR). The

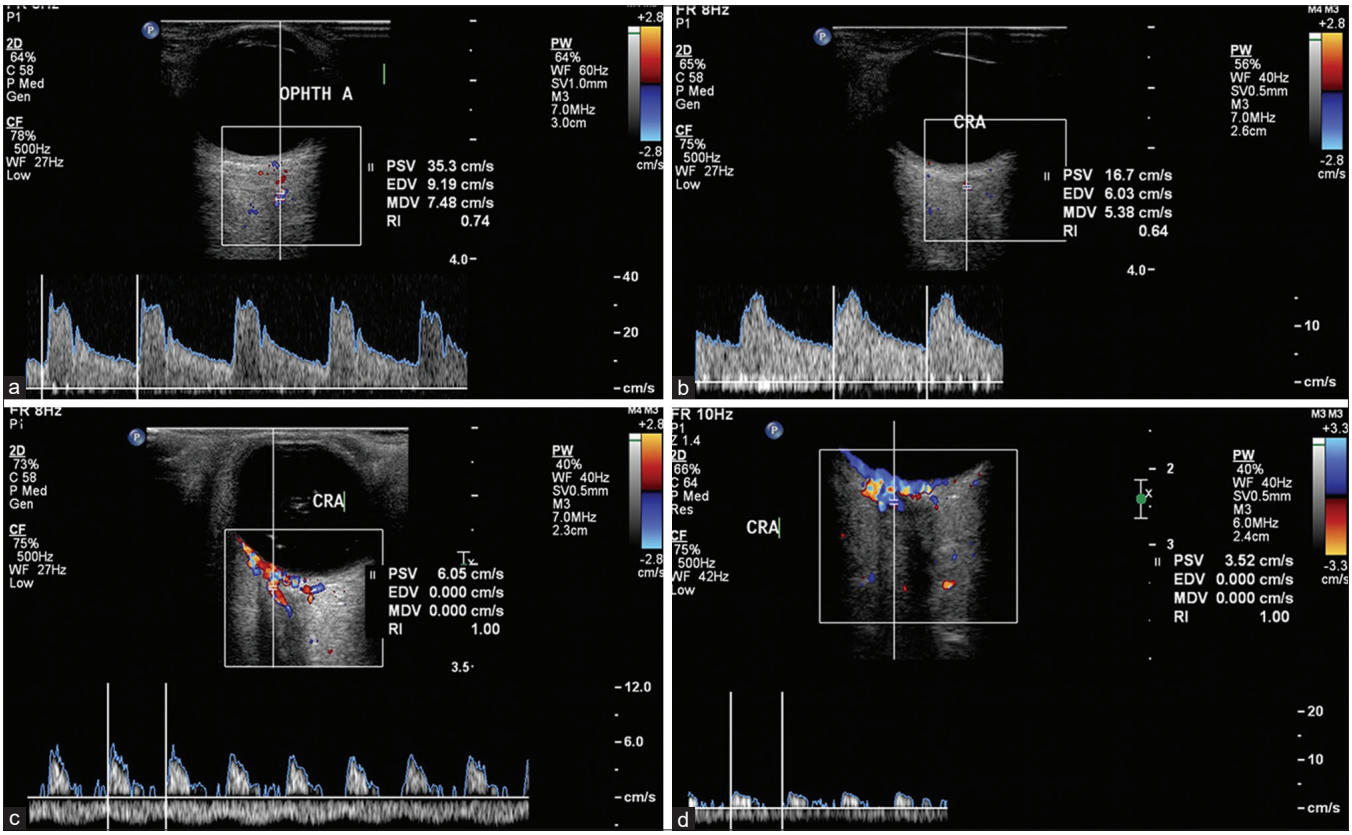


Figure 1: (a) Color Doppler image (CDI) showing normal color flow and spectral waveform in ophthalmic artery with preserved M wave pattern; (b) CDI showing normal color flow and spectral waveform in central retinal artery; (c) CDI showing low-velocity monophasic flow in central retinal artery in a patient with moderate NPDR; (d) CDI showing monophasic spectral waveform with absent diastolic flow in central retinal artery in a patient with severe NPDR

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eye with worse retinopathy was included in the study. If both eyes had equal retinopathy, the right eye was assigned to the study.

Color Doppler sonography of the eye was performed for cases and controls by a masked expert Radiologist using a color Doppler unit and a 12 to 5 MHZ linear-array transducer (Philips iU22 xMATRIX ultrasound system). The patients were examined in the supine position and care was taken to avoid any undue pressure on the eye. Examiner's hand was rested on the orbital margin and sterile coupling gel was applied over closed eyelids. The Doppler signal was registered online after the cursor was positioned on the region of interest. To reduce errors in measurement of velocities, angle correction was applied to the pulsed Doppler recordings.

The peak systolic velocity (PSV) and end-diastolic velocity (EDV) readings were obtained from the ophthalmic artery (OA), central retinal artery (CRA), and the posterior ciliary artery (PCA). Since the velocity wave in a central retinal vein (CRV) is not in direct relation to the cardiac cycle its hemodynamic parameters were expressed as maximum (V max) and minimum (V min) blood velocity. Signals from the ophthalmic artery were obtained from the medial aspect of the eyeball, superior to the optic nerve. The central retinal artery and vein were located within the optic nerve, around 10 mm behind the eyeball. [Fig. 1] Signals from the temporal branch of the short posterior ciliary artery were located lateral to the optic nerve.

With the help of PSV and EDV, we calculated the vascular resistance expressed by the resistivity index and pulsatile index using Pourcelot's formula.

Resistive index (RI) = (PSV-EDV)/PSV; Pulsatile index (PI) = (PSV-EDV)/Vmean

Statistical analysis

For statistical analysis diabetic patients were further divided into two groups; Group 1 included patients with no retinopathy, mild NPDR, and moderate NPDR while Group 2 consisted of patients with severe NPDR and PDR.

Statistical analysis was performed using statistical package for social sciences (SPSS, SPSS Inc, Chicago, IL, USA) version 24. Data were expressed as mean ± standard deviation. The normality of data distribution was tested by the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) with a *post hoc* Bonferroni was used for the analysis of normally distributed continuous data. Kruskal-Wallis analysis was used for analyzing non-normally distributed data. A comparison between hemodynamic parameters was calculated by ANOVA followed by Tukey-Kramer *post hoc* comparison. Pearson's coefficient was used to study the correlation between variables.

Table 1: Demographic and clinical characteristics of controls and diabetic subjects

Characteristic	Controls (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
Age (years)	56.5±9.00	55.5±8.60	51.9±5.64	0.209
Body mass index (kg/m^2)	23.34±3.62	24.81±3.15	24.53±3.88	0.141
Median Systolic BP (mmHg)	110 (20)	120 (18.75)	125 (12.5)	0.076
Median Diastolic BP (mmHg)	70 (30)	76.5 (10)	70 (10)	0.259
Median duration of diabetes (years)	-	3 (8)	10 (11)	0.115
Glycosylated hemoglobin (%)	5.81±0.29	9.09±2.13	10.86±2.33	0.014*

BP=Blood pressure; *P<0.05

Table 2: Hemodynamic parameters of retrobulbar blood vessels among controls and patient groups

Hemodynamic parameters (cm/s)	Control (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
OA PSV	29.72±8.10	30.48±9.78	28.76±12.84	0.838
OA EDV	6.87±2.69	6.34±3.41	5.09±3.50	0.166
OA RI	0.75±0.06	0.79±0.06	0.83±0.05	0.000*
OA PI	1.58±0.27	1.74±0.30	1.86±0.25	0.002*
CRA PSV	12.42±3.85	10.40±4.87	8.42±2.92	0.004*
CRA EDV	2.73±1.17	2.33±1.27	1.27±0.94	0.000*
CRA RI	0.77±0.06	0.74±0.16	0.85±0.11	0.019*
CRA PI	1.63±0.30	1.65±0.50	2.08±0.65	0.004*
PCA PSV	15.44±5.61	12.81±3.83	14.95±5.15	0.054
PCA EDV	4.19±2.23	2.97±1.29	3.33±1.63	0.011*
PCA RI	0.73±0.06	0.75±0.08	0.78±0.08	0.061
PCA PI	1.45±0.24	1.58±0.37	1.68±0.45	0.044*
CRV V max	6.14±1.63	6.12±1.66	5.85±2.07	0.853
CRV V min	3.51±0.97	3.34±0.89	3.02±1.05	0.243
CRV RI	0.41±0.11	0.44±0.11	0.46±0.08	0.329
CRV PI	0.60±0.24	0.65±0.25	0.70±0.17	0.350

OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=Maximum blood velocity; V min=Minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

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Binary logistic regression analysis was performed to determine independent risk factors for retinopathy. All tests for statistical significance were two-tailed and performed assuming a type I error probability of <0.05.

Results

Demographic and clinical parameters

The study included 52 males and 48 females. The age of patients ranged from 41 to 76 years. The duration of diabetes ranged from 6 months to 20 years. Among the diabetic patients, 29 were on oral hypoglycemic agents, 9 were on insulin, and 12 were on combination therapy. Group 1 (n = 36) consisted of 16 patients with no DR, 13 with mild NPDR, and 7 with moderate NPDR. Group 2 (n = 14) consisted of 6 patients with severe NPDR and 8 with PDR. There were no statistically significant differences in the age, body mass index and blood pressure between controls and diabetic groups. Glycosylated hemoglobin (HbA1c) levels were found to be significantly higher in Group 2 diabetic patients (P = 0.014). No significant difference was noted in the duration of diabetes among diabetic groups. The demographic and clinical characteristics of controls and diabetic subjects are presented in Table 1.

Hemodynamic parameters

Table 2 shows the hemodynamic parameters of retrobulbar blood vessels among controls and patient groups.

Ophthalmic artery

Significant differences were noted in the RI and PI values of the ophthalmic artery between controls and diabetic patients with various DR grades. OA flow velocities did not vary between groups.

Table 3: Pearson correlation coefficients between the duration of diabetes, HbA1c, and hemodynamic parameters

Variable	Duration of diabetes	HbA1c
OA PSV	0.021 (0.885)	-0.120 (0.407)
OA EDV	-0.141 (0.328)	-0.055 (0.706)
OA RI	0.314* (0.026)	0.097 (0.504)
OA PI	0.299* (0.035)	0.078 (0.588)
CRA PSV	-0.135 (0.349)	-0.269 (0.059)
CRA EDV	-0.464* (0.001)	-0.299*(0.035)
CRA RI	0.375* (0.007)	-0.046 (0.753)
CRA PI	0.487* (0.000)	0.131 (0.370)
PCA PSV	-0.052 (0.722)	0.170 (0.239)
PCA EDV	-0.234 (0.102)	0.196 (0.172)
PCA RI	0.332* (0.019)	-0.075 (0.606)
PCA PI	0.321*(0.023)	-0.085 (0.556)
CRV V max	0.134 (0.354)	0.128 (0.375)
CRV V min	-0.143 (0.322)	-0.058 (0.687)
CRV RI	0.390* (0.005)	0.172 (0.233)
CRV PI	0.415*(0.003)	0.210 (0.144)

Corresponding P values are shown in brackets. HbA1c=Glycosylated hemoglobin; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=maximum blood velocity; V min=minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

Central retinal artery and vein

The hemodynamic parameters of CRA were significantly different between controls, DR Group 1, and Group 2 whereas no such difference was seen in the CRV. *Post hoc* comparison revealed a significantly reduced PSV and EDV of CRA (P = 0.005 and 0.000, respectively) along with increased PI (P = 0.003) in DR group 2 compared to controls. Patients with group 2 DR also demonstrated a significant decrease of EDV and increased RI of CRA compared to group 1 (P = 0.015). PSV, EDV, and RI of CRA did not differ significantly between controls and DR group 1.

Posterior ciliary artery

The EDV and PI of PCA were found to vary significantly between controls and diabetic groups. Significant reduction in PSV and EDV of PCA was noted in DR group 1 compared to controls (P = 0.046 and P = 0.010, respectively after *post hoc* comparison).

Relation between duration of diabetes, HbA1c, and hemodynamic parameters

Duration of diabetes correlated positively with the resistive and pulsatile indices of all retrobulbar vessels studied namely ophthalmic artery, CRA, PCA, and CRV. HbA1c levels, as well as the duration of diabetes, were found to correlate negatively with EDV of CRA. No correlation was found between duration of diabetes and HbA1c levels (R = 0.183, P = 0.203). Table 3 shows the relationship between duration of diabetes, HbA1c levels, and hemodynamic parameters.

Binary logistic regression analysis revealed that HbA1c levels and RI of OA were independent risk factors of retinopathy.

Discussion

The main observations of our study are as follows: (1) RI in the ophthalmic artery was significantly higher in both DR groups than the control group and was an independent risk factor of retinopathy, (2) Patients with Group 1 DR had decreased blood flow velocity (PSV and EDV) in the posterior ciliary artery compared to control group, (3) Patients with Group 2 DR had significantly reduced EDV and increased RI in the CRA compared to Group 1, (4) Compared to the control group, patients with Group 2 DR had slower flow velocities and increased PI in CRA but RI values did not vary significantly.

Our finding of decreased EDV in the posterior ciliary artery in patients with Group 1 DR supports the hypothesis that choroidal blood flow is affected in the early stages of DR. Laser Doppler flowmetry has shown decreased choroidal blood flow in the foveal region in early DR.[14] Choroidal vascularity index (CVI) analysis by swept-source optical coherence tomography (OCT) has also shown reduced CVI in patients with mild/moderate NPDR.[15] Cao *et al.* have reported a 4 times more choriocapillary loss in diabetic eyes compared to non-diabetic eyes in histopathologic studies.[16] Ferrara *et al.* have demonstrated the loss of intermediate and large blood vessels in the Sattler's layer and Haller's layers in diabetic eyes in choroidal OCT.[17] Our observation of decreased EDV in the PCA suggests increased peripheral vascular resistance of the choroidal circulation in early DR and is in concordance with these studies. Dimitrova *et al.* reported decreased EDV and increased RI in PCA in patients

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with background DR compared to controls in CDI of diabetic patients performed in sitting posture.[6] The authors have suggested that erect posture during CDI measurement is essential to appreciate significant alterations in posterior ciliary circulation. Supine posture has been suggested to increase EDV and decrease the RI.[18] As autoregulation and autonomic nervous responses are altered in diabetics, the correlation of change in circulatory parameters between normal individuals and diabetics associated with postural variation may not be expected to be equal. However, CDI was performed in supine posture in our patients. Our observations could be attributed to the shorter duration of diabetes in our patients than theirs.

RI in OA was higher in both DR groups compared with controls in our study. These findings are similar to those of previous studies.[5,19] Basturk *et al.* evaluated the association between the resistivity index of orbital arteries and DR in 91 types 2 diabetics with microalbuminuria using CDI. The authors have demonstrated increased RI values of all orbital arteries in patients with DR compared to those without DR and controls. RI value of ≥ 0.72 in OA indicated the presence of DR with 78.4% sensitivity and 70% specificity.[10] Our finding of increased RI in the OA in both DR groups can be attributed to the statistically nonsignificant reduction in EDV observed in diabetic subjects because of downstream vascular changes in the retina and choroid. We did not observe significant differences in CRA parameters between controls and DR Group 1. Hence, we believe that the OA hemodynamic parameters in our study probably represent alterations in choroidal blood flow. It has been suggested that of the total ocular blood supply from OA, less than 10% flows to the retina while the remainder is directed to choroid via the ciliary circulation due to the high metabolic demand of photoreceptors.[20] Moreover, the diastolic blood flow of PCA is higher than CRA since these vascular channels of choroid have low resistance.[21]

Table 4: Various published studies on ocular circulation in diabetic patients using color Doppler imaging

Study	Country	Sample size	Duration of diabetes (years)	Findings
Arai *et al.*, 1998[9]	Japan	74 Controls: 22 NDR: 27 DR: 25	NA	Significant increase in RI of CRA in patients with DR compared to those without DR and controls (P<0.01)
MacKinnon *et al.*, 2000[5]	Scotland	62 Controls: 17 NDR/BDR: 24 PPDR/PDR: 21	NDR/BDR: 10 PPDR/PDR: 12	Significant decrease in both PSV and EDV of CRA in the PPDR/PDR group compared to NDR/BDR and control group (P<0.05) while RI showed no significant change. RI of OA was increased in diabetics compared to controls.
Dimitrova *et al.* , 2001[6]	Japan	95 Controls: 22 NDR: 38 BDR: 35	NDR: 9.0±7.2 BDR: 18. 9±11.2	Significant decrease in EDV and increase in RI of PCA in BDR group compared to controls (P=0.01 and 0.0003, respectively)
Gracner *et al.*, 2004[8]	Slovenia	66 Controls: 22 NPDR: 22 SNPDR/PDR: 22	13.5±9.31	Significant increase in PSV of OA in the SNPDR/PDR group compared to controls. Significant increase in RI of PCA in SNPDR/PDR group compared to NPDR and controls
Sullu *et al.*, 2005[7]	Turkey	34 Controls: 20 PDR: 14	13.5±6.1	Decrease in mean EDV and increase in RI of both OA and CRA in PDR compared to controls (P<0.05)
Baydar *et al.*, 2007[11]	Australia	44 (65 eyes) Controls: 11 Diabetics: 33	4.94±3.90	The RI of CRA was significantly higher in the control group than in the NDR group. RI of OA was significantly higher in NDR than in the moderate NPDR group.
Basturk *et al.*, 2012[10]	Turkey	118 Controls: 27 NDR: 40 DR: 51	NDR: 6.87±4.37 DR: 8.23±2.55	Patients with DR had significantly higher mean RIs of OA, CRA and PCA compared to those without DR and controls (P<0.001)
Karami *et al.*, 2012[30]	Iran	123 Controls: 25 BDR: 74 PPDR: 24	BDR: 5.6±0.7 PPDR: 12.6±1.1	RI of OA and CRV were significantly higher in patients with DR compared to controls (P<0.005)
Our study	India	100 Controls: 50 NDR/mild/ moderate NPDR: 36 SNPDR/PDR: 14	NDR/mild/moderate NPDR: 8 SNPDR/PDR: 11	RI of OA was significantly increased in diabetics compared to controls. Significant decrease in both PSV and EDV of PCA in mild/ moderate NPDR compared to controls (P<0.05) Significantly reduced EDV and increased RI of CRA in SNPDR/PDR compared to mild/moderate NPDR group (P=0.015)

NDR=No diabetic retinopathy; BDR=Background diabetic retinopathy; PPDR=Preproliferative diabetic retinopathy; NPDR=Nonproliferative diabetic retinopathy; SNPDR=Severe nonproliferative diabetic retinopathy; PDR=Proliferative diabetic retinopathy; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; RI=Resistivity index; NA=Not available

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Khatri *et al.* assessed the correlation between RI of OA and topographic alterations of retinal pigment epithelium (RPE) on spectral-domain optical coherence tomography (SD-OCT).[22] In their study involving 75 patients with type 2 diabetes and 24 controls, a significant increase in OA RI was noted with the severity of DR ($F = 14.23$, $P < 0.001$). A positive correlation was noted between RI of OA and grades of RPE alterations on SD-OCT suggesting that decreased blood flow contributes to RPE dysfunction. Studies demonstrating early retinal damage in experimental diabetes have reported morphological changes in RPE and animal studies have also shown reduced choroidal blood flow in young diabetic animals suggesting that blood flow deficit could be an early pathological change in DR.[23,24] Although it is difficult to propose a causal association it can be postulated that changes in choroidal circulation (as reflected by increased RI of OA) lead to downregulation of antiangiogenic PEDF (pigment epithelial-derived factor) and upregulation of VEGF (vascular endothelial growth factor) thereby contributing to progression of DR. Differential expression and secretion of these growth factors derived from the retinal pigment epithelium have been suggested to lead to nonproliferative and proliferative DR.[25]

Patients with Group 2 DR had significantly reduced EDV in the CRA compared to Group 1 DR as well as healthy controls. Our observations are in line with previous studies.[5,6,8] These can be attributed to the increased peripheral vascular resistivity with the progression of DR. Pathological findings in DR such as capillary basement membrane thickening, retinal capillary leukostasis, loss of retinal capillaries, and altered blood rheology support this circulatory feature.[2,26,27]

Another observation of our study was that DR Group 2 had significantly reduced flow velocity in the CRA but an increase in RI was not statistically significant compared to the control group. This can be due to the concomitant decrease of both PSV and EDV in patients with Group 2 DR. Neudorfer *et al.* evaluated the long-term changes in the retrobulbar circulation of 138 eyes of diabetic subjects at a 10-year follow-up.[28] Patients were divided into four groups, namely, nondiabetic controls, diabetics without DR, nonproliferative DR, and proliferative DR. RI values of CRA and PCA had increased in the two no retinopathy groups and in the nonproliferative DR group, with a surprising nonsignificant decrease in the proliferative DR group. The authors have concluded that an increase of resistance in the retrobulbar blood vessels due to DR can decrease over time and may even be reversed. It has been suggested that the decrease in resistance reflects the beginning of an increase in retinal blood flow with the progression of DR due to the short-circuiting of the obstructed retinal capillary network.[28] This is supported by histopathologic studies, which have shown areas of capillary closure being traversed by preferential dilated anastomotic channels.[29]

Our finding of a positive correlation between duration of diabetes and RI of all retrobulbar vessels was similar to the observations of Karami *et al.*[30] We found negative correlation between EDV of CRA and duration of diabetes as well as HbA1c levels ($R = -0.464$, $P = 0.001$; $R = -0.299$, $P = 0.035$, respectively). Poor glycemic control (as reflected by increased HbA1c) and longer duration of diabetes can be associated with increased peripheral vascular resistance and thus reduced EDV in CRA. Our findings of HbA1c and RI of OA as independent risk factors of DR are similar to that of Basturk *et al.*[10]

Table 4 shows the various published studies on ocular circulation in diabetic patients using CDI.

Our study has several strengths. None of the diabetic patients had associated vascular diseases. Hypertension as well as antihypertensive medications significantly alter the resistive indices of blood vessels and therefore confound the results. We intentionally excluded these patients to study the influence of diabetes alone on ocular hemodynamics. A single masked, experienced radiologist performed all the Doppler examinations gently with the least pressure on the globe thus eliminating interobserver variability. The presence and severity of DR were graded based on seven field stereo fundus photography for accuracy.

To our knowledge, this study is the first of its kind in a pure cohort of diabetics from India.

There are a few limitations to our study. The sample size was small which accounts for the limited subgroup analysis. CDI provides information on blood flow velocity only and absolute volumetric blood flow cannot be quantified since it is not possible to study the diameter of retrobulbar vessels with CDI accurately. In spite of this limitation, blood velocity is probably a good indicator of blood flow within a given vessel.[31,32] Moreover, measurement of retinal blood flow is difficult and no accurate method to quantitatively assess perfusion is currently used clinically.

Conclusion

In conclusion, significant changes in the blood flow velocities and RI were observed in the retrobulbar vessels, especially in an ophthalmic artery in diabetics compared to controls. RI could be potentially useful for early diagnosis and follow-up of DR. CDI with results of increased resistance or decreased flow can be of help to identify diabetic individuals at higher risk for developing severe DR. This is especially useful in patients with opaque media where conventional methods are of little use.

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Conflicts of interest

There are no conflicts of interest.

References

1. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.

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2. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye (Lond) 2009;23:1496-508.
3. Aburn NS, Sergott RC. Orbital colour Doppler imaging. Eye (Lond) 1993;7:639-47.
4. Dimitrova G, Kato S. Color Doppler imaging of retinal diseases. Surv Ophthalmol 2010;55:193-214.
5. MacKinnon JR, McKillop G, O'Brien C, Swa K, Butt Z, Nelson P. Colour Doppler imaging of the ocular circulation in diabetic retinopathy. Acta Ophthalmol Scand 2000;78:386-9.
6. Dimitrova G, Kato S, Tamaki Y, Yamashita H, Nagahara M, Sakurai M, *et al.* Choroidal circulation in diabetic patients. Eye (Lond) 2001;15:602-7.
7. Sullu Y, Hamidova R, Beden U, Yakupov K, Canbaz S, Danaci M. Effects of pars plana vitrectomy on retrobulbar haemodynamics in diabetic retinopathy. Clin Exp Ophthalmol 2005;33:246-51.
8. Gracner T. Ocular blood flow velocity determined by color Doppler imaging in diabetic retinopathy. Ophthalmologica 2004;218:237-42.
9. Arai T, Numata K, Tanaka K, Kiba T, Kawasaki S, Saito T, *et al.* Ocular arterial flow hemodynamics in patients with diabetes mellitus. J Ultrasound Med 1998;17:675-81.
10. Basturk T, Albayrak R, Ulas T, Akcay M, Unsal A, Toksoy M, *et al.* Evaluation of resistive index by color Doppler imaging of orbital arteries in type II diabetes mellitus patients with microalbuminuria. Ren Fail 2012;34:708-12.
11. Baydar S, Adapinar B, Kebapci N, Bal C, Topbas S. Colour Doppler ultrasound evaluation of orbital vessels in diabetic retinopathy. Australas Radiol 2007;51:230-5.
12. Pandey SK, Sharma V. World diabetes day: Battling the emerging epidemic of diabetic retinopathy. Indian J Ophthalmol 2018;66:1652-3.
13. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, *et al.* Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Global Diabetic Retinopathy Project Group. Ophthalmology 2003;110:1677-82.
14. Nagaoka T, Kitaya N, Sugawara R, Yokota H, Mori F, Hikichi T, *et al.* Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. Br J Ophthalmol 2004;88:1060-3.
15. Kim M, Ha MJ, Choi SY, Park YH. Choroidal vascularity index in type-2 diabetes analyzed by swept-source optical coherence tomography. Sci Rep 2018;8:70.
16. Cao J, McLeod S, Merges CA, Lutty GA. Choriocapillaris degeneration and related pathologic changes in human diabetic eyes. Arch Ophthalmol 1998;116:589-97.
17. Ferrara D, Waheed NK, Duker JS. Investigating the choriocapillaris and choroidal vasculature with new optical coherence tomography technologies. Prog Retin Eye Res 2016;52:130-55.
18. Evans DW, Harris A, Garrett M, Chung HS, Kagemann L. Glaucoma patients demonstrate faulty autoregulation of ocular blood flow during posture change. Br J Ophthalmol 1999;83:809-13.
19. Tamaki Y, Nagahara M, Yamashita H, Kikuchi M. Blood velocity in the ophthalmic artery determined by color Doppler imaging in normal subjects and diabetics. Jpn J Ophthalmol 1993;37:385-92.
20. Cioffi GA, Granstam E, Alm A. Ocular circulation. In: Kaufman PL, Alm A, editors. Adler's Physiology of the Eye. 10th ed. St. Louis, MO: Mosby; 2003. p. 747-73.
21. Güven D, Ozdemir H, Hasanreisoglu B. Hemodynamic alterations in diabetic retinopathy. Ophthalmology 1996;103:1245-9.
22. Khatri M, Saxena S, Kaur A, Bhasker SK, Kumar M, Meyer CH. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retina Vitreous 2018;4:12.
23. Decanini A, Karunadharma PR, Nordgaard CL, Feng X, Olsen TW, Ferrington DA. Human retinal pigment epithelium proteome changes in early diabetes. Diabetologia 2008;51:1051-61.
24. Muir ER, Rentería RC, Duong TQ. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. Invest Ophthalmol Vis Sci 2012;53:6488-94.
25. Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO. Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 2003;22:1-29.
26. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. Microcirculation 2007;14:25-38.
27. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008;2:1130-8.
28. Neudorfer M, Kessner R, Goldenberg D, Lavie A, Kessler A. Retrobulbar blood flow changes in eyes with diabetic retinopathy: A 10-year follow-up study. Clin Ophthalmol 2014;8:2325-32.
29. Garner A. Histopathology of diabetic retinopathy in man. Eye (Lond) 1993;7:250-3.
30. Karami M, Janghorbani M, Dehghani A, Khaksar K, Kaviani A. Orbital Doppler evaluation of blood flow velocities in patients with diabetic retinopathy. Rev Diabet Stud 2012;9:104-11.
31. Hansen NB, Stonestreet BS, Rosenkrantz TS, Oh W. Validity of Doppler measurements of anterior cerebral artery blood flow velocity: Correlation with brain blood flow in piglets. Pediatrics 1983;72:526-31.
32. Taylor GA, Short BL, Walker LK, Traystman RJ. Intracranial blood flow: Quantification with duplex Doppler and color Doppler flow US. Radiology 1990;176:231-6.

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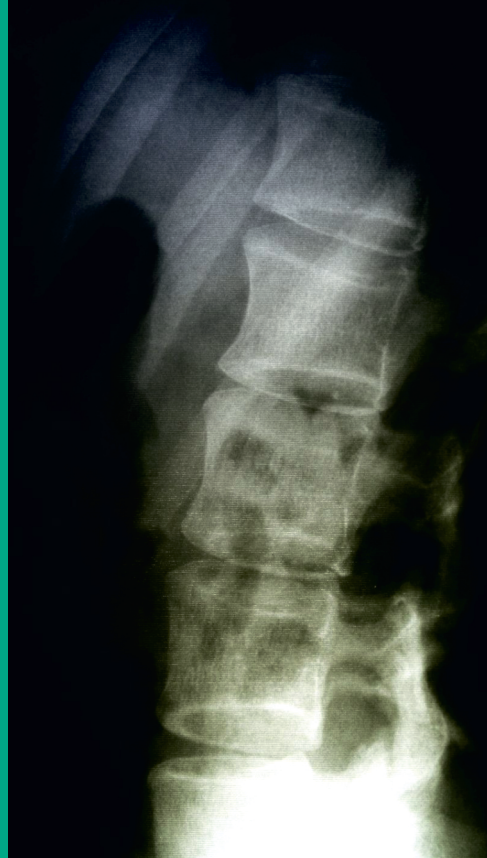
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Original Article

Utility of ultrasound elastography in evaluation of thyroid nodules and correlation with cytology

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Abstract

Background: Ultrasound elastography (USE) has emerged as a potentially useful tool in the evaluation of thyroid nodules. The aim of this study was to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with fine-needle aspiration cytology (FNAC) analysis as reference standard.

Materials and Methods: This was a cross-sectional prospective study carried out at a tertiary care center in South India between May 2014 and March 2015. Consecutive patients with solid nodule in the thyroid gland on conventional ultrasound (US) underwent USE, followed by US-guided FNAC of the thyroid nodule. The findings of B-mode images and USE were correlated with cytology. Statistical analysis was performed using SPSS version 24. Descriptive statistics were presented as mean ± standard deviation. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value of USE were calculated.

Results: A total of sixty patients with sixty solid thyroid nodules were enrolled in the study. The mean age of the patients was 48.27 ± 16.10 years. About 83.3% were females. The frequency of benign nodules on cytopathologic analysis was 60% (n = 36). Seventy-five percent of the malignant lesions had irregular or poorly defined borders with lobulated margins on US. The sensitivity, specificity, positive predictive value, and negative predictive value of USE were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as reference standard. Higher elastography scores were found to be significantly associated with malignant cytology (P = 0.0001).

Conclusion: Elastography, with its fairly high diagnostic accuracy and correlation with malignant cytology, can be an effective noninvasive adjunctive tool in distinguishing benign from malignant thyroid nodules.

Keywords: Elastography, thyroid nodules, ultrasound

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INTRODUCTION

The prevalence of thyroid nodules which are clinically palpable is about 2%–6% in the general population.[1] Although majority of these nodules are benign, up to 7% can be malignant.[2,3]

On palpation, a hard or firm nature is associated with an increased risk of malignancy. However, palpation is a highly subjective method and its value is limited in patients with multinodular goiter or deep-seated nodules. Ultrasonography (USG) is the most commonly used imaging modality to diagnose thyroid nodules. The utility of

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USG in differentiating benign from malignant lesions has been established by several studies.[4-6] However, no USG feature has been shown to have both high sensitivity and high specificity in detecting malignancy in thyroid nodules.[7] Elastography is a newly developed noninvasive dynamic imaging technique analogous to manual palpation. This technique uses ultrasound (US) to estimate tissue stiffness by measuring the degree of distortion under application of an external force.[8] Several studies have used US elastography (USE) for evaluation of thyroid disorders.[9-11]

Fine-needle aspiration cytology (FNAC) is an efficient tool in distinguishing benign nodules that can be managed conservatively from those with suspicious or malignant features that warrant further intervention. Limited data exist on the utility of elastography in differentiating benign from malignant lesions of the thyroid and its correlation with FNAC, especially from India. The aim of this study was to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with FNAC analysis as reference standard.

MATERIALS AND METHODS

This prospective study was carried out at a tertiary care center in South India between May 2014 and March 2015. Consecutive patients of either gender who were found to have thyroid swelling/goiter on clinical examination and referred for USG were considered. Only those with solid thyroid nodules at least 8 mm in size on conventional US were included in the study. Cystic lesions with no solid component, multinodular goiters with coalescent nodules, and nodules with eggshell calcification were excluded from the study. A large nodule involving more than 75% of thyroid lobe volume was also excluded because of inadequate normal thyroid tissue to be used as reference. The institutional review board approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants.

With a patient in supine position and neck fully extended, B-mode US of the thyroid gland was performed with SIEMENS ACUSON S2000 machine using high-frequency probes 9 L4 and 14 L5. US features studied included echogenicity, regularity of borders, presence of halo, vascular pattern, and calcification. Thyroid imaging reporting and data system (TIRADS) scoring was used to categorize the lesions[12] [Table 1].

USE was performed with SIEMENS ACUSON S2000 at the same time as the B-mode examination. The probe was placed over the region such that the lesion is in the center of the image, holding the scan plane perpendicular to the skin surface. At least 5 mm of normal adjacent tissue was included to assess the lesion stiffness in relation to the average elasticity of surrounding tissue. Patients were asked to hold their breath and avoid swallowing during examination in order to minimize the motion of the thyroid gland. The elasticity images were obtained by appropriate compression, followed by decompression of the transducer. The images were displayed on split-screen mode with US grayscale images on the left and USE images on the right. Multiple frames were acquired, and the best-fit B mode-USE image pairs were selected for examination [Figures 1 and 2]. The targeted lesion was scored as 1–4, using strain elastographic scores proposed by Asteria *et al*.[9] [Table 2].

US-guided FNAC of the thyroid nodule was done using a 22G needle, and the lesion was aspirated at least twice with the freehand technique. The aspirated contents were expelled onto glass slides and immediately fixed with 95% ethyl alcohol. The slides were stained with modified Papanicolaou staining and examined under light microscopy. The cytological results were classified

Table 1: Thyroid image reporting and data system scoring of thyroid nodules

Category	Description
TIRADS 1	Normal thyroid gland
TIRADS 2	Benign conditions
TIRADS 3	Probably benign nodules (<5% malignant)
TIRADS 4	Suspicious nodules (5%-80% malignancy rate)
TIRADS 5	Probably malignant nodules (malignancy rate >80%)
TIRADS 6	Biopsy-proven malignant nodules

TIRADS – Thyroid image reporting and data system

Table 2: Asteria's criteria of scoring of thyroid nodules on elastography

Elasticity Score	Description
Score of 1	Elasticity in the entire examined area
Score of 2	Elasticity in a large part of the examined area
Score of 3	Stiffness in a large part of the examined area
Score of 4	Nodule without elasticity

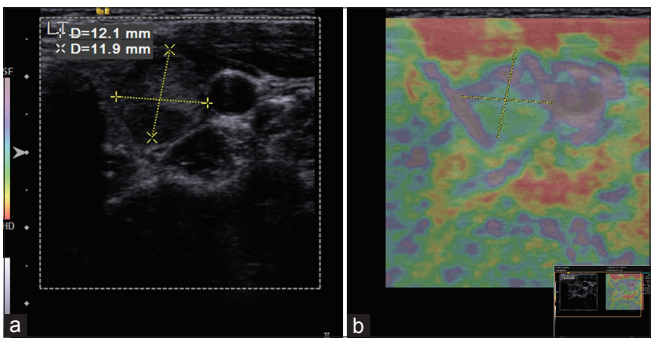


Figure 1: Thyroid nodule images obtained on conventional ultrasound (a) and ultrasound elastography with elasticity score 2 (b)

as benign, indeterminate/suspicious (follicular lesions), and malignant according to standard pathological criteria.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago. IL, USA) version 24. Descriptive statistics were presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages and compared using the Chi-square test. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were calculated. Statistical significance was set at $P < 0.05$.

For statistical purposes, USE score of 2 was strongly predictive of a benign lesion and therefore considered sample negative for malignancy. Scores of 3 and 4 describing stiffness in a large part of the examined area and nodule without elasticity, respectively, were considered as positive for malignancy in our study. With regard to cytology, two groups were considered for evaluation: benign and malignant. Patients with fine-needle aspiration (FNA) reports of indeterminate/suspicious (follicular lesions) were also included in the malignant group for statistical purposes.

RESULTS

A total of sixty patients with sixty solid thyroid nodules were enrolled in the study. The age of the patients ranged from 19 to 85 years (mean: 48.27 ± 16.10 years). About 83.3% (n = 50) were female and 16.7% (n = 10) were male. The frequency of benign nodules on cytopathologic analysis was 60% (n = 36), whereas 40% (n = 24) were nonbenign (follicular lesions and malignant tumors). Of the 24 nonbenign thyroid nodules, 20 had been reported as papillary carcinoma, 2 as medullary carcinoma, and 2 as follicular neoplasm. Table 3 shows the presenting clinical features and cytologic diagnoses of patients with thyroid nodules.

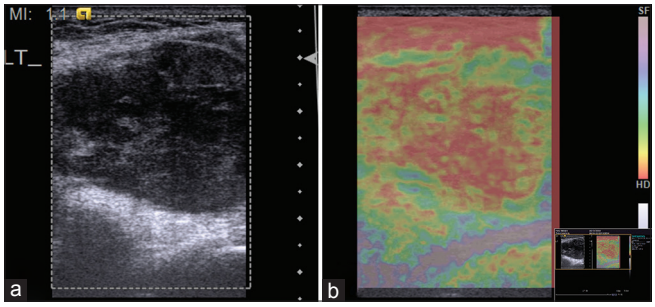


Figure 2: Heterogeneous nodule on conventional ultrasound (a) and ultrasound elastography with elasticity score 4 (b)

Seventy-five percent of the malignant lesions had irregular or poorly defined borders with lobulated margins on US and 68% had microcalcification. Seventy-seven percent of the benign nodules were hyperechoic. On TIRADS scoring, score of 2 was found in 24 cases, all benign lesions; score 3 in 14 cases, 8 benign and 6 malignant; score 4 in 12 cases, 4 benign and 8 malignant; and score 5 in 10 cases, all malignant lesions. Table 4 shows the correlation of USE scores and FNAC diagnoses of patients.

The sensitivity, specificity, positive predictive value, and negative predictive value of US elastography were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as a reference standard [Table 5]. Higher elastography scores were found to be significantly associated with malignant cytology ($P = 0.0001$).

DISCUSSION

Thyroid nodules are common in the general population, and the reported prevalence varies depending on the identification method. Apart from serum markers and FNAC, USG plays an important role in the detection and evaluation of thyroid nodules. Being noninvasive conventional US provides information regarding the nodule dimensions, structure as well as parenchymal changes and is widely used to determine which nodules should be biopsied. Microcalcifications, hypoechogenicity,

Table 3: Clinical presentation of thyroid nodules

Clinical feature	Benign	Malignant	Total number of patients, *n* (%)
Neck swelling	26	2	28 (46.7)
Weight loss	2	8	10 (16.7)
Dysphagia	2	6	8 (13.3)
Pain	6	2	8 (13.3)
Hoarseness of voice	0	6	6 (10)

Table 4: Correlation of elastography scores and cytologic diagnoses of patients with thyroid nodules

Elastography score	Benign	Malignant	Total
2	28	2	30
3	8	2	10
4	-	20	20
Total	36	24	60

Table 5: 2×2 table for calculation of sensitivity, specificity, positive predictive value, and negative predictive value of ultrasound elastography taking cytology as a reference standard

USE	Malignancy (positive on FNA)	Malignancy (negative on FNA)	Total
Positive	22 (true positive)	8 (false positive)	30
Negative	2 (false negative)	28 (true negative)	30
Total	24	36	60

FNA – Fine-needle aspiration; USE – Ultrasound elastography

irregular or microlobulated margins, taller than wide shape, and increased intranodular vascularity have been reported as independent risk factors for malignancy.[13,14] Although these features are characterized by high specificity, their positive predictive value is lowered by their relatively low sensitivity.[15]

USE is based on the principle that the softer parts of tissues deform easier than the harder parts, under compression, thereby allowing an objective determination of tissue consistency. Malignancy is often associated with changes in the mechanical properties of a tissue, and USE has been used to differentiate benign from malignant lesions in the breast, liver, and prostate.[16-18] The two general criteria for evaluating the efficacy of USE are elasticity scores and strain ratio. Although elastography has been suggested to be of great use as an adjunctive tool in the diagnosis of thyroid cancer, studies have reported conflicting evidence on its additional value in predicting thyroid malignancy.[19-21]

This study was planned to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with FNAC analysis as a reference standard. The mean age of presentation for benign nodules was 44.31 ± 14.28 years, whereas that for malignant lesions was 54.21 ± 17.13 years ($P = 0.018$) A majority of our cases diagnosed with thyroid nodules were females (83.3%). Thyroid nodules are reported to be four times more common in women than men, and their frequency increases with age and low iodine intake.[22] The female preponderance has been explained by the hormonal influences of estrogen and progesterone, as increasing nodule size and new nodule development have been demonstrated to be related to pregnancy and multiparity.[23]

Of the sixty nodules studied, 60% were benign ($n = 36$) and 40% ($n = 24$) were nonbenign. Of the 36 benign nodules, 26 were colloid nodules and 10 were adenomatoid nodules. The 24 nonbenign lesions included 20 papillary carcinomas, 2 medullary carcinomas, and 2 follicular neoplasms. The most common clinical presentation was painless mass in the neck which was encountered in almost 47% of the patients. Majority of the patients who presented with significant weight loss, dysphagia, and hoarseness of voice were found to have malignant lesions. Dysphagia, dysphonia, reports of rapid growth, male gender, prior head-and-neck irradiation, and presentation at extremes of age have been reported as significant elements in the patient's history, which increase the likelihood of malignancy.[24]

Our study found that 75% of malignant lesions had irregular or poorly defined borders with lobulated margins, whereas 68% of malignant lesions had microcalcification. Seventy-seven percent of the benign nodules were hyperechoic, whereas 23% were hypoechoic. None of the hyperechoic nodules was malignant. Although none of these US features is sufficient to differentiate benign lesions from malignant ones, studies have shown that a combination of at least two of the features is better helpful in pointing out a subset of lesions at high risk for malignancy.[25,26]

With regard to TIRADS score, all the nodules with TIRADS score of 2 ($n = 24$) had benign FNA characteristics, whereas all the patients with TIRADS score of 5 ($n = 10$) were found to have malignant lesions on FNA. Among TIRADS 3 lesions ($n = 14$), 57% were benign, and among TIRADS 4 lesions ($n = 12$), 33% were found to be benign on FNA.

On US elastography, score of 2 was found in 30 nodules, 28 benign and 2 malignant; score 3 in 10 cases, 8 benign and 2 malignant; and score 4 in 20 cases, all malignant. The sensitivity, specificity, positive predictive value, and negative predictive value of US elastography were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as a reference standard.

The elastography scores of 3–4 were achieved in 22 of the 24 malignant nodules in our study. Among the 20 nodules with cytologic diagnosis of papillary carcinoma, 18 were correctly scored as malignant by USE, thus achieving 90% diagnostic accuracy in terms of confirming the malignant etiology. Only two nodules were incorrectly given a USE score of 2. Both of the two nodules with medullary carcinoma thyroid had been scored as malignant on USE (score 4). We had eight false-positive nodules (score 3) which can be attributed to the fibrotic changes and calcification that lead to hardness of the nodule under study.

Our observations are in line with that of Mehrotra *et al.* who have reported the sensitivity and specificity of USE for thyroid malignancy to be 90% and 79.6%, respectively.[27] Of the 147 nodules examined by USE and US-guided FNAC, 122 were nonneoplastic, 5 were benign neoplasm, 10 had indeterminate cytologies, and 10 had malignant neoplasms. The observed positive and negative predictive values were 24.3% and 99.1%, respectively. In contrast, we found the positive predictive value of elastography to be 73.3% in our study. This can be explained by the high prevalence of malignant lesions among our study population.

The sensitivity and specificity of elastography based on elastography scores have been reported to vary from

86% to 97% and 66%–100% from various studies.[8,9,28] The observed wide differences are due to the fact that these studies involve different groups of populations, referral bias (patients referred for FNAC/surgery), varied inclusion and exclusion criteria, different machines as well as criteria for USE scoring. A recent comprehensive meta-analysis to investigate the diagnostic value of qualitative elastography in distinguishing benign from malignant thyroid nodules included 20 studies involving 3973 thyroid nodules.[29] Pooled results of elastography have shown a summary sensitivity of 85% (95% confidence interval [CI], 79%–90%) and specificity of 80% (95% CI, 73%–86%). The respective pooled negative predictive and positive predictive values were 97% (95% CI, 94%–98%) and 40% (95% CI, 34%–48%), respectively.

Despite the potential limitations of USE such as intra/interobserver variability and nodule characteristics such as calcification and follicular carcinoma (which appears soft), USE is considered to be reliable in the diagnosis of benign nodules in view of the high negative predictive values observed in most of the studies including ours.

FNAC is an essential diagnostic modality in the evaluation of thyroid nodules and features in all the guidelines in patient management.[30] Although the use of FNAC in the evaluation of thyroid nodules provides reliable results, it is invasive and the potential for complications such as postprocedural hematoma, increased patient morbidity, cost, and possibility of nondiagnostic specimen does exist. Unnecessary/repeat FNACs and FNA-induced changes can result in false-positive results on thyroid cytology and false-negative results or understaging of the tumor on thyroid pathology.[31,32]

Higher elastography scores were found to be significantly associated with malignant cytology ($P = 0.0001$) in our study. Merino *et al*. have also noted a statistically significant association between elasticity scores and cytological results, suggesting that malignant nodules could be excluded by elastography.[33]

Limitations of our study include small sample size and the fact that observations are from a single center.

CONCLUSION

With scanty literature comparing elastography with FNAC available from this part of the world, our study highlights that elastography has a fairly high diagnostic accuracy and correlation with malignant cytology. It can be an effective noninvasive adjunctive tool in distinguishing benign from malignant thyroid nodules and reduce the burden of unnecessary invasive procedures.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Dean DS, Gharib H. Epidemiology of thyroid nodules. Best Pract Res Clin Endocrinol Metab 2008;22:901-11.
2. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, *et al*. Management of thyroid nodules detected at US: Society of radiologists in ultrasound consensus conference statement. Radiology 2005;237:794-800.
3. Hoang JK, Lee WK, Lee M, Johnson D, Farrell S. US features of thyroid malignancy: Pearls and pitfalls. Radiographics 2007;27:847-60.
4. Jena A, Patnayak R, Prakash J, Sachan A, Suresh V, Lakshmi AY. Malignancy in solitary thyroid nodule: A clinicoradiopathological evaluation. Indian J Endocrinol Metab 2015;19:498-503.
5. Bonavita JA, Mayo J, Babb J, Bennett G, Oweity T, Macari M, *et al*. Pattern recognition of benign nodules at ultrasound of the thyroid: Which nodules can be left alone? AJR Am J Roentgenol 2009;193:207-13.
6. Remonti LR, Kramer CK, Leitão CB, Pinto LC, Gross JL. Thyroid ultrasound features and risk of carcinoma: A systematic review and meta-analysis of observational studies. Thyroid 2015;25:538-50.
7. Listewnik MH, Birkenfeld B, Piwowarska-Bilska H, Cichoń-Bańkowska K, Iglińska-Wagner L, Watrak W, *et al*. The application of SPECT/CT scintigraphy with MIBI-Tc^{99}(m) in the diagnosis of thyroid nodules – A preliminary report. Endokrynol Pol 2010;61:422-6.
8. Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: New developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab 2007;92:2917-22.
9. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al*. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
10. Hu X, Liu Y, Qian L. Diagnostic potential of real-time elastography (RTE) and shear wave elastography (SWE) to differentiate benign and malignant thyroid nodules: A systematic review and meta-analysis. Medicine (Baltimore) 2017;96:e8282.
11. Hahn SY, Shin JH, Ko EY, Bae JM, Choi JS, Park KW. Complementary role of elastography using carotid artery pulsation in the ultrasonographic assessment of thyroid nodules: A prospective study. Korean J Radiol 2018;19:992-9.
12. Horvath E, Majlis S, Rossi R, Franco C, Niedmann JP, Castro A, *et al*. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab 2009;94:1748-51.
13. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, *et al*. Risk of malignancy in nonpalpable thyroid nodules: Predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab 2002;87:1941-6.
14. Moon WJ, Jung SL, Lee JH, Na DG, Baek JH, Lee YH, *et al*. Benign and malignant thyroid nodules: US differentiation-multicenter retrospective study. Radiology 2008;247:762-70.
15. Popoveniuc G, Jonklaas J. Thyroid nodules. Med Clin North Am 2012;96:329-49.
16. Chang JM, Won JK, Lee KB, Park IA, Yi A, Moon WK. Comparison of shear-wave and strain ultrasound elastography in the differentiation of benign and malignant breast lesions. AJR Am J Roentgenol

2013;201:W347-56.
17. Kim JE, Lee JY, Bae KS, Han JK, Choi BI. Acoustic radiation force impulse elastography for focal hepatic tumors: usefulness for differentiating hemangiomas from malignant tumors. Korean J Radiol 2013;14:743-53.
18. Miyanaga N, Akaza H, Yamakawa M, Oikawa T, Sekido N, Hinotsu S, *et al*. Tissue elasticity imaging for diagnosis of prostate cancer: A preliminary report. Int J Urol 2006;13:1514-8.
19. Moon HJ, Sung JM, Kim EK, Yoon JH, Youk JH, Kwak JY. Diagnostic performance of gray-scale US and elastography in solid thyroid nodules. Radiology 2012;262:1002-13.
20. Veyrieres JB, Albarel F, Lombard JV, Berbis J, Sebag F, Oliver C, *et al*. A threshold value in Shear Wave elastography to rule out malignant thyroid nodules: A reality? Eur J Radiol 2012;81:3965-72.
21. Trimboli P, Guglielmi R, Monti S, Misischi I, Graziano F, Nasrollah N, *et al*. Ultrasound sensitivity for thyroid malignancy is increased by real-time elastography: A prospective multicenter study. J Clin Endocrinol Metab 2012;97:4524-30.
22. Mazzaferri EL. Management of a solitary thyroid nodule. N Engl J Med 1993;328:553-9.
23. Kung AW, Chau MT, Lao TT, Tam SC, Low LC. The effect of pregnancy on thyroid nodule formation. J Clin Endocrinol Metab 2002;87:1010-4.
24. Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-71.
25. Papini E. The dilemma of non-palpable thyroid nodules. J Endocrinol Invest 2003;26:3-4.
26. Frasoldati A, Valcavi R. Challenges in neck ultrasonography: Lymphadenopathy and parathyroid glands. Endocr Pract 2004;10:261-8.
27. Mehrotra P, McQueen A, Kolla S, Johnson SJ, Richardson DL. Does elastography reduce the need for thyroid FNAs? Clin Endocrinol (Oxf) 2013;78:942-9.
28. Ghajarzadeh M, Sodagari F, Shakiba M. Diagnostic accuracy of sonoelastography in detecting malignant thyroid nodules: A systematic review and meta-analysis. AJR Am J Roentgenol 2014;202:W379-89.
29. Nell S, Kist JW, Debray TP, de Keizer B, van Oostenbrugge TJ, Borel Rinkes IH, *et al*. Qualitative elastography can replace thyroid nodule fine-needle aspiration in patients with soft thyroid nodules. A systematic review and meta-analysis. Eur J Radiol 2015;84:652-61.
30. Ahn SS, Kim EK, Kang DR, Lim SK, Kwak JY, Kim MJ. Biopsy of thyroid nodules: Comparison of three sets of guidelines. AJR Am J Roentgenol 2010;194:31-7.
31. Eze OP, Cai G, Baloch ZW, Khan A, Virk R, Hammers LW, *et al*. Vanishing thyroid tumors: A diagnostic dilemma after ultrasonography-guided fine-needle aspiration. Thyroid 2013;23:194-200.
32. Canberk Ş, Fırat P, Schmitt F. Pitfalls in the cytological assessment of thyroid nodules. Turk Patoloji Derg 2015;31 Suppl 1:18-33.
33. Merino S, Arrazola J, Cárdenas A, Mendoza M, De Miguel P, Fernández C, *et al*. Utility and interobserver agreement of ultrasound elastography in the detection of malignant thyroid nodules in clinical care. AJNR Am J Neuroradiol 2011;32:2142-8.

Original Article

Role of Transrectal Ultrasound Elastography in the Diagnosis of Prostate Carcinoma

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Abstract

Background: The purpose of this study was to evaluate the diagnostic value of transrectal real- time strain elastography (RTE) in identifying prostatic carcinoma (PCa). **Materials and Methods:** 60 patients suspected of having PCa based on abnormal digital rectal examination and raised prostate specific antigen levels underwent transrectal ultrasound (TRUS), color Doppler (CD) and RTE. Elastograms were scored on a five point scale based on distribution of strain in relation to hypoechoic area on TRUS. Twelve core systematic biopsy as well as targeted biopsy was performed from suspicious areas on TRUS and RTE. Diagnostic performance of sonoelastography was evaluated using histopathology as reference standard. **Results:** Histopathology revealed cancer in 28 out of 60 patients (47%) studied. Gleason score ranged from 6 to 9. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TRUS in detecting prostate cancer were 78.57%, 81.25%, 78.57%, and 81.25%, respectively. On CD evaluation 87.5% (n = 28) of benign lesions showed symmetric, radial flow compared to 14.3% (n = 4) of malignant lesions ($P < 0.0001$). The sensitivity and specificity of RTE was 89.29% and 56.25% with PPV and NPV being 58.13% and 82.35%, respectively. Higher elastography score was found to be significantly associated with malignant histopathology ($P = 0.004$). Cancer detection rate with RTE was greater for tumors with higher Gleason score. **Conclusion:** RTE was found to have better sensitivity than TRUS as well as combination of TRUS and CD. Although less specific, RTE can be an effective adjuvant tool to TRUS for guidance of biopsy and improve detection rate of PCa.

Keywords: Elastography, prostate cancer, ultrasonography

Introduction

Prostate carcinoma (PCa) is the second most frequently diagnosed cancer in males all over the world and is a major healthcare concern associated with significant morbidity and mortality. The burden of PCa is estimated to grow to 1.7 million new cases and 499000 new deaths by 2030 as a result of growth and aging of the global population.[1]

Digital rectal examination (DRE), screening for prostate specific antigen (PSA), and imaging techniques such as transrectal ultrasound (TRUS) and magnetic resonance imaging (MRI) are the methods commonly employed for detection of PCa. DRE is limited by its low sensitivity, subjective nature, and depends on the experience of the clinician. Although PSA testing has been widely used for screening of prostatic cancer, it is not disease specific and can be elevated in patients with prostatitis as well as benign prostatic hyperplasia.[2,3] In a patient with suspicion of prostatic cancer, current guidelines involve histopathologic examination of systematic biopsy cores to confirm or rule out cancer.[4] However, the conventional biopsy protocol misses significant cancer in a large proportion of patients.[5] Further many insignificant cancers that do not need immediate treatment are also detected leading to overdiagnosis and treatment.[6] Use of TRUS for detecting prostate cancer has limitations such as poor specificity of focal abnormalities, frequent multifocality of cancer within the prostate, and substantial percentage of isoechoic prostatic carcinoma (PCa).[7] Although MRI results are promising, it is expensive and not always available. In view of these limitations, improved noninvasive imaging modalities that

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can provide better characterization of prostatic lesions and increase detection of prostatic cancer are needed.

Ultrasound elastography (USE) is a dynamic technique that uses ultrasound to estimate tissue stiffness by measuring the degree of distortion under application of an external force. As cancerous tissue has greater stiffness than a benign lesion USE has been used to differentiate malignant from benign lesions in breast, thyroid, liver, and cervix. Recent studies have demonstrated the utility of sonoelastography in detecting prostate cancer.[8-10]

Data on the usefulness of transrectal strain elastography (SE) in evaluation of prostatic cancer and its correlation with histopathology are relatively few, especially from India. The purpose of the present study was to evaluate the usefulness and diagnostic performance of transrectal SE in identifying prostatic cancer with histopathologic diagnosis obtained by needle biopsy of prostate as reference standard.

Materials and Methods

This prospective study was performed in a tertiary care center in South India between December 2014 and September 2016. 60 consecutive patients who were referred to the department of Radiology with suspicion of PCa based on abnormal DRE and elevated PSA levels (>4 ng/ml) were enrolled. The institutional review board approved the study protocol and the protocol was in compliance with tenets of the Declaration of Helsinki (IRB approval Number: 14/399). Informed consent was obtained from all participants. Exclusion criteria consisted of patients with bleeding disorders, inflammatory bowel disease and surgical absence of rectum or ileoanal pouch. All patients underwent TRUS (grey scale and Doppler) and SE as well as needle biopsy of prostate in the same session.

TRUS of prostate was done using an endocavitary transducer (Siemens Accuson S3000, Siemens Healthineers, Erlangen, Germany) with 9–4 MHz range and 174 degree field of vision, capable of user selectable multi hertz imaging. Xylocaine gel was applied over a probe cover applied onto the probe. All patients were examined in the left lateral decubitus position. Prostate was imaged in both axial and sagittal planes with assessment of volume, echogenicity, surface, calcification, vascularity, and the presence of nodules. Each nodule was assessed for size, margins, location in the gland, morphology, and echogenicity.

Glands with homogeneous echotexture or minimal heterogeneity on TRUS were labeled as benign while those with ill-defined echotexture abnormality, focal bulge or hypoechoic mass were categorized as malignant. For Color Doppler (CD) imaging, capsular/periurethral flow and symmetric radial flow from capsular branches were considered benign while asymmetric or increased flow with disorganized pattern was labeled malignant.[11]

Using the same probe transrectal real-time strain elastography (RTE) of prostate was done by a single experienced radiologist. The probe was placed over the region such that the lesion is in the center of the image. Elastograms were obtained by slight compression and decompression of the prostate. The appropriate frequency and pressure of the compression/decompression was optimized with the help of real time display of quality index. Elastograms obtained with quality index equal to or >60 were only included. At least a 5 mm thickness of normal adjacent tissue was included, to assess the lesion stiffness in relation with the average elasticity of the surrounding tissue. A standardized color coding system was used, where red indicated soft, more compliant areas and blue indicated stiff areas. Green color indicated average strain in the region of interest. The elastograms along with conventional B-mode images were displayed side by side on split screen mode. Multiple frames were acquired and the best fit B mode-elastography image pairs were selected for examination. The elasticity patterns were scored using a five point scale proposed by Kamoi *et al*.[10] [Table 1] The cut off for malignancy was set between scores of 2 and 3 [Figures 1-4].

Sampling of the prostate was performed either in the sagittal or in the axial plane. Biopsies were taken using an 18 G biopsy gun under strict aseptic precautions. Extended core biopsy protocol (12 cores) was used. Additional targeted biopsy was carried out on suspicious areas identified by B mode US and elastography. The grade (degree of aggressiveness) of prostate cancer was evaluated on the basis of the Gleason score. No major complications were encountered after the procedure.

The findings of TRUS, Doppler and RTE were interpreted by consensus by two experienced radiologists who were blinded to patients' clinical profile and biopsy results. The histopathologic results were considered as the reference standard, and utility of RTE was evaluated.

Statistical analysis

Data was entered in the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. The diagnostic sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated with histopathologic analysis as reference standard. For analyzing the combined diagnostic performance of tests, the test was considered positive if either of them was

Table 1: Sonoelastography scoring system proposed by Kamoi *et al*.

Elastography score	Description
Score 1	Normal - homogeneous strain
Score 2	Probably normal - symmetric heterogeneous strain
Score 3	Indeterminate - asymmetric focal stiff lesion which is not related to any hypoechoic area
Score 4	Probably carcinoma (strain at the periphery of the hypoechoic lesion with sparing of the center
Score 5	Definitely carcinoma (no strain in the entire hypoechoic lesion or in the surrounding area)

suspicious for malignancy and negative if neither showed suspicious features. Pearson Chi-square test was used to study the association between categorical variables. $P < 0.05$ was considered as statistically significant.

Results

The age of the patients ranged from 53 to 88 years (Mean: 69.16 ± 9.40 years). Serum PSA levels ranged between 4.3 and 178.6 ng/ml (Mean 48.54 ± 56.69 ng/ml). No Significant difference in PSA levels were noted between patients with carcinoma and benign lesions of the prostate ($P = 0.083$). In 60 patients, 784 biopsy cores were harvested out of which 720 was according to the extended core biopsy protocol and 64 were targeted (36 and 28 targeted to areas found suspicious on B-mode US and RTE, respectively). Histopathology revealed cancerous focus in at least one biopsy core in 28/60 (47%) patients. Gleason score ranged from 6 to 9 (Mean: 7.42 ± 1.13). Among the 32 patients with benign histopathology results, 20 (62.5%) were found to have benign prostatic hyperplasia and 12 (37.5%) had prostatitis. The mean prostatic volume was 38.20 ± 18.25 cm^3 (range: 8–89 cm^3). The mean age of patients with benign and malignant lesions were 66.78 ± 9.56 years and 71.89 ± 8.59 years, respectively ($P = 0.035$).

Out of the 60 patients, 28 (47%) were suspected of having prostate cancer by TRUS. The sensitivity, specificity, PPV, and NPV of TRUS in detecting prostate cancer were 78.57%, 81.25%, 78.57%, and 81.25%, respectively, with histopathology as reference standard. On CD evaluation 87.5% ($n = 28$) of benign lesions showed symmetric, radial flow compared to 14.3% ($n = 4$) of malignant lesions ($P < 0.0001$).

Table 2 shows the correlation of elastography scores and histopathologic diagnoses of lesions. Out of the 32 patients

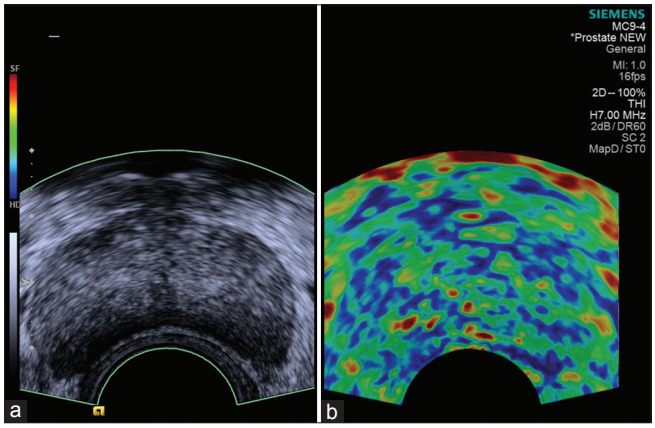


Figure 1: (a) Transrectal ultrasonography image showing mild enlargement of prostate. (b) Sonoelastography showing heterogeneous appearance with symmetrical mosaic pattern of green and blue (Score 2 – probably normal). Histopathology revealed hyperplasia of prostate with benign glandular pattern

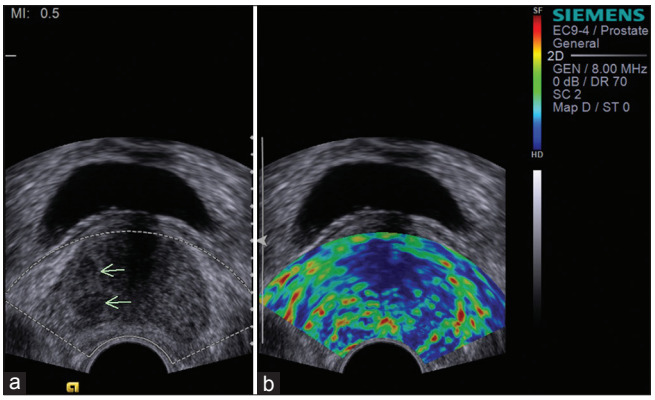


Figure 2: (a) Transrectal ultrasonography image showing hypoechoic lesion in the right peripheral zone (arrows) of prostate. (b) Sonoelastography showing focal asymmetric stiff lesion not related to hypoechoic area (score 3 – indeterminate). Histopathology was suggestive of adenocarcinoma prostate

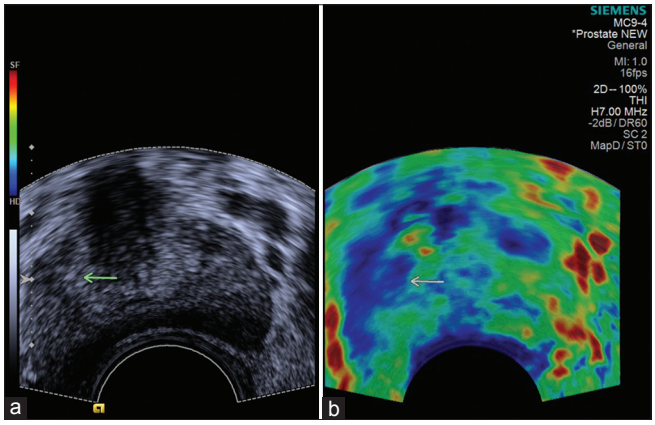


Figure 3: (a) Transrectal ultrasonography image showing hypoechoic lesion in the right peripheral zone (arrow) of prostate. (b) Sonoelastography showing stiffness in the center of the lesion and strain at the periphery; the peripheral part of lesion appears green and the central part appears blue (score 4 – probably carcinoma). Histopathology confirmed adenocarcinoma prostate

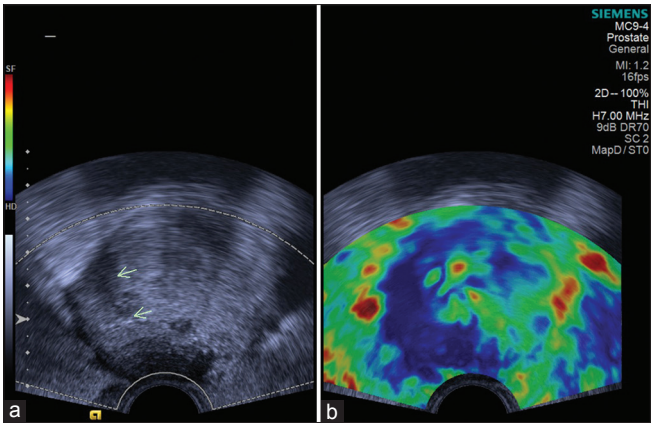


Figure 4: (a) Transrectal ultrasonography image showing hypoechoic lesion with irregular margin in the right peripheral zone (arrows) of prostate. (b) Sonoelastography showing stiffness in the entire hypoechoic lesion and in the surrounding area; the entire lesion appears blue (score 5 – definitely carcinoma). Histopathology confirmed adenocarcinoma prostate

with benign histopathology, 14 were judged as negative for malignancy at RTE. There were 3 false negative patients on elastography with score of 2. The sensitivity and specificity of RTE was 89.29% and 56.25% with positive and NPV of 58.13% and 82.35%, respectively. Higher elastography score was found to be significantly associated with malignant histopathology ($P = 0.004$).

Table 3 shows the relation between Gleason score and elastography scores. Approximately 84% of patients with a Gleason score ≤6 were detected by RTE whereas for scores 8–9, the detection rate was 100%.

The diagnostic performance of TRUS, combined modalities (TRUS and Doppler) and RTE are shown in Table 4.

Discussion

In this prospective study from South India, we evaluated the diagnostic performance of transrectal real-time elastography (RTE) in detection of prostatic cancer in patients with abnormal DRE and elevated PSA levels. RTE showed superior sensitivity of nearly 90% versus sensitivity of 79% by TRUS and 86% by a combination of TRUS and CD ultrasound.

Table 2: Correlation between elastography score and histopathologic diagnosis of lesions

Elastogram pattern	Benign ($n=32$)	Malignant ($n=28$)	Total ($n=60$)
1	0	0	0
2	14	3	17
3	18	4	22
4	0	9	9
5	0	12	12

Table 3: Relation between Gleason score and elastography scores

Gleason score	ES 1	ES 2	ES 3	ES 4	ES 5
≤6	0	1	0	2	3
7	0	2	2	6	2
8	0	0	0	1	1
9	0	0	2	0	6

ES: Elastography score

Table 4: Diagnostic performance of transrectal ultrasound, combined modalities (transrectal ultrasound and Doppler) and real-time strain elastography

Performance measure	TRUS	TRUS and doppler	RTE
Sensitivity (%)	78.57	85.71	89.29
Specificity (%)	81.25	75	56.25
Positive predictive value (%)	78.57	75	58.13
Negative predictive value (%)	81.25	85.71	82.35

TRUS: Transrectal ultrasound, RTE: Real-time strain elastography

Ever since the description of principles of elastography by Ophir *et al*., elasticity imaging has been applied to a wide variety of tissues such as breast, thyroid, liver, lymph nodes and prostate.[12]

The two US elastography techniques in clinical use are SE and shear wave elastography. Several studies have evaluated the utility of SE in diagnosing prostatic cancer and have shown varied results. While few have compared the diagnostic accuracy with needle biopsy of prostate,[13-15] others have used histopathologic examination of radical prostatectomy specimen as the reference standard.[8,9,16] The heterogeneous results can be attributed to the varied inclusion criteria, demographic characteristics of patients, serum PSA levels, location of tumors, reference standard, number of biopsy cores, systematic or targeted biopsies and Gleason score of the tumors examined.

The age of patients with malignancy of prostate was significantly higher compared to those with benign lesions in our study ($P = 0.035$). Age is considered to be an established risk factor for prostatic cancer and study of age-specific incidence curves have shown that risk of prostate cancer begins to rise sharply after 55 years of age and peaks at 70–74, declining slightly thereafter.[17]

In our study, TRUS revealed hypoechoic/focal lesions in 28 patients (47%). Out of these, 22 were confirmed as adenocarcinoma prostate while 6 turned out to be benign on histopathology. The results from our study in terms of specificity and PPV of TRUS in detecting prostatic cancer are similar to that of Ferrari *et al*. who had evaluated 84 patients suspected of prostatic cancer by TRUS, RTE and transperineal prostate biopsy.[18] B-mode US demonstrated 56% sensitivity, 80% specificity, 70% PPV and 67% NPV in diagnosing prostatic cancer. A hypoechoic lesion in the peripheral zone of prostate is suggested to be more likely malignant, the proposed cause being replacement of normal loose glandular tissue by a packed mass of tumor cells which results in fewer reflecting interfaces. Hyperplasia, prostatitis, fibrosis and cysts of prostate are other lesions that appear hypoechoic on TRUS.[19] Nearly 25%–40% of prostate cancers are isoechoic and missed by TRUS.[20]

CD and Power Doppler (PD) have been reported to be useful in detecting isoechoic tumors which may be missed by conventional ultrasound.[21,22] In 2 patients with prostatic cancer in our study, Doppler evaluation revealed asymmetric, increased flow while TRUS was not suspicious for malignancy. Few authors have shown that increased CD signal correlates positively with grading and staging of prostate cancer as well as risk of recurrence after treatment.[23,24]

On CD evaluation, 87.5% ($n = 28$) of benign lesions showed symmetric, radial flow compared to 14.3% ($n = 4$) of malignant lesions ($P < 0.0001$) in our study. A combination of TRUS and Doppler revealed superior diagnostic performance with sensitivity and NPV of nearly 86% compared to TRUS. However, benign lesions like prostatitis can also show

increased flow on CD and CD does not reveal microscopic vessels of prostatic cancer that do not possess enough flow to cause Doppler shift.[25,26] Despite these limitations, our findings indicate that CD imaging can be a useful adjunct to improve tumor detection by TRUS.

Prostatic cancer tends to be stiffer than benign lesions due to replacement of normal glandular tissue by neoplastic cells.[27] Stiffer tissues demonstrate less strain to deformation, and RTE helps in differentiation of benign and malignant tissues based on distribution of strain. Miyagawa *et al*. evaluated the usefulness of RTE in detecting prostatic cancer prior to systematic biopsy and have noted a diagnostic sensitivity of 72.6% for elastography and 89.5% for combination of TRUS and elastography.[14] Kamoi *et al*. used a five point subjective scale based on degree and distribution of strain in relation to hypoechoic area and found that RTE had 68% sensitivity and 81% specificity in detecting prostatic cancer. The combination of RTE with PD US further increased the sensitivity to 78%.[10]

Using the cutoff value of 3 (suggesting focal asymmetric lesion without strain unrelated to hypoechoic lesion) proposed by Kamoi *et al*., RTE was found to have sensitivity of 89.29% and specificity of 56.25% in our study. Of 43 patients with RTE findings suspicious for carcinoma, 25 were confirmed to be malignant on histopathology. Benign entities such as benign prostatic hyperplasia, adenomyomatosis, fibrosis and prostatitis can be associated with increased tissue stiffness and therefore may be difficult to distinguish from carcinoma prostate. This can be responsible for the relatively low specificity and PPV observed in our study. Because of the high frequency of false-positive results, RTE should be used in combination with TRUS to increase the detection rate.

In 5 of 28 patients with prostatic cancer (17.9%), B mode US was normal but RTE was suggestive of carcinoma. While systematic biopsy picked up malignancy in 3 patients, targeted biopsy based on RTE findings resulted in detection of cancer in 2 patients. The role of RTE targeted biopsy in detecting prostatic cancer has been reported by several authors. Pallwein *et al*. compared a 5-core RTE targeted biopsy with a 10-core systematic biopsy and concluded that cancer detection rate per patient was not significantly higher for targeted approach compared to systematic approach but an RTE targeted core was 2.9 fold more likely to be positive for cancer than a systematic core.[28] Salomon *et al*. analyzed the incremental detection rate of 4-core RTE targeted biopsy in addition to randomized 10-core in 1024 patients.[29] Additional use of RTE resulted in an incremental detection rate of 18.3%. 34 patients who harbored high-grade PCa (Gleason score ≥4) were diagnosed by RTE targeted biopsy only. A recent meta-analysis on the role of RTE targeted biopsy has shown that the detection rate of malignancy is enhanced when systematic biopsies are combined with RTE targeted biopsies.

Further RTE targeted biopsy can make a nearly equivalent diagnosis with fewer cores.[30]

In our study RTE detected 5 of 6 patients with prostatic cancer of Gleason score ≤6, 10 of 12 with score 7 and all with Gleason score 8 or 9. Our observations are similar to that of other authors who have noted that RTE detection rate of prostate cancer with higher Gleason score was greater than that of prostatic cancer with a lower Gleason score.[9,31] This might be explained by the greater cell density in high grade tumors resulting in stiffer tissue. In contrast, Tsutsumi *et al*. have noted a lower RTE detection rate for high grade tumors.[32]

Our study has few limitations. The sample size was small and no interobserver variability was studied since the RTE findings were interpreted by consensus. Another main limitation of RTE is that the degree of manual compression influences the elasticity image and may compromise diagnostic accuracy of RTE. However all examinations were performed by a single experienced radiologist and only images with optimal compression as displayed by the quality index were included in the study. Recent literature suggests that multiparametric MRI has greater sensitivity and specificity for detection of clinically significant prostate cancer.[33]

Although the incidence of prostatic cancer is low in Asian countries compared to the west, demographic and epidemiological transition in recent times have resulted in increase in the burden of various cancers including prostate in India. Prostate is reported to be the second leading site of cancer among males in several cities in India and cancer projection data estimate that the number of cases will become doubled by 2020.[34]

Conclusion

In this initial study from South India, RTE was found to have better sensitivity than TRUS as well as combination of TRUS and CD. With its greater sensitivity and NPV, RTE can be an effective adjuvant tool to guide biopsy and thereby increase the detection rate of prostatic cancer. Further prospective studies with larger sample size are needed before recommending RTE along with conventional TRUS for routine use in the evaluation of suspected prostatic malignancy in India.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Banu NA, Azim FA, Kamal M, Rumi MA, Barua AR, Khan KH. Inflammation and glandular proliferation in hyperplastic prostates: Association with prostate specific antigen value. Bangladesh Med Res Counc Bull 2001;27:79-83.
3. Ferrero Doria R, Pérez Flores D, Terrer Artes C, Guzmán Martínez-Valls PL, Morga Egea JP, Tomás Ros M, *et al*. Impact of prostatic benign hyperplasia and prostatic inflammation on the increase of prostate specific antigen levels. Actas Urol Esp 1997;21:100-4.

4. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, *et al*. EAU guidelines on prostate cancer. part 1: Screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 2014;65:124-37.
5. Lecornet E, Ahmed HU, Hu Y, Moore CM, Nevoux P, Barratt D, *et al*. The accuracy of different biopsy strategies for the detection of clinically important prostate cancer: A computer simulation. J Urol 2012;188:974-80.
6. Borza T, Konijeti R, Kibel AS. Early detection, PSA screening, and management of overdiagnosis. Hematol Oncol Clin North Am 2013;27:1091-110, vii.
7. Lemaître L, Villers A, Mouton D, Puech P. Transrectal ultrasound and biopsy of the prostate. J Radiol 2006;87:201-9.
8. Pallwein L, Mitterberger M, Struve P, Pinggera G, Horninger W, Bartsch G, *et al*. Real-time elastography for detecting prostate cancer: Preliminary experience. BJU Int 2007;100:42-6.
9. Sumura M, Shigeno K, Hyuga T, Yoneda T, Shiina H, Igawa M. Initial evaluation of prostate cancer with real-time elastography based on step-section pathologic analysis after radical prostatectomy: A preliminary study. Int J Urol 2007;14:811-6.
10. Kamoi K, Okihara K, Ochiai A, Ukimura O, Mizutani Y, Kawauchi A, *et al*. The utility of transrectal real-time elastography in the diagnosis of prostate cancer. Ultrasound Med Biol 2008;34:1025-32.
11. Halpern EJ, Ramey JR, Strup SE, Frauscher F, McCue P, Gomella LG. Detection of prostate carcinoma with contrast-enhanced sonography using intermittent harmonic imaging. Cancer 2005;104:2373-83.
12. Ophir J, Céspedes I, Ponnekanti H, Yazdi Y, Li X. Elastography: A quantitative method for imaging the elasticity of biological tissues. Ultrason Imaging 1991;13:111-34.
13. König K, Scheipers U, Pesavento A, Lorenz A, Ermert H, Senge T. Initial experiences with real-time elastography guided biopsies of the prostate. J Urol 2005;174:115-7.
14. Miyagawa T, Tsutsumi M, Matsumura T, Kawazoe N, Ishikawa S, Shimokama T, *et al*. Real-time elastography for the diagnosis of prostate cancer: Evaluation of elastographic moving images. Jpn J Clin Oncol 2009;39:394-8.
15. Brock M, von Bodman C, Palisaar RJ, Löppenberg B, Sommerer F, Deix T, *et al*. The impact of real-time elastography guiding a systematic prostate biopsy to improve cancer detection rate: A prospective study of 353 patients. J Urol 2012;187:2039-43.
16. Tsutsumi M, Miyagawa T, Matsumura T, Endo T, Kandori S, Shimokama T, *et al*. Real-time balloon inflation elastography for prostate cancer detection and initial evaluation of clinicopathologic analysis. AJR Am J Roentgenol 2010;194:W471-6.
17. Gann PH. Risk factors for prostate cancer. Rev Urol 2002;4 Suppl 5:S3-S10.
18. Ferrari FS, Scorzelli A, Megliola A, Drudi FM, Trovarelli S, Ponchietti R. Real-time elastography in the diagnosis of prostate tumor. J Ultrasound 2009;12:22-31.
19. Sarkar S, Das S. A review of imaging methods for prostate cancer detection. Biomed Eng Comput Biol 2016;7:1-5.
20. Shinohara K, Wheeler TM, Scardino PT. The appearance of prostate cancer on transrectal ultrasonography: Correlation of imaging and pathological examinations. J Urol 1989;142:76-82.
21. Newman JS, Bree RL, Rubin JM. Prostate cancer: Diagnosis with color Doppler sonography with histologic correlation of each biopsy site. Radiology 1995;195:86-90.
22. Okihara K, Kojima M, Nakanouchi T, Okada K, Miki T. Transrectal power Doppler imaging in the detection of prostate cancer. BJU Int 2000;85:1053-7.
23. Cornud F, Belin X, Piron D, Chrétien Y, Flam T, Casanova JM, *et al*. Color Doppler-guided prostate biopsies in 591 patients with an elevated serum PSA level: Impact on Gleason score for nonpalpable lesions. Urology 1997;49:709-15.
24. Ismail M, Petersen RO, Alexander AA, Newschaffer C, Gomella LG. Color Doppler imaging in predicting the biologic behavior of prostate cancer: Correlation with disease-free survival. Urology 1997;50:906-12.
25. Sharma S. Imaging and intervention in prostate cancer: Current perspectives and future trends. Indian J Radiol Imaging 2014;24:139-48.
26. Taverna G, Morandi G, Seveso M, Giusti G, Benetti A, Colombo P, *et al*. Colour Doppler and microbubble contrast agent ultrasonography do not improve cancer detection rate in transrectal systematic prostate biopsy sampling. BJU Int 2011;108:1723-7.
27. Krouskop TA, Wheeler TM, Kallel F, Garra BS, Hall T. Elastic moduli of breast and prostate tissues under compression. Ultrason Imaging 1998;20:260-74.
28. Pallwein L, Mitterberger M, Struve P, Horninger W, Aigner F, Bartsch G, *et al*. Comparison of sonoelastography guided biopsy with systematic biopsy: Impact on prostate cancer detection. Eur Radiol 2007;17:2278-85.
29. Salomon G, Drews N, Autier P, Beckmann A, Heinzer H, Hansen J, *et al*. Incremental detection rate of prostate cancer by real-time elastography targeted biopsies in combination with a conventional 10-core biopsy in 1024 consecutive patients. BJU Int 2014;113:548-53.
30. Tu X, Qiu S, Chang T, Jin K, Bao Y, Yang L, *et al*. The role of real-time elastography-targeted biopsy in the detection and diagnosis of prostate cancer: A systematic review and meta-analysis. Medicine (Baltimore) 2018;97:e0220.
31. Pallwein L, Mitterberger M, Pinggera G, Aigner F, Pedross F, Gradl J, *et al*. Sonoelastography of the prostate: Comparison with systematic biopsy findings in 492 patients. Eur J Radiol 2008;65:304-10.
32. Tsutsumi M, Miyagawa T, Matsumura T, Kawazoe N, Ishikawa S, Shimokama T, *et al*. The impact of real-time tissue elasticity imaging (elastography) on the detection of prostate cancer: Clinicopathological analysis. Int J Clin Oncol 2007;12:250-5.
33. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, *et al*. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): A paired validating confirmatory study. Lancet 2017;389:815-22.
34. Jain S, Saxena S, Kumar A. Epidemiology of prostate cancer in India. Meta Gene 2014;2:596-605.

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Original Article

Utility of Ultrasound Elastography to Differentiate Benign from Malignant Cervical Lymph Nodes

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Abstract

Background: The purpose of this study was to evaluate the usefulness of strain elastography and acoustic radiation force impulse (ARFI) imaging in the differentiation of benign and malignant cervical lymph nodes (LNs). **Materials and Methods:** In this prospective study, 50 enlarged cervical LNs (33 benign and 17 malignant) were examined by B-mode ultrasound (US), color Doppler, and strain elastography. Elastographic patterns (1–5) were categorized based on distribution of hard area within LN. The shear wave velocity (SWV) of LNs was evaluated by ARFI imaging. Diagnostic performance of sonoelastographic parameters was compared taking histopathology of LN as a reference standard. Optimal cutoff value of the mean SWV values for predicting malignancy was determined using receiver operating characteristic curve analysis. **Results:** Among US parameters, borders of LN had the highest diagnostic accuracy (80%), while echogenicity had the least (48%). Majority of benign LNs ($n = 31$) had elastography patterns 1 and 2, while majority of malignant LNs ($n = 16$) had patterns 3–5 ($P = 0.000$). The sensitivity, specificity, and accuracy of elastography were 94.1%, 93.9%, and 94%, respectively. The mean SWV of benign LNs (1.670 ± 0.367 m/s) differed significantly from malignant LNs (2.965 ± 0.826 m/s; $P = 0.000$). A cutoff value of 2.05 m/s predicted malignancy with 88.2% sensitivity and 84.8% specificity and gave an area under the curve of 0.949 (95% confidence interval: 0.70–1.20). **Conclusion:** Elastography has high diagnostic accuracy in differentiating benign and malignant cervical LNs and can be potentially useful in selecting the LN with high probability of malignancy, on which fine-needle aspiration cytology/biopsy can be performed.

Keywords: Acoustic radiation force impulse imaging, elastography, lymph nodes, ultrasound

Introduction

Cervical lymphadenopathy, a common condition encountered by physicians in clinical practice, could be caused by a variety of pathologic processes such as infections, vasculitis, and malignancies such as lymphoma and metastases. Metastatic cervical lymphadenopathy is a common problem in head and neck oncology. Depending on the primary site, up to 80% of patients with upper aerodigestive mucosal malignancy have been reported to have cervical nodal metastasis at presentation.[1] The differentiation of malignant from benign lymph nodes (LNs) is essential because it predicts the patient prognosis and helps in decision-making and planning of management.[2]

High-resolution ultrasound (US) is the extensively used imaging technique for evaluating the morphology and internal architecture of cervical LNs. Color Doppler sonography is used to assess the pattern of intranodal vessels, blood flow velocity, and vascular resistance.[3] Although Gray-scale sonography in combination with Doppler has been suggested to be useful in differentiating metastatic LNs from reactive ones based on various criteria, there is no single US criterion for diagnosing malignant LNs with satisfactory sensitivity and specificity.[4,5]

Real-time elastography (RTE) is a novel, noninvasive imaging modality that assesses tissue elasticity by comparing local tissue displacement from US signals before and after the application of a compressive force.[6] Stiff tissues show less deformation (strain) than soft tissues under the compression of a transducer. Since malignant tissues are stiffer than their benign counterparts at many

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sites, several reports have documented the utility of US elastography in differentiating malignant from benign lesions in the breast, prostate, liver, thyroid, and cervix.[7-11] There are two types of elastography techniques, namely strain elastography and shear wave-based elastography. Recent studies have shown the usefulness of strain elastography in differential diagnosis of benign and malignant cervical LNs.[12-14] However, some authors have questioned its additional value over conventional US in predicting malignancy and decision-making in terms of selection of LNs for biopsy.[15-17] Shear wave elastography with acoustic radiation force impulse (ARFI) imaging is an emerging sonographic technique in the assessment of malignant cervical nodes.[18,19]

Data on the utility of elastography in the evaluation of cervical LNs are relatively few, especially from India. The purpose of the present study was to evaluate the utility and diagnostic performance of strain elastography compared to conventional B-mode US and color Doppler in the differentiation of benign and malignant cervical LNs. We also investigated the usefulness of ARFI imaging in differentiating benign and malignant LNs.

Materials and Methods

This prospective study was performed at a tertiary care center in South India between August 2016 and February 2018. Fifty LNs of 50 consecutive patients who were referred for ultrasonogram of enlarged cervical LNs were enrolled. The Institutional review board (IRB) approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki (IRB Approval No. 16/334). Informed consent was obtained from all participants. Patients with suboptimal image quality and those with completely necrotic nodes without solid component were not included. Patients who had received specific treatment (chemotherapy/radiotherapy) were also excluded from the study.

High-resolution US imaging was performed in the supine position with a high-frequency linear probe 9 L4 (Siemens Accuson S3000, Siemens Healthineers, Erlangen, Germany) by a single expert radiologist, blinded to patient's clinical profile. In gray-scale US, the LNs were evaluated for short-axis diameter and short-to-long axis diameter ratio (S/L ratio) in the longitudinal plane, borders (regular or irregular), echogenicity (homogeneous/heterogeneous), and hilum (present or absent). Intranodal vascular pattern was evaluated using color Doppler imaging. Absent flow signals or blood flow limited to the hilum was categorized as benign. Nonhilar blood flow with spotted, peripheral, or mixed types of vascularization was categorized as malignant. RTE was performed for the targeted node using the same probe. Manual light compression and decompression of the target LN by the transducer were performed to achieve an optimal image. Patients were asked to hold their breath and avoid swallowing during the examination to minimize motion of LNs. The elastographic box contained the LN and adjacent sternocleidomastoid muscle for each patient. Elastograms obtained with quality factor score ≥60 were included in the study. Color map settings with red coded as hard area and blue coded as soft area were used. The images were displayed on split screen mode with US gray-scale images on the left and elastogram images on the right. The best-fit B-mode US elastogram image pairs were selected for analysis. The elasticity patterns were categorized on a 5-point scale described by Alam *et al*.[12] [Table 1]. LNs showing type 1 and 2 elastography patterns were considered benign, while those with types 3, 4, and 5 were categorized as malignant.

For ARFI imaging, the targeted LN on B-mode image was identified using a region of interest, characterized by a fixed box [Figures 1-5]. Three measurements of shear wave velocity (SWV) were performed, and the average was calculated. SWV was expressed in meter/second.

Table 1: Elastographic patterns of lymph nodes

Patterns	Description
1	Absent or very small hard area
2	Small, scattered hard areas, hard area <45% of LN
3	Hard area ≥45% of LN
4	Peripheral hard area and central soft area
5	Hard area occupying entire LN

LN: Lymph node

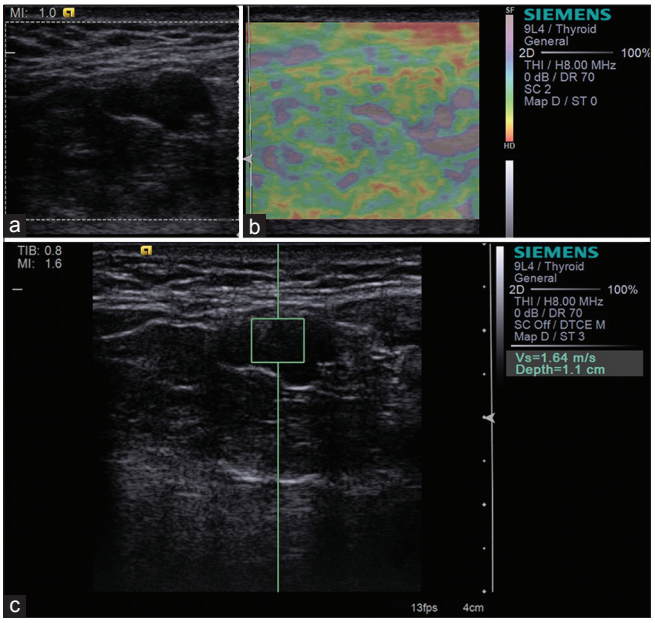


Figure 1: (a) B-mode ultrasound showing oval hypoechoic lymph node with irregular margins and absent hilum, (b) elastography image showing absent or very small hard area corresponding to pattern 1, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 1.64 m/s in the boxed area. Histopathology was suggestive of tuberculous lymphadenitis

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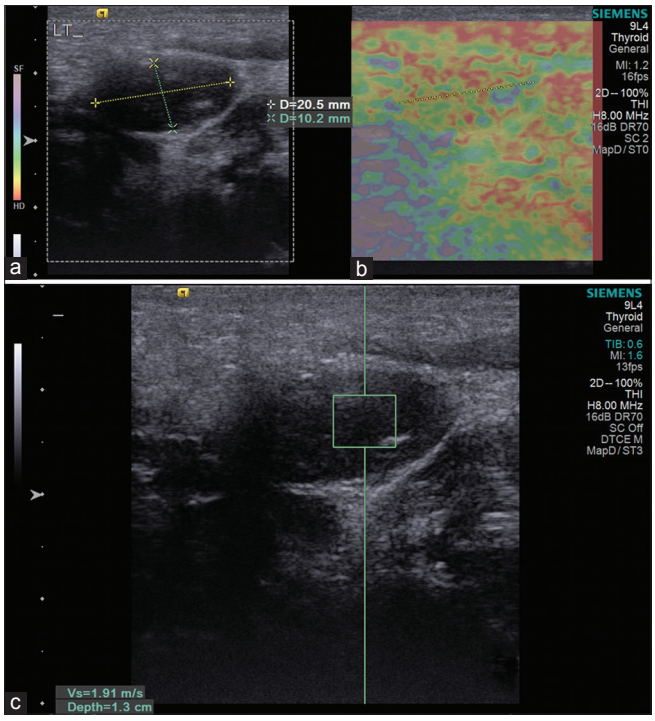


Figure 2: (a) B-mode ultrasound showing oval hypoechoic lymph node, (b) elastography image showing small, scattered hard areas corresponding to pattern 2, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 1.91 m/s in the boxed area. Histopathology was suggestive of reactive lymphadenitis

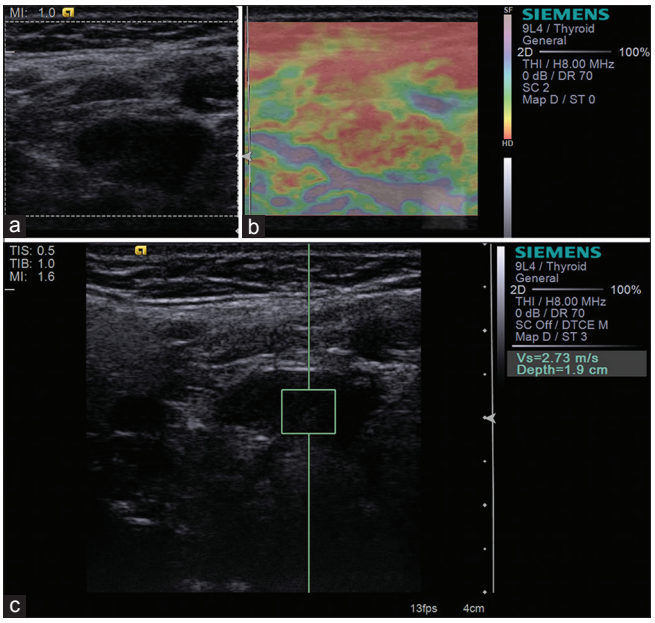


Figure 3: (a) B-mode ultrasound showing oval hypoechoic lymph node with loss of fatty hilum, (b) elastography image showing hard area ≥45% of lymph node corresponding to pattern 3, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 2.73 m/s in the boxed area. Histopathology revealed metastatic lymph node from carcinoma breast

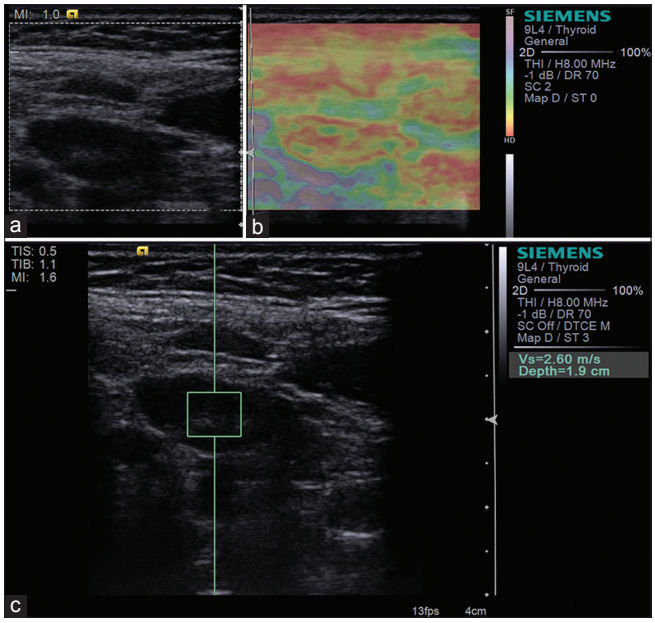


Figure 4: (a) B-mode ultrasound showing oval hypoechoic lymph node with loss of fatty hilum, (b) elastography image showing peripheral hard area and central soft area corresponding to pattern 4, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 2.60 m/s in the boxed area. Histopathology revealed metastatic lymph node from mucoepidermoid carcinoma parotid

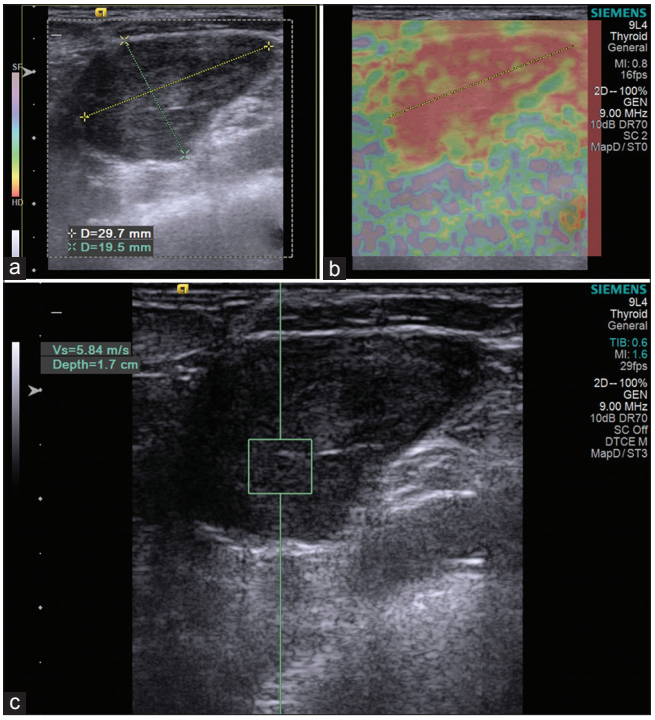


Figure 5: (a) B-mode ultrasound showing hypoechoic lymph node with irregular margins and absent hilum, (b) elastography image showing hard area occupying entire lymph node corresponding to pattern 5, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 5.84 m/s in the boxed area. Histopathology revealed metastatic lymph node from small cell carcinoma lung

Following elastography, all patients underwent surgical excision of the LN after cutaneous marking under US guidance. Histopathologic analysis of the LN was taken as the diagnostic standard of reference.

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Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. Chi-square test was used to know the association between categorical variables. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated with histopathologic analysis as reference standard. For analyzing the combined diagnostic performance of B-mode and Doppler, scores were assigned for five criteria: S/L ratio (ratio ≤0.5, score of 1: >0.5, 2), hilum (present, 1; absent, 2), borders (regular, 1; irregular, 2), echogenicity (homogenous, 1; heterogeneous, 2), and blood flow (hilar, 1; nonhilar, 2). The score for each LN was determined by adding the individual scores. A statistically supported cutoff line was set between scores 6 and 7. LNs with score <6 were categorized as benign, and 7–10 were categorized as malignant. Student *t*-test was performed to evaluate for significant difference in SWV values between benign and malignant LNs. A receiver operating characteristic (ROC) curve analysis was applied to obtain the optimal cutoff value for SWV to differentiate between benign and malignant LNs. Statistical significance was set at $P < 0.05$.

Results

The age of the patients ranged from 12 to 75 years (mean: 45.4 ± 16.9 years). About 52% (n = 26) were female and 48% (n = 24) were male. Of the 50 LNs examined, 33 (66%) were benign and 17 (34%) were malignant on histopathology. Of the 33 benign LNs, 27 had been reported as reactive and six as tuberculous lymphadenitis. Among the 17 malignant nodes, 15 were metastatic and two were due to lymphoma. Metastatic nodes were secondary to carcinoma lung, 4; parotid, 2; thyroid, 1; stomach, 1; breast, 1; poorly differentiated carcinoma from occult primary, 1; and head-and-neck squamous cell carcinoma, 5.

The US characteristics of the LNs and their diagnostic sensitivity, specificity, PPVs and NPV s, and accuracy are shown in Table 2. There were no significant differences between the benign and malignant LNs with respect to S/L ratio and echogenicity ($P = 0.273$ and $P = 0.623$, respectively). Hilum was found to be abnormal in all the 17 malignant LNs studied. Almost 81.8% of benign LNs (n = 27) had regular borders, whereas only 23.5% (n = 4) of malignant LNs had regular borders (P = 0.000). Among all B-mode parameters, borders of LN had the highest diagnostic accuracy (80%), while echogenicity was found to have the least accuracy (48%). On color Doppler evaluation, nearly 72% (n = 24) of benign nodes showed hilar flow compared to 35% (n = 6) of malignant nodes (P = 0.010).

Table 3 shows the correlation of elastographic pattern and histopathologic diagnosis of LNs. Majority of benign LNs (n = 31) had elastography patterns 1 and 2, while majority of malignant LNs (n = 16) had patterns 3–5. Higher elastographic pattern was significantly associated with malignant histopathology (P = 0.000).

The diagnostic performance of combined modalities (B-mode and color Doppler) and elastography is shown in Table 4.

The mean SWV of benign LNs was 1.670 ± 0.367 m/s. Metastatic LNs were significantly stiffer with mean SWV of 2.965 ± 0.826 m/s (P = 0.000). Figure 6 shows the ROC curve for SWVs measured using ARFI imaging in the differentiation of benign and malignant LNs. The best cutoff value for differentiating benign from malignant LNs was estimated to be 2.05 m/s with sensitivity of 88.2%, specificity of 84.8%, and area under the curve equal to 0.949 (95% confidence interval [CI]: 0.70–1.20).

Table 2: Ultrasound characteristics of lymph nodes and their diagnostic performance

US criteria	Benign LNs (n=33)	Malignant LNs (n=17)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
S/L ratio							
≤0.5	15 (45.4)	5 (29.4)	70.5	45.4	40	75	54
>0.5	18 (54.6)	12 (70.6)					
Hilum							
Present	22 (66.7)	0	100	66.6	60	100	78
Absent	11 (33.3)	17 (100)					
Borders							
Regular	27 (81.8)	4 (23.5)	68.4	87.1	76.4	81.8	80
Irregular	6 (18.2)	13 (76.5)					
Echogenicity							
Homogeneous	16 (48.5)	7 (41.2)	41.1	51.5	30.4	62.9	48
Heterogeneous	17 (51.5)	10 (58.8)					
Color Doppler							
Hilar	24 (72.8)	6 (35.3)	64.7	72.7	55	80	70
Nonhilar	9 (27.2)	11 (64.7)					

US: Ultrasound, LNs: Lymph nodes, S/L ratio: Ratio of short-axis diameter to long-axis diameter, PPV: Positive predictive value, NPV: Negative predictive value

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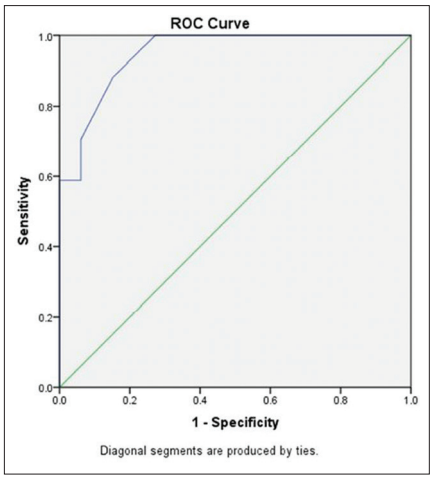


Figure 6: Receiver operating characteristic curve for shear wave velocities measured using acoustic radiation force impulse imaging in the differentiation of benign and malignant lymph nodes. The area under the curve was 0.949

Discussion

In this prospective study from India, we evaluated the performance of RTE in classification of benign and malignant cervical LNs. Our results indicate that RTE has a high specificity and diagnostic accuracy for identification of malignant LNs.

Although B-mode US is the most extensively used method for classification of cervical LNs because of its noninvasiveness and easy accessibility, specific criteria for distinguishing benign and malignant LNs are yet unclear. The association of signs often produces a highly suggestive appearance in most cases. In our study, the accuracy rate in distinguishing benign and malignant LNs was greatest for borders of LN (80%) and least for echogenicity (48%).

There were no significant differences between the benign and malignant LNs with respect to S/L ratio in our study. In contrast, several studies have noted S/L ratio >0.5 or a rounded shape to be associated with malignancy.[12,20] However, shape of LN can be misleading as nodes in nonmalignant conditions such as tuberculosis and Rosai–Dorfman disease have also been described as round.[21]

Our criterion of regular versus irregular borders revealed a diagnostic accuracy of 80%. Some other studies have categorized the LN borders as sharp and unsharp.[22,23] In a study by Khanna *et al*. from India, irregular margins were found in 7% of reactive LNs versus 55% of metastatic and 66% of tubercular nodes.[24] The absence of visible hilum due to replacement or effacement is considered as an important criterion for malignant LNs and has been reported by several authors.[12,13,25] In our study, all the 17 malignant LNs had no visible hilum, thus showing 100% sensitivity and NPV.

We observed a statistically significant difference in the vascular patterns between benign and malignant LNs ($P = 0.010$). Our observations can be comparable to that of Misra *et al*. and several other authors who have noted significant difference in vascularity between benign and malignant LNs.[13,26] In our study, the accuracy of vascularity of LN in predicting malignancy was 70%.

Table 3: Correlation of elastographic pattern and histopathologic diagnosis of lymph nodes

Elastogram pattern	Benign ($n=33$)	Malignant ($n=17$)	Total ($n=50$)
1	5	0	5
2	26	1	27
3	1	2	3
4	1	4	5
5	0	10	10

Table 4: Diagnostic performance of elastography and combined evaluation (B-mode and color Doppler)

Performance measure	Elastography	Combined B-mode and Doppler
Sensitivity (%)	94.1	94.1
Specificity (%)	93.9	81.2
PPV (%)	88.9	72.7
NPV (%)	96.9	96.4
Accuracy (%)	94	86

PPV: Positive predictive value, NPV: Negative predictive value

Even though combined B-mode US and Doppler could be potentially useful in differentiating reactive from malignant LNs, this modality may not have definite diagnostic value because of some overlapping sonographic appearances of benign and malignant nodes. Fine-needle aspiration cytology (FNAC) in the evaluation of cervical LNs has been reported to have a sensitivity of 82% and specificity of 97%.[27] However, FNAC is invasive and does not always provide a conclusive diagnostic result. Core needle biopsy from cervical LNs is also difficult owing to their relatively small size and potential injury to adjacent vascular structures.

RTE being noninvasive, has been suggested to be an adjunctive imaging tool to conventional US and could potentially reduce unnecessary biopsies.[28] Few prospective and retrospective studies concerning strain elastography of cervical LNs have been published in the literature.[29] While some have performed the measurement of elasticity scores (ES), some have measured both ES and strain ratio.

In our study, we used a 5-point scale described by Alam *et al*. to categorize the elasticity patterns. Accordingly, LNs showing types 1 and 2 elastography pattern were considered benign, while those with types 3, 4, and 5 were categorized as malignant. Nearly, 93.9% of benign LNs ($n = 31$) had a score of 1 or 2, while 94.1% of malignant LNs ($n = 16$) had a score of 3–5. The sensitivity, specificity, and accuracy of elastography were 94.1%, 93.9%, and 94%, respectively.

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Alam *et al*. evaluated 85 cervical LNs (53 metastatic and 32 reactive) using 5-point elasticity scale and documented 83% sensitivity, 100% specificity, and 89% accuracy in differentiating metastatic from reactive LNs.[12] Bhatia *et al*. evaluated 74 cervical LNs (37 malignant and 37 benign) using RTE.[17] Dynamic cine loops of elasticity imaging were qualitatively scored on a 4-point elasticity scale by three independent observers. Median ES for benign and malignant nodes was 2 and 3, respectively. A cutoff ES score of ≥2 achieved only 62.2% sensitivity, 83.8% specificity, and 73% accuracy for malignancy. Fair to good interobserver agreement was noted in their study.

Higher accuracy rates observed in our study can be attributed to the fact that there were very few cases of lymphoma ($n = 2$) and tuberculous adenitis ($n = 6$) in our study. Lymphomas have been reported to show low stiffness from an elastographic point of view because of the large size and homogeneous structure of LNs which can produce false-negative results for malignancy.[30] Granulomatous lymphadenitis associated with tuberculosis may demonstrate calcification, scarring, or necrosis and therefore show areas of greater stiffness (false positive).[14]

Ying *et al*. performed a meta-analysis to evaluate the performance of RTE for diagnosis of malignant LNs.[29] Nine studies including 835 LNs were analyzed. The pooled sensitivity and specificity for ES in diagnosing malignant LNs were 74% (95% CI: 66%–81%) and 90% (95% CI: 82%–94%), respectively.

In the present study, we were able to obtain greater specificity, PPV, and accuracy with RTE than conventional US and Doppler for detecting malignant LNs from diverse primary sites. Hence, RTE with its fairly high specificity and diagnostic accuracy can reduce unnecessary invasive procedures for diagnosis of malignant LNs. However, it cannot replace the conventional B-mode US and Doppler because it is dependent on the machine as well as operator's expertise since excessive compression can cause nonaxial displacement and change the tissue stiffness. This in turn can affect the accuracy of the software's correlation algorithms. Furthermore, elastography is not useful for calcified LNs and those with cystic degeneration.

Relatively, few clinical studies have evaluated ARFI in the assessment of malignant cervical LNs.

In our study, ARFI imaging revealed higher SWV for malignant LNs than benign ones. The cutoff value of 2.05 m/s had sensitivity of 88.2% and specificity of 84.8% in identifying malignant LNs. Our results are comparable to that of Fujiwara *et al*. who evaluated SWVs of 42 cervical LNs using ARFI.[31] Of the numerous cutoff values tested, a SWV cutoff value of 1.9 m/s best distinguished between reactive and malignant LNs. Their criterion of SWV >1.9 m/s had 95.0% sensitivity and 81.8% specificity. According to a recent meta-analysis of nine ARFI imaging studies that included 1084 LNs, the pooled sensitivity and specificity values for detecting malignancies were 87% (95% CI: 83%–91%) and 88% (95% CI: 82%–92%), respectively.[19]

Our study has few limitations. The sample size was small, and only enlarged LNs were included. Strain elastography using free hand compression is dependent on the compression technique, and excessive compression can alter tissue stiffness. A single expert radiologist performed all the elastograms, and no interobserver variability was studied. However, studies have reported fair to almost perfect interobserver agreement in strain elastography.[6]

Head-and-neck cancers account for nearly one-third of the cancer burden in India, and it is a well-known fact that status of cervical LN is the single most important prognostic factor in squamous cell carcinoma of the head and neck.[32] To the best of our knowledge, this is the first study from India to document the diagnostic performance of elastography in evaluation of cervical lymphadenopathy, and it has shown encouraging results.

Conclusion

Elastography has high diagnostic accuracy in differentiating benign and malignant cervical LNs and can be an effective noninvasive adjunctive tool in the evaluation of cervical lymphadenopathy. It is potentially useful in selecting the LN with high probability of malignancy, on which FNAC/biopsy can be performed, thereby reducing sampling errors. ARFI imaging can be a useful technique for differentiating benign from malignant cervical LNs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Chong V. Cervical lymphadenopathy: What radiologists need to know. Cancer Imaging 2004;4:116-20.
2. Na DG, Lim HK, Byun HS, Kim HD, Ko YH, Baek JH. Differential diagnosis of cervical lymphadenopathy: Usefulness of color Doppler sonography. AJR Am J Roentgenol 1997;168:1311-6.
3. Ghafoori M, Azizian A, Pourrajabi Z, Vaseghi H. Sonographic evaluation of cervical lymphadenopathy; comparison of metastatic and reactive lymph nodes in patients with head and neck squamous cell carcinoma using gray scale and Doppler techniques. Iran J Radiol 2015;12:e11044.
4. Leboulleux S, Girard E, Rose M, Travagli JP, Sabbah N, Caillou B, *et al.* Ultrasound criteria of malignancy for cervical lymph nodes in patients followed up for differentiated thyroid cancer. J Clin Endocrinol Metab 2007;92:3590-4.
5. Gupta A, Rahman K, Shahid M, Kumar A, Qaseem SM, Hassan SA, *et al.* Sonographic assessment of cervical lymphadenopathy: Role of high-resolution and color Doppler imaging. Head Neck 2011;33:297-302.
6. Choi YJ, Lee JH, Baek JH. Ultrasound elastography for evaluation of cervical lymph nodes. Ultrasonography 2015;34:157-64.
7. Thomas A, Fischer T, Frey H, Ohlinger R, Grunwald S, Blohmer JU, *et al.* Real-time elastography – An advanced method of ultrasound: First results in 108 patients with breast lesions. Ultrasound Obstet Gynecol 2006;28:335-40.

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8. Taylor LS, Rubens DJ, Porter BC, Wu Z, Baggs RB, di Sant'Agnese PA, *et al.* Prostate cancer: Three-dimensional sonoelastography for *in vitro* detection. Radiology 2005;237:981-5.
9. Friedrich-Rust M, Ong MF, Herrmann E, Dries V, Samaras P, Zeuzem S, *et al.* Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. AJR Am J Roentgenol 2007;188:758-64.
10. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al.* US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
11. Thomas A, Kümmel S, Gemeinhardt O, Fischer T. Real-time sonoelastography of the cervix: Tissue elasticity of the normal and abnormal cervix. Acad Radiol 2007;14:193-200.
12. Alam F, Naito K, Horiguchi J, Fukuda H, Tachikake T, Ito K. Accuracy of sonographic elastography in the differential diagnosis of enlarged cervical lymph nodes: Comparison with conventional B-mode sonography. AJR Am J Roentgenol 2008;191:604-10.
13. Turgut E, Celenk C, Tanrivermis Sayit A, Bekci T, Gunbey HP, Aslan K. Efficiency of B-mode ultrasound and strain elastography in differentiating between benign and malignant cervical lymph nodes. Ultrasound Q 2017;33:201-7.
14. Teng DK, Wang H, Lin YQ, Sui GQ, Guo F, Sun LN. Value of ultrasound elastography in assessment of enlarged cervical lymph nodes. Asian Pac J Cancer Prev 2012;13:2081-5.
15. Lo WC, Cheng PW, Wang CT, Liao LJ. Real-time ultrasound elastography: An assessment of enlarged cervical lymph nodes. Eur Radiol 2013;23:2351-7.
16. Acu L, Oktar SÖ, Acu R, Yücel C, Cebeci S. Value of ultrasound elastography in the differential diagnosis of cervical lymph nodes: A comparative study with B-mode and color Doppler sonography. J Ultrasound Med 2016;35:2491-9.
17. Bhatia KS, Cho CC, Yuen YH, Rasalkar DD, King AD, Ahuja AT. Real-time qualitative ultrasound elastography of cervical lymph nodes in routine clinical practice: Interobserver agreement and correlation with malignancy. Ultrasound Med Biol 2010;36:1990-7.
18. Suh CH, Choi YJ, Baek JH, Lee JH. The diagnostic performance of shear wave elastography for malignant cervical lymph nodes: A systematic review and meta-analysis. Eur Radiol 2017;27:222-30.
19. Zhang P, Zhang L, Zheng S, Yu C, Xie M, Lv Q. Acoustic radiation force impulse imaging for the differentiation of benign and malignant lymph nodes: A systematic review and meta-analysis. PLoS One 2016;11:e0166716.
20. Ahuja AT, Ying M, Ho SY, Antonio G, Lee YP, King AD, *et al.* Ultrasound of malignant cervical lymph nodes. Cancer Imaging 2008;8:48-56.
21. Ahuja AT, Ying M. Sonographic evaluation of cervical lymph nodes. AJR Am J Roentgenol 2005;184:1691-9.
22. Ying M, Ahuja A, Brook F. Sonographic appearances of cervical lymph nodes: Variations by age and sex. J Clin Ultrasound 2002;30:1-1.
23. Ahuja A, Ying M. An overview of neck node sonography. Invest Radiol 2002;37:333-42.
24. Khanna R, Sharma AD, Khanna S, Kumar M, Shukla RC. Usefulness of ultrasonography for the evaluation of cervical lymphadenopathy. World J Surg Oncol 2011;9:29.
25. Lyshchik A, Higashi T, Asato R, Tanaka S, Ito J, Hiraoka M, *et al.* Cervical lymph node metastases: Diagnosis at sonoelastography – Initial experience. Radiology 2007;243:258-67.
26. Misra D, Panjwani S, Rai S, Misra A, Prabhat M, Gupta P, *et al.* Diagnostic efficacy of color Doppler ultrasound in evaluation of cervical lymphadenopathy. Dent Res J (Isfahan) 2016;13:217-24.
27. Haque MA, Talukder SI. Evaluation of fine needle aspiration cytology (FNAC) of lymph node in mymensingh. Mymensingh Med J 2003;12:33-5.
28. Tan SM, Teh HS, Mancer JF, Poh WT. Improving B mode ultrasound evaluation of breast lesions with real-time ultrasound elastography – A clinical approach. Breast 2008;17:252-7.
29. Ying L, Hou Y, Zheng HM, Lin X, Xie ZL, Hu YP, *et al.* Real-time elastography for the differentiation of benign and malignant superficial lymph nodes: A meta-analysis. Eur J Radiol 2012;81:2576-84.
30. Lenghel LM, Bolboaca SD, Botar-Jid C, Baciut G, Dudea SM. The value of a new score for sonoelastographic differentiation between benign and malignant cervical lymph nodes. Med Ultrason 2012;14:271-7.
31. Fujiwara T, Tomokuni J, Iwanaga K, Ooba S, Haji T. Acoustic radiation force impulse imaging for reactive and malignant/metastatic cervical lymph nodes. Ultrasound Med Biol 2013;39:1178-83.
32. Shah SB, Sharma S, D'Cruz AK. Head and neck oncology: The Indian scenario. South Asian J Cancer 2016;5:104-5.

Original Article

Utility of Ultrasound Strain Elastography to Differentiate Benign from Malignant Lesions of the Breast

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Abstract

Background: The purpose of this study was to determine the utility and diagnostic performance of strain elastography (SE) in differentiating benign from malignant lesions of the breast. **Materials and Methods:** In this prospective study, 50 palpable breast masses in 50 patients were examined by mammography, B-mode ultrasound (US) and SE. Lesions were categorized using Breast Imaging Reporting and Data System (BIRADS) scoring based on mammographic and sonographic features. Elasticity scores were assessed on a five-point scale based on the distribution of strain, and the lesion size on SE imaging and B-mode (elasticity imaging/B mode [EI/B] ratio) was compared. Findings were correlated with the BIRADS assessment and diagnostic performance of sonoelastography was evaluated taking histopathology as reference standard. **Results:** Histopathology revealed 29 (58%) malignant and 21 (42%) benign lesions. Infiltrative ductal carcinoma and fibroadenoma were the most common malignant and benign lesions, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of SE was 100%, 76.1%, 85.2%, 100%, and 90%, respectively. Higher elasticity score was significantly associated with malignant histopathology ($P < 0.00001$). The mean EI/B ratio for malignant lesions was 1.36 ± 0.24 while that of benign lesions was 1.03 ± 0.30 ($P = 0.000$). **Conclusion:** Real-time SE of the breast, with its superior sensitivity and specificity, could provide improved characterization of benign and malignant breast masses compared with mammography and conventional US. Due to greater diagnostic accuracy, SE can be an effective adjunctive tool to B-mode US in predicting malignancy of breast, as well as in reducing the need for biopsies in benign breast lesions.

Keywords: Breast, elasticity imaging technique, sonoelastography

Introduction

Breast cancer is the most common malignancy in women all over the world and continues to remain a leading cause of cancer-related death in females. The incidence of breast cancer has been increasing steadily in both developing and developed countries.[1] Increased life expectancy, urbanization, changes in lifestyle, and delay in diagnosis contribute to the increasing incidence as well as mortality in the low- and middle-income countries. Early detection is critical to improve the breast cancer outcome and survival.

Current imaging techniques available for screening of breast cancer include mammography, ultrasonography, and magnetic resonance imaging (MRI). Although mammography is the current standard breast screening technique, its sensitivity is reduced in high-density breast tissue, in smaller tumors, and in young women.[2] Breast ultrasonography is widely used in the evaluation and categorization of breast lesions, owing to its high sensitivity. However, inability to image deeper lesions, limited ability to distinguish isoechoic lesions from surrounding fat, low specificity and increased biopsy rates resulting in patient discomfort and anxiety are some potential limitations of breast ultrasound (US).[3] Even though MRI is useful for screening of subjects with high breast cancer risk, its use for routine screening is limited because of high cost, time consumption and lack of widespread availability as well as need for experienced radiologists.

US elastography (USE) is a noninvasive imaging modality complimentary to US that detects tissue elasticity by the

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application of an external stress. As malignant tissues are firmer than benign ones, the estimate of tissue stiffness by USE can aid in the differentiation between benign and malignant lesions. Recent studies have reported the utility of USE in the characterization of lesions in various tissues, such as thyroid, liver, prostate, lymph nodes, and breast.[4-8] It has been suggested that current elastography systems can evaluate histological information by depicting the distribution of tissue stiffness or strain which might have the potential to predict the therapeutic effect of treatment with anticancer agents.[9]

Published literature on the usefulness of strain elastography (SE) in the evaluation of breast lesions and its correlation with histopathology is limited, especially from India. The purpose of this study was to determine the utility and diagnostic performance of SE in differentiating benign from malignant lesions of the breast with histopathology as the reference standard.

Materials and Methods

This prospective study was conducted at a tertiary care center in South India from December 2014 to December 2017. The institutional review board approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants. Fifty consecutive female patients aged 40 years and above referred to the department of radiology for the evaluation of palpable breast lump and sonography-guided biopsy were included in the study. Exclusion criteria consisted of those with cystic lesions of breast, history of prior biopsy, postsurgical status, prosthetic breast implants, and subcentimeter lesions. All patients underwent mammography, B-mode US, and SE followed by US-guided biopsy in the same session. All examinations and biopsy were performed by a single expert radiologist with over 10 years of experience in sonography.

Mammography was performed with SIEMENS MAMMOMAT 3000. Mediolateral oblique and craniocaudal views were taken in all patients, and in addition, coned-down or magnification views were used when necessary.

Ultrasonography of the breast was performed with SIEMENS ACUSON S3000 using high-frequency probe 18 L6. Sonographic features studied included size of the lesion, shape, margins, orientation, internal echogenicity, vascularity, calcification, and posterior acoustic shadowing. The lesions were categorized using the Breast Imaging Reporting and Data System (BIRADS) scoring based on mammographic and sonographic features as the following categories – category 2: benign; category 3: probably benign; category 4: suspicious for malignancy; and category 5: highly suggestive of malignancy.[10] BIRADS category 1 lesion was excluded from the study. Categories 2 and 3 were considered negative for malignancy and categories 4 and 5 were labeled as malignant.

SE of the breast was performed using the same probe in the supine position. The probe was placed over the region such that the lesion was at the center of the image. Holding the scan plane perpendicular to the skin surface, a slight compression–decompression movement was applied. The appropriate pressure and frequency of compression/decompression were optimized with the help of real-time display of quality index. Elastograms with quality index equal to or greater than 60 were included. The region of interest (ROI) included the lesion with subcutaneous layers and pectoralis muscle. It was ensured that the target tissue occupied no more than 25% of the total area of ROI. At least 5 mm thickness of normal adjacent tissue was included to assess the lesion stiffness in relation with average elasticity of surrounding tissue. Color map settings with blue coded as hard area and green coded as soft area were used. The images were displayed on a split screen mode with B-mode images on the left and elastographic images on the right. The best fit B-mode elastographic image pairs were selected for analysis. [Figures 1-4] The longest dimension of the lesion on elastographic image was compared with that of the corresponding B-mode image, and elasticity imaging/B mode ratio (EI/B ratio) was calculated.

The elasticity patterns were scored using the TSUKUBA scoring system [Table 1] described by Itoh *et al*.[11] The elasticity scores 1, 2, and 3 were considered benign, while scores 4 and 5 were labeled as malignant.

Biopsy of the lesion was performed under US guidance using an 18G tru-cut needle. Histopathology results were taken as the reference standard.

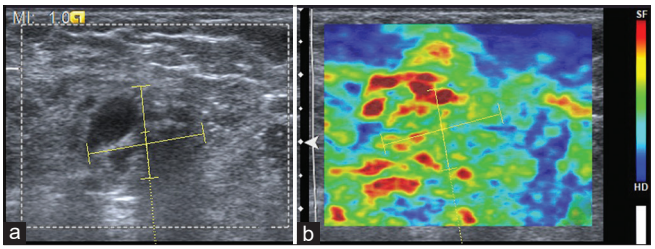


Figure 1: (a) B-mode ultrasound showing ill-defined hypoechoic lesion which was categorized as Breast Imaging Reporting and Data System category 4. (b) With strain elastography, entire hypoechoic lesion is deformable and evenly shaded green as surrounding breast tissue, corresponding to elasticity score 1. Histopathology was suggestive of nonspecific mastitis

Table 1: TSUKUBA elasticity score

Elasticity score	Description
1	Even strain in the entire lesion
2	Parts of the hypoechoic lesion does not show strain
3	Periphery of the hypoechoic lesion shows strain, no strain at the center
4	Entire lesion shows no strain
5	Entire lesion and surrounding area shows no strain

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 24. Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. Chi-square test was used to know the association between categorical variables. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated using 2 × 2 tables with histopathological analysis as the reference standard. Statistical significance was set at $P < 0.05$.

Results

Patients were aged between 40 and 72 years (mean: 48.96 ± 7.52 years). The mean age of the patients with benign and malignant lesions was 45.4 ± 5.47 and 51.5 ± 7.84 years, respectively ($P = 0.001$). On histopathological evaluation, 29 (58%) were found to be malignant and 21 (42%) were benign. Infiltrative ductal carcinoma and fibroadenoma were the most common malignant and benign lesions, respectively. Of the 29 malignant lesions, 17 (59%) were infiltrative ductal carcinoma, 11 (38%) were invasive lobular carcinoma and 1 (3%) was mucinous carcinoma. Among the 21 benign lesions, 7 (33%) were fibroadenoma, 4 (19%) were atypical ductal hyperplasia, 3 (15%) were breast abscess, 2 (9%) were sclerosing adenosis, and 2 (9%) were nonspecific mastitis. Granulomatous mastitis, complex cyst, and benign phyllodes tumor accounted for 1 (5%) each.

Mammography of 14 patients (28%) did not show any demonstrable lesion due to dense breast with predominantly fibroglandular tissue. 41% of the malignant lesions (12/29) had spiculated appearance on mammography, and microcalcification was encountered in 34% (10/29). 14% (4/29) showed architectural distortion.

Based on the BIRADS assessment, three were assigned BIRADS category 3, 18 were assigned category 4, and 29 were assigned category 5. The sensitivity, specificity, PPV, NPV, and accuracy of BIRADS assessment were 96.5%, 9.5%, 59.5%, 66.6%, and 60%, respectively, with histopathology as the reference standard.

Qualitative SE using elasticity scores had a sensitivity of 100% and specificity of 76.1% in detecting malignancy. The PPV and NPV were 85.2% and 100%, respectively, with diagnostic accuracy of 90%. Higher elasticity score was significantly associated with malignant histopathology ($P < 0.00001$). The mean EI/B ratio for malignant lesions was 1.36 ± 0.24 while that of benign lesions was 1.03 ± 0.30 ($P = 0.000$). Table 2 shows the correlation between histopathology, BIRADS assessment, and elasticity scoring among the lesions.

Discussion

In this prospective study, we evaluated the diagnostic performance of SE in predicting malignancy in lesions of breast. Qualitative SE with elasticity scoring was found to have a superior sensitivity, specificity, and diagnostic accuracy compared to conventional BIRADS assessment.

USE estimates the stiffness of tissues based on the degree of deformation of a tissue in response to an externally applied force. SE is a qualitative technique that uses either manual compression or an acoustic radiation force impulse push pulse to deform tissues.

Several authors have reported the additional value of SE to improve the specificity of conventional US in the differentiation

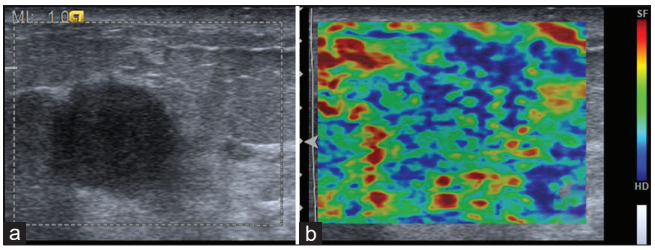


Figure 2: (a) B-mode ultrasound showing poorly marginated round hypoechoic lesion categorized as Breast Imaging Reporting and Data System category 3. (b) Strain elastographic image showing deformability of the peripheral portion of the lesion with stiff tissue in the center corresponding to elasticity score 3. Histopathology was suggestive of fibroadenoma

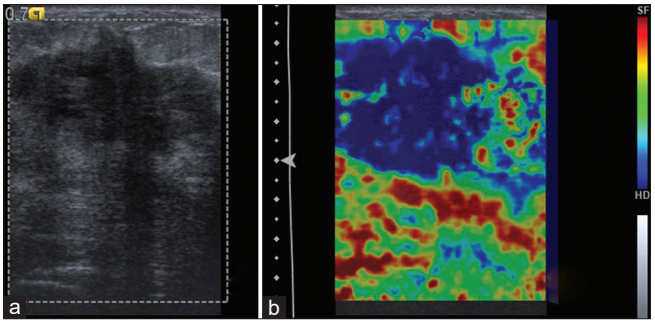


Figure 3: (a) B-mode ultrasound showing ill-defined hypoechoic lesion categorized as Breast Imaging Reporting and Data System 4. (b) With strain elastography, entire lesion is stiff, corresponding to elasticity score 4. Histopathology was suggestive of calcified fibroadenoma

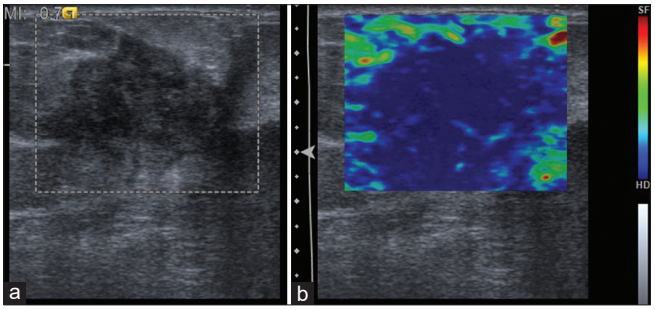


Figure 4: (a) B-mode ultrasound showing ill-defined hypoechoic lesion categorized as Breast Imaging Reporting and Data System 5. (b) With strain elastography, entire lesion and surrounding area are stiff, corresponding to elasticity score 5. Histopathology was suggestive of infiltrative ductal carcinoma

Table 2: Correlation between histopathology, Breast Imaging Reporting and Data System assessment and elasticity scoring among the lesions

Histopathology	BIRADS category	Elasticity score					Total (n=50)
		1	2	3	4	5	
Benign	3			2			2
	4	1	1	8	1	2	13
	5	1	1	2		2	6
Malignant	3					1	1
	4					5	5
	5					23	23

BIRADS: Breast Imaging Reporting and Data System

of benign and malignant breast masses and decrease the need for biopsies. While few have used EI/B ratio,[12] others have used five-point color scale and strain ratio[13,14] in the interpretation of SE images of the breast. However, the superior diagnostic performance of SE over B-mode US is controversial and factors such as operator experience, use of varied cutoffs, tumor size, and histologic subtype have been shown to influence the outcome.[15,16] Elasticity scoring had a sensitivity of 100% and specificity of 76.1%% in detecting malignancy among breast lesions in our study. The mean size of benign and malignant lesions was 17.06 ± 3.7 and 16.77 ± 3.1 mm, respectively. No significant difference in size was noted between benign and malignant lesions (P = 0.767). The superior diagnostic performance observed in our study can be attributed to the fact that most of the lesions (40/50) were smaller than 2 cm in size. Giuseppetti *et al*. have reported a better diagnostic performance of elastosonography in lesions smaller than 2 cm.[15] This is because a larger lesion has more chances of having hemorrhage or necrosis which might alter the tissue stiffness inside the lesion. Parajuly *et al*., based on their experience, have observed that lesions more than 5 cm should not be recruited in a study.[17]

Our results are comparable to that of Schaefer *et al*. and Atabey *et al*., who evaluated the performance of SE in breast lesions using a five-point scoring system.[18,19] With a cutoff between elasticity scores 3 and 4, elastography provided higher sensitivity and NPV. The authors have concluded that elastography can be an effective adjunct imaging modality in differential diagnosis of breast lesions, before the decision to biopsy a lesion in certain cases.[19]

In our study, 34 lesions were categorized as malignant on SE. Among these, 85% (29/34) were confirmed as malignancy on histopathology. Five benign lesions which had been categorized as malignant on SE were fibroadenoma (1), atypical ductal hyperplasia (3), and sclerosing adenosis (1) associated with calcification, which might have increased the stiffness of lesion. The false-positive rate was 23.8% in our study. In contrast, Zhi *et al*. have noted a false-positivity rate of 4.3% with SE in their study. Calcification and organized hemorrhage in benign lesions were responsible for false-positive diagnoses on SE.[3]

All the lesions with elasticity score less than or equal to 3 turned out to be negative for malignancy in our study. There were 13 benign lesions that presented as BIRADS category 4 necessitating biopsy. With a cutoff between 3 and 4 on SE, 10 of these 13 (76.9%) were true negatives on SE. Our observations are in line with recent literature, suggesting that SE can be useful in downgrading BIRADS category 4 lesions and reduce benign biopsy rates.[20]

Seven out of 18 BIRADS category 4 lesions and 25/29 BIRADS category 5 lesions had elasticity score 5. Among these, 87.8% turned out to be malignant, suggesting that elasticity scoring can be a useful predictor of malignancy. Satake *et al*. evaluated the ability of sonoelastography in predicting malignancy in breast masses classified as BIRADS category 4 or 5. Elasticity score was found to be a significant predictor of malignancy in BIRADS 4 masses (P = 0.002). However, elasticity score was not a significant predictor of malignancy in BIRADS category 5 lesions.[21]

A recent meta-analysis by Gong *et al*. on real-time elastography (RTE) in the differentiation of benign and malignant lesions of the breast that included 22 studies evaluating 4713 breast nodules reported an overall mean sensitivity and specificity of 0.834 and 0.842, respectively for elasticity scoring.[22] The authors have stated that RTE has high sensitivity and specificity in differentiating benign from malignant lesions and therefore can potentially reduce unnecessary breast biopsies. The mean EI/B ratio for malignant lesions was 1.36 ± 0.24 while that of benign lesions was 1.03 ± 0.30 (P = 0.000, P < 0.00001) in our study. Several studies have shown that tumors appear larger on elastography than on B-mode US.[23,24] A cutoff value of less than 1 for benign lesions and greater than or equal to 1 for malignant lesions has been shown to have high sensitivity and specificity for the characterization of lesions.[12] The variable dimension in elastography has been attributed to the desmoplastic reaction occurring in many cancers of the breast. Malignant lesions have been reported to appear significantly larger on strain imaging presumably because strain is uniquely sensitive to desmoplasia surrounding the lesion.[25]

Our study has few limitations. The sample size was limited and a single experienced radiologist performed all the examinations. SE is an operator-dependent technique and steady compression is needed for optimal images. However, in our study only images with optimal compression as displayed by the quality index were included. Intra- or inter-observer variability was not taken into account.

To the best of our knowledge, this is the first study on the utility of SE among the Indian population. The cancer projection data from India show that the number of breast cancer cases will become almost double by 2020.[26] Owing to the fact that the density of Indian breasts is different from that of the Western population[27] and density of breast parenchyma

can significantly influence the diagnostic performance of elastography, there is a need for more studies from this part of the world.

Conclusion

In this initial study from India, we conclude that real-time SE of the breast, with its superior sensitivity and specificity, could provide improved characterization of benign and malignant breast masses compared with mammography and conventional US. Due to greater diagnostic accuracy, SE can be an effective adjunctive tool to B-mode US in predicting malignancy of breast as well as decreasing unnecessary biopsies in clinical practice.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lin L, Yan L, Liu Y, Yuan F, Li H, Ni J. Incidence and death in 29 cancer groups in 2017 and trend analysis from 1990 to 2017 from the Global Burden of Disease Study. J Hematol Oncol 2019;12:96.
2. Zhao H, Zou L, Geng X, Zheng S. Limitations of mammography in the diagnosis of breast diseases compared with ultrasonography: A single-center retrospective analysis of 274 cases. Eur J Med Res 2015;20:49.
3. Zhi H, Ou B, Luo BM, Feng X, Wen YL, Yang HY. Comparison of ultrasound elastography, mammography, and sonography in the diagnosis of solid breast lesions. J Ultrasound Med 2007;26:807-15.
4. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al*. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
5. Friedrich-Rust M, Ong MF, Herrmann E, Dries V, Samaras P, Zeuzem S, *et al*. Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. AJR Am J Roentgenol 2007;188:758-64.
6. Kamoi K, Okihara K, Ochiai A, Ukimura O, Mizutani Y, Kawauchi A, *et al*. The utility of transrectal real-time elastography in the diagnosis of prostate cancer. Ultrasound Med Biol 2008;34:1025-32.
7. Turgut E, Celenk C, Tanrivermis Sayit A, Bekci T, Gunbey HP, Aslan K. Efficiency of B-mode ultrasound and strain elastography in differentiating between benign and malignant cervical lymph nodes. Ultrasound Q 2017;33:201-7.
8. Thomas A, Fischer T, Frey H, Ohlinger R, Grunwald S, Blohmer JU, *et al*. Real-time elastography--an advanced method of ultrasound: First results in 108 patients with breast lesions. Ultrasound Obstet Gynecol 2006;28:335-40.
9. Barr RG, Nakashima K, Amy D, Cosgrove D, Farrokh A, Schafer F, *et al*. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 2: Breast. Ultrasound Med Biol 2015;41:1148-60.
10. Lazarus E, Mainiero MB, Schepps B, Koelliker SL, Livingston LS. BI-RADS lexicon for US and mammography: Interobserver variability and positive predictive value. Radiology 2006;239:385-91.
11. Itoh A, Ueno E, Tohno E, Kamma H, Takahashi H, Shiina T, *et al*. Breast disease: Clinical application of US elastography for diagnosis. Radiology 2006;239:341-50.
12. Barr RG, Destounis S, Lackey LB 2nd, Svensson WE, Balleyguier C, Smith C. Evaluation of breast lesions using sonographic elasticity imaging: A multicenter trial. J Ultrasound Med 2012;31:281-7.
13. Stachs A, Hartmann S, Stubert J, Dieterich M, Martin A, Kundt G, *et al*. Differentiating between malignant and benign breast masses: Factors limiting sonoelastographic strain ratio. Ultraschall Med 2013;34:131-6.
14. Zhao QL, Ruan LT, Zhang H, Yin YM, Duan SX. Diagnosis of solid breast lesions by elastography 5-point score and strain ratio method. Eur J Radiol 2012;81:3245-9.
15. Giuseppetti GM, Martegani A, Di Cioccio B, Baldassarre S. Elastosonography in the diagnosis of the nodular breast lesions: Preliminary report. Radiol Med 2005;110:69-76.
16. Chang JM, Won JK, Lee KB, Park IA, Yi A, Moon WK. Comparison of shear-wave and strain ultrasound elastography in the differentiation of benign and malignant breast lesions. AJR Am J Roentgenol 2013;201:W347-56.
17. Parajuly SS, Lan PY, Yun MB, Gang YZ, Hua Z. Diagnostic potential of strain ratio measurement and a 5 point scoring method for detection of breast cancer: Chinese experience. Asian Pac J Cancer Prev 2012;13:1447-52.
18. Schaefer FK, Heer I, Schaefer PJ, Mundhenke C, Osterholz S, Order BM, *et al*. Breast ultrasound elastography – Results of 193 breast lesions in a prospective study with histopathologic correlation. Eur J Radiol 2011;77:450-6.
19. Atabey AO, Arıbal E, Ergelen R, Kaya H. Value of strain elastography ultrasound in differentiation of breast masses and histopathologic correlation. J Breast Health 2014;10:234-8.
20. Cho N, Moon WK, Park JS, Cha JH, Jang M, Seong MH. Nonpalpable breast masses: Evaluation by US elastography. Korean J Radiol 2008;9:111-8.
21. Satake H, Nishio A, Ikeda M, Ishigaki S, Shimamoto K, Hirano M, *et al*. Predictive value for malignancy of suspicious breast masses of BI-RADS categories 4 and 5 using ultrasound elastography and MR diffusion-weighted imaging. AJR Am J Roentgenol 2011;196:202-9.
22. Gong X, Xu Q, Xu Z, Xiong P, Yan W, Chen Y. Real-time elastography for the differentiation of benign and malignant breast lesions: A meta-analysis. Breast Cancer Res Treat 2011;130:11-8.
23. Isermann R, Grunwald S, Hatzung G, Könsgen-Mustea D, Behrndt PO, Geaid AA, *et al*. Breast lesion sizing by B-mode imaging and sonoelastography in comparison to histopathological sizing – A prospective study. Ultraschall Med 2011;32 Suppl 1:S21-6.
24. Stachs A, Pandjaitan A, Martin A, Stubert J, Hartmann S, Gerber B, *et al*. Accuracy of tumor sizing in breast cancer: A comparison of strain elastography, 3-D ultrasound and conventional B-mode ultrasound with and without compound imaging. Ultrasound Med Biol 2016;42:2758-65.
25. Insana MF, Pellot-Barakat C, Sridhar M, Lindfors KK. Viscoelastic imaging of breast tumor microenvironment with ultrasound. J Mammary Gland Biol Neoplasia 2004;9:393-404.
26. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol 2017;13:289-95.
27. Singh T, Khandelwal N, Singla V, Kumar D, Gupta M, Singh G, *et al*. Breast density in screening mammography in Indian population – Is it different from western population? Breast J 2018;24:365-8.

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Analysis of different pathologies presenting as acute abdomen and performance of MDCT in making the diagnosis with clinical, intra-operative and histopathology correlation

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Abstract
Background: Acute abdomen is one of the most common emergencies presenting in the emergency room which requires immediate attention. Making an accurate diagnosis is of paramount importance in managing these patients, since most of these patients frequently require a surgical intervention. The divergence of patient population and the underlying pathology in acute abdomen calls for high quality imaging studies to make quick and precise diagnosis. Multi-detector computed tomography is an ideal tool in this regard.
Materials and Methods: It is a prospective study conducted on 73 patients who presented with acute abdomen and subsequently underwent multi-detector computed tomography to ascertain the underlying pathology. The radiological findings in computed tomography were correlated with clinical, intra-operative and histopathological findings whenever available.
Results: In our study the performance of multi-detector computed tomography, when compared with diagnosis made based on intra-operative findings, post-operative histopathology and clinical findings showed sensitivity of 97% and specificity of 75%. Overall Positive Predictive value of 98.5% and negative predictive value of 60% and accuracy of 96%.
Conclusion: Since most of the patients presenting with acute abdomen have non-specific and overlapping clinical findings, making an accurate clinical diagnosis is challenging. In this scenario MDCT comes as a handy tool with high accuracy and good sensitivity and specificity. The results obtained in our study are comparable with other studies conducted worldwide.

Keywords: Acute abdomen, MDCT performance, acute abdominal pain

Introduction
Acute abdomen is a clinical entity in which patients present with sudden onset of intense abdominal pain necessitating emergency medical/surgical management [1]. Majority of these patients present to the emergency department. The underlying pathology in acute abdomen could be anything ranging from acute inflammatory conditions involving the appendix, gallbladder, pancreas to non-specific pain usually associated with mesenteric adenitis, especially in children. In a given patient there are numerous differential diagnoses and making a single specific diagnosis is seldom seen and it is very difficult, given the overlap of presenting symptoms and clinical examination findings in various pathologies. Further evaluation with laboratory blood tests and radiological investigations is part of management of these patients.

Patients presenting with acute abdomen are in severe pain and very sick. Hence it is essential to make a prompt and accurate diagnosis in these patients [2]. Most of these patients are initially evaluated with abdominal radiograph and ultrasound. The role of these basic radiological investigations is limited by various factors. Major limiting factors are two dimensional nature of radiographs. In ultrasound limiting factors are inability of the patient to cooperate for the study due to severe pain, thick body habitus of the patient, excessive bowel gas and lack of adequately filled urinary bladder. However radiograph has fairly good specificity in diagnosing pneumoperitoneum. It gives useful information in only in less than 50% of patients [2].

Ultrasound has its own advantages. It is cheap, easily available, no need to administer contrast and it is free of ionizing radiation, hence better suited for children and pregnant women. Ultrasound is a valuable tool in the assessment of pathology related to gallbladder and acute ureteric colic, especially when the obstruction is at the vesico-ureteric junction, which is by far the most common site.

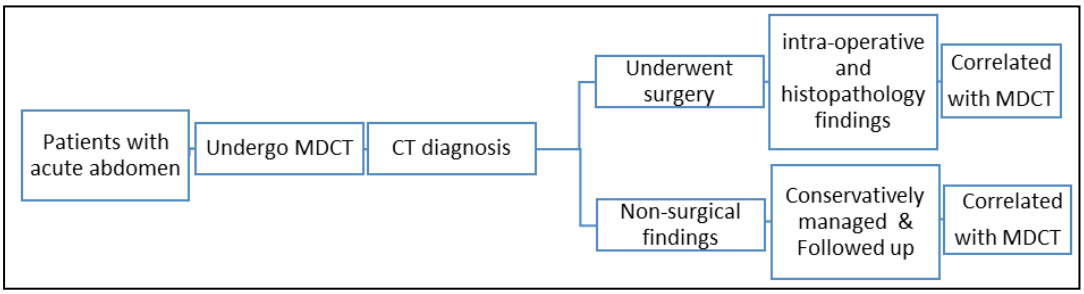
Though these basic radiological investigations do indeed provide valuable information, in a good number of patients computed tomography (CT) examination is essential. Multi-Detector CT (MDCT) is a widely accepted primary investigation of choice in these patients [3, 4, 5]. The scanning is fast, (acquisition time of 2-3 minutes), and yields specific diagnosis. In MDCT, multiple images can be acquired in a single tube rotation. The whole abdomen and pelvis can be scanned within a single breath hold at a slice thickness of less than a millimeter (0.3-1mm). These sub millimeter sections can be used to obtain good quality reconstructed images like multi-planar reconstructions (MPR) maximum intensity projection (MIP) and 3dimentional (3D) reconstruction**.** MDCT is a great tool for comprehensive evaluation of entire abdomen. There is excellent visualization of the solid organs, hallow viscera, vasculature, peritoneum and retroperitoneal structures along with lung bases.

Our study aims at analyzing the incidence and frequency of various pathologies presenting as acute abdomen in a tertiary care hospital and the accuracy of 128 slice MDCT in making a correct diagnosis by correlating the CT diagnosis with intra-operative and post-operative histopathology diagnosis, whenever available and clinical diagnosis if the patient does not undergo surgery. Intra-operative and post-operative histopathology are considered as gold standard.

Materials and Methods

It is a prospective study performed on 73 subjects who presented with acute pain abdomen, who were referred to radiology department for MDCT abdomen. The duration of this study was from May 2015 to June 2016. Prior to the commencement of the study, necessary clearance was obtained from the institutional ethics committee and written informed consent was taken from the participating patients. Inclusion criteria: Patients who are presenting with clinical symptoms of acute abdomen and undergoing MDCT. Exclusion criteria: Patients who have contraindication to iodinated contrast media either due to contrast allergy or due to impaired renal function. Patients lost to follow up.

CT protocol: MDCT was performed with Siemens SOMATOM definition edge 128 slice scanner. Oral iodinated contrast was not given in patients who are kept nil by mouth in view of immediate surgical exploration, who cannot tolerate oral liquids (pancreatitis), suspected high degree of bowel obstruction, and suspected vascular pathology where the study is done in abdominal angiogram protocol (oral contrast interferes with vascular reconstructions). All the other patients were administered oral and rectal contrast as per routine protocol. All patients underwent a non-contrast study followed by contrast study. Approximately 80-90ml (1-2ml/kg body weight) iodinated non-ionic contrast was given intravenously using a pressure injector at 4ml/sec rate. Images were obtained at arterial (20-25sec), venous (50-60seconds) and delayed phases (>120 sec) using bolus tracking and automatic triggering of acquisition. Raw data was acquired with slice thickness of 0.625mm with a pitch of 0.8-1.5. The images were reconstructed in 5mm thickness for viewing purpose and in 1mm thickness for doing various reconstructions [6].



Study design

Results

In this study 73 patients with acute abdomen underwent MDCT. The findings of MDCT were correlated with the intraoperative findings and histopathological findings in patients who had undergone surgery. In conservatively managed patients the MDCT findings were correlated with clinical course. Table 1 shows the age distribution in the study population.

Table 1: Age group wise distribution of acute abdomen in the study population

Age (years)	Frequency	Percentage
0 to 10	4	5
11 to 20	6	8
21 to 30	8	11
31 to 40	16	22
41 to 50	12	16
51 to 60	9	12
61 to 70	11	15
71 to 80	6	8
91 to 100	1	1
Total	73	100

The sex distribution of the study population is shown in figure 1.

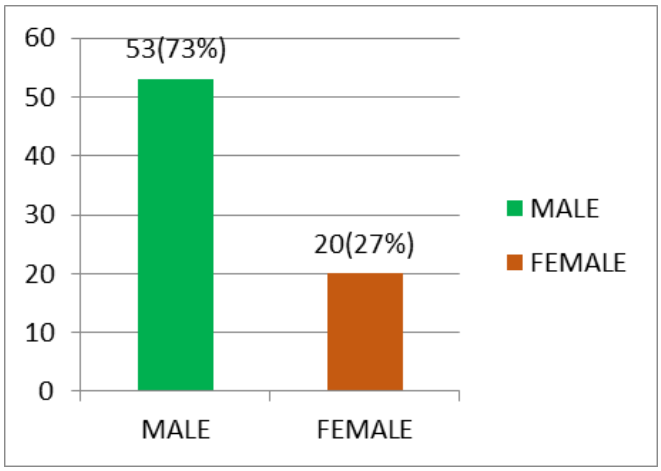


Fig 1: Gender distribution of acute abdomen.

Table 2 shows the frequency and percentage of the various pathologies detected among the study population.

Table 2: Distribution of various pathologies in study population.

No	Pathology	Frequency	Percentage
1	Appendicitis	16	22
2	Bowel obstruction	13	18
3	Acute pancreatitis	11	15
4	Perforation	10	14
5	Urolithiasis	5	7
6	Cholecystitis	4	5
7	Bowel ischemia	2	3
8	Aortic dissection	1	1
9	Diverticulitis	2	3
10	Aortic aneurysm	2	3
11	Intussusception	2	3
12	Volvulus	2	3
13	Nonspecific pain	3	4
	Total	73	100

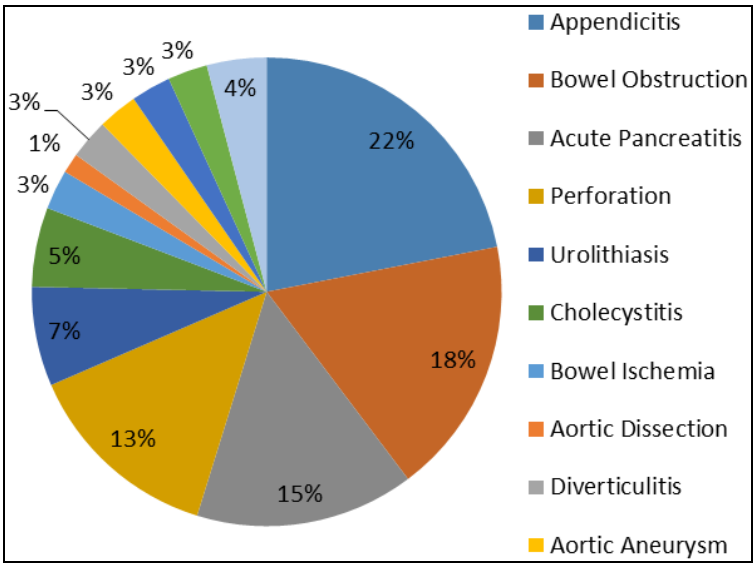


Fig 2: Pie chart showing percentage of various pathologies in acute abdomen

The pie chart (fig. 2) shows the percentage of patients affected by various pathologies, observed in the study population.

Table 3 shows the gender wise number of patients affected by various pathologies.

Table 3: Gender wise distribution of pathologies.

No	Pathology	Frequency	Male	Female
1	Appendicitis	16	10	6
2	Bowel Obstruction	13	9	4
3	Acute Pancreatitis	11	11	0
4	Perforation	10	7	3
5	Urolithiasis	5	3	2
6	Bowel Ischemia	2	2	0
7	Aortic Dissection	1	1	0
8	Cholecystitis	4	4	0
9	Diverticulitis	2	2	0
10	Aortic Aneurysm	2	2	0
11	Volvulus	2	0	2
12	Intussusception	2	1	1
13	Non-Specific Abdominal Pain	3	1	2
	Total	73	53	20

Demographics of few most common pathologies.

1. **Appendicitis:** Age and sex wise distribution of appendicitis in the study population is shown in figure 3.

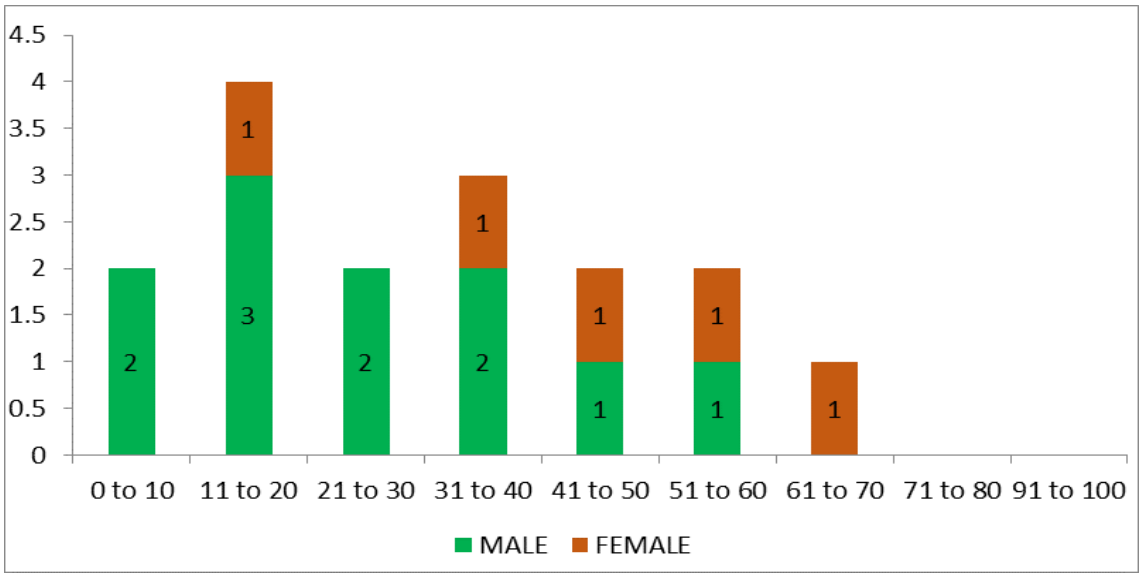


Fig 3: Gender and age wise distribution-appendicitis

2. **Bowel obstruction:** Age and sex wise distribution of bowel obstruction in the study population is shown in figure 4.

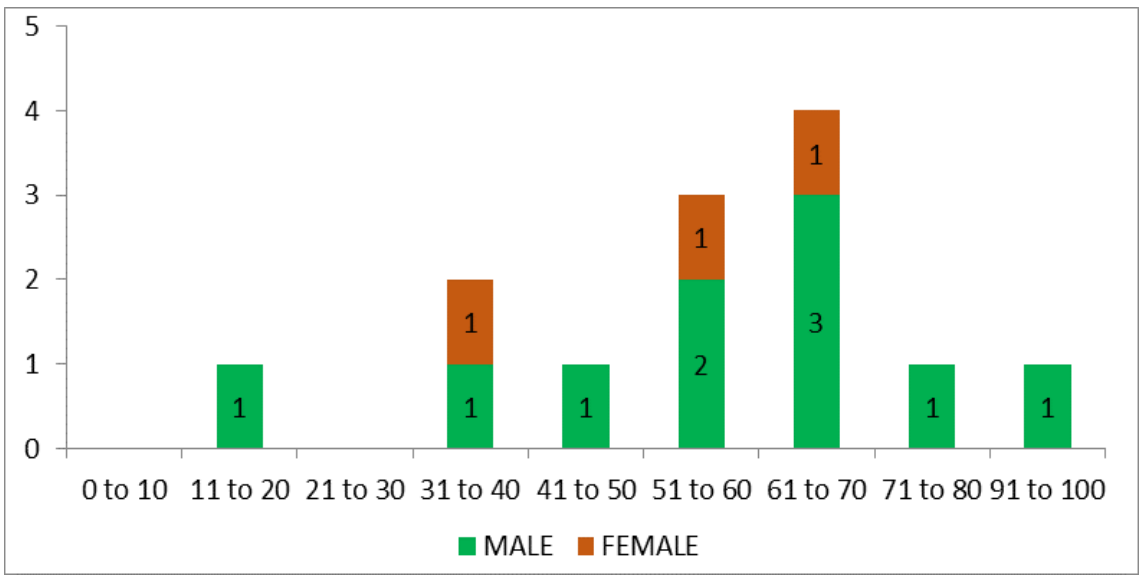


Fig 4: Gender and age wise distribution of bowel obstruction

3. **Pancreatitis:** Age and sex wise distribution of pancreatitis in the study population is shown in figure 5.

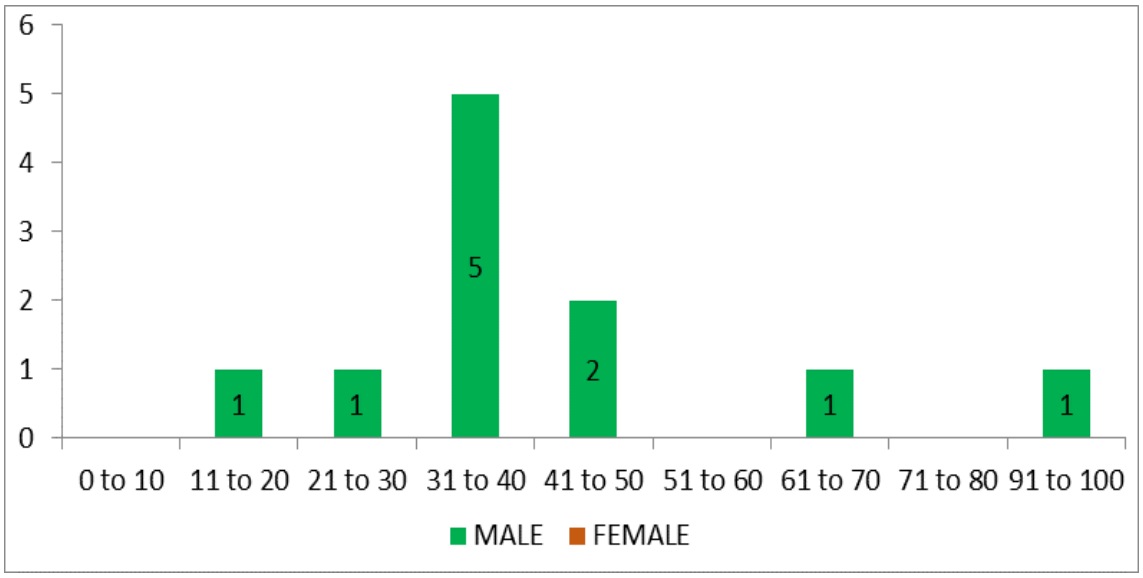


Fig 5: Gender and age wise distribution of pancreatitis

4. **Bowel perforation:** Age and sex wise distribution of bowel perforation in the study population is shown in figure 6.

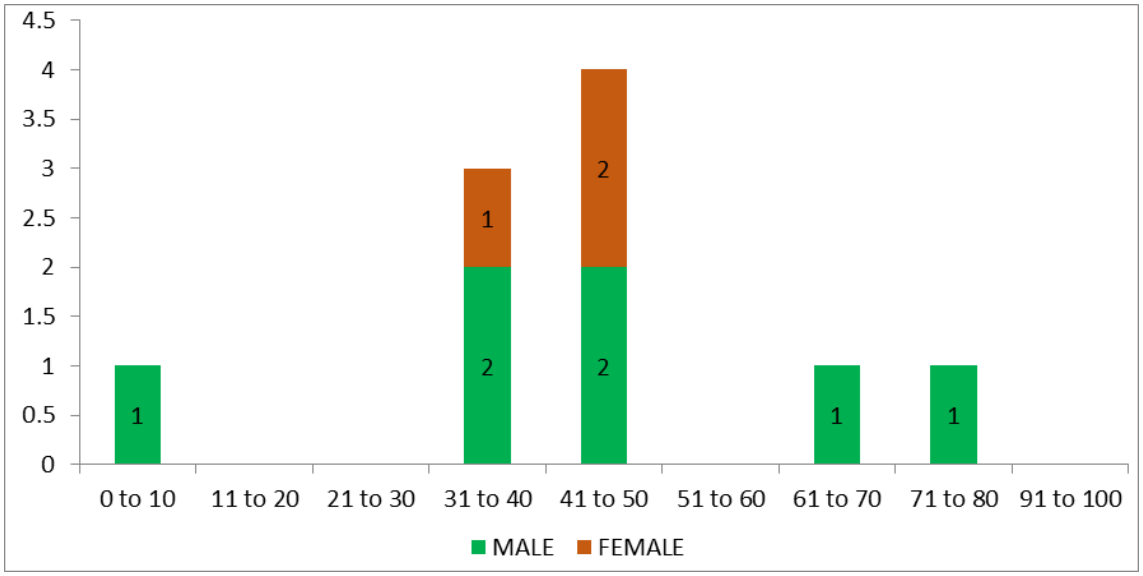


Fig 6: Gender and Age wise distribution of bowel perforation

Table 4 shows the overall diagnostic performance of MDCT compared with intra-operative and post-operative HPE diagnosis and clinical diagnosis.

Table 4: Overall diagnostic performance of MDCT

MDCT performance in acute abdomen	
Sensitivity	97.10%
Specificity	75.00%
Positive Predictive Value	98.53%
Negative Predictive Value	60.00%

Discussion

The current generation of the MDCT scanners with cutting edge technology have become the main stay of evaluation of patients with acute abdomen. The technology enables acquisition of isotropic images with exquisite spatial resolution and significantly reduced radiation exposure. The multi-row multi-detector spiral scanning technology has reduced scanning time significantly, leading to improved output and reduced movement related artefacts. The large volumetric data obtained in the axial plane allows reformations into any required plane with same resolution [7]. The advances in reformation techniques have made the process automatic which saves time. Further increase in the computing speed has facilitated faster radiological interpretation in critically ill patients. The shear speed and accuracy of CT in acute abdomen has made the role of plain radiography nearly obsolete.

In our study group of 73 patients 53 were males and 20 were females, ranging from 5 to 93 years. We analyzed various pathologies presenting as acute abdomen. Most common pathology found in our study was acute appendicitis, seen in 22% followed by bowel obstruction in 18%, acute pancreatitis 15% and bowel perforation 14%. Among which appendicitis is the most common cause and is consistent with most of the studies carried out internationally [8].

However we did not encounter any definitive gynecologic pathology, which may be due to the fact that patients with gynecological pathology were primarily evaluated by ultrasound which itself can yield a specific diagnosis.

In our study 16 patients had CT findings suggestive of acute appendicitis and related conditions (Figure 7). CT diagnosis in one out of these16 cases was non-concordant with intraoperative surgical findings. Intra-operatively it was found to be ileo-cecal tuberculosis which was confirmed by histopathological examination. The actual CT findings in this case were masked by clumping of bowel loops in the right iliac fossa, which made visualization of appendix difficult. This was erroneously diagnosed as inflammatory appendicular mass.

The sensitivity and Positive Predictable Value in acute appendicitis were 100% and 93.75% respectively. Intra operative findings very well correlated with CT findings except for one case. This is consistent with study conducted by Rao PM *et al.* [9] which shows 91% to 100% sensitivity for CT in the diagnosis of appendicitis. In another study done by Mawiah H. Alshebromi *et al.* [10] CT showed high sensitivity of 100% and poor specificity of mere 16%.



Fig 7: Axial and coronal MPR images showing a typical case of appendicitis with thickened and enhancing appendix (arrow in a). Coronal MPR (arrow in b) in same patient

In our study 13 out of 73 cases were diagnosed to have bowel obstruction. The various etiologies of bowel obstruction are stricture, adhesion, hernia and mesenteric band [11]. The sensitivity and positive predictable value were 100%. Our results are comparable with Mallo *et al.* [12] in which sensitivity of MDCT in diagnosis of bowel obstruction was 81% to 100% and specificity 68% to 100%. A study conducted by Suri S *et al.* [13] shows that CT is highly sensitive in determining level and cause of obstruction. This is made possible by the exquisite coronal and sagittal reformatted MPR images [14] which are very useful in identifying the transitional zone (Figure 8a). MPR images in any oblique or orthogonal plane are good in identifying intussusception and the underlying pathology as well (Figure 8b)

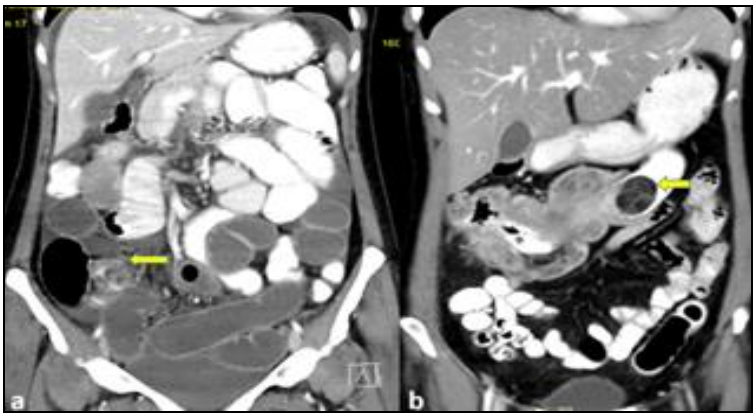


Fig 8: Case of acute bowel obstruction. Coronal MPR shows the transitional zone, in this case it was adhesion causing obstruction (a); Colo-colic intussusception and the underlying pathology, lipoma are shown well in coronal MPR (b).

We encountered 11 cases of pancreatitis out of 73 cases. In all the cases MDCT correctly identified the pathology. In all of the cases diagnosis was confirmed by abnormal levels of elevated pancreatic enzymes. The sensitivity of MDCT in acute pancreatitis was 100%. Comparable results were shown by B. Gianni *et al.* [15]. This is a disease entity where CT clearly scores over ultrasound, because visualization of pancreas itself, in most of the cases of pancreatitis is interfered by bowel gas. Estimation of pancreatic necrosis is impossible with ultrasound, which is the key factor in management and prognostication. CT also is better in identifying and quantifying the peripancreatic collections, pseudo cysts and identifying air in emphysematous pancreatitis (Figure 9).

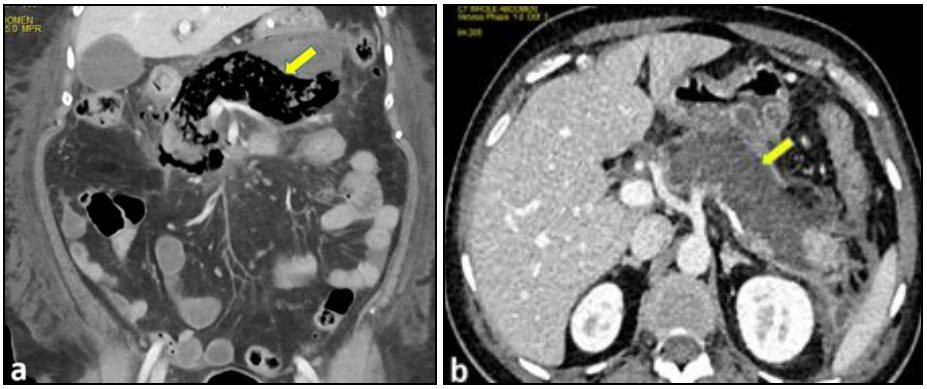


Fig 9: Coronal MPR showing extensive air replacing the entire pancreas, suggestive of emphysematous pancreatitis (arrow in a). Axial post contrast image shows extensive necrosis of the pancreatic parenchyma (arrow in b)

In our study 8 out of 10 cases of confirmed perforation were correctly diagnosed by MDCT. Two missed cases presented very late in the course of the disease, nearly after a week. Because of this delay in presentation these cases lacked the typical imaging features. Hence the perforation had already been sealed off and the pneumoperitoneum had been absorbed, the diagnosis was missed. In hallow viscus perforation the site of perforation can be exactly identified with MDCT. The early and occult perforations and sealed off perforations can be made out with MDCT, due to its ability to detect even smallest amount of free air. The sensitivity and accuracy for bowel perforation in our study was 80%, which was comparable to the study done by Sung Hwan Kim *et al.* and other investigators [16, 17, 18] who gave an accuracy of 82% to 90% for predicting site of perforation by CT.

In our study 5 out of 73 cases had urolithiasis, all the 5 cases were correctly identified by MDCT with sensitivity of 100%. The above findings are comparable with study performed by Isabelle Boulay *et al.* and others [19, 20, 21]. Leschka Sebastian *et al.* [5] state that thin slices and good multi-planar reconstructions helps in identifying even sub-millimeter calculi (Figure 10). This is complemented by the fact that nearly 100% of the renal calculi are radio-opaque.



Fig 10: Coronal MPR image showing the dilated calyces, and ureter in its entirety with an obstructing calculus in the mid ureter (arrow).

In our study 4 out of 73 cases had CT findings suggestive of cholecystitis with sensitivity of 100% which is comparable to other international studies. However the limitation of CT in gall bladder pathology is its failure to detect radio-lucent calculi, which could be the underlying predisposing factor for cholecystitis. Ultrasound and Magnetic Resonance Imaging (MRI) have better sensitivity in identifying Cholelithiasis. MDCT clearly performs better than ultrasound in detecting the complications like emphysematous cholecystitis and gangrenous cholecystitis. MDCT is the most sensitive modality in identifying complications related to cholecystitis as stated by L. Turturici *et al.* [22]

In 3 out of the 73 patients no significant abnormalities were detected by MDCT. And this was the case even in diagnostic laparoscopy. These patients were considered to have non-specific abdominal pain. The pathology in these patients presumed to be due to transient pain caused by intestinal colic or pain caused by a passing ureteric calculus, which would have completely passed out from the urinary bladder also, hence not seen in CT.

In our study the overall accuracy, sensitivity, specificity and positive and negative predictable values of MDCT were 96%, 97%, 75% and 98% and 60% respectively which were comparable to the study results of Monica Mangini *et al.* [23]

Conclusion

Acute abdomen is a commonly encountered, usually a surgical emergency. In our study the most commonly encountered pathologies were appendicitis, bowel

obstruction, bowel perforation and acute pancreatitis. It is essential to make a speedy and accurate diagnosis in these patients to decide on the management as to subject them to surgery or treat conservatively. For this purpose we need to employ a robust diagnostic tool. The clinical examination and laboratory parameters have very poor sensitivity and specificity, hence cannot be dependent upon. Very good sensitivity and specificity of MDCT makes it an ideal tool in the evaluation of acute abdomen. Recent advances in hardware, software and computation speed have made MDCT a powerful diagnostic tool in acute abdomen. MDCT scores over all other imaging modalities because of its speed and versatility.

Limitations of the study: Our study employed relatively small sample size, mainly because many patients were taken up for surgery based on ultrasound/radiography findings alone without CT. Though ultrasound may not give a specific diagnosis, these patients were taken up for surgery without CT, in the background of a given clinical scenario, like presence of guarding and rigidity in a suspected case of hallow viscus perforation where ultrasound showed free fluid and radiograph showed pneumoperitoneum which necessitated immediate laparotomy. Baring few specific conditions like gallbladder pathology and gynecological pathology MDCT is the primary modality of choice for evaluating acute abdomen.

References

1. Silen W. Cope's early diagnosis of the acute abdomen, 19th Ed. New York: Oxford University Press, 1996.
2. Suhail Rafiq *et al.*, Multidetector computerized tomography in acute abdomen, International Surgery Journal. 2019; 6(7):2353-2357.
3. Trott AT, Lucas RH. Acute abdominal pain. In: Rose P, ed. Emergency medicine, 4th ed. St. Louis: Mosby, 1998, 1888-1903.
4. Bolog N, Popiel A, Oancea I, Mangrau A, Beuran M, Nicolau E. Multidetector row spiral CT (MDCT) in bowel related non-traumatic abdominal emergencies. J gastroenterol. 2003; 12:319-324.
5. Frauenfelder T, Wildermuth S, Marincek B, Boehm T. Nontraumatic emergent abdominal vascular conditions: Advantages of multidetector row CT and three-dimensional imaging. Radiographics. 2004; 24:481-496.
6. Leschka Sebastian, Alkadhi H, Wildermuth S, Marincek B. Multidetector computed tomography of acute abdomen. Eur Radiol 2005; 15(12):2435–2447.
7. Adriano Massimiliano Priola *et al.*, Accuracy of 64-row multi detector CT in the diagnosis of surgically treated acute abdomen, Clinical Imaging 37 (2013) 902–907.
8. Radiologic Clinics of North America RSS. -11-1. 2015; 53(6): i-134.
9. Rao PM, Rhea JT, Novelline RA. Sensitivity and specificity of the individual CT signs of appendicitis: experience with 200 helical appendiceal CT examinations. J Comput Assist Tomogr. 1997; 21:686-692.
10. Mawiah H. Alshebromi, Sensitivity and specificity of computed tomography and ultrasound for the prediction of acute appendicitis at King Fahad Specialist Hospital in Buraidah, Saudi Arabia, Saudi Med J. 2019; 40(5):458-462.
11. Durgesh Kumar Saini, Poras Chaudhary, Chikkala Kanak Durga, Kiran Saini: Role of multislice computed tomography in evaluation and management of intestinal obstruction Post graduate Institute of Medical Education and Research, Dr. Ram Manohar Lohia Hospital, New Delhi, India Clinics and Practice. 2013; 3:e20.
12. Mallo RD, Salem R, Lalani T. Computed tomography diagnosis of ischemia and complete obstruction in small bowel obstruction: a systematic review. J Gastro in test Surg. 2005; 9:690-4.
13. Suri S, Gupta S, Sudhakar PJ, Venkataramu NK, Sood B, Wig JD. Comparative evaluation of plain films, ultrasound and CT in the diagnosis of intestinal obstruction. Acta Radiol. 1999; 40:422-8.
14. Ginanni B, Ruschi F, Turturici L, Donatelli G, Scionti A, Bianchi F *et a*. Orsitto; Pisa/IT: Multidetector-CT (MDCT) findings in Acute Pancreatitis (AP) 10.1594/ecr2014/C-2071
15. Sung Hwan Kim *et al.*: Gastro intestinal tract perforation: MDCT findings according to the perforation sites, Koren J radiol, 2009, 10(1).
16. Imuta M *et al.* Multidetector CT findings suggesting a perforation site in the gastrointestinal tract: analysis in surgically confirmed 155 patients Radiat Med. EPUB. 2007; 25(3):113-8.
17. Hainaux B, Agneessens E, Bertinotti R *et al*. Accuracy of MDCT in predicting site of gastrointestinal tract perforation. AJR Am J Roentgenol, 2006, 1.
18. Isabelle Boulay *et al.*: Ureteral Calculi: Diagnostic Efficacy of Helical CT and Implications for Treatment of Patients, AJR. 1999; 172:1485-1490.
19. Katz DS, Lane MJ, Sommer FG. Unenhanced helical CT of ureteral stones: incidence of associated urinary tract findings. AJR Am J Roentgenol. 1996; 166:1319-1322.
20. Smith RC, Verga M, McCarthy S, Rosenfield AT. Diagnosis of acute flank pain: value of unenhanced helical CT. AJR Am J Roentgenol. 1996; 166:97-101.
21. Turturici L, Ginanni B, Donatelli G, Ruschi F, Scionti A, Bianchi F *et al*. Orsitto; Pisa/IT: Acute Cholecystitis: Ultrasonography and CT indications in the emergency department; 10.1594/ecr2014/C-2123
22. Monica Mangini *et al.*: Non-traumatic acute bowel disease: differential diagnosis with 64-row MDCT, Emerg Radiol. 2008; 15:171-178.

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Evaluation of acoustic radiation force impulse (ARFI) elastography as a tool for non invasive detection and grading of liver fibrosis with histopathology correlation

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Abstract
Background: Chronic insult caused by viral infection, autoimmune disease, alcohol and other toxins can damage the liver parenchyma and ultimately lead to liver fibrosis and cirrhosis. Acoustic Radiation Force Impulse (ARFI) elastography is emerging as a valuable tool in the detection and grading of fibrosis in liver.
Materials and Methods: It is a prospective comparative study performed in 36 patients between December 2016 and March 2018 who presented with deranged liver function test and referred for ultrasonography, ARFI elastography of liver followed by liver biopsy. The findings of Elastography were correlated with histopathology.
Results: When compared with histopathology ARFI had a sensitivity of 82%, specificity of 85%, positive predictive value of 90% and negative predictive value of 75% in detecting liver fibrosis when we used optimum cut values of ARFI.
Conclusion: ARFI elastography is a valuable tool in non-invasively detecting liver fibrosis and can be included as part of routine ultrasonic examination protocol of liver. ARFI technology allows the quantification of liver fibrosis and can be effectively used to stage fibrosis. Hence ARFI can be used as an alternative to liver biopsy and it might substitute liver biopsy in future.

Keywords: Elastography, ARFI, liver fibrosis grading

Introduction
The incidence of chronic liver parenchymal disease is constantly on the raise. The major underlying causes are alcoholism and viral hepatitis [1]. In response to continuous inflammation and injury there is fibrosis [2, 3, 4] and regeneration which result in liver cirrhosis and the attendant complications like portal hypertension, variceal bleed, ascites, liver failure, hepatic encephalopathy and even development of hepatocellular carcinoma[1]. Generally cirrhosis is considered to be irreversible, however accumulating evidence suggests that it could be reversible to some extent, especially when targeted therapy is given [1, 3, 5]. Hence, early detection and grading the severity of liver fibrosis is of utmost importance for treatment. The degree of hepatic fibrosis is an important factor which influences the treatment and determines the prognosis of the underlying liver disease [1]. Therefore, there is tremendous need for quantitative assessment and grading of hepatic fibrosis. The method used traditionally for assessing the degree of liver fibrosis is liver biopsy. Which is considered as gold standard method for determining the extent of liver fibrosis. However it is an invasive procedure which is associated with patient discomfort and occasionally severe complications like bleeding, infection and rarely death [1]. Histopathological examination (HPE) of liver fibrosis is greatly dependent on the size of the sample and segment from which the sample is obtained. Generally a biopsy specimen of size 1.5-2cm is required by the pathologist for accurate evaluation. The distribution of fibrosis within the liver is very non-uniform and sampling errors are bound to happen. [4, 6, 7]These disadvantages associated with liver biopsy have been the thrust behind pursuing alternate methods to assess liver fibrosis. ARFI Elastography is a recently introduced technology to evaluate tissue stiffness non-invasively.

The technology of ARFI Elastography involves the mechanical excitation of tissue using short-duration acoustic pulses (push pulses) in a region of interest, producing shear waves that spread away from the region of interest, perpendicular to the acoustic push pulse, generating localized, micron-scale displacement of the tissue [8, 9]. Simultaneously, detection waves of lower intensity than that of the push pulse (1:100) are generated. By recording the shear wave front at several locations and correlating these measurements with the elapsed time, the shear wave velocity (SWV) can be quantified in meter per second unit. Generally, the stiffer a region in the tissue, the greater the SWV as it travels through this region. This technique can be used in various organs and it is well suited for liver in view of the size and relative superficial location of the organ. The advantage of this technology is that it is incorporated in an ultrasound machine and the operator can use the ultrasound to measure the stiffness of the tissue of interest in real time.

Materials and Methods

It is a prospective study performed from December 2016 to March 2018. Before starting the study necessary clearance was obtained from the institutional ethics committee and informed written consent was obtained from the patients. The study population includes 36 patients with clinical and laboratory features of altered liver function who were referred to radiology department for further evaluation with ultrasound. All the patients underwent liver sonography, ARFI Elastography and subsequently, with in a period of two weeks, liver biopsy. The findings of Elastography were correlated with HPE. Inclusion criteria: patients under evaluation for altered liver function parameters, nonalcoholic-steatohepatitis, chronic viral infections, patients undergoing liver biopsy for assessment of fibrosis. Exclusion criteria: Patients with bleeding disorders, altered coagulation parameters, patients unable to hold their breath and uncooperative. Ultrasound protocol: Examination was performed in SEIMENS S2000 machine in supine position. B mode Ultrasound: to assess the size, echogenicity and echo-texture of liver. ARFI Elastography: Total of 8 measurements of shear wave velocity were taken, at least 2 from each segment of right lobe. Measurement was calculated by placing a 0.5 x 1cm box at least 1.5 to 2cm deep to the liver capsule (Fig. 1) and away from major vessels. The velocity was calculated in meter per second and the mean and median velocity of all 8 measurements were used to grade the stiffness of the liver.

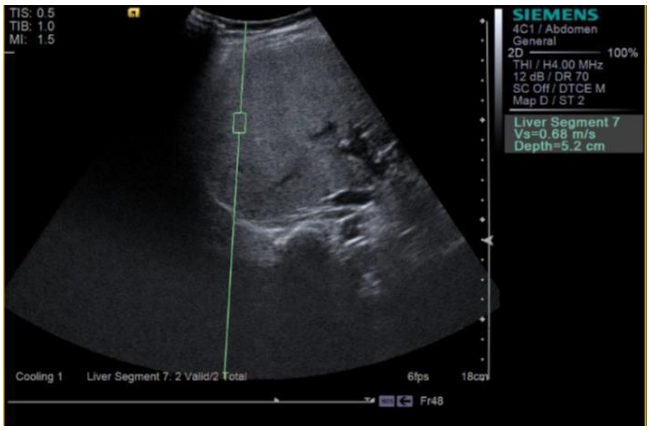


Fig 1: Measurement of ARFI by placing an ROI in each segment of the right lobe of liver.

Biopsy: Biopsy of the liver was obtained using 18G fully automatic gun (Bard MaxCore) with 22mm long cutting needle harvesting a 20mm long core. The specimen was fixed in formalin and sent for HPE. Fibrosis was scored in histopathology using the METAVIR scoring system. The cut of value [10] of shear wave velocities corresponding to METVIR grades is shown in Table 1:

Table 1: METAVIR Score and corresponding mean SWI in ARFI

Metavir Score	Histopathology	Mean Shear Wave Velocity
F0	No fibrosis	1.09+/- 0.42m/s
F1	Portal fibrosis without septa	1.22+/- 0.41m/s
F2	Portal fibrosis with few septa	1.37+/- 0.48m/s
F3	Numerous septa without cirrhosis	1.70+/- 0.59m/s
F4	Cirrhosis	2.23+/- 0.71m/s

Results

In this study 36 patients were evaluated with Ultrasonography and Elastography, the findings were compared with the HPE findings. In our study out of 36 patients 10 (28%) patients were female and 26 (72%) were male who presented with liver disease and altered liver function tests with different underlying pathologies like Nonalcoholic steatohepatitis (NASH), chronic viral hepatitis, storage disease and Cryptogenic cirrhosis. Our study had patients ageing from 11 years to 60 years with an average of 30 years. We had 14 (39%) patients below the age of 30 and 22(61%) patients more than 30 years of age. The distribution of various grades of fibrosis in the study population is shown in figure 2. The number of patients in the study population with various grades of fibrosis in ARFI and HPE is shows in table 2.

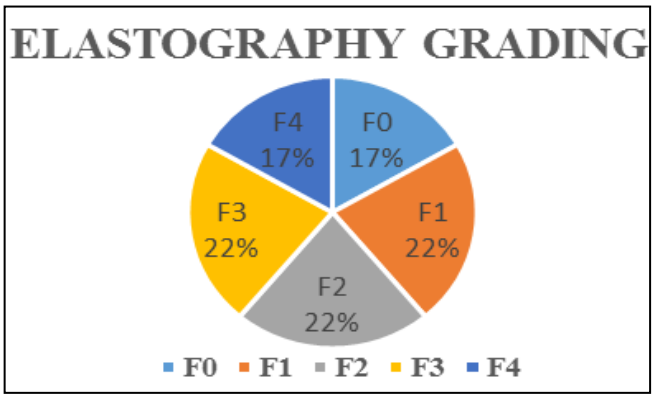


Fig 2: Distribution of various degrees of fibrosis in the study population in ARFI.

Table 2: Grading of fibrosis

	ARFI		HPE	
	Frequency	Percent	Frequency	Percent
F0	6	17	10	28
F1	8	22	4	11
F2	8	22	8	22
F3	8	22	4	11
F4	6	17	10	28
Total	36	100	36	100

B mode ultrasound findings of liver are shown in Table 3. Out of the 36 patients 4 had normal appearing liver, majority showed diffuse fat infiltration in the liver and 8 patients had altered echo-texture of liver parenchyma.

Table 3: Ultrasound findings in study population

Frequency		Percent
Normal	4	11
Steatosis	24	67
Altered echo texture	8	22
Total	36	100

In our study of 36 patients, ARFI showed clinically insignificant fibrosis in 16 patients with a METAVIR score of F0, F1 which was commonly seen with patients of acute hepatitis and nonalcoholic steatohepatitis. Clinically significant fibrosis (F2, F3, and F4) was seen in 20 patients with a majority of them affected by chronic viral hepatitis. (Tables 4).

Biopsy showed clinically insignificant fibrosis (F0, F1) in 14patients (39%) and clinically significant fibrosis (F2, F3, F4) in 22 patients (61%). Of the 14 patients who had insignificant fibrosis, different underlying etiologies such as NASH, storage diseases and autoimmune hepatitis were found, patients with significant fibrosis had viral hepatitis. (Tables 4).

Table 4: Significance of fibrosis in ARFI and HPE

	ARFI		HPE	
	Frequency	Percent	Frequency	Percent
Significant fibrosis	20	56	22	61
Insignificant fibrosis	16	44	14	39
Total	36	100	36	100

The cross tabulation comparing various grades of fibrosis in ARFI and HPE are shown in table 5.

Table 5: Elastography Grade Vs Histopathology Grade Cross Tabulation

			HPE Grade					Total
			F0	F1	F2	F3	F4	
ARFI Elastography Grade	F0	Count	2	0	2	0	2	6
		% with ARFI grade	33.3	0	33.3	0	33.3	100%
	F1	Count	4	4	0	0	0	8
		% with ARFI grade	50%	50%	0	0	0	100%
	F2	Count	4	0	4	0	0	8
		% with ARFI grade	50%	0	50%	0	0	100%
	F3	Count	0	0	2	4	2	8
		% with ARFI grade	0	0	25%	50%	25%	100%
	F4	Count	0	0	0	0	6	6
		% with ARFI grade	0	0	0	0	100%	100%
Total		Count	20	8	8	4	10	36
		% with ARFI grade	27.8%	11.1%	22.2%	11.1%	27.8%	100%

Table 6: Chi-Square Test

P value	
Pearson Chi-Square	0.028

The Chi square test shows a P = .028 which is statistically significant and proves elastography as a reliable tool for prediction of fibrosis and grading of liver fibrosis.

The cross tabulation comparing significant and insignificant fibrosis in ARFI and HPE is shown in table 7.

Table7: Significant Vs insignificant fibrosis in elastography and histopathology cross tabulation

			Histopathology		Total
			Significant	Insignificant	
ARFI	Significant fibrosis	Count	18	2	20
		% with ARFI	90%	10%	100%
	Insignificant fibrosis	Count	4	12	16
		% with ARFI	25%	75%	100%
		Count	22	14	36
		% with ARFI	61.1%	38.9%	100%

Table 8: Chi-Square Test

	P value
Pearson Chi-Square	0.005

The Chi square test shows a P = .005which is statistically significant.

Table 9: Sensitivity and specificity calculation

ARFI	Histopathology	
	Disease	No disease
Test positive	18(a)	2(b)
Test negative	4(c)	12(d)

Sensitivity: 82%, Specificity: 85%

Positive predictive value: 90%, Negative predictive value: 75%

Discussion

Generally fibrosis is thought to be an irreversible process till the discovery of hepatic stellate cells (HSC) in the 1980s. HSCs play an important role in fibrosis. Following chronic liver injury HSC under inflammatory milieu proliferate and activate to produce pro inflammatory and fibrogenic factors. Activated HSC produce large amounts of extracellular matrix (ECM). The extracellular matrix accumulates and distorts the liver architecture and forms a fibrous scar along with regeneration of hepatic tissue which subsequently lead to formation of the nodules. Studies carried out in the 1990s demonstrated that hepatic fibrosis was a reversible process if the cause was to be treated [2, 3, 4]. Since then researchers have taken keen interest in developing therapies for reversing/slowing down progression and severity of liver fibrosis. Thus, assessing the stage and severity of fibrosis precisely has taken the center stage in the management of chronic liver disease. In fibrosis and cirrhosis the pathological changes in tissue lead to alteration of mechanical properties like stiffness which can be measures noninvasively. The gold standard for assessing liver fibrosis

is biopsy and HPE. Liver biopsy, though safe, is an invasive procedure with a small risk of complications like severe hemorrhage. The risk of significant bleed is more when there is alteration of the coagulation parameters or in the presence of significant ascites. Both these risk factors are seen more frequently in patients with liver parenchymal disease, which in the first place is the indication for doing a biopsy. Percutaneous liver biopsy yields only a tiny fragment of the liver (1 in 50000 of the total volume of the liver) [11] which may lead to errors in staging of fibrosis, since there can be non-uniform distribution of fibrosis with in the liver. Bedossa *et al* [12] proved that the chance of under diagnosis of the stage of fibrosis, when the length of biopsy core is less than 2.5 cm can reach up to 25%. They have also proved that in almost half of the cases where liver fragments obtained from both the lobes of liver in the same session showed different stages of fibrosis. Hence in many ways liver biopsy is a flawed gold standard. For the past 10-15 years, enormous efforts have been made by several groups for establishing a reliable and reproducible noninvasive marker for liver fibrosis as a substitute to the invasive liver biopsy technique. In our study of 36 patients we have attempted to study the efficacy of ARFI compared to histopathology. The accuracy of ARFI elastography for assessing the severity of liver fibrosis and grading was evaluated. Calculating the sensitivity, specificity, positive predictive value and negative predictive value of the noninvasive test ARFI demonstrated a good sensitivity and specificity. Accurately grading and quantifying the severity of hepatic fibrosis secondary to an underlying disease is of great importance for planning appropriate treatment strategies. Studies comparing the non-invasive methods for evaluating the severity of fibrosis in chronic liver disease to liver biopsy are being performed to assess if these techniques would replace the invasive test. Virtual touch tissue imaging application enables ARFI technology to evaluate the deep tissues. SWI in meter per second can be quantified in a region of interest with ARFI technology in a precise anatomical location, which represents the elasticity property of the tissue. In our study we encountered 4 false negative patients. One of them was hepatitis B patient and another one patient with Hepatitis C. In these cases we presume values taken in the areas of necrosis may have contributed to low values. Another 2 patients were cases of alcoholic hepatitis, in whom the steatosis may have contributed to the falsely low values. We also had 2 false positive patients encountered in our study. One of them had hepatitis B acute stage and another one patient had hepatitis A. Hepatitis is known to have both inflammation and fibrosis, one of which may predominate depending upon the stage of disease [13, 14]. In acute stage because of inflammation, we can get erroneous high values [15, 16]. There are no possible means to differentiate the high values due to inflammation from that due to fibrosis. This may explain the false positivity in the hepatitis patients.

A study by Takahashi *et al*. [17] of 80 patients with mixed etiologies using ARFI (Siemens Healthcare), had a sensitivity and specificity of 91% and 80% respectively for F>2 stage of fibrosis. This is in concordance with the results in our study. In a similar study by Sporea at al. [18] using 114 patients with mixed etiologies, also had a sensitivity and specificity of 89% and 68% respectively. We attempted to study whether ARFI was sensitive in patients with NASH, who form the majority of the mixed etiology liver disease without viral hepatitis group. 10%-15% of NASH patients show a progression in the severity of fibrosis and architectural remodeling, and liver cirrhosis is seen in 15%-25% of cases. Three to 5% of people with fatty liver may develop cirrhosis. Cirrhosis due to NASH and cryptogenic cirrhosis have risk of developing HCC. HCC may rarely occur in a non-cirrhotic NASH patient. Hence lies the importance of detecting early fibrosis in patients with NASH [18, 19]. In a study conducted by Fraqueli *et al* [20] using 200 patients with mixed etiology liver diseases other than viral hepatitis, the sensitivity and specificity of ARFI was 72% and 84% respectively. In a study of 106 patients with HBV and HCV conducted by Friedrich-Rust *et al* [21] the sensitivity and specificity was 69% and 92% respectively. In our analysis of 28 HBV and HCV patients, we had a sensitivity of 90% and specificity of 75%. The liver stiffness assessment is challenging in patients with viral hepatitis because of the nature of underlying disease. There may be increased necro-inflammatory changes in the acute phase of the disease which undergoes fibrosis later on. The necrotic areas will give lower stiffness values compared to the other areas of inflammation thus may decrease overall sensitivity. The higher sensitivity in our study may be because these patients were assessed in the advanced stage of the disease. Review of previous publications and our own study has shown that US elastography definitely has a role in the staging of fibrosis where it has the potential of replacing liver biopsy. Another upcoming and exciting non-invasive modality to evaluate liver fibrosis is MR Elastography which has the advantage of avoiding sampling errors and operator variability. New MR imaging contrast agents that target specifically the collagen and other extracellular matrix macromolecules may be developed in the near future. A collagen-specific MR imaging contrast agent and also contrast agents that selectively target hepatic stellate cells and other cells involved in the pathogenesis of liver fibrosis may permit imaging of fibrogenesis or fibrolysis. All such data may provide valuable information for guiding antifibrotic therapy development and monitoring patients in clinical trials. Thus these noninvasive methods may help in the assessing the severity of fibrosis.

Conclusion

Acoustic Radiation Force Impulse (ARFI) elastography is a promising noninvasive tool to quantify liver fibrosis. It also has the advantage of routine ultrasound assessment of the liver during the same session of elastography study. ARFI measurements are done in real time under vision. As many number of measurements as the operator wishes can be obtained in a single study. The assessment can be repeated frequently as and when required. The technique has good sensitivity and specificity and less operator dependent when compared with other ultrasound techniques as the calculation is done automatically by the system and there are tools which are useful to obtain a good quality study. The technique has good sensitivity and specificity which has the potential to replace invasive biopsy. Limitations: Our study is limited by the relatively small sample size. The small sample size is primarily due to limitation in the number of patients undergoing biopsy. ARFI elastography is a relatively new technology that has not been extensively investigated, hence large prospective studies are needed to ascertain its efficacy and to form strict cut off values for the shear wave velocity. Different technologies and

measurement calculations are adopted by different ultrasound venders. Because of this standard values are not available for comparison across different ultrasound systems [22], which needs to be addressed by various ultrasound manufacturing vendors and an amicable consensus to be arrived at.

References

1. Jain VA, Dixit RA, Chowdhury V. Can acoustic radiation force impulse elastography be a substitute for liver biopsy in predicting liver fibrosis? Clinical Radiology. 2016; 71:869-875.
2. Friedman SL. Hepatic fibrosis: overview. Toxicology. 2008; 254(3):120-129.
3. Wallace K, Burt AD, Wright MC. Liver fibrosis. Biochem Genet. 2008; 411(1):1-18.
4. Afdhal NH, Nunes D. Evaluation of liver fibrosis: a concise review. Am J Gastroenterology. 2004; 99 (6):1160-1174.
5. Ruediger S. Goertz, Luise Gaßmann, Deike Strobel, Dane Wildner, Acoustic Radiation Force Impulse (ARFI) Elastography in Autoimmune and Cholestatic Liver Diseases, Annals of Hepatology. 2019; 18(1):23-29.
6. Haaga, John RCT, MRI of the whole body, 5th edition. Philadelphia: Mosby/Elsevier. 2009; 2:1455-1460.
7. Heiken JP. Liver. In: Lee JK, Sagel SS, Stanley RJ, Heiken JP. Computed Body Tomography with MRI correlation, 3rd ed, Philadelphia: Lippincott Williams and Wilkins. 1998; 1:701-777.
8. Kanzler S, Baumann M, Schumacher P, Dries V, Bayer E, Gerken G *et al*. Prediction of progressive liver fibrosis in hepatitis C infection by serum and tissue levels of transforming growth factor-beta. J Viral Hepat. 2001; 8:430-437.
9. Nelson DR, Gonzalez-Peralta RP, Qian K, Xu Y, Marousis CG, Davis GL *et al*. Transforming growth factor-beta 1 in chronic hepatitis C. J Viral Hepat. 1997; 4:29-35.
10. Simona Bota, Ioan Sporea, Roxana Şirli, Alina Popescu, How useful are ARFI elastography cut-off values proposed by metaanalysis for predicting the significant fibrosis and compensated liver cirrhosis? Medical Ultrasound. 2015: 17(2):200-205.
11. Emily Carey, DO, William D. Carey, MD, Noninvasive tests for liver disease, fibrosis, and cirrhosis: Is liver biopsy obsolete? Cleveland clinic Journal of Medicine. 2010; 7(8):7.
12. Pierre Bedossa, Delphine Darg`ere, Valerie Paradis, Sampling Variability of Liver Fibrosis in Chronic Hepatitis C, Hepatology. 2003; 38:1449-1457.
13. Chen S, Sanchez W, Callstrom MR, Gorman B, Lewis JT, Sanderson SO *et al*. Assessment of liver viscoelasticity by using shear waves induced by ultrasound radiation force. Radiology. 2013; 266:964-970.
14. Davies G, Koenen M. Acoustic radiation force impulse elastography in distinguishing hepatic haemangiomata from metastases: preliminary observations. Br J Radiol. 2011; 84:939-943.
15. Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B *et al*. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. J Hepatol. 2011; 55:666-672.
16. Takahashi H, Ono N, Eguchi Y, Eguchi T, Kitajima Y *et al*. Evaluation of acoustic radiation force impulse for fibrosis staging of chronic liver disease: a pilot study. Liver Int. 2010; 30:538-545.
17. Sporea I, Sirli RL, Deleanu A, Popescu A, Fosca M, Danila M *et al*. Acoustic radiation force impulse elastography as compared to transient elastography and liver biopsy in patients with chronic hepatopathies. Ultraschall Med. 2011; 32:S46-S52.
18. Hashimoto E, Yatsuji S, Tobari M, Taniai M, Torii N, Tokushige K *et al*. Hepatocellular carcinoma in patients with non alcoholicsteatohepatitis. J Gastroenterol. 2009; 44:89-95.
19. Timothy J. Hall, Milkowski y, Brian Garra, Paul carrson *et al* RSNA/QIBA: Shear wave speed as a biomarker for liver fibrosis staging.
20. Fraquelli M, Rigamonti C, Casazza G *et al*, Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease, Gut. 2007; 56:968-973.
21. Mireen Friedrich-Rust MD, Katrin Wunder MD, Susanne Kriener MD, Liver Fibrosis in Viral Hepatitis: Noninvasive Assessment with Acoustic Radiation Force Impulse Imaging versus Transient Elastography, Radiology: August. 2009; 252(2):595-604.
22. 12m Hittalamani IM, Lakhkar BB, Pattanashetti RC, Lakhkar BN. Acoustic radiation force impulse elastography of liver as a screening tool for liver fibrosis in alcoholic liver disease. Indian J Radio Imaging. 2019; 29:190-4.

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Case Report

Hereditary hemorrhagic telangiectasia of liver: Pathophysiology with role of radiology in diagnosis and treatment

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Abstract

Hereditary hemorrhagic telangiectasia (HHT) or Osler–Weber–Rendu syndrome is a rare condition which can result in significant *systemic and hepatobiliary* abnormalities. Liver involvement in HHT consists primarily of the consequence of various intrahepatic shunts. Even though these vascular shunts are present in the majority of patients with HHT, symptoms occur only in *minority* with clear predilection to *female gender*. The symptoms and imaging findings of liver vascular malformations can be easily overlooked or misdiagnosed which can result in delay in treatment or potentially harmful vascular interventions. In this case report, we discuss the pathophysiology of HHT in liver involvement, role of imaging in diagnosis, and the possible role of interventional radiologist in the treatment.

Key words: Embolization for shunt reduction; hereditary hemorrhagic telangiectasia; intrahepatic shunts; liver vascular malformation

Introduction

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder seen approximately in one in 5000–8000 individuals.[1] It presents with vascular malformations (VM) in the nose, skin, liver, gastrointestinal tract, brain, and lung. Telangiectasia is the VM seen in HHT where there is direct communication between the arterioles and venules without the intervening capillaries. This abnormal communication can result in shunts between various vascular beds in the solid organs and hallow viscera. Definitive clinical diagnosis of HHT can be made based on the presence of at least three of four Curacao criteria,[2] published in 2000. They are 1. Epistaxis, 2. Cutaneous or mucosal VM, 3. Presence of visceral telangiectasia, and 4. Positive family history. Liver involvement is seen in approximately 75% of people with HHT.[2] Although only a small 8%–10% of these patients develops symptomatic liver pathology, the rest remains asymptomatic.

Case Report

We are presenting a patient who presented with upper gastrointestinal (GI) bleed and epistaxis with anemia. On further evaluation, she was found to have telangiectasia in the nose and multiple angioectasias in the stomach and in the duodenum in upper GI endoscopy. This

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triad of findings, 1. Spontaneous recurrent epistaxis, 2. Telangiectasia of nose, and 3. Visceral telangiectasia, fulfill 3 Curacao criteria to qualify for definitive diagnosis of HHT. Further evaluation with contrast-enhanced computed tomography (CECT) was done to rule out chronic liver parenchymal disease. The CECT revealed heterogenous enhancement of the periphery of the liver in the arterial phase [Figure 1A] which becomes homogenous in the venous phase [Figure 1B]. In addition, there was moderately enlarged proper hepatic artery measuring 10 mm in diameter (normal <7 mm) and abnormally dilated intrahepatic branches of the hepatic artery and early filling of the portal venous branches [Figures 2A, B and 3]. There were abnormal tortuous vascular channels along the ligamentum teres with early filling of the branches of portal vein [Figures 3 and 4]. There were faintly enhancing tortuous vascular channels along the intrahepatic branches of the hepatic artery. There was slight reduction in the volume of the left lobe of liver. The surface of the liver was smooth. All these imaging features were suggestive of intrahepatic arterio-portal shunts with possible secondary chronic liver parenchyma disease. She was treated symptomatically with cauterization of the nasal telangiectasia, nasal packing, and blood transfusion for anemia. Few small telangiectasia lesions in the stomach showed active bleed during endoscopy and these lesions were treated with argon plasma coagulation.

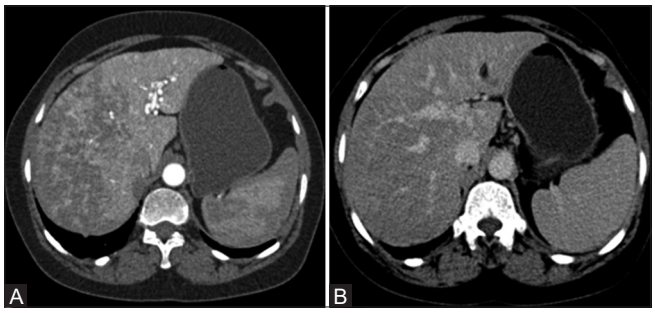


Figure 1 (A and B): Contrast-enhanced CT axial section shows heterogenous enhancement of the liver parenchyma in the arterial phase (A) which becomes homogenous in the venous phase (B)

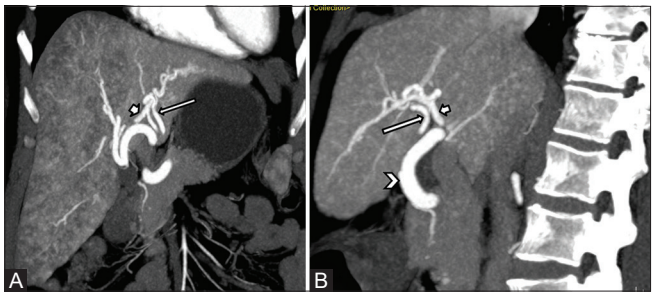


Figure 3 (A and B): Coronal (A) and sagittal (B) maximum intensity projection (MIP) images showing enlarged hepatic artery branches—long thin arrows (in A and B)—and early filling of the portal venous branches—short thick arrow (in a and B). The enlarged main hepatic artery is marked with arrow head in B

Discussion

In HHT, clinical symptoms occur in approximately 8% of the people.[3] There is a distinct female preponderance in symptomatic patients, with majority of the patients presenting in their fourth decade.[3] Presenting features are secondary to high-output cardiac failure (most common),[3] portal hypertension, GI bleed either from varices due to portal hypertension or angioectasia of the mucosa of the GI tract, and biliary disease. Other less common forms of clinical presentation in the liver and GIT are portosystemic encephalopathy and abdominal angina. The basic pathophysiology in liver involvement is development of intrahepatic VMs. Liver vascularity is unique by the way of presence of dual arterial supply and presence of dormant, potential portosystemic shunts, like paraumbilical vein, which can open up in any form of disturbance in the equilibrium of liver circulation.

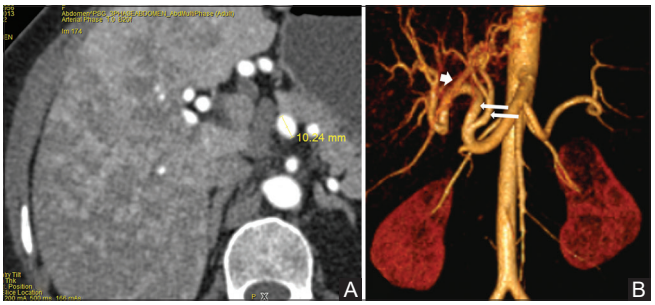


Figure 2 (A and B): Axial CT image in the arterial phase acquisition (A) and 3D volume rendered image (B) showing enlarged hepatic artery proper, measuring more than 10 mm. There is enlarged proper hepatic artery and its branches (long thin arrows in B) and early filling of the portal venous branch (short thick arrow in B) seen with fuzzy margins

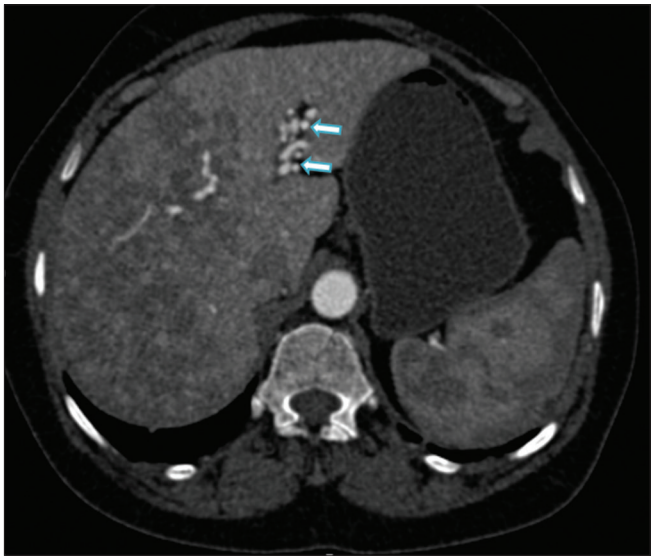


Figure 4: Axial CT image showing multiple tortuous early filling portal venous branches along the ligamentum teres (arrows) seen along with the left hepatic artery branches

The VMs in the liver can lead to three different types of vascular shunts.[3,4] These are 1. Arterio-venous (AV) shunt (between the hepatic artery and hepatic vein), 2. Arterio-portal (AP) shunt (between the hepatic artery and portal vein), and 3. Portal venous (PV) shunt (between portal vein and hepatic vein).[1] Shunts are usually extensive. The shunts can be visualized by imaging studies if they are macroscopic. Frequently they can be microscopic and not seen in imaging. Each predominant type of shunt gives rise to distinct clinical manifestation.

AV shunt

This is the most common symptomatic VM. It usually affects women. The presenting clinical feature is related to high-output cardiac failure.[1] AP shunt can also involve liver leading to biliary ischemia and ischemic necrosis of the bile duct[5] due to steal phenomenon. The loss of integrity of the bile duct due to ischemia can lead to biliomas and intrahepatic biliary cysts mimicking Caroli's disease. In chronic cases, *secondary sclerosing cholangitis* develops with stricture and abnormal dilatation of intrahepatic bile ducts. Biliary ischemia-related complications are seen *exclusively* in women.[3]

AP shunt

This is the second most common shunt after AV shunting. Shunting of blood from the hepatic artery to the portal vein causes *portal hypertension*. Secondary to portal hypertension, there can be ascites, varices formation, and GI bleed. In addition, there can be formation of focal nodular hyperplasia and *nodular regenerative hyperplasia in the liver*. It equally affects men and women. The diagnosis is made based on hepatic venous pressure gradient which should be more than or equal to 10 mmHg.

PV shunt

This is the least common type of shunt and usually causes high-output cardiac failure. Rarely, this can cause focal nodular hyperplasia and nodular regenerative hyperplasia in the liver which can lead to portal hypertension and cirrhosis.

Diagnosis of HHT is made based on the Curacao criteria.[2] Imaging is indicated to confirm the diagnosis if three of the four clinical criteria are not met. In addition, imaging has to be done to look for associated complications like liver cirrhosis. In a known case of HTT, the presence of ascites, GI bleed, and abdominal pain should raise the suspicion of liver involvement and imaging should be performed. Doppler study can show abnormal tortuous vessels in the liver. CECT and MRI can reveal multiple liver VMs. There can be enlarged hepatic artery, normal ≤7 mm,[1] heterogenous enhancement of the liver in the arterial phase of contrast study which becomes homogenous in the venous phase.

CT is the recommended *imaging modality* of choice. In CECT, the type of shunt can be determined in two-third of patients. The majority of patients with liver disease will show AV shunt. Biliary disease is generally seen in the late phase of the disease. The gold standard to establish the type of shunt is angiography.[3] Angiography can clearly demonstrate the early, subtle VMs and mesenteric steal syndrome. However, in the majority of the patients, the diagnosis can be established with less invasive imaging methods like CECT.

Treatment

No treatment needed in asymptomatic patients with liver involvement. Primarily, the management is medical with treatment of anemia and correcting coagulation abnormalities.[6] High-output cardiac failure and complications of portal hypertension are treated in the standard way like treating cardiac failure and portal hypertension due to other etiologies. It should be noted that trans-jugular intrahepatic porta systemic shunt (TIPS) creation may not be effective in the treatment of GI bleed secondary to telangiectasia of the GI tract. Shunt reduction procedures like embolization may be considered in patients with cardiac failure and mesenteric ischemia.[7] However, these procedures are associated with high morbidity and mortality and have only transient therapeutic effect. In carefully selected patients, few sessions of endovascular embolization may be effective in reducing portosystemic shunt and alleviating mesenteric ischemia. Ultimately patients might require liver transplantation, which can be curative.

Conclusion

HHT is a rare cause of vascular dysplasia involving many organs and systems. Although the majority of the patients remain asymptomatic, high index of suspicion should be kept not to miss liver involvement by the disease. Radiologists should be aware of the imaging findings and possible treatment options like shunt reduction embolization offered by us. Life-threatening complications like high-output cardiac failure not responding to maximum medical therapy and severe biliary ischemia should be recognized and these patients should be referred for an early liver transplant.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ginon I, Decullier E, Finet G, Cordier JF, Marion D, Saurin JC, *et al*. Hereditary hemorrhagic telangiectasia, liver vascular malformations and cardiac consequences. Eur J Intern Med 2013;24:e35-9.

2. Leitão P, Carvalho A, Guerra C, Gonçalves J, Ramos I. Congestive cirrhosis in Osler-Weber-Rendu syndrome: A rare case report. Radiol Case Rep 2018;13:51-4.
3. Garcia-Tsao G. Liver involvement in hereditary hemorrhagic telangiectasia (HHT). J Hepatol 2007;46:499-507.
4. Brandt E, Haghighat L, Garcia-Tsao G, Henderson K, Pollak J, Proctor D, *et al*. Three types of intrahepatic shunts presenting in a single patient with hereditary hemorrhagic telangiectasia. J Am Coll Cardiol 2018;71. doi: 10.1016/S0735-1097(18)32934-6.
5. Vlachou PA, Colak E, Koculym A, Kirpalani A, Kim TK, Hirschfield GM, *et al*. Improvement of ischemic cholangiopathy in three patients with hereditary hemorrhagic telangiectasia following treatment with bevacizumab. J Hepatol 2013;59:186-9.
6. Govani FS, Shovlin CL. Hereditary haemorrhagic telangiectasia: A clinical and scientific review. Eur J Hum Genet 2009;17:860-71.
7. Weinstein J, Lao J. Staged embolization of a complex hepatic arterio-portal fistula, Posters and Exhibits, JVIR, Educational Exhibit Abstract No. 466.

Original Article

Evaluation of Resistive Index of Orbital Vessels using Color Doppler Imaging in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Resistive index (RI), derived from color Doppler imaging (CDI), is a marker of vascular resistance used widely in varied clinical settings. The aim of this study was to analyze the association between RIs of the orbital vessels in a pure cohort of type 2 diabetic patients with or without retinopathy using CDI. **Materials and Methods:** Fifty patients having type 2 diabetes and 50 age-matched controls were evaluated in this prospective study. Diabetic retinopathy (DR) was diagnosed based on seven-field stereo fundus photography and diabetic patients were divided into two. Patients with no DR ($n = 26$) were taken as Group 1, while patients with DR ($n = 24$) were taken as Group 2. CDI was performed and the RIs of the ophthalmic artery (OA), posterior ciliary artery (PCA), central retinal artery (CRA), and central retinal vein (CRV) were measured. **Results:** Significant differences were observed in the mean RI values of all orbital arteries between controls and patients with DR ($P < 0.05$). Comparison of RI values between controls and Group 1 showed no significant differences. Mean RI values of the PCA and CRA were found to be significantly higher in the patients in Group 2 than in Group 1 ($P = 0.03$ and $P < 0.001$, respectively). The duration of diabetes correlated with the mean RI of all the orbital vessels. RI of the CRA was a reliable predictive indicator for DR ($P = 0.001$). **Conclusion:** RIs of the orbital arteries are significantly higher in patients with DR. RI of the orbital vessels can be a potentially useful biomarker in the early diagnosis and follow-up of patients with DR.

Keywords: Color Doppler ultrasonography, diabetic retinopathy, vascular resistance

Introduction

Color Doppler imaging (CDI) is a convenient noninvasive method that gives information about the velocity of blood and vascular resistance at various sites of complex vasculature using simultaneous B mode ultrasound and Doppler images. The resistive index (RI), calculated from CDI, is a universally used measure of resistance to the arterial blood flow. In the recent past, RIs of the orbital arteries have been studied extensively to gain diagnostic and prognostic insight into a variety of clinical conditions, such as chronic obstructive pulmonary disease, hypertension, chronic kidney disease, and diabetes-related micro and macrovasculopathies.[1-5]

Usefulness of RI of the retrobulbar vessels in risk stratification and management of ophthalmic diseases such as diabetic retinopathy (DR), age-related macular degeneration, glaucoma, retinal vein occlusion, and thyroid-associated orbitopathy has been documented in the literature.[6,7]

Several studies have evaluated the RI of the retrobulbar vessels in diabetic patients with or without retinopathy and have shown inconsistent results. While few studies have shown significant correlation between RI of the orbital vessels and severity of DR,[8,9] few others suggest that the hemodynamic alterations observed in the orbital arteries reflect macrovascular changes and are not related to DR.[5,10] The observed inconsistencies can be attributed partly to the differences in sample sizes, patient characteristics, duration of diabetes, methods of assessment of DR, and probe positions used for color Doppler

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measurement. Further, most of these studies included patients with comorbidities such as hypertension and coronary artery disease as well as those patients on cardiac or antihypertensive drugs, which might alter the vascular resistance.

The aim of this study was to analyze the association between RIs of the orbital vessels in a pure cohort of type 2 diabetic patients with or without retinopathy using CDI.

Materials and Methods

This prospective study was conducted between March 2017 and May 2019 from a tertiary care institution in South India. The institutional human ethical committee approval was obtained before the initiation of the study, and the principles of Declaration of Helsinki were adhered to. Informed, written consent was obtained from all the study participants. Fifty consecutive patients of type 2 diabetes (as per definition of the American Diabetes Association) were recruited for the study. Exclusion criteria were the presence of associated systemic hypertension, nephropathy, dyslipidemia, and cardiovascular diseases. Patients on medications known to affect systemic hemodynamics such as angiotensin-converting enzyme inhibitors, calcium channel blockers, and antimigraine drugs were excluded. Patients with a history of any ocular disease that might affect ocular blood flow such as retinal diseases, high myopia, glaucoma, previous ocular surgery, or panretinal photocoagulation were also excluded from the study. A control group of 50 age-matched subjects were included. None of the controls had any ocular or systemic disease.

Data regarding age, gender, body mass index (BMI), duration of diabetes and antidiabetic medications were obtained from all the study participants. Blood pressure and serum glycosylated hemoglobin (HbA1c) levels were determined.

All participants underwent a comprehensive eye examination and the presence/absence of retinopathy was diagnosed through dilated fundus examination and 30° stereoscopic fundus photograph of seven standard early treatment DR study fields using a digital fundus camera (TRC-50 DX Mydriatic Retinal Camera; Topcon Medical Systems, Oakland, NJ, USA). Diabetic patients were divided based on retinopathy into two groups. Group 1 contained patients with no DR; Group 2 included patients with DR. One eye (right eye, unless another ocular pathology was identified) was included from each study participant.

A masked expert radiologist performed CDI of the eye for all study participants using a color Doppler unit and a 12–5 MHZ linear-array transducer (Philips iU22 xMATRIX ultrasound system). The CDI was performed with patients lying in the supine position with a 30° head tilt and any undue pressure on the eye was avoided. Examiner's hand was rested on the orbital margin, and sterile coupling gel was applied over closed eyelids. Angle of the transducer was taken as 30°–60° during the examination. CDI was performed after exclusion of any other ocular or orbital pathologies by B mode, and optic nerve was considered as a reference for all further measurements. The velocity of blood flow in the ophthalmic artery (OA) was measured 20–30 mm behind the globe after crossing of the optic nerve in the nasal aspect. Blood flow velocity in the central retinal vein (CRV) and central retinal artery (CRA) was measured within the optic nerve around 5 mm behind the posterior margin of the eyeball. Blood flow velocity from the temporal branch of the short posterior ciliary artery (PCA) was determined approximately 5–10 mm posterior to the globe. The peak systolic velocity (PSV) and end diastolic velocity (EDV) measurements were taken for OA, CRA, PCA, and CRV. With the help of PSV and EDV, we calculated the vascular resistance based on the RI using Pourcelot's formula:

$$RI = (PSV - EDV)/PSV.$$

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) Version 24. Data were reported as mean ± standard deviation. The normality of data distribution was assessed by Kolmogorov–Smirnov test. One-way analysis of variance with a *post hoc* Bonferroni was used for analysis of normally distributed continuous data. Kruskal–Wallis analysis was used for analyzing nonnormally distributed data. Bivariate analysis using Pearson's correlation coefficient was used to study the relationship of RIs with biochemical and demographic variables of patients with diabetes. Receiver operating characteristic (ROC) curve analysis was used to explore the predictive indicators of DR. A $P < 0.05$ (two-tailed) was considered statistically significant.

Results

The study consisted of 51 males and 49 females. The age of the participants ranged from 41 to 76 years. Duration of diabetes ranged between 6 months and 20 years. Among the diabetic patients studied, 26 had no DR and constituted Group 1. Group 2 consisted of 24 patients with DR. In Group 2, three patients had mild nonproliferative DR (NPDR) characterized by only few microaneurysms, seven had moderate NPDR (presence of more than just microaneurysms but less than severe NPDR), six had severe NPDR (presence of more than 20 intraretinal hemorrhages in each of four quadrants or definite venous beading in 2+ quadrants or prominent intraretinal microvascular abnormalities in 1+ quadrant), and eight had proliferative DR (presence of neovascularization of disc or elsewhere in the retina).

There were no statistically significant differences in the age, BMI, and blood pressure between controls and diabetic groups. Duration of diabetes and HbA1c levels were found to be significantly increased in Group 2 patients with DR ($P < 0.001$). Significant differences were observed in the RI values of all orbital arteries between controls and diabetic groups. Table 1 shows the demographic data and clinical attributes of controls and diabetic subjects.

Subgroup analysis with *post hoc* comparison revealed significantly higher RI values in OA, PCA, and CRA in Group 2 compared to controls ($P < 0.001$, $P < 0.001$, and $P = 0.03$, respectively). Comparison of RI values between Group 1 and controls showed no significant differences. When diabetic Groups 1 and 2 were compared, statistically significant differences were observed in the mean RI values of CRA and PCA ($P < 0.001$ and $P = 0.03$, respectively).

In bivariate analysis, the mean RI values of all orbital vessels, namely OA, CRA, PCA, and CRV, had a significant correlation with the duration of diabetes ($R = 0.396$, $P < 0.001$; $R = 0.282$, $P = 0.005$; $R = 0.334$, $P = 0.001$; and $R = 0.290$, $P = 0.003$, respectively) [Figure 1]. In addition, the mean RI of the OA correlated significantly with HbA1c levels ($R = 0.321$, $P = 0.001$) [Figure 2].

ROC analysis showed that the RI of CRA was a definitive predictive indicator for DR. RI of the CRA ≥ 0.79 indicated the existence of DR with a sensitivity of 75% and a specificity of 73% (area under the curve = 0.780 [95% confidence interval: 0.650–0.909]) [Figure 3].

Discussion

DR, a common microvascular complication of diabetes, is one of the leading causes for preventable blindness in people of the working age.[11] There has been conflicting evidence on the ocular hemodynamics as to whether blood flow is increased or decreased in diabetics. Curtis *et al*. have proposed a hemodynamic framework which states that there is an initial reduction in the perfusion of retina before the onset of DR which later increases as DR progresses.[12]

The various hematological changes observed in diabetes such as increased aggregation of erythrocytes and platelets, increased blood viscosity, and reduced deformability of

Table 1: Demographic and clinical characteristics of controls and diabetic subjects

Characteristic	Controls (n=50)	Group 1 (n=26)	Group 2 (n=24)	P
Mean age (years)	56.58±8.81	54.85±8.52	54.42±7.84	0.52
Mean body mass index (kg/m^2)	23.35±3.62	25.33±2.69	24.09±3.88	0.07
Median systolic BP (mmHg)	110 (110-130)	120 (110-126.25)	120 (110-130)	0.16
Median diastolic BP (mmHg)	70 (70-80)	76.5 (70-80)	70 (70-80)	0.27
Median duration of diabetes (months)	-	24 (6-72)	120 (48-144)	<0.001
Mean glycosylated hemoglobin (%)	5.81±0.30	8.62±1.91	10.64±2.29	<0.001
Mean RI of OA	0.76±0.06	0.79±0.06	0.82±0.06	<0.001
Mean RI of CRA	0.77±0.07	0.72±0.17	0.84±0.11	0.001*
Mean RI of PCA	0.73±0.06	0.74±0.08	0.79±0.08	0.004*
Mean RI of CRV	0.42±0.12	0.44±0.12	0.46±0.11	0.29

*P<0.05. BP: Blood pressure, OA: Ophthalmic artery, CRA: Central retinal artery, PCA: Posterior ciliary artery, CRV: Central retinal vein, RI: Resistivity index

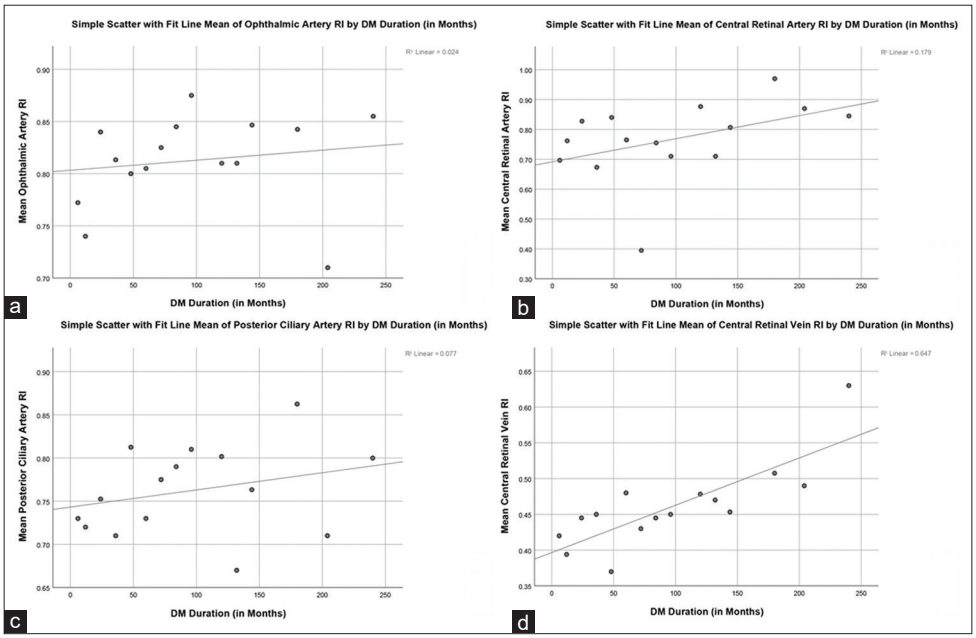


Figure 1: Scatter diagram showing correlation between duration of diabetes and resistive index of (a) ophthalmic artery, (b) central retinal artery, (c) posterior ciliary artery, and (d) central retinal vein

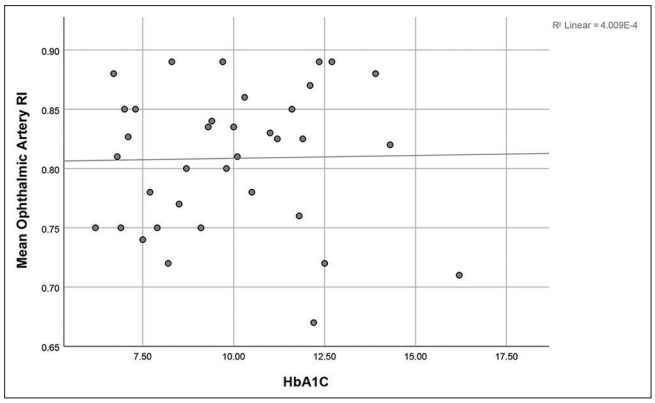


Figure 2: Scatter diagram showing correlation between mean resistive index of ophthalmic artery and glycosylated hemoglobin

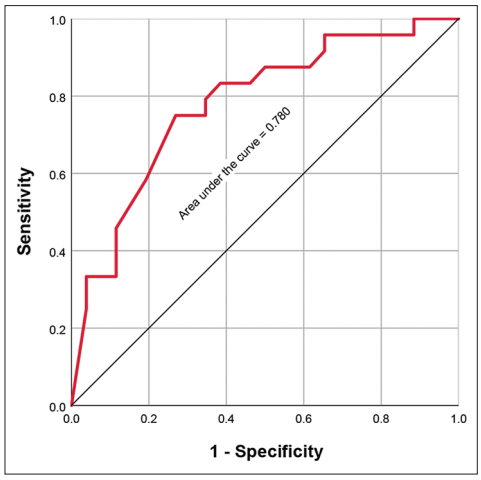


Figure 3: Receiver operating characteristic curve analysis of resistive index of central retinal artery and diabetic retinopathy (area under the curve was 0.780)

erythrocytes contribute to increased peripheral vascular resistance.[13] RI, an indirect measure of vascular resistance, can be useful to identify individuals at higher risk for developing DR. Further, RI, being independent of Doppler angle, has been shown to be reliable and reproducible with least coefficient of variation.[14]

Our study results demonstrate that significant changes occur in the RIs of the orbital vessels in patients with diabetes. The orbital vascular resistance (as reflected by RI) was found to be significantly increased in patients with DR as compared to controls and those in Group 1. Our observations are in agreement with certain other published studies.[15,16]

We observed no significant differences in the mean RI values between controls and those without DR. Our findings are similar to that of Basturk *et al*., who evaluated the RI of the orbital arteries in type 2 diabetics with microalbuminuria. No significant differences were observed in the mean RIs of OA, CRA, and PCA between controls and no DR group.[9]

In contrast, Dimitrova *et al*. have noted higher RI in the PCA in patients with no DR than controls.[17] However, their study included patients with hypertension and longer duration of diabetes than ours. In patients with hypertension, the RI of the PCA is reported to be high and correlates with duration of hypertension.[18] Moreover, CDI was performed in erect posture, which might have influenced the results. Few other investigators have noted increased RI values in the OA and CRA in patients with diabetes but without DR compared to that of controls, suggesting that even before the appearance of overt DR, there is increased resistance to blood flow in the peripheral vascular bed.[19,20]

We found significantly greater RI in the PCA and CRA ($P = 0.03$ and $P < 0.001$, respectively) in patients with DR compared to those without DR. While the inner layers of the retina are perfused by the CRA, the choroidal vasculature (derived from PCA) is responsible for the nourishment of outer retina. The various pathological changes observed in DR, such as loss of pericytes, thickening of the capillary basement membrane, and vascular smooth muscle cells, lead to compromised arteriolar integrity and autoregulation of blood flow. Loss of capillaries is critical to the pathogenesis of progressive retinal ischemia in DR and is a ubiquitous retinal finding from histopathological specimens.[21] Dropout of capillaries could be responsible for the increased vascular resistance observed in the RI of CRA and PCA in patients with DR. Further, the RI of the CRA was found to be a reliable predictive indicator of DR in our study. In this context, our findings are partly in agreement with that of Krasnicki *et al*., who have noted significant differences in the RI of CRA in subjects with DR and concomitant coronary artery disease.[5] The CRA, one of the major feeding vessels of the retina, reflects the identified vascular changes that have been associated with DR more sensitively. It has been suggested that hemodynamics in OA, a medium-sized muscular artery with many branches, is unlikely to be influenced by isolated alterations in blood flow in one of the branches.[22]

However, with progression of DR, the RI of the OA can eventually increase due to ensuing vascular changes related to diabetes in both retinal and choroidal vasculature.

Our finding of positive correlation between RI of all orbital vessels and the duration of diabetes is in concordance with that of Karami *et al*.[23] With prolonged duration of diabetes, alterations in blood vessel diameter and perfusion pressure occur due to disturbed autoregulatory mechanisms, subsequently leading to changes in the resistance of all retrobulbar vessels. We identified a statistically significant correlation between RI of the OA and the levels of HbA1c ($P = 0.001$). Clermont *et al*. have demonstrated reduction in the blood flow to retina with increasing HbA1c in subjects with type 1 diabetes without DR.[24]

Nagaoka *et al*. evaluated subjects with type 2 diabetes for changes in the retinal circulatory parameters using laser Doppler velocimetry and found significantly higher HbA1c in patients with the least retinal blood flow quartile, suggesting that poor glycemic control is associated with lowering of the retinal blood flow.[25]

Our study has several strengths. None of the patients had any associated vascular diseases such as hypertension. The presence of DR was assessed through seven-field stereo fundus photography for accuracy. A single experienced radiologist performed the CDI studies of the entire study participants, thereby eliminating interobserver variability. All examinations were performed with least pressure on the globe.

Limitations of our study include cross-sectional design and a small sample size. However, we intentionally excluded patients with other comorbid conditions to focus the effects of diabetes on retrobulbar circulation. Subgroup analysis of patients with different stages of retinopathy could not be performed because of limited sample size.

Conclusion

RIs of the orbital arteries are significantly higher in subjects with DR. RI of the orbital vessels can be a potentially useful biomarker in the early diagnosis and follow-up of patients with DR. This may be particularly helpful in patients with opacities in the ocular media.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ozer T, Altin R, Ugurbas SH, Ozer Y, Mahmutyazicioglu K, Kart L. Color Doppler evaluation of the ocular arterial flow changes in chronic obstructive pulmonary disease. Eur J Radiol 2006;57:63-8.
2. Reddy R. Usefulness of color Doppler imaging of orbital arteries in young hypertensive patients. Proc (Bayl Univ Med Cent) 2019;32:514-9.
3. Rokni Yazdi H, Faraji S, Ahmadi F, Shahmirzae R. Color Doppler indices of orbital arterial flow in end-stage renal disease patients; are the changes related to chronic hemodialysis or chronic renal failure? Iran J Radiol 2012;9:12-6.
4. Basturk T, Akcay M, Albayrak R, Unsal A, Ulas T, Koc Y. Correlation between the resistive index values of renal and orbital arteries. Kidney Blood Press Res 2012;35:332-9.
5. Krasnicki P, Dmuchowska DA, Proniewska-Skretek E, Dobrzycki S, Mariak Z. Ocular haemodynamics in patients with type 2 diabetes and coronary artery disease. Br J Ophthalmol 2014;98:675-8.
6. Dimitrova G, Kato S. Color Doppler imaging of retinal diseases. Surv Ophthalmol 2010;55:193-214.
7. Walasik-Szemplińska D, Pauk-Domańska M, Sanocka U, Sudoł-Szopińska I. Doppler imaging of orbital vessels in the assessment of the activity and severity of thyroid-associated orbitopathy. J Ultrason 2015;15:388-97.
8. Khatri M, Saxena S, Kaur A, Bhasker SK, Kumar M, Meyer CH. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retina Vitreous 2018;4:12.
9. Basturk T, Albayrak R, Ulas T, Akcay M, Unsal A, Toksoy M, *et al*. Evaluation of resistive index by color Doppler imaging of orbital arteries in type II diabetes mellitus patients with microalbuminuria. Ren Fail 2012;34:708-12.
10. Ino-ue M, Azumi A, Yamamoto M. Ophthalmic artery blood flow velocity changes in diabetic patients as a manifestation of macroangiopathy. Acta Ophthalmol Scand 2000;78:173-6.
11. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.
12. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye (Lond) 2009;23:1496-508.
13. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008;2:1130-8.
14. Neudorfer M, Kessner R, Goldenberg D, Lavie A, Kessler A. Retrobulbar blood flow changes in eyes with diabetic retinopathy: A 10-year follow-up study. Clin Ophthalmol 2014;8:2325-32.
15. MacKinnon JR, McKillop G, O'Brien C, Swa K, Butt Z, Nelson P. Colour Doppler imaging of the ocular circulation in diabetic retinopathy. Acta Ophthalmol Scand 2000;78:386-9.
16. Gracner T. Ocular blood flow velocity determined by color Doppler imaging in diabetic retinopathy. Ophthalmologica 2004;218:237-42.
17. Dimitrova G, Kato S, Tamaki Y, Yamashita H, Nagahara M, Sakurai M, *et al*. Choroidal circulation in diabetic patients. Eye (Lond) 2001;15:602-7.
18. Akal A, Ulas T, Goncu T, Karakas E, Karakas O, Kurnaz F, *et al*. Evaluation of resistive index using color Doppler imaging of orbital arteries in geriatric patients with hypertension. Indian J Ophthalmol 2014;62:671-4.
19. Arai T, Numata K, Tanaka K, Kiba T, Kawasaki S, Saito T, *et al*. Ocular arterial flow hemodynamics in patients with diabetes mellitus. J Ultrasound Med 1998;17:675-81.
20. Tamaki Y, Nagahara M, Yamashita H, Kikuchi M. Blood velocity in the ophthalmic artery determined by color Doppler imaging in normal subjects and diabetics. Jpn J Ophthalmol 1993;37:385-92.
21. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. Microcirculation 2007;14:25-38.
22. Harris A, Joos K, Kay M, Evans D, Shetty R, Sponsel WE, *et al*. Acute IOP elevation with scleral suction: Effects on retrobulbar haemodynamics. Br J Ophthalmol 1996;80:1055-9.
23. Karami M, Janghorbani M, Dehghani A, Khaksar K, Kaviani A. Orbital Doppler evaluation of blood flow velocities in patients with diabetic retinopathy. Rev Diabet Stud 2012;9:104-11.
24. Clermont AC, Aiello LP, Mori F, Aiello LM, Bursell SE. Vascular endothelial growth factor and severity of nonproliferative diabetic retinopathy mediate retinal hemodynamics *in vivo*: A potential role for vascular endothelial growth factor in the progression of nonproliferative diabetic retinopathy. Am J Ophthalmol 1997;124:433-46.
25. Nagaoka T, Sato E, Takahashi A, Yokota H, Sogawa K, Yoshida A. Impaired retinal circulation in patients with type 2 diabetes mellitus: Retinal laser Doppler velocimetry study. Invest Ophthalmol Vis Sci 2010;51:6729-34.

Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction

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ORIGINAL PAPER

Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction

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Abstract

Background Acute ureteric colic is one of the most commonly encountered acute abdominal conditions. Diagnosis of obstruction caused by ureteric calculus occasionally becomes challenging if there is inadequate dilation of the urinary tract proximal to obstruction. In such a situation, intra-renal artery Doppler parameters can be used as a diagnostic tool. Our study compared intra-renal arterial Doppler in patients with obstructed and non-obstructed kidneys presenting within 24 h of onset of symptoms of unilateral acute renal colic.

Results The resistivity index (RI) in the segmental arteries of all 54 patients with obstructed kidneys was significantly higher than in those with non-obstructed kidney: 0.75 vs. 0.56, with a p value less than 0.001, a sensitivity of 85% and a specificity of 93%.

Conclusion Doppler ultrasound is a useful diagnostic tool in the evaluation of acute renal obstruction, thus enabling practitioners to avoid ordering unnecessary CT and exposing patients to ionizing radiation. An RI value > 0.7 has good sensitivity and specificity in our study. The results obtained in the study were comparable to those of pioneer studies conducted worldwide.

Keywords Acute renal colic · Renal artery Doppler · Resistive index

Abbreviations

ARC	Acute renal failure
ER	Emergency room
USG	Ultrasonography
US	Ultrasound
RI	Resistive index
CT	Computed tomography.
NSAID	Non-steroidal anti-inflammatory drugs
GFR	Glomerular filtration rate
eGFR	Estimated glomerular filtration rate.
Tc^{99m} DTPA	Technetium$^{-99\,m}$- Diethylene-triamine-penta-acetate
CDUS	Color Doppler Ultrasound
MPR	Multiplanar reconstruction
ROC	Receiver operating characteristic curve
PCS	Pelvicalyceal system

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Background

Acute renal colic (ARC) is one of the common causes of patients presenting to the emergency room (ER). It accounts for 30–35% of urologic emergencies [1, 2]. The recurrence rate over a period of 5 years is 30–40% [2]. Ultrasonography (US) remains the most commonly used modality in the initial evaluation of patients presenting with acute abdomen, especially in children [3], and the utility of ultrasound in the diagnosis of ARC cannot be overstated in cases where ARC is one of the most common pathologies presenting as acute abdomen. Absence of significant dilatation of the pelvicalyceal system, in spite of severe obstruction being present, is a well-recognized finding in some patients. In some

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studies, the absence of significant dilation in ARC has been reported in up to 30% [4] of patients. The dilated collecting system seen in US need not always be due to obstruction in the downstream urinary tract. These conditions include a baggy extra-renal type of pelvis, a compound upper pole calyx, due to over-distended urinary bladder, vesico-ureteric reflux, congenital type of mega calyces, infection and residual dilatation due to previous obstruction. Very rarely, pseudo-aneurysms of intra renal arteries can mimic renal pelvi-ectasis. This kind of vascular pathology can also be easily diagnosed with CDUS [5]. Acute complete ureteric obstruction is associated with changes in renal blood flow, as well as with an increase in renal pelvic pressure. In the first few hours, renal blood flow increases, and after 3 to 5 h, renal blood flow decreases, probably because of efferent arteriolar vasoconstriction produced by prostaglandins and other vasoactive substances [6, 7]. These changes can be effectively demonstrated by the RI of intra-renal arteries, as measured by Doppler ultrasound.

Methods

A prospective study was done on 54 patients who were referred to the department of radiology with clinical features of ARC for further evaluation with ultrasound or non-contrast CT. The study period is from January 2015 to December 2017. Prior to the commencement of the study, necessary clearance was obtained from the institutional ethics committee, and informed written consent was obtained from all the participating patients.

Inclusion criteria

All patients with symptoms of acute ureteric obstruction presenting to the emergency department within 24 h of onset of unilateral ARC were included.

Exclusion criteria

(a) Patients with history of renal parenchymal disease, chronic renal obstruction, renal trauma, or bilateral renal obstruction; (b) patients with a single kidney; and (c) patients in whom ureteric obstruction could not be confirmed either with USG or with CT were excluded.

All patients underwent gray-scale ultrasonography (US) and Doppler evaluation of intra-renal arteries. Computed tomography (CT) was done for those patients in whom US could not confirm the cause of obstruction.

Evaluation by ultrasound and Doppler study

The kidney on the side of obstruction was treated as the case kidney, and the contralateral normal (non-obstructed) kidney served as control. All patients underwent B-mode US and color doppler ultrasound (CDUS) with Philips iU22 using a C 1–5 transducer and Siemens Acuson S3000 using a 6C1 HD transducer. The presence or absence of pelvicalyceal system dilatation in each kidney was assessed using the gray-scale images. At least three Doppler spectra were obtained from inter-lobar arteries along the border of the medullary pyramids, and their mean was taken (Fig. 1).

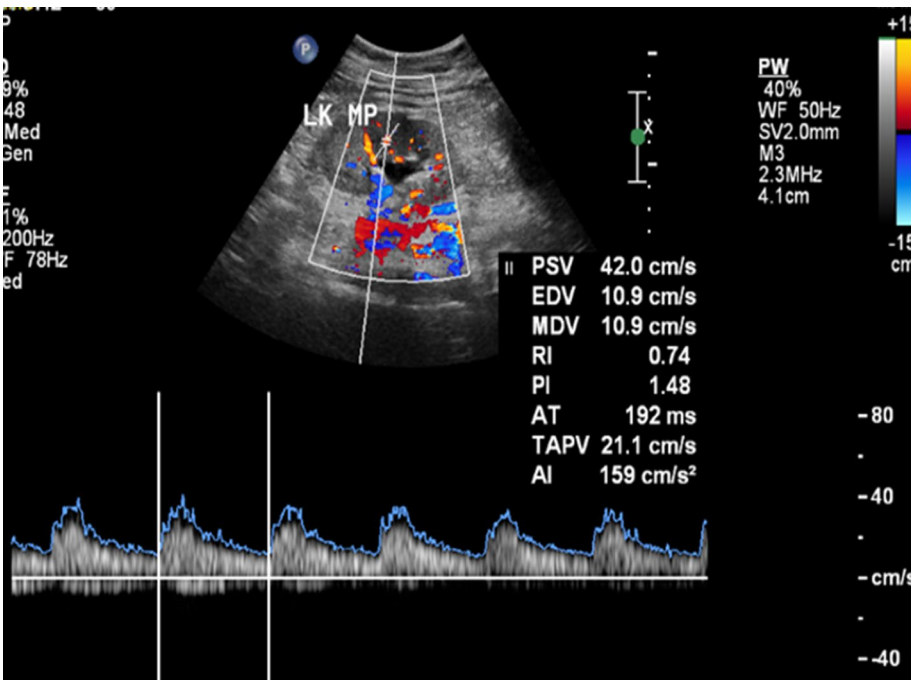


Fig. 1 Doppler US demonstrating an RI value of 0.74 in the mid pole of left kidney (obstructed kidney)

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The Doppler waveforms were made using the lowest pulse repetition frequency possible without aliasing. This maximized the size of the Doppler spectrum and decreased the percentage error in the measurements. In addition, the lowest possible wall filter for each ultrasound scanner was used. The Doppler sample width was set at 2–3 mm. The renal RI was calculated using the standard formula. The mean RI value was calculated for each kidney.

Evaluation by computed tomography

Siemens SOMATOM Definition Edge 128 slice Computerized Tomography (CT) equipment was used for the study. Non-enhanced CT of the abdomen, extending from the dome of the diaphragm to the pubic symphysis, was performed in all patients in whom the site and cause of obstruction could not be confirmed with US. Raw data was acquired with 0.6 mm thickness and pitch of 0.8. Reconstruction from the raw data was done in 5 mm thickness for viewing purposes and in 1 mm thickness for coronal and sagittal multi-planar reconstruction (MPR) (Fig. 2). The sites of ureteric obstructions were identified. Those patients not confirmed to have obstructions by CT were excluded from the study.

Statistical methods

All data were systematically collected, tabulated and analyzed using Microsoft Excel and Strata 6 for Windows. The Student *t* test was used in univariate analysis for the continuous variables, and Chi square test was used for analysis of noncontiguous variables. A *p* value of less than 0.05 was considered to be statistically significant.

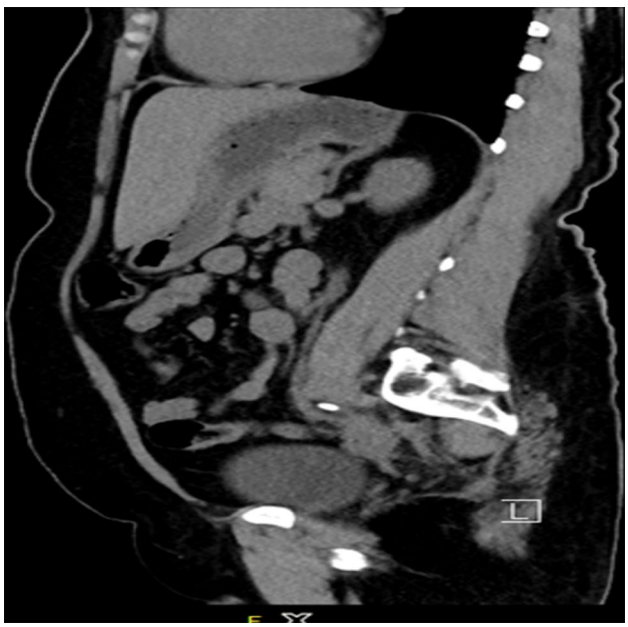


Fig. 2 Sagittal reconstruction of non-enhanced CT showing a calculus in the left distal ureter who presented with acute left renal colic

Results

This study evaluated 54 patients who had presented to the EMD with unilateral ARC within 24 h of the onset of symptoms. All the patients underwent B-mode US, CDUS and CT if the obstruction could not be confirmed in US. The presence or absence of PCS dilatation in each kidney was assessed using the grey-scale images. In this study population, 34 patients were males and 20 were females, as shown (Fig. 3). The mean age of the male patients was 46.8 years and that of the female patients was 38.5 years. The age range of the male patients was 19–79 years, and that of the female ones was 18–61 years.

The modalities used in the diagnosis and the site of obstruction are shown in Tables 1, 2.

Out of 54 patients, 26 cases proximal obstructions, while 28 had distal obstructions. The mean RI of the obstructed kidneys was higher in cases of distal obstruction (0.79 ± 0.004) than in those of proximal obstruction (0.76 ± 0.006).

Of the 54 patients, 45 patients had mild hydronephrosis, FIVE patients had moderate hydronephrosis and four patients did not have any dilatation of the collecting system despite the presence of ureteric obstruction (Fig. 4).

Of the 54 patients who were included in this study, 27 patients had elevated renal parameters, but did not have history of chronic renal impairment (Table 3).

In our study, we found that the RI in obstructed kidneys was significantly higher than in unobstructed kidneys (0.75 vs. 0.56; $p < 0.001$). The RI was higher in obstructed kidneys in all cases. The difference in RI between the obstructed and unobstructed kidneys (delta RI) ranged from 0.09 to 0.38, with a mean delta RI of 0.24 (Table 4).

The RI values in obstructed kidneys ranged from 0.68 to 0.84 (Fig. 5). The mean RI value in obstructed kidneys was 0.75, compared to 0.56 in unobstructed kidneys.

The group statistics of RI are shown in Tables 5, 6. The receiver operating characteristic curve (ROC) curve depicting the sensitivity of RI in ureteric obstruction is shown in Fig. 6.

By using a cut off RI value of > 0.70 in acute unilateral renal obstruction, the sensitivity obtained in the study was 85% and the specificity was 93% (Table 7).

Discussion

Grayscale ultrasound is the investigation method of choice in the initial evaluation of ARC, as it is easily available, portable and involves no ionizing radiation [8, 9]. However,

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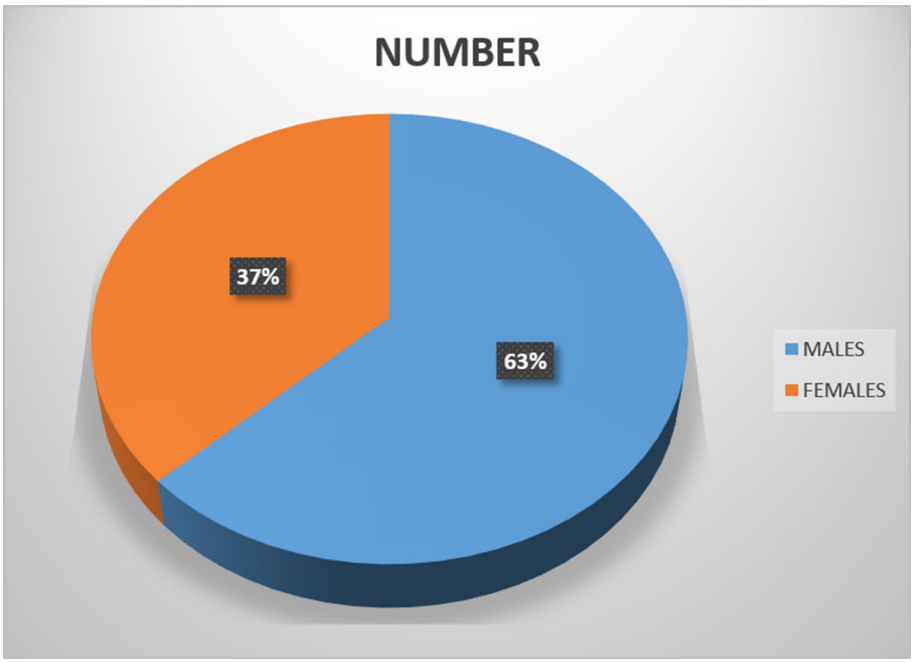


Fig. 3 Sex distribution in the study population

Table 1 Modality used in diagnosing the obstruction

Modality	Number	Percentage
CT and US	44	81%
US	10	19%
Total	54	100%

Table 2 Site of obstruction in the study population

Site of calculus	Frequency
Proximal ureter	26
Distal ureter	28

urinary system dilatation seen on conventional gray-scale USG has been shown to be sensitive (90%), but not specific (65–84%), in the diagnosis of renal obstruction.

The most sensitive, gold standard test for diagnosing obstructive uropathy is renal scintigraphy. However the availability of scintigraphy and radiation associated with it continue to be problematic [10]. Traditionally, the evidence of renal obstruction provided by US has been indirect and dependent on the anatomical criterion of dilatation of the pelvicalyceal system and ureter proximal to the level of obstruction. However, US fails to reveal hydronephrosis in acute obstruction of the kidney in up to 35% of cases [11]. More direct functional evidence of obstruction usually requires scintigraphy, but recently Doppler US techniques have been used to obtain functional information in suspected renal obstruction [11]. US imaging may miss (false negative) the diagnosis of obstruction in a variety of situations. Mild dilatation may be overlooked or considered clinically insignificant. Some patients with obstructive renal failure may show no pelvicalyceal system (PCS) dilatation. The reasons for this are unclear; in some patients, it may be related to dehydration or due to decompression of the pelvicalyceal system by rupture of a calyceal fornix [12]. It is commonly explained by urologists to be caused by the use of non-steroidal anti-inflammatory drugs (NSAIDs), by dehydration or by the renin angiotensin effect [13]. If that is true, such impairment will be detected through measurement of glomerular filtration rate (GFR). Measurement of GFR is commonly done using Technetium c-99 m diethylene-triamine-penta-acetate (DTPA), and does not require blood or urine sampling [14]. There is a growing evidence that renal impairment in acute unilateral ureteric obstruction is due to absorption of obstructed urine, with its high amount of creatinine, back into systemic circulation. This is considered false renal impairment with normal GFR [15]. In a study, El-Shazly et al. [16] measured renal function through measurement of estimated GFR (eGFR) usingTc-99 m DTPA. The authors showed that differences between eGFR on admission and after passage of stone or relieve of obstruction were non-significant. Their results support the hypothesis of false impairment in this clinical scenario. This tends to change the practice, as there is no need to embark on urgent intervention to relieve the ureteric obstruction, and conservative medical management is justifiable.

Routine B-mode US can be falsely positive or negative in acute ureteric obstruction [10]. PCS dilatation may be missed if the system is filled with blood clots, calculus, tumors or pus, in which case the usual anechoic appearance

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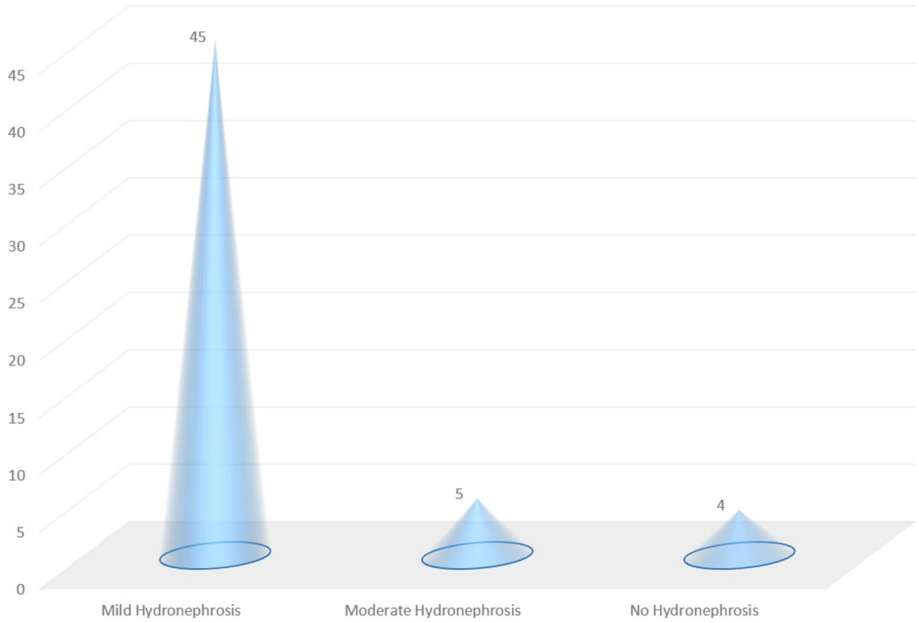


Fig. 4 Degree of hydronephrosis in the study population

Table 3 Number of patients with elevated serum urea and creatinine

No. of patients	Serum urea and creatinine
27	Increased
27	Normal

Table 4 Resistive indices in obstructed and unobstructed kidneys

Kidney	RI
Obstructed	0.75 ± 0.08
Non-obstructed	0.56 ± 0.09
Delta RI	0.24 ± 0.08

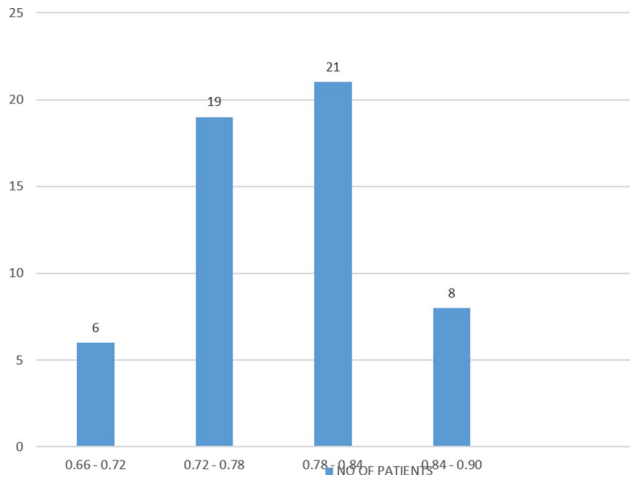


Fig. 5 Mean RI values in obstructed kidneys

Table 5 Group statistics

	Frequency	mean RI	SD	*P* value
Obstructed kidneys	54	0.75	0.08	0.000*
Unobstructed kidneys	54	0.56	0.09	

*Statistically significant ($P < 0.05$)

Table 6 Group statistics

	Mean RI value		*p* value
	< 0.70	> 0.70	
Obstructed kidneys	5 (9.3%)	49 (90.7%)	0.000*
Unobstructed kidneys	48 (88.9%)	6 (11.1%)	

*Statistically significant ($p < 0.05$)

of the PCS is lost. Intermittent ureteric obstruction, particularly that caused by ureteric calculi, may also lead to a failure to visualize dilatation of collecting system with US. On the other hand, in an attempt not to miss the diagnosis of obstruction in patients with only mild PCS dilatation, the false positive rate was found to be as high as 26% Causes of a false positive diagnosis include: (i) dilatation of non-obstructed PCS, when there are anatomical variants such as extra-renal pelvis; (ii) over-distended urinary bladder, with or without diuresis; (iii) vesico-ureteric reflux (VUR), (iv) residual dilatation of PCS due to previous obstruction or infection; (v) advanced pregnancy; and (vi) central renal fluid collections other than in the PCS, including in normal vessels, renal artery aneurysms and para-pelvic cysts.

Acute unilateral ureteric obstruction results in a complex sequence of changes in renal blood flow and ureteric

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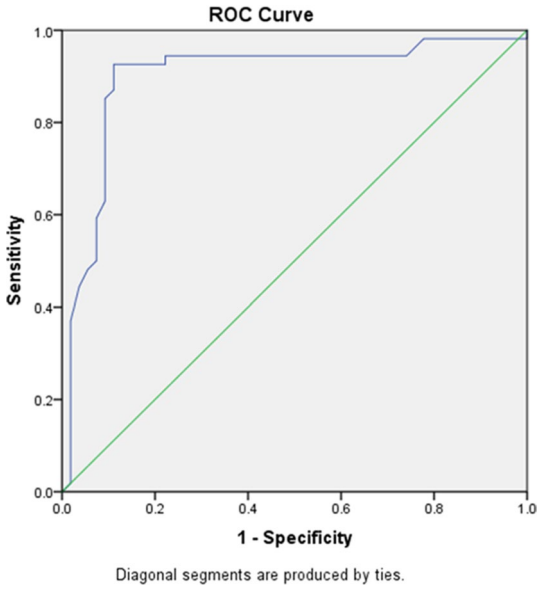


Fig. 6 ROC curve showing the sensitivity and specificity of resistive index

Table 7 Sensitivity and Specificity of RI with cut off of 0.70

Cut off RI	Sensitivity	Specificity
> 0.70	85%	93%

pressure [17]. In the first two hours, the renal blood flow increases, because of afferent arteriole vasodilatation and the ureteric pressure increases. From two to six hours after obstruction, the renal blood flow decreases, secondary to vasoconstriction of the efferent arterioles, and the ureteric pressure remains elevated. Subsequently, at 6 to 18 hours, the renal blood flow remains reduced because of vasoconstriction of the afferent arterioles, and the ureteric pressure decreases.

The role of renal CDUS in the evaluation of ARC has been vigorously debated. Rodgers et al. [18] found an elevated RI in acutely obstructed kidneys, especially when compared with the RI in normal contralateral kidneys and with a control group of healthy subjects. Similar results were obtained by Platt et al. [19] in a study of 23 patients with acute unilateral ureteric obstruction. However, others have reported that Doppler ultrasound is highly insensitive for detecting ARC. Tublin et al. [20] correlated the results of Doppler USG with those of urography in 32 patients presenting with symptoms of renal colic. The RI value of normal kidneys is usually less than 0.7 [21, 22]. When the published discriminatory thresholds for obstruction (mean RI ≥ 0.70 and ΔRI ≥ 0.10) were applied, the sensitivity and specificity of Doppler USG were only 44% and 82%, respectively.

In our study of 54 patients, we found that the RI in obstructed kidneys was significantly higher than in the unobstructed kidneys (0.75 vs. 0.56; $p < 0.001$). The RI was higher in obstructed kidneys in all cases. The difference between the obstructed and unobstructed kidneys (delta RI) ranged from 0.09 to 0.38, with a mean delta RI of 0.24.

Saboo et al. [6] stated in their study that delta RI ≥ 0.006 was highly sensitive and specific, and with a higher delta RI specificity remained the same whereas the sensitivity fell rapidly. Our results correlate well with those reported in earlier studies by Badr et al. [23] and Miletic et al. [24].

We also studied the effect of the level of obstruction on RI values. When we compared levels of ureteric obstruction (proximal vs distal), distal obstructions had a higher mean RI value (0.79) than proximal obstructions (0.76), and the values were not statistically significant. However, the study done by de Toledo *et al.* [25] showed that patients with proximal ureteric obstructions have RIs higher than those with distal obstructions.

We also analyzed the utility of CDUS in patients who did not show dilatation of the PCS on ultrasound. Patients who did not have PCS dilatation on US were later confirmed to have obstruction on CT. RI values were higher in all these patients (0.71), with a delta RI of 0.24. Discriminatory RI ≥ 0.70, which is higher than the delta RI found in the study done by Platt JF et al. [26], was found to be highly sensitive (85%) and specific (93%) in this study.

Conclusion

Using the discriminatory threshold RI of ≥ 0.70, the overall sensitivity of CDUS in diagnosing acute ureteric obstruction is 85% and the specificity is 93%. CDUS is a useful tool for demonstrating altered renal perfusion in acute ureteric obstructions before pelvicalyceal system dilatation occurs, thus increasing the sensitivity and specificity. Gray-scale US should therefore, be complemented by CDUS as an adjunctive test in patients presenting with acute renal colic, as it is a very simple, noninvasive and easy-to-calculate parameter. The utility of this tool cannot be overemphasized in special cases like pregnant women and children, in whom a dilated pelvicalyceal system without acute obstruction is encountered more frequently. It is mandatory to differentiate obstructive and non-obstructive causes with US alone in this subset of patients, as further evaluation with CT is not desirable due to the hazard of ionizing radiation. Given the high propensity of any group of patients with urinary tract calculi to undergo frequent imaging studies due to recurrent ureteric colic and the presence of significant residual hydronephrosis, we should strive hard to minimize radiation dosage by avoiding frequent CT studies. At the same time, maintaining acceptable diagnostic accuracy is of paramount importance.

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Limitations of the study: US is an operator-dependent examination and the results are affected by patients' body habitus and availability of good window. An increase in the RI of intra-renal vessels is also found in case of vascular or parenchymal diseases (renal vein thrombosis, acute renal insufficiency, etc.). The CDUS changes can return to normal after 48 h [27], after which its utility is doubtful with reduced sensitivity and specificity.

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Author contributions VM: Conception, Design, Acquisition, Analysis and Interpretation of data, Review. KMS: Design, Acquisition, analysis, Interpretation. NE: Concept, Design, Review. BD: Concept, Design. All the authors have read the manuscript and approve the manuscript fully for publication.

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Compliance with ethical standards

Conflict of interest None.

Ethics approval Obtained from the Institutional Human Ethics committee (IHEC) of PSG institute of Medical sciences and Research, Coimbatore, Tamilnadu, India. Reference Number: 15/417.

Consent to participate It was obtained from each patient at the time of Ultrasound Scan and CT (wherever applicable) along with general consent prescribed by the institution. This consent is a verbal consent since no additional procedure was done or drugs were administered for the purpose of the study. The ultrasound and CT Scan (wherever applicable) were done at the request of the referring physician and only those studies performed at the request of the referring clinician were utilized for the study. It is approved by the institutional ethics committee.

Consent for publication Obtained from all the authors all the authors have read and approved the manuscript.

Availability of data and material Available.

References

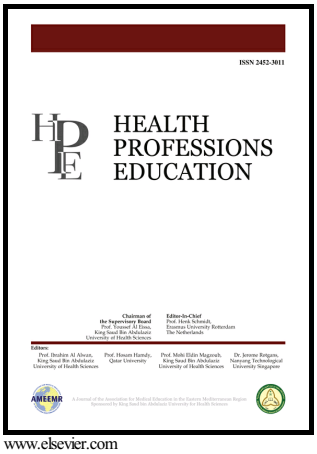
1. Piazzese EMS, Mazzeo GI, Galipo S, Fiumara Canfora F, Angio LG (2012) The renal resistive index as a predictor of acute hydronephrosis in patients with renal colic. J Ultrasound 15:239246
2. Sterrett S, Stephen N (2009) Emergency room follow-up trends in urolithiasis: single-center report. Urology 73:1195–1198. https://doi.org/10.1016/j.urology.2008.07.057
3. di Giacomo V, Trinci M, van der Byl G, Catania VD, Calisti A, Miele V (2014) Ultrasound in newborns and children suffering from non-traumatic acute abdominal pain: imaging with clinical and surgical correlation. J Ultrasound. https://doi.org/10.1007/s40477-014-0087-4
4. Ravindernath ML, Mahender Reddy G (2017) Mean resistive index as a prognostic tool for hydronephrosis in patients with acute renal colic: a study in a tertiary care. Int J Adv Med 4(2):329–333. https://doi.org/10.18203/2349-3933.ijam20170996
5. Tufano A, Minelli R, Rossi E, Brillantino C, Di Serafino M, Zeccolini M, Cantisani V, Vallone G (2017) Inferior epigastric artery pseudoaneurysm secondary to port placement during a robot-assisted laparoscopic radical cystectomy. J Ultrasound. https://doi.org/10.1007/s40477-020-00442-1
6. Saboo SS, Soni SS, Saboo SH, Chinapuvvula NR, Kaza S (2007) Doppler sonography in acute renal obstruction. Indian J Radiol Imaging 17:188–192
7. Azmi H (2003) Duplex doppler sonography in patients with acute renal colic: prospective study and literature review. Int Urol Nephrol 35(2):135–140
8. Jindal G, Ramchandani P (2007) AFP secondary to urolithiasis: radiologic evaluation and alternate diagnoses. Radiol Clin N Am 45(2007):395–410. https://doi.org/10.1016/j.rcl.2007.04.001
9. Apoku N, Ayoola O et al (2015) Ultrasound evaluation of obstructive uropathy and its hemodynamic responses in southwest Nigeria. Int Braz J Urol. 41:556–561. https://doi.org/10.1590/S1677-5538.IBJU.2014.0197
10. Ruiz-Martíneza A, Sierra-Díazb E et al (2019) Renal Doppler ultrasound resistive index vs renal scintigraphy with 99mTc-DTPA as diagnostic test for ureteropelvic junction obstruction in children. Actas Urol Esp 43(8):419–424. https://doi.org/10.1016/j.acuro.2019.02.005
11. Granata A, Zanoli L, Clementi S, Fatuzzo P, Fiorini F (2014) Resistive intrarenal index: myth or reality? Br J Radiol 87:20140004. https://doi.org/10.1259/bjr.20140004
12. Arumuham V, Brodie A, Bycroft J (2019) The management of urolithiasis. Surgery 37:7. https://doi.org/10.1016/j.mpsur.2019.04.003
13. Al-Ani A, Al-Jalham K, Ibrahim T, Majzoub A, Al-Rayashi M, Hayati A, Mubarak W et al (2015) Factors determining renal impairment in unilateral ureteral colic secondary to calcular disease: a prospective study. Int Urol Nephrol 47(7):1085–1090
14. Prigent A, Cosgriff P, Gates GF, Granerus G, Fine EJ, Itoh K, Peters M et al (1999) Consensus report on quality control of quantitative measurements of renal function obtained from the renogram: international consensus committee from the scientific committee of radionuclides in nephrourology. Semin Nucl Med 29(2):146–159. https://doi.org/10.1016/s0001-2998(99)80005-1
15. Rosenzweig B, Pinthus JH, Kleinmann N, Joffe E, Erlich T, Fridman E, Winkler H et al (2015) The relative contribution of urine extravasation to elevate plasma creatinine levels in acute unilateral ureteral obstruction. Can Urol Assoc J 9(7–8):428–433. https://doi.org/10.5489/cuaj.2804
16. El-Shazlya M, Aziza M et al (2016) Acute unilateral ureteric obstruction in young men with high serum creatinine: is it true or false renal impairment? World J Nephrol Urol 5(3):51–53
17. Sayani R, Ali M et al (2012) Functional evaluation of the urinary tract by duplex Doppler ultrasonography in patients with acute renal colic. Internat J Nephrol Renov Dis 5:15–21. https://doi.org/10.2147/IJNRD.S27628
18. Rodgers PM, Bates JA, Irving HC (1992) Intrarenal Doppler ultrasound studies in normal and acutely obstructed kidneys. Br J Radiol 65(771):207–212. https://doi.org/10.1259/0007-1285-65-771-207
19. Platt J, Ellis J, Rubin J (1991) Examination of native kidneys with duplex Doppler ultrasound. Semin Ultrasound CT MR 12:308–318
20. Tublin ME, Bude RO, Platt JF (2003) The resistive index in renal Doppler sonography: where do we stand? AJR Am J Roentgenol 180(4):885–892. https://doi.org/10.2214/ajr.180.4.1800885
21. Drudi FM, Liberatore M, Cantisani V et al (2014) Role of color Doppler ultrasound in the evaluation of renal transplantation from living donors. J Ultrasound. https://doi.org/10.1007/s40477-014-0077-6

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22. Spatola L, Andrulli S (2015) Doppler ultrasound in kidney diseases: a key parameter in clinical long-term follow-up. J Ultrasound. https://doi.org/10.1007/s40477-016-0201-x
23. Badr K, Brenner B (1988) Renal circulatory and nephron function in experimental obstruction of the urinary tract. In: Brenner BM, Lazarus J (eds) Acute renal failure. Churchill Livingstone, New York, pp 91–118
24. Miletic D, Fuckar Z, Sustic A, Mozetic V, Smokvina A, Stancic M (1998) Resistance and pulsatility indices in acute renal obstruction. J Clin Ultrasound 26(2):79–84
25. Toledo De et al (2017) Doppler-duplex ultrasound in renal colic. Eu J Radiol 23(2):143–1481996
26. Platt JF, Rubin JM, Ellis J (1989) Distinction between obstructive and nonobstructive pyelocaliectasis with duplex Doppler sonography. AJR Am J Roentgenol 153(5):997–1000. https://doi.org/10.2214/ajr.153.5.997
27. Gul H, Habib I et al (2014) Diagnostic accuracy of renal arterial resistive index in acute renal colic. KJMS 7:1

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Impact of a Comprehensive Early Clinical Exposure Program

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Impact of a Comprehensive Early Clinical Exposure Program

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Abstract

Purpose: To study the impact of an early clinical exposure program designed to provide a wide variety (cognitive, affective and psychomotor) of learning experiences for the preclinical year students.

Method: One hundred and fifty preclinical students were posted in small groups to selected departments – Transfusion medicine, Catheterization lab, Simulation Lab, Radiology, Neurology, Nephrology, Respiratory Medicine and General Surgery, . Each student had atleast 10 hours of clinical exposure under this program. The program was evaluated through a series of pre and post test questionnaires, which were designed based on the learning objectives of each session. Students were also asked to give their written open comments about the program.

Results: There was a significant increase in the post-test scores when compared to the pretest scores for all the sessions. Analysis of the open feedback showed that the program had significant impact on the cognitive, psychomotor and affective domains. "Application of basic

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sciences in clinical practice", "motivation to learn", "got familiar with various specialties", "insight about what the patient undergoes" were the themes identified from the open comments.

Conclusion: Implementation of an early clinical exposure program has been left to the discretion of individual institutions. The innovative use of Early Clinical Exposure program to teach/learn clinical skills like phlebotomy and Basic Life Support had been well appreciated by the students. The present design involving a variety of learning experiences has been successful in introducing the various dimensions of medical profession like scientific, ethical, interpersonal, professional and social to the new entrants in addition to enhancing their motivation to learn. A well designed ECE program could be the ideal first step in the making of a holistic doctor.

Keywords: attitude, learning, simulation lab, medical education, curriculum.

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1. Introduction

In India, students enter medical schools at an early age with little priming towards the profession. Entry of students in most of the medical colleges is entirely based on their academic performance with no weighting for humanistic attitude and communication skills. [1, 2] Due to lack of adequate exposure, most students do not realize what to expect in the medical profession. A few enter the medical college purely due to parental pressure. [3] Large classes, intense competition, exploding vocabulary and voluminous content delivered over a short time are some of the challenges faced by the first year MBBS students. [3, 4] Insufficient level of guidance and problems pertaining to transition from school to college life can also be included to this list of problems faced by the students during this period. Some students find the abrupt shift of medium of instruction from vernacular language to English difficult to cope. All these, along with the traditional curriculum, where the students are engaged in long hours of classroom and laboratory teaching with no exposure to the clinical setup, plays a major role in dousing the enthusiasm of the upcoming doctor. Under these circumstances, students often find their preclinical year to be tough, dreary and most importantly, they fail to understand the relevance of basic sciences in the clinical setting.

There has been an urgent call for curricular reforms with emphasis towards vertical and horizontal integration and competency based training.[5] Many parts of the world have responded to these needs by shifting away from discipline-based curriculum. In line with these changes, Medical Council of India (MCI) developed the 'Vision 2015' document. MCI vision 2015 aims to produce a new generation of medical graduates of global standards through curricular reforms. "The roles of this new age doctor are recognized as clinician, communicator, life-long learner, team leader and professional".[6] Required curricular changes begin early in the form of a

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foundation course; early clinical exposure; properly designed integrated teaching and programs to develop the correct attitude and communication skills (ATCOM module). [6]

Early clinical exposure (ECE) needs to be a coordinated effort by the preclinical, paraclinical and clinical faculty. If implemented effectively, it has the potential to improve motivation for learning and promote deep learning, better understanding and longer retention of the knowledge. It can facilitate the students to understand the application of basic sciences in clinical practice and aid in effective learning of clinical skills. It could also serve as a platform for students to improve their communication skills and get initiated into inculcating professionalism at a very early and impressionable stage of their medical education.[7] Above all, it provides an opportunity for them to see the illness from the patient's perspective. All this can foster the development of a holistic doctor who will be competent, communicative, humanistic and empathetic towards the patients.

To offer this experience, 'Clinical observership' program was started in PSG Institute of Medical Sciences and Research (PSGIMSR) in the year 2000. In this program, the first year MBBS students had the opportunity to spend half a day in the following selected specialties in small groups: cardiology, gastroenterology, radiology, nephrology and transfusion medicine. This initiative was highly appreciated by the students.[8] This program has now been modified and expanded to include more departments and implemented readily to suit the mandates from MCI as Early Clinical Exposure. The impact of this program was evaluated.

2. Methods

2.1 Participants

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One hundred and fifty first year MBBS students participated in early clinical exposure program conducted in the year 2015-16. Students were divided into 3 batches (A, B, C) based on their roll numbers. Each batch was further divided into 5 small teams comprising of 10 students. Each team spent an hour (from 4pm to 5 pm) in the clinical departments every day of the week in turns, ensuring that there were no more than 10 students in an ECE session. Though attendance for the ECE program was compulsory as per MCI and University guidelines, participating in the program evaluation and giving feedback was strictly on voluntary basis. Informed consent was obtained from the participants. The study was approved by the Institutional Human Ethics committee (No: 15/343)

2.2 Materials

The program was implemented in two cycles of three weeks duration. Five clinical departments were covered in each cycle. The first cycle of ECE sessions were conducted in the months of Nov-Dec 2015 and included rotations in Clinical simulation laboratory (for phlebotomy training), blood bank, and departments of neurology, radiology and surgery. During the second rotation of the program, which was conducted in Mar 2016, the students were posted once again in Clinical simulation laboratory (for Basic life support training) and radiology in addition to the departments of nephrology, respiratory medicine and cardiology (Catheterization Laboratory).

2.3 Procedure

An orientation session for the students was conducted by the program coordinator prior to starting the rotations, wherein the objectives of the program, clear directives regarding the conduct of the sessions and the expected goals of this innovative venture were highlighted.

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Clinical department postings were coordinated by a faculty in each department. The designate faculty drew out the learning objectives for this session based on the student's prior knowledge and availability of clinical materials. This arrangement was kept uniform for the entire class. Based on the learning objectives, questionnaires were formulated for all the sessions and used as pretest and posttest for the students. Students who wished to participate in the program evaluation gave informed consent, took part in the pretest and post test and were also encouraged to give their written open comments anonymously.

2.4 Data collection and analysis:

The questionnaires were designed in a five point Likert scale with 6-10 items to assess the effectiveness of ECE sessions. The scores used were 1 = not aware, 2 = not sure, 3 = know vaguely, 4 = know with reasonable clarity and 5 = know confidently. The questionnaire was meant to assess the impact of the program on the students. This was achieved by comparing the scores obtained by the students in pre-post tests. The scores were expressed as mean ± standard deviation and were compared by paired't' test using IBM SPSS software version 19.

The open comments regarding the program were analyzed through inductive approach. [9] Specific themes were identified and the comments were assigned to the appropriate theme.

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3. Results

Among the one hundred and fifty participants, one hundred and forty four participants submitted their filled in pre and post test questionnaires. There was a significant increase in the post-test scores when compared to the pretest scores for all ECE sessions (Table 1).

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Table 1- Comparison of pretest and posttest scores of ECE sessions

ECE Session	Pretest score (n=144)	Post test score (n=144)	P value
Radiology	28.69±6.11	36.65±6.62	<0.001
Neurology	21.44±5.77	30.14±7.55	<0.001
Surgery	22.57±5.93	34.31±7.76	<0.001
Simulation Lab (Phlebotomy)	15.54±4.75	24.06±4.60	<0.001
Blood Bank	7.94±2.31	9.14±2.67	<0.001
Nephrology	16.33±2.84	28.51±5.11	<0.001
Respiratory Medicine	15.3±12.59	23.27±4.74	<0.001
Simulation lab (Basic Life Support)	16.11±5.87	29.19±5.93	<0.001
Cardiology	18.08±5.44	29±5.33	<0.001

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One hundred and eight students had given their written open comments anonymously. The review of the open comments has been tabulated in Table 2.

Table 2 – Summary of open written comments

General	'Knew about various departments/ specialties in the hospital, their functioning & infrastructure' 'Made us familiar with various fields'

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Knowledge	'Learnt a lot, better way of learning' 'Able to correlate theory with practical work' 'Learnt the importance of basic science and its application in clinical medicine'
Psychomotor domain	'CPR - empowered us' 'Learnt and taught CPR to my family members' 'Observed live procedures, saw instruments, drugs'
Affective domain	'Insight about what patient undergoes' 'Learnt about the duties of a doctor' 'Learnt the importance of human life' 'Learnt how to handle a patient' 'Learnt to behave kindly towards patients'
Motivating	'Interesting motivating inspiring useful worthy awesome' 'invoked self interest' 'made us feel proud to be a doctor' 'My dream to walk into the hospital as doctor made true'
Suggestions	'Program timing can be increased' 'one more cycle' 'timing can be shifted to morning hours' 'can include all departments in hospital especially cardiothoracic surgery, oncology, gynecology, clinical lab and opportunity to interact with patients and staff'

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4. Discussion

Regulatory bodies of the medical education have spelled out clearly that the medical education needs to be geared to train professionals capable of providing holistic care to patients with compassion.[6] Early clinical exposure, if implemented effectively could very well initiate the changes in the medical education system in the right direction to achieve the stipulated goal.

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There have been no clear directives to implement this program in terms of content, duration and mode of delivery. This has left the challenging responsibility of designing ECE entirely to the individual institutions, and the resultant programs would certainly depend upon the resources and initiatives available. Reports on the details of the conduct of ECE are scanty.[11-13] Interactive lectures, case scenarios, case based learning and occasional hospital visits have been implemented by a few of the institutions, as part of ECE.[11-13] In PSGIMSR, this program has been implemented as postings to selected departments of not less than an hour's duration per session. Each student gets an exposure of at least ten hours of this experience. The posting involves a variety of experiences like observing an interventional or investigative procedure, seeing a patient in ward or observing the doctor-patient interaction in the outpatient clinics, observing the demonstration of clinical signs, learning and practicing skills like phlebotomy or Basic Life Support (BLS) on manikins in the Clinical simulation laboratory.

First year MBBS students have a demanding schedule with enormous course content being delivered in just 10-11 months. In such a crucial situation finding time for ECE is a challenge. Last hour of the working day was selected for this program without interfering the conventional teaching sessions. Keeping the program at the end of the day also gave an opportunity to extend the session beyond the allotted hour when required and accommodated the availability of the patients for a particular procedure.

Selection of departments were done with an intention to expose the students to a variety of learning experiences involving all the three domains of learning- cognitive, psychomotor and affective. The central theme was to make students relate the basic science subjects they were learning in lecture classes to the clinical practice. The program also included super specialty departments like cardiology, nephrology and neurology for which the students would not have

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the opportunity to be exposed in their regular curriculum. This provided a chance for the new entrants to interact with veteran specialists and super specialists, thus giving the students a peek at the prospects that lay ahead of them.

The departments to which the students were posted in each rotation were selected based on the subject matter covered in basic sciences until the time of the program. For example, the first rotation concurred with the completion of lower limb in anatomy, hence visiting the surgery department to see a case of varicose veins/ peripheral vascular disease was more pertinent than visiting Catheterization Lab. Catheterization Lab was reserved for the second rotation, which was scheduled when the students were learning or just finished learning thorax and abdomen in anatomy.

The learning objectives for each session were chosen carefully in view of student's prior knowledge, the availability of clinical material and relevance. In departments like nephrology, radiology, simulation lab, blood bank the content was fixed and all students were uniformly exposed. For example in nephrology, the students witnessed a patient undergoing dialysis. The principle of dialysis, biochemical alterations in the blood before and after dialysis was discussed. In other departments like respiratory medicine, surgery, neurology the experience was more impromptu, based on the availability of cases. In cardiology, the objective was to give the students an experience of observing coronary angiogram followed by a discussion on the relevant anatomical aspects. If there were no patients scheduled for angiogram at the time of ECE session they were allowed to view the recorded procedure.

Most students appreciated the ECE experience as 'inspiring', 'motivating', 'interesting' 'awesome', 'useful', 'good way of learning', 'evoked self interest'. They felt it helped them see

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the relevance of basic science in clinical practice. Interested students also contacted the clinicians in the specialty of their choice later and pursued their interest in the subject. For students who enter the medical profession without prior exposure, this program provided a bird's eye view of the opportunities that lay ahead of them as evidenced from their comments like 'Knew about various departments/ specialties in the hospital, their functioning & infrastructure' 'Made us familiar with various fields'

In the Clinical simulation Laboratory students had instructions in Phlebotomy in the first rotation and hands-on training in BLS in the second rotation. BLS enables a person to recognize and support people in life threatening emergencies until full medical care can be provided. Any trained person can perform this if the need arises. Introducing BLS training as early as possible is considered essential and may help to increase their eagerness to learn and passion for the profession.[10,14] ECE could be the ideal platform to introduce BLS training to the medical students. Students in their feedback expressed that learning BLS empowered them and made them feel more confident.

The program seemed to have helped students to develop a humanistic attitude towards the patients as evidenced by their comments like –'developed an insight about what the patient undergoes', 'learnt how to behave kindly with patients', 'learnt the importance of human life'. They also commented that they gained an insight about the various roles/duties of a doctor. Students were of the opinion that more departments need to be incorporated in the program and the duration of the program should be increased. Authors fully endorse these views and feel that time factor could probably be a major restriction for expanding this program as per the students' wishes.

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Conclusion:

ECE can serve as a platform for providing the preclinical year students a cosmic variety of experiences involving all the three domains of learning. It can also be adapted as the teaching strategy to introduce the various dimensions of medical profession like scientific, ethical, interpersonal, professional and social. This program if implemented effectively has the potential to be the ideal first step in the making of a holistic doctor.

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References:

1. Aggarwal S, Sharma V. The problems of medical education in a developing economy: The case of India. *Ann Trop Med Public Health*. 2012;5:627-629.
2. Solanki A, Kashyap S. Medical education in India: Current challenges and the way forward. *Med Teach*. 2014;36:1027-1031.
3. Mandal A, Ghosh A, Sengupta G, Das N, Mukherjee S, Bera T. Factors affecting the performance of undergraduate medical students: A perspective. *Indian J Community Med*. 2012;37:126-129
4. Swaminathan A, Viswanathan S, Gnanadurai T, Ayyavoo S, Manickam T. Perceived stress and sources of stress among first-year medical undergraduate students in a private medical college – Tamil Nadu. *Natl J Physiol Pharm Pharmacol*. 2016;6:9-14.

Accepted manuscript

ACCEPTED MANUSCRIPT

5. DM, Cooke M, O'Brien BC. Calls for reform of medical education by the Carnegie Foundation for the Advancement of Teaching: 1910 and 2010. *Irby Acad Med*. 2010;85:220-227.
6. Vision 2015 Medical Council of India.
7. Yardley S, Littlewood S, Margolis S, Scherpbier A, Spencer J, Ypinazar V et al. What has changed in the evidence for early experience? Update of a BEME systematic review. *Med Teach*. 2010;32:740-746.
8. Kumar PA and Kumar PN. Clinical observership: A desirable adjunct to preclinical training. *AMJ*. 2011;4:294-299.
9. Burnard P, Gill P, Stewart K, Treasure E, Chadwick B. Analysing and presenting qualitative data. *BDJ*. 2008;204:429-432.
10. Das M, Elzubeir M. First aid and basic life support skills training early in the medical curriculum: curriculum issues, outcomes, and confidence of students. *Teach Learn Med*. 2001;13:240-246.
11. Tayade MC , Bhimani N , Kulkarni NB, Dandekar KN. The impact of Early Clinical Exposure on First M.B.B.S. Students. *IJHBR*. 2014;2:176-81.
12. Rawekar A, Jagzape A, Srivastava T, Gotarkar S. Skill Learning Through Early Clinical Exposure: An Experience of Indian Medical School. *JCDR*. 2016;10:JC01-JC04.
13. Sathishkumar S, Thomas N, Tharion E, Neelakantan N, Vyas R. Attitude of medical students towards Early Clinical Exposure in learning endocrine physiology. *BMC Med Educ*. 2007;7:30. Published online 2007 Sep 5. doi: 10.1186/1472-6920-7-30

Accepted manuscript

ACCEPTED MANUSCRIPT

14. Athipathy V, Kumar PA, Tolstoy R, Jeyakrishnan P, Mohanasundaram P, Reddy A. Basic life support through early clinical exposure: Students' perspective. *AMJ* 2016;9:319–323.

Keywords: attitude, learning, simulation lab, medical education, curriculum.

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Original Research Article

Gallbladder motility in children with chronic functional abdominal pain

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ABSTRACT

Background: Chronic abdominal pain is a common gastrointestinal symptom in children that significantly lowers their quality of life. In adults, Gall bladder (GB) hypomotility / dyskinesia is associated with many functional abdominal disorders but there is scarcity of evidence on its role in childhood gastro-intestinal disorders. Aim of the study is to evaluate the GB motility in children with chronic functional abdominal pain (FAP).
Methods: Children aged 5-15 years with chronic abdominal pain fulfilling ROME-III criteria and healthy controls were included and all study participants were subjected to ultrasonographic evaluation of the gall bladder volume in fasting state and post - Fatty test meal (FTM) and its ejection fraction was calculated.
Results: Sixtysix children including 31 with chronic FAP underwent sonographic evaluation for GB motility. The mean ejection fraction (EF) of cases and controls were 51.72±17.76% and 57.3±23.26% (p value - 0.158). The mean EF of cases with upper abdominal pain and lower/peri-umbilical abdominal pain were 41.7±17.1% and 57.2±15.9% respectively (*p* value < 0.0001). Increasing BMI had no significant association with GB motility among the participants.
Conclusions: There is lack of significant association between children with chronic FAP and GB hypomotility. Children with upper abdominal pain have lesser GB EF, suggesting a possible abnormal GB motility.

Keywords: Childhood functional abdominal pain, Gallbladder, Ultrasonography

INTRODUCTION

Chronic abdominal pain is a common gastrointestinal symptom in children presenting to outpatient department. In United states and Europe, its prevalence rates ranges from 0.3 – 19% among school-going children and accounts for about 2% to 4% of all pediatric office visits.[1,2] Though it is not associated with any organic gastrointestinal pathology, children with FAP have a significantly lower quality of life and it contributes for school – absenteeism.[3] In 1999, the term abdominal pain related-functional gastrointestinal disorders in pediatric ROME II criteria, which included functional dyspepsia, functional abdominal pain (FAP), irritable bowel syndrome and abdominal migraine. These terminologies were further redefined based on duration of symptoms before diagnosis in ROME III criteria.[3]Many functional gastrointestinal disorders like functional dyspepsia, irritable bowel syndrome and disorders of Gall bladder (GB) has been studied for any possible association with abnormalities in GB motility, but the results are inconclusive.[4] In adults, GB hypomotility/dyskinesia is well established in idiopathic constipation and functional dyspepsia, but there is scarcity of evidence on its role in childhood functional abdominal disorders.[5,6] Our study

was designed to study the GB motility in children with functional abdominal pain.

METHODS

This crosssectional study was done in the outpatient department of pediatrics, PSG institute of medical sciences from January 2016 to August 2017. Children aged 5-15 years attending the OPD of the institution with chronic abdominal pain fulfilling ROME-III criteria, without any obvious cause for abdominal pain and with no previous abdominal surgeries were included in the study. Healthy children who visited the hospital for routine health visit or vaccination were included as controls. Institutional Human ethical committee approved the study and all study participants were included after obtaining informed consent. A detailed history including dietary and allergy history was obtained and clinical assessment was done. They were documented in a pre-designed data collection tool.

A total of 31 cases and 35 controls were recruited in the study and all study participants were subjected to ultrasonographic evaluation of the gall bladder in fasting state. A radiologist experienced in pediatric Sonography performed the evaluation using Philips iU22 xMATRIX ultrasound system. The Gall bladder was assessed for its area, thickness and volume, where area was derived by trace method and maximum GB width and length were measured using transverse and longitudinal images of the GB.

The volume of GB was calculated in fasting and 30-minutes following a fatty test meal using Dodd's formula, Volume (in cm^3) = 0.52 x [L x W x A], where L is the greatest length, W- greatest transverse width, and A-greatest anteroposterior diameter of the GB. The gallbladder ejection fraction (EF) for each examination was obtained from two-volume data using the following equation. Where V_0 = gallbladder volume in fasting state, Vn = gallbladder volume after the fatty test meal,

$$EF\ (\%) = [(Vo-Vn) / Vo] \times 100$$

For the purpose of this study, Children with ejection fraction of less than 40% were considered to have gallbladder hypomotility.

Statistical analysis was done using statistical package for the social science version-19 (SPSS) software. The fasting and post-prandial GB volume was expressed as mean ± SD and students t test was used to compare their means between groups. Chi-squared testing was applied to look for possible association between EF and BMI. A p-value less than 0.05 was considered significant.

RESULTS

There were a total of 66 children included in this study. The mean age of cases and controls were 10.14±2.71 years and 11.54±1.94 years respectively. The mean height of the cases group was 132.9±15.9 (range – 100 to 165cms) and that of controls was 145.14±11.6 (range – 110 to 159cms). The mean weight of cases and controls were 27.3 ± 10.5 Kg and 33.8 ± 6.6 Kg respectively. The mean body mass index (BMI) was 14.9±2.8 kg/m^2 (range of 11.3 to 26.3 kg/m^2) in case group and 15.9±1.8 kg/m^2 (range from 11.9 to 19.5 kg/m^2) in the control group. Out of 31 cases there were 11 boys (35.5%) and 20 (64.5%) girls respectively and in 35 controls there were 23 (65.7%) boys and 12 (34.3%) girls respectively (Table 1).

Table 1: Baseline characteristics in the study groups.

Clinical parameters	Cases (n=31)	Controls (n=35)	P value*
Age (in years)	10.14±2.71	11.54±1.94	0.08
Gender ratio	1:2	2:1	-
Height (in m)	1.34±0.15	1.43±0.11	0.06
Weight (in Kg)	27.3±10.8	34.31±6.3	0.03
BMI (Kg/m^2)	14.60±3.1	16.59±1.60	0.03
Abdominal pain			
Predominantly Upper (%)	11 (40%)	-	
Predominantly Lower (%)	19 (60%)		

*Student's T-Test

The mean fasting and post-prandial gallbladder volumes of cases and controls were 8.20±3.97 (cm^3), 7.52±3.13(cm^3) and 3.51±1.57 (cm^3) and 2.94±1.59 (cm^3) respectively. Though the fasting GB volumes were greater than post-test meal, in both groups, yet there was no statistical significance (P value – 0.390) (Table 2). The mean ejection fraction of cases and controls were 51.72±17.76% and 57.37±23.26% (p value – 0.158).

Table 2: Comparison of GB Volume and Ejection fraction in study groups before- and following a fatty test meal (FTM).

Groups	GB Volume (in mm^3)		Ejection Fraction (%)
	Fasting	Post-FTM	
Cases	8.20±3.97	7.52±3.13	51.95±18.02
Controls	3.51±1.57	2.94±1.59	57.04±23.03
P-value*	0.797	0.127	0.239

*Student's T-Test

The ejection fraction of GB were compared among children with functional abdominal pain who presented predominantly with upper or lower / peri-umbilical regions, to identify any possible association between the site of abdominal pain with GB motility. The mean ejection fractions of cases with upper abdominal pain and lower/peri-umbilical abdominal pain were 41.7±17.1% and 57.2±15.9% respectively (p value <0.0001), but the former did not qualify for the definition of GB hypomotility i.e. EF<40% (Table 3).

Table 3: Comparison of GB Volume and Ejection fraction based on the site of abdominal pain.

Site of Abdominal pain	GB Volume (in mm^3)		Ejection Fraction (%)
	Fasting	Post-FTM	
Predominantly Upper	7.41±3.13	4.17±2.15	41.73±17.13
Predominantly Lower	8.67±4.40	3.13±1.0	57.87±16.12
P-value*	0.497	0.25	0.019

*Student's T-Test

In order to identify any possible association between increasing BMI and GB contractility among cases, they were sub-grouped as, BMI<16 and BMI ≥ 16. There was equal number of children (n=4) in each subgroup with EF<40%, suggestive of GB dysmotility and their mean EF were 54.28 ± 17.99 % and 48.19 ± 17.53 % respectively (*p*-value < 0.001) (Figure 1).

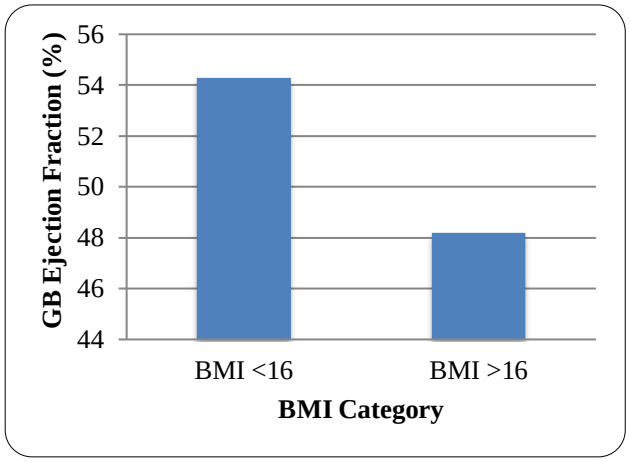


Figure 1: GB ejection fraction among bases grouped based on BMI.

DISCUSSION

In our study, we found no significant difference in the gall bladder volume (in fasting state and post Fatty test meal) among the cases and controls with equal number of children in both groups with GB ejection fraction <40%. Children who presented with upper abdominal pain had significantly lesser ejection fraction than those with lower abdominal pain. Many adult studies have recognized GB hypomotility in obese patients than non-obese patients. Sari et al found a positive correlation between fasting gallbladder volume and body mass index and body fat weight in obese women than non-obese women, but no such studies were available for comparison in children.[7] In the present study there existed no significant association in GB dysmotility and increasing BMI among cases.

The exact pathogenesis for FAP remains unclear, but visceral hypersensitivity, abnormal brain - gut interaction, immune dysregulation and altered gut motility has been postulated for the occurrence of the symptoms.[8,9] Gall bladder dyskinesia has been associated with various gastrointestinal symptoms like bloating, dyspepsia, post prandial epigastric pain and upper abdominal fullness.[10]

Mehra et alm observed gall bladder hypomotility in children with functional constipation, thus strengthening the claim of GB dyskinesia as a possible claim for functional abdominal symptoms in children.[11] Several imaging modalities like oral choledochocystography and syntigraphy are utilized to assess GB contraction but Sonography has been reliably used for assessing the GB area and volume, their changes following a fatty meal.[12] Ultrasonography could be preferred for its noninvasive nature and no radiation exposure that accompanies other modalities.

In our study we found 8 (26.7%) of the children with FAP healthy and 9 (25%) controls with mean GB Ejection fraction <40% suggesting that GB hypomotility could possibly be unrelated for the abdominal pain. Interestingly children who had predominant upper abdominal pain had low GB Ejection fraction compared to the children with lower abdominal pain (41.73±17.13% vs 57.87±16.12%) as noted by Hofeltd et al, in their study where a majority of children (96.67%) who underwent laproscopic cholecystectomy for biliary dyskinesia had upper quadrant pain.[13] A similar study with more subjects with FAP localized predominantly to upper abdominal could establish an association with GB motility.

Few limitations in our study are firstly the children were consecutively enrolled and sample size of the study is too small for drawing strong conclusions. Secondly the co-existing infection like H. pylori or parasitic infection of gut could possibly have an impact on the abdominal pain that was under evaluation. Nonetheless the present study is the first of its kind that has meticulously evaluated the GB volume and contractility in children with FAP and has found a possible association of GB dyskinesis with upper abdominal pain.

CONCLUSION

To conclude, abnormality in GB motility could be a cause for FAP presenting predominantly as upper abdominal pain in children thus necessitating its evaluation and a larger well-controlled study might help in better understanding of the entity.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Chitkara DK, Rawat DJ, Talley NJ. The epidemiology of childhood recurrent abdominal

pain in Western countries: a systematic review. The Am J gastroenterol. 2005;100(8):1868.
2. Spee LA, Lisman-Van Leeuwen Y, Benninga MA, Bierma-Zeinstra SM, Berger MY. Prevalence, characteristics, and management of childhood functional abdominal pain in general practice. Scandinavian journal of primary health care. 2013;31(4):197-202.
3. Youssef NN, Atienza K, Langseder AL, Strauss RS. Chronic abdominal pain and depressive symptoms: analysis of the national longitudinal study of adolescent health. Clinic Gastroenterol Hepatol. 2008;6(3):329-32.
4. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterol. 2006;130(5):1527-37.
5. Hemingway D, Neilly JB, Finlay IG. Biliary dyskinesia in idiopathic slow-transit constipation. Diseases of the colon & rectum. 1996;39(11):1303-7.
6. Velanovich V. Biliary dyskinesia and biliary crystals: a prospective study. The Am surg. 1997;63(1):69-74.
7. Sari R, Balci MK, Coban E, Karayalcin U. Sonographic evaluation of gallbladder volume and ejection fraction in obese women without gallstones. J clinic ultrasound. 2003;31(7):352-7.
8. Halac U, Noble A, Faure C. Rectal sensory threshold for pain is a diagnostic marker of irritable bowel syndrome and functional abdominal pain in children. J ped. 2010;156(1):60-5.
9. Bakker MJ, Boer F, Benninga MA, Koelman JH, Tijssen MA. Increased auditory startle reflex in children with functional abdominal pain. J ped. 2010;156(2):285-91.
10. Carr JA, Walls J, Bryan LJ, Snider DL. The treatment of gallbladder dyskinesia based upon symptoms: results of a 2-year, prospective, nonrandomized, concurrent cohort study. Surg Laparoscop Endoscop Percutaneous Tech. 200;19(3):222-6.
11. Mehra R, Sodhi KS, Saxena A, Thapa BR, Khandelwal N. Sonographic evaluation of gallbladder motility in children with chronic functional constipation. Gut and liver. 2015;9(3):388.
12. Dodds WJ, Groh WJ, Darweesh RM, Lawson TL, Kishk SM, Kern MK. Sonographic measurement of gallbladder volume. Am J roentgenol. 1985;145(5):1009-11.
13. Hofeldt M, Richmond B, Huffman K, Nestor J, Maxwell D. Laparoscopic cholecystectomy for treatment of biliary dyskinesia is safe and effective in the pediatric population. The Am surg. 2008;74(11):1069-72.

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Original Article

Evaluation of retrobulbar circulation in type 2 diabetic patients using color Doppler imaging

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Purpose: To investigate the retrobulbar circulatory parameters in type 2 diabetes mellitus patients with color Doppler imaging (CDI) and compare the results with nondiabetic controls. **Methods:** This prospective study included 50 type 2 diabetic patients and 50 age-matched controls. Seven field stereo fundus photography was used to diagnose and classify diabetic retinopathy (DR). Diabetic patients were further divided into two: Group 1, consisted of patients with no DR, mild and moderate non-proliferative DR (n = 36); Group 2, severe nonproliferative and proliferative DR (n = 14). CDI was performed using Philips iU22 xMATRIX ultrasound. The peak systolic velocity (PSV), end-diastolic velocity (EDV), resistivity index (RI) and pulsatile index (PI) of ophthalmic (OA), posterior ciliary artery (PCA), and central retinal artery (CRA) along with central retinal vein (CRV) were recorded. **Results:** RI in the ophthalmic artery was significantly higher in both DR groups than the control group (P = 0.000). Diabetic Group 1 had decreased blood flow velocity (PSV and EDV) in PCA compared to controls (P = 0.046 and P = 0.010, respectively). Group 2 DR had significantly reduced EDV and increased RI in CRA compared to Group 1 (P = 0.015). Binary logistic regression analysis revealed glycosylated hemoglobin and RI of OA to be independent risk factors of DR. **Conclusion:** Significant changes in resistivity index and flow velocities were observed in the retrobulbar vessels, especially in ophthalmic artery in diabetics compared to controls. CDI with results of increased resistance or decreased flow could be useful to predict individuals at higher risk for developing severe DR.

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Key words: Blood flow velocities, color Doppler ultrasonography, diabetes mellitus, retrobulbar

Ocular manifestations of diabetes are numerous and among them, diabetic retinopathy (DR) is a major clinical issue for ophthalmologists. DR is the most frequent cause of preventable blindness in working-age adults (20–74 years) and is therefore considered as a major public health issue.[1] Though treatable in appropriate stages, the prevention of retinal complications of diabetes and consequent visual impairment is vital to reduce the burden of DR. There is a felt need for better diagnostic modalities and surrogate markers in the evaluation and management of DR.

In spite of decades of research, the mechanisms behind the onset and progression of DR still remain unclear. From a hemodynamic point of view, evidence has shown that there is an early reduction in blood flow to retina before the onset of DR followed by a gradual increase as the retinopathy progresses.[2] Color Doppler imaging (CDI) is an established, safe, and noninvasive ultrasonic method useful for qualitatively and quantitatively assessing the blood flow velocities and indirect blood flow.[3] CDI has been suggested to be valuable in the diagnosis and management of several ocular diseases with altered hemodynamics such as glaucoma, retinal vascular diseases, ischemic optic neuropathy, and ocular ischemic syndrome.[4] An analysis of retrobulbar hemodynamics in diabetes using CDI can provide insight into the pathogenesis and prospective treatment for DR as well as predict those at higher risk of developing vision threatening DR. Several authors have evaluated the circulatory changes in diabetics using CDI and reported conflicting results. While few studies have reported a reduction in the retrobulbar blood flow velocities,[5-7] others have noted an increase[8] or no change.[9] Studies on the resistive index (RI) of orbital vessels which is a measure of peripheral vascular resistance have also shown significant discrepancies.[10,11] Factors such as demographic variables, duration of diabetes, methods of DR assessment, positions of CDI, and sample sizes account for the observed discrepancies. Moreover, most studies have not excluded patients with hypertension and those on medications affecting systemic blood flow which can influence the results. Studies on orbital hemodynamic alterations among type 2 diabetics from India are relatively few. The diabetic population in India is close to hitting the alarming mark of 69.9 million by 2025 and 80 million by 2030, denoting an expected increase of 266%. Hence, India is deemed as the world's capital of diabetes.[12] With increasing prevalence of diabetes and DR, it is important to know the hemodynamic alterations among diabetics from the Indian population.

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The objective of this study was to evaluate the flow velocities and vascular resistance of orbital blood vessels using CDI in a pure cohort of type 2 diabetic patients from India and compare the results with healthy controls.

Methods

This prospective hospital-based study was carried out at a tertiary care institution from south India between March 2017 and April 2019. The study included consecutive patients of type 2 DM (as per definition of the American Diabetes Association) attending the ophthalmology outpatient clinic for the screening of retinopathy. Exclusion criteria were history of any ocular disease that might affect ocular blood flow such as glaucoma, nondiabetic retinal vascular occlusion, ocular inflammation, high myopia, previous ocular surgery, or panretinal photocoagulation. Patients with systemic hypertension, dyslipidemia, nephropathy, and cardiovascular diseases were excluded from the study. Pregnant and breastfeeding women were not included. Patients taking medications known to affect systemic hemodynamics such as Angiotensin-converting enzyme inhibitors, calcium channel blockers, and anti-migraine drugs were also excluded.

50 age-matched healthy subjects were enrolled as a control group. The control subjects were recruited from individuals attending the department of ophthalmology for a routine eye examination. They were not on any medication and did not have ocular or systemic disease. The study was conducted according to the principles of the Declaration of Helsinki and the study protocol was approved by the institutional ethics committee. Informed consent was obtained from all participants.

Information regarding age, gender, body mass index, duration of diabetes, antidiabetic medications, and blood pressure were obtained from all participants. Serum glycosylated hemoglobin (HbA1c) was determined from fasting venous blood sample.

All study participants underwent a comprehensive ophthalmic examination including dilated fundus with slit-lamp biomicroscope using a 90 diopter lens and indirect ophthalmoscopy. A 30° stereoscopic fundus photograph of 7 standard early treatment diabetic retinopathy study (ETDRS) fields was taken from both eyes of diabetic patients using a digital fundus camera (TRC-50 DX Mydriatic retinal camera; Topcon Medical Systems, Oakland, NJ).

The presence, as well as the severity of diabetic retinopathy, was determined by grading of color fundus photographs following the international clinical disease severity scale for diabetic retinopathy which is based on the findings of WESDR (Wisconsin Epidemiological Study of Diabetic Retinopathy) and ETDRS.[13] The diabetic patients were assigned to one of the following groups namely no retinopathy, mild nonproliferative diabetic retinopathy (NPDR), moderate NPDR, severe NPDR, and proliferative diabetic retinopathy (PDR). The

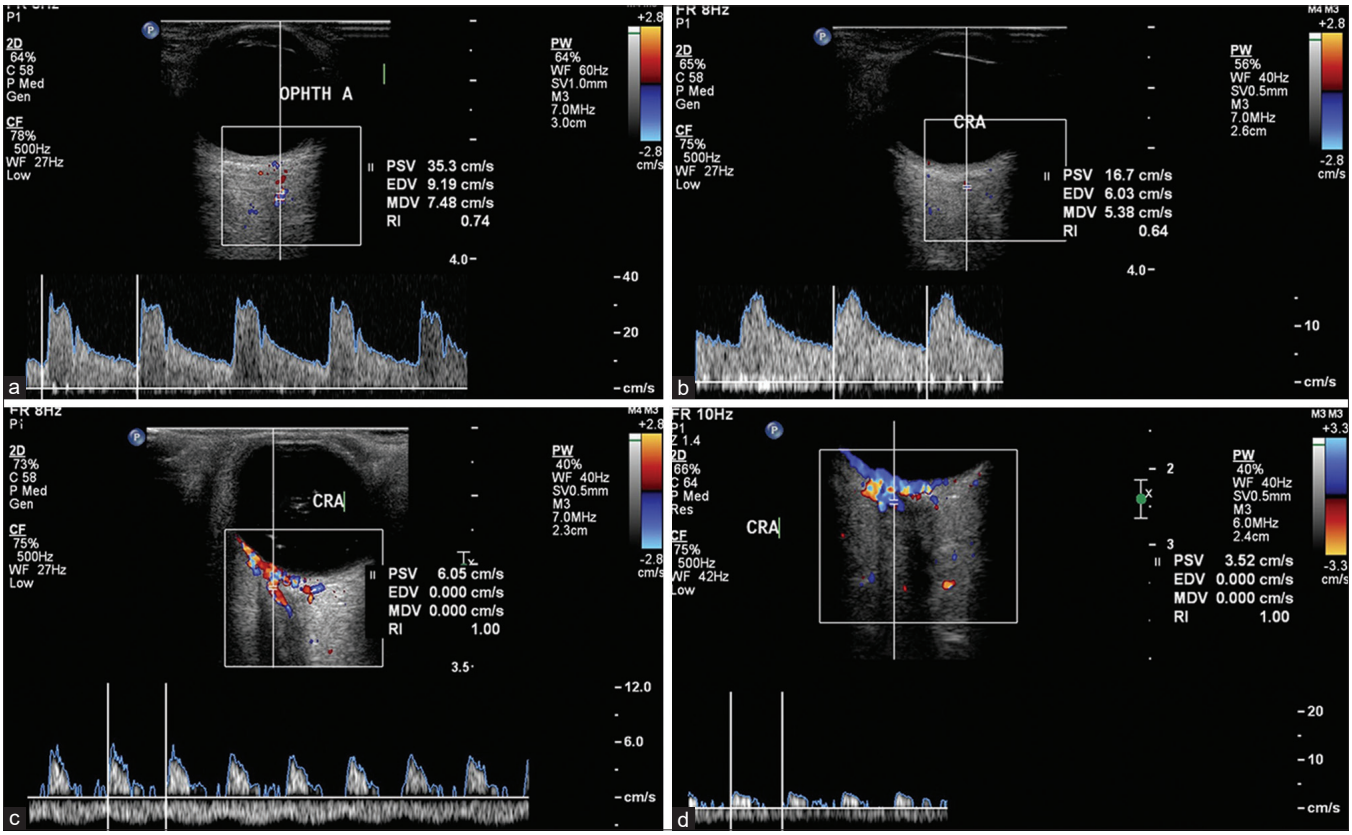


Figure 1: (a) Color Doppler image (CDI) showing normal color flow and spectral waveform in ophthalmic artery with preserved M wave pattern; (b) CDI showing normal color flow and spectral waveform in central retinal artery; (c) CDI showing low-velocity monophasic flow in central retinal artery in a patient with moderate NPDR; (d) CDI showing monophasic spectral waveform with absent diastolic flow in central retinal artery in a patient with severe NPDR

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eye with worse retinopathy was included in the study. If both eyes had equal retinopathy, the right eye was assigned to the study.

Color Doppler sonography of the eye was performed for cases and controls by a masked expert Radiologist using a color Doppler unit and a 12 to 5 MHZ linear-array transducer (Philips iU22 xMATRIX ultrasound system). The patients were examined in the supine position and care was taken to avoid any undue pressure on the eye. Examiner's hand was rested on the orbital margin and sterile coupling gel was applied over closed eyelids. The Doppler signal was registered online after the cursor was positioned on the region of interest. To reduce errors in measurement of velocities, angle correction was applied to the pulsed Doppler recordings.

The peak systolic velocity (PSV) and end-diastolic velocity (EDV) readings were obtained from the ophthalmic artery (OA), central retinal artery (CRA), and the posterior ciliary artery (PCA). Since the velocity wave in a central retinal vein (CRV) is not in direct relation to the cardiac cycle its hemodynamic parameters were expressed as maximum (V max) and minimum (V min) blood velocity. Signals from the ophthalmic artery were obtained from the medial aspect of the eyeball, superior to the optic nerve. The central retinal artery and vein were located within the optic nerve, around 10 mm behind the eyeball. [Fig. 1] Signals from the temporal branch of the short posterior ciliary artery were located lateral to the optic nerve.

With the help of PSV and EDV, we calculated the vascular resistance expressed by the resistivity index and pulsatile index using Pourcelot's formula.

Resistive index (RI) = (PSV-EDV)/PSV; Pulsatile index (PI) = (PSV-EDV)/Vmean

Statistical analysis

For statistical analysis diabetic patients were further divided into two groups; Group 1 included patients with no retinopathy, mild NPDR, and moderate NPDR while Group 2 consisted of patients with severe NPDR and PDR.

Statistical analysis was performed using statistical package for social sciences (SPSS, SPSS Inc, Chicago, IL, USA) version 24. Data were expressed as mean ± standard deviation. The normality of data distribution was tested by the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) with a *post hoc* Bonferroni was used for the analysis of normally distributed continuous data. Kruskal-Wallis analysis was used for analyzing non-normally distributed data. A comparison between hemodynamic parameters was calculated by ANOVA followed by Tukey-Kramer *post hoc* comparison. Pearson's coefficient was used to study the correlation between variables.

Table 1: Demographic and clinical characteristics of controls and diabetic subjects

Characteristic	Controls (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
Age (years)	56.5±9.00	55.5±8.60	51.9±5.64	0.209
Body mass index (kg/m^2)	23.34±3.62	24.81±3.15	24.53±3.88	0.141
Median Systolic BP (mmHg)	110 (20)	120 (18.75)	125 (12.5)	0.076
Median Diastolic BP (mmHg)	70 (30)	76.5 (10)	70 (10)	0.259
Median duration of diabetes (years)	-	3 (8)	10 (11)	0.115
Glycosylated hemoglobin (%)	5.81±0.29	9.09±2.13	10.86±2.33	0.014*

BP=Blood pressure; *P<0.05

Table 2: Hemodynamic parameters of retrobulbar blood vessels among controls and patient groups

Hemodynamic parameters (cm/s)	Control (*n*=50)	Group 1 (*n*=36)	Group 2 (*n*=14)	*P*
OA PSV	29.72±8.10	30.48±9.78	28.76±12.84	0.838
OA EDV	6.87±2.69	6.34±3.41	5.09±3.50	0.166
OA RI	0.75±0.06	0.79±0.06	0.83±0.05	0.000*
OA PI	1.58±0.27	1.74±0.30	1.86±0.25	0.002*
CRA PSV	12.42±3.85	10.40±4.87	8.42±2.92	0.004*
CRA EDV	2.73±1.17	2.33±1.27	1.27±0.94	0.000*
CRA RI	0.77±0.06	0.74±0.16	0.85±0.11	0.019*
CRA PI	1.63±0.30	1.65±0.50	2.08±0.65	0.004*
PCA PSV	15.44±5.61	12.81±3.83	14.95±5.15	0.054
PCA EDV	4.19±2.23	2.97±1.29	3.33±1.63	0.011*
PCA RI	0.73±0.06	0.75±0.08	0.78±0.08	0.061
PCA PI	1.45±0.24	1.58±0.37	1.68±0.45	0.044*
CRV V max	6.14±1.63	6.12±1.66	5.85±2.07	0.853
CRV V min	3.51±0.97	3.34±0.89	3.02±1.05	0.243
CRV RI	0.41±0.11	0.44±0.11	0.46±0.08	0.329
CRV PI	0.60±0.24	0.65±0.25	0.70±0.17	0.350

OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=Maximum blood velocity; V min=Minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

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Binary logistic regression analysis was performed to determine independent risk factors for retinopathy. All tests for statistical significance were two-tailed and performed assuming a type I error probability of <0.05.

Results

Demographic and clinical parameters

The study included 52 males and 48 females. The age of patients ranged from 41 to 76 years. The duration of diabetes ranged from 6 months to 20 years. Among the diabetic patients, 29 were on oral hypoglycemic agents, 9 were on insulin, and 12 were on combination therapy. Group 1 (n = 36) consisted of 16 patients with no DR, 13 with mild NPDR, and 7 with moderate NPDR. Group 2 (n = 14) consisted of 6 patients with severe NPDR and 8 with PDR. There were no statistically significant differences in the age, body mass index and blood pressure between controls and diabetic groups. Glycosylated hemoglobin (HbA1c) levels were found to be significantly higher in Group 2 diabetic patients (P = 0.014). No significant difference was noted in the duration of diabetes among diabetic groups. The demographic and clinical characteristics of controls and diabetic subjects are presented in Table 1.

Hemodynamic parameters

Table 2 shows the hemodynamic parameters of retrobulbar blood vessels among controls and patient groups.

Ophthalmic artery

Significant differences were noted in the RI and PI values of the ophthalmic artery between controls and diabetic patients with various DR grades. OA flow velocities did not vary between groups.

Table 3: Pearson correlation coefficients between the duration of diabetes, HbA1c, and hemodynamic parameters

Variable	Duration of diabetes	HbA1c
OA PSV	0.021 (0.885)	-0.120 (0.407)
OA EDV	-0.141 (0.328)	-0.055 (0.706)
OA RI	0.314* (0.026)	0.097 (0.504)
OA PI	0.299* (0.035)	0.078 (0.588)
CRA PSV	-0.135 (0.349)	-0.269 (0.059)
CRA EDV	-0.464* (0.001)	-0.299*(0.035)
CRA RI	0.375* (0.007)	-0.046 (0.753)
CRA PI	0.487* (0.000)	0.131 (0.370)
PCA PSV	-0.052 (0.722)	0.170 (0.239)
PCA EDV	-0.234 (0.102)	0.196 (0.172)
PCA RI	0.332* (0.019)	-0.075 (0.606)
PCA PI	0.321*(0.023)	-0.085 (0.556)
CRV V max	0.134 (0.354)	0.128 (0.375)
CRV V min	-0.143 (0.322)	-0.058 (0.687)
CRV RI	0.390* (0.005)	0.172 (0.233)
CRV PI	0.415*(0.003)	0.210 (0.144)

Corresponding P values are shown in brackets. HbA1c=Glycosylated hemoglobin; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; V max=maximum blood velocity; V min=minimum blood velocity; RI=Resistivity index; PI=Pulsatility index; *P<0.05

Central retinal artery and vein

The hemodynamic parameters of CRA were significantly different between controls, DR Group 1, and Group 2 whereas no such difference was seen in the CRV. *Post hoc* comparison revealed a significantly reduced PSV and EDV of CRA (P = 0.005 and 0.000, respectively) along with increased PI (P = 0.003) in DR group 2 compared to controls. Patients with group 2 DR also demonstrated a significant decrease of EDV and increased RI of CRA compared to group 1 (P = 0.015). PSV, EDV, and RI of CRA did not differ significantly between controls and DR group 1.

Posterior ciliary artery

The EDV and PI of PCA were found to vary significantly between controls and diabetic groups. Significant reduction in PSV and EDV of PCA was noted in DR group 1 compared to controls (P = 0.046 and P = 0.010, respectively after *post hoc* comparison).

Relation between duration of diabetes, HbA1c, and hemodynamic parameters

Duration of diabetes correlated positively with the resistive and pulsatile indices of all retrobulbar vessels studied namely ophthalmic artery, CRA, PCA, and CRV. HbA1c levels, as well as the duration of diabetes, were found to correlate negatively with EDV of CRA. No correlation was found between duration of diabetes and HbA1c levels (R = 0.183, P = 0.203). Table 3 shows the relationship between duration of diabetes, HbA1c levels, and hemodynamic parameters.

Binary logistic regression analysis revealed that HbA1c levels and RI of OA were independent risk factors of retinopathy.

Discussion

The main observations of our study are as follows: (1) RI in the ophthalmic artery was significantly higher in both DR groups than the control group and was an independent risk factor of retinopathy, (2) Patients with Group 1 DR had decreased blood flow velocity (PSV and EDV) in the posterior ciliary artery compared to control group, (3) Patients with Group 2 DR had significantly reduced EDV and increased RI in the CRA compared to Group 1, (4) Compared to the control group, patients with Group 2 DR had slower flow velocities and increased PI in CRA but RI values did not vary significantly.

Our finding of decreased EDV in the posterior ciliary artery in patients with Group 1 DR supports the hypothesis that choroidal blood flow is affected in the early stages of DR. Laser Doppler flowmetry has shown decreased choroidal blood flow in the foveal region in early DR.[14] Choroidal vascularity index (CVI) analysis by swept-source optical coherence tomography (OCT) has also shown reduced CVI in patients with mild/moderate NPDR.[15] Cao *et al.* have reported a 4 times more choriocapillary loss in diabetic eyes compared to non-diabetic eyes in histopathologic studies.[16] Ferrara *et al.* have demonstrated the loss of intermediate and large blood vessels in the Sattler's layer and Haller's layers in diabetic eyes in choroidal OCT.[17] Our observation of decreased EDV in the PCA suggests increased peripheral vascular resistance of the choroidal circulation in early DR and is in concordance with these studies. Dimitrova *et al.* reported decreased EDV and increased RI in PCA in patients

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with background DR compared to controls in CDI of diabetic patients performed in sitting posture.[6] The authors have suggested that erect posture during CDI measurement is essential to appreciate significant alterations in posterior ciliary circulation. Supine posture has been suggested to increase EDV and decrease the RI.[18] As autoregulation and autonomic nervous responses are altered in diabetics, the correlation of change in circulatory parameters between normal individuals and diabetics associated with postural variation may not be expected to be equal. However, CDI was performed in supine posture in our patients. Our observations could be attributed to the shorter duration of diabetes in our patients than theirs.

RI in OA was higher in both DR groups compared with controls in our study. These findings are similar to those of previous studies.[5,19] Basturk *et al.* evaluated the association between the resistivity index of orbital arteries and DR in 91 types 2 diabetics with microalbuminuria using CDI. The authors have demonstrated increased RI values of all orbital arteries in patients with DR compared to those without DR and controls. RI value of ≥ 0.72 in OA indicated the presence of DR with 78.4% sensitivity and 70% specificity.[10] Our finding of increased RI in the OA in both DR groups can be attributed to the statistically nonsignificant reduction in EDV observed in diabetic subjects because of downstream vascular changes in the retina and choroid. We did not observe significant differences in CRA parameters between controls and DR Group 1. Hence, we believe that the OA hemodynamic parameters in our study probably represent alterations in choroidal blood flow. It has been suggested that of the total ocular blood supply from OA, less than 10% flows to the retina while the remainder is directed to choroid via the ciliary circulation due to the high metabolic demand of photoreceptors.[20] Moreover, the diastolic blood flow of PCA is higher than CRA since these vascular channels of choroid have low resistance.[21]

Table 4: Various published studies on ocular circulation in diabetic patients using color Doppler imaging

Study	Country	Sample size	Duration of diabetes (years)	Findings
Arai *et al.*, 1998[9]	Japan	74 Controls: 22 NDR: 27 DR: 25	NA	Significant increase in RI of CRA in patients with DR compared to those without DR and controls (P<0.01)
MacKinnon *et al.*, 2000[5]	Scotland	62 Controls: 17 NDR/BDR: 24 PPDR/PDR: 21	NDR/BDR: 10 PPDR/PDR: 12	Significant decrease in both PSV and EDV of CRA in the PPDR/PDR group compared to NDR/BDR and control group (P<0.05) while RI showed no significant change. RI of OA was increased in diabetics compared to controls.
Dimitrova *et al.* , 2001[6]	Japan	95 Controls: 22 NDR: 38 BDR: 35	NDR: 9.0±7.2 BDR: 18. 9±11.2	Significant decrease in EDV and increase in RI of PCA in BDR group compared to controls (P=0.01 and 0.0003, respectively)
Gracner *et al.*, 2004[8]	Slovenia	66 Controls: 22 NPDR: 22 SNPDR/PDR: 22	13.5±9.31	Significant increase in PSV of OA in the SNPDR/PDR group compared to controls. Significant increase in RI of PCA in SNPDR/PDR group compared to NPDR and controls
Sullu *et al.*, 2005[7]	Turkey	34 Controls: 20 PDR: 14	13.5±6.1	Decrease in mean EDV and increase in RI of both OA and CRA in PDR compared to controls (P<0.05)
Baydar *et al.*, 2007[11]	Australia	44 (65 eyes) Controls: 11 Diabetics: 33	4.94±3.90	The RI of CRA was significantly higher in the control group than in the NDR group. RI of OA was significantly higher in NDR than in the moderate NPDR group.
Basturk *et al.*, 2012[10]	Turkey	118 Controls: 27 NDR: 40 DR: 51	NDR: 6.87±4.37 DR: 8.23±2.55	Patients with DR had significantly higher mean RIs of OA, CRA and PCA compared to those without DR and controls (P<0.001)
Karami *et al.*, 2012[30]	Iran	123 Controls: 25 BDR: 74 PPDR: 24	BDR: 5.6±0.7 PPDR: 12.6±1.1	RI of OA and CRV were significantly higher in patients with DR compared to controls (P<0.005)
Our study	India	100 Controls: 50 NDR/mild/ moderate NPDR: 36 SNPDR/PDR: 14	NDR/mild/moderate NPDR: 8 SNPDR/PDR: 11	RI of OA was significantly increased in diabetics compared to controls. Significant decrease in both PSV and EDV of PCA in mild/ moderate NPDR compared to controls (P<0.05) Significantly reduced EDV and increased RI of CRA in SNPDR/PDR compared to mild/moderate NPDR group (P=0.015)

NDR=No diabetic retinopathy; BDR=Background diabetic retinopathy; PPDR=Preproliferative diabetic retinopathy; NPDR=Nonproliferative diabetic retinopathy; SNPDR=Severe nonproliferative diabetic retinopathy; PDR=Proliferative diabetic retinopathy; OA=Ophthalmic artery; CRA=Central retinal artery; PCA=Posterior ciliary artery; CRV=Central retinal vein; PSV=Peak systolic velocity; EDV=End diastolic velocity; RI=Resistivity index; NA=Not available

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Khatri *et al.* assessed the correlation between RI of OA and topographic alterations of retinal pigment epithelium (RPE) on spectral-domain optical coherence tomography (SD-OCT).[22] In their study involving 75 patients with type 2 diabetes and 24 controls, a significant increase in OA RI was noted with the severity of DR ($F = 14.23$, $P < 0.001$). A positive correlation was noted between RI of OA and grades of RPE alterations on SD-OCT suggesting that decreased blood flow contributes to RPE dysfunction. Studies demonstrating early retinal damage in experimental diabetes have reported morphological changes in RPE and animal studies have also shown reduced choroidal blood flow in young diabetic animals suggesting that blood flow deficit could be an early pathological change in DR.[23,24] Although it is difficult to propose a causal association it can be postulated that changes in choroidal circulation (as reflected by increased RI of OA) lead to downregulation of antiangiogenic PEDF (pigment epithelial-derived factor) and upregulation of VEGF (vascular endothelial growth factor) thereby contributing to progression of DR. Differential expression and secretion of these growth factors derived from the retinal pigment epithelium have been suggested to lead to nonproliferative and proliferative DR.[25]

Patients with Group 2 DR had significantly reduced EDV in the CRA compared to Group 1 DR as well as healthy controls. Our observations are in line with previous studies.[5,6,8] These can be attributed to the increased peripheral vascular resistivity with the progression of DR. Pathological findings in DR such as capillary basement membrane thickening, retinal capillary leukostasis, loss of retinal capillaries, and altered blood rheology support this circulatory feature.[2,26,27]

Another observation of our study was that DR Group 2 had significantly reduced flow velocity in the CRA but an increase in RI was not statistically significant compared to the control group. This can be due to the concomitant decrease of both PSV and EDV in patients with Group 2 DR. Neudorfer *et al.* evaluated the long-term changes in the retrobulbar circulation of 138 eyes of diabetic subjects at a 10-year follow-up.[28] Patients were divided into four groups, namely, nondiabetic controls, diabetics without DR, nonproliferative DR, and proliferative DR. RI values of CRA and PCA had increased in the two no retinopathy groups and in the nonproliferative DR group, with a surprising nonsignificant decrease in the proliferative DR group. The authors have concluded that an increase of resistance in the retrobulbar blood vessels due to DR can decrease over time and may even be reversed. It has been suggested that the decrease in resistance reflects the beginning of an increase in retinal blood flow with the progression of DR due to the short-circuiting of the obstructed retinal capillary network.[28] This is supported by histopathologic studies, which have shown areas of capillary closure being traversed by preferential dilated anastomotic channels.[29]

Our finding of a positive correlation between duration of diabetes and RI of all retrobulbar vessels was similar to the observations of Karami *et al.*[30] We found negative correlation between EDV of CRA and duration of diabetes as well as HbA1c levels ($R = -0.464$, $P = 0.001$; $R = -0.299$, $P = 0.035$, respectively). Poor glycemic control (as reflected by increased HbA1c) and longer duration of diabetes can be associated with increased peripheral vascular resistance and thus reduced EDV in CRA. Our findings of HbA1c and RI of OA as independent risk factors of DR are similar to that of Basturk *et al.*[10]

Table 4 shows the various published studies on ocular circulation in diabetic patients using CDI.

Our study has several strengths. None of the diabetic patients had associated vascular diseases. Hypertension as well as antihypertensive medications significantly alter the resistive indices of blood vessels and therefore confound the results. We intentionally excluded these patients to study the influence of diabetes alone on ocular hemodynamics. A single masked, experienced radiologist performed all the Doppler examinations gently with the least pressure on the globe thus eliminating interobserver variability. The presence and severity of DR were graded based on seven field stereo fundus photography for accuracy.

To our knowledge, this study is the first of its kind in a pure cohort of diabetics from India.

There are a few limitations to our study. The sample size was small which accounts for the limited subgroup analysis. CDI provides information on blood flow velocity only and absolute volumetric blood flow cannot be quantified since it is not possible to study the diameter of retrobulbar vessels with CDI accurately. In spite of this limitation, blood velocity is probably a good indicator of blood flow within a given vessel.[31,32] Moreover, measurement of retinal blood flow is difficult and no accurate method to quantitatively assess perfusion is currently used clinically.

Conclusion

In conclusion, significant changes in the blood flow velocities and RI were observed in the retrobulbar vessels, especially in an ophthalmic artery in diabetics compared to controls. RI could be potentially useful for early diagnosis and follow-up of DR. CDI with results of increased resistance or decreased flow can be of help to identify diabetic individuals at higher risk for developing severe DR. This is especially useful in patients with opaque media where conventional methods are of little use.

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Conflicts of interest

There are no conflicts of interest.

References

1. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.

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2. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye (Lond) 2009;23:1496-508.
3. Aburn NS, Sergott RC. Orbital colour Doppler imaging. Eye (Lond) 1993;7:639-47.
4. Dimitrova G, Kato S. Color Doppler imaging of retinal diseases. Surv Ophthalmol 2010;55:193-214.
5. MacKinnon JR, McKillop G, O'Brien C, Swa K, Butt Z, Nelson P. Colour Doppler imaging of the ocular circulation in diabetic retinopathy. Acta Ophthalmol Scand 2000;78:386-9.
6. Dimitrova G, Kato S, Tamaki Y, Yamashita H, Nagahara M, Sakurai M, *et al.* Choroidal circulation in diabetic patients. Eye (Lond) 2001;15:602-7.
7. Sullu Y, Hamidova R, Beden U, Yakupov K, Canbaz S, Danaci M. Effects of pars plana vitrectomy on retrobulbar haemodynamics in diabetic retinopathy. Clin Exp Ophthalmol 2005;33:246-51.
8. Gracner T. Ocular blood flow velocity determined by color Doppler imaging in diabetic retinopathy. Ophthalmologica 2004;218:237-42.
9. Arai T, Numata K, Tanaka K, Kiba T, Kawasaki S, Saito T, *et al.* Ocular arterial flow hemodynamics in patients with diabetes mellitus. J Ultrasound Med 1998;17:675-81.
10. Basturk T, Albayrak R, Ulas T, Akcay M, Unsal A, Toksoy M, *et al.* Evaluation of resistive index by color Doppler imaging of orbital arteries in type II diabetes mellitus patients with microalbuminuria. Ren Fail 2012;34:708-12.
11. Baydar S, Adapinar B, Kebapci N, Bal C, Topbas S. Colour Doppler ultrasound evaluation of orbital vessels in diabetic retinopathy. Australas Radiol 2007;51:230-5.
12. Pandey SK, Sharma V. World diabetes day: Battling the emerging epidemic of diabetic retinopathy. Indian J Ophthalmol 2018;66:1652-3.
13. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, *et al.* Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Global Diabetic Retinopathy Project Group. Ophthalmology 2003;110:1677-82.
14. Nagaoka T, Kitaya N, Sugawara R, Yokota H, Mori F, Hikichi T, *et al.* Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. Br J Ophthalmol 2004;88:1060-3.
15. Kim M, Ha MJ, Choi SY, Park YH. Choroidal vascularity index in type-2 diabetes analyzed by swept-source optical coherence tomography. Sci Rep 2018;8:70.
16. Cao J, McLeod S, Merges CA, Lutty GA. Choriocapillaris degeneration and related pathologic changes in human diabetic eyes. Arch Ophthalmol 1998;116:589-97.
17. Ferrara D, Waheed NK, Duker JS. Investigating the choriocapillaris and choroidal vasculature with new optical coherence tomography technologies. Prog Retin Eye Res 2016;52:130-55.
18. Evans DW, Harris A, Garrett M, Chung HS, Kagemann L. Glaucoma patients demonstrate faulty autoregulation of ocular blood flow during posture change. Br J Ophthalmol 1999;83:809-13.
19. Tamaki Y, Nagahara M, Yamashita H, Kikuchi M. Blood velocity in the ophthalmic artery determined by color Doppler imaging in normal subjects and diabetics. Jpn J Ophthalmol 1993;37:385-92.
20. Cioffi GA, Granstam E, Alm A. Ocular circulation. In: Kaufman PL, Alm A, editors. Adler's Physiology of the Eye. 10th ed. St. Louis, MO: Mosby; 2003. p. 747-73.
21. Güven D, Ozdemir H, Hasanreisoglu B. Hemodynamic alterations in diabetic retinopathy. Ophthalmology 1996;103:1245-9.
22. Khatri M, Saxena S, Kaur A, Bhasker SK, Kumar M, Meyer CH. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retina Vitreous 2018;4:12.
23. Decanini A, Karunadharma PR, Nordgaard CL, Feng X, Olsen TW, Ferrington DA. Human retinal pigment epithelium proteome changes in early diabetes. Diabetologia 2008;51:1051-61.
24. Muir ER, Rentería RC, Duong TQ. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. Invest Ophthalmol Vis Sci 2012;53:6488-94.
25. Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO. Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 2003;22:1-29.
26. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. Microcirculation 2007;14:25-38.
27. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008;2:1130-8.
28. Neudorfer M, Kessner R, Goldenberg D, Lavie A, Kessler A. Retrobulbar blood flow changes in eyes with diabetic retinopathy: A 10-year follow-up study. Clin Ophthalmol 2014;8:2325-32.
29. Garner A. Histopathology of diabetic retinopathy in man. Eye (Lond) 1993;7:250-3.
30. Karami M, Janghorbani M, Dehghani A, Khaksar K, Kaviani A. Orbital Doppler evaluation of blood flow velocities in patients with diabetic retinopathy. Rev Diabet Stud 2012;9:104-11.
31. Hansen NB, Stonestreet BS, Rosenkrantz TS, Oh W. Validity of Doppler measurements of anterior cerebral artery blood flow velocity: Correlation with brain blood flow in piglets. Pediatrics 1983;72:526-31.
32. Taylor GA, Short BL, Walker LK, Traystman RJ. Intracranial blood flow: Quantification with duplex Doppler and color Doppler flow US. Radiology 1990;176:231-6.

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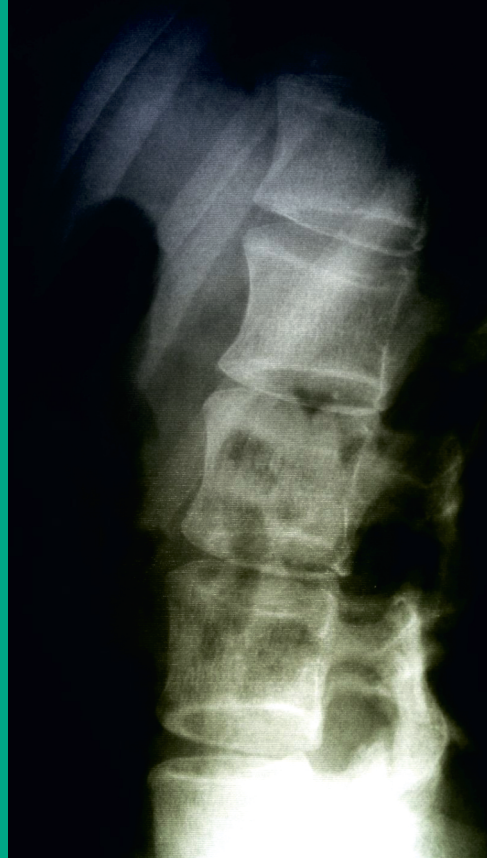
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Original Article

Utility of ultrasound elastography in evaluation of thyroid nodules and correlation with cytology

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Abstract

Background: Ultrasound elastography (USE) has emerged as a potentially useful tool in the evaluation of thyroid nodules. The aim of this study was to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with fine-needle aspiration cytology (FNAC) analysis as reference standard.

Materials and Methods: This was a cross-sectional prospective study carried out at a tertiary care center in South India between May 2014 and March 2015. Consecutive patients with solid nodule in the thyroid gland on conventional ultrasound (US) underwent USE, followed by US-guided FNAC of the thyroid nodule. The findings of B-mode images and USE were correlated with cytology. Statistical analysis was performed using SPSS version 24. Descriptive statistics were presented as mean ± standard deviation. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value of USE were calculated.

Results: A total of sixty patients with sixty solid thyroid nodules were enrolled in the study. The mean age of the patients was 48.27 ± 16.10 years. About 83.3% were females. The frequency of benign nodules on cytopathologic analysis was 60% (n = 36). Seventy-five percent of the malignant lesions had irregular or poorly defined borders with lobulated margins on US. The sensitivity, specificity, positive predictive value, and negative predictive value of USE were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as reference standard. Higher elastography scores were found to be significantly associated with malignant cytology (P = 0.0001).

Conclusion: Elastography, with its fairly high diagnostic accuracy and correlation with malignant cytology, can be an effective noninvasive adjunctive tool in distinguishing benign from malignant thyroid nodules.

Keywords: Elastography, thyroid nodules, ultrasound

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INTRODUCTION

The prevalence of thyroid nodules which are clinically palpable is about 2%–6% in the general population.[1] Although majority of these nodules are benign, up to 7% can be malignant.[2,3]

On palpation, a hard or firm nature is associated with an increased risk of malignancy. However, palpation is a highly subjective method and its value is limited in patients with multinodular goiter or deep-seated nodules. Ultrasonography (USG) is the most commonly used imaging modality to diagnose thyroid nodules. The utility of

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USG in differentiating benign from malignant lesions has been established by several studies.[4-6] However, no USG feature has been shown to have both high sensitivity and high specificity in detecting malignancy in thyroid nodules.[7] Elastography is a newly developed noninvasive dynamic imaging technique analogous to manual palpation. This technique uses ultrasound (US) to estimate tissue stiffness by measuring the degree of distortion under application of an external force.[8] Several studies have used US elastography (USE) for evaluation of thyroid disorders.[9-11]

Fine-needle aspiration cytology (FNAC) is an efficient tool in distinguishing benign nodules that can be managed conservatively from those with suspicious or malignant features that warrant further intervention. Limited data exist on the utility of elastography in differentiating benign from malignant lesions of the thyroid and its correlation with FNAC, especially from India. The aim of this study was to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with FNAC analysis as reference standard.

MATERIALS AND METHODS

This prospective study was carried out at a tertiary care center in South India between May 2014 and March 2015. Consecutive patients of either gender who were found to have thyroid swelling/goiter on clinical examination and referred for USG were considered. Only those with solid thyroid nodules at least 8 mm in size on conventional US were included in the study. Cystic lesions with no solid component, multinodular goiters with coalescent nodules, and nodules with eggshell calcification were excluded from the study. A large nodule involving more than 75% of thyroid lobe volume was also excluded because of inadequate normal thyroid tissue to be used as reference. The institutional review board approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants.

With a patient in supine position and neck fully extended, B-mode US of the thyroid gland was performed with SIEMENS ACUSON S2000 machine using high-frequency probes 9 L4 and 14 L5. US features studied included echogenicity, regularity of borders, presence of halo, vascular pattern, and calcification. Thyroid imaging reporting and data system (TIRADS) scoring was used to categorize the lesions[12] [Table 1].

USE was performed with SIEMENS ACUSON S2000 at the same time as the B-mode examination. The probe was placed over the region such that the lesion is in the center of the image, holding the scan plane perpendicular to the skin surface. At least 5 mm of normal adjacent tissue was included to assess the lesion stiffness in relation to the average elasticity of surrounding tissue. Patients were asked to hold their breath and avoid swallowing during examination in order to minimize the motion of the thyroid gland. The elasticity images were obtained by appropriate compression, followed by decompression of the transducer. The images were displayed on split-screen mode with US grayscale images on the left and USE images on the right. Multiple frames were acquired, and the best-fit B mode-USE image pairs were selected for examination [Figures 1 and 2]. The targeted lesion was scored as 1–4, using strain elastographic scores proposed by Asteria *et al*.[9] [Table 2].

US-guided FNAC of the thyroid nodule was done using a 22G needle, and the lesion was aspirated at least twice with the freehand technique. The aspirated contents were expelled onto glass slides and immediately fixed with 95% ethyl alcohol. The slides were stained with modified Papanicolaou staining and examined under light microscopy. The cytological results were classified

Table 1: Thyroid image reporting and data system scoring of thyroid nodules

Category	Description
TIRADS 1	Normal thyroid gland
TIRADS 2	Benign conditions
TIRADS 3	Probably benign nodules (<5% malignant)
TIRADS 4	Suspicious nodules (5%-80% malignancy rate)
TIRADS 5	Probably malignant nodules (malignancy rate >80%)
TIRADS 6	Biopsy-proven malignant nodules

TIRADS – Thyroid image reporting and data system

Table 2: Asteria's criteria of scoring of thyroid nodules on elastography

Elasticity Score	Description
Score of 1	Elasticity in the entire examined area
Score of 2	Elasticity in a large part of the examined area
Score of 3	Stiffness in a large part of the examined area
Score of 4	Nodule without elasticity

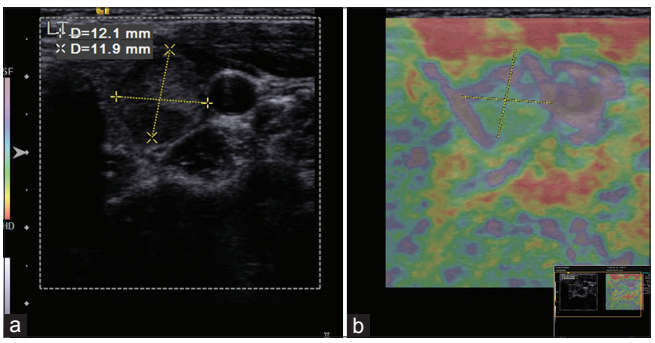


Figure 1: Thyroid nodule images obtained on conventional ultrasound (a) and ultrasound elastography with elasticity score 2 (b)

as benign, indeterminate/suspicious (follicular lesions), and malignant according to standard pathological criteria.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago. IL, USA) version 24. Descriptive statistics were presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages and compared using the Chi-square test. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were calculated. Statistical significance was set at $P < 0.05$.

For statistical purposes, USE score of 2 was strongly predictive of a benign lesion and therefore considered sample negative for malignancy. Scores of 3 and 4 describing stiffness in a large part of the examined area and nodule without elasticity, respectively, were considered as positive for malignancy in our study. With regard to cytology, two groups were considered for evaluation: benign and malignant. Patients with fine-needle aspiration (FNA) reports of indeterminate/suspicious (follicular lesions) were also included in the malignant group for statistical purposes.

RESULTS

A total of sixty patients with sixty solid thyroid nodules were enrolled in the study. The age of the patients ranged from 19 to 85 years (mean: 48.27 ± 16.10 years). About 83.3% (n = 50) were female and 16.7% (n = 10) were male. The frequency of benign nodules on cytopathologic analysis was 60% (n = 36), whereas 40% (n = 24) were nonbenign (follicular lesions and malignant tumors). Of the 24 nonbenign thyroid nodules, 20 had been reported as papillary carcinoma, 2 as medullary carcinoma, and 2 as follicular neoplasm. Table 3 shows the presenting clinical features and cytologic diagnoses of patients with thyroid nodules.

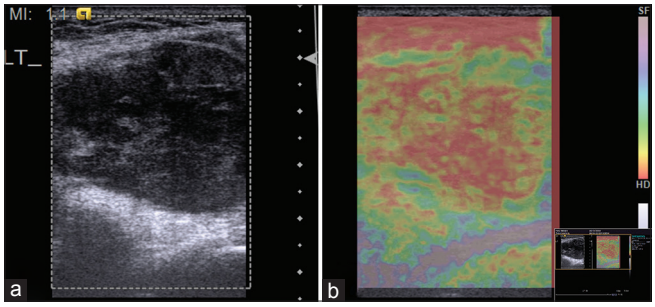


Figure 2: Heterogeneous nodule on conventional ultrasound (a) and ultrasound elastography with elasticity score 4 (b)

Seventy-five percent of the malignant lesions had irregular or poorly defined borders with lobulated margins on US and 68% had microcalcification. Seventy-seven percent of the benign nodules were hyperechoic. On TIRADS scoring, score of 2 was found in 24 cases, all benign lesions; score 3 in 14 cases, 8 benign and 6 malignant; score 4 in 12 cases, 4 benign and 8 malignant; and score 5 in 10 cases, all malignant lesions. Table 4 shows the correlation of USE scores and FNAC diagnoses of patients.

The sensitivity, specificity, positive predictive value, and negative predictive value of US elastography were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as a reference standard [Table 5]. Higher elastography scores were found to be significantly associated with malignant cytology ($P = 0.0001$).

DISCUSSION

Thyroid nodules are common in the general population, and the reported prevalence varies depending on the identification method. Apart from serum markers and FNAC, USG plays an important role in the detection and evaluation of thyroid nodules. Being noninvasive conventional US provides information regarding the nodule dimensions, structure as well as parenchymal changes and is widely used to determine which nodules should be biopsied. Microcalcifications, hypoechogenicity,

Table 3: Clinical presentation of thyroid nodules

Clinical feature	Benign	Malignant	Total number of patients, *n* (%)
Neck swelling	26	2	28 (46.7)
Weight loss	2	8	10 (16.7)
Dysphagia	2	6	8 (13.3)
Pain	6	2	8 (13.3)
Hoarseness of voice	0	6	6 (10)

Table 4: Correlation of elastography scores and cytologic diagnoses of patients with thyroid nodules

Elastography score	Benign	Malignant	Total
2	28	2	30
3	8	2	10
4	-	20	20
Total	36	24	60

Table 5: 2×2 table for calculation of sensitivity, specificity, positive predictive value, and negative predictive value of ultrasound elastography taking cytology as a reference standard

USE	Malignancy (positive on FNA)	Malignancy (negative on FNA)	Total
Positive	22 (true positive)	8 (false positive)	30
Negative	2 (false negative)	28 (true negative)	30
Total	24	36	60

FNA – Fine-needle aspiration; USE – Ultrasound elastography

irregular or microlobulated margins, taller than wide shape, and increased intranodular vascularity have been reported as independent risk factors for malignancy.[13,14] Although these features are characterized by high specificity, their positive predictive value is lowered by their relatively low sensitivity.[15]

USE is based on the principle that the softer parts of tissues deform easier than the harder parts, under compression, thereby allowing an objective determination of tissue consistency. Malignancy is often associated with changes in the mechanical properties of a tissue, and USE has been used to differentiate benign from malignant lesions in the breast, liver, and prostate.[16-18] The two general criteria for evaluating the efficacy of USE are elasticity scores and strain ratio. Although elastography has been suggested to be of great use as an adjunctive tool in the diagnosis of thyroid cancer, studies have reported conflicting evidence on its additional value in predicting thyroid malignancy.[19-21]

This study was planned to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with FNAC analysis as a reference standard. The mean age of presentation for benign nodules was 44.31 ± 14.28 years, whereas that for malignant lesions was 54.21 ± 17.13 years ($P = 0.018$) A majority of our cases diagnosed with thyroid nodules were females (83.3%). Thyroid nodules are reported to be four times more common in women than men, and their frequency increases with age and low iodine intake.[22] The female preponderance has been explained by the hormonal influences of estrogen and progesterone, as increasing nodule size and new nodule development have been demonstrated to be related to pregnancy and multiparity.[23]

Of the sixty nodules studied, 60% were benign ($n = 36$) and 40% ($n = 24$) were nonbenign. Of the 36 benign nodules, 26 were colloid nodules and 10 were adenomatoid nodules. The 24 nonbenign lesions included 20 papillary carcinomas, 2 medullary carcinomas, and 2 follicular neoplasms. The most common clinical presentation was painless mass in the neck which was encountered in almost 47% of the patients. Majority of the patients who presented with significant weight loss, dysphagia, and hoarseness of voice were found to have malignant lesions. Dysphagia, dysphonia, reports of rapid growth, male gender, prior head-and-neck irradiation, and presentation at extremes of age have been reported as significant elements in the patient's history, which increase the likelihood of malignancy.[24]

Our study found that 75% of malignant lesions had irregular or poorly defined borders with lobulated margins, whereas 68% of malignant lesions had microcalcification. Seventy-seven percent of the benign nodules were hyperechoic, whereas 23% were hypoechoic. None of the hyperechoic nodules was malignant. Although none of these US features is sufficient to differentiate benign lesions from malignant ones, studies have shown that a combination of at least two of the features is better helpful in pointing out a subset of lesions at high risk for malignancy.[25,26]

With regard to TIRADS score, all the nodules with TIRADS score of 2 ($n = 24$) had benign FNA characteristics, whereas all the patients with TIRADS score of 5 ($n = 10$) were found to have malignant lesions on FNA. Among TIRADS 3 lesions ($n = 14$), 57% were benign, and among TIRADS 4 lesions ($n = 12$), 33% were found to be benign on FNA.

On US elastography, score of 2 was found in 30 nodules, 28 benign and 2 malignant; score 3 in 10 cases, 8 benign and 2 malignant; and score 4 in 20 cases, all malignant. The sensitivity, specificity, positive predictive value, and negative predictive value of US elastography were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as a reference standard.

The elastography scores of 3–4 were achieved in 22 of the 24 malignant nodules in our study. Among the 20 nodules with cytologic diagnosis of papillary carcinoma, 18 were correctly scored as malignant by USE, thus achieving 90% diagnostic accuracy in terms of confirming the malignant etiology. Only two nodules were incorrectly given a USE score of 2. Both of the two nodules with medullary carcinoma thyroid had been scored as malignant on USE (score 4). We had eight false-positive nodules (score 3) which can be attributed to the fibrotic changes and calcification that lead to hardness of the nodule under study.

Our observations are in line with that of Mehrotra *et al.* who have reported the sensitivity and specificity of USE for thyroid malignancy to be 90% and 79.6%, respectively.[27] Of the 147 nodules examined by USE and US-guided FNAC, 122 were nonneoplastic, 5 were benign neoplasm, 10 had indeterminate cytologies, and 10 had malignant neoplasms. The observed positive and negative predictive values were 24.3% and 99.1%, respectively. In contrast, we found the positive predictive value of elastography to be 73.3% in our study. This can be explained by the high prevalence of malignant lesions among our study population.

The sensitivity and specificity of elastography based on elastography scores have been reported to vary from

86% to 97% and 66%–100% from various studies.[8,9,28] The observed wide differences are due to the fact that these studies involve different groups of populations, referral bias (patients referred for FNAC/surgery), varied inclusion and exclusion criteria, different machines as well as criteria for USE scoring. A recent comprehensive meta-analysis to investigate the diagnostic value of qualitative elastography in distinguishing benign from malignant thyroid nodules included 20 studies involving 3973 thyroid nodules.[29] Pooled results of elastography have shown a summary sensitivity of 85% (95% confidence interval [CI], 79%–90%) and specificity of 80% (95% CI, 73%–86%). The respective pooled negative predictive and positive predictive values were 97% (95% CI, 94%–98%) and 40% (95% CI, 34%–48%), respectively.

Despite the potential limitations of USE such as intra/interobserver variability and nodule characteristics such as calcification and follicular carcinoma (which appears soft), USE is considered to be reliable in the diagnosis of benign nodules in view of the high negative predictive values observed in most of the studies including ours.

FNAC is an essential diagnostic modality in the evaluation of thyroid nodules and features in all the guidelines in patient management.[30] Although the use of FNAC in the evaluation of thyroid nodules provides reliable results, it is invasive and the potential for complications such as postprocedural hematoma, increased patient morbidity, cost, and possibility of nondiagnostic specimen does exist. Unnecessary/repeat FNACs and FNA-induced changes can result in false-positive results on thyroid cytology and false-negative results or understaging of the tumor on thyroid pathology.[31,32]

Higher elastography scores were found to be significantly associated with malignant cytology ($P = 0.0001$) in our study. Merino *et al*. have also noted a statistically significant association between elasticity scores and cytological results, suggesting that malignant nodules could be excluded by elastography.[33]

Limitations of our study include small sample size and the fact that observations are from a single center.

CONCLUSION

With scanty literature comparing elastography with FNAC available from this part of the world, our study highlights that elastography has a fairly high diagnostic accuracy and correlation with malignant cytology. It can be an effective noninvasive adjunctive tool in distinguishing benign from malignant thyroid nodules and reduce the burden of unnecessary invasive procedures.

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Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Dean DS, Gharib H. Epidemiology of thyroid nodules. Best Pract Res Clin Endocrinol Metab 2008;22:901-11.
2. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, *et al*. Management of thyroid nodules detected at US: Society of radiologists in ultrasound consensus conference statement. Radiology 2005;237:794-800.
3. Hoang JK, Lee WK, Lee M, Johnson D, Farrell S. US features of thyroid malignancy: Pearls and pitfalls. Radiographics 2007;27:847-60.
4. Jena A, Patnayak R, Prakash J, Sachan A, Suresh V, Lakshmi AY. Malignancy in solitary thyroid nodule: A clinicoradiopathological evaluation. Indian J Endocrinol Metab 2015;19:498-503.
5. Bonavita JA, Mayo J, Babb J, Bennett G, Oweity T, Macari M, *et al*. Pattern recognition of benign nodules at ultrasound of the thyroid: Which nodules can be left alone? AJR Am J Roentgenol 2009;193:207-13.
6. Remonti LR, Kramer CK, Leitão CB, Pinto LC, Gross JL. Thyroid ultrasound features and risk of carcinoma: A systematic review and meta-analysis of observational studies. Thyroid 2015;25:538-50.
7. Listewnik MH, Birkenfeld B, Piwowarska-Bilska H, Cichoń-Bańkowska K, Iglińska-Wagner L, Watrak W, *et al*. The application of SPECT/CT scintigraphy with MIBI-Tc^{99}(m) in the diagnosis of thyroid nodules – A preliminary report. Endokrynol Pol 2010;61:422-6.
8. Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: New developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab 2007;92:2917-22.
9. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al*. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
10. Hu X, Liu Y, Qian L. Diagnostic potential of real-time elastography (RTE) and shear wave elastography (SWE) to differentiate benign and malignant thyroid nodules: A systematic review and meta-analysis. Medicine (Baltimore) 2017;96:e8282.
11. Hahn SY, Shin JH, Ko EY, Bae JM, Choi JS, Park KW. Complementary role of elastography using carotid artery pulsation in the ultrasonographic assessment of thyroid nodules: A prospective study. Korean J Radiol 2018;19:992-9.
12. Horvath E, Majlis S, Rossi R, Franco C, Niedmann JP, Castro A, *et al*. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab 2009;94:1748-51.
13. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, *et al*. Risk of malignancy in nonpalpable thyroid nodules: Predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab 2002;87:1941-6.
14. Moon WJ, Jung SL, Lee JH, Na DG, Baek JH, Lee YH, *et al*. Benign and malignant thyroid nodules: US differentiation-multicenter retrospective study. Radiology 2008;247:762-70.
15. Popoveniuc G, Jonklaas J. Thyroid nodules. Med Clin North Am 2012;96:329-49.
16. Chang JM, Won JK, Lee KB, Park IA, Yi A, Moon WK. Comparison of shear-wave and strain ultrasound elastography in the differentiation of benign and malignant breast lesions. AJR Am J Roentgenol

2013;201:W347-56.
17. Kim JE, Lee JY, Bae KS, Han JK, Choi BI. Acoustic radiation force impulse elastography for focal hepatic tumors: usefulness for differentiating hemangiomas from malignant tumors. Korean J Radiol 2013;14:743-53.
18. Miyanaga N, Akaza H, Yamakawa M, Oikawa T, Sekido N, Hinotsu S, *et al*. Tissue elasticity imaging for diagnosis of prostate cancer: A preliminary report. Int J Urol 2006;13:1514-8.
19. Moon HJ, Sung JM, Kim EK, Yoon JH, Youk JH, Kwak JY. Diagnostic performance of gray-scale US and elastography in solid thyroid nodules. Radiology 2012;262:1002-13.
20. Veyrieres JB, Albarel F, Lombard JV, Berbis J, Sebag F, Oliver C, *et al*. A threshold value in Shear Wave elastography to rule out malignant thyroid nodules: A reality? Eur J Radiol 2012;81:3965-72.
21. Trimboli P, Guglielmi R, Monti S, Misischi I, Graziano F, Nasrollah N, *et al*. Ultrasound sensitivity for thyroid malignancy is increased by real-time elastography: A prospective multicenter study. J Clin Endocrinol Metab 2012;97:4524-30.
22. Mazzaferri EL. Management of a solitary thyroid nodule. N Engl J Med 1993;328:553-9.
23. Kung AW, Chau MT, Lao TT, Tam SC, Low LC. The effect of pregnancy on thyroid nodule formation. J Clin Endocrinol Metab 2002;87:1010-4.
24. Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-71.
25. Papini E. The dilemma of non-palpable thyroid nodules. J Endocrinol Invest 2003;26:3-4.
26. Frasoldati A, Valcavi R. Challenges in neck ultrasonography: Lymphadenopathy and parathyroid glands. Endocr Pract 2004;10:261-8.
27. Mehrotra P, McQueen A, Kolla S, Johnson SJ, Richardson DL. Does elastography reduce the need for thyroid FNAs? Clin Endocrinol (Oxf) 2013;78:942-9.
28. Ghajarzadeh M, Sodagari F, Shakiba M. Diagnostic accuracy of sonoelastography in detecting malignant thyroid nodules: A systematic review and meta-analysis. AJR Am J Roentgenol 2014;202:W379-89.
29. Nell S, Kist JW, Debray TP, de Keizer B, van Oostenbrugge TJ, Borel Rinkes IH, *et al*. Qualitative elastography can replace thyroid nodule fine-needle aspiration in patients with soft thyroid nodules. A systematic review and meta-analysis. Eur J Radiol 2015;84:652-61.
30. Ahn SS, Kim EK, Kang DR, Lim SK, Kwak JY, Kim MJ. Biopsy of thyroid nodules: Comparison of three sets of guidelines. AJR Am J Roentgenol 2010;194:31-7.
31. Eze OP, Cai G, Baloch ZW, Khan A, Virk R, Hammers LW, *et al*. Vanishing thyroid tumors: A diagnostic dilemma after ultrasonography-guided fine-needle aspiration. Thyroid 2013;23:194-200.
32. Canberk Ş, Fırat P, Schmitt F. Pitfalls in the cytological assessment of thyroid nodules. Turk Patoloji Derg 2015;31 Suppl 1:18-33.
33. Merino S, Arrazola J, Cárdenas A, Mendoza M, De Miguel P, Fernández C, *et al*. Utility and interobserver agreement of ultrasound elastography in the detection of malignant thyroid nodules in clinical care. AJNR Am J Neuroradiol 2011;32:2142-8.

Original Article

Role of Transrectal Ultrasound Elastography in the Diagnosis of Prostate Carcinoma

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Abstract

Background: The purpose of this study was to evaluate the diagnostic value of transrectal real- time strain elastography (RTE) in identifying prostatic carcinoma (PCa). **Materials and Methods:** 60 patients suspected of having PCa based on abnormal digital rectal examination and raised prostate specific antigen levels underwent transrectal ultrasound (TRUS), color Doppler (CD) and RTE. Elastograms were scored on a five point scale based on distribution of strain in relation to hypoechoic area on TRUS. Twelve core systematic biopsy as well as targeted biopsy was performed from suspicious areas on TRUS and RTE. Diagnostic performance of sonoelastography was evaluated using histopathology as reference standard. **Results:** Histopathology revealed cancer in 28 out of 60 patients (47%) studied. Gleason score ranged from 6 to 9. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TRUS in detecting prostate cancer were 78.57%, 81.25%, 78.57%, and 81.25%, respectively. On CD evaluation 87.5% (n = 28) of benign lesions showed symmetric, radial flow compared to 14.3% (n = 4) of malignant lesions ($P < 0.0001$). The sensitivity and specificity of RTE was 89.29% and 56.25% with PPV and NPV being 58.13% and 82.35%, respectively. Higher elastography score was found to be significantly associated with malignant histopathology ($P = 0.004$). Cancer detection rate with RTE was greater for tumors with higher Gleason score. **Conclusion:** RTE was found to have better sensitivity than TRUS as well as combination of TRUS and CD. Although less specific, RTE can be an effective adjuvant tool to TRUS for guidance of biopsy and improve detection rate of PCa.

Keywords: Elastography, prostate cancer, ultrasonography

Introduction

Prostate carcinoma (PCa) is the second most frequently diagnosed cancer in males all over the world and is a major healthcare concern associated with significant morbidity and mortality. The burden of PCa is estimated to grow to 1.7 million new cases and 499000 new deaths by 2030 as a result of growth and aging of the global population.[1]

Digital rectal examination (DRE), screening for prostate specific antigen (PSA), and imaging techniques such as transrectal ultrasound (TRUS) and magnetic resonance imaging (MRI) are the methods commonly employed for detection of PCa. DRE is limited by its low sensitivity, subjective nature, and depends on the experience of the clinician. Although PSA testing has been widely used for screening of prostatic cancer, it is not disease specific and can be elevated in patients with prostatitis as well as benign prostatic hyperplasia.[2,3] In a patient with suspicion of prostatic cancer, current guidelines involve histopathologic examination of systematic biopsy cores to confirm or rule out cancer.[4] However, the conventional biopsy protocol misses significant cancer in a large proportion of patients.[5] Further many insignificant cancers that do not need immediate treatment are also detected leading to overdiagnosis and treatment.[6] Use of TRUS for detecting prostate cancer has limitations such as poor specificity of focal abnormalities, frequent multifocality of cancer within the prostate, and substantial percentage of isoechoic prostatic carcinoma (PCa).[7] Although MRI results are promising, it is expensive and not always available. In view of these limitations, improved noninvasive imaging modalities that

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can provide better characterization of prostatic lesions and increase detection of prostatic cancer are needed.

Ultrasound elastography (USE) is a dynamic technique that uses ultrasound to estimate tissue stiffness by measuring the degree of distortion under application of an external force. As cancerous tissue has greater stiffness than a benign lesion USE has been used to differentiate malignant from benign lesions in breast, thyroid, liver, and cervix. Recent studies have demonstrated the utility of sonoelastography in detecting prostate cancer.[8-10]

Data on the usefulness of transrectal strain elastography (SE) in evaluation of prostatic cancer and its correlation with histopathology are relatively few, especially from India. The purpose of the present study was to evaluate the usefulness and diagnostic performance of transrectal SE in identifying prostatic cancer with histopathologic diagnosis obtained by needle biopsy of prostate as reference standard.

Materials and Methods

This prospective study was performed in a tertiary care center in South India between December 2014 and September 2016. 60 consecutive patients who were referred to the department of Radiology with suspicion of PCa based on abnormal DRE and elevated PSA levels (>4 ng/ml) were enrolled. The institutional review board approved the study protocol and the protocol was in compliance with tenets of the Declaration of Helsinki (IRB approval Number: 14/399). Informed consent was obtained from all participants. Exclusion criteria consisted of patients with bleeding disorders, inflammatory bowel disease and surgical absence of rectum or ileoanal pouch. All patients underwent TRUS (grey scale and Doppler) and SE as well as needle biopsy of prostate in the same session.

TRUS of prostate was done using an endocavitary transducer (Siemens Accuson S3000, Siemens Healthineers, Erlangen, Germany) with 9–4 MHz range and 174 degree field of vision, capable of user selectable multi hertz imaging. Xylocaine gel was applied over a probe cover applied onto the probe. All patients were examined in the left lateral decubitus position. Prostate was imaged in both axial and sagittal planes with assessment of volume, echogenicity, surface, calcification, vascularity, and the presence of nodules. Each nodule was assessed for size, margins, location in the gland, morphology, and echogenicity.

Glands with homogeneous echotexture or minimal heterogeneity on TRUS were labeled as benign while those with ill-defined echotexture abnormality, focal bulge or hypoechoic mass were categorized as malignant. For Color Doppler (CD) imaging, capsular/periurethral flow and symmetric radial flow from capsular branches were considered benign while asymmetric or increased flow with disorganized pattern was labeled malignant.[11]

Using the same probe transrectal real-time strain elastography (RTE) of prostate was done by a single experienced radiologist. The probe was placed over the region such that the lesion is in the center of the image. Elastograms were obtained by slight compression and decompression of the prostate. The appropriate frequency and pressure of the compression/decompression was optimized with the help of real time display of quality index. Elastograms obtained with quality index equal to or >60 were only included. At least a 5 mm thickness of normal adjacent tissue was included, to assess the lesion stiffness in relation with the average elasticity of the surrounding tissue. A standardized color coding system was used, where red indicated soft, more compliant areas and blue indicated stiff areas. Green color indicated average strain in the region of interest. The elastograms along with conventional B-mode images were displayed side by side on split screen mode. Multiple frames were acquired and the best fit B mode-elastography image pairs were selected for examination. The elasticity patterns were scored using a five point scale proposed by Kamoi *et al*.[10] [Table 1] The cut off for malignancy was set between scores of 2 and 3 [Figures 1-4].

Sampling of the prostate was performed either in the sagittal or in the axial plane. Biopsies were taken using an 18 G biopsy gun under strict aseptic precautions. Extended core biopsy protocol (12 cores) was used. Additional targeted biopsy was carried out on suspicious areas identified by B mode US and elastography. The grade (degree of aggressiveness) of prostate cancer was evaluated on the basis of the Gleason score. No major complications were encountered after the procedure.

The findings of TRUS, Doppler and RTE were interpreted by consensus by two experienced radiologists who were blinded to patients' clinical profile and biopsy results. The histopathologic results were considered as the reference standard, and utility of RTE was evaluated.

Statistical analysis

Data was entered in the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. The diagnostic sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated with histopathologic analysis as reference standard. For analyzing the combined diagnostic performance of tests, the test was considered positive if either of them was

Table 1: Sonoelastography scoring system proposed by Kamoi *et al*.

Elastography score	Description
Score 1	Normal - homogeneous strain
Score 2	Probably normal - symmetric heterogeneous strain
Score 3	Indeterminate - asymmetric focal stiff lesion which is not related to any hypoechoic area
Score 4	Probably carcinoma (strain at the periphery of the hypoechoic lesion with sparing of the center
Score 5	Definitely carcinoma (no strain in the entire hypoechoic lesion or in the surrounding area)

suspicious for malignancy and negative if neither showed suspicious features. Pearson Chi-square test was used to study the association between categorical variables. $P < 0.05$ was considered as statistically significant.

Results

The age of the patients ranged from 53 to 88 years (Mean: 69.16 ± 9.40 years). Serum PSA levels ranged between 4.3 and 178.6 ng/ml (Mean 48.54 ± 56.69 ng/ml). No Significant difference in PSA levels were noted between patients with carcinoma and benign lesions of the prostate ($P = 0.083$). In 60 patients, 784 biopsy cores were harvested out of which 720 was according to the extended core biopsy protocol and 64 were targeted (36 and 28 targeted to areas found suspicious on B-mode US and RTE, respectively). Histopathology revealed cancerous focus in at least one biopsy core in 28/60 (47%) patients. Gleason score ranged from 6 to 9 (Mean: 7.42 ± 1.13). Among the 32 patients with benign histopathology results, 20 (62.5%) were found to have benign prostatic hyperplasia and 12 (37.5%) had prostatitis. The mean prostatic volume was 38.20 ± 18.25 cm^3 (range: 8–89 cm^3). The mean age of patients with benign and malignant lesions were 66.78 ± 9.56 years and 71.89 ± 8.59 years, respectively ($P = 0.035$).

Out of the 60 patients, 28 (47%) were suspected of having prostate cancer by TRUS. The sensitivity, specificity, PPV, and NPV of TRUS in detecting prostate cancer were 78.57%, 81.25%, 78.57%, and 81.25%, respectively, with histopathology as reference standard. On CD evaluation 87.5% ($n = 28$) of benign lesions showed symmetric, radial flow compared to 14.3% ($n = 4$) of malignant lesions ($P < 0.0001$).

Table 2 shows the correlation of elastography scores and histopathologic diagnoses of lesions. Out of the 32 patients

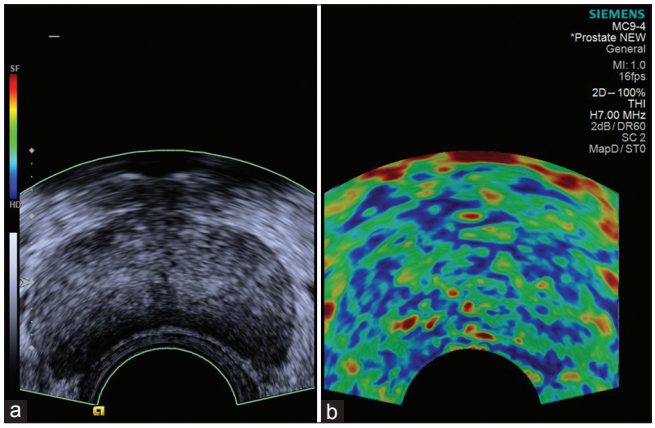


Figure 1: (a) Transrectal ultrasonography image showing mild enlargement of prostate. (b) Sonoelastography showing heterogeneous appearance with symmetrical mosaic pattern of green and blue (Score 2 – probably normal). Histopathology revealed hyperplasia of prostate with benign glandular pattern

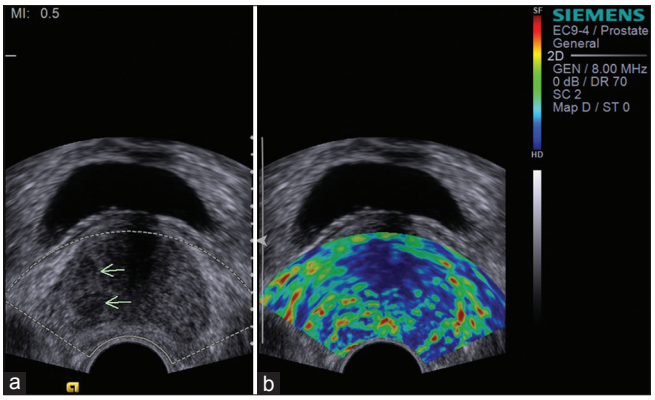


Figure 2: (a) Transrectal ultrasonography image showing hypoechoic lesion in the right peripheral zone (arrows) of prostate. (b) Sonoelastography showing focal asymmetric stiff lesion not related to hypoechoic area (score 3 – indeterminate). Histopathology was suggestive of adenocarcinoma prostate

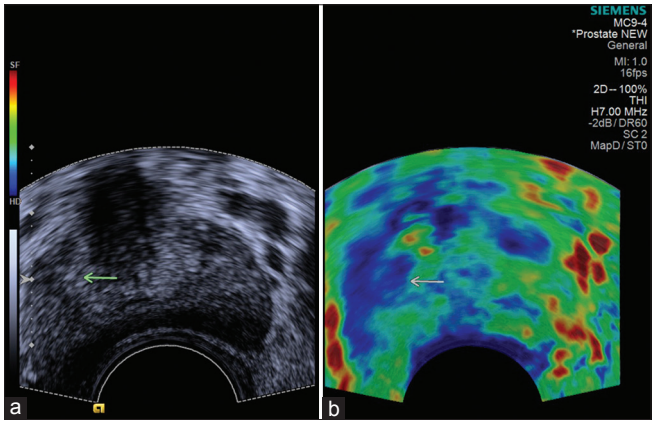


Figure 3: (a) Transrectal ultrasonography image showing hypoechoic lesion in the right peripheral zone (arrow) of prostate. (b) Sonoelastography showing stiffness in the center of the lesion and strain at the periphery; the peripheral part of lesion appears green and the central part appears blue (score 4 – probably carcinoma). Histopathology confirmed adenocarcinoma prostate

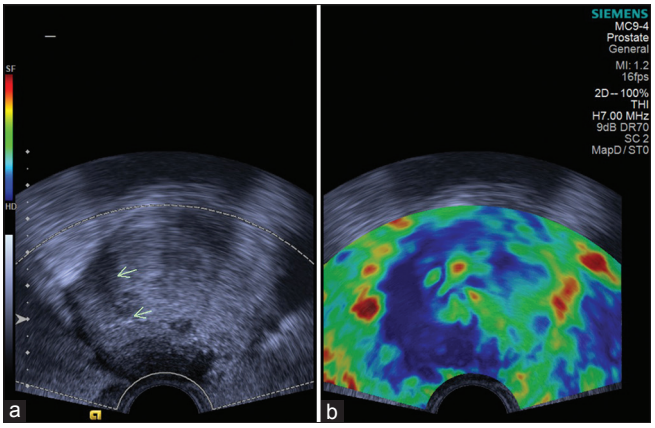


Figure 4: (a) Transrectal ultrasonography image showing hypoechoic lesion with irregular margin in the right peripheral zone (arrows) of prostate. (b) Sonoelastography showing stiffness in the entire hypoechoic lesion and in the surrounding area; the entire lesion appears blue (score 5 – definitely carcinoma). Histopathology confirmed adenocarcinoma prostate

with benign histopathology, 14 were judged as negative for malignancy at RTE. There were 3 false negative patients on elastography with score of 2. The sensitivity and specificity of RTE was 89.29% and 56.25% with positive and NPV of 58.13% and 82.35%, respectively. Higher elastography score was found to be significantly associated with malignant histopathology ($P = 0.004$).

Table 3 shows the relation between Gleason score and elastography scores. Approximately 84% of patients with a Gleason score ≤6 were detected by RTE whereas for scores 8–9, the detection rate was 100%.

The diagnostic performance of TRUS, combined modalities (TRUS and Doppler) and RTE are shown in Table 4.

Discussion

In this prospective study from South India, we evaluated the diagnostic performance of transrectal real-time elastography (RTE) in detection of prostatic cancer in patients with abnormal DRE and elevated PSA levels. RTE showed superior sensitivity of nearly 90% versus sensitivity of 79% by TRUS and 86% by a combination of TRUS and CD ultrasound.

Table 2: Correlation between elastography score and histopathologic diagnosis of lesions

Elastogram pattern	Benign ($n=32$)	Malignant ($n=28$)	Total ($n=60$)
1	0	0	0
2	14	3	17
3	18	4	22
4	0	9	9
5	0	12	12

Table 3: Relation between Gleason score and elastography scores

Gleason score	ES 1	ES 2	ES 3	ES 4	ES 5
≤6	0	1	0	2	3
7	0	2	2	6	2
8	0	0	0	1	1
9	0	0	2	0	6

ES: Elastography score

Table 4: Diagnostic performance of transrectal ultrasound, combined modalities (transrectal ultrasound and Doppler) and real-time strain elastography

Performance measure	TRUS	TRUS and doppler	RTE
Sensitivity (%)	78.57	85.71	89.29
Specificity (%)	81.25	75	56.25
Positive predictive value (%)	78.57	75	58.13
Negative predictive value (%)	81.25	85.71	82.35

TRUS: Transrectal ultrasound, RTE: Real-time strain elastography

Ever since the description of principles of elastography by Ophir *et al*., elasticity imaging has been applied to a wide variety of tissues such as breast, thyroid, liver, lymph nodes and prostate.[12]

The two US elastography techniques in clinical use are SE and shear wave elastography. Several studies have evaluated the utility of SE in diagnosing prostatic cancer and have shown varied results. While few have compared the diagnostic accuracy with needle biopsy of prostate,[13-15] others have used histopathologic examination of radical prostatectomy specimen as the reference standard.[8,9,16] The heterogeneous results can be attributed to the varied inclusion criteria, demographic characteristics of patients, serum PSA levels, location of tumors, reference standard, number of biopsy cores, systematic or targeted biopsies and Gleason score of the tumors examined.

The age of patients with malignancy of prostate was significantly higher compared to those with benign lesions in our study ($P = 0.035$). Age is considered to be an established risk factor for prostatic cancer and study of age-specific incidence curves have shown that risk of prostate cancer begins to rise sharply after 55 years of age and peaks at 70–74, declining slightly thereafter.[17]

In our study, TRUS revealed hypoechoic/focal lesions in 28 patients (47%). Out of these, 22 were confirmed as adenocarcinoma prostate while 6 turned out to be benign on histopathology. The results from our study in terms of specificity and PPV of TRUS in detecting prostatic cancer are similar to that of Ferrari *et al*. who had evaluated 84 patients suspected of prostatic cancer by TRUS, RTE and transperineal prostate biopsy.[18] B-mode US demonstrated 56% sensitivity, 80% specificity, 70% PPV and 67% NPV in diagnosing prostatic cancer. A hypoechoic lesion in the peripheral zone of prostate is suggested to be more likely malignant, the proposed cause being replacement of normal loose glandular tissue by a packed mass of tumor cells which results in fewer reflecting interfaces. Hyperplasia, prostatitis, fibrosis and cysts of prostate are other lesions that appear hypoechoic on TRUS.[19] Nearly 25%–40% of prostate cancers are isoechoic and missed by TRUS.[20]

CD and Power Doppler (PD) have been reported to be useful in detecting isoechoic tumors which may be missed by conventional ultrasound.[21,22] In 2 patients with prostatic cancer in our study, Doppler evaluation revealed asymmetric, increased flow while TRUS was not suspicious for malignancy. Few authors have shown that increased CD signal correlates positively with grading and staging of prostate cancer as well as risk of recurrence after treatment.[23,24]

On CD evaluation, 87.5% ($n = 28$) of benign lesions showed symmetric, radial flow compared to 14.3% ($n = 4$) of malignant lesions ($P < 0.0001$) in our study. A combination of TRUS and Doppler revealed superior diagnostic performance with sensitivity and NPV of nearly 86% compared to TRUS. However, benign lesions like prostatitis can also show

increased flow on CD and CD does not reveal microscopic vessels of prostatic cancer that do not possess enough flow to cause Doppler shift.[25,26] Despite these limitations, our findings indicate that CD imaging can be a useful adjunct to improve tumor detection by TRUS.

Prostatic cancer tends to be stiffer than benign lesions due to replacement of normal glandular tissue by neoplastic cells.[27] Stiffer tissues demonstrate less strain to deformation, and RTE helps in differentiation of benign and malignant tissues based on distribution of strain. Miyagawa *et al*. evaluated the usefulness of RTE in detecting prostatic cancer prior to systematic biopsy and have noted a diagnostic sensitivity of 72.6% for elastography and 89.5% for combination of TRUS and elastography.[14] Kamoi *et al*. used a five point subjective scale based on degree and distribution of strain in relation to hypoechoic area and found that RTE had 68% sensitivity and 81% specificity in detecting prostatic cancer. The combination of RTE with PD US further increased the sensitivity to 78%.[10]

Using the cutoff value of 3 (suggesting focal asymmetric lesion without strain unrelated to hypoechoic lesion) proposed by Kamoi *et al*., RTE was found to have sensitivity of 89.29% and specificity of 56.25% in our study. Of 43 patients with RTE findings suspicious for carcinoma, 25 were confirmed to be malignant on histopathology. Benign entities such as benign prostatic hyperplasia, adenomyomatosis, fibrosis and prostatitis can be associated with increased tissue stiffness and therefore may be difficult to distinguish from carcinoma prostate. This can be responsible for the relatively low specificity and PPV observed in our study. Because of the high frequency of false-positive results, RTE should be used in combination with TRUS to increase the detection rate.

In 5 of 28 patients with prostatic cancer (17.9%), B mode US was normal but RTE was suggestive of carcinoma. While systematic biopsy picked up malignancy in 3 patients, targeted biopsy based on RTE findings resulted in detection of cancer in 2 patients. The role of RTE targeted biopsy in detecting prostatic cancer has been reported by several authors. Pallwein *et al*. compared a 5-core RTE targeted biopsy with a 10-core systematic biopsy and concluded that cancer detection rate per patient was not significantly higher for targeted approach compared to systematic approach but an RTE targeted core was 2.9 fold more likely to be positive for cancer than a systematic core.[28] Salomon *et al*. analyzed the incremental detection rate of 4-core RTE targeted biopsy in addition to randomized 10-core in 1024 patients.[29] Additional use of RTE resulted in an incremental detection rate of 18.3%. 34 patients who harbored high-grade PCa (Gleason score ≥4) were diagnosed by RTE targeted biopsy only. A recent meta-analysis on the role of RTE targeted biopsy has shown that the detection rate of malignancy is enhanced when systematic biopsies are combined with RTE targeted biopsies.

Further RTE targeted biopsy can make a nearly equivalent diagnosis with fewer cores.[30]

In our study RTE detected 5 of 6 patients with prostatic cancer of Gleason score ≤6, 10 of 12 with score 7 and all with Gleason score 8 or 9. Our observations are similar to that of other authors who have noted that RTE detection rate of prostate cancer with higher Gleason score was greater than that of prostatic cancer with a lower Gleason score.[9,31] This might be explained by the greater cell density in high grade tumors resulting in stiffer tissue. In contrast, Tsutsumi *et al*. have noted a lower RTE detection rate for high grade tumors.[32]

Our study has few limitations. The sample size was small and no interobserver variability was studied since the RTE findings were interpreted by consensus. Another main limitation of RTE is that the degree of manual compression influences the elasticity image and may compromise diagnostic accuracy of RTE. However all examinations were performed by a single experienced radiologist and only images with optimal compression as displayed by the quality index were included in the study. Recent literature suggests that multiparametric MRI has greater sensitivity and specificity for detection of clinically significant prostate cancer.[33]

Although the incidence of prostatic cancer is low in Asian countries compared to the west, demographic and epidemiological transition in recent times have resulted in increase in the burden of various cancers including prostate in India. Prostate is reported to be the second leading site of cancer among males in several cities in India and cancer projection data estimate that the number of cases will become doubled by 2020.[34]

Conclusion

In this initial study from South India, RTE was found to have better sensitivity than TRUS as well as combination of TRUS and CD. With its greater sensitivity and NPV, RTE can be an effective adjuvant tool to guide biopsy and thereby increase the detection rate of prostatic cancer. Further prospective studies with larger sample size are needed before recommending RTE along with conventional TRUS for routine use in the evaluation of suspected prostatic malignancy in India.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Banu NA, Azim FA, Kamal M, Rumi MA, Barua AR, Khan KH. Inflammation and glandular proliferation in hyperplastic prostates: Association with prostate specific antigen value. Bangladesh Med Res Counc Bull 2001;27:79-83.
3. Ferrero Doria R, Pérez Flores D, Terrer Artes C, Guzmán Martínez-Valls PL, Morga Egea JP, Tomás Ros M, *et al*. Impact of prostatic benign hyperplasia and prostatic inflammation on the increase of prostate specific antigen levels. Actas Urol Esp 1997;21:100-4.

4. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, *et al*. EAU guidelines on prostate cancer. part 1: Screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 2014;65:124-37.
5. Lecornet E, Ahmed HU, Hu Y, Moore CM, Nevoux P, Barratt D, *et al*. The accuracy of different biopsy strategies for the detection of clinically important prostate cancer: A computer simulation. J Urol 2012;188:974-80.
6. Borza T, Konijeti R, Kibel AS. Early detection, PSA screening, and management of overdiagnosis. Hematol Oncol Clin North Am 2013;27:1091-110, vii.
7. Lemaître L, Villers A, Mouton D, Puech P. Transrectal ultrasound and biopsy of the prostate. J Radiol 2006;87:201-9.
8. Pallwein L, Mitterberger M, Struve P, Pinggera G, Horninger W, Bartsch G, *et al*. Real-time elastography for detecting prostate cancer: Preliminary experience. BJU Int 2007;100:42-6.
9. Sumura M, Shigeno K, Hyuga T, Yoneda T, Shiina H, Igawa M. Initial evaluation of prostate cancer with real-time elastography based on step-section pathologic analysis after radical prostatectomy: A preliminary study. Int J Urol 2007;14:811-6.
10. Kamoi K, Okihara K, Ochiai A, Ukimura O, Mizutani Y, Kawauchi A, *et al*. The utility of transrectal real-time elastography in the diagnosis of prostate cancer. Ultrasound Med Biol 2008;34:1025-32.
11. Halpern EJ, Ramey JR, Strup SE, Frauscher F, McCue P, Gomella LG. Detection of prostate carcinoma with contrast-enhanced sonography using intermittent harmonic imaging. Cancer 2005;104:2373-83.
12. Ophir J, Céspedes I, Ponnekanti H, Yazdi Y, Li X. Elastography: A quantitative method for imaging the elasticity of biological tissues. Ultrason Imaging 1991;13:111-34.
13. König K, Scheipers U, Pesavento A, Lorenz A, Ermert H, Senge T. Initial experiences with real-time elastography guided biopsies of the prostate. J Urol 2005;174:115-7.
14. Miyagawa T, Tsutsumi M, Matsumura T, Kawazoe N, Ishikawa S, Shimokama T, *et al*. Real-time elastography for the diagnosis of prostate cancer: Evaluation of elastographic moving images. Jpn J Clin Oncol 2009;39:394-8.
15. Brock M, von Bodman C, Palisaar RJ, Löppenberg B, Sommerer F, Deix T, *et al*. The impact of real-time elastography guiding a systematic prostate biopsy to improve cancer detection rate: A prospective study of 353 patients. J Urol 2012;187:2039-43.
16. Tsutsumi M, Miyagawa T, Matsumura T, Endo T, Kandori S, Shimokama T, *et al*. Real-time balloon inflation elastography for prostate cancer detection and initial evaluation of clinicopathologic analysis. AJR Am J Roentgenol 2010;194:W471-6.
17. Gann PH. Risk factors for prostate cancer. Rev Urol 2002;4 Suppl 5:S3-S10.
18. Ferrari FS, Scorzelli A, Megliola A, Drudi FM, Trovarelli S, Ponchietti R. Real-time elastography in the diagnosis of prostate tumor. J Ultrasound 2009;12:22-31.
19. Sarkar S, Das S. A review of imaging methods for prostate cancer detection. Biomed Eng Comput Biol 2016;7:1-5.
20. Shinohara K, Wheeler TM, Scardino PT. The appearance of prostate cancer on transrectal ultrasonography: Correlation of imaging and pathological examinations. J Urol 1989;142:76-82.
21. Newman JS, Bree RL, Rubin JM. Prostate cancer: Diagnosis with color Doppler sonography with histologic correlation of each biopsy site. Radiology 1995;195:86-90.
22. Okihara K, Kojima M, Nakanouchi T, Okada K, Miki T. Transrectal power Doppler imaging in the detection of prostate cancer. BJU Int 2000;85:1053-7.
23. Cornud F, Belin X, Piron D, Chrétien Y, Flam T, Casanova JM, *et al*. Color Doppler-guided prostate biopsies in 591 patients with an elevated serum PSA level: Impact on Gleason score for nonpalpable lesions. Urology 1997;49:709-15.
24. Ismail M, Petersen RO, Alexander AA, Newschaffer C, Gomella LG. Color Doppler imaging in predicting the biologic behavior of prostate cancer: Correlation with disease-free survival. Urology 1997;50:906-12.
25. Sharma S. Imaging and intervention in prostate cancer: Current perspectives and future trends. Indian J Radiol Imaging 2014;24:139-48.
26. Taverna G, Morandi G, Seveso M, Giusti G, Benetti A, Colombo P, *et al*. Colour Doppler and microbubble contrast agent ultrasonography do not improve cancer detection rate in transrectal systematic prostate biopsy sampling. BJU Int 2011;108:1723-7.
27. Krouskop TA, Wheeler TM, Kallel F, Garra BS, Hall T. Elastic moduli of breast and prostate tissues under compression. Ultrason Imaging 1998;20:260-74.
28. Pallwein L, Mitterberger M, Struve P, Horninger W, Aigner F, Bartsch G, *et al*. Comparison of sonoelastography guided biopsy with systematic biopsy: Impact on prostate cancer detection. Eur Radiol 2007;17:2278-85.
29. Salomon G, Drews N, Autier P, Beckmann A, Heinzer H, Hansen J, *et al*. Incremental detection rate of prostate cancer by real-time elastography targeted biopsies in combination with a conventional 10-core biopsy in 1024 consecutive patients. BJU Int 2014;113:548-53.
30. Tu X, Qiu S, Chang T, Jin K, Bao Y, Yang L, *et al*. The role of real-time elastography-targeted biopsy in the detection and diagnosis of prostate cancer: A systematic review and meta-analysis. Medicine (Baltimore) 2018;97:e0220.
31. Pallwein L, Mitterberger M, Pinggera G, Aigner F, Pedross F, Gradl J, *et al*. Sonoelastography of the prostate: Comparison with systematic biopsy findings in 492 patients. Eur J Radiol 2008;65:304-10.
32. Tsutsumi M, Miyagawa T, Matsumura T, Kawazoe N, Ishikawa S, Shimokama T, *et al*. The impact of real-time tissue elasticity imaging (elastography) on the detection of prostate cancer: Clinicopathological analysis. Int J Clin Oncol 2007;12:250-5.
33. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, *et al*. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): A paired validating confirmatory study. Lancet 2017;389:815-22.
34. Jain S, Saxena S, Kumar A. Epidemiology of prostate cancer in India. Meta Gene 2014;2:596-605.

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Original Article

Utility of Ultrasound Elastography to Differentiate Benign from Malignant Cervical Lymph Nodes

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Abstract

Background: The purpose of this study was to evaluate the usefulness of strain elastography and acoustic radiation force impulse (ARFI) imaging in the differentiation of benign and malignant cervical lymph nodes (LNs). **Materials and Methods:** In this prospective study, 50 enlarged cervical LNs (33 benign and 17 malignant) were examined by B-mode ultrasound (US), color Doppler, and strain elastography. Elastographic patterns (1–5) were categorized based on distribution of hard area within LN. The shear wave velocity (SWV) of LNs was evaluated by ARFI imaging. Diagnostic performance of sonoelastographic parameters was compared taking histopathology of LN as a reference standard. Optimal cutoff value of the mean SWV values for predicting malignancy was determined using receiver operating characteristic curve analysis. **Results:** Among US parameters, borders of LN had the highest diagnostic accuracy (80%), while echogenicity had the least (48%). Majority of benign LNs ($n = 31$) had elastography patterns 1 and 2, while majority of malignant LNs ($n = 16$) had patterns 3–5 ($P = 0.000$). The sensitivity, specificity, and accuracy of elastography were 94.1%, 93.9%, and 94%, respectively. The mean SWV of benign LNs (1.670 ± 0.367 m/s) differed significantly from malignant LNs (2.965 ± 0.826 m/s; $P = 0.000$). A cutoff value of 2.05 m/s predicted malignancy with 88.2% sensitivity and 84.8% specificity and gave an area under the curve of 0.949 (95% confidence interval: 0.70–1.20). **Conclusion:** Elastography has high diagnostic accuracy in differentiating benign and malignant cervical LNs and can be potentially useful in selecting the LN with high probability of malignancy, on which fine-needle aspiration cytology/biopsy can be performed.

Keywords: Acoustic radiation force impulse imaging, elastography, lymph nodes, ultrasound

Introduction

Cervical lymphadenopathy, a common condition encountered by physicians in clinical practice, could be caused by a variety of pathologic processes such as infections, vasculitis, and malignancies such as lymphoma and metastases. Metastatic cervical lymphadenopathy is a common problem in head and neck oncology. Depending on the primary site, up to 80% of patients with upper aerodigestive mucosal malignancy have been reported to have cervical nodal metastasis at presentation.[1] The differentiation of malignant from benign lymph nodes (LNs) is essential because it predicts the patient prognosis and helps in decision-making and planning of management.[2]

High-resolution ultrasound (US) is the extensively used imaging technique for evaluating the morphology and internal architecture of cervical LNs. Color Doppler sonography is used to assess the pattern of intranodal vessels, blood flow velocity, and vascular resistance.[3] Although Gray-scale sonography in combination with Doppler has been suggested to be useful in differentiating metastatic LNs from reactive ones based on various criteria, there is no single US criterion for diagnosing malignant LNs with satisfactory sensitivity and specificity.[4,5]

Real-time elastography (RTE) is a novel, noninvasive imaging modality that assesses tissue elasticity by comparing local tissue displacement from US signals before and after the application of a compressive force.[6] Stiff tissues show less deformation (strain) than soft tissues under the compression of a transducer. Since malignant tissues are stiffer than their benign counterparts at many

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sites, several reports have documented the utility of US elastography in differentiating malignant from benign lesions in the breast, prostate, liver, thyroid, and cervix.[7-11] There are two types of elastography techniques, namely strain elastography and shear wave-based elastography. Recent studies have shown the usefulness of strain elastography in differential diagnosis of benign and malignant cervical LNs.[12-14] However, some authors have questioned its additional value over conventional US in predicting malignancy and decision-making in terms of selection of LNs for biopsy.[15-17] Shear wave elastography with acoustic radiation force impulse (ARFI) imaging is an emerging sonographic technique in the assessment of malignant cervical nodes.[18,19]

Data on the utility of elastography in the evaluation of cervical LNs are relatively few, especially from India. The purpose of the present study was to evaluate the utility and diagnostic performance of strain elastography compared to conventional B-mode US and color Doppler in the differentiation of benign and malignant cervical LNs. We also investigated the usefulness of ARFI imaging in differentiating benign and malignant LNs.

Materials and Methods

This prospective study was performed at a tertiary care center in South India between August 2016 and February 2018. Fifty LNs of 50 consecutive patients who were referred for ultrasonogram of enlarged cervical LNs were enrolled. The Institutional review board (IRB) approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki (IRB Approval No. 16/334). Informed consent was obtained from all participants. Patients with suboptimal image quality and those with completely necrotic nodes without solid component were not included. Patients who had received specific treatment (chemotherapy/radiotherapy) were also excluded from the study.

High-resolution US imaging was performed in the supine position with a high-frequency linear probe 9 L4 (Siemens Accuson S3000, Siemens Healthineers, Erlangen, Germany) by a single expert radiologist, blinded to patient's clinical profile. In gray-scale US, the LNs were evaluated for short-axis diameter and short-to-long axis diameter ratio (S/L ratio) in the longitudinal plane, borders (regular or irregular), echogenicity (homogeneous/heterogeneous), and hilum (present or absent). Intranodal vascular pattern was evaluated using color Doppler imaging. Absent flow signals or blood flow limited to the hilum was categorized as benign. Nonhilar blood flow with spotted, peripheral, or mixed types of vascularization was categorized as malignant. RTE was performed for the targeted node using the same probe. Manual light compression and decompression of the target LN by the transducer were performed to achieve an optimal image. Patients were asked to hold their breath and avoid swallowing during the examination to minimize motion of LNs. The elastographic box contained the LN and adjacent sternocleidomastoid muscle for each patient. Elastograms obtained with quality factor score ≥60 were included in the study. Color map settings with red coded as hard area and blue coded as soft area were used. The images were displayed on split screen mode with US gray-scale images on the left and elastogram images on the right. The best-fit B-mode US elastogram image pairs were selected for analysis. The elasticity patterns were categorized on a 5-point scale described by Alam *et al*.[12] [Table 1]. LNs showing type 1 and 2 elastography patterns were considered benign, while those with types 3, 4, and 5 were categorized as malignant.

For ARFI imaging, the targeted LN on B-mode image was identified using a region of interest, characterized by a fixed box [Figures 1-5]. Three measurements of shear wave velocity (SWV) were performed, and the average was calculated. SWV was expressed in meter/second.

Table 1: Elastographic patterns of lymph nodes

Patterns	Description
1	Absent or very small hard area
2	Small, scattered hard areas, hard area <45% of LN
3	Hard area ≥45% of LN
4	Peripheral hard area and central soft area
5	Hard area occupying entire LN

LN: Lymph node

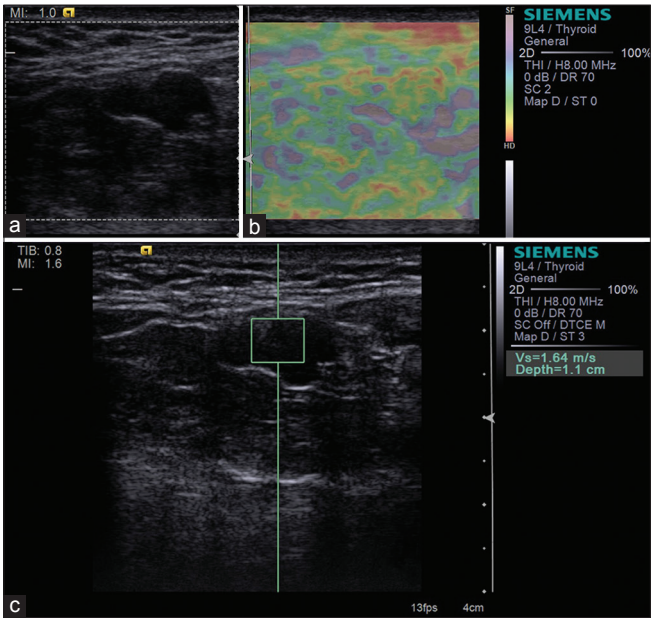


Figure 1: (a) B-mode ultrasound showing oval hypoechoic lymph node with irregular margins and absent hilum, (b) elastography image showing absent or very small hard area corresponding to pattern 1, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 1.64 m/s in the boxed area. Histopathology was suggestive of tuberculous lymphadenitis

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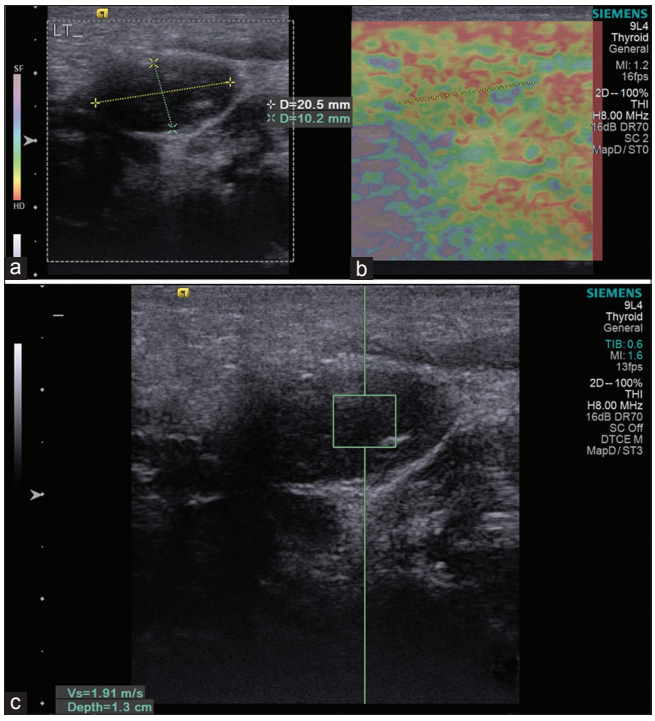


Figure 2: (a) B-mode ultrasound showing oval hypoechoic lymph node, (b) elastography image showing small, scattered hard areas corresponding to pattern 2, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 1.91 m/s in the boxed area. Histopathology was suggestive of reactive lymphadenitis

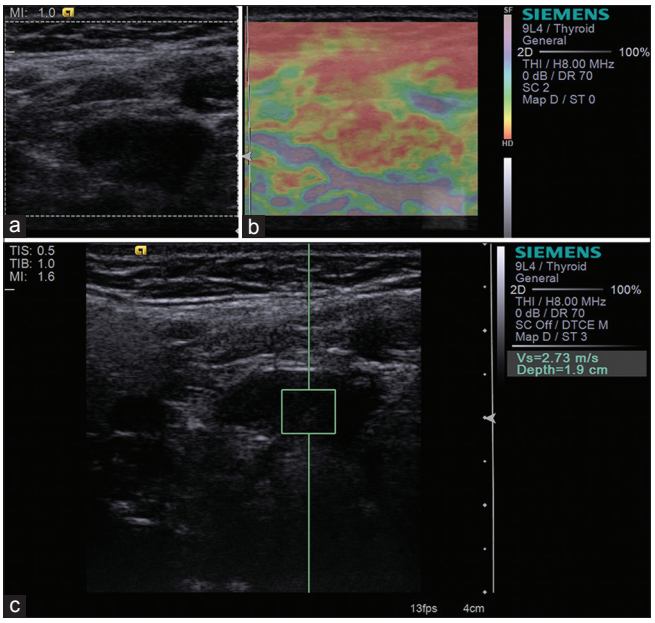


Figure 3: (a) B-mode ultrasound showing oval hypoechoic lymph node with loss of fatty hilum, (b) elastography image showing hard area ≥45% of lymph node corresponding to pattern 3, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 2.73 m/s in the boxed area. Histopathology revealed metastatic lymph node from carcinoma breast

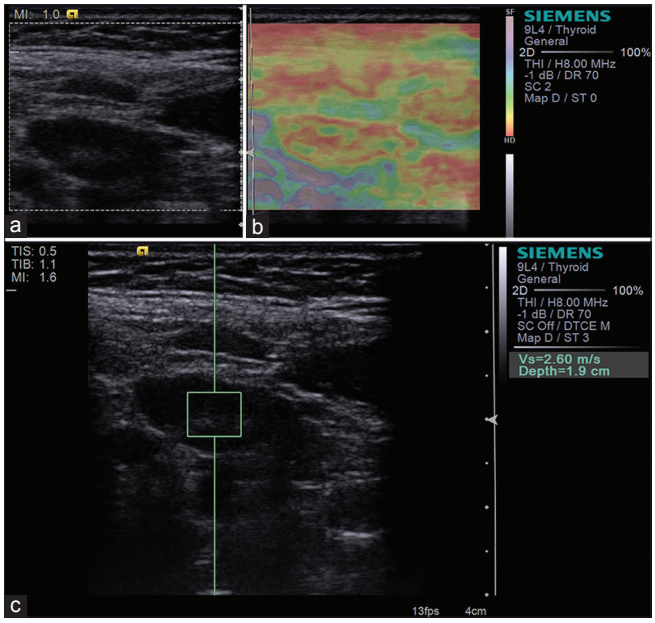


Figure 4: (a) B-mode ultrasound showing oval hypoechoic lymph node with loss of fatty hilum, (b) elastography image showing peripheral hard area and central soft area corresponding to pattern 4, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 2.60 m/s in the boxed area. Histopathology revealed metastatic lymph node from mucoepidermoid carcinoma parotid

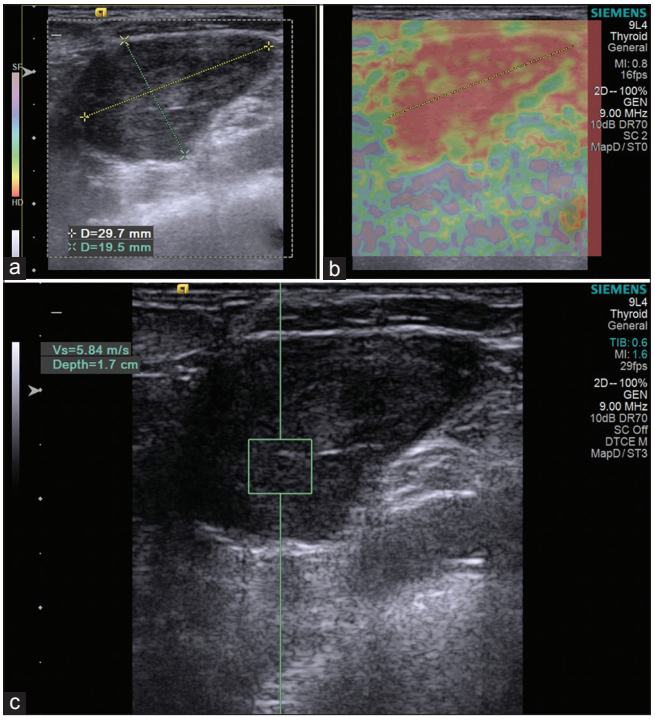


Figure 5: (a) B-mode ultrasound showing hypoechoic lymph node with irregular margins and absent hilum, (b) elastography image showing hard area occupying entire lymph node corresponding to pattern 5, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 5.84 m/s in the boxed area. Histopathology revealed metastatic lymph node from small cell carcinoma lung

Following elastography, all patients underwent surgical excision of the LN after cutaneous marking under US guidance. Histopathologic analysis of the LN was taken as the diagnostic standard of reference.

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Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. Chi-square test was used to know the association between categorical variables. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated with histopathologic analysis as reference standard. For analyzing the combined diagnostic performance of B-mode and Doppler, scores were assigned for five criteria: S/L ratio (ratio ≤0.5, score of 1: >0.5, 2), hilum (present, 1; absent, 2), borders (regular, 1; irregular, 2), echogenicity (homogenous, 1; heterogeneous, 2), and blood flow (hilar, 1; nonhilar, 2). The score for each LN was determined by adding the individual scores. A statistically supported cutoff line was set between scores 6 and 7. LNs with score <6 were categorized as benign, and 7–10 were categorized as malignant. Student *t*-test was performed to evaluate for significant difference in SWV values between benign and malignant LNs. A receiver operating characteristic (ROC) curve analysis was applied to obtain the optimal cutoff value for SWV to differentiate between benign and malignant LNs. Statistical significance was set at $P < 0.05$.

Results

The age of the patients ranged from 12 to 75 years (mean: 45.4 ± 16.9 years). About 52% (n = 26) were female and 48% (n = 24) were male. Of the 50 LNs examined, 33 (66%) were benign and 17 (34%) were malignant on histopathology. Of the 33 benign LNs, 27 had been reported as reactive and six as tuberculous lymphadenitis. Among the 17 malignant nodes, 15 were metastatic and two were due to lymphoma. Metastatic nodes were secondary to carcinoma lung, 4; parotid, 2; thyroid, 1; stomach, 1; breast, 1; poorly differentiated carcinoma from occult primary, 1; and head-and-neck squamous cell carcinoma, 5.

The US characteristics of the LNs and their diagnostic sensitivity, specificity, PPVs and NPV s, and accuracy are shown in Table 2. There were no significant differences between the benign and malignant LNs with respect to S/L ratio and echogenicity ($P = 0.273$ and $P = 0.623$, respectively). Hilum was found to be abnormal in all the 17 malignant LNs studied. Almost 81.8% of benign LNs (n = 27) had regular borders, whereas only 23.5% (n = 4) of malignant LNs had regular borders (P = 0.000). Among all B-mode parameters, borders of LN had the highest diagnostic accuracy (80%), while echogenicity was found to have the least accuracy (48%). On color Doppler evaluation, nearly 72% (n = 24) of benign nodes showed hilar flow compared to 35% (n = 6) of malignant nodes (P = 0.010).

Table 3 shows the correlation of elastographic pattern and histopathologic diagnosis of LNs. Majority of benign LNs (n = 31) had elastography patterns 1 and 2, while majority of malignant LNs (n = 16) had patterns 3–5. Higher elastographic pattern was significantly associated with malignant histopathology (P = 0.000).

The diagnostic performance of combined modalities (B-mode and color Doppler) and elastography is shown in Table 4.

The mean SWV of benign LNs was 1.670 ± 0.367 m/s. Metastatic LNs were significantly stiffer with mean SWV of 2.965 ± 0.826 m/s (P = 0.000). Figure 6 shows the ROC curve for SWVs measured using ARFI imaging in the differentiation of benign and malignant LNs. The best cutoff value for differentiating benign from malignant LNs was estimated to be 2.05 m/s with sensitivity of 88.2%, specificity of 84.8%, and area under the curve equal to 0.949 (95% confidence interval [CI]: 0.70–1.20).

Table 2: Ultrasound characteristics of lymph nodes and their diagnostic performance

US criteria	Benign LNs (n=33)	Malignant LNs (n=17)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
S/L ratio							
≤0.5	15 (45.4)	5 (29.4)	70.5	45.4	40	75	54
>0.5	18 (54.6)	12 (70.6)					
Hilum							
Present	22 (66.7)	0	100	66.6	60	100	78
Absent	11 (33.3)	17 (100)					
Borders							
Regular	27 (81.8)	4 (23.5)	68.4	87.1	76.4	81.8	80
Irregular	6 (18.2)	13 (76.5)					
Echogenicity							
Homogeneous	16 (48.5)	7 (41.2)	41.1	51.5	30.4	62.9	48
Heterogeneous	17 (51.5)	10 (58.8)					
Color Doppler							
Hilar	24 (72.8)	6 (35.3)	64.7	72.7	55	80	70
Nonhilar	9 (27.2)	11 (64.7)					

US: Ultrasound, LNs: Lymph nodes, S/L ratio: Ratio of short-axis diameter to long-axis diameter, PPV: Positive predictive value, NPV: Negative predictive value

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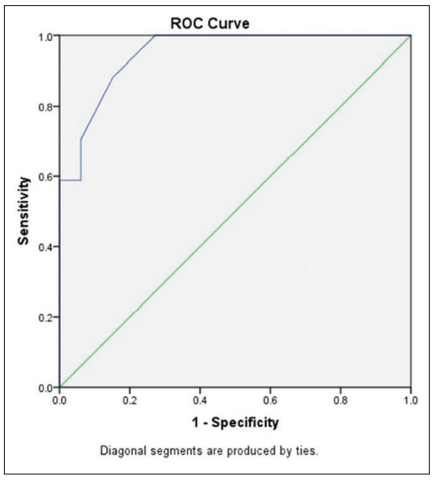


Figure 6: Receiver operating characteristic curve for shear wave velocities measured using acoustic radiation force impulse imaging in the differentiation of benign and malignant lymph nodes. The area under the curve was 0.949

Discussion

In this prospective study from India, we evaluated the performance of RTE in classification of benign and malignant cervical LNs. Our results indicate that RTE has a high specificity and diagnostic accuracy for identification of malignant LNs.

Although B-mode US is the most extensively used method for classification of cervical LNs because of its noninvasiveness and easy accessibility, specific criteria for distinguishing benign and malignant LNs are yet unclear. The association of signs often produces a highly suggestive appearance in most cases. In our study, the accuracy rate in distinguishing benign and malignant LNs was greatest for borders of LN (80%) and least for echogenicity (48%).

There were no significant differences between the benign and malignant LNs with respect to S/L ratio in our study. In contrast, several studies have noted S/L ratio >0.5 or a rounded shape to be associated with malignancy.[12,20] However, shape of LN can be misleading as nodes in nonmalignant conditions such as tuberculosis and Rosai–Dorfman disease have also been described as round.[21]

Our criterion of regular versus irregular borders revealed a diagnostic accuracy of 80%. Some other studies have categorized the LN borders as sharp and unsharp.[22,23] In a study by Khanna *et al*. from India, irregular margins were found in 7% of reactive LNs versus 55% of metastatic and 66% of tubercular nodes.[24] The absence of visible hilum due to replacement or effacement is considered as an important criterion for malignant LNs and has been reported by several authors.[12,13,25] In our study, all the 17 malignant LNs had no visible hilum, thus showing 100% sensitivity and NPV.

We observed a statistically significant difference in the vascular patterns between benign and malignant LNs ($P = 0.010$). Our observations can be comparable to that of Misra *et al*. and several other authors who have noted significant difference in vascularity between benign and malignant LNs.[13,26] In our study, the accuracy of vascularity of LN in predicting malignancy was 70%.

Table 3: Correlation of elastographic pattern and histopathologic diagnosis of lymph nodes

Elastogram pattern	Benign ($n=33$)	Malignant ($n=17$)	Total ($n=50$)
1	5	0	5
2	26	1	27
3	1	2	3
4	1	4	5
5	0	10	10

Table 4: Diagnostic performance of elastography and combined evaluation (B-mode and color Doppler)

Performance measure	Elastography	Combined B-mode and Doppler
Sensitivity (%)	94.1	94.1
Specificity (%)	93.9	81.2
PPV (%)	88.9	72.7
NPV (%)	96.9	96.4
Accuracy (%)	94	86

PPV: Positive predictive value, NPV: Negative predictive value

Even though combined B-mode US and Doppler could be potentially useful in differentiating reactive from malignant LNs, this modality may not have definite diagnostic value because of some overlapping sonographic appearances of benign and malignant nodes. Fine-needle aspiration cytology (FNAC) in the evaluation of cervical LNs has been reported to have a sensitivity of 82% and specificity of 97%.[27] However, FNAC is invasive and does not always provide a conclusive diagnostic result. Core needle biopsy from cervical LNs is also difficult owing to their relatively small size and potential injury to adjacent vascular structures.

RTE being noninvasive, has been suggested to be an adjunctive imaging tool to conventional US and could potentially reduce unnecessary biopsies.[28] Few prospective and retrospective studies concerning strain elastography of cervical LNs have been published in the literature.[29] While some have performed the measurement of elasticity scores (ES), some have measured both ES and strain ratio.

In our study, we used a 5-point scale described by Alam *et al*. to categorize the elasticity patterns. Accordingly, LNs showing types 1 and 2 elastography pattern were considered benign, while those with types 3, 4, and 5 were categorized as malignant. Nearly, 93.9% of benign LNs ($n = 31$) had a score of 1 or 2, while 94.1% of malignant LNs ($n = 16$) had a score of 3–5. The sensitivity, specificity, and accuracy of elastography were 94.1%, 93.9%, and 94%, respectively.

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Alam *et al*. evaluated 85 cervical LNs (53 metastatic and 32 reactive) using 5-point elasticity scale and documented 83% sensitivity, 100% specificity, and 89% accuracy in differentiating metastatic from reactive LNs.[12] Bhatia *et al*. evaluated 74 cervical LNs (37 malignant and 37 benign) using RTE.[17] Dynamic cine loops of elasticity imaging were qualitatively scored on a 4-point elasticity scale by three independent observers. Median ES for benign and malignant nodes was 2 and 3, respectively. A cutoff ES score of ≥2 achieved only 62.2% sensitivity, 83.8% specificity, and 73% accuracy for malignancy. Fair to good interobserver agreement was noted in their study.

Higher accuracy rates observed in our study can be attributed to the fact that there were very few cases of lymphoma ($n = 2$) and tuberculous adenitis ($n = 6$) in our study. Lymphomas have been reported to show low stiffness from an elastographic point of view because of the large size and homogeneous structure of LNs which can produce false-negative results for malignancy.[30] Granulomatous lymphadenitis associated with tuberculosis may demonstrate calcification, scarring, or necrosis and therefore show areas of greater stiffness (false positive).[14]

Ying *et al*. performed a meta-analysis to evaluate the performance of RTE for diagnosis of malignant LNs.[29] Nine studies including 835 LNs were analyzed. The pooled sensitivity and specificity for ES in diagnosing malignant LNs were 74% (95% CI: 66%–81%) and 90% (95% CI: 82%–94%), respectively.

In the present study, we were able to obtain greater specificity, PPV, and accuracy with RTE than conventional US and Doppler for detecting malignant LNs from diverse primary sites. Hence, RTE with its fairly high specificity and diagnostic accuracy can reduce unnecessary invasive procedures for diagnosis of malignant LNs. However, it cannot replace the conventional B-mode US and Doppler because it is dependent on the machine as well as operator's expertise since excessive compression can cause nonaxial displacement and change the tissue stiffness. This in turn can affect the accuracy of the software's correlation algorithms. Furthermore, elastography is not useful for calcified LNs and those with cystic degeneration.

Relatively, few clinical studies have evaluated ARFI in the assessment of malignant cervical LNs.

In our study, ARFI imaging revealed higher SWV for malignant LNs than benign ones. The cutoff value of 2.05 m/s had sensitivity of 88.2% and specificity of 84.8% in identifying malignant LNs. Our results are comparable to that of Fujiwara *et al*. who evaluated SWVs of 42 cervical LNs using ARFI.[31] Of the numerous cutoff values tested, a SWV cutoff value of 1.9 m/s best distinguished between reactive and malignant LNs. Their criterion of SWV >1.9 m/s had 95.0% sensitivity and 81.8% specificity. According to a recent meta-analysis of nine ARFI imaging studies that included 1084 LNs, the pooled sensitivity and specificity values for detecting malignancies were 87% (95% CI: 83%–91%) and 88% (95% CI: 82%–92%), respectively.[19]

Our study has few limitations. The sample size was small, and only enlarged LNs were included. Strain elastography using free hand compression is dependent on the compression technique, and excessive compression can alter tissue stiffness. A single expert radiologist performed all the elastograms, and no interobserver variability was studied. However, studies have reported fair to almost perfect interobserver agreement in strain elastography.[6]

Head-and-neck cancers account for nearly one-third of the cancer burden in India, and it is a well-known fact that status of cervical LN is the single most important prognostic factor in squamous cell carcinoma of the head and neck.[32] To the best of our knowledge, this is the first study from India to document the diagnostic performance of elastography in evaluation of cervical lymphadenopathy, and it has shown encouraging results.

Conclusion

Elastography has high diagnostic accuracy in differentiating benign and malignant cervical LNs and can be an effective noninvasive adjunctive tool in the evaluation of cervical lymphadenopathy. It is potentially useful in selecting the LN with high probability of malignancy, on which FNAC/biopsy can be performed, thereby reducing sampling errors. ARFI imaging can be a useful technique for differentiating benign from malignant cervical LNs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Chong V. Cervical lymphadenopathy: What radiologists need to know. Cancer Imaging 2004;4:116-20.
2. Na DG, Lim HK, Byun HS, Kim HD, Ko YH, Baek JH. Differential diagnosis of cervical lymphadenopathy: Usefulness of color Doppler sonography. AJR Am J Roentgenol 1997;168:1311-6.
3. Ghafoori M, Azizian A, Pourrajabi Z, Vaseghi H. Sonographic evaluation of cervical lymphadenopathy; comparison of metastatic and reactive lymph nodes in patients with head and neck squamous cell carcinoma using gray scale and Doppler techniques. Iran J Radiol 2015;12:e11044.
4. Leboulleux S, Girard E, Rose M, Travagli JP, Sabbah N, Caillou B, *et al.* Ultrasound criteria of malignancy for cervical lymph nodes in patients followed up for differentiated thyroid cancer. J Clin Endocrinol Metab 2007;92:3590-4.
5. Gupta A, Rahman K, Shahid M, Kumar A, Qaseem SM, Hassan SA, *et al.* Sonographic assessment of cervical lymphadenopathy: Role of high-resolution and color Doppler imaging. Head Neck 2011;33:297-302.
6. Choi YJ, Lee JH, Baek JH. Ultrasound elastography for evaluation of cervical lymph nodes. Ultrasonography 2015;34:157-64.
7. Thomas A, Fischer T, Frey H, Ohlinger R, Grunwald S, Blohmer JU, *et al.* Real-time elastography – An advanced method of ultrasound: First results in 108 patients with breast lesions. Ultrasound Obstet Gynecol 2006;28:335-40.

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8. Taylor LS, Rubens DJ, Porter BC, Wu Z, Baggs RB, di Sant'Agnese PA, *et al.* Prostate cancer: Three-dimensional sonoelastography for *in vitro* detection. Radiology 2005;237:981-5.
9. Friedrich-Rust M, Ong MF, Herrmann E, Dries V, Samaras P, Zeuzem S, *et al.* Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. AJR Am J Roentgenol 2007;188:758-64.
10. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al.* US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
11. Thomas A, Kümmel S, Gemeinhardt O, Fischer T. Real-time sonoelastography of the cervix: Tissue elasticity of the normal and abnormal cervix. Acad Radiol 2007;14:193-200.
12. Alam F, Naito K, Horiguchi J, Fukuda H, Tachikake T, Ito K. Accuracy of sonographic elastography in the differential diagnosis of enlarged cervical lymph nodes: Comparison with conventional B-mode sonography. AJR Am J Roentgenol 2008;191:604-10.
13. Turgut E, Celenk C, Tanrivermis Sayit A, Bekci T, Gunbey HP, Aslan K. Efficiency of B-mode ultrasound and strain elastography in differentiating between benign and malignant cervical lymph nodes. Ultrasound Q 2017;33:201-7.
14. Teng DK, Wang H, Lin YQ, Sui GQ, Guo F, Sun LN. Value of ultrasound elastography in assessment of enlarged cervical lymph nodes. Asian Pac J Cancer Prev 2012;13:2081-5.
15. Lo WC, Cheng PW, Wang CT, Liao LJ. Real-time ultrasound elastography: An assessment of enlarged cervical lymph nodes. Eur Radiol 2013;23:2351-7.
16. Acu L, Oktar SÖ, Acu R, Yücel C, Cebeci S. Value of ultrasound elastography in the differential diagnosis of cervical lymph nodes: A comparative study with B-mode and color Doppler sonography. J Ultrasound Med 2016;35:2491-9.
17. Bhatia KS, Cho CC, Yuen YH, Rasalkar DD, King AD, Ahuja AT. Real-time qualitative ultrasound elastography of cervical lymph nodes in routine clinical practice: Interobserver agreement and correlation with malignancy. Ultrasound Med Biol 2010;36:1990-7.
18. Suh CH, Choi YJ, Baek JH, Lee JH. The diagnostic performance of shear wave elastography for malignant cervical lymph nodes: A systematic review and meta-analysis. Eur Radiol 2017;27:222-30.
19. Zhang P, Zhang L, Zheng S, Yu C, Xie M, Lv Q. Acoustic radiation force impulse imaging for the differentiation of benign and malignant lymph nodes: A systematic review and meta-analysis. PLoS One 2016;11:e0166716.
20. Ahuja AT, Ying M, Ho SY, Antonio G, Lee YP, King AD, *et al.* Ultrasound of malignant cervical lymph nodes. Cancer Imaging 2008;8:48-56.
21. Ahuja AT, Ying M. Sonographic evaluation of cervical lymph nodes. AJR Am J Roentgenol 2005;184:1691-9.
22. Ying M, Ahuja A, Brook F. Sonographic appearances of cervical lymph nodes: Variations by age and sex. J Clin Ultrasound 2002;30:1-1.
23. Ahuja A, Ying M. An overview of neck node sonography. Invest Radiol 2002;37:333-42.
24. Khanna R, Sharma AD, Khanna S, Kumar M, Shukla RC. Usefulness of ultrasonography for the evaluation of cervical lymphadenopathy. World J Surg Oncol 2011;9:29.
25. Lyshchik A, Higashi T, Asato R, Tanaka S, Ito J, Hiraoka M, *et al.* Cervical lymph node metastases: Diagnosis at sonoelastography – Initial experience. Radiology 2007;243:258-67.
26. Misra D, Panjwani S, Rai S, Misra A, Prabhat M, Gupta P, *et al.* Diagnostic efficacy of color Doppler ultrasound in evaluation of cervical lymphadenopathy. Dent Res J (Isfahan) 2016;13:217-24.
27. Haque MA, Talukder SI. Evaluation of fine needle aspiration cytology (FNAC) of lymph node in mymensingh. Mymensingh Med J 2003;12:33-5.
28. Tan SM, Teh HS, Mancer JF, Poh WT. Improving B mode ultrasound evaluation of breast lesions with real-time ultrasound elastography – A clinical approach. Breast 2008;17:252-7.
29. Ying L, Hou Y, Zheng HM, Lin X, Xie ZL, Hu YP, *et al.* Real-time elastography for the differentiation of benign and malignant superficial lymph nodes: A meta-analysis. Eur J Radiol 2012;81:2576-84.
30. Lenghel LM, Bolboaca SD, Botar-Jid C, Baciut G, Dudea SM. The value of a new score for sonoelastographic differentiation between benign and malignant cervical lymph nodes. Med Ultrason 2012;14:271-7.
31. Fujiwara T, Tomokuni J, Iwanaga K, Ooba S, Haji T. Acoustic radiation force impulse imaging for reactive and malignant/metastatic cervical lymph nodes. Ultrasound Med Biol 2013;39:1178-83.
32. Shah SB, Sharma S, D'Cruz AK. Head and neck oncology: The Indian scenario. South Asian J Cancer 2016;5:104-5.

Original Article

Utility of Ultrasound Strain Elastography to Differentiate Benign from Malignant Lesions of the Breast

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Abstract

Background: The purpose of this study was to determine the utility and diagnostic performance of strain elastography (SE) in differentiating benign from malignant lesions of the breast. **Materials and Methods:** In this prospective study, 50 palpable breast masses in 50 patients were examined by mammography, B-mode ultrasound (US) and SE. Lesions were categorized using Breast Imaging Reporting and Data System (BIRADS) scoring based on mammographic and sonographic features. Elasticity scores were assessed on a five-point scale based on the distribution of strain, and the lesion size on SE imaging and B-mode (elasticity imaging/B mode [EI/B] ratio) was compared. Findings were correlated with the BIRADS assessment and diagnostic performance of sonoelastography was evaluated taking histopathology as reference standard. **Results:** Histopathology revealed 29 (58%) malignant and 21 (42%) benign lesions. Infiltrative ductal carcinoma and fibroadenoma were the most common malignant and benign lesions, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of SE was 100%, 76.1%, 85.2%, 100%, and 90%, respectively. Higher elasticity score was significantly associated with malignant histopathology ($P < 0.00001$). The mean EI/B ratio for malignant lesions was 1.36 ± 0.24 while that of benign lesions was 1.03 ± 0.30 ($P = 0.000$). **Conclusion:** Real-time SE of the breast, with its superior sensitivity and specificity, could provide improved characterization of benign and malignant breast masses compared with mammography and conventional US. Due to greater diagnostic accuracy, SE can be an effective adjunctive tool to B-mode US in predicting malignancy of breast, as well as in reducing the need for biopsies in benign breast lesions.

Keywords: Breast, elasticity imaging technique, sonoelastography

Introduction

Breast cancer is the most common malignancy in women all over the world and continues to remain a leading cause of cancer-related death in females. The incidence of breast cancer has been increasing steadily in both developing and developed countries.[1] Increased life expectancy, urbanization, changes in lifestyle, and delay in diagnosis contribute to the increasing incidence as well as mortality in the low- and middle-income countries. Early detection is critical to improve the breast cancer outcome and survival.

Current imaging techniques available for screening of breast cancer include mammography, ultrasonography, and magnetic resonance imaging (MRI). Although mammography is the current standard breast screening technique, its sensitivity is reduced in high-density breast tissue, in smaller tumors, and in young women.[2] Breast ultrasonography is widely used in the evaluation and categorization of breast lesions, owing to its high sensitivity. However, inability to image deeper lesions, limited ability to distinguish isoechoic lesions from surrounding fat, low specificity and increased biopsy rates resulting in patient discomfort and anxiety are some potential limitations of breast ultrasound (US).[3] Even though MRI is useful for screening of subjects with high breast cancer risk, its use for routine screening is limited because of high cost, time consumption and lack of widespread availability as well as need for experienced radiologists.

US elastography (USE) is a noninvasive imaging modality complimentary to US that detects tissue elasticity by the

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application of an external stress. As malignant tissues are firmer than benign ones, the estimate of tissue stiffness by USE can aid in the differentiation between benign and malignant lesions. Recent studies have reported the utility of USE in the characterization of lesions in various tissues, such as thyroid, liver, prostate, lymph nodes, and breast.[4-8] It has been suggested that current elastography systems can evaluate histological information by depicting the distribution of tissue stiffness or strain which might have the potential to predict the therapeutic effect of treatment with anticancer agents.[9]

Published literature on the usefulness of strain elastography (SE) in the evaluation of breast lesions and its correlation with histopathology is limited, especially from India. The purpose of this study was to determine the utility and diagnostic performance of SE in differentiating benign from malignant lesions of the breast with histopathology as the reference standard.

Materials and Methods

This prospective study was conducted at a tertiary care center in South India from December 2014 to December 2017. The institutional review board approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants. Fifty consecutive female patients aged 40 years and above referred to the department of radiology for the evaluation of palpable breast lump and sonography-guided biopsy were included in the study. Exclusion criteria consisted of those with cystic lesions of breast, history of prior biopsy, postsurgical status, prosthetic breast implants, and subcentimeter lesions. All patients underwent mammography, B-mode US, and SE followed by US-guided biopsy in the same session. All examinations and biopsy were performed by a single expert radiologist with over 10 years of experience in sonography.

Mammography was performed with SIEMENS MAMMOMAT 3000. Mediolateral oblique and craniocaudal views were taken in all patients, and in addition, coned-down or magnification views were used when necessary.

Ultrasonography of the breast was performed with SIEMENS ACUSON S3000 using high-frequency probe 18 L6. Sonographic features studied included size of the lesion, shape, margins, orientation, internal echogenicity, vascularity, calcification, and posterior acoustic shadowing. The lesions were categorized using the Breast Imaging Reporting and Data System (BIRADS) scoring based on mammographic and sonographic features as the following categories – category 2: benign; category 3: probably benign; category 4: suspicious for malignancy; and category 5: highly suggestive of malignancy.[10] BIRADS category 1 lesion was excluded from the study. Categories 2 and 3 were considered negative for malignancy and categories 4 and 5 were labeled as malignant.

SE of the breast was performed using the same probe in the supine position. The probe was placed over the region such that the lesion was at the center of the image. Holding the scan plane perpendicular to the skin surface, a slight compression–decompression movement was applied. The appropriate pressure and frequency of compression/decompression were optimized with the help of real-time display of quality index. Elastograms with quality index equal to or greater than 60 were included. The region of interest (ROI) included the lesion with subcutaneous layers and pectoralis muscle. It was ensured that the target tissue occupied no more than 25% of the total area of ROI. At least 5 mm thickness of normal adjacent tissue was included to assess the lesion stiffness in relation with average elasticity of surrounding tissue. Color map settings with blue coded as hard area and green coded as soft area were used. The images were displayed on a split screen mode with B-mode images on the left and elastographic images on the right. The best fit B-mode elastographic image pairs were selected for analysis. [Figures 1-4] The longest dimension of the lesion on elastographic image was compared with that of the corresponding B-mode image, and elasticity imaging/B mode ratio (EI/B ratio) was calculated.

The elasticity patterns were scored using the TSUKUBA scoring system [Table 1] described by Itoh *et al*.[11] The elasticity scores 1, 2, and 3 were considered benign, while scores 4 and 5 were labeled as malignant.

Biopsy of the lesion was performed under US guidance using an 18G tru-cut needle. Histopathology results were taken as the reference standard.

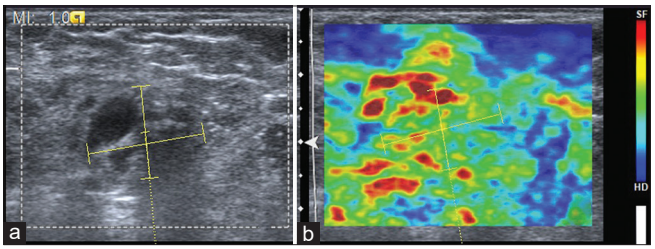


Figure 1: (a) B-mode ultrasound showing ill-defined hypoechoic lesion which was categorized as Breast Imaging Reporting and Data System category 4. (b) With strain elastography, entire hypoechoic lesion is deformable and evenly shaded green as surrounding breast tissue, corresponding to elasticity score 1. Histopathology was suggestive of nonspecific mastitis

Table 1: TSUKUBA elasticity score

Elasticity score	Description
1	Even strain in the entire lesion
2	Parts of the hypoechoic lesion does not show strain
3	Periphery of the hypoechoic lesion shows strain, no strain at the center
4	Entire lesion shows no strain
5	Entire lesion and surrounding area shows no strain

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 24. Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. Chi-square test was used to know the association between categorical variables. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated using 2 × 2 tables with histopathological analysis as the reference standard. Statistical significance was set at $P < 0.05$.

Results

Patients were aged between 40 and 72 years (mean: 48.96 ± 7.52 years). The mean age of the patients with benign and malignant lesions was 45.4 ± 5.47 and 51.5 ± 7.84 years, respectively ($P = 0.001$). On histopathological evaluation, 29 (58%) were found to be malignant and 21 (42%) were benign. Infiltrative ductal carcinoma and fibroadenoma were the most common malignant and benign lesions, respectively. Of the 29 malignant lesions, 17 (59%) were infiltrative ductal carcinoma, 11 (38%) were invasive lobular carcinoma and 1 (3%) was mucinous carcinoma. Among the 21 benign lesions, 7 (33%) were fibroadenoma, 4 (19%) were atypical ductal hyperplasia, 3 (15%) were breast abscess, 2 (9%) were sclerosing adenosis, and 2 (9%) were nonspecific mastitis. Granulomatous mastitis, complex cyst, and benign phyllodes tumor accounted for 1 (5%) each.

Mammography of 14 patients (28%) did not show any demonstrable lesion due to dense breast with predominantly fibroglandular tissue. 41% of the malignant lesions (12/29) had spiculated appearance on mammography, and microcalcification was encountered in 34% (10/29). 14% (4/29) showed architectural distortion.

Based on the BIRADS assessment, three were assigned BIRADS category 3, 18 were assigned category 4, and 29 were assigned category 5. The sensitivity, specificity, PPV, NPV, and accuracy of BIRADS assessment were 96.5%, 9.5%, 59.5%, 66.6%, and 60%, respectively, with histopathology as the reference standard.

Qualitative SE using elasticity scores had a sensitivity of 100% and specificity of 76.1% in detecting malignancy. The PPV and NPV were 85.2% and 100%, respectively, with diagnostic accuracy of 90%. Higher elasticity score was significantly associated with malignant histopathology ($P < 0.00001$). The mean EI/B ratio for malignant lesions was 1.36 ± 0.24 while that of benign lesions was 1.03 ± 0.30 ($P = 0.000$). Table 2 shows the correlation between histopathology, BIRADS assessment, and elasticity scoring among the lesions.

Discussion

In this prospective study, we evaluated the diagnostic performance of SE in predicting malignancy in lesions of breast. Qualitative SE with elasticity scoring was found to have a superior sensitivity, specificity, and diagnostic accuracy compared to conventional BIRADS assessment.

USE estimates the stiffness of tissues based on the degree of deformation of a tissue in response to an externally applied force. SE is a qualitative technique that uses either manual compression or an acoustic radiation force impulse push pulse to deform tissues.

Several authors have reported the additional value of SE to improve the specificity of conventional US in the differentiation

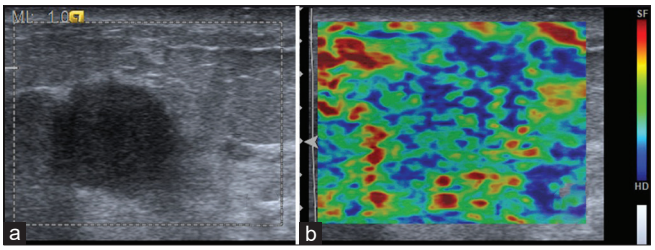


Figure 2: (a) B-mode ultrasound showing poorly marginated round hypoechoic lesion categorized as Breast Imaging Reporting and Data System category 3. (b) Strain elastographic image showing deformability of the peripheral portion of the lesion with stiff tissue in the center corresponding to elasticity score 3. Histopathology was suggestive of fibroadenoma

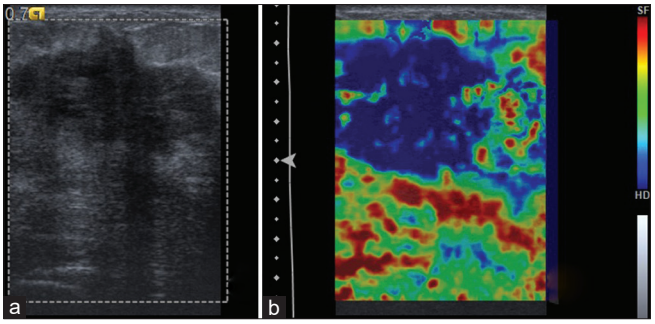


Figure 3: (a) B-mode ultrasound showing ill-defined hypoechoic lesion categorized as Breast Imaging Reporting and Data System 4. (b) With strain elastography, entire lesion is stiff, corresponding to elasticity score 4. Histopathology was suggestive of calcified fibroadenoma

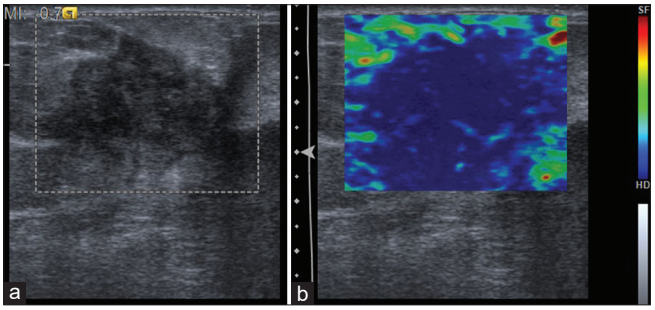


Figure 4: (a) B-mode ultrasound showing ill-defined hypoechoic lesion categorized as Breast Imaging Reporting and Data System 5. (b) With strain elastography, entire lesion and surrounding area are stiff, corresponding to elasticity score 5. Histopathology was suggestive of infiltrative ductal carcinoma

Table 2: Correlation between histopathology, Breast Imaging Reporting and Data System assessment and elasticity scoring among the lesions

Histopathology	BIRADS category	Elasticity score					Total (n=50)
		1	2	3	4	5	
Benign	3			2			2
	4	1	1	8	1	2	13
	5	1	1	2		2	6
Malignant	3					1	1
	4					5	5
	5					23	23

BIRADS: Breast Imaging Reporting and Data System

of benign and malignant breast masses and decrease the need for biopsies. While few have used EI/B ratio,[12] others have used five-point color scale and strain ratio[13,14] in the interpretation of SE images of the breast. However, the superior diagnostic performance of SE over B-mode US is controversial and factors such as operator experience, use of varied cutoffs, tumor size, and histologic subtype have been shown to influence the outcome.[15,16] Elasticity scoring had a sensitivity of 100% and specificity of 76.1%% in detecting malignancy among breast lesions in our study. The mean size of benign and malignant lesions was 17.06 ± 3.7 and 16.77 ± 3.1 mm, respectively. No significant difference in size was noted between benign and malignant lesions (P = 0.767). The superior diagnostic performance observed in our study can be attributed to the fact that most of the lesions (40/50) were smaller than 2 cm in size. Giuseppetti *et al*. have reported a better diagnostic performance of elastosonography in lesions smaller than 2 cm.[15] This is because a larger lesion has more chances of having hemorrhage or necrosis which might alter the tissue stiffness inside the lesion. Parajuly *et al*., based on their experience, have observed that lesions more than 5 cm should not be recruited in a study.[17]

Our results are comparable to that of Schaefer *et al*. and Atabey *et al*., who evaluated the performance of SE in breast lesions using a five-point scoring system.[18,19] With a cutoff between elasticity scores 3 and 4, elastography provided higher sensitivity and NPV. The authors have concluded that elastography can be an effective adjunct imaging modality in differential diagnosis of breast lesions, before the decision to biopsy a lesion in certain cases.[19]

In our study, 34 lesions were categorized as malignant on SE. Among these, 85% (29/34) were confirmed as malignancy on histopathology. Five benign lesions which had been categorized as malignant on SE were fibroadenoma (1), atypical ductal hyperplasia (3), and sclerosing adenosis (1) associated with calcification, which might have increased the stiffness of lesion. The false-positive rate was 23.8% in our study. In contrast, Zhi *et al*. have noted a false-positivity rate of 4.3% with SE in their study. Calcification and organized hemorrhage in benign lesions were responsible for false-positive diagnoses on SE.[3]

All the lesions with elasticity score less than or equal to 3 turned out to be negative for malignancy in our study. There were 13 benign lesions that presented as BIRADS category 4 necessitating biopsy. With a cutoff between 3 and 4 on SE, 10 of these 13 (76.9%) were true negatives on SE. Our observations are in line with recent literature, suggesting that SE can be useful in downgrading BIRADS category 4 lesions and reduce benign biopsy rates.[20]

Seven out of 18 BIRADS category 4 lesions and 25/29 BIRADS category 5 lesions had elasticity score 5. Among these, 87.8% turned out to be malignant, suggesting that elasticity scoring can be a useful predictor of malignancy. Satake *et al*. evaluated the ability of sonoelastography in predicting malignancy in breast masses classified as BIRADS category 4 or 5. Elasticity score was found to be a significant predictor of malignancy in BIRADS 4 masses (P = 0.002). However, elasticity score was not a significant predictor of malignancy in BIRADS category 5 lesions.[21]

A recent meta-analysis by Gong *et al*. on real-time elastography (RTE) in the differentiation of benign and malignant lesions of the breast that included 22 studies evaluating 4713 breast nodules reported an overall mean sensitivity and specificity of 0.834 and 0.842, respectively for elasticity scoring.[22] The authors have stated that RTE has high sensitivity and specificity in differentiating benign from malignant lesions and therefore can potentially reduce unnecessary breast biopsies. The mean EI/B ratio for malignant lesions was 1.36 ± 0.24 while that of benign lesions was 1.03 ± 0.30 (P = 0.000, P < 0.00001) in our study. Several studies have shown that tumors appear larger on elastography than on B-mode US.[23,24] A cutoff value of less than 1 for benign lesions and greater than or equal to 1 for malignant lesions has been shown to have high sensitivity and specificity for the characterization of lesions.[12] The variable dimension in elastography has been attributed to the desmoplastic reaction occurring in many cancers of the breast. Malignant lesions have been reported to appear significantly larger on strain imaging presumably because strain is uniquely sensitive to desmoplasia surrounding the lesion.[25]

Our study has few limitations. The sample size was limited and a single experienced radiologist performed all the examinations. SE is an operator-dependent technique and steady compression is needed for optimal images. However, in our study only images with optimal compression as displayed by the quality index were included. Intra- or inter-observer variability was not taken into account.

To the best of our knowledge, this is the first study on the utility of SE among the Indian population. The cancer projection data from India show that the number of breast cancer cases will become almost double by 2020.[26] Owing to the fact that the density of Indian breasts is different from that of the Western population[27] and density of breast parenchyma

can significantly influence the diagnostic performance of elastography, there is a need for more studies from this part of the world.

Conclusion

In this initial study from India, we conclude that real-time SE of the breast, with its superior sensitivity and specificity, could provide improved characterization of benign and malignant breast masses compared with mammography and conventional US. Due to greater diagnostic accuracy, SE can be an effective adjunctive tool to B-mode US in predicting malignancy of breast as well as decreasing unnecessary biopsies in clinical practice.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Lin L, Yan L, Liu Y, Yuan F, Li H, Ni J. Incidence and death in 29 cancer groups in 2017 and trend analysis from 1990 to 2017 from the Global Burden of Disease Study. J Hematol Oncol 2019;12:96.
2. Zhao H, Zou L, Geng X, Zheng S. Limitations of mammography in the diagnosis of breast diseases compared with ultrasonography: A single-center retrospective analysis of 274 cases. Eur J Med Res 2015;20:49.
3. Zhi H, Ou B, Luo BM, Feng X, Wen YL, Yang HY. Comparison of ultrasound elastography, mammography, and sonography in the diagnosis of solid breast lesions. J Ultrasound Med 2007;26:807-15.
4. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al*. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
5. Friedrich-Rust M, Ong MF, Herrmann E, Dries V, Samaras P, Zeuzem S, *et al*. Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. AJR Am J Roentgenol 2007;188:758-64.
6. Kamoi K, Okihara K, Ochiai A, Ukimura O, Mizutani Y, Kawauchi A, *et al*. The utility of transrectal real-time elastography in the diagnosis of prostate cancer. Ultrasound Med Biol 2008;34:1025-32.
7. Turgut E, Celenk C, Tanrivermis Sayit A, Bekci T, Gunbey HP, Aslan K. Efficiency of B-mode ultrasound and strain elastography in differentiating between benign and malignant cervical lymph nodes. Ultrasound Q 2017;33:201-7.
8. Thomas A, Fischer T, Frey H, Ohlinger R, Grunwald S, Blohmer JU, *et al*. Real-time elastography--an advanced method of ultrasound: First results in 108 patients with breast lesions. Ultrasound Obstet Gynecol 2006;28:335-40.
9. Barr RG, Nakashima K, Amy D, Cosgrove D, Farrokh A, Schafer F, *et al*. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 2: Breast. Ultrasound Med Biol 2015;41:1148-60.
10. Lazarus E, Mainiero MB, Schepps B, Koelliker SL, Livingston LS. BI-RADS lexicon for US and mammography: Interobserver variability and positive predictive value. Radiology 2006;239:385-91.
11. Itoh A, Ueno E, Tohno E, Kamma H, Takahashi H, Shiina T, *et al*. Breast disease: Clinical application of US elastography for diagnosis. Radiology 2006;239:341-50.
12. Barr RG, Destounis S, Lackey LB 2nd, Svensson WE, Balleyguier C, Smith C. Evaluation of breast lesions using sonographic elasticity imaging: A multicenter trial. J Ultrasound Med 2012;31:281-7.
13. Stachs A, Hartmann S, Stubert J, Dieterich M, Martin A, Kundt G, *et al*. Differentiating between malignant and benign breast masses: Factors limiting sonoelastographic strain ratio. Ultraschall Med 2013;34:131-6.
14. Zhao QL, Ruan LT, Zhang H, Yin YM, Duan SX. Diagnosis of solid breast lesions by elastography 5-point score and strain ratio method. Eur J Radiol 2012;81:3245-9.
15. Giuseppetti GM, Martegani A, Di Cioccio B, Baldassarre S. Elastosonography in the diagnosis of the nodular breast lesions: Preliminary report. Radiol Med 2005;110:69-76.
16. Chang JM, Won JK, Lee KB, Park IA, Yi A, Moon WK. Comparison of shear-wave and strain ultrasound elastography in the differentiation of benign and malignant breast lesions. AJR Am J Roentgenol 2013;201:W347-56.
17. Parajuly SS, Lan PY, Yun MB, Gang YZ, Hua Z. Diagnostic potential of strain ratio measurement and a 5 point scoring method for detection of breast cancer: Chinese experience. Asian Pac J Cancer Prev 2012;13:1447-52.
18. Schaefer FK, Heer I, Schaefer PJ, Mundhenke C, Osterholz S, Order BM, *et al*. Breast ultrasound elastography – Results of 193 breast lesions in a prospective study with histopathologic correlation. Eur J Radiol 2011;77:450-6.
19. Atabey AO, Arıbal E, Ergelen R, Kaya H. Value of strain elastography ultrasound in differentiation of breast masses and histopathologic correlation. J Breast Health 2014;10:234-8.
20. Cho N, Moon WK, Park JS, Cha JH, Jang M, Seong MH. Nonpalpable breast masses: Evaluation by US elastography. Korean J Radiol 2008;9:111-8.
21. Satake H, Nishio A, Ikeda M, Ishigaki S, Shimamoto K, Hirano M, *et al*. Predictive value for malignancy of suspicious breast masses of BI-RADS categories 4 and 5 using ultrasound elastography and MR diffusion-weighted imaging. AJR Am J Roentgenol 2011;196:202-9.
22. Gong X, Xu Q, Xu Z, Xiong P, Yan W, Chen Y. Real-time elastography for the differentiation of benign and malignant breast lesions: A meta-analysis. Breast Cancer Res Treat 2011;130:11-8.
23. Isermann R, Grunwald S, Hatzung G, Könsgen-Mustea D, Behrndt PO, Geaid AA, *et al*. Breast lesion sizing by B-mode imaging and sonoelastography in comparison to histopathological sizing – A prospective study. Ultraschall Med 2011;32 Suppl 1:S21-6.
24. Stachs A, Pandjaitan A, Martin A, Stubert J, Hartmann S, Gerber B, *et al*. Accuracy of tumor sizing in breast cancer: A comparison of strain elastography, 3-D ultrasound and conventional B-mode ultrasound with and without compound imaging. Ultrasound Med Biol 2016;42:2758-65.
25. Insana MF, Pellot-Barakat C, Sridhar M, Lindfors KK. Viscoelastic imaging of breast tumor microenvironment with ultrasound. J Mammary Gland Biol Neoplasia 2004;9:393-404.
26. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol 2017;13:289-95.
27. Singh T, Khandelwal N, Singla V, Kumar D, Gupta M, Singh G, *et al*. Breast density in screening mammography in Indian population – Is it different from western population? Breast J 2018;24:365-8.

Original Article

Evaluation of Resistive Index of Orbital Vessels using Color Doppler Imaging in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Resistive index (RI), derived from color Doppler imaging (CDI), is a marker of vascular resistance used widely in varied clinical settings. The aim of this study was to analyze the association between RIs of the orbital vessels in a pure cohort of type 2 diabetic patients with or without retinopathy using CDI. **Materials and Methods:** Fifty patients having type 2 diabetes and 50 age-matched controls were evaluated in this prospective study. Diabetic retinopathy (DR) was diagnosed based on seven-field stereo fundus photography and diabetic patients were divided into two. Patients with no DR ($n = 26$) were taken as Group 1, while patients with DR ($n = 24$) were taken as Group 2. CDI was performed and the RIs of the ophthalmic artery (OA), posterior ciliary artery (PCA), central retinal artery (CRA), and central retinal vein (CRV) were measured. **Results:** Significant differences were observed in the mean RI values of all orbital arteries between controls and patients with DR ($P < 0.05$). Comparison of RI values between controls and Group 1 showed no significant differences. Mean RI values of the PCA and CRA were found to be significantly higher in the patients in Group 2 than in Group 1 ($P = 0.03$ and $P < 0.001$, respectively). The duration of diabetes correlated with the mean RI of all the orbital vessels. RI of the CRA was a reliable predictive indicator for DR ($P = 0.001$). **Conclusion:** RIs of the orbital arteries are significantly higher in patients with DR. RI of the orbital vessels can be a potentially useful biomarker in the early diagnosis and follow-up of patients with DR.

Keywords: Color Doppler ultrasonography, diabetic retinopathy, vascular resistance

Introduction

Color Doppler imaging (CDI) is a convenient noninvasive method that gives information about the velocity of blood and vascular resistance at various sites of complex vasculature using simultaneous B mode ultrasound and Doppler images. The resistive index (RI), calculated from CDI, is a universally used measure of resistance to the arterial blood flow. In the recent past, RIs of the orbital arteries have been studied extensively to gain diagnostic and prognostic insight into a variety of clinical conditions, such as chronic obstructive pulmonary disease, hypertension, chronic kidney disease, and diabetes-related micro and macrovasculopathies.[1-5]

Usefulness of RI of the retrobulbar vessels in risk stratification and management of ophthalmic diseases such as diabetic retinopathy (DR), age-related macular degeneration, glaucoma, retinal vein occlusion, and thyroid-associated orbitopathy has been documented in the literature.[6,7]

Several studies have evaluated the RI of the retrobulbar vessels in diabetic patients with or without retinopathy and have shown inconsistent results. While few studies have shown significant correlation between RI of the orbital vessels and severity of DR,[8,9] few others suggest that the hemodynamic alterations observed in the orbital arteries reflect macrovascular changes and are not related to DR.[5,10] The observed inconsistencies can be attributed partly to the differences in sample sizes, patient characteristics, duration of diabetes, methods of assessment of DR, and probe positions used for color Doppler

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measurement. Further, most of these studies included patients with comorbidities such as hypertension and coronary artery disease as well as those patients on cardiac or antihypertensive drugs, which might alter the vascular resistance.

The aim of this study was to analyze the association between RIs of the orbital vessels in a pure cohort of type 2 diabetic patients with or without retinopathy using CDI.

Materials and Methods

This prospective study was conducted between March 2017 and May 2019 from a tertiary care institution in South India. The institutional human ethical committee approval was obtained before the initiation of the study, and the principles of Declaration of Helsinki were adhered to. Informed, written consent was obtained from all the study participants. Fifty consecutive patients of type 2 diabetes (as per definition of the American Diabetes Association) were recruited for the study. Exclusion criteria were the presence of associated systemic hypertension, nephropathy, dyslipidemia, and cardiovascular diseases. Patients on medications known to affect systemic hemodynamics such as angiotensin-converting enzyme inhibitors, calcium channel blockers, and antimigraine drugs were excluded. Patients with a history of any ocular disease that might affect ocular blood flow such as retinal diseases, high myopia, glaucoma, previous ocular surgery, or panretinal photocoagulation were also excluded from the study. A control group of 50 age-matched subjects were included. None of the controls had any ocular or systemic disease.

Data regarding age, gender, body mass index (BMI), duration of diabetes and antidiabetic medications were obtained from all the study participants. Blood pressure and serum glycosylated hemoglobin (HbA1c) levels were determined.

All participants underwent a comprehensive eye examination and the presence/absence of retinopathy was diagnosed through dilated fundus examination and 30° stereoscopic fundus photograph of seven standard early treatment DR study fields using a digital fundus camera (TRC-50 DX Mydriatic Retinal Camera; Topcon Medical Systems, Oakland, NJ, USA). Diabetic patients were divided based on retinopathy into two groups. Group 1 contained patients with no DR; Group 2 included patients with DR. One eye (right eye, unless another ocular pathology was identified) was included from each study participant.

A masked expert radiologist performed CDI of the eye for all study participants using a color Doppler unit and a 12–5 MHZ linear-array transducer (Philips iU22 xMATRIX ultrasound system). The CDI was performed with patients lying in the supine position with a 30° head tilt and any undue pressure on the eye was avoided. Examiner's hand was rested on the orbital margin, and sterile coupling gel was applied over closed eyelids. Angle of the transducer was taken as 30°–60° during the examination. CDI was performed after exclusion of any other ocular or orbital pathologies by B mode, and optic nerve was considered as a reference for all further measurements. The velocity of blood flow in the ophthalmic artery (OA) was measured 20–30 mm behind the globe after crossing of the optic nerve in the nasal aspect. Blood flow velocity in the central retinal vein (CRV) and central retinal artery (CRA) was measured within the optic nerve around 5 mm behind the posterior margin of the eyeball. Blood flow velocity from the temporal branch of the short posterior ciliary artery (PCA) was determined approximately 5–10 mm posterior to the globe. The peak systolic velocity (PSV) and end diastolic velocity (EDV) measurements were taken for OA, CRA, PCA, and CRV. With the help of PSV and EDV, we calculated the vascular resistance based on the RI using Pourcelot's formula:

$$RI = (PSV - EDV)/PSV.$$

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) Version 24. Data were reported as mean ± standard deviation. The normality of data distribution was assessed by Kolmogorov–Smirnov test. One-way analysis of variance with a *post hoc* Bonferroni was used for analysis of normally distributed continuous data. Kruskal–Wallis analysis was used for analyzing nonnormally distributed data. Bivariate analysis using Pearson's correlation coefficient was used to study the relationship of RIs with biochemical and demographic variables of patients with diabetes. Receiver operating characteristic (ROC) curve analysis was used to explore the predictive indicators of DR. A $P < 0.05$ (two-tailed) was considered statistically significant.

Results

The study consisted of 51 males and 49 females. The age of the participants ranged from 41 to 76 years. Duration of diabetes ranged between 6 months and 20 years. Among the diabetic patients studied, 26 had no DR and constituted Group 1. Group 2 consisted of 24 patients with DR. In Group 2, three patients had mild nonproliferative DR (NPDR) characterized by only few microaneurysms, seven had moderate NPDR (presence of more than just microaneurysms but less than severe NPDR), six had severe NPDR (presence of more than 20 intraretinal hemorrhages in each of four quadrants or definite venous beading in 2+ quadrants or prominent intraretinal microvascular abnormalities in 1+ quadrant), and eight had proliferative DR (presence of neovascularization of disc or elsewhere in the retina).

There were no statistically significant differences in the age, BMI, and blood pressure between controls and diabetic groups. Duration of diabetes and HbA1c levels were found to be significantly increased in Group 2 patients with DR ($P < 0.001$). Significant differences were observed in the RI values of all orbital arteries between controls and diabetic groups. Table 1 shows the demographic data and clinical attributes of controls and diabetic subjects.

Subgroup analysis with *post hoc* comparison revealed significantly higher RI values in OA, PCA, and CRA in Group 2 compared to controls ($P < 0.001$, $P < 0.001$, and $P = 0.03$, respectively). Comparison of RI values between Group 1 and controls showed no significant differences. When diabetic Groups 1 and 2 were compared, statistically significant differences were observed in the mean RI values of CRA and PCA ($P < 0.001$ and $P = 0.03$, respectively).

In bivariate analysis, the mean RI values of all orbital vessels, namely OA, CRA, PCA, and CRV, had a significant correlation with the duration of diabetes ($R = 0.396$, $P < 0.001$; $R = 0.282$, $P = 0.005$; $R = 0.334$, $P = 0.001$; and $R = 0.290$, $P = 0.003$, respectively) [Figure 1]. In addition, the mean RI of the OA correlated significantly with HbA1c levels ($R = 0.321$, $P = 0.001$) [Figure 2].

ROC analysis showed that the RI of CRA was a definitive predictive indicator for DR. RI of the CRA ≥ 0.79 indicated the existence of DR with a sensitivity of 75% and a specificity of 73% (area under the curve = 0.780 [95% confidence interval: 0.650–0.909]) [Figure 3].

Discussion

DR, a common microvascular complication of diabetes, is one of the leading causes for preventable blindness in people of the working age.[11] There has been conflicting evidence on the ocular hemodynamics as to whether blood flow is increased or decreased in diabetics. Curtis *et al*. have proposed a hemodynamic framework which states that there is an initial reduction in the perfusion of retina before the onset of DR which later increases as DR progresses.[12]

The various hematological changes observed in diabetes such as increased aggregation of erythrocytes and platelets, increased blood viscosity, and reduced deformability of

Table 1: Demographic and clinical characteristics of controls and diabetic subjects

Characteristic	Controls (n=50)	Group 1 (n=26)	Group 2 (n=24)	P
Mean age (years)	56.58±8.81	54.85±8.52	54.42±7.84	0.52
Mean body mass index (kg/m^2)	23.35±3.62	25.33±2.69	24.09±3.88	0.07
Median systolic BP (mmHg)	110 (110-130)	120 (110-126.25)	120 (110-130)	0.16
Median diastolic BP (mmHg)	70 (70-80)	76.5 (70-80)	70 (70-80)	0.27
Median duration of diabetes (months)	-	24 (6-72)	120 (48-144)	<0.001
Mean glycosylated hemoglobin (%)	5.81±0.30	8.62±1.91	10.64±2.29	<0.001
Mean RI of OA	0.76±0.06	0.79±0.06	0.82±0.06	<0.001
Mean RI of CRA	0.77±0.07	0.72±0.17	0.84±0.11	0.001*
Mean RI of PCA	0.73±0.06	0.74±0.08	0.79±0.08	0.004*
Mean RI of CRV	0.42±0.12	0.44±0.12	0.46±0.11	0.29

*P<0.05. BP: Blood pressure, OA: Ophthalmic artery, CRA: Central retinal artery, PCA: Posterior ciliary artery, CRV: Central retinal vein, RI: Resistivity index

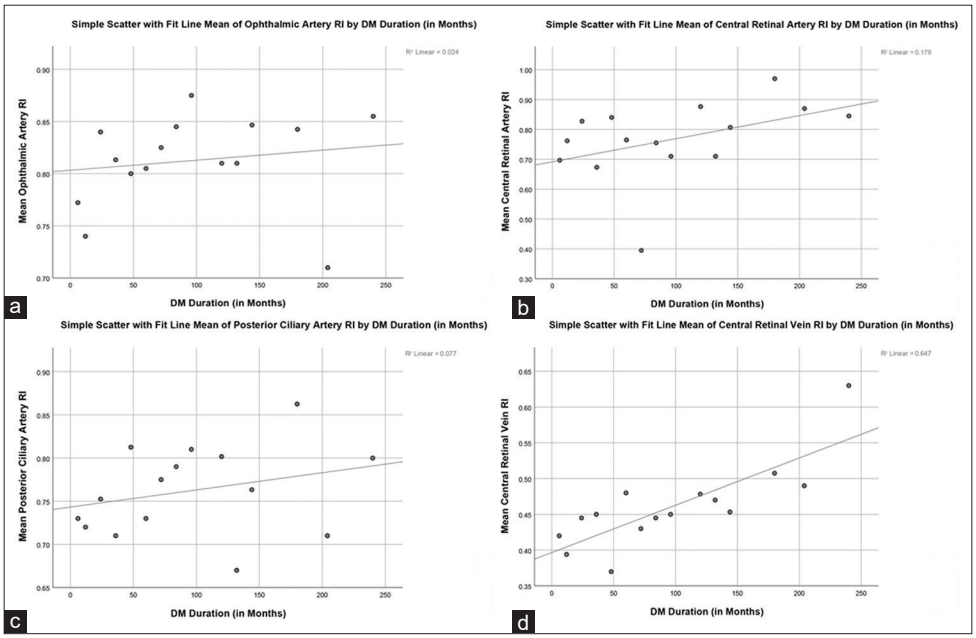


Figure 1: Scatter diagram showing correlation between duration of diabetes and resistive index of (a) ophthalmic artery, (b) central retinal artery, (c) posterior ciliary artery, and (d) central retinal vein

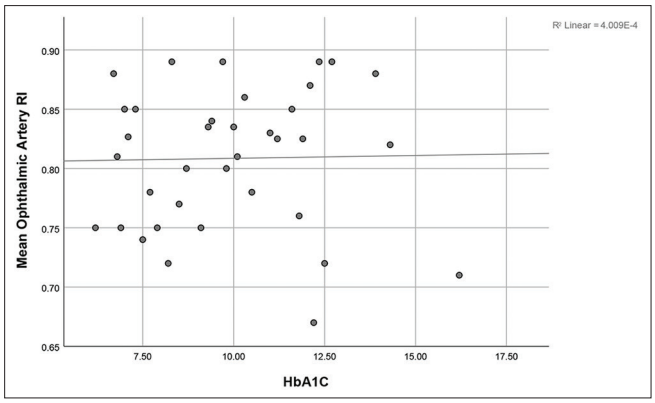


Figure 2: Scatter diagram showing correlation between mean resistive index of ophthalmic artery and glycosylated hemoglobin

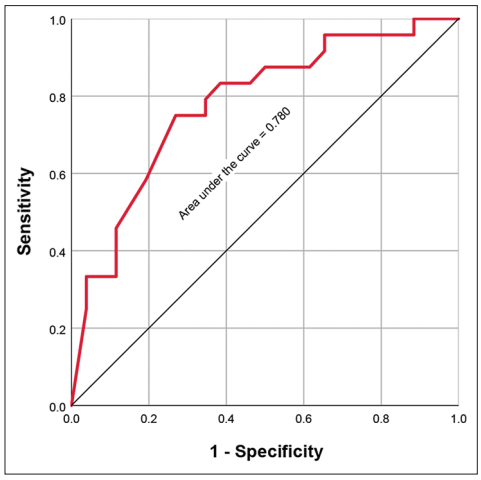


Figure 3: Receiver operating characteristic curve analysis of resistive index of central retinal artery and diabetic retinopathy (area under the curve was 0.780)

erythrocytes contribute to increased peripheral vascular resistance.[13] RI, an indirect measure of vascular resistance, can be useful to identify individuals at higher risk for developing DR. Further, RI, being independent of Doppler angle, has been shown to be reliable and reproducible with least coefficient of variation.[14]

Our study results demonstrate that significant changes occur in the RIs of the orbital vessels in patients with diabetes. The orbital vascular resistance (as reflected by RI) was found to be significantly increased in patients with DR as compared to controls and those in Group 1. Our observations are in agreement with certain other published studies.[15,16]

We observed no significant differences in the mean RI values between controls and those without DR. Our findings are similar to that of Basturk *et al*., who evaluated the RI of the orbital arteries in type 2 diabetics with microalbuminuria. No significant differences were observed in the mean RIs of OA, CRA, and PCA between controls and no DR group.[9]

In contrast, Dimitrova *et al*. have noted higher RI in the PCA in patients with no DR than controls.[17] However, their study included patients with hypertension and longer duration of diabetes than ours. In patients with hypertension, the RI of the PCA is reported to be high and correlates with duration of hypertension.[18] Moreover, CDI was performed in erect posture, which might have influenced the results. Few other investigators have noted increased RI values in the OA and CRA in patients with diabetes but without DR compared to that of controls, suggesting that even before the appearance of overt DR, there is increased resistance to blood flow in the peripheral vascular bed.[19,20]

We found significantly greater RI in the PCA and CRA ($P = 0.03$ and $P < 0.001$, respectively) in patients with DR compared to those without DR. While the inner layers of the retina are perfused by the CRA, the choroidal vasculature (derived from PCA) is responsible for the nourishment of outer retina. The various pathological changes observed in DR, such as loss of pericytes, thickening of the capillary basement membrane, and vascular smooth muscle cells, lead to compromised arteriolar integrity and autoregulation of blood flow. Loss of capillaries is critical to the pathogenesis of progressive retinal ischemia in DR and is a ubiquitous retinal finding from histopathological specimens.[21] Dropout of capillaries could be responsible for the increased vascular resistance observed in the RI of CRA and PCA in patients with DR. Further, the RI of the CRA was found to be a reliable predictive indicator of DR in our study. In this context, our findings are partly in agreement with that of Krasnicki *et al*., who have noted significant differences in the RI of CRA in subjects with DR and concomitant coronary artery disease.[5] The CRA, one of the major feeding vessels of the retina, reflects the identified vascular changes that have been associated with DR more sensitively. It has been suggested that hemodynamics in OA, a medium-sized muscular artery with many branches, is unlikely to be influenced by isolated alterations in blood flow in one of the branches.[22]

However, with progression of DR, the RI of the OA can eventually increase due to ensuing vascular changes related to diabetes in both retinal and choroidal vasculature.

Our finding of positive correlation between RI of all orbital vessels and the duration of diabetes is in concordance with that of Karami *et al*.[23] With prolonged duration of diabetes, alterations in blood vessel diameter and perfusion pressure occur due to disturbed autoregulatory mechanisms, subsequently leading to changes in the resistance of all retrobulbar vessels. We identified a statistically significant correlation between RI of the OA and the levels of HbA1c ($P = 0.001$). Clermont *et al*. have demonstrated reduction in the blood flow to retina with increasing HbA1c in subjects with type 1 diabetes without DR.[24]

Nagaoka *et al*. evaluated subjects with type 2 diabetes for changes in the retinal circulatory parameters using laser Doppler velocimetry and found significantly higher HbA1c in patients with the least retinal blood flow quartile, suggesting that poor glycemic control is associated with lowering of the retinal blood flow.[25]

Our study has several strengths. None of the patients had any associated vascular diseases such as hypertension. The presence of DR was assessed through seven-field stereo fundus photography for accuracy. A single experienced radiologist performed the CDI studies of the entire study participants, thereby eliminating interobserver variability. All examinations were performed with least pressure on the globe.

Limitations of our study include cross-sectional design and a small sample size. However, we intentionally excluded patients with other comorbid conditions to focus the effects of diabetes on retrobulbar circulation. Subgroup analysis of patients with different stages of retinopathy could not be performed because of limited sample size.

Conclusion

RIs of the orbital arteries are significantly higher in subjects with DR. RI of the orbital vessels can be a potentially useful biomarker in the early diagnosis and follow-up of patients with DR. This may be particularly helpful in patients with opacities in the ocular media.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ozer T, Altin R, Ugurbas SH, Ozer Y, Mahmutyazicioglu K, Kart L. Color Doppler evaluation of the ocular arterial flow changes in chronic obstructive pulmonary disease. Eur J Radiol 2006;57:63-8.
2. Reddy R. Usefulness of color Doppler imaging of orbital arteries in young hypertensive patients. Proc (Bayl Univ Med Cent) 2019;32:514-9.
3. Rokni Yazdi H, Faraji S, Ahmadi F, Shahmirzae R. Color Doppler indices of orbital arterial flow in end-stage renal disease patients; are the changes related to chronic hemodialysis or chronic renal failure? Iran J Radiol 2012;9:12-6.
4. Basturk T, Akcay M, Albayrak R, Unsal A, Ulas T, Koc Y. Correlation between the resistive index values of renal and orbital arteries. Kidney Blood Press Res 2012;35:332-9.
5. Krasnicki P, Dmuchowska DA, Proniewska-Skretek E, Dobrzycki S, Mariak Z. Ocular haemodynamics in patients with type 2 diabetes and coronary artery disease. Br J Ophthalmol 2014;98:675-8.
6. Dimitrova G, Kato S. Color Doppler imaging of retinal diseases. Surv Ophthalmol 2010;55:193-214.
7. Walasik-Szemplińska D, Pauk-Domańska M, Sanocka U, Sudoł-Szopińska I. Doppler imaging of orbital vessels in the assessment of the activity and severity of thyroid-associated orbitopathy. J Ultrason 2015;15:388-97.
8. Khatri M, Saxena S, Kaur A, Bhasker SK, Kumar M, Meyer CH. Resistive index of ophthalmic artery correlates with retinal pigment epithelial alterations on spectral domain optical coherence tomography in diabetic retinopathy. Int J Retina Vitreous 2018;4:12.
9. Basturk T, Albayrak R, Ulas T, Akcay M, Unsal A, Toksoy M, *et al*. Evaluation of resistive index by color Doppler imaging of orbital arteries in type II diabetes mellitus patients with microalbuminuria. Ren Fail 2012;34:708-12.
10. Ino-ue M, Azumi A, Yamamoto M. Ophthalmic artery blood flow velocity changes in diabetic patients as a manifestation of macroangiopathy. Acta Ophthalmol Scand 2000;78:173-6.
11. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010;376:124-36.
12. Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye (Lond) 2009;23:1496-508.
13. Cho YI, Mooney MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol 2008;2:1130-8.
14. Neudorfer M, Kessner R, Goldenberg D, Lavie A, Kessler A. Retrobulbar blood flow changes in eyes with diabetic retinopathy: A 10-year follow-up study. Clin Ophthalmol 2014;8:2325-32.
15. MacKinnon JR, McKillop G, O'Brien C, Swa K, Butt Z, Nelson P. Colour Doppler imaging of the ocular circulation in diabetic retinopathy. Acta Ophthalmol Scand 2000;78:386-9.
16. Gracner T. Ocular blood flow velocity determined by color Doppler imaging in diabetic retinopathy. Ophthalmologica 2004;218:237-42.
17. Dimitrova G, Kato S, Tamaki Y, Yamashita H, Nagahara M, Sakurai M, *et al*. Choroidal circulation in diabetic patients. Eye (Lond) 2001;15:602-7.
18. Akal A, Ulas T, Goncu T, Karakas E, Karakas O, Kurnaz F, *et al*. Evaluation of resistive index using color Doppler imaging of orbital arteries in geriatric patients with hypertension. Indian J Ophthalmol 2014;62:671-4.
19. Arai T, Numata K, Tanaka K, Kiba T, Kawasaki S, Saito T, *et al*. Ocular arterial flow hemodynamics in patients with diabetes mellitus. J Ultrasound Med 1998;17:675-81.
20. Tamaki Y, Nagahara M, Yamashita H, Kikuchi M. Blood velocity in the ophthalmic artery determined by color Doppler imaging in normal subjects and diabetics. Jpn J Ophthalmol 1993;37:385-92.
21. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. Microcirculation 2007;14:25-38.
22. Harris A, Joos K, Kay M, Evans D, Shetty R, Sponsel WE, *et al*. Acute IOP elevation with scleral suction: Effects on retrobulbar haemodynamics. Br J Ophthalmol 1996;80:1055-9.
23. Karami M, Janghorbani M, Dehghani A, Khaksar K, Kaviani A. Orbital Doppler evaluation of blood flow velocities in patients with diabetic retinopathy. Rev Diabet Stud 2012;9:104-11.
24. Clermont AC, Aiello LP, Mori F, Aiello LM, Bursell SE. Vascular endothelial growth factor and severity of nonproliferative diabetic retinopathy mediate retinal hemodynamics *in vivo*: A potential role for vascular endothelial growth factor in the progression of nonproliferative diabetic retinopathy. Am J Ophthalmol 1997;124:433-46.
25. Nagaoka T, Sato E, Takahashi A, Yokota H, Sogawa K, Yoshida A. Impaired retinal circulation in patients with type 2 diabetes mellitus: Retinal laser Doppler velocimetry study. Invest Ophthalmol Vis Sci 2010;51:6729-34.

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Case Report

Pseudotumor Cerebri Complicating Multisystem Inflammatory Syndrome in a Child

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Abstract

Purpose: To report a case of pseudotumor cerebri (PTC) in a child associated with multisystem inflammatory syndrome in children (MIS-C), associated with presumed coronavirus disease 2019.

Methods: A previously healthy 11-year-old female child presented with a 4-day history of fever, headache, vomiting, and loose stools. Laboratory investigations revealed neutrophilic leukocytosis, and markers of inflammation (C-reactive protein, ferritin, and interleukin-6) were significantly elevated. Pharyngeal swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by polymerase chain reaction was negative while anti-SARS-CoV-2 antibody was highly reactive. Ophthalmic evaluation for transient visual obscurations during hospital course revealed swelling of the optic disc in both eyes. Spectral-domain optical coherence tomography and ultrasonography confirmed the ophthalmoscopic findings. There was no neurologic deficit. Magnetic resonance imaging of the brain and magnetic resonance venogram revealed no structural lesion. The opening pressure of cerebrospinal fluid (CSF) was 336 mm of water, and CSF composition was normal.

Results: A diagnosis of PTC associated with MIS-C was made, and the child was treated with oral acetazolamide. Edema of the optic disc regressed following therapy, and the child is under follow-up.

Conclusions: PTC can occur in association with MIS-C. Clinicians need to be aware of this potential neuro-ophthalmic complication in MIS-C. Prompt diagnosis and treatment can prevent visual loss.

Keywords: COVID-19, Multisystem inflammatory syndrome in children, Pseudotumor cerebri

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Introduction

Pseudotumor cerebri syndrome (PTCS) is a disorder characterized by increased intracranial pressure of unclear etiology that carries significant morbidity and limited therapeutic options. PTCS can be primary otherwise called idiopathic intracranial hypertension (IIH) or secondary, attributable to a number of medical conditions and drugs. This neurologic syndrome predominantly affects obese women of reproductive age group, and clinical features include symptoms and signs of raised intracranial pressure with normal cerebrospinal fluid (CSF) composition and no structural abnormality on brain imaging.[1] PTCS is relatively uncommon in children and is associated with variable risk factors and clinical presentation.[2] We report a case of PTCS in a child associated with multisystem inflammatory syndrome in children (MIS-C), temporally associated with coronavirus disease 2019 (COVID-19).

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Case Report

An 11-year-old girl with no significant past medical history was brought to the emergency department with complaints of fever over the past 4 days associated with headache, vomiting,

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abdominal pain, and loose stools. There was associated lethargy but no history of seizures, altered sensorium, or respiratory symptoms. There were no sick contacts. At admission, the child had a temperature of 39°C, heart rate of 112 beats/min, and oxygen saturation of 100% on room air. Body mass index was 16 kg/m^2. Physical examination was unremarkable except for bilateral conjunctival congestion. Investigations revealed decreased hemoglobin, leukocytosis with neutrophilia, and elevated inflammatory markers including ferritin, interleukin-6 (IL-6), and C-reactive protein. Blood and urine cultures were negative, and liver function tests were found to be normal. Nasopharyngeal and oropharyngeal swabs for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by real-time polymerase chain reaction (RT-PCR) were negative. Serologic testing for SARS-CoV-2 antibodies by chemiluminescence immunoassay was highly reactive (observed titer – 212, ≥1 being labeled reactive) suggestive of SARS-CoV-2 infection. Table 1 shows the laboratory parameters. Echocardiogram was normal. On day 3 of admission, the child complained of transient obscurations of vision lasting for a few seconds associated with change in posture, and was referred for ophthalmic consultation.

On examination, uncorrected visual acuity was 20/20, and color vision was normal in both eyes. Anterior segment examination was unremarkable, and pupillary responses were normal. Extraocular movements were full. Fundus examination revealed optic disc swelling in both eyes [Figure 1a]. Neurologic examination was otherwise normal. Visual field evaluation by automated perimetry (Humphrey field analyzer) was found to be unreliable due to extensive fixation losses. Spectral-domain optical coherence tomography (OCT) showed increased thickness of peripapillary retinal nerve fiber layer (RNFL) in both eyes in all the four quadrants confirming edema of the optic disc [Figure 2a]. Magnetic resonance imaging of the brain and magnetic resonance venography (MRV) revealed normal ventricles, brain parenchyma, and venous sinuses. Transorbital ultrasound revealed bulging optic disc in both eyes. The optic nerve sheath diameter (ONSD) measurements obtained 3 mm posterior to the globe were found to be 5.6 mm in the right eye and 6 mm in the left eye [Figure 3a]. Lumbar puncture revealed a CSF opening pressure of 336 mm of water. CSF cultures were negative, and biochemistry was normal.

Since the child was hemodynamically stable and not hypoxic (SpO_2 100% on room air) at admission as well as during hospital course, she was managed with empiric broad-spectrum antibiotic therapy (intravenous ceftriaxone 2 g for 7 days) along with supportive care for fever (paracetamol) and loose stools (intravenous fluid replacement). As serial IL-6 and serum ferritin showed declining trend, the child was treated only with supportive measures. A diagnosis of pseudotumor cerebri (PTC) associated with MIS-C was made, and the child was started on oral acetazolamide 500 mg twice daily. By 4 weeks, headache and transient visual obscurations had subsided. Visual acuity remained 20/20, and fundus examination revealed resolving disc edema. The dose of oral acetazolamide was reduced to 250 mg thrice daily. At her last visit, 7 weeks since her initial presentation, she was found to have visual acuity of 20/20 and resolved disc edema in both eyes [Figure 1b]. Follow-up OCT revealed normalization of peripapillary RNFL thickness [Figure 2b]. The ONSD measurements were found to be 4 mm in both eyes at follow-up [Figure 3b]. The dose of acetazolamide has been tapered further, and she is under follow-up.

Table 1: Laboratory parameters of the patient and reference range

Parameter	Value	Reference range
Hemoglobin (g/dL)	10.5	11.5-15.5
White blood cell count (10^3/µL)	17	5-13
Neutrophil (%)	91.1	45-65
Lymphocyte (%)	7.5	32-52
Erythrocyte sedimentation rate (mm/h)	31	4-12
C-reactive protein (mg/dL)	39	<0.6
Serum ferritin (ng/mL)	352.4	7-140
IL-6 (pg/mL)	197.8	<7
NT-proBNP (pg/mL)	1163	<125
D-dimer (mg/L FEU)	1.48	<0.5
Anti-SARS-CoV-2 titer	212	<1

IL-6: Interleukin-6, NT-proBNP: N-terminal-pro-brain natriuretic peptide, Anti-SARS-CoV-2: Antibody against severe acute respiratory syndrome coronavirus 2

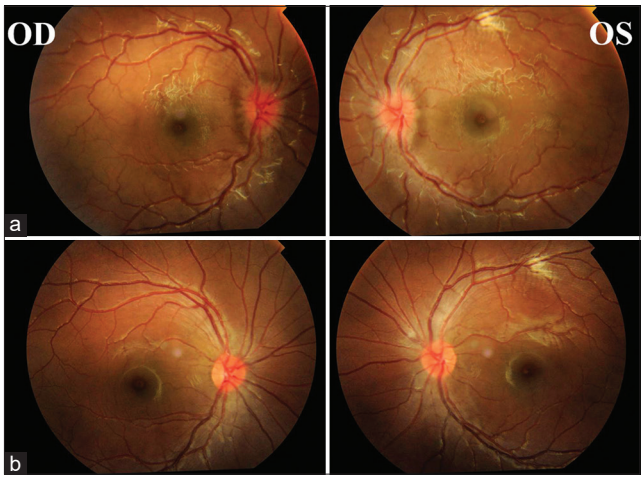


Figure 1: (a) Fundus photograph both eyes showing edema of optic disc. (b) Fundus photograph both eyes showing resolved disc edema at follow-up

Discussion

MIS-C, also called pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2, is a life-threatening condition that can lead to multi-organ dysfunction and long-term sequelae. The clinical features of MIS-C include severe illness necessitating hospitalization, fever, and involvement of two or more organ systems, in combination with laboratory evidence of inflammation and laboratory or epidemiologic evidence of SARS-CoV-2 infection.[3] Our

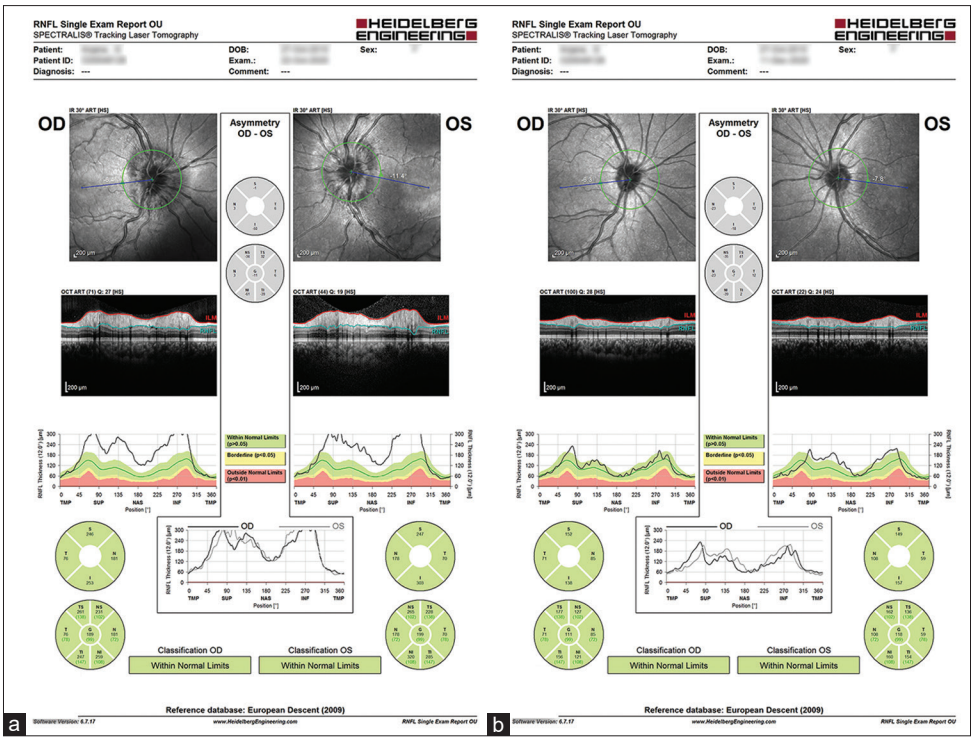


Figure 2: (a) Spectral-domain optical coherence tomography (SD-OCT) showing increased thickness of peripapillary retinal nerve fiber layer (RNFL) in both eyes. (b) SD-OCT both eyes at follow-up showing normalization of peripapillary RNFL thickness

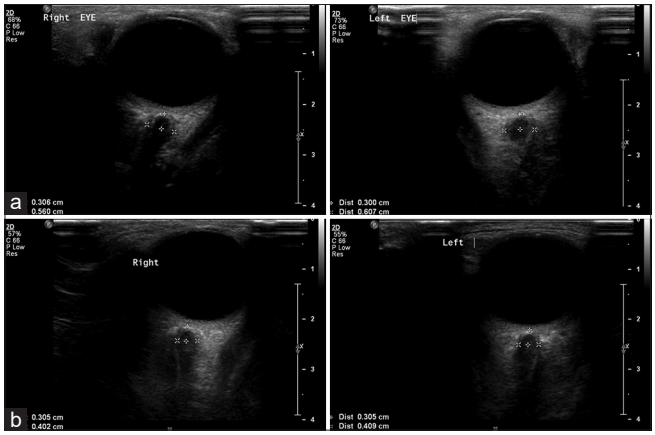


Figure 3: (a) Transorbital ultrasound showing increased optic nerve sheath diameter (ONSD) 3 mm posterior to inner scleral surface; 5.6 mm in the right eye and 6 mm in the left eye. (b) Transorbital ultrasound at follow-up showing normalization of ONSD; 4 mm in both eyes

patient was found to meet all the criteria specified in the Centers for Disease Control and Prevention and World Health Organization case definitions of MIS-C.[4,5]

The pathophysiology of MIS-C is unclear, and possible mechanisms include T-cell or antibody recognition of self-antigens resulting in autoantibodies, immune response against viral antigens expressed on infected cells and formation of immune complexes activating inflammation as well as viral superantigen sequences which activate host immune cells.[6] Dysregulated hyperinflammation is a critical event in the pathogenesis of MIS-C, and this condition is frequently associated with gastrointestinal symptoms and cardiovascular dysfunction.[7]

Although neurologic manifestations have been documented during acute phase of infection with SARS-CoV-2,[8,9] involvement of the central nervous system is uncommon in MIS-C. The neurologic features of MIS-C have been strongly suggested to represent a postinfectious immune response, and manifestations reported include headache, encephalopathy, and aseptic meningitis.[10]

We had not performed RT-PCR for SARS-CoV-2 on CSF since our patient showed evidence of previous infection with SARS-CoV-2 rather than current infection (pharyngeal swab was negative while antibodies to SARS-CoV-2 were present). Only one-third of cases of MIS-C have been reported to be positive by RT-PCR for SARS-CoV-2. The delayed presentation of MIS-C relative to the pandemic curve, a low proportion of cases who were SARS-CoV-2 positive by RT-PCR, and a high proportion who were antibody positive suggest that this inflammatory syndrome is not mediated by direct viral invasion but coincides with the development of acquired immune responses to SARS-CoV-2.[6] Previous studies have noted the absence of SARS-CoV-2 RNA in CSF analysis of COVID-19 patients with neurological symptoms suggesting that indirect mechanisms are responsible for neurologic manifestations in these patients.[11,12]

Although the symptoms of PTCS in children are similar to that of adults, the demographics vary, and children are reported to

have a higher incidence of secondary PTC. A distinct seasonal variation has been reported in the clinical presentation of IIH in prepubertal children, suggesting a possible association between IIH and concurrent infections in this age group.[13] Agraz *et al*. have noted a high incidence of atopy in their study cohort of children with PTC, suggesting that autoimmune component may play a role in pediatric population.[2]

To the best of our knowledge, four similar cases of PTCS associated with MIS-C (two case reports and a case series of two patients) have been reported in literature.[14-16] Although two patients had received therapy with doxycycline and steroids, the short course and recent initiation preclude the possibility of these medications contributing to increased intracranial pressure.

The mechanism behind the occurrence of PTCS in association with MIS-C is unclear. It has been postulated that increased intracranial pressure reflects systemic inflammation related to SARS-CoV-2 infection, resulting in central nervous system effects.[16] It is of interest to note that obesity, the most striking risk factor for PTCS, is associated with dysregulation of several inflammatory cytokines and aberrant glucocorticoid metabolism through manipulation of the enzyme 11-beta-hydroxysteroid dehydrogenase (11β-HSD).[17] 11β-HSD is a bidirectional enzyme that regulates prereceptor corticosteroid availability and glucocorticoid availability in the central nervous system that is one of the factors pivotal to intracranial pressure homeostasis. Among the two isoforms, 11β-HSD1 activates cortisol from cortisone while the isoform 11β-HSD2 inactivates cortisol. It is well established that several inflammatory cytokines such as tumor necrosis factor-α and ILs 1 and 6 are potent activators of 11β-HSD1.[18]

We speculate that PTC associated with MIS-C can be multifactorial, and one another possibility could be aberrant glucocorticoid metabolism as a result of cytokine storm and 11β-HSD1 activity. Increased 11β-HSD1 activity has been shown to influence the pathogenesis of PTC through manipulation of CSF dynamics at the level of choroidal plexus as well as arachnoidal granulations.[19] Further studies are warranted in this regard.

Few cases of isolated intracranial hypertension in association with active SARS-CoV-2 infection have been reported in adults. The proposed pathophysiologic mechanisms for intracranial hypertension include acute encephalitis, venous sinus thrombosis, intracranial venous congestion due to inflammation as a result of SARS-CoV-2 infection, and the associated coagulopathy as well as hyperviscosity state leading to impaired absorption of CSF.[20,21] The absence of clinical features such as altered sensorium/seizures and normal brain parenchyma evident on neuroimaging in our patient ruled out encephalitis. Normal MRV ruled out venous sinus thrombosis in our patient.

The other possible etiologies of swelling of optic disc in the setting of SARS-CoV-2 infection include optic neuritis, ischemic optic neuropathy, papillophlebitis, and retinal vein occlusion. The presence of normal visual acuity and color vision along with normal pupillary responses and the absence of associated retinal hemorrhages or venous tortuosity in our patient made us exclude these causes.

Prompt diagnosis and treatment of PTCS is essential since it can lead to irreversible visual loss. Although the pathophysiology remains unclear, clinicians should be aware of this potential complication with MIS-C. Fundus examination is valuable as part of a multidisciplinary approach toward management of MIS-C.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed

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Conflicts of interest

There are no conflicts of interest.

References

1. Friedman DI, Liu GT, Digre KB. Revised diagnostic criteria for the pseudotumor cerebri syndrome in adults and children. Neurology 2013;81:1159-65.
2. Agraz D, Morgan LA, Fouzdar Jain S, Suh DW. Clinical features of pediatric idiopathic intracranial hypertension. Clin Ophthalmol 2019;13:881-6.
3. Nakra NA, Blumberg DA, Herrera-Guerra A, Lakshminrusimha S. Multi-System Inflammatory Syndrome in Children (MIS-C) following SARS-CoV-2 infection: Review of clinical presentation, hypothetical pathogenesis, and proposed management. Children (Basel) 2020;7:69.
4. Centers for Disease Control and Prevention. Emergency Preparedness and Response: Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19). Health Advisory. Available from: https://emergency.cdc.gov/han/2020/han00432.asp. [Last accessed on 2020 Dec 26].
5. World Health Organization. Multisystem Inflammatory Syndrome in Children and Adolescents with COVID-19: Scientific Brief; 2020. Available from: https://www.who.int/publications-detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19. [Last accessed on 2020 Dec 26].
6. Jiang L, Tang K, Levin M, Irfan O, Morris SK, Wilson K, *et al.* COVID-19 and multisystem inflammatory syndrome in children and adolescents. Lancet Infect Dis 2020;20:e276-88.
7. Radia T, Williams N, Agrawal P, Harman K, Weale J, Cook J, *et al.* Multi-system inflammatory syndrome in children & adolescents (MIS-C): A systematic review of clinical features and presentation. Paediatr Respir Rev. 2021;38:51-57.
8. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, *et al.* Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020;77:683-90.
9. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, *et al.* Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun 2020;87:18-22.
10. Chen TH. Neurological involvement associated with COVID-19 infection in children. J Neurol Sci 2020;418:117096.
11. Bellon M, Schweblin C, Lambeng N, Cherpillod P, Vazquez J,

Lalive PH, *et al.* Cerebrospinal fluid features in SARS-CoV-2 RT-PCR positive patients. Clin Infect Dis 2020;ciaa1165.
12. Neumann B, Schmidbauer ML, Dimitriadis K, Otto S, Knier B, Niesen WD, *et al.* Cerebrospinal fluid findings in COVID-19 patients with neurological symptoms. J Neurol Sci 2020;418:117090.
13. Distelmaier F, Tibussek D, Schneider DT, Mayatepek E. Seasonal variation and atypical presentation of idiopathic intracranial hypertension in pre-pubertal children. Cephalalgia 2007;27:1261-4.
14. Verkuil LD, Liu GT, Brahma VL, Avery RA. Pseudotumor cerebri syndrome associated with MIS-C: A case report. Lancet 2020;396:532.
15. Parsons E, Timlin M, Starr C, Fries A, Wells R, Studer M, *et al.* MIS-C in February 2020 and implications of genomic sequencing for SARS-CoV-2. J Pediatric Infect Dis Soc 2020;10:695-97.
16. Baccarella A, Linder A, Spencer R, Jonokuchi AJ, King PB, Maldonado-Soto A, *et al.* Increased intracranial pressure in the setting of multisystem inflammatory syndrome in children, associated with COVID-19. Pediatr Neurol 2021;115:48-9.
17. Sinclair AJ, Ball AK, Burdon MA, Clarke CE, Stewart PM, Curnow SJ, *et al.* Exploring the pathogenesis of IIH: An inflammatory perspective. J Neuroimmunol 2008;201-202:212-20.
18. Chapman KE, Coutinho AE, Zhang Z, Kipari T, Savill JS, Seckl JR. Changing glucocorticoid action: 11β-hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. J Steroid Biochem Mol Biol 2013;137:82-92.
19. Sinclair AJ, Walker EA, Burdon MA, van Beek AP, Kema IP, Hughes BA, *et al.* Cerebrospinal fluid corticosteroid levels and cortisol metabolism in patients with idiopathic intracranial hypertension: A link between 11beta-HSD1 and intracranial pressure regulation? J Clin Endocrinol Metab 2010;95:5348-56.
20. Svedung Wettervik T, Kumlien E, Rostami E, Howells T, von Seth M, Velickaite V, *et al.* Intracranial pressure dynamics and cerebral vasomotor reactivity in coronavirus disease 2019 patient with acute encephalitis. Crit Care Explor 2020;2:e0197.
21. Silva MT, Lima MA, Torezani G, Soares CN, Dantas C, Brandão CO, *et al.* Isolated intracranial hypertension associated with COVID-19. Cephalalgia 2020;40:1452-8.

Original article

Correlation of diffusion tensor imaging with clinical and neurological findings in patients with cervical spondylotic myelopathy

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ABSTRACT:

Background: Cervical spondylosis is a common degenerative disease of spine generally seen in old age, frequently causing compression of spinal cord and /or nerve roots by protruding disc or osteophytes. Magnetic Resonance Imaging (MRI) is the modality of choice for evaluation of patients with features of cervical spondylotic myelopathy. In addition to routine MRI sequences, advanced MR techniques like diffusion tensor imaging (DTI) can be used to obtain specific information about the spinal cord.

Materials and methods: It is a prospective observational study conducted in 30 patients who presented with clinical and neurological features of cervical spondylotic myelopathy, who had undergone MRI.

Result: All the patients had undergone routine MRI along with diffusion tensor imaging. The Apparent Diffusion Coefficient (ADC) and Fractional Anisotropy (FA) values of the spinal cord were measured at the level of normal spinal canal dimension and at the level where there is narrowing of the spinal canal. The ADC values were found to be high and the FA values were low at the level of the spinal canal stenosis in comparison with values measured at the level of normal spinal canal dimension, which were statistically significant.

Conclusion: DTI parameters showed significant correlation with the clinical parameters, suggesting that DTI can be used to detect microscopic changes related to compressive myelopathy, before changes are apparent in the routine MR sequences. This can enable us to intervene early to avoid irreversible damage to the spinal cord.

Key words: Cervical spondylotic myelopathy, Diffusion tensor imaging, Fractional anisotropy.

Introduction

Cervical spondylosis is a common degenerative disease of spine generally seen in old age, frequently causing compression of spinal cord and /or nerve roots by various pathological entities like protruding disc, osteophytes and thickening of ligamentous structures around the spinal cord.[1] Spondylotic myelopathy refers to compression of the spinal cord by the above mentioned structures leading to impairment of spinal cord function.[2] Cervical spondylotic myelopathy (CSM) is usually seen after the 5th decade. Patients with CSM usually present with symptoms of numbness and weakness in limbs, pain over shoulder and neck, incoordination. Magnetic Resonance Imaging (MRI) of cervical spine is non-invasive and accurate imaging tool in assessing the structural changes of spinal cord due to its high contrast and soft tissue resolution[3]. It helps to ascertain the site and the

severity of degenerative compressive myelopathy. It is also useful to exclude any other pathology, like neoplasm which can cause compressive myelopathy and mimic degenerative disc disease.

Classical MRI finding seen in compressive myelopathy is focal T2 hyperintensity in spinal cord at the level of a compressive lesion. This signal change is suggestive of either spinal cord oedema in acute cases, or gliosis and myelomalacia in chronic cases[4,5]. Oedema is associated with swelling of the spinal cord and chronic changes on the other hand show thinning of the spinal cord. Other imaging modalities like Computed Tomography (CT) and radiography play a complementary role in the evaluation of CSM. Due to its inherent high spatial resolution CT is better than MRI in the assessment of cortical bone, osteophytes and other calcified ligamentous structures. These structures can also cause spinal canal compromise. Plain radiography is still being used as an initial tool for evaluating degenerative disc changes[6]. The ultimate aim of doing imaging studies is to identify the spinal cord abnormalities early and take remedial measures to prevent further damage[7].

Aims:

1. To compare Fractional Anisotropy (FA) and Apparent Diffusion Coefficient (ADC) values of the spinal cord obtained at the level with above and below the level of spinal canal stenosis.
2. To compare the Diffusion Tensor Imaging (DTI) parameters with Nurick score and neurological examination findings.
3. To detect the early microscopic level structural abnormalities of spinal cord using DTI before overt, irreversible changes manifest in routine MR sequences.

Materials and methods:

It is a prospective observational study performed in 30 patients who were referred by the department of neurology. These patients presented with clinical symptoms and neurological findings suggestive of cervical myelopathy. The study period was from January 2018 to March 2019 at the department of Radiology and imaging sciences PSG Institute of Medical Sciences & Research. Prior to commencement of the study, clearance has been obtained from Institutional Human Ethical Committee and informed written consent has been taken from the participants. Patients with clinical and neurological features of CSM were included in the study. Patients with following abnormalities were excluded from the study: 1.focal or diffuse signal abnormality in the spinal cord. 2. History of cervical spine injury / surgery. 3. Focal lesions in the brain which may be the underlying pathology causing the neurological abnormality.

Prior to undergoing MRI all these patients were assessed symptom wise for the severity of CSM using Nurick score and neurological examination was performed to assess the power of lower and upper limbs. In Nurick score patients were categorized from grade 0 to 3, based on clinical history alone, where grade 0 represented mild form of disease with only symptoms suggestive of nerve root involvement (radiculopathy), grade 3 represents more severe neurological deficit where the patients are wheel chair bound with no independent mobility (myelopathy). On clinical examination muscle power of the limbs was assessed using standard grade 1 to grade 5.

All the patients underwent MRI in Siemens 1.5 Tesla 16 channel machine.

MRI protocol:

1. Routine non contrast T1 and T2 weighted images to look for overt signal changes in T2 Weighted Images.
2. DTI acquisition using EPI yielding the following set of images:

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1. Diffusion weighted imaging, 2.Trace weighted, 3.FA and ADC mapping 4.Tensor imaging.

DTI parameters:

Diffusion directions measured – 20.

The b factors – 0, 100 and 1000

TR/ TE - 3600 /94ms

Field of view - 230mm

Slice thickness - 5mm.

Images were analysed using Siemens Syngo via work station. Initially presence of any focal or diffuse spinal cord lesions was excluded. After that dimension of the spinal canal was measured at all the vertebral level. Subsequently DTI analysis was performed by drawing region of interest at the level of maximum spinal canal stenosis, one vertebral level above the spinal canal stenosis and one level below (Figure 1a). DTI Parameters assessed were FA and ADC, the values of which were displayed as a table in the system (Figure 1b) along with images of visual maps of ADC (Figure 2a) and FA (Figure 2b).

Statistical analysis:

The data has been analysed using SPSS 23.0 version. To describe the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean & standard deviation (SD) were used for continuous variables. To find the significant difference in the multivariate analysis for repeated measures the repeated measures of ANOVA was used with Bonferroni correction to control the type I error on multiple comparison. To assess the relationship between the variables Pearson's Correlation was used. In all the above statistical tools the probability value .05 is considered as statistically significant.

Results:

A total of 30 patients were enrolled in our study of which 20 were men and 10 were women (Figure 3). Nurick Score distribution is given in the table 1.

All the patients had muscle power grade between III and V. (Figure 4). No patient in our study had muscle power less than 3.

Diffusion tensor parameters (ADC and FA) were measured at 3 levels of the spinal cord (Figure 1a). One measurement at the level of the spinal canal stenosis and 2 measurements (above and below the level of stenosis) at the level of normal canal dimension. The values of ADC and FA measured at different levels of the spinal cord are given in table 2.

Pairwise comparison of ADC and FA was performed and results are given in table 3 and table 4. Patients were found to have high ADC and low FA values at the level of stenosis when compared with ADC and FA values at the level of no stenosis. The differences were found to be statistically highly significant with a p value of 0.0005 (ANOVA test was used). There was negative correlation between the mean FA and ADC at the level of stenosis with r value of -0.424 and p value of 0.019. There was negative correlation between the mean FA and ADC at the level of no stenosis with r value of -0.0545 and p value of 0.002. There is negative correlation between Nurick score (clinical grading) and muscle power (neurological examination) r value of -0.07 and P value of 0.001.

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Correlation of FA and ADC with muscle power:

At the level of stenosis:

There was a positive correlation between muscle power and mean FA value with r value of 0.2 and p value 0.1. There is a negative correlation between muscle power and mean ADC value at the level of stenosis with r value of -0.1 and p value 0.1 and p value of 0.3.

At the level of no stenosis:

There is a positive correlation between muscle power grade and mean FA value at the non- stenosis level with r value of 0.18 and p value 0.32 and p value of 0.4. There is a negative correlation between muscle power grade and mean ADC value at the non-stenosis level upper with r value of -0.25 and p value 0.17.

Correlation of FA and ADC with Nurick score:

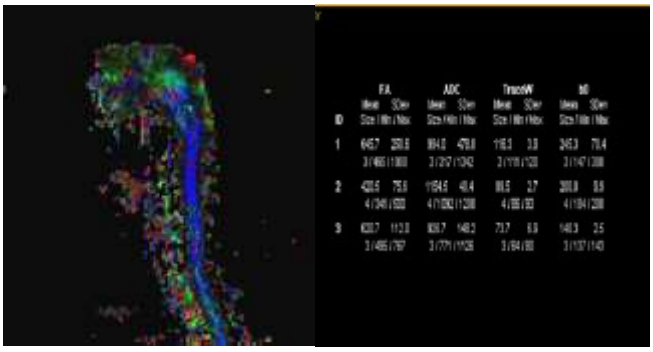
At the level of stenosis:

FA and Nurick score has negative correlation with r value of -0.4 and p value of .0005. ADC and Nurick score has a positive correlation with r value of -0.1 and p value of – 0.2

At the level of no stenosis:

FA and Nurick score had positive correlation with r value of 0.1 and p value of –0.4. ADC and Nurick score had negative correlation r value of -0.05 and p value of– 0.7

ROC analysis of FA in predicting microscopic level structural cord abnormality in the spinal cord at the level of the spinal canal stenosis. (Figure 5) was done. Subsequently test result variables (table 5) and sensitivity and specificity (table 6) were calculated. Likewise ROC analysis of ADC in predicting microscopic level structural cord abnormality was done (Figure 6). Test result variables (table 7) and sensitivity and specificity (table 8) were also calculated for ADC. Both FA and ADC showed good sensitivity and specificity in predicting the early microscopic level changes in the spinal cord caused by degenerative disease, ADC was slightly more specific than FA.

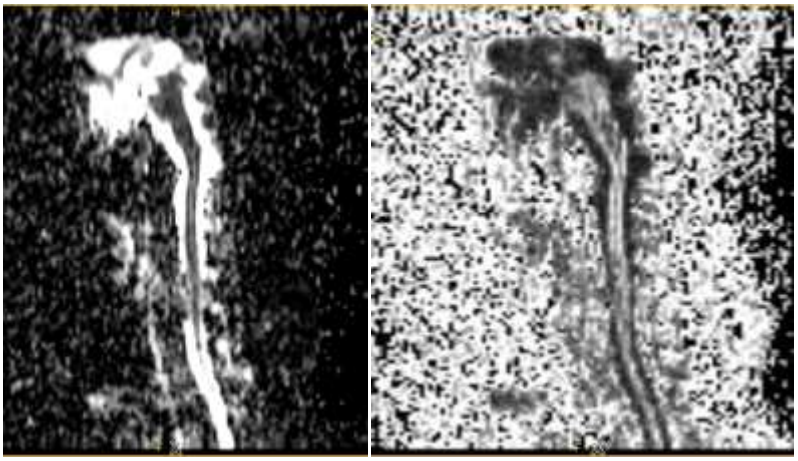


a **b**

Figure 1. Method of FA and ADC measurement.

Three circular Region of Interest (ROI) are drawn “at”, “above” and “below” the level of stenosis (a) yielding the numeric values of each level in a table (b).

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a b

Figure 2. ADC (a) and FA (b) maps obtained with DTI.

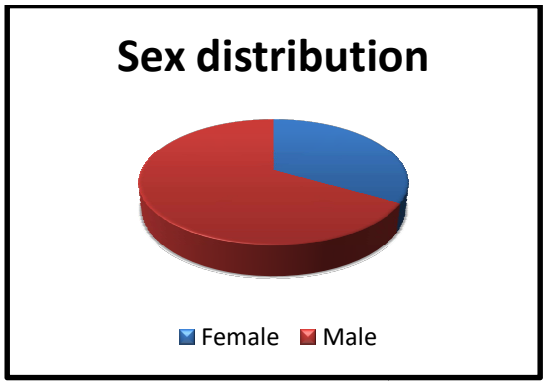


Figure 3. Sex distribution in the study population.

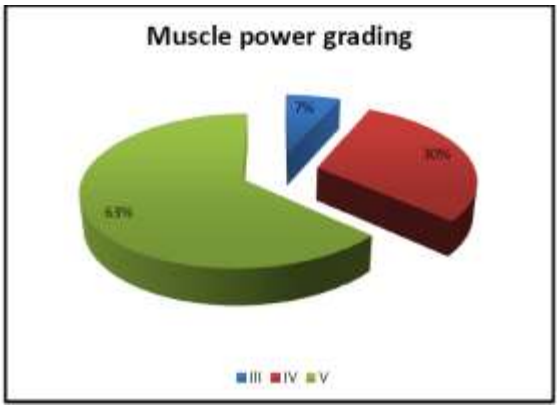


Figure 4. Muscle power distribution in the study population.

Majority of the patients had a muscle power of 5. Remaining patients had muscle power of 4 and 3.

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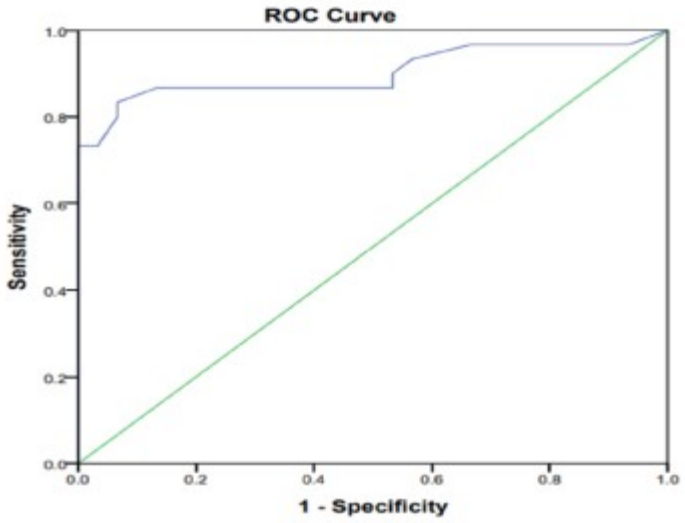


Figure 5: ROC analysis of FA in predicting the microscopic structural abnormality in the spinal cord.

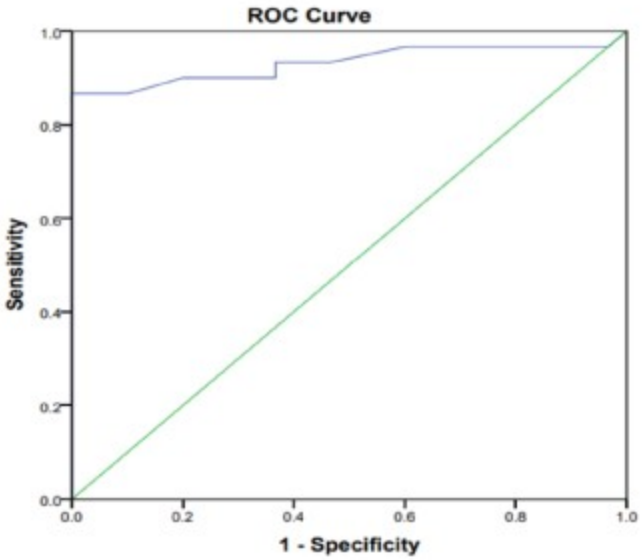


Figure 6: ROC analysis of ADC in predicting the microscopic structural abnormality in the spinal cord.

Table1: Nurick Score distribution in the study population:

Nurick Score	Number	Percent
0	2	6.7
1	9	30.0
2	9	30.0
3	10	33.3
Total	30	100.0

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Table 2: Values of ADC and FA measured at different levels of the spinal cord.

	ADC		FA	
Level of spinal cord	Mean	SD	Mean	SD
Stenotic Level	1.3470	.23997	.5067	.09484
Non-stenotic level upper	1.0141	.09272	.6670	.07957
Non-stenotic level lower	1.0120	.08919	.63443	.07566

Table 3: Pairwise comparison of ADC

(I) ADC		Mean Difference (I-J)	Std. Error	Sig.[b]
Mean ADC stenotic level	Mean ADC non-stenotic level upper	.333*	.045	.0005
	Mean ADC non-stenotic level lower	.335*	.043	.0005
Mean ADC non-stenotic level upper	Mean ADC stenotic level	-.333*	.045	.000
	Mean ADC non-stenotic level lower	.002	.021	1.000
Mean ADC non-stenotic level lower	Mean ADC stenotic level	-.335*	.043	.000
	Mean ADC non-stenotic level upper	-.002	.021	1.000

Based on estimated marginal means.

*The mean difference is significant at the .05 level.

b. Adjustments for multiple comparisons: Bonferroni.

Table 4: Pairwise comparison of FA:

(I) FA		Mean Difference (I-J)	Std. Error	Sig.[b]
Mean FA stenotic level	Mean FA non-stenotic level upper	.160*	.019	.0005
	Mean FA non-stenotic level lower	.128*	.023	.0005
Mean FA non-stenotic level upper	Mean FA stenotic level	-.160*	.019	.000
	Mean FA non-stenotic level lower	.033	.017	.219
Mean FA non-stenotic level lower	Mean FA stenotic level	-.128*	.023	.000
	Mean FA non-stenotic level upper	-.033	.017	.219

Based on estimated marginal means.

*The mean difference is significant at the .05 level.

b. Adjustments for multiple comparisons: Bonferroni.

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Table 5: Test result variables of FA

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.902	.045	.0005	.815	.990

Table 6: Sensitivity and specificity calculation of FA.

Predictor	Cut off	AUROC	Sensitivity	Specificity
FA	0.57	0.902	86.7%	86.7%

Table 7: Test result variables of ADC.

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.932	.039	.0005	.857	1.000

Table 8: Sensitivity and specificity calculation of ADC.

Predictor	Cut off	AUROC	Sensitivity	Specificity
ADC	1.1	0.932	86.7%	90.0%

Discussion:

MRI has revolutionized the evaluation of pathology related to spinal cord. Exceptionally high contrast resolution and soft tissue resolution place MRI way above other imaging modalities in the evaluation of spinal cord.[2] Recent advances in respect to high field strength and fast and robust sequences have greatly contributed to the superior performance of MRI[8]. Very small yet important structures like the nerve roots and the thin ligaments need to be assessed in almost all patients which can harbour the pathology. However significant degree of anatomical and structures abnormalities need to be present before they are visible in routine MRI sequences, primarily in the spinal cord[6]. Hence the need for imaging technique which is similar to a biomarker to pick up early microscopic level changes in the neural structures, specially the spinal cord. Role of DTI in the evaluation of the brain pathologies has been proved and it is being used routinely, however its usage in the evaluation of spinal cord is still evolving.

The application of DTI in the evaluation of spinal cord is hindered by various factors. Spinal cord is a very narrow structure with a high elasticity and situated in anatomically complex location, which are the factors

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making evaluation difficult. Other factors like CSF pulsation, motion artefacts and breathing related artefacts make usage of DTI difficult as a routine sequence. Newer techniques like reduced field of view with oblique spin echo and OVS (outer volume suppression) have been used to overcome the limitations and better images can be obtained. All these improved techniques provide high resolution images not only in the cervical cord but also the entire spinal cord.

Budzik et al[9] studied the feasibility of DTI and fiber tracking in CSM. FA values showed correlation with the patients' clinical scores. High signal intensity of the spinal cord on T2WI did not correlated either with the DTI parameters or with the clinical symptoms, suggesting that FA is better in depicting the early structural abnormality of the spinal cord when compared with T2W imaging[10, 11]. But they did not study the relationship of DTI scalars with cervical canal width. In our study all the patients had spinal canal stenosis, in that about 73% of the patients had relative narrowing and 27% has absolute narrowing.

In our study degenerative changes like disc bulge, protrusion, osteophytes and ligament thickening led to spinal canal narrowing. These pathological changes caused disruption of the white matter tracts and other secondary changes in cervical cord. Secondary changes like cord edema and macrophage proliferation lead to increased diffusivity of water molecules with in the spinal cord parenchyma, especially in the white matter which can alter the FA and ADC values[12]. These changes were apparent much before macroscopic changes appear in routine T1 and T2W images.**UzeyirAhmadli et al**[13] investigated the utility of DTI sequence in patients with CSM symptoms for early detection of microscopic changes in the spinal cord at the level of narrowed spinal canal without any T2 signal alterations. FA values with in the cord were reduced and ADC increased at site of canal stenosis when compared with non-stenotic segments above the level of canal stenosis. However no significant difference was noted in FA and ADC values between stenotic segment and non-stenotic segment distal to the level of stenosis.

In our study there was statistically significant reduction in the FA values and increase in the ADC values at the site of stenosis when compared to the non-stenotic levels (upper and lower). There was no statistically significant reduction in FA value noted at the non-stenotic levels (upper and lower). The reduction in FA value at the stenotic level is presumably due to reduced neuronal fibers with increase in the extracellular space or due to extracellular edema which can result in increased diffusivity of water molecules and consequently facilitated diffusion.

Jones et al[14] reported a significant correlation between FA and modified Japanese orthopedic association score and Nurick score, and reported that patients who had a higher FA at the compressed level of spinal cord tended to have a better recovery of functionality after surgery when compared to patients undergoing surgery who had a lower FA value pre operatively. In our study there was significant correlation between the patient’s symptoms and the DTI parameters. We graded clinical symptoms using only Nurick score, modified Japanese orthopedic association score was not used.

FA and Nurick score had a negative correlation with r value of -0.4 and p value –0.0005, which implies that the FA values tend to be less when the Nurick score is on the higher side. ADC and Nurick score had a positive correlation with r value of-0.1 and p value of – 0.2, which is equal to higher ADC means higher Nurick score.There was also a significant correlation between the DTI parameters and muscle power. There was positive correlation between muscle power and mean FA value at the level of stenosis with r value of 0.2 and p

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value of 0.1. There is a negative correlation between muscle power and mean ADC value at the level of stenosis with r value of -0.1 and p value of 0.3. ROC was calculated between the stenotic and non stenotic level. The mean was calculated between the upper and lower non-stenotic level and it was compared with the stenotic level. FA showed a sensitivity of 86.7% and specificity was 86.7% when the cut was kept at 0.57 (Table 6). ADC showed a sensitivity of 86.7% and specificity was 90.0%, when the cut off is kept at 1.1. (Table 8).

Conclusion:

- Our study showed significant correlation between the patients' clinical symptoms and DTI parameters.
- DTI has the potential to detect microscopic structural changes in the spinal cord, before the T2 signal changes appear on conventional MRI.
- DTI can be used as a tool for detecting early changes in cervical spondylotic myelopathy, which would prognosticate the clinical and surgical outcome[14, 15].
- DTI technique should be routinely used on patients with clinical symptoms of cervical spondylotic myelopathy.

Limitations:

- The sample size of the study population was relatively small
- The long-term follow-up of the patients were not done
- It was impossible to determine the percentage of patients with early cervical spondylotic myelopathy who would have advanced to T2 hyperintensity changes in spinal cord, when left untreated.
- Cost and the availability of MRI.

Further studies have to be done on large group with long term follow up of those patients to determine the potential utility of quantitative diffusion tensor imaging.

Conflicts of interest:

There are no conflicts of interest to declare.

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References:

1. Crandall, Paul H., and Ulrich Batzdorf. "Cervical spondylotic myelopathy."Journal of neurosurgery 25.1 (1966): 57
2. Montgomery, D. M., and R. S. Brower. "Cervical spondylotic myelopathy. Clinical syndrome and natural history." The Orthopedic clinics of North America23.3 (1992): 487-493.
3. Al-Mefty, Ossama, et al. "Myelopathic cervical spondylotic lesions demonstrated by magnetic resonance imaging." Journal of neurosurgery 68.2 (1988): 217-222.

4. Chen, Chi-Jen, et al. "Intramedullary High Signal Intensity on T2-Weighted MR Images in Cervical Spondylotic Myelopathy: Prediction of Prognosis with Type of Intensity 1." Radiology 221.3 (2001): 789-794.
5. Fernández de Rota, Juan Jose, et al. "Cervical spondylotic myelopathy due to chronic compression: the role of signal intensity changes in magnetic resonance images." Journal of Neurosurgery: Spine 6.1 (2007): 17-22.
6. Freeman, T. B., and C. R. Martinez. "Radiological evaluation of cervical spondylotic disease: Limitation of magnetic resonance imaging for diagnosis and preoperative assessment." Perspect Neurol Surg 3.91 (1992): 34-54.
7. Suri, Ashish, et al. "Effect of intramedullary signal changes on the surgical outcome of patients with cervical spondylotic myelopathy." The Spine Journal3.1 (2003): 33-45.
8. Fulong Dong, Wu Y, Song P "A preliminary study of 3.0-T magnetic resonance diffusion tensor imaging in cervical spondylotic myelopathy".Eur Spine J. 2018 Aug;27(8):1839-1845.
9. Budzik JF, Balbi V, Le Thuc V, Duhamel A, Assaker R, Cotten A "Diffusion tensor imaging and fibre tracking in cervical spondylotic myelopathy" Eur Radiol.2011 Feb;21(2):426-33.
10. Kara B, Celik A, Karadereler S, Ulusoy L, "The role of DTI in early detection of cervical spondylotic myelopathy: a preliminary study with 3-T MRI" Neuroradiology. 2011 Aug;53(8):609-16.
11. Mori S, Zhang JPrinciples of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 2006 Sep 7;51(5):527-39
12. Toktas ZO, Tanrıkulu B, Koban O, Kilic T, Konya D "Diffusion tensor imaging of cervical spinal cord: A quantitative diagnostic tool in cervical spondylotic myelopathy". J Craniovertebr Junction Spine. 2016 Jan-Mar;7(1):26-30
13. Uzeyir Ahmadli, Nils H Ulrich ,Yao Yuqiang, Daniel Nanz Early detection of cervical spondylotic myelopathy using diffusion tensor imaging: Experiences in 1.5-tesla magnetic resonance imaging. Neuroradiol J. 2015 Oct; 28(5): 508–514.
14. Jones JG, Cen SY, Lebel RM, Hsieh PC, Law M "Diffusion tensor imaging correlates with the clinical assessment of disease severity in cervical spondylotic myelopathy and predicts outcome following surgery". AJNR Am J Neuroradiol. 2013 Feb;34(2):471-8
15. Zheng W, Chen H, Wang N, Liang Y, Xiao W "Application of Diffusion Tensor Imaging Cutoff Value to Evaluate the Severity and Postoperative Neurologic Recovery of Cervical Spondylotic Myelopathy". World Neurosur. 2018 Oct;118: e849-e855.

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Technical Note

Assessment of Adult Diagnostic Reference Levels for Panoramic Radiography in Tamil Nadu Region

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Abstract

Aim: The aim of this study was to calculate dose area product (DAP) and to determine diagnostic reference level (DRL) for adult panoramic procedures in Tamil Nadu. **Materials and Methods:** In this study, air kerma on the front side of the secondary collimator was measured with a Black Piranha, RTI Electronics, Sweden and multiplied with the corresponding exposed area to calculate DAP. The obtained DAP values were further analyzed, and DRL was calculated using the Microsoft Excel software. The study was carried out with regular adult exposure parameters. **Results:** The mean, range, and 3rd quartile values for 67 panoramic scanners in Tamil Nadu, India, were calculated as 94 mGycm^2, 41 mGycm^2–165 mGycm^2, and 114.3 mGycm^2, respectively. The results are comparable with other international studies. **Conclusion:** The present study suggests that further optimization can be achieved in many centers by the recruitment of professionally qualified radiographers and conducting periodic training programs on the optimization of exposure parameters. Considering this as the first study for the dental DRL assessment, further studies are suggested to establish national dental DRL in India.

Keywords: Dental radiography, diagnostic reference level, dose area product, radiation protection

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Introduction

Dental radiographic examinations and their associated risks have long been neglected by the dental professionals and the institutions because of the relatively low levels of radiation doses involved,[1] compared to other diagnostic radiology disciplines. However, with the rapid increase in the number of dental facilities, in the last several years, across the country, especially in smaller towns, a huge number of children developing dental problems are undergoing dental X-ray imaging.[2] With the introduction of newer types of dental X-ray units such as the cone-beam (CB) dental computed tomography (CT) and increasing frequency of imaging with such advanced systems, the doses involved in dental imaging have gone up very steeply and are steadily bringing dental radiology into the renewed focus of the professional bodies and regulators. In the last decade, much stricter regulations have been introduced in the field of dental radiography, but not much attention has been given to this discipline, in India, so far. This work is an effort, in this direction, to assess doses in dental radiography facilities and their optimization, in the southern part of India, which can later be extended to other parts of India.

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Diagnostic reference level (DRL) is a practical tool that can be used for the optimization of medical exposures.[3-6] A representative number of panoramic X-ray units in the region have to be selected for the establishment of regional DRL in panoramic dental radiography. By comparing the hospital dose with the regional DRL, any hospital in the region can assess whether the doses are consistently higher or lower compared to the regional DRL and then take corrective action. DRL along with the mean observed patient dose values would help to identify the good and bad panoramic practices in dental radiography. It can be used for the elimination of unnecessary radiation exposure that does not contribute to the clinical purpose of the image.[7] The measurement of patient radiation doses during diagnosis procedures is important[8,9] for the assessment of patient risk.

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By establishing zone-wise DRL, in the South, North, East, West, and Central parts of the country and consolidating the data national level DRL can be set. Thus, the objective of the present study, initiated by PSG Institute of Medical Sciences and Research in consultation with and grant from the Atomic Energy Regulatory Board (AERB), is to establish DRL for adult panoramic radiography in the region of Tamil Nadu and compare them with the internationally recommended DRLs.

Materials and Methods

Panoramic scanners

Sixty-seven panoramic dental X-ray machines, including 22 different models were randomly selected based on workload from dental facilities in the Tamil Nadu region, and measurements were performed without the presence of the patient in place. Before commencing the measurements in the hospitals, a questionnaire was sent to collect the radiation safety status, detector type, quality assurance (QA) status, and exposure parameters used for the adult panoramic scans. Measurements were taken after doing the QA testing of the respective scanner. Mechanical, electrical, and radiation quality tests were done for the respective scanners as per the AERB to reconfirm the positional accuracy of the dosimeter. The beam area of the scanner was measured by using a computed radiography (CR) cassette and verified with the machine manual given by the manufacturer. Table 1 shows the average exposure parameters used with each model of panoramic scanners and their active detector beam area.

The CR cassette was kept in front of the secondary collimator and exposed for 1 s to measure the beam area. The procedure was repeated for all the selected machines involved in this study and confirmed the area of the detector.

Dosimeter

A solid-state dosimeter (RTI Black Piranha 557), from RTI electronics, Sweden, that has an active width of 3 mm, was used for the measurement of radiation dose.

Aligning Piranha dosimeter at the secondary collimator is a difficult task as it is heavy and single strap of Transpore tape may not be enough to hold it safely during the measurement. Since there is no laser light in some of the panoramic scanners to replicate the tube slit position at the detector side, positioning of solid-state dosimeter along the narrow tube slit is a tedious task. Thus, the initial positioning of dosimeter may not show an accurate result. Graphs shown in Ocean software helps to identify the inaccurate and accurate position of dosimeter after the radiation exposure [Figure 1]. Piranha holder, specifically configured to hold Piranha 557 dosimeter during the panoramic scan, helps to hold the dosimeter tightly during the scan time [Figure 2]. Minute positional alignment of dosimeter is also possible using piranha holder.

Experiment technique

The Piranha 557 dosimeter used in this study is more convenient to use as compared to the detector used by Lee *et al*.[5] in their study which had an active sensor width of 1.5 mm since its active sensor width is 3 mm. The "OCEAN 2014 connect" software enables the black Piranha to connect directly to the computer through bluetooth. During the measurement, the black Piranha solid-state dosimeter was attached in front of the secondary collimator with a Piranha holder [Figure 3].

Table 1: Average exposure parameters used for different scanners and beam area

Number of scanners	Name of the scanner	Beam area (cm^2)	kVp	mA	Time (s)
1	Newtom Go 3D	0.60*14.6	78	8	12.7
1	Rotograph Evo D	0.60*14.60	72	6	14.4
1	Villa Sistemi, Rotograph plus	0.60*14.3	78	10	17.0
1	HYPERION X-7	0.60*14.60	67	5	9.1
1	Xmind Novus	0.61*14.75	70	7	9.0
1	Gendex Dental Systems	0.60*15.10	70	10	16.0
1	X-MIND PANO D+	0.61*14.75	73	10	17.6
1	CS 9300	0.50*14.9	75	12	14.3
1	GME Pantograph 10	0.61*14.75	75	10	17.0
1	Xtropan 2000	0.60*15.00	70	12	17.0
2	ADVAPEX - DENTOPAN-10	0.69*15.10	70	10	14.0
2	Planmeca ProOne	0.60*14.6	69	7	9.8
2	Soredex	0.61*14.75	73	10	9.0
3	Planmeca proline -EC	0.50*13.6	66	11	16.0
3	ORTHOPHOS XG 3	0.325*13	67	13	14.1
3	Genoray Co. Ltd, EXTOR-P	0.64*15.00	72	11	12.0
4	CS 8100	0.50*13.12	73	11	10.7
5	ASAHI IIIECM roentgen	0.69*15.10	73	10	12.0
6	Vatech Pax-i	0.60*15.04	72	9	13.2
6	Planmeca Promax	0.60*14.70	67	9	15.8
6	ORTHOPHOS XG 5	0.325*13	66	12	14.1
15	Kodak 8000	0.40*12.80	71	10	13.7

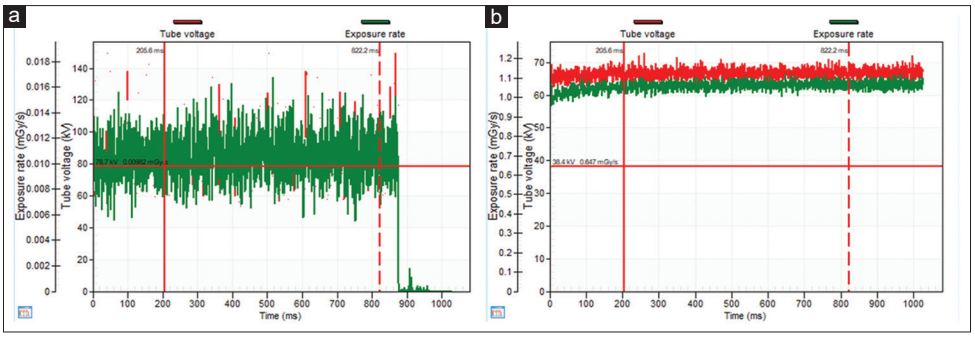


Figure 1: Graphical representation shown in Piranha Ocean software after inaccurate positioning (a) and accurate positioning (b) of dosimeter



Figure 2: Panoramic Piranha holder with Piranha dosimeter

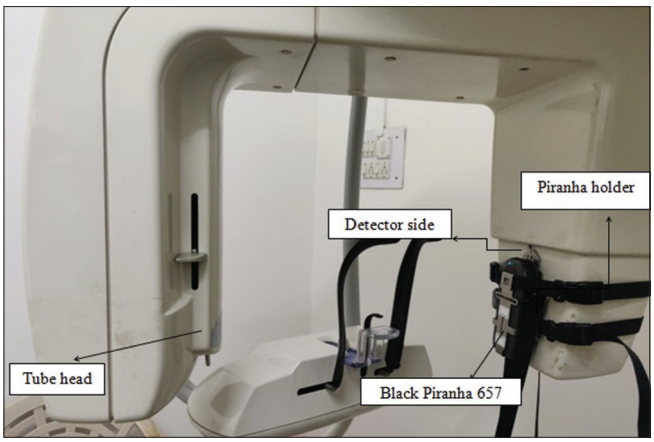


Figure 3: Pictorial representation of experimental procedure

Once the panoramic scanner passed AERB QA test, then that particular machine is included for the DRL study. The regular kVp, mA, and time used for the adult panoramic scan were loaded in the panoramic scanner. Measurements were taken for 20 patient protocols. Figure 4 shows the screenshot image of the reading shown in the software after the exposure. Exposures were repeated without the presence of patients, and radiation doses were noted.

The product of the measured dose with the measured area gives the dose area product (DAP) values. After calculating DAP from 67 panoramic dental scanners, statistical parameters such as average, 3rd quartile values were calculated using Microsoft Excel software. The 3rd quartile value can be suggested as DRL, according to Radiation Protection Document No. 109 of the European Commission.[10]

Results

Verification of DAP calculation was made by comparing the machine DAP and calculated DAP [Figure 5]. Of 38 displayed values from different panoramic scanners, the observed deviation from machine value was well within ± 18%. The maximum deviation observed between machine DAP and calculated DAP was −17.68%.

The assessed DRL value, along with the calculated DAP values, for 67 panoramic radiography units are given in Figure 6. The calculated DAP values ranged from 41 mGycm^2 to 165 mGycm^2. The lowest and highest DAP values for all procedures were calculated at scanners based on digital and analog type detectors, respectively. However, in many digital type scanners, high radiation doses were observed.

Mean exposure parameters, along with the mean, range, and the 3rd quartile of the DAP values for analog and digital mode of detectors are given in Table 2. There was nearly 3.6-fold difference between the highest (148 mGycm^2) and lowest (41 mGycm^2) DAP values for the digital detector units. Almost 2.5-fold difference was observed for analog type units between the lowest (65 mGycm^2) and highest (165 mGycm^2).

The DRL was calculated from DAP values using Microsoft Excel software by the formula "PERCENTILE, (array, k)," where array represents the DAP values and *k* represents the percentile value (in the present case *k* is 0.75).

From the findings of the present study, the suggested DRL for adult panoramic procedures in Tamil Nadu region was 114.3 mGycm^2.

Discussion

Unlike other dental X-ray units like intraoral and CBCT scanners, the panoramic unit produces a narrow slit beam and tracks a circular path around the patient's head to

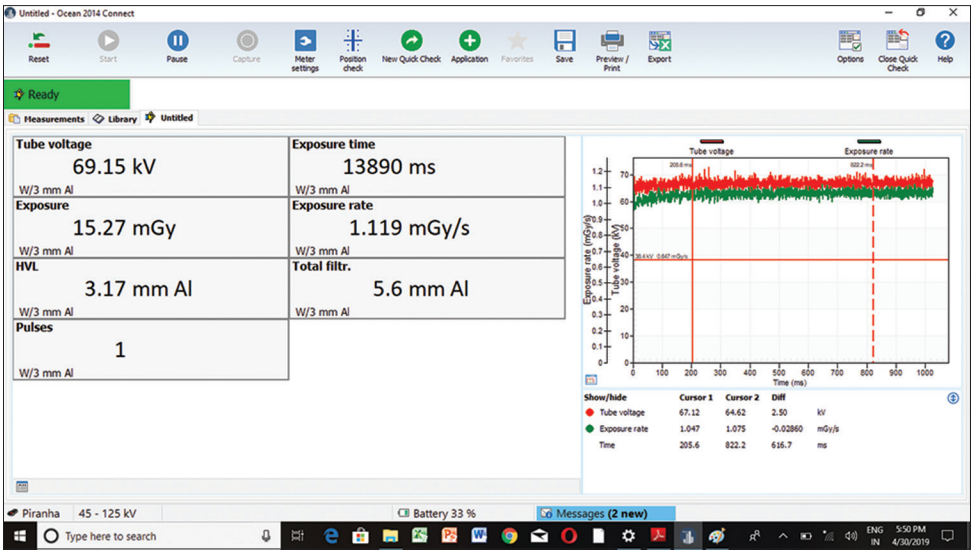


Figure 4: Screenshot of the reading obtained in Ocean 2014 Connect software for exposure parameters of 70 kVp, 10 mA and 13.9 s

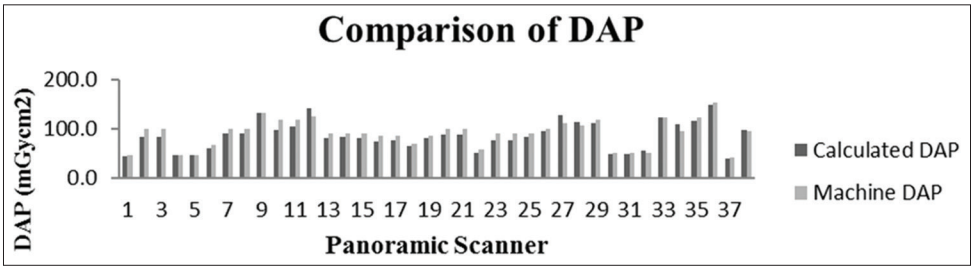


Figure 5: Comparison of machine dose area product and calculated dose area product

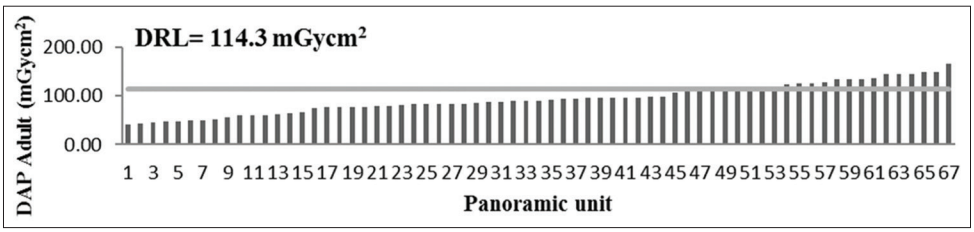


Figure 6: Proposed adult diagnostic reference level and calculated dose area product values of different panoramic units

Table 2: Mean exposure parameters along with range, mean and 3rd quartile of dose area product values for digital and analogue types of units

Type of unit	Exposure mean (kV)	Mean (mA)	Mean exposure time (s)	DAP range (mGycm²)	Mean DAP (mGycm²)	DRL (mGycm²)
Analogue	71.2	10.3	14.4	65-165	105.9	125.5
Digital	70.1	10.0	13.2	41-148	90.7	112.4
Total	70.3	10.0	13.5	41-165	93.6	114.3

DRL: Diagnostic reference level, DAP: Dose area product

obtain the dental X-ray image. For the accurate radiation dose measurement process during panoramic procedure, the dosimeter has to follow the narrow slit beam. Any misalignment of dosimeter may create a huge error. Moreover, the fact that the beam slit is broader at the detector side of the scanner makes it comparatively easy to align the dosimeter for radiation dose measurement. The agreement between the calculated DAP values and machine displayed DAP values further confirms the reasonable accuracy of the machine displayed values [Figure 5]. The observed deviation may be attributed to the difference in the type of dosimeter used, time slab of measurement and experimental technique. For better accuracy, the display values can be calibrated in terms of the measured values.

Figure 7 shows the comparison of other countries' dental panoramic DRL with the present study, the Tamil Nadu DRL. Similar results were published by different authors such as Tierris *et al*.[11] and Han *et al*.[12]

Tierris *et al*.[11] found 101 mGycm^2 and 117 mGycm^2, respectively, as their mean and 3rd quartile values. Careful analysis of the results published by them indicates that there are 18 units that have DAP > 100 mGycm^2 and in that, 8 units have DAP in between 200 mGycm^2 and 250 mGycm^2. Therefore, the DRL of their study was affected by the scanners with higher doses. The average exposure parameter (72.4 kVp, 10.5 mA, and 15.2 s) calculated by them was slightly higher than the average calculated values of the present study (70.3 kVp, 10.0 mA, and 13.5 s). The present study has 23 scanners which are having DAP >100 mGycm^2 and the highest DAP value calculated was 165 mGycm^2.

Han *et al*.[12] have measured DAP using DAP meter (VacuDAP-OEM, Vacutec, Germany) on 42 panoramic scanners. The maximum DAP observed from their study also crossed 200 mGycm^2 with an average of 98.2 mGycm^2 and a corresponding 3rd quartile value of 120.3 mGycm^2. Their average kVp and mAs calculated from different panoramic scanners ranged from 68–82.3 kVp and 110.7–211.2 mAs, respectively. The present study kVp and mAs ranged from 66–78 kVp and 46–204 mAs. Hence, the high DRL in Han *et al*.[12] is influenced by larger exposure parameters.

Williams and Montgomery[13] have assessed DRL from 16 panoramic X-ray units using thermoluminescence dosimeters. The mean DAP and corresponding 3rd quartile values for standard adult examination are 113 and 139 mGycm^2, respectively. The difference in the type of dosimeter and sample size may have influenced their DRL value. This may be the reason for variation in their DRL as compared with the present study.

The DRL assessed by Poppe *et al*.[14] and Hodolli *et al*.[15] are 101.4 and 93 mGycm^2, respectively. A close analysis of the results of their studies shows that the majority of their panoramic X-ray units have DAP lesser than 100 mGycm^2. Therefore, the DRLs of their studies are affected by the scanners with lower doses. The average exposure parameters calculated by Poppe *et al*.[14] and Hodolli *et al*.[15] are 73.1 kVp, 11.6 mA, and 15.3 s and 72.8 kVp, 12.8 mA, and 16.2 s, respectively. Although the exposure parameters are higher than the present study average values, their DAP values are lesser than the current study calculated values. The difference in tube inherent filtration and manufacturing date can result in lesser DAP readings for their studies.

In short DRL values obtained in the present study agree with the corresponding results reported by other countries. The difference in patient physical parameter, equipment exposure parameter, tube inherent filtration, type of dosimeter used for measurement were some of the reasons for slight variation between the DRLs as assessed by the present study and by other investigators referred to here. Although the method used in the present study produces comparable results, direct use of DAP meter is a much easier method.

In setting the DRL, special consideration has to be given for digital type detector scanners for further dose optimization. In the present study, almost 33% of digital detectors were having DAP >100 mGycm^2, whereas 38% analog-type detectors had DAP >100 mGycm^2. The current study also found that many clinics and hospitals were using higher exposure parameters while using digital type detectors and comparable or lesser parameters for analog type scanners. There is always a trend to increase the dose, as higher doses can reduce the image noise to an extent. Thus, high image quality can be obtained easily.[16] Periodic QA is suggested to assess the image quality and for the optimization of exposure parameters. Higher doses obtained from this study indicate that more attention is required toward the radiation protection aspects of digital radiography. While operating the panoramic X-ray units, the operators should ensure that the patient is not

Table 3: Mean exposure parameters for scanners which were operated by professionally and not professionally qualified radiographers

Type	kVp	mA	Time (s)	Average DAP
Scanners operated by professionally qualified and experienced radiographers	67	8	13	74
Scanners NOT operated by professionally qualified and experienced radiographers	71	11	14	102

DAP: Dose area product

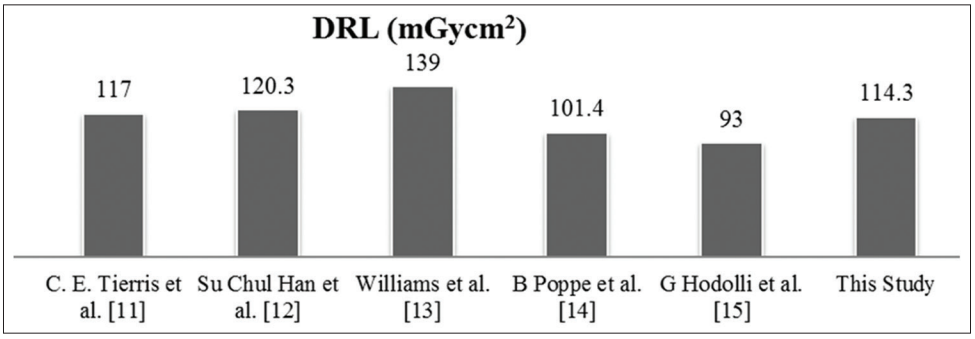


Figure 7: Comparison of other country panoramic diagnostic reference level and the present study diagnostic reference level

exposed to a dose higher than necessary when using digital image receptors.

Table 3 shows the difference in exposure parameters and average DAP values between those panoramic scanners, which were operated by professionally qualified and not professionally qualified operators.

Conclusion

This paper shows dose measurements carried out in almost 50% of the panoramic scanners in the Tamil Nadu region of South India to establish adult panoramic DRL. Although the black Piranha dosimeter consumes time to position it along the narrow slit of the panoramic scanner, its active width of 3 mm enables in reducing the positioning error comparatively. The obtained results were shown to be comparable to another country DRLs. However, the calculated DAP of certain scanners is higher than the assessed DRL value indicating the fact that the exposure parameters used in those scanners are rather high and can be further optimized. Exposure parameters have suggested to further optimize the dose to prevent patient from receiving unnecessary radiation dose from these scanners. Further, it is suggested to conduct training programs for radiographers and dentists to follow the principle of as low as reasonably achievable (ALARA).

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Conflicts of interest

There are no conflicts of interest.

References

1. Kaeppler G, Dietz K, Herz K, Reinert S. Factors influencing the absorbed dose in intraoral radiography. Dentomaxillofac Radiol 2007;36:506-13.
2. Hujoel P, Hollender L, Bollen AM, Young JD, McGee M, Grosso A. Head-and-neck organ doses from an episode of orthodontic care. Am J Orthod Dentofacial Orthop 2008;133:210-7.
3. Vañó E, Miller DL, Martin CJ, Rehani MM, Kang K, Rosenstein M, *et al.* ICRP publication 135: Diagnostic reference levels in medical imaging. Ann ICRP 2017;46:1-44.
4. International Atomic Energy Agency. Diagnostic Reference Levels (DRLs) in Medical Imaging; 2018. Available from: https://www.iaea.org/resources/rpop/health-professionals/nuclear-medicine/diagnostic-nuclear-medicine/diagnostic-reference-levels-in-medical-imaging. [Last accessed on 2018 Jun 15].
5. Lee JS, Kim YH, Yoon SJ, Kang BC. Reference dose levels for dental panoramic radiography in Gwangju, South Korea. Radiat Prot Dosimetry 2010;142:184-90.
6. Doyle P, Martin CJ, Robertson J. Techniques for measurement of dose width product in panoramic dental radiography. Br J Radiol 2006;79:142-7.
7. International Commission on Radiological Protection. Radiological Protection and Safety in Medicine. ICRP Publication 73 Ann. ICRP 26 (2). Oxford: Pergamon Press; 1996.
8. Underhill TE, Kimura K, Chilvarquer I, McDavid WD, Langlais RP, Preece JW, *et al.* Radiobiologic risk estimation from dental radiology. Part II. Cancer incidence and fatality. Oral Surg Oral Med Oral Pathol 1988;66:261-7.
9. Gibbs SJ, Pujol A, McDavid WD, Welander U, Tronje G. Patient risk from rotational panoramic radiography. Dentomaxillofac Radiol 1988;17:25-32.
10. European Commission. Guidance on diagnostic reference levels (DRLs) for medical exposures. Radiation protection 109. Office for Official Publications of the European Communities, Luxembourg; 1999.
11. Tierris CE, Yakoumakis EN, Bramis GN, Georgiou E. Dose area product reference levels in dental panoramic radiology. Radiat Prot Dosimetry 2004;111:283-7.
12. Han S, Lee B, Shin G, Choi J, Kim J, Park C, *et al.* Dose area product measurement for diagnostic reference levels and analysis of patient dose in dental radiography. Radiat Prot Dosimetry 2012;150:523-31.
13. Williams JR, Montgomery A. Measurement of dose in panoramic dental radiology. Br J Radiol 2000;73:1002-6.
14. Poppe B, Looe HK, Pfaffenberger A, Chofor N, Eenboom F, Sering M, *et al.* Dose-area product measurements in panoramic dental radiology. Radiat Prot Dosimetry 2007;123:131-4.
15. Hodolli G, Kadiri S, Nafezi G, Bahtijari M, Syla N. Diagnostic reference levels at intraoral and dental panoramic examinations. Int J Radiat Res 2019;17:147-50.
16. International Commission on Radiological Protection. Managing Patient Dose in Digital Radiology. ICRP Publication 93 Ann. ICRP 34 (1). Oxford: Pergamon Press; 2004.

Original Article

Role of Transrectal Ultrasound Elastography in the Diagnosis of Prostate Carcinoma

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Abstract

Background: The purpose of this study was to evaluate the diagnostic value of transrectal real- time strain elastography (RTE) in identifying prostatic carcinoma (PCa). **Materials and Methods:** 60 patients suspected of having PCa based on abnormal digital rectal examination and raised prostate specific antigen levels underwent transrectal ultrasound (TRUS), color Doppler (CD) and RTE. Elastograms were scored on a five point scale based on distribution of strain in relation to hypoechoic area on TRUS. Twelve core systematic biopsy as well as targeted biopsy was performed from suspicious areas on TRUS and RTE. Diagnostic performance of sonoelastography was evaluated using histopathology as reference standard. **Results:** Histopathology revealed cancer in 28 out of 60 patients (47%) studied. Gleason score ranged from 6 to 9. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TRUS in detecting prostate cancer were 78.57%, 81.25%, 78.57%, and 81.25%, respectively. On CD evaluation 87.5% (n = 28) of benign lesions showed symmetric, radial flow compared to 14.3% (n = 4) of malignant lesions ($P < 0.0001$). The sensitivity and specificity of RTE was 89.29% and 56.25% with PPV and NPV being 58.13% and 82.35%, respectively. Higher elastography score was found to be significantly associated with malignant histopathology ($P = 0.004$). Cancer detection rate with RTE was greater for tumors with higher Gleason score. **Conclusion:** RTE was found to have better sensitivity than TRUS as well as combination of TRUS and CD. Although less specific, RTE can be an effective adjuvant tool to TRUS for guidance of biopsy and improve detection rate of PCa.

Keywords: Elastography, prostate cancer, ultrasonography

Introduction

Prostate carcinoma (PCa) is the second most frequently diagnosed cancer in males all over the world and is a major healthcare concern associated with significant morbidity and mortality. The burden of PCa is estimated to grow to 1.7 million new cases and 499000 new deaths by 2030 as a result of growth and aging of the global population.[1]

Digital rectal examination (DRE), screening for prostate specific antigen (PSA), and imaging techniques such as transrectal ultrasound (TRUS) and magnetic resonance imaging (MRI) are the methods commonly employed for detection of PCa. DRE is limited by its low sensitivity, subjective nature, and depends on the experience of the clinician. Although PSA testing has been widely used for screening of prostatic cancer, it is not disease specific and can be elevated in patients with prostatitis as well as benign prostatic hyperplasia.[2,3] In a patient with suspicion of prostatic cancer, current guidelines involve histopathologic examination of systematic biopsy cores to confirm or rule out cancer.[4] However, the conventional biopsy protocol misses significant cancer in a large proportion of patients.[5] Further many insignificant cancers that do not need immediate treatment are also detected leading to overdiagnosis and treatment.[6] Use of TRUS for detecting prostate cancer has limitations such as poor specificity of focal abnormalities, frequent multifocality of cancer within the prostate, and substantial percentage of isoechoic prostatic carcinoma (PCa).[7] Although MRI results are promising, it is expensive and not always available. In view of these limitations, improved noninvasive imaging modalities that

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can provide better characterization of prostatic lesions and increase detection of prostatic cancer are needed.

Ultrasound elastography (USE) is a dynamic technique that uses ultrasound to estimate tissue stiffness by measuring the degree of distortion under application of an external force. As cancerous tissue has greater stiffness than a benign lesion USE has been used to differentiate malignant from benign lesions in breast, thyroid, liver, and cervix. Recent studies have demonstrated the utility of sonoelastography in detecting prostate cancer.[8-10]

Data on the usefulness of transrectal strain elastography (SE) in evaluation of prostatic cancer and its correlation with histopathology are relatively few, especially from India. The purpose of the present study was to evaluate the usefulness and diagnostic performance of transrectal SE in identifying prostatic cancer with histopathologic diagnosis obtained by needle biopsy of prostate as reference standard.

Materials and Methods

This prospective study was performed in a tertiary care center in South India between December 2014 and September 2016. 60 consecutive patients who were referred to the department of Radiology with suspicion of PCa based on abnormal DRE and elevated PSA levels (>4 ng/ml) were enrolled. The institutional review board approved the study protocol and the protocol was in compliance with tenets of the Declaration of Helsinki (IRB approval Number: 14/399). Informed consent was obtained from all participants. Exclusion criteria consisted of patients with bleeding disorders, inflammatory bowel disease and surgical absence of rectum or ileoanal pouch. All patients underwent TRUS (grey scale and Doppler) and SE as well as needle biopsy of prostate in the same session.

TRUS of prostate was done using an endocavitary transducer (Siemens Accuson S3000, Siemens Healthineers, Erlangen, Germany) with 9–4 MHz range and 174 degree field of vision, capable of user selectable multi hertz imaging. Xylocaine gel was applied over a probe cover applied onto the probe. All patients were examined in the left lateral decubitus position. Prostate was imaged in both axial and sagittal planes with assessment of volume, echogenicity, surface, calcification, vascularity, and the presence of nodules. Each nodule was assessed for size, margins, location in the gland, morphology, and echogenicity.

Glands with homogeneous echotexture or minimal heterogeneity on TRUS were labeled as benign while those with ill-defined echotexture abnormality, focal bulge or hypoechoic mass were categorized as malignant. For Color Doppler (CD) imaging, capsular/periurethral flow and symmetric radial flow from capsular branches were considered benign while asymmetric or increased flow with disorganized pattern was labeled malignant.[11]

Using the same probe transrectal real-time strain elastography (RTE) of prostate was done by a single experienced radiologist. The probe was placed over the region such that the lesion is in the center of the image. Elastograms were obtained by slight compression and decompression of the prostate. The appropriate frequency and pressure of the compression/decompression was optimized with the help of real time display of quality index. Elastograms obtained with quality index equal to or >60 were only included. At least a 5 mm thickness of normal adjacent tissue was included, to assess the lesion stiffness in relation with the average elasticity of the surrounding tissue. A standardized color coding system was used, where red indicated soft, more compliant areas and blue indicated stiff areas. Green color indicated average strain in the region of interest. The elastograms along with conventional B-mode images were displayed side by side on split screen mode. Multiple frames were acquired and the best fit B mode-elastography image pairs were selected for examination. The elasticity patterns were scored using a five point scale proposed by Kamoi *et al*.[10] [Table 1] The cut off for malignancy was set between scores of 2 and 3 [Figures 1-4].

Sampling of the prostate was performed either in the sagittal or in the axial plane. Biopsies were taken using an 18 G biopsy gun under strict aseptic precautions. Extended core biopsy protocol (12 cores) was used. Additional targeted biopsy was carried out on suspicious areas identified by B mode US and elastography. The grade (degree of aggressiveness) of prostate cancer was evaluated on the basis of the Gleason score. No major complications were encountered after the procedure.

The findings of TRUS, Doppler and RTE were interpreted by consensus by two experienced radiologists who were blinded to patients' clinical profile and biopsy results. The histopathologic results were considered as the reference standard, and utility of RTE was evaluated.

Statistical analysis

Data was entered in the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. The diagnostic sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated with histopathologic analysis as reference standard. For analyzing the combined diagnostic performance of tests, the test was considered positive if either of them was

Table 1: Sonoelastography scoring system proposed by Kamoi *et al*.

Elastography score	Description
Score 1	Normal - homogeneous strain
Score 2	Probably normal - symmetric heterogeneous strain
Score 3	Indeterminate - asymmetric focal stiff lesion which is not related to any hypoechoic area
Score 4	Probably carcinoma (strain at the periphery of the hypoechoic lesion with sparing of the center
Score 5	Definitely carcinoma (no strain in the entire hypoechoic lesion or in the surrounding area)

suspicious for malignancy and negative if neither showed suspicious features. Pearson Chi-square test was used to study the association between categorical variables. $P < 0.05$ was considered as statistically significant.

Results

The age of the patients ranged from 53 to 88 years (Mean: 69.16 ± 9.40 years). Serum PSA levels ranged between 4.3 and 178.6 ng/ml (Mean 48.54 ± 56.69 ng/ml). No Significant difference in PSA levels were noted between patients with carcinoma and benign lesions of the prostate ($P = 0.083$). In 60 patients, 784 biopsy cores were harvested out of which 720 was according to the extended core biopsy protocol and 64 were targeted (36 and 28 targeted to areas found suspicious on B-mode US and RTE, respectively). Histopathology revealed cancerous focus in at least one biopsy core in 28/60 (47%) patients. Gleason score ranged from 6 to 9 (Mean: 7.42 ± 1.13). Among the 32 patients with benign histopathology results, 20 (62.5%) were found to have benign prostatic hyperplasia and 12 (37.5%) had prostatitis. The mean prostatic volume was 38.20 ± 18.25 cm^3 (range: 8–89 cm^3). The mean age of patients with benign and malignant lesions were 66.78 ± 9.56 years and 71.89 ± 8.59 years, respectively ($P = 0.035$).

Out of the 60 patients, 28 (47%) were suspected of having prostate cancer by TRUS. The sensitivity, specificity, PPV, and NPV of TRUS in detecting prostate cancer were 78.57%, 81.25%, 78.57%, and 81.25%, respectively, with histopathology as reference standard. On CD evaluation 87.5% ($n = 28$) of benign lesions showed symmetric, radial flow compared to 14.3% ($n = 4$) of malignant lesions ($P < 0.0001$).

Table 2 shows the correlation of elastography scores and histopathologic diagnoses of lesions. Out of the 32 patients

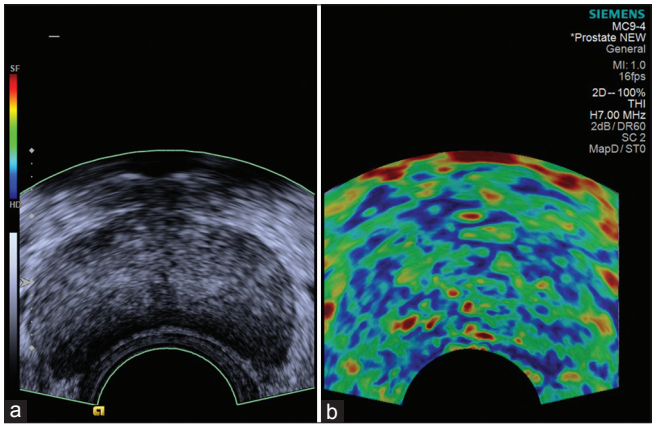


Figure 1: (a) Transrectal ultrasonography image showing mild enlargement of prostate. (b) Sonoelastography showing heterogeneous appearance with symmetrical mosaic pattern of green and blue (Score 2 – probably normal). Histopathology revealed hyperplasia of prostate with benign glandular pattern

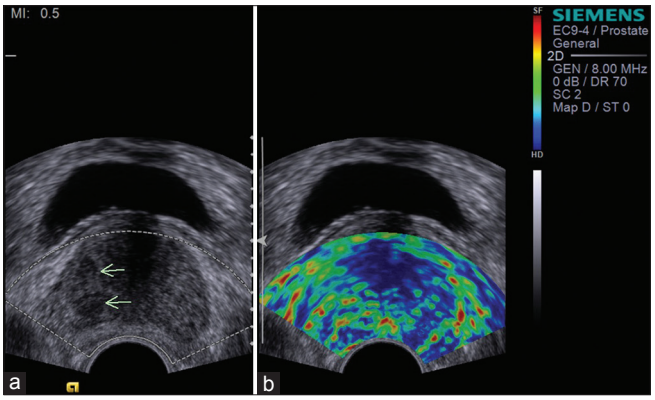


Figure 2: (a) Transrectal ultrasonography image showing hypoechoic lesion in the right peripheral zone (arrows) of prostate. (b) Sonoelastography showing focal asymmetric stiff lesion not related to hypoechoic area (score 3 – indeterminate). Histopathology was suggestive of adenocarcinoma prostate

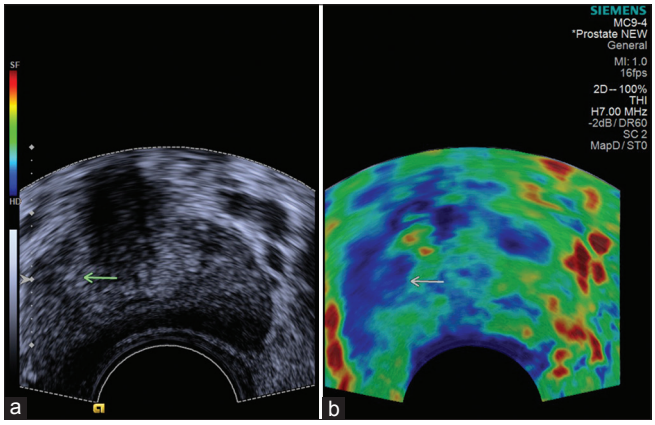


Figure 3: (a) Transrectal ultrasonography image showing hypoechoic lesion in the right peripheral zone (arrow) of prostate. (b) Sonoelastography showing stiffness in the center of the lesion and strain at the periphery; the peripheral part of lesion appears green and the central part appears blue (score 4 – probably carcinoma). Histopathology confirmed adenocarcinoma prostate

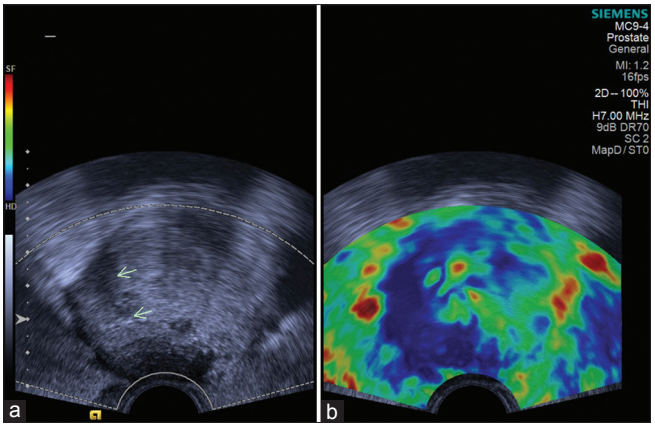


Figure 4: (a) Transrectal ultrasonography image showing hypoechoic lesion with irregular margin in the right peripheral zone (arrows) of prostate. (b) Sonoelastography showing stiffness in the entire hypoechoic lesion and in the surrounding area; the entire lesion appears blue (score 5 – definitely carcinoma). Histopathology confirmed adenocarcinoma prostate

with benign histopathology, 14 were judged as negative for malignancy at RTE. There were 3 false negative patients on elastography with score of 2. The sensitivity and specificity of RTE was 89.29% and 56.25% with positive and NPV of 58.13% and 82.35%, respectively. Higher elastography score was found to be significantly associated with malignant histopathology ($P = 0.004$).

Table 3 shows the relation between Gleason score and elastography scores. Approximately 84% of patients with a Gleason score ≤6 were detected by RTE whereas for scores 8–9, the detection rate was 100%.

The diagnostic performance of TRUS, combined modalities (TRUS and Doppler) and RTE are shown in Table 4.

Discussion

In this prospective study from South India, we evaluated the diagnostic performance of transrectal real-time elastography (RTE) in detection of prostatic cancer in patients with abnormal DRE and elevated PSA levels. RTE showed superior sensitivity of nearly 90% versus sensitivity of 79% by TRUS and 86% by a combination of TRUS and CD ultrasound.

Table 2: Correlation between elastography score and histopathologic diagnosis of lesions

Elastogram pattern	Benign ($n=32$)	Malignant ($n=28$)	Total ($n=60$)
1	0	0	0
2	14	3	17
3	18	4	22
4	0	9	9
5	0	12	12

Table 3: Relation between Gleason score and elastography scores

Gleason score	ES 1	ES 2	ES 3	ES 4	ES 5
≤6	0	1	0	2	3
7	0	2	2	6	2
8	0	0	0	1	1
9	0	0	2	0	6

ES: Elastography score

Table 4: Diagnostic performance of transrectal ultrasound, combined modalities (transrectal ultrasound and Doppler) and real-time strain elastography

Performance measure	TRUS	TRUS and doppler	RTE
Sensitivity (%)	78.57	85.71	89.29
Specificity (%)	81.25	75	56.25
Positive predictive value (%)	78.57	75	58.13
Negative predictive value (%)	81.25	85.71	82.35

TRUS: Transrectal ultrasound, RTE: Real-time strain elastography

Ever since the description of principles of elastography by Ophir *et al*., elasticity imaging has been applied to a wide variety of tissues such as breast, thyroid, liver, lymph nodes and prostate.[12]

The two US elastography techniques in clinical use are SE and shear wave elastography. Several studies have evaluated the utility of SE in diagnosing prostatic cancer and have shown varied results. While few have compared the diagnostic accuracy with needle biopsy of prostate,[13-15] others have used histopathologic examination of radical prostatectomy specimen as the reference standard.[8,9,16] The heterogeneous results can be attributed to the varied inclusion criteria, demographic characteristics of patients, serum PSA levels, location of tumors, reference standard, number of biopsy cores, systematic or targeted biopsies and Gleason score of the tumors examined.

The age of patients with malignancy of prostate was significantly higher compared to those with benign lesions in our study ($P = 0.035$). Age is considered to be an established risk factor for prostatic cancer and study of age-specific incidence curves have shown that risk of prostate cancer begins to rise sharply after 55 years of age and peaks at 70–74, declining slightly thereafter.[17]

In our study, TRUS revealed hypoechoic/focal lesions in 28 patients (47%). Out of these, 22 were confirmed as adenocarcinoma prostate while 6 turned out to be benign on histopathology. The results from our study in terms of specificity and PPV of TRUS in detecting prostatic cancer are similar to that of Ferrari *et al*. who had evaluated 84 patients suspected of prostatic cancer by TRUS, RTE and transperineal prostate biopsy.[18] B-mode US demonstrated 56% sensitivity, 80% specificity, 70% PPV and 67% NPV in diagnosing prostatic cancer. A hypoechoic lesion in the peripheral zone of prostate is suggested to be more likely malignant, the proposed cause being replacement of normal loose glandular tissue by a packed mass of tumor cells which results in fewer reflecting interfaces. Hyperplasia, prostatitis, fibrosis and cysts of prostate are other lesions that appear hypoechoic on TRUS.[19] Nearly 25%–40% of prostate cancers are isoechoic and missed by TRUS.[20]

CD and Power Doppler (PD) have been reported to be useful in detecting isoechoic tumors which may be missed by conventional ultrasound.[21,22] In 2 patients with prostatic cancer in our study, Doppler evaluation revealed asymmetric, increased flow while TRUS was not suspicious for malignancy. Few authors have shown that increased CD signal correlates positively with grading and staging of prostate cancer as well as risk of recurrence after treatment.[23,24]

On CD evaluation, 87.5% ($n = 28$) of benign lesions showed symmetric, radial flow compared to 14.3% ($n = 4$) of malignant lesions ($P < 0.0001$) in our study. A combination of TRUS and Doppler revealed superior diagnostic performance with sensitivity and NPV of nearly 86% compared to TRUS. However, benign lesions like prostatitis can also show

increased flow on CD and CD does not reveal microscopic vessels of prostatic cancer that do not possess enough flow to cause Doppler shift.[25,26] Despite these limitations, our findings indicate that CD imaging can be a useful adjunct to improve tumor detection by TRUS.

Prostatic cancer tends to be stiffer than benign lesions due to replacement of normal glandular tissue by neoplastic cells.[27] Stiffer tissues demonstrate less strain to deformation, and RTE helps in differentiation of benign and malignant tissues based on distribution of strain. Miyagawa *et al*. evaluated the usefulness of RTE in detecting prostatic cancer prior to systematic biopsy and have noted a diagnostic sensitivity of 72.6% for elastography and 89.5% for combination of TRUS and elastography.[14] Kamoi *et al*. used a five point subjective scale based on degree and distribution of strain in relation to hypoechoic area and found that RTE had 68% sensitivity and 81% specificity in detecting prostatic cancer. The combination of RTE with PD US further increased the sensitivity to 78%.[10]

Using the cutoff value of 3 (suggesting focal asymmetric lesion without strain unrelated to hypoechoic lesion) proposed by Kamoi *et al*., RTE was found to have sensitivity of 89.29% and specificity of 56.25% in our study. Of 43 patients with RTE findings suspicious for carcinoma, 25 were confirmed to be malignant on histopathology. Benign entities such as benign prostatic hyperplasia, adenomyomatosis, fibrosis and prostatitis can be associated with increased tissue stiffness and therefore may be difficult to distinguish from carcinoma prostate. This can be responsible for the relatively low specificity and PPV observed in our study. Because of the high frequency of false-positive results, RTE should be used in combination with TRUS to increase the detection rate.

In 5 of 28 patients with prostatic cancer (17.9%), B mode US was normal but RTE was suggestive of carcinoma. While systematic biopsy picked up malignancy in 3 patients, targeted biopsy based on RTE findings resulted in detection of cancer in 2 patients. The role of RTE targeted biopsy in detecting prostatic cancer has been reported by several authors. Pallwein *et al*. compared a 5-core RTE targeted biopsy with a 10-core systematic biopsy and concluded that cancer detection rate per patient was not significantly higher for targeted approach compared to systematic approach but an RTE targeted core was 2.9 fold more likely to be positive for cancer than a systematic core.[28] Salomon *et al*. analyzed the incremental detection rate of 4-core RTE targeted biopsy in addition to randomized 10-core in 1024 patients.[29] Additional use of RTE resulted in an incremental detection rate of 18.3%. 34 patients who harbored high-grade PCa (Gleason score ≥4) were diagnosed by RTE targeted biopsy only. A recent meta-analysis on the role of RTE targeted biopsy has shown that the detection rate of malignancy is enhanced when systematic biopsies are combined with RTE targeted biopsies.

Further RTE targeted biopsy can make a nearly equivalent diagnosis with fewer cores.[30]

In our study RTE detected 5 of 6 patients with prostatic cancer of Gleason score ≤6, 10 of 12 with score 7 and all with Gleason score 8 or 9. Our observations are similar to that of other authors who have noted that RTE detection rate of prostate cancer with higher Gleason score was greater than that of prostatic cancer with a lower Gleason score.[9,31] This might be explained by the greater cell density in high grade tumors resulting in stiffer tissue. In contrast, Tsutsumi *et al*. have noted a lower RTE detection rate for high grade tumors.[32]

Our study has few limitations. The sample size was small and no interobserver variability was studied since the RTE findings were interpreted by consensus. Another main limitation of RTE is that the degree of manual compression influences the elasticity image and may compromise diagnostic accuracy of RTE. However all examinations were performed by a single experienced radiologist and only images with optimal compression as displayed by the quality index were included in the study. Recent literature suggests that multiparametric MRI has greater sensitivity and specificity for detection of clinically significant prostate cancer.[33]

Although the incidence of prostatic cancer is low in Asian countries compared to the west, demographic and epidemiological transition in recent times have resulted in increase in the burden of various cancers including prostate in India. Prostate is reported to be the second leading site of cancer among males in several cities in India and cancer projection data estimate that the number of cases will become doubled by 2020.[34]

Conclusion

In this initial study from South India, RTE was found to have better sensitivity than TRUS as well as combination of TRUS and CD. With its greater sensitivity and NPV, RTE can be an effective adjuvant tool to guide biopsy and thereby increase the detection rate of prostatic cancer. Further prospective studies with larger sample size are needed before recommending RTE along with conventional TRUS for routine use in the evaluation of suspected prostatic malignancy in India.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Banu NA, Azim FA, Kamal M, Rumi MA, Barua AR, Khan KH. Inflammation and glandular proliferation in hyperplastic prostates: Association with prostate specific antigen value. Bangladesh Med Res Counc Bull 2001;27:79-83.
3. Ferrero Doria R, Pérez Flores D, Terrer Artes C, Guzmán Martínez-Valls PL, Morga Egea JP, Tomás Ros M, *et al*. Impact of prostatic benign hyperplasia and prostatic inflammation on the increase of prostate specific antigen levels. Actas Urol Esp 1997;21:100-4.

4. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, *et al*. EAU guidelines on prostate cancer. part 1: Screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 2014;65:124-37.
5. Lecornet E, Ahmed HU, Hu Y, Moore CM, Nevoux P, Barratt D, *et al*. The accuracy of different biopsy strategies for the detection of clinically important prostate cancer: A computer simulation. J Urol 2012;188:974-80.
6. Borza T, Konijeti R, Kibel AS. Early detection, PSA screening, and management of overdiagnosis. Hematol Oncol Clin North Am 2013;27:1091-110, vii.
7. Lemaître L, Villers A, Mouton D, Puech P. Transrectal ultrasound and biopsy of the prostate. J Radiol 2006;87:201-9.
8. Pallwein L, Mitterberger M, Struve P, Pinggera G, Horninger W, Bartsch G, *et al*. Real-time elastography for detecting prostate cancer: Preliminary experience. BJU Int 2007;100:42-6.
9. Sumura M, Shigeno K, Hyuga T, Yoneda T, Shiina H, Igawa M. Initial evaluation of prostate cancer with real-time elastography based on step-section pathologic analysis after radical prostatectomy: A preliminary study. Int J Urol 2007;14:811-6.
10. Kamoi K, Okihara K, Ochiai A, Ukimura O, Mizutani Y, Kawauchi A, *et al*. The utility of transrectal real-time elastography in the diagnosis of prostate cancer. Ultrasound Med Biol 2008;34:1025-32.
11. Halpern EJ, Ramey JR, Strup SE, Frauscher F, McCue P, Gomella LG. Detection of prostate carcinoma with contrast-enhanced sonography using intermittent harmonic imaging. Cancer 2005;104:2373-83.
12. Ophir J, Céspedes I, Ponnekanti H, Yazdi Y, Li X. Elastography: A quantitative method for imaging the elasticity of biological tissues. Ultrason Imaging 1991;13:111-34.
13. König K, Scheipers U, Pesavento A, Lorenz A, Ermert H, Senge T. Initial experiences with real-time elastography guided biopsies of the prostate. J Urol 2005;174:115-7.
14. Miyagawa T, Tsutsumi M, Matsumura T, Kawazoe N, Ishikawa S, Shimokama T, *et al*. Real-time elastography for the diagnosis of prostate cancer: Evaluation of elastographic moving images. Jpn J Clin Oncol 2009;39:394-8.
15. Brock M, von Bodman C, Palisaar RJ, Löppenberg B, Sommerer F, Deix T, *et al*. The impact of real-time elastography guiding a systematic prostate biopsy to improve cancer detection rate: A prospective study of 353 patients. J Urol 2012;187:2039-43.
16. Tsutsumi M, Miyagawa T, Matsumura T, Endo T, Kandori S, Shimokama T, *et al*. Real-time balloon inflation elastography for prostate cancer detection and initial evaluation of clinicopathologic analysis. AJR Am J Roentgenol 2010;194:W471-6.
17. Gann PH. Risk factors for prostate cancer. Rev Urol 2002;4 Suppl 5:S3-S10.
18. Ferrari FS, Scorzelli A, Megliola A, Drudi FM, Trovarelli S, Ponchietti R. Real-time elastography in the diagnosis of prostate tumor. J Ultrasound 2009;12:22-31.
19. Sarkar S, Das S. A review of imaging methods for prostate cancer detection. Biomed Eng Comput Biol 2016;7:1-5.
20. Shinohara K, Wheeler TM, Scardino PT. The appearance of prostate cancer on transrectal ultrasonography: Correlation of imaging and pathological examinations. J Urol 1989;142:76-82.
21. Newman JS, Bree RL, Rubin JM. Prostate cancer: Diagnosis with color Doppler sonography with histologic correlation of each biopsy site. Radiology 1995;195:86-90.
22. Okihara K, Kojima M, Nakanouchi T, Okada K, Miki T. Transrectal power Doppler imaging in the detection of prostate cancer. BJU Int 2000;85:1053-7.
23. Cornud F, Belin X, Piron D, Chrétien Y, Flam T, Casanova JM, *et al*. Color Doppler-guided prostate biopsies in 591 patients with an elevated serum PSA level: Impact on Gleason score for nonpalpable lesions. Urology 1997;49:709-15.
24. Ismail M, Petersen RO, Alexander AA, Newschaffer C, Gomella LG. Color Doppler imaging in predicting the biologic behavior of prostate cancer: Correlation with disease-free survival. Urology 1997;50:906-12.
25. Sharma S. Imaging and intervention in prostate cancer: Current perspectives and future trends. Indian J Radiol Imaging 2014;24:139-48.
26. Taverna G, Morandi G, Seveso M, Giusti G, Benetti A, Colombo P, *et al*. Colour Doppler and microbubble contrast agent ultrasonography do not improve cancer detection rate in transrectal systematic prostate biopsy sampling. BJU Int 2011;108:1723-7.
27. Krouskop TA, Wheeler TM, Kallel F, Garra BS, Hall T. Elastic moduli of breast and prostate tissues under compression. Ultrason Imaging 1998;20:260-74.
28. Pallwein L, Mitterberger M, Struve P, Horninger W, Aigner F, Bartsch G, *et al*. Comparison of sonoelastography guided biopsy with systematic biopsy: Impact on prostate cancer detection. Eur Radiol 2007;17:2278-85.
29. Salomon G, Drews N, Autier P, Beckmann A, Heinzer H, Hansen J, *et al*. Incremental detection rate of prostate cancer by real-time elastography targeted biopsies in combination with a conventional 10-core biopsy in 1024 consecutive patients. BJU Int 2014;113:548-53.
30. Tu X, Qiu S, Chang T, Jin K, Bao Y, Yang L, *et al*. The role of real-time elastography-targeted biopsy in the detection and diagnosis of prostate cancer: A systematic review and meta-analysis. Medicine (Baltimore) 2018;97:e0220.
31. Pallwein L, Mitterberger M, Pinggera G, Aigner F, Pedross F, Gradl J, *et al*. Sonoelastography of the prostate: Comparison with systematic biopsy findings in 492 patients. Eur J Radiol 2008;65:304-10.
32. Tsutsumi M, Miyagawa T, Matsumura T, Kawazoe N, Ishikawa S, Shimokama T, *et al*. The impact of real-time tissue elasticity imaging (elastography) on the detection of prostate cancer: Clinicopathological analysis. Int J Clin Oncol 2007;12:250-5.
33. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, *et al*. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): A paired validating confirmatory study. Lancet 2017;389:815-22.
34. Jain S, Saxena S, Kumar A. Epidemiology of prostate cancer in India. Meta Gene 2014;2:596-605.

Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction

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ORIGINAL PAPER

Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction

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Abstract

Background Acute ureteric colic is one of the most commonly encountered acute abdominal conditions. Diagnosis of obstruction caused by ureteric calculus occasionally becomes challenging if there is inadequate dilation of the urinary tract proximal to obstruction. In such a situation, intra-renal artery Doppler parameters can be used as a diagnostic tool. Our study compared intra-renal arterial Doppler in patients with obstructed and non-obstructed kidneys presenting within 24 h of onset of symptoms of unilateral acute renal colic.

Results The resistivity index (RI) in the segmental arteries of all 54 patients with obstructed kidneys was significantly higher than in those with non-obstructed kidney: 0.75 vs. 0.56, with a p value less than 0.001, a sensitivity of 85% and a specificity of 93%.

Conclusion Doppler ultrasound is a useful diagnostic tool in the evaluation of acute renal obstruction, thus enabling practitioners to avoid ordering unnecessary CT and exposing patients to ionizing radiation. An RI value > 0.7 has good sensitivity and specificity in our study. The results obtained in the study were comparable to those of pioneer studies conducted worldwide.

Keywords Acute renal colic · Renal artery Doppler · Resistive index

Abbreviations

ARC	Acute renal failure
ER	Emergency room
USG	Ultrasonography
US	Ultrasound
RI	Resistive index
CT	Computed tomography.
NSAID	Non-steroidal anti-inflammatory drugs
GFR	Glomerular filtration rate
eGFR	Estimated glomerular filtration rate.
Tc^{99m} DTPA	Technetium$^{-99\,m}$- Diethylene-triamine-penta-acetate
CDUS	Color Doppler Ultrasound
MPR	Multiplanar reconstruction
ROC	Receiver operating characteristic curve
PCS	Pelvicalyceal system

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Background

Acute renal colic (ARC) is one of the common causes of patients presenting to the emergency room (ER). It accounts for 30–35% of urologic emergencies [1, 2]. The recurrence rate over a period of 5 years is 30–40% [2]. Ultrasonography (US) remains the most commonly used modality in the initial evaluation of patients presenting with acute abdomen, especially in children [3], and the utility of ultrasound in the diagnosis of ARC cannot be overstated in cases where ARC is one of the most common pathologies presenting as acute abdomen. Absence of significant dilatation of the pelvicalyceal system, in spite of severe obstruction being present, is a well-recognized finding in some patients. In some

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studies, the absence of significant dilation in ARC has been reported in up to 30% [4] of patients. The dilated collecting system seen in US need not always be due to obstruction in the downstream urinary tract. These conditions include a baggy extra-renal type of pelvis, a compound upper pole calyx, due to over-distended urinary bladder, vesico-ureteric reflux, congenital type of mega calyces, infection and residual dilatation due to previous obstruction. Very rarely, pseudo-aneurysms of intra renal arteries can mimic renal pelvi-ectasis. This kind of vascular pathology can also be easily diagnosed with CDUS [5]. Acute complete ureteric obstruction is associated with changes in renal blood flow, as well as with an increase in renal pelvic pressure. In the first few hours, renal blood flow increases, and after 3 to 5 h, renal blood flow decreases, probably because of efferent arteriolar vasoconstriction produced by prostaglandins and other vasoactive substances [6, 7]. These changes can be effectively demonstrated by the RI of intra-renal arteries, as measured by Doppler ultrasound.

Methods

A prospective study was done on 54 patients who were referred to the department of radiology with clinical features of ARC for further evaluation with ultrasound or non-contrast CT. The study period is from January 2015 to December 2017. Prior to the commencement of the study, necessary clearance was obtained from the institutional ethics committee, and informed written consent was obtained from all the participating patients.

Inclusion criteria

All patients with symptoms of acute ureteric obstruction presenting to the emergency department within 24 h of onset of unilateral ARC were included.

Exclusion criteria

(a) Patients with history of renal parenchymal disease, chronic renal obstruction, renal trauma, or bilateral renal obstruction; (b) patients with a single kidney; and (c) patients in whom ureteric obstruction could not be confirmed either with USG or with CT were excluded.

All patients underwent gray-scale ultrasonography (US) and Doppler evaluation of intra-renal arteries. Computed tomography (CT) was done for those patients in whom US could not confirm the cause of obstruction.

Evaluation by ultrasound and Doppler study

The kidney on the side of obstruction was treated as the case kidney, and the contralateral normal (non-obstructed) kidney served as control. All patients underwent B-mode US and color doppler ultrasound (CDUS) with Philips iU22 using a C 1–5 transducer and Siemens Acuson S3000 using a 6C1 HD transducer. The presence or absence of pelvicalyceal system dilatation in each kidney was assessed using the gray-scale images. At least three Doppler spectra were obtained from inter-lobar arteries along the border of the medullary pyramids, and their mean was taken (Fig. 1).

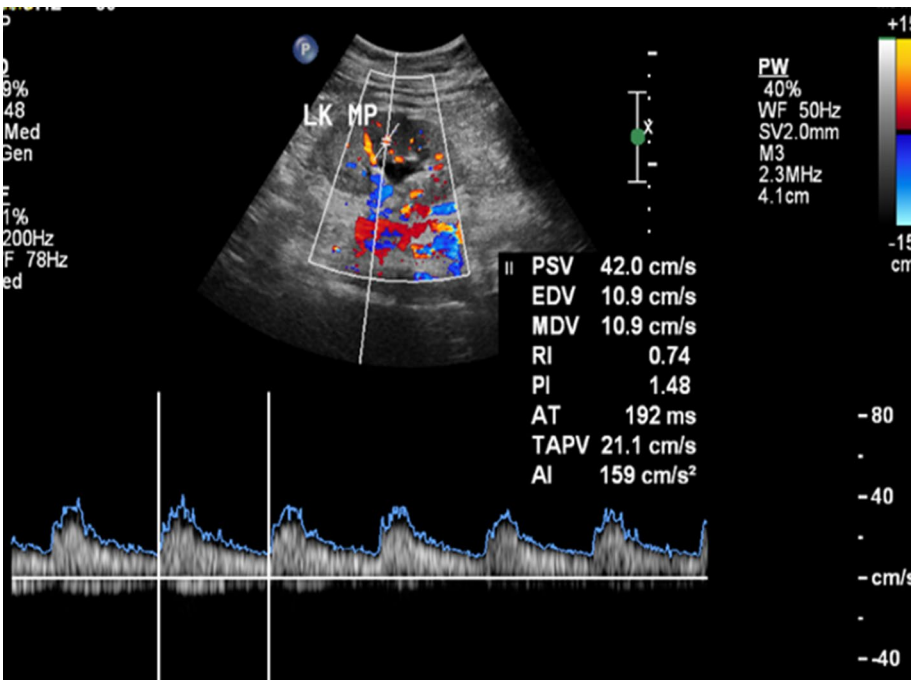


Fig. 1 Doppler US demonstrating an RI value of 0.74 in the mid pole of left kidney (obstructed kidney)

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The Doppler waveforms were made using the lowest pulse repetition frequency possible without aliasing. This maximized the size of the Doppler spectrum and decreased the percentage error in the measurements. In addition, the lowest possible wall filter for each ultrasound scanner was used. The Doppler sample width was set at 2–3 mm. The renal RI was calculated using the standard formula. The mean RI value was calculated for each kidney.

Evaluation by computed tomography

Siemens SOMATOM Definition Edge 128 slice Computerized Tomography (CT) equipment was used for the study. Non-enhanced CT of the abdomen, extending from the dome of the diaphragm to the pubic symphysis, was performed in all patients in whom the site and cause of obstruction could not be confirmed with US. Raw data was acquired with 0.6 mm thickness and pitch of 0.8. Reconstruction from the raw data was done in 5 mm thickness for viewing purposes and in 1 mm thickness for coronal and sagittal multi-planar reconstruction (MPR) (Fig. 2). The sites of ureteric obstructions were identified. Those patients not confirmed to have obstructions by CT were excluded from the study.

Statistical methods

All data were systematically collected, tabulated and analyzed using Microsoft Excel and Strata 6 for Windows. The Student *t* test was used in univariate analysis for the continuous variables, and Chi square test was used for analysis of noncontiguous variables. A *p* value of less than 0.05 was considered to be statistically significant.

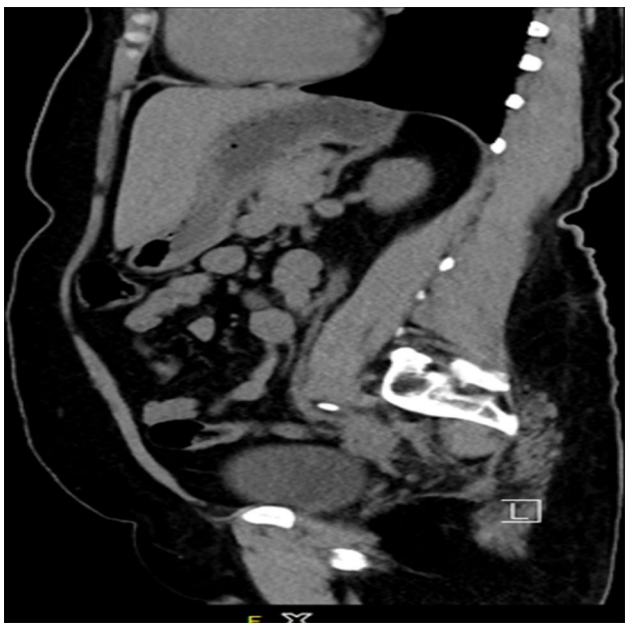


Fig. 2 Sagittal reconstruction of non-enhanced CT showing a calculus in the left distal ureter who presented with acute left renal colic

Results

This study evaluated 54 patients who had presented to the EMD with unilateral ARC within 24 h of the onset of symptoms. All the patients underwent B-mode US, CDUS and CT if the obstruction could not be confirmed in US. The presence or absence of PCS dilatation in each kidney was assessed using the grey-scale images. In this study population, 34 patients were males and 20 were females, as shown (Fig. 3). The mean age of the male patients was 46.8 years and that of the female patients was 38.5 years. The age range of the male patients was 19–79 years, and that of the female ones was 18–61 years.

The modalities used in the diagnosis and the site of obstruction are shown in Tables 1, 2.

Out of 54 patients, 26 cases proximal obstructions, while 28 had distal obstructions. The mean RI of the obstructed kidneys was higher in cases of distal obstruction (0.79 ± 0.004) than in those of proximal obstruction (0.76 ± 0.006).

Of the 54 patients, 45 patients had mild hydronephrosis, FIVE patients had moderate hydronephrosis and four patients did not have any dilatation of the collecting system despite the presence of ureteric obstruction (Fig. 4).

Of the 54 patients who were included in this study, 27 patients had elevated renal parameters, but did not have history of chronic renal impairment (Table 3).

In our study, we found that the RI in obstructed kidneys was significantly higher than in unobstructed kidneys (0.75 vs. 0.56; $p < 0.001$). The RI was higher in obstructed kidneys in all cases. The difference in RI between the obstructed and unobstructed kidneys (delta RI) ranged from 0.09 to 0.38, with a mean delta RI of 0.24 (Table 4).

The RI values in obstructed kidneys ranged from 0.68 to 0.84 (Fig. 5). The mean RI value in obstructed kidneys was 0.75, compared to 0.56 in unobstructed kidneys.

The group statistics of RI are shown in Tables 5, 6. The receiver operating characteristic curve (ROC) curve depicting the sensitivity of RI in ureteric obstruction is shown in Fig. 6.

By using a cut off RI value of > 0.70 in acute unilateral renal obstruction, the sensitivity obtained in the study was 85% and the specificity was 93% (Table 7).

Discussion

Grayscale ultrasound is the investigation method of choice in the initial evaluation of ARC, as it is easily available, portable and involves no ionizing radiation [8, 9]. However,

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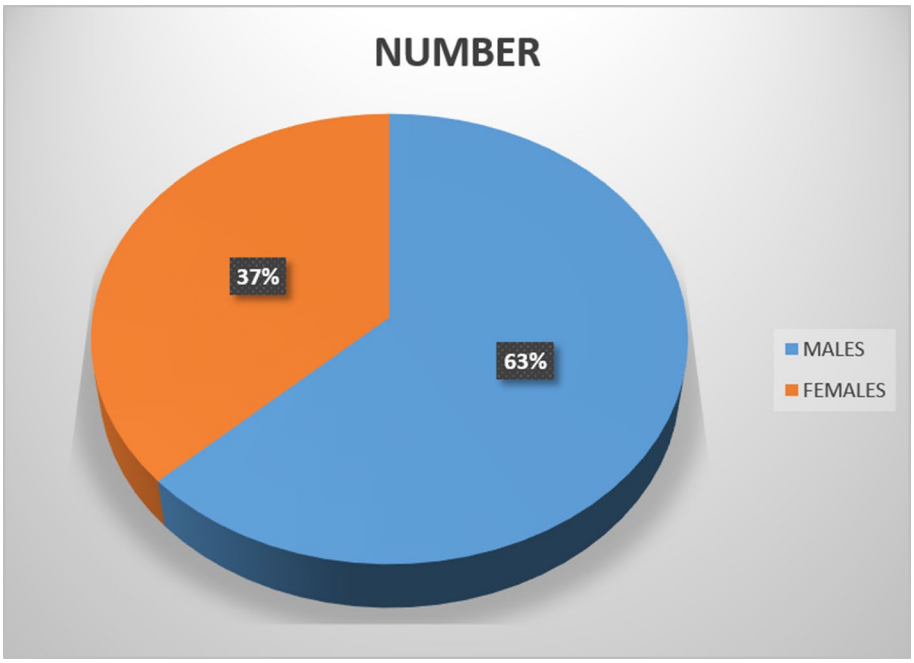


Fig. 3 Sex distribution in the study population

Table 1 Modality used in diagnosing the obstruction

Modality	Number	Percentage
CT and US	44	81%
US	10	19%
Total	54	100%

Table 2 Site of obstruction in the study population

Site of calculus	Frequency
Proximal ureter	26
Distal ureter	28

urinary system dilatation seen on conventional gray-scale USG has been shown to be sensitive (90%), but not specific (65–84%), in the diagnosis of renal obstruction.

The most sensitive, gold standard test for diagnosing obstructive uropathy is renal scintigraphy. However the availability of scintigraphy and radiation associated with it continue to be problematic [10]. Traditionally, the evidence of renal obstruction provided by US has been indirect and dependent on the anatomical criterion of dilatation of the pelvicalyceal system and ureter proximal to the level of obstruction. However, US fails to reveal hydronephrosis in acute obstruction of the kidney in up to 35% of cases [11]. More direct functional evidence of obstruction usually requires scintigraphy, but recently Doppler US techniques have been used to obtain functional information in suspected renal obstruction [11]. US imaging may miss (false negative) the diagnosis of obstruction in a variety of situations. Mild dilatation may be overlooked or considered clinically insignificant. Some patients with obstructive renal failure may show no pelvicalyceal system (PCS) dilatation. The reasons for this are unclear; in some patients, it may be related to dehydration or due to decompression of the pelvicalyceal system by rupture of a calyceal fornix [12]. It is commonly explained by urologists to be caused by the use of non-steroidal anti-inflammatory drugs (NSAIDs), by dehydration or by the renin angiotensin effect [13]. If that is true, such impairment will be detected through measurement of glomerular filtration rate (GFR). Measurement of GFR is commonly done using Technetium c-99 m diethylene-triamine-penta-acetate (DTPA), and does not require blood or urine sampling [14]. There is a growing evidence that renal impairment in acute unilateral ureteric obstruction is due to absorption of obstructed urine, with its high amount of creatinine, back into systemic circulation. This is considered false renal impairment with normal GFR [15]. In a study, El-Shazly et al. [16] measured renal function through measurement of estimated GFR (eGFR) usingTc-99 m DTPA. The authors showed that differences between eGFR on admission and after passage of stone or relieve of obstruction were non-significant. Their results support the hypothesis of false impairment in this clinical scenario. This tends to change the practice, as there is no need to embark on urgent intervention to relieve the ureteric obstruction, and conservative medical management is justifiable.

Routine B-mode US can be falsely positive or negative in acute ureteric obstruction [10]. PCS dilatation may be missed if the system is filled with blood clots, calculus, tumors or pus, in which case the usual anechoic appearance

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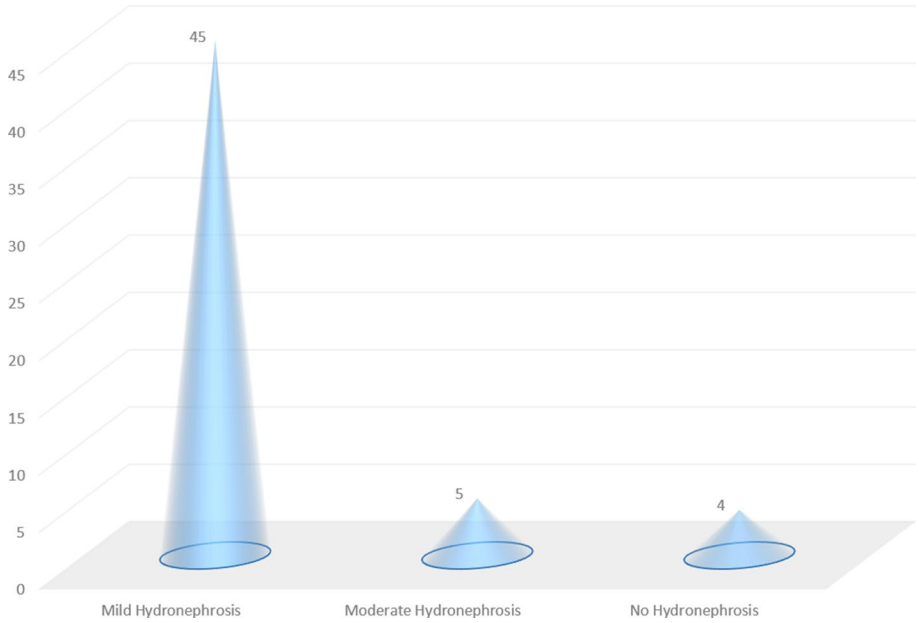


Fig. 4 Degree of hydronephrosis in the study population

Table 3 Number of patients with elevated serum urea and creatinine

No. of patients	Serum urea and creatinine
27	Increased
27	Normal

Table 4 Resistive indices in obstructed and unobstructed kidneys

Kidney	RI
Obstructed	0.75 ± 0.08
Non-obstructed	0.56 ± 0.09
Delta RI	0.24 ± 0.08

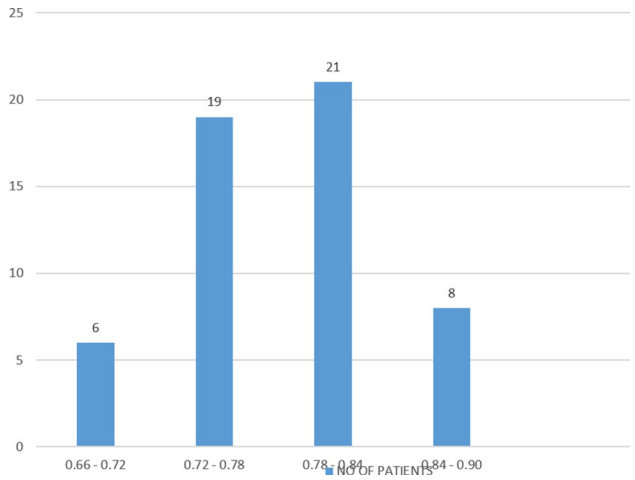


Fig. 5 Mean RI values in obstructed kidneys

Table 5 Group statistics

	Frequency	mean RI	SD	*P* value
Obstructed kidneys	54	0.75	0.08	0.000*
Unobstructed kidneys	54	0.56	0.09	

*Statistically significant ($P < 0.05$)

Table 6 Group statistics

	Mean RI value		*p* value
	< 0.70	> 0.70	
Obstructed kidneys	5 (9.3%)	49 (90.7%)	0.000*
Unobstructed kidneys	48 (88.9%)	6 (11.1%)	

*Statistically significant ($p < 0.05$)

of the PCS is lost. Intermittent ureteric obstruction, particularly that caused by ureteric calculi, may also lead to a failure to visualize dilatation of collecting system with US. On the other hand, in an attempt not to miss the diagnosis of obstruction in patients with only mild PCS dilatation, the false positive rate was found to be as high as 26% Causes of a false positive diagnosis include: (i) dilatation of non-obstructed PCS, when there are anatomical variants such as extra-renal pelvis; (ii) over-distended urinary bladder, with or without diuresis; (iii) vesico-ureteric reflux (VUR), (iv) residual dilatation of PCS due to previous obstruction or infection; (v) advanced pregnancy; and (vi) central renal fluid collections other than in the PCS, including in normal vessels, renal artery aneurysms and para-pelvic cysts.

Acute unilateral ureteric obstruction results in a complex sequence of changes in renal blood flow and ureteric

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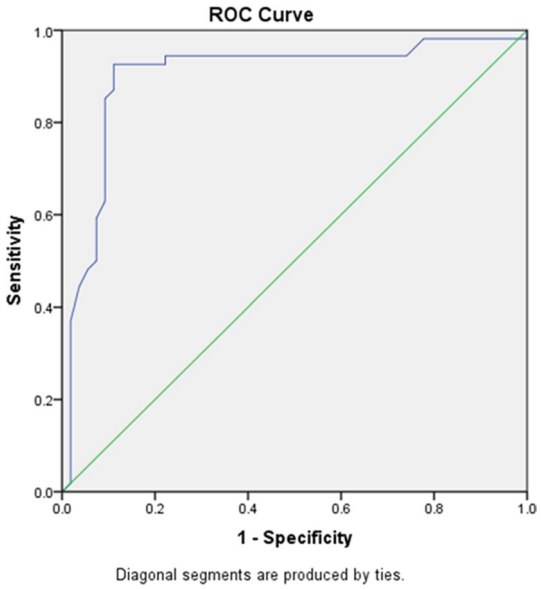


Fig. 6 ROC curve showing the sensitivity and specificity of resistive index

Table 7 Sensitivity and Specificity of RI with cut off of 0.70

Cut off RI	Sensitivity	Specificity
> 0.70	85%	93%

pressure [17]. In the first two hours, the renal blood flow increases, because of afferent arteriole vasodilatation and the ureteric pressure increases. From two to six hours after obstruction, the renal blood flow decreases, secondary to vasoconstriction of the efferent arterioles, and the ureteric pressure remains elevated. Subsequently, at 6 to 18 hours, the renal blood flow remains reduced because of vasoconstriction of the afferent arterioles, and the ureteric pressure decreases.

The role of renal CDUS in the evaluation of ARC has been vigorously debated. Rodgers et al. [18] found an elevated RI in acutely obstructed kidneys, especially when compared with the RI in normal contralateral kidneys and with a control group of healthy subjects. Similar results were obtained by Platt et al. [19] in a study of 23 patients with acute unilateral ureteric obstruction. However, others have reported that Doppler ultrasound is highly insensitive for detecting ARC. Tublin et al. [20] correlated the results of Doppler USG with those of urography in 32 patients presenting with symptoms of renal colic. The RI value of normal kidneys is usually less than 0.7 [21, 22]. When the published discriminatory thresholds for obstruction (mean RI ≥ 0.70 and ΔRI ≥ 0.10) were applied, the sensitivity and specificity of Doppler USG were only 44% and 82%, respectively.

In our study of 54 patients, we found that the RI in obstructed kidneys was significantly higher than in the unobstructed kidneys (0.75 vs. 0.56; $p < 0.001$). The RI was higher in obstructed kidneys in all cases. The difference between the obstructed and unobstructed kidneys (delta RI) ranged from 0.09 to 0.38, with a mean delta RI of 0.24.

Saboo et al. [6] stated in their study that delta RI ≥ 0.006 was highly sensitive and specific, and with a higher delta RI specificity remained the same whereas the sensitivity fell rapidly. Our results correlate well with those reported in earlier studies by Badr et al. [23] and Miletic et al. [24].

We also studied the effect of the level of obstruction on RI values. When we compared levels of ureteric obstruction (proximal vs distal), distal obstructions had a higher mean RI value (0.79) than proximal obstructions (0.76), and the values were not statistically significant. However, the study done by de Toledo *et al.* [25] showed that patients with proximal ureteric obstructions have RIs higher than those with distal obstructions.

We also analyzed the utility of CDUS in patients who did not show dilatation of the PCS on ultrasound. Patients who did not have PCS dilatation on US were later confirmed to have obstruction on CT. RI values were higher in all these patients (0.71), with a delta RI of 0.24. Discriminatory RI ≥ 0.70, which is higher than the delta RI found in the study done by Platt JF et al. [26], was found to be highly sensitive (85%) and specific (93%) in this study.

Conclusion

Using the discriminatory threshold RI of ≥ 0.70, the overall sensitivity of CDUS in diagnosing acute ureteric obstruction is 85% and the specificity is 93%. CDUS is a useful tool for demonstrating altered renal perfusion in acute ureteric obstructions before pelvicalyceal system dilatation occurs, thus increasing the sensitivity and specificity. Gray-scale US should therefore, be complemented by CDUS as an adjunctive test in patients presenting with acute renal colic, as it is a very simple, noninvasive and easy-to-calculate parameter. The utility of this tool cannot be overemphasized in special cases like pregnant women and children, in whom a dilated pelvicalyceal system without acute obstruction is encountered more frequently. It is mandatory to differentiate obstructive and non-obstructive causes with US alone in this subset of patients, as further evaluation with CT is not desirable due to the hazard of ionizing radiation. Given the high propensity of any group of patients with urinary tract calculi to undergo frequent imaging studies due to recurrent ureteric colic and the presence of significant residual hydronephrosis, we should strive hard to minimize radiation dosage by avoiding frequent CT studies. At the same time, maintaining acceptable diagnostic accuracy is of paramount importance.

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Limitations of the study: US is an operator-dependent examination and the results are affected by patients' body habitus and availability of good window. An increase in the RI of intra-renal vessels is also found in case of vascular or parenchymal diseases (renal vein thrombosis, acute renal insufficiency, etc.). The CDUS changes can return to normal after 48 h [27], after which its utility is doubtful with reduced sensitivity and specificity.

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Author contributions VM: Conception, Design, Acquisition, Analysis and Interpretation of data, Review. KMS: Design, Acquisition, analysis, Interpretation. NE: Concept, Design, Review. BD: Concept, Design. All the authors have read the manuscript and approve the manuscript fully for publication.

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Compliance with ethical standards

Conflict of interest None.

Ethics approval Obtained from the Institutional Human Ethics committee (IHEC) of PSG institute of Medical sciences and Research, Coimbatore, Tamilnadu, India. Reference Number: 15/417.

Consent to participate It was obtained from each patient at the time of Ultrasound Scan and CT (wherever applicable) along with general consent prescribed by the institution. This consent is a verbal consent since no additional procedure was done or drugs were administered for the purpose of the study. The ultrasound and CT Scan (wherever applicable) were done at the request of the referring physician and only those studies performed at the request of the referring clinician were utilized for the study. It is approved by the institutional ethics committee.

Consent for publication Obtained from all the authors all the authors have read and approved the manuscript.

Availability of data and material Available.

References

1. Piazzese EMS, Mazzeo GI, Galipo S, Fiumara Canfora F, Angio LG (2012) The renal resistive index as a predictor of acute hydronephrosis in patients with renal colic. J Ultrasound 15:239246
2. Sterrett S, Stephen N (2009) Emergency room follow-up trends in urolithiasis: single-center report. Urology 73:1195–1198. https://doi.org/10.1016/j.urology.2008.07.057
3. di Giacomo V, Trinci M, van der Byl G, Catania VD, Calisti A, Miele V (2014) Ultrasound in newborns and children suffering from non-traumatic acute abdominal pain: imaging with clinical and surgical correlation. J Ultrasound. https://doi.org/10.1007/s40477-014-0087-4
4. Ravindernath ML, Mahender Reddy G (2017) Mean resistive index as a prognostic tool for hydronephrosis in patients with acute renal colic: a study in a tertiary care. Int J Adv Med 4(2):329–333. https://doi.org/10.18203/2349-3933.ijam20170996
5. Tufano A, Minelli R, Rossi E, Brillantino C, Di Serafino M, Zeccolini M, Cantisani V, Vallone G (2017) Inferior epigastric artery pseudoaneurysm secondary to port placement during a robot-assisted laparoscopic radical cystectomy. J Ultrasound. https://doi.org/10.1007/s40477-020-00442-1
6. Saboo SS, Soni SS, Saboo SH, Chinapuvvula NR, Kaza S (2007) Doppler sonography in acute renal obstruction. Indian J Radiol Imaging 17:188–192
7. Azmi H (2003) Duplex doppler sonography in patients with acute renal colic: prospective study and literature review. Int Urol Nephrol 35(2):135–140
8. Jindal G, Ramchandani P (2007) AFP secondary to urolithiasis: radiologic evaluation and alternate diagnoses. Radiol Clin N Am 45(2007):395–410. https://doi.org/10.1016/j.rcl.2007.04.001
9. Apoku N, Ayoola O et al (2015) Ultrasound evaluation of obstructive uropathy and its hemodynamic responses in southwest Nigeria. Int Braz J Urol. 41:556–561. https://doi.org/10.1590/S1677-5538.IBJU.2014.0197
10. Ruiz-Martíneza A, Sierra-Díazb E et al (2019) Renal Doppler ultrasound resistive index vs renal scintigraphy with 99mTc-DTPA as diagnostic test for ureteropelvic junction obstruction in children. Actas Urol Esp 43(8):419–424. https://doi.org/10.1016/j.acuro.2019.02.005
11. Granata A, Zanoli L, Clementi S, Fatuzzo P, Fiorini F (2014) Resistive intrarenal index: myth or reality? Br J Radiol 87:20140004. https://doi.org/10.1259/bjr.20140004
12. Arumuham V, Brodie A, Bycroft J (2019) The management of urolithiasis. Surgery 37:7. https://doi.org/10.1016/j.mpsur.2019.04.003
13. Al-Ani A, Al-Jalham K, Ibrahim T, Majzoub A, Al-Rayashi M, Hayati A, Mubarak W et al (2015) Factors determining renal impairment in unilateral ureteral colic secondary to calcular disease: a prospective study. Int Urol Nephrol 47(7):1085–1090
14. Prigent A, Cosgriff P, Gates GF, Granerus G, Fine EJ, Itoh K, Peters M et al (1999) Consensus report on quality control of quantitative measurements of renal function obtained from the renogram: international consensus committee from the scientific committee of radionuclides in nephrourology. Semin Nucl Med 29(2):146–159. https://doi.org/10.1016/s0001-2998(99)80005-1
15. Rosenzweig B, Pinthus JH, Kleinmann N, Joffe E, Erlich T, Fridman E, Winkler H et al (2015) The relative contribution of urine extravasation to elevate plasma creatinine levels in acute unilateral ureteral obstruction. Can Urol Assoc J 9(7–8):428–433. https://doi.org/10.5489/cuaj.2804
16. El-Shazlya M, Aziza M et al (2016) Acute unilateral ureteric obstruction in young men with high serum creatinine: is it true or false renal impairment? World J Nephrol Urol 5(3):51–53
17. Sayani R, Ali M et al (2012) Functional evaluation of the urinary tract by duplex Doppler ultrasonography in patients with acute renal colic. Internat J Nephrol Renov Dis 5:15–21. https://doi.org/10.2147/IJNRD.S27628
18. Rodgers PM, Bates JA, Irving HC (1992) Intrarenal Doppler ultrasound studies in normal and acutely obstructed kidneys. Br J Radiol 65(771):207–212. https://doi.org/10.1259/0007-1285-65-771-207
19. Platt J, Ellis J, Rubin J (1991) Examination of native kidneys with duplex Doppler ultrasound. Semin Ultrasound CT MR 12:308–318
20. Tublin ME, Bude RO, Platt JF (2003) The resistive index in renal Doppler sonography: where do we stand? AJR Am J Roentgenol 180(4):885–892. https://doi.org/10.2214/ajr.180.4.1800885
21. Drudi FM, Liberatore M, Cantisani V et al (2014) Role of color Doppler ultrasound in the evaluation of renal transplantation from living donors. J Ultrasound. https://doi.org/10.1007/s40477-014-0077-6

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22. Spatola L, Andrulli S (2015) Doppler ultrasound in kidney diseases: a key parameter in clinical long-term follow-up. J Ultrasound. https://doi.org/10.1007/s40477-016-0201-x
23. Badr K, Brenner B (1988) Renal circulatory and nephron function in experimental obstruction of the urinary tract. In: Brenner BM, Lazarus J (eds) Acute renal failure. Churchill Livingstone, New York, pp 91–118
24. Miletic D, Fuckar Z, Sustic A, Mozetic V, Smokvina A, Stancic M (1998) Resistance and pulsatility indices in acute renal obstruction. J Clin Ultrasound 26(2):79–84
25. Toledo De et al (2017) Doppler-duplex ultrasound in renal colic. Eu J Radiol 23(2):143–1481996
26. Platt JF, Rubin JM, Ellis J (1989) Distinction between obstructive and nonobstructive pyelocaliectasis with duplex Doppler sonography. AJR Am J Roentgenol 153(5):997–1000. https://doi.org/10.2214/ajr.153.5.997
27. Gul H, Habib I et al (2014) Diagnostic accuracy of renal arterial resistive index in acute renal colic. KJMS 7:1

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RESEARCH ARTICLE

WILEY

Deeply supervised U-Net for mass segmentation in digital mammograms

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Abstract

Mass detection is a critical process in the examination of mammograms. The shape and texture of the mass are key parameters used in the diagnosis of breast cancer. To recover the shape of the mass, semantic segmentation is found to be more useful rather than mere object detection (or) localization. The main challenges involved in the mass segmentation include: (a) low signal to noise ratio (b) indiscernible mass boundaries, and (c) more false positives. These problems arise due to the significant overlap in the intensities of both the normal parenchymal region and the mass region. To address these challenges, deeply supervised U-Net model (DS U-Net) coupled with dense conditional random fields (CRFs) is proposed. Here, the input images are preprocessed using CLAHE and a modified encoder-decoder-based deep learning model is used for segmentation. In general, the encoder captures the textual information of various regions in an input image, whereas the decoder recovers the spatial location of the desired region of interest. The encoder-decoder-based models lack the ability to recover the non-conspicuous and spiculated mass boundaries. In the proposed work, deep supervision is integrated with a popular encoder-decoder model (U-Net) to improve the attention of the network toward the boundary of the suspicious regions. The final segmentation map is also created as a linear combination of the intermediate feature maps and the output feature map. The dense CRF is then used to fine-tune the segmentation map for the recovery of definite edges. The DS U-Net with dense CRF is evaluated on two publicly available benchmark datasets CBIS-DDSM and INBREAST. It provides a dice score of 82.9% for CBIS-DDSM and 79% for INBREAST.

KEYWORDS

conditional random fields, deep supervision, mammograms, mass segmentation

1 | INTRODUCTION

According to Global Cancer Observatory Database (GLOBOCAN) released in the year 2018, breast cancer is contributing to over 25.4% of all the new cases diagnosed.[1] Screening programs are organized by the government to aid in the early diagnosis of the disease. Mammograms are widely accepted as the primary screening tool for breast cancer. A mammogram is an X-ray image of the breast, which capture the changes in the breast tissue. Presence of mass and microcalcification in the mammograms characterize the disease.[2,3] The

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detection of these regions is difficult as their pixel intensities often correlate with the normal tissue.

Computer-aided detection and computer-aided diagnosis tools[3] are used to lessen the burden of the radiologist in the process of diagnosis. They interpret digitally captured medical images and provide useful information about the suspicious regions. They employ artificial intelligence and image processing techniques for the task of detection and analysis. CAD systems are constantly evolving with the advent of new techniques in the domain to provide accurate results. This paper deals with mass segmentation: a key step in the diagnosis of breast cancer. Earlier studies on mass segmentation employ region growing[4] or contour-based techniques.[5] The shortcomings of the above-mentioned approaches are the initial seed selection and the initial contour selection.[2] With the recent success of the deep neural networks in most of the vision-oriented challenges, there has been a spurt of research into their applicability for medical diagnosis.[6-9] There are two different approaches applied for lesion identification: Object Detection and Semantic Segmentation. In object detection, mass locations are approximated by a bounding box and the network is trained to predict its coordinates. Cascade of belief networks[10] and region-based convolutional neural networks (CNNs)[11] have previously been explored for mass detection. The limitation of the object detection technique is that it requires additional processing to recover the object boundary. In the semantic segmentation, every pixel is classified either as a background or mass region. Fully CNN[12] is popularly used for the task wherein the fully connected layers of CNN are replaced with up sampling layers to restore the spatial context of the lesion. Though FCN flared well in most of the recognition tasks, it provides a coarser output. U-Net[13] employed a symmetrical encoder and decoder path with the concept of skip connections to recover the region of interest (ROI) context in the image. U-Net outperformed other models significantly when the image possess higher signal to noise ratio (SNR). The proposed work improves the U-Net model with deep supervision and channel attention to prevent the loss of information at the encoder.

The main contributions of the work include:

1. A lightweight U-Net architecture is proposed with improved attention to the boundaries of the suspicious regions. The output of the encoder layer is monitored for proper attention of the mass boundaries and the output of the decoder layer is monitored for proper attention of the mass regions. This leads to faster convergence and lesser false positives.
2. A learnable fusion layer is used to combine the output of the intermediate layers with output of the last layer. This prevents the loss of information at the encoder.
3. Exponential logarithm dice loss function is used to improve the segmentation of the smaller objects. It is an unbiased metric, which treat the objects equally irrespective of their size.
4. Unlike other works, the proposed model uses dedicated preprocessing and post processing modules to improve the segmentation result. CLAHE is used to enhance the contrast of the input image and dense conditional random field (CRF) is used to recover the boundary of the detected regions from the final segmentation map of deeply supervised U-Net model (DS U-Net).

2 | METHOD

2.1 | Overview

The proposed model employs an end-to-end architecture for mass segmentation in mammograms. The model is trained over the images from CBIS-DDSM and INBREAST dataset (Figure 1). It starts with preprocessing the images using CLAHE to improve SNR. Then, it segments the mass regions in the preprocessed image using DS U-Net. Finally, it applies dense CRF to recover the shape of the mass. Figure 2 shows the various components involved in the proposed architecture.

2.2 | Preprocessing

Preprocessing is often neglected when the deep neural networks perform the task of segmentation. Since mammogram images exhibit low SNR, the detection becomes infeasible without proper preprocessing. From the literature, it is found that the histogram equalization

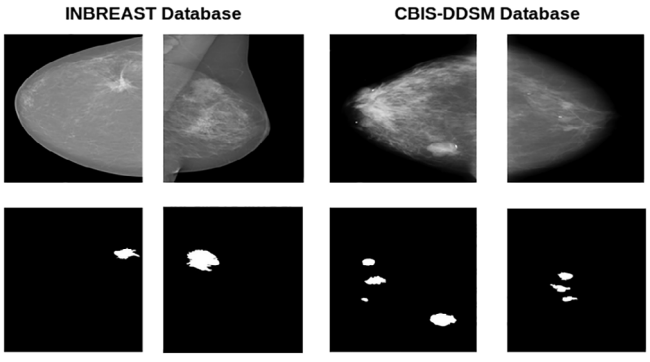


FIGURE 1 Mammogram images with ground truth indicating mass. First row: full field of view mammogram images in INBREAST and CBIS-DDSM dataset. Second row: manually segmented mass regions

technique and its variants are beneficial for the mammogram images.[14,15]

In the proposed work, CLAHE is used to improve the contrast in the mammogram image.[11] It splits the image into nonoverlapping blocks of size N * N and computes the transformation function for each of these blocks separately using histogram equalization. Then, for every pixel in the image, four nearest blocks are considered and their respective transformation mapping is used to find the resultant pixel intensity. Applying histogram equalization to homogenous regions can introduce noise in the resultant image. To remove the noise, Intensity values with frequency count greater than clip limit (threshold) are redistributed across other bins with lesser frequency count. This technique is found to improve the edges in the image. Figure 3 shows the sample images from INBREAST and CBIS-DDSM which are enhanced using CLAHE.

2.3 | Deeply supervised U-net

Deep supervision[16-18] brings in transparency to the intermediate hidden layers of deep neural networks. It uses the error factor of intermediate feature maps with respect to the ground truth in the training objective criterion. This improves the robustness of the neural network in both the segmentation and the classification tasks.[16] In the segmentation network, the output of intermediate layers is up-sampled and compared with ground truth to quantize their error margin.

U-Net[13] has become the de-facto standard for biomedical image segmentation. It employs an encoder decoder-based architecture. Encoder consists of convolution layers for retrieving contextual information and pooling layers for down-sampling the images. Downsampling aids in the retrieval of higher-level contextual information and also offers translational invariance. U-Net has a symmetrical decoder path which up-samples the feature map so as to recover the spatial context of the detections. Skip connections are pathways which carry the spatial information from the encoder to the decoder. The proposed work has extended over the idea of Chen et al[19] and Mishra et al[13] to use the intermediate layers of the encoder and the decoder to focus on the boundaries and regions simultaneously. In addition, the proposed model utilizes Squeeze Excitation blocks[12,20] to reinforce the channels of higher importance before combining the feature maps from the skip connection and the decoder. Figure 4 shows the complete architecture of proposed model. The DS U-Net is used to improve the recovery of the object boundary and significantly reduce the number of false positives.

The encoder block applies repeated 3*3 convolution on the input tensor as shown in Figure 5A. After every convolution, batch normalization is applied followed by ReLU activation. The decoder block (Figure 5C) up-samples the high-level features from the previous layer using bilinear interpolation and combines it with spatial context (low level features) from the encoder block. Subsequently, 3*3 convolution is applied before passing the tensor to the next layer. After every stage of the decoder,

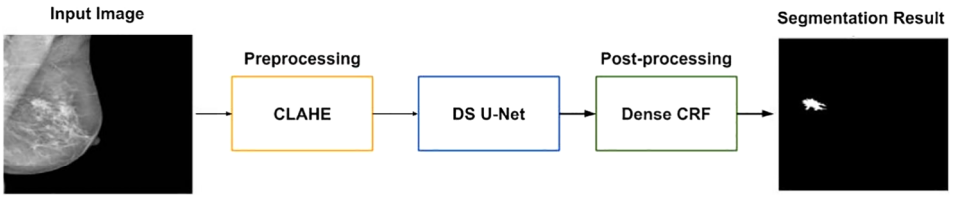


FIGURE 2 Proposed architecture for mass segmentation in mammograms [Color figure can be viewed at wileyonlinelibrary.com]

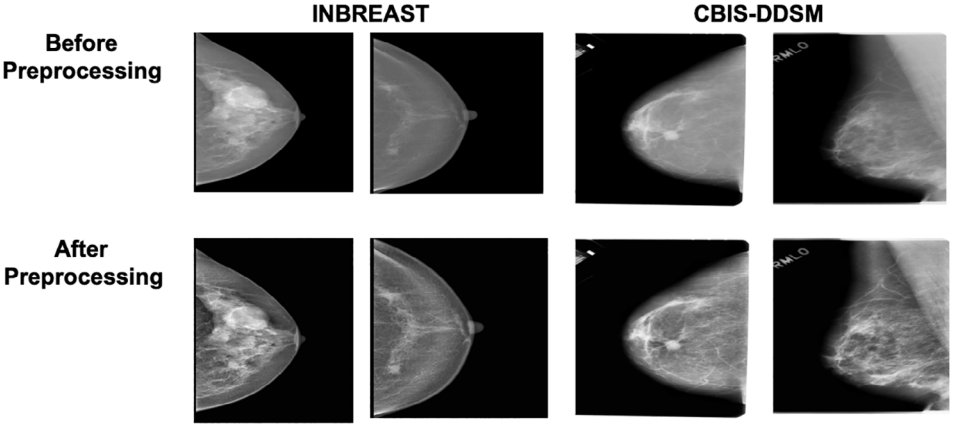


FIGURE 3 Preprocessed mammogram images using CLAHE

a drop out layer with the probability of 0.5 is used to avoid overfitting. The high-level features and the low-level features are subjected to channel attention before being fed as the input to the decoder block. Channel attention reinforces the feature maps with salient features using two-layered network. The first layer contains nodes equal to 1/16th of the number of input feature maps and the second layer contains nodes equal to the number of input feature maps. The network operates over the average intensity value of the input channels as shown in Figure 5B. Blocks A1 to A6 are attention blocks, which supervises the encoder and decoder layers as shown in Figure 4. It applies 1*1 convolution to reduce the dimensions and bilinear upsampling to enlarge the size of the intermediate tensor to match the output dimensions (Figure 5B). Fusion layer produces the end segmentation result as the linear combination of the outputs from the attention blocks and the output from the final decoder layer (D_4) as shown in Equation (1).

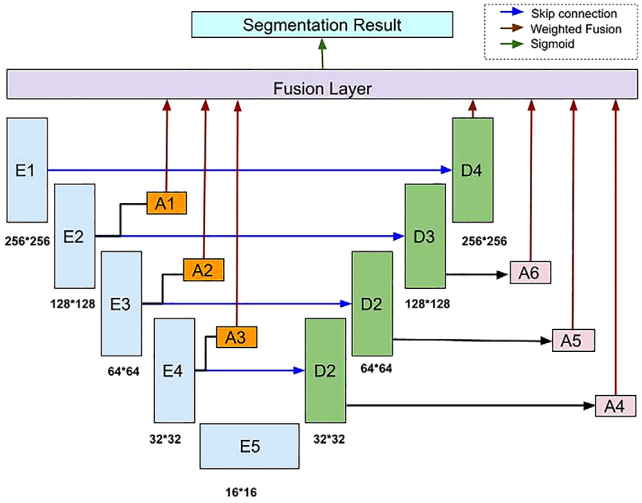


FIGURE 4 Deeply supervised U-Net: auxiliary blocks A1, A2, and A3 are providing attention to the boundaries, and auxiliary blocks A4, A5, and A6 are used to discriminate the entire mass region from the background [Color figure can be viewed at wileyonlinelibrary.com]

$$\hat{y} = \text{sigmoid}\left(\sum_{i=1}^{6} h_i O(A_i) + h_D O(D_4)\right) \quad (1)$$

Here, $\hat{y}$ represents the final segmentation result, O(.) represents the output of the block provided in the parenthesis and h $\{h_D, h_1, h_2 \ldots . h_6\}$ represents the weights associated with the output of final decoder block and the attention blocks. The objective criterion is modified as in Equation (2) so that the attention blocks A1 to A3 can steer the network toward boundary identification and blocks A4 to A6 can steer the network toward region identification.

$$L(\hat{y}, y_B, y_M; \theta) = L_f(\hat{y}, y_M) + \text{Auxiliaryloss} \quad (2)$$

$$\text{Auxiliary loss} = \left(1 - \frac{t}{T}\right)^d \left(\sum_{i=1}^{3} L_{A_i}(O(A_i), y_B) + \sum_{i=4}^{6} L_{A_i}(O(A_i), y_M) + L_{D_4}(O(D_4), y_M)\right) \quad (3)$$

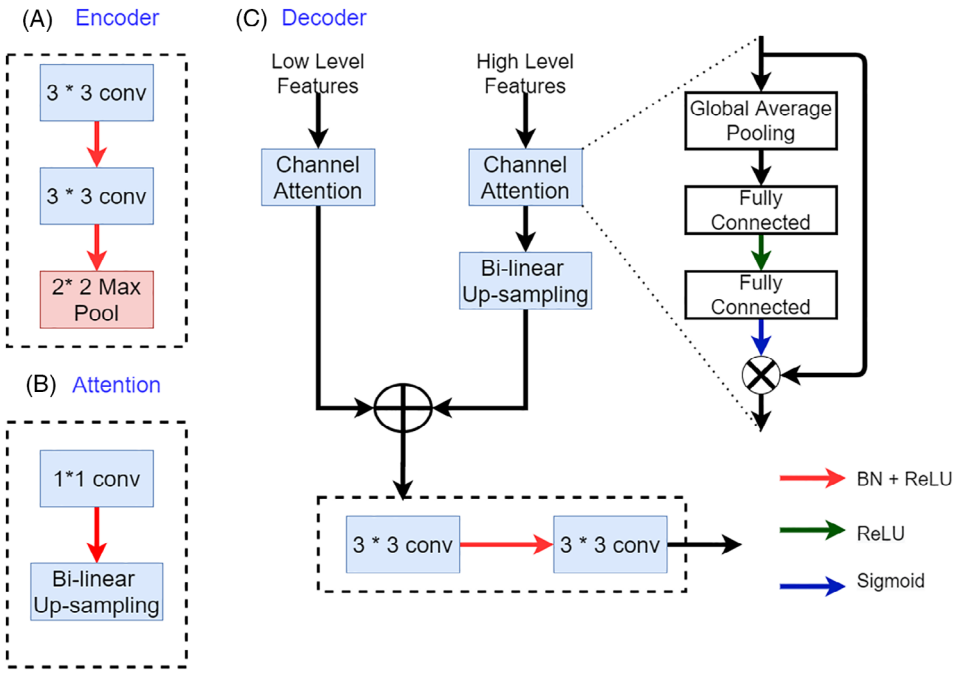


FIGURE 5 Various blocks of DS U-Net architecture, A, encoder, B, attention, and, C, decoder [Color figure can be viewed at wileyonlinelibrary.com]

Here, L represents the loss function which is discussed in section D, θ represents the parameters of the network which include (W_{encoder},W_{decoder}, $W_{\text{attention}}$,h), Y_B represents the ground truth labeled with only the boundary pixels of the suspicious mass and Y_M represents the ground truth labeled for the entire region of the suspicious mass.

A factor of $\left(1-\frac{t}{T}\right)^d$ is used for auxiliary loss to reduce the impact of attention layers as the number of iterations (t) approach the total number of epochs T.[17] It ensures that the intermediate activations are not modified to a larger extent at the later stage of the training process. d is a constant which is set to a value of 2. The boundary pixels are extracted by performing morphological erosion on the region mask with disk shaped structuring element (value of radius is set to 3) and subtracting the resultant from the original image. The boundary mask obtained after the morphological operation is shown in Figure 6.

2.4 | Loss function

Sorensen dice coefficient is the popularly used metric to measure the similarity between two images. To gauge the differences between the prediction and ground truth, error value of (1-Dice coefficient) is used. The size of the object can influence this value as it just takes into account the percentage of misclassified pixels. In particular, this error function results in higher value even with fewer number of misclassifications for smaller objects. Thus, the network may be biased toward the object of larger sizes upon using this loss function. To achieve a balanced loss whereby the network focuses on both the objects with low and high prediction accuracy equally, exponential logarithm dice loss is used.[21]

Let y_i represents the ground truth of i^{th} pixel which takes either a value of 0 (or) 1. A value of 0 indicates the background (normal region) and a value of 1 indicates the mass (abnormal region). Let p_i represents the prediction probability of i^{th} pixel belonging to mass. The exponential logarithm dice loss is computed as in Equation (4), where M denotes the total number of training samples in the mini-batch.

$$\mathcal{L}=\frac{\sum_{k=1}^{M}\left(-\ln(\text{Dice}_k)\right)^{\gamma}}{M} \tag{4}$$

The dice score for image k is calculated as $\text{Dice}_k=\frac{\sum_{i=1}^{N}2y_i*p_i+\varepsilon}{\sum_{i=1}^{N}y_i+\sum_{i=1}^{N}p_i+\varepsilon}$. ε(epsilon) value of 1e-6 is used for numerical stability. γ value in the loss is used to adjust the non-linearity between the dice score value and the error. In the experiments, γ value of 0.3 is used[21] as it offers a decreasing gradient with dice score values less than 0.5 and an increasing gradient with dice score greater than 0.5. This helps the loss function to behave differently for objects of varying sizes.

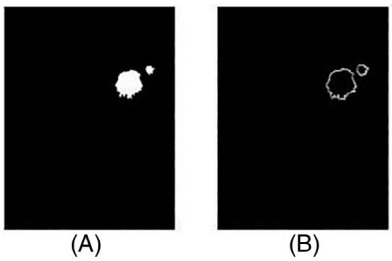


FIGURE 6 Segmentation masks. A, Region mask. B, Boundary mask

2.5 | Post-processing

DS U-Net precisely identifies the location of the mass in a given mammogram image. As the shape of the detected lesions can aid in the diagnosis of the disease, post-processing is applied to recover the entire context of the detected lesions. Probabilistic graphical models have proven to be effective in modeling the neighborhood dependencies. Therefore, it possesses higher discriminatory power to differentiate the foreground pixels from the background pixels. Hence, CRFs is used to fine-tune the resulting segmentation map of Deep Supervised U-Net model. Works by Chen et al[22] and Zheng et al[23] showed that state of the art segmentation results can be obtained by incorporating the dense CRF model. The model accepts the raw intensity values of input image and the unary potentials (sigmoid probabilities of DS U-Net) to generate the final probability map as shown in Figure 7.

CRF model treats the individual pixels X_u as random variables which can take values from the set $L = 0, 1$ where 0 indicate background and 1 indicate foreground (suspicious mass region). CRF inference assigns a configuration $X=X_1,X_2,X_3.....X_{N^2}$ for every random variable in the image I such that the energy given in Equation (5) is minimized.

$$\begin{aligned}E(X,I)&=\sum\nolimits_{uV}\Phi_u(X_u=x\,|\,I)\\&+\sum\nolimits_{\{u,v\}\xi}\Psi_{u,v}(X_u=x,X_v=y|I)\end{aligned} \tag{5}$$

The term $\Phi_u$denotes the unary cost associated with assigning label x for the variable X_u. This cost is usually obtained from the classifier. The interdependencies

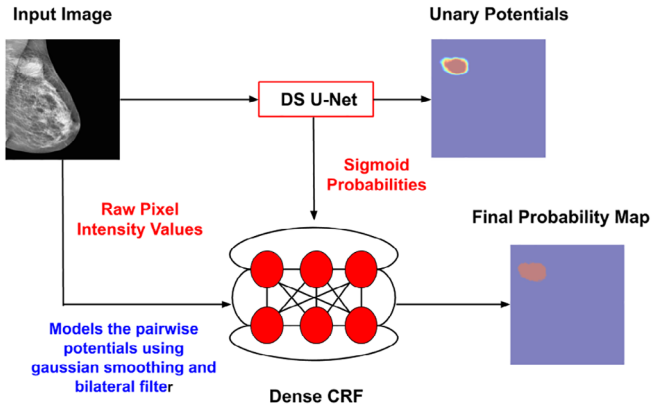


FIGURE 7 Dense CRF model process flow [Color figure can be viewed at wileyonlinelibrary.com]

between the pixels are modeled using pairwise cost $\Psi_{u,\,v}$ which accounts for the cost of assigning labels x and y for the pixels X_u and X_v, respectively. The common pairwise cost used is Pott's model which provides a cost of 0 when the neighboring pixels are assigned with same label and a value of 1 otherwise. Further, certain assumptions should also be made on how the pixels connect with each other. There are two popular configurations used: Grid CRF and fully connected CRF. Fully connected CRF assume that every pixel in the image is connected with every other pixel whereas Grid CRF assumes that every pixel is connected only to its adjacent pixels. Dense CRF leverages on fully connected CRF to model the neighborhood dependencies. CRF has been formulated on the basis of the Gibbs distribution as given in Equation (6).

$$P(X\,|\,I) = \frac{1}{Z(I)}\exp(-E(X,I)) \tag{6}$$

The learning phase of CRF is defined as the computation of marginal probabilities for every random variable X_u and for every pair of random variables (X_u, X_v) which are connected by edges, that is, all pixels except itself $u \neq v$ in a fully connected graph. Detection of true distribution for the unary Φ and pairwise potentials Ψ are infeasible. Mean field inference approximates the p(X|I) using a simpler distribution Q(X|I) and iteratively reduces the Kullback Leibler divergence between Q(X|I) and p(X|I). In the proposed model, unary potentials Φ are obtained from DS U-Net and the pairwise potentials Ψ are modeled using the sum of two Gaussian kernels as suggested in Reference 24: Gaussian smoothing kernel $\exp\left(-\frac{||p_u-p_v||^2}{2\sigma_\alpha^2}\right)$ and Gaussian preserved bilateral filtering kernel $\exp\left(-\frac{||p_u-p_v||^2}{2\sigma_\alpha^2}-\frac{||I_u-I_v||^2}{2\sigma_\beta^2}\right)$. Here, $p_u$and p_V denote the position of the pixels. I_u and I_v denote the intensity of the pixels. Weights are associated with the kernels to control the significance of the filters.

Thus, the pairwise potentials in CRF is formulated as $\Psi_{u,\,v}(X_u, X_v) = \mu(X_u, X_v)k(f_u, f_v)$ where μ denotes the label similarity using common Pott's model and k denotes the sum of Gaussian kernels. Mean Field updates for assigning a label l for the random variable X_u is provided in Equation (7).

$$Q_u(X_u = l\,|\,I) = \frac{1}{Z_u}\exp -\Phi_u(I) - \sum\nolimits_{l'L}\mu(l,l') \sum\nolimits_{m=1}^{2} w^{(m)} \sum\nolimits_{v\neq u} k^{(m)}(f_u,f_v)Q_u(l') \tag{7}$$

Here, $w^{(m)}$ indicate the strength of the filter m. In the experiments, weights for Gaussian smoothing and bilateral filters are considered as 1 and 5, respectively. Number of iterations T_0 for convergence is set as 50.

III Experiment

2.6 | Datasets

The model is evaluated on two popularly available benchmark datasets: INbreast[25] and CBIS-DDSM.[26] INbreast contains 107 full field digital mammography (FFDM) images with mass findings. These images are available in DICOM format and are of size 3328 * 4084 (or) 2560 * 3328 with 14-bit resolution. The boundary pixels of the mass are manually annotated and provided in XML format. Because of the smaller size of the dataset, a 5-fold cross-validation is performed on the dataset to gauge the model's performance.

CBIS-DDSM dataset contains curated mammogram images from DDSM, a largest available mammogram database. They provide separate training and test set for mass detection and microcalcification detection. The dataset comprises of FFDM images along with the segmentation mask and the cropped ROI for every suspicious finding in DICOM format. For the experiments, a curated set of 689 images is used for training and a set of 168 images is used for testing.[27] There are multiple ROI detections in an image which are provided as separate masks in the dataset. They are merged into a single mask before training. Both the datasets contain significant amount of benign cases[27] and they also include masses of varying sizes as shown in Figure 1.

2.7 | Experimental framework

All the experiments are carried out using NVIDIA GTX 1080 Ti GPU and the models are implemented in keras. Adam[28] optimizer with warm restart is used for the training

Algorithm 1

Mean field inference for dense CRF

Input:

X_u Grayscale intensity of the Input image at pixel position u.

$U_u(l)$ Output of last decoding layer of DS U-net for the random variable u and label l.

Output:

$Q_u(l)$ Probability of the pixel X_u computed using Dense CRF

Initialize parameters

$\boldsymbol{W}$ - a vector of size 1 x m where m denotes the number of filter kernels $\boldsymbol{\mu}$ - compatibility matrix

Initialize $\boldsymbol{Q_u(l)}$ with the softmax on unary potentials $U_u(l)$

while T< T_0 (T_0 denotes the number of iterations)

For every filter m in the set {Gaussian smoothing filter, Gaussian bilateral filter}

$\bar{Q}_u(l)$ + = w[m] * sum of the filter coefficients

when operated on pixels other than u

$\bar{Q}_u(l)$ = sum the potentials of the labels l' that are consistent with l according to $\boldsymbol{\mu}$

$\bar{Q}_u(l) = -U_u(l)$-$\bar{Q}_u(l)$

$\bar{Q}_u(l)$ = Compute softmax over $\bar{Q}_u(l)$ considering pixel value of all the other labels

end while

process with the batch size as 4. The models are trained for about 200 epochs with 0.001 as the initial learning rate. Here, the learning rate is decreased using cosine annealing for a defined number of iterations known as a cycle and it is reinitialized to a maximum value after every cycle. The parameters of the model are randomly assigned as the other initializers failed to converge. The intensity values in the image are scaled to a fixed range between 0 and 1 before being fed as the input to the network.

2.8 | Metrics

To measure the correctness of the prediction, two types of metrics are used: pixel level metrics and region level metrics. Pixel level metrics include accuracy, sensitivity, and specificity. The **Accuracy** measures overall pixel accuracy, that is, percentage of correctly classified pixels, **Sensitivity** measures the percentage of correct predictions corresponding to pixels constituting the mass region in the ground truth, **Specificity** measures the percentage of correct predictions corresponding to pixels constituting the normal region in the prediction map.

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \tag{8}$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}. \tag{9}$$

Here, true positive (TP) denotes the count of pixels which are classified correctly as belonging to mass. False positive (FP) denotes the count of normal pixels classified as mass and false negative (FN) denotes the count of pixels which are incorrectly classified as normal pixels. Sensitivity will be low when there are more FN. It happens due to under-segmentation wherein the mass occupies a very small region that cannot be properly captured by the model. Specificity is affected when there are large number of FP. False positives are the consequence of over-segmentation wherein the normal parenchymal regions are detected as mass.

Object level metrics use the size and the boundary of positive predictions for evaluation. Jaccard coefficient, dice score, relative area difference (ΔA), and Hausdorff distance are used for evaluating the regions in the resulting prediction map.

$$\text{Jaccard} = \frac{\text{TP}}{\text{TP} + \text{FP} + \text{FN}} \tag{10}$$

$$\text{Dice score} = \frac{2\text{TP}}{2\text{TP} + \text{FP} + \text{FN}} \tag{11}$$

$$\Delta A = \frac{|(TP + FP) - (TP + FN)|}{(TP + FN)}. \quad (12)$$

Jaccard and **dice score** measure the amount of overlap between ground truth (GT) and prediction map. A value of 0 indicates that there is no overlap between prediction and GT. A value of 1 indicates an exact match between GT and prediction. Jaccard is an estimate of average performance of the model and Dice score is an estimate of worst-case performance of the model. **Relative area difference** is used to measure the differences in the size of prediction against the ground truth. To measure the maximum deviation along the boundary between GT and predictions, **Hausdorff distance** metric is used. It measures the longest distance between any two closest points that lie in the boundary of ground truth and prediction.

$$H = \max(h(\mathrm{GT}, \mathrm{pred}), h(\mathrm{pred}, \mathrm{GT})) \quad (13)$$

Here, $h(\mathrm{A}, \mathrm{B}) = \max(\min(\|a - b\|))a \in A, \quad b \in B$ where A and B represent the set of boundary pixels. The lower values of H indicate accurate segmentation.

2.9 | Experimental results

The proposed work is aimed at improving the segmentation of lesions in whole mammograms. An ablation study is conducted to determine the effectiveness of pre-processing and post-processing techniques. To measure the performance of the model, experiments are repeated thrice and the average value of the metrics are considered.

2.9.1 | Impact of preprocessing

Mammograms exhibit low contrast when compared with other medical images. To improve the contrast in the mammograms, CLAHE is used. Since the images in CBIS-DDSM are obtained from four different scanners, they exhibit varying contrast. Hence, selective preprocessing is used, that is, CLAHE is applied only to those images which exhibit poor global contrast. The global contrast in an image is estimated using root mean square (RMS) metric. RMS measures the variance of the normalized gray level values in an image as specified in Equation (14).

$$\mathrm{RMS} = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2} \quad (14)$$

where x_i is a normalized gray level value $0 \le x_i \le 1$, $\bar{x}$ is the mean normalized gray level and n denotes the total number of pixels in an image. The block size and the clip limit for CLAHE are considered as (128128) and 0.01, respectively.

Table 1 depicts the performance of DS U-Net for both the datasets w/ & w/o preprocessing. There is a significant improvement in all the metrics for both the datasets when preprocessed images are used in the model construction. In particular, the dice score is increased by a factor of 4% and 1.6% for CBIS-DDSM and INbreast, respectively.

2.9.2 | Impact of postprocessing

Postprocessing is applied on the prediction maps obtained from the DS U-Net model using dense CRF. It is used to recover the boundary of the abnormal region. This stage is critical as the region boundary can offer crucial information about the malignancy of the region. Dense CRF uses the probability of pixels from DS U-Net as unary potential and models the pairwise potential as the sum of Gaussian smoothing and bilateral filters. After a grid search over different kernel sizes and filter strength, ideal values are determined. The kernel size of (7,7) for Gaussian smoothing filter and (3,3) for bilateral filter provided the optimal performance.

Table 2 depicts the performance of DS U-Net after postprocessing. Hausdorff distance is the only metric which accounts for the shape of the object out of all the considered metrics. A significant reduction of 0.05 is observed for Hausdorff distance metric after applying dense CRF over the prediction maps of DS U-Net. As the

TABLE 1 Evaluation metric values observed for DS U-Net model trained using images w/ and w/o preprocessing

Dataset	Pre-processing	Dice score	Jaccard coefficient	Accuracy	Sensitivity	Specificity	Hausdorff distance	ΔA
CBIS-DDSM	No	78.7	83.5	99.7	78.1	99.8	2.7	29.2
	Yes	82.7	85.7	99.7	83.1	99.8	2.48	21.9
INBREAST	No	77	83.2	98.7	78.01	99.47	1.97	31.5
	Yes	78.6	83.4	99	81.1	99.3	1.91	33.5

TABLE 2 Evaluation metric values observed for DS U-Net model w/ and w/o postprocessing (post processing is carried out on the model output over the preprocessed images)

Dataset	Models	Dice score	Jaccard coefficient	Accuracy	Sensitivity	Specificity	Hausdorff distance	ΔA
CBIS-DDSM	DS U-Net	82.7	85.7	99.7	83.1	99.8	2.48	21.9
	DS U-Net with dense CRF	82.7	85.7	99.7	84.1	99.8	2.43	22.7
INBREAST	DS U-Net	78.6	83.4	99	81.1	99.3	1.91	33.5
	DS U-Net with dense CRF	79	83.4	98.11	81	98.4	1.86	31.1

TABLE 3 Comparison of proposed model (DS U-Net with dense CRF) with the other popular segmentation architectures for whole mammograms

Dataset	Models	Dice score	Sensitivity	ΔA	Hausdorff distance
CBIS-DDSM	U-Net[13]	73.6	79.4	42.7	3.38
	AU-Net[27]	80.9	84.5	29.9	2.97
	DS U-Net + Dense CRF	82.7	84.1	22.7	2.43
INBREAST	U-Net[13]	69.3	70.4	44	4.54
	AU-Net[27]	79.1	80.8	37.6	4.04
	DS U-Net + Dense CRF	79	81	31.1	1.86

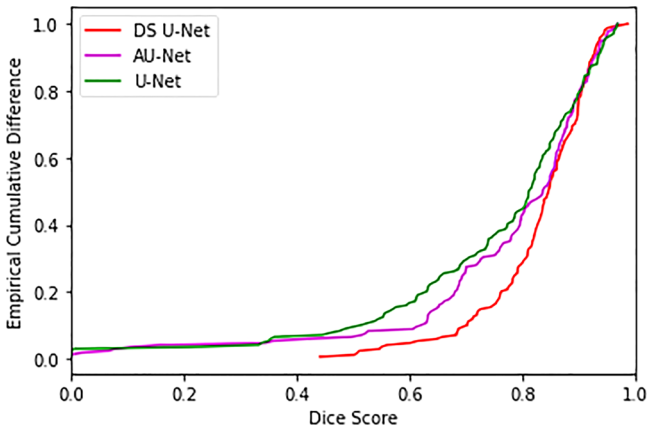


FIGURE 8 Empirical cumulative difference plot of dice score metric for the test images belonging to the CBIS-DDSM dataset [Color figure can be viewed at wileyonlinelibrary.com]

technique only changes the class label for a smaller percentage of pixels which lie along the boundary of the detected regions, it does not affect the value of other metrics as reported in the table.

2.9.3 | Comparison with other state-of-art models

In order to compare the effectiveness of the proposed model, AU-Net[27] and U-Net[13] models are considered. The U-Net model uses skip connections to carry information from the encoder stage to the decoder stage for recovering the spatial context. This information is then combined with the feature maps of the decoder through concatenation. But it is found to be ineffective and also attributes to certain information loss.[27] This problem is addressed in AU-Net by utilizing an asymmetrical network backbone and attention guided dense up-sampling. AU-Net offers better accuracy than the U-Net model at the cost of increased computational complexity. DS U-Net addresses the loss of information by employing deep supervision over the intermediate activations. It also uses channel attention to retrieve important information from the feature maps. The computational complexity of DS U-Net is reduced by employing feature addition instead of concatenation. Table 3 depicts the performance of all the three models. It clearly shows that the DS U-Net provides substantial improvement in all the key metrics when compared with other models. In particular, the dice score of DS U-Net shows an improvement of 1.8% in comparison with the AU-Net model for the CBIS-DDSM dataset. There is also a huge reduction in the Hausdorff distance and ΔA by 0.54 and 7.2, respectively, for the CBIS-DDSM dataset when compared with the AU-Net model. Dice score values of all the models are inspected further using CDF (Figure 8).

The qualitative improvement of the proposed model over the others is shown in Figures 9 and 10. The

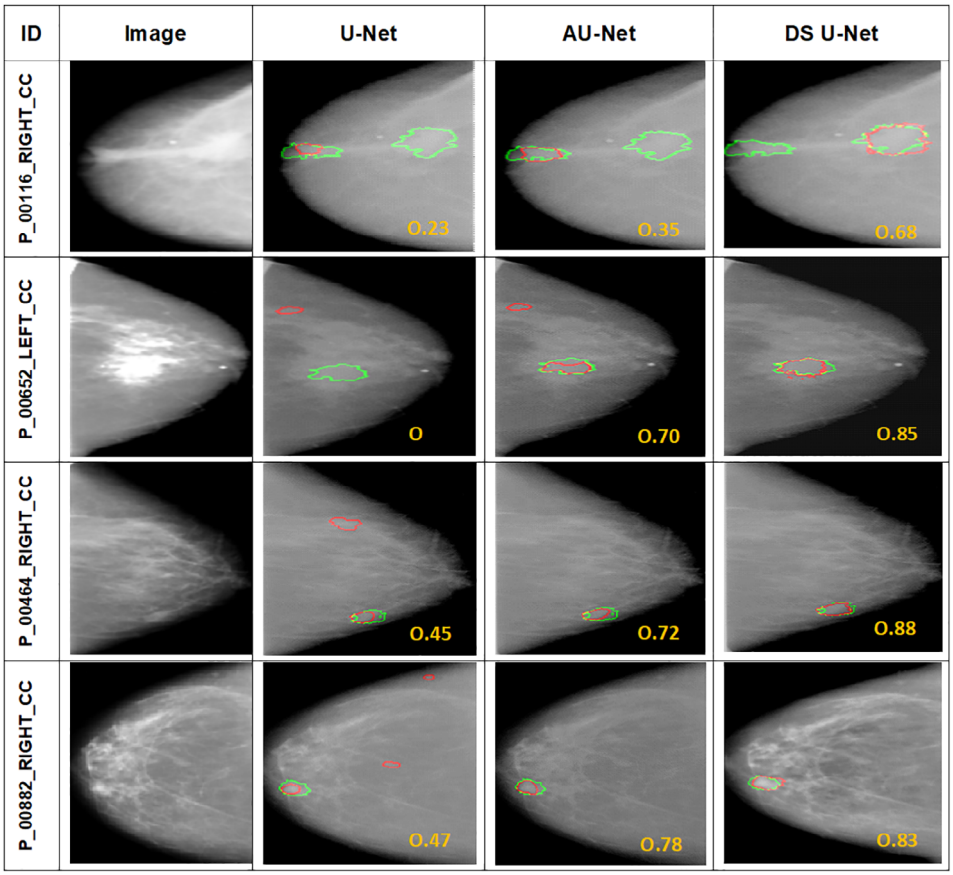


FIGURE 9 Prediction results for sample images from the CBIS-DDSM dataset. (The green line marks the boundary of ground truth and the red line marks the boundary of prediction. Dice score for predictions of the model is specified at the bottom right corner of the image.) [Color figure can be viewed at wileyonlinelibrary.com]

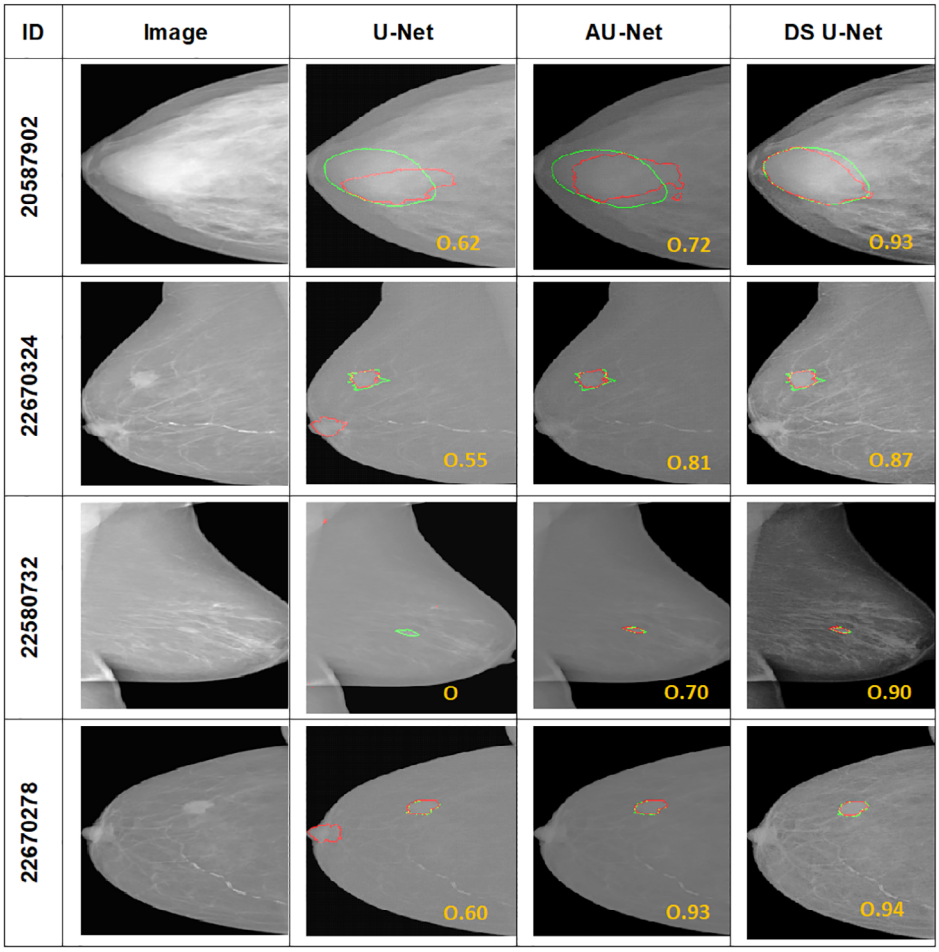


FIGURE 10 Prediction results for sample images from the INbreast dataset. (The green line marks the boundary of ground truth and the red line marks the boundary of prediction. The dice score for predictions of the model is specified at the bottom right corner of the image.) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Computational complexity of various models

Model	U-net	AU-Net	DS U-Net
Number of trainable parameters (in millions)	31	75.4	31.6

TABLE 5 Overview of semantic segmentation architectures used in the literature for segmentation of mass in digital mammograms

Segmentation models	Architectural modifications	Dataset	Images	Dice score	Sensitivity
PSPNet[29,30]	Spatial pyramid pooling	Private dataset	380	67.85	72.02
DeepLabV3+[29,31]	Atorus convolution and an efficient decoder	Private dataset	380	68.27	70.72
FC-DenseNet[29]	Dense connections	Private dataset	380	73.55	79.68
ASPP-FC-DenseNet[29]	Atorus convolution with dense connections	Private dataset	380	76.97	79.83
Attention Net[32]	Attention gates	DDSM dataset	400	77.32	68.01
Attention DenseNet[29]	Dense blocks and Attention Gates	DDSM dataset	400	81.36	76.19
AU-Net[27]	Complex up-sampling with asymmetrical encoder and decoder blocks	CBIS-DDSM	857	80.9	84.5

prediction result of all the models for sample images from both the datasets are depicted. The boundary of the ground truth is delineated using lines in green color and the boundary of the predicted regions are delineated using lines in red color. From the figures, it is apparent that the U-Net model fails to detect lesions of smaller size.

To better understand the overlap of predictions and ground truth across the entire set of images in CBIS-DDSM dataset, empirical cumulative difference of dice score is plotted (Figure 8). It shows the frequency count of every possible dice score in the obtained predictions. The DS U-Net locates lesion in the images quite comfortably as the graph starts with dice score of 0.4, that is, the model does not have any predictions with a dice score less than 0.4. The graph of the DS U-Net also shows a steeper increase after the dice score 0.8. This is indicative of the fact that most of the model predictions are precise.

Table 4 specifies the number of trainable parameters of the considered models. The DS U-Net uses a smaller number of convolution layers in the encoder and the decoder blocks compared with AU-Net. This subsequently reduces the training and inference time. It also performs better than AU-Net with half the number of parameters involved in it.

Table 5 provides an overview of other popular semantic segmentation architectures that are used for mass segmentation in the digital mammograms. Most of the works were carried out on a dataset with not more than 400 images. This work uses 857 images from the CBIS-DDSM dataset.

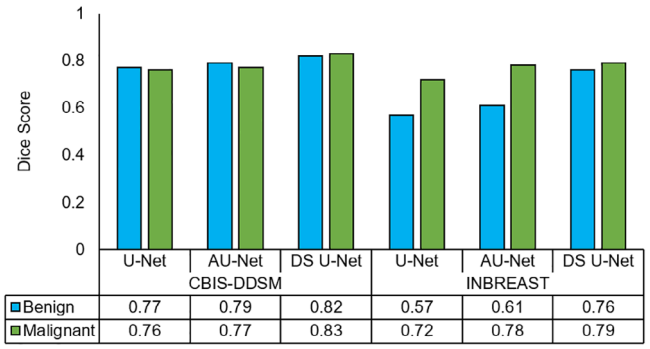


FIGURE 11 Average dice score of different models with respect to lesion type [Color figure can be viewed at wileyonlinelibrary.com]

Effectiveness of the model in the segmentation of benign lesions

The existing CAD system fails to detect lesions at benign stage, as the lesions are more subtle in nature. To assess the effectiveness of the model in detecting the lesions at benign stage, the model's average dice score for benign and malignant lesions are determined. Figure 11 depicts the performance of AU-Net, U-Net and DS U-Net model for both the datasets. The DS U-

Net provides a similar dice score for both the lesion categories. There is also a significant improvement in the average dice score of DS U-Net for benign lesions when compared with AU-Net. This strengthens the inference that DS U-Net works better for mammograms when compared to other models.

3 | CONCLUSION

In this work, an end-to-end framework is created for the mass segmentation in digital mammograms. It uses CLAHE to improve the contrast of the input mammogram, deep supervised U-Net to segment the lesions and dense CRF to recover the entire context of the lesions. The DS U-Net has improved the U-Net model with channel attention and deep supervision for faster convergence and increased performance. Several pixel level and region level metrics are used to evaluate the performance of the model. The proposed architecture is found to perform better when compared with AU-Net and U-Net. In future, this model can also be explored for the segmentation of other medical images.

ACKNOWLEDGMENTS

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DATA AVAILABILITY STATEMENT

The CBIS-DDSM data that support the findings of this study are openly available in Mass-Training and Mass-Test folders at https://doi.org/10.7937/K9/TCIA.2016.7O02S9CY, reference number [26]. The INBREAST data used in the study can be obtained on request to the authors of the article DOI: 10.1016/j.acra.2011.09.014, reference number [25]

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REFERENCE

1. Cancer Facts & Figures (GLOBOCAN). https://gco.iarc.fr/. Accessed September 2020.
2. Matthias E, Alexander H. CADx of mammographic masses and clustered microcalcifications: A review. *Medical Physics*. 2009; 36(6Part1):2052–2068. http://dx.doi.org/10.1118/1.3121511.
3. Jiang Y, Nishikawa RM, Schmidt RA, Metz CE, Giger ML, Doi K. Improving breast cancer diagnosis with computer-aided diagnosis. *Acad Radiol*. 1999;6:22-33.
4. Kupinski MA, Giger ML. Automated seeded lesion segmentation on digital mammograms. *IEEE Trans Med Imaging*. 1998; 17(4):510-517.
5. Brake GM, Karssemeijer N. Segmentation of suspicious densities in digital mammograms. *Med Phys*. 2001;28(2):259-266.
6. Litjens G, Kooi T, Bejnordi BE, et al. A survey on deep learning in medical image analysis. *Med Image Anal*. 2017;42:60-88. https://doi.org/10.1016/j.media.2017.07.005.
7. Zhu Q, Du B, Yan P. Boundary-weighted domain adaptive neural network for prostate MR image segmentation. *IEEE Trans Med Imag*. 2020;39(3):753-763. https://doi.org/10.1109/TMI.2019.2935018.
8. Zhu Q, Bo D, Turkbey B, Choyke P, Yan P. Exploiting interslice correlation for MRI prostate image segmentation, from recursive neural networks aspect. *Complexity*. 2018;2018:1-10. https://doi.org/10.1155/2018/4185279.
9. Zhu Q, Du B, Wu J, Yan P. A deep learning health data analysis approach: automatic 3D prostate MR segmentation with densely-connected volumetric convnets. Paper presented at: 2018 International Joint Conference on Neural Networks (IJCNN), Rio de Janeiro; 2018. pp. 1-6. https://doi.org/10.1109/IJCNN.2018.8489136.
10. Dhungel N, Carneiro G, Bradley AP. Automated mass detection in mammograms using cascaded deep learning and random forests. *Digital Image Computing: Techniques and Applications (DICTA), 2015 International Conference*. New York, NY: IEEE; 2015:1-8.
11. Joseph J, Sivaraman J, Periyasamy R, Simi VR. An objective method to identify optimum clip-limit and histogram specification of contrast limited adaptive histogram equalization for MR images. *Biocybernet Biomed Eng*. 2017;37(3):489-497. https://doi.org/10.1016/j.bbe.2016.11.006.
12. Hu J, Shen L, Sun G. Squeeze-and-excitation networks. Paper presented at: 2018 IEEE/CVF Conference on Computer Vision and Pattern Recognition; 2018. pp. 7132-7141. https://doi.org/10.1109/CVPR.2018.00745
13. Ronneberger O, Fischer P, Brox T. U-net: convolutional networks for biomedical image segmentation. Paper presented at: International Conference on Medical Image Computing and Computer-Assisted Intervention; 2015. pp. 234-241.
14. Makandar A, Halalli B. Breast cancer image enhancement using median filter and CLAHE. *Int J Sci Eng Res*. 2015;6(4):462-464.
15. Sundaram M, Ramar K, Arumugam N, Prabin G. Histogram modified local contrast enhancement for mammogram images. *Applied Soft Computing*. 2011;11(8):5809–5816.
16. Lee C-Y, Xie S, Gallagher P, Zhang Z, Zhuowen T. Deeply supervised nets. *Proc Machine Learning Res*. 2015;38:562-570.
17. Mishra D, Chaudhury S, Sarkar M, Soin AS. Ultrasound image segmentation: a deeply supervised network with attention to boundaries. *IEEE Trans Biomed Eng*. 2019;66(6):1637-1648.
18. Zhu Q, Du B, Turkbey B, Choyke PL, Yan P. Deeply-supervised CNN for prostate segmentation. Paper presented at: 2017 International Joint Conference on Neural Networks (IJCNN); (2017. https://doi.org/10.1109/ijcnn.2017.7965852
19. Chen H, Qi X, Cheng J-Z, Heng P-A, et al. Deep contextual networks for neuronal structure segmentation. Paper presented at:

13th AAAI Conference on Artificial Intelligence; 2016. pp. 1167-1173.
20. Zhu W, Huang Y, Tang H, Qian Z, Du N, Fan W, Xie X. AnatomyNet: deep 3D squeeze and excitation UNets for fast and fully automated whole volume anatomical segmentation. arXiv: 1808.05238; 2018. pp. 1-14.
21. Wong KCL, Moradi M, Tang H, Syeda-Mahmood T. 3D segmentation with exponential logarithmic loss for highly unbalanced object sizes. In: Frangi A, Schnabel J, Davatzikos C, Alberola-López C, Fichtinger G, eds. *Medical Image Computing and Computer Assisted Intervention – MICCAI 2018. MICCAI 2018. Lecture Notes in Computer Science*. Vol 11072. Switzerland: Springer; 2018. https://doi.org/10.1007/978-3-030-00931-1_70.
22. Chen LC, Papandreou G, Kokkinos I, Murphy K, Yuille AL. Semantic image segmentation with deep convolutional nets and fully connected CRFs. Paper presented at: ICLR; 2015.
23. Zheng S, Jayasumana S, Romera-Paredes B, Vineet V, Su Z, Du D, Huang C Torr P. Conditional random fields as recurrent neural networks. Paper presented at: IEEE ICCV; 2015.
24. Krahenbuhl P, Koltun V. Efficient inference in fully connected crfs with Gaussian edge potentials. Paper presented at: NIPS; 2011.
25. Moreira Inês C., Amaral Igor, Domingues Inês, Cardoso António, Cardoso Maria João, Cardoso Jaime S. INbreast. *Academic Radiology*. 2012;19 (2):236–248. http://dx.doi.org/10.1016/j.acra.2011.09.014.
26. Lee RS, Gimenez F, Hoogi A, Miyake KK, Gorovoy M, Rubin DL. A curated mammography data set for use in computer-aided detection and diagnosis research. *Scientific Data*. 2017;4:170177.
27. Sun H, Li C, Liu B, et al. AUNet: attention-guided dense-upsampling networks for breast mass segmentation in whole mammograms. *Physics in Medicine and Biology*. 2020;65(5): 055005. https://doi.org/10.1088/1361-6560/ab5745.
28. Reddi SJ, Kale S, Kumar S. *On the convergence of adam and beyond*. Paper presented at: ICLR 2018 Conference.
29. Hai J, Qiao K, Chen J, et al. Fully convolutional densenet with multiscale context for automated breast tumor segmentation. *Journal of Healthcare Engineering*. 2019;2019:1-11. https://doi.org/10.1155/2019/8415485.
30. Zhao H, Shi J, Qi X, Wang X, Jia J. Pyramid scene parsing network. Proceedings of 30th IEEE Conference on Computer Vision and Pattern Recognition, CVPR; January 2017, pp. 6230-6239. https://doi.org/10.1109/CVPR.2017.660
31. Chen L, Zhu Y, Papandreou G, Schroff F, Aug CV. Encoder-decoder with atrous separable convolution for semantic image segmentation. arXiv; 2018. http://arxiv.org/abs/1802.02611.
32. Li S, Dong M, Du G, Mu X. Attention dense-U-net for automatic breast mass segmentation in digital mammogram. *IEEE Access*. 2019;7:59037-59047. https://doi.org/10.1109/ACCESS.2019.2914873.

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Original Article

Radiology Section

Qualitative and Quantitative Assessment of Enhancement and Washout Characteristics of HCC and non HCC Hypervascular Liver Tumours

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Introduction: The image based diagnosis of Hepatocellular Carcinoma (HCC) relies on assessment of arterial enhancement and venous or delayed phase washout. This assessment is based on visual analysis; however, studies quantifying such enhancement and washout are lacking.

Aim: To qualitative assessment of the imaging characteristics of HCC and non HCC hypervascular liver tumours followed by quantitative assessment of the enhancement and washout kinetics, and also to derive objective values that may help in differentiating HCC from non HCC on Computed Tomography (CT).

Materials and Methods: The present study was carried out in PSG hospitals, Coimbatore, Tamil Nadu, India. Retrospective analysis of hypervascular liver lesions was performed over a study period of four years (January 2012 to February 2016). These lesions were divided into two groups namely; HCC and non HCC. All non HCCs were pathologically proven, while HCC cases included those diagnosed on histopathology or typical imaging findings based on American Association for the Study of Liver Diseases (AASLD) (2010) and Liver Imaging Reporting and Data Systems (LIRADS) (2014) diagnostic criteria along with an elevated Alpha Fetoprotein (AFP) of more than 400 IU/mL.

A qualitative analysis of the enhancement and washout patterns was done followed by quantitative analysis using Regions of Interest (ROI) measurement of attenuation in Hounsfield units (HU). The quantitative parameters assessed were; Mean Attenuation (MA) of lesions in different phases, Attenuation Gain Percentage (AGP) on arterial phase, Arterial Phase Attenuation Change (AAC), Attenuation Loss Percentage in venous and delayed phases (ALP-V, ALP-D), Percentage Attenuation Ratio in venous and delayed phases (PAR-V, PAR-D) and Relative Washout Ratios from arterial to venous/delayed (RWR A-V, RWR A-D) and from venous to delayed phases (RWR V-D). Sensitivity and specificity were calculated for qualitative washout.

For quantitative variables comparative analysis between HCCs and non HCCs was done using Student's t-test. ANOVA was used to compare different categories. ROC curves were drawn for values that were significantly different to establish a cutoff value.

Results: A total of 120 lesions were evaluated consisting of HCCs (n=88) and non HCCs (n=32). Qualitatively majority (81) of HCCs demonstrated arterial enhancement and washout with eight cases of these demonstrating subtle washout requiring settlement by consensus. Washout were absent in seven cases. True mimics of HCC on qualitative analysis were hepatic adenomas, few cases of metastasis and hepatoblastoma were also there. The overall sensitivity of the washout appearance for predicting HCC was 92% while specificity was 78%. On quantitative analysis a PAR-V≥106 has sensitivity of 70% and specificity of 75%, RWR-V-D≥41.79 has sensitivity of 75% and specificity of 70% for HCC detection. Additionally significant difference was seen between RWR V-D of HCC when compared to cholangiocarcinomas (p-value 0.035) and metastasis (p-value 0.026) and also between PAR-V of HCC and metastasis (p-value 0.033).

Conclusion: The qualitative analysis combined with quantitative assessment of washout using percentage attenuation ratio in venous phase and relative washout ratio from venous to delayed phase offers good sensitivity and specificity in image based diagnosis of HCC.

Keywords: Hepatocellular carcinoma, Percentage attenuation ratio, Relative washout ratio

INTRODUCTION

The diagnosis of HCC on imaging relies on subjective assessment of arterial enhancement and venous or delayed phase washout. The arterial phase enhancement is attributed to an increase in intralesional flow with relative reduction in portal flow which occurs as the lesion progresses from dysplastic nodule to HCC [1,2]. Venous or delayed phase washout is decrease in attenuation of the lesion relative to background liver. Factors attributed to washout include early drainage of contrast material as compared to parenchyma and retention of contrast by surrounding fibrotic parenchyma [1-3]. There is ample literature on arterial enhancing mimics of HCC [1,3-7]. Arterial enhancing metastases, cholangiocarcinomas are known, similarly washout, a hallmark of HCC has been described in other tumours [5-7]. According to some studies Intrahepatic Mass-forming Cholangiocarcinoma (IMCC) showing arterial enhancement, was the most common lesion misinterpreted as HCC in preliver transplant imaging work up in recipients [5,9]. In cirrhotic livers lesions like regenerative nodules, dysplastic nodules, arterioportal or venous shunts and confluent fibrosis may also mimic this appearance [2,5-9]. Owing to typical enhancement characteristics of HCC, radiological imaging plays a crucial role in diagnosis and this combined with certain clinical and biochemical characteristics have been adopted as practice guideline for diagnosing HCC over the world. Differentiating HCC from other hypervascular tumours is important since HCC has therapeutic implications and prognostic factors which are very different from other lesions [2,8,10].

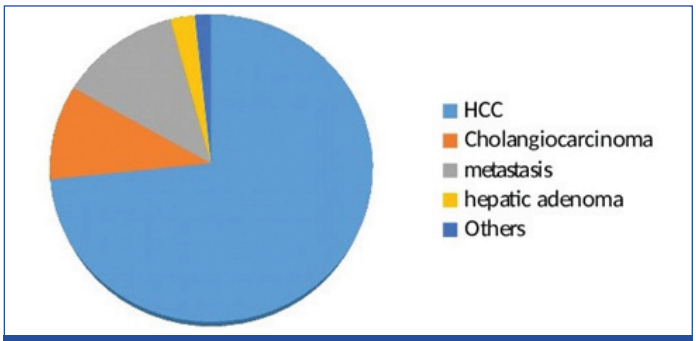
The assessment for hypervascularity of tumours and washout or persistent enhancement relies heavily on radiologist's subjective visual assessment. Only few studies have attempted to quantitate these variables and define the washout phenomenon [2,10]. In one

study, parameters such as relative contrast washout ratio between the arterial phase and delayed phase and attenuation ratio of the lesion to the background liver were studied and the attenuation ratio of the lesion to the background liver in the delayed phase was found to have excellent sensitivity and specificity for HCC detection and good correlation with subjective assessment [2]. In yet another study, an attenuation difference of greater than 10 HU between the arterial and delayed phase yielded optimal sensitivity and specificity for detecting HCC [10]. Certain studies have also quantitatively assessed the washout pattern on Magnetic Resonance Imaging (MRI), with a study evaluating the signal to noise ratio and liver to lesion contrast [11]. Overall the available literature is limited in this aspect with variable results. In present study, we analysed a large retrospective cohort of hypervascular liver tumours which were consecutively diagnosed over a period of four years in our hospital. We performed a qualitative assessment of the imaging characteristics of HCC and non HCC hypervascular liver tumours followed by quantitative assessment of the enhancement, washout kinetics and derived objective values that help in differentiating HCC from non HCC on CT. To the best of our knowledge this is the first such Indian cohort of hypervascular liver tumours being subjected to such qualitative and quantitative assessment.

MATERIALS AND METHODS

Institutional Ethics Committee approval was obtained and requirement for informed consent was waived after considering the retrospective nature of study. An extensive search of our Hospital Information System/Picture Archiving And Communication System (HIS/PACS-Vepro Medical Systems) for liver lesions on CT using keywords Hepatic, liver lesion, hepatic lesion, space occupying lesion, mass, HCC, Hepatocellular carcinoma and each of the individual common malignant and benign liver lesions revealed a total of 389 patients from January 2012 to February 2016 (four years duration). All Multidetector Computed Tomography (MDCT) examinations were performed on 64 slice MDCT (Somatom 64, Seimens healthcare) or 128 slice (Somatom Definition edge, Siemens healthcare) helical scanners. All studies were carried out in craniocaudal direction with patient supine with average 120 KV and 250-450 mA, values varying depending on patients build. Unenhanced images were obtained followed by administration of IV contrast (Iopromide 350-370 mg/mL), power injector was used for injection of contrast at a rate of 4 mL/sec. Automated bolus tracking was used. Images were obtained in arterial, venous and delayed phases, typically at 18-28 seconds, 55-65 seconds and 300 seconds after initiation of contrast administration.

Hypervascular tumours on arterial phase were studied; thus, all hypo or avascular lesions were excluded. Hypodense lesions with thin peripheral enhancement, the pattern which is most common in metastatic lesions, were also excluded. The other exclusion criteria were: i) HCCs without histopathology and not meeting the criteria mentioned below in confirmation of tumor subtype, ii) Diffusely infiltrative HCCs as enhancement pattern and washout characteristics are difficult to assess in them, iii) HCCs with changes related to treatments like thermal ablation or transarterial chemoembolisation, iv) Non HCCs without definitive histological diagnosis, v) Very small tumours less than one cm in size as placing ROI for measurements is difficult in them, vi) Benign lesions like hemangiomas, abscess and cysts were also excluded as they show fairly typical enhancement patterns and in most no histopathological confirmation is done. Histopathological confirmation was available for all non HCCs and HCC (35 cases). 53 HCCs, all in at risk population having underlying cirrhosis were diagnosed based on AASLD guidelines (2010) and LI-RADS (version 2014). First AASLD criteria was applied (a lesion of more than 2 cm showing arterial phase hyperenhancement and subsequent washout, in presence of cirrhosis and elevated AFP levels, we used AFP cutoff of >400 IU/mL to enhance specificity), tumours meeting these criteria are virtually diagnostic of HCC [13]. All of these 53 cases were LI-RADS 5 category (indicating 100% certainty of HCC), with 34 cases being LR-5, 17 cases LR-5V with macrovascular invasion and two cases being LR-5us (being 1-2 cm in size and being visible as discrete nodules on ultrasound) [14]. Two HCCs were histological variants, one a fibrolamellar HCC and another cholangiocellular variant. After application of exclusion and inclusion criteria we arrived at a study population of 120 patients (86 males and 34 females) mean age 58.5 years, range (3-78 years). There were 88 (73%) HCCs, 12 (10%) mass forming cholangiocarcinomas, 15 (12.5%) metastasis, 3 (2%) adenomas, 1 (0.08%) hepatoblastoma and 1 (0.08%) angiosarcoma [Table/Fig-1]. For purpose of comparability the tumours were divided in two broad groups of HCC and non HCC.

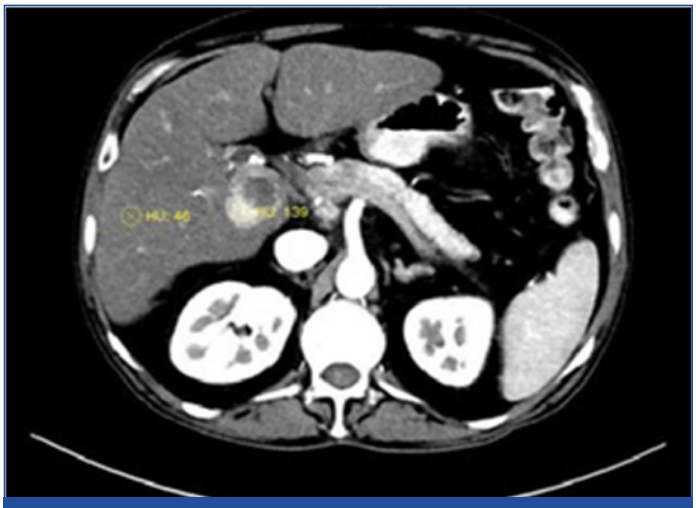


[Table/Fig-1]: Distribution of tumours.

A subjective or qualitative assessment was performed by two radiologists; having nine and five years of experience in abdominal imaging. After confirming the hypervascular nature of the tumour on the arterial phase assessment of subjective washout was done and presence or absence of washout on venous or delayed phases was noted. Presence of any delayed hyperenhancing central stroma was also noted. The examining radiologists were blinded to the results of histopathology and final diagnosis. Discrepancies if any were settled by consensus.

The quantitative analysis was done by one radiologist. The attenuation were measured in HU by drawing circular or oval ROI over the lesion. The ROIs were drawn to include maximally enhancing areas on arterial phase and ranged from 10 to 20 mm with similar ROI drawn on corresponding venous and delayed phase images. The necrotic areas of the tumour, major intratumoral feeding vessels or intratumoral arterioportal shunts were excluded [Table/Fig-2]. In case of large tumours with variable areas of enhancement, multiple ROIs were drawn in the enhancing regions of the tumour and averaged. Two ROIs were also obtained in the adjacent normal background liver in all the phases, taking care to avoid blood vessels. In case of multiple lesions, largest lesion was selected.

The following quantitative indices were calculated 1) Mean attenuation(MA) of lesions in different phases 2) Attenuation gain



[Table/Fig-2]: Hepatocellular carcinoma in caudate lobe with an eccentric necrotic component, ROI placement was done in the enhancing tumour component .

percentage on arterial phase (AGP): SOL a-BL a / BL a x100), Arterial phase attenuation change (AAC): SOL a –BL a (these indices assess the arterial enhancement of the lesions). 3) Attenuation loss percentage in venous and delayed phases (ALP-V, ALP-D): BL v/d-SOL v/d/ BL v/d x100; Percentage attenuation ratio in venous and delayed phases (PAR-V, PAR-D): 100x BL v/d/SOL v/d; Relative wash out ratio (RWR A-V, RWR A-D) from arterial to venous and delayed phases: 100x (SOL a-SOL v/d)/SOL a and from venous to delayed phases(RWR V-D): 100 x (SOLv-SOL d)/ SOL v.(these variables define the washout characteristics). (SOL a and BL a were attenuation of space occupying lesion and background liver attenuation on arterial phase respectively, SOL v/d and BL v/d were lesion and background liver attenuation on venous and delayed phases).

STATISTICAL ANALYSIS

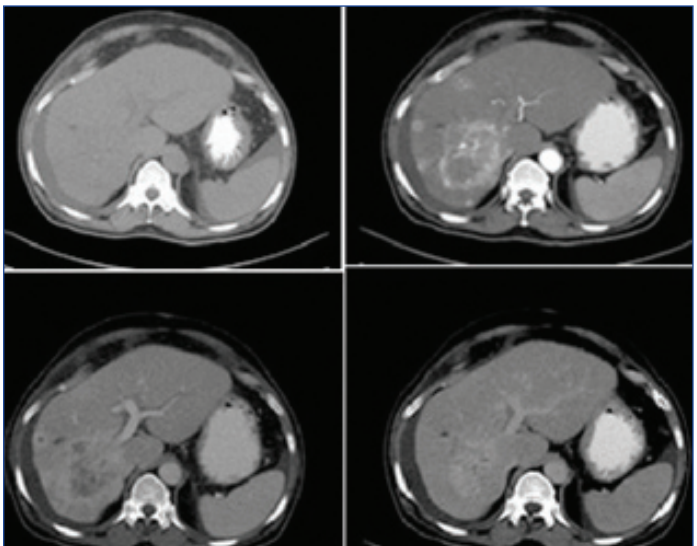
Statistical analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). Sensitivity and specificity for washout appearance were calculated. For quantitative variables comparative analysis between HCCs and non HCCs was done using Student's t-test. ANOVA analysis was used for comparison between HCC and individual categories in Non HCC (cholangiocarcinomas, metastasis and others). ROC curves were drawn for values that were significantly different to establish a cutoff value.

RESULTS

The demographic and qualitative characteristics of the HCC and non HCC are as in [Table/Fig-3]. On qualitative analysis all HCCs showed homogenous or patchy enhancement on arterial phase. There was excellent interobserver agreement between the two radiologists for hypervascularity (Kappa value of 0.91) and presence of washout (Kappa value of 0.86) and discrepancies if any, were settled by consensus. Washout was noted on venous phase in 62/88 (69.3%) patients while on delayed phase additional 19 patients showed washout (81/88,92%). Overall 92% HCCs demonstrated washout in either venous or delayed phase. There was lack of subjective washout as perceived by both radiologists in 7/88 (8%) tumours [Table/Fig-4]. Of these however, two tumours did demonstrate a difference in quantitative washout if absolute values of HU obtained from ROI between the lesion and background liver were considered (though the difference was less than 10 HU). In addition in 8 (9%) tumours the washout was perceived as subtle and required either extensive window setting adjustments (in five cases) or settlement by consensus (three cases). The confounding factors encountered affecting visual assessment were presence of 1) steatosis in the background liver with lesion appearing hyperdense relative to the surrounding fatty liver in two cases, 2) presence of enhancing peripheral pseudocapusle in five cases and a delayed enhancing central fibrous stroma (noted in one case of fibrolamellar HCC). The metastases were the commonest non HCC lesion. Most of the hypervascular metastases were from adenocarcinomas with known or unknown primary sites. A qualitative washout appearance similar to HCC was noted in three cases (20%). Amongst the carcinoid metastasis or neuroendocrine tumours in present study sparing one which washed out the other two were hyper or isodense to parenchyma. Both cases of sarcoma metastasis (n=2) had peripheral enhancement, while washout was seen in one, the other was seen filling in from periphery to the centre. Thus, most of metastatic lesions showed no washout in the venous or delayed phase except one case of transitional cell carcinoma, one of neuroendocrine tumour and one sarcoma. Most of the adenocarcinoma metastases were isodense to parenchyma on delayed phase. The metastasis from gastrointestinal stromal tumour had peripheral fill in pattern.

	HCC	Non HCC	p-value
Females	21	13	0.107
Mean age (years)	63.8	54.03	0.000
Size <3 cm	18 (20.45%)	3 (9.37%)	0.1868
Mean size (cm)	6.55±4.17	7.00±3.7	0.5916
Washout present (%)	81 (92%)	7 (21.8%)	0.001

[Table/Fig-3]: Demographic and qualitative parameters of HCC and Non HCC. The difference is significant at p<0.05
HCC*- Hepatocellular carcinoma



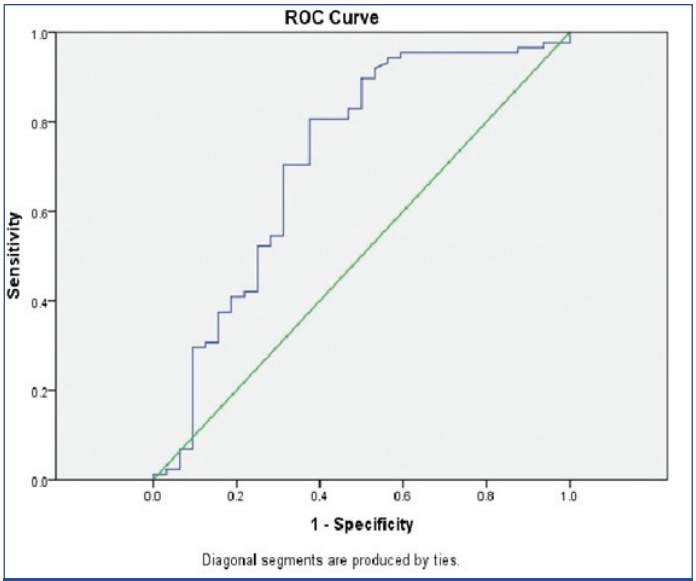
[Table/Fig-4]: Histopathologically proven hepatocelluar carcinoma, multinodular in alcohol related cirrhosis, showing progressive enhancement pattern with lack of subjective or quantitative washout.

Amongst cholangiocarcinomas, none demonstrated washout on delayed phase, all the lesions appeared isodense, hyperdense or showed central enhancing stroma in the delayed phase. The true HCC mimics on qualitative analysis were the three cases of hepatic adenomas and one case of hepatoblastoma which had an enhancement and washout appearance similar to HCC. The only case of angiosarcoma showed hyperdense appearance on delayed phase. Thus, hepatic adenomas, few hypervascular non adenocarcinoma metastasis and hepatoblastoma showed washout appearance in venous or delayed phase; thus, mimicking HCC. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%.

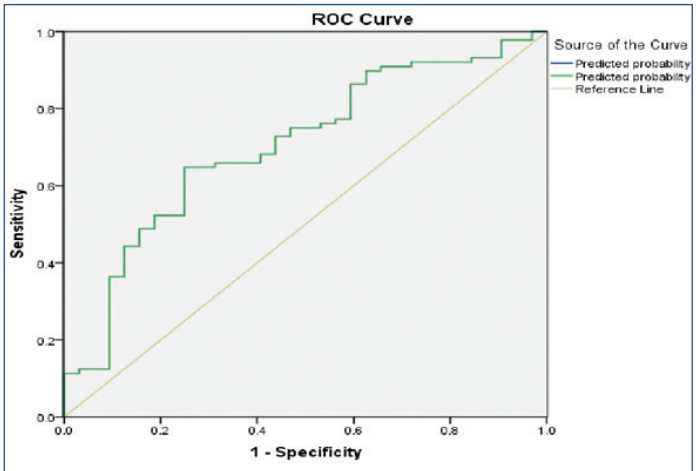
The difference between the mean attenuation of the HCCs and non HCCs was significant in noncontrast (p-value 0.01), arterial (p-value 0.002) and delayed phases (p-value 0.013), while the difference was not significant in the venous phase (p-value 0.614). The quantitative indices are as listed in [Table/Fig-5]. There was no significant difference between the two groups in attenuation change in arterial phase (AAC), while AGP was significant (p-value 0.018). Of the washout parameters, in venous phase significant difference (p-value 0.002) was found in PAR-V in venous phases with no significant difference seen between the two groups in PAR-D. The washout parameters assessed were relative washout from arterial to venous or delayed phase (RWR A-V, A-D) and from venous to delayed phase (RWR-V-D). The relative washout from arterial to venous or delayed phases was (RWR A-V,A-D) was found to be significant (p-value 0.022, 0.004). Washout from venous to delayed phase (RWR-V-D) was also significant between the two groups (p-value 0.000). ALP-V that is attenuation loss percentage in venous phase was also significant (p-value 0.001). However, area under curve was significant for RWR V-D and PAR-V only (r-value >0.7) and analysis of ROC curves for PAR-V and RWR-V-D revealed good sensitivity and specificity for detection of HCC [Table/Fig-6,7].

	HCC (Mean)	Non HCC (Mean)	p-value
MA-NCCT	66.31	52.71	0.01
MA-Arterial	105.96	89.8	0.002
MA-Venous	89.34	91.73	0.614
MA-Delayed	51.75	63.09	0.013
AAC	13.5	2.46	0.070
AGP	27.9	7.17	0.018
PAR-V	116.29	101.89	0.002
PAR-D	117.33	116.09	0.810
RWR A-V	9.3	-6.6	0.022
RWR A-D	45.05	25.6	0.004
RWR V-D	43.27	31.38	<0.001
ALP-V	10.83	-1.16	0.001
ALP-D	12.01	9.73	0.487

[Table/Fig-5]: Details of quantitative variables assessed.
MA-NCCT*-Mean Attenuation Non Contrast CT
AAC*- Arterial phase attenuation change, AGP*- Attenuation gain percentage, PAR-V*- Percentage attenuation ratio-venous phase, PAR-D*- Percentage attenuation ratio-delayed phase, RWR A-V*- Relative washout ratios arterial-ratio, RWR A-D*- Relative washout ratios arterial-delayed, RWR V-D*- Relative washout ratios venous-phase, PLP-V*- Attenuation loss percentage-venous phase, ALP-D*- Attenuation loss percentage-delayed phase



[Table/Fig-6]: Receiver operating curve (ROC) for RWR-V-D, area under curve >0.7



[Table/Fig-7]: Receiver operating curve (ROC) for PAR-V, area under curve >0.7

On individual comparison of HCC with metastasis, cholangiocarcinoma and others category, significant difference was seen between RWR V-D of HCC (mean 43.27) when compared to cholangiocarcinomas (mean 31.2, p value 0.035) and metastasis (mean 33.63, p value 0.026).

DISCUSSION

Studies show that combination of arterial hypervascularity and washout appearance of HCC on MDCT offers a specificity approaching 100% for HCC >20 mm in at risk populations. According to the AASLD guidelines (2010) a lesion of more than 10 mm showing arterial phase hyperenhancement and subsequent washout, in presence of cirrhosis can be considered as HCC and treated as such. Similar guidelines have been proposed by European Association for the Study of the Liver (EASL) in 2012 [13-15]. More recently LIRADS (2014) has been developed for categorising liver lesions in at risk population; this classification aims to facilitate clear communication between different personnel involved in care of these patients. The lesions falling in LR-5 category are nearly certain to be HCCs and consists of hypervascular lesions of more than 2 cm with one or more combinations of washout, capsule, threshold growth. In addition macrovascular mainly portal vein invasion constitutes LR-5V category [14].

A comprehensive quantitative analysis may help in providing further parameters that assists differentiation between HCC and non HCC. A quantitative assessment may also reduce interobserver variability and lay foundation for development of diagnostic algorithms and computed aided detection parameters. To the best of our knowledge, this is also the first study of this kind in India with no previous Indian cohort of hypervascular liver tumours having being subjected to this kind of qualitative and quantitative assessment.

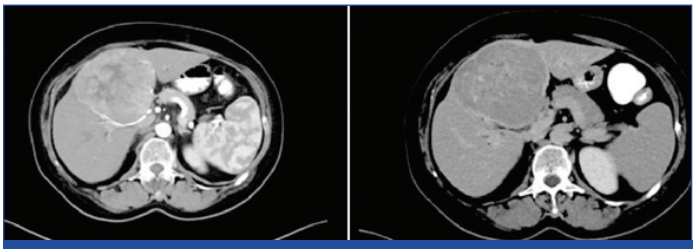
HCCs showed uniform or nonuniform enhancement with most showing washout appearance. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%. The venous phase washout was seen in 69.3% patients while in delayed phase this number increased to 92%. About 8% of HCC did not washout at all while another 9% had a subtle washout. Latter could be resolved subjectively itself and washout even though subtle could be resolved qualitatively with a consensus agreement of two experienced radiologists. Thus, a qualitative washout assessment in a tertiary level setup with high volumes and experienced radiological staff does hold an excellent sensitivity and a reasonable specificity for HCC detection. The specificity may approach 100% if additionally presence of imaging features of cirrhosis is taken into account since none of the non HCCs had underlying cirrhosis. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%.

On quantitative evaluation there was significant difference in the attenuation between the two groups on noncontrast, arterial and delayed phases. Of the arterial parameters the AAC was found to be significantly different between the HCCs and non HCCs. However, when comparing the HCCs with individual subgroups of non HCCs the significance could not be elicited. The AGP was found to be significantly different between the two groups however, it merely represents arterial hypervascularity and doesn't quantitate washout. The PAR as suggested by Liu Y et al., is a measurement of washout appearance of HCC, measured as a ratio of liver and lesion attenuation [2]. The values for PAR in venous phase showed significant difference between the HCC and non HCC while in delayed phase the difference was not statistically significant. The mean PAR-V of HCC was higher than Non HCC. The absence of difference of PAR in delayed phase is probably because there were lesions like adenoma and certain metastases that washed out significantly more than HCC from venous to delayed phase as compared to HCC resulting in almost similar values of PAR-D. A cutoff of PAR-V of 106 provided 70% sensitivity in diagnosing HCC. Relative Washout Ratio (RWR) from arterial to delayed and venous phase was significantly different between the two groups but seems irrelevant as many of the values were negative, since maximum enhancement was obtained in venous phase in many tumours. Considering the fact that attenuation of the lesion was maximum in

venous phase, the calculated RWR from venous to delayed phase (RWR-V-D) showed that the HCC washout significantly more than non HCC and this temporal parameter of washout from venous to delayed phase seems more relevant than ascertaining washout from arterial to venous or arterial to delayed phase. As discussed earlier the washout phenomena is an appearance based on a lot of factors and RWR from venous to delayed phase seems to be one of the parameters that may differentiate the HCC and non HCC negating the effect of liver parenchymal attenuation. It is likely that gradient of loss of attenuation of lesion from venous to delayed phase is much more significant. The mean values of RWR-V-D of HCC (43.27) and non HCC (31.38) were significantly different and a cutoff of 41.79 offered a sensitivity of 75%.

When comparing individual groups there was a significant difference between the mean RWR-V-D values of HCC (mean 43.27) versus hypervascular IMCC (mean 31.2) and metastasis (mean 33.63). Loyer EM et al., compared the kinetics of HCC versus IMCC and found that HCC loose enhancement from venous to delayed phase while IMCC gain enhancement from the corresponding phases, however they included all cases of cholangiocarcinomas and not just the hypervascular variants [16]. Liu Y et al., used PAR ratio as a simple tool to measure the washout appearance [2]. In present study it is apparent that there are some arterial phase enhancing mimics showing the washout appearance complicating the oversimplified guidelines. We also had a much larger study population. In present study PAR-V was significant offering good sensitivity and specificity. This is in contradistinction to the study by Liu Y et al., which found PAR-D to be significant [2]. RWR-V-D was also significantly different between two groups and this gradient may actually help in cases that are not so straight forward.

In present study we found some true HCC mimics; however, which have similar qualitative appearance and quantitative indices like HCC, something which has not been highlighted in the other studies. These mimics were hepatocellular adenomas, hepatoblastoma and certain hypervascular metastasis [Table/Fig-8]. Hypervascular liver metastases from neuroendocrine tumours mimicking hepatocellular carcinoma in enhancement and washout pattern has been described in literature [16,17]. These mimics should be considered as differentials for HCC arising in noncirrhotic liver. The value of quantitative indices also appears to be doubtful for these mimics and appeared to be similar in both subgroups. Although, proving this statistically was difficult in present study due to the small sample size of the mimics. Thus, while a qualitative assessment combined with some useful quantitative parameters, can provide a noninvasive image based diagnosis in majority of cases in the settings of cirrhosis, the same may not hold true for HCC in noncirrhotic livers. A thorough clinicoradiological assessment can however, point to correct diagnosis with hepatocellular adenoma having distinct age group and predispositions, hepatoblastoma usually arising in pediatric age groups and metastatic lesions usually being accompanied by their primary known tumours, though biopsy may be needed for confirmation. Nevertheless a thorough qualitative assessment combined with assessment of certain useful quantitative indices mentioned above, can permit confident noninvasive image based diagnosis for a majority of HCCs in most radiological setup.



[Table/Fig-8]: Neuroendocrine tumour metastatic to liver, showing arterial phase enhancement and delayed phase washout, a pattern indistinguishable from HCC.

LIMITATION

There were some limitations of present study, firstly the lack of histopathology for all HCCs and the use of AASLD guidelines and LIRADS classification with AFP levels for identifying the same (partly because obtaining a biopsy is now almost unethical in patients with cirrhosis meeting the classic imaging diagnostic criteria), but we were stringent in including patients with image based diagnosis, only cirrhotic patients with AFP of more than 400 IU/mL with washout were included. The incidence of cholangiocarcinomas which is known to be the most common mimic would be very low in this group and is unlikely to alter the results. The other limitations included, HCCs less than 1 cm which were not included in our data set. Thus, their behavior was not assessed, heterogeneity within the non HCC group and the sample size of the non HCC group was small compared to HCC. Retrospective nature of analysis also limits the evaluation.

CONCLUSION

While there are number of guidelines which include the more popular likes of AASLD and LI-RADS for diagnosis of HCCs and categorising liver lesions; yet, most of these continue to be subjective in nature with lack of quantification of hypervascularity or washout. A quantitative analysis defining the washout is useful additional determinant adding objectivity to image based diagnosis. Quantitative variables like PAR-V (threshold value 106), RWR-V-D (threshold of 41.79) complement the qualitative analysis, for diagnosis of HCC and have future implications like incorporation into guidelines and development of diagnostic algorithms for computed aided detection software packages.

REFERENCES

[1] Kamaya A, Maturen KE, Tye GA, Liu Y, Parti N, Desser T. Hypervascular liver lesions; Semin Ultrasound CT MR. 2009;30(5):387-407.
[2] Liu Y, Shin L, Brooke J, Kamaya A. Quantitatively defining washout in hepatocellular carcinoma. AJR Am J Roentgenol. 2013;200(1):84-89.
[3] Khosa F, Khan AN, Eisenberg RL. Hypervascular liver lesions on MRI. AJR Am J Roentgenol. 2011;197(2):W204-20.
[4] Murakami T, Tsurusaki M. Hypervascular benign and malignant liver tumors that require differentiation from hepatocellular carcinoma: Key points of imaging diagnosis. Liver Cancer. 2014;3(2):85-96.
[5] Kim SA, Lee JM, Lee KB, Kim SH, Yoon SH, Han JK, et al. Intrahepatic mass-forming cholangiocarcinomas: enhancement patterns at multiphasic CT, with special emphasis on arterial enhancement pattern-correlation with clinicopathologic findings. Radiology. 2011;260(1):148-57.
[6] Oliver J, Baron R, Federle M, Jones B, Sheng R. Hypervascular liver metastases: Do unenhanced and hepatic arterial phase CT images affect tumor detection ?. Radiology. 1997;205(3):709-15.
[7] Kim TK, Lee E, Jang HJ. Imaging findings of mimickers of hepatocellular carcinoma. Clin Mo Hepatol. 2015;21(4):326-43.
[8] Kang Y, Lee JM, Kim SH, Han JK, Choi BI. Intrahepatic Mass-forming cholangiocarcinoma: Enhancement patterns on gadoxetic acid-enhanced MR images. Radiology. 2012;264(3):751-60.
[9] Luca A, Caruso S, Milazzo M, Mamone G, Marrone G, Miraglia R, et al. Multidetector-row computed tomography (MDCT) for the diagnosis of hepatocellular carcinoma in cirrhotic candidates for liver transplantation: prevalence of radiological vascular patterns and histological correlation with liver explants. Eur Radiol. 2010;20(4):898-907.
[10] Tan CH, Thng CH, Low AS, Tan VK, Hartono S, Koh TS, et al. Washout of hepatocellular carcinoma carcinoma; quantitative region of interest analysis on CT. Ann Acady Med, Singapore. 2011;40(6):269-75.
[11] Frericks BB, Loddenkemper C, Huppertz A, Valdeig S, Stroux A, Seja M, et al. Qualitative and quantitative evaluation of hepatocellular carcinoma and cirrhotic liver enhancement using Gd-EOB-DTPA. AJR Am J Roentgenol. 2009;193(4):1053-60.
[12] McEvoy SH, McCarthy CJ, Lavelle LP, Moran DE, Cantwell CP, Skehan SJ, et al. Hepatocellular carcinoma: illustrated guide to systematic radiologic diagnosis and staging according to guidelines of the american association for the study of liver diseases. RadioGraphics. 201333(6):1653-68.
[13] Song DS, Bae SH. Changes of guidelines diagnosing hepatocellular carcinoma during the last ten-year period. Clin Mol hepatol. 2012;18(3):258-67.
[14] Rakhmonova G, Choi J, Kim M. Liver imaging reporting and data system (LI-RADS) version 2014: understanding and application of the diagnostic algorithm. Clin Mole Hepatol. 2016;22(2):296-307.
[15] Cruite I, Tang A, Sirlin C. Imaging-based diagnostic systems for hepatocellular carcinoma. AJR Am J Roentgenol. 2013;201(1):41-55.

[16] Loyer EM, Chin H, Dubrow RA, David CL, Eftekhari F, Charnsangavej C. Hepatocellular carcinoma and intrahepatic peripheral cholangiocarcinoma: enhancement patterns with quadruple phase helical CT-A comparative study. Radiology. 1999;212(3):866-75.

[17] Laroia ST, Sasturkar S, Rastogi A, Pamecha V. Solitary hypervascular liver metastasis from neuroendocrine tumor mimicking hepatocellular cancer: All that glitters is not gold. Indian J Nucl Med. 2015:30(1):42-46 .

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Original article

Correlation of splenic ARFI with endoscopic findings of esophageal varices in chronic liver parenchymal disease

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Abstract:

Background: Variceal bleed the major cause of mortality in cirrhosis. Upper gastrointestinal (GI) endoscopy remains the main stay of diagnosis and treatment in variceal bleed. Splenic elastography using Acoustic Radiation Force Impulse (ARFI) technique can be used to non-invasively predict presence and severity of esophageal varices.

Material and methods: It is a hospital based prospective case control study, done in 100 patients with chronic liver parenchymal disease, who had undergone upper GI endoscopy. Fifty consecutive patients with esophageal varies and 50 consecutive patients without esophageal varices were included in the study and subjected to splenic ARFI. Data was analyzed with SPSS version 20.0 software using chi-square test or Fischer exact test, independent sample-t test. The receiver operating characteristics (ROC) curve was plotted and the area under the curve was used to determine the cut off points for statistically significant variables.

Results: Spleen ARFI showed significant correlation between cirrhotics with esophageal varices and without varices groups. The cut off of ≥ 3.16 m/s (AUROC: 0.906) precisely detects varices with sensitivity of 94% and specificity of 92%. Spleen ARFI also correlates with severity of varices. Cut off of ≥ 3.29 m/s (AUROC: 0.874) can confidentially differentiate low grade from high grade varices in cirrhotics with sensitivity of 85% and specificity of 100%.

Conclusions: Amongst the noninvasive parameters analyzed in this study, spleenic ARFI exclusively showed highest sensitivity and specificity in the detection of varices and differentiation of high grade varices from low grade varices.

Key words: Splenic ARFI, cirrhosis, Endoscopy, Esophageal varices.

Introduction:

Diverse insults on the liver lead to a common endpoint as cirrhosis. Cirrhosis is pathologically characterized by extensive fibrosis, regenerative nodules and distortion of hepatic parenchymal architecture [1]. Alcoholism and viral hepatitis have been the most common etiologies leading to cirrhosis, however nonalcoholic fatty liver disease is emerging as an important underlying pathology.

Complications of cirrhosis can be categorized as portal hypertension related and portal hypertension unrelated complications. Most common portal hypertension related complications is development of esophageal varices and variceal bleeding [2]. Till date, invasive upper gastrointestinal endoscopy (UGI scopy) remains the mainstay

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for the detection and grading of esophageal varices [3]. In addition to diagnostic advantage, UGI scopy plays a major role in treatment such as band ligation and sclerotherapy. Despite its diagnostic and therapeutic utility, UGI scopy is an invasive procedure, has its inherent complications such as bleeding, perforation, infection and cardio-respiratory complications related to sedation [4].

With current advances in the field of Ultrasonography, estimation of elasticity of the tissue has been made possible with Elastography. It is analogous to manual palpation and in fact offers better information, based on the principle that pathology changes the stiffness of the tissue. Shear Wave Elastography (SWE) is a technique of elastography that uses "shear wave" generated within tissue secondary to the acoustic push, the so called Acoustic Radiation Force Impulse (ARFI). In this technique as no external compression is required and a push impulse can be directed anywhere, it is possible to attain a fine elastogram of entire organ. Additionally, quantification of elasticity of particular region of interest based on shear wave velocity (SWV) is possible [5].

Elasticity of the spleen will be decreased in cirrhosis due to portal venous congestion and hyperplasia of splenic tissue; hence the splenic stiffness will be increased. Portal venous congestion results in esophageal varices. Screening UGI scopy is recommended in all cirrhotic patients in accordance with recent guidelines as primary prophylaxis against variceal hemorrhage[6]. Implementing these guidelines is confronted with cost, complications and invasiveness of UGI scopy. Hence, it necessitates accurate and noninvasive methods for detecting and grading the severity of esophageal varices.

Aim:

To evaluate splenic ARFI as a noninvasive tool for detection of esophageal varices and grading of varices as to high and low grades in chronic liver parenchymal disease.

Materials and methods:

It is a prospective case control study done on 100 patients over a period of 14 months, who were referred to the department of radiology with a diagnosis of chronic liver parenchymal disease. Subsequently all these patients underwent UGI scopy and were divided into two groups. Consecutive 50 patients without esophageal varices comprised the first group and 50 patients with esophageal varices comprised second group. Study was undertaken after obtaining necessary clearance from the institutional human ethics committee. Patients who are of less than 18 years of age and who did not undergo UGI scopy were excluded from the study. All the patients were subjected to splenic ARFI and UGI scopy.

Splenic ARFI protocol:

Splenic ARFI was performed with Siemens Acuson S2000^{TM} / Siemens Acuson S3000 ^{TM} ultrasound systems with ARFI enabled 6C1 / 4C1 curvilinear transducers. Patient was made to lie in right lateral decubitus position and assessment was done by intercostal approach. In order to cover uniformly the spleen, we adopted the stratified random sampling method used in the previous studies [7] (Figure 1). Spleen will be subdivided into cranial external(1), cranial central(2), cranial internal(1), intermediate external(1), intermediate central(1), intermediate internal(1), caudal external(1), caudal central(1) and caudal internal(1) segments. Numbers in the parenthesis represents the number of ARFI values obtained in each segment. In breath hold position, region of interest measuring 10mm in depth and 5mm in width was placed in parenchyma devoid of visible blood vessels and >1 cm below the level of spleen capsule and ARFI evaluation was done (Figure 2). Total of 10 successful measurements were obtained for each patient. Mean ARFI values were automatically calculated and expressed

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in meters per second (m/s).

The data obtained was coded and entered in Microsoft Excel Spreadsheet. Data was analyzed using SPSS version 20.0 statistical software. Categorical variables were expressed as percentages and the comparative analysis was done using chisquare test or Fischer exact test. Continuous variables were expressed as mean ±standard deviation (SD) and the comparative analysis was done by independentsample 't' test. The receiver operating characteristics (ROC) curve was plotted and the area under the curve was determined to the cut off points for statistically significant variables. A probability value (p value) of less than or equal to 0.05 at 95% confidence interval was considered as statistically significant.

Various radilogical parameters like liver size, portal vein diamater, portal vein flow velocity, spleen size, liver ARFI and splenic ARFI were analysed for their value as predictors of presence or absence of esophageal varices, if present, to differentiate high grade varices from low grade varices using various statistical methods as mentioned above.

Results:

TABLE 1: SEX DISTRIBUTION

SEX	WITH EVs		WITHOUT EVs	
	NUMBER	%	NUMBER	%
MALE	36	72	38	76
FEMALE	14	28	12	24
TOTAL	50	100	50	100

TABLE 2: ETIOLOGY DISTRIBUTION IN CIRRHOTICS WITH EVs

ETIOLOGY	WITH EVs		WITHOUT EVs	
	NUMBER	%	NUMBER	%
ALCOHOL	37	74	35	70
NAFLD	6	12	6	12
AUTOIMMUNE	6	12	9	18
METABOLIC	1	2	0	0
TOTAL	50	100	50	100

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TABLE 3: SIGNIFICANCE OF RADIOLOGICAL PREDICTORS BETWEEN CIRRHOTICS WITH EVs AND WITHOUT EVs

PREDICTOR	INDEPENDENT 't' TEST		
	WITH EVs MEAN ± SD	WITHOUT EVs MEAN ± SD	P value
LIVER SIZE	140.50 ± 20.97	138.36 ± 19.62	0.599
PORTAL VEIN DIAMETER	12.54 ± 2.46	12.28 ± 2.06	0.569
PORTAL VEIN FLOW VELOCITY	18.91 ± 6.23	20.28 ± 4.56	0.216
SPLEEN SIZE	**147.54 ± 26.5**	**138.66 ± 16.90**	**0.049***
PLATELET COUNT SPLEEN SIZE RATIO	**588.52 ± 274.58**	**972.86 ± 177.99**	**0.000***
LIVER ARFI	3.03 ± 0.78	2.92 ± 0.69	0.458
SPLEEN ARFI	**3.33 ± 0.23**	**2.99 ± 0.17**	**0.000***

*** P= < 0.05 is statistically significant**

Spleen size, PSR and spleen ARFI showed statistical significance between cirrhotics with EVs and cirrhotics without EVs groups.

TABLE 4: SIGNIFICANCE OF RADIOLOGICAL PREDICTORS BETWEEN LOW GRADE EVs AND HIGH GRADE EVs

PREDICTORS			
	LOW GRADE MEAN ± SD	HIGH GRADE MEAN ± SD	P value
LIVER SIZE	144.91 ± 23.39	136.74 ± 18.27	0.172
PORTAL VEIN DIAMETER	12.22 ± 2.33	12.81 ± 2.58	0.399
PV FLOW VELOCITY	18.69 ± 7.09	19.10 ± 5.52	0.819
SPLEEN SIZE	140.70 ± 31.17	153.37 ± 20.60	0.092
PLATELET SPLEEN SIZE RATIO	651.63 ± 223.87	534.75 ± 305.22	0.135
LIVER ARFI	2.99 ± 0.65	3.07 ± 0.90	0.698
SPLEEN ARFI	**3.22 ± 0.36**	**3.42 ± 0.28**	**0.001***

*** P= < 0.05 IS STATISTICALLY SIGNIFICANT**

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Spleen ARFI solely independently shows statistical significance between low grade EVs and high grade EVs subgroups.

TABLE 5: ROC ANALYSIS: SPLEEN ARFI IN EVs DETECTION

PREDICTOR	CUT OFF	AUROC	SENSITIVITY	SPECIFICITY
SPLEEN ARFI	≥ 3.16	0.906	94%	92%

ROC analysis of spleen ARFI (Figure 3) in detecting EVs in this study showed maximum area under the curve of 0.906 at a cutoff of ≥ 3.16 with maximum sensitivity of 94% and specificity of 92%.

TABLE 6: ROC ANALYSIS: SPLENIC ARFI IN HIGH GRADE AND LOW GRADE EVs

PREDICTOR	CUT OFF	AUROC	SENSITIVITY	SPECIFICITY
SPLEEN ARFI	≥ 3.29	0.874	85%	100%

ROC analysis of spleen ARFI (Figure 4) in delineating high grade and low grade EVs depicted a cutoff of ≥ 3.29 with maximum area under the curve of 0.874 at cutoff of with sensitivity of 85% and specificity of 100%.



Figure: 1. Stratified random sampling method. Spleen will be subdivided into cranial external(1), cranial central(2), cranial internal(1), intermediate external(1), intermediate central(1), intermediate internal(1), caudal external(1), caudal central(1) and caudal internal (1) segments. Numbers in the parenthesis represents the number of ARFI values obtained in each segment.



Figure: 2. In breath hold position, region of interest measuring 10mm in depth and 5mm in width was placed in parenchyma devoid of visible blood vessels and >1 cm below the level of spleen capsule and ARFI evaluation was done.

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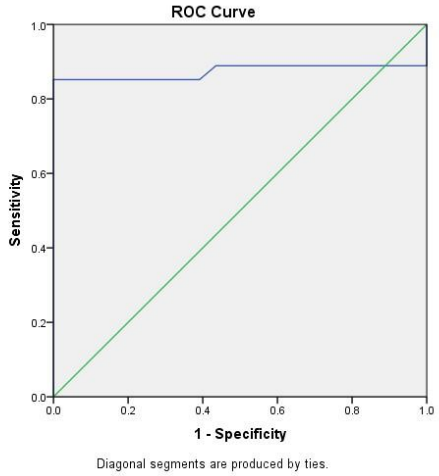


Figure: 3. ROC analysis of spleen ARFI in detecting EVs showed maximum area under the curve of 0.906 at a cutoff of ≥ 3.16 with maximum sensitivity of 94% and specificity of 92%.

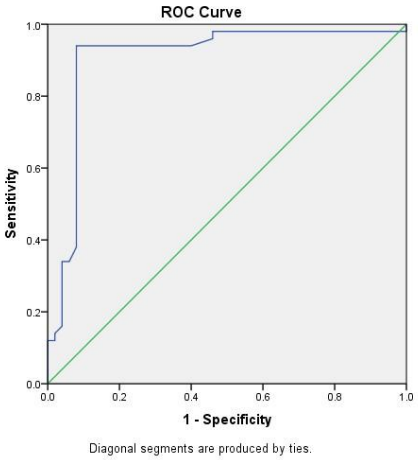


Figure: 4. ROC analysis of spleen ARFI in delineating high grade and low grade EVs depicted a cutoff of ≥ 3.29 with maximum area under the curve of 0.874 at cutoff of with sensitivity of 85% and specificity of 100%

Discussion:

Recent guidelines endorse that endoscopic screening should be done for all cirrhotic patients at the time of diagnosis to recognize those at high risk of bleeding and possibly to benefit from primary prophylaxis [6]. Implementation of these guidelines imposes huge burden on UGI scopy units and frequent testing over time can decrease the compliance of patient. By noninvasive prediction of the presence of EVs, one can recommend the appropriate category of patients to undergo invasive UGI scopy, thereby reducing unnecessary endoscopic examinations and burden on endoscopy units.

Previous studies have correlated various radiological and laboratory parameters either alone or in combination with the presence or absence of EVs in cirrhotics. Although, the efficiency of parameters or combination of parameters reported in each study differ amongst them, due to the heterogeneous nature of the studies. Good predictive value of several non-endoscopic variables for the presence or absence of EVs have been documented

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by many previous studies, but the data is limited from our part of the country. This study was performed to assess the effectiveness of various clinical, laboratory and radiological parameters, in reliably detecting the existence of EVs and high grade EVs in cirrhotic patients.

In the present study, there is no significant association of demographic parameters between the two study groups. Previous studies by Zaman et al [8] and Cherian et al [9], showed that Child Pugh class B or C cirrhotic patients, as compared to class A cirrhotics, have high propensity towards the detection of EVs on UGI scopy. In contrary, our study inferred no significant association between Child Pugh class and the presence of EVs (p = 0.799). In addition, we assessed MELD scores and found significant affiliation with presence of EVs, in congruence to the results of previous studies by De Mattos et al [10] , Tafarel et al [11] and Chandail VS et al [12].

Our study showed no significant variation in either portal vein diameter or portal vein flow velocity between cirrhotic patients with EVs and without EVs. Our study in contradiction to previous studies inferred that there is no significant difference in liver stiffness measured by elastography between cirrhotics with EVs and cirrhotics without EVs (p= 0.458).

In agreement with previous studies, our study also showed significant difference in the values of spleen stiffness measured by ARFI between cirrhotics with EVs and without EVs (p= 0.000). We acquired the optimal cutoff of ≥ 3.16m/s (AUROC: 0.906) with sensitivity of 94% and specificity of 92%, thereby validating spleen ARFI as a non-invasive radiological marker for the presence of EVs in cirrhotics.

In the present study, we furthermore evaluated the utility of the above discussed noninvasive clinical, laboratory and radiological parameters in the detection of high grade EVs in cirrhotics. Of all the noninvasive parameters analyzed, spleen ARFI exclusively showed significant difference between the two subgroups (p= 0.001). We derived the optimal cutoff of ≥ 3.29m/s (AUROC: 0.874) with sensitivity of 85% and specificity of 100%, which is in very close approximation with spleen ARFI cutoff of ≥ 3.30m/s obtained by Takuma et al[13]. Thereby substantiating spleen ARFI as a non-invasive radiological marker for the presence of high grade EVs in cirrhotics. In other words, the presence of high grade EVs can be reliably ruled out with the spleen ARFI cutoff of < 3.29m/s in patients with chronic liver parenchymal disease. Our study was limited by only few patients with grade IV EVs being included in this study. It is due to the lower prevalence of grade IV EVs, as in part by the practice of generous prophylactic and therapeutic measures which delays or eliminates progression of low grade EVs to high grade EVs. Inter observer variability is not assessed in this study. Future studies on this context should focus on multicenter design with evaluation of equipment of various manufacturers and assessment of inter observer variability.

Conclusions:

Amongst the noninvasive parameters analyzed in this study, spleenic ARFI exclusively showed highest sensitivity and specificity in the detection of varices and differentiation of high grade varices from low grade varices.

References :

1. Sangster GP, Previgliano CH, Nader M, Chwoschtschinsky E, and Heldmann MG. MDCT Imaging Findings of Liver Cirrhosis: Spectrum of Hepatic and Extrahepatic Abdominal Complications. HPB Surg 2013;129396:1-12.

2. Schuppan D, Afdhal NH. Liver Cirrhosis. Lancet 2008;371(9615):838-851.

3. Singh SP, Panigrahi MK. Spectrum of Upper Gastrointestinal Hemorrhage in Coastal Odisha. Trop Gastroenterol

2013;34(1):14-17.

4. Riley S, Alderson SD, Birmingham. Complications of upper gastrointestinal endoscopy. BSG Guidelines in Gastroenterology 2006 Nov:7-13.

5. Taljanovic MS, Gimber LH, Becker GW, Latt LD, Klauser AS, Melville DM et al. Shear-Wave Elastography: Basic Physics and Musculoskeletal Applications. Radiographics 2017 May-Jun;37(3):855-870.

6. Kim HY, Jin EH, Kim W, Lee JY, Woo H , Oh S et al. The Role of Spleen Stiffness in Determining the Severity and Bleeding Risk of Esophageal Varices in Cirrhotic Patients. Medicine (Baltimore) 2015 Jun;94(24):e1031.

7. Rizzo L, Attanasio M, Pinzone MR et al. A New Sampling Method for Spleen Stiffness Measurement Based on Quantitative Acoustic Radiation Force Impulse Elastography for Noninvasive Assessment of EsophagealVarices in Newly Diagnosed HCV-Related Cirrhosis. BioMed Research International 2014;Article ID 365982:1-8.

8. Zaman A, Hapke R, Flora K, Rosen HR, Benner K. Factors predicting the presence of esophageal or gastric varices in patients with advanced liverdisease. Am J Gastroenterol 1999 Nov;94(11):3292-3296

9. Cherian JV, Deepak N, Ponnusamy RP, Somasundaram A, Jayanthi V. Non-invasive predictors of esophageal varices. Saudi J Gastroenterol 2011 Jan-Feb;17(1):64-8.

10. de Mattos AZ, de Mattos AA, Daros LF, Musskopf MI. Aspartate aminotransferase-to-platelet ratio index (APRI) for the non-invasive prediction of esophageal varices. Ann Hepatol 2013 Sep-Oct;12:910-4.

11. Tafarel JR, Tolentino LH, Correa LM, Bonilha DR, Piauilino P, Martins FP et al. Prediction of esophageal varices in hepatic cirrhosis by noninvasive markers. Eur J Gastroenterol Hepatol 2011;23:754-58.

12. Chandail VS, Kotwal SK, Koul S, Gupta R, Mahajan A. Non-invasive markers for prediction of varices in patients with portal hypertension. Int J Res Med Sci 2017;5:1007-10.

13. Takuma Y, Nouso K, Morimoto Y, Tomokuni J, Sahara A, Toshikuni N et al. Measurement of spleen stiffness by acoustic radiation force impulse imaging identifies cirrhotic patients with esophageal varices. Gastroenterology 2013 Jan;144(1):92-101.

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Original Article

Utility of Ultrasound Elastography to Differentiate Benign from Malignant Cervical Lymph Nodes

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Abstract

Background: The purpose of this study was to evaluate the usefulness of strain elastography and acoustic radiation force impulse (ARFI) imaging in the differentiation of benign and malignant cervical lymph nodes (LNs). **Materials and Methods:** In this prospective study, 50 enlarged cervical LNs (33 benign and 17 malignant) were examined by B-mode ultrasound (US), color Doppler, and strain elastography. Elastographic patterns (1–5) were categorized based on distribution of hard area within LN. The shear wave velocity (SWV) of LNs was evaluated by ARFI imaging. Diagnostic performance of sonoelastographic parameters was compared taking histopathology of LN as a reference standard. Optimal cutoff value of the mean SWV values for predicting malignancy was determined using receiver operating characteristic curve analysis. **Results:** Among US parameters, borders of LN had the highest diagnostic accuracy (80%), while echogenicity had the least (48%). Majority of benign LNs ($n = 31$) had elastography patterns 1 and 2, while majority of malignant LNs ($n = 16$) had patterns 3–5 ($P = 0.000$). The sensitivity, specificity, and accuracy of elastography were 94.1%, 93.9%, and 94%, respectively. The mean SWV of benign LNs (1.670 ± 0.367 m/s) differed significantly from malignant LNs (2.965 ± 0.826 m/s; $P = 0.000$). A cutoff value of 2.05 m/s predicted malignancy with 88.2% sensitivity and 84.8% specificity and gave an area under the curve of 0.949 (95% confidence interval: 0.70–1.20). **Conclusion:** Elastography has high diagnostic accuracy in differentiating benign and malignant cervical LNs and can be potentially useful in selecting the LN with high probability of malignancy, on which fine-needle aspiration cytology/biopsy can be performed.

Keywords: Acoustic radiation force impulse imaging, elastography, lymph nodes, ultrasound

Introduction

Cervical lymphadenopathy, a common condition encountered by physicians in clinical practice, could be caused by a variety of pathologic processes such as infections, vasculitis, and malignancies such as lymphoma and metastases. Metastatic cervical lymphadenopathy is a common problem in head and neck oncology. Depending on the primary site, up to 80% of patients with upper aerodigestive mucosal malignancy have been reported to have cervical nodal metastasis at presentation.[1] The differentiation of malignant from benign lymph nodes (LNs) is essential because it predicts the patient prognosis and helps in decision-making and planning of management.[2]

High-resolution ultrasound (US) is the extensively used imaging technique for evaluating the morphology and internal architecture of cervical LNs. Color Doppler sonography is used to assess the pattern of intranodal vessels, blood flow velocity, and vascular resistance.[3] Although Gray-scale sonography in combination with Doppler has been suggested to be useful in differentiating metastatic LNs from reactive ones based on various criteria, there is no single US criterion for diagnosing malignant LNs with satisfactory sensitivity and specificity.[4,5]

Real-time elastography (RTE) is a novel, noninvasive imaging modality that assesses tissue elasticity by comparing local tissue displacement from US signals before and after the application of a compressive force.[6] Stiff tissues show less deformation (strain) than soft tissues under the compression of a transducer. Since malignant tissues are stiffer than their benign counterparts at many

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sites, several reports have documented the utility of US elastography in differentiating malignant from benign lesions in the breast, prostate, liver, thyroid, and cervix.[7-11] There are two types of elastography techniques, namely strain elastography and shear wave-based elastography. Recent studies have shown the usefulness of strain elastography in differential diagnosis of benign and malignant cervical LNs.[12-14] However, some authors have questioned its additional value over conventional US in predicting malignancy and decision-making in terms of selection of LNs for biopsy.[15-17] Shear wave elastography with acoustic radiation force impulse (ARFI) imaging is an emerging sonographic technique in the assessment of malignant cervical nodes.[18,19]

Data on the utility of elastography in the evaluation of cervical LNs are relatively few, especially from India. The purpose of the present study was to evaluate the utility and diagnostic performance of strain elastography compared to conventional B-mode US and color Doppler in the differentiation of benign and malignant cervical LNs. We also investigated the usefulness of ARFI imaging in differentiating benign and malignant LNs.

Materials and Methods

This prospective study was performed at a tertiary care center in South India between August 2016 and February 2018. Fifty LNs of 50 consecutive patients who were referred for ultrasonogram of enlarged cervical LNs were enrolled. The Institutional review board (IRB) approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki (IRB Approval No. 16/334). Informed consent was obtained from all participants. Patients with suboptimal image quality and those with completely necrotic nodes without solid component were not included. Patients who had received specific treatment (chemotherapy/radiotherapy) were also excluded from the study.

High-resolution US imaging was performed in the supine position with a high-frequency linear probe 9 L4 (Siemens Accuson S3000, Siemens Healthineers, Erlangen, Germany) by a single expert radiologist, blinded to patient's clinical profile. In gray-scale US, the LNs were evaluated for short-axis diameter and short-to-long axis diameter ratio (S/L ratio) in the longitudinal plane, borders (regular or irregular), echogenicity (homogeneous/heterogeneous), and hilum (present or absent). Intranodal vascular pattern was evaluated using color Doppler imaging. Absent flow signals or blood flow limited to the hilum was categorized as benign. Nonhilar blood flow with spotted, peripheral, or mixed types of vascularization was categorized as malignant. RTE was performed for the targeted node using the same probe. Manual light compression and decompression of the target LN by the transducer were performed to achieve an optimal image. Patients were asked to hold their breath and avoid swallowing during the examination to minimize motion of LNs. The elastographic box contained the LN and adjacent sternocleidomastoid muscle for each patient. Elastograms obtained with quality factor score ≥60 were included in the study. Color map settings with red coded as hard area and blue coded as soft area were used. The images were displayed on split screen mode with US gray-scale images on the left and elastogram images on the right. The best-fit B-mode US elastogram image pairs were selected for analysis. The elasticity patterns were categorized on a 5-point scale described by Alam *et al*.[12] [Table 1]. LNs showing type 1 and 2 elastography patterns were considered benign, while those with types 3, 4, and 5 were categorized as malignant.

For ARFI imaging, the targeted LN on B-mode image was identified using a region of interest, characterized by a fixed box [Figures 1-5]. Three measurements of shear wave velocity (SWV) were performed, and the average was calculated. SWV was expressed in meter/second.

Table 1: Elastographic patterns of lymph nodes

Patterns	Description
1	Absent or very small hard area
2	Small, scattered hard areas, hard area <45% of LN
3	Hard area ≥45% of LN
4	Peripheral hard area and central soft area
5	Hard area occupying entire LN

LN: Lymph node

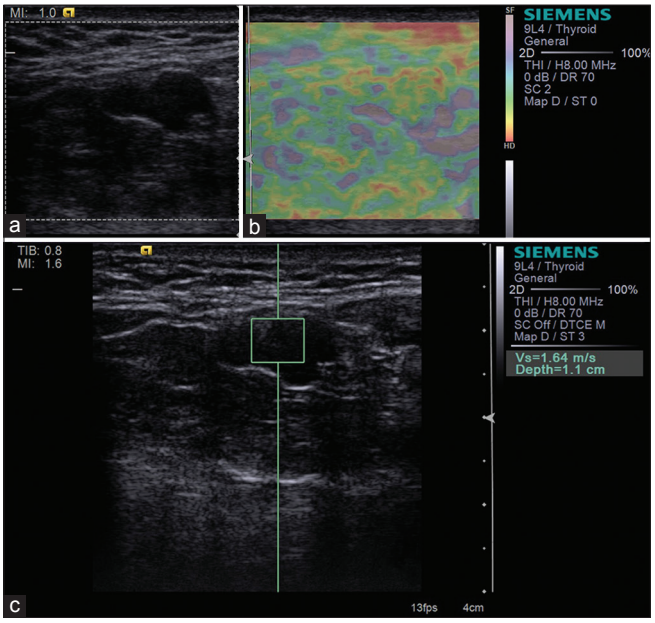


Figure 1: (a) B-mode ultrasound showing oval hypoechoic lymph node with irregular margins and absent hilum, (b) elastography image showing absent or very small hard area corresponding to pattern 1, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 1.64 m/s in the boxed area. Histopathology was suggestive of tuberculous lymphadenitis

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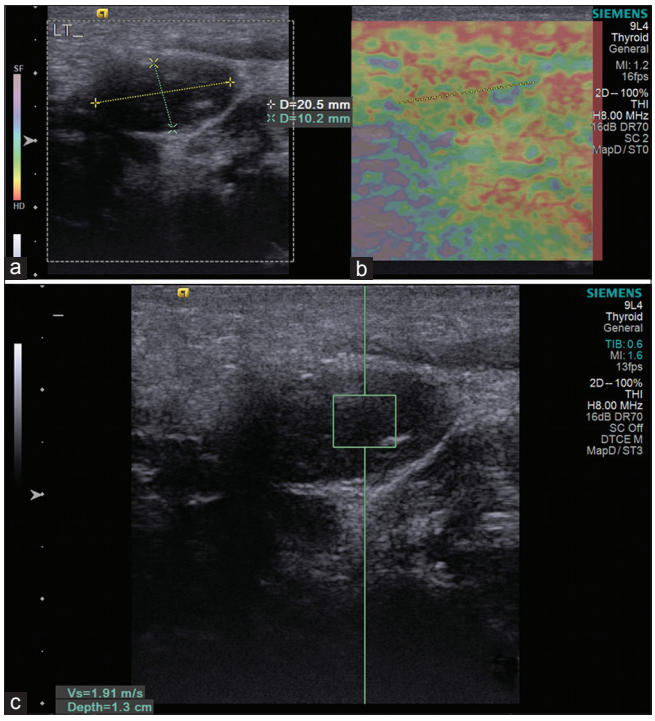


Figure 2: (a) B-mode ultrasound showing oval hypoechoic lymph node, (b) elastography image showing small, scattered hard areas corresponding to pattern 2, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 1.91 m/s in the boxed area. Histopathology was suggestive of reactive lymphadenitis

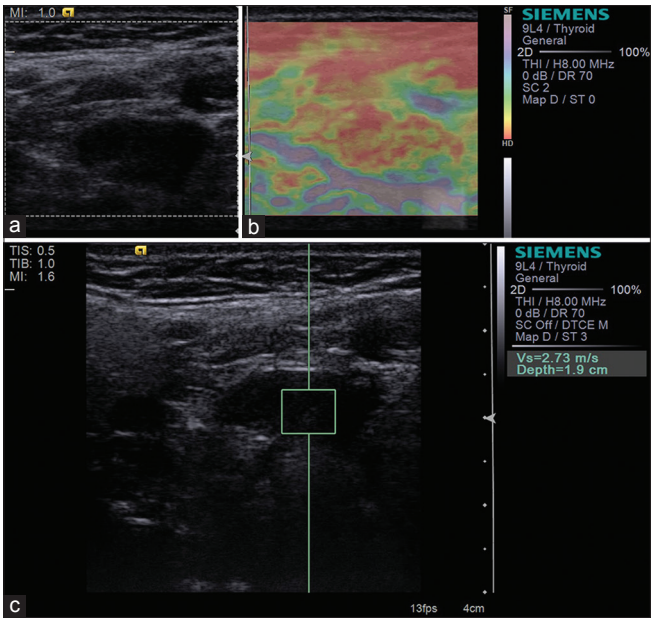


Figure 3: (a) B-mode ultrasound showing oval hypoechoic lymph node with loss of fatty hilum, (b) elastography image showing hard area ≥45% of lymph node corresponding to pattern 3, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 2.73 m/s in the boxed area. Histopathology revealed metastatic lymph node from carcinoma breast

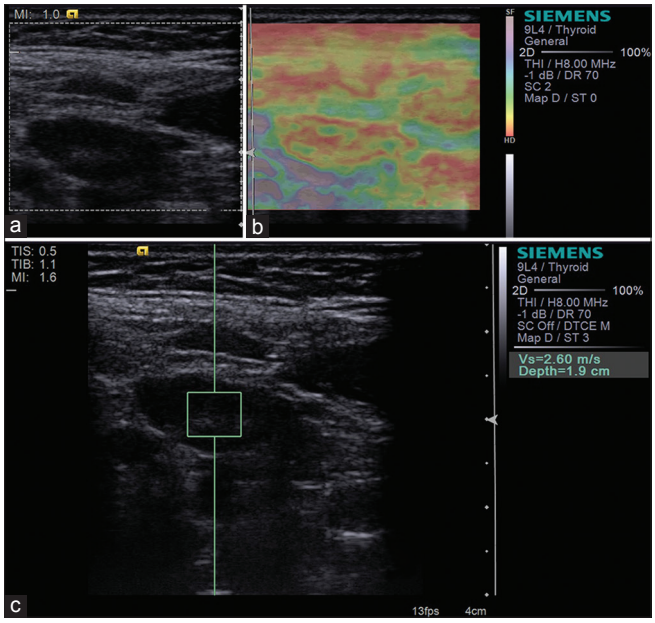


Figure 4: (a) B-mode ultrasound showing oval hypoechoic lymph node with loss of fatty hilum, (b) elastography image showing peripheral hard area and central soft area corresponding to pattern 4, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 2.60 m/s in the boxed area. Histopathology revealed metastatic lymph node from mucoepidermoid carcinoma parotid

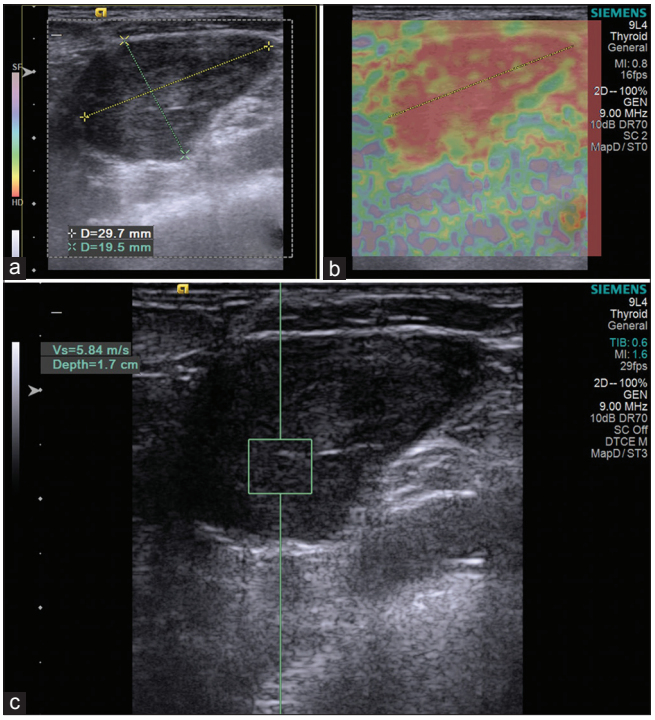


Figure 5: (a) B-mode ultrasound showing hypoechoic lymph node with irregular margins and absent hilum, (b) elastography image showing hard area occupying entire lymph node corresponding to pattern 5, and (c) acoustic radiation force impulse imaging shows a shear wave velocity of 5.84 m/s in the boxed area. Histopathology revealed metastatic lymph node from small cell carcinoma lung

Following elastography, all patients underwent surgical excision of the LN after cutaneous marking under US guidance. Histopathologic analysis of the LN was taken as the diagnostic standard of reference.

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Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 24 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages. Chi-square test was used to know the association between categorical variables. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated with histopathologic analysis as reference standard. For analyzing the combined diagnostic performance of B-mode and Doppler, scores were assigned for five criteria: S/L ratio (ratio ≤0.5, score of 1: >0.5, 2), hilum (present, 1; absent, 2), borders (regular, 1; irregular, 2), echogenicity (homogenous, 1; heterogeneous, 2), and blood flow (hilar, 1; nonhilar, 2). The score for each LN was determined by adding the individual scores. A statistically supported cutoff line was set between scores 6 and 7. LNs with score <6 were categorized as benign, and 7–10 were categorized as malignant. Student *t*-test was performed to evaluate for significant difference in SWV values between benign and malignant LNs. A receiver operating characteristic (ROC) curve analysis was applied to obtain the optimal cutoff value for SWV to differentiate between benign and malignant LNs. Statistical significance was set at $P < 0.05$.

Results

The age of the patients ranged from 12 to 75 years (mean: 45.4 ± 16.9 years). About 52% (n = 26) were female and 48% (n = 24) were male. Of the 50 LNs examined, 33 (66%) were benign and 17 (34%) were malignant on histopathology. Of the 33 benign LNs, 27 had been reported as reactive and six as tuberculous lymphadenitis. Among the 17 malignant nodes, 15 were metastatic and two were due to lymphoma. Metastatic nodes were secondary to carcinoma lung, 4; parotid, 2; thyroid, 1; stomach, 1; breast, 1; poorly differentiated carcinoma from occult primary, 1; and head-and-neck squamous cell carcinoma, 5.

The US characteristics of the LNs and their diagnostic sensitivity, specificity, PPVs and NPV s, and accuracy are shown in Table 2. There were no significant differences between the benign and malignant LNs with respect to S/L ratio and echogenicity ($P = 0.273$ and $P = 0.623$, respectively). Hilum was found to be abnormal in all the 17 malignant LNs studied. Almost 81.8% of benign LNs (n = 27) had regular borders, whereas only 23.5% (n = 4) of malignant LNs had regular borders (P = 0.000). Among all B-mode parameters, borders of LN had the highest diagnostic accuracy (80%), while echogenicity was found to have the least accuracy (48%). On color Doppler evaluation, nearly 72% (n = 24) of benign nodes showed hilar flow compared to 35% (n = 6) of malignant nodes (P = 0.010).

Table 3 shows the correlation of elastographic pattern and histopathologic diagnosis of LNs. Majority of benign LNs (n = 31) had elastography patterns 1 and 2, while majority of malignant LNs (n = 16) had patterns 3–5. Higher elastographic pattern was significantly associated with malignant histopathology (P = 0.000).

The diagnostic performance of combined modalities (B-mode and color Doppler) and elastography is shown in Table 4.

The mean SWV of benign LNs was 1.670 ± 0.367 m/s. Metastatic LNs were significantly stiffer with mean SWV of 2.965 ± 0.826 m/s (P = 0.000). Figure 6 shows the ROC curve for SWVs measured using ARFI imaging in the differentiation of benign and malignant LNs. The best cutoff value for differentiating benign from malignant LNs was estimated to be 2.05 m/s with sensitivity of 88.2%, specificity of 84.8%, and area under the curve equal to 0.949 (95% confidence interval [CI]: 0.70–1.20).

Table 2: Ultrasound characteristics of lymph nodes and their diagnostic performance

US criteria	Benign LNs (n=33)	Malignant LNs (n=17)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
S/L ratio							
≤0.5	15 (45.4)	5 (29.4)	70.5	45.4	40	75	54
>0.5	18 (54.6)	12 (70.6)					
Hilum							
Present	22 (66.7)	0	100	66.6	60	100	78
Absent	11 (33.3)	17 (100)					
Borders							
Regular	27 (81.8)	4 (23.5)	68.4	87.1	76.4	81.8	80
Irregular	6 (18.2)	13 (76.5)					
Echogenicity							
Homogeneous	16 (48.5)	7 (41.2)	41.1	51.5	30.4	62.9	48
Heterogeneous	17 (51.5)	10 (58.8)					
Color Doppler							
Hilar	24 (72.8)	6 (35.3)	64.7	72.7	55	80	70
Nonhilar	9 (27.2)	11 (64.7)					

US: Ultrasound, LNs: Lymph nodes, S/L ratio: Ratio of short-axis diameter to long-axis diameter, PPV: Positive predictive value, NPV: Negative predictive value

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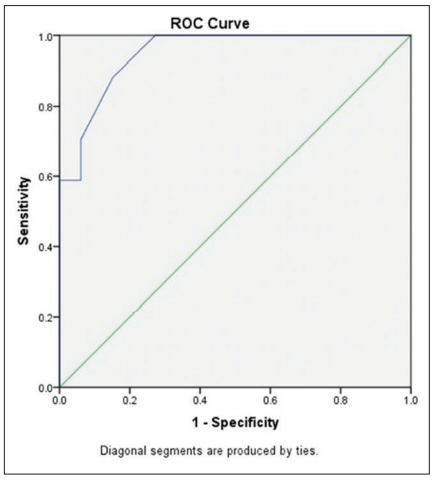


Figure 6: Receiver operating characteristic curve for shear wave velocities measured using acoustic radiation force impulse imaging in the differentiation of benign and malignant lymph nodes. The area under the curve was 0.949

Discussion

In this prospective study from India, we evaluated the performance of RTE in classification of benign and malignant cervical LNs. Our results indicate that RTE has a high specificity and diagnostic accuracy for identification of malignant LNs.

Although B-mode US is the most extensively used method for classification of cervical LNs because of its noninvasiveness and easy accessibility, specific criteria for distinguishing benign and malignant LNs are yet unclear. The association of signs often produces a highly suggestive appearance in most cases. In our study, the accuracy rate in distinguishing benign and malignant LNs was greatest for borders of LN (80%) and least for echogenicity (48%).

There were no significant differences between the benign and malignant LNs with respect to S/L ratio in our study. In contrast, several studies have noted S/L ratio >0.5 or a rounded shape to be associated with malignancy.[12,20] However, shape of LN can be misleading as nodes in nonmalignant conditions such as tuberculosis and Rosai–Dorfman disease have also been described as round.[21]

Our criterion of regular versus irregular borders revealed a diagnostic accuracy of 80%. Some other studies have categorized the LN borders as sharp and unsharp.[22,23] In a study by Khanna *et al*. from India, irregular margins were found in 7% of reactive LNs versus 55% of metastatic and 66% of tubercular nodes.[24] The absence of visible hilum due to replacement or effacement is considered as an important criterion for malignant LNs and has been reported by several authors.[12,13,25] In our study, all the 17 malignant LNs had no visible hilum, thus showing 100% sensitivity and NPV.

We observed a statistically significant difference in the vascular patterns between benign and malignant LNs ($P = 0.010$). Our observations can be comparable to that of Misra *et al*. and several other authors who have noted significant difference in vascularity between benign and malignant LNs.[13,26] In our study, the accuracy of vascularity of LN in predicting malignancy was 70%.

Table 3: Correlation of elastographic pattern and histopathologic diagnosis of lymph nodes

Elastogram pattern	Benign ($n=33$)	Malignant ($n=17$)	Total ($n=50$)
1	5	0	5
2	26	1	27
3	1	2	3
4	1	4	5
5	0	10	10

Table 4: Diagnostic performance of elastography and combined evaluation (B-mode and color Doppler)

Performance measure	Elastography	Combined B-mode and Doppler
Sensitivity (%)	94.1	94.1
Specificity (%)	93.9	81.2
PPV (%)	88.9	72.7
NPV (%)	96.9	96.4
Accuracy (%)	94	86

PPV: Positive predictive value, NPV: Negative predictive value

Even though combined B-mode US and Doppler could be potentially useful in differentiating reactive from malignant LNs, this modality may not have definite diagnostic value because of some overlapping sonographic appearances of benign and malignant nodes. Fine-needle aspiration cytology (FNAC) in the evaluation of cervical LNs has been reported to have a sensitivity of 82% and specificity of 97%.[27] However, FNAC is invasive and does not always provide a conclusive diagnostic result. Core needle biopsy from cervical LNs is also difficult owing to their relatively small size and potential injury to adjacent vascular structures.

RTE being noninvasive, has been suggested to be an adjunctive imaging tool to conventional US and could potentially reduce unnecessary biopsies.[28] Few prospective and retrospective studies concerning strain elastography of cervical LNs have been published in the literature.[29] While some have performed the measurement of elasticity scores (ES), some have measured both ES and strain ratio.

In our study, we used a 5-point scale described by Alam *et al*. to categorize the elasticity patterns. Accordingly, LNs showing types 1 and 2 elastography pattern were considered benign, while those with types 3, 4, and 5 were categorized as malignant. Nearly, 93.9% of benign LNs ($n = 31$) had a score of 1 or 2, while 94.1% of malignant LNs ($n = 16$) had a score of 3–5. The sensitivity, specificity, and accuracy of elastography were 94.1%, 93.9%, and 94%, respectively.

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Alam *et al*. evaluated 85 cervical LNs (53 metastatic and 32 reactive) using 5-point elasticity scale and documented 83% sensitivity, 100% specificity, and 89% accuracy in differentiating metastatic from reactive LNs.[12] Bhatia *et al*. evaluated 74 cervical LNs (37 malignant and 37 benign) using RTE.[17] Dynamic cine loops of elasticity imaging were qualitatively scored on a 4-point elasticity scale by three independent observers. Median ES for benign and malignant nodes was 2 and 3, respectively. A cutoff ES score of ≥2 achieved only 62.2% sensitivity, 83.8% specificity, and 73% accuracy for malignancy. Fair to good interobserver agreement was noted in their study.

Higher accuracy rates observed in our study can be attributed to the fact that there were very few cases of lymphoma ($n = 2$) and tuberculous adenitis ($n = 6$) in our study. Lymphomas have been reported to show low stiffness from an elastographic point of view because of the large size and homogeneous structure of LNs which can produce false-negative results for malignancy.[30] Granulomatous lymphadenitis associated with tuberculosis may demonstrate calcification, scarring, or necrosis and therefore show areas of greater stiffness (false positive).[14]

Ying *et al*. performed a meta-analysis to evaluate the performance of RTE for diagnosis of malignant LNs.[29] Nine studies including 835 LNs were analyzed. The pooled sensitivity and specificity for ES in diagnosing malignant LNs were 74% (95% CI: 66%–81%) and 90% (95% CI: 82%–94%), respectively.

In the present study, we were able to obtain greater specificity, PPV, and accuracy with RTE than conventional US and Doppler for detecting malignant LNs from diverse primary sites. Hence, RTE with its fairly high specificity and diagnostic accuracy can reduce unnecessary invasive procedures for diagnosis of malignant LNs. However, it cannot replace the conventional B-mode US and Doppler because it is dependent on the machine as well as operator's expertise since excessive compression can cause nonaxial displacement and change the tissue stiffness. This in turn can affect the accuracy of the software's correlation algorithms. Furthermore, elastography is not useful for calcified LNs and those with cystic degeneration.

Relatively, few clinical studies have evaluated ARFI in the assessment of malignant cervical LNs.

In our study, ARFI imaging revealed higher SWV for malignant LNs than benign ones. The cutoff value of 2.05 m/s had sensitivity of 88.2% and specificity of 84.8% in identifying malignant LNs. Our results are comparable to that of Fujiwara *et al*. who evaluated SWVs of 42 cervical LNs using ARFI.[31] Of the numerous cutoff values tested, a SWV cutoff value of 1.9 m/s best distinguished between reactive and malignant LNs. Their criterion of SWV >1.9 m/s had 95.0% sensitivity and 81.8% specificity. According to a recent meta-analysis of nine ARFI imaging studies that included 1084 LNs, the pooled sensitivity and specificity values for detecting malignancies were 87% (95% CI: 83%–91%) and 88% (95% CI: 82%–92%), respectively.[19]

Our study has few limitations. The sample size was small, and only enlarged LNs were included. Strain elastography using free hand compression is dependent on the compression technique, and excessive compression can alter tissue stiffness. A single expert radiologist performed all the elastograms, and no interobserver variability was studied. However, studies have reported fair to almost perfect interobserver agreement in strain elastography.[6]

Head-and-neck cancers account for nearly one-third of the cancer burden in India, and it is a well-known fact that status of cervical LN is the single most important prognostic factor in squamous cell carcinoma of the head and neck.[32] To the best of our knowledge, this is the first study from India to document the diagnostic performance of elastography in evaluation of cervical lymphadenopathy, and it has shown encouraging results.

Conclusion

Elastography has high diagnostic accuracy in differentiating benign and malignant cervical LNs and can be an effective noninvasive adjunctive tool in the evaluation of cervical lymphadenopathy. It is potentially useful in selecting the LN with high probability of malignancy, on which FNAC/biopsy can be performed, thereby reducing sampling errors. ARFI imaging can be a useful technique for differentiating benign from malignant cervical LNs.

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Conflicts of interest

There are no conflicts of interest.

References

1. Chong V. Cervical lymphadenopathy: What radiologists need to know. Cancer Imaging 2004;4:116-20.
2. Na DG, Lim HK, Byun HS, Kim HD, Ko YH, Baek JH. Differential diagnosis of cervical lymphadenopathy: Usefulness of color Doppler sonography. AJR Am J Roentgenol 1997;168:1311-6.
3. Ghafoori M, Azizian A, Pourrajabi Z, Vaseghi H. Sonographic evaluation of cervical lymphadenopathy; comparison of metastatic and reactive lymph nodes in patients with head and neck squamous cell carcinoma using gray scale and Doppler techniques. Iran J Radiol 2015;12:e11044.
4. Leboulleux S, Girard E, Rose M, Travagli JP, Sabbah N, Caillou B, *et al.* Ultrasound criteria of malignancy for cervical lymph nodes in patients followed up for differentiated thyroid cancer. J Clin Endocrinol Metab 2007;92:3590-4.
5. Gupta A, Rahman K, Shahid M, Kumar A, Qaseem SM, Hassan SA, *et al.* Sonographic assessment of cervical lymphadenopathy: Role of high-resolution and color Doppler imaging. Head Neck 2011;33:297-302.
6. Choi YJ, Lee JH, Baek JH. Ultrasound elastography for evaluation of cervical lymph nodes. Ultrasonography 2015;34:157-64.
7. Thomas A, Fischer T, Frey H, Ohlinger R, Grunwald S, Blohmer JU, *et al.* Real-time elastography – An advanced method of ultrasound: First results in 108 patients with breast lesions. Ultrasound Obstet Gynecol 2006;28:335-40.

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8. Taylor LS, Rubens DJ, Porter BC, Wu Z, Baggs RB, di Sant'Agnese PA, *et al.* Prostate cancer: Three-dimensional sonoelastography for *in vitro* detection. Radiology 2005;237:981-5.
9. Friedrich-Rust M, Ong MF, Herrmann E, Dries V, Samaras P, Zeuzem S, *et al.* Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. AJR Am J Roentgenol 2007;188:758-64.
10. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al.* US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
11. Thomas A, Kümmel S, Gemeinhardt O, Fischer T. Real-time sonoelastography of the cervix: Tissue elasticity of the normal and abnormal cervix. Acad Radiol 2007;14:193-200.
12. Alam F, Naito K, Horiguchi J, Fukuda H, Tachikake T, Ito K. Accuracy of sonographic elastography in the differential diagnosis of enlarged cervical lymph nodes: Comparison with conventional B-mode sonography. AJR Am J Roentgenol 2008;191:604-10.
13. Turgut E, Celenk C, Tanrivermis Sayit A, Bekci T, Gunbey HP, Aslan K. Efficiency of B-mode ultrasound and strain elastography in differentiating between benign and malignant cervical lymph nodes. Ultrasound Q 2017;33:201-7.
14. Teng DK, Wang H, Lin YQ, Sui GQ, Guo F, Sun LN. Value of ultrasound elastography in assessment of enlarged cervical lymph nodes. Asian Pac J Cancer Prev 2012;13:2081-5.
15. Lo WC, Cheng PW, Wang CT, Liao LJ. Real-time ultrasound elastography: An assessment of enlarged cervical lymph nodes. Eur Radiol 2013;23:2351-7.
16. Acu L, Oktar SÖ, Acu R, Yücel C, Cebeci S. Value of ultrasound elastography in the differential diagnosis of cervical lymph nodes: A comparative study with B-mode and color Doppler sonography. J Ultrasound Med 2016;35:2491-9.
17. Bhatia KS, Cho CC, Yuen YH, Rasalkar DD, King AD, Ahuja AT. Real-time qualitative ultrasound elastography of cervical lymph nodes in routine clinical practice: Interobserver agreement and correlation with malignancy. Ultrasound Med Biol 2010;36:1990-7.
18. Suh CH, Choi YJ, Baek JH, Lee JH. The diagnostic performance of shear wave elastography for malignant cervical lymph nodes: A systematic review and meta-analysis. Eur Radiol 2017;27:222-30.
19. Zhang P, Zhang L, Zheng S, Yu C, Xie M, Lv Q. Acoustic radiation force impulse imaging for the differentiation of benign and malignant lymph nodes: A systematic review and meta-analysis. PLoS One 2016;11:e0166716.
20. Ahuja AT, Ying M, Ho SY, Antonio G, Lee YP, King AD, *et al.* Ultrasound of malignant cervical lymph nodes. Cancer Imaging 2008;8:48-56.
21. Ahuja AT, Ying M. Sonographic evaluation of cervical lymph nodes. AJR Am J Roentgenol 2005;184:1691-9.
22. Ying M, Ahuja A, Brook F. Sonographic appearances of cervical lymph nodes: Variations by age and sex. J Clin Ultrasound 2002;30:1-1.
23. Ahuja A, Ying M. An overview of neck node sonography. Invest Radiol 2002;37:333-42.
24. Khanna R, Sharma AD, Khanna S, Kumar M, Shukla RC. Usefulness of ultrasonography for the evaluation of cervical lymphadenopathy. World J Surg Oncol 2011;9:29.
25. Lyshchik A, Higashi T, Asato R, Tanaka S, Ito J, Hiraoka M, *et al.* Cervical lymph node metastases: Diagnosis at sonoelastography – Initial experience. Radiology 2007;243:258-67.
26. Misra D, Panjwani S, Rai S, Misra A, Prabhat M, Gupta P, *et al.* Diagnostic efficacy of color Doppler ultrasound in evaluation of cervical lymphadenopathy. Dent Res J (Isfahan) 2016;13:217-24.
27. Haque MA, Talukder SI. Evaluation of fine needle aspiration cytology (FNAC) of lymph node in mymensingh. Mymensingh Med J 2003;12:33-5.
28. Tan SM, Teh HS, Mancer JF, Poh WT. Improving B mode ultrasound evaluation of breast lesions with real-time ultrasound elastography – A clinical approach. Breast 2008;17:252-7.
29. Ying L, Hou Y, Zheng HM, Lin X, Xie ZL, Hu YP, *et al.* Real-time elastography for the differentiation of benign and malignant superficial lymph nodes: A meta-analysis. Eur J Radiol 2012;81:2576-84.
30. Lenghel LM, Bolboaca SD, Botar-Jid C, Baciut G, Dudea SM. The value of a new score for sonoelastographic differentiation between benign and malignant cervical lymph nodes. Med Ultrason 2012;14:271-7.
31. Fujiwara T, Tomokuni J, Iwanaga K, Ooba S, Haji T. Acoustic radiation force impulse imaging for reactive and malignant/metastatic cervical lymph nodes. Ultrasound Med Biol 2013;39:1178-83.
32. Shah SB, Sharma S, D'Cruz AK. Head and neck oncology: The Indian scenario. South Asian J Cancer 2016;5:104-5.

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Is CT Cisternography in CSF Rhinorrhea Really Antiquated? Clinical Review of 14 Cases with Pictorial Depiction

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ABSTRACT

CSF rhinorrhea is a complex problem due to the intricacy of anatomy of the cranio-facial region and precision required in diagnosis and management. Detection of accurate site of leak is of paramount importance in this era of minimally invasive procedures. CT Cisternography provides us with both the site of leak and visualization of defect. CT Cisternography of late has lost its appeal with advent of Magnetic Resonance Cisternography, which is usually performed in combination with High Resolution Contrast Tomography (HRCT). In our series we pictorially depict various findings of CSF leak on CT Cisternography particularly emphasizing on its role in current scenario.

Keywords: Cerebrospinal fluid (CSF), Computed Tomography (CT), Computed Tomography Cisternography (CT C), High resolution Contrast Tomography (HRCT), Magnetic Resonance Cisterongraphy (MR C)

INTRODUCTION

CSF (Cerebrospinal Fluid) rhinorrhea was first reported as a physiological phenomenon by Galen in 18th century [1]. However, it was in mid-19th century that the complications of CSF leak like meningitis and abscess formation were identified, thus emphasizing the need for intervention [2-4].

An osseous and dural defect in the region of skull base resulting in communication between subarachnoid space and extracranial space usually the paranasal sinuses results in CSF rhinorrhea. CSF rhinorrhea is complex problem due to intricacy of regional anatomy and precision required in diagnosis and management. With advent of minimally invasive surgical technique exact localization is of paramount importance. Imaging modalities for detection of CSF rhinorrhea have evolved over time and include radionuclide study, CT Cisternography (CT C), completely noninvasive Magnetic resonance Cisternography (MR C), Contrast enhanced MR Cisternography and varying combination of these.

CT Cisternography is an imaging modality that involves injection of iodinated contrast into the spinal subarachnoid space and then imaging the cranial subarachnoid space using HRCT. It demonstrates the bony defect and also shows the leak. Of late it has fallen in disrepute due to the advent of MRI cisternography which is noninvasive and comprises of thin section heavily T2W contiguous images. As MRI is inadequate to demonstrate bony defect, it is usually combined with HRCT PNS to detect the bony defect.

We shall herein present a series of 14 cases of CSF Rhinorrhea in whom CT Cisternogram was performed, emphasizing on the current role of CT Cisternogram, along with review of clinical and imaging findings.

MATERIALS & METHODS

We retrospectively searched for cases of CSF rhinorrhea evaluated with CT Cisternography done over the past three years from September 2013 to September 2016 in the Hospital Information System-Picture Archiving and Communication System (HIS-PACS) system of our hospital. After excluding those with inadequate clinical and surgical records we found about 14 patients.

All the patients were evaluated by the otolaryngologist and neurologists of our hospital, the CSF leak was confirmed by clinical and biochemical investigations of nasal discharge.

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Imaging was performed during clinically active CSF leak, as reported by the patient. In all cases, high-resolution CT and contrast-enhanced CT Cisternography were performed on the same day.

CT scan was carried out on 128 slice Somatom definition edge, Siemens Health care, Erlangen., Germany. All the patients initially underwent non-contrast high-resolution CT of paranasal sinuses from the level of hard palate superiorly including the frontal sinuses in cranio-caudal direction using slice thickness of 1 mm. Sub-millimetric reconstructions (0.5 mm) in bone and soft tissue kernels were performed.

Prior to starting the procedures pledgets one in each nostril were introduced. After lumbar puncture, 8-10 mL of an iodinated nonionic low-osmolar contrast agent (iopropamide/iohexol, 300 mg/ml iodine) was instilled into thecal sac. The patients were then placed in knee elbow position with dependent flexed head to provoke CSF leak andcontrast enhanced CT scan was performed with collimation similar to non-contrast CT.

The images were evaluated by two radiologists (7- and 15-years' experience) and discrepancies if any were settled by consensus.

On plain study note was made of site of visible defects, fractures in case of trauma, presence of pneumoencephalous and sinus opacification /extracranial fluid in communication with CSF.

On contrast study an increase in 50% of HU value of the paranasal sinus fluid /extra cranial fluid as compared to non-contrast study [2], a column of contrast extending from subarachnoid space to the extra cranial space (paranasal sinus) or opacification of the nasal pledget was considered positive for leak. Pooling of contrast material at a fracture site with deformity of a local sulcus as described by Fagerlund and Liliequist [5] was considered positive. Additonally, pneumoencephlous in communication with para nasal sinus air was also considered positive for CSF leak.

The demographic data, clinical presentation, treatment details of surgical or conservative treatment were evaluated and tabulated.

RESULTS

We retrospectively evaluated 14 patients who underwent CT Cisternography from the year 2013 to 2016. The medical records, surgical details, investigations and CT Cisternography images were evaluated. Of the 14 patients 9 were females mean age was 45.5 with age ranging from 29 years to 56 years. The procedure was tolerated well in most patients with minor headache controlled by NSAIDS and head rising in most, while the headache was severe in one patient warranting admission for 2 days post procedure. The demographic distribution, imaging and surgical details are as depicted in **Tables 1 and 2**.

All patients presented with typical history of persistent clear nasal discharge, which could not be sniffed back, and which increased on forward bending. The duration of symptoms ranged from 1 day to one and a half year. 2 of 14 patients one with spontaneous leak and the other with post traumatic leak had signs and symptoms suggestive of meningitis. The cause was non traumatic in 11 of 14 patients, while in others the cause was trauma accidental (n=3) or iatrogenic (n=1). Over all the cribriform plate and sphenoid sinuses were equally involved (n=5).

Of the patients with non-traumatic leak, thecribriform plate (n=5/11) was most commonly involved, the next common site was sphenoid sinus (n=4/10). Left cribriform plate was more frequently involved than left (n=3/5). In all patients with leak fromcribriformplate the defect was clearly appreciated and a continuous column of contrast extending from the subarachnoid space to the extra cranial space (intofrontoethmoidal recess) was noted with opacification of nasal swab **(Figure 1)**. One patient had two sites of leak from both the Fovea Ethmoidalis and Cribriform plate **(Figure 2)**, while in the other two no leak or defect could be appreciated. Of the four cases of sphenoid defect, one patient had defect in the inferolateral wall also known as Sternsberg Canal **(Figure 3)** and other three in the posterior wall **(Figure 4)**. These leaks were seen as continuous extension of contrast from subarachnoid space to the sinus **(Figures 2 and 3)**. In one patient significant spontaneous penumoencephalus prevented opacification of subarachnoid space. The defect was easily identified on HRCT with subarachnoid air seen communicating with para nasal sinus air **(Figure 5)**. All the patients with cribriform leak or absence of obvious leak on CT Cisternogram were treated conservatively and it subsided spontaneously, while those involving sphenoid sinuses were treated surgically **(Table 1)**.

The post trauma group of the 3 patients comprised of one iatrogenic leak and two post traumatic leaks. One patient presented immediately post trauma and CT Cisterogram was done within a week of trauma. This patient had fracture of anterior and middle cranial fossa and pneumoencephaolus with extra dural extravasation of contrast reaching upto the fracture with deformity of an adjacent sulcus **(Figure 6)**. This patient was treated with fracture fixation and duroplasty. The other patient has past history of road accident and nasal bleed. He presented with one-and-a-half-year history of CSF rhinorrhea. CT Cisternogram showed multiple defects in frontal sinus and roof of sphenoid and ethmoid sinus. Leak was however seen from the roof of ethmoid and sphenoid sinus as increase in density of sinus fluid by

Table 1. Clinical, imaging and treatment details of all 14 patients.

No.	1	2	3	4	5	6	7
AGE	30	48	43	40	29	56	52
GENDER	M	M	F	M	M	F	M
PRESENTATION	Nasal discharge	Nasal discharge	Retroviral positive, meningitis	Hypotensive headache, discharge	Left nasal discharge	Right nasal discharge	Nasal discharge
DURATION	4mths	2mths	15days	1week	18mths	1 mth	15days
CAUSE	Non traumatic	Non traumatic	Non traumatic	Traumatic	Delayed Traumatic	Non traumatic	Non -Traumatic
SITE OF DEFECT ON PLAIN	None	None	Right Cribriform plate	1) Multiple fractures of inner table of left frontal sinuses 2) Left Cribriform plate	1) Left frontal sinus encephalocele 2) Roof of posterior ethmoid 3) Sphenoid air cells	Posterior wall of sphenoid sinus	Posterior wall of sphenoid
SITE OF LEAK ON CTC	None	None	Right Cribriform plate	Distorted sulcus sign near fracture of left frontal sinus inner table	Leak from ethmoid and sphenoid roof defect, increase in density of	Leak into sphenoid sinus, sinus opacification	Spontaneous pneumoencephalous in continuity with sphenoid sinus air through defect in posterior sphenoid wall, no opacification of
PROCEDURAL COMPLICATION	Persistent headache required admission	None	None	None	None	None	None
TREATMENT	Lost to follow-up	Conservative	Conservative	Fixation of fracture with duroplasty and exteriorisation of frontal air cells.	Endoscopic endonasal CSF leak repair with fascia lata	Endoscopic repair, meningocele cauterised, fat graft from thigh, middle turbinate	Fascialata graft, endoscopic repair

8	54	F	Meningitis , Nasal discharge	6days	Non traumatic	Right cribriform plate	Right Cribriform plate	None	Conservative	
9	54	F	Nasal discharge post-surgery	2days	Traumatic, Iatrogenic	1)Right lateral wall sphenoid sinus with adjacent sinus opacification 2) Right	Opacification of right mastoid air cells and Eustachian tube	None	Conservative	
10	38	F	Nasal discharge	6mths	Non traumatic	Right inferolateral sphenoid sinus defect	Leak into the sphenoid sinus via inferolateral wall defect	None	Fascialata graft +middle turbinate graft,	
11	50	F	Nasal discharge	15days	Non traumatic	Posterior wall sphenoid	Leak into the sphenoid sinus via posterior wall defect	None	Endoscopic transphenoidal surgery recommended/ patient not	
12	49	F	Nasal discharge	20days	Non traumatic	Left Cribriform plate	Left Cribriform plate	None	Conservative	
13	38	F	Nasal discharge	20days	Non traumatic	Left Cribriform plate	Left Cribriform plate	None	Conservative	
14	56	F	Nasal discharge	1mth	Non traumatic	1) Left cribriform plate 2) Left fovea ethmoidals	1) Left cribriform plate 2) Left fovea ethmoidali	None	Conservative	

M: Male, F: Female, mths : Months

more than 50% **(Figure 7)**. Frontal sinus encephalocele was also noted in this patient **(Figure 7)**. This leak was also surgically treated. We had one patient of iatrogenic cause of CSF rhinorrhea post microsurgery for trigeminal neuralgia. In this patient there was a right occipito-mastoid defect seen with extravasation of contrast into the defect and mastoid aircells, further extension into the middle ear and subsequently into the Eustachian tube and right nasopharynx were seen **(Figure 8)**. In this patient another defect was appreciated in the right lateral wall of sphenoid sinus with adjacent sinus opacification **(Figure 8)**.

DISCUSSION

CSF Rhinorrhea was classified by Oyammainto two groups traumatic (including iatrogenic) and non-traumatic [6]. He further subdivided the latter into nontraumatic with normal pressure and nontraumatic with CSF hypertension. The high-pressure leaks were those in whom the leak was secondary to congenital defects, infections or tumors resulting in raised intracranial tension [6,7]. While the other category that is low pressure leak is now identified as spontaneous leak and is thought to be a manifestation of benign intracranial hypertension considering its association with empty sella [7].

In literature few authors report traumatic CSF leak to be most common [8,9]. However, in our study we found the non-traumatic category to be common, which is consistent with research findings [10,11]. However, this could be because of the fact that plain HRCT is usually done for patients with significant head injury usually

Table 2. Etiology and site of leak.

	n
Females	9/14
Mean age (range)	45.5yrs (29-56yrs)
Site of leak Cribriform Sphenoid Fovea ethmoidalis Occpitomastoid Frontal	 6 5 1 1 1
Cause Traumatic Non traumatic	 3 11
Multiple defects	4/14
Multiple leaks	2/14
No leak no defect/sinus Opacification	2/14
No leak only defect present	1/14
Post procedure complication	1/14
Surgical treatment done/suggested	6/14

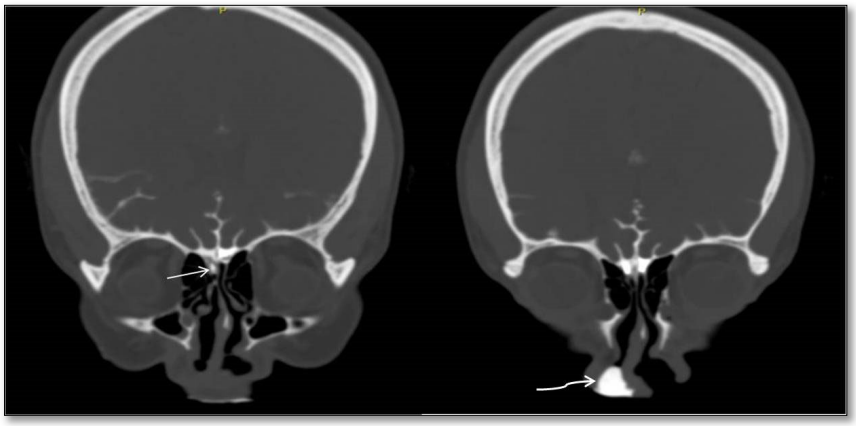


Figure 1. Linear column of contrast extending from the subarchnoid space through defect in right cribriform plate into the superior meatus (right frontoethmoidal recess) (white arrow).

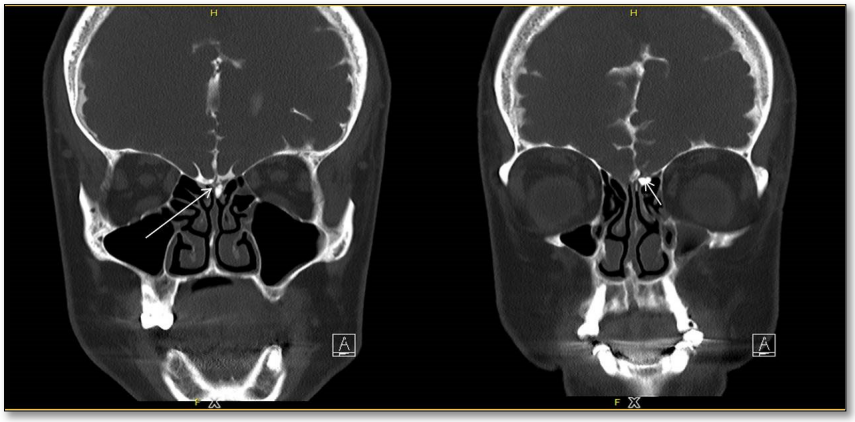


Figure 2. Two defects in Left cribriform plate (Long arrow) and left fovea ethmoidalis (Short arrow).

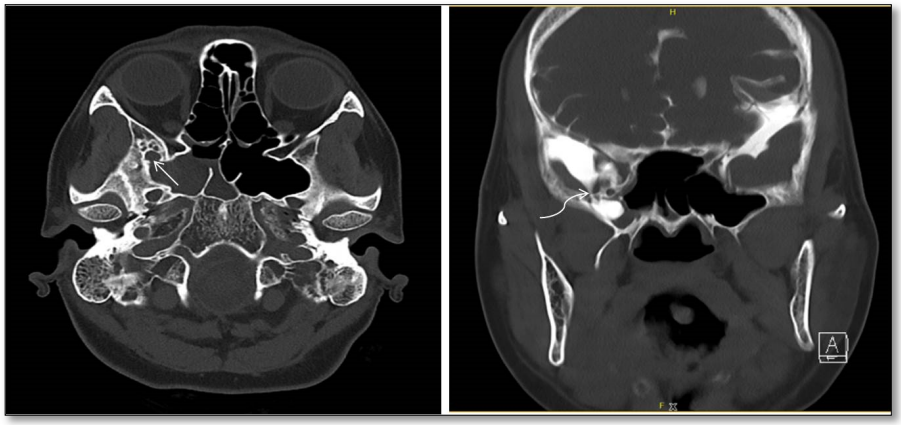


Figure 3. Defect measuring 3.5mm noted in the inferolateral wall of sphenoid sinus on the right side with minimal thinning of adjacent greater wing of sphenoid Sternbergs canal – persistent lateral cranio pharyngeal canal connecting intracranial space and sphenoid sinus due to congenital fusion defect between basisphenoid and greater wing of sphenoid .Contrast leak is seen through the defect from the base of right middle cranial fossa,pooling at the right sphenoid sinus (curved arrow).

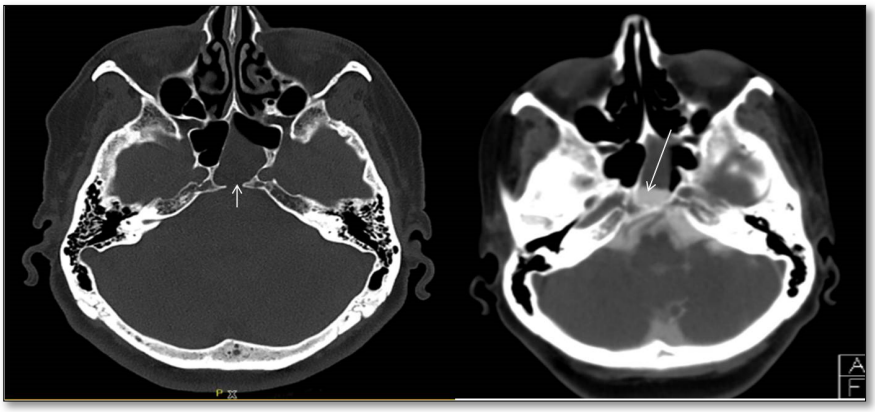


Figure 4. 5mm bony defect (short arrow) at that level in the posterior wall of left sphenoid sinus just above the clivus with CSF leak through defect. into sphenoid sinus (long arrow).

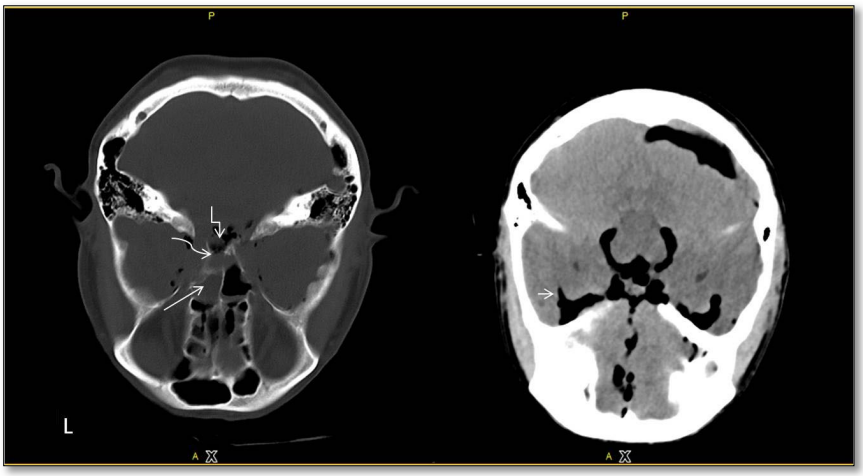


Figure 5. Extensive spontaneous pneumocephalus (short arrow) with fluid level in left sphenoid sinus (long arrow) with 6mm defect in the posterior wall of the sphenoid sinus above the clivus (curved arrow) with air pocket in the prepontine subarachnoid space communicating with the sphenoid sinus (elbow arrow) .No demonstrable flow of contrast from spinal to the brain subarachnoid space.

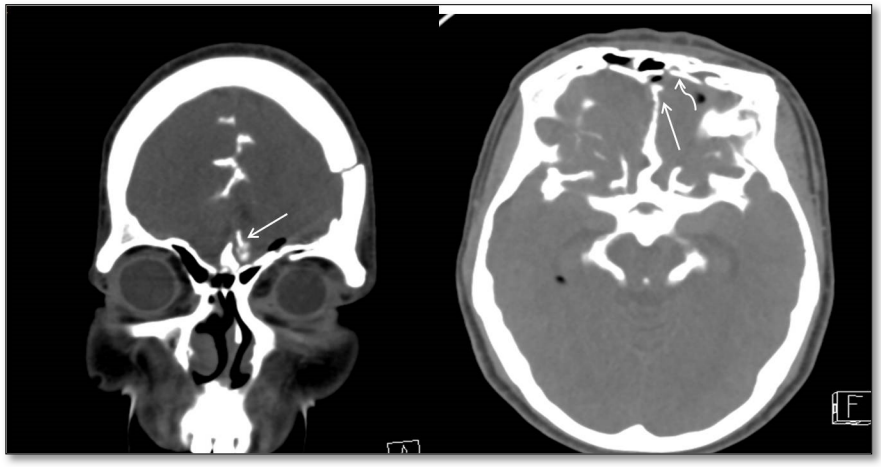


Figure 6. Contrast seen extending upto the fractures (curved arrow) in the inner table of left frontal sinus (straight arrow), distorted sulcus (straight arrow) with small pneumocephalus (curved arrow).

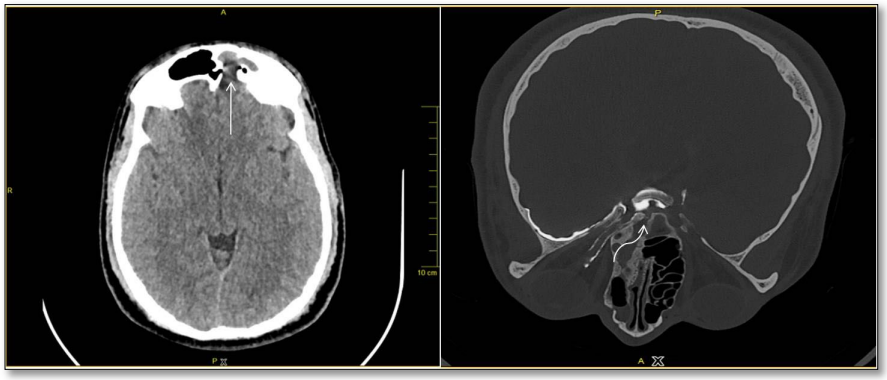


Figure 7. Defect in the inner table of left frontal sinus with herniation of brain tissue (straight arrow) but cisternogram showing CSF leak in the defect in the roof of the left sphenoid sinus and left posterior ethmoid sinus (curved arrow).



Figure 8. Post-surgery Right occipito-mastoid defect (long arrow), opacification of mastoid air cells and Eustachain tube (short arrow,defect in right lateral wall of sphenoid (curved arrow).

immediately after trauma, it usually suffices to detail the site of fractures in such cases and CT Cisternography is not feasible in acute settings.

The commonest site of leak was cribriform plate followed by sphenoid bone which was similar to other findings [8]. This is probably due to the fact that cribriform plate is thinnest and weakest portion of anterior cranial fossa [8,12]. All patients had typical findings of persistent nasal discharge which could not be sniffed back and increased on forward bending. Only two patients presented with meningitis, while hypotensive headache was present in all. Meningitis in the two cases was probably the reason of CSF rhinorrhea. Raised intracranial tension due to meningitis

was probably the cause for CSF leak in these patients rather than meningitis being a complication CSF leak.

Patients with CSF rhinorrhea pose a diagnostic challenge for both the otolaryngologists and radiologists. In this era of minimally invasive procedures where small skull base defects are routinely repaired using nasal endoscopes having limited field of view for, accurate localization of the leak is mandatory [3,8]. Also, symptoms in many patients with CSF leak resolve spontaneously or with conservative management [8]. Hence, we see that decision regarding management depends on the location and etiology. Plethora of imaging modalities have been described and discarded in an attempt to find the gold standard. The studies that are available for evaluation of CSF rhinorrhea are radionuclide study, HRCT alone, HRCT with CT cisternography, MRI cisternography with HRCT PNS and recently described CEMR cisternography. Of these the radionuclide study owing to its extremely low specificity is considered obsolete and is reserved only in cases where the diagnosis itself is in question [2,13]. There are few studies which say that HRCT alone suffices [14], but more than one site of leak and more than one defect are not rare. CT Cisternography has overtime undergone a drastic revolution due to the thin slice capabilities of current scanners and use of water-soluble contrast. It combines best of both worlds, that is it provides excellent localization of osseous defect and also shows the actual extra-cranial pooling of contrast. Of late it has lost its appeal with advent of MRI as it is slightly invasive in terms of requirement of lumbar puncture, patient discomfort in the form of post procedural headache, theoretical possibility of opening up of the defects, introducing infection, lumbar CSF leak and its low sensitivity in detecting the site in inactive leaks [3,15-17]. At present the most recommended modality is MRI Cisternography, HRCT PNS or combination of both which are non-invasive and quick [3,17]. In most of our patients we were able to isolate the site of leak with only mild patient discomfort. Post procedure complication rate was low and only one patient developed significant headache requiring admission.

We found that in patients who presented with recent trauma had multiple fractures on HRCT and pneumocephalus, which could interfere with visualization of leak on MRI. While with Cisternography though we could not see leakage into sinus of leak we could infer the possible site of leak using the distorted sulcal sign. Similarly, in a post-traumatic leak presenting late we found that the site of leak was different from the site of obvious defect or site of sinus opacification. In our only case of iatrogenic CSF leak post otomastoidectomy we additionally found a small defect in lateral wall of sphenoid sinus with adjacent sphenoid sinus opacification. However, on cisternography we found the leak to be into the mastoid air cells and subsequently into the naspharynx via Eustachian tube.

In non-traumatic category we found congenital defects in few patients. Sternberg's *Canal* which represents persistent lateral cranio-pharyngeal canal connecting intracranial space and sphenoid sinus due to congenital fusion defect between basi-sphenoid and greater wing of sphenoid was seen in one patient [18]. Additionally, defects in posterior wall were seen in three patients which requires surgical intervention as the sizes of defect were significant. In one patient placement of patient in knee elbow position probably induced spontaneous pneumocephalous, which prevented opacification of intracranial subarachnoid space. However, we could infer the site of leak as the subarachnoid air was seen in communication with sinus air through defect in posterior wall of sphenoid sinus. Other patients in non-traumatic group had leak from cribriform plate (seen as a column of contrast extending from subarachnoid space to extracranial space) and were treated conservatively. Multiple sites of leak from cribriform plate and fovea ethmoidalis were noted in one patient. In two patients no defects or leak could be appreciated probably due to inactivity of the leak during procedure.

Thus, in patients in both traumatic and non-traumatic categories wherein multiple defects, multiple sites of leak are anticipated CT Cisternogram provides concrete evidence regarding site of leak.

The major limitation of our study was lack of MRI for all patients due to which comparative analysis was not possible. Most of the post trauma patients rarely undergo CT Cisterography in acute settings and hence were not included in the study.

CONCLUSION

In conclusion, despite many limitations of CT Cisternography, it still holds importance as a problem-solving tool for detection of exact site of leak in patients where multiple sites of leak or multiple defects are suspected. Additionally, we have demonstrated spectrum of possible imaging findings of CSF leak on CT Cisternography.

REFERENCES

1. Hess JM, Jonnalagadda SS, Slavin JL (2016) Dairy foods: Current evidence of their effects on bone, cardiometabolic, cognitive and digestive health. Compr Rev Food Sci Food Saf 15: 251-268.

2. Zlab MK, Moore GF, Daly DT, Yonkers AJ (1992) Cerebrospinal fluid rhinorrhea: A review of the literature. Ear Nose Throat J 71: 314-317.

3. Stone JA, Castillo M, Neelon B, Mukherji SK (1999) Evaluation of CSF leaks: High-resolution CT compared with contrast-enhanced CT and radionuclide cisternography. AJNR Am J Neuroradiol 20: 706-712.

4. Shetty PG, Shroff MM, Sahani DV, Kirtane MV (1998) Evaluation of high-resolution CT and MR cisternography in the diagnosis of cerebrospinal fluid fistula. AJNR Am J Neuroradiol 19: 633-639.

5. Eljamel MS, Fay PM (1990) Acute traumatic Cerebrospinal fluid rhinorrhoea: The risk of intracranial infection. Brit J Neurosurg 11: 205-207.

6. Fagerlund M, Lilequist B (1987) Intermittent cerebrospinal liquorrhea: Cerebral computed tomography in the non-drop period. Acta Radiol 28: 189-192.

7. Ommaya AK, Di Chiro G, Baldwin M, Pennybacker JB (1968) Non-traumatic cerebrospinal fluid rhinorrhoea. J Neurol Neurosurg Psychiatr 31(3): 214-225.

8. Schuknecht B, Simmen D, Briner HR, Holzmann D (2008) Nontraumatic skull base defects with spontaneous CSF rhinorrhoea and arachnoid herniation: Imaging findings and correlation with endoscopic sinus surgery in 27 patients. Am J Neuroradiol 29: 542-549.

9. Kirtane MV, Gautham K, Upadhyaya SR (2005) Endoscopic CSF rhinorrhoea closure: Our experience in 267 cases. Otolaryngol Head Neck Surg 132: 208-212.

10. Sannareddy RR, Rambabu K, Kumar VE, Gnana RB, Ranjan A (2014) Endoscopic management of CSF rhinorrhea. Neurol India 62: 532-539.

11. Hegazy HM, Carrau RL, Snyderman CH, Kassam A, Zweig J (2000) Transnasal endoscopic repair of cerebrospinal fluid rhinorrhea: A meta-analysis. Laryngoscope 110: 1166-1172.

12. Psaltis AJ, Schlosser RJ, Banks CA, Yawn J, Soler ZM (2012) A systematic review of the endoscopic repair of cerebrospinal fluid leaks. Otolaryngol Head Neck Surg 147: 196-203.

13. Lloyd KM, DelGaudio JM, Hudgins PAP (2008) Imaging of skull base cerebrospinal fluid leaks in adults. Radiology 248: 725-736.

14. Mathias T, Levy J, Fatakia A, McCoul ED (2016) Contemporary approach to the diagnosis and management of cerebrospinal fluid rhinorrhea. Ochsner J 16: 136-142.

15. Lloyd MN, Kimber PM, Burrows EH (1994) Post-traumatic cerebrospinal fluid rhinorrhoea: Modern high-definition computed tomography is all that is required for the effective demonstration of the site of leakage. Clin Radiol 49: 100-103.

16. Schuknecht B, Simmen D, Briner HR, Holzmann D (2008) Nontraumatic skull base defects with spontaneous CSF rhinorrhoea and arachnoid herniation: Imaging findings and correlation with endoscopic sinus surgery in 27 patients. Am J Neuroradiol 29: 542-549.

17. Colquhoun IR (1993) CT cisternography in the investigation of cerebrospinal fluid rhinorrhoea. Clin Radiol 47: 403-408.

18. Mostafa BE, Khafagi A (2004) Combined HRCT and MRI in the Detection of CSF Rhinorrhea. Skull Base 14: 157-162.

19. Bendersky DC, Landriel FA, Ajler PM, Hem SM, Carrizo AG (2011) Sternberg's canal as a cause of encephalocele within the lateral recess of the sphenoid sinus: A report of two cases. Surg Neurol Int 2: 171.

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Original Article

Radiology Section

Qualitative and Quantitative Assessment of Enhancement and Washout Characteristics of HCC and non HCC Hypervascular Liver Tumours

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Introduction: The image based diagnosis of Hepatocellular Carcinoma (HCC) relies on assessment of arterial enhancement and venous or delayed phase washout. This assessment is based on visual analysis; however, studies quantifying such enhancement and washout are lacking.

Aim: To qualitative assessment of the imaging characteristics of HCC and non HCC hypervascular liver tumours followed by quantitative assessment of the enhancement and washout kinetics, and also to derive objective values that may help in differentiating HCC from non HCC on Computed Tomography (CT).

Materials and Methods: The present study was carried out in PSG hospitals, Coimbatore, Tamil Nadu, India. Retrospective analysis of hypervascular liver lesions was performed over a study period of four years (January 2012 to February 2016). These lesions were divided into two groups namely; HCC and non HCC. All non HCCs were pathologically proven, while HCC cases included those diagnosed on histopathology or typical imaging findings based on American Association for the Study of Liver Diseases (AASLD) (2010) and Liver Imaging Reporting and Data Systems (LIRADS) (2014) diagnostic criteria along with an elevated Alpha Fetoprotein (AFP) of more than 400 IU/mL.

A qualitative analysis of the enhancement and washout patterns was done followed by quantitative analysis using Regions of Interest (ROI) measurement of attenuation in Hounsfield units (HU). The quantitative parameters assessed were; Mean Attenuation (MA) of lesions in different phases, Attenuation Gain Percentage (AGP) on arterial phase, Arterial Phase Attenuation Change (AAC), Attenuation Loss Percentage in venous and delayed phases (ALP-V, ALP-D), Percentage Attenuation Ratio in venous and delayed phases (PAR-V, PAR-D) and Relative Washout Ratios from arterial to venous/delayed (RWR A-V, RWR A-D) and from venous to delayed phases (RWR V-D). Sensitivity and specificity were calculated for qualitative washout.

For quantitative variables comparative analysis between HCCs and non HCCs was done using Student's t-test. ANOVA was used to compare different categories. ROC curves were drawn for values that were significantly different to establish a cutoff value.

Results: A total of 120 lesions were evaluated consisting of HCCs (n=88) and non HCCs (n=32). Qualitatively majority (81) of HCCs demonstrated arterial enhancement and washout with eight cases of these demonstrating subtle washout requiring settlement by consensus. Washout were absent in seven cases. True mimics of HCC on qualitative analysis were hepatic adenomas, few cases of metastasis and hepatoblastoma were also there. The overall sensitivity of the washout appearance for predicting HCC was 92% while specificity was 78%. On quantitative analysis a PAR-V≥106 has sensitivity of 70% and specificity of 75%, RWR-V-D≥41.79 has sensitivity of 75% and specificity of 70% for HCC detection. Additionally significant difference was seen between RWR V-D of HCC when compared to cholangiocarcinomas (p-value 0.035) and metastasis (p-value 0.026) and also between PAR-V of HCC and metastasis (p-value 0.033).

Conclusion: The qualitative analysis combined with quantitative assessment of washout using percentage attenuation ratio in venous phase and relative washout ratio from venous to delayed phase offers good sensitivity and specificity in image based diagnosis of HCC.

Keywords: Hepatocellular carcinoma, Percentage attenuation ratio, Relative washout ratio

INTRODUCTION

The diagnosis of HCC on imaging relies on subjective assessment of arterial enhancement and venous or delayed phase washout. The arterial phase enhancement is attributed to an increase in intralesional flow with relative reduction in portal flow which occurs as the lesion progresses from dysplastic nodule to HCC [1,2]. Venous or delayed phase washout is decrease in attenuation of the lesion relative to background liver. Factors attributed to washout include early drainage of contrast material as compared to parenchyma and retention of contrast by surrounding fibrotic parenchyma [1-3]. There is ample literature on arterial enhancing mimics of HCC [1,3-7]. Arterial enhancing metastases, cholangiocarcinomas are known, similarly washout, a hallmark of HCC has been described in other tumours [5-7]. According to some studies Intrahepatic Mass-forming Cholangiocarcinoma (IMCC) showing arterial enhancement, was the most common lesion misinterpreted as HCC in preliver transplant imaging work up in recipients [5,9]. In cirrhotic livers lesions like regenerative nodules, dysplastic nodules, arterioportal or venous shunts and confluent fibrosis may also mimic this appearance [2,5-9]. Owing to typical enhancement characteristics of HCC, radiological imaging plays a crucial role in diagnosis and this combined with certain clinical and biochemical characteristics have been adopted as practice guideline for diagnosing HCC over the world. Differentiating HCC from other hypervascular tumours is important since HCC has therapeutic implications and prognostic factors which are very different from other lesions [2,8,10].

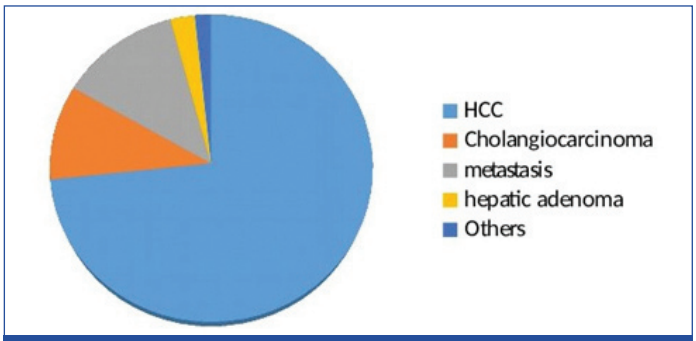
The assessment for hypervascularity of tumours and washout or persistent enhancement relies heavily on radiologist's subjective visual assessment. Only few studies have attempted to quantitate these variables and define the washout phenomenon [2,10]. In one

study, parameters such as relative contrast washout ratio between the arterial phase and delayed phase and attenuation ratio of the lesion to the background liver were studied and the attenuation ratio of the lesion to the background liver in the delayed phase was found to have excellent sensitivity and specificity for HCC detection and good correlation with subjective assessment [2]. In yet another study, an attenuation difference of greater than 10 HU between the arterial and delayed phase yielded optimal sensitivity and specificity for detecting HCC [10]. Certain studies have also quantitatively assessed the washout pattern on Magnetic Resonance Imaging (MRI), with a study evaluating the signal to noise ratio and liver to lesion contrast [11]. Overall the available literature is limited in this aspect with variable results. In present study, we analysed a large retrospective cohort of hypervascular liver tumours which were consecutively diagnosed over a period of four years in our hospital. We performed a qualitative assessment of the imaging characteristics of HCC and non HCC hypervascular liver tumours followed by quantitative assessment of the enhancement, washout kinetics and derived objective values that help in differentiating HCC from non HCC on CT. To the best of our knowledge this is the first such Indian cohort of hypervascular liver tumours being subjected to such qualitative and quantitative assessment.

MATERIALS AND METHODS

Institutional Ethics Committee approval was obtained and requirement for informed consent was waived after considering the retrospective nature of study. An extensive search of our Hospital Information System/Picture Archiving And Communication System (HIS/PACS-Vepro Medical Systems) for liver lesions on CT using keywords Hepatic, liver lesion, hepatic lesion, space occupying lesion, mass, HCC, Hepatocellular carcinoma and each of the individual common malignant and benign liver lesions revealed a total of 389 patients from January 2012 to February 2016 (four years duration). All Multidetector Computed Tomography (MDCT) examinations were performed on 64 slice MDCT (Somatom 64, Seimens healthcare) or 128 slice (Somatom Definition edge, Siemens healthcare) helical scanners. All studies were carried out in craniocaudal direction with patient supine with average 120 KV and 250-450 mA, values varying depending on patients build. Unenhanced images were obtained followed by administration of IV contrast (Iopromide 350-370 mg/mL), power injector was used for injection of contrast at a rate of 4 mL/sec. Automated bolus tracking was used. Images were obtained in arterial, venous and delayed phases, typically at 18-28 seconds, 55-65 seconds and 300 seconds after initiation of contrast administration.

Hypervascular tumours on arterial phase were studied; thus, all hypo or avascular lesions were excluded. Hypodense lesions with thin peripheral enhancement, the pattern which is most common in metastatic lesions, were also excluded. The other exclusion criteria were: i) HCCs without histopathology and not meeting the criteria mentioned below in confirmation of tumor subtype, ii) Diffusely infiltrative HCCs as enhancement pattern and washout characteristics are difficult to assess in them, iii) HCCs with changes related to treatments like thermal ablation or transarterial chemoembolisation, iv) Non HCCs without definitive histological diagnosis, v) Very small tumours less than one cm in size as placing ROI for measurements is difficult in them, vi) Benign lesions like hemangiomas, abscess and cysts were also excluded as they show fairly typical enhancement patterns and in most no histopathological confirmation is done. Histopathological confirmation was available for all non HCCs and HCC (35 cases). 53 HCCs, all in at risk population having underlying cirrhosis were diagnosed based on AASLD guidelines (2010) and LI-RADS (version 2014). First AASLD criteria was applied (a lesion of more than 2 cm showing arterial phase hyperenhancement and subsequent washout, in presence of cirrhosis and elevated AFP levels, we used AFP cutoff of >400 IU/mL to enhance specificity), tumours meeting these criteria are virtually diagnostic of HCC [13]. All of these 53 cases were LI-RADS 5 category (indicating 100% certainty of HCC), with 34 cases being LR-5, 17 cases LR-5V with macrovascular invasion and two cases being LR-5us (being 1-2 cm in size and being visible as discrete nodules on ultrasound) [14]. Two HCCs were histological variants, one a fibrolamellar HCC and another cholangiocellular variant. After application of exclusion and inclusion criteria we arrived at a study population of 120 patients (86 males and 34 females) mean age 58.5 years, range (3-78 years). There were 88 (73%) HCCs, 12 (10%) mass forming cholangiocarcinomas, 15 (12.5%) metastasis, 3 (2%) adenomas, 1 (0.08%) hepatoblastoma and 1 (0.08%) angiosarcoma [Table/Fig-1]. For purpose of comparability the tumours were divided in two broad groups of HCC and non HCC.

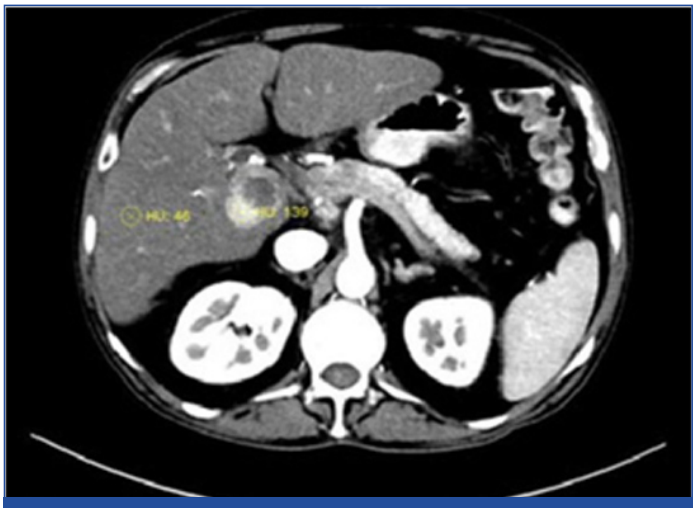


[Table/Fig-1]: Distribution of tumours.

A subjective or qualitative assessment was performed by two radiologists; having nine and five years of experience in abdominal imaging. After confirming the hypervascular nature of the tumour on the arterial phase assessment of subjective washout was done and presence or absence of washout on venous or delayed phases was noted. Presence of any delayed hyperenhancing central stroma was also noted. The examining radiologists were blinded to the results of histopathology and final diagnosis. Discrepancies if any were settled by consensus.

The quantitative analysis was done by one radiologist. The attenuation were measured in HU by drawing circular or oval ROI over the lesion. The ROIs were drawn to include maximally enhancing areas on arterial phase and ranged from 10 to 20 mm with similar ROI drawn on corresponding venous and delayed phase images. The necrotic areas of the tumour, major intratumoral feeding vessels or intratumoral arterioportal shunts were excluded [Table/Fig-2]. In case of large tumours with variable areas of enhancement, multiple ROIs were drawn in the enhancing regions of the tumour and averaged. Two ROIs were also obtained in the adjacent normal background liver in all the phases, taking care to avoid blood vessels. In case of multiple lesions, largest lesion was selected.

The following quantitative indices were calculated 1) Mean attenuation(MA) of lesions in different phases 2) Attenuation gain



[Table/Fig-2]: Hepatocellular carcinoma in caudate lobe with an eccentric necrotic component, ROI placement was done in the enhancing tumour component .

percentage on arterial phase (AGP): SOL a-BL a / BL a x100), Arterial phase attenuation change (AAC): SOL a –BL a (these indices assess the arterial enhancement of the lesions). 3) Attenuation loss percentage in venous and delayed phases (ALP-V, ALP-D): BL v/d-SOL v/d/ BL v/d x100; Percentage attenuation ratio in venous and delayed phases (PAR-V, PAR-D): 100x BL v/d/SOL v/d; Relative wash out ratio (RWR A-V, RWR A-D) from arterial to venous and delayed phases: 100x (SOL a-SOL v/d)/SOL a and from venous to delayed phases(RWR V-D): 100 x (SOLv-SOL d)/ SOL v.(these variables define the washout characteristics). (SOL a and BL a were attenuation of space occupying lesion and background liver attenuation on arterial phase respectively, SOL v/d and BL v/d were lesion and background liver attenuation on venous and delayed phases).

STATISTICAL ANALYSIS

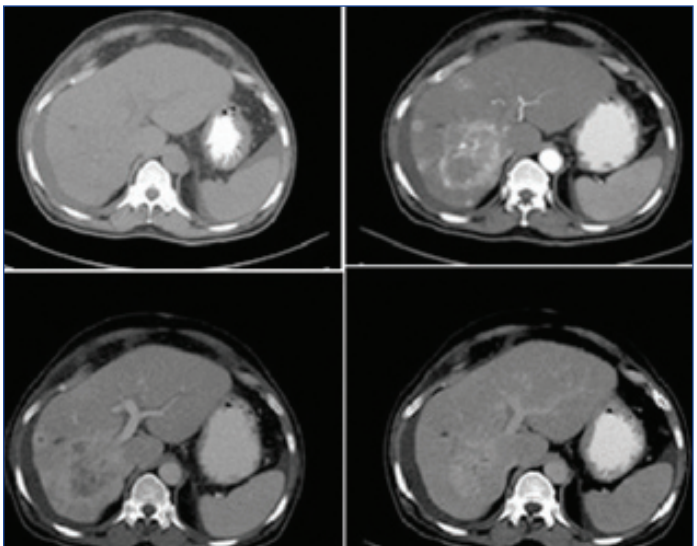
Statistical analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). Sensitivity and specificity for washout appearance were calculated. For quantitative variables comparative analysis between HCCs and non HCCs was done using Student's t-test. ANOVA analysis was used for comparison between HCC and individual categories in Non HCC (cholangiocarcinomas, metastasis and others). ROC curves were drawn for values that were significantly different to establish a cutoff value.

RESULTS

The demographic and qualitative characteristics of the HCC and non HCC are as in [Table/Fig-3]. On qualitative analysis all HCCs showed homogenous or patchy enhancement on arterial phase. There was excellent interobserver agreement between the two radiologists for hypervascularity (Kappa value of 0.91) and presence of washout (Kappa value of 0.86) and discrepancies if any, were settled by consensus. Washout was noted on venous phase in 62/88 (69.3%) patients while on delayed phase additional 19 patients showed washout (81/88,92%). Overall 92% HCCs demonstrated washout in either venous or delayed phase. There was lack of subjective washout as perceived by both radiologists in 7/88 (8%) tumours [Table/Fig-4]. Of these however, two tumours did demonstrate a difference in quantitative washout if absolute values of HU obtained from ROI between the lesion and background liver were considered (though the difference was less than 10 HU). In addition in 8 (9%) tumours the washout was perceived as subtle and required either extensive window setting adjustments (in five cases) or settlement by consensus (three cases). The confounding factors encountered affecting visual assessment were presence of 1) steatosis in the background liver with lesion appearing hyperdense relative to the surrounding fatty liver in two cases, 2) presence of enhancing peripheral pseudocapusle in five cases and a delayed enhancing central fibrous stroma (noted in one case of fibrolamellar HCC). The metastases were the commonest non HCC lesion. Most of the hypervascular metastases were from adenocarcinomas with known or unknown primary sites. A qualitative washout appearance similar to HCC was noted in three cases (20%). Amongst the carcinoid metastasis or neuroendocrine tumours in present study sparing one which washed out the other two were hyper or isodense to parenchyma. Both cases of sarcoma metastasis (n=2) had peripheral enhancement, while washout was seen in one, the other was seen filling in from periphery to the centre. Thus, most of metastatic lesions showed no washout in the venous or delayed phase except one case of transitional cell carcinoma, one of neuroendocrine tumour and one sarcoma. Most of the adenocarcinoma metastases were isodense to parenchyma on delayed phase. The metastasis from gastrointestinal stromal tumour had peripheral fill in pattern.

	HCC	Non HCC	p-value
Females	21	13	0.107
Mean age (years)	63.8	54.03	0.000
Size <3 cm	18 (20.45%)	3 (9.37%)	0.1868
Mean size (cm)	6.55±4.17	7.00±3.7	0.5916
Washout present (%)	81 (92%)	7 (21.8%)	0.001

[Table/Fig-3]: Demographic and qualitative parameters of HCC and Non HCC. The difference is significant at p<0.05
HCC*- Hepatocellular carcinoma



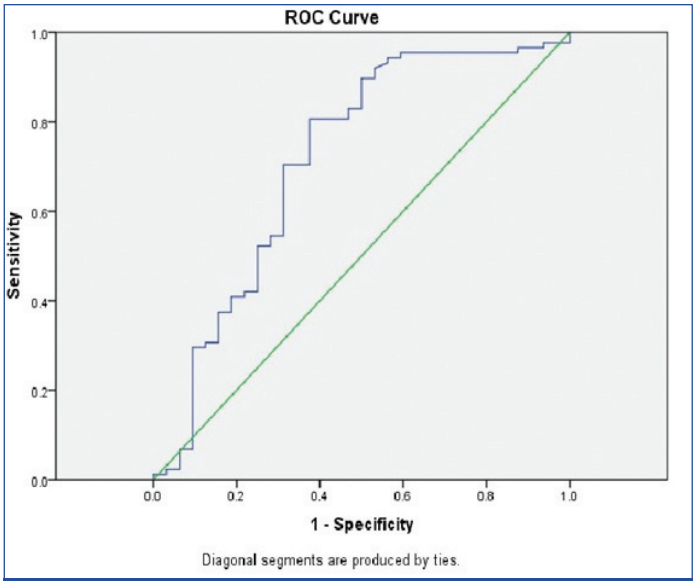
[Table/Fig-4]: Histopathologically proven hepatocelluar carcinoma, multinodular in alcohol related cirrhosis, showing progressive enhancement pattern with lack of subjective or quantitative washout.

Amongst cholangiocarcinomas, none demonstrated washout on delayed phase, all the lesions appeared isodense, hyperdense or showed central enhancing stroma in the delayed phase. The true HCC mimics on qualitative analysis were the three cases of hepatic adenomas and one case of hepatoblastoma which had an enhancement and washout appearance similar to HCC. The only case of angiosarcoma showed hyperdense appearance on delayed phase. Thus, hepatic adenomas, few hypervascular non adenocarcinoma metastasis and hepatoblastoma showed washout appearance in venous or delayed phase; thus, mimicking HCC. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%.

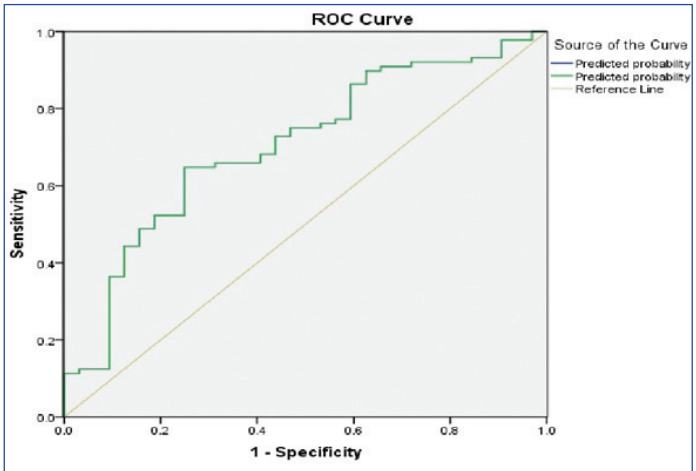
The difference between the mean attenuation of the HCCs and non HCCs was significant in noncontrast (p-value 0.01), arterial (p-value 0.002) and delayed phases (p-value 0.013), while the difference was not significant in the venous phase (p-value 0.614). The quantitative indices are as listed in [Table/Fig-5]. There was no significant difference between the two groups in attenuation change in arterial phase (AAC), while AGP was significant (p-value 0.018). Of the washout parameters, in venous phase significant difference (p-value 0.002) was found in PAR-V in venous phases with no significant difference seen between the two groups in PAR-D. The washout parameters assessed were relative washout from arterial to venous or delayed phase (RWR A-V, A-D) and from venous to delayed phase (RWR-V-D). The relative washout from arterial to venous or delayed phases was (RWR A-V,A-D) was found to be significant (p-value 0.022, 0.004). Washout from venous to delayed phase (RWR-V-D) was also significant between the two groups (p-value 0.000). ALP-V that is attenuation loss percentage in venous phase was also significant (p-value 0.001). However, area under curve was significant for RWR V-D and PAR-V only (r-value >0.7) and analysis of ROC curves for PAR-V and RWR-V-D revealed good sensitivity and specificity for detection of HCC [Table/Fig-6,7].

	HCC (Mean)	Non HCC (Mean)	p-value
MA-NCCT	66.31	52.71	0.01
MA-Arterial	105.96	89.8	0.002
MA-Venous	89.34	91.73	0.614
MA-Delayed	51.75	63.09	0.013
AAC	13.5	2.46	0.070
AGP	27.9	7.17	0.018
PAR-V	116.29	101.89	0.002
PAR-D	117.33	116.09	0.810
RWR A-V	9.3	-6.6	0.022
RWR A-D	45.05	25.6	0.004
RWR V-D	43.27	31.38	<0.001
ALP-V	10.83	-1.16	0.001
ALP-D	12.01	9.73	0.487

[Table/Fig-5]: Details of quantitative variables assessed.
MA-NCCT*-Mean Attenuation Non Contrast CT
AAC*- Arterial phase attenuation change, AGP*- Attenuation gain percentage, PAR-V*- Percentage attenuation ratio-venous phase, PAR-D*- Percentage attenuation ratio-delayed phase, RWR A-V*- Relative washout ratios arterial-ratio, RWR A-D*- Relative washout ratios arterial-delayed, RWR V-D*- Relative washout ratios venous-phase, PLP-V*- Attenuation loss percentage-venous phase, ALP-D*- Attenuation loss percentage-delayed phase



[Table/Fig-6]: Receiver operating curve (ROC) for RWR-V-D, area under curve >0.7



[Table/Fig-7]: Receiver operating curve (ROC) for PAR-V, area under curve >0.7

On individual comparison of HCC with metastasis, cholangiocarcinoma and others category, significant difference was seen between RWR V-D of HCC (mean 43.27) when compared to cholangiocarcinomas (mean 31.2, p value 0.035) and metastasis (mean 33.63, p value 0.026).

DISCUSSION

Studies show that combination of arterial hypervascularity and washout appearance of HCC on MDCT offers a specificity approaching 100% for HCC >20 mm in at risk populations. According to the AASLD guidelines (2010) a lesion of more than 10 mm showing arterial phase hyperenhancement and subsequent washout, in presence of cirrhosis can be considered as HCC and treated as such. Similar guidelines have been proposed by European Association for the Study of the Liver (EASL) in 2012 [13-15]. More recently LIRADS (2014) has been developed for categorising liver lesions in at risk population; this classification aims to facilitate clear communication between different personnel involved in care of these patients. The lesions falling in LR-5 category are nearly certain to be HCCs and consists of hypervascular lesions of more than 2 cm with one or more combinations of washout, capsule, threshold growth. In addition macrovascular mainly portal vein invasion constitutes LR-5V category [14].

A comprehensive quantitative analysis may help in providing further parameters that assists differentiation between HCC and non HCC. A quantitative assessment may also reduce interobserver variability and lay foundation for development of diagnostic algorithms and computed aided detection parameters. To the best of our knowledge, this is also the first study of this kind in India with no previous Indian cohort of hypervascular liver tumours having being subjected to this kind of qualitative and quantitative assessment.

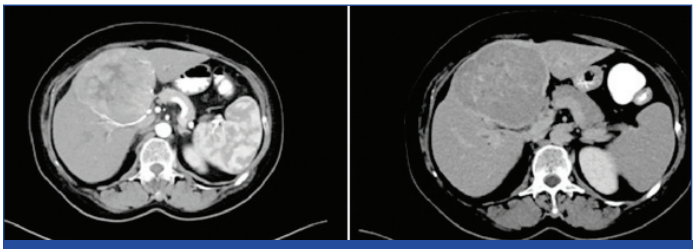
HCCs showed uniform or nonuniform enhancement with most showing washout appearance. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%. The venous phase washout was seen in 69.3% patients while in delayed phase this number increased to 92%. About 8% of HCC did not washout at all while another 9% had a subtle washout. Latter could be resolved subjectively itself and washout even though subtle could be resolved qualitatively with a consensus agreement of two experienced radiologists. Thus, a qualitative washout assessment in a tertiary level setup with high volumes and experienced radiological staff does hold an excellent sensitivity and a reasonable specificity for HCC detection. The specificity may approach 100% if additionally presence of imaging features of cirrhosis is taken into account since none of the non HCCs had underlying cirrhosis. The overall sensitivity of the qualitative washout appearance for predicting HCC was 92% while specificity was 78%.

On quantitative evaluation there was significant difference in the attenuation between the two groups on noncontrast, arterial and delayed phases. Of the arterial parameters the AAC was found to be significantly different between the HCCs and non HCCs. However, when comparing the HCCs with individual subgroups of non HCCs the significance could not be elicited. The AGP was found to be significantly different between the two groups however, it merely represents arterial hypervascularity and doesn't quantitate washout. The PAR as suggested by Liu Y et al., is a measurement of washout appearance of HCC, measured as a ratio of liver and lesion attenuation [2]. The values for PAR in venous phase showed significant difference between the HCC and non HCC while in delayed phase the difference was not statistically significant. The mean PAR-V of HCC was higher than Non HCC. The absence of difference of PAR in delayed phase is probably because there were lesions like adenoma and certain metastases that washed out significantly more than HCC from venous to delayed phase as compared to HCC resulting in almost similar values of PAR-D. A cutoff of PAR-V of 106 provided 70% sensitivity in diagnosing HCC. Relative Washout Ratio (RWR) from arterial to delayed and venous phase was significantly different between the two groups but seems irrelevant as many of the values were negative, since maximum enhancement was obtained in venous phase in many tumours. Considering the fact that attenuation of the lesion was maximum in

venous phase, the calculated RWR from venous to delayed phase (RWR-V-D) showed that the HCC washout significantly more than non HCC and this temporal parameter of washout from venous to delayed phase seems more relevant than ascertaining washout from arterial to venous or arterial to delayed phase. As discussed earlier the washout phenomena is an appearance based on a lot of factors and RWR from venous to delayed phase seems to be one of the parameters that may differentiate the HCC and non HCC negating the effect of liver parenchymal attenuation. It is likely that gradient of loss of attenuation of lesion from venous to delayed phase is much more significant. The mean values of RWR-V-D of HCC (43.27) and non HCC (31.38) were significantly different and a cutoff of 41.79 offered a sensitivity of 75%.

When comparing individual groups there was a significant difference between the mean RWR-V-D values of HCC (mean 43.27) versus hypervascular IMCC (mean 31.2) and metastasis (mean 33.63). Loyer EM et al., compared the kinetics of HCC versus IMCC and found that HCC loose enhancement from venous to delayed phase while IMCC gain enhancement from the corresponding phases, however they included all cases of cholangiocarcinomas and not just the hypervascular variants [16]. Liu Y et al., used PAR ratio as a simple tool to measure the washout appearance [2]. In present study it is apparent that there are some arterial phase enhancing mimics showing the washout appearance complicating the oversimplified guidelines. We also had a much larger study population. In present study PAR-V was significant offering good sensitivity and specificity. This is in contradistinction to the study by Liu Y et al., which found PAR-D to be significant [2]. RWR-V-D was also significantly different between two groups and this gradient may actually help in cases that are not so straight forward.

In present study we found some true HCC mimics; however, which have similar qualitative appearance and quantitative indices like HCC, something which has not been highlighted in the other studies. These mimics were hepatocellular adenomas, hepatoblastoma and certain hypervascular metastasis [Table/Fig-8]. Hypervascular liver metastases from neuroendocrine tumours mimicking hepatocellular carcinoma in enhancement and washout pattern has been described in literature [16,17]. These mimics should be considered as differentials for HCC arising in noncirrhotic liver. The value of quantitative indices also appears to be doubtful for these mimics and appeared to be similar in both subgroups. Although, proving this statistically was difficult in present study due to the small sample size of the mimics. Thus, while a qualitative assessment combined with some useful quantitative parameters, can provide a noninvasive image based diagnosis in majority of cases in the settings of cirrhosis, the same may not hold true for HCC in noncirrhotic livers. A thorough clinicoradiological assessment can however, point to correct diagnosis with hepatocellular adenoma having distinct age group and predispositions, hepatoblastoma usually arising in pediatric age groups and metastatic lesions usually being accompanied by their primary known tumours, though biopsy may be needed for confirmation. Nevertheless a thorough qualitative assessment combined with assessment of certain useful quantitative indices mentioned above, can permit confident noninvasive image based diagnosis for a majority of HCCs in most radiological setup.



[Table/Fig-8]: Neuroendocrine tumour metastatic to liver, showing arterial phase enhancement and delayed phase washout, a pattern indistinguishable from HCC.

LIMITATION

There were some limitations of present study, firstly the lack of histopathology for all HCCs and the use of AASLD guidelines and LIRADS classification with AFP levels for identifying the same (partly because obtaining a biopsy is now almost unethical in patients with cirrhosis meeting the classic imaging diagnostic criteria), but we were stringent in including patients with image based diagnosis, only cirrhotic patients with AFP of more than 400 IU/mL with washout were included. The incidence of cholangiocarcinomas which is known to be the most common mimic would be very low in this group and is unlikely to alter the results. The other limitations included, HCCs less than 1 cm which were not included in our data set. Thus, their behavior was not assessed, heterogeneity within the non HCC group and the sample size of the non HCC group was small compared to HCC. Retrospective nature of analysis also limits the evaluation.

CONCLUSION

While there are number of guidelines which include the more popular likes of AASLD and LI-RADS for diagnosis of HCCs and categorising liver lesions; yet, most of these continue to be subjective in nature with lack of quantification of hypervascularity or washout. A quantitative analysis defining the washout is useful additional determinant adding objectivity to image based diagnosis. Quantitative variables like PAR-V (threshold value 106), RWR-V-D (threshold of 41.79) complement the qualitative analysis, for diagnosis of HCC and have future implications like incorporation into guidelines and development of diagnostic algorithms for computed aided detection software packages.

REFERENCES

[1] Kamaya A, Maturen KE, Tye GA, Liu Y, Parti N, Desser T. Hypervascular liver lesions; Semin Ultrasound CT MR. 2009;30(5):387-407.
[2] Liu Y, Shin L, Brooke J, Kamaya A. Quantitatively defining washout in hepatocellular carcinoma. AJR Am J Roentgenol. 2013;200(1):84-89.
[3] Khosa F, Khan AN, Eisenberg RL. Hypervascular liver lesions on MRI. AJR Am J Roentgenol. 2011;197(2):W204-20.
[4] Murakami T, Tsurusaki M. Hypervascular benign and malignant liver tumors that require differentiation from hepatocellular carcinoma: Key points of imaging diagnosis. Liver Cancer. 2014;3(2):85-96.
[5] Kim SA, Lee JM, Lee KB, Kim SH, Yoon SH, Han JK, et al. Intrahepatic mass-forming cholangiocarcinomas: enhancement patterns at multiphasic CT, with special emphasis on arterial enhancement pattern-correlation with clinicopathologic findings. Radiology. 2011;260(1):148-57.
[6] Oliver J, Baron R, Federle M, Jones B, Sheng R. Hypervascular liver metastases: Do unenhanced and hepatic arterial phase CT images affect tumor detection ?. Radiology. 1997;205(3):709-15.
[7] Kim TK, Lee E, Jang HJ. Imaging findings of mimickers of hepatocellular carcinoma. Clin Mo Hepatol. 2015;21(4):326-43.
[8] Kang Y, Lee JM, Kim SH, Han JK, Choi BI. Intrahepatic Mass-forming cholangiocarcinoma: Enhancement patterns on gadoxetic acid-enhanced MR images. Radiology. 2012;264(3):751-60.
[9] Luca A, Caruso S, Milazzo M, Mamone G, Marrone G, Miraglia R, et al. Multidetector-row computed tomography (MDCT) for the diagnosis of hepatocellular carcinoma in cirrhotic candidates for liver transplantation: prevalence of radiological vascular patterns and histological correlation with liver explants. Eur Radiol. 2010;20(4):898-907.
[10] Tan CH, Thng CH, Low AS, Tan VK, Hartono S, Koh TS, et al. Washout of hepatocellular carcinoma carcinoma; quantitative region of interest analysis on CT. Ann Acady Med, Singapore. 2011;40(6):269-75.
[11] Frericks BB, Loddenkemper C, Huppertz A, Valdeig S, Stroux A, Seja M, et al. Qualitative and quantitative evaluation of hepatocellular carcinoma and cirrhotic liver enhancement using Gd-EOB-DTPA. AJR Am J Roentgenol. 2009;193(4):1053-60.
[12] McEvoy SH, McCarthy CJ, Lavelle LP, Moran DE, Cantwell CP, Skehan SJ, et al. Hepatocellular carcinoma: illustrated guide to systematic radiologic diagnosis and staging according to guidelines of the american association for the study of liver diseases. RadioGraphics. 201333(6):1653-68.
[13] Song DS, Bae SH. Changes of guidelines diagnosing hepatocellular carcinoma during the last ten-year period. Clin Mol hepatol. 2012;18(3):258-67.
[14] Rakhmonova G, Choi J, Kim M. Liver imaging reporting and data system (LI-RADS) version 2014: understanding and application of the diagnostic algorithm. Clin Mole Hepatol. 2016;22(2):296-307.
[15] Cruite I, Tang A, Sirlin C. Imaging-based diagnostic systems for hepatocellular carcinoma. AJR Am J Roentgenol. 2013;201(1):41-55.

[16] Loyer EM, Chin H, Dubrow RA, David CL, Eftekhari F, Charnsangavej C. Hepatocellular carcinoma and intrahepatic peripheral cholangiocarcinoma: enhancement patterns with quadruple phase helical CT-A comparative study. Radiology. 1999;212(3):866-75.

[17] Laroia ST, Sasturkar S, Rastogi A, Pamecha V. Solitary hypervascular liver metastasis from neuroendocrine tumor mimicking hepatocellular cancer: All that glitters is not gold. Indian J Nucl Med. 2015:30(1):42-46 .

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Congenital Biotinidase Deficiency-Clinching the Diagnosis with Classic Imaging Features

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Abstract: *Congenital biotinidase deficiency (CBD) is a rare inborn error of metabolism, resulting in biotin deficiency. The recognition of clinical, laboratory and magnetic resonance imaging findings can help us to arrive at a correct diagnosis at the earliest which can enable institution of appropriate early treatment and prevention of late complications. We present the typical MRI findings in a 3 ½ month old baby who presented with multiple episodes of seizures which finally turned out to be profound biotinidase deficiency.*

Key Words*: Congenital biotinidase deficiency, recurrent seizures, skin manifestations, bilateral symmetrical diffusion restriction, cerebral atrophy.*

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I. Introduction

Congenital biotinidase deficiency (CBD) is a rare autosomal recessive congenital neuro- metabolic disorder,[1] which leads to profound deficiency of biotin which is an essential cofactor for many enzymes[2] Biotin deficiency can lead to neurological, cutaneous and visual problems.-4 Making a diagnosis based on clinical manifestations alone is difficult. Brain imaging especially Magnetic Resonance Imaging (MRI) with advanced MR techniques like diffusion weighted imaging and MR spectroscopy (MRS) has typical features which can clinch the diagnosis. As MRI plays a key role in making the diagnosis at an early stage of the disease it should be considered in all patients who present with clinical features suggestive of metabolic disorders.

II. Case Report

A 3 ½ months old baby girl presented with complaints of recent onset of multiple episodes of seizures, lethargy, hypotonia, skin peeling and alopecia. The antenatal and birth history were unremarkable. The baby was born of a non-consanguineous marriage. Baby was immunized up to the age. The baby was developmentally normaltill 3 months of age and attained social smile at 2 months. Head control has not yet been attained. The baby started to develop recurrent episodes of seizure for 15 days and regression of previously attained social smile. At presentation the child weighted 5.1kg. Head circumference measured about 40cm.In view of multiple episodes of seizures and lethargy, baby was admitted in neonatal intensive care ward and started on empirical antibiotic therapy. Routine blood investigations revealed markedly elevated lactate (11.6mmol/L), reduced bicarbonate (2.7mmol/L) and pH of 6.9. Further blood investigations showed elevated ammonia (200microgram/dl.) and urine ketones(3+). Blood culture and cerebrospinal fluid (CSF) culture were negative. In view of multiple episodes of seizures, skin manifestations, alopecia, severe metabolic acidosis and hyperammonemia, the diagnostic possibility of inborn errors of metabolism was suspected and referred to department of radiology for further evaluation with MRI of the brain.



Figure 1: Clinical photograph of the child showing severe alopecia and skin peeling.

MRI of the brainrevealed prominent cortical sulci and fissures, mildly dilated ventricles– suggesting loss of brain volume. In addition there wereconfluent areas of T2/ fluid attenuated inversion recovery (FLAIR) sequence hyperintensities with T1 hypointensities involving bilateral fronto-parietal white matter, bilateral cerebellar white matter, and cerebellar vermis. There were also bilateral symmetrical areas of diffusion restriction seen in corona radiata, posterior limb of internal capsule, cerebellar white matter, cerebellar vermis, midbrain and left medial temporal gyri. In addition to the above findings, subdural hygroma noted along bilateral frontal convexities (more on the left side). No evidence of mass lesion / hemorrhage seen within brain parenchyma. MRS showed elevated lactate and glycine. Based on the above MRI findings, the diagnostic possibility of inborn errors of metabolism such as CBD, Maple syrup urine disease (MSD)andLeigh's syndrome (LS)were suggested.

With the combination of clinical and radiological findings, biotinidase deficiency was considered as most probablediagnosisand baby was started on oral biotin.Subsequently the diagnosis was confirmed with tandem mass spectroscopy which revealed profound biotinidase deficiency. The baby responded well to oral biotin with reduction of lactate and ammonia and rise in bicarbonate and pH levels.

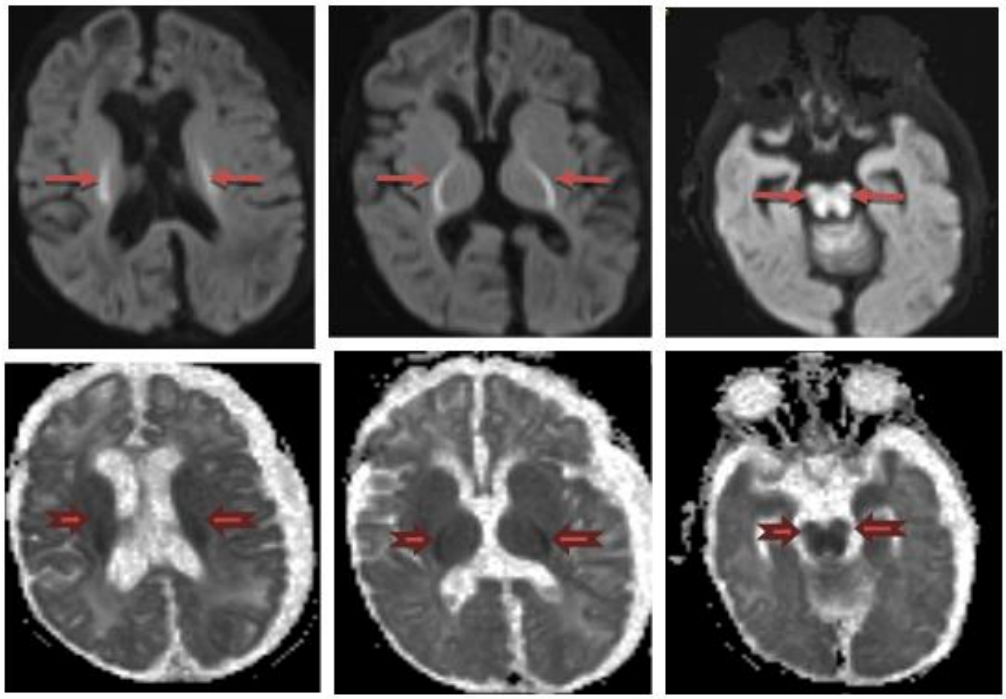


Figure 2: Diffusion weighted images (top row) and Apparent Diffusion Co-efficient (ADC) maps (bottom row) showing bilaterally symmetrical areas of diffusion restriction in the corona radiata, posterior limbs of internal capsule and midbrain which appear hyperintense in DWI (horizontal regular arrows in top row) and hypointense (horizontal notched arrows in bottom row) in ADC.

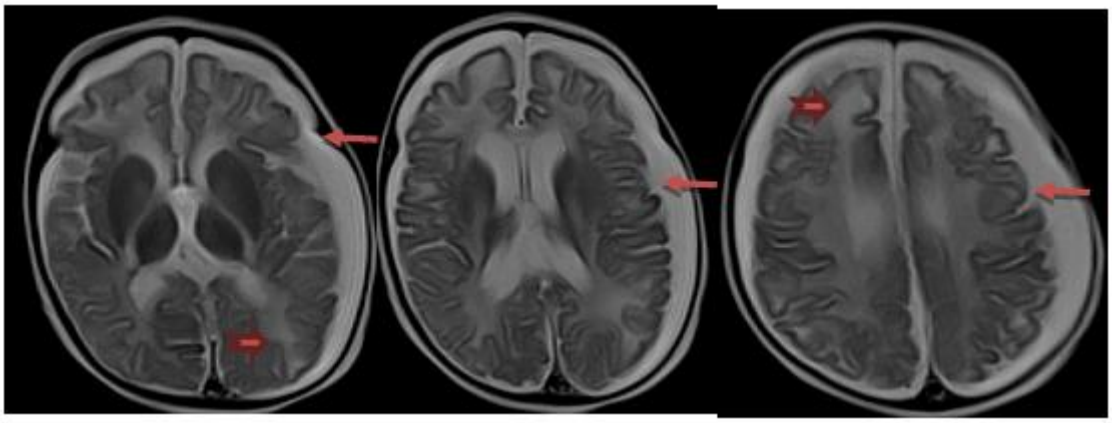


Figure 3: T2Weighted images showing bilateral prominent subdural hygroma (horizontal regular arrows) and abnormal hyperintense areas in the frontal and parieto-occipital white matter (Notched arrow)

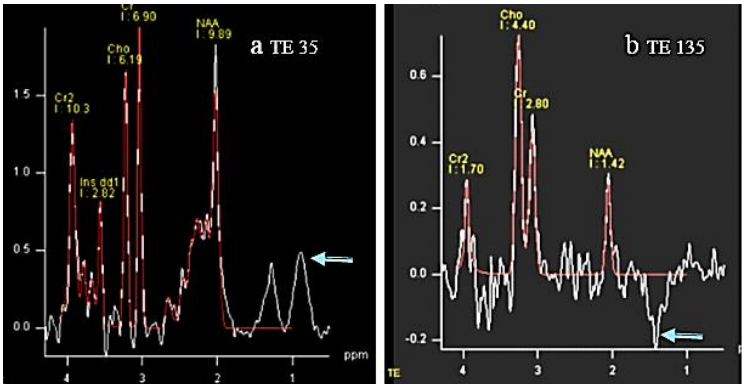


Figure 4: Magnetic resonance imaging H1 spectroscopy images (a, b) acquired with short time to echo (TE 35ms) and intermediate time to echo (TE 135ms). With short TE there is doublet peak at 1.3ppm (Horizontal arrow in a) and reversal of doublet in intermediate TE (horizontal arrow in b), consistent with lactate peak.

III. Discussion

CBD a neurocutaneous disorder,is a rare inborn error of metabolism with autosomal recessiveinheritancein which the body is unable to breakdown the conjugated form of biotin (vitaminB7)and clinical biotin deficiency[4]. This condition manifests due to mutation in biotinidase(BTD) gene at chromosome 3q25. The estimated prevalence of profound biotinidase deficiency (<10% of normal level) is approximately 1: 137000 newborns. The partial biotinidasedeficiency(<30%of normal) is estimated at 1:60,000 newborns[1]. The symptoms manifest in early to mid-infancy (mean age of onset – 3.5 months) [5].

Biotinidase deficiency causes biotin deficiency through multiple mechanisms such as, prevention of release of biotin bound to protein in food particles thus preventing absorption of biotin from intestine, impaired recycle of biotin from carboxylases and histones. The biotin serves as a co-factor for many enzymes such as pyruvate carboxylase, acetyl co-A carboxylase, propionyl Co-A carboxylase, beta methylcrotonyl-coA carboxylase and hence the enzyme biotinidase plays a role in fatty acid synthesis, amino acid catabolism and gluconeogenesis. The substrates accumulated in case of biotinidase deficiency are 3-hydroxy isovaleric acid, 3-methyl crotonylglycine, methylcitrate and 3-hydroxy propionate[6].When biotin is deficient,the activity of pyruvatecarboxylase in CNS falls, leading to localized lactic acidosis. The alopecia and dermatitis in these patients are possibly due to depletion of fatty acids as a result of deficient acetyl –CoA carboxylase.

Clinically, the babies present with multiple episodes of seizures, lethargy, hypotonia, ataxia, developmental delays, coma, alopecia, skin rash (mucocutaneous candidiasis) [2]. Hearing loss and decreased visual acuity are the late complications and are seen less frequently. Biochemically, there will be severe metabolic acidosis, ketosis, hyperammonemia, lactic acidosis and abnormal organic acids in urine. Ketosis and skin manifestations are the atypical features.

Radiologically, MRI will reveal, features of delayed myelination likeabnormalT2/FLAIR hyperintensities with T1 hypointensities in bilateral fronto-parietal white matter, bilateral cerebellar white matter, and cerebellar vermis. There can be bilateral symmetrical areas of diffusion restriction involving corona radiata, posterior limb of internal capsule, cerebellar white matter, cerebellar vermis, anterior midbrain, medial

temporal gyri, hippocampi and parahippocampal gyrus[1].The changes of restricted diffusion will be seen more in the myelinated white matter fibers. MR spectroscopy will show elevated lactateandglycine and decreased N-Acetylaspartate and choline. Some studies have shown involvement of the corpus callosum as well, which was not seen in our study[4].

There are few other conditions in which the MRI findings can closely mimic CBD, which should be considered in the differential diagnoses. They are 1.Maple syrup urine disease[7] (MSUD)–here the onset of clinical signs and symptoms is relatively early, during first week of life with poor feeding, vomiting, dystonia and seizures. Skin manifestations will be absent. Biochemically – ketoacidosis and characteristic maple syrup odor in urine. In MRI, diffusion restriction is seen in deep cerebellar white matter, dorsal brain stem, cerebralpeduncles, and posterior limb of internal capsule, basal ganglia and thalami. Basal ganglia and thalami are typically spared in CBD. 2.Leigh'ssyndrome (LS) – mitochondrial disorder which presents later, towards the end of first year with hypotonia, psychomotor deterioration, ataxia, ptosis and swallowing difficulties without any skin manifestations[8]. In MRI grey matter is more frequently involved, diffusion restriction can be less pronounced and seen only in acute phase in corpus striatum, sub thalamic nucleus, deep cerebellar nuclei, pons, medulla and periaqueductal grey matter. In MRS there will be large lactate peakand small NAA peak.

IV. Conclusion

When a baby presents in early infancy with multiple episodes of seizures, skin manifestations, elevated lactate, metabolic acidosis, ketosis and hyperammonemia with typical MRI findings of bilateral symmetrical areas of diffusion restriction and brain atrophy, the likely diagnosis ofCBD has to be considered which will help to institute proper and timely treatment and thereby avoiding complications and long term sequeale of permanent neuronal damage. Since the structural changes are reversible, it is of paramount importance to make correct and prompt diagnosis of CBD.

Conflicts of interest: Nil
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Bibliography

[1]. Ranjan RS, Taneja S, Singh A, Gupta V. Congenital biotinidase deficiency – MRI findings in two cases. Indian J Radiol Imaging, 2019;29:99-103.
[2]. Matthias R. Baumgartner, Pediatric Neurology Part III, Biotinidase deficiency Handbook of Clinical Neurology 2013.
[3]. Shamina Rahman, Susan standing, R Neil Dalton, Michael G Pike, Late presentation of biotinidase deficiency with acute visual loss and gait disturbance: Developmental medicine and child neurology, 1997, 39: 830-831.
[4]. Karimzadeh P, Ahmadabadi F, Jafari N, Jabbehdari S, Alaee MR, Ghofrani M, Taghdiri MM, Tonekaboni SH. Biotinidase Deficiency: A Reversible Neurometabolic Disorder (An Iranian Pediatric Case Series). Iran J Child Neurol. 2013 Autumn; 7(4):47-52.
[5]. AmitavaDasgupta,, Biotin and other interferences in immunoassay, Chapter 2 - Biotin: Pharmacology, Pathophysiology, and Assessment of Biotin Status, , Pages 17-35, Elsevier 2019
[6]. Jeffrey M. Chinsky, Robert D. Steiner, Developmental-Behavioral Pediatrics (Fourth Edition), Elsevier 2009.
[7]. Aditi Jain, K. Jagdeesh, Ranoji Mane, and SaurabhSingla, Imaging in Classic Form of Maple Syrup Urine Disease: A Rare Metabolic Central Nervous System, J ClinNeonatol. 2013 Apr-Jun; 2(2): 98–100. PMID: 24049754.
[8]. AL Kartikasalwah et al. Leigh syndrome: MRI findings in two children Biomed Imaging Interv J 2010; 6(1):e6.

Original article

Correlation of splenic ARFI with endoscopic findings of esophageal varices in chronic liver parenchymal disease

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Abstract:

Background: Variceal bleed the major cause of mortality in cirrhosis. Upper gastrointestinal (GI) endoscopy remains the main stay of diagnosis and treatment in variceal bleed. Splenic elastography using Acoustic Radiation Force Impulse (ARFI) technique can be used to non-invasively predict presence and severity of esophageal varices.

Material and methods: It is a hospital based prospective case control study, done in 100 patients with chronic liver parenchymal disease, who had undergone upper GI endoscopy. Fifty consecutive patients with esophageal varies and 50 consecutive patients without esophageal varices were included in the study and subjected to splenic ARFI. Data was analyzed with SPSS version 20.0 software using chi-square test or Fischer exact test, independent sample-t test. The receiver operating characteristics (ROC) curve was plotted and the area under the curve was used to determine the cut off points for statistically significant variables.

Results: Spleen ARFI showed significant correlation between cirrhotics with esophageal varices and without varices groups. The cut off of ≥ 3.16 m/s (AUROC: 0.906) precisely detects varices with sensitivity of 94% and specificity of 92%. Spleen ARFI also correlates with severity of varices. Cut off of ≥ 3.29 m/s (AUROC: 0.874) can confidentially differentiate low grade from high grade varices in cirrhotics with sensitivity of 85% and specificity of 100%.

Conclusions: Amongst the noninvasive parameters analyzed in this study, spleenic ARFI exclusively showed highest sensitivity and specificity in the detection of varices and differentiation of high grade varices from low grade varices.

Key words: Splenic ARFI, cirrhosis, Endoscopy, Esophageal varices.

Introduction:

Diverse insults on the liver lead to a common endpoint as cirrhosis. Cirrhosis is pathologically characterized by extensive fibrosis, regenerative nodules and distortion of hepatic parenchymal architecture [1]. Alcoholism and viral hepatitis have been the most common etiologies leading to cirrhosis, however nonalcoholic fatty liver disease is emerging as an important underlying pathology.

Complications of cirrhosis can be categorized as portal hypertension related and portal hypertension unrelated complications. Most common portal hypertension related complications is development of esophageal varices and variceal bleeding [2]. Till date, invasive upper gastrointestinal endoscopy (UGI scopy) remains the mainstay

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for the detection and grading of esophageal varices [3]. In addition to diagnostic advantage, UGI scopy plays a major role in treatment such as band ligation and sclerotherapy. Despite its diagnostic and therapeutic utility, UGI scopy is an invasive procedure, has its inherent complications such as bleeding, perforation, infection and cardio-respiratory complications related to sedation [4].

With current advances in the field of Ultrasonography, estimation of elasticity of the tissue has been made possible with Elastography. It is analogous to manual palpation and in fact offers better information, based on the principle that pathology changes the stiffness of the tissue. Shear Wave Elastography (SWE) is a technique of elastography that uses "shear wave" generated within tissue secondary to the acoustic push, the so called Acoustic Radiation Force Impulse (ARFI). In this technique as no external compression is required and a push impulse can be directed anywhere, it is possible to attain a fine elastogram of entire organ. Additionally, quantification of elasticity of particular region of interest based on shear wave velocity (SWV) is possible [5].

Elasticity of the spleen will be decreased in cirrhosis due to portal venous congestion and hyperplasia of splenic tissue; hence the splenic stiffness will be increased. Portal venous congestion results in esophageal varices. Screening UGI scopy is recommended in all cirrhotic patients in accordance with recent guidelines as primary prophylaxis against variceal hemorrhage[6]. Implementing these guidelines is confronted with cost, complications and invasiveness of UGI scopy. Hence, it necessitates accurate and noninvasive methods for detecting and grading the severity of esophageal varices.

Aim:

To evaluate splenic ARFI as a noninvasive tool for detection of esophageal varices and grading of varices as to high and low grades in chronic liver parenchymal disease.

Materials and methods:

It is a prospective case control study done on 100 patients over a period of 14 months, who were referred to the department of radiology with a diagnosis of chronic liver parenchymal disease. Subsequently all these patients underwent UGI scopy and were divided into two groups. Consecutive 50 patients without esophageal varices comprised the first group and 50 patients with esophageal varices comprised second group. Study was undertaken after obtaining necessary clearance from the institutional human ethics committee. Patients who are of less than 18 years of age and who did not undergo UGI scopy were excluded from the study. All the patients were subjected to splenic ARFI and UGI scopy.

Splenic ARFI protocol:

Splenic ARFI was performed with Siemens Acuson S2000^{TM} / Siemens Acuson S3000 ^{TM} ultrasound systems with ARFI enabled 6C1 / 4C1 curvilinear transducers. Patient was made to lie in right lateral decubitus position and assessment was done by intercostal approach. In order to cover uniformly the spleen, we adopted the stratified random sampling method used in the previous studies [7] (Figure 1). Spleen will be subdivided into cranial external(1), cranial central(2), cranial internal(1), intermediate external(1), intermediate central(1), intermediate internal(1), caudal external(1), caudal central(1) and caudal internal(1) segments. Numbers in the parenthesis represents the number of ARFI values obtained in each segment. In breath hold position, region of interest measuring 10mm in depth and 5mm in width was placed in parenchyma devoid of visible blood vessels and >1 cm below the level of spleen capsule and ARFI evaluation was done (Figure 2). Total of 10 successful measurements were obtained for each patient. Mean ARFI values were automatically calculated and expressed

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in meters per second (m/s).

The data obtained was coded and entered in Microsoft Excel Spreadsheet. Data was analyzed using SPSS version 20.0 statistical software. Categorical variables were expressed as percentages and the comparative analysis was done using chisquare test or Fischer exact test. Continuous variables were expressed as mean ±standard deviation (SD) and the comparative analysis was done by independentsample 't' test. The receiver operating characteristics (ROC) curve was plotted and the area under the curve was determined to the cut off points for statistically significant variables. A probability value (p value) of less than or equal to 0.05 at 95% confidence interval was considered as statistically significant.

Various radilogical parameters like liver size, portal vein diamater, portal vein flow velocity, spleen size, liver ARFI and splenic ARFI were analysed for their value as predictors of presence or absence of esophageal varices, if present, to differentiate high grade varices from low grade varices using various statistical methods as mentioned above.

Results:

TABLE 1: SEX DISTRIBUTION

SEX	WITH EVs		WITHOUT EVs	
	NUMBER	%	NUMBER	%
MALE	36	72	38	76
FEMALE	14	28	12	24
TOTAL	50	100	50	100

TABLE 2: ETIOLOGY DISTRIBUTION IN CIRRHOTICS WITH EVs

ETIOLOGY	WITH EVs		WITHOUT EVs	
	NUMBER	%	NUMBER	%
ALCOHOL	37	74	35	70
NAFLD	6	12	6	12
AUTOIMMUNE	6	12	9	18
METABOLIC	1	2	0	0
TOTAL	50	100	50	100

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TABLE 3: SIGNIFICANCE OF RADIOLOGICAL PREDICTORS BETWEEN CIRRHOTICS WITH EVs AND WITHOUT EVs

PREDICTOR	INDEPENDENT 't' TEST		
	WITH EVs MEAN ± SD	WITHOUT EVs MEAN ± SD	P value
LIVER SIZE	140.50 ± 20.97	138.36 ± 19.62	0.599
PORTAL VEIN DIAMETER	12.54 ± 2.46	12.28 ± 2.06	0.569
PORTAL VEIN FLOW VELOCITY	18.91 ± 6.23	20.28 ± 4.56	0.216
SPLEEN SIZE	**147.54 ± 26.5**	**138.66 ± 16.90**	**0.049***
PLATELET COUNT SPLEEN SIZE RATIO	**588.52 ± 274.58**	**972.86 ± 177.99**	**0.000***
LIVER ARFI	3.03 ± 0.78	2.92 ± 0.69	0.458
SPLEEN ARFI	**3.33 ± 0.23**	**2.99 ± 0.17**	**0.000***

*** P= < 0.05 is statistically significant**

Spleen size, PSR and spleen ARFI showed statistical significance between cirrhotics with EVs and cirrhotics without EVs groups.

TABLE 4: SIGNIFICANCE OF RADIOLOGICAL PREDICTORS BETWEEN LOW GRADE EVs AND HIGH GRADE EVs

PREDICTORS			
	LOW GRADE MEAN ± SD	HIGH GRADE MEAN ± SD	P value
LIVER SIZE	144.91 ± 23.39	136.74 ± 18.27	0.172
PORTAL VEIN DIAMETER	12.22 ± 2.33	12.81 ± 2.58	0.399
PV FLOW VELOCITY	18.69 ± 7.09	19.10 ± 5.52	0.819
SPLEEN SIZE	140.70 ± 31.17	153.37 ± 20.60	0.092
PLATELET SPLEEN SIZE RATIO	651.63 ± 223.87	534.75 ± 305.22	0.135
LIVER ARFI	2.99 ± 0.65	3.07 ± 0.90	0.698
SPLEEN ARFI	**3.22 ± 0.36**	**3.42 ± 0.28**	**0.001***

*** P= < 0.05 IS STATISTICALLY SIGNIFICANT**

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Spleen ARFI solely independently shows statistical significance between low grade EVs and high grade EVs subgroups.

TABLE 5: ROC ANALYSIS: SPLEEN ARFI IN EVs DETECTION

PREDICTOR	CUT OFF	AUROC	SENSITIVITY	SPECIFICITY
SPLEEN ARFI	≥ 3.16	0.906	94%	92%

ROC analysis of spleen ARFI (Figure 3) in detecting EVs in this study showed maximum area under the curve of 0.906 at a cutoff of ≥ 3.16 with maximum sensitivity of 94% and specificity of 92%.

TABLE 6: ROC ANALYSIS: SPLENIC ARFI IN HIGH GRADE AND LOW GRADE EVs

PREDICTOR	CUT OFF	AUROC	SENSITIVITY	SPECIFICITY
SPLEEN ARFI	≥ 3.29	0.874	85%	100%

ROC analysis of spleen ARFI (Figure 4) in delineating high grade and low grade EVs depicted a cutoff of ≥ 3.29 with maximum area under the curve of 0.874 at cutoff of with sensitivity of 85% and specificity of 100%.



Figure: 1. Stratified random sampling method. Spleen will be subdivided into cranial external(1), cranial central(2), cranial internal(1), intermediate external(1), intermediate central(1), intermediate internal(1), caudal external(1), caudal central(1) and caudal internal (1) segments. Numbers in the parenthesis represents the number of ARFI values obtained in each segment.



Figure: 2. In breath hold position, region of interest measuring 10mm in depth and 5mm in width was placed in parenchyma devoid of visible blood vessels and >1 cm below the level of spleen capsule and ARFI evaluation was done.

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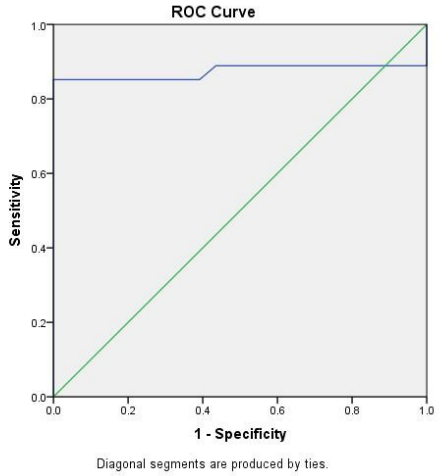


Figure: 3. ROC analysis of spleen ARFI in detecting EVs showed maximum area under the curve of 0.906 at a cutoff of ≥ 3.16 with maximum sensitivity of 94% and specificity of 92%.

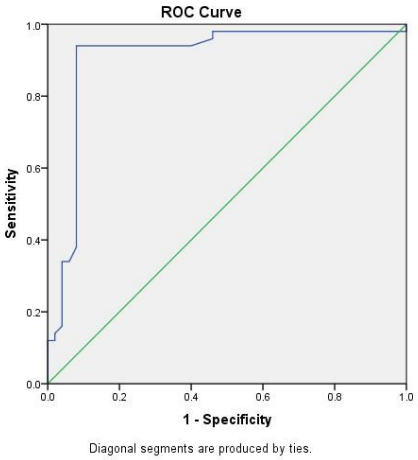


Figure: 4. ROC analysis of spleen ARFI in delineating high grade and low grade EVs depicted a cutoff of ≥ 3.29 with maximum area under the curve of 0.874 at cutoff of with sensitivity of 85% and specificity of 100%

Discussion:

Recent guidelines endorse that endoscopic screening should be done for all cirrhotic patients at the time of diagnosis to recognize those at high risk of bleeding and possibly to benefit from primary prophylaxis [6]. Implementation of these guidelines imposes huge burden on UGI scopy units and frequent testing over time can decrease the compliance of patient. By noninvasive prediction of the presence of EVs, one can recommend the appropriate category of patients to undergo invasive UGI scopy, thereby reducing unnecessary endoscopic examinations and burden on endoscopy units.

Previous studies have correlated various radiological and laboratory parameters either alone or in combination with the presence or absence of EVs in cirrhotics. Although, the efficiency of parameters or combination of parameters reported in each study differ amongst them, due to the heterogeneous nature of the studies. Good predictive value of several non-endoscopic variables for the presence or absence of EVs have been documented

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by many previous studies, but the data is limited from our part of the country. This study was performed to assess the effectiveness of various clinical, laboratory and radiological parameters, in reliably detecting the existence of EVs and high grade EVs in cirrhotic patients.

In the present study, there is no significant association of demographic parameters between the two study groups. Previous studies by Zaman et al [8] and Cherian et al [9], showed that Child Pugh class B or C cirrhotic patients, as compared to class A cirrhotics, have high propensity towards the detection of EVs on UGI scopy. In contrary, our study inferred no significant association between Child Pugh class and the presence of EVs (p = 0.799). In addition, we assessed MELD scores and found significant affiliation with presence of EVs, in congruence to the results of previous studies by De Mattos et al [10] , Tafarel et al [11] and Chandail VS et al [12].

Our study showed no significant variation in either portal vein diameter or portal vein flow velocity between cirrhotic patients with EVs and without EVs. Our study in contradiction to previous studies inferred that there is no significant difference in liver stiffness measured by elastography between cirrhotics with EVs and cirrhotics without EVs (p= 0.458).

In agreement with previous studies, our study also showed significant difference in the values of spleen stiffness measured by ARFI between cirrhotics with EVs and without EVs (p= 0.000). We acquired the optimal cutoff of ≥ 3.16m/s (AUROC: 0.906) with sensitivity of 94% and specificity of 92%, thereby validating spleen ARFI as a non-invasive radiological marker for the presence of EVs in cirrhotics.

In the present study, we furthermore evaluated the utility of the above discussed noninvasive clinical, laboratory and radiological parameters in the detection of high grade EVs in cirrhotics. Of all the noninvasive parameters analyzed, spleen ARFI exclusively showed significant difference between the two subgroups (p= 0.001). We derived the optimal cutoff of ≥ 3.29m/s (AUROC: 0.874) with sensitivity of 85% and specificity of 100%, which is in very close approximation with spleen ARFI cutoff of ≥ 3.30m/s obtained by Takuma et al[13]. Thereby substantiating spleen ARFI as a non-invasive radiological marker for the presence of high grade EVs in cirrhotics. In other words, the presence of high grade EVs can be reliably ruled out with the spleen ARFI cutoff of < 3.29m/s in patients with chronic liver parenchymal disease. Our study was limited by only few patients with grade IV EVs being included in this study. It is due to the lower prevalence of grade IV EVs, as in part by the practice of generous prophylactic and therapeutic measures which delays or eliminates progression of low grade EVs to high grade EVs. Inter observer variability is not assessed in this study. Future studies on this context should focus on multicenter design with evaluation of equipment of various manufacturers and assessment of inter observer variability.

Conclusions:

Amongst the noninvasive parameters analyzed in this study, spleenic ARFI exclusively showed highest sensitivity and specificity in the detection of varices and differentiation of high grade varices from low grade varices.

References :

1. Sangster GP, Previgliano CH, Nader M, Chwoschtschinsky E, and Heldmann MG. MDCT Imaging Findings of Liver Cirrhosis: Spectrum of Hepatic and Extrahepatic Abdominal Complications. HPB Surg 2013;129396:1-12.

2. Schuppan D, Afdhal NH. Liver Cirrhosis. Lancet 2008;371(9615):838-851.

3. Singh SP, Panigrahi MK. Spectrum of Upper Gastrointestinal Hemorrhage in Coastal Odisha. Trop Gastroenterol

2013;34(1):14-17.

4. Riley S, Alderson SD, Birmingham. Complications of upper gastrointestinal endoscopy. BSG Guidelines in Gastroenterology 2006 Nov:7-13.

5. Taljanovic MS, Gimber LH, Becker GW, Latt LD, Klauser AS, Melville DM et al. Shear-Wave Elastography: Basic Physics and Musculoskeletal Applications. Radiographics 2017 May-Jun;37(3):855-870.

6. Kim HY, Jin EH, Kim W, Lee JY, Woo H , Oh S et al. The Role of Spleen Stiffness in Determining the Severity and Bleeding Risk of Esophageal Varices in Cirrhotic Patients. Medicine (Baltimore) 2015 Jun;94(24):e1031.

7. Rizzo L, Attanasio M, Pinzone MR et al. A New Sampling Method for Spleen Stiffness Measurement Based on Quantitative Acoustic Radiation Force Impulse Elastography for Noninvasive Assessment of EsophagealVarices in Newly Diagnosed HCV-Related Cirrhosis. BioMed Research International 2014;Article ID 365982:1-8.

8. Zaman A, Hapke R, Flora K, Rosen HR, Benner K. Factors predicting the presence of esophageal or gastric varices in patients with advanced liverdisease. Am J Gastroenterol 1999 Nov;94(11):3292-3296

9. Cherian JV, Deepak N, Ponnusamy RP, Somasundaram A, Jayanthi V. Non-invasive predictors of esophageal varices. Saudi J Gastroenterol 2011 Jan-Feb;17(1):64-8.

10. de Mattos AZ, de Mattos AA, Daros LF, Musskopf MI. Aspartate aminotransferase-to-platelet ratio index (APRI) for the non-invasive prediction of esophageal varices. Ann Hepatol 2013 Sep-Oct;12:910-4.

11. Tafarel JR, Tolentino LH, Correa LM, Bonilha DR, Piauilino P, Martins FP et al. Prediction of esophageal varices in hepatic cirrhosis by noninvasive markers. Eur J Gastroenterol Hepatol 2011;23:754-58.

12. Chandail VS, Kotwal SK, Koul S, Gupta R, Mahajan A. Non-invasive markers for prediction of varices in patients with portal hypertension. Int J Res Med Sci 2017;5:1007-10.

13. Takuma Y, Nouso K, Morimoto Y, Tomokuni J, Sahara A, Toshikuni N et al. Measurement of spleen stiffness by acoustic radiation force impulse imaging identifies cirrhotic patients with esophageal varices. Gastroenterology 2013 Jan;144(1):92-101.

Original article

Correlation of diffusion tensor imaging with clinical and neurological findings in patients with cervical spondylotic myelopathy

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ABSTRACT:

Background: Cervical spondylosis is a common degenerative disease of spine generally seen in old age, frequently causing compression of spinal cord and /or nerve roots by protruding disc or osteophytes. Magnetic Resonance Imaging (MRI) is the modality of choice for evaluation of patients with features of cervical spondylotic myelopathy. In addition to routine MRI sequences, advanced MR techniques like diffusion tensor imaging (DTI) can be used to obtain specific information about the spinal cord.

Materials and methods: It is a prospective observational study conducted in 30 patients who presented with clinical and neurological features of cervical spondylotic myelopathy, who had undergone MRI.

Result: All the patients had undergone routine MRI along with diffusion tensor imaging. The Apparent Diffusion Coefficient (ADC) and Fractional Anisotropy (FA) values of the spinal cord were measured at the level of normal spinal canal dimension and at the level where there is narrowing of the spinal canal. The ADC values were found to be high and the FA values were low at the level of the spinal canal stenosis in comparison with values measured at the level of normal spinal canal dimension, which were statistically significant.

Conclusion: DTI parameters showed significant correlation with the clinical parameters, suggesting that DTI can be used to detect microscopic changes related to compressive myelopathy, before changes are apparent in the routine MR sequences. This can enable us to intervene early to avoid irreversible damage to the spinal cord.

Key words: Cervical spondylotic myelopathy, Diffusion tensor imaging, Fractional anisotropy.

Introduction

Cervical spondylosis is a common degenerative disease of spine generally seen in old age, frequently causing compression of spinal cord and /or nerve roots by various pathological entities like protruding disc, osteophytes and thickening of ligamentous structures around the spinal cord.[1] Spondylotic myelopathy refers to compression of the spinal cord by the above mentioned structures leading to impairment of spinal cord function.[2] Cervical spondylotic myelopathy (CSM) is usually seen after the 5th decade. Patients with CSM usually present with symptoms of numbness and weakness in limbs, pain over shoulder and neck, incoordination. Magnetic Resonance Imaging (MRI) of cervical spine is non-invasive and accurate imaging tool in assessing the structural changes of spinal cord due to its high contrast and soft tissue resolution[3]. It helps to ascertain the site and the

severity of degenerative compressive myelopathy. It is also useful to exclude any other pathology, like neoplasm which can cause compressive myelopathy and mimic degenerative disc disease.

Classical MRI finding seen in compressive myelopathy is focal T2 hyperintensity in spinal cord at the level of a compressive lesion. This signal change is suggestive of either spinal cord oedema in acute cases, or gliosis and myelomalacia in chronic cases[4,5]. Oedema is associated with swelling of the spinal cord and chronic changes on the other hand show thinning of the spinal cord. Other imaging modalities like Computed Tomography (CT) and radiography play a complementary role in the evaluation of CSM. Due to its inherent high spatial resolution CT is better than MRI in the assessment of cortical bone, osteophytes and other calcified ligamentous structures. These structures can also cause spinal canal compromise. Plain radiography is still being used as an initial tool for evaluating degenerative disc changes[6]. The ultimate aim of doing imaging studies is to identify the spinal cord abnormalities early and take remedial measures to prevent further damage[7].

Aims:

1. To compare Fractional Anisotropy (FA) and Apparent Diffusion Coefficient (ADC) values of the spinal cord obtained at the level with above and below the level of spinal canal stenosis.
2. To compare the Diffusion Tensor Imaging (DTI) parameters with Nurick score and neurological examination findings.
3. To detect the early microscopic level structural abnormalities of spinal cord using DTI before overt, irreversible changes manifest in routine MR sequences.

Materials and methods:

It is a prospective observational study performed in 30 patients who were referred by the department of neurology. These patients presented with clinical symptoms and neurological findings suggestive of cervical myelopathy. The study period was from January 2018 to March 2019 at the department of Radiology and imaging sciences PSG Institute of Medical Sciences & Research. Prior to commencement of the study, clearance has been obtained from Institutional Human Ethical Committee and informed written consent has been taken from the participants. Patients with clinical and neurological features of CSM were included in the study. Patients with following abnormalities were excluded from the study: 1.focal or diffuse signal abnormality in the spinal cord. 2. History of cervical spine injury / surgery. 3. Focal lesions in the brain which may be the underlying pathology causing the neurological abnormality.

Prior to undergoing MRI all these patients were assessed symptom wise for the severity of CSM using Nurick score and neurological examination was performed to assess the power of lower and upper limbs. In Nurick score patients were categorized from grade 0 to 3, based on clinical history alone, where grade 0 represented mild form of disease with only symptoms suggestive of nerve root involvement (radiculopathy), grade 3 represents more severe neurological deficit where the patients are wheel chair bound with no independent mobility (myelopathy). On clinical examination muscle power of the limbs was assessed using standard grade 1 to grade 5.

All the patients underwent MRI in Siemens 1.5 Tesla 16 channel machine.

MRI protocol:

1. Routine non contrast T1 and T2 weighted images to look for overt signal changes in T2 Weighted Images.
2. DTI acquisition using EPI yielding the following set of images:

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1. Diffusion weighted imaging, 2.Trace weighted, 3.FA and ADC mapping 4.Tensor imaging.

DTI parameters:

Diffusion directions measured – 20.

The b factors – 0, 100 and 1000

TR/ TE - 3600 /94ms

Field of view - 230mm

Slice thickness - 5mm.

Images were analysed using Siemens Syngo via work station. Initially presence of any focal or diffuse spinal cord lesions was excluded. After that dimension of the spinal canal was measured at all the vertebral level. Subsequently DTI analysis was performed by drawing region of interest at the level of maximum spinal canal stenosis, one vertebral level above the spinal canal stenosis and one level below (Figure 1a). DTI Parameters assessed were FA and ADC, the values of which were displayed as a table in the system (Figure 1b) along with images of visual maps of ADC (Figure 2a) and FA (Figure 2b).

Statistical analysis:

The data has been analysed using SPSS 23.0 version. To describe the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean & standard deviation (SD) were used for continuous variables. To find the significant difference in the multivariate analysis for repeated measures the repeated measures of ANOVA was used with Bonferroni correction to control the type I error on multiple comparison. To assess the relationship between the variables Pearson's Correlation was used. In all the above statistical tools the probability value .05 is considered as statistically significant.

Results:

A total of 30 patients were enrolled in our study of which 20 were men and 10 were women (Figure 3). Nurick Score distribution is given in the table 1.

All the patients had muscle power grade between III and V. (Figure 4). No patient in our study had muscle power less than 3.

Diffusion tensor parameters (ADC and FA) were measured at 3 levels of the spinal cord (Figure 1a). One measurement at the level of the spinal canal stenosis and 2 measurements (above and below the level of stenosis) at the level of normal canal dimension. The values of ADC and FA measured at different levels of the spinal cord are given in table 2.

Pairwise comparison of ADC and FA was performed and results are given in table 3 and table 4. Patients were found to have high ADC and low FA values at the level of stenosis when compared with ADC and FA values at the level of no stenosis. The differences were found to be statistically highly significant with a p value of 0.0005 (ANOVA test was used). There was negative correlation between the mean FA and ADC at the level of stenosis with r value of -0.424 and p value of 0.019. There was negative correlation between the mean FA and ADC at the level of no stenosis with r value of -0.0545 and p value of 0.002. There is negative correlation between Nurick score (clinical grading) and muscle power (neurological examination) r value of -0.07 and P value of 0.001.

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Correlation of FA and ADC with muscle power:

At the level of stenosis:

There was a positive correlation between muscle power and mean FA value with r value of 0.2 and p value 0.1. There is a negative correlation between muscle power and mean ADC value at the level of stenosis with r value of -0.1 and p value 0.1 and p value of 0.3.

At the level of no stenosis:

There is a positive correlation between muscle power grade and mean FA value at the non- stenosis level with r value of 0.18 and p value 0.32 and p value of 0.4. There is a negative correlation between muscle power grade and mean ADC value at the non-stenosis level upper with r value of -0.25 and p value 0.17.

Correlation of FA and ADC with Nurick score:

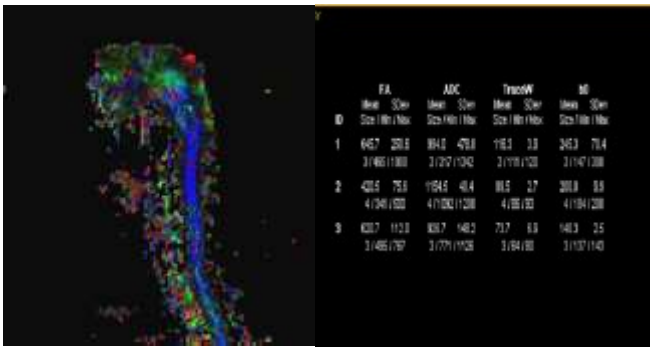
At the level of stenosis:

FA and Nurick score has negative correlation with r value of -0.4 and p value of .0005. ADC and Nurick score has a positive correlation with r value of -0.1 and p value of – 0.2

At the level of no stenosis:

FA and Nurick score had positive correlation with r value of 0.1 and p value of –0.4. ADC and Nurick score had negative correlation r value of -0.05 and p value of– 0.7

ROC analysis of FA in predicting microscopic level structural cord abnormality in the spinal cord at the level of the spinal canal stenosis. (Figure 5) was done. Subsequently test result variables (table 5) and sensitivity and specificity (table 6) were calculated. Likewise ROC analysis of ADC in predicting microscopic level structural cord abnormality was done (Figure 6). Test result variables (table 7) and sensitivity and specificity (table 8) were also calculated for ADC. Both FA and ADC showed good sensitivity and specificity in predicting the early microscopic level changes in the spinal cord caused by degenerative disease, ADC was slightly more specific than FA.

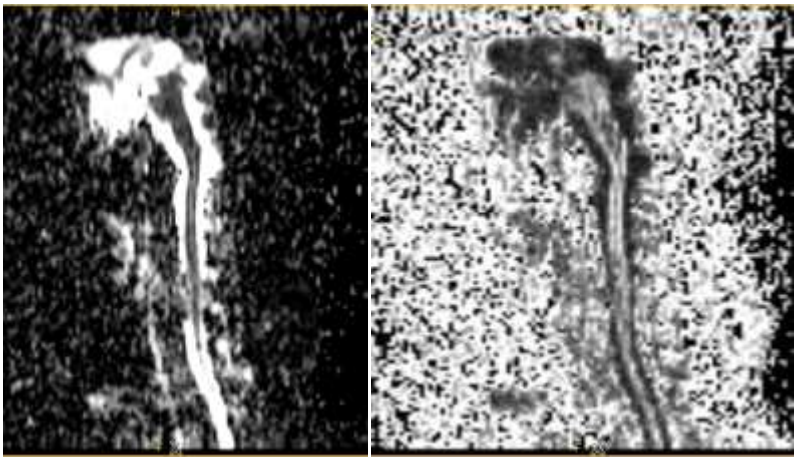


a **b**

Figure 1. Method of FA and ADC measurement.

Three circular Region of Interest (ROI) are drawn “at”, “above” and “below” the level of stenosis (a) yielding the numeric values of each level in a table (b).

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a b

Figure 2. ADC (a) and FA (b) maps obtained with DTI.

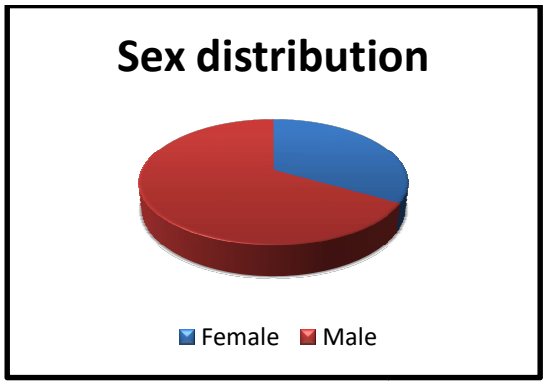


Figure 3. Sex distribution in the study population.

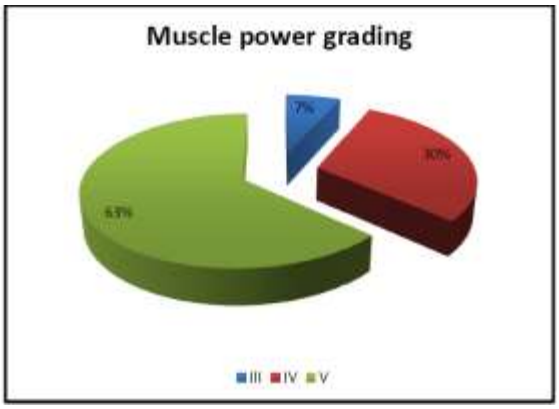


Figure 4. Muscle power distribution in the study population.

Majority of the patients had a muscle power of 5. Remaining patients had muscle power of 4 and 3.

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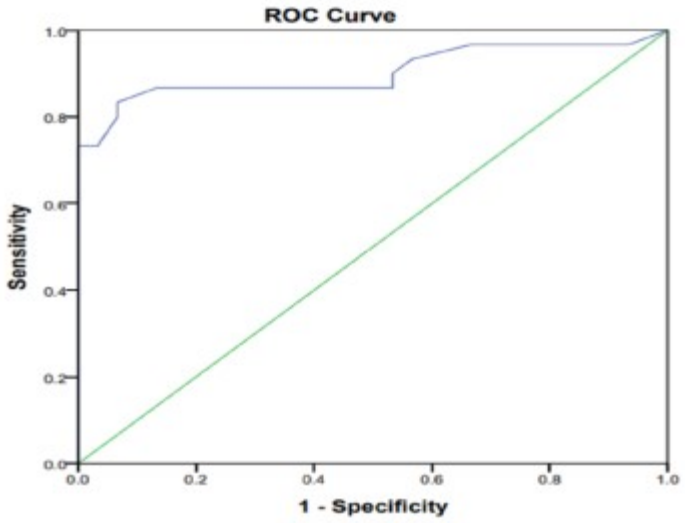


Figure 5: ROC analysis of FA in predicting the microscopic structural abnormality in the spinal cord.

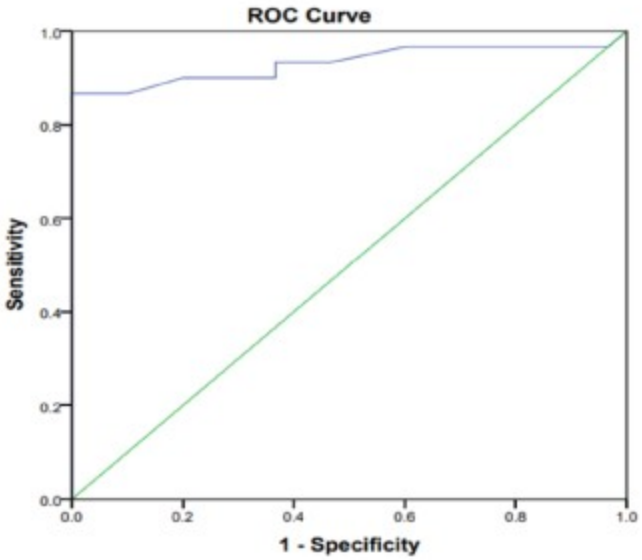


Figure 6: ROC analysis of ADC in predicting the microscopic structural abnormality in the spinal cord.

Table1: Nurick Score distribution in the study population:

Nurick Score	Number	Percent
0	2	6.7
1	9	30.0
2	9	30.0
3	10	33.3
Total	30	100.0

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Table 2: Values of ADC and FA measured at different levels of the spinal cord.

	ADC		FA	
Level of spinal cord	Mean	SD	Mean	SD
Stenotic Level	1.3470	.23997	.5067	.09484
Non-stenotic level upper	1.0141	.09272	.6670	.07957
Non-stenotic level lower	1.0120	.08919	.63443	.07566

Table 3: Pairwise comparison of ADC

(I) ADC		Mean Difference (I-J)	Std. Error	Sig.[b]
Mean ADC stenotic level	Mean ADC non-stenotic level upper	.333*	.045	.0005
	Mean ADC non-stenotic level lower	.335*	.043	.0005
Mean ADC non-stenotic level upper	Mean ADC stenotic level	-.333*	.045	.000
	Mean ADC non-stenotic level lower	.002	.021	1.000
Mean ADC non-stenotic level lower	Mean ADC stenotic level	-.335*	.043	.000
	Mean ADC non-stenotic level upper	-.002	.021	1.000

Based on estimated marginal means.

*The mean difference is significant at the .05 level.

b. Adjustments for multiple comparisons: Bonferroni.

Table 4: Pairwise comparison of FA:

(I) FA		Mean Difference (I-J)	Std. Error	Sig.[b]
Mean FA stenotic level	Mean FA non-stenotic level upper	.160*	.019	.0005
	Mean FA non-stenotic level lower	.128*	.023	.0005
Mean FA non-stenotic level upper	Mean FA stenotic level	-.160*	.019	.000
	Mean FA non-stenotic level lower	.033	.017	.219
Mean FA non-stenotic level lower	Mean FA stenotic level	-.128*	.023	.000
	Mean FA non-stenotic level upper	-.033	.017	.219

Based on estimated marginal means.

*The mean difference is significant at the .05 level.

b. Adjustments for multiple comparisons: Bonferroni.

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Table 5: Test result variables of FA

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.902	.045	.0005	.815	.990

Table 6: Sensitivity and specificity calculation of FA.

Predictor	Cut off	AUROC	Sensitivity	Specificity
FA	0.57	0.902	86.7%	86.7%

Table 7: Test result variables of ADC.

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.932	.039	.0005	.857	1.000

Table 8: Sensitivity and specificity calculation of ADC.

Predictor	Cut off	AUROC	Sensitivity	Specificity
ADC	1.1	0.932	86.7%	90.0%

Discussion:

MRI has revolutionized the evaluation of pathology related to spinal cord. Exceptionally high contrast resolution and soft tissue resolution place MRI way above other imaging modalities in the evaluation of spinal cord.[2] Recent advances in respect to high field strength and fast and robust sequences have greatly contributed to the superior performance of MRI[8]. Very small yet important structures like the nerve roots and the thin ligaments need to be assessed in almost all patients which can harbour the pathology. However significant degree of anatomical and structures abnormalities need to be present before they are visible in routine MRI sequences, primarily in the spinal cord[6]. Hence the need for imaging technique which is similar to a biomarker to pick up early microscopic level changes in the neural structures, specially the spinal cord. Role of DTI in the evaluation of the brain pathologies has been proved and it is being used routinely, however its usage in the evaluation of spinal cord is still evolving.

The application of DTI in the evaluation of spinal cord is hindered by various factors. Spinal cord is a very narrow structure with a high elasticity and situated in anatomically complex location, which are the factors

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making evaluation difficult. Other factors like CSF pulsation, motion artefacts and breathing related artefacts make usage of DTI difficult as a routine sequence. Newer techniques like reduced field of view with oblique spin echo and OVS (outer volume suppression) have been used to overcome the limitations and better images can be obtained. All these improved techniques provide high resolution images not only in the cervical cord but also the entire spinal cord.

Budzik et al[9] studied the feasibility of DTI and fiber tracking in CSM. FA values showed correlation with the patients' clinical scores. High signal intensity of the spinal cord on T2WI did not correlated either with the DTI parameters or with the clinical symptoms, suggesting that FA is better in depicting the early structural abnormality of the spinal cord when compared with T2W imaging[10, 11]. But they did not study the relationship of DTI scalars with cervical canal width. In our study all the patients had spinal canal stenosis, in that about 73% of the patients had relative narrowing and 27% has absolute narrowing.

In our study degenerative changes like disc bulge, protrusion, osteophytes and ligament thickening led to spinal canal narrowing. These pathological changes caused disruption of the white matter tracts and other secondary changes in cervical cord. Secondary changes like cord edema and macrophage proliferation lead to increased diffusivity of water molecules with in the spinal cord parenchyma, especially in the white matter which can alter the FA and ADC values[12]. These changes were apparent much before macroscopic changes appear in routine T1 and T2W images.**UzeyirAhmadli et al**[13] investigated the utility of DTI sequence in patients with CSM symptoms for early detection of microscopic changes in the spinal cord at the level of narrowed spinal canal without any T2 signal alterations. FA values with in the cord were reduced and ADC increased at site of canal stenosis when compared with non-stenotic segments above the level of canal stenosis. However no significant difference was noted in FA and ADC values between stenotic segment and non-stenotic segment distal to the level of stenosis.

In our study there was statistically significant reduction in the FA values and increase in the ADC values at the site of stenosis when compared to the non-stenotic levels (upper and lower). There was no statistically significant reduction in FA value noted at the non-stenotic levels (upper and lower). The reduction in FA value at the stenotic level is presumably due to reduced neuronal fibers with increase in the extracellular space or due to extracellular edema which can result in increased diffusivity of water molecules and consequently facilitated diffusion.

Jones et al[14] reported a significant correlation between FA and modified Japanese orthopedic association score and Nurick score, and reported that patients who had a higher FA at the compressed level of spinal cord tended to have a better recovery of functionality after surgery when compared to patients undergoing surgery who had a lower FA value pre operatively. In our study there was significant correlation between the patient’s symptoms and the DTI parameters. We graded clinical symptoms using only Nurick score, modified Japanese orthopedic association score was not used.

FA and Nurick score had a negative correlation with r value of -0.4 and p value –0.0005, which implies that the FA values tend to be less when the Nurick score is on the higher side. ADC and Nurick score had a positive correlation with r value of-0.1 and p value of – 0.2, which is equal to higher ADC means higher Nurick score.There was also a significant correlation between the DTI parameters and muscle power. There was positive correlation between muscle power and mean FA value at the level of stenosis with r value of 0.2 and p

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value of 0.1. There is a negative correlation between muscle power and mean ADC value at the level of stenosis with r value of -0.1 and p value of 0.3. ROC was calculated between the stenotic and non stenotic level. The mean was calculated between the upper and lower non-stenotic level and it was compared with the stenotic level. FA showed a sensitivity of 86.7% and specificity was 86.7% when the cut was kept at 0.57 (Table 6). ADC showed a sensitivity of 86.7% and specificity was 90.0%, when the cut off is kept at 1.1. (Table 8).

Conclusion:

- Our study showed significant correlation between the patients' clinical symptoms and DTI parameters.
- DTI has the potential to detect microscopic structural changes in the spinal cord, before the T2 signal changes appear on conventional MRI.
- DTI can be used as a tool for detecting early changes in cervical spondylotic myelopathy, which would prognosticate the clinical and surgical outcome[14, 15].
- DTI technique should be routinely used on patients with clinical symptoms of cervical spondylotic myelopathy.

Limitations:

- The sample size of the study population was relatively small
- The long-term follow-up of the patients were not done
- It was impossible to determine the percentage of patients with early cervical spondylotic myelopathy who would have advanced to T2 hyperintensity changes in spinal cord, when left untreated.
- Cost and the availability of MRI.

Further studies have to be done on large group with long term follow up of those patients to determine the potential utility of quantitative diffusion tensor imaging.

Conflicts of interest:

There are no conflicts of interest to declare.

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References:

1. Crandall, Paul H., and Ulrich Batzdorf. "Cervical spondylotic myelopathy."Journal of neurosurgery 25.1 (1966): 57
2. Montgomery, D. M., and R. S. Brower. "Cervical spondylotic myelopathy. Clinical syndrome and natural history." The Orthopedic clinics of North America23.3 (1992): 487-493.
3. Al-Mefty, Ossama, et al. "Myelopathic cervical spondylotic lesions demonstrated by magnetic resonance imaging." Journal of neurosurgery 68.2 (1988): 217-222.

4. Chen, Chi-Jen, et al. "Intramedullary High Signal Intensity on T2-Weighted MR Images in Cervical Spondylotic Myelopathy: Prediction of Prognosis with Type of Intensity 1." Radiology 221.3 (2001): 789-794.
5. Fernández de Rota, Juan Jose, et al. "Cervical spondylotic myelopathy due to chronic compression: the role of signal intensity changes in magnetic resonance images." Journal of Neurosurgery: Spine 6.1 (2007): 17-22.
6. Freeman, T. B., and C. R. Martinez. "Radiological evaluation of cervical spondylotic disease: Limitation of magnetic resonance imaging for diagnosis and preoperative assessment." Perspect Neurol Surg 3.91 (1992): 34-54.
7. Suri, Ashish, et al. "Effect of intramedullary signal changes on the surgical outcome of patients with cervical spondylotic myelopathy." The Spine Journal3.1 (2003): 33-45.
8. Fulong Dong, Wu Y, Song P "A preliminary study of 3.0-T magnetic resonance diffusion tensor imaging in cervical spondylotic myelopathy".Eur Spine J. 2018 Aug;27(8):1839-1845.
9. Budzik JF, Balbi V, Le Thuc V, Duhamel A, Assaker R, Cotten A "Diffusion tensor imaging and fibre tracking in cervical spondylotic myelopathy" Eur Radiol.2011 Feb;21(2):426-33.
10. Kara B, Celik A, Karadereler S, Ulusoy L, "The role of DTI in early detection of cervical spondylotic myelopathy: a preliminary study with 3-T MRI" Neuroradiology. 2011 Aug;53(8):609-16.
11. Mori S, Zhang JPrinciples of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 2006 Sep 7;51(5):527-39
12. Toktas ZO, Tanrıkulu B, Koban O, Kilic T, Konya D "Diffusion tensor imaging of cervical spinal cord: A quantitative diagnostic tool in cervical spondylotic myelopathy". J Craniovertebr Junction Spine. 2016 Jan-Mar;7(1):26-30
13. Uzeyir Ahmadli, Nils H Ulrich ,Yao Yuqiang, Daniel Nanz Early detection of cervical spondylotic myelopathy using diffusion tensor imaging: Experiences in 1.5-tesla magnetic resonance imaging. Neuroradiol J. 2015 Oct; 28(5): 508–514.
14. Jones JG, Cen SY, Lebel RM, Hsieh PC, Law M "Diffusion tensor imaging correlates with the clinical assessment of disease severity in cervical spondylotic myelopathy and predicts outcome following surgery". AJNR Am J Neuroradiol. 2013 Feb;34(2):471-8
15. Zheng W, Chen H, Wang N, Liang Y, Xiao W "Application of Diffusion Tensor Imaging Cutoff Value to Evaluate the Severity and Postoperative Neurologic Recovery of Cervical Spondylotic Myelopathy". World Neurosur. 2018 Oct;118: e849-e855.

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Analysis of different pathologies presenting as acute abdomen and performance of MDCT in making the diagnosis with clinical, intra-operative and histopathology correlation

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Abstract
Background: Acute abdomen is one of the most common emergencies presenting in the emergency room which requires immediate attention. Making an accurate diagnosis is of paramount importance in managing these patients, since most of these patients frequently require a surgical intervention. The divergence of patient population and the underlying pathology in acute abdomen calls for high quality imaging studies to make quick and precise diagnosis. Multi-detector computed tomography is an ideal tool in this regard.
Materials and Methods: It is a prospective study conducted on 73 patients who presented with acute abdomen and subsequently underwent multi-detector computed tomography to ascertain the underlying pathology. The radiological findings in computed tomography were correlated with clinical, intra-operative and histopathological findings whenever available.
Results: In our study the performance of multi-detector computed tomography, when compared with diagnosis made based on intra-operative findings, post-operative histopathology and clinical findings showed sensitivity of 97% and specificity of 75%. Overall Positive Predictive value of 98.5% and negative predictive value of 60% and accuracy of 96%.
Conclusion: Since most of the patients presenting with acute abdomen have non-specific and overlapping clinical findings, making an accurate clinical diagnosis is challenging. In this scenario MDCT comes as a handy tool with high accuracy and good sensitivity and specificity. The results obtained in our study are comparable with other studies conducted worldwide.

Keywords: Acute abdomen, MDCT performance, acute abdominal pain

Introduction
Acute abdomen is a clinical entity in which patients present with sudden onset of intense abdominal pain necessitating emergency medical/surgical management [1]. Majority of these patients present to the emergency department. The underlying pathology in acute abdomen could be anything ranging from acute inflammatory conditions involving the appendix, gallbladder, pancreas to non-specific pain usually associated with mesenteric adenitis, especially in children. In a given patient there are numerous differential diagnoses and making a single specific diagnosis is seldom seen and it is very difficult, given the overlap of presenting symptoms and clinical examination findings in various pathologies. Further evaluation with laboratory blood tests and radiological investigations is part of management of these patients.

Patients presenting with acute abdomen are in severe pain and very sick. Hence it is essential to make a prompt and accurate diagnosis in these patients [2]. Most of these patients are initially evaluated with abdominal radiograph and ultrasound. The role of these basic radiological investigations is limited by various factors. Major limiting factors are two dimensional nature of radiographs. In ultrasound limiting factors are inability of the patient to cooperate for the study due to severe pain, thick body habitus of the patient, excessive bowel gas and lack of adequately filled urinary bladder. However radiograph has fairly good specificity in diagnosing pneumoperitoneum. It gives useful information in only in less than 50% of patients [2].

Ultrasound has its own advantages. It is cheap, easily available, no need to administer contrast and it is free of ionizing radiation, hence better suited for children and pregnant women. Ultrasound is a valuable tool in the assessment of pathology related to gallbladder and acute ureteric colic, especially when the obstruction is at the vesico-ureteric junction, which is by far the most common site.

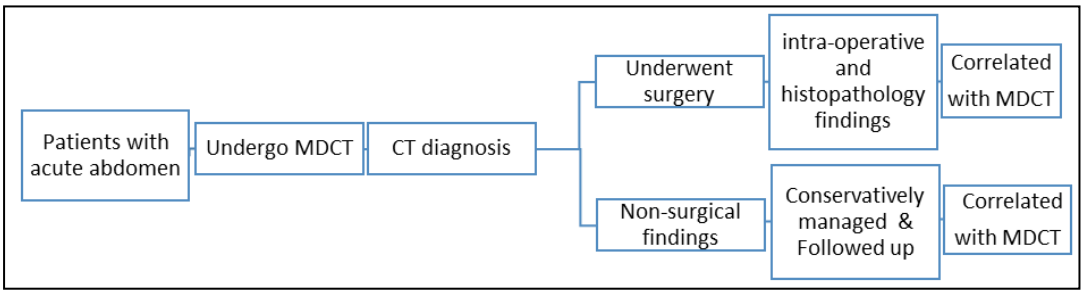
Though these basic radiological investigations do indeed provide valuable information, in a good number of patients computed tomography (CT) examination is essential. Multi-Detector CT (MDCT) is a widely accepted primary investigation of choice in these patients [3, 4, 5]. The scanning is fast, (acquisition time of 2-3 minutes), and yields specific diagnosis. In MDCT, multiple images can be acquired in a single tube rotation. The whole abdomen and pelvis can be scanned within a single breath hold at a slice thickness of less than a millimeter (0.3-1mm). These sub millimeter sections can be used to obtain good quality reconstructed images like multi-planar reconstructions (MPR) maximum intensity projection (MIP) and 3dimentional (3D) reconstruction**.** MDCT is a great tool for comprehensive evaluation of entire abdomen. There is excellent visualization of the solid organs, hallow viscera, vasculature, peritoneum and retroperitoneal structures along with lung bases.

Our study aims at analyzing the incidence and frequency of various pathologies presenting as acute abdomen in a tertiary care hospital and the accuracy of 128 slice MDCT in making a correct diagnosis by correlating the CT diagnosis with intra-operative and post-operative histopathology diagnosis, whenever available and clinical diagnosis if the patient does not undergo surgery. Intra-operative and post-operative histopathology are considered as gold standard.

Materials and Methods

It is a prospective study performed on 73 subjects who presented with acute pain abdomen, who were referred to radiology department for MDCT abdomen. The duration of this study was from May 2015 to June 2016. Prior to the commencement of the study, necessary clearance was obtained from the institutional ethics committee and written informed consent was taken from the participating patients. Inclusion criteria: Patients who are presenting with clinical symptoms of acute abdomen and undergoing MDCT. Exclusion criteria: Patients who have contraindication to iodinated contrast media either due to contrast allergy or due to impaired renal function. Patients lost to follow up.

CT protocol: MDCT was performed with Siemens SOMATOM definition edge 128 slice scanner. Oral iodinated contrast was not given in patients who are kept nil by mouth in view of immediate surgical exploration, who cannot tolerate oral liquids (pancreatitis), suspected high degree of bowel obstruction, and suspected vascular pathology where the study is done in abdominal angiogram protocol (oral contrast interferes with vascular reconstructions). All the other patients were administered oral and rectal contrast as per routine protocol. All patients underwent a non-contrast study followed by contrast study. Approximately 80-90ml (1-2ml/kg body weight) iodinated non-ionic contrast was given intravenously using a pressure injector at 4ml/sec rate. Images were obtained at arterial (20-25sec), venous (50-60seconds) and delayed phases (>120 sec) using bolus tracking and automatic triggering of acquisition. Raw data was acquired with slice thickness of 0.625mm with a pitch of 0.8-1.5. The images were reconstructed in 5mm thickness for viewing purpose and in 1mm thickness for doing various reconstructions [6].



Study design

Results

In this study 73 patients with acute abdomen underwent MDCT. The findings of MDCT were correlated with the intraoperative findings and histopathological findings in patients who had undergone surgery. In conservatively managed patients the MDCT findings were correlated with clinical course. Table 1 shows the age distribution in the study population.

Table 1: Age group wise distribution of acute abdomen in the study population

Age (years)	Frequency	Percentage
0 to 10	4	5
11 to 20	6	8
21 to 30	8	11
31 to 40	16	22
41 to 50	12	16
51 to 60	9	12
61 to 70	11	15
71 to 80	6	8
91 to 100	1	1
Total	73	100

The sex distribution of the study population is shown in figure 1.

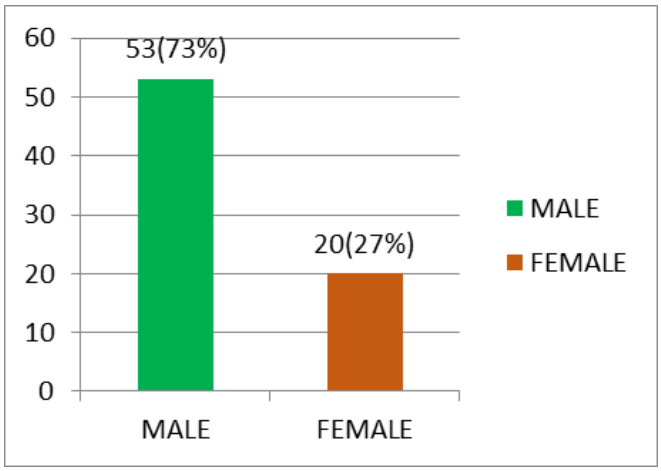


Fig 1: Gender distribution of acute abdomen.

Table 2 shows the frequency and percentage of the various pathologies detected among the study population.

Table 2: Distribution of various pathologies in study population.

No	Pathology	Frequency	Percentage
1	Appendicitis	16	22
2	Bowel obstruction	13	18
3	Acute pancreatitis	11	15
4	Perforation	10	14
5	Urolithiasis	5	7
6	Cholecystitis	4	5
7	Bowel ischemia	2	3
8	Aortic dissection	1	1
9	Diverticulitis	2	3
10	Aortic aneurysm	2	3
11	Intussusception	2	3
12	Volvulus	2	3
13	Nonspecific pain	3	4
	Total	73	100

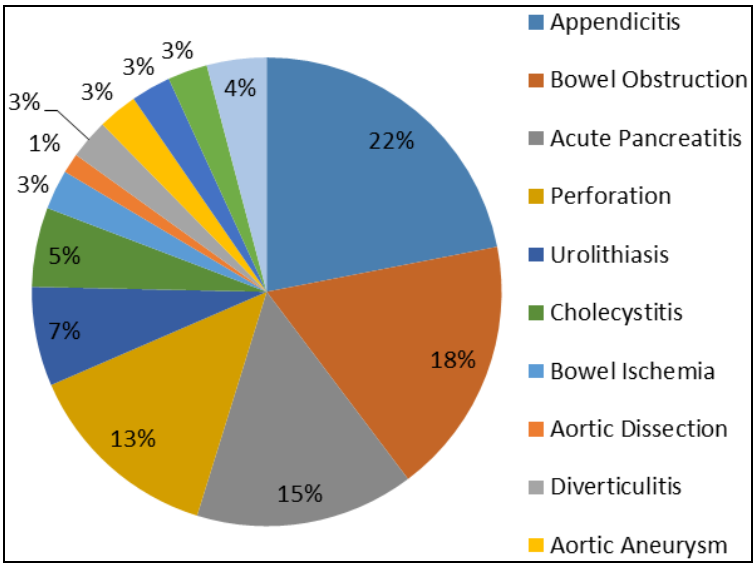


Fig 2: Pie chart showing percentage of various pathologies in acute abdomen

The pie chart (fig. 2) shows the percentage of patients affected by various pathologies, observed in the study population.

Table 3 shows the gender wise number of patients affected by various pathologies.

Table 3: Gender wise distribution of pathologies.

No	Pathology	Frequency	Male	Female
1	Appendicitis	16	10	6
2	Bowel Obstruction	13	9	4
3	Acute Pancreatitis	11	11	0
4	Perforation	10	7	3
5	Urolithiasis	5	3	2
6	Bowel Ischemia	2	2	0
7	Aortic Dissection	1	1	0
8	Cholecystitis	4	4	0
9	Diverticulitis	2	2	0
10	Aortic Aneurysm	2	2	0
11	Volvulus	2	0	2
12	Intussusception	2	1	1
13	Non-Specific Abdominal Pain	3	1	2
	Total	73	53	20

Demographics of few most common pathologies.

1. **Appendicitis:** Age and sex wise distribution of appendicitis in the study population is shown in figure 3.

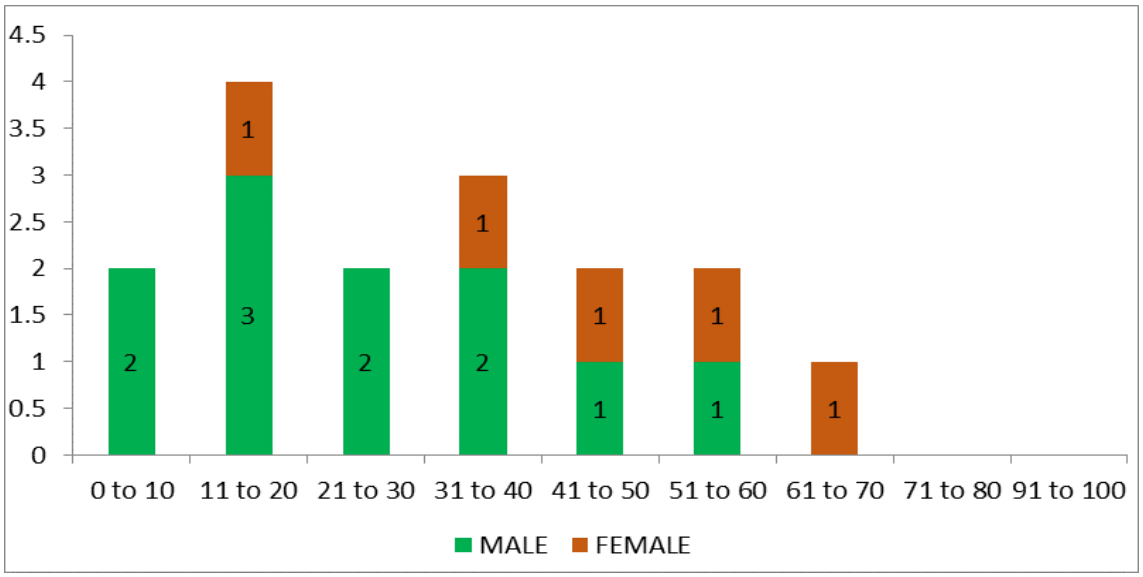


Fig 3: Gender and age wise distribution-appendicitis

2. **Bowel obstruction:** Age and sex wise distribution of bowel obstruction in the study population is shown in figure 4.

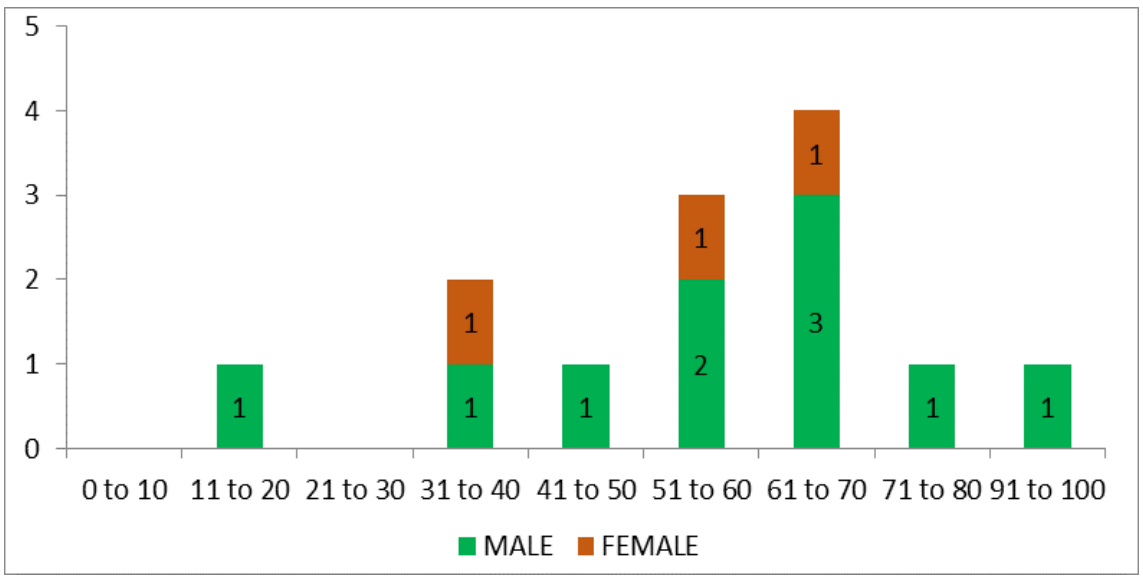


Fig 4: Gender and age wise distribution of bowel obstruction

3. **Pancreatitis:** Age and sex wise distribution of pancreatitis in the study population is shown in figure 5.

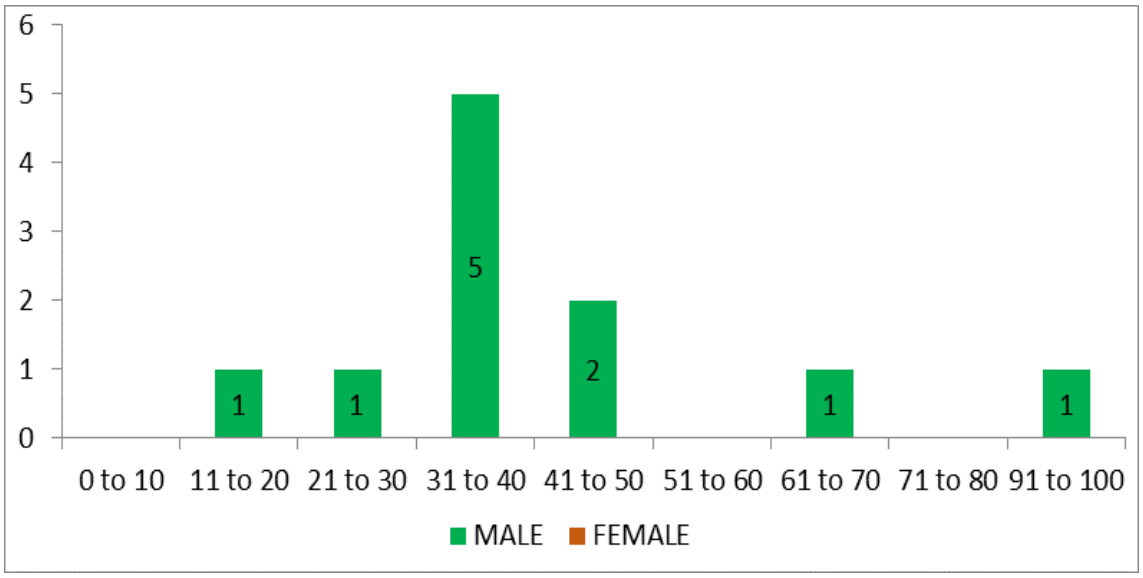


Fig 5: Gender and age wise distribution of pancreatitis

4. **Bowel perforation:** Age and sex wise distribution of bowel perforation in the study population is shown in figure 6.

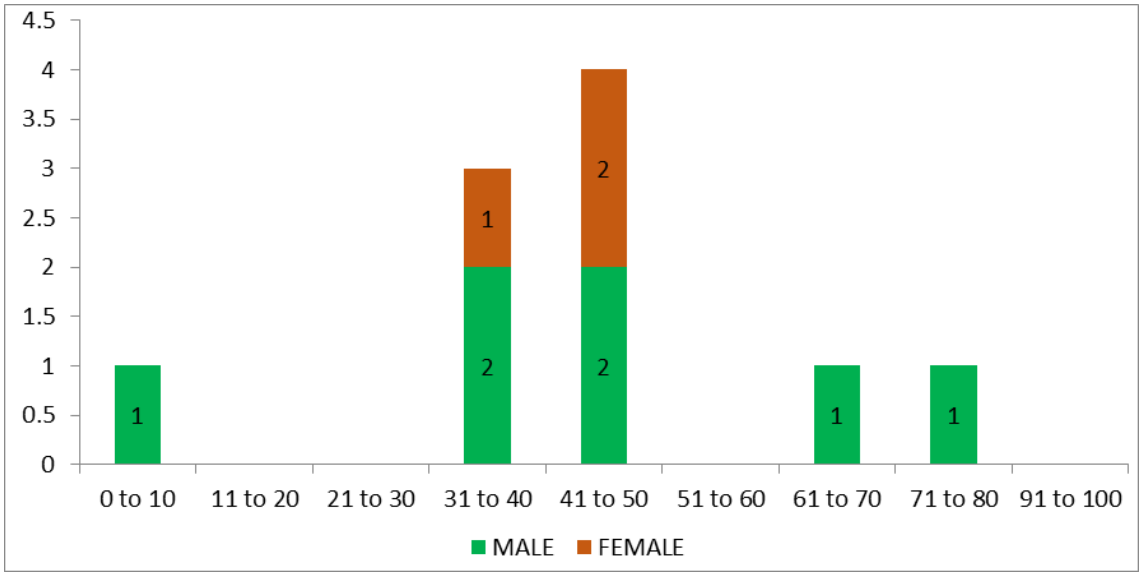


Fig 6: Gender and Age wise distribution of bowel perforation

Table 4 shows the overall diagnostic performance of MDCT compared with intra-operative and post-operative HPE diagnosis and clinical diagnosis.

Table 4: Overall diagnostic performance of MDCT

MDCT performance in acute abdomen	
Sensitivity	97.10%
Specificity	75.00%
Positive Predictive Value	98.53%
Negative Predictive Value	60.00%

Discussion

The current generation of the MDCT scanners with cutting edge technology have become the main stay of evaluation of patients with acute abdomen. The technology enables acquisition of isotropic images with exquisite spatial resolution and significantly reduced radiation exposure. The multi-row multi-detector spiral scanning technology has reduced scanning time significantly, leading to improved output and reduced movement related artefacts. The large volumetric data obtained in the axial plane allows reformations into any required plane with same resolution [7]. The advances in reformation techniques have made the process automatic which saves time. Further increase in the computing speed has facilitated faster radiological interpretation in critically ill patients. The shear speed and accuracy of CT in acute abdomen has made the role of plain radiography nearly obsolete.

In our study group of 73 patients 53 were males and 20 were females, ranging from 5 to 93 years. We analyzed various pathologies presenting as acute abdomen. Most common pathology found in our study was acute appendicitis, seen in 22% followed by bowel obstruction in 18%, acute pancreatitis 15% and bowel perforation 14%. Among which appendicitis is the most common cause and is consistent with most of the studies carried out internationally [8].

However we did not encounter any definitive gynecologic pathology, which may be due to the fact that patients with gynecological pathology were primarily evaluated by ultrasound which itself can yield a specific diagnosis.

In our study 16 patients had CT findings suggestive of acute appendicitis and related conditions (Figure 7). CT diagnosis in one out of these16 cases was non-concordant with intraoperative surgical findings. Intra-operatively it was found to be ileo-cecal tuberculosis which was confirmed by histopathological examination. The actual CT findings in this case were masked by clumping of bowel loops in the right iliac fossa, which made visualization of appendix difficult. This was erroneously diagnosed as inflammatory appendicular mass.

The sensitivity and Positive Predictable Value in acute appendicitis were 100% and 93.75% respectively. Intra operative findings very well correlated with CT findings except for one case. This is consistent with study conducted by Rao PM *et al.* [9] which shows 91% to 100% sensitivity for CT in the diagnosis of appendicitis. In another study done by Mawiah H. Alshebromi *et al.* [10] CT showed high sensitivity of 100% and poor specificity of mere 16%.



Fig 7: Axial and coronal MPR images showing a typical case of appendicitis with thickened and enhancing appendix (arrow in a). Coronal MPR (arrow in b) in same patient

In our study 13 out of 73 cases were diagnosed to have bowel obstruction. The various etiologies of bowel obstruction are stricture, adhesion, hernia and mesenteric band [11]. The sensitivity and positive predictable value were 100%. Our results are comparable with Mallo *et al.* [12] in which sensitivity of MDCT in diagnosis of bowel obstruction was 81% to 100% and specificity 68% to 100%. A study conducted by Suri S *et al.* [13] shows that CT is highly sensitive in determining level and cause of obstruction. This is made possible by the exquisite coronal and sagittal reformatted MPR images [14] which are very useful in identifying the transitional zone (Figure 8a). MPR images in any oblique or orthogonal plane are good in identifying intussusception and the underlying pathology as well (Figure 8b)

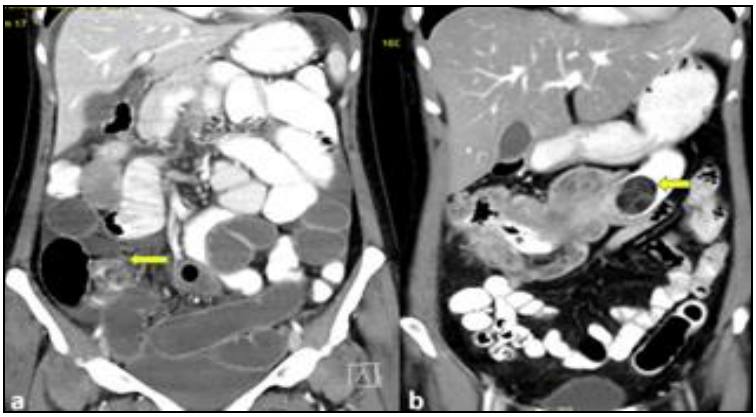


Fig 8: Case of acute bowel obstruction. Coronal MPR shows the transitional zone, in this case it was adhesion causing obstruction (a); Colo-colic intussusception and the underlying pathology, lipoma are shown well in coronal MPR (b).

We encountered 11 cases of pancreatitis out of 73 cases. In all the cases MDCT correctly identified the pathology. In all of the cases diagnosis was confirmed by abnormal levels of elevated pancreatic enzymes. The sensitivity of MDCT in acute pancreatitis was 100%. Comparable results were shown by B. Gianni *et al.* [15]. This is a disease entity where CT clearly scores over ultrasound, because visualization of pancreas itself, in most of the cases of pancreatitis is interfered by bowel gas. Estimation of pancreatic necrosis is impossible with ultrasound, which is the key factor in management and prognostication. CT also is better in identifying and quantifying the peripancreatic collections, pseudo cysts and identifying air in emphysematous pancreatitis (Figure 9).

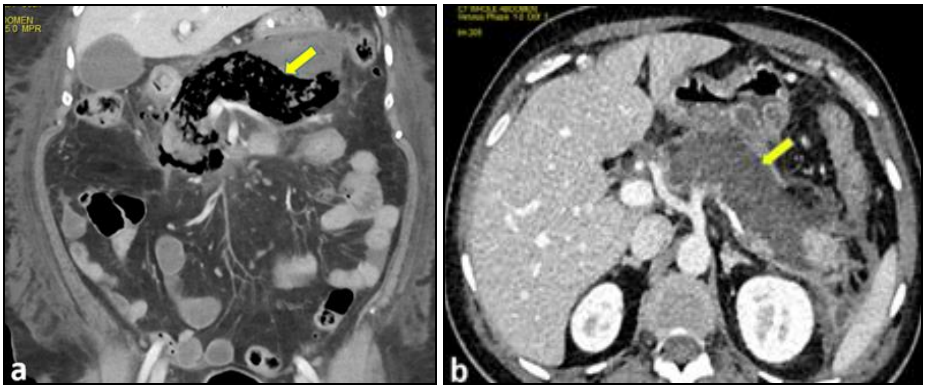


Fig 9: Coronal MPR showing extensive air replacing the entire pancreas, suggestive of emphysematous pancreatitis (arrow in a). Axial post contrast image shows extensive necrosis of the pancreatic parenchyma (arrow in b)

In our study 8 out of 10 cases of confirmed perforation were correctly diagnosed by MDCT. Two missed cases presented very late in the course of the disease, nearly after a week. Because of this delay in presentation these cases lacked the typical imaging features. Hence the perforation had already been sealed off and the pneumoperitoneum had been absorbed, the diagnosis was missed. In hallow viscus perforation the site of perforation can be exactly identified with MDCT. The early and occult perforations and sealed off perforations can be made out with MDCT, due to its ability to detect even smallest amount of free air. The sensitivity and accuracy for bowel perforation in our study was 80%, which was comparable to the study done by Sung Hwan Kim *et al.* and other investigators [16, 17, 18] who gave an accuracy of 82% to 90% for predicting site of perforation by CT.

In our study 5 out of 73 cases had urolithiasis, all the 5 cases were correctly identified by MDCT with sensitivity of 100%. The above findings are comparable with study performed by Isabelle Boulay *et al.* and others [19, 20, 21]. Leschka Sebastian *et al.* [5] state that thin slices and good multi-planar reconstructions helps in identifying even sub-millimeter calculi (Figure 10). This is complemented by the fact that nearly 100% of the renal calculi are radio-opaque.



Fig 10: Coronal MPR image showing the dilated calyces, and ureter in its entirety with an obstructing calculus in the mid ureter (arrow).

In our study 4 out of 73 cases had CT findings suggestive of cholecystitis with sensitivity of 100% which is comparable to other international studies. However the limitation of CT in gall bladder pathology is its failure to detect radio-lucent calculi, which could be the underlying predisposing factor for cholecystitis. Ultrasound and Magnetic Resonance Imaging (MRI) have better sensitivity in identifying Cholelithiasis. MDCT clearly performs better than ultrasound in detecting the complications like emphysematous cholecystitis and gangrenous cholecystitis. MDCT is the most sensitive modality in identifying complications related to cholecystitis as stated by L. Turturici *et al.* [22]

In 3 out of the 73 patients no significant abnormalities were detected by MDCT. And this was the case even in diagnostic laparoscopy. These patients were considered to have non-specific abdominal pain. The pathology in these patients presumed to be due to transient pain caused by intestinal colic or pain caused by a passing ureteric calculus, which would have completely passed out from the urinary bladder also, hence not seen in CT.

In our study the overall accuracy, sensitivity, specificity and positive and negative predictable values of MDCT were 96%, 97%, 75% and 98% and 60% respectively which were comparable to the study results of Monica Mangini *et al.* [23]

Conclusion

Acute abdomen is a commonly encountered, usually a surgical emergency. In our study the most commonly encountered pathologies were appendicitis, bowel

obstruction, bowel perforation and acute pancreatitis. It is essential to make a speedy and accurate diagnosis in these patients to decide on the management as to subject them to surgery or treat conservatively. For this purpose we need to employ a robust diagnostic tool. The clinical examination and laboratory parameters have very poor sensitivity and specificity, hence cannot be dependent upon. Very good sensitivity and specificity of MDCT makes it an ideal tool in the evaluation of acute abdomen. Recent advances in hardware, software and computation speed have made MDCT a powerful diagnostic tool in acute abdomen. MDCT scores over all other imaging modalities because of its speed and versatility.

Limitations of the study: Our study employed relatively small sample size, mainly because many patients were taken up for surgery based on ultrasound/radiography findings alone without CT. Though ultrasound may not give a specific diagnosis, these patients were taken up for surgery without CT, in the background of a given clinical scenario, like presence of guarding and rigidity in a suspected case of hallow viscus perforation where ultrasound showed free fluid and radiograph showed pneumoperitoneum which necessitated immediate laparotomy. Baring few specific conditions like gallbladder pathology and gynecological pathology MDCT is the primary modality of choice for evaluating acute abdomen.

References

1. Silen W. Cope's early diagnosis of the acute abdomen, 19th Ed. New York: Oxford University Press, 1996.
2. Suhail Rafiq *et al.*, Multidetector computerized tomography in acute abdomen, International Surgery Journal. 2019; 6(7):2353-2357.
3. Trott AT, Lucas RH. Acute abdominal pain. In: Rose P, ed. Emergency medicine, 4th ed. St. Louis: Mosby, 1998, 1888-1903.
4. Bolog N, Popiel A, Oancea I, Mangrau A, Beuran M, Nicolau E. Multidetector row spiral CT (MDCT) in bowel related non-traumatic abdominal emergencies. J gastroenterol. 2003; 12:319-324.
5. Frauenfelder T, Wildermuth S, Marincek B, Boehm T. Nontraumatic emergent abdominal vascular conditions: Advantages of multidetector row CT and three-dimensional imaging. Radiographics. 2004; 24:481-496.
6. Leschka Sebastian, Alkadhi H, Wildermuth S, Marincek B. Multidetector computed tomography of acute abdomen. Eur Radiol 2005; 15(12):2435–2447.
7. Adriano Massimiliano Priola *et al.*, Accuracy of 64-row multi detector CT in the diagnosis of surgically treated acute abdomen, Clinical Imaging 37 (2013) 902–907.
8. Radiologic Clinics of North America RSS. -11-1. 2015; 53(6): i-134.
9. Rao PM, Rhea JT, Novelline RA. Sensitivity and specificity of the individual CT signs of appendicitis: experience with 200 helical appendiceal CT examinations. J Comput Assist Tomogr. 1997; 21:686-692.
10. Mawiah H. Alshebromi, Sensitivity and specificity of computed tomography and ultrasound for the prediction of acute appendicitis at King Fahad Specialist Hospital in Buraidah, Saudi Arabia, Saudi Med J. 2019; 40(5):458-462.
11. Durgesh Kumar Saini, Poras Chaudhary, Chikkala Kanak Durga, Kiran Saini: Role of multislice computed tomography in evaluation and management of intestinal obstruction Post graduate Institute of Medical Education and Research, Dr. Ram Manohar Lohia Hospital, New Delhi, India Clinics and Practice. 2013; 3:e20.
12. Mallo RD, Salem R, Lalani T. Computed tomography diagnosis of ischemia and complete obstruction in small bowel obstruction: a systematic review. J Gastro in test Surg. 2005; 9:690-4.
13. Suri S, Gupta S, Sudhakar PJ, Venkataramu NK, Sood B, Wig JD. Comparative evaluation of plain films, ultrasound and CT in the diagnosis of intestinal obstruction. Acta Radiol. 1999; 40:422-8.
14. Ginanni B, Ruschi F, Turturici L, Donatelli G, Scionti A, Bianchi F *et a*. Orsitto; Pisa/IT: Multidetector-CT (MDCT) findings in Acute Pancreatitis (AP) 10.1594/ecr2014/C-2071
15. Sung Hwan Kim *et al.*: Gastro intestinal tract perforation: MDCT findings according to the perforation sites, Koren J radiol, 2009, 10(1).
16. Imuta M *et al.* Multidetector CT findings suggesting a perforation site in the gastrointestinal tract: analysis in surgically confirmed 155 patients Radiat Med. EPUB. 2007; 25(3):113-8.
17. Hainaux B, Agneessens E, Bertinotti R *et al*. Accuracy of MDCT in predicting site of gastrointestinal tract perforation. AJR Am J Roentgenol, 2006, 1.
18. Isabelle Boulay *et al.*: Ureteral Calculi: Diagnostic Efficacy of Helical CT and Implications for Treatment of Patients, AJR. 1999; 172:1485-1490.
19. Katz DS, Lane MJ, Sommer FG. Unenhanced helical CT of ureteral stones: incidence of associated urinary tract findings. AJR Am J Roentgenol. 1996; 166:1319-1322.
20. Smith RC, Verga M, McCarthy S, Rosenfield AT. Diagnosis of acute flank pain: value of unenhanced helical CT. AJR Am J Roentgenol. 1996; 166:97-101.
21. Turturici L, Ginanni B, Donatelli G, Ruschi F, Scionti A, Bianchi F *et al*. Orsitto; Pisa/IT: Acute Cholecystitis: Ultrasonography and CT indications in the emergency department; 10.1594/ecr2014/C-2123
22. Monica Mangini *et al.*: Non-traumatic acute bowel disease: differential diagnosis with 64-row MDCT, Emerg Radiol. 2008; 15:171-178.

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Evaluation of acoustic radiation force impulse (ARFI) elastography as a tool for non invasive detection and grading of liver fibrosis with histopathology correlation

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Abstract
Background: Chronic insult caused by viral infection, autoimmune disease, alcohol and other toxins can damage the liver parenchyma and ultimately lead to liver fibrosis and cirrhosis. Acoustic Radiation Force Impulse (ARFI) elastography is emerging as a valuable tool in the detection and grading of fibrosis in liver.
Materials and Methods: It is a prospective comparative study performed in 36 patients between December 2016 and March 2018 who presented with deranged liver function test and referred for ultrasonography, ARFI elastography of liver followed by liver biopsy. The findings of Elastography were correlated with histopathology.
Results: When compared with histopathology ARFI had a sensitivity of 82%, specificity of 85%, positive predictive value of 90% and negative predictive value of 75% in detecting liver fibrosis when we used optimum cut values of ARFI.
Conclusion: ARFI elastography is a valuable tool in non-invasively detecting liver fibrosis and can be included as part of routine ultrasonic examination protocol of liver. ARFI technology allows the quantification of liver fibrosis and can be effectively used to stage fibrosis. Hence ARFI can be used as an alternative to liver biopsy and it might substitute liver biopsy in future.

Keywords: Elastography, ARFI, liver fibrosis grading

Introduction
The incidence of chronic liver parenchymal disease is constantly on the raise. The major underlying causes are alcoholism and viral hepatitis [1]. In response to continuous inflammation and injury there is fibrosis [2, 3, 4] and regeneration which result in liver cirrhosis and the attendant complications like portal hypertension, variceal bleed, ascites, liver failure, hepatic encephalopathy and even development of hepatocellular carcinoma[1]. Generally cirrhosis is considered to be irreversible, however accumulating evidence suggests that it could be reversible to some extent, especially when targeted therapy is given [1, 3, 5]. Hence, early detection and grading the severity of liver fibrosis is of utmost importance for treatment. The degree of hepatic fibrosis is an important factor which influences the treatment and determines the prognosis of the underlying liver disease [1]. Therefore, there is tremendous need for quantitative assessment and grading of hepatic fibrosis. The method used traditionally for assessing the degree of liver fibrosis is liver biopsy. Which is considered as gold standard method for determining the extent of liver fibrosis. However it is an invasive procedure which is associated with patient discomfort and occasionally severe complications like bleeding, infection and rarely death [1]. Histopathological examination (HPE) of liver fibrosis is greatly dependent on the size of the sample and segment from which the sample is obtained. Generally a biopsy specimen of size 1.5-2cm is required by the pathologist for accurate evaluation. The distribution of fibrosis within the liver is very non-uniform and sampling errors are bound to happen. [4, 6, 7]These disadvantages associated with liver biopsy have been the thrust behind pursuing alternate methods to assess liver fibrosis. ARFI Elastography is a recently introduced technology to evaluate tissue stiffness non-invasively.

The technology of ARFI Elastography involves the mechanical excitation of tissue using short-duration acoustic pulses (push pulses) in a region of interest, producing shear waves that spread away from the region of interest, perpendicular to the acoustic push pulse, generating localized, micron-scale displacement of the tissue [8, 9]. Simultaneously, detection waves of lower intensity than that of the push pulse (1:100) are generated. By recording the shear wave front at several locations and correlating these measurements with the elapsed time, the shear wave velocity (SWV) can be quantified in meter per second unit. Generally, the stiffer a region in the tissue, the greater the SWV as it travels through this region. This technique can be used in various organs and it is well suited for liver in view of the size and relative superficial location of the organ. The advantage of this technology is that it is incorporated in an ultrasound machine and the operator can use the ultrasound to measure the stiffness of the tissue of interest in real time.

Materials and Methods

It is a prospective study performed from December 2016 to March 2018. Before starting the study necessary clearance was obtained from the institutional ethics committee and informed written consent was obtained from the patients. The study population includes 36 patients with clinical and laboratory features of altered liver function who were referred to radiology department for further evaluation with ultrasound. All the patients underwent liver sonography, ARFI Elastography and subsequently, with in a period of two weeks, liver biopsy. The findings of Elastography were correlated with HPE. Inclusion criteria: patients under evaluation for altered liver function parameters, nonalcoholic-steatohepatitis, chronic viral infections, patients undergoing liver biopsy for assessment of fibrosis. Exclusion criteria: Patients with bleeding disorders, altered coagulation parameters, patients unable to hold their breath and uncooperative. Ultrasound protocol: Examination was performed in SEIMENS S2000 machine in supine position. B mode Ultrasound: to assess the size, echogenicity and echo-texture of liver. ARFI Elastography: Total of 8 measurements of shear wave velocity were taken, at least 2 from each segment of right lobe. Measurement was calculated by placing a 0.5 x 1cm box at least 1.5 to 2cm deep to the liver capsule (Fig. 1) and away from major vessels. The velocity was calculated in meter per second and the mean and median velocity of all 8 measurements were used to grade the stiffness of the liver.

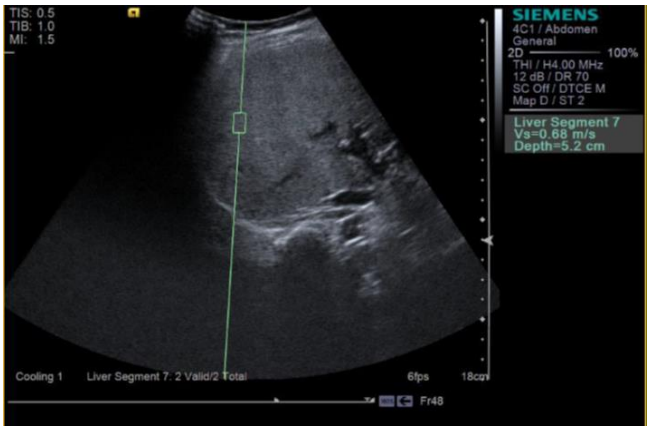


Fig 1: Measurement of ARFI by placing an ROI in each segment of the right lobe of liver.

Biopsy: Biopsy of the liver was obtained using 18G fully automatic gun (Bard MaxCore) with 22mm long cutting needle harvesting a 20mm long core. The specimen was fixed in formalin and sent for HPE. Fibrosis was scored in histopathology using the METAVIR scoring system. The cut of value [10] of shear wave velocities corresponding to METVIR grades is shown in Table 1:

Table 1: METAVIR Score and corresponding mean SWI in ARFI

Metavir Score	Histopathology	Mean Shear Wave Velocity
F0	No fibrosis	1.09+/- 0.42m/s
F1	Portal fibrosis without septa	1.22+/- 0.41m/s
F2	Portal fibrosis with few septa	1.37+/- 0.48m/s
F3	Numerous septa without cirrhosis	1.70+/- 0.59m/s
F4	Cirrhosis	2.23+/- 0.71m/s

Results

In this study 36 patients were evaluated with Ultrasonography and Elastography, the findings were compared with the HPE findings. In our study out of 36 patients 10 (28%) patients were female and 26 (72%) were male who presented with liver disease and altered liver function tests with different underlying pathologies like Nonalcoholic steatohepatitis (NASH), chronic viral hepatitis, storage disease and Cryptogenic cirrhosis. Our study had patients ageing from 11 years to 60 years with an average of 30 years. We had 14 (39%) patients below the age of 30 and 22(61%) patients more than 30 years of age. The distribution of various grades of fibrosis in the study population is shown in figure 2. The number of patients in the study population with various grades of fibrosis in ARFI and HPE is shows in table 2.

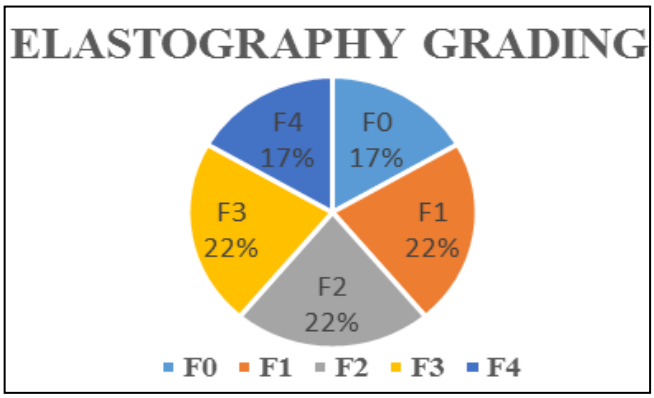


Fig 2: Distribution of various degrees of fibrosis in the study population in ARFI.

Table 2: Grading of fibrosis

	ARFI		HPE	
	Frequency	Percent	Frequency	Percent
F0	6	17	10	28
F1	8	22	4	11
F2	8	22	8	22
F3	8	22	4	11
F4	6	17	10	28
Total	36	100	36	100

B mode ultrasound findings of liver are shown in Table 3. Out of the 36 patients 4 had normal appearing liver, majority showed diffuse fat infiltration in the liver and 8 patients had altered echo-texture of liver parenchyma.

Table 3: Ultrasound findings in study population

Frequency		Percent
Normal	4	11
Steatosis	24	67
Altered echo texture	8	22
Total	36	100

In our study of 36 patients, ARFI showed clinically insignificant fibrosis in 16 patients with a METAVIR score of F0, F1 which was commonly seen with patients of acute hepatitis and nonalcoholic steatohepatitis. Clinically significant fibrosis (F2, F3, and F4) was seen in 20 patients with a majority of them affected by chronic viral hepatitis. (Tables 4).

Biopsy showed clinically insignificant fibrosis (F0, F1) in 14patients (39%) and clinically significant fibrosis (F2, F3, F4) in 22 patients (61%). Of the 14 patients who had insignificant fibrosis, different underlying etiologies such as NASH, storage diseases and autoimmune hepatitis were found, patients with significant fibrosis had viral hepatitis. (Tables 4).

Table 4: Significance of fibrosis in ARFI and HPE

	ARFI		HPE	
	Frequency	Percent	Frequency	Percent
Significant fibrosis	20	56	22	61
Insignificant fibrosis	16	44	14	39
Total	36	100	36	100

The cross tabulation comparing various grades of fibrosis in ARFI and HPE are shown in table 5.

Table 5: Elastography Grade Vs Histopathology Grade Cross Tabulation

			HPE Grade					Total
			F0	F1	F2	F3	F4	
ARFI Elastography Grade	F0	Count	2	0	2	0	2	6
		% with ARFI grade	33.3	0	33.3	0	33.3	100%
	F1	Count	4	4	0	0	0	8
		% with ARFI grade	50%	50%	0	0	0	100%
	F2	Count	4	0	4	0	0	8
		% with ARFI grade	50%	0	50%	0	0	100%
	F3	Count	0	0	2	4	2	8
		% with ARFI grade	0	0	25%	50%	25%	100%
	F4	Count	0	0	0	0	6	6
		% with ARFI grade	0	0	0	0	100%	100%
Total		Count	20	8	8	4	10	36
		% with ARFI grade	27.8%	11.1%	22.2%	11.1%	27.8%	100%

Table 6: Chi-Square Test

P value	
Pearson Chi-Square	0.028

The Chi square test shows a P = .028 which is statistically significant and proves elastography as a reliable tool for prediction of fibrosis and grading of liver fibrosis.

The cross tabulation comparing significant and insignificant fibrosis in ARFI and HPE is shown in table 7.

Table7: Significant Vs insignificant fibrosis in elastography and histopathology cross tabulation

			Histopathology		Total
			Significant	Insignificant	
ARFI	Significant fibrosis	Count	18	2	20
		% with ARFI	90%	10%	100%
	Insignificant fibrosis	Count	4	12	16
		% with ARFI	25%	75%	100%
		Count	22	14	36
		% with ARFI	61.1%	38.9%	100%

Table 8: Chi-Square Test

	P value
Pearson Chi-Square	0.005

The Chi square test shows a P = .005which is statistically significant.

Table 9: Sensitivity and specificity calculation

ARFI	Histopathology	
	Disease	No disease
Test positive	18(a)	2(b)
Test negative	4(c)	12(d)

Sensitivity: 82%, Specificity: 85%

Positive predictive value: 90%, Negative predictive value: 75%

Discussion

Generally fibrosis is thought to be an irreversible process till the discovery of hepatic stellate cells (HSC) in the 1980s. HSCs play an important role in fibrosis. Following chronic liver injury HSC under inflammatory milieu proliferate and activate to produce pro inflammatory and fibrogenic factors. Activated HSC produce large amounts of extracellular matrix (ECM). The extracellular matrix accumulates and distorts the liver architecture and forms a fibrous scar along with regeneration of hepatic tissue which subsequently lead to formation of the nodules. Studies carried out in the 1990s demonstrated that hepatic fibrosis was a reversible process if the cause was to be treated [2, 3, 4]. Since then researchers have taken keen interest in developing therapies for reversing/slowing down progression and severity of liver fibrosis. Thus, assessing the stage and severity of fibrosis precisely has taken the center stage in the management of chronic liver disease. In fibrosis and cirrhosis the pathological changes in tissue lead to alteration of mechanical properties like stiffness which can be measures noninvasively. The gold standard for assessing liver fibrosis

is biopsy and HPE. Liver biopsy, though safe, is an invasive procedure with a small risk of complications like severe hemorrhage. The risk of significant bleed is more when there is alteration of the coagulation parameters or in the presence of significant ascites. Both these risk factors are seen more frequently in patients with liver parenchymal disease, which in the first place is the indication for doing a biopsy. Percutaneous liver biopsy yields only a tiny fragment of the liver (1 in 50000 of the total volume of the liver) [11] which may lead to errors in staging of fibrosis, since there can be non-uniform distribution of fibrosis with in the liver. Bedossa *et al* [12] proved that the chance of under diagnosis of the stage of fibrosis, when the length of biopsy core is less than 2.5 cm can reach up to 25%. They have also proved that in almost half of the cases where liver fragments obtained from both the lobes of liver in the same session showed different stages of fibrosis. Hence in many ways liver biopsy is a flawed gold standard. For the past 10-15 years, enormous efforts have been made by several groups for establishing a reliable and reproducible noninvasive marker for liver fibrosis as a substitute to the invasive liver biopsy technique. In our study of 36 patients we have attempted to study the efficacy of ARFI compared to histopathology. The accuracy of ARFI elastography for assessing the severity of liver fibrosis and grading was evaluated. Calculating the sensitivity, specificity, positive predictive value and negative predictive value of the noninvasive test ARFI demonstrated a good sensitivity and specificity. Accurately grading and quantifying the severity of hepatic fibrosis secondary to an underlying disease is of great importance for planning appropriate treatment strategies. Studies comparing the non-invasive methods for evaluating the severity of fibrosis in chronic liver disease to liver biopsy are being performed to assess if these techniques would replace the invasive test. Virtual touch tissue imaging application enables ARFI technology to evaluate the deep tissues. SWI in meter per second can be quantified in a region of interest with ARFI technology in a precise anatomical location, which represents the elasticity property of the tissue. In our study we encountered 4 false negative patients. One of them was hepatitis B patient and another one patient with Hepatitis C. In these cases we presume values taken in the areas of necrosis may have contributed to low values. Another 2 patients were cases of alcoholic hepatitis, in whom the steatosis may have contributed to the falsely low values. We also had 2 false positive patients encountered in our study. One of them had hepatitis B acute stage and another one patient had hepatitis A. Hepatitis is known to have both inflammation and fibrosis, one of which may predominate depending upon the stage of disease [13, 14]. In acute stage because of inflammation, we can get erroneous high values [15, 16]. There are no possible means to differentiate the high values due to inflammation from that due to fibrosis. This may explain the false positivity in the hepatitis patients.

A study by Takahashi *et al*. [17] of 80 patients with mixed etiologies using ARFI (Siemens Healthcare), had a sensitivity and specificity of 91% and 80% respectively for F>2 stage of fibrosis. This is in concordance with the results in our study. In a similar study by Sporea at al. [18] using 114 patients with mixed etiologies, also had a sensitivity and specificity of 89% and 68% respectively. We attempted to study whether ARFI was sensitive in patients with NASH, who form the majority of the mixed etiology liver disease without viral hepatitis group. 10%-15% of NASH patients show a progression in the severity of fibrosis and architectural remodeling, and liver cirrhosis is seen in 15%-25% of cases. Three to 5% of people with fatty liver may develop cirrhosis. Cirrhosis due to NASH and cryptogenic cirrhosis have risk of developing HCC. HCC may rarely occur in a non-cirrhotic NASH patient. Hence lies the importance of detecting early fibrosis in patients with NASH [18, 19]. In a study conducted by Fraqueli *et al* [20] using 200 patients with mixed etiology liver diseases other than viral hepatitis, the sensitivity and specificity of ARFI was 72% and 84% respectively. In a study of 106 patients with HBV and HCV conducted by Friedrich-Rust *et al* [21] the sensitivity and specificity was 69% and 92% respectively. In our analysis of 28 HBV and HCV patients, we had a sensitivity of 90% and specificity of 75%. The liver stiffness assessment is challenging in patients with viral hepatitis because of the nature of underlying disease. There may be increased necro-inflammatory changes in the acute phase of the disease which undergoes fibrosis later on. The necrotic areas will give lower stiffness values compared to the other areas of inflammation thus may decrease overall sensitivity. The higher sensitivity in our study may be because these patients were assessed in the advanced stage of the disease. Review of previous publications and our own study has shown that US elastography definitely has a role in the staging of fibrosis where it has the potential of replacing liver biopsy. Another upcoming and exciting non-invasive modality to evaluate liver fibrosis is MR Elastography which has the advantage of avoiding sampling errors and operator variability. New MR imaging contrast agents that target specifically the collagen and other extracellular matrix macromolecules may be developed in the near future. A collagen-specific MR imaging contrast agent and also contrast agents that selectively target hepatic stellate cells and other cells involved in the pathogenesis of liver fibrosis may permit imaging of fibrogenesis or fibrolysis. All such data may provide valuable information for guiding antifibrotic therapy development and monitoring patients in clinical trials. Thus these noninvasive methods may help in the assessing the severity of fibrosis.

Conclusion

Acoustic Radiation Force Impulse (ARFI) elastography is a promising noninvasive tool to quantify liver fibrosis. It also has the advantage of routine ultrasound assessment of the liver during the same session of elastography study. ARFI measurements are done in real time under vision. As many number of measurements as the operator wishes can be obtained in a single study. The assessment can be repeated frequently as and when required. The technique has good sensitivity and specificity and less operator dependent when compared with other ultrasound techniques as the calculation is done automatically by the system and there are tools which are useful to obtain a good quality study. The technique has good sensitivity and specificity which has the potential to replace invasive biopsy. Limitations: Our study is limited by the relatively small sample size. The small sample size is primarily due to limitation in the number of patients undergoing biopsy. ARFI elastography is a relatively new technology that has not been extensively investigated, hence large prospective studies are needed to ascertain its efficacy and to form strict cut off values for the shear wave velocity. Different technologies and

measurement calculations are adopted by different ultrasound venders. Because of this standard values are not available for comparison across different ultrasound systems [22], which needs to be addressed by various ultrasound manufacturing vendors and an amicable consensus to be arrived at.

References

1. Jain VA, Dixit RA, Chowdhury V. Can acoustic radiation force impulse elastography be a substitute for liver biopsy in predicting liver fibrosis? Clinical Radiology. 2016; 71:869-875.
2. Friedman SL. Hepatic fibrosis: overview. Toxicology. 2008; 254(3):120-129.
3. Wallace K, Burt AD, Wright MC. Liver fibrosis. Biochem Genet. 2008; 411(1):1-18.
4. Afdhal NH, Nunes D. Evaluation of liver fibrosis: a concise review. Am J Gastroenterology. 2004; 99 (6):1160-1174.
5. Ruediger S. Goertz, Luise Gaßmann, Deike Strobel, Dane Wildner, Acoustic Radiation Force Impulse (ARFI) Elastography in Autoimmune and Cholestatic Liver Diseases, Annals of Hepatology. 2019; 18(1):23-29.
6. Haaga, John RCT, MRI of the whole body, 5th edition. Philadelphia: Mosby/Elsevier. 2009; 2:1455-1460.
7. Heiken JP. Liver. In: Lee JK, Sagel SS, Stanley RJ, Heiken JP. Computed Body Tomography with MRI correlation, 3rd ed, Philadelphia: Lippincott Williams and Wilkins. 1998; 1:701-777.
8. Kanzler S, Baumann M, Schumacher P, Dries V, Bayer E, Gerken G *et al*. Prediction of progressive liver fibrosis in hepatitis C infection by serum and tissue levels of transforming growth factor-beta. J Viral Hepat. 2001; 8:430-437.
9. Nelson DR, Gonzalez-Peralta RP, Qian K, Xu Y, Marousis CG, Davis GL *et al*. Transforming growth factor-beta 1 in chronic hepatitis C. J Viral Hepat. 1997; 4:29-35.
10. Simona Bota, Ioan Sporea, Roxana Şirli, Alina Popescu, How useful are ARFI elastography cut-off values proposed by metaanalysis for predicting the significant fibrosis and compensated liver cirrhosis? Medical Ultrasound. 2015: 17(2):200-205.
11. Emily Carey, DO, William D. Carey, MD, Noninvasive tests for liver disease, fibrosis, and cirrhosis: Is liver biopsy obsolete? Cleveland clinic Journal of Medicine. 2010; 7(8):7.
12. Pierre Bedossa, Delphine Darg`ere, Valerie Paradis, Sampling Variability of Liver Fibrosis in Chronic Hepatitis C, Hepatology. 2003; 38:1449-1457.
13. Chen S, Sanchez W, Callstrom MR, Gorman B, Lewis JT, Sanderson SO *et al*. Assessment of liver viscoelasticity by using shear waves induced by ultrasound radiation force. Radiology. 2013; 266:964-970.
14. Davies G, Koenen M. Acoustic radiation force impulse elastography in distinguishing hepatic haemangiomata from metastases: preliminary observations. Br J Radiol. 2011; 84:939-943.
15. Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B *et al*. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. J Hepatol. 2011; 55:666-672.
16. Takahashi H, Ono N, Eguchi Y, Eguchi T, Kitajima Y *et al*. Evaluation of acoustic radiation force impulse for fibrosis staging of chronic liver disease: a pilot study. Liver Int. 2010; 30:538-545.
17. Sporea I, Sirli RL, Deleanu A, Popescu A, Fosca M, Danila M *et al*. Acoustic radiation force impulse elastography as compared to transient elastography and liver biopsy in patients with chronic hepatopathies. Ultraschall Med. 2011; 32:S46-S52.
18. Hashimoto E, Yatsuji S, Tobari M, Taniai M, Torii N, Tokushige K *et al*. Hepatocellular carcinoma in patients with non alcoholicsteatohepatitis. J Gastroenterol. 2009; 44:89-95.
19. Timothy J. Hall, Milkowski y, Brian Garra, Paul carrson *et al* RSNA/QIBA: Shear wave speed as a biomarker for liver fibrosis staging.
20. Fraquelli M, Rigamonti C, Casazza G *et al*, Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease, Gut. 2007; 56:968-973.
21. Mireen Friedrich-Rust MD, Katrin Wunder MD, Susanne Kriener MD, Liver Fibrosis in Viral Hepatitis: Noninvasive Assessment with Acoustic Radiation Force Impulse Imaging versus Transient Elastography, Radiology: August. 2009; 252(2):595-604.
22. 12m Hittalamani IM, Lakhkar BB, Pattanashetti RC, Lakhkar BN. Acoustic radiation force impulse elastography of liver as a screening tool for liver fibrosis in alcoholic liver disease. Indian J Radio Imaging. 2019; 29:190-4.

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Case Report

Hereditary hemorrhagic telangiectasia of liver: Pathophysiology with role of radiology in diagnosis and treatment

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Abstract

Hereditary hemorrhagic telangiectasia (HHT) or Osler–Weber–Rendu syndrome is a rare condition which can result in significant *systemic and hepatobiliary* abnormalities. Liver involvement in HHT consists primarily of the consequence of various intrahepatic shunts. Even though these vascular shunts are present in the majority of patients with HHT, symptoms occur only in *minority* with clear predilection to *female gender*. The symptoms and imaging findings of liver vascular malformations can be easily overlooked or misdiagnosed which can result in delay in treatment or potentially harmful vascular interventions. In this case report, we discuss the pathophysiology of HHT in liver involvement, role of imaging in diagnosis, and the possible role of interventional radiologist in the treatment.

Key words: Embolization for shunt reduction; hereditary hemorrhagic telangiectasia; intrahepatic shunts; liver vascular malformation

Introduction

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder seen approximately in one in 5000–8000 individuals.[1] It presents with vascular malformations (VM) in the nose, skin, liver, gastrointestinal tract, brain, and lung. Telangiectasia is the VM seen in HHT where there is direct communication between the arterioles and venules without the intervening capillaries. This abnormal communication can result in shunts between various vascular beds in the solid organs and hallow viscera. Definitive clinical diagnosis of HHT can be made based on the presence of at least three of four Curacao criteria,[2] published in 2000. They are 1. Epistaxis, 2. Cutaneous or mucosal VM, 3. Presence of visceral telangiectasia, and 4. Positive family history. Liver involvement is seen in approximately 75% of people with HHT.[2] Although only a small 8%–10% of these patients develops symptomatic liver pathology, the rest remains asymptomatic.

Case Report

We are presenting a patient who presented with upper gastrointestinal (GI) bleed and epistaxis with anemia. On further evaluation, she was found to have telangiectasia in the nose and multiple angioectasias in the stomach and in the duodenum in upper GI endoscopy. This

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triad of findings, 1. Spontaneous recurrent epistaxis, 2. Telangiectasia of nose, and 3. Visceral telangiectasia, fulfill 3 Curacao criteria to qualify for definitive diagnosis of HHT. Further evaluation with contrast-enhanced computed tomography (CECT) was done to rule out chronic liver parenchymal disease. The CECT revealed heterogenous enhancement of the periphery of the liver in the arterial phase [Figure 1A] which becomes homogenous in the venous phase [Figure 1B]. In addition, there was moderately enlarged proper hepatic artery measuring 10 mm in diameter (normal <7 mm) and abnormally dilated intrahepatic branches of the hepatic artery and early filling of the portal venous branches [Figures 2A, B and 3]. There were abnormal tortuous vascular channels along the ligamentum teres with early filling of the branches of portal vein [Figures 3 and 4]. There were faintly enhancing tortuous vascular channels along the intrahepatic branches of the hepatic artery. There was slight reduction in the volume of the left lobe of liver. The surface of the liver was smooth. All these imaging features were suggestive of intrahepatic arterio-portal shunts with possible secondary chronic liver parenchyma disease. She was treated symptomatically with cauterization of the nasal telangiectasia, nasal packing, and blood transfusion for anemia. Few small telangiectasia lesions in the stomach showed active bleed during endoscopy and these lesions were treated with argon plasma coagulation.

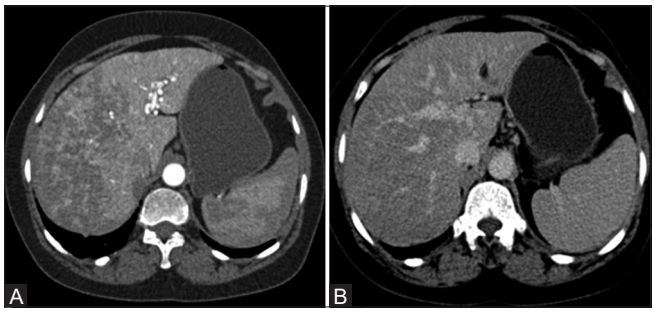


Figure 1 (A and B): Contrast-enhanced CT axial section shows heterogenous enhancement of the liver parenchyma in the arterial phase (A) which becomes homogenous in the venous phase (B)

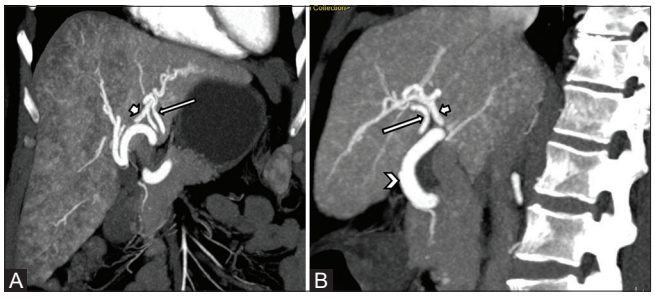


Figure 3 (A and B): Coronal (A) and sagittal (B) maximum intensity projection (MIP) images showing enlarged hepatic artery branches—long thin arrows (in A and B)—and early filling of the portal venous branches—short thick arrow (in a and B). The enlarged main hepatic artery is marked with arrow head in B

Discussion

In HHT, clinical symptoms occur in approximately 8% of the people.[3] There is a distinct female preponderance in symptomatic patients, with majority of the patients presenting in their fourth decade.[3] Presenting features are secondary to high-output cardiac failure (most common),[3] portal hypertension, GI bleed either from varices due to portal hypertension or angioectasia of the mucosa of the GI tract, and biliary disease. Other less common forms of clinical presentation in the liver and GIT are portosystemic encephalopathy and abdominal angina. The basic pathophysiology in liver involvement is development of intrahepatic VMs. Liver vascularity is unique by the way of presence of dual arterial supply and presence of dormant, potential portosystemic shunts, like paraumbilical vein, which can open up in any form of disturbance in the equilibrium of liver circulation.

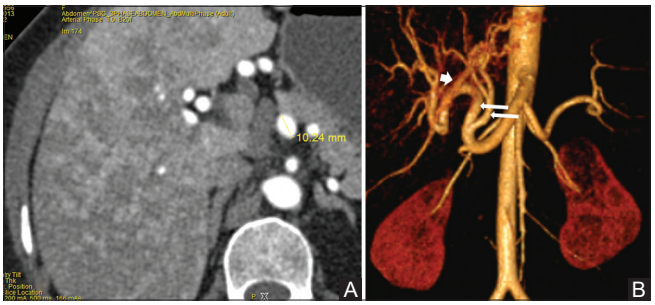


Figure 2 (A and B): Axial CT image in the arterial phase acquisition (A) and 3D volume rendered image (B) showing enlarged hepatic artery proper, measuring more than 10 mm. There is enlarged proper hepatic artery and its branches (long thin arrows in B) and early filling of the portal venous branch (short thick arrow in B) seen with fuzzy margins

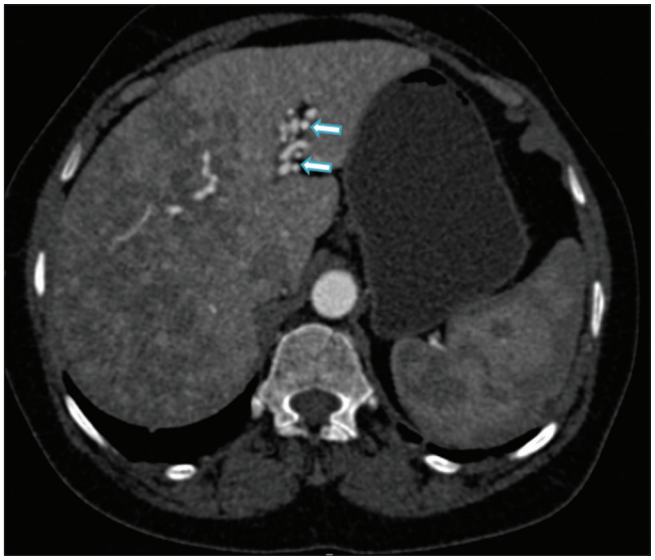


Figure 4: Axial CT image showing multiple tortuous early filling portal venous branches along the ligamentum teres (arrows) seen along with the left hepatic artery branches

The VMs in the liver can lead to three different types of vascular shunts.[3,4] These are 1. Arterio-venous (AV) shunt (between the hepatic artery and hepatic vein), 2. Arterio-portal (AP) shunt (between the hepatic artery and portal vein), and 3. Portal venous (PV) shunt (between portal vein and hepatic vein).[1] Shunts are usually extensive. The shunts can be visualized by imaging studies if they are macroscopic. Frequently they can be microscopic and not seen in imaging. Each predominant type of shunt gives rise to distinct clinical manifestation.

AV shunt

This is the most common symptomatic VM. It usually affects women. The presenting clinical feature is related to high-output cardiac failure.[1] AP shunt can also involve liver leading to biliary ischemia and ischemic necrosis of the bile duct[5] due to steal phenomenon. The loss of integrity of the bile duct due to ischemia can lead to biliomas and intrahepatic biliary cysts mimicking Caroli's disease. In chronic cases, *secondary sclerosing cholangitis* develops with stricture and abnormal dilatation of intrahepatic bile ducts. Biliary ischemia-related complications are seen *exclusively* in women.[3]

AP shunt

This is the second most common shunt after AV shunting. Shunting of blood from the hepatic artery to the portal vein causes *portal hypertension*. Secondary to portal hypertension, there can be ascites, varices formation, and GI bleed. In addition, there can be formation of focal nodular hyperplasia and *nodular regenerative hyperplasia in the liver*. It equally affects men and women. The diagnosis is made based on hepatic venous pressure gradient which should be more than or equal to 10 mmHg.

PV shunt

This is the least common type of shunt and usually causes high-output cardiac failure. Rarely, this can cause focal nodular hyperplasia and nodular regenerative hyperplasia in the liver which can lead to portal hypertension and cirrhosis.

Diagnosis of HHT is made based on the Curacao criteria.[2] Imaging is indicated to confirm the diagnosis if three of the four clinical criteria are not met. In addition, imaging has to be done to look for associated complications like liver cirrhosis. In a known case of HTT, the presence of ascites, GI bleed, and abdominal pain should raise the suspicion of liver involvement and imaging should be performed. Doppler study can show abnormal tortuous vessels in the liver. CECT and MRI can reveal multiple liver VMs. There can be enlarged hepatic artery, normal ≤7 mm,[1] heterogenous enhancement of the liver in the arterial phase of contrast study which becomes homogenous in the venous phase.

CT is the recommended *imaging modality* of choice. In CECT, the type of shunt can be determined in two-third of patients. The majority of patients with liver disease will show AV shunt. Biliary disease is generally seen in the late phase of the disease. The gold standard to establish the type of shunt is angiography.[3] Angiography can clearly demonstrate the early, subtle VMs and mesenteric steal syndrome. However, in the majority of the patients, the diagnosis can be established with less invasive imaging methods like CECT.

Treatment

No treatment needed in asymptomatic patients with liver involvement. Primarily, the management is medical with treatment of anemia and correcting coagulation abnormalities.[6] High-output cardiac failure and complications of portal hypertension are treated in the standard way like treating cardiac failure and portal hypertension due to other etiologies. It should be noted that trans-jugular intrahepatic porta systemic shunt (TIPS) creation may not be effective in the treatment of GI bleed secondary to telangiectasia of the GI tract. Shunt reduction procedures like embolization may be considered in patients with cardiac failure and mesenteric ischemia.[7] However, these procedures are associated with high morbidity and mortality and have only transient therapeutic effect. In carefully selected patients, few sessions of endovascular embolization may be effective in reducing portosystemic shunt and alleviating mesenteric ischemia. Ultimately patients might require liver transplantation, which can be curative.

Conclusion

HHT is a rare cause of vascular dysplasia involving many organs and systems. Although the majority of the patients remain asymptomatic, high index of suspicion should be kept not to miss liver involvement by the disease. Radiologists should be aware of the imaging findings and possible treatment options like shunt reduction embolization offered by us. Life-threatening complications like high-output cardiac failure not responding to maximum medical therapy and severe biliary ischemia should be recognized and these patients should be referred for an early liver transplant.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ginon I, Decullier E, Finet G, Cordier JF, Marion D, Saurin JC, *et al*. Hereditary hemorrhagic telangiectasia, liver vascular malformations and cardiac consequences. Eur J Intern Med 2013;24:e35-9.

2. Leitão P, Carvalho A, Guerra C, Gonçalves J, Ramos I. Congestive cirrhosis in Osler-Weber-Rendu syndrome: A rare case report. Radiol Case Rep 2018;13:51-4.
3. Garcia-Tsao G. Liver involvement in hereditary hemorrhagic telangiectasia (HHT). J Hepatol 2007;46:499-507.
4. Brandt E, Haghighat L, Garcia-Tsao G, Henderson K, Pollak J, Proctor D, *et al*. Three types of intrahepatic shunts presenting in a single patient with hereditary hemorrhagic telangiectasia. J Am Coll Cardiol 2018;71. doi: 10.1016/S0735-1097(18)32934-6.
5. Vlachou PA, Colak E, Koculym A, Kirpalani A, Kim TK, Hirschfield GM, *et al*. Improvement of ischemic cholangiopathy in three patients with hereditary hemorrhagic telangiectasia following treatment with bevacizumab. J Hepatol 2013;59:186-9.
6. Govani FS, Shovlin CL. Hereditary haemorrhagic telangiectasia: A clinical and scientific review. Eur J Hum Genet 2009;17:860-71.
7. Weinstein J, Lao J. Staged embolization of a complex hepatic arterio-portal fistula, Posters and Exhibits, JVIR, Educational Exhibit Abstract No. 466.

Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction

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ORIGINAL PAPER

Doppler sonographic evaluation of resistive index of intra-renal arteries in acute ureteric obstruction

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Abstract

Background Acute ureteric colic is one of the most commonly encountered acute abdominal conditions. Diagnosis of obstruction caused by ureteric calculus occasionally becomes challenging if there is inadequate dilation of the urinary tract proximal to obstruction. In such a situation, intra-renal artery Doppler parameters can be used as a diagnostic tool. Our study compared intra-renal arterial Doppler in patients with obstructed and non-obstructed kidneys presenting within 24 h of onset of symptoms of unilateral acute renal colic.

Results The resistivity index (RI) in the segmental arteries of all 54 patients with obstructed kidneys was significantly higher than in those with non-obstructed kidney: 0.75 vs. 0.56, with a p value less than 0.001, a sensitivity of 85% and a specificity of 93%.

Conclusion Doppler ultrasound is a useful diagnostic tool in the evaluation of acute renal obstruction, thus enabling practitioners to avoid ordering unnecessary CT and exposing patients to ionizing radiation. An RI value > 0.7 has good sensitivity and specificity in our study. The results obtained in the study were comparable to those of pioneer studies conducted worldwide.

Keywords Acute renal colic · Renal artery Doppler · Resistive index

Abbreviations

ARC	Acute renal failure
ER	Emergency room
USG	Ultrasonography
US	Ultrasound
RI	Resistive index
CT	Computed tomography.
NSAID	Non-steroidal anti-inflammatory drugs
GFR	Glomerular filtration rate
eGFR	Estimated glomerular filtration rate.
Tc^{99m} DTPA	Technetium$^{-99\,m}$- Diethylene-triamine-penta-acetate
CDUS	Color Doppler Ultrasound
MPR	Multiplanar reconstruction
ROC	Receiver operating characteristic curve
PCS	Pelvicalyceal system

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Background

Acute renal colic (ARC) is one of the common causes of patients presenting to the emergency room (ER). It accounts for 30–35% of urologic emergencies [1, 2]. The recurrence rate over a period of 5 years is 30–40% [2]. Ultrasonography (US) remains the most commonly used modality in the initial evaluation of patients presenting with acute abdomen, especially in children [3], and the utility of ultrasound in the diagnosis of ARC cannot be overstated in cases where ARC is one of the most common pathologies presenting as acute abdomen. Absence of significant dilatation of the pelvicalyceal system, in spite of severe obstruction being present, is a well-recognized finding in some patients. In some

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studies, the absence of significant dilation in ARC has been reported in up to 30% [4] of patients. The dilated collecting system seen in US need not always be due to obstruction in the downstream urinary tract. These conditions include a baggy extra-renal type of pelvis, a compound upper pole calyx, due to over-distended urinary bladder, vesico-ureteric reflux, congenital type of mega calyces, infection and residual dilatation due to previous obstruction. Very rarely, pseudo-aneurysms of intra renal arteries can mimic renal pelvi-ectasis. This kind of vascular pathology can also be easily diagnosed with CDUS [5]. Acute complete ureteric obstruction is associated with changes in renal blood flow, as well as with an increase in renal pelvic pressure. In the first few hours, renal blood flow increases, and after 3 to 5 h, renal blood flow decreases, probably because of efferent arteriolar vasoconstriction produced by prostaglandins and other vasoactive substances [6, 7]. These changes can be effectively demonstrated by the RI of intra-renal arteries, as measured by Doppler ultrasound.

Methods

A prospective study was done on 54 patients who were referred to the department of radiology with clinical features of ARC for further evaluation with ultrasound or non-contrast CT. The study period is from January 2015 to December 2017. Prior to the commencement of the study, necessary clearance was obtained from the institutional ethics committee, and informed written consent was obtained from all the participating patients.

Inclusion criteria

All patients with symptoms of acute ureteric obstruction presenting to the emergency department within 24 h of onset of unilateral ARC were included.

Exclusion criteria

(a) Patients with history of renal parenchymal disease, chronic renal obstruction, renal trauma, or bilateral renal obstruction; (b) patients with a single kidney; and (c) patients in whom ureteric obstruction could not be confirmed either with USG or with CT were excluded.

All patients underwent gray-scale ultrasonography (US) and Doppler evaluation of intra-renal arteries. Computed tomography (CT) was done for those patients in whom US could not confirm the cause of obstruction.

Evaluation by ultrasound and Doppler study

The kidney on the side of obstruction was treated as the case kidney, and the contralateral normal (non-obstructed) kidney served as control. All patients underwent B-mode US and color doppler ultrasound (CDUS) with Philips iU22 using a C 1–5 transducer and Siemens Acuson S3000 using a 6C1 HD transducer. The presence or absence of pelvicalyceal system dilatation in each kidney was assessed using the gray-scale images. At least three Doppler spectra were obtained from inter-lobar arteries along the border of the medullary pyramids, and their mean was taken (Fig. 1).

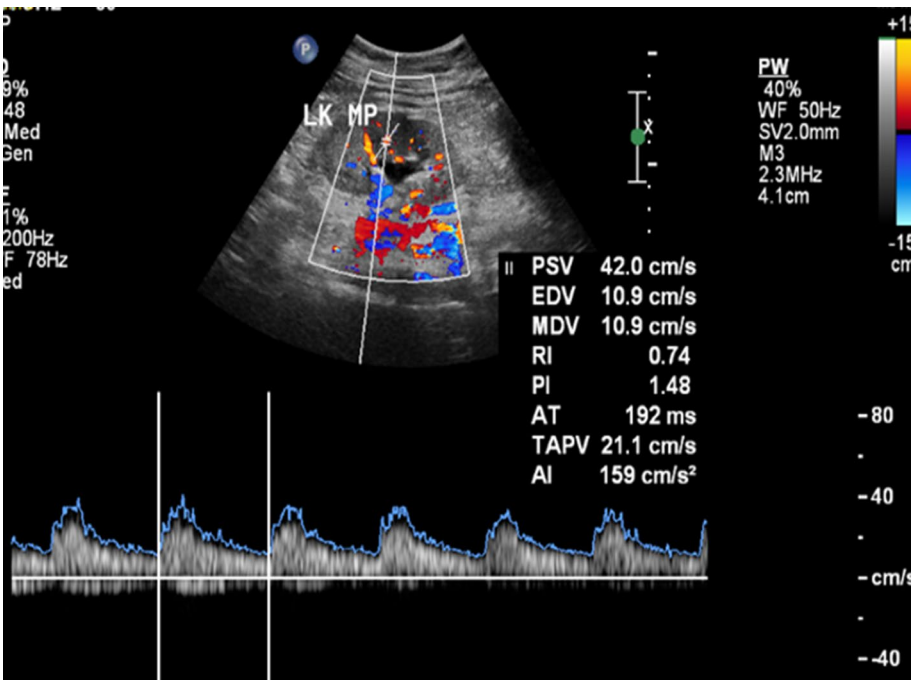


Fig. 1 Doppler US demonstrating an RI value of 0.74 in the mid pole of left kidney (obstructed kidney)

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The Doppler waveforms were made using the lowest pulse repetition frequency possible without aliasing. This maximized the size of the Doppler spectrum and decreased the percentage error in the measurements. In addition, the lowest possible wall filter for each ultrasound scanner was used. The Doppler sample width was set at 2–3 mm. The renal RI was calculated using the standard formula. The mean RI value was calculated for each kidney.

Evaluation by computed tomography

Siemens SOMATOM Definition Edge 128 slice Computerized Tomography (CT) equipment was used for the study. Non-enhanced CT of the abdomen, extending from the dome of the diaphragm to the pubic symphysis, was performed in all patients in whom the site and cause of obstruction could not be confirmed with US. Raw data was acquired with 0.6 mm thickness and pitch of 0.8. Reconstruction from the raw data was done in 5 mm thickness for viewing purposes and in 1 mm thickness for coronal and sagittal multi-planar reconstruction (MPR) (Fig. 2). The sites of ureteric obstructions were identified. Those patients not confirmed to have obstructions by CT were excluded from the study.

Statistical methods

All data were systematically collected, tabulated and analyzed using Microsoft Excel and Strata 6 for Windows. The Student *t* test was used in univariate analysis for the continuous variables, and Chi square test was used for analysis of noncontiguous variables. A *p* value of less than 0.05 was considered to be statistically significant.

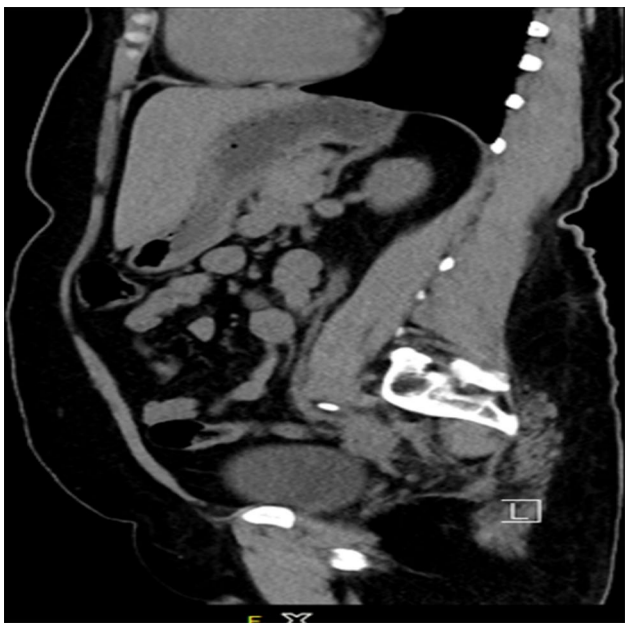


Fig. 2 Sagittal reconstruction of non-enhanced CT showing a calculus in the left distal ureter who presented with acute left renal colic

Results

This study evaluated 54 patients who had presented to the EMD with unilateral ARC within 24 h of the onset of symptoms. All the patients underwent B-mode US, CDUS and CT if the obstruction could not be confirmed in US. The presence or absence of PCS dilatation in each kidney was assessed using the grey-scale images. In this study population, 34 patients were males and 20 were females, as shown (Fig. 3). The mean age of the male patients was 46.8 years and that of the female patients was 38.5 years. The age range of the male patients was 19–79 years, and that of the female ones was 18–61 years.

The modalities used in the diagnosis and the site of obstruction are shown in Tables 1, 2.

Out of 54 patients, 26 cases proximal obstructions, while 28 had distal obstructions. The mean RI of the obstructed kidneys was higher in cases of distal obstruction (0.79 ± 0.004) than in those of proximal obstruction (0.76 ± 0.006).

Of the 54 patients, 45 patients had mild hydronephrosis, FIVE patients had moderate hydronephrosis and four patients did not have any dilatation of the collecting system despite the presence of ureteric obstruction (Fig. 4).

Of the 54 patients who were included in this study, 27 patients had elevated renal parameters, but did not have history of chronic renal impairment (Table 3).

In our study, we found that the RI in obstructed kidneys was significantly higher than in unobstructed kidneys (0.75 vs. 0.56; $p < 0.001$). The RI was higher in obstructed kidneys in all cases. The difference in RI between the obstructed and unobstructed kidneys (delta RI) ranged from 0.09 to 0.38, with a mean delta RI of 0.24 (Table 4).

The RI values in obstructed kidneys ranged from 0.68 to 0.84 (Fig. 5). The mean RI value in obstructed kidneys was 0.75, compared to 0.56 in unobstructed kidneys.

The group statistics of RI are shown in Tables 5, 6. The receiver operating characteristic curve (ROC) curve depicting the sensitivity of RI in ureteric obstruction is shown in Fig. 6.

By using a cut off RI value of > 0.70 in acute unilateral renal obstruction, the sensitivity obtained in the study was 85% and the specificity was 93% (Table 7).

Discussion

Grayscale ultrasound is the investigation method of choice in the initial evaluation of ARC, as it is easily available, portable and involves no ionizing radiation [8, 9]. However,

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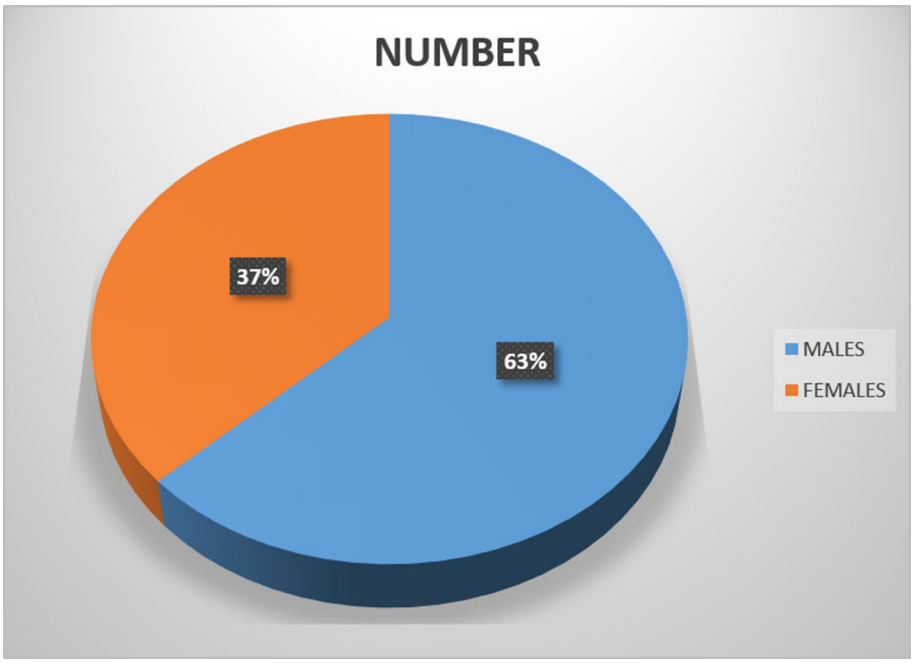


Fig. 3 Sex distribution in the study population

Table 1 Modality used in diagnosing the obstruction

Modality	Number	Percentage
CT and US	44	81%
US	10	19%
Total	54	100%

Table 2 Site of obstruction in the study population

Site of calculus	Frequency
Proximal ureter	26
Distal ureter	28

urinary system dilatation seen on conventional gray-scale USG has been shown to be sensitive (90%), but not specific (65–84%), in the diagnosis of renal obstruction.

The most sensitive, gold standard test for diagnosing obstructive uropathy is renal scintigraphy. However the availability of scintigraphy and radiation associated with it continue to be problematic [10]. Traditionally, the evidence of renal obstruction provided by US has been indirect and dependent on the anatomical criterion of dilatation of the pelvicalyceal system and ureter proximal to the level of obstruction. However, US fails to reveal hydronephrosis in acute obstruction of the kidney in up to 35% of cases [11]. More direct functional evidence of obstruction usually requires scintigraphy, but recently Doppler US techniques have been used to obtain functional information in suspected renal obstruction [11]. US imaging may miss (false negative) the diagnosis of obstruction in a variety of situations. Mild dilatation may be overlooked or considered clinically insignificant. Some patients with obstructive renal failure may show no pelvicalyceal system (PCS) dilatation. The reasons for this are unclear; in some patients, it may be related to dehydration or due to decompression of the pelvicalyceal system by rupture of a calyceal fornix [12]. It is commonly explained by urologists to be caused by the use of non-steroidal anti-inflammatory drugs (NSAIDs), by dehydration or by the renin angiotensin effect [13]. If that is true, such impairment will be detected through measurement of glomerular filtration rate (GFR). Measurement of GFR is commonly done using Technetium c-99 m diethylene-triamine-penta-acetate (DTPA), and does not require blood or urine sampling [14]. There is a growing evidence that renal impairment in acute unilateral ureteric obstruction is due to absorption of obstructed urine, with its high amount of creatinine, back into systemic circulation. This is considered false renal impairment with normal GFR [15]. In a study, El-Shazly et al. [16] measured renal function through measurement of estimated GFR (eGFR) usingTc-99 m DTPA. The authors showed that differences between eGFR on admission and after passage of stone or relieve of obstruction were non-significant. Their results support the hypothesis of false impairment in this clinical scenario. This tends to change the practice, as there is no need to embark on urgent intervention to relieve the ureteric obstruction, and conservative medical management is justifiable.

Routine B-mode US can be falsely positive or negative in acute ureteric obstruction [10]. PCS dilatation may be missed if the system is filled with blood clots, calculus, tumors or pus, in which case the usual anechoic appearance

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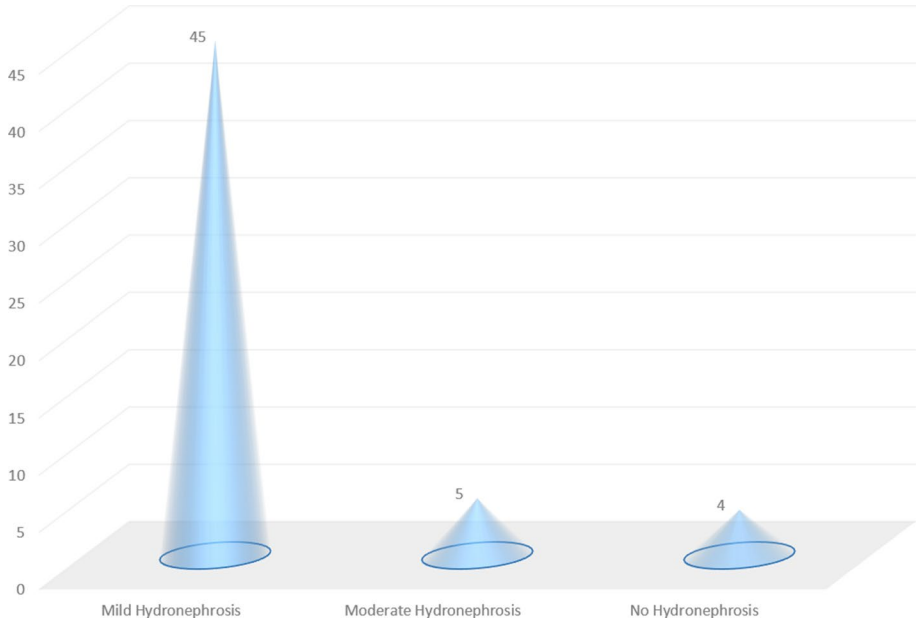


Fig. 4 Degree of hydronephrosis in the study population

Table 3 Number of patients with elevated serum urea and creatinine

No. of patients	Serum urea and creatinine
27	Increased
27	Normal

Table 4 Resistive indices in obstructed and unobstructed kidneys

Kidney	RI
Obstructed	0.75 ± 0.08
Non-obstructed	0.56 ± 0.09
Delta RI	0.24 ± 0.08

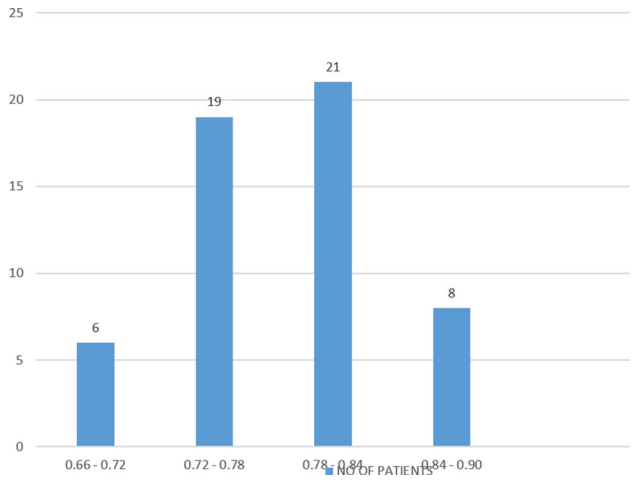


Fig. 5 Mean RI values in obstructed kidneys

Table 5 Group statistics

	Frequency	mean RI	SD	*P* value
Obstructed kidneys	54	0.75	0.08	0.000*
Unobstructed kidneys	54	0.56	0.09	

*Statistically significant ($P < 0.05$)

Table 6 Group statistics

	Mean RI value		*p* value
	< 0.70	> 0.70	
Obstructed kidneys	5 (9.3%)	49 (90.7%)	0.000*
Unobstructed kidneys	48 (88.9%)	6 (11.1%)	

*Statistically significant ($p < 0.05$)

of the PCS is lost. Intermittent ureteric obstruction, particularly that caused by ureteric calculi, may also lead to a failure to visualize dilatation of collecting system with US. On the other hand, in an attempt not to miss the diagnosis of obstruction in patients with only mild PCS dilatation, the false positive rate was found to be as high as 26% Causes of a false positive diagnosis include: (i) dilatation of non-obstructed PCS, when there are anatomical variants such as extra-renal pelvis; (ii) over-distended urinary bladder, with or without diuresis; (iii) vesico-ureteric reflux (VUR), (iv) residual dilatation of PCS due to previous obstruction or infection; (v) advanced pregnancy; and (vi) central renal fluid collections other than in the PCS, including in normal vessels, renal artery aneurysms and para-pelvic cysts.

Acute unilateral ureteric obstruction results in a complex sequence of changes in renal blood flow and ureteric

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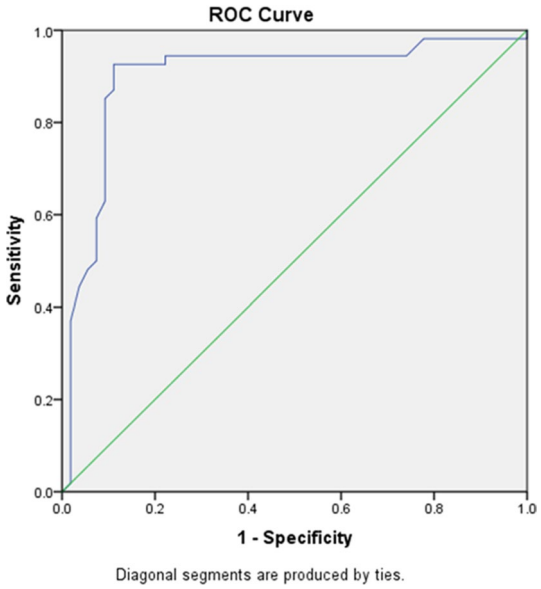


Fig. 6 ROC curve showing the sensitivity and specificity of resistive index

Table 7 Sensitivity and Specificity of RI with cut off of 0.70

Cut off RI	Sensitivity	Specificity
> 0.70	85%	93%

pressure [17]. In the first two hours, the renal blood flow increases, because of afferent arteriole vasodilatation and the ureteric pressure increases. From two to six hours after obstruction, the renal blood flow decreases, secondary to vasoconstriction of the efferent arterioles, and the ureteric pressure remains elevated. Subsequently, at 6 to 18 hours, the renal blood flow remains reduced because of vasoconstriction of the afferent arterioles, and the ureteric pressure decreases.

The role of renal CDUS in the evaluation of ARC has been vigorously debated. Rodgers et al. [18] found an elevated RI in acutely obstructed kidneys, especially when compared with the RI in normal contralateral kidneys and with a control group of healthy subjects. Similar results were obtained by Platt et al. [19] in a study of 23 patients with acute unilateral ureteric obstruction. However, others have reported that Doppler ultrasound is highly insensitive for detecting ARC. Tublin et al. [20] correlated the results of Doppler USG with those of urography in 32 patients presenting with symptoms of renal colic. The RI value of normal kidneys is usually less than 0.7 [21, 22]. When the published discriminatory thresholds for obstruction (mean RI ≥ 0.70 and ΔRI ≥ 0.10) were applied, the sensitivity and specificity of Doppler USG were only 44% and 82%, respectively.

In our study of 54 patients, we found that the RI in obstructed kidneys was significantly higher than in the unobstructed kidneys (0.75 vs. 0.56; $p < 0.001$). The RI was higher in obstructed kidneys in all cases. The difference between the obstructed and unobstructed kidneys (delta RI) ranged from 0.09 to 0.38, with a mean delta RI of 0.24.

Saboo et al. [6] stated in their study that delta RI ≥ 0.006 was highly sensitive and specific, and with a higher delta RI specificity remained the same whereas the sensitivity fell rapidly. Our results correlate well with those reported in earlier studies by Badr et al. [23] and Miletic et al. [24].

We also studied the effect of the level of obstruction on RI values. When we compared levels of ureteric obstruction (proximal vs distal), distal obstructions had a higher mean RI value (0.79) than proximal obstructions (0.76), and the values were not statistically significant. However, the study done by de Toledo *et al.* [25] showed that patients with proximal ureteric obstructions have RIs higher than those with distal obstructions.

We also analyzed the utility of CDUS in patients who did not show dilatation of the PCS on ultrasound. Patients who did not have PCS dilatation on US were later confirmed to have obstruction on CT. RI values were higher in all these patients (0.71), with a delta RI of 0.24. Discriminatory RI ≥ 0.70, which is higher than the delta RI found in the study done by Platt JF et al. [26], was found to be highly sensitive (85%) and specific (93%) in this study.

Conclusion

Using the discriminatory threshold RI of ≥ 0.70, the overall sensitivity of CDUS in diagnosing acute ureteric obstruction is 85% and the specificity is 93%. CDUS is a useful tool for demonstrating altered renal perfusion in acute ureteric obstructions before pelvicalyceal system dilatation occurs, thus increasing the sensitivity and specificity. Gray-scale US should therefore, be complemented by CDUS as an adjunctive test in patients presenting with acute renal colic, as it is a very simple, noninvasive and easy-to-calculate parameter. The utility of this tool cannot be overemphasized in special cases like pregnant women and children, in whom a dilated pelvicalyceal system without acute obstruction is encountered more frequently. It is mandatory to differentiate obstructive and non-obstructive causes with US alone in this subset of patients, as further evaluation with CT is not desirable due to the hazard of ionizing radiation. Given the high propensity of any group of patients with urinary tract calculi to undergo frequent imaging studies due to recurrent ureteric colic and the presence of significant residual hydronephrosis, we should strive hard to minimize radiation dosage by avoiding frequent CT studies. At the same time, maintaining acceptable diagnostic accuracy is of paramount importance.

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Limitations of the study: US is an operator-dependent examination and the results are affected by patients' body habitus and availability of good window. An increase in the RI of intra-renal vessels is also found in case of vascular or parenchymal diseases (renal vein thrombosis, acute renal insufficiency, etc.). The CDUS changes can return to normal after 48 h [27], after which its utility is doubtful with reduced sensitivity and specificity.

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Author contributions VM: Conception, Design, Acquisition, Analysis and Interpretation of data, Review. KMS: Design, Acquisition, analysis, Interpretation. NE: Concept, Design, Review. BD: Concept, Design. All the authors have read the manuscript and approve the manuscript fully for publication.

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Compliance with ethical standards

Conflict of interest None.

Ethics approval Obtained from the Institutional Human Ethics committee (IHEC) of PSG institute of Medical sciences and Research, Coimbatore, Tamilnadu, India. Reference Number: 15/417.

Consent to participate It was obtained from each patient at the time of Ultrasound Scan and CT (wherever applicable) along with general consent prescribed by the institution. This consent is a verbal consent since no additional procedure was done or drugs were administered for the purpose of the study. The ultrasound and CT Scan (wherever applicable) were done at the request of the referring physician and only those studies performed at the request of the referring clinician were utilized for the study. It is approved by the institutional ethics committee.

Consent for publication Obtained from all the authors all the authors have read and approved the manuscript.

Availability of data and material Available.

References

1. Piazzese EMS, Mazzeo GI, Galipo S, Fiumara Canfora F, Angio LG (2012) The renal resistive index as a predictor of acute hydronephrosis in patients with renal colic. J Ultrasound 15:239246
2. Sterrett S, Stephen N (2009) Emergency room follow-up trends in urolithiasis: single-center report. Urology 73:1195–1198. https://doi.org/10.1016/j.urology.2008.07.057
3. di Giacomo V, Trinci M, van der Byl G, Catania VD, Calisti A, Miele V (2014) Ultrasound in newborns and children suffering from non-traumatic acute abdominal pain: imaging with clinical and surgical correlation. J Ultrasound. https://doi.org/10.1007/s40477-014-0087-4
4. Ravindernath ML, Mahender Reddy G (2017) Mean resistive index as a prognostic tool for hydronephrosis in patients with acute renal colic: a study in a tertiary care. Int J Adv Med 4(2):329–333. https://doi.org/10.18203/2349-3933.ijam20170996
5. Tufano A, Minelli R, Rossi E, Brillantino C, Di Serafino M, Zeccolini M, Cantisani V, Vallone G (2017) Inferior epigastric artery pseudoaneurysm secondary to port placement during a robot-assisted laparoscopic radical cystectomy. J Ultrasound. https://doi.org/10.1007/s40477-020-00442-1
6. Saboo SS, Soni SS, Saboo SH, Chinapuvvula NR, Kaza S (2007) Doppler sonography in acute renal obstruction. Indian J Radiol Imaging 17:188–192
7. Azmi H (2003) Duplex doppler sonography in patients with acute renal colic: prospective study and literature review. Int Urol Nephrol 35(2):135–140
8. Jindal G, Ramchandani P (2007) AFP secondary to urolithiasis: radiologic evaluation and alternate diagnoses. Radiol Clin N Am 45(2007):395–410. https://doi.org/10.1016/j.rcl.2007.04.001
9. Apoku N, Ayoola O et al (2015) Ultrasound evaluation of obstructive uropathy and its hemodynamic responses in southwest Nigeria. Int Braz J Urol. 41:556–561. https://doi.org/10.1590/S1677-5538.IBJU.2014.0197
10. Ruiz-Martíneza A, Sierra-Díazb E et al (2019) Renal Doppler ultrasound resistive index vs renal scintigraphy with 99mTc-DTPA as diagnostic test for ureteropelvic junction obstruction in children. Actas Urol Esp 43(8):419–424. https://doi.org/10.1016/j.acuro.2019.02.005
11. Granata A, Zanoli L, Clementi S, Fatuzzo P, Fiorini F (2014) Resistive intrarenal index: myth or reality? Br J Radiol 87:20140004. https://doi.org/10.1259/bjr.20140004
12. Arumuham V, Brodie A, Bycroft J (2019) The management of urolithiasis. Surgery 37:7. https://doi.org/10.1016/j.mpsur.2019.04.003
13. Al-Ani A, Al-Jalham K, Ibrahim T, Majzoub A, Al-Rayashi M, Hayati A, Mubarak W et al (2015) Factors determining renal impairment in unilateral ureteral colic secondary to calcular disease: a prospective study. Int Urol Nephrol 47(7):1085–1090
14. Prigent A, Cosgriff P, Gates GF, Granerus G, Fine EJ, Itoh K, Peters M et al (1999) Consensus report on quality control of quantitative measurements of renal function obtained from the renogram: international consensus committee from the scientific committee of radionuclides in nephrourology. Semin Nucl Med 29(2):146–159. https://doi.org/10.1016/s0001-2998(99)80005-1
15. Rosenzweig B, Pinthus JH, Kleinmann N, Joffe E, Erlich T, Fridman E, Winkler H et al (2015) The relative contribution of urine extravasation to elevate plasma creatinine levels in acute unilateral ureteral obstruction. Can Urol Assoc J 9(7–8):428–433. https://doi.org/10.5489/cuaj.2804
16. El-Shazlya M, Aziza M et al (2016) Acute unilateral ureteric obstruction in young men with high serum creatinine: is it true or false renal impairment? World J Nephrol Urol 5(3):51–53
17. Sayani R, Ali M et al (2012) Functional evaluation of the urinary tract by duplex Doppler ultrasonography in patients with acute renal colic. Internat J Nephrol Renov Dis 5:15–21. https://doi.org/10.2147/IJNRD.S27628
18. Rodgers PM, Bates JA, Irving HC (1992) Intrarenal Doppler ultrasound studies in normal and acutely obstructed kidneys. Br J Radiol 65(771):207–212. https://doi.org/10.1259/0007-1285-65-771-207
19. Platt J, Ellis J, Rubin J (1991) Examination of native kidneys with duplex Doppler ultrasound. Semin Ultrasound CT MR 12:308–318
20. Tublin ME, Bude RO, Platt JF (2003) The resistive index in renal Doppler sonography: where do we stand? AJR Am J Roentgenol 180(4):885–892. https://doi.org/10.2214/ajr.180.4.1800885
21. Drudi FM, Liberatore M, Cantisani V et al (2014) Role of color Doppler ultrasound in the evaluation of renal transplantation from living donors. J Ultrasound. https://doi.org/10.1007/s40477-014-0077-6

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22. Spatola L, Andrulli S (2015) Doppler ultrasound in kidney diseases: a key parameter in clinical long-term follow-up. J Ultrasound. https://doi.org/10.1007/s40477-016-0201-x
23. Badr K, Brenner B (1988) Renal circulatory and nephron function in experimental obstruction of the urinary tract. In: Brenner BM, Lazarus J (eds) Acute renal failure. Churchill Livingstone, New York, pp 91–118
24. Miletic D, Fuckar Z, Sustic A, Mozetic V, Smokvina A, Stancic M (1998) Resistance and pulsatility indices in acute renal obstruction. J Clin Ultrasound 26(2):79–84
25. Toledo De et al (2017) Doppler-duplex ultrasound in renal colic. Eu J Radiol 23(2):143–1481996
26. Platt JF, Rubin JM, Ellis J (1989) Distinction between obstructive and nonobstructive pyelocaliectasis with duplex Doppler sonography. AJR Am J Roentgenol 153(5):997–1000. https://doi.org/10.2214/ajr.153.5.997
27. Gul H, Habib I et al (2014) Diagnostic accuracy of renal arterial resistive index in acute renal colic. KJMS 7:1

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Determination Of Gestational Age In Third Trimester Using Foetal Transcerebellar Diameter And Assessment Of Foetal Growth Using Tcd/Ac Ratio

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Abstract:
Objectives:*To evaluate the role of fetal transcerebellar diameter (TCD) as an independent parameter in predicting the gestational age in the third trimester of pregnancy. To derive nomogram from sonographically measured foetal transcerebellar diameter for estimating the gestational age in Indian population.*
Materials and methods:*Hospital based prostective study.148 healthy pregnant patient in third trimester were included in the study who underwent obstetric sonography using GE Voluson P8 machine. In all the patients following parameters were obtained - BPD, HC, AC, FL, TCD, foetal heart rate, estimated foetal weight, AFI and placental position. Transverse cerebellar diameter is obtained in the axial plane in the cerebellar view. Gestational age for the measured TCD is obtained from the reference chart by Hill et.al.,TCD/AC ratio was calculated according to the following formula: TCD/AC ratio (%): TCD (mm)/AC (mm) x 100*
Conclusion:*TCD correlates with clinical gestational age as the first best parameter and also positively correlates with BPD, HC, AC and FL. So TCD can be reliably be used for gestational age assessment in third trimester. TCD/AC ratio remains fairly constant in third trimester with value of 14.59 +/- 2SD, So the ratio is independent of gestational age and can be used in assessing the fetal growth in pregnancies with unknown dating.*

Keywords: *Foetal growth, Gestational age,TCD/AC ratio, Third trimester, Transcerebellar diameter.*

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I. Introduction

Ultrasound assessment of gestational age is becoming increasingly important[1]. Obstetric surveillance and management of complicated pregnancy and decisions regarding the optimal timing of delivery remains paramount important. Estimation of gestational age using ultrasonographically derived fetal parameters is perhaps the cornerstone in obstetrics and is an important component in the management of pregnancies [2].

Many parameters are being used for establishing gestational age, for example biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) (1). Identification of aberrant growth as well as growth disturbance across the spectrum of GA in both healthy fetuses and those with genetic abnormalities will be helpful for optimal fetal surveillance [2]. Evaluation of the posterior fossa of the fetal cranium has been accepted as part of routine obstetric ultrasound examination. Parameters such as BPD, HC and AC compute the gestational age more correctly in the earlier gestation.

Fetal transcerebellar diameter can predict the gestational age reliably at any trimester since cerebellum is not liable to change in its form and also its size. The fetal cerebellum grows progressively along with gestational age[1]. The cerebellum and posterior fossa are aligned perpendicular to the plane of maximum extrinsic compression and withstand deformation by extrinsic pressure than the parietal bones [3]. So fetal transcerebellar diameter has remained consistently superior in predicting GA in second and third trimester, both singleton and twin gestations and in variation of fetal head shape such as dolicocephaly and brachycephaly and in normal fetus and in intrauterine growth restricted fetus (IUGR). Fetal transcerebellar diameter to abdominal circumference ratio is used to determine the correct gestational age and also to predict intrauterine growth restriction (IUGR) [1,2].

II. Objectives

(i) To evaluate the role of fetal transcerebellar diameter as an independent parameter in predicting the gestational age in the third trimester of pregnancy.
(ii) To derive nomogram from sonographically measured foetal transcerebellar diameter for estimating the gestational age in Indian population.

III. Inclusion and Exclusion Criteria

3.1 Inclusion criteria:

Healthy women with uncomplicated pregnancy between the 25^{th} week of gestation and term.

3.2 Exclusion criteria:

Unknown or inaccurate date of last menstrual period (LMP), irregular menstrual cycles, oligohydramnios.

Polyhydramnios, diabetic mother, pregnancy induced hypertension, pre eclampsia, dolichocephalic and brachycephalic skull, multiple gestation fetal chromosomal abnormalities, fetal anomalies intrauterine growth restriction and any other known maternal and foetal abnormality.

IV. Materials and Methods

4.1 Hardware

GE Voluson P8.

4.2 Methods

Hospital based prostective study. Ethical clearance was obtained from the institutional ethical review committee of Karpagam faculty of medical science research, Coimbatore. All the statutory requirements under PC-PNDT act were followed. All relevant clinical history was obtained and the correct LMP was confirmed.

148 healthy pregnant patient included in the study who underwent obstetric sonography in Department of Radiology, and Karpagam faculty of medical science research, Coimbatore. In all the patients following parameters were obtained - BPD, HC, AC, FL, TCD, foetal heart rate, estimated foetal weight, AFI and placental position.

Transverse cerebellar diameter is obtained in the axial plane in the cerebellar view i.e with a slight rotation of the transducer approximately 30^0 from the conventional thalamic plane where the biparietal diameter is measured using the cavum septi pellucidi, third ventricle and thalami as landmarks. In this plane posterior fossa with cerebellum is visualized. The cisterna magna is just posterior to the cerebellum. This plane provides the widest transcerebellar diameter.

Gestational age for the measured TCD is obtained from the reference chart "Predicted menstrual age for transverse cerebellar diameter of 14 mm to 56 mm"by Hill *et.al*., in his study "Transverse cerebellar diameter as a predictor of menstrual age" in 1990. TCD/AC ratio was calculated according to the following formula: TCD/AC ratio (%): TCD (mm)/AC (mm) x 100



Figure 1: The caliper on the edge of the cerebellar hemisphere demonstrates the method to obtain transcerebellar diameter.

4.3 Statistical analysis

The following methods of statistical analysis have been used in this study.

The results for each parameter (numbers and percentages) for discrete data and averaged (mean + standard deviation) for continuous data are presented in Table and Figure.

4.3.1 An evaluation was made of the linear relationship between two variables using Pearson's correlation. Pearson Correlation technique used to test the direction and strength of the relationship between gestational age obtained using transverse cerebellar diameter vs individual parameters such as biparietal diameter, head circumference, abdominal circumference and femoral length. It uses the statistic R which falls between -1 and +1. The + and – signs are used for positive linear correlations and negative linear correlations, respectively.A correlation greater than 0.8 is generally described as strong, whereas a correlation less than 0.5 are generally described as weak.

The coefficient of determination, r^2, is useful because it gives the proportion of the variance (fluctuation) of one variable that is predictable from the other variable. It is a measure that allows us to determine how certain one can be in making predictions from a certain model/graph.

4.3.2 The student't' test was used to determine whether there was a statistical difference between the parameters measured. Student's t test is as follows:

. $t=\dfrac{\bar{x}_1-\bar{x}_2}{s\sqrt{\dfrac{1}{n_1}+\dfrac{1}{n_2}}}$ ~~~ $t_{n1+n2-2}$ Where $s^2=\dfrac{(n_1-1){s_1}^2+(n_2-1){s_2}^2}{(n_1+n_2-2)}$

In the above test the "p" value of less than 0.05 was accepted as indicating statistical significance. Data analysis was carried out using Statistical Package for Social Science (SPSS, V 10.5)

V. Results

The age of 148 patient included in the study was in the range of 17 to 36 years with the mean age of 26.6 years.

5.1 Third trimester correlation

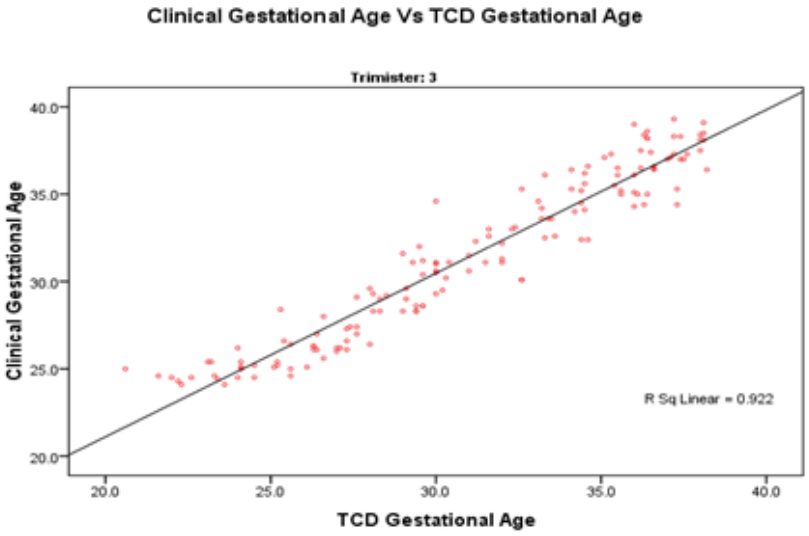


Figure 2. A scatter diagram showing the correlation and regression analysis of the TCD with CGA in third trimester. Here TCD is correlated with CGA by 92.2 %.

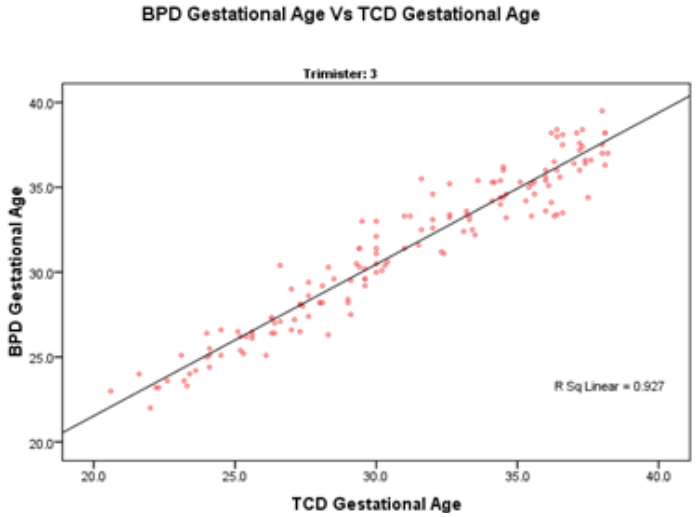


Figure 3. A scatter diagram showing the correlation and regression analysis of the TCD with BPD in third trimester. Here TCD is correlated with BPD by 92.7 %.

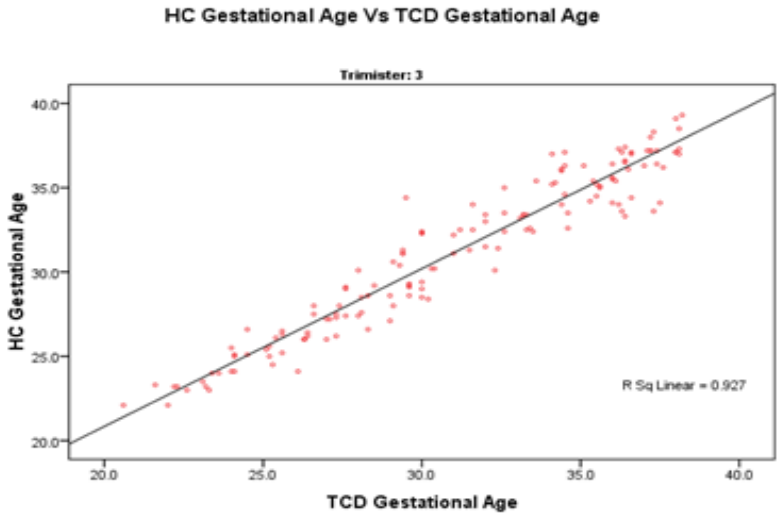


Figure 4. A scatter diagram showing the correlation and regression analysis of the TCD with HC in third trimester. Here TCD is correlated with HC by 92.7 %.

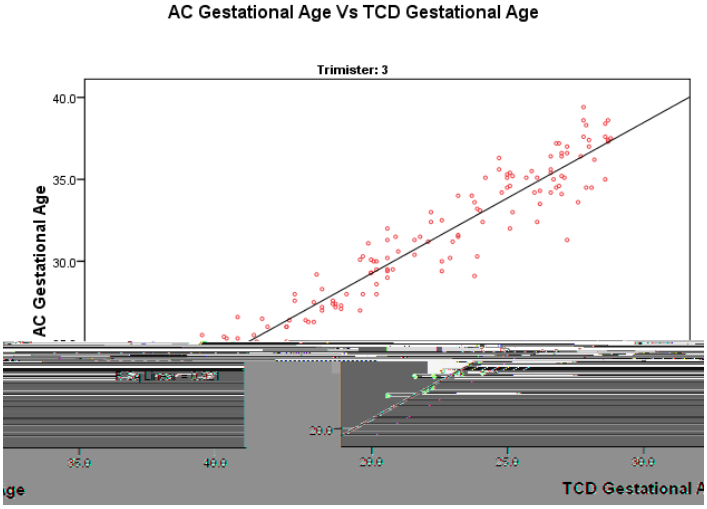


Figure 5. A scatter diagram showing the correlation and regression analysis of the TCD with AC in third trimester. Here TCD is correlated with AC by 92.1 %.

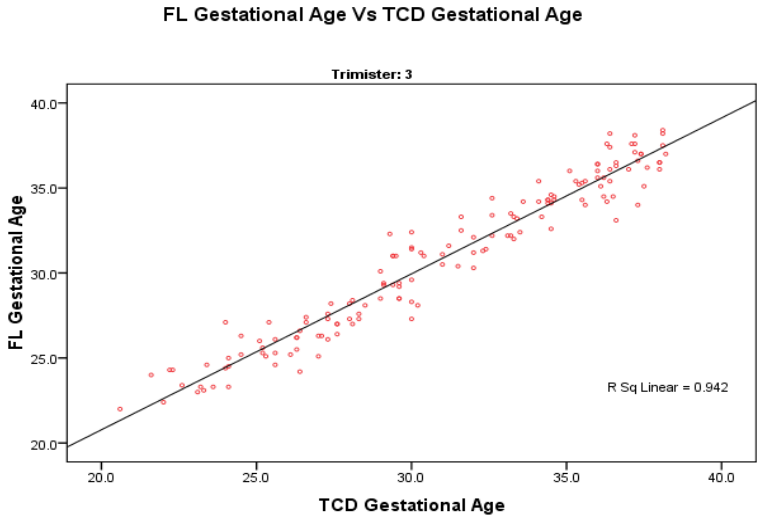


Figure 6. A scatter diagram showing the correlation and regression analysis of the TCD with FL in third trimester. Here TCD is correlated with FL by 94.2 %.

TCD is correlated with Clinical GA, BPD, HC, AC and FL in third trimester by 92.2%, 92.7%, 92.7%, 92.1% and 94.2% respectively.

TABLE 1. Correlation co-efficient of TCD with CGA, BPD, HC, AC and FL in third trimester

Combination Of Parameters	***Pearson's Correlation Co-Efficient (r)***	***Significance***
TCD VS CGA	0.960	P<0.001
TCD VS BPD	0.963	P<0.001
TCD VS HC	0.963	P<0.001
TCD VS AC	0.960	P<0.001
TCD VS FL	0.970	P<0.001

This table reveals the association between the foetal measurements and TCD. The correlation was best for TCD vs FL (r : 0.970). The correlation for TCD vs CGA, BPD, HC and AC was almost similar (r : 0.960, 0.963, 0.963 and 0.970 respectively). All the correlations were statistically significant.

TABLE 2. Correlation co-efficient of CGA with BPD, HC, AC, FL and TCD in third trimesters

Combination of Parameters	***Pearson's Correlation Co-Efficient (r)***	***Significance***
CGA VS BPD	0.937	P<0.001
CGA VS HC	0.947	P<0.001
CGA VS AC	0.952	P<0.001
CGA VS FL	0.960	P<0.001
CGA VS TCD	0.960	P<0.001

The above table shows the association between foetal measurements with CGA. The correlation was best for CGA vs FL and CGA vs TCD (r : 0.960). All the correlations were statistically significant.

5.2 TCD/AC Ratio:

TCD/AC remain fairly constant, between 25th week and term with values of TCD/AC ratio 14.59 +/- 2SD (SD: 1.14). There was a strong linear relationship between TCD and AC.

Table 3. Nomogram

TCD (mm)	***Gestational age***	***TCD (mm)***	***Gestational age***
29	25.3	43	33.4
30	25.7	44	34.1
31	26.5	45	34.4
32	27.2	46	34.7
33	27.8	47	34.9

34	28.6	48	35.7
35	29.2	49	36.1
36	29.7	50	36.4
37	30.3	51	36.8
38	31.0	52	37.2
39	31.4	53	37.4
40	31.9	54	37.8
41	32.5	55	38.0
42	33.2	56	38.1

VI. Discussion

In this study of 148 healthy women with uncomplicated pregnancy shows correlation between the gestational age and TCD. This has also been proven by various other studies. There is a linear relationship between the cerebellar growth and the gestational age in the third (24 wks to term) trimester. This relationship of foetal cerebellar growth and gestational age is statistically significant. In the present study the correlation of TCD with other foetal biometric parameters such as BPD, HC, AC and FL were statistically significant with the P value of 0.963, 0.963, 0.960 and 0.970 respectively. TCD correlate with the clinical gestational age with the statistically significant P value of 0.960. So in the present study TCD correlates well with gestational age with high correlation coefficient of 0.96 in the third trimester along with FL, as the first accurate parameters for assessing the gestational age.

The fetal cerebellum visualized as early as 10-20 postmenstrual weeks, grows in linear pattern in second trimester but its curve flattens in third trimester [1]. The normal foetal TCD increase with advancing gestational age and exhibit a more than two fold increase in size during the second half of pregnancy [4]. Normal sonographic anatomy of the developing cerebellum can be misinterpreted for pathological conditions [5,6]. So caution is warranted in making an early diagnosis of foetal cerebellar dysgenesis [5].

Sonographic transverse cerebellar diameter is not affected by the alteration in the shape of the foetal skull which affects the biparietal diameter and increase the variability [7-9]. TCD correlates well with gestational age and is more useful indicator of the accurate gestational age in case of dolichocephaly or brachycephaly and facilitate antenatal detection of congenital disorders [8]. Difference in actual gestational age and gestational age predicted by TCD is 0-4 days for 17-21 weeks, 0-2 days for 22-28 weeks, > 5 days for 29-36 weeks and 9 days for 37 weeks [10]. Foetal TCD correlates well with the clinical gestational age in third trimester also [1,10].

The foetal TCD / AC ratio is a gestational age independent method of assessing foetal growth [1,11-13]. In normal pregnant women the TCD / AC ratio was fairly constant with the mean of 13.68 +/- 0.96 irrespective of gestational age [1,12,13]. A value exceeding 2 SD of the mean was significantly associated with birth of a small for gestational age infants but not useful for identifying large for gestational age infant [12]. In the present study we found that TCD/AC ratio remains fairly constant in third trimester with value of 14.59 +/- 2SD (SD: 1.141). There was a strong linear relationship between TCD and AC. TCD/AC ratio was 13.6 +/- 0.95% in study done by Campbell *et. al.* in 1991 [13]. Meyer and his colleagues described that TCD/AC ratio is 13.69+/-0.94% in there study in 1993 [12]. According to studies by Malik *et. al*. in 2003 [14] and 2006 [1] TCD/AC ratios were 0.14064 +/-0.059 and 0.13 respectively.

The foetal TCD is not independent of the ethnic origin of the patient [15,16]. A study in 2001 on 153 normal pregnant women on Thai population concludes that growth rate of TCD was slightly less than that of a western study after 28 weeks of gestation [16]. In our study, all the patients were of Indian origin and the normogram for predicting gestational age from TCD was obtained. The values were compared with a study conducted by Hill *et. Al* [17]and it was observed that the values reported in our study are slightly smaller. This is probably due to the difference in the ethnic origin of patients.

The foetal TCD is useful for prediction of gestational age at the extremes of foetal growth [18-20]. There is relative preservation of normal cerebellar growth in growth restricted fetuses and a similar rate of growth in singleton and multifoetal gestation. In most of growth restricted fetuses, except for TCD all other biometric parameters will be < 10^{th} percentile [18]. The difference between actual and predicted gestational age based on TCD in IUGR foetus is within 3 days in 97.5% in the second trimester and 93.3% in the third trimester. In large for date foetus the difference between actual and predicted gestational age based on TCD is within 3 days was 100% in both second and third trimesters [19].

VII. Conclusion

TCD correlates gestational age as the first best parameter and also positively correlates with BPD, HC, AC and FL.So TCD can be reliably be used for gestational age assessment in third trim. TCD/AC ratio is independent of gestational age and can be used in assessing the fetal growth in pregnancies with unknown

dating. The results of present study show that transcerebellar diameter along with other parameters can improve the accuracy of antenatal sonography as well as sensitivity and specificity for fetal growth abnormalities.

References

[1]. Ghazala Malik, Fareesa Waqar, Abdul Ghaffar and Huma Zaidi, Determination of gestational age by transverse cerebellar diameter in third trimester of pregnancy, JCPSP, 2006, 16(4), 249-252.
[2]. Martin R. Chavez et. al., Fetal transcerebellar diameter measurement for prediction of gestsational age at the extremes of fetal growth, J Ultrasound Med,2007, 26, 1167-1171.
[3]. Mahony, B. S., Callen, P. and Filly, A. R., The distal femoral epiphysisal ossification center in the assessment of third trimester menstrual age: sonographic identification and measurement, Radiology,1985, 155, 201-204.
[4]. Vinkesteijn, A. S. M., Mulder, P. G. H. and Wladimiroff, J. W., Fetal transverse cerebellar diameter measurements in normal and reduced fetal growth, Ultrasound in obstetrics and gynecology, 2000, 15(1) 47-51.
[5]. Catherine J. Babcook et. al., Sonograhic anatomy of the developing cerebellum: normal embryology canresemble pathology, AJR, 1996, 166, 427-433.
[6]. Brayann Bromley et. al., Closure of the cerebellar vermis: evaluation with second trimester US, Radiology, 1994, 193, 761-763.
[7]. Mikovic Z. et. al., Growth of the fetal cerebellum in normal pregnancy, Jugosl Ginekol Perinatol, 1989, 29, 5-6, 157-160.
[8]. Hata, K. et. al., Ultrasonographic measurement of the fetal transverse cerebellum in utero, Gynecol obstet invest, 1989, 28(2), 111-112.
[9]. Goldstein, I., Reece, E. A., Pilu, G., Bovicelli, L. and Hobbins, J. C., Cerebellar measurements with ultrasonography in the evaluation of fetal growth and development, Am J Obstet gynecol, 1987, 156(5) 1065-1069.
[10]. Chavez et. al., Fetal transcerebellar diameter measurement with particular emphasis in the third trimester: a reliable perictor of gestational age, Am J Obstet gynecol, 1995, 191(3) 979-984.
[11]. Meyer, W. J. et. al., The fetal transverse cerebellar diameter / abdominal circumference ratio: a gestational age – independent method of assessing fetal size, J Ultrasound Med, 1993, 12(7) 379-382.
[12]. Meyer et. al., Ultrasonographic detection of abnormal fetal growth with the gestational age independent transverse cerebellar diameter / abdominal circumference ratio, Am J Obstet gynecol, 1994, 171(4) 1057-1063.
[13]. Winston A. Campbell et. al., Transverse cerebellar diameter / abdominal circumference ratio throughout pregnancy: a gestational age – independent method to assess fetal growth, Am J Obstet gynecol, 1991, 77(6) 893-895.
[14]. Malik, R., Pandya, V. K. and Shrivastava, P., Gestational age estimation using transcerebellar diameter with grading of fetal cerebellum and evaluation of TCD/AC ratio as a gestational age independent parameter, Ind J Radiol Imag, 2003, 13(1) 95-97.
[15]. Jacquemyn, Y., Sys, SU and Verdonk, P., Fetal transverse cerebellar diameter in different ethnic groups, J Perinat Med, 2000, 28(1) 14-19.
[16]. Uerpairojkit, B. et. al., Fetal transverse cerebellar diameter in Thai population groups, J Med Assoc Thai, 2001, 84(1) S346-351.
[17]. Hill, L. M., Guzick D. and Fries J., Transverse cerebellar diameter as a predictor of menstrual age, Obstet Gynecol, 1990, 75, 983.
[18]. Goldstein et. al., Cerebellar growth in normal and growth restricted fetuses of multiple gestation, Am J Obstet gynecol, 1995, 173(4) 1343-1348.
[19]. Martin R. Chavez et. al., Fetal transcerebellar diameter measurement for prediction of gestsational age at the extremes of fetal growth, J Ultrasound Med, 2007, 26, 1167-1171.
[20]. Guan B., Surveillance of fetal growth and fetal cerebellar transverse diameter by ultrasonographic measurement, Zhonghua Yi Xue Za Zhi, 1992, 72(2) 65-67.

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Original Article

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New Generation Contrast Media and Renal Saftey

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Abstract

Background: With the advancement in radiological interventions and diagnostic imaging iodinated contrast has an important role to play. Research and advancement in contrast media safety makes this pharmaceutical agent safe to use in daily practice. Our study is done to assess the safety of present generation iodinated contrast media over the kidneys in patients with heterogenous clinical setting undergoing contrast enhanced CT with serum creatinine and e-GFR as a tool for renal function assessment. The study concludes low-osmolar non – ionic contrast is safe in general population; however, we would advise judicious use of intravenous low-osmolar non-ionic contrast in patients with associated comorbid conditions due to mild but insignificant rise in serum creatinine values. **Subjects and Methods:** ?. **Results:** ?. **Conclusion:** ?.

Keywords: Contrast induced nephropathy; low-osmolar contrast media.

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Abbrevations: e-GFR = estimated glomerular filtration rate, sr. creatitine = serum creatinine, DM = Diabetes mellitus, HTN = Hypertension, CT = Computed tomography, CIN = Contrast induced nephropathy.

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Introduction

The role of iodinated contrast in medical diagnostic imaging and intervention procedures are inevitable. Their value has been long recognised as attested by their increased usage in various diagnostic and intervention procedures. Under normal circumstance nearly all the intravenous contrast media are eliminated through kidneys. The purpose of this study is to evaluate the renal safety of present-day contrast media in patients with heterogenous clinical settings using serum creatinine and e-GFR.

Subjects and Methods

This is a prospective study done with 158 patients at A.J. Institute of Medical sciences between 2011-2014 undergone computed tomography with Low-osmolar non-ionic iodinated contrast media (Iopromide) with iodine strength 350mg/ml and is given as per the standard regime i.e., 1 - 2 ml/ kg. Sr, creatinine is evaluated in the subjects blood sample collected 4-6hrs before and 48-72hrs after procedure. eGFR is calculated using Cockcroft-Gault formula1.

Collected data were also analysed for sensitivity, specificity, positive predictive value and chi-square test. P-value <0.05 are considered significant and P – value >0.05 are considered insignificant.

Results

Table 1: Influence of Age Over Creatinine Variation

Creatinine Variation	Age in Years							Total
	19 – 29	30 – 39	40 – 49	50 – 59	60 – 69	70 – 79	80 – 90	
0 – 5 %	2	3	9	6	5	2	0	27
6 – 10 %	1	3	5	13	9	5	0	36
11 – 15 %	4	5	7	14	11	4	1	46
16 – 20 %	6	2	12	2	7	3	1	33
21 – 25 %	2	3	4	1	5	1	0	16
Total	15	16	37	36	37	15	2	158

CHI – Square Test Value: 26.70 P-value : 0.31

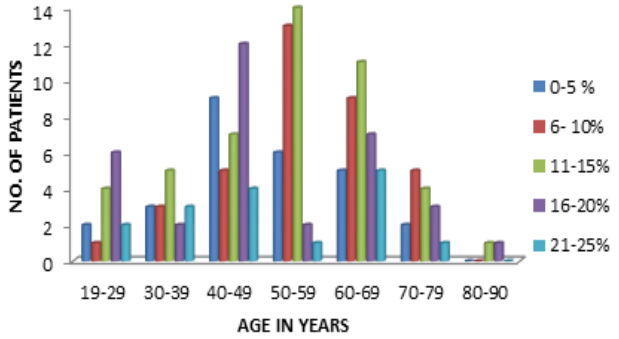


Figure 1: Influence of Age Over Creatinine Variation

The above table and column chart [Figure 1] represent the comparison of age with the creatinine variation to know the influence of age over the variation in creatinine value. The statistical analysis using Pearson Chi-Square test with value of 26.70 and P-value 0.31 confirms that age does not have direct influence on percentage of creatinine variation when using non-ionic contrast.

Table 2: Comorbid Conditions Over Creatinine Variation

Creatinine variation	Comorbid Conditions				Total
	Absent	DM	HTN	BOTH	
0 – 5 %	27	0	0	0	27
6 – 10 %	24	8	0	4	36
11 – 15 %	32	7	1	6	46
16 – 20 %	22	10	1	0	33
21 – 25 %	2	9	0	5	16
Total	107	34	2	15	158

CHI – Square Test Value: 44.70 P – VALUE: 0.0001

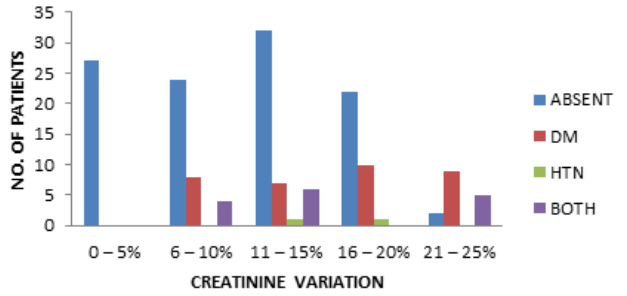


Figure 2: Creatinine Variation.

The above table and column chart [Figure 2] analysis the incidence of contrast induced nephropathy and percentage of creatinine variation among the study population with associated comorbid conditions. There is no incidence of contrast induced nephropathy among the study population with associated comorbid conditions. However, the statistical analysis with Pearson Chi-Square test value 44.70 and P-value 0.0001 confirms that subjects with comorbid conditions have direct influence on percentage variation on creatinine value. It is evident from the column chart that majority of population among the higher percentage (21 – 25%) creatinine variation group are with Diabetes or both Diabetes and Hypertension.

Discussion

Contrast-induced nephrotoxicity (CIN) is a sudden deterioration in renal function following the recent intravascular administration of iodinated contrast medium in the absence of another nephrotoxic event. Low and iso-osmolar contrast is relatively safe in comparison with high-osmolar contrast media.[2,3] Unfortunately, very few published studies adequately isolate patients undergoing contrast enhanced CT in whom iodinated contrast medium exposure is the only nephrotoxic event.[4-7] CIN appears within 48 hours after the administration of radiographic contrast media and is maintained for 2–5 days.[8] An overall incidence of CIN in the general population is reported to be 0 –2.3%.[9]

There are no standard criteria for the diagnosis of CIN; criteria used in the past have included percent change in the baseline serum creatinine (e.g., an increase of variously 25% to 50%) and absolute elevation from baseline serum creatinine (e.g., an increase of variously 0.5 to 2.0 mg/dL).[10,11] One of the most commonly used criteria has been an absolute increase of 0.5 mg/dL.

The risk of developing contrast-induced nephropathy is influenced by diabetes, hypertension, age and reduced eGFR.[12] The risk of CIN was found to be 0.6% in patients with eGFR greater than 40 mL/min/1.73m2 and 4.6% in patients with an eGFR of 30 to 40 mL/min/1.73m2.[13] The CIN rate was 7.8% with an eGFR > 30 mL/min/1.73m2.

We in our study have aimed at analysing changes in renal functions in patients with heterogenous clinical settings undergoing computed tomography with low-osmolar iodinated contrast media keeping serum creatinine and eGFR as baseline investigation.

The major preventive action against CIN is to ensure adequate hydration.[14] We during our study maintained adequate hydration with 0.9% normal saline infusion at 100 mL/hr, beginning 6 to 12 hours before and continuing 4 to 12 hours after intravascular iodinated contrast medium administration for the inpatient and oral hydration for outpatients with comorbidity such as diabetes and hypertension.[15]

Among the 158-study population 110(69.6%) were male patients and 48 (30.4%) were female patients with wide range of age distribution. Among the 158 patients 34 are diabetic, 2 are hypertensive, 15 had both diabetes and hypertension, rest of the 107 patients did not had any comorbidities. The percentage increase in serum creatinine did not had any direct relation to the age or sex. However, the statistical analysis [Table 2] with value 44.70 and P-value 0.0001 confirms that subjects with comorbid conditions have direct influence on percentage variation on creatinine value but insignificant rise to term it as contrast induced nephropathy.

Conclusion

After a thorough evaluation of 158 patients with heterogenous clinical setting who has undergone contrast enhanced computed tomography with lowosmolar non-ionic intravenous contrast, we can conclude that incidence of contrast induced nephropathy among the general population is negligible and the use of lowosmolar non – ionic contrast is safe in such patients.

However, we would advise judicious use of intravenous lowosmolar non-ionic contrast in patients with associated comorbid conditions like Diabetes mellitus and hypertension, since we saw a mild but insignificant rise in serum creatinine values in such patients.

References

1. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16:31-41.
2. Barrett BJ, Carlisle EJ. Metanalysis of the relative nephrotoxicity of high- and low-osmolality iodinated contrast media. Radiology 1993;

188:171-178.
3. Pugh ND. Ha Pugh ND. H Pugh ND. Haemodynamic and rheological effects of contrast media: the role of viscosity and osmolality. Eur Radiol 1996; 6: S13-S15.
4. BaeKT. Intravenous contrast medium administration and scan timing at CT: considerations and approaches. Radiology 2010; 256:32-61.
5. Vergara M, Seguel S. Adverse reactions to contrast media in CT: effects of temperature and ionic property. Radiology 1996; 199:363-366.
6. Herts BR, Schneider E, Poggio ED, Obuchowski NA, Baker ME. Identifying outpatients with renal insufficiency before contrast enhanced CT by using estimated glomerular filtration rates versus serum creatinine levels. Radiology 2008; 248:106-113
7. Katzberg RW, Newhouse JH. Intravenous contrast medium-induced nephrotoxicity: is the medical risk as great as we have come to believe? Radiology 2010; 256:21-28.
8. Kolonko A, Wiecek A. Contrast-associated nephropathy: old clinical problem and new therapeutic perspectives. Nephrol Dial Transplant 1998; 13:803–806
9. Lasser EC, Lyon SG, Berry CC. Reports on contrast media reactions: analysis of data from reports to the US Food and Drug Administration. Radiology 1997; 203: 605–610.
10. Katzberg RW. Urography into the 21st century: new contrast media, renal handling, imaging characteristics, and nephrotoxicity. Radiology 1997;204:297–312
11. Parfrey PS, Griffiths SM, Barrett BJ, et al. Contrast material-induced renal failure in patients with diabetes mellitus, renal insufficiency, orboth. N Engl J Med 1989;320:143–153
12. ACR manual on contrast media 2018; Version 10.3: 33-44.
13. Thomsen HS, Morcos SK. Risk of contrast-medium-induced nephropathy in high-risk patients undergoing MDCT--a pooled analysis of two randomized trials. Eur Radiol 2009; 19:891-897.
14. Erley CM. Does hydration prevent radiocontrast-induced acute renal failure? Nephrol Dial Transplant 1999; 14:1064–1066
15. Solomon RJ, Natarajan MK, Doucet S, et al. Cardiac Angiography in Renally Impaired Patients (CARE) study: a randomized double-blind trial of contrast-induced nephropathy in patients with chronic kidney disease. Circulation 2007; 115:3189-3196.

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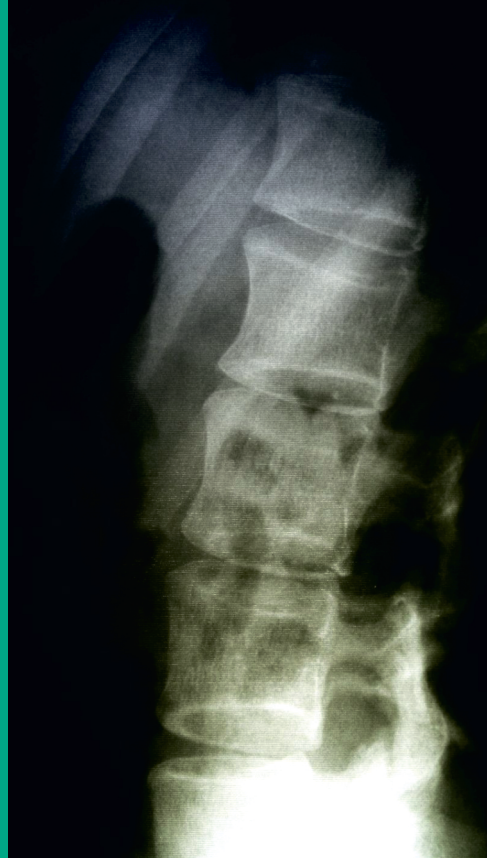
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Original Article

Utility of ultrasound elastography in evaluation of thyroid nodules and correlation with cytology

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Abstract

Background: Ultrasound elastography (USE) has emerged as a potentially useful tool in the evaluation of thyroid nodules. The aim of this study was to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with fine-needle aspiration cytology (FNAC) analysis as reference standard.

Materials and Methods: This was a cross-sectional prospective study carried out at a tertiary care center in South India between May 2014 and March 2015. Consecutive patients with solid nodule in the thyroid gland on conventional ultrasound (US) underwent USE, followed by US-guided FNAC of the thyroid nodule. The findings of B-mode images and USE were correlated with cytology. Statistical analysis was performed using SPSS version 24. Descriptive statistics were presented as mean ± standard deviation. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value of USE were calculated.

Results: A total of sixty patients with sixty solid thyroid nodules were enrolled in the study. The mean age of the patients was 48.27 ± 16.10 years. About 83.3% were females. The frequency of benign nodules on cytopathologic analysis was 60% (n = 36). Seventy-five percent of the malignant lesions had irregular or poorly defined borders with lobulated margins on US. The sensitivity, specificity, positive predictive value, and negative predictive value of USE were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as reference standard. Higher elastography scores were found to be significantly associated with malignant cytology (P = 0.0001).

Conclusion: Elastography, with its fairly high diagnostic accuracy and correlation with malignant cytology, can be an effective noninvasive adjunctive tool in distinguishing benign from malignant thyroid nodules.

Keywords: Elastography, thyroid nodules, ultrasound

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INTRODUCTION

The prevalence of thyroid nodules which are clinically palpable is about 2%–6% in the general population.[1] Although majority of these nodules are benign, up to 7% can be malignant.[2,3]

On palpation, a hard or firm nature is associated with an increased risk of malignancy. However, palpation is a highly subjective method and its value is limited in patients with multinodular goiter or deep-seated nodules. Ultrasonography (USG) is the most commonly used imaging modality to diagnose thyroid nodules. The utility of

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USG in differentiating benign from malignant lesions has been established by several studies.[4-6] However, no USG feature has been shown to have both high sensitivity and high specificity in detecting malignancy in thyroid nodules.[7] Elastography is a newly developed noninvasive dynamic imaging technique analogous to manual palpation. This technique uses ultrasound (US) to estimate tissue stiffness by measuring the degree of distortion under application of an external force.[8] Several studies have used US elastography (USE) for evaluation of thyroid disorders.[9-11]

Fine-needle aspiration cytology (FNAC) is an efficient tool in distinguishing benign nodules that can be managed conservatively from those with suspicious or malignant features that warrant further intervention. Limited data exist on the utility of elastography in differentiating benign from malignant lesions of the thyroid and its correlation with FNAC, especially from India. The aim of this study was to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with FNAC analysis as reference standard.

MATERIALS AND METHODS

This prospective study was carried out at a tertiary care center in South India between May 2014 and March 2015. Consecutive patients of either gender who were found to have thyroid swelling/goiter on clinical examination and referred for USG were considered. Only those with solid thyroid nodules at least 8 mm in size on conventional US were included in the study. Cystic lesions with no solid component, multinodular goiters with coalescent nodules, and nodules with eggshell calcification were excluded from the study. A large nodule involving more than 75% of thyroid lobe volume was also excluded because of inadequate normal thyroid tissue to be used as reference. The institutional review board approved the study protocol, and the protocol complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants.

With a patient in supine position and neck fully extended, B-mode US of the thyroid gland was performed with SIEMENS ACUSON S2000 machine using high-frequency probes 9 L4 and 14 L5. US features studied included echogenicity, regularity of borders, presence of halo, vascular pattern, and calcification. Thyroid imaging reporting and data system (TIRADS) scoring was used to categorize the lesions[12] [Table 1].

USE was performed with SIEMENS ACUSON S2000 at the same time as the B-mode examination. The probe was placed over the region such that the lesion is in the center of the image, holding the scan plane perpendicular to the skin surface. At least 5 mm of normal adjacent tissue was included to assess the lesion stiffness in relation to the average elasticity of surrounding tissue. Patients were asked to hold their breath and avoid swallowing during examination in order to minimize the motion of the thyroid gland. The elasticity images were obtained by appropriate compression, followed by decompression of the transducer. The images were displayed on split-screen mode with US grayscale images on the left and USE images on the right. Multiple frames were acquired, and the best-fit B mode-USE image pairs were selected for examination [Figures 1 and 2]. The targeted lesion was scored as 1–4, using strain elastographic scores proposed by Asteria *et al*.[9] [Table 2].

US-guided FNAC of the thyroid nodule was done using a 22G needle, and the lesion was aspirated at least twice with the freehand technique. The aspirated contents were expelled onto glass slides and immediately fixed with 95% ethyl alcohol. The slides were stained with modified Papanicolaou staining and examined under light microscopy. The cytological results were classified

Table 1: Thyroid image reporting and data system scoring of thyroid nodules

Category	Description
TIRADS 1	Normal thyroid gland
TIRADS 2	Benign conditions
TIRADS 3	Probably benign nodules (<5% malignant)
TIRADS 4	Suspicious nodules (5%-80% malignancy rate)
TIRADS 5	Probably malignant nodules (malignancy rate >80%)
TIRADS 6	Biopsy-proven malignant nodules

TIRADS – Thyroid image reporting and data system

Table 2: Asteria's criteria of scoring of thyroid nodules on elastography

Elasticity Score	Description
Score of 1	Elasticity in the entire examined area
Score of 2	Elasticity in a large part of the examined area
Score of 3	Stiffness in a large part of the examined area
Score of 4	Nodule without elasticity

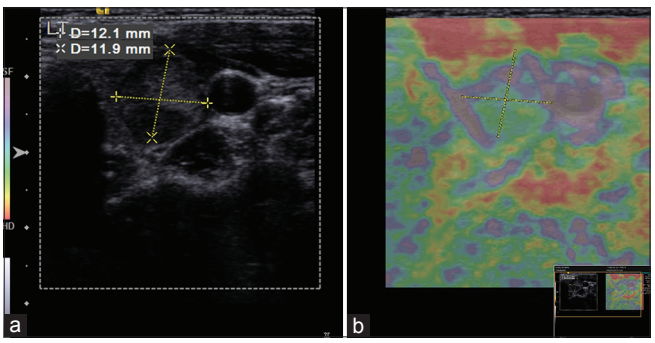


Figure 1: Thyroid nodule images obtained on conventional ultrasound (a) and ultrasound elastography with elasticity score 2 (b)

as benign, indeterminate/suspicious (follicular lesions), and malignant according to standard pathological criteria.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago. IL, USA) version 24. Descriptive statistics were presented as mean ± standard deviation. Qualitative variables were presented in the form of frequency and percentages and compared using the Chi-square test. The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were calculated. Statistical significance was set at $P < 0.05$.

For statistical purposes, USE score of 2 was strongly predictive of a benign lesion and therefore considered sample negative for malignancy. Scores of 3 and 4 describing stiffness in a large part of the examined area and nodule without elasticity, respectively, were considered as positive for malignancy in our study. With regard to cytology, two groups were considered for evaluation: benign and malignant. Patients with fine-needle aspiration (FNA) reports of indeterminate/suspicious (follicular lesions) were also included in the malignant group for statistical purposes.

RESULTS

A total of sixty patients with sixty solid thyroid nodules were enrolled in the study. The age of the patients ranged from 19 to 85 years (mean: 48.27 ± 16.10 years). About 83.3% (n = 50) were female and 16.7% (n = 10) were male. The frequency of benign nodules on cytopathologic analysis was 60% (n = 36), whereas 40% (n = 24) were nonbenign (follicular lesions and malignant tumors). Of the 24 nonbenign thyroid nodules, 20 had been reported as papillary carcinoma, 2 as medullary carcinoma, and 2 as follicular neoplasm. Table 3 shows the presenting clinical features and cytologic diagnoses of patients with thyroid nodules.

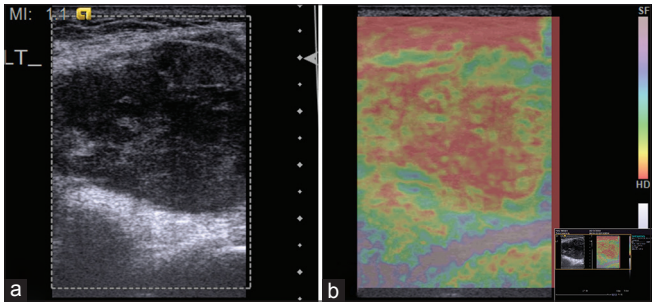


Figure 2: Heterogeneous nodule on conventional ultrasound (a) and ultrasound elastography with elasticity score 4 (b)

Seventy-five percent of the malignant lesions had irregular or poorly defined borders with lobulated margins on US and 68% had microcalcification. Seventy-seven percent of the benign nodules were hyperechoic. On TIRADS scoring, score of 2 was found in 24 cases, all benign lesions; score 3 in 14 cases, 8 benign and 6 malignant; score 4 in 12 cases, 4 benign and 8 malignant; and score 5 in 10 cases, all malignant lesions. Table 4 shows the correlation of USE scores and FNAC diagnoses of patients.

The sensitivity, specificity, positive predictive value, and negative predictive value of US elastography were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as a reference standard [Table 5]. Higher elastography scores were found to be significantly associated with malignant cytology ($P = 0.0001$).

DISCUSSION

Thyroid nodules are common in the general population, and the reported prevalence varies depending on the identification method. Apart from serum markers and FNAC, USG plays an important role in the detection and evaluation of thyroid nodules. Being noninvasive conventional US provides information regarding the nodule dimensions, structure as well as parenchymal changes and is widely used to determine which nodules should be biopsied. Microcalcifications, hypoechogenicity,

Table 3: Clinical presentation of thyroid nodules

Clinical feature	Benign	Malignant	Total number of patients, *n* (%)
Neck swelling	26	2	28 (46.7)
Weight loss	2	8	10 (16.7)
Dysphagia	2	6	8 (13.3)
Pain	6	2	8 (13.3)
Hoarseness of voice	0	6	6 (10)

Table 4: Correlation of elastography scores and cytologic diagnoses of patients with thyroid nodules

Elastography score	Benign	Malignant	Total
2	28	2	30
3	8	2	10
4	-	20	20
Total	36	24	60

Table 5: 2×2 table for calculation of sensitivity, specificity, positive predictive value, and negative predictive value of ultrasound elastography taking cytology as a reference standard

USE	Malignancy (positive on FNA)	Malignancy (negative on FNA)	Total
Positive	22 (true positive)	8 (false positive)	30
Negative	2 (false negative)	28 (true negative)	30
Total	24	36	60

FNA – Fine-needle aspiration; USE – Ultrasound elastography

irregular or microlobulated margins, taller than wide shape, and increased intranodular vascularity have been reported as independent risk factors for malignancy.[13,14] Although these features are characterized by high specificity, their positive predictive value is lowered by their relatively low sensitivity.[15]

USE is based on the principle that the softer parts of tissues deform easier than the harder parts, under compression, thereby allowing an objective determination of tissue consistency. Malignancy is often associated with changes in the mechanical properties of a tissue, and USE has been used to differentiate benign from malignant lesions in the breast, liver, and prostate.[16-18] The two general criteria for evaluating the efficacy of USE are elasticity scores and strain ratio. Although elastography has been suggested to be of great use as an adjunctive tool in the diagnosis of thyroid cancer, studies have reported conflicting evidence on its additional value in predicting thyroid malignancy.[19-21]

This study was planned to assess the efficacy of elastography in differentiating benign from malignant thyroid nodules with FNAC analysis as a reference standard. The mean age of presentation for benign nodules was 44.31 ± 14.28 years, whereas that for malignant lesions was 54.21 ± 17.13 years ($P = 0.018$) A majority of our cases diagnosed with thyroid nodules were females (83.3%). Thyroid nodules are reported to be four times more common in women than men, and their frequency increases with age and low iodine intake.[22] The female preponderance has been explained by the hormonal influences of estrogen and progesterone, as increasing nodule size and new nodule development have been demonstrated to be related to pregnancy and multiparity.[23]

Of the sixty nodules studied, 60% were benign ($n = 36$) and 40% ($n = 24$) were nonbenign. Of the 36 benign nodules, 26 were colloid nodules and 10 were adenomatoid nodules. The 24 nonbenign lesions included 20 papillary carcinomas, 2 medullary carcinomas, and 2 follicular neoplasms. The most common clinical presentation was painless mass in the neck which was encountered in almost 47% of the patients. Majority of the patients who presented with significant weight loss, dysphagia, and hoarseness of voice were found to have malignant lesions. Dysphagia, dysphonia, reports of rapid growth, male gender, prior head-and-neck irradiation, and presentation at extremes of age have been reported as significant elements in the patient's history, which increase the likelihood of malignancy.[24]

Our study found that 75% of malignant lesions had irregular or poorly defined borders with lobulated margins, whereas 68% of malignant lesions had microcalcification. Seventy-seven percent of the benign nodules were hyperechoic, whereas 23% were hypoechoic. None of the hyperechoic nodules was malignant. Although none of these US features is sufficient to differentiate benign lesions from malignant ones, studies have shown that a combination of at least two of the features is better helpful in pointing out a subset of lesions at high risk for malignancy.[25,26]

With regard to TIRADS score, all the nodules with TIRADS score of 2 ($n = 24$) had benign FNA characteristics, whereas all the patients with TIRADS score of 5 ($n = 10$) were found to have malignant lesions on FNA. Among TIRADS 3 lesions ($n = 14$), 57% were benign, and among TIRADS 4 lesions ($n = 12$), 33% were found to be benign on FNA.

On US elastography, score of 2 was found in 30 nodules, 28 benign and 2 malignant; score 3 in 10 cases, 8 benign and 2 malignant; and score 4 in 20 cases, all malignant. The sensitivity, specificity, positive predictive value, and negative predictive value of US elastography were 91.7%, 77.77%, 73.33%, and 93.3%, respectively, with FNAC as a reference standard.

The elastography scores of 3–4 were achieved in 22 of the 24 malignant nodules in our study. Among the 20 nodules with cytologic diagnosis of papillary carcinoma, 18 were correctly scored as malignant by USE, thus achieving 90% diagnostic accuracy in terms of confirming the malignant etiology. Only two nodules were incorrectly given a USE score of 2. Both of the two nodules with medullary carcinoma thyroid had been scored as malignant on USE (score 4). We had eight false-positive nodules (score 3) which can be attributed to the fibrotic changes and calcification that lead to hardness of the nodule under study.

Our observations are in line with that of Mehrotra *et al.* who have reported the sensitivity and specificity of USE for thyroid malignancy to be 90% and 79.6%, respectively.[27] Of the 147 nodules examined by USE and US-guided FNAC, 122 were nonneoplastic, 5 were benign neoplasm, 10 had indeterminate cytologies, and 10 had malignant neoplasms. The observed positive and negative predictive values were 24.3% and 99.1%, respectively. In contrast, we found the positive predictive value of elastography to be 73.3% in our study. This can be explained by the high prevalence of malignant lesions among our study population.

The sensitivity and specificity of elastography based on elastography scores have been reported to vary from

86% to 97% and 66%–100% from various studies.[8,9,28] The observed wide differences are due to the fact that these studies involve different groups of populations, referral bias (patients referred for FNAC/surgery), varied inclusion and exclusion criteria, different machines as well as criteria for USE scoring. A recent comprehensive meta-analysis to investigate the diagnostic value of qualitative elastography in distinguishing benign from malignant thyroid nodules included 20 studies involving 3973 thyroid nodules.[29] Pooled results of elastography have shown a summary sensitivity of 85% (95% confidence interval [CI], 79%–90%) and specificity of 80% (95% CI, 73%–86%). The respective pooled negative predictive and positive predictive values were 97% (95% CI, 94%–98%) and 40% (95% CI, 34%–48%), respectively.

Despite the potential limitations of USE such as intra/interobserver variability and nodule characteristics such as calcification and follicular carcinoma (which appears soft), USE is considered to be reliable in the diagnosis of benign nodules in view of the high negative predictive values observed in most of the studies including ours.

FNAC is an essential diagnostic modality in the evaluation of thyroid nodules and features in all the guidelines in patient management.[30] Although the use of FNAC in the evaluation of thyroid nodules provides reliable results, it is invasive and the potential for complications such as postprocedural hematoma, increased patient morbidity, cost, and possibility of nondiagnostic specimen does exist. Unnecessary/repeat FNACs and FNA-induced changes can result in false-positive results on thyroid cytology and false-negative results or understaging of the tumor on thyroid pathology.[31,32]

Higher elastography scores were found to be significantly associated with malignant cytology ($P = 0.0001$) in our study. Merino *et al*. have also noted a statistically significant association between elasticity scores and cytological results, suggesting that malignant nodules could be excluded by elastography.[33]

Limitations of our study include small sample size and the fact that observations are from a single center.

CONCLUSION

With scanty literature comparing elastography with FNAC available from this part of the world, our study highlights that elastography has a fairly high diagnostic accuracy and correlation with malignant cytology. It can be an effective noninvasive adjunctive tool in distinguishing benign from malignant thyroid nodules and reduce the burden of unnecessary invasive procedures.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Dean DS, Gharib H. Epidemiology of thyroid nodules. Best Pract Res Clin Endocrinol Metab 2008;22:901-11.
2. Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, *et al*. Management of thyroid nodules detected at US: Society of radiologists in ultrasound consensus conference statement. Radiology 2005;237:794-800.
3. Hoang JK, Lee WK, Lee M, Johnson D, Farrell S. US features of thyroid malignancy: Pearls and pitfalls. Radiographics 2007;27:847-60.
4. Jena A, Patnayak R, Prakash J, Sachan A, Suresh V, Lakshmi AY. Malignancy in solitary thyroid nodule: A clinicoradiopathological evaluation. Indian J Endocrinol Metab 2015;19:498-503.
5. Bonavita JA, Mayo J, Babb J, Bennett G, Oweity T, Macari M, *et al*. Pattern recognition of benign nodules at ultrasound of the thyroid: Which nodules can be left alone? AJR Am J Roentgenol 2009;193:207-13.
6. Remonti LR, Kramer CK, Leitão CB, Pinto LC, Gross JL. Thyroid ultrasound features and risk of carcinoma: A systematic review and meta-analysis of observational studies. Thyroid 2015;25:538-50.
7. Listewnik MH, Birkenfeld B, Piwowarska-Bilska H, Cichoń-Bańkowska K, Iglińska-Wagner L, Watrak W, *et al*. The application of SPECT/CT scintigraphy with MIBI-Tc^{99}(m) in the diagnosis of thyroid nodules – A preliminary report. Endokrynol Pol 2010;61:422-6.
8. Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: New developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab 2007;92:2917-22.
9. Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, *et al*. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523-31.
10. Hu X, Liu Y, Qian L. Diagnostic potential of real-time elastography (RTE) and shear wave elastography (SWE) to differentiate benign and malignant thyroid nodules: A systematic review and meta-analysis. Medicine (Baltimore) 2017;96:e8282.
11. Hahn SY, Shin JH, Ko EY, Bae JM, Choi JS, Park KW. Complementary role of elastography using carotid artery pulsation in the ultrasonographic assessment of thyroid nodules: A prospective study. Korean J Radiol 2018;19:992-9.
12. Horvath E, Majlis S, Rossi R, Franco C, Niedmann JP, Castro A, *et al*. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab 2009;94:1748-51.
13. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, *et al*. Risk of malignancy in nonpalpable thyroid nodules: Predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab 2002;87:1941-6.
14. Moon WJ, Jung SL, Lee JH, Na DG, Baek JH, Lee YH, *et al*. Benign and malignant thyroid nodules: US differentiation-multicenter retrospective study. Radiology 2008;247:762-70.
15. Popoveniuc G, Jonklaas J. Thyroid nodules. Med Clin North Am 2012;96:329-49.
16. Chang JM, Won JK, Lee KB, Park IA, Yi A, Moon WK. Comparison of shear-wave and strain ultrasound elastography in the differentiation of benign and malignant breast lesions. AJR Am J Roentgenol

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Technical Note

Assessment of Regional Pediatric Computed Tomography Dose Indices in Tamil Nadu

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Abstract

The aim of this article is to assess Tamil Nadu pediatric computed tomography (CT) diagnostic reference levels (DRLs) by collecting radiation dose data for the most commonly performed CT examinations. This work was performed for thirty CT scanners installed in various parts of the Tamil Nadu region. The patient cohort was divided into two age groups: <1 year, and 1–5 years. CT dose indices were measured using a 10 cm^3 pencil ion chamber with pediatric head and body polymethyl methacrylate phantoms. Dose data such as volumetric CT dose index ($CTDI_v$) and dose length product (DLP) on a minimum of twenty average-sized pediatric patients in each category were recorded to calculate a mean site $CTDI_v$ and DLP value. The rounded 75th percentile was used to calculate a pediatric DRL for each hospital, and then region by compiling all results. Data were collected for 3600 pediatric patients. Pediatric CT DRL for two age groups: <1 year ($CTDI_v$ and DLP of head [20 mGy, 352 mGy.cm], chest [7 mGy, 120 mGy.cm] and abdomen [12 mGy, 252 mGy.cm]), and 1–5 years ($CTDI_v$ and DLP of head [38 mGy, 505 mGy.cm], chest [8 mGy, 132 mGy.cm] and abdomen [14 mGy, 270 mGy.cm]) for select procedures have been calculated. Proposed pediatric DRLs of $CTDI_v$ and DLP for head procedure were lower, and for chest and abdomen procedures were higher than European pediatric DRLs for both age groups.

Key words: Diagnostic reference level, pediatric computed tomography, pediatric computed tomography diagnostic reference level, volumetric computed tomography dose index

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Introduction

Children are being referred for computed tomography (CT) scan due to the latter's speed, accuracy, flexibility, and accessibility. This has been mainly due to the introduction of fast spiral scanning, which obviates the need for sedation to keep children motionless, thereby permitting scans of younger or less cooperative children.[1] In the US, about 33% of all pediatric CT procedures are in children aged 10 or younger, with 17% of children aged five or younger.[2] At these ages, the organs and tissues are inherently more radiation sensitive to the oncogenic effects due to the larger quantity of cells that are separating and reproducing.[3-5] The International Commission on Radiological Protection (ICRP) states that children are 2–3 times more radiosensitive as compared to adult patients, due to the effect of cell division in developing organs and the longer anticipated lifetime for developing cancer cells.[6,7] The radiation-induced effects are also elevated in pediatric patients due to wider and enlarged cellular circulation of red bone marrow, and their higher postexposure life expectancy.[8,9] With this sharp radio-sensitivity now well documented, and to better inform radiographers about the significance of limiting the unwanted radiation dose to pediatric patients[10] the European Commission (EC) has, in its recommendations published in the year 2000, stressed the need for optimization of pediatric CT radiation dose.[11] The Food and Drug Administration (FDA) has also published related recommendations in 2002[12] about pediatric radiation dose optimization. While the use of CT scan for pediatric cases has increased unlike adult examination protocols, most of the times the exposure parameters used in

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2013;201:W347-56.
17. Kim JE, Lee JY, Bae KS, Han JK, Choi BI. Acoustic radiation force impulse elastography for focal hepatic tumors: usefulness for differentiating hemangiomas from malignant tumors. Korean J Radiol 2013;14:743-53.
18. Miyanaga N, Akaza H, Yamakawa M, Oikawa T, Sekido N, Hinotsu S, *et al*. Tissue elasticity imaging for diagnosis of prostate cancer: A preliminary report. Int J Urol 2006;13:1514-8.
19. Moon HJ, Sung JM, Kim EK, Yoon JH, Youk JH, Kwak JY. Diagnostic performance of gray-scale US and elastography in solid thyroid nodules. Radiology 2012;262:1002-13.
20. Veyrieres JB, Albarel F, Lombard JV, Berbis J, Sebag F, Oliver C, *et al*. A threshold value in Shear Wave elastography to rule out malignant thyroid nodules: A reality? Eur J Radiol 2012;81:3965-72.
21. Trimboli P, Guglielmi R, Monti S, Misischi I, Graziano F, Nasrollah N, *et al*. Ultrasound sensitivity for thyroid malignancy is increased by real-time elastography: A prospective multicenter study. J Clin Endocrinol Metab 2012;97:4524-30.
22. Mazzaferri EL. Management of a solitary thyroid nodule. N Engl J Med 1993;328:553-9.
23. Kung AW, Chau MT, Lao TT, Tam SC, Low LC. The effect of pregnancy on thyroid nodule formation. J Clin Endocrinol Metab 2002;87:1010-4.
24. Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-71.
25. Papini E. The dilemma of non-palpable thyroid nodules. J Endocrinol Invest 2003;26:3-4.
26. Frasoldati A, Valcavi R. Challenges in neck ultrasonography: Lymphadenopathy and parathyroid glands. Endocr Pract 2004;10:261-8.
27. Mehrotra P, McQueen A, Kolla S, Johnson SJ, Richardson DL. Does elastography reduce the need for thyroid FNAs? Clin Endocrinol (Oxf) 2013;78:942-9.
28. Ghajarzadeh M, Sodagari F, Shakiba M. Diagnostic accuracy of sonoelastography in detecting malignant thyroid nodules: A systematic review and meta-analysis. AJR Am J Roentgenol 2014;202:W379-89.
29. Nell S, Kist JW, Debray TP, de Keizer B, van Oostenbrugge TJ, Borel Rinkes IH, *et al*. Qualitative elastography can replace thyroid nodule fine-needle aspiration in patients with soft thyroid nodules. A systematic review and meta-analysis. Eur J Radiol 2015;84:652-61.
30. Ahn SS, Kim EK, Kang DR, Lim SK, Kwak JY, Kim MJ. Biopsy of thyroid nodules: Comparison of three sets of guidelines. AJR Am J Roentgenol 2010;194:31-7.
31. Eze OP, Cai G, Baloch ZW, Khan A, Virk R, Hammers LW, *et al*. Vanishing thyroid tumors: A diagnostic dilemma after ultrasonography-guided fine-needle aspiration. Thyroid 2013;23:194-200.
32. Canberk Ş, Fırat P, Schmitt F. Pitfalls in the cytological assessment of thyroid nodules. Turk Patoloji Derg 2015;31 Suppl 1:18-33.
33. Merino S, Arrazola J, Cárdenas A, Mendoza M, De Miguel P, Fernández C, *et al*. Utility and interobserver agreement of ultrasound elastography in the detection of malignant thyroid nodules in clinical care. AJNR Am J Neuroradiol 2011;32:2142-8.

THE ILL EFFECTS IN THE ORAL HEALTH AMONG THE ADULTS DUE TO THE CONSUMPTION OF AERATED DRINKS

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ABSTRACT

BACKGROUND

Excessive consumption of carbonated soft drink is detrimental to oral and general health and the popularity of the aerated drinks among adults has grown exponentially. This study aims at determining the prevalence of aerated drinks among students in PSG Institute of management. The focus of the study is mainly on the aerated drinks consumption pattern, frequency of consumption, reason and the following symptoms.

MATERIALS AND METHODS

The study was conducted using questionnaire. About 200 students in PSG Institute of Management were taken for the study and the answer to the questions were analysed.

RESULTS

The results showed that out of the total participants 93% reported consuming aerated drinks out of which 84% of participants were seen to consume aerated drink for a period of more than 3 years. About 55% of participants were found to have symptoms like sensitivity. About 41% of them were found to have anterior tooth discoloration and 47% of them were found to have bleeding gums. About 52% of them were found to have mouth dryness consuming aerated drinks. Hence, the participants who took larger quantity of aerated drinks for a longer period of time were found to have more than three symptoms of the study conducted (sensitivity, anterior tooth discoloration, bleeding gums and mouth dryness).

CONCLUSION

The above study clearly shows that there is an increase in proportion of consumption of aerated drinks among young adult, thus resulting in increased ill effect in the oral health.

KEYWORDS

Aerated Drinks Consumption, Oral Health, Practices Students-PSG Institute of Management.

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BACKGROUND

The aerated drinks are being marketed for their taste, energy, refreshment and for hydration for which these products are not indented, popularity of these drinks among adults and adolescents has grown exponentially. Worryingly, they consume them socially as well as during physical activity. They are high in sugar and are acidic products; marketing ignores the potential harmful effect of these soft drinks.

Aerated soft drinks containing inherent acid and sugar have both acidogenic and cariogenic potential. Many studies showed a positive relationship between caries and dental erosion and the consumption of soft drinks.[1]

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Consumption of aerated drink for a longer period of the time found to have many potential oral and health hazards.

The aim of the study is to evaluate the prevalence of aerated drinks consumption among group of adult in PSG Institute of Management and to analyse the out coming symptoms like sensitivity, discoloration in the anterior teeth (rampant carries), mouth dryness and bleeding gums.

MATERIALS AND METHODS

A total number of 200 students in PSG Institute of Management were planned to be included in the study, out of which only 180 students gave their consent to participant in the study and completed the questionnaire. A time period of 45 minutes were given to the participant for completing the questionnaire. The questionnaire mainly focused on the consumption of the aerated drinks, quantity frequency period of consumption and the symptoms like sensitivity discoloration in the anterior teeth, bleeding gums, dryness of mouth and the participant dental visit.

After obtaining the result from the questionnaire, the participants were grouped as group I (250 mL), group II (500 mL), group III (750 mL) (all of them consuming the aerated drinks at least 250 mL once in a week).

The inclusive criteria of this study are the young healthy adult of age group between 18-23 years who consumed 250 mL of aerated drinks once in a week.

Exclusion criteria of this study are nondrinkers, occasional drinkers and participants who took less than 250 mL of aerated drinks per week. So, out of 180 students who participated in the study, only 136 of the participants were in the inclusion criteria and they were subjected to the statistical analysis.

RESULTS

The frequency of the parameters is summarised in terms of percentage, proportions, tabulated and illustrated as histograms.

Statistical Analysis

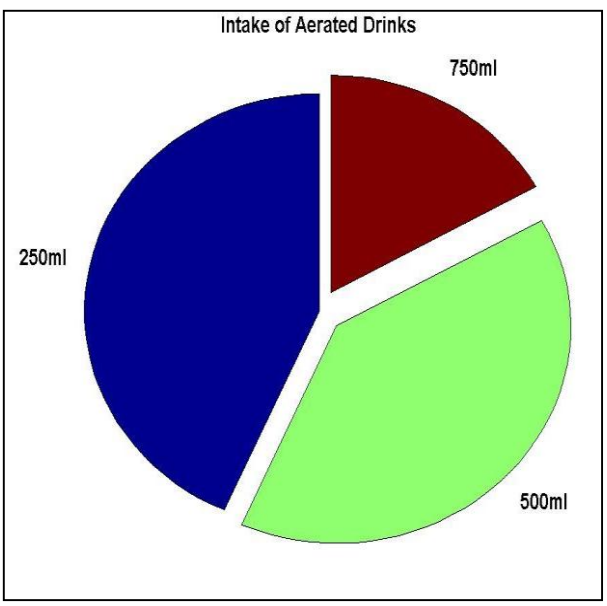


Figure 1. Intake of Aerated Drinks

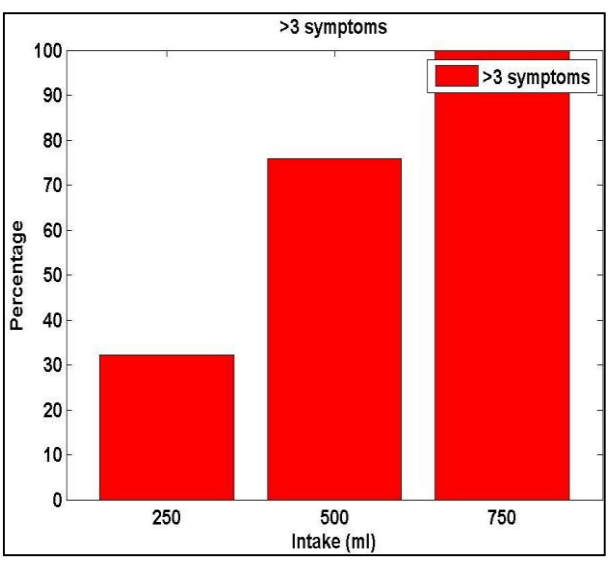


Figure 2. >3 Symptoms

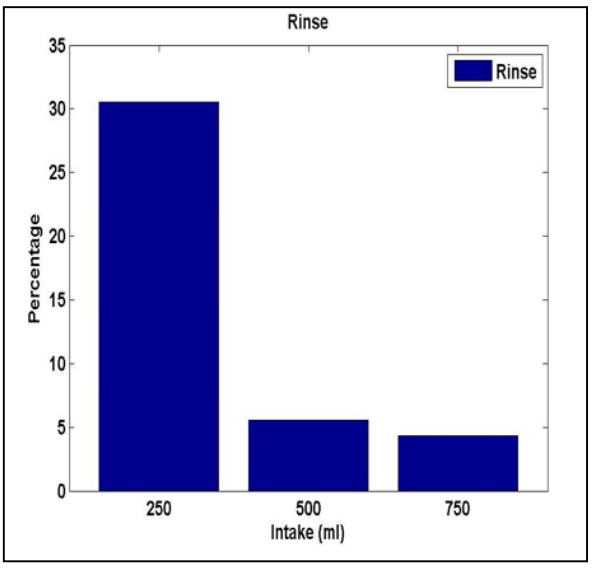


Figure 3. Rinse

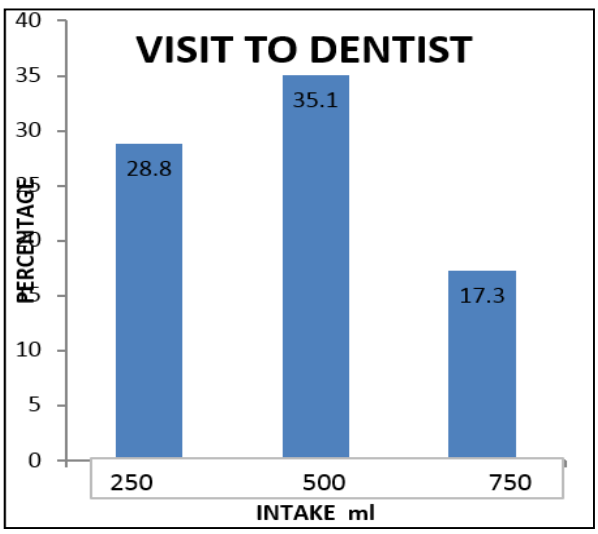


Figure 4. Visit to Dentist

Category	250 mL	500 mL	750 mL
Number	59	54	23
Rinse	18	3	1
>3 symptoms	19	41	23
Dental visits	17	19	4

Table 1

The statistical analysis of the study conducted states that 17% of the participants who took about 750 mL of aerated drinkers were found to be 100% positive for more than three of the symptoms considered (sensitivity, bleeding gums, mouth dryness, discoloration in the anterior teeth) than compared to the other two groups in the participants who consumed 250 mL, 32% of them were affected with more than three symptoms and in the 500 mL were 76%. The dia[2] shows that the participants with 250 mL consumption rinsed the mouth immediately hence preventing the drop in PH, hence less ill effects than compared to the other two groups. It is also clearly seen from dia[3] that the participant with 750 mL had less dental visit than compared to their counterparts.

DISCUSSION

Soft drinks have many potential health hazards .The inherent acid and sugar have both acidogenic and cariogenic potential resulting in dental caries and potential enamel erosion.[4] Most soft drinks contain one or more food acidulates; phosphoric and citric acid are common, but malic, tartaric and other organic acid also may be present.[5] The presence of these polybasic acid in beverages have ability to chelate calcium at higher PH, this mean that they can be very erosive to dental enamel.[2] In few cases, the slowly progressing caries suddenly may become rampant, which may be due to the result from frequent exposure to erosive acids.[3] Drinking method also has significant impact on dental erosion, holding the drink longer in the mouth lead to more pronounced PH drop in saliva.[6,7] When the pH level in mouth goes below 5.5 (i.e., the critical pH value), the acids begin to breakdown the enamel on teeth. The longer the teeth are exposed to a low salivary pH, the more likely the development of dental caries.[8] Statistically, frequency of consumption have shown significant differences in susceptibility to erosion, which appear to emerge over time with increase intake of acidic beverage.[9,10] In 60% of the soft drinks, caffeine is present as an flavour addictive.[11] Caffeine is a central nervous system stimulant with diuretic properties and also reduce salivary flow by direct effect upon the salivary gland through effect on the autonomous nervous system or through diuresis and dehydration.[12] Hence, with over time increase, intake of soft drink affect the salivary gland and decreases salivary secretion. The combination of sugar and caffeine may encourage frequent and perpetual pattern of consumption leading to early initiation and rapid progression of dental caries.[13] In rare cases, severe reduction in salivary secretion may also be seen, which in turn result in reduction in cleansing action leading to plaque formation ultimately leading to gingivitis.[14] The statistical analysis of the above conducted study clearly shows that increase in intake of aerated drink were seen to affect the oral health both directly and indirectly.

CONCLUSION

The above study proves that increase in proportion of consumption of aerated drink among young adult causes increased ill effect of oral health than compared to the nondrinkers resulting in discoloration, sensitivity, dental caries and gingivitis. This insist that all dental practitioners should educate their patient about the ill effect of aerated drinks on the teeth and should design health promotion initiatives with this in mind.

REFERENCES

[1] Johansson AK, Johansson A, Birkhed D, et al. Dental erosion, soft-drink intake, and oral health in young Saudi men, and the development of a system for assessing erosive anterior tooth wear. Acta Odontol Scand 1996;54(6):369-378.

[2] Cairns AM, Watson M, Creanor SL, et al. The PH and titratable acidity of a range of diluting drinks and their potential effect on dental erosion. J Dent 2002;30(7-8):313-317.

[3] McIntyre JM. Erosion. Aust Prosthodont J 1992;6:17-25.

[4] Cheng R, Yang H, Shao MY, et al. Dental erosion and severe tooth decay related to soft drinks: a case report and literature review. J Zhejiang Univ Sci B 2009;10(5):395-399.

[5] Rugg-Gonn AJ, Nunn JH. Diet and dental erosion. Nutrition diet and oral health. Hong Kong: Oxford University Press 1999.

[6] Johansson AK, Lingstrom P, Imfeld T, et al. Influence of drinking method on tooth- surface PH in relation to dental erosion: Eur J Oral Sci 2004;112(6):484-489.

[7] Majaver YN, Javidi N, Manshaee K. Influence of soft drink on salivary PH- 1986-2002. The Chinese Journal of Dental Research 2008;11(1):52-55.

[8] Takahashi N. Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. International Congress Series Elsevier 2005;1284:103-112.

[9] Hunter ML, West NX, Hughes JA, et al. Erosion of deciduous and permanent dental hard tissue in the oral environment. J Dent 2000;28(4):257-263.

[10] von Fraunhofer JA, Rogers MM. Effects of sports drinks and other beverages on dental enamel. Gen Dent 2005;53(1):28-31.

[11] Keast RS, Riddell LJ. Caffeine as a flavor addictive in soft drinks. Appetite 2007;49(1):255-259.

[12] Scully C. Drug effects on salivary gland: dry mouth. Oral Dis 2009;9(4):165-176.

[13] Majewski RF. Dental caries in adolescent associated with caffeinated carbonated beverages. Pediatri Dent 2001;23(3):198-203.

[14] Mizutani S, EKuni D, Tomofuji T. et al. Relationship between xerostomia and gingival condition in young adults. Journal of Periodontal Research 2015;50(1):74-79.

PREVALENCE OF DENTAL CARIES AMONG SCHOOL CHILDREN IN SULUR - COIMBATORE

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ABSTRACT

BACKGROUND

Dental caries is a universal health problem with involving the people globally of all regions and society. The agonising fact is that despite several efforts towards total eradication, dental caries is still prevalent. As the prevalence of dental caries is very high among school children and there is a paucity of such data in Coimbatore and the literature review does not reveal many such studies from this area, the study was conducted in the school going children in Sulur.

MATERIALS AND METHODS

All the students were screened visually using torch with the help of mouth mirror and probe and the observation recorded. A health screening camp was conducted for the students in private school, Sulur, February 20-25, 2017, by a team of doctors from PSG UHTC. A total of 1945 students were screened. The students health details have been entered in their health card and those requiring further evaluation have been counseled and the nursing staff at school has been requested to facilitate and guide for followup. All the students were screened visually using torch with the help of mouth mirror and probe and the observation recorded. All the students who were present in school during 20th to 25th were screened and considered as the inclusion criteria. The exclusion criteria were the absentees during this period. The students were made to sit in an ordinary chair in broad daylight facing away from the sunlight and examined in their school. Data were compiled in an excel worksheet and the percentage calculated.

RESULTS

A total of 1945 students were screened, of which, 541 students were found to have dental problems that is about 28% of the total screening done. The percentage of dental caries were found to be higher compared to other dental diseases like deep caries, malalignment, malocclusion and calculus. The percentage of dental caries was found to be higher in the females about 78% than the male for whom it was about 72%. The percentage of deep caries in female was 12% and male was 13%. The malocclusion in female 14% and 1% MFA (myofunctional appliance), male were about 15%. The percentage of calculus in female was 8% and male 13% and the follow up procedure for these conditions will be restoration, extraction, braces and MFA appliance and scaling.

CONCLUSION

The high prevalence rate shows further follow up and awareness among teachers, parents and students regarding dental caries and dental hygiene is needed. Awareness among students can be generated by the school teachers because they are role model for the students.

KEYWORDS

Dental Caries, Malalignment, Malocclusion, Calculus.

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BACKGROUND

Oral health is an integral part of general health. In the developing countries like India, changing lifestyle and dietary pattern are distinctly expanding caries incidence. Dental caries is a disease with multifactorial cause. The incidence pattern of dental caries varies not only with age, sex, socioeconomic status, race, geographical location, food habits and oral hygiene practices, but also within the oral cavity. The seriousness and societal costs of dental caries in preschool children are enormous. The prevention of dental caries has long been considered as an important task for the health profession. Scientific research continues to make progress in identifying the best practices for diagnosing, treating and preventing dental caries.

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MATERIALS AND METHODS

A health screening camp was conducted for the students in private school, Sulur, February 20-25, 2017, by a team of doctors from PSG UHTC. A total of 1945 students were screened. The students health details have been entered in their health card and those requiring further evaluation have been counseled and the nursing staff at school has been

Indian Dental Association Tamilnadu State Branch

ORIGINAL RESEARCH

'Comparison of TMA, STAINLESS STEEL and TIMOLIUM for friction, load deflection and surface characteristic"

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Associate Professor, PSG Institute of Medical Science & Research, Coimbatore.

Abstract:

OBJECTIVE

The present study aims at characterizing and comparing the mechanical property and surface characteristic of two arch wire alloys in orthodontics, stainless steel and TMA with newly introduced Timolium.

METHOD

An INSTRON 2366 testing machine was used for frictional characteristic and three points bend testing. Scanning electron microscope was used for surface evaluation and x-ray fluorescence for elemental analysis of Timolium wire specimen.

RESULT

Stainless steel was the strongest archwire alloy, and less friction at the archwire-bracket interface. TMA wires exhibited better load deflection characteristics with less stiffness than the other two wires. The surface of TMA appeared rough and exhibited very high values for friction at the archwire-bracket interface. Timolium appeared to be an alpha-beta titanium alloy composed of titanium, aluminum, and vanadium and intermediate in nature for all the parameters evaluated.

CONCLUSION

Timolium with its smooth surface, reduced friction and better strength can be considered as an introductive breakthrough in clinical orthodontic practice.

KEYWORDS

Friction, load deflection, scanning electron microscope, stainless steel, TMA, Timolium.

Introduction:

Gold alloy was one of the earliest orthodontic wire used .The gold wires have decreased usage in orthodontics because of their low yield strength and increasing costs. In 1940s austenitic stainless steel with adequate spring back, good formability, and moderate cost began to displace gold as primary alloy for orthodontic wires. Elgiloy, a chrome cobalt nickel alloy with excellent corrosion resistance was used. Nickel titanium alloy was introduced in orthodontics several years ago and find its application where deflection and low forces are required. These wires are not amenable to joining operations. With the introduction of b-titanium alloy distinctive features of good spring back, low force delivery, good formability, weld ability, high corrosion resistance, and excellent bio compatibility were available for orthodontist. The disadvantages are surface roughness and high friction. In 1995 a new TMA featuring dramatically reduced friction for a superior sliding mechanics was introduced by Burstone. Timolium ,a patented vanadium based high performance aerospace alloy, has been designed to produce a much smoother surface texture than other titanium molybdenum wires(TMA).Timolium as claimed by the manufactures has almost the same frictional resistance and half the stiffness of stainless steel making it ideal choice for finishing, aligning as well as leveling and torqueing through all phases of treatment.

MATERIALS AND METHOD

The following materials were used in the study

1. 0.016"x 0.022" stainless steel (ormco corporation Giendora CA) – 15 numbers.
2. 0.016"x0.022" TMA (ormco corporation Glendora CA) – 15 numbers.
3. 0.016"x0.022" Timolium (TP labs, Indiana polis IN) – 15 numbers.
4. 0.018" Edgewise canine brackets (American ortho.) – 45 numbers.
5. 0.010" ligature wires (ortho organizer).
6. Cold cure acrylic.
7. Stainless poles 5mm in diameter.
8. Instron machine with model number 2366.

METHOD

FRICTION ANALYSIS

In this study Tidy's (30) frictional test designs were used to stimulate canine retraction. The force acting on the surface of the tooth was simulated by single equivalent force acting at the center of resistance of the root. [27] . Four 0.018" slot edge wise bracket were fixed, at 8mm intervals with a 16mm space for a movable canine bracket at the center. A power arm of 10mm length from bracket slot was fixed at the base of each canine bracket. This distance was chosen according to Burstone finding relating to the location of center of resistance of canine. (3)

The wires stainless steel, titanium molibdinum and Timolium, were then evaluated for friction in the 0.018" slot edge wise brackets.

This jig assembly was mounted on to the lower jaw of instron machine. The canine bracket was then ligated to the test wires. The stainless steel ligatures 0.010" were initially fully tightened and then unwound by three turns; loose ligation was checked by rocking the brackets to confirm that there is play between both the spans of the bracket and the archwire.(21) the bracket was then moved over the archwire with the help of a 0.016"stainless steel wire which was suspended from a special jig which was attached to the upper jaw of the instron machine which was moved in the upward direction, at cross head speed of 5mm/min. In each test they were moved not less than 2.5mm across the central space and the load cell reading were recorded on the digital display.

The differences between load cell reading and load on the power arm thus represents the frictional resistance of the given material.

LOAD DEFLECTION ANALYSIS

The three point bending test was conducted as described by miura et al. (9)

A single bracket was attached on a steel pole, acting like a dental unit placed on a movable stage, so that the bracket span could be set at 14mm. the test wire was held in place with a ligature wire in the slot with a known quantity of force. This jig assembly was mounted on to the lower jaw of the instron machine.

A steel pole of 5mm in diameter was placed in an acrylic block, which was mounted on to the upper jaw of the instron machine. The steel pole which was attached to the upper jaw of instron machine was moved down so that mid portion of the wire segment was deflected to 2mm at the speed of 0.1mm/min under the pressure from the metal pole. The load cell reading in digital display was noted at 0.5mm, 1mm, 1.5mm and 2mm deflection.

The above tests were divided in to three groups comprising of 15 wires each.

Group 1 - 0.016"x0.022" stainless steel wires.

Group 2 - 0.016"x0.022" TMA wires.

Group 3 -0.016"x0.022" Timolium TM wires.

STATISTICAL ANALYSIS

Mean and standard deviation were estimated from the samples for each study group. The groups were subjected to one –way ANOVA to test the level of significance among the test groups. Multiple range tests by Tusky –HSD procedure was employed to identify the significant group if the p –value in the one- way ANOVA was significant.

In the present study, $p<0.05$ was considered as the level of significance.

SURFACE CHARASTERISTIC

In surface, characteristic study each wire was studied before and after sliding through the bracket with the help of a scanning electron microscope a 1cm specimen of each alloy wire was mounted and these wires were then scanned and viewed on the monitor at different magnification and representative micrographs (750 x) of the alloys were obtained.

RESULT

The result of the frictional resistance of the three wires which were analyzed in the study using instron testing machine are tabulated as follows (Table-1); and is graphically plotted in(chart -1)

Table-1

Frictional resistance between stainless steel, TMA, Timonium.

Group	Number of sample	Mean ± (grams)
I	15	203.2 ± 24.2
II	15	301.3 ± 27.5
III	15	222.5 ± 32.9

The above values were subjected to statistical analysis and tabulated as below (Table- 2)

Table-2

ONE-WAY ANOVA AND MRT

GROUP	ANOVA	Multiple range test
	P-Value	Significant group at 5%level
I	<0.001/(sig)	TMA VS STAINLESS STEEL
II		TMA VS TIMOLIUM
III		

- ANOVA test revealed a statistically significant difference among the three groups of the wires analyzed.
- Multiple range tests revealed a statistically significant difference between group-I and group-II and group-III.

The results of the three groups were analyzed for load deflection is tabulated as follows (Table 3); and are graphically represented in (chart 2).

Table-3

Load deflection between stainless steel, TMA and Timolium.

Group	Number of samples	Mean ± S. D (grams)
I	15	1576.3 ± 46.3
II	15	1189.3 ± 19.2
III	15	1319.6 ± 281

The above values were then subjected to statistical analysis and tabulated as below (Table 4)

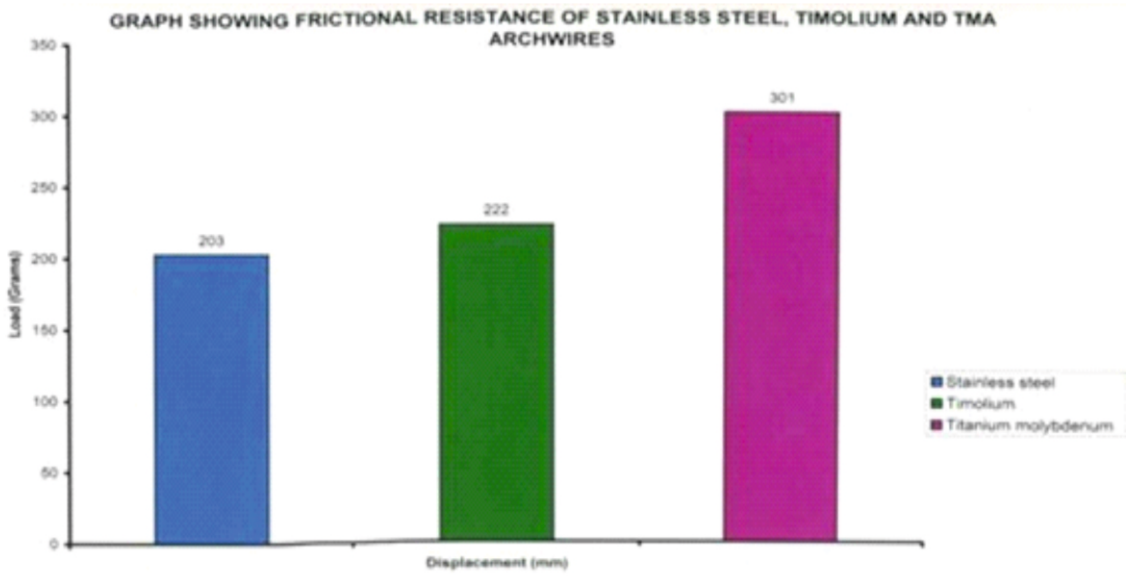
Table-4

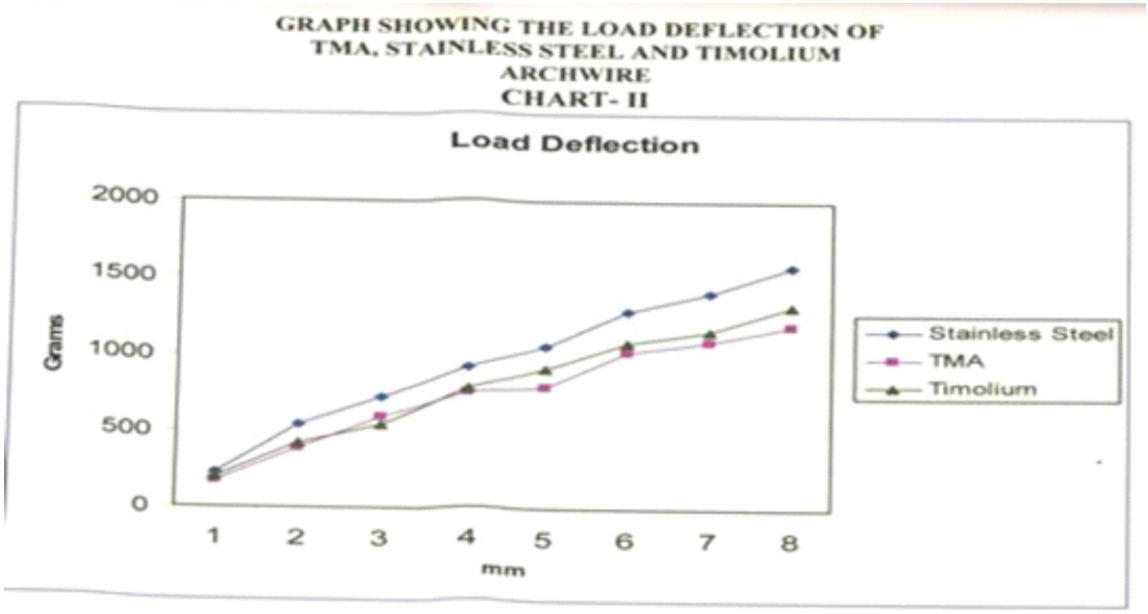
ONE-WAY ANOVA AND MRT

Group	ANOVA	Multiple range test
	P-Value	Significant group at 5%level
I	<0.0001/(Sig)	STAINLESS STEEL VS TMA
II		STAINLESS STEEL VS TIMOLIUM
III		TIMOLIUM VS TMA

- ANOVA test revealed a statistically significant difference among the three groups of wires analyzed.
- Multiple range tests revealed that there was a significant difference between Timolium and TMA wires.

All the three groups of wires which were



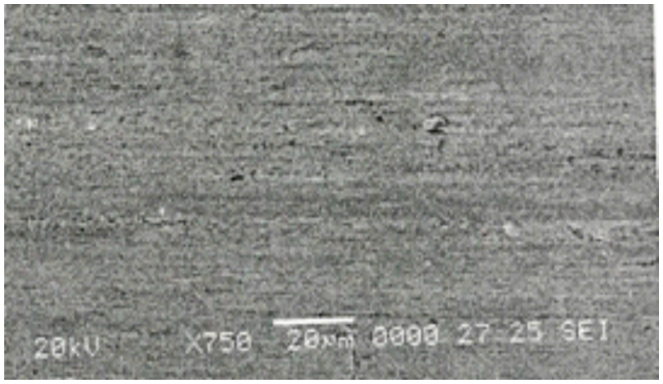


evaluated for the surface characteristic using scanning electron microscopy both before and after sliding of wire in the bracket, reveals that in

STAINLESSSTEEL (FIGURE -1)

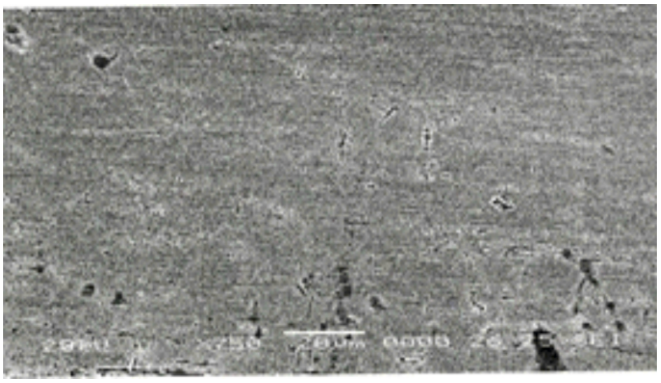
BEFORE SLIDING OF WIRE

(a)



AFTER SLIDING OF WIRE

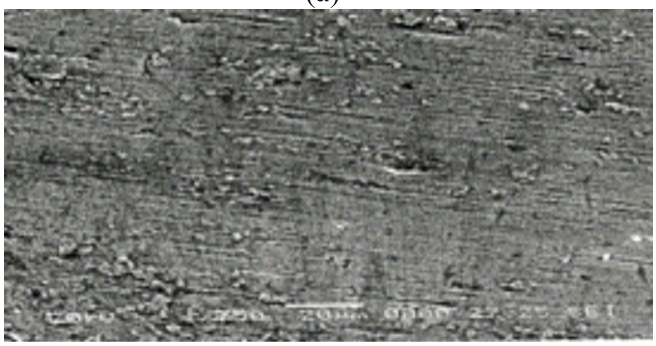
(b)



TIMOLIUM (FIGURE-2)

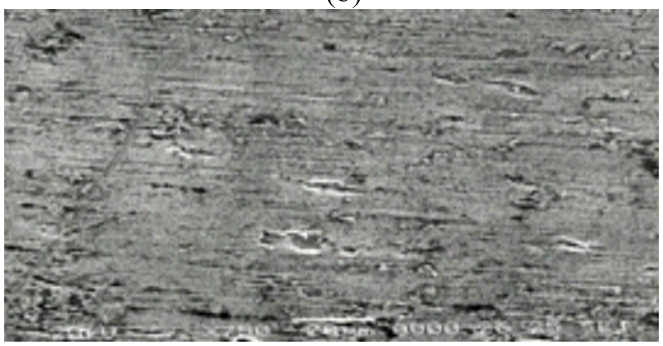
BEFORE SLIDING OF WIRE

(a)



AFTER SLIDING OF WIRE

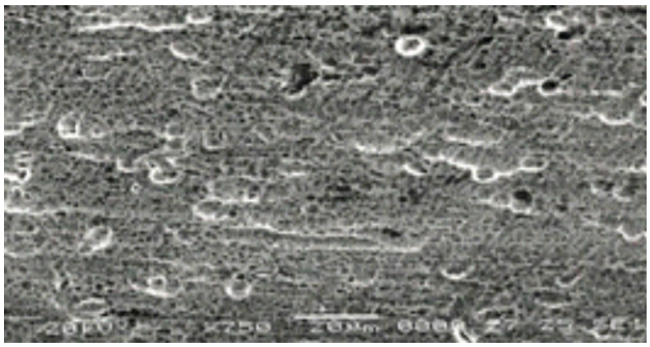
(b)



TMA (FIGURE-3)

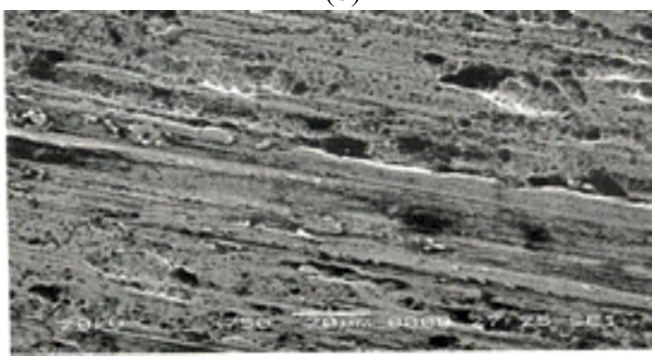
BEFORE SLIDING OF WIRE

(a)



AFTER SLIDING OF WIRE

(b)

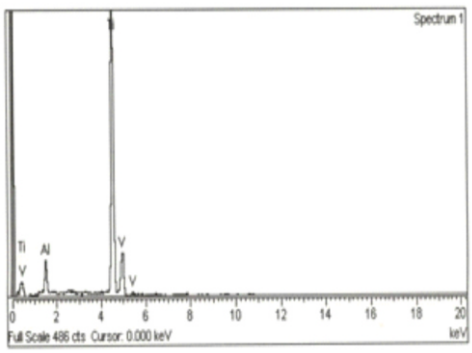


The composition of the timolium wires in (Table-5) and graphically represented in (Chart- 3.)

Table – 5

Element	Weight%	Atomic%
TiK	91.70	87.97
ALK	5.67	9.65
VK	2.63	2.37
Totals	100.00	

Chart -III



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DISCUSSION

Characterization of arch wire alloy forms an initial step towards understanding arch wire behavior in biomechanical requirement of the clinical situation from the plethora of the material available.

Frictional force has long been an important consideration in orthodontic mechanotherapy. It is a well-known fact that any force needed to retract teeth must overcome friction. (13)

Friction is a function of the relative roughness of two surfaces in contact [23].

Both static and kinetic sliding friction arises in any orthodontic situation of intended displacement, overtime, of an arch wire through a bracket (or) a bracket along an arch wire. (1)

Profit (2000) (26) reported that 50 per cent of the force necessary to initiate tooth movement required to overcome the regarding force generated between brackets, arch wires and ligatures. This implies that only 50 percent of the force applied reaches the tooth and its supporting tissues. The absolute value for the optimum force required to produce "biological tooth movement" is extremely difficult to quantity and it has been demonstrated that increasing the force increases the rate of orthodontic tooth movement up to a point (Andreasn and Quevedo,(1970)(1); Frank and Nikolai,(1980)(8); Garner et al; (1986)(10), Beyond this point tooth movement fails thus, the concept of optimal force. (8)

Kinetic friction was measured rather than static friction because of the variable nature of the frictional process. The result shows that the frictional resistance of Timolium lies between stainless steel and TMA with range being close to that of stainless steel.

The present study clearly indicates a greater friction when TMA wires are used, with a mean value of 301.3 (±) 27.5 grams. The least friction arch wire with stainless steel, with a mean value of 203.2 (±) 32.9 grams.

The result of the study conducted by Cash et al., (2004)(5) is similar to the present study.

The study conducted by Garner et al., (1986)(10), Dresche et al., (1989)(7), Kusy and Whitley, Kapila et al.,(1990)(14), Angolkar et al., (1990),(2) is similar to the present study stating that beta titanium arch wires developed frictional forces greater than stainless steel.

Surface evaluation of an arch wire alloy is important because of its influence on working characteristic as well as working potential. (18) Scanning electron microscopic evaluation of surface characteristics revealed a smooth surface with horizontal lines for Timolium arch wires as in accordance to, pradeep babu[30] . Stainless Steel exhibited vertically oriented cracks, and TMA, a very rough surface as reported extensively in the literature [4, 3, 6].

A modified version of the three-point test by Miura et al(9) was performed to evaluate the load deflection properties, the most important parameter determining the biological nature of tooth movement.(4) The result indicated beta titanium with a mean of 1189.3 (±) 19.2 grams. Stainless steel was more rigid among three arch wire alloy with very high loading value and less spring back properties with a mean of 1576.3 (±) 46.3 grams. Timolium was intermediate in nature 1319.6 (±) 281 grams.

According to cristiane [33] , Oltjen et al., (1970)[22] , vijayalakshmi RD [34] Timolium possesses comparatively low stiffness, better strength and behave as an intermediate between stainless steel and TMA.

Elemental analysis of Timolium with the help of EDS revealed the alloy, with

combination of alpha and beta phase of titanium alloy, exhibits an unusual combination of strength and surface smoothness.

CONCLUSION

In the study performed shows that in frictional studies, Timolium has almost has the same surface friction as stainless steel, unlike TMA (Titanium molybdenum) which have much higher frictional characteristics. This reduced frictional property of the Timolium helps in efficient canine retraction.

Timolium has the half the stiffness midway between TMA and stainless Steel making it ideal choice for finishing, aligning as well as leveling and torqueing throughout all phases of treatment.

Timolium bends easily in "T" boots and" loops" because it has a higher stiffness than TMA and fewer surface defects.

Timolium with its smooth surface, reduced friction and better strength can be considered as an introductive breakthrough in clinical orthodontic practice.

REFERENCES

1. Andereasen, Quevedo "Evaluation of frictional force in the 0.022x0.28 edgewise brackets in vitro"Journal of biomechanics 3:151-160:1970.
2. Angolkar et al., "Evaluation of friction between ceramic brackets and orthodontic wires of four alloy" American journal of orthodontics and dentofacial orthopedics 98:499-506, 1990:
3. Burstone, Pryputneiwlcz."Holographic determination of centers of rotation produced by orthodontic forces" American journal of orthodontics and dentofacial orthopedics 1980; 77:396-405.
4. Burstone. "Variable modulas orthodontics American journal of orthodontics and dentofacial orthopedics 1981; 80:1-10:1981.
5. Cash, Curtis Garrigi A-majo and MC Donald, "A comparative study of the static and kinetic frictional resistance of titanium molybdenum alloy arch wires in stainless steel brackets" European journal of orthodontics 26:105-11 2004.
6. Downing, Cabe, Gorden "A study of frictional forces between orthodontic bracket and arch wires" British journal of orthodontics. Nov 21 (4):349-57, 1994.
7. Drescher, Bonravel And Schumacher "Frictional force between bracket and arch wire" American journal of orthodontics and dentofacial orthopedics Nov:397-404 1989.
8. Frank, Nikoli "A comparative study of frictional resistance between orthodontic bracket and arch wire" American journal of orthodontics and dentofacial orthopedics 78:593-609,1980.
9. Fugio Miura, Masakuni Mugi, Yoshiakiohora, Hitoshi Hamanaka. "The super elastic property of Japanese NITI alloy wire for use in orthodontics" American journal of orthodontics and dentofacial orthopedics jul:1-10,1986.
10. Garner, Allai, Moore "A comparison of frictional force during simulated canine retraction of a continuous edge wise arch wire" American journal of orthodontic and dentofacial orthopedics 90:199-203,1986, Hartel, Schmuth, Bouravel, Drescher "The surface roughness of orthodontic wire-a laser optical and profilometric study" Schweiz monatssechr zahnmed 102(10):1195-202:1992.
11. Johnson "Relative Stiffness of beta titanium arch wires" Angle orthod jan 73 (3):259-67:2003.
12. Kapila And Sachdeva "Mechanical property and clinical application of orthodontic wires" Amreican journal of

orthodontics and dentofacial orthopedics Aug.96:100-109:1989.

13. Kapila, Angolkar, Duncanson, Nanda "Evaluation of friction between edgewise stainless steel brackets and orthodontic wires of four alloys" American journal of orthodontics and dentofacial orthopedics 98:117-126,1990.
14. Kapila, Angolkar, Duncanson, Nanda "Evaluation of friction between edgewise stainless steel brackets and orthodontic wires of four alloys" American journal of orthodontics and dentofacial orthopedics 98:117-126,1990.
15. Kusy, Whitley "Effect of sliding velocity on the co-efficient of friction in a model orthodontic system" Dental materials 5:235-240,1989.
16. Kusy, Whitley" Co-efficient of friction for arch wire in stainless steel and polycrystalline alumina bracket slot: 1the dry state. American journal of orthodontics and dentofacial orthopaedics 98:300-312, 1990.
17. Kusy, Whitley "Effect of surface roughness on the co-efficient of friction in modern orthodontic system"Journal of biomechanics23 (9):913-25.
18. Kusy, Whitley, Prewitt "Comparison of the frictional co-efficient for selected archwire bracket slot combination in dry and wet state". Angle orthodontist Nov 4:293-302, 1991.
19. Katherine Kula,Ceib Phillps,Annagibilaro William,Profit "Effect of ion implantation of TMA archwire on therate of orthodontic sliding space closure" American journal of orthodontics and dentofacial orthopedics Nov:577-580,1998.
20. Loftus, Artun, Nicholls, Alonzo, Stoner, "Evaluation of friction during sliding tooth ovement in various bracket-arch wire combination" American journal of orthodontics and dentofacial orthopaedics Sep116:336-45,1999.
21. Max Hain, Ashish Dhopatkar, Peterruck "The effect of ligation method on friction in sliding mechanics"AMJ. Orthodontics and dentofcial orthopedics April:416-422,2003.
22. Oltjen, Manville, Duncanson, Joydeepghosh, 'Stiffness-deflection behavior of selected orthodontic wire" Angle orthodontist No.3:209-218,1997.
23. Michel Perger, Reg L Eadix, Gray Kenneth, Narasimha Prasad, "The friction and wear pattern of orthodontic bracket and arch wire in dry state" American journal of orthodontics and dentofacial orthopaedics 118:662-74,2000.
24. Nanda R Editor "Biomechanics in clinical orthodontics" Philadelphia WBsaunders p 190,1997.
25. Nicolus J. "Frictional forces in fixed orthodontic appliance" Dent Practit 18:362-66,1968.
26. Proffit "Contemporary orthodontics" 2nd Edn.CV Mosby St.LouisP-345,2000.
27. Pourbain, "Atlas of Electrochemical equilibrium in aqueous solution" Oxford UK:pergmon press:213-22,1960.
28. Sunil Kapila, Angulkar, Duncanson and Nanda. "Evaluation of friction between stainless steel brackets and wires": American journal of orthodontics and dentofacial orthopedics Aug:117-126,1990.
29. Taneja, Duncanson, Khajotia, Nanda: "Deactivation force-deflection behavior of multistranded stainless steel wire": American journal of orthodontics and dentofacial orthopaedics Jui:124(1)61-8,2003.
30. Tidy D.C,"Frictional forces in fixed appliance"American journal of

orthodontics and dentofacial orthopaedics;96:249-254.

31. Vaughan, Duncanson,Nanda and currier,"Relative kinetic frictional forces between sintered stsinless brackets and orthodontic wires":American journal of orthodontics and dentofacial orthopaedics;212-218.1995.
32. Evaluating the surface characteristic of stainless steel,TMA,Timolium and Titanium –niobium wires: An invivo scanning electron microscope study:K Pradeep Babu1 ,V nagakeerthi2 ,Deepika madathody3 ,A Lakshmi prasanna4 ,Vidhya Gopinath5 ,M Senthil Kumar6 ,A Nanda Kumar7 : The journal of contemporary Dentalpractice,may 2016;17(5):372-376.
33. Fofces in stainless steel, Timolium and TMA intrusion arches,with different bending Magnitudes: Cristiane Aparecida de Assis Claro1 ;Jorge Abrao2 ;silvia Augusta Braga Reis1 ;Braz.oralres.Vol 21 no.2 sao .paulo Apr/June2007.
34. A comparative evaluation of Metallurgical properties of stainless steel and TMA archwires with Timolium and titanium niobium archwire-An invitro study: Vijayalakshmi RD,Nagachandran KS,Kummi p,Jayakumar p.Indian J Dent Res.2009 oct-Dec;20(4):448-52.

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Evaluation for prevalence of malocclusion among adults and need for orthodontic treatment: A cross sectional study

Dr. K Abhirami and Dr. G Valli

Abstract
Aim: The aim of this cross-sectional study was to find the percentage of prevalence of malocclusion and the type of occlusal trait from urban –rural population of age group 17-23 in a college in Coimbatore district.
Materials and Method: A descriptive cross -sectional study with purposive sampling method.
Statistical test used: Examinations were computerized and analyzed using statistical package for social science version 24. Chi square test was used for computing statistical significance.
Result: The difference in prevalence of malocclusion in college students from rural and urban area was significant with the prevalence being more in rural population.
Conclusion: Malocclusion is widely spread among young adult population with greater prevalence in students from rural area, with Angles class I to be the most predominant type of malocclusion. The type of occlusal trait in rural students was proclination and the urban students had maxillary anterior crowding.

Keywords: Crowding, malocclusion, over jet, overbite, prevalence

1. Introduction
In such a diverse country like India a large variation in prevalence of malocclusion exist in various regions of our country. The data on orthodontic awareness and treatment need is very scanty. Malocclusion is undoubtedly a public concern in young population that require the orthodontic treatment need in India. There is no record of the earlier reports to the prevalence of malocclusion, the occlusal trait and orthodontic treatment need in college going students of age 17-23 in Coimbatore district, Tamil Nadu state, India.
With the greater attention to aesthetics in recent years there is a notable increase in orthodontic treatment need in demand as a consequence of a higher perception rate of malocclusion [1, 2].
In a vast country like India there is a wide range of variation in the prevalence of malocclusion like 20.4% in Shimla city in Himachal Pradesh [3], 38.7% in Davangree Karnataka [4], 52% in Nalgonda Telengana [5], 66% in Jaipur [14], 80% in Ahmednagar in Maharashtra [6], 83.3% in Kozhikode district in kerela [7], 83.3% in Hyderabad in Telengana [8], 87.4% in Leh [9].
The aim of this study was to record prevalence of malocclusion and to define difference in occlusal trait in urban and rural population and the orthodontic treatment need.

2. Materials and Method
2.1. Study Design: Descriptive cross-sectional study. A health screening camp of dental, opthalmology and general medicine was conducted for students in a private college in Coimbatore from 22-1-2018 to 22-2-2018 and about 3500 students were screened by a team of doctors from PSGUHTC. Two examiners were involved in this research study, examiner A and examiner B. The study was carried out for a period of 28 days approximately examining 125 students per day. Demographic data including name, age, gender and permanent address of the candidate were recorded. The Inclusion criteria were subjects with permanent dentition, with no remaining deciduous dentition, subjects with age group of 17-23, Students present during the period of oral examination. Students with missing 1st molar, facial trauma, history of previous or ongoing orthodontic treatment were excluded from the study.

3. Ethical clearance
The study protocol was submitted to the institutional ethical committee [PSGIMSR] and clearance was obtained Project no: 18/205. July 30th 2018.

4. Training and calibration of examiner
Oral examination was performed by two trained and calibrated examiners. Before the survey, both the examiners and recording clerks were participated in a training and clinical calibration program in the department. Following this training, 10% of the children were examined by each of the two investigators to assess inter - examiner reliability and Kappa Values of 0. 87 and 0.88 were found respectively. There was good agreement between the examinations by the same examiner. The interview and examination of a single study subject took 3 to 4 minutes. Each subject was examined by a single examiner with aid of natural light. With head in natural position with a metallic ruler. Torch was used whenever required. This was measured to.5mm measurements, if unsure lower score was considered. The criterion was limited to occlusal anterior-posterior (A-P) relationship, crowding, over jet, overbite, midline diastema.[10]

5. Sample Design
Probability sampling method.

5.1. Sample size
Sample size was calculated with the reference to Mahajen *et al.* [16] using prevalence of malocclusion as 82%.
Sample size was calculated using formula n = $4xpq/d^2$ and found to be n= 103.

6. Method of registration
Sagital occlusal relationship was recorded using angles classification system as class I, II, III. According to occlusal relationship of 1st molar and other occlusal traits such as overjet, over bite based on the method of Bioerk *et al.* [11] for registration of Malocclusion in centric occlusion. Overbite was recorded as normal, followed by deep bite (>2/3) of lower incisors were covered by upper incisors) and open bite as no overlap (based on incisors overlap relation in the vertical dimension).over jet was recorded as normal (0-4mm),increased (>4mm) and deficient (<0mm),depending on the horizontal distance between the labial surface of the upper and lower incisors, and crowding was considered as present when there was over lapping of one tooth with respect to the other tooth.

7. Result
Prevalence of malocclusion in rural and urban population in a private college in south Indian population in Coimbatore district of age group 17-23 were taken for the study, the total sample size was 3313 students, out of which 1452 from rural area and 1861 were from urban area. The difference in prevalence of malocclusion in rural and urban students (88%vs82%) was significant statistically with (p =0.0001) (Table-1).

Table 1: Prevalence of Malocclusion in college students from urban - rural area

Occlusion	Rural		Urban		Total		P-Value
	n	%	n	%	n	%	
Normal	233	16.05	410	22.04	643	19.4	<0.0001*
Malocclusion	1219	83.95	1451	77.96	2670	80.5	
Total	1452	100	1861	100	3313	100	

(*P<0.05 significant, CI: 0.56-0.81)

The distribution of occlusal traits in urban and rural area: 19% had class I normal occlusion.16% of rural and 22% of urban population had class I normal occlusion with the statistically significant difference between rural and urban population (p=0.0001) class I sagittal occlusion was found in 85% of the subjects, class II in 11% and class III in 4% with the statistically significant difference (p=0.0001) between two groups. (Table-2)

Table 2: (AP relation) Prevalence of occlusal trait in college students from urban - rural area.

Occlusion	Rural						Urban						P-Value
	I		II		III		I		II		III		
A-P relation	n	%	n	%	n	%	n	%	n	%	n	%	<0.0001*
	1276	88	130	9	46	3	1526	82	251	13	84	5	

(*P<0.05 significant)

Normal over jet was seen in 43.5%, excessive in 33% and reduced in 23.5%. The difference between the urban and rural population was statistically significant (p=0.0001) with rural population having more of increased over jet. (Table-3)

Table 3: Prevalence of overjet in college students from urban - rural area.

Overjet	Urban		Rural		P-Value
	n	%	n	%	
Normal	763	41	668	56	<0.0001*
Excessive	539	29	537	37	
Reduced	559	301	247	17	

(*P<0.05 significant)

Normal overbite was seen in 55.5%, deep in 28.5% and reduced in 16% of total sample. The difference between urban and rural population was statistically significant (p=0.0001) with urban population having more of deep bite. (Table -4)

Table 4: Prevalence of overbite in college students from urban - rural area.

Overbite	Urban		Rural		P-value
	n	%	n	%	
Normal	911	49	908	62	<0.0001*
Excessive	688	37	287	20	
Reduced	262	14	257	18	

(*P<0.05 significant)

The frequency of crowding was 57.5%, urban population had 64% and rural had 51%. The difference between the urban and rural population was statistically significant (p=0.0001) with urban population having more of anterior crowding. (Table-5)

Table 5: Prevalence of Crowding in college students from urban - rural area.

Crowding	Urban		Rural		P-value
	n	%	n	%	
Present	1189	64	738	51	<0.0001*
Absent	672	56	714	49	

(*P<0.05 significant, CI 0.50 -0.67)

Midline diastema was present 17%, urban had 11%, rural had 23%. The difference between the urban and rural population was statistically significant (p=0.0001) with rural population having more of midline diastema. (Table-6)

Table 6: Prevalence of diastema in college students from urban - rural area.

Diastema	Urban		Rural		P-value
	n	%	n	%	
Present	207	11	334	23	<0.0001*
Absent	1654	89	1118	77	

(*P<0.05 significant, CI 1.96 – 2.89)

8. Discussion

Malocclusion has often been referred to as" disease of civilization" signifying that it is found (or at least reported) primarily in urbanized populations. This call for such studies involving the urban- rural population.

This cross-sectional study aims at finding the percentage of prevalence and type of malocclusion in students of age group 17-23 from urban and rural population in a college in Coimbatore district, Tamil Nadu state, India.

The prevalence of malocclusion in this study was to be around 84% which is in similar finding to by Ojass kumar *et al*. [8] Retna Kumari Narayanan *et al*. [7] Roopa Siddegowda *et al*. [15] Mahajen *et al*. [16].

In the present study prevalence of malocclusion was significant with increased overjet and midline diastema in rural area which is similar to the study conducted by Amit Rekhi *et al*. [19], who stated that rural population had midline diastema of 26.97% and increased overjet.

In the present study crowding was found to be the predominant type of malocclusion in urban population similar to the study conducted by Divakar karanth *et al*. [12] and Ravi Kumar Gudipaneni *et al*. [13].

In the present study class II type of malocclusion to be predominant in urban population similar to the study conducted by Kaur *et al*. [17], Dila Baz Khan *et al*. [18]

The most common type of malocclusion in this study was Angle's class I in both rural and urban population (88% and 82%) followed by crowding (51% and 64%). The least prevalent malocclusion was Angle's class III (3% and 5%). The result of the study regarding the most common type of malocclusion were in agreement with a study carried out by Patoli and Rashid [20] who reported Angle's Class I to be the most prevalent malocclusion (88.8%) followed by crowding (50%). However the result of this study were contradicted by Abu Alhaija *et al*. [21] who reported Angles class II as the most commonly occurring malocclusion.

This study is limited as it has only recorded malocclusion in age group 17-23 years which cannot be generalized to the entire population.

9. Conclusion

Malocclusion is wide spread in population examined at Coimbatore, India. Prevalence of malocclusion was more in rural population when compared with urban population. Crowded incisors were most common feature associated with class I malocclusion. In future further efforts should be made on a larger scale to obtain a base line data to find out the orthodontic treatment need.

10. Reference

1. Perillu L, Masucci C, Ferro F, Baccetti TA, picella D: Prevalence of orthodontic treatment need in southern Italian school children Eur J Orthod. 2010; 32(1):49-53.
2. Shobhasundareswaran, Praveen kizhakool: Prevalence and gender distribution of malocclusion among13-15-year-old adolescents of Kerela, South India Epidimiological work Indian j Dent Res. 2019; 30(3):455-461.
3. VK Bhardwaj, Veeresha KL, KR Sharma. Prevalence of malocclusion and orthodontic treatment needs among 16 and 17 years old school going children in Shimla city, Himachal Pradesh Indian j Dent Res. 2011; 22(4):556-560.
4. Sureshbabu AM, Chandu GN, Shafulla Md. Prevalence of malocclusion and orthodontic treatment needs among 13-15 year old school going children of Davangere city, Karnataka Journal of Indian Association of Public health Dentistry. 2005; 5(6):32-35.
5. Rajendra Reddy E, Manjula M, Sreelakshmi N, Thabitharani Rajesh Aduri S, Dharamraj Patil B. Prevalence of Malocclusion among 6 to 10 year old Nalgonda school children J Int OralHealth. 2013; 5(6):49-54.
6. Prashant Mishra, Nilesh Mote, Sumeet Mishra, Richa Mishra, Jyoti Rajbhar, Olavo Neil-Pravara: Prevalence of Different Types of Malocclusion in Young Adults, In Ahmednagar District, Maharashtra Med Rev, 2018, 10(1).
7. Retna Kumari Narayanan, Jeseem MT, Anupam Kumar TV: Prevalence of Malocclusion among 10-12-year-old Schoolchildren in Kozhikode District, Kerala: An Epidemiological Study Int J Clin Pediatr Dent. 2016; 9(1):50-55.
8. Ojass Kumar S, Tahseen Sultana, Santosh Bharadwaj S. Srujan Kumar GS, Manoj l. Assessment of Malocclusion Prevalence and Different Variables Associated with in Children Aged 10-12 Years Belonging to Hyderabad City, Telangana Sch. Acad. J Biosci. 2017; 5(11):804-808.
1. Satinder Pal Singh, Vinay Kumar and Phunchok Narboo. Prevalence of Malocclusion among Children and Adolescents in Various School of Leh Region journal of orthodontics and endodontics. 2015; 1(2):15.
9. Onyeaso CO: Prevalence of Malocclusion among adolescents in Ibandan, Nigeria, AMJ orthod Dentofacial orthop. 2004: 126:604-7.
10. Bjoerk A, Krebs A, Solow B. A method for epidemiological registration of malocclusion. Acta Odontol Scand. 1964; 22:27-41.
11. Iman Bugaighis, Divakar Karanth. The prevalence of malocclusion in urban Libyan School children, journal of orthodontic society. 2013; 2(1):1-6.
12. Ravi Kumar Gudipaneni, Raed Aldahmeshi F, Santosh Patil R, Mohammad Khursheed Alam. The prevalence of malocclusion and the need for orthodontic treatment among adolescents in the northern border region of Saudi Arabia: and epidemiological study, BMC Oral Health. 2018; 18(16):2-6.
13. Trehan M, Chugh VK, Sharma S. Prevalence of malocclusion in Jaipur, India. Int J Clin Pediatr Dent. 2009; 2(1):23-25.
14. Roopa Siddegowda, Rani Satish M. The prevalence of malocclusion and its gender distribution among Indian school children: An epidemiological survey, SRM journal of research in dental science. 2014; 5(4):224-229.
15. Nanika Mahajan, Bhanu Kotwal, Sharad Kharyal, Vinod Tomar, Abhiroop Singh Jamwal. Prevalence of Different Types of Malocclusion in the patients visiting Goverment Dental College, Jammu in India, International Journal of Scientific Study. 2017; 15(6):54-56.
16. Kaur H, Pavithra US, Abraham R. Prevalence of malocclusion among adolescents in south Indian

population, Int Soc Prev Community Dent. 2013; 3(2):97-102.
17. Dila Baz Khan, Saqib Ali, Imdadullah. An evaluation of malocclusion in rural and urban school children of district Peshawar, J kcd. 2014; 4(2):10-13.
18. Amit Rekhi, Amit Mehra, Yashika Saini. Assessment of the severity of malocclusion and treatment needs orthodontic among 16-24 –years –old rural population of Dehradun, India. A cross sectional study year. 2016; 14(1):57-62.
19. Patoli S, Rashid F. Prevalence of malocclusion in lethrar-a suburb of Islamabad. PODJ. 2011; 31:365-6.
20. Abu Alhaija ES, Al-Khateeb SN, Al Nimri KS. Preva-lence of malocclusion in 13-15 year old North Jordanian school children: Community Dent Health. 2005; 22(4):266-7.

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Dental Health Status among College Students and the Treatment Needs

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ABSTRACT

Aim: The aim of this cross-sectional study was to investigate the oral health status and normative need of college students both from urban –rural population of age group 17-23.

Materials and method: A descriptive cross-sectional study was done among 3312 college students belonging to urban –rural population of age group 17-23 in Coimbatore district, Tamil Nadu using purposive sampling method. Data entered in Microsoft excel sheet and analyzed using statistical package for social science (SPSS) version 24. Chi-square test was used for computing statistical significance

Result: Overall dental caries prevalence accounted to 70% (rural-72.12% urban-68.43%) with a mean DT of (1.14) in the rural population than urban population (1.09) and majority of the student's required one surface restoration. Periodontal status of the students as measured by community periodontal index (CPI) showed that majority of the student participant (32.66%) had calculus necessitating the need for oral prophylaxis, with the mean CPI index of (1.13) rural than (0.94) Urban.

Conclusion: In this study the dental caries prevalence and the periodontal problem was more in the rural population than the urban population with the one surface restoration and oral prophylaxis as the predominant treatment need.

Key words: College students, Oral health, Urban, Rural, Dental caries, Periodontal disease

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INTRODUCTION

Oral diseases are the most common of the chronic diseases and are important in public health problems because of their prevalence, their impact on individuals, society, and the expense of their treatment [1]. On September 6, 2016, a new definition of oral health was approved by the FDI (World Dental Federation General Assembly) [2]. The new definition acknowledges the multifaceted nature and attributes of oral health. In developing countries were the resources are sparse it is of utmost importance to plan primordial preventive strategies rather than treating oral diseases and data regarding the prevalence of oral diseases gives us comprehensive picture to identify the target group and impart preventive modalities and there is a paucity of such data of this age group (17-23) in Coimbatore, Tamil Nadu, India.

College life is a crucial period of transition with personal responsibility. Students in this period can be targeted for preventing dental diseases and building future oral health [3] voluminous literature pertaining to oral health status of school children [4,5] is available, but data pertaining to young adults, especially to college students are sparse. Thus, analysing epidemiological data of age groups not customarily studied [world health organisation (WHO) index age groups] give the exact picture of the current oral health status and normative needs of the population. The aim of this study was to accesses the oral health status among and the normative needs among college students in Coimbatore in Tamil Nadu.

MATERIALS AND METHOD

Study Design: Descriptive cross-sectional

study. A health screening camp of dental, ophthalmology and general medicine was conducted for students in a private college in Coimbatore from 22-1-2018 to 22-2-2018 and about 3500 students were screened by a team of doctors from PSGUHTC. Two examiners were involved in this research study, examiner A and examiner B. The study was carried out for a period of 28 days approximately examining 125 students per day. Demographic data including name, age, gender, and permanent address of the candidate were recorded. The Inclusion criteria were subjects with permanent dentition, with no remaining deciduous dentition, subjects with age group of 17-23, Students present during the period of oral examination. The students taken for the study were 3312. The study protocol was submitted to the institutional ethical committee [PSGIMSR] and clearance was obtained Project proposal number-18/223 August 10th 2018.

Oral examination was performed by two trained and calibrated examiners. Before the survey, both the examiners and recording clerks were participated in a training and clinical calibration program in the department. Following this training, 10% of the students were examined by each of the two investigators to assess inter-examiner reliability and Kappa Values of 0. 87 and 0.88 were found, respectively. The interview and examination of a single study subject took 3 to 4 minutes. The study was done using probability sampling method and the sample size of calculated reference to Punitha et al. [6] using prevalence of caries as 36.7%. Sample size was calculated using formula $n=4 \times pq/d^2$ and found to be n=372.

Method of registration

Oral examination was done using the WHO Basic Oral Health Assessment [7] form with plane mouth mirror and WHO periodontal probe. Clinical examination was conducted using natural light with the participant sitting on a chair with a head rest. The examination was performed in a classroom. The examination area was arranged in such a way to allow one person at a time apart from the examiner and the recorder. The recorder was made to stand close to the examiner so that the instructions and the code could be easily heard, and the examiner could see that the findings were being recorded correctly. The data were coded and analyzed using the SPSS version 24 software the level of statistical significance was kept at $p<0.05$. The descriptive statistics were calculated. Chi-square test was used.

RESULT

A total of 3312 (urban-1888; 57%, rural -1424; 43%) participated in this study. The average DMFT among the target group was 1.92 with urban having DMFT score of 1.95 rural with 1.80 (Table 1).

In this study the highest score as measured by CPI showed that a total of 21.13% of the student had a bleeding as their highest score, 32.66% had calculus indicating the need for oral hygiene instruction and oral prophylaxis and 5.07% had a pocket of 4-5mm necessitating surgical interventions (Table 2). The community–wise distribution according to the highest score as measured by CPI showed that35.74% rural and 30.34% urban had calculus as the highest score.

Mean number of sextants with bleeding was higher in urban (0.21), whereas the mean number of sextants with calculus and shallow pocket was more in rural (0.35) compared to urban (0.30) (Table 3).

Association of CPI Index with community (urban/rural) shows that rural population has increased periodontal diseases than urban, and it is statistically significant $p < .000$ with 95% CI (0.255-0.121) (Table 4).

Loss of attachment according to the highest score showed that loss of attachment of 0-3mm was seen in 94.41%, 4-5mm in 3.38%, 6-8mm in 1.20% of the population (Table 5).

Mean number of sextants with score 1 was more in rural than urban (0.04; 0.02) (Table 6).

Association of Loss of attachment with community (urban/rural) shows that rural population has increased Loss of attachment than urban and it is statistically significant $p < .003$ with 95% CI (0.048-0.010) (Table 7).

Among the study participant 0.3% had prosthesis in the upper arch and 0.1% in the lower arch, out of which 0.3% had bridge denture and 0.1% had both bridge and partial denture.

Table 1: Distribution of study participants according to dental caries status.

	Urban	Rural	Total	P value	95% Confidence interval
Caries prevalence	1292 (68.43%)	1027 (72.12%)	2319 (70%)	-	-
DT	1.09 ± 0.92	1.14 ± 0 .90	1.11 ± 0.91	0.094	-0.117-0.009
MT	0.27 ± 0.86	0.24 ± 0.86	0.26 ± 0.86	0.226	-0.023-0.096
FT	0.59 ± 1.36	0.5 ± 1.26	0.55 ± 1.32	0.051	0.000-0.182
DMFT	1.95 ± 1.97	1.80 ± 1.86	1.92 ± 1.92	0.271	-0.058-0.205
DMFT-decayed, missed and filled teeth; DT-decayed teeth; FT-filled teeth; MT-missing teeth.					

Table 2: Periodontal status according to the highest score as measured by CPI.

	Urban	Rural	Total
Dentate	1888	1424	3312
Score 0	836 (44.27%)	521 (36.58%)	1357 (40.97%)
Score 1	402 (21.29%)	298 (20.92%)	700 (21.13%)
Score 2	573 (30.34%)	509 (35.74%)	1082 (32.66%)
Score 3	77 (4.07%)	91 (6.39%)	168 (5.07%)
Score 4	0 (0%)	5 (0.35%)	5 (0.15%)
[Score 0- Normal, score 1- Bleeding gums, score 2-Calculus, Score 3-4-5 mm pocket depth] [Chi square value- 34.191, P value-.000(Significant)]			

Table 3: Distribution according to mean number of sextants with highest score as measured by CPI.

	Urban	Rural	Total
Score 0	0.44	0.36	0.41
Score 1	0.21	0.2	0.21
Score 2	0.3	0.35	0.32
Score 3	0.04	0.06	0.05
Score 4	-	0.003	0.001

Table 4: Association of CPI Index with community (urban/rural).

Distribution	Number	Mean (SD)	F-value	P-value	95% Confidence interval
Urban	1888	0.94 ± 0.952	8.31	0	0.255- 0.121
Rural	1424	1.13 ± 0.997			

Table 5: Loss of attachment according to the highest score.

	Urban	Rural	Total
Dentate	1888	1424	3312
Score 0	1821 (96.45%)	1339 (94.03%)	3160 (94.41%)
Score 1	48 (2.54%)	64 (4.49%)	112 (3.38%)
Score 2	19 (1%)	21 (1.47%)	40 (1.20%)
Chi square value-11.119, P value-0.004 (Significant)			

Table 6: Loss of attachment according to the mean number of sextants with the highest score.

	Urban	Rural	Total
Score 0	0.96	0.94	0.95
Score 1	0.02	0.04	0.03
Score 2	0.01	0.01	0.01

Table 7: Association of Loss of attachment with community (urban/rural).

Distribution	Number	Mean (SD)	F-value	P-value	95% Confidence interval
Urban	1888	0.05 ± 0.252	33.52	0.003	0.048- 0.010
Rural	1424	0.07 ± 0.314			

DISCUSSION

Oral health is an essential component of general health and overall well-being of an individual. Oral cavity and its surrounding structures that are free of any diseases are indicative of good oral health. This not only makes a person look and feel good, it is equally relevant in maintaining oral function [8-10]. Oral disease is one of the most common public health issues worldwide with significant socio-economic impacts, and yet it is frequently neglected in public health policy [11].

Schools and colleges play an immense role in the growth of young minds not only for learning new information but also by having long-lasting effects [12]. In India despite improvements in infrastructure and dental manpower, oral health care remains to be neglected entity as it is not considered to be life threatening [13].

As there is no national level oral health policy and fee for services is still the most common mode of payment for dental service preventive strategies hold priority as the most economical way for improving oral health of the community. To advocate preventive measures, it is essential to assess the complete presence of disease burden in the community.

This cross-sectional study aims at finding the percentage and prevalence of dental caries, periodontal diseases in students of age group 17-23 from urban and rural population in a college in Coimbatore district, Tamil Nadu State, India.

Overall caries percentage in the study conducted by DharV et al. [14], Saravanan S et al. [15], Niyanta Joshi et al. [16], Farooqi FA et al. [17], Kumar S et al. [18] was found similar to the present study around 70%

The mean DMFT of the study conducted by theFarooqi et al. [17], Kumar et al. [18] is like the present study (1.94 ± 2.0) and (1.94).

The study conducted by Mohammed A.AL-Rafee et al. [19], Sriailapanan P, et al. [20], Soroye et al. [21], Sanadhya et al. [22], Akinyamoju et al. [23], showed rural population had more caries prevalence which is supporting to the present study.

The study conducted by Maserejian NN et al. [24], Patro et al. [25], David J et al [26] showed Urban population to have more Dental caries prevalence contradicting to the present study.

The study conducted by Kalsbeek H et al. [27] (24%) (Score-1), Hannan et al [28] (33.5%) (score-2) periodontal index was like the present study.

The study conducted by Arvidson et al. [29], Baiju et al. [30], Vadiakas et al. [31] concluded that rural population had more periodontal diseases than urban population like the present study.

The study conducted by Popoola et al. [32], Sane [33] had mean plaque index (1.12 ± 0.41) which was like the present study (1.13 ± 0.997). The study conducted by Zhang S et al. [34] showed that only 7% of the population had normal periodontium which was contradicting to the present study. The limitations of this study are inherent to cross-sectional studies, which do not allow establishing a relationship of cause and effect, and it only addresses the prevalence and severity of the disease without looking at sociodemographic variables such as parental education, economic status or family size. Furthermore, the current paper only reports the dental caries and the periodontal status and does not examine the oral hygiene practice or other individual reasons for the above dental morbidity.

CONCLUSIONS

The caries prevalence and the periodontal diseases severity were higher in students from rural areas than the students from the urban area. The DMFT index was higher in students from urban area than the students from rural area; this is because the FT value was higher in the urban area indicating the awareness and availability of dental care.Dental health care needs are high in rural areas than in urban areas.

RECOMMENDATION

It is recommended that continuous scientific monitoring, design, and effective implementation of preventive and restorative programs to be carried out by dental surgeons and included in national wide program like RBSK (RASHTRIYA BAL SWASTHYA KARYAKRAM).

REFERENCES

1. Sheiham A. Oral health, general health and quality of life. Bull World Health Organ. 2005; 83:644.
2. Glick M, Williams DM, Kleinman DV, et al. A new definition for oral health developed by the FDI world dental federation opens the door to a universal definition of oral health. J Public Health Dent 2017; 77:3–5.
3. Sharda AJ, Shrtty S. Relationship of periodontal status and dental caries status with oral health knowledge, attitude and behaviour among professional students in India. Int J oral Sci 2009; 1:196-206.
4. Rodrigues JS, Damle SG. Prevalence of dental caries and treatment need in 12-15-year-old municipal school children of Mumbai. Indian Soc Pedod Prev Dent 1998; 16:31-36.
5. Kundu H, Patthi B, Singla A, et al. Dental caries scenario among 5,12 and 15 –year-old children in India-A retrospective analysis. J Clin Diagn Res 2015; 9:1-5.

6. Punitha VC, Amudhan A, Sivaprakasam P, et al. Role of dietary habits and diet in caries occurrence and severity among urban adolescent school children. J Pharm Bioallied Sci 2015; 7:296–300.
7. World Health Organisation. Oral health surveys–Basic methods. 4th Edn Geneva: World Health Organisation: 1997.
8. Kumar H, Behura SS, Ramachandra S, et al. Oral health knowledge, attitude, and practices among dental and medical students in eastern India-A comparative study. J Int Soc Prev Community 2017; 7:58-63.
9. Abdollahi M, Radfar M. A review of drug-induced oral reactions. J Contemp Dent Pract 2003; 4:10-31.
10. Puy CL. The role of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Oral Cir Bucal 2006; 11:449-455.
11. Jin LJ, Lamster IB, Greenspan JS, et al. Global burden of oral diseases: Emerging concepts, management and interplay with systemic health. Oral Dis 2016; 22:609–619.
12. Dileep CP, Basavaraj P, Jaayaprakash K. Survey on Knowledge, attitude and practice about the hygiene among teachers in the Ksnpurcity. J Indian Assoc Public Health Dent 2006; 8:57-59.
13. Ahuja NK, Parmar R. Demographics and current scenario with respect to dentist, dental institutions and dental practices in India. Indian J Dent Sci 2011; 3:8-11.
14. Dhar V, Jain A, Van Dyke TE, et al. Prevalence of dental caries and treatment needs in the school-going children of rural areas in Udaipur district. J Indian Soc Pedod Prev Dent. 2007; 25:119–121.
15. Saravanan S, Kalyani V, Vijayarani MP, et al. Caries prevalence and treatment needs of rural school children in Chidambaram Taluk, Tamil Nadu, South India. Indian J Dent Res 2008; 19:186–190.
16. Joshi N, Sujan SG, Joshi K, et al. Bhavna dave prevalence, severity and related factors of dental caries in school going children of Vadodara city– An epidemiological study. J Int Oral Health 2013; 5:40-48.
17. Farooqi FA, Khabeer A, Moheet IA, et al. Prevalence of dental caries in primary and permanent teeth and its relationship with tooth brushing habits among schoolchildren in eastern Saudi Arabia. Saudi Med J 2015; 36:737–742.
18. Kumar S, Tadakamadla J, Duraiswamy P, et al. Dental caries and its socio-behavioral predictors-An exploratory cross-sectional study. J Clin Pediatr Dent 2016; 40:186–192.
19. Al-Rafee MA, AlShammery AR, AlRumikan AS, et al. A comparison of dental caries in urban and rural children of the Riyadh Region of Saudi Arabia. Front Public Health 2019; 7:195.
20. Srisilapanan P, Nirunsittirat A, Roseman J. Trends over time in dental caries status in urban and rural Thai children. J Clin Exp Dent 2017; 9.
21. Soroye MO, Adegbulugbe CI. Oral health status, knowledge of dental caries aetiology, and dental clinic attendance: A comparison of secondary school students in the rural and urban areas of Lagos. Port Harcourt Med J 2016; 10:42-49.
22. Sanadhya S, Aapaliya P, Jain S, et al. Assessment and comparison of clinical dental status and its impact on oral health-related quality of life among rural and urban adults of Udaipur, India: A cross-sectional study. J Basic Clin Pharmacy 2015; 6:52-58.
23. Akinyamoju CA, Dairo DM, Adeoye IA, et al. Dental caries and oral hygiene status: Survey of schoolchildren in rural communities, Southwest Nigeria. Nigerian Postgrad Med J 2018; 25:239-245.
24. Maserejian NN, Tavares MA, Hayes C, et al. Rural and urban disparities in caries prevalence in children with unmet dental needs: The New England Children's Amalgam Trial. J Public Health Dent 2008; 68:7-13.
25. Patro BK, Kumar RB, Goswami A, et al. Prevalence of dental caries among adults and elderly in an urban resettlement colony of New Delhi. Indian J Dent Res 2008; 19:95-98.
26. David J, Wang NJ, Astrøm AN, et al. Dental caries and associated factors in 12-year-old schoolchildren in Thiruvananthapuram, Kerala, India. Int J Paediatr Dent 2005; 15:420-428.
27. Kalsbeek H, Helderman WHP. Oral health in 13–15-year-old schoolchildren in Palmyra, Syrian Arab Republic. Leiden, The Netherland: TNO Prevention and Health 2004.
28. Hannan MA, Chowdhury MTH, Khan MAI, et al. Prevalence of gingivitis, plaque accumulation and decayed, missing and filled teeth among slum population in Bangladesh. Med Res Counc Bull 2014; 40:47-51.
29. Arvidson-Bufano UB, Holm AK. Dental health in urban and rural areas of central and western Bangladesh. Trpoical Dent J 1990; 13:81-86.
30. Baiju RM, Peter E, Nayar BR, et al. Prevalence and predictors of early periodontal disease among adolescents. J Indian Society Periodontol 2019; 23:356-361.
31. Vadiakas G, Oulis CJ, Tsinidou K, et al. Oral hygiene, and periodontal status of 12 and 15-year-old Greek adolescents: A national pathfinder survey. Eur Archives Paediat Dent 2012; 13:11-20.
32. Popoola BO, Dosumu EB, Ifesanya JU. Periodontal status and treatment need among adolescents in Ibadan, Southwestern Nigeria. Braz J Oral Sci 2015; 14:117-121.
33. Sane AS, Nikhbakht-Nasrabadi A. Periodontal health status and treatment needs in Iranian adolescent population. Arch Iran Med 2005; 8:290-294.
34. Zhang S, Xu B, Liu J, et al. Dental and periodontal status of 12-year-old Dai school children in Yunnan Province, China: A cross-sectional study. BMC Oral Health 2015; 15:117.

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Case Report

Mucormycosis during Coronavirus Disease Pandemic: A Diagnosis we Cannot Afford to Miss

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ABSTRACT

Mucormycosis is a very aggressive, devastating opportunistic fungal infection commonly involving nasal, maxillary,orbital, and cerebral regions. In the second wave of the coronavirus disease 2019 (COVID-19) pandemic, a high frequency of mucormycosis infections has been seen because of the triad of COVID-19, systemic steroids, and preexisting diabetes mellitus. A patient might present with a simple mobile tooth/teeth with multiple gingival abscesses Necrotic bone could be seen in the palate. Blackish discoloration might be present in the oral mucosa. There might be extraoral swelling . If rhino-oculo cerebral mucormycosis is diagnosed, then there are two immediate treatment modalities to be followed. Both surgical and medical treatment are necessary. Surgical is thorough debridement of the necrotic tissue. To prevent the occurrence of mucormycosis, meticulous glycemic control and rational use of steroids and antibiotics for the shortest possible time with adequate attention toward the ventilators and tubing are necessary.

KEYWORDS: *COVID-19, dentistry, gingival abscesses, mucormycosis*

Mucormycosis is a very aggressive, devastating opportunistic fungal infection commonly involving nasal, maxillary,orbital, and cerebral regions. It is acquired primarily through inhalation of spores through the nasal route. It is a rapidly progressive fungal disorder and any delay in identification and management leads to high morbidity and mortality.

The fungus belongs to the order Mucorales and subphylum Mucormycotina and it is angioinvasive that causes occlusion of blood vessels, which leads to tissue necrosis. Other forms of clinical presentation are gastrointestinal, bone, and joint infection and pulmonary, cutaneous, and disseminated mucormycosis.

In the second wave of the coronavirus disease-2019 (COVID-19) pandemic, a high frequency of mucormycosis infections has been seen because of the triad of COVID-19, systemic steroids, and preexisting diabetes mellitus (DM). This could be due to any or all of the following reasons COVID 19 associated illness requiring high and prolonged steroid use leading to worsening of preexisting DM and immunosuppression; elevated ferritin leading to high iron load, acidosis, and endothelial damage and the use of multiple broad spectrum antibiotics to prevent or treat secondary infections.[1,2] Since India has the second-highest number of COVID-19 cases,[3] the second-highest number of DM

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patients,[4] and the highest incidence of mucormycosis,[5] it is expected that mucormycosis will be seen in India in huge numbers, especially in COVID-19 patients.

Predisposing Factors

- Uncontrolled DM
- Concurrent/recently treated COVID-19
- Inappropriate use of steroids
- High doses of steroids
- Using for prolonged periods.
- Immunocompromised individuals
- Malignancy
- Transplant recipients.
- People under long-standing oxygen therapy
- Prolonged intensive care unit (ICU) stays
- People under mechanical ventilation
- Prolonged use of broad-spectrum antibiotics
- Nosocomial-contaminated dirty linen, ICU instruments, and materials - if not properly sterilized.

Clinical Presentation

A patient might present with a simple mobile tooth/teeth with multiple gingival abscesses [Figure 1]. Necrotic bone could be seen in the palate. Blackish discoloration might be present in the oral mucosa. There might be extraoral swelling [Figure 2]. We should not misdiagnose it as simple periodontitis or cellulitis if the patient is/was COVID positive and diabetic and was given steroids. Strongly suspect mucormycosis and evaluate the patient or refer to a higher center. The patient might have other symptoms such as headache, myalgia, and fever and nasal symptoms such as nasal discharge often bloody, brownish, or blackish. Diminished vision or proptosis might be present. There could be altered sensorium in case of cerebral involvement.



Figure 1: Multiple gingival abscesses in a diabetic patient who was coronavirus disease positive before 15 days and was given steroids (Picture Courtesy – Dr. S. Sanjeev Kumar, Ambur)

Diagnosis

Clinically, there will be necrotic bone with blackish eschar. Computerized tomography and/or magnetic resonance imaging might show erosion of bone involving nasal, paranasal sinuses, maxilla, ethmoid, and base of the skull. KOH smear will show the presence of nonseptate, ribbon-like hyphae (at least 6–16 μm wide) with obliteration of the vessels. On histopathological examination, we could find angioinvasion, coagulation necrosis, hemorrhagic infarction, infiltration by neutrophils, and perineural invasion. Fungal culture done with routine media between 30°C and 37°C might show grayish–black or cotton-white colony.[6]

Treatment Algorithm

If rhino–oculo cerebral mucormycosis is diagnosed, then there are two immediate treatment modalities to be followed. Both surgical treatment and medical treatment are necessary. Surgical is thorough debridement of the necrotic tissue. Functional endoscopic sinus surgery is done if there is nasal and paranasal sinus involvement without involving maxilla or palate or zygoma. If the maxilla is involved, then maxillectomy is done. If the orbit is involved, then orbital exenteration is done. If the frontal bone is involved, then debridement or cranialization is done. The osteomyelitic bone is removed and the necrotic tissue is debrided.

Medical treatment is with injection amphotericin B (1–1.5 mg/kg/day) or injection liposomal amphotericin B 5–10 mg/kg/day for 14–21 days.

Conclusion

As the disease is known to be really very aggressive, mortality is generally >50%.[7] The mortality increases



Figure 2: Extraoral swelling in a patient who is diabetic and was coronavirus disease positive before 20 days

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to an unbelievably high 80%, with an intracerebral involvement of the disease.[8] Larger studies are the need of the hour in mucormycosis in COVID-19 to know prognostic markers and outcomes.

Due to the rapid increase in cases of mucormycosis in COVID-19, there is an immense need to improve awareness among treating physicians and dentists about early diagnosis and treatment. There is marked variability in the frequency of mucormycosis cases reported in different regions in pre- and post-COVID times. A nationwide registry and larger studies could help in providing useful data. Abnormalities in the immune system in COVID-19, sensitivity patterns of fungus, and the reasons for the sudden surge in cases in the second wave of the pandemic need to be studied. To prevent the occurrence of mucormycosis, meticulous glycemic control, rational use of steroids and antibiotics for the shortest possible time with adequate attention toward the ventilators and tubing are necessary. The microbiologists should also monitor the ward, ICU, and hospital environment to account for fungal diseases and take regular swabs and culture them.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. John TM, Jacob CN, Kontoyiannis DP. When uncontrolled diabetesmellitus and severe COVID-19 converge: The perfect storm for mucormycosis. J Fungi (Basel) 2021;7:298.
2. Rubino F, Amiel SA, Zimmet P, Alberti G, Bornstein S, Eckel RH, *et al.* New-onset diabetes in COVID-19. N Engl J Med 2020;383:789-90.
3. WHO Coronavirus (COVID 19) Dashboard, Data Table Page 1. (https://covid19.who.int/)
4. Unnikrishnan R, Anjana R, Mohan V. Diabetes mellitus and its complications in India. Nat Rev Endocrinol 2016;12:357-70.
5. Prakash H, Chakrabarti A. Global Epidemiology of Mucormycosis. J Fungi (Basel) 2019;5:1.
6. Management Protocol for Mucormycosis AIIMS, Rishikesh. Multidisciplinary Mucor management team_Version 1.0_16.05.2021:7.
7. Patel A, Kaur H, Xess I, Michael JS, Savio J, Rudramurthy S, *et al*. A multicentre observational study on the epidemiology, risk factors, management and outcomes of mucormycosis in India. Clin Microbiol Infect 2020;26:944.e9- 944.e15.
8. Cagnoni PJ. Liposomal amphotericin B versus conventional amphotericin B in the empirical treatment of persistently febrile neutropenic patients. J Antimicrob Chemother 2002;49 Suppl 1:81-6.

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CASE REPORT

A Simple and Innovative Technique for Prosthetic Rehabilitation of Continuous Maxillary and Orbital Defect

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ABSTRACT

Orbital exenteration along with hemimaxillectomy for malignant tumor of maxilla usually results in continuous orbital and maxillary defect. A removable maxillofacial prosthesis with adequate retention and stability during functional movement along with good esthetics is the key for successful rehabilitation of such patients. This case report describes a novel and yet cost-effective method for retention of silicone orbital prosthesis using acrylic resin base attached to maxillary obturator using pin and socket of an electric plug which results in better retention of both the prosthesis.

Keywords: Continuous defect, Obturator, Orbital prosthesis.

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INTRODUCTION

Surgical resection for orofacial malignancy is extremely debilitating in nature because of its deep psychological impact with severe disfigurement and functional impairment. It often results in communication between the oral and nasal cavity that causes difficulty in swallowing and speech, nasal regurgitation, and unesthetics facial appearance. Such defects are usually rehabilitated by a facial prosthesis to restore function and appearance along with an intraoral obturator to restore speech and swallowing.[1] Various methods used for retention of such prosthesis include spectacle frame,[2] anatomic retention using acrylic conformer relined by a soft liner,[3] adhesives,[4] stud attachments, magnets,[5] and osseointegrated implant.[6] With use of spectacle retained prosthesis, it becomes mandatory for the patient to wear spectacles during use of prosthesis. Limitations of adhesives include possible allergic reactions, decreased efficacy with use, and need for daily application and removal. Although osseointegrated implants may provide the most reliable prosthesis retention, additional surgeries, expenses, inadequate bone, and prior radiation to the area may contraindicate their use. Other means are use of magnets or buttons to attach orbital prosthesis with obturator.[7] Limitations of magnets include high cost, corrosion, and loss of magnetism with time. This case report describes an innovative technique for retention of silicone orbital prosthesis to maxillary obturator with use of pin and socket of an electric plug.

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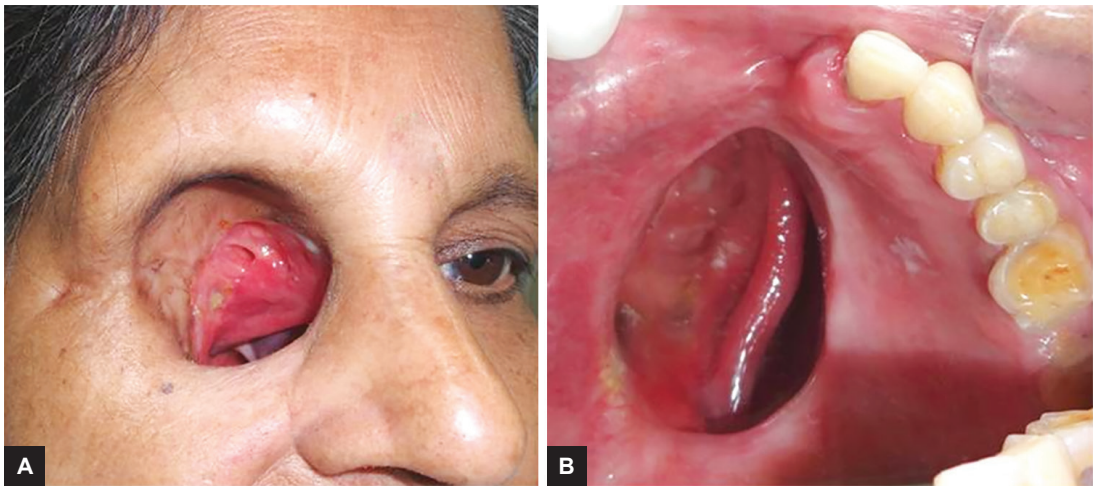
Corresponding Author: Vinayak Bharate, Lecturer, Department of Prosthodontics, Dr. D. Y. Patil Dental College & Hospital Pune, Maharashtra, India, Phone: +918826810845, e-mail: vinayakbharate@gmail.com.

CASE REPORT

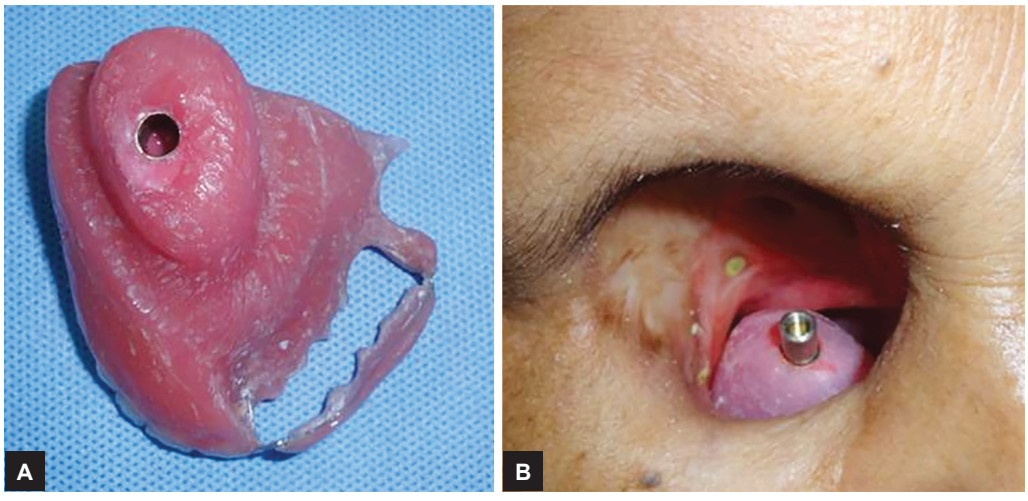
A 55-year-old female patient reported to the Department of Prosthodontics, CDER, AIIMS, New Delhi, with a chief complaint of missing right eye and regurgitation of food since last 3 months (Figs 1A and B). Patient had underwent orbital exenteration and hemimaxillectomy of the right side because of squamous cell carcinoma eroding the right orbital floor, posterior wall of maxilla, infratemporal fossa, right hard palate, medial wall of maxilla, right nasal cavity, and right ethmoidal air sinuses. This led to the formation of right orbital defect which was continuous with intraoral maxillary defect. For prosthetic rehabilitation of this patient, silicone orbital prosthesis attached to a hollow closed bulb obturator with the help of electric pin and socket was planned.

Impression Procedure for Obturator and Orbital Defect

Primary impression of intraoral maxillary defect was made with alginate and poured with dental stone (type III). Maxillary cast was then surveyed and undesirable undercuts were blocked. Custom tray was thus fabricated, border molding of the defect was done and final impression was made with light body addition silicone. On final working, model of obturator record base was fabricated, jaw relation was made followed by try in patient to check



Figs 1A and B: Orbital defect and intraoral maxillary defect



Figs 2A and B: Intraoral maxillary obturator

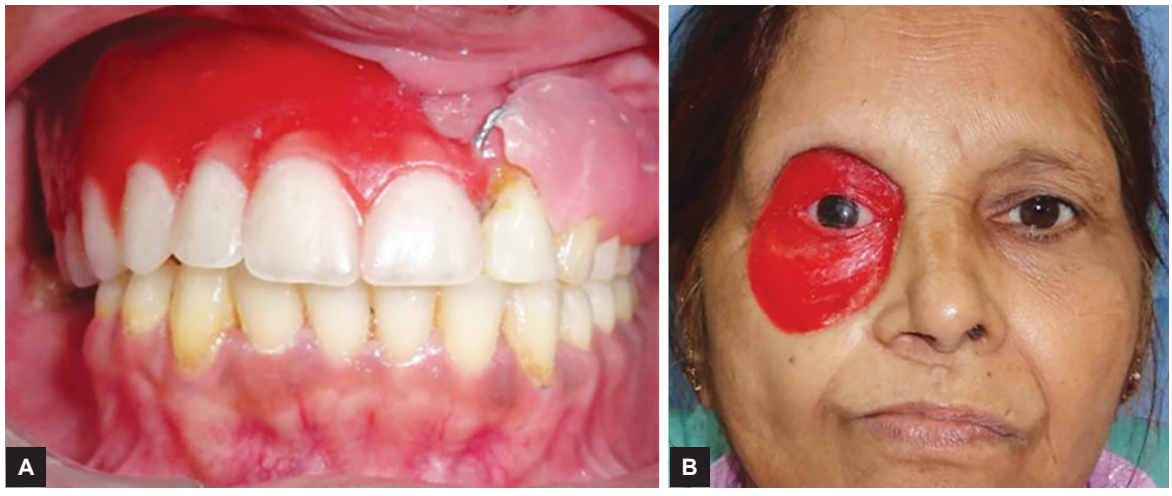
esthetics and function. After try-in was completed, the orbital impression was made with trial denture in place covering the intraoral defect. Patient was draped for impression and extraoral marking was made on the face with indelible pencil so that it can replicate exact position of anatomical landmarks over the cast. Custom tray was fabricated with the help of impression compound with additional relief holes. Alginate was used as a primary impression material. Final impression of orbital defect was made with light body addition silicone with custom acrylic tray.

Attachment of Pin and Socket to Acrylic Conformer and Obturator

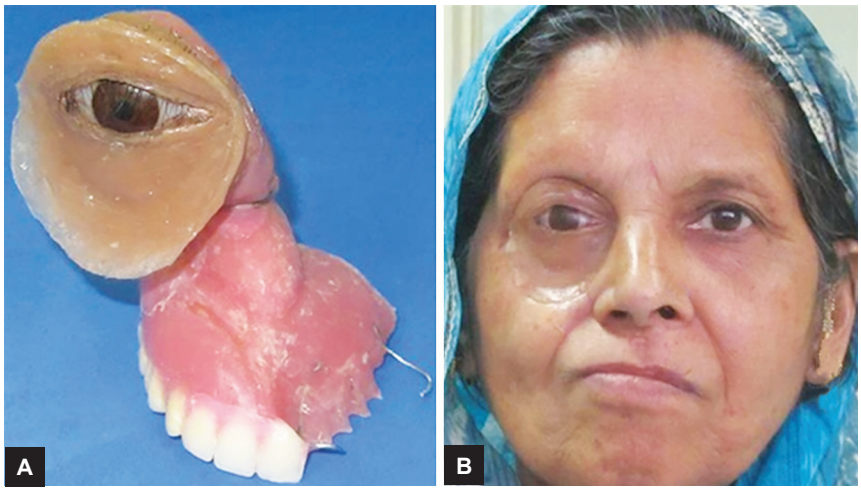
An acrylic conformer of orbital defect was fabricated and trial was made. To provide a mechanical undercut for the retention of orbital prosthesis, a 4 mm thick sprue wax was attached to the base of acrylic conformer. An obturator plate made of cold cure acrylic resin was also adjusted in patient's mouth. On the base of acrylic conformer, a pin of electric plug was attached and a corresponding socket was attached to the top of bulb of the maxillary obturator and fit of both the parts were checked for easy insertion and removal of prosthesis. Flasking, dewaxing, and packing with heat cure acrylic resin was carried out for both acrylic conformer and obturator. Processed heat cure bases were tried on the patient for accurate adaptation, retention, and stability during functional movements (Figs 2A and B).

Fabrication of Orbital Prosthesis

To replicate pupil-iris size, color, contour, and sclera shape of contralateral eye accurately, a customized ocular prosthesis was fabricated. Its final orientation was first refined on the working model with the help of recorded facial measurement and then tried on patients face. Missing structures like eyelids and associated anatomical structures were sculpted in modelling wax. Final completed wax pattern was tried on the patient face for evaluation of ocular orientation gaze, eyelid aperture, size, and volume



Figs 3A and B: Trial of obturator and orbital prosthesis



Figs 4A and B: Final silicone eye prosthesis and intraoral obturator

of sclera visible and prosthesis margin fit as compared to the contralateral eye (Figs 3A and B). Patient's opinion was also taken regarding the esthetics acceptability of prosthesis. This was followed by flasking, dewaxing, and packing in room temperature vulcanizing (RTV) silicone. Retrieved orbital prosthesis was characterized and was delivered to the patient with intraoral obturator in place (Figs 4A and B).

DISCUSSION

Surgical resection of the maxilla usually results in communication between the oral and nasal cavity that causes difficulty in swallowing, nasal regurgitation, unintelligible speech, and unesthetic appearance. Surgical reconstruction in such patient may add major financial burden and also has certain limitations, such as donor site morbidity, risk of recurrence, and compromise in esthetics. Therefore, prosthetic rehabilitation is usually preferred for such defects as it is quite inexpensive, allows for periodic examination and cleaning, and is also an alternative to surgery in medically compromised patients.

For rehabilitation of such patient, selection of biologically and functionally acceptable material for fabrication of prosthesis with economically feasible retentive aid should be used. For fabrication of orbital prosthesis, Factor II RTV silicone was used as it has better marginal adaptation, adequate edge strength, more lifelike appearance and better color stability as compared to other available material for maxillofacial prosthesis fabrication.[8] In present case, to retain the obturator and orbital prosthesis together economic and household electric pin and socket was used.

With the use of osseointegrated implant and magnets, a much better final outcome with improved retention and stability would have been achieved but this was not possible because of financial constraints. Advantages of this technique are adequate retention, economical, tissue tolerance, noninvasive, and comfort for use and wear.

CONCLUSION

Orbital exenteration along with hemimaxillectomy with an ablative surgery as a treatment procedure for malignant

tumor of maxilla resulting in continuous maxillary and orbital defect severely affects the vital functions, such as mastication, speech, swallowing, deglutition, and facial esthetics. For rehabilitation of such defects, removable maxillofacial prosthesis with adequate retention and stability during function along with good esthetics is necessary. A simple and economic attachment which provides adequate retention for both orbital and obturator prosthesis made up of a pin and socket of an electric plug, was used in this case.

REFERENCES

1. Brignoni R, Dominici JT. An intraoral-extraoral combination prosthesis using an intermediate framework and magnets: a clinical report. J Prosthet Dent 2001 Jan;85(1):7-11.
2. McClelland RC. Facial prosthesis following radical maxillofacial surgery. J Prosthet Dent 1977 Sep;38(3):327-330.
3. Fattore L, Edmonds DC. A technique for the obturation of anterior maxillary defects with accompanying midfacial tissue loss. J Prosthet Dent 1987 Aug;58(2):203-205.
4. Wolfaardt JF, Tam V, Faulkner MG, Prasad N. Mechanical behavior of three maxillofacial prosthetic adhesive systems: a pilot project. J Prosthet Dentist 1992 Dec;68(6):943-949.
5. Boucher LJ, Heupel EM. Prosthetic restoration of a maxilla and associated structures. J Prosthet Dent 1966 Jan-Feb;16(1): 154-168.
6. Nishimura RD, Roumanas E, Moy PK, Sugai T. Nasal defects and osseointegrated implants: UCLA Experience. J Prosthet Dent 1996 Dec;76(6):597-602.
7. Lemon JC, Chambers MS. Locking retentive attachment for an implant-retained auricular prosthesis. J Prosthet Dent 2002 Mar;87(3):336-338.
8. Rodrigues S, Shenoy VK, Shenoy K. Prosthetic rehabilitation of a patient after partial rhinectomy: a clinical report. J Prosthet Dent 2005 Feb;93(2):125-128.

Original Article

Effect of denture soft liner on mandibular ridge resorption in complete denture wearers after 6 and 12 months of denture insertion: A prospective randomized clinical study

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Abstract

Purpose: Soft liners act as a cushion between the denture base and the residual ridge. Hence, it is important to study their effect on resorption of mandibular denture bearing area. Therefore, the purpose of this study was to evaluate the influence of soft denture liner on mandibular ridge resorption after 1 year in completely denture wearers.

Materials and Methods: Twenty-eight completely edentulous patients having age between 45 and 60 years with well-formed ridges in class I jaw relations were selected as per the inclusion and exclusion criterion. Randomization chart was used to enroll participants in experimental and control groups who were given mandibular dentures with and without soft denture liner, respectively. Vertical measurements were made on orthopantomograph and analyzed using Adobe Photoshop 7.0 software at five points, i.e., one at central incisor and two points at right and left first premolars and two in each first molar region.

Results: On application of repeated measures analysis of variance, both groups showed a significant change in bone height after denture delivery ($P < 0.05$). Intergroup comparison (Wilcoxon rank sum test) of bone height in different regions at various time intervals showed statistically significant difference in bone levels ($P < 0.05$) from baseline to 6 months and baseline to 12months ($P < 0.01$), while the difference was statistically not significant during 6 and 12 months' period.

Conclusion: The use of soft denture liner significantly reduces the residual ridge resorption in complete denture wearers as compared to conventional denture wearers (without denture liner) over a period of 1 year.

Keywords: Complete denture, edentulous ridge, residual ridge resorption, soft liner

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INTRODUCTION

Residual ridge resorption is a chronic, progressive, irreversible, and disabling disease, and it has probably multifactorial origin.[1] Bone loss varies from patient to patient, but more significant changes occur in the mandibular arch. Tallgren[2] and Atwood and Coy[3] found

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that the mean ratio of anterior maxillary to anterior mandibular (residual ridge reduction [RRR]) was 1:4. This is because mandibular ridge bears higher functional forces transmitted through the dentures than the maxillary ridge because of its smaller area and less advantageous shape of the lower basal seat.[4]

Residual ridge resorption, especially in the mandible, may affect denture stability and retention.[5,6] Gross resorption of the edentulous mandibular alveolar process over a period of time resulting in excessive loss of the denture bearing ridge and mostly the overlying thin and atrophic mucosa over it makes it more difficult to withstand the masticatory load.[7,8] The mucosa gets impinged between the sharp ridge and the denture resulting in severe pain and discomfort to the patient.[8]

The difficulties encountered in such patients during function can be reduced with the use of either implants or resilient denture liners.[5,6,9] Although implants have been reported as highly effective,[10] they are not a viable solution for all edentulous patients because of unfavorable underlying bone, relevant medical, psychological, and financial constraints.[11] On the other hand, treatment with the resilient denture liners has only few limitations, being the nonsurgical application procedure, and have low treatment cost. The flexibility, resiliency, and the shock absorbency of the resilient denture liner materials help in impact reduction and distribution of masticatory load to the ridge.[12-15]

The use of soft liners became popular in dentistry because they have many clinical advantages. These materials have the ability to help in healing of the inflamed mucosa,[16-18] distribute the functional load in the support area of the prostheses[19,20] and improve their adaptation and retention.[21] According to the literature, due to cushioning effect provided by soft liners, lesser amount of forces are transferred to the underlying bone during various functions as compared to one without soft liners.[13-15,22] This leads to a reduction in residual ridge resorption. Although the idea of the resilient liners was introduced several years ago, most of the studies were either laboratory[22] or clinical observations for their efficacy. As intraoral environment is the most precise testing, clinical studies are a must to draw definitive conclusions.[23] Currently, the literature is scanty regarding the evidence of effect resilient liners on residual ridge resorption in complete denture wearers. Therefore, this study was designed to evaluate the effect of soft denture liners on the mandibular ridge resorption in complete denture wearers till 1 year of their application.

MATERIALS AND METHODS

This clinical study was carried out in the Department of Prosthodontics, CDER, All India Institute of Medical Sciences, New Delhi after approval from institutional ethics committee (IESC/T-255/01.06.2012). A total number of 28 completely edentulous patients within the age group of 45–60 years following the inclusion criteria; edentulous patients for the last 6 months, having class I jaw relation, well-developed ridges with firm mucosa and no previous denture experience were selected to participate in the study. Patient suffering with any systemic disorder which may influence bone metabolism and having class II or class III jaw relationship were excluded. Participants were thoroughly informed about the whole procedure, need of follow-up visits, radiographs required, and written informed consent was obtained.

The sample size was calculated based on literature review[24] as two groups, parallel, repeated measures with resorption as the primary outcome variable measured at 6 months' and 12 months' postdenture insertion. Keeping in mind the possibility to have dropouts and to achieve 90% power with detectable difference at 95% confidence interval, 14 participants were enrolled in each of the two groups as per stratified (male/female) block randomization which was based on computer-generated numbers given by the statistician.

For participants in experimental group (n = 14, 7 males and 7 females), maxillary dentures were fabricated in heat cured acrylic resin (Travelon, Dentsply India, Mumbai, India) while mandibular denture was lined with heat cured acrylic denture soft liner (Permasoft, Dentsply International, West Philadelphia Street York, PA, USA) at the time of packing. Participants in control group (n = 14, 7 males and 7 females) were provided with conventional maxillary and mandibular complete dentures fabricated

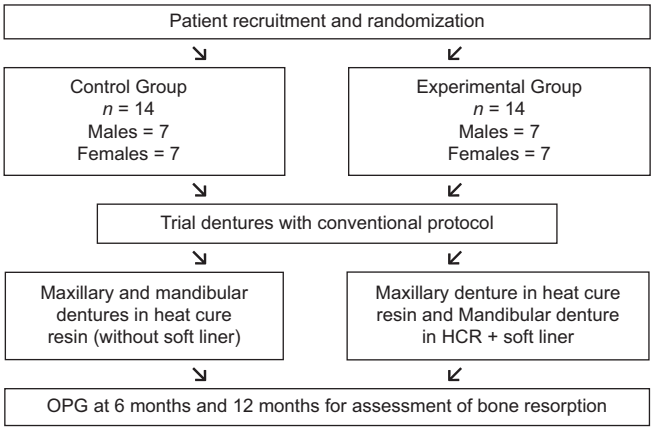


Figure 1: Flow chart of methodology

in heat cured acrylic resin (without soft liner) [Figure 1]. Bilateral balanced occlusal scheme was given in all the participants. The radiopaque markers were placed over the tooth surfaces in mandibular dentures after dewaxing at five different points (one in central incisor region and one in 1st premolar and 1st molar region on both sides) and secured in position with glue. Then the mold was packed in heat cured acrylic resin. Besides this, in experimental group, wax spacer of uniform two mm thickness was adapted over the mandibular cast and the remaining space was packed with heat cured acrylic resin. Wax spacer was replaced after trial closure with heat cured acrylic soft denture liner and denture was cured using the standard procedure. After processing, dentures were remounted for occlusal adjustment. Following this, dentures were finished, polished, and delivered to the participants.

For each participant, three panoramic radiographs (orthopantomogram [OPG]) were taken to assess the amount of bone resorption; immediately, at 6 months and at 12 months after denture insertion.

To assure the reproducibility between successive films, all the radiographs were taken on same machine (OPG machine details) by a trained radiographer using standard protocol and blinded about the use of soft denture liner. For the measurements, a reference plane was drawn touching the inferior border of the mandible. On this line, 90° tangents were drawn from the lower border of the radiopaque markers in five different regions; 2 in 1st premolar, 2 in 1st molar, and 1 in midline [Figure 2]. The distances from the radiopaque markers to the reference plane were measured with the help of Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA, USA). All the measurements were performed by two independent and blinded examiners to minimize the operator-based bias and error. In case of different values, measurements were verified again.

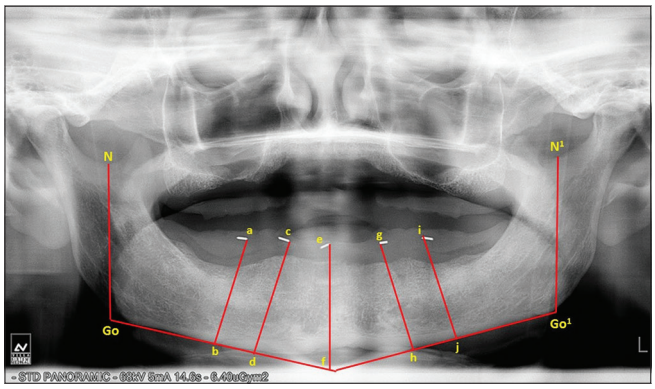


Figure 2: Orthopantomogram showing the measurement of bone height from the radiopaque marker to the reference point at the base of the mandible. Go and Go1 = mandibular right and left gonion, N and N1 = right and left mandibular notch, N-Go and N1-Go1 = fixed reference line, a, c, e, g, i = radiopaque markers

The magnification error for each participant was corrected by measuring the distance from the mandibular notch to the gonion on each radiograph. Measurements recorded immediately after denture insertions were considered as baseline values, and measurements made at 6 months and 12 months were adjusted according to the baseline radiograph.

The following equation represents an example of correction factor:

$$\text{Correction factor} = \frac{\text{Notch to gonion distance at denture insertion}}{\text{Notch to gonion after 6 months or 12 months}}$$

The values on each side were multiplied by the correction factor. To avoid any horizontal error, right and left sides of mandible were treated as separate and multiplied with respective factors.[24]

The data were analyzed using Statistical Package for Social Sciences (version 19, IBM, Chicago, IL, USA). Quantitative variables were assessed for approximate normality and summarized by mean and standard deviation. Wilcoxon rank-sum test was used to compare nonparametric variables in between the two groups. Within group change in the quantitative variables was assessed by repeated measure two-way analysis of variance followed by *post hoc* with Bonferroni test. In this study, the confidence interval was set at 95% and $P < 0.05$ was considered as statistically significant.

RESULTS

A total of 28 (14 male/14 female) participants with mean age of 53.93 ± 4.25 years were rehabilitated with maxillary and mandibular complete dentures. The mean mandibular bone height from radiopaque markers at five different points in the right posterior, anterior and left posterior regions to a reference plane in mandible was measured in both groups over a period of 12 months [Table 1]. The mean change in bone height in right posterior, anterior and left posterior region in control group from baseline to 12 months was 1.4 ± 0.14 mm (4.9%), 1.4 ± 0.2 mm (5.2%), and 1.7 ± 0.05 mm (5.9%) respectively and in experimental group, this was 0.7 ± 0.08 mm (2.3%), 0.6 ± 0.06 mm (2.5%), and 0.9 ± 0.08 mm (3.2%), respectively. Results showed decrease in bone height at

all time intervals in both groups, and the change was statistically significant ($P < 0.05$) [Table 2].

The comparison of bone height between the two groups in different regions at various time intervals showed significant difference in bone levels ($P < 0.05$) at baseline to 6 months and baseline to 12 months' period ($P < 0.01$), while the difference for 6 and 12 months' period was statistically not significant in right posterior ($P = 0.43$) and anterior region ($P = 0.16$) [Table 3].

DISCUSSION

Resorption of the residual ridge has been estimated with various radiographic techniques such as lateral cephalometric and panoramic radiographs. Panoramic radiography is widely used because an image of both the jaws can be produced on one film with a relatively lesser radiation exposure, and is common in routine examinations, especially for edentulous patients before the construction of complete dentures.[25-27] Magnification is one of the major problems with panoramic radiography. Larheim and Svanaes[28] investigated the precision of measurements of mandibular linear dimensions in panoramic radiographs and found that the variability of vertical measurements made from repeated panoramic radiographs is small when patients are properly positioned in the panoramic apparatus. According to Xie *et al*.,[29] if reference lines and measured points are located in the same vertical plane or in approximately the same plane as the teeth, variations in vertical measurements in the mandible and maxilla are within a small range.

Enlow *et al*.[30] described the distribution of surface fields of resorption and deposition in all parts of the edentulous mandible and stated that residual ridge resorption is usually more rapid in the premolar and molar region than the anterior region of the mandible, due to the lower position of the reversal line in the posterior region. Hence, it is especially important to record the resorption in anterior and posterior region of the mandible. The results of this study revealed statistically significant rate of RRR at all five reference points of measurement in mandible in all the participants. After 6 months of denture delivery, the bone loss ranged from 0.3 to 1.2 mm as compared to that during 6–12-month period (0.3–0.5 mm) in both groups in all the regions. According to Tallgren[2] and Kovacic *et al*.,[31] RRR was greater during the first few months after the tooth extraction than later. According to Karaagaçlioglu and Ozkan,[32] duration of edentulousness is one of the most important factor in contributing to the severity of mandibular bone loss. Mandibular resorption rate was greatest in the earlier stages of edentulism and slowed with the longevity of edentulism.[33]

Participants in the experimental group showed 50% lesser bone loss in all the three regions compared to the control group over a period of 12 months. After 1 year period, bone loss in experimental group ranged from 0.3 to 0.9 mm and in control group were 0.5–1.7 mm. Similar results were obtained by Elcharkawi and elMahdy[24] when comparing the bone loss in mandible after 6 months of denture delivery with and without soft liners. Lesser resorption with soft liner may be due to equal distribution of stresses under the denture and

Table 1: Mean mandibular bone level at five different points from base of the mandible up to the radio-opaque marker at different time intervals (*n*=14)

Area	Group 1 (control)			Group 2 (experimental)		
	Baseline	6 months	12 months	Baseline	6 months	12 months
Right posterior						
Molar	27.1±2.3	26.2±2.2	25.6±2.1	29.4±3.4	29±3.5	28.6±3.5
Premolar	30.2±2.6	29.3±2.6	28.8±2.6	30.1±5.6	29.7±5.6	29.4±5.6
Anterior-incisor	25.2±3.1	24.3±2.8	23.7±2.6	25.7±4.1	25.4±4.1	25.1±4.1
Left posterior						
Molar	28.3±3.2	27.1±3.2	26.6±3.2	28.4±2.9	27.6±2.8	27.2±2.9
Premolar	31.8±3.5	30.4±3.6	29.9±3.7	30.5±2.8	30.2±2.9	29.9±2.9

Table 2: Comparison of mean bone level in different regions within the group at different time intervals

Groups (*n*=14)	Regions	Bone level (mean±SD) in mm			Overall (*P*)	*Post hoc* comparison (*P*)*		
		Baseline	6 months	12 months		0-6 months	6-12 months	0-12 months
Group 1 (control)	Right posterior	28.6±2.4	27.7±2.3	27.2±2.3	0.01	0.01	0.01	0.01
	Anterior	25.2±3.1	24.3±2.8	23.7±2.6	0.01	0.01	0.01	0.01
	Left posterior	30.1±2.6	28.8±2.6	28.3±2.7	0.01	0.01	0.01	0.01
Group 2 (experimental)	Right posterior	29.7±4.2	29.3±4.2	29±4.3	0.01	0.01	0.01	0.01
	Anterior	25.7±4.1	25.4±4.1	25.1±4.1	0.01	0.01	0.01	0.01
	Left posterior	29.4±2.7	28.9±2.7	28.5±2.8	0.01	0.01	0.01	0.01

*$P<0.05$ - statistically significant. SD: Standard deviation

Table 3: Comparison of bone loss between the two groups in different regions at various time intervals

Regions	Time interval (months)	Group 1 (mm) (%)	Group 2 (mm) (%)	*P**
Right posterior	0-6	0.9±0.05 (3.1)	0.4±0.05 (1.3)	0.01
	6-12	0.5±0.12 (1.7)	0.3±0.04 (0.8)	0.43
	0-12	1.4±0.14 (4.9)	0.7±0.08 (2.3)	0.01
Anterior	0-6	0.9±0.13 (3.1)	0.3±0.04 (0.9)	0.01
	6-12	0.5±0.1 (2.2)	0.3±0.06 (1.2)	0.16
	0-12	1.4±0.2 (5.2)	0.6±0.06 (2.5)	0.01
Left posterior	0-6	1.2±0.06 (3.7)	0.6±0.09 (1.6)	0.01
	6-12	0.5±0.04 (1.6)	0.3±0.03 (1.1)	0.01
	0-12	1.7±0.05 (5.9)	0.9±0.08 (3.2)	0.01

*$P<0.05$ - statistically significant; Wilcoxon rank-sum test

lesser amount of force transmitted to underlying bone due to cushioning effect provided by viscoelastic property of soft liners.[13] Thus, the use of the resilient reliner material considerably minimized the stress in the alveolar bone and mucosa.[33-35] According to Lima *et al*.,[34] the thickness of the soft liner plays a significant role in the stress distribution especially in patient with thin mucosa, 2 mm thick soft liner relined denture shows lower stress distribution to bone as compared to denture with no soft liner or soft liner of variable thickness (0.5,1, 1.5, 2.5 mm). Taguchi *et al*.[36] stated that acrylic resilient liner material showed a higher viscoelastic behavior than silicone material after applying the stress, and have the ability of stress distribution or stress relaxation.

Contrary to this study, Al-Noori and Said[37] found no significant difference in bone resorption with and without soft liner in complete denture patients. This may be due to difference in the study design, i.e., their subjects were edentulous at least 1 year before denture delivery as compared to the present study in which patients were edentulous for 6 months only.

The main limitation of this study is the short study period. Cushioning effect of soft liner decreases over time; so they have to be replaced repeatedly.[38] Further studies can be carried out to evaluate the effect of different types of soft liner materials on ridge resorption and relining of complete dentures for patients with compromised ridges.

CONCLUSION

The use of complete denture with soft denture liner significantly reduces the residual ridge resorption as compared to the one without denture liner. Although acrylic denture liner bonds chemically to denture base, only problem that arises is the longevity of the denture liner and its long-term maintenance. The risk-benefit ratio should be analyzed before the use of this material.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Atwood DA. Reduction of residual ridges: A major oral disease entity. J Prosthet Dent 1971;26:266-79.
2. Tallgren A. The continuing reduction of the residual alveolar ridges in complete denture wearers: A mixed-longitudinal study covering 25 years. J Prosthet Dent 1972;27:120-32.
3. Atwood DA, Coy WA. Clinical, cephalometric, and densitometric study of reduction of residual ridges. J Prosthet Dent 1971;26:280-95.
4. Tallgren A. Alveolar bone loss in denture wearers as related to facial morphology. Acta Odontol Scand 1970;28:251-70.
5. Zarb GA, Schmitt A. The longitudinal clinical effectiveness of osseointegrated dental implants: The Toronto study. Part I: Surgical results. J Prosthet Dent 1990;63:451-7.
6. Zarb GA, Schmitt A. The longitudinal clinical effectiveness of osseointegrated dental implants: The Toronto study. Part III: Problems and complications encountered. J Prosthet Dent 1990;64:185-94.
7. Reich KM, Huber CD, Lippnig WR, Ulm C, Watzek G, Tangl S. Atrophy of the residual alveolar ridge following tooth loss in an historical population. Oral Dis 2011;17:33-44.
8. Meyer RA. Management of denture patients with sharp residual ridges. J Prosthet Dent 1966;16:431-7.
9. Zarb GA, Schmitt A. The longitudinal clinical effectiveness of osseointegrated dental implants: The Toronto Study. Part II: The prosthetic results. J Prosthet Dent 1990;64:53-61.
10. Feine JS, Carlsson GE, Awad MA, Chehade A, Duncan WJ, Gizani S, *et al*. The McGill consensus statement on overdentures. Mandibular two-implant overdentures as first choice standard of care for edentulous patients. Gerodontology 2002;19:3-4.
11. Hayakawa I, Hirano S, Takahashi Y, Keh ES. Changes in the masticatory function of complete denture wearers after relining the mandibular denture with a soft denture liner. Int J Prosthodont 2000;13:227-31.
12. Shim JS, Watts DC. An examination of the stress distribution in a soft-lined acrylic resin mandibular complete denture by finite element analysis. Int J Prosthodont 2000;13:19-24.
13. Murata H, Haberham RC, Hamada T, Taguchi N. Setting and stress relaxation behavior of resilient denture liners. J Prosthet Dent 1998;80:714-22.
14. Murata H, Taguchi N, Hamada T, Kawamura M, McCabe JF. Dynamic viscoelasticity of soft liners and masticatory function. J Dent Res 2002;81:123-8.
15. Williamson RT. Clinical application of a soft denture liner: A case report. Quintessence Int 1995;26:413-8.
16. Bates JF, Smith DC. Evaluation of indirect resilient liners for dentures: Laboratory and clinical tests. J Am Dent Assoc 1965;70:344-53.
17. Goll G, Smith DE, Plein JB. The effect of denture cleansers on temporary soft liners. J Prosthet Dent 1983;50:466-72.
18. Nikawa H, Iwanaga H, Hamada T, Yuhta S. Effects of denture cleansers on direct soft denture lining materials. J Prosthet Dent 1994;72:657-62.
19. Sinobad D, Murphy WM, Huggett R, Brooks S. Bond strength and rupture properties of some soft denture liners. J Oral Rehabil 1992;19:151-60.
20. Aydin AK, Terzioglu H, Akinay AE, Ulubayram K, Hasirci N. Bond strength and failure analysis of lining materials to denture resin. Dent Mater 1999;15:211-8.
21. Eick JD, Craig RG, Peyton FA. Properties of resilient denture liners in simulated mouth conditions. J Prosthet Dent 1962;12:1043-52.
22. Surapaneni H, Ariga P, Haribabu R, Ravi Shankar Y, Kumar VH,

Attili S. Comparative evaluation of tensile bond strength between silicon soft liners and processed denture base resin conditioned by three modes of surface treatment: An *in vitro* study. J Indian Prosthodont Soc 2013;13:274-80.
23. Rodrigues S, Shenoy V, Shetty T. Resilient liners: A review. J Indian Prosthodont Soc 2013;13:155-64.
24. elCharkawi HG, elMahdy AS. The effect of resilient layer and occlusal reactive complete dentures on the residual alveolar ridge. J Prosthet Dent 1988;59:598-602.
25. Xie Q, Wolf J, Ainamo A. Quantitative assessment of vertical heights of maxillary and mandibular bones in panoramic radiographs of elderly dentate and edentulous subjects. Acta Odontol Scand 1997;55:155-61.
26. Xie Q, Ainamo A, Tilvis R. Association of residual ridge resorption with systemic factors in home-living elderly subjects. Acta Odontol Scand 1997;55:299-305.
27. Saglam AA. The vertical heights of maxillary and mandibular bones in panoramic radiographs of dentate and edentulous subjects. Quintessence Int 2002;33:433-8.
28. Larheim TA, Svanaes DB. Reproducibility of rotational panoramic radiography: Mandibular linear dimensions and angles. Am J Orthod Dentofacial Orthop 1986;90:45-51.
29. Xie Q, Soikkonen K, Wolf J, Mattila K, Gong M, Ainamo A. Effect of head positioning in panoramic radiography on vertical measurements: An *in vitro* study. Dentomaxillofac Radiol 1996;25:61-6.
30. Enlow DH, Bianco HJ, Eklund S. The remodeling of the edentulous mandible. J Prosthet Dent 1976;36:685-93.
31. Kovacic I, Celebic A, Zlataric DK, Petricevic N, Bukovic D, Bitanga P, *et al*. Decreasing of residual alveolar ridge height in complete denture wearers. A five year follow up study. Coll Antropol 2010;34:1051-6.
32. Karaagaçlioglu L, Ozkan P. Changes in mandibular ridge height in relation to aging and length of edentulism period. Int J Prosthodont 1994;7:368-71.
33. Parkinson CF. Similarities in resorption patterns of maxillary and mandibular ridges. J Prosthet Dent 1978;39:598-602.
34. Lima JB, Orsi IA, Borie E, Lima JH, Noritomi PY. Analysis of stress on mucosa and basal bone underlying complete dentures with different reliner material thicknesses: A three-dimensional finite element study. J Oral Rehabil 2013;40:767-73.
35. Kawano F, Ohguri T, Koran III A, Matsumoto N, Ichikawa T. Influence of lining design of three processed soft denture liners on cushioning effect. J Oral Rehabil 1999;26:962-8.
36. Taguchi N, Murata H, Hamada T, Hong G. Effect of viscoelastic properties of resilient denture liners on pressures under dentures. J Oral Rehabil 2001;28:1003-8.
37. Al-Noori AK, Said GA. The Effect of soft lining materials on mandibular residual ridge resorption for edentulous patient. Al Rafidain Dent J 2013;13:66-71.
38. Kimoto S, Kimoto K, Murakami H, Gunji A, Ito N, Kawai Y. Survival analysis of mandibular complete dentures with acrylic-based resilient liners. Gerodontology 2013;30:187-93.

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ORIGINAL RESEARCH PAPER

Periodontology

KEY WORDS:

AWARENESS OF ORAL HYGIENE AMONG PATIENTS ATTENDING DENTAL OUT PATIENT DEPARTMENT IN A TERTIARY HOSPITAL IN COIMBATORE –A CROSS SECTIONAL STUDY

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ABSTRACT

Periodontitis is one of the leading oral infections in India. Prevalence of periodontitis was 86% as per world oral health report in 2003 and has been ever increasing. Factors like neglected or under awareness of oral hygiene and oral hygiene, practices play a critical role in the pathogenesis of periodontitis .Moreover it is related to many systemic diseases. Present cross sectional study is done to evaluate the oral hygiene awareness and practices among patients attending dental OPD in PSG hospital, Coimbatore. The study is done to add on to the scientific base and to improve the awareness of oral hygiene and practices among Coimbatore population .The results show that though there is improvement in oral hygiene practices, awareness about oral prophylaxis and follow up is lacking among the study population.

Introduction

Health is wealth and oral health is window to overall health[1].In our country it is reported that awareness in oral hygiene for general wellbeing is under rated even in areas with adequate access to oral health care. Epidemiological studies suggest high prevalence of periodontitis from poor oral hygiene practices and calculus deposition[2]. Albandar in an overview concluded that subjects of Asian ethnicity had the third highest prevalence of periodontitis[3].

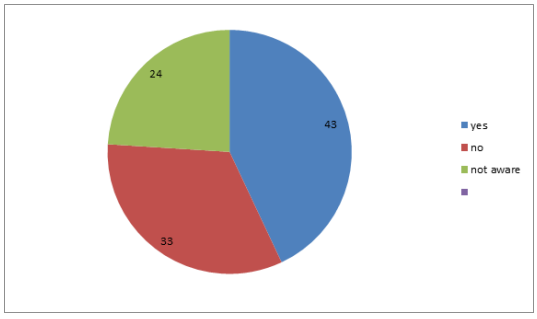
Periodontitis is one of the main inflammatory dental disease which is related to many systemic diseases bi-directionally like diabetes[4],cardiovascular disease[5],rheumatoid arthritis[6], alziemers disease[7,8],osteoporosis etc.[9][10].periodontal disease also leads to early tooth loss at an early age which leads to compromise in mastication and psychological problems in addition to affecting systemic health. Oral hygiene awareness is crucial as it aids towards effective plaque control, which is the corner stone to maintain periodontal health [11].Therefore, more efforts are needed to encourage greater use of preventive and therapeutic services. To achieve this goal, it is important to gain information regarding the awareness levels and values related to periodontal and gingival health. Literature on awareness of oral hygiene practices and aids among patients visiting dental clinics for treatment is negligible. This study is carried out with an aim to determine the oral hygiene status, knowledge about the practices and aids used in maintaining the oral hygiene among patients visiting dental outpatient department.

Methodology

Ethical clearance was obtained from the Institutional Board. Patients were selected randomly, Data was collected from 200 patients visiting dental out patient department after getting consent to participate in the survey. The inclusion criteria was patients above 18years and the exclusion criteria was medically compromised patients and those who did not consent to the survey. Questionnaire comprising 14 questions was used to collect the data. Questions relating to knowledge and attitude towards oral hygiene practices and aids were asked in face in face interview. Brushing techniques were explained to the patients as an attempt to improve the awareness about the oral hygiene habits. Completed questionnaire were then analyzed statistically to obtain the results in terms of percentage.

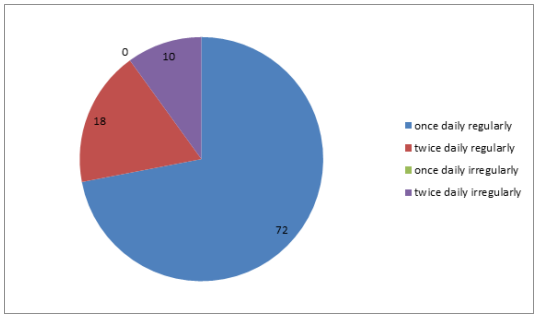
Results

Of the participants, 62% were male and 38% were female. About 43%feel oral health maintenance is important for general health, 33% do not agree and 24% were not aware of it[Figure1]



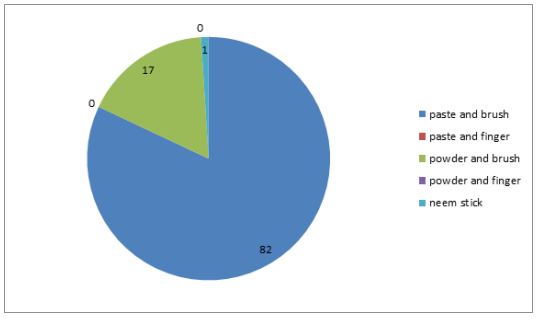
Figure[1] Oral health and general wellbeing

72% of people brush once regularly,18%brush twice regularly and 10% brush twice irregularly[Figure2]



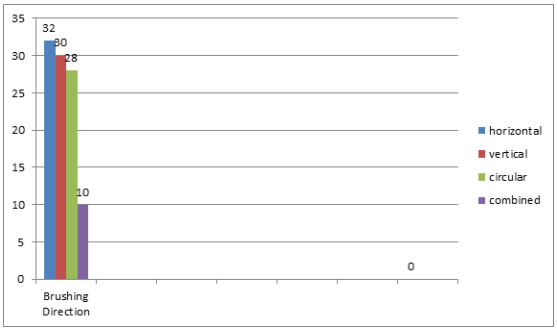
Figure[2]Brushing frequency

Paste and brush is used by 82% of people,17% use powder and brush and 1% used neem stick,figure[3].



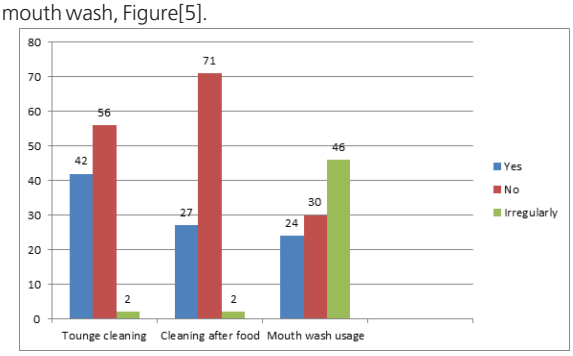
Figure[3] Frequency of population using brush and paste

About 32%of people use horizontal tooth brushing technique,30% use vertical brushing technique,28% use circular brushing technique and 10% use combined brushing techniques, figure[4].



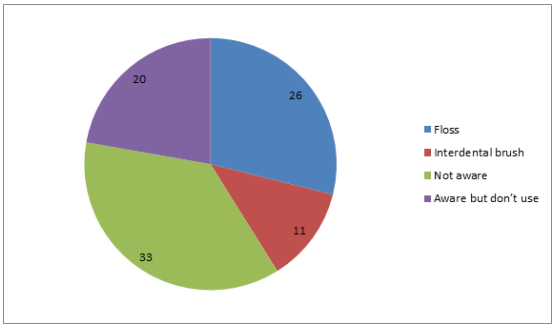
Figure[4] Brushing technique

Forty two percent of people clean their tongue daily and 56% of people don't and 2% clean irregularly ,figure[5]. Twenty seven percent of people clean their mouth after eating and 71% don't and 2% clean irregularly, Figure[5]. Twenty four percent of people use mouth wash regularly,46% use irregularly and 30% don't use mouth wash, Figure[5].



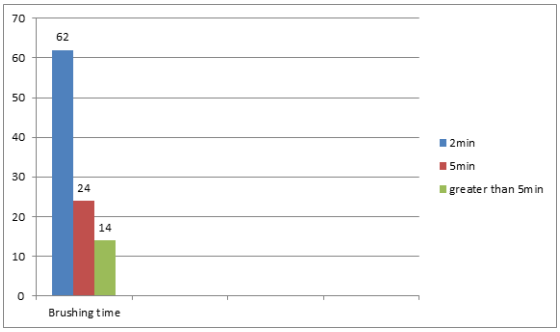
Figure[5] Frequency of tongue cleaning, cleaning after food intake and mouth wash usage

Regarding usage of interdental aids 26%use floss, 11%use interdental brush,43% are not aware if such aids and 20% are aware but don't use inter dental aids, Figure[6].



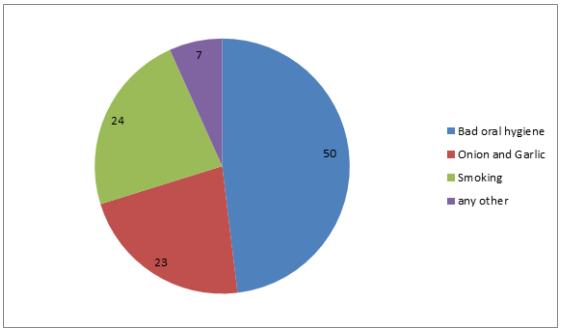
Figure[6] Percentage of people using interdental aids

Brushing time followed is 2min by 62%,5 minutes by 24 % and more than 5min by 14%,figure[7].



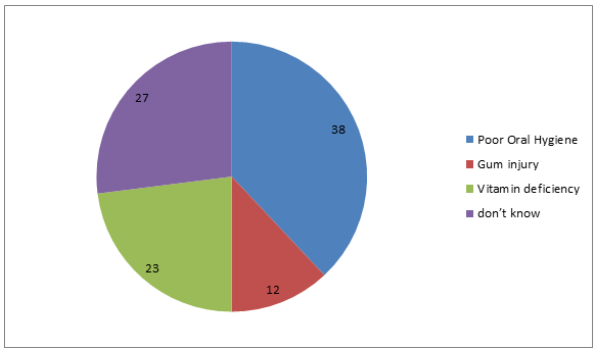
Figure[7] Brushing time percentage

Around 50%think bad oral hygiene is the cause for bad breath, 23% feel onion and garlic is the prime cause,20% feel it is because of smoking and 7% feel there is no specific reason, Figure[8].



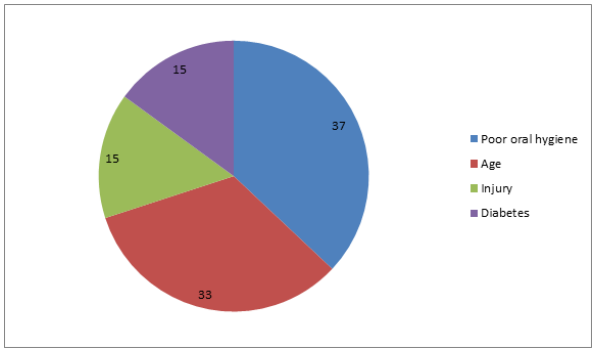
Figure[8] Frequency of people with bad breath

About 38% of people agree that poor oral hygiene is responsible for bleeding gums,12% say it is due to gum injury,23% say it is due to vitamin deficiency and 27% don't know the reason, Figure[9]



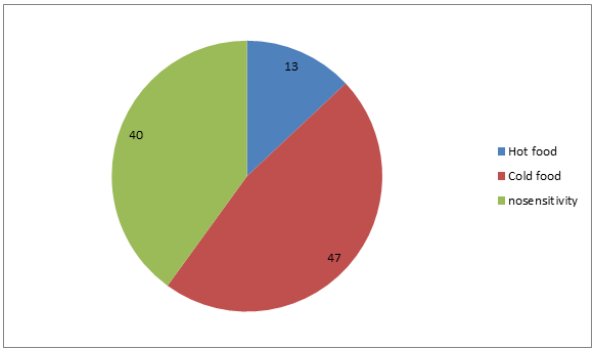
Figure[9] Percentage of people noticed gum bleeding

Loosening of teeth is due to poor oral hygiene say 37% of people, 33% say it is due to age,15% say it is due to injury and 15% agree the reason is diabetes, Figure[10].



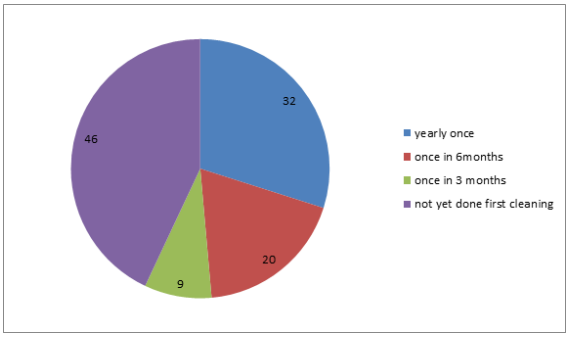
Figure[10] Percentage of people with loosening of teeth

Hot food causes sensitivity in 13%,cold food causes sensitivity in 47% and 40% do not have sensitivity, Figure[11]



Figure[11] Percentage of people with tooth sensitivity

Oral prophylaxis is done by 32% of people once in a year ,13% of people do it once in 6months , 09% do it once in 3 months and 46% are yet to clean their teeth ,figure[12]



Figure[12] Percentage of people undergone oral prophylaxis

Discussion

Oral health is necessary for general wellbeing. Due to lack of awareness ,oral hygiene is ignored in India were the population is estimated to be more than 1.35billion.27% of this population live in urban area. The present study is conducted in tertiary care hospital dental department where a combined population from village and urban area come under one roof for various treatments. Among the study subjects,62% were literate and 38% were illiterate. For the illiterate subjects ,the questions were explained in their mother tongue and their answers were collected.

Forty three percent of subjects agreed that oral health is needed for overall health ,33% did not agree and 24% were not aware about the fact, Figure[1]. Our results shows that the level of awareness is much low compared to 60.4% in the study conducted by Sukhvinder et al in 2014[12].

Only 18% of subjects brushed twice daily,72 % brushes once daily and 10% brushes twice irregularly, Figure[2], which is also low compared to 58% of police recruits in a study by Dilip et al [13] but coincides with the study done by Kuppusamy VL et al where twice brushing was 17%[14].

Paste and brush was used by 82% of population which coincides with study done by Subha et al [15].Using tooth brush decreases the frequency of tooth wear Bhardwaj et al [16].Seventeen percent use powder for brushing which coincided with study by Sukhvinder[12] which again contribute to tooth wear, Figure[3].

Thirty-two percent used horizontal technique,30%used vertical method,28% used circular technique and 10% used combined technique. It is less when compared to study done by Zhu et al and Jain et al[17 ,18].Basically subjects were not very clear with the techniques existing for brushing. Awareness about the techniques if improved can reduce the tooth and periodontal diseases related to abrasion, Figure[4].

Only 32% clean their tongue regularly and 27% wash their mouth after food which is due to lack of awareness, Figure[6]. Thirty-seven percent of population use either dental floss or inter dental aid which is a huge transformation from 0% in study conducted by Hanaa M. Jamjoon in 2001[19]. This may related to the factors like oral health care media advertisements, Figure[6].

Around 62% brushes for 2 minutes,24% for 5minutes and 14% for more than 5minutes similar to study by Sukhvinder[16].Beneficial role of long brushing time for more than 2minutes in plaque removal is yet to be studied, figure[7].

More than 30% of study population agreed that poor oral hygiene is the reason for bad breath, bleeding gums and loosening of teeth which is similar to study by Ali et al[20] and MM Dayakar[21],Figure[8,9,10].

Mouth wash regular usage was 24% and irregular usage was 46% mainly as alternative for malodor, figure[11].Almost 50% agreed with cold food as the cause for sensitivity. The reason for sensitivity can be gum recession, caries, hypersensitivity which was not examined in the present study. About 46% of population are yet to do their first ever oral prophylaxis which is an alarming situation, Figure[12] . This study is similar to one by Peterson et al and Al Hussaini et al[22 and 23].This mind set of not reaching dentist for regular checkup and oral prophylaxis may be due to a generalized view that oral hygiene is separate entity from overall health in general population.

Limitation

Since the study is hospital based and only 200 subjects were involved, further studies involving general public and more involving population has to be carried out to further improvise our knowledge about oral hygiene practices. This will help to organize awareness programs at various levels to inbuilt the mindset that good oral hygiene is needed for overall wellbeing.

Conclusion

The results show that though usage of tooth brush and paste has increased, our general population are still reluctant about oral care, nearly 50% are yet to do their first ever oral prophylaxis. Awareness has to be created regarding regular dental visit and oral prophylaxis which can improve the life time of natural teeth and also help to reduce overall inflammatory reactions as periodontitis is related to many systemic diseases[1,4,5,6,7,8,9,10].Studies at various level and with different sample groups have to be conducted in India and compared in order to improvise our oral hygiene awareness and practice programs which will definitely improve oral health.

Conflict of interest – Nil

REFERENCES

1. Boppml et al.The Surgeon Generals report on oral health dental hygiene:You can depend on us.J Dent Hyg.2001;75:263.[PubMed]
2. CorbertEf et al .Periodontal diseases in Asians. J Int Acad Periodontol 2006;8:13-44
3. Albandar JM, Rams TE. Global epidemiology of periodontal diseases: An overview. Periodontology 2000 2002;29:7-10.
4. Po-Chun Chang Interrelationships of periodontitis and diabetes: A review of the current literature. Journal of Dental Sciences (2012) 7, 272e282
5. Tonetti MS, Van Dyke TE Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases.J Periodontol. 2013 Apr;84(4 Suppl):S24-9.
6. Vilana Maria Adriano Araújo, Relationship between Periodontitis and Rheumatoid Arthritis: Review of the Literature.Mediators Inflamm. 2015; 2015: 259074
7. Francisco B. Teixeira Periodontitis and Alzheimer's Disease: A Possible Comorbidity between Oral Chronic Inflammatory Condition and Neuroinflammation Front Aging Neurosci. 2017; 9: 327
8. Mark Ide Periodontitis and Cognitive Decline in Alzheimer's Disease PLOS ONE | DOI:10.1371/journal.pone.0151081 March 10, 2016
9. Chin-Wei (Jeff) Wang1 and Laurie K. McCauley1 Osteoporosis and Periodontitis Curr Osteoporos Rep. 2016 December ; 14(6): 284–291. doi:10.1007/s11914-016-0330-3.
10. Jemim kim Periodontal disease and systemic conditions: a bidirectional relationship Odontology. 2006 September ; 94(1): 10–21.
11. Johani HA. Oral Hygiene Practica among Saudi Patients in Jeddah.Cario Dent J.2008;24:395-401.
12. Sukhvinder Singh Oberoi et al, Evaluating awareness regarding oral hygiene practices and exploring gender differences among patients attending for oral prophylaxisJ Indian Soc Periodontol. 2014 May-Jun; 18(3): 369–374
13. Dilip CL .Health status,treatment requirements,knoweldge and attitude towards oral ealth of police recruits in Karnataka.J Indian Assoc Public Health Dent .2005;5:20-34.
14. Kuppuswamy VL et al Oral hygiene status, knowledge, perceptions and practices among school settings in rural South India. Oral Health Dent Manag. 2014 Mar;13(1):146-54.
15. Subha. M Oral Health Awareness among the General Population in Chennai Research J. Pharm. and Tech. 10(11): November 2017
16. Vinay Kumar Bhardwaj. Tooth brushing behaviours and dental abrasion among the population in Shimla, Himachal Pradesh in India: A cross-sectional study. Journal of Cranio-Maxillary Diseases /Vol 3/ Issue 2/July 2014.
17. Zhu L, Petersen PE, Wang HY, Bian JY, Zhang BX. Oral health knowledge, attitudes and behaviour of adults in China. Int Dent. J 2005; 55:231-41
18. Nitika Jain, Dipika Mitra, K. P. Ashok, Jyothi Dundappa, SwetaSoni, Sameer Ahmed. Oral hygiene-awareness and practice among patients attending oPd at Vyas dental College and Hospital, Jodhpur. Journal of Indian Society of Periodontology - Vol 16, Issue 4, Oct-Dec 2012
19. Jamjoom HM. Preventive Oral Health Knoweledge and Practise in Jeddah,Saudhi Arabia .J KAU:Me Sci .2001;9:17-25.
20. Ali NS, Khan M, Butt M, Riaz S. Implications of practices and perception on oral hygiene in patients attending a tertiary care hospital. J Pak Dent Assoc. 2012;1:20–3.

21. Mundoor Manjunath Dayakar A survey about awareness of periodontal health among the students of professional colleges in Dakshina Kannada DistrictJ Indian Soc Periodontol. 2016 Jan-Feb; 20(1): 67–71.
22. Petersen PE,Hadi R,Al-Zaabi FS,Hussein JM,Behbehani JM,Skougaard MR,et al .Dental Knowledge,attitudes and behaviours among kuwaiti mothers and school teachers.J Pedod.1990;14:158-64.
23. Al-Hussaini R,Al-Kandari M,Hamadi T,Al-Mutawa,Honkala S,Memon A.Dental health knowledge,attitudes and behaviours among students at kuwait university health sciences centre. Med Princ Pract.2003;12:260-5.

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Management of Three Rooted Mandibular First Molar with Six Canals: A Case Report

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Abstract

The objective of dentists is to preserve teeth affected by either caries, trauma, or any other pathological condition. Teeth with deep carious lesions often require endodontic treatment to preserve their form and function. Sound knowledge of root canal anatomy, endodontic pathology, and proper treatment protocol are vital in endodontic treatment success. Currently, there has been an ongoing trend of case reports that highlight the presence of extra canals, which in turn, cautions the clinician to be more prudent. Any missed canal during root canal therapy is the common cause of treatment failure. This case report emphasizes the fact that proper understanding of pulp anatomy combined with the use of modern diagnostic aids and proper treatment protocol is essential for treatment success. This case report describes successful management of three rooted mandibular first molar with extra canals.

Categories: Pain Management, Dentistry, Anatomy
Keywords: mandibular first molar, additional canal, mid-mesial canal

Introduction

The success of endodontic therapy depends on the thorough elimination of infected pulp tissue, microorganisms, and complete three-dimensional sealing of the root canal space. Managing aberrant canals can be difficult, but inability or failure to identify extra canals can lead to treatment failure due to incomplete debridement of the root canal system. The clinician should thus have a thorough knowledge of not only the normal anatomy of the root canal system but possible root canal variations for successful endodontic treatment. Vertucci proposed a standardized method for classifying root canal anatomical variations and divided canal configuration into eight different types based on number of canals from orifices to apex [1]. Weine introduced a clinically significant classification (Type I to Type IV) of the root canal anatomy [2], and new additional canal types were also reported by Sert and Bayirli [3]. The earliest permanent posterior tooth to erupt, mandibular first molar, is most frequently involved in endodontic procedures. Mandibular first molar typically has two roots with the most prevalent root canal system configuration found in mesial and distal roots being Vertucci classification Type IV (two canals run separately from orifice to apex) and Type I (one canal runs from orifice to apex), respectively, but it is common to find other complexities with regard to number of root and root canals. This case report describes successful identification and management of three rooted mandibular first molar with six canals. Three canals were present in mesial root revealing Type XV Sert and Bayirli canal configuration, and the two distal roots

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had Type II Vertucci canal configuration in one distal root and Type I Vertucci canal configuration in another.

Case Presentation

A 35-year-old male patient of South Indian origin reported with continuous throbbing pain in a lower left back tooth. The patient was referred by a general dentist who had performed emergency access opening to relieve acute pain in the left mandibular first molar, which was diagnosed with acute irreversible pulpitis one week prior. The patient had a non-contributory medical history. Clinical examination revealed that the left lower first molar (tooth # 36) had access opening done and restored with temporary cement, and the tooth was mildly tender on percussion. Tooth mobility and periodontal probing around the tooth was within physiologic limits. A thorough inspection and interpretation of the baseline intra-oral peri-apical radiograph (Figure *1*) was made at the time when the patient presented. The radiograph revealed an unclear view of the canal space as well as variation in the outline of the distal root contour, suggesting possibility of complex root anatomy. Based on history, clinical and radiographic examination, plan for completion of root canal treatment was decided.



FIGURE 1: Baseline digital radiograph

The tooth was anesthetized with an inferior alveolar nerve block using 2% lignocaine with 1:80,000 epinephrine (Lignox 2%, Indoco Remedies, Ltd.). Under rubber dam isolation, the temporary restoration was removed, and the pulpal floor was carefully examined with magnification loupe (3.5 x), and three canals (mesiobuccal, mesiolingual, distal canal) were easily identified.

Careful exploration using a DG16 Endodontic Explorer (Manipal Instruments, Manipal, Karnataka, India) was done along the groove connecting mesiobuccal and mesiolingual orifice, and a third mid-mesial canal was found. The distal canal was positioned a little buccally, indicating the possibility of an extra canal, and a dark line was observed between the distal

canal orifice and the distolingual corner of the pulp chamber floor. The conventional access was modified by extending the access cavity distolingually and a second distolingual canal orifice was detected. Exploration was carried out along the groove extending between distolingual and distobuccal canals, and an additional distobuccal canal was found. Thus, the pulpal floor revealed six distinct root canal orifices (Figure 2): three were detected mesially (mesiobuccal, middle mesial, and mesiolingual) and three distally.

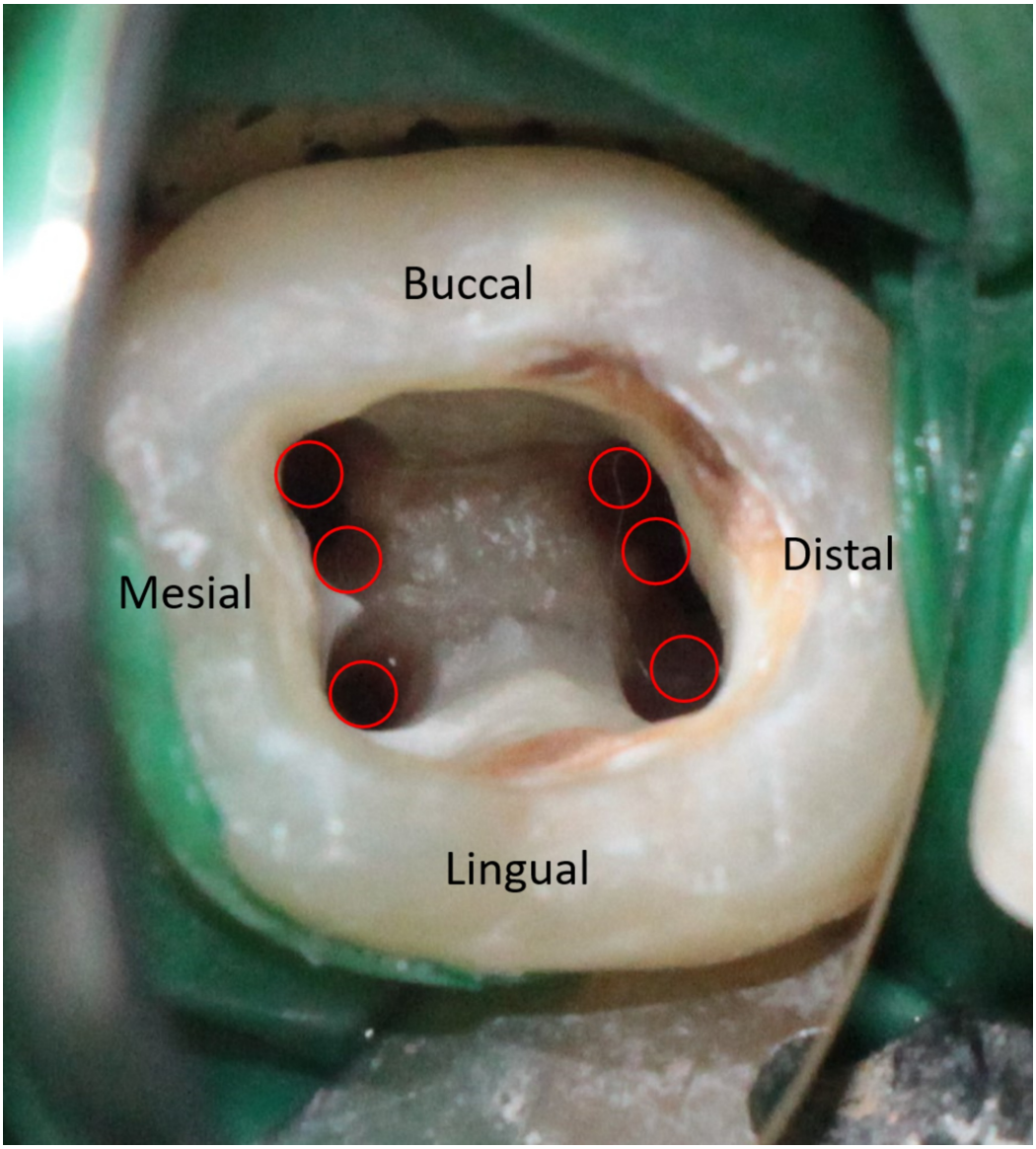


FIGURE 2: Access opening showing six distinct canal orifices

After canal identification and negotiation, coronal enlargement was done with the help of Ni-Ti ProTaper gold orifice shaper (SX; Dentsply, Maillefer, Ballaigues, Switzerland) to improve the straight-line access of canals. The working length was then determined by electronic apex locator (Root Zx II, J Morita, Japan) and was verified with a radiograph (Figure 3). Interpretation of working length radiograph confirmed the presence of an additional distolingual root. Of the six distinct root canal orifices detected, three were detected mesially (mesiobuccal, middle mesial, and mesiolingual), indicating the Type XV (3-2) Sert and Bayirli canal configuration, and two canals were present in distobuccal root (distobuccal 1 and distobuccal 2) with Type II Vertucci canal configuration, and one independent canal in distolingual root.

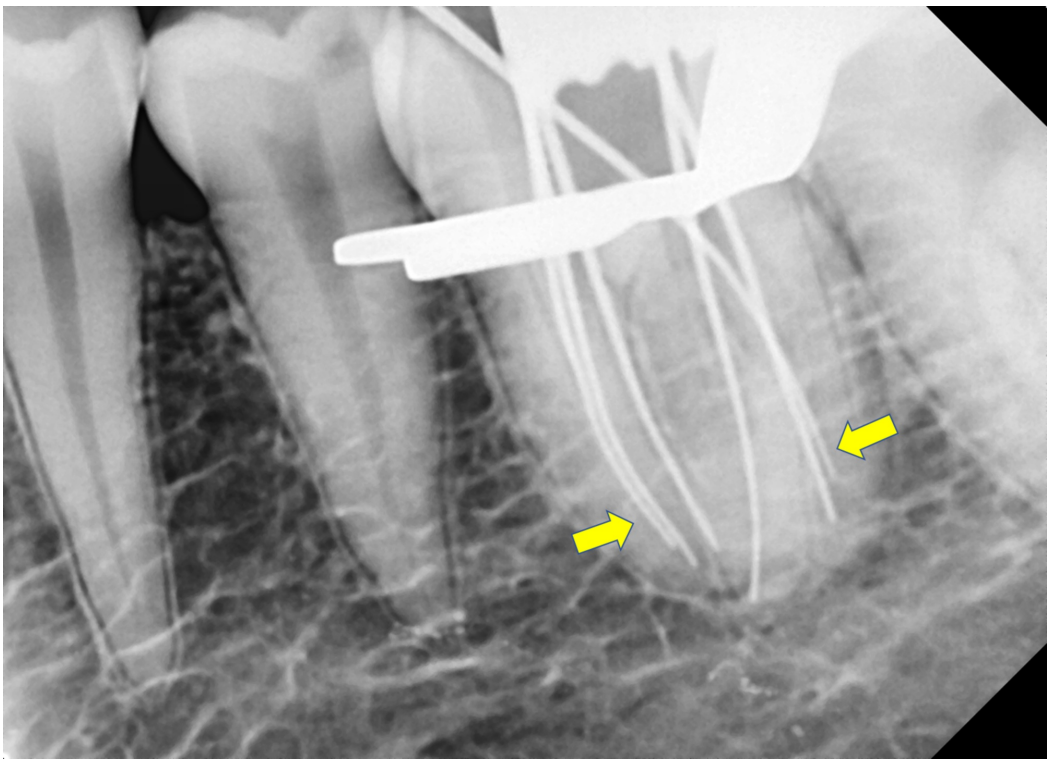


FIGURE 3: Working length radiograph showing six canals and extra distolingual root

Following working length determination, cleaning and shaping were done using Protaper Ni-Ti gold files using the crown down technique. All the instrumentation was performed with copious irrigation using 5.25% sodium hypochlorite and 17% ethylenediaminetetraacetic acid, alternatively. Irrigants were activated by sonic system (MM1500 Sonic Air, Micro Mega International, Besancon, France). Calcium hydroxide intracanal medicament (Vitapex, Neo-Dental International, Federal Way, WA) was placed in root canals, and access cavity was restored with temporary cement. At the second visit, after one week, calcium hydroxide medicament was removed, and a final rinse was done using sodium hypochlorite. Root canals were dried with absorbent points, and master cone was selected and positioned in respective canals, and radiograph was evaluated (Figure *4*). Canals were then obturated with warm vertical compaction technique, and calcium hydroxide-based sealer (Sealapex, Kerr Dental, Orange, CA) was used. Post-obturation X-ray was evaluated (Figure *5*), and at the six-month follow-up visit the patient was asymptomatic.

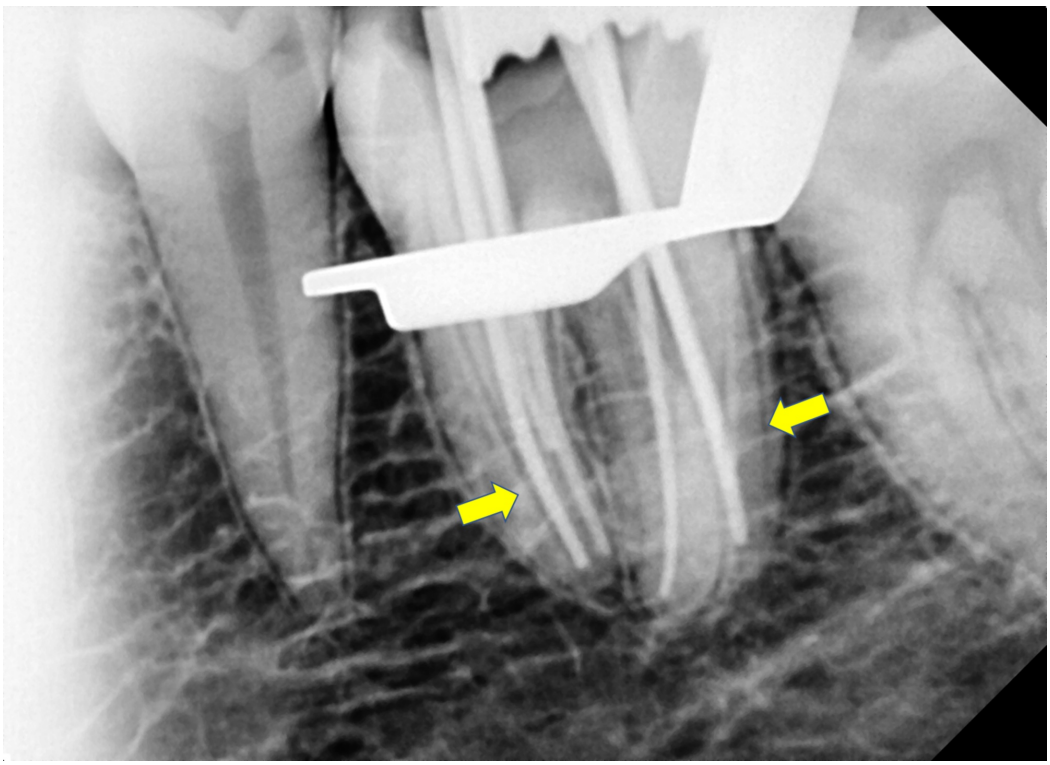


FIGURE 4: Master cone radiograph

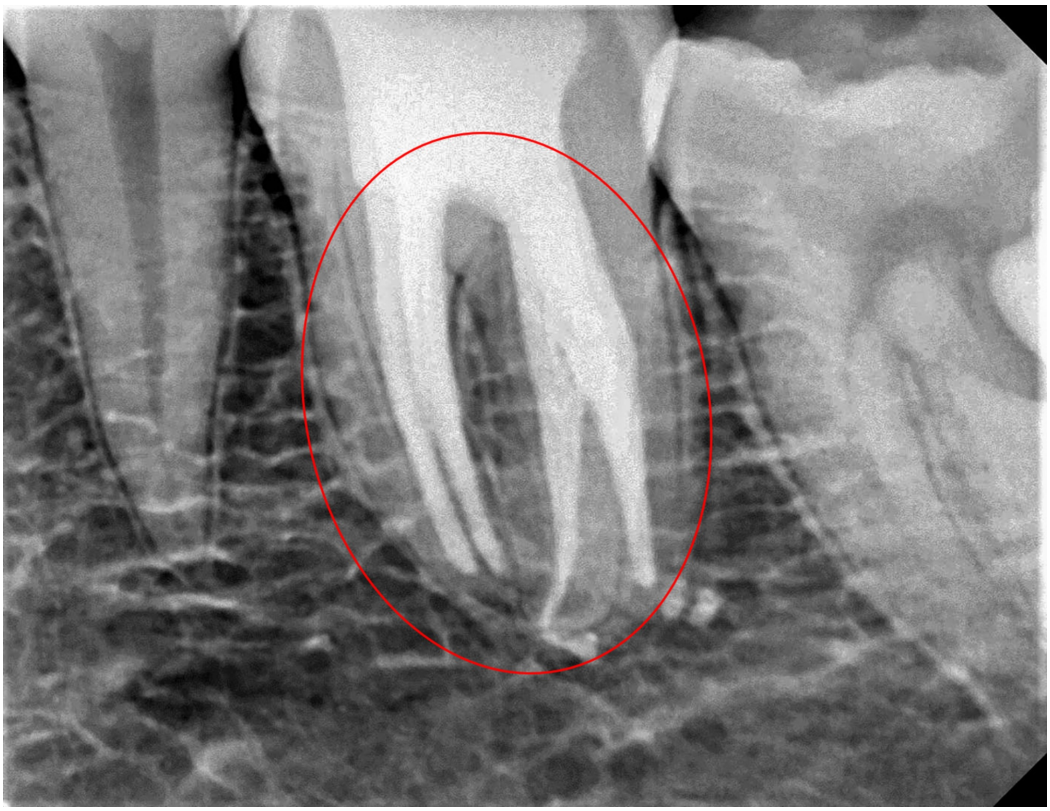


FIGURE 5: Post-obturation radiograph

Discussion

Various authors have reported the existence of mandibular first molar with additional root and extra canals [4,5]. Although preoperative radiograph has a key role in endodontic treatment, a minimum of two diagnostic periapical radiographs should be taken at different angulation for careful evaluation of the root canal morphology and to avoid chances of missing extra canals [6]. Krasner and Rankow proposed specific laws: the law of symmetry, the law of color change, and the laws of orifice location to locate canal orifices in an efficient manner [7]. Proper knowledge of root canal anatomy and its variations combined with use of various diagnostic aids such as spiral computed tomography, cone beam computed tomography, magnification devices, microscopes, performing sodium hypochlorite test will facilitate the identification of additional root and root canals.

The uniqueness of this case is the presence of six distinct root canal orifices and canals with extra distolingual root (radix entomolaris) in mandibular first molar. The mesial roots had canals with Type XV (3-2) as per Sert and Bayirli canal configurations, that is, the middle mesial canal merged with the mesiobuccal canal just above the apical third and exited through a common foramen. The mesiolingual canal was independent type with separate orifices and apical portal of exits.

In the distal root, distal buccal 1 and distobuccal 2 canal joined in its apical third having a mutual exit. The distolingual root had a separate canal. The presence of two distal roots is unusual but does occur. This additional root that can usually be found distolingually was first mentioned in literature by Carabelli and was called “radix entomolaris” [8]. A literature search showed that the prevalence of radix entomolaris in the mandibular first molar was reported to be 5.3% in the Indian population, 13.3% in South Indian by Chourasia et al. and Chandra et al., respectively [9,10]. Access cavity modifications are necessary (from triangle to rectangle or rhomboidal) to locate the additional canals in both mesial and distal roots. It has been reported that among the three-rooted mandibular first molars studied by the authors, the distolingual orifice was located 2.93 mm lingual to the distobuccal orifice, and the access form should be modified to a trapezoidal shape to expose all orifices [11].

Barker et al. and Vertucci and Williams first demonstrated the existence of a third root canal, in the mesial root of mandibular molars [12,13]. Since then, several authors have reported the existence of middle mesial canals. It has been hypothesized that the connective pulp tissue is likely to be compressed by the accumulation of secondary dentin during the growth of the root, which can, at times, form vertical walls, creating three independent mesial root canals [14]. Studies reveal that the incidence of negotiable middle mesial canals was found to be higher in younger patients [15,16]. In a clinical evaluation of 100 mandibular first and second molars, Pomeranz et al. found that 12 molars had mid-mesial canals in their mesial roots and classified them into three morphologic types as follows: fin, confluent, and independent, with the most prevalent anatomy being “fin” type [16]. However, Nosrat et al. reported that among the mandibular molar studied by the authors, 46.7% of teeth anatomy was confluent type, while Karapinar-Kazandag et al. found that in all the teeth studied by the authors, mid-mesial canal anatomy showed “confluent” anatomy [15,17]. The extra-mesial canal orifice found between the two main mesial canals is generally hidden by a dentinal projection of the pulp chamber wall, and this layer of dentin is differentiated from the pulp chamber floor by its lighter color. The mean diameter of mid-mesial canal orifice is reported to be 0.16 mm, which is three times less than the diameter of the two main orifices [18]. Enlargement of the middle mesial canal is more limited than for either the mesiobuccal or mesiolingual canal to avoid lateral perforation because the middle mesial canal is usually found in the narrowest portion mesiodistally of this root [16]. In addition, variation in location of the mid-mesial canal with respect to main canals has been reported by various authors. Karapinar-Kazandag et al. reported a higher number of additional mesial canals located closer to the mesiolingual canal in their study [17]. In a study

evaluating the frequency of middle mesial canals in a North Indian population, the authors found that among 258 mandibular first molars, in 67% of the cases the mid-mesial canal orifice was located in the middle of the mesiobuccal and mesiolingual canal orifices, 20% had the orifice closer to the mesiolingual canal, while the remaining 12% had the orifices located closer to the mesiobuccal canal [19]. Differences in observation by various authors may be attributed to difference in study methodology, population studied, and number of teeth included in the study.

The use of modern diagnostic tools can prevent an inadvertent search for extra canals that can lead to excessive removal of tooth structure and perforations. In this case report, use of magnification loupes aided in the proper detection of extra canals. In addition, proper treatment protocol was followed that led to success of endodontic treatment.

Conclusions

A thorough understanding of root canal anatomy is the key to successful root canal therapy. It is mandatory that clinicians should have a full understanding of the canal configuration and be aware of anatomical variations. Judicious interpretation of the radiographs, definite access preparation, and proper exploration of the pulp chamber under magnification, followed by proper cleaning and shaping of canals, are the fundamental prerequisites for a favorable outcome.

Additional Information

Disclosures

Human subjects: Consent was obtained by all participants in this study. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References

1. Vertucci FJ: Root canal anatomy of the human permanent teeth . Oral Surg Oral Med Oral Pathol. 1984, 58:589-599. 10.1016/0030-4220(84)90085-9
2. Weine FS: Endodontic Therapy, Sixth Edition. Mosby, St. Louis; 2004.
3. Sert S, Bayirli G: Evaluation of the root canal configurations of the mandibular and maxillary permanent teeth by gender in the Turkish population. J Endod. 2004, 30:391-398. 10.1097/00004770-200406000-00004
4. de Pablo OV, Estevez R, Péix Sánchez M, Heilborn C, Cohenca N: Root anatomy and canal configuration of the permanent mandibular first molar: a systematic review. J Endod. 2010, 36:1919-1931. 10.1016/j.joen.2010.08.055
5. Ballullaya SV, Vemuri S, Kumar PR: Variable permanent mandibular first molar: review of literature. J Conserv Dent. 2013, 16:99-110. 10.4103/0972-0707.108176
6. Cantatore G, Berutti E, Castellucci A: Missed anatomy: frequency and clinical impact . Endod Topics. 2006, 15:3-31. 10.1111/j.1601-1546.2009.00240.x
7. Krasner P, Rankow HJ: Anatomy of the pulp-chamber floor . J Endod. 2004, 30:5-16. 10.1097/00004770-200401000-00002
8. Carabelli G: Systematisches Handbuch Der Zahnheilkunde . Braumuller und Seidel, Vienna; 1844.
9. Chourasia HR, Meshram GK, Warhadpande M, Dakshindas D: Root canal morphology of mandibular first permanent molars in an Indian population. Int J Dent. 2012, 2012:6.

10.1155/2012/745152

10. Chandra SS, Chandra S, Shankar P, Indira R: Prevalence of radix entomolaris in mandibular permanent first molars: a study in a South Indian population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011, 112:77-82. 10.1016/j.tripleo.2011.02.016
11. Gu Y, Lu Q, Wang H, Ding Y, Wang P, Ni L: Root canal morphology of permanent three-rooted mandibular first molars—part I: pulp floor and root canal system. J Endod. 2010, 36:990-994. 10.1016/j.joen.2010.02.030
12. Barker BC, Lockett BC, Parsons KC: The demonstration of root canal anatomy . Aust Dent J. 1969, 14:37-41. 10.1111/j.1834-7819.1969.tb03330.x
13. Vertucci FJ, Williams RG: Root canal anatomy of the mandibular first molar . J N J Dent Assoc. 1974, 45:27-28.
14. Martínez-Berná A, Badanelli P: Mandibular first molars with six root canals . J Endod. 1985, 11:348-352. 10.1016/S0099-2399(85)80043-1
15. Nosrat A, Deschenes RJ, Tordik PA, Hicks ML, Fouad AF: Middle mesial canals in mandibular molars: incidence and related factors. J Endod. 2015, 41:28-32. 10.1016/j.joen.2014.08.004
16. Pomeranz HH, Eidelman DL, Goldberg MG: Treatment considerations of the middle mesial canal of mandibular first and second molars. J Endod. 1981, 7:565-568. 10.1016/S0099-2399(81)80216-6
17. Karapinar-Kazandag M, Basrani BR, Friedman S: The operating microscope enhances detection and negotiation of accessory mesial canals in mandibular molars. J Endod. 2010, 36:1289-1294. 10.1016/j.joen.2010.04.005
18. Versiani MA, Ordinola-Zapata R, Keleş A, Alcin H, Bramante CM, Pécora JD, Sousa-Neto MD: Middle mesial canals in mandibular first molars: a micro-CT study in different populations . Arch Oral Biol. 2016, 61:130-137. 10.1016/j.archoralbio.2015.10.020
19. Sherwani OA, Kumar A, Tewari RK, Mishra SK, Andrabi SM, Alam S: Frequency of middle mesial canals in mandibular first molars in North Indian population - an in vivo study. Saudi Endod J. 2016, 6:66-70. 10.4103/1658-5984.180618

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EVALUATION OF SURFACE TENSION OF THREE ROOT CANAL SEALERS USING PENDANT DROP METHOD

Dental Science

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ABSTRACT

The aim of this study was to evaluate the surface tension values of established and potential endodontic sealers.Densities of zinc oxide eugenol ,AH Plus and Bioceramic sealers were calculated using pendant drop method.Surface tension was evaluated using rame hart goniometer with DROP image analyser software[Finn Knut Hansen1990-2006].The results show that surface tension of zinc oxide eugenol was the least followed by AH plus and Bioceramic sealer.Bioceramic sealer showed the highest surface tension of 47.8 Nm.From other studies it is known that bioceramic sealer has the highest dentinal tubular penetration.Various factors like dentin roughness,wetness,type of irrigant used,viscosity ,hydrophilicity of root canal sealers,temperature,dentinal tubule texture and density etc influence the penetration of sealers.Further studies are needed to evaluate the role of surface tension of root canal sealers as a potent influencer in dentinal tubule penetration for enodontic success.

KEYWORDS

surface tension, pendant drop, root canal sealers, goniometer.

INTRODUCTION

In endodontics the most commonly used filling method involves gutta percha with root canal sealers. Root canal sealers play a pivotal role in endodontic success by sealing the the void space between the core material and dentinal wall,effectively penetrationg and sealing the accessory canals and also acts as a lubricant thus preventing secondary infection[1].Various factors influence the dentinal tubule penetration and overall sealing scenario of root canal sealers like composition of the sealer,flow,bond strength,dentinal tubule diameter and orientation,rate of insertion of material[2], physiochemical properties of dentin [3,4,5].etc .An important factor influencing the flow ,wettability,adhesion and penetration of root canal sealer is the surface tension of the sealer.Surface tension is defined as 'the force between molecules that produces a tendency for the liquid surface area to decrease.This force tends to limit the adhesion and penetration ability of the liquid. It has inverse relationship with wettability[6,7] that is when the surface tansion is low,wettability will be high and inturn penetration and adhesion will be high. Having an array of endodontic sealers available in the market makes the decision for ideal sealer always difficult.Though many studies have been done on the various entities like sealer penetration,contact angle ,microleakage ,shrinkage,influence of irrigants,instruments used during biomechanical preparation,smear layer presence etc for the success of endodontic treatment.Evaluation of surface tension of sealers which influences the flow,wettability,penetration and adhesion have not yet been studied.

This study aims at finding the missing strength of sealers that is the surface tension among there endodontic sealers Zinc oxide eugenol (with grossman formula),AH plus Dentsply)and Bioceramic sealer(smart paste bio).Zinc oxide eugenol which the gold standard for sealer ,AH Plus which is near to gold standard in Resin based sealers and recently introduced calcium silico phosphate based bioceramic sealer(smart paste bio).Surface tension is evaluated to find out its influence in sealer penetration and adhesion to create an effective monoblock.

MATERIALS AND METHODS

Densitometer was used to determine the density of experimental sealers. Weight of 2ml syringe was calculated.sealers were mixed according to the manufacturers instructions and mass was calculated for 1ml of the sealer.density was calculated from the formula density=mass/volume.Surface tension was calculated using pendant drop method [figure 1]in rame harte goniometer [figure1.2]with DROP image analyser software.Fitting the drop to the shape method was used for surface tension calculation. Five values were taken for each sealer and mean of it was determined.

RESULTS

Mean value of surface tension as determined by rame hart goniometer using pendant drop method with DROP image analyser software is given in table1. The unit of surface tension is Nm/m.

DISCUSSION

Requirement for An ideal root canal sealer includes it being tacky which helps for good adhesion between it and the canal wall when set,creating a hermetic seal [8].Low surface tension decreases contact angle thereby increasing the wettability which inturn favours good sealer penetration and adhesion to dentinal tubules[9,10,11,12].

Surface tension is measured by pendent drop technique at room temperatures to nullify any discrepancy due to temperature.Geometry of a drop was analyzed optically using DROP image analyser software[Finn Knut Hansen 1990-2006].Pendent Drop method was used in the study because of high accuracy,ability to trace to national standards,apparatus cleanliness doesnot affect the measurement,small volume can be used to measure surface tension[13].

The reason for selecting Zinc oxide Eugenol sealer is that it is the gold standard still even with the introduction of a plethora of root canal selers. AH plus though has improved compostion still lacks the ability to create monoblock effect and the recently introduced bioceramic sealer has promising composition with setting in the presence of water .It is a known factor that dentinal tuble contains water and irrigants which we use also influence the adhesion of the sealer to the dentin.

From the results of this study,it is seen that Zinc oxide eugenol (25.13Nm/m)has low surface tension followed by AH Plus(33Nm/m) and Bioceramic sealer(47.8Nm/m).which inturn predicts the wetting and dentinal tuble penetration to be high for Zinc Oxide Eugenol followed by AH Plus and Bioceramic(Smart seal bio) sealers. studies done by Jardim Del Monaco R et al ,Wang Y et al reveal that Bioceramic sealers have better dentinal tubule penetration than AH PLUS sealer[14,15] ,while studies done by SK Arikalta et al say AH Plus has better dentinal tubule penetration and interfacial adaptation than Bioceramic sealer [16]while studies done by Afaf Al Hadad et al reveals equal Dentinal tubule Pentration[17].In the same way bioceramic sealer has superior wetting than AH plus sealer as studied by Jung-Hong Ha et al[20] .studies done by ss pawar et al,Srinidhi.V.Ballullaya et al state that bioceramic sealer has better adhesion and adaptation than AH plus and Zinc Oxide eugenol sealer [18,19]whereas studies done by Ola M Sakr et al state that bioceramic sealer has more dye leakage than AH 26 sealer[21].

Though the surface tension is low for Zinc Oxide Eugenol and AH Plus sealers,their sealer penetration adaptation,dye leakage are comparatively low than Bioceramic sealer.The reason for this variation may be the method of biomechanical preparation,dentin sirface roughness,dentin tubule orientation and diameter,irrigants

used,force of condensation etc.This study suggest for studies on surface tension of sealers to explore its role as a potential influencer in dentinal tubule penetration and adaptation which again is foremost for endodontic success.

Limitations

In all the studies mentioned as referrences,different bioceramic sealers from different companies were used .Therefore culminating the results under a broad category ,bioceramic sealers will result in false impact about the sealer.More studies have to be carried out on these sealers to find out the ideal root canal sealer.

Table 1.Surface Tension of experimental sealers in Nm/m

Sealer	Surface tension valuesNm/m
Zinc oxide eugenol	25.13
AH plus	33
Bioceramic	47.8



Figure 1 Pendant Drop of Sealer



Figure 1.2 Rame Hart Goniometer

REFERENCES

1. Salz U, Poppe D, Sbicego S, Roulet JF. Sealing properties of a new root canal sealer. International Endodontic Journal. 2009; 42:1084-1089.
2. Dag O. Endodontic topics. 2005; 12: 25-38.
3. Tuncer and SafaTuncer. Effect of Different FinalIrrigation Solutions on Dentinal Tubule Penetration Depth and Percentage of Root Canal Sealer. Journal Of Endodontics 2012;38:860-863.
4. Branstetter and J.A. Von Fraunhofer.The physical properties and sealing action of endodontic sealer cements: a review of the literature.Journal ofendodontics 1982; 8: 126-30.
5. Balguerie, Lucas van dresluis, Karen valleys, Marie gurgel –georgelin, and Franck diemer.Sealer penetration and adaptation in the dentinal tubules: A scanning electron Microscopic study.Journal of Endodontics2011;37:1576-79.
6. Giardino,EmanueleAmbu, Carlo Becce,LiaRimondiniand Marco Morra. Surface Tension Comparison of Four Common Root CanalIrrigants and Two New Irrigants Containing Antibiotic. Journal OfEndodontics2006;32:1091-1093.
7. Shafrin and William A.Zisman. Constitutive relations in the wetting of low energy surface and the theory of the retraction method of preparing monolayers.1960;64:519-524.
8. Grossman LI. Endodontic practice. 10th ed. 1982
9. DeAssis,Maira do Prado and RenataA.Simao.Evaluation of the Interaction between Endodontic Sealers and Dentin treated with Different Irrigant Solutions. Journal OfEndodontics 2011;37:1550-1552
10. Tuncer and SafaTuncer. Effect of Different FinalIrrigation Solutions on Dentinal Tubule Penetration Depth and Percentage of Root Canal Sealer. Journal Of Endodontics 2012;38:860-863.
11. Giardino,EmanueleAmbu, Carlo Becce,LiaRimondiniand Marco Morra.Surface Tension Comparison of Four Common Root Canal Irrigants and Two New Irrigants Containing Antibiotic. Journal OfEndodontics2006;32:1091-1093.
12. Shafrin and William A.Zisman.Constitutive relations in the wetting of low energy surface and the theory of the retraction method of preparing monolayers.1960;64:519-524.
13. Surface and colloidal science Robert J. Good ,Robert R. Stromberg volume 11
14. Rodrigo Jardim Del Monaco, Marcelo Tavares de Oliveira ,Adriano Fonseca de Lima ,Ricardo Scarparo Navarro, influence of Nd:YAG laser on the penetration of a bioceramic root canal sealer into dentinal tubules: A confocal analysisPLOS ONE August 22, e:0202295
15. Wang Y, Liu S, Dong Y (2018) In vitro study of dentinal tubule penetration and filling quality of bioceramic sealer. PLoS ONE 13(2): e0192248
16. Arikatla Sampath Kumar, Chalasani Uma, Mandava Jyothi, Yelisela Rajiv Kumar Interfacial adaptation and penetration depth of bioceramic endodontic sealers 2018 volume21(4) 373-377
17. Afaf AL-HADDAD, Noor Hayaty ABU KASIM and Zeti Adura CHE AB AZIZ Interfacial adaptation and thickness of bioceramic-based root canal sealers Dental Materials Journal 2015; 34(4): 516–521
18. Suprit Sudhir Pawar, Madhu Ajay Pujar, and Saleem Dadapeer Makandar Evaluation of the apical sealing ability of bioceramic sealer, AH plus & epiphany: An in vitro studyJ Conserv Dent. 2014 Nov-Dec; 17(6): 579–582.
19. Srinidhi V. Ballullaya1, Vusurumarthi Vinay2, Jayaprakash Thumu3, Srihari Devalla4, Indira Priyadarshini Bollu5, Sagarika Balla6 Stereomicroscopic Dye Leakage Measurement of Six Different Root Canal Sealers J Clin Diagn Res. 2017 Jun;11(6):ZC65-ZC68
20. Jung-Hong Ha,1 Hyeon-Cheol Kim,2 Young Kyung Kim,1 and Tae-Yub Kwon3,An Evaluation of Wetting and Adhesion of Three Bioceramic Root Canal Sealers to Intraradicular Human DentinMaterials (Basel). 2018 Aug; 11(8): 1286
21. Ola M. Sakr, Manal M. Abdelhafeez, Salsabil M. AlKhalifah and Sha'eah Mohammad AlWehaiby. 2017. Evaluation of Microleakage in Endodontically Treated Teeth with Two Different Types of Sealers: Bioceramic Based Sealer and AH26; An in vitro Study. Int.J.Curr.Microbiol.App.Sci. 6(12): 3556-3564

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INVITRO COMPARATIVE EVALUATION OF CONTACT ANGLE OF THREE ROOT CANAL SEALERS

Dental Science

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ABSTRACT

AIM: The aim of this study was to evaluate the contact angle of three endodontic sealers zinc oxide eugenol,AH plus [epoxy resin based] and bioceramic [calcium silico phosphate based] sealers.

METHODOLOGY: Working length of 21 samples of human mandibular premolars were determined followed by chemomechanical preparation. The specimens were then decoronated,split longitudinally, flattened, smoothened and stored in distilled water. Half of the dentin discs were treated with 5ml of 3%NaOCl followed by 17%EDTA and 2%chlorhexidinefor 1min each.The samples were air dried and divided into 3 groups based on the experimental sealers used. A total of 14 samples were assigned for each group with 7 samples per subgroup. In each sample 1drop [0.1 ml] of sealer was deposited and the contact angleat 0,5,10,15,20,25,30 seconds after the initial application of the sealers were recorded at room temperature.

RESULT: contact angle values were low for zinc oxide eugenol followed by AH Plus and Bioceramic sealer.

CONCLUSION: The present study revealed that lower contact angle along with smear layer removal enhanced the wettability of root canal sealers.

KEYWORDS

Wettability, Contact Angle, Smear Layer,dentin

INTRODUCTION

In endodontic practice, the success of root canal therapy mainly depends on the elimination of pathogenic microorganisms and their byproducts followed by three dimensional obturation of the root canal space.To achive this challenging task,a biologically acceptable solid or semisolid obturating material in conjuction with root canal sealers has been advocated in endodontic literatures.

Guttapercha is the most widely used core material for obturation till date. Its property of inertness,plasticity and solvent solubility have made it an ideal candidate for root canal filling. However it has a major disadvantage of not adhering to dentinal walls of the root canal space. This neccessiates the use of endodontic sealer mandatory[5,19]

Adhesion of root canal sealers is highly dependent on the wettablility of dentin .Wettability is a measure of surface reactivity or adhesiveness of substrate. It can be expressed interms of angle between the liquid drop and the plane surface of solid on which it rests .This angle is referred to as **contact angle** [4,10,12,14,22,32].Contact angle has inverse relationship with wettability,that is,the lower the contact angle,the greater the surface free energy and hence the greater the adhesion[8,12,32]

There are a number of factors which influence wettability of endodontic sealers.They are

1. The physiochemical properties of root dentin(surface free energy). [3,6,32]
2. The influence of irrigant solutions(sodium hypochlorite, EDTA,c hlorhexidineetc) that are used during the chemomechanical preparation.[2,26,32]
3. The dentin moisture condition and dentinal tubular density.[20]
4. Presence of smear layer due to biomechanical preparation ofroot canal.[17,29]
5. Hydrophobicity, Hydrophilicity, viscosity of sealers,film thic kness, obturation technique and the temperature at which the proc edure is performed.[3]

For good adhesion of root canal sealers,wettability[surface free energy] of dentin should be high and contact angle of sealers should be low.

Different endodontic sealers have different contact angle .In the present day, a plethora of sealers are made available in the market, one may find it difficult to choose a good sealer. [33]Every manufacturers claim that their product is the ideal one, but till date none of the sealers have proved to be the ideal except for a few which can come closer to be ideal.

The aim of this present study was to compare and evaluate the contact angle of three endodontic sealers zinc oxide eugenol,AH plus [epoxy resin based] and bioceramic [calcium silico phosphate based] sealers in the presence and absence of smear layer at 0,5,10,15,20,25,30 seconds after initial application of the sealers with goniometer.

MATERIALS AND METHODS

MATERIALS USED

SEALERS:

1. ZINC OXIDE EUGNOL SEALER (grossman formula)
2. AH PLUS SEALER(DENTSPLY)
3. BIOCERAMIC SEALER(SMART PASTE BIO)

IRRIGANTS:

Distilled water,3% sodium hypochlorite(PRIME DENTAL PROD UCTS),2% chlorhexidine(Asep-RC),17% EDTA (Den tm ark),2%sterile paper points(Dentsply)

ARMAMENTARIUM

carborundum disk,Laboratory Lathe (sugunas enterprises),Sand paper(3M ESPE.,1000 grit roughness),Glass slab ,Cement spatula,Plastic spatula,kfiles (MANI, INC.,size 15 – 45), Finger spreaders(MANI, INC.,size 15- 30),

SPECIAL EQUIPMENTS:

Goniometer(Easy Drop –KRUSS DSA 204)

SAMPLE SIZE

60decoronated human mandibular premolars were included in this study 21 decoronated human mandibular premolars were longitudinally sectioned to obtain 42 root dentin samples for contact angle measurement.

SAMPLE SELECTION

INCLUSION CRITERIA:

Single rooted mandibular premolars with single root canal, complete root formation and intact root surface.

EXCLUSION CRITERIA:

Teeth with root resorption , Root canal treated teeth , Teeth with root facture

STUDY METHOD

PREPARATION AND GROUPING OF THE SAMPLE FOR CONTACT ANGLE MEASUREMENT

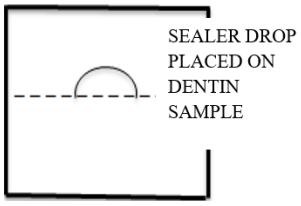
From the stored collection of human mandibular premolars extracted for orthodontic purpose,21 samples were assigned for contact angle measurement. Working length was determined. Chemomechanical preparation of the specimens was done using k files till apical size 30,the specimens were irrigated with initial rinse protocol using 5ml of 3%NaOCl between each instrument,then the specimens were decoronated. The remaining root segments were then split longitudinally through the root canal in buccolingual direction using carborundum disk at low speed under water coolent.Buccal and lingual

surface of the samples were further flattened and smoothened using 1000 grit sand paper to obtain smooth and flat suface for contact angle measurement . The samples were then stored in distilled water until use. In one half of the specimens,canal space of the dentin sections were treated with the following irrigation protocol:5ml of 3%sodium hypochlorite followed by 5ml of 17%EDTA and5ml of 2%chlorhexidineas a final rinse. The dentin sections were rinsed with each irrigant solution for 1min.The samples were gently air dried before contact angle measurement.The samples were then divided into 3 groups based on the experimental sealers used. A total of 14 samples were assigned for each group with 7 samples per subgroup. The contact angles were measured at varied time intervals of 0,5,10, 15,2 0 ,25,30seconds in room temperature.
The groups were divided as follows

MEASUREMENT OF CONTACT ANGLE OF SEALERS ON DENTIN SURFACE

The samples were mounted flat on glass slab for measurement of contact angle.

In each sample 1drop [0.1 ml] of sealer [mixed as per manufacture instructions] was deposited on the dentin surface and then with help of software, images of the drop was captured and analysed to provide values of contact angles at time intervals of 0,5,10,15,20,25,30 seconds after the initial application of the sealers and the values were recorded.



STATISTICALANALYSIS

Kruskal - Wallis and Mann Whitney tests were used for intra and inter group analysis

CONACT ANGLE MEASUREMENT

The contact angle values(in degrees)were measured using goniometer.The observation revealed that the values decreased upto 30 seconds from the time of application in all the groups.

Table 1 shows the mean standard deviation Contact angle value in degrees for all the groups

Table 2 shows inter group p-value comparison for contact angle measurement in the presence of and after the removal of smear layer.
Table3 shows intra group p-value comparison for contact angle measurement.

DISCUSSION

For better penetration of sealer and adhesion into dentinal tubules, the wettability should be high. wettability of sealer is high when the contact angle formed by the sealer on the dentinal surface is low. The moisture content,temperature,the contents present in the dentinal tubules also influences the wettability and sealer penetration.[35]

In the current study contact angle of Zinc oxide eugenol,AH plus and bioceramic sealers were compared. Bioceramic sealer was compared with Zinc oxide eugenol and AH plus sealers because Zinc oxide eugenol sealer has been the gold standard for all the sealers and AH plus sealer is found to be a benchmark amongst the recently introduced resin based sealers.[33]

Middle third of root dentin is used in this study to measure contact angle ,as the dentin tubule density is high in this region.From the literature it is evident that the diameter of open dentinal tubules in the middle third of root dentin was large enough to promote deeper sealer penetration.[3,9,35]Additionally greater forces may be expected to be applied during lateral condensation in this part of dentin when compared to coronal and apical third of root dentin.[35]Apical third of the root section may not be ideal to perform the present study because it contains less tubules with smaller diameter and few of them frequently found obliterated.[3]

Irrigation regime used in this study was similarto the clinical situation as recommended by Zehnder 2006where intermittent rinse with saline was used between the irrigants -3%sodium hypochlorite,17%EDTA and2%Chlorhexidinegluconate.[2,26,32]

It has been proved in the literature that 3% sodium hypochlorite has antimicrobial activity and pulp dissolving capacity, hence it was recommended as a routine irrigant in the irrigation protocol.[1,25]Moreover it also has the collagen dissolving property which enhances the adhesion of smear layer to dentinal wall. Because of this reason subsequent irrigation with 17% EDTA, a chelating agent has been recommended to favour the removal of smear layer.[11,21,29]. Smear layer is defined as layer of irregular,amorphous microcrystalline and organic particle debris that is found on root canal walls and in dentinal tubules after root canal instrumentation.It was first described by Mc comb and smith in 1975. It has two layers,superficial layer and deep layer [smear plugs]where the material is packed into dentinal tubules .Smear layer if present will impede the sealer adhesion and penetration by decreasing the wettability of the sealers and also favoursmicroleakage contributing to endodontic treatment failure.[17]

Final rinse was carried out with2%Chlorhexidinegluconate, a cationic bisbiguanidebecause of its antimicrobial property that acts by abso rbing onto or disrupting the cell wall causing leakage of intracellular components[1], additionaly it exhibits substantivity in the root canal for some time after being used as final rinse[25].It was found from the previous studies that chlorhexidinegluconatewhen used as the final irrigant increases the surface free energy of dentin and this ensures better wetting,flow and penetration of theendodontic sealers[23].

From the literature search, it was observed that clinical significance of the precipitate (HEDTA)2(H2CHX)3formed by the interaction of EDTA with chlorhexidinehas not been established[23].From the wettability data obtained in the previous studies (de Assis,Bohn &Ilie),it was confirmed that this precipitate decreases the wettability effect of EDTA.In the current study dentin was irrigated with each irrigant for one minute[31].

The contact angle was measured for 30seconds (with 5 seconds interval) from the initial application of the sealer to dentin using sessile drop method[30].This represents the clinical time frame necessary to apply a freshly mixed sealer to the root canal wall prior to obturation (Bohn&Ilie 2014). It is worth mentioning here that contact angle measurement was carried out on a flat dried dentinal surface.Contact angle measurements require a solid surface with a clean,rigid and highly smooth surface[2,12].From the previous studies it is known that blot drying dentin with paper points lead to the formation of irregular fluid film on the dentinal surface making the measurement critical[16,20]. Therefore,in this study dentin discs were gentlyair dried to create a standard surface condition while maintaining the hydration in the deeper layers of dentin to facilitate the contact angle measurement.The dentin discs were not totally dessicatedas it is well known that on dessication, the exposed collagen matrix caneven collapse[24].

Any volumetric change could affect the contact angle value.To avoid this,sealer droplet volume was standardized with micropipette.0.1ml of the sealer was used to calculate the contact angle[8,13,15, 18,34].Room temperature was maintained at 25C during the measurement of contact angle in air conditioned room[22]..Roughness created by the instrumentation technique also influences the contact angle values[18,22].To prevent the influence of the instrument used during the canal preparation, dentin surfaces were smoothened using 1000 grit sand paper.

In the present study dentin sections treated with distilled water was used as control group because of the presence of smear layer in it.All the sealers were mixed individually according to manufactures instructions before the measurement.Contact angle was measured in the presence and absence of smear layer in all the groups. As the irrigation regime and time was standardized,it was expected that surface free energy of dentin is the same for all the groups and surface tension of the sealer has major influence on the contact values obtained[4,31].

The contact angle measurement decreased from the time of application to final measurement in all the groups(Table 1,2). This may be related

to the spreading and setting properties of the sealers used in the study[6].The inter and intra group comparison was highly significant expect for inter group comparison between zinc oxide eugenol and AH plus sealers at 10 and 15 seconds in the presence of smear layer. This may be attributed to the long working time of zinc oxide eugenol and AH plus sealers.

The contact angles measurement obtained in the current study reveals that zinc oxide eugenol has the least values followed by AHplus and Bioceramic sealer for both the test and control groups.From the values obtained (Table 1,2), it was clear that absence of smear layer in the test groups has favoured the wettability which was in accordance with the previous studies[8,12,22,28,35].The results were consistent with the surface tension values of the sealers.The contact angle of bioceramic sealer obtained in the study was high compared to other two experimental sealers which could be attributed to the viscosity and spreading properties of the sealers.

Bioceramic sealer sets in the presence of dentinal tubule fluid.It was also found that Bioceramic sealer exhibited better adaptation to dentinal walls . They produce non resorbable hydroxyapatite during setting reaction forming a chemical bond with dentin walls.This may be the cause for the better adaptation and penetration of bioceramic sealer over zinc oxide eugenol and AH plus sealers observed in the current study.

CONCLUSION

Within the limitations of this in-vitro study, it can be concludedthat:

1. Zinc oxide Eugenol sealer showed the lowest **contact angle values**followed by AH Plus and Bioceramic sealer(ZnoEu< AH plus <Bioceramic sealer).
2. For all the sealers, smear layer removal from the canal influenced the reduction of the contact angle

From the current study, it is evident that lower contact angle with smear layer removal improves the wettability of sealers on dentin substrate.The role of other factors like dentin moisture condition and roughness, obturation technique, viscosity, film thickness,capillary action of sealers etc which influence the wettability were not evaluated in the current study. Hence further clinical studies relating to the above factors are required for the identification of ideal sealer to achieve long term endodontic success.

Table1 the mean standard deviation of contact angles in degrees for all the groups

Group	Sealer	Initial Rinse	Storage	Final Rinse
I A	Zinc oxide eugenol	5ml of 3%NaOCl	Distilled water	Distilled water
I B	Zinc oxide eugenol	5ml of 3%NaOCl	Distilled water	5ml of 3%NaOCl 5ml of 17%EDTA &5ml of 2%CHX
IIA	AH plus	5ml of 3%NaOCl	Distilled water	Distilled water
IIB	AH plus	5ml of 3%NaOCl	Distilled water	5ml of 3%NaOCl 5ml of 17%EDTA &5ml of 2%CHX
IIIA	Bioceramic	5ml of 3%NaOCl	Distilled water	Distilled water
III B	Bioceramic	5ml of 3%NaOCl	Distilled water	5ml of 3%NaOCl 5ml of 17%EDTA &5ml of 2%CHX

Table2 Inter group pvalue comparison for contact angle measur ement in the presence and after the removal of smear layer

Seconds	Zinc oxide eugenol and AH plus		Bioceramic and zinc oxide eugenol		Bioceramic and AHplus	
	Smear layer	No smear layer	Smear layer	No smear layer	Smear layer	No smear layer
0	0.033	0.002	0.002	0.002	0.002	0.002
5	0.002	0.001	0.001	0.001	0.001	0.001
10	0.18	0.001	0.002	0.001	0.002	0.001
15	0.68	0.013	0.001	0.002	0.002	0.001
20	0.001	0.002	0.001	0.002	0.001	0.002
25	0.001	0.002	0.001	0.002	0.001	0.001
30	0.001	0.002	0.002	0.002	0.001	0.001

p -value <0.05 significant

Table 3: Intra group comparison for contact angle values p– value

sealer	Subgroups	p – value						
		0sec	5sec	10sec	15sec	20sec	25sec	30sec
Zinc oxide eugenol	Irrigant	.000	.000	.000	.000	.000	.000	.000
	Control							
AH plus	Irrigant	.000	.000	.000	.000	.000	.000	.000
	Control							
Bioceramic	Irrigant	.000	.000	.000	.000	.000	.000	.000
	Control							

p-value<0.05 is significant

REFERENCES

1. Akisue, Viviane S.Tomita, GiulioGavini, and Jose Antonio Poli de Figueiredo.Effect of the combination of sodium hypochlorite and chlorhexidine on dentinal permeability and scanning electron microscopy precipitate observation.Journal of Endodontics 2010;36:847-50. 1
2. Attal, Erik Asmussen, Michael Degrange.Effects of surface treatment on the free surface energy of dentin.Dent Mater 1994;10:259-264. 2
3. Balguerie, Lucas van dresluis, Karen valleys, Marie gurgel –georgelin, and Franck diemer.Sealer penetration and adaptation in the dentinal tubules: A scanning electron Microscopic study.Journal of Endodontics2011;37:1576-79. 3
4. Bohn and N.IlieWetting behaviour of silicone – and resin based root canal sealers. International Endodontic Journal 2014;47:542-549. 4
5. Branstetter and J.A. Von Fraunhofer The physical properties and sealing action of endodontic sealer cements: a review of the literature. Journal ofendodontics 1982; 8: 126-30.5
6. Chadha, Sonalitanej, Mohitkumar, Sumitgupta.An in vitro comparative evaluation of depth of tubular penetration of three resin- based root canal sealers. Journal of conservative dentistry 2012; 15 :18-21. 6
7. Chandra,Padmanabhan Shankar and Rajamani Indira.Depth of Penetration of Four Resin Sealers into Radicular Dentinal Tubules A Confocal Microscopic Study. Journal OfEndodontics 2012;38:1412-1416. 7
8. DeAssis,Maira do Prado and RenataA.Simao.Evaluation of the Interaction between Endodontic Sealers and Dentin treated with Different Irrigant Solutions. Journal OfEndodontics 2011;37:1550-1552. 8
9. De-Deus,Karina Di Giorgi and Rivial Antonio.Interfacial adaptation of Epiphany self – adhesive sealer to root Dentin.Oral surgry, oral medicine, oral pathology,oral radiology and Endodontology2011;111:381-386. 9
10. Dilipkumar,Shafath Ahmed,M.Dhivya.Comparison of efficiency of various cleansing techniques on dentin wettability using contact angle test. Indian Journal OfInterdisiplinary Dentistry 2012; 1:250-254. 10
11. Dotto, Rosanamaria Coelho travassos, Elias pandonormotcy de olivira, Manoeledurado de lima machado and Jose luiz martins.Evaluvation of ethylenediaminetetraacetic acid (EDTA) solution and gel for smear layer removal.Australian Endodontic Journal 2007;33: 62- 65. 11
12. Extrand. Contact angles and their hysteresis as a measure of liquid –solid Adhesion.Langmuir 2004;20:4017-4021. 12
13. Good and M.N.Koo.The Effect of Drop Size on Contact Angle.Journal of Colloid and Interface Science 1979;71:283-292.13
14. Hu,Junqi Ling and Yan Gao.Effects of Irrigation Solutions on Dentin Wettability and Roughness.Journal OfEndodontics 2010;36:1064-1067. 14
15. Iwanami,TakatomoYoshika, Mitsuhiro Sunakawa,ChihiroKobayash.Spreading of root canal irrigants on root dentine.Australian Endodontic Journal 2007;33:66-72. 15
16. Khorasani, H.Mirzadeh,Z. Kermani.Wettability of porous polydimethylsiloxane surface morphology Study.Applied Surface Science 2005;242:339-345. 16
17. Kokkas,Asteriosch.Boutsioukis,LeonidasP.Vassiliadis and Christos K.Stavrianos. The influence of smear layer on the Dentinal Tubule Penetration Depth by Three Different Root Canal Sealers:An In Vitro Study. Journal OfEndodontics 2004;30:100-102. 17
18. Kontakiotis,Giorgos N. Tzanetakis and AlexiosL.Loizides.A comparative study of contact angles of four different root canalSealers. Journal OfEndodontics 2007;33(3):299-302. 18
19. Lee,Michael C.Williams,Jean J.Camps and David.Pashley.Adhesion of Endodontic Sealers to Dentin and Gutta-Percha. Journal OfEndodontics 2002;28:684-688. 19
20. Nagas, M.OzgurUyanik, AyhanEymiril, Cehereli ,PekkaK.Vallittu,Lippo V.J. Lassila and VeliDurmaz. Dentin Moisture Conditions Affect the Adhesion of Root Canal Sealers. Journal OfEndodontics 2011:1-5. 20
21. Nakashima and RintaroTerata. Effect of pH modified EDTA solution to the properties of Dentin.Journal OfEndodontics 2005;31;47-49. 21
22. , Danielle Ferreira de Assis,Brenda P.F.A Gomes andRenataA.Simao.Effect of disinfectant solutions on the surface free energy and wettability of filling material. Journal OfEndodontics 2011;37:980-982. 22
23. Rasimick, Michelle Nekich,Megan , Barry L. Musikant, and Allan S. Deutsch.Interaction between Prado chlorhexidinedigluconate and EDTA.Journal of endodontics2008; 34:1521-3. 23
24. Rosales, G.W. Marshall, S.J. Marshall, L.G.Watanabe ,M. Toledano, M.A. Cabrerizo and R.Osorio.Acid –etching and Hydration influence on dentin roughnesss and Wettability.J Dent Res 1999;78:1554-1559. 24
25. Rosenthal, LaizSpanberg and Kamran Safari .Chlorhexinesubstantivity in root canal dentin. Oral surgery, oral medicine, oral pathology,oral radiology andEndodon tology2004 ; 98:488-92. 25
26. Saleh,I.E.Ruyter,M.Haapasalo and D.Ostavik. The effects of den tine pretreatment on the adhesion of root –canal sealers.International Endodontic Journal 2002;35:859-866. 26
27. Sen, B. Piskin& N.Baran.The effect of tubular penetration of root canal sealers on dye Microleakage. International Endodontic journal -1996; 29: 23-28. 27
28. Shafrin and William A.Zisman.Constitutive relations in the wetting of low energy surface and the theory of the retraction method of preparing monolayers.1960;64:519-524. 28
29. Shahravan, AliakbarHaghdoost, AlirezaAdl, Hessamrahimi and Fahimehsh adifa r.Effect of smear layer on sealing abiity of canal obturation : A systematic review and meta – analysis.Journal of endodontics2007; 33:96-105. 29
30. Shang,Markus Flurry ,James B.Harsh,Richard L.Zollars.Comparison of different methods to measure contact angles of soil colloidsJournal of Colloid and Interface Science 2008;328:299-307. 30
31. Teixeria,M.C.S.Felippe and W.T.Felippe. The effect of application time of EDTA and NaOCl on intracanalsmearlayer removal :an SEM analysis. International Endodontic Journal 2005;38:285-290. 31
32. Tuncer and SafaTuncer. Effect of Different FinalIrrigation Solutions on Dentinal Tubule

Penetration Depth and Percentage of Root Canal Sealer Journal Of Endodontics 2012;38:860-863. 32
33. Tyagi,Priyesh Mishra,ParimalaTyagi.Evolution of root canal sealers: an insight story. European Journal Of General Dentistry 2013;2:199-218. 33
34. Vafei,M.Z.Podowski.Analysis of the realationshipbetween liquid droplet size and contact angle. Advances in colloid and Interface Science 2005;113:133-146. 34
35. Vassiliadis,Spyros A. Sklavounos and Christos K.Stavrianos. Depth of Penetration and Appearance of Grossman Sealer in the Dentinal Tubules:An In Vivo Study.Journal OfEndodontics 1994; 20: 373-376. 35
36. Wu,A.J.Degee and P.R.Wesselink.Effect of tubule orientation in the cavity wall on the seal of dental filling materials :an in vitro study.International Endodontic Journal 1998;31:326-332. 36

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COMPARATIVE EVALUATION OF LATERAL PENETRATION DEPTH OF ROOT CANAL SEALERS UNDER CONFOCAL LASER SCANNING MICROSCOPE

Dental Science

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ABSTRACT

AIM: This in vitro study was conducted to evaluate the lateral depth of penetration of zinc oxide eugenol (with grossman formula),AH Plus (Dentsply)and Bioceramic sealers(smart paste bio) into the dentinal tubules using confocal laser scanning microscope.

MATERIALS AND METHODS: Thirty nine recently extracted single-rooted mandibular premolars were taken. The working length was determined. Chemomechanical preparation done using k file to apical size 30. Between each instrument, the canal was irrigated with 5ml of 3%NaOCl.Three teeth were reserved as control and were not subjected to any procedure.In the rest of the samples, the canal were irrigated with 5ml of 3%sodium hypochlorite followed by 5ml of 17%EDTA and as a final rinse,5ml of 2%chlorhexidinewas used.Then the canals were dried with sterile paper points.The samples were divided into 3 groups (12 samples per group) based on the sealers used and were obturated with guttapercha cones using lateral condensation technique. All the sealers were mixed individualy with rhodamineB dye before obturation. Sections were prepared from middle third of the root for observation under confocal laser microscope

RESULTS: Bioceramic sealer(smart paste bio) resulted in greater sealer penetration into dentinal tubules compared to AH plus and zinc oxide eugenol sealers.

KEYWORDS

rhodamineB dye, sealer penetration, confocal laser scanning microscope

INTRODUCTION

Endodontic success is achieved by three dimensional hermetic seal in the main canal and the lateral dentinal tubules[1].In india most commonly used system to obturate root canal space is lateral condensation or the single cone technique.In both the techniques guttapercha is used with different root canal sealers available for obturation. Regardless of the obturation techniques gutta percha has no adhesive property[2]Good sealer penetration into the dentinal tubules with tight adaptation is therefore mandatory to prevent microleakage ,reinfection and aid in endodontic success[6,19,20].Various sealers exhibit different physiochemical properties which influences the penetration depth and interfacial adaptation[3].Untill today no endodontic sealer has proven to achieve all the properties of ideal endodontic sealer.In this study we have evaluated the penetration depth of Zinc oxide eugenol, AH plus(Dentsply) with newer endodontic sealer ,Bioceramic sealer(smart paste bio) under confocal laser scanning microscope.

METHODOLOGY

Thirty nine recently extracted single-rooted mandibular premolars were taken .The working length was determined. Chemomechanical preparation done using k file to apical size 30. Between each instrument, the canal was irrigated with 5ml of 3%NaOCl.Three teeth were reserved as control and were not subjected to any procedure.In the rest of the samples, the canal were irrigated with 5ml of 3%sodium hypochlorite followed by 5ml of 17%EDTA and as a final rinse, 5ml of 2%chlorhexidinewas used.Then the canals were dried with sterile paper points.The samples were divided into 3 groups (12 samples per group) based on the sealers used and were obturated with guttapercha cones using lateral condensation technique. All the sealers were mixed individualy with rhodamineB dye before obturation. Sections were prepared from middle third of the root for observation under confocal laser microscope .

The specimens were grouped as follows ,Group1:Zinc Oxide Eugenol sealer, Group 2:AH Plus sealer,Group3:Bioceramic sealer The samples were mounted flat on glass slab and viewed under confocal laser scanning microscope to determine the sealer penetration depth into the dentinal tubules [fig 1].

RESULTS

Sealer penetration depth was measured in micrometer using confocal laser microscope.The values are given in Table 1.

Table 2 shows the mean penetration depth of the experimental sealers.
Table 3 shows sealer penetration into dentinal tubules(inter group comparison)

STATISTICALANALYSIS

For sealer penetration measurement,One way analysis of difference (ANOVA) was used to study the overall mean within groups. The mean and standard deviation were calculated. The p- value showed significant difference among the groups [Table 3].

DISCUSSION

Quantification of sealer penetration depth was carried under confocal laser scanning microscope.

It has no special specimen processing step and the observations can be made close to normal conditions.Moreover CLSM works on noninvasive approach and has less potential to produce technique artifacts[18].CLSM offers a lot of advantage over SEM (scanning electron microscope), these include the ability to control depth of field, elimination or reduction of background information away from the focal plane (which leads to image degradation), and the capability to collect serial optical sections even from thick specimens[6]Scanning electron microscopic observation requires additional gold sputtering of the samples which is time consuming[18].

All the sealers were mixed according to the manufactures instructions and labeled with RhodamineB isothiocyanate fluorescent dye to facilitate the observation. Since there was no specific method devised to incorporate this dye into the dentinal sealers in the literature,[6,9]in this current study to maintain the standard amount of dye mixed,10 parts of sealer was manually mixed with 1 part of dye powder so that approximate concentration of 0.1% of the dye was obtained.The quantity of dye mixed with the sealer was negligiblethat it will not alter the physio chemical properties of sealer.[6,9]

Irrigation regime used in this study was similarto the clinical situation as recommended by Zehnder 2006where intermittent rinse with saline was used between the irrigants -3%sodium hypochlorite,17%EDTA and2%Chlorhexidinegluconate.[3,11,12,13]

Lateral compaction technique was used to obturate the samples as adding a core of laterally condensed guttapercha gives better control of obturation and helps to press the sealer into the dentinal tubules. [5,9,14,15] Three teeth served as negative control.The control teeth were neither irrigated with the standardised irrigation protocolnor obturated. Sections from the middle third of the root was used for observation in this study.[7,16]Cross section of the root was used in this study because in longitudinal sections overall penetration of the sealers into the dentinal tubules cannot be viewed.[10]

Zinc oxide eugenol and AHplus sealers showed a minimum penetration depth of 91.20um and 71.56 um and a maximum penetration depth of 257.55um and 158.66 um respectively. The penetration depth of Bioceramic sealer obtained from this study shows a minimum depth of 238.59um and a maximum depth of 409.67 um. The intra and inter group comparison was significant [Table3].

The results of the study show that the Bioceramic sealer has equal and highest penetration depth compared to other two sealers.Additional observation was that bioceramic sealer showed high penetration depth in all the samples.

Zinc oxide eugenol sealer showed deeper penetration (159.21±95.23 micrometer) than AH plus sealer (115.49±43.17micrometer) in contrast to previous studies by Chadha et al, Chandra et al and kokkas[5,6,8].In those studies it was reasoned out that capillary action played a major role in the deeper penetration of AH plus sealer.[5,6,8]White et al found that both Zinc oxide eugenol and AH plus sealer had equal penetration[6]. On the other hand, Vassiliadis et al in his landmark in vivo study on the penetration of zinc oxide eugenol sealer found that the sealer penetrated to a maximum of 900micrometer [14].Silicone oil being one of the components of AH plus sealer may be responsible for its hydrophobicity. This may give the possible explanation for the minimum penetration depth of AH plus sealer[5,15]. In thecase of zinc oxide eugenol sealer, its penetration was profoundly influenced by the filling technique,particle size and flow[10,15].Other possible reasons the different result between zinc oxide eugenol and AH plus sealer penetration depth in the current study may be possibly due to film thickness, flow and viscosity of the sealer mix used in the obturation[4,5].

Bioceramic sealer sets in the presence of dentinal tubule fluid.It was also found that Bioceramic sealer exhibited better adaptation to dentinal walls and penetration into dentin tubules (323.35±86.32 micrometer). This study results coincides with Roula El Hachem[17]. They produce non resorbable hydroxyapatite during setting reaction forming a chemical bond with dentin walls.This may be the cause for the better adaptation and penetration of bioceramic sealer over zinc oxide eugenol and AH plus sealers observed in the current study.

CONCLUSION

1. Bioceramic sealer displayed better tubular penetration followed by Zinc oxide Eugenol and AH Plus(Bioceramic sealer >ZnOEu> AH plus).
2. For all the sealers, smear layer removal from the canal increased the tubular penetration.

Table 1: sealer penetration depth into dentinal tubules in micrometer

Sample no	Group 1(zinc oxide eugenol sealer)	Group 2 (AH plus Sealer)	Group 3 (Bioceramic Sealer)
1.	97.36	87.44	264.67
2.	91.2	97.79	272.23
3.	94.08	84.62	238.59
4.	111.4	72.32	389.88
5.	110.61	71.56	296.57
6.	181.97	152.26	400.32
7.	142.67	128.21	409.67
8.	148.68	147.08	387.83
9.	244.34	134.78	320.51
10.	254.44	158.66	275.29
11.	257.55	155.61	342.45
12.	176.21	95.5	282.15

Three teeth assingned as control (to check for smear layer removal) was neither subjected to irrigation protocol nor obturated.

Table 2: Sealer penetration into dentinal tubules

SEALERS	MEAN±STANDARD DEVIATION
ZINC OXIDE EUGENOL	159.21±95.23
AH PLUS	115.49±43.17
BIOCERAMIC SEALER	323.35±86.32

Table 3: Sealer penetration into dentinal tubules inter group comparison

SEALER	Mean ± Standard Deviation	p-value	Overall p - value
Zinc Oxide Eugenol	159.21±95.23	0.369	0.000
Ahplus	115.49±43.17		
Bioceramic	323.35±86.32	0.000	
Zinc Oxide Eugenol	159.21±95.23		
Bioceramic	323.35±86.32	0.000	
Ahplus	115.49±43.17		

p-value<0.05 is significant

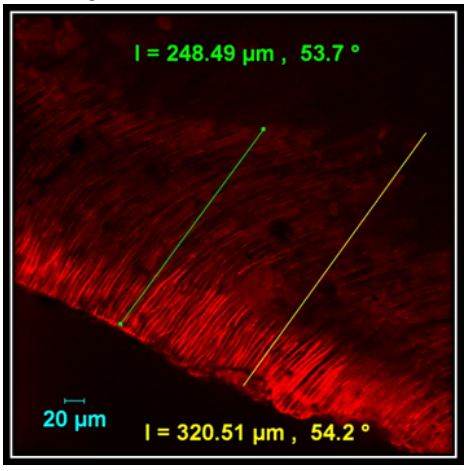


Fig 1:sealer Penetration Depth Under Confocal Laser Microscope

REFERENCES

1. Ingle J, Newton C, West JG, et al. Obturation of the radicular space. In: Ingle J, Bakland L, eds. Endodontics. 2002; 5th ed. Hamilton Ontario, Canada: B C Decker Inc. Ltd. 571–668.
2. Endodontists AAO: Guide to Clinical Endodontics, 2004; Ed 4. Chicago, American Association of Endodontists.
3. Attal, Erik Asmussen, Michael Degrange.Effects of surface treatment on the free surface energy of dentin.Dent Mater 1994;10:259-264.
4. Balguerie, Lucas van dresluis, Karen valleys, Marie gurgel –georgelin, and Franck diemer. Sealer penetration and adaptation in the dentinal tubules: A scanning electron Microscopic study.Journal of Endodontics2011;37:1576-79.
5. Chadha, Sonalitanej, Mohitkumar, Sumitgupta.An in vitro comparative evaluation of depth of tubular penetration of three resin- based root canal sealers. Journal of conservative dentistry 2012; 15 :18-21.
6. Chandra,Padmanabhan Shankar and Rajamani Indira.Depth of Penetration of Four Resin Sealers into Radicular Dentinal Tubules A Confocal Microscopic Study. Journal OfEndodontics 2012;38:1412-1416.
7. De-Deus,Karina Di Giorgi and Rivial Antonio.Interfacial adaptation of Epiphany self – adhesive sealer to root Dentin.Oral surgry, oral medicine, oral pathology,oral radiology and Endodontology2011;111:381-386.
8. Kokkas,Asteriosch.Boutsioukis,LeonidasP.Vassiliadis and Christos K.Stavrianos. The influence of smear layer on the Dentinal Tubule Penetration Depth by Three Different Root Canal Sealers:An In Vitro Study. Journal OfEndodontics 2004;30:100-102.
9. kuci,TayfunAlacam, Ozeryavas, ZeynapErgul –ulger and Guvenkayaoglu. Sealer penetration into dentinal tubules in the presence or absence of smear layer: A confocal laser scanning microscopic study. Journal of Endodontics, 2014; 40: 1627-8.
10. Mamootil and H.H. Messer. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo.International Endodontic Journal2007;40:873-881.
11. Sadeghi and Tania Safari. The effect of different irrigation regimes on the apical seal of two endodontic sealers.Iranian Endodontic Journal 2008;3:119-22.
12. Saleh,I.E.Ruyter,M.Haapasalo and D.Ostavik. The effects of den tine pretreatment on the adhesion of root –canal sealers.International Endodontic Journal 2002;35:859-866.
13. Tuncer and SafaTuncer . Effect of Different FinalIrrigation Solutions on Dentinal Tubule Penetration Depth and Percentage of Root Canal Sealer.Journal Of Endodontics 2012;38:860-863.
14. Vassiliadis,Spyros A. Sklavounos and Christos K.Stavrianos.Depth of Penetration and Appearance of Grossman Sealer in the Dentinal Tubules:An In Vivo Study.Journal OfEndodontics 1994; 20: 373-376.
15. Weis, P.Parashos and H.H.Messer. Effect of obturation technique on the sealer cement thickness and dentinal tubule penetration.International Endodontic Journal 2004;37:653-663.
16. Wu,A.J.Degee and P.R.Wesselink. Effect of tubule orientation in the cavity wall on the seal of dental filling materials :an in vitro study.International Endodontic Journal 1998;31:326-332.
17. Roula El Hachem,Issam Khalil,Guy Le Brun et al. Dentinal tubule penetration of AH Plus, BC Sealer and a novel tricalcium silicate sealer: a confocal laser scanning microscopy study Clinical Oral InvestigationsApril 2019, Volume 23, Issue 4, pp 1871–1876.
18. de –Deus, EduradoDiogoGurgel –Filho, Claudio maniglia,- Ferreira, Taubycoutinho-filho. Influence of the filling technique on depth of tubular penetration of root canal sealers: a scanning electron microscopy study.BrazJOralSci 2004; 3:433-38
19. Branstetter and J.A. Von Fraunhofer. The physical properties and sealing action of endodontic sealer cements: a review of the literature.Journal ofendodontics 1982; 8: 126-30.
20. Lee,Michael C.Williams,Jean J.Camps and David.Pashley.Adhesion of Endodontic Sealers to Dentin and Gutta-Percha. Journal OfEndodontics 2002;28:684-688.

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Review Article

Physiatrist's Assessment and Management of Diabetic Foot

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ABSTRACT

The incidence of diabetes is increasing day by day in India. By the year 2030, India will be the world capital of diabetes. Foot ulcerations, infections and Charcot neuropathic osteoarthropathy are three serious foot complications of diabetes mellitus that can too frequently lead to gangrene and lower limb amputation. Although not all foot complications can be prevented, dramatic reductions in their frequency have been achieved through the implementation of team approach to diabetic patient management. A multidisciplinary team approach is recommended for individuals with diabetic foot ulcers. Foot ulcers and wound care may require care by a podiatrist, orthopedic surgeon, vascular surgeon and physiatrist experienced in the management of individuals with diabetes. A Physiatrist is a medical doctor who specializes in physical medicine and rehabilitation. Physiatrists diagnose and treat both acute and chronic pain, and specialize in a variety of nonsurgical treatment for the musculoskeletal system. Initial screening for peripheral arterial disease (PAD) should include a history of claudication and an assessment of pedal pulse. Ankle brachial index (ABI) to be obtained in as many patients with PAD being asymptomatic. The neurologic examination recommended is designed to identify loss of protective sensation by simple clinical tests like use of a 10 G monofilament, tests of pinprick sensation, ankle reflex assessment, vibration testing using a 128HZ tuning fork and testing vibration perception threshold with a Biothesiometer. Patients with diabetes and high risk foot conditions should be educated about proper care of the foot and the selection of appropriate footwear. Plantar pressure is measured using podia scan and areas of high plantar pressure are identified. Footwear which offers pressure relief over these high plantar pressure areas is then prescribed. Interim footwear to be used by the patients who have forefoot and hind foot ulcers which require off loading the ulcer areas till the ulcer is healed.

Key words: Ankle brachial Index, Biothesiometry, Podia scan, Ideal diabetic footwear

INTRODUCTION

The incidence of diabetes is increasing day by day in India. In India, diabetic foot ulcers affect 15% of diabetics during their lifetime. Evidence from published literature shows 100,000 leg amputations are done per year due to diabetes related problems which results in an expense of approximately $1,960 for complete treatment of diabetic foot ulcers. Out of 62 million diabetics in India, 25% develop diabetic foot ulcers, of which 50% become infected, requiring hospitalization while 20% need amputation. Diabetic foot ulcers contribute to approximately 80% of all non-traumatic amputations in India, annually. [(1)]

The risk factors for the diabetic foot ulceration include vascular disease, peripheral neuropathy, limited joint mobility, foot deformities, abnormal foot pressures, minor trauma, history of ulceration or amputation, and impaired visual acuity. Early recognition and management of risk factors can prevent or delay adverse outcomes and the goal of the Physiatrist is to prevent limb loss among diabetics and maintain their quality of life.

FOOT CARE RECOMMENDATIONS [(2)]

- Perform a comprehensive foot evaluation at least annually to identify risk factors for ulcers and amputations.
- Patients with evidence of sensory loss or prior ulceration or amputation should have their feet inspected at every visit.
- Obtain a prior history of ulceration, amputation, Charcot foot, angioplasty or vascular surgery, cigarette smoking, retinopathy, and renal disease and assess current symptoms of neuropathy (pain, burning, numbness) and vascular disease (leg fatigue, claudication).
- The examination should include inspection of the skin, assessment of foot deformities, neurological assessment (10-g monofilament testing with at least one other assessment: pinprick, temperature, vibration), and vascular assessment including pulses in the legs and feet.
- Patients with symptoms of claudication or decreased or absent pedal pulses should be referred for ankle-brachial index and for further vascular assessment as appropriate.
- A multidisciplinary approach is recommended for individuals with foot ulcers and high-risk feet (e.g., dialysis patients and those with Charcot foot or prior ulcers or amputation).
- Refer patients who smoke or who have histories of prior lower-extremity complications, loss of protective sensation, structural abnormalities, or peripheral arterial disease to foot care specialists for ongoing preventive care and lifelong surveillance.
- Provide general preventive foot self-care education to all patients with diabetes.
- The use of specialized therapeutic footwear is recommended for high-risk patients with diabetes including those with severe neuropathy, foot deformities, or history of amputation.

VASCULAR EXAMINATION:

Peripheral vascular disease rarely leads to foot ulcerations directly. However, once ulceration develops, arterial insufficiency will result in prolonged healing and imparts an elevated risk for amputation. Vascular assessment would include inspection and assessment of pedal pulses. Initial screening for PAD should include a history of decreased walking speed, leg fatigue, claudication, and an assessment of the pedal pulses. Ankle-brachial index testing should be performed in patients with symptoms or signs of PAD. [(3)] The Ankle Brachial Index (ABI) is the ratio of the blood pressure in the lower legs to the blood pressure in the arms. Compared to the arm, lower blood pressure in the leg is an indication of blocked arteries (peripheral vascular disease). The ABI is calculated by dividing the systolic blood pressure at the ankle by the systolic blood pressures in the arm. The following can be used as a guide to interpreting results of ankle brachial index [(4)]

1. Normal ABI ranges from **1.0 — 1.4**
    a. Pressure is normally higher in the ankle than the arm.
2. Values **above 1.4** suggest a non compressible calcified vessel.
    a. In diabetic or elderly patients, the limb vessels may be fibrotic or calcified. In this case, the vessel may be resistant to collapse by the blood pressure cuff, and a signal may be heard at high cuff pressures. The persistence of a signal at a high pressure in these individuals results in an artifactually elevated blood pressure value.
3. A value **below 0.9** is considered diagnostic of PAD.
4. Values **less than 0.5** suggests severe PAD.
    a. Individuals with such severe disease may not have sufficient blood flow to heal a fracture or surgical wound; they should be considered for revascularization if they have a non-healing ulcer.

Figure 1: Interpretation of the Ankle Brachial Index

Refer patients with significant symptoms or a positive ABI for further vascular assessment and consider exercise, medications, and surgical options. Patients

with ABI 0.6 to 0.9 will be taught Buerger's exercise and prescribed vasodilators like Cilostazol 100 mg twice daily until symptoms improve. Cilostazol is a quinolinone derivative that inhibits specific cellular phosphadiesterases, which cause arterial vasodilation and inhibition of platelet function and makes it a valuable as a therapy of intermittent claudication and as a means of secondary prevention of stroke. [(5)] Patients with ABI 0.5 – 0.8 and below 0.5 to undergo non-invasive arterial studies like Doppler study and referred for revascularization procedures to prevent limb loss because arterial perfusion is a vital component for ulcer healing.

NEUROLOGICAL EXAMINATION:

The neurologic examination recommended is designed to identify loss of protective sensation (LOPS) rather than early neuropathy. The clinical examination to identify LOPS is simple and requires no expensive equipment. Five simple clinical tests (use of a 10-g monofilament, vibration testing using a 128-Hz tuning fork, tests of pinprick sensation, ankle reflex assessment, and testing vibration perception threshold with a biothesiometer), are considered useful in the diagnosis of LOPS in the diabetic foot.

Use of the vibration perception threshold (VPT) is a simple way of detecting large-fiber dysfunction, thus identifying individuals with diabetes at risk of ulceration. [(6-7)] The Bio-thesiometer is an instrument designed to measure simply and accurately the threshold of appreciation of vibration in human subjects. It is essentially an "electrical tuning fork" whose amplitude may be set to any predetermined level or whose amplitude may be gradually increased until the threshold of vibratory sensation is reached. The Bio-thesiometer is not only far superior to a tuning fork in accuracy, but will detect neurological changes that are not disclosed with a tuning fork. The results of the vibration perception threshold (VPT) testing are interpreted as follows: [(7)]

Table 1: Interpretation of vibration perception threshold values:

Vibration Pressure Threshold	Interpretation/ Grading for risk of developing foot ulcer
< 15 V	Normal
15 – 20 V	Mild
20 -25 V	Moderate
> 25 V	Severe

The risk of developing a neuropathic ulcer is much higher if a person has a Bio-thesiometer reading greater than 30-40 volts.

Patients with diabetes and high-risk foot conditions should be educated regarding their risk factors and appropriate management. Patients at risk should understand the implications of the LOPS, the importance of foot monitoring on a daily basis, the proper care of the foot, including nail and skin care, and the selection of appropriate footwear. Patients' understanding of these issues and their physical ability to conduct proper foot surveillance and care should be assessed. Patients with visual difficulties, physical constraints preventing movement, or cognitive problems that impair their ability to assess the condition of the foot and to institute appropriate responses will need other people, such as family members, to assist in their care. Patients who are found to have diabetic neuropathy are treated with antidepressants, anticonvulsants, topical agents, opiate analgesics and vitamin B12 supplementation.

PLANTAR PRESSURE MEASUREMENT:

High plantar foot pressure has been identified as a significant risk factor for ulcerations. A large number of studies have suggested that plantar pressures are high in people with diabetic peripheral neuropathy (DPN) and in people with a history of diabetic foot ulcers. [(8-17)] Measurement of these foot pressures is possible utilizing a variety of modalities. Several computerized systems can provide quantitative measurement of plantar foot pressure. These measurements are important in identifying areas of the foot at risk for ulceration and possibly in the evaluation of orthotic adjustments. Podia scan, which consists of

Harris mat, special scanner copier and printer, image analysis software and patient reporting software is not as sophisticated, but can provide a qualitative measurement of plantar foot pressures, both dynamic and static. When the patient steps over the Harris mat, greater local foot forces deposit more ink and detects the area of greatest concern for ulceration. Thus the Harris mat produces a weight bearing imprint of the foot and the podia scan produces qualitative multi color output and makes the pressure reading easier.

DIABETIC FOOTWEARS:

People with neuropathy or evidence of increased plantar pressure (e.g., erythema, warmth, callus, or measured pressure) may be adequately managed with well-fitted walking shoes or athletic shoes that cushion the feet and redistribute pressure. [(18)] Proper footwear is an important part of an overall treatment program for people with diabetes. Ideal diabetic footwear will relieve areas of excessive pressure, reduce shock and shear, accommodate, stabilize and support deformities and accommodate shoe inserts. There are two types of therapeutic shoes for diabetes. They are depth shoes and custom molded shoe. Depth shoe provides a minimum of 3/16" (4.7mm) of additional depth used to accommodate custom – molded or customized inserts. People with bony deformities (e.g., hammertoes, prominent metatarsal heads, bunions) may need extra-wide or depth shoes. Custom–molded shoe is constructed over a positive model/mold of a patient's foot. People with extreme bony deformities (e.g., Charcot foot) who cannot be accommodated with commercial therapeutic footwear may need custom-molded shoes. Footwear which are mass produced and then modified to fit an individual is called customized footwear. Medically indicated temporary footwear that permits ambulation and facilitates wound healing or post trauma recovery for the involved foot are called interim footwear. There are fore foot pressure relieving foot wear , hind foot pressure relieving footwear and rocker bottom footwear for relieving pressures at forefoot, hind foot and mid foot respectively.

PREVENTION:

The following are the important attributes of a foot ulcer prevention program undertaken within the framework of the multidisciplinary team.

- PODIATRIC CARE

Regular visits, examinations, and foot care
Risk assessment
Early detection and aggressive treatment of new lesions

- PROTECTIVE SHOES

Adequate room to protect from injury; well cushioned walking sneakers, extra depth, custom molded shoes
Special modifications as necessary

- PRESSURE REDUCTION

Cushioned insoles, custom orthoses, padded hosiery
Pressure measurements-computerized or Harris Mat

- PROPHYLACTIC SURGERY

Correct structural deformities- hammertoes, bunions, Charcot
Prevent recurrent ulcers over deformities
Intervene at appropriate time

- PREVENTIVE EDUCATION

Patient education – need for daily inspection and necessity for early intervention
Physician education – significant foot lesions, importance of regular foot examination, and current concepts of diabetic foot management.

CONCLUSION

Ulceration, infection, gangrene and lower extremity amputation are complications often encountered in patients with diabetes mellitus. These often result in extensive morbidity, repeated hospitalizations and mortality to the patient. All diabetic foot complications cannot be prevented but it is indeed possible to dramatically reduce their incidence through appropriate management and prevention program. The multidisciplinary team approach to diabetic foot disorders is the optimal method to achieve favorable rates of

limb salvage in the high risk diabetic patient. Foot care programs emphasizing preventive management can reduce the incidence of foot ulceration through modification of self car practices, appropriate evaluation of risk factors, and the formulation of treatment protocols aimed at early intervention, limb prevention and the prevention of new lesions. The physiatrist should play an integral role in this scheme, providing ongoing surveillance, education and management of new or impending lesions.

REFERENCES

1. Ghosh P, Valia R. Burden of Diabetic Foot Ulcers in India: Evidence Landscape from Published Literature. Value in Health. 2017 Oct 1;20(9):A485.
2. Cefalu WT, Berg EG, Saraco M, Petersen MP, Uelmen S, Robinson S. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes-2019. Diabetes Care. 2019 Jan 1;42:S124-38.
3. Aboyans V, Criqui MH, Abraham P, Allison MA, Creager MA, Diehm C, Fowkes FG, Hiatt WR, Jönsson B, Lacroix P, Marin B. Measurement and interpretation of the ankle-brachial index: a scientific statement from the American Heart Association. Circulation. 2012 Dec 11;126(24):2890-909.
4. Cameron HA, Waller PC, Ramsay LE. Drug treatment of intermittent claudication: a critical analysis of the methods and findings of published clinical trials, 1965-1985. Br J Clin Pharmacol 1988; 26: 569-76
5. Boulton AJ, Vinik AI, Arezzo JC, et al. Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care. 2005; 28(4): 956-962.
6. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010;33(10):2285-2293.
7. Tchen P, Chiu HC. Vibratory perception threshold in diabetic neuropathy. J Formos Med Assoc. 1990;89(1):23-29.
8. Veves A, Murray HJ, Young MJ, Boulton AJM. The risk of foot ulceration in diabetic patients with high foot pressure: a prospective study. Diabetologia. 1992;35:660–663.
9. Frykberg RG, Lavery LA, Pham H, Harvey C, Harkless L, Veves A. Role of neuropathy and high foot pressures in diabetic foot ulceration. Diabetes Care. 1998;21: 1714–1719.
10. Pham H, Armstrong DG, Harvey C, Harkless LB, Giurini JM, Veves A. Screening techniques to identify people at high risk for diabetic foot ulceration: a prospective multicenter trial. Diabetes Care. 2000;23:606–611.
11. Hokkam EN. Assessment of risk factors in diabetic foot ulceration and their impact on the outcome of the disease. Prim Care Diabetes. 2009;3: 219–224.
12. Stokes IA, Faris IB, Hutton WC. The neuropathic ulcer and loads on the foot in diabetic patients. Acta Orthop Scand. 1975;46: 839–847.
13. Ctercteko GC, Dhanendran M, Hutton WC, Le Quesne LP. Vertical forces acting on the feet of diabetic patients with neuropathic ulceration. Br J Surg. 1981;68:608–614.
14. Waaijman R, Bus SA. The interdependency of peak pressure and pressure-time integral in pressure studies on diabetic footwear: no need to report both parameters. Gait Posture. 2012;35:1–5.
15. Fernando ME, Crowther RG, Pappas E, Lazzarini PA, Cunningham M, Sangla KS, Buttner P, Golledge J. Plantar pressure in diabetic peripheral neuropathy patients with active foot ulceration, previous ulceration and no history of ulceration: a meta-analysis of observational studies. PLoS ONE. 2014; 9:e99050.
16. Boulton AJ, Hardisty CA, Betts RP, Franks CI, Worth RC, Ward JD, Duckworth T. Dynamic foot pressure and other studies as diagnostic and management aids in diabetic neuropathy. Diabetes Care. 1983;6:26–33.
17. Armstrong DG, Peters EJ, Athanasiou KA, Lavery LA. Is there a critical level of plantar foot pressure to identify patients at risk for neuropathic foot ulceration? J Foot Ankle Surg. 1998;37:303–307.
18. Fonseca VA, Pendergrass M, McDuffie RH, Fonseca VA. Diabetes in clinical practice. New Orleans: springer; 2010.

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Original Research Article

Morbidity profile of truck drivers: a South Indian cross-sectional study

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ABSTRACT

Background: Recent studies have shown that workers on the transport industry are more predisposed to cardiovascular morbidity and other health morbidities due to their improper diet and sedentary lifestyle. This study was aimed at assessing the common morbidity profile among the truck drivers and associated demographic and clinical parameters considered relevant to the development of cardiovascular disease.
Methods: This was a cross sectional study conducted among 175 truck drivers aged 18-60 years at the Walayar check post, Coimbatore, southern India. After getting informed consent, a detailed physical examination was conducted. Socio demographic information was also elicited.
Results: It was observed that 50% of the truck drivers were overweight and obese. Hypertensives were 41.1% and diabetics were 12%. About 27.4% had raised systolic blood pressure (BP) and 29.1% had raised diastolic BP. Low back pain was observed in 34.3%, hip pain in 24.6%, knee pain in 9.7%, shoulder pain in 5.4%, diminished vision in 4.6%, giddiness in 2.3% and hearing defects in 1.1% of the truck drivers.56.6% were current smokers and 66.3% were taking alcohol consumption more than 4 times a month. Older age was found to be a significant independent predictor [odds ratio OR 1.04 (95% CI 1.03-1.05)] for the total number of cardio vascular disease morbidities.
Conclusions: We observed a higher morbidity among the truck drivers and observed that periodic health appraisals are needed.

Keywords: Truck driver, Morbidity, Hypertension

INTRODUCTION

Non communicable diseases are considered as one of the major health and development challenges of 21st century. The social and economic effect of increased morbidity and mortality associated with cardiovascular disease is a great challenge that has to be encountered in these decades.[1,2] Recent studies done in other countries have shown that workers in the transport industry are more predisposed to cardiovascular morbidity due to their improper diet and sedentary behavior and unhealthy life styles. The commonly seen morbidities reported among truck drivers were diabetes mellitus, obesity, hypertension and heart disease.[3-6] However, most of the research done in India among the truck drivers, have focused only on their sexual health. To the extent of our knowledge, very few studies were done in India to assess other co morbidities like cardiovascular morbidity profile. Many studies done in other parts of the country have reported that professional truck drivers and other road transport professionals have a higher risk of ischemic heart disease.[7-9]

Objectives

Considering the importance of truck drivers to our country's economy, more studies are needed to assess their morbidity profile in general and associated risk factors. Considering the inherent risks associated with the profession of truck driving and scarcity of studies aimed at evaluating the cardiovascular risk of morbidity profile in their profession, this study was aimed to assess the morbidity profile among truck drivers in general and to assess the independent predictors of the total number of cardiovascular risk factors identified among them. Thus, this study focused on truck drivers, whose working conditions favor the development of a set of cardiovascular risk factors and simultaneously impose difficulties in accessing health care and adhering to life style changes that enhance quality of life. The results would provide important information to be used at personal and community level to develop strategies and actions targeted at the prevention of cardio vascular disease and the promotion of general health of these workers.

METHODS

Study design, setting and participants

A cross sectional study was conducted on 25th August 2016 among 175 truck drivers aged 18-70 years at the Walayar check post, Coimbatore, Southern India. Ethical clearance was obtained from Institute Human ethics committee, PSG IMS&R. We also got permission from highway transport authorities. The study was conducted at Walayar check post which is located on a national highway of South India on 23rd August 2016 between 10am to 4pm. Drivers who came with valid driving license for submission of documents at the check post and those who gave informed consent were included. A detailed physical examination was conducted. Socio demographic questions were also included. Truck drivers with known congenital heart disease and genetic disorders were excluded. The truck drivers who exhibited risk factors were informed about their condition and advised to attend health care services.

Variables and measurement

Demographic details were collected first followed by measurement of systolic and diastolic blood pressures using mercury sphygmomanometer, two times over a period of 10 minutes and the mean value was calculated. Digital weighing machine was used to measure weight nearest to 0.1 kg. Height was measured with bare foot nearest to 0.1 cm using calibrated stadiometer. Random blood sugar was also measured. The following criteria and definitions were used: family history of CVD: when the individual mentions the occurrence of heart disease in a first degree relative, history of chronic diseases: truck drivers were asked whether they had been previously been diagnosed with any chronic diseases and what medicines they were taking, subjects were considered physically active if they are performing aerobic (walking, biking, dancing and racing) or anaerobic activities (body building) at least 150 minutes in a week and moderately active if they do 10 to 150 minutes in a week and insufficient if they do less than 10 minutes in a week, subjects were considered active smokers if they use tobacco daily in any quantity or at quit smoking in the previous 12 months. Subjects were considered non-smokers if they had not used tobacco for the past 12 months. For determining the body mass index (BMI) the truck drivers were weighed (in kg) without shoes on a 150kg mechanical anthropometric scale and height in meter. The BMI levels were classified according to the WHO guidelines; subjects were normal if their BMI was 18.5-24.5 kg/m^2, overweight if their BMI was 25.0-29.9 kg/m^2.[11] Grade 1 obese if the BMI was 30.0-34.9kg/m^2, grade 2 obese if the BMI was 35.0-39.9 kg/m^2 and grade 3 obese if the BMI was ≥40.0 kg/m^2. Hypertension was defined as systolic blood pressure greater than or equal to 140 mm Hg or diastolic blood pressure greater than or equal to 90 mmHg. Self-reported history of previously known disease was also included.

Sample size

With an expected prevalence of hypertension as 45% and 20% allowable error of prevalence, the minimum sample size required for the study was 122.[1] With a non-response rate of 20%, the total sample required for this study was 153. A total 175 truck drivers were studied.

Statistical methods

Data were analyzed using SPSS 19.Initially data were analyzed descriptively. The categorical variables were represented by absolute frequencies. The numerical variables were summarized with averages, minima, maxima and standard deviations. The existence of association between two categorical variables was verified using Chi square test. Student t test was used to compare the averages of the numerical variables between the groups of two independent samples. Mean (SD) were calculated for continuous variables and percentages for categorical variables. 95% confidence interval was calculated for prevalence assuming normal approximation. As the total number of morbidity is expected to follow Poisson regression, we have initially checked this assumption by analyzing whether mean of the total number of CVD risk factors is approximately equal to variance using Kolmogrov Smirnov Z test. After observing the goodness of fit, Poisson regression model was performed to determine the independent predictors of the total number of cardiovascular risk factors of the truck drivers. This includes diabetes mellitus, hypertension and overweight including obesity. The independent predictors considered were age, smoking, alcohol consumption, family history of CVD and duration of physical activity. A significant level of 5% used for all statistical tests.

RESULTS

Descriptive data

Table 1 shows some characteristics among the 175 truck drivers studied. The population consist young adults with mean age of 39.38 years with a range of 22 years- 67 years. 50% of the subjects were aged 40 years and above, 137 (78.3%) were ever married, 112 (64%) had education only up to 10th standard, 56% were current smokers, 66.3% consume alcohol more than 4 times a month and 42% had family history of CVD.

Table 1: Baseline demographic characteristics (n=175).

Variable	%	Minimum	Maximum	Mean	Median
Age (years)		22	67	39.38	39.00
Physical activity (in minutes per week)		0	460	93.25	0.00
Weight (kg)		46	100	73.23	73.00
Height (cm)		144	185.50	169.55	170.00
Body mass index (kg/m^2)		16.90	36.13	25.47	24.96
Systolic blood pressure (mmHg)		70	190	127.80	130.00
Diastolic blood pressure (mmHg)		55	120	80.29	80.00
Family history of CVD	41.7				
Smoking	56.0				
Alcohol consumption	66.3				

Table 2: Morbidity profile observed.

	Number (n=175)	%
Hypertension	72	41.1
Diabetes	21	12
Overweight/ obesity	86	49.1
Low back pain	60	34.3
Hip pain	43	24.6
Knee pain	17	9.7
Shoulder pain	10	5.4
Diminished vision	8	4.6
Giddiness	4	2.3
Hearing defects	2	1.1

Table 3: Distribution of hypertension according to body mass index.

Hypertension	BMI≤ 25 kg/m^2 (n=89)		BMI 25-29.99 kg/m^2 (n=72)		BMI≥ 30 kg/m^2 (n=14)	
	N	%	N	%	N	%
Systolic only	18	20.2	25	34.7	5	35.7
Diastolic only	19	21.3	24	33.3	8	57.1
Systolic + diastolic	27	30.3	35	48.6	8	57.1
General hypertension	28	31.5	36	50.0	8	57.1

Table 4: Distribution of Study subjects based on systolic and diastolic blood pressure.

Classification	Cut point	Number	%
Optimal	SBP<120	28	16
Normal	SBP 120-129	40	22.9
High normal	SBP 130-139	59	33.7
Hypertension	SBP ≥140	48	27.4
Optimal	DBP<80	49	28
Normal	DBP 80-84	73	41.7
High normal	DBP 85-89	2	1.1
Hypertension	DBP ≥90	51	29.1

Outcome data

The analysis of the parameters obtained during the clinical evaluation shows that (Table 2), 41.1% were having hypertension, 34.3% were having low back pain, 24.6% were having hip pain and 12 5% were diabetes. It was observed that 41.1% were overweight (Table 3) and 8.05 were obese. It was also observed that 1 subject was underweight. It was also observed that the mean BMI (Table 1) of 25.47 kg/m^2 was found to be in the overweight range (25 kg/m^2-29.99 kg/m^2). We are observing an increasing trend in the blood pressure according to the increase in BMI (Table 4).

Table 5: Factors associated with the total number of morbidity using Poisson regression model.

Variable	Odds ratio	95% CI	P value
Higher age	1.03	1.02 -1.05	p<0.001
Positive family history	1.04	0.83 -1.30	0.69
Smoking	1.04	0.83 -1.34	1.32
Alcohol consumption	0.82	0.65	1.04
Physical activity	1.00	0.99 -1.00	1.00

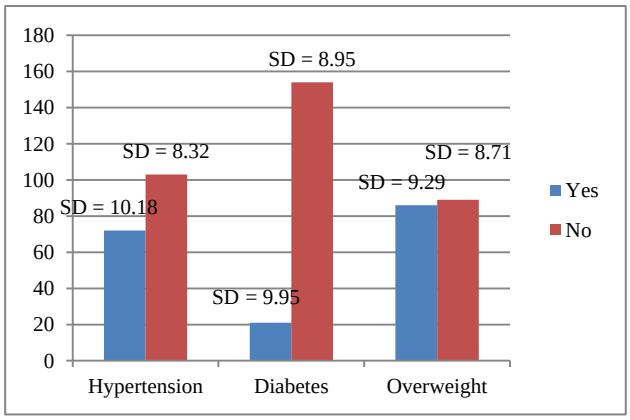


Figure 1: Mean (SD) of age (years) among cardio vascular disease morbidity.

In our study, 59 truck drivers (33.7%) were considered high normal and 48 truck drivers (27.4%) were hypertensive considering systolic blood pressure. Based on diastolic blood pressure, 2 drivers (1.1%) were considered as high normal and 51 drivers (29.1%) were hypertensive. The prevalence of overweight and diabetes were higher in this study population compared to the recent study at Hyderabad in the year 2014. The prevalence of hypertension was similar to the reported prevalence of Hyderabad.[10]

Main results

It was observed that the mean is approximately equal to variance. From the results from Kolmogov Smirnov test, it is concluded that the total number of morbidity follows Poisson distribution (z=0.665, p=0.768) and hence decided to Poisson regression analysis. On using Poisson regression model, Age was found as a significant independent predictor [OR 1.03 (95% CI 1.02-1.05)] for the total number of cardio vascular disease morbidity. It was also observed that mean age of truck drivers with risk factors were between 42.45 years to 46.95 years which is the most productive years putting them at risk of CVD morbidity (Table 5).

Other analyses

On further analysis it was observed that, a significant difference in the mean (SD) age of truck drivers between those who have hypertension 42.48 (10.18) compared with those who do not have hypertension 37.21 (8.32) (p<0.001). A similar difference in the age was observed for diabetes 46.95 (9.95) versus non diabetes 38.35 (8.95) (p<0.001) and for overweight and obesity 42.45 (9.29) versus others 36.41(8.71) (p<0.001).

DISCUSSION

The aim of the present study was to characterize a population of truck drivers with regard to demographic and clinical variables and the presence of cardiovascular risk factors. Our study highlights the need to retrospectively evaluate and systematize the presence of various risk factors in the population of long distance truck drivers. The main finding of our study is that truck drivers have a high prevalence of multiple factors that put them at high risk of cardiovascular events. This is an alarming prospect given the morbidity and mortality rates in the south Indian population and the risks these events pose to truck drivers and others who use roads.

Our study results showed that there is a high prevalence of behavioral risk factors such as overweight, obesity, hypertension, diabetes among the truck drivers and these risk factors were higher at older ages. A number of studies done on other parts other country have demonstrated that high prevalence of obesity among workers in the transport industry. According to Moreno et al., this category of workers in Brazil has a higher incidence of obesity, physical inactivity, inadequate diet, smoking habits, high levels of cholesterol and glycemia, hypertension, and obstructive sleep apnea in comparison to the general population in Brazil.[11,12] A study carried out in the United States involving more than 600 thousand workers found the highest prevalence of obesity to be among male employees who work as highway truck drivers.[13] A representative sample of the Australian in productive age compared ten different functional categories with regards to the risk of obesity and found that male employees of the transportation industry had a higher risk of overweight and obesity.[14] In our study, it was observed that nearly one third of the truck drivers were having low back pain (LBP). It has been pointed out by several studies that the factors leading occupational

LBP among truck drivers include exposure to vibration, repeatedly lifting heavy objects, strained postures, and sitting for work for many hours.[15] In a recent study conducted at Japan it was observed that 52.9% of the truck drivers were having LBP. Most drivers pointed out that vibration and road shock account for the LBP. Troup et al. noted that muscle fatigue due to mechanical stress such as road shock transmitted to the body and a restricted sitting posture contribute to truck drivers' LBP. From the viewpoint of prophylaxis, an improvement in the working condition is needed to reduce the incidence of drivers' LBP to some extent.

In this study we observed higher prevalence of hypertension among truck drivers. According to World Health Organization, systolic blood pressure greater than 115 mm Hg accounts 62% of cases of cardiovascular disease and 49% of cases of ischemic heart disease.[16] Moreover, arterial hypertension is one of the major risk factors of cerebro vascular accident. Many studies have also demonstrated an increase in the prevalence of systemic hypertension among professional drivers.[17,18]

In our study older age was found to be an independent predictor of the total cardiovascular morbidity profile life span. With aging, there is an increased acquisition of several CVD risk factors in an individual's life span. Several studies have shown that when age and other risk factors are used jointly to examine an individual's risk of CVD, it has been found that the contribution of age in the multivariate models may be a reflection of the intensity and duration of exposure to the traditional CVD risk factors.[19-23] Avoidance of these traditional risk factors at younger ages can reduce CVD risk associated with age per se.

People employed in the transport sector form a considerable workforce of our nation.[24-28] Intersectorial consideration between transport department and health department is needed to protect and provide health to transport employees. Thorough periodical medical examinations are to be conducted to these truck drivers and necessary steps are to be taken. In addition strict guidelines about diet are to be formulated to the truck drivers. In addition it is observed that the truck drivers must be educated at their younger age preferably early thirties to prevent overweight and obesity and thereby to reduce hypertension. Regular health education programs are to be conducted regarding various non-communicable diseases and attendance to such programs should be made compulsory. Strict guidelines about diet are to be formulated and implemented in all the eateries catering to the employees. Various recreational facilities, counseling centers with psychologists are to be established in all depots to help the employees cope with their stress.

Limitations

Our study has some limitations. First, this study does not allow the determination of causal associations since we used cross sectional study design. Another limitation of our study is that, we have not quantified the smoking and alcohol intake. Fasting blood sugar values were not studied. Because we were looking for a cluster of cardio vascular risk factors, another limitation of this study was the lack of measurements on lipid profiles or inflammatory markers.

CONCLUSION

In conclusion, we observed a higher morbidity among the truck drivers' ages. The data in the present study allow us to draw conclusion that truck drivers are highly vulnerable to developing CVD because of the high prevalence of variety of risk factors. Furthermore we must consider that the truck drivers we studied were adult men and that cardiovascular risk factors could decrease their maximum age and it affects their economy, the lives of the truck drivers and individuals who use the roads.

Recommendations and generalizability

More national studies are needed to look into what percentages of road traffic accidents involving human error and what percentages involve medical conditions involving cardiovascular conditions. Accordingly guidelines for the minimum standards required for fitness to drive those drivers with CVD. However, the driving restrictions may have disadvantage in the form of psychosocial effects on recipients and their family members. Instead, regular health education programs and appropriate healthcare action based on comprehensive public policies that focus on the truck drivers work environment and monitoring of these factors must be established at the national level. Accordingly various recreational facilities and counseling centers are to be established in highways for the truck drivers. Finally, intersectorial coordination between transport department and health department is needed to protect and promote health of transport employees.

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REFERENCES

1. Udayar SE, Sampath S, Arun D, Sravan S. Epidemiological study of cardiovascular risk factors among public transport drivers in rural area of Chittoor district of Andhra Pradesh. Int J Community Med Public Health. 2017;2(4):415-20.
2. Sharvanan EU, Konduru RK, Praveen BA Pawan K, sivachandran V, Srinivas T. Study of cardiovascular risk factors among transport drivers in rural area of Andhra Pradesh. Int J Res Health Sci. 2014;2(1):420-6.
3. Hirata RP, Sampaio LM, Leitão Filho FS, Braghiroli A, Balbi B, Romano S, et al. General characteristics and risk factors of cardiovascular disease among interstate bus drivers. Scientific World J. 2012;2012.
4. Tűchsen F, Andersen O, Costa G, Filakti H, Marmot MG. Occupation and ischemic heart disease in the European community: a comparative study of occupations at potential high risk. Am J Industrial Med. 1996;30:407-14.
5. Shin SY, Lee CG, Song HS, Kim SH, Lee HS, Jung MS, et al. Cardiovascular Disease Risk of Bus Drivers in a City of Korea. Ann Occupational Environ Med.1996;30:407-14.
6. Bigert C, Klerdal K, Hammer N, Hallqvist J, Gustavsson P. Time trends in the incidence of myocardial infarction among professional drivers in Stockholm 1977-96. Occupational Environ Med. 2004;61(12):987-91.
7. Malinauskiene V. Truck driving and risk of myocardial infarction. Przeglad lekarski. 2003;60:89-90.
8. Albright CL, Winkleby MA, Ragland DR, Fisher J, Syme SL. Job strain and prevalence of hypertension in a biracial population of urban bus drivers. Am J Public Health. 1992;82(7):984-9.
9. Ragland DR, Winkleby MA, Schwalbe J, Holman BL, Morse L, Syme SL, Fisher JM. Prevalence of hypertension in bus drivers. AAOHN J. 1989;37(2):71-9.
10. Sharma PK, Ganguly E. Morbidity profile of long distance truck drivers in Hyderabad city, India. J Dr. NTR Univ Health Sci. 2014;10;3(4):234.
11. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363:157-63.
12. Moreno CR, Louzada FM, Teixeira LR, Borges F, Lorenzi-Filho G. Short sleep is associated with obesity among truck drivers. Chronobiol Int. 2006;23(6):1295-303.
13. Caban AJ, Lee DJ, Fleming LE, Gómez-Marín O, LeBlanc W, Pitman T. Obesity in US workers: The national health interview survey, 1986 to 2002. Am J Public Health. 2005;95(9):1614-22.
14. Allman-Farinelli MA, Chey T, Merom D, Bauman AE. Occupational risk of overweight and obesity: an analysis of the Australian Health Survey. J Occupational Med Toxicol. 2010;5(1):14.
15. Troup JDG. Drivers bavk pain its prevention: A review of the postural, vibratory and muscular factors, together with the problem of transmitted road-shock. Applied Ergonomics. 1978: 207-214.
16. Kelsey JL, Hardy RJ: Driving of motor vehicles as a risk factor for acute herniated lumbar intervertebral disc. Am J Epidemiol. 1975;102:63-73.
17. Kelsey JL: An epidemiological study of acute herniated lumbar intervertebral disc. Rheumatol Rehab. 1975;14:144-59.
18. Frymoyer JW, Pope MH, Clements JH. Risk factors in LBP: An epidemiological survey. J Bone Joint Surg. 1983;65:213-8.
19. Bovenzi M, Zadini A. Self-reported Low Back Symptoms in Urban Bus Drivers Exposed to Whole-Body Vibration. Spine. 1992;17:1048-59.
20. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors and impact of urbanization. Circulation. 2001;104:2746-53.
21. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet. 2006;367(9524):1747-57.
22. Bigert C, Gustavasson P, Hallqvis J, Hogstedt C, Lewne M, Plato N, et al. Myocardial infarction among professional drivers. Epidemiology. 2003;14(3):333-9.
23. Mathew Anil C, Veyilmuthu R, Dhanush M, PremAnand ES, Xavier ChristuRajan V. Factors associated with hypertension among truck drivers: a cross sectional study at a check post on a national highway in South India. Int J Med Res Health Sci. 2017;6(5):126-9.
24. Allman-Farinelli MA, Chey T, Merom D, Bauman AE. Occupational risk of overweight and obesity: an analysis of the Australian Health Survey. J Occupational Med Toxicol. 2010;5(1):14.
25. Miyamoto M, Shirai Y, Nakayama Y, Gemebun Y, Kaneda K. An epidemiologic study of occupational low back pain in truck drivers. J Nippon Med School. 2000;67(3):186-90.
26. Ragland DR, Winkleby MA, Schwalbe J, Holman BL, Morse L, Syme SL, et al. Prevalence of hypertension in bus drivers. Int J Epidemiol. 1987;16(2):208-14.
27. Winkleby MA, Ragland DR, Fisher JM, Syme SL. Excess risk of sickness and disease in bus drivers: a review and synthesis of epidemiological studies. Int J Epidemiol. 1988;17(2):255-62.
28. Gersh BJ, Sliwa K, Mayosi BM, Yusuf S. Novel therapeutic concepts the epidemic of cardiovascular disease in the developing world: global implications. Eur Heart J. 2010;31(6):642-8.

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Original Research Article

Effect of Therapeutic Exercise Vs Retro Walking In Individuals with Knee Osteoarthritis

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ABSTRACT

Background: Osteoarthritis (OA) is a degenerative joint disease and one of the major health problems that cause functional impairment and reduced quality of life. There is osteophyte formation remodeling of subarticular bone, ligamentous laxity, and synovial inflammation weakening of periarticular muscles. These changes may occur as a result of an imbalance in the equilibrium between the break down and repair of joint tissue. Hamstring tightness and quadriceps weakness are the major problems in osteoarthritis individuals. Tightness of hamstring muscle and quadriceps weakness can cause pain in patients with osteoarthritis. Stretching of hamstring and strengthening of quadriceps muscle can improve the pain and strength of the muscles around the knee joint. Retro walking can help in strengthening of the quadriceps muscles.
Methods: It was a Quasi experimental study with comparison between 2 groups consisting 30 participants (males and females) 15 in each group, with osteoarthritis. Group A received Hamstring stretch and quadriceps strengthening exercises and Group B received Retro walking, for 9 sessions, 3 days/week for a period of 3 weeks. Outcome measure was WOMAC and NRS measured before and after intervention.
Results: The results suggest that participants in Group B had significant improvement in NRS and WOMAC than Group A.
Conclusions: Both group A and group B showed significant results and displayed efficient improvement in decreasing pain and functional disability in patients with knee osteoarthritis.

Key words: Retro walking, Knee Osteoarthritis, Exercises, Pain and disability.

INTRODUCTION

Osteoarthritis (OA) is a non-inflammatory progressive disorder of movable joints, particularly weight bearing joints. Exact cause of OA is not known. However it is strongly believed that it occurs due to aging or wear and tear or degenerative changes in the joints. [1] Risk factors for OA knee includes age, gender, obesity, occupation, sports, osteoporosis, previous trauma, irregularity in joint surfaces, internal derangement, heredity, leisure and diseases leaving articular cartilage damage. [2] There is increased risk of development or progression of disease due to greater or uncontrolled loading on the joint; therefore, quadriceps strength needs to be considered in the study of knee OA. A reduced quadriceps strength has been shown to be associated with the presence of OA in the knee. [3]

The major symptoms present in osteoarthritis are pain and functional disability. [4] The knee is most common site for osteoarthritis with characteristic sign like pain, stiffness, tenderness, swelling, crepitus, and loss of movement, valgus or varus deformity, locking of the knee, on

auscultation of joint- scratching, crepitus and later on loud crackling sound. In addition, they have a lot of functional limitation when sitting and standing or going up and down stairs. [5, 6]

There is osteophyte formation remodeling of subarticular bone, ligamentous laxity, synovial inflammation and weakening of periarticular muscles. These changes may occur as a result of an imbalance in the equilibrium between the break down and repair of joint tissue.

According to Framingham study, among the individuals aged above 45 years the prevalence of radiographic knee OA was 19.2%, and in those above 80 years, it is 43.7%. [7] In rural India the incidence is 5.78% which is about 30% of all rheumatological problems. In India there is increased knee arthritis than western population. [8]

Management of knee arthritis is mainly concentrated on reducing pain, increasing joint ROM and improving extensor muscle strength, neglecting ligamentous and muscular tightening which affects lower limbs function and gait. The initial conservative management includes - Risk factor education of obesity, avoidance of ground level activities, Non steroidal anti inflammatory drugs, Intraarticular injections of sodium hyaluronate. The most surgical management done is total knee replacement in most advanced cases. [9]

Physiotherapy as the main choice of conservative management; which includes various strategies such as manual therapy, exercises, patellar taping and electrical modalities with or without thermal modalities as measures for pain reduction. [11] Recently, closed kinematic chain exercises have drawn much attention in the management of knee arthritis. [12] Studies suggest that these exercises are more effective and functional than the traditionally employed open kinematic chain exercises. [13] Closed kinematic chain exercises for knee joint can be incorporated in many ways; one of them is Retro‑walking. [12] Retro‑walking is walking backwards. [11] Since there is propulsion in backward direction and reversal of leg movement in Retro‑walking, different muscle activation patterns from those in forward walking are required. [14] Retrowalking significantly lowers peak patellofemoral joint compressive force and a significantly slower rate of loading has been found during backward walking. Consequently, trauma to the articular cartilage is reduced during retrowalking. [1] Retrowalking could be utilized as a mode of training during knee rehabilitation since excessive loading of the joint and overstretching of the ligaments are prevented whilst quadriceps strength. [15]

Strength of the quadriceps musculature is one of the intrinsic factors that have been shown to affect the knee joint functions. It is evident that lower extremity strength has a major role in knee joint shock attenuation during weight bearing activities. [10]

MATERIALS AND METHODS

The research design used for the study was Quasi experimental study with comparison between 2 groups. Participants received 9 sessions, 3 days per week for a period of 3 weeks. Each treatment session lasted for 30 minutes. Both male and female participants with a clinical diagnosis of unilateral or bilateral osteoarthritis of knee referred to department of PMR, from the department of orthopaedics, PSG Hospitals. The sample size was 30. Sample design was Convenience sampling. Selection Criteria used for this study was as follows- The inclusion criteria for the study:- Male and female participants, clinically diagnosed with osteoarthritis of knee by orthopaedician, Age more than 40 years, Participants having Pre test NRS score more than 5 score, Participants with unilateral or bilateral involvement of knee, Willingness to participate in the study. The exclusion criteria for the study were Participants with History of lower limb or spinal surgeries, Neurological disorder (motor and sensory loss), Pathologies related to hip, knee and

spine, Cardio vascular problems with increased heart rate, Other musculoskeletal disorders associated with knee joint, IT band, adductor muscle and sartorius muscle tightness.

The participants were screened from outpatient department of PMR department, after finding their suitability according to the inclusion and exclusion criteria, they were requested to participate in the study. The participants were briefed about the nature of study, the duration of intervention and the intervention being used in the language best understood by the participants. They were encouraged to clarify queries regarding the study, if any. An informed written consent form, previously approved by the Institutional Ethical Committee was then obtained from the participants. The demographic data was obtained and a detailed assessment of 30 participants was done on the basis of name, age, gender, height, weight, BMI, present chief complaints, past history, pain history, and medical history. Participants were subsequently allocated in a group according to the selection criteria. The outcome measures NRS and WOMAC were assessed before the intervention as given below;

- Pain was assessed with the help of Numerical Rating Scale, an 11-point numeric scale with 0 representing one pain extreme (e.g. "no pain") and 10 representing the other pain extreme (e.g. "pain as bad as you can imagine" and "worst pain imaginable"). [16]
- Physical function was assessed with the help of Western Ontario and McMaster Universities Osteoarthritis Index, is a widely used, proprietary set of standardized questionnaire used by health professionals to evaluate the condition of patients with osteoarthritis of the knee and hip, including pain, stiffness, and physical functioning of the joints. [17]

The two groups were as follows: Group A (Conventional Group) and Group B (Experimental Group). In group A Hamstring stretch and quadriceps strengthening with 5 seconds hold and a rest interval of 2 seconds for 10 repetitions for each exercise for a period of 3 weeks, 3 days per week was given. In group B retro walking with a rest (if need) for a period of 3 weeks, 3 days per week was given.

Procedure:

In Group A

Conventional Physiotherapy treatment for this group was given static quadriceps exercise, dynamic quadriceps exercise, straight leg raise, prone knee bending, and simple hamstring stretches, for 10 repetitions with 5 seconds hold followed by 2 seconds rest for each exercise for a period of 3 days per week for duration of 3 weeks. Home program for 2nd and 3rd week: same exercises.

In Group B

Procedure for retro walking: The participants initially were made to walk 4 steps retrowalk and were observed for any discomfort. If no discomfort then, participant was made to retrowalk for 10 minutes per session. The participants will be first familiarized with the retrowalking on flat surface (distance 20 meters) such that during retrowalking, the toes strike the ground first instead of the heel. Practice session was made for retrowalking with support of wall. The participants received retrowalking on flat surface (distance 20 meters) at their maximum pace with support of the wall for 10 minutes per session. The session included 4 minutes of retrowalking following 2 minutes of rest time and then again 4 minutes of retrowalking. The therapist was walking besides the participant. The protocol followed was retrowalking for 10 minutes, 3 days/ week for duration of 3 weeks. Home program for 2nd and 3rd week: same walking pattern.

Ethical clearance

The study followed the ethical standards of institutional human ethics committee, PSG IMS&R. [(Ref. no. PSG/IHEC/2018/ Appr/Exp/165) (project no.18/073)].

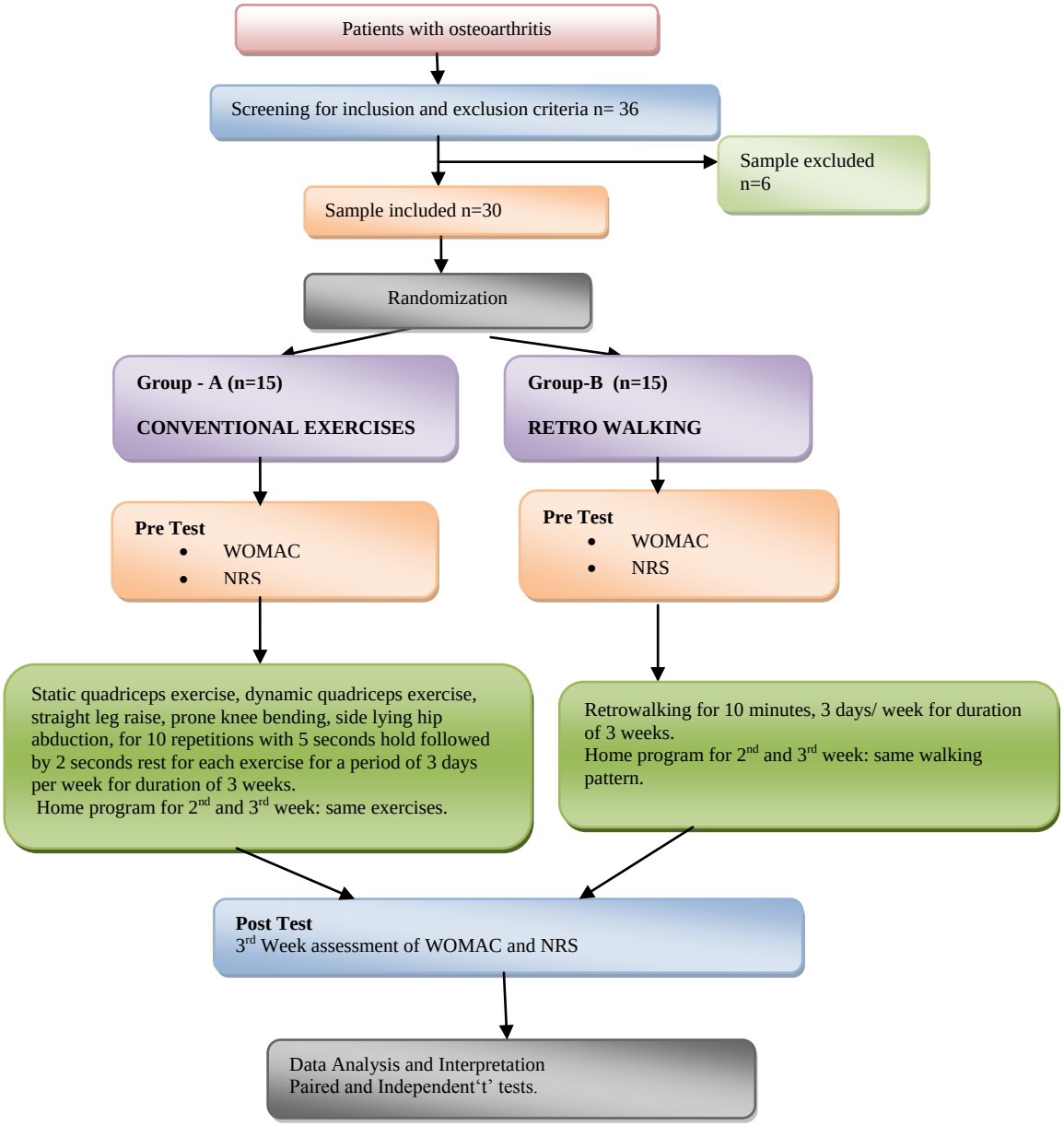


Fig.1 Flow chart representing the procedure of selection of participants

RESULTS

Study variables were done before starting physiotherapy sessions on both groups and at the end of the study, study variables include NRS and WOMAC.

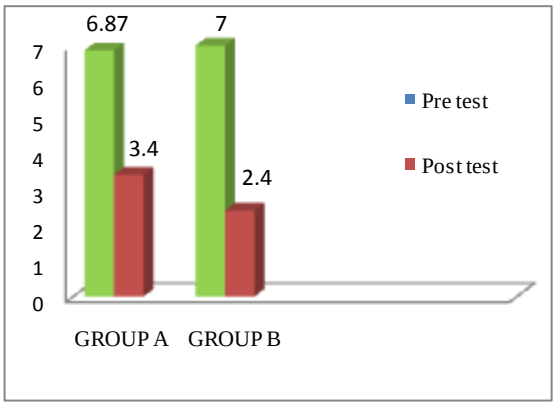
Data analysis: Statistical analysis was performed on the data obtained from 30 patients. Data was analyzed using Statistical Package for Social Sciences [SPSS] software [version 16.0]. Descriptive statistics for all outcome measures were expressed as mean, standard deviations and test of significance such as paired 't' test used for comparing data within each group and independent 't' test for comparing between the groups. Data was considered statistically significant with $p<0.05$ and highly significant with $p<0.01$.

Pre and post mean difference of pain (NRS) shows 3.47 ± 0.74 in group A and 4.60 ± 0.83 in group B. As $p<0.05$ there is significant difference between two groups.

Pre and post mean difference values of WOMAC shows 15.01 ± 4.51 in group A and 20.15 ± 5.29 in group B which shows a significant difference between two groups.

Table 1: Paired "t" test values for OA knee (NRS)

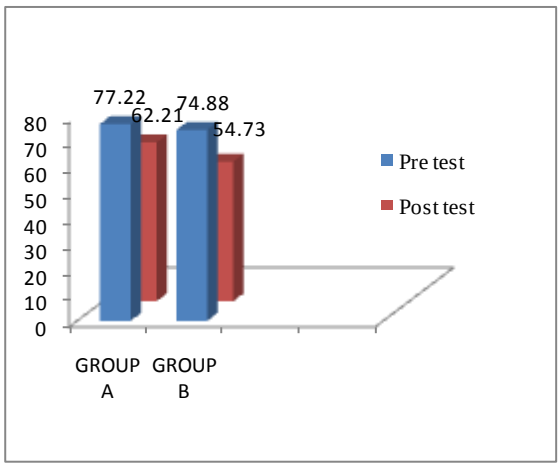
Group	Mean	Mean Difference	Standard Deviation	"t" Value	"p" Value
Group A Pre Test Post Test	 6.87 3.40	3.47	0.74	18.07	P<0.05
Group B Pre Test Post Test	 7.00 2.40	4.60	0.83	21.51	P<0.05



Graph 1: Mean values of NRS

Table 2: Paired "t "Test values for OA Knee (WOMAC)

Groups	Mean	Mean Difference	Standard Deviation	"t" Value	"p" Value
Group A Pre Test Post Test	 77.22 62.21	15.01	4.51	12.90	P<0.05
GROUP B PRE TEST POST TEST	 74.88 54.73	20.15	5.29	14.76	P<0.05



Graph 2: Mean values of WOMAC

Table 3: Independent "t" test for OA knee

Outcome Measures	Mean Difference	"t" Value	"p" Value
NPRS	1.00	4.33	P<0.05
WOMAC	7.49	4.53	P<0.05

DISCUSSION

The purpose of the study was to determine the effects of retro walking over conventional physiotherapy treatment in osteoarthritis of knee joint. According to many studies the backward walking increases the hamstring activation which generates reduced patello femoral and lower tibiofemoral compression load stress and ACL strain, and therefore backward walking reverses the shear forces in knee joint. [18] Results of the present study showed that retrowalking is more effective in reducing pain and disabilities as compared to conventional physiotherapy treatment.

Reduction of pain and disability was seen in both the groups. But a much better improvement was seen in group B. Despite the fact that patients in the group A performed conventional exercises to reduce pain and to improve physical function, changes were identified. The result of this study also suggests that retro walking positively influences pain and disability in the group B.

The outcome measures used for assessment were the Numerical pain Rating Scale (NRS) and the Western Ontario and

McMaster Arthritis Index (WOMAC). In the group B, the mean value for NRS was reduced at the end of 3rd week, showing a significant reduction in pain. The mean value for WOMAC was also reduced at the end of 3rd week, showing a significant reduction in pain, stiffness and disability.

CONCLUSION

Participants who underwent retrowalking showed significant reduction in pain, stiffness and disability at the end of the treatment period when compared to participants with conventional physiotherapy. Therefore, retrowalking can be considered beneficial reduction in pain, stiffness and disability in knee osteoarthritis individuals. The result of this study also suggests that retro walking positively influences pain and disability in group B.

Suggestions

- The study can be extended to large sample size.
- The duration of the study can be increased.
- Activities of daily living and recreational activities of patients were not taken into account. There were few dropouts that can be strategically improved in future.

ACKNOWLEDGEMENT

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REFERENCES

1. Deepti N Wadhwa, Deepali N Hande. Effects of Retrowalking on Osteoarthritis of Knee in Geriatric Population. (IOSR-JSPE). 2016; 3(2):37-43.
2. Khatri S M. Basics of Orthopedic Physiotherapy. Jaypee Brothers Medical Publishers. New Delhi. 2013, chapter no. 6 Arthritis, 119-126.
3. Shahnawaz Anwer, Ahmad Alghadir. Effect of Isometric Quadriceps Exercise on Muscle Strength, Pain, and Function in Patients with Knee Osteoarthritis: A Randomized Controlled Study. J. Phys. Ther. Sci.2014; 26: 745–748.
4. Young Dae Yuna, Hee Joon Shinb, Sung Joong Kimc, Sang Wan Limd, SukJu Choie. The Effects of Resistance Exercise and Balance Exercise on Proprioception and WOMAC Index of Patients with Degenerative Knee Osteoarthritis. International academy of Physical Therapy Research,1: 169-175, (2010).
5. MundermannA, Dyrby CO, Andriacchi TP. Secondary gait changes in patients with medial compartment knee OA : Increased load at the ankle, knee and hip during walking ,arthritis Rheum .2005;52:2835-44
6. Maheshwari J. Essential‟s orthopedics 3rd edition (revised) Mehta publishers New Delhi June 2010 chapter no.35 Degenerative Disorders, 252-254.
7. Anna Litwic et al. Epidemiology and burden of osteoarthritis.Br Med Bull.2013; 105:185-199.
8. G. S. Kulkarni. Text book of orthopaedics and trauma. 2nd edition (vol 4); 2008.
9. Meena .v, shanthi .c, madhavi .k. Effectiveness of pnf stretching versus static stretching on pain and hamstring flexibility following moist heat in individuals with knee osteoarthritis. Int j physiother. 2016; 3(5):529-534.
10. Minor MA: Exercise in the treatment of osteoarthritis. Rheum Dis Clin North Am, 1999, 25: 397–415, viii. [Medline] [CrossRef]
11. Nor AM, Lyn KS. Effects of passive joint mobilization on patients with knee osteoarthritis. Sains Malays 2011;40: 1461‑5.
12. Chen LY, Su FC, Chiang PY. Kinematic and EMG analysis of backward walking on treadmill. Conf Proc IEEE Eng Med Biol Soc 2000; 2:825‑7.
13. Cipriani DJ, Armstrong CW, Gaul S. Backward walking at three levels of treadmill inclination: An electromyographic and kinematic analysis. J Orthop Sports Phys Ther 1995; 22:95‑102.
14. Kumar TR, Ashraf M. The effect of backward walking treadmill training on kinematics of the trunk and lower limbs. Serb J Sports Sci 2009; 3:121‑7.
15. Brink M. The effect of backward locomotion as a rehabilitation program on functional ability of patients following knee injury. Stellenbosch University. 2010.

16. P Ornetti, M Dougados, S Paternotte, I Logeart, L Gossec. Validation of a numerical rating scale to assess functional impairment in hip and knee osteoarthritis: comparison with the WOMAC function scale. Ann Rheum Dis 2011; 70: 740-746.
17. N Bellamy. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). American College of Rheumatology. 2015. 12-22
18. Farhin Mulla, Amrutkuvar Pawar, Trupti Warude. Effect of Reverse Treadmill Walking and Low Intensity Cycle Ergometry in Chronic Knee Osteoarthritis Subjects-Comparative Study. International Journal of Science and Research (IJSR) 2017; 6(3): 671-675.

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Original Research Article

Efficacy of Swiss Ball Therapy in Balance Rehabilitation of Hemiplegic Stroke Participants

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ABSTRACT

Background: Impaired balance is one of the most common problems in post stroke patients. Thus the subject may unable to maintain balance in sitting and standing or to move in weight bearing posture without loss of balance. Treatment focusing on balance impairment is important to achieve functional independence at the earliest following stroke.
Methodology: Quasi experimental design - pretest and posttest designs with two groups at Department of Physical Medicine & Rehabilitation, PSG Hospitals, Coimbatore, Tamil Nadu, India. A total of 30 stroke patients in the age group of 45 to 60 years who satisfied the selection criteria were randomly assigned into two groups. Group A received conventional therapy along with balance training using Swiss ball for 60 minutes/session and Group B received conventional therapy along with balance training on mat and stool for 60 minutes/session. Outcomes were measured with Berg balance scale.
Results: All participants in Group A and Group B showed significant improvement in Berg balance scale with a mean difference of 13.6 and 8.7 respectively, calculated 't' value using the paired test for group A and B were 39.3 and 15.9 ($P<0.05$) respectively. When comparing between the groups using independent 't' test, the Berg balance scale scores showed mean difference of 4.6 and 't' value of 5.4 ($P<0.05$)
Conclusion: This study revealed that there was a significant improvement in balance following balance training on Swiss ball than on bed and stool among hemi paretic patients.

Key Words: Swiss ball, Static balance, Dynamic balance, Berg Balance Scale

INTRODUCTION

Stroke is a rapidly developing clinical signs of focal cerebral dysfunction; lasting more than 24 hours or leading to death with no apparent cause other than the vascular origin. [1]

Balance is frequently disturbed following stroke with impairments in steadiness, symmetry and dynamic stability. [2] The automatic adjustment of muscle tension that occurs normally in preparation for and during a movement task, termed automatic postural tone is impaired in stroke patients. Thus the patient may lack ability to stabilize the proximal joints and trunk appropriately resulting in postural malalignment, impaired balance and risk of falling. Commonly problems may exist when reacting to a destabilizing external force or during self initiated movement. [3]

Patients with stroke typically demonstrate asymmetry with most of weight in sitting or standing shifted to non-paretic side. Hemiplegic subjects typically fall in

side of weakness. [4] They also have increase postural sway in standing. Balance problem during dressing activities increases the likelihood of stroke survivors having a fall.

In preface of stroke balance training is considered vital because it helps in achieving optimal posture, gait and thus can hasten the patients progression to near normal functional activities.

A cordial therapeutic intervention along with a good patient co-operation has led to early balance improvisation in patients. [5] Swiss ball therapy along with other physical therapy intervention will help in early regaining of balance when compared to conventional therapy interventions. [6, 7]

In this study we aimed at improving the balance through Swiss ball exercises along with other conventional balance training. So with the improvement of balance it's able to achieve a good and independent functional mobility, gait and ADL activities. The Berg Balance Scale is a used as an assessment tool to identify balance impairment. [8] Functional activities such as reaching, bending, transferring, and standing are evaluated on the test to evaluate balance.

METHODOLOGY

STUDY DESIGN

Pretest and posttest design with a comparison group – a quasi experimental study.

STUDY SETTING

Physiotherapy division, Department of Physical Medicine and Rehabilitation, PSG Hospitals

NUMBER OF SUBJECTS

A total of 30 hemiplegic participants who met inclusion criteria were randomly assigned into Experimental group (Group A – 15 subjects) and Control group (Group B – 15 subjects).

INCLUSION CRITERIA

Age: 45-60 years; Gender: male; Ischemic stroke for the first time; Functional balance grade – fair

EXCLUSION CRITERIA

Cognitive deficit; Bilateral cerebellar stroke; Sensory disorders; Cardiovascular system instability; Tumors and infections; Orthopedic conditions.

INSRTUMRNATION USED

Swiss Ball; Mattress; Couch; Stool; Bolsters; Balance board; Measuring tape.

OUTCOME MEASURES

Berg Balance Scale.

PROCEDURE

The purpose of the study was explained to the subjects, verbal description of all procedures and test was given to them and a written consent was obtained from the patients who fulfilled the inclusion and exclusion criteria and randomly assigned to two groups. An assessor who was blinded to the group allocation took the outcome measurements using Berg Balance Scale. Initial assessment was taken on the first day before therapy. Intervention was given to each group separately for a total of 10 sessions (5 sessions / week). Post assessment was taken after 10 sessions of physiotherapy treatment using the same outcome measure. Comparison of pretest and posttest values within the group and between the groups was done finally.

INTERVENTION

The subjects in the experimental group (Group A) received conventional therapy along with balance training using Swiss ball for 60 minutes per session, the subjects in control group (Group - B) received conventional therapy and balance training activities on mat and stool. Exercises performed by both the groups are given in figure 1.

DATA ANALYSES

Paired 't' test was used to find the difference within the group between pre and post intervention scores in the outcome measure. Independent 't' test was used to find the difference between the groups in the outcome measure. The statistical significance for this study was set as $p < 0.05$.

Figure 1: Illustrates treatment protocol

CONVENTIONAL PHYSICAL THERAPY
Normalization of muscle tone: Slow sustained stretching for biceps and wrist flexors, hamstrings and calf muscles; Weight bearing activities in forearm supported prone lying, prone kneeling, half kneeling, sitting and standing. Range of Motion exercises: Passive and active assisted exercises for the affected upper and lower limb - 15 repetitions each exercises.
GROUP A (Balance training using swiss ball)
Supine lying position: Pelvic bridging bilateral and unilateral; lower trunk rotation; lower limb flexion and extension. Sitting position: Static sitting balance training - patients should sit on the ball and have both feet touch the floor with hips, knees and ankles at 90-90-90 degree position and can work on achievement of an upright erect posture; Trunk forward and lateral reach; Upper and lower trunk flexion-extension and lateral flexion activities; Marching activities; Trunk rotation and PNF chopping and lifting activities. 15 repetitions each activities.
GROUP B (Balance training using mat and stool):
Supine lying position: Pelvic bridging bilateral and unilateral; lower trunk rotation; lower limb flexion and extension. Sitting position: Static sitting balance training - patients should sit on the stool and have both feet touch the floor with hips, knees and ankles at 90-90-90 degree position and can work on achievement of an upright erect posture; Trunk forward and lateral reach; Upper and lower trunk flexion-extension and lateral flexion activities; Marching activities; Trunk rotation and PNF chopping and lifting activities. 15 repetitions each activities.

RESULTS

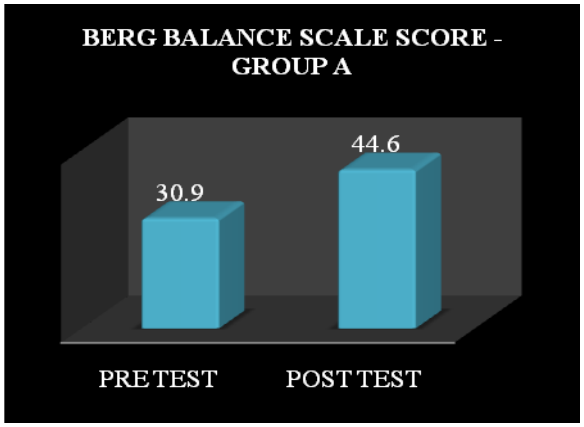
The aim of the study is to find out whether balance training using Swiss ball when given along with conventional balance training improves balance in all day activities among post stroke patients. As shown in Table 1, all participants in group A&B, showed significant improvement in Berg balance scale score with a mean difference of 13.6 and 8.7 respectively. The calculated 't' value by using the Paired 't' test for group A&B were 39.3 and 15.9 respectively which was greater than the table 't' value of 2.14; $p<0.05$. This shows that there is a significant improvement in balance in both the groups. As shown in Table 2, when comparing the groups using Independent 't' test, Berg balance scale score showed mean difference of 4.7 and the 't' value of 7.5;$P<0.05$, which was greater than the table 't' value of 2.04. This results shows that there is a statistically significant improvement in group A which underwent balance training using Swiss ball along with conventional balance training.

Table 1: Mean, mean difference and Paired 't' test values for Berg balance scale of groups A&B.

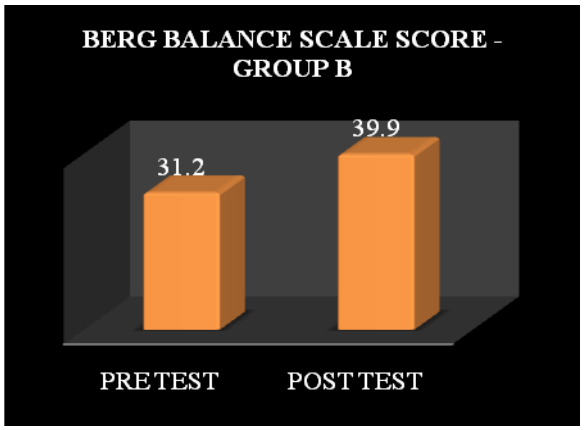
Groups	Means	Mean difference	't' value	'p' value
Group A				
Pre test	30.9	13.6	39.3	p < 0.05
Post test	44.6			
Group B				
Pre test	31.2	8.7	15.9	p < 0.05
Post test	39.9			

Table 2: Mean difference and Independent "t" Test values for Berg balance scale of groups A&B.

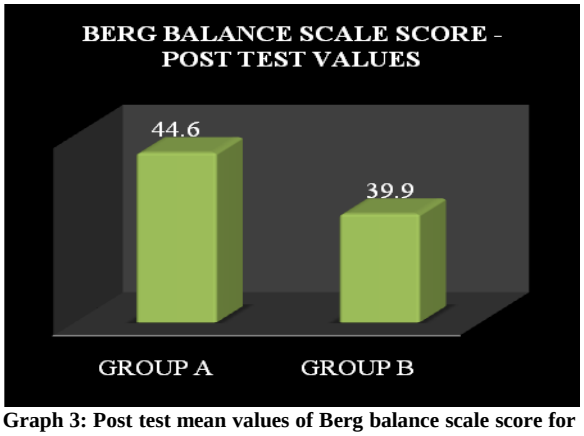
Outcome measure	Mean difference	't' value	'p' value
Berg balance scale	4.6	5.4	p < 0.05



Graph 1: Pre test and post test mean values of Berg balance scale score for group – A.



Graph 2: Pre test and post test mean values of Berg balance scale score for group – B.



Graph 3: Post test mean values of Berg balance scale score for groups – A & B.

DISCUSSION

In stroke patients who can able to walk, the majority of falls were reported only during walking, an activity that requires dynamic and reactive balance for example turning, transferring, and stair climbing because of reduced balance.

Conventional physical therapy uses only traditional balance training protocols on a stable surface that is less demanding for the post stroke patients to improve balance, but the stroke participants need more of balance to prevent the fall related complications.

The Swiss ball, as a movable surface, provides the patient with some uncertainty in terms of stability. A sudden movement of the ball requires the patient to be able to make a quick, unanticipated postural response, to realign the centre of gravity in relation the base of support. Swiss ball stimulates body's natural motor reflex and encourages body to react as a whole integrated unit by increasing the muscle recruitment, thereby increasing the efficiency of the muscles for effective postural mechanism.

A significant improvement in balance the experimental group (balance training using Swiss ball) is due to the postural perturbation provided by the movement of the Swiss ball to which the trunk muscles responded reactively in order to maintain the desired postural stability.

Duncan et. al., (2009) in his study has stated that activity of the trunk muscle was greater when exercises are performed on a Swiss ball than in exercises performed on stable surface in healthy adults. [6] Karthik Babu et. al., (2011) in their study found that there was a significant improvement in lateral flexion of trunk when trained with Swiss ball than in plinth. The results were similar to our findings that balance improvement in Swiss ball training is much significant than conventional balance training. [9]

In Berg balance scale the components such as trunk rotation, turning and placing alternate step on stool scores improved much in Swiss ball training group than the conventional balance training group. This is due to the fact that with Swiss ball therapy coordination improved much more because of perturbations on unstable surface resulted in greater muscle recruitment.

CONCLUSION

The intent of this study was to find out the effect of Swiss ball therapy in balance rehabilitation of hemiplegic stroke participants. From the results of this study we can state that Swiss ball when administered along with conventional physical therapy will be more effective in improving balance.

REFERENCES

1. Susan B. O' Sullivan, Thomas J. Schmitz, Physical medicine and rehabilitation, fifth edition, Jaypee publication, chapter 18, Pg. No. 705.
2. F.Horak. Clinical assessment of balance disorders. *Gait & Posture*, Volume 6, Issue 1, August 1997 Pages 76-84.
3. Sarah F Tyson, Marie Hanley, Jay Chillala, Andrea Selley and Raymond C Tallis. Balance disability after stroke. Phys Ther. 2006;86:30–38.
4. Jocelyn E Harris, Janice J Eng, Daniel S Marigold, Craig D Tokuno and Cheryl L Louis. Relationship of Balance and Mobility to Fall Incidence in People With Chronic Stroke. Phys Ther. Vol. 85, No. 2, February 2005, pp. 150-158.
5. Chun Chen, MD; Pao-Tsai Cheng, MD; Chia-Ling Chen, MD, PhD; Shih-Ching Chen, MD; Chia-Ying Chung, MD; Tu-Hsueh Yeh, MD. Effects of Balance Training on Hemiplegic Stroke Patients. Med J 2002;25: 583-90.
6. Duncan M. Muscle activity of the upper and lower rectus abdominis during exercises performed on and off a Swiss ball. J Body w Mov Ther. 2009 Oct; 13(4): 364-7.
7. Akshatha Nayak, Karthik babu S, Vijayakumar K, Does Training on Swiss Ball Improve Trunk Performance after Stroke? - A single blinded, quasi experimental study design. Indian Journal of Physiotherapy and Occupational Therapy - An International Journal. 2012; 6(1): 172-5.

8. Ted J Stevenson. Detecting change in patients with stroke using the Berg Balance Scale. Australian Journal of Physiotherapy 47: 29-38.
9. Karthik babu S, Nayak A, Vijayakumar K, Misri Z K, Suresh BV, Ganesan S. Comparison of physio ball and plinth trunk exercises regimens on trunk control and functional balance in patients with acute stroke: A pilot randomized controlled trial. Clin Rehabil. 2011 Aug 1; 25(8):709-19.

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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

MICROCURRENT THERAPY AS A POTENTIAL MEASURE FOR TREATMENT OF PRESSURE ULCER - A CASE REPORT

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Microcurrent Therapy,
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ABSTRACT

Background and Purpose: Electrical stimulation has been used in clinical practice to accelerate wound healing for which positive results have been reported in clinical studies. The Purpose of the case report is to document the clinical effects of application of microcurrent stimulation in healing of pressure ulcer in a chronic bedridden patient. **Case Description:** The Patient was a 53 year old male who was diagnosed to have Cerebro vascular accident with Intraventricular hemorrhage and subsequent hydrocephalus underwent external ventricular drainage and Ventriculo peritoneal shunting before one year. Current evaluation demonstrated primary impairments of muscle weakness in right upper and lower limbs associated with activity limitation of bed mobility and ambulation. The patient also had secondary impairments of grade IV pressure ulcer in the sacral region. A plan of care was developed to focus on the management of primary and secondary impairments along with application of microcurrent electrical stimulation for management of pressure ulcer. **Outcomes:** The patient had benefits with significant reduction in the surface area of the pressure ulcer following 8 weeks of microcurrent stimulation. The Pressure Ulcer Scale for Healing (PUSH) showed a significant change from a score of 15 at week one, to a score of 6 at the end of 8 weeks. **Discussion:** Pressure ulcers are one of the most common issues in the elderly and immobile individuals, which constitute a major psychological and physiological burden with reduced quality of life. Among the treatment options, this research report focuses on the beneficial effects of Microcurrent electrical stimulation, based on its results in pain control, edema control and wound healing.

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INTRODUCTION

Microcurrent therapy (MCT) is a form of low-intensity, direct current that delivers monophasic or biphasic pulsed micro amperage currents across the skin, below the sensation threshold which is a novel treatment for different pain disorders and wounds (Koopman, 2009). It has been used to regenerate the internal bioelectrical activity of injured tissues and has been consistent with that of endogenous currents that act at the cellular level. Application of microcurrent can influence the behaviour of cells involved in healing processes such as migration, proliferation and production of proteins and cytokines. At the tissue level, it can promote angiogenesis, neural sprouting and increase the rate of tissue synthesis (Poltawski, 2009). Microcurrent therapy results in accelerated healing of wounds compared with sham therapy.

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Sufficient literature has reported positive outcomes as the result of application of microcurrent therapy in a range of pain syndromes, venous ulcers and open wounds. In this case report we have provided description about application effects of microcurrent therapy for healing of pressure ulcer wounds. A pressure ulcer, also known as pressure sore, decubitus ulcer, or bedsore, is defined as a localized injury to the skin and/ or underlying tissue occurring most often over a bony prominence and caused by pressure, shear, or friction forces alone or in combination. Pressure ulcers represent a major burden to patients and caretakers and have a detrimental effect on patients' quality of life (Grey, 2006). Pressure ulcers are classified into four categories: Stage I describes intact skin with nonblanchable erythema; Stage II pressure ulcers have partial-thickness skin damage with possible blister formation, but no subcutaneous tissues visible; Stage III pressure ulcers have full-thickness skin loss with subcutaneous fat exposed but no muscles, bones, or tendons visible; Stage IV pressure ulcers have tissue loss with exposure of muscles, bones, tendons, or vital organs (Dealey, 2006).

Pressure ulcer prevention aims to eliminate or reduce factors that predispose a person to pressure ulcer development. Strategies commonly used include other than regular risk assessment, use of special pressure relieving support surfaces, regular repositioning and turning, local skin care, nutrition support, removal of necrotic tissue, prevention of skin maceration, control of infections, which encourages soft tissue repair (Reddy, 2006). The purpose of the case report is to identify the effects of application of microcurrent therapy along with routine care for healing of pressure ulcer.

CASE DESCRIPTION: The patient was a 53 years old male who had been present with systemic hypertension over a period of 1 year, diagnosed to have an episode of cerebro vascular accident – interventricular hemorrhage, intracerebral hemorrhage and a subsequent episode of hydrocephalus before 1 year. He subsequently underwent conservative management for CVA and surgical treatment for hydrocephalus with external ventricular drainage followed by ventriculo peritoneal shunting performed by a neurosurgeon. The Patient underwent post surgical care and inpatient rehabilitation for a period of 85 days and was discharged to home from a tertiary care hospital. After a period of 6 months, the patient was admitted for provision of rehabilitation care. During that time, patient is confined to bed with complaints of weakness of muscles of both upper and lower extremities, decreased bed mobility, pressure ulcer in the sacral region and was completely dependent for performing activities of daily living (ADL). A review of systems demonstrated normal higher mental functions along with impairments in motor, sensory and integumentary functions. He had severe neurological impairments with a score of 40/56 in Scandinavian Stroke Scale (SSS), had modified dependence in functional activities with a score of 80/126 on the Functional Independent Measure (FIM).

Integumentary system examination revealed that the patient has grade IV pressure ulcer in the sacral region – Full thickness skin and tissue loss with exposure of muscles, tendons, bones and vital organs. The pressure ulcer scale for healing (PUSH tool) is a commonly used tool developed by the NPUAP, which grades pressure ulcers based on size of wound, wound bed tissue type, and exudate amount.(6)The pressure ulcer scale for healing (PUSH tool) Version 3.0 was used to assess the extent of ulcer. The patient had a PUSH score of 15 initially with a score of 10 in the Length X Width component as the wound had a total surface area of 46 cm^2, a score of 2 in the exudates component with moderate amount of exudate and a score of 3 in the tissue type component with slough tissues.

Intervention: The patient underwent regular physiotherapy treatment with short term goals to improve voluntary motor control, bed mobility and transfers, long term goals being set towards improvement of locomotor ability and independent activities of daily living. For the treatment of pressure ulcer, regular positioning care and saline dressing were the primary treatment choice along with application of microcurrent therapy. An intelect combination therapy unit was used to deliver microcurrents through reusable carbon-impregnated silicon rubber electrodes, with intensity within range of 20 – 50μA and negative polarity. The electrodes are placed such that it covers the surface area of the wound. The patient received microcurrent therapy for a duration of 20 minutes per session per day, six days a week for eight weeks. The patient underwent positioning care with regular change of position for every 2 hours and wound care with saline dressing for two times a day. During the course of treatment, the patient did not have/report any adverse events.

Table 1. Pressure Ulcer Scale for Healing (PUSH) weekly scores

Variables	On Initial Assessment	2nd week	4th week	6th week	8th week
Length X width	10	9	8	7	5
Exudate amount	2	1	1	0	0
Tissue type	3	3	3	2	1
PUSH total score	15	13	12	9	6

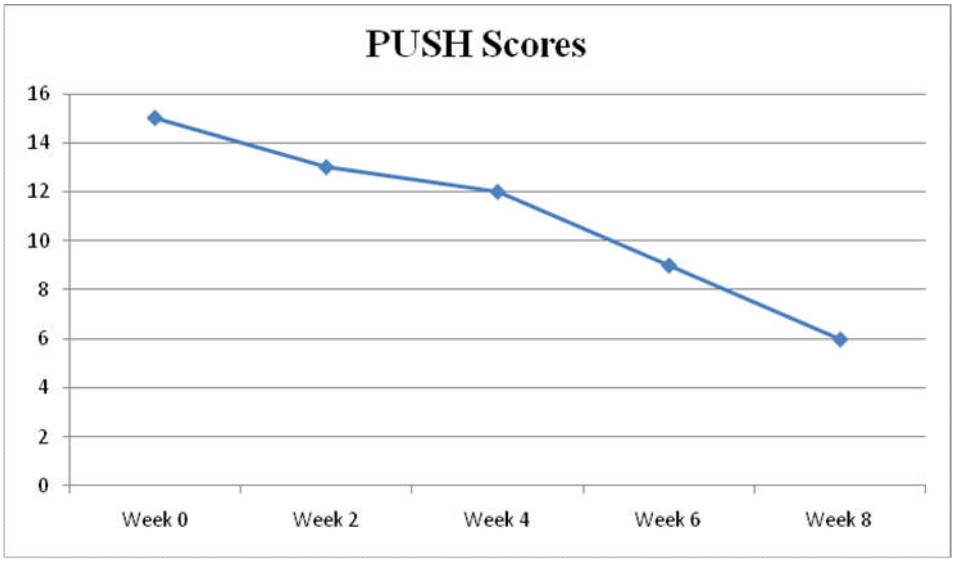


Figure 1. Graphical representation of PUSH scores monitored weekly

Outcome Measures

The pressure ulcer scale for healing (PUSH tool) Version 3.0 was used to assess the healing of ulcer. The PUSH model provides an accurate, simple, and clinically useful way to measure progress toward wound healing. Assessment of the ulcer characteristics include surface area, exudate amount (none, light, moderate, and heavy), and surface appearance (necrotic tissue, slough, granulation tissue, epithelial tissue, and closed). Each parameter is scored, and the sum of the three yields a total wound status score.

RESULTS

The pressure ulcer wound was monitored every week with PUSH score. At the end of six weeks of application of microcurrent electrical stimulation, the following results were obtained: (i) reduction in the wound surface area from the initial size of 46 cm^2 to 2.5 cm^2, ii) the exudates from the wound were completely absent, iii) Wound was completely covered with epithelial tissue from the initial stages of slough tissue. The findings resulted in a change of total PUSH score from 15 at day 1 to a score of 6 at the end of eight weeks.[1]

DISCUSSION

In this case report, we have reported the effects of microcurrent electrical stimulation on healing of pressure ulcer in an individual with severe mobility impairments. Previous studies have documented that adequate mobility is one of the efficient strategy in the process of healing of pressure ulcer.(5)To accelerate wound healing process in an individual with impaired mobility, the intervention strategies such as utilisation of pressure relieving support surfaces, adequate nutrition, wound care and regular positioning care is alone inadequate. In scenarios where endogenous healing has failed or healing has become obsolete, adjunct use of microcurrent therapy which is the transcutaneous delivery of miniature pulsating current that mimics the currents generated in the body at the cellular level, also known to stimulate cellular physiology and growth may be favorable (7)[2.]

Along with the use of conventional saline dressing and regular positioning, microcurrent therapy have resulted in reduction in wound surface area, improvement in tissue healing and growth in the wound site, demonstrated by a gradual decrease in the PUSH score through the weeks. At the end of eight weeks of microcurrent application, the total PUSH score had shown a change of 9 points from the time of initial assessment, a magnitude of change of 60%. The wound surface area has been reduced by 94%, with a change from initial size of 46cm^2 to 2.6 cm^2 at the end of eight weeks. Microcurrent application have been identified to physiologically help in re-establishment of tissue bio-electricity by imitating endogenous electrical signals that can guide cellular behavior and the proposed mechanisms of galvanotaxis which is attraction of positively/negatively charged cells towards the opposite pole of an electric field, thereby increasing transportation, cell migration, proliferation and synthesis of new tissue which are essential in the healing process (8,9).

Experimental studies involving the use of microcurrent electrical stimulation in larger specific population targeting wound healing are suggested. Therefore, the microcurrent electrical stimulation is considered an alternative for wound treatment specifically with in those with failed natural healing, reparation difficulties, devitalized tissue like pressure, diabetic, stasis and arterial ulcers.

Conclusion

These results indicate that microcurrent therapy has a positive effect in healing of pressure ulcers and it is preferable because of its easy application, acceleration in the healing and low cost.

Conflict of Interest: Ethical approval has been obtained for the case report from the Institute of Human ethics committee, PSG Institute of Medical Sciences and Research.

REFERENCES

Balakatounis KC., Angoules AG. 2008. Low-intensity electrical stimulation in wound healing: review of the efficacy of externally applied currents resembling the current of injury. Eplasty.8.

Dealey C., Lindholm C. 2006. Pressure ulcer classification. InScience and practice of pressure ulcer management (pp. 37-41). Springer, London.

Grey JE., Harding KG., Enoch S. 2006. Pressure ulcers. Bmj. Feb 23;332(7539):472-5.

Koopman JS., Vrinten DH., van Wijck AJ. 2009. Efficacy of microcurrent therapy in the treatment of chronic nonspecific back pain: a pilot study. The Clinical journal of pain. Jul 1;25(6):495-9.

Lessiani G., Galati V., Franzone G., Iodice P. 2014. Efficacy of modulated microcurrent stimulation in pressure ulcers treatment: A monocentric, prospective, double-blind, randomized study. *J Nov Physiother.,* 4(224):2.

Nair HK. 2018. Microcurrent as an adjunct therapy to accelerate chronic wound healing and reduce patient pain. Journal of wound care. May 2;27(5):296-306.

Poltawski L., Watson T. 2009. Bioelectricity and microcurrent therapy for tissue healing – a narrative review.Physical Therapy Reviews., 14(2):104–14.

Reddy M., Gill SS., Rochon PA. 2006. Preventing pressure ulcers: a systematic review. Jama. Aug 23;296(8):974-84.

Stotts NA., Rodeheaver GT., Thomas DR., Frantz RA., Bartolucci AA., Sussman C., Ferrell BA., Cuddigan J., Maklebust J. 2001. An instrument to measure healing in pressure ulcers: development and validation of the pressure ulcer scale for healing (PUSH). The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. Dec 1;56(12):M795-9.

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Physiatrist's Management of Diabetic Foot Ulcer

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ABSTRACT

Diabetes is a metabolic disease that affects multiple systems. Even within the nervous system the manifestations of this disease can be wide and varied. Foot disorders are a main source of morbidity and a leading cause of hospitalization for person with diabetes mellitus. Ulceration, infection, gangrene and amputation are significant complications of diabetes. Although not all foot complications can be prevented, dramatic reductions in their frequency have been obtained through the implementation of multidisciplinary team approach to patient management. The objectives of diagnosis and treatment of diabetic foot sequelae center around maintaining the patient as an ambulatory, productive member of society or returning the patient to that state as quickly and safely as possible. This may at any time require the expertise of a number of different specialists on the diabetic foot care team and a Physiatrist is one among them. The physiatrist by virtue of his or her training is uniquely suited to serve as a primary member of a multidisciplinary team for the management of diabetic foot disorders and able to recognize impending diabetic foot complications. Total Contact Cast is considered the Gold standard to assist healing for active diabetic ulcerations by providing weight (pressure) redistribution and friction/shear control. Multiple randomized controlled clinical studies have been conducted which illustrate that the majority of diabetic foot ulcers heal with the use of TCC in a mean time of 35-52 days for 85-95% of patients.

Keywords: Diabetes mellitus, Diabetic Ulcer, Total contact cast

INTRODUCTION

Patients with diabetes are prone to multiple complications such as diabetic foot ulcer. Diabetic foot ulcer is a common complication of diabetes that has shown an increasing trend over previous decades. In total, it is estimated that 15% of patients with diabetes will suffer from diabetic foot ulcer during their lifetime. [1] Although accurate figures are difficult to obtain for the prevalence of diabetic foot ulcer, the prevalence of this complication ranges from 4%-27%. [2-4]

To date, diabetic foot ulcer is considered as a major source of morbidity and a leading cause of hospitalization in patients with diabetes. It is estimated that approximately 20% of hospital admissions among patients with diabetes are the result of diabetic foot ulcer. [5] Indeed, diabetic foot ulcer can lead to infection, gangrene, amputation, and even death if necessary care is not provided. [6] On the other hand, once diabetic foot ulcer has developed, there is an increased risk of ulcer progression that may ultimately lead to amputation. Overall, the rate of lower limb amputation in patients with DM is 15 times higher than patients without diabetes. It is estimated that approximately 50% - 70% of all lower limb amputations are due to diabetic foot ulcer. [1] In addition, it is reported that every 30 second one leg is amputated due to diabetic foot ulcer in worldwide. [2]

Foot ulceration is the most common single precursor to lower extremity amputations among persons with diabetes. As a result of neuropathy, the foot can become deformed. This happens through two routes of reasons. The first is that the neuropathy causes paralysis of small muscles of foot, which result in clawing of the toes. Clawing of the toes causes prominence of the metatarsal heads in the

bottom of the foot as well as the knuckles on the dorsum of top of the foot. The neuropathy also causes diminished sensation. As the prominent metatarsal heads on the plantar aspect of the foot are subjected to increased pressure because of diminished sensation, the skin will begin to hypertrophy and become callused. The callused skin can be subjected to shear forces. The forces will cause a separation between the layers of the skin, which will fill with fluid, which can then become contaminated and infected. The pressure can also cause primary breakdown of the skin in these areas and the result is a foot ulcer. Once the initial breakdown and contamination occurs, the foot then can go on to significant problems of infection. Thus increased biomechanical stress is one of the most important pathways leading to ulceration in patients with neuropathic and neuroischemic foot ulcers.

Traditionally, diabetic foot ulcers are treated with regular dressings with frequent debridement with minimal weight bearing on the affected foot. The key element of any treatment program designed to heal these wounds is effective reduction in pressure (offloading). Offloading of the wound is one of the key elements in treating diabetic foot ulcers. Several offloading devices are available, such as walkers, half shoes, orthoses, felted foam and the total contact casting (TCC). Though offloading can be achieved by several orthotics, most of these are removable and strict compliance is not achieved thus reducing their effectiveness.

TOTAL CONTACT CASTING:

TCC is a casting technique that is used to heal diabetic foot ulcers. The cast is used to heal diabetic foot ulcers by distributing weight along the entire plantar aspect of the foot. It is applied in such a way as to intimately contact the exact contour of the foot. Hence the designation "Total Contact Cast". By relieving the bony prominent areas of pressure, the ulcers are permitted to heal if the cast is applied in such a way that the patient can remain ambulatory during the treatment of the ulcer.

Pressure is expressed in terms of force or pounds over the area per square inch. Therefore, if the weight-bearing area is enlarged the pressure per unit of weight bearing area diminished. In this way the pressure area is enlarged and the pressure per unit of the weight bearing area is diminished. So the pressure which has been concentrated on the bony prominences is distributed over the entire plantar aspect of the foot, allowing reversal of the mechanism that caused the ulcer to occur. Total Contact Cast minimizes ground reactive pressure and shear (friction), eliminates the propulsive phase of gait, shortens stride length, allows for healing while ambulating and forces patient compliance.

Total contact cast has the advantage that it cannot be easily removed by the patient and limits activity which promotes rapid healing of ulcers. When correctly applied, it has proven not only to interrupt the chain of events in the pathogenic pathway that produces ulceration but it also changes the histology of the ulcer shifting it from a chronic inflammatory state to a more evoluting condition. [7] Healing of ulcers using TCC takes approximately 6-8 weeks, during which the cast is replaced on a weekly basis. The cast is removed when complete healing is achieved, after which is recommended that the diabetic patient wear specially designed shoes that will relieve pressure over the ulcer healed area.

CAST APPLICATION:

Application of the cast should be as quick and smooth as possible to avoid lamination and thus weakening of the cast. The patient is kept in prone position. Placing the patient prone is the easiest way to apply the cast. Also the patient is more comfortable as he/she will not get cramps in the thigh or hip. The prone position allows the assistant to hold the appropriate position of the foot to the leg, usually ninety degrees to the leg. This position also allows the gastro-soleus complex to shift proximally

on the leg thereby removing their bulk allowing for the cast to fit more snugly. The cast should end at the widest point of the calf and should not end the cast at the knee level as this could impinge the common peroneal nerve.

Table 1: Materials required for Total Contact Casting (TCC)

Materials required	
3" or 4" stockinette 1- 1 " tape Adhesive felt- 4" wide x 20-30" long 3" or 4" webril 1-3" roll plaster casting tape 2-4" rolls plaster casting tape 1-2" roll synthetic casting tape 1-3" roll synthetic casting tape 2-4" rolls synthetic casting tape	1 walking heel Silicone (clear-site) protectors Foam toe protectors Precut plywood foot plate

PROCEDURE:

Total contact casting is a precise procedure that requires skillful application. The ulcer is debrided in standard fashion and if a dressing has to be applied, it is held in position with one inch paper tape. Circumferential dressings are not recommended. The patient is placed in the prone position. Bony prominences, the posterior heel and achilles tendon are padded. One fourth inch adhesive felt is applied to the leg and foot starting anteriorly on the tibial tuberosity crossing the ankle, ending on the dorsum of the foot. Stockinet is placed from the toes to just below the knee. One to two inches are left past the toes, folded dorsally and held in position with one-inch tape. Folds are created at the ankle and the excess material is cut away and the ends of the stockinet are taped with one inch tape. One fourth inch foam is placed over the toes and past the metatarsophalangeal joints dorsally and plantarly to protect the toes and limit MPJ tendonous motion. The assistant stands lateral to the extremity and holds the foot 90 degrees to the leg while the knee is flexed 90 degrees. One thin roll of webril is snugly placed over the foot and leg with minimal overlap. One or two rolls of plaster rolls are placed from the distal aspect of the toes ending just below the fibular head. The toes should be fully covered with the plaster so that they do not move once the cast dries. A 5" x 30" splint double folded at the distal 1/3 is now applied plantarly from the toes ending posteriorly on the leg. The precut plywood is placed on the plantar aspect of the foot. It should at least meet the entire plantar aspect of the foot. Multiple precut 3" to 4" pieces of plaster material are used to fill voids in areas where the foot does not meet the wood so that absolutely no rocking of the wood on the foot is noted. Four-inch synthetic rolls are used to cover the foot and leg. The walking heel is centered in the middle of the foot in a line just distal to the long axis of the tibia. The foot and leg are held in this position for a minimum of 5 minutes to allow the cast to dry. The patient may now be placed in the supine position and sits for another 20 minutes while the cast dries. It is preferred to maintain non weight bearing for 24 hours until the cast sets up well. The cast is changed as often as necessary but at least weekly to check for healing and other ulcer formation. [8]

INDICATIONS FOR TCC:

The patient must be non-infected, have an adequate blood supply to heal (ABI >0.7) and the ulcer must be Wagner Grade I or II.

CONTRA INDICATIONS FOR TCC:

Acute infection, severe ischemia, claustrophobia, Wagner Grade III or IV ulcers and wounds that are probe to bone, tendon or capsule.

DISADVANTAGES:

Although a TCC seems a highly attractive offloading modality, several disadvantages have been reported: new ulcers may occur, daily wound care is not possible, mobility is impaired, costs may be relatively high, and specialized staff seems necessary. [9] The TCC technique is not very effective in reducing elevated plantar pressures at the heel region. In some cases the ulcer may not heal during cast treatment (cast failure). In case of prolonged casting, joint rigidity and muscular atrophy have been documented. [10]

CONCLUSION

Diabetic foot ulcer healing may be delayed if the ulcer is subjected to constant pressure while walking. Total contact casting provides optimal wound healing conditions by ensuring that the ulcer is not further traumatized and also prevents the development of new ulcers in the bony prominences of the foot. Hence TCC is an excellent procedure for healing diabetic foot ulcers. The Physiatrist plays an integral role in the management of new or impending diabetic foot lesions.

REFERENCES

1. Leone S, Pascale R, Vitale M, Esposito S. Epidemiology of diabetic foot Infez Med. 2012;20 Suppl 1:8–13.
2. Richard JL, Schuldiner S. Epidemiology of diabetic foot problems. La Revue de medecine interne. 2008 Sep;29:S222-30.
3. Nather A, Bee CS, Huak CY, Chew JL, Lin CB, Neo S, Sim EY. Epidemiology of diabetic foot problems and predictive factors for limb loss. Journal of Diabetes and its Complications. 2008 Mar 1;22(2): 77-82.
4. Bakri FG, Allan AH, Khader YS, Younes NA, Ajlouni KM. Prevalence of diabetic foot ulcer and its associated risk factors among diabetic patients in Jordan. Jordan Medical Journal. 2012 Apr;171(785):1-6.
5. Fard AS, Esmaelzadeh M, Larijani B. Assessment and treatment of diabetic foot ulcer. International journal of clinical practice. 2007 Nov;61(11):1931-8.
6. Snyder RJ, Hanft JR. Diabetic foot ulcers-effects on quality of life, costs, and mortality and the role of standard wound care and advanced-care therapies in healing: a review. Ostomy/wound management. 2009 Nov 1;55(11):28.
7. Piaggesi A, Macchiarini S, Rizzo L, Palumbo F, Tedeschi A, Nobili LA, Leporati E, Scire V, Teobaldi I, Del Prato S. An off-the-shelf instant contact casting device for the management of diabetic foot ulcers: a randomized prospective trial versus traditional fiberglass cast. Diabetes care. 2007 Mar 1;30(3):586-90.
8. Jimenez A. Total Contact Casting. Update. 2003:282.
9. Armstrong DG, Lavery LA. Evidence-based options for off-loading diabetic wounds. Clinics in podiatric medicine and surgery. 1998 Jan;15(1):95-104.
10. Caravaggi C, Faglia E, De Giglio R, Mantero M, Quarantiello A, Sommariva E, Gino M, Pritelli C, Morabito A. Effectiveness and safety of a nonremovable fiberglass off-bearing cast versus a therapeutic shoe in the treatment of neuropathic foot ulcers: a randomized study. Diabetes care. 2000 Dec 1;23(12):1746-51.

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Effectiveness of Intense Sensory Motor Stimulation on Improving Functional Recovery in Post Stroke Individuals

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ABSTRACT

Introduction: Rehabilitation measures help to restore lost activities, improve quality of life and decrease the long term economic cost of stroke. Realistic goal setting, interdisciplinary team approach and the active participation of both patient and family members are important for the success of stroke rehabilitation. The primary objective of this study is to investigate the effects of intense sensory motor stimulation, given in the form of electrical stimulation, massage and re-education exercise programme, in augmenting recovery in stroke patients.
Methods: A prospective single group pre and post test study design was conducted with a total of 29 hemiparetic patients who provided informed consent. All the participants received the therapeutic measures of electrical stimulation, massage and re-education exercises including gait training and ADL retraining until discharge. The Neurological and Functional recovery were measured using Scandinavian Stroke Scale score (SSS) and Functional Independence Measure score (FIM) respectively.
Results: An Analysis of variance 'F' Value of 37.31 and 43.94 which are significant at 0.01 level shows that there is a significant difference between scores at admission, discharge and follow up in SSS and FIM scores respectively. The Pearson correlation coefficient 'r' value of 0.62, 0.81 and 0.97 at three time periods suggests that a moderate to strong negative correlation exists between SSS and FIM scores which are significant at 0.01 levels.
Conclusion: A significant level of neurological and functional recovery was obtained in the study group during the time of discharge and in the follow up period indicative of effectiveness of intense sensory motor stimulation in augmenting motor recovery in stroke patients.

Keywords: Sensory Motor Stimulation, Functional Recovery, Stroke Patients

INTRODUCTION

Stroke is the most disabling chronic condition. The prevalence rate for hemiplegia in South India was reported to be 56.9 per 10000. [1,2] WHO defined stroke as "rapidly developed clinical signs of focal or global disturbance of cerebral function; lasting more than 24 hours; leading to death with no apparent cause other than vascular origin". [3] The 24 hours threshold in the definition excludes transient ischemic attacks (TIA). Modern advances in stroke research aid better management of stroke patients. In the past, recovery from motor deficit after a stroke was a puzzling scientific question. Now it has been found out that, neuronal organization, dynamic changes in the somatotopia of primary motor cortex, recruitment of remote motor cortices, and participation of associative cortices are clearly part of rearrangement process. [4] It is likely that such mechanisms represent the basis of clinical recovery of stroke patients.

Rehabilitation measures help to restore lost activities, improve quality of life and decrease the long term economic cost of stroke. [5] Realistic goal setting,

interdisciplinary team approach and active participation of both patient and family members are important for the success of stroke rehabilitation. [6] It is desirable to start rehabilitation programmes to stroke patients once they are medically stable. [7] Various programmes of treatment, for patients with motor control problems, were developed in the past based on neurophysiological and developmental research of the time as well as careful observation of the responses made by patients when being handled.

Review of literature shows that there will be natural, spontaneous neurological recovery after stroke. The mechanism to explain recovery is neuroplasticity. Brain plasticity is the ability of the nervous system to modify its structural and functional organization. The two most plausible forms of plasticity are collateral sprouting of new synaptic connections and unmasking of previously latent functional pathways. [8] Experimental evidence indicates that plasticity can be altered by several external conditions, including pharmacological agents, electrical stimulation and environmental stimulation. [9]

A comprehensive critical review of medical literature reveals that rehabilitation interventions have variable relationships with functional outcome after stroke. 1) Increased functional skills on admission to rehabilitation, early initiation of rehabilitation services, and rehabilitation in an interdisciplinary setting after stroke appear to have strong relationship with improved functional outcome at hospital discharge and follow-up. 2) The use of specialized type of therapy services and greater intensity of therapy services appear to have a weak relationship with improved functional outcome at hospital discharge and follow-up. 3) The current literature is too limited to allow an assessment of the relationship of specific types of non-inpatient rehabilitation services after stroke and functional outcome. [10-12]

Current stroke rehabilitation practice throughout the world is based on a number of approaches that were developed independently by several pioneers in the 1950's. Prior to the 1950's the physical treatment of the patients following stroke focused on encouraging the patients to use the unaffected side of the body to compensate for the disabilities of the affected side. The affected limbs were not a prime focus for treatment.

During the 1950's a number of new physiotherapy approaches emerged. These were developed by practitioners who were dissatisfied with the results of practice at the time and were interested in treating the paresis resulting from stroke. The work of many of these pioneers e.g. Bobath [1969], Brunnstrom[1961, 1970], Knott & Voss [1968] and Rood [1969] remains influential today. They developed their programmes primarily from observation and experience. Some of the approaches were associated with neuro physiological concepts current at that time; but others were developed observation alone and then a suitable theory was adapted to explain the effects.

Thus, several different physiotherapy approaches are used in the management of the patients who have suffered a stroke but these approaches are mutually exclusive and a rarely delivered in the uniform purist manner. The theoretical basis of the individual approaches remains unsubstantiated. All of the approaches aim to improve motor control and all of them demand that the therapist has a specialized training in the individual concept.

Many different electrical stimulation applications have been explored since the pioneering work of Liberson et al on foot drop. [13] So in this study electrical stimulation is used to augment motor recovery in patients being rehabilitated after acute stroke, in addition to olive oil massage, and re-education exercise programme.

The primary objective of the study is to investigate the effects of intense sensory motor stimulation, given in the form of electrical stimulation, massage and re-education exercise programme, in

augmenting recovery in stroke patients. The secondary objective of the study is to investigate the relationship between neurological and functional recovery in stroke patients.

METHODS

DESIGN

A prospective single group pre and post test study design. A total of 29 hemiparetic patients admitted to Government Institute of Rehabilitation Medicine, Chennai Medical college, Chennai were recruited. The participants received the intervention until they were discharged.

PARTICIPANTS

All stroke patients who were admitted to the Government Institute of Rehabilitation Medicine, Chennai Medical College, Chennai were screened and recruited on providing consent. No specific selection criteria with reference to age, side, and severity of paralysis, aphasia, neglect, incontinence and cognitive function were applied. But stroke cases of longer duration were not included in this study. On admission to the rehabilitation facility, age, and gender were documented for each patient as were the type and side of the cerebral lesion. The duration between stroke onset and rehabilitation were also recorded.

INTERVENTION

A) ELECTRICAL STIMULATION

The muscles stimulated in the upper limb are deltoid, supraspinatus, biceps, triceps, common flexors of forearm, common extensors of forearm, intrinsic muscle of hand. The muscles stimulated in the lower limb are Gluteus maximus, Quadriceps and Tibialis anterior. Each muscle is stimulated for 10 minutes. The active electrode is kept over the motor point of the muscle to be stimulated. The reference electrode is kept over the nearby bony point. An Electrical Stimulator which provides faradic current with a pulse width of 300 ms, intensity of 100 v and a frequency of 50Hz was used. The muscle contraction/relaxation time ratio is set at 5/10 sec. The patients received electrical stimulation daily for a duration of 1 ½ - 2 hours. [14-18]

B) MASSAGE AND RANGE OF MOTION EXERCISES

After electrical stimulation, olive oil massage was done to the affected upper and lower limbs. Effleurage or stroking massage involves lightly running the hand over the skin. This was performed with either superficial or deep pressure and provides different effects depending on the depth of treatment. Deep stroking involves mechanical effects and should be performed with continuous contact of the hands from distal to proximal on the extremities, the back, or the neck. Once the proximal extent is reached the hands can be drawn back to the distal position applying light contact or no contact at al. [19]

Then PROM exercises were done to the affected upper and lower extremities to prevent the occurrence of joint contracture. Once the patient developed volitional movement, strengthening programme was instituted with optimal movement pattern. ADL training to the upper limbs and gait training to the lower limbs were also administered.

OUTCOME MEASURES

After thorough neurological examination, CT brain was done to confirm the diagnosis. Then the neurological status was assessed using Scandinavian Stroke Scale score [20] and functional status was evaluated using Functional Independence Measure score. [21] Then the treatment programme was started. Outcomes were assessed in every two weeks. The patients were followed up to 6 months.

STATISTICAL ANALYSIS

To analyze the data, two types of statistical techniques are used. They are descriptive statistics and inferential statistics. The averages of baseline characteristics were calculated using mean and standard deviation. One way ANOVA was used to analyse the differences in neurological recovery and functional recovery at three different time periods

(admission, discharge and follow up). Paired‘t’ test was used to identify the difference in the scores between admission, discharge and follow up. The relationship between functional recovery and neurological recovery at the time of admission, discharge and follow up were assessed using Pearson correlation coefficient ‘r’.

RESULTS

The descriptive statistics of the mean scores of Scandinavian Stroke Scale (SSS) and Functional Independence Measure (FIM) is represented in Table 1. In both measures, the scores on admission is found to be less when compared to those at discharge and follow up. The standard deviation also seems to be within the normal limits. It does not skew very much. So this distribution can be considered as normal distribution.

Table 1 The mean and standard deviation of Scandinavian Stroke Scale (SSS) scores and Functional Independence Measure (FIM) scores of stroke patients at different stages

S.no	Stages	No. of cases	SSS Mean ± SD	FIM Mean ± SD
1	On admission	29	32.82 ±9.16	75.79 ± 19.22
2	On discharge	29	47.9 ± 7.068	105.17 ± 12.75
3	On follow up	29	53.13 ± 6.85	115.37 ± 12.00

An Analysis of variance results to identify the difference in the SSS scores at admission, discharge and follow up, shows that the F value 37.31 found to be significant at 0.01 level indicates, that stroke level of different stages are significantly different from one another. The mean scores of SSS increased from 32.82 ±9.16 on admission to a score of 47.9 ± 7.06 at discharge and increased further on follow up to a score of 53.13 ± 6.85. Similarly ANOVA results of FIM scores shows that the F value of 43.94 which is significant at 0.01 level reveals that, there is significant difference in the functional independence levels among the three levels. The mean score ascends from 75.79 ± 19.22 at the time of admission to 105.17 ± 12.75 at the time of discharge and to a score of 115.37 ± 12.00 at follow-up. Both the scores show an improvement in the order of follow-up > discharge > admission.

Table 2 ANOVA results of Scandinavian Stroke Scale scores in different time periods of stroke patients is shown below

S. No	Source of variations	Sum of squares	DF	Mean square	F	Level of significance “p”
1.	Between groups	6269.63	2	3134.81		
2.	Within groups	5366.27	84	63.88	37.31	>0.01

Table 3 ANOVA results of Functional Independence Measure scores of stroke patients at different levels

S. No	Source of variations	Sum of squares	DF	Mean square	F	Level of significance “p”
1.	Between groups	24499.13	2	12249.56		
2.	Within groups	23415.73	84	278.75	43.94	>0.01

To identify whether there is any statistically important difference exists between the time of admission and discharge, discharge and follow up, student‘t’ test was used. Between admission and discharge, the SSS scores had ‘t’ value 6.79 which reveals that the difference is significant. Similarly the FIM scores had ‘t’ value 6.87 which is significant at 0.01 level indicates that the patients have significantly improved at discharge.

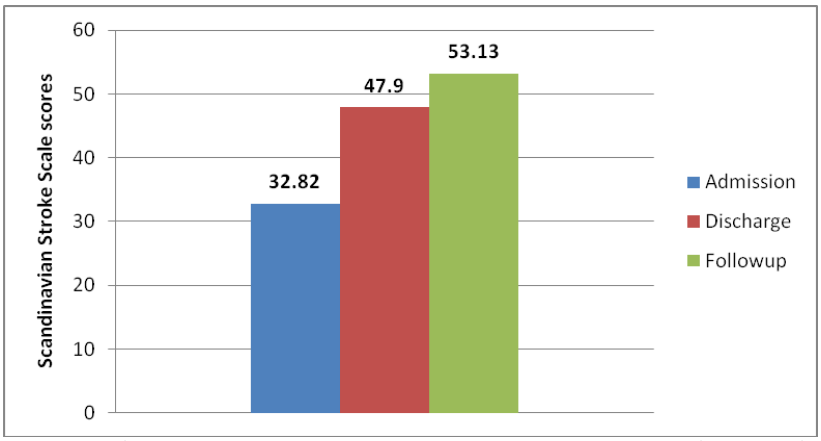
Table 4 The mean, standard deviation and ‘t’ value of Scandinavian Stroke Scale scores and Functional Independence Measure scores on admission and discharge

S. No	Outcome	On admission Mean ± SD	On discharge Mean ± SD	‘t’ value	‘p’ value
1.	SSS	32.82 ± 9.16	47.90 ± 7.68	6.79	>0.01
2.	FIM	75.79 ± 19.22	109.17± 12.75	6.87	>0.01

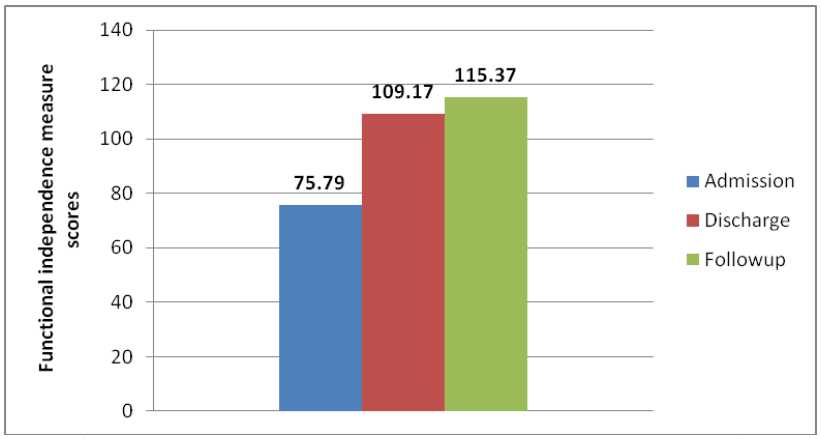
On comparison of scores between discharge and admission, both SSS and FIM scores were found to show a statistically significant difference among them with 't' values of 9.58 and 9.42 respectively. Hence the scores of SSS and FIM differed significantly between three time periods of evaluation.

Table 5 The mean, standard deviation and 't' value of Scandinavian Stroke Scale scores and Functional Independence Measure scores on admission and follow up

S. No	Outcome	On admission Mean ± SD	On follow up Mean ± SD	't' value	'p' value
1.	SSS	32.82 ± 9.16	53.13 ± 6.85	9.58	>0.01
2.	FIM	75.79 ± 19.22	115.37± 12.00	9.42	>0.01



Graph 1: Representation of Scandinavian stroke scale scores on admission, discharge and follow up of stroke patients



Graph 2: Representation of Functional Independence Measure scores on admission, discharge and follow up of stroke patients

Since the stroke level has got direct effects on the motor functions, the Pearson Correlation Coefficient was used to identify the relationship between the magnitude of the stroke and the level of functional independency at the time of admission, at the time of discharge and at the time of follow up.

Table 7 Correlation coefficient 'r' between Scandinavian Stroke Scale score and Functional Independence Measure score on admission, discharge and follow up

	On Admission	On Discharge	On Follow up
Correlation coefficient 'r' between SSS and FIM scores	0.62	0.81	0.97
Level of Significance	p>0.01	p>0.01	p>0.01

Based on the strength of correlation coefficient, the 'r' value of 0.62 between the scores at the time of admission and 'r' value of 0.81 at the time of discharge and 'r' value of 0.97 at the time of follow-up which are significant at 0.01 level indicate that a moderate to strong negative correlation exists between SSS and FIM scores. The above correlation

coefficient value suggests that, when the severity of stroke level increases, the functional independence level significantly decreases. Whereas, when there is improvement of the stroke status, the functional independence level will also increase significantly.
Since the total number of cases in certain conditions is found to be less, the non parametric tests were used to analyze the data. The percentage was calculated for each condition and the same was discussed.

Table 8 Shows the rate neurological recovery [SSS score] and functional recovery [FIM score] in cases of stroke at different levels of brain

S. No	Topography	On admission		On discharge		On follow up	
		SSS	FIM	SSS	FIM	SSS	FIM
1	Cortical level	57%	60%	85%	85%	93%	93%
2	Internal capsular level	53%	40%	85%	78%	85%	86%
3	Brainstem level	63%	72%	82%	92%	100%	99%

From the above table it is learnt that the neurological and functional recovery on discharge and follow-up are fair if the stroke is at the level of the internal capsule. The neurological and functional recovery on discharge and follow up are better if the stroke is at the levels of cortex or brainstem.

DISCUSSION

The above results indicate that the condition of stroke has significantly improved at the time of discharge and follow up when compared to the condition at the time of admission. It is possible to say that the different forms of "intense sensory motor stimulation" would have contributed much for the recovery of the stroke patients. Similarly even after the discharge, the improved condition was maintained significantly. This has proven the efficiency of the intense sensory motor stimulation in stroke rehabilitation. In the follow up it was found that most patients were able to maintain their functions and to make further improvements. Also their psychological well-being was also quite good.

The benefits of this recovery were maintained for a longtime, perhaps because all patients continued to do exercise. Some patients continued with their assigned exercise programme at the hospital and others continued exercise programme at home.

The above findings prove that the functional independence of stroke patients have improved very much at the time of discharge and follow up. The possible reason could be due to the attack of stroke, the movements of the extremities were lost. That could be the possible reason for their low level of functional independency. Since the stroke level is highly related with the motor function, it would have retarded the physical function. [22] Once the severity of the stroke level is decreased, the functional independency will also improve significantly. Another possibility could be the beneficial effects of exercise therapy for the upper limbs and motor training for the lower limbs, which could have improved the functional independency of the stroke patients during discharge and follow up. These results are in accordance with the intensive task specific training to improve lower limb function and gait in acute stroke individuals. [23, 24]

The management of stroke, so long a "Cinderella condition", is changing rapidly as new developments appear for acute treatment, rehabilitation and secondary prevention. Stroke is no longer an untreatable or unpredictable condition and it is vital that hospitals design appropriate systems to manage patients in an interdisciplinary environment. [25]

Though this study size was relatively small, a large treatment effect was demonstrated. In our study, the maximum improvement in the motor recovery was observed at 3 months. These results improved again slightly and remained constant up to the follow up period. Significant improvement in motor recovery in the stroke patients indicates that "intense

sensory motor stimulation" is an effective method of stroke rehabilitation to augment motor recovery. Electrical stimulation is a relatively low cost intervention. Concerns that subjects would find the treatment uncomfortable or inconvenient were unfounded. All subjects reported that the sensation of the muscle contraction following electrical stimulation and the resultant movement of the limb were encouraging. No stimulation induced complications requiring medical attention such as burns, falls or fractures occurred for any patient. A few subjects showed minor skin irritation transiently. Thus intense sensory motor stimulation programme could achieve a better functional outcome in stroke patients and could shorten the hospital stay for rehabilitation.

CONCLUSION

The following conclusions are drawn from the above study:

1. There was a significant level of neurological and functional recovery in the study group during the time of discharge and in the follow up period, when compared to their neurological and functional status at the time of admission. This shows the effectiveness of intense sensory motor stimulation in augmenting motor recovery in stroke patients.
2. The improvements in the neurological and functional status observed at the time of discharge were maintained during the follow-up period. Stroke cases with severe neurological damage and severe functional impairment as evidenced by low scores in the Scandinavian Stroke Scale score and FIM score on admission, required longer duration of treatment.
3. In the hemorrhagic stroke cases, the neurological and functional recovery at the time of discharge and follow up are less, when compared to thrombotic and embolic stroke cases. The neurological and functional recovery on discharge and follow up are better if the lesion is at the level of the cortical than at the capsular level.

REFERENCES

1. Bonita R. Epidemiology of stroke. *Lancet*. 1992; 339(8789):342-344.
2. Dalal P, Bhattacharjee M, Vairale J, Bhat P. UN millennium development goals: Can we halt the stroke epidemic in India?. Ann Indian Acad Neurol 2007;10:130-6
3. Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaborative study. *Bull World Health Organ*. 1980; 58(1):113-130.
4. Chollet F, Loubinoux I, Carel C, et al. Mechanisms of motor recovery after cerebrovascular accident. Revue Neurologique. 1999; 155(9):718-724.
5. Bohannon RW, Andrews AW, Smith MB. Rehabilitation goals of patients with hemiplegia. Int J Rehabil Res 1988; 11:181-3.
6. Kotila M, Waltimo O, Niemi ML, Laaksonen R, Lempinen M. The profile of recovery from stroke and factors influencing outcome. *Stroke*. 1984;15(6):1039-1044.
7. Watson LD, Quinn DA. Stages of stroke: a model for stroke rehabilitation. *Br J Nurs*. 1998;7(11):631-640.
8. Bach y Rita P. Central nervous system lesions: sprouting and unmasking in rehabilitation. *Arch Phys Med Rehabil*. 1981; 62(9):413-417.
9. Bach-y-Rita P. Brain plasticity as a basis of the development of rehabilitation procedures for hemiplegia. *Scand J Rehabil Med*. 1981; 13(2-3):73-83.
10. Heinemann AW, Roth EJ, Cichowski K, Betts HB. Multivariate analysis of improvement and outcome following stroke rehabilitation. *Arch Neurol*. 1987;44(11):1167-1172.
11. Ottenbacher KJ, Jannell S. The results of clinical trials in stroke rehabilitation research. *Arch Neurol*. 1993; 50(1):37-44.
12. Trialists'Collaboration SU. How do stroke units improve patient outcomes? A collaborative systematic review of the randomized trials. Stroke. 1997 Nov;28(11): 2139-44.
13. Liberson WT, Holmquest HJ, Scot D, Dow M. Functional electrotherapy: stimulation of the peroneal nerve synchronized with the

swing phase of the gait of hemiplegic patients. *Arch Phys Med Rehabil*. 1961; 42:101-105.
14. Faghri PD, Rodgers MM, Glaser RM, Bors JG, Ho C, Akuthota P. The effects of functional electrical stimulation on shoulder subluxation, arm function recovery, and shoulder pain in hemiplegic stroke patients. *Arch Phys Med Rehabil*. 1994; 75(1):73-79.
15. Peckham PH, Creasey GH. Neural prostheses: clinical applications of functional electrical stimulation in spinal cord injury. *Paraplegia*. 1992; 30(2): 96-101.
16. Rushton DN. Functional electrical stimulation. *Physiol Meas*. 1997; 18(4): 241-275.
17. Low and reed electrotherapy explained principles and practice: edition 2. Electrical stimulation of nerve and muscle. P.63.
18. Weingarden HP, Kizony R, Nathan R, Ohry A, Levy H. Upper limb functional electrical stimulation for walker ambulation in hemiplegia: a case report. *Am J Phys Med Rehabil*. 1997; 76(1):63-67.
19. Hofkosh JM (1985) Classical massage. In Basmajian JV (ed) Manipulation, Traction and Massage. Williams & Wilkins, Baltimore, pp 263–269.
20. Lindenstrøm E, Boysen G, Waage Christiansen L, à Rogvi Hansen B, Würtzen Nielsen P: Reliability of Scandinavian Neurological Stroke Scale. Cerebrovasc Dis 1991;1:103-107.
21. Franchignoni F, Tesio L, Martino MT, Benevolo E, Castagna M. Length of stay of stroke rehabilitation inpatients: prediction through the functional independence measure. *Ann Ist Super Sanita*. 1998; 34(4): 463-467.
22. Oczkowski W, Barreca S. Neural network modeling accurately predicts the functional outcome of stroke survivors with moderate disabilities. Arch Phys Med Rehabil 1997; 78:340-5.
23. Richards CL, Malouin F, Wood-Dauphinee S, Williams JI, Bouchard JP, Brunet D. Task-specific physical therapy for optimization of gait recovery in acute stroke patients. *Arch Phys Med Rehabil*. 1993;74(6):612-620.
24. Kwakkel G, Wagenaar RC, Koelman TW, Lankhorst GJ, Koetsier JC. Effects of intensity of rehabilitation after stroke. A research synthesis. *Stroke*. 1997; 28(8): 1550-1556.
25. Appelros P, Nydevik I. [For whom is training after stroke most beneficial? Selection method exists and should be used]. *Lakartidningen*. 1999; 96(19): 2343-2347.

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RESEARCH ARTICLE

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Improved ICA algorithm for ECG feature extraction and R-peak detection

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Summary

Electrocardiogram (ECG) signal transmission and monitoring plays a paramount role in long-term cardiac monitoring and analysis to provide remote health care in time, especially for the postoperative people and people in remote areas. The accuracy of ECG signals is of fundamental importance in cardiac diagnosis like R-peak detection. So we need to incorporate analytical methods in existing healthcare systems, to capture more meaningful ECG components and to represent physical cardiac sources more clearly. With this aim, hardware optimized FCAICA (fast confluence adaptive independent component analysis) algorithm is proposed to extract pure ECG components from the ECG mixtures. The extracted signals are then subjected to R-peak detection for further analysis. The proposed improved fast confluence adaptive independent component analysis (IFCAICA) method occupies less hardware resources, consumes low power, improves accuracy, and sensitivity in R-peaks detection. In 0.18 nm technology, the IFCAICA consumes 10.13 mW of power and operates with 3.4 MHz operating frequency.

KEYWORDS

ECG, independent component analysis, long-term monitoring, R-peak detection, VLSI

1 | INTRODUCTION

Electrocardiogram (ECG) is a conventional means of a biomedical signal for the finding heart abnormality. It is well known that ECG signals acquired noninvasively are widely used in clinical area for patient health monitoring including pacemaker follow-up and long-term monitoring.[1] In this industrialized world, millions of people die annually due to various coronary heart diseases and lack of timely detection of abnormal heart rhythms. To detect the heart abnormalities in time, the ECG measures should provide the cardiac signals without any mixtures or other disturbances. Real-time ECG monitoring system is a medical telemetry instrument used by medical personnel to monitor patients' ECG signal while allowing the patients to remain fully mobile and comfortable. It uses wearable ECG sensors to measure electrical signals generated from the patients' heart in hospitals. Figure 1 shows the architecture of Cloud-based remote cardiac monitoring system with the proposed IFCAICA (improved fast confluence adaptive independent component analysis) technique for future implementation in hospitals.

ECG signals are collected using wearable ECG electrodes from postoperative patients or remote patients and their data are sent to a preprocessing unit. This subsystem performs an important task of removing high-frequency noise. This filtered signal is then fed to the proposed independent component analyzer which extracts the original ECG components from its mixtures. The extracted signals are then sent to the Cloud after processing and the cardiac activity of the patient

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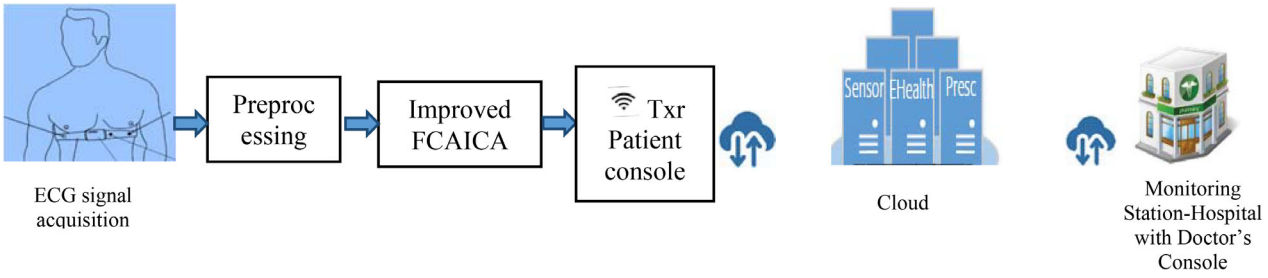


FIGURE 1 Cloud-based architecture for remote cardiac monitoring system [Colour figure can be viewed at wileyonlinelibrary.com]

can be monitored from the Cloud. So the entire system encompasses the subsystems for ECG acquisition, ECG extraction/enhancement using ICA, processing of ECG, and sending it to Cloud for transmission and storage. This entire system will developed in tie-up with PSG hospitals. In this article, an optimal IFCAICA technique is proposed for ECG extraction and R-peaks are estimated using QRS detection algorithm. Independent component analysis (ICA) is a blind source separation (BSS) technique, used to find the original sources from the mixtures when there is no knowledge about the sources.[2] It extracts the essential structure of the data from the acquired signal.[3,4] Here N electrodes are considered with sources represented by $S_1, S_2 \ \ldots \ S_N$. When ECG data are recorded from these electrodes, recorded signals are not pure source signals but they are mixture of each other. These generated signals are represented by $X = (x_1, x_2 \ \ldots \ x_n)$ which are linear combination of statistically independent sources. With an unknown nonsingular mixing matrix A of size $N \times N$, the observed mixture is mathematically represented as $X = \mathrm{AS}$ and is given by (1)

$$\begin{pmatrix} x_1(t) \\ x_2(t) \\ . \\ . \\ . \\ x_N(t) \end{pmatrix} = \mathrm{A} \begin{pmatrix} s_1(t) \\ s2(t) \\ . \\ . \\ . \\ s_N(t) \end{pmatrix} + n \tag{1}$$

Neglecting the noise n, the sources are estimated by multiplying the mixtures X with a demixing matrix B. This demixing data model B is estimated using ICA algorithms by formulating an objective function or contrast function and then minimizing or maximizing it. In order to realize the real-time, long-term ECG monitoring, and to optimize device size and power, the proposed algorithm has been implemented in very large scale integration (VLSI) technology and accuracy is improved using floating-point arithmetic operations. Recent works done on ECG signal using ICA techniques are described in Chapter II. Chapter III explains the improved FCAICA (fast confluence adaptive independent component analysis) with its internal hardware architecture. ECG waveforms, extracted results using the proposed method, R-peak detection and comparison results are provided in the chapter IV. Chapter V concludes the article explaining the applications and future work.

Main objectives of this article are:

- To develop improved FCAICA unit to extract the sources from the ECG mixtures.
- To perform R-peak detection on extracted ECG.
- To provide accurate ECG signal to the physicians for continuous remote monitoring with lesser hardware resources and power.

2 | RELATED WORK

According to the report from World Health Organization (WHO), the overall death rate due to cardiovascular diseases (CVD) is significant. Increase in death rates due to CVD is attributed to the collective amount of patients with obesity, smoking habit, diabetes mellitus, and other lifestyle changes. Some of the CVD among many others is atrial and ventricular arrhythmias, that happen due to cardiac rhythm disturbances. Ventricular fibrillation and flutter arrhythmias

are life threatening and they result in hemodynamic collapse, cardiac arrest, and sudden cardiac death.[5] Many methods that are used to filter out the noise and artifacts from ECG were only partially successful. The filters often lead to a reduction in the amplitudes of the component waves and the QRS complex. As some noise and artifacts are random in nature and have a wide range of frequency content, the filters fail to remove the interference when they are within the same frequency range as the cardiac signal. Principal component analysis (PCA) and ICA methods occupy a specific place in higher order statistical methods for superior feature extraction and ECG interpretation. It has been already proved that ICA is a valuable tool for enhancing EEG/ECG signals, suppressing artifacts, and irrelevant noises produced by eye blinks and muscle activity. Artifact and noise removal is the first step in ECG signal processing. ICA was used for removing breathing artifact from ECG and promising results were provided which led authors to apply ICA technique for more noise separation.[6] ICA-based architecture for BSS separation of linearly mixed signals was developed by using well established MIT-BIH database.[7] Some motion artifacts are ectopic and hence they cannot be detected easily by conventional methods. PCA-ICA–based algorithm was developed for motion artifact removal.[8] Carrying the idea forward, the author, Sarfraz used ICA methods for motion artifact removal along with ECG feature extraction with two-lead design.[9] In 2010, Romero extended the use of PCA and ICA for noise reduction and motion artifacts removal in ambulatory conditions where noise level rises and signal quality greatly reduces relative to activity. BSS for post-nonlinear mixtures and general nonlinear mixtures was discussed by Reference 10. It is then found that, when the mixtures as well as separation structure are structurally constrained, nonlinear BSS problem can be solved appropriately. The prior information on sources helps in simplification of the algorithms or reduction in indeterminacies in the solutions.

Authors in Reference 11 described the process of BSS technique for ICA. This article mainly aimed at separation of the source components from complex mixtures of signals based on statistical assumptions. Authors in Reference 12 extended a conventional constrained ICA framework for communication and signal processing. In Reference 13, the ICA with cyclo-stationary constraints (ICA-CC) and ICA with spatial constraints (ICA-SC) were described. Authors in Reference 14 designed an expectation maximization algorithm for noisy ICA. The authors of this article have proved that the proposed model was able to perform the BSS with the noisy ICA model. They also recommended ICA method for harmonic separation in which, the least square method was used to determine the amplitudes and phases of harmonic components. The authors suggested that the ICA was one of the most widely used techniques for BSS problems and demonstrated fast ICA algorithm to solve the BSS problems.

The authors in Reference 15 presented a comparative study of a fixed-point algorithm on a fixed-point platform with respect to another floating-point processor. ICA algorithm has been tested on a 32-bit fixed point and a 32-bit floating-point digital signal processors (DSPs). DSP processors have been increasingly important in the biomedical signal processing and research. Most of the research publications in this area are focused on offline processing of the biomedical signals. These algorithms cannot be applied to real-time essential features extraction and artifact removal processes.[16] They also impose constraints on area, power, speed, and cost of the processors. VLSI technological advancement has created a significant impact on biomedical signal processing.[17]

Joint approximation diagonalization of Eigen-matrices (JADE) ICA algorithm for determination of order of ICA components was described in Reference 18. This method was successfully applied to the ECG data and it provides potential solution for online ECG processing. This technique alleviates the limitation of ICA that one has to depend on visual review of the ICA components for advanced processing.

The ICA method with PCA variance estimator proposed in Reference 19 cleans the noisy, corrupted ECG signals and finds the true, false, and actual R-peaks. In this method, JADE algorithm was applied to the three-channel ECG data and it is observed that the approach is quite effective in extracting the original components and the method provides improved signal to noise ratio. In Reference 20, authors combined parameterization and multidimensional ICA approach and showed higher accuracy and automation. ECG analysis may be done with morphological basis or statistical model or neural model. Among these methods, ICA neural model has been successful in ECG analysis that separates ECG data recorded with multiple electrodes into independent components. Though the JADE algorithm performs better compared to Infomax algorithm, TDSEP (temporal de-correlation source separation) algorithm and MRMI-SIG (maximization of Reny's mutual information) algorithms, JADE is very fast for low dimensionality data. TDSEP is rapid but does not always reach the critical SIR (signal to interference ratio) when thermal noise is added, whereas Fast ICA have the best computation time for all data sets and is faster.[21] So FCAICA was developed based on Fast ICA algorithm and has been taken as a reference for the proposed work. The Fast confluence adaptive ICA algorithm has been shown to offer better performance in finding demixing matrix B, compared to other ICA algorithms in term of convergence speed and signal to noise ratio.[22,23] This method optimizes the contrast function by adaptively changing the weight vectors based on the fitness value. It has been successfully applied to separate signals from mixtures of super Gaussian and sub-Gaussian signals. This article proposes improved FCAICA algorithm to produce the best score function with lesser hardware resources for

separating the mixtures before QRS extraction. Among different ECG feature extraction techniques, QRS detection plays a significant role in ECG analysis. QRS detection should consider not only the physiological variability of the QRS complexes of the waves, but also the causes of various types of noise that can be present in the ECG signal. Noise sources include muscle noise, powerline interference and artifacts due to electrode motion, baseline wander, and T waves with high-frequency characteristics related to QRS complexes. This improved FCAICA algorithm is applied to remove such noises from the ECG signal.

3 | ECG SIGNAL SEPARATION USING IMPROVED FCAICA AND R-PEAK DETECTION

This section presents the detailed description of the improved FCAICA algorithm and R-peak detection technique. We consider that, there exist n unknown ECG signals, S_i, $i = 1, 2, \ldots n$ which are mutually independent. It is supposed that the original signal is linearly mixed together with a mixing matrix $A_{n\times n}$ and provides mixture X.

$$X = AS \tag{2}$$

where $S = [S_1, \ldots S_n]$ and $X = [x_1, \ldots x_n]$ are n dimensional source and mixed signals, A represents the interference matrix. The estimates of signals $Y = [y_1, \ldots y_n]$ are found by,

$$Y = X.B \tag{3}$$

where B is the $n \times n$ separating or demixing matrix whose columns are found using the proposed algorithm. In this research work, the acquired ECG signals are applied to a conversion unit which performs the real number to IEEE-754 floating point number conversion. The converted data are then stored in a memory. After that, these signals are passed into a preprocessing block, where the redundant data are eliminated by data centering and whitening techniques. Preprocessing reduces the correlation between the signal and so algorithm estimates the components faster. Then, the iterative improved FCAICA algorithm is implemented to extract the original signal from the ECG mixtures and the R-peak detection algorithm is applied.

3.1 | Signal preprocessing

In signal separation, data centering and whitening are essential preprocessing steps. Centering is a simple operation that refers to subtracting the mean of the mixtures from each observed sample $m = \epsilon\{X\}$ to produce zero mean outputs. After centering the signals, new white vectors are obtained by whitening the signals. Whitening transformation mainly uses Eigen value decomposition of the covariance matrix. The whitening step includes the normalization of the variances to one. It is performed by using covariance, Eigen value, and Eigen vector calculators. The observed signal X is linearly transformed and thus a new whitened signal Z is obtained. The whitening process reduces the correlation between the signals to be estimated. The whitening matrix is found by using,

$$W = [D^{-1/2}E^T]\,X \tag{4}$$

where W represents the whitening matrix, E is the orthogonal matrix of eigenvector found from the covariance matrix, D is the diagonal matrix of Eigen values associated with each Eigen vector S.

$$Z = WX \tag{5}$$

The whitening process is completed by finding whitened vector Z.

3.2 | Improved fast confluence adaptive ICA

The next process is to perform iteration to meet convergence and signal extraction. The algorithm presented below finds the 2×2 separation matrix for extracting the two ECG signals from their mixtures.

Algorithm 1. Fast confluence adaptive ICA

Step 1: Form a weight matrix W_M by assuming N sub matrices or column vectors as,

$$W_M = \{w_1, w_2, w_3, w_4, \ \dots \ w_N\}$$

where $w_i = (w_{i1}, w_{i2}, \ \dots w_{in})^T$

w_{ij} represents the jth weight of ith column vector and n is the number of sources. N defines the size of the search space to obtain globally best solution.

Step 2: Find norm of each vector and divide it by corresponding norms

Step 3: Find the updated weight vector W_{new} for all the weights in W_M.

$$w_{\text{new}i}(k+1) \leftarrow E\{Z(w(k)\overline{Z}^T)^3\} - 3w_i(k) \tag{6}$$

where Z represents the whitened speech vector.

Step 4: Calculate the fitness value

$$\boldsymbol{\Phi} i = w_i(k+1) - w_i(k) \tag{7}$$

Step 5: If convergence is achieved, the corresponding vector is one of the columns of demixing matrix. If it is not converged go to the next step.

Step 6: Divide the N weight vectors into M groups ($N = 2 \times M$) with 2 ($n = 2$) weights in each group.

Step 7: In each group,

If $\boldsymbol{\Phi} i(k) < \boldsymbol{\Phi} i\ (k+1)$ **then**

$$\text{ref}_i(k) = \boldsymbol{\Phi} i(k)$$

else

$$\text{ref}_i(k) = \boldsymbol{\Phi} i(k+1)$$

end

Step 8: **If** $\text{ref}_i(k)$is positive

$$w_i(k) = w_i(k) - C;$$

where C represents a random non-negative floating point number between "0" and "1."

else

$$w_i(k) = w_i(k) + C$$

end

Step 9: Repeat from step 3 to find $w_i(k+1)$ until $\boldsymbol{\Phi} i \approx 0$.

The first step in this algorithm initializes the weight vectors. The assumed weights are normalized, updated, and then the fitness value is calculated. Then, the convergence-checking unit is used to check the fitness value. On convergence, the iteration process is terminated and the data are sent for separation matrix estimation. Otherwise, the fitness parameter is checked for having a positive or negative value. If the fitness value is negative, the addition operation is performed on the weight vectors with a non-negative floating-point number. Otherwise, the subtraction is performed on the weight vectors. Then this process is repeated from step 3 until convergence is achieved. Once the convergence is achieved, the vector

with best fitness value is used as one of the columns of demixing matrix. As the matrix is considered to be orthogonal, second column of demixing matrix is easily found. Since there can only be one vector possible, which is orthonormal to first column of B, without any loss of generality, demixing matrix can be found. Then, the two signals are estimated by combining the separation matrix and original mixture. This method provides much simpler computation, lower chip area, and lower power consumption during VLSI implementation. In traditional methods, random weight vectors generated from random number generator unit are directly given to input of weight vector update system, when the convergence is not met. In previous FCAICA method, the weight vectors are updated in adaptive manner by an adaptive optimization unit according to the fitness value by performing the sampling formation of weight update. The optimization unit consists of subtraction module and comparator units, which are mainly used to update the weight values based on a kurtosis function given in (6). Comparator unit is used to decide the traversal direction of weight vectors. In proposed IFCAICA, the number of arithmetic units used in iterative process has been reduced. Figures 2 and 3 show the architecture of weight update units of FCAICA and proposed IFCAICA algorithms, respectively. FCAICA contains four adders and nine multipliers. In improved FCAICA, the number of adders and multipliers are reduced to three and six, respectively. This IFCAICA unit efficiently separates the source signals with lesser resources. Here, $W1(k-1)$ and $W2(k-2)$ represent the input weight vectors, k indicates the iteration number, and $W1(k)$ and $W2(k)$ are the output weight vectors.

Here, memory1 and memory2 act as internal registers used to store the whitened data. As area, power, and speed are the major design issues in VLSI circuits, this research work concentrates on them. Reconfigurable components (RCs) of improved FCAICA algorithm such as, weight vector updating unit, convergence checking unit, and separation matrix calculator units are individually developed, optimized, and are integrated together to form a complete module. The estimates found using proposed FCAICA algorithm are then applied for R-peak detection.

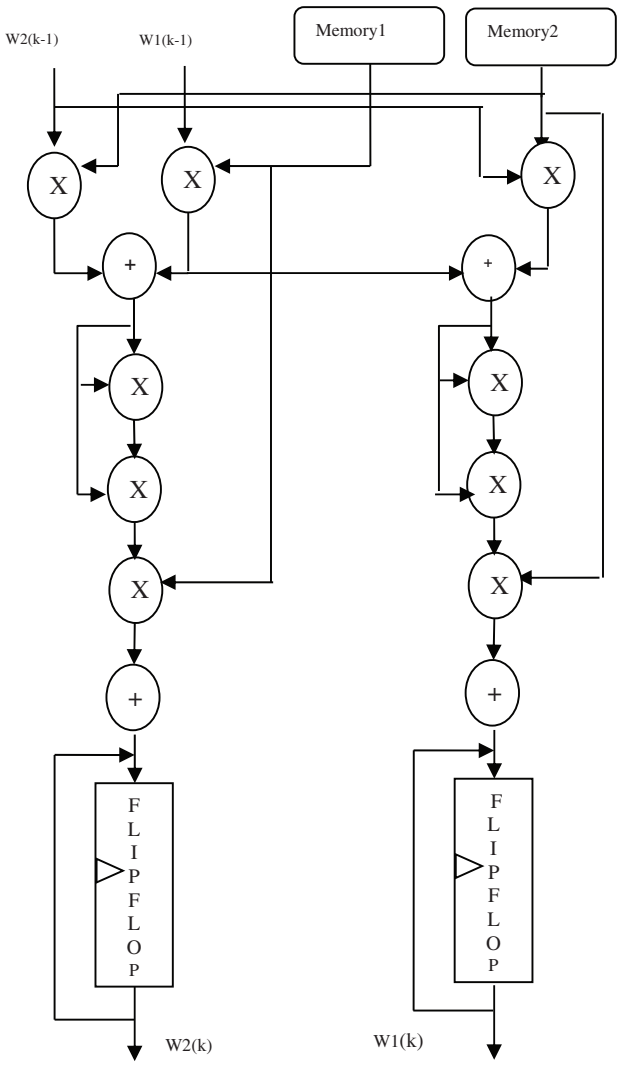
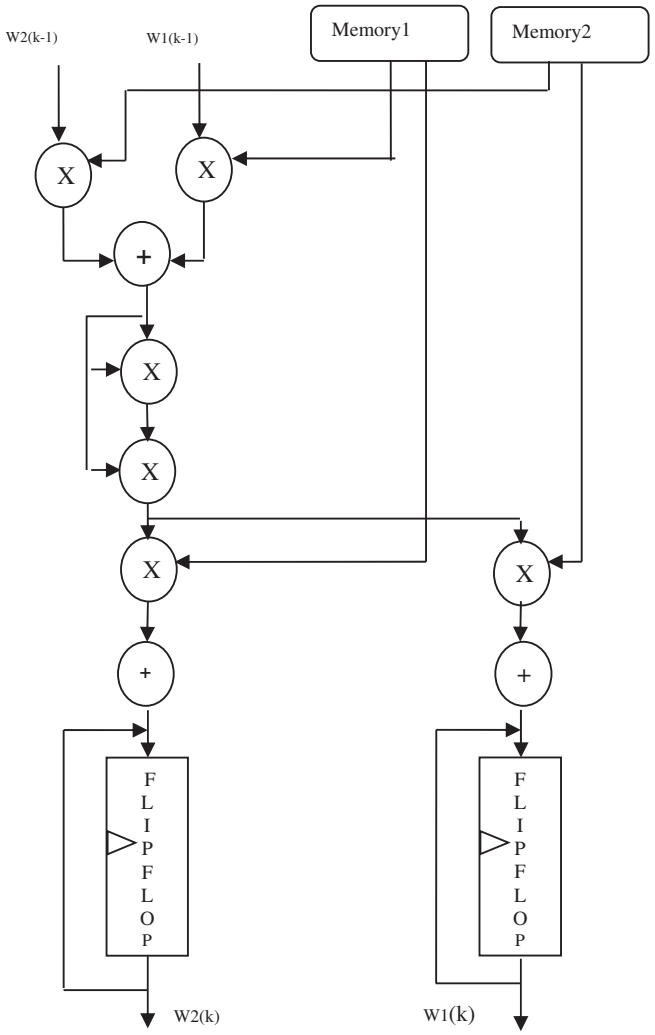


FIGURE 2 Weight update unit in FCAICA

FIGURE 3 Proposed weight update unit in IFCAICA



3.3 | R-peak detection algorithm

The widely used and often cited QRS detection algorithm proposed by Pan Tompkins[24] is used to calculate the R-peaks after sources are estimated using the proposed IFCAICA technique. This method recognizes the peaks with high detection sensitivity. The algorithm adjusts parameters periodically according to changes in ECG waves. The simulation, implementation results, and comparison results are provided in the next chapter.

4 | PERFORMANCE ANALYSIS

This section provides the experimental results of the proposed system. Extensive simulations were carried out on ECG dataset to validate the performance of IFCAICA. The ECG data provided in this result are of a 68 years old male with sampling rate of 1 KHz. The leads shown are V1 and V5.

4.1 | Results of ECG

The ECG signals acquired from the patient in two-channel case are shown in Figure 4, where the *x*-axis represents the number of samples and the *y*-axis represents the amplitude in microvolts. After reading the input signals and preprocessing, the proposed improved FCAICA algorithm is applied to separate the original signals from the mixtures. The centering and whitening processes are performed to eliminate the redundancy present in the input signals. This experiment is carried out with 11 600 samples. The reconstructed ECG signals are shown in Figure 5.

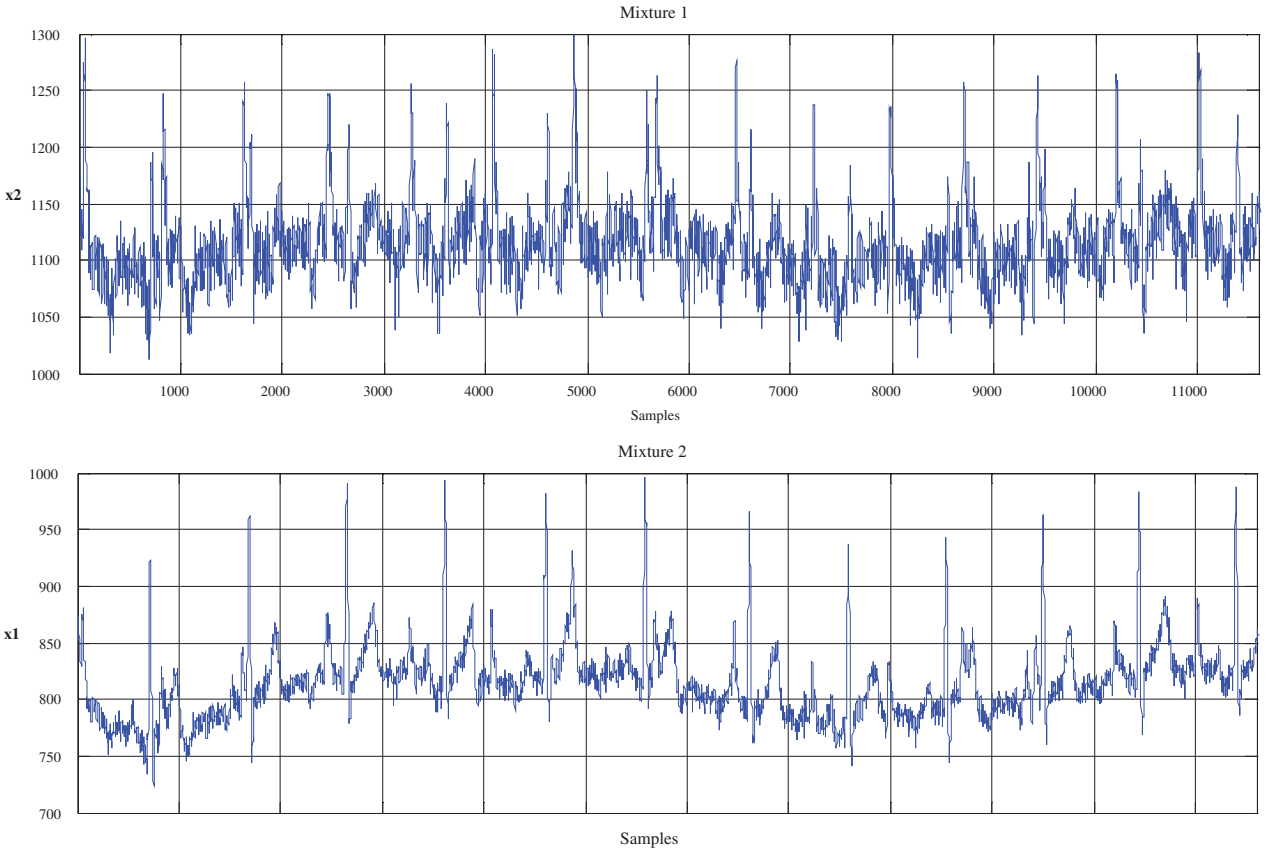


FIGURE 4 ECG mixtures [Colour figure can be viewed at wileyonlinelibrary.com]

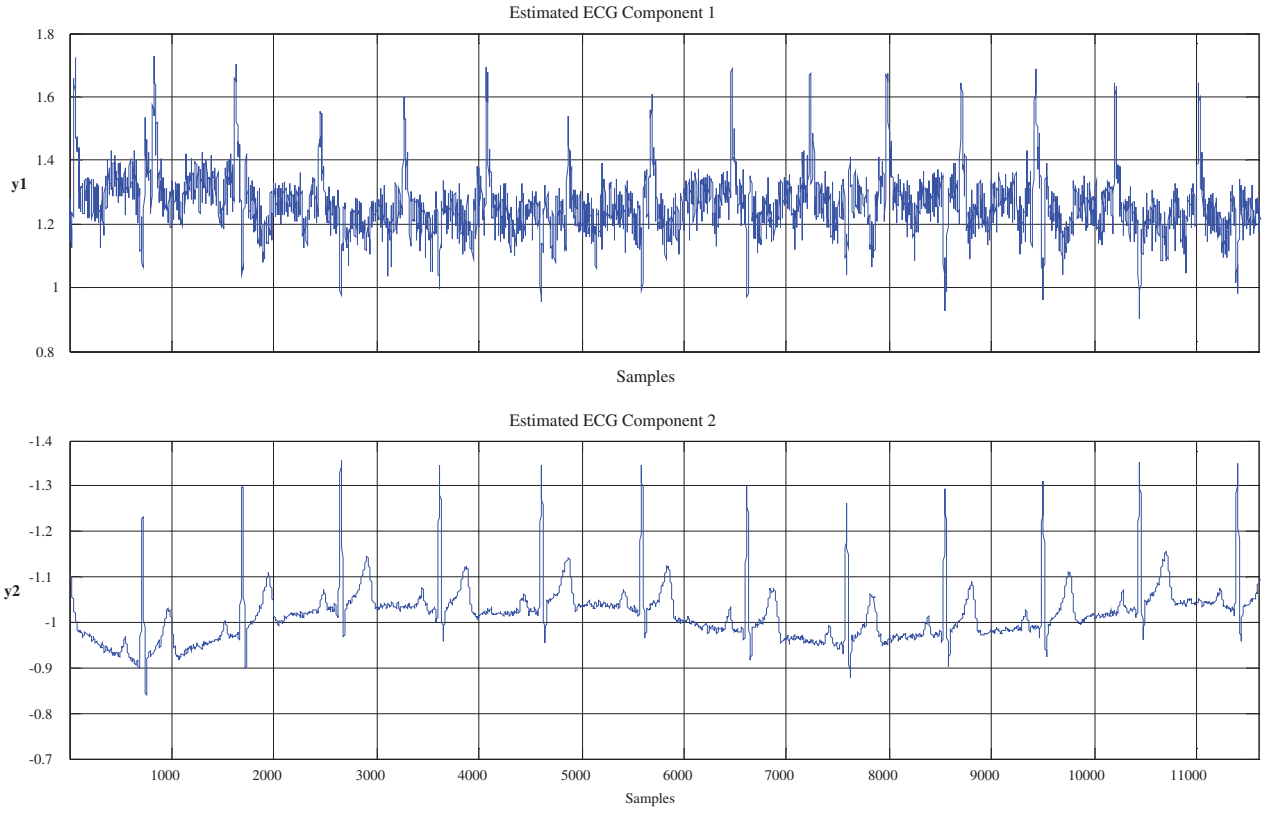


FIGURE 5 Reconstructed ECG [Colour figure can be viewed at wileyonlinelibrary.com]

4.2 | R-Peak detection

For a complete long-term cardiac monitoring and classification system, R-peak detection is required as the primary step. The performance of the classification may be affected if R-wave detection results with errors. A number of investigation works published in the literature realized high detection rates of more than 99.5% remarkably.[25,26] So, it is straightforward to incorporate the R-wave detection algorithms into the proposed scheme to develop a fully automatic heartbeat classification system. The results obtained with the R-peak detection algorithm after ICA processing are shown in Figure 6.

4.3 | Sensitivity analysis with R-Peak detection

The effectiveness of the proposed method is also validated with six-leads of ECG data (V1-V6), by evaluating the sensitivity using true and false peaks before and after applying proposed ICA method. Table 1 provides the details of true positive, false positive, and false negative peaks found in ECG components before applying ICA. After the application of ICA, true positive, false positive, and false negative values are found to be reduced and are shown in Table 2.

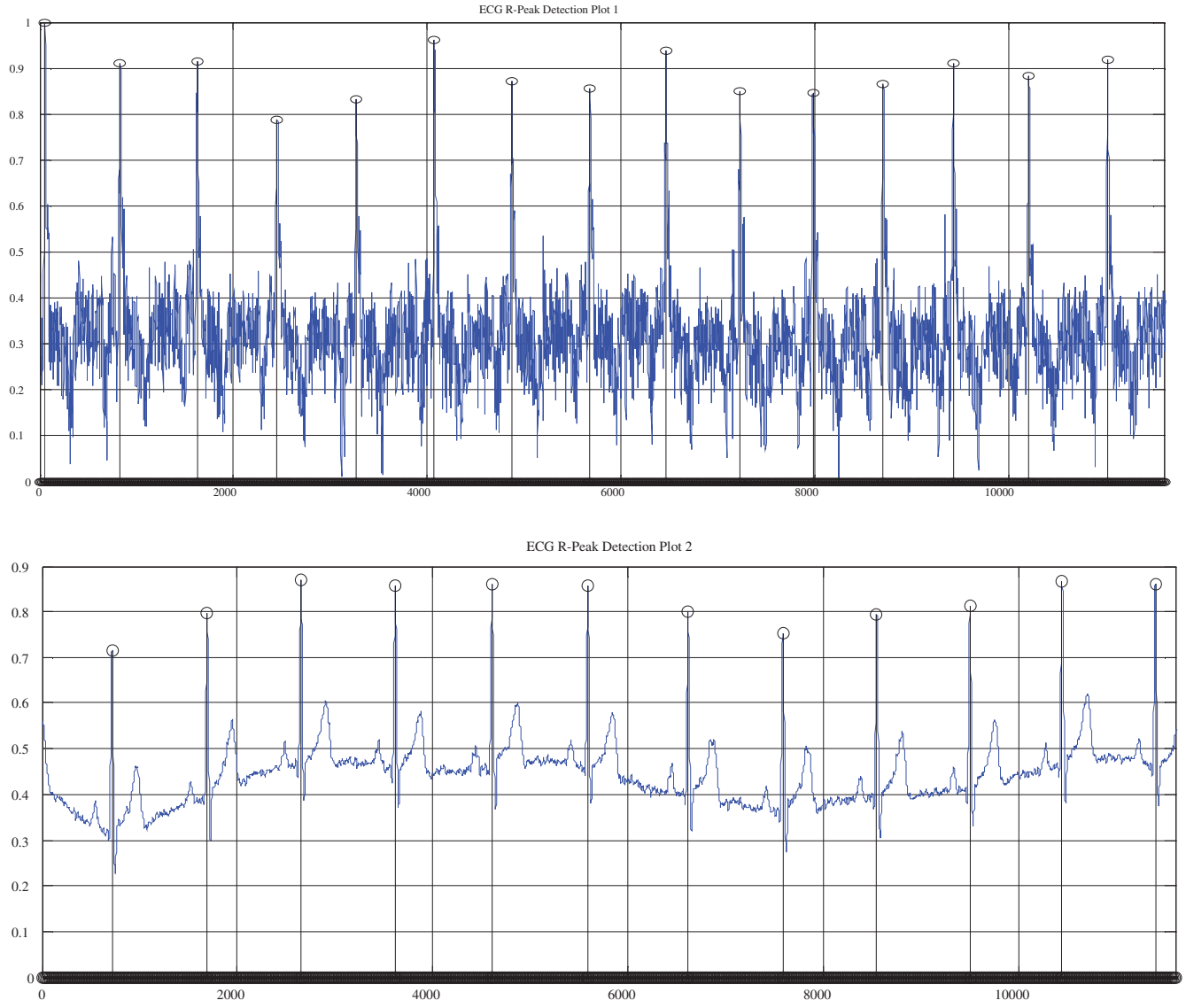


FIGURE 6 Results of R-peak detection [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Sensitivity before applying ICA

Lead	Actual R-peaks	True positive (TP)	False positive (FP)	False negative (FN)
V1	150	150	8	1
V2	150	150	9	1
V3	150	149	6	3
V4	150	149	7	2
V5	150	150	7	1
V6	150	148	3	2
Total		896	40	10

Note: Sensitivity $= \frac{TP}{(TP+FN)} = 97.5\%$.

TABLE 2 Sensitivity after applying ICA

Lead	Actual R-peaks	True positive (TP)	False positive (FP)	False negative (FN)
V1	150	150	71	5
V2	150	147	32	4
V3	150	149	14	3
V4	150	148	25	3
V5	150	149	20	2
V6	150	146	14	5
Total		889	176	22

Note: Sensitivity $= \frac{TP}{(TP+FN)} = 98.89\%$.

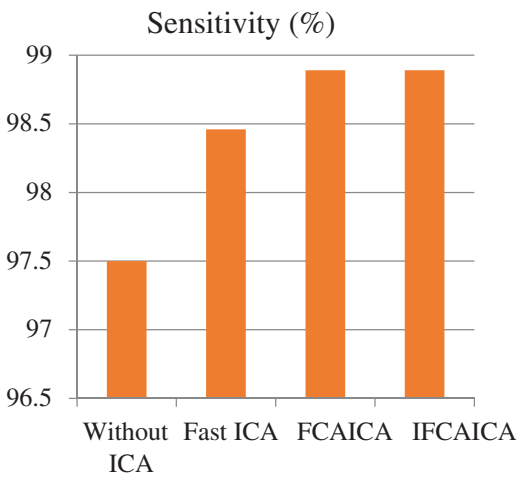


FIGURE 7 Sensitivity analysis [Colour figure can be viewed at wileyonlinelibrary.com]

The R-peak estimator with the proposed ICA algorithm gives a sensitivity of 98.89%, whereas before applying ICA the sensitivity is 97.5% and it is illustrated in Figure 7. The results are derived by taking 100 000 samples for all the algorithms. The leads used in this analysis are V1 to V6. Comparing the results of Jade algorithm in the literature that has a sensitivity of 98.07[19] with the Fast ICA algorithm, FCAICA algorithm, and proposed IFCAICA technique, it is proved that the proposed method performs better in terms of sensitivity and hence provides better accuracy in separation. The performance metrics of R-peak detection algorithm with performance metrics true positive (TP), false positive (FP), and false negative(FN) values are plotted in total and shown in Figure 8. As the proposed ICA method implicitly incorporates higher order statistics through a nonlinear function to achieve independence, it provides better results compared to other algorithms.

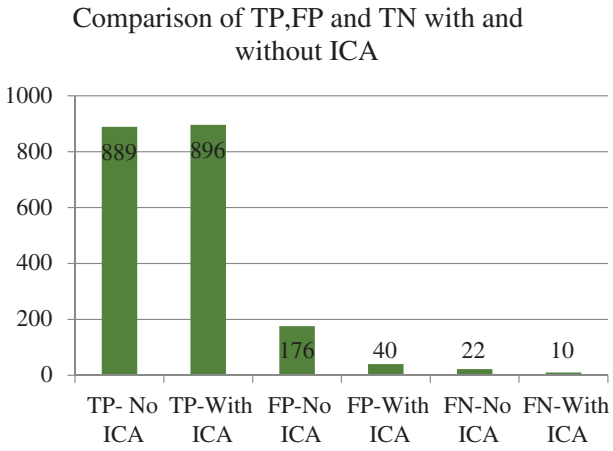


FIGURE 8 Performance metrics [Colour figure can be viewed at wileyonlinelibrary.com]

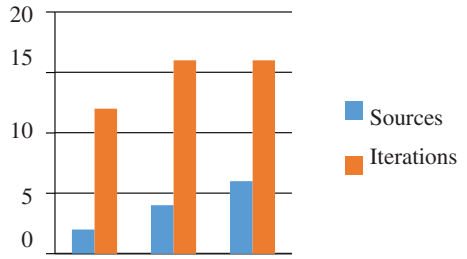


FIGURE 9 Number of sources vs iterations [Colour figure can be viewed at wileyonlinelibrary.com]

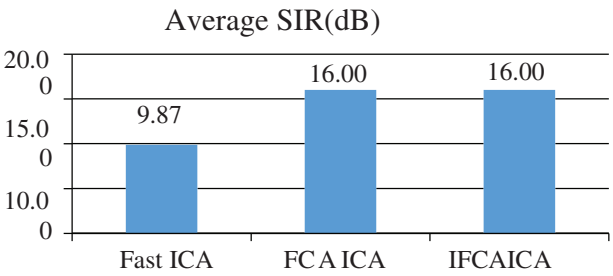


FIGURE 10 SIR analysis of ICA algorithms [Colour figure can be viewed at wileyonlinelibrary.com]

4.4 | Iterations and SIR

The simulation was carried out to find the number of iterations needed for convergence of the algorithm. At first two leads were used and then it was extended to six leads (V1-V6).The average number of iterations are 12, 16, 16 with the sources 2, 4, and 6, respectively. The number of iterations taken to extract the independent components using proposed IFCICA technique is shown in Figure 9. The average SIR value obtained using this algorithm for two-lead case is 16 dB which is fine enough to extract the required ECG information. The usage of floating point arithmetic operations significantly improves the SIR of separation system. The SIR obtained for IFCAICA is compared with Fast ICA and FCAICA which is shown in Figure 10.

4.5 | Comparative analysis-VLSI implementation

Once all the performance metrics are calculated for evaluation of the algorithm, hardware implementation is done. Experimental results are analyzed, evaluated, and hardware results are obtained using the Cadence tools NCsim, rc, and encounter. GDSII (graphical data stream information interchange) file is created in 180 nm technology for VLSI implementation. Comparative analysis among the existing and proposed methods is done. Figure 11 depicts the cell instances, area, total power consumption, and maximum operating frequency of Fast ICA, FCAICA, and improved FCAICA algorithms. IFCAICA provides better results compared to FCAICA in terms of area, power, and maximum frequency. Since the operating frequency of FCAICA is high compared to other algorithms, it separates the mixture with a maximum

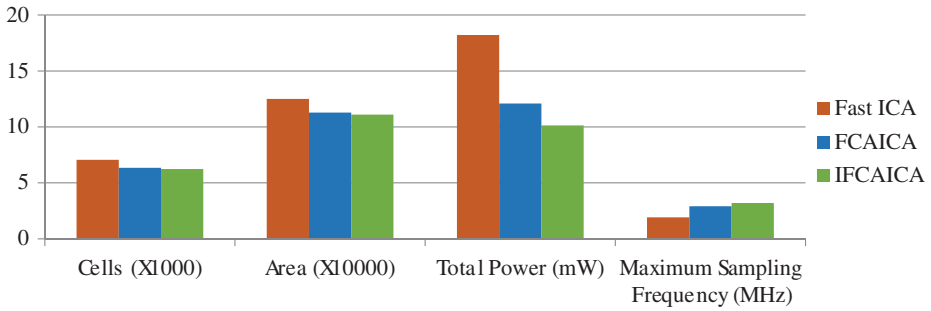


FIGURE 11 Comparative analysis between existing and proposed methods [Colour figure can be viewed at wileyonlinelibrary.com]

possible speed. Due to the reduced complexity of adaptive optimization unit of algorithm, the resource utilization and power consumption are reduced, and the operating speed is increased.

It is observed from these results that, there is an improvement of 16% optimization in power and 14.7% improvement in speed (operating frequency) achieved when compared to FCAICA algorithm. Fast ICA results are also shown for reference.

5 | CONCLUSION AND FUTURE WORK

The main aim of development of this algorithm is to extract pure ECG component from the mixture signals with lesser hardware resources and improved speed. This technique can be used in the development of a healthcare system for continuous monitoring of clean ECG signal when the patient is away from the hospital. Wireless connectivity enables us to transmit the acquired ECG signals directly to the Cloud server. The physicians could use these data from cloud for further analysis and clinical review. The experimental results show that the proposed improved FCAICA algorithm occupies lesser hardware compared to existing techniques. The sensitivity of the algorithm for R-peak detection is also good. So it can be well applied for ECG signal separation. The proposed IFCAICA algorithm may also be enhanced for other signals such as EEG and spread spectrum signals.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Rundo F, Conoci S, Ortis A, Battiato S. An advanced bio-inspired PhotoPlethysmoGraphy (PPG) and ECG pattern recognition system for medical assessment. *Sensors*. 2018;18(2):405. https://doi.org/10.3390/s18020405.
2. Hyvärinen A. Independent component analysis: recent advances. *Philos Trans Roy Soc A*. 2012;371:1-5.
3. Liang Y, Harris J, Naqvi SM, Chen G, Chambers JA. Independent vector analysis with a generalized multivariate Gaussian source prior for frequency domain blind source separation. *Sig Process*. 2014;105:175-184.
4. Nam KW, Ji YS, Han J, et al. Clinical evaluation of the performance of a blind source separation algorithm combining beamforming and independent component analysis in hearing aid use. *Speech Commun*. 2013;55:544-552.
5. Huikuri HV, Castellanos A, Myerburg RJ. Sudden death due to cardiac arrhythmias. *N Engl J Med*. 2001;345:1473-1482.
6. Wisbeck JO, Barros AK, Yy AKB, Ojeda RG. Application of ICA in the separation of breathing artefacts in ECG signals. *Proc International Conference on Neural Information Processing*, *Japan*; 1998:211-214.
7. Barros AK, Mansour A, Ohnishi N. Removing artefacts from electrocardiographic signals using independent components analysis. *Neurocomputing*. 1998;22:173-186.
8. Romero I. PCA-based noise reduction in ambulatory ECGs. *Proc. IEEE International Conference on Computing in Cardiology*, *UK*, 2010:677-680.
9. Sarfraz M, Li F. Independent component analysis for motion artefacts removal from electrocardiogram. *Glob Perspect Artif Intell*. 2016;1:49-55.
10. Jutten C, Karhunen J. Advances in Nonlinear Blind Source Separation. *Proc. 4th International Symposium on Independent Component Analysis and Blind Signal Separation*, *Japan*; 2003:245-256.
11. Kulchandani J, Dangarwala KJ. Blind source separation via independent component analysis: algorithms and applications. *Int J Comput Sci Inform Technol*. 2014;5:6739-6743.

12. Wang X, Huang Z, Zhou Y, Ren X. Approaches and applications of semi-blind signal extraction for communication signals based on constrained independent component analysis: the complex case. *Neurocomputing*. 2013;101:204-216.
13. Zhang HF, Li LP. Expectation-maximization algorithm for noisy independent component analysis. *J Univ Electron Sci Technol China*. 2012;41:527-531.
14. Ai Y, Zhang H. Harmonic separation based on independent component analysis method. *J Comput*. 2013;8:433-440.
15. Patil D, Das N, Routray A. Implementation of fast-ICA: a performance based comparison between floating point and fixed point DSP platform. *Measure Sci Rev*. 2011;11:118-124.
16. Shyu K-K, Lee M-H, Wu Y-T, Lee P-L. Implementation of pipelined fast ICA on FPGA for real-time blind source separation. *IEEE Trans Neural Netw*. 2008;19(6):958-970.
17. Morales A, García E, Castillo MA, Carvajal J, Banqueri AJP. Flexible ECG acquisition system based on analog and digital reconfigurable devices. *Arch Intern Med*. 2011;165(2):261-270.
18. He T, Clifford G, Tarassenko L. Application of independent component analysis in removing artefacts from the electrocardiogram. *Neural Comput Appl*. 2006;15(2):106-116.
19. Chawla MPS. Parameterization and R-peak error estimations of ECG signals using independent component analysis. *Comput Math Methods Med*. 2007;8:263-285.
20. Chawla MPS. Detection of indeterminacies in corrected ECG signals using parameterized multidimensional independent component analysis. *Comput Math Methods Med*. 2009;10:85-115.
21. Mantini D, Hild KE, Alleva G, Comani S. Performance comparison of independent component analysis algorithms for fetal cardiac signal reconstruction: a study on synthetic fMCG data. *Phys Med Biol*. 2006;51:1033-1046.
22. Jayasanthi M, Muniraj NJR. ECG extraction by improved independent component analysis. *Asian J Inform Technol*. 2016;15:2638-2644.
23. Jayasanthi M, Muniraj NJR. Jammer suppression in spread spectrum communication using novel independent component analysis approach. *AEU Int J Electron Commun*. 2016;70:998-1005.
24. Pan J, Tompkins WJ. A real-time QRS detection algorithm. *IEEE Trans Biomed Eng*. 1985;32(3):230–236.
25. Lagerholm M, Peterson G, Braccini G, Edenbrandt L, Sornmo L. Clustering ECG complex using Hermite functions and self-organizing maps. *IEEE Trans Biomed Eng*. 2000;47:838-848.
26. Afonso VX, Tompkins WJ, Nguyen TQ, Luo SE. ECG beat detection using filter banks. *IEEE Trans BiomedEng*. 1999;46:192-202.

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Effectiveness of transcutaneous electrical nerve stimulation in relieving labour pain

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Abstract

Background: TENS by neuro-physiological means seems to help in relieving labour pain. Most of the pregnant women are not aware of the coping strategies for labour pain. As a result they tend to be restless and stress themselves by shouting or screaming due to pain. By using TENS, women in labour can save their energy without stressing themselves and make use of the saved energy for pushing the fetus during the second stage of labour. The objective was to analyze the effect of TENS on relieving labour pain among the postnatal mothers who had used TENS to cope up with the labour pain.

Methods: Data collected from 1041 women [Primipara (n=702) and Multipara (n=339)] who used TENS to cope up pain throughout the labour was used for this retrospective study.

Results: 88% of women had vaginal delivery and only 12% of women had cesarean section. More than 50% of both primiparous and multiparous women who had normal and vacuum assisted vaginal delivery experienced excellent pain relief using TENS. Almost 40 % of both primiparous and multiparous women who had forceps delivery and lower segment cesarean section experienced excellent pain relief using TENS. Except 2% of women with normal vaginal delivery, none of them stated that there was no relief of pain using TENS.

Conclusions: TENS had a very good effect in coping up the labour pain and could be used during the first and second stages of labour.

Keywords: Labour pain, Pain gate theory, TENS

Introduction

The pain experienced in labour is affected by the processing of multiple physiological and psychosocial factors. Transcutaneous electrical nerve stimulation (TENS) is widely used for chronic or postoperative pain control, either replacing or complementing analgesic drugs, and is based on the gate theory of pain proposed by Melzack and Wall in 1965. According to this theory, the modulation of pain perception induced by TENS is attributed to the recruitment of A-beta (Aβ) afferent fibers in the posterior horn of the spinal cord, which would prevent or hamper the activation of the pain-conducting small A-delta (Aδ) fibers. It is further thought that by reducing anxiety, increasing a sense of control and by providing distraction, TENS increases a woman's sense of well-being and thereby reduces pain in labour (Brucker 1984; Findley 1999; Gentz 2001; Simkin 2004). The first report on the use of TENS in obstetrics came from Scandinavia in the 1970's, where it was introduced as a non-pharmacological resource for pain relief during labor.[1] TENS by neuro-physiological means seems to help in relieving pain during the first stage of labour. It

fulfils the criteria of an efficacious, simple to administer method of pain relief with no side effects on the mother and the baby. It would be a useful addition to the present methods of pain relief in labour.[2]

Most of the pregnant women are not aware of the coping strategies for labour pain. As a result, they tend to be restless and stress themselves by shouting or screaming due to pain. It would be better to make them understand about the labour pain beforehand, usually at their final review at obstetrics and gynecology (OG) outpatient department (OPD) and also teach them the method of operating the obstetric TENS equipment to cope up with the labour pain. This would help the women in labour to save their energy without stressing themselves and make use of the saved energy for pushing the fetus during the second stage of labour.

There was no evidence from previous studies with larger sample size and usage of TENS throughout the first and second stage of labour. Hence, this study was conducted with an objective to analyse the effect of TENS on relieving labour pain among the postnatal mothers who had used TENS to cope up with the labour pain during their first and second stages of labour.

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Design and sample size

This is a retrospective study and the data collected from both the primipara and multipara mothers who had used TENS for coping up labour pain throughout labour was used for this study. Ethical clearance was obtained from Institutional Human Ethical Committee, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. Out of 2595 women who delivered between February 2015 and February 2016, the data of 1041 women [Primipara (n=702) and Multipara (n=39)] were taken for this study.

The data of 1554 women could not be included, 3 women had assisted breech delivery, 2 women underwent vaginal birth after cesarean (VBAC), 403 underwent elective cesarean, 10 women had twin gestation, 142 women had already had an experience of using TENS for previous delivery, 95 women had immediate progression of labour and 309 women were not willing to use TENS to cope up labour pain.

The data of all the participants showed that they had undergone the awareness programme on the coping strategies of labour pain and about the method of application of TENS equipment when they had visited OG OPD during their final review. The data was then segregated into normal vaginal delivery (NVD), forceps delivery (FD), vacuum assisted vaginal delivery (VAVD) and lower segment cesarean section (LSCS). The feedback forms on relief of labour pain which were collected from the mothers on the first post natal day were used for data analysis.

Intervention

ELLE Obstetrics TENS equipment had been used for this study. The technique of TENS application involved placement of two pairs of flat electrodes on both side of the woman's thoracic and sacral spines (T10-L1 during the first stage of labour) and (S1-S4 during second stage of labour) as pain is felt at the dermatomal distribution T10 and Ll.

During full dilation and stretching of the birth canal, pudendal nerve is stimulated at S2-S4 level. These electrodes provided continuous, low-intensity electrical impulses or stimuli from ELLE TENS (battery- operated) device. The pulse frequency used was between 16 Hz and 100 Hz with pulse duration of 150 µs. Two modes are available on the ELLE TENS, each with a burst and boost function (Table 1).

Mode 1 is primarily used for the earlier stages of labour. Mode 2 is used when the labour advanced and when the contractions became more frequent. The burst function button is used in between contractions which promotes secretion of endorphins and encephalins. The boost function button was used during contractions. This gives an extra surge of power required to combat the pain during contractions. The labour ward therapist fixed the electrodes and the women operated the device themselves. The labour ward therapist ensured the proper use of the device and the position of the electrodes frequently.

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Outcome measures and statistical analysis

The pain index and degree of pain relief were used as outcome measures. The percentage of pain relief was assessed both by the postnatal women and by the therapist (Table 2 and 3). Pain index was calculated by multiplying the pain relief scores assessed both by the postnatal women and therapist. The degree of pain relief was graded accordingly by the therapist in-charge of the obstetrics and gynecological physiotherapy (Table 4).

□□□□2 □□□r □□□□□rd □□□□□r □□□□□□r □□□□□□ □□□□r □□□□□

□r □□□□□□□□□□r □□□□	□□r
0-25 %	1
26-50 %	2
51-75 %	3
76-100 %	4

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Moaning or shouting during contraction	0-25%	1
Wincing during contraction	26-50%	2
Restless with discomfort	51-75%	3
Comfortable and sleeping	76-100%	4

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1-4	No relief
5-8	Fair relief
9-12	Good relief
13-16	Excellent relief

Subjective assessment of labour pain (by the postnatal women)

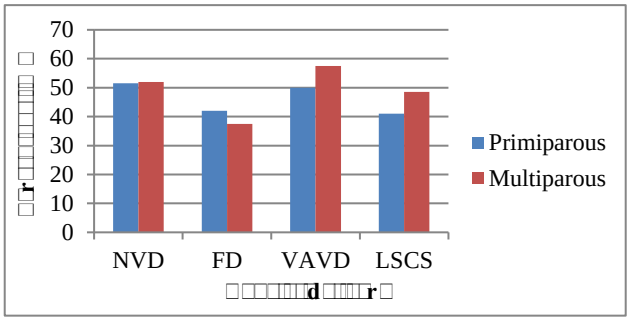
This variable was standardized by visual analog scale. The distance marked were calculated as a percentage of line length (from 0-100%). Scores were given according to the percentage of pain relief.

Observer assessment of labour pain (by the therapist)

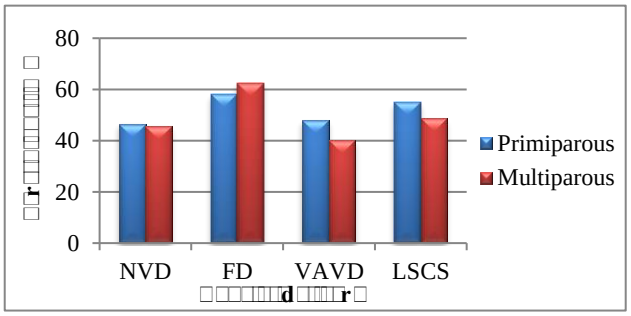
This grading of percentage of pain relief and scoring was done by the labour ward therapist who attended the pregnant women during labour.

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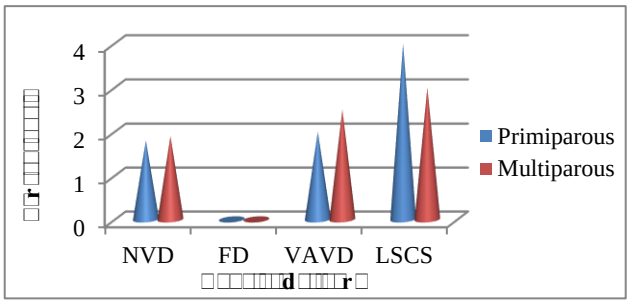
Among the 1041 pregnant women who had used TENS for coping up labour pain, it was found that only 12% of women underwent cesarean section and 88% of women had vaginal delivery (NVD, FD and VAVD), out of which 58% were primigravida women. The average duration of application of TENS for the primiparous women was 6.19±0.46 hours (Mean and SD) and for that of the multiparous women was 6.09±1.26 hours (Mean and SD).



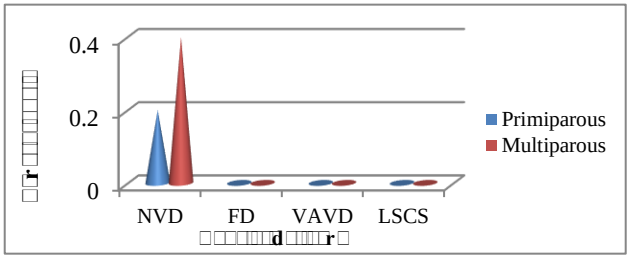
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NVD	740 (71%)	482 (65%)	258 (35%)	24.76±3.32	26.88±3.89	6.09±4.86	5.29±4.40
FD	27 (3%)	19 (70%)	8(30%)	24.15±2.26	27.62±2.66	6.01±3.95	4.74±2.54
VAVD	144(14%)	104 (72)	40 (28%)	25.10±3.37	27.1±3.78	5.79±4.52	7.21±6.03
LSCS	130 (12%)	97(75%)	33(25%)	25.82±3.94	27.42±4.86	6.85±5.46	7.10±5.21

NVD: Normal vaginal delivery; FD: Forceps delivery; VAVD: Vacuum assisted vaginal delivery; LSCS: Lower segment cesarean section.

Normal vaginal delivery

It was analyzed from the data that, 740 mothers had normal vaginal delivery. Among them, 482 were primiparous (24.76±3.32 years) and 258 (26.88±3.89 years) were mulitparous. More than 50% of both primiparous and multiparous women (51.5% and 52% respectively) experienced excellent pain relief using TENS (Figure 1) and more than 45% of both primiparous and multiparous women (46.5% and 45.7% respectively) had good pain relief (Figure 2). Fair relief and no relief of pain by using TENS have been stated by less than 2 % of the NVD mothers (Figure 3 and 4).

Forceps delivery

Only twenty seven mothers had forceps delivery. Among the 19 primiparous (24.15±2.26 years) and 8 multiparous (27.62±2.66 years) women who had FD, 42% of the primiparous women and 37.5% of multiparous women experienced excellent pain relief using TENS (Figure 1). More than 55% of both primiparous and multiparous women experienced good pain relief by using TENS (Figure 2). None of them have stated that they had fair relief or no relief after using TENS (Figure 3 and 4).

Vacuum assisted vaginal delivery

One hundred and forty four mothers [primiparous (n=104; 25.10±3.37 years) and multiparous (n=40; 27.1±3.78 years)] had VAVD. 52% of primiparous and 55% of multiparous women had excellent pain relief (Figure 1). 48% of primiparous and 40 % of multiparous women experienced good relief (Figure 2). Less than 3 % of them had stated fair relief and none of the VAVD mothers had stated that they had no relief of pain by using TENS to cope up labour pain (Figure 3 and 4).

Lower segment caesarean section

One hundred and thirty mothers had lower segment cesarean section. Among them 97 mothers were primiparous (25.82±3.94 years) and 33 were multiparous (27.42±4.86 years). 41% of primiparous and 48.5% of multiparous mothers experienced excellent relief (Figure 1). 55% of primiparous and 48.5% of multiparous women experienced good relief of pain (Figure 2). Less than 4% of them experienced fair relief of pain by using TENS. None of the LSCS participants had stated that there was no pain relief using TENS for coping up labour pain (Figure 3 and 4). These results are summarised in Table 5.

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The present study evaluated the relief of pain by the mothers who had used TENS for labour. It was observed in this study that 50.3 % of the mothers had experienced excellent pain relief and 47.4% of the mothers had experienced good pain relief by using TENS. The knowledge about effectiveness of TENS for labour pain given to the antenatal women during their last review had provided confidence among the women to use the TENS equipment during labour. This correlated with a study which had stated that by reducing anxiety, increasing a sense of control and by providing distraction, TENS increases a woman's sense of well-being and thereby reduces pain in labour.[3] A study included 14 randomized controlled trials comparing women receiving TENS during labour versus routine care or placebo devices. The authors demonstrated little difference in satisfaction with pain relief or in pain ratings between the TENS and control groups. However, approximately two-thirds of women who had received TENS reported that they would prefer to use it again for future labour and one another study states that 74% of the parturient in their experimental group reported being satisfied with TENS, and 100% of them would prefer to use this method again for future childbirths.[4,5]

The findings of the present study are similar to the previous studies with respect to the mode of delivery for the women who had used TENS during labour. In present study about 88% had vaginal delivery (including NVD, FD and VAVD) and 12 % had emergency caesarean. Thomas et al reported that in their intervention group, 80.3% vaginal births, 8.3% forceps/vacuum and 11.4% caesarean sections. Normal delivery occurred in all women in TENS groups in comparison to 98% in tramadol group and 99% in control group.[6] Use of TENS

was associated with the shortest first and second stages of labour, and highest rate of spontaneous delivery.[7]

The pulse frequency of the ELLE TENS device which was used in this study ranged between 16 Hz and 100 Hz with pulse duration of 150 μs. The parameters used in present study were similar to the few other related studies, Fayis F El-shamy and Anees G Saleh have applied TENS (Medsana, China) on acupuncture points for 30 minutes, at a frequency of 100 Hz with a burst frequency of 2 Hz, pulse width 150 μs, and intensity according to patients tolerance.[5,8] They have stated that different opioid peptides have been released in central nervous system by TENS application. Encephalins and β-endorphins are released with TENS application of 2Hz, while dynorphin is released in the spinal cord with 100-Hz, a combination of both frequencies allows synergistic interaction among the three endogenous opioid peptides and provides a powerful analgesic effect. Santana et al, had applied TENS continuously for 30 minutes starting at the beginning of the active phase of labour (4 cm of cervical dilation).[5] The TENS unit produced a modified biphasic asymmetric pulse and was set to a pulse width of 100 ms and a frequency of 100 Hz.

Elle TENS machine has been used in our study. Lai Fong HO et al have used the same machine and have stated that we can encourage the labouring women to mobilise even when they are using the TENS machine, which will help in shortening the latent or first stage of labour.[7] Moreover, it also reduces the incidence of neonatal respiratory suppression caused by pethidine.

In present study, the mean duration of application of TENS for the NVD was 6.09±4.86 hours [(Mean and SD); primiparous] and 5.29±4.40 hours [(Mean and SD); multiparous]. Most of the studies have used TENS for the early stage of labour for 30 minutes.[5,8] Experienced practitioners state that TENS may be more effective if initiated in early labour, presumably to allow for a build-up in endorphin production before the pain becomes severe.[9] TENS provides continuous, low-intensity electrical impulses or stimuli and during the contractions the woman increases the stimulation from low to high intensity by turning the control knobs on the device. High intensity should be maintained for at least 1 minute to facilitate release of endorphins.[10] Similarly one another study has stated that during labour, low-intensity electrical stimulation is given continuously or is applied by the woman herself as the contraction begins. This stimulation blocks the afferent fibers, preventing pain from traveling to the spinal cord synapses from the uterus. As labour and descent progresses, the electrodes are moved to stimulate the S2 and S4 level. High intensity stimulation is generally needed to control the pain at this stage.[11] In present study, the TENS was given continuously during labour with the women operating the machine, switching to boost and burst modes during contractions and in between contractions respectively. The electrodes were applied at the level of T10-L1 during first stage of labour and S2-S4 during second stage of labour. Duration of application of TENS for the primipara women was comparatively more than that of the multipara women who had ND and FD with a mean difference of 0.8 and 1.27 hours respectively. The current study evaluated the perception of labour pain of 1041 pregnant women and analyzed the effect of TENS to cope up labour pain. This study has strong evidence to state that usage of TENS had good effect in reducing the labour pain for both primigravida and multigravida women.

Limitation

The intensity of pain depends upon several factors. The most important being the emotional stability of the women.[8] It also depends upon the pain threshold of women. As it is a retrospective study, the pain threshold of the individual woman could not be identified. Correlation of pain threshold and pain index could be included in future studies. Lack of control group is another limitation of this study. Comparing the women using TENS for labour pain with the women who had sedation could yield good evidence for the effect of TENS for labour pain. Further high quality randomized controlled trials could be done to support the evidence of usage of TENS for labour pain.

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It is concluded that TENS had a very good effect in coping up the labour pain and could be used during the first and second stage of labour by both the primigravida and multigravida women. The awareness of labour pain and usage of TENS for labour pain forms the important concept in the childbirth education programme.

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1. Mello LFD, Nobrega LF, Lemos A. Transcutaneous electrical stimulation for pain relief during labor: a systematic review and meta-analysis. Rev Bras Fisioter. 2011;15(3);175-84.
2. Padma, Prasanna A, Urala. Transcutaneous electrical nerve stimulation and labour pain. Bahrain Med Bull 2000;22(1):1-8.

3. Jones L, Othman M, Dowswell T, Alfirevic Z, Gates S, Newburn M, et al. Pain management for women in labour: an overview of systematic reviews. Cochrane Database of Systematic Reviews. 2012;3:CD009234.
4. Bedwell C, Dowswell T, Neilson JP, Lavender T. The use of transcutaneous electrical nerve stimulation (TENS) for pain relief in labour: a review of the evidence. Midwifery. 2011;27(5):e149-8.
5. Santana LS, Gallo RBS, Ferreira CHJ, Duarte G, Quintana SM, Marcolin AC. Transcutaneous electrical nerve stimulation (TENS) reduces pain and postpones the need for pharmacological analgesia during labour: a randomised trial. J Physiotherapy. 2016;62:29-34.
6. Thakur R, Patidar R. Comparative study of transcutaneous electrical nerve stimulation (TENS) and tramadol hydrochloride for pain relief in labor. J Obstet Gynecol Ind. 2004;54(4):346-50.
7. Ho LF, Lee ILY, Gy Ma G, "Intrapartum transcutaneous electrical nerve stimulation for pain relief and outcome of labour", Hong Kong J Gynaecol Obstet Midwifery. 2011;11:54-8.
8. El-shamy FF, Saleh AG. Effect of electroacupoint in treatment of pain during first stage of labour. J Adv Biol. 2014;4(1):328-32.
9. Simkin P, Bolding A. Update on nonpharmacologic approaches to relieve labor pain and prevent suffering. J Midwifery Wom Health. 2004;49(6).
10. Alden KR, Lowdermilk DL, Cashion MC, Perry SE. Maternity and women's health care. 10th Ed. Elsevier Health Sciences: 2013.
11. Pillitteri A. Maternal and child health nursing: care of the childbearing and childrearing family. Lippincott Williams and Wilkins: 2013.

Ramamoorthy V, Sumathi G, Mahalakshmi V, Seetha P. Effect of transcutaneous electrical nerve stimulation on labour pain relief among primigravida and multigravida mothers. Int J Reprod Contracept Obstet Gynecol 2017;6:980-5.

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Factors Associated with Hypertension Among Truck Drivers: A Cross Sectional Study at A Check Post on A National Highway in South India

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ABSTRACT

Background: *Workers in the transport industry are at greater risk for cardio vascular diseases (CVD) due to their increased diet and sedentary behavior.* ***Aims:*** *The aim of the present study is to estimate the prevalence of hypertension among truck drivers and its associated factors.* ***Methods and materials:*** *This study was conducted at Walayar check post which is located on a National highway of South India in August 2016. A detailed physical examination was conducted by a team of medical doctors. Socio demographic information were also elicited.* ***Results:*** *The prevalence of overweight was 50% and the prevalence of hypertension was 40%. In multivariate data analysis, only history of chronic disease was significantly associated with hypertension. Overweight was not significantly associated with hypertension when controlled for age, duration of driving, smoking, marital status, and history of chronic disease (odds ratio=1.987, P=0.054). On further stratified analysis, it was found that higher age (odds ratio=3.160 P<0.001) and longer duration of driving (odds ratio=1.076 P<0.001) were significantly associated with overweight. Overweight (odds ratio=2.210 P<0.05) and history of chronic disease (odds ratio=10.178 P<0.001) were significantly associated with high blood pressure after removing both age and duration of driving from this model. Receiver operating characteristic curve analysis relating age with overweight identified 38 years as the optimum cut off value.* ***Conclusions:*** *The truck drivers must be educated at their younger age preferably early thirties to prevent overweight and thereby to reduce hypertension.*

Keywords: Truck drivers, hypertension, overweight, obesity

INTRODUCTION

The social and economic effect of increased morbidity and mortality associated with cardiovascular diseases (CVD) is a great challenge in both developed and developing countries [1-3]. Many studies have demonstrated that there is a strong correlation between risk factors of cardiovascular diseases and occupational factors [4-7]. Workers in the transport industry are at greater risk of an increased diet and sedentary behavior [8,9]. Truck drivers in India have to travel long distances in their life time on an extensive spread of national and state high ways that range from well-engineered roads to complete absence of concrete roads. Their occupation pre-disposes them to multitude of risk factors such as prolonged sitting and driving, tight running schedules, reduced rest breaks, traffic congestion, the sedentary nature of job and resultant physical, psychological, and behavioral problems [8-10]. Thus, professional drivers in particular have higher mortality, morbidity and absenteeism rates due to obesity. Hypertension is one of the main outcomes of the disease and is common among professional drivers. Considering the inherent risks associated with the profession of truck drivers, the importance of truck drivers to the country's economy and scarcity of studies aimed at evaluating the cardiovascular risk profile among these professionals, the present study is aimed to assess the prevalence of hypertension and the factors associated with hypertension among long distance truck drivers.

METHODS

Study design

This was a cross sectional study conducted at Walayar check post which is located on a National highway of South India on August 2016.

Ethics

Ethical clearance for the study was obtained from Institutional Ethical Committee, PSGIMS&R, Coimbatore.

Sample size: With an expected prevalence of hypertension as 45% [9] and with 20% allowable error, the sample size required for this study was estimated as 122. With a non-response of 20%, the total sample was 153. We totally studied 175 truck drivers.

Inclusion and exclusion criteria

Drivers who came with valid driving license for submission of documents at the check post on the day of survey and those who gave informed consent were included. Drivers with known congenital heart disease and genetic disorders were excluded.

Methodology

A detailed physical examination was conducted by a team of medical doctors. Blood pressure was measured on right arm in sitting position using digital sphygmomanometer. Two readings were taken at 3 minutes interval and the mean of the two was recorded. The following variables were also evaluated: socio demographic characteristics (age, education, family history of CVD, prior diagnosis of chronic disease), physically active, eating patterns, consumption of alcohol or other stimulants and smoking habits.

The following criteria and definitions were used [9]:

- Family history of CVD: when the individual mentions the occurrence of heart disease in a first degree relative.
- History of chronic diseases: truck drivers were asked whether they had been previously being diagnosed with any chronic diseases and what medicines they were taking.
- Subjects were considered physically active if they are performing aerobic (walking, biking, dancing, and racing) or anaerobic activities (body building) at least 150 minutes in a week and moderately active if they do 10 to 150 minutes in a week and insufficient if they do less than 10 minutes in a week.
- Subjects were considered active smokers if they use tobacco daily in any quantity or at quit smoking in the previous 12 months. Subjects were considered non-smokers if they had not used tobacco for the past 12 months.
- For determining the body mass index (BMI) the truck drivers were weighed (in kg) without shoes on a 150 kg mechanical anthropometric scale and height in meter. The BMI levels were classified according to the WHO guidelines [11]; subjects were normal if their BMI was 18.5 kg/m^2 to 24.5 kg/m^2, overweight if their BMI was 25.0 kg/m^2 to 29.9 kg/m^2, grade 1 obese if the BMI was 30.0 kg/m^2 to 34.9 kg/m^2, grade 2 obese if the BMI was 35.0 kg/m^2 to39.9 kg/m^2 and grade 3 obese if the BMI $\geq$ 40.0 kg/m^2.

Statistics

Data were analysed using SPSS (19). Mean (SD) were calculated for continuous variables and percentages were calculated for categorical variables. Logistic regression analysis was done to estimate the odds ratio and its statistical significance. Hypertension was defined as present in those with systolic blood pressure greater than or equal to 140 mm Hg or those with diastolic blood pressure greater than or equal to 90 mm Hg. For statistical analysis, BMI>25 kg/m^2 were considered as overweight. Variables significantly associated ($P<0.2$) with hypertension were subjected to multivariate regression analysis. However, due to multicollinearity, stratified analysis was further done to observe the association. Receiver operating characteristic curve analysis was then done to find the optimum cut off value.

RESULTS

The mean age of the study participants were 39.38 years with the minimum age of 22 years and the maximum of 67

years. The prevalence of overweight was 50% and the prevalence of hypertension was 40% (Table 1). In univariate analysis, positive family history, overweight, history of chronic disease, smoking, longer duration of driving, marital status and older age were significantly associated with hypertension (P<0.2) (Table 2). However, in the multivariate analysis, only history of chronic disease was found to be significant. Overweight was not significantly associated with hypertension when controlled for age, duration of driving, smoking, marital status and history of chronic disease (odds ratio=1.987; P=0.054). On further analysis, it was found that higher age (odds ratio=3.160; P<0.001) and longer duration of driving (odds ratio=1.076; P<0.001) were significantly associated with overweight. Overweight (odds ratio=2.210; P<0.05) and history of chronic disease (odds ratio=10.178; P<0.001) were significantly associated with high blood pressure after removing both age and duration of driving from this model. Receiver operating characteristic curve analysis relating age with overweight identified 38 years as the optimum cut off value. Hence it is concluded that the truck drivers must be educated at their younger age preferably early thirties to prevent overweight and thereby to reduce hypertension.

Table 1 Prevalence of hypertension and obesity among the study population

Condition	Number N=175	%
Hypertension	70	40
Raised systolic blood pressure	48	27.4
Raised diastolic blood pressure	51	29.1
Overweight/Obesity	86	49.1

Table 2 Variables associated with hypertension

Variable	Status	Number	Prevalence	Odds ratio	P value
Positive family history	Yes	73	45.2	1.449	0.234
	No	102	36.3	1	
Overweight	Yes	86	50	2.296	0.008*
	No	89	30.3	1	
Family history of chronic disease	Yes	25	84	10.821	0.000*
	No	150	32.7	1	
Smoking	Yes	98	44.9	1.558	0.136*
	No	77	33.8	1	
Old age	Yes	87	47.1	1.813	0.056*
	No	88	33	1	
Marital status (Married)	Yes	137	45.3	3.1	0.007*
	No	38	21.1	1	
Physical activity (Active)	Yes	49	38.8	0.931	0.837
	No	126	40.5	1	
Duration of driving (hours)	-	-	-	1.072	0.000*
P<0.2					

DISCUSSION

A number of studies have demonstrated that the high prevalence of obesity among workers in the transport industry. According to Moreno, et al. this category of workers has a higher incidence of obesity, physical inactivity, inadequate diet, smoking habits, higher levels of cholesterol and hypertension [12,13]. A study carried out in the United States involving more than six hundred thousand workers found the highest prevalence of obesity to be among male employees who work in the transport services [14]. The worldwide prevalence of hypertension was estimated at 1 billion individuals with approximately 7.1 million deaths occurring per year due to this condition [9]. According to the World Health Organization, systolic blood pressure greater than 115 mm Hg accounts for 62% of cases of cardiovascular disease and 49% of cases of ischemic heart disease. Moreover, arterial hypertension is one of the major risk factors of cerebrovascular accident. In our study, we observed a prevalence of 41%, with a prevalence of raised systolic blood pressure of 27.4% and a prevalence of raised diastolic blood pressure of 29.1% among truck drivers.

We observed that the truck drivers must be educated at their younger age particularly early thirties to prevent overweight and obesity and thereby to reduce hypertension. Young adulthood is a vulnerable period for weight gain and the health consequences of becoming obese during the life span are of serious concern. Unhealthy dieting habits include high sugar sweetened beverage consumption, lower vegetable intake and greater consumption of foods prepared outside

the home, which may place young adult long distance truck drivers at increased risk for overweight and obesity. To improve their nutrition and health, as they progress through the life cycle, it requires approaches specifically targeted to this group. Strategies and programs for obesity prevention of truck drivers should include both individual level and population level approaches. However, the evidence base for prevention of weight gain and halting overweight and obesity in young adulthood truck drivers is currently limited.

CONCLUSION

The data in the present study allow us to draw conclusion that long distance truck drivers are highly vulnerable to develop CVD because of the high prevalence of obesity and hypertension observed. The accumulation and association of risk factors and unique features of this profession indicate that traditional actions could not change the scenario. Appropriate health care actions based on comprehensive policies that focus on the truck drivers work environment and monitoring of these factors must be established. More research into programs tailored to the needs of young truck drivers is warranted.

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REFERENCES

[1] Gersh, Bernard. "The epidemic of cardiovascular disease in the developing world: Global implications." *Circulation* 126.Suppl 21 (2012): A606-A606.

[2] Yusuf, S., et al. "Global burden of cardiovascular diseases-part I: General considerations, the epidemiologic transition, risk factors, and impact of urbanization." *Quarterly Journal of Cardiology* 7.2 (2002): 132-139.

[3] Lopez, Alan D., et al. "Global and regional burden of disease and risk factors, 2001: Systematic analysis of population health data." *The Lancet* 367.9524 (2006): 1747-1757.

[4] Winkleby, Marilyn A., et al. "Excess risk of sickness and disease in bus drivers: A review and synthesis of epidemiological studies." *International Journal of Epidemiology* 17.2 (1988): 255-262.

[5] Albright, Cheryl L., et al. "Job strain and prevalence of hypertension in a biracial population of urban bus drivers." *American Journal of Public Health* 82.7 (1992): 984-989.

[6] Ragland, David R., et al. "Prevalence of hypertension in bus drivers." *AAOHN journal* 37.2 (1989): 71-79.

[7] Bigert, C., et al. "Time trends in the incidence of myocardial infarction among professional drivers in Stockholm 1977-1996." *Occupational and environmental medicine* 61.12 (2004): 987-991.

[8] Udayar, Sharvanan Eshwaran, et al. "Epidemiological study of cardiovascular risk factors among public transport drivers in rural area of Chittoor district of Andhra Pradesh." *International Journal of Community Medicine and Public Health* 2.4 (2017): 415-420.

[9] Hirata, Raquel Pastréllo, et al. "General characteristics and risk factors of cardiovascular disease among interstate bus drivers." *The Scientific World Journal* 2012 (2012).

[10] Sangaleti, Carine Teles, et al. "Prevalence of cardiovascular risk factors among truck drivers in the South of Brazil." *BMC public health* 14.1 (2014): 1063.

[11] Barba, Corazon, et al. "Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies." *The lancet* 363.9403 (2004): 157.

[12] Moreno, C. R. C., et al. "High risk for obstructive sleep apnea in truck drivers estimated by the Berlin questionnaire: Prevalence and associated factors." *Chronobiology international* 21.6 (2004): 871-879.

[13] Moreno, C. R. C., et al. "Short sleep is associated with obesity among truck drivers." *Chronobiology international* 23.6 (2006): 1295-1303.

[14] Caban, Alberto J., et al. "Obesity in US workers: The national health interview survey, 1986 to 2002." *American Journal of Public Health* 95.9 (2005): 1614-1622.

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Original Article

Comparison of Functional Ramp Walk Test and 6-min Walk Test in Healthy Volunteers: A New Approach in Functional Capacity Evaluation

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Abstract

Objective: Inclined surfaces or ramps are the common obstacles faced by elderly and cardiopulmonary disabled in accessing public amenities. Ramp walking is one of the most common functional demands to be met by a common man in the industrialized world. To assess the functional (uphill walking) capacity, we need a different functional stress test over the routinely used 6-min walk test (6MWT). Hence, a new 3-min steep ramp walk test (3MRWT) was constructed to meet the demands similar to an uphill walk and to provide more functional stress than routinely used 6MWT. **Methodology:** The observational, crossover study design was adopted for this study. Fifteen healthy participants (8 males, 7 females) performed both tests in a randomized order with a washout time of 6 h in between the tests. Walking distance to both ramp and ground, heart rate, blood pressure, saturation (S_pO_2), dyspnea, and fatigue with Borg exertion scale were compared prior and after the two walk tests. **Results:** The average distances covered in 6MWT were 510.5 ± 55.06 and 440.65 ± 25.08 meters and in 3MRWT were 270.18 ± 30.8, 230.05 ± 15.06 meters for male and female respectively. The difference between 3MRWT and 6MWT distances covered by the participants was statistically significant ($t = 0.893$). The mean difference between the heart rate, saturation and perceptions were highly significant ($P < 0.001$). **Conclusion:** The study results show that 3MRWT is valid over routinely administered 6MWT and may provide greater functional stress (uphill or ramp walk capacity) in a shorter duration in healthy individuals in assessing the maximal functional capacity in a ramp or uphill walking.

Keywords: 3-min ramp walk test, 6-min walk test, functional capacity, submaximal exercise test, valve replacement

Introduction

Inclined surface or ramps are the common obstacles faced by elderly and cardiopulmonary disabled in accessing public amenities. Ramp walking is one of the most common functional demands to be met by an ordinary man in the industrialized world. This component is usually neglected or overseen in human performance laboratories in the measurement of functional capacities.

Walk tests have been a fascinating attraction in the measurement of functional capacity in the modern clinical arena; 2-min walk test (2MWT), 6MWT, and 12MWT are the most common. Various exercise tests in clinical use represent increasing complexity – 6MWT, stair climbing, shuttle walk test (SWT), detection of exercise-induced asthma, and cardiac stress test – the latter being more complex than the former.[1] However, it may not check the ability of the individual to walk in slope or uphill. For the functional stress test, still step tests are utilized, but the validity is always questionable. Laboratory-based ramp walk protocols in a treadmill are not readily accessible, costly, needs expertise, and cumbersome for functional capacity testing in primary care centers.

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Uphill or ramp walking demands balance in elderly. It also requires more energy and induces much hemodynamic stress than level-walking. Walk tests are convenient, cost little, and are easy to complete. In cardiac rehabilitation, symptom-limited exercise testing with bicycle or treadmill is used traditionally.[1] However, this might not be suitable for evaluating elderly patients who have problems with balance, neuromuscular coordination, vision, or gait.[2]

To assess the functional capacity, we need a short-term operational stress test over the gold standard 6MWT and to provide an operational stress test with better sensitivity. Hence, we developed a 3-min steep ramp walk test (3MRWT) which is fulfilling the criteria of the practical walk test. Although ramp walking can be trained using the treadmill, it is not feasible for the Indian patients as they are not familiar with the treadmill and their fear may also show variation in the functional assessment. When developing the new functional walk test, it should produce a similar cardiopulmonary response or higher response than with gold standard 6MWT, so we proposed this study to compare 3MRWT with 6MWT in normal healthy individuals.

We hypothesized that none of the available field tests (either walk test or step test) measures real hemodynamic parameters or the functional stress of the persons climbing slopes or ramps. The purpose of this study was to develop a self-paced ramp walk field test to assess the functional capacity in healthy individuals.

Methodology

Our study was initiated after getting approval from the institutional human ethics committee. The participants were assigned for two tests with washout time of 6 h between the tests [Figure 1] after getting informed consent in their language (Tamil and English). This study was conducted in the ward belonging to Department of Cardiothoracic and Vascular Surgery, Pulmonary Medicine department of PSG Hospitals, Coimbatore, Tamil Nadu, India.

Participants

Healthy volunteers were recruited through advertisement in hospital notice board and local institutional weekly newspaper, Ninety five people volunteered for the study. From them, 22 volunteers were selected by picking from a bowl having names of all volunteers. Participants aged 25-65 years, both male and female were included in the study. Participants with a known history of unstable cardiac disorders such as recent angina, cardiac surgeries <2 months, and uncompensated pulmonary ailments – current asthma, bronchitis exacerbations <3 weeks were excluded from the study. The participants with current orthopedic diseases such as osteoarthritis, neurogenic claudication, new vertebral disc, facet problems <3 weeks, and fractures <2 months were excluded from the study. Furthermore, participants with movement and balance disorders such as stroke, Parkinson's, recent surgeries on lower limbs and trunk <2 months, visually challenged, balance disturbances such as vertigo were excluded from the study. Height and weight were checked using calibrated measures for all the participants.

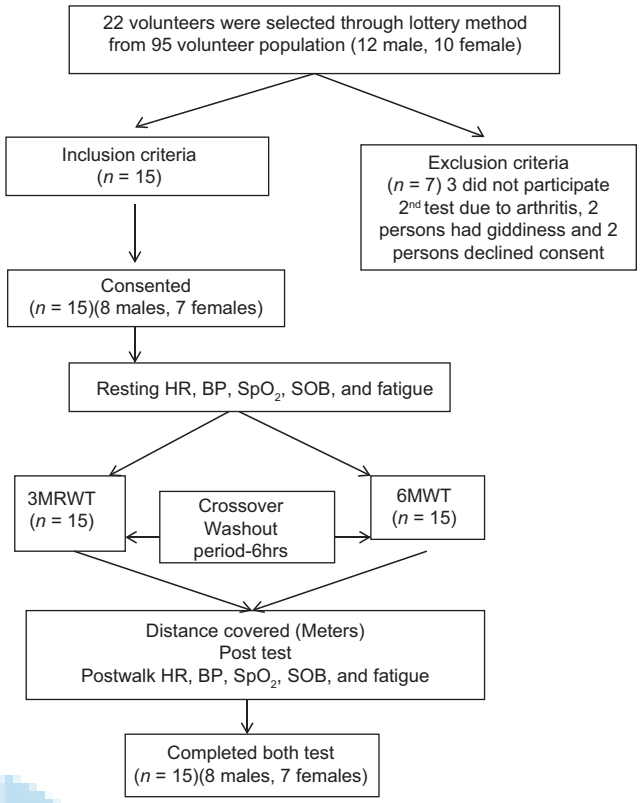


Figure 1: Flow of participants

6-min walk test

This is a self-paced walk test. It was conducted as per the American Thoracic Society (ATS) guidelines (2002). This test involves the participant to walk as far as possible in 6 min around two cones placed at 30 m. The encouragement words were delivered as per ATS guidelines at the end of every 1 min. During every lap, saturation, pulse rate, and exertion level were noted. Baseline and postwalk blood pressure was also noted.[3]

3-min Ramp walk test

This test was developed according to the standardization of 6MWT by ATS and American association of Respiratory Care, 2008. The standardization of the ramp parameters was done after auditing 15 hospitals and discussion with hospital-based Civil Engineers and contractors by the authors. The standardization of the ramp across hospitals are as follows: 20-m ramp with an elevation of 30°, 10-feet length for 1-foot steep raise, and 57 feet elevation [Figure 2]. The participant was instructed to walk in the ramp up and down for 3 min. 3MRWT is self-paced walk test such as 6-min level walk test in gauging the uphill walk capacity of the individual. Total time of test was set at 3 min because the investigators anticipated a similar exertion in walking uphill of 3 min rather than walking level ground 6 min. The encouragement similar to 6MWT was given during each minute of the test during ramp walk up and down.

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Outcome measures

The data collected during the tests were pulse rate, SpO2, and after the tests distance walked, blood pressure changes. Symptoms of breathlessness and fatigue were evaluated through modified rate of perceived exertion scale.

Data analysis and interpretation

We summarize data as mean ± standard deviation (SD) and tested between group differences using unpaired *t*-test. We tested within-group change from baseline by paired *t*-test. $P < 0.05$ was considered statistically significant. Analysis was done using SPSS 17.0 software (SPSS Statistical Package, 2007, Chicago, IL, USA).

Results

Fifteen participants including 7 females and 8 males completed the study. All participants found it easy to pace themselves and no difficulties were encountered in administering both tests. The characteristics of the participants are described in Table 1. Both males and females performed well in the both walk tests [Figure 3]. Male participants walked better in both trials; it may be due to heterogeneity in anthropometry, physical activity, and motivation. Both genders showed a better performance in the 3MRWT rather than the 6MWT. The distance covered in both tests for male and female, respectively, in 6MWT was 510.5 ± 55.06, 440.65 ± 25.08 meters and in 3MRWT was 270.18 ± 30.8, 230.05 ± 15.06 m.

Table 2 summarizes the changes in cardiorespiratory parameters during the 3MRWT and 6MWT. It shows the mean ± SD values of SpO_2, heart rate, systolic and diastolic blood pressure, at the

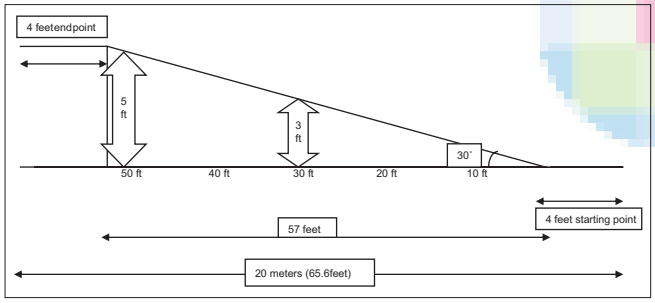


Figure 2: Schematic depiction of ramp parameters

Table 1: Characteristics of the participants

Demographics	Mean±SD
Age, years	43.67±11.62
Height, cm	154.4±7.88
Weight, kg	65.27±10.32
BMI, kg/m^2	25.91±3.83
SpO_2 (baseline), %	98.4±0.65
HR (baseline), beats/min	77.86±8.15
Mean arterial BP (baseline), mmHg	102±9.96

SD=Standard deviation, BP=Blood pressure, HR=Heart rate, BMI=Body mass index, SPO_2=Oxygen saturation

Table 2: Change in cardrespiratory parameters during 3-min ramp walk test and 6-min walk test

	3 MRWT	6 MWT	Paired *t*	Significance (*P*)
HR (beats/min)				
Baseline	78±9	78±8	3.802	0.002
End	121±18	112±14		
Change from baseline	43±13	34±10		
Mean arterial BP (mmHg)				
Baseline	97±8	102±10	2.598	0.021
End	102±7	103±6		
Change from baseline	6±6	1±8		
Saturation (%)				
Baseline	98±1	98±1	1.702	0.111
End	97±1	97±1		
Change from baseline	−2±1	−2±1		

3MRWT=3-min steep ramp walk test, 6MWT=6-min walk test, BP=Blood pressure, HR=Heart rate

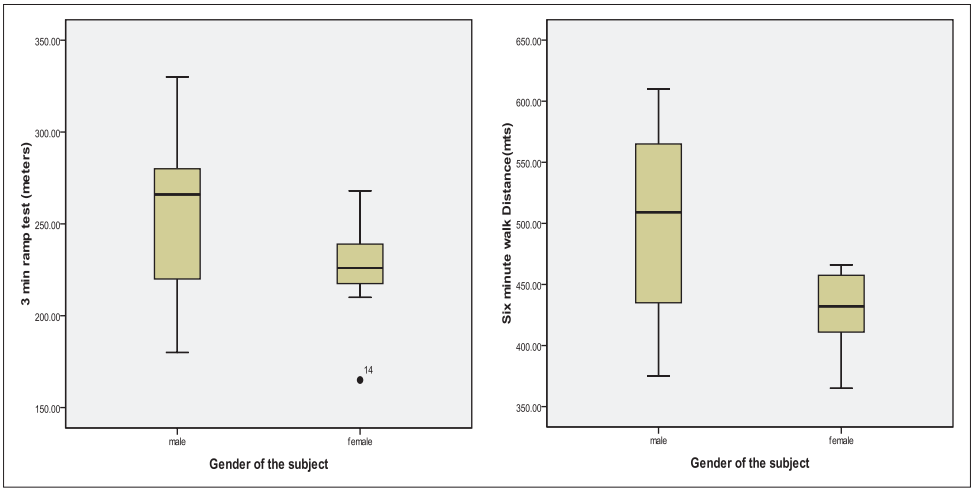


Figure 3: Gender difference in distance walked

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beginning and the end of each exercise test. As compared to the 6MWT, the mean change in heart rate and arterial blood pressure were higher after the 3MRWT. These differences were statistically significant. However, the change in oxygen saturation was not significantly different. Moreover, no difference was observed in the symptoms of shortness of breath and fatigue after the two tests, since all of them were healthy participants.

This study results show that there is a significant difference between 3MRWT and 6MWT in assessing the maximal functional capacity of healthy individuals.

Discussion

We presented here preliminary results on normal healthy individuals to find a short-term functional stress test over the gold standard 6MWT and to provide an operational stress test with better sensitivity. Hence, we developed a 3MRWT which is fulfilling the criteria of the practical walk test. Our new functional walk test produced a higher cardiopulmonary response than routinely employed 6MWT.

Like the 6MWT, 3MRWT was also easy to administer, better tolerated, and reflects activities of daily living better than the other walk tests. It could be a useful measure of functional capacity to assess people with at least moderately severe impairment. The test could be used for preoperative and postoperative evaluations and for measuring the response to therapeutic interventions in patients with cardiac and pulmonary diseases.[3]

A recent review by De Feo *et al*., found that the assessment of the functional physical capacity by 6MWT early after cardiothoracic surgery may be useful to adopt the modalities of the training program to obtain the best result at the most appropriate training intensity.[4] Likewise, our 3MRWT also may be helpful in rehabilitation area to assess functional capacity and to guide operational activities.

In a randomized controlled trial involving patients with chronic obstructive pulmonary disease (COPD), cardiorespiratory performance during 6MWT and SWT were compared. Patients performed two 6MWTs and two SWTs in a randomized order on the same day. In this trial, SWT and 6MWT induced a similar cardiorespiratory performance in patients recovering from acute exacerbation of COPD. Both tests were reproducible and feasible even in hospitalized patients.[5] This trial formed the basis of our study.

In another randomized, double-crossover experimental study in patients with COPD showed that course length (10 m vs. 30 m) substantially influences the performance of patients in a 6MWT. The statistical and clinically meaningful difference in 6MWD in patients with COPD, singly depending on the length of the walk course, highlights a practical problem. Existing reference equations cannot be applied to predict the walking distance in the frequently used 6MWT on a 10 m course for people with COPD, due to a substantial overestimation. Unique reference equations for the 6MWT on a 10-m course seem necessary.[6]

The tests were performed only once to demonstrate the changes in cardiorespiratory parameters. For the results to be more significant, double cross-over method can be adopted. The participants of the study were not equally recruited according to the gender category (8 males, and 7 females). Each gender and age group may show some variation during the test. Further, the results may not be extrapolated to clinical populations. Due to lesser sample size, inherent variation in the baseline is inevitable and the washout period shall be prolonged.

This study can be conducted with composite sample across the country as a multicenter trial with healthy individuals, cardiorespiratory conditions such as COPD, coronary artery bypass grafting, and lung surgeries and presurgery screening, outcome measure in a cardiopulmonary rehabilitation and other functional capacity evaluation areas, exercise testing areas to prove the sensitivity of 3MRWT.

This study suggests that the staged incremental response may be similar in both tests that imply that 3MRWT may be an efficient possibly as equivalent to the gold standard 6MWT in clinical exercise testing arena. Thus, we propose that 3MRWT may provide a greater hemodynamic stress in the smaller time frame. As 3MWT takes smaller time frame, it may be reproducible that can be allowed for future studies to investigate.

Conclusion

In summary, 3MRWT might be an efficient alternative to the 6MWT in eliciting better cardiorespiratory stress responses to assess the maximal functional capacity. The 3MRWT is simple, easy to administer, requires no equipment, time and cost saving, safe, and is reproducible.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Wasserman K, Hanson JE, Sue DY, *et al*. Principles of Exercise Testing and Interpretation. 3rd ed. Philadelphia: Lippincott, Williams & Wilkins; 1999.
2. Weisman IM, Zeballos RJ. An integrated approach to the interpretation of cardiopulmonary exercise testing. Clin Chest Med 1994;15:421-45.
3. ATS board of directors, guidelines for the six minute walk test. Am J Respir Crit care Med 2002;166:111-7.
4. De Feo S, Tramarin R, Lorusso R, Faggiano P. Six-minute walking test after cardiac surgery: Instructions for an appropriate use. Eur J Cardiovasc Prev Rehabil 2009;16:144-9.
5. Vagaggini B, Taccola M, Severino S, Marcello M, Antonelli S, Brogi S, *et al.* Shuttle walking test and 6-minute walking test induce a similar cardiorespiratory performance in patients recovering from an Acute Exacerbation of Chronic Obstructive Pulmonary Disease. Respiration 2003;70:579-84.
6. Beekman E, Mesters I, Hendriks EJ, Klaassen MP, Gosselink R, van Schayck OC, *et al.* Course length of 30 metres versus 10 metres has a significant influence on six-minute walk distance in patients with COPD: An experimental crossover study. J Physiother 2013;59:169-76.